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Design, Synthesis and Biological Evaluation of New Anti-VZV Agents

Marco Migliore

A thesis submitted to Cardiff University for the Degree of

PHILOSOPHIAE DOCTOR

The Welsh School of Pharmacy

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.....to my Father

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Abstract

An introduction to this work presented in this thesis involves an overview of nucleic acids and the use of nucleoside analogues in antiviral therapy.

Bicyclic furano pyrimidine nucleosides (BCNA)s were discovered by the McGuigan group as potent and selective inhibitors of Varicella-Zoster Virus. A brief description of Structure Activity Relationships of this class of compounds is presented, identifying a long lipophilic chain as a specific requirements for antiviral activity.

We herein report the synthesis and biological evaluation of a new series aimed to further investigate the specific requirement for biological acitivity.

Two sites of BCNAs were modified on the lead compound, the side chain and the sugar moiety.

A series bearing electron-donating and electron-withdrawing aryl groups was synthesised in order to investigate the role of the position of the substitutent.

Then, the phosphoramidate approach was applied to the lead compound in order to broaden the spectrum of activity, which was limited only to VZV.

Modifications of the sugar moiety include the inversion of all the stereo-centres of the lead compound, obtaining the L-enantiomer, and the inversion of the stereochemistry at the C-1' obtaining thus the α-derivative. Furthermore the replacement of the furano ring of the sugar with a cyclopentane was thought as a good strategy in order to increase the resistance to enzymatic cleavage.

Given the poor bioavailability of the lead compound, the valyl ester was synthesised making also the hydrochloric and succinate salts to increase the chemical stability and water solubility

Finally, using the intrinsic fluorescence of these derivatives, a cell study was carried out in order to investigate the distribution of the compound inside the cell.

Table of Contents

Abbreviations and Acronyms

3TC	(Lamivudine
5-FU	5-Fluorouracil
Ac	Acetyl
ACN	Acetonitrile
ACV	Acyclovir
AIBN	2,2'-Azoisobutyronitrile
Ara-A	Vidarabine
AUC	Area Under the Curve
AZT	Zidovudine
BCNA	Bicyclo nucleoside analogue
Bn	Benzyl
Boc	Butyloxycarbonyl
BVaraU	Sorivudine
BVdU	Brivudin
BVU	*E*-5-(2-bromovinyl)uracil
Bz	Benzoyl
CAN	Ceric ammonium nitrate
CBV	Carbovir
CDI	Carbonyl diimidazole
CHN	Elemental Analysis
LogP	Calculated LogP
CMV	CytomegaloVirus
d4T	Stavudine
DBAD	Ditertbutylazodicarboxilate
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
ddC	Zalcitabine
ddI	Didanosine
DIBAH	Diisobutylaluminium hydride
DIPEA	Diisopropylethylamine

DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
DP	Diphosphate
DPD	Dihydro pyrimidine dehydrogenase
EBV	Epstein-Barr virus
EDU	5-ethynyl-2'-deoxyuridine
eq.	Equivalents
FCV	Famcyclovir
FddA	Iodenosine
Fmoc	Fluorenylmethyloxycarbonyl
FTC	Emtricitabine
GCV	Ganciclovir
HCMV	Human cytomegalo virus
HIV	Human immunodeficiency virus
HMDS	Hexamethyldisilazane
HPLC	High performance liquid chromatography
HPMPC	Cidofovir
HSV	Herpes simplex virus
IDA	Isotopic dilution assay
IDU	Idoxyuridine
MCPBA	Metachloro perbenzoic acid
MP	Monophosphate
NDP	Nucleoside diphosphate
NMR	Nuclear magnetic resonance
PCV	Pencyclovir
PIs	Protease inhibitors
PMEA	Phosphonyl methoxy ethyl adenine
PK	Phospho kinase
PK-	Phospho kinase deficient
ppm	parts per million
PS	Polymer Support
Pyr	Pyridine
RNA	Ribonucleic acid
SARs	Structure Activity Relationships

SI	Selectivity Index
TBAB	Tetrabutylammonium bicarbonate
TBDMS	^{t}Butyldimethylsilyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TK	Thymidine kinase
TK-	Thymidine kinase deficient
TLC	Thin layer chromatography
TMS	Trimethylsilyl
Tos	Tosyl
TP	Triphosphate
TPase	Thymidine phosphorylase
TPP	Triphenylphosphine
UPase	Uridine phosphorylase
VZV	Varicella zoster virus

Chapter 1: Introduction

1.1 Constituents of DNA and RNA: Nucleosides and Nucleotides[1]

The nucleic acids DNA and RNA are very suitable carriers of genetic information due their covalent structures. These macromolecules are *linear polymers* built up from similar units connected end to end. Each monomer unit within the polymer is a *nucleotide* that consists of three components: a sugar, a heterocyclic base and a phosphate group. The sequence of bases in the polymer uniquely characterizes a nucleic acid and constitutes a form of linear information.

The sugar in deoxyribonucleic acid (DNA) is deoxyribose. The deoxy prefix indicates that the 2'-carbon atom of the sugar lacks the oxygen atom. The sugars in nucleic acids are linked to one another by phosphodiester bridges. Specifically, the 3'-hydroxyl (3'-OH) group of the sugar moiety of one nucleotide is esterified to a phosphate group, which is joined to the 5'-hydroxyl group of the adjacent sugar.

Two of the bases of DNA are derivatives of purine, adenine (A) and guanine (G) and two of pyrimidine, cytosine (C) and thymine (T). Ribonucleic acid (RNA) differs from that of DNA in two respects. Firstly, the sugar units are riboses rather than deoxyriboses. Secondly, thymine (T) is replaced by the base uracil (U).

A unit consisting of a nitrogen base bonded to a sugar is referred to as a nucleoside. The four nucleoside units in RNA are called adenosine, guanosine, cytidine, and uridine, whereas those in DNA are called deoxyadenosine, deoxyguanosine, deoxycytidine, and thymidine. In each case, N-9 of a purine or N-1 of a pyrimidine is attached to C-1' of the sugar. The base lies above the plane of sugar when the structure is written in the standard orientation; that is, the configuration of the N-glycosidic linkage is β. The site of attachment in naturally occurring nucleotides is the hydroxyl group attached to C-5' of the sugar. A compound formed by the attachment of a phosphate group to the C-5' of a nucleoside sugar is called a nucleoside 5'-phosphate or a 5'-nucleotide (fig 1.1).

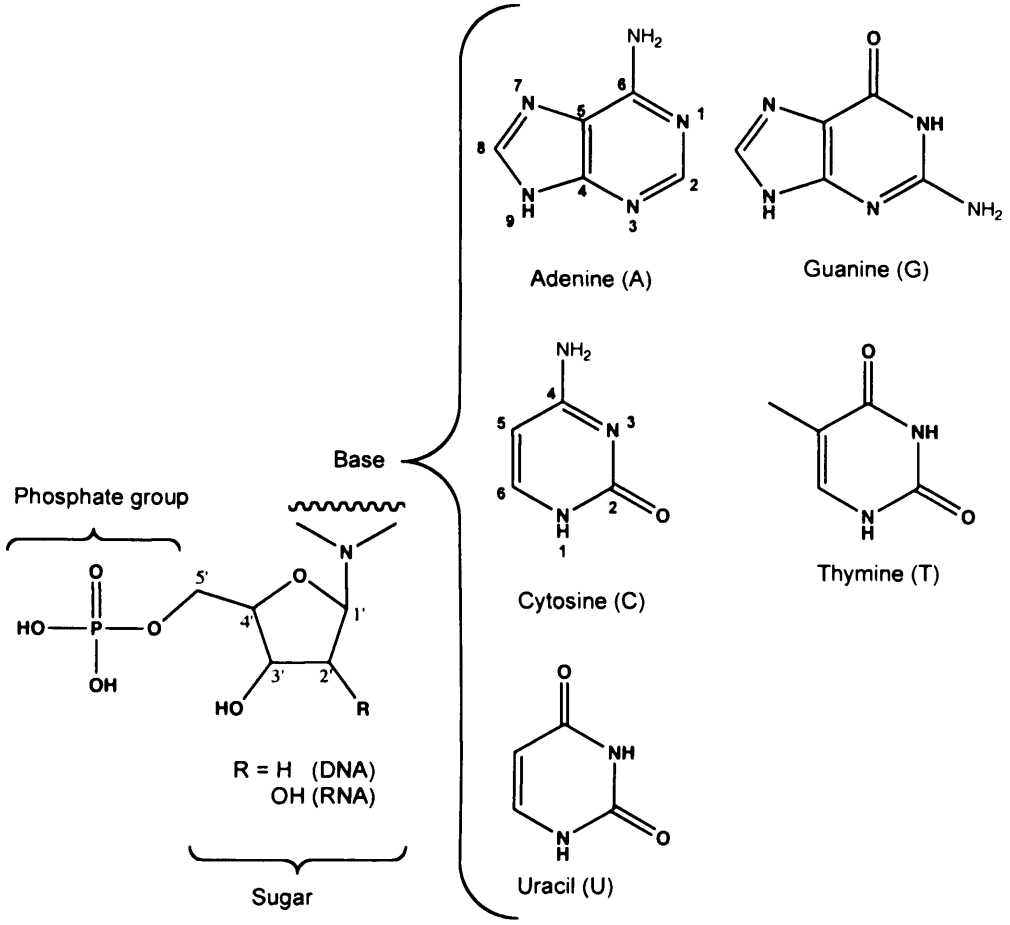

Fig 1.1: structure of nucleotides in DNA and RNA

1.2 The double helix is stabilised by hydrogen bonds and hydrophobic interactions[1]

The formation of specific base pairs was discovered during the determination of the three-dimensional structure of DNA by the use of X-ray diffraction. The characteristics of these diffraction patterns indicated that DNA is formed of two chains that wind in a regular helical structure. From these data and others, Watson and Crick deduced a structural model for DNA that accounted for the diffraction pattern.

The features of the Watson - Crick Model of DNA deduced from the diffraction patterns are:

1. Two helical polynucleotide chains are coiled around a common axis and run in opposite directions.

2. The sugar-phosphate backbones are on the outside and the purine and pyrimidine bases lie on the inside of the helix.

3. The bases are nearly perpendicular to the helix axis, and adjacent bases are distant 3.4 Å. The helical structure repeats every 34 Å, so there are 10 bases per turn of helix.
4. The diameter of the helix is 20 Å.

Watson and Crick discovered that by hydrogen bonds guanine could be paired with cytosine and adenine with thymine to form base pairs that have essentially the same shape (fig 1.2). These base pairs are held together by specific hydrogen bonds, which stabilise the helix because of their large numbers in a DNA molecule. These *base-pairing rules* account for the observation, originally made by Erwin Chargaff in 1950, that the ratios of adenine to thymine and of guanine to cytosine are nearly 1:1 in the DNAs of all species studied.

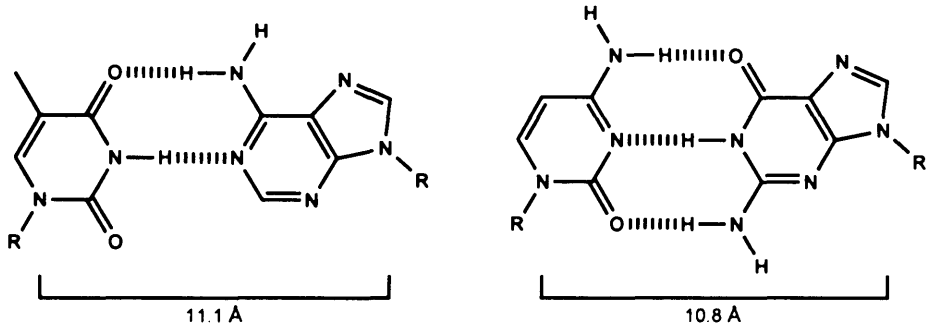

Fig 1.2: base pairs

Inside the helix, the bases are stacked one on top of another. The stacking of base pairs contributes to the stability of the double helix in two ways. First, the double helix is stabilised by the hydrophobic effect. The hydrophobic bases cluster in the interior of the helix away from the surrounding water, whereas the polar surfaces are exposed to water. The stacked base pairs attract one another through Van der Waals forces. In addition, base stacking in DNA is favored by the conformations of the somewhat rigid five-membered rings of the backbone sugars.

1.3 DNA replication

Replication is the fundamental molecular process to pass genes from one generation to the next by copying a double strand of DNA. It starts with the unwinding and separation of DNA creating the replication fork (fig 1.3). The two strands of parent DNA are used as a template for the synthesis of new DNA by the DNA-polymerase[2] (three different polymerases have been found in eukaryotic cells, DNA-polymerase I, II and III)[3] adding deoxynucleoside 5'-

triphosphates at the terminal 3'-OH of the growing DNA strand. A primer with a free 3'-OH is needed for this process, which is synthesised by the RNA-polymerase primase at the replication fork. Once this primer is made, DNA synthesis can begin, elongating the polynucleotide chain originating with the RNA primer. Due to the fact that the two strands of DNA are antiparallel, the leading strand can be copied continuously down its entire length in direction 5'→3'. However, the other parent strand, called lagging strand, must be copied discontinuously in short fragments (Okazaki fragments)[4] as the DNA unwinds in the direction 5'→3'. Both the strands are synthesised by DNA polymerase III, which also has nuclease activity with error correction capacity. DNA polymerase I removes the primer and fills the gaps between the Okazaki fragments. Finally, DNA ligase joins the fragments.

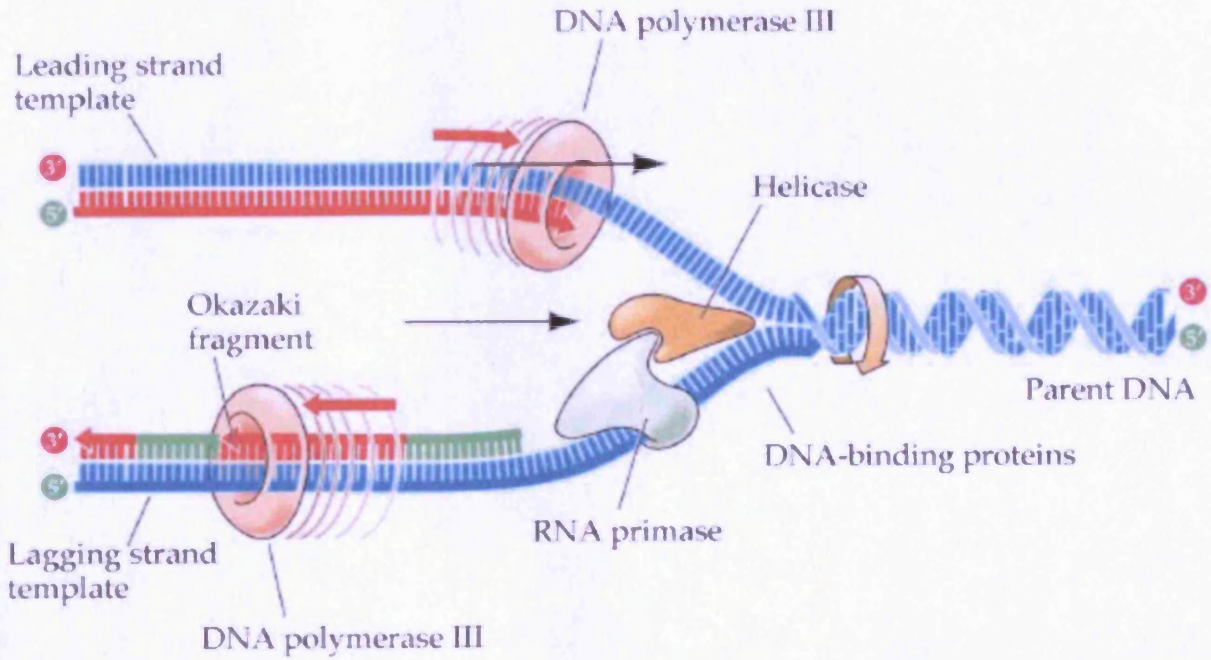

Fig 1.3: replication fork[5]

1.4 Nucleoside analogues as antiviral agents

Viruses can be defined as packets of infectious nucleic acid surrounded by protective coats.[6] Unlike bacteria or eukaryotic cells, viruses are unable to generate metabolic energy or to synthesise proteins. They also differ from cells in having either DNA or RNA, but not both. Due to the extreme simplicity of their reproductive apparatus, they are obligate parasites, that is, they multiply exclusively within living cells, forcing the host cell to synthesise viral proteins and viral nucleic acids, followed by final assembly into new virions. A virus is biologically silent outside the cell, and it becomes biologically active and pathogenic only when it has entered the host cell. The viral genome always encodes one or more enzymes, which have vital functions in the replication of the viral nucleic acids. A chemotherapeutic agent that could selectively interact with such viral enzymes, thus disrupting the synthesis of viral DNA or RNA at non-toxic concentration for the host cell, would have the potential as an antiviral drug.

When a virus takes over the host cell's biosynthetic machinery, the replication of its genome is fundamental to viral reproduction. It is not surprising that nucleoside analogues, interacting primarily with the replication of the viral genome, constitute the major class of compounds that exhibit significant in vitro and in vivo antiviral activity. Viral polymerases use nucleoside triphosphates as monomeric substrates, in the same way as cellular DNA polymerases use deoxynucleoside triphosphates for the replication of cellular DNA. This means that a nucleoside analogue must be available in the triphosphate form to interact with viral polymerases. Most nucleoside derivatives are phosphorylated within the cell by viral or cellular kinases[7] to the 5'-monophosphate analogue, which is further converted into the triphosphate form, the active form of the drug. Once the nucleoside is converted into the triphosphate form, it could act either as an inhibitor of the viral polymerase or as a substrate for the viral polymerase and thus being incorporated in the growing viral DNA. These two mechanisms of action may sometimes coexist in the same antiviral nucleoside analogue. [8]

Selective inhibition of the viral polymerase at non-toxic concentrations for the host cell may be achieved by selective activation by the viral kinase, but not by the cellular kinase (i.e. acyclovir in HSV-1) or by activation in both infected and non-infected cells, but then the triphosphate form being a good substrate only for the viral polymerase and not for the cellular DNA polymerase (i.e. AZT in HIV).

1.5 Pyrimidine analogues phosphorylated by viral thymidine kinases

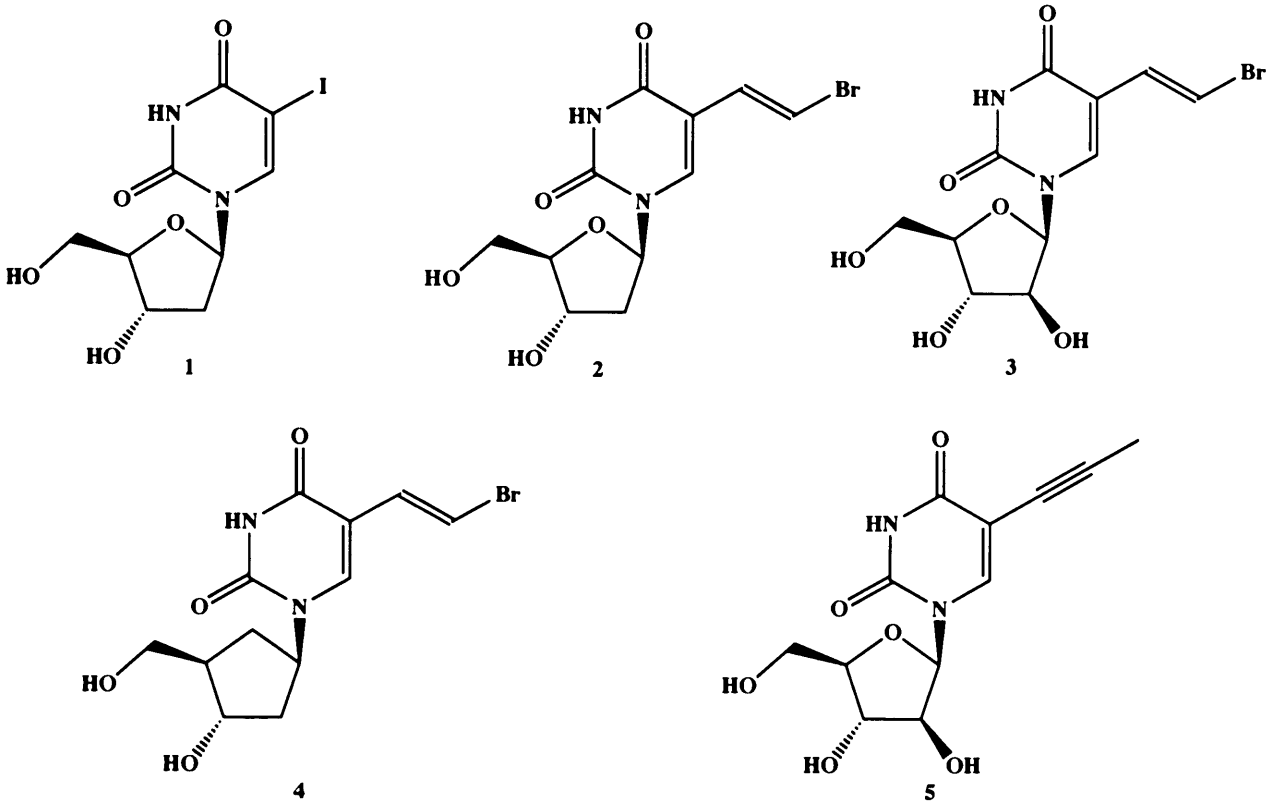

Fig 1.4: 5-substitued uridine nucleosides

Iodoxuridine (5-Iodo-2'-deoxyuridine, IDU, **1**) was the first antiviral nucleoside - discovered in the late fifties[9] - that showed to be an effective treatment of HSV infections.[10] In HSV infected cells, IDU is phosphorylated by the viral kinase to monophosphate, which is further converted to the active triphosphate. IDU triphosphate acts as an inhibitor/substrate for viral DNA polymerase. In fact the replacement of thymidine by IDU in the viral DNA seems to be primarily responsible for the antiviral activity of IDU.[8] IDU is phosphorylated by normal cellular enzymes as well as by virally encoded enzymes, ultimately producing IDU-triphosphate in normal cells, and thus incorporating IDU into cellular DNA resulting in toxicity.[9,11] The unfavourable therapeutic index of IDU prompted the search for more selective and less toxic anti-herpes agents. These studies resulted in the discovery of potent inhibitors of HSV and VZV, like Brivudine[12] (5-[(E)-(2-bromovinyl)]-2'-deoxyuridine, BVdU, **2**) which is selectively phosphorylated by the viral thymidine kinase of HSV-1 and VZV, to the mono- and diphosphate form[13,14], then cellular enzymes mediate the third

phosphorylation. HSV-2 TK is unable to phosphorylate BVdU-monophosphate to the corresponding diphosphate, and this accounts for the observed selectivity of the compound against HSV-1.[13]

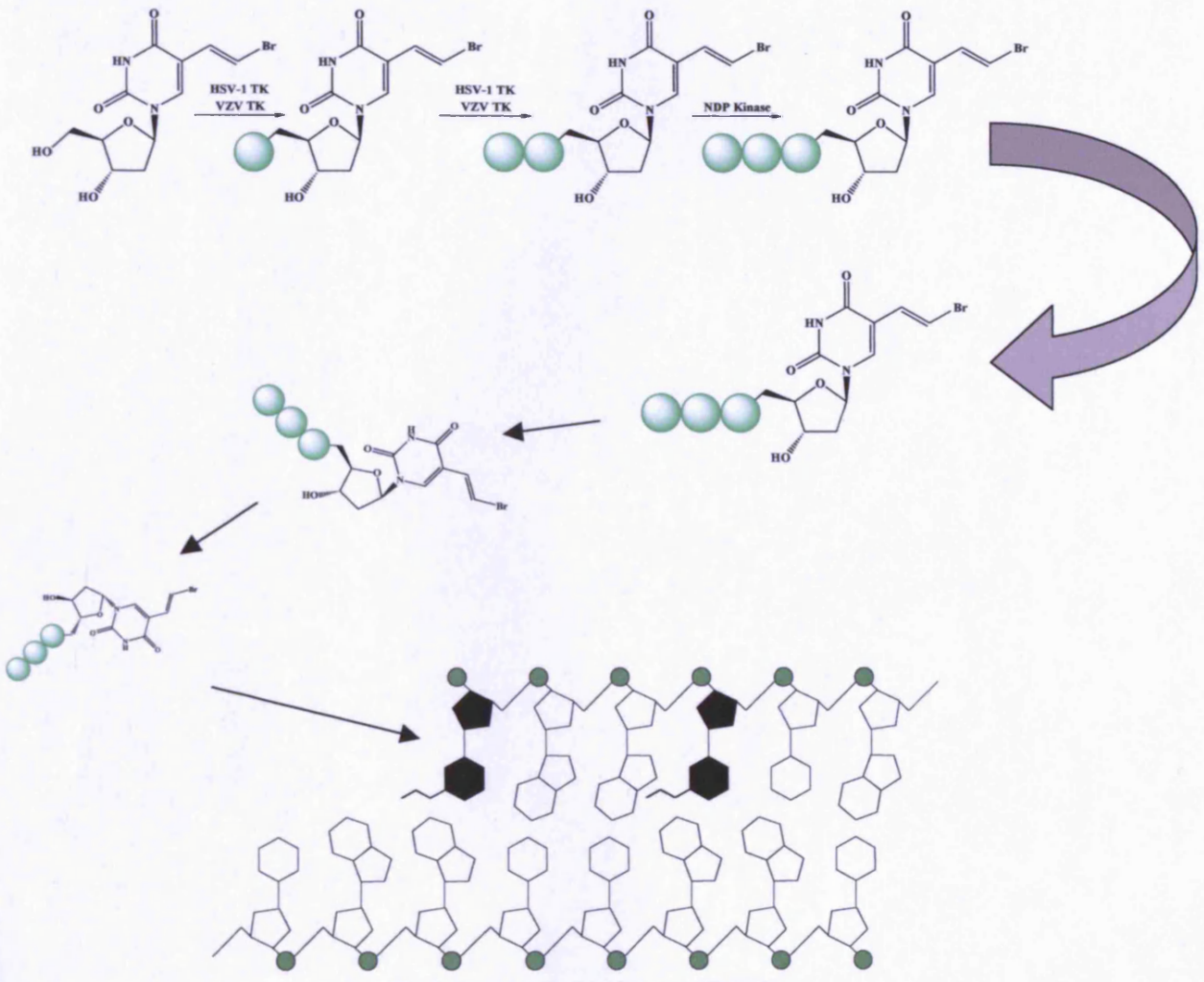

Fig 1.5: activation pathway of BVDU

BVdU-triphosphate may act either as a competitive inhibitor of viral DNA or as a substrate for this enzyme. When acting as a substrate, the compound is integrated into viral DNA affecting stability and functioning of the viral DNA.[15,16] Sorivudine (5-[(E)-(2-bromovinyl)]-uracil arabinoside, BVaraU, **3**) exhibits high potency against HSV-1 and particularly good activity against VZV.[17] As BVdU, BVaraU shows a considerable specificity in its antiviral activity: it is highly potent against HSV-1, but not HSV-2.[18] The carbocyclic analogue of BVdU (**4**) also proved to be a potent and selective inhibitor of HSV-1 and VZV, but not as good as the parent nucleoside analogue.[19]

Another antiviral nucleoside analogue, the activity of which depends on selective activation by the viral TK, is Netivudine[20] ((1-β-D-arabinofuranosyl)-5-prop-1-ynyl-uracil, 882C, **5**). This compound is a potent and selective inhibitor of VZV replication. As with the other pyrimidine analogues, it is selectively converted to the monophosphate form by VZV TK, which also mediates the second phosphorylation. HSV-TK is able to convert netivudine to netivudine-MP, but then does not catalyse the following conversion to the diphosphate, which explains the selectivity of the compound for VZV.[21]

1.6 Pyrimidine analogues phosphorylated by cellular thymidine kinase

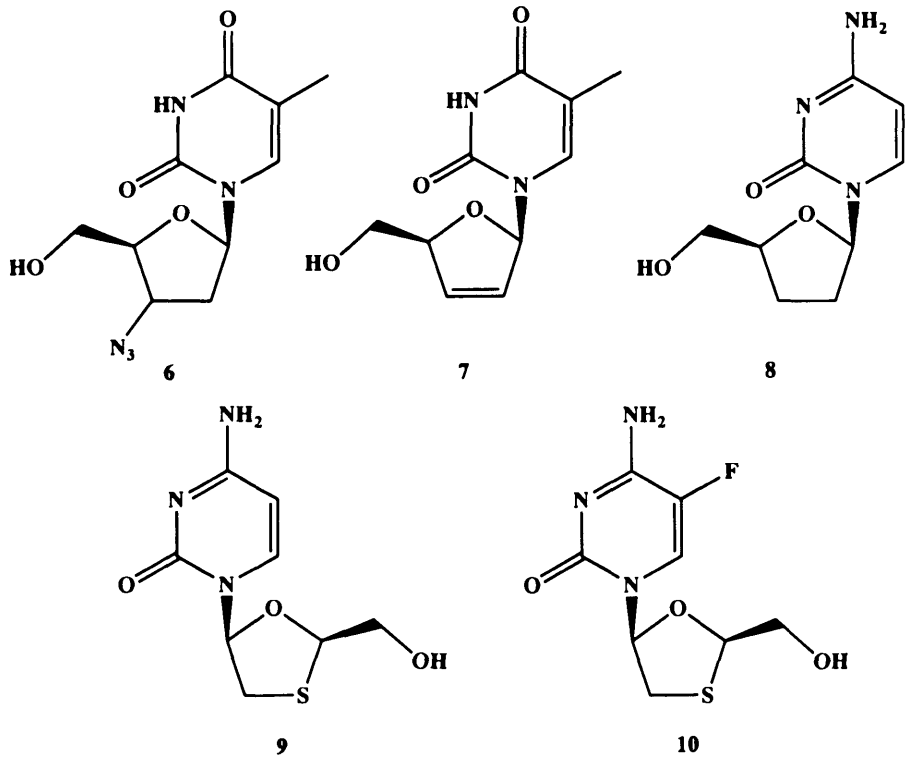

Fig 1.6: Anti-HIV dideoxypyrimidine nucleosides

Not every virus encodes its own specific TK and in the case of HIV, a nucleoside analogue must be a good substrate for the cellular kinases, in order to be phosphorylated to the triphosphate form. An example of a virus that does not encode for a specific TK is HIV. Since the anti-HIV nucleosides need to be phosphorylated by the cellular kinases in order to be active, only minor modifications in the structure of natural nucleosides would be tolerated in order to be a good substrate and to be activated to the triphosphate form.

Azidothymidine (AZT, zidovudine **6**) is the prototype of the pyrimidine dideoxynucleoside analogues that dominate the treatment of HIV infection.[22] Its initial discovery led to the development of several other related nucleosides with antiviral activity against HIV, such as Stavudine[23] (d4T, didehydrodideoxythymidine, **7**) and Zalcitabine[24] (ddC, dideoxycytidine, **8**). In addition, several cytidine analogues with the "unnatural" L-configuration have been found to selectively inhibit HIV replication and these compounds are now in the market: 2',3'-dideoxy-3'-thiacytidine (Lamivudine,[25] 3TC, **9**), and its 5-fluoro-substituted counterpart 2',3'-dideoxy-5-fluoro-3'-thiacytidine (Emtricitabine,[26] FTC, **10**).

All pyrimidine dideoxynucleoside analogues exhibit the same mechanism of action: they need to be phosphorylated to the triphosphate form and then they can act as inhibitors/substrates/chain terminators at the HIV reverse transcriptase level. This mechanism of action, originally demonstrated for AZT,[27] seems to be applicable to several other dideoxynucleoside analogues, including the L-dideoxycytidine analogues.[28,29]

1.7 Acyclic guanosine analogues

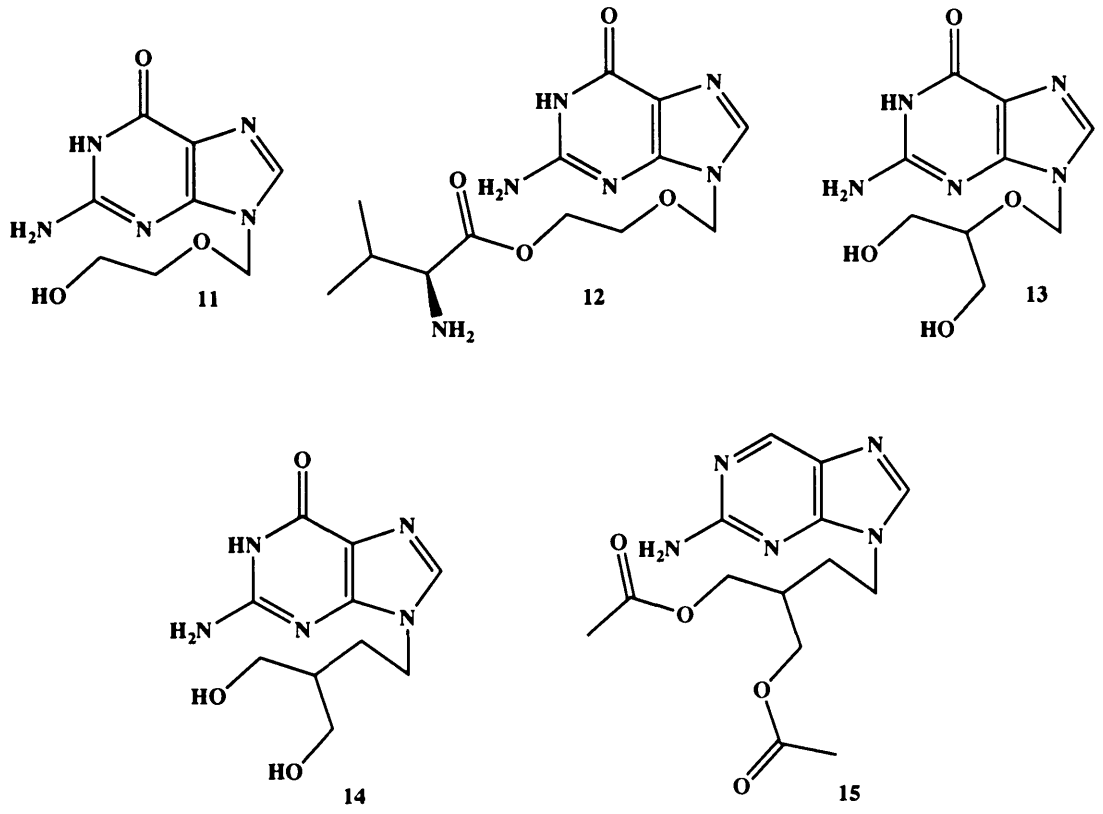

Fig 1.7: Acyclic guanosine nucleosides

The first acyclic nucleoside with selective antiviral activity against HSV-1, HSV-2 and VZV was acyclovir[30] (ACV, **11**), becoming the treatment of choice for most of the herpesvirus infections. Acyclovir is highly active against HSV, even if it is 50 fold less active against VZV.[31] The compound is recognised by the viral TK[32] and it is selectively phosphorylated in infected cells. The monophosphate is then further phosphorylated by the cellular kinases to its active form of triphosphate.[33,34]

Acyclovir triphosphate inhibits viral DNA synthesis by acting as a substrate for viral DNA polymerase.[31] Furthermore, while being a potent inhibitor of viral DNA polymerase, ACV-triphosphate is a poor inhibitor of cellular DNA polymerases.[31] Thus the selectivity of acyclovir is achieved at two different levels: the compound is activated in infected cells only, and its active form is only recognised by the viral enzyme. ACV-triphosphate also acts as substrate for viral DNA polymerase.[35] When acting as a substrate of the viral DNA polymerase, it is incorporated into the growing DNA strand acting as a chain terminator.[36]

A big disadvantage of acyclovir is its poor oral bioavailability. To overcome this problem valacyclovir[37] (**12**), the L-valyl-ester of acyclovir, has been developed as a prodrug. Valacyclovir is hydrolysed in the body to acyclovir, giving a greater oral bioavailability compared to acyclovir itself.

Ganciclovir[35] (GCV, **13**) is highly active against HSV-1 and HSV-2, only poorly active against VZV, but more active than ACV against HCMV (Human Cytomegalovirus). Although GCV is phosphorylated by the viral TK in HSV infected cells, HCMV does not code for a thymidine kinase. However, it is known that HCMV encodes a protein, the gene product UL97, responsible for the rate-determining initial phosphorylation of GCV.[38] This enzymatic difference of HCMV compared to other herpesviruses explains why the majority of anti-HSV and anti-VZV nucleosides often prove to be inactive against HCMV. GCV-TP appears to act both as a faulty substrate and as an inhibitor of HCMV DNA polymerase.[39]

The corresponding carbocyclic equivalent of ganciclovir is penciclovir[40] (PCV, **14**), which is reported to be similar to that of ACV, in that it shows potent activity against HSV and VZV, but only weak activity against HCMV.[41]

Famciclovir[42] (FCV, **15**), is an orally bioavailable prodrug of penciclovir that is metabolised to PCV by de-acetylation and 6-oxidation of the purine ring.

1.8 Purine Analogues

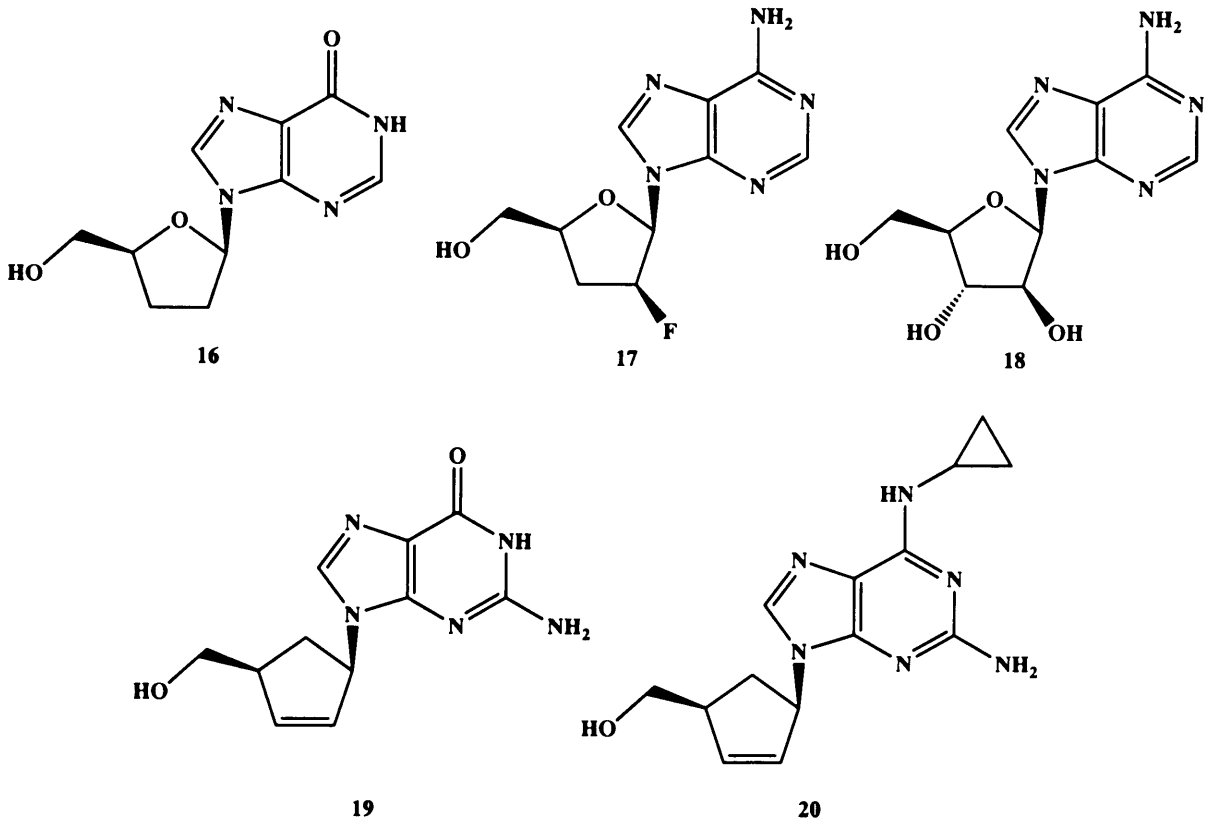

Fig 1.8: Purine analogues

Other purine analogues have been developed as antiviral agents, in particular some dideoxynucleoside analogues show to be active against HIV. Didanosine[43] (2',3'-dideoxyinosine, ddI, **16**) is an alternative treatment to AZT[44] (**7**). ddI is phosphorylated to ddIMP , converted to ddAMP and then converted to ddATP which is the active species.[45] ddI was one of the first purine analogues and other purine derivatives have been synthesised. Iodenosine[46] (2'-fluoro-2',3'-dideoxyarabinosyladenine, FddA, **17**) was rationally designed as a more chemically and enzymatically stable anti-AIDS drug than its parent compound didanosine. Vidarabine[47] (9-β-D-arabinosyladenine, Ara-A, **18**) was the first nucleoside antiviral analog to be given systemically and was the first agent to be licensed for the treatment of systematic herpes virus infection in man, although it has limited potency. Carbovir[48] (carbocyclic analog of 2',3'-dideoxy-2',3'-didehydroguanosine, CBV, **19**) is a potent inhibitor of HIV, but the poor bioavailability and the toxicity limited its potential clinical use.[49] Abacavir[50] (1592U89, **20**) was originally synthesised to overcome the poor availability and it can be considered as a prodrug of CBV. Within the cell it is converted to

CBV-MP and further into CBV-TP, which is the active metabolite against HIV.[51] The anti-HIV mechanism of the purine dideoxynucleoside analogues is similar to that of the pyrimidine dideoxynucleoside analogues. They are converted into the triphosphate form and then they can interact with HIV reverse transcriptase as inhibitor / substrate / chain terminator.

1.9 Acyclic nucleoside analogue phosphonates

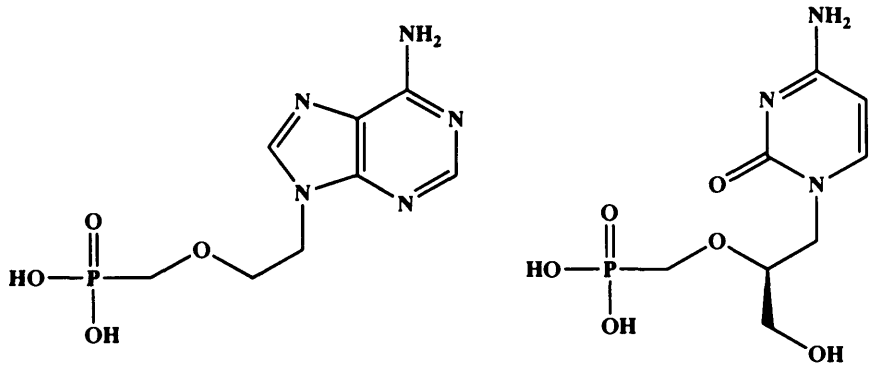

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Fig 1.9: Acyclic nucleoside phosphonates

The activity of acyclic nucleosides analogues depends on the phosphorylation steps, a process that it does not occur in cells deficient of kinases. A rational approach to by-pass this requirement is to develop a monophosphate form of the acyclic nucleosides, but it has limited use as a drug for two main reasons. First, the charge of the phosphate group prevents the drug crossing the cell membrane and second shows instability in vivo due to the rapid hydrolysis. To overcome this problem, a new class of nucleoside analogues has been develop of introducing a phosphonate moiety that is far more chemically and metabolically stable than the phosphate moiety.

PMEA[52] (**21**) is active against HSV, EBV and HIV. Its diphosphate acts as a potent inhibitor of HSV DNA-polymerase as well as chain terminator when it is incorporated into viral DNA. Cidofovir[53] (HPMPC, **22**) shows a broad-spectrum antiviral acivity, being active versus all members of herpes family including HSV-TK$^-$, VZV-TK$^-$ and CMV-UL96$^-$.

1.10 Non-nucleoside analogues

Although nucleosides provide a major class of antiviral treatment some non-nucleoside therapies have been developed against herpes viruses, HIV and flu.

Foscarnet[54] (**23**), an organic analog of inorganic pyrophosphate, selectively inhibits the pyrophosphate binding site on viral DNA polymerases at concentrations that do not affect human DNA polymerases. Because foscarnet is not activated by thymidine kinase, it maintains activity in some viruses that have lost thymidine kinase activity to gain resistance to aciclovir or ganciclovir. Therefore, foscarnet is often used in aciclovir or ganciclovir resistant disease.

Amantadine[55] (**24**) is an amphiphilic primary amine that has both prophylactic and therapeutic efficacy against type A influenza virus infections. This compound blocks the M2 ion channel, and thus prevents the passage of H+ ions that are required for the necessary acidity to allow for the viral uncoating process.[56]

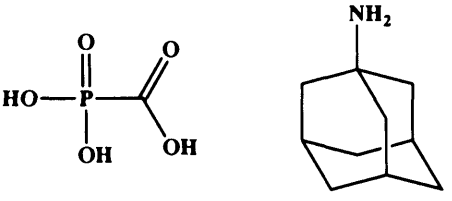

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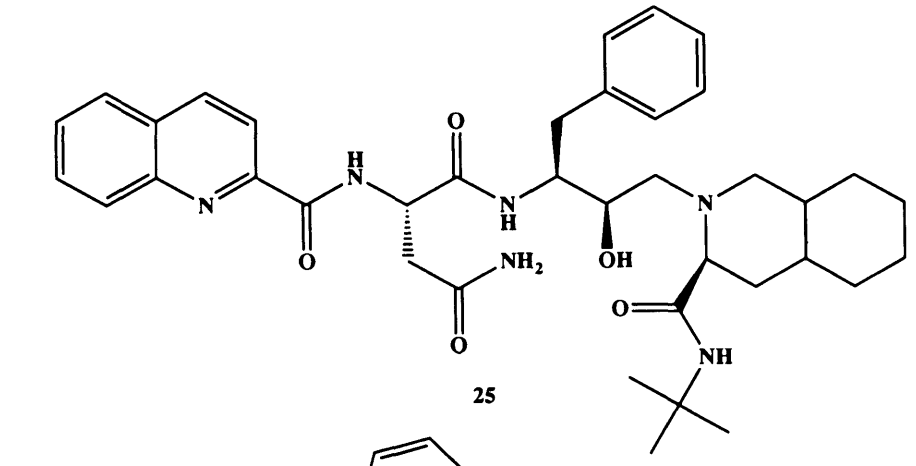
Fig 1.10: Non-nucleoside antivirals

Protease Inhibitors (PIs) are reversible competitive inhibitors of HIV protease that are able to displace HIV polyproteins acting as peptide-like molecules, binding onto HIV proteases to prevent the accumulation of structural proteins required for new virion formation.[57] Immature virions normally arise after assembly and packaging of these structural polyproteins. HIV-1 protease cleaves these precursor proteins to produce the final structural proteins of a mature virion core and to activate reverse transcriptase for new infection cycles. Since protease is necessary for the production of mature virions, its inhibition renders the virus noninfectious as the body rapidly clears immature virions.[57] Although PI therapy does not prevent destruction of already infected CD4+ lymphocytes, further spread of infection to uninfected cells is prevented. Currently, there are eight available HIV-1 PIs: Saquinavir (**25**), Ritonavir (**26**), Indinavir (**27**), Nelfinavir (**28**), Amprenavir, Atazanavir, Lopinavir and Tripanavir.



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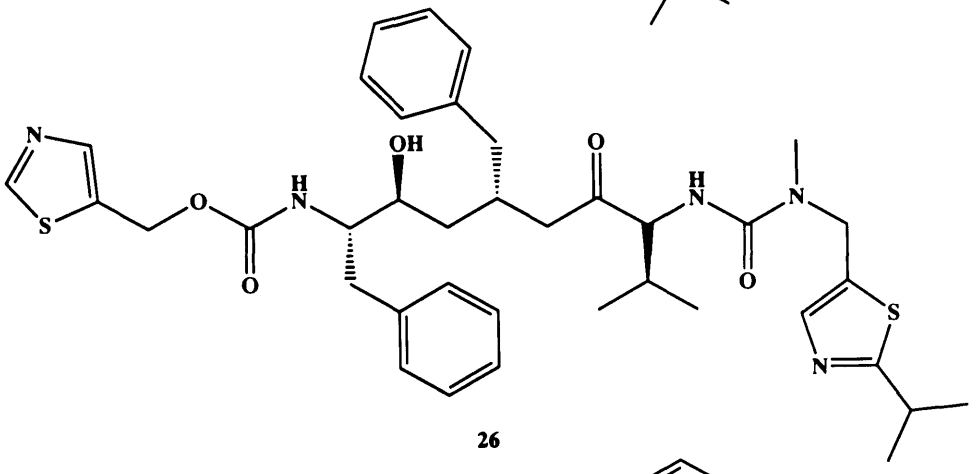

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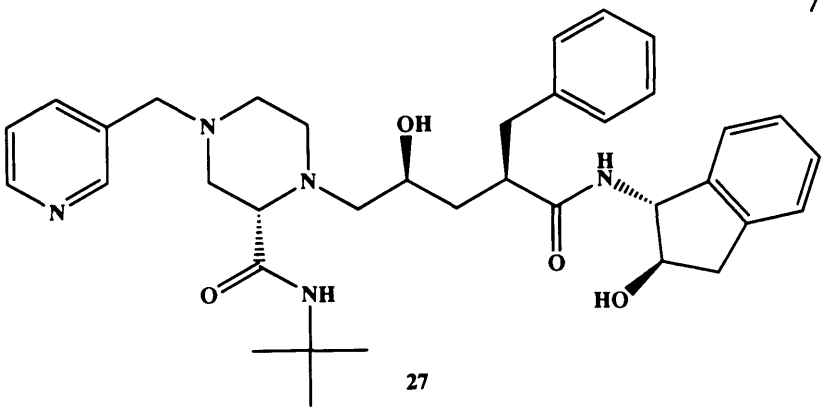

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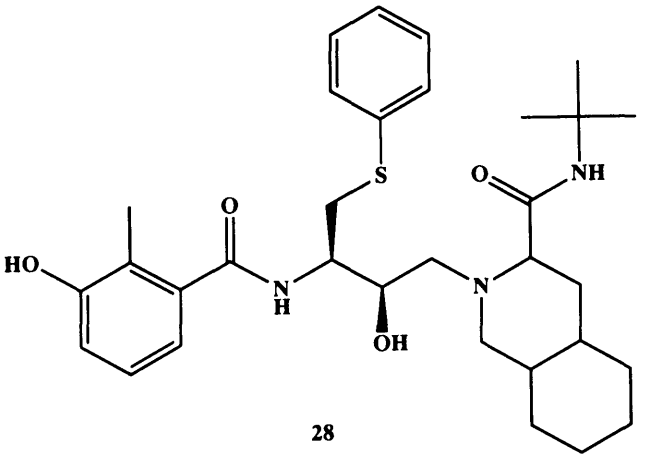

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Fig 1.11: HIV Protease Inhibitors

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Chapter 2: BCNAs

2.1 Varicella Zoster Virus

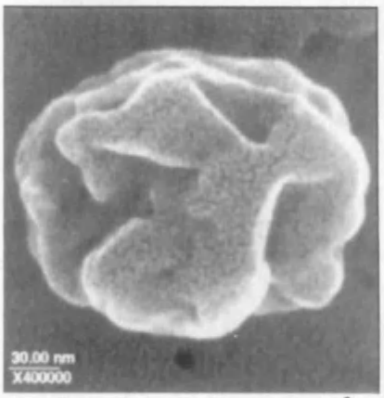


Fig 2.1: Varicella Zoster Virus[2]

VZV is one of the eight members of the Herpes-viridae family, more specifically it is classified with herpes simplex viruses (HSV). Although HSV replicates in cells of numerous animal species, VZV has a narrow host range including only human and simian origin. The VZV particle is 125-175 nm in diameter, and has a lipid envelope bearing glycoprotein spikes.[1]

VZV is the cause of both Varicella (chickenpox) and herpes Zoster (shingles). Chickenpox is the primary manifestation of VZV infection and 90% of cases occur by 10 years of age. The virus becomes permanently established in sensory ganglia, persists in latent form, and recurs when reactivated as herpes Zoster. VZV reactivation occurs in up to 15% of those who have had Varicella manifesting by a vesicular rash, affecting the sensory nerve.[3]

The drugs of first choice for the treatment of herpes Zoster by oral administration are ACV **11** and its prodrug valacyclovir **12**, while vidarabine **18** has been used intravenously. These drugs have a beneficial effect on reducing acute pain caused from shingles and on speeding healing.[4]

Valacyclovir **12** and famciclovir **15** have been licensed for the treatment of herpes-zoster virus infections.[5] Their pharmacokinetic profile is better than ACV and their half-life is longer, making of these compounds better drugs for treatment of VZV.[6]

BVDU **2** and BVaraU **3** are the most potent inhibitors of VZV that have reported to date, inhibiting VZV replication in cell culture at an EC_{50} less than 10 μM. BVDU is a substrate for thymidine phosphorylase that metabolises BVDU to BVU [(E)-5-(2-bromovinyl)uracil] and 2-deoxyribose-1-phosphate.[7] The rapid conversion to BVU, which is inactive as an antiviral agent, reduces the antiviral potency of BVDU. However, BVU can be re-converted to BVDU, through a pentosyl transfer reaction with any 5-substituted 2'-deoxyuridine, including also thymidine, as the pentosyl donor, restoring in part the antiviral activity of the compound.[8] Moreover BVU is a potent inhibitor of dihydropyrimidine dehydrogenase (DPD), the enzyme

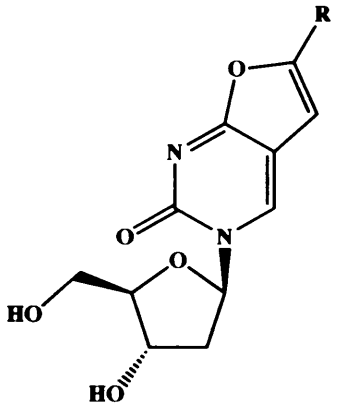
responsible for the catabolism of pyrimidines. DPD is needed for the degradation of 5-fluorouracil, an anti-cancer agent, and BVU increases its half-life. The combination BVDU with 5-fluorouracil results in an increase in plasma levels of 5-fluorouracil,[9] and it has lead to fatalities in the clinic.

2.2 BCNAs : Discovery of a new class of anti-VZV agents

A number of nucleosides with potent biological properties have arisen by substitution at the 5-position of deoxyuridine analogues. In particular 5-alkynyl analogues have shown to be potent inhibitors of herpes viruses.[10] This class of nucleosides is rather cytotoxic and its viral selectivity is low.[11]

During the research program, aimed to improving the biological activity of 5-alkynyl-2'-deoxyuridine analogues with long alkyl side chains, a new class of antiviral nucleoside derivatives bearing an unusual bicyclic base was discovered in our laboratory.[12]

The synthesis of 5-alkynyl-2'-deoxyuridine involves Sonogashira coupling of terminal alkynes with 5-iodo nucleosides. An unwanted by-product in such coupling reaction is the fluorescent furano-pyrimidine, observed by a number of researchers as a more polar spot on TLC. [13,14,15] The treatment of the target 5-alkynyl nucleoside with methanol, triethylamine and copper iodide leads to the total conversion to the fluorescent spot **25**.[12]



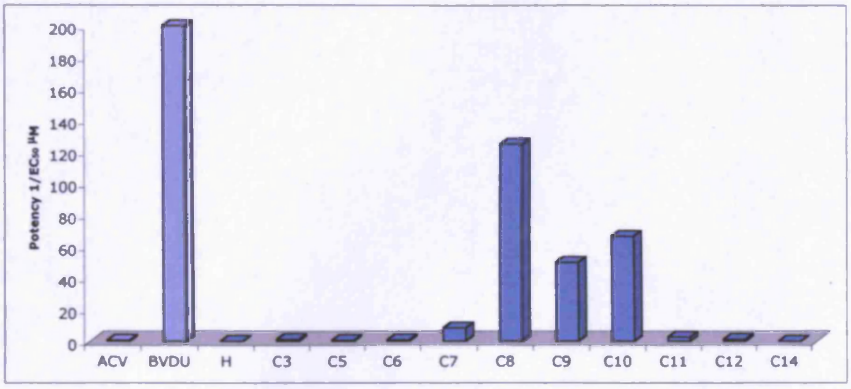
29a R = H
29b R = C_3H_7
29c R = C_4H_9
29d R = C_5H_{11}
29e R = C_6H_{13}
29f R = C_7H_{15}
29g R = C_8H_{17}
29h R = C_9H_{19}
29i R = $C_{10}H_{21}$
29j R = $C_{11}H_{23}$
29k R = $C_{12}H_{25}$
29l R = $C_{14}H_{29}$

The previously only well characterized by-products in the 2'-deoxy series were the compounds **29a** and **29c**. However the parent compound **29a** was inactive against HSV, CMV, VZV. A novel series of **29**, bearing a long alkyl side-chain, has been synthesised and evaluated for their ability to inhibit the replication of herpes viruses (HSV-1, HSV-2, VZV, CMV).

The bicyclic nucleosides show considerable potency and selectivity against VZV and the length of the side-chain plays a crucial role in the activity. In fact the optimal length is between 8 and 10 carbon atoms, with an EC_{50} of 7-20 nM (**29g-i**). These compound are ca. 300 fold more active than ACV and roughly equipotent with BVDU.[16]

Furthermore the low cytotoxicity (CC_{50}> 50 μM) results in a high selectivity index (SI) >5000. The structure-activity relationships (SAR) regarding the long alkyl chain is clear: a

short chain ≤C6 leads to little antiviral activity, C7, C11 and C12 confer a moderate activity, while C8-10 give a high potency.[15]



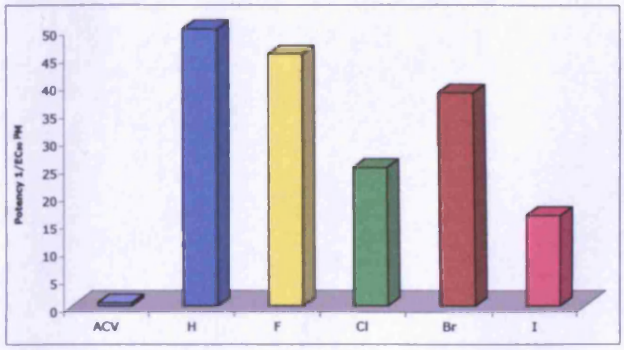
		EC_{50} μM[a]				MCC μM[b]	CC_{50} μM[c]
		OKA	YS	TK⁻ 07	TK⁻ YS		
29a	H	28	33	>200	>200	>200	>200
29b	C3	0.8	0.5	>200	>200	>200	>200
29d	C5	1.8	3.5	>50	>50	>50	>50
29e	C6	1.3	2.8	>50	>50	200	>200
29f	C7	0.12	0.33	>50	>50	>50	>50
29g	C8	0.008	0.024	>50	>50	>50	>50
29h	C9	0.02	0.02	>200	>200	>200	>200
29i	C10	0.015	0.008	>50	>50	>50	>50
29j	C11	0.37	0.3	>50	>50	200	>200
29k	C12	0.8	1.2	>50	>50	50	>200
29l	C14	>50	>50	>50	>200	>200	>200
BVDU		0.005	0.005	>200	>200	>200	>200
ACV		2.9	1	74	125	>200	>200

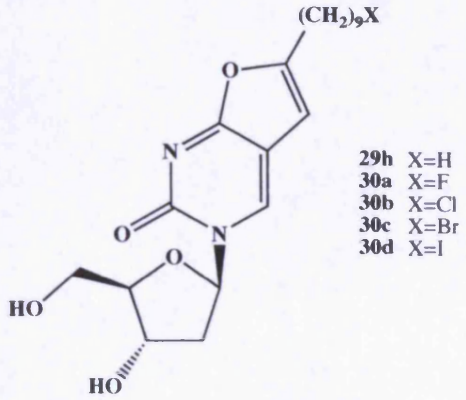
Table 2.1

a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

2.2.1 Terminal Halogen Substitution

Given the apparent necessity for a long alkyl chain, a new series retaining this moiety has been synthesised introducing a halogen in the ω-position to probe SARs in this region. However, compounds **30a-d** do not show any significant difference from the parent compound **29h**.[15, 17]





29h X=H
30a X=F
30b X=Cl
30c X=Br
30d X=I

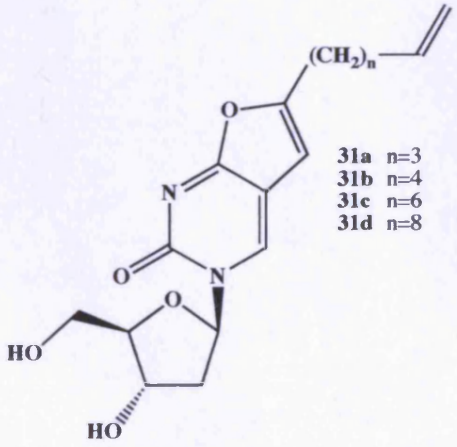
		EC_{50} μM[a]				MCC μM[b]	CC_{50} μM[c]
		OKA	**YS**	**TK⁻ 07**	**TK⁻ YS**		
29h	H	0.02	0.02	>200	>200	>200	>200
30a	F	0.022	0.014	>20	>20	>50	200
30b	Cl	0.04	0.02	>20	>50	>200	200
30c	Br	0.026	0.025	>50	50	>200	>50
30d	I	0.061	0.034	>50	>50	>50	>200
ACV		2.9	1	74	125	>200	>200

Table 2.2

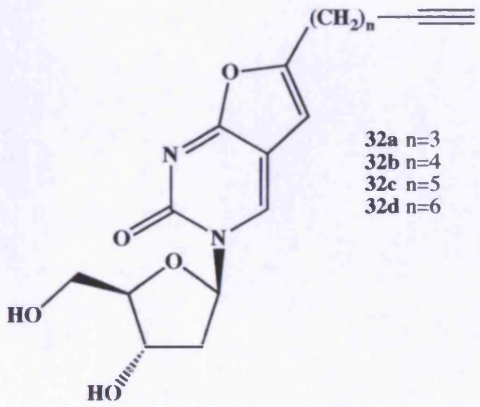
a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

2.2.2 Terminal Unsaturation

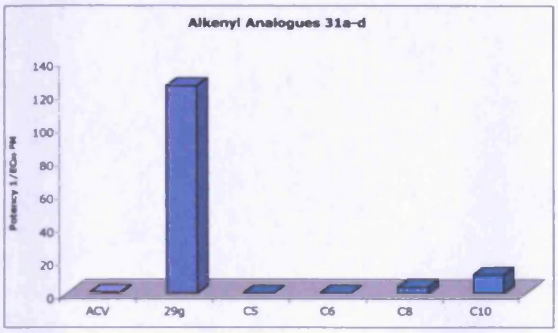
A range of medium to long chain ω-alkenyl (**31a-d**) and ω-alkynyl (**32a-d**) derivatives have been prepared, but although these compounds retain antiviral activity at non-toxic concentration, they are less active than the corresponding alkyl analogues.[18] (data on the C8 alkyl **29g** are shown for reference).

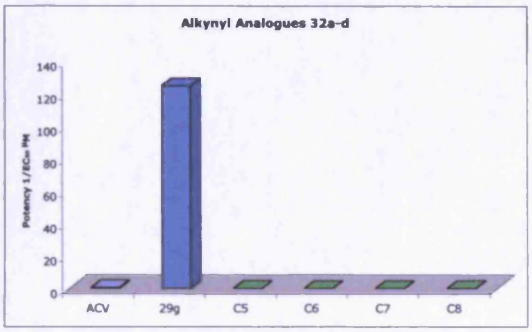


31a n=3
31b n=4
31c n=6
31d n=8



32a n=3
32b n=4
32c n=5
32d n=6





		EC_{50} μM[a]				MCC μM[b]	CC_{50} μM[c]
		OKA	**YS**	**TK⁻ 07**	**TK⁻ YS**		
31a	C5	>200	>200	>200	>200	>200	>200
31b	C6	14	13	>200	>200	>200	>200
31c	C8	0.27	0.06	>200	>50	>200	>200
31d	C10	0.09	0.1	>200	>200	>50	>200
32a	C5	8	10	>200	>200	>200	>200
32b	C6	25	33	>200	>200	>200	>200
32c	C7	79	37	>200	>200	>200	>200
32d	C8	5	4	>200	>200	>200	>200
29g		0.008	0.024	>50	>50	>50	>50
ACV		2.9	1	74	125	>200	>200

Table 2.3

a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

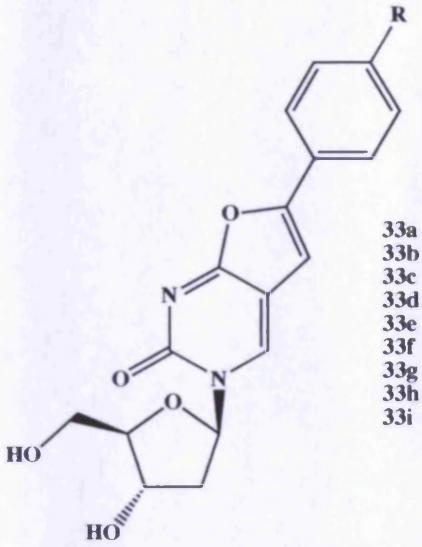
An important role for the activity is given by lipophilicity. A calculated figure LogP of between 2.5-3.5 appears optimal. In fact comparing the octyl **29g** to the corresponding alkenyl **31c** and alkynyl **32d**, it is notable that the presence of an unsaturation has a very negative effect on the activity following the trend Alkyl >> Alkenyl > Alkynyl. Partly, this may be explained by the impact of the insaturation on log P.

		EC_{50}	
	LogP	**OKA**	**YS**
29g	3.01	0.008	0.024
31c	2.52	0.27	0.06
32d	1.65	5	4

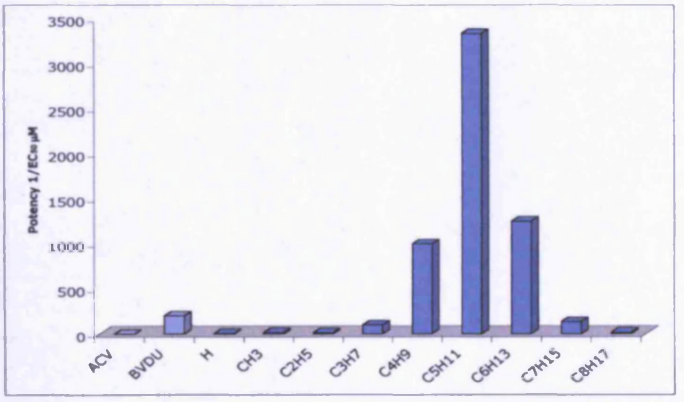
Table 2.4: LogP vs EC_{50} (8 carbons chain)

2.2.3 Alkylaryl analogues

In order to introduce a conformational constraint in the alkyl chain keeping a high logP, some p-alkylaryl compounds have been synthesised (**33a-i**). The introduction of a phenyl moiety between the base and the alkyl chain resulted in an increasing of potency up to subnanomolar concentration for the pentylphenyl **33f,** which is 10,000 fold more active than ACV and ca. 20 fold more active than BVDU with no cytotoxicity.[19]



33a R=H
33b R=CH_3
33c R=C_2H_5
33d R=C_3H_7
33e R=C_4H_9
33f R=C_5H_{11}
33g R=C_6H_{13}
33h R=C_7H_{15}
33i R=C_8H_{17}

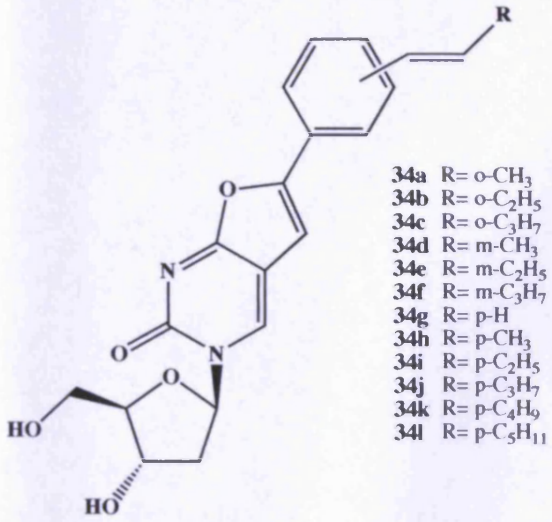


		EC_{50} µM[a]				MCC µM[b]	CC_{50} µM[c]
		OKA	**YS**	**TK⁻ 07**	**TK⁻ YS**		
33a	H	0.16	0.28	>200	>162	>20	>200
33b	C1	0.06	0.06	103	>200	>200	>200
33c	C2	0.07	0.09	>50	>50	>20	123
33d	C3	0.008	0.01	>50	>20	>50	188
33e	C4	0.001	0.0008	>20	>20	>200	>200
33f	C5	0.0003	0.0001	>5	>5	>50	>200
33g	C6	0.0008	0.0002	>5	>5	>20	18
33h	C7	0.0074	0.0057	>5	>5	5	18
33i	C8	0.065	0.05	>20	>20	>20	>200
BVDU		0.005	0.005	>200	>200	>200	>200
ACV		2.9	1	74	125	>200	>200

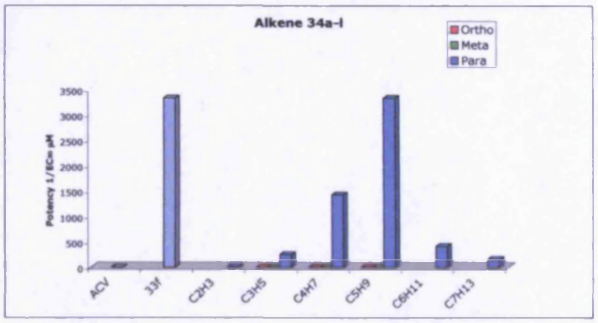
Table 2.5

a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

The further substitution of the phenyl ring constraining the side chain with an unsaturation on the α-position has been done, moving also the substituent in the various positions other than para (**34a-l**).



34a R= o-CH_3
34b R= o-C_2H_5
34c R= o-C_3H_7
34d R= m-CH_3
34e R= m-C_2H_5
34f R= m-C_3H_7
34g R= p-H
34h R= p-CH_3
34i R= p-C_2H_5
34j R= p-C_3H_7
34k R= p-C_4H_9
34l R= p-C_5H_{11}



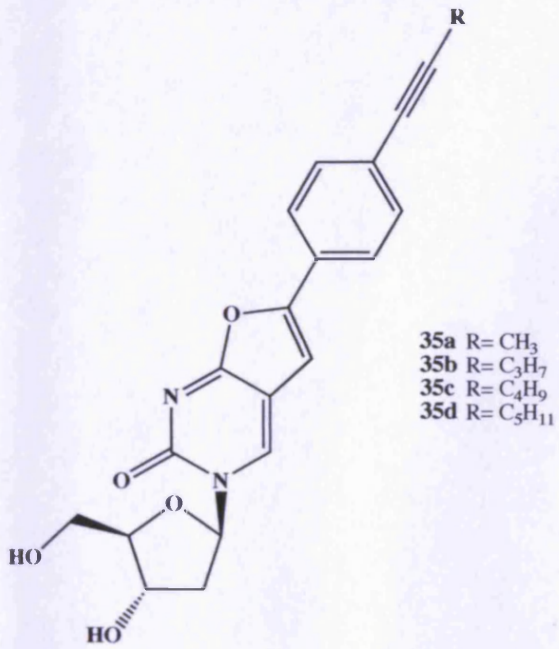
			EC_{50} µM[a]				MCC µM[b]	CC_{50} µM[c]
			OKA	**YS**	**TK⁻ 07**	**TK⁻ YS**		
Ortho	**34a**	C3	12	-	>80	-	80	102
	34b	C4	33	-	>80	-	400	103
	34c	C5	8.4	-	>16	-	80	41
Meta	**34d**	C3	13	58	>200	>200	>200	>200
	34e	C4	1.9	2.3	>20	>15	>20	68
	34f	C5	1.1	1.3	>20	>20	>20	>200
Para	**34g**	C2	0.07	0.08	41	-	200	93
	34h	C3	0.003	0.003	>5	>5	>5	>200
	34i	C4	0.0003	0.0006	>5	>5	>5	>200
	34j	C5	0.00011	0.0009	1.1	>5	>5	>200
	34k	C6	0.0024	0.0069	>16	-	80	>200
	34l	C7	0.0061	0.0084	>16	-	80	>200
	33f		0.0003	0.0001	>5	>5	>50	>200
	ACV		2.9	1	74	125	>200	>200

Table 2.6

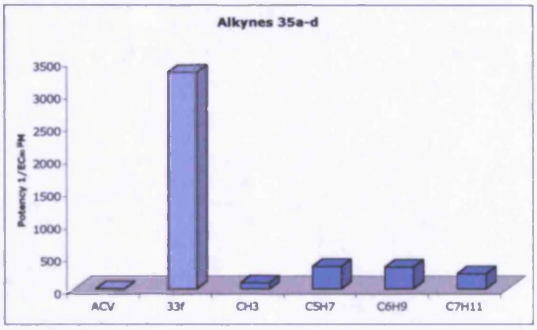
a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

For this series is very clear that the substitution on the positions ortho and meta is not acceptable, while the para-subtitution follows the trend of the corresponding saturated series **33**. In fact the pentenyl **34j** shows potency equal to the pentyl analogue **33f**.

The introduction of a triple bond at the α-position, and a further constrain of the chain, lead to the alkynyl series **35a-d**, which resulted in a loss of activity.[20]



35a R= CH_3
35b R= C_3H_7
35c R= C_4H_9
35d R= C_5H_{11}



		EC_{50} μM[a]				MCC μM[b]	CC_{50} μM[c]
		OKA	**YS**	**TK⁻ 07**	**TK⁻ YS**		
35a	C3	0.011	0.0071	>2	>2	>2	>200
35b	C5	0.0029	0.0018	>1.4	>5	>5	>200
35c	C6	0.003	0.005	35	-	>50	200
35d	C7	0.0043	0.0053	>20	-	>20	>50
33f		0.0003	0.0001	>5	>5	>50	>200
ACV		2.9	1	74	125	>200	>200

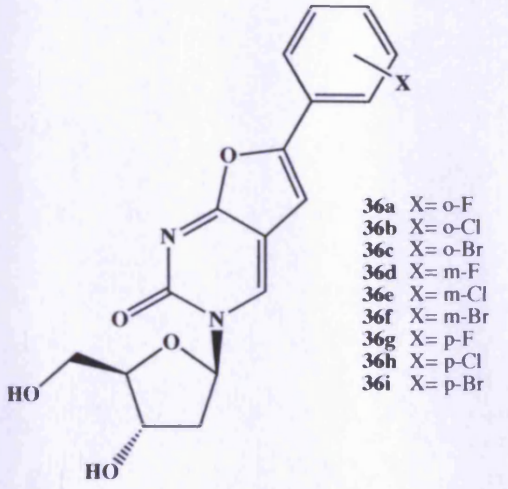
Table 2.7

a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

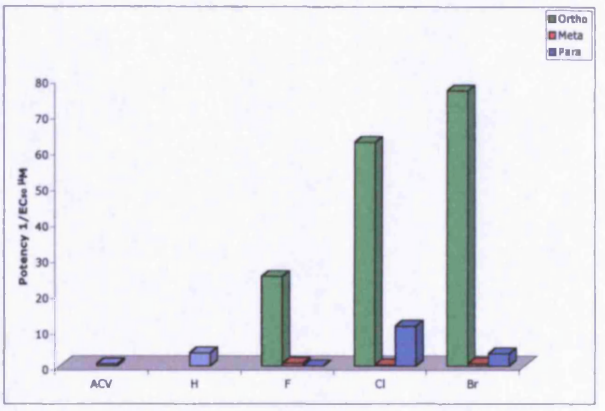
2.2.4 Haloaryl derivatives

A different moiety from alkyl chains on the phenyl ring has been introduced leading to haloaryl analogues (**36a-I**).

The SARs of this series is not very clear, but it is evident that the introduction of a halogen in the phenyl ring affects the antiviral activity suggesting that the decreasing order of the potency is ortho > para > meta.[21]



36a X= o-F
36b X= o-Cl
36c X= o-Br
36d X= m-F
36e X= m-Cl
36f X= m-Br
36g X= p-F
36h X= p-Cl
36i X= p-Br



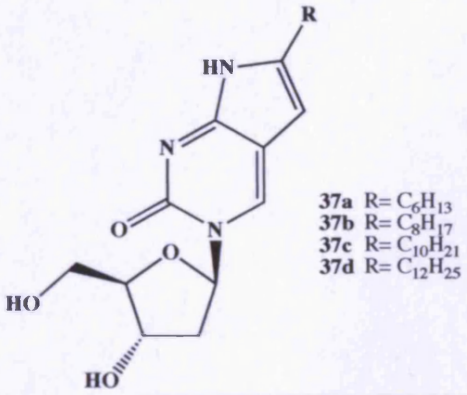
			EC_{50} μM[a]				MCC μM[b]	CC_{50} μM[c]
			OKA	**YS**	**TK⁻ 07**	**TK⁻ YS**		
Ortho	**36a**	F	0.04	0.03	>20	-	-	>200
	36b	Cl	0.016	0.011	>20	>20	>20	137
	36c	Br	0.01	0.02	>5	>5	>5	>200
Meta	**36d**	F	1.5	2.4	>200	>200	>200	>200
	36e	Cl	2	1.8	>20	>20	>20	>200
	36f	Br	1.3	1.2	>5	>5	>5	36
Para	**36g**	F	>50	>50	>50	>50	200	171
	36h	Cl	0.09	0.08	>20	>20	>20	>200
	36i	Br	0.29	0.2	>5	>5	>2	96
	33a	H	0.16	0.28	>200	162	>20	>200
	ACV		2.9	1	74	125	>200	>200

Table 2.8

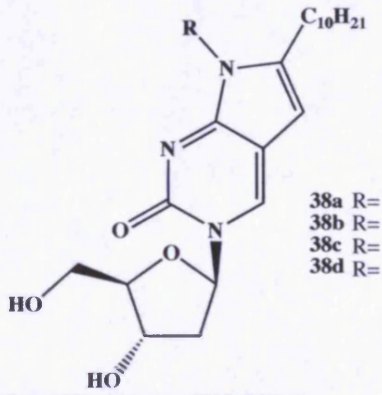
a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

2.2.5 Base modification: pyrrolo and thiophene analogues

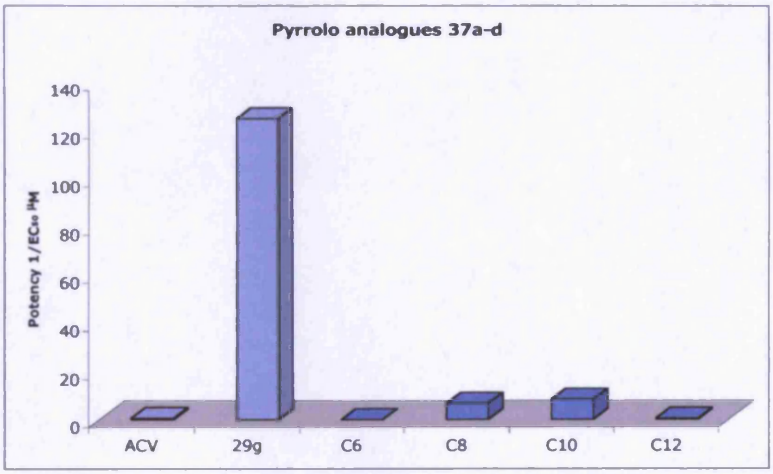
It was of interest to probe modifications in the base, synthesising two novel series bearing a nitrogen[22] rather than oxygen in the furan ring (**37a-d**) and (**38a-d**).



37a R= C_6H_{13}
37b R= C_8H_{17}
37c R= $C_{10}H_{21}$
37d R= $C_{12}H_{25}$



38a R= CH_3
38b R= C_2H_5
38c R= C_3H_7
38d R= C_4H_9



		EC_{50} µM[a]				MCC µM[b]	CC_{50} µM[c]
		OKA	**YS**	**TK⁻ 07**	**TK⁻ YS**		
37a	C6	>50	>50	>50	>50	>50	>50
37b	C8	0.15	0.48	>20	>20	20	>50
37c	C10	0.12	0.15	>200	>200	>20	>200
37d	C12	2.4	14.5	73	80	>200	>200
38a	Me	6	8	>50	>20	>50	179
38b	Et	>5	>5	>5	>5	20	34
38c	Pr	>5	>5	>5	>5	20	47
36d	Bu	7.1	7	>50	23	200	57
29g		0.008	0.024	>50	>50	>50	>50
ACV		2.9	1	74	125	>200	>200

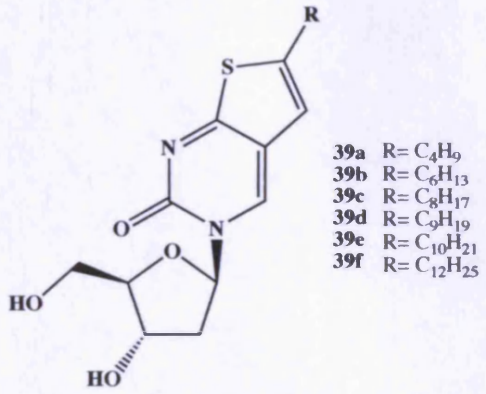
Table 2.9

a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

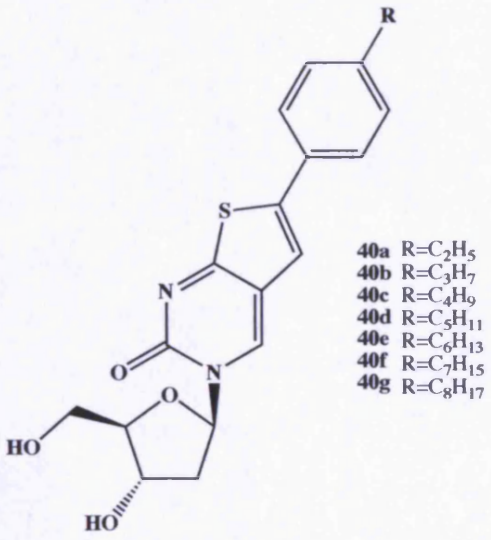
The replacement of the ring oxygen with nitrogen results in a loss of activity compared to the corresponding octyl derivatives **29g**. Although the reasons of this poor activity are not very clear, one possible explanation is the change of a H-bond acceptor [O] with a H-bond donator [NH].[15] The introduction of an alkyl chain on the nitrogen (**38a-d**) led to a further loss of activity (data not shown), clearly suggesting that replacement of the oxygen with nitrogen is not tolerated and the introduction of an alkyl chain on the nitrogen is not accepted.[20]

The next change was the substitution of the oxygen with sulphur, synthesising a series bearing an alkyl[23] (**39a-f**) and alkyl-phenyl moiety[24] (**40a-g**).

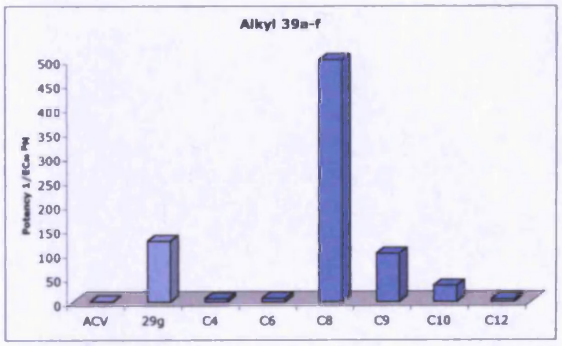
The introduction of sulphur lead to variations in activity depending on the series, in fact it is interesting that that in the alkyl series **39** this modification is well tolerated leading in general to a slight improvement on the antiviral activity[21] while in the alkyl-phenyl series **40** lead to a reduction in potency.[22]

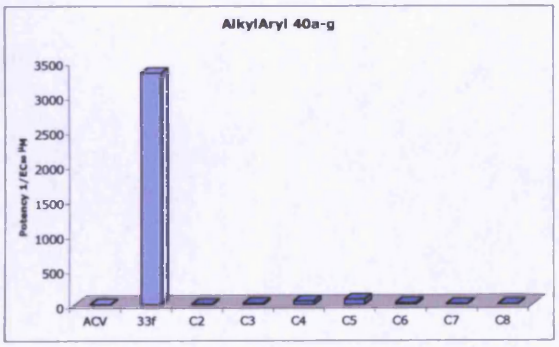


39a R= C_4H_9
39b R= C_6H_{13}
39c R= C_8H_{17}
39d R= C_9H_{19}
39e R= $C_{10}H_{21}$
39f R= $C_{12}H_{25}$



40a R=C_2H_5
40b R=C_3H_7
40c R=C_4H_9
40d R=C_5H_{11}
40e R=C_6H_{13}
40f R=C_7H_{15}
40g R=C_8H_{17}





		EC_{50} μM[a]				MCC μM[b]	CC_{50} μM[c]
		OKA	**YS**	**TK⁻ 07**	**TK⁻ YS**		
39a	C4	0.16	0.15	>200	153	>100	>200
39b	C6	0.14	0.14	>50	>50	125	>200
39c	C8	0.002	0.005	>5	>5	20	53
39d	C9	0.01	0.01	>20	>20	>20	>20
39e	C10	0.03	0.06	>5	>5	20	54
39f	C12	0.2	0.3	>5	>5	12	49
40a	C2	0.2	0.15	20	-	>200	>200
40b	C3	0.06	0.09	>16	-	80	>200
40c	C4	0.02	0.028	>3.2	-	50	>200
40d	C5	0.014	0.025	20	-	50	>200
40e	C6	0.043	0.08	>50	-	200	>200
40f	C7	0.18	0.27	>20	-	50	>200
40g	C8	3.4	-	>20		>20	>200
29g		0.008	0.024	>50	>50	>50	>50
33f		0.0003	0.0001	>5	>5	>50	>200
ACV		2.9	1	74	125	>200	>200

Table 2.10

a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

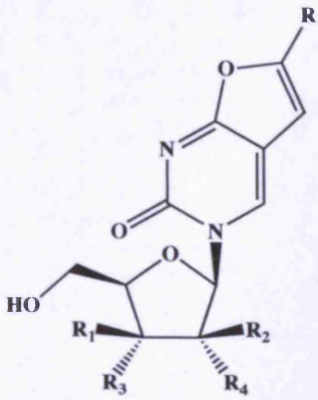
2.2.6 Sugar Modifications

So far, every modification that has been made was carried out on a 2'-deoxyribose nucleosides, without altering the sugar moiety. As the antiviral effect is strictly dependent on the kinase activity, a deoxyribose nucleoside is the ideal substrate for the enzyme and only small modifications are tolerated in order to have a good antiviral activity.[15] Thus, different sugar-derivatives have been synthesised bearing different alkyl chains just to probe the SARs on the sugar moiety.

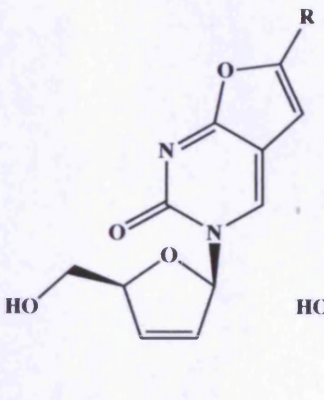
The introduction of a hydroxyl group in position 2' results in a loss of activity (ara **41a** and ribo **41b**).[15] The removing of the 3'-OH from the sugar led to a loss of activity for the d4[25] (**41c**) and dd[26] (**41d**), but the latter surprisingly gained a modest anti-HCMV activity.[27]

The shifting of the 3'-OH to position 2' (3'-dRibo **41e**) is not well tolerated, as this compound is poorly active.[28] The corresponding analogue of ACV has been synthesised (**41f**), showing that this compound retains antiviral activity comparable to ACV and that the presence of a 3'-OH is beneficial for activity.[29] However, the 3'-OH has to be in Ribo position, as the dXylo derivative (**41g**) does not have an activity as good as the corresponding dRibo analogue (**29g**).[30]

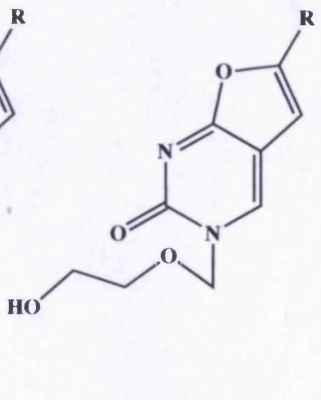
From this data, it seems that for a good activity, the dRibo is the best sugar accepted as a good substrate, confirming that even very small modifications are not very well tolerated.



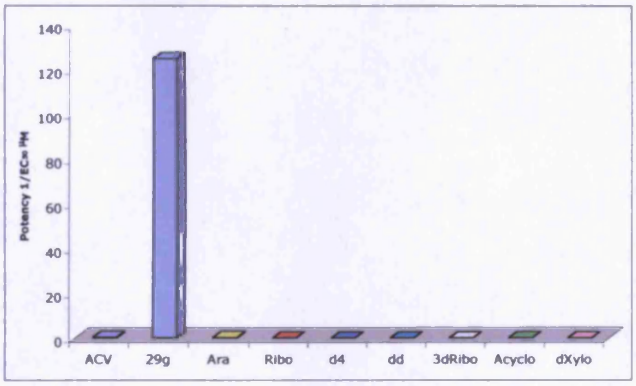
41a R=C_8H_{17} R_1=R_4=H R_2=R_3=OH
41b R=C_8H_{17} R_1=R_2=H R_3=R_4=OH
41d R=C_8H_{17} R_1=R_2=R_3=R_4=H
41e R=C_8H_{17} R_1=R_2=R_3=H R_4=OH
41g R=$C_{10}H_{21}$ R_1=OH R_2=R_3=R_4=H



41c R= $C_{10}H_{21}$



41f R= C_8H_{17}

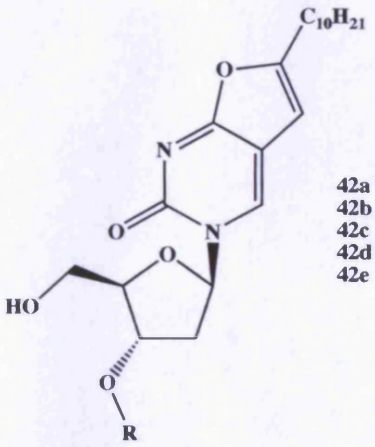


		EC_{50} µM[a]				MCC µM[b]	CC_{50} µM[c]
		OKA	YS	TK⁻ 07	TK⁻ YS		
41a	Ara	3.6	3.3	200	67	>200	>200
41b	Ribo	23	21	>20	>20	50	>50
41c*	d4	>50	>50	>50	>50	200	16
41d	dd	40	20	>200	>50	>200	>200
41e	3dRibo	>50	-	>200	-	>50	>50
41f	Acyclo	11	-	>16	-	>80	>200
41g*	DXylo	24	>20	>50	>50	>50	>22
29g	dRibo	0.008	0.024	>50	>50	>50	>50
ACV		2.9	1	74	125	>200	>200

Table 2.11

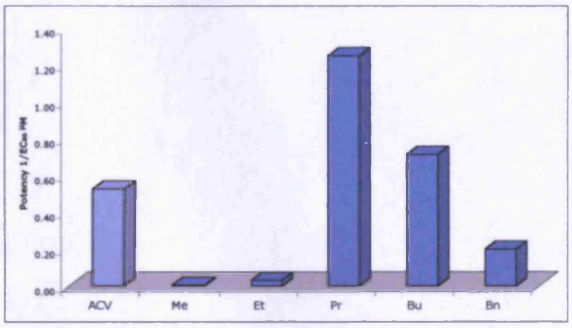
a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%
* C10 derivatives

2.2.7 Modifications at 3'-position



42a R= CH_3
42b R= C_2H_5
42c R= C_3H_7
42d R= C_4H_9
42e R= Bn

Many anti-viral nucleosides have the requirement for a free 3'-hydroxyl function for biological activity, in contrast to compounds such as AZT, ddC, d4T that act as chain terminators. Thus, 3'-O-alkyl ethers have been prepared[15,23] (**42a-e**), and the loss of activity compared to the parent analogue **29g** supports the hypothesis that a free 3'-OH is needed for a good antiviral effect.



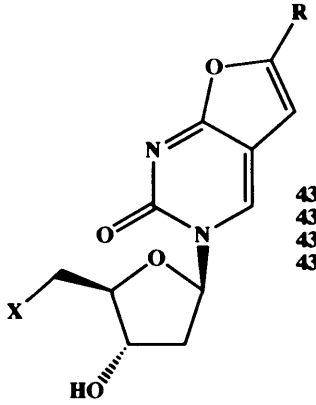
		EC_{50} µM[a]				MCC µM[b]	CC_{50} µM[c]
		OKA	**YS**	**TK⁻ 07**	**TK⁻ YS**		
42a	Me	>200	>200	>200	>200	>200	>200
42b	Et	100	93	>200	>50	>200	-
42c	Pr	1.2	0.64	>20	8.8	>20	-
42d	Bu	1.4	26	>50	>50	>200	>200
42e	Bn	>50	>50	>50	>20	>50	-
29g	H	0.008	0.024	>50	>50	>50	>50
ACV		2.9	1	74	125	>200	>200

Table 2.12

a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

2.2.8 Modifications at 5'-position

The mechanism of action of the BCNAs is still not fully understood, but it is clear the presence of a viral TK-mediated phosphorylation is needed. To further prove this theory, compounds that cannot be phosphorylated at the 5'-position have been synthesised (**43a-d**) resulting in a drastic loss of activity. This lack of activity can be seen as further evidence of a mechanism of anti-VZV action involving an obligate 5'-phosphorylation.[31]



43a R= C_8H_{17} X= H
43b R= C_8H_{17} X= Cl
43c R= PhC_5H_{11} X= H
43d R= PhC_5H_{11} X= Cl

		EC_{50} μM[a]				MCC μM[b]	CC_{50} μM[c]
		OKA	YS	TK⁻ 07	TK⁻ YS		
43a	H	14	-	>3.2	-	80	120
43b	Cl	15	-	>200	-	>200	>200
43c	H	>5	>5	>5	>5	20	95
43d	Cl	>5	-	>5	-	20	>50
29g	OH	0.008	0.024	>50	>50	>50	>50
33a	OH	0.0003	0.0001	>5	>5	>50	>200
ACV		2.9	1	74	125	>200	>200

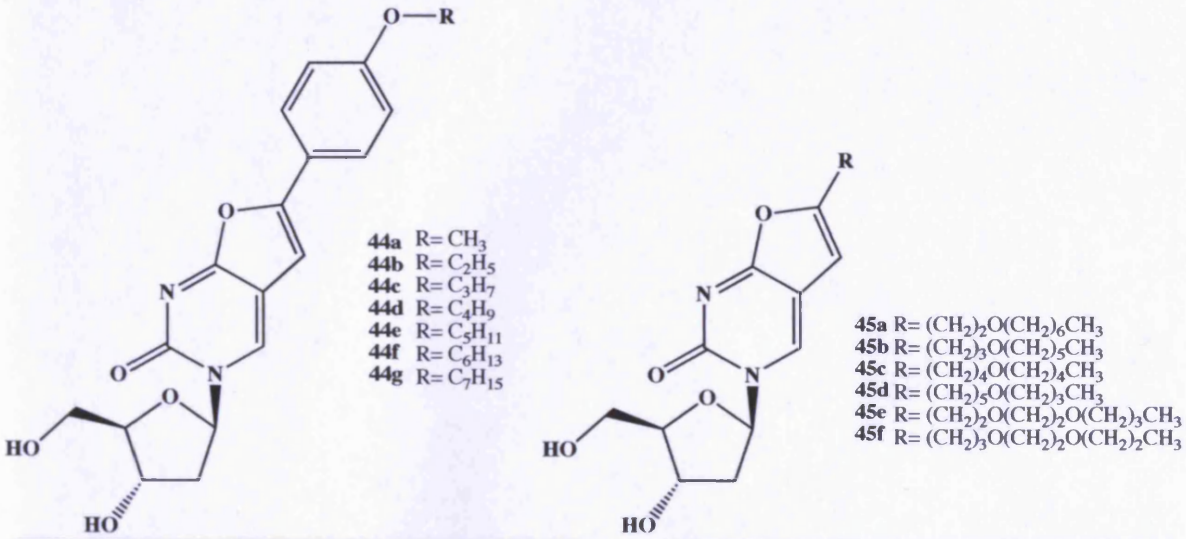
Table 2.13

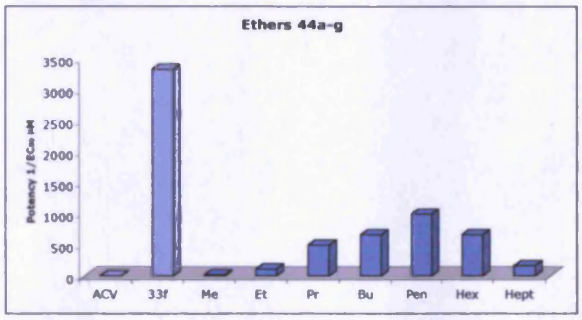
a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

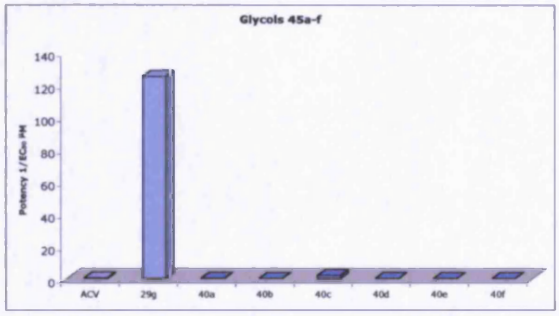
2.2.9 Enhancement of water solubility: Ether, Glycol and Pyridyl analogues

The alkyl side chain and resulting high logP of this class of compounds is a requirement for the activity, and this might be useful to cross cell membranes and for a topical formulation for the treatment of shingles. However, the range of logP for optimal activity is between 2.5 and 3.5 implying very low water solubility. In order to overcome this problem for a possible oral formulation, the introduction of ether or glycol moiety (**44a-g**[32,33], **45a-f**[34]) would increase the water solubility due to the presence of oxygen atoms in an alkyl chain. In fact compounds **44**

and **45** are more water-soluble than the corresponding alkane, ca 100 fold for ethers and 1000 fold for glycols,[32] but this is accompanied by a considerable reduction of activity.[31,32]





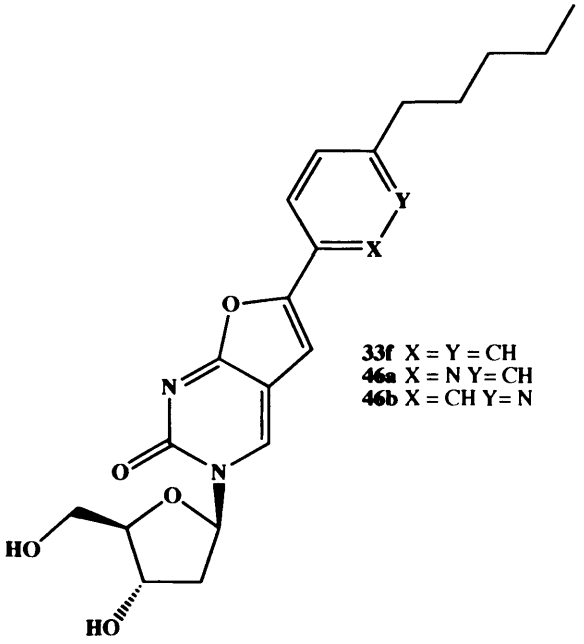


		EC_{50} μM[a]				MCC μM[b]	CC_{50} μM[c]
		OKA	**YS**	**TK⁻ 07**	**TK⁻ YS**		
44a	C1	0.05	0.045	>50	>50	200	>200
44b	C2	0.009	0.012	45	>50	>50	>200
44c	C3	0.002	0.002	11	>50	>200	>200
44d	C4	0.0015	0.0021	-	-	>200	>200
44e	C5	0.001	0.001	3.2	>20	>20	>200
44f	C6	0.0015	0.0014	>5	>20	>20	>200
44g	C7	0.006	0.006	>50	>20	>20	>200
45a	2	5.9	8.1	>50	>50	200	>200
45b	3	17	22	>50	>50	200	>200
45c	4	0.5	0.75	>50	>50	>200	>200
45d	5	7.9	9.3	>50	>50	>200	>200
45e	2,2	>200	>200	>200	>200	>200	>200
45f	3,2	120	97	>200	>200	>200	>200
29g		0.008	0.024	>50	>50	>50	>50
33a		0.0003	0.0001	>5	>5	>50	>200
ACV		2.9	1	74	125	>200	>200

Table 2.14

a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

For this reason, the substitution of the phenyl ring of the compound **33f** with a pyridine ring has been performed, decreasing the lipophilicity of the molecule without producing any major modification on the lead compound. The logP of the compounds **46a-b** is less than **33f**, and their activity is so low that it clearly shows that the replacement of the phenyl ring with a pyridyl ring is extremely damaging for the activity.[35]



33f X = Y = CH
46a X = N Y= CH
46b X = CH Y= N

	CLogP	EC_{50} μM[a]				MCC μM[b]	CC_{50} μM[c]
		OKA	YS	TK⁻ 07	TK⁻ YS		
46a	2.23	>80	-	>80	-	400	>200
46b	2.02	0.1	-	>50	-	80	>200
33f	3.52	0.0003	0.0001	>5	>5	>50	>200
ACV	-2.42	2.9	1	74	125	>200	>200

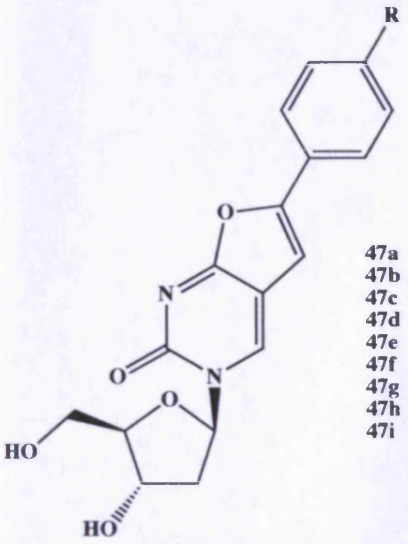
Table 2.15

a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

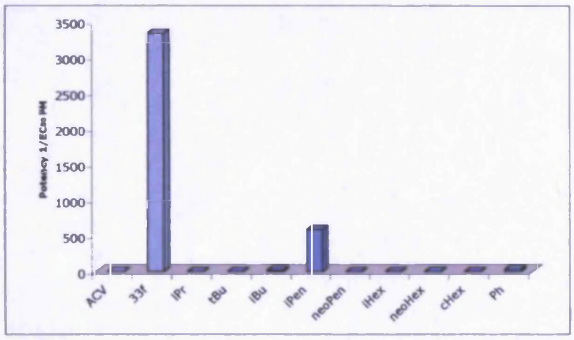
2.2.10 Side Chain Modifications: branched chain derivatives

Taking into account that polar groups in the side chain decrease activity and that the presence of a phenyl ring results in a significant boost, the introduction of a lipophilic branched-chain has been useful for an investigation on the steric hindrance effect and conformational restriction keeping the logP in the range of optimal antiviral activity (**47a-i**). However, the

introduction of this branching on the side chain results in a decreasing of activity showing that a non-branched alkane is one of the requirements for good activity.[36]



47a R= iPr
47b R= tBu
47c R= iBu
47d R= iPen
47e R= neoPen
47f R= iHex
47g R= neoHex
47h R= cHex
47i R= Ph



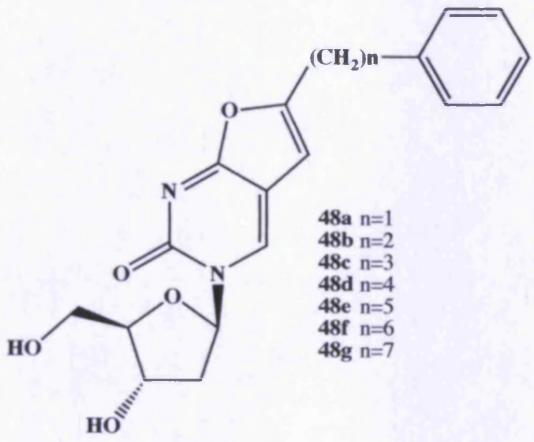
		EC_{50} μM[a]				MCC μM[b]	CC_{50} μM[c]
		OKA	**YS**	**TK⁻ 07**	**TK⁻ YS**		
47a	^{i}Pr	6.9	7.9	>20	>50	>50	>200
47b	^{t}Bu	>50	>20	>50	>50	>50	>200
47c	^{i}Bu	0.081	0.051	>200	>2	>200	>200
47d	^{i}Pen	0.0022	0.0011	>50	>2	>50	>200
47e	^{neo}Pen	>80	-	>80	-	400	180
47f	^{i}Hex	>80	-	>80	-	400	200
47g	^{neo}Hex	0.27	-	>80	-	>80	>200
47h	^{c}Hex	0.8	2.8	>20	>20	>20	>200
47i	Ph	0.031	0.032	>5	>5	>200	>200
33a		0.0003	0.0001	>5	>5	>50	>200
ACV		2.9	1	74	125	>200	>200

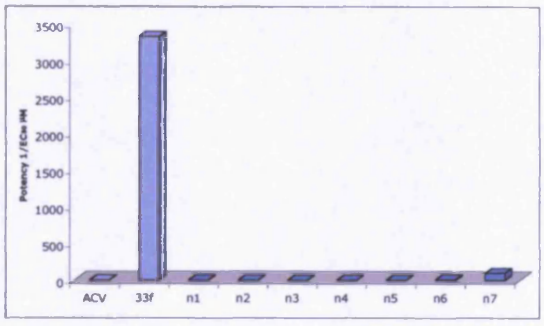
Table 2.16

a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

2.2.11 ArylAlkyl substitution

Another substitution is the shifting of the phenyl ring to the end of the alkyl chain. Despite compounds **48a-g** being chemically isomeric with **33b-h**, there is a dramatic reduction of antiviral activity, proving that logP is not the only main requirement for being active.[37,24]
It would thus appear that conjugation between the two aryl rings is essential for biological activity.





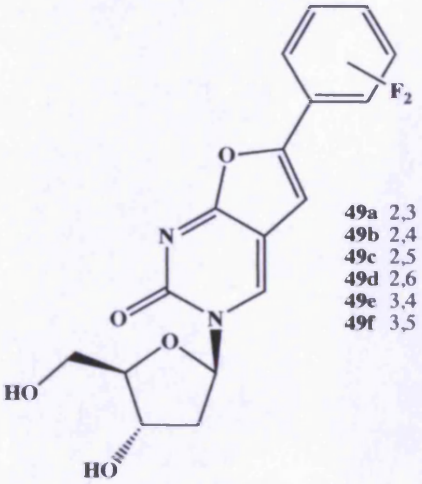
	n	EC_{50} μM[a]				MCC μM[b]	CC_{50} μM[c]
		OKA	YS	TK^- 07	TK^- YS		
48a	1	>20	>20	>20	>20	50	>100
48b	2	>50	>50	>50	>50	200	>200
48c	3	2	1.4	>200	>200	>200	>200
48d	4	28	26	>200	>200	>200	>200
48e	5	9.4	9.2	>20	>20	50	84
48f	6	0.1	0.15	>20	-	50	2.7
48g	7	0.01	0.022	200	-	>200	<2
33a		0.0003	0.0001	>5	>5	>50	>200
ACV		2.9	1	74	125	>200	>200

Table 2.17

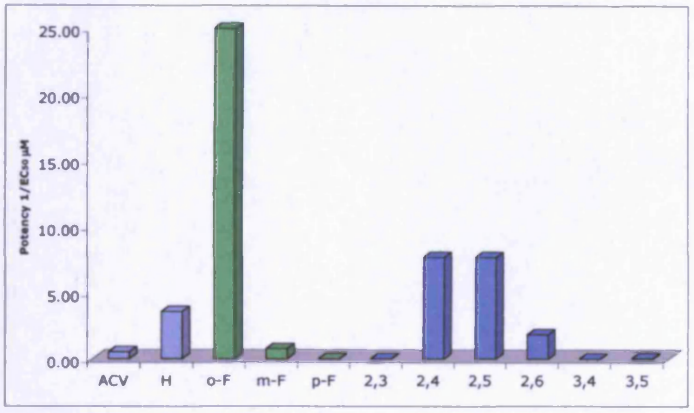
a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

2.2.12 Difluorophenyl analogues

To further study the effect of substitution on the phenyl ring, a series of difluoro analogues have been synthesised (**45a-f**). It is clear that the presence of fluorine in position 3 of the phenyl ring reduces the activity regardless of the position of the second fluorine.[38] For comparison the mono-fluorinated derivatives **36a**, **36d** and **36g** are included.



49a 2,3
49b 2,4
49c 2,5
49d 2,6
49e 3,4
49f 3,5



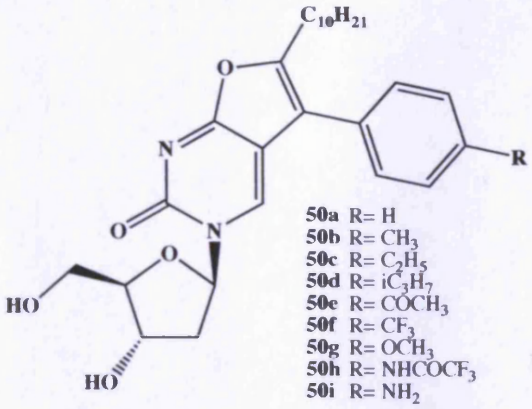
		EC_{50} µM[a]				MCC µM[b]	CC_{50} µM[c]
		OKA	YS	TK⁻ 07	TK⁻ YS		
49a	2,3	>80	-	>80	-	400	>200
49b	2,4	<0.13	-	184	-	>200	>200
49c	2,5	<0.13	-	>3.2	-	16	29
49d	2,6	0.54	-	>200	-	>200	>200
49e	3,4	>80	-	>80	-	400	>200
49f	3,5	19	-	>80	-	200	>200
36a	2	0.04	0.03	>20	-	-	>200
36d	3	1.5	2.4	>200	>200	>200	>200
36g	4	>50	>50	>50	>50	200	171
33a	H	0.16	0.28	>200	>162	>20	>200
ACV		2.9	1	74	125	>200	>200

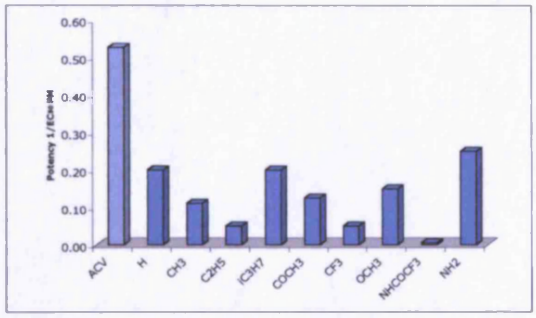
Table 2.18

a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

2.2.13 6-Alkyl-5-Aryl derivatives

The presence of a phenyl group with an alkyl side chain is beneficial for the activity. Moving of the phenyl ring from position 6 to position 5 was interesting in order to obtain information on the steric demands at active site of the enzyme (**49a-i**). The biological data show that this kind of substitution is extremely damaging for the antiviral activity with a 1000 fold loss of activity.[39]





		EC$_{50}$ μM[a]				MCC μM[b]	CC$_{50}$ μM[c]
		OKA	**YS**	**TK$^-$ 07**	**TK$^-$ YS**		
50a	H	>5	>5	>5	>5	20	37
50b	Me	9	9	>5	>5	>20	89
50c	Et	>20	>20	>20	>20	50	>200
50d	^{i}Pr	>5	>5	>5	>5	20	>200
50e	CH_3CO	8	5	>50	>50	200	>200
50f	CF_3	>20	>20	>20	20	50	>200
50g	OMe	6.7	4.4	-	>20	>20	>200
50h	$NHCOCF_3$	>200	>50	>50	>50	>200	>200
50i	NH_2	40	3.7	-	8.1	>50	67
ACV		2.9	1	74	125	>200	>200

Table 2.19

a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

2.2.14 Summary of SARs

Fig 2.2 is the plot of LogP versus activity of all the series above described. From this graph it can be seen that all the compounds cover a wide range of LogP, but when inside the range of 2.5-4.0 the activity is maximum suggesting that the optimal LogP for this kind of compounds is inside this window.

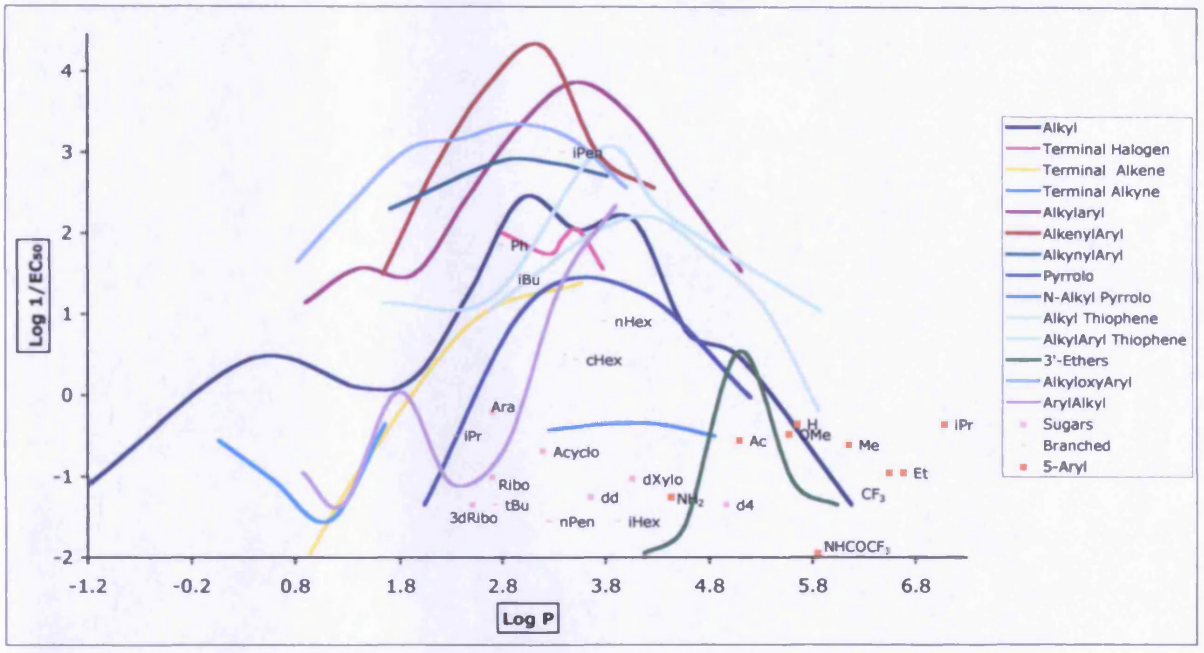


Fig 2.2: LogP vs Activity

2.3 Mechanism of action of BCNAs

Unlike the BCNAs, all other anti-VZV drugs known so far have been shown to have antiviral activity against other herpes-viruses as well. Recent pharmacological studies revealed that the BCNAs are recognised as substrates for phosphorylation by VZV-TK.[40] However, in contrast to BVDU, HSV-1 and HSV-2 TK do not recognise the BCNAs as substrates for phosphorylation, and the BCNA-5'-monophosphate is not a substrate for the dTMP kinase activity of HSV-1 TK. Furthermore the BCNAs are not recognised substrates by the cellular TK, therefore, the unique recognition of the BCNAs by VZV TK, but not by HSV TK or any other cellular TK, forms the basis of the unique specificity of the BCNAs for VZV.[41] Interestingly, when NDP kinase was added to a mixture containing BCNAs and VZV TK, no trace of BCNA-TP could be detected.[39] Although it cannot be excluded that the BCNA-DPs can be recognized by other NDP kinase isoenzymes or by other cellular enzymes, the mechanism of anti-VZV activity of the novel class of BCNAs may be entirely different from that of BVDU (being a inhibitor of viral DNA polymerase and incorporated into viral DNA when it is converted into BVDU-5'-triphosphate).

Pyrimidine nucleoside analogues are susceptible to hydrolysis to their free base by the pyrimidine nucleoside catabolic enzyme uridine phosphorylase (UPase) or thymidine phosphorylase (TPase). The free bases usually do not have any significant therapeutic activity. Thus, the UPase and TPase enzymes inactivate the antiviral pyrimidine nucleoside analogues and BVDU is a well-known example of an anti-herpes virus drug. The BCNAs were found to be resistant to the phosphorolytic cleavage by TPase, and thus are expected to be relatively stable in biological fluids as human plasma does not catabolise BCNAs to their free bases. [42] BVU and related compounds are known to be good inhibitors of human DPD,[43] the catabolic enzyme responsible of degradation of natural pyrimidines and pyrimidine analogues such as 5-FU. Given the potential complication of co-administration of BVdU and 5-FU, it is of clinical importance to know whether the free base of BCNAs can inhibits this enzyme. In contrast to BVU (IC_{50} 10μM against human liver DPD), free BCNA bases are completely inactive in inhibiting DPD at 100-250 μM. In addition, plasma levels of 5-FU were not affected by free BCNA base.[41]

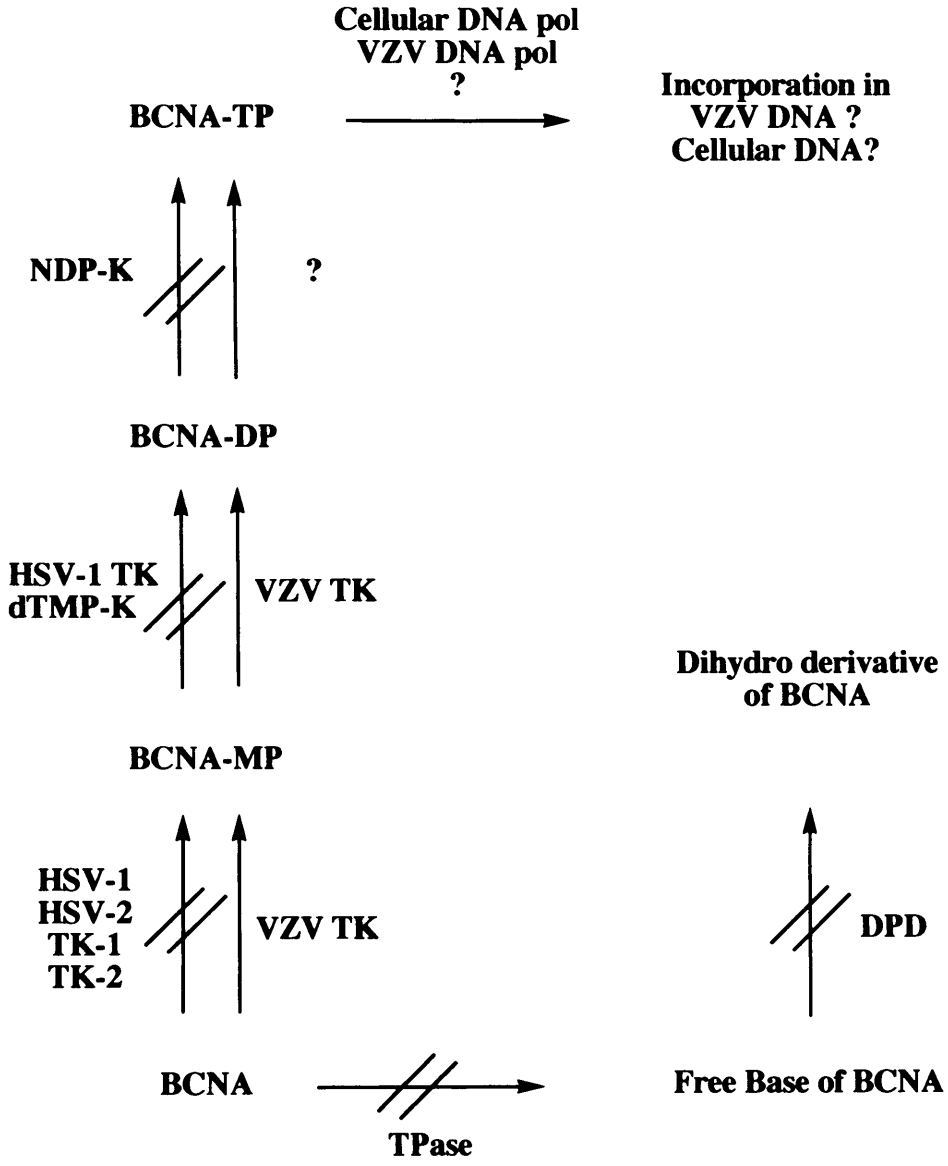

Cellular DNA pol
VZV DNA pol
?
BCNA-TP
Incorporation in
VZV DNA ?
Cellular DNA?
NDP-K
?
BCNA-DP
HSV-1 TK
dTMP-K
VZV TK
Dihydro derivative
of BCNA
BCNA-MP
HSV-1
HSV-2
TK-1
TK-2
VZV TK
DPD
BCNA
Free Base of BCNA
TPase

Fig 2.

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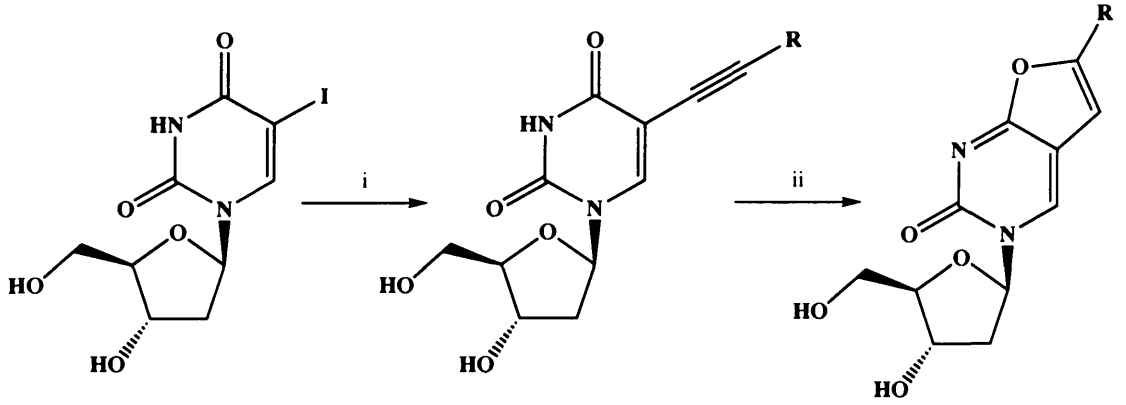
Chapter 3: Improvement of the properties of BCNAs

The extraordinary potency BCNAs exhibit against VZV had never been reported before, in fact this new class of anti-VZV agents is ca 300-fold that of ACV for the early lead with long alkyl side chains (C8-C10) and equipotent to BVDU[1], and the profile becomes even more pronounced when the alkyl side chain is replaced by a p-alkylphenyl.[2]

The most potent analogue of this family is the p-pentylphenyl derivative, which exhibited activity against VZV at subnanomolar concentrations, with no cytotoxicity observed at concentrations of 200 μM. Thus, pentylphenyl **33f** shows an antiviral selectivity of over 1,000,000 and is ca. 10,000 times more potent than acyclovir against VZV. BCNAs are completely specific for VZV, being inactive against other viruses.[1,2]

3.1 Improvement of the chemistry to BCNAs

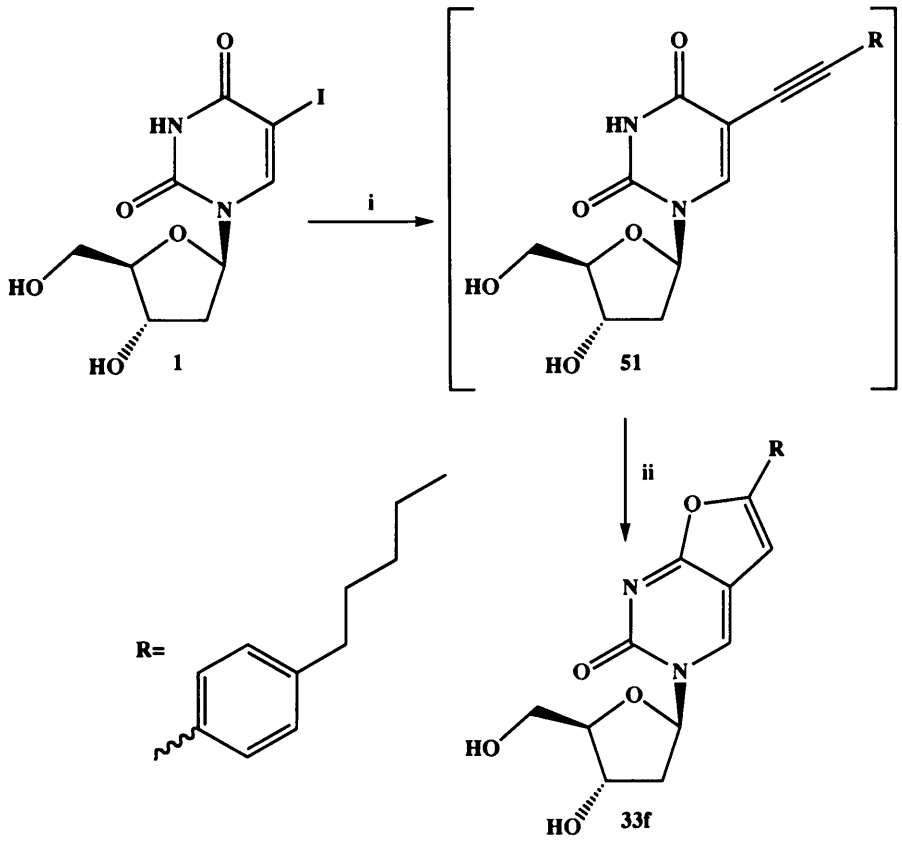
Many analogues have been synthesised in order to have a global view of SARs of this kind of compounds.[3,4,5] Furthermore the pharmacology of the BCNAs has been studied in order to understand the mechanism of action of this new class of anti-viral, which now is not completely clear. After a preliminary selection, several derivatives have been identified as possible clinical candidates, and a scale up production process was needed for the synthesis of these compounds, free from any column chromatography purification. The early synthesis of BCNAs is performed by a Pd-coupling of 5-iodo-2'-deoxyuridine with the appropriate alkyne, and then converted into the bicyclic system in the presence of copper iodide and a base[6,7] (scheme 3.1).



Scheme 3.1: *i) DMF, alkyne, Tetrakis Pd(PPh$_3$)$_4$, CuI, DIPEA. ii) MeOH, TEA, CuI.*

The work up of this reaction is quite long and tedious involving the use of large amount of solvents for low scale synthesis. Finally the overall yield of these reactions is usually less than 25% after column chromatography followed by a trituration with an organic solvent.

Other synthetic routes have been reported in order to synthesise BCNAs, involving microwave catalysis to reduce the time of the Sonogashira cross-coupling[8] and the use of silver nitrate as catalyst to cyclise the coupled compound at room temperature.[9] Although these methods give a good yield, the same work up and purification by column chromatography is needed to isolate the desired compound and in some cases protection of starting material and de-protection of the product are necessary in order to avoid the formation of side-products, adding two more steps in the synthetic route. In order to remove the chromatographic purification, a Pd-coupling of 5-iodo-2'-deoxyuridine with an alkyne under Sonogashira conditions has been performed, and then the coupled compound has been cyclised in the same pot, warming the mixture in the presence of a base. The most potent derivative pentylphenyl **33f** has been used in this new synthetic route as a sample (scheme 3.2).



Scheme 3.2: *i) DMF, 4-pentylethynylbenzene, Tetrakis Pd(PPh$_3$)$_4$, CuI, DIPEA. ii) CuI, TEA, Δ.*

After solubilisation of IDU **1** in DMF, a catalytic amount of tetrakis Pd^0 was added and allowed to react before 3 eq. of 4-pentylphenylacetylene were added, followed by the addition of a catalytic amount of copper iodide and 2 eq. of diisopropylethylamine (DIPEA). Under these conditions the reaction does not show any by-product, in fact when leaving it overnight four spots were seen on TLC: excess of alkyne that did not react, triphenylphosphine from the decomposition of tetrakis, tetrakis and the coupled compound **51**. The rate of the reaction can be increased by heating to 45 °C allowing the reaction to complete in less than one hour. **51** was then converted to **33f** by addition of a further catalytic amount of copper iodide and a large excess of triethylamine, heating at 80 °C for 2-6 hours. It was noticed that the purity of copper iodide regulated the rate of cyclisation, in fact impure copper led to a slower cyclisation, which in some cases lasted 24 hours, while a purer reagent led to complete cyclisation in less than two hours. The solvents were removed under high vacuum keeping the temperature under 50 °C and the residue was washed with DCM and the resulting precipitate was filtered to give **33f** more than 99% pure by HPLC with yields in the range of 50%-85%. This synthesis can be applied to 1g scale reactions up to 20g scale without any significant loss in yield and any column chromatography purification. External contract laboratories have now used this process more or less as here up to Kg scale.

3.2 Bioavailability

The high logP of these compounds helps the membrane permeation, but it may present a problem due to low water solubility, significantly reducing the bioavailability. In fact the solubility of **33f** in water is ca. 0.9 mg/l[10] and **29g** is just slightly more soluble.[11] A potential candidate could be found on the alkoxyphenyl series **44**, in particular **44c** represents a good compromise between solubility and activity.[11] These three compounds **33f**, **29g** and **44c** have been successfully re-synthesised in high yield and purity over 99% on HPLC with the new synthetic route (fig. 3.1).

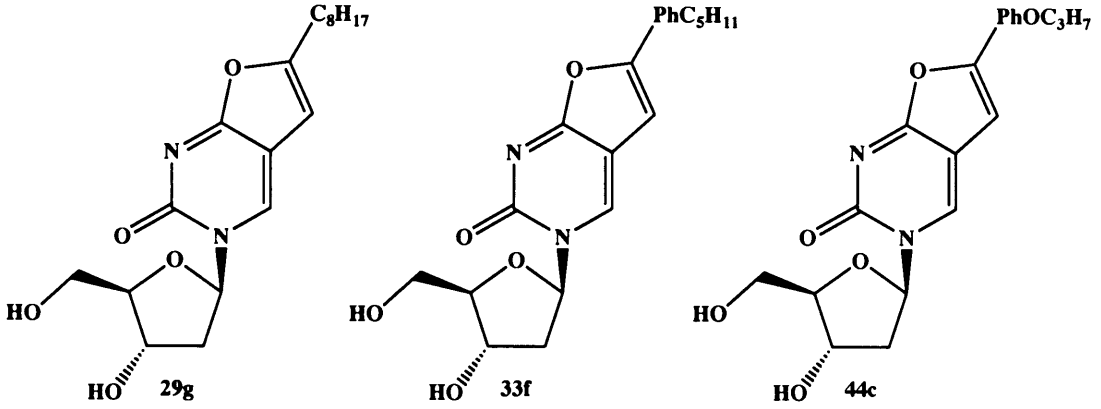


Fig 3.1: potential BCNA derivatives for pre-clinical studies

Preliminary studies of rodent bioavailability on these compounds were performed by external laboratories, showing that **29g** exhibits the best bioavailability with an estimated value less than 25%, followed by **33f** with a value less than 15%. Instead the more water-soluble **44c** shows a bioavailability value of 0% (table 3.1).

	EC_{50} μM[a]	Solubility mg/l	Bioavailability %F
29g	0.008	1.9	<25
33f	0.0003	0.9	<15
44c	0.002	4.7	0

Tab 3.1: Solubility, Potency and Bioavailability data
a) effective concentration required to reduce virus plaque formation by 50%

The poor bioavailability of **29g** and **33f** was somehow expected, due probably to the low water solubility of this kind of compounds. It was surprising that no trace of **44c** was detected, assuming that a rapid metabolism converts **44c** into a compound that is immediately eliminated.

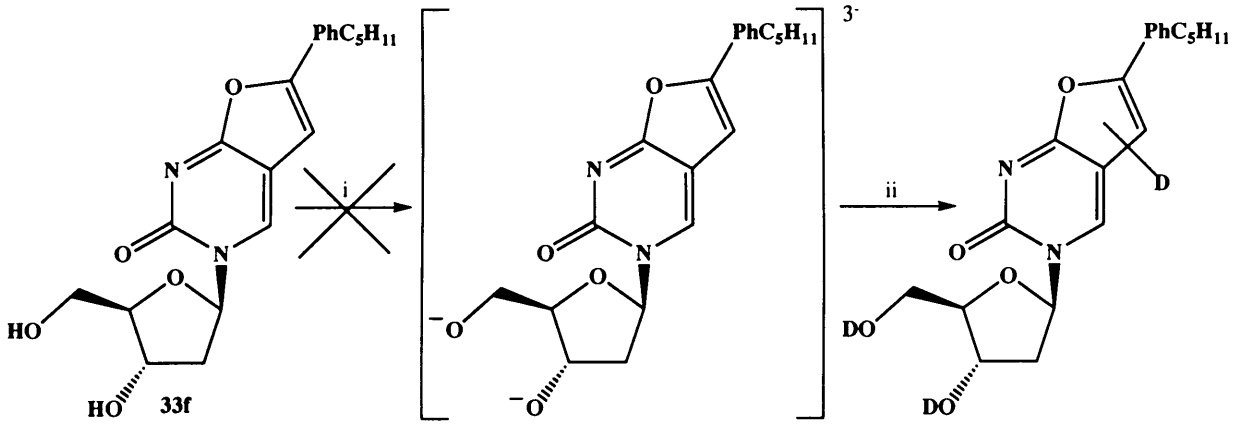
3.3 Biological Tracker: Isotope Dilution Assay (IDA)

The use of an external standard to measure bioavailability can lead to a significant error that in some cases can reach even 30%[12]. Therefore an internal standard would be more suitable for this kind of assay as the maximum error often is not more than 1%. The internal standard used in this kind of assay has to possess the same biological properties but different mass. An isotopic version of the target compound matches all the characteristics required. BCNA levels of **33f** can be measured by this assay, the Isotope Dilution Assay (IDA), based on stable isotope dilution mass spectrometry. A known amount of a deuterated analogue of **33f** is used as internal standard and added to the sample before sample processing. After analysis by HPLC and mass spectroscopy, the ratio of relative intensities of the signal of BCNA and its deuterated analog in the mass spectrum is used to determine the exact concentration of BCNA in the sample.

In order to synthesise this isotopic tracker, a stable deuterium-carbon bond has to be formed in order to obtain the target molecule with the requested features.

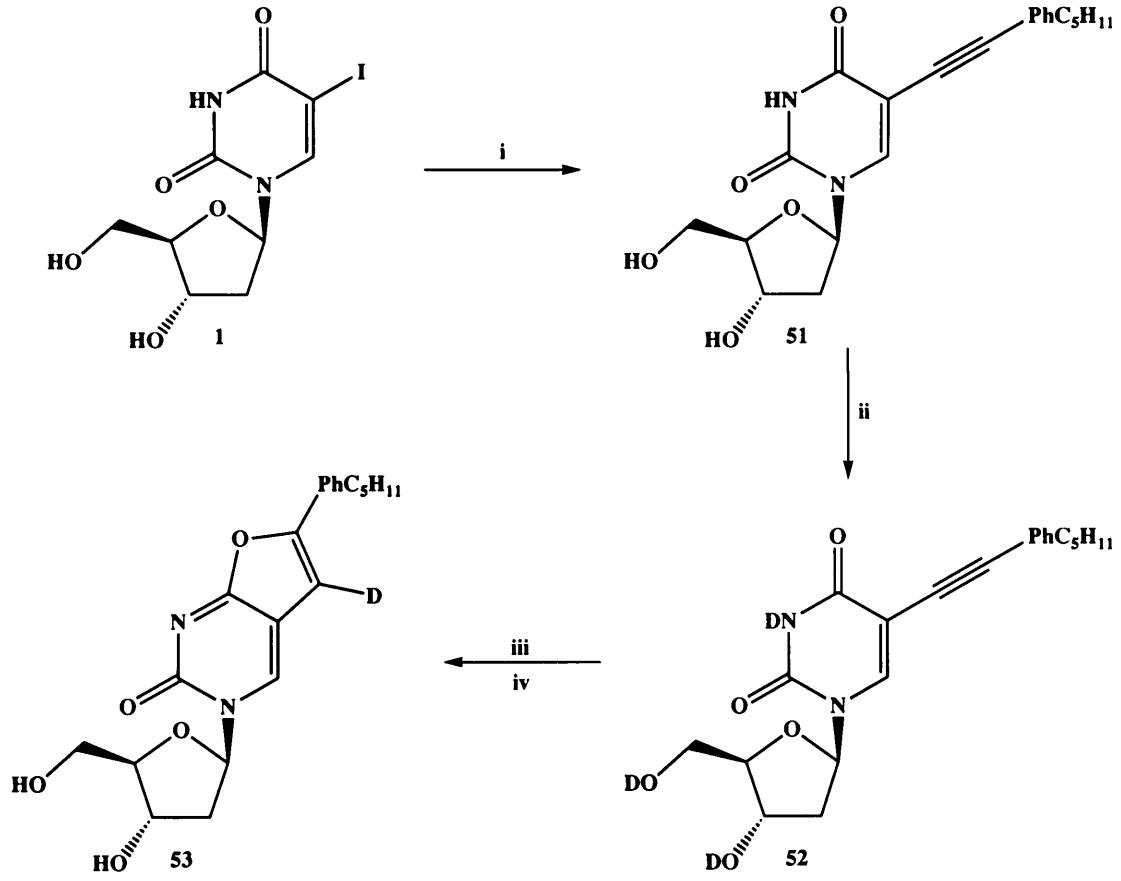
A first attempt was made treating **33f** with ^{t}butyl lithium. Three equivalents were used in order to deprotonate the two hydroxyl groups and one carbon on the molecule.

Treatment of the reaction mixture with deutereted water should have led to the desired compound (scheme 3.3). When **33f** was treated with ^{t}BuLi the TLC showed signs of decomposition of the starting material.



Scheme 3.3: *i) pyridine, ^{t}BuLi. ii) D_2O*

Another attempt was made coupling IDU with 4-pentylphenylacetylene under Sonogashira conditions (scheme 3.4). When the starting material was completely consumed the solvent was evaporated and the residue washed with cold DCM obtaining a precipitate that was identified as compound **51** but not fully characterised. Compound **51** was then dissolved in deuterated methanol (CH_3OD) in order to convert the exchangeable hydrogens with deuterium and then the solvent removed under high vacuum. This operation was repeated until no trace of OH and NH was present (checked by ^{1}H-NMR) to obtain compound **52** that was dissolved in a mixture CH_3OD and triethylamine (7:3) in the presence of catalytic amount of copper iodide under reflux to obtain compound **53**, deuterated on position 5 of the base.



Scheme 3.4: *i) DMF, 4-pentylphenylacetylene, Tetrakis Pd(PPh$_3$)$_4$, CuI, DIPEA. ii) CH$_3$OD. iii) CH$_3$OD, TEA, CuI, Δ. iv) MeOH.*

The success of the reaction was verified by mass spectrometry, ^{1}H-NMR, ^{2}H-NMR and ^{13}C-NMR. The mass spectrum of compound **33f** presents a peak at 398 m/z, while the spectrum of **53** shows a peak at 399 m/z. Evidence of deuteration can be seen in then ^{1}H-NMR. The spectrum of **33f** presents a singlet at 7.20 ppm that represents the signal of H-5, the substitution of the hydrogen with deuterium results in the disappearance of this signal as shown in fig. 3.2.

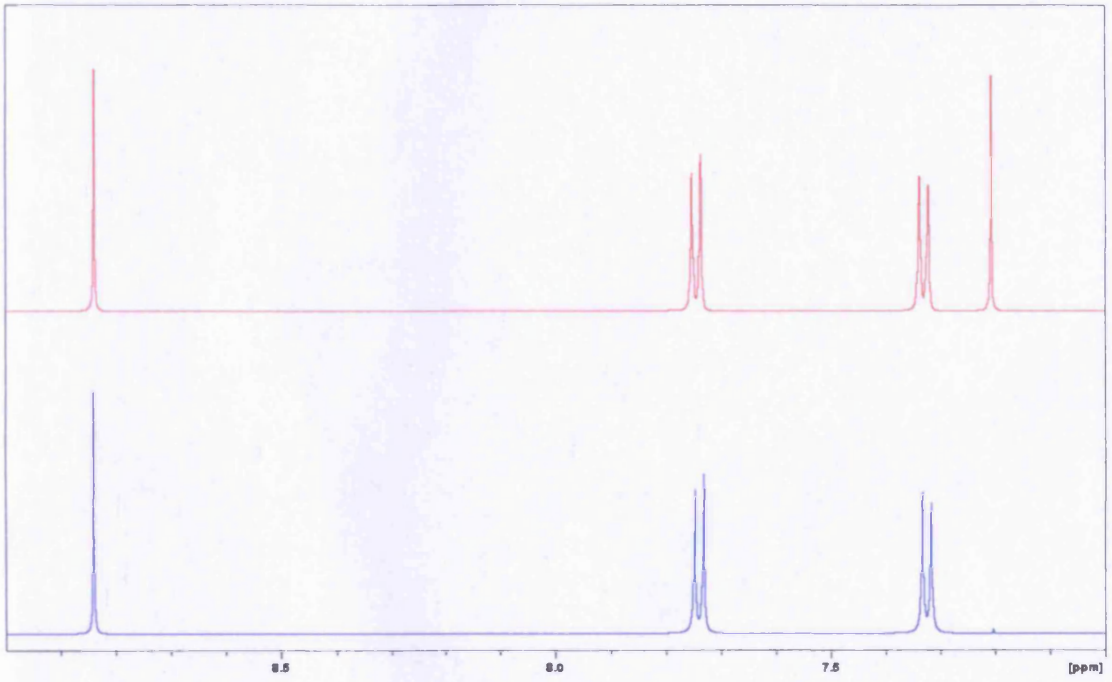


Fig. 3.2: red spectrum **33f**, blue spectrum **53**

Regarding the ^{13}C-NMR, the signal of the carbon that bears the deuterium atom was so small that it was not possible to detect it clearly. The PENDANT spectrum of **53** showed a weak broad 'up' signal at 98.48 ppm, where in **33f** shows a sharp intense 'down' signal at 98.65 ppm. This happens because deuterium increases the relaxing time of the carbon to which it is attached and the resulting effect is more dramatic than in quaternary or carbonyl carbons.[13] Furthermore the chemical shift is slightly different (fig 3.3). This small difference in chemical shift is often observed in ^{19}F-NMR of molecules containing chlorine or bromine atoms. Isotopes do not show any difference in their electronic environment within the Born-Oppenheimer approximation, but they show different chemical shifts due to different vibrational levels.[14]

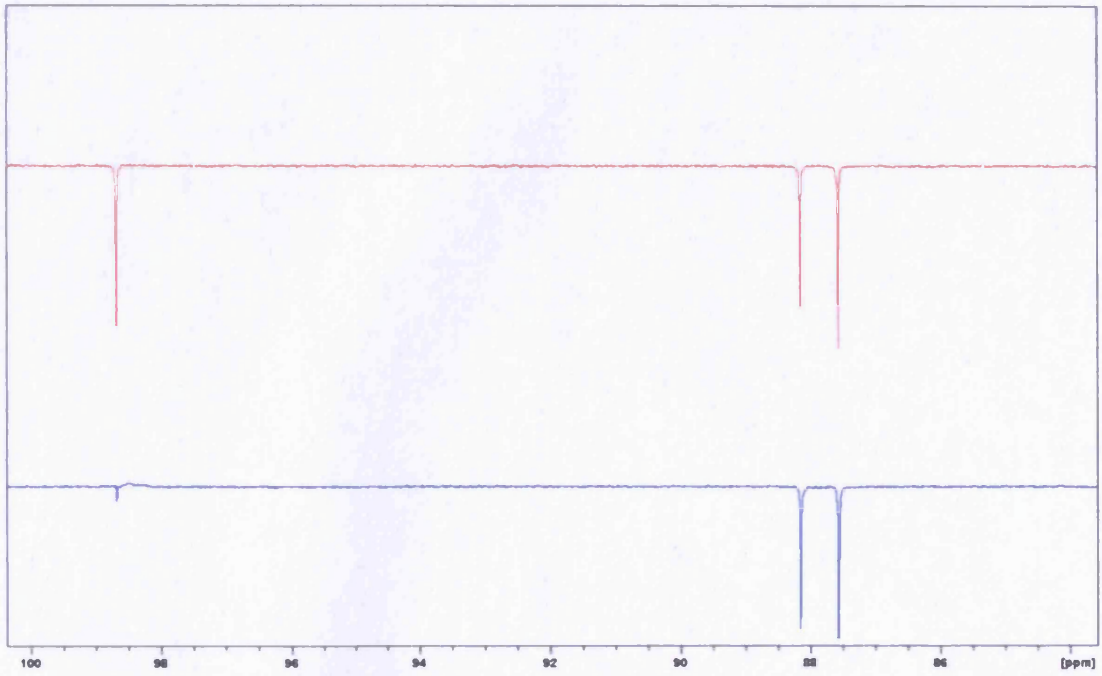


Fig 3.3: red spectrum **33f**, blue spectrum **53**

A further analysis to prove the deuteration was given by the ^{2}H-NMR experiment. The spectrum in fig 3.4 shows the presence of a peak at 7.20 ppm indicating the success of reaction. Usually ^{2}H-NMR experiments are run in fluorinated solvents as a lock signal, but in this case the spectrum has been acquired without lock in normal DMSO as the magnetic field was stable enough for the duration of the experiment.

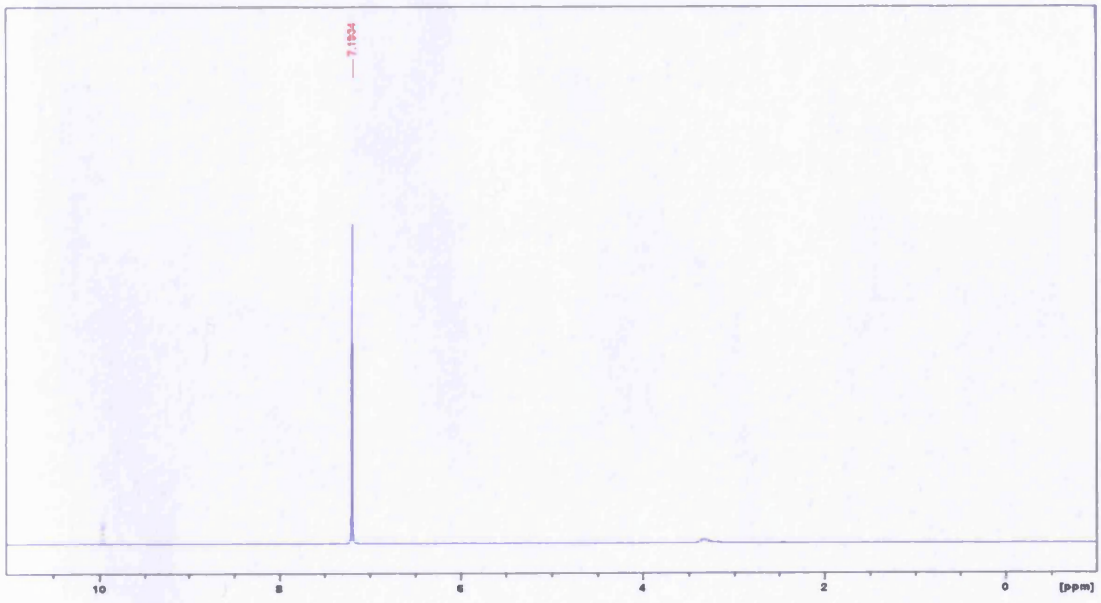
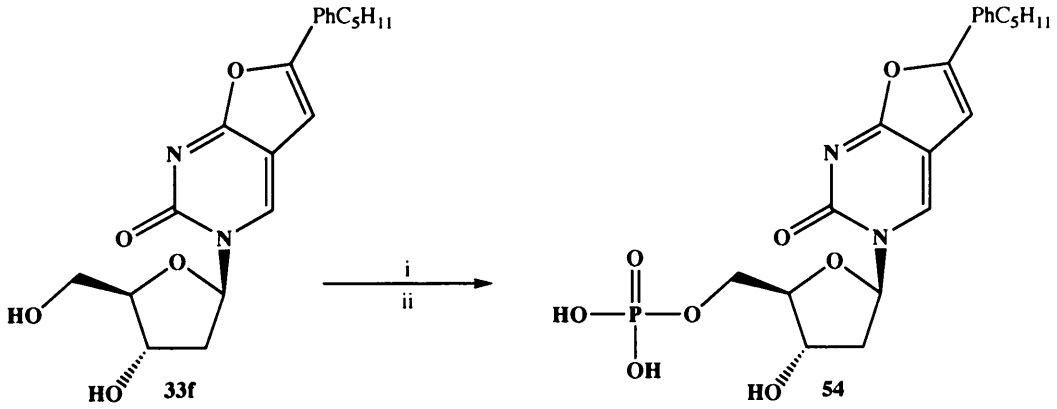


Fig 3.4: 2H-NMR of **53**

3.4 Phosphate prodrug

Since the above mentioned ether analogue results were not satisfactory, the monophosphate of **33f** has been thought as a solution, as the presence of a charge should increase significantly the water solubility. This strategy has been used for fludarabine[15], an anti-cancer nucleoside used against chronic lymphocytic leukaemia. This drug is administrated as a monophosphate salt, which is rapidly de-phosphorylated in human plasma and once it is inside the cell, it is converted into tri-phosphate inhibiting the ribonucleotide reductase and DNA-polymerase. Based on this strategy, the monophosphate analogue of **33f** was synthesised following the Yoshikawa procedure obtaining the compound **54** as the tributylammonium salt (scheme 3.3). Compound **54** has been reported before,[16] but the replacement of ammonium bicarbonate with tetrabutylammonium bicarbonate resulted in an easier purification by column chromatography. As expected, compound **54** is >100 fold more water-soluble than parent drug **33f**, but despite the solubility enhancement, the bioavailability did not show any significant improvement over **33f**.

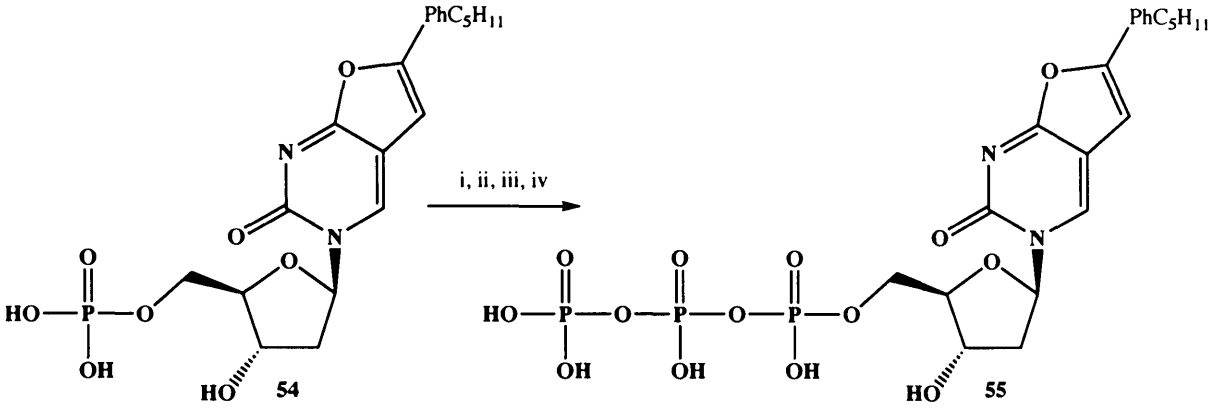


Scheme 3.3: *i) $PO(OEt)_3$, $POCl_3$. ii) $HNBu_3HCO_3$, H_2O*

In a previous work,[15] the triphosphate analogue of **33f** was successfully synthesised. The conversion of **54** into **55** (scheme 3.4) led to the formation of a more polar fluorescent by-product, and while **55** has been isolated and purified in moderate yield, the purification of this second fluorescent spot was difficult and it could not be possible to isolate it in pure form but it has been obtained in a mixture with the tri-phosphate **55**. This mixture, called X, has been sent to test exhibiting a better inhibition against the viral DNA-polymerase then **55**. In order to identify and isolate this X-compound, **55** was synthesised again reproducing the exact

conditions reported, using **54** as starting material (scheme 3.4). Only **55** was detected, isolated in pure form and fully characterised, matching with the data reported.[15]

The identity of the putative more active inhibitor in the mixture is currently unclear.



Scheme 3.4: *i) DMF, CDI. ii) MeOH. iii) tributylammoniumpyrophosphate. iv) TBAB.*

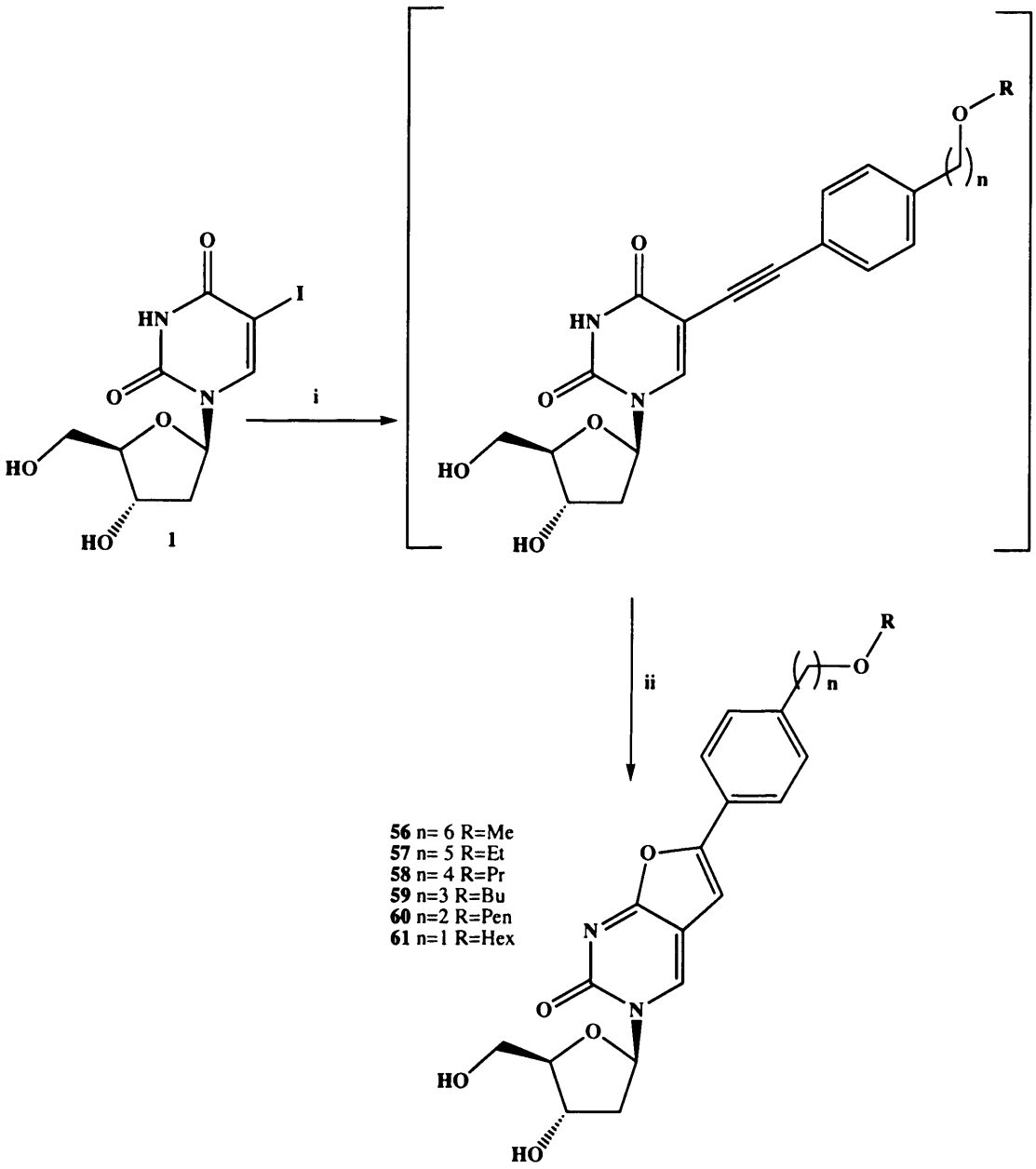
3.5 Ether analogues: enhanced solubility

It is known that the intrinsic lipophilicity of the lead compounds has a negative effect on the water solubility, affecting directly the bioavailability. The introduction of one or two atoms of oxygen in the alkyl chain has been reported, leading to the alkoxyphenyl series **44**[11,17] and glycol series **45**.[18] The first one retains a good antiviral activity but it is rapidly metabolised in vivo as the best compound **44c** was found to have bioavailability of ca 0%, while the second instead shows a dramatic loss of activity.

The modification of the alkoxyphenyl series **44** would be a good strategy in order to enhance the water solubility whilst keeping a high antiviral activity. The shifting of the oxygen through the side chain should retain the water solubility without affecting the antiviral activity converting the aromatic ether into an aliphatic ether, making it harder to metabolise.

A new series of arylalkyl ethers **56-61** has been synthesised,[10] keeping fixed the total length of the aliphatic chain at 8 atoms and moving the oxygen along it.

The synthesis involves the Pd-coupling of IDU with the corresponding acetylene under Sonogashira conditions and then cyclising the ethynyl analogue in the presence of triethylamine at 80 °C (scheme 3.5).

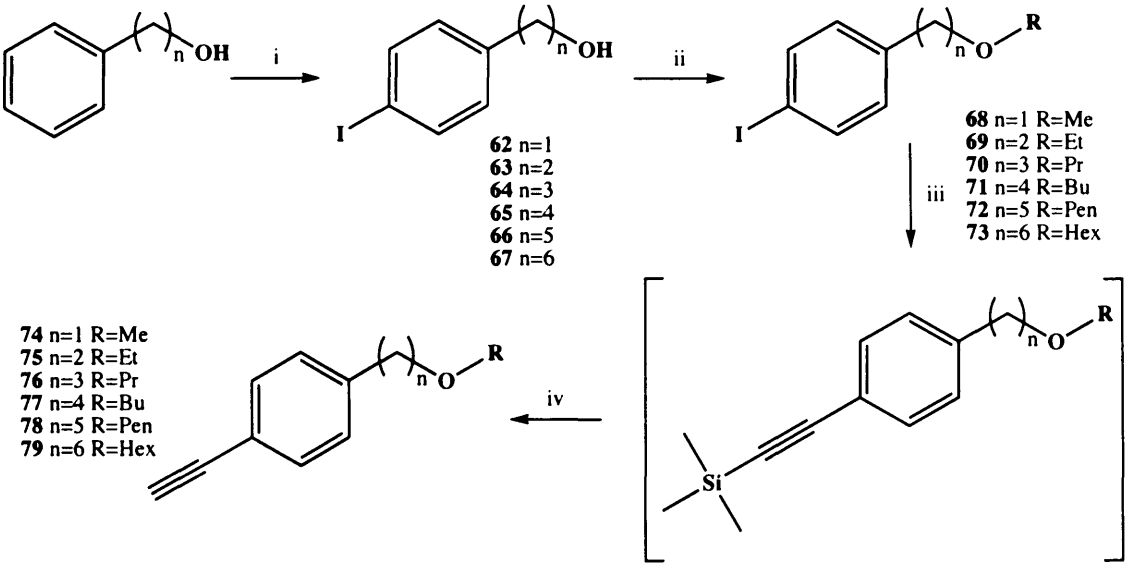


Scheme 3.5: *i) DMF, 4-ethynylalkylbenzene, Tetrakis Pd(PPh$_3$)$_4$, CuI, DIPEA. ii) CuI, TEA,*

The 4-ethynylalkylbenzene derivatives were not commercially available, and they had to be synthesised starting from the appropriate alcohol, according to scheme 3.6.

The alcohol was protected by acetylation of the hydroxyl group in the presence of a catalytic amount of concentrated sulphuric acid, in a solution of acetic acid at 100 °C. Then the aromatic ring was halogenated by reaction of iodine with sodium iodate. The crude of this reaction, after extraction, was treated with 10% sodium methoxide in methanol in order to deprotect the acetyl group, leading to the iodophenyl alcohols **62-67** in high yield. Although this reaction shows some regioselectivity for the para position, ^{1}H-NMR showed traces of the

ortho by-product (<10%). The separation of the two regioisomers by column chromatography was not achieved and the mixtures were used for the next step without any further purification. Compounds **62-67** were treated with sodium hydride and then coupled with the corresponding alkyl mesylate under reflux in THF to obtain the ethers **68-73**. These iodo-ethers underwent a Pd-coupling with TMS-acetylene, and the elimination of TMS was performed in the presence of TBAF, yielding acetylenes **74-79**.



Scheme 3.6: *i) a. CH_3COOH, H_2SO_4 b. I_2, $NaIO_3$ c. MeONa, MeOH. ii) THF, NaH, Alkyl-mesylate. iii) CH_3CN, Tetrakis $PdCl_2$, CuI, DIPEA. iv) TBAF.*

3.6 Biological results and solubility studies

The synthesised ethers **56-61** were evaluated for their ability to inhibit the replication of VZV according to previously described methods.[19] Data are shown in table 3.1 for the activity of **56-61** versus two strains of thymidine kinase-competent (TK^+) VZV and also two strains of thymidine kinase deficent (TK^-) VZV with data also included for the reference anti-herpetics ACV **11** and **33f**.

The main purpose for preparing this new series was to enhance the water solubility whilst retaining lipophilicity and antiviral potency. Water-solubility studies have been carried out on this series[10] alongside parent pentylphenyl **33f** (table 3.1).

Although these compounds retain full antiviral activity at non-toxic concentration, the introduction of an atom of oxygen in the alkyl chain did not achieve significant water

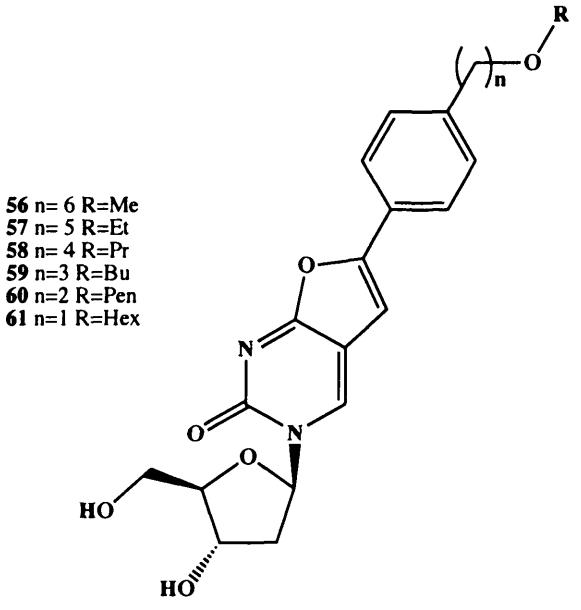
solubility enhancement. Along this new series, only compound **56** exhibits water solubility greater than **33f**, but the enhancement obtained is not significant.

In contrast to earlier reported ether and glycol series, where solubility enhancement were achieved to the detriment of activity, and aryloxy series, where bioavailability was close to 0%, this new series retains full antiviral activity with no significant improvement of water solubility.

				EC_{50} μM[a]					
	R	ClogP	Sol. mg/l	**VZV OKA**	**VZV YS**	**TK⁻ VZV 07**	**TK⁻ VZV YS**	MCC μM[b]	CC_{50} μM[c]
56[10]	Me	2.78	2.13	0.001	0.0084	>20	>0.5	100	>50
57[10]	Et	2.64	1.01	-	-	-	-	-	-
58[10]	Pr	2.64	0.74	0.0002	0.0005	>20	>20	20	>50
59	Bu	2.64	0.96	0.012	0.01	20	-	50	>200
60[10]	Pen	2.79	-	-	-	-	-	-	-
61	Hex	3.33	0.66	0.038	0.072	>100	>100	>100	>50
33f	-	3.52	0.9	0.0003	0.0001	>5	>5	>20	>200
ACV	-	-2.42	2500[20]	2.9	1	74	125	>200	>200

a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration required to alter microscopically detectable cell morphology
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

Table 3.1



56 n= 6 R=Me
57 n= 5 R=Et
58 n= 4 R=Pr
59 n=3 R=Bu
60 n=2 R=Pen
61 n=1 R=Hex

Fig 3.5: Ether series

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Chapter 4: Substitution at the phenyl moiety

The investigation of the effect of halogen substitution on different sites of the aryl moiety of BCNAs has been reported before. The SARs of this series are not very clear, but it is evident that the introduction of a halogen in the phenyl ring affects the antiviral activity. Comparison of the different regioisomers reveals a marked dependence on the nature and position of the halogen for the anti-viral activity. In general the decreasing order of the potency is ortho > para > meta. [1] Although this series is less active against VZV than pentylphenyl **33f**, the ortho derivatives **36a-c** are more potent than the phenyl parent **33a** (fig 4.1).

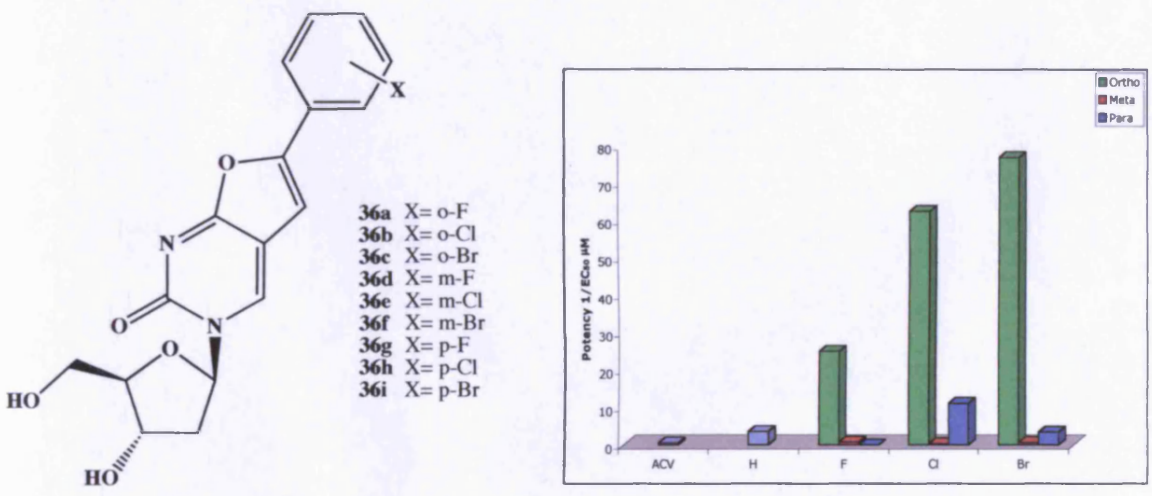


Fig 4.1: Halophenyl derivatives

In view of these results, the ortho position has been further investigated in a previous study,[2] showing that the introduction of polar and non-polar groups at this position of the phenyl moiety appears very damaging for the potency (table 4.1) and that the target enzyme does not tolerate such kind of substitutions.[2] The introduction of a very large substituent causes a significant reduction of biological activity as can be observed with isopropyl derivative **81**, which is 30 time less potent than ethyl derivative **80**.

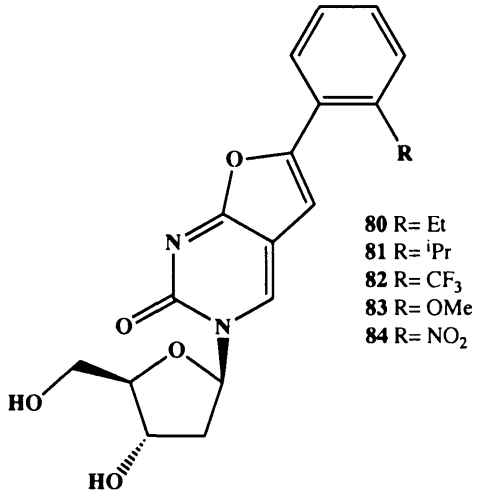


Fig 4.2: Ortho derivatives

		EC_{50} μM[a]				MCC μM[b]	CC_{50} μM[c]
	R	VZV OKA	VZV YS	TK⁻ VZV 07	TK⁻ VZV YS		
80	Et	0.22	0.33	>200	-	>200	>200
81	^{i}Pr	6.7	-	>80	-	400	165
82	CF_3	6.7	7.6	>80	-	400	>200
83	OMe	0.08	0.09	>200	-	>200	>200
84	NO_2	4.8	-	>16	-	80	88
33a	H	0.16	0.28	>200	>162	>20	>200
ACV	-	2.9	1	74	125	>200	>200

Table 4.1

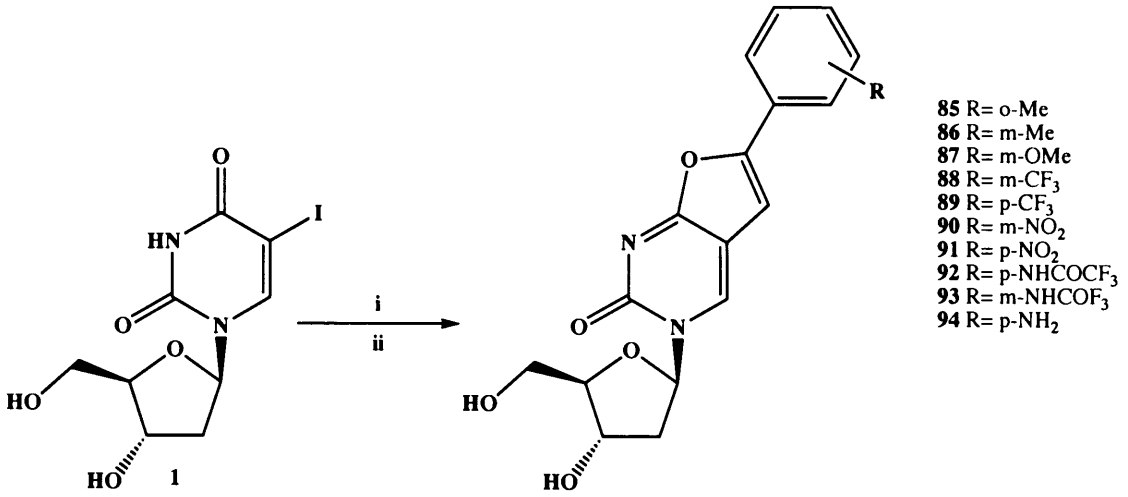
a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration required to alter microscopically detectable cell morphology
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

4.1 Synthesis of target molecules

In order to further probe and complete the SARs in this region of the molecule, the synthesis of a new series of BCNAs was planned, introducing the same kind of substituents in the meta and para position.

The standard procedure for the synthesis of target BCNAs was applied to the compounds where the acetylenes were commercially available, involving a Pd-catalysed coupling reaction between IDU (**1**) and the appropriate acetylene (scheme 4.1). Only for compound **85**, the isolation of the intermediate was needed in order to cyclise it in dioxane in the presence of 1

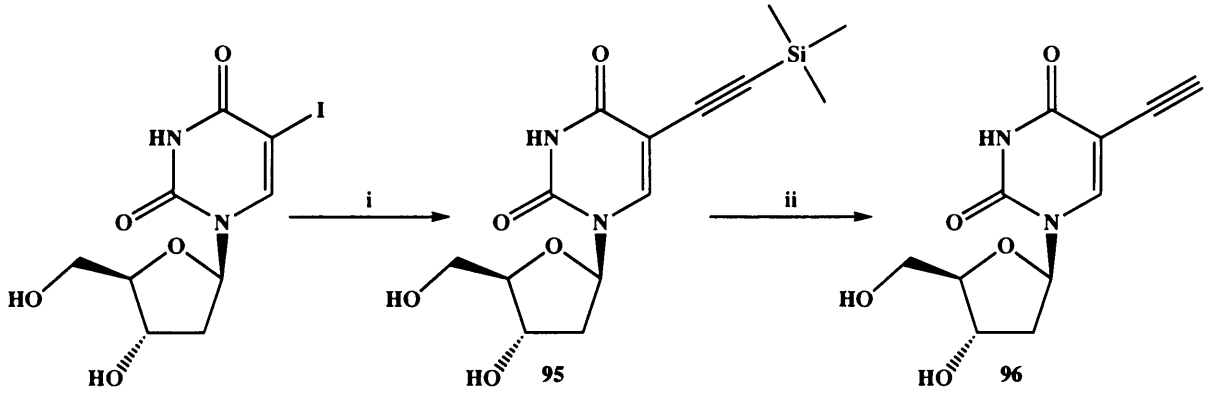
eq. of copper iodide and large excess of TEA under reflux as reported for the ortho derivatives.[2]



Scheme 4.1: *i) DMF, Arylacetylene, Tetrakis Pd(PPh$_3$)$_4$, CuI, DIPEA. ii) a: TEA, CuI. b(85 only): Dioxane, CuI.*

4.1.1 Nitro-Derivatives

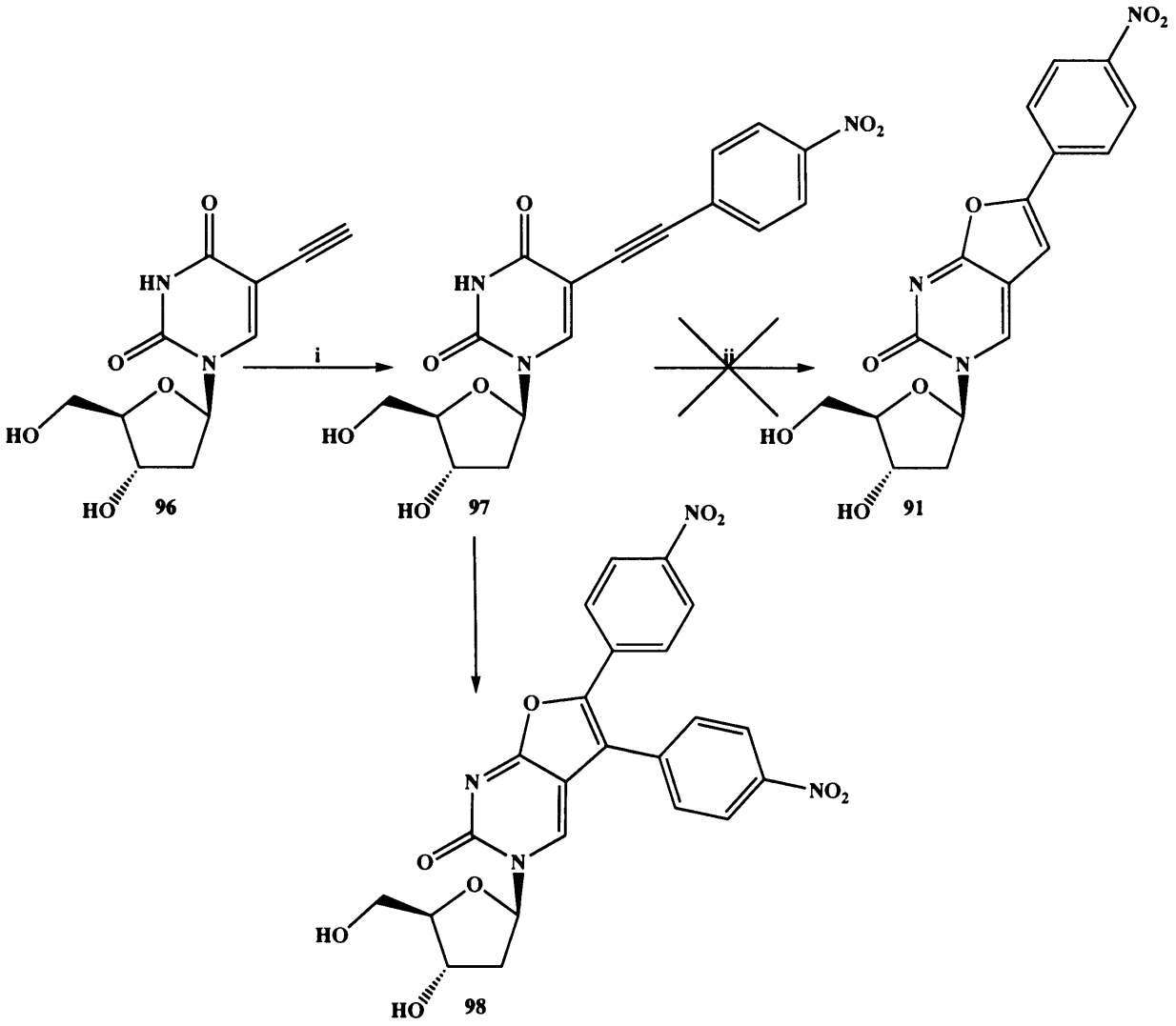
The acetylene required to synthesise the nitro derivatives **90** and **91** were not commercially available, therefore a new synthetic pathway was planned (scheme 4.2). IDU was coupled with trimethylsilyl acetylene under Sonogashira conditions to obtain 5-(trimethylsilyl-ethynyl)-2'-deoxyuridine **95**, which was de-protected with methanolic ammonia to give EDU **96** in 65% overall yield. In this reaction, the use of acetonitrile rather than DMF was found to be more convenient in terms of purification by column chromatography.



Scheme 4.2: *i) ACN, TMS-acetylene, Tetrakis Pd(PPh$_3$)$_4$, CuI, DIPEA. ii) MeOH/NH$_3$*

The standard Pd-catalysed coupling was applied to EDU **96** (scheme 4.3), which was reacted with 3 eq. of p-iodonitrobenzene. At this stage the TLC showed the total conversion of **96**

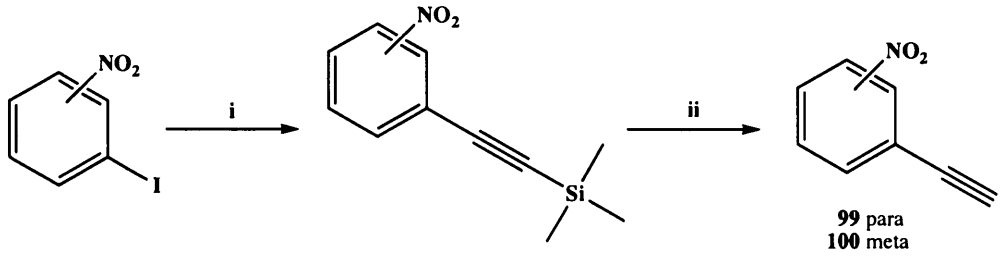
into **97**. The addition of TEA and further copper iodide under heating did not lead to the formation of **91**, but the intermediate **97** was totally converted into the di-substituted derivative **98**. Evidences for the formation of **98** were given by mass spectra, NMR and CHN, which are consistent with the proposed structure.



Scheme 4.3: *i) DMF, p-iodontrobenzene, Tetrakis Pd(PPh3)4, CuI, DIPEA. ii) CuI, TEA.*

A second attempt was made, carrying out the same reaction using this time 3 eq. of EDU and 1 eq. of p-iodonitrobenzene. This time compound **91** was obtained and no trace of **98** was observed. However, the purification of **91** represented a problematic job due to the presence of impurities with an r.f. similar to **91** and it was not possible to obtain it in pure form after two chromatographic columns. In view of this result, 4-ethynylnitrobenzene **99** and 3-ethynylnitrobenzene **100** were synthesised, coupling the corresponding iodonitrobenzenes with TMS-acetylene followed by deprotection with methanolic ammonia in high yield after

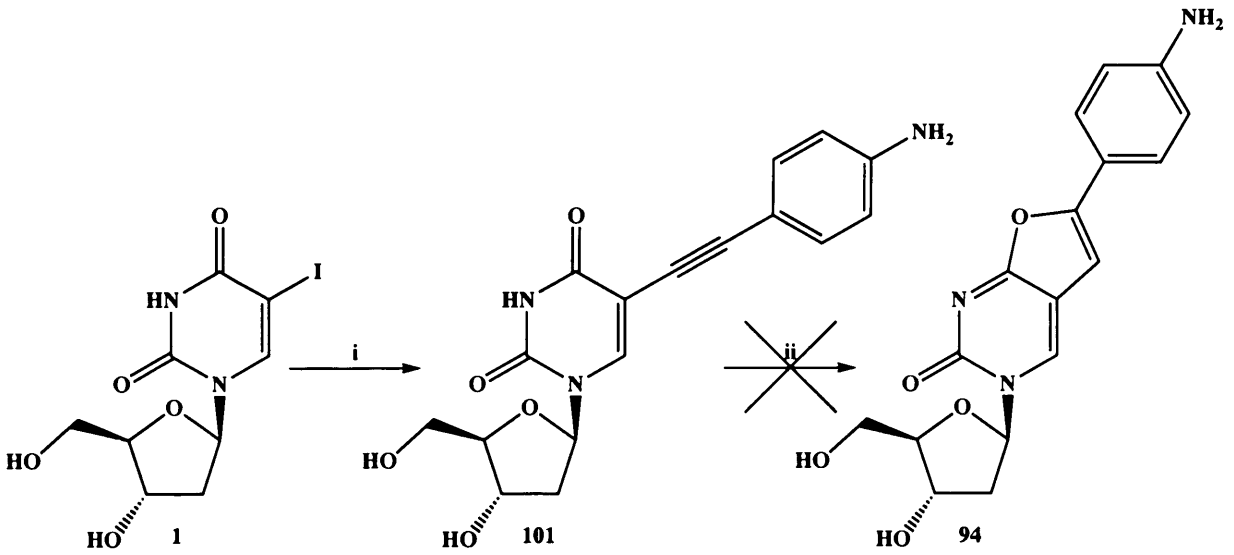
purification by flash column chromatography (scheme 4.4). These acetylenes were used for the coupling with IDU **1** using the standard procedure.



Scheme 4.4: : *i) DMF, TMS-acetylene, Tetrakis Pd(PPh$_3$)$_4$, CuI, DIPEA. ii) MeOH/NH$_3$*

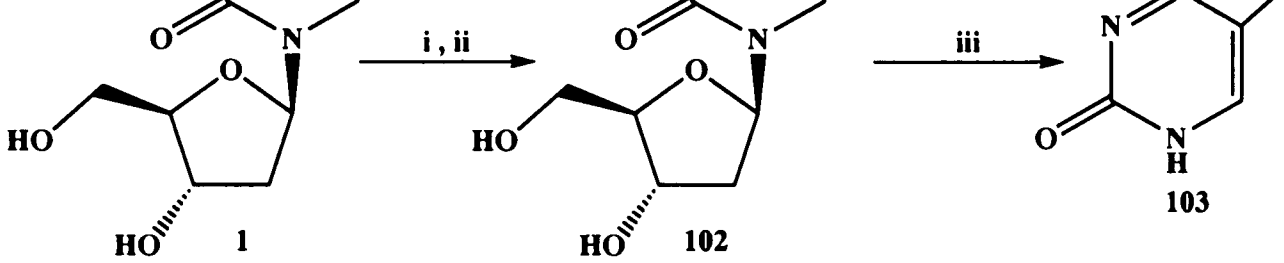
4.1.2 Amino derivatives

The synthesis of amino derivatives was previously attemped[2,3] using different synthetic routes without reaching the target molecules. According to scheme 4.5, IDU **1** was totally converted into **101** when coupled with 4-ethynylaniline, but when the cyclisation was attempted, the TLC showed multiple spots and the most yellow fluorescent product was isolated and identified as a mixture of unknown compounds.[3]



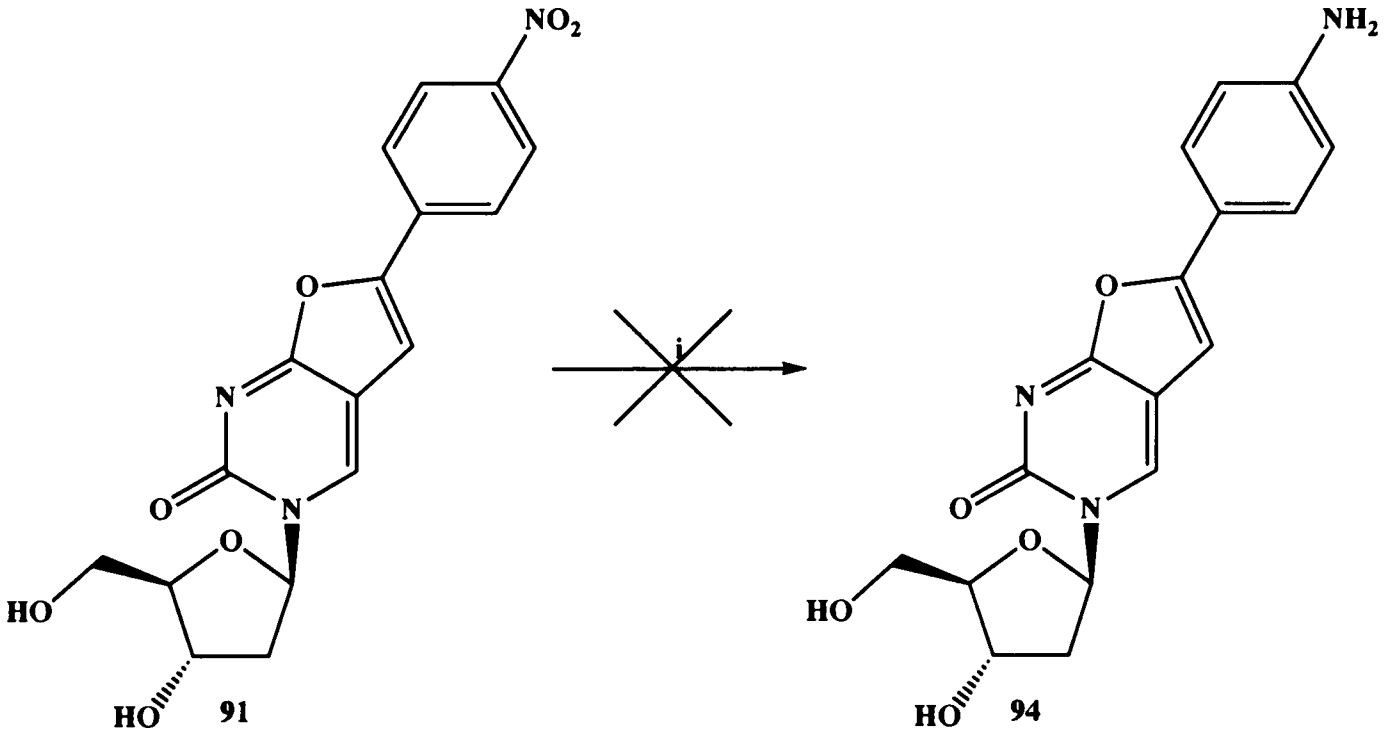
Scheme 4.5: *i) DMF, 4-ethynylaniline, Tetrakis Pd(PPh$_3$)$_4$, CuI, DIPEA. ii) CuI, TEA.*

The protection of the amino group with BOC was though to be a solution to prevent the formation of by-products. The elimination of the protecting group would have carried out after achieving the cyclisation of the nucleoside (scheme 4.6). The coupling of IDU with 4-ethynyl-BOC-aniline followed by cyclisation led to compound **102** without formation of any by-product. However the deprotection of the BOC in acidic conditions failed to give the target compound **94**, resulting in a cleavage of the glycosidic bond and yielding compound **103**.[3]



Scheme 4.6: *i) DMF, 4-ethynyl-BOC-aniline, Tetrakis Pd(PPh$_3$)$_4$, CuI, DIPEA. ii) CuI, TEA. iii) TFA,*

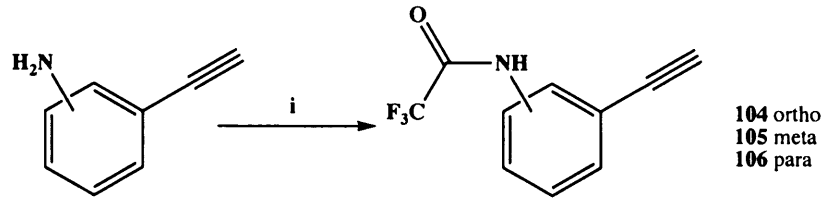
An alternative synthetic pathway was tried, involving the reduction of the nitro grou into an amino group using hydrazine in the presence of Pd on carbon at reflux[4] (schem Unfortunately this reduction was found to be too aggressive and decomposition of non-fluorescent spots was observed on TLC.



Scheme 4.7: *i) EtOH, N_2H_4, 5% Pd/C*

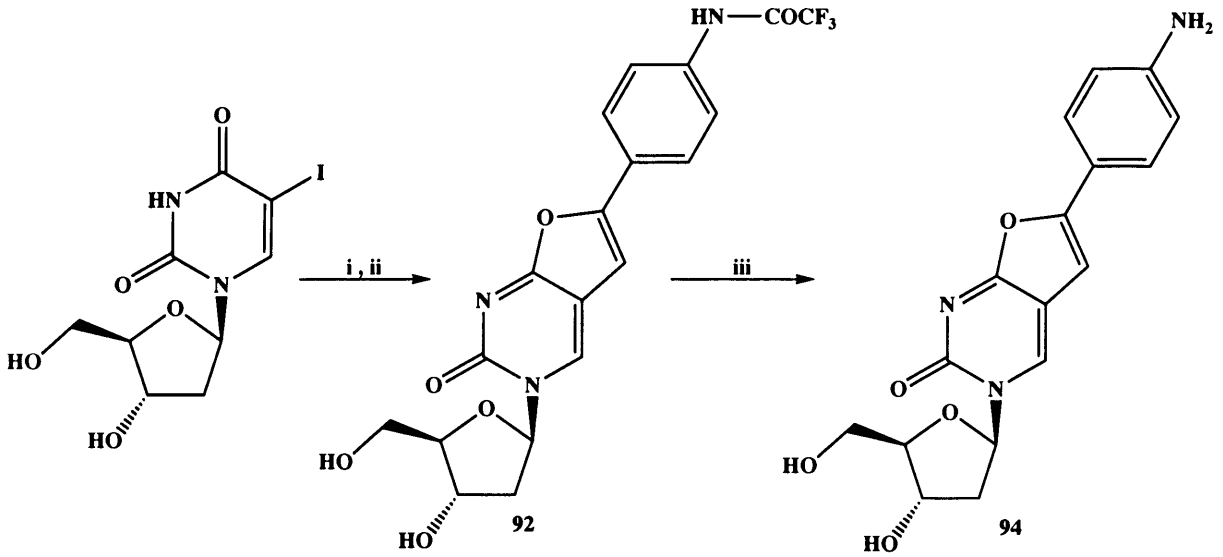
After the successful cyclisation reaction with a protected amino group **102**, an alte protecting group has been sought in order to achieve the deprotection in milder con The trifluoroacetyl group represented a good solution as it can be easily removed i

conditions. According to scheme 4.8, ethynylanilines were reacted with trifluoroacetic anhydride, giving the protected acetylenes **104-106**.



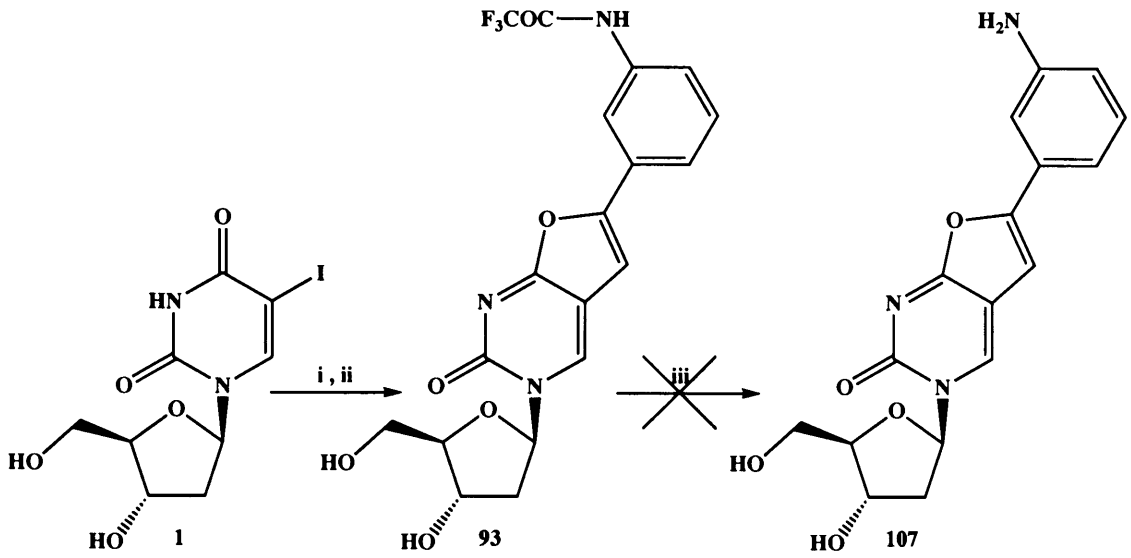
Scheme 4.8: *i) THF, trifluoroacetic anhydride*

Compound **106** was then used for the coupling with IDU under standard conditions followed by cyclisation in order to obtain compound **92**. Mild hydrolysis of the latter, in the presence of 1 eq. of potassium carbonate in methanol, gave the desired compound **94** in high yield (scheme 4.9).



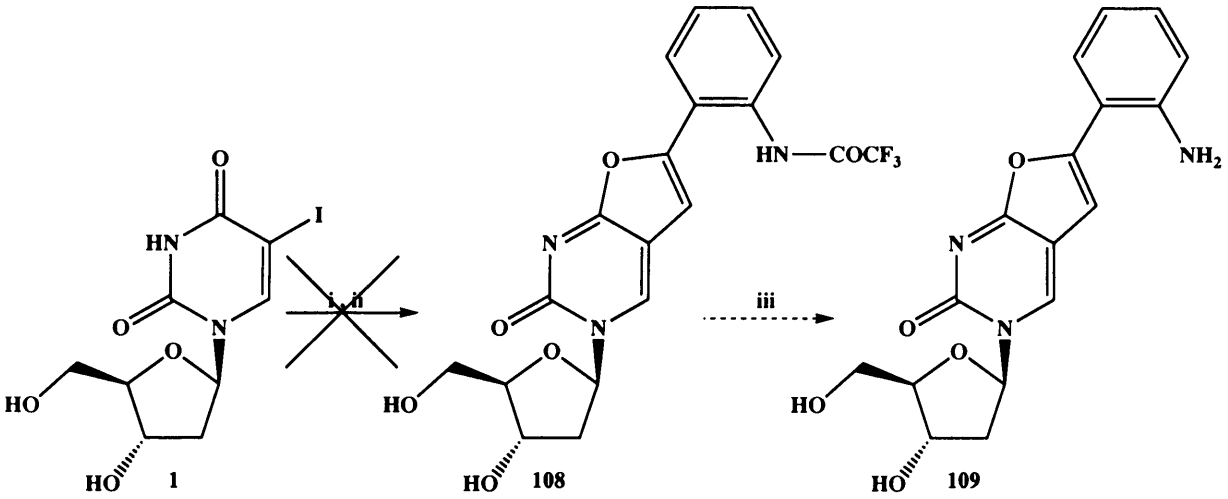
Scheme 4.9: *i) DMF, 106, Tetrakis Pd(PPh$_3$)$_4$, CuI, DIPEA. ii) CuI, TEA. iii) MeOH/K$_2$CO$_3$.*

The synthetic route used to prepare **94** was used also to synthesise the meta (**107**) and ortho (**109**) derivatives. Compound **93** has been obtained in 40% overall yield according scheme 4.10, but when the deprotection was attempted, compound **93** decomposed into non-fluorescent unidentified compounds. Another attempt of deprotection was made using methanolic ammonia obtaining the same kind of decomposition.



Scheme 4.10: *i) DMF, **105**, Tetrakis Pd(PPh$_3$)$_4$, CuI, DIPEA. ii) CuI, TEA. iii) MeOH/K$_2$CO$_3$.*

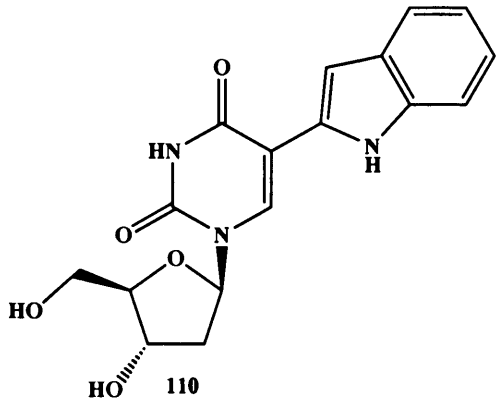
At the same time the synthesis of the ortho derivative **109** was tried following the scheme 4.11.



Scheme 4.11: *i) DMF, **104**, Tetrakis Pd(PPh$_3$)$_4$, CuI, DIPEA. ii) CuI, TEA. iii) MeOH/K$_2$CO$_3$.*

Coupling IDU with acetylene **104** led to clean formation of the coupled compound that would have been cyclised after addition of copper iodide and triethylamine to obtain **108**. Although on TLC a fluorescent spot was observed, the cyclisisation did not form the expected furan ring, but it occurred via the amido group onto the carbon-carbon triple bond to generate an indolic system, removing at the same time the protecting group and obtaining compound **110** (fig 4.3). Evidence of this cyclised by-product was given by ^{1}H-NMR, confirmed from the presence of two different NH signals over 11 ppm, one given by the pyrimidine ring and the other by the indole ring. Compound **110** is reported in the literature[5] involving a 5 step

synthesis starting from IDU. Herein one-pot synthesis of **110** has been reported starting from the same starting material.

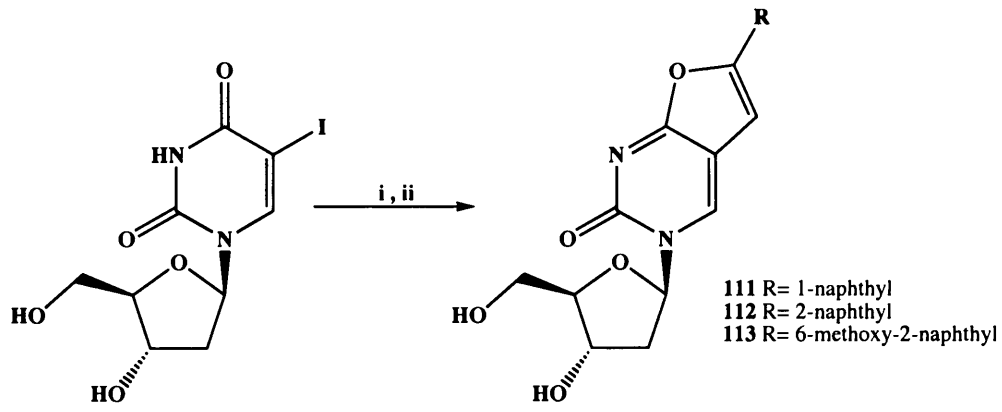


110

Fig 4.3: 5-(2-indolyl)-2'-deoxyuridine **110**

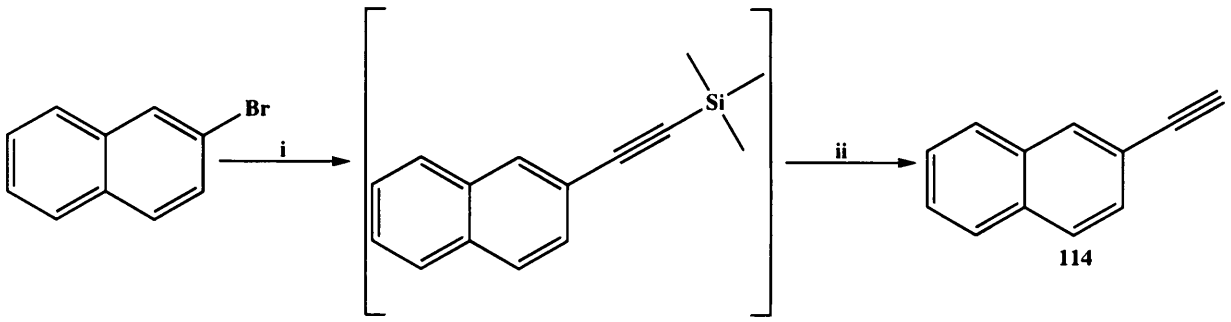
4.2 Naphthyl derivatives

It was of interest to further examine the effects of extending the conjugation of the bicyclic furano system. The substitution of the pentyl chain of **33f** with a phenyl moiety led to the biphenyl derivative **47i**[6] that was found to be less active but still active in the nM range (0.031 μM). In order to further investigate this kind of substitution, a new series of BCNAs bearing a naphthyl moiety have been synthesised. The standard procedure was applied in order to synthesise compounds **111-113**, coupling IDU with the corresponding naphthyl acetylenes (scheme 4.12).



Scheme 4.12: *i) DMF, arylacetylene, Tetrakis Pd(PPh_3)$_4$, CuI, DIPEA. ii)* ***a****: CuI, TEA.* ***b****(only 111): Acetone, TEA, $AgNO_3$.*

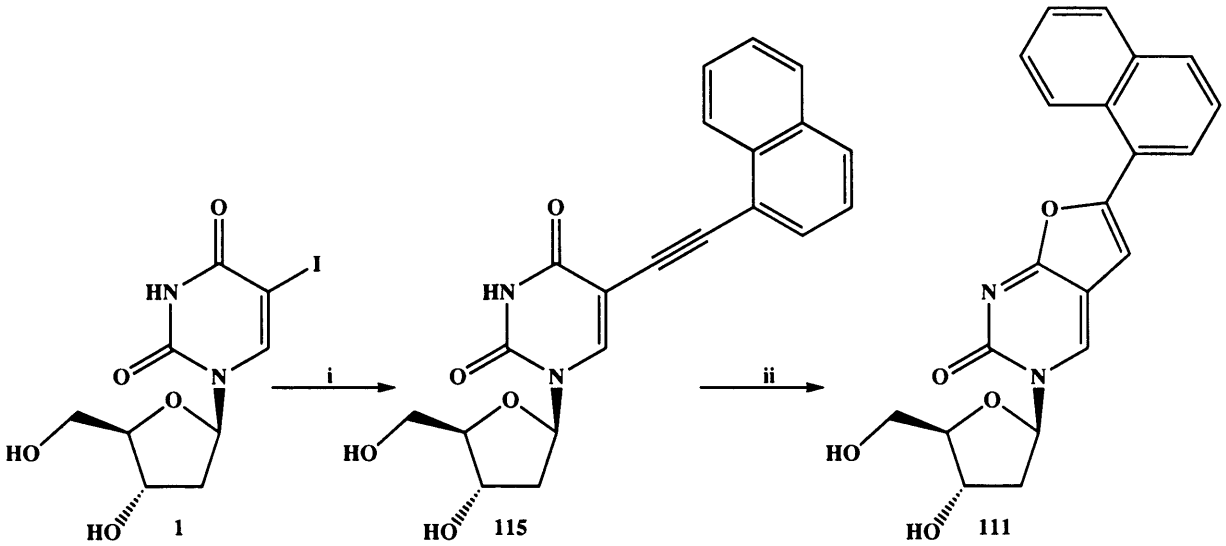
2-ethynylnaphthalene **114**, needed for the synthesis of **112**, was not commercially available, but it was easily synthesised starting from 2-bromonaphthalene in quantitative yield (scheme 4.13).



Scheme 4.13: *i) ACN, TMS-acetylene, Tetrakis Pd(PPh$_3$)$_4$, CuI, DIPEA. ii) TBAF.*

While compound **112** and **113** were obtained in good yield, compound **111** presented some difficulties during the synthesis. Appling the standard condition to synthesise compound **111**, it was observed the total conversion of IDU into compound **115** (scheme 4.14), but when copper iodide and TEA were added no reaction occurred, recovering compound **115** in almost quantitative yield after column chromatography. Synthesis of **111** was attempted in the past, without obtaining the target molecule due to many complications encountered carrying out the reaction and purification.[6] Cyclisation of **115** was then performed in dioxane, as for ortho derivatives, heating at reflux in the presence of 1 eq. of copper iodide and a large excess of TEA, but after 24 hours the TLC did not show any trace of target compound. The reaction was then left under reflux for other 6 days, adding at regular intervals fresh copper iodide, after which TLC showed only a trace of a fluorescent spot that was quantified by HPLC resulting in less than 2% of the potential product. The most probable reason of the failed cyclisation is steric hindrance of the naphthyl ring.

The failure of the ring closure under standard conditions, led to use another method in order to complete the cyclisation. In the literature many methods are reported for this kind of cyclisation, but most of them are similar to one used so far. Agrofoglio reported a ring closure that occurs at room temperature in the presence of silver nitrate.[7] When compound **115** was reacted with silver nitrate at room temperature a fluorescent spot was observed after 24 hours, although most of the starting material was still present. After this promising result, compound **115** was treated with silver nitrate and TEA in methanol at reflux (scheme 4.14). After 6 hours compound **115** was completely converted into **111** without any formation of by-products or decomposition.



Scheme 4.14: *i) DMF, 1-ethynylnaphthalene, Tetrakis $Pd(PPh_3)_4$, CuI, DIPEA. ii) Acetone, TEA, $AgNO_3$.*

4.3 Biological results

The synthesised compound **85-94** and **111-113** were evaluated for their ability to inhibit the replication of VZV in cell culture. Table 4.2 contains data relating to two strains of thymidine kinase-competent (TK^+) VZV and also two strains of thymidine kinase-deficient (TK^-) VZV, with data also given for ACV, **33a** (phenyl) and **33f** (pentylphenyl) as reference compounds.

		EC_{50} μM[a]					
	R	**OKA**	**YS**	**TK^- 07**	**TK^- YS**	MCC μM[b]	CC_{50} μM[c]
85	o-Me	0.12	0.09	>100	>100	>20	>50
86	m-Me	1.62	1.79	>100	>100	>20	>50
87	m-OMe	4.6	0.71	>100	>100	>50	>50
88	m-CF_3	5.6	4.6	>100	>100	>50	>50
89	p-CF_3	2.7	2	87	>100	>50	>50
90	m-NO_2	>20	-	>20	>20	100	8.4
91	p-NO_2	2	-	>4	>20	20	6.9
92	p-$NHCOCF_3$	9	-	>100	>100	>100	>50
93	m-$NHCOCF_3$	-	-	-	-	-	-
98	bis 5,6-p-NO_2	>20	-	>20	-	100	>50
94	p-NH_2	>20	-	>100	-	>100	>50
111	1-Naph	-	-	-	-	-	-
112	2-Naph	>100	>100	>100	>100	>100	>50
113	MeO-Naph	>20	-	>20	-	>100	>50
33a	H	0.16	0.28	>200	>162	>20	>200
33f	p-Pentyl	0.0003	0.0001	>5	>5	>20	>200
ACV	-	2.9	1	74	125	>200	>200

Table 4.2

a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration required to alter microscopically detectable cell morphology
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

Compounds **85-94** were found to be less active than lead compound **33f**, although they show an activity comparable to ACV and compound **33a**. The SAR for this series is very complex, but in general the substitution in meta position always presents a negative effect on biological activity, while the para derivatives, as expected, are the most active followed by the ortho, suggesting that this position seems to be tolerated. Furthermore, the nature of the substitutent

affects the potency. In fact non-polar electron-donating groups (Me and MeO) were found to be the most potent derivatives of this series, while introducing an electron-withdrawing group, such as NO_2 or CF_3, the potency dramatically drops (fig 4.2).

Interesting to notice that the trifluoroacetamido derivatives **92**, **93** and the amino derivative **94**, although they have electron-donating groups on the phenyl ring, were found to be less active than ACV.

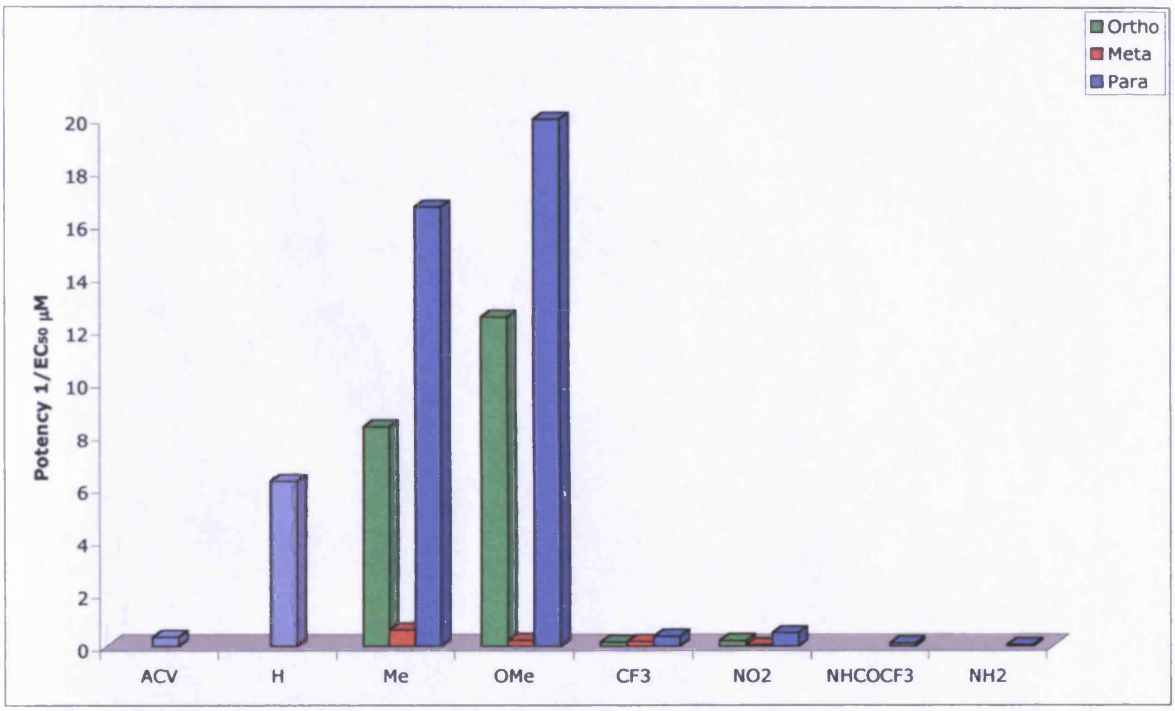


Fig 4.2: Ortho-Meta-Para Derivatives

Regarding the naphthyl derivatives **112** and **113**, the extension of the conjugation with the introduction a naphthyl ring is extremely damaging for the activity, as this compound can be said to be inactive. One possible explanation for the inactivity of these compounds could be given by steric hindrance due to the size of the naphthyl system, suggesting that some flexibility is required for antiviral activity.

4.4 Conformational Studies

The difficulty to cyclise the ortho derivatives lead us to investigate the influence of a substitutent on the planarity of the phenyl ring respected to the bicyclo pyrimidine. First a molecular modelling study was carried out on compounds that bear a methyl group on the phenyl ring in position ortho **85**, meta **86** and para **86**, calculating the dihedral angle between the carbon in ortho to the phenyl and the C6 of the bicyclo (fig 4.3).

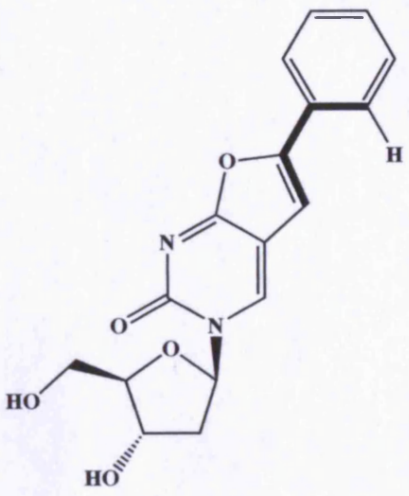


Fig 4.3: Dihedral angle calculated

Using a computational stochastic search, it was calculated that dihedral angle of the para derivative **33b** was 17°, the phenyl ring is almost co-planar to the bicyclo pyrimidine (fig 4.4). In this compound there is an extensive conjugation between the bicyclo and phenyl.

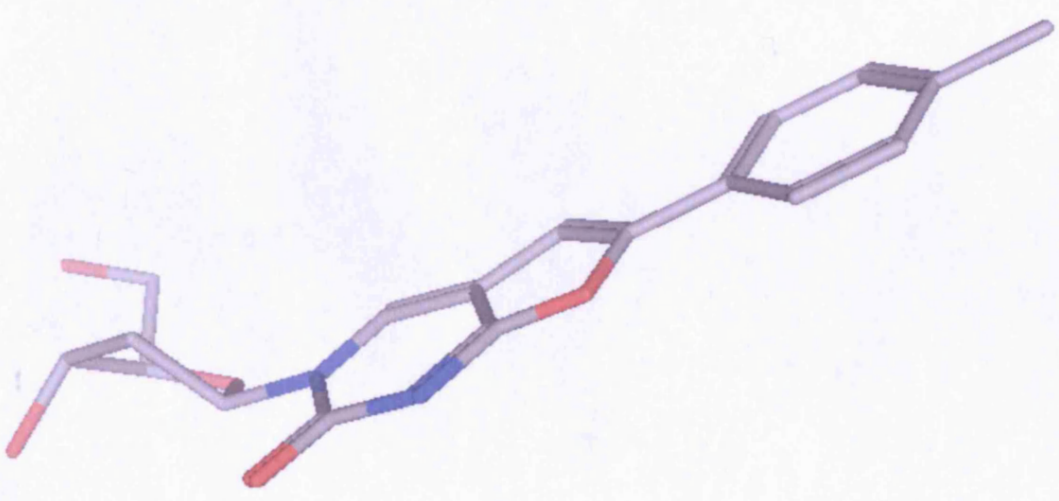


Fig 4.4: Para 17°

The meta derivative **86** was found be very similar to para derivative showing a rotation of 18°(fig 4.5), in this case there is still an extensive conjugation.

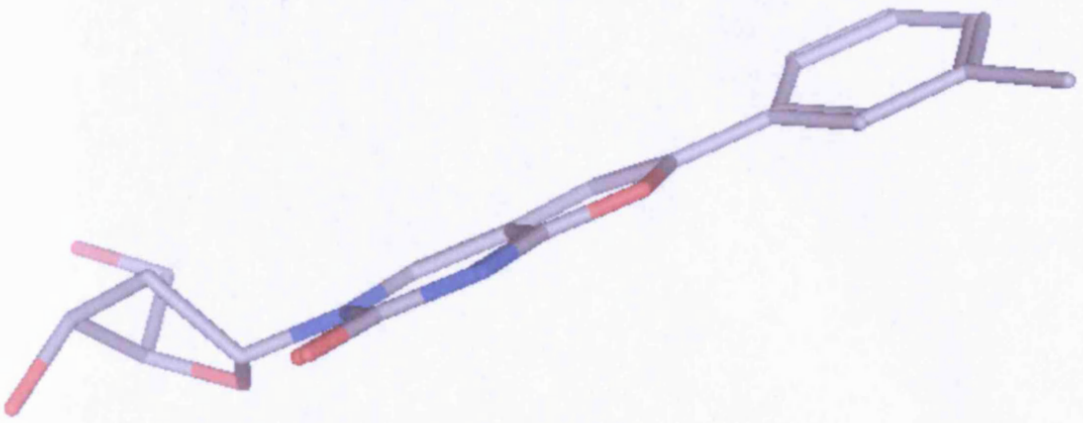


Fig 4.5: Meta 18°

Regarding the ortho derivative **85**, a massive rotation of the phenyl ring was predicted measuring a dihedral angle of 50°. This significant distortion should affect significantly the conjugation and electronic properties of the molecule (fig 4.6).

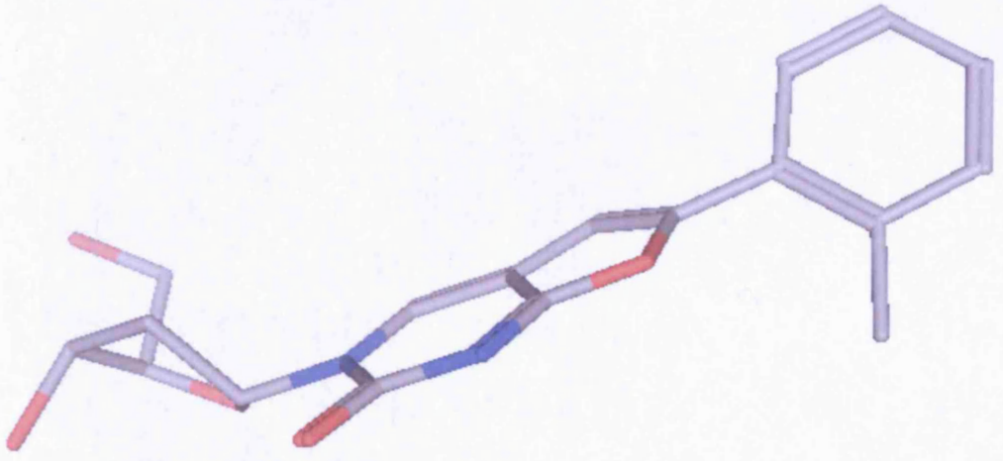


Fig 4.6: Ortho 50°

UV and fluorescence were carried out in order to support these predicted conformations.

4.4.1 UV

With the rotation of the phenyl ring the conjugation between the bicyclo and phenyl decreases affecting consequently the UV spectra of the derivatives. In order to confirm this hypothesis, the UV spectra of **85**, **86** and **33b** were recorded at a fixed concentration of 95μM in MeOH.

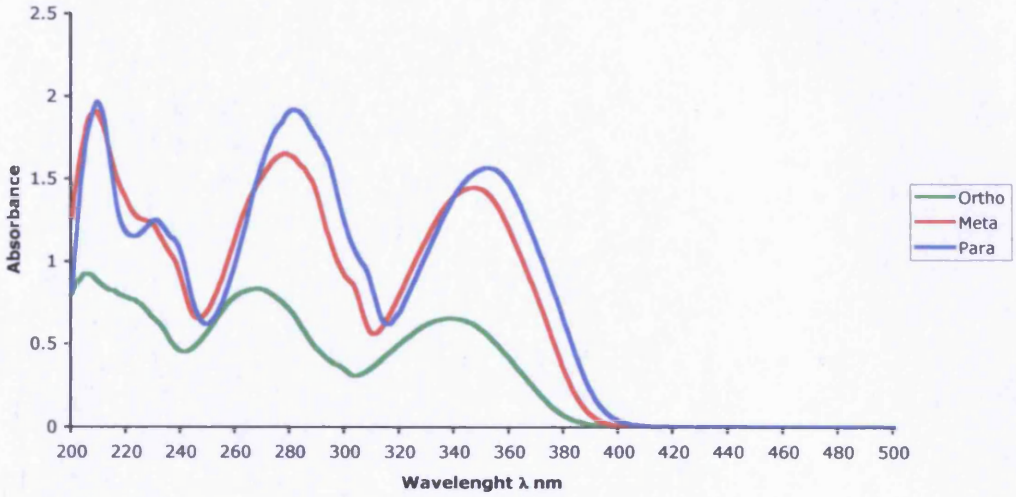


Fig 4.7: UV spectrum of **85**, **86**, **33f**

When the conjugation is perturbed the parameters that are affected are the wavelength λ of the maximum of absorbance and the extinction coefficient ε.[8] These parameters for compounds **85**, **86** and **33f** are summarised on table 4.3.

	Peak 1 (Log ε_o)	**Peak 2 (Log ε_o)**	**Peak 3 (Log ε_o)**
Ortho	338 (5.84)	268 (5.95)	206 (5.99)
Meta	349 (6.18)	279 (6.24)	208 (6.30)
Para	352 (6.22)	281 (6.31)	209 (6.31)

Table 4.3

As predicted, the para and meta derivatives show the maximum conjugation, presenting the same electronic properties, even if there is a small difference that shows that the para derivative is more conjugated.

Regarding the ortho derivative, the maximum of absorbance is at shorter wavelengths, but the significant difference can be observed on extinction coefficient. In fact the absorbance is

halved compared to the other two compounds giving a further prove that this compound presents a lesser conjugation.

4.4.2 Fluorescence

Given the intrinsic fluorescence of BCNAs, the spectra of the three derivatives were recorded. A "fluorescence excitation spectrum" is obtained by scanning the excitation monochromator at a fixed emission wavelength. Thus for each compound a 3μM solution in MeOH was scanned within the range of 190 nm to 650 nm at a fixed emission wavelength of 395 nm (fig 4.8).

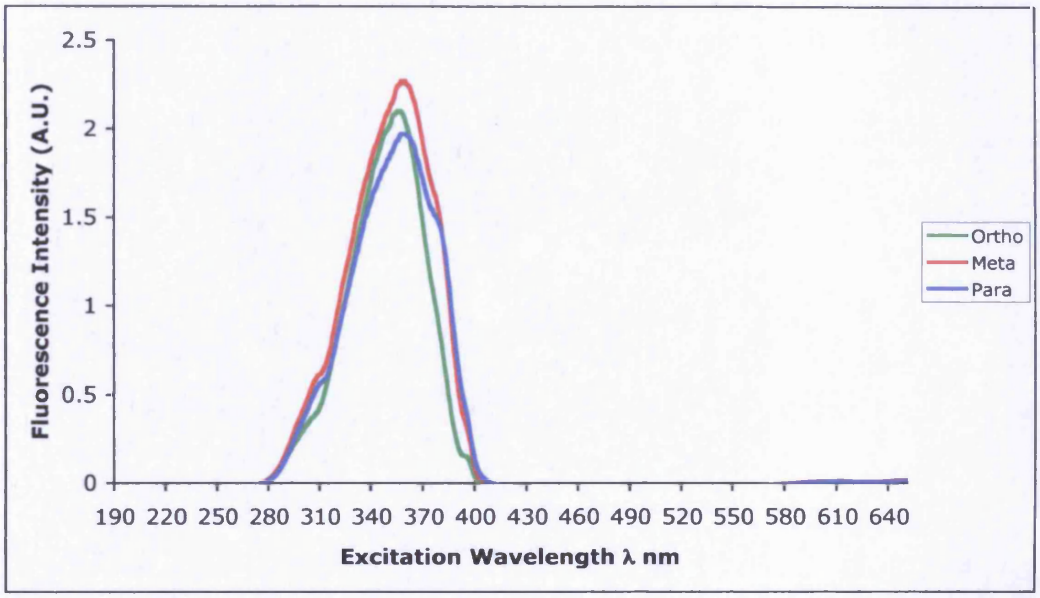


Fig 4.8: Fluorescence excitation spectra

The fluorescence intensity is reported in arbitrary units, due to the need for calibration with a series of standards to obtain quantitative information. As fig 4.8 shows, the optimal excitation wavelength is slightly different for the 3 derivatives. As expected the order found is para (359 nm), meta (357 nm) and ortho (355 nm), which are close to the values of 366 nm of the UV detecting lamp.

When the emission monochromator is scanned at a fixed excitation wavelength, an "emission" or "fluorescence" spectrum is recorded. Thus, the same solutions were scanned at the fixed excitation wavelength of 359 nm for para, 357 nm for meta and 355 nm for ortho (fig 4.9).

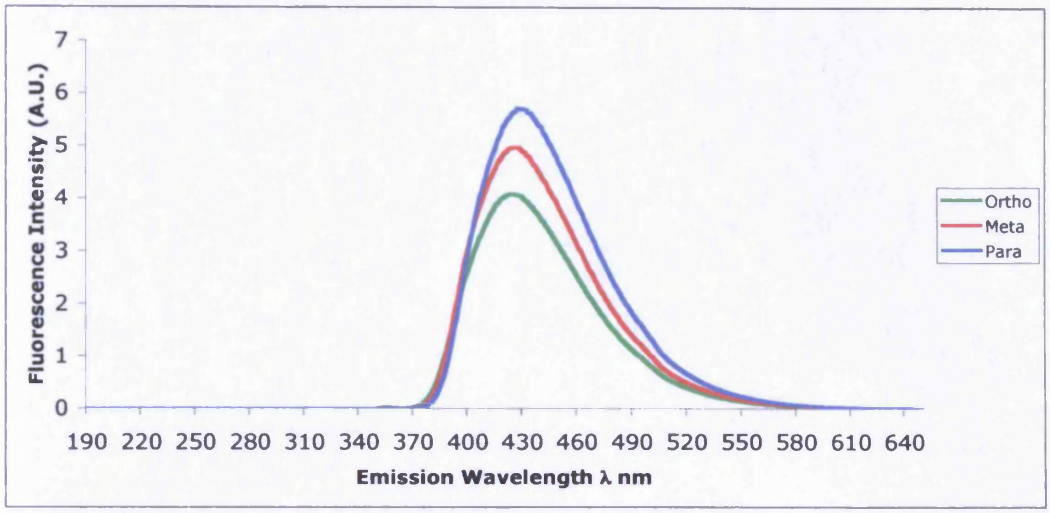


Fig 4.9: Fluorescence emission spectra

The change observed is not as evident as for the UV, the emission spectra for each compound are very similar, suggesting that the rotation of the phenyl does not affect in significant way the fluorescence of the compounds.

	Wavelength nm	Intensity
Ortho	424	4.09
Meta	426	4.97
Para	427	5.72

Table 4.4

References

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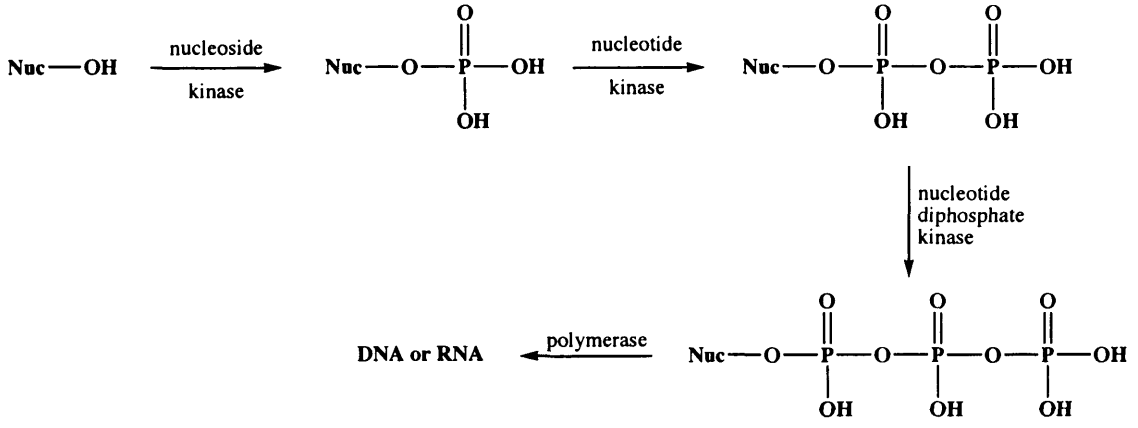
Chapter 5: Protide Approach on BCNAs

5.1 Protide approach: background

Nucleoside analogues have been a subject of numerous studies as antiviral and anticancer agents.[1] In order to show a biological activity they must be converted into nucleotides by viral and/or cellular kinases. This activation process follows the pathway utilized by natural nucleosides. In the first step nucleosides are phosphorylated to give monophosphates. The latter are converted to diphosphates by the action of nucleotide kinases. In the next step, they are further phosphorylated to triphosphates with the aid of NDP kinases. Triphosphates of nucleoside analogues then compete with their natural counterparts for DNA or RNA polymerases. They can either act as terminators of the growing polynucleotide chain or get incorporated into the chain thus modifying the properties of the nucleic acid.



Scheme 5.1: mechanism of activation of nucleosides.

Selectivity of the antiviral effect is then achieved by a differential inhibition of viral incorporation and cellular polymerases by the corresponding nucleoside triphosphates. It is then clear that intracellular introduction of nucleoside analogues as phosphorylated metabolites could avoid difficulties associated with the use of non-phosphorylated analogues and even activate inactive or less active compounds. Such an approach obviates the crucial first phosphorylation step and delivers the monophosphate into the cells.[2] However, nucleotides are highly polar charged species which are unable to cross the cellular membrane and moreover the ready degradation with phosphatases makes the use of free nucleotide

analogues not useful. Therefore, much of the recent efforts were focussed on finding suitable prodrugs of monophosphates of nucleoside analogues (pronucleotides).

It is clear that necessary requirements for achieving a successful pronucleotides are:

1) resistance to degradation by phosphatases

2) capability of penetrating the cellular membrane

3) delivery of the nucleotide analogues inside the cell.

Three groups of pronucleotide approaches, each with a different mechanism of action, are emerging as promising platforms against HIV.

The SATE pronucleotides elaborated by Perigaud,[3] the Phosphoramidates derived from amino acids introduced by McGuigan[4] and, finally, CycloSal designed by Meier[5] (fig 5.1). In the case of amino acid phosphoramidates, the studies were largely focussed on phenyl phosphoralaninates of AZT and d4T. Extensive structure–activity relationship studies[4,6,7,8,9] indicated that a combination of alanine ester and phenyl phosphate provides for the most effective agents. Consequently, the Phosphoramidate technology was used by several groups of investigators as antiviral pronucleotides[10,11,12,13,14,15] and potential antitumor agents.[16,17]

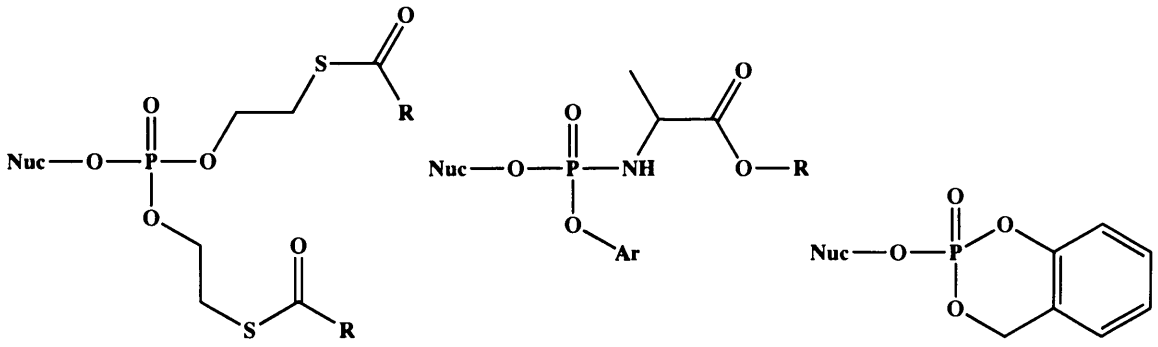
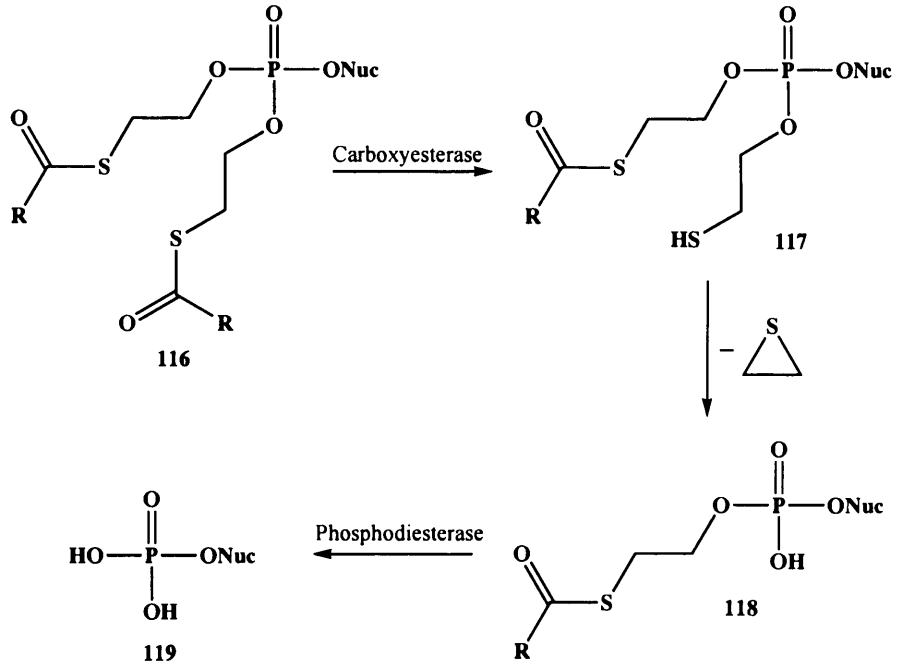


Fig 5.1: Pronucleotides approaches

5.2 SATE-Nucleotides

The SATE approach is based on the enzymatic activity of carboxyesterases, used to trigger the nucleotide delivery. The esterase cleaves a thioester moiety of **116** to yield a carboxylic acid as well as the thioethyl phosphate diester **117** which undergoes a spontaneous fragmentation to episulphide and the phosphate monoester **118**. Then a phosphodiesterases cleaves the other ester to yield the free nucleotide **119** (scheme 5.1).[18] This approach was

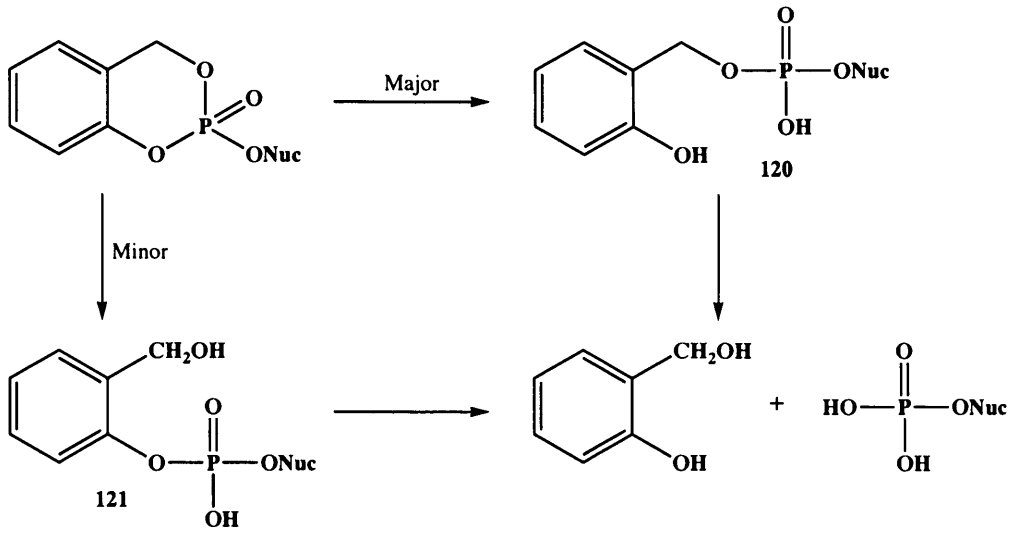
successfully applied to d4T[19] and AZT[20] in order to by-pass thymidine kinase and ddA to by-pass adenosine deaminase.[20]



Scheme 5.2: Activation of SATE pronucleotide

5.3 CycloSal Nucleotide

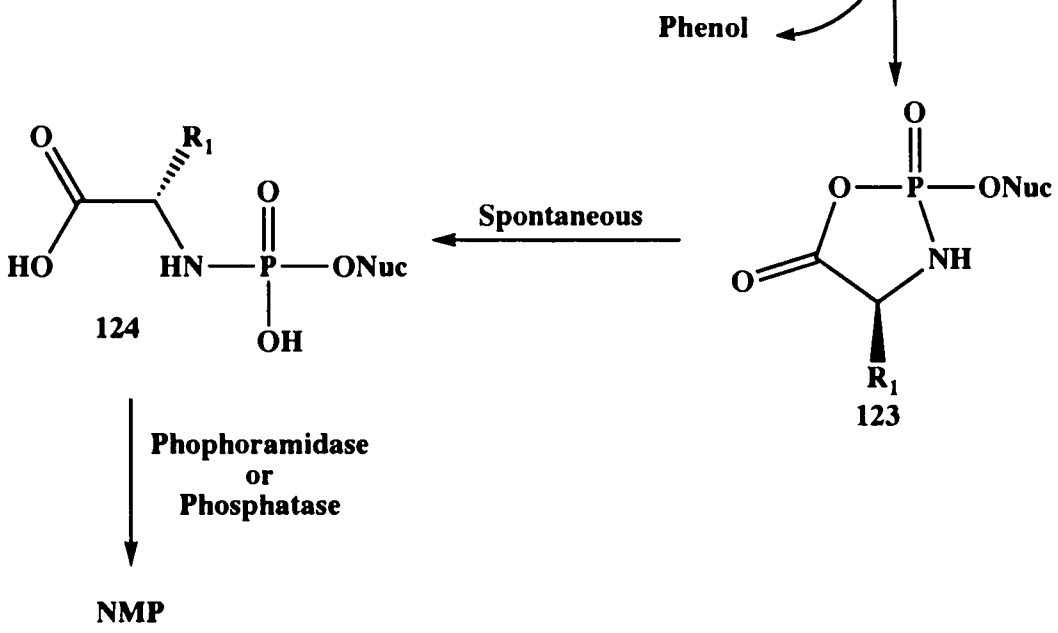
The CycloSal nucleotide approach does not rely on enzymatic activation, but the release of the free nucleoside and the carrier is achieved by a controlled, chemically induced hydrolysis involving a successive coupled cleavage of the phenyl and benzyl esters of the phosphotriester.[21] The rationale of this prodrug is based on the different hydrolysis properties of the phenyl and benzyl phosphate esters. As the negative charge arising from the cleavage of the phenolic moiety can be delocalised on the aromatic ring, the phenyl ester is the most labile and then selectively cleaved to 2-hydroxybenzylphosphodiester **120**. In contrast, the alternative cleavage of the ester to 2-hydroxymethylphenyl phosphodiester **121** is unfavourable. As consequence of the first step, the remaining masking group (P-O_{Benzyl}) is activated by the strong electron-donating hydroxy group in the ortho position, resulting in a spontaneous cleavage of the di-ester to yield the free nucleotide and salicyl alcohol. This approach was successfully applied to d4T,[22,23] acyclovir,[24] BVdU[25,26] and ddA.[27] As for SATE, in the case of d4T the CycloSal prodrug was designed to by-pass thymidine kinase, while the ddA analogue was used to by-pass adenosine deaminase.



Scheme 5.3: Activation of CycloSal pronucleotide

5.4 Aryl Phosphoramidate Approach

Aryl phosphoramidates are a class of membrane soluble prodrugs characterised by a phosphate moiety linked to a nucleoside, a phenyl group and to an amino acid ester. This approach was applied to different nucleoside analogues (ddU,[28] AZT,[29] d4T,[30] ddA,[31] PMPA,[32] PMEA,[32] BVdU,[33] etc.). The mechanism of activation of phosphoramidate analogues was the object of extensive studies. In the bioactivation of phosphoramidate prodrug, the first step is the hydrolysis of the carboxylic ester function of the amino acid moiety by an enzyme with esterase activity to yield the derivative **122**. This enzyme is thought to belong to class of proteases, such as catepsin B and proteinase K.[34,35] The cleavage of the ester moiety is followed by an intramolecular nucleophilic attack of the phosphorus by the carboxylate, with spontaneous elimination of phenol forming a five-membered cyclic anhydride **123**. Immediate hydrolysis of **123** gives the aminoacyl phosphoramidate diester **124**, which was described as an important depot form for the free nucleotide.[36] Compound **124** then undergoes the cleavage of the P-N bond mediated by a phosphoramidase or phosphatase to delivery the free nucleotide.[36]



Scheme 5.4: Activation of Phosphoramidate pronucleotide

In order to study the SARs of phosphoramidate analogues, many compound synthesised modifying the different components that build the prodrug.

Ester moiety: modifying this part of the molecule the activation of the cascade of eve be modulated in relation to the activity of the esterase.

Aryl moiety: the introduction of electron-withdrawing groups can be used to enha leaving group ability of the aryl moiety, increasing the formation of the key ami intermediate. Instead, electron-donating groups decrease the rate of formation intermediate.

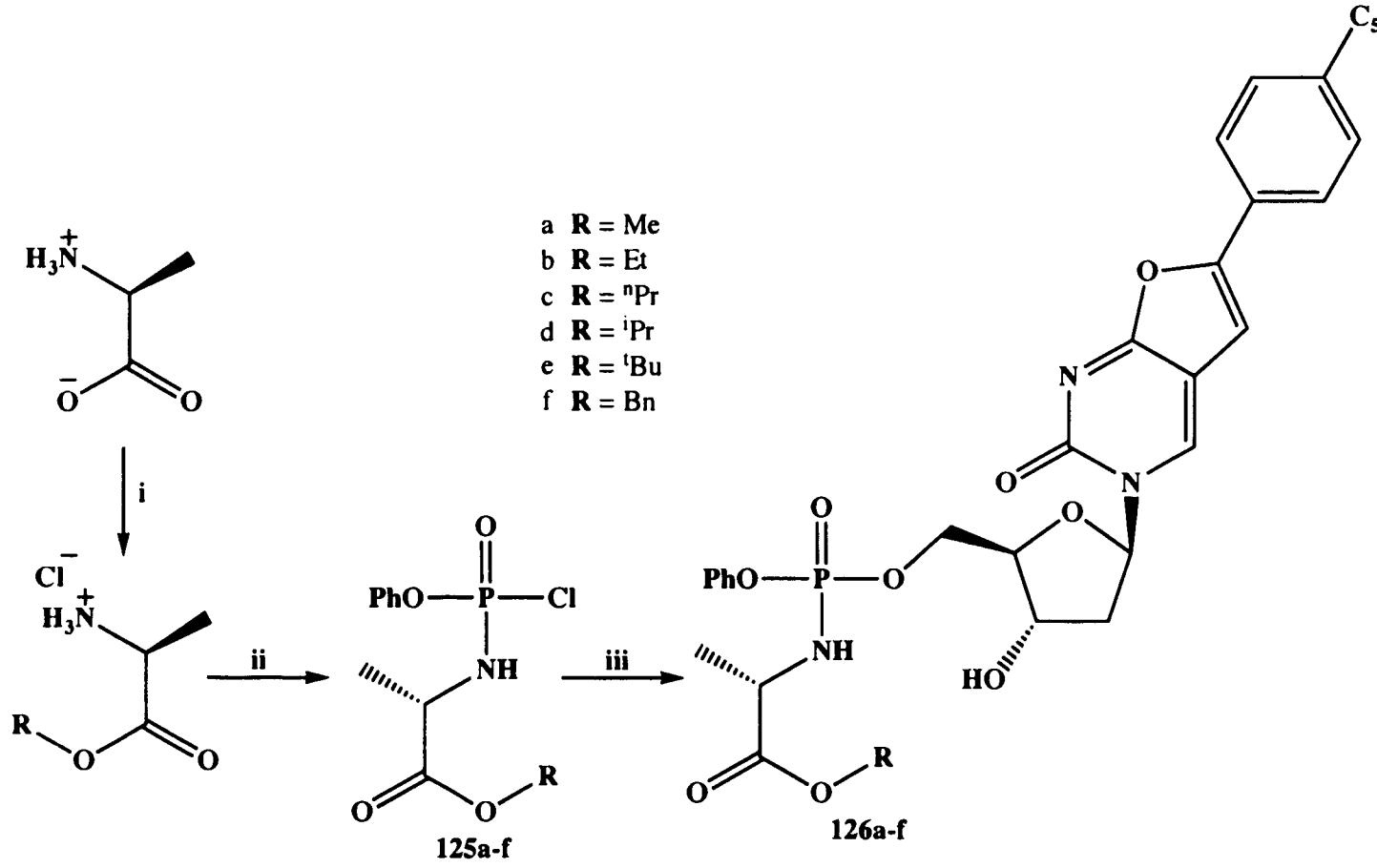
Amino Acid: introduction of different amino acids has a different effect on both the and the phosphoramidase activity, affecting the rate of cleavage of the phosphoramidate and the rate of the subsequent cleavage to monophosphate.

As a result of these modifications, it was observed that the introduction of an α-ami ester is essential for biological activity[37] and that replacement with an alkyl amine or β acids resulted in a complete lack of activity.[8] Among the natural amino acids, L-alan found to be most effective, while L-valine resulted in extremely poor activity.[37] Furth

The phosphoramidate approach was applied to BNCA 33I, to investigate if this strateg
improve the biological properties. Biochemical studies pointed out that the BCNAs,
being efficiently phosphorylated by VZV-TK, are not good substrates for cellular kin
well as for HSV-1 TK. This observation is taken as explanation of the extrao
specificity of this class of compounds as anti-VZV agents. Due to their extremely se
mechanism of activation, these analogues rely only on activation by the viral TK f
biological activity and failing that they would become inactive.

Moreover, it was expected that the phosphoramidate approach could convert the analo
into an active compound against other viruses that are not able to phosphorylate E
Furthermore, it was of interest to observe if the extraordinary potency of BCNAs was r
also in infected cells unable to express VZV-TK (TK^-), where normally BCNAs were
to be inactive.

thionyl chloride easily afforded the corresponding ester salts in quantitative yield.



Scheme 5.5: *i) ROH $SOCl_2$. ii) DCM, $PhOPOCl_2$, TEA. iii) Pyr, **33f**, NMI.*

The commercially available phenyl dichlorophosphate was reacted with L-alanine est at low temperature and in the presence of TEA to give the phenyl(L-alaninyl phosphorochloridates (**125a-f**), which were used in the final step after purification by chromatography. The phosphorochloridates could be used also as a crude, witho column chromatography, but it was observed that the purification process was found t an important enhancement during the coupling stage, improving significantly the yield. The ^{31}P-NMR spectra of phosphorochloridates **125a-f** showed the presence of two sig showed in table 5.1. This is due to the lack of stereoselectivity of this reaction that c the achiral phosphorus of the substrate into a chiral centre, giving a diasteromeric mixt ratio 1:1.

125f	Bn	68%	7.97, 7.60

Table 5.1: Phenyl phosphorochloridates

The final coupling between **33f** and the target phosphorochloridates was performed ac to Van Boom procedure[41] in the presence of 1-methylimidazole (NMI), which a coupling reagent. Due to the chirality of the phosphorus centre, the final phosphora **126a-f** were prepared as mixture of two diasteroisomers in a ratio 1:1. No separatio two diateroisomers was performed.

In the case of the methyl, n-propyl and i-propyl derivatives, the 3',5'-diphosphor derivatives **126m-o** were isolated in consistent yield to allow the characterisati biological evaluation. This side reaction occurred because 4 eq. of phosphorochlorida reacted with **33f** as the usage of less amount resulted in a poor reactivity of the substra

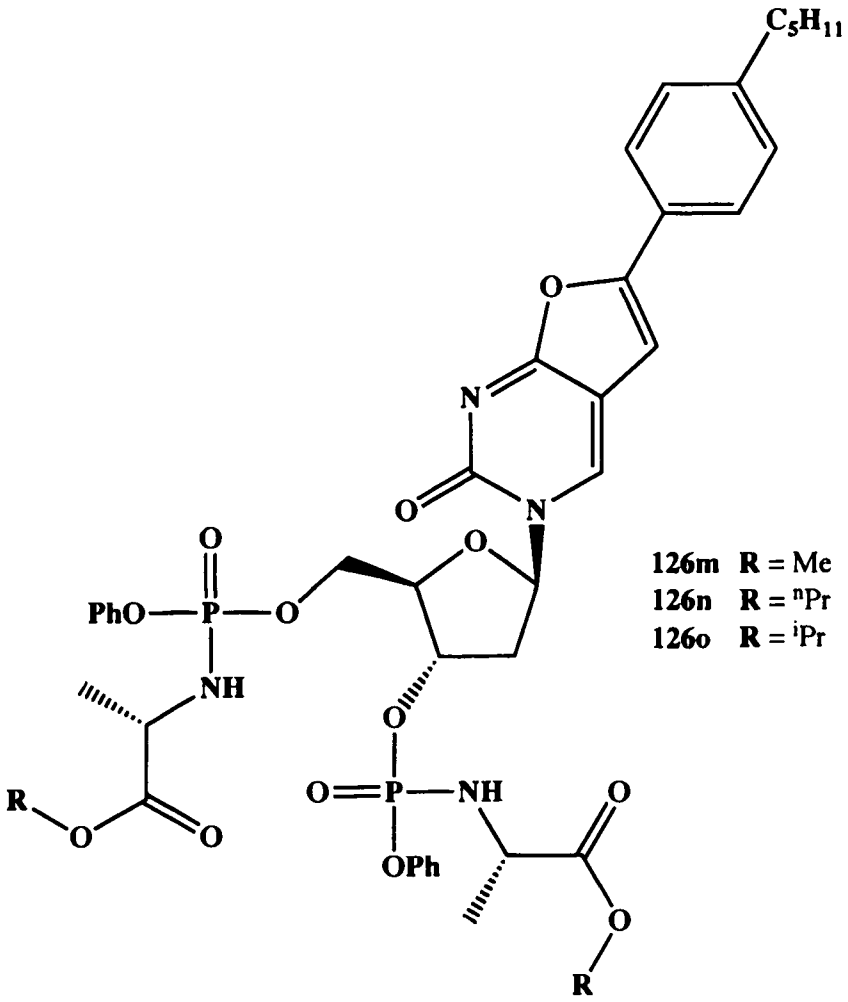


Fig 5.2: 3',5'-diphosphoramidates

The low yield of the coupling reaction with the phosphorochloridates is due to an extensive purification process, which involved repeated column chromatographies and usage of preparative TLC.

Compound	R	Yield %	^{31}P-NMR δ
126a	Me	12%	4.41, 4.17
126b	Et	6%	4.45, 4.36
126c	^{n}Pr	11%	4.61, 4.43
126d	^{i}Pr	15%	4.49, 4.43
126e	^{t}Bu	17%	4.59, 4.52
126f	Bn	24%	4.22, 3.49

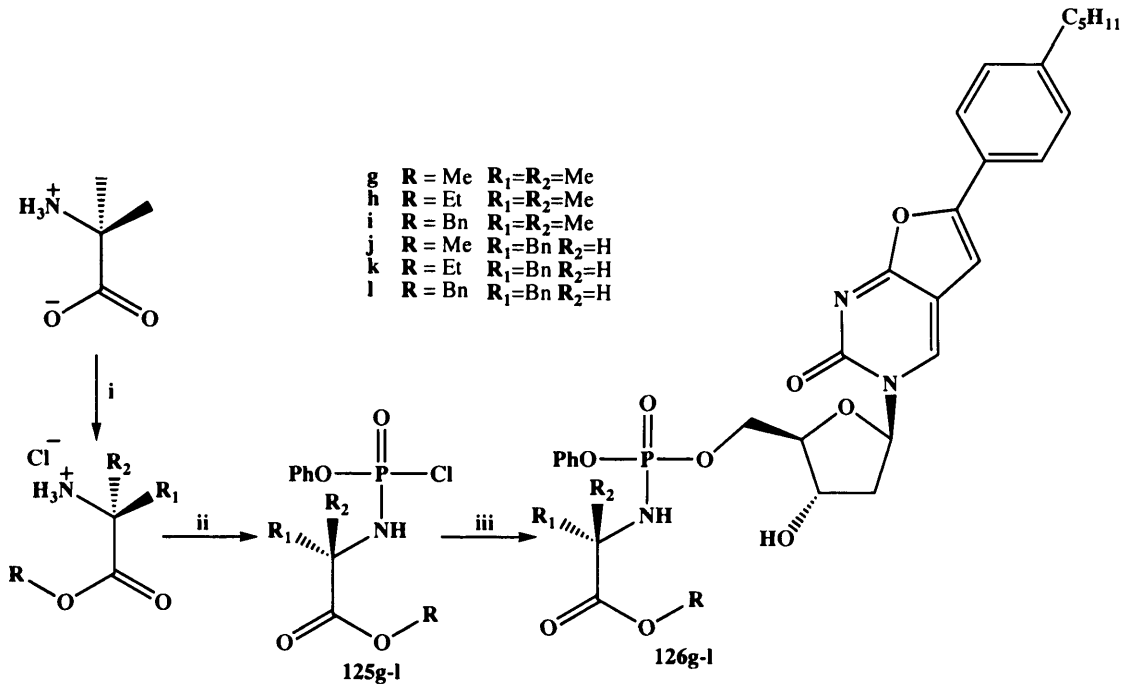
Table 5.2: Phenyloxy phosphoramidates

5.7 Amino Acid variations

In order to identify any structure activity relationships and improve the biological activity, other derivatives containing modifications to the amino acid moiety were prepared. In fact, the nature of the amino acid group seemed from the previous SARs to be of fundamental importance for the biological activity of phosphoramidates. A different amino acid core would modulate the kinetics of the esterase- and phosphoramidase- type enzyme, leading to different biological results.

In order to understand the role of the amino acid, the natural amino acid phenylalanine (Phe) and the unnatural amino acid dimethylglycine (DMG) were employed as methyl, ethyl and benzyl esters.

The procedure used to prepare compounds **126g-l** was based on the same chemistry previously described (scheme 5.6). The amino acid ester salts were commercially available or readily prepared by reaction of the appropriate alcohol with either thionyl chloride or tosylic acid in toluene using Dean-Stark apparatus.[42]



Scheme 5.6: *i) a:ROH, $SOCl_2$. b:Toluene, TosOH, ROH. ii) DCM, $PhOPOCl_2$, TEA. iii) Pyr, **33f**, NMI.*

Compound	R	Amino Acid	Yield (%)	^{31}P-NMR δ
125g	Me	DMG	54%	6.97
125h	Et	DMG	45%	7.05
125i	Bn	DMG	77%	6.98
125j	Me	Phe	63%	9.43, 9.25
125k	Et	Phe	58%	9.36, 9.26
125l	Bn	Phe	46%	9.10, 9.07

Table 5.3

Phenyl dichlorophosphate was coupled to corresponding amino acid ester salts under standard conditions to give the phosphorochloridate **125g-l**. As for the L-alanine analogues, also compounds **125g-l** were purified by column chromatography in order to improved to yield on the final step. Compounds **125g-i** were obtained as enantiomeric mixture given the presence of only one signal on ^{31}P-NMR. Compounds **125j-i** were instead obtained as mixture of two diasteroisomers in a ratio 1:1.

The coupling of the synthesised phosphorochloridates with **33f** was performed as previously described obtaining the target phosphoramidates **126g-l**. Also in this case the diphosphoramidate adduct was detected, but this time it was not possible to isolate it in consistent yield.

Compound	R	Amino Acid	Yield %	^{31}P-NMR δ
126g	Me	DMG	22%	3.01, 2.93
126h	Et	DMG	19%	3.01, 2.87
126i	Bn	DMG	15%	4.61, 4.43
126j	Me	Phe	12%	4.49, 4.43
126k	Et	Phe	13%	4.59, 4.52
126l	Bn	Phe	17%	4.22, 3.49

Table 5.4: Phenyl Phosphoramidates amino acid variation

5.8 Biological evaluation

The synthesised compound **126a-l** were evaluated for their ability to inhibit the replication of VZV in tissue culture. Table 5.5 contains data relating to two strains of thymidine kinase-competent (TK^+) VZV and also two strains of thymidine kinase-deficient (TK^-) VZV, with data also given for ACV and **33f** as reference compounds.

				EC_{50} μM[a]					
	Amino Acid	Ester	LogP	OKA	YS	TK^- 07	TK^- YS	MCC μM[b]	CC_{50} μM[c]
126a	Ala	Me	5.53	0.031	0.024	>16	-	80	41.5
126b	Ala	Et	6.06	0.010	0.009	>16	-	80	21
126c	Ala	^{n}Pr	6.59	0.0066	0.0046	>3.2	-	16	9
126d	Ala	^{i}Pr	6.37	0.029	0.014	>3.2	-	16	9.3
126e	Ala	^{t}Bu	6.76	0.473	0.485	>3.2	-	16	8.6
126f	Ala	Bn	7.24	0.0067	0.0072	>3.2	-	80	34
126g	DMG	Me	5.84	0.100	0.230	15	-	≥20	>50
126h	DMG	Et	6.37	0.036	0.045	>4	-	≥4	27
126i	DMG	Bn	7.55	0.010	0.024	>4	-	≥4	26
126j	Phe	Me	6.95	0.015	0.032	>4	-	20	24
126k	Phe	Et	7.47	0.016	0.036	>4	-	20	12
126l	Phe	Bn	8.66	0.010	0.020	>4	-	20	20
126m*	Ala	Me	7.30	0.43	-	>100	-	>100	>100
126n*	Ala	^{n}Pr	9.42	0.040	0.030	>40	-	>40	>20
126o*	Ala	^{i}Pr	8.98	0.180	0.118	>40	-	≥40	>50
ACV	-	-	-2.42	2.9	1	74	125	>200	>200
33f	-	-	3.52	0.0003	0.0001	>5	>5	>50	>200

Table 5.5: Biological Data. *Diphosphoramidate derivatives

a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

Although the synthesised phosphoramidates did not show any enhancement of the activity of the parent nucleoside **33f**, they still retain a good potency, being in some cases over 400 fold more active than ACV. This reduction of activity is probably due to the fact that the phosphorylation of **33f** by VZV-TK is much more efficient than the activation of the prodrug.

For VZV-TK competent cell lines (OKA and YS), lipophilicity seems to play an important role for the biological activity of phosphoramidates: the activity of these compounds increased when the chain of the ester was extended, increasing consequently the LogP, reaching the maximum with the n-propyl ester **126c** and the benzyl ester **126f**. However, the lipophilicity is not the only factor that affects the biological activity of this class of compounds, in fact the introduction of a branched ester such as i-propyl, lead to a significant reduction of activity suggesting that the enzyme responsible for the activation of the prodrug prefers primary esters. This hypothesis is supported by the fact that the introduction of a very bulky ester like the t-butyl is extremely damaging for the activity if compared to the other phosphoramidates.

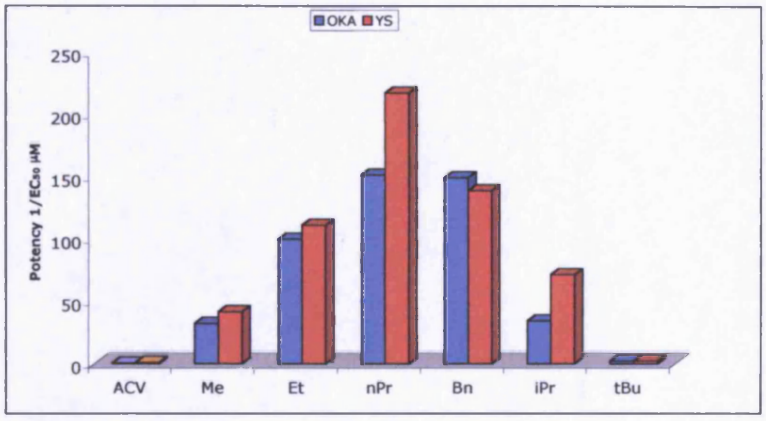


Fig 5.2: Alanine Phosphoramidates

The replacement of L-alanine with the unnatural amino acid dimethylglycine showed that the antiviral activity follows the same trend seen for the previous series, even though it is clear that this amino acid slightly reduces the biological results.

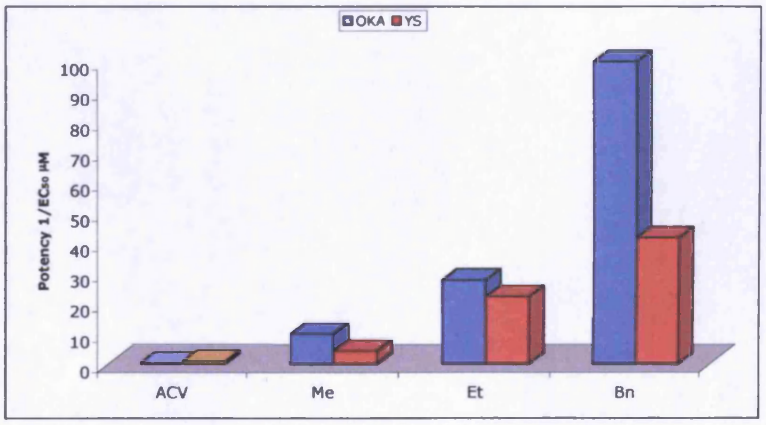


Fig 5.3: dimethylglycine Phosphoramidates

The introduction of an amino acid such as phenylalanine raised significantly the lipophilicity of the phosphoramidates and it seems that the activity of such derivatives does not show any dependence on LogP.

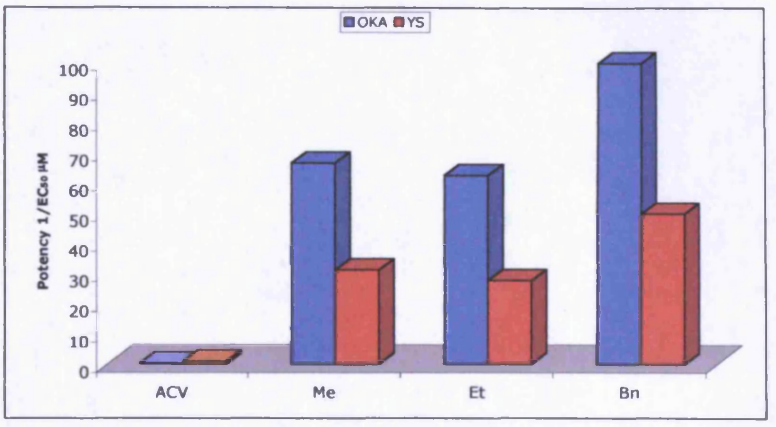


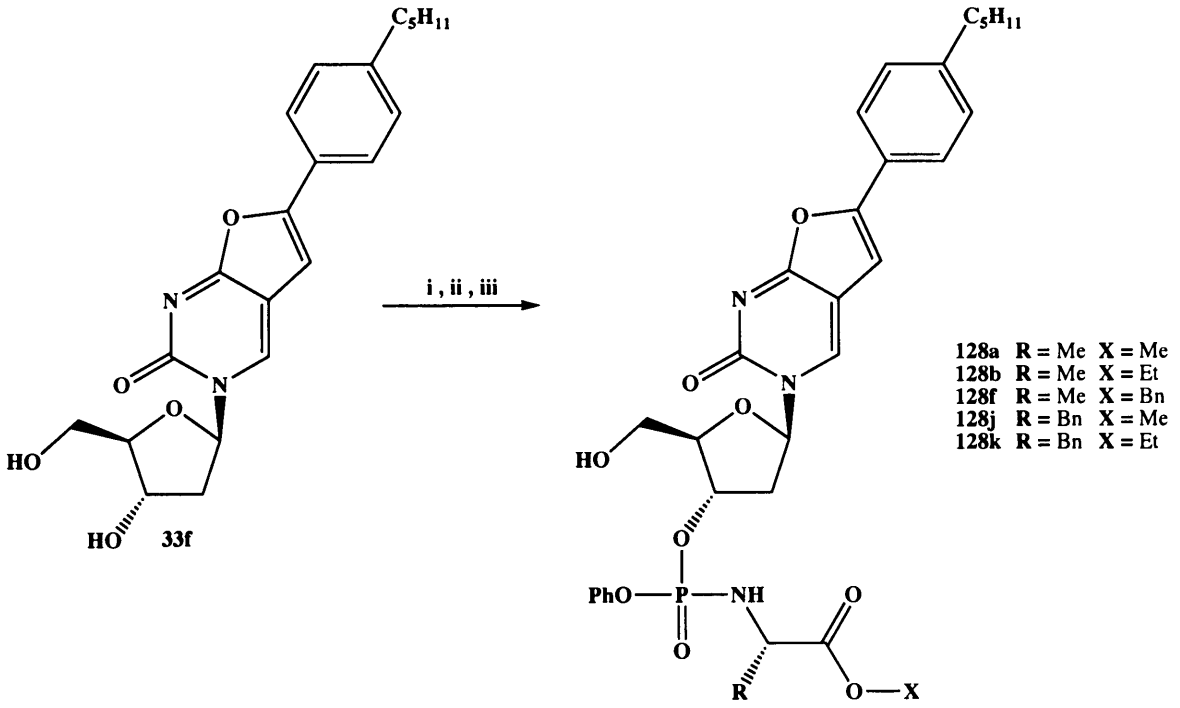
Fig 5.4: Phenylalanine Phosphoramidates

Regarding the VZV-TK deficient cell line, all phosphoramidates were found active at concentrations greater than 3μM. This dramatic loss of activity is probably due to the fact that this cell line does not express VZV-TK and then incapable to phosphorylate BCNA-MP to diphosphate. This suggests that the VZV-TK plays a fundamental role for activation of BCNA, and that this important enzyme for the activation of this class of compounds performs the second phosphoryation, or that BCNA protides function as BCNA prodrugs, not of NMP and thus require VZV-TK for the first phosphorylation.

This series was also tested against HCMV, HSV-1, HSV-2, HIV, but no antiviral effect was observed below 20μM for any of the viruses considered.

5.9 3'-Phosphoramidates

The diphosphoramidates **126m-o**, isolated as by-products, were tested and found to be active below μM concentrations against VZV. This interesting result lead us to think that the phosphoramidates in 3' position of BCNA could show some activity, and thus a few derivatives were synthesised according to scheme 5.6. In order to achieve a 3' selectivity, the position 5' of the sugar was protected with dimethoxytrityl group (DMT). Then the tritylated BCNA **127** was reacted with the corresponding phosphorochloridate under standard conditions to obtain the target 3'-phosphoramidate derivatives **128**. The use of DMT was found very useful because it was instantaneously removed after acid extraction in order to remove the NMI, circumventing the deprotection step.



Scheme 5.7: *i) Pyr, DMT-Cl. ii) THF, **125a,b,f,j,k**, NMI. iii) DOWEX H+*

As table 5.6 shows, the general yield of this new series of phosphoramidates is higher then the previous one. For these analogues no extensive purification was needed as the compound was easily obtained with just one flash column chromatography. Also in this case the 3'-phosphoramidates synthesised showed two peaks by ^{31}P-NMR, due to the presence of two diasteroisomeric compounds in a ratio 1:1 and no separation was performed.

Compound	Ester	Amino Acid	Yield (%)	^{31}P-NMR δ
128a	Me	Ala	25%	3.14, 2.21
128b	Et	Ala	32%	3.13, 2.05
128f	Bn	Ala	56%	3.00, 1.92
128j	Me	Phe	52%	3.19, 2.49
128k	Et	Phe	58%	3.23, 2.46

Table 5.6: 3'-Phosphoramidate

5.10 Biological Evaluation

The synthesised compound **128a,b,f,j,k** were evaluated for their ability to inhibit the replication of VZV in tissue culture. Table 5.7 contains data relating to two strains of thymidine kinase-competent (TK^+) VZV and also two strains of thymidine kinase-deficient (TK^-) VZV, with data also given for ACV, BVdU and **33f** as reference compounds.

				EC_{50} μM[a]					
	Amino Acid	Ester	LogP	OKA	YS	TK^- 07	TK^- YS	MCC μM[b]	CC_{50} μM[c]
128a	Ala	Me	4.64	0.011	0.0094	>20	-	≥5	>50
128b	Ala	Et	5.17	0.015	-	>100	-	-	-
128f	Ala	Bn	6.35	0.005	0.0059	>20	-	>20	>50
128j	Phe	Me	5.81	0.0041	0.0050	>20	-	≥20	>50
128k	Phe	Et	6.34	<0.009*	<0.032*	>20	-	≥20	>50
ACV	-	-	-2.42	2.9	1	74	125	>200	>200
BVdU	-	-	-0.82	0.005	0.005	-	-	>200	>200
33f	-	-	3.52	0.0003	0.0001	>5	>5	>50	>200

Table 5.7: Biological Data. * Preliminary data, to be repeated

As shown in table 5.7, the 3'-phosphoramidates were found to have an activity comparable to 5'-phosphoramidates. This result is not easy to interpret, a possible explanation is that the phosphoramidate is converted to the phosphate inside the cell and then it is hydrolysed to parent drug, which is phosphorylated again by the VZV-TK. As for the previous series, in VZV-TK deficient cell line this series also lacks of biological activity, suggesting again the viral TK is fundamental for the biological activity. Also this series was tested against HCMV,

HSV-1, HSV-2 and HIV. Against HCMV these derivatives were found to active at concentrations above 4μM, while for the other viruses biological data are still awaited.

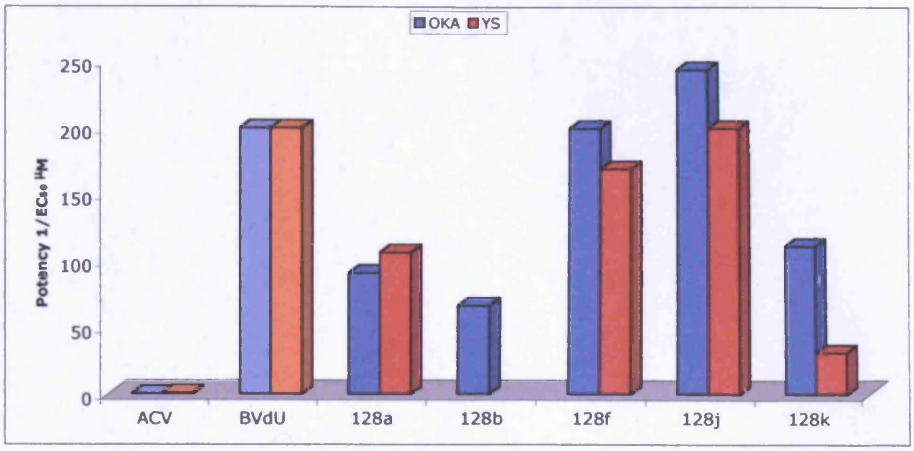


Fig 5.5: 3'-Phosphoramidates

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Chapter 6: Modified Stereochemistry on BCNA

6.1 L-Nucleosides

L-nucleosides are the enantiomers of the natural nucleosides that have an inverted configuration of at all chiral centres. By analogy with the natural D-nucleosides, the nucleobase is designated to be in β-orientation if it is cis to the 4'-hydroxymethyl group in the sugar moiety (fig 6.1). At molecular level, biologically active nucleosides have the natural β-D-configuration as most amino acids have the L-configuration. Enzyme reactions are normally highly stereospecific, acting mainly on one enantiomer, such D-nucleosides and L-amino acids. It was believed that optically active compounds, such as natural nucleosides, would be considerably more active than 'unnatural' L-enantiomers.[1]

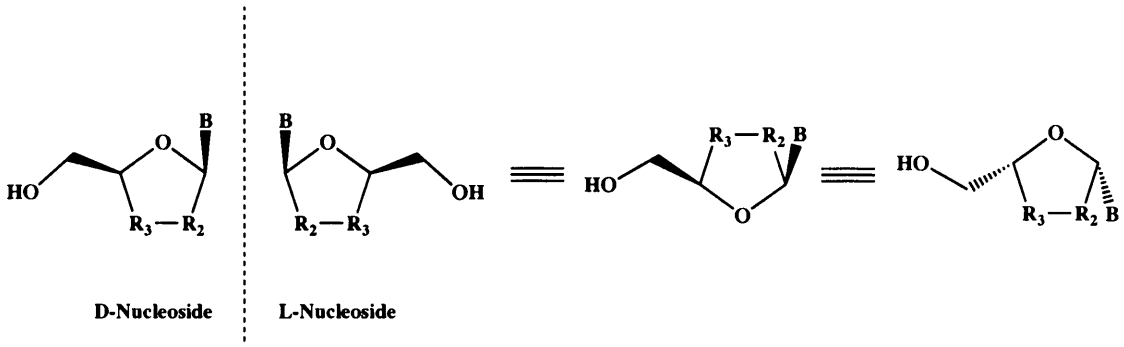


Fig 6.1: D,L-Nuclesides

Although the first L-nucleoside was synthesised in the 1960s, little attention was given to L-nucleosides until the emergence of Lamividine **9**. This nucleoside was synthesised for the first time as a racemic mixture ((±)-2',3'-dideoxy-3'-thiacytidine (BCH-189)) which shows a good anti-HIV activity.[2] Subsequently, the resolution of (±)-BCH-189 showed not only that the anti-HIV activity resided in both enantiomers, but also that the L-enantiomer (3TC, Lamivudine, **9**) was more active then its D-counterpart and that the cytotoxicity resided mainly in the latter.[2]

The explanation for the different activity and toxicological profiles of (+)-BCH-189 and (-)-BCH-189 (3TC) is given to their metabolic and pharmacodynamic properties. Lamivudine is resistant to enzymatic deamination, while (+)-BCH-189 is deaminated to 2',3'-dideoxy-3'-thiauridine by deoxycytidine deaminase. Both enantiomers are substrates of deoxycytidine

kinase that converts them to the monophosphate form. Further phosphorylation to diphosphate and triphosphate is performed by deoxycytidylate kinase and NDP kinase respectively. Since the first phosphorylation is the key step in their conversion to the active triphosphates, the major affinity of Lamivudine for deoxycytidine kinase gives a good explanation to its greater activity over its D-enantiomer.[2]

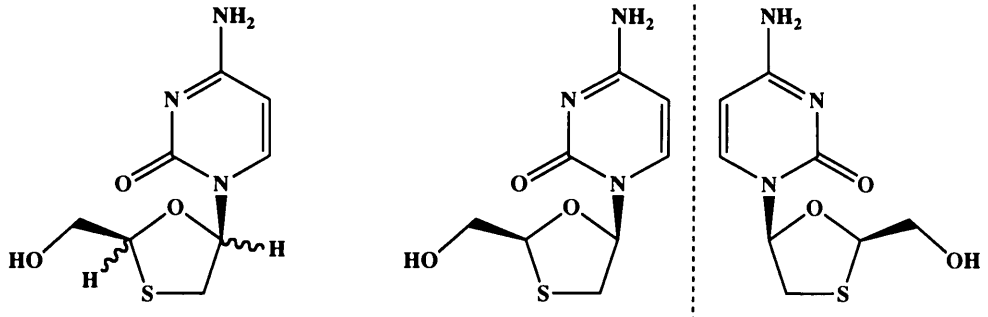


Fig 5.2: BCH-189 and 3TC

The interest in L-nucleosides was encouraged by the expectation that L-nucleosides would be recognised not by mammalian enzymes, but by virus-encoded or bacterial enzymes. The most surprising aspect of L-nucleosides was the fact that some cellular enzymes are able to phosphorylate L-nucleosides to the triphosphate form, providing antiviral/biological activities.[1] The physical and chemical properties of L-nucleosides are identical to with those of D-counterparts except for optical rotation. Their pharmacological properties, in a chiral environment, can be different for each enantiomer. The explanation of these differences can be given by active transportation of nucleosides into the cells, anabolic and catabolic enzymes that act on these compounds and interaction with the viral/cellular target enzymes.[1]

5.2 L-Nucleosides as anti-Herpes agents

HSV-1 TK is a virus-encoded enzyme essential for viral infection and reactivation that does not have such precise specificity as the human TK, in fact it can phosphorylate also deoxy-cytidine and a variety of purine and pyrimidine analogues, which represent most of the anti-herpetic drugs in the clinic.

Recently it has been reported that L-thymidine is not recognised by human TK but that it acts as a good substrate for HSV-1 TK, reducing proliferation in HeLa cells, with little or no toxic effect on host cells.[3]

IdU is an effective antiviral agent, but its therapeutic value is limited by the fact that also human TK recognises it as a substrate. Instead BVdU shows a remarkable potency, although it was found to have potential drawbacks due to interactions with other drugs.

L-IDU and L-BVdU show an inhibitory activity against HSV-1 TK similar to that of their D-counterparts, while being inactive against human TK (table 5.1).[3]

	IC_{50} μM		
	HSV-1 TK	**HeLa TK**	**SI**
D-IDU	0.12	6.05	50
L-IDU	0.35	>2000	>5000
D-BVdU	0.07	>600	>8000
L-BVdU	0.26	>600	>2000

Table 6.1

In view of these results, the L-enantiomer **129** of the most potent anti-VZV analogue pentyl phenyl **33f** was synthesised in order to explore the possibility if this compound may possess different biological properties from **33f**.

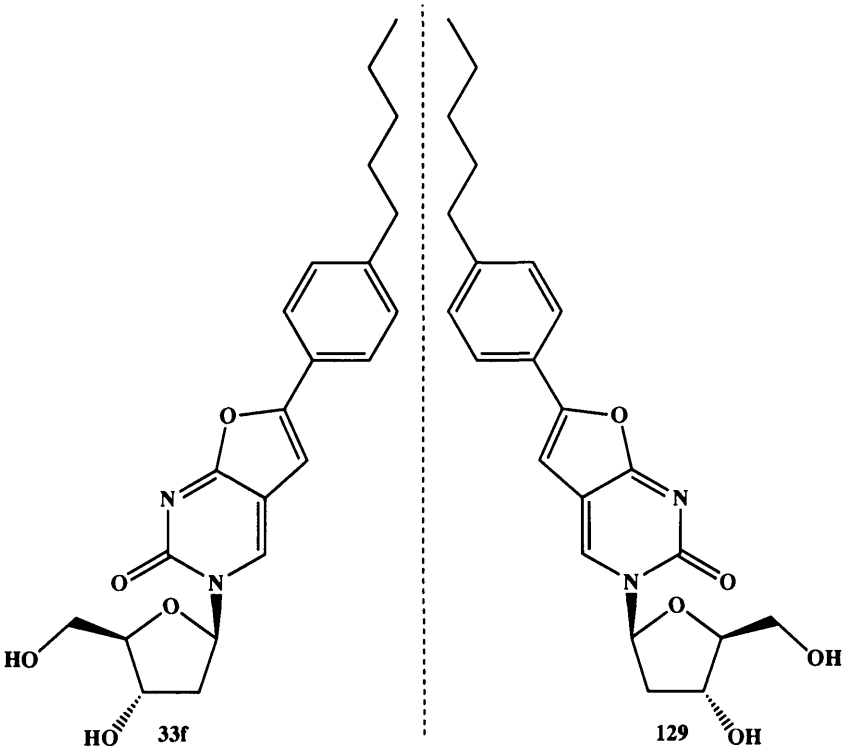
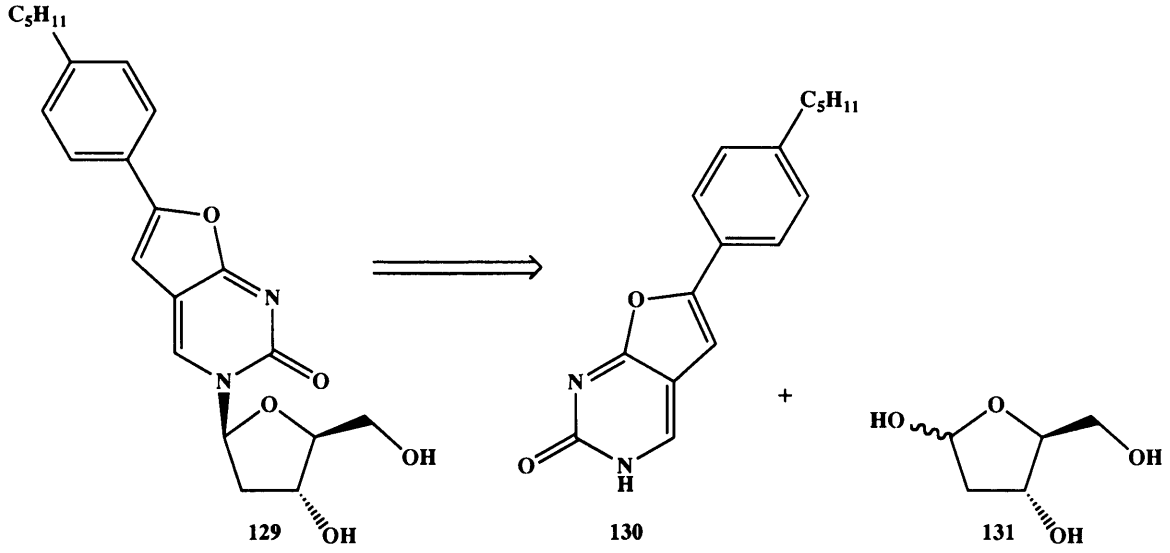


Fig 6.3: D- and L- derivatives of pentylphenyl of BCNA

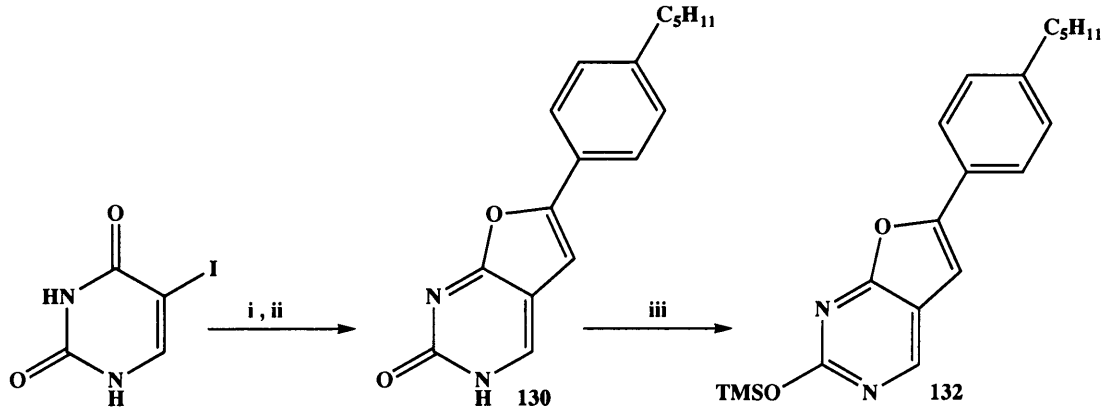
6.3 Synthesis of target L-Nucleoside

Since it was not possible to start from L-5-iodo-2'-deoxyuridine or L-2'-deoxyuridine, it was necessary to plan a new synthetic route for compound **129**. After a retrosynthetic analysis (scheme 6.1), it was though that target compound could be synthesised by condensation of the unusual nucleobase **130** with L-deoxyribose **131** under Vorbrüggen conditions.



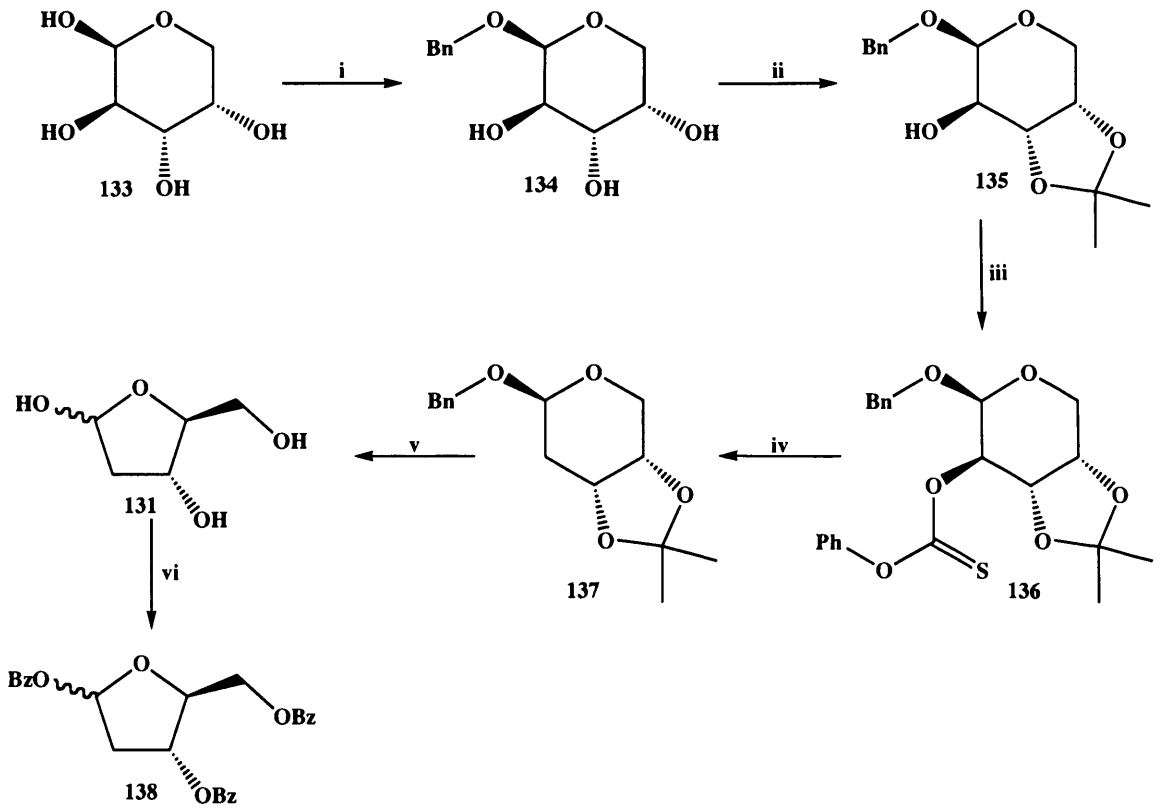
Scheme 6.1

Compound **130** was easily obtained in good yield reacting 5-iodouracil with 4-pentylethynylbenzene under standard conditions. The derivative thus obtained was impure of metallic Pd, that was not possible to remove either by crystallisation, due to the insolubility of **130** even in boiling DMSO, nor column chromatography as it crystallised on silica. It was decided to use **130** without any further purification for the successive step. The silylation, required from Vorbrüggen procedure, was performed in boiling HMDS obtaining **132** in high yield (scheme 6.2).



Scheme 6.2: *i), DMF, 4-pentylethynylbenzene, Tetrakis Pd(PPh$_3$)$_4$, CuI, DIPEA. ii) CuI, TEA. iii) HMDS.*

Given the high cost and lack of a prompt source of L-deoxyribose, the sugar **131** was synthesised according to the scheme 6.3.[4]



Scheme 6.3: *i) BnOH, $HCl_{(g)}$. ii) Acetone, Dimethoxypropane, TosOH. iii) DCM, Phenyl-chloro-thionoformate, Pyr. iv) Toluene, AIBN, Tributyl-tinhydride. v) H_2O, TFA. vi) DCM, BzCl, Pyr.*

The inexpensive β-L-arabinopyranose was reacted with benzyl alcohol in the presence of gaseous hydrochloric acid in order to obtain compound **134** in high yield. The protection of the anomeric OH with a benzyl group led to three main advantages. Firstly, the introduction of a chromophore allows the visualisation of the compound on TLC in order to monitor the successive reactions more easily. Secondly, the benzyl group can be removed in mild conditions and thirdly, the reaction is stereospecific giving exclusively the β-anomer **134**, simplifying the interpretation of NMR spectra of the following derivatives. Treatment of **134** with an excess of dimethoxypropane in the presence of a catalytic amount of tosylic acid afforded **135** in very high yield. The successive step was the reduction of the free hydroxy group to alkane by the Barton-McCombie deoxygenation. In order to perform this reaction it was necessary to convert **135** into a Barton ester **136**, that it was done in quantitative yield, and then the deoxygenation was carried out through a radical reduction with tributyltin

hydride obtaining **137** in very high yield. Elimination of the protecting groups with diluted aqueous trifluoroacetic acid afforded L-deoxyribose **131** (44% overall yield).

In order to perform the Vorbüggen condensation, L-deoxyribose was fully protected with benzoyl groups affording compound **138**. It was noticed that carrying out the reaction at different temperatures, a mixture of isomers was observed, all having the mass of tribenzoyl-L-deoxyribose. L-deoxyribose can exist in four possible forms, the α and β anomers combined with the furano and pyrano forms (fig 6.4).

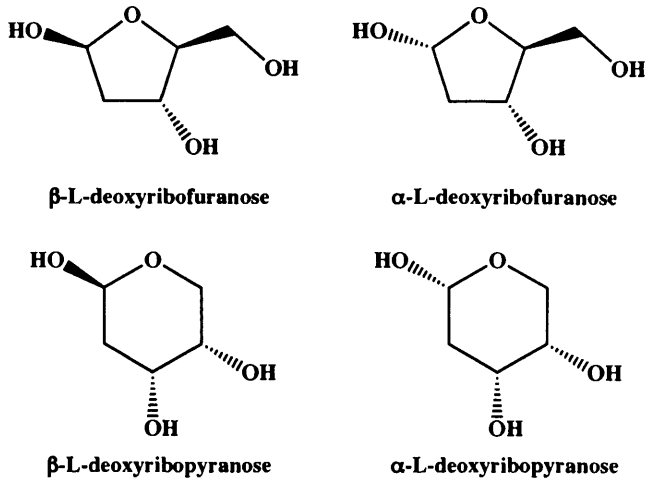


Fig 6.4: possible forms of L-deoxyribose

A brief study on the protection of **131** was performed in order to understand the role of the temperature on the ratio of pyrano and furano forms. When carried out at 30 °C, the reaction led to the exclusive formation of the furano form, but decreasing the temperature the ratio between pyrano and f increased at the point that at –30 °C only the pyrano form was detected (table 6.2). Regarding the ratio between α and β, no significant difference was observed along the different experiments, always obtaining a mixture α / β in a ratio ca 1:1 in the case of the furano form and only the α anomer for the pyrano form.

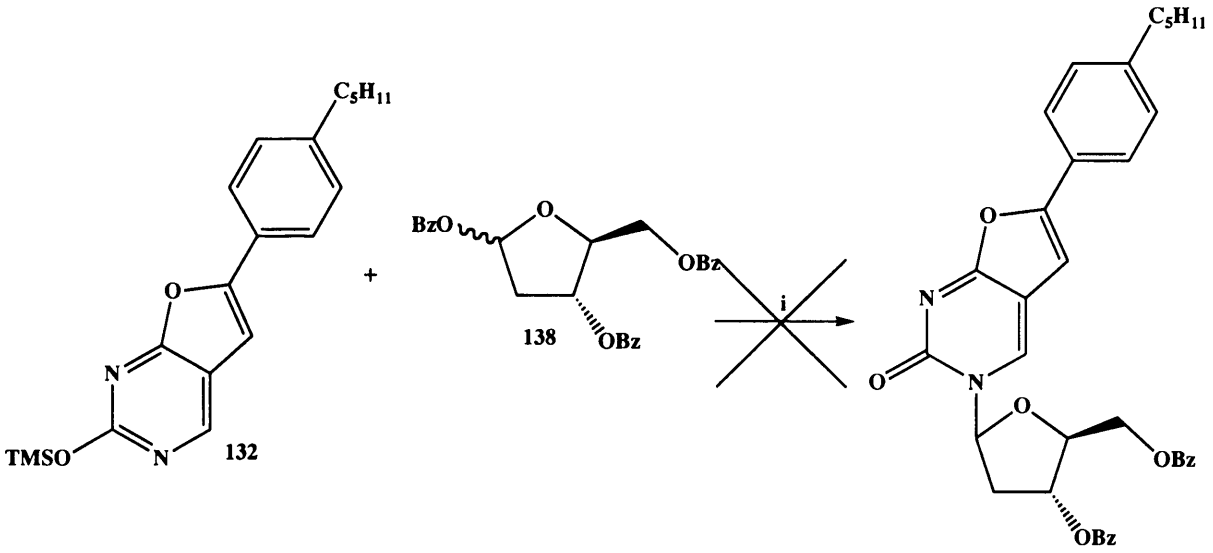
Temperature (°C)	Pyrano / Furano Ratio[a]
30	0 / 100
15	30 /70
-10	65 /35
-30	100 / 0

a) Approximate ratio measured by ^{1}H-NMR

Table 6.2

The coupling between the protected sugar **138** and the silylated base **132** was performed under Vorbüggen conditions according to scheme 6.4.

The TLC analysis of the reaction showed the total conversion of **132** into a more polar fluorescent compound. After purification by column chromatography it was found that the isolated spot was the free base **130**. It was though that the catalyst could be responsible, being too reactive at room temperature and decomposing the silylated base **132**. The reaction was then performed at low temperature (<5°C) resulting again in the formation of the free base **130**. The use of another catalyst such as TMS-triflate was not found useful for this reaction and the free base **130** was isolated again. An explanation for these failures is probably due to the fact that the silyl group is very labile in the presence of a Lewis acid, inactivating the base and then quenching the reaction.



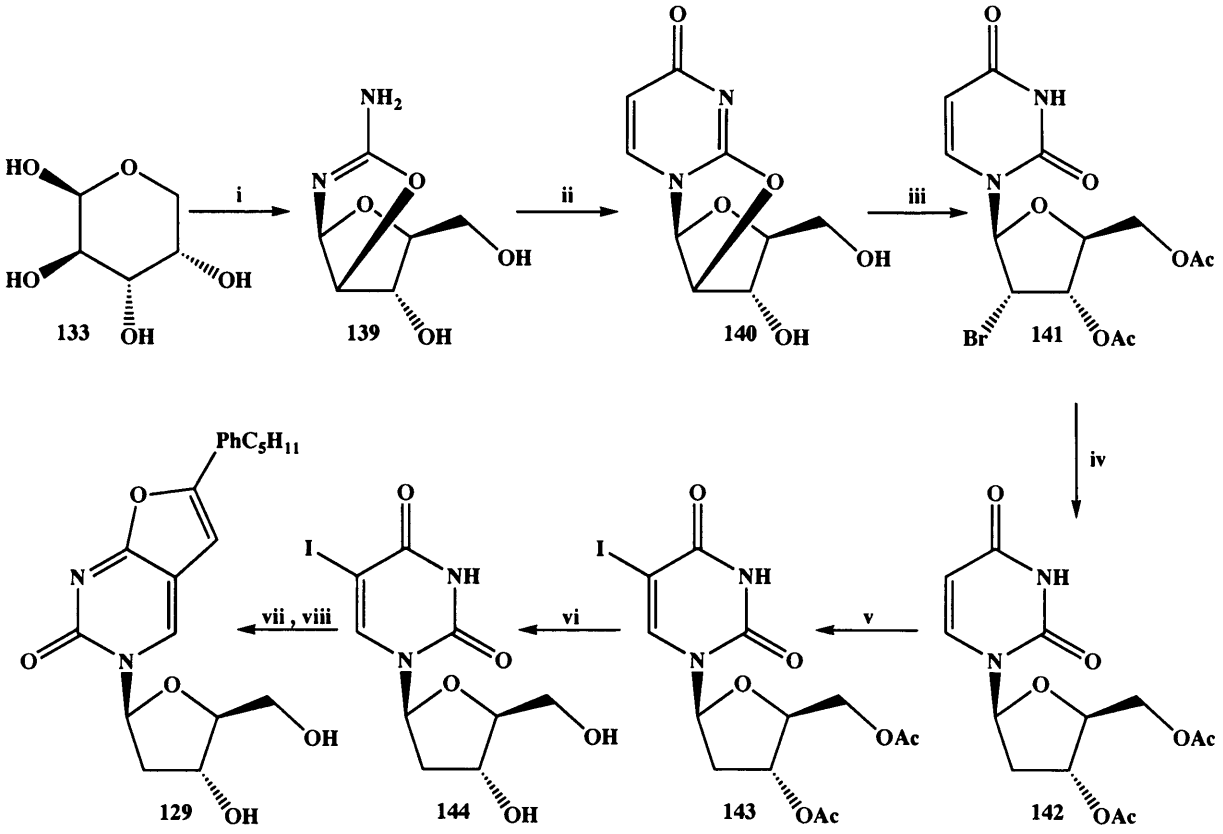
Scheme 6.4: i) DCM, $SnCl_4$

Assuming that the base **130** cannot be coupled with the sugar **136** in a traditional way, an alternative route was planned using a linear synthetic pathway rather than a convergent synthesis (scheme 6.5).[5,6,7,8] The target of this new route was to synthesise L-5-iodo-2'-deoxyurine **144** in order to react it with 4-pentylethynylbenzene under the standard reaction.

β-L-arabinopyranose was reacted with cyanamide in basic environment to give the oxazoline **139**. When refluxed with methyl propiolate in aqueous ethanol, compound **139** afforded in high yield $O^{2,2'}$-anhydro-L-uridine **140,** that was treated with acetyl bromide under reflux in order to protect the hydroxy groups and break the 2,2' anhydro-bond, obtaining compound **141**. The bromo derivative was de-halogenated with tributyltin hydride to afford the protected

2'-deoxy-L-uridine **142**. The selective iodination of position 5 of the base was achieved using ceric ammonium nitrate and molecular iodine in acetic acid at reflux to isolate in very high yield **143**. Deprotection of the acetyl groups was performed with sodium methoxyde to obtain L-IDU **144** in quantitative yield.

The coupling of L-IDU with 4-pentylethynylbenzene was performed as for the D-analogue, affording in good yield nucleoside **129** (17% overall yield).



Scheme 6.5: *i) MeOH, cyanamide, H_20 / NH_3. ii) 50% EtOH, methyl propiolate. iii) ACN, AcBr. iv)Toluene, AIBN, TBTH. v) AcOH, I_2, CAN. vi) MeOH, MeONa. vii) DMF, 4-pentylethynylbenzene, Tetrakis $Pd(PPh_3)_4$, CuI, DIPEA. viii) TEA, CuI.*

This procedure was found to be quicker and less problematic than the previous route, considering the fact that no column chromatography was performed during the synthetic pathway, as the intermediates were at least 95% pure by ^{1}H-NMR. Another point was the selectivity of this route over the previous one. In fact with the first route a mixture of α and β nucleosides would have be obtained, while with the latter only the β anomer was obtained.

The spectroscopic data acquired (NMR, Mass, CHN, HPLC, optical rotation) were consistent with the structure expected and perfectly matched with its D-enantiomer **33f**, apart from the optical rotation that showed an $[\alpha]_D^{20}$ of +80 for **33f** and –80 for **129**, confirming that the L-enantiomer was obtained.

6.4 α-Nucleosides

Although a few α–nucleosides were found to occur naturally, all nucleotides found in nucleic acids and essentially all nucleosides and nucleotides occurring elsewhere are in the β configuration. For this reason α anomers were assumed to be biologically inert, and only few have been examined for biological activity.[9] Although growth-inhibitory activity was reported for some α nucleosides of adenine analogues, the only α nucleoside that was studied extensively as a biologically active agent is α–thiodeoxyguanosine **145**, which is phosphorylated and incorporated into DNA by terminal addition.[9] During examination of a variety of adenosine analogues, it was found that 9-α-D-arabinofuranosyl adenine **147** and and in particular 9-α-D-arabinofuranosyl-8-azaadenine **146** showed an antiviral activity against HSV, where the β anomer not only had no antiviral activity, but was found to be also to be cytotoxic.[9] Recently, a new class of α–benzimidazole nucleosides was found to be generally active against HMCV, in particular compound **148** is active at under μM concentrations with very low or no cytotoxicity.[10] However, at least in this case the agents appear to have a non-nucleoside mechanism of action.

As this kind of modification has never been attempted on BCNAs, it was of interest to synthesise a α-BCNA analogue bearing the pentylphenyl moiety as side chain.

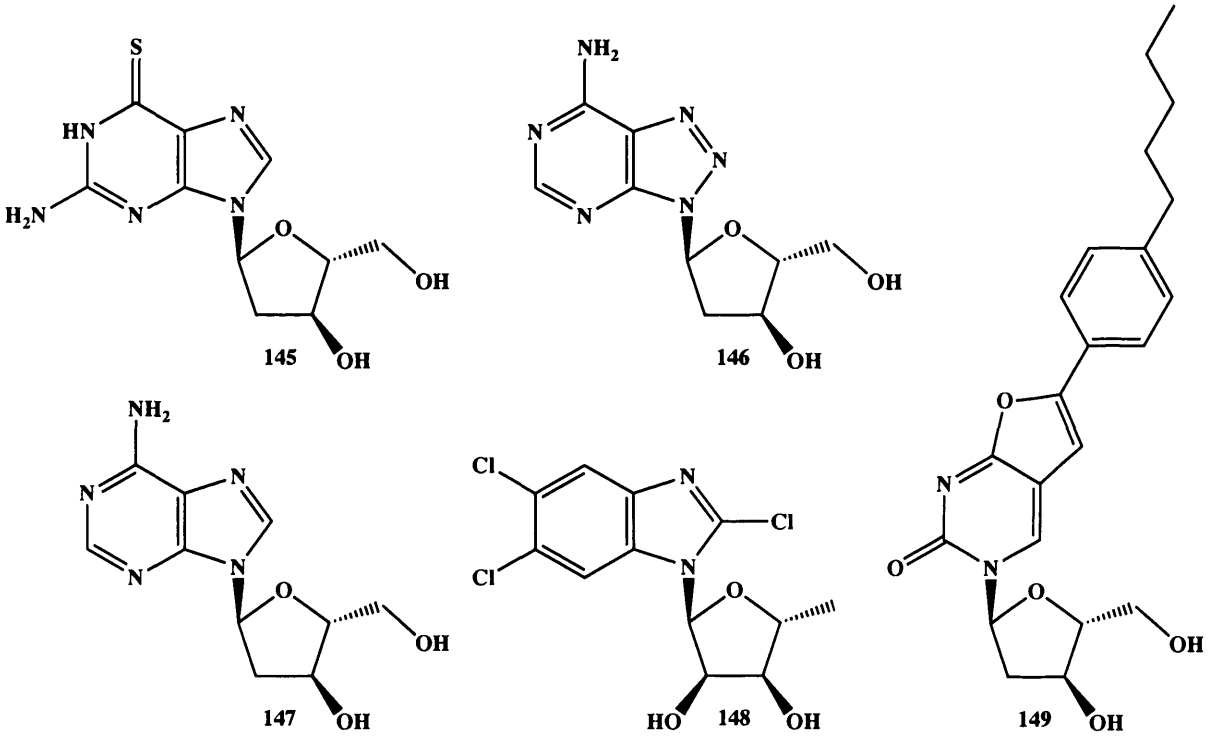
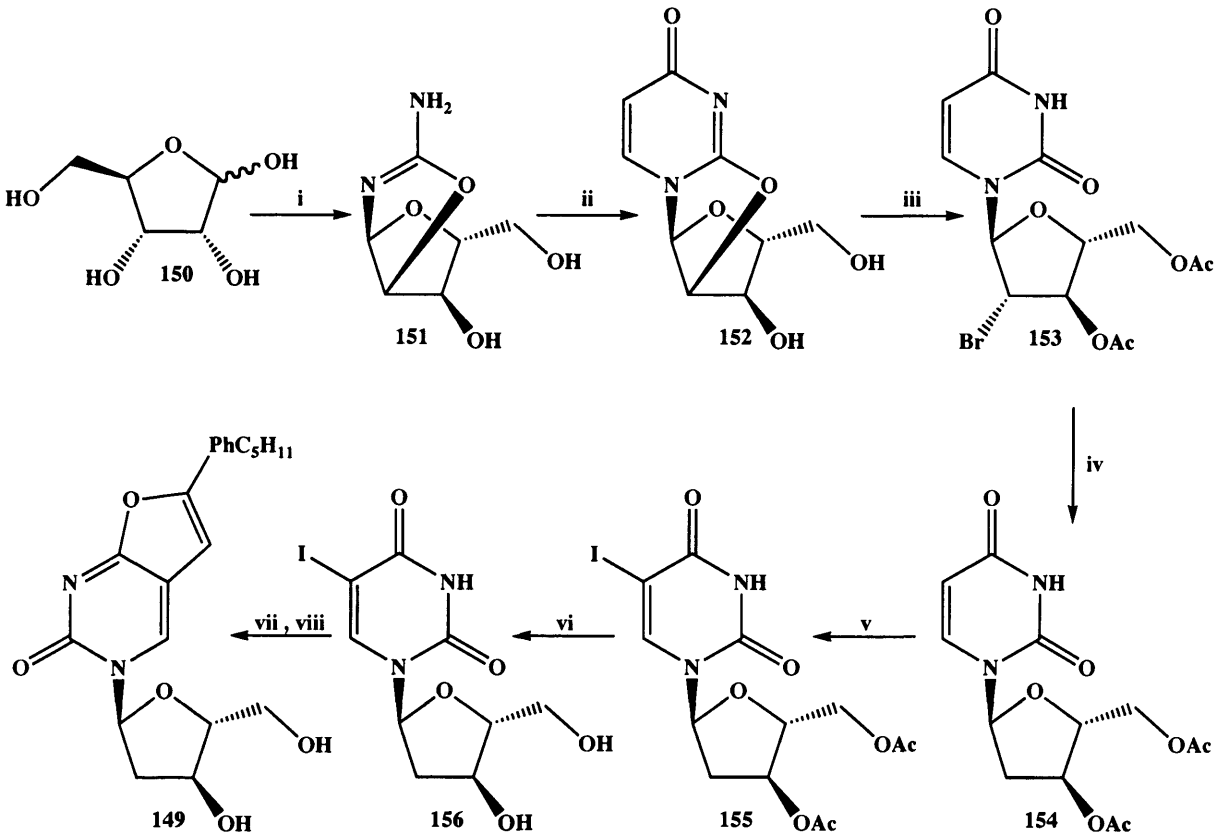


Fig 6.5: α purine analogues and target α-BCNA **136**

6.5 Synthesis of α-BCNA

The synthetic route used to prepare the α-BCNA was the same as for the L-derivative **129**, but instead of starting from L-β-arabinose, D-ribose **150** was used as starting material (scheme 6.6). The conversion of D-reibose into oxazoline **151**, was performed as described for the L-derivative, but the reaction instead of lasting 15 hours, lasted 3 days. As the TLC analysis of this reaction was inconclusive, the reaction was monitored by ^{13}C-NMR until total conversion of **152**. The pK_a of ribose is 12.04, a value slightly lesser than arabinose (pK_a 12.43),[11] and this small difference (ca 3 fold less acidic) could explain the slower rate of the formation of **151**. The successive reactions were performed without encountering any difficulties, obtaining every intermediate in high yield. Also in this case no column chromatography was needed for the synthesis of the intermediates that were at least 95-97% pure by ^{1}H-NMR.



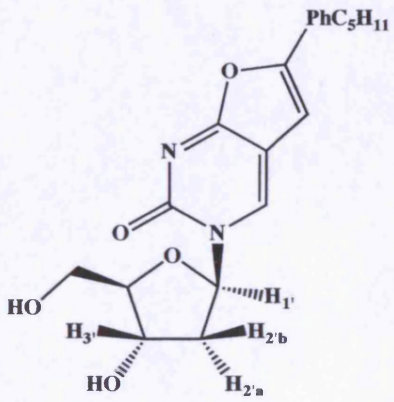
Scheme 6.6: *i) MeOH, cyanamide, H_20 / NH_3. ii) 50% EtOH, methyl propiolate. iii) ACN, AcBr. iv) Toluene, AIBN, TBTH. v) AcOH, I_2, CAN. vi) MeOH, MeONa. vii) DMF, 4-pentylethynylbenzene, Tetrakis $Pd(PPh_3)_4$, CuI, DIPEA. viii) TEA, CuI.*

All data acquired (NMR, Mass, CHN) for compound **149** were consistent with the structure expected. However, ^{1}H-NMR of **33f** and **149** were compared together, showing a first glance only few differences in chemical shifts. An in-depth analysis of compound **149** and **33f** carried out by NMR showed significant differences between these two derivatives in the sugar moiety. The exact assignment of ^{1}H-NMR peaks was done on the basis of COSY (H-H correlation) and a NOESY experiment performed on both compounds.

For compound **33f**, where the stereochemistry is known, the most relevant data emerging from the observation of the NOESY spectrum are the cross-peaks between the H-1'/H-2'$_a$ and H-2'$_a$/OH-3', assigning to H-2'$_a$ the α-configuration. H-2'$_b$ was assigned to be in β-configuration as no cross-peak was observed neither with OH-3' nor with H-1', furthermore the cross-peak H-2'$_b$/H-3' is stronger then the one H-2'$_a$/H-3' (fig 6.6).

The major piece of evidence for the stereochemistry of compound **148** is the presence of the cross peak H-1'/H-2'$_b$ and the absence of the cross peak H-2'$_b$/OH-3', suggesting that H-2'$_b$ and H-1' are in β configuration and that the stereochemistry at 1'-carbon is the one of the expected structure. The configuration of H-2'$_a$ was assigned to be α due to the presence of the cross peak OH-3'/H-2'$_a$ and absence of the cross peak H-2'$_a$/H-1'.

It was observed also that the base has a shielding effect on the 2' proton in cis, in fact in compound **33f** the β-proton is the most shielded while in compound **149** is the α-proton. Furthermore the base has a deshielding effect on the 4' proton, in the β-nucleoside the proton is at 3.95 ppm while in the α-nucleoside is at 4.43 ppm. The other protons of the sugar are not affected. These effects of shielding and deshielding are due probably to the aromatic nature of the base resulting in anisotropic effects that affect the chemical shift of the protons involved.



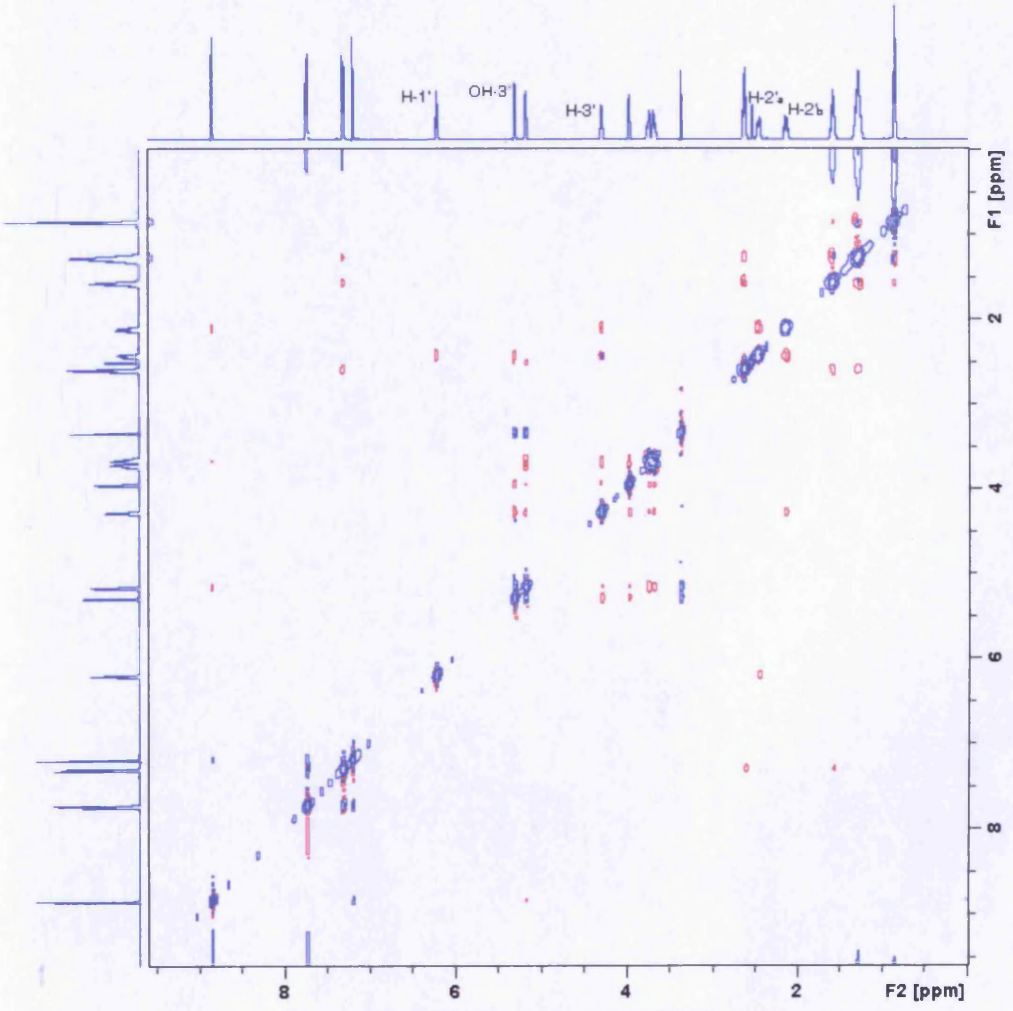


Fig 6.6: Noesy spectrum of **33f**

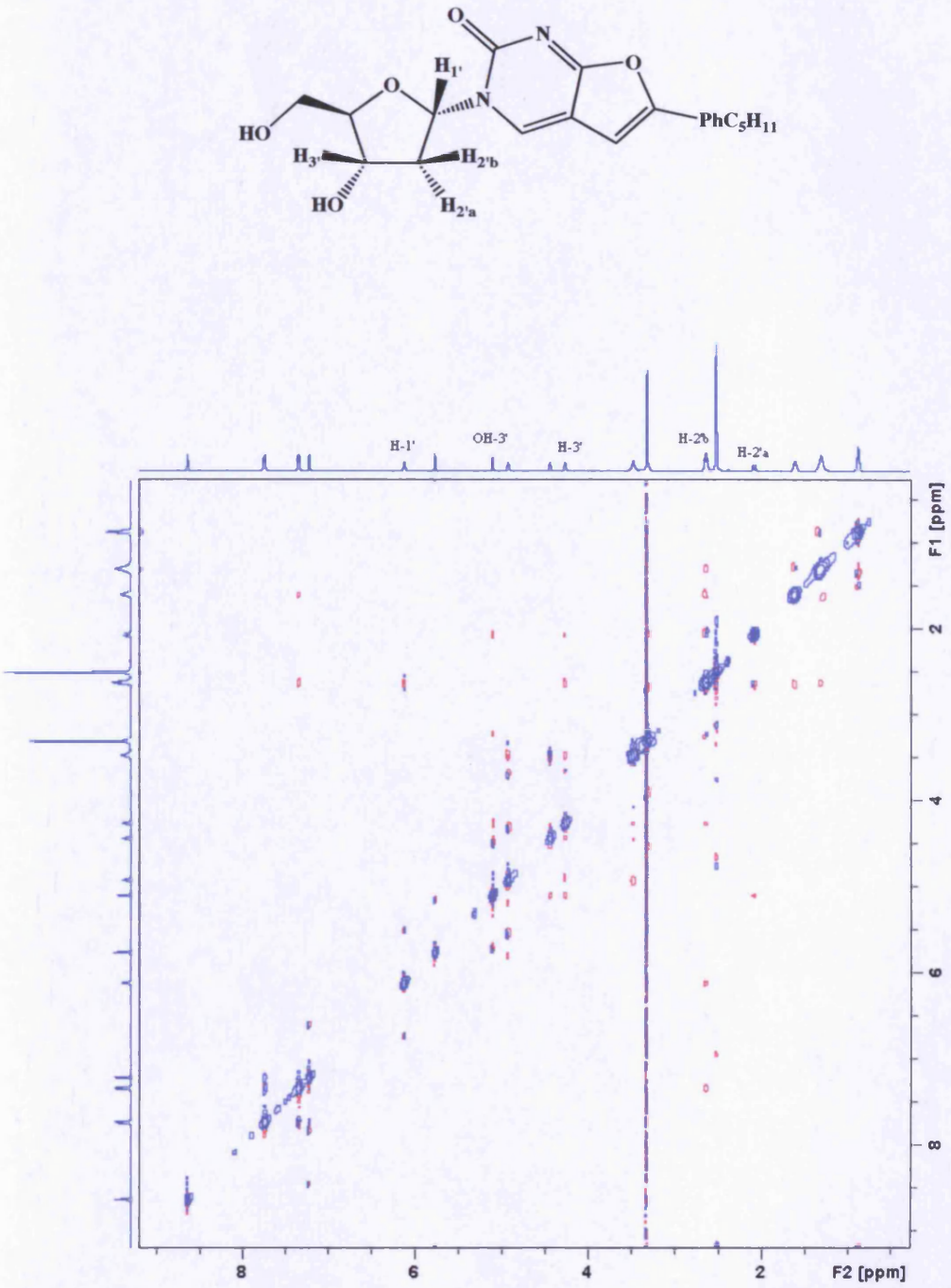


Fig 6.7: Noesy spectrum of **148**

6.6 Biological evaluation

The L-nucleoside **129** and α-nucleoside **149** were evaluated in vitro for their ability to inhibit the replication of VZV, according to reported methods.[12] Data are shown in table 5.3 for the activity of **129** and **149** versus two strains of TK-competent (TK^+) VZV and two strains of TK-deficient (TK^-) virus. Data are included for the reference compounds ACV, **33f** and the modified sugar derivatives **41a-41g** that bear an alkyl chain (table 6.3). Cytotoxicity data are also included for each compound in two assays.

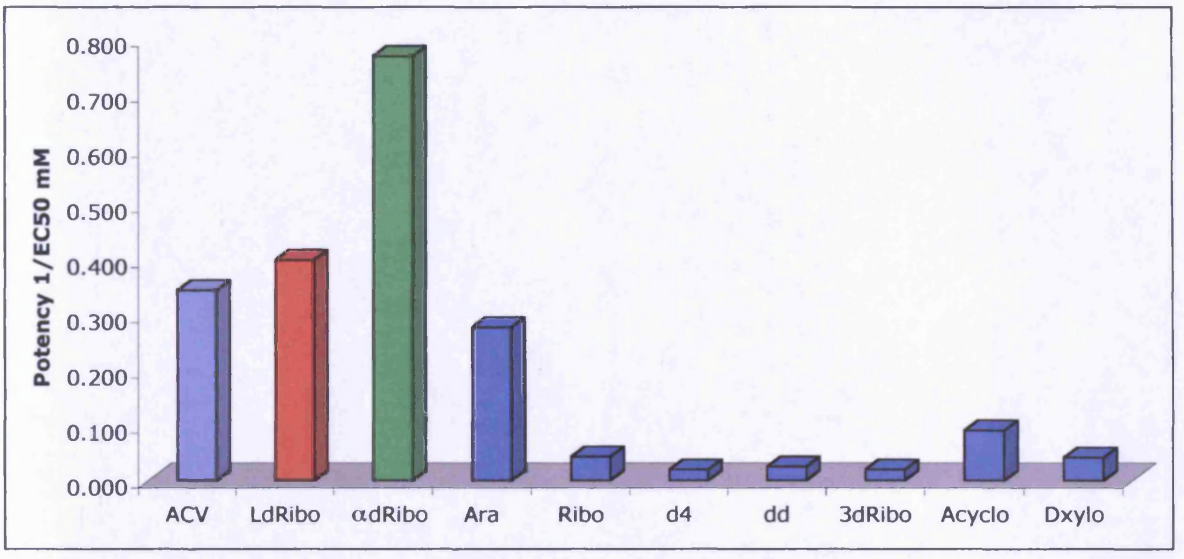


Fig 6.8: Biological data for modified sugar derivatives

		EC_{50} μM[a]				MCC μM[b]	CC_{50} μM[c]
		OKA	**YS**	**TK^- 07**	**TK^- YS**		
129	L-dRibo	2.5	1	>5	>5	>20	>50
149	α-dRibo	1.3	3.1	13	-	>100	100
41a	Ara	3.6	3.3	200	67	>200	>200
41b	Ribo	23	21	>20	>20	50	>50
41c	d4	>50	>50	>50	>50	200	16
41d	dd	40	20	>200	>50	>200	>200
41e	3dRibo	>50	-	>200	-	>50	>50
41f	Acyclo	11	-	>16	-	>80	>200
41g	dXylo	24	>20	>50	>50	>50	>22
33f	dRibo	0.0003	0.0001	>5	>5	>50	>200
ACV		2.9	1	74	125	>200	>200

a) effective concentration required to reduce virus plaque formation by 50%
b) minimal cytotoxic concentration
c) 50% cytotoxic concentration required to inhibit cell growth by 50%

Table 6.3

The L-enantiomer still retains a certain anti-VZV activity, although it is considerably less active then parent nucleoside analogue **33f** at non-cytotoxic concentrations. Like the L-derivative, also compound **149** retains an anti-VZV activity being two fold more active than the L-derivative, but still less active than parent nucleoside **33f**. Along all the modified sugar series, compound **129** and **149** were found to be the most active. In fact, compound **129** is equipotent to ACV while **149** is twice more active.

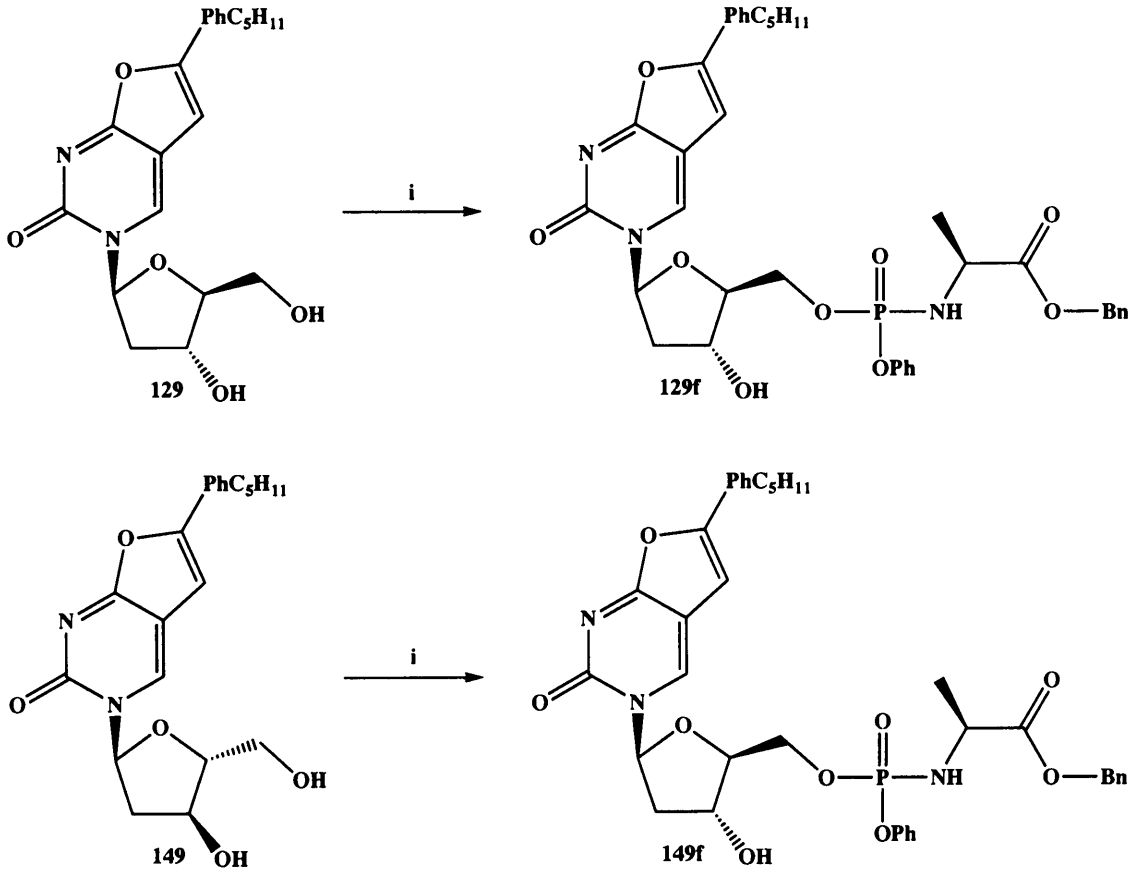
As a rule, also these compounds entirely lost antiviral activity against TK^- VZV strains, pointing to the requirement for VZV TK-mediated phosphorylation for activation.

Concluding, the SARs of the modified sugar derivatives clearly suggest that any modification of the sugar moiety leads to a dramatic loss of activity and that any modification of the D-β-2'-deoxyribosyl moiety is not tolerated.

6.7 Phosphoramidates

Given the dramatic loss of activity of compounds **129** and **149**, the phosphoramidate approach was applied to these nucleosides in order to increase the antiviral effect and to observe if the high selectivity of VZV-TK applies also to the second phosphorylation.

The standard procedure to prepare the phosphoramidates was applied, synthesising just one phosphoramidate for each nucleoside: phenol alanine benzylester (Scheme 6.7). The corresponding nucleoside was reacted with the phosphochloridate **125f** in the presence of NMI and as predicted the yield of this reaction was not very high: 20% for **129f** and 23% for **149f**. These two derivatives were tested against VZV and other viruses as for the parent drugs, but biological data are still awaited.



Scheme 6.7: *i) Pyr, 125f, NMI*

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Chapter 6: Modified Stereochemistry on BCNA

6.1 L-Nucleosides

L-nucleosides are the enantiomers of the natural nucleosides that have an inverted configuration of at all chiral centres. By analogy with the natural D-nucleosides, the nucleobase is designated to be in β-orientation if it is cis to the 4'-hydroxymethyl group in the sugar moiety (fig 6.1). At molecular level, biologically active nucleosides have the natural β-D-configuration as most amino acids have the L-configuration. Enzyme reactions are normally highly stereospecific, acting mainly on one enantiomer, such D-nucleosides and L-amino acids. It was believed that optically active compounds, such as natural nucleosides, would be considerably more active than 'unnatural' L-enantiomers.[1]

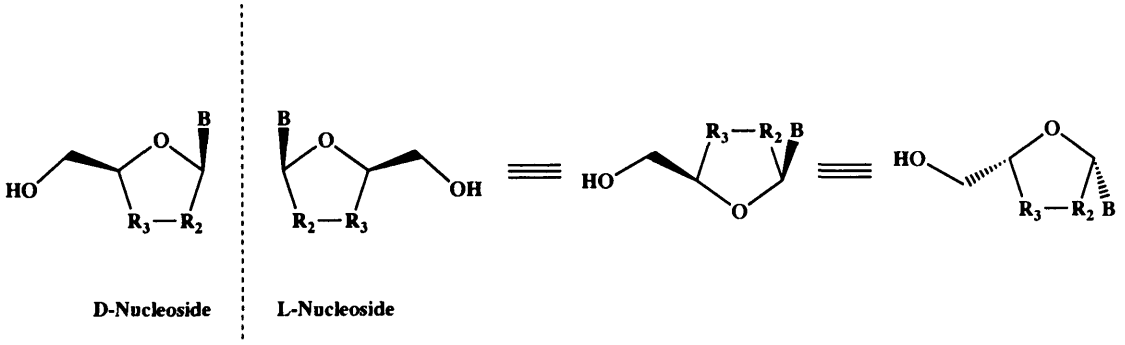


Fig 6.1: D,L-Nuclesides

Although the first L-nucleoside was synthesised in the 1960s, little attention was given to L-nucleosides until the emergence of Lamividine **9**. This nucleoside was synthesised for the first time as a racemic mixture ((±)-2',3'-dideoxy-3'-thiacytidine (BCH-189)) which shows a good anti-HIV activity.[2] Subsequently, the resolution of (±)-BCH-189 showed not only that the anti-HIV activity resided in both enantiomers, but also that the L-enantiomer (3TC, Lamivudine, **9**) was more active then its D-counterpart and that the cytotoxicity resided mainly in the latter.[2]

The explanation for the different activity and toxicological profiles of (+)-BCH-189 and (-)-BCH-189 (3TC) is given to their metabolic and pharmacodynamic properties. Lamivudine is resistant to enzymatic deamination, while (+)-BCH-189 is deaminated to 2',3'-dideoxy-3'-thiauridine by deoxycytidine deaminase. Both enantiomers are substrates of deoxycytidine

Chapter 7: Carbocyclic BCNA

The research for nucleoside analogues as selective inhibitors of kinases and polymerases whitout any cytotoxicity, for the control of viral disease and cancer, is the subject of intense research.[1,2,3] Nucleoside analogues that are substrates for cellular kinases, but resistant to other host enzymes such as phosphorylases, are fundamental for the development of new useful therapeutic agents. The replacement of the ring oxygen in sugar moiety of the nucleoside with a methylene unit (CH_2) led to carbocyclic nucleoside analogues that were found to be extremely resistant to phosphorylases and cleavage at the glycosidic bond.[4]

Some natural carbanucleosides were found to be biologically active, in fact the adenosine analogues Aristeromycin **157**[5] and Neoplacine A **158**[6] displayed antibiotic and anticancer activity. Subsequently, other synthetic carbocyclic analogues with useful therapeutic effects were discovered. In particular Carbovir **19**[7] and its analogue Abacavir **20**[8] were found to be inhibitors of HIV.

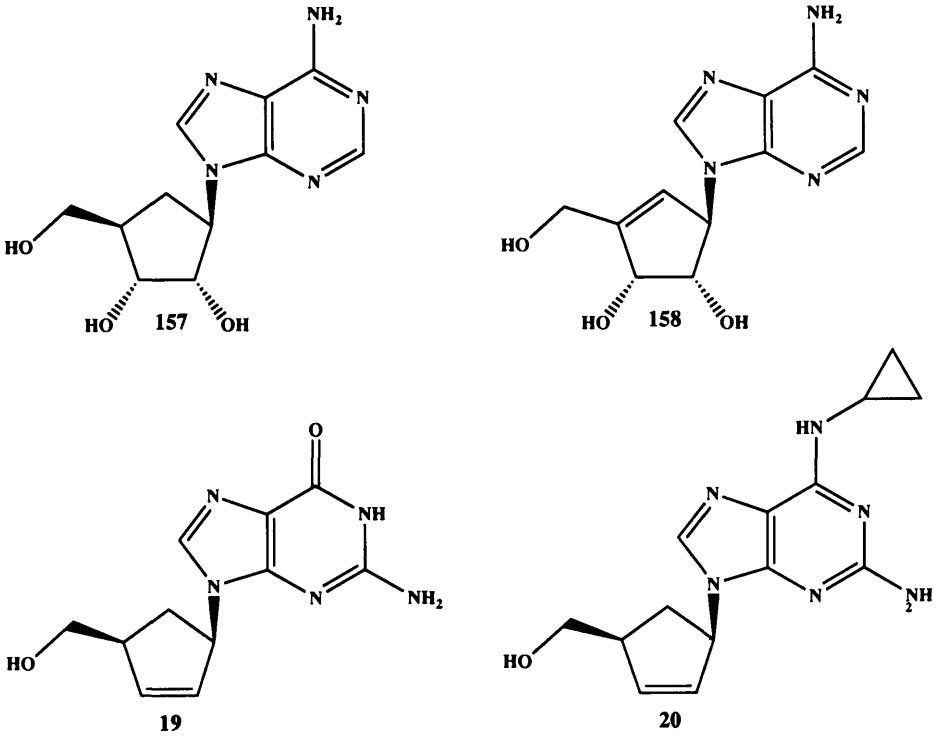


Fig 7.1: Purine carbocycle nucleosides

Among anti-herpetic drugs, the corresponding carbocycle analogues of IDU **159** and BVdU **4** were found to be potent inhibitors of HSV-1 in vitro.[9] The replacement of the furan ring of the

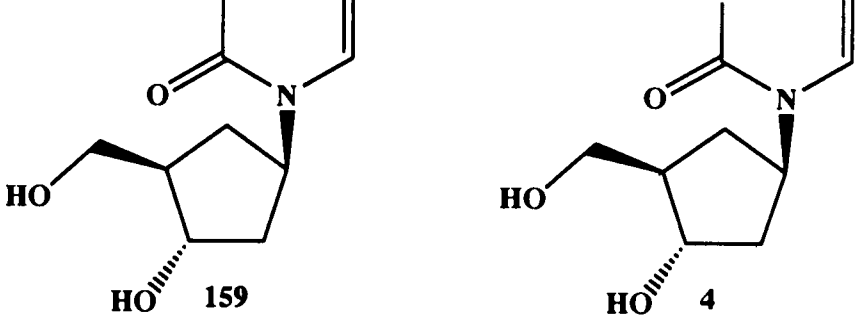


Fig 7.2: anti-HSV carbocycle nucleosides

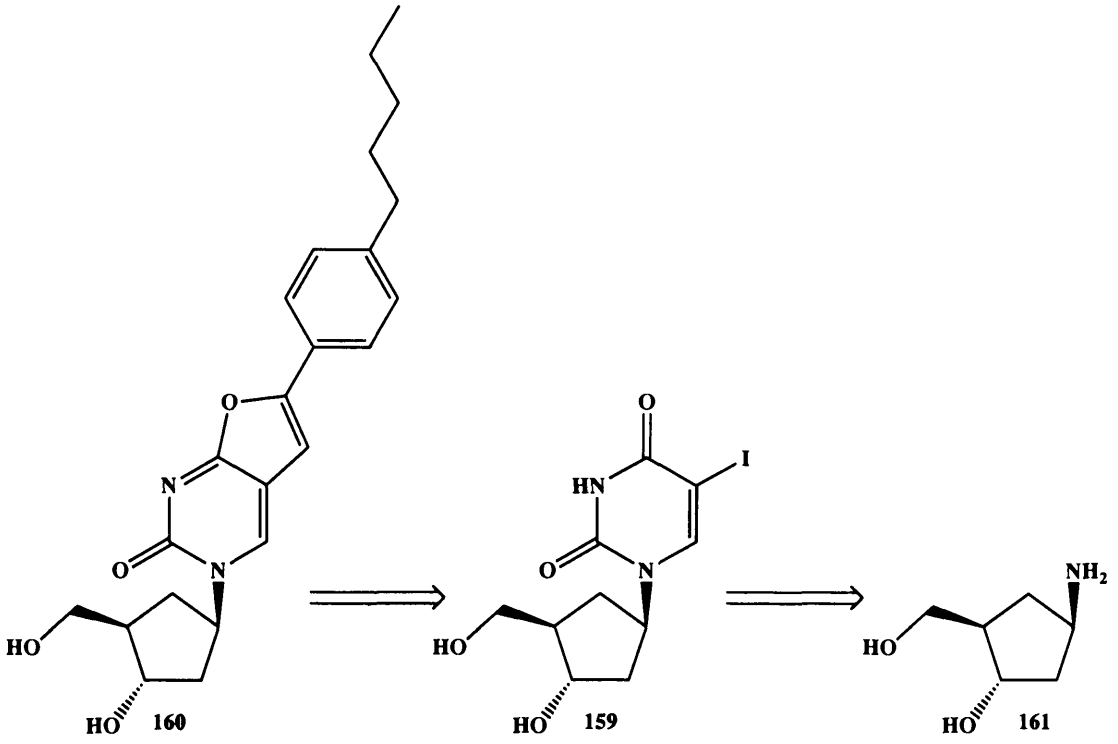
	MIC (μg/ml)		
	HSV-1	HSV-2	TK⁻ HSV-1
BVdU	0.01	2	≥400
IDU	0.1	0.4	≥400
C-BVdU	0.05	7	>400
C-IDU	0.07	10	>400

Table 7.1

7.1 Carbocyclic analogue of BCNA

As mentioned above, the carbosugars make the nucleosides extremely resistant cleavage by the TPases, due to the increase of the stability of the glycosidic bond. Alt the BCNAs were found to be extremely resistant to phosphorylases in vitro, administered in vivo, a substantial cleavage of the glycosidic bond was detected. Furthe this class of nucleosides is more susceptible to acidic cleavage than the other nucle This aspect could represent a problem, especially in a possible oral formulation compounds encounter an acidic environment generated by the stomach, resulting in a potentially base cleavage and reduction of bioavailability.

Bearing in mind the retention of biological activity of C-BVdU and C-IDU against herpes virus, the carbocycle analogue of **33f** was synthesised after a retrosynthetic analysis of target molecule **160** (scheme 7.1).

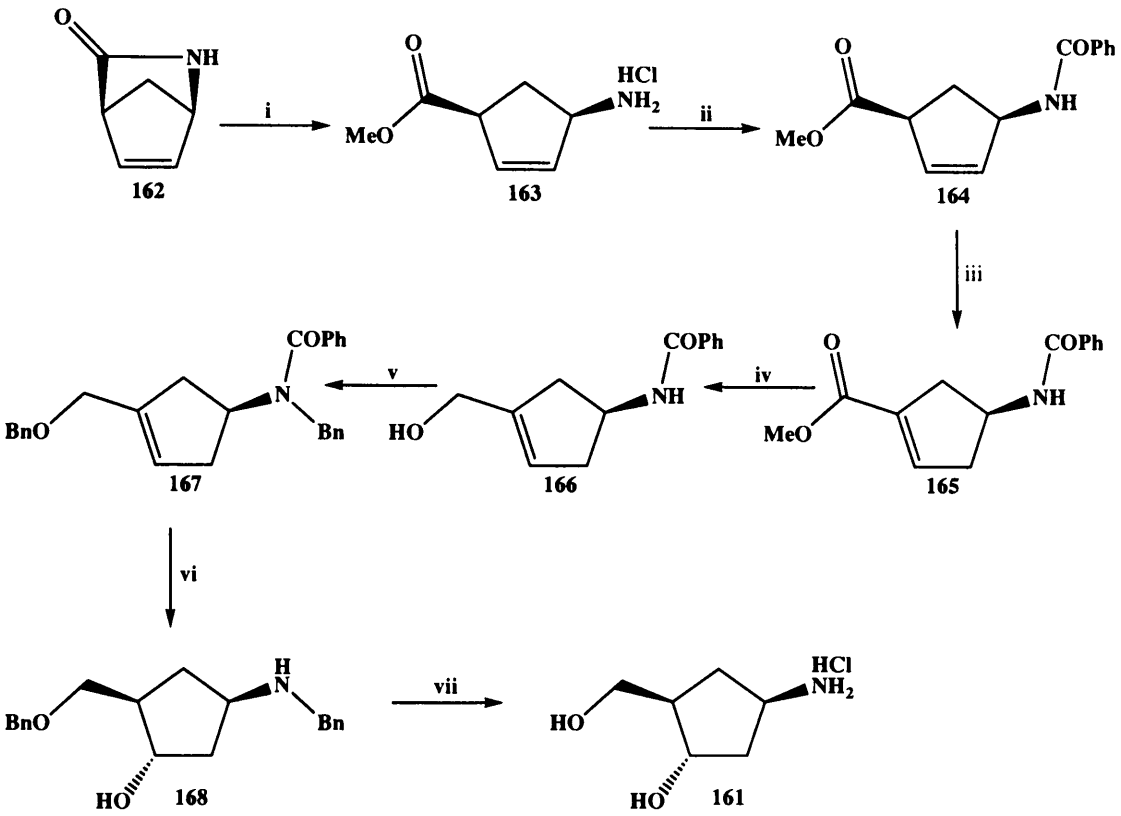


Scheme 7.1

Compound **160** can be synthesised reacting C-IDU **159** with pentylphenyl acetylene under Sonogashira conditions. C-IDU **159**, instead, was obtained starting from the amino-cyclopentane derivative **161** in two steps reaction. However, the most challenge part of the synthesis was the preparation of the amino-cyclopentane derivative **161**, which was obtained following two different routes.

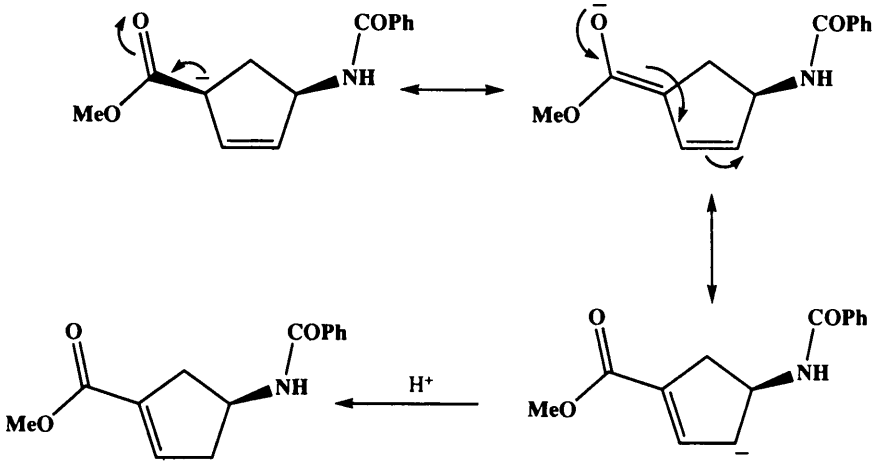
7.2 Synthesis of Carbosugar: Route I

As a first attempt for the synthesis of the carbosugar **161**, a modified version of the method described by Bray[11] was adopted. (scheme 7.2).



Scheme 7.2: *i) MeOH, $SOCl_2$. ii) BzCl, Pyr. iii) DBU, DCM. iv) $AlCl_3$, DIBAH, DCM, Toluene. v) BnBr, K_2CO_3, $Bu_4N^+HSO_4^-$, ^{t}ButylMethyl Ether. vi) BH_3•Dimethylsulfide, 2-Methyl-2-Butene, THF, NaOH, H_2O_2. vii) Pd/C, ^{i}PrOH, H_2O.*

Starting from the commercially available Vince lactam **162**, the amido bond was rapidly cleaved under strong acidic conditions converting substrate into methyl ester by methanolysis obtaining compound **163** in quantitative yield. The protection of the amino group with benzoyl chloride in pyridine was easily achieved, isolating in high yield compound **164**. The isomerisation of **164** into **165** was performed in the presence of a strong base such as DBU. The proposed mechanism for the shifting of the double bond is showed in scheme 7.3. DBU removes the proton in α to the carbonylic carbon, generating a carbanion, which is stabilised by resonance. The successive protonation occurs on the conjugated species forming the compound **165** in quantitative yield.

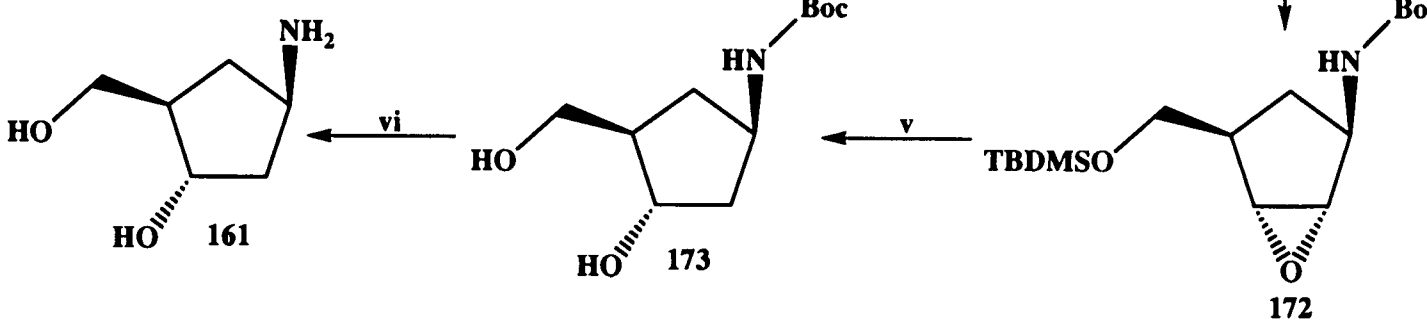


Scheme 7.3

Reduction of the ester **165** using a wide variety of hydride based reducing agents ($LiAlH_4$, Red-Al, Vitride) led to complex mixtures of products as a result of competitive 1,2 vs 1,4 reduction.[11] In addition, treatment of the substrate with DIBAH, although 1,2 selective, led to partial reduction of the amide.[11] The use of one eq. of $AlCl_3$ enhances significantly the chemoselectivity of DIBAH towards reduction of the ester.[11] Under these conditions, the target allylic alcohol **166** was isolated without any detectable over-reduction. The benzylation of compound **166** led to compound **167**, and enhanced the steric hindrance around the β face. The hydroboration was then achieved using the hindered disiamylborane providing the 3α-hydroxy derivative **168**. Under the same conditions hydroboration of **166** leads to a loss of selectivity.[11] Finally, palladium catalysed hydrogenolysis of the benzyl groups afforded the required amino diol **161** (41% overall yield).

7.3 Synthesis of Carbosugar: Route II

Even though the route I was found to be efficient, the work up of some reactions was laborious and complicated. A new route was then planned in order to simplify the synthesis of the carbosugar **161**. Vince lactam **162** was protected with Boc group in order to obtain **169** in quantitative yield.[12] The introduction of Boc group in the amido function brings two main advantages.



Scheme 7.4: *i) Boc anhydride, TEA, CH_2Cl_2. ii) MCPBA, DCM. iii) $NaBH_4$, MeOH. iv) TBDMS-Cl, Imia DCM. v) Red-Al, Toluene. vi) H_2O.*

First the successive epoxidation with 2 eq. of MCPBA gave exclusively the *exo* epoxide in excellent yield, according with Katagiri.[13] This selectivity is given by the bulky natu Boc, in fact without the Boc the reaction led to a mixture of *exo* and *endo* derivativ described by Dominguez (scheme 7.5).[14]

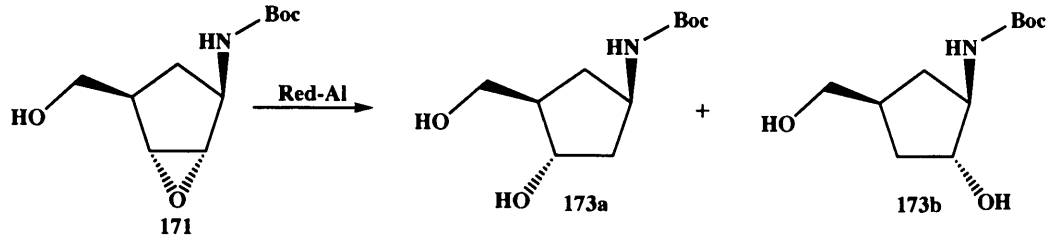
Scheme 7.5

Secondly, the amido function of the free lactam does not suffer reduction by so borohydride, but when a carbonyl group like the Boc is introduced, the reduction o without affecting the epoxide ring, converting the amido bond into amino-alcohol **171**. reaction is known as RAC reaction (Reductive Amido-bond Cleavage).[13]

Treatment of **171** with Red-Al gave a mixture of the two regioisomers **173a** and **173b** ratio of 3 :1 (scheme 7.6).[14]

In view of the fact that the regioselectivity of the ring-opening of epoxide **171** could be d in favour of compound **173a**, a bulky protecting group like TBDMS was introduce position 5. Reaction of **171** with ^{t}butyldimethylsilyl chloride followed by reduction with Al gave compound **173** as the sole product.[14] Although the silyl group is cleaved durin

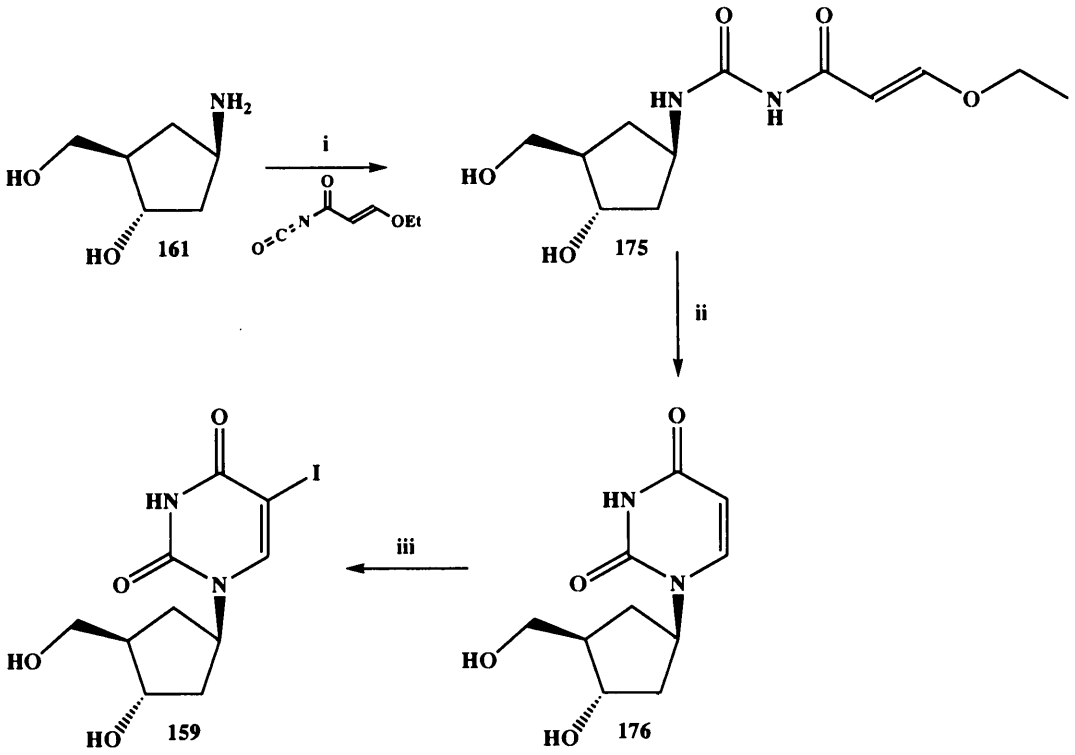
reduction, it is clearly in place at the stage of the ring-opening since total control of the regiochemistry is observed. Removing of Boc group in refluxing water afforded **161** in quantitative yield (64% overall yield).



Scheme 7.6

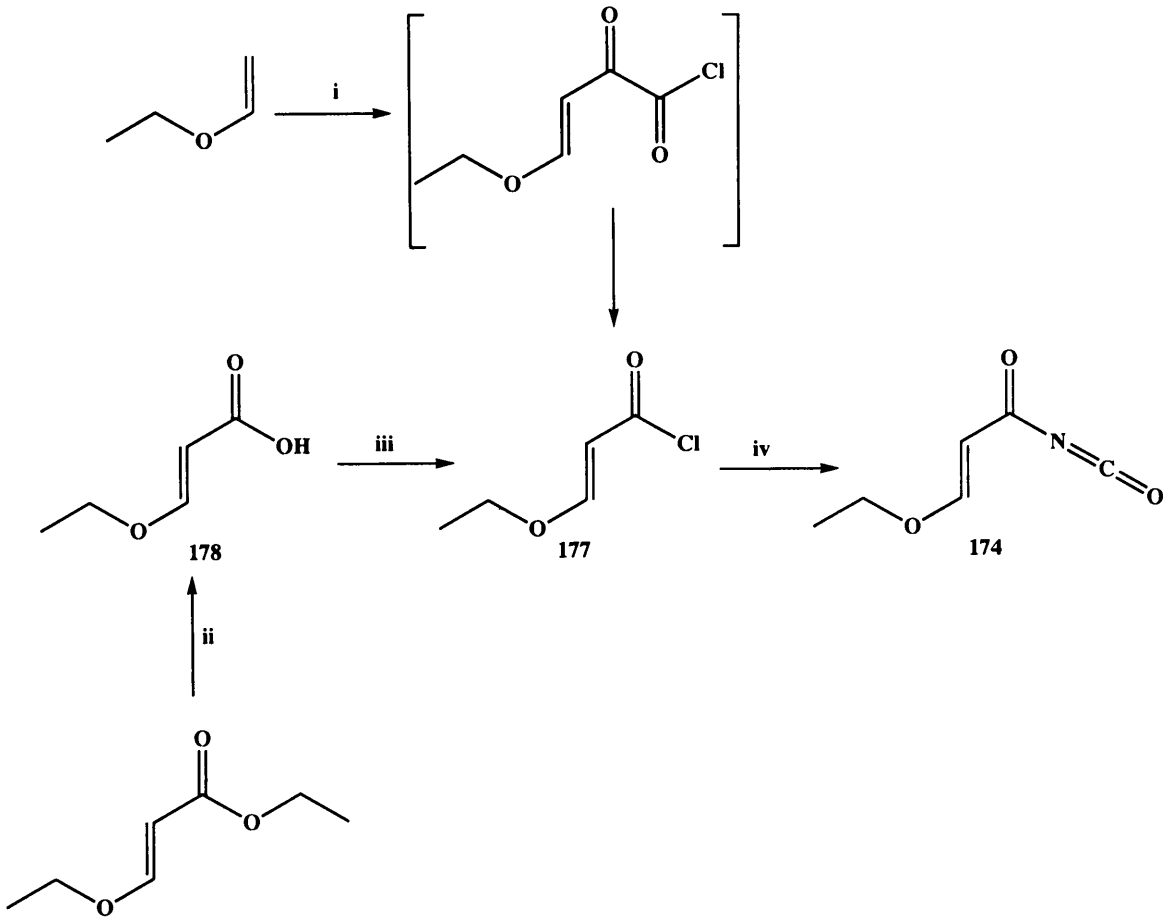
7.4 Synthesis of C-IDU

The synthesis of C-IDU **159** was achieved reacting the amino-diol **161** with ethoxyacrylisocyanate **174** (scheme 7.8) in the presence of DBU affording the urea derivative **175** in good yield.[15] Cyclisation of **175** in 2M H_2SO_4 at reflux was completed in good yield (55%).[16] Iodination was performed with molecular iodine in the presence of one eq. of CAN in acetic acid obtaining C-IDU **159** in good yield (53%).[17]



Scheme 7.7: *i) DBU, ethoxyacrylisocyanate 174, DMF. ii) H_2SO_4. iii) I_2, CAN, AcOH.*

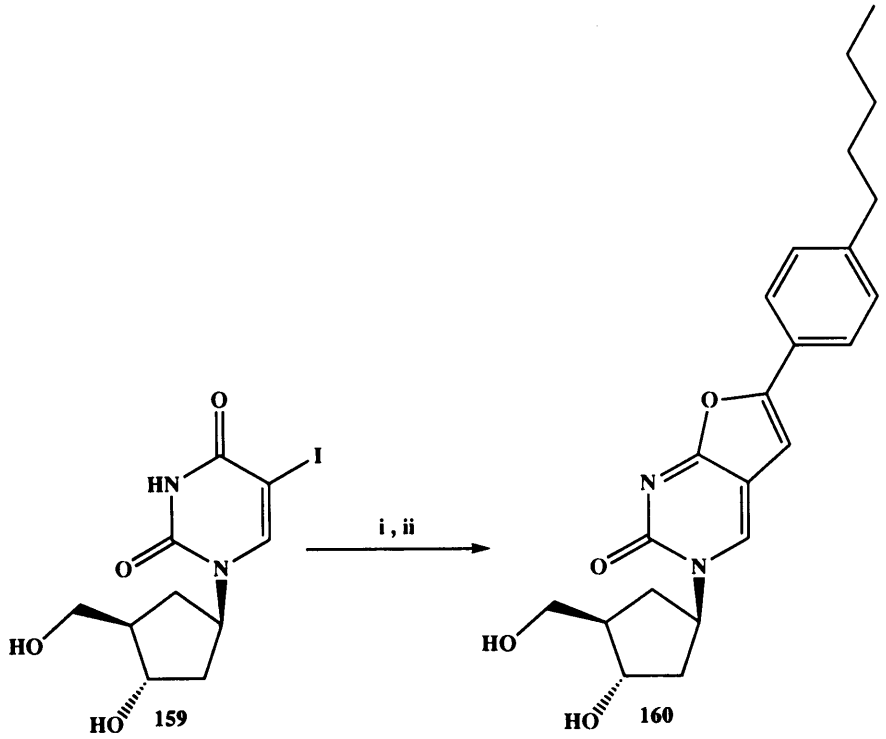
Compound **174** was synthesised according the scheme 7.8, adopting two possible routes.
In the first route ethyl-vinyl ether is reacted with oxalyl chloride to give an intermediate that is decarbonylated to compound **177** by heating at reflux. The acyl chloride thus obtained was purified by vacuum distillation.[16] Although only one reaction was involved for the preparation of **177** decomposition problems were encountered during the distillation and an alternative route was followed.
In the second route, starting from the commercially available ethyl-ethoxyacrylate, the substrate was hydrolysed in basic condition in aqueous NaOH, and the resulting acid **178** was then converted into **177** by thionyl chloride.[18] The final step was performed in the presence of silver isocyanate at reflux to obtain compound **176** needed for the synthesis of C-IDU.[16]



Scheme 7.8: *i) Oxalyl chloride. ii) H_2O, NaOH. iii) $SOCl_2$, DCM. iv) AgNCO, Benzene.*

7.5 Synthesis of C-BCNA

The synthesis of Carba-BCNA **160** was easily performed as described for the other BCNAs. In fact reacting C-IDU **159** with pentylphenyl acetylene under Sonogashira conditions followed by cyclisation gave in moderate yield (32%) the corresponding carbocyclic derivative **160** (scheme 7.9).



Scheme 7.9: *i) DMF, 4-pentylethynylbenzene, Tetrakis Pd(PPh$_3$)$_4$, CuI, DIPEA. ii) TEA, CuI.*

7.6 Biological results and NMR study

The C-BCNA **160** was evaluated for its ability to inhibit the replication of VZV in tissue culture. Table 7.2 contains data relating to two strains of thymidine kinase-competent (TK^+) VZV and also two strains of thymidine kinase-deficient (TK^-) VZV, with data also given for ACV and **33f** as reference compounds.

	EC_{50}					
	OKA	**YS**	**TK⁻07**	**TK⁻ YS**	**MCC**	**CC_{50}**
160	0.28	-	-	-	>100	>100
33f	0.0003	0.0001	>5	>5	>20	>200
ACV	2.9	1	74	125	>200	>200

Table 7.2

As it can be seen on table 7.2, the replacement of the furanose oxygen of the sugar with a methylene group led to a significant (c.a. 1000 fold) loss of activity, making the C-BCNA poorly active against VZV.

A possible explanation centres on a possible hydrogen bond in the active site that is eliminated on compound **160**, but this hypothesis is not very strong as the carbocyclic analogue of BVdU exhibits the same activity as the corresponding nucleoside.

It was thought then that the interaction between the enzyme and the carbocycle was hindered somehow by several factors, like the conformation of the sugar or the base.

In fact it was demonstrated that the conformation of the sugar ring plays a critical role in determining the affinity of kinases and polymerases for nucleosides.[19] An unrestricted furanose ring adopts a number of conformations that can be described by the value of *P* in a pseudorotation cycle (fig 7.2) that depends on the sugar torsion angles (ν0-ν4) of the furanose ring. By convention, a phase angle $P = 0°$ corresponds to a North (*N*) conformation possessing a symmetrical twist form $^3T_2$, while an angle of $P = 180°$ corresponds to the South (*S*) conformation [$_3T^2$]. Because nucleosides and nucleotides can crystallise in either *N* or *S* conformation and in solution there is a dynamic equilibrium, the sugar does not show any preference for either state and the energy barrier for interconversion is quite low.[19] When a nucleoside or nucleotide binds to an enzyme, it is expected an imposition of a specific conformation on the sugar ring (*N* or *S*) for optimal fit that should affect energy binding and/or catalytic activity. In fact in the specific case of HSV-TK, it was found that the enzyme

prefers nucleosides with the *S* conformation where the *N* nucleosides were found to be inactive or poorly active.[20] Given the structural similarity of the VZV-TK with HSV-TK, it was assumed that also VZV-TK prefers the *S* to the *N* conformation.

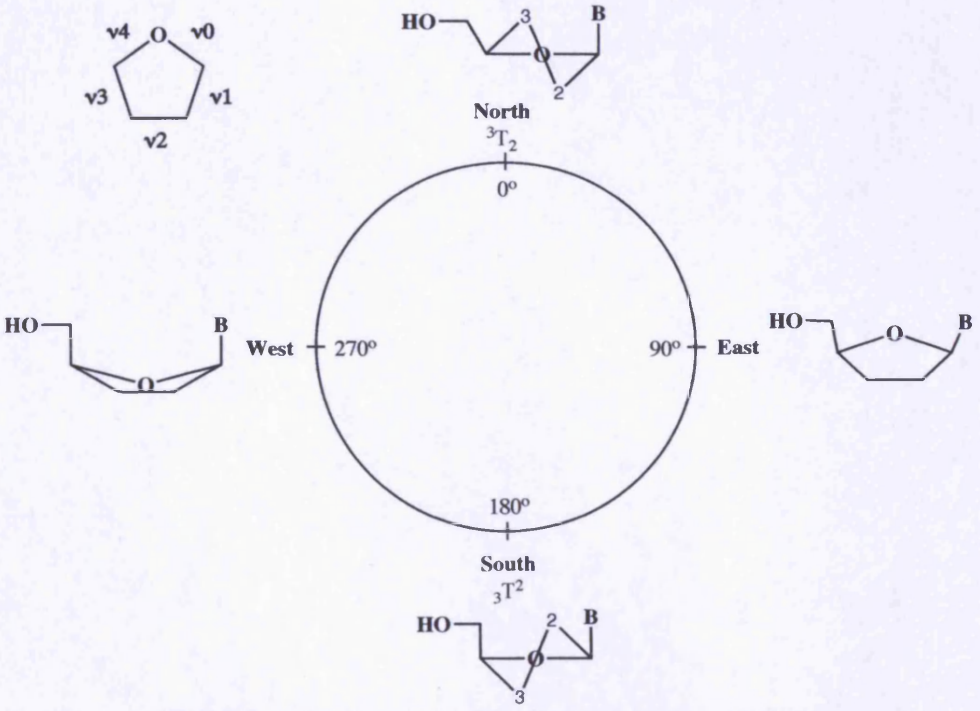


Fig 7.2: Pseudoration cycle of the furanose ring in nucleosides

Thus a conformational study of **33f** and **160** was carried out in order to investigate how the conformation of these two very similar analogues can affect the antiviral activity. After a conformational search based on a stochastic method a computational model was obtained. It was found that the two compounds adopt different conformations on the sugar: the parent compound **33f** adopts the *S* conformation (fig 7.3) whereas the carbocycle derivative **160** adopts the *N* conformation (fig 7.4).

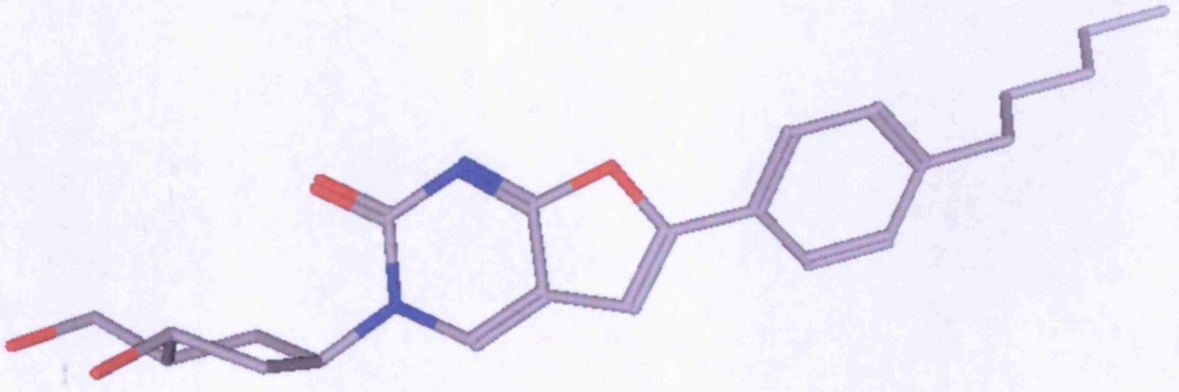


Fig 7.4: C-BCNA **160** in North conformation

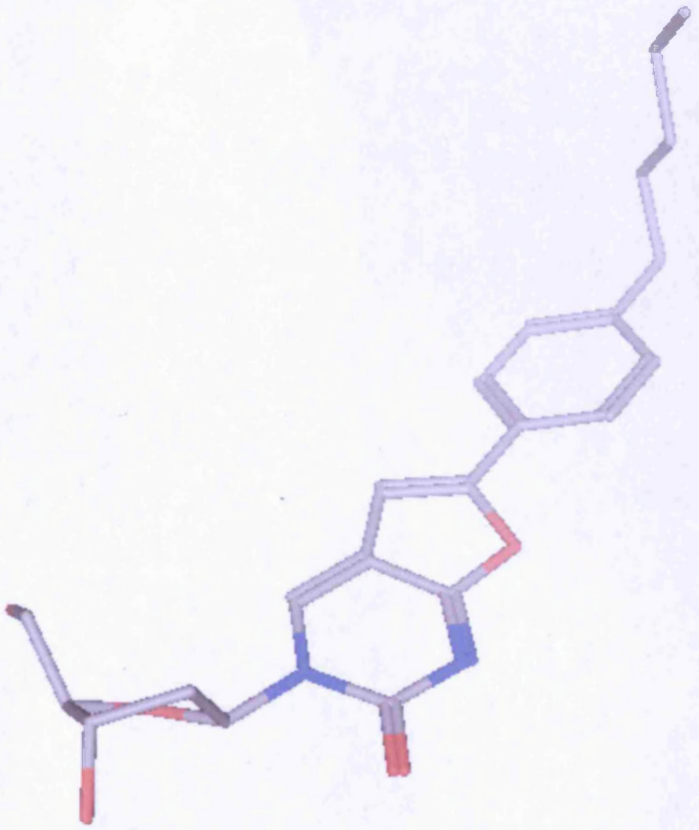


Fig 7.3: BCNA **33f** in South conformation

The dominant sugar pucker can be identified based on $J_{1'-2'\beta}$ and $J_{3'-4'}$ coupling constants.[21] The percentage of C3'-*endo* (*S*) versus C2'-endo (*N*) are calculated from the following equations:

$$\%\,S = \frac{J_{1',2'\beta}}{J_{1',2'\beta}+J_{3',4'}} \times 100 \qquad \%\,N = \frac{J_{3',4'}}{J_{1',2'\beta}+J_{3',4'}} \times 100$$

The NMR spectra of both compounds were analysed and the J values required were measured for some anti-herpetic nucleosides in order to obtain the wanted percentages (table 7.3).

	%N	%S	K_{eq} (*N/S*)	$J_{1',2'\beta}$	$J_{3',4'}$
33f	39	61	**0.64**	6.1	3.9
160	82	18	**8.22**	1.6	7.4
IDU	32	68	**0.47**	6.6	3.1
BVdU	33	67	**0.51**	6.5	3.3

Table 7.3: Percentage of N vs S conformer and equilibrium constant (N/S) at 25°C

It was also reported that these compounds do not undergo a significant temperature dependent conformational change,[22] in fact when heated at 37 °C no significant change on J values was observed on compounds **33f** and **160**. As it can be seen, the experimental data show that for BCNA **33f** the preferred conformation is South (K_{eq} <1) supporting the hypothesis that VZV-TK prefers this conformation and that C-BCNA **160** adopts the North conformation (K_{eq} >1) that is not tolerated by the VZV enzyme, giving a positive explanation why this compound exhibits lower activity than the parent analogue.

In order to further support these data, NOESY spectra were recorded determining also the *syn* / *anti* conformations of the base for both compounds.

The parent **33f** shows an *anti* conformation as the H-4 of the base presents strong interactions with the H-2'β. Moreover medium interactions were observed with the H-5' and OH-5', plus a medium-weak interaction with H-3'. From these data is clear that the H-4 faces above the sugar and that the strong interaction with the H-2'β and the weak interaction with the H-3' indicates a predominant south conformation. Furthermore, the H-1' shows a medium interaction with OH-3' that supports the south conformation plus a very weak interaction with H-4 implying the *anti* conformation.

Regarding the carbocycle **160**, the strong interaction of H-4 with H-1' proves the *syn* conformation as no other relevant interactions with the rest of the sugar were detected, implying that the H-4 is faraway from the β-face of the sugar. The H-1' instead shows strong interactions with H-2' and H-6' as expected, plus other two: one strong with the H-4' and one weak with the H-3'. The H-2'β does not show any interactions with H-4 confirming the *syn* conformation. The lack of interactions between H-1' and OH-3' means that these protons are very distant and this situation can occur only when the sugar is in north conformation.

Concluding, it was though that a minor change like the replacement of the furanose oxygen of the sugar with a methylene would not affected the shape of the molecule and hence the biological activity. Instead it was found that this substitution deeply affects of the molecule inverting the preferred conformation of the sugar from south to north. Moreover, this switch changed also the conformation of the base from *anti* to *syn* conformation making of this compound a poor substrate for the VZV-TK.

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than 1,000,000. These encouraging biological properties led to an extensive pre-cli programme, where several problems were encountered. The major issue encountered represented by very low absorption and substantial enzymatic cleavage resulting bioavailability value less than 15%. The same problem of poor availability emerged with ACV[1] that was solved synthesising amino acid prodrugs. In particular valacyc **12** increased the bioavailability of ACV from 20% to 55%.[1] Different prodrugs o were synthesised in order to improve the pharmacokinetic properties (Fig 8.1).

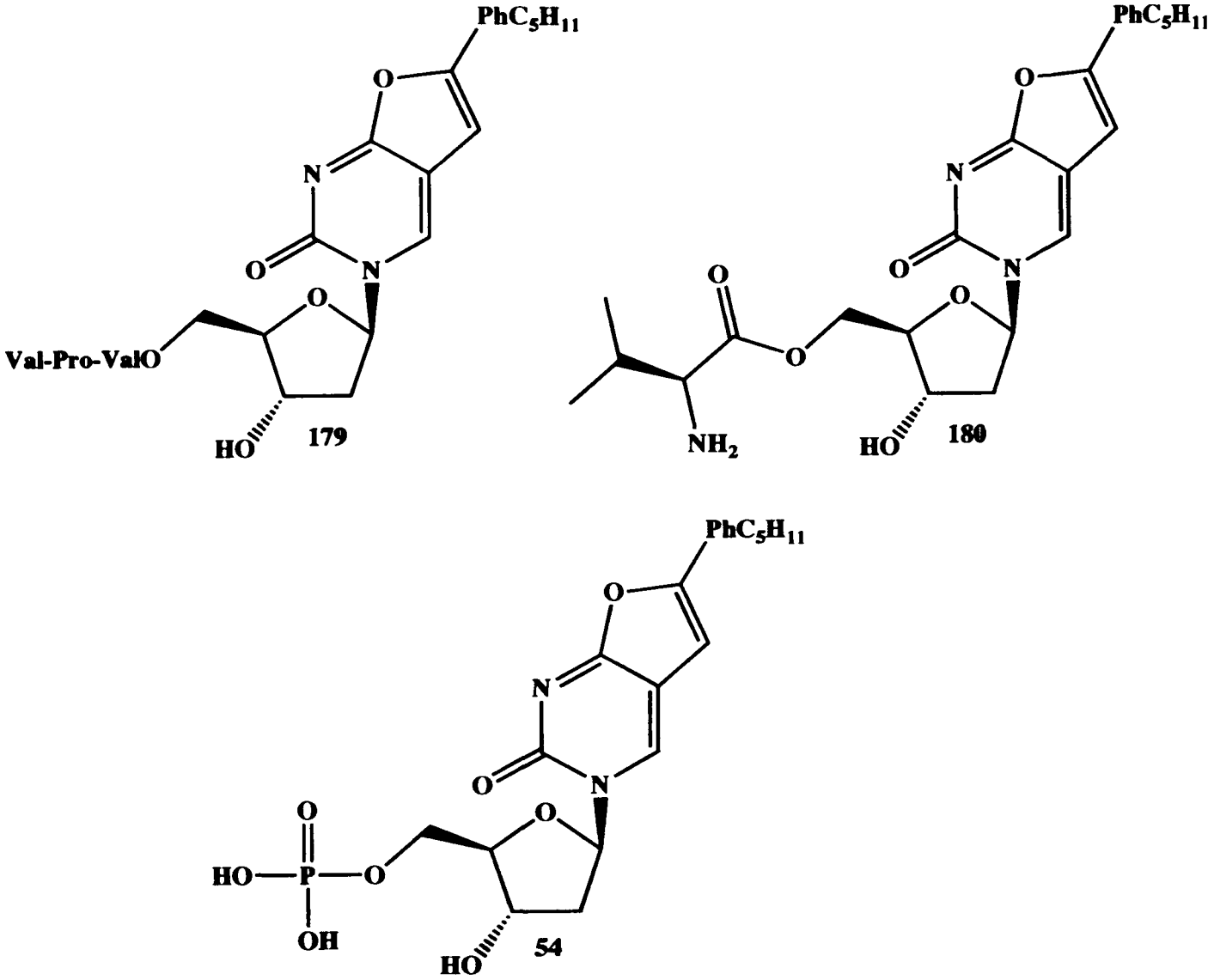


Fig 8.1: Prodrugs of BCNA

Three kinds of prodrugs of the BCNA **33f** were developed in collaboration with external labs: tripeptide (Val-Pro-Val) ester **179**, valinyl ester **180** and monophosphate **54** (fig. 8.1). After a preliminary study it was found that the antiviral activity of these compounds did not show any variation from the parent **33f**. Regarding bioavailability, it was found that the tripeptide was the best compound, being 20 times better than the parent. The valinyl ester instead exhibited a lower bioavailability than tripeptide, but still 10 times higher than **33f**. The phosphate **54** did not show any significant improvement in pharmacokinetic properties, and the problematic synthesis was an additional reason for discarding it. The tripeptide **179**, although it shows an excellent bioavailability is expensive to synthesise, thus the Valinyl ester **180** emerged as excellent candidate also because it represents a good compromise between cost, synthesis and biological properties. The bioavailability results are summarised on table 8.1 and fig 8.2.

Time	33f	MP* (54)	Val (180)	Val-Pro-Val (180)
15'	0.43	1.72	2.61	8.25
30'	0.62	2.1	1.93	11.9
45'	0.85	3.4	7.5	17.3
60'	0.68	2.57	4.46	10.3
120'	1.25	2.22	3.19	2.38
180'	0.79	1.1	1.42	0.86

Table 8.1: Concentrations vs time, *estimated values

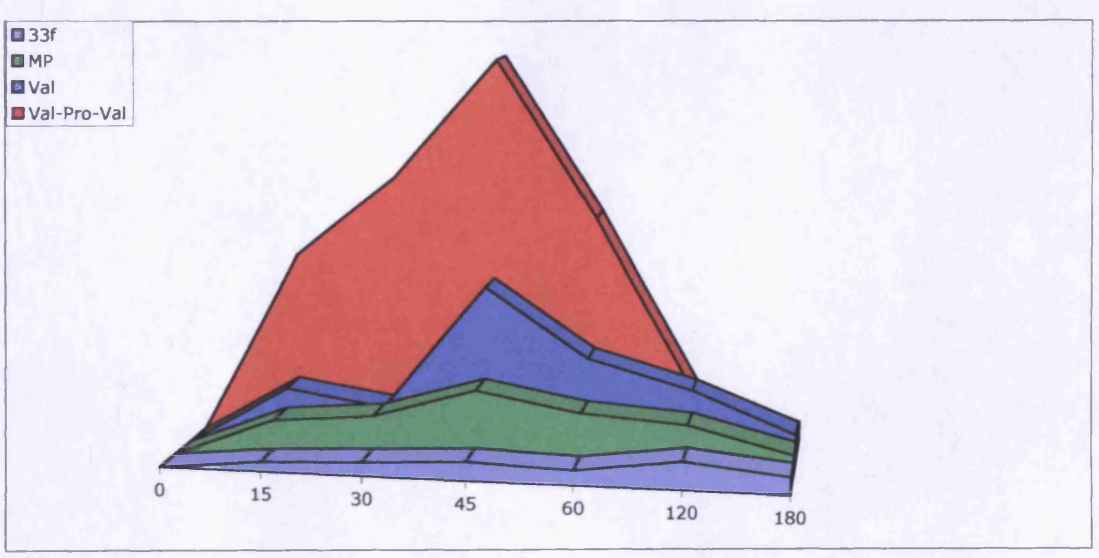
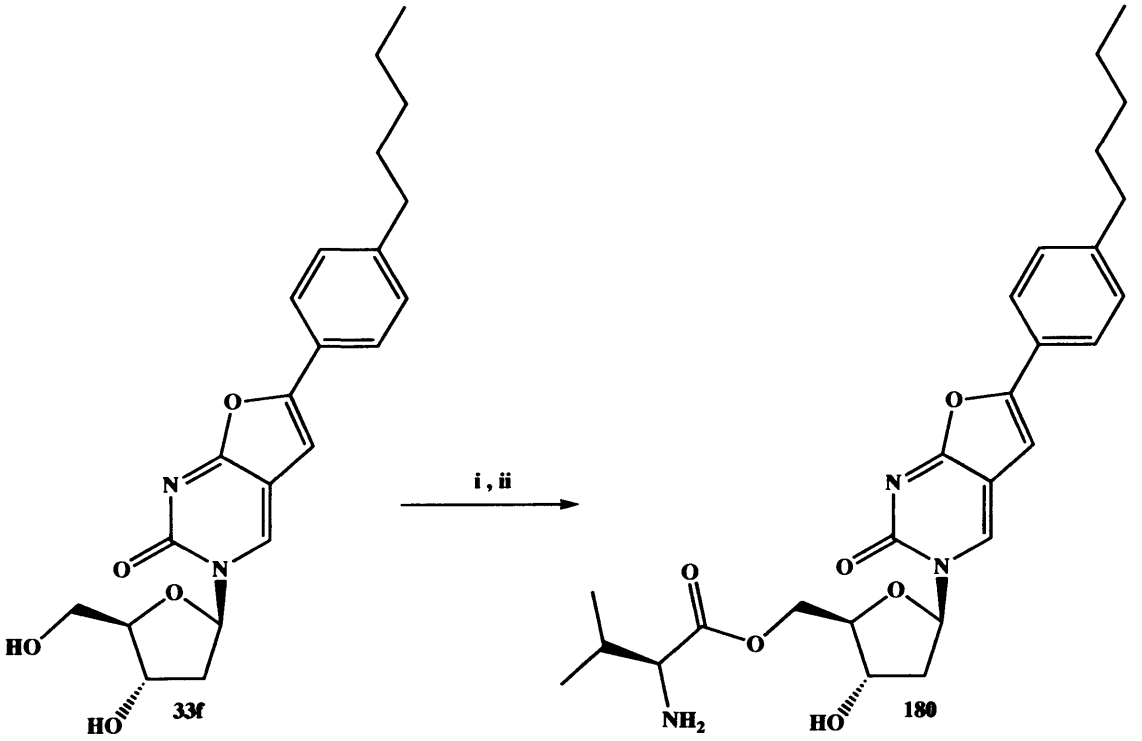


Fig 8.2: AUC diagram

8.2 Synthesis of Val-BCNA

The preparation of compound **180** was performed condensing the parent nucleoside **33f** with Fmoc-L-Valine under Mitsunobu conditions (scheme 8.1). In these conditions the reaction was shown to be highly selective towards the 5'-OH as it is a primary alcohol, and the formation of the 3'-derivative and the 3',5'-diester were observed on TLC but not isolated. Once coupled with the amino acid, the Fmoc group was readily removed by using two eq. of piperidine. The major problem encountered in this second step was the cleavage of the ester bond resulting in a difficult purification during the chromatographic process due to the similarilty of the r.f. of the produg **180** and parent drug **33b**. It was noticed that it is possible to isolate the Fmoc-intermediate after the first step and then remove the protecting group, but it was found more convenient to carry on the two steps in one pot, as only one chromatographic column was performed instead of two.



Scheme 8.1: *i) DMF, DBAB, TPP-PS,Fmoc-L-valine. ii) Piperidine.*

In order to increase the water solubility of this prodrug, the hydrochloric and succinate salts were synthesised reacting **180** with one eq. of the appropriate acid. Compound **180** was found to be 20 times more soluble than parent nucleoside. Conversion of **180** into hydrochloric salt increased the solubility of 25 fold, becoming 500 times more soluble

than parent nucleoside **33f**. Instead the succinate salt resulted in a significant boost in solubility, 1500 times more soluble than **180** and 30,000 times than **33f** (table 8.3). Furthermore it was found that formation of salts increased significantly the stability of this compound under "stressing" conditions in particular the hydrochloric salt resulted the most stable compound along the valyl series. The HCl salt, called FV100, has been selected for clinical evaluation.

	Solubility mg/ml	Enhancement
33f	0.001	-
Val-BCNA (8.2)	0.020	20
Val-BCNA•HCl	0.490	490
Val-BCNA•Succ	30	30,000

Table 8.3

8.3 Cell Study

When irradiated in a range of wavelengths of 340-380 nm, **33b** or **180** show an intense fluorescence clearly visible (fig 8.3). This phenomenon allowed us to start a biological study aiming to track the compound, in particular the distribution, inside the cell. Normally cells do not absorb in this range and thus they are virtually invisible.

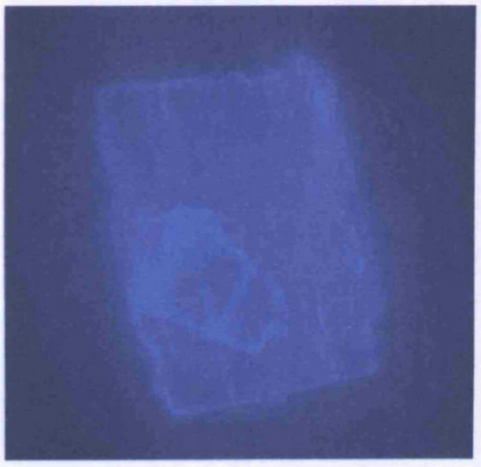


Fig 8.3: An irradiated crystal of **33b**

When HeLa cells (cervical carcinoma epithelial cells) are treated with an 8μM solution of **33b** for 1 hour, it can be seen that the compound is uniformly distributed in the cytoplasm, in particular it was observed that the derivative is localised in a structural component of the cell like the cytoskeleton. It was also noticed that the compound did not diffuse inside the nucleus (fig 8.4).

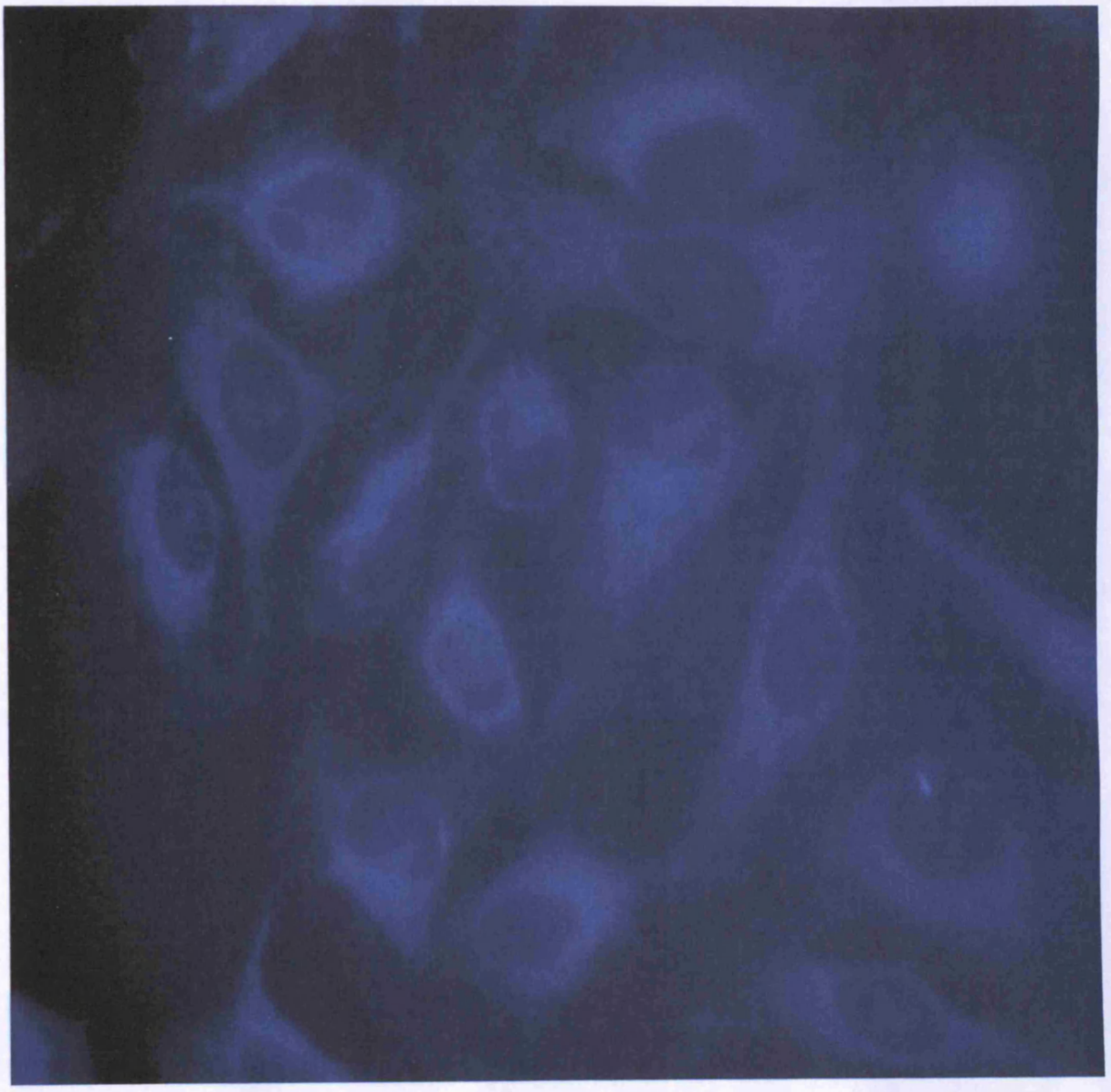


Fig 8.4: cells treated with **33b** after 1 hour (HeLa)

Treating the cells with **180** for 1 hour, it was observed a different distribution as fig 8.5 clearly shows. The compound is trapped inside small vesicles scattered around the cell, which are probable lysosomes. This effect is due to the presence of the amino group in the compound that is protonated in the acidic environment of lysosome and therefore it cannot cross the membrane of the vesicle for passive diffusion getting trapped. Again the presence of the compound inside the nucleus was not detected.

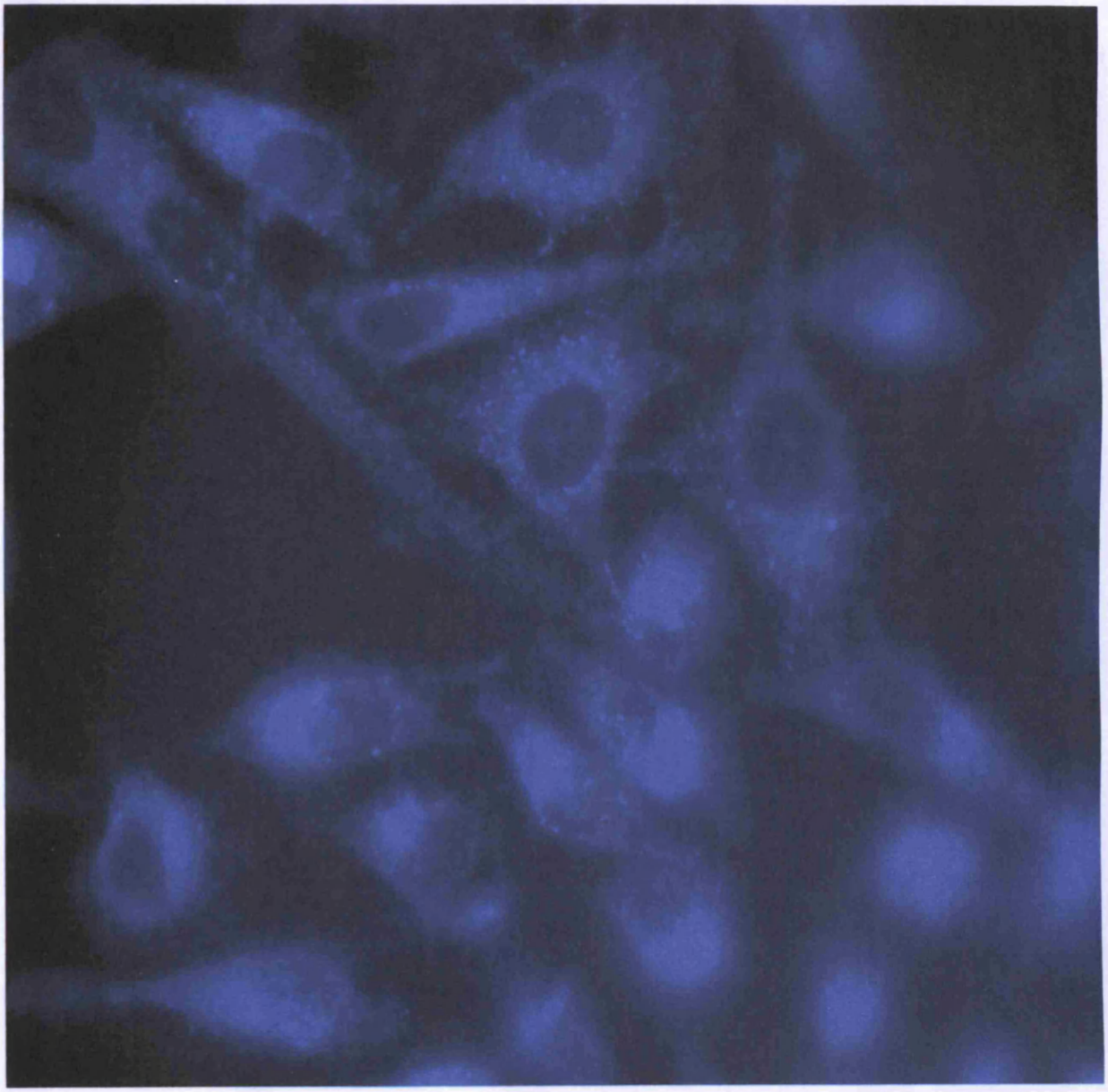


Fig 8.5: cells treated with **180** after 1 hour (HeLa)

In order to understand the mechanism of formation of these vesicles, more experiments were performed in different conditions.

Administering **180** to HeLa cells and observing after 2 minutes, it can be seen that the vesicles are not formed yet and that **180** is uniformly distributed in the cytoplasma like the parent drug **33b** (fig 8.6). This fact implies that the formation of the vesicles is not an immediate process but it is time dependent.

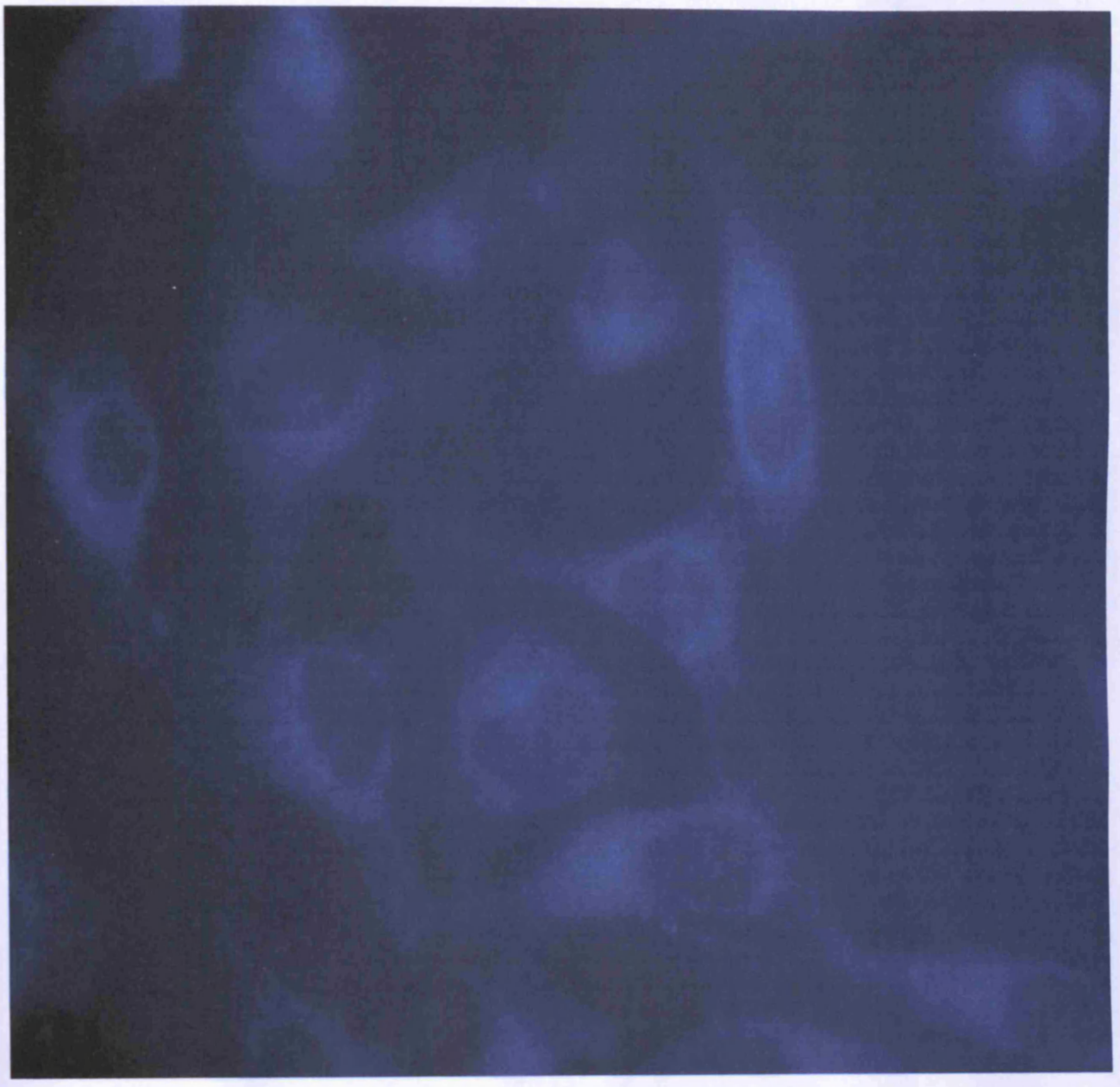


Fig 8.6: cells treated with **180** after 2 minutes (HeLa)

Carrying out the experiment at 0 °C for 1 hour, it was found that formation of the vesicles is significantly slowed. At this temperature the surface of the membrane is not fluid as at 37 °C, but it is rather dense preventing the compound to cross efficiently the lipid bilayer. From this result it can hypothesised that the transport of **180** inside the vesicles is a passive phenomenon and not active. Further studies are still ongoing in order to clarify the global process.

Fig 8.7: cells treated with **180** after 1 hour at 0 °C (HeLa)

In order to prove that the formation of vesicles is not dependent on the type of cells used, another cell line completely different from HeLa was used. The experiment was carried out exactly as for the HeLa cells using KG1a cell line (acute myeloid leukaemia cells).
As fig 8.8 shows, the KG1a cells present fluorescent vesicles, confirming that the cell line used does not affect the kind of distribution in the cytoplasma of **180**.

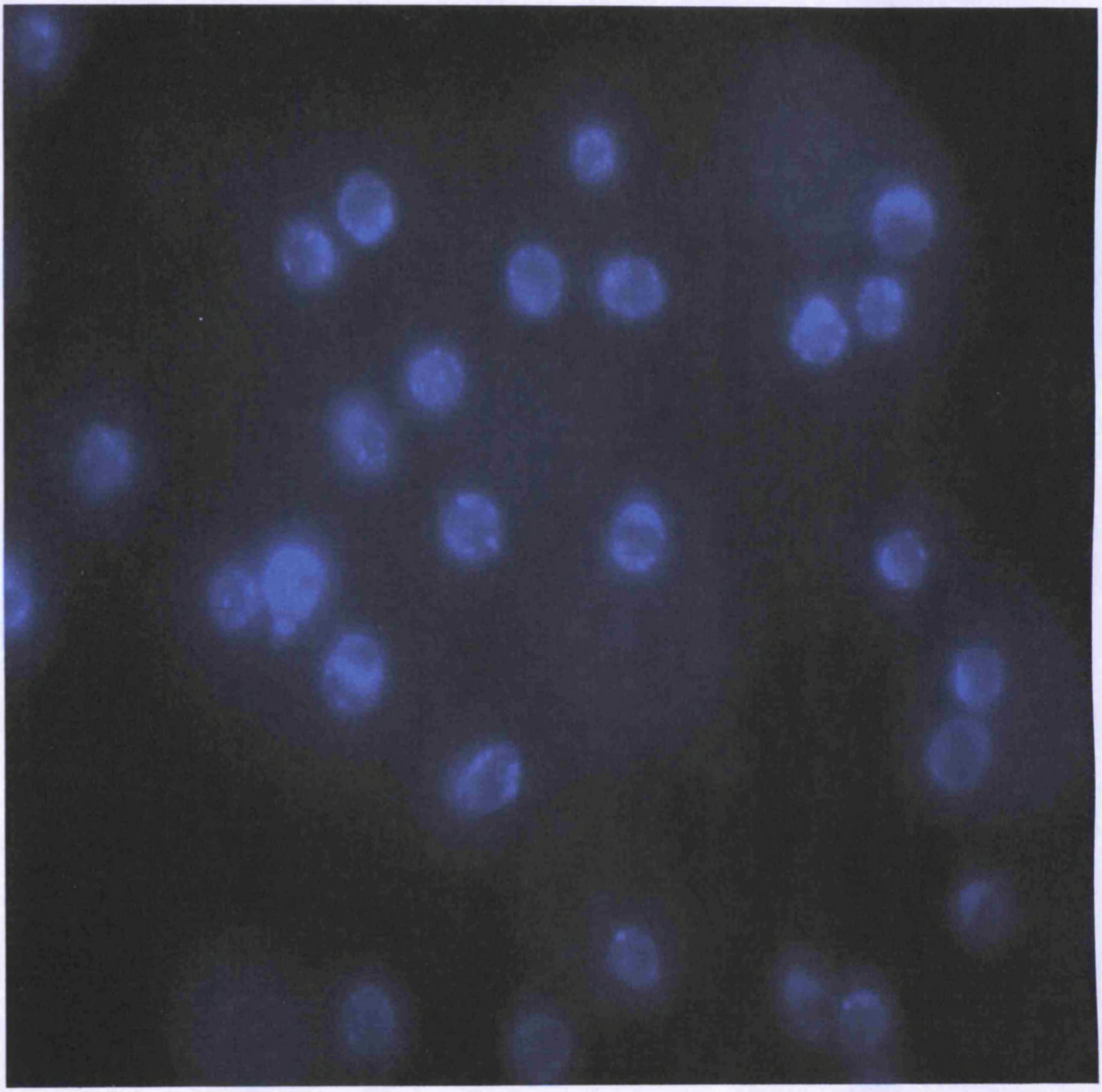
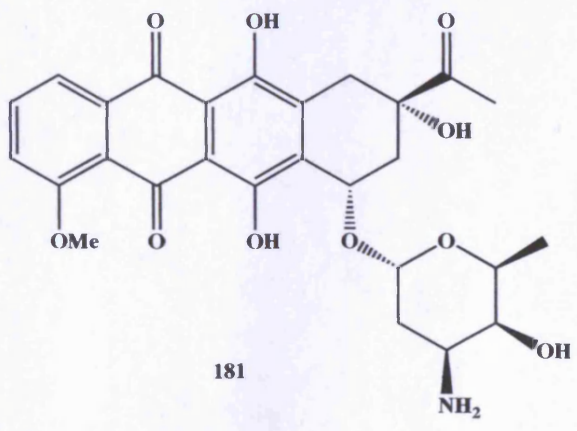


Fig 8.8: cells treated with **180** after 1 hour (KG1a)

The same kind of vesicles can be observed on the cells where the anticancer drug daunorubicine **181** was administered. As for compound **180**, daunorubicine is trapped inside the lysosomes and it cannot cross the membrane due to the positive charge on the amino group generated by the low pH of this organelle (fig 8.10).



181

Fig 8.9: Daunorubicine

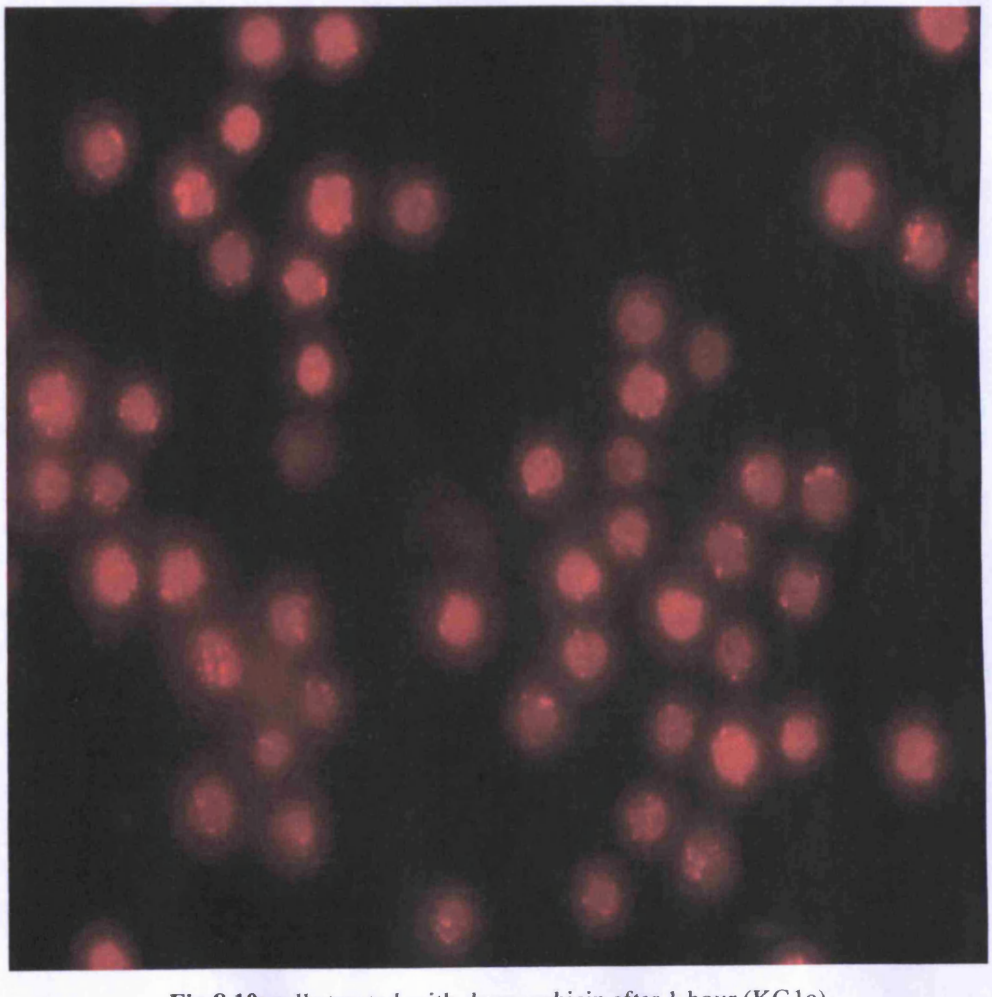
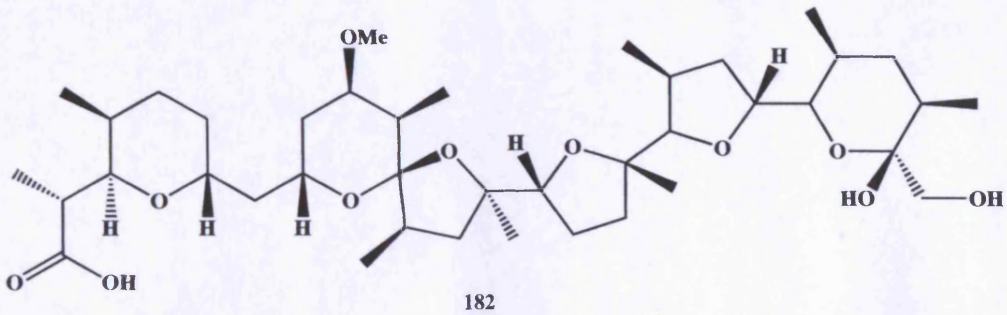


Fig 8.10: cells treated with daunorubicin after 1 hour (KG1a)

Administering the antibiotic nigericin **182** to KG1a cells, the proton pump of the lysosomes is inhibited and thus the pH gradient between the vesicle and cytoplasma is nullified. In these conditions daunorubicin cannot be protonated and then trapped inside the lysosomes diffusing around the cytoplasma (fig 8.12). This experiment was used as a positive control.



182

Fig 8.11: Nigericin

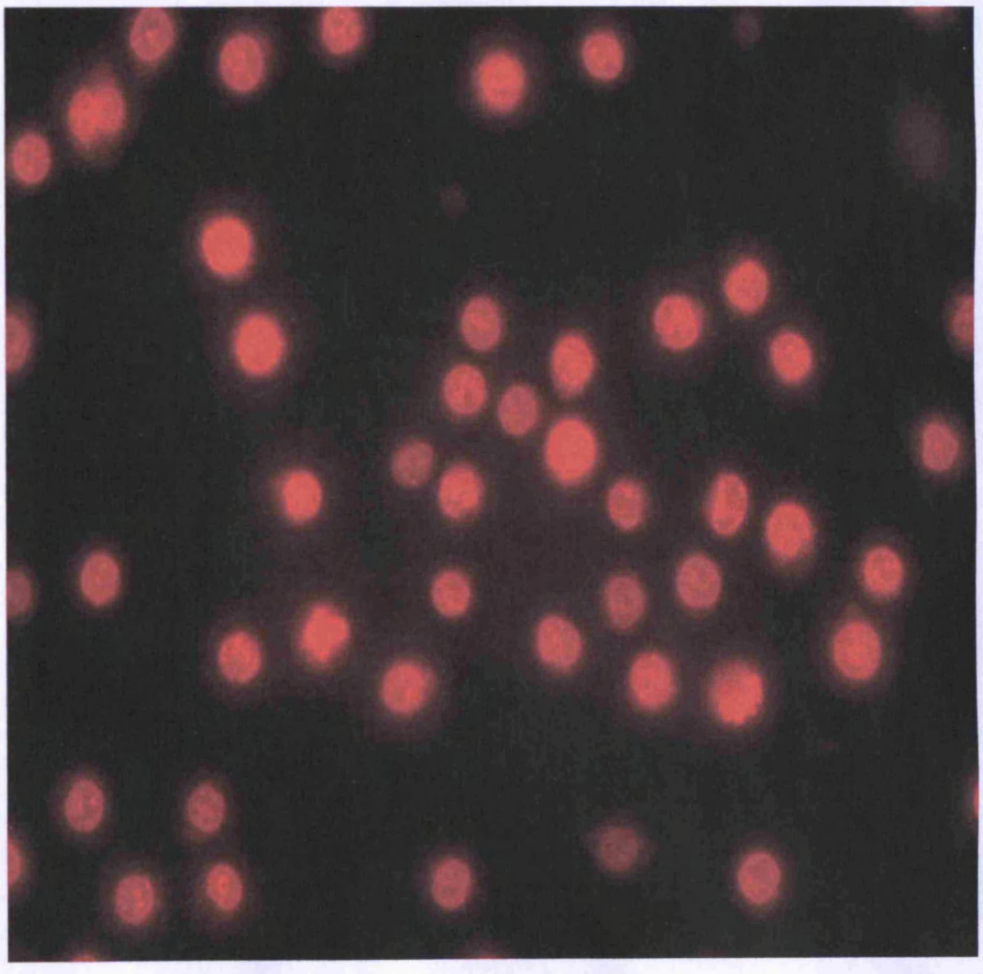


Fig 8.12: Daunorubicine + Nigericine

Carrying out the same experiment, KG1a cells were treated first with nigericin and then with **180**. It was observed that no vesicles were formed, and that the compound was well distributed in the cytosplasma, exactly like daunorubicine (Fig 8.13). To notice that also in this cell line compound **180** has never been detected inside the nucleus.

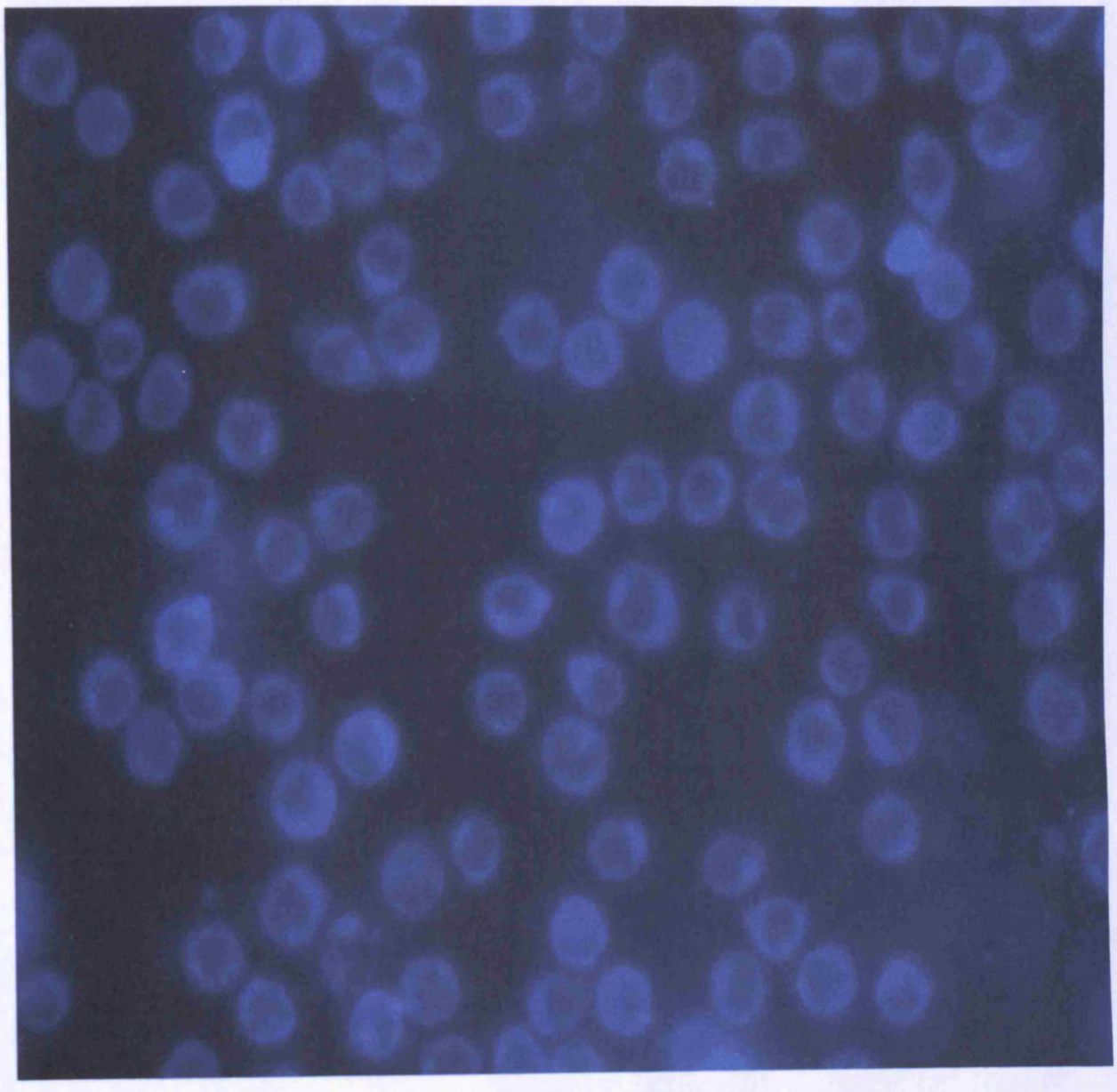


Fig 8.13: **180** + Nigericin (K1Ga)

References

[1] Soul Lawton J., Seaber E., On N., Wootton R., Rolan P., Posner J.; Absolute biovalability and metabolic disposition of valacyclovir, the L-valyl ester of acyclovir, following oral administration to humans, *Antimicrobial Agents and Chemotherapy*, **1995**, 39, 2759-2764.

Conclusions

This thesis is a report of a further development of BCNAs as inhibitors of VZV.

Given the high potency of the lead compound and the possibility that this compound could go into clinical trial, it was needed to have a substantial amount of nucleoside for pharmacological purposes. Therefore the chemistry of BCNAs was further improved, reducing time and removing any column chromatography from the synthesis. This new process was successfully used by external labs up to Kg scale.

In order to enhance the bioavailability of the lead compound a few strategies were used. The first one was the introduction of an ether side chain, with full retention of the antiviral activity but with no significant increase of water solubility. Then three prodrugs were synthesised, that include the monophosphate, the valinyl ester and a tripeptide ester. All of three were found to have a bioavailability better than lead with the order monophosphate, valinyl ester and finally tripeptide ester. The valinyl ester in particular represented a compromise between easy of synthesis access and biological properties making of this compound and its hydrochloric salt (FV-100) a good candidate for the clinical trial.

Then a series of mono-substituted phenyl derivatives was synthesised bearing electron-withdrawing and electron-donating groups in position ortho, meta and para. From the introduction of such groups it emerged that electron-withdrawing groups and meta substitution are damaging for biological activity while electron-donating groups group improve activity.

Given the general lack of activity of BCNAs against other viruses, the phosphoramidate protide approach was applied to this class of compounds resulting in loss of activity vs the lead. Moreover these derivatives were found not to be active against other viruses suggesting that VZV is the main target of BCNAs.

The L-enantiomer and the α-anomer of BCNA were synthesised in order to broaden the spectrum of activity, but these two derivatives were found to be poorly active against VZV, therefore the phosphoramidate technology was applied to them. Biological results for the other viruses are still awaited.

The replacement of the oxygen of the sugar ring with a methylene group led to the C-BCNA which was found to be chemically more stable then lead. This kind of substitution deeply affected

the biological activity in negative way. The explanation why this compound shows an activity 1000 fold lesser than parent may be that the main conformation of the sugar of C-BCNA is North while the lead compound shows a predominant South conformation. Furthermore the conformation of the base of C-BCNA is syn while for BCNA is anti, making of this compound a poor substrate for VZV-TK as inhibition data suggest.

Finally a cell study was carried out in order to investigate of the distribution of the lead C1743 and it prodrug FV-100 inside the cell. CF1743 was rapidaly uptaken and homogeneously distributed around the cytosol, while FV-100 was found to be segregated into vesicles, probably lysosomes. Interesting to notice that no compound was observed inside the nucleus, suggesting that the mechanism of action of BCNAs is more complex than it appears. Further studies on this field are still on going.

Chapter 9

General experimental details

Solvents and reagents

The following anhydrous solvents were bought from Aldrich® with sureseal stopper: Chloroform ($CHCl_3$), dichloromethane (DCM), diethyl ether (Et_2O), 1,4-dioxane, N,N-dimethylformamide (DMF), N-methylimidazole (NMI), pyridine (Pyr), tetrahydrofuran (THF), triethylamine (TEA). All reagents commercially available were used without further purification.

Thin Layer Chromatography (TLC)

Precoated, aluminium backed plates (60 F_{254}, 0.2 mm thickness, Merck) were visualised under both short (254 nm) and long wave (365 nm) ultraviolet light.
Preparitive TLC plates (20x20 cm, 500-2000 μm) were purchased from Merck.

Column Chromatography (CC)

Column chromatography processes were carried out using silica gel supplied by Fluka (silica gel 60, 35-70 μm, 220-440 mesh). Glass columns were slurry packed using the appropriate eluent and samples were applied either as a concentrated solution in the same eluent or pre-adsorbed on silica gel.

High Performance Liquid Chromatography (HPLC)

Analytical procedures were run on a Varian ProStar instrument (LC work station, Varian Prostar 355 LC detector) using a Polaris C18-A 10 u column; elution was performed using a mobile phase consisting of water / acetonitrile in gradient (Water 100→0 in 20 minutes).

Nuclear Magnetic Resonance (NMR)

^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz), ^{31}P-NMR (202 MHz), ^{19}F-NMR (470 MHz) were recorder on a Bruker Avance 500 spectrometer at 25°C. Spectra were calibrated to the residual signal of the deuterated solvent used. ^{13}C-NMR, ^{31}P-NMR and ^{19}F-NMR were

proton-decoupled. Chemical shifts are given in parts per million (ppm) and coupling constants (J) in Hertz.

Abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), qn (quintet), sx (sextet), m (multiplet), bs (broad signal), dd (doublet of doublets), dt (doublet of triplets), ddd (doublet of doublets of doublets).

Mass Spectroscopy (MS)

Mass spectra were recorded on a microTOFLC Bruker Daltonics spectrometer and/or performed as a service by the University of Birmingham.

Elemental Analysis (CHN)

CHN microanalysis was performed as a service by the School of Pharmacy at the University of London.

Optical Rotation

The optical rotation was measured on a Bellingham+Stanley Ltd. ADP-220 polarimeter.

Molecular Modeling Studies

The computational studies were performed by using MOE 2006.08 Chemical Computing Group (Inc. Montreal, Quebec, Canada).

Standard Procedures and Synthesis

For convenience, standard procedures are given, since similar procedures were adopted for the synthesis of phosphorochloridates, phosphoramidates and BCNAs.

Standard Procedure A: synthesis of phosphorochloridates

Anhydrous TEA (2.8 ml, 2.0 eq.) was added dropwise at –78 °C to a stirred solution of phenyl dichlorophosphate (2.11 g, 1.0 eq.) and the appropriate amino acid ester salt (10 mmol) in 30 ml of anhydrous DCM under an argon atmosphere. Following the addition, the reaction mixture was allowed to slowly warm to room temperature. Formation of desired compound was monitored by ^{31}P-NMR. The solvent was removed under reduced pressure and the residue was resuspended in anhydrous Et_2O and filtered under nitrogen. The filtrate was

reduced to dryness to give the crude product which was purified by flash column chromatography (Hexane / Ethyl Acetate 1:1).

Standard Procedure B: synthesis of phosphoramidates

To a stirring solution of **33f** (0.2 g, 0.5 mmol) and the appropriate phosphorochloridate (4 eq.) in 5 ml of anhydrous Pyr at 0 °C was added dropwise over 1 minute NMI (0.4 ml, 10 eq.). After 5 minutes, the reaction was let to rise to room temperature and stirred for 2 hours. Water was added to quench the reaction. The solvent was removed under reduced pressure and the oil was dissolved in DCM, washed with HCl 0.5 M three times. The organic layer was dried over $MgSO_4$, filtered, reduced to dryness and purified by column chromatography ($CHCl_3$ / MeOH).

Standard Procedure C: synthesis of 3'-phosphoramidates

To a stirring solution of **127** (0.35 g, 0.5 mmol) and the appropriate phosphorochloridate (4 eq.) in 5 ml of anhydrous THF at 0 °C was added dropwise over 1 minute NMI (0.4 ml, 10 eq.). After 5 minutes, the reaction was allowed to rise to room temperature and stirred for 2 hours. Water was added to quench the reaction and DOWEX 50Wx8-100 was added and the mixture was stirred for 30 minutes. The resin was filtered and the solvent was removed under reduced pressure and the oil was dissolved in DCM, washed with HCl 0.5 M three times. The organic layer was dried over $MgSO_4$, filtered, reduced to dryness and purified by column chromatography ($CHCl_3$ / MeOH).

Standard Procedure D: synthesis of BCNAs

To a stirred solution of IDU **1** (1.06 g, 3 mmol) in dry DMF (15 m) at room temperature, the appropriate acetylene (3 eq.), *tetrakis*(triphenylphosphine)Pd^0 (0.35 g, 0.1 eq.), copper(I) iodide (0.12 g, 0.2 eq.) and DIPEA (1 ml, 2 eq.) were added. The reaction mixture was stirred for 15 hours at room temperature, after which time TEA (15 ml) and further copper(I) iodide (0.12g 0.2 eq.) were added. The reaction mixture was then heated at 80 °C and stirred for 4-6 hours. The solvent was removed under reduced pressure and the resulting residue was dissolved in DCM and left at 0 °C for 15 hours. The resulting precipitate was filtered and washed with DCM.

Cell Cultures

HeLa cells. (cervical carcinoma epithelial cells, adherent cells) were cultured under standard conditions in 35mm MatTek live.

HeLa cells were maintained at 37 °C and 5% CO_2 in a humidified incubator in D-MEM/1000g/ml glucose supplemented with 10% Foetal calf serum, 100 IU/mL penicillin and 100 μg/mL streptomycin. All cell culture reagents were from Invitrogen. For microscopy experiments, $1.2x10^5$ cells were plated on to 35mm glass bottomed culture dishes (MatTek Corporation, Ashland, USA) and incubated under tissue culture conditions for 16-20 hours. The cells were then washed 2x with D-MEM phenol red free medium and incubated for 1 hour (2 minutes) at 37 °C (or 0 °C) with 8μM of **180**. The media was removed and the cells washed 2x in clear media prior to adding 1ml of clear medium containing 20mM HEPES buffer pH 7.4. The fluorescence of the compound in the living cells was then immediately analysed on a Leica DMIRB inverted fluorescent microscope equipped with a 340-380nm band pass filter and a 40x oil objective.

KG1a cells. (acute myeloid leukaemia cells, non-adherent)

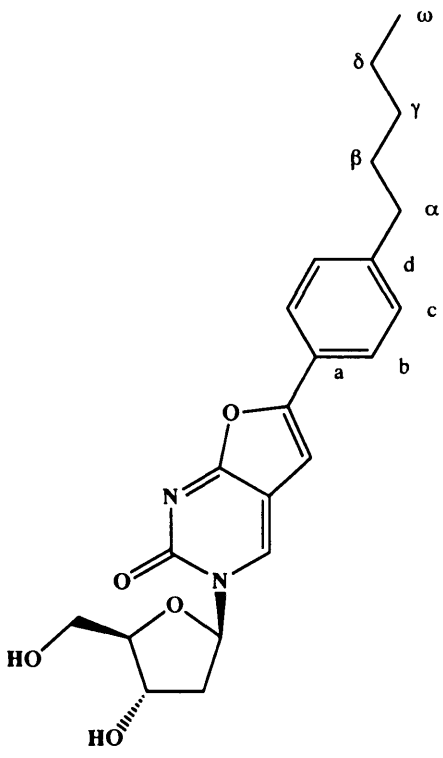
Cells were maintained at 37 °C in a humidified 5% CO_2 incubator. KG1a cells were maintained at a confluency of $0.5\text{-}1x10^6$ in RPMI 1640 media supplemented with 10% foetal calf serum (FCS), 100 IU/ml penicillin and 100μg/ml streptomycin. $5x10^5$ cells were centrifuged at incubated at 1500 x g and resuspended in 5-00μl media containing x compound. The cells were then centrifuged for 1 min at 4°C and resuspended in 1ml clear media (see above). This procedure was repeated 2x and finally the cell pellet was resuspended in 25μl clear media. 2μl aliquots of this was loaded on to Hendley slides and imaged as described above on the fluorescent microscope.

Nigericin

For experiments using nigericin the cells were pretreated with 20μM nigericin for 30 min prior to the addition of the compound (Daunorubicine, **180**). The cells were then processed for fluorescence microscopy as described.

33f 3-(2-deoxy-β-D-ribofuranosyl)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf1743]



Prepared according to Standard Procedure D using 4-ethynylpentylbenzene.

White solid: 1.02 g, 85%

^{1}H-NMR (DMSO, 500 MHz) : δ 8.84 (1H, s, H-4), 7.75 (2H, d, J 7.6 Hz, Ph_b), 7.33 (2H, d, J 7.6 Hz, Ph_c), 7.22 (1H, s, H-5), 6.20 (1H, t, J 5.7 Hz, H-1'), 5.28 (1H, d, J 4.2 Hz, OH-3'), 5.15 (1H, t, J 5.1 Hz, OH-5'), 4.27 (1H, dq, J 6.1 Hz 4.2 Hz, H-3'), 3.94 (1H, q, J 3.8 Hz, H-4'), 3.72 (1H, ddd, J 12.1 Hz 5.1 Hz 3.6 Hz, H-5'a), 3.65 (1H, ddd, J 12.1 Hz 5.1 Hz 4.2 Hz, H-5'b), 2.61 (2H, t, J 7.5 Hz, $CH_2\alpha$), 2.42 (1H, ddd, J 13.5 Hz 6.3 Hz 4.1 Hz, H-2'$_\alpha$), 2.11 (1H, dt, J 13.5 Hz 6.1 Hz, H-2'$_\beta$), 1.59 (2H, qn, J 7.5 Hz, $CH_2\beta$), 1.35-1.24 (4H, m, $CH_2\delta$ + $CH_2\gamma$), 0.86 (3H, t, J 7.1 Hz, $CH_3\omega$)

^{13}C-NMR (DMSO, 125 MHz): δ 171.02 (C7a), 153.90 (C6), 153.76 (C2), 144.02 (Ph-C_d), 137.77 (C4), 128.93 (Ph-C_c), 125.86 (Ph-C_a), 124.50 (Ph-C_b), 106.88 (C4a), 98.62 (C5), 88.16 (C4'), 87.57 (C1'), 69.55 (C3'), 60.68 (C5'), 42.26 (C2'), 34.89 (Cα), 30.80 (Cγ), 30.33 (Cβ), 21.89 (Cδ), 13.84 (Cω)

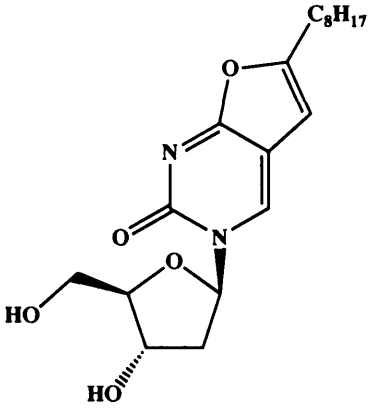
Anal. Calcd for $C_{22}H_{26}N_2O_5$: C 66.32, H 6.58, N 7.03, O 20.08. Found C 66.28, H 6.60, N 6.99, O 20.13

MS: 421 (M+Na)

$[\alpha]_d^{20}$: +80° (*c* 4.0 , DMSO)

29g 3-(2-deoxy-β-D-ribofuranosyl)-6-octyl-furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf1368]



Prepared according to Standard Procedure D using 1-decyne.

White solid: 0.6 g 57%

^{1}H-NMR (DMSO, 500 MHz): 8.68 (1H, s, H-4), 6.43 (1H, s, H-5), 6.17 (1H, t, J 6.21 Hz, H-1'), 5.29 (1H, d, J 4.30 Hz, OH-3'), 5.12 (1H, t, J 5.24 Hz, OH-5'), 4.24 (1H, dq, J 5.91 Hz 4.09 Hz, H-3'), 3.91 (1H, q, J 3.82 Hz, H-4'), 3.68 (1H, ddd, J 12.07 Hz 5.42 Hz 3.70 Hz, H-5'), 3.62 (1H, ddd, J 12.07 Hz 5.30 Hz 4.06 Hz, H-5'), 2.64 (2H, t, J 7.27 Hz, $CH_2\alpha$), 2.38 (1H, ddd, J 13.43 Hz 6.06 Hz 4.20 Hz, H-2'$_\alpha$), 2.05 (1H, dt, J 13.43 Hz 6.15 Hz, H-2'$_\beta$), 1.61 (2H, qn, J 7.32 Hz, $CH_2\beta$), 1.33-1.24 (10H, m, 5x CH_2), 0.85 (3H, t, J 6.58 Hz, $CH_3\omega$)

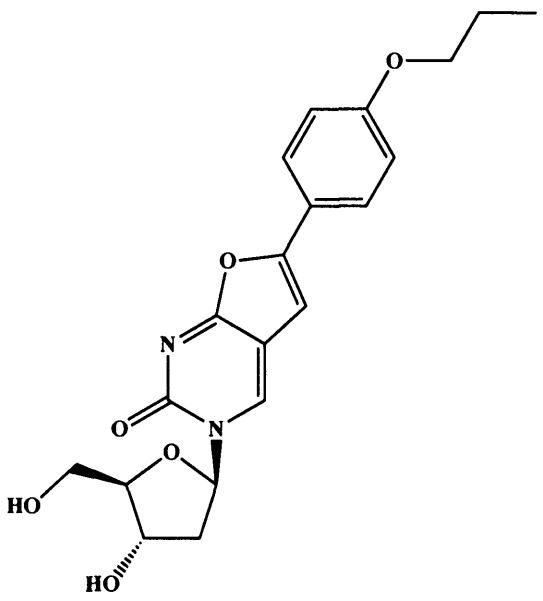
^{13}C-NMR (DMSO, 125 MHz): 171.03 (C7a), 158.27 (C6), 153.75 (C2), 136.72 (C4), 106.33 (C4a), 99.72 (C5), 88.07 (C4'), 87.34 (C1'), 69.64 (C3'), 60.75 (C5'), 41.17 (C2'), 31.21 28.59 28.55 28.33 (4xCH_2), 27.32 (Cα), 26.35 (Cβ), 22.04 (CH_2), 13.91 (Cω)

Anal. Calcd for $C_{19}H_{28}N_2O_5$: C 62.62, H 7.74, N 7.69, O 21.95. Found C 62.60, H 7.78, N 7.79, O 21.83

MS: 387 (M+Na)

44c 3-(2-deoxy-β-D-ribofuranosyl)-6-(4-propoxyphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf1948]



Prepared according to Standard Procedure D using 4-ethynylpropoxybenzene.

White solid 0.71 g 61%

^{1}H-NMR (DMSO, 500 MHz): 8.79 (1H, s, H-4), 7.76 (2H, d J 8.85 Hz, Ph), 7.10 (1H, s, H-5), 7.06 (2H, d J 8.85 Hz, Ph), 6.20 (1H, t, J 6.15 Hz, H-1'), 5.29 (1H, d, J 4.35 Hz, OH-3'), 5.16 (1H, t, J 5.25 Hz, OH-5'), 4.26 (1H, dq, J 5.95 Hz 4.15 Hz, H-3'), 4.00 (2H, t, J 5.20 Hz, $CH_2\alpha$), 3.94 (1H, q, J 3.60 Hz, H-4'), 3.71 (1H, ddd, J 12.04 Hz 5.24 Hz 3.69 Hz, H-5'), 3.64 (1H, ddd, J 12.04 Hz 5.24 Hz 3.88 Hz, H-5'), 2.41 (1H, ddd, J 13.50 Hz 6.31 Hz 4.28 Hz, H-2'), 2.10 (1H, dt, J 13.50 Hz 6.13 Hz, H-2'), 1.76 (2H, sx, J 7.46 Hz, $CH_2\beta$), 1.00 (3H, t, J 6.97 Hz, $CH_3\gamma$)

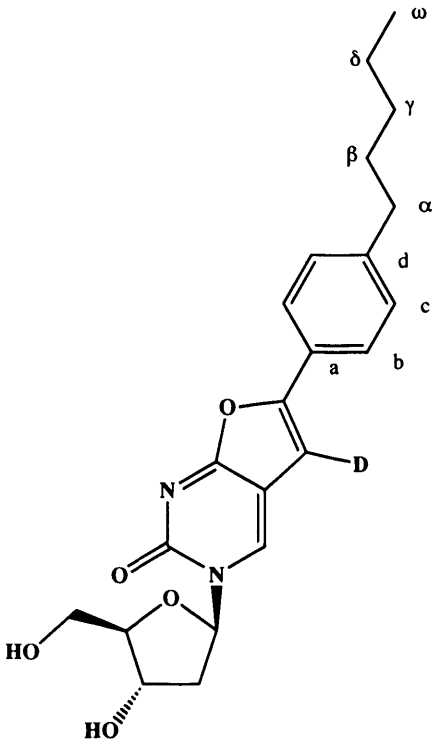
^{13}C-NMR (DMSO, 125 MHz) : 171.03 (C7a), 159.64 (C6), 153.93 (Ph-C_d), 153.76 (C2), 137.19 (C4), 126.22 (Ph-C_b), 120.77 (Ph-C_a), 115.02 (Ph-C_c), 107.11 (C4a), 97.24 (C5), 88.12 (C4'), 87.49 (C1'), 69.57 (C3'), 69.14 (Cα), 60.70 (C5'), 41.24 (C2'), 21.93 (Cβ), 10.31 (Cγ)

Anal. Calcd for $C_{20}H_{22}N_2O_6$: C 62.17, H 5.74, N 7.25, O 24.84. Found C 62.21, H 5.78, N 7.32, O 24.69

MS: 409 (M+Na)

53 3-(2-deoxy-β-D-ribofuranosyl)-5-deutero-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2572]



To a stirred solution of IDU **1** (1.06 g, 3 mmol) in dry DMF (15 m) at room temperature, 4-ethynylpentylbenzene (1.55 g, 3 eq.), *tetrakis*(triphenylphosphine)Pd^0 (0.35 g, 0.1 eq.), copper(I) iodide (0.12 g, 0.2 eq.) and DIPEA (1 ml, 2 eq.) were added. The reaction mixture was stirred for 15 hours at room temperature. The solvent was removed under reduced pressure and the resulting residue was dissolved in DCM and left at 0°C for 5 hours. The resulting precipitate was filtered and washed with DCM. The precipitate was the dissolved in anhydrous CH_3OD stirred under argon for 30 minutes and then dried under reduced pressure. This operation was performed three times. The residue was then dissolved in anhydrous CH_3OD (15 ml) and TEA (15 ml) plus copper(I) iodide (0.12g 0.2 eq.) were added. The reaction mixture was then heated at 60°C and stirred for 2 hours. The solvents were removed under reduced pressure and the resulting residue was suspended in MeOH and stirred for 15 minutes. The solvent was removed and the residue was washed with cold DCM to obtain the titled compound.

White solid: 0.60 g, 50%

^{1}H-NMR (DMSO, 500 MHz): δ 8.84 (1H, s, H-4), 7.75 (2H, d, J 7.6 Hz, H-Ph_b), 7.33 (2H, d, J 7.6 Hz, H-Ph_c), 6.20 (1H, t, J 5.7 Hz, H-1'), 5.28 (1H, d, J 4.2 Hz, OH-3'), 5.15 (1H, t, J 5.1 Hz, OH-5'), 4.27 (1H, dq, J 6.1 Hz 4.2 Hz, H-3'), 3.94 (1H, q, J 3.8 Hz, H-4'), 3.72 (1H, ddd, J 12.1 Hz 5.1 Hz 3.6 Hz, H-5'a), 3.65 (1H, ddd, J 12.1 Hz 5.1 Hz 4.2 Hz, H-5'b), 2.61 (2H, t, J 7.5 Hz, $CH_2\alpha$), 2.42 (1H, ddd, J 13.5 Hz 6.3 Hz 4.1 Hz, H-2'$_\alpha$), 2.11 (1H, dt, J 13.5 Hz 6.1 Hz, H-2'$_\beta$), 1.59 (2H, qn, J 7.5 Hz, $CH_2\beta$), 1.35-1.24 (4H, m, $CH_2\delta$ + $CH_2\gamma$), 0.86 (3H, t, J 7.1 Hz, $CH_3\omega$)

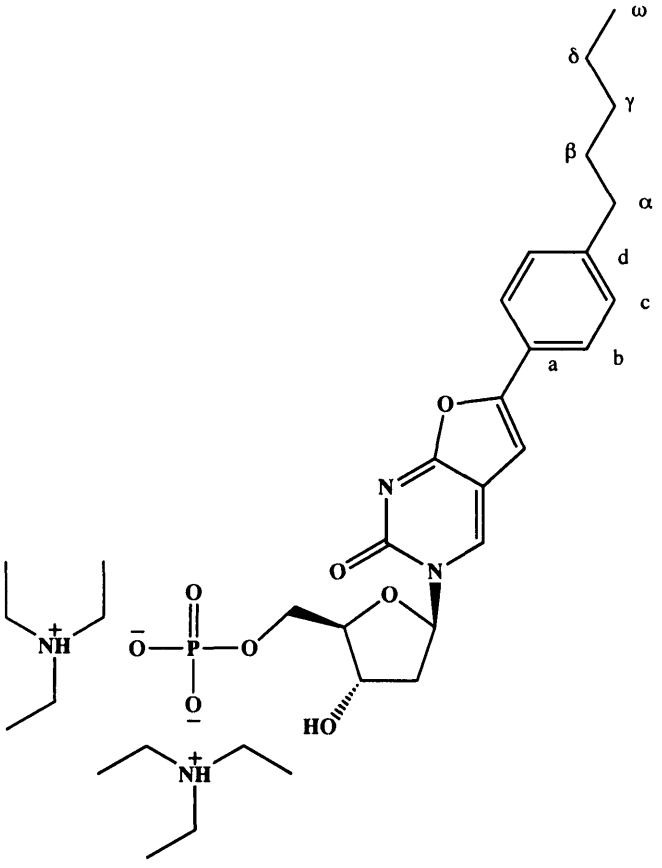
^{2}H-NMR (DMSO, 76 MHz): δ 7.22 (1D, s, D-5)

^{13}C-NMR (DMSO, 125 MHz): δ 171.02 (C7a), 153.90 (C6), 153.76 (C2), 144.02 (Ph-C_d), 137.77 (C4), 128.93 (Ph-C_c), 125.86 (Ph-C_a), 124.50 (Ph-C_b), 106.88 (C4a), 98.62 (bs C5), 88.16 (C4'), 87.57 (C1'), 69.55 (C3'), 60.68 (C5'), 42.26 (C2'), 34.89 (Cα), 30.80 (Cγ), 30.33 (Cβ), 21.89 (Cδ), 13.84 (Cω)

MS: 422 (M+Na)

54 3-(2-deoxy-β-D-ribofuranosyl)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one-5'-monophosphate triethylammonium salt

[Cf2359]



Under an argon atmosphere, to a stirred suspension of **33f** (0.64g, 1.6 mmol) in triehyl phosphate (5 ml), $POCl_3$ (0.22 ml, 1.5 eq.) was added at 0°C, and the reaction stirred at the same temperature for 2 hours. After this time a triethylammonium bicarbonate 1M solution was added until the effervescence stopped. The mixture was concentrated under reduced pressure, the residue was purified by column chromatography (^{i}PrOH:TEA 9:1 to ^{i}PrOH:TEA:H_2O 9:1:2) to obtain the titled compound.

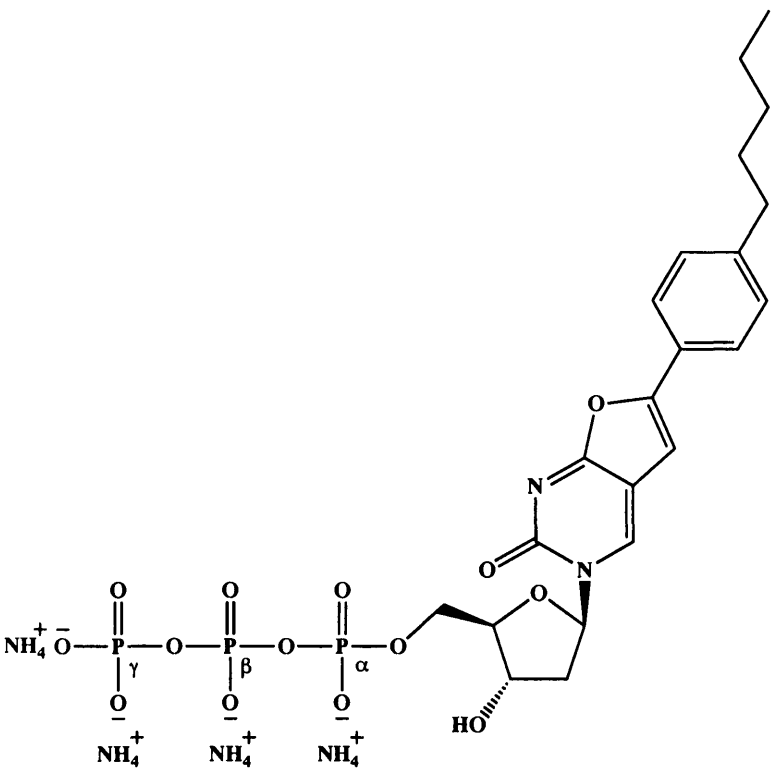
White solid 0.44 g 40%

^{31}P-NMR (DMSO, 202 MHz): δ -0.36

^{1}H-NMR (DMSO, 500 MHz): δ 9.05 (1H, s, H-4), 7.64 (2H, d, J 7.6 Hz, H-Ph_b), 7.24 (2H, d, J 7.6 Hz, H-Ph_c), 7.22 (1H, s, H-5), 6.18 (1H, m, H-1'), 4.33-4.32 (1H, m, H-3'), 4.01 (2H, m, H-5'), 3.96-3.90 (1H, m, H-4'), 2.56 (2H, t, J 7.7 Hz, CH_2α), 2.42-2.34 (1H, m, H-2'$_\alpha$), 2.13-2.05 (1H, m, H-2'$_\beta$), 1.60-1.51 (2H, m, CH_2β), 1.33-1.24 (4H, m, CH_2δ + CH_2γ), 0.86 (3H, t, J 6.8 Hz, CH_3ω)

55 3-(2-deoxy-β-D-ribofuranosyl)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one-5'-triophosphate ammonium salt

[Cf2423]

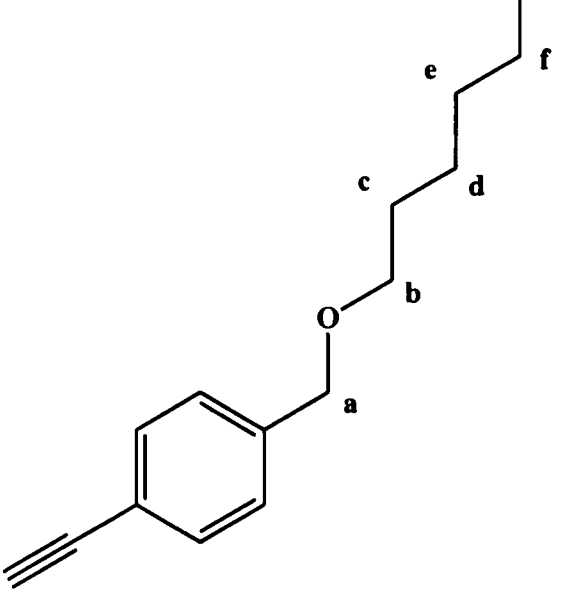


To a solution of **55** (0.14 g, 0.2 mmol) in anhydrous DMF (3 ml), 1,1'-carbonylimidazole (0.16 g, 5 eq.) dissolved in anhydrous DMF (3 ml) was added. After 4 hours MeOH (2.5 ml) was added and the mixture was stirred for 30 minutes. After this time tributylammonium pyrophosphate (0.78 g, 3 eq.) was added and the reaction was stirred for 24 hours. The solvent was removed under reduced pressure and the residue was dissolved in a minimum amount of ammonium bicarbonate 0.4M solution and the purified by column chromatography (^{i}PrOH:NH_3 7:1:2 to ^{i}PrOH:NH_3:H_2O 6:1:3) to obtain the titled compound.

White solid: 0.10 g, 71%

^{31}P-NMR (D_2O, 202 MHz): δ -23.1 (t, J 20 Hz, Pβ), -11.6 (d, 19.9 Hz, Pα), -10.23 (d, J 20 Hz, Pγ)

^{1}H-NMR(H_2O, 500 MHz): δ 8.50 (1H, s, H-4), 7.44 (2H, d, J 7.6 Hz, H-Ph_b), 7.1 (2H, d, J 7.6 Hz, H-Ph_c), 6.90 (1H, s, H-5), 6.03 (1H, m, H-1'), 4.51-4.41 (1H, m, H-3'), 4.25-4.04 (3H, m, H-4'+H-5'), 2.51-2.35 (3H, m, CH_2α+H-2'$_\alpha$), 2.20-2.15 (1H, m, H-2'$_\beta$), 1.42 -1.37 (2H, m, CH_2β), 1.11-1.01 (4H, m, CH_2δ + CH_2γ), 0.72 (3H, t, J 7.1 Hz, CH_3ω)



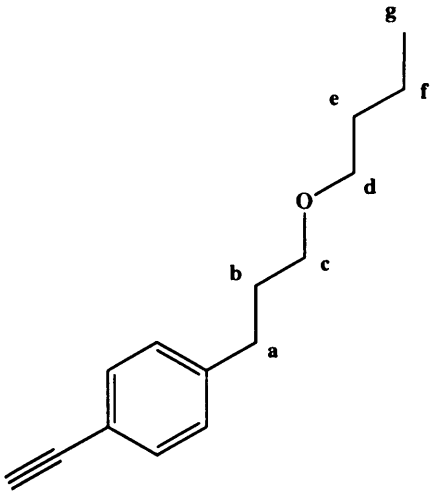
30 minutes and then poured into ice. The solutio extracted with DCM, and then the orgain layer was w with a saturated solution of $NaHCO_3$. The residue wa dissolved in a 10% solution of MeONa/MeOH (20n heated at 70°C for 2 hours. The solvent was removed reduce pressure and then dissolved in DCM and w with H_2O. The solvent was then removed to compound **62**. To a solution of **62** in THF (100 ml), under argon atmosphere, was slowly added NaH (1.6 g, 2 eq. [60% dispersion]). A minutes stirring, hexyl methane sulfonate was added and the reaction was heated at 70 4 hours. The reaction was quenched by addition of water and extracted with CHC solvent was removed under reduce pressure to yield the crude compound **68**.

To a stirred solution of crude **68** in dry DMF (30 m) at room temperature, TMS-acetyle ml, 2 eq.), *tetrakis*(triphenylphosphine)Pd^0 (2.31 g, 0.1 eq.), copper(I) iodide (0.76 g, 0 and DIPEA (6.97 ml, 2 eq.) were added. The mixrure was stirred 15 hours at temperature, after which a 1M solution of TBAF (25 ml) was added and stirred at th temperature for 1 hour. The solvent was removed under high vacuum and the residu purified by column chromatography (Hexane : Ethyl Acetate 9:1) to yield the title comp

Yellow oil: 1.38 g, 32%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.49 (2H, d, J 7.6 Hz, Ph-H_3), 7.30 (2H, d, J 7.6 Hz, P 4.51 (2H, s, H-a), 3.49 (2H, t, J 7.0 Hz, H-b), 3.10 (1H, s, C≡CH), 1.58-1.54 (2H, m 1.45-1.30 (6H, m, H-d+H-e+H-f), 0.92 (3H, t, 6.7 Hz, H-g).

76 4-ethynyl-(3-butoxypropyl)benzene



A mixture of glacial acetic acid (13.5 ml) and I_2 (2.5 g 0.5 eq.) was heated at 100°C. Concentrated sulphuric acid (1.4 ml) was added, followed by 3-phenyl-1-propanol (2.72 ml, 20 mmol). A solution of $NaIO_3$ (0.99 g, 0.25 eq.) in H_2O (6 ml) was added dropwise. The reaction was stirred at 100°C for 30 minutes and then poured into ice. The solution was extracted with DCM, and then the orgain layer was washed with a saturated solution of $NaHCO_3$. The residue was then dissolved in a 10% solution of MeONa/MeOH (20ml) and heated at 70°C for 2 hours. The solvent was removed under reduce pressure and then dissolved in DCM and washed with H_2O. The solvent was then removed to obtain compound **64**. To a solution of **64** in THF (100 ml), at 0°C under argon atmosphere, was slowly added NaH (1.6 g, 2 eq. [60% dispersion]). After 5 minutes stirring, butyl methane sulfonate was added and the reaction was heated at 70°C for 4 hours. The reaction was quenched by addition of water and extracted with $CHCl_3$. The solvent was removed under reduce pressure to yield the crude compound **70**.

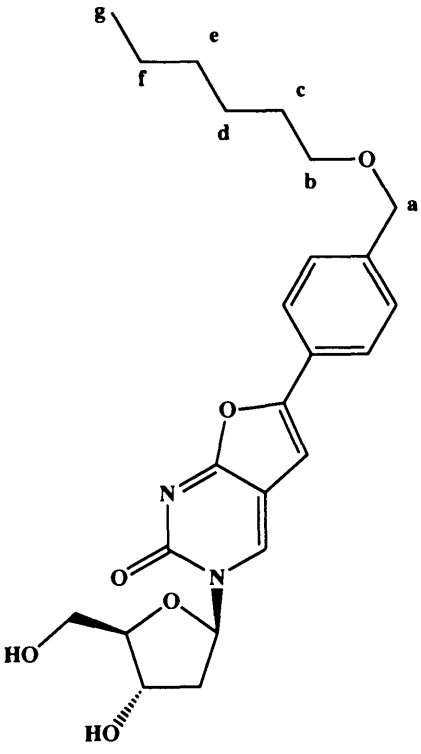
To a stirred solution of crude **70** in dry DMF (30 m) at room temperature, TMS-acetylene (5.6 ml, 2 eq.), *tetrakis*(triphenylphosphine)Pd^0 (2.31 g, 0.1 eq.), copper(I) iodide (0.76 g, 0.2 eq.) and DIPEA (6.97 ml, 2 eq.) were added. The mixrure was stirred 15 hours at room temperature, after which a 1M solution of TBAF (25 ml) was added and stirred at the same temperature for 1 hour. The solvent was removed under high vacuum and the residue was purified by column chromatography (Hexane : Ethyl Acetate 9:1) to yield the title compound.

Yellow oil: 1.71 g, 39%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.48 (2H, d, J 7.7 Hz, Ph-H_3), 7.22 (2H, d, J 7.7 Hz, Ph-H_2), 3.47-3.38 (4H, m, H-c+H-d), 3.10 (1H, s, C≡CH), 2.70 (2H, t, J 7.4 Hz, H-a), 1.99-1.91 (2H, m, H-b), 1.67-1.63 (2H, m, H-e), 1.50-1.47 (2H, m, H-f), 1.02 (3H, t, 7.3 Hz, H-g).

59 3-(2-deoxy-β-D-ribofuranosyl)-6-(4-hexoxymethylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2522]



Prepared according to Standard Procedure D using **74**.

White solid: 0.46 g, 35%

^{1}H-NMR (CD_3OD, 500 MHz): δ 9.00 (1H, s, H-4), 7.81 (2H, d, J 7.8 Hz, H-Ph_b), 7.43 (2H, d, J 7.8 Hz, H-Ph_c), 7.12 (1H, s, H-5), 6.32 (1H, t, J 5.5 Hz, H-1'), 4.52 (2H, s, H-a), 4.45-4.40 (1H, m, H-3'), 4.11-4.08 (1H, m, H-4'), 3.95-3.78 (2H, m, H-5'), 3.52 (2H, t, J 6.78, H-b), 2.63 (1H, m, H-2'$_\alpha$), 2.23 (1H, m, H-2'$_\beta$), 1.61 (2H, m, H-c), 1.49-1.25 (6H, m, H-d+ H-e+H-f), 0.91 (3H, t, J 6.87 Hz, H-g)

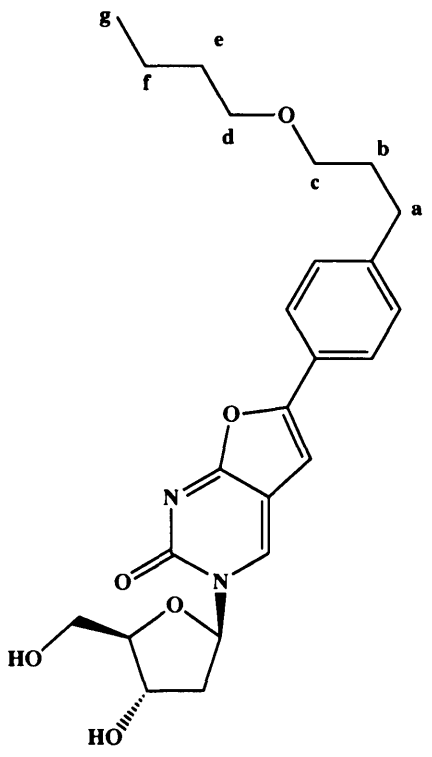
^{13}C-NMR (CD_3OD, 125 MHz): δ 171.02 (C7a), 156.87 (C6), 153.76 (C2), 141.97 (Ph), 139.45 (C4), 129.31 (Ph), 128.96 (Ph), 125.94 (Ph), 109.87 (C4a), 99.72 (C5), 90.00 (C4'), 89.77 (C1'), 73.30 (Ca), 71.75 (Cb), 71.37 (C3'), 62.31 (C5'), 42.90 (C2'), 32.83 (Cc), 30.76 (Cd), 26.98 (Ce), 23.70 (Cf), 14.40 (Cg)

Anal. Calcd for $C_{23}H_{28}N_2O_6$: C 64.47, H 6.59, N 6.54, O 22.40. Found C 64.40, H 6.60, N 6.70, O 22.30

MS: 451 (M+Na)

61 3-(2-deoxy-β-D-ribofuranosyl)-6-(4-(butoxypropyl)phenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2325]



Prepared according to Standard Procedure D using **76**.

White solid: 0.46 g, 35%

^{1}H-NMR (CD_3OD, 500 MHz): δ 8.97 (1H, s, H-4), 7.75 (2H, d, J 8.3 Hz, H-Ph_b), 7.34 (2H, d, J 8.3 Hz, H-Ph_c), 7.04 (1H, s, H-5), 6.34 (1H, t, J 5.9 Hz, H-1'), 4.45 (1H, m, H-3'), 4.10 (1H, q, J 3.7 Hz, H-4'), 3.93 (1H, dd, J 12.1 Hz 3.3 Hz, H-5'a), 3.83 (1H, dd, J 12.1 hz 3.9 Hz, H-5'b), 3.48-3.43 (4H, m, H-c+H-d), 2.76 (2H, t, J 7.3 Hz, H-a), 2.64 (1H, ddd, J 13.6 Hz 6.4 Hz 4.6 Hz, H-2'$_\alpha$), 2.25 (1H, dt, J 13.6 Hz 6.0 Hz, H-2'$_\beta$), 1.94-1.87 (2H, m, H-b), 1.58 (2H, qn, J 6.6 Hz, H-e), 1.43 (2H, sx, J 7.5 Hz, H-f), 0.96 (3H, t, J 7.3 Hz, H-g)

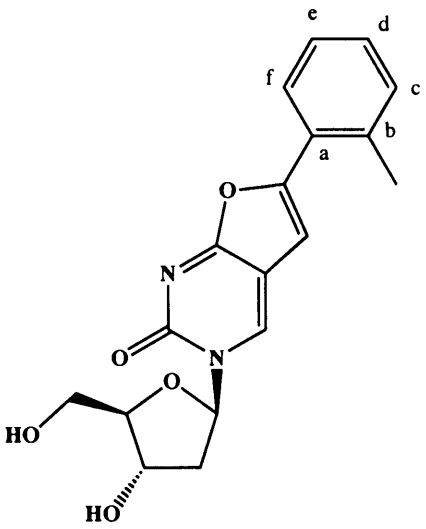
^{13}C-NMR (CD_3OD, 125 MHz): δ 172.95 (C7a), 157.24 (C6), 156.85 (C2), 145.59 (Ph), 139.11 (C4), 130.28 (Ph), 127.52 (Ph), 126.01 (Ph), 109.97 (C4a), 99.00 (C5), 89.96 (C4'), 89.75 (C1'), 71.72 (Cc), 71.38 (C3'), 70.82 (Cd), 62.32 (C5'), 42.89 (C2'), 33.23 (Cc), 32.33 (Ce), 20.44 (Ca+Cf), 14.26 (Cg)

Anal. Calcd for $C_{23}H_{28}N_2O_6$: C 64.47, H 6.59, N 6.54, O 22.40. Found C 64.38, H 6.56, N 6.75, O 22.31

MS: 451 (M+Na)

85 3-(2-deoxy-β-D-ribofuranosyl)-6-(*o*-toluyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2533]



To a stirred solution of IDU **1** (1.06 g, 3 mmol) in dry DMF (15 m) at room temperature, 2-ethynyl-toluene (3 eq.), *tetrakis*(triphenylphosphine)Pd^0 (0.35 g, 0.1 eq.), copper(I) iodide (0.12 g, 0.2 eq.) and DIPEA (1 ml, 2 eq.) were added. The reaction mixture was stirred for 15 hours at room temperature. The solvent was removed under reduced pressure and the resulting residue was dissolved in DCM and left at 0°C for 1 hour. The resulting precipitate was filtered and washed with DCM. The precipitate was then dissolved in 1,4-dioxane (15 ml) and copper(I) iodide (0.60 g, 1 eq.) and TEA (15 ml) were added. The reaction was stirred at reflux for 3 hours, after which the solvents were removed under reduced pressure and the residue crystallized from MeOH to obtain the titled compound.

White solid: 0.39 g, 56%

^{1}H-NMR (DMSO, 500 MHz): δ 8.87 (1H, s, H-4), 7.73 (1H, bs, Ph_f), 7.37 (3H, m, Ph_c+Ph_d+P_e), 7.01 (1H, s, H-5), 6.21 (1H, t, J 6.2 Hz, H-1'), 5.29 (1H, bs, OH-3'), 5.14 (1H, bs, OH-5'), 4.30-4.23 (1H, m, H-3'), 3.98-3.92 (1H, m, H-4'), 3.74-3.68 (1H, m, J 11.6 Hz, H-5'a), 3.68-3.62 (1H, m, J 11.6 Hz, H-5'b), 2.51 (3H, s, CH_3), 2.47-2.40 (1H, m, H-2'$_\alpha$), 2.11 (1H, dt, 12.5 Hz, H-2'$_\beta$)

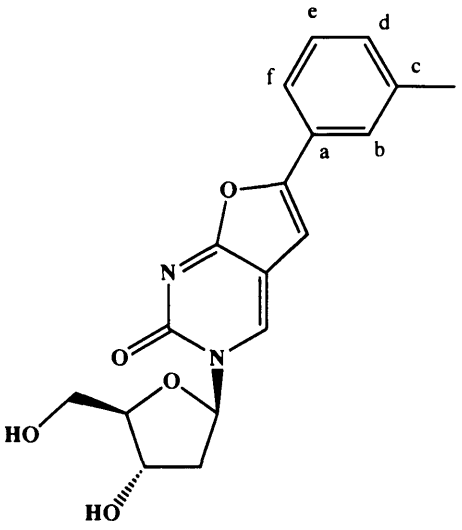
^{13}C-NMR (DMSO, 125 MHz): δ 170.62 (C7a), 153.81 (C6), 153.11 (C2), 144.02 (Ph-C_d), 138.42 (C4), 135.58, (Ph-C_a), 131.44 (Ph), 129.24 (Ph), 127.70 (Ph-C_b), 127.33 (Ph-C_f), 126.37 (Ph-C_b), 106.78 (C4a), 103.2 (C5), 88.23 (C4'), 87.65 (C1'), 69.68 (C3'), 60.79 (C5'), 41.27 (C2'), 21.45 (CH_3)

Anal. Calcd for $C_{18}H_{18}N_2O_5$: C 63.15, H 5.30, N 8.18, O 23.37. Found C 63.08, H 5.40, N 8.00, O 23.52

MS: 365 (M+Na)

86 3-(2-deoxy-β-D-ribofuranosyl)-6-(*m*-toluyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2534]



Prepared according to Standard Procedure D using 3-ethynyltoluene.

White solid: 0.76 g, 74%

^{1}H-NMR (DMSO, 500 Mhz): δ 8.87 (1H, s, H-4), 7.67 (1H, s, Ph_b), 7.64 (1H, d, J 7.7 Hz, Ph_f), 7.39 (1H, t, J 7.7 Hz, Ph_e), 7.26 (1H, d, J 9.3 Hz, Ph_d), 7.25 (1H, s, H-5), 6.20 (1H, t, J 6.1 Hz, H-1'), 5.29 (1H, d, J 4.3 Hz, 3'-OH), 5.17 (1H, t, J 5.2 Hz, 5'-OH), 4.29-4.23 (1H, m, H-3'), 3.94 (1H, q, J 3.8 Hz, H-4'), 3.72 (1H, ddd, J 12.0 Hz 5.2 Hz 3.6 Hz, H-5'a), 3.65 (1H, ddd, J 12.0 Hz 5.2 Hz 3.6 Hz, H-5'b), 2.43 (1H, ddd, J 13.5 Hz 6.3 Hz 4.3 Hz, H-2'$_\alpha$), 2.39 (3H, s, CH_3), 2.12 (1H, dt, J 13.5 Hz 6.0 Hz, H-2'$_\beta$)

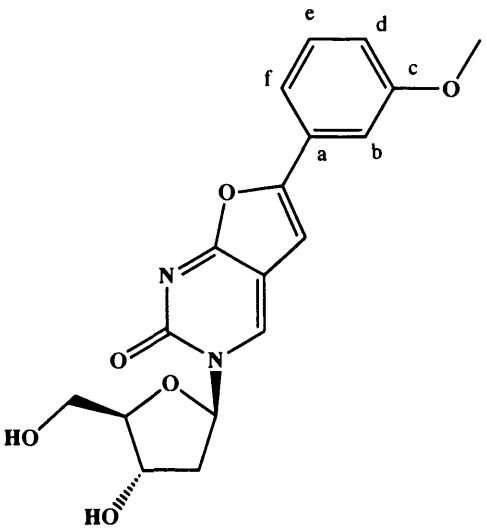
^{13}C-NMR (DMSO) : δ 171.01 (C7a), 153.76 (C6 C2), 138.40 (Ph-C_a) 138.11(C4), 130.09 (Ph-C_d), 128.97 (Ph-C_e), 128.28 (Ph-C_c), 124.88 (Ph-C_b), 121.76 (Ph-C_f), 106.76 (C4a), 99.38 (C5), 88.15 (C4'), 87.60 (C1'), 69.50 (C3'), 60.65 (C5'), 41.23 (C2'), 20.93 (CH_3)

Anal. Calcd for $C_{18}H_{18}N_2O_5$: C 63.15, H 5.30, N 8.18, O 23.37. Found C 63.10, H 5.42, N 8.05, O 23.43

MS: 365 (M+Na)

87 3-(2-deoxy-β-D-ribofuranosyl)-6-(*m*-anisolyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2490]



Prepared according to Standard procedure D using 3-ethynylanisole.

White solid: 0.74 g, 69%

^{1}H-NMR (DMSO, 500 Mhz): δ 8.90 (1H, s, H-4), 7.45-7.39 (2H, m, Ph), 7.39-7.35 (1H, m, Ph), 7.34 (1H, s, H-5), 6.20 (1H, t, J 6.2 Hz, H-1'), 5.31 (1H, d, J 4.4 Hz, 3'-OH), 5.19 (1H, t, J 4.8 Hz, 5'-OH), 4.31-4.23 (1H, m, H-3'), 3.94 (1H, q, J 3.8 Hz, H-4'), 3.84 (3H, s, OCH_3), 3.78-3. 59 (2H, m, H-5'), 2.43 (1H, ddd, J 13.7 Hz 6.4 Hz 4.5 Hz, H-2'$_{\alpha}$), 2.12 (1H, dt, J 13.7 Hz 5.9 Hz, H-2'$_{\beta}$)

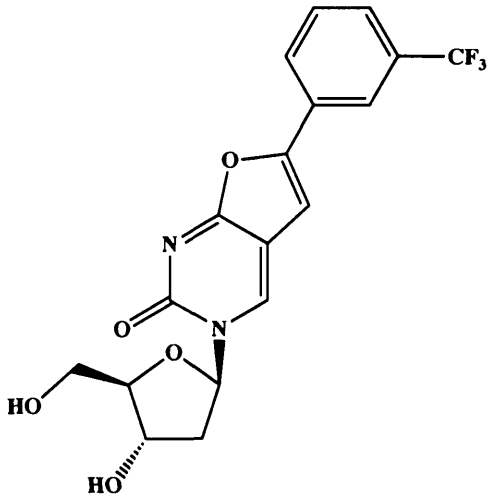
^{13}C-NMR (DMSO, 125 MHz): δ 171.35 (C7a), 160.03 (Ph-C_c), 154.12 (C2), 153.78 (C6), 138.67 (C4), 130.66 (Ph-C_a), 130.03 (Ph-C_e), 117.27 (Ph-C_d), 115.70 (Ph-C_b), 109.95 (Ph-C_f), 107.06 (C4a), 99.38 (C5), 88.35 (C4'), 88.01 (C1'), 69.81 (C3'), 60.98 (C5'), 55.68 (OCH_3), 41.62 (C2')

Anal. Calcd for $C_{18}H_{18}N_2O_6$: C 60.33, H 5.06, N 7.82, O 26.79. Found C 60.01, H 5.24, N 8.09, O 26.66

MS: 381 (M+Na)

88 3-(2-deoxy-β-D-ribofuranosyl)-6-(*m*-α,α,α-trifluorotoluyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2488]



Prepared according to Standard procedure D using 3-ethynyl-α,α,α-trifluorotouene

White solid: 0.63 g, 53%

^{1}H-NMR (DMSO, 500 MHz): δ 8.94 (1H, s, H-4), 8.19-8.08 (2H, m, Ph), 7.81-7.69 (2H, m, Ph), 7.54 (1H, s, H-5), 6.17 (1H, t, J 6.0 Hz, H-1'), 5.30 (1H, d, J 4.0 Hz, 3'-OH), 5.22 (1H, t, J 5.0 Hz, 5'-OH), 4.30-4.21 (1H, m, H-3'), 3.98-3.90 (1H, m, H-4'), 3.78-3.58 (2H, m, H-5'), 2.49-2.36 (1H, m, H-2'$_\alpha$), 2.18-2.06 (1H, m, H-2'$_\beta$)

^{13}C-NMR (DMSO, 125 MHz): δ 171.32 (C7a), 154.09 (Ph), 152.15 (C2), 139.53 (C4), 130.69 (Ph), 130.11 (Ph), 129.82 (Ph), 128.63 (Ph), 125.95 (q, J 270 Hz, CF_3), 121.24 (Ph), 106.81 (4a), 102.07 (C5), 88.54 (C4'), 88.11 (C1'), 69.71 (C3'), 60.91 (C5'), 41.61 (C2')

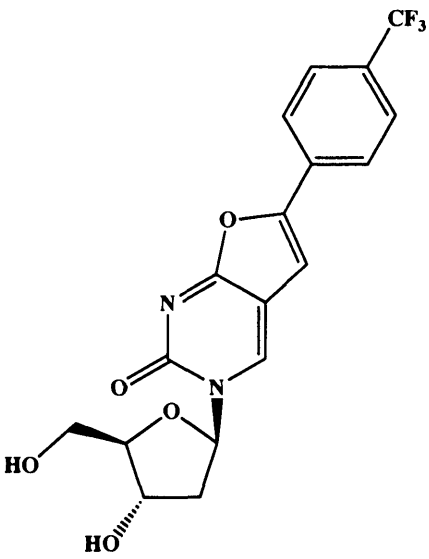
^{19}F-NMR (DMSO, 482 MHz): δ -61.76

Anal. Calcd for $C_{18}H_{15}N_2O_5F_3$: C 54.55, H 3.81, N 7.07, O 20.19. Found C 54.26, H 4.01, N 7.10

MS: 419 (M+Na)

89 3-(2-deoxy-β-D-ribofuranosyl)-6-(*p*-α,α,α-trifluorotoluyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2487]



Prepared according to Standard procedure D using 4-ethynyl-α,α,α-trifluorotouene.

White solid: 0.75 g, 63%

^{1}H-NMR (DMSO, 500 MHz): δ 8.96 (1H, s, H-4), 8.03 (2H, d, J 9.0 Hz, Ph), 7.84 (2H, d, J 9.0 Hz, Ph), 7.51 (1H, s, H-5), 6.19 (1H, t, J 6.0 Hz, H-1'), 5.32 (1H, d, J 4.0 Hz, 3'-OH), 5.21 (1H, t, J 5.1 Hz, 5'-OH), 4.33-4.22 (1H, m, H-3'), 3.98-3.90 (1H, m, H-4'), 3.78-3.58 (2H, m, H-5'), 2.51-2.36 (1H, m, H-2'$_\alpha$), 2.20-2.07 (1H, m, H-2'$_\beta$)

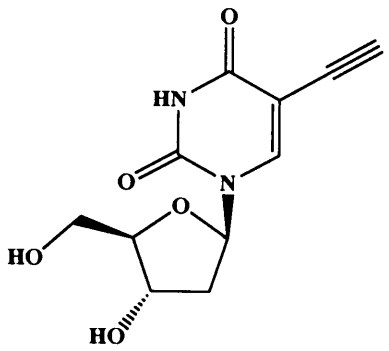
^{13}C-NMR (DMSO, 125 MHz): δ 171.35 (C7a), 154.09 (Ph), 152.15 (C2), 139.76 (C4), 132.51 (Ph), 129.8 (Ph), 126.37 (Ph), 126.16 (Ph), 125.42 (q, J 268 Hz, CF_3), 106.76 (4a), 102.75 (C5), 88.59 (C4'), 88.17 (C1'), 69.79 (C3'), 60.96 (C5'), 41.62 (C2')

^{19}F-NMR (DMSO, 482 MHz): δ -61.72

Anal. Calcd for $C_{18}H_{15}N_2O_5F_3$: C 54.55, H 3.81, N 7.07, O 20.19. Found C 54.31, H 3.98, N 7.01

MS: 419 (M+Na)

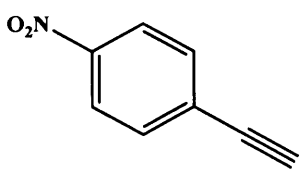
96 5-ethynyl-2'-deoxyuridine



To a stirred solution of IDU **1** (1.06 g, 3 mmol) in dry ACN (15 m) at room temperature, the TMS-acetylene (1.3 ml, 3 eq.), *tetrakis*(triphenylphosphine)Pd^0 (0.35 g, 0.1 eq.), copper(I) iodide (0.12 g, 0.2 eq.) and DIPEA (1 ml, 2 eq.) were added. The reaction mixture was stirred for 15 hours at room temperature, after which time a 1M solution of TBAF in THF (6 ml, 2 eq.) was added. The solvent was removed under reduced pressure and the resulting residue was purified by column chromatography ($CHCl_3$: MeOH 95:5) to afford 0.57 g (76%) of the title compound.

^{1}H-NMR (DMSO, 500 MHz): δ 11.64 (1H, bs, NH), 8.31 (1H, s, H-6), 6.11 (1H, dd, J 6.5 Hz 3.9 Hz, H-1'), 5.27 (1H, d, J 3.8 Hz, 3'-OH), 5.18 (1H, t, J 4.5 Hz, 5'-OH), 4.24 (1H, m, H-3'), 4.10 (1H, s, CH), 3.80 (1H, m, H-4'), 3.60 (2H, m, H-5'), 2.20-2.10 (2H, m, H-2')
^{13}C-NMR (DMSO, 125 MHz): δ 162.0 (C4), 150.0 (C2), 144.9 (C6), 98.0 (C5), 88.0 (C4'), 85.2 (C1'), 84.0 (C), 76.8 (CH), 70.3 (C3'), 61.2 (C5'), 40.6 (C2')

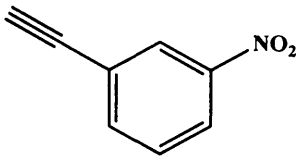
99 4-ethynylnitrobenzene



To a stirred solution of 4-iodonitrobenzene (2.50 g, 10 mmol) in dry ACN (50 m) at room temperature, the TMS-acetylene (4.2 ml, 3 eq.), *tetrakis*(triphenylphosphine)Pd^0 (1.60 g, 0.1 eq.), copper(I) iodide (0.38 g, 0.2 eq.) and DIPEA (2.6 ml, 2 eq.) were added. The reaction mixture was stirred for 15 hours at room temperature, after which time a 1M solution of

TBAF in THF (20 ml, 2 eq.) was added. The solvent was removed under reduced pressure and the resulting residue was purified by column chromatography (Hexane : Ethyl Acetate 8:3) to afford 1.35 g (92%) of the title compound.
MS: 148 (M+H)

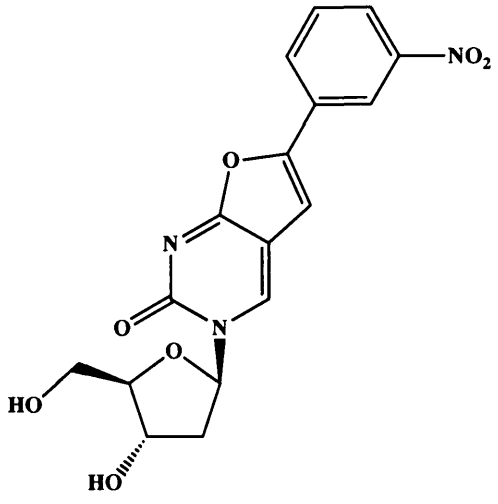
100 3-ethynylnitrobenzene



To a stirred solution of 3-iodonitrobenzene (2.50 g, 10 mmol) in dry ACN (50 m) at room temperature, TMS-acetylene (4.2 ml, 3 eq.), *tetrakis*(triphenylphosphine)Pd^0 (1.60 g, 0.1 eq.), copper(I) iodide (0.38 g, 0.2 eq.) and DIPEA (2.6 ml, 2 eq.) were added. The reaction mixture was stirred for 15 hours at room temperature, after which time a 1M solution of TBAF in THF (20 ml, 2 eq.) was added. The solvent was removed under reduced pressure and the resulting residue was purified by column chromatography (Hexane : Ethyl Acetate 8:3) to afford 1.09 g (74%) of the title compound.
MS: 148 (M+H)

90 3-(2-deoxy-β-D-ribofuranosyl)-6-(*m*-nitrophenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2493]



Prepared according to Standard Procedure D using 3-ethynylnitrobenzene **100**.
Yellow solid: 0.39 g, 35%
^{1}H-NMR (DMSO, 500 MHz): δ 8.99 (1H, s, H-4), 8.58 (1H, m, Ph), 8.28 (2H, m, Ph), 7.80 (1H, t, J 9.0 Hz, Ph), 7.60 (1H, s, H-5), 6.19 (1H, t, J 6.0 Hz, H-1'), 5.32 (1H, d, J 4.0 Hz, 3'-OH), 5.21 (1H, t, J 5.1 Hz, 5'-OH), 4.33-4.20 (1H, m, H-3'), 3.99-3.91 (1H, m, H-4'), 3.78-3.58 (2H, m, H-5'), 2.50-2.38 (1H, m, H-2'$_\alpha$), 2.20-2.07 (1H, m, H-2'$_\beta$)

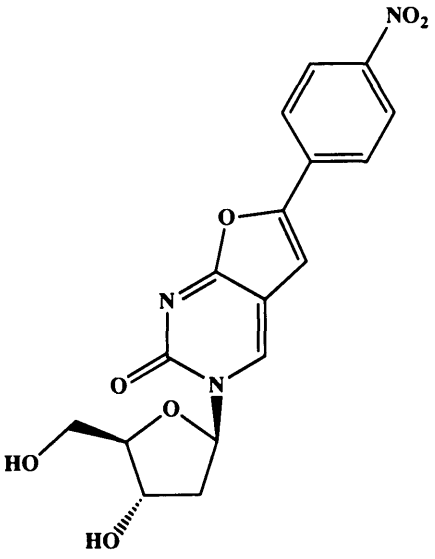
^{13}C-NMR (DMSO, 125 MHz): δ 171.76 (C7a), 154.54 (C6), 151.90 (C2), 149.27 (Ph), 140.38 (C4), 131.66 (Ph), 131.37 (Ph), 130.83 (Ph), 124.42 (Ph), 124.42 (Ph), 107.18 (4a), 103.35 (C5), 89.05 (C4'), 88.64 (C1'), 70.18 (C3'), 61.37 (C5'), 42.07 (C2')

Anal. Calcd for $C_{17}H_{15}N_3O_7$: C 54.69, H 4.05, N 11.26, O 30.00. Found C 54.38, H 3.98, N 11.53, O 30.11

MS: 396 (M+Na)

91 3-(2-deoxy-β-D-ribofuranosyl)-6-(*m*-nitrophenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2492]



Prepared according to Standard Procedure D using 4-ethynylnitrobenzene **99**.

Yellow solid: 0.47 g, 42%

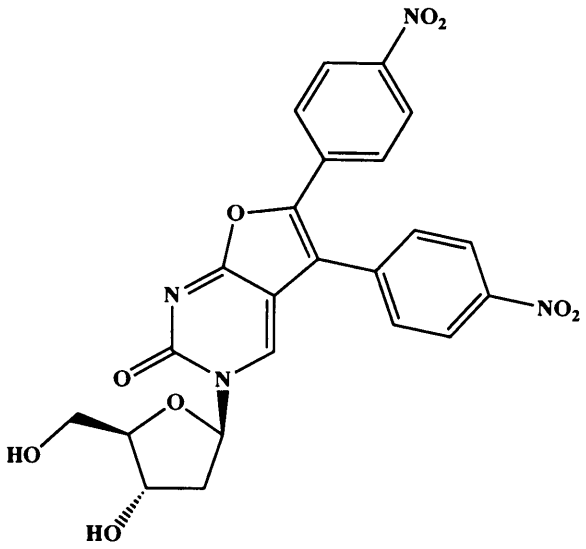
^{1}H-NMR (DMSO, 500 MHz): δ 9.01 (1H, s, H-4), 8.33 (2H, d, J 9.0 Hz, Ph), 8.09 (2H, d, J 9.0 Hz, Ph), 7.63 (1H, s, H-5), 6.19 (1H, t, J 6.0 Hz, H-1'), 5.32 (1H, d, J 4.4 Hz, 3'-OH), 5.21 (1H, t, J 6.0 Hz, 5'-OH), 4.35-4.20 (1H, m, H-3'), 3.99-3.91 (1H, m, H-4'), 3.78-3.58 (2H, m, H-5'), 2.50-2.38 (1H, m, H-2'$_\alpha$), 2.20-2.07 (1H, m, H-2'$_\beta$)

^{13}C-NMR (DMSO, 125 MHz): δ 171.40 (C7a), 154.03 (C6), 151.51 (C2), 147.42 (Ph), 140.43 (C4), 134.65 (Ph), 125.73 (Ph), 124.76 (Ph), 106.69 (4a), 104.58 (C5), 88.62 (C4'), 88.27 (C1'), 69.73 (C3'), 60.92 (C5'), 41.61 (C2')

Anal. Calcd for $C_{17}H_{15}N_3O_7$: C 54.69, H 4.05, N 11.26, O 30.00. Found C 54.42, H 4.05, N 11.12, O 30.41

MS: 396 (M+Na)

98 3-(2-deoxy-β-D-ribofuranosyl)-6-(*m*-nitrophenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one



To a stirred solution of **96** (0.76 g, 3 mmol) in dry ACN (15 m) at room temperature, 4-iodonitrobenzene (2.24 g, 3 eq.), *tetrakis*(triphenylphosphine)Pd^0 (0.35 g, 0.1 eq.), copper(I) iodide (0.12 g, 0.2 eq.) and DIPEA (1 ml, 2 eq.) were added. The reaction mixture was stirred for 15 hours at room temperature, after which time TEA (15 ml) and further copper(I) iodide (0.12 g 0.2 eq.) were added. The reaction mixture was then heated at 80 °C and stirred for 4-6 hours. The solvent was removed under reduced pressure and the resulting residue was dissolved in DCM and left at 0 °C for 15 hours. The resulting precipitate was filtered and washed with DCM to obtain 1.08 g (73%) of the title compound.

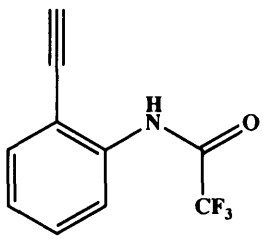
^{1}H-NMR (DMSO, 500 MHz): δ 9.22 (1H, s, H-4), 8.38 (2H, d, J 8.5 Hz, Ph), 8.27 (2H, d, J 9.0 Hz, Ph), 7.78 (2H, d, J 9.0 Hz, Ph), 7.77 (2H, d, 8.5 Hz, Ph), 6.15 (1H, dd, J 6.5 Hz 3.9 Hz, H-1'), 5.32 (1H, d, J 4.8 Hz, 3'-OH), 5.23 (1H, t, J 4.1 Hz, 5'-OH), 4.34-4.24 (1H, m, H-3'), 3.88 (1H, dt, J 5.4 Hz 2.7 Hz, H-4'), 3.77-3.57 (2H, m, H-5'), 2.44 (1H, qn, J 6.7 Hz, H-2'$_\alpha$), 2.27-2.17 (1H, m, H-2'$_\beta$)

^{13}C-NMR (DMSO, 125 MHz): δ 170.20 (C7a), 154.03 (C6), 147.81 (C2), 147.7 (C4a), 146.9 (C5), 140.43 (C4), 136.55, 134.42, 130.73, 128.36, 125.00, 124.78, 117.25, 107.21 (Ph), 88.12 (C4'), 87.97 (C1'), 69.93 (C3'), 59.62 (C5'), 41.51 (C2')

Anal. Calcd for $C_{17}H_{15}N_3O_7$: C 55.87, H 3.67, N 11.33, O 29.12. Found C 55.98, H 3.96, N 11.69, O 28.79

MS: 517 (M+Na)

104 2-ethynyl-N-trifluoroacetylaniline



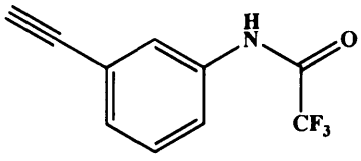
2-ethynylaniline (2.34 g, 20 mmol) was dissolved in 10 ml of THF, trifluoroacetic anhydride (was added dropwise at 0 °C and the mixture was stirred for 2 hours. The solvent was removed under vaccum and the residue was washed with Hexane to afford 3.67 g (86%)of **104** as yellow solid.

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.63 (1H, bs, NH), 8.22 (1H, d, J 8.3 Hz, H-6), 7.40 (1H, dd, J 7.8 Hz 1.6 Hz, H-3), 7.31 (1H, dt, J 8.0 Hz 1.6 Hz, H-4), 7.07 (1H, dt, J 7.7 Hz 1.0 Hz, H-5), 3.48 (1H, s, CH)

^{13}C-NMR ($CDCl_3$, 500 MHz): δ 154.69 (CO, q, J 38.8 Hz), 136.79 (C1), 132.38 (C3), 130.44 (C4), 125.50 (C5), 119.74 (C6), 115.61 (CF_3, q, J 288.8 Hz), 112.21 (C2), 85.74 (C), 77.87 (CH)

^{19}F-NMR (CDCl3, 470 MHz): δ -75.75

105 3-ethynyl-N-trifluoroacetylaniline



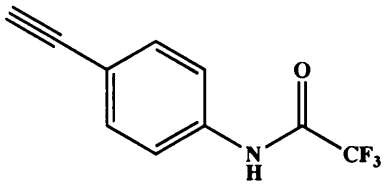
3-ethynylaniline (2.34 g, 20 mmol) was dissolved in 10 ml of THF, trifluoroacetic anhydride (was added dropwise at 0 °C and the mixture was stirred for 2 hours. The solvent was removed under vaccum and the residue was washed with Hexane to afford 3.67 g (86%)of **104** as yellow solid.

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.01 (1H, bs, NH), 7.62 (2H, s, H-2), 7.50 (1H, d, J 7.2 Hz, H-6), 7.31-7.24 (2H, m, H-4+H-5), 3.13 (1H, s, CH)

^{13}C-NMR ($CDCl_3$, 500 MHz): δ 155.25 (CO, q, J 37.6 Hz), 135.12 (C1), 130.08 (C5), 129.35 (C4), 124.22 (C2), 123.41 (C3), 121.20 (C6), 115.69 (CF_3, q, J 288.0 Hz), 82.40 (C), 78.34 (CH)

^{19}F-NMR (CDCl3, 470 MHz): δ -75.75

106 4-ethynyl-N-trifluoroacetylaniline



4-ethynylaniline (2.34 g, 20 mmol) was dissolved in 10 ml of THF, trifluoroacetic anhydride (was added dropwise at 0 °C and the mixture was stirred for 2 hours. The solvent was removed under vaccum and the residue was washed with Hexane to afford 3.67 g (86%)of **104** as yellow solid.

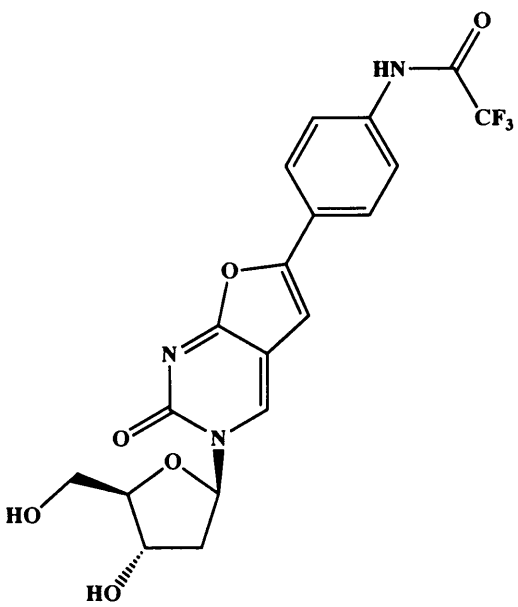
^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.95 (1H, bs, NH), 7.58 (2H, d, J 8.4 Hz, H-3), 7.54 (1H, d, J 8.4 Hz, H-2), 3.13 (1H, s, CH)

^{13}C-NMR ($CDCl_3$, 500 MHz): δ 155.05 (CO, q, J 38.1 Hz), 135.4 (C1), 133.23 (C3), 120.19 (C2), 120.14 (C4), 115.69 (CF_3, q, J 287.8), 82.63 (C), 77.95 (CH)

^{19}F-NMR (CDCl3, 470 MHz): δ -75.71

92 3-(2-deoxy-β-D-ribofuranosyl)-6-(*p*-N-trifluoroacetylanilinyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2541]



Prepared according to Standard Procedure D using 4-ethynyltrifluoroacetylaniline.

White solid: 0.85 g, 65%

^{1}H-NMR (DMSO, 500 MHz): δ 11.42 (1H, bs, NH), 8.86 (1H, s, H-4), 7.89-7.77(4H, m, Ph), 7.23 (1H, s, H-5), 6.19 (1H, t, J 6.0 Hz, H-1'), 5.30 (1H, d, J 4.0 Hz, 3'-OH), 5.17 (1H, t, J 5.0 Hz, 5'-OH), 4.29-4.25 (1H, m, H-3'), 3.96-3.93 (1H, m, H-4'), 3.78-3.60 (2H, m, H-5'), 2.48-2.38 (1H, m, H-2'$_\alpha$), 2.16-2.07 (1H, m, H-2'$_\beta$)

^{13}C-NMR (DMSO, 125 MHz): δ 171.00 (C7a), 154.48 (q, J 37.6 Hz, NHCO), 153.74 (C6), 153.05 (C2), 138.12 (C4), 137.16 (Ph), 125.41 (Ph), 125.26 (Ph), 121.20 (Ph), 115.63 (q, J 288.2 Hz, CF_3), 106.78 (4a), 99.37 (C5), 88.16 (C4'), 87.62 (C1'), 69.50 (C3'), 60.65 (C5'), 41.24 (C2')

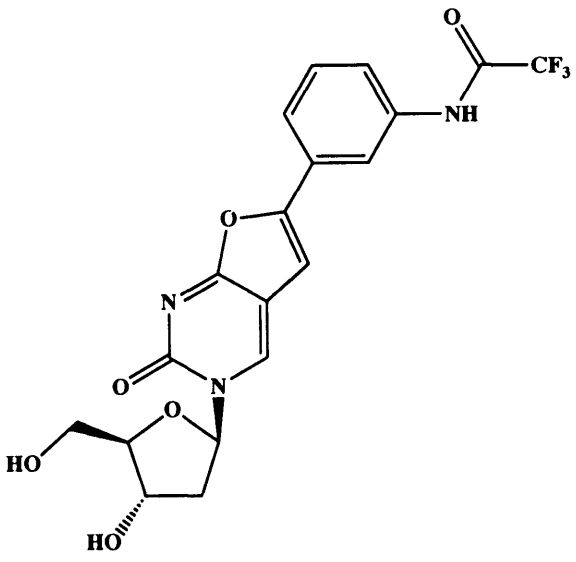
^{19}F-NMR (DMSO, 482 MHz): δ -73.87

Anal. Calcd for $C_{19}H_{16}N_3O_6F_3$: C 51.94, H 3.67, N 9.56, O 21.85. Found C 51.80, H 4.00, N 9.45

MS: 462 (M+Na)

93 3-(2-deoxy-β-D-ribofuranosyl)-6-(*m*-trifluoroacetylanilinyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2559]



Prepared according to Standard Procedure D using 3-ethynyltrifluoroacetylaniline.

White solid: 0.42 g, 32%

^{1}H-NMR (DMSO, 500 MHz): δ 11.41 (1H, bs, NH), 8.90 (1H, s, H-4), 8.13 (1H, m, Ph), 7.68 (2H, d, J 7.6 Hz, Ph), 7.55 (1H, t, 7.9 Hz, Ph), 7.30 (1H, s, H-5), 6.19 (1H, t, J 6.0 Hz, H-1'), 4.29-4.23 (1H, m, H-3'), 3.98-3.92 (1H, m, H-4'), 3.71 (1H, dd, J 12.2 Hz 3.5 Hz, H-5'a), 3.65 (1H, dd, J 12.2 Hz 3.7 Hz, H-5'b), 2.46-2.40 (1H, m, H-2'$_\alpha$), 2.12 (1H, dt, J 13.8 Hz 6.2 Hz, H-2'$_\beta$)

^{13}C-NMR (DMSO, 125 MHz): δ 170.96 (C7a), 154.68 (q, J 36.25 Hz, CONH), 153.74 (C6), 152.76 (C2), 138.67 (C4), 137.05 (Ph), 130.03 (Ph), 129.07 (Ph), 122.50 (d, J 13.75 Hz, Ph), 115.63 (q, J 288.2 Hz, CF_3), 106.40 (4a), 100.4 (C5), 88.16 (C4'), 87.70 (C1'), 69.50 (C3'), 60.65 (C5'), 41.22 (C2')

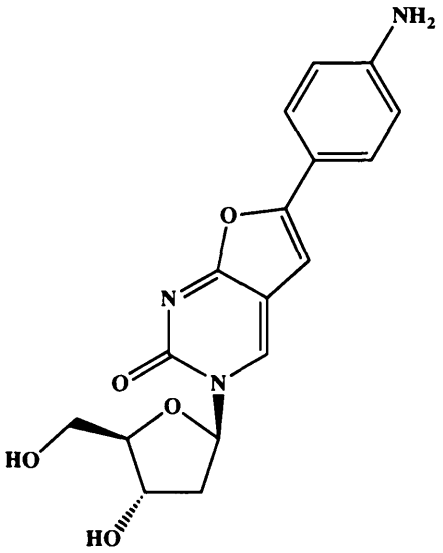
^{19}F-NMR (DMSO, 482 MHz): δ -73.93

Anal. Calcd for $C_{19}H_{16}N_3O_6$: C 51.94, H 3.67, N 9.56, O 21.85. Found C 51.78, H 3.50, N 9.68

MS: 462 (M+Na)

94 3-(2-deoxy-β-D-ribofuranosyl)-6-(*p*-anilinyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[2542]



Compound **92** (0.13 g, 0.3 mmol) was dissolved in a mixture MeOH / H_2O (5:1) and K_2CO_3 (0.1 g , 2.5 eq.) was added. The mixture was stirred for 15 hours at room temperature.

The solvent was removed under reduced pressure and the residue was purified by column chromatography (CHCl3:MeOH 9:1) to obtain the titled compound.

White solid: 0.08 g, 80%

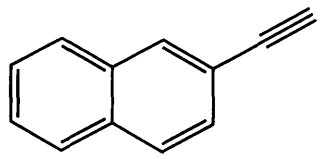
^{1}H-NMR (DMSO, 500 MHz): δ 8.74 (1H, s, H-4), 7.65-7.50 (4H, m, Ph), 6.99 (1H, s, H-5), 6.19 (1H, t, J 6.2 Hz, H-1'), 5.26 (2H, bs, OH-3'+ OH-5'), 4.29-4.25 (1H, m, H-3'), 3.96-3.91 (1H, m, H-4'), 3.74-3.60 (2H, m, H-5'), 2.44-2.36 (1H, m, H-2'$_\alpha$), 2.14-2.06 (1H, m, H-2'$_\beta$)

^{13}C-NMR (DMSO, 125 MHz): δ 171.07 (C7a), 155.00 (C6), 153.85 (C2), 136.58 (C4), 127.16 (Ph), 124.60 (Ph), 123.81 (Ph), 119.20 (Ph), 107.45 (4a), 96.19 (C5), 88.08 (C4'), 87.42 (C1'), 69.61 (C3'), 60.72 (C5'), 41.20 (C2')

Anal. Calcd for $C_{17}H_{17}N_3O_5F_3$: C 59.47, H 4.99, N 12.24, O 23.30. Found C 59.50, H 4.87, N 12.00, O 23.63

MS: 366 (M+Na)

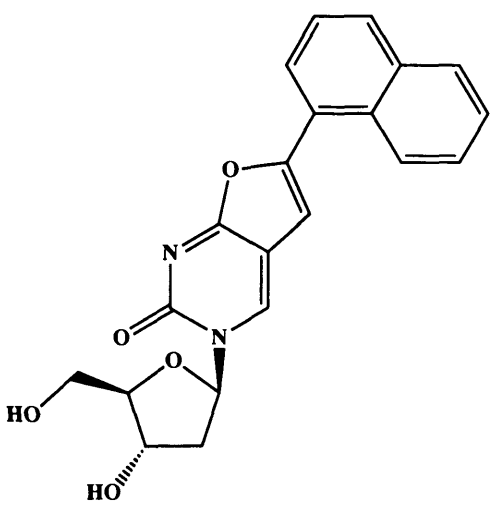
114 2-ethynyl naphthalene



To a stirred solution of 2-bromonaphthalene (4.14 g, 20 mmol) in dry ACN (100 m) at room temperature, TMS-acetylene (8.5 ml, 3 eq.), *tetrakis*(triphenylphosphine)Pd^0 (3.2 g, 0.1 eq.), copper(I) iodide (0.76 g, 0.2 eq.) and DIPEA (5.2ml, 2 eq.) were added. The reaction mixture was stirred for 15 hours at room temperature, after which time a 1M solution of TBAF in THF (40 ml, 2 eq.) was added. The solvent was removed under reduced pressure and the resulting residue was purified by column chromatography (Hexane : Ethyl Acetate 8:3) to afford 2.65 g (87%) of the title compound.
MS: 153(M+H)

111 3-(2-deoxy-β-D-ribofuranosyl)-6-(1-naphthyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[2577]



To a stirred solution of IDU **1** (1.06 g, 3 mmol) in dry DMF (15 m) at room temperature, 1-ethynylnaphtalene (1.37 g, 3 eq.), *tetrakis*(triphenylphosphine)Pd^0 (0.35 g, 0.1 eq.), copper(I) iodide (0.12 g, 0.2 eq.) and DIPEA (1 ml, 2 eq.) were added. The reaction mixture was stirred for 15 hours at room temperature. The solvent was removed under reduced pressure and the resulting residue was dissolved in DCM and left at 0°C for 5 hours. The resulting precipitate was filtered and washed with DCM. The precipitate was then dissolved in anhydrous Acetone (15 ml) and TEA (15 ml) plus silver nitrate (0.51g, 1 eq.) were added. The reaction mixture was then heated at 60°C and stirred for 5 hours. The solvents were removed under reduced pressure and the resulting residue was purified by column chromatography to yield the titled compound.

White solid: 0.40 g, 35%

^{1}H-NMR (DMSO, 500 MHz): δ 8.94 (1H, s, H-4), 8.35 (1H, d, J 8.2 Hz, Naph), 8.11-8.04 (2H, m, Naph), 7.90 (1H, d, J 7.2 Hz, Naph), 7.70-7.61 (3H, m, Naph), 7.23 (1H, s, H-5), 6.23 (1H, t, J 6.1 Hz, H-1'), 5.31 (1H, d, J 4.3 Hz, OH-3'), 5.17 (1H, t, J 5.2 Hz, OH-5'), 4.32-4.26 (1H, m, H-3'), 4.00-3.95 (1H, m, H-4'), 3.77-3.63 (2H, m, H-5'), 2.50-2.43 (1H, m, H-2'$_\alpha$), 2.14 (1H, dt, J 13.4 Hz 6.2 Hz, H-2'$_\beta$)

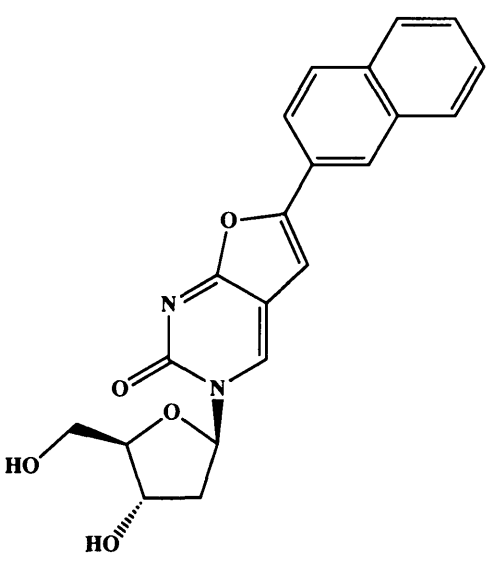
^{13}C-NMR (DMSO, 125 MHz): δ 171.06 (C7a), 153.80 (C6), 153.17 (C2), 138.62 (C4), 133.46 (Naph), 130.29 (Naph), 129.532 (Naph), 128.81 (Naph), 127.42 (Naph), 127.25 (Naph), 126.49 (Naph), 125.80 (Naph), 125.49 (Naph), 124.68 (Naph), 106.62 (C4a), 103.96 (C5), 88.25 (C4'), 87.72 (C1'), 69.67 (C3'), 60.79 (C5'), 41.31

Anal. Calcd for $C_{21}H_{18}N_2O_5$: C 66.66, H 4.79, N 7.40, O 21.14. Found C 66.20, H 5.10, N 7.79, O 20.91

MS: 401 (M+Na)

112 3-(2-deoxy-β-D-ribofuranosyl)-6-(2-naphthyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2520]



Prepared according to Standard Procedure D using 2-ethynylnaphtalene.

Yellow solid: 0.84 g, 74%

^{1}H-NMR (DMSO, 500 MHz) : δ 8.81 (1H, s, H-4), 8.33 (1H, s, Naph), 8.09-7.95 (4H, m, Naph), 7.60-7.56 (2H, m, Naph), 7.40 (1H, s, H-5), 6.20 (1H, t, J 5.7 Hz, H-1'), 5.28 (1H, d, J 4.0 Hz, OH-3'), 5.20 (1H, t, J 5.2 Hz, OH-5'), 4.29-4.25 (1H, m, H-3'), 3.96-3.92 (1H, m, H-4'), 3.72 (1H, ddd, J 12.1 Hz 5.1 Hz 3.6 Hz, H-5'a), 3.65 (1H, ddd, J 12.1 Hz 5.1 Hz 4.2 Hz, H-5'b), 2.45 (1H, ddd, J 13.4 Hz 6.2 Hz 4.0 Hz, H-2'$_\alpha$), 2.11 (1H, dt, J 13.4 Hz 6.0 Hz, H-2'$_\beta$)

^{13}C-NMR (DMSO, 125 MHz): δ 171.15 (C7a), 153.79 (C6), 153.63 (C2), 138.28 (C4), 133.01 (Naph), 132.81 (Naph), 128.75 (Naph), 128.47 (Naph), 127.70 (Naph), 127.05 (Naph),

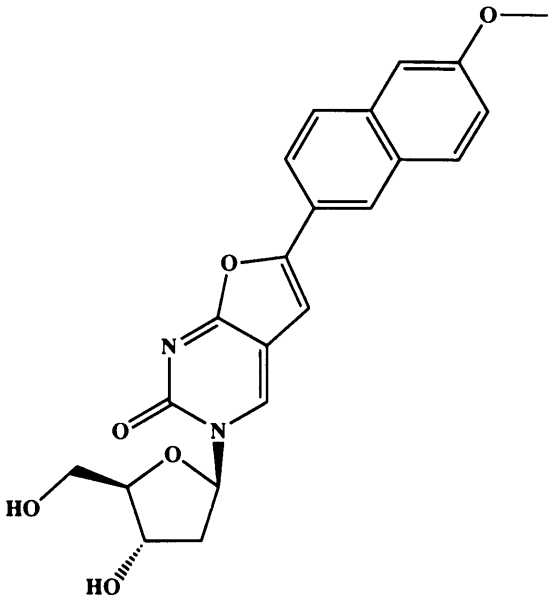
126.98 (Naph), 125.70 (Naph), 123.41 (Naph), 122.22 (Naph), 106.85 (C4a), 100.32 (C5), 88.18 (C4'), 87.65 (C1'), 69.50 (C3'), 60.66 (C5'), 41.25

Anal. Calcd for $C_{21}H_{18}N_2O_5$: C 66.66, H 4.79, N 7.40, O 21.14. Found C 66.28, H 4.80, N 7.69, O 21.23

MS: 401 (M+Na)

112 3-(2-deoxy-β-D-ribofuranosyl)-6-(2-naphthyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2523]



Prepared according to Standard Procedure D using 2-ethynylnaphtalene.

Yellow solid: 0.81 g, 66%

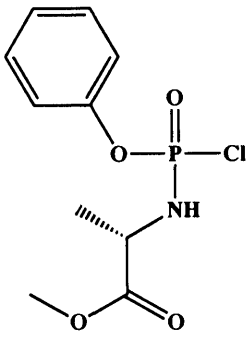
^{1}H-NMR (DMSO, 500 MHz): δ 8.75 (1H, s, H-4), 8.23 (1H, s, Naph), 7.98 (1H, d, J 9.3 Hz, Naph), 7.88 (2H, s, Naph), 7.38 (1H, d, J 2.3 Hz, Naph), 7.30 (1H, s, H-5), 7.21 (1H, dd, J 9.2 Hz 2.7, Naph), 6.21 (1H, t, J 6.0 Hz, H-1'), 5.28 (1H, d, J 4.0 Hz, OH-3'), 5.20 (1H, t, J 5.3 Hz, OH-5'), 4.29-4.25 (1H, m, H-3'), 3.96-3.92 (1H, m, H-4'), 3.90 (3H, s, OCH_3), 3.72 (1H, ddd, J 12.1 Hz 5.1 Hz 3.6 Hz, H-5'a), 3.65 (1H, ddd, J 12.1 Hz 5.1 Hz 4.2 Hz, H-5'b), 2.45 (1H, ddd, J 13.1 Hz 6.0 Hz 4.1 Hz, H-2'$_\alpha$), 2.11 (1H, dt, J 13.1 Hz 6.0 Hz, H-2'$_\beta$)

^{13}C-NMR (DMSO, 125 MHz): δ 171.13 (C7a), 158.19 (Naph), 154.00 (C6), 153.80 (C2), 137.75 (C4), 134.56 (Naph), 130.05 (Naph), 128.13 (Naph), 127.59 (Naph), 123.43 (Naph), 123.39 (Naph), 122.71 (Naph), 119.41 (Naph), 106.99 (C4a), 106.23 (Naph), 99.11 (C5), 88.16 (C4'), 87.59 (C1'), 69.54 (C3'), 60.69 (C5'), 55.30 (OCH_3), 41.25

Anal. Calcd for $C_{22}H_{20}N_2O_6$: C 64.70, H 4.94, N 6.86, O 23.51. Found C 64.46, H 4.80, N 6.80, O 23.94

MS: 431 (M+Na)

125a Phenyl(methoxy-L-Alaninyl)phosphochloridate



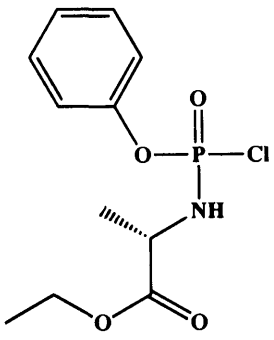
Prepared according to standard procedure A using Alanine methylester hydrochloride.

Colourless oil: 1.66 g, 60%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.40-7.18 (5H, m, Ph), 4.95-4.73 (1H, bs, NH), 4.32-4.10 (1H, m, Ala-CH), 3.82 3.78 (3H, 2 s, est CH_3), 1.58-1.44 (3H, m, Ala-CH_3).

^{31}P-NMR ($CDCl_3$, 202 MHz): δ 8.08, 7.88

125b Phenyl(ethoxy-L-Alaninyl)phosphochloridate



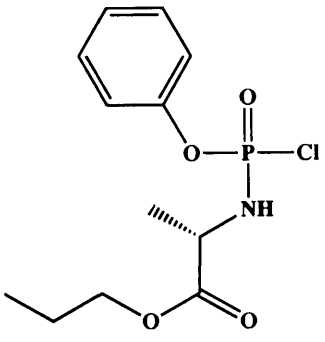
Prepared according to standard procedure A using Alanine ethylester hydrochloride.

Colourless oil: 2.33 g, 80%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.30-7.18 (5H, m, Ph), 4.95-4.83 (1H, bs, NH), 4.22-4.01 (3H, m, Ala-CH, est CH_2), 1.44-1.39 (3H, m, Ala-CH_3), 1.22-1.10 (3H, m, est CH_3)

^{31}P-NMR ($CDCl_3$, 202 MHz): δ 7.97, 7.60

125c Phenyl(n-propoxy-L-Alaninyl)phosphochloridate



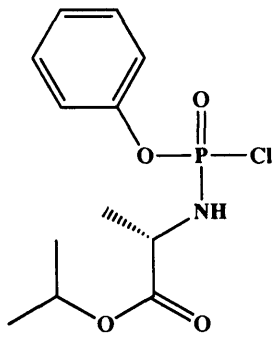
Prepared according to standard procedure A using Alanine n-propylester hydrochloride

Colourless oil: 1.98 g, 80%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.45-7.18 (5H, m, Ph), 5.32-5.15 (1H, bs, NH), 4.22-4.07 (3H, m, Ala-CH, est CH_2), 1.85-1.62, (2H, m, est CH_2), 1.61-1.50 (3H, m, Ala-CH_3), 0.98-0.85 (3H, m, est CH_3)

^{31}P-NMR ($CDCl_3$, 202 MHz): δ 7.80, 7.50

125d Phenyl(i-propoxy-L-Alaninyl)phosphochloridate



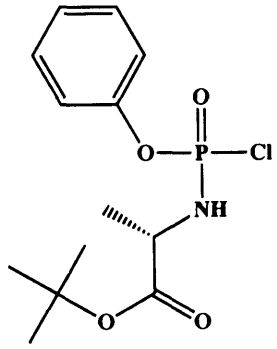
Prepared according to standard procedure A using Alanine i-propylester hydrochloride

Colourless oil: 2.17 g, 71%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.45-7.18 (5H, m, Ph), 5.32-5.10 (1H, m, est CH), 4.72-4.57 (1H, bs, NH), 4.22-4.07 (1H, m, Ala-CH) 1.62-1.58, (3H, m, Ala-CH_3), 1.39-1.28 (6H, m, est CH_3)

^{31}P-NMR ($CDCl_3$, 202 MHz): δ 7.90, 7.50

125e Phenyl(t-butyloxy-L-Alaninyl)phosphochloridate



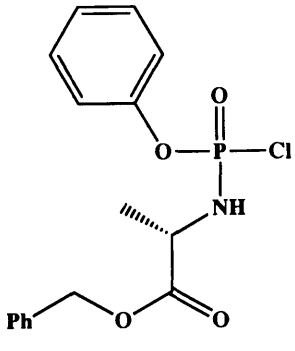
Prepared according to standard procedure A using Alanine t-butylester hydrochloride

Colourless oil: 1.73 g, 54%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.48-7.23 (5H, m, Ph), 4.65-4.48 (1H, bs, NH), 4.22-4.00 (1H, m, Ala-CH) 1.60-1.57, (12H, m, est CH_3, Ala-CH_3)

^{31}P-NMR ($CDCl_3$, 202 MHz): δ 8.08, 7.79

125f Phenyl(benzyloxy-L-Alaninyl)phosphochloridate



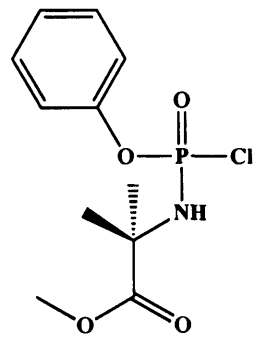
Prepared according to standard procedure A using Alanine benzylester hydrochloride

Colourless oil: 2.40 g, 68%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.42-7.20 (10H, m, Ph+Bn), 5.30-5.21(2H, m, Bn-CH_2), 4.50-4.35 (1H, m, NH), 4.35-4.20 (1H, m, Ala-CH) 1.61-1.53 (3H, m, Ala-CH_3)

^{31}P-NMR ($CDCl_3$, 202 MHz): δ 7.97, 7.60

125g Phenyl(methoxy-dimethylglycinyl)phosphochloridate



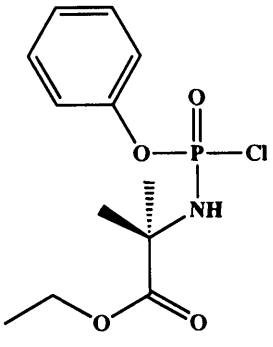
Prepared according to standard procedure A using Dimethylglycine methylester hydrochloride.

Colourless oil: 1.57 g, 54%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.41-7.19 (5H, m, Ph), 4.97 (1H, d, J 10.0 Hz, NH), 3.79 (3H, s, est CH_3), 1.70 (3H, s, DMG-CH_3), 1.68 (3H, s, DMG-CH_3).

^{31}P-NMR ($CDCl_3$, 202 MHz): δ 6.97

125h Phenyl(ethoxy-dimethylglycinyl)phosphochloridate



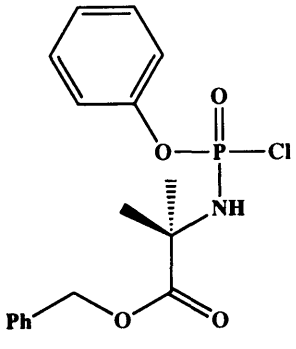
Prepared according to standard procedure A using Dimethylglycine ethylester hydrochloride.

Colourless oil: 1.37 g, 45%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.38-7.16 (5H, m, Ph), 5.04 (1H, d, J 10.3 Hz, NH), 4.21 (2H, q, J 7.3 Hz, est CH_2), 1.67 (3H, s, DMG-CH_3), 1.66 (3H, s, DMG-CH_3), 1.27 (3H, t, J 7.1 Hz, est CH_3)

^{31}P-NMR ($CDCl_3$, 202 MHz): δ 7.05

125i Phenyl(ethoxy-dimethylglycinyl)phosphochloridate



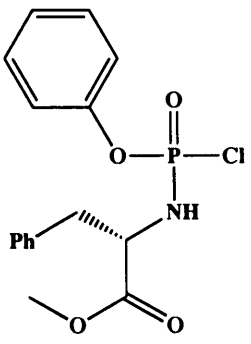
Prepared according to standard procedure A using Dimethylglycine benzylester hydrochloride.

Colourless oil: 2.83 g, 77%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.51-7.26 (10H, m, Ph+ Bn), 5.30 (2H, s, Bn-CH_2), 5.08 (1H, d, J 9.8 Hz, NH), 1.80 (3H, s, DMG-CH_3), 1.78 (3H, s, DMG-CH_3)

^{31}P-NMR ($CDCl_3$, 202 MHz): δ 6.98

125j Phenyl(methoxy-phenylalaninyl)phosphochloridate



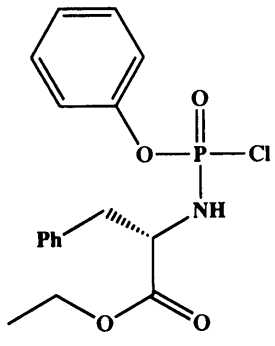
Prepared according to standard procedure A using phenylalanine methylester hydrochloride.

Colourless oil: 2.23 g, 63%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.46-7.16 (10H, m, Phe-Ph+Ph), 4.68-4.38 (2H, m, Phe-CH+NH), 3.78 3.75 (3H, 2s, est CH_3), 3.21-3.16 (2H, m, Phe-CH_2)

^{31}P-NMR ($CDCl_3$, 202 MHz): δ 9.43, 9.25

125k Phenyl(ethoxy-phenylalaninyl)phosphochloridate



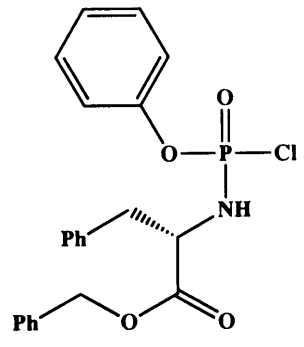
Prepared according to standard procedure A using phenylalanine ethylester hydrochloride.

Colourless oil: 2.13 g, 58%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.41-7.12 (10H, m, Phe-Ph+Ph), 4.50-4.24 (2H, m, Phe-CH+NH), 4.24-4.07 (2H, m, est CH_2), 3.18-3.11 (2H, m, Phe-CH_2), 1.30-1.18 (3H, m, est CH_3)

^{31}P-NMR ($CDCl_3$, 202 MHz): δ 9.36, 9.26

125j Phenyl(benzyloxy-phenylalaninyl)phosphochloridate



Prepared according to standard procedure A using phenylalanine benzylester hydrochloride.

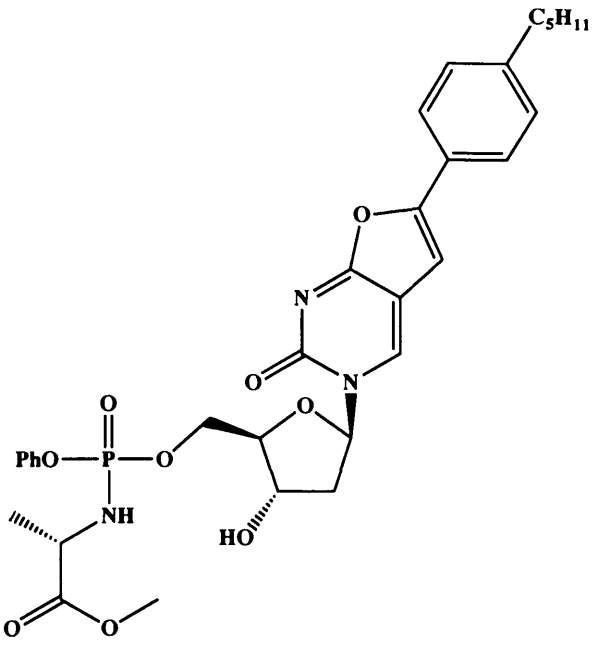
White solid: 2.13 g, 46%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.41-7.01 (15H, m, Phe-Ph+Ph+Bn), 5.21-5.15 (2H, m, Bn-CH_2), 4.62-4.39 (1H, m, Phe-CH), 4.33-4.15 (1H, m, NH), 3.24-3.07 (2H, m, Phe-CH_2)

^{31}P-NMR ($CDCl_3$, 202 MHz): δ 9.10, 9.07

126a 3-(2-deoxy-β-D-ribofuranosyl-5-[phenyl-(methoxy-L-alaninyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2451]



Prepared according to Standard Procedure B using **125a**

White foam: 0.04 g, 12%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.60 8.55 (1H, 2s, H-4), 7.69-7.62 (2H, m, BCNA-Ph), 7.40-7.10 (7H, m, BCNA-Ph+Ph), 6.60 6.58 (1H, 2s, H-5), 6.43-6.30 (1H, m, H-1'), 4.62-3.89 (5H, m, H-3'+H-4'+ Ala-CH+ H-5'), 3.76 3.70 (3H, 2s, est CH_3), 2.90-2.74 (1H, m, H-2'$_\alpha$), 2.71-2.60 (2H, m, $CH_2\alpha$), 2.20-2.06 (1H, m, H-2'$_\beta$), 1.74-1.59 (2H, m, $CH_2\beta$), 1.49-1.22 (7H, m, $CH_2\gamma$+$CH_2\delta$+Ala+CH_3), 0.97-0.84 (3H, m, $CH_3\omega$)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 173.1 173.0 (Ala-CO), 170.6 (C2), 154.8 (C7a), 153.7 (C6), 149.3 149.2 (Ph), 144.0 (C4a), 134.9 (C5), 128.8 128.0, 124.9, 124.3, 123.8, 119.1, 119.0, 107.4 (Ph), 96.2 96.1 (C4), 87.2 (C1'), 84.4 84.3 (C4'), 68.9 (C3'), 64.5 (C5'), 51.7 (est CH_3), 49.4 49.2 (Ala-CH), 40.5 (C2'), 34.8 ($CH_2\alpha$), 30.4 ($CH_2\gamma$), 29.9 ($CH_2\beta$), 21.5 ($CH_2\delta$), 19.9 19.8 (Ala-CH_3), 13.0 ($CH_3\omega$)

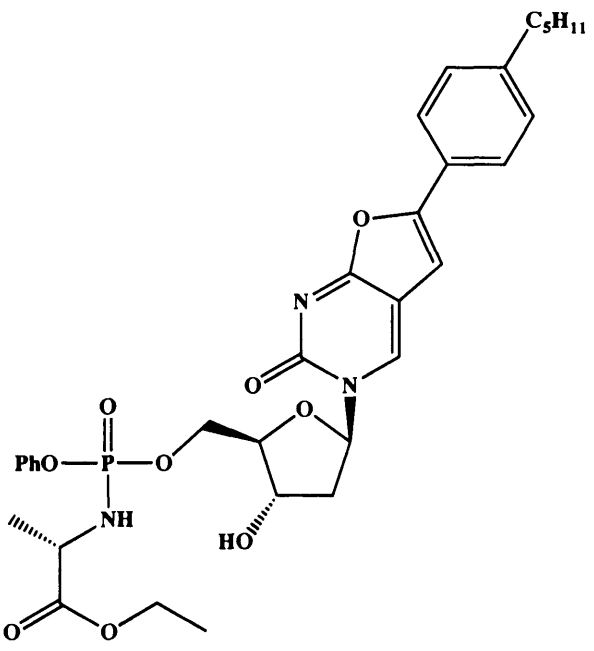
^{31}P-NMR ($CDCl_3$, 202 MHz): δ 4.41, 4.17

Anal. Calcd for $C_{32}H_{38}N_3O_9P$: C 60.09, H 5.99, N 6.57, O 22.51. Found C 60.46, H 5.67, N 6.80

MS: 662 (M+Na)

126b 3-(2-deoxy-β-D-ribofuranosyl-5-[phenyl-(ethoxy-L-alaninyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2452]



Prepared according to Standard Procedure B using **125b**

White foam: 0.02 g, 6%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.60 8.59 (1H, 2s, H-4), 7.69-7.58 (2H, m, BCNA-Ph), 7.40-7.07 (7H, m, BCNA-Ph+Ph), 6.72 6.59 (1H, 2s, H-5), 6.43-6.30 (1H, m, H-1'), 4.60-3.65 (7H, H-3'+H-4'+ Ala-CH+ H-5'+est CH_2), 2.90-2.74 (1H, m, H-2'$_\alpha$), 2.71-2.60 (2H, m, $CH_2\alpha$), 2.20-2.06 (1H, m, H-2'$_\beta$), 1.78-1.55 (2H, m, $CH_2\beta$), 1.49-1.12 (10H, m, $CH_2\gamma$+$CH_2\delta$+Ala-CH_3+est CH_3), 1.01-0.81 (3H, m, $CH_3\omega$)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 172.6 172.5 (Ala-CO), 170.5 (C2), 154.9 154.7 (C7a), 153.9 (C6), 149.5 149.4 149.3 (Ph), 143.9 (C4a), 135.2 135.0 (C5), 128.9 128.0, 127.8, 124.9, 124.8, 124.3, 124.2, 123.8, 119.2, 119.1, 107.4 (Ph), 96.4 96.2 (C4), 87.4 (C1'), 84.7 84.6 (C4'), 69.1 69.0 (C3'), 65.0 64.8 64.7 (C5'), 60.8 60.7 (est CH_2), 49.5 49.3 (Ala-CH), 40.6 (C2'), 34.8 ($CH_2\alpha$), 30.4 ($CH_2\gamma$), 29.9 ($CH_2\beta$), 21.5 ($CH_2\delta$), 19.9 19.8 (Ala-CH_3), 13.1 13.0 ($CH_3\omega$), 9.9 (est CH_3)

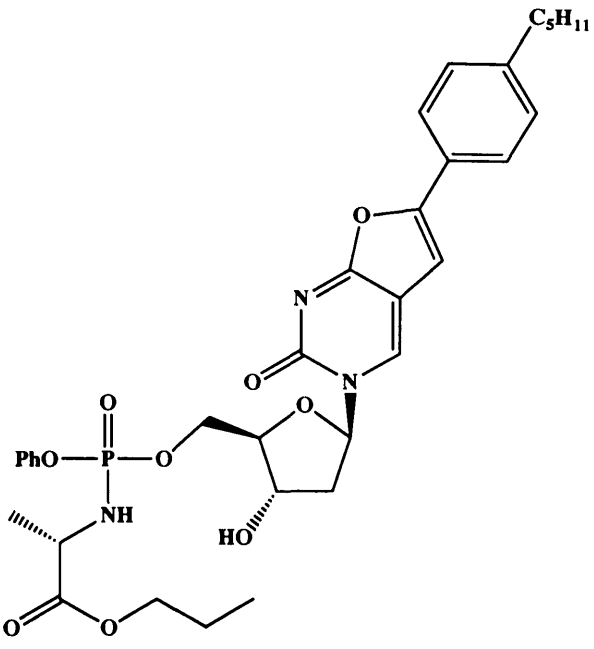
^{31}P-NMR ($CDCl_3$, 202 MHz): δ 4.45 4.36

Anal. Calcd for $C_{33}H_{40}N_3O_9P$: C 60.64, H 6.17, N 6.43, O 22.03. Found C 60.65, H 6.47, N 6.85

MS: 676 (M+Na)

126c 3-(2-deoxy-β-D-ribofuranosyl-5-[phenyl-(n-propoxy-L-alaninyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2453]



Prepared according to Standard Procedure B using **125c**

White foam: 0.04 g, 11%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.49 8.47 (1H, 2s, H-4), 7.56-7.46 (2H, m, BCNA-Ph), 7.29-6.95 (7H, m, BCNA-Ph+Ph), 6.50 6.45 (1H, 2s, H-5), 6.35-6.20 (1H, m, H-1'), 4.61-3.20 (8H, H-3'+H-4'+ Ala-CH+ H-5'+est CH_2+ NH), 2.80-2.62 (1H, m, H-2'$_\alpha$), 2.60-2.40 (2H, m, $CH_2\alpha$), 2.10-1.90 (1H, m, H-2'$_\beta$), 1.65-1.40 (4H, m, $CH_2\beta$+ est CH_2), 1.40-1.12 (7H, m, $CH_2\gamma$+$CH_2\delta$+Ala-CH_3), 0.91-0.65 (6H, m, $CH_3\omega$+est CH_3)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 174.2 (Ala-CO), 172.0 (C2), 156.3 (C7a), 155.1 (C6), 150.5 (Ph), 145.4 (C4a), 136.5 (C5), 130.3, 129.4, 126.7, 125.8, 125.7, 120.6, 120.5, 109.0 (Ph), 97.8 97.6 (C4), 88.7 88.6 (C1'), 86.0 (C4'), 70.1 (C3'), 67.8 67.7 (est CH_2), 66.0 (C5'), 51.0 50.9 (Ala-CH), 42.5 (C2'), 36.2 ($CH_2\alpha$), 31.9 ($CH_2\gamma$), 31.3 ($CH_2\beta$), 22.9 ($CH_2\delta$), 22.2 (est CH_2), 21.3 (Ala-CH_3), 14.4 ($CH_3\omega$), 10.6 (est CH_3)

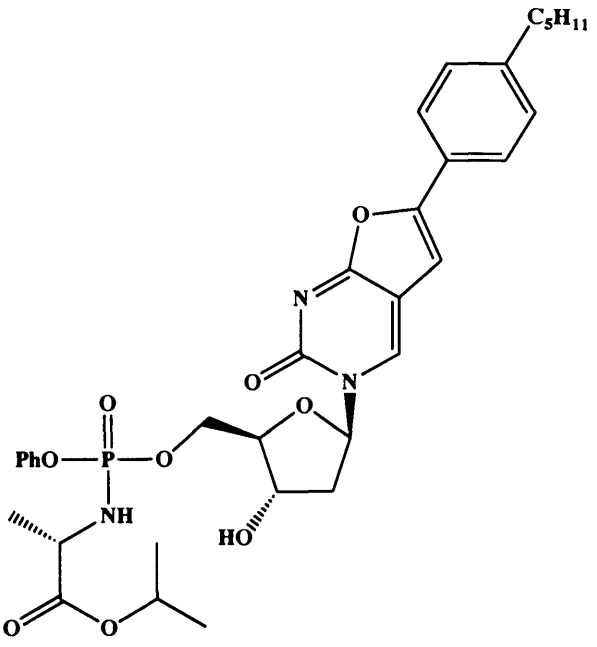
^{31}P-NMR ($CDCl_3$, 202 MHz): δ 4.61 4.43

Anal. Calcd for $C_{34}H_{42}N_3O_9P$: C 61.16, H 6.34, N 6.29, O 21.57. Found C 60.86, H 6.43, N 6.01

MS: 690 (M+Na)

126d 3-(2-deoxy-β-D-ribofuranosyl-5-[phenyl-(i-propoxy-L-alaninyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2454]



Prepared according to Standard Procedure B using **125d**

White foam: 0.05 g, 15%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.60 8.55 (1H, 2s, H-4), 7.69-7.62 (2H, m, BCNA-Ph), 7.40-7.10 (7H, m, BCNA-Ph+Ph), 6.60 6.58 (1H, 2s, H-5), 6.43-6.30 (1H, m, H-1'), 5.10-4.88 (1H, m, est CH), 4.62-3.89 (5H, H-3'+H-4'+ Ala-CH+ H-5'), 2.88-2.70 (1H, m, H-2'$_\alpha$), 2.70-2.53 (2H, m, $CH_2\alpha$), 2.18-2.00 (1H, m, H-2'$_\beta$), 1.74-1.51 (2H, m, CH_2), 1.49-1.05 (13H, m, $CH_2\gamma$+$CH_2\delta$+Ala-CH_3+ est CH_3), 1.01-0.81 (3H, m, CH_3)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 173.5 173.4 (Ala-CO), 172.0 (C2), 156.2 156.1 (C7a), 155.3 (C6), 150.7 150.8 (Ph), 145.3 (C4a), 136.6 136.4 (C5), 130.2, 129.4, 126.3, 125.6, 125.2, 120.7, 120.6, 108.9 (Ph), 97.8 97.7 (C4), 88.8 88.7 (C1'), 86.1 (C4'), 70.4 70.3 (est CH), 69.9 69.8 (C3'), 66.2 (C5'), 51.0 50.8 (Ala-CH), 42.0 (C2'), 36.2 ($CH_2\alpha$), 31.9 ($CH_2\gamma$), 31.3 ($CH_2\beta$), 22.9 ($CH_2\delta$), 22.2 22.1 (est CH_3), 21.3 (Ala-CH_3), 14.4 ($CH_3\omega$)

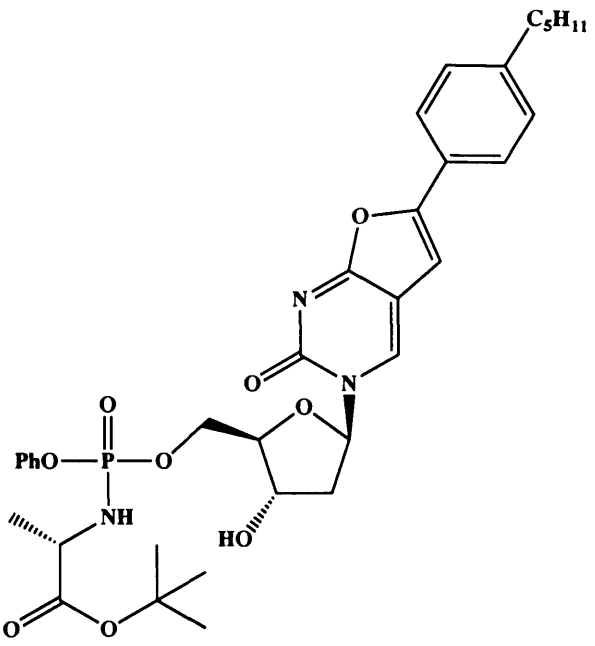
^{31}P-NMR ($CDCl_3$, 202 MHz): δ 4.49 4.43

Anal. Calcd for $C_{34}H_{42}N_3O_9P$: C 61.16, H 6.34, N 6.29, O 21.57. Found C 60.06, H 6.33, N 6.41

MS: 690 (M+Na)

126e 3-(2-deoxy-β-D-ribofuranosyl-5-[phenyl-(t-butoxy-L-alaninyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2455]



Prepared according to Standard Procedure B using **125e**

White foam: 0.06 g, 17%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.60 8.55 (1H, 2s, H-4), 7.69-7.62 (2H, m, BCNA-Ph), 7.40-7.10 (7H, m, BCNA-Ph+Ph), 6.60 6.58 (1H, 2s, H-5), 6.43-6.30 (1H, m, H-1'), 4.62-3.89 (6H, H-3'+H-4'+ Ala-CH+ H-5'+NH), 2.90-2.74 (1H, m, H-2'$_\alpha$), 2.71-2.60 (2H, m, $CH_2\alpha$), 2.20-2.06 (1H, m, H-2'$_\beta$), 1.74-1.59 (2H, m, CH_2), 1.49-1.22 (16H, m, $CH_2\gamma$+$CH_2\delta$+Ala-CH_3+ est CH_3), 0.97-0.84 (3H, m, CH_3)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 171.7 171.6 (Ala-CO), 170.6 (C2), 154.9 154.7 (C7a), 153.8 (C6), 149.5 149.4 149.3 (Ph), 143.9 (C4a), 135.0 134.8 (C5), 128.8, 128.0, 124.9, 124.3, 124.2, 123.9, 119.2, 119.1, 107.5 (Ph), 96.4 96.2 (C4), 87.3 87.1 (C1'), 84.6 84.5 (C4'), 81.4 81.3 (est C), 69.0 68.8 (C3'), 64.7 (C5'), 49.9 49.8 49.7 (Ala-CH), 40.7 40.5 (C2'), 34.8 ($CH_2\alpha$), 30.4 ($CH_2\gamma$), 29.9 ($CH_2\beta$), 26.9 (est CH_3), 21.5 ($CH_2\delta$), 20.1 20.0 (Ala-CH_3), 13.0 ($CH_3\omega$)

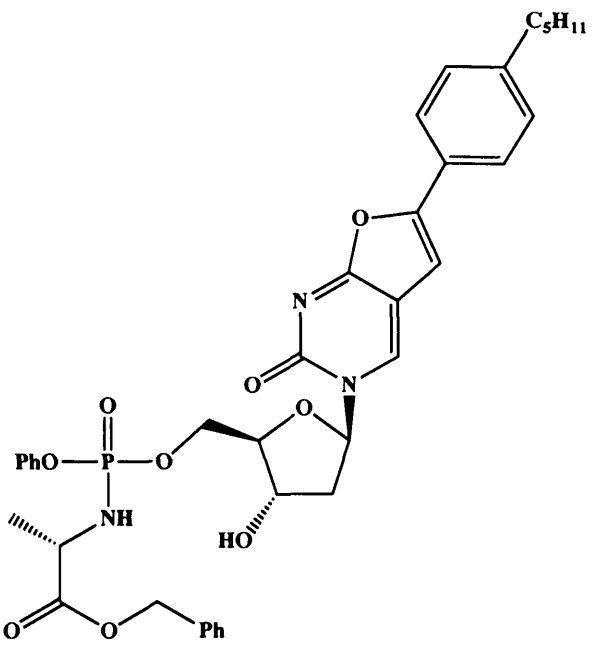
^{31}P-NMR ($CDCl_3$, 202 MHz): δ 4.59 4.52

Anal. Calcd for $C_{35}H_{44}N_3O_9P$: C 61.66, H 6.51, N 6.16, O 21.12. Found C 61.70, H 6.48, N 6.10

MS: 704 (M+Na)

126f 3-(2-deoxy-β-D-ribofuranosyl-5-[phenyl-(benzyloxy-L-alaninyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2300]



Prepared according to Standard Procedure B using **125f**

White foam: 0.09 g, 24%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.45 8.34 (1H, 2s, H-4), 7.56-7.48 (2H, m, BCNA-Ph), 7.27-7.00 (12H, m, BCNA-Ph+Ph+Bn), 6.47 6.45 (1H, 2s, H-5), 6.27-6.21 (1H, m, H-1'), 5.07-4.98 (2H, m, Bn-CH_2), 4.44-3.88 (6H, m, H-3'+H-4'+Ala-CH+H-5'+NH), 2.73-2.60 (1H, m, H-2'$_\alpha$), 2.59-2.50 (2H, m, $CH_2\alpha$), 2.05-1.85 (1H, m, H-2'$_\beta$), 1.60-1.50 (2H, m, $CH_2\beta$), 1.36-1.09 (7H, m, $CH_2\gamma$+$CH_2\delta$+Ala+CH_3), 0.87-0.80 (3H, m, $CH_3\omega$)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 172.9 172.8 (Ala-CO), 170.6 (C2), 154.8 (C7a), 153.7 (C6), 146.4 146.3 (C4a), 138.7 138.5 (C5), 130.9, 130.8, 130.2, 129.6, 129.4, 129.3, 129.2, 126.3, 125.9, 125.8, 121.6, 121.5, 121.3, 121.2, 104.7, 104.5 (Ph), 99.2 99.1 (C4), 90.3 90.2 (C1'), 87.8 87.7 87.6 (C4'), 71.7 71.6 (C3'), 68.1 68.0 (Bn-CH_2), 67.5 67.4 67.3 67.2 (C5'), 51.9 51.7 (Ala-CH), 42.8 42.7 (C2'), 36.7 ($CH_2\alpha$), 32.6 ($CH_2\gamma$), 32.2 ($CH_2\beta$), 23.6 ($CH_2\delta$), 20.3 (Ala-CH_3), 14.4 ($CH_3\omega$)

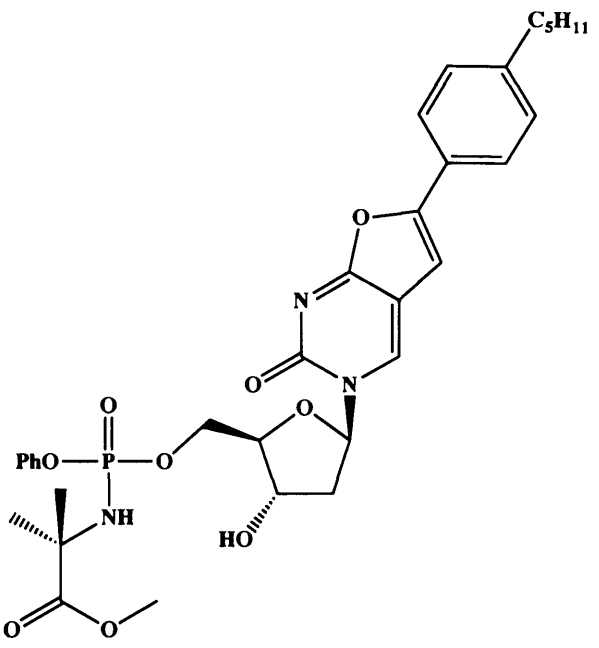
^{31}P-NMR ($CDCl_3$, 202 MHz): δ 4.22, 3.49

Anal. Calcd for $C_{38}H_{42}N_3O_9P$: C 63.77, H 5.91, N 5.57, O 20.12. Found C 63.80, H 5.90, N 5.85

MS: 738 (M+Na)

126g 3-(2-deoxy-β-D-ribofuranosyl-5-[phenyl-(methoxy-dimethylglycinyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2481]



Prepared according to Standard Procedure B using **125g**

White foam: 0.07 g, 22%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.63 8.55 (1H, 2s, H-4), 7.72-7.59 (2H, m, BCNA-Ph), 7.41-7.20 (7H, m, BCNA-Ph+Ph), 6.61 6.59 (1H, 2s, H-5), 6.48-6.37 (1H, m, H-1'), 4.84-4.73 (1H, bs, NH), 4.64-4.42 (2H, m, H-3'+H-4'), 4.41-4.29 (2H, m, H-5'), 3.76 (3H, 2, est CH_3), 2.94-2.76 (1H, m, H-2'$_\alpha$), 2.73-2.61 (2H, m, $CH_2\alpha$), 2.18-2.02 (1H, m, H-2'$_\beta$), 1.75-1.56 (8H, m, $CH_2\beta$+ DMG-CH_3), 1.47-1.32 (4H, m, $CH_2\gamma$+CH_2), 0.95-0.84 (3H, m, $CH_3\omega$)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 176.4 176.2 176.1 (DMG-CO), 172.0 (C2), 156.3 156.1 (C7a), 155.2 (C6), 151.1 151.0 150.9 (Ph), 145.4 145.3 (C4a), 136.6 136.3 (C5), 130.2, 130.1, 129.4, 126.3, 125.6, 125.3, 120.7, 120.6, 108.9 (Ph), 97.8 97.6 (C4), 88.8 88.6 (C1'), 86.1 86.0 (C4'), 70.6 70.4 (C3'), 66.4 66.3 (C5'), 57.7 57.6 (DMG-C), 53.4 (est CH_3), 42.1 42.0 (C2'), 36.2 ($CH_2\alpha$), 31.9 ($CH_2\gamma$), 31.4 ($CH_2\beta$), 27.5 27.4 (DMG-CH_3), 22.9 ($CH_2\delta$), 14.4 ($CH_3\omega$)

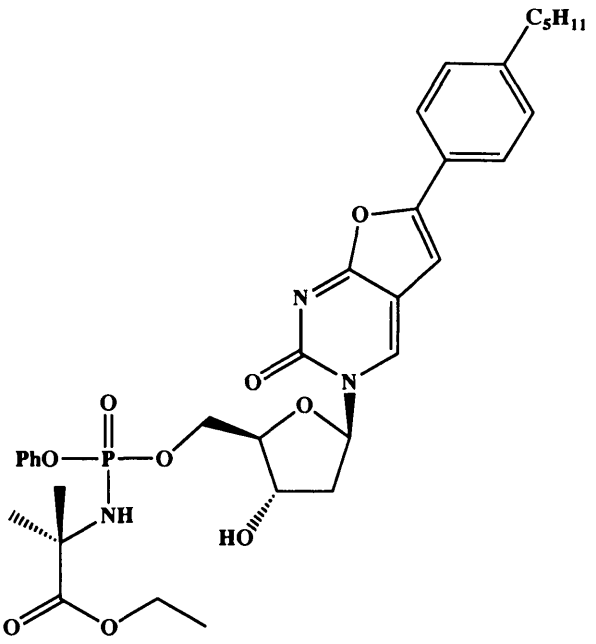
^{31}P-NMR ($CDCl_3$, 202 MHz): δ 3.01, 2.93

Anal. Calcd for $C_{33}H_{40}N_3O_9P$: C 60.64, H 6.17, N 6.43, O 22.03. Found C 60.58, H 6.20, N 6.80

MS: 676 (M+Na)

126h 3-(2-deoxy-β-D-ribofuranosyl-5-[phenyl-(ethoxy-dimethylglycinyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2482]



Prepared according to Standard Procedure B using **125h**

White foam: 0.06 g, 19%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.51 8.40 (1H, 2s, H-4), 7.61-7.53 (2H, m, BCNA-Ph), 7.22-6.99 (7H, m, BCNA-Ph+Ph), 6.50 (1H, 2s, H-5), 6.35-6.26 (1H, m, H-1'), 4.52-4.04 (7H, m, NH+H-3'+H-4'+H-5'+est CH_2), 2.85-2.62 (1H, m, H-2'$_\alpha$), 2.62-2.49 (2H, m, $CH_2\alpha$), 2.06-1.82 (1H, m, H-2'$_\beta$), 1.65-1.41 (8H, m, $CH_2\beta$+ DMG-CH_3), 1.40-1.09 (7H, m, $CH_2\gamma$+$CH_2\delta$+est CH_3), 0.95-0.91 (3H, m, $CH_3\omega$)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 175.9 175.7 175.6 (DMG-CO), 172.0 171.9 (C2), 156.2 156.1 (C7a), 155.3 155.2 (C6), 151.1 151.0 150.9 (Ph), 145.4 145.3 (C4a), 136.7 136.4 (C5), 130.2, 130.1, 130.0, 129.4, 126.3, 125.5, 125.2, 120.7, 120.6, 120.5, 108.9 108.8 (Ph), 97.8 97.6 (C4), 88.8 88.7 (C1'), 86.2 86.1 (C4'), 70.6 70.4 (C3'), 66.4 (C5'), 62.4 (est CH_2), 57.5 (DMG-C), 42.1 42.0 (C2'), 36.2 ($CH_2\alpha$), 31.9 ($CH_2\gamma$), 31.4 ($CH_2\beta$), 27.4 27.3 (DMG-CH_3), 22.9 ($CH_2\delta$), 14.5 14.4 ($CH_3\omega$), 14.3 (est CH_3)

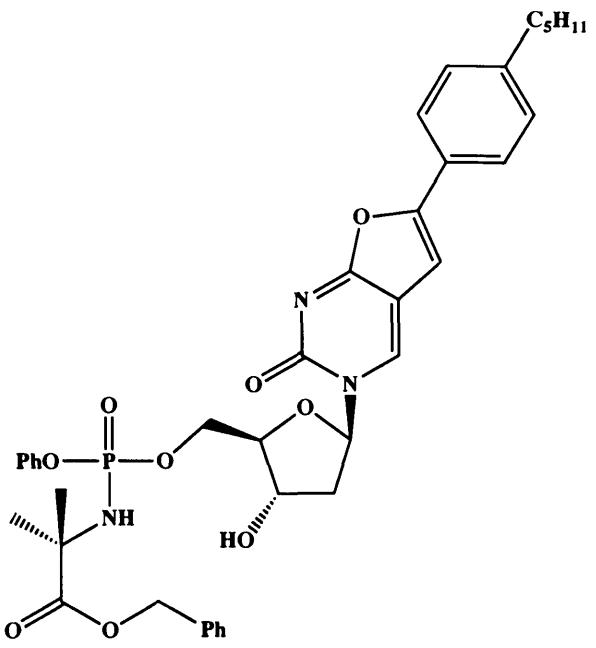
^{31}P-NMR ($CDCl_3$, 202 MHz): δ 3.01, 2.87

Anal. Calcd for $C_{34}H_{42}N_3O_9P$: C 61.16, H 6.34, N 6.29, O 21.57. Found C 60.98, H 6.30, N 6.09

MS: 690 (M+Na)

126i 3-(2-deoxy-β-D-ribofuranosyl-5-[phenyl-(benzyloxy-dimethylglycinyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2483]



Prepared according to Standard Procedure B using **125i**

White foam: 0.05 g, 15%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.54 8.46 (1H, 2s, H-4), 7.62-7.51 (2H, m, BCNA-Ph), 7.37-6.99 (12H, m, BCNA-Ph+Ph+Bn), 6.52 6.49 (1H, 2s, H-5), 6.40-6.25 (1H, m, H-1'), 4.92-4.72 (2H, m, Bn-CH_2), 4.54-4.17 (5H, m, NH+H-3'+H-4'+H-5'), 2.86-2.65 (1H, m, H-2'$_\alpha$), 2.65-2.53 (2H, m, $CH_2\alpha$), 2.08-1.90 (1H, m, H-2'$_\beta$), 1.68-1.49 (8H, m, $CH_2\beta$+ DMG-CH_3), 1.40-1.22 (4H, m, $CH_2\gamma$+$CH_2\delta$), 0.96-0.80 (3H, m, $CH_3\omega$)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 174.3 174.2 (DMG-CO), 170.5 (C2), 154.9 154.7 (C7a), 153.9 (C6), 149.5 (Ph), 143.9 (C4a), 135.3 135.0 (C5), 134.3, 134.2, 128.8, 128.7, 127.9, 127.6, 127.4, 127.0, 126.9, 124.8, 124.7, 124.2, 123.8, 119.3, 119.2, 107.6 (Ph), 96.4 96.2 (C4), 87.3 87.1 (C1'), 84.6 (C4'), 68.7 68.4 (C3'), 66.6 (C5'), 64.7 (Bn-CH_2), 56.2 (DMG-C), 40.5 40.3 (C2'), 34.8 ($CH_2\alpha$), 30.4 ($CH_2\gamma$), 29.9 ($CH_2\beta$), 26.1 26.0 25.9 (DMG-CH_3), 21.5 ($CH_2\delta$), 13.0 ($CH_3\omega$)

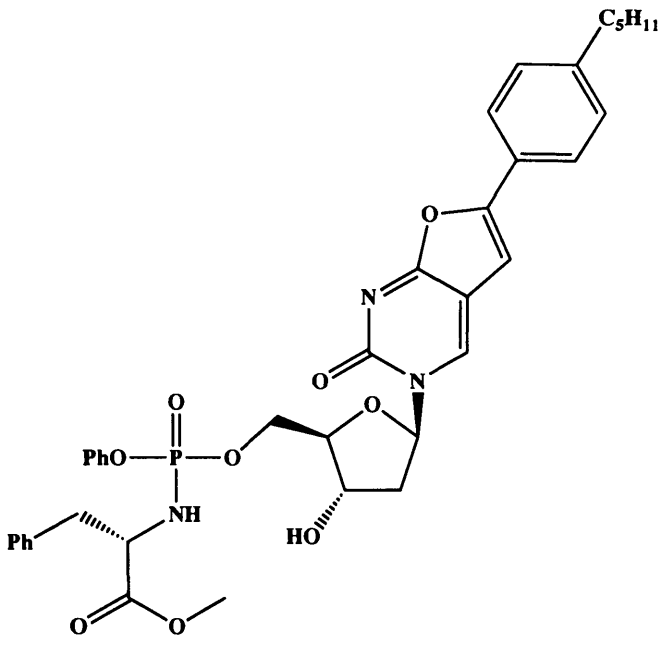
^{31}P-NMR ($CDCl_3$, 202 MHz): δ 3.16, 3.10

Anal. Calcd for $C_{39}H_{44}N_3O_9P$: C 64.19, H 6.08, N 5.57, O 19.73. Found C 64.35, H 6.38, N 5.15

MS: 752 (M+Na)

126j 3-(2-deoxy-β-D-ribofuranosyl-5-[phenyl-(methoxy-phenylalaninyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2484]



Prepared according to Standard Procedure B using **125j**

White foam: 0.04 g, 12%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.54 8.51 (1H, 2s, H-4), 7.70-7.56 (2H, m, BCNA-Ph), 7.32-7.04 (12H, m, BCNA-Ph+Ph+Phe-Ph), 6.60 6.50 (1H, 2s, H-5), 6.44-6.29 (1H, m, H-1'), 4.54-3.89 (6H, m, NH+H-3'+H-4'+H-5'+Phe-CH), 3.68 3.65 (3H, 2s, est CH_3), 3.17-2.60 (5H, m, Phe-CH+H-2'$_\alpha$+$CH_2\alpha$), 2.15-1.89 (1H, m, H-2'$_\beta$), 1.77-1.59 (2H, m, CH_2), 1.46-1.30 (4H, m, $CH_2\gamma$+CH_2), 1.03-0.90 (3H, m, $CH_3\omega$)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 173.4 173.2 173.1 (Phe-CO), 171.9 (C2), 156.2 156.1 (C7a), 155.3 (C6), 150.9 150.7 (Ph), 145.4 145.3 (C4a), 136.5 136.3 (C5), 130.2, 130.1, 129.9, 129.5, 129.4, 129.1, 127.6, 127.5, 126.3, 125.6, 125.5, 125.3, 120.7, 120.6, 120.5, 120.4, 108.9 (Ph), 97.9 97.7 (C4), 89.0 (C1'), 86.2 (C4'), 70.6 70.4 (C3'), 66.3 (C5'), 56.7 56.3 (Phe-CH), 52.8 (est CH_3), 42.0 (C2'), 40.6 (Phe-CH_2), 36.2 ($CH_2\alpha$), 31.9 ($CH_2\gamma$), 31.4 ($CH_2\beta$), 22.9 ($CH_2\delta$), 14.4 ($CH_3\omega$)

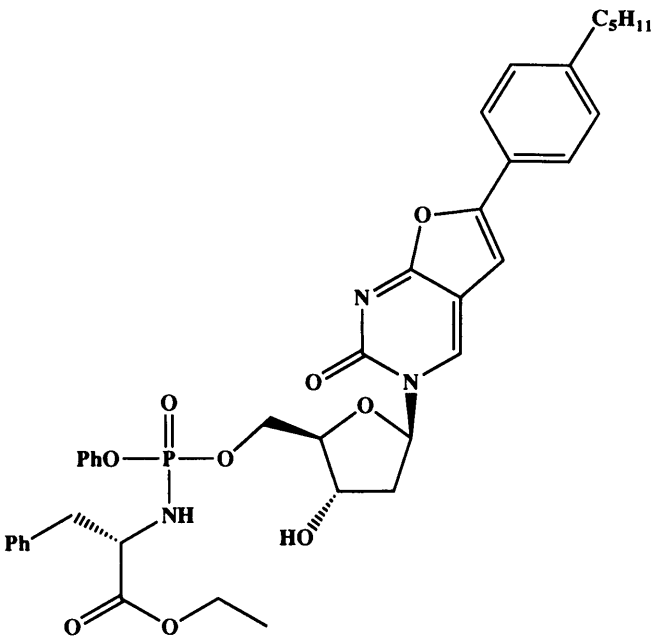
^{31}P-NMR ($CDCl_3$, 202 MHz): δ 4.34, 4.15

Anal. Calcd for $C_{38}H_{42}N_3O_9P$: C 63.77, H 5.91, N 5.87, O 20.12. Found C 63.58, H 5.96, N 6.12

MS: 738 (M+Na)

126k 3-(2-deoxy-β-D-ribofuranosyl-5-[phenyl-(ethoxy-dimethylglycinyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2485]



Prepared according to Standard Procedure B using **125k**

White foam: 0.05 g, 13%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.57 8.53 (1H, 2s, H-4), 7.69-7.56 (2H, m, BCNA-Ph), 7.36-6.93 (12H, m, BCNA-Ph+Ph+Phe-Ph), 6.60 6.51 (1H, 2s, H-5), 6.45-6.32 (1H, m, H-1'), 4.57-3.97 (8H, m, NH+H-3'+H-4'+H-5'+Phe-CH+est CH_2), 3.24-2.53 (5H, m, Phe-CH+H-2'$_\alpha$+$CH_2\alpha$), 2.17-1.84 (1H, m, H-2'$_\beta$), 1.80-1.51 (2H, m, $CH_2\beta$), 1.48-1.28 (4H, m, $CH_2\gamma$+$CH_2\delta$), 1.25-1.12 (3H, m, est CH_3), 1.03-0.88 (3H, m, $CH_3\omega$)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 173.0 172.9 172.8 (Phe-CO), 171.9 (C2), 156.2 156.0 (C7a), 155.3 (C6), 150.8 (Ph), 145.3 (C4a), 136.7 136.5 136.4 (C5), 130.2, 130.1, 129.9, 129.4, 129.0, 127.6, 127.5, 126.3, 125.6, 125.5, 125.3, 120.7, 120.6, 120.5, 120.4, 108.9 (Ph), 97.9 97.8 (C4), 88.9 (C1'), 86.3 86.2 86.1 (C4'), 70.6 70.4 (C3'), 66.2 (C5'), 62.0 (est CH_2), 56.7 56.3 (Phe-CH), 42.1 42.0 (C2'), 40.7 (Phe-CH_2), 36.2 ($CH_2\alpha$), 31.9 ($CH_2\gamma$), 31.3 ($CH_2\beta$), 22.9 ($CH_2\delta$), 14.4 (est CH_3), 14.2 ($CH_3\omega$)

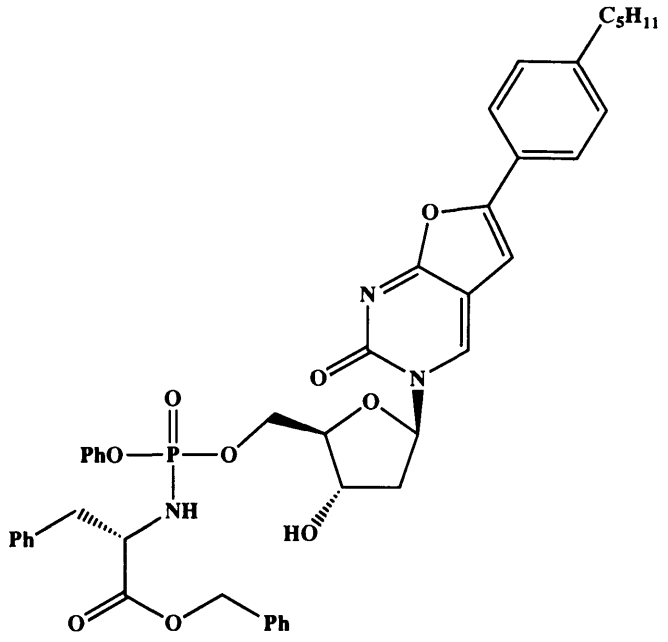
^{31}P-NMR ($CDCl_3$, 202 MHz): δ 4.44, 4.19

Anal. Calcd for $C_{39}H_{44}N_3O_9P$: C 64.19, H 6.08, N 5.76, O 19.73. Found C 64.43, H 6.10, N 5.42

MS: 752 (M+Na)

126j 3-(2-deoxy-β-D-ribofuranosyl-5-[phenyl-(benzyloxy-phenylalaninyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2484]



Prepared according to Standard Procedure B using **125j**

White foam: 0.07 g, 17%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.54 8.52 (1H, 2s, H-4), 7.69-7.57 (2H, m, BCNA-Ph), 7.43-7.00 (17H, m, BCNA-Ph+Ph+Phe-Ph+Bn), 6.58 6.51 (1H, 2s, H-5), 6.45-6.31 (1H, m, H-1'), 5.20-5.01 (2H, m, Bn-CH_2), 4.51-4.01 (6H, m, NH+H-3'+H-4'+H-5'+Phe-CH), 3.10-2.87 (2H, m, Phe-CH), 2.81-2.63 (3H, m, H-2'$_\alpha$+$CH_2\alpha$), 2.00-1.84 (1H, m, H-2'$_\beta$), 1.67-1.51 (2H, m, $CH_2\beta$), 1.38-1.19 (4H, m, $CH_2\gamma$+$CH_2\delta$), 0.94-0.80 (3H, m, $CH_3\omega$)

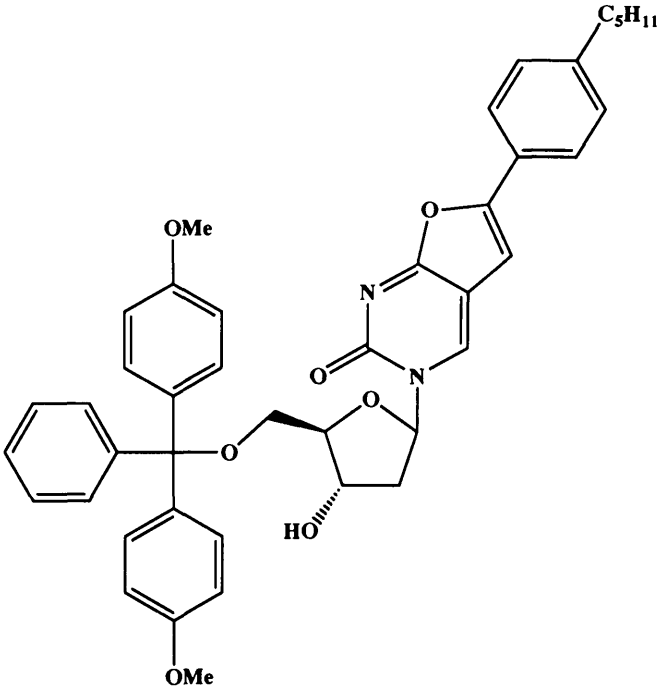
^{13}C-NMR ($CDCl_3$, 125 MHz): δ 171.3 171.2 (Phe-CO), 170.5 (C2), 154.8 154.6 (C7a), 153.8 (C6), 149.4 (Ph), 143.8 (C4a), 134.6 134.5 (C5), 133.9, 128.8, 128.7, 128.5, 127.9, 127.6, 127.5, 127.4, 126.1, 124.8, 124.2, 124.1, 123.8, 119.2, 119.1, 119.0, 107.4 (Ph), 96.4 96.3 (C4), 87.4 (C1'), 84.6 84.5 (C4'), 69.1 69.0 (C3'), 66.3 (C5'), 64.8 (Bn-CH_2), 55.2 54.8 (Phe-CH), 40.5 (Phe-CH_2), 39.2 (C2'), 34.8 ($CH_2\alpha$), 30.4 ($CH_2\gamma$), 29.9 ($CH_2\beta$), 21.5 ($CH_2\delta$), 13.0 ($CH_3\omega$)

^{31}P-NMR ($CDCl_3$, 202 MHz): δ 4.40, 4.28

Anal. Calcd for $C_{44}H_{46}N_3O_9P$: C 66.74, H 5.86, N 5.31, O 18.19. Found C 66.68, H 5.96, N 5.01

MS: 814 (M+Na)

127 3-(2'-Deoxy-β-D-ribofuranosyl-5'-[bis(4-methoxyphenyl)-phenyl]-6-(4-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one



33b (0.5 g, 1.25 mmol) was dissolved in pyridine (10 ml), dimethoxytrirylchlorode (0.51 g, 1.2 eq.) was added at 0°C and the solution was stirred overnight at room temperature. The solvents were evaporated under high vacuum and the residue was purified by column chromatography pre-washed with TEA ($CHCl_3$: MeOH: Et_3N 95:5) to obtain 0.56 g (64%) as orange solid.

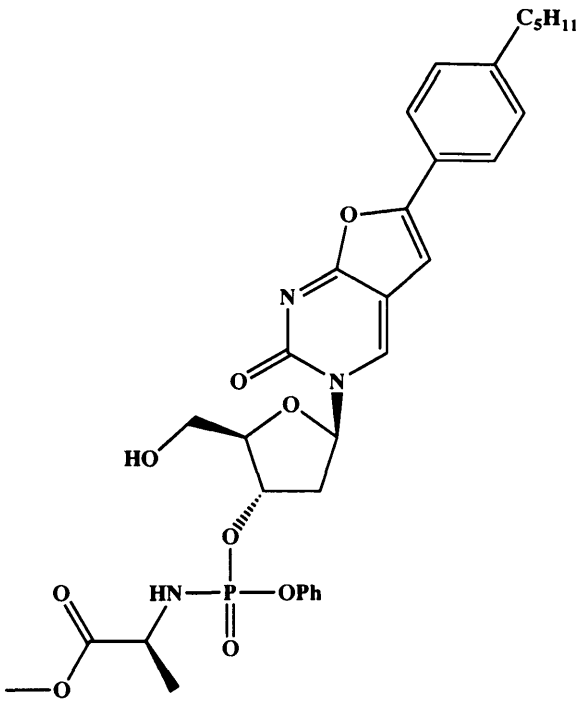
^{1}H-NMR (DMSO, 500 Mhz): δ 9.05 (1H, s, H-4), 7.58-6.87 (13H, m, Trityl), 6.25 (1H, dd, J 6.7 Hz 3.6 Hz, H-1'), 5.80 (1H, s, H-5), 4.72 (1H, q, J 6.7 Hz, H-3'), 4.12 (1H, dt, J 5.7 Hz 2.9 Hz, H-4'), 3.76 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 3.59 (1H, dd, J 11.0 Hz 3.0 Hz, H-5'), 3.53 (1H, dd, J 11.0 Hz 2.7 Hz, H-5'), 2.70-2.63 (3H, m, $CH_2\alpha$ + H-2'$_\alpha$), 2.46 (1H, ddd, J 13.8 Hz 6.5 Hz 3.7 Hz, H-2'), 1.66 (2H, qn, J 7.5 Hz, $CH_2\beta$), 1.41-1.33 (4H, m, $CH_2\gamma$ + $CH_2\delta$), 0.93 (3H, t *J* 6.8 Hz, $CH_3\omega$)

^{13}C-NMR (DMSO, 125 MHz) : 172.73 (C7a), 160.33 (C), 160.26 (Ph), 156.70 (Ph), 156.57 (Ph), 153.89 (C=O), 153.75 (C6), 146.21 (Ph), 145.61 (C4a), 138.84 (C4), 137.18, 136.72, 131.58, 131.38, 130.17, 129.70, 129.26, 128.31, 127.14, 125.82, 125.59, 114.53, 114.51, 109.55 (Ph), 98.75 (C5), 89.59 (C1'), 88.45 (Ph-C_q), 87.85 (C4'), 69.89 (C3'), 63.14 (C5'), 55.89 (OCH_3), 55.87 (OCH_3), 42.93 (C2'), 36.81 ($CH_2\alpha$), 32.66 ($CH_2\gamma$), 32.15 ($CH_2\beta$), 23.65 ($CH_2\delta$), 14.64 ($CH_3\omega$)

MS: 723 (M+Na)

128a 3-(2-deoxy-β-D-ribofuranosyl-3-[phenyl-(methoxy-L-alaninyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2511]



Prepared according to Standard Procedure C using **125a**

White foam: 0.08 g, 25%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.74-8.69 (1H, 2s, H-4), 7.54-6.66 (10H, m, BCNA-Ph+Ph+H-5), 6.24-6.20 (1H, m, H-1'), 5.12-5.05(1H, m, H-3'), 4.20-3.49 (8H, m. H-4'+ H-5'+Ala-CH+est CH_3+NH), 2.85-2.27 (4H, m, H-2'$_\alpha$+$CH_2\alpha$), 1.54-1.43 (2H, m, $CH_2\beta$), 1.30-1.11 (7H, m, $CH_2\gamma$+$CH_2\delta$+Ala-CH_3), 0.82-0.80 (3H, m, $CH_3\omega$)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 173.1 173.0 172.9 172.8 (Ala-CO), 170.5 (C2), 155.1 155.0 (C7a), 163.8 (C6), 149.3 149.2 (Ph), 144.1 144.0 (C4a), 135.7 135.5 (C5), 128.9, 128.8, 128.5, 128.4 127.96,124.77, 124.3 123.9, 119.6 119.3, 119.2, 119.0, 118.9, 114.4, 107.8, 107.7 (Ph), 96.3 (C4), 87.1 86.9 (C1'), 85.4 (C4'), 75.8 75.3 73.9 73.1 (C3'), 60.2 59.8 (C5'), 51.7 51.3 (est CH_3), 49.4 49.1 (Ala-CH), 39.2 39.1 39.0 (C2'), 34.8 ($CH_2\alpha$), 30.4 ($CH_2\gamma$), 29.9 ($CH_2\beta$), 21.5 ($CH_2\delta$), 19.6 19.5(Ala-CH_3), 12.9 ($CH_3\omega$).

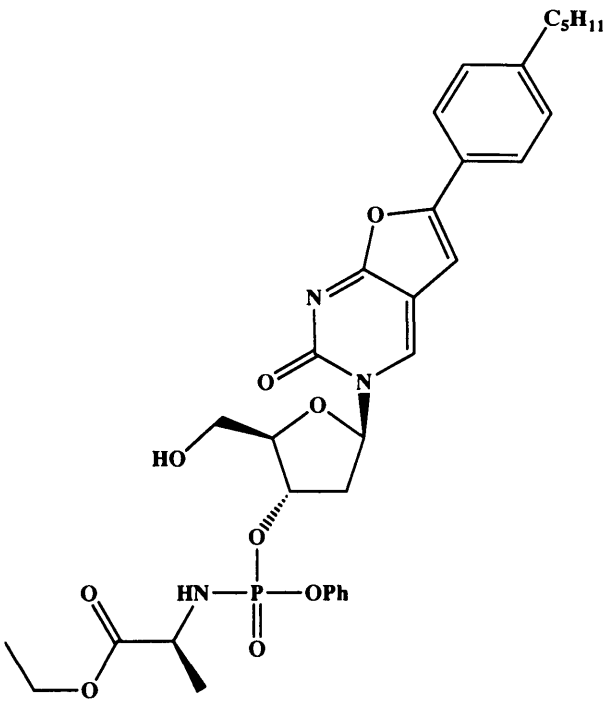
^{31}P-NMR ($CDCl_3$, 202 MHz): δ 3.14, 3.21

Anal. Calcd for $C_{32}H_{38}N_3O_9P$: C 60.09, H 5.99, N 6.57, O 22.51. Found C 60.10, H 5.80, N 6.42

MS: 662 (M+Na)

128b 3-(2-deoxy-β-D-ribofuranosyl-3-[phenyl-(ethoxy-L-alaninyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2510]



Prepared according to Standard Procedure C using **125b**

White foam: 0.10 g, 32%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.66-8.61 (1H, 2s, H-4), 7.56-7.06 (9H, m, BCNA-Ph+Ph), 6.60-6.59 (1H, 2s, H-5), 6.28-6.22 (1H, m, H-1'), 5.14-5.09 (1H, m, H-3'), 4.17-3.40 (7H, m. H-4'+ H-5'+Ala-CH+est CH_2+NH), 2.83-2.22 (4H, m, H-2'$_\alpha$+$CH_2\alpha$), 1.59-1.41 (2H, m, $CH_2\beta$), 1.36-1.11 (10H, m, $CH_2\gamma$+$CH_2\delta$+Ala-CH_3+est CH_3), 0.86-0.81 (3H, m, $CH_3\omega$)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 173.8 173.7 173.5 173.4 (Ala-CO), 171.8 171.7 (C2), 155.9 155.8 (C7a), 154.8 (C6), 150.4, 150.3, 145.0, 144.9, 136.4, 136.3,129.9, 129.8, 128.9, 125.9, 125.8, 125.3, 125.2, 124.9, 120.4, 120.3, 120.0, 119.9, 108.5, 108.4 (Ph), 97.17 (C4), 87.9 87.8 (C1'), 86.4 86.3 86.2 86.1 (C4'), 76.1 76.0 74.4 74.3 (C3'), 61.8 61.7 61.2 60.7 (C5' + est CH_2), 50.4 50.2 (Ala-CH), 40.3 40.0 (C2'), 35.8 ($CH_2\alpha$), 31.4 ($CH_2\gamma$), 30.9 ($CH_2\beta$), 22.5 ($CH_2\delta$), 20.7 20.6 (Ala-CH_3), 14.1 ($CH_3\omega$), 14.0 (est CH_3)

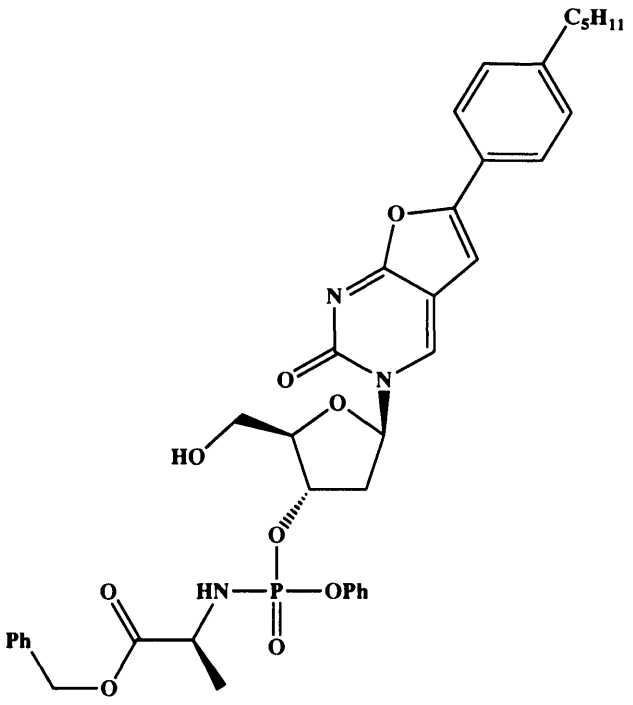
^{31}P-NMR ($CDCl_3$, 202 MHz): δ 3.13, 2.05

Anal. Calcd for $C_{33}H_{40}N_3O_9P$: C 60.64, H 6.17, N 6.43, O 22.03. Found C 60.45, H 6.15, N 6.12

MS: 676 (M+Na)

128f 3-(2-deoxy-β-D-ribofuranosyl-3-[phenyl-(benzyloxy-L-alaninyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2512]



Prepared according to Standard Procedure C using **125f**

White foam: 0.20 g, 56%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.66-8.61 (1H, 2s, H-4), 7.54-7.04 (14H, m, BCNA-Ph+ Phe-Ph+Bn), 6.59-6.57 (1H, 2s, H-5), 6.25-6.21 (1H, m, H-1'), 5.15-4.98 (3H, m, H-3'+Bn-CH_2), 4.27-3.75 (5H, m, H-4'+H5'+Ala-CH+NH), 2.82-2.20 (4H, m, H-2'+$CH_2\alpha$), 1.57-1.51 (2H, m, $CH_2\beta$), 1.30-1.13 (7H, m, $CH_2\gamma$+$CH_2\delta$+Ala-CH_3), 0.83-0.77 (3H, m, $CH_3\omega$)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 173.6 173.5 173.4 (Ala-CO), 171.7 171.6 (C2), 155.9 (C6), 154.90 154.83, 150.37, 150.32 (Ph), 145.0 144.9 (C4a), 136.5 136.4 (C5), 135.2, 135.1, 129.9 129.7 128.9 128.7 128.6 128.4 128.3 128.2 128.1 125.9 125.8 125.3 124.9 120.4 120.3 120.0 119.9 108.5 108.4 (Ph), 97.2 (C4), 87.8 (C1'), 86.3 (C4'), 76.1 76.0 74.8 74.7 (C3'), 67.4 67.2 (Bn-CH_2), 50.5 50.2 (Ala-CH), 40.3 40.1 (C2'), 35.8 ($CH_2\alpha$), 31.5 ($CH_2\gamma$), 30.9 ($CH_2\beta$), 22.5 ($CH_2\delta$), 20.6 20.5 20.4 (Ala-CH_3), 14.0 ($CH_3\omega$)

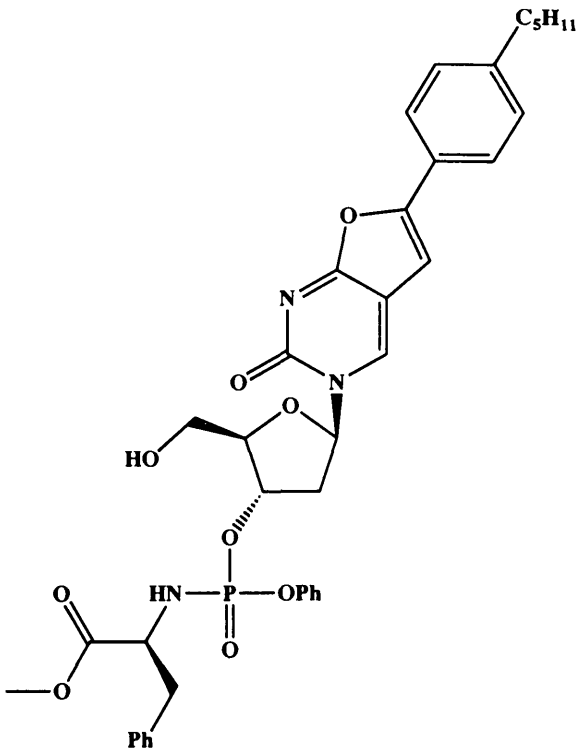
^{31}P-NMR ($CDCl_3$, 202 MHz): δ 3.00, 1.92

Anal. Calcd for $C_{38}H_{42}N_3O_9P$: C 63.77, H 5.91, N 5.87, O 20.12. Found C 63.90, H 6.05, N 5.59

MS: 738 (M+Na)

128j 3-(2-deoxy-β-D-ribofuranosyl-3-[phenyl-(methoxy-L-phenylalaninyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2513]



Prepared according to Standard Procedure C using **125j**

White foam: 0.18 g, 52%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.59-8.56 (1H, 2s, H-4), 7.70-7.07 (14H, m, BCNA-Ph+Ph+Phe-Ph), 6.70-6.69 (1H, 2s, H-5), 6.30-6.22 (1H, m, H-1'), 5.06-4.86 (1H, m, H-3'), 4.29-3.51 (8H, m, H-4'+H-5'+Phe-CH+est CH_3+NH), 3.14-2.17 (6H, m, H-2'+$CH_2\alpha$+Phe-CH_2), 1.69-1.62 (2H, m, $CH_2\beta$), 1.40-1.32 (4H, m, $CH_2\gamma$+CH_2), 0.94-0.91 (3H, m, $CH_3\omega$)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 172.8 (Phe-CO), 171.8 (C2), 156.1 156.0 (C7a), 154.6 (C6), 150.3 (Ph), 145.1 (C4a), 135.9 135.8 (C5), 135.5, 129.8, 129.4, 129.0, 128.7, 127.4, 124.3, 125.9, 125.4, 125.3, 125.0, 124.9, 120.3, 120.2, 120.0, 108.4, 108.2 (Ph), 96.9 (C4), 87.8 87.5 (C1'), 85.7 (C4'), 75.1 73.8 (C3'), 61.0 60.6 (C5'), 56.0 55.6 (Phe-C), 52.5 52.4 (est CH_3), 40.3 40.2 (Phe-CH_2), 40.2 40.1 39.8 39.5 (C2'), 34.8 ($CH_2\alpha$), 31.4 ($CH_2\gamma$), 30.9 ($CH_2\beta$), 22.5 ($CH_2\delta$), 14.0 ($CH_3\omega$)

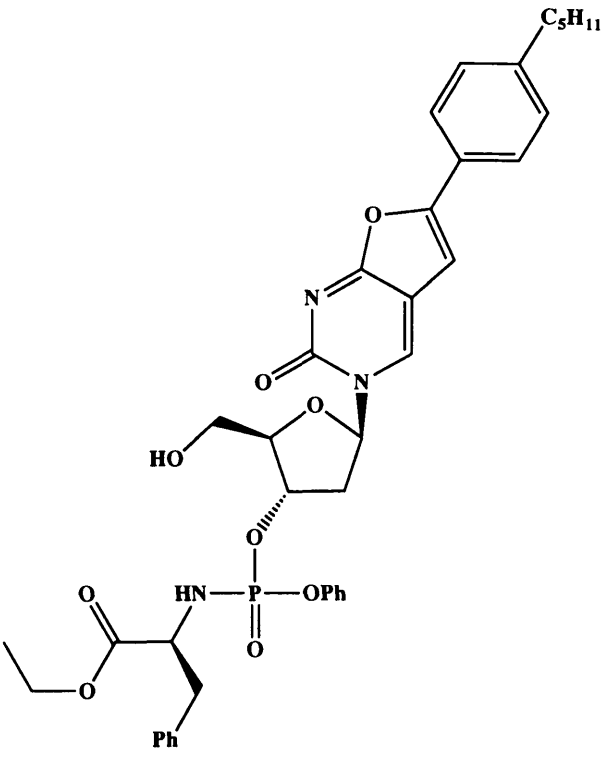
^{31}P-NMR ($CDCl_3$, 202 MHz): δ 3.19, 2.49

Anal. Calcd for $C_{38}H_{42}N_3O_9P$: C 63.77, H 5.91, N 5.87, O 20.12. Found C 63.85, H 5.69, N 5.55

MS: 738 (M+Na)

128k 3-(2-deoxy-β-D-ribofuranosyl-3-[phenyl-(ethoxy-L-phenylalaninyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2514]



Prepared according to Standard Procedure C using **125k**

White foam: 0.21 g, 58%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.52-8.50 (1H, 2s, H-4), 7.59-6.98 (14H, m, BCNA-Ph+Ph+Phe-Ph), 6.60-6.59 (1H, 2s, H-5), 6.22-6.12 (1H, m, H-1'), 5.00-4.77 (1H, m, H-3'), 4.16-3.58 (7H, m, H-4'+H-5'+Phe-CH+est CH_2+NH), 3.30-2.06 (6H, m, H-2'+$CH_2\alpha$+Phe-CH_2), 1.60-1.53 (2H, m, $CH_2\beta$), 1.31-1.00 (7H, m, $CH_2\gamma$+CH_2+est CH_3), 0.86-0.81 (3H, m, $CH_3\omega$)

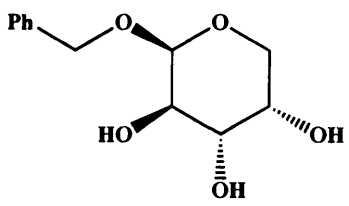
^{13}C-NMR ($CDCl_3$, 125 MHz): δ 172.4 172.3 (Phe-CO), 171.8 (C2), 156.1 156.0 (C7a), 154.7 154.6 (C6), 150.3 (Ph), 145.1 145.0 (C4a), 136.0, 135.9, 135.7, 135.4, 129.8, 129.5, 129.0, 128.6, 127.3, 127.2, 125.9, 125.4, 125.3, 124.3, 120.3, 120.2, 120.0, 119.9, 108.4, 108.2 (Ph), 97.0 (C4), 87.8 87.5 (C1'), 85.9 85.8 85.7 (C4'), 75.3 73.8 (C3'), 61.8 60.7 (C5'), 61.1 60.6 (est CH_2), 56.1 55.6 (Phe-C), 40.4 40.3 (Phe-CH_2), 40.2 40.1 39.9 39.5 (C2'), 35.8 ($CH_2\alpha$), 31.4 ($CH_2\gamma$), 30.9 ($CH_2\beta$), 22.5 ($CH_2\delta$), 14.0 14.0 ($CH_3\omega$)

^{31}P-NMR ($CDCl_3$, 202 MHz): δ 3.23, 2.46

Anal. Calcd for $C_{39}H_{44}N_3O_9P$: C 64.19, H 6.08, N 5.76, O 19.73. Found C 64.00, H 6.09, N 5.95

MS: 752 (M+Na)

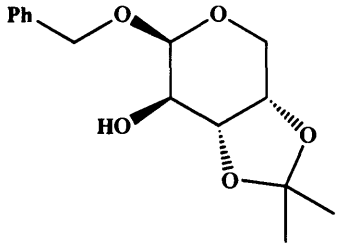
134 1-*O*-Benzyl-β-L-Arabinopyranoside



Benzyl alcohol (50 mL) was saturated with hydrogen chloride for 40 min at 0°C, L-arabinose (10 g, 66.6 mmol) was added, and the mixture was stirred at room temperature overnight. The solution was concentrated and Ethyl Acetate was slowly added while stirring for precipitation. Filtration of the resulting solid, washing with Ethyl Acetate, and drying under nitrogen air gave 16.00 g (100%) of desired compound as a white precipitate, which was used for the next step without further purification.

^{1}H-NMR (DMSO, 500 MHz): δ 7.40-7.27 (5H, m, Ph), 4.76 (1H, d, J 2.3 Hz, H-1), 4.66 (1H, d, J 12.4 Hz, Bn-CH_2), 4.46 (1H, d, J 12.4 Hz, Bn-CH_2), 4.25 (3H, bs, OH-2 + OH-3+ OH-4), 3.73 (1H, m, H-4), 3.68 (1H, dd, J 11.8 Hz 1.8 Hz, H-5), 3.64-3.63 (2H, m, H-2 + H-3), 3.47 (1H, dd, J 11.8 Hz 2.9 Hz, H-5')
^{13}C-NMR (DMSO, 125 MHz): δ 138.18 (Ph-C1), 128.13 (Ph-C4), 127.40 (Ph-C3), 127.27 (Ph-C2), 98.90 (C1), 69.06 (C2), 68.62 (C3), 68.40 (Bn-CH_2), 68.25 (C4), 63.27 (C5)

135 1-*O*-Benzyl-3,4-*O*-isopropylidene-β-L-Arabinopiranoside

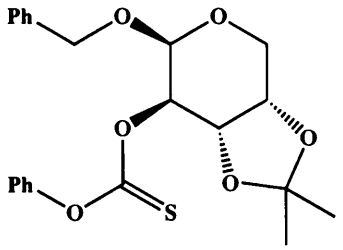


A mixture of **134** (2.0 g, 8.3 mmol), 2,2-dimethoxypropane (2.6 mL, 2.5 eq.) and *p*-TsOH•H_2O (0.1 g, 0.05 eq.) in acetone (20 mL) was stirred for 1 h. The reaction mixture was then neutralized with Et_3N and evaporated under reduced pressure to give the wanted compound as a yellow syrup, which was filtered through a short silica gel pad and washing with Hexane : Ethyl Acetate 3:1 to obtain 2.2 g (95%) of titled compound as white solid.

^{1}H-NMR (DMSO, 500 MJz): δ 7.41-7.28 (5H, m, Ph), 5.12 (1H, d, J 6.6 Hz, OH-2), 4.74 (1H, d, J 3.3, H-1), 4.68 (1H, d, J 12.3 Hz, Bn-CH_2), 4.47 (1H, d, J 12.3 Hz, Bn-CH_2), 4.23 (1H, dd, J 5.6 Hz 1.8 Hz, H-4), 4.05 (1H, dd, J 7.7 Hz 5.7 Hz, H-3), 3.88 (1H, dd, J 13.3 Hz 2.8 Hz, H-5), 3.83 (1H, dd, 13.3 Hz 1.2 Hz, H-5), 3.51 (1H, ddd, J 7.7 Hz 6.6 Hz 3.3 Hz, H-2), 1.41 (3H, s, CH_3), 1.28 (3H, s, CH_3)

^{13}C-NMR (DMSO, 125 MHz): δ 137.75 (Ph-C1'), 128.18 (Ph-C4') , 127.49 (Ph-C3'), 127.42 (Ph-C2'), 107.63 (C) , 98.09 (C1), 75.59 (C3), 72.72 (C4), 69.78 (C2), 68.56 (Bn-CH_2), 58.56 (C5), 28.16 (CH_3), 26.22 (CH_3)

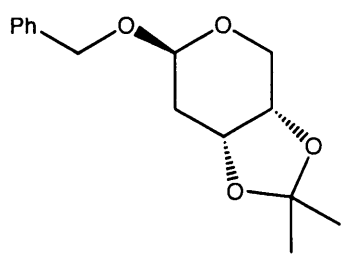
136 1-*O*-Benzyl-3,4-*O*-isopropylidene-2-*O*-phenoxythiocarbonyl-β-L-Arabinopiranoside



To a stirred solution of **135** (2 g, 7.1 mmol) and pyridine (2.5 ml, 4.3 eq.) in anhydrous DCM (60 ml), *O*-phenylchlorothionoformate (1.4 ml, 2.3 eq.) was added at room temperature. The resulting yellow solution was stirred overnight at room temperature under argon atmosphere. After evaporation of the solvent, the residue was purified by column chromatography using Hexane : Ethyl Acetate 10:1 as eluent to give 2.85 g (96%) of pure compound as yellow oil.

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.45-7.29 (8H, m, Bn-Ph +CSO-Ph), 7.12-7.09 (2H, m, CSO-Ph), 5.48 (1H, dd, J 8.1 Hz 3.4 Hz, H-2), 5.28 (1H, d, J 3.4, H-1), 4.81 (1H, d, J 12.3 Hz, Bn-CH_2), 4.62 (1H, d, *J* 12.3 Hz, Bn-CH_2), 4.60 (1H, dd, J 8.1 Hz 5.6 Hz, H-3), 4.35 (1H, dt, J 5.6 Hz 1.8 Hz, H-4), 4.09 (2H, m, H-5), 1.61 (3H, s, CH_3), 1.42 (3H, s, CH_3)
^{13}C-NMR ($CDCl_3$, 125 MHz): δ 194.83 (C=S), 153.45 (PhOCS-C1), 137.75 (Bn-C1), 129.47 128.53 127.96 127.64 126.57 121.86 (Bn-Ph + PhO), 109.67 (C), 94.21 (C1), 81.19 (C2), 73.71 (C4), 72.64 (C3), 69.56 (Bn-CH_2), 58.94 (C5), 28.01 (CH_3), 26.39 (CH_3)

137 1-*O*-Benzyl-3,4-*O*-isopropylidene-2-deoxy-β-L-Arabinopiranoside

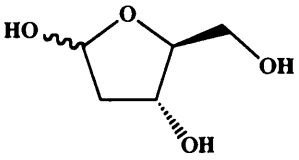


To a stirred solution of **136** (2.85 g, 6.8 mmol) in anhydrous and degassed toluene (30 ml), a solution of *n*Bu_3SnH (4.3 ml, 2.3 eq.) and ACCN (0.33 g, 0.2 eq.) were added over 1 hour. After the addiction the solution was stirred at reflux for 1 hour. The resulting clear solution was evaporated and purified by column chromatography Hexane : Ethy lAcetate 6:1 to give 1.46 g (81%) of the desired compound as a colourless oil.

^{1}H-NMR ($CDCl_3$): δ 7.29-6.73 (5H, m, Ph), 4.92 (1H, dd, J 6.0 Hz 4.6 Hz, H-1), 4.70 (1H, d, J 11.9 Hz,Bn-CH_2), 4.43 (1H, d, J 11.9 Hz, Bn-CH_2), 4.40 (1H, dt, J 6.5 Hz 4.9 Hz , H-3), 4.09 (1H, dt, J 6.4 Hz 2.5 Hz, H-4), 3.83 (1H, dd, J 13.0 Hz 2.8 Hz, H-5) 3.71 (1H, dd, J 13.0 Hz 2.4 Hz, H-5), 2.11 (1H, dt, J 14.8 Hz 4.8 Hz, H-2), 1.80 (1H, ddd, 14.8 Hz 6.1 Hz, 4.6 Hz, H-2), 1.44 (3H, s, CH_3), 1.27 (3H, s, CH_3)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 137.78 (Bn-C1), 129.64 (Bn-C4), 128.47 (Bn-C3), 127.75 (Bn-C2), 108.84 (C) , 95.78 (C1), 72.06 (C4), 69.96 (C3), 69.41 (Bn-CH_2), 61.24 (C5), 31.49 (C2), 27.25 (CH_3), 25.45 (CH_3)

131 2-deoxy-L-ribose

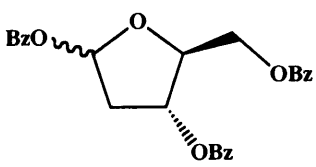


A mixture of **137** (1.46 g, 5.5 mmol) in 4% of trifluoroacetic acid was stirred at 40°C overnight. The reaction mixture was poured into a separatory funnel and washed with Ethyl Acetate three times. The aqueous layer was filtered through Amberlite Ira400 (HCO_3^-) and evaporated to dryness. The remaining syrup was co-evaporated with toluene twice to give 0.44 g (60%) of 2-Deoxy-L-Ribose.

^{1}H-NMR (DMSO, 500 MHz): δ 6.10 (1H, d J 4.9 Hz, OH-1), 4.97 (1H, dt, J 4.9 Hz 2.8 Hz, H-1), 4.47 (1H, br, OH-5), 4.45 (1H, br, OH-3), 3.83 (1H, dt, J 7.4 Hz 3.6 Hz, H-3), 3.63 (1H, dd, 11.0 Hz 2.9 Hz, H-5), 3.50 (1H, m, H-4), 3.44 (1H, dd, J 11.0 Hz 6.0 Hz, H-5), 1.78 (1H, ddd, J 12.6 Hz 7.9 Hz 2.8 Hz), 1.47 (1H, ddd, J 12.6 Hz 5.4 Hz 3.8 Hz)

^{13}C-NMR (DMSO, 125 MHz): δ 91.25 (C1), 67.35 (C4), 65.05 (C3), 63.05 (C5), 35.56 (C2)

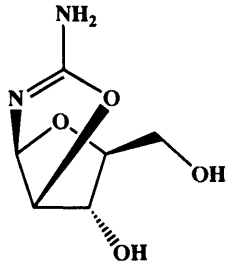
138 1,3,5-Tri-O-benzoyl-2-deoxy-L-ribose



A mixture of **131** (0.50 g, 3.7 mmol) and benzoyl chloride (2.1 g, 4.0 eq.) in dry dichloromethane (10 cm3) was stirred for 30 minutes at -15°C. Then pyridine (4 ml) in dry DCM (8 ml) was added dropwise over a period of 1 hour at 15°C. After the addition, the mixture was stirred for 1 h over the same temperature range, washed twice with water, dried over MgSO and filtered. After removing the solvent under reduced pressure the residue was purified by column chromatography Hexane : Ethyl Acetate 1:1.

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.10-7.16 (15H, m, Bz), 6.62-6.59 (1H, m, H-1'), 5.78-5.72 (1H, m, H-3'), 5.65-5.48 (1H, m, H-4'), 4.28-4.20 (1H, m, H-5'), 4.13-4.07 (1H, m, H-5'), 2.62-2.54 (1H, m, H-2'), 2.33-2.25 (1H, m, H-2')
13C-NMR ($CDCl_3$, 125 MHz): δ 165. 82, 164.85, 162.37 (Bz-CO), 136.13, 134.52, 133.61, 133.36, 133.28, 130.58, 130.04, 129.89, 129.82, 129.70, 129.57, 129.54, 128.88, 128.63, 128.54, 128.41, 128.34, 123.79 (Bz-Ph), 92.53 (C1'), 68.00 (C4'), 66.34 (C3'), 63.42 (C5'), 30.39 (C2')

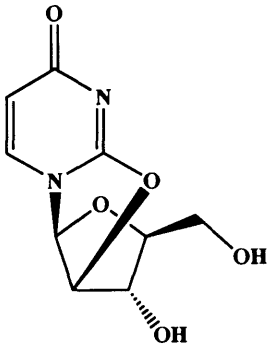
139 2-amino-β-L-arabinofurano[1',2':4,5]oxazoline



A mixture of L-arabinose (17g, 113 mmol), cyanamide (10 g, 2 eq.), MeOH (30 ml) and 6M NH_4OH (5 ml) was stirred at room temperature for 48 hours and then kept at –10°C for 15 hours. The product was collected by filtration, washed with MeOH and Et_2O and dried under vacuum to obtain 14.75 g (75%) of white solid.

^{1}H-NMR (DMSO, 500 MHz): δ 6.27 (2H, bs, NH_2), 5.67 (1H, d, J 5.5 Hz, H-1'), 5.39 (1H, bs, OH-3'), 4.68 (1H, bs, OH-5'), 4.53 (1H, d, J 5.5 Hz, H-2'), 4.02-3.99 (1H, m, H-3'), 3.67-3.62 (1H, m, H-4'), 3.31-3.23 (2H, m, H-5')
13C-NMR (DMSO, 125 MHz): δ 162.18 (C-NH_2), 99.95 (C1'), 88.04 (C2'), 84.58 (C4'), 75.62 (C3'), 61.55 (C5')

140 $O^{2,2'}$-Anhydro-L-uridine



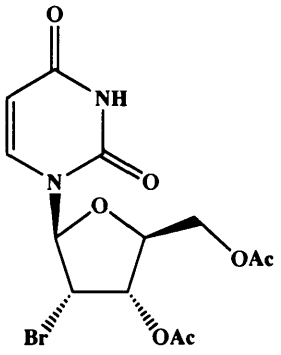
A solution of **139** (12g, 69 mmol) and methyl propiolate (12 ml, 2 eq.) in 50% aqueous ethanol (180 ml) was refluxed for 5 hours and then dried under reduced pressure to obtain a residue that was taken with acetone and left at 0°C for 2 hours. The resulting white precipitate filtrated and washed with acetone to afford 10 g (65%) of the titled compound.

^{1}H-NMR (DMSO, 500 MHz): δ 7.83 (1H, d, J 7.4 Hz, H-5), 6.31 (1H, d, J 5.8 Hz, H-1'), 5.87 (1H, d, J 4.3 Hz, OH-3'), 5.84 (1H, d, J 7.4 Hz, H-6), 5.20 (1H, d, J 5.5 Hz, H-2'), 4.97 (1H, t,

J 5.0 Hz, OH-5'), 4.41-4.37 (1H, m, H-3'), 4.08 (1H, t, J 4.1 Hz, H-4'), 3.31-3.25 (1H, m, H-5'), 3.23-3.16 (1H, m, H-5')

^{13}C-NMR (DMSO, 125 MHz): δ 171.10 (C4), 159.76 (C2), 136.77 (C5), 108.59 (C6), 89.97 (C1'), 89.16 (C4'), 88.71 (C2'), 74.71 (C3'), 60.81 (C5')

141 3', 5'-diacetyl-2'-bromo-2'-deoxy-L-uridine

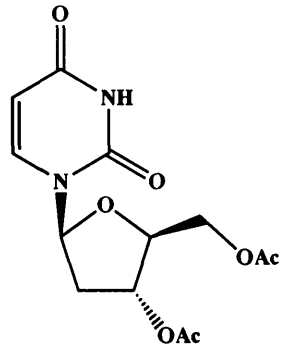


Compound **140** (2.26 g, 10 mmol) was suspended in ACN (50 ml) and heated at reflux. AcBr (6.7 ml, 9 eq.) was added dropwise. The mixture was stirred at reflux for 1 hours, then the sovent was removed under reduced pressure and the residue was washed with Et_2O to obtain the titled compound as orange solid 3.90 g (100%).

^{1}H-NMR (DMSO, 500 MHz): δ 11.52 (1H, bs, NH), 7.70 (1H, d, J 8.2 Hz, H-5), 6.15 (1H, d, 7.2 Hz, H-1'), 5.78 (1H, d, J 8.2 Hz, H-6), 5.28-5.23 (1H, m, H-3'), 5.01 (1H, t, 6.5 Hz, H-2'), 4.36-4.23 (3H, m, H-4'+H-5'), 2.14 (3H, s, CH_3), 2.08 (3H, s, CH_3)

^{13}C-NMR (DMSO, 125 MHz): δ 170.10 (Ac-CO), 169.24 (Ac-CO), 162.74 (C4), 150.46 (C2), 140.04 (C5), 102.86 (C6), 88.81 (C1'), 79.64 (C4'), 71.09 (C3'), 62.78 (C5'), 47.48 (C2'), 20.51 (Ac-CH_3), 20.50 (Ac-CH_3)

142 3', 5'-diacetyl-2'-deoxy-L-uridine

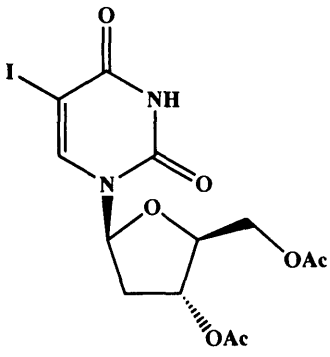


To a stirred solution of **141** (3.90 g , 10 mmol) in anhydrous and degassed toluene (30 ml), a solution of nBu$_3$SnH (4.3 ml, 2.3 eq.) and AIBN (0.33 g , 0.2 eq.) were added. After the addiction the solution was stirred at reflux for 2 hours. The resulting clear solution was evaporated and Et_2O was slowly added to obtain a precipitate. The solid was filtered and dried to obtain 2.99 g (96%) of the titled compound.

^{1}H-NMR (DMSO, 500 MHz): δ 11.52 (1H, bs, NH), 7.70 (1H, d, J 8.2 Hz, H-5), 6.15 (1H, d, 7.2 Hz, H-1'), 5.78 (1H, d, J 8.2 Hz, H-6), 5.17-5.19 (1H, m, H-3'), 4.29-4.20 (1H, m, H-4'), 4.19-4.08 (2H, m, H-5'), 2.29-2.48 (2H, m, H-2'), 2.11 (3H, S, CH_3), 2.02 (3H, s, CH_3)

^{13}C-NMR (DMSO, 125 MHz): δ 170.20 (Ac-CO), 169.56 (Ac-CO), 161.74 (C4), 151.56 (C2), 139.34 (C5), 103.86 (C6), 85.12 (C1'), 81.76 (C4'), 75.23 (C3'), 63.12 (C5'), 42.12 (C2'), 21.10 (Ac-CH_3), 20.76 (Ac-CH_3)

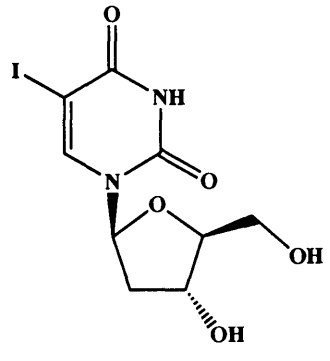
143 3', 5'-diacetyl-2'-deoxy-5-iodo-L-uridine



To solution of **142** (2.99 g, 9.60 mmol) in glacial AcOH was added I_2 (1.22 g, 0.5 eq.) and CAN (2.63 g, 0.5 eq.) and the mixture was then stirred at 80°C for 1 hours. The solvent was removed under reduced pressure and the residue was dissolved in DCM and washed with H_2O and brine. The organic layer was dried over $MgSO_4$ and evaporated to afford 4.00 g (95%) of the titled compound.

^{1}H-NMR (DMSO, 500 MHz): δ 11.74 (1H, bs, NH), 8.05 (1H, s, H-6), 6.11 (1H, t, J = 7.1 Hz, H-l'), 5.17-5.19 (1H, m, H-3'), 4.20-4.29 (3H, m, H-4'+H-5'), 2.29-2.48 (2H, m, H-2'), 2.10 (3H, s, CH_3), 2.04 (3H, s, CH_3)
^{13}C-NMR (DMSO, 125 MHz): δ 170.30 (Ac-CO), 170.10 (Ac-CO), 159.64 (C4), 149.79 (C2), 143.70 (C6), 85.42 (C4'), 82.36 (C1'), 74.54 (C3'), 68.93 (C5), 63.78 (C5'), 38.38 (C2'), 21.11 (Ac-CH_3), 20.96 (Ac-CH_3)

144 2'-deoxy-5-iodo-L-uridine



Compound **143** (4.00 g, 9.1 mmol) was dissolved in 1M MeONa / MeOH solution (100 ml) and then stirred for 2 hours. The solution was concentrated under reduced pressure and diluited with H_2O (100 ml). DOWEX H^+ was added and the solution was stirred for 30 minutes. The resin was filtered and washed with H_2O, then the solution was dried under reduced pressure to obtain 2.75 g (85%) of the titled compound.

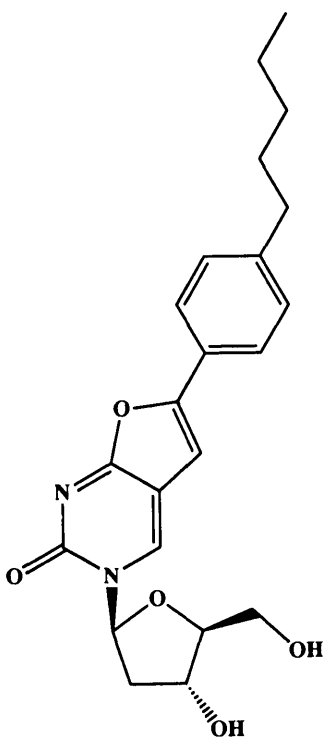
^{1}H-NMR (DMSO, 500 MHz): δ 11.67 (1H, bs, NH), 8.40 (1H, s, H-6), 6.10 (1H, t, J 6.6 Hz, H-1'), 5.24 (1H, d, J 4.4 Hz, OH-3'), 5.13 (1H, t, J 4.9 Hz, OH-5'), 4.26-4.22 (1H, m, H-3'),

3.80 (1H, q, J 3.3 Hz, H-4'), 3.63 (1H, ddd, J 11.9 Hz 4.9 Hz, 3.3 Hz, H-5'), 3.57 (1H, ddd, J 11.9 Hz 4.9 Hz 3.3 Hz, H-5'), 2.17-2.08 (2H, m, H-2')

^{13}C-NMR (DMSO, 125 MHz): δ 160.46 (C4), 150.08 (C2), 145.03 (C6), 87.51 (C4'), 84.64 (C1'), 70.00 (C3'), 69.18 (C5), 60.81 (C5'), 40.16 (C2')

129f 3-(2-deoxy-β-L-ribofuranosyl)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2543]



Prepared according to Standard Procedure D (**144** instead of IDU) using 4-ethynylpentylbenzene. Even if the compound was pure on NMR, the solid was purified by column chromatography ($CHCl_3$: MeOH 8:2).

White solid: 0.54 g, 45%

^{1}H-NMR (DMSO, 500 MHz): δ 8.84 (1H, s, H-4), 7.75 (2H, d, J 7.6 Hz, Ph_b), 7.33 (2H, d, J 7.6 Hz, Ph_c), 7.22 (1H, s, H-5), 6.20 (1H, t, J 5.7 Hz, H-1'), 5.28 (1H, d, J 4.2 Hz, OH-3'), 5.15 (1H, t, J 5.1 Hz, OH-5'), 4.27 (1H, dq, J 6.1 Hz 4.2 Hz, H-3'), 3.94 (1H, q, J 3.8 Hz, H-4'), 3.72 (1H, ddd, J 12.1 Hz 5.1 Hz 3.6 Hz, H-5'a), 3.65 (1H, ddd, J 12.1 Hz 5.1 Hz 4.2 Hz, H-5'b), 2.61 (2H, t, J 7.5 Hz, $CH_2\alpha$), 2.42 (1H, ddd, J 13.5 Hz 6.3 Hz 4.1 Hz, H-2'$_\alpha$), 2.11 (1H, dt, J 13.5 Hz 6.1 Hz, H-2'$_\beta$), 1.59 (2H, qn, J 7.5 Hz, $CH_2\beta$), 1.35-1.24 (4H, m, $CH_2\delta$ + $CH_2\gamma$), 0.86 (3H, t, J 7.1 Hz, $CH_3\omega$)

^{13}C-NMR (DMSO, 125 MHz): δ 171.02 (C7a), 153.90 (C6), 153.76 (C2), 144.02 (Ph-C_d), 137.77 (C4), 128.93 (Ph-C_c), 125.86 (Ph-C_a), 124.50 (Ph-C_b), 106.88 (C4a), 98.62 (C5), 88.16 (C4'), 87.57 (C1'), 69.55 (C3'), 60.68 (C5'), 42.26 (C2'), 34.89 (Cα), 30.80 (Cγ), 30.33 (Cβ), 21.89 (Cδ), 13.84 (Cω)

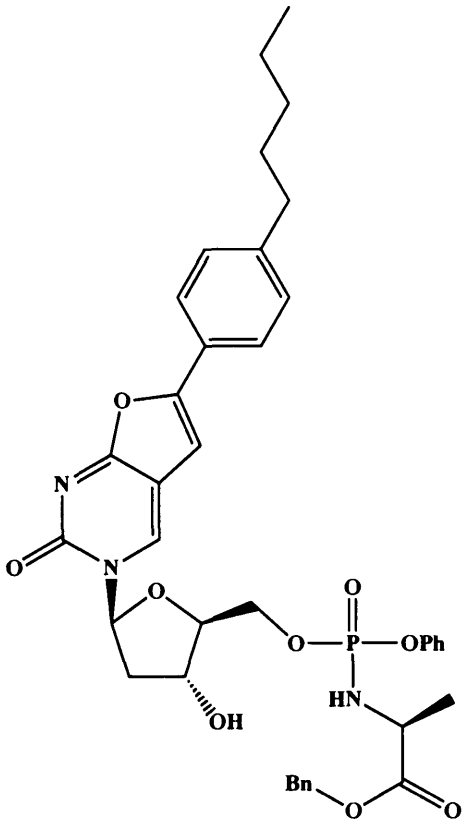
Anal. Calcd for $C_{22}H_{26}N_2O_5$: C 66.32, H 6.58, N 7.03, O 20.08. Found C 66.30, H 6.56, N 7.10, O 20.25

MS: 421 (M+Na)

$[\alpha]_d^{20}$: -80° (*c* 4.0 , DMSO)

129f 3-(2-deoxy-β-L-ribofuranosyl-5-[phenyl-(benzyloxy-L-alaninyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2552]



Prepared according to Standard Procedure C (**129** instead of **33f**) using **125f**

White foam: 0.07 g, 20%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.45 8.34 (1H, 2s, H-4), 7.56-7.48 (2H, m, BCNA-Ph), 7.27-7.00 (12H, m, BCNA-Ph+Ph+Bn), 6.47 6.45 (1H, 2s, H-5), 6.27-6.21 (1H, m, H-1'), 5.07-4.98 (2H, m, Bn-CH_2), 4.44-3.88 (6H, m, H-3'+H-4'+Ala-CH+H-5'+NH), 2.73-2.60 (1H, m, H-2'$_\alpha$), 2.59-2.50 (2H, m, $CH_2\alpha$), 2.05-1.85 (1H, m, H-2'$_\beta$), 1.60-1.50 (2H, m, $CH_2\beta$), 1.36-1.09 (7H, m, $CH_2\gamma$+$CH_2\delta$+Ala+CH_3), 0.87-0.80 (3H, m, $CH_3\omega$)

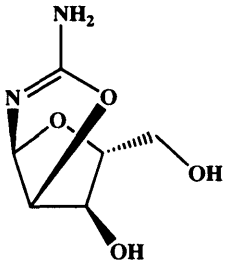
^{13}C-NMR ($CDCl_3$, 125 MHz): δ 172.9 172.8 (Ala-CO), 170.6 (C2), 154.8 (C7a), 153.7 (C6), 146.4 146.3 (C4a), 138.7 138.5 (C5), 130.9, 130.8, 130.2, 129.6, 129.4, 129.3, 129.2, 126.3, 125.9, 125.8, 121.6, 121.5, 121.3, 121.2, 104.7, 104.5 (Ph), 99.2 99.1 (C4), 90.3 90.2 (C1'), 87.8 87.7 87.6 (C4'), 71.7 71.6 (C3'), 68.1 68.0 (Bn-CH_2), 67.5 67.4 67.3 67.2 (C5'), 51.9 51.7 (Ala-CH), 42.8 42.7 (C2'), 36.7 ($CH_2\alpha$), 32.6 ($CH_2\gamma$), 32.2 ($CH_2\beta$), 23.6 ($CH_2\delta$), 20.3 (Ala-CH_3), 14.4 ($CH_3\omega$)

^{31}P-NMR ($CDCl_3$, 202 MHz): δ 4.23, 3.50

Anal. Calcd for $C_{38}H_{42}N_3O_9P$: C 63.77, H 5.91, N 5.57, O 20.12. Found C 63.65, H 5.82, N 5.97

MS: 738 (M+Na)

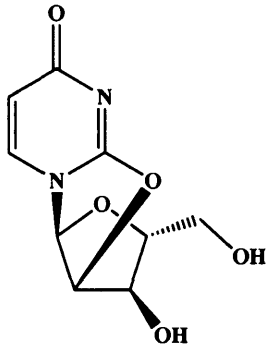
151 2-amino-α-D-ribofurano[1',2':4,5]oxazoline



A mixture of D-ribose (17g, 113 mmol), cyanamide (10 g, 2 eq.), MeOH (30 ml) and 6M NH_4OH (5 ml) was stirred at room temperature for 72 hours and then kept at –10°C for 15 hours. The product was collected by filtration, washed with MeOH and Et_2O and dried under vacuum to obtain 17.00 g (86%) of white solid.

^{1}H-NMR (DMSO, 500 MHz): δ 6.25 (2H, bs, NH_2), 5.78 (1H, d, J 3.4 Hz, H-1'), 5.15 (1H, bs, OH-3'), 4.63-4.52 (2H, bs, OH-5'+H-2'), 3.76-3.68 (1H, m, H-3'), 3.68-3.61 (1H, m, H-5'), 3.44-3.36 (1H, m, H-5'), 3.30-3.25 (1H, m, H-4')
13C-NMR (DMSO, 125 MHz): δ 163.74 (C-NH_2), 98.16 (C1'), 80.69 (C2'), 77.70 (C4'), 71.09 (C3'), 60.35 (C5')

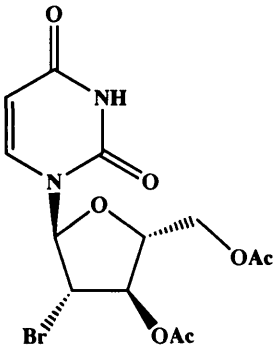
152 $O^{2,2'}$-Anhydro-α-D-uridine



A solution of **151** (12g, 69 mmol) and methyl propiolate (12 ml, 2 eq.) in 50% aqueous ethanol (180 ml) was refluxed for 5 hours and then dried under reduced pressure to obtain a residue that was taken with acetone and left at 0°C for 2 hours. The resulting white precipitate filtrated and washed with acetone to afford 11.53 g (75%) of the titled compound.

^{1}H-NMR (DMSO, 500 MHz): δ 7.85 (1H, d, J 7.0 Hz, H-5), 6.20 (1H, d, J 5.0 Hz, H-1'), 5.89 (1H, d, J 7.0 Hz, H-6), 5.74 (1H, d, J 6.5 Hz, OH-3'), 5.24 (1H, t, J 5.4 Hz, H-2'), 4.85 (1H, t, J 5.5 Hz, OH-5'), 4.06 (1H, q, J 6.5 Hz, H-3'), 3.73-3.67 (1H, m, H-5'), 3.60-3.54 (1H, m, H-4'), 3.51-3.44 (1H, m, H-5')
^{13}C-NMR (DMSO, 125 MHz): δ 171.03 (C4), 160.68 (C2), 136.82 (C5), 108.78 (C6), 88.57 (C1'), 81.43 (C2'), 80.76 (C4'), 69.82 (C3'), 59.48 (C5')

153 3', 5'-diacetyl-2'-bromo-2'-deoxy-α-D-uridine

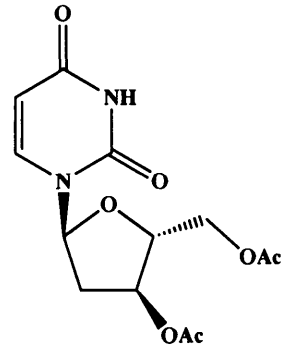


Compound **152** (2.26 g, 10 mmol) was suspended in ACN (50 ml) and heated at reflux. AcBr (6.7 ml, 9 eq.) was added dropwise. The mixture was stirred at reflux for 1 hours, then the sovent was removed under reduced pressure and the residue was washed with Et_2O to obtain the titled compound as orange solid 3.90 g (100%).

^{1}H-NMR (DMSO, 500 MHz): δ 11.46 (1H, bs, NH), 7.80 (1H, d, J 8.0 Hz, H-5), 6.22 (1H, d, 5.3 Hz, H-1'), 5.67 (1H, d, J 8.0 Hz, H-6), 5.43 (1H, t, J 5.0 Hz, H-3'), 4.85 (1H, t, 5.2 Hz, H-2'), 4.62 (1H, q, J 6.0 Hz, H-4'), 4.33-4.25 (2H, m, H-5'), 2.06 (6H, s, CH_3)

^{13}C-NMR (DMSO, 125 MHz): δ 169.99 (Ac-CO), 169.46 (Ac-CO), 162.97 (C4), 150.40 (C2), 140.47 (C5), 101.95 (C6), 90.32 (C1'), 81.19 (C4'), 77.46 (C3'), 63.69 (C5'), 48.60 (C2'), 20.54 (Ac-CH_3), 20.50 (Ac-CH_3)

154 3', 5'-diacetyl-2'-deoxy-α-D-uridine

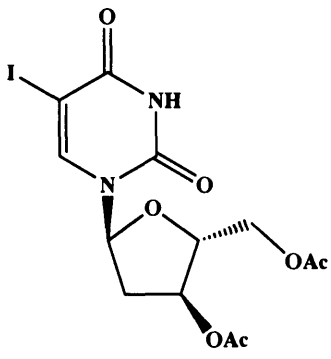


To a stirred solution of **153** (3.90 g , 10 mmol) in anhydrous and degassed toluene (30 ml), a solution of nBu$_3$SnH (4.3 ml, 2.3 eq.) and AIBN (0.33 g , 0.2 eq.) were added. After the addiction the solution was stirred at reflux for 2 hours. The resulting clear solution was evaporated and Et_2O was slowly added to obtain a precipitate. The solid was filtered and dried to obtain 2.99 g (96%) of the titled compound.

^{1}H-NMR (DMSO, 500 MHz): δ 11.29 (1H, bs, NH), 7.68 (1H, d, J 7.7 Hz, H-5), 6.12 (1H, d, 6.1 Hz, H-1'), 5.62 (1H, d, J 7.7 Hz, H-6), 5.14 (1H, d, J 6.6 Hz, H-3'), 4.67 (1H, t, J 5.1 Hz, H-4'), 4.16-4.07 (2H, m, H-5'), 2.77 (1H, dt, J 14.8 Hz 6.9 Hz, H-2'), 2.16 (1H, d, J 14.8 Hz, H-2'), 2.06 (3H, s, CH_3), 1.99 (3H, s, CH_3)

^{13}C-NMR (DMSO, 125 MHz): δ 170.10 (Ac-CO), 169.82 (Ac-CO), 163.26 (C4), 150.31 (C2), 140.47 (C5), 100.87 (C6), 86.31 (C1'), 83.42 (C3'), 73.83 (C4'), 63.42 (C5'), 37.16 (C2'), 20.77 (Ac-CH_3), 20.55 (Ac-CH_3)

155 3', 5'-diacetyl-2'-deoxy-5-iodo-α-D-uridine

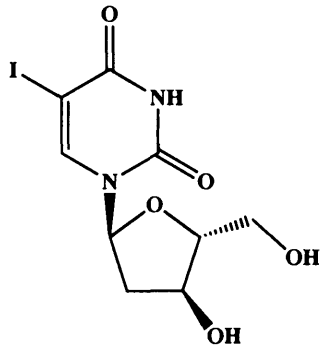


To solution of **154** (2.99 g, 9.60 mmol) in glacial AcOH was added I_2 (1.22 g, 0.5 eq.) and CAN (2.63 g, 0.5 eq.) and the mixture was then stirred at 80°C for 1 hours. The solvent was removed under reduced pressure and the residue was dissolved in DCM and washed with H_2O and brine. The organic layer was dried over $MgSO_4$ and evaporated to afford 4.00 g (95%) of the titled compound.

^{1}H-NMR (DMSO, 500 MHz): δ 11.68 (1H, bs, NH), 8.05 (1H, s, H-6), 6.15 (1H, t, J = 7.1 Hz, H-l'), 5.17 (1H, d, J 5.5 Hz, H-3'), 4.77 (1H, t, J 4.6 Hz, H-4'), 4.15-4.03 (2H, m, H-5'), 2.77 (1H, dt, J 14.8 Hz 6.8 Hz, H-2'), 2.22 (1H, d, J 14.8 Hz, H-2'), 2.06 (3H, s, CH_3), 2.04 (3H, s, CH_3)

^{13}C-NMR (DMSO, 125 MHz): δ 170.08 (Ac-CO), 169.49 (Ac-CO), 160.58 (C4), 150.03 (C2), 144.88 (C6), 86.47 (C1'), 83.55 (C4'), 73.85 (C3'), 68.50 (C5), 63.37 (C5'), 37.08 (C2'), 20.92 (Ac-CH_3), 20.56 (Ac-CH_3)

156 2'-deoxy-5-iodo-α-D-uridine



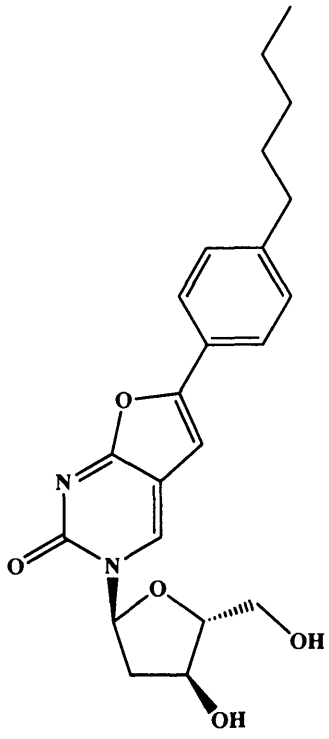
Compound **155** (4.00 g, 9.1 mmol) was dissolved in 1M MeONa / MeOH solution (100 ml) and then stirred for 2 hours. The solution was concentrated under reduced pressure and diluited with H_2O (100 ml). DOWEX H^+ was added and the solution was stirred for 30 minutes. The resin was filtered and washed with H_2O, then the solution was dried under reduced pressure to obtain 2.75 g (85%) of the titled compound.

^{1}H-NMR (DMSO, 500 MHz): δ 11.62 (1H, bs, NH), 8.31 (1H, s, H-6), 6.10 (1H, t, J 7.0 Hz, H-1'), 5.40 (1H, bs, OH-3'), 4.81 (1H, bs, OH-5'), 4.24 (1H, d, J 4.8 Hz, H-3'), 4.21-4.16 (1H, m, H-4'), 3.40-3.34 (2H, m, H-5'), 2.57 (1H, dt, J 14.3 Hz 6.4 Hz, H-2'), 1.92 (1H, d, J 14.3 Hz, H-2')

^{13}C-NMR (DMSO, 125 MHz): δ 160.57 (C4), 150.20 (C2), 146.03 (C6), 89.67 (C4'), 86.19 (C1'), 70.51 (C3'), 68.32 (C5), 61.68 (C5'), 39.99 (C2')

149 3-(2-deoxy-α-D-ribofuranosyl)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2553]



Prepared according to Standard Procedure D (**155** instead of IDU) using 4-ethynylpentylbenzene. Even if the compound was pure on NMR, the solid was purified by column chromatography ($CHCl_3$: MeOH 8:2).

White solid: 0.42 g, 35%

^{1}H-NMR (DMSO, 500 MHz): δ 8.63 (1H, s, H-4), 7.73 (2H, d, J 6.9 Hz, Ph_b), 7.33 (2H, d, J 6.9 Hz, Ph_c), 7.21 (1H, s, H-5), 6.11 (1H, d, J 6.8 Hz, H-1'), 5.10 (1H, d, J 2.6 Hz, OH-3'), 4.92 (1H, t, J 5.7 Hz, OH-5'), 4.43 (1H, t, J 4.6 Hz, H-4'), 4.28-4.24 (1H, m, H-3'), 3.52-3.42 (2H, m, H-5'), 2.67-2.59 (3H, m, H-$2'_\beta$+$CH_2\alpha$), 2.07 (1H, d, J 14.5 Hz, H-$2'_\alpha$), 1.60 (2H, qn, J 7.4 Hz, $CH_2\beta$), 1.37-1.22 (4H, m, $CH_2\delta$ + $CH_2\gamma$), 0.87 (3H, t, J 6.8 Hz, $CH_3\omega$)

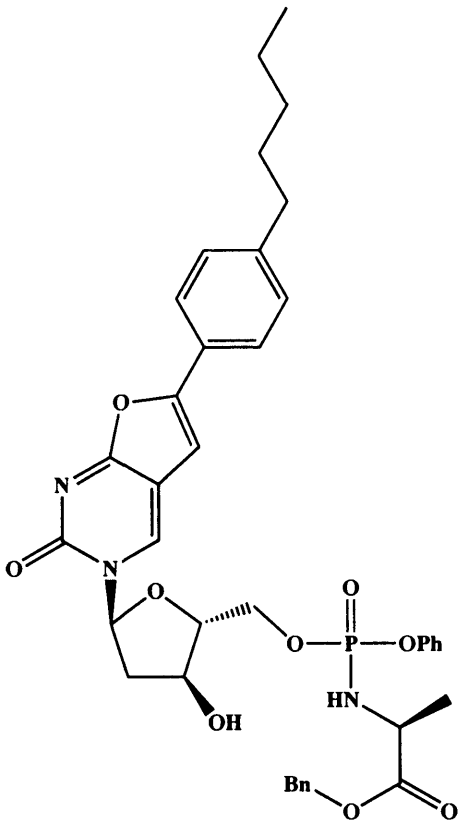
^{13}C-NMR (DMSO, 125 MHz): δ 171.03 (C7a), 153.83 (C6), 153.50 (C2), 143.94 (Ph-C_d), 138.84 (C4), 128.99 (Ph-C_c), 125.96 (Ph-C_a), 124.45 (Ph-C_b), 106.13 (C4a), 98.84 (C5), 90.65 (C4'), 89.57 (C1'), 70.61 (C3'), 61.68 (C5'), 40.75 (C2'), 34.88 (Cα), 30.80 (Cγ), 30.36 (Cβ), 21.89 (Cδ), 13.87 (Cω)

Anal. Calcd for $C_{22}H_{26}N_2O_5$: C 66.32, H 6.58, N 7.03, O 20.08. Found C 66.21, H 6.36, N 7.26, O 20.38

MS: 421 (M+Na)

149f 3-(2-deoxy-α-D-ribofuranosyl-5-[phenyl-(benzyloxy-L-alaninyl)]phosphate)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2552]



Prepared according to Standard Procedure C (**149** instead of **33f**) using **125f**

White foam: 0.08 g, 23%

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.22 8.19 (1H, 2s, H-4), 7.43-7.38 (2H, m, BCNA-Ph), 7.31-7.05 (12H, m, BCNA-Ph+Ph+Bn), 6.45 6.43 (1H, 2s, H-5), 6.14-6.01 (1H, m, H-1'), 5.14-5.04 (2H, m, Bn-CH_2), 4.64-4.57 (1H, m, H-4'), 4.43-4.35 (1H, m, H-3'), 4.16-3.94 (3H, m, Ala-CH+H-5'), 3.73-3.55 (1H, m, NH), 2.62-2.45 (4H, m, CH_2α+H-2'), 1.62-1.50 (2H, m, CH_2β), 1.35-1.31 (3H, m, Ala-CH_3), 1.31-1.22 (4H, m, CH_2δ+CH_2γ), 0.86-0.79 (3H, m, CH_3ω)

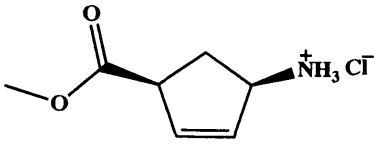
^{13}C-NMR ($CDCl_3$, 125 MHz): δ 173.34 173.30 173.28 173.24 (Ala-CO), 171.45 (C2), 155.63 155.60 (C7a), 154.66 154.62 (C6), 150.61 150.53 150.48 (C4a), 144.77 136.88, 135.21 135.20 129.82, 129.80, 128.81, 128.69, 128.66, 128.57, 128.53, 128.28, 128.22, 125.76, 125.21, 125.17, 124.75, 120.22, 120.18, 120.12, 120.08, 107.65, 107.59 (Ph), 96.93 (C4), 91.02 91.00 (C1'), 88.34 88.28 88.23 (C4'), 71.92 71.87 (C3'), 67.40 67.65 (Bn-CH_2), 66.58 66.53 66.51 66.47 (C5'), 50.45 50.34 (Ala-CH), 40.98 40.90 (C2'), 35.83 (CH_2α), 31.48 (CH_2γ), 30.94 (CH_2β), 22.53 (CH_2δ), 20.92 20.88 (Ala-CH_3), 14.02 (CH_3ω)

^{31}P-NMR ($CDCl_3$, 202 MHz): δ 2.62, 2.36

Anal. Calcd for $C_{38}H_{42}N_3O_9P$: C 63.77, H 5.91, N 5.57, O 20.12. Found C 63.82, H 5.96, N 5.47

MS: 738 (M+Na)

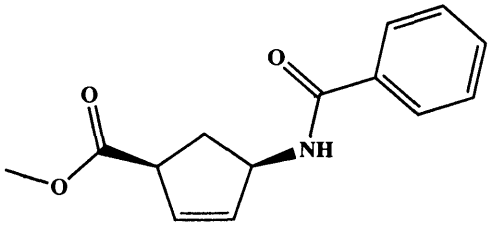
163 cis-4-amino-2-cyclopentenecarboxilic acid methyl ester hydrochloride



$SOCl_2$ (8 ml) was slowly added to MeOH (50 ml) at 0°C, then 5.45 g (50 mmol) of Vince lactame **162** was added and the mixture was stirred for 2 hours at the same temperature. The solvent was removed under reduced pressure to obtain 8.88 g (100%) of the titled compound as white solid.

^{1}H-NMR (DMSO, 500 MHz): δ 8.44 (3H, bs, NH_3), 6.07 (1H, dd, J 5.5 Hz 2.2 Hz, H-2), 5.90 (1H, dd, J 5.5 Hz 2.4 Hz, H-3), 4.21-4.12 (1H, m, H-1), 3.72-3.67 (1H, m, H-4), 3.65 (3H, s, OCH_3), 2.60-2.52 (1H, m, H-6), 2.00-1.92 (1H, m, H-6)
^{13}C-NMR (DMSO, 125 MHz): δ 172.68 (C-5), 134.18 (C-2), 130.44 (C-3), 55.18 (C-1), 51.89 (OCH_3), 48.93 (C-4), 31.21 (C6)

164 cis-4-benzoylamino-2-cyclopentenecarboxilic acid methyl ester

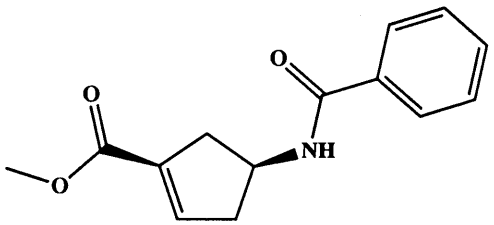


Compound **163** (8.8 g, 50 mmol) was dissolved in Pyr (50 ml) and benzoyl chloride (6.4 ml, 1.1 eq.) was added dropwise at 0°C. The mixture was then stirred at room temperature for 3 hours after which it was poured in ice. When the ice complete melted the precipitate was filtered, washed with water and dried under vaccum to obtain 11.04 g (90%) of the title compound as a white solid.

^{1}H-NMR (DMSO, 500 MHz): δ 8.53 (1H, d, J 7.0 Hz, NH), 7.88 (2H, d, J 7.5 Hz, Ph-H_2), 7.52 (1H, t, 7.5 Hz, Ph-H_4), 7.45 (2H, t, 7.5 Hz, Ph-H_3), 5.94-5.90 (1H, m, H-2), 5.90-5.85

(1H, m, H-3), 5.03 (1H, q, J 7.8 Hz, H-1), 3.66 (3H, s, OCH_3), 3.64-3.59 (1H, m, H-4), 2.58 (1H, dt, J 13.0 Hz 7.8 Hz, H-6), 1.94 (1H, dt, J 13.0 Hz 7.8 Hz, H-6)
^{13}C-NMR (DMSO, 125 MHz): δ 173.59 (C-5), 165.80 (CON), 134.47 (C-3), 134.24 (Ph-C), 131.12 (Ph-C_4), 130.60 (C-2), 128.14 (Ph-H_3), 127.31 (Ph-C_2), 54.91 (C-1), 51.69 (OCH_3), 48.46 (C-4), 33.64 (C-6)

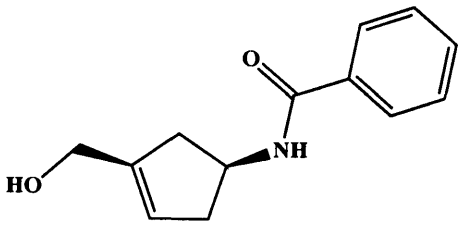
165 4-(benzoylamino)-1-cyclopentenecarboxilic acid methyl ester



Compound **164** (11.04 g, 45 mmol) was dissolved in DCM (55 ml) and DBU (9.6 ml, 1.45 eq.) was added. The mixture was stirred at room temperature for 15 hours after which it was cooled at 0°C and ice (17 g), H_2O (11 ml) and $H_2SO_{4(C)}$ (3.3 ml) were added. The organic phase was separated and washed with water. The organic layer was evaporated under reduced pressure and the solid was triturated with Et_2O filtered and dried to obtain 11 g (100%) of the title compound as a yellow solid.

^{1}H-NMR (DMSO, 500 MHz): δ 8.60 (1H, d, J 6.2 Hz, NH), 7.86 (2H, d, J 7.5 Hz, Ph-H_2), 7.52 (1H, t, 7.5 Hz, Ph-H_4), 7.45 (2H, t, 7.5 Hz, Ph-H_3), 6.76-6.70 (1H, m, H-3), 4.66-4.58 (1H, m, H-1), 3.69 (3H, s, OCH_3), 2.95-2.84 (2H, m, H-2+H-6), 2.61-2.49 (2H, m, H-2+H-6)
^{13}C-NMR (DMSO, 125 MHz): δ 165.98 (CON), 164.45 (C-5), 141.82 (C-3), 134.39 (Ph-C), 133.83 (C-4), 131.07 (Ph-C_4), 128.12 (Ph-H_3), 127.27 (Ph-C_2), 51.29 (OCH_3), 48.83 (C-1), 40.10 (C6), 33.64 (C2)

166 N-[3-(Hydroxymethyl)-3-cyclopentenyl]-benzamide



Compound **165** (11 g, 45 mmol) was suspended in a mixture of DCM (50 ml) and Toluene (25 ml) and the suspension was colled at 0°C. $AlCl_3$ (6 g, 1 eq.) was added and the reaction was mixture was stirred for 20 minutes to give a cloudy solution. The resultant mixture was then cooled to 0°C and a 25% solution of DIBAH in toluene (69 ml, 2 eq.) was added at a rate such that the reaction mixture remained in the temperature range of 0°C-10°C. Upon completion of the additionof DIBAH the reaction mixture was stirred for 10 minutes at 0°C. After this time the mixture was slowly added to a solution of 4M HCl at room temperature. Toluene was added and stirring continued for a further 30 minutes. The reaction mixture was the cooled to 0°C and stirred at this temperature for 1 hour. The product was harvested by filtration and washed with toluene, 2M HCl and H_20 and dried under vacuum to yield the title compound (8.9 g, 90%).

^{1}H-NMR (DMSO, 500 MHz): δ 8.50 (1H, d, J 6.8 Hz, NH), 7.85 (2H, d, J 7.8 Hz, Ph-H_2), 7.51 (1H, t, 7.8 Hz, Ph-H_4), 7.45 (2H, t, 7.8 Hz, Ph-H_3), 5.52-5.48(1H, m, H-3), 4.58 (1H, sx, J 6.8 Hz, H-1), 4.02-3.93 (2H, m, H-5), 2.72-2.57 (2H, m, H-2+H-6), 2.39-2.25 (2H, m, H-2+H-6)

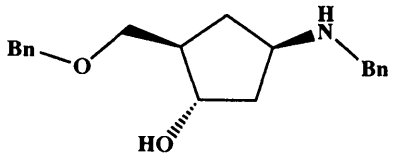
^{13}C-NMR (DMSO, 125 MHz): δ 165.99 (CO), 143.66 (C-4), 134.60 (Ph-C), 130.95 (Ph-C_4), 128.10 (Ph-H_3), 127.25 (Ph-C_2), 121.38 (C-3), 59.99 (C-5), 49.45 (C-1), 39.14 (C2), 38.76 (C6)

Compound **166** (8.9 g, 41 mmol) was added to t-butyl methyl ether (90 ml) and the resu suspension stirred at room temperature while potassium carbonate (34 g), tet butylammonium hydrogen sulphate (2.1 g) and sodium hydroxide (11.5 g) were a sequentially. Stirring was continued while the internal temperature was raised to reflux hot water bath. Reflux was continued for a further 25 minutes and then benzyl bromide ml, 2.2 eq.) was added dropwise to the refluxing suspension. Reflux was continued f further 160 minutes. Heating was discontinued and methanol (1 ml) added over 30 sec The mixture was then cooled and distilled water (5 times 18 ml) added over 4 minutes. mixture was further cooled with a cold water bath, transferred to a glass separating flask the lower aqueous phase removed. The aqueous phase was extracted with t-butyl methyl e and the organic phases combined and washed with distilled water. The organic phase dried and the residue was purified by column chromatography (Hexane Ethyl Acetate 9 : obtain the title compound (14.05 g, 86%) as a yellow oil.

^{1}H-NMR (DMSO, 500 MHz): δ 7.55-7.21 (15H, m, Ph+2xBn), 5.53 (1H, bs, H-3), 4.62 Bs, Bn+H-1), 4.38 (2H, s, Bn), 3.90 (2H, s, H-5), 2.40 (4H, bs, H-2+H-6)

^{13}C-NMR (DMSO, 125 MHz): δ 172.21 (CO), 141.74 (C-4), 140.26, 138.98, 131.14, 131 130.43, 130.21, 130.10, 129.59, 129.37, 129.18, 128.49, 128.16 (Ar), 126.59 (C-3), 7 (Bn-CH_2), 69.96 (C-5), 60.00 (bs, C-1), 46.62 (Bn-CH_2), 38.86 (bs, C2), 38.42 (bs, C6)

168 4-benzylamino-2-Benzyloxymethyl-1-cyclopentanol

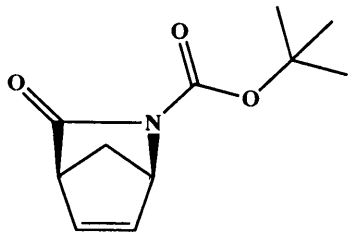


2-methyl-2-butene (25 ml) was added to tetrahydrofuran (25 ml) and the resulting solution stirred under nitrogen and cooled to 0°C. Borane dimethylsulphide complex (10.6 ml) was then added at a rate such that the temperature of the mixture did not exceed 24°C. This solution was then stirred and treated with a solution of **167** (14.05 g, 35 mmol) in tetrahydrofuran (28 ml) over 40 minutes. Stirring was continued for 24 h at room temperature and then the reaction quenched by cooling the solution to 0°C and adding dropwise a mixture of water (1.5 ml) and tetrahydrofuran (14 ml). The solution was re-cooled to 0°C and then 3M sodium hydroxide (35 ml) added over 12 minutes. The solution/emulsion obtained was cooled to –20°C and hydrogen peroxide (30%, 36 ml) added dropwise over ca 2 hours such that an internal temperature of <30°C was maintained. After 20 minutes a solution of sodium sulphite (7 g) in water (28 ml) was added over 70 minutes. Following the final addition of hydrogen peroxide the mixture was left under nitrogen overnight. The mixture was then stirred and methyl isobutyl ketone (70 ml) was added. The phases were separated and the aqueous phase was extracted with methyl isobutyl ketone. The combined organic phases were washed with water and the organic phase reduced to ca 70 ml volume. The resulting yellow solution was stirred and cooled to 4°C and then concentrated hydrochloric acid (3 ml) was added. The cooling bath was removed and after 30 minutes t-butyl methyl ether (70 ml) was added dropwise. The pH was lowered to 1-2 during the addition of t-butyl methyl ether by further addition of concentrated hydrochloric acid. The resulting suspension was stirred for ca 1 hour with ice/water cooling and then filtered. The residual white solid was washed with t-butyl methyl ether and then dried to give the title compound (7.5 g, 63%).

^{1}H-NMR (DMSO, 500 MHz): 9.6 (2H, broad, NH_2), 7.7-7.2 (10H, m, 2xBn), 4.90 (1H, broad, OH-3), 4.48 (2H, m, Bn), 4.10 (2H, m, Bn), 3.98 (1H, m, H-3), 3.62-3.53 (1H, m, H-1), 3.53-3.35 (2H, m, H-5), 2.30-2.20 (1H, m, H-6), 2.10-.1.82 (3H, m, H-2+H-4), 1.54-1.47 (1H, m, H-6)

^{13}C-NMR (DMSO, 125 MHz): δ 138.52 (Bn-C), 132.17 (Bn-C), 130.00, 128.78, 128.56, 128.22, 127.43, 127.36 (Bn), 72.05 (Bn-CH_2), 71.32 (C-5), 71.17 (C-3), 55.35 (C-1), 48.85 (Bn-CH_2), 46.56 (C-4), 37.24 (C2), 31.01 (C6)

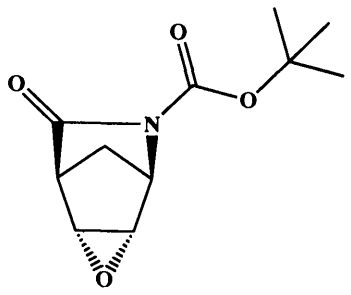
169 N-Boc-2-azabicyclo [2.2.1]hept-5-en-3-one



A solution of Boc anhydride (14.4 g, 1.1 eq.) in THF (10 ml) was added dropwise to a stirred solution of Vince lactam **162** (6 g, 55 mmol), TEA (12 ml) and DMAP (0.67 g, 0.1 eq.) in THF (50 ml) at room temperature. The reaction was stirred for 4 hours and the solvent was then removed under reduced pressure. The residue was dissolved in Ethyl Acetate and washed with H_20 and brine. The organic layer was separated dried over $MgSO_4$ and evaporated under reduced pressure to give 11.5 g (100%) of the title compound as an orange solid.

^{1}H-NMR (DSMO, 500 MHz): δ 6.98 (1H, dd, J 5.1 Hz 1.9 Hz, H-2), 6.75 (1H, dt, J 3.5 Hz 1.8 Hz, H-3), 4.91-4.89 (1H, m, H-1), 3.32 (1H, bs, H-4), 2.30 (1H, bs, H-6), 2.08-2.05 (1H, m, H-6), 1.42 (9H, s, Boc)
^{13}C-NMR (DMSO, 125 MHz): δ 175.42 (C-5), 149.82 (Boc-CO), 140.40 (C-2), 138.15 (C-3), 81.47 (Boc-C), 62.10 (C-1), 54.29 (C-6), 53.89 (C-4), 27.61 (Boc-CH_3)

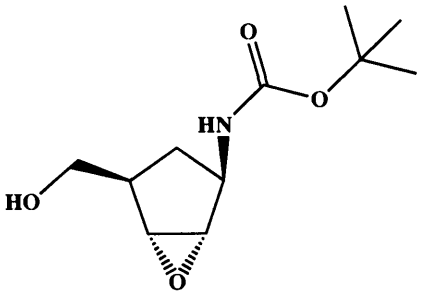
170 N-Boc-2-azabicyclo [2.2.1]hept-5-an-3-one-2,3-epoxide



Compound **169** (11.5 g, 55 mmol) was dissolved in 100 ml of DCM and MCPBA 60% (32 g, 2 eq.), was addedand the mixture was stirred for 48 hours. The precipitate formed was filtered and the resulting solution was washed with a saturated solution of $NaHCO_3$ until complete remotion of the perbenzoic acid. The solvent was removed under reduced pressure to obtain 10.53 g (85%) of the title compound as a white solid.

^{1}H-NMR (DSMO, 500 MHz): δ 4.64-4.62 (1H, m, H-1), 3.95 (1H, dd, J 3.6 Hz 1.2 Hz, H-2), 3.82 (1H, dd, J 3.6 Hz 1.5 Hz, H-3), 3.07 (1H, qn, J 1.5 Hz, H-4), 1.70 (1H, dt, J 10.3 Hz 1.8 Hz, H-6), 1.62 (1H, dt, J 10.3 Hz 1.6 Hz, H-6), 1.49 (9H, s, Boc)
^{13}C-NMR (DMSO, 125 MHz): δ 172.90 (C-5), 149.14 (Boc-CO), 82.02 (Boc-C), 58.02 (C-1), 52.46 (C-2), 49.35 (C-3), 47.60 (C-4), 27.60 (Boc-CH_3), 26.22 (C-6)

171 N-Boc-4-hydroxymethyl-cyclopentylamine-2,3-epoxide

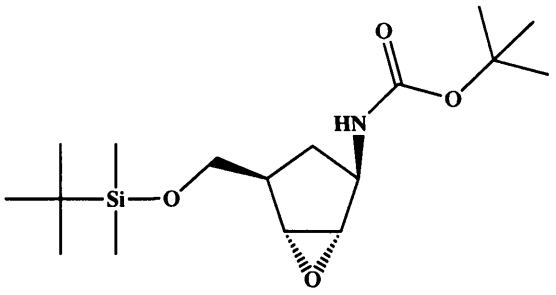


Compound **171** (9.00 g, 40 mmol), was dissolved in MeOH (100 ml) and $NaBH_4$ (4.54 g, 3 eq.) was added at 0°C. the mixture was stirred at the same temperature for 2 hours after which the excess of $NaBH_4$ was neutralised by addition of NH_4Cl solution. The solvent was removed

under vaccum and dissolved in DCM, washed with H_20 and dried to obtain the title compound as colourless crystals (9.17 g, 100%).

^{1}H-NMR (DSMO, 500 MHz): δ 6.70 (1H, d, J 6.7 Hz, NH), 5.03 (1H, bs, OH-5), 3.92 (1H, t, J 7.9 Hz, H-1), 3.53-3.36 (3H, m, H-5+H-2), 3.34-3.30 (1H, m, H-3), 2.24 (1H, dt, J 8.8 Hz 5.0 Hz, H- 4), 1.80 (1H, dt, J 13.9 Hz 8.8 Hz, H-6), 1.39 (9H, s, Boc), 1.29 (1H, d, J 13.9 Hz, H-6)
^{13}C-NMR (DMSO, 125 MHz): δ 154.79 (Boc-CO), 77.98 (Boc-C), 61.53 (C-5), 59.30 (C-2), 58.15 (C-3), 49.79 (C-1), 40.86 (C-4), 31.64 (C-6), 27.62 (Boc-CH_3)

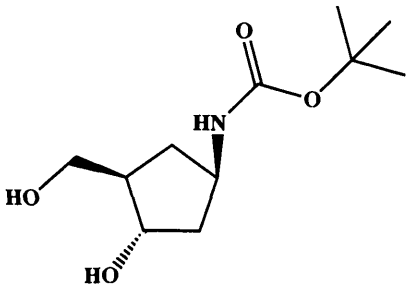
172 N-Boc-4-^{t}butyldimethylsilyloxymethyl-cyclopentylamine-2,3-epoxide



To a solution of **171** (9.17 g, 40 mmol) in DCM (100 ml), imidazole (2.73 g, 40 mmol) and TBDMS-Cl were added. The mixture was stirred for 15 hours at room temperature. The solution was then washed with 0.5M solution of citric acid and the organic layer was dried over $MgSO_4$, evaporated to give the title compound as clear oil (13.74 g, 100%).

^{1}H-NMR (DSMO, 500 MHz): δ 6.49 (1H, d, J 7.2 Hz, NH), 3.92 (1H, t, J 7.2 Hz, H-1), 3.70-3.60 (2H, m, H-5), 3.43-3.41 (1H, m, H-2), 3.34 (1H, bs, H-3), 2.31-2.25 (1H, m, H- 4), 1.85-1.75 (1H, dt, J 13.9 Hz 8.7 Hz, H-6), 1.39 (9H, s, Boc), 1.30 (1H, d, J 13.9 Hz, H-6), 0.89 (9H, s, TBDMS), 0.09 (3H, s, TBDMS), 0.08 (3H, s, TBDMS)
^{13}C-NMR (DMSO, 125 MHz): δ 154.72 (Boc-CO), 77.98 (Boc-C), 63.85 (C-5), 59.10 (C-2), 58.03 (C-3), 49.85 (C-1), 40.97 (C-4), 31.74 (C-6), 27.62 (Boc-CH_3), 25.85 (TBDMS-CH_3), 18.07 (TBDMS-C), -5.46 (TBDMS-CH_3)

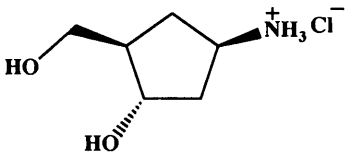
173 N-Boc-4-hydroxymethyl-3-hydroxy-1-cyclopentylamine



Compound **172** (13.74 g, 40 mmol) was dissolved in toluene (50 ml) and 65 % RED-Al (25 ml, 2 eq.) was added at 0°C. The mixture was stirred for 2 hour and then a saturated solution of NH_4Cl was added and stirred for 30 minutes. The precipate was filtred and the solvents were removed under reduced pressure to give the title compound as a white solid (7.03 g, 76%).

^{1}H-NMR (DSMO, 500 MHz): δ 6.75 (1H, d, J 7.2 Hz, NH), 4.55, (2H, bs, OH-3+OH-5), 3.92-3.68 (2H, m, H-1+H-3), 3.49-3.21 (2H, m, H-5), 2.05-1.93 (1H, m, H-2), 1.79-1.71 (1H, m, H-4), 1.71-1.63 (1H, m, H-6), 1.54-1.49 (1H, m, H-6), 1.35 (9H, s, Boc), 1.07-0.99 (1H, m, H-2)
^{13}C-NMR (DMSO, 125 MHz): δ 154.99 (Boc-CO), 77.26 (Boc-C), 71.75 (C-3), 63.10 (C-5), 49.19 (C-4), 48.91 (C-1), 41.07 (C-6), 38.44 (C-2), 25.84

161 4-Amino-2-hydroxymethyl-1-cyctopentanol



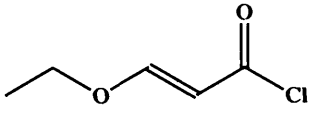
METHOD A: Compound **168** (5 g, 16 mmol) was dissolved in isopropanol (12.5 mL) and water (4 mL). Charcoal (2.5 g) was added and the mixture stirred for 30 minutes. After this time celite (1.25 g) was added and the mixture filtered through a bed of celite. The filter bed was washed with a mixture of isopropanol (6 mL) and water (2 mL) twice. The filtrate and washes were combined. 5% Palladium on charcoal (5 g) was charged into a dry flask. The flask was purged with nitrogen and the combined filtrate and washes from above were added.

The resulting mixture was stirred and heated to 50°C under an atmosphere of hydrogen for 3 hours. After this time the reaction mixture was allowed to cool and then filtered through a pad of celite. The filter pad was then washed with mixtures of isopropanol and water. The combined filtrate and washings were evaporated to approximately 20 mL. n-Butanol (10 mL) was added and the mixture re-evaporated under reduced pressure to 3 mL. Methanol (2 mL) containing concentrated hydrochloric acid (2 mL) was added followed by n-butanol (10 mL). The solvent was removed under reduced pressure to yield a gum. This was taken up in methanol and the volume reduced under pressure to the point at which crystallisation had just begun. Acetone (10 mL) was added slowly and filtration followed by washing with n-butanol/acetone (1:1) and acetone yielded after drying, under reduced pressure, the title compound (2 g, 95%).

METHOD B: Compound **173** (7.03 g, 30 mmol) was dissolved in 100 ml of H_2O and the solution was refluxed for 24 hours. The solvent was removed under vaccum to yield 3.98 g (100%) of the title compound as brown crystals.

1H-NMR (DMSO, 500 MHz): δ 8.20 (3H, bs, NH_3), 4.90-4.50 (2H, bs, OH-3+OH-5), 3.94 (1H, bs, H-3); 3.55-3.48 (1H, m, H-1), 3.48-3.21 (2H, m, H-5), 2.17-2.09 (1H, m, H-6); 1.95-1.70 (3H, m, H-2+H-4), 1.34-1.27 (1H, m, H-6)
^{13}C-NMR (DMSO, 125 MHz): δ 71.44 (C-3), 62.40 (C-5), 49.20 (C-4), 48.71 (C-1), 39.00 (C2), 32.32 (C6)

177 Ethoxy-acryloyl chloride

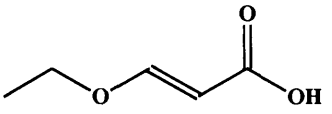


METHOD A: Ethyl vinyl ether (9.6 ml, 100 ml) was slowly added to oxalyl chloride (12.9 ml, 1.5 eq.) at 0°C. The reaction mixture was maintaned for 2 hours at)°C and then warmed at room temperature for 12 hours. Excess of oxalyl chloride was distilled off, and the residue was heated at 120°C for 30 minutes and then purified by vaccum distillation, affording **177** (7.53 g, 53%).

METHOD B: **178** (7 g, 60 mmol) was dissolved in 50 ml of DCM and $SOCl_2$ (8.7 ml, 120 mmol) was added dropwise. The mixture was then stirred at reflux for 2 hours. The solution was concentreated under reduced pressure to obtain the title compound **177** (8 g, 100%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.76 (1H, d, J 12.1 Hz, H-3), 5.49 (1H, d, J 12.1 Hz, H-2), 4.05 (2H, q, J 7.2 Hz, CH_2), 1.38 (3H, t, J 7.2 Hz, CH_3)
^{13}C-NMR ($CDCl_3$, 125 MHz): δ 168.25 (C-3), 164.51 (C-1), 102.80 (C-2), 68.89 (CH_2), 14.35 (CH_3)

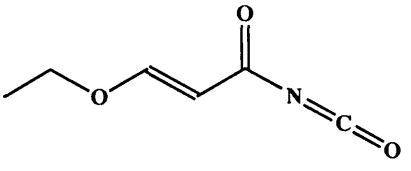
178 Ethoxy-acrylic acid



A mixture of ethoxyethyl acrylate (10 g, 52 mmol), H_20 (20 ml) and NaOH (2.8 g, 1.1 eq.) was stirred at 110°C for 30 minutes. The resulting clear solution was cooled to room temperature and acidified by careful addition of $HCl_{(C)}$ (5 ml). The product was extracted with Wthyl Acetate and H_20 and the combined extracts were dried over $MgSO_4$ and concentrated under reduced pressure to yield 7.00 g (87%) of the title acid.

^{1}H-NMR (DMSO, 500 MHz): δ 11.82 (1H, bs, OH), 7.52 (1H, d, J 12.6 Hz, H-3), 5.14 (1H, d, J 12.6 Hz, H-2), 3.94 (2H, q, J 6.7 Hz, CH_2), 1.24 (3H, t, J 6.7 Hz, CH_3)
^{13}C-NMR (DMSO, 125 MHz): δ 168.17 (C-3), 166.22 (C-1), 96.71 (C-2), 66.58 (CH_2), 14.30 (CH_3)

174 Ethoxy-acryloyl isocyanate

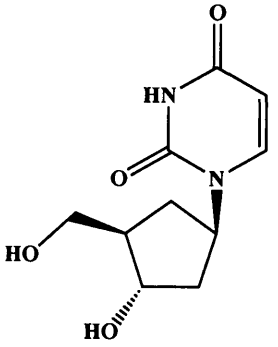


Dry silver cyanate (16.27 g, 108 mmol) was refluxed in dry benzene (80 ml) for 30 minutes, and a solution of **177** (7.3 g, 0.5 eq.) in dry benzene (20 ml) was added dropwise. After

addition of **177** was complete, the mixture was stirred under reflux for 30 minutes before allowing the solid to settle out. The supernatant was then decanted and used directly in the next reaction.

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.87 (1H, d, J 12.3 Hz, H-3), 5.50 (1H, d, J 12.3 Hz, H-2), 4.05 (2H, q, J 7.1 Hz, CH_2), 1.51 (3H, t, J 7.1 Hz, CH_3)
^{13}C-NMR ($CDCl_3$, 125 MHz): δ 166.89 (C-3), 164.31 (C-1), 128.90 (NCO), 100.92 (C-2), 67.70 (CH_2), 14.48 (CH_3)

176 1-(3-hydroxy-4-(hydroxymethyl)cyclopentyl)uracil

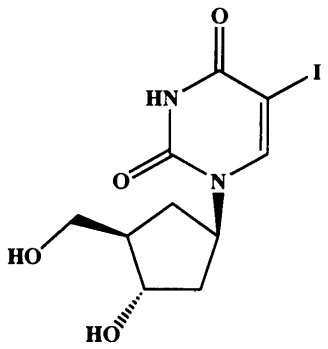


To a solution of **161** (2.01 g, 12 mmol) in dimethylformamide (15 mL) containing DBU (2 mL, 12 mmol) and 4-Å molecular sieves (3 g) at -20 °C was added a 0.5M benzenic solution of **176** (37 ml, 1.7 eq.). The mixture was stirred at -15 °C for 1 h and then left to warm to room temperature. After 18 hours the mixture was filtered and the filtrate evaporated. The residue was purified by column chromatography on silica gel, eluting with chloroform-methanol (9: 1) to give the intermediate acryloylurea **174** (2.48 g, 76%) as a brown solid. A solution of the acryloylurea **174** (2.48 g, 9.1 mmol) in 5% sulfuric acid (50 mL) was refluxed for 3 hours. The mixture was cooled to room temperature and adjusted to pH 7 with 2N sodium hydroxide. The solution was evaporated under reduced pressure and the residue dried and then extracted with ethanol. The combined ethanolic solution was evaporated under reduced pressure and purified by column chromatography ($CHCl_3$ MeOH 9:1) to give 1.48 g (72%) of the title compound.

^{1}H-NMR (DMSO, 500 MHz): δ 10.30 (1H, bs, NH), 7.70 (1H, d, J 7.9 Hz, H-5), 5.56 (1H, d, J 7.9 Hz, H-6), 4.94 (1H, qn, J 8.9 Hz, H-1'), 4.83 (1H, bs, OH-3'), 4.71 (1H, bs, OH-5'),

4.09-4.02 (1H, m, H-3'), 3.51-3.35 (2H, m, H-5'), 2.12-2.00 (1H, m, H-2'), 1.95-1.72 (3H, m, H+4'+H-6'), 1.43-1.34 (1H, m, H-2')
^{13}C-NMR (DMSO, 125 MHz):δ 163.11 (C-2), 151.00 (C-4), 142.29 (C-5), 101.33 (C-6), 71.37 (C-3'), 62.57 (C-5'), 53.61 (C-1'), 48.85 (C-4'), 38.92 (C-2'), 32.32 (C-6')

159 1-(3-hydroxy-4-(hydroxymethyl)cyclopentyl)-5-iodo-uracil

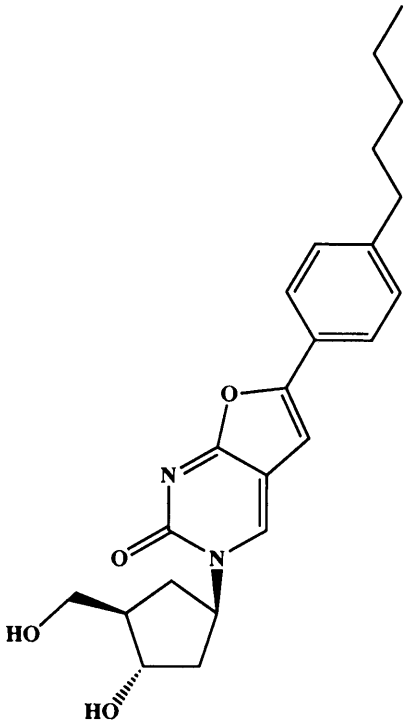


To solution of **174** (1.48 g, 6.5 mmol) in glacial AcOH was added I_2 (0.83 g, 0.5 eq.) and CAN (1.78 g, 0.5 eq.) and the mixture was then stirred at 80°C for 1 hours. The solvent was removed under reduced pressure and the residue was purified by column chromatography to afford 1.21g (53%) of the titled compound.

^{1}H-NMR (DMSO, 500 MHz): δ 9.51 (1H, bs, NH), 9.51 (1H, s, H-6), 4.96-4.87 (1H, m, H-1'), 4.71 (1H, bs, OH-3'), 4.60 (1H, bs, OH-5'), 4.1-3.95 (1H, m, H-3'), 3.43-3.29 (2H, m, H-5'), 2.11-2.00 (1H, m, H-2'), 1.87-1.68 (3H, m, H+4'+H-6'), 1.48-1.41 (1H, m, H-2')
^{13}C-NMR (DMSO, 125 MHz):δ 165.39 (C-2), 160.49 (C-4), 146.64 (C-6), 71.61 (C-3'), 68.32 (C5), 63.25 (C-5'), 55.61 (C-1'), 48.85 (C-4'), 38.92 (C-2'), 32.32 (C-6')

3-(3-hydroxy-4-(hydroxymethyl)cyclopentyl)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[Cf2566]



Prepared according to Standard Procedure D (**159** instead of IDU) using 4-ethynylpentylbenzene. The compund was purified by column chromatography ($CHCl_3$:MeOH 95:5) to afford 0.38 g (32%) of the title compound as brown solid. A small portion (100 mg) was further purified by preparative HPLC (H_2O: ACN 0:100 in 20 minutes) to afford 40 mg of pure compound as white solid.

^{1}H-NMR (DMSO, 500 MHz): δ 8.65 (1H, s, H-4), 7.74 (2H, d, J 8.1 Hz, Ph_b), 7.32 (2H, d, J 8.1 Hz, Ph_c), 5.21 (1H, m, H-1'), 4.79 (1H, d, J 4.1 Hz, OH-3'), 4.64 (1H, t, J 4.8 Hz, OH-5'), 4.06 (1H, dq, J 7.4 Hz 4.1 Hz, H-3'), 3.57-3.51 (1H, m, H-5'), 3.47-3.41 (1H, m, H-5'), 2.62 (2H, t, J 7.4 Hz, $CH_2\alpha$), 2.26 (1H, ddt, J 12.6 Hz 7.9 Hz 1.3 Hz, H-6'$_b$), 2.06 (1H, ddd, J 12.9 Hz 9.9 Hz 6.7 Hz, H-2'$_b$), 2.01-1.92 (2H, m, H-2'$_a$+H-4'), 1.59 (1H, qn, J 7.7 Hz, $CH_2\beta$), 1.51 (1H, ddd, J 12.6 Hz 9.9 Hz 9.1 Hz. H-6'$_a$), 1.36-1.22 (4H, m, $CH_2\gamma$+$CH_2\delta$), 0.87 (3H, t, J 6.8 Hz, $CH_3\omega$)

^{13}C-NMR (DMSO, 500 MHz): δ 170.46 (C7a), 154.62 (C6), 153.66 (C2), 144.00 (Ph-C_d), 140.00 (C4), 128.97 (Ph-C_c), 125.92 (Ph-C_a), 124.52 (Ph-C_b), 106.99 (C4a), 98.51 (C5),

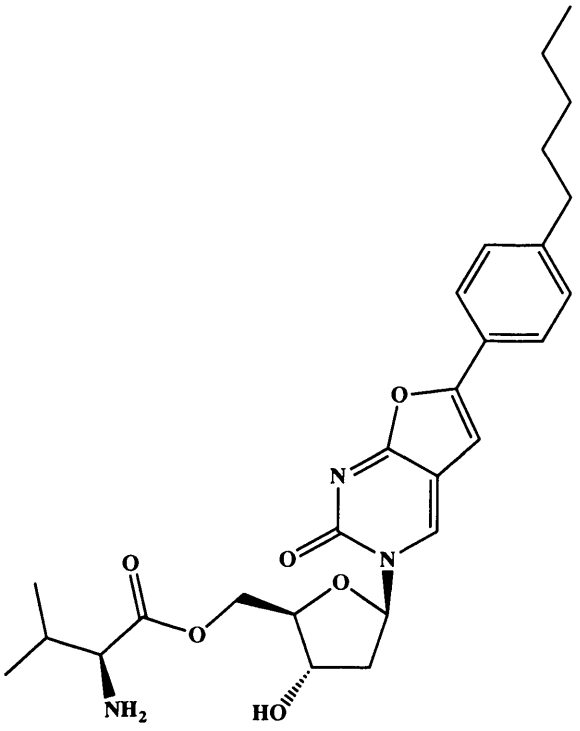
71.43 (C-3'), 62.64 (C5'), 56.94 (C-1'), 49.17 (C-4'), 39.71 (C2'), 34.88 (Cα), 33.32 (C-6'), 30.80 (Cγ), 30.36 (Cβ), 21.89 (Cδ), 13.87 (Cω)

Anal. Calcd for $C_{23}H_{28}N_2O_4$: C 69.67, H 7.12, N 7.07, O 16.14. Found C 69.70, H 7.15, N 7.00, O 16.20

MS: 419 (M+Na)

180 3-(2-deoxy-α-D-ribofuranosyl-5-[valynyl)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one

[FV100]



33f (200 mg, 0.5 mmol) was dissolved in dry DMF (5ml), followed by the addition of polymer-bound triphenylphosphine [370 mg, 1.1 mmol, (3 mmol p/g resin)] and di-*tert*-butyl azodicarboxylate (DBAD) (231 mg , 1.0 mmol) to the mixture and stirred for 20 minutes. A solution of Fmoc-Val-OH (340 mg, 1.0 mmol) in DMF (5 ml) was added dropwise over a period of 30 minutes. The reaction mixture was stirred at room temperature under an argon atmosphere until complete disappearance of the starting material (overnight). The resin was filtered off and washed with ethyl acetate. Piperidine (1 ml, 10 mmol) was added to the solution and stirred for 10 minutes. The solvent was removed under reduced pressure without

warming over 35°C and the residue was dissolved in ethyl acetate (20 mL), washed with 10% $NaHCO_3$ (3 x 20 mL) and brine (2 x 20 mL). The final residue was purified by column chromatography (gradient CH_2Cl_2 : MeOH 100% 98% 95% 90%), to give 137 mg of **180** (55% yield) as a yellow solid.

^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 8.3 (1H, s, H-4), 7.55 (2H, d, J 7.6 Hz, Ph), 7.15 (2H, d, J 7.6 Hz, Ph), 6.6 (1H, s, H-5), 6.25 (1H, t, J 6.0 Hz, H-1'), 4.45-4.30 (4H, m, H-3'+H-4'+H-5'), 3.23 (1H, d, J 5.0 Hz, Val-CH), 2.84-2.75 (1H, m, H-2'$_\alpha$), 2.53 (2H, t, J 7.6 Hz, $CH_2\alpha$), 2.17-2.09 (1H, m, H-2'$_\beta$), 2.00-1.90 (1H, m, Val-CH), 1.58-1.49 (2H, m, $CH_2\beta$), 1.31-1.20 (4H, m, $CH_2\gamma$+$CH_2\delta$), 0.89 (3H, d, J 6.6 Hz, Val-CH_3), 0.84 (3H, d, J 6.6 Hz, Val-CH_3), 0.82 (3H, t, 7.0 Hz, $CH_3\omega$)

^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 175.16 (Val-CO), 171.62 (C7a), 156.26 (C6), 154.89 (C2), 145.19 (Ph), 135.29 (C4), 129.02 (Ph), 125.69 (Ph), 124.95 (Ph), 108.60 (C4a), 96.82 (C5), 88.73 (C1'), 85.08 (C4'), 70.90 (C3'), 64.19 (C5'), 60.19 (Val-CH), 41.91 (C2'), 35.82 (Cα), 32.32 (Val-CH), 31.44 (Cγ), 30.89 (Cβ), 22.50 (Cδ), 19.30 (Val-CH_3), 17.24 (Val-CH_3), 13.99 (Cω)

HPLC: H_2O/CH_3CN gradient 0-100 in 15 min: Rt 12.47 min (99.1%)

Anal. Calcd for $C_{27}H_{35}N_3O_6$: C 65.17%, H 7.09%, N 8.44%. Found C 64.87%, H 7.29%, N 7.91%

MS: 520 (M+Na)

3-(2-deoxy-α-D-ribofuranosyl-5-[valynyl)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one hydrochloric salt

[FV100 HCl]

180 (0.3 g, 0.6 mmol) was dissolved in 3 ml of THF. Under vigorous stirring 1M HCl (1 ml) was added dropwise at 0°C and the mixture were stirred for 10 minutes. The solvents were dried under reduce pressure to obtain 0.32 g (100%) of yellow oil that solidified with addition of Et_2O.

^{1}H-NMR (DMSO, 500 MHz) δ: 8.60 (4H, bs, NH_3+H-4), 7.76 (2H, d, J 6.8 Hz, Ph), 7.34 (2H, d, J 6.8 Hz, Ph), 7.23 (1H, s, H-5), 6.24 (1H, t, J 6.2 Hz, H-1;), 4.53-4.45 (2H, m, H-5'), 4.32-4.26 (1H, m, H-3'), 4.16-4.12 (1H, m, H-4'), 3.95 (1H, bs, Val-CH), 2.63 (2H, t, J 7.5

Hz, $CH_2\alpha$), 2.47-2.40 (1H, m, H-2'$_\alpha$), 2.30-2.23 (1H, m, H-2'$_\beta$), 2.21-2.14 (1H, m, Val-CH), 1.64-1.55 (2H, m, $CH_2\beta$), 1.36-1.23 (4H, m, $CH_2\gamma$+$CH_2\delta$), 0.99 (3H, d, J 6.3 Hz, Val-CH_3), 0.96 (3H, d, J 6.3 Hz, Val-CH_3), 0.87 (3H, t, J 6.5 Hz, $CH_3\omega$)

^{13}C-NMR (DMSO, 125 MHz) δ: 175.16 (Val-CO), 171.13 (C7a), 168.88 (C6), 153.97 (C2), 153.70 (C6), 144.18 (Ph), 137.94 (C4), 129.04 (Ph), 125.77 (Ph), 124.58 (Ph), 107.22 (C4a), 98.75 (C5), 87.71 (C1'), 84.13 (C4'), 69.73 (C3'), 65.26 (C5'), 57.35 (Val-CH), 40.18 (C2'), 34.88 (Cα), 30.88 (Val-CH), 30.39 (Cγ), 29.38 (Cβ), 21.91 (Cδ), 18.26 (Val-CH_3), 17.55 (Val-CH_3), 13.90 (Cω)

HPLC: H_2O/CH_3CN gradient 0-100 in 15 min : Rt 12.47 mins (98%)

Anal. Calcd for $C_{27}H_{36}ClN_3O_6$: C 60.72%, H 6.79%, 7.87%. Found C 60.21%, H 6.67%, N 7.34%

MS: 498 (M+H)

3-(2-deoxy-α-D-ribofuranosyl-5-[valynyl)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one succinate salt

[FV100 Succinate]

180 (0.3 g, 0.6 mmol) was dissolved in 3 ml of THF. Under vigorous stirring succinic acid (1 ml) was added dropwise at 0°C and the mixture were stirred for 10 minutes. The solvents were dried under reduce pressure to obtain 0.32 g (100%) of yellow oil that solidified with addition of Et_2O.

^{1}H-NMR (DMSO, 500 MHz) δ: 8.58 (1H, s, H-4), 7.73 (2H, d, J 7.5 Hz, Ph), 7.34 (2H, d, J 7.5 Hz, Ph), 7.50 (1H, s, H-5), 6.21 (1H, t, J 6.2 Hz, H-1;), 4.41-4.31 (2H, m, H-5'), 4.27-4.21 (1H, m, H-3'), 4.15-4.10 (1H, m, H-4'), 3.32 (1H, d, J 5.0 Hz, Val-CH), 2.61 (2H, t, J 7.5 Hz, $CH_2\alpha$), 2.47-2.40 (1H, m, H-2'$_\alpha$), 2.38 (4H, s, Succ), 2.24-2.16 (1H, m, H-2'$_\beta$), 1.92-1.83 (1H, m, Val-CH), 1.63-1.53 (2H, m, $CH_2\beta$), 1.35-1.18 (4H, m, $CH_2\gamma$+$CH_2\delta$), 0.91-0.82 (9H, m, 2xVal-CH_3+$CH_3\omega$)

^{13}C-NMR (DMSO, 125 MHz) δ: 174.03 (Val-CO), 173.91 (Succ-CO), 171.09 (C7a), 153.97 (C2), 153.69 (C6), 144.15 (Ph), 137.68 (C4), 129.02 (Ph), 125.77 (Ph), 124.55 (Ph), 107.12 (C4a), 98.60 (C5), 87.74 (C1'), 84.61 (C4'), 69.83 (C3'), 63.89 (C5'), 59.23 (Val-CH), 40.53 (C2'), 34.89 (Cα), 31.42 (Val-CH), 30.80 (Cγ), 30.38 (Cβ), 29.46 (Succ-CH_2), 21.91 (Cδ), 18.84 (Val-CH_3), 17.52 (Val-CH_3), 13.88 (Cω)

MS: 498 (M+H)