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Doctor of Philosophy

By

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The Design, Synthesis and Evaluation of some novel antiviral nucleosides.

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My warmest thanks go to my Family. A special huge thanks to Jon, Mum, Dad, Ian and Dawn for all of their love and support. I love you all xxx.

Abstract

An introduction to the work within this thesis is presented in two chapters. The first provides an overview of viruses, in particular the herpes viruses Varicella-Zoster (VZV) and Human Cytomegalovirus (HMCV) and of nucleic acids and the role of nucleosides in the treatment of these viruses. The second provides a summary of the extensive structure activity relationship investigations previously carried out within our laboratories on a novel class of antiviral nucleosides with an unusual bicyclic base (BCNAs), that have been shown to be highly selective and potent inhibitors of VZV.

These studies showed three main sites for modification: 1) the sugar moiety, 2) the bicyclic base and 3) the side chain. With this in mind, further modifications at each of these sites has been investigated.

Modifications to the sugar included the synthesis of some 3'alkyl ether analogues bearing a pentylphenyl side chain, and the synthesis of amino acid prodrugs have been attempted.

The effect of difluoro substitution on the phenyl ring of the side chain has been investigated, with the synthesis of all six possible analogues. These produced varied and interesting antiviral activities.

Substitution of the *furo* oxygen with sulfur, to give thieno analogues bearing varying length alkylphenyl side chains was achieved to give base modifications. This series of analogues was found to retain antiviral activity against VZV, although slightly less active than the parent furo compounds. Thionation of the 2 carbonyl group of the bicyclic base was also attempted.

Some bicyclic nucleoside analogues have also shown anti-HCMV activity and further investigations into this class of compounds with the synthesis of analogues bearing a *Pyrro* bicyclic base are included.

An investigation into the pH stability of the BCNAs was also carried out and the parent analogue bearing a pentylphenyl side chain was found to be stable at a range of pHs.

Finally, with the X-ray crystal structure of VZV thymidine kinase published we began a preliminary investigation into the possible interaction of the BCNAs with this enzyme using molecular modelling.

Contents

Chapter 1: Introduction

1.0 Viruses[1,2]

Viruses are intracellular parasites. Lacking the necessary 'machinery' to replicate themselves, they invade and take over the host cell. These virus particles are called virions and are composed of two parts: a nucleic acid core either in the form of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) which is surrounded by a protective protein shell called a capsid. In some viruses, such as the herpesviruses, this is further protected by a lipid envelope with glycoprotein spikes on the surface (fig 1.0).

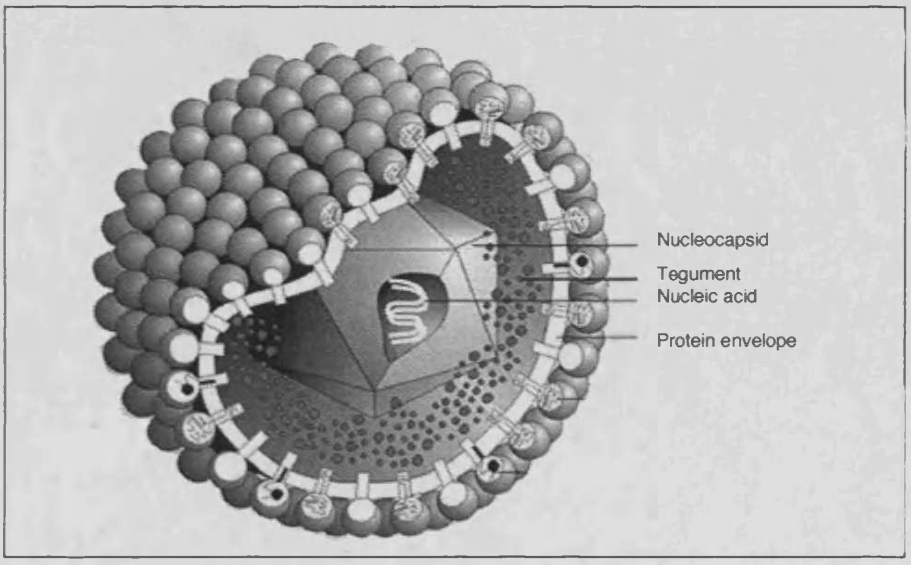


Fig 1.01: Diagram of the structure of a herpes virion.
(www.biografix.de/.../ english/html/2/h_2b2a.htm)

1.01 Herpesviruses[3,4]

There are eight herpes viruses which have been identified to affect humans: HSV-1, HSV-2, varicella zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), and the three human herpes viruses HHV-6, HHV-7 and HHV-8.

As well as structure, this family of viruses also share a number of biological properties. They share the ability to remain latent in the host and viral replication invariably results in the destruction of the host cell. The shared ability to remain in the host allows for later reactivation of the virus.

1.02 Varicella zoster virus (VZV)[5,6]

VZV is responsible for two well known diseases: chicken pox and shingles. Primary infection with VZV results in chicken pox. This is a highly contagious condition most commonly seen in childhood and spread through respiration. It then usually follows a benign course affecting nearly every human worldwide during childhood[7] manifesting itself as an itchy pox like rash on the skin (Fig 1.02).

In most cases the virus will remain dormant for life but, due to its ability to reactivate, the virus can cause a secondary disease called shingles. The precise method of this ability to remain dormant and the reactivation of the herpes viruses are still poorly understood. The incidence of shingles is increased in the elderly and immuno-compromised patients and therefore its reactivation is linked to a weakening of the immune system. As a result, complications can arise in patients with shingles, the most common complaint being an extremely unbearable itchy and severely painful rash (Fig 1.02). A severe and debilitating effect of shingles is caused by damage and inflammation of the nerves. Called post-herpatic neuralgia the resulting pain can last for months or even years after the patient presents with shingles.

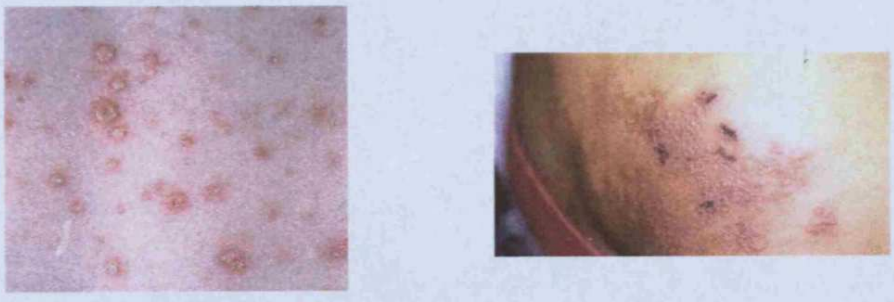


Fig 1.02: Pictures of chicken pox and shingles
(http://www.dermnet.com/image.cfm?) / www.pg.com/science/ skincare/Skin_tws_59.htm

1.03 Cytomegalovirus (CMV)[6,8]

Like the varicella zoster virus, CMV shares the common characteristics of structure and latent ability with the herpes virus. Unlike VZV, which is transmitted by the respiratory route, CMV requires close contact and is spread through bodily secretions such as blood.

Like VZV, CMV is ubiquitous in the human population with an estimated 50-90% of persons becoming infected by puberty depending on country of origin. However, as the virus is mostly asymptomatic, primary infection, viral shedding, latency and reactivation of the virus can occur without any disease symptoms and most people are not aware that they have been infected with CMV.

Severe complications can arise in congenital infections and in patients whose immune system is compromised. Congenital infection can be extremely serious if the infant is born to a previously seronegative mother who is primarily infected with CMV whilst pregnant. This can result in a high risk that the virus will be passed to the fetus and can result in the newborn presenting with a symptomatic infection which can be fatal or lead to birth defects. However, in mothers who are seropositive to CMV, the antibodies they have produced are passed to the fetus and although the child will also be born infected with CMV they very rarely produce any symptomatic disease.

CMV can also be extremely serious in immunocompromised patients. When not controlled by the immune system, CMV is very invasive and infects many organs of the body, which can result in life threatening systemic disease.

At present, the majority of chemotherapeutic agents used for the treatment against viruses are nucleoside analogues.

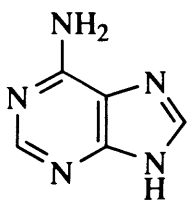
1.1 Nucleosides and Nucleotides[9,10]

Nucleosides are the building blocks of DNA and RNA. They consist of a nitrogenous base and a sugar. These are different in DNA and RNA. The sugar moiety in DNA is deoxyribose while in RNA it is ribose (Fig 1.03).

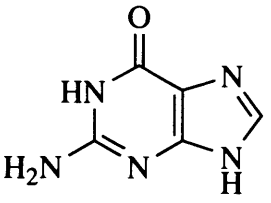
Deoxyribose ribose

Fig 1.03: Structures of the sugars found in DNA and RNA

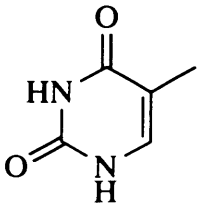
There are five bases found in DNA or RNA: two purines, adenine and guanine, and three pyrimdines, thymine, cytosine, and uracil (Fig 1.04). Whilst adenine, guanine and cytosine are all found in DNA and RNA, thymine is only present in DNA and uracil is only found in RNA.



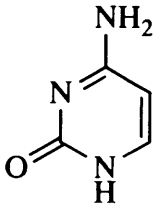
Adenine



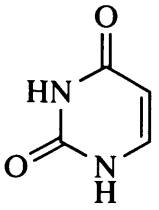
Guanine



Thymine



Cytosine



Uracil

Fig 1.04: Structures of the bases found in DNA and RNA

A nucleoside is formed through a glycosidic bond that binds with β configuration, the C-1' position of the ribose or deoxyribose sugar to the N-1 of the pyrimidine bases or N-9 of the purine bases.

A nucleotide is a phosphate ester of a nucleoside. This is linked to a hydroxyl group of another nucleotide to give a chain of nucleotides that form the backbone of DNA. In DNA and RNA the nucleotides are linked by a phosphate ester between the 5' hydroxyl group of one nucleoside and the 3' hydroxyl group of another nucleoside to create a chain. The sequence of these nucleotides make up a code, which is responsible for storing and transmitting genetic information (Fig 1.05).

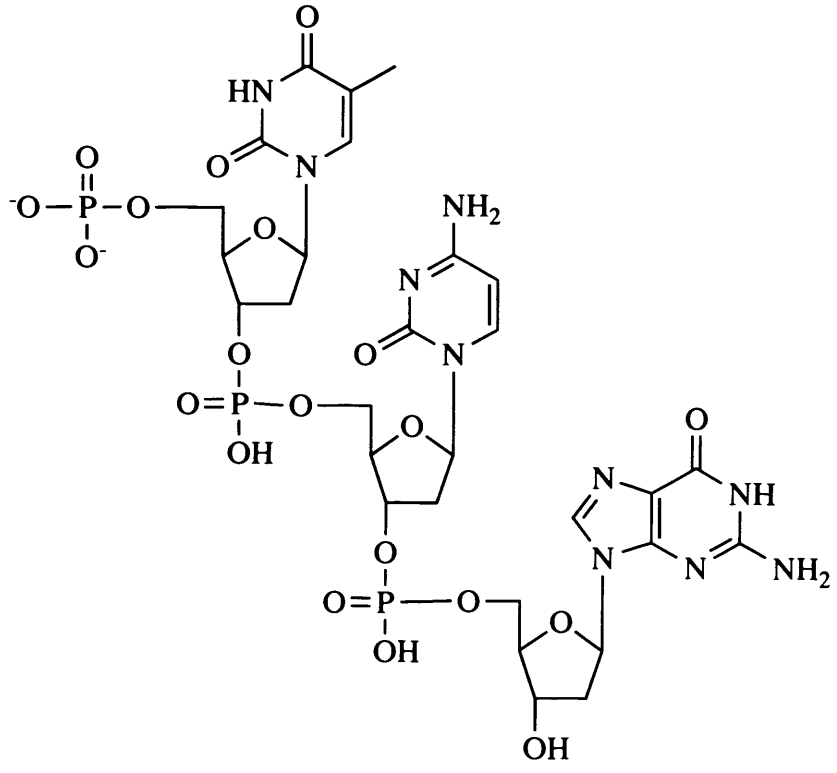


Fig 1.05: Example of single strand of DNA chain

In the cell, DNA exists as two strands of nucleotides intertwined and coiled around a central axis to form a double helix (Fig1.06). This structure was first proposed by Watson and Crick in 1953[11], after they studied X-ray diffraction photographs of DNA fibres.

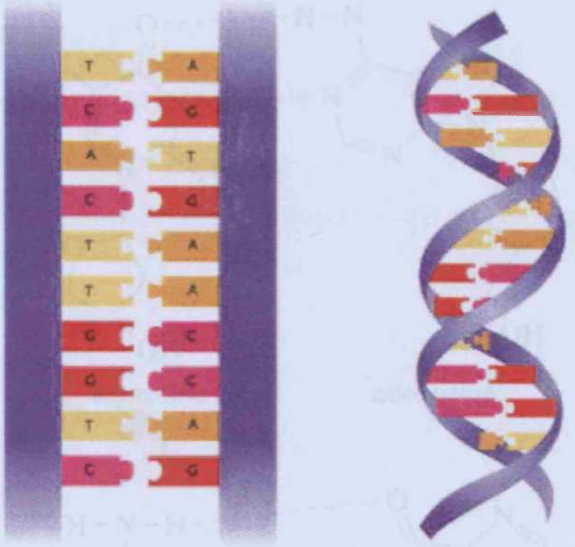


Fig 1.06: Double helix structure of DNA
(http://info.cancerresearchuk.org/images/gpimages/ys_DNA_4and5)

From this, they discovered that the two DNA strands run in opposite directions with the 5'-phosphate end of each chain found at opposite ends of the coil. The sugar phosphate moieties are found on the external part of the helix and provide the regular helical shape. The bases face inwards, stacked in flat planes roughly perpendicular to the long axis of the helix. In fact, DNA resembles a flexible ladder with, wrapped helically around a central axis.

The space in between the two chains provides just enough room in the central core for one purine and one pyrimidine base. Two purines are too wide to occupy this space while two pyrimidine bases are too narrow to fit this space exactly.

The pair of bases form hydrogen bonds that hold the helix together. It was discovered that adenine is always paired with thymine and guanine is always paired with cytosine[12] (Fig 1.07).

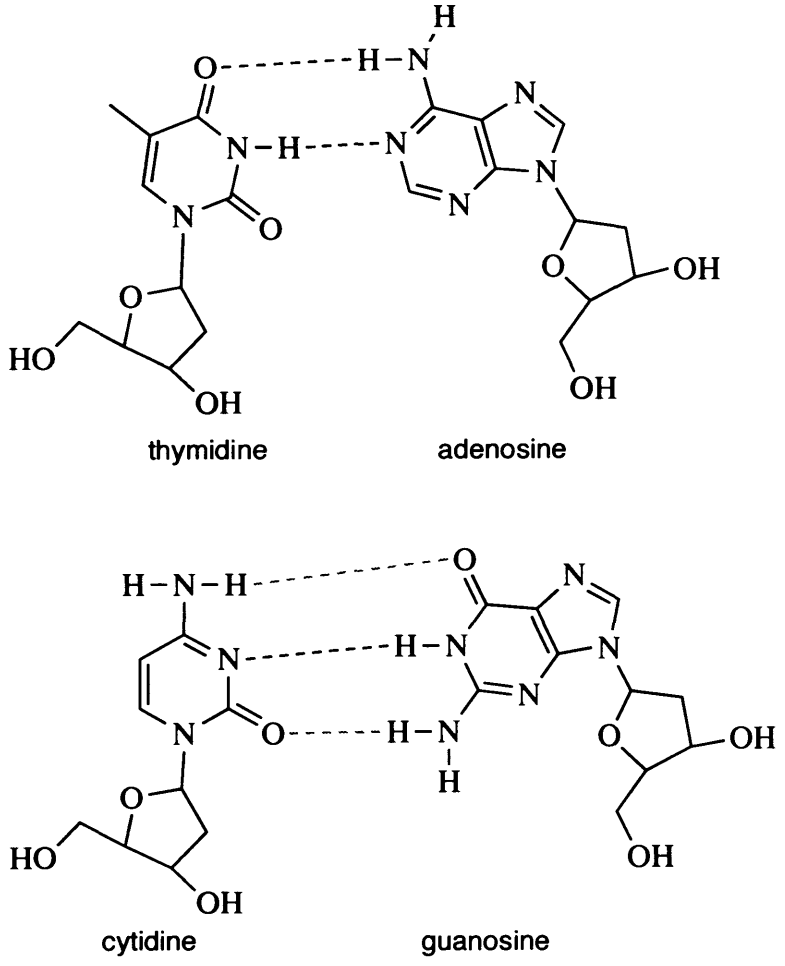


Fig 1.07: Base pairing seen in DNA

1.11 DNA Replication[13,14]

Replication is the process by which new DNA is synthesised and genetic information is passed on from one generation to the next. In the replication of DNA the two strands unwind and separate. However, they do not completely separate before replication begins. Instead the synthesis of new DNA strands takes place concurrently with the unwinding of the parent DNA. In this way some of the energy required to break the hydrogen bonds of the parent DNA comes

from the formation of the new ones. The site of this unwinding and synthesis is called a replication fork.

DNA polymerase is the enzyme responsible for the synthesis of new DNA. The synthesis of new DNA strands occurs in the direction of 5' → 3' only. The substrates for DNA polymerase are the deoxynucleotide triphosphates. These are incorporated into the DNA chain by nucleophilic attack of the 3' oxygen onto the α phosphate (Fig 1.08). The nucleotide incorporated is determined by the nucleotide in the template strand.

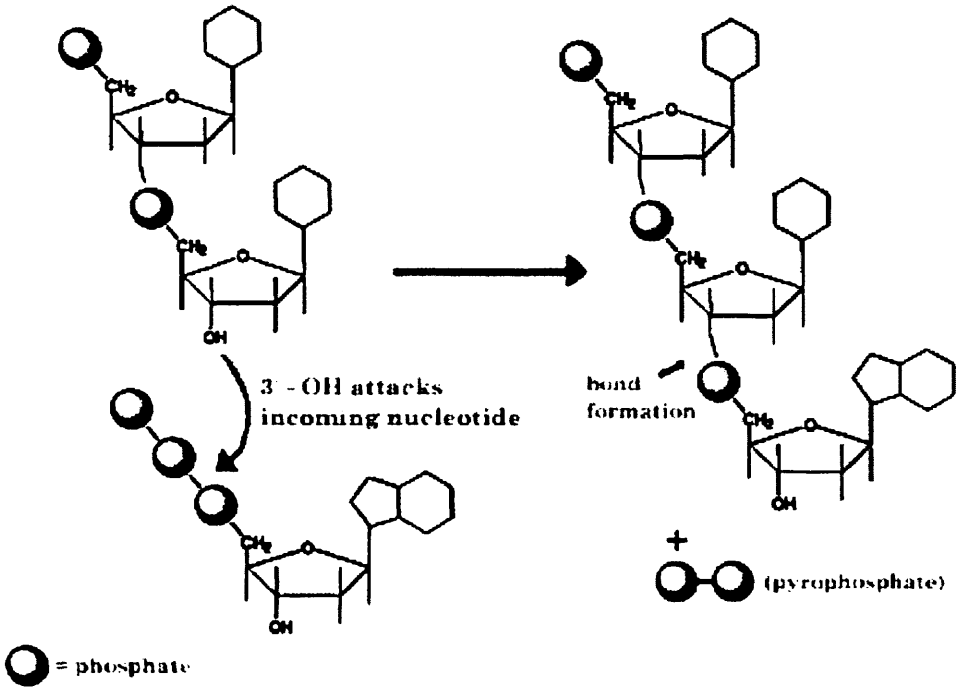


Fig 1.08: DNA elongation
(www.rhodes.edu/.../ mbprotected/dnastructure.html)

The ability of DNA polymerase to only synthesise new DNA in the 5' → 3' direction means that only one of the new strands can be constructed continuously towards the replication fork. As this strand is continuously formed, it is called the leading strand. The other strand is synthesised discontinuously and

called the lagging strand. This means that this strand is formed in fragments (Okazaki fragments) as the template is exposed.

Both strands are synthesised by the action of the family of enzymes, the DNA polymerases.

1.2 Antiviral therapy

Viruses are essentially biologically inert on their own outside the cell and are only active once inside a host cell. Potential targets for antiviral drug development can be found at various points in the viral cell cycle (Fig 1.09) including viral attachment, entry, uncoating, DNA or RNA replication, protein biosynthesis, assembly and release. Yet the search for selective antiviral agents proved challenging. However, herpes viruses encode for a number of unique enzymes which play a vital role in the synthesis of viral nucleic acids[6].

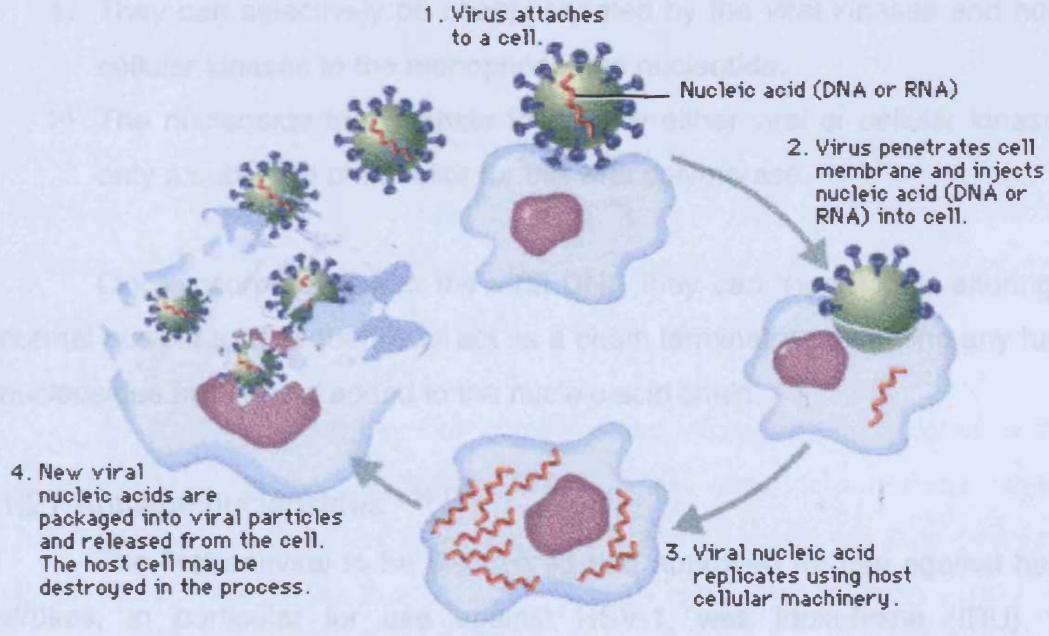


Fig 1.09: Diagram of viral replication

(http://images.encarta.msn.com/xrefmedia/aencmed/targets/illus/ilt/T028557A.gif)

Therefore, an agent which can interfere in this process by selective inhibition of viral enzymes causing disruption to viral DNA or RNA synthesis without causing toxic affects to the host cell, would make a potential antiviral drug.

It is not surprising therefore that nucleosides, which are the building blocks of nucleic acids, have a major role in the design and synthesis of antiviral agents and make up the major class of antiviral agents to date.

In the same way as cellular DNA polymerases, viral polymerases use nucleoside triphosphates as the substrates for building the viral nucleic acids. The nucleoside is first converted to the monophosphate, which is further converted to the di and then the tri phosphates.

Nucleoside analogues are able to act as antiviral agents in two main ways:

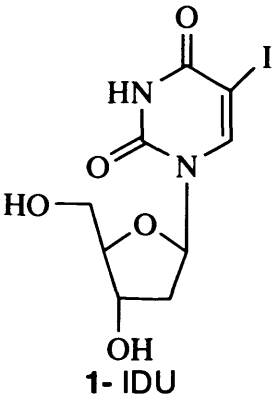
1) They can selectively be phosphorylated by the viral kinases and not the cellular kinases to the monophosphate nucleotide.
2) The nucleoside triphosphate formed by either viral or cellular kinases is only a substrate or inhibitor for the viral polymerase.

Once incorporated into the viral DNA they can then act by altering the normal nucleic acid sequence or act as a chain terminator preventing any further nucleosides from being added to the nucleic acid chain.

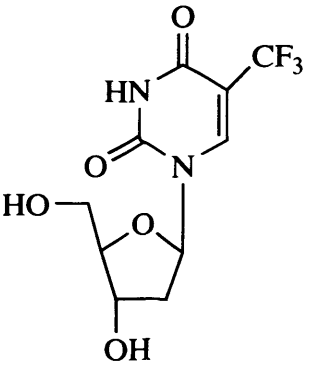
1.21 Antiviral nucleosides[15,16,17]

The first antiviral to be discovered and approved for use against herpes viruses, in particular for use against HSV-1, was Idoxuridine (IDU) **1**, a pyrimidine analogue substituted at the 5 position with an iodine atom. [18]

IDU is phosphorylated by thymidine kinase to its monophosphate, which is then converted further to the active triphosphate. However this active species was found to be a substrate for both viral and cellular DNA polymerase reducing its activity as a selective antiviral. Toxicity seen in bone marrow also prevented its use systemically and IDU is approved only for topical use against herpes keratitis. Another analogue, trifluorothymidine (TFT) **2** substituted at the 5 position with trifluromethyl was found to have a similar profile.



1- IDU



2 – TFT

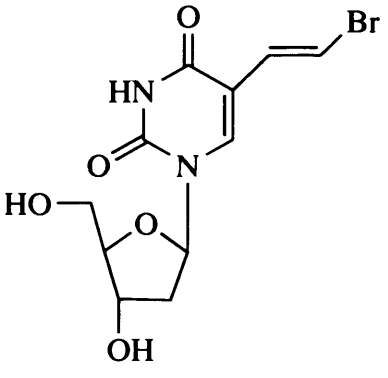
This discovery led to the synthesis and analysis of other pyrimidine nucleoside analogues substituted at the 5 position. This led to one of the most potent antiviral agents to date, Brivudine (BVDU) **3**.[19] This was found to be a selective inhibitor of HSV-1. The thymidine kinase of HSV-1 selectively phosphorylates Brivudine to its mono and diphosphate forms. This is then further phosphorylated by cellular kinases to its active triphosphate form.

It has been shown, that some pyrimidine analogues substituted at the 5-position show selective phosphorylation by viral encoded thymidine kinase[20]. Therefore they are only active against viruses that encode for their own kinase enzymes such as the herpes viruses HSV-1 and VZV, which can explain their selectivity for these viruses.

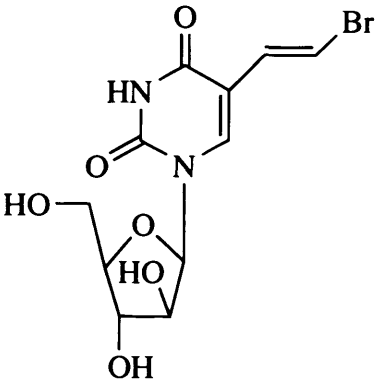
However unlike IDU, the triphosphate of Brivudin was found to be a selective substrate for HSV-1 DNA polymerase. Brivudin can act as an antiviral agent against DNA polymerase in two ways. It can act as a competitive inhibitor mimicking the natural substrate thymidine triphosphate. Alternatively it can act as a substrate for DNA polymerase and be incorporated into the DNA chain, which can affect the stability or function of the viral DNA.

Other analogues of Brivudin with modifications to the sugar moiety such as the arabino **4** or carbocyclic **5** derivatives were also found to show good activity against HSV-1. The arabino analogue (BvaraU) **4** was also found to exhibit antiviral activity against VZV as did the carbocyclic analogue[21].

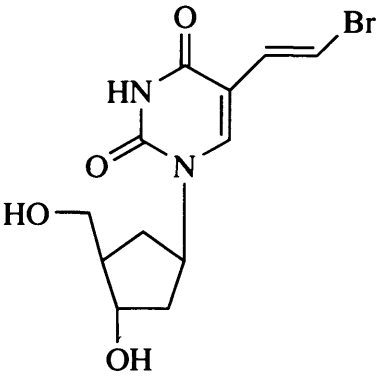
Despite exhibiting excellent antiviral potency, Brivudine is only licensed in a few countries. During metabolism the glycosidic bond is cleaved to the free base (5-bromovinyluracil). This base was found to interfere with the degradation of the anti-cancer drug 5-fluorouracil, which results in an increased exposure to 5-fluorouracil, which may intensify its toxicity. This led to fatal events when BVDU and 5-fluorouracil were co-administered.



3 – Brivudine



4 – BvaraU



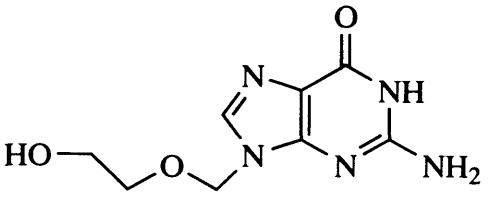
5 – cBVDU

The most successful nucleoside analogues for the treatment of herpes viruses, which are also specifically phosphorylated in the first instance by viral kinases are the class of acyclic nucleosides. These are nucleoside analogues in which the cyclic sugar moiety has been broken.

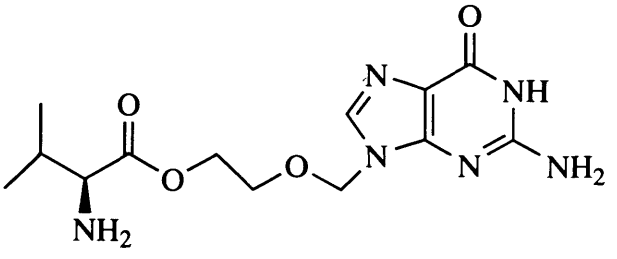
The first acyclic nucleoside shown to have selective herpes virus activity particularly against HSV-1, HSV-2 and VZV was aciclovir (ACV) **6**[22]. This, like the pyrimidine analogues is selectively phosphorylated to its monophosphate by viral thymidine kinase. This is then further converted to its active triphosphate form by cellular kinases.

The aciclovir triphosphate mimics the natural substrate deoxyguanosine triphosphate and is incorporated into the growing DNA strand by the viral DNA polymerase. This causes chain termination of the DNA strand. Aciclovir lacks a second hydroxyl group to allow incorporation of a further nucleoside and therefore elongation of the DNA chain further is not possible.

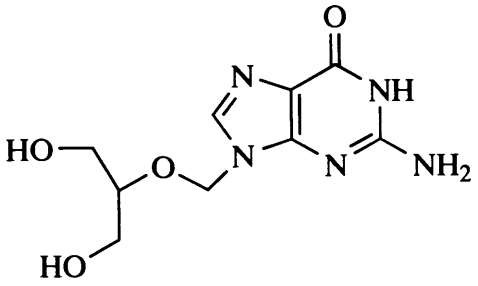
Aciclovir, while showing great activity against HSV-1 and some activity against VZV, has a poor bioavailability profile, approximately 20-30% after oral administration[23]. This was overcome with the synthesis of the L-valyl ester prodrug to give valaciclovir **7,** which increased the bioavailability to approximately 60%[24]. To date aciclovir has been used as the treatment of choice for most of the herpes infections.



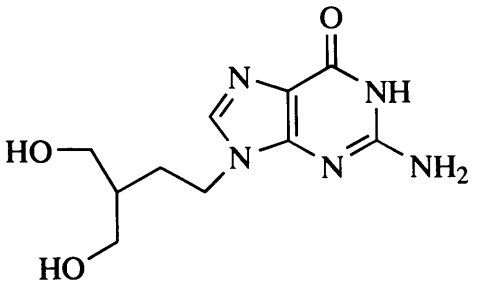
6 – Aciclovir



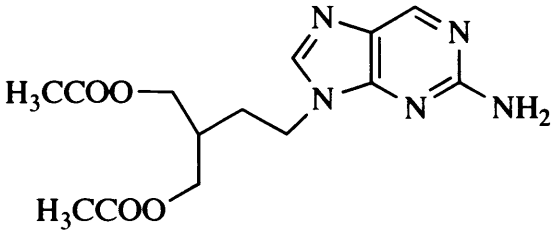
7- Valaciclovir



8 – Ganciclovir



9- Penciclovir



10- Famciclovir

Another acyclic analogue that has been successfully marketed as an antiviral drug is Ganciclovir **8**[25]. This analogue is structurally very similar to aciclovir but differs in that it has a second hydroxyl group, which can mimic the 3'-hydroxyl group of a nucleoside.

Ganciclovir was found to be active against all herpes virus but more active than aciclovir against Epstein Barr virus and CMV. Due to extremely poor bioavailability, administration by intravenous injection of ganciclovir is usually necessary making it an unfavourable treatment against HSV. However its profile against CMV treatment in AIDS and transplant patients makes it marketable for these conditions, with administration most commonly given in hospital.

Other acyclic analogues which have made it to the market are penciclovir **9** and its prodrug Famciclovir **10**[26,27]. These are the carbocylic analogues of ganciclovir lacking the ether oxygen in the ether acyclic chain. Like ACV, penciclovir inhibits DNA polymerase following conversion to its triphosphate

form. However, with the second hydroxyl group present, it does not act as a chain terminator like acyclovir but acts as a competitive inhibitor of viral DNA polymerase.

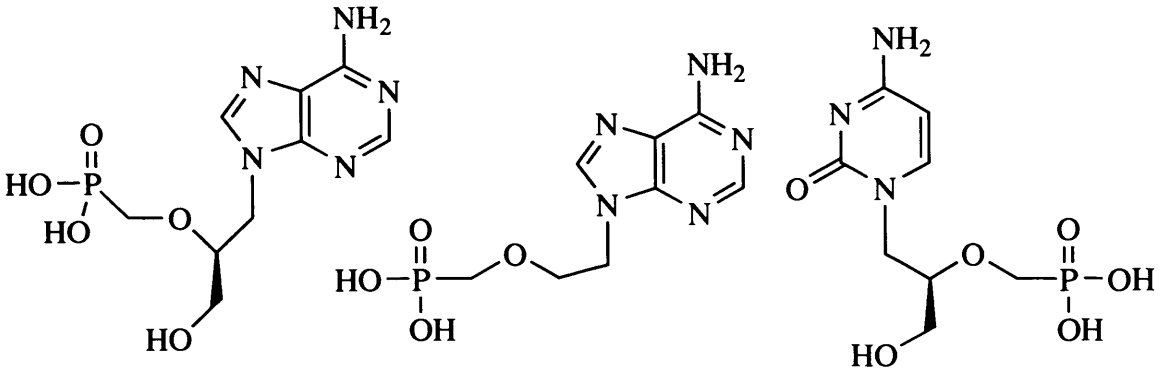
Penciclovir is active against HSV-1, HSV-2 and VZV. Its triphosphate has been found to be more stable than the triphosphate of acyclovir and has been shown to be effective against a small percentage of ACV resistant HSV strains[28]. It is used topically to treat herpes labialis in immunocompromised patients.

Famciclovir is the orally bioavailable prodrug of penciclovir and is used in the United States for the treatment of acute VZV zoster infection and genital herpes. Its high oral bioavailability gives it an advantage over acyclovir as less frequent dosing is required.

As already stated, in order to become active, antiviral agents must interact with viral enzymes, and the nucleosides must successfully be phosphorylated in the cell. This involves three activation steps with the triphosphate formation needed to convey antiviral activity. Some DNA viruses that do not encode for their own thymidine kinase and some mutant strains of HSV and VZV, which are lacking this enzyme, are not sensitive to these nucleoside analogues that require selective phosphorylation to the mono phosphate by viral TK.

It would therefore be advantageous if this initial phosphorylation step could be bypassed. The free monophosphates are unable to make successful prodrugs for this approach, the phosphorous-oxygen bond is readily cleaved by phosphatases in the cell.

This led to the synthesis of several phosphonate analogues of the acyclic nucleosides. The first of these HPMPA **11** was found to have a broad activity against DNA viruses including activity against HSV-1 and 2, EBV, VZV and HCMV[29].



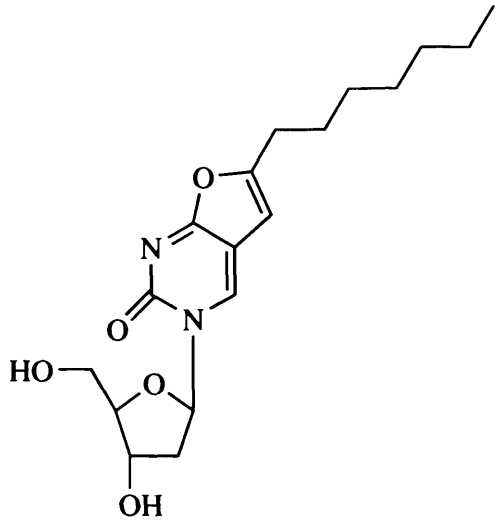
11 – HPMPA **12** – PMEA **13** - HPMPC

This was closely followed by the aciclovir phosphonate PMEA **12** and the cytosine analogue HPMPC **13**. Synthesised concurrently with HPMPA, PMEA was also found to have anti-herpes activity and in addition was found to have activity against HIV[30].

HPMPC is closely related to HPMPA in that they both contain a hydroxyl group in the phosphonate chain, but the adenosine has been replaced with cytosine. This analogue was shown also to have a broad spectrum of activity, being virtually active against all herpes viruses[31].

These analogues are then able to enter the cell where they bypass the initial phosphorylation requiring only two steps to be converted to their active triphosphate form[32]. As they do not require initial activation by the viral enzyme thymidine kinase, these analogues would be expected to be active in viruses that lack this enzyme. In fact, this was shown to be the case and these compounds could be used in the treatment of ACV resistant strains of herpes virus infections[33].

Within our laboratories a new class of nucleoside analogues bearing an unusual bicyclic base and long side chain **14** were found to be highly selective and potent inhibitors of VZV. This activity, as well as the extensive SAR investigations carried out following this discovery, are discussed in more detail in the following chapter.

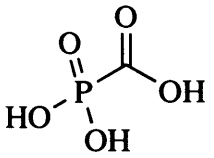


14

1.22 Non Nucleoside treatments

Although nucleosides provide a major class of antiviral treatments some non-nucleoside therapies have been marketed for use against the herpes viruses.

A pyrophosphate analogue, Foscarnet is available for the treatment of herpes viruses, which, like the nucleoside analogues already discussed, acts to inhibit the viral DNA polymerase. However, unlike the nucleosides, this does not require activation but inhibits the DNA polymerase directly[34]. It is primarily used for the treatment of HCMV infections when Ganciclovir treatment has shown no effective response and it would also be possible for use against ACV resistant strains of HSV and VZV[15].



Foscarnet

A varicella zoster virus vaccine has also been manufactured and is licensed for use in some countries including the United States for the prevention of chicken pox. However, its use is a controversial issue and the lasting effects on the epidemiology of the virus and affect on the incidences of shingles are still highly debated. As a result other countries have yet to decide whether the cost effectiveness of the vaccine and the mild nature of childhood infection warrants wide spread vaccination[35].

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Chapter 2: Bicyclic Nucleoside Analogues (BCNAs)

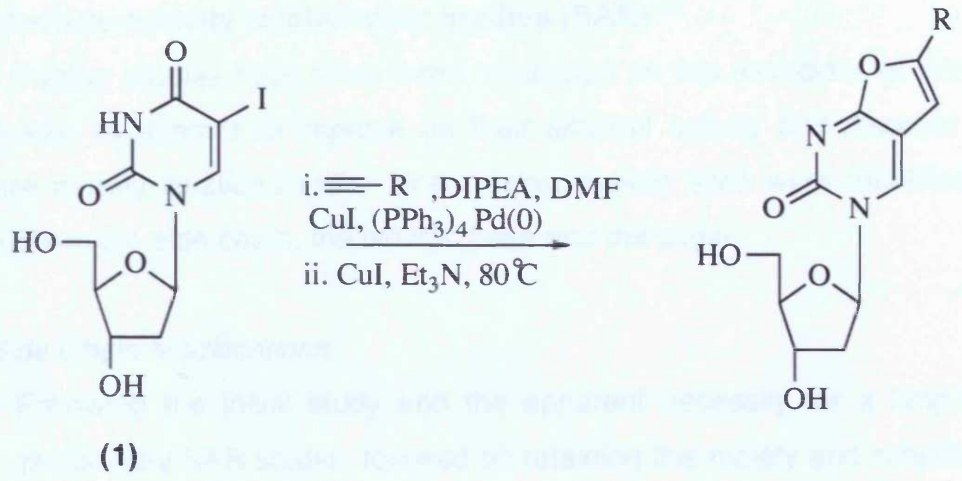
2.0 Introduction

During previous investigations aimed at synthesising 5-alkynyl-2'-deoxyuridines with long alkyl side chains, it was discovered by us, that a side product of the reaction, a nucleoside analogue bearing an unusual bicyclic base moiety, showed antiviral activity.[1]

5-alkynyl-2'-deoxyuridines are synthesised by a Pd/Cu co-catalysed reaction of a terminal alkyne with 5-iodo-2'-deoxyuridine (IDU) (1)[2,3]. During the synthesis of these analogues, M.J. Robins et al. had noted the formation of these by-products, which can be observed on TLC by the presence of a slower running fluorescent spot. Under these conditions the yields of the bicyclic product were usually low (less than 10%) although they could be synthesised successfully by heating at reflux a mixture of 5-alkynyl-2'deoxyuridine and copper iodide (CuI) in a solution of triethylamine and methanol. [2]

Our research group was able to successfully prepare the bicyclic analogues in a one-pot procedure, by addition of an appropriate amount of CuI and triethylamine to the reaction mixture after the alkyne coupling and heating the reaction to 80°C. [1] **(Scheme 2.01)**

Prior to our discovery that these bicyclic nucleoside analogues with a long alkyl side chain showed antiviral activity, only a few of these compounds had been isolated and characterised. [2,4,5,6] Of these, only the parent compound (R=H), has available biological data and was found to be inactive as an antiviral[4].



(1)

Scheme 2.01: One pot synthesis of the bicyclic nucleoside analogues

A lead series of BCNAs bearing varying lengths of alkyl side chain was prepared by us and evaluated for their antiviral activity against a number of viruses and found to selectively inhibit VZV[1]. The leads were found to be approximately 300 fold more potent than ACV against VZV and showed no cytotoxicity. As shown in Fig. 2.01, the antiviral activity of these compounds seems to be dependent on the length of the side chain with the most potent analogue having a chain length of eight to ten carbons.

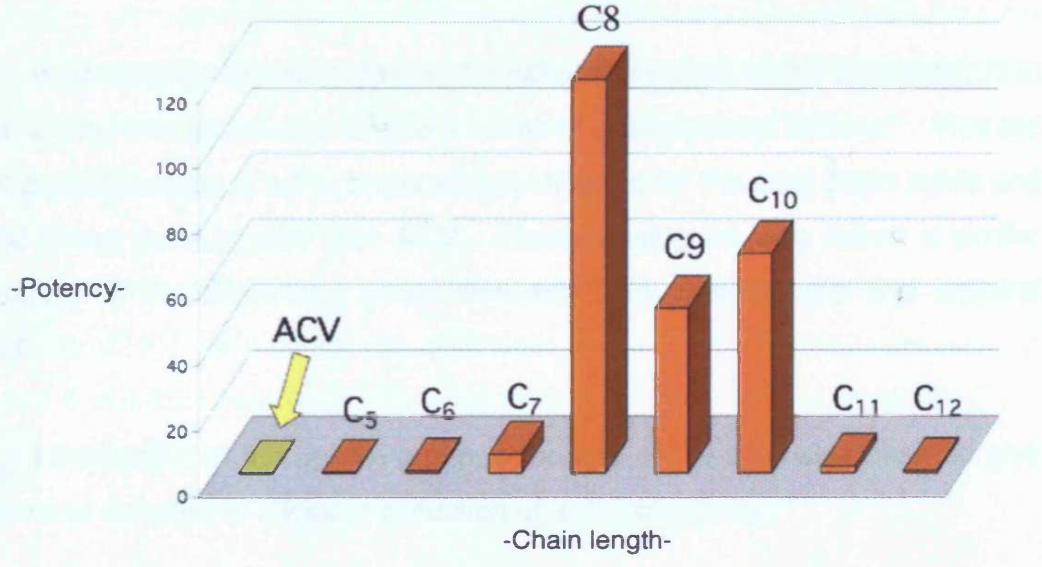


Fig 2.01: Antiviral properties of lead structures bearing an alkyl side chain (potency = $1/EC_{50}$ in μM)

2.1 Structure activity relationship studies (SAR)[7,8]

Further studies have since been conducted on this new class of antiviral nucleosides to attempt to improve on their antiviral activity and discover any structure activity relationships. Three main possible sites were identified for modification: the side chain, the bicyclic base and the sugar.

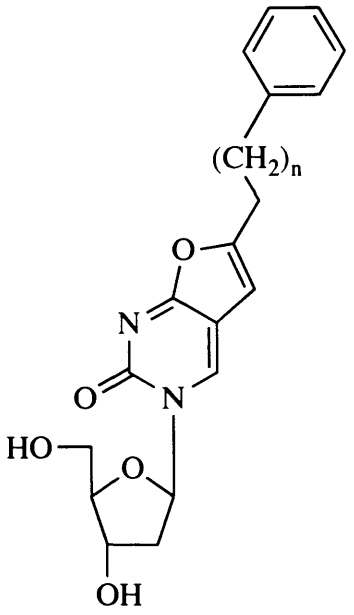
2.11 Side Chain Modifications

Following the initial study and the apparent necessity for a long alkyl chain, preliminary SAR studies focused on retaining this moiety and substituting at the terminus with polar groups or unsaturation.

Analogues of the *n*-decyl BCNA bearing a terminal halo atom were prepared and found to retain full antiviral activity equipotent with the leads structures bearing an alkyl side chain[9]. In contrast replacement of the terminal methyl with a hydroxyl group resulted in decreased activity[7]. A range of terminal alkenyl and alkynyl analogues was also prepared. It was found that in general, while unsaturation at the terminus was well tolerated in the case of longer chain alkenes, it was not so in the case of the alkynes[10]. Although, very long alkynyl chains were not available and so the SAR of these analogues was limited.

In an attempt to restrict the conformational freedom of the alkyl side chain a phenyl ring was introduced to give a series of *p*-alkylphenyl BCNAs[11]. This led to enhanced potency of >100 times of that obtained for the alkyl chain leads and 10,000 times more potent than ACV. These analogues also follow a similar correlation to the alkyl lead series between side chain length and antiviral activity.

Introduction of the phenyl group to the end of the alkyl side chain to give **15** however resulted in a loss or reduction of antiviral activity. [12]



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As a result of the necessity for a long side chain, these analogues have a relatively high lipophilicity. To try to increase the water solubility of these compounds, one or two of the methylene units in the alkyl side chain were replaced by an oxygen to give the ether or glycol derivatives[13]. However this resulted in a reduction in antiviral activity against VZV. Alkyloxyphenyl analogues were also prepared and found to retain high antiviral activity against VZV whilst also having increased water solubility[14]. The alkyloxyphenyl analogues retained lipophilicity comparable to the parents, while the ether/glycol chains resulted in reduced lipophilicity and potency indicating a need for an optimal calculated log P of 2.5 to 3.5*.

To further investigate an increase in water solubility whilst maintaining the lipophilicity of the side chain, the phenylalkyl was replaced with a pyridylalkyl side chain, but this also resulted in a reduction of antiviral activity against VZV[15].

* calculated using ChemDraw Ultra. **2004.** CambridgeSoft.

(E)-alkene and alkyne phenyl side chains were prepared and the biological results showed that the *p*-alkenes and alkynes were well tolerated, maintaining antiviral activity in the nanomolar range[16]. Only alkene *ortho* and *meta* analogues were prepared which showed that, the *meta* analogues had reduced activity but maintained potency comparable with aciclovir (*ca.* 2 μM) while the *ortho* analogues showed a further reduction in antiviral activity against VZV (*ca.* 15 μM).

2.12 Sugar modifications

Following the discovery that BvaraU, the arabinosyl derivative of BVDU (a herpes antiviral agent) is as potent as the deoxyribose derivative[17], replacement of the 2'deoxyribose sugar of the alkyl BCNAs with ribose and arabinose was investigated[8].

In contrast with BVaraU, the arabinosyl derivatives of the bicyclic nucleosides were found to be considerably less potent than the parent nucleoside (*ca.* 300 fold). Substitution of the sugar with ribose led to even further loss of antiviral activity, with the ribosyl BCNA approximately 3000 fold less active than its 2'-deoxy derivative[8].

Investigations then led to substitution of the 3' hydroxyl group on the sugar moiety of these BCNAs. Introduction of a fluorine in the 3'-position resulted in a complete loss of antiviral activity[18]. The 2',3'-didedoxy analogues were also prepared and found to be completely inactive as antivirals against VZV. However this change to the sugar moiety resulted in activity and selectivity against another herpes family virus, CMV[19]. These modifications seemed to suggest that the 3'-hydroxyl was a necessary requirement for the conveyance of antiviral activity of these BCNAs.

However, one series of analogues have produced ambiguous results to contradict this view. Some 3' alkyl ethers of the alkyl BCNAs were synthesised and while most resulted in no or only partial antiviral activity against VZV, one analogue, the 3'-O-propyl was found to be significantly active against VZV[20], although approximately 35 times less active than the corresponding BCNA parent.

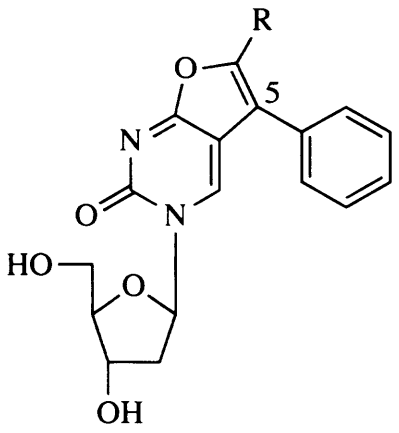
Substitution of the 5' hydroxyl group with a chlorine atom was also investigated and found to result in a loss of antiviral activity, [15] indicating that this hydroxyl group is also essential for the BCNA series to possess antiviral activity against VZV.

2.13 Bicyclic base modifications

The third moiety available for modification is the bicyclic base. Introduction of nitrogen to replace the *furo* oxygen in the alkyl BCNAs to give the *pyrro* bicyclic base was found to be detrimental to antiviral activity[21].

Introduction of a sulfur in the same position in the bicyclic base however, was well tolerated and the *thieno* analogues were found to retain full antiviral activity against VZV when compared to the parent *furo* alkyl BCNAs[22].

Other base modifications have involved substitution at the 5 position of the bicyclic base. Some 5 aryl substituted analogues of the alkyl BCNAs (**16**) have been prepared and tested for their antiviral activity. However these were not well tolerated and resulted in either loss or reduction in antiviral activity against VZV[12].



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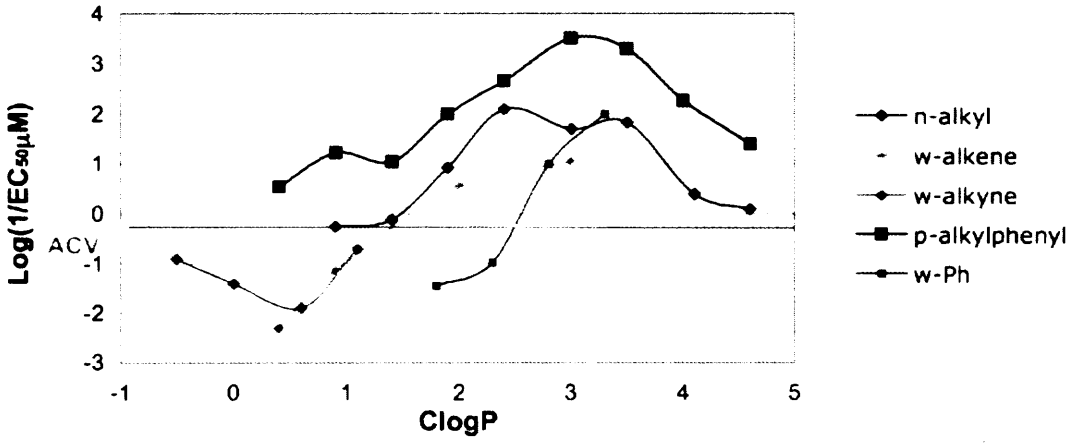
Following these studies, as well as a necessity for a long side chain to convey antiviral activity, the ClogP of the BCNAs also seems to be of significance with an optimal value between 2.5 and 3.5 **(Graph 2.01)**.



Graph 2.01: Correlation between Clog P and potency (potency = log $1/EC_{50}$)

2.2 Mechanism of action

The mechanism of action of these BCNAs against VZV is still not fully understood. A summary of information gathered so far is shown below **(Fig 2.02)**. From this data it can be seen that the VZV thymidine kinase (TK) plays a crucial role. The compounds are active only in VZV cells that express VZV TK. They are completely devoid of antiviral activity in VZV cells that are deficient of VZV TK.

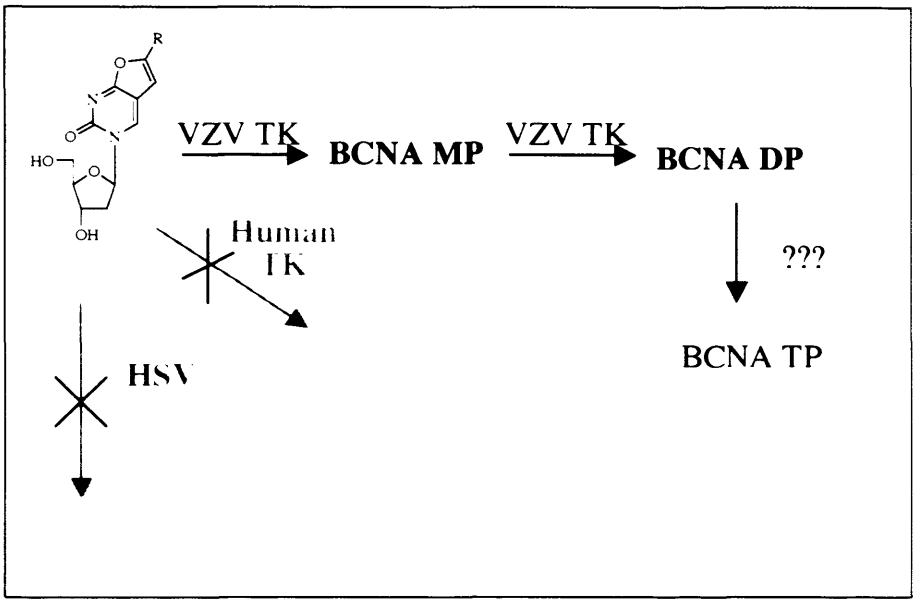


Fig (2.02): Summary of the action of the BCNAs

The phosphorylation of these analogues has been detected[8] and this, with the observations above, show the absolute requirement for VZV-TK mediated phosphorylation for antiviral activity.

More over, the BCNAs have shown complete selectivity for VZV. They are not active against any other herpes viruses. Enzymatic studies have also shown their phosphorylation to be selectively induced by VZV TK, they are not phosphorylated by human TK or HSV TK[8].

More recently, these analogues have been shown to express even more selectivity. They are active only towards human VZV. Recent studies of these analogues have shown them to be inactive against simian VZV showing complete selectivity towards human VZV[23], despite apparent phosphorylation by simian TK.

2.3 Summary:

Following discovery of the BCNAs as potent and selective antiviral agents against VZV, three main sites for modifications were identified and have been pursued to investigate the mechanism of action and SAR of these compounds (Fig 2.03).

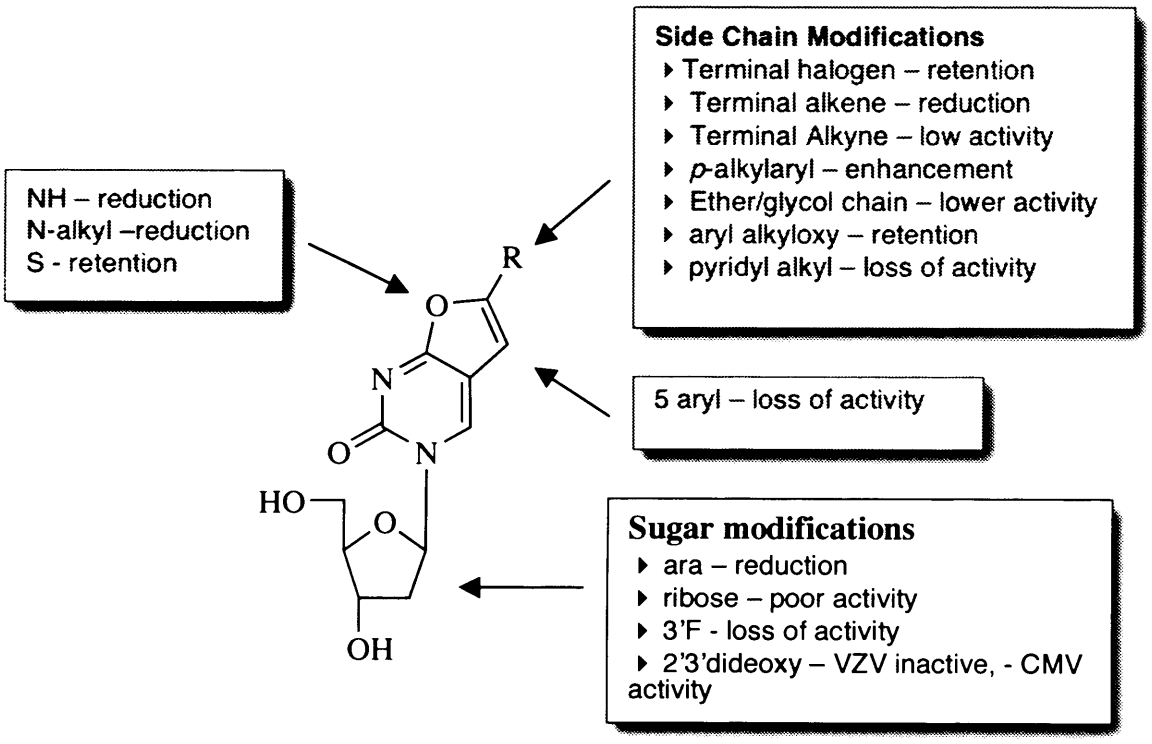


Fig 2.03: Summary of SAR studies on the BCNAs to date.

Aim of Study:

As shown above three main sites for modification of the BCNAs can be pursued. Discussed herein, is the synthesis of a series of modifications for each site and their affect on antiviral activity. More recently the crystal structure of VZV TK has been published[24] and molecular modelling to investigate the interaction of the BCNAs with this enzyme is also discussed.

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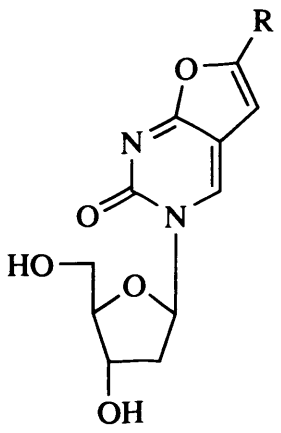
Chapter 3 : Modifications to the Bicyclic Base:

3.0 Replacement of the furo oxygen with sulfur.

As shown in chapter 2, previous SAR studies on the bicyclic base moiety of the alkyl BCNAs **(17)** replaced the *furano* oxygen atom with a nitrogen or sulfur atom to give the *pyrrole* **(18)** or *thieno* **(19)** analogues respectively.



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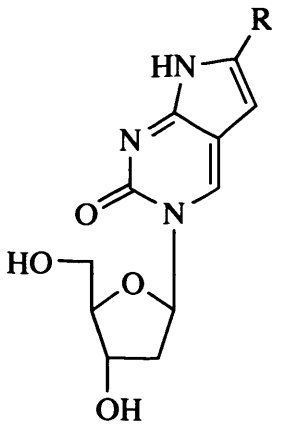

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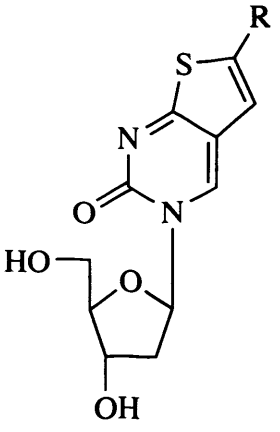
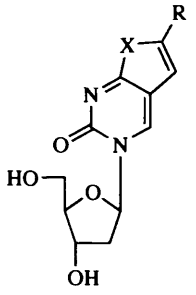


(19)

As shown in the table below **(Table 3.01)**, the presence of a hydrogen donor group such as the *pyrrole* does not seem to be well tolerated to convey antiviral activity against VZV[1] for the alkyl BCNA family. The *thieno* analogues, (which are hydrogen acceptors) however, were well tolerated and retained the full antiviral activity seen with the *furo* parent nucleosides.[2]

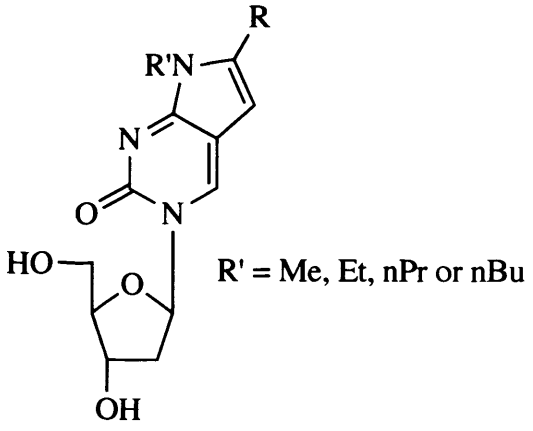
Table 3.01: Antiviral activity and cytotoxicity of previous *furo, pyrrole* and *thieno* alkyl BCNAs.



Cmp Cf	X	R	EC_{50} (μM)[a] VZV OKA	VZV YS	VZV TK- 07	VZV TK- YS	MCC (μM)[b]	CC_{50} (μM)[c]
1368	O	C_8H_{17}	0.008	0.024	>50	>50	>50	>50
1369	O	$C_{10}H_{21}$	0.015	0.008	>50	>50	>50	>50
1395	NH	C_6H_{13}	>50	>50	>50	>50	>50	>50
1397	NH	C_8H_{17}	0.15	0.38	>20	>20	20	>50
1681	NH	$C_{10}H_{25}$	2*	2*	>20	>20	≥20	>200
1450	NH	$C_{12}H_{25}$	3.7	14	76	31	>200	>200
2010	S	C_4H_9	0.16	0.16	≥200	152	≥200	>200
2011	S	C_6H_{13}	0.14	0.14	>50	≥50	125	>200
2012	S	C_8H_{17}	0.003	0.005	>20	>20	>20	>20
2013	S	$C_{12}H_{25}$	0.25	0.3	>5	>5	12	49

a. EC_{50}, 50% effective concentration required to reduce viral plaque formation by 50%. b. MCC, minimal cytotoxic concentration required to alter microscopically detectable cell morphology. c. CC_{50}, 50% cytotoxic concentration required to inhibit Hel cell growth by 50 %. * Varying degrees of inhibition were noted at concentrations of 2 μM or higher. No complete inhibition was observed at higher concentrations tested (20 μM).

Compounds containing an alkyl group on the *pyrrole* moiety **(20)** were also synthesised and found to be inactive or had low antiviral activity (data not shown). This would support the theory of hydrogen bonding, as the alkyl chain could sterically prevent the nitrogen atom from forming hydrogen bonds.



(20)

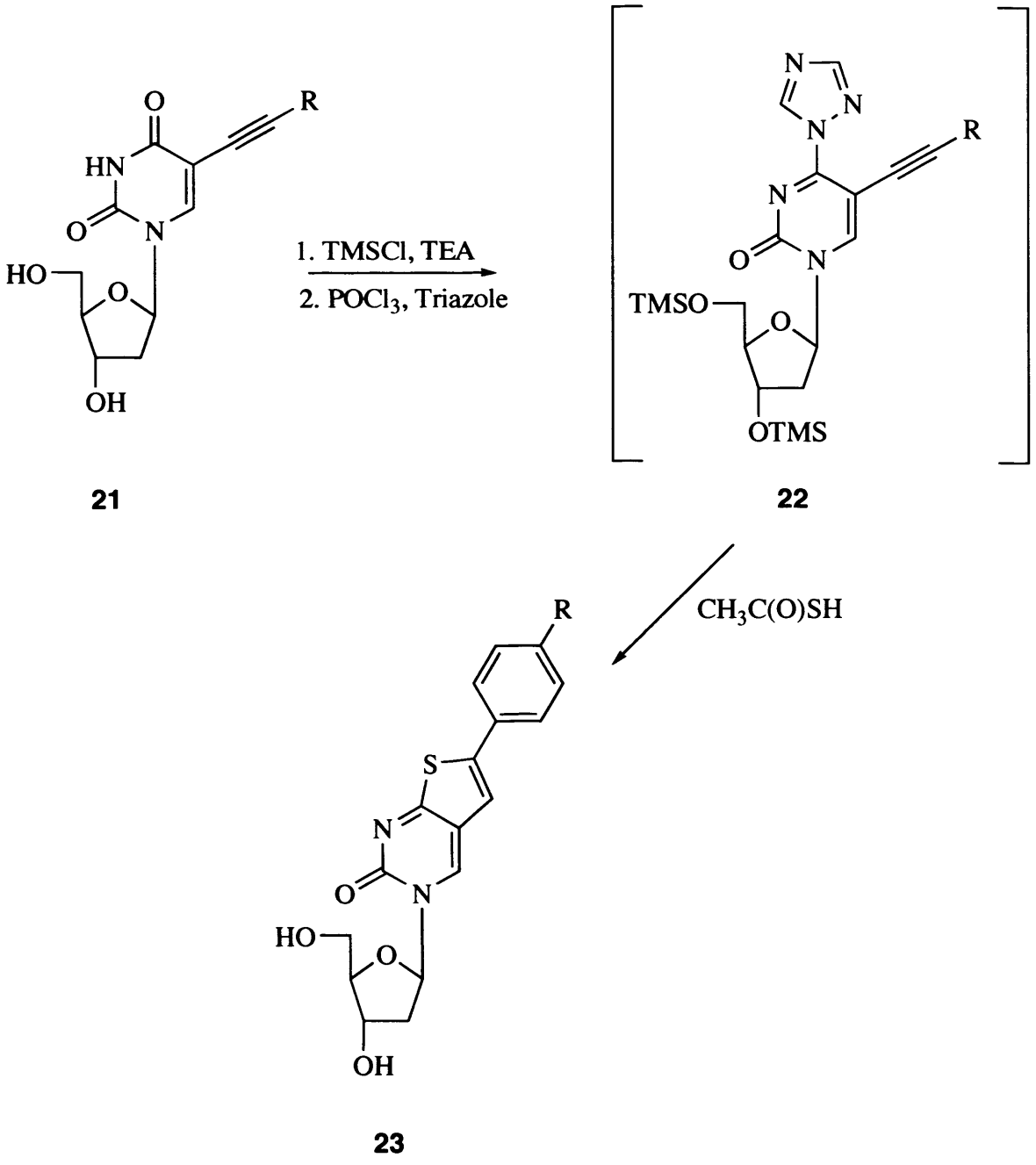
3.1 Aim of study

Considering this information, and with the aim to further study the SAR of these compounds and attempt to improve antiviral activity, we herein report the synthesis and biological evaluation of *thieno* analogues of the most potent BCNAs to date, which bear an alkylphenyl side chain.[3]

3.2 Synthesis of *thieno* analogues.

The target compounds were synthesised using the same established procedure as followed for the synthesis of the alkyl side chain *thieno* analogues[2] (Scheme 3.01). The 5-alkynyl-2'-deoxyuridine starting materials **(21)** were not commercially available, but were prepared as previously published *via* the coupling of IDU and the corresponding acetylene.[4] The free hydroxyl groups were protected with chlorotrimethylsilane in the presence of triethylamine, followed by addition of phosphorus oxychloride and triazole to form the 4-triazole intermediates **(22)**.

Addition of thiolacetic acid was expected to result in the formation of 4-thio derivatives, as had been observed in other nucleoside analogues,[5,6] which would then have been cyclised following the established methods of previous BCNAs to form the *thieno* analogues. However, addition of thiolacetic acid to the 5-alkynyl derivatives results in direct cyclisation to form the desired *thieno* products **(23)**. It is unclear exactly how this cyclisation occurs in acidic media and without a copper catalyst, when the parent nucleosides require both a basic media and the presence of a copper catalyst in order to cyclise.

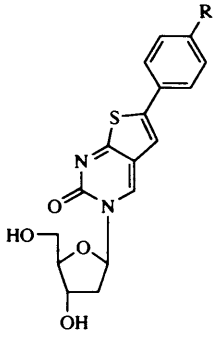


Scheme 3.01: General synthesis of the *thieno* bicyclic nucleosides

Alkylphenyl *thieno* analogues, where R = ethyl - butyl **(23a-c)** were prepared using this method. Analogues with longer alkyl chains (pentyl – octyl) were also synthesised within the group by Luis Sevillano.[7] The alkynyl intermediates **(21a-c)** were dissolved in acetonitrile, and triethylamine and chlorotrimethylsilane were added and stirred at room temperature until no

starting material was detected by TLC. Phosphorus oxychloride and triazole were added at 0 °C and stirred for 5 hr. Saturated sodium bicarbonate solution was added and the product extracted with dichloromethane. The organic layer was concentrated, the residue dissolved in acetonitrile and stirred in the presence of thiolacetic acid overnight at room temperature.

It was observed that for the analogues with alkyl chain lengths butyl-octyl **(23c-23g)**, a white precipitate was formed after stirring overnight with thiolacetic acid. Filtration followed by washing or recrystallisation of the precipitate with methanol resulted in the collection of the pure products in reasonable yields (41%-72%). Where a precipitate was not observed, the products were obtained following column chromatography, which may have resulted in the much lower yields (6 % and 2 %) obtained for these compounds.



Cmpd	R	Cf	Purification	Yield (%)
23a	Et	2266	Column chromatography	2%
23b	nPr	2267	Column chromatography	6%
23c	nBu	2268	Trituration	72%
23d*	nPnt	2269	Trituration	44%
23e*	nHex	2270	Trituration	58%
23f*	nHept	2271	Trituration	33%
23g*	nOct	2272	Trituration	41%

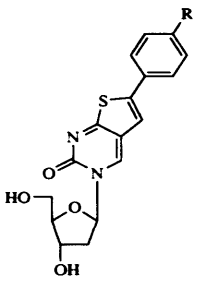
* prepared by Luis Sevillano

The products were characterised by ^{1}H and ^{13}C NMR. Formation of the *thieno* moiety was indicated by the shift of the H5 peak from 7.2 ppm (as seen with the *furo* parent) to *ca.* 7.5 ppm in the ^{1}H NMR, and the C5 peak from 100 ppm to 115 ppm in the ^{13}C NMR. Where possible, further characterisation and analysis was conducted by mass spectrometry and elemental analysis.

3.3 Biological Evaluation

The *thieno* analogues were tested for their ability to inhibit the replication of VZV against both TK competent and TK deficient VZV, the results of which are shown in Table 3.02.

Table 3.02: Biological evaluation of *thieno* analogues bearing an alkyl phenyl side chain.



	Cmpd	Cf	R	EC_{50} (μM)[a] VZV OKA	VZV YS	VZV TK- YS	MCC (μM)[b]	CC_{50} (μM)[c]
	23 a	2266	C_2H_5	0.15	0.2	20	>200	>200
	23 b	2267	C_3H_7	0.06	0.09	>16	80	>200
	23 c	2268	C_4H_9	0.020	0.028	>3.2	50	>200
	23 d	2269	C_5H_{11}	0.014	0.025	20	50	>200
	23 e	2270	C_6H_{13}	0.043	0.08	>50	200	>200
	23 f	2271	C_7H_{15}	0.18	0.27	>20	50	>200
	23 g	2272	C_8H_{17}	3.4	-	>20	≥20	>200
	Parent *furo*		C_5H_{11}	0.0003	0.0001	>5	≥20	>200
	ACV	-	-	1.5 ± 0.6	1.1 ± 0.1	40 ± 5	>200	>400

a. EC_{50}, 50% effective concentration required to reduce viral plaque formation by 50%. b. MCC, minimal cytotoxic concentration required to alter microscopically detectable cell morphology. c. CC_{50}, 50% cytotoxic concentration required to inhibit Hel cell growth by 50 %.

The data in the table shows that all of these *thieno* analogues display antiviral activity against VZV. However they are not as potent as their *furo* parent nucleoside bearing the same side chain. On comparison with the most potent anti-VZV BCNAs to date bearing a pentylphenyl side chain and the new corresponding *thieno* analogue, it can be seen that this new series of compounds is *ca.* 50-fold less active than their *furo* parent nucleosides.

Thus while, the previously synthesised *thieno* analogues bearing an alkyl side chain retained full antiviral activity against VZV,[2] these new *thieno* analogues bearing an alkylphenyl side chain did not.

However, these new analogues remain potent and selective inhibitors of VZV, with the most potent compound **(23d)** showing an activity against VZV of *ca.* 100 times that of the current treatment aciclovir. As seen with previous series of BCNAs, these analogues also follow the correlation between chain length and antiviral activity, with the most active analogue bearing a pentylphenyl side chain (**23 d** 0.014μM), the same as seen with the parent nucleosides.

As the target of these BCNAs is still not known, it is not possible to determine exactly how these *thieno* analogues differ from the *furo* parent nucleosides or the previous *thieno* analogues to result in reduced antiviral activity against VZV. It is possible, that this could be an effect of the steric properties of the sulfur atom on the alkylphenyl ring in the side chain and therefore possibly its position and binding within its active site.

To probe global SAR, the structures of the *furo* parent (carbon atoms coloured grey) and *thieno* nucleoside (carbon atoms coloured green) bearing a pentylphenyl side chain were both drawn and minimised using the computer software package MOE®.[8]

MOE®, which stands for, Molecular Operating Environment is a software package used for computational chemistry, and will be discussed in more depth later (chapter 8). The minimised structures were then superimposed keeping the pyridine rings of both molecules fixed (**Fig 3.01**).

As the picture indicates, the replacement of the oxygen atom in the bicyclic base with a sulfur atom has an effect on the side chain with the phenylalkyl side chain being twisted away from the sulfur atom. Further to this, measurements were calculated of the distance between the oxygen or sulfur atom and the nearest phenyl CH-carbon and its angle with the side chain (Table 3.03), which showed that as expected both the distance and the angle were increased by the *thieno* analogue.

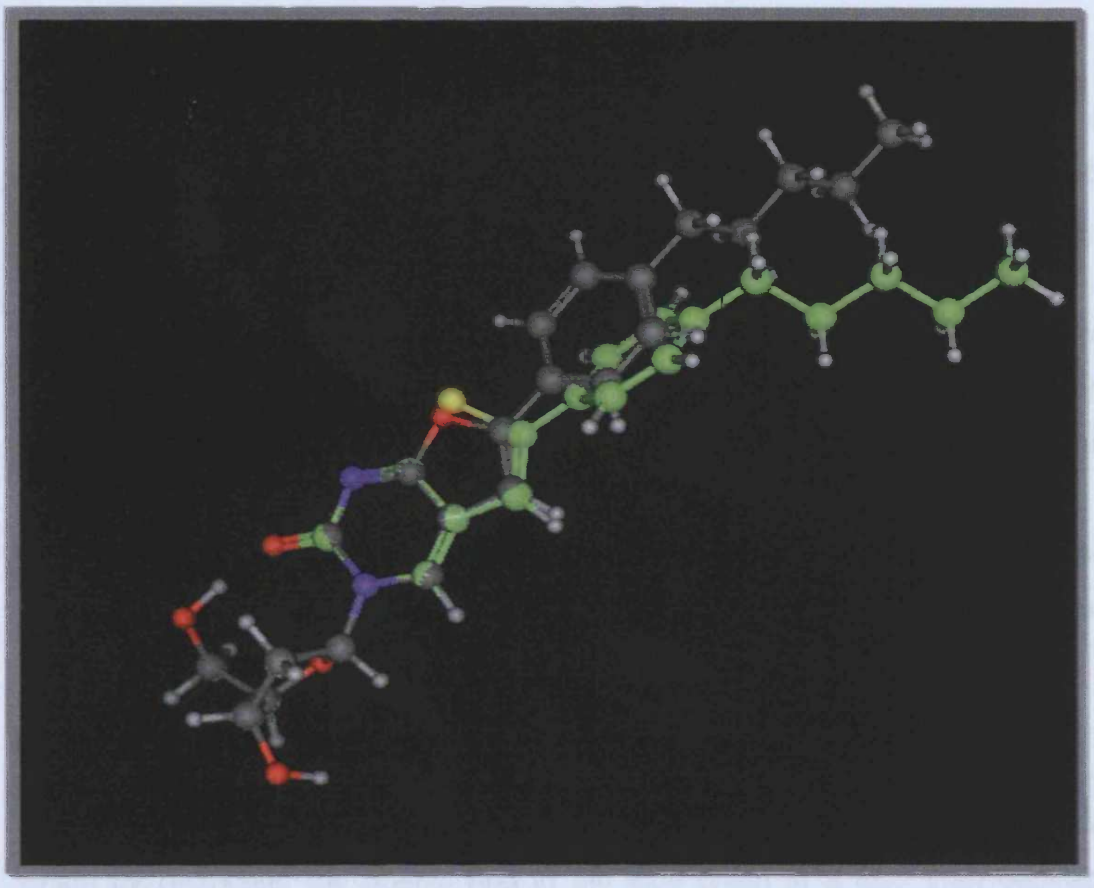
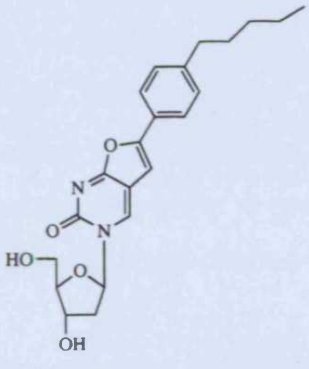
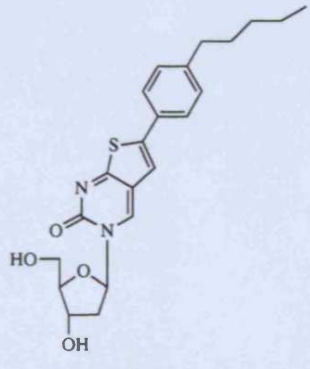


Fig 3.01: Superimposed image of Cf1743 and Cf2269



Cf1743



Cf2269

Table 3.03: Computational measurements

compound	Distance (A°)	Angle
Cf (*furo*)	2.83	116.9°
Cf (*thieno*)	3.30	120.1°

3.4 Conclusion

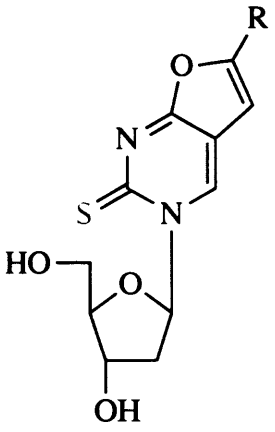
A new series of *thieno* bicyclic pyrimidine nucleosides bearing an alkylphenyl side chain have been synthesised and tested for their ability to inhibit the replication of VZV *in vitro.*

The results from the biological evaluation showed that while these *thieno* analogues showed antiviral activity against VZV, they were not as potent as their *furo* parent nucleosides. These results differ from previous *thieno* analogues bearing an alkyl side chain, which had been found to retain the full activity of their *furo* parent nucleosides against VZV. However, these new *thieno* BCNAs still remain amongst the most potent and selective antiviral nucleoside analogues against VZV *in vitro.*

The modelling of these compounds and comparison with the lead *furo* analogue suggest that the sulfur atom may affect the orientation of the side chain by restricting the phenyl ring in the side chain in a less than desirable position.

3.5 Thionation of the C-2 Carbonyl

2-Thiopyrimidine nucleoside analogues bearing a halogen atom and more recently an alkyl chain at the C5 position have been synthesised and found to possess antiviral activity.[9,10,11] It was considered interesting, therefore, to convert the carbonyl group at the 2 position of the bicyclic nucleosides to the thiocarbonyl analogues **(24)**.



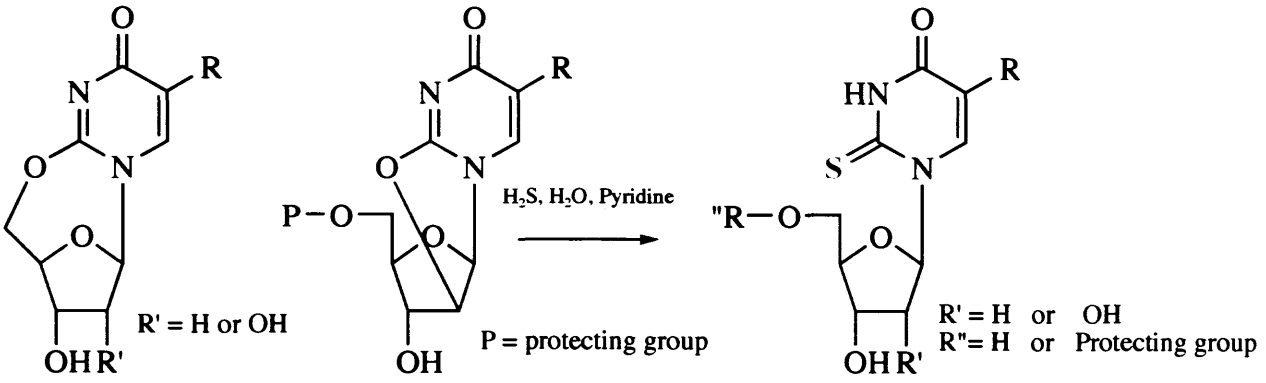
(24)

Previous 2-thiocarbonyl nucleosides were often synthesised following a multi-step synthesis which first required the preparation of the hydroxyl protected chloro sugar. This was then coupled with the 2-thio pyrimidine base to give the corresponding 2-thio nucleosides. Deprotection of the sugar followed by reaction at the 5-position to give the halo or alkyl compounds was then required to give the active 5-substituted-2-thiopyrimidines[12].

Thiocarbonyls can be prepared directly from the corresponding ketone or aldehyde, following reaction with thionating agents such as hydrogen sulfide, phosphorus pentasulfide and Lawessons reagent. It would therefore be advantageous if the 2-carbonyl of the BCNAs could be directly converted to the thio analogue.

3.6 Thionating agents

Hydrogen sulfide (H_2S) in the presence of an acid catalyst has successfully been applied to the synthesis of 2-thiocarbonyl nucleosides[13,14,15] from their corresponding 2,5' **(25)** or 2,2' **(26)** anhydro derivative **(Scheme 3.02)**.



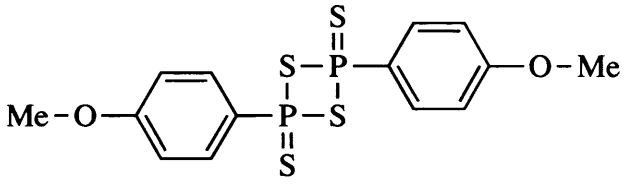
(25) **(26)**

Scheme 3.02: Thionation with H_2S

However, even though this reagent usually results in good yields and clean products, the difficulties associated with its use and the extra synthetic steps required for the preparation of the anhydro derivative means that this reagent would not be the most advantageous in the synthesis of the 2-thio BCNAs.

Phosphorus pentasulfide and Lawessons reagent are now more commonly used for direct thionation of carbonyl groups due to their ease of use over H_2S. The inorganic reagent phosphorus pentasulfide (P_4S_{10}) has extensively been used to form thiocarbonyls in good yields with simple purification of the products. However, problems can arise from its insolubility in most organic solvents.

As a result, the use of Lawessons reagent[16] **(27)** has become a more popular choice of thionating. It is thought to be an organic analogue of phosphorus pentasulfide and has the advantage of being more soluble in organic solvents and reacts in equimolar quantities with a variety of aldehydes and ketones to give the corresponding thio analogues in reasonable yields.



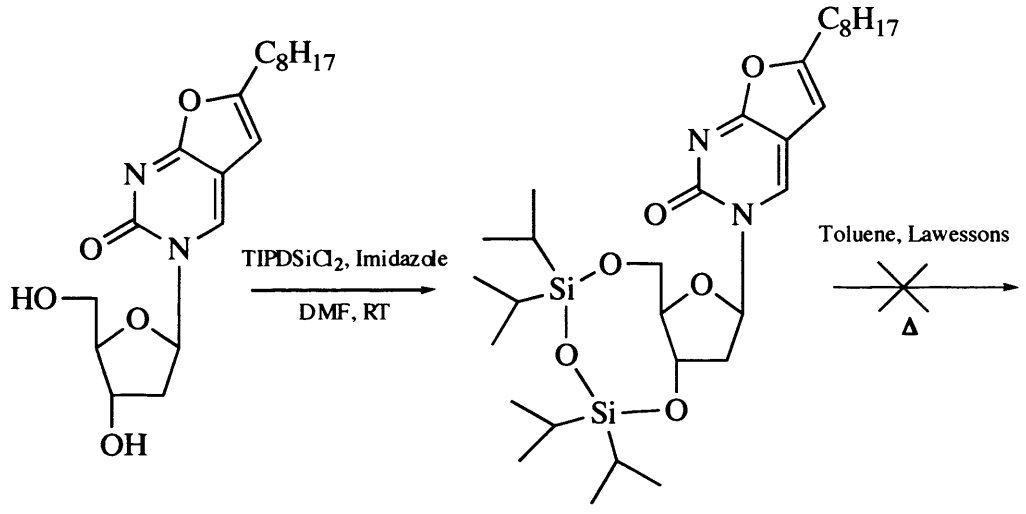
(27) – Lawessons reagent.

3.7 Synthesis

An investigation into the synthesis of pyrimidine analogues found thionation to be regioselective at the 4-position when using Lawessons reagent.[17] Therefore, in order to thionate the 2-positon of nucleosides the carbonyl at position 4 must be protected.[18]

It was thought, that in the case of the BCNAs where the oxygen at the 4-position is part of the *furo* ring, and in effect protected, that thionation at the 2-position might be possible. Due to ease and advantages of use, it was decided to first attempt the synthesis using Lawessons reagent on BCNAs already prepared and available within the laboratory.

To prevent possible thionation of the free hydroxyl groups, the parent nucleoside **(28)** was dissolved in DMF and stirred overnight in the presence of 1,3,dichloro-1,1,3,3-tetra-isopropyldisiloxane and imidazole to give the di-hydroxyl protected compound **(29)** in 42% yield **(Scheme 3.03)**.

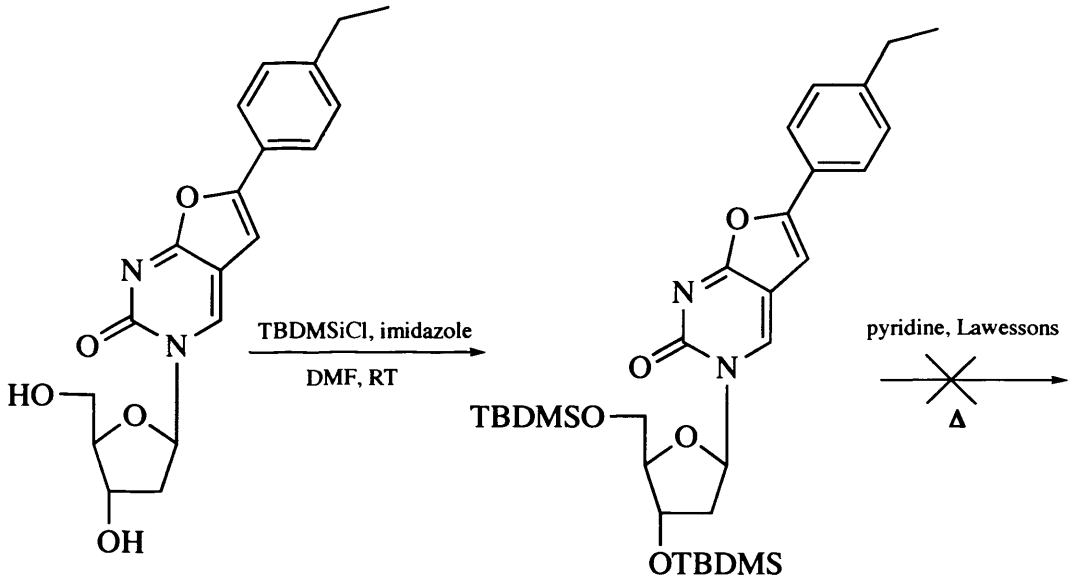


(28) **(29)**

Scheme 3.03: Protection and Thionation – attempt 1

This was then heated for 6 hr at reflux in toluene in the presence of Lawessons reagent. After this time, as well as a large quantity of remaining starting material, TLC analysis observed the formation of a product which was more polar that the starting material. This would not be expected of the desired product, which should be more lipophilic. TLC analysis of the product *vs* the parent BCNA **(28)** showed that this reaction had caused deprotection of the hydroxyl groups.

Following the synthesis of thiopyrimidine analogues, it was suggested that the use of pyridine as solvent was preferential to toluene in thionation reactions.[17] Due to the cleavage of the tetra-isopropyldisiloxane protecting group used previously, the BCNA **(30)** was first protected with chloro-*tert*butyldimethylsilane **(Scheme 3.04)** to give the hydroxyl protected analogue **(31)** in good yield (76%).



(30) **(31)**

Scheme 3.04: Attempt 2 at thionation

This was then dissolved in pyridine containing Lawessons reagent and heated at 100 °C for 16 hr, after which time, no reaction was seen by TLC. A further amount of Lawessons reagent was added and stirring continued at 100 °C for 3 days. Still no reaction was observed after TLC analysis. It was suggested that the retention factor (Rf) of the starting material and the desired product might be so similar by TLC that they would not be differentiated. Subsequent purification and analysis of the product collected however, showed it to be reclaimed starting material (76%).

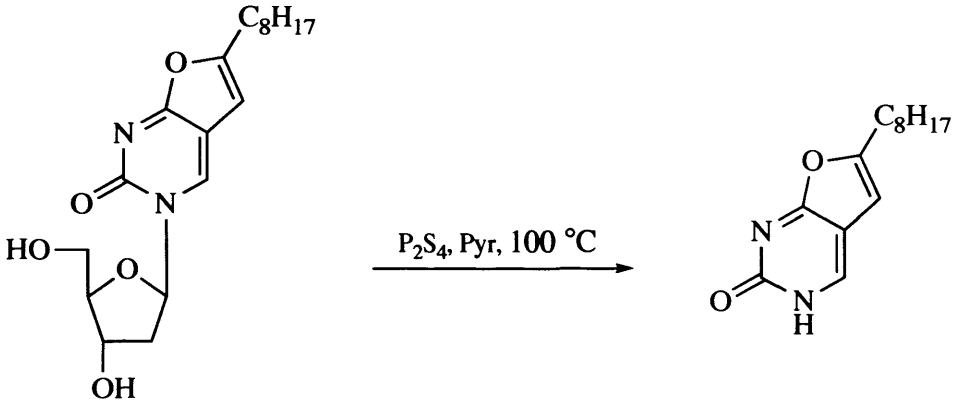
These unsuccessful attempts could be due to the aforementioned regioselectivity of the carbonyl at the 4-position. It was therefore decided to attempt the synthesis of the desired compounds using phosphorus pentasufide.

The hydroxyl protected nucleoside **(31)** was dissolved in pyridine and stirred for 24 hr at 100 °C in the presence of phosphorus pentasulfide. After this time, no starting material was seen remaining by TLC. However, again the

formation of a more polar product was observed, which TLC analysis showed to be the unprotected nucleoside **(30).**

Following this cleavage of the hydroxyl protecting groups, it was decided to attempt thionation directly onto the nucleoside with free hydroxyl groups **(Scheme 3.05)**. The nucleoside **(28)** was dissolved in pyridine and stirred at 100 °C for 36 hr in the presence of phosphorus pentasulfide.

In this case, a more lipophilic fluorescent product was observed by TLC. The product was collected with some impurities, after precipitation from the reaction mixture during removal of the solvent *in vacuo*. Analysis of this solid by nmr and mass spectrometry showed the product to be the cleaved base **(32)**.



(28) **(32)**

Scheme 3.05 : Attempted thionation resulting in base cleavage

Very recently within our laboratories, other researchers using high temperatures and pyridine as the solvent have seen this instability of the BCNA's[19]. To determine if this affect is a result solely of the high temperature and pyridine or due to the individual reaction conditions the most potent BCNA to date bearing a pentylphenyl side chain was dissolved in pyridine and stirred at to 100 °C.

3.8 Conclusion

The direct thionation of the carbonyl at position 2 of the bicyclic nucleosides with Lawessons reagent and phosphorus pentasulfide has been attempted and shown to be unsuccessful. Where a reaction was seen, this resulted in a cleavage of the base or removal of the 3',5' OH protecting groups.

As the desired analogues could not be obtained following direct thionation, the synthesis of these compounds would require a multistep synthesis involving protection of the carbonyl at the 4 position. Problems with coupling and cyclisation of nucleosides containing a thiocarbonyl group have also previously been encountered within our group[20] and therefore further attempts to obtain the desired analogues were not attempted.

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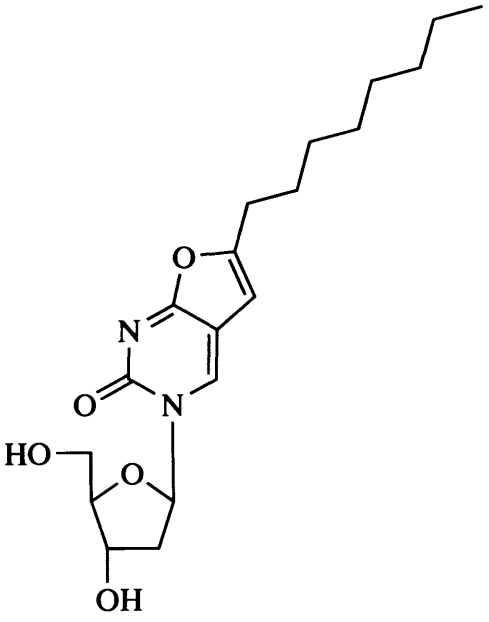
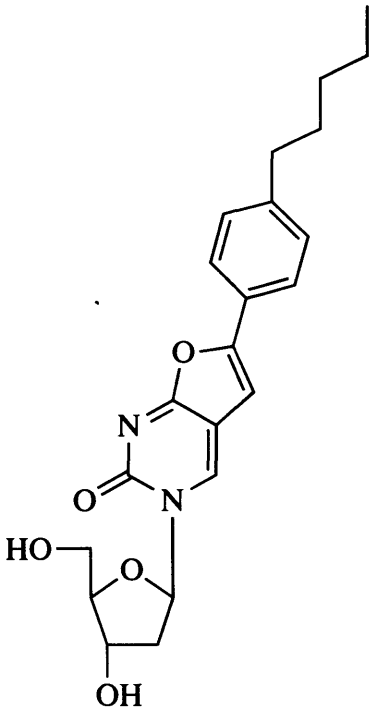
Chapter 4. Side Chain Modification

4.0 Introduction

The insertion of a phenyl ring into the alkyl side chain of the lead BCNA **CF1368** to give **Cf1743,** led to an increase of *ca.* 100 fold in antiviral potency against VZV compared to the alkyl analogues[1], and to our most potent BCNA to date.



Cf 1368



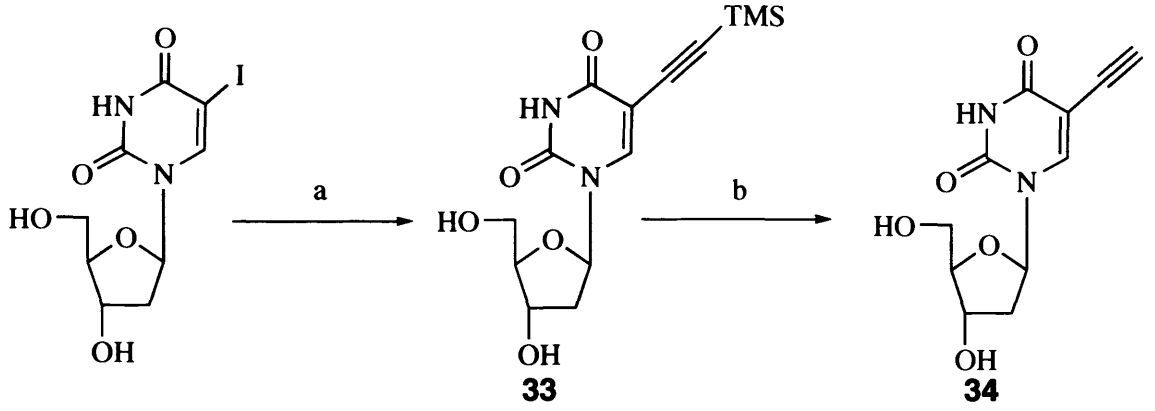
Cf 1743

To further investigate this modification, structure activity relationship studies were carried out by synthesising analogues with halogen-substituted phenyls in the side chain. Previous investigations on the alkyl lead compound had shown that terminal substitution of the alkyl chain with a halogen atom was well tolerated with respect to anti-VZV activity [2].

The *para*-substituted bromo and chloro phenyl analogues were prepared using the established Pd/Cu catalysed one pot coupling and cyclisation reaction and tested for their antiviral activity against VZV.[3] It was noted that these analogues conveyed antiviral activity comparable to that of the parent bearing a phenyl ring with no substitution.

These studies were then extended to include the *para*-fluoro analogue and the *ortho* and *meta* substituted isomers.[4] In this case, not all of the acetylene derivatives of the appropriate halophenyl were commercially available and where not available the appropriate halo iodobenzene derivative was used and coupled with 5-ethynyl-2'-deoxyuridine (EDU).

EDU **(34)** is prepared by Pd/Cu catalysed coupling of IDU with trimethylsilylacetylene[5] followed by deprotection of the trimethylsilyl group with ammonia in methanol (Scheme 4.01). The desired analogues can then be prepared by coupling and cyclisation of EDU with the corresponding halo iodo-benzene.[6]

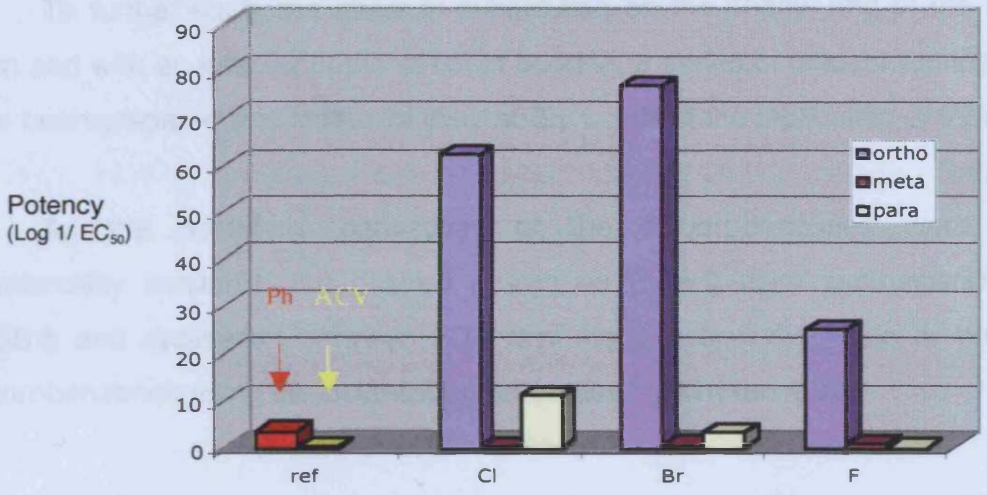


a.) Acetonitrile, Trimethylsilyl acetylene, tetrakis (triphenylphosphine)palladium(0), CuI, Et_3N, reflux, 5hr. b.) methanol/ammonium hydroxide (10%) (1:1), 5 hr.

Scheme 4.01: Synthesis of EDU

The analogues were tested for their antiviral activity against VZV and the results compared with that obtained for the parent analogue bearing an unsubstituted phenyl ring in the side chain and also to aciclovir. These showed, that in general, the *para*-substituted analogues retained antiviral activity, the *meta*-substituted analogues displayed activity against VZV but were less active than the parent phenyl compound, while the *ortho*-substituted analogues showed an increase in activity by *ca.* 10 fold. These results are highlighted in Graph 4.01 below.

Graph 4.01: Antiviral activity of halophenyl substituted BCNAs



In contrast to the general findings and previous aryl compounds, the *para*-fluoro analogue was found to be completely inactive as an antiviral. This result is unexpected. Fluorine is often used as a replacement for hydrogen in SAR studies and is shown to cause minimal steric effect at the active site.[7] It may have different electronic properties to hydrogen, which may alter its chemical reactivity and lipophilicity, but the fluorine in the *ortho* and *meta* position followed the potency of the other halogens, and ClogP calculations* of the *para* analogue showed that the introduction of the fluorine has little impact on the lipophilicity.

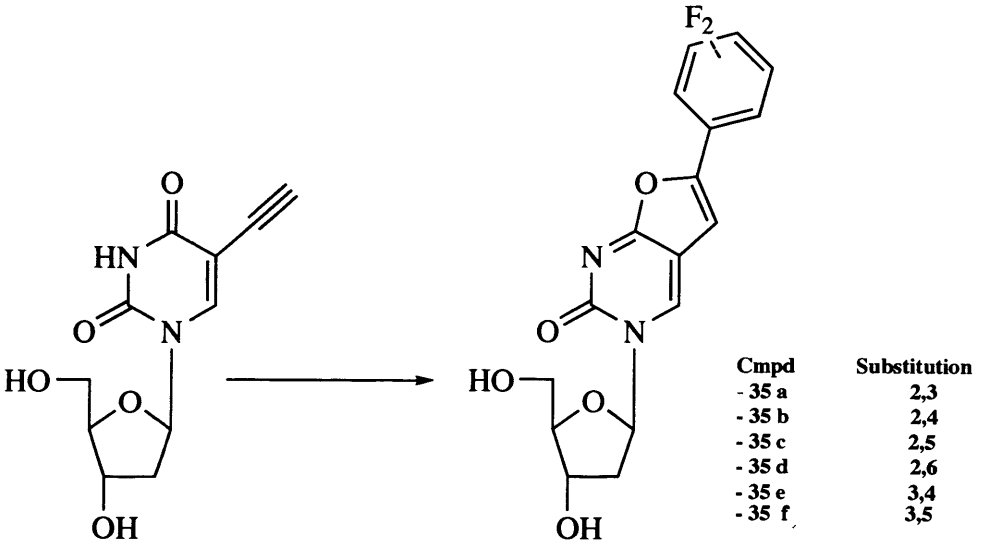
* Calculated using ChemDraw 7

The result is even more intriguing following enzymatic studies of this compound. The *para*-fluoro analogue has been found to be the best BCNA inhibitor of VZV thymidine kinase, an enzyme important in viral replication.[8] Thus although the mechanistic origins of this inactivity are currently unknown, it is hypothesised that the fluorine atoms size and properties allow it to enter and bond in the active site of VZV TK without being metabolised or that further phosphorylation to the diphosphate is blocked in this case.

4.1 Aim of study: Difluorophenyl analogues

To further study the effect of substitution on the phenyl ring of the side chain and with an interest in the effect of fluorine, a series of difluoro analogues have been prepared and tested for their ability to inhibit the replication of VZV.

As the acetylene derivatives of the difluorobenzenes were not commercially available, the desired analogues (**35a-f**) were synthesised via coupling and cyclisation between EDU and the corresponding iodo or bromo difluorobenzenes using the established procedures (**Scheme 4.02**).



Cmpd	Substitution
- 35 a	2,3
- 35 b	2,4
- 35 c	2,5
- 35 d	2,6
- 35 e	3,4
- 35 f	3,5

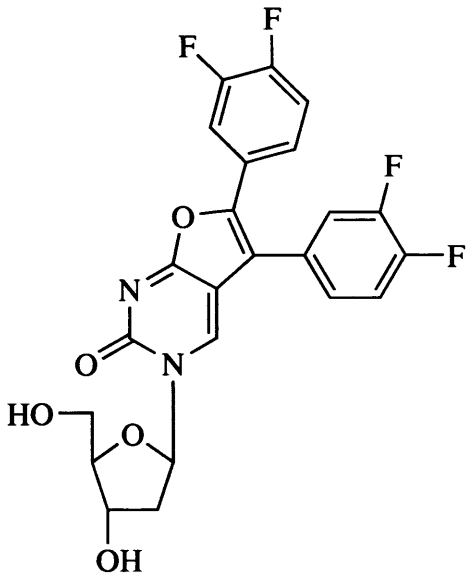
Scheme 4.02: Synthesis of the difluoro analogues.

The synthesis of EDU adopting the procedure described above (**Scheme 4.01),** resulted in a problematic purification, but afforded the product in reasonable yield (60%). It has been suggested that synthesis, using acetonitrile as the solvent and the use of dichlorobisdiphenylphosphine palladium (II) results in better yields and easier purification.[9] Use of this method provided EDU (75%) after simple purification.

The synthesis and purification of these difluoro analogues has proved less straightforward than expected. The differing substitution on the phenyl ring resulted in the products having variable solubility, which in some cases made their purification and characterisation difficult.

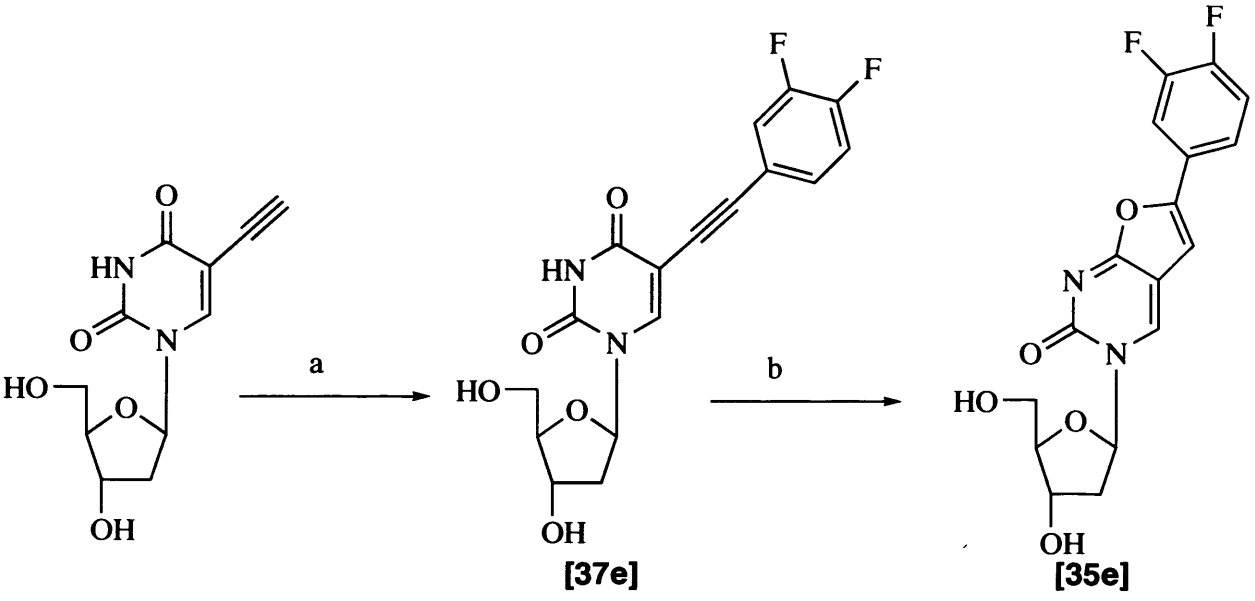
Where possible the iodo derivative of the benzyl to be coupled is used, as the bromo derivatives have been found to be less reactive[10] and can result in lower yields.[9] Only one iodo difluorobenzene was commercially available and therefore the synthesis of the 3,4-difluoro analogue **[35e]** was attempted first using the established procedure (as shown above).

The desired analogue **(35e),** initially seemed to have been synthesised and purified with ease in good yield (76%). However, the structural analysis did not confer with the desired product. Most notably the CH peak at position 5 was missing from both the ^{1}H and ^{13}C nmr spectra and integration for the aryl hydrogens was twice of that expected. Mass spectral analysis later also indicated the presence of an additional di-fluorophenyl moiety which along with the absence of the C-5 proton in ^{1}H nmr, confirmed the product to be the 5,6-disubstituted analogue **[36]** (46% corrected).



[36]

This type of substitution has been seen previously[11,12] and following suggestions, synthesis of the desired analogue was attempted using a two-step procedure instead of the usual one pot reaction. This required isolation and purification of the coupled product **(37e)**, which was then dissolved in a mixture of methanol and triethylamine in the presence of copper iodide to afford the cyclised product **(Scheme 4.03)**.



[37e] **[35e]**

a. DMF, 3,4-difluoro,1-iodobenzene, tetrakis (triphenylphosphine)palladium(0), CuI, DIPEA, RT, Overnight. b. methanol/triethyl amine (7:3), CuI

Scheme 4.03: Synthesis of the difluorophenyl BCNAs

Following this procedure the coupled compound **[37e]** was obtained in a reasonable yield (56%), which was subsequently cyclised to give the desired analogue **[35e]** as a pure white powder in excellent yield (93%).

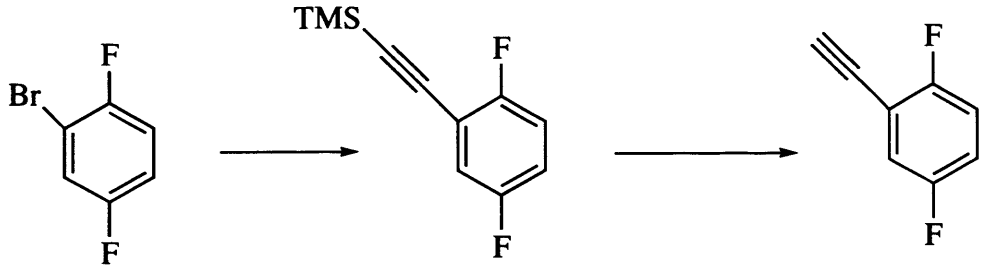
The 2,4-difluorophenyl, 2,5-difluorophenyl and 3,5-difluorophenyl analogues were prepared using the standard one pot synthesis[1] of the BCNAs, following coupling of EDU with the corresponding bromo derivatives.

The reaction of 2,4-difluoro-1-bromobenzene with EDU seemed to proceed well, with TLC analysis of the reaction mixture detecting no trace of EDU still present. However, the reaction mixture was darker than usual and a number of side products were observed on the TLC plate. Removal of the solvent produced a tarry mixture, which was difficult to purify by column chromatography but, by following this with trituration in methanol the product **(35 b)** could be obtained although in low yield (9%).

After the overnight coupling of EDU with 3,5-difluoro-1-bromobenzene, TLC analysis of the reaction mixture showed the presence of remaining EDU. The reaction was continued through to the cyclisation step as it was assumed that the addition of further CuI and the temperature would force the reaction to completion. However, TLC analysis of the final reaction showed EDU still remaining, but also the fluorescent presence of the product. Following purification by column chromatography and trituration with methanol, the desired product (**35f**) was obtained (7.5%).

Synthesis of the 2,5-difluorophenyl analogue also proved difficult. Visualisation of the reaction mixture by TLC analysis was difficult due to multiple spots and after column chromatography the product was obtained in low yield (1.4%). This product was also observed to have poor solubility. It was difficult to dissolve in DMSO in order to complete full structural analysis. In fact, due to this and the low yield, obtaining a carbon NMR proved most troublesome and could not be obtained.

Following the difficulties observed in obtaining these compounds other alternatives to their synthesis were sought. In order to increase yields, the synthesis of the corresponding difluororophenylacetylene derivative was attempted **(Scheme 4.04)**. It was thought that this could then be coupled with IDU to give the compounds in improved yields.



Scheme 4.04: Synthesis of acetylene derivative

This reaction may have its own complications however. Previous synthesis attempted on 1,4-dibromobenzene showed that the reaction can be difficult to follow by TLC and the product can have the same or an extremely similar R_f to the starting material.[9]

The synthesis of the acetylene derivative of 2,5-difluoro-1-bromobenzene was attempted by reaction with trimethylsilylacetylene. After stirring at room temperature overnight, TLC analysis showed the possible presence of a spot with a very similar R_f to the starting material as well remaining starting material. Following purification by column chromatography the product was found to degrade to give a black crude residue. This quick degradation made NMR analysis impossible and made it impossible to determine if the product formed was indeed the desired derivative.

This gave the only option of continuing with the difluoro-bromobenzene derivatives and their coupling with EDU, despite the low yields. However, in order to try to make purification easier the method adopted for the synthesis of

EDU using acetonitrile as solvent and dichlorobis(diphenylphosphine)palladium (II) as the catalyst was used.

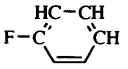
The synthesis of the remaining two analogues, the 2,3-difluorophenyl **(35a)** and 2,6-diflourophenyl **(35d)** were attempted in these conditions. However, these conditions did not improve the synthesis or purification of these compounds, both being obtained in 2% yield.

4.2 NMR Analysis

The presence of the fluorine atoms in these molecules provided some interesting nmr patterns and splittings not previously seen with the BCNAs. In the ^{1}H nmr, those compounds synthesised with one of the fluorine atoms in the *ortho* / 2-position of the phenyl ring, were found to couple with the H-5 proton producing a doublet for the H-5 at approximately 7.2 ppm and with a coupling constant *(J)* value of 2.9 Hz.

For mono substituted fluoro benzene compounds, it is possible to determine the position of the fluorine atom using ^{1}H nmr coupling constant values of the phenyl protons. The three corresponding protons should couple with the fluorine to give ^3J (H-F) = 9.0 Hz, ^4J (H-F) = 5.7 Hz and ^5J (H-F) = 0.2 Hz[13] **(Table 4.01)**. The addition of an extra fluorine atom in the phenyl ring of the side chain of these BCNAs produced complicated multiplets and it was not possible to prove the positions of the fluorine atoms in the phenyl ring from the ^{1}H nmr.

Table 4.01: Literature coupling constants (*J*) for the couplings of fluorine

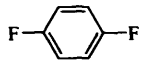
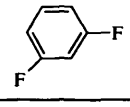
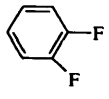
		(H-F)			(C-F)				
	coupling	^3J	^4J	^5J	^1J	^2J	^3J	^4J	^5J
	Hz	9.0	5.7	0.2	245	32	4	1	0

The influence of the fluorine atoms was also seen in the ^{13}C nmr spectra. All of the phenyl carbons were influenced by the fluorine atoms causing multiplets that, in some cases made identification of specific carbon peaks difficult. The C-F phenyl carbon nmr peaks were found as broad multiplets and were seen downfield from the C-H phenyl carbon peaks.

As seen in the ^{1}H nmr the C-5 position was shown to interact with a fluorine at position 2 in the phenyl side chain, where present, to produce a doublet, (J = *ca.*12 Hz,).

Due to the effect of the two fluorine atoms, it was not possible to fully prove the position of the fluorine atoms on the phenyl ring using ^{1}H or ^{13}C nmr, however, it can be possible using the ^{19}F nmr spectra. Difluorinated benzenes have been shown to have different chemical shifts depending on the substitution positions of the fluorine atoms (see table 4.02 below).[13]

Table 4.02 : Literature chemical shifts of difluorinated benzenes

Compound	δ
	-119
	-110
	-139

The difluorophenyl BCNAs synthesised are consistent with this data. The 2,3-difluoro and 3,4-difluoro analogues with CF bonds directly next to each other were seen as multiplets at -139 ppm and -137 ppm respectively. The 3,5-difluoro, 2,6-difluoro and 2,4-difluoro analogues which are spaced apart by one carbon atom on the ring, were also consistent with the above table, found at –

108.9, -110.4 and -108.3 ppm respectively, as was the only analogue spaced by two carbons, the 2,5-difluoro analogue producing a multiplet of peaks at –117.7 ppm. It was not possible however, to distinguish between the difluoro analogues any more than this.

From this spectral analysis it can be concluded that the compounds obtained are indeed the desired compounds with the fluorine atoms in the correct positions around the phenyl ring in the side chain.

4.3 Biological results

All of the desired analogues **(35a to 35g)** and also the 5,6-disubsituted BCNA **(36)** were tested for their antiviral activity against VZV; the results of which are shown in the table below **(Table 4.03)**. For comparative purposes the compounds were compared to the parent BCNA bearing an unsubstituted phenyl ring in the side chain [Cf 1837].

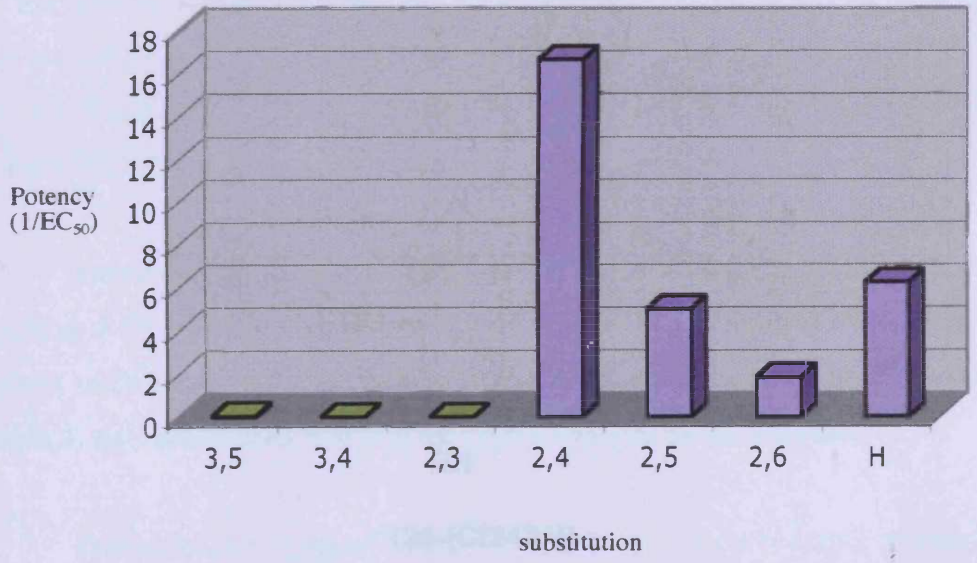
The effect of these substitutions are also shown in graph 4.02 below showing potency vs. substitution, and are also compared to the parent BCNA. Some of the analogues showed antiviral activity against VZV, but as can be seen in the chart those containing a meta substitution were found be either less potent than the parent or showed no activity at all. This is similar to the effect that mono halogen substitution had on antiviral activity and seems to be independant of the position of the second fluorine atom on the phenyl ring.

Table 4.03: Antiviral activity of difluorophenyl BCNAs

Compound	F position	EC_{50} [a] (μM) VZV OKA	EC_{50} [a] (μM) VZV TK-	MCC[b] (μM)	CC_{50}[c] (μM)
35a [Cf2421]	2,3	>80	>80	400	>200
35b [Cf2367]	2,4	<0.13	184	≥200	>200
35c [Cf2371]	2,5	<0.13	>3.2	16	29
35d [Cf2370]	2,6	0.54	>200	>200	>200
35e [Cf2372]	3,4	>80	>80	400	>200
35f [Cf2369]	3,5	19	>80	200	>200
35[Cf2368]	3,4 (5,6disub)	38	32	≥200	>200
Cf1837	H	0.28	>200	>200	>200

a. EC_{50}, 50% effective concentration required to reduce viral plaque formation by 50%. b. MCC, minimal cytotoxic concentration required to alter microscopically detectable cell morphology. c. CC_{50}, 50% cytotoxic concentration required to inhibit Hel cell growth by 50 %.

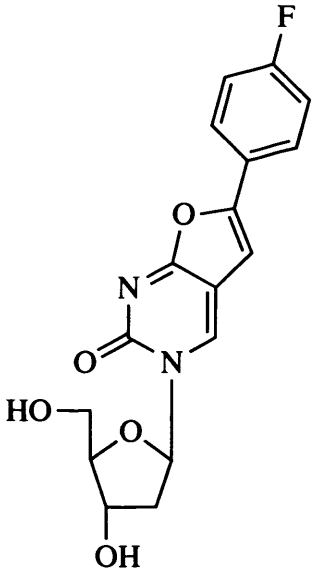
Graph 4.02 Potency vs. difluoro substitution



Apart from the 2,3-difluorophenyl analogue which contains a *meta* positioned fluorine, the remaining compounds with a fluorine in the ortho position **(35b, 35c and 35d)** more or less retain the antiviral activity as seen with the parent phenyl BCNA.

What is unexpected is the presence of antiviral activity of the 2,4-difluorophenyl analogue, which contains a fluorine atom in the para position. This is surprising, as the previous mono substituted analogue with a fluorine in the *para* position was shown to be completely inactive as an antiviral against VZV[4].

With this unexpected result, the *para* fluorophenyl analogue **(38 [Cf2421])** was resynthesised and tested for its antiviral activity against VZV. 4-fluoro-ethynylbenzene was coupled and cyclised with IDU using the published one pot method for the synthesis of BCNAs[14] to give the desired compound as a white powder (20%).



(38-[Cf2421])

To further analyse the purity of the compound before sending for biological testing, HPLC analysis was performed (appendix i). The compound has a retention time (Rt) of *ca.* 5.9 min (eluent: gradient 70% H_2O in acetontirile increased to 50% H_2O in acetonitrile after 30 min). The presence of a second peak could also be seen (*ca.* Rt 33.5min) but found to be a minor impurity (less than 10%).

Biological analysis of this compound was found to concur with the original findings with no activity against VZV at concentrations up to 100 μM.

4.4 Conclusion

Six new bicyclic nucleoside analogues substituted at the 6-position with a difluorophenyl moiety as well as one 5,6-disubsituted analogue have been prepared and analysed for their ability to inhibit the replication of VZV *in vitro*.

It was found, that those analogues with a fluorine in the *meta* / position 3 on the phenyl side chain had reduced antiviral activity against VZV regardless of the substitution of a second fluorine atom, while those analogues with a fluorine atom in the *ortho* / position 2 of the phenyl ring retained activity compared to the parent BCNA which has an unsubstituted phenyl ring in the side chain.

Interestingly, analogue **(35b)** with one of the fluorine atoms in the *para* / position 4 of the phenyl ring was found to be active, retaining the activity of the parent of BCNA whilst the mono substituted analogue with a fluorine in the *para* position was previously found to be totally inactive as an antiviral.

These results suggest that there are more complex factors at play here in the ability of BCNAs to display antiviral activity against VZV.

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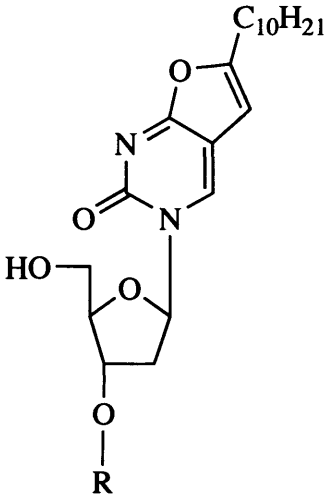
Chapter 5- Sugar Modifications

5.1 Introduction

Some earlier modifications to the sugar moiety of the anti-VZV BCNAs focused on replacement of the 3' or 5' hydroxyl groups with chlorine or fluorine.[1,2] This resulted in a loss of antiviral activity against VZV indicating the need for both of these hydroxyl groups to be present in order for the BCNAs to convey antiviral activity.

A series of 3'-alkyl ethers of the alkyl BCNAs have also been prepared within our laboratory.[3] Analogues where R= Me, Et, nPr and nBu **(37)** were synthesised and tested for their ability to inhibit the replication of VZV *in vitro* **(Table 5.01)**.



(39)

The results showed that as the length of the chain increased from Me to nPr, the antiviral activity against VZV apparently increases but as the chain increases further to nBu the analogue loses antiviral activity. The 3'-O-nPr analogue was shown to be the most active (0.55 μM), however, this is *ca.* 36 fold less active than the parent compound (R=H).

Table 5.01: Biological results of 3' ether analogues of the alkyl BCNAs

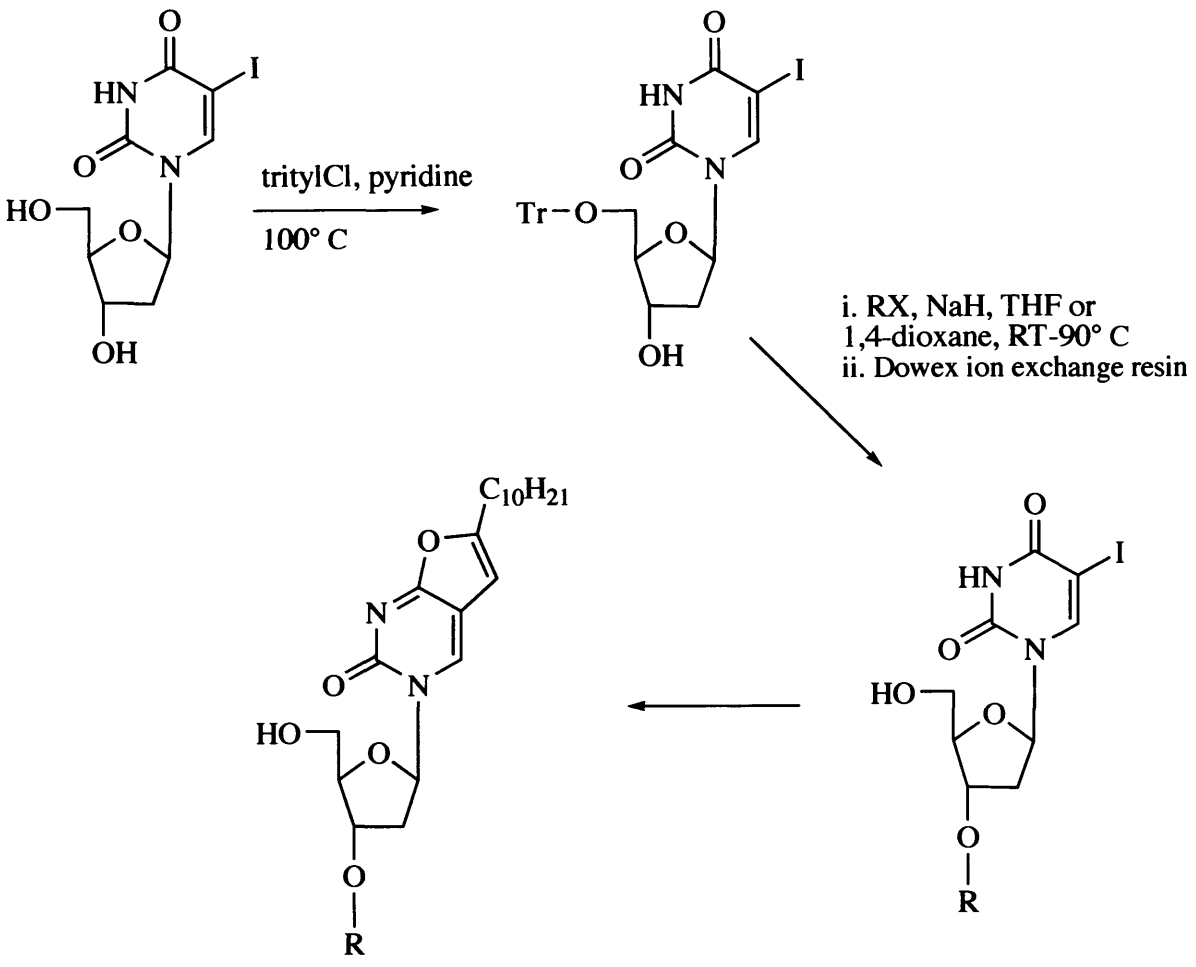
R=	Cf	EC_{50} (μM)[a] VZV YS	EC_{50} (μM) [a] VZV OKA	TK^-
Me	1723	>200	>200	>200
Et	1727	33	126	>50
nPr	1728	0.55	1.1	>200
nBu	1730	1.4 *	26	>50
Bn	1729	>5	nd	>5
H	1369	0.015	0.008	

a. EC_{50}, 50% effective concentration required to reduce viral plaque formation by 50%. * no end point reached.

The results obtained are surprising. As stated above previous studies on modification to the 3' position have led us to believe that the 3' hydroxyl group is necessary for efficient phosphorylation and therefore antiviral activity. Some of these 3' alkylethers have shown antiviral activity suggesting that this may not necessarily be the case.

We wished to investigate sugar modifications further. However the synthesis of these compounds requires starting from IDU for each of the analogues made. Therefore, the initial aim of this study was to find a more efficient route in the synthesis of modified sugar analogues starting from a parent BCNA.

The established method **(Scheme 5.01)** starting from IDU requires a minimum of eleven steps, which first requires protection of the 5' hydroxy group with trityl chloride. This is followed by formation of the 3' ether using a classical Williamson ether method, which involves the nucleophilic substitution of an alkyl halide with sodium alkoxide. The use of Dowex ion exchange resin after the reaction to destroy the excess of sodium hydride also resulted in the deprotection of the trityl group. Coupling and cyclisation of this product yielded the desired BCNAs.

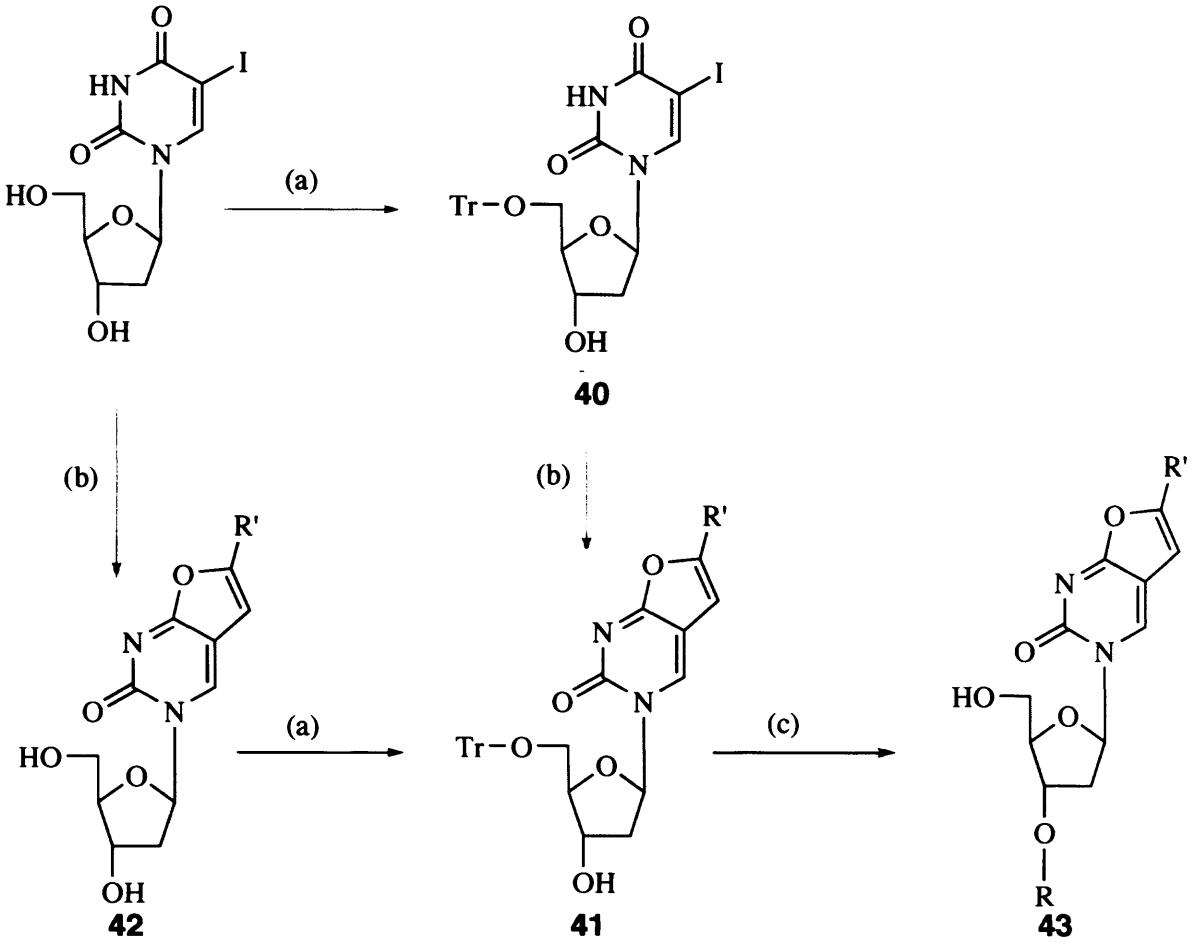


Scheme 5.01: Route 1 to the 3'O-alkyl ether BCNAs.

By first synthesising the 5' hydroxyl protected bicyclic nucleoside, the number of reaction steps to produce the desired analogues would be reduced. There are two possible routes to reach the protected analogue shown below **(Scheme 5.02).**

The first (shown with red arrows), follows the first step as seen in the established procedure with the synthesis of 5' tritylIDU **(40)**. This is then coupled and cyclised at the 5-position to give the desired protected BCNA **(41).** The second (shown with blue arrows), forms the cyclised nucleoside **(42)** first,

followed by protection of the 5' hydroxyl group with trityl chloride to give the desired analogue **(41),** which in both routes could then be used to give the 3'alkylether analogues **(43)**.



(a.) TrCl / pyridine (b.) i. $(PPh_3)_4Pd(0)$, CuI, DIPEA, DMF, ii. CuI, Et_3N, (c) NaH, XI

Scheme 5.02: Possible alternative routes to 3'alkylether BCNAs

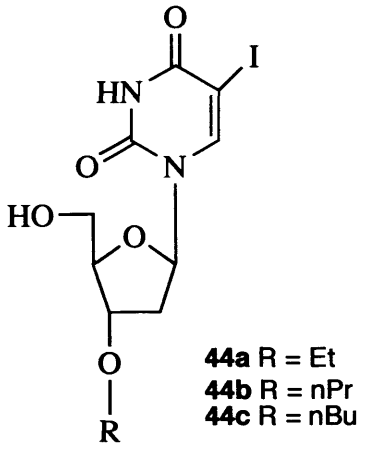
Protection of the 5' hydroxyl group of IDU with trityl chloride produced the desired compound **40** in good yields (80%). This was then coupled and cyclised with 1-decyne to give the desired compound **41** bearing an alkyl side chain of 8 carbons in length (64%).

The alternative route (in blue) was attempted concurrently, with the BCNA **(42)** bearing an octyl side chain ($R'=C_8H_{17}$) obtained using the established procedure from IDU (34%).[4] Protection of the 5' hydroxyl group of the BCNAs directly had not previously been attempted and in this case proved to be difficult. The reaction of **42** ($R=C_8H_{17}$) with trityl chloride using the same procedure to protect the 5' hydroxyl of IDU resulted in degradation to produce a black tarry mixture and numerous spots were observed on TLC. This also made purification difficult and using this route the desired compound **41** was obtained in poor yield (14%).

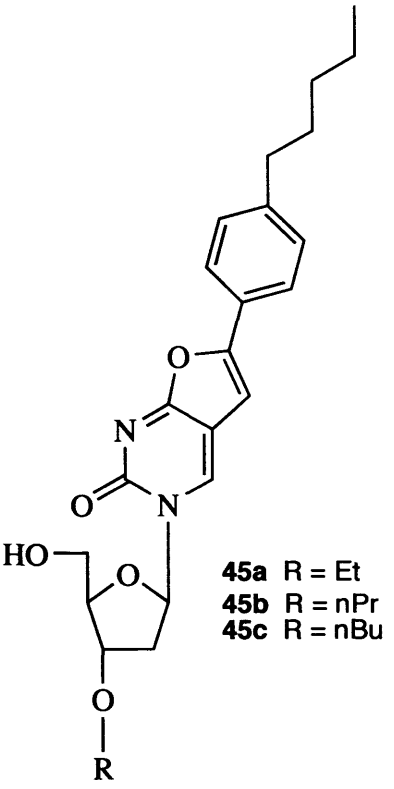
The final step of both routes requires the etherification of the 3' hydroxyl group. The same procedure as used previously within our group for the synthesis of the 3'-O-alkyl analogues of IDU was attempted with the 5'-trityl BCNA **(41).** This involves a classical Williamson ether method using sodium hydride. However these reaction conditions resulted in cleavage of the bicyclic base. This shows that the original route with reaction of 5'-protected IDU is most successful.

With this in mind, it was decided to synthesise 3'-O-alkyl analogues of the most potent BCNAs to date bearing a pentylphenyl side chain[5] using the established method. The synthesis of analogues where R= Et, nPr and nBu **(45a-c)** has been attempted.

Trityl IDU was re-synthesised and placed in the appropriate conditions in the presence of the desired alkyl halide to give the corresponding 3'O-alkyl analogues **(44a-c)**. Reaction with iodoethane resulted in low yield (13 %). TLC detected unreacted starting material and the product also required recrystallisation following column chromatography to obtain a pure white powder. This may have contributed to the low yield. The reaction was repeated twice to give further amounts of the derivative **44a** (27%) desired in order to proceed.



44a R = Et
44b R = nPr
44c R = nBu



45a R = Et
45b R = nPr
45c R = nBu

Using the established procedure[4], **44a** was then coupled and cyclised with 4-n-pentylphenylacetylene to give the desired final product **45a** as a white powder (27%).

The 3'O-Butyl analogue **44c** was obtained in the minimal required amount necessary for the next step after two attempts (max yield 28%). During one of the reaction attempts, the presence of a second product was observed by TLC. ^{1}H NMR analysis of the product obtained showed the absence of an NH peak, which indicates that the derivative has also been alkylated at the 3-N position.

44c was then coupled and cyclised with 4-n-pentylphenylacetylene to give the desired final analogue **45c** (17%). The low yield can be attributed to the amount of starting material. Others within the group have suggested that using below 600 mg of starting material for this reaction results in low yields and often makes purification and isolation of any product formed extremely difficult.

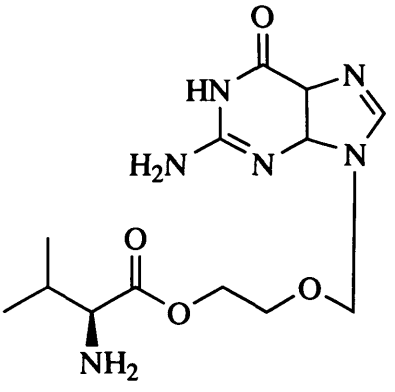
The 3'-O-propyl analogue **44b** proved a lot more difficult. Repeated attempts at this reaction resulted in an impure product that showed the absence of a NH peak in the proton NMR. Thus target compound **45b** was not available.

The final products obtained (**43a** and **43c)** were sent for biological testing against their ability to inhibit the replication of VZV. The intermediates **42a** and **42c** were also sent for biological testing.

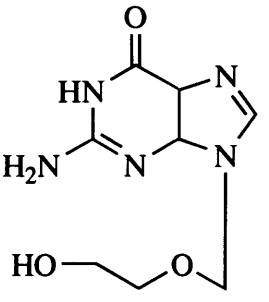
So far only biological data for the intermediate **44a** has been received and shown to have poor antiviral activity against VZV with and EC_{50} of 179 μM. The biological data for the other compounds are still awaited.

5.2 Amino Acid prodrugs

Following the success of Valaciclovir **(46)**, a prodrug of the anti-herpes nucleoside analogue acyclovir **(47),** we decided to investigate the synthesis of similar analogues of the BCNAs.



(46) Valaciclovir

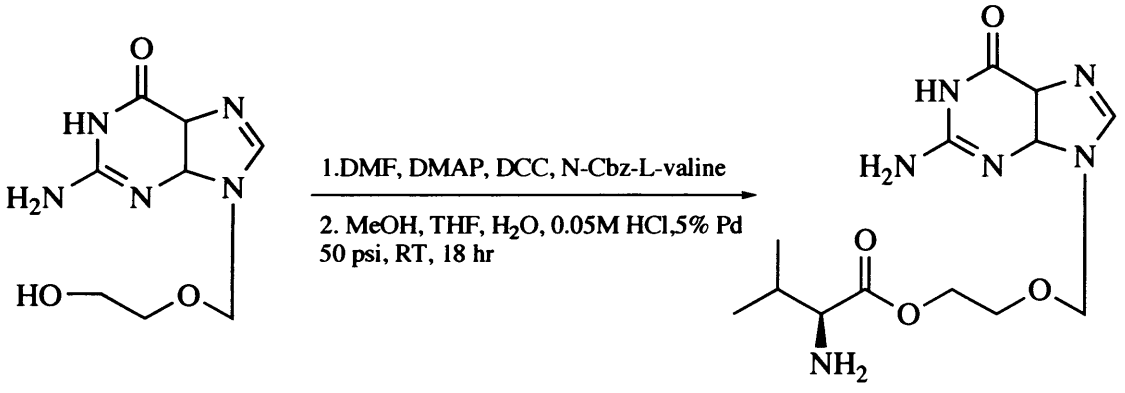


(47) Aciclovir

In an investigation for a prodrug of aciclovir, eighteen amino acid esters were synthesised. These were examined for their *in vitro* antiviral activity against HSV-1 and their ability to work as prodrugs evaluated by measuring the amount of aciclovir recovered in rats urine.[6]

They found that the L-amino acids were better prodrugs than either the D amino acids or a mixture of the D,L isomers with the L-valyl ester (valaciclovir) performing as the best prodrug. The administration of valaciclovir resulted in an increased bioavailability when compared to the administration of acyclovir alone. 54 % of the administered dose of valaciclovir was excreted as acyclovir compared to 12-20% after the administration of acyclovir. Amino acid prodrugs of penciclovir and ganciclovir have also been shown to increase the bioavailability when compared to the administration of the parent nucleoside analogues alone.[7,8]

Valciclovir is prepared following a two step synthesis starting from acyclovir (**Scheme 5.03**). Esterification between the 5'hydroxyl and N-protected valine is first performed in the presence of dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP). Deprotection using catalytic dehydrogenation in the presence of HCl gives the product as the hydrochloride salt.

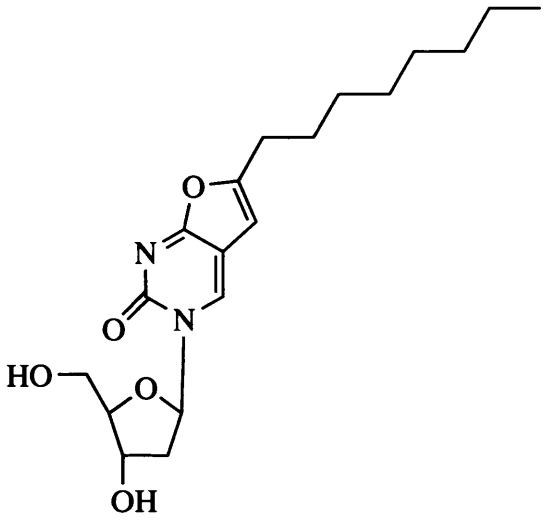


Scheme 5.03: Synthesis of valaciclovir

This method was therefore adopted in the attempt to prepare the amino acid prodrugs of the BCNA bearing an octyl side chain.

5.3 Synthesis

The starting bicyclic nucleoside bearing an octyl side chain was prepared using the established procedure starting with IDU[4] to give the desired analogue **(42)** (32%).



(42)

Esterification of the 5' hydroxyl group of **42** was the attempted using the above method with dicyclocarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP). After 24 hours, TLC analysis showed that starting material still remained and that three fluorescent products were being formed (**a b** and **c**). The reaction was recharged with DCC, DMAP and N-Cbz-L-valine and stirring continued for a further 6 days.

After this time TLC analysis showed still the strong presence of starting material (**Fig 5.01**). Separation of the products proved slightly more problematic than expected. Initial column chromatography was only successful in separating the lower running of the products (R_f 0.3= **a**).

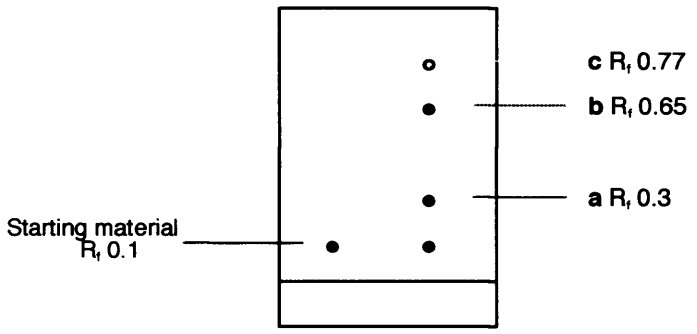
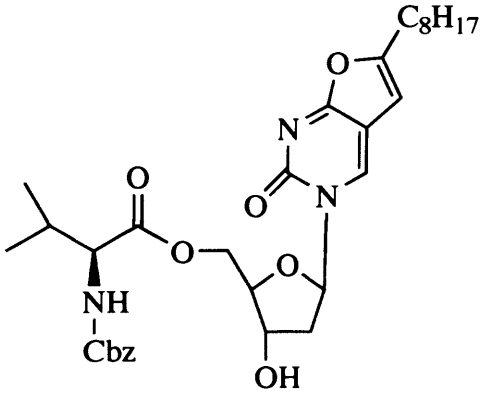


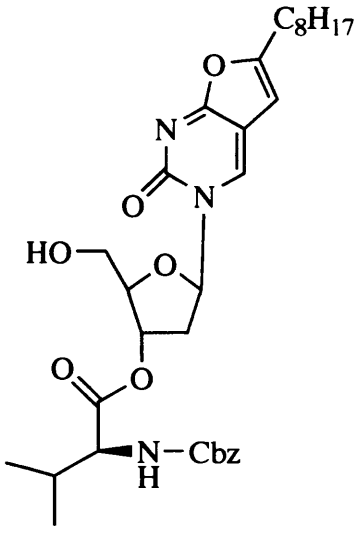
Fig 5.01: Diagram of the TLC observed

Further purification of the **b/c** mixture using a slower running eluent (60% ethylacetate in hexane) allowed separation of the two products with **c** being obtained as a foam. The product **b** was obtained with slight impurities of **c** and required further purification by preparative TLC.

Mass spectra showed that both products **a** and **b** possessed the required mass of the desired analogue. These are most likely therefore the desired 5'-ester and the 3'-amino acid substituted analogues (**48** and **49** respectively).

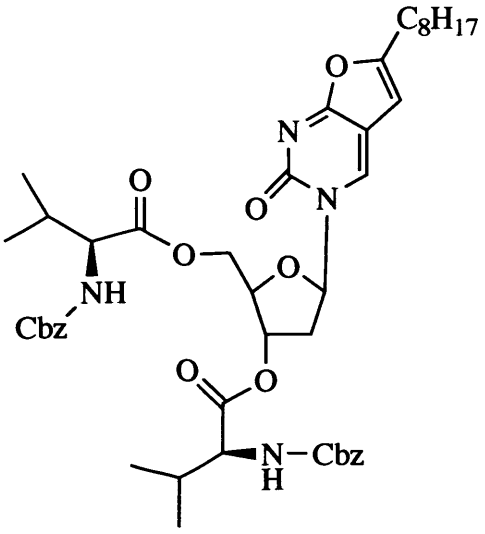


(48)



(49)

The final product **c** had a mass greater than these. The most likely third product is the 3',5'-disubstituted analogue **(50)**. The mass obtained for this product concurs with this to give a mass of 852.9 [MNa^+].



(50)

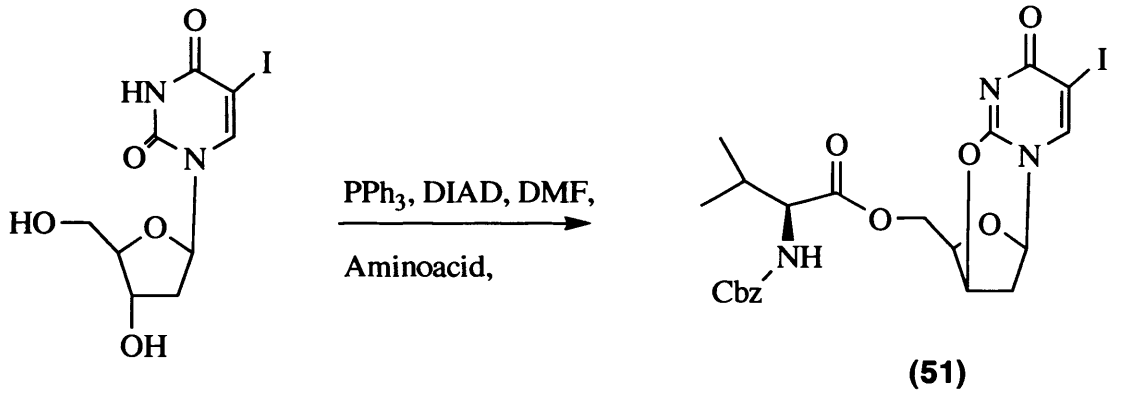
NMR analysis also confirmed these structures as those of the three products formed. Most notably by the disappearance of one or both of the hydroxyl groups.

Obtaining the final prodrug requires deprotection of the amino acid moiety. Therefore, analogue **48** was subjected to the hydrogenation conditions shown above. After shaking overnight, the mixture was filtered over celite. TLC analysis of the filtrate solution showed no starting material and instead one other fluorescent spot was observed. Concentration of the filtrate followed by trituration with acetone gave an off white powder. NMR analysis found no presence of sugar peaks and TLC analysis with the free base confirmed that this was the product formed.

With deprotection conditions of the carboxylbenzyl group too severe for the BCNAs we decided to attempt the synthesis of these analogues using an amino acid with an alternative protecting group. L-alanine in which the amine is protected with t-butoxycarbonyl was available within our laboratories and can be removed in acidic conditions.

Following our results using DCC and DMAP, which found the BCNA to be relatively unreactive we decided to attempt a Mitsunobu type esterification using triphenylphosphine and DIAD. However, even after 48 hours no reaction was observed by TLC. The 5' hydroxyl group of the BCNAs had also been found to be unreactive previously to tritylation, a protection reaction that usually proceeds without complications.

The Mitsunobu procedure has been successful with the nucleoside 5-fluoro-2'deoxyuridine[9] and we therefore attempted the reaction using IDU as the starting material (**Scheme 5.04**). After stirring overnight the presence of one main product was seen to have formed. Following purification by column chromatography the product was obtained in reasonable yield (43%). The NMR analysis however did not confer with the desired analogue. Instead investigation of the NMR suggested the formation of a 2-3'dihydro derivative **(51)**.



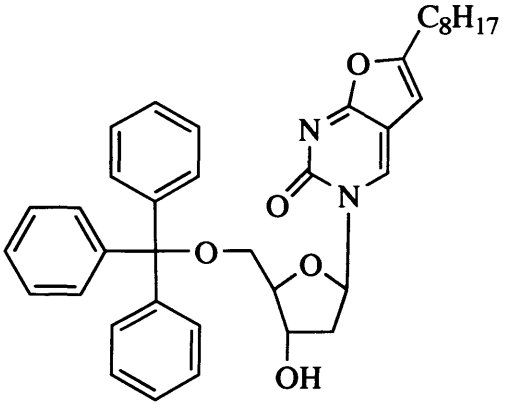
(51)

Scheme 5.04: Attempted synthesis using Mitsunobu reaction conditions.

More recently valinyl ester prodrugs β-L-2'deoxycytidine (LdC) have been prepared.[10] 3'-mono, 5'-mono and 3',5'-disubstituted amino acid ester prodrugs of this nucleoside were prepared using multiple additions of DCC and DMAP. To achieve the mono substituted analogues selective protection of the other hydroxyl group was first required.

This study also found that of the three analogues synthesised: the 3'-mono ester, the 5'-mono ester and the 3',5-'diester, the 3'-mono had the best pharmacokinetic profile with an oral bioavailability of 84.6%.

Following this and with the 5' hydroxyl protected IDU already available from previous synthesis we attempted the synthesis of the 3'amino acid ester prodrug. First 5'-trityl-IDU was coupled and cyclised with 1-decyne to give the 5'protected BCNA **(52)** bearing an octyl side chain in good yield (51 %).



(52)

This was then reacted with the amino acid in the presence of DCC and DMAP and stirred overnight. Additional DCC, DMAP and amino acid was added after this time as starting material was seen to remain and the reaction mixture stirred for a further 48 hours. After which time no starting material was seen by TLC.

Column chromatography over a smaller amount of silica was used to obtain the main fluorescent spot as an oil with minor impurities. This was dissolved in methanol without analysis and excess amberlite added. It was hoped that the amberlite, which is used to deprotect the trityl group may also be sufficiently acidic to also remove the t-butoxy protecting group of the amino acid. However, in this case it also proved difficult to deprotect the 5' trityl and a mixture of a small amount of 5' deprotected as well as 5' protected was obtained.

Deprotection of both the trityl and t-boc are usually carried out in acidic conditions. However when this was attempted using 5% and 1 % trifluoroacetic acid in dichloromethane or 5 % HCl in dichloromethane the result was cleavage of the bicyclic base.

There are numerous other esterification methods available that could be investigated, but unfortunately time constraints meant that this investigation was not continued further.

[1] G.M. Luoni; C. McGuigan; G. Andrei; R. Snoeck; E. De Clercq and J. Balzarini. Bicyclic nucleoside inhibitors of Varicella-zoster virus modified on the sugar moiety: 3' and 5' derivatives. *Antiviral. Chem. Chemother.* **2004**, 15, 333-341.

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[3] R.N. Pathirana. *Internal Report.* Cardiff University. **1999**.

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[5] C. McGuigan, H. Baruki, S. Blewett, A. Carangio, J. T. Erichsen, G. Andrei, R. Snoeck, E. De Clercq and J. Balzarini. Highly potent and selective inhibition of varicella-zoster virus by bicyclic furopyrimidine nucleosides bearing an aryl side chain. *J. Med. Chem.* **2000,** 43:4993-4997.

[6] L.M. Beauchamp; G.F. Orr; P. De Miranda; T. Burnette; and T.A. Krenitsky. Amino acid ester prodrugs of acyclovir. *Antiviral. Chem. Chemother.* **1992**, 3, 157-164.

[7] DK. Kim; N. Lee; YW. Kim; K. Chang; GJ. Im; WS Choi and K. H. Kim. Synthesis and evaluation of amino acid esters of 6-deoxypenciclovir as potential prodrugs of penciclovir. *Bioorg. Med. Chem.* **1999**, 7, 419-424.

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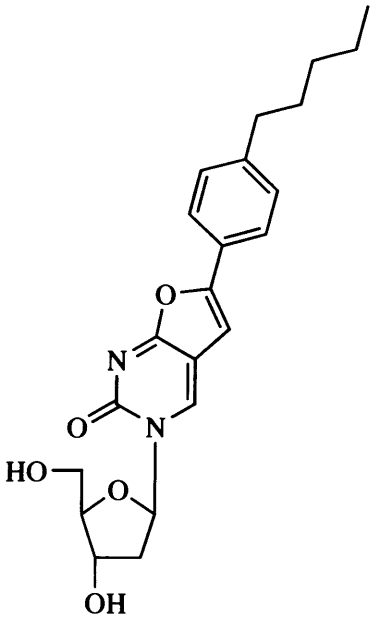
[10] S. Benzaria; C. Pierra; D. Bardior; E. Creeton-Scott; E. G. bridges; X.-J. Zhou; D. Standring and G. Gosselin. Monoval-Ldc: Efficient prodrug of 2'-deoxy-β-L-cytidine (L-dc), a potent and selective anti-HBV agent. *Nucleosides. Nucleotides.* **2003**, 22, 1003-1006.

Chapter 6: PH stability study

The BCNAs have been shown to be susceptible to base cleavage under certain basic and acidic conditions.[1,2] If they are to be successful as an oral antiviral, they need to be stable under the range physiological pH. Subjected to the acid conditions of the stomach (pH 1-3) they then pass through the small and large intestines where they can experience more basic pH (5-8).[3]

To determine this stability, we commenced a study, placing the lead BCNA to date, with a pentylphenyl side chain **[Cf1743]** in varying pH aqueous solutions. Hydrion™ Buffer salts were obtained from Aldrich© and prepared according to instructions. For a full investigation a range of pH from 2 to 11 were used.



[Cf1743]

A suspension of Cf1743 (approx 5 mg) in the appropriate pH solution (25 mL) was stirred at 37°C for 48 hr. The stability of the compound was determined by TLC analysis at 2 hr, 24 hr and 48 hr intervals. Degradation of the BCNA

causing base cleavage would result in the observance of a more polar spot on the TLC plate. (see **Table 6.01)**

Table 6.01: Stability study results

pH	2 hr	24 hr	36 hr
2[a]	X	X	X
4[a]	X	X	X
7[b]	X	X	X
9[c]	X	X	X
11[d]	X	X	X

X no degradation
√ degradation

Buffer salts : a. potassium biphthalate, b. sodium phosphate dibasic, c. sodium bicarbonate, d. sodium phosphate tribasic.

As can be seen in the above table no base cleavage was observed at the complete range of pH used, pH 2, 4, 7, 9 and 11.

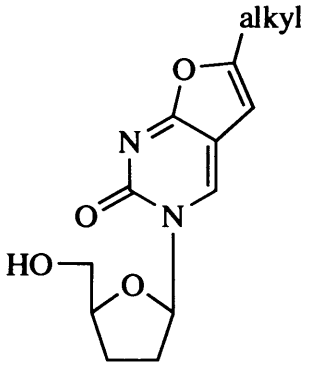
Chapter 7: Non-nucleoside bicyclic analogues

7.0 Introduction

Following the discovery of the BCNAs as potent and selective inhibitors of VZV,[1] our research group conducted an extensive investigation into the structure activity relationships of these compounds. As stated in chapter 2 three main sites were identified and modified. These are: the bicyclic base, the side chain and the sugar.

All alterations to the sugar moiety, which included substitutions of the hydroxyl groups with a halogen,[2,3] and replacement of the 2'-deoxy sugar with alternative sugars resulted in a reduction or loss of antiviral activity against VZV.

However the 2'3'-dideoxy analogue **(53)** whilst losing anti-viral activity against VZV expressed μM antiviral activity against another herpes virus, cytomegalovirus (CMV). These compounds were also found to be inactive against the other herpes viruses HSV-1 and HSV-2.[4]



53

Cpd	Chain length	HCMV EC_{50} (μM)
Cf1815	C_8H_{17}	1.5
Cf2004	C_9H_{19}	1.2
Cf1821	$C_{10}H_{21}$	2.6

7.1 Structure Activity Relationships

After this discovery, a series of modifications to this new series of analogues was started. With the success of the *p*-alkylphenyl BCNAs and their enhancement of antiviral activity against VZV a series of 2'3'-dideoxy analogues bearing a *p*-alkylphenyl side chain were synthesised and tested for their antiviral activity.

However in contrast to the parent BCNAs against VZV, this substitution of the side chain did not result in an enhancement of activity of these analogues against CMV. Also, unlike the alkyl 2'3'-dideoxy analogues, which were inactive against VZV, this new series was found to retain μM activity against VZV.[5]

With the lack of activity seen with the p-alkylphenyl dideoxy analogues the investigation turned back to investigating the SAR and possible mechanism of action of the alkyl analogues against CMV.

Studies conducted by the Rega Institute for medical research (Leuven Belgium) found that this new series of compounds behaved unlike other classical nucleoside antivirals, which inhibit DNA polymerase. Time of addition experiments showed that these compounds seem to be acting at an earlier stage of the viral cell cycle. This is similar to information reported on the mechanism of action of previous non-nucleoside inhibitors of CMV.[6,7]

With this new series of compounds apparently acting as non-nucleosides we investigated their need to retain a nucleoside structure. Retaining the bicyclic base and alkyl side chain, a new series of analogues were synthesised in which the sugar moiety was replaced by an alkyl chain, tetrahydrofuran, isopentyl or cyclopentyl groups (**Fig 7.01**).[5,8,9,10]

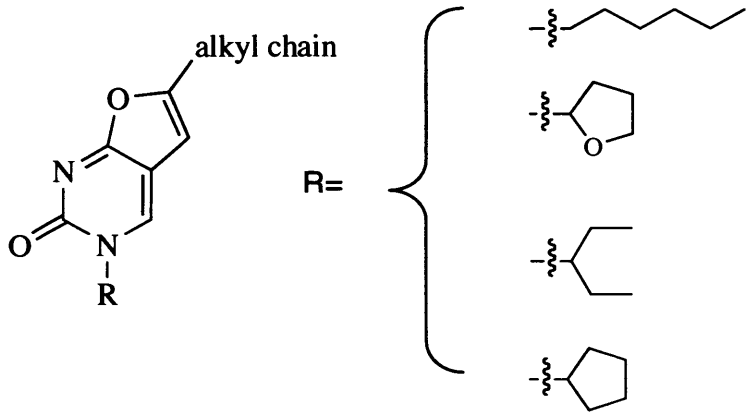


Fig 7.01: Potential anti-CMV compounds synthesised

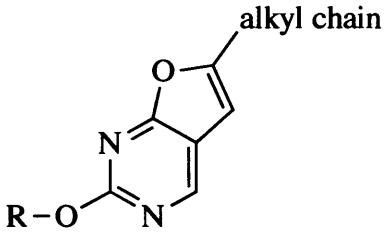
The need for a long alkyl side chain, which has been seen with the VZV BCNAs was also investigated by synthesising the above analogues with differing chain lengths of 4, 6, 7 and 10 carbons.

None of the analogues showed an improved activity against CMV compared tp the 2'3'-dideoxy lead analogue. In fact, most showed a complete lack of antiviral activity. The cyclopentyl analogue bearing a heptyl side chain however retained μM antiviral activity against CMV (5.0 μM).

The results also showed preference for a heptyl side chain with those analogues bearing shorter or longer side chains not tolerated. However the results seen with the analogues bearing a decyl side chain could be as a result of their poor solubility. In some cases, antiviral data could not be obtained due to the insolubility of the analogues.[8]

The synthesis of these analogues also results in substitution at the 2 carbonyl position of the bicyclic base **(54)**. These analogues were also isolated and tested for their anti-CMV ability. These analogues, like their N-alkylated

counterparts were found to be inactive except for the derivative bearing a cyclopentyl moiety. This analogue was surprisingly found to be as active (3.0 μM) as its N-alkylated partner indicating that these new analogues do not need to strictly adhere to a nucleoside structure in order to have antiviral activity against CMV.



54

7.2 Synthesis

The free bicyclic base is first prepared using the standard one pot procedure as used for the coupling and cyclisation of the BCNAs[1]. 5-iodouracil was coupled and cyclised with the appropriate terminal alkyne in the presence of CuI and a catalytic amount of Pd(0) to give the desired free bicyclic base **(Fig. 7.02)**.

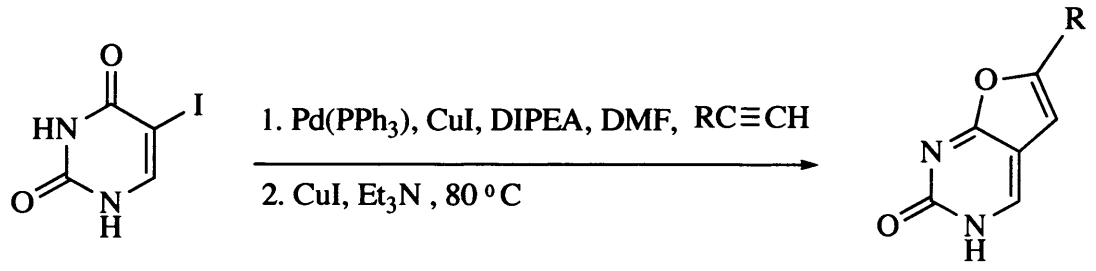


Fig 7.02: Synthesis of the free base

The free base is extremely insoluble which makes obtaining the pure compound by column chromatography difficult. However it is obtained with reasonable purity for the next synthetic step by trituration and filtration with methanol.

The free bases are then reacted with the appropriate alkyl halide in the presence of potassium carbonate in DMF at 80 °C to give the desired N-alkylated analogues. As stated above this reaction also results in the formation of the O-alkylated analogues (**Fig 7.03**).

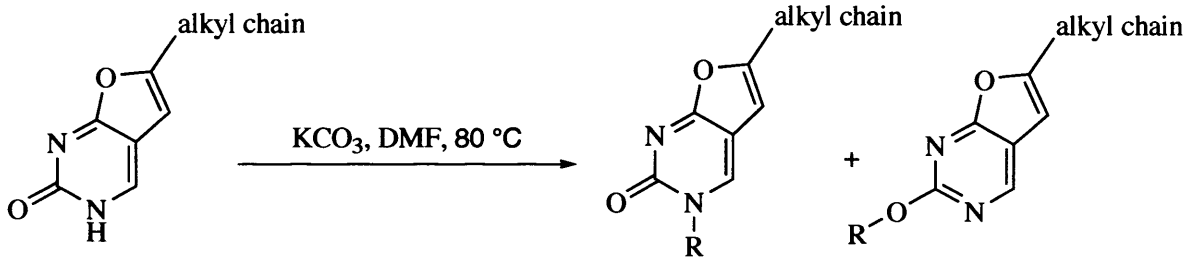
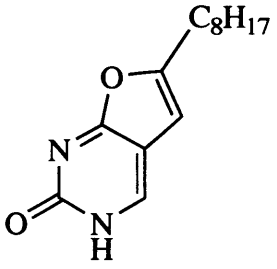


Fig 7.03: Substitution of the free base

7.3 Aim

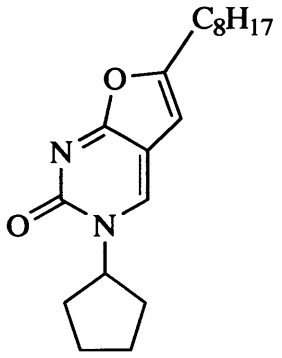
As shown above, all investigations to date have targeted the side chain or substitution on the base. Therefore we now report an investigation into the SAR of the bicyclic base moiety and replacement of the *furano* oxygen with nitrogen to give the *pyrrolo* analogues.

The free base bearing an octyl side chain was synthesised as above starting from 5-iodouracil and decyne to give the desired analogue **(55)** in reasonable yield (68%).

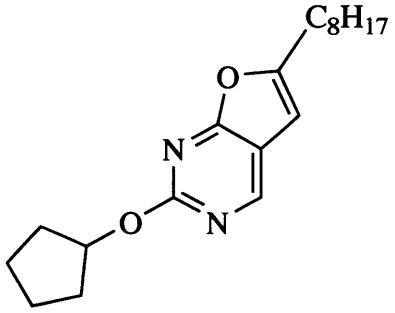


(55)

The free base **55** was substituted with cyclopentyl bromide to give the N-alkylated **(56)** and O-alkylated **(57)** analogues in 16% and 83% yields respectively.



(56)



(57)

It was hoped that by employing the same method used for the synthesis of the *pyrrolo* analogues of the BCNAs,[11] a number of 3-N and 2-O analogues with various alkyl substituted amines in the bicyclic base could be synthesised (**Fig 7.04**). This requires the desired derivatives **56** and **57** being heated in a sealed tube containing the desired amine and methanol.

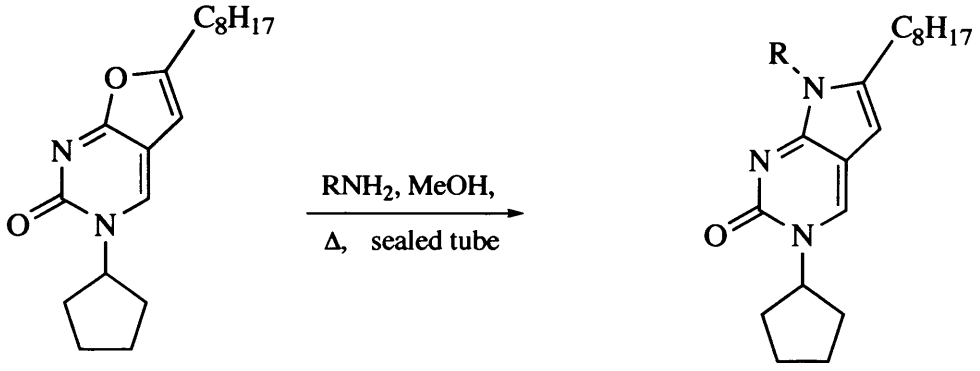
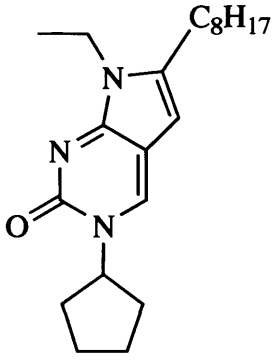


Fig 7.04: Example of *pyrollo synthesis*

The analogues **56** and **57** were first suspended in a solution of ammonia (33%) in methanol (1:1) and heated at 50 °C overnight in a sealed tube. However in both cases no reaction was seen. The reaction was continued stirring at 50 °C for a further 24 hours. Still no reaction was seen observed.

The reaction was also attempted using methylamine (40%) instead of ammonia. Again no reaction was seen with either of the analogues.

The analogues **56** and **57**, were also placed in a solution of ethylamine (70%) and methanol and stirred in a sealed tube at 60 °C for 24 hours. After this time while no reaction was observed for **57,** no starting material was seen remaining by TLC for the **56** derivative and one other fluorescent spot was observed The work up of this reaction was continued and after purification the desired analogue (**58)** was obtained (47%).



58

From this it was deduced that mechanistic properties might prevent the O-alkylated analogues from being prepared using this procedure. Therefore further reactions focused only on the N-alkylated analogue **56**.

Following the success of obtaining the ethyl substituted *pyrrolo* analogue, synthesis of the unsubstitued analogue was also attempted. In this case a greater volume of ammonia (7 mL) to methanol (3 mL) was used and the reaction stirred at 80 °C for 24 hr. After this time a faint fluorescent spot was observed by TLC and stirring continued for approximately a further 72 hours. Still no further conversion of the starting material was observed after this time and possibly due to the low quantity of product formed no product was obtained.

7.4 NMR

Both the ^{1}H NMR and the ^{13}C NMR of the product **58** obtained showed a clear difference with the NMR obtained of the starting materials. From initial observance of the ^{1}H NMR an additional peak at *ca.* 4.0 ppm can be observed which on further investigation was shown to be the CH_2 group of the ethyl moiety, more downfield due to the effect of the nitrogen atom.

The effect of the substitution on the bicyclic system in the ^{13}C NMR can also clearly be seen. On first observations the C-7a carbon, which is usually found at *ca.* 171 ppm for both the BCNAs and CMV *furo* bicyclic analogues is not observed in the carbon spectra for this analogue.

From this effect of the nitrogen, it was not possible to fully characterise the carbon spectra. Therefore a heteronuclear multi-bond correlation (HMBC) spectra was obtained and analysed (**Fig 7.05**).

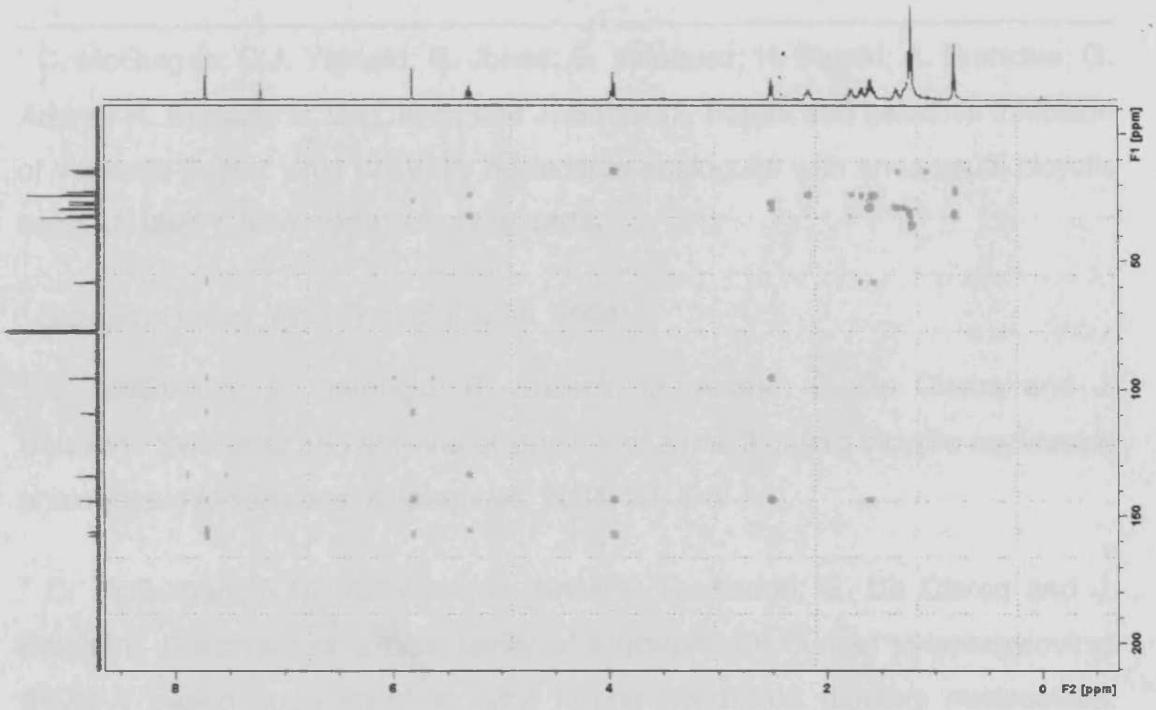


Fig 7.05: HMBC of **58**

This shows that that the nitrogen has indeed affected the chemical shift of the two adjacent carbons (C-7a and C-6), which are found more upfield in the carbon spectra. The C-7a peak was shifted from 171 ppm to 158 ppm while the peak corresponding to the C-6 carbon was found at 144 ppm instead of the usual 156 ppm.

7.5 Summary

The attempted synthesis of *pyrrolo* analogues of this new class of anti-CMV nonnucleosides has resulted in the formation of one analogue bearing an ethyl substituted amine. This has been sent for antiviral evaluation to determine is ability to inhibit the replication of CMV and the results are awaited.

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[10] H. Weldon. *Internal Report.* Cardiff University. **2002.**

[11] C. McGuigan; R.N. Pathirana; G. Jones; G. Andrei; R. Snoeck; E. De Clercq and J. Balzarini. Anti-varicella-zoster virus bicyclic nucleosides: replacement of furo by pyrro base reduces antiviral potency. *Antiviral. Chem. Chemother.* **2000,** 11, 343-348.

Chapter 8: Molecular modelling studies.

8.0 Introduction

Until recently the research and development of successful new drug treatments has relied in some part on luck. Discovery of natural products as efficacious compounds has led to the biological screening of a huge variety of more natural resources in the search for more potential biologically active compounds.

The development of potentially new active compounds where no information on the drug active site is known resulted in investigations into the structure activity relationship (SARs) of compounds. This method is one of the earliest approaches to structure based design of new potential compounds, and relies upon finding a correlation between the physiochemical properties of a compound and its activity. Graphic representation of the physical properties versus activity predicts the general features needed for activity. This allows the medicinal chemist to rationally design further modifications in their attempt to optimize a drug. However this process can be extremely time consuming requiring the synthesis of various analogues and awaiting their biological analysis.

Using modern techniques it can now be possible to isolate the active site of a drug and an increasing number have had their structure determined by x-ray crystallography or NMR.

Using molecular modeling it is possible to investigate how a drug or biological substrate interacts in the active site. Having the structure of the active site known, also allows for the virtual screening of chemical structures of a known or designed database. This is much quicker than the traditional biological screening which require the time of assays to be performed. By docking these structures with the active site it is possible to determine structures that could

potentially be more active by possessing a higher affinity with the target enzyme than the natural ligand.

The docking of a compound into the active site of an enzyme requires finding the favourable conformation of the molecule by taking into account the interactions calculated between the ligand and its host. This can be expressed physically by determination of an energy minimum of the ligand-enzyme complex.

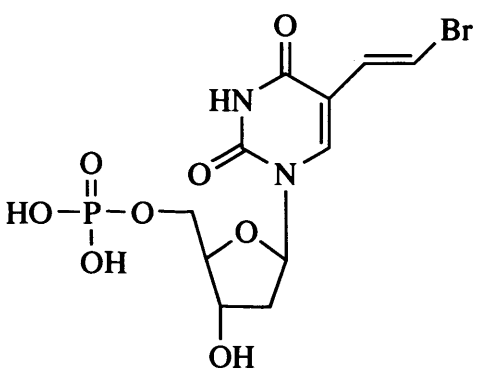
8.1 Aim

As shown earlier a new class of nucleoside analogues bearing an unusual bicyclic base and long side chain have been found to be potent and selective antiviral agents against VZV.[1]

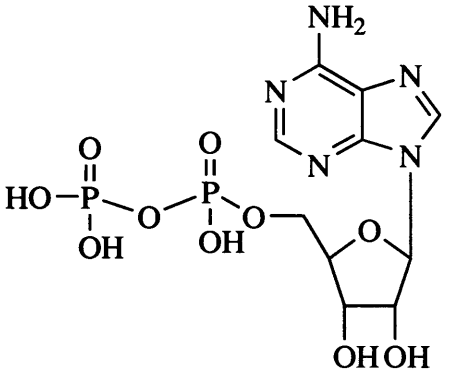
This selectivity has been shown to involve the viral encoded enzyme, thymidine kinase (TK). This enzyme is responsible for the initial phosphorylation step to give the monophosphate, during activation of a nucleoside into its active phosphate forms.

Only VZV encoded TK can successfully phosphorylate the BCNAs. It has been shown that in this case the TK is responsible for phosphorylation of the BCNAs to both the mono and di phosphate forms. Neither HSV-1 TK shown to be structurally similar to VZV TK or cellular TK are able to perform this function, which suggests that this enzyme plays a role in the selectivity of these compounds[2]. However, care must be taken when interpreting these results when compared to antiviral activity as the activity of these compounds is obtained through antiviral assays in tissue culture, which means a series of steps are being assessed.

Recently, the crystal structure of VZV TK complexed with BVDU monophosphate (BVDU-MP) and ADP has been solved and made available on the protein data bank (PDB accession number 10SN).[3,4] It was therefore of interest to investigate the interaction of the BCNAs with this enzyme using molecular modelling.



BVDU-MP



ADP

8.2 Molecular modelling

In order to view, analyse and perform computational experiments with a known active site, a number of software packages are available. We have used MOE™, (molecular operating environment).[5]

The crystal structure of VZV TK was isolated as two dimers bonded by a sulfur bridge to give a tetramer structure containing four BVDU and four ADP ligands. This structure as obtained from the protein data bank is shown below (Fig 8.01).

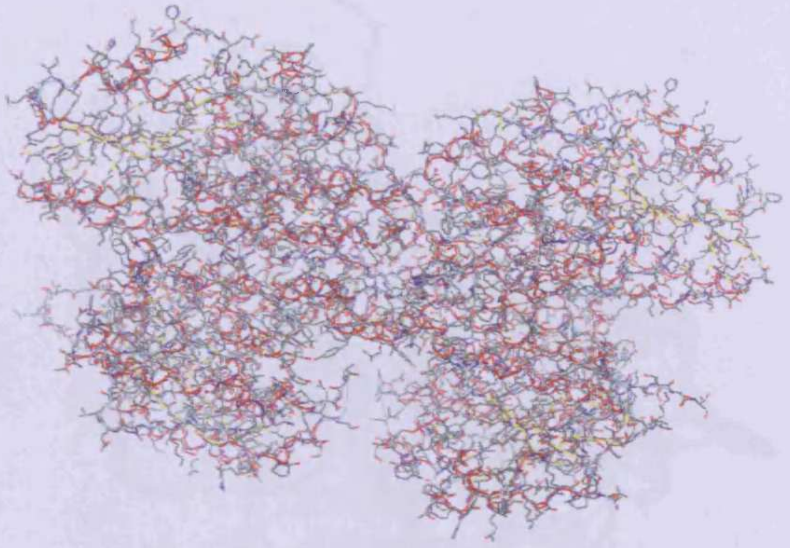


Fig 8.01: Crystal structure of VZV-TK – shows tetramer formation through crystallisation

Analysis of the structure of VZV-TK and comparison with HSV-TK found that HSV-1-TK and VZV-TK are structurally extremely similar.[3] This similarity is shown below (**Fig. 8.02**) which, shows the backbone of VZV-TK in green ribbon format superimposed onto the backbone of HSV-1 TK (accession number 1EK2) shown in red ribbon format.

However the structure of the active site receptor of these two enzymes has been analysed in more depth by L.E. Bird et al and shown to have significant difference which would affect the binding of substrates.

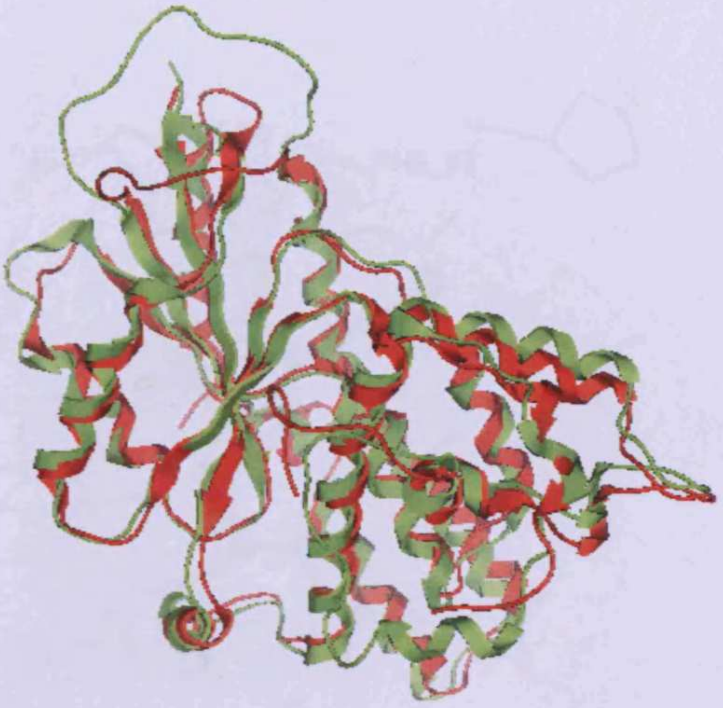


Fig. 8.02 –Figure showing structural similarity of VZV-TK with HSV-1 TK.

The active site of the TK enzymes can be seen as containing two main binding domains: the ADP binding site and the pyrimidine receptor site. In VZV TK, the adenine base is found sandwiched between the two residues Arg-183 and Pro-302 and hydrogen bonds are formed between the N-6 of adenine and the residue Gln 300 (**Fig 8.03**).

The BVDU ligand is found in the pyrimidine binding pocket in its monophosphate form showing that a single turnover of the enzyme has already occurred. The carbonyl oxygen at position 4 and the N-3 of the BVDU form hydrogen bonds with the Gln 90. The pyrimidine base of the nucleoside is stacked between two the two aromatic residues Phe-93 and Phe-139 (**Fig 8.04**) forming van der Waals interactions (not shown). Additional van der Waals contacts are seen with Ile-62.

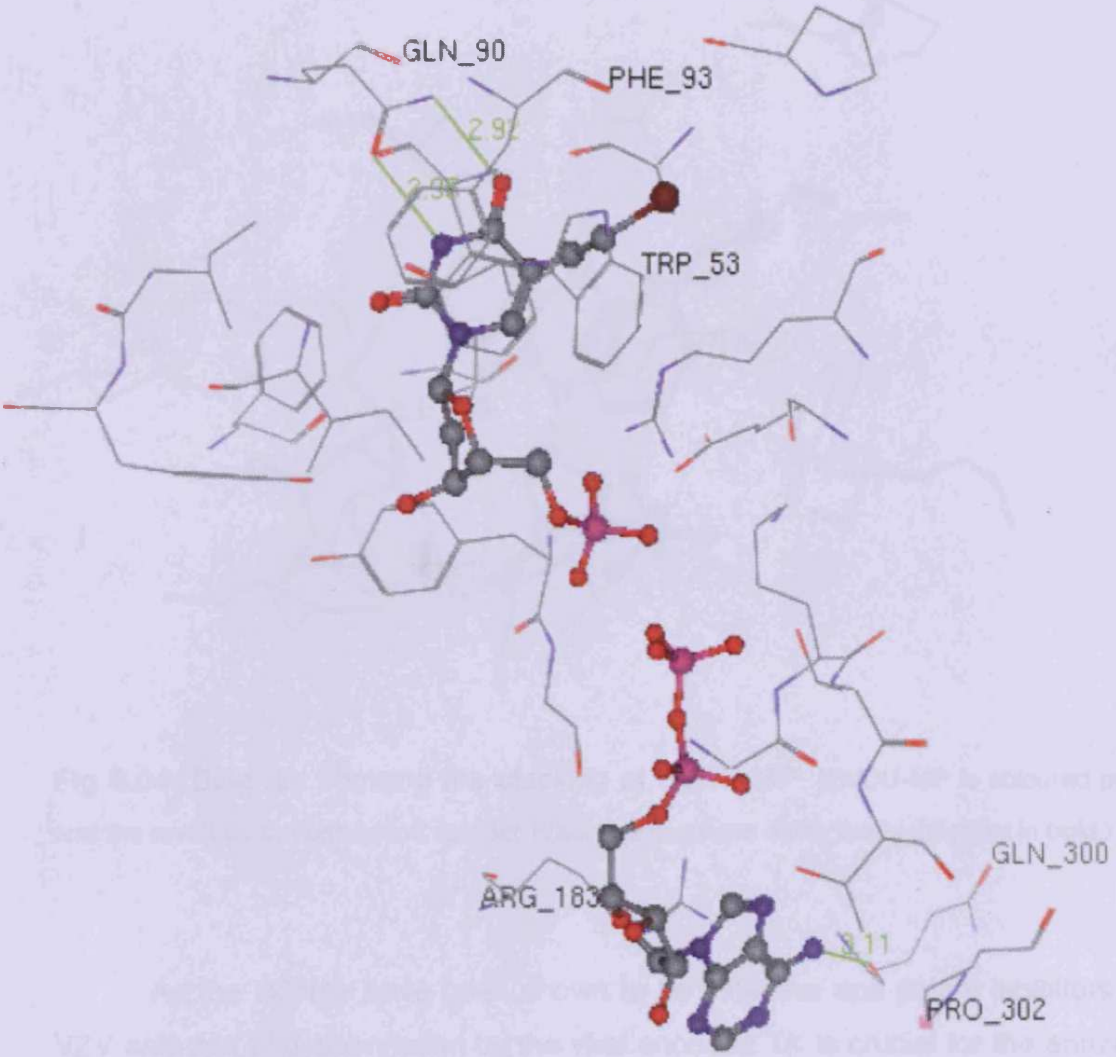


Fig 8.03: Diagram showing binding of BVDU-MP and ATP in the active site of VZV-TK

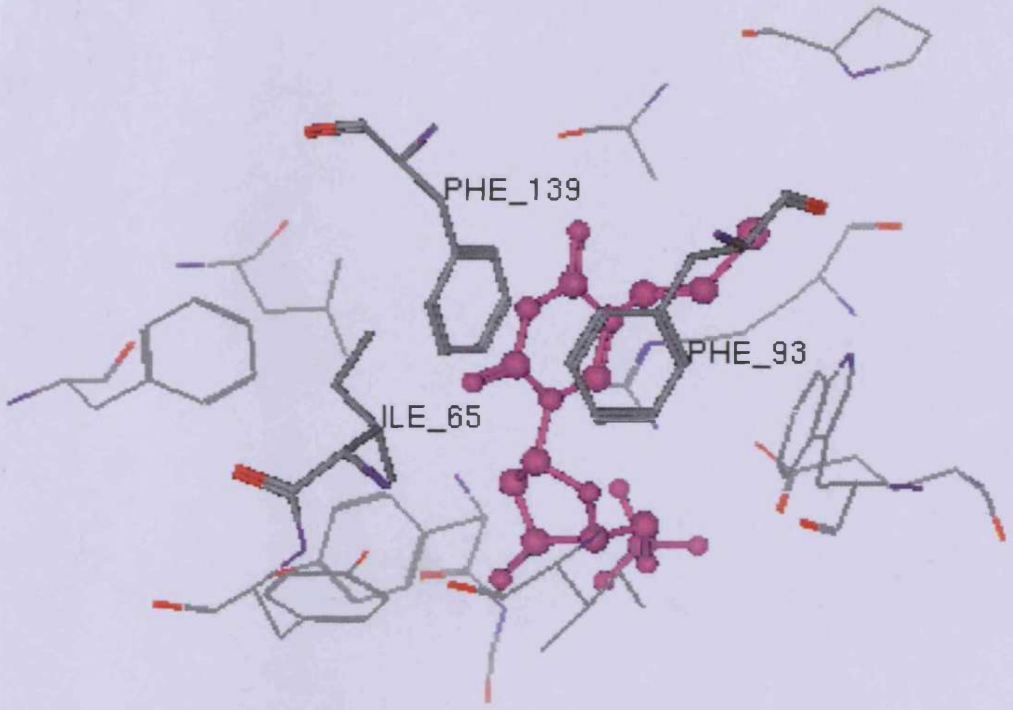


Fig 8.04: Diagram showing the stacking of BVDU-MP. (BVDU-MP is coloured pink and the residues through which van der Waals contacts are made are highlighted in bold.)

As the BCNAs have been shown to be selective and potent inhibitors of VZV and that phosphorylation by the viral encoded TK is crucial for the antiviral activity of these compounds, we were interested in investigating how the BCNAs may interact with this enzyme.

A database of 28 compounds was constructed from the BCNAs synthesised to date, covering a range of modifications (**Fig. 8.05**). These were then all preliminarily docked with the receptor.

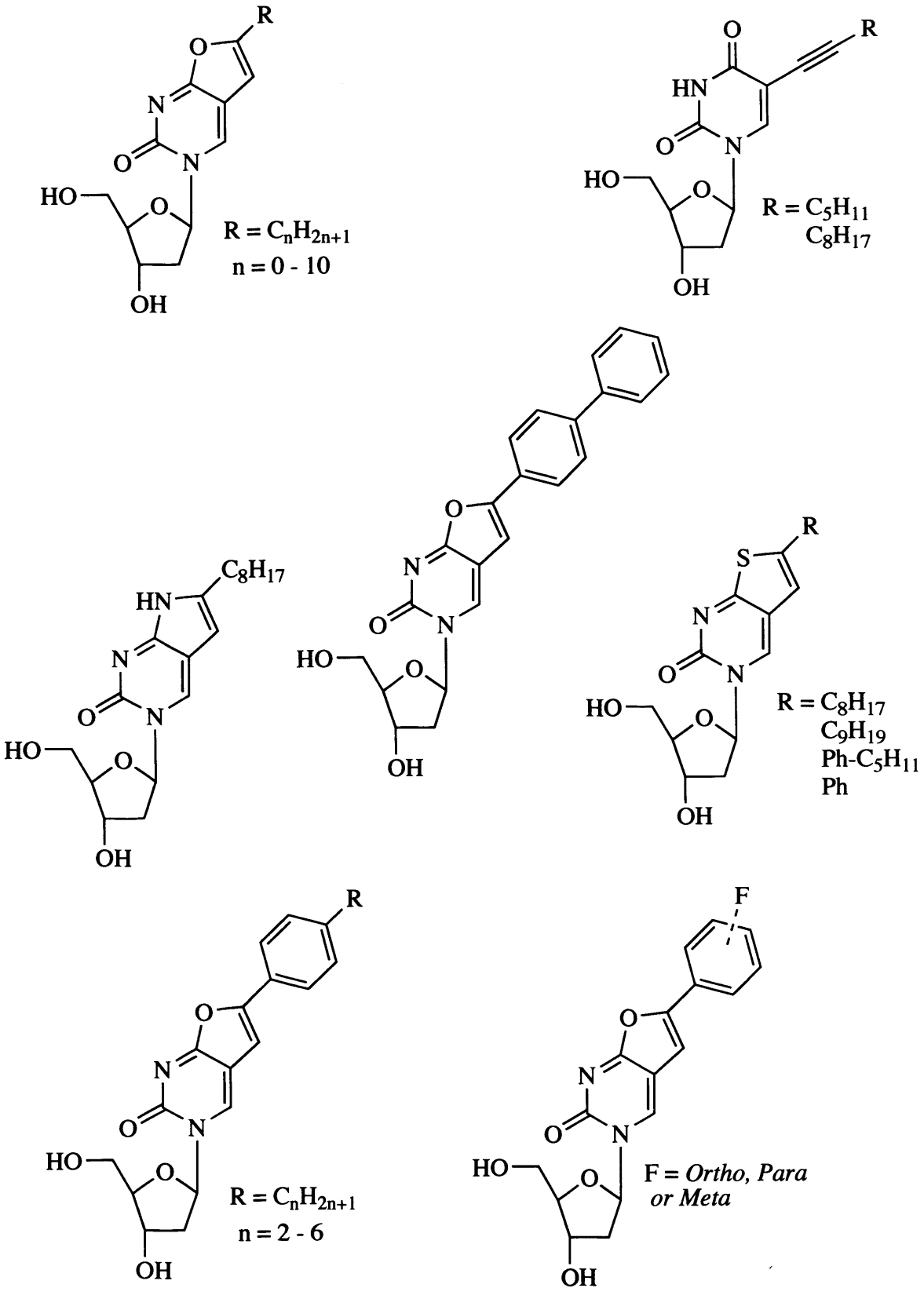


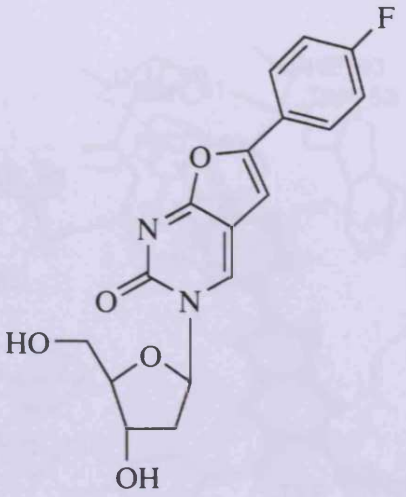
Fig 8.05: Database of compounds chosen for initial dockings.

Molecular docking is used to predict the structure of the complex formed between the ligand and the receptor. It uses algorithms to generate a number of possible structures and a means to score them so the user can identify those of most interest. The algorithm used with MOE-dock is simulated annealing which conducts searches within a specified 3D box and seeks to optimize the spatial and electrostatic interactions between the ligand and the protein by gradually reducing the allowed movement of the ligand. The disadvantage of this type of docking is that the result depends on the initial placement of the ligand.

Before docking, it is necessary to first prepare the structure of the protein and define the active site. The suitable file was downloaded from the protein databank (accession number 10SN), a single protein chain selected (one enzyme) and the BVDU-MP and ADP plus any water molecules removed. The backbone of the enzyme was then fixed and hydrogen atoms were added and minimized. The structures of the compounds to be docked were minimized and the active docking site selected and the docking of the compounds began.

Our initial dockings of the database produced a minimal five conformation results for each of the analogues. These were assessed visually to see which were most suitable, by analysis of their position in the pyrimidine pocket and of the sugar in comparison to where ATP would approach.

Of the 28 compounds one of the most suitable candidates for further analysis was the *para*-fluoro analogue **38**. Of the analogues chosen for analysis this compound has a more rigid structure and lacks the side chain, which due to its size and steric bulk would make their docking more difficult. This can explain why this structure has docked more successfully. This analogue although not an active antiviral, has also been shown to be the best inhibitor of VZV-TK of all the BCNAs.



38

A more thorough docking was then completed using this analogue alone and over 100 conformations of possible ligand-protein complexes were obtained. These were ranked according to those most favourable, with those conformations requiring the least energy ranked the highest.

The two top conformations are shown below along with the residues that are most likely to produce interactions (**Fig. 8.06a and Fig 8.06b**).

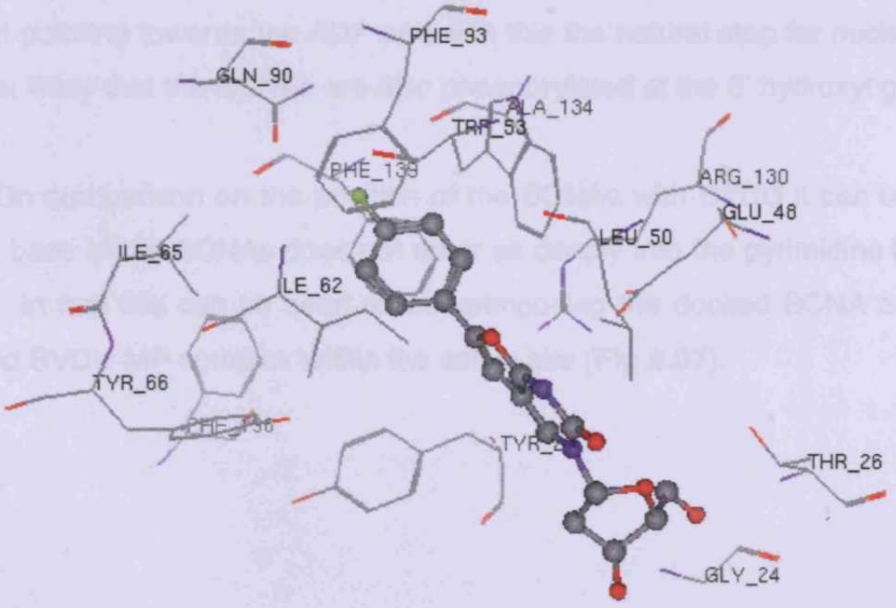


Fig 8.06a : Docking of 38 with VZV-TK

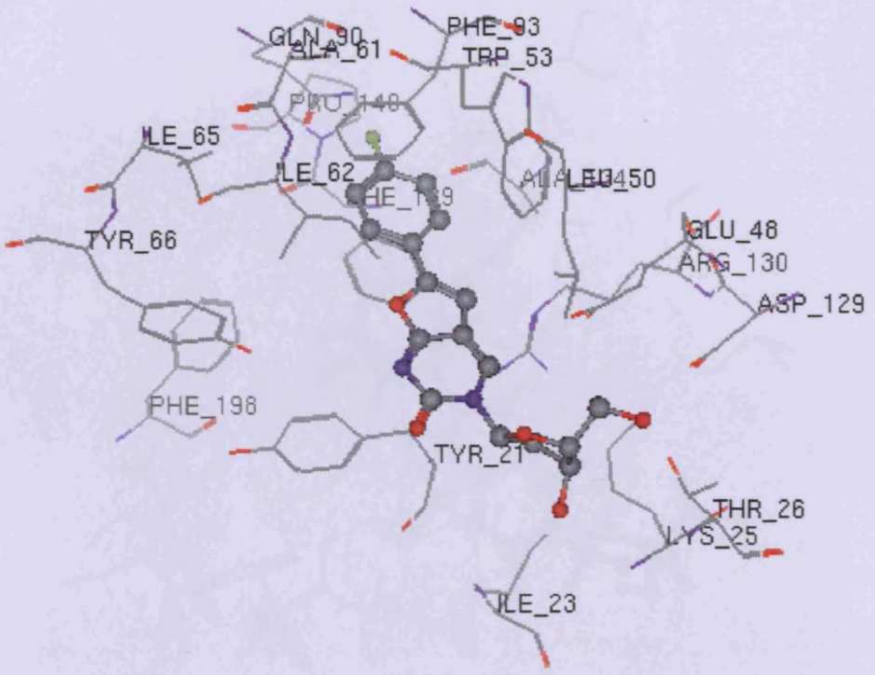


Fig. 8.06b: - Diagram showing **38** docked with VZV-TK

As can be seen in the diagrams the sugar can adopt different conformations. This can result in one conformation having the 3' hydroxyl facing towards the ATP binding site and favourable for phosphorylation. However as the majority of results from the other dockings result with the expected 5' hydroxyl pointing towards the ADP and with this the natural step for nucleosides it is most likely that the BCNAs are also phosporylated at the 5' hydroxyl group.

On comparison on the position of the BCNAs with BVDU it can be seen that the base of the BCNAs does not enter as deeply into the pyrimidine binding pocket. In fact this can be seen on superimposing the docked BCNA onto the identified BVDU-MP complex within the active site (**Fig 8.07**).

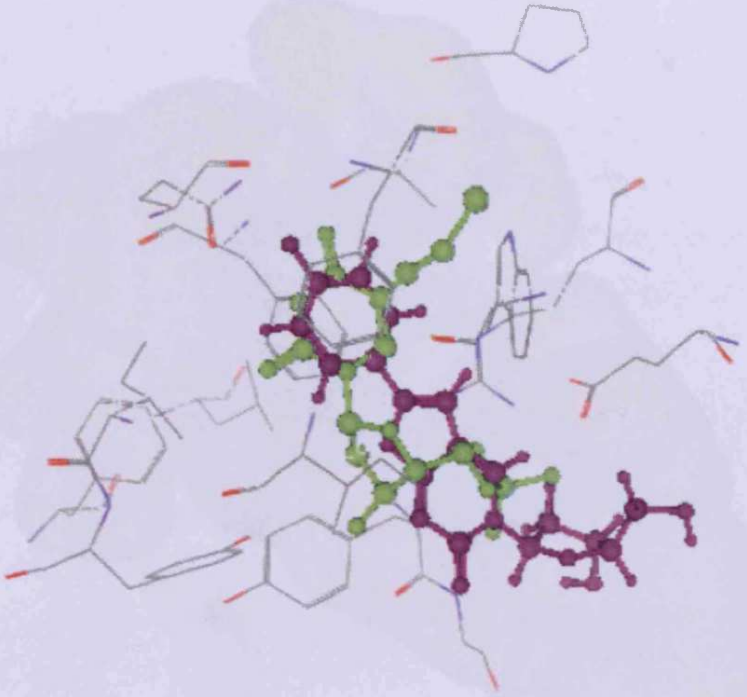


Fig. 8.07: Diagram showing position of BCNA compared to that of the isolated BVDU-MP. (BVDU in green and BCNA in purple)

In fact the phenyl ring of the side chain is found stacked between the two residues Phe_139 and Phe_93, which are usually seen to form van der waals contacts with the pyrimidine base of BVDU-MP.

A molecular surface diagram of the receptor allows a view of the space available for a potential ligand to occupy and what contacts are most likely to occur (hydrophilic or hydrophobic). A 3D molecular structure of this into which BVDU-MP has been placed is shown below to illustrate this. This 3D image is formed of three colours. Red represents exposed protein areas, blue and green show hydrophilic and hydrophobic areas respectively.

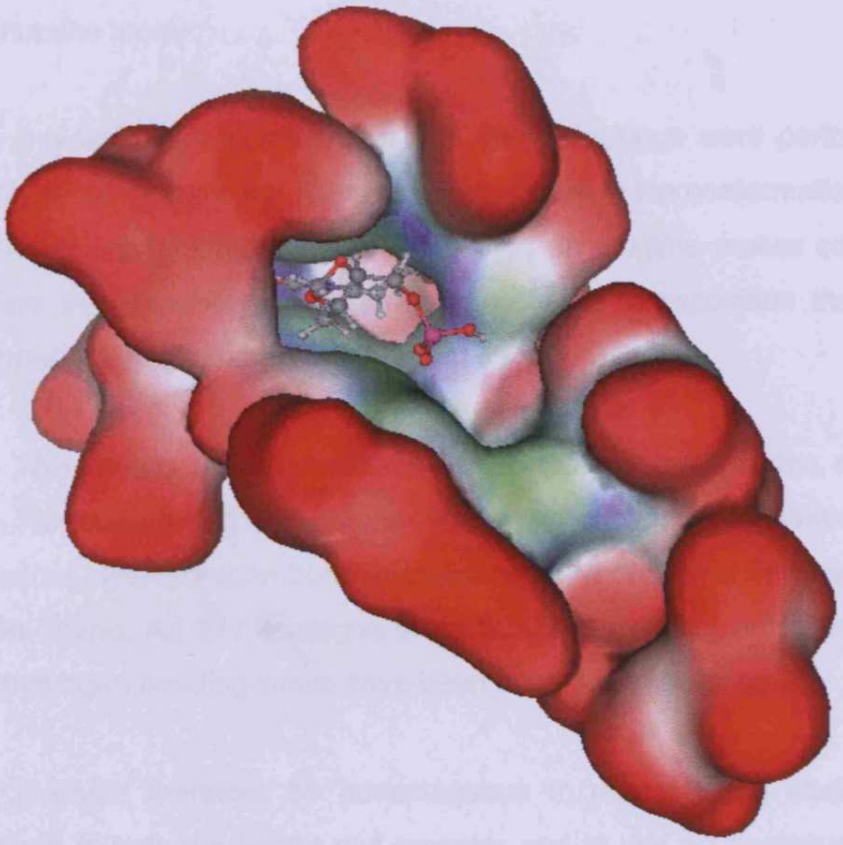


Fig. 8.08: Molecular surface of the receptor site of VZV-TK containing the ligand BVDU-MP

As Fig 8.08 is representative of the receptor and not the enzyme as a whole it is important to note that these colourings can be used only as a guide. The red areas in this case will not all be exposed proteins as most will be surrounded by the rest of the protein residues. However it is still a useful tool in analysing the receptor site.

The vinylic bromine side chain of BVDU is found to protude into a side pocket within the pyrimidine receptor and it can be seen that this pocket is just big enough to accommodate the BVDU ligand. This space is not big enough to accommodate the alkyl side chain of the BCNAs. In fact, the phenyl ring of the side chain of the docked BCNA is in the pyrimidine pocket (isolated from the crystal structure following the first phosphorylation of BVDU) as far as is

possible. This would cause a steric clash if an alkyl chain were present instead of the fluorine atom.

It is important to remember that these dockings were performed giving flexibility to the ligand only. The enzyme was fixed in the conformation as seen in the isolated crystal structure. In reality when an enzyme makes contact with a substrate the residues of the enzyme are able to reposition themselves to accommodate the ligand and make contacts.

This means that in the case of the BCNAs the enzyme may shift its shape, forming a larger or new binding pockets to accommodate these nucleosides. No hydrogen bond interactions were observed between the BCNA and the ligand. As this analogue in particular is a known inhibitor of VZV-TK some hydrogen bonding would have been expected to be seen.

It would therefore be advantageous to repeat these studies allowing movement of both the ligand and receptor and to use an analogue bearing an alkyl chain attached to the phenyl ring of the side chain. Unfortunately phosphorylation data of these compounds were not available at this time but it would also of interest to compare any results obtained with this data to see if any correlation could be formed.

Regrettably limited availability of time and difficulties associated with the modelling of alkyl chains meant that this and other analysis of the BCNAs bearing an alkyl chain has not been further investigated.

8.3 Summary

This initial investigation into the interaction of the BCNAs with VZV-TK has shown that these compounds most likely bind in the receptor similar to other nucleosides. However, due to steric interactions between the BCNA and the enzyme, it has been seen in this case that the phenyl ring of the side chain, instead of the base moiety, forms contacts within the pyrimidine binding site. This does not allow space for the presence of an alkyl chain on the BCNAs, yet these analogues have been shown to be phoshphorylated by VZV-TK.

This may be a result of using a fixed conformation of the receptor which has been formed after contact with BVDU and it would therefore be interesting in the future to repeat this analysis giving the receptor flexibility.

[1] C. McGuigan; C.J. Yarnold; G. Jones; S. Velaquez; H. Baruki; A. Brancale; G. Andrei; R. Snoeck; E. De Clercq and J. Balzarini. Potent and selective inhibition of Varicella-Zoster virus (VZV) by nucleoside analogues with an unusual bicyclic base. *J. Med. Chem.* **1999,** 42, 4479-4484.

[2] J. Balzarini and C. McGuigan. Chemotherapy of varicella-zoster virus by a novel class of highly specific anti-VZV bicyclic pyrimidine nucleosides. *Biochem. Biophys. Acta.* **2002,** 287-295.

[3] L.E. Bird; J. Ren; A. Wright; K.D. Leslie; B. Degreve; J. Balzarini and D.K. Stammer. Crystal structure of varicella zoster virus thymidine kinase. *J. Bio. Chem.* **2003,** 27, 24680-24687.

[4] http://www.rcsb.org/pdb/

[5] MOE. **2004**. Version 3. Chemical Computing group Inc.

EXPERIMENTAL PROCEDURES

The structure of all final compounds were determined by ^{1}H, ^{13}C and where appropriate ^{19}F NMR. Where possible further evaluation with mass spectrometry and elemental analysis were also performed.

General Methods

Thin layer chromatograph

Thin layer chromatography (TLC) was performed on commercially available silica gel 60 F_{254} aluminium backed plates supplied by Merck. Separation of components was visualised using an ultra violet lamp (254nm and 366nm). Preparative TLC was performed on glass backed, PK6F silica gel 60-A plates, (500μm or 1000μm thickness), supplied by Whatman.

Column Chromatography

Glass columns were slurry packed in the appropriate eluent under gravity, with silica gel (C-gel 60A, 40-60μm, Phase Sep, U.K). Samples were applied as a concentrated solution, in the same eluent, or pre-absorbed onto silica. Fractions containing the product were detected by TLC, "pooled", and concentrated in vacuo. Flash column chromatography was performed using an electrical pump.

NMR Spectroscopy

^{1}H and ^{13}C NMR spectra were obtained using a Bruker Avance DPX300 spectrometer using frequencies of 300 MHz for ^{1}H NMR, 75MHz for ^{13}C NMR and 282 MHz for ^{19}F NMR. All spectra were auto calibrated to the deuterated solvent reference peak and all ^{13}C NMR spectra obtained were proton decoupled. Abbreviations are used in the assignment of the NMR signals: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad signal).

Mass Spectrometry

Mass spectra was performed as a service by Birmingham University.

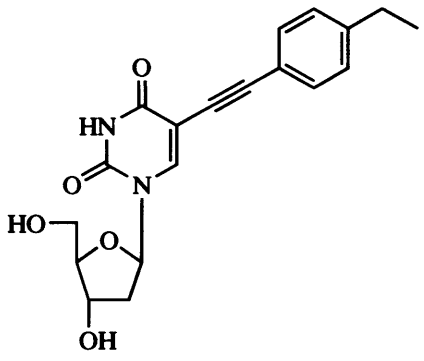
Elemental Analysis

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

Solvents and Reagents

All solvents used were anhydrous and used as supplied. All reagents were used as received. All Glassware used was oven dried at 130 °C for several hours and allowed to cool under a stream of nitrogen before use.

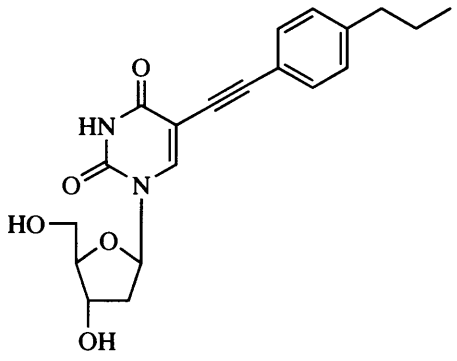
5-(4-n-ethylphenylacetylene)-2'-deoxyuridine (21a)



To a stirred solution of IDU (1.08 g, 3.05 mmol) in DMF (20 mL), were added, 4-n-ethylphenylacetylene (1 g, 7.96 mmol, 2.5 eq), copper iodide (108 mg, 0.56 mmol, 0.2 eq.), tetrakis-(triphenylphosphine)palladium(0) (331 mg, 0.29 mmol, 0.1 eq), and DIPEA (1 mL, 742 mg, 5.75 mmol, 1.9 eq). The mixture was stirred overnight at room temperature, under a nitrogen atmosphere. After this time, the reaction mixture was concentrated *in vacuo*, and the resulting residue was dissolved in dichloromethane/methanol (1:1) (15 mL), whereupon an excess of Amberlite IRA-400 (HCO_3^- form) was added and stirred for 30 min. The resin was filtered and washed with methanol, and the combined filtrate was evaporated to dryness. The product was obtained after trituration with dichloromethane. Filtration followed by a wash with dichloromethane (2 x 5 mL) gave the product as a white powder, (350 mg, 32%).

^{1}H NMR (d_6 DMSO; 300MHz) δ 11.71 (1H, s, NH), 8.36 (1H, s, H-6), 7.39 (2H, d, J = 8.11 Hz, 2 x Phenyl-CH), 7.25 (2H, d, J = 8.19 Hz, 2 x Phenyl-CH), 6.14 (1H, t, J = 6.48 Hz, H-1'), 5.29 (1H, br, s, 3'OH), 5.19 (1H, br, s, 5'OH), 4.26 (1H, m, H-3'), 3.81 (1H, m, H-4'), 3.62 (2H, m, H-5'), 2.63 (2H, m, α-CH_2), 2.16 (2H, m, H-2'), 1.18 (3H, t, J = 7.57 Hz, CH_3); ^{13}C NMR (d_6 DMSO; 75Mhz) 15.6 (CH_3), 28.4 (CH_2), 40.7 (C-2'), 61.2 (C-5'), 70.3 (C-3'), 82.2 (α-alkynyl), 85.1, 87.9 (C-1' and C—4'), 92.3 (ß-alkynyl), 98.7 (C-5), 120.0 (2 x Ph), 128.5 (*ipso*-C), 131.5 (2 x Ph), 144.0 (*para*-C), 144.9 (C-6), 150.3 (C-2), 162.6 (C-4).

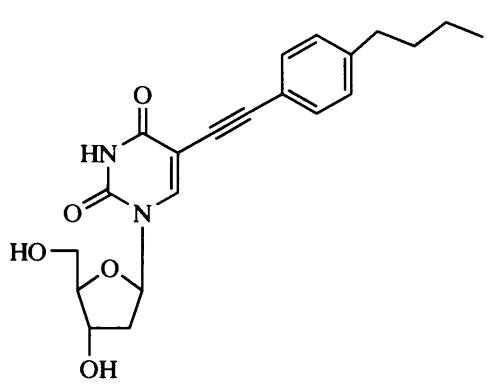
5-(4-n-propylphenylacetylene)-2'-deoxyuridine (21b)



This was prepared as described for **(21a)**, from IDU (1.01g, 2.85 mmol), DMF (10mL), 4-n-propyl-phenylacetylene (1.00g, 6.49mmol, 2.4 eq), Tetrakis(triphenylphosphine)palladium(0) (320 mg, 0.28 mmol, 0.1eq), copper iodide (106 mg, 0.55 mmol, 0.2eq) and DIPEA (1mL, 740 mg, 5.75 mmol, 2.0 eq). The product was obtained after purification by column chromatography (eluent: ethyl acetate), followed by trituration with dichloromethane to give a white powder (470 mg, 31%).

^{1}H NMR (d_6 DMSO; 300MHz) δ 11.71 (1H, s, NH), 8.37 (1H, s, H-6), 7.38 (2H, d, *J* = 8.01 Hz, 2 x Phenyl-CH), 7.23 (2H, d, *J* = 8.08 Hz, 2 x Phenyl-CH), 6.14 (1H, t, *J* = 6.53 Hz, H-1'), 5.28 (1H, m, 3'OH), 5.19 (1H, m, 5'OH), 4.26 (1H, m, H-3'), 3.80 (1H, m, H-4'), 3.63 (2H, m, H-5'), 2.54 (2H, m, H-2'), 2.17 (2H, m, CH_2), 1.60 (2H, m, CH_2), 0.89 (3H, t, *J* = 0.02 Hz, CH_3); ^{13}C NMR (d_6 DMSO; 75Mhz) δ 13.9 (CH_3), 24.2 and 37.4 (2 x CH_2), 40.7 (C-2'), 61.2 (C-5'), 70.3 (C-3'), 82.2 (α-alkynyl), 85.1, 87.9 (C-1' and C-4'), 92.3 (β-alkynyl), 98.7 (C-5), 120.0 (*ipso*-C), 129.1 and 131.4 (4 x Phenyl-CH), 143.3 (*para*-C), 144.0 (C-6), 149.8 (C-2), 161.8 (C-4).

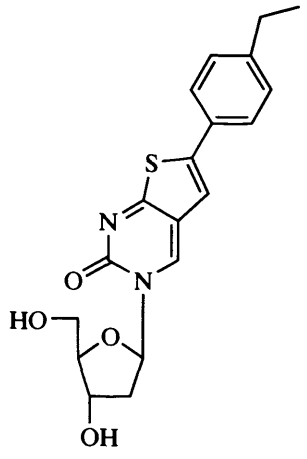
5-(4-n-butylphenylacetylene)-2'-deoxyuridine (21c)



This was prepared as described for **21a**, from IDU (2.67g, 7.54 mmol), DMF (20 mL), 4-n-butyl-phenylacetylene (2.99 g, 18.89 mmol, 2.5 eq), tetrakis (triphenylphosphine) palladium(0) (868 mg, 0.75 mmol, 0.1 eq), Copper iodide (154 mg, 0.81 mmol, 0.11 eq), DIPEA (2.6 mL, 1.93 g, 14.96 mmol,

2.0 eq). Purification by column chromatography (eluent; 10% methanol in dichloromethane) gave the product as a white powder (1.53 g, 53 %).
^{1}H NMR (d_6 DMSO; 300MHz) δ 11.67 (1H, s, NH), 8.34 (1H, s, H-6), 7.36 (2H, d, *J* = 7.96 Hz, 2 x Phenyl-CH), 7.21 (2H, d, *J* = 8.05 Hz, 2 x Phenyl-CH), 6.12 (1H, t, *J* = 6.48 Hz, H-1'), 5.26 (1H, m, 3'OH), 5.16 (1H, m, 5'OH), 4.24 (1H, m, H-3'), 3.79 (1H, m, H-4'), 3.59 (2H, m, H-5'), 2.58 (2H, t, *J* = 7.60 Hz, αCH$_2$), 2.14 (2H, m, H-2'), 1.51 (2H, m, CH$_2$), 1.27(2H, m, CH$_2$), 0.87 (3H, t, *J* = 7.30 Hz, CH$_3$); ^{13}C NMR (d_6 DMSO; 75Mhz) δ 14.1 (CH$_3$), 22.1 (CH$_2$), 33.2 (βCH$_2$), 35.0 (αCH$_2$), 40.5 (C-2'), 61.2 (C-5'), 70.3 (C-3'), 82.1 (α-alkynyl), 85.1, 87.9 (C-1' and C-4'), 92.3 (β-alkynyl), 98.7 (C-5), 120.0 (*ipso*-C), 129.0 and 131.5 (4 x Phenyl-CH), 143.6 (C-4), 144.0 (*para*-C), 161.8 (C-4).

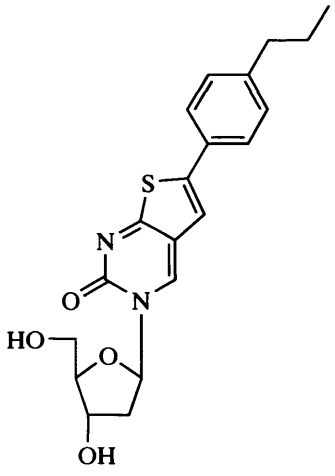
3-(2-deoxy-β-D-ribofuranosyl)-6-(4-n-ethylphenyl)-2,3,dihydrothieno [2,3,d]pyrimidine-2-one (23a) [Cf 2266]



To a solution of **21a** (280 mg, 0.79 mmol) in acetonitrile (12 mL), were added, chloro-trimethylsilane (500 uL, 428 mg, 3.93 mmol, 5.0 eq), triethylamine (1.2 mL, 871 mg, 8.63 mmol, 10.9 eq). The mixture was stirred at room temperature for 3 hr under a nitrogen atmosphere. Phosphorous oxychloride ($POCl_3$) (150 μL, 247 mg, 1.6 eq), and triazole (487 mg, 7.06 mmol, 8.9 eq,) were added at 0°C and the reaction continued stirring for 5 hr at 0°C under a nitrogen atmosphere. $NaHCO_3$ sat. solution was added and the mixture was extracted with dichloromethane. The organic layer was dried over $MgSO_4$, and the solvent was evaporated. The residue was dissolved in acetonitrile (10 mL) and thiolacetic acid (200 uL, 213 mg, 2.80 mmol, 3.5 eq) was added. The mixture was stirred overnight at room temperature under a nitrogen atmosphere. The solvent was removed *in vacuo* and the crude was obtained after trituration with acetone to give the pure product (6.1 mg, 2%).

^{1}H NMR (d_6 DMSO; 300MHz) δ 8.99 (1H, s, H-4), 7.62 (2H, d, *J*=8.16 Hz, 2 x Phenyl-CH), 7.50 (1H, s, H-5), 7.32 (2H, d, *J* = 8.18 Hz, 2 x Phenyl-CH), 6.14 (1H, t, *J* = 6.05 Hz, H-1'), 5.36, (1h, br, s, 3'OH), 5.21 (1H, br, s, 5'OH), 4.25 (1H, m, H-3'), 3.97 (1H, m, H-4'), 3.68 (2H, m, H-5'), 2.65 (2H, m, αCH_2), 2.45 (1H, m, H-2'), 2.14 (1H, m, H-2'), 1.20 (3H, t, *J* = 7.55 Hz, CH_3); ^{13}C NMR (d_6 DMSO; 75Mhz) δ 15.8 (CH_3), 28.3 (CH_2), 41.5 (C-2'), 61.0 (C-5'), 69.9 (C-3'), 88.3, 88.8 (C-1' and C-4'), 115.4 (C-5), 119.4 ($C\text{-}4_a$), 126.4 and 129.0 (4 x Phenyl-CH), 130.4 (ipso-C), 138.2 (C-4), 139.8 (para-C), 145.2 (C-6), 152.0 (C-2), 171.8 ($C\text{-}7_a$).

3-(2-deoxy-β-D-ribofuranosyl)-6-(4-n-propylphenyl)-2,3,dihydrothieno-[2,3,d] pyrimidine-2-one (23b) [Cf2267]

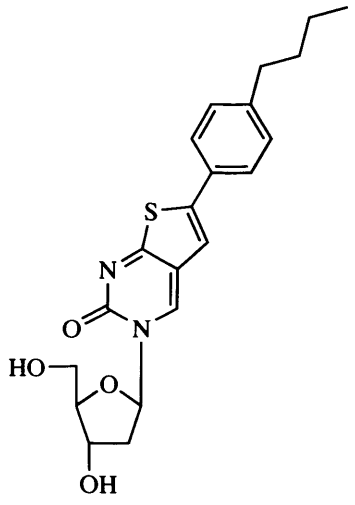


This was prepared as described for **23a** using, **21b** (430 mg, 1.16 mmol), in acetonitrile (15 mL), Chloro-trimethylsilane (740 μL, 633 mg, 5.97 mmol, 5.1 eq), triethylamine (1.8 mL, 1.31 g, 12.94 mmol, 11.2 eq), $POCl_3$ (220 μL, 362 mg, 2.36 mmol, 2.0 eq,) and triazole (729 mg, 10.55 mmol, 9.1 eq). Followed by acetonitrile (15 mL) and thiolacetic acid (330 μL, 351 mg, 4.62 mmol, 4.0 eq). After purification by dry loaded column chromatography (eluent; 5% methanol in dichloromethane) to give the product as a white powder (25 mg, 6%).

^{1}H NMR (d_6 DMSO; 300MHz) δ 8.98 (1H, s, H-4), 7.61 (2H, d, *J* = 8.12 Hz, 2 x Phenyl-CH), 7.50 (1H, s, H-5), 7.30 (2H, d, *J* = 8.13 Hz, 2 x Phenyl-CH), 6.14 (1H, t, *J* = 5.99 Hz, H-1'), 5.33 (1H, d, *J* = 4.25 Hz, 3'OH), 5.17 (1H, m, 5'OH), 4.25 (1H, m, H-3'), 3.97 (1H, m, H-4'), 3.67 (2H, m, H-5'), 3.58 (2H, t, *J* = 7.52 Hz, αCH_2), 2.45 (1H, m, H-2'), 2.14 (1H, m, H-2'), 1.61 (2H, m, CH_2), 0.91 (3H, t, *J* = 7.31 Hz, CH_3); ^{13}C NMR (d_6 DMSO; 75Mhz) δ 14.0 (CH_3), 24.3 (CH_2), 37.2 (αCH_2), 41.5 (C-2'), 61.0 (C-5'), 69.9 (C-3'), 88.3, 88.8 (C-1' and C-4'), 115.4 (C-5'), 119.5 (C-4a), 126.1 , 129.1, 129.6, and 130.4 (4 x Phenyl-CH), 131.4 (*ipso-*

C), 138.2 (C-4), 129.8 (C-6), 143.5 (*para*-C), 152.1 (C-2), 177.8 (C-7_a); [MS ES+]; m/z 409.1 (100%, [M + Na]$^+$), 293.1 (40 %, [Base + Na]$^+$. FAB m/e 409.1204 (MNa$^+$ $C_{21}H_{24}N_2O_4NaS$ requires 409.1198); Found: C, 61.53%; H, 5.57; N, 7.30. $C_{20}H_{22}N_2O_4S$ requires: C, 62.16%; H, 5.74%; N,7.25%.

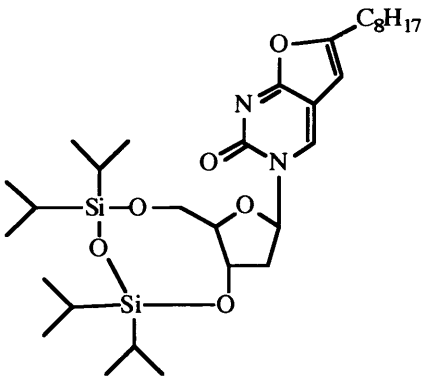
3-(2-deoxy-β-D-ribofuranosyl)-6-(4-n-butylphenyl)-2,3,dihydrothieno-[2,3,d] pyrimidine-2-one (23c) [Cf2268]



This was prepared as described for **23a** using, **21c,** (418 mg, 1.09 mmol), in acetonitrile (14 mL), Chloro-trimethylsilane (690 uL, 590 mg, 5.4 mmol, 5.0 eq), triethylamine (1.72 mL, 1.25 g, 12.36 mmol, 11.3 eq), $POCl_3$ (200 uL, 329 mg, 2.15 mmol, 2.0 eq), Triazole (690 mg, 9.99 mmol, 9.2 eq). Followed by additional acetonitrile (14 mL) and thiolacetic acid (0.28 mL, 298 mg, 3.92 mmol, 3.6 eq). A precipitate was observed in the reaction mixture, which was filtered and washed with dichloromethane and methanol to give the product as a white powder (313 mg, 72%).

^{1}H NMR (d_6 DMSO; 300MHz) δ 8.97 (1H, s, NH), 7.60 (2H, d, *J*=6.83 Hz, 2 x Phenyl-CH), 7.49 (1H, s, H-5), 7.30 (2H, d, *J*=6.29 Hz, 2 X Phenyl-CH), 6.12 (1H, br, s, H-1'), 5.32 (1H, br, s, 3'OH), 5.18 (1H, br, s, 5'OH), 4.25 (1H, m, H-3'), 3.97 (1H, m, H-4'), 3.68 (2H, m, H-5'), 2.61 (2H, br, s, αCH_2), 2.50 (1H, m, H-2'), 2.14 (1H, m, H-2'), 1.57 (2H, m, CH_2), 1.31 (2H, m, CH_2), 0.91 (3H, br, s, CH_3); ^{13}C NMR (d_6 DMSO; 75Mhz) δ 14.1 (CH_3), 22.1 (CH_2), 33.3 (CH_2), 34.8 (αCH_2), 41.6 (C-2'), 61.1 (C-5'), 69.9 (C-3'), 88.3 and 88.8 (C-1' and C-4'), 115.4 (C-5), 119.4 (C-4a), 126.2 (2 x Phenyl-CH), 129.5 (2 x Phenyl-CH), 130.4 (*ipso*-C), 138.2 (C-4), 139.8 (*para*-C), 143.8 (C-6), 152.0 (C-2), 177.8 (C-7a). [MS ES+]; m/z 423.2 (100%, [M + Na]$^+$), 307.1 (31%, [Base + Na]$^+$). FAB m/e 423.1342 (MNa$^+$ $C_{21}H_{24}N_2O_4NaS$ requires 423.1354); Found: C, 62.53%; H, 5.98%; N, 7.17%, $C_{21}H_{24}N_2O_4S$ requires: C, 62.98%; H, 6.04%; N,6.99%.

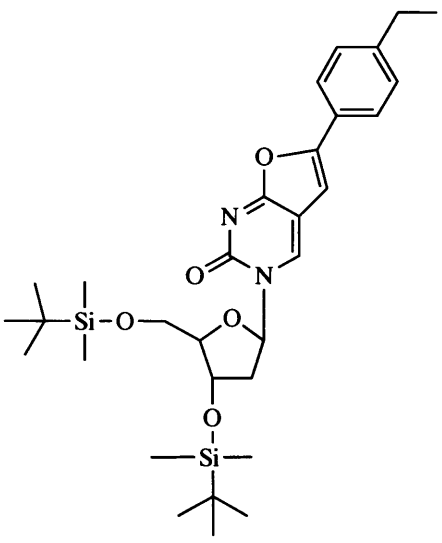
3-(2'deoxy-(3,5'-O-tetraisopropyldisilyloxanyl)-β-D-ribofuranosyl)-2,3-dihydrofuro-6-octyl[2,3,*d*]pyrimidine-2-one (29)



To a stirred solution of **42** (560 mg, 1.37 mmol) in DMF (15 mL), were added, 1,3,dichloro-1,1,3,3-tetra-isopropyldisiloxane (864 mg, 853 μL, 2.74 mmol, 1.2 eq), and imidazole (229 mg, 3.37 mmol, 2.5 eq). This mixture was stirred overnight at room temperature under and atmosphere of nitrogen. After which time, was added, water (50 mL) which was subsequently extracted with diethyl ether. The organic layer was collected, dried over $MgSO_4$, filtered and the filtrate concentrated *invacuo*. The crude was purified using flash chromatography (eluent: 30% ethyl acetate in petroleum ether), to give the product as a white solid (350 mg, 42%).

^{1}H NMR (d_6 DMSO; 300MHz) δ 8.46 (1H, s, H-4), 6.14 (1H, d, *J* = 6.79 Hz, H-1'), 6.07 (1H, s, H-5), 4.37 (1H, m, H-3'), 4.23 – 3.85 (3H, br, m, H-4' and H-5), 2.64 (3H, m, αCH_2 and H-2'), 2.40 (1H, m, H-2'), 1.68 (2H, t, *J* = 6.71 Hz, βCH_2), 0.89 – 1.32 (41H, m, 9 x CH_3, 4 x CH and 5 x CH_2); ^{13}C NMR (d_6 DMSO; 75Mhz) δ 12.8, 13.2, 13.4 and 13.8 (4 x CH), 17.3, 17.4, 17.7, 17.8, 17.9 (8 x CH_3), 23.1, 27.2, 28.7, 29.4, 29.6, 32.2 (7 x CH_2), 40.0 (C-2'), 59.9 (C-5'), 66.2 (C-3'), 85.7 and 86.9 (H-1' and H-4'), 99.2 (H-5), 107.9 (C-4a), 135.2 (C-4), 155.1 (C-2), 160.2 (C-6), 172.1 (C-7a).

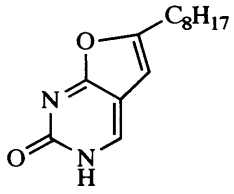
3-(2-deoxy-3,5-bis[*tert*-butyldimethylsilanyloxy)-β-D-ribofuranosyl)–6-[4-ethylphenyl] -2,3-dihydrofuro[2,3-d]pyrimidine-2-one (31)



To a stirred solution of 3-(2-deoxy-β-D-ribofuranosyl)-6-[4-ethylphenyl]- 2,3-dihydro-[2,3,d] pyrimidine-2-one (370 mg, 1.04 mmol,) in DMF, were added, imidazole (900 mg, 13.24 mmol, 13.2 eq) and *tert*-butyl-chlorodimethylsilane (505 mg, 3.35 mmol, 3.2 eq). The mixture was stirred overnight under a nitrogen atmosphere at room temperature. The solvent was removed *in vacuo* and the residue purified by column chromatography, (eluent: 30% methanol in dichloromethane to give the product as a white powder (510 mg, 84%).

^{1}H NMR ($CDCl_3$; 300MHz) δ 8.72 (1H, s, H-4), 7.74 (2H, 2 x Phenyl-CH), 7.33 (2H, 2 x Phenyl-CH), 6.65 (1H, s, H-5), 6.40 (1H, dd, *J* = 4.64 Hz, H-1'), 4.45 (1H, m, H-3'), 4.06-3.87 (3H, m, H-4' and H-5'), 2.74 (1H, m, H-2'), 2.25 (1H, m, H-2'), 1.31 (2H, q, αCH_2), 1.00-0.92 (18H, m, 2 x $(CH_3)_3$), 0.22-0.10 (15H, m, 4 x $SiCH_3$ and CH_3); ^{13}C NMR (d_6 $CDCl_3$; 75Mhz) δ -5.0, -4.9, -4.5 and -4.1 (2 x $(CH_3)_3$), 15.8 (CH_3), 18.4 and 18.9 (2 x Si-C-), 26.1 and 26.4 (4 x CH_3), 29.2 (αCH_2), 43.0 (C-2'), 62.23 (C-5'), 70.42 (C-3'), 88.4 and 88.5 (C-1' and C-4'), 97.2 (H-5), 108.3 (C-4a), 125.4 (2 x Phenyl-CH), 126.4 (*ipso*-C), 128.9 (2 x Phenyl-CH), 136.3 (C-4), 146.7 (*para*-C), 155.1 (C-2), 156.2 (C-6), 172.1 (C-7a).

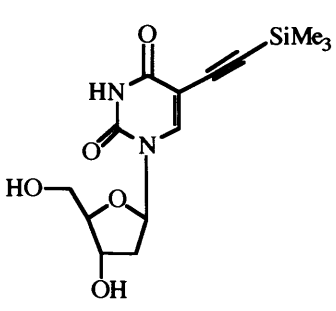
6-Octyl-3H-furo[2,3-*d*]pyrimidine-2-one (32)



To a solution of 3-(2'deoxy-β-D-ribofuranosyl)-2,3-dihydrofuro-6-octyl[2,3*d*]pyrimidine-2-one (456 mg, 1.24 mmol) in pyridine (20 mL) was added phosphorous pentasulfide (3.10 g, 16.32 mmol, 13.2 eq.) and the mixture stirred at 100 °C for 36 hr. The solvent was removed *invacuo* and the residue subjected to column chromatography (eluent: 10% methanol in dichloromethane) to give the product (289 mg, 94 %).

^{1}H NMR ($CDCl_3$; 300MHz) δ 11.97 (1H, s, NH), 8.13 (1H, s, H-4), 6.35 (1H, s, H-5), 2.61 (2H, t, J = 7.09 Hz, αCH_2), 1.57 (2H, m, CH_2), 1.13 (10H, m, 5 x CH_2), -3.08 (3H, br, s, CH_3); [MS ES+]; m/z 271.2 (100 %, [Base + Na]$^+$).

5-(2-trimethylsilyl-1-ethylnyl)-2'deoxyuridine (33)



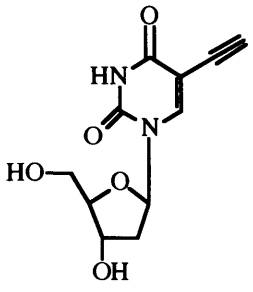
Via Route A: To a solution of 5-iodo-2'deoxyuridine (4.18 g, 11.81 mmol) in DMF (40 mL), were added, trimethylsilylacetylene (4.5 mL, 3.13 g, 31.91 mmol, 2.7 eq), tetrakis(triphenylphosphine)palladium(0) (1.17 g, 1.01 mmol, 0.1 eq), CuI (416 mg, 2.19 mmol, 0.2eq) and DIPEA (4.8 mL, 3.56 g, 27.55 mmol, 2.3 eq). The mixture was stirred overnight at room temperature under a nitrogen atmosphere. After which time, the solvent was removed *in vacuo*, the residue dissolved in methanol/dichloromethane (1:1) (20 mL), excess Amberlite IRA-400 (HCO_3^- form) was added and stirred for 30 min. The resin was then filtered, washed with methanol and the filtrate concentrated. The residue was then purified by flash column chromatography (eluent: methanol/dichloromethane (1:9), to give the pure product (2.30 g, 60%).

Via route B. To a solution of IDU (5.08 g, 14.35mmol) in acetonitrile (20 mL), were added, trimethylsilylacetylene (6 mL, 5.14 g, 47.29 mmol, 3.3 eq), dichlorobisdiphenylphosphine palladium (II) (576 mg, 0.81 mmol, 0.1 eq), CuI (504 mg, 2.64 mmol, 0.2 eq), DIPEA (6.0 mL, 4.45 g, 34.51 mmol, 2.4 eq). The

mixture was heated at reflux under a nitrogen atmosphere for 5 hr. After which time, the solvent was removed *in vacuo*, the residue dissolved in methanol/dichloromethane (1:1) (20 mL), excess Amberlite IRA-400 (HCO_3^- form) was added and stirred for 30 min. The resin was then filtered, washed with methanol and the filtrate concentrated. The residue was then purified by flash column chromatography (eluent: 10% methanol in dichloromethane), to give the pure product (3.53 g, 75%).

^{1}H NMR (d_6 DMSO; 300MHz) δ 11.66 (1H, s, NH), 8.28 (1H, s, H-4), 6.10 (1H, t, *J* = 6.43 Hz, H-1'), 5.27 (1H, d, *J* = 4.21 Hz, 3'OH), 5.14 (1H, m, 5'OH), 4.24 (1H, m, H-3'), 3.80 (1H, m, H-4'), 3.61 (2H, m, H-5'), 2.14 (2H, m, H-2'), 1.11 (9H, m, $(CH_3)_3$); ^{13}C NMR (d_6 DMSO; 75Mhz) 0.0 (CH_3), 40.2 (C-2'), 60.8 (C-5'), 70.0 (C-3'), 84.8 (C-1'), 87.6 (C-4'), 97.1 (β-alkynyl), 98.0 (α-alkynyl), 98.3 (C-5), 144.8 (C-6), 149.4 (C-2),161.5 (C-4).

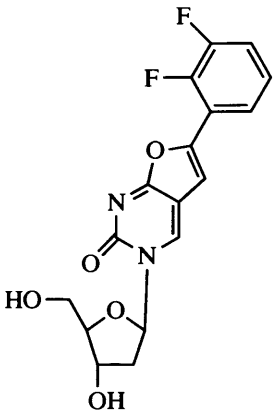
5-ethynyl-2'deoxyuridine (EDU) (34)



A mixture of **(33)** (1.86 g, 6 mmol) dissolved in a solution of methanol/ammonia (10%) (1:1), was stirred at room temperature for 5 hr. After which time, the solvent was removed *in vacuo* and residue purified by washing with methanol and filtration to give the product as a white powder (1.10 g, 76%).

^{1}H NMR (d_6-DMSO; 300 MHz); δ 11.64 (1H, s, NH), 8.31 (1H, s, H-6), 6.11 (1H, t, *J* = 6.50 Hz, H-1'), 5.26 (1H, d, *J* = 4.03 Hz, 3'-OH), 5.16 (1H, t, *J* = 4.68 Hz, 5'-OH), 4.24 (1H, m, H-3'), 4.11 (1H, s, ethynyl-CH), 3.80 (1H, m, H-4'), 3.60 (2H, m, H-5'), 2.13 (2H, m, H-2'); ^{13}C NMR (d_6-DMSO; 75 MHz): 40.6 (C-2'), 61.2 (C-5'), 70.3 (C-3'), 76.8 (α-alkynyl), 84.0 (β-alkynyl), 85.2 (C-1'), 88.0 (C-4'), 98.0 (C-5), 144.9 (C-6), 150.0 (C-2), 162.0 (C-4)

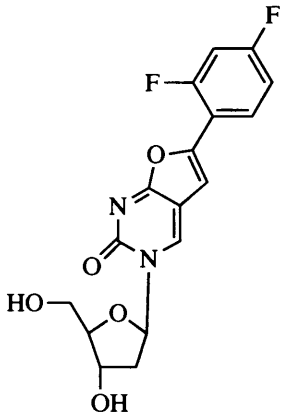
3-(2-deoxy-β-D-ribofuranosyl)-6-(2,3difluorophenyl)-2,3,dihydrofuro-[2,3-d] pyrimidine-2-one (35a) [Cf2372]



To a solution of EDU **(34)** (880 mg, 3.49 mmol) in acetonitrile (15 mL) was added, 1,bromo-2,3,difluorophenyl (1 mL, 1.72 g, 8.93 mmol, 2.6 eq), Dichlorobis (triphenylphosphine)palladium (II) (249 mg, 0.35 mmol, 0.1 eq.), CuI (137 mg, 0.72 mmol, 0.1 eq.) amd DIPEA (1.2 mL, 890 mg, 6.9 mmol, 2.0 eq.). This was stirred at reflux for 6 hr under an atmosphere of nitrogen, after which time, the solvent was removed *invacuo*. The residue was dissolved in methanol/dichloromethane (20 mL,) (1:1), excess amberlite HCO_3^- form was added and the mixture stirred for 30 min. The resin was then filtered, washed with methanol and the filtrate concentrated and purified by column chromatography, (eluent: 5 % methanol in dichloromethane). The pure product was obtained as a white powder (15 mg, 2 %).

^{1}H NMR (d_6 DMSO; 300 MHz); δ 8.97 (1H, s, H-4), 7.67 (1H, m, Phenyl-CH), 7.52 (1H, m, Phenyl-CH), 7.41 (1H, m, phenyl-CH), 7.25 (1H, d, *J* = 2.87 Hz, H-5), 6.19 (1H, t, *J* = 6.08 Hz, H-1'), 5.34 (1H, d, *J* = 4.28 Hz, 3'OH), 5.17 (1H, t, *J* = 4.79 Hz, 5'OH), 4.27 (1H, m, H-3'), 3.98 (1H, m, H-4'), 3.69 (2H, m, H-5'), 2.46 (1H, m, H-2'), 2.13 (1H, m, H2'); ^{13}C NMR (d_6 DMSO; 75 MHz): 41.6 (C-2'), 61.2 (C-5'), 70.1 (C-3'), 88.3 and 88.7 (C-1' and C-4'), 105.8 and 105.9 (C-5), 106.6 (C4a), 118.2 and 118.5 (Phenyl-CH,), 118.9 and 119.1 (*ipso-C*),122.2 (Phenyl-CH), 126.0 and 126.1 (Phenyl-CH), 140.2 (C-4), 145.3 and 145.5 (phenyl-CF), 146.8 (C-6), 148.8 and 149.0 (Phenyl-CF), 154.1 (C-2), 170.8 (C-7a); ^{19}F NMR (d_6-DMSO; 282 Mhz): -139.72 (1F, m, Phenyl-CF), -138.7 (1F, m, Phenyl-CF). [MS ES+]; m/z 387 (100 %, [Mass + Na^+], 271 (24 %, [Base + Na^+]. FAB m/e 387.0760 (MNa^+ $C_{17}H_{14}F_2N_2O_5Na$ requires 387.0768).

3-(2-deoxy-β-D-ribofuranosyl)-6-(2,4difluorophenyl)-2,3,dihydrofuro-[2,3-d] pyrimidine-2-one (35b) [Cf2367]

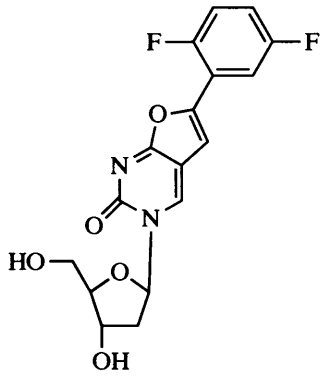


To a solution of EDU **(34)** (1.09 g, 4.33 mmol) in DMF (20 mL) were added, 2,4-difluoro,1-iodobenzene (1.3 mL, 2.61 g, 10.81 mmol, 2.5 eq.), tetrakis(triphenylphosphine) palladium(0) (570 mg, 0.49 mmol, 0.1 eq.), copper iodide (166 mg, 0.87 mmol, 0.2 eq.) and DIPEA (1.5 mL, 1.11 g, 8.63 mmol, 2.0 eq.). This mixture was stirred at room temperature, overnight under an atmosphere of nitrogen. After which time, additional copper iodide (163 mg, 0.85 mmol, 0.2 eq.) and triethylamine (10 mL) were added and the mixture stirred at reflux for 6 hr. The solvent was removed *invacuo* and the residue dissolved in methanol/dichloromethane (30 mL) (1:1), an excess of amberlite HCO_3^- form added and stirred for 30 minutes. The resin was filtered, washed with methanol and the filtrate concentrated. This was purified by column chromatography (eluent: 5% methanol in dichloromethane) to give the product as a white powder (143 mg, 9%).

^{1}H NMR (d_6-DMSO; 300 MHz); δ 8.90 (1H, s, H-4), 7.88 (1H, m, Ph), 7.50 (1H, m, Phenyl-CH), 7.27 (1H, m, Phenyl-CH), 7.09 (1H, d, *J* = 2.99 Hz, H-5), 6.17 (1H, t, *J* = 6.06 Hz, H-1'), 5.33 (1H, d, *J* = 4.24 Hz, 3'OH), 5.16 (1H, t, *J* = 5.17 Hz, 5'OH), 4.26 (1H, m, H-3'), 3.95 (1H, m, H-4'), 3.67 (2H, m, H-5'), 2.44 (1H, m, H-2'), 1.10 (1H, m, H-2'); ^{13}C NMR (d_6 DMSO; 75 MHz); δ 40.5 (C-2'), 60.1 (C5'), 68.94 (C-3'), 87.2 and 87.6 (C-1' and C-4'), 103.0 and 103.1 (C-5, *J* = 16.95 Hz), 104.1, 104.5 and 104.8 (Phenyl-CH), 105.6 (C4a), 111.7, 111.7, 112.0 and 112.9 (Phenyl-CH), 112.6, 112.7, 112.8 and 112.8 (*ipso*-C), 127.3, 127.3, 127.4 and 127.5 (Phenyl-CH), 138.4 (C4), 146.1 and 146.1 (C-6, *J* = 2.25 Hz), 153.0 (C2), 156.6, 156.7, 159.8, 159.9, 160.0, 160.1,163.2 and 163.4 (2 x Phenyl-CF), 169.7 (C7a). ^{19}F NMR (d_6-DMSO; 282 MHz): δ -108.53 (1F, m, Phenyl-CF), -108.04 (1F, m, Phenyl-CF). [MS ES+]; m/z 387.1 (100 %, [Mass + Na^+], 271.1 (39 %, [Base + Na^+]. FAB m/e 387.0758 (MNa^+ $C_{17}H_{14}F_2N_2O_5Na$

requires 387.0768); Found: C, 56.28%; H, 3.86; N, 7.63, $C_{17}H_{14}F_2N_2O_5$ requires: C, 56.05%; H, 3.87%; N,7.69%.

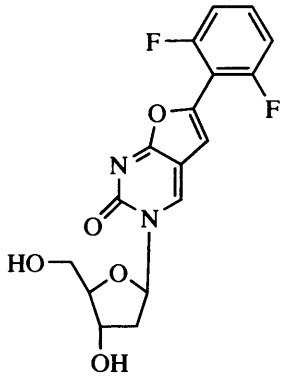
3-(2-deoxy-β-D-ribofuranosyl)-6-(2,5difluorophenyl)-2,3,dihydrofuro-[2,3-d] pyrimidine-2-one (35c) [CF2371]



To a solution of EDU **(34)** (1.20 g, 4.76 mmol) in DMF (10 mL) were added, 2-Bromo-1,4,-difluorobenzene (2.3 g, 11.92 mmol, 2.5 eq.), copper iodide (182 mg, 0.95 mmol, 0.2 eq.) tetrakis (triphenylphosphine) palladium(0) (590 mg, 0.51 mmol, 0.1 eq.) and DIPEA (20 mL, 1.48 g, 11.5 mmol, 2.4 eq.). The mixture was stirred at room temperature, overnight under an atmosphere of oxygen. After which time, the solvent was removed *invacuo,* the residue dissolved in methanol/dichloromethane (20 mL) (1:1), an excess of amberlite IRA-400 (HCO_3^- form) added and the mixture stiired for 30 min. The resin was filtered, washed with methanol, the filtrate concentrated and purified by column chromatography (eluent: 5% methanol in dichloromethane) followed by recrystallisation in methanol to give the pure product (82 mg, 5 %).

^{1}H NMR (d_6 DMSO; 300 MHz); δ 8.96 (1H, s, H-4), 7.64 (1H, m, Ph), 7.50 (1H, m, Phenyl-CH), 7.36 (1H, m, Phenyl-CH), 7.24 (1H, d, J = 2.97 Hz, H-5), 6.28 (1H, t, J = 6.08 Hz, H-1'), 5.34 (1H, m, 3'OH), 5.16 (1H, m, 5'OH), 4.26 (1H, m, H-3'), 3.98 (1H, m, H-4'), 3.69 (2H, m, H-5'), 2.46 (1H, m, H-2'), 2.12 (1H, H-2'); ^{19}F NMR (d_6-DMSO; 282 Mhz): δ -118.28 (1F, m, Phenyl-CF), -117.61 (1F, m, Phenyl-CH). Found: C, 55.85%; H, 4.14%; N, 7.50%, $C_{17}H_{14}F_2N_2O_5$ requires: C, 56.05%; H, 3.87%; N,7.69%.

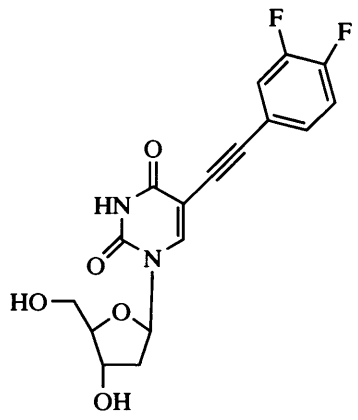
3-(2-deoxy-β-D-ribofuranosyl)-6-(2,6difluorophenyl)-2,3,dihydrofuro-[2,3-d] pyrimidine-2-one (35d) [CF2370]



To a suspension of EDU (1.03 g, 4.09 mmol) in acetonitrile (15 mL) were added, 1-bromo-2,6-difluorobenzene (1.2 mL, 2.06 g, 10.67 mmol 2.6 eq), Dichlorobis(triphenylphosphine)palladium (II) (297 mg, 0.42 mmol, 1.2 eq.), CuI (174 mg, 0.91 mmol, 0.2 eq.) and DIPEA (1.4 mL, 1.04 g, 8.05 mmol, 2.0 eq.). The mixture was stirred at reflux for 4 1/2 hr under an atmosphere of nitrogen, after which time, CuI (152 mg, 0.70 mmol, 0.2 eq) and Et_3N (10 mL) were added and stirring at reflux continued for a further 5 hr. The solvent was removed *in vacuo*, the residue dissolved in methanol/dichloromethane (1:1) (20 mL), an excess of amberlite IRA-400 HCO_3^- form was added and stirred for 30 min. The resin was filtered, washed with methanol and the filtrate concentrated to give a brown residue which was purified by column chromatography (eluent: 5% methanol in dichloromethane) to give the product as a white powder (40 mg, 2 %).

^1H NMR (d_6 DMSO; 300 MHz); 8.98 (1H, s, H-4), 7.61 (1H, m, Phenyl-CH), 7.43 (2H, m, 2 x Phenyl-CH), 7.19 (1H, s, H-5), 6.19 (1H, t, *J* = 5.99 Hz, H-1'), 5.33 (1H, d, *J* = 4.04 Hz, 3'OH), 5.17 (1H, t, *J* = 5.25 Hz, 5'OH), 4.14 (1H, m, H-3'), 3.96 (1H, m, H-4'), 3.68 (2H, m, H-5'), 2.46 (1H, m, H-2'), 2.13 (1H, m, H-2'); ^{13}C NMR (d_6 DMSO; 75 MHz); δ 48.6 (C-2'), 60.8 (C-5'), 69.6 (C-3'), 87.9 and 88.3 (C-1' and C-4'), 105.5 (C-4a), 106.6 and 106.7 (C-5), 107.2, 107.2, 107.3, 112.6, 112.6, 112.7 and 112.8 (2 x Phenyl-CH), 131.9, 132.0 and 132.1 (*para*-CH), 139.8 (C-4), 143.1 (C-6), 153.7 (C-2), 158.1, 158.1, 160.1 and 160.1 (2 x Phenyl-CF), 170.7 (C-7a); ^{19}F NMR (d_6-DMSO; 282 MHz): δ -110.4 (2F, m, 2 x Ph-CF). [MS ES+]; m/z 387.1 (100 %, [Mass + Na^+], 271.0 (37 %, [Base + Na^+]. FAB m/e 387.0775 (MNa^+ $C_{17}H_{14}F_2N_2O_5Na$ requires 387.0768).

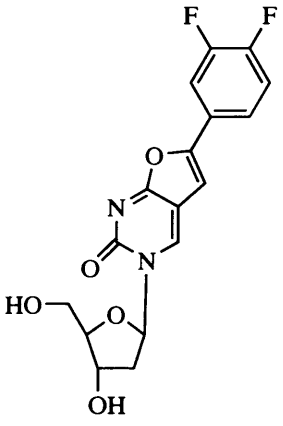
5-(3,4difluorophenyl)ethynyl-2'-deoxyuridine (37e)



To a solution of EDU (1.03 g, 4.09 mmol) in DMF (10 mL), were added, 1,2difluoro-4-iodobenzene (1.45 mL, 2.89 g, 12.04 mmol, 2.9 eq), CuI (140 mg, 0.73 mmol, 0.2 eq.), tetrakis (triphenylphosphine) palladium(0) (560 mg, 0.48 mmol, 0.1 eq.) and DIPEA (1.65 mL, 1.22 g, 9.49 mmol, 2.3 eq). The mixture was stirred overnight at room temperature under an atmosphere of nitrogen. After which time, the solvent was removed, the residue dissolved in methanol/dichloromethane (1:1) (20 mL), excess amberlite IRA-400 (HCO_3^- form) was added and stirred for 30 min. The resin was filtered, washed with methanol and the filtrate concentrated to give a brown residue, which was purified by column chromatography (eluent: 5% methanol in dichloromethane) to give the product as a white powder (830 mg, 56%).

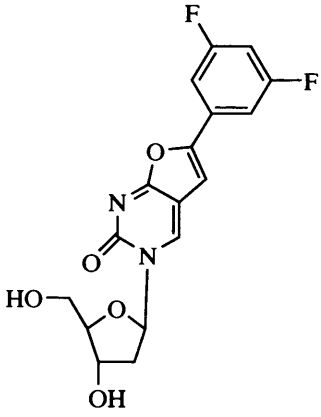
^{1}H NMR (d_6 DMSO; 300 MHz); δ 11.75 (1H, s, NH), 8.46 (1H, s, H-6), 7.50 (3H, m, 3 x Phenyl-CH), 6.14 (1H, t, *J* = 6.41 Hz), 5.30 (1H, d, *J* = 4.33 Hz, 3'OH), 5.22 (1H, t, *J* = 4.74 Hz, 5'OH), 4.28 (1H, t, *J* = 4.01 Hz, H-3'), 3.84 (1H, m, H-4'), 3.64 (2H, m, H-5'), 2.19 (2H, m, H-2'); ^{13}C NMR (d_6 DMSO; 75 MHz); δ 40.7 (C-2'), 61.1 (C-5'), 70.2 (C-3'), 83.6 (α-alkynyl), 85.3 (C-1'), 88.0 (C-4'), 90.0 (ß-alkynyl), 97.9 (C-5), 109.5 (*ipso*-C), 118.6 (d, *J* = 17.41 Hz, Phenyl-CH), 120.5 (d, *J* = 18.42 Hz, Phenyl-CH), 129.1 (Phenyl-CH), 144.8 (C-6), 148.0 (m, 1 x Phenyl-CF), 149.8 (C-2), 151.3 (m, 1 x Phenyl-CF), 161.7 (C-4); ^{19}F NMR (d_6-DMSO; 470 MHz); δ - 137.6 and -136.4 (2F, m, 2 x Phenyl-CF).

3-(2-deoxy-β-D-ribofuranosyl)-6-(3,4difluorophenyl)-2,3,dihydrofuro-[2,3-d] pyrimidine-2-one (35e) [CF2421]



37e (760 mg, 2.09 mmol) was dissolved in a mixture of methanol and triethyl amine (7:3) (50 mL) and was added, CuI (74 mg, 0.39 mmol, 0.2 eq.) and the mixture was stirred at reflux, under an atmosphere of nitrogen for 5 hr. After which time, the precipitate formed was filtered and washed with methanol to give the product (710 mg, 93%). ^{1}H NMR (d_6 DMSO; 300 MHz); δ 8.94 (1H, s, H-4), 7.98 (1H, m, 1 x Phenyl-CH), 7.64 (2H, m, 2 x Phenyl-CH), 7.39 (1H, s, H-5), 6.19 (1H, t, *J* = 5.91 Hz, H-1'), 5.32 (1H, d, *J* = 4.28 Hz, 3'OH), 5.21 (1H, t, *J* = 5.08 Hz, 5'OH), 4.27 (1H, m, H-3'), 3.95 (1H, m H-4'), 3.69 (2H, m, H-5'), 2.44 (1H, m, H-2'), 2.13 (1H, m, H-2'); ^{13}C NMR (d_6 DMSO; 75 MHz); δ 42.3 (C-2), 61.7 (C-5'), 70.5 (C-3'), 88.8 and 89.3 (C-1' and C-4'), 102.0 (C-5), 107.6 (C-4a), 114.9 (d, *J* = 19.42 Hz, Ph), 119.2 (d, *J* = 17.77 Hz, Phenyl-CH), 122.7 (m, Phenyl-CH), 127.1 (m, *ipso*-C), 140.0 (C-4), 149.3 (m , Phenyl-CF), 152.6 (m, Phenyl-CF), 154.8 (C-2), 172.0 (C-7a); ^{19}F NMR (d_6-DMSO; 282 MHz); δ -136.35 - -138.25; [MS ES+]; m/z 387 (100 %, [Mass + Na^+], 271 (34 %, [Base + Na^+]. FAB m/e 387.0776 (MNa^+ $C_{17}H_{14}F_2N_2O_5Na$ requires 387.0768).

3-(2-deoxy-β-D-ribofuranosyl)-6-(3,5difluorophenyl)-2,3,dihydrofuro-[2,3-d] pyrimidine-2-one (35f)[CF2369]

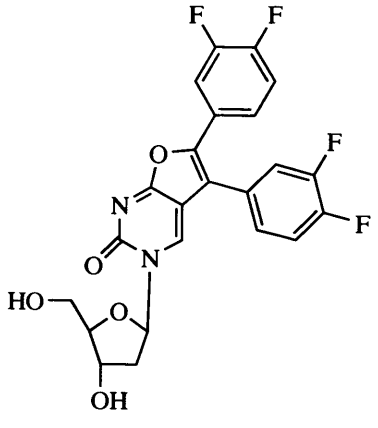


To a solution of EDU (1.09 g, 4.33 mmol) dissolved in DMF (15 mL), were added, 1-bromo-3,5difluorobenzene (1.5 mL, 2.51 g, 13.01 mmol, 3.0 eq.), tetrakis (triphenylphosphine) palladium(0) (570 mg, 0.49 mmol, 0.1 eq.), CuI (167 mg, 0.87 mmol, 0.2 eq.) and DIPEA (1.5 mL, 1.12 g, 8.66 mmol, 2.0 eq.). The mixture was stirred overnight at room temperature under an atmosphere of nitrogen after which time was added, CuI

(190 mg, 0.99 mmol, 0.2 eq) and Et_3N (10 mL) and the mixture stirred at reflux for 7 hr. The solvent was removed *in vacuo* and the residue dissolved in methanol/dichloromethane (1:1) (30 mL), excess amberlite IRA-400 (HCO_3^- form) was added and stirred for 30 min. The resin was filtered, washed with methanol and the filtrate concentrated to give a brown residue which was purified by column chromatography (eluent: 5% methanol in dichloromethane), followed by trituration with methanol to give the product (117 mg, 8%).

^1H NMR (d_6 DMSO; 300 MHz); δ 8.95 (1H, s, H-4), 7.56 (2H, d, J = 6.46 Hz, 2 x Phenyl-CH), 7.48 (1H, s, H-5), 7.31 (1H, t, J = 9.23 Hz, Phenyl-CH), 6.14 (1H, t, J = 5.92 Hz, H-1'), 5.29 (1H, d, J = 4.14 Hz, 3'OH), 5.19 (1H, t, J = 4.87 Hz, 5'OH), 4.22 (1H, m, H-3'), 3.91 (1H, m, H-4'), 3.71-3.61 (2H, br, m, H-5') 2.40 (1H, m, H-2'), 2.10 (1H, m, H-2'); ^{13}C NMR (d_6-DMSO; 75 MHz); δ 41.6 (C-2'), 60.9 (C-5'), 69.1 (C-3'), 88.2 and 88.6 (C-1' and C-4'), 102.6 (C-5), 104.9 (m, *para*-CH), 106.6 (C-4a), 108.1 (m, 2 x Phenyl-CH), 132.0 (*ipso*-C), 139.9 (C-4), 151.4 (m, C-6), 154.1 (C-2), 161.5 (m, Phenyl-CF), 164.8 (m, Phenyl-CF), 171.2 (C-7a); ^{19}F NMR (d_6-DMSO; 282 Mhz): δ -108.9 (m, 2 x Phenyl-CF); [MS ES+]; m/z 387.3 (100 %, [Mass + Na^+], 271.2 (41 %, [Base + Na^+]. FAB m/e 387.0772 (MNa^+ $C_{17}H_{14}F_2N_2O_5Na$ requires 387.0768). Found: C, 55.67%; H, 3.87; N, 7.57, $C_{17}H_{14}F_2N_2O_5$ requires: C, 56.05%; H, 3.87%; N,7.69%.

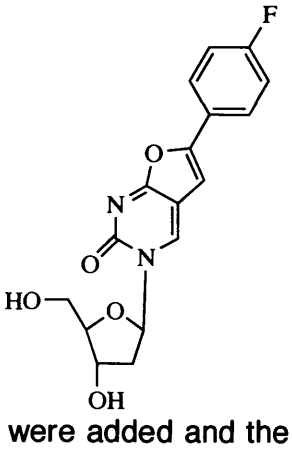
3-(2'deoxy-ß-D-ribofuranosyl)-5-(3,4dufluorophenyl)-6-(3,4difluorophenyl)-2,3-dihydrofuro[2,3-*d*]pyrmidine-2-one (36) [Cf2368]



To a solution of EDU (1.09 g, 4.33 mmol) in DMF (15 mL) was added, 3,4difluoro-1-iodobenzene (1.5 mL, 2.99 g, 12.44 mmol, 2.9 eq), tetrakis (triphenylphosphine) palladium(0) (565 mg, 0.49 mmol, 0.1 eq.), CuI (154 mg, 0.81 mmol, 0.2 eq.) and DIPEA (1.5 mL, 1.11 g, 8.63 mmol, 2.0 eq). The mixture was stirred at room temperature overnight under an atmosphere of nitrogen after

which time, was added CuI (156 mg, 0.82 mmol, 0.2 eq and Et_3N (10 mL) and the mixture stirred at reflux for 4 hr. The solvent was removed *in vacuo,* the residue dissolved in methanol/dichloromethane (1:1) (40 mL), excess amberlite IRA-400 (HCO_3^- form) was added and the mixture stirred for 30 min. The resin was then filtered, washed with methanol and the filtrate concentrated and purified by column chromatography (eluent: 5% methanol in dichloromethane) followed by trituration with methanol to give the pre product 1.20 g, 76%).
^1H NMR (d_6 DMSO; 300 MHz); δ 9.05 (1H, s, H-6), 7.62 – 7.35 (7H, m, 6 x Phenyl-CH and H-5), 6.18 (1H, m, H-1'), 5.32 (1H, d, *J* = 4.21 Hz, 3'OH), 5.13 (1H, m, 5'OH), 4.30 (1H, m, H-3') 3.90 (1H, m, H-4'), 3.66 (2H, m, H-5'), 2.46 (1H, m, H-2'), 2.21 (1H, m, H-2'); ^{13}C NMR (d_6 DMSO; 75 MHz); δ 41.2 (C-2'), 59.6 (C-5), 68.1 (C-3'), 87.7 and 88.8 (C-1' and C-4'), 107.2 (C-4a), 114.1, 115.8, 115.9, 118.2,118.3, 118.4, 118.5, 118.6, 118.7, 124.1, 125.6, 126.2 and 126.6 (C-5, 6 x Phenyl-CH and 2 x *ipso*-C), 139.0 (C-4), 146.0 (C-2), 148.8 and 150.8 (2 x Phenyl-CF), 153.7 (C-6) and 169.6 (C-7a); ^{19}F NMR (d_6-DMSO; 282 Mhz): δ -137.2 (4F, m, 4 x Ph-CF). [MS ES+]; m/z 499.1 (100 %, [Mass + Na^+], 383.1 (36 %, [Base + Na^+].

3-(2'deoxy-ß-D-ribofuranosyl)6-(4-fluorophenyl)-2,3-dihydrofuro[2,3*d*] pyrmidine-2-one (38)[Cf2450]

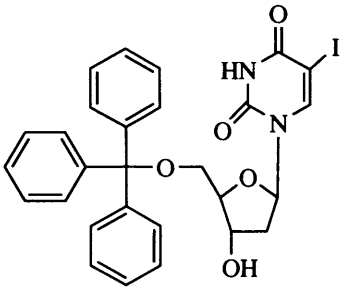


To a solution of IDU (2.98 g, 8.42 mmol) in DMF (30 mL) were added, 4-fluoro-1-ethynylbenzene (1g, 8.33 mmol, 1.0 eq.), DIPEA (2.5 mL, 1.86 g, 14.35 mmol, 1.7 eq.), tetrakis (triphenylphosphine)palladium(0) 979 mg, 0.85 mmol, 0.1 eq.), CuI (275 mg, 1.44 mmol, 0.17 eq.) and the mixture stirred at room temperature overnight. After which time, CuI (283 mg, 1.48 mmol, 0.18 eq.) and triethylamine (3 mL) were added and the mixture stirred at reflux for 5 hr. The solvent was removed *in vacuo*, the residue dissolved in methanol/dichloromethane (50:50), excess amberlite IRA-400 (HCO_3^- form) was added and stirred for 30 min. The resin

was filtered, washed with methanol and the filtrate concentrated. Column chromatography (eluent: 5 % methanol in dichloromethane) afforded the desired product as a white solid (555.4 mg, (20%).

^{1}H NMR (d_6 DMSO; 300MHz) δ 8.89 (1H, s, H4), 7.91 (2H, m, Phenyl-CH), 7.37 (2H, m, Phenyl-CH), 7.29 (1H, s, H-5), 6.19 (1H, t, *J* = 6.03 Hz, H-1'), 5.33 (1H, d, *J* = 4.29 Hz, 3'OH), 5.22 (1H, t, *J* = 0.02 Hz, 5'O H), 4.27 (1H, m, H-3'), 3.94 (1H, m, H-4'), 3.68 (2H, m, H-5'), 2.43 (1H, m, H-2'), 2.11 (1H, m, H-2'); ^{13}C NMR (d_6 DMSO; 75Mhz) 41.8 (C-2'), 60.9 (C-5'), 69.8 (C-3'), 87.9, 88.5 (C-1', C-4'), 100.2 (C-5), 107.5 (C-4a), 116.5 (Phenyl-CH), 127.2 (*ipso*-C), 127.9 (Phenyl-CH), 136.4(C-4), 153.2 (*para*-C), 154.1 (C-6), 160.2 (C-2), 171.4 (C-7a); (^{19}F NMR (d_6-DMSO; 282 MHz); δ 111.53 (1F, m, Phenyl-CF). m/z 369.0 (100%, [M + Na])$^+$, 253.0 (24 %, [Base + Na]$^+$. FAB m/e 369.0864 (MNa$^+$ $C_{17}H_{15}FN_2O_5$ Requires 369.0863).

5-iodo-5'-O-trityl-2'-deoxyuridine (40)

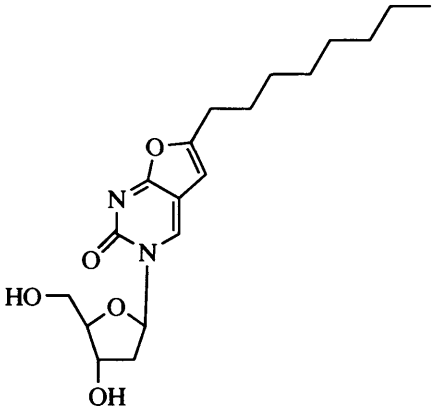


To a stirred solution of 5-iodo-2'deoxyuridine (2.98 g, 8.42 mmol) in pyridine (30 mL), was added trityl chloride (2.92 g, 10.47 mmol, 1.2 eq). The mixture was stirred for 5.5 h, at 100 °C, under a nitrogen atmosphere. After this time, the reaction mixture was concentrated in vacuo and the crude product purified by flash column chromatography (eluent: ethyl acetate/ dichloromethane (1:4)). The appropriate fractions were combined and the solvent was removed in vacuo to give 5-iodo-5'-O-trityl-2'-deoxyuridine as the pure product (4.01 g, 80%).

^{1}H NMR (d_6 DMSO; 300MHz) δ 11.80 (1H, s, NH), 8.05 (1H, s, H-6), 7.2-7.5 (15H, broad m, Ph), 6.06 (1H, dd, *J* = 6.61 Hz, H-1'), 5.38 (1H, d, *J* = 4.43 Hz, 3'-OH), 4.28 (1H, m, H-3'), 3.94 (1H, m, H-4'), 3.24 (2H, m, H-5'), 2.24 (2H, m, H-2' and H-2'); ^{13}C NMR (d_6 DMSO; 75 MHz) δ 40.2 (C-2'), 64.2 (C-5'), 70.8 (C-

3'), 85.1 and 86.0 (C-1' and C-4'), 86.7 (C-Ph_3), 127.5, 128.4, 128.6 (Phenyl-CH), 146.6 (C-6), 150.4 and 160.9 (C-2 and C-4).

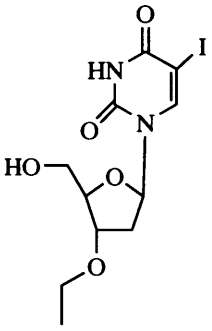
3-(2-deoxy-β-D-ribofuranosyl)-6-octyl-2,3,dihydrofuro-[2,3,d]pyrimidine-2-one (42) [Cf1368]



To a solution of IDU (3.31 g, 9.35 mmol) in DMF (30 mL) were added, DIPEA (3.0 mL,16.95 mmol, 1.8 eq.), 1-decyne (4.8 mL, 25.41 mmol, 2.7 eq.), tetrakis(triphenylphosphine)Pd(0) (837 mg, 0.85 mmol, 0.1 eq) and CuI (324 mg, 1.70 mmol, 0.2 eq.) and the mixture stirred overnight at room temperature under an atmosphere of nitrogen. Et_3N (15 mL) and CuI (322 mg, 1.69 mmol, 0.2 eq.) were added and the mixture stirred at 80 °C for 6 hr. The solvent was removed *in vacuo*, the residue dissolved in methanol/dichloromethane (1;1) (20 mL), excess amberlite (HCO_3^- form) was added and stirred for 30 min. The resin was filtered, washed with methanol and the filtrate concentrated. Column chromatography (gradient eluent: dichloromethane increase to 5% methanol in dichloromethane) yielded the product as a white powder (1.10 g, 32%).

^{1}H NMR (d_6 DMSO; 300MHz) δ 8.67 (1H, s, H-4), 6.43 (1H, s, H-5), 6.17 (1H, dd, J = 6.16 Hz, H-1'), 5.29 (1H, d, J = 4.26 Hz, 3'OH), 5.13 (1H, t, J = 5.11 Hz, 5'OH), 4.23 (1H, m, H-3'), 3.91 (1H, m, H-4), 3.65 (2H, m, H-5'), 2.64 (2H, t, J = 7.27 Hz, αCH_2), 2.44 (1H, m, H-2'), 2.07 (1H, m, H-2'), 1.62 (2H, m, βCH_2), 1.26 (10H, m, 5xCH_2), 0.85 (3H, t, J = 6.44 Hz, CH_3); ^{13}C NMR (d_6 DMSO; 75 MHz), δ 14.3 (CH_3), 26.7, 27.7, 28.7, 28.9, 29.0 (5 x CH_2), 31.6 (βCH_2), 39.4 (αCH_2), 41.5 (C-2'), 61.1 (C-5'), 70.0 (C-3'), 87.7, 88.4 (C-1' and C-4'), 100.1 (C-5), 106.7 (C-4a), 137.1 (C-4), 154.1 (C-2), 158.6 (C-6), 171.5 (C-7a).

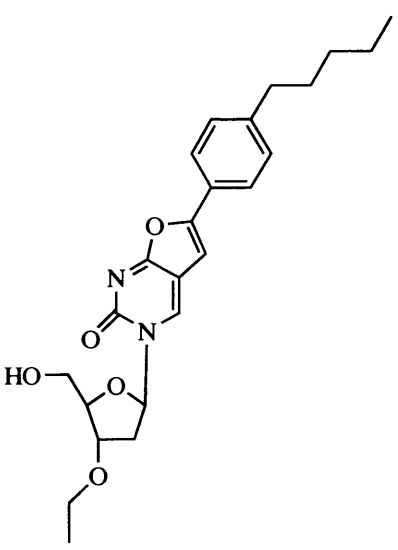
5-iodo-3'O-ethyl-2'-deoxyuridine (44a)



To a stirred solution of 5-iodo-5'-O-trityl-2'-deoxyuridine **1** (2 g, 3.36 mmol) in anhydrous tetrahydrofuran (THF) (35 mL), was added sodium hydride (269 mg, 60%, 6.71 mmol, 2 eq). The mixture was stirred for 30 min at room temperature under a nitrogen atmosphere. After this time, iodoethane (634 mg, 325 μL, 4.06 mmol, 1.2 eq) dissolved in THF (15mL) was added dropwise over a period of 1 h. The resulting mixture was stirred for 8 h at 50 °C. Excess sodium hydride was removed on addition of Dower 50W ion-exchange resin. The resin was filtered and the filtrate was concentrated in vacuo. The residue was extracted with ethyl ac etate and the extract washed with brine (3 x 50 mL) and dried over magnesium sulphate. The solvent was removed in vacuo and the crude product purified by flash column chromatography (eluent: petroleum ether/ ethyl acetate (3:7)). The appropriate fractions were combined, and the solvent removed in vacuo to give 5-iodo-3'-O-trityl-2'-deoxyuridine as the pure product (210 mg, 16%).

^{1}H NMR (d_6 DMSO; 300MHz) δ 11.71 (1H, s, NH), 8.38 (1H, s, H-6), 6.05 (1H, dd, J = 7.10 Hz, H-1'), 5.22 (1H, t, J = 4.75 Hz, 5'OH), 4.05 (1H, m, H-3'), 3.93 (1H, m, H-4'), 3.60 (2H, m, H-5'), 3.46 (2H, q, J = 6.89 Hz, $\underline{CH_2}CH_3$), 2.20 (2H, m, H-2'), 1.13 (3H, t, J = 6.96 Hz, $CH_2\underline{CH_3}$).

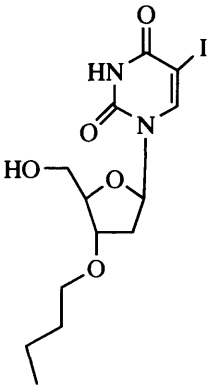
3-(2-deoxy-3-ethyl-β-D-ribofuranosyl)-6-(4-n-pentylphenyl)-2,3,dihydrofuro-[2,3,d]pyrimidine-2-one (45a) [Cf2517]



To a solution of **44a** (1.00 g, 2.62 mmol) in DMF (20 mL) were added, 4-n-pentylphenylacetylene (1.3 mL, 1.15 g, 5.22 mmol, 2.0 eq), tetrakis (triphenylphosphine) palladium(0) (312 mg, 0.27 mmol, 0.1 eq.), copper iodide (110 mg, 0.58 mmol, 0.2 eq.) and DIPEA (910 μL, 675 mg, 5.23 mmol, 2.0 eq.). The mixture was stirred at room temperature, overnight under an atmosphere of nitrogen. After which time was added, copper iodide (110 mg, 0.58 mmol, 0.2 eq.) and triethylamine (10 mL) and the mixture stirred at 100 °C for 5 hr. The solvent was removed in vacuo, the residue dissolved in methanol / dichloromethane (1:1) (20 mL) and stirred in the presence of excess amberlite (HCO_3^- form). The resin was filtered washed, with methanol, the filtrate concentrated and purified by column chromatography (eluent: gradient dichloromethane increased to 5% methanol in dichloromethane) to give the desired product (248 mg, 28%).

H nmr (d_6 DMSO; 300MHz) δ 8.82 (1H, s, H-4), 7.75 (2H, d, J = 8.07 Hz, Phenyl-CH), 7.34 (2H, d, J = 8.13 Hz, Phenyl-CH), 7.22 (1H, s, H-5), 6.16 (1H, t, J = 6.30 Hz, H-1'), 5.27 (1H, t, J = 5.19 Hz, 5'OH), 4.09 (1H, m, H-3'), 3.68 (1H, m, H-4'), 3.44-3.53 (5H, br, m, H-5', CH_2 and C-2'), 2.62, (2H, t, J = 7.55 Hz, αCH_2), 2.14 (1H, m, H-2'), 1.60 (2H, m, CH_2), 1.28 (4H, m, 2 x CH_2), 1.15 (3H, t, J = 6.95 MHz, CH_3), 0.87 (3H, t, J = 6.72 Hz, CH_3); ^{13}C NMR (d_6 DMSO; 75 MHz), δ 14.3 (CH_3), 15.6 (CH_3), 22.3, 31.8, 31.2, 35.3 and 38.8 (4 x CH_2 and C-2'), 61.4 and 64.3 (CH_2 and C-5'), 78.4 (C-3'), 86.2 and 88.1 (C-1' and C-4'), 99.0 (C-5), 107.4 (C-4a), 124.9 (Phenyl-CH), 126.2 (ipso -C), 129.4 (Phenyl-CH), 138.2 (C-4), 144.5 (para-C), 154.1 and 154. 3 (C-2 and C-6), 171.4 (C-7a).

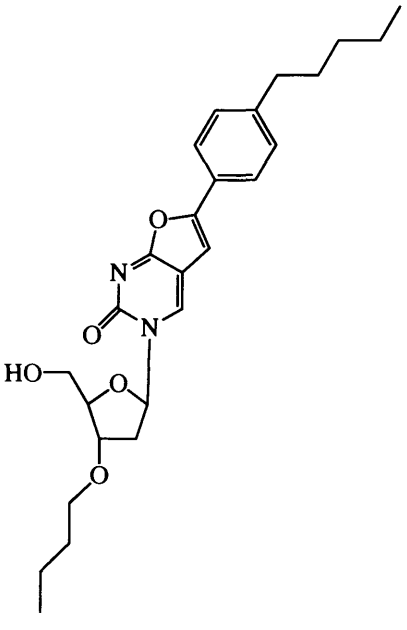
5-iodo-3'O-butyl-2'-deoxyuridine (44c)



To a solution of IDU (2.10 g, 3.52 mmol) in dioxane (30 mL) was added, NaH (60%, 269 mg, 6.72 mmol, 1.9 eq.) and iodobutane 1.4 mL, 2.26 g, 12.30 mmol, 2.5 eq.). The mixture was stirred at 90 °C overnight. After which time a precipitate was seen. The solvent was removed *in vacuo* and the residue dissolved in methanol, dowex 50W ion-exchange resin was added and stirred for 45 min. The resin was filtered, the filtrate concentrated and purified by column chromatography (eluent: gradient dichloromethane increased to 5 % methanol in dichloromethane) to give the desired product (402 mg, 28 %).

H nmr (d_6 DMSO; 300MHz) δ11.66 (1H, s, NH), 8.35 (1H, s, H-6), 6.04 (1H, t, *J* = 6.78 Hz, H-1'), 5.20 (1H, t, *J* = 4.38 Hz, 5'-OH), 4.06 (1H, m, H-3'), 3.96 (1H, m, H-4'), 3.64 (2H, m, H-5'), 3.44 (2H, CH_2), 2.23 (2H, m H-2'), 1.51 (2H, m, CH_2), 1.40-1.15 (2H, m, CH_2), 0.88 (3H, t, *J* = 6.58 Hz, CH_3).

3-(2-deoxy-3-butyl-β-D-ribofuranosyl)-6-(4-n-pentylphenyl)-2,3,dihydrofuro-[2,3,d]pyrimidine-2-one (45c) [Cf2516]

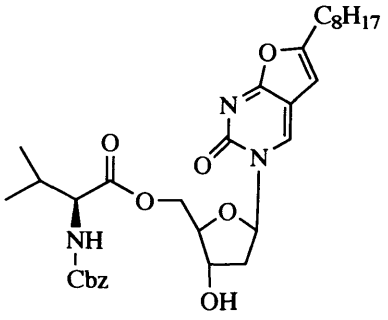


To a solution of **44c** (400mg, 0.98 mmol) in DMF (10 mL) were added, 4-n-pentylphenylacetylene (480 μL, 425 mg, 2.46 mmol, 2.5 eq.), tetrakis (triphenylphosphine) palladium(0) (115 mg, 0.10 mmol, 0.1 eq.), copper iodide (39 mg, 0.20 mmol, 0.2 eq.) and DIPEA (340 μL, 252 g, 1.96 mmol, 2.0 eq.). The mixture was stirred at room temperature, overnight under an atmosphere of nitrogen. After which time was added, copper iodide (39 mg, 0.20 mmol, 0.2 eq.) and triethylamine (3 mL) and the mixture stirred at 100 °C for 5 hr. The solvent was removed *in vacuo*, the residue dissolved in methanol /

dichloromethane (1:1) (20 mL) and stirred in the presence of excess amberlite (HCO_3^- form). The resin was filtered washed, with methanol, the filtrate concentrated and purified by column chromatography (eluent: gradient dichloromethane increased to 5% methanol in dichloromethane) to give the desired product (248 mg, 28%).

H NMR (d_6 DMSO; 300MHz) δ 8.81 (1H, s, H-4), 7.74 (2H, d, *J* = 8.20 Hz, Phenyl-CH), 7.33 (2H, d, *J* = 8.25 Hz, Phenyl-CH), 7.21 (1H, s, H-5), 6.15 (1H, t, *J* = 6.35 Hz, H-1'), 5.23 (1H, t, *J* = 5.23 Hz, 5'OH), 4.07 (2H, br, s, O$\underline{CH_2}$), 3.70 (2H, m, H-3' and H-4'), 3.45 (3H, m, H-5' and H-2'), 2.62 (2H, t, *J* = 7.63 Hz, αCH_2), 2.15 (1H, m, H-2'), 1.61 (2H, m, CH_2), 1.52 (2H, m, CH_2), 1.27 -1.37 (6H, m, 3 x CH_2), 0.89 (6H, 2 x CH_3); ^{13}C NMR (d_6 DMSO; 75 MHz), δ 13.7 (CH_3), 13.8 (CH_3), 18.8, 21.9, 30.3, 30.7, 31.3, 34.9 (6 x CH_2), 38.3 (C-2'), 61.1 (C-5'), 68.3 (CH_2), 78.2 (C-3'), 85.8 and 87.7 (C-1' and C-4'), 98.6 (C-5), 107.1 (C-4a), 124.5 (Phenyl-CH), 129.0 (Phenyl-CH), 137.8 (C-4), 144.2 (C-2),162.3 (C-6), 171.1 (C-7a).

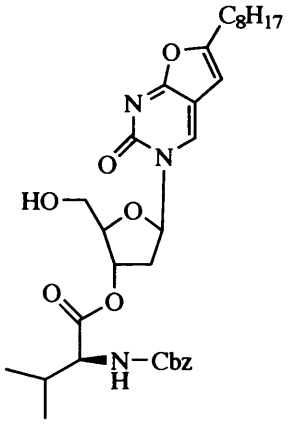
3-(2-deoxy-5-O-[N-Cbz-L-valinate]-β-D-ribofuranosyl)-2,3-dihydrofuran-6-octyl[2,3,d]pyrimidine-2-one (48)



To a stirred solution of **42** (2.08 g, 5.71 mmol) in DMF (150 mL), were added, DMAP (139 mg, 1.14 mmol, 1.1eq), N-Cbz-L-valine (1.89 g, 7.53 mmol, 1.3 eq) and DCC (1.70 g, 8.25 mmol, 1.4 eq). This mixture was stirred under a nitrogen atmosphere at room temperature for 24 h. The mixture was recharged with additional, DMAP (134 mg, 1.09 mmol, 0.2 eq), N-Cbz-L-valine (1.87 g, 7.45 mmol, 1.3 eq) and DCC (1.70 g, 8.25 mmol, 1.4 eq), and stirring continued for 6 days. The solvent was removed *in vacuo* and the product obtained after column chromatography (eluent: 1% methanol in dichloromethane (770 mg, 23%).

^{1}H NMR (d_6 DMSO; 300MHz) δ 8.39 (1H, s, H-4), 7.84 (1H, d, *J* = 7.63 Hz, NH), 7.32 (5H, m, Phenyl-CH), 6.41 (1H, s, H-5), 6.22 (1H, dd, *J* = 6.31 Hz, H-1') 5.50 (1H, d, *J* = 4.01 Hz, 3'-OH), 5.05 (2H, d, *J* = 12.49 Hz, $\underline{CH_2}$Ph), 4.22-4.38 (3H, m, H-3' and H-5'), 4.16 (1H, m, H-4'), 3.93 (1H, dd, *J* = 7.17 Hz, $\underline{CH}CH(CH_3)_2$), 2.62, (2H, t, *J* = 7.08 Hz, αCH_2), 2.09 (1H, m, H-2'), 2.11 (1H, m, H2'), 1.98 (1H, m, $CH\underline{CH}(CH_3)_2$), 1.59 (2H, m, βCH_2), 1.25 (10H, m, 5 x CH_2), 0.87 (9H, m, 3 x CH_3); ^{13}C NMR (d_6-DMSO; 75 MHz) δ 14.3 (CH_3), 18.8 and 19.3 (2 x CH_3), 22.4, 26.7, 27.6, 28.7, 28.9 and 29.0 (6 x CH_2), 29.85 ($CH\underline{CH}(CH_3)_2$), 31.58 (αCH_2), 41.23 (C-2'), 60.49 ($\underline{CH}CH(CH_3)_2$), 64.69 (C-5'), 65.98 ($\underline{CH_2}$Ph), 70.57 (C-3'), 84.99 (C-4'), 87.91 (C-1'), 100.25 (C-5), 107.08 ($C\text{-}4_a$), 128.11, 128.24 and 128.69 (Phenyl-CH), 136.68 (C-4), 137.10 (Phenyl-C), 154.09 (C-2), 156.86 (C-6), 158.61($\underline{C}OOCH_2$Ph), 171.63 ($C\text{-}7_a$), 172.25 ($(CH_3)_2CHCH\underline{C}OO$).

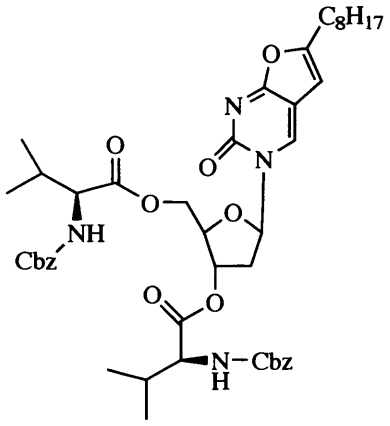
3-(2-deoxy-3,-O-[N-Cbz-L-Valinate]-β-D-ribofuranosyl)-6-octyl-2,3-dihydrofuran- [2,3,d]pyrimidine-2-one (49)



Obtained from the reaction above **(48)**, following additional column chromatography (eluent: 60 % ethyl acetate in hexane) and preparative TLC (eluent: 10 % methanol in dichloromethane to give the product (70 mg, 3%).

^{1}H NMR (d_6 DMSO; 300MHz) δ 8.64 (1H, s, H-4), 7.81 (1H, d, *J* = 7.82 Hz, NH), 7.35 (5H, m, Phenyl-CH), 6.46 (1H, s, H-5), 6.22 (1H, m, H-1'), 5.28 (2H, m, 5'-OH), 5.09 and 5.04 (2H, d, *J* = 12.49 Hz, $\underline{CH_2}$Ph), 4.11 (1H, m, H-4) 3.97 (1H, dd, *J* = 7.02 Hz, $\underline{CH}CH(CH_3)_2$), 3.67 (2H, m, H-5'), 2.66 (2H, t, *J* = 7.21 Hz, αCH_2), 2.54 (1H, m, H-2'), 2.29 (1H, m, H-2'), 2.09 (1H, m, $CH\underline{CH}(CH_3)_2$), 1.60 – 1.25 (10H, m, 5 x CH_2), 0.92 (6H, d, *J* = 6.72 Hz, $CHCH(\underline{CH_3})_2$), 0.86 (3H, t, *J* = 6.49, CH_3). MS (ES^+) M/Z 620 (100%, $[MNa]^+$), 248 (40%, [base H]$^+$).

3-(2-deoxy-3,5-O-[N-Cbz-L-Valinate]-β-D-ribofuranosyl)-6-octyl-2,3-dihydrofuran [2,3,d]pyrimidine-2-one (50)

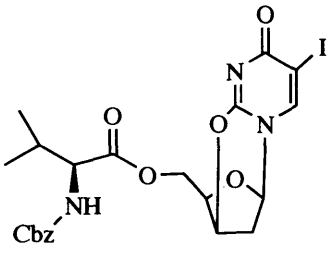


Obtained from the reaction above **48.** The product was obtained by further column chromatography (eluent: 60 % ethyl acetate in hexane (6:4)) and preparative TLC (eluent: 10 % methanol in dichloromethane) to give the product as a foam (460 mg, 10%)

^{1}H NMR (d_6 DMSO; 300MHz) δ 8.37 (1H, s, H-4), 7.84 (2H, d, *J* = 7.48 Hz, 2 x NH), 7.36 (10H, m, Phenyl-CH), 6.40 (1H, s, H-5), 6.19 (1H, dd, *J* = 6.40 Hz, H-1'), 5.22 (1H, m, H-3'), 5.04 (4H, m, 2 x $\underline{CH_2}$Ph), 4.33 (2H, m, H-5'), 3.89 – 4.07 (3H, m, H-4' and 2 x $(CH_3)_2CH\underline{CH}$), 2.62 (2H, t, *J* = 7.05 Hz, αCH_2), 2.58 (1H, m, H-2'), 2.37 (1H, m, H-2'), 2.08 (1H, m, $(CH_3)_2\underline{CH}CH$), 1.95 (1H, m, $(CH_3)_2\underline{CH}CH$), 1.15 – 1.26 (10H, m, 5 x CH_2), 0.84 – 0.94 (15H, m, 5 x CH_3); ^{13}C NMR (d_6-DMSO; 75 MHz) δ 14.3 (CH_3), 18.8 and 19.3 ($(CH_3)_2$), 22.4, 26.7, 27.6, 28.6, 29.0 and 29.00 (6 x CH_2), 29.7 and 30.0 ($CH\underline{CH}(CH_3)_3$, 31.6 (αCH_2), 39.0 (C-2'), 60.2 and 60.6 ($\underline{CH}CH(CH_3)_2$), 64.6 (C-5'), 66.0 and 66.1 ($\underline{CH_2}$Ph), 75.1 (C-3'), 82.5 (C-4'), 88.0 (C-1'), 100.3 (C-5), 107.3 (C-4a), 128.2, 128.7 and 128.7 (Phenyl-CH), 136.58 (C-4), 137.22 (Phenyl-C), 154. 07 (C-2), 156.87 (C-6), 158.77 ($\underline{C}OOCH_2$Ph), 171.78 (C-7a), 172.09 ($(CH_3)_2CHCH\underline{C}OO$);

MS (ES^+) M/Z 853 (100% $[MNa]^+$), 831 (60% $[MH]^+$), 247 (70% $[base]^+$).

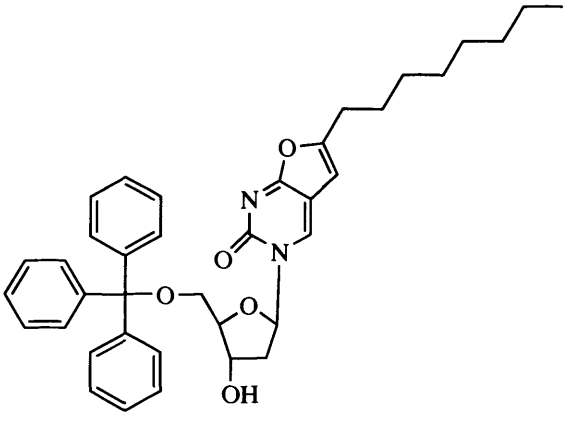
5-iodo-3'-2anhydro-2'-deoxyuridine (51)



To a solution of triphenylphosphine (4.34 g, 16.56 mmol, 5.3 eq.), in DMF (20 mL) dropwise at 0°C, DIAD (3.2 mL, 3.29 g, 16.26 mmol, 5.2 eq and the misxtures stirred for 30 min. After this time a solution of IDU (1.10 g, 3.11 mmol) and Boc-L-alanine (1.03 g, 5.44 mmol, 1.8 eq.) in DMF (15 mL) were added and stirring continued at 0°C for a further 20 min before allowing to reach room temperature and stirring overnight. The product was obtained following column chromatography (eluent: 10 methanol in dichloromethane) (1.58 g, 43 %).

^{1}H NMR (d_6 DMSO; 300MHz) δ 8.32 (1H, s, H-6), 7.30 (1H, d, *J* = 7.15 Hz, NH), 5.96 (1H, d, *J* = 3.63 Hz, H-1'), 5.37 (1H, s, H-3'), 4.31-4.43 (2H, m, H-4' and H-5'), 4.12 (1H, m, H-5'), 3.96 (1H, m, C<u>H</u>CH_3), 2.66 (1H, m, H-2'), 2.52 (1H, m, H-2'), 1.35 (9H, s, $(CH_3)_3$), 1.22 (3H, d, *J* = 7.31 Hz, CH_3CH); ^{13}C NMR (d_6 DMSO; 75 MHz) δ 17.1 (CH_3), 28.5 ($(CH_3)_3$), 32.8 (C-2'), 49.21 (<u>C</u>HCH_3), 62.9 (C-5'), 78.0 (C-3'), 81.1 (C-5), 82.5 (C-4'), 87.6 (C-1'), 145.7 (C-6), 154.3 (CH<u>C</u>OO), 155.6 (C-2), 167.2 (C-4), 173.2 (NH<u>C</u>O).

3-(2-deoxy-5-trityl-β-D-ribofuranosyl)- 6-octyl-2,3-dihydrofuro- [2,3-*d*] pyrimidine-2-one (52)



via 5-iodo-5'-O-trityl-2'-deoxyuridine

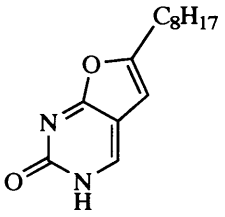
To a stirred solution of 5-iodo-5'-O-trityl-2'-deoxyuridine (570 mg, 0.96 mmol) in THF (25 mL) were added, diisopropylethylamine (371 mg, 500 μL, 2.88 mmol, 3 eq), 1-decyne (709 mg, 925 μL, 5.13 mmol, 5 eq), tetrakis(triphenylphosphine)palladium(0) (166 mg, 0.144 mmol, 0.15 eq) and copper iodide (55 mg, 2.88 mmol, 3 eq). The above solution was stirred for 20 h at room temperature. After this time, were

added, copper iodide (53 mg, 2.77 mmol, 2.9 eq) and triethylamine (12 mL). This mixture was stirred for 4 h at 60 °C. The reaction mixture was then concentrated in vacuo, and the resulting residue was dissolved in methanol/dichloromethane (1:1) (40 mL), whereupon Amberlite IRA-400 (HCO_3^- form) was added and stirred for 30 min. The resin was filtered and washed with methanol, and the combined filtrate was evaporated to dryness. The crude product was purified by flash column chromatography, (eluent: petroleum ether/ethyl acetate (1:3)) to produce 3-(2'deoxy-5'trityl-β-D-ribofuranosyl)-6-octyl-2,3-dihydrofuro- [2,3-*d*]pyrimidine-2-one as a white solid (370 mg, 64%).

via 3-(2-deoxy-β-D-ribofuranosyl)-2,3-dihydrofuro-6-octyl[2,3-*d*]pyrimidine-2-one: To a stirred solution of 3-(2-deoxy-β-D-ribofuranosyl)-2,3-dihydrofuro-6-octyl[2,3*d*]pyrimidine-2-one (570 mg, 1.56 mmol) in pyridine (10 mL), was added trityl chloride (468 mg, 1.68 mmol, 1 eq). The mixture was stirred for 6 h at 100 °C under a nitrogen atmosphere. After this time, the solvent was removed in vacuo and the residue extracted with dichloromethane, washed with brine (3 x 50 mL) and dried over magnesium sulphate. The solvent was removed in vacuo and the crude purified by flash chromatography (eluent: methanol/dichloromethane (0.5:9.5)), to give the product, (130 mg, 14%).

^{1}H NMR (d_6-DMSO; 300MHz) δ 8.52 (1H, s, H-4), 7.2-7.6 (15H, m, Phenyl-CH), 6.15 (1H, dd, *J* = 5.06 Hz, H-1'), 5.70 (1H, s, H-5), 5.44 (1H, d, *J* = 4.82 Hz, 3'OH), 4.38 (1H, m, H-3'), 4.02 (1H, m, H-4'), 3.27 (2H, m, H-5'), 2.60 (2H, t, *J* = 7.26, αCH_2), 2.44 (1H, m, H-2'), 2.22 (1H, m, H-2'), 1.57 (2H, br, βCH_2), 1.24 (10H, m, 5 x CH_2), 0.85 (3H, t, *J* = 6.35 Hz, CH_3).

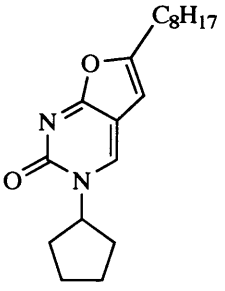
6-Octyl-3-*H*-furo[2,3-*d*]pyrimdine-2-one (55)



To a solution of 5-iodouracil (5.09 g, 21.39 mmol) in DMF (50 mL) was added, CuI (805 mg, 4.21 mmol, 0.2 eq.), tetrakis (triphenylphosphine)palladium(0) (960 mg, 0.83 mmol, 0.04 eq.), DIPEA (7.3 mL, 5.43 g, 41.99 mmol, 2.0 eq.) and 1-decyne (11.4 mL, 8.73 g, 63.15 mmol, 3.0 eq.) and stirred overnight at room temperature overnight under an atmosphere of nitrogen. CuI (806 mg, 4.22 mmol, 0.2 eq) and Et_3N (25 mL) were added and the mixture stirred at reflux for 5 hr. After which time, the solvent was reduced to approximately 30 mL *in vacuo* and then filtered and washed with methanol to give a grey powder (3.6 g, 68 %).

NMR data shown above for the cleaved base **(32)**

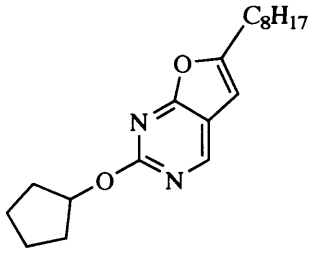
6-octyl-3-cyclopentyl-3*H*-furo[2,3-*d*]pyrimidine-2-one (56)



To a suspension of **(55)** (3.60 g, 14.50 mmol) in DMF (60 mL) was added KCO_3 (4.01 g, 29.01 mmol, 2.0 eq) and cyclopentyl bromide (3.1 mL, 4.31 g, 28.91 mmol, 2.0 eq.) and the above mixture was stirred at reflux overnight. The solvent was removed *in vacuo* and the residue dissolved in dichloromethane and extracted with a saturated solution of sodium chloride. The organic extracts were collected dried on magnesium sulphate, filtered and the filtrate concentrated and purified by column chromatography (gradient eluent: dichloromethane increased to 5 % methanol in dichloromethane) to give the product (720 mg, 16 %).

^1H NMR ($CDCl_3$; 300 Mhz) δ 7.87 (1H, s, H-4), 6.17 (1H, s, H-5), 5.34 (1H, m, CH), 2.73 (2H, t, J = 7.35 Hz, αCH_2), 2.35 (2H, m, βCH_2), 1.85-1.70 (10H, br, m, 5 x CH_2), 1.36 (8H, 4 x CH_2), 0.97 (3H, m, CH_3); ^{13}C NMR ($CDCl_3$; 75Mhz) δ 14.5 (CH_3), 23.1, 24.1, 27.2, 28.7, 29.5, 29.6, 29.6, 32.3 and 32.8 (9 x CH_2), 59.7 (CH), 98.9 (C-5), 108.3 (C-4a), 135.6 (C-4), 156.2 (C-6), 160.4 (C-2) 171.6 (C-7a).

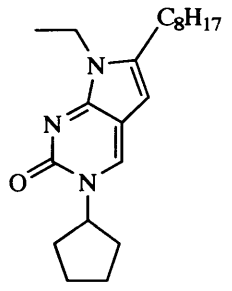
6-octyl-2-cyclopentyl-3*H*-furo[2,3-*d*]pyrimidine-2-one (57)



The desired analogue was obtained from the above reaction as a white powder (3.82 g, 83 %).

^{1}H NMR ($CDCl_3$; 300 Mhz) δ 8.68 (1H, s, H-4), (1H, s, H-5), 5.68 (1H, br, s, CH), 2.84 (2H, t, J = 7.50 Hz, αCH_2), 1.72 – 2.15 (10H, m, βCH_2 and 4 x CH_2), 1.39 (10H, m, 5 x CH_2), 0.98 (3H, t, J = 6.44 Hz, CH_3); ^{13}C NMR ($CDCl_3$; 75Mhz) δ 14.5 (CH_3), 23.1, 24.2, 27.6, 28.8, 29.5, 29.6, 29.7, 32.3 and 33.2 (9 x CH_2), 80.4 (CH), 99.5 (C-5), 114.0 (C-4a), 150.9 (C-4), 159.0 (C-6), 162.6 (C-2), 168.8 (C-7a).

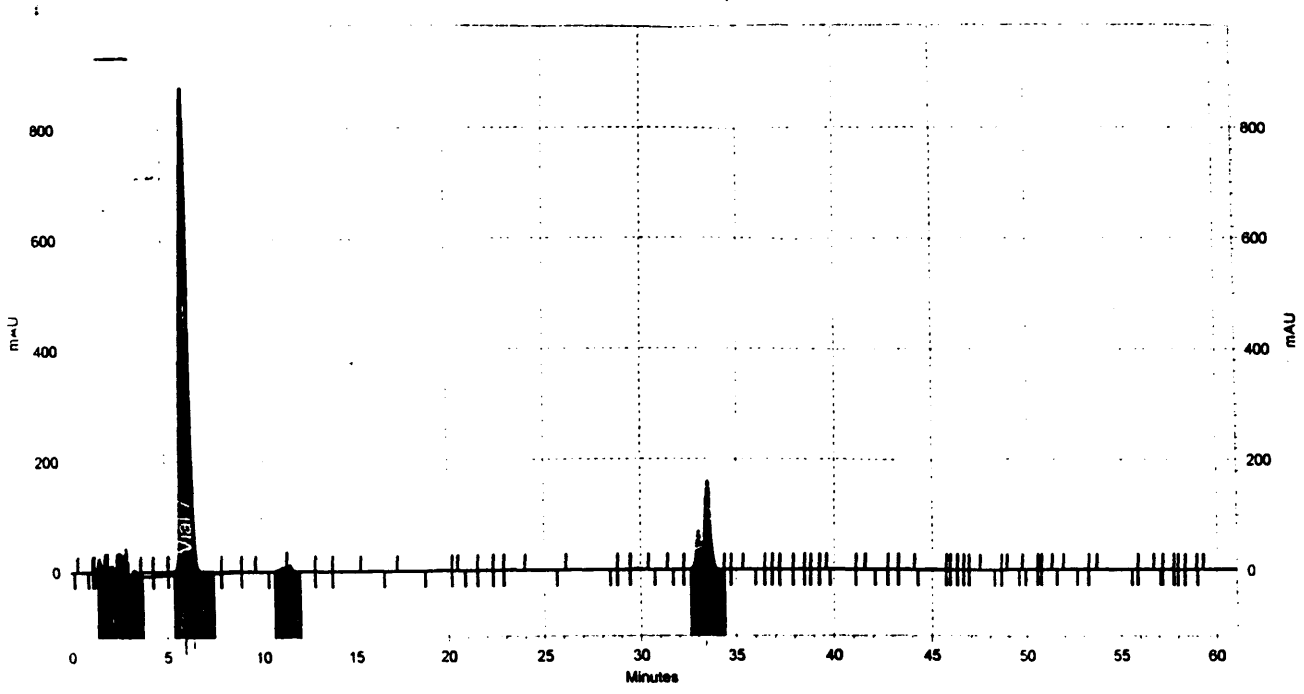
6-octyl-3*H*-N-ethyl-pyrrolo[2,3-*d*]pyrimidine-2-one (58)



56 (100 mg, 0.32 mmol) was suspended in a solution of ethyl amine (70%) (6 mL) and methanol (5 mL) and heated at 60 °C in a sealed tube for 24 hr. The mixture was transferred to a round bottom flask and the solvent removed *in vacuo*. The residue was dissolved in dioxane, toluene (25 mL) was added and the mixture heated under reflux using dean stark apparatus in the presence of *p*-toluenesulfonyl chloride (5.8 mg, 0.03 mmol, 0.1 eq.).

^{1}H NMR ($CDCl_3$; 300Mhz) δ 7.82 (1H, s, H-4,), 5.92 (1H, s, H-5), 5.41 (2H, t, dd, J = 7.91 Hz, CH), 4.08 (2H, q, J = 7.15 Hz, N-$\underline{CH_2}CH_3$), 2.62 (2H, t, J = 7.53 Hz, αCH_2), 2.28 (2H, m, βCH_2), 1.63-1.91 (8H, br, m, 4 x CH_2), 1.33 (13H, m, 5 x CH_2 and 1 x CH_3), 0.93 (3H, t, J = 6.62 Hz, CH_3); ^{13}C NMR ($CDCl_3$; 75Mhz) δ 14.5 (CH_3), 15.5 (CH_3), 23.1, 24.6, 27.1, 28.0, 29.6, 29.8, 30.0, 32.2, 33.0 and 36.5 (10 x CH_2), 58.7 (CH), 96.1 (C-5), 109.7 (C-4a), 134.4 (C-4), 144.2 (C-6), 156.5 (C-2), 157.9 (C-7a).

Annette Angell



—— C:\CLASS-VP\Data\Federica\ana115, Detector A-252 nm

0

70% H_2O

30% CH_3CN

30'

50% H_2O

50% CH_3CN

0%

100% CH_3CN