DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL NUCLEOTIDE PRODRUGS AS POTENTIAL ANTI HEPATITIS C VIRUS AGENTS

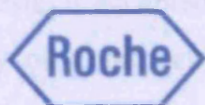CARDIFF UNIVERSITY

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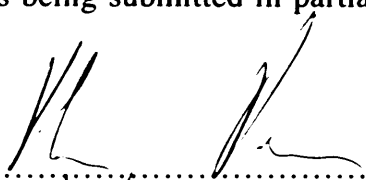Date.........11/02/2007..............

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Acknowledgements

Abstract

Hepatitis C virus (HCV) represents the leading cause of liver disease. Most of the patented potential anti-HCV agents are nucleosides. Usually, to be active, nucleosides need to be phosphorylated three times in order to generate the corresponding 5'-triphosphate inside cells. Our group has developed the phosphoramidate approach that bypasses the first phosphorylation step and releases the 5'-monophosphate intracellulary.

Part of the presented work was focused on the synthesis and the biological evaluation of phosphoramidates of novel 4'-modifed nucleosides (AZC, AZU, AZA, *dipentanoyl*-AZC and ETU).

Phosphoramidates with different natural (L-alanine, L-valine, glycine, L-leucine, L-isoleucine, L-methionine, L-proline, L-valine and L-phenylalanine) and unnatural (cyclopentylglycine, dimethylglycine, β-alanine, N-methylglycine, L-ethylaspartate and D-alanine) amino acid were synthesised.

Octyl, dodecyl (never synthesised before), methyl, ethyl, isopropyl, benzyl, 2-butyl, butyl and *tert*-butyl are all the variation on the ester part of the phosphoramidate structures synthesised.

α- and β-naphthyl, 8-quinoline and different substituents on the phenyl ring (*para*-chloro, 3,4-dichloro, *para*-methyl and *para*-methoxy) are the major examples of aryl moiety variations applied to these nucleosides.

The protection of the 2'- and 3'-positions with cyclopentylidene and subsequent deprotection significantly increased the overall yield of these reactions.

The optimisation of the synthesis of AZA was achieved and it represents the first example of a 4'-modified ribo-purine nucleoside active against HCV. The application of our technology to AZA and AZU converted an inactive nucleoside into a sub-micromolar active phosphoramidate. The separation of the two diastereoisomers was achieved for some of the AZC phosphoramidates synthesised.

A series of novel phosphoramidates of 2'-methyl modified purine (adenosine and guanosine) were synthesised using the 2'-, 3'-protection previously optimised. The aryl moiety (α-naphthyl and phenyl) and ester (methyl, ethyl, *tert*-butyl, benzyl) variations were explored using L-alanine as amino acid. A general enhancement of activity was observed applying the phosphoramidate technology to these nucleosides.

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Chapter One

Nucleoside analogues in the treatment of HCV infections

1.1 Hepatitis C Virus (HCV)

1.1.2 General characteristics of HCV

The Hepatitis C Virus (HCV) was identified for the first time in 1989 as a single-stranded positive sense RNA virus of the flaviviridae family.[1]

According to the WHO (World Health Organisation), more than 170 million people are chronically infected by this virus, which is the main cause of liver disease.[2] This includes over 4 million Americans, and is projected to cost a staggering $400 billion or $100,000 per patient for lifetime healthcare maintenance (excluding liver transplantation costs). The Centre for Disease Control (CDC) estimates that HCV costs the United States more than $600 million annually. By 2015 as many as 40,000 Americans are expected to die annually as a consequence of HCV-related complications; already the prevalence of HCV infections is four times greater than HIV in the United States and the death rate from hepatitis C is expected to surpass that of AIDS in the near future. WHO has estimated the percentage of people for each continent that are currently infected by HCV (**Table 1.1**).[2]

WHO Region	Total Population (Millions)	Hepatitis C Prevalence (Rate%)	Infected Population (Millions)
Africa	602	5.3	31.9
Americas	785	1.7	13.1
Eastern Mediterranean	466	4.6	21.3
Europe	858	1.03	8.9
South-East Asia	1500	2.15	32.2
Western Pacific	1600	3.9	62.2
Total	5811	3.1	169.7

Table 1.1. HCV estimated world infection prevalence[2]

The most reliable data suggests that 3.1% of the worldwide population is infected by HCV, with the highest percentage in Africa and Eastern Mediterranean countries (**Table 1.1**). Approximately 85% of individuals initially infected with this virus will become chronically infected, of which 20–30% progress onto liver cirrhosis. The other 15% of HCV infected individuals simply have an acute infection that resolves spontaneously in a few weeks or months.[3, 4] The major routes of transmission of this virus are injecting drug use, transfusion, occupational exposure and sexual transmission (**Graph 1.1**).[5]

Graph 1.1. Sources of infection for people with HCV[2]
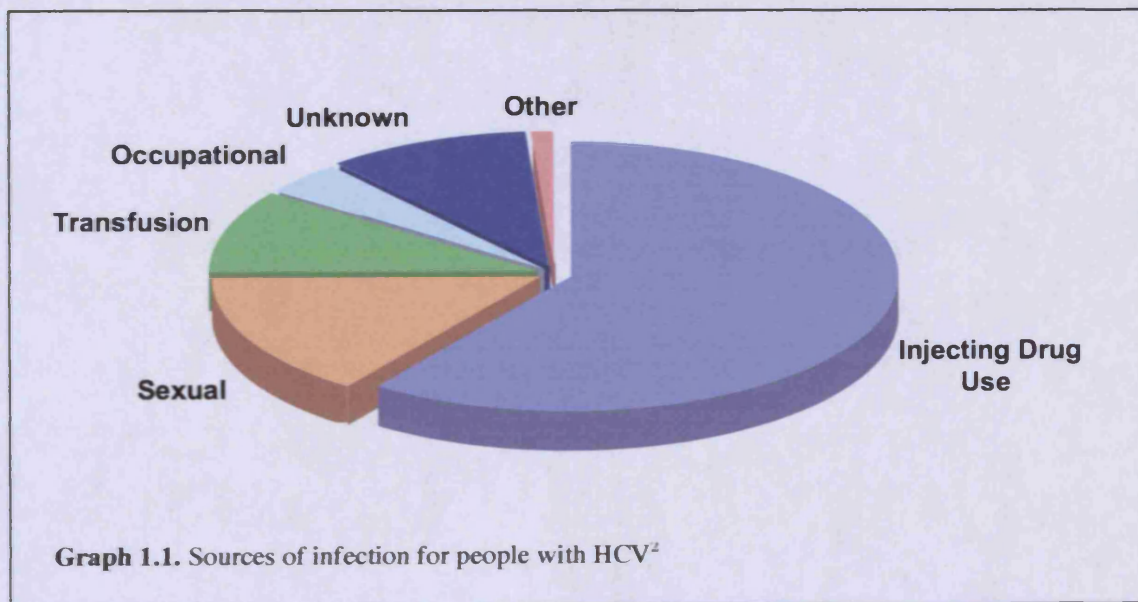
1.1.2 HCV structure

The structural components of HCV comprise the core, which contains the genome, and two heavily glycosylated envelope glycoproteins E1 and E2 (**Figure 1.1**).[6, 7]

The envelope glycoprotein 1 (E1) and 2 (E2) are referred to as hypervariable regions 1 and 2 since they are susceptible to an extraordinarily high mutation rate.[6]

Figure 1.1. HCV organisation
http://www.rit.edu/~japfaa/infectious.html
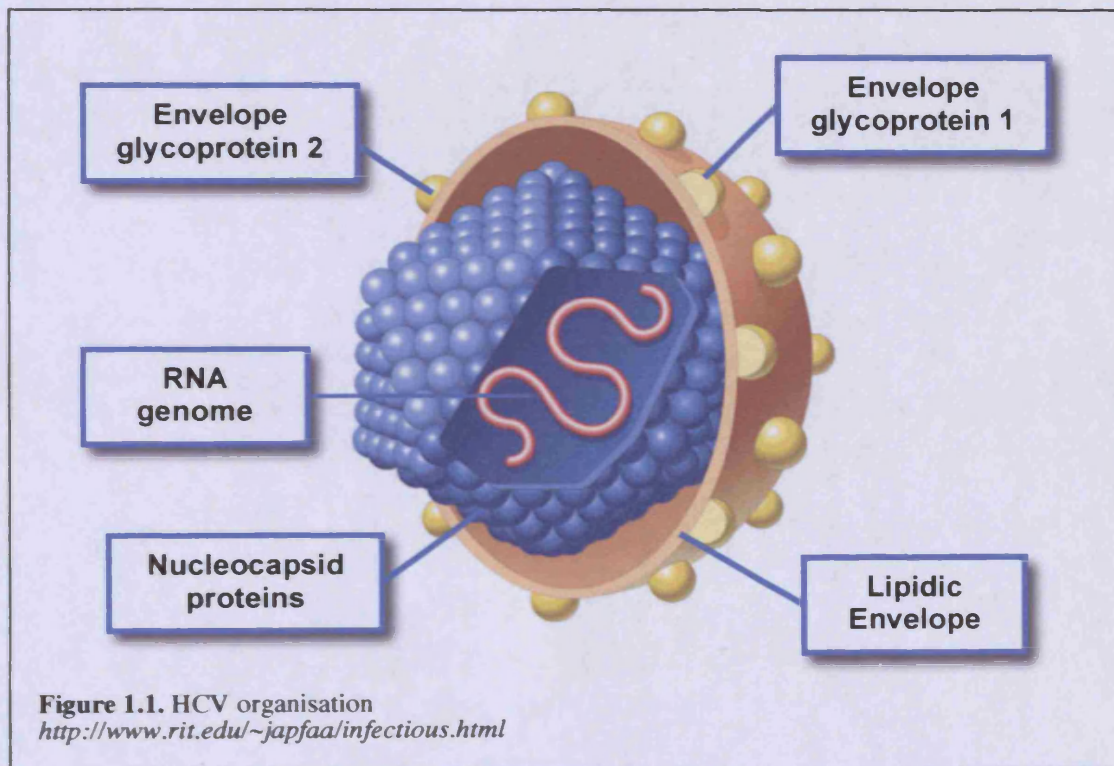
In the HCV genome, which is composed of 9600 nucleotides,[1] there are three different regions: regions encoding for structural and non-structural proteins, and the 5'- and 3'- Non-Translated Regions (5'-NTR and 3'-NTR, **Figure 1.2**) at the extremities of the HCV genome.[16, 17]

Figure 1.2. HCV: genome organisation
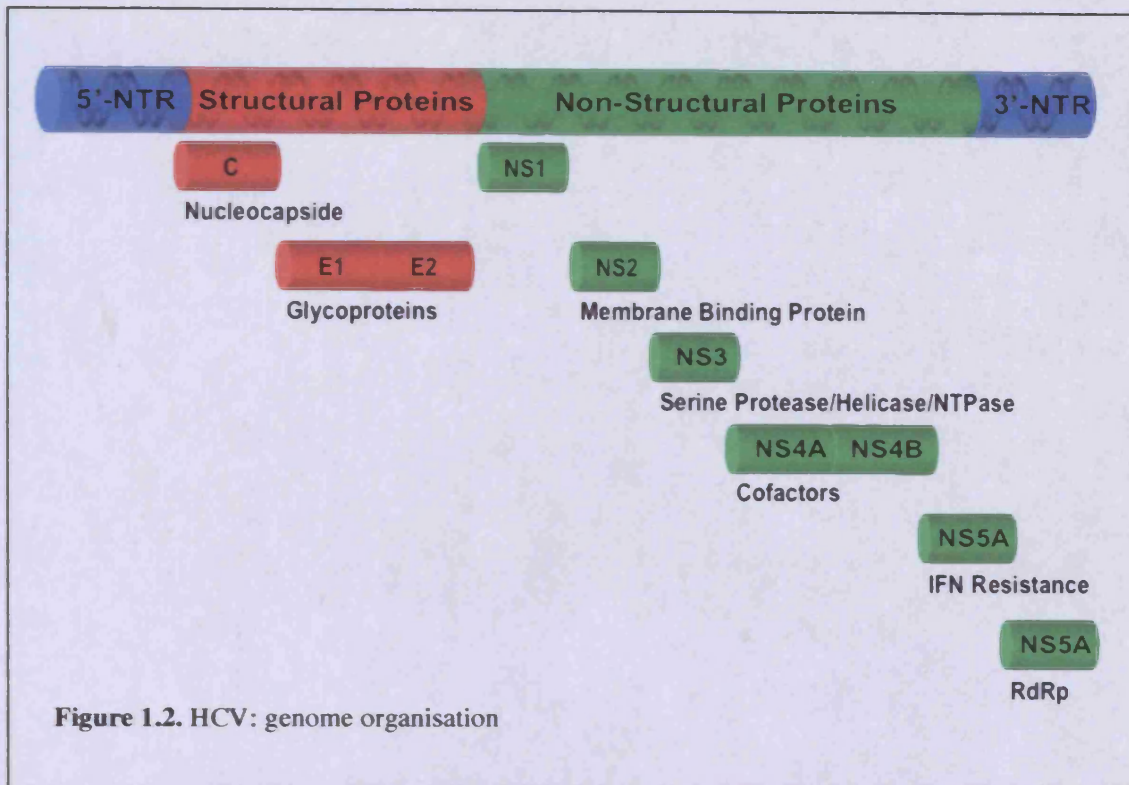
The 5'NTR is also called the Internal Ribosome Entry Site (IRES) and it allows the transcription of the viral genome by the ribosome of the host cell.[6, 7] The 5'-NTR contains 341 nucleosides organised in different domains. The AUG triplet, present in the fifth domain, is structurally important because it is the initiator sequence for the interaction of the ribosome of the host cell.

The region encoding the polyprotein precursor is divided into different domains: C, E1 and E2 (which encode for structural proteins) and NS1, NS2, NS3, NS4A/B and NS5A/B (which encode for non-structural proteins).[8, 9]

C, E1 and E2 have a hydrophobic C terminus. This feature is thought to be important for the membrane association and the cleavage from the polyprotein by the host signal peptidases. The C domain encodes for a non-glycosylated protein C that, complexed with genomic RNA, forms the nucleocapsid:[8, 9] this is also important as it represents a signal sequence that directs the E1 and E2 to the lumen of the endoplasmic reticulum (ER). Adjacent to C, there are two domains E1 and E2 that code for the envelope glycoproteins E1 and E2.[8, 9] Once synthesised, E2 is modified by N-linked glycosylation in the ER.

The NS1 domain function is actually unknown, while the NS2 encodes for a membrane-binding protein.[10]

The NS3 encodes for three different proteins:[11, 12, 13] serine protease (located at the N-terminus),[11, 12] helicase[13] and nucleotide triphosphatase (located at the C-terminus).[11, 12] The serine protease activity is responsible for the cleavage of polyproteins to release the NS3/4A, NS4A/B, NS4B/5A and NS5A/B.[11, 12] Furthermore, helicase unwinds duplex RNA structures that are essential during the virus replication.[11, 12]

The NS4 has two different domains: NS4A and NS4B. NS4A is an essential cofactor of the NS3 proteinase and is required for efficient polyprotein processing. The function of NS4B is not yet known. NS5A is a highly phosphorylated protein. The level of phosphorylation is influenced by NS4A via direct interaction with NS5A.[21] The role that NS5A may play in RNA replication is so far unknown; presumably it is an important regulator of replication and is possibly responsible for interferon resistance.[14, 15]

The last domain (NS5B) encodes for RNA-dependent RNA-polymerase (RdRp), which is very important during viral replication,[16] and a key target of anti-HCV therapy. The RdRp has a molecular mass of 68 kDa.[16]

1.1.3 HCV replication

The hypothetical model of HCV replication starts from the interaction between the viral envelope glycoprotein E2 and a protein of the host cell called CD81 (**Figure 1.3**).[17, 18, 19]

Figure 1.3. HCV replication
http://www.genelabs.com/research/HCV_LifeCycle.jpg
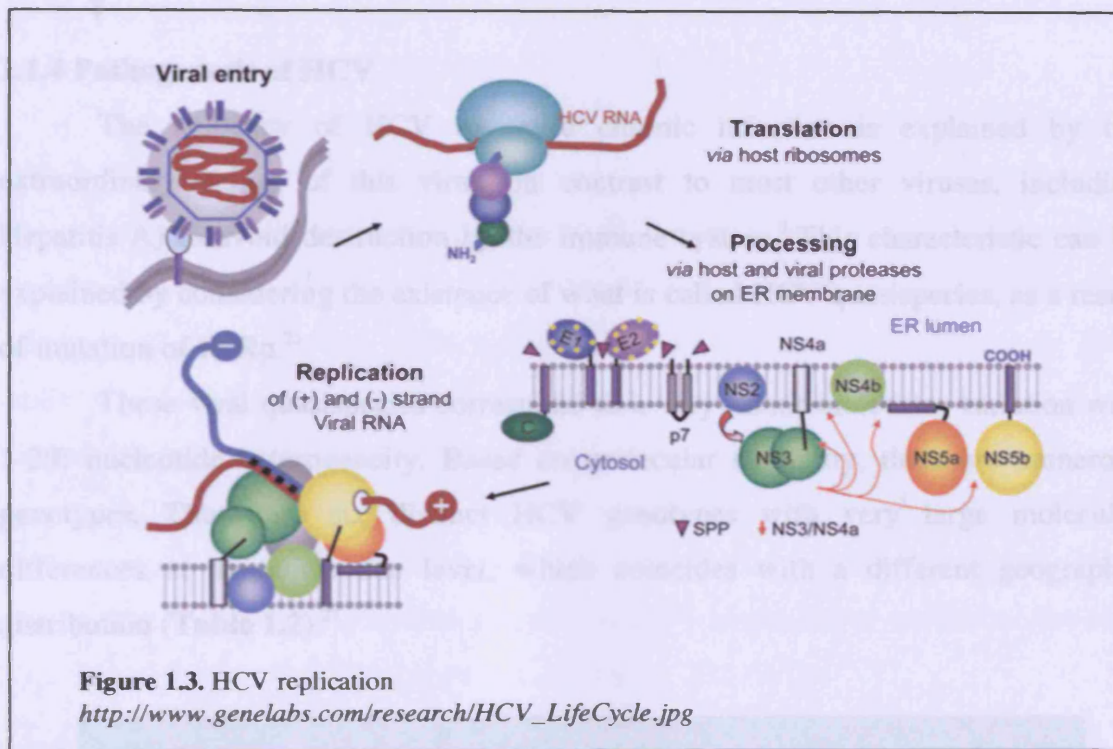
CD-81 is a receptor expressed on hepatocytes and B lymphocytes. CD-81 is a membrane-associated protein in which there is a Large Extracellular Loop (LEL) that can bind to the HCV, but the mechanism of internalisation of the virus is unknown.[18]

The viral genome (plus-RNA) is then released into the cell and enters into the cytoplasm. Due to the presence of the 5'-NRT, the viral genomes can bind to the ribosomes and express the encoding polyprotein precursors.[19] The result is the synthesis of a polyprotein that is cleaved by the viral serine protease (coded by NS3 subunit) to release all the viral proteins that remain tightly associated with membranes of the endoplasmic reticulum.[20, 21]

The plus-RNA is also used for the synthesis of minus-RNA by the replicase formed by a complex between NS3, NS4A and NS5B.[21]

This new RNA strand is used as a template for the production (by RdRp) of an excess amount of positive strand (plus-RNA)[21] that interacts with the structural proteins to be encapsidated.[21]

Viruses are encapsulated, by budding, into the lumen of the ER and then exported out of the host cells through transit in the Golgi complex.

1.1.4 Pathogenesis of HCV

The tendency of HCV to cause chronic infection is explained by the extraordinary ability of this virus (in contrast to most other viruses, including Hepatitis A) to avoid destruction by the immune system.[3] This characteristic can be explained by considering the existence of what is called HCV quasispecies, as a result of mutation of RdRp.[21]

These viral quasispecies correspond to a very minor molecular variation with 1-2% nucleotide heterogeneity. Based on molecular similarity, there are numerous genotypes. There are six distinct HCV genotypes with very large molecular differences at the nucleotide level, which coincides with a different geographic distribution (**Table 1.2**).[21]

Genotype	Geography
Type 1	Japan/China/Russia/US
Type 2	Western Europe
Type 3	North America
Type 4	Central and North Africa/ Middle East
Type 5	South Africa
Type 6	South-East Asia

Table 1.2. HCV genotypes and their geographical distribution

Indeed, HCV like several other RNA viruses, does not circulate in infected individuals as a homogeneous population of identical viral entities, but as a pool of genetically distinct but closely related variants.[21]

The principal effect of virus infection in humans is liver damage (cirrhosis and hepatocellular carcinoma),[21,22] but the causes are not completely understood. The virus itself probably does not cause liver cell damage directly. Indeed, the level of the virus in the blood does not correlate with the actual liver damage seen in a liver biopsy.[22]

Liver damage in chronic HCV is probably caused by the interplay between the virus and the immune system, which includes cytotoxic lymphocytes and specific inflammatory messengers (cytokines).[21]

Several extra-hepatic conditions are associated with chronic Hepatitis C.[23] These conditions are not very common and their occurrence does not correlate with the severity of the underlying liver disease.[23] The most widely described associated condition is cryoglobulinaemia.[24,25] This condition is due to the presence of abnormal antibodies (cryoglobulins) produced by HCV stimulation of lymphocytes. These antibodies can deposit in small blood vessels, thereby causing inflammation of the vessels in tissues throughout the body.[24,25,26]

Patients with cryoglobulinaemia can have quite a variety of symptoms. These symptoms may include weakness, joint pain or swelling (arthralgia or arthritis), a raised purple skin rash (palpable purpura) usually in the lower portion of the legs, swelling of the legs and feet due to loss of protein in the urine.[26]

In addition, these patients may develop Raynaud's phenomenon, in which the fingers and toes change colour (white, then purple, then red) and become painful in cold temperatures.[26]

1.1.5 Current treatment

Until now, the only marketed treatment for patients with Hepatitis C are conventional interferon α-2b (IFN), sometimes combined with ribavirin (1, **Figure 1.4**), pegylated interferon, and combined pegylated α-2b interferon and ribavirin.

By attaching the polyethylene glycol, the size of the interferon increases so it takes longer for the body to excrete. It also helps protect the interferon molecule from metabolism.[27] Previous studies have shown that pegylated alpha interferons are more effective in producing a sustained viral response in patients with chronic hepatitis C than their non-pegylated counterparts.[27]

Normally interferon stays in the body for about 24 hours; PEG-interferon will remain in the system for up to seven days at higher levels, permitting the drug to be injected only once a week. For this reason, pegylated alpha interferons significantly improve the quality of life for patients as the frequency of administration is dramatically reduced.

Pegylated interferon is used alone (monotherapy) or in some cases, in combination therapy when taken with an antiviral ribavirin (Rebetol ®). In both cases, it is administered intravenously since it is a protein, and needs to enter the bloodstream directly. Hepatitis C patients exhibit only a 20% response to treatment with alpha-interferon; though as many as 40 to 50% may initially respond, most will typically relapse.[27]

IFN has been shown to possess direct antiviral, antiproliferative, and immunomodulatory activities.[27] However, the exact mechanism of action of IFN in the treatment of chronic HCV infection is not known.

Our knowledge of the mechanism of action of ribavirin is no better than that of IFN. Ribavirin is not a potent direct antiviral agent *in vitro*, with an EC_{50} value in the high micromolar (μmol/L) range against most viruses. When ribavirin is combined with interferon, published reports indicate that the response rate improves two to ten fold.[27] The mechanism of action of ribavirin is unknown but at present there are 4 proposed mechanisms.

They can be divided into two groups.[27] The first group consists of two possible indirect mechanisms:

1. Enhancement of host T-cell-mediated immunity against viral infection through switching the T-cell phenotype from type 2 to type 1 and

2. Inhibition of the host enzyme human inosine monophosphate dehydrogenase (IMPDH).

The second group consists of two other hypotheses for the direct inhibition of HCV:

1. Inhibition of NS5B-encoded RNA-dependent RNA polymerase (RdRp) by ribavirin;

2. A RNA mutagen that drives a rapidly mutating RNA virus over the threshold to "error catastrophe".[21]

Interferon α-2b   **Ribavirin (1)**

Figure 1.4. Structure of Interferon α-2b and Ribavirin
http://www.stanford.edu/group/virus/flavi/2004gallo/drug.html
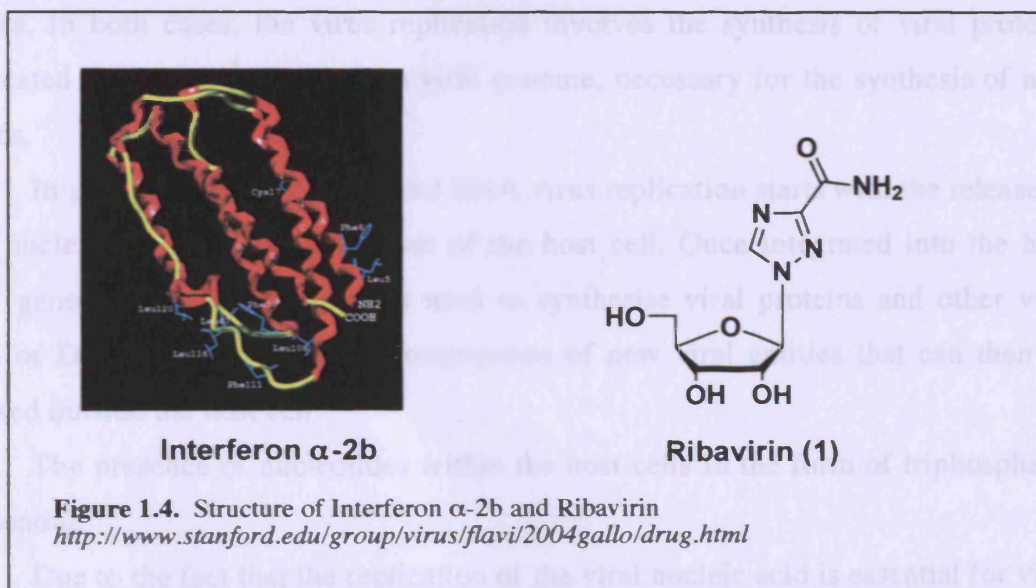
Therapy is initially effective in only a fraction of affected patients and it appears that eradication of infection is unusual in patients infected with the genotypes most common in the United States and most areas of Europe.[3]

1.2 Rationale for the use of nucleoside analogues in antiviral therapy

In general, a virus can be defined as a non-cellular biological entity. Viruses consist of nucleic acid surrounded by protein; some animal viruses are also surrounded by a membrane. Inside the infected cell, the virus uses the synthetic capability of the host to produce progeny virus.[28]

All viruses are incapable of self-replication. Their life cycle strictly depends on the presence of a host cell capable of synthesising the viral proteins and replicating the viral genome.

In general, viruses can be divided into two major categories: RNA and DNA viruses. In both cases, the virus replication involves the synthesis of viral proteins implicated in the duplication of the viral genome, necessary for the synthesis of new virions.

In general terms, the DNA and RNA virus replication starts with the release of viral nucleic acid into the cytoplasm of the host cell. Once integrated into the host cells' genome, the viral genome is used to synthesise viral proteins and other viral RNA or DNA necessary for the construction of new viral entities that can then be released outside the host cell.

The presence of nucleotides within the host cells in the form of triphosphates is essential.

Due to the fact that the replication of the viral nucleic acid is essential for viral reproduction, 50% of the antiviral drugs in current use or under development in the UK target enzymes involved in the viral replication and, in particular, viral RNA/DNA polymerases, which have been preferential targets for antiviral chemotherapy. One of the major antiviral classes is represented by nucleoside analogues. However to interact with these enzymes, nucleosides have to be converted to the corresponding triphosphate species by viral or human kinases.

A nucleoside triphosphate by itself is not a desirable drug candidate as its high charge content can preclude cell penetration. Thus, nucleoside analogues are used as prodrugs of the bioactive triphosphate form.[7]

However, as a consequence of the structure similarity with the natural nucleosides, the possibility of direct interaction with cellular DNA replication can give rise to toxicity problems.

These modified nucleosides can act in two different ways: chain terminators stopping the polymerisation of the viral genome (DNA or RNA) or enzyme inhibitors, where the major targets are the RNA/DNA polymerase or the viral protease, both mechanisms of action may coexist in the same nucleoside analogue.

1.3 Nucleoside analogues and their activation mechanism

Nucleoside analogues represent an important class of antiviral and anticancer drugs many of which have already been marketed (**Figure 1.5**).

BVdU (2)   AZT (3)   Gemcytabine (4)

Aciclovir (5)   IDU (6)   AraC (7)

Figure 1.5. Examples of marketed nucleosides
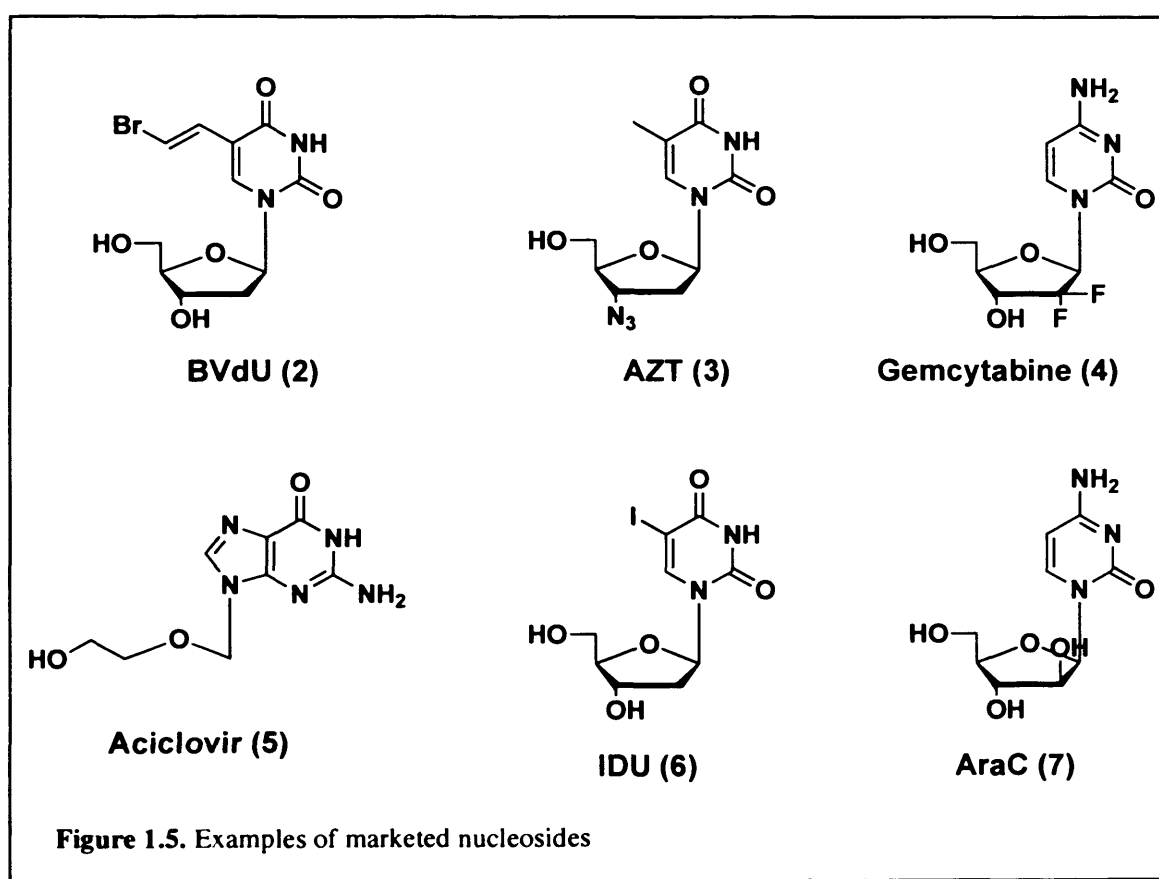
Generally, in order to be active, these nucleosides need to be phosphorylated three times. Although, there are example of nucleosides (e.g. AraC) in which the active form are represented by their diphosphate species. In general, nucleoside kinase (NK) is responsible for the first phosphorylation to give the 5'-monophosphate (5'-MP) and nucleoside monophosphate kinase (NMPK) for the second to give the 5'-diphosphate (5'-DP).[29-33]

The last phosphorylation is due to the presence of nucleoside diphosphate kinase (NDPK) to give the 5'-triphosphate (5'-TP), which represents the active species in most cases. Following intracellular conversion to the nucleoside analogue 5'-TP, the compounds act by selectively inhibiting the RNA/DNA-polymerase of the virus (**Figure 1.6**) and/or incorporation into the growing nucleic acid chain and downstream disruption of function.[34]

Figure 1.6. Scheme of nucleoside activation
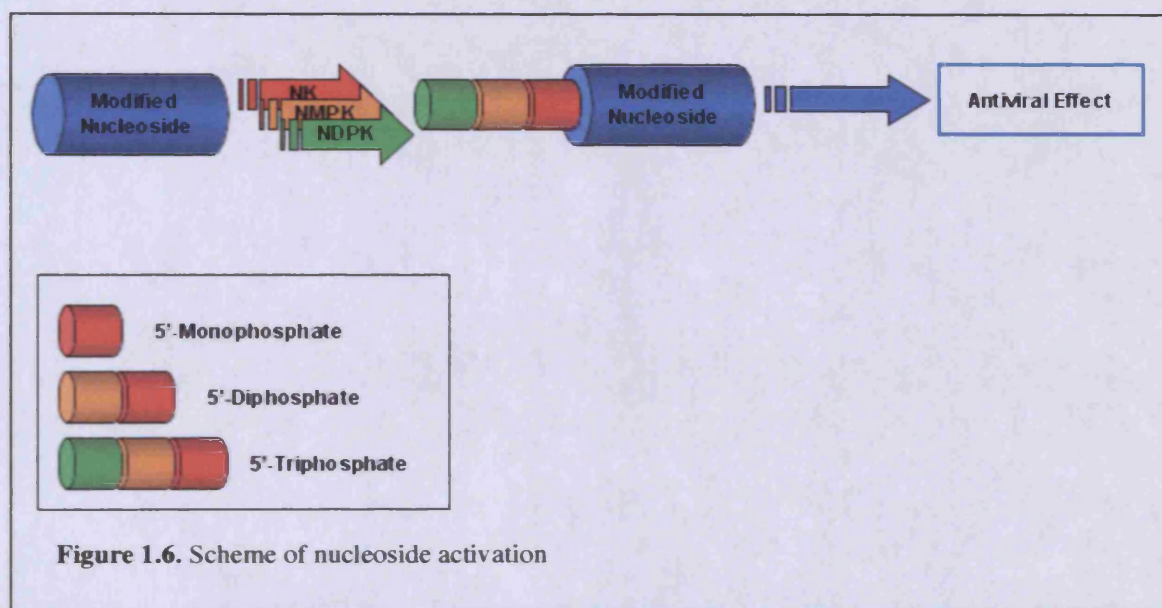
Due to the structural differences and the high selectivity of the substrate, the first phosphorylation of the modified nucleoside is often inefficient. Therefore administration of 5'-MP should bypass this limiting step. Unfortunately, because of the high polarity, these compounds (nucleoside 5'-monophosphates) are not able to easily penetrate the cellular membrane.

However, the phosphate moiety offers a suitable site to attach a degradable lipophilic carrier residue, which allows an increased level of penetration of the cell membrane or transfer across cell membrane.

1.4 Nucleoside analogues with potential HCV activity

Many pharmaceutical companies are investing in the discovery of new approaches against HCV and the principal class of compounds are RNA- and DNA-polymerase inhibitors (**Figure 1.7**).

Figure 1.7. Some nucleoside RNA-polymerase inhibitors currently under investigation
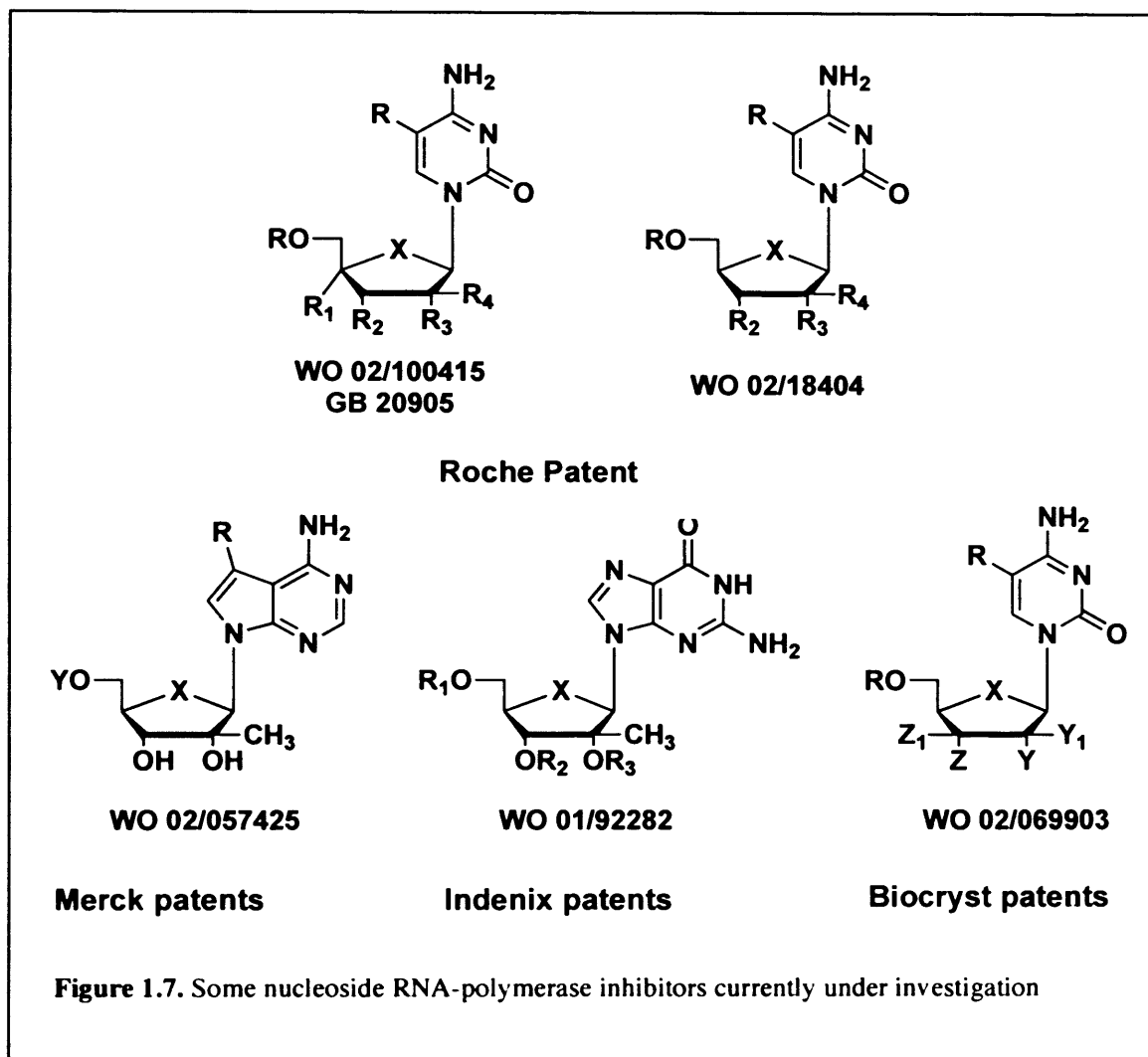
The reason to focus the research programme on nucleosides is due to a number of reasons: (1) they show good selectivity as antivirals, because their mechanism of action can cause the inhibition of RNA synthesis that provides the basis of HCV replication (2) the target for all these nucleoside analogues is RdRp, as this enzyme is essential for the HCV replication.

1.5 Prodrug approach

The concept of prodrug was first introduced by Albert[35] as a pharmacologically inactive derivative of an active drug which could be used to modify the properties of drugs. Numerous prodrugs have been designed and developed to overcome pharmaceutical and pharmacokinetics barriers in clinical drug application. A classical prodrug design often represents a non-specific chemical approach to mask undesirable drug properties such as limited bioavailability, lack of site specificity and chemical instability. According to Wermuth,[36] prodrugs can be divided into two main groups: bioprecursor and carrier-linked prodrugs.

In the case of bioprecursor prodrugs, molecules containing latent functionality can be converted into an active drug. A bioprecursor cannot be converted to the active drug by simple cleavage of the group: commonly the types of activation consist of oxidation (most common method), reduction and phosphorylation (antiviral agent).

In the case of carrier-linked prodrugs, drugs are attached through a metabolically labile chemical linkage to another molecule designated as the "pro-moiety". The "pro-moiety" alters the physical properties of the drug. Carrier-linked prodrugs can be divided into bipartite, tripartite and mutual prodrugs.

A bipartite prodrug is a compound where the pro-moiety is a carrirer, acting like a "masking-group", which liberates the active drug.

A tripartite prodrug is a drug modified by a two component "masking-group"; a carrier connected to a linker arm, which is connected to the drug itself. The mechanism of liberation involves first an enzymatic reaction to cleave the carrier. The remaining inactive compound undergoes a spontaneous cleavage of the linker to release the bioactive drug.

In a mutual prodrug, the carrier, released after the cleavage, is a bioactive drug as well.

1.6 Protide approaches

The protide approach is a prodrug approach based on the concept that the modified nucleotide can be derivatised with certain masking groups to increase the lipophilicity; allowing the nucleotide prodrugs to efficiently cross the cell membrane. The masking groups used for the protide approach must be non-toxic and should be good leaving groups that can be enzymatically or chemically cleaved.

Once inside the cell, the protide can be hydrolysed to release the 5'-MP, which can then be further converted to the active 5'-TP (**Figure 1.8**).

Figure 1.8. Protide approach for modified nucleotide
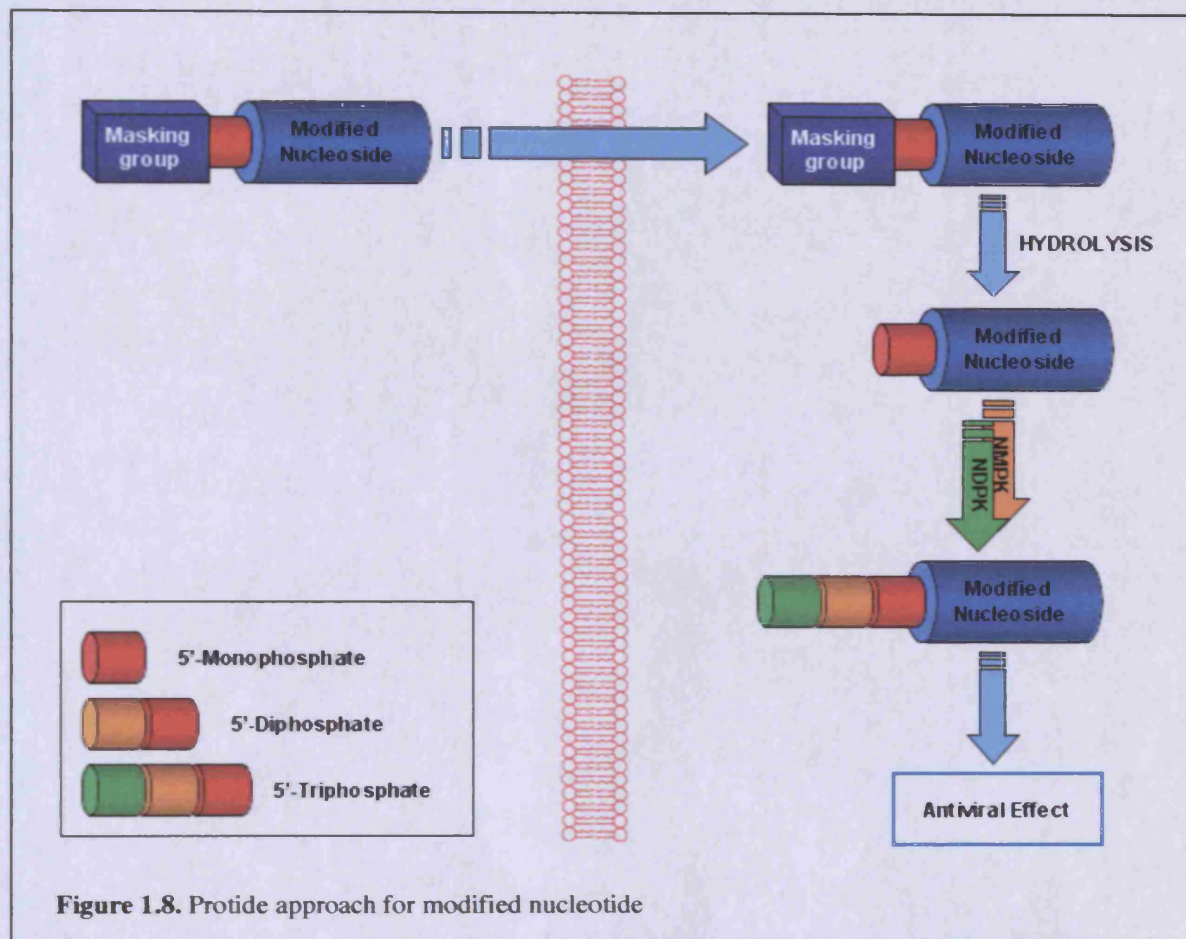
Several researchers, starting from these principles, have applied different pronucleotide (prodrug of nucleoside) approaches. Some recent successful approaches include: bis(pivaloxymethyl)-phosphotriester-nucleoside [Bis(POM)- (**8**)],[37-39] bis(isopropyloxycarbonyloxymethyl)-phosphotriester-nucleoside [Bis(POC)- (**9**)],[40, 41] bis(*S*-[2-hydroxyethylsulfidyl]-2-thioethyl-phosphotriester-nucleoside [Bis(SDTE)- (**10**)] and bis(*S*-acyl-2-thioethyl)-phosphotriester-nucleoside [Bis(SATE)- (**12**)],[42-45] *cyclo*saligenyl nucleoside [*cyclo*sal-nucleoside (**11**)],[46-50] phosphoramidate diesters (**13**) [51, 52] and nucleoside aryl phosphoramidates (**14**) (**Figure 1.9**). [53-58]

Figure 1.9. Pronucleotide approaches
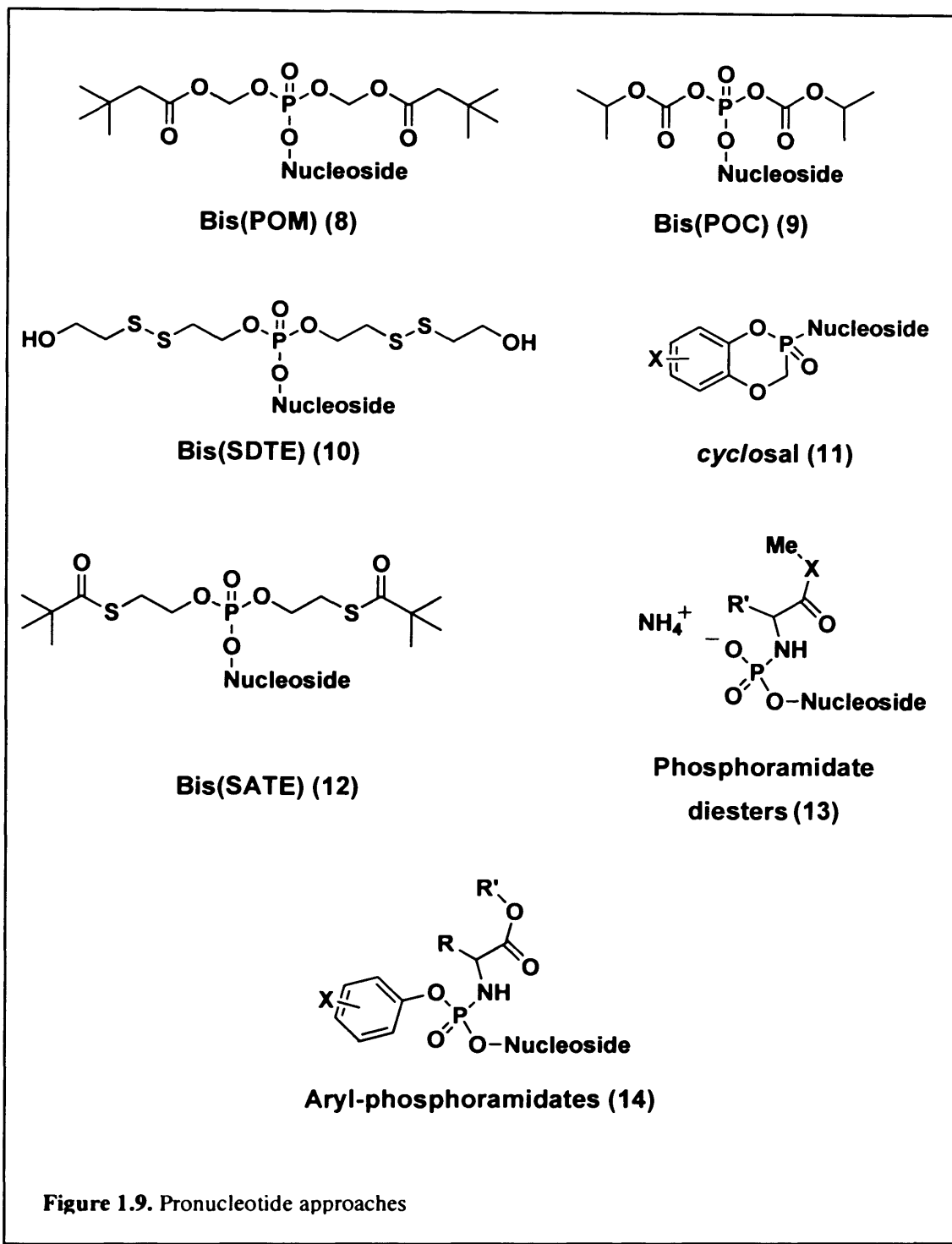
1.6.1 Bis(POM) approach

The Bis(POM) approach (**8**), developed by Farquhar *et al.*,[37-39] is one of the possible ways to deliver the 5'-monophosphate nucleoside into the cell. The general structure is a Bis(pivoxyl) nucleoside. The mechanism of release of 5'-monophosphate (5'-MP) is thought to follow an initial enzymatic reaction by an esterase that cleaves one of the two POM groups to give the hydroxymethyl analogue,

which spontaneously dissociates with elimination of formaldehyde to give the phosphodiester. Cleavage of the second POM group by cellular carboxyesterase regenerates the parent nucleoside 5'-monophosphate (5'MP).

This approach has been successfully applied in order to deliver the anti-cancer drug 5-fluoro-2'-deoxyuridinemonophosphate (5-FdUMP) and the anti-herpes and anti-HIV PMEA (9-(2-phosphomethoxypropyl)adenine).[37-39]

Preliminary *in vitro* studies on Bis(POM)-PMEA have shown a high increase of bioavailability. Moreover, studies with radiolabelled Bis(POM)-PMEA showed a considerable increase (100 fold) of cellular uptake of PMEA. PMEA was subsequently tested *in vivo* for oral bioavailability in monkeys: the results obtained showed a bioavailability greater then 40%. For this reason Bis(POM)-PMEA was selected for human clinical trials. A drawback of this approach is the requirement of a second identical activation reaction, because the intermediate phosphodiester is a poorer substrate for the activating carboxyesterase. Moreover, the delivery of one molecule of the nucleoside results in the liberation of two equivalents of potentially toxic formaldehyde and pivalinic acid.

Nevertheless, the Bis(POM)-PMEA has been approved for the treatment of HBV (Viread™).

1.6.2 Bis(POC) approach

The Bis(POC) approach (9) has been developed by Fridland *et al.*,[40, 41] and it represents the possible solution of all the problems involved in the Bis(POM) approach (8). Using this protide approach, it is possible to deliver the monophosphate without liberation of potentially toxic substrates.

The mechanism of action is again a carboxyesterase-catalysed cleavage of the isopropyl ester to give isopropanol and the intermediate diester that fragments into carbon dioxide, formaldehyde and a mono-(POC)phosphate ester that is subsequently degraded to yield the corresponding nucleoside monophosphate. In contrast to the Bis(POM)-approach, this approach avoids the formation of two equivalents of pivalonic acid that can cause toxicity. The Bis(POC)-approach was applied to the anti-HIV PMPA and has shown 30% bioavailability in dogs in repeat 5-day dosage of 60 mg/kg/day.[40]

1.6.3 Bis(SATE) Bis(SDTE) approaches

Bis(SATE) and Bis(SDTE) approaches have been developed by Imbach and Gosselin.[42-45] In particular, the Bis(SDTE)-approach has been applied to PMEA and an *in vitro* study showed an enhancement of activity most probably due to an increase of bioavailability for these derivatives. Their mechanism of activation is similar to that shown for the Bis(POM) and Bis(POC)-approaches. It starts with the carboxyesterase catalyzed reaction for Bis(SATE) releasing pivalonic acid and the corresponding thioethylester. The product spontaneously eliminates episulfide to yield the intermediate phosphodiester. As in the case of Bis(POM)- and Bis(POC)- approaches, the Bis(SATE)- derivative requires a second identical enzyme-catalyzed activation step to release the corresponding 5'-monophosphate.

The Bis(SDTE)-nucleoside activation mechanism, on the other hand, starts with a reductase enzyme that cleaves the disulfide bond with the formation of thioethanol and thioethyltriester. From this point, the mechanism of activity is the same as that described for the Bis(SATE)-approach.

Bis(SDTE)- and Bis(SATE)-nucleoside approaches have been successfully applied to 5-FdUMP, PMEA, dduMP and AZTMP.[42-45]

1.6.4 *Cyclo*sal approach

The *cyclo*sal approach has been developed by Chris Meier *et al.*, [46-50] and is another possible way to improve the biological properties of nucleoside analogues. It has been applied to several nucleosides with variable results.

The phenyl ester bond should be the most labile while the concurrent cleavage of the benzyl ester in the *cyclo*sal structure to yield the 2-hydroxymethyl-phenylphosphodiester is unfavourable because the phosphate ester in the ortho position of the benzyl ester stabilises this bond.

The hydrolysis should proceed as follows: in the initial step the phenyl ester bond is cleaved selectively because the negative charge could be delocalised in the aromatic ring leading to the 2-hydroxy-benzylphosphodiester. As a consequence, the substituent *ortho* to the benzyl group switches from an acceptor (phosphate) to a donor (hydroxyl) group and this induces the cleavage of the diester and subsequently releases 5'-monophosphate nucleoside and the salicylic alcohol spontaneously.

The main difference to the other mentioned prodrug approaches is that this concept requires only one activation step to deliver the corresponding monophosphate nucleoside. Moreover, compared to the Bis(POM)-, Bis(POC)-, Bis(SATE)- and Bis(SDTE)- approaches, this concept does not result in the formation and release of toxic intermediates such as formaldehyde, episulfide and pivalinic acid.

The *cyclo*sal approach has been applied to different nucleosides:[46-50] d4T (stavudine), AZT, FdU, d4A (2',3'-dideoxy-2',3'-didehydroadenosine). One of the most important advantages of this approach is the water solubility of the *cyclo*sal derivatives.

1.6.5 Phosphoramidate monoester approach

Phosphoramidate monoesters essentially developed by Wagner *et al.*[51,52] are a prodrug approach for nucleosides where only one of the phosphate charges is masked by an amino acid ester. This is an adaptation of the approach of McGuigan *et al.*.[53-58] Aromatic amino acid ester phosphoramidate monoesters of AZT [51] and FLT (3'-fluoro-dideoxythymidine)[52] were synthesised and found to be potent, non toxic and water soluble anti-HIV-1 agents.

Due to the remaining charge, these compounds are considerably water soluble and indefinitely stable in human blood.[51,52]

1.7 Aryl-phosphoramidate

1.7.1 General characteristics

The aryl-phosphoramidate approach, developed by McGuigan *et al.*, is one example of the protide approach and represents a route to the delivery of the 5'-mono-phosphates of modified nucleosides.

The applications of our technology to nucleosides such as d4T[53-57] and abacavir[58] represent the most successful examples.

d4T (15)   **Abacavir (16)**

Figure 1.10. d4T and Abacavir.
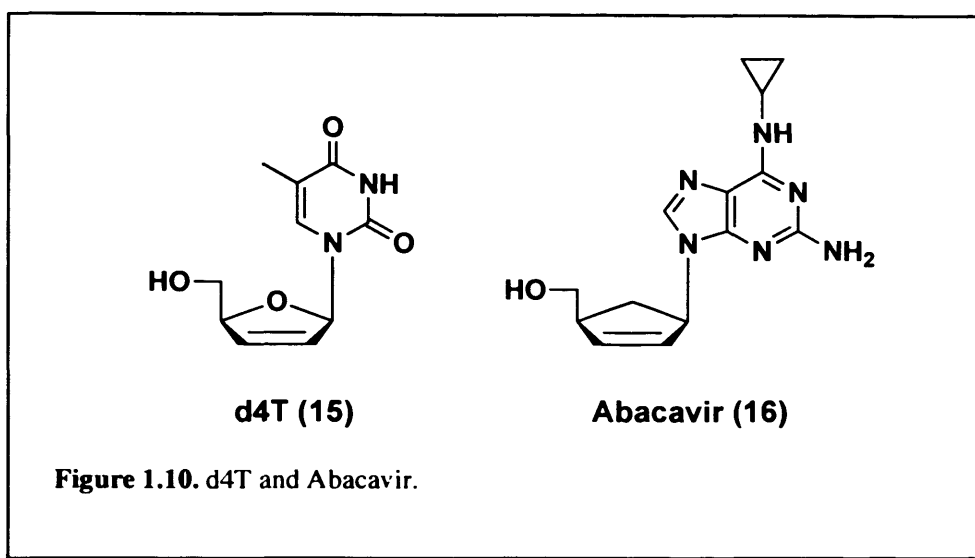
1.7.2 Proposed mechanism of activation

This prodrug approach allows the pronucleotide to penetrate the membrane by passive diffusion and, once in the cell, releases the active species (5'-MP). The putative activation mechanism of these compounds comprises three steps (**Figure 1.11**).[59]

The activation is thought to follow an initial esterase-type catalysis, cleaving the ester.[59] The esterase involved in this first step has not yet been identified but from ^{31}P-NMR studies it has been possible to observe the activity of a pig liver esterase on a number of aryl-phosphoramidates.

The second step is an intramolecular nucleophilic attack of the carboxylic group on the phosphorus to give a cyclic intermediate (**18**). This intermediate has never been isolated due to its probable instability; it is presumably rapidly hydrolysed to the corresponding amino acyl phosphoramidate diester (**19**).

Figure 1.11. Putative activation mechanism of aryl phosphoramidates
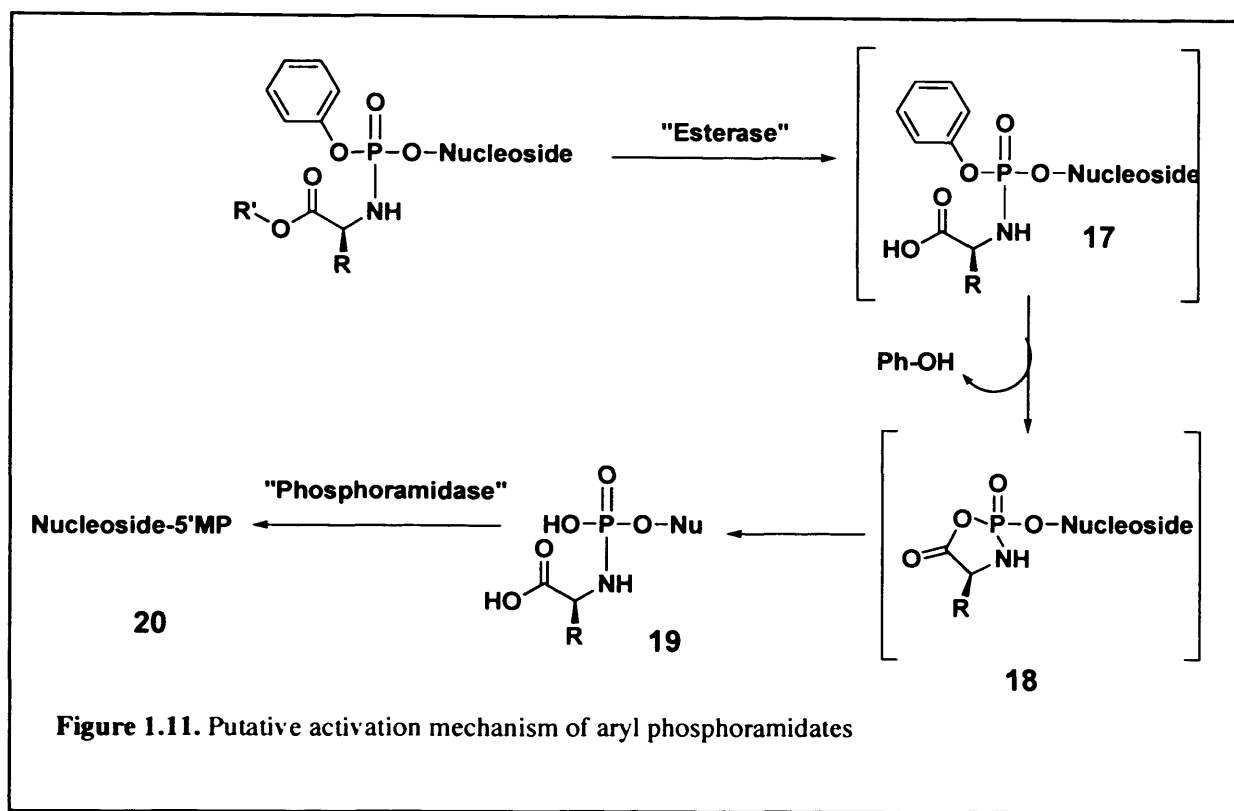
The final step is an enzymatic cleavage of the amino acid to give the nucleoside 5'-MP (**20**).

Results from *in vitro* antiviral tests found d4T-phosphoramidates to be 10 times more potent than the parent nucleoside in wild-type CEM/O and MY-4 cells.[54] Most importantly, the d4TMP phosphoramidate completely retained the full biological activity in mutant thymidine kinase-deficient CEM-TK⁻ cells and suppressed HIV-1 infection in natural peripheral blood lymphocytes. This result proved that d4TMP was delivered intracellulary making the observed activity of the compound entirely independent of thymidine kinase (TK) suggesting that phosphoramidate approach was successful as a TK-bypass.[54] The metabolism of d4T phosphoramidate was analysed by HPLC. The formation of compound **19** was observed proving the postulated phosphoramidate metabolism (**Figure 1.11**).

1.7.3 Previous successful examples

There is a variety of evidence that suggests the aryl-phosphoramidate approach is the key to increasing the activity of the modified nucleosides. For example, d4A phosphoramidate (**21**) has shown a 1000-fold boost in activity in HIV-2 CEM cells

[TK⁺/TK⁻] compared to the parent nucleoside (**22**) (**Figure 1.12**). Moreover, the same nucleoside (**22**) is inactive against Hepatitis B virus, while the phosphoramidate (**21**) is active at sub-μM levels.[53]

Figure 1.12. d4A and parent phosphoramidate
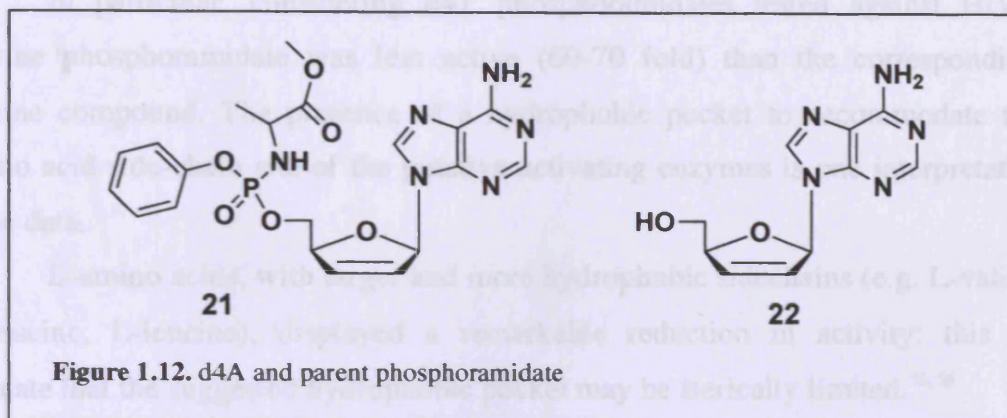
1.7.4 Possible modification sites

For the corresponding aryl-phosphoramidates of a particular nucleoside analogue, there are three possible modification sites that have been investigated and optimised the aryl moiety, ester and amino acid (**Figure 1.13**).

Figure 1.13. Possible aryl phosphoramidate modification
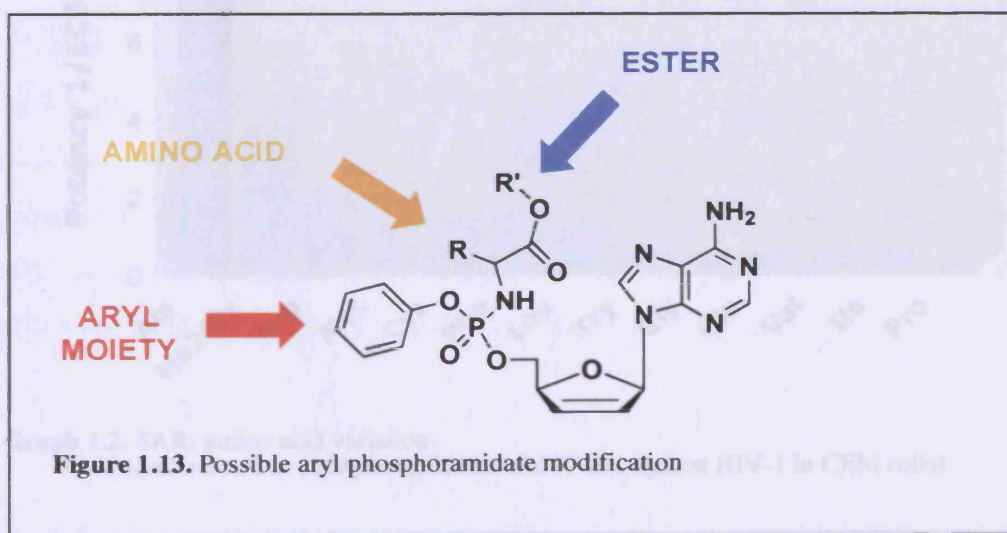
1.7.4.1 Amino acid variation

In the past, amino acid variations have been the subject of an intense study. The amino acid that has shown the broadest activity, for a variety of nucleosides and biological targets has been L-alanine (**Graph 1.2**).[55]

In particular, considering d4T phosphoramidates tested against HIV, the glycine phosphoramidate was less active (60-70 fold) than the corresponding L-alanine compound. The presence of a hydrophobic pocket to accommodate the L-amino acid side-chain site of the putative activating enzymes is one interpretation of these data.

L-amino acids, with larger and more hydrophobic sidechains (e.g. L-valine, L-isoleucine, L-leucine), displayed a remarkable reduction in activity: this might indicate that the suggested hydrophobic pocket may be sterically limited.[55, 56]

Graph 1.2. SAR: amino acid variation
(data related to phosphoramidates of d4T and against HIV-1 in CEM cells)
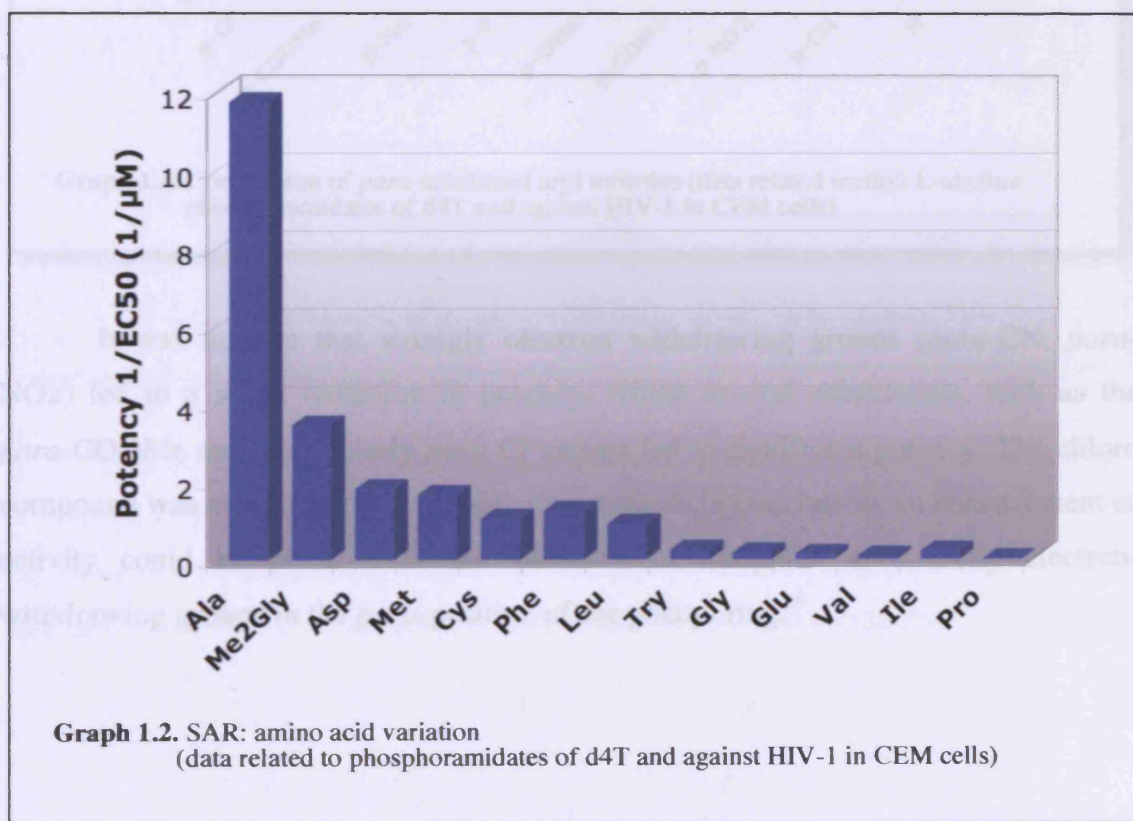
1.7.4.2 Aryl moiety variation

The second possible modification site is the aryl moiety. Studies exploring substitution in the *para*-position on d4T aryl-phosphoramidates showed that a chloro group enhanced the anti-HIV activity (**Graph 1.3**).[56]

Graph 1.3. Comparison of *para*-substitued aryl moieties (data related methyl-L-alanine phosphoramidates of d4T and against HIV-1 in CEM cells)
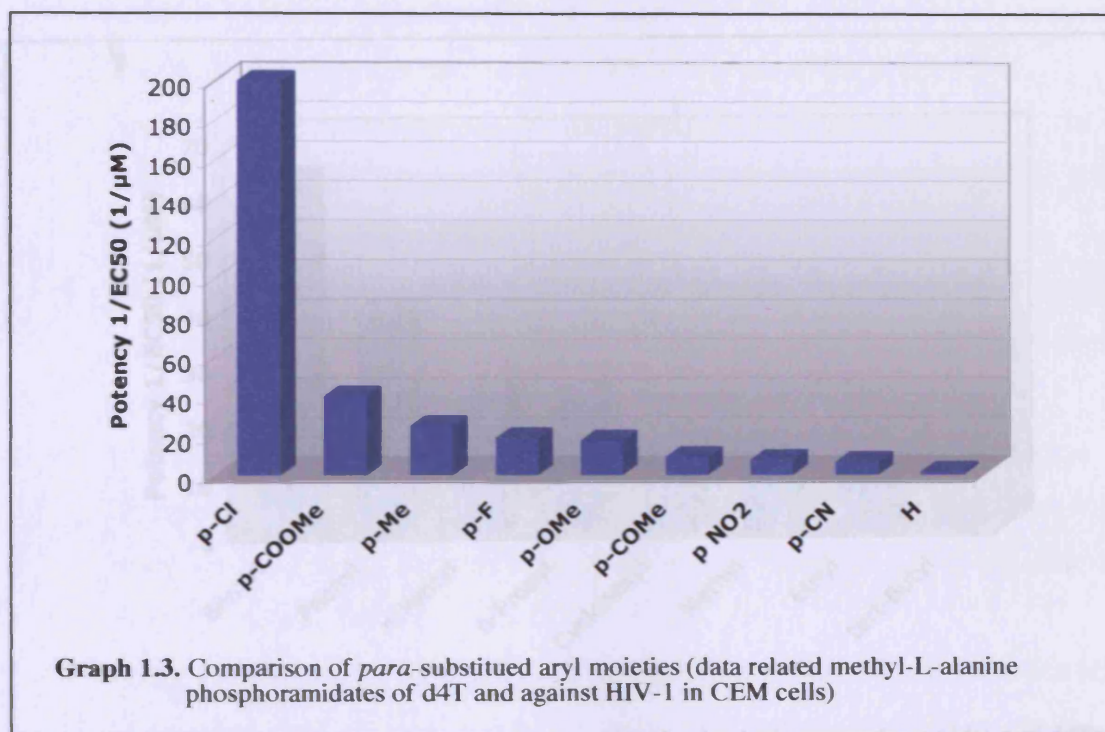
It was notable that strongly electron withdrawing groups (*para*-CN, *para*-NO2) led to a slight reduction in potency, whilst several substituents, such as the *para*-COOMe and, particularly *para*-Cl groups led to significant potency. The chloro compound was also the most lipophilic unit studied. In conclusion, an enhancement of activity could be obtained in the presence of lipophilic and mildly electron-withdrawing groups in the *para*-position of the phenyl ring.[59]

1.7.4.3 Ester variation

The ester of the amino acid provides another possible modification site. Previous work suggests the benzyl ester to be the best ester in many cases (**Graph 1.4**).[57]

Graph 1.4. Comparison of different esters (date related to L-alanine-phosphoramidates of d4T against HIV-1 in CEM cells)[57]
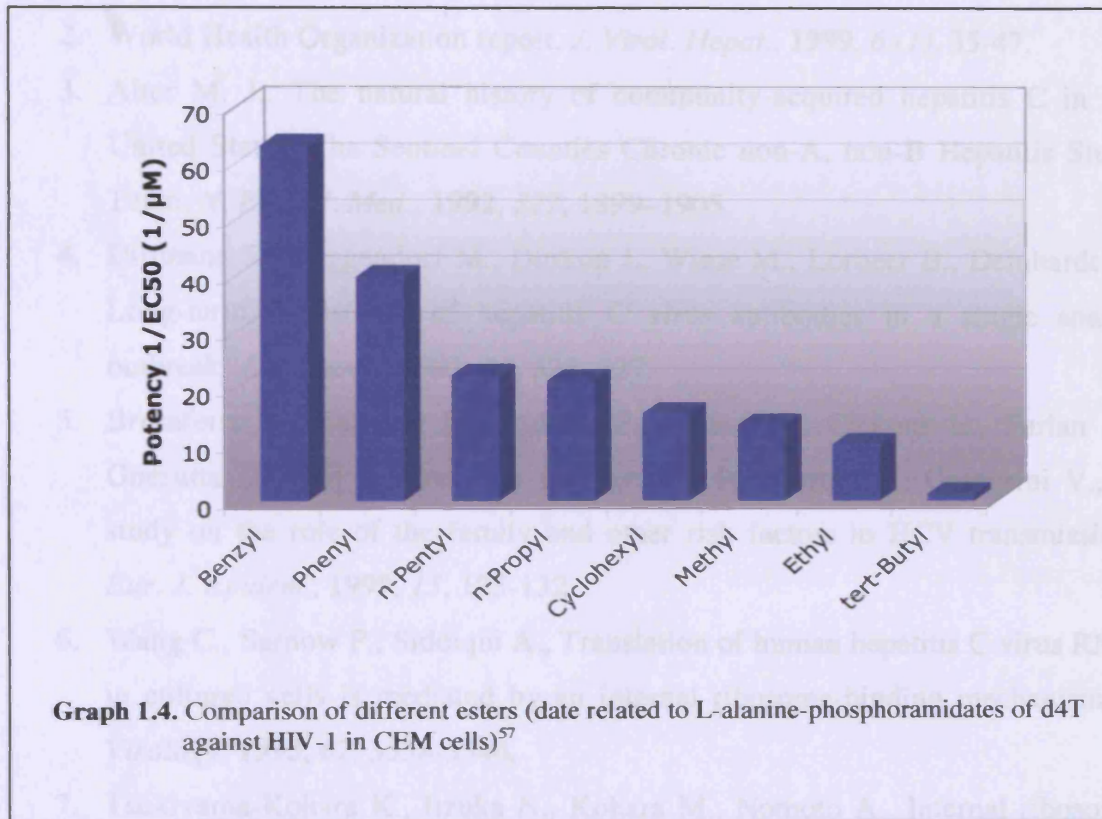
The benzyl ester was noted to be highly potent. The *tert*-butyl ester analogue of d4T was >50 times less potent than the benzyl ester parent compound. This trend can be explained considering that the *tert*-butyl ester might be a poor substrate for the esterase involved in the activation mechanism of the phosphoramidates.[57]

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Chapter Two

Aim of the work

2.1 Collaboration with Roche

In collaboration with Roche Bioscience, the phosphoramidate approach technology was applied to different 4'-modified ribo-nucleosides (**Figure 2.1**) in order to explore the structure activity relationship (SAR) of the three different modification sites (amino acid, ester and aryl moiety); the results of which are illustrated in this thesis.

Figure 2.1. Roche nucleosides
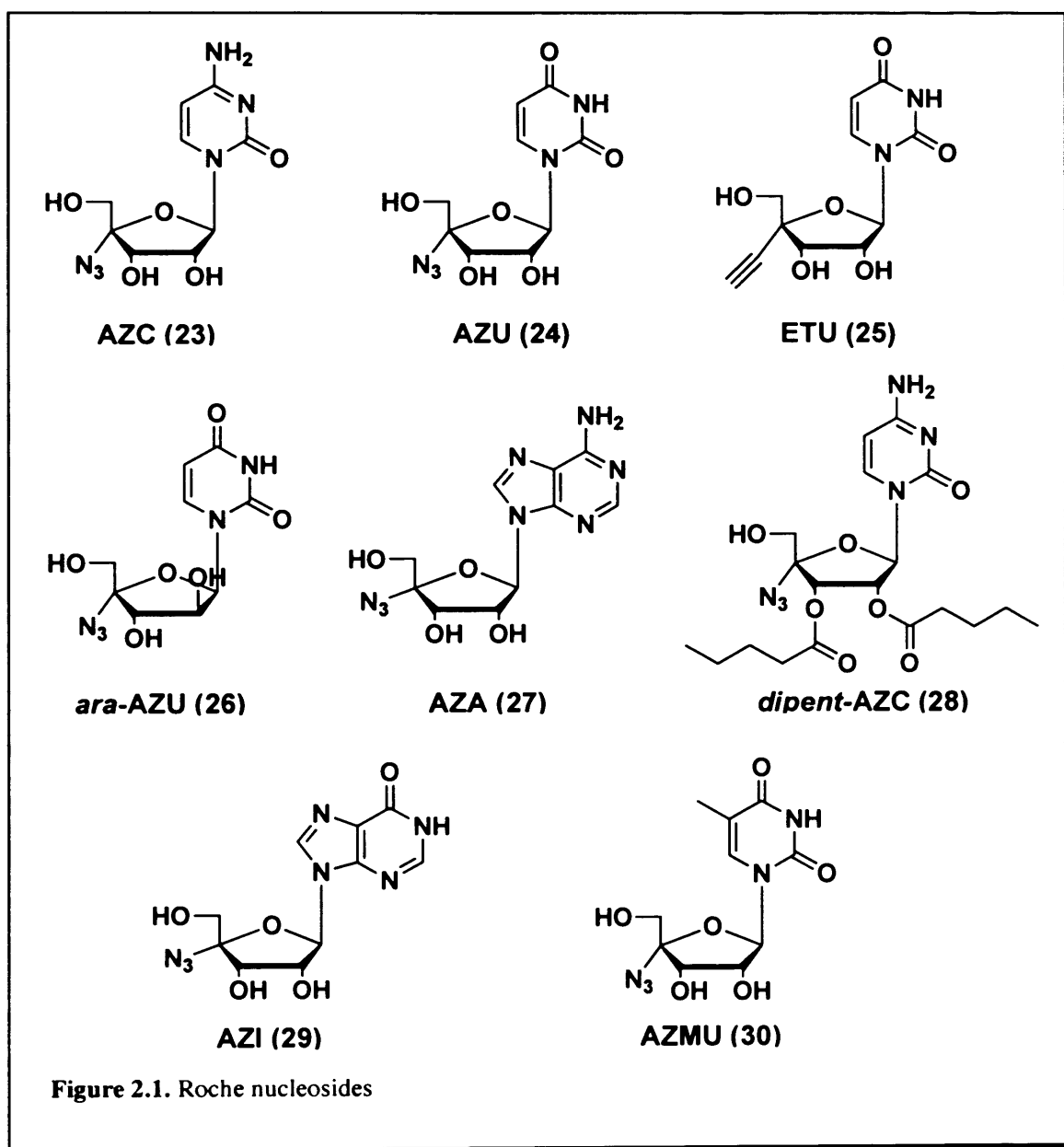
The activity of 4'-azidocytidine (**23**) and 4'-azidouridine (**24**) and their corresponding 5'-triphosphate species were reported (**Table 2.1**).[1]

	EC_{50} (µM) Nucleoside	IC_{50} (µM) 5'-Triphosphate
AZC	1.28	0.29
AZU (24)	>100	0.22

Table 2.1. Activity of Roche nucleosides
EC_{50}= the molar concentration of nucleoside which produces 50% of the maximum possible response.
IC_{50}= the molar concentration of 5'-triphosphate which produces 50% of RdRp inhibition.

AZU was found to be completely inactive; in contrast the corresponding 5'-triphosphate showed good activity as an inhibitor of RdRp. This difference in behaviour was correlated with inefficient metabolism, required to convert the nucleoside into the corresponding active species (5'-triphosphate, see **Chapter One**). Moreover recent studies have demonstrated that AZC-monophosphate is incorporated into the RNA of HCV during its replication and the inhibition of further RNA elongation is the result of this process. [1]

2.2 β-2'-methylnucleosides

The application of the phosphoramidate approach applied to different 2'-modified nucleosides will be illustrated. In the literature there are several examples of reported 2'-modified nucleosides. [2-5]
The active form of these modified nucleosides is represented by the corresponding 5'-triphosphate species that can interact with the viral RdRp. This interaction allows these species to be incorporated into the growing chain of viral RNA and consequently cease the HCV replication. Consequently, in order to be active, the modified nucleoside has two requisites: it should be a good substrate for RdRp to be incorporated into the viral RNA and simultaneously, it should be conformationally different than the natural substrate to affect the termination of the viral RNA. The aim of this project was the initial synthesis of 2'-methyladenosine and 2'-

methylguanosine; subsequently, based on the previously explored SAR, the synthesis a series of phosphoramidates was planned.

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Chapter Three

Aryloxyphosphoramidates of cytidine analogues

3.1. Roche nucleosides

3.1.1 Target enzymes

Within the HCV genome, the helicase, serine protease (encoded by NS3 subunit) and RNA dependent RNA polymerase (RdRp encoded by NS5B) are essential in the virus replication.[1] For this reason, they have to be considered possible target enzymes in the design of an anti-HCV drug.

The helicase unwinds stable structures in the RNA template, facilitating replication. The serine protease is responsible for the cleavage of polyproteins once they are synthesised. Inhibition of these enzymes can impede viral replication.

We focused our attention on RdRp: it acts on single stranded minus-RNA producing complementary strands starting from the nucleoside 5'-triphosphate, incorporating 5'-monophosphate species in the RNA structure and releasing a diphosphate molecule (**Figure 3.1**).
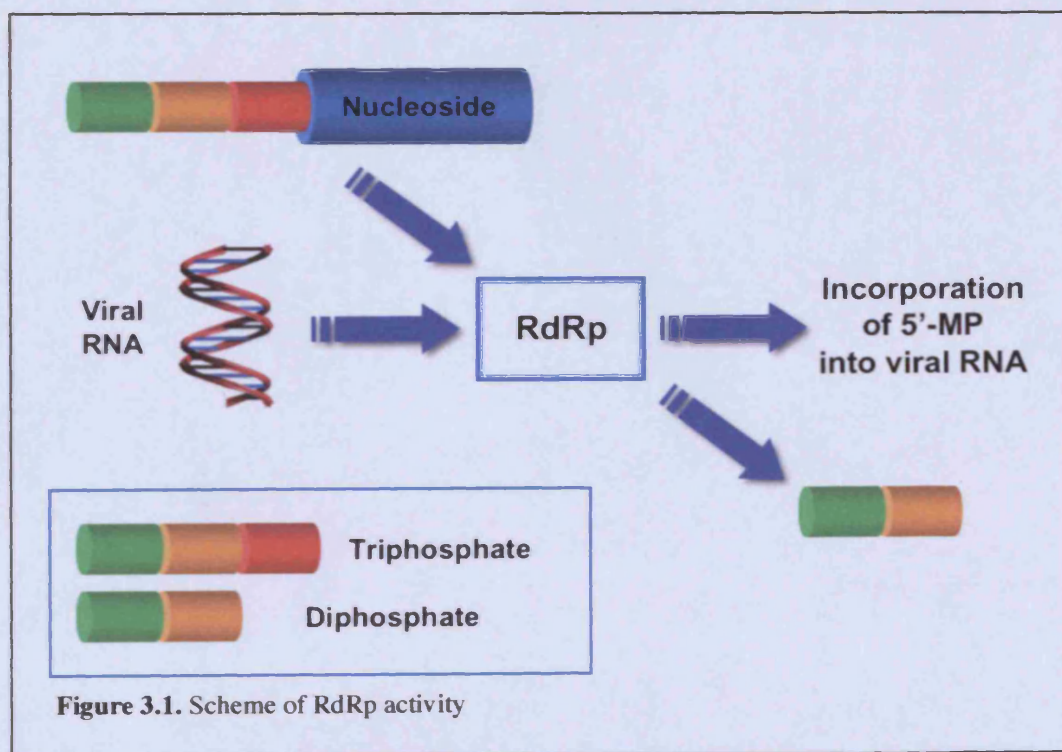
Figure 3.1. Scheme of RdRp activity

The inhibition of RdRp will cease HCV replication. HCV encodes for a specific RdRp therefore, if successful, a new class of nucleoside can be effective against all of the 6 genotypes of HCV so far isolated.

3.1.2 Roche Biosciences Database

Roche Biosciences has patented and screened 4'-modified ribo-nucleosides that were designed as chain terminators of the growing RNA chain (**Figure 3.2**). The presence of a bulky group in the 4'-position might confer steric and/or conformational sugar modification and consequently it may impede the RNA elongation. Moreover, to be incorporated into viral RNA these nucleosides need to be phosphorylated to the corresponding 5'-triphosphosphate. A previous study demonstrated that the corresponding 2'-deoxy-nucleoside analogues (in particular 4'-azidothymidine) showed activity against mutant HIV in the same range of AZT, and they were found to be active at sub-micromolar level.[3]

Figure 3.2. Roche Biosciences patents
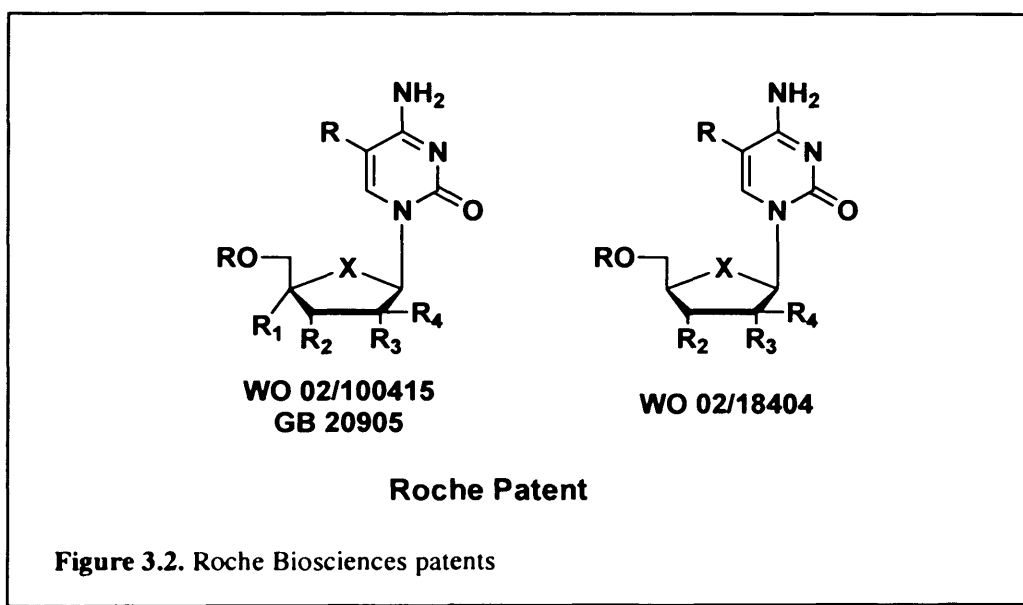
The groups introduced were alkynyl, azido, cyano, alkyl, alkenyl, allyl, and alkoxy. The majority (95%) of the compounds synthesised were inactive against HCV.[4] The aim of the collaboration between our research group and Roche Biosciences was to apply the aryl-phosphoramidate approach to different Roche 4'-modified ribo-nucleosides

3.2 Suggested synthetic pathway

The phosphoramidate synthesis is a three step reaction: first the synthesis of the aryldichlorophosphate (33) starting from the appropriate phenol/naphthol (31) and POCl$_3$ (32), followed by the coupling of 33 with the corresponding amino acid ester (34). The obtained phosphochloridate (35) is then reacted with the appropriate nucleoside to give the desired arylphosphoramidate (36) (Figure 3.3). [6,7]
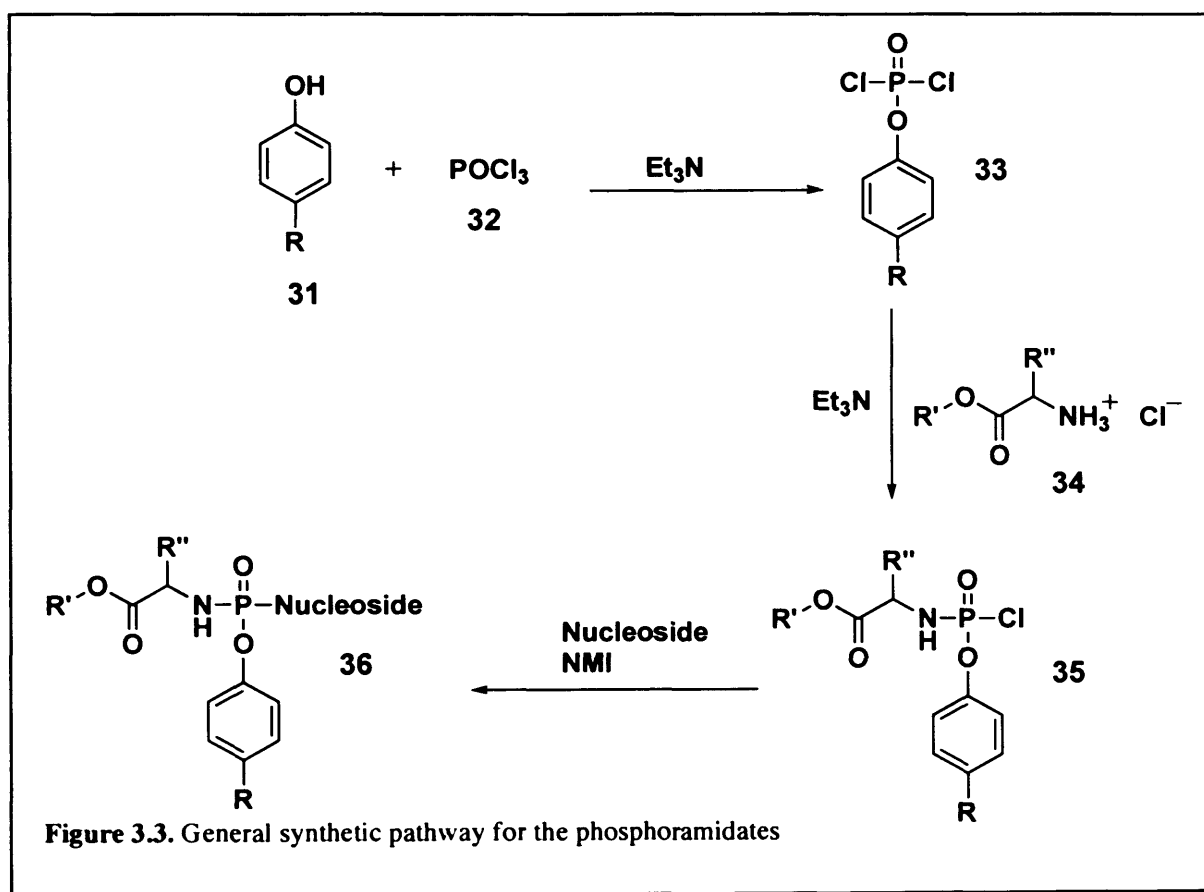
Figure 3.3. General synthetic pathway for the phosphoramidates

3.3 Phenyl phosphoramidates of 4'-azidocytidine

As mentioned in **Chapter One**, in the aryloxyphosphoramidates there are three possible modification sites: namely the aryl moiety, the lateral chain and the ester of the amino acid. In the first instance, we focused our attention on the amino acid ester using phenyl as the aryl moiety. Some of the amino acid esters were not commercially available. In this case were synthesised.

3.3.1 Synthesis of amino acid esters

The synthesis of the amino acid esters not commercially available is outlined below. There are two possible synthetic routes: the first was used for high-boiling alcohols. In this case, the amino acid (**37**) and the appropriate alcohol (**38**), in the presence of p-toluene sulphonic acid (pTSA), were stirred at reflux overnight to give the corresponding amino acid ester tosylate salts (**39**) (**Figure 3.4**).
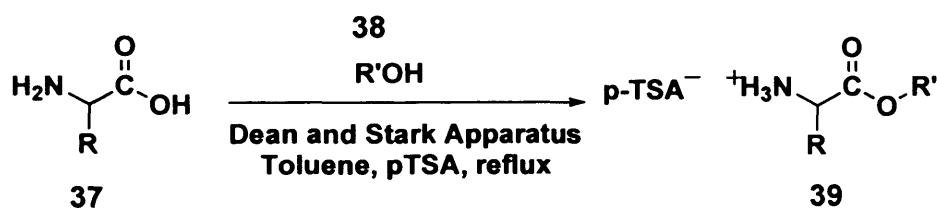
Figure 3.4. General synthetic pathway for the synthesis of amino acid esters with high-boiling alcohols (R= chain of the amino acid and R'= ester)

The second synthetic route was used for low-boiling alcohols: in this case the amino acid (**37**) was heated at reflux overnight with the appropriate alcohol in the presence of thionyl chloride to give the amino acid ester hydrochloride salts (**40**) (**Figure 3.5**).
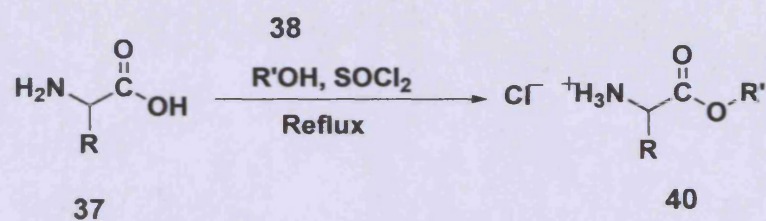
Figure 3.5. General synthetic pathway for the synthesis of amino acid esters with low-boiling alcohols (R= chain of the amino acid and R'= ester)

Not all the esters synthesised gave a solid product: in these cases, the ester hydrochloride salt was used in the subsequent reaction as an oil. The yields of the obtained products are reported in the table below (**Table 3.1**).

Cpd no.	Amino acid	Ester	Salt	Yield
41[8]	cyclopentylgly	benzyl	tosylate	60%
42[8]	cyclopentylgly	ethyl	hydrochloride	56%
43[8]	cyclopentylgly	isopropyl	hydrochloride	56%
44	L-isoleucine	ethyl	hydrochloride	39%
45[9]	D-alanine	butyl	tosylate	62%
46[10]	D-alanine	dodecyl	tosylate	67%
47[9]	L-alanine	butyl	tosylate	67%

Table 3.1. Yields of amino acid esters synthesised

3.3.2 Synthesis of phosphorylating agents for coupling with 4'-azidocytidine

The synthetic route for the synthesis of phosphochloridate (**49**) was developed by McGuigan *et al.*, [6, 11, 12, 13] and involved a coupling reaction between commercially available phenyl-dichlorophosphate (**48**) and the appropriate amino acid salt (**39/40**) in the presence of triethylamine (TEA) at -78 °C overnight (**Figure 3.6**).

Figure 3.6. General synthetic pathway for the synthesis of phosphorylating agents
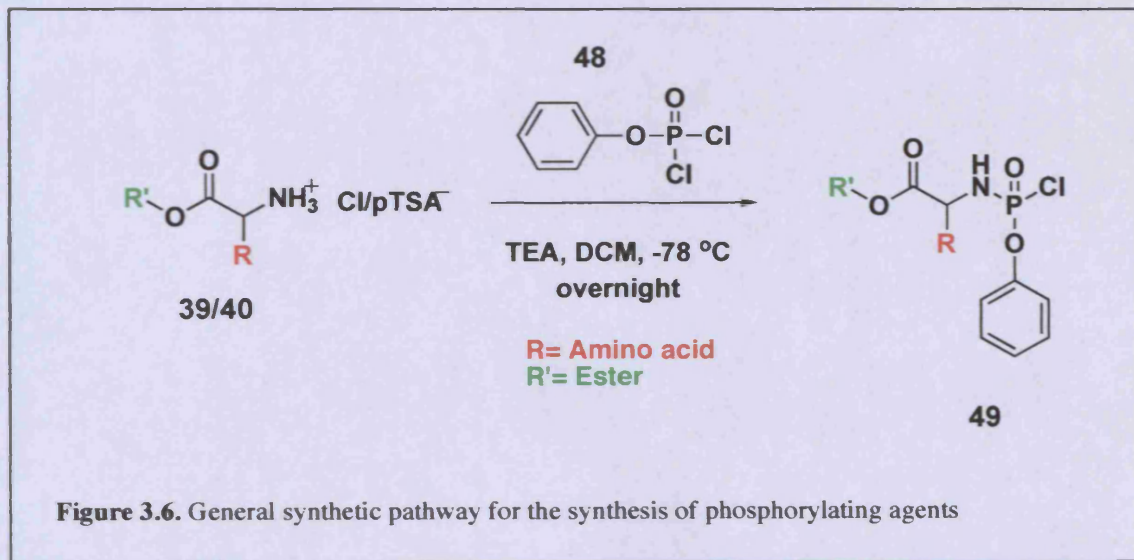
Due to the possible instability of the compounds (**49**) the purification involved a rapid flash column chromatography using EtOAc/Hexane (7:3) as eluent.

This reaction was not stereoselective, so the result was the formation of two stereoisomers. In the case of chiral amino acids, the splitting of the ^{31}P-NMR signal was noted. The observed chemical shifts were between 7.7 and 10.6 ppm (**Table 3.2**).
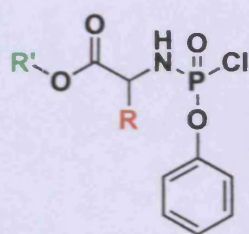
R'=Ester

R= Amino acid
R'= Ester

Cpd no.	Amino acid	Ester	^{31}P (ppm)
50[8]	cyclopentylgly	benzyl	7.72
51[8]	cyclopentylgly	ethyl	8.09
52[8]	cyclopentylgly	isopropyl	8.16
53	L-isoleucine	ethyl	10.64, 10.01
54[8]	D-alanine	benzyl	9.29, 9.05
55	D-alanine	*tert*-butyl	9.48, 9.30
56	D-alanine	butyl	9.45, 9.29
57	D-alanine	dodecyl	9.38, 9.15
58	L-alanine	butyl	9.40, 9.19
59	L-ethyl-asp	ethyl	9.78, 9.54

Table 3.2. ^{31}P Chemical shifts of phosphorochloridates synthesised

Compounds **50**, **51** and **52** showed only one signal in the ^{31}P-NMR as a consequence of the absence of a chiral centre in the α-position of the amino acid.

3.3.3 Initial synthesis of arylphosphoramidates in the presence of NMI

As mentioned previously, the most common pathway for the synthesis of the arylphosphoramidates (**60**) comprised the use of *N*-methyl-imidazole (NMI) as activator of the phosphorochloridate (**49**) (**Figure 3.7**).[6]

Figure 3.7. Mechanism of activation of phosphorochloridate in the presence of NMI
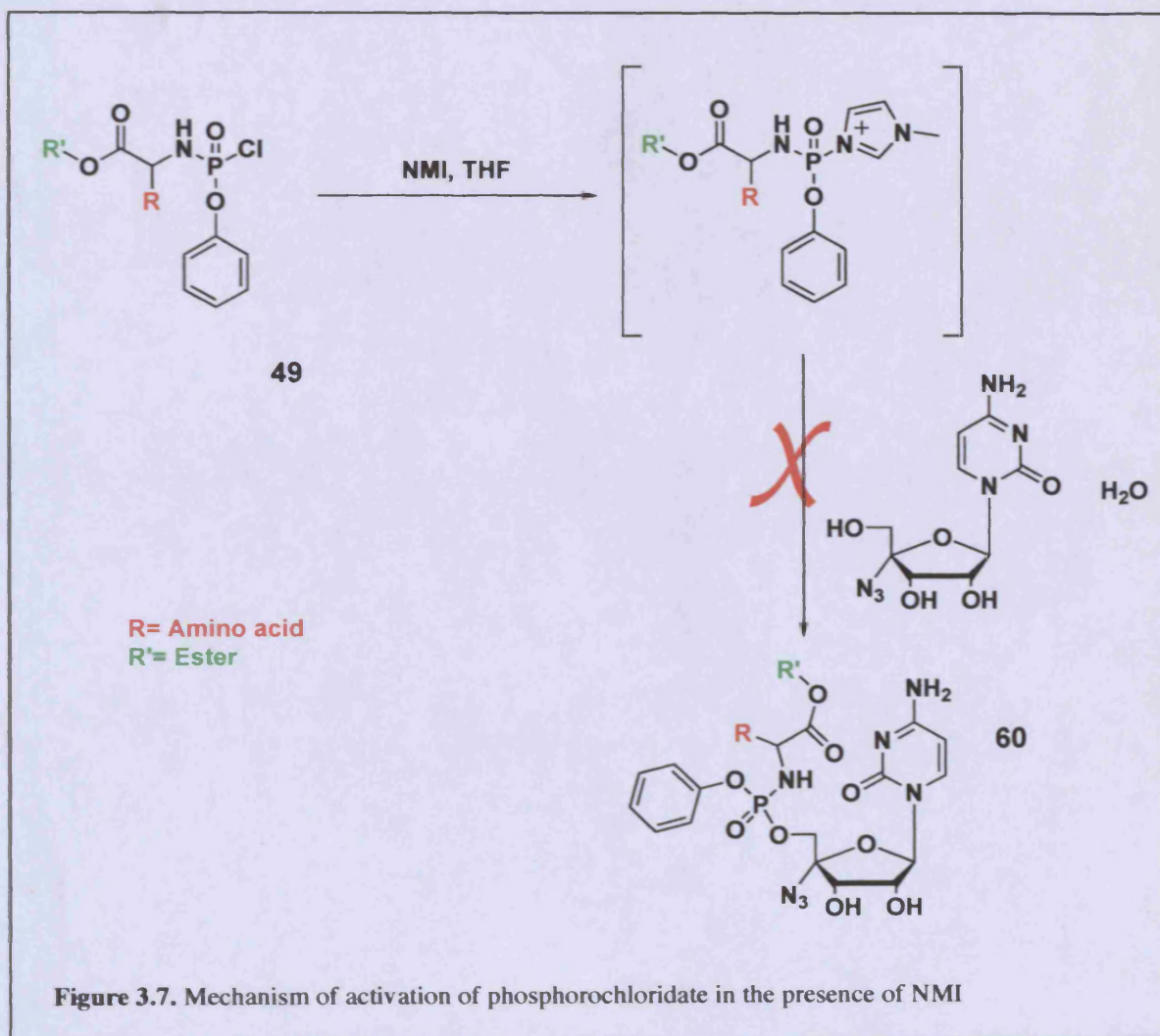
Unfortunately using the standard procedure developed in our group, [8, 9] there was no evidence of the presence of the desired product.

To increase the reactivity of the nucleoside, the same procedure was tried with different equivalents of NMI (5-10 equivalents) and phosphorochloridate (P-Cl, **49**) (3-8 equivalents) at -78 °C and room temperature, but in these cases also there was no evidence of the presence of phosphoramidates (**60**).

4'-Azidocytidine (**23**) had a low solubility in THF (used as solvent for the reaction). In an attempt to increase the solubility, other solvents (acetonitrile, pyridine) were tried by other members of the group without satisfactory results.

4'-Azidocytidine (**23**) was synthesised as a monohydrate and as a consequence, the molecule of water might react with the phosphorochloridate before the nucleoside, to give the product of hydrolysis of the phosphorochloridate.

In order to have the nucleoside as dry as possible, 4'-azidocytidine was azeotroped with pyridine. Unfortunately, also in this case the reaction did not show any presence of product. The low reactivity observed may be a consequence of the presence of the azido group in the 4'-position, reducing easy access of the electrophile to the 5'-position.

3.3.4 Alternative synthesis of phosphoramidates

A completely different approach was proposed, exploiting the higher reactivity of the phenyldichlorophosphate (P-Cl$_2$) as a source of phosphate. It was planned as a one pot reaction: the first step was nucleophilic substitution of 4'-azidocytidine on P-Cl$_2$ with the formation of an unstable intermediate (**61**) that was coupled with the corresponding amino acid ester to give the desired product (**62**) (**Figure 3.8**).

Figure 3.8. Proposed phosphoramidate synthetic route
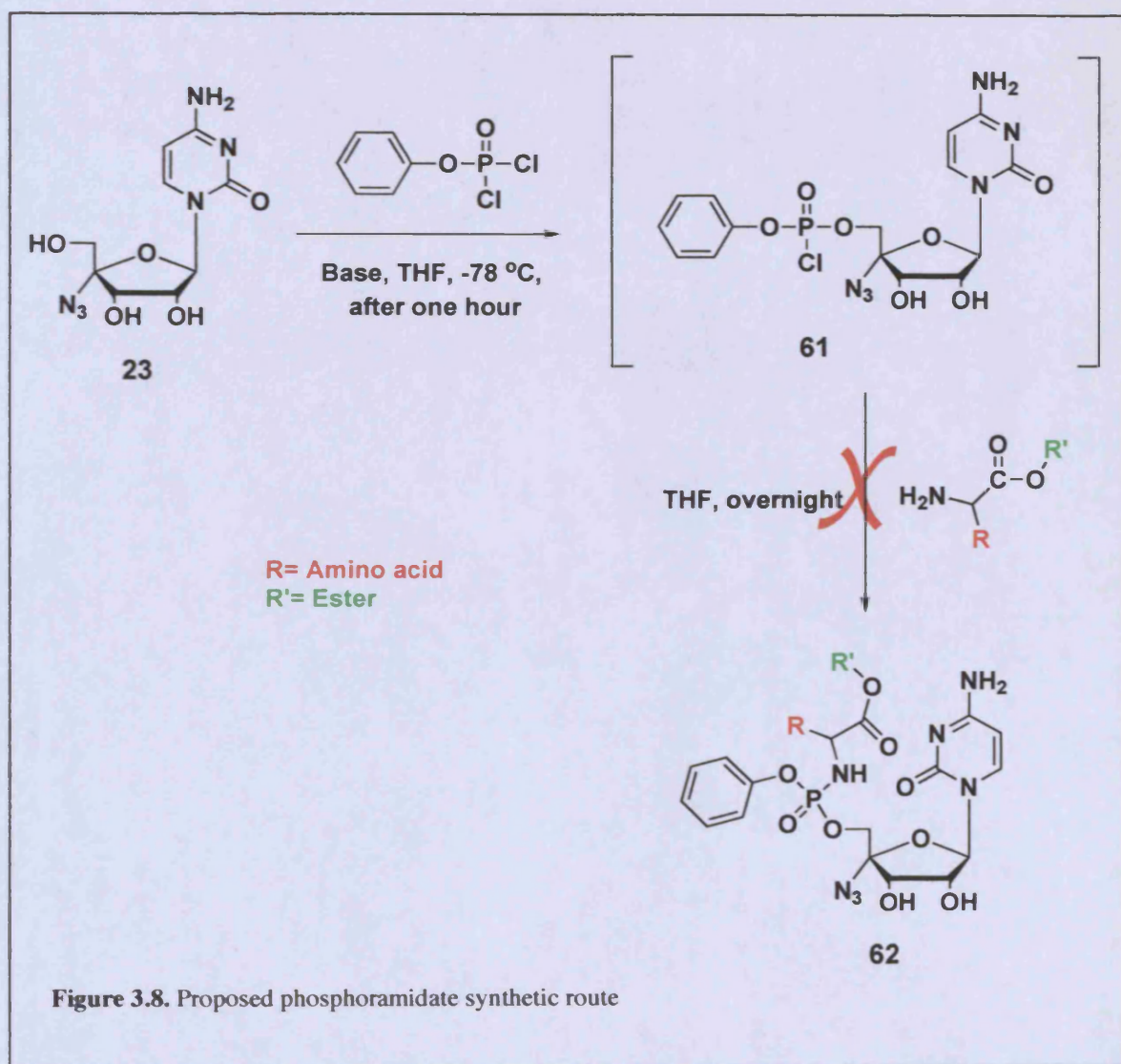
Several conditions were tried but the desired product was not isolated (**Table 3.3**). In all these attempts, the nucleophilic attack of 4'-azidocytidine (**23**) with P-Cl$_2$ was probably achieved. The ^{31}P-NMR of the reaction showed the presence of a single peak at 4.2 ppm, instead of 3.5 ppm (chemical shift of P-Cl$_2$).

Eq. of AZC	Eq. of P-Cl₂	Base	Eq. of Base	Amino acid	Ester	Temp.
1	1	NMI	1.5 eq	cyclopentylgly	ethyl	-78° C
1	3	NMI	3 eq	cyclopentylgly	ethyl	r.t.
1	1	TEA	2 eq	cyclopentylgly	ethyl	r.t
1	3	NMI	3 eq	L-phenylalanine	ethyl	r.t.

Table 3.3. Conditions tried with the new synthetic scheme

However, due to its possible instability, this intermediate (**61**) was not isolated and characterised and, without further purification, the amino acid ester was added to the reaction mixture, and stirred overnight. Analysis of the crude product of the reaction (TLC and MS) did not show any evidence of the presence of the phosphoramidate. It was assumed that the chemical shift observed did not derive from the hypothesised nucleophilic substitution of the phenyl-dichlorophosphate with the nucleoside, but from the complex between P-Cl₂ and NMI or TEA. With the challenges to clearly understand why this reaction was not working, this synthetic pathway was abandoned.

3.3.5 Synthesis of phenyl phosphoramidate in the presence of ᵗBuMgCl

The method of Uchiyama was investigated next.[13] This approach is based on the treatment of nucleoside with 1 equivalent of a strong organometallic base, such as a solution of *tert*-butylmagnesium chloride (tBuMgCl), to form the corresponding metal alkoxide (**63**) (**Figure 3.9**) followed by the coupling reaction with the appropriate phosphochloridate.[13] Due to the presence of a ribo-sugar with 3 hydroxy groups in the 2'-, 3'- and 5'-positions possible regioselectivity problems might be encountered. In the first instance, 1.5 eq of Grignard reagent was used. Unfortunately, due to the really low yield, the desired product was only observed by MS.

Considering that 4'-azidocytidine (**23**) was synthesised as a monohydrate derivative, the same reaction was repeated in the presence of 2.5 eq of ᵗBuMgCl. In

this case, from TLC, it was possible to identify the desired phosphoramidate which was isolated.
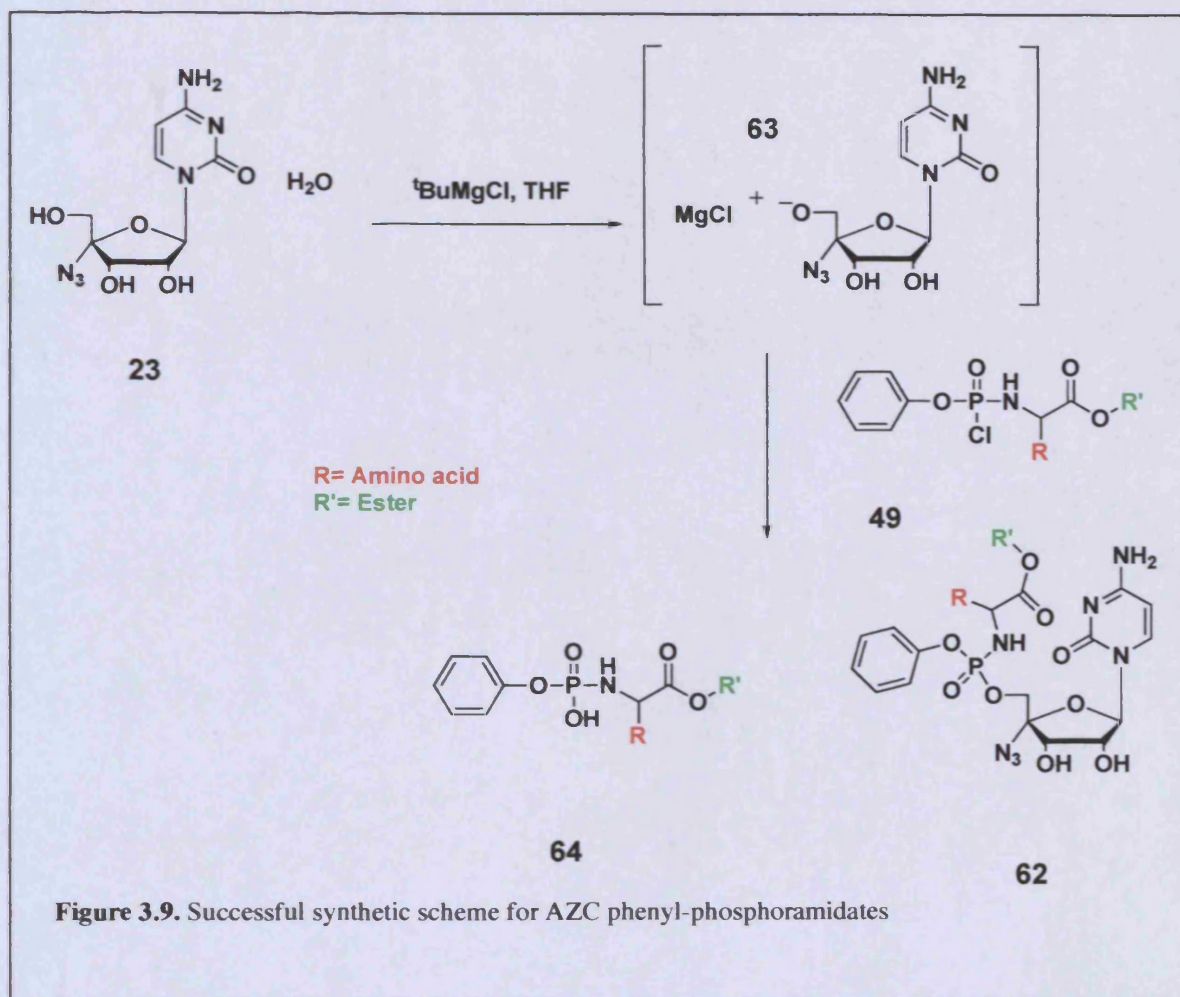
Figure 3.9. Successful synthetic scheme for AZC phenyl-phosphoramidates

The general synthetic pathway started with the addition of 2.5 equivalents of 'BuMgCl to a solution containing 1 equivalent of 4'-azidocytidine in THF. After 15 minutes, the appropriate phenylphosphorochloridate (**49**) was added and stirred overnight (**Figure 3.9**). The reaction was quenched by the addition of an aqueous solution of NH_4Cl. The phosphoramidates synthesised are reported in **Table 3.4**.

Nevertheless, the purification required at least one chromatography column and two preparative TLC plates to give the pure aryl phosphoramidate (**62**). The presence of a product caused by the hydrolysis of the phenylphosphochloridate was observed (**64**), isolated and fully characterised: by ^{31}P-NMR it appears as a broad peak at 0.1 ppm.
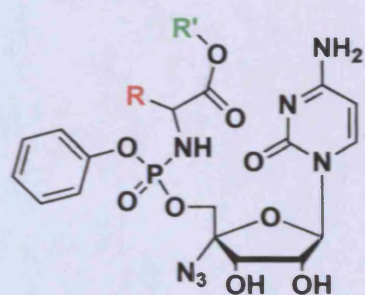
Cpd no.	Amino acids	Esters	^{31}P ppm
65	cyclopentylgly	benzyl	3.70, 367
66	cyclopentylgly	ethyl	3.74
67	cyclopentylgly	isopropyl	3.77, 3.75
68	L-isoleucine	ethyl	5.55, 5.30
69	D-alanine	benzyl	4.80, 4.26
70	D-alanine	*tert*-butyl	4.91, 4.45
71	D-alanine	butyl	4.73, 4.55
72	D-alanine	dodecyl	4.80, 4.33
73	L-ethyl-asp	ethyl	4.67, 4.40
74	L-alanine	butyl	4.82 4.32

R= Amino Acid
R'= Ester

Table 3.4. List of phenyl phosphoramidates synthesised

Due to the high number of purification steps, the initial yields were reasonably low (5-10%).

The use of tBuMgCl as coupling agent might cause a non-regiospecific reaction with 2'- and 3'- phosphorylation, forming side products. However after analysing ^{13}C-NMR spectra, the isolated product was the aryloxy-phosphoramidate in the 5'-position with no traces of other regioisomers.

Figure 3.10. Example of ^{13}C-NMR of aryloxy-phosphoramidate
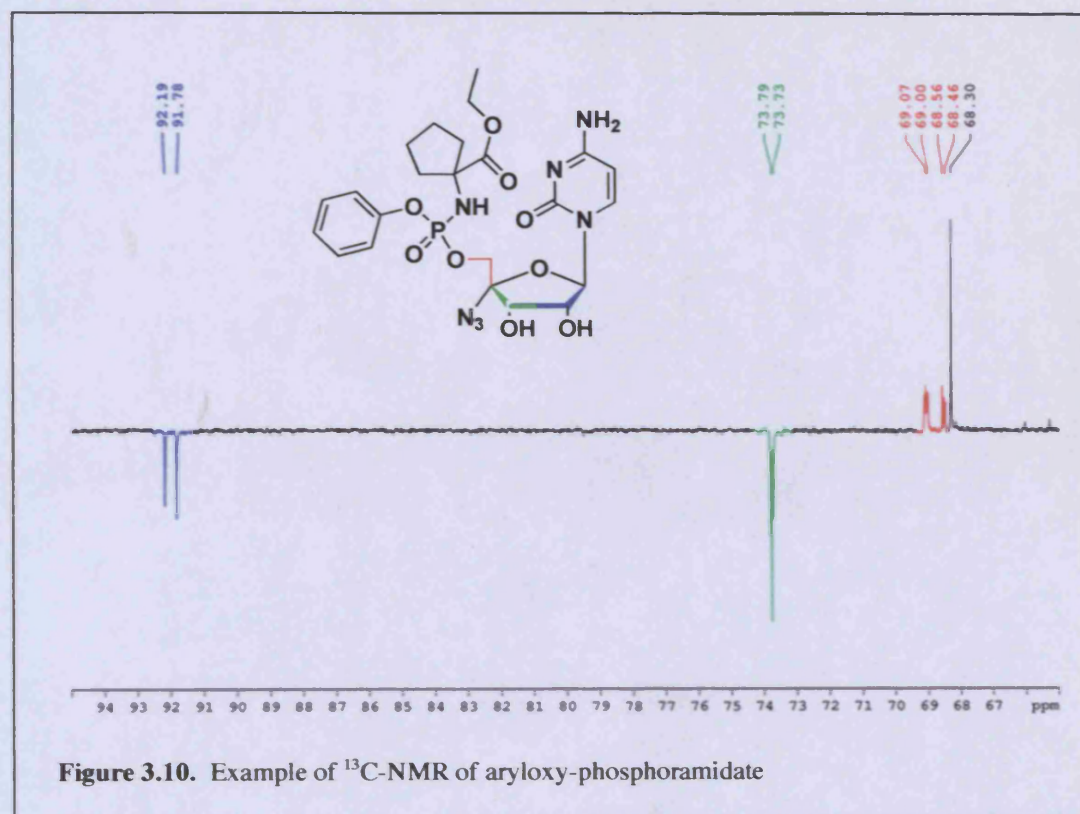
Only the CH_2 group in the 5'-position (red) displayed 4 signals as a consequence of the presence of the two diastereoisomers and the coupling with the phosphorus. The CH in the 2'- (blue) and 3'-position (green) had only two signals each (**Figure 3.10**), due to the presence of the two diastereoisomers.

3.3.6 Biological activity of phenyl phosphoramidates of 4'-azidocytidine

All the compounds synthesised were evaluated *in vitro* using a replicon assay by Sophie Le Pogam and Isabel Najera in the lab of Klaus Klumpp in Roche Biosciences (Palo Alto, California).

3.3.6.1 Replicon assay

The replicon assay used a subgenomic HCV replicon composed of the HCV 5'-NTR, the gene encoding for renilla luciferase (Re-Luc, a protein obtained from Renilla), fused with neomycin phosphotransferase (NPTII) and a part of HCV replicon that is constituted by the subunits NS3, NS4B, NS5A and NS5B and the HCV 3'-NTR (**Figure 3.11**).[14, 15]

Figure 3.11. Structure of the replicon for the assay
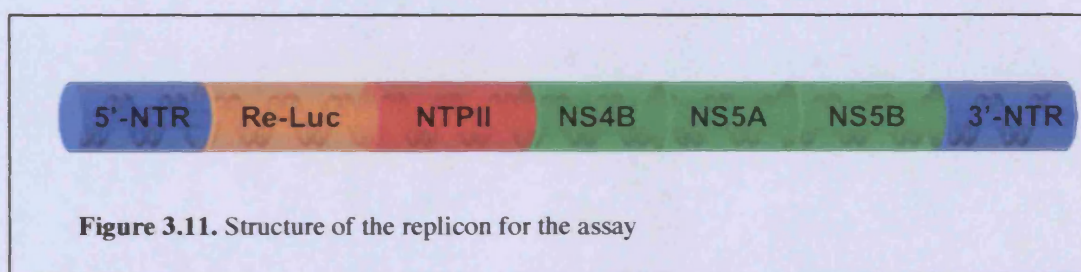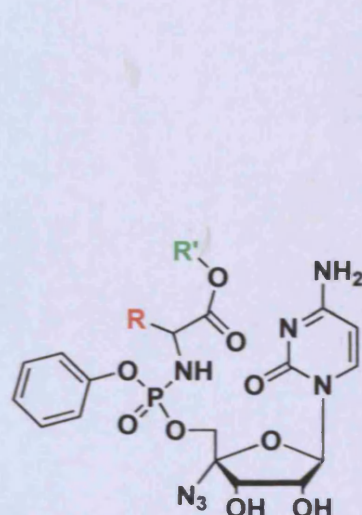
The cell line used for this assay was the human hepatoma cell (Huh-7).[14, 15] This strand of RNA, after *in vitro* transcription, was incorporated into Huh-7, and neomycin (conferred by subunit NTPII) resistant colonies were isolated and expanded.

The activity of renilla luceferase expressed by the replicon, reflects its RNA level in the cells, so indirectly the IC_{50} of aryl phosphoramidate was measured as inhibition of luciferase activity. The advantage of this kind of assay is that using this particular protein (luciferase) and only a part of HCV replicon, there is a high replication of the virus that could simulate the real behaviour of the virus in the host liver cells.

Different mutations have been inducted on the HCV subunits and in particular in the NS3, NS5A and NS5B to improve the activity of this method.[14, 15]

3.3.6.2 Biological activity of phenyl phosphoramidate of 4'-azidocytidine

All the compounds synthesised were evaluated against HCV *in vitro*. **Table 3.5** displays all the reported data for the phenyl phosphoramidates.

Cpd no.	Amino acids	Esters	EC_{50} (µM)	CC_{50} (µM)
65	cyclopentylgly	benzyl	9.0	>100
66	cyclopentylgly	ethyl	<100	>100
67	cyclopentylgly	isopropyl	<100	>100
68	L-isoleucine	ethyl	>100	>100
69	D-alanine	benzyl	0.9	>100
70	D-alanine	*tert*-butyl	<100	>100
71	D-alanine	dodecyl	>100	>100
72	D-alanine	butyl	0.92	>100
73	L-ethyl-asp	ethyl	<100	>100
74	L-alanine	butyl	10.8	>100
23	4'-azidocytidine		1.28	>100

5'-triphosphate-AZC= 0.29 µM IC_{50}

R= Amino Acid
R'= Ester

Table 3.5. Biological data of AZC phenyl phosphoramidates
IC_{50}= The concentration of phosphoramidate, which produces 50% of the maximum possible effect
CC_{50}= The concentration of phosphoramidate, which produces 50% of toxic effect

There is no significant difference in terms of activity comparing 4'-azidocytidine (**23**) and its corresponding most active synthesised phosphoramidate (**69**). This effect might indicate that, once in the cells, these phosphoramidates may be hydrolysed releasing the nucleoside and not the 5'-monophosphate, without any possibility to bypass the first phosphorylation. Another possible explanation might be related to the activation mechanism of the 4'-azidocytidine itself. The first

phosphorylation might not be the limiting step for this nucleoside so its corresponding phosphoramidates, once inside the cells, release the 5'-monophosphate that is a poor substrate for the second or third phosphorylation.

3.3.6.3 Ester variation

The ester of the amino acid carries out an important role in the phosphoramidate metabolism; they have to be a good substrate for the "esterase" in order to be easily removed since their cleavage represents the first step in the 5'-monophosphate release (see **Chapter One**).

In collaboration with other researchers in our group, the ester variation has been explored in the L-alanine phosphoramidate series (**Graph 3.1**).

Graph 3.1. Ester variation for L-alanine AZC phosphoramidates (ethyl, benzyl, 2-butyl, *tert*-butyl, isopropyl and methyl phosphoramidates were synthesized by other researchers of our group)
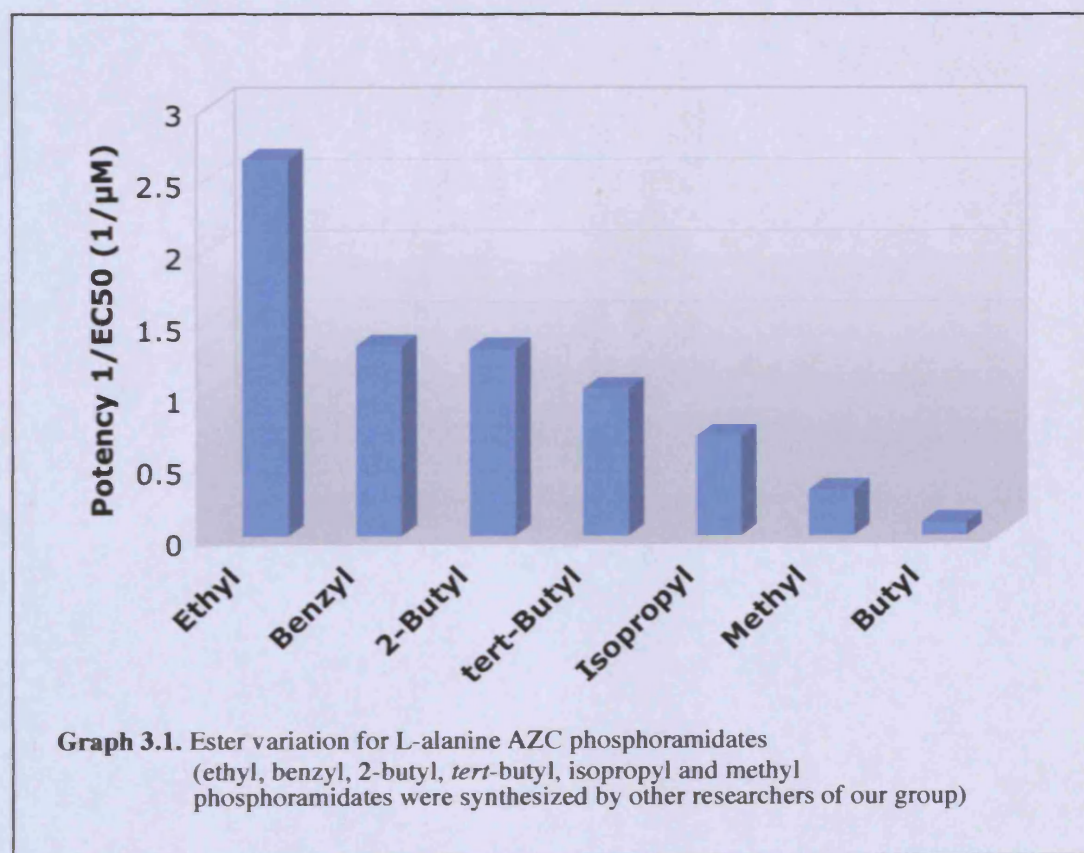
As previously observed,[17] the presence of a good substrate for the esterase such as the ethyl and benzyl group increases the activity. More bulky esters such as butyl, isopropyl and *tert*-butyl decrease the activity. The presence of the 2-butyl ester

does not significantly reduce the activity even though this group does not usually represent a good substrate for the esterase. Surprisingly the *tert*-butyl ester phosphoramidate showed reasonable activity in contrast with methyl and butyl ester derivatives that were poorly active.

3.3.6.4 Amino acid variation

Another important part in the phosphoramidate structure is represented by the amino acid. Also in this case the presence of a good substrate for the esterase can efficiently deliver the 5'-monophosphate. For the 4'-azidocytidine the amino acid variation has been investigated. The activity of different benzyl phosphoramidates is reported in **Graph 3.2**.

Graph 3.2. Amino acid variation for AZC phosphoramidates
(all benzyl ester phosphoramidates, L-alanine, glycine, dimethylgycine, phenylalanine, leucine and valine were synthesised by other researcher in our group)
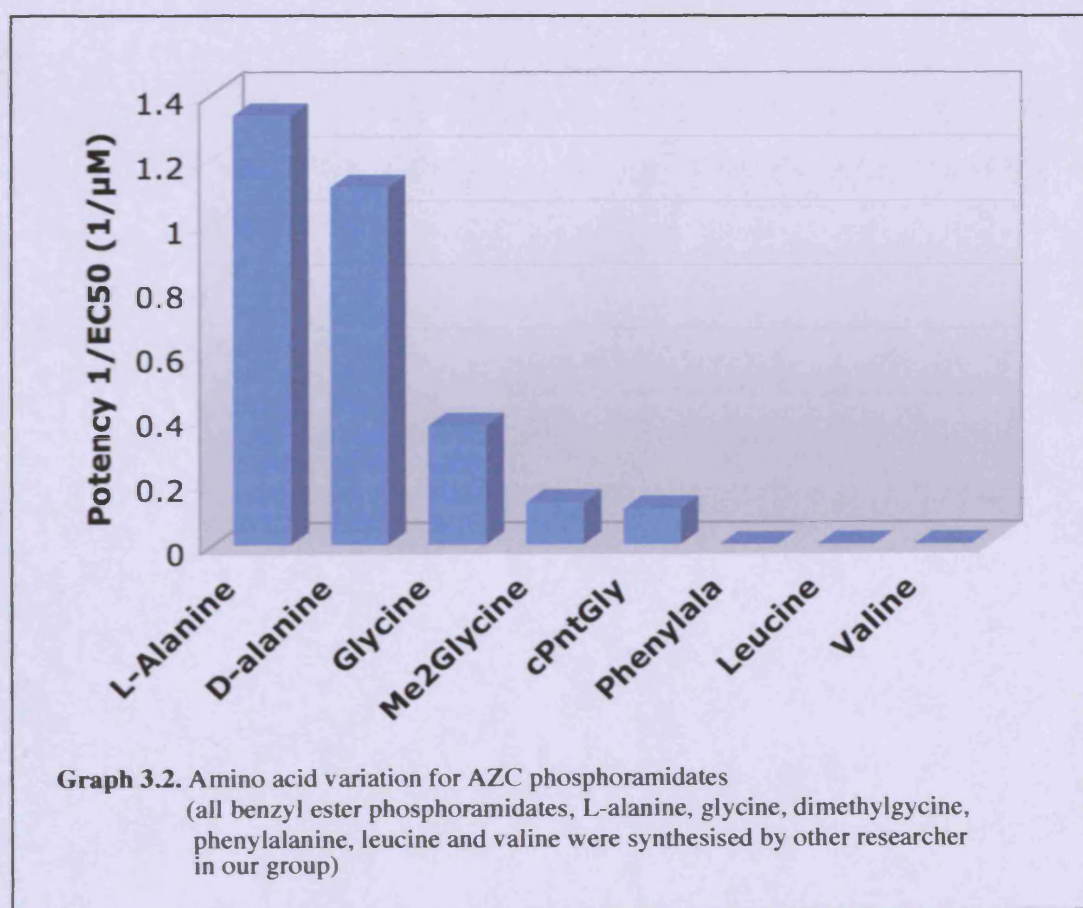
As predicted and observed in the past,[18] it is clear that L-alanine is the most active amino acid.

Of note was the inactivity of cyclopentylglycine, which was unexpected and not observed in previous projects. [18]

In contrast with the data for d4T phosphoramidates,[6] the D-alanine phosphoramidate showed similar activity to the corresponding L-isomer. A 3-5 fold reduction in activity was found on replacing L-alanine with glycine and dimethylglycine. This might indicate that the presence of the methyl group with the L-alanine configuration does not confer any advantage over the corresponding simple hydrogen of glycine or the D-alanine isomer. Moreover, L-amino acids, with larger and more hydrophobic side chains (e.g. L-valine, L-isoleucine, L-leucine), showed a remarkable reduction in activity: this might indicate the presence of a small hydrophobic pocket in the putative activating enzyme ("esterase" and "phosphoramidase").

3.4. Substituted phenyl phosphoramidates of 4'-azidocytidine

In previous work it was noticed that the presence of different substituents on the aryl moieties can modify the biological activity.[11] In order clearly to define how the activity is influenced by the presence of a particular substituent the Topliss tree concept has been applied to ethyl L-leucine 4'-azidocytidine phosphoramidate.

The Topliss tree methodology has been reported as an operational scheme for analogue design that may help to find the best substituent and their corresponding relative position on the aryl moiety of an active compounds (**Figure 3.12**).[20]
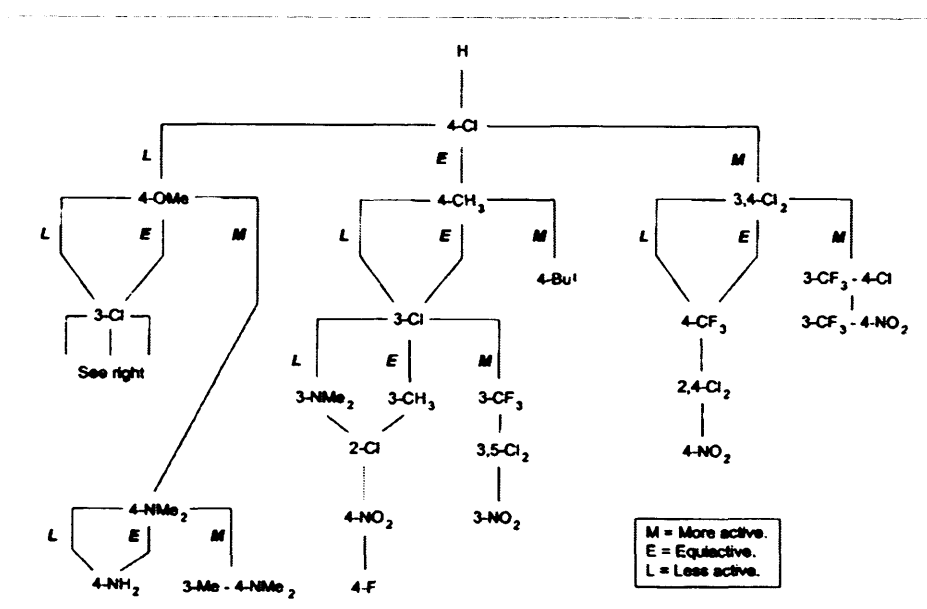
Figure 3.12. Topliss tree

The Topliss tree is based on the effect of electron-withdrawing and electron-releasing groups on the aryl moiety. In the first instance, the preparation of *para*-chloro on the phenyl ring is suggested. If this new compound is more active than the parent compound (M) this means that the electron-withdrawing effect can give an increase in activity; for this reason, it would be advantageous to synthesise the 3,4-dichloro, increasing the inductive effect on the ring.

If it is equally active (E) this means that the presence of this group does not affect the activity, and so it would be better to introduce the methyl group in the *para* position.

If it is less active (L) the choice is to synthesise the *p*-methoxy, because, this means that the presence of an electron-withdrawing group can give a decrease in

activity, so it is possible that the presence of an electron-releasing group can help the activity. The Topliss tree methodology was applied to the 4'-azidocytidine phosphoramidate with ethyl-L-leucine as the amino acid ester, which was previously synthesised by other member of our group and tested against HCV.

3.4.1 Synthesis of phosphorylating agent for coupling with amino acids

In order to synthesise phosphoramidates with substituents on the aryl moiety, it was necessary to synthesise arylphosphorodichloridates that represented the starting materials for the couplings with the appropriate amino acid. The general synthetic pathway, used for the synthesis of aryldichlorophosphates, was developed by McGuigan *et al.*[6-7] Phosphorus oxychloride ($POCl_3$, **75**) and the appropriate phenol derivative (**76**) were coupled in the presence of TEA to give the corresponding aryldichlorophosphate (**77**) (**Figure 3.13**).

Figure 3.13. General synthetic pathway for the synthesis of aryldichlorophosphate (R and R'= substituents on the aryl moiety)
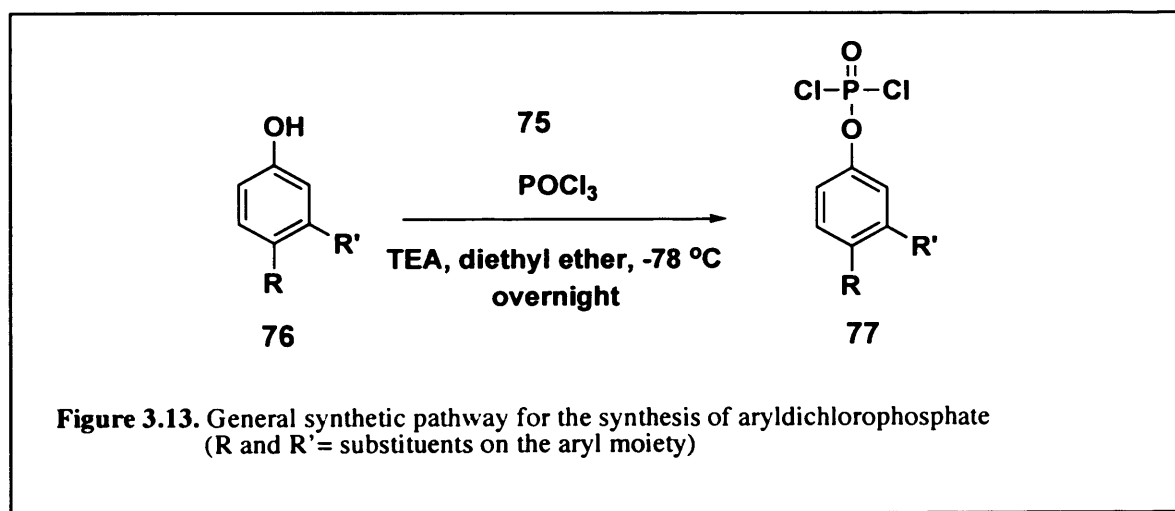
Because of the substantial instability of these compounds, the crude product of the reaction was quickly filtered to remove the triethylammonium salt. The reactions succeeded with reasonable yield and high purity. The observed chemical shifts in ^{31}P-NMR spectra were between 4.8 and 5.5 ppm (**Table 3.6**).

Cpd no.	R	R'	Yield	[31]P (ppm)
78[8]	chloro	H	40%	5.00
79	chloro	chloro	45%	4.79
80	methyl	H	72%	4.98
81[11]	methoxy	H	67%	5.45

Table 3.6. Yields for the aryl-dichlorophosphates synthesised

3.4.2 Synthesis of phosphorylating agents for coupling with 4'-azidocytidine

As mentioned in section **3.3.2** the synthetic route for the synthesis of phosphorylating agents for coupling with the modified nucleosides was developed by McGuigan *et al.*,[1-4] involving the coupling of the appropriate substituted phenylphosphorodichloridate with L-leucine ethyl ester hydrochloride in the presence of triethylamine (TEA) at -78 °C overnight. Due to the possible instability of the compounds the purification involved a filtration through a filtration funnel to remove the triethylammonium salt. As a result of the presence of two possible phosphorus configurations, it was possible to see signal splitting in the ^{31}P-NMR spectra (**Table 3.7**).
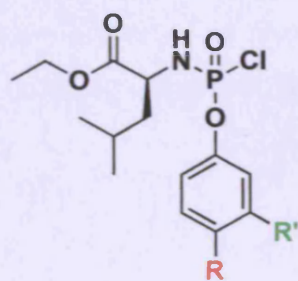
Cpd no.	R	R'	[31]P (ppm)
82	chloro	H	9.75, 9.73
83	chloro	chloro	9.84, 9.78
84	methyl	H	10.06, 9.63
85	methoxy	H	10.54, 10.22

Table 3.7. [31]P Chemical shifts of L-leucine ethyl ester phosphorochloridates

3.4.3 Synthesis and biological activity of phosphoramidates of 4'-azidocytidine with substituents on the aryl moiety

Four compounds with different substituents present in the first row of the Topliss tree (*p*-chloro, 3,4 dichloro, *p*-methyl and *p*-methoxy) were synthesised simultaneously. The synthetic pathway used has been previously described (see section **3.3.5**) and involved the synthesis of an alkoxide intermediate in the presence of an excess of tBuMgCl followed by coupling with the appropriate phosphorochloridate (**Figure 3.14**).

Figure 3.14. Synthesis of substituted phenyl AZC phosphoramidates
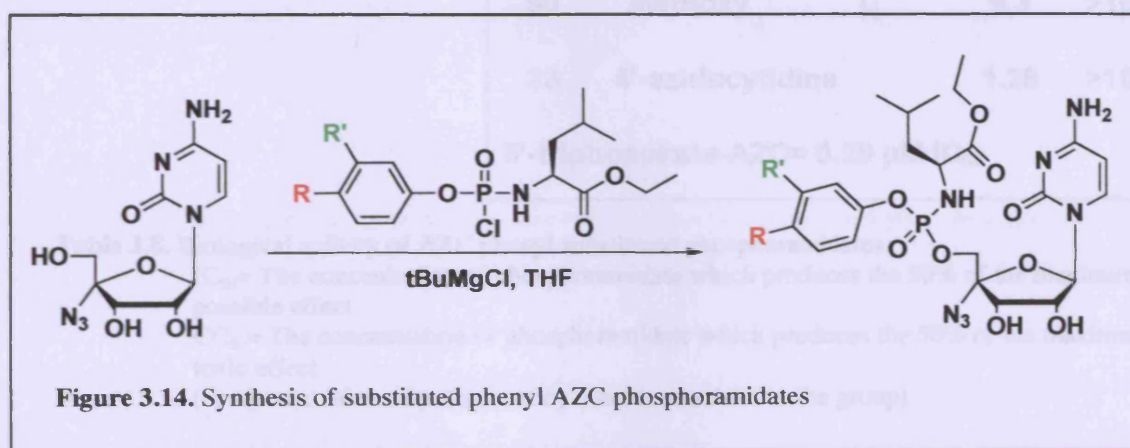
The phosphoramidates synthesised had yields between 3% and 14%. These compounds were tested *in vitro* in the replicon assay (see section **3.3.6.1**) against HCV (**Table 3.8**).[14, 15]
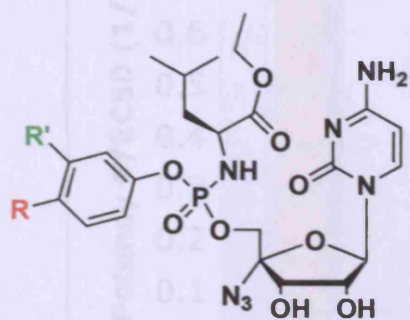
Cpd no.	R'	R'	EC$_{50}$ (µM)	CC$_{50}$ (µM)
86	H	H	>100	>100
87	chloro	H	2.52	>100
88	chloro	chloro	2.32	>100
89	methyl	H	4.3	>100
90	methoxy	H	9.3	>100
23	4'-azidocytidine		1.28	>100
5'-triphosphate-AZC= 0.29 µM IC$_{50}$				

Table 3.8. Biological activity of AZC phenyl substituted phosphoramidates
IC$_{50}$= The concentration of phosphoramidate which produces the 50% of the maximum possible effect
CC$_{50}$= The concentration of phosphoramidate which produces the 50% of the maximum toxic effect
(compound **86** was synthesised by other researchers in the group)

For AZC conversion of an inactive unsubstituted phosphoramidate to substituted phosphoramidates active at the μM level was achieved (**Graph 3.3**).

In particular, the effect of an inductive group such as *para*-chloro (**87**) and 3,4-dichloro (**88**) on the activity was higher when compared to the presence of an electron-donating group such as *para*-methyl (**89**) and *para*-methoxy (**90**). However, the difference of the effect between an electron-withdrawing (**87, 88**) or electron-donating group (**89, 90**) is not significant.

Graph 3.3. Topliss tree applied to L-leucine ethyl ester phosphoramidates
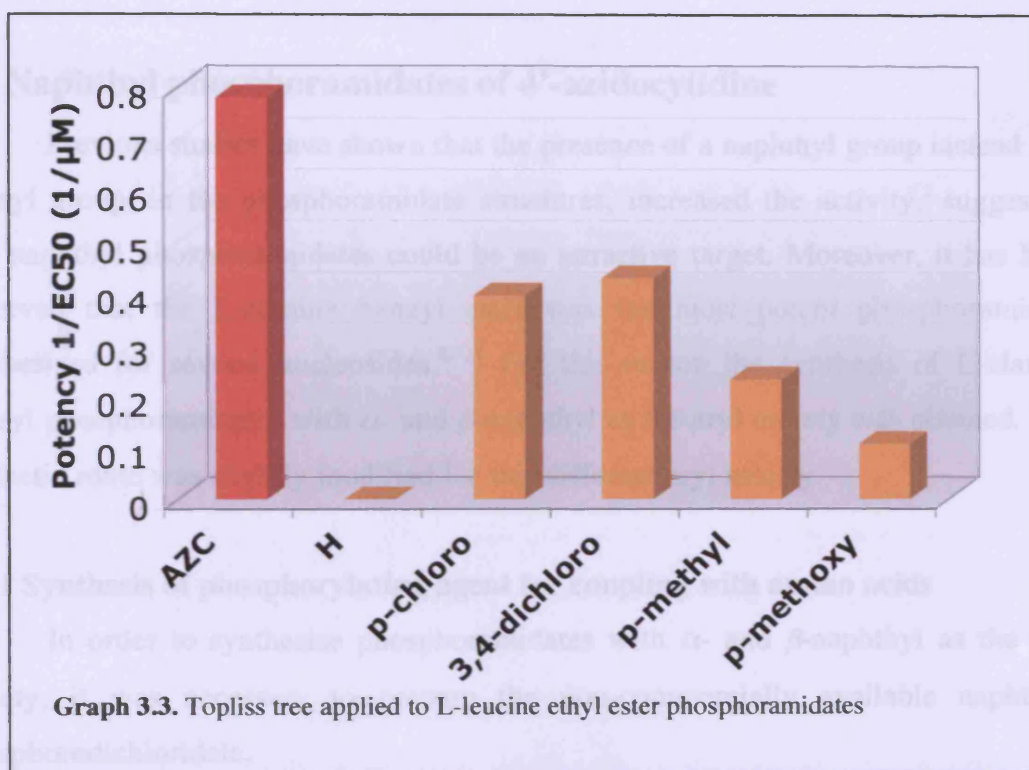
Most importantly the activity of the parent nucleoside (4'-azidocytidine, **23**) was higher compared to the substituted and unsubstituted phosphoramidates, that might be a direct consequence of the inefficient phosphoramidate metabolism to release the corresponding 5'-monophosphate.

3.5 Naphthyl phosphoramidates of 4'-azidocytidine

Previous studies have shown that the presence of a naphthyl group instead of a phenyl group, in the phosphoramidate structures, increased the activity,[1] suggesting that naphthyl phosphoramidates could be an attractive target. Moreover, it has been observed that the L-alanine benzyl ester was the most potent phosphoramidate synthesised for several nucleosides.[6, 11] For this reason the synthesis of L-alanine benzyl phosphoramidates with α- and β-naphthyl as the aryl moiety was planned. The synthetic route was slightly modified for this different aryl moiety.

3.5.1 Synthesis of phosphorylating agent for coupling with amino acids

In order to synthesise phosphoramidates with α- and β-naphthyl as the aryl moiety, it was necessary to prepare the non-commercially available naphthyl-phosphorodichloridate.

The synthesis has been previously described in section **3.3.1**: the same procedure was used, the only difference being that in this case the starting materials were α- and β-naphthol. The purification methods required exactly the same procedure previously described.

The ^{31}P-chemical shift and the yield of the synthesised phosphorodichloridates (**91, 92**) are reported in **Table 3.9**.

O
‖
Cl–P–Cl
|
O
|
Ar
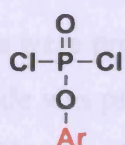
Ar= Aryl moiety

Cpd no.	Aryl moiety	Yield	[31]P (ppm)
91[19]	α-naphthyl	90%	3.91
92[19]	β-naphthyl	87%	3.60

Table 3.9. Yields of the synthesis of α-naphthyl and β-naphthyl-phosphorodichloridates

3.5.2 Synthesis of phosphorylating agents for coupling with 4'-azidocytidine

For the synthesis of these intermediates, we applied the previously described procedure for the synthesis of phosphorochloridates (see section **3.3.2**).

The purification method involved rapid column chromatography in the presence of a mixture of hexane/EtOAc (7:3) as eluent.

Due to the non-regiospecific reaction, the synthesised products were a mixture of two diastereoisomers with a signal splitting observed in ^{31}P-NMR spectra (**Table 3.10**).
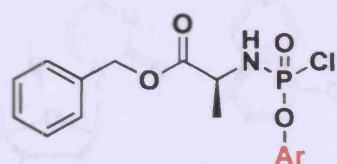
Cpd no.	Aryl moiety	^{31}P (ppm)
93[19]	α-naphthyl	8.58, 8.25
94[19]	β-naphthyl	8.32, 8.10

Table 3.10. α-Naphthyl and β-naphthyl-phosphorochloridate

3.5.3 Synthesis of α-naphthyl 4'-azidocytidine phosphoramidates

In an attempt to increase the yield, the same phosphoramidate reaction was performed on 2',3'-protected 4'-azidocytidine. Protection in the 2'- and 3'-positions, increased the solubility of the nucleoside in THF, therefore increasing its availability to react with the phosphorochloridate.

3.5.3.1 Synthesis of 2',3'-protected α-naphthyl 4'-azidocytidine phosphoramidates

In an attempt to increase the solubility of the nucleoside in THF and to reduce the number of chromatography columns required for the purification, the 2'- and 3'-positions were protected with cyclopentylidene. The corresponding 2'- and 3'-rptected nucleoside was provided by Roche.

The coupling of 2',3'-cyclopentylidene 4-azidocytidine (**96**) was carried out using the Uchiyama procedure previously described (**Figure 3.15**).

The most important achievement was the complete solubilisation of the nucleoside in THF. The reaction was stirred overnight and the purification usually required just one chromatography column. As a consequence, the yields obtained were higher (72-85%) than those observed for the unprotected 4'-azidocytidine (3-14%).

Figure 3.15. Synthesis of 2',3'-cyclopentylidene α-naphthyl AZC phosphoramidates
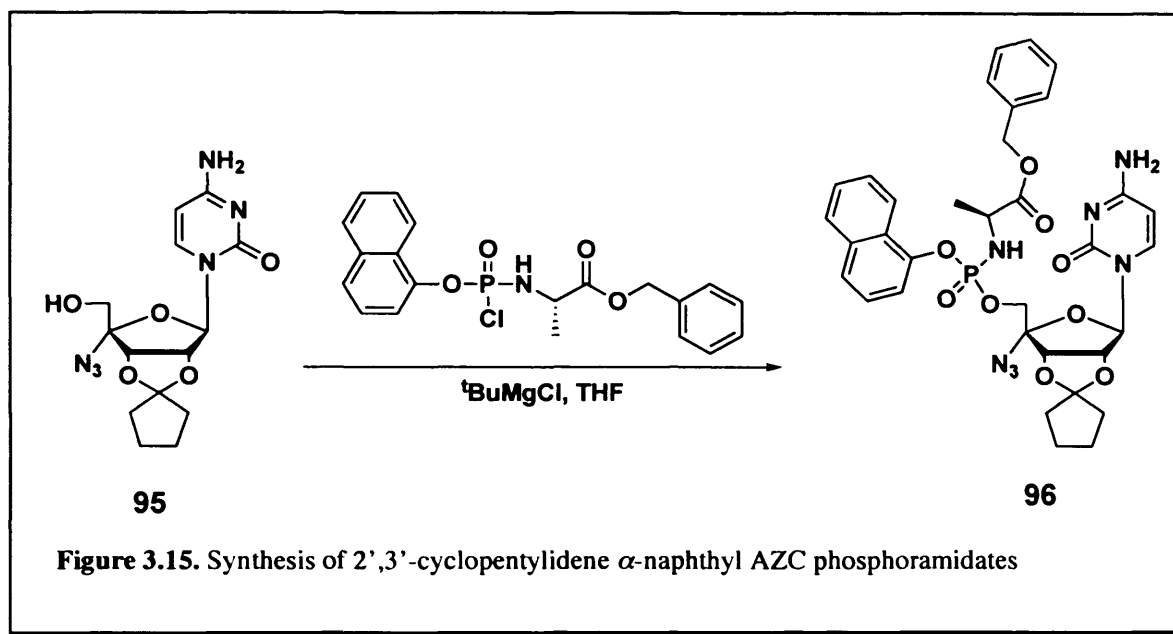
3.5.3.2   Separation   of   2',3'-cyclopentylidene-AZC   α-naphthyl-phosphoramidate diastereoisomers

The synthetic pathway used for the synthesis of the 4'-azidocytidine phosphoramidates was not stereospecific: and the product obtained was a mixture of compounds in which the P-centre had two possible configurations (two diastereoisomers). In order to explore the possibility of different activity between the two diastereoisomers, their separation was attempted using semi-preparative HPLC.

The mobile phase used was a mixture of acetonitrile and water in a ratio of 1:1. The analytical chromatogram obtained showed an almost complete separation of the two peaks (**Figure 3.16**).
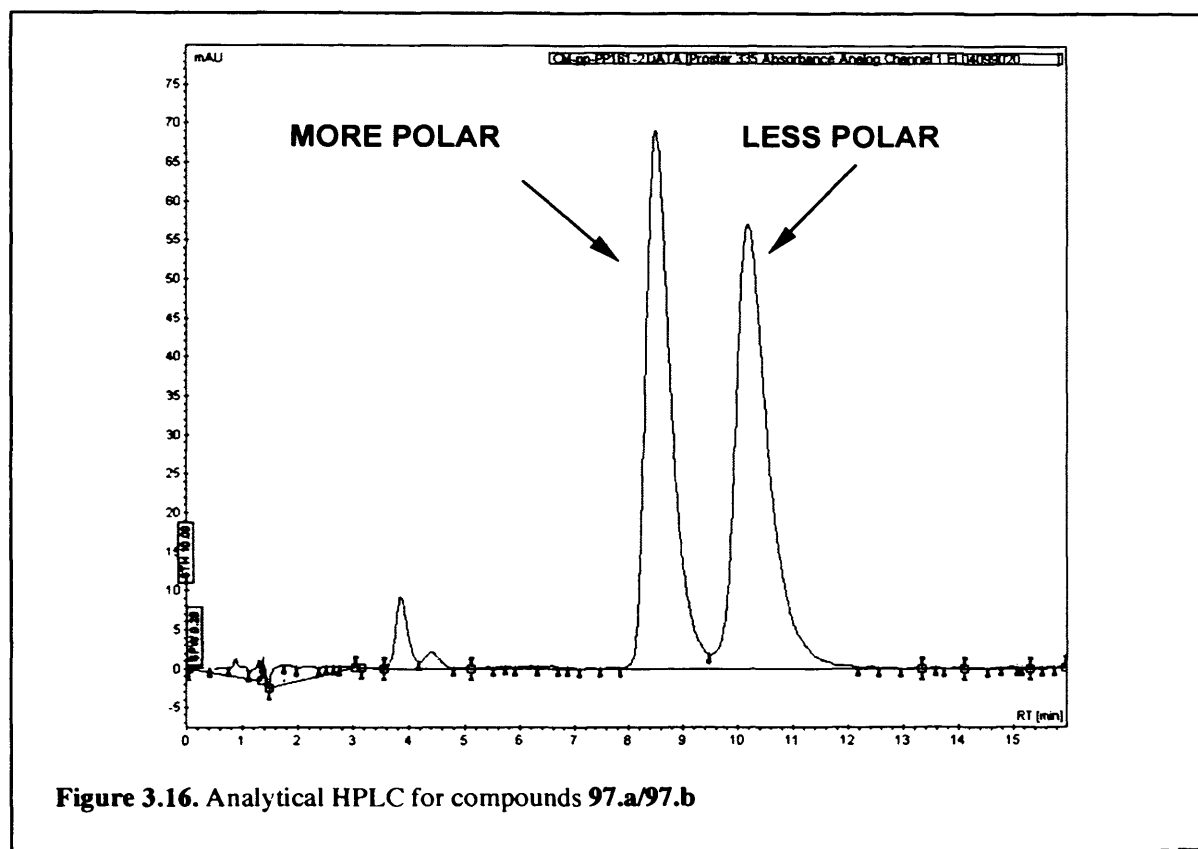
Figure 3.16. Analytical HPLC for compounds **97.a/97.b**

The two diastereoisomers (**97.a/97.b**) had retention times between 8 and 12 minutes. Also in this case the UV spectra of the two peaks were absolutely identical. The same method was then adapted to the preparative column, in order to separate a reasonable quantity of material.

Figure 3.17 shows a region of the ¹H-NMR spectra of the separated diastereoisomers. An important observation is the presence of only one diastereoisomer in each fraction. In particular, the proton in the 5- and 1'-positions gives, in both cases, a doublet, due to the coupling, respectively, with the proton in the 6- and 2'-position.
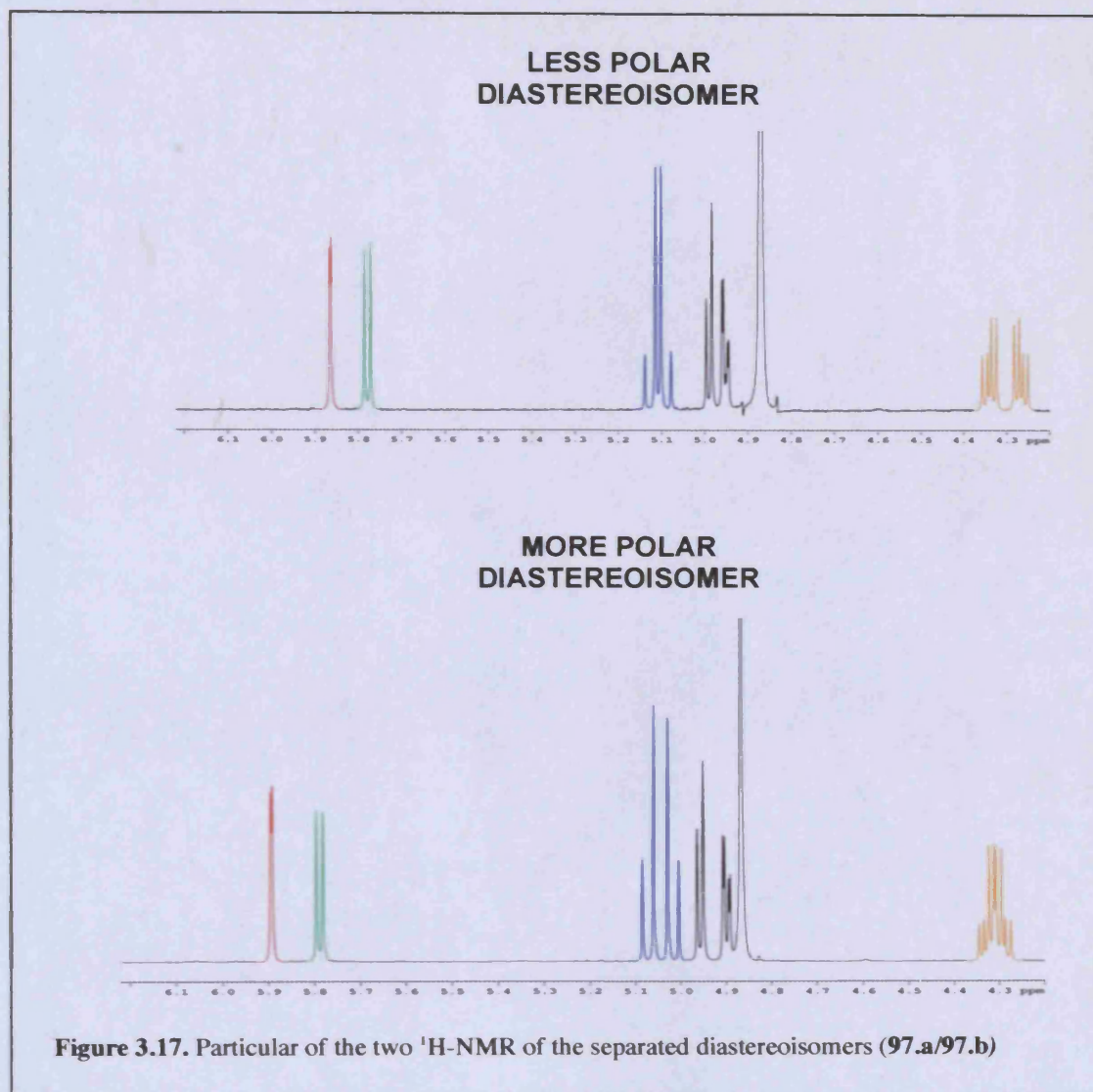
Figure 3.17. Particular of the two ^{1}H-NMR of the separated diastereoisomers (**97.a/97.b**)

Moreover, the CH_2-protons in the 5'-position (orange), in the case of the less polar diastereoisomer the signal is a doublet of doublets that become a multiplet in the case of the more polar diastereoisomer (**Figure 3.17**). The same observation can be made with the benzylic protons (blue): in the first case (less polar diastereoisomer) the signal is an AB system; in contrast for the more polar compound it is a doublet of doublets (**Figure 3.17**). Consequently, the conformations of 5'-position and benzyl group might be affected by the different phosphorous configuration of the two diastereoisomers.

3.5.3.3 Deprotection of the 2',3'-positions

The cleavage of the acetal group in the 2' and 3'-positions was performed in the presence of an 80% aqueous solution of formic acid. The reaction was stirred at room temperature for 4 hours (**Figure 3.18**).

97.a/97.b   **98.a/98.b**
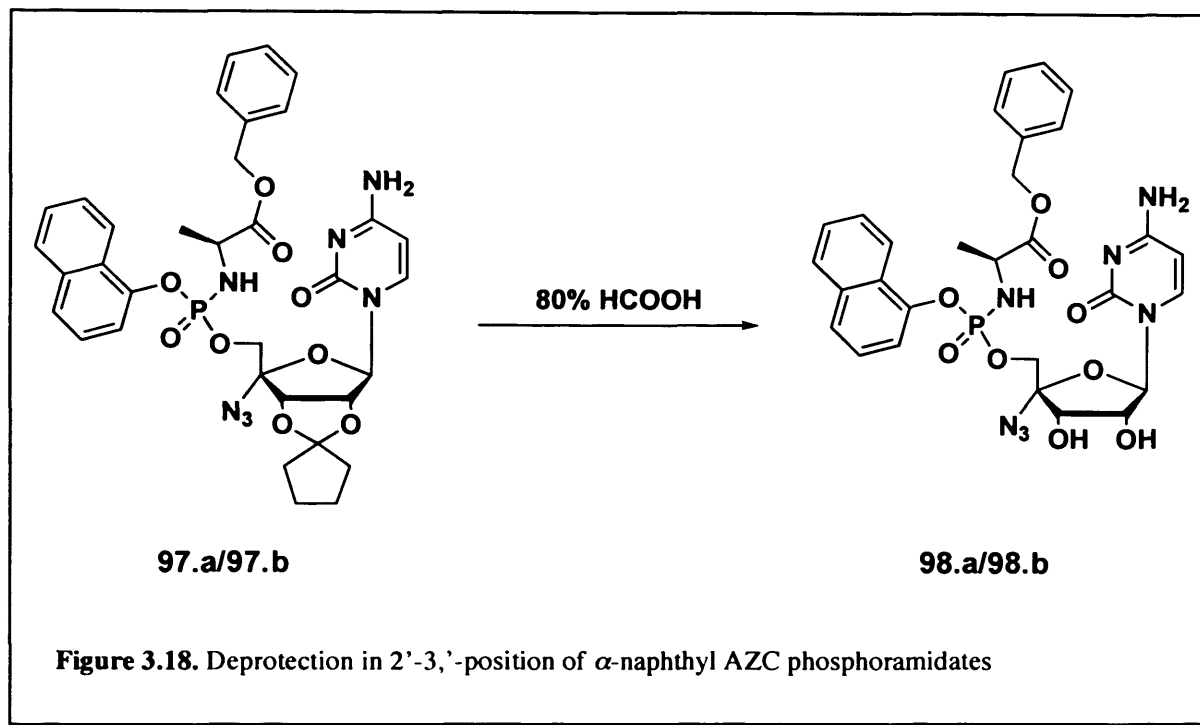
Figure 3.18. Deprotection in 2'-3,'-position of α-naphthyl AZC phosphoramidates

The reaction produced the desired compound (**98.a/98.b**) and another more polar compound that might derive from the degradation of the phosphoramidate. The purification method required only one chromatography column with reasonably high yields (80% and 82%).

3.5.4 Synthesis and HPLC separation β-naphthyl 4'-azidocytidine phosphoramidates

The β-naphthyl phosphoramidate of L-alanine benzyl ester was synthesised using the Uchiyama procedure described in section **3.3.5**. The yield for this reaction (5%) was comparable with that obtained for the phenyl phosphoramidate synthesis. Using the HPLC method optimised for the separation of α-naphthyl phosphoramidates, the two diastereoisomers were partially to separate. After HPLC,

two main fractions were isolated with different ratios of diastereoisomers (**Table 3.11**).

	Diastereoisomer 1	Diastereoisomer 2	Cpd no.
Fraction 1	6.3	1	99.a
Fraction 2	1	7.8	99.b

Table 3.11. Ratio of the two fractions isolated

3.5.5 Biological activity of naphthyl phosphoramidates of 4'-azidocytidine

All the naphthyl phosphoramidates have been tested *in vitro* using a replicon assay (see section **3.3.6.1**) against HCV (**Table 3.12**).[11, 12]
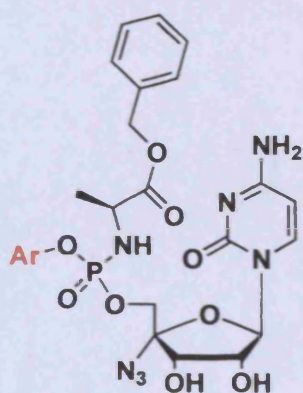
Ar= Aryl moiety

Cpd no.	Aryl moiety	EC_{50} (μM)	CC_{50} (μM)
98.a	α-naphthyl	0.82	>100
98.b	α-naphthyl	0.84	>100
99.a	β-naphthyl	2.86	>100
99.b	β-naphthyl	4.27	>100
23	4-azidocytidine	1.28	>100
5'-triphosphate-AZC= 0.29 μM IC_{50}			

Table 3.12. Biological activity of AZC naphthyl phosphoramidates
IC_{50}= The concentration of phosphoramidate which produces the 50% of the maximum possible effect
CC_{50}= The concentration of phosphoramidate which produces the 50% of the maximum toxic effect

When the corresponding phenyl phosphoramidates of 4'-azidocytidine were compared to the nucleoside itself a reduction of 5-fold in activity was observed, that might be due to the poor interaction of the phenyl moiety with one of the enzymes involved in the release mechanism of the 5'-monophosphate (see section **1.7.2**).

Graph 3.4. Biological activity of AZC-phosphoramidates with different aryl moieties
α-naphthyl 1= less polar diasteroisomer
α-naphthyl 2= more polar diasteroisomer
β-naphthyl 1= fraction 1
β-naphthyl 2= fraction 2
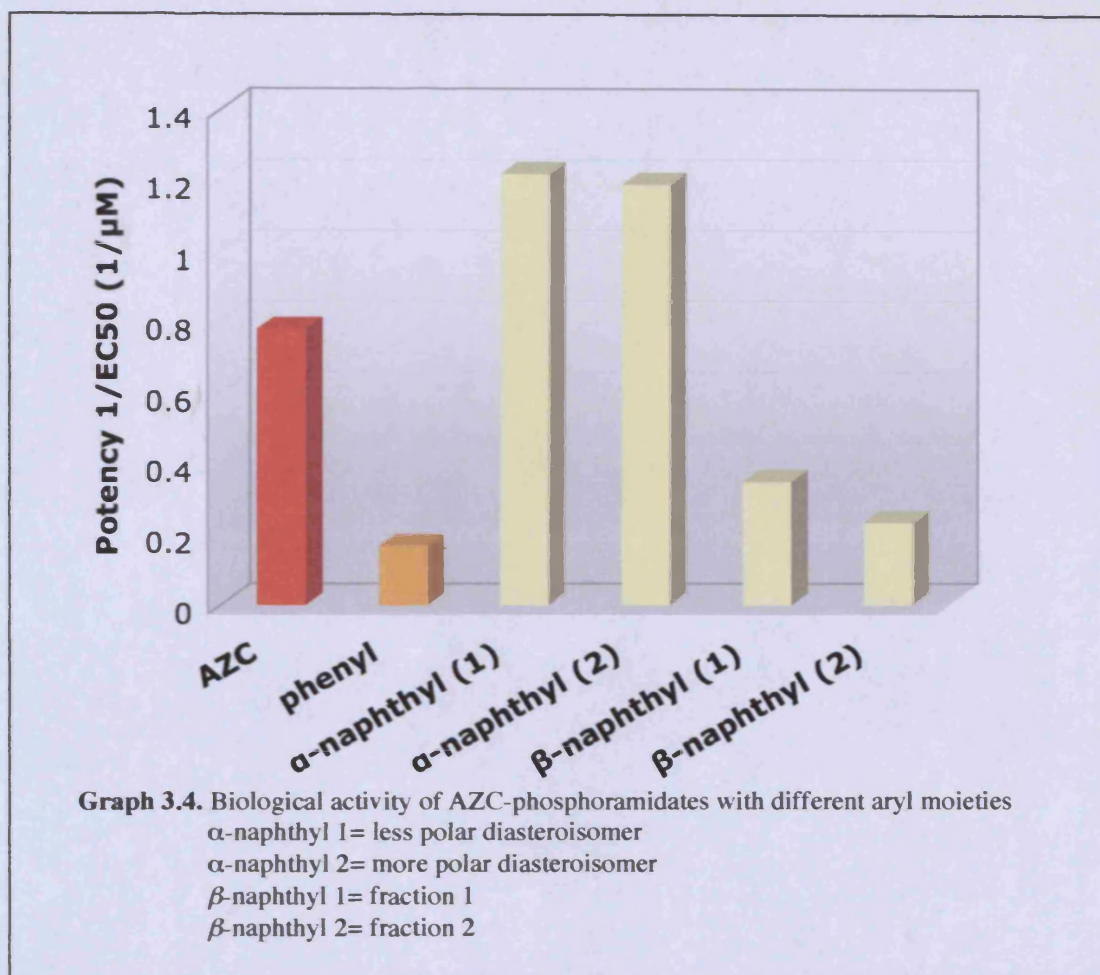
The comparison between the phenyl and its corresponding α-naphthyl phosphoramidate shows a 7-fold increase in activity for the naphthyl analogue (**Graph 3.4**). This behaviour might be explained considering the phosphoramidate metabolism, in which the corresponding alkoxide of the aryl moiety is released as a side product (see section **1.7.2**). Consequently the release of a more stabilised product such as the naphthol alkoxide might facilitate the entire process. Another possible explanation for the increase in activity is the increase of lipophilicity seen going from a phenyl group to a naphthyl-group. However, this cannot explain the different behaviour between α-and β-naphthyl-phosphoramidates. It may be more likely that the active site of one of the enzymes involved in the metabolism of the phosphoramidate might have a lipophilic pocket capable of accommodating a phenyl group or α-naphthyl-group, while, the β-naphthyl-group having a different orientation in space may be a poor substrate for these enzymes.

3.6 *dipentanoyl*-AZC aryl phosphoramidates

Following the successful work done with protected AZC (see section **3.3.1**), it was decided to synthesise phosphoramidates of a derivative of 4'-azidocytidine with a biologically cleavable protecting group. The presence of the pentanoyl group in the 2'- and 3'-positions was suggested in order to increase the lipophilicity of the nucleoside and subsequently its availability in the reaction solvent (THF). Moreover this protecting group could be biologically cleaved inside the cells by esterase activity. A new class of phosphoramidates (**100**) derived from 2',3'-dipentanoate 4'-azidocytidine was planned (**Figure 3.19**).

Figure 3.19. Scheme of *dipentanoyl*-AZC phosphoramidate synthesis
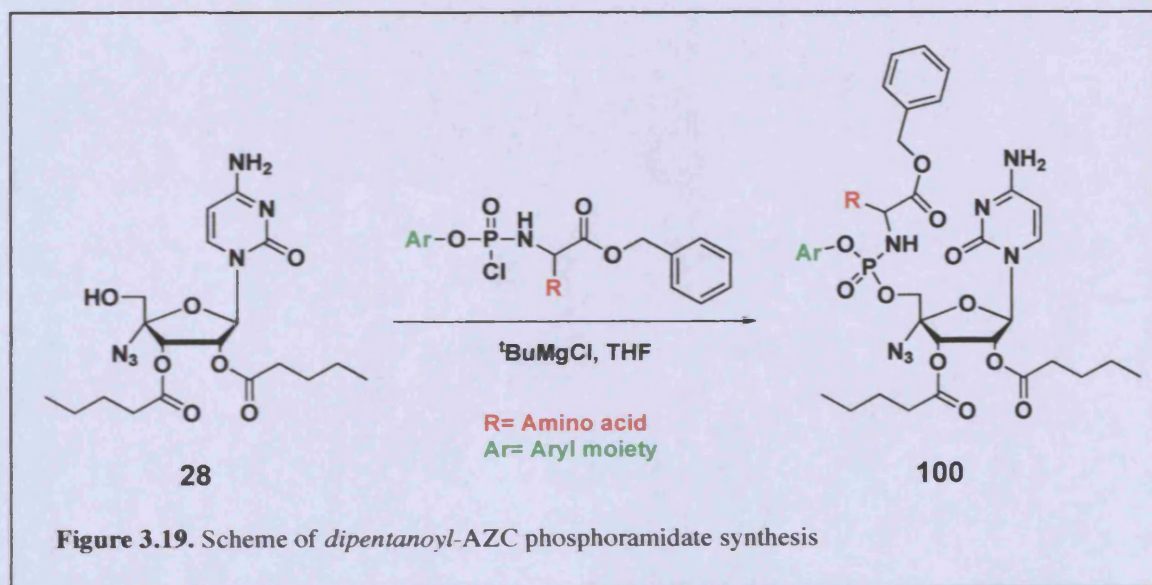
Using the usual synthetic pathway it was possible to synthesise the phosphoramidates reported in **Table 3.13**.
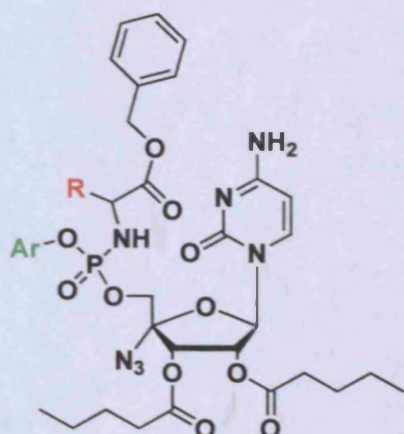
Cpd No.	Amino acids	Aryl moiety	^{31}P (ppm)
101	D-alanine	phenyl	4.60, 4.13
102.a	L-alanine	α-naphthyl	3.55
102.b	L-alanine	α-naphthyl	3.54
103	L-alanine	β-naphthyl	3.32, 3.28

R= Lateral chain
Ar= Aryl Moiety

Table 3.13. *dipentanoyl*-AZC phosphoramidates synthesised

3.6.1 Separation of *dipentanoyl*-AZC α-naphthyl-phosphoramidates diastereoisomers

The separation of **102.a/102.b** was partially successful using HPLC and an isocratic solvent system: 57% acetonitrile 43% water. The analytical chromatogram showed a good separation between the two diastereoisomers (**Figure 3.20**).
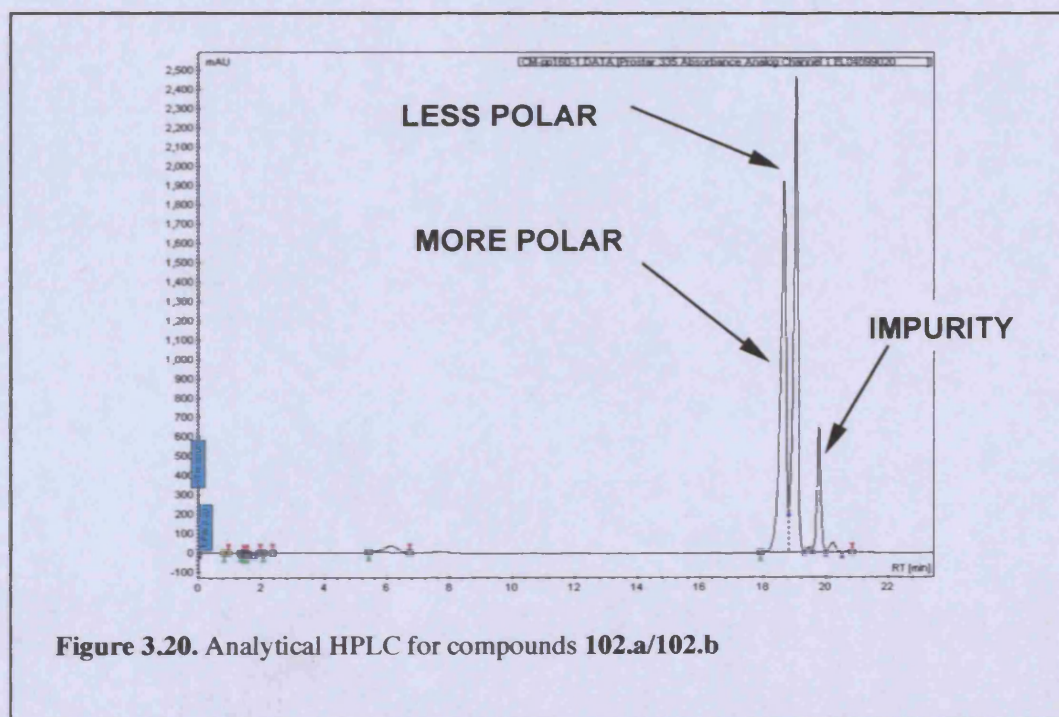
Figure 3.20. Analytical HPLC for compounds **102.a/102.b**

It was possible to distinguish the two diastereoisomers (retention time between 18 and 19 minutes) and a third impurity (retention time 20 minutes). Starting from these promising results, the same method was applied to a preparative HPLC that allowed a separation of a larger quantity of compound (20 and 30 mg respectively). Analytical HPLC was performed on the two fractions separately (**Figure 3.21**).
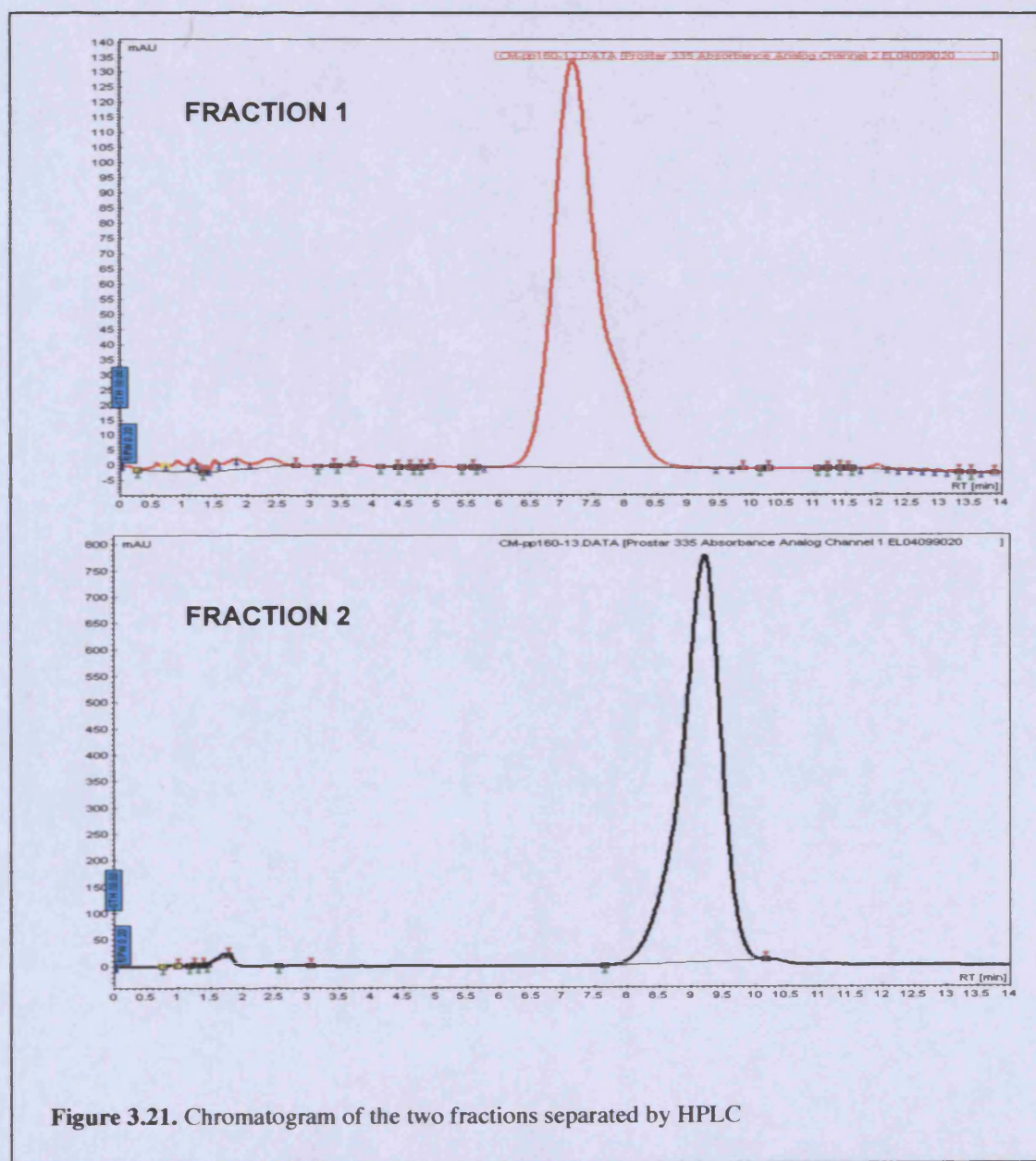
Figure 3.21. Chromatogram of the two fractions separated by HPLC

In both the chromatograms there is prevalence of one diastereoisomer. In the ^{1}H-NMR spectra of these two fractions there is a noted difference between the signals of the two diastereoisomers. In the first fraction the more shielded protons are predominant (blue), instead, in the second fraction the deshielded protons (red) have the highest intensity (**Figure 3.22**).

Moreover, the main signal of the benzylic protons is a doublet of doublets (blue) in the first fraction; for the diastereoisomers in the second one the main signal is an AB system (**Figure 3.22**). From this observation, it might be possible that the different phosphorus configuration of these two compounds affects also the 3D conformation of the rest of the molecule.
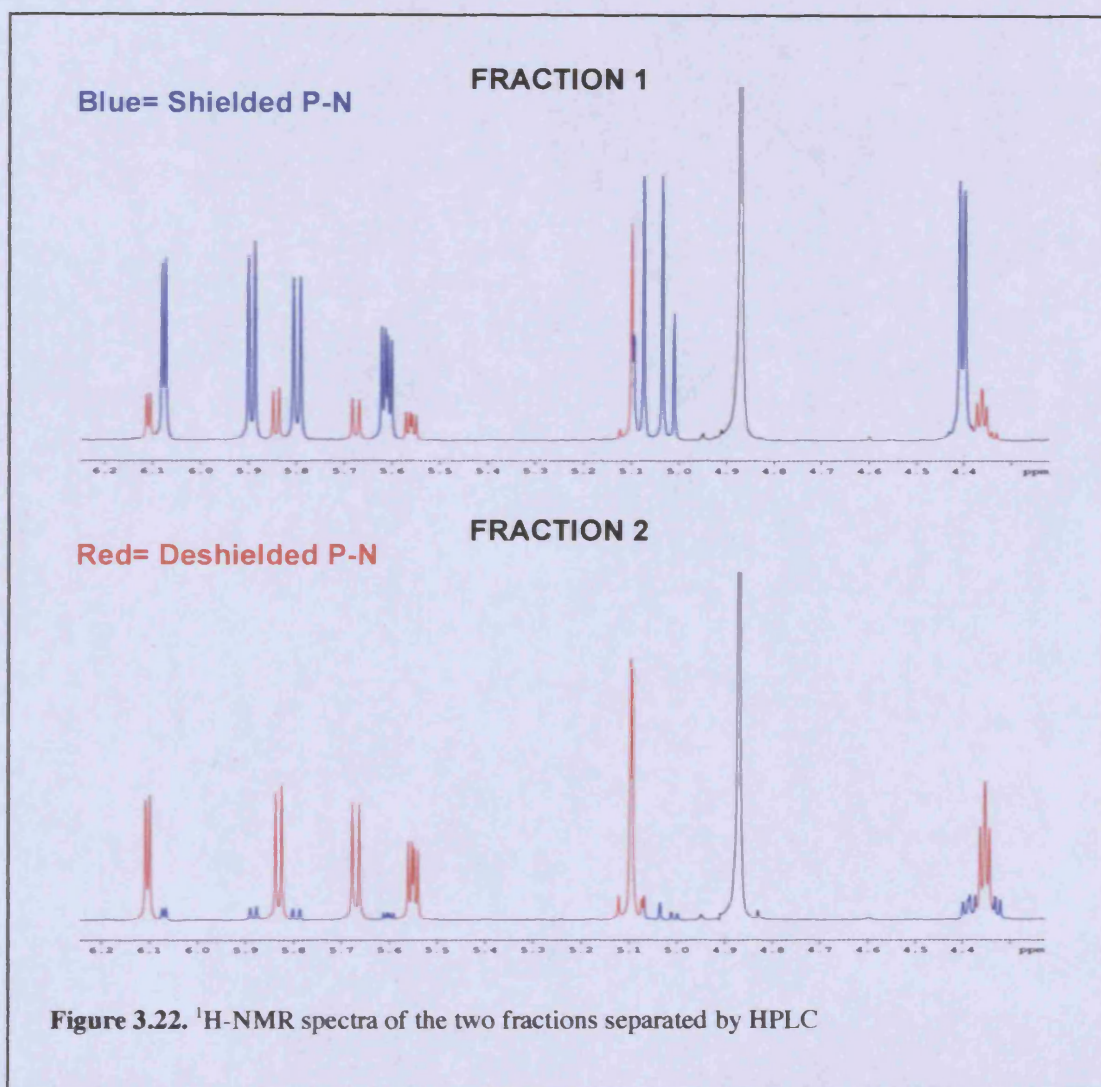
Figure 3.22. ^{1}H-NMR spectra of the two fractions separated by HPLC

From the ^{1}H-NMR spectra integration, we determined the ratio between the two diastereoisomers in the two fractions (**Table 3.14**).

	Diastereoisomer 1	Diastereoisomer 2	Cpd no.
Fraction 1	3.4	1	102.a
Fraction 2	1	10	102.b

Table 3.14. Ratio of diastereoisomers in the two fractions isolated

3.6.2 Biological activity of phosphoramidates of *dipentanoyl*-4'-azidocytidine

All the naphthyl phosphoramidates were tested *in vitro* using the replicon assay (see section **3.3.6.1**) against HCV (**Table 3.15**).
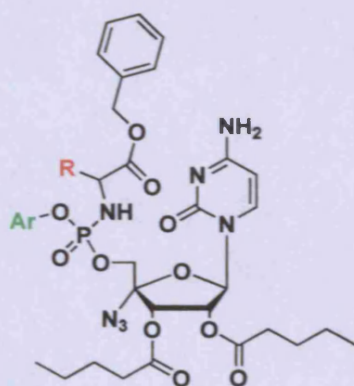
R= Lateral chain
Ar= Aryl Moiety

Cpd No.	Amino acid	Aryl moiety	EC$_{50}$ (µM)	CC$_{50}$ (µM)
101	D-alanine	phenyl	1.2	>100
102.a	L-alanine	α-naphthyl	0.37	>100
102.b	L-alanine	α-naphthyl	0.96	>100
103	L-alanine	β-naphthyl	0.47	>100
5'-triphosphate-AZC= 0.29 µM IC$_{50}$				

Table 3.15. Biological activity of pent-AZC phosphoramidates synthesised
IC$_{50}$= The concentration of phosphoramidate which produces the 50% of the maximum possible effect
CC$_{50}$= The concentration of phosphoramidate which produces the 50% of the maximum toxic effect

Comparing the two pure and separated *dipentanoyl*-4'-azidocytidine (**102.a/102.b**) phosphoramidate diastereoisomers and 4'-azidocytidine, there is no difference in terms of biological activity (**98.a/98.b**). Moreover, as observed for the α-naphthyl, the two diastereoisomers do not show a significant difference in terms of activity. Surprisingly the activity of the α-naphthyl (**102.a/102.b**) and the β-naphthyl (**103**) derivatives are comparable and in the case of compound **102.b** (pure α-naphthyl

diastereoisomer) the activity is lower than compound **103**. The biological activity of the L-alanine phenyl phosphoramidate of *dipent*-4'-azidocytidine is 0.39 μM,[18] only 3 fold higher than the activity of the corresponding D-alanine derivative. The presence of a methyl group in the lateral chain of the amino acid may be important for the activity, but the enzyme involved in phosphoramidate metabolism does not appear to require a particular conformation in that position.

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Chapter Four

Aryloxyphosphoramidates of uridine analogues

4.1 4'-Azidouridine phenyl phosphoramidates

As illustrated in **Chapter Two**, the aim of the collaboration with Roche Biosciences was to apply the aryl-phosphoramidate technology to different 4' modified nucleoside analogues. 4'-Azidouridine (**24**) was tested against HCV in the replicon assay and was found completely inactive, whereas the corresponding 5'-triphosphate had activity at 0.22 μM against RdRp.[1] The activity of 4'-azidouridine triphosphate and the inactivity of the corresponding nucleoside might indicate that 4'-azidouridine (**24**) is poorly phosphorylated by the kinases (see **Chapter One**). One possibility to overcome this problem was the delivery into the cell of the corresponding 5'-monophosphate via phosphoramidate technology.

4.1.1 Synthesis of 4'-azidouridine phosphoramidates

Some of the chosen amino acid esters were not commercially available and needed to be synthesised.

4.1.1.1 Synthesis of amino acid esters

The synthesis of amino acid esters has been described in **Chapter Three** (**Figure 3.4** and **3.5**). All the amino acid esters reported as tosylate salts were synthesised using Dean Stark apparatus; while, all the hydrochloride amino acid esters were synthesised in the presence of thionyl chloride. In **Table 4.1** the yields of the amino acid esters synthesised are reported.

Cpd no.	Amino acid	Ester	Salt	Yield
104[14]	D-alanine	isopropyl	Tosylate	64%
105	D-alanine	2-butyl	tosylate	76%
106	D-alanine	octyl	tosylate	75%

Table 4.1. Yields of amino acid esters synthesised

4.1.1.2 Synthesis of phosphorylating agents for coupling with 4'-azidouridine

This synthetic route has already been described in **Chapter Three** (**Figure 3.6**): a reaction between the commercially available phenyldichlorophosphate and the appropriate amino acid salt.[2-5] The ^{31}P-NMR chemical shifts were observed between 8.49 and 9.41 ppm (**Table 4.2**).

R= Amino acid
R'= Ester

Cpd no.	Amino acid	Ester	^{31}P (ppm)
107[14]	D-alanine	isopropyl	9.41, 9.09
108	D-alanine	2-butyl	8.49
109	D-alanine	octyl	9.20, 9.00

Table 4.2. ^{31}P Chemical shifts of phosphorochloridates synthesised

4.1.1.3 Synthesis of phenyl-AZU phosphoramidates

As the only successful access to 4'-azidocytidine aryloxyphosphoramidates was in the presence of tBuMgCl (see **Chapter Three**), a series of 4'-azidouridine phosphoramidates was synthesised using the same strategy (**Figure 4.1**).[8]

On comparison with the synthesis of 4'-azidocytidine phosphoramidates there were two substantial differences: due to its higher lipophilicity 4'-azidouridine was more soluble in THF and there were no traces after the first chromatography column of P-OH (**64**, product of hydrolysis of phosphorochloridate) and consequently the purification method was much easier.
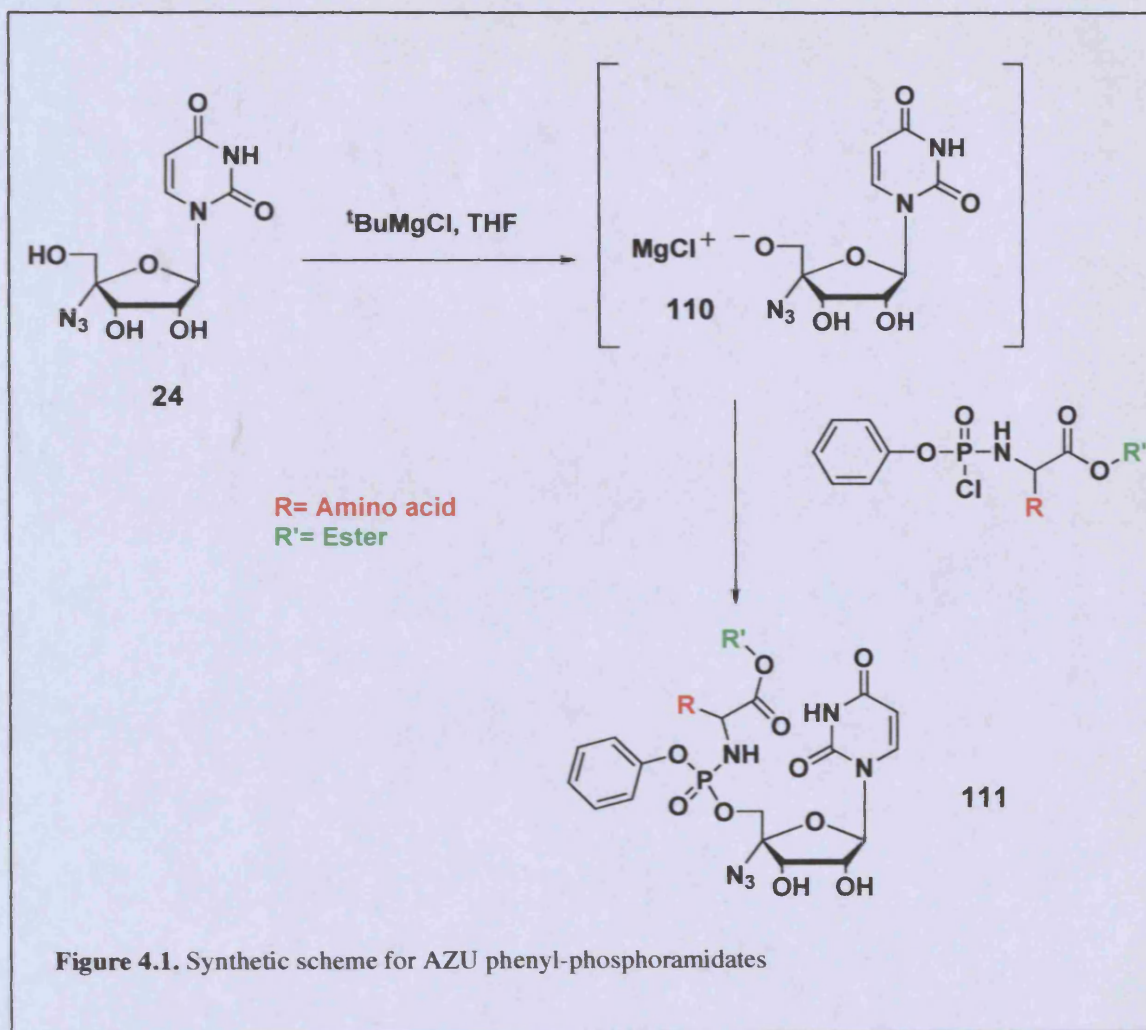
Figure 4.1. Synthetic scheme for AZU phenyl-phosphoramidates

The yields observed using this nucleoside were between 10 and 20% with the synthesised phosphoramidates reported in **Table 4.3.**
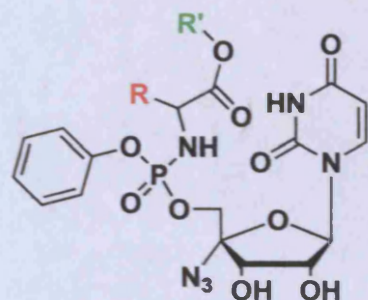
R= Amino acid
R'= Ester

Cpd no.	Amino acids	Esters	^{31}P (ppm)
112	Cyclopentylgly	benzyl	3.77, 3.74
113	Cyclopentylgly	ethyl	3.85, 3.83
114	Cyclopentylgly	isopropyl	3.87, 3.83
115	D-alanine	benzyl	4.89, 4.29
116	D-alanine	dodecyl	4.92, 4.38
117	D-alanine	butyl	4.81, 4.59
118	D-alanine	2-butyl	4.97, 4.41
119	D-alanine	octyl	4.35, 4.20
120	D-alanine	isopropyl	4.97, 4.41
121	L-alanine	butyl	4.91, 4.35

Table 4.3. ^{31}P Chemical shifts of AZU phosphoramidates

4.1.2 Synthesis of phenyl phosphoramidates via protected 4'-azidouridine

In order to increase the solubility of the nucleosides and consequently the average yield of the phosphoramidates, the 2'- and 3'-positions were protected with an acetal group that could be easily cleaved in acid conditions. The corresponding 2'- and 3'-protected nucleoside was provided by Roche. In particular, the cyclopentylidene 4'-azidouridine was used to generate a second series of 4'-azidouridine phosphoramidates. Some of the chosen amino acid esters were not commercially available and therefore needed to be synthesised.

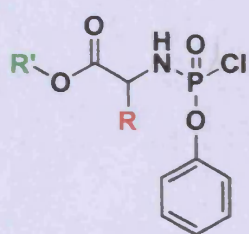### 4.1.2.1 Synthesis of amino acid esters

A second series of amino acid esters were synthesised using the previously described procedure (section **4.1.1**). The amino acid esters generated are reported in **Table 4.4**.

Cpd no.	Amino acid	Ester	Salt	Yield
122[5]	α,α-dimethylgly	benzyl	Tosylate	42%
123[5]	α,α-dimethylgly	ethyl	hydrochloride	73%
124[5]	L-proline	ethyl	hydrochloride	77%
125[14]	β-alanine	ethyl	hydrochloride	65%
126[6]	N-methyl-glycine	ethyl	hydrochloride	78%
127[14]	L-alanine	isopropyl	hydrochloride	85%

Table 4.4. Amino acid esters synthesised

4.1.2.2 Synthesis of phosphorylating agents for coupling with protected 4'-azidouridine

The synthesis of phosphorochloridates involved a coupling reaction between the commercially available phenyldichlorophosphate and the corresponding amino acid ester in the presence of TEA at -78°C overnight. Due to the possible instability of these compounds, the purification method involved a rapid chromatography column using, as eluent, a mixture of EtOAc/hexane 3:7. The phosphorochloridates synthesised are reported in **Table 4.5**. [2-5]
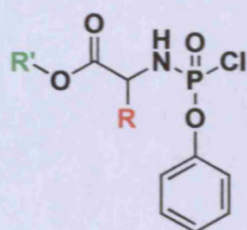
R= Amino acid
R'= Ester

Cpd no.	Amino acid	Ester	^{31}P (ppm)
128[5]	L-alanine	methyl	7.88
129[5]	L-alanine	ethyl	7.94
130[14]	L-alanine	isopropyl	8.22
131[7]	L-alanine	*tert*-butyl	8.07
132[5]	L-alanine	benzyl	7.79, 7.75
133[14]	glycine	benzyl	9.15
134[14]	L-valine	benzyl	9.48, 8.95
135[5]	α,α-dimethylgly	benzyl	5.53
136[5]	α,α-dimethylgly	ethyl	5.58
137[14]	L-phenylalanine	benzyl	7.91, 7.85
138[14]	L-phenylalanine	ethyl	8.03, 7.95
139[5]	L-leucine	ethyl	8.46, 8.22
140[14]	L-proline	ethyl	7.83, 7.76
141	L-methionine	ethyl	8.70, 8.45
142[14]	β-alanine	ethyl	10.00
143[5]	N-methyl-glycine	ethyl	11.19
144	ethyl-aspartate	ethyl	9.78, 9.54

Table 4.5. ^{31}P Chemical shifts of phosphorochloridates synthesised

4.1.2.3 Synthesis of phenyl phosphoramidates via protected 4'-azidouridine

The coupling of 2',3'-cyclopentylidene 4'-azidouridine (**145**) with the corresponding phosphorochloridate was carried out using the Uchiyama procedure through an alkoxide intermediate to give the corresponding phosphoramidate (**146**).[3, 6] As mentioned before (**Chapter Three**) the deprotection of the 2'- and 3'-position was performed in the presence of an aqueous solution of 80% formic acid to give the desired phenyl phosphoramidates of 4–azidouridine (**147**) (**Figure 4.4**).
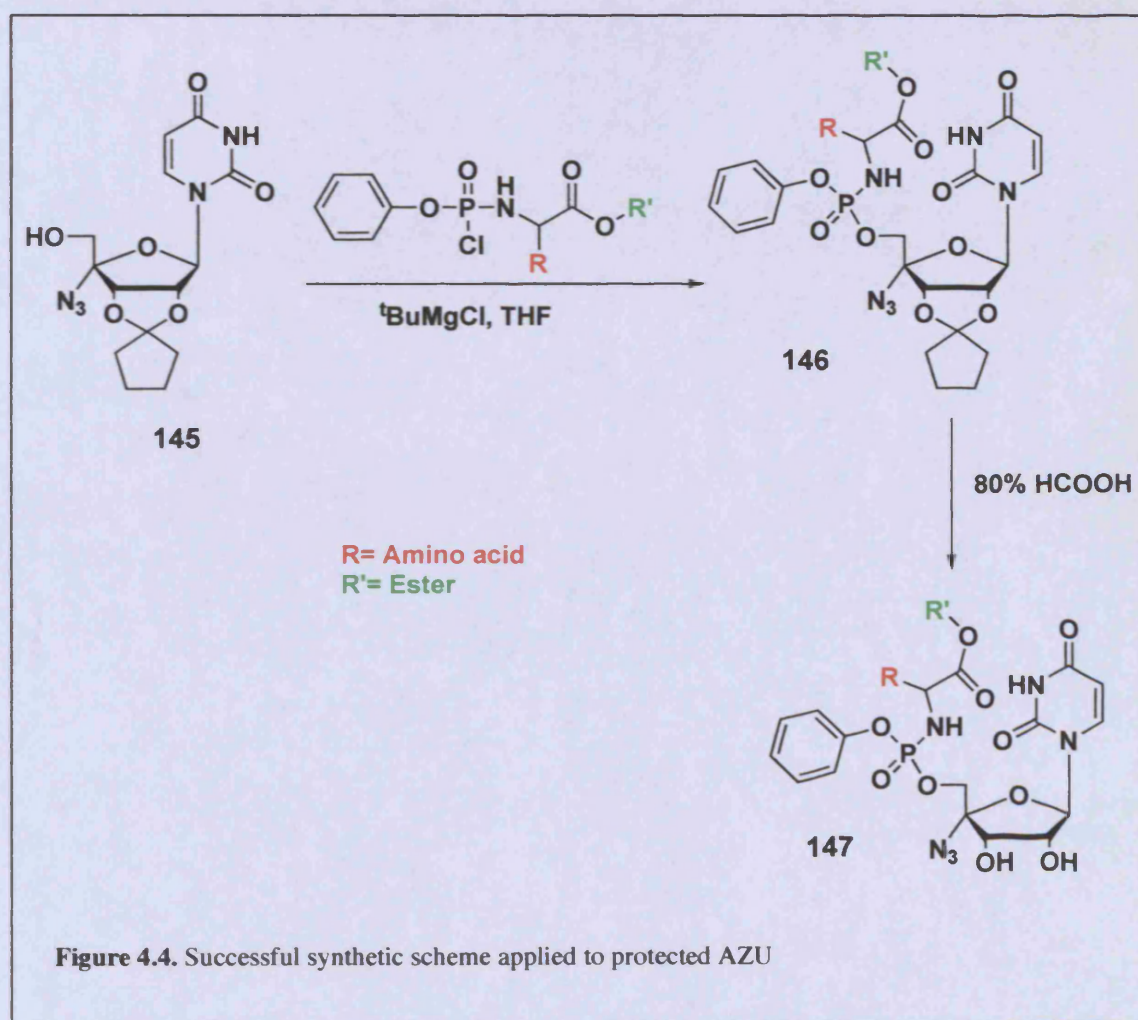
Figure 4.4. Successful synthetic scheme applied to protected AZU

For both reactions (coupling and deprotection), the purification method required one chromatography column giving an overall yield between 70 and 80%. **Table 4.6** reports the phosphoramidates synthesised using this synthetic pathway.

Cpd no.	Amino acids	Esters	^{31}P (ppm)
148	L-alanine	methyl	3.50, 3.31
149	L-alanine	ethyl	3.56, 3.35
150	L-alanine	isopropyl	3.59, 3.38
151	L-alanine	*tert*-butyl	3.63, 3.59
152	L-alanine	benzyl	3.53, 3.28
153	glycine	benzyl	3.53, 3.28
154	L-valine	benzyl	4.45, 4.14
155	α,α-dimethylgly	benzyl	1.86, 1.83
156	α,α-dimethylgly	ethyl	1.90, 1.87
157	L-phenylalanine	benzyl	3.21, 3.02
158	L-phenylalanine	ethyl	3.28, 3.06
159	L-leucine	ethyl	3.83, 3.47
160	L-proline	ethyl	1.60, 1.25
161	L-methionine	ethyl	3.81, 3.48
162	β-alanine	ethyl	3.33, 3.27
163	N-methyl-glycine	ethyl	5.12, 4.93
164	ethyl-aspartate	ethyl	3.66, 3.39

R= Amino acid
R'= Ester

Table 4.6. Phosphoramidates synthesised via protected AZU

The presence of the cyclopentylidene group in the 2'- and 3'-positions significantly increased the solubility of 4'-azidouridine and consequently the concentration of the nucleoside in the reaction solvent (THF). The purification method required one chromatography column for each step (coupling and deprotection) and

the yields observed were significantly higher compared to the same reaction performed with the unprotected 4'-azidouridine (**24**).

4.1.3 Biological activity of phenyl phosphoramidates of 4'-azidouridine

All the phosphoramidates synthesised were tested *in vitro* in the replicon assay (see section **3.3.6.1**).[9, 10]

4.1.3.1 Biological activity of L-alanine phenyl phosphoramidates of 4'-azidouridine

Historically, L-alanine phosphoramidates have in general shown the best antiviral and anticancer activity.[11] In **Chapter Three** a similar result was shown for 4'-azidocytidine. Starting from these promising results, L-alanine phosphoramidates with different substituents were prepared to explore the SAR in the ester position. In **Table 4.7** the biological activities of the synthesised L-alanine phosphoramidates are reported.

R= Ester

Cpd no.	Esters	EC_{50} (µM)	CC_{50} (µM)
121	butyl	1.2	>100
148	methyl	2.5	>100
149	ethyl	1.3	>100
150	isopropyl	0.96	>100
151	*tert*-butyl	5.1	>100
152	benzyl	0.61	>100
24	4'-azidouridine	>100	>100

5'-triphosphate-AZU= 0.22 µM IC_{50}

Table 4.7. Biological data of L-alanine AZU phenyl phosphoramidates
EC_{50}= The concentration of phosphoramidate which produces the 50% of the maximum possible effect
CC_{50}= The concentration of phosphoramidate which produces the 50% of the maximum toxic effect

The application of our phosphoramidate technology has converted an inactive nucleoside (4'-azidouridine, **24**) into sub-μM active compounds.

As expected, the benzyl ester showed the best activity. The poor activity of *tert*-butyl derivative might be a direct consequence of the inefficiency of one or both of the enzymes ("esterase" and "phosphoramidase") involved in the phosphoramidate metabolism.

Methyl, ethyl and isopropyl derivatives did not show a significant difference in potency (**Graph 4.1**).

Graph 4.1. Activity of AZU L-alanine phosphoramidates
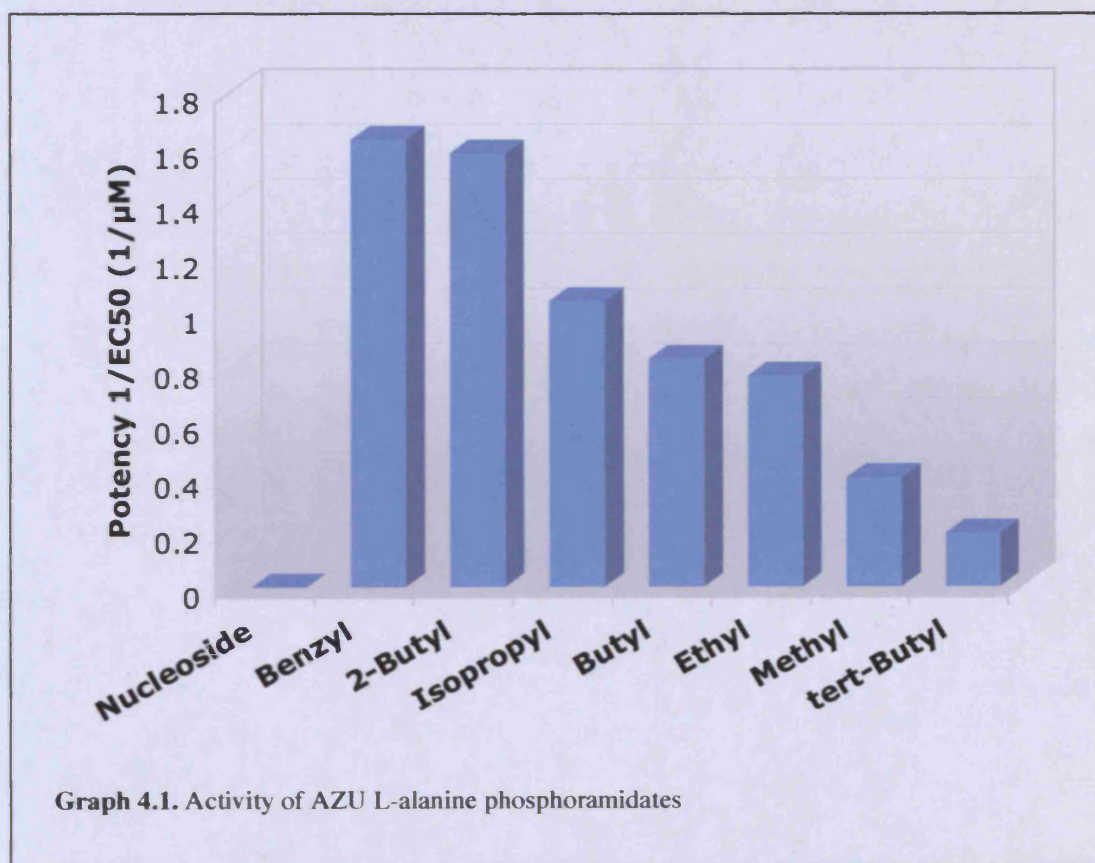
The 2-butyl phosphoramidate showed almost the same activity as the corresponding benzyl derivative, but interestingly, the L-alanine ethyl ester phosphoramidate showed poor activity.

The butyl derivative showed an activity similar to the isopropyl and ethyl analogues.

4.1.3.2 Biological activity of D-alanine phenyl phosphoramidates of 4'-azidouridine

In previous published works, D-alanine phosphoramidates showed poor or at least modest activity, indicating a possible stereoselective metabolism of phosphoramidates.[12] Initial biological results of 4'-azidocytidine phosphoramidates, in contrast, showed that L-alanine and D-alanine phosphoramidates had comparable activity; for this reason we decided to investigate the possible variation of the ester in a series of D-alanine phosphoramidates. The biological activities observed are reported in **Table 4.8**.
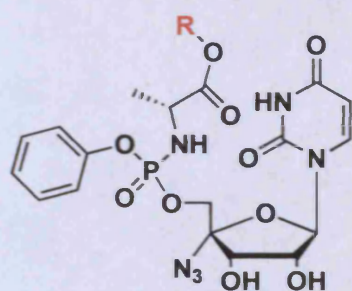
R= Ester

Cpd no.	Ester	EC_{50} (μM)	CC_{50} (μM)
115	benzyl	1.24	>100
116	dodecyl	16.3	>100
117	butyl	>100	>100
118	2-butyl	>100	>100
119	octyl	28.8	>100
120	isopropyl	100	>100
24	4'-azidouridine	>100	>100
5'-triphosphate-AZU= 0.22 μM IC_{50}			

Table 4.8. Biological data of D-alanine AZU phenyl phosphoramidates
EC_{50}= The concentration of phosphoramidate which produces the 50% of the maximum possible effect
CC_{50}= The concentration of phosphoramidate which produces the 50% of the maximum toxic effect

From these data, the phosphoramidate bearing a benzyl ester showed the best activity possibly because the benzyl group might be a good substrate for the esterase enzyme involved in the activation mechanism of phosphoramidates (see **Chapter Three**).

Graph 4.2. Ester variation for D-alanine phosphoramidates
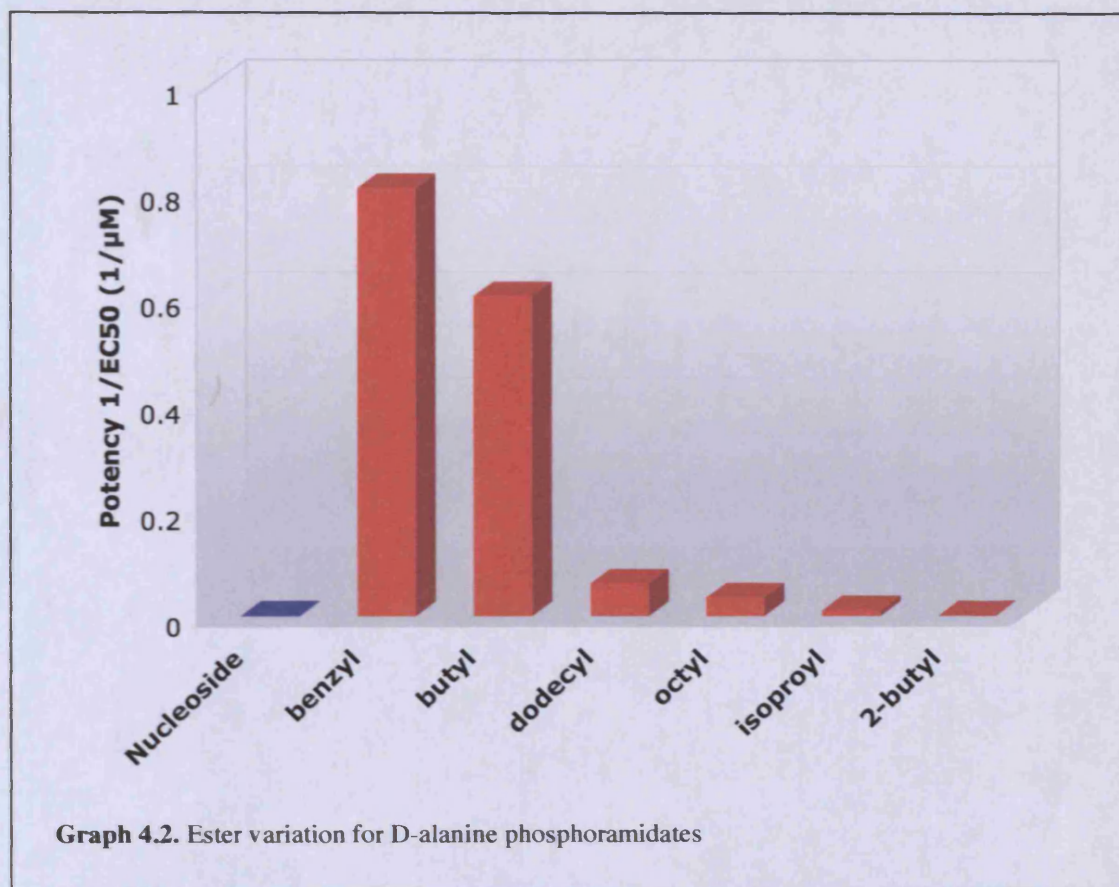
The dodecyl and octyl ester derivatives have never been made in the past (**Graph 4.2**). A long and lipophilic chain should represent a poor substrate for the enzyme involved in the phosphoramidate metabolism. The presence of modest activity might be an indication that the cleavage of the ester, by the corresponding enzyme was not the limiting step during the release of the 5'-monophosphate form. In the case of the D-alanine series the isopropyl and 2-butyl ester derivative showed poor activity, as a consequence of possible steric hindrance within the active site close to the ester bond.

4.1.3.3 Biological activity of phenyl phosphoramidates of 4'-azidouridine

In order to investigate possible biological activity variations on changing the amino acid unit, a series of different amino acid ester phosphoramidates was synthesised and tested in the replicon assay (**Table 4.9**).
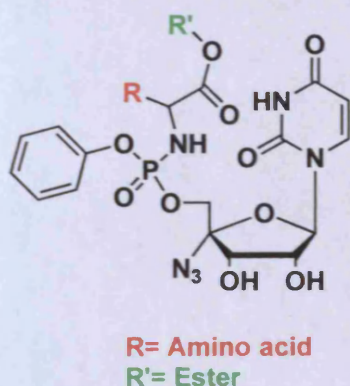
R= Amino acid
R'= Ester

| Cpd no.	Amino acid	Ester	EC_{50} (µM)	CC_{50} (µM)
112	cyclopentylgly	benzyl	<100	>100
113	cyclopentylgly	ethyl	>100	>100
114	cyclopentylgly	isopropyl	100	>100
153	glycine	benzyl	1.6	>100
154	L-valine	benzyl	<100	>100
155	α,α-dimethylgly	benzyl	3.4	>100
156	α,α-dimethylgly	ethyl	10.3	>100
157	L-phenylalanine	benzyl	<100	>100
158	L-phenylalanine	ethyl	1.37	>100
159	L-leucine	ethyl	2.3	>100
160	L-proline	ethyl	6.0	>100
161	L-methionine	ethyl	14.0	>100
162	β-alanine	ethyl	>100	>100
163	N-methyl-glycine	ethyl	>100	>100
164	ethylaspartate	ethyl	>100	>100
24	4'-azidouridine		>100	>100

5'-triphosphate-AZU= 0.22 µM IC_{50}

Table 4.9. Biological data of AZU phenyl phosphoramidates
EC_{50}= The concentration of phosphoramidate which produces the 50% of the maximum possible effect
CC_{50}= The concentration of phosphoramidate which produces the 50% of the maximum toxic effect

The inactivity of phosphoramidates incorporating unnatural amino acids such as β-alanine and *N*-methyl-glycine might indicate that the presence of an α-amino acid and a secondary amino group were prerequisites for the phosphoramidate metabolism. Also in this case all the cyclopentylglycine phosphoramidates are inactive, confirming a behaviour observed previously for the 4'-azidocytidine phosphoramidate (see **Chapter Three**).

Subsequently, the biological activities of different amino acid benzyl esters have been compared, for both 4'-azidouridine and 4'-azidocytidine phosphoramidates (**Graph 4.3**).

Graph 4.3. Amino acid variation for AZU and AZC benzyl phosphoramidates
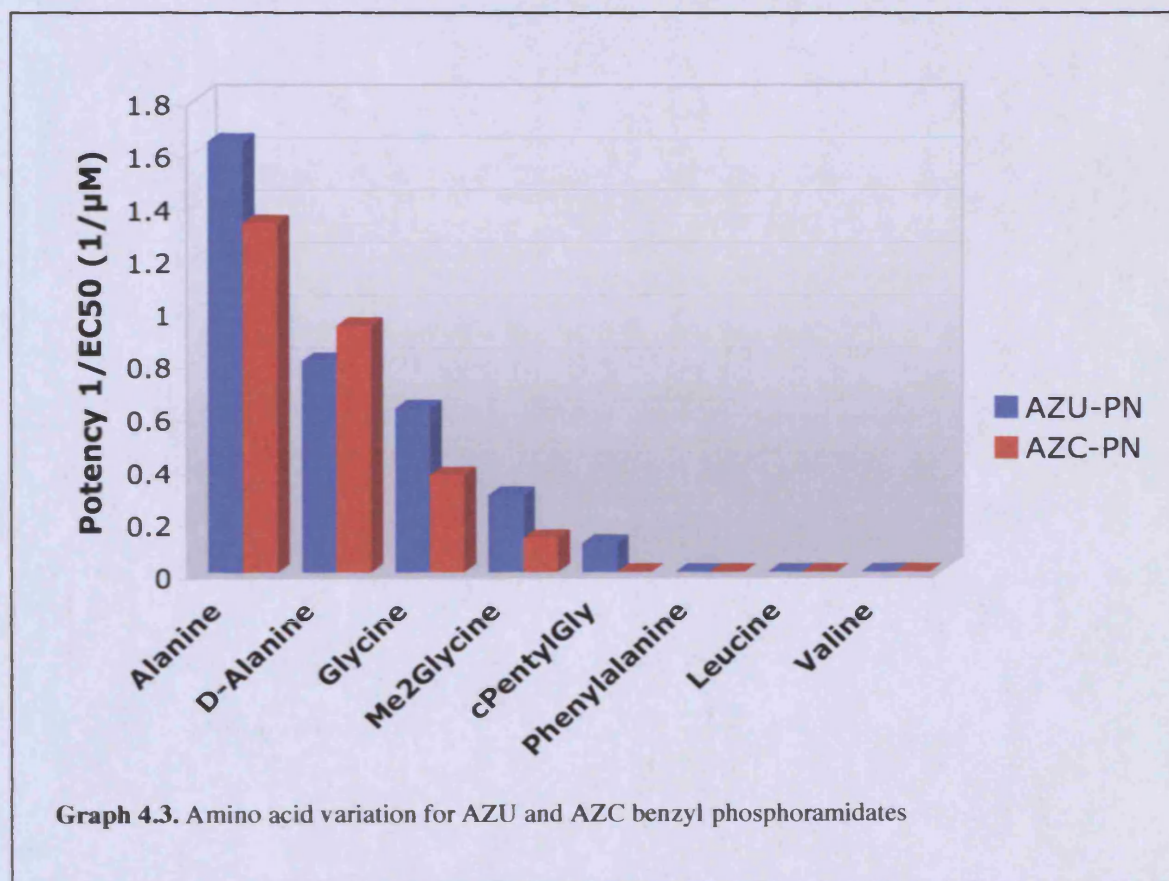
The D-alanine phosphoramidate was 2 fold less active than the corresponding L-isomer. Almost the same reduction in activity was found by replacing L-alanine with glycine and dimethylglycine. This indicates that the presence of the methyl group of the L-alanine unit does not confer any advantage over the glycine or D-alanine. Moreover, the fact that D-alanine and glycine are equipotent might indicate the

presence of a non-stereospecific mechanism in the release of the 5'-monophosphate. Considering other L-amino acids with larger and more hydrophobic side chains (e.g. L-valine, L-isoleucine, L-leucine), a notable reduction of activity was observed: this might indicate the presence of a small hydrophobic pocket in the putative activating enzyme ("esterase" and "phosphoramidase").

The possible correlation between the activity of the phosphoramidates and the lipophilicity of the amino acid present in these structures has been analysed and reported in **Graph 4.4**.

Graph 4.4. Correlation between AZU-PN activity ClogP and number of carbon (all the amino acids are benzyl ester)
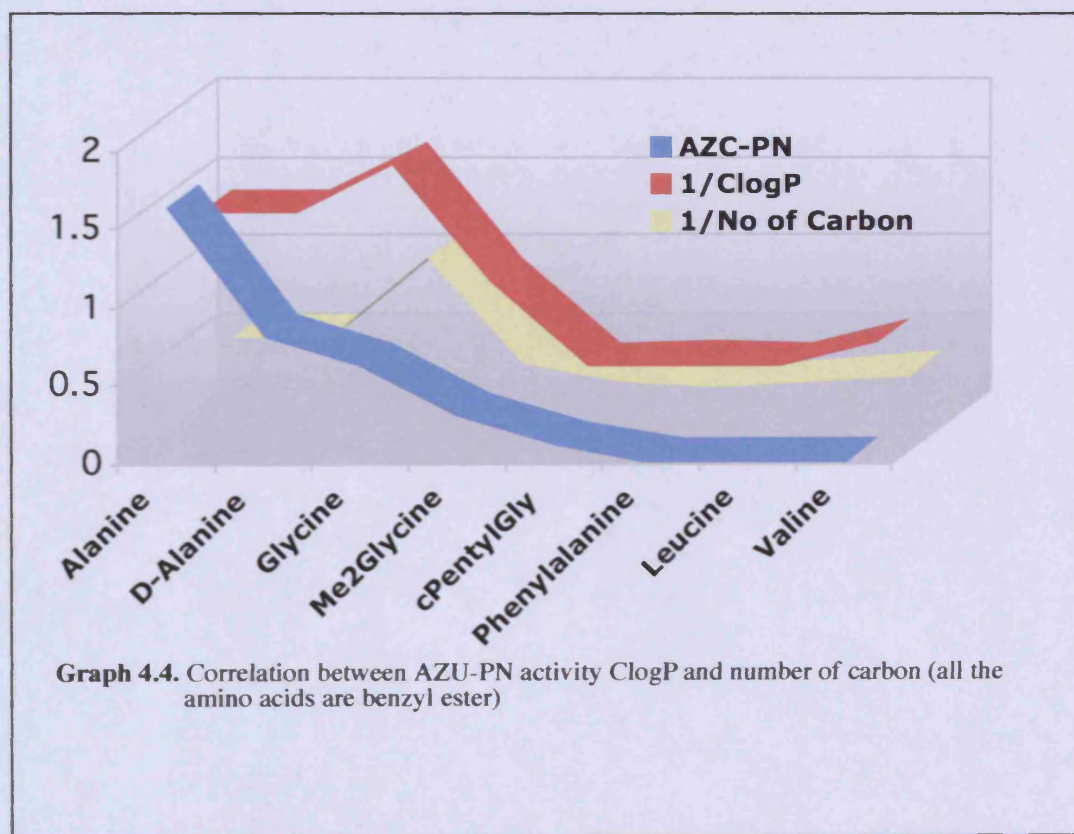
ClogP expresses the value of lipophilicity of a molecule that might be correlated to the percentage of carbon in the molecules.

Substantial reduction in activity is observed when the ClogP reaches a value of ~1. In this series of phosphoramidates (**Graph 4.4**) the variation of ClogP is owing to the lipophilicity of the lateral chain of the amino acid.

The trend of activity may result from size and length of the lateral chain of each amino acid. In **Graph 4.4**, the reciprocal of the number of the carbons in the

lateral chain for each amino acid is indicated and is an expression of the value of the steric hindrance: more lipophilic amino acid (e.g. phenylalanine, leucine, valine) have a lower value compared to more hydrophilic amino acid (e.g. L- and D-alanine, glycine and dimethylglycine).

The trend of the activity and the reciprocal of the number of the carbons showed similar profile, indicating that the active site of one of the enzyme, involved in the release of the 5'-monophosphate might only tolerate small lateral chains such as the methyl of L- and D-alanine.

4.2 Substituted phenyl phosphoramidates of 4'-azidouridine

In order to compare the effect of electon-donating and electron-withdrawing substituents on 4'-azidouridine and 4'-azidocytidine phosphoramidates, the Topliss tree concept has been applied to ethyl L-leucine 4'-azidouridine phosphoramidates.[13]

As mentioned in **Chapter Three**, the Topliss tree is a methodology designed to find the best substituent that can be present on the aryl moiety of a general phosphoramidate system.[13]

4.2.1 Synthesis of phosphorylating agents for coupling with 4-azidouridine

In order to synthesise the appropriate phosphrochloridate required for the coupling with 4'-azidouridine, the compounds previously mentioned in **Chapter Three** (see section **3.4.1** and **3.4.2**) were resynthesised.

4.2.2 Synthesis and biological activity of phosphoramidates of 4'-azidouridine with substituents on the aryl moiety

Four possible substituents present in the first row of the Topliss tree (*p*-chloro, 3,4 di-chloro, *p*-methyl and *p*-methoxy) were synthesised. The synthesis as previously described (see section **3.3.5**) was an application of the Uchiyama procedure in which the synthesis of the 5'-alkoxide of 4'-azidouridine was performed using an excess of tBuMgCl followed by coupling with the appropriate phosphorochloridate (**Figure 4.4**).[2-5]
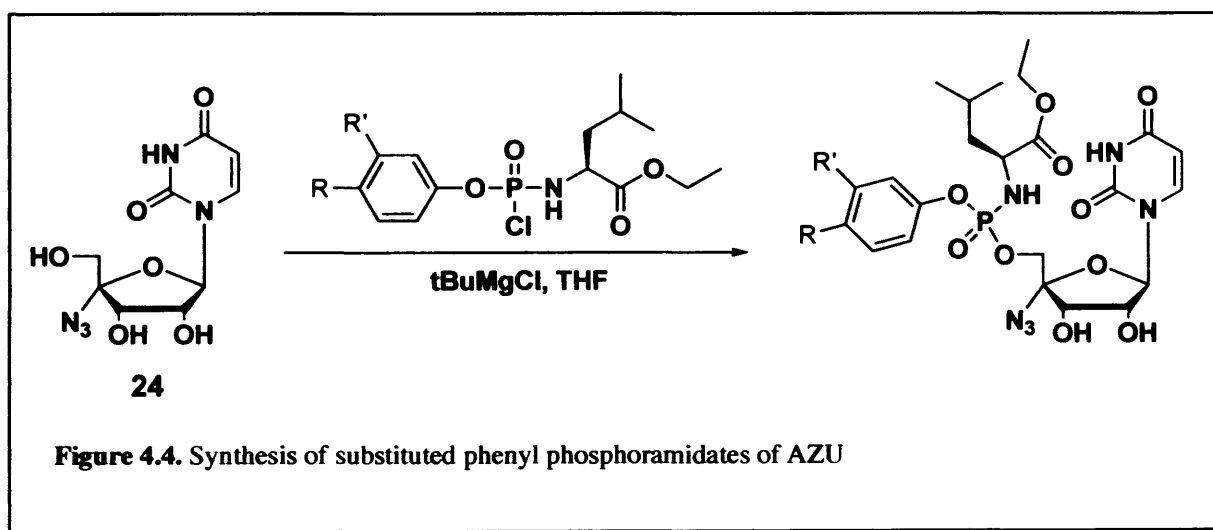
Figure 4.4. Synthesis of substituted phenyl phosphoramidates of AZU

All the compounds synthesised were tested *in vitro* in the replicon assay (see section **3.3.6.1**) against HCV (**Table 4.10**).[9, 10]

The unsubstituted phosphoramidates showed an $EC_{50}= 2.3$ μM which represents an activity superior to the substituted (electron-donating and electron-withdrawing groups) derivatives (**Table 4.10**).
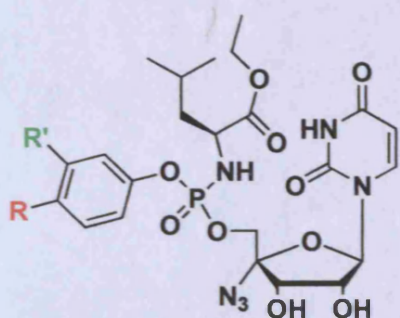
Cpd no.	R'	R"	EC_{50} (μM)	CC_{50} (μM)
165	chloro	H	<100	>100
166	chloro	chloro	15.1	>100
167	methyl	H	2.1	>100
168	methoxy	H	<100	>100
159	H	H	2.3	>100
24	4'-azidouridine		1.28	>100
5'-triphosphate-AZU= 0.22 μM IC_{50}				

Table 4.10. Biological activity of AZU phenyl substituted phosphoramidates
$EC_{50}=$ The concentration of phosphoramidate which produces the 50% of the maximum possible effect
$CC_{50}=$ The concentration of phosphoramidate which produces the 50% of the maximum toxic effect

In the case of *para*-chloro and *para*-methoxy the activity was totally absent (**Graph 4.5**). The presence of an inductive group (*p*-chloro and 3,4-dichloro) slightly reduced the observed activity. However, there is not a great difference, which indicates that the presence of an electron-withdrawing or electron-donating group might not influence the activity significantly.

Graph 4.5. Topliss tree applied to AZU L-leucine ethyl ester phosphoramidates
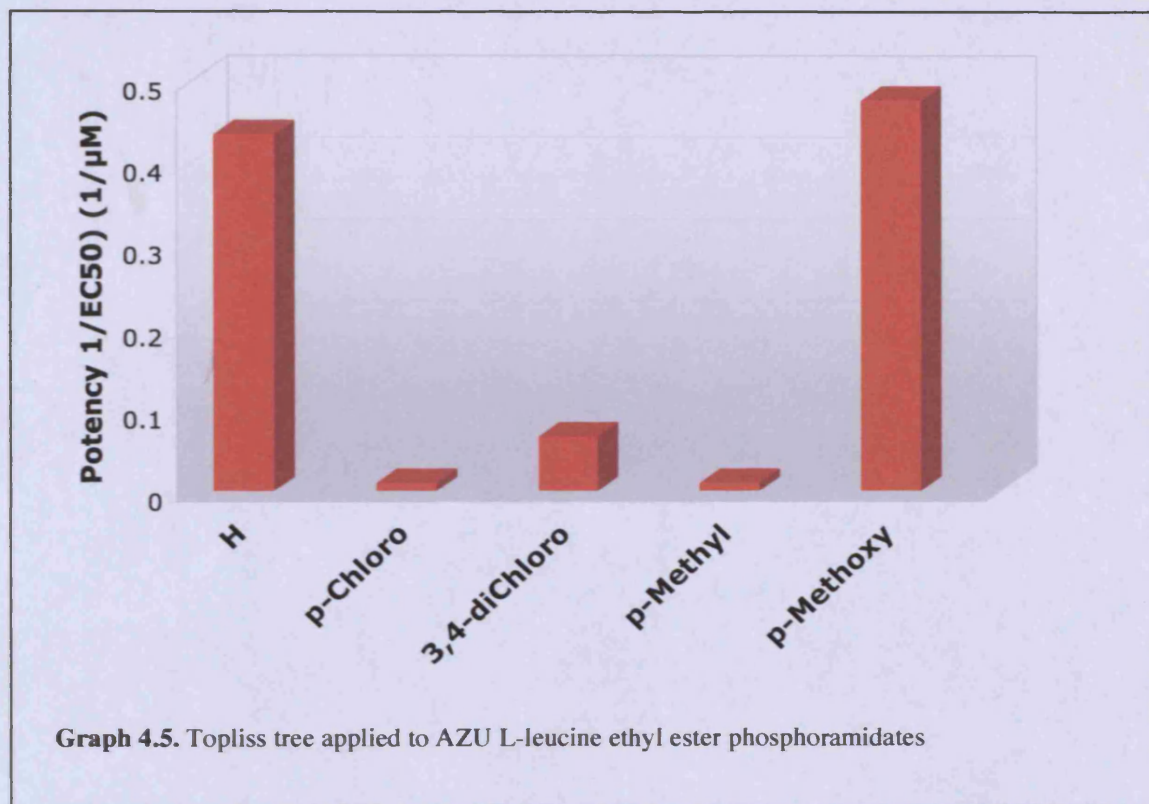
A possible explanation might be correlated with the instability observed during the synthesis of the two unsubstituted phosphoramidates of 4'-azidocytidine and 4'-azidouridine. As a consequence of this instability, the phosphoramidates tested were already partially decomposed invalidating the biological results. For this reason the Topliss tree was not further investigated.

4.3 Naphthyl and quinolinyl phosphoramidates of 4'-azidouridine

Previous studies have shown that the presence of a more lipophilic aryl moiety can increase the biological activity of phosphoramidates.[14] For this reason we decided to synthesise a small series of phosphoramidates with α- and β-naphthyl as the aryl moiety.

4.3.1 Synthesis of phosphorylating agent for coupling with amino acids
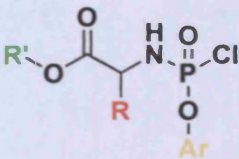
The synthesis of the phosphorylating agent for coupling with the amino acid was performed following the procedure already described in **Chapter Three** (see section **3.5.1**)

4.3.2 Synthesis of phosphorylating agents for coupling with 4'-azidouridine

The previously described procedure for the synthesis of phosphorochloridate (see section **3.3.6**) was applied .[2-5]

The instability of these compounds necessitated a rapid purification method by chromatography column using a mixture of hexane/EtOAc (7:3) as eluent.

Due to the non-stereospecific reaction, the synthesised products were a mixture of two diastereoisomers with a signal splitting in the ^{31}P-NMR spectra (**Table 4.11**).

Cpd no.	Amino acid	Ester	Aryl moiety	^{31}P (ppm)
169	cyclopentylgly	benzyl	α-naphthyl	7.05
170	cyclopentylgly	ethyl	α-naphthyl	6.90
171[14]	D-alanine	isopropyl	α-naphthyl	7.88, 7.37
172[14]	D-alanine	benzyl	α-naphthyl	7.40, 7.17

R= Amino acid
R'= Ester
Ar= Aryl moiety

Table 4.11. Naphthyl phosphorochloridates synthesised

4.3.3 Synthesis of α- and β-naphthyl 4'-azidouridine phosphoramidates

The synthesis of naphthyl phosphoramidates of 4'-azidouridine was carried out using the Uchiyama procedure in the presence of an excess of tBuMgCl in THF (see section **3.3.5**).[3, 6]

In the first instance, L-alanine benzyl ester with α- and β-naphthyl as the aryl moiety were synthesised, with yields of 9% and 5% respectively.
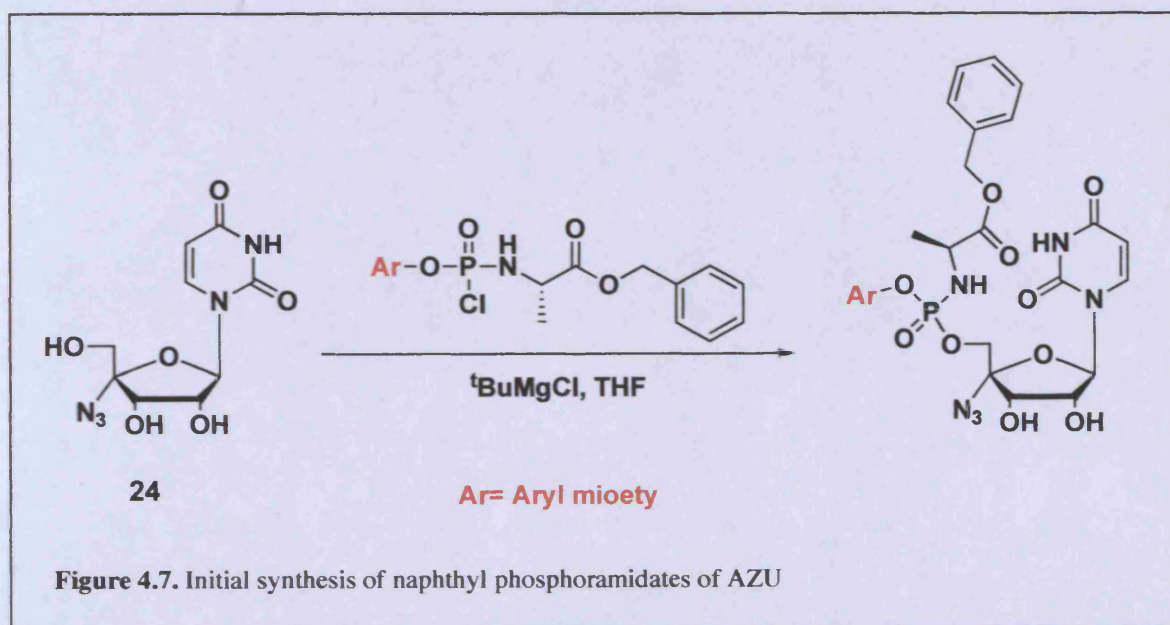
Figure 4.7. Initial synthesis of naphthyl phosphoramidates of AZU

In an attempt to increase the yield, the same phosphoramidate reaction was performed with 2',3'-protected 4'-azidouridine (**145**) and following the results obtained with the protection of 4'-azidocytidine, the cyclopentylidene group was utilised (**Figure 4.8**).

Figure 4.8. Synthesis of 2',3'-cyclopentylidene 4'-azidouridine phosphoramidates
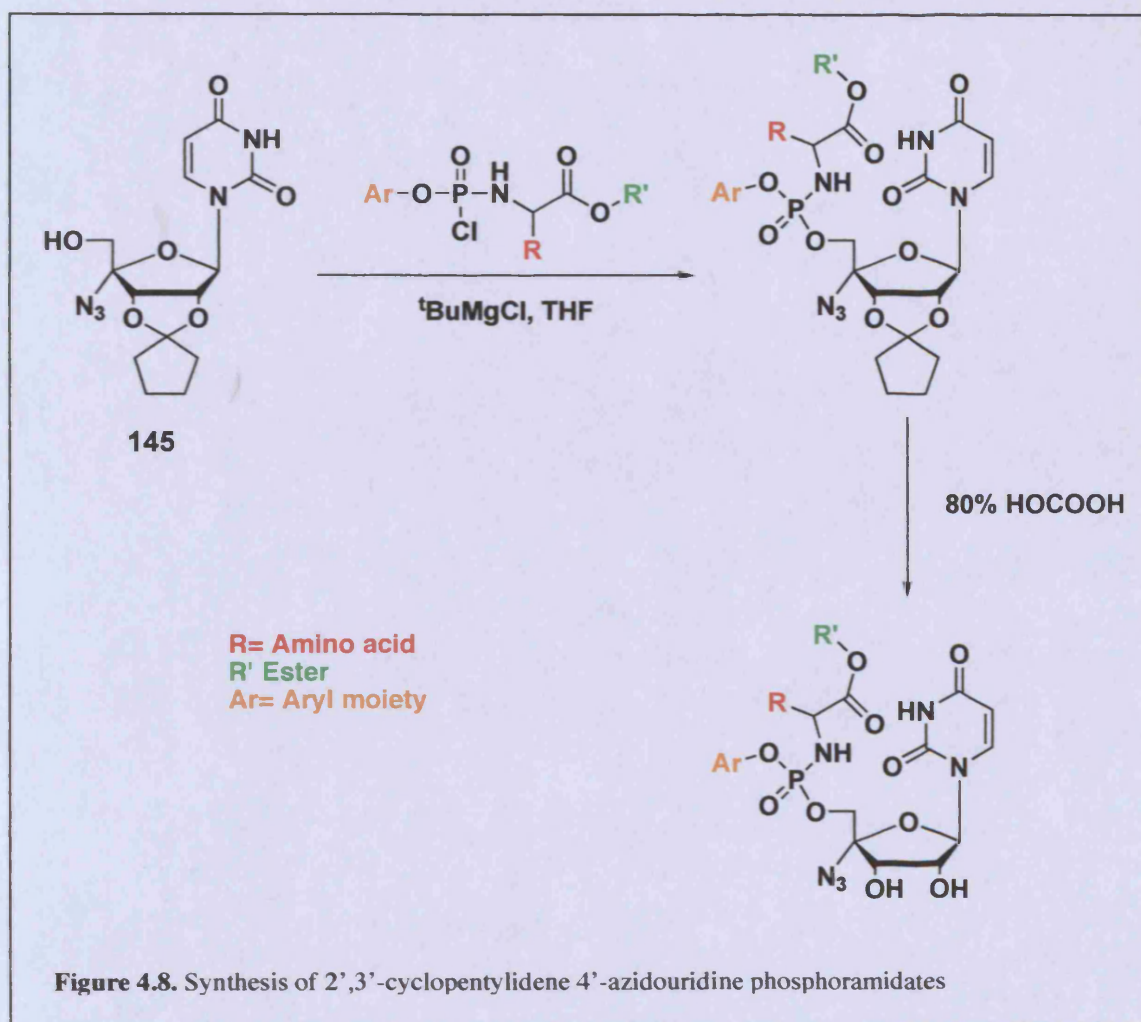
The reaction was stirred overnight and the purification usually required only one chromatography column with a significant increase in the overall yields.

The phosphoramidates synthesised using the protected and unprotected 4'-azidouridine are reported in **Table 4.12**.
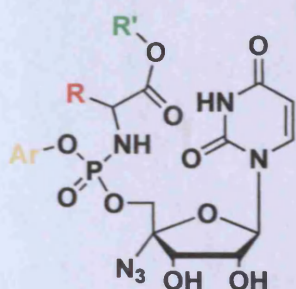
Cpd no.	Amino acid	Ester	Aryl moiety	^{31}P (ppm)
173	L-alanine	benzyl	α-naphthyl	3.94, 3.76
174	L-alanine	benzyl	β-naphthyl	3.78, 336
175	cyclopentylgly	benzyl	α-naphthyl	3.10, 3.06
176	cyclopentylgly	ethyl	α-naphthyl	3.23, 3.15
177	D-alanine	isopropyl	α-naphthyl	3.73, 360
178	D-alanine	benzyl	α-naphthyl	3.80, 360

R= Amino acid
R'= Ester
Ar= Aryl moiety

Table 4.12. AZU naphthyl phosphoramidates synthesised and their ^{31}P chemical shift

Due to the non-stereoselectivity of the coupling reaction, a mixture of two phosphate diastereoisomers was obtained as indicated by the splitting in the ^{31}P-NMR and ^{1}H-NMR. The observed ^{31}P-NMR chemical shifts were between 7.7 and 10.6 ppm.

4.3.4 Synthesis of 8-quinolinyl phosphoramidate of 4'-azidouridine

The synthesis of a phosphoramidate with 8-hydroxy-quinoline as an aryl moiety was planned, because this group represents an isostere of the naphthol group and their calculated pK_a values are similar ($pK_a\sim5$). The pK_a value is important in the phosphoramidate metabolism because one of the side products of this process is the corresponding phenolate of the aryl moiety: as a consequence a group with a similar pK_a might have similar efficiency in the release of 5'-monophosphate.

In this case the first attempt was conducted using the standard procedure, but it was not possible to isolate the desired compound (**179**). Due to the possible high instability of the 8-hydroxy-quinolinyl group in the reaction conditions, the synthesis was performed using a modification to the standard procedure (**Figure 4.9**).

Figure 4.9. Synthetic pathway for the synthesis of 8-quinolinyl phosphoramidate
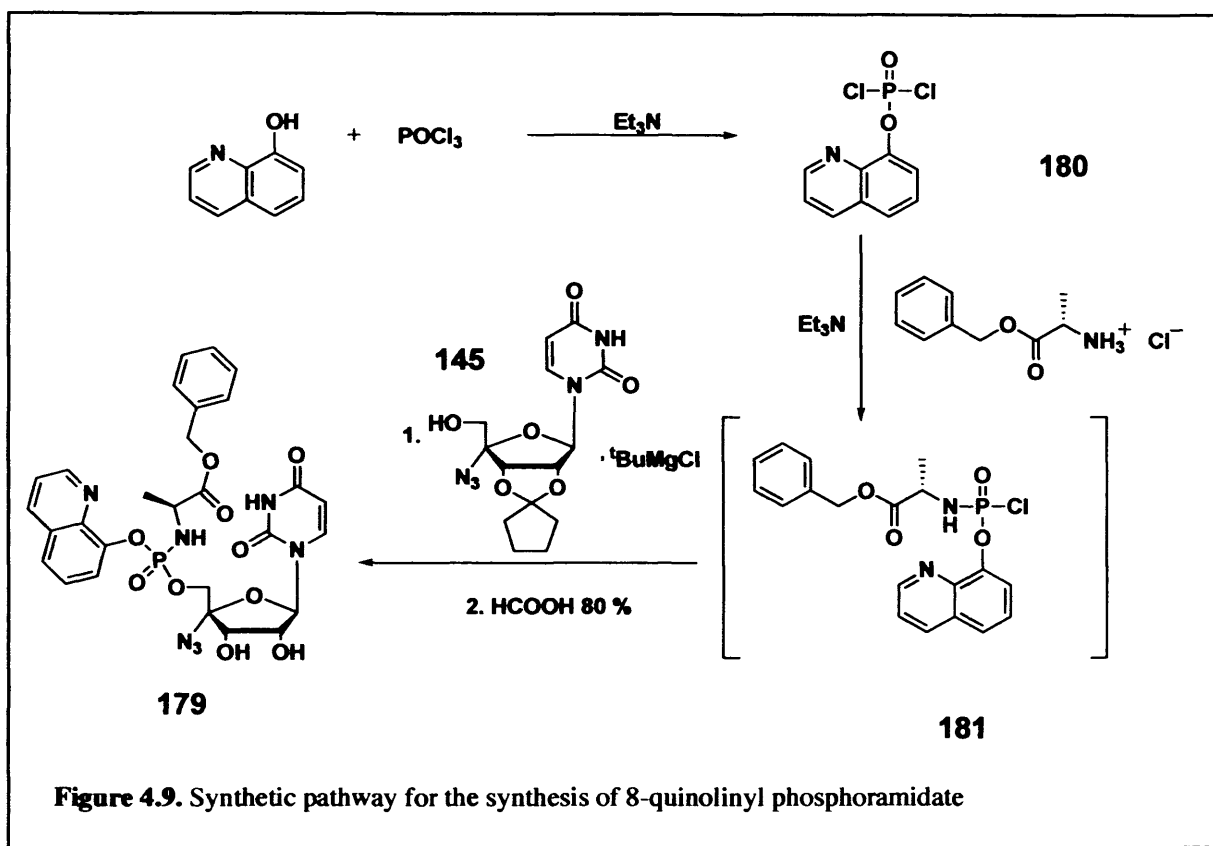
Thus, the phosphorodichloridate (**180**) synthesis was carried out using the standard procedure described before with a yield of 75 %

The following coupling reaction with L-alanine benzyl ester hydrochloride was performed under the standard condition, and the phosphorochloridate (**181**), was used for the next step with 2,3'-cyclopentylidene 4-'azidouridine (**125**) to give the corresponding phosphoramidate. The purification of this compound was carried out using HPLC.

The deprotection using the standard procedure (solution of 80% HCOOH) was performed. The desired product (**179**) was obtained in reasonable yield. For this compound the splitting of signals in ^{31}P- (4.01, 3.95) and ^{1}H-NMR spectra was observed.

4.3.5 Biological activity of naphthyl and 8-quinolinyl phosphoramidates of 4'-azidouridine

All the naphthyl and 8–quinolinyl phosphoramidates of 4'-azidouridine were tested in the replicon assay and the obtained data are reported in **Table 4.13**.[9, 10]
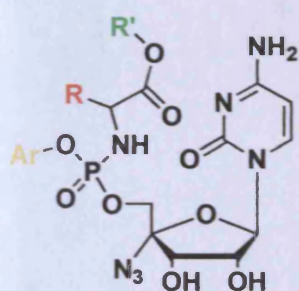
Cpd no.	Amino acid	Ester	Aryl moiety	EC_{50} (μM)	EC_{50} (μM)
173	L-alanine	benzyl	α-naphthyl	0.19	>100
174	L-alanine	benzyl	β-naphthyl	3.5	>100
175	cyclopentylgly	benzyl	α-naphthyl	15.9	>100
176	cyclopentylgly	ethyl	α-naphthyl	>100	>100
177	D-alanine	isopropyl	α-naphthyl	>100	>100
178	D-alanine	benzyl	α-naphthyl	1.0	>100
179	L-alanine	benzyl	8-quinolinyl	7.62	>100
24	4'-azidouridine			>100	>100

5'-triphosphate-AZU= 0.22 μM IC_{50}

R= Amino acid
R'= ester
Ar= Aryl moiety

Table 4.13. Biological results of AZU naphthyl and 8-quinoinyl phosphoramidates

The cyclopentylglycine phosphoramidates (**175**, **176**) displayed poor activity, while for D-alanine derivatives, the isopropyl was found to be completely inactive, the benzyl ester showed an activity of 1.0 μM.

4.3.5.1 L-alanine naphthyl and 8-quinolinyl phosphoramidates of 4'-azidouridine

The β-naphthyl phosphoramidate showed a considerable increase in activity compared to the inactive nucleoside (**24**), but most importantly the α-naphthyl derivative had an activity at 0.1 μM with an increase of >1000 fold compared to the corresponding nucleoside (4'-azidouridine, **24**) (**Graph 4.6**).

Graph 4.6. Biological activity of AZU phosphoramidates with different aryl moiety
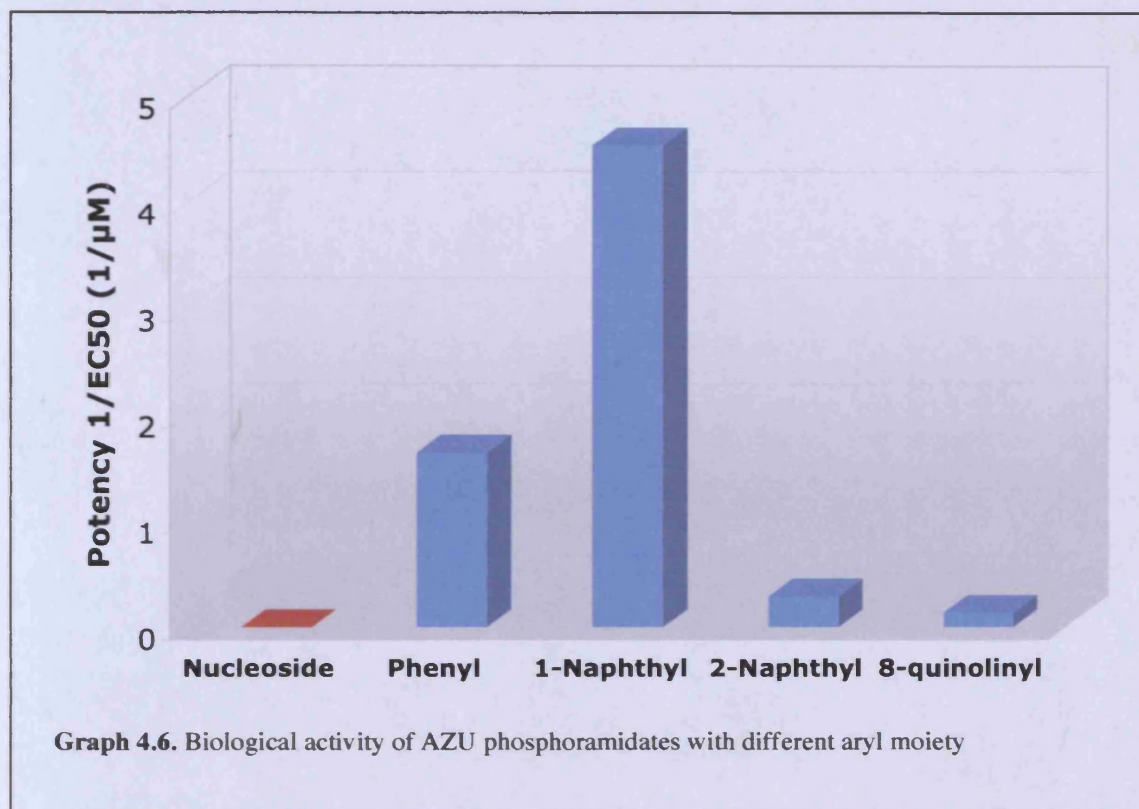
Comparing the α-naphthyl (**173**) and its corresponding phenyl phosphoramidate (**152**) the enhancement of the activity is 2.5-fold. This significant increase in activity has not been found for the β-naphthyl derivatives. An explanation for this different behaviour might be related to the nature of the active site of one of the enzymes (esterase) in which there might be a lipophilic pocket capable of interacting with a phenyl group or α-naphthyl-group (π-π interaction). The β-naphthyl-group, on the other hands represents a poor substrate for the enzyme, due to its different orientation.

The biological data obtained for the 8-quinolinyl phosphoramidate (**179**) indicated that this compound is less active than the corresponding α-naphthyl and phenyl derivatives. Initially, the biological activity for this compound was expected to be comparable with the α-naphthyl derivative. However, the problems of instability encountered during the synthesis of this phosphoramidate (**179**) might have caused partial degradation during the test for the biological activity.

4.4 4'-Ethynyluridine phosphoramidates

Another 4'-modification made by Roche was the introduction of an ethynyl group on the uridine structure (25). In the replicon assay 4'-ethynyluridine was found to be completely inactive. As explained for the 4'-azidouridine (24), the possible explanation for this involved the poor interaction between this nucleoside and the intracellular TK (see **Chapter One**).[1] If the first phosphorylation in particular, was the rate limiting step in this process, phosphoramidate methodology would represent a possible solution to efficiently deliver the 5'-monophosphate into the cells. The synthesis of the D-alanine benzyl ester phosphoramidates of 4'-ethynyluridine (180) was performed using the conditions already described for the 4'-azidocytidine (23) and the 4'-azidouridine (24) (Figure 4.10).
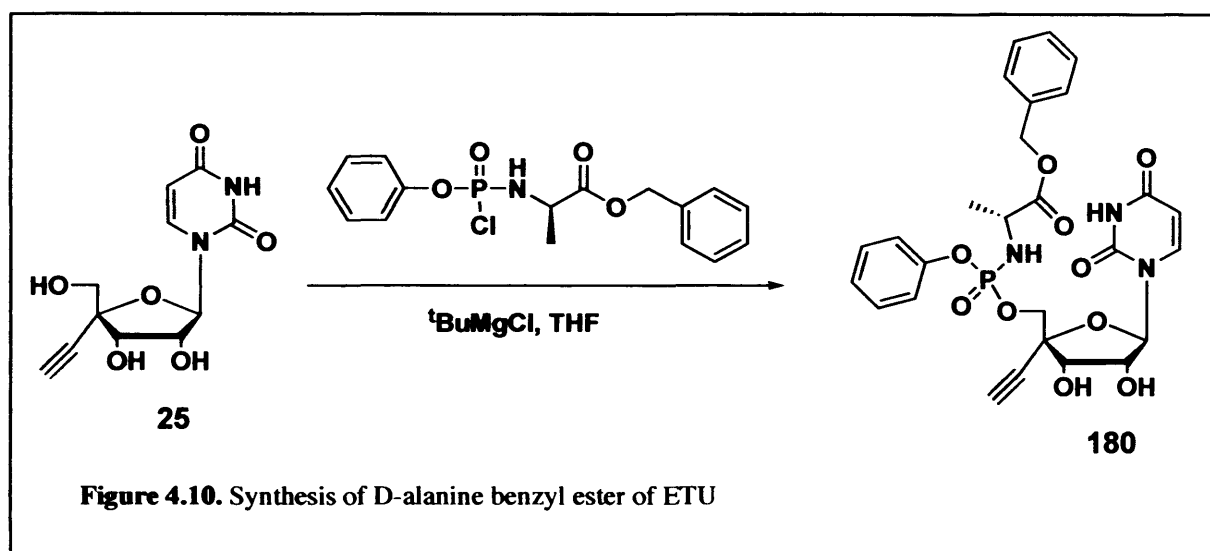
Figure 4.10. Synthesis of D-alanine benzyl ester of ETU

The procedure was the same used for the synthesis of 4'-azidocytidine (23) and 4'-azidouridine (24) phosphoramidates. The purification required one column and one preparative TLC without any traces of P-OH (64). The phosphoramidates were synthesised in reasonable yield. Preliminary biological data on the 4'-ethynyluridine phosphoramidates showed complete inactivity of these compounds; consequently other phosphoramidates were not synthesised. A possible explanation of this inactivity might be related to nucleoside activation to the corresponding 5'-triphosphate: in this case, the limiting step may not be the first but the second or the third phosphorylation; consequently the delivery of 5'-monophosphate through the phosphoramidate methodology does not bypass this limiting step.

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Chapter Five

Aryloxyphosphoramidates of adenosine analogues

5.1 Synthesis of 4'-azido-adenosine (AZA)

The synthesis of 4'-azidoadenosine was planned in order to apply the arylphosphoramidate approach to a purine nucleoside with the same structural modification as the previous examples (4'-azidocytidine **23**, 4'-azidouridine **24** and 4'-ethynyluridine **27**).

5.1.1 Initial synthetic pathway for the synthesis of 4'-azido-adenosine

4'-Azidoadenosine represents the first example of a modified purine tested against HCV in this project. The proposed synthetic pathway is shown in **Figure 5.1.**[1]

a. Ph₃P, I₂, dioxane, pyridine; b. tBuOK, Pyridine; c. ICl, NaN₃, DMF;
d. BzCl, Pyridine; e. 1. MCPBA, CH₂Cl₂/H₂O; 2. MeONa, MeOH

Figure 5.1. Synthetic pathway for AZA
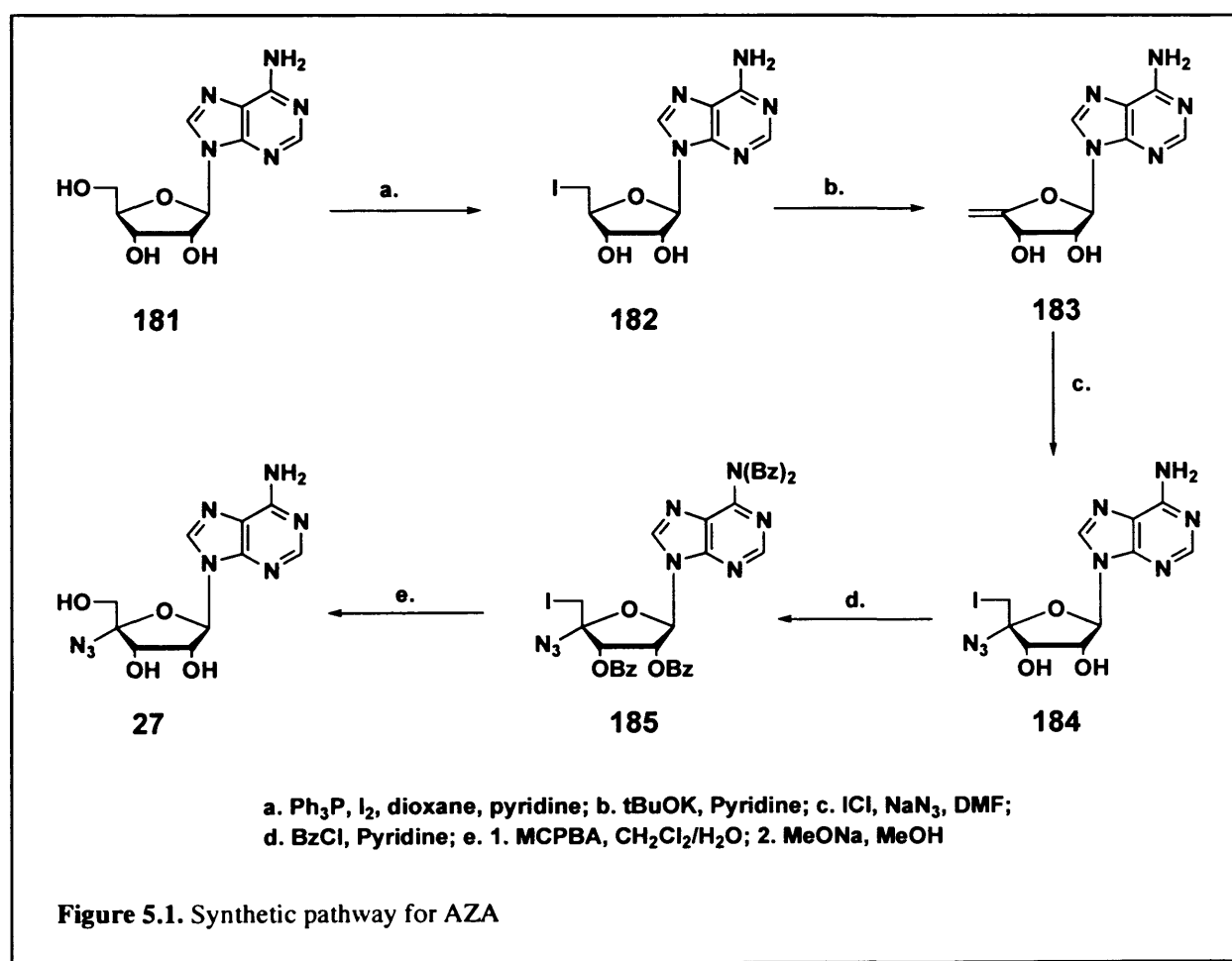
5.1.2 Iodination of adenosine

The first attempt was performed using the same conditions as reported in literature.[1] Due to a low solubility of the starting material (181) in dioxane-pyridine, the yield for this first reaction was 3%; for this reason other conditions were tried.[2,3]

The first alternative was to use triphenylphosphine, iodine and an excess of imidazole in NMP (N-methyl-pyrrolidinone), which afforded 26% of an impure compound contaminated with triphenylphosphonium oxide.[2]

In order to use a different source of iodine, according to the literature, the iodination in the 5'-position was attempted using CI_4 and triphenylphosphine in a solution of hexamethylphosphoric acid triamide (HMPT).[3] Unfortunately, difficulty to remove the solvent and the formation of many product as indicated by TLC, were clear signs against a possible use of this reaction on a large scale.

Another attempt of the literature procedure was performed this time using pyridine as the solvent. In this case, after purification by column chromatography, the yield was 90%. Unfortunately, from the ^{1}H-NMR spectra the presence of triphenylphosponium oxide was observed.

The reaction was repeated on a larger scale starting from 15 g of adenosine. It afforded, after purification by column chromatography, a yield greater than 100%. The possible explanation was the presence, also in this case, of triphenylphosphonium oxide, confirmed by the presence of a signal at 25 ppm in the ^{31}P-NMR spectra.

In order to avoid the use of a column for the purification, the first step was repeated, with the same conditions previously described, followed by an extraction ($EtOAc/H_2O$). Due to the higher lipophilicity of triphenylphosphine, its percentage in the product was higher than before. Several attempts with crystallisation were tried with different solvents: ethanol, isopropanol, ethanol/chloroform, ethanol/ethylacetate, ethanol/hexane, without any suitable results.

In order to overcome this problem, the same reaction was performed using triphenylphosphine supported on resin (polystyrene, PS-triphenylphosphine). In this case the desired product was obtained pure and in reasonable yield. Due to the high polarity of 5'-deoxy-5'-iodoadenosine (182) the use of large amounts of EtOAc was necessary to fully extract the compound. In order to make the protocol reliable on a larger scale, the same reaction was tried using as starting material the 2',3'-isopropylidene protected adenosine (186) with PS-triphenylphosphine. In this case,

the reaction proceeded at 50 °C (**Figure 5.2**). After purification by extraction, the pure desired product (**187**) was obtained in poor yield (13%). TLC indicated the formation of a polar compound that had been noticed in all the previous attempts; in the earlier cases it represented only a side product of the reaction and did not significantly affect the yield. This compound, isolated and fully characterised, was the product of intramolecular nucleophilic substitution at the 5'-position involving the nitrogen in the 3-position (**188**).
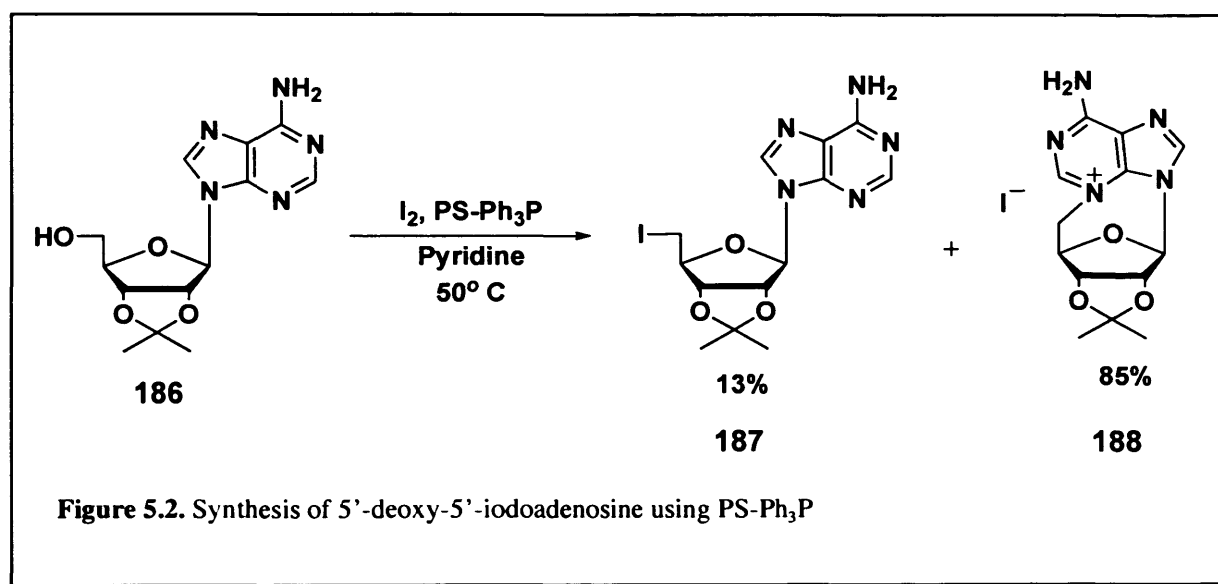

Figure 5.2. Synthesis of 5'-deoxy-5'-iodoadenosine using PS-Ph₃P

This side product (**188**) has been reported in the literature for a compound having a good leaving group at the 5'-position (such as triphenylphosphonium oxide or tosylate).[4,5,6]

The reaction was repeated on a large scale using chromatography column as the purification method.

5.1.3 Elimination of HI from 5'-deoxy-5'-iodoadenosine

The second step was the elimination in 4'-5' position and it was performed using tBuOK in pyridine (**Figure 5.1**).[2] Compared to the use of MeONa/MeOH, this method gave rise to an easier purification protocol. The main problem in this reaction was the unreliable yield (between 15% and 80%). By reducing the concentration of tBuOK, from 4.5 to 3.0 equivalents, and increasing the volume of pyridine, it was possible to avoid the formation of a black compound that contaminated the product and that was not easily removed by column chromatography. Nevertheless, in this

way it was possible to obtain the desired product (**183**) in more reliable yield (70-80%).

5.1.4 Stereoselective and regioselective addition of IN₃

The key step in this synthesis was the regio- and stereo-selective addition of IN_3 in the 4'- and 5'-positions (**Figure 4.1**), performed *in situ* by synthesis of IN_3 starting from NaN_3 and ICl. In this preparation, iodine added preferentially to the β-face of the double bond to give an iodonium intermediate which was opened on the α-side at the 4'-position by azide ion. There was no evidence of the presence of the 4'-epimer.

This step was successful only the first time that this reaction was attempted. In order to repeat this reaction again, the temperature was increased, unsuccessfully. New reagents were bought, but also in this case the TLC of the reaction did not show any presence of the desired product. The reason for the unreliability of this reaction (which was reasonably successful in the pyrimidine series) remains unclear.

5.1.5 Protection with benzoyl chloride

The fourth step was performed as reported in the literature.[1] The lower yield may be correlated with the presence of salts impossible to detect by NMR that might reduce the actual weight of the starting material.

5.1.6 Displacement of iodine and deprotection of 2',3'-hydroxy and 6-amino benzoyl derivatives
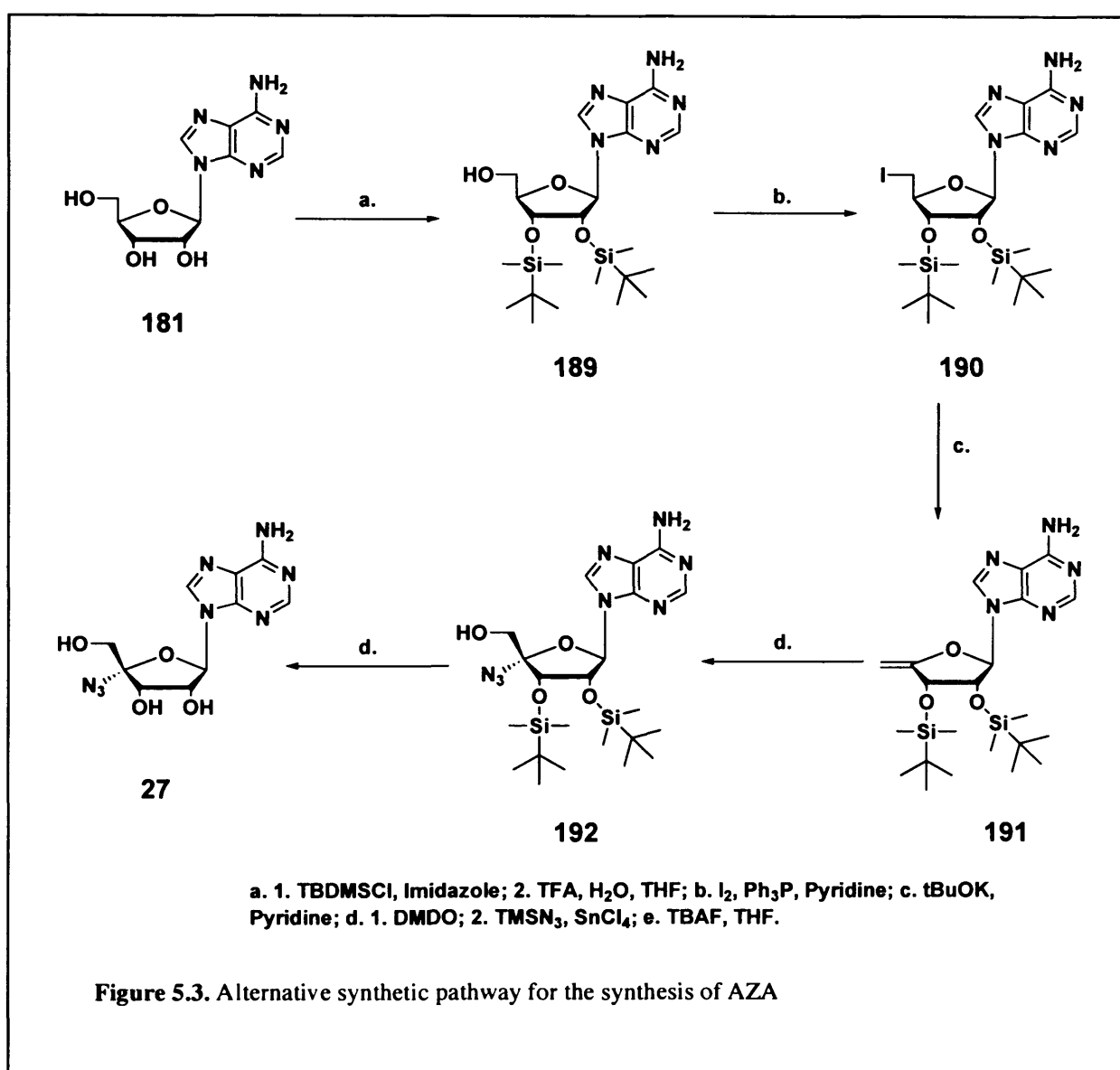
The choice of *meta*-chloroperbenzoic acid (mCPBA)[1] for the last step was made because the literature reported several studies about the displacement of iodine with subsequent deprotection with the more successful one employed using a one pot oxidation with mCPBA, followed by deprotection using NaOMe in MeOH.[1]

The presence of water, in the reaction enviroment, would oxidise the iodine to the corresponding hypervalent species improving the leaving group ability of iodine. In order to avoid the formation of several side products, the literature reported the displacement of the iodine and the subsequent deprotection as a one pot reaction.[1] Following this procedure, the overall yield of these two reactions combined was 50%

and it gave enough AZA to synthesise one phosphoramidate and its corresponding 5'-monophosphate.

5.2 Alternative pathway for the synthesis of 4'-azidoadenosine

With the previous synthetic pathway it was possible to synthesise only one phosphoramidate and the corresponding 5'-monophosphate. In order to synthesise more AZA, an alternative synthetic pathway was proposed (**Figure 5.3**).

a. 1. TBDMSCl, Imidazole; 2. TFA, H_2O, THF; b. I_2, Ph_3P, Pyridine; c. tBuOK, Pyridine; d. 1. DMDO; 2. $TMSN_3$, $SnCl_4$; e. TBAF, THF.

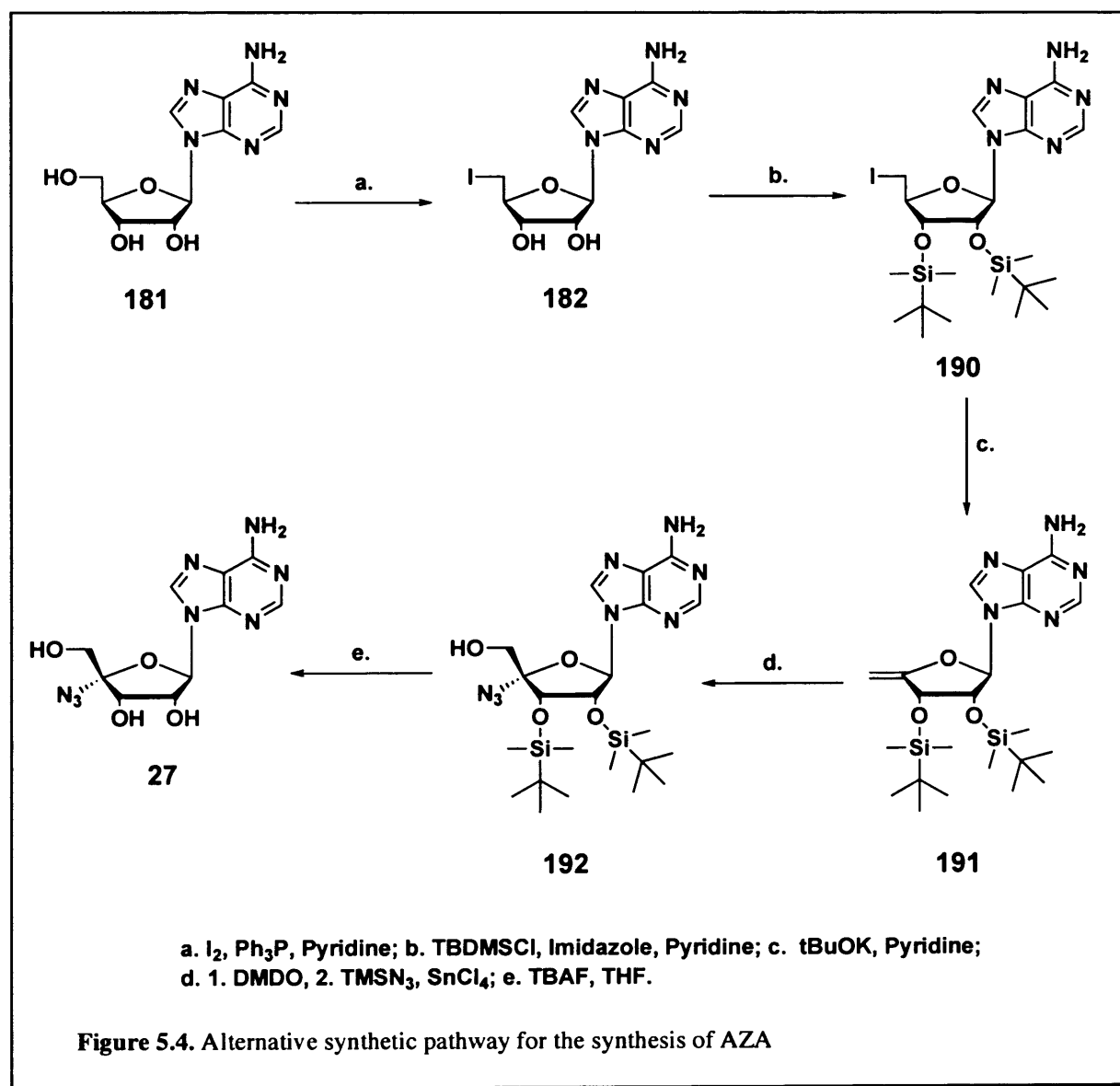**Figure 5.3.** Alternative synthetic pathway for the synthesis of AZA

The first step of this alternative synthetic pathway was the complete silylation of adenosine (in 2'-, 3'- and 5'-positions)[7] followed by selective deprotection at the

5'-position using a solution of TFA/H$_2$O/THF 4:1:1.[8] The reaction was attempted on a small scale, and gave a pure product with quantitative yield. The iodination was repeated twice. In the first attempt PS-Ph$_3$P was used, and in second instance Ph$_3$P was employed, in both cases, from TLC it was possible to see the presence of significant amounts of starting material.

5.2.1 Modified synthetic pathway for the synthesis of 4'-azidoadenosine

This alternative synthetic scheme was partially modified, with the iodination as first step followed by protection in the 2',3'-positions (**Figure 5.4**).

a. I$_2$, Ph$_3$P, Pyridine; b. TBDMSCl, Imidazole, Pyridine; c. tBuOK, Pyridine; d. 1. DMDO, 2. TMSN$_3$, SnCl$_4$; e. TBAF, THF.

Figure 5.4. Alternative synthetic pathway for the synthesis of AZA

5.2.2 Iodination and 2',3'-silylation of adenosine

The first step of this alternative synthetic pathway involved the selective iodination at the 5'-position of adenosine followed by protection at the 2',3'-positions using the *tert*-butyldimethylsilyl group.

In this case, the first purification method used was chromatography column which gave a product contaminated with triphenylphosphinum oxide. In order to avoid this problem, the first two steps (iodination of the 5'-position and protection in the 2',3'-position) were combined together (**Figure 5.5**).
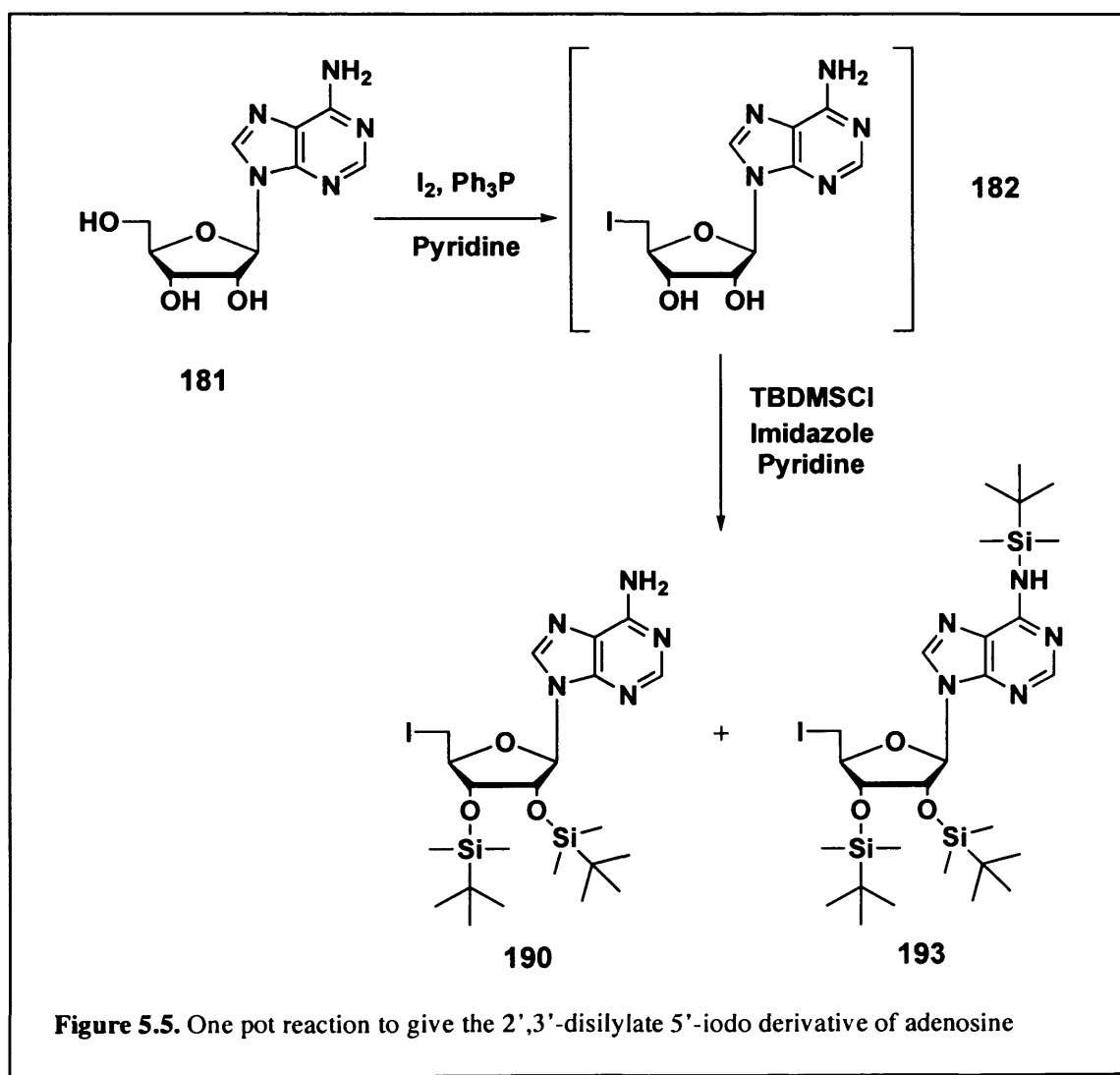

Figure 5.5. One pot reaction to give the 2',3'-disilylate 5'-iodo derivative of adenosine

On a small scale this one pot procedure afforded the desired product (**190**) with an overall yield of 52% with the presence (13% yield) of the complete product of silylation in 2'-, 3'- and 6-position (**193**). In attempt to increase the yield, the *tert-*

butyldimethyl silyl chloride was substituted with *tert*-buthyldimethylsilyltrifluoro methanesulfonate. This variation was applied successfully on a small scale and then repeated on a large scale with a significant increase in the overall yield.

5.2.3 Elimination of HI from 5'-deoxy-5'-iodoadenosine

Following previously described methodology (see section **5.1.3**) a pure white product was isolated in high yield. This reaction was attempted on a small scale and subsequently on a large scale affording, respectively, 88% and 70% yield (**Figure 5.4**).

5.2.4 Epoxidation in the 4'-, 5'-position and regio- and stereo-selective ring opening
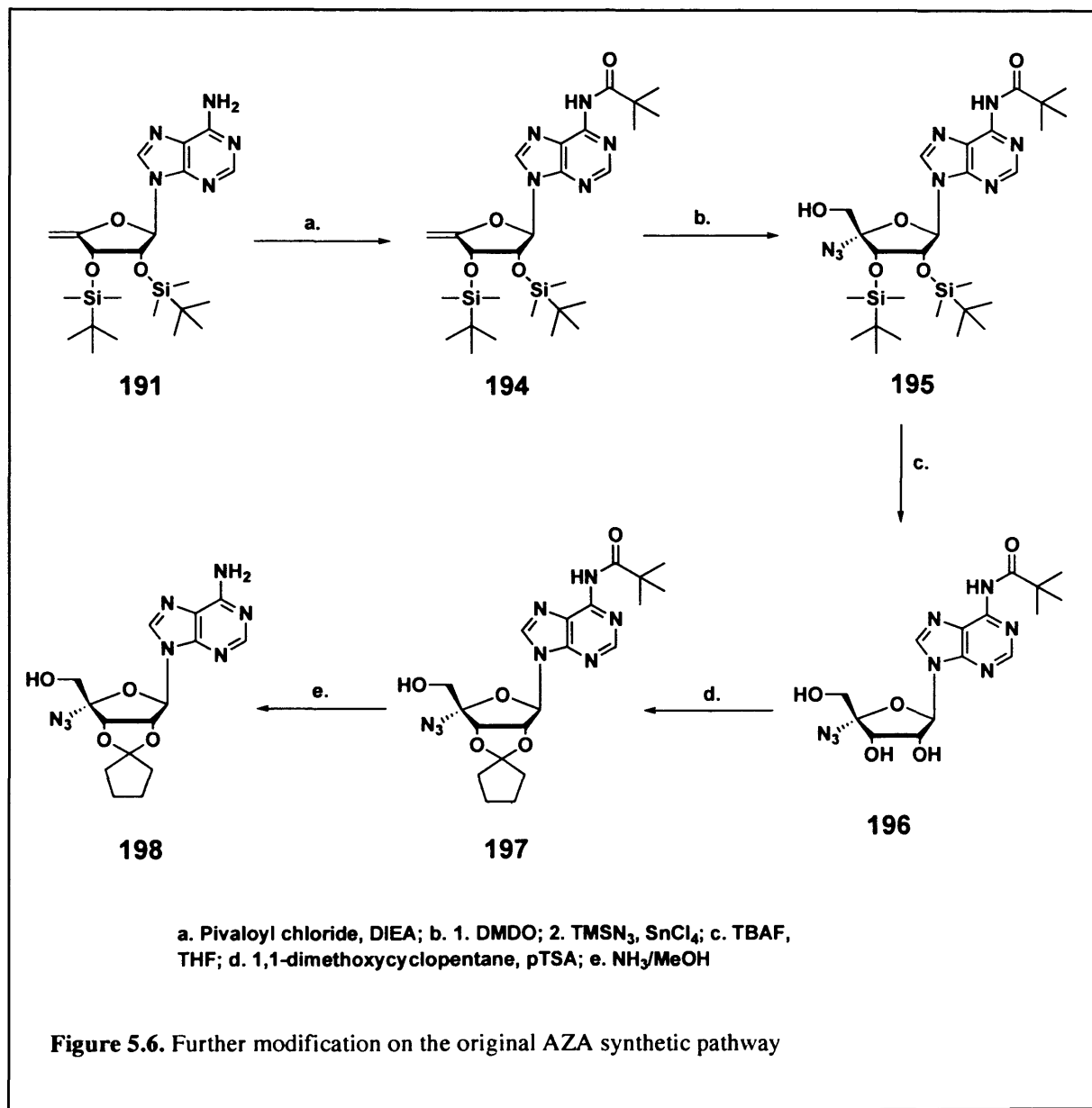
In the literature the synthesis of the epoxide followed by the regio- and stereo-selective ring opening has been well reported for several nucleosides. [9]

The synthesis of the epoxide derivative was performed using a 0.1 M solution of dimethyldioxirane (DMDO) in acetone.

The synthesis of DMDO was achieved starting from oxone® (monopersulphate compound) in the presence of water and acetone at basic pH. The DMDO was distilled as a 0.1 M solution of acetone.

5.2.5 Further modification on the 4'-azidoadenosine synthetic pathway

In an attempt to avoid the possible N^6-oxidation of adenosine during the epoxidation reaction, the previous synthetic pathway was further modified with the protection of the 6-NH_2 with a pivaloyl group (**Figure 5.6**). The reaction was attempted on a small and larger scale, affording respectively 95% and 93% (**194**).

a. Pivaloyl chloride, DIEA; b. 1. DMDO; 2. TMSN₃, SnCl₄; c. TBAF, THF; d. 1,1-dimethoxycyclopentane, pTSA; e. NH₃/MeOH

Figure 5.6. Further modification on the original AZA synthetic pathway

The subsequent epoxidation using DMDO afforded the desired product used in the next step. The regio- and stereo-selective ring opening was performed using azido-trimethilsylane and SnCl₄. In the literature it has been reported that the use of other

Lewis acids, such as $TiCl_4$ or $EtAlCl_2$, gave mainly the starting material with low yield.[9] The work up for this reaction was the neutralisation of the Lewis acid using an aqueous solution of $NaHCO_3$, which resulted in an emulsion that was eliminated by filtration through a celite pad. Subsequent purification by chromatography column afforded the pure product (**195**) in an overall yield between 39 and 54%.

5.2.5.1 Deprotection of hydroxy groups in the 2'- and 3'-positions

The deprotection of the two hydroxy groups in the 2'- and 3'-positions (**Figure 5.6**) was performed using the standard procedure to cleave the silyl group: a solution of tetrabutylammonium fluoride (TBAF) in THF at room temperature. After purification by column the desired product (**195**) was obtained with 70% yield; however from the analysis of the ^{1}H-NMR spectra the presence of tetrabutylammonium protons were observed with a calculated percentage of 5% of the impurity.

5.2.5.2 Protection of hydroxy groups in the 2'- and 3'-positions

From previous work on AZC (**23**) and AZU (**24**) (see **Chapter Three** and **Chapter Four**), the protection of hydroxy groups in the 2'- and 3'-positions was important in order to obtain a higher yield and easier purification during the phosphoramidate synthesis. Considering the previous successful results, the cyclopentylidene group was chosen. The protection reaction was performed using 1,1-dimethoxycyclopentane as solvent and 1.5 equivalents of *p*-toluene sulphonic acid (**Figure 5.6**). The purification method was chromatography column, which on a small and large scale gave the pure product (**197**) in yields of 71 and 67% respectively.

5.2.5.3 Deprotection of the 6-NH_2

Due to the presence of the pivaloyl group, the last step of this synthesis was the deprotection of the 6-NH_2. This reaction was performed in methanolic ammonia overnight (**Figure 5.6**).

Purification by chromatography column afforded the final product in 80% yield. Starting from 15 g of adenosine, 800 mg of the 2',3'-protected 4'-azidoadenosine (**198**) was obtained.

5.3 *α*-naphthyl 4'-azidoadenosine phosphoramidates

The 2',3'-protected 4'-azidoadenosine represented the staring material for the synthesis of a small series of phosphoramidate.
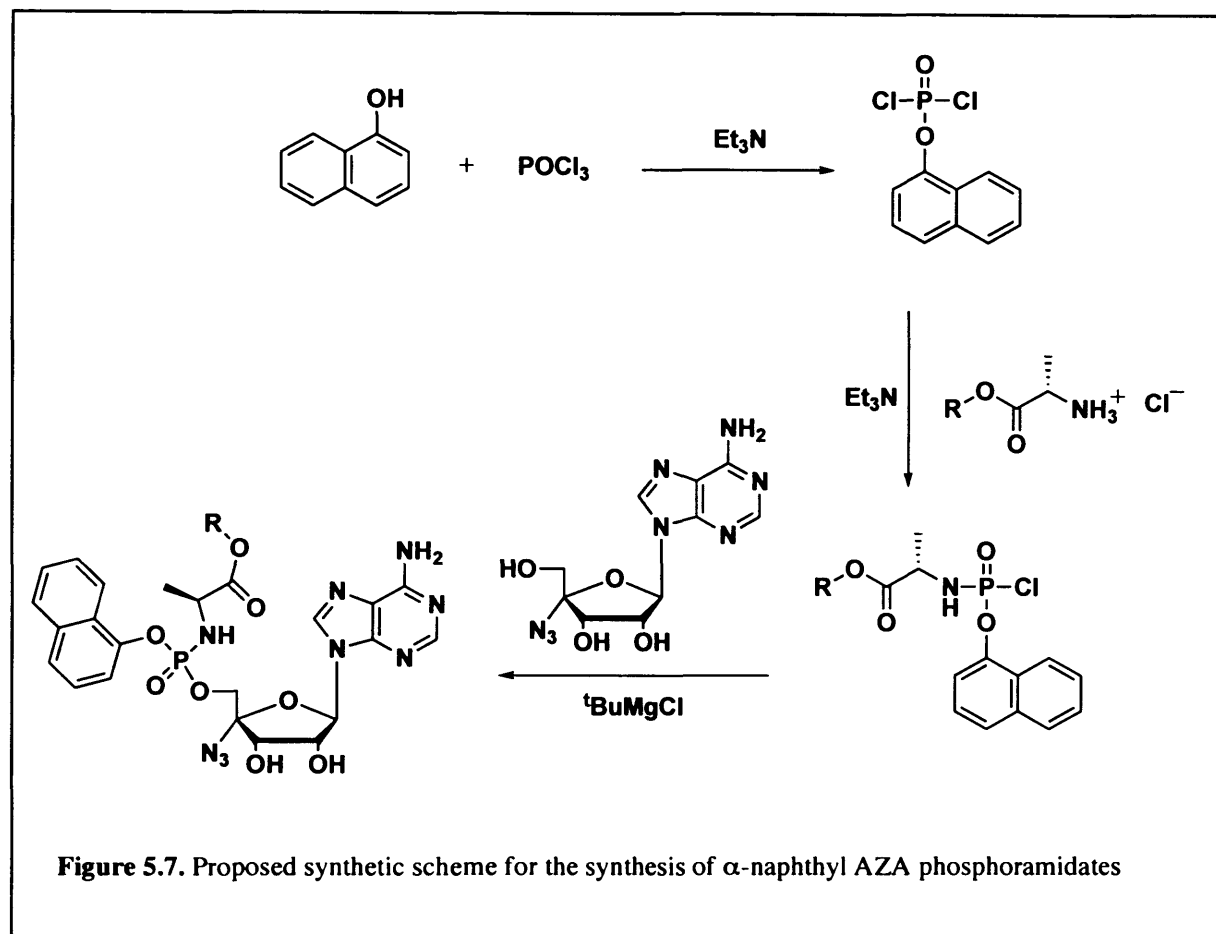

Figure 5.7. Proposed synthetic scheme for the synthesis of α-naphthyl AZA phosphoramidates

A series of 4'-azidoadenosine *α*-naphthyl phosphoramidates was targeted (**Figure 5.7**) using the Uchiyama procedure.[10]

### 5.3.1 Synthesis of phosphorylating agent for coupling with amino acids

The synthesis of the *α*-naphthyl dichlorophosphate was repeated following the conditions previously described in **Chapter Three** and **Chapter Four**.

5.3.2 Synthesis of phosphorylating agents for coupling with 4'-azidocytidine

The SAR previously explored with 4'-azidocytidine and 4'-azidouridine phosphoramidates (see **Chapter Three** and **Chapter Four**) showed that the presence of L-alanine as amino acid is an essential requisite in order to have good activity. For

this reason a small series of 4'-azidoadenosine α-naphthyl phosphoramidates was planned.

The phosphorylating agents used for coupling with 4'-azidoadenosine (**27**) were synthesised using the same procedure as described in **Chapter Three** and **Chapter Four**.[12, 13, 14] The synthesised phosphorochloridates are reported in **Table 5.1**.
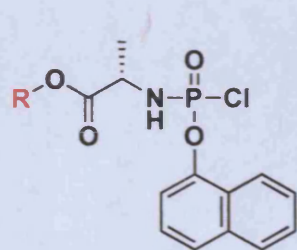
Cpd no.	Ester	Yield	^{31}P (ppm)
200[11]	ethyl	86%	8.42, 8.39
201[11]	*tert*-butyl	89%	8.48, 8.19

R= Ester

**Table 5.1.** Phosphorochloridate synthesised

5.3.3 Synthesis of α-naphthyl 4'-azidoadenosine phosphoramidates

In the first instance, the synthesis of naphthyl phosphoramidates of 4'-azidoadenosine (**202**) was performed in the presence of 4'-azidoadenosine, using the Uchiyama procedure using an excess of tBuMgCl in THF (see **Chapter Three**).[10] The first phosphoramidate synthesised was the α-naphthyl L-alanine benzyl ester (**Figure 5.8**).

27   'BuMgCl, THF   202

**Figure 5.8.** Initial synthesis of naphthyl phosphoramidates of AZA
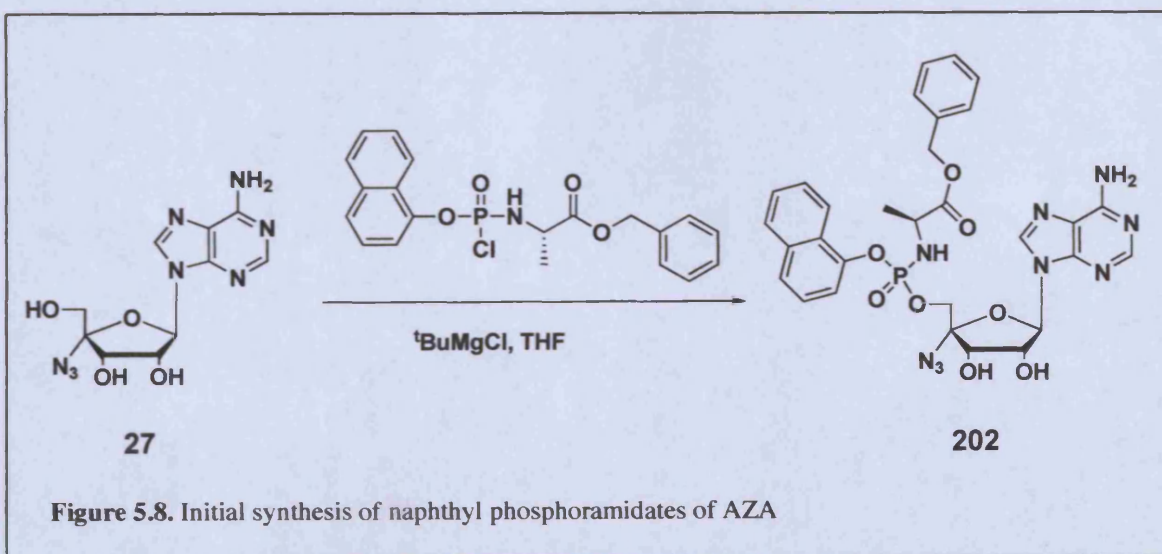
The major problem was the presence of what has been defined P-OH (**64**) after the first column (see **Chapter Three**). The full purification format required one chromatography column, one preparative TLC and one preparative HPLC. Due to the high number of purification steps, the yield of product was low (6%).

In an attempt to increase the yield and simplify the purification method, the same phosphoramidate reaction was performed with 2',3'-protected 4'-azidoadenosine (**198**). Following the successful results obtained with the protection of 4'-azidocytidine and 4'-azidouridine, cyclopentylidene was the group introduced in the 2'- and 3'-positions (**Figure 5.9**).

Figure 5.9. Synthesis of 2',3'-cyclopentylidene AZA phosphoramidates
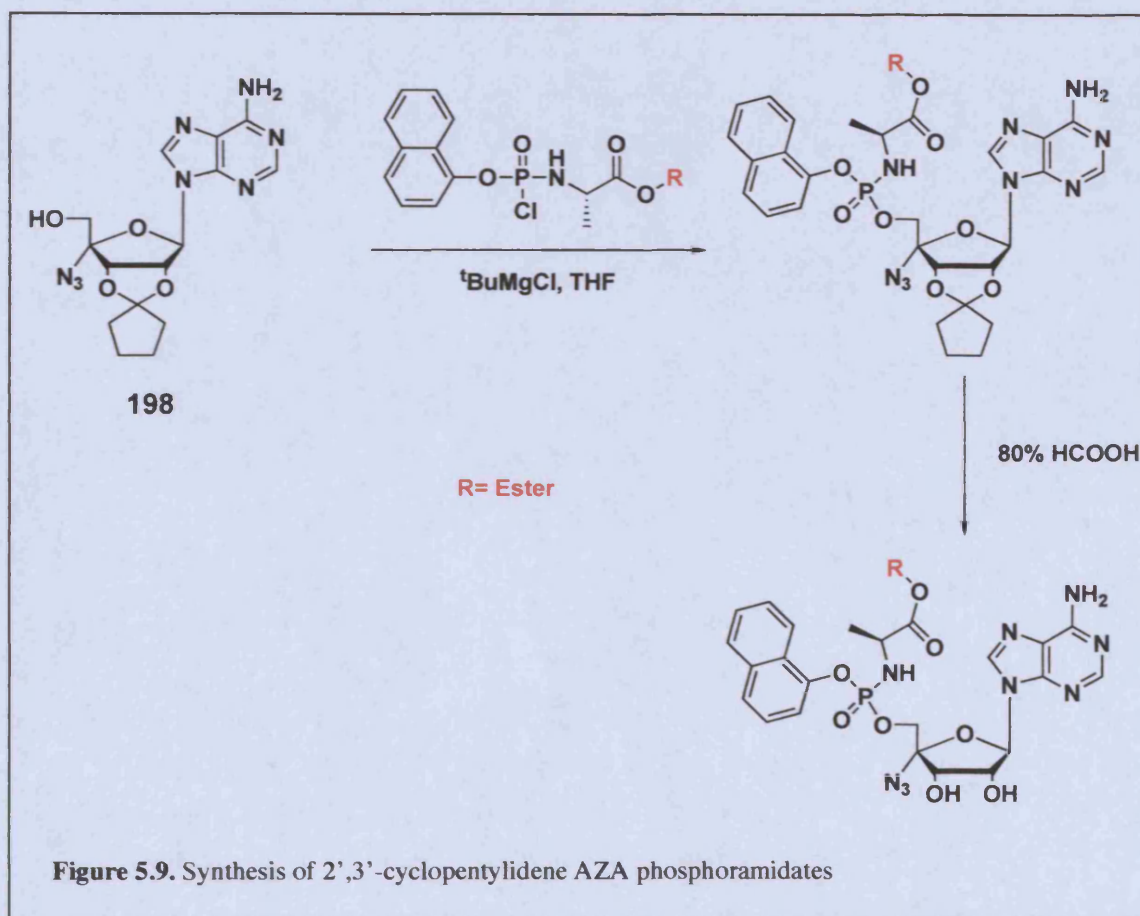
The reaction was stirred overnight and the purification usually required one chromatography column with good overall yields. The followed deprotection reaction was performed using an aqueous solution of formic acid (**Figure 5.9**).

The phosphoramidates synthesised are reported in **Table 5.2**.
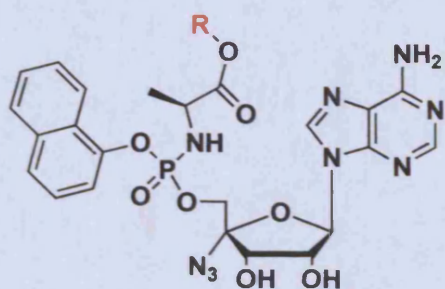
Cpd No.	Ester	^{31}P (ppm)
203	ethyl	3.72, 3.68
204	*tert*-butyl	3.85, 3.73

Table 5.2. α-Naphthyl phosphoramidates synthesised with 2',3'-cyclopentylidene AZA

5.4 Phenyl 4'-azidoadenosine phosphoramidates

In order to compare the difference of activity between the presence of an α-naphthyl and phenyl unit as the aryl moiety, a series of phenyl 4'-azidoadenosine phosphoramidates was planned. Also in this case, L-alanine was used as the amino acid and benzyl, ethyl and *tert*-butyl as esters.

5.4.1 Synthesis of phosphorylating agents for coupling with 4'-azidoadenosine

The non-commercially available phosphorochloridates were synthesised using the previously described procedure (see **Chapter Three**), in the presence of phenyldichlorophosphate, the appropriate amino acid ester and with an excess of triethylamine (**Figure 5.12**).

The phosphochloridates necessary for coupling with 4'-azidoadenosine were resynthesised using the procedure described in **Chapter Three** and **Chapter Four**. [12-14]
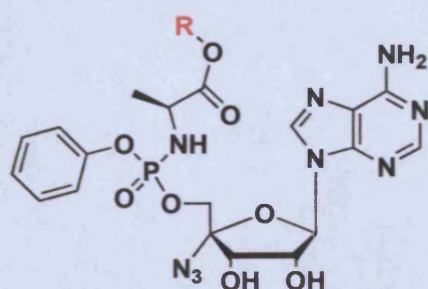
5.4.2 Synthesis of protected AZA phosphoramidates

In order to avoid problems such as low solubility and difficult purification, the phenyl phosphoramidates of 4'-azidoadenosine were synthesised via the corresponding 2',3'-cyclopentylidene nucleoside (**Figure 5.10**) using the Uchiyama procedure with an excess of tBuMgCl in THF (see **Chapter Three** and **Chapter Four**),[10] It required only one chromatography column to obtain pure 2',3'-protected 4'-azidoadenosine phosphoramidates.

The deprotection was carried out with a 80% solution of HCOOH at room temperature for 4 hours (**Figure 5.10**).

Figure 5.10. Synthesis of 2',3'-cyclopentylidene AZA phosphoramidates
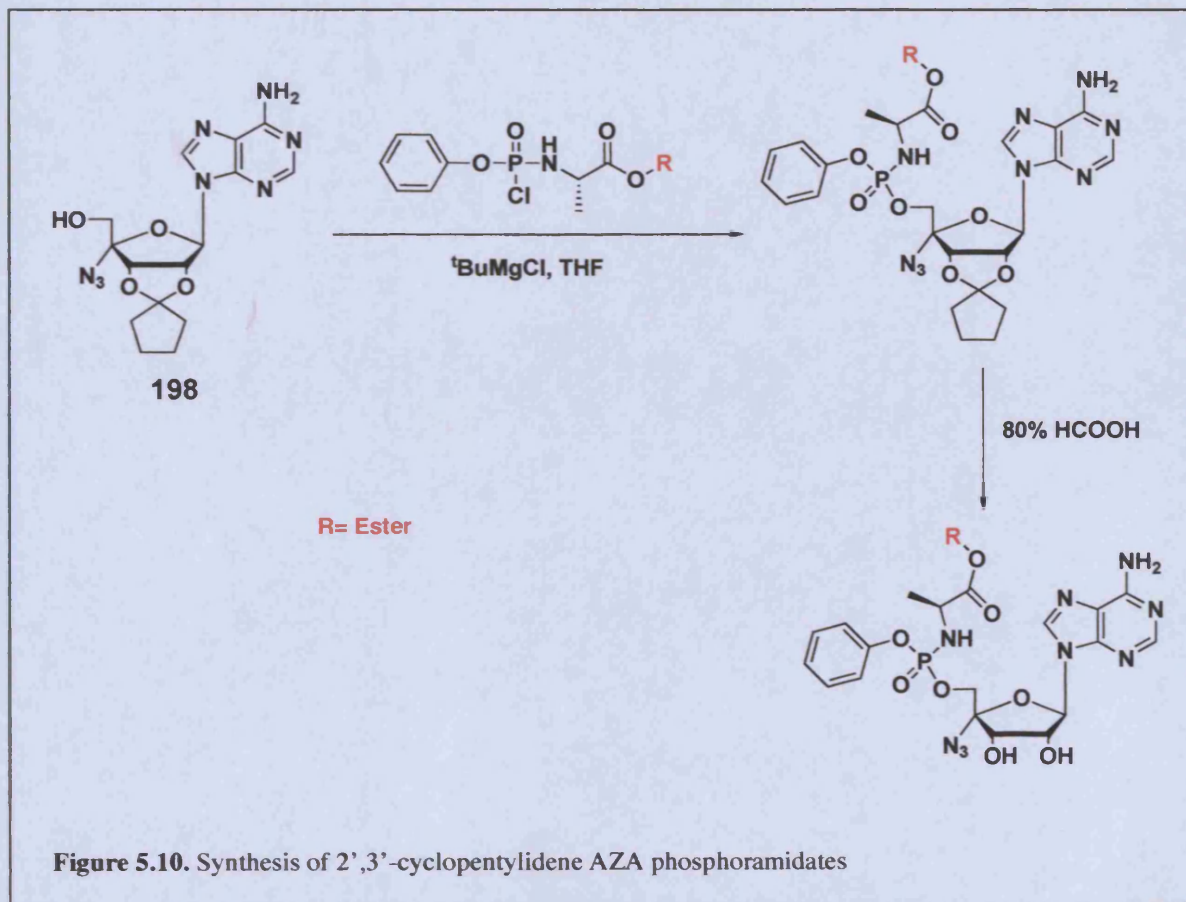
The purification of the deprotection reaction required only one column. The overall yield was around 70-80%. In this way it was possible to synthesise the phosphoramidates reported in **Table 5.4**.


Cpd No.	Esters	^{31}P (ppm)
206	benzyl	3.38, 3.21
207	ethyl	3.44, 3.28
208	*tert*-butyl	3.42

Table 5.4. Phenyl Phosphoramidates synthesised using the 2',3'-protected AZA

5.5 Biological activity of aryloxy phosphoramidates of 4'-azidoadenosine

All the α-naphthyl and phenyl phosphoramidates have been tested *in vitro* using a replicon assay (see section **3.3.6.1**) against HCV (**Table 5.5**).[15, 16]

R= Ester
Ar= Aryl moiety
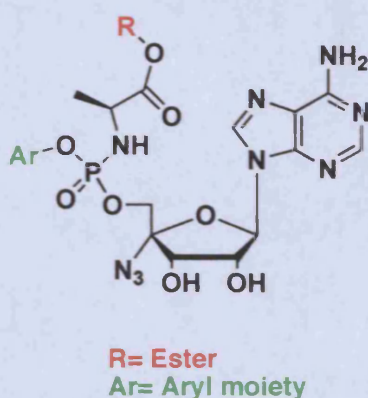
Cpd no.	Ester	Aryl moiety	EC_{50} (μM)	CC_{50} (μM)
202	benzyl	α-naphthyl	0.22	>100
203	ethyl	α-naphthyl	0.59	>100
204	*tert*-butyl	α-naphthyl	>100	>100
206	benzyl	phenyl	4.00	>100
207	ethyl	phenyl	1.50	>100
208	*tert*-butyl	phenyl	>100	>100
27	4'-azidoadenosine		>100	>100

Table 5.5. Biological activity of AZA naphthyl phosphoramidates
EC_{50}= The concentration of phosphoramidate which produces the 50% of the maximum possible effect
CC_{50}= The concentration of phosphoramidate which produces the 50% of the maximum toxic effect

The 4'-azidoadenosine (**27**) was found to be completely inactive and non-toxic, indicating that this nucleoside might be a poor substrate for the kinases and consequently the concentration into the cell of its corresponding active form (5'-triphosphate) is very low.

The corresponding ethyl and benzyl ester phosphoramidates were active against HCV. These data confirm that the phosphoramidate approach is a valuable technology that allows the conversion of inactive nucleoside (**27**) into sub-μM active compounds (**202**, **203**) bypassing the first phosphorylation that is often the rate limiting step in the activation mechanism of nucleosides.

The comparison between phenyl and its corresponding α-naphthyl phosphoramidates shows a significant (18-fold) increase for the benzyl ester (**202** and **206**) (**Graph 5.1**).

Graph 5.1. Biological activity of AZA phosphoramidates synthesised
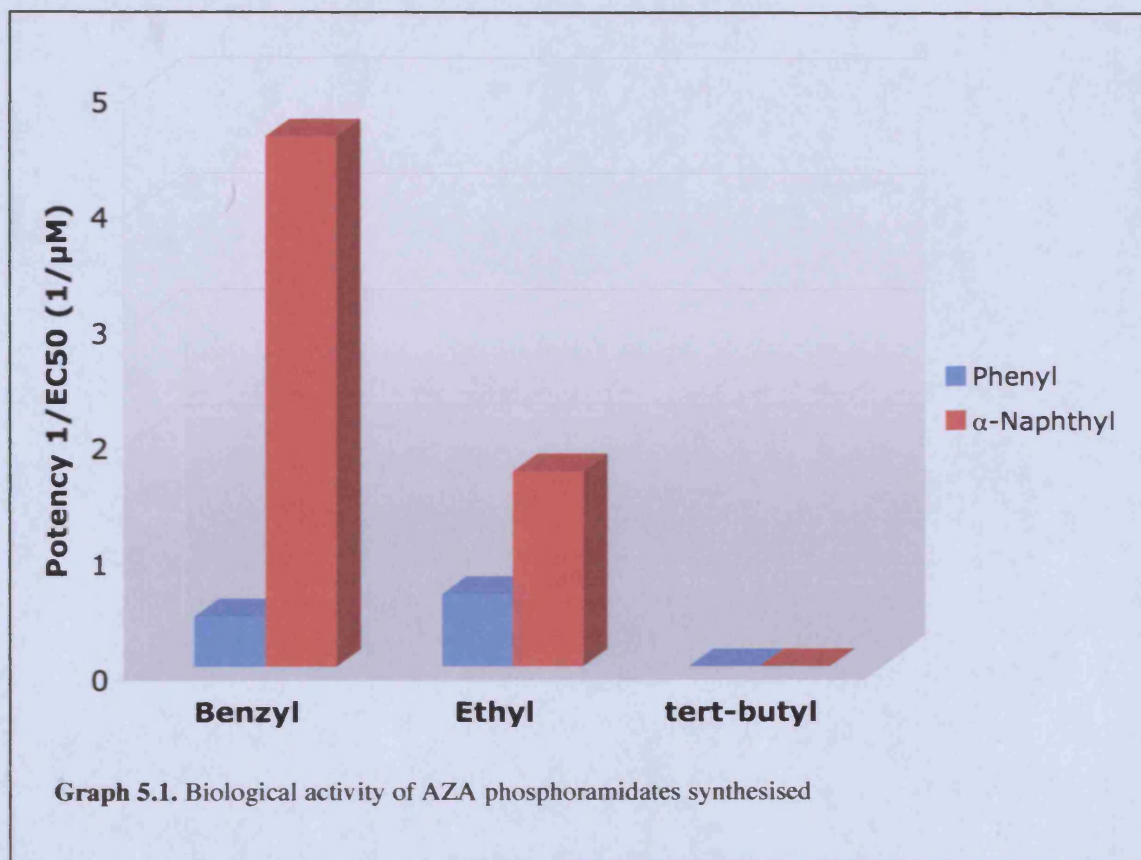
This increase of activity might be the result of a decrease in the pK_a value and the increase in lipophilicity that might facilitate the passive diffusion of phosphoramidates. The two *tert*-butyl phosphoramidates synthesised, by way of contrast were not active as a consequence of being poor substrates for the esterase involved in the monophosphate release.

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Chapter Six

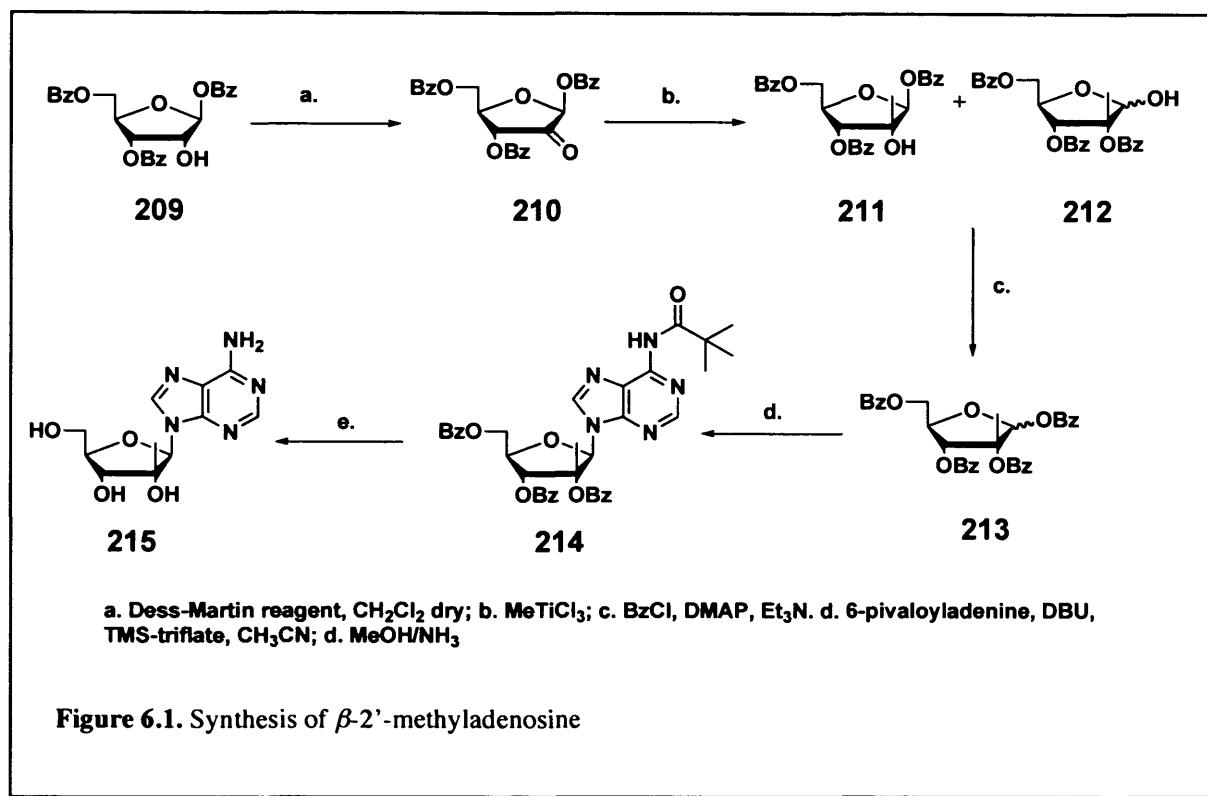## Aryloxyphosphoramidates of β-2'-methylnucleosides

6.1 β-2'-methylnucleosides

Several examples of modified nucleosides have been reported with potential anti-HCV activity. [1, 2, 3, 4] In most cases, the modification was made at the 2'- or 3'-positions. The active forms of these nucleosides are represented by their corresponding 5'-triphosphate that (interacting with the active site of the RdRp) are incorporated into the growing chain of the viral RNA and consequently cease the HCV replication.

The 2'-methylpurines (adenosine and guanosine) have been demonstrated to be potent anti-HCV agents:[1, 2] β-2'-methyladenosine showed IC_{50}= 1.9 μM (inhibition of RdRp), EC_{50}= 0.26 μM against HCV, and the detected level of its corresponding 5'-triphosphate was 105 Intracellular NTP/pmol per 10^6 cell. β-2'-methylguanosine, instead, showed IC_{50}= 0.13 μM (inhibition of RdRp), EC_{50}= 3.5μM against HCV, but most importantly the detected level of its corresponding 5'-triphosphate was rather poor (Intracellular NTP/pmol per 10^6 cell= 0.2). This might be an indication that this nucleoside was a poor substrate for the kinases responsible for the conversion into its corresponding active species (5'-triphosphate). We decide to apply the aryloxyphosphoramidate approach to these two modified nucleosides in order to explore the possibility of further increasing their activity against HCV. [1, 2]

6.2 Synthesis of *β*-2'-methyladenosine

The synthesis of *β*-2'-methyladenosine was planned following the reported procedure (**Figure 6.1**).[1,2]

a. Dess-Martin reagent, CH_2Cl_2 dry; b. $MeTiCl_3$; c. BzCl, DMAP, Et_3N. d. 6-pivaloyladenine, DBU, TMS-triflate, CH_3CN; d. $MeOH/NH_3$

Figure 6.1. Synthesis of *β*-2'-methyladenosine

6.2.1 Oxidation of the 2-position

The oxidation in the 2-position (**Figure 6.1**) of the starting material (**209**) was performed in the presence of the synthesised Dess-Martin reagent in anhydrous conditions overnight at room temperature to give a yield of 95%.[5]

6.2.2 Stereoselective addition of the methyl group at the *β*-2-position

The stereoselective addition of a methyl group in the *β*-2-position (**Figure 6.1**) was performed in the presence of methyl titanium trichoride synthesised *in situ* from an anhydrous solution of titanium tetrachloride and methyl magnesium bromide in diethyl ether at -78° C. The keto-derivative (**210**) was added as a solution in diethyl ether. The purification method required one column chromatography to give a mixture of **211** and **212**. The formation of **212** was due to an intramolecular trans-benzoylation to give a mixture of *α*- and *β*-derivatives (**212**). Compound **211** and **212** were isolated

as a mixture and used for the next step without further purification. Also in this case the yield observed (70%) was in the same range reported in the literature.[5]

6.2.3 Benzoylation of tribenzoate β-2-methyl ribosugar

In order to achieve a stereoselective coupling with the corresponding base (N^6-pivaloyladenine) it was necessary to have a fully protected sugar. For this reason a benzoylation reaction was performed on the mixture of **211** and **212** (**Figure 6.1**) using benzoyl chloride triethylamine and DMAP as an activator in anhydrous conditions.[5] The reaction was stirred overnight and the desired compound was obtained after purification by column chromatography. The yield (41%) observed was reasonably low relating to the presence of salts in the mixture of **211** and **212** that cannot be seen in the ^{1}H-NMR spectra.

6.2.4 Coupling reaction between 1,2,3,5-tetrabenzoate β-2-methyl ribosugar and N^6-protected adenine

In order to avoid problems of selectivity in N^7- and N^9-positions, the adenine was protected in N^6-position with the pivaloyl group (**217**) using standard conditions (**Figure 6.2**).

Figure 6.2. Synthesis of pivaloyl adenine
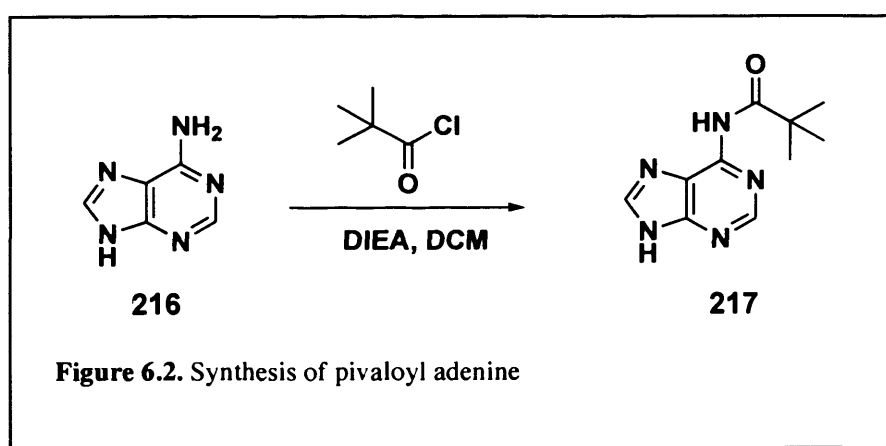
The following coupling reaction (**Figure 6.1**) to synthesise the fully protected nucleoside (**214**) was performed with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and trimethylsilyl trifluoromethanesulfonate (TMS-triflate). DBU is a strong base and, in this case it was used to remove the proton in the N^7-position of the corresponding

adenine. TMS-triflate, instead, was used in order to increase the reactivity in the 1-position of **213**. The reaction proceeded as described in the literature and the purification method required one chromatography column to give the pure nucleoside analogue (**214**) in reasonably high yield (49%).

Heteronuclear multiple bond correlation (HMBC) showed the correlation between C4 and H1', confirming the presence of the N^9-regioisomer.

Nuclear Overhauser Enhancement Spectroscopy (NOESY), instead, showed no correlation between the proton of H1' and the three protons of the methyl group in the 2'-position confirming the presence of the β-nucleoside; instead, the correlation between H2' and the three protons of the methyl group in the 2'-position confirmed the presence of the methyl group in the β-position.

6.2.5 Deprotection of N^6-pivaloyl-2',3',5'-tetrabenzoate β-2-methyladenosine

The final step was removal of the protecting groups using methanolic ammonia (**Figure 6.1**) at room temperature in a sealed tube overnight. The purification required one column chromatography to give the desired product (**215**) in 72% yield.

6.3 α-naphthyl β-2'-methyladenosine phosphoramidates

Considering the previously explored SAR (**Chapter Three, Chapter Four** and **Chapter Five**), a series of L-alanine α-naphthyl phosphoramidates of β-2'-methyladenosine was planned.

6.3.1 Synthesis of 2',3'-cyclopentylidene β-2'-methyladenosine

In our previous work (**Chapter Three, Chapter Four** and **Chapter Five**) the presence of a protecting group in the 2'- and 3'-positions improved the selectivity of reaction in the 5'-position and increases the solubility of the nucleoside in THF. For this reason the synthesised β-2'-methyladenosine was protected in the 2'- and 3'-position with the cyclopentylidene group. N^6-pivaloyl-2',3',5'-tetrabenzoateβ-2'-methyladenosine (**214**) was selectively deprotected in the 2'- 3'- and 5'-positions whilst keeping the pivaloyl group in the N^6-position. This reaction was performed in the presence of a 1 M solution of NaOH in ethanol and pyridine (**Figure 6.3**). The next step involved the introduction of the cyclopentylidene group in 2'- and 3'-positions in the presence of 1,1-dimethoxycyclopentane and a catalytic amount of p-TSA with a yield of 53% (**Figure 6.3**). The final step was the deprotection of the 6-amino group using methanolic ammonia (**Figure 6.3**) to give the desired product (**220**) in quantitative yield.

a. 1 M NaOH, ethanol, pyridine; b. 1,1-dimethoxypentane, p-TSA; c. MeOH/NH₃

Figure 6.3. Synthesis of 2',3'-protected β-2'-methyladenosine
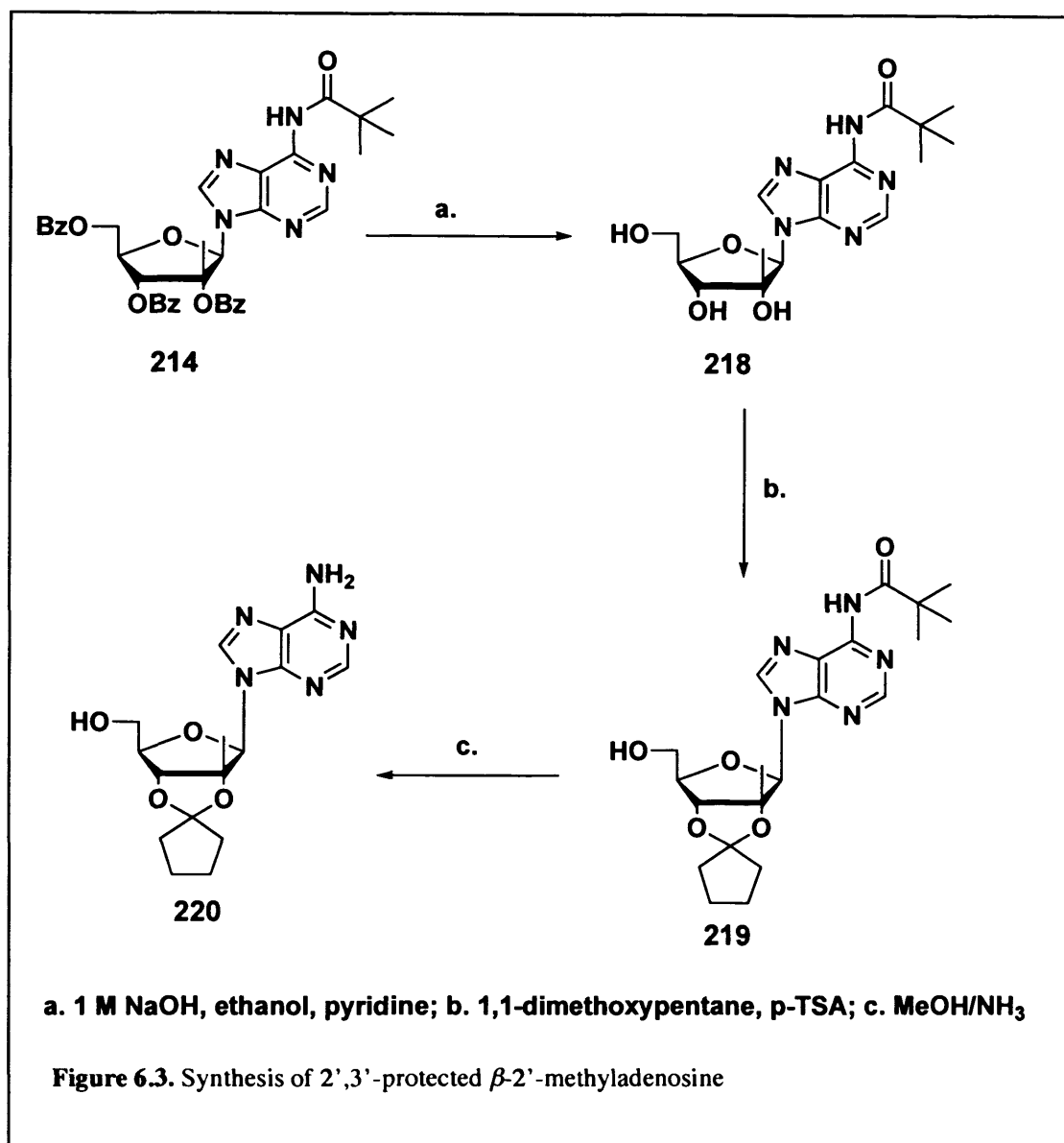
6.3.2 Synthesis of phosphorylating agents for coupling with *β*-2'-methyladenosine

The SAR previously explored (**Chapter Three, Chapter Four** and **Chapter Five**) showed that the presence of L-alanine as the amino acid is essential to enhance biological activity. For this reason a small series of β-2'-methyladenosine L-alanine α-naphthyl phosphoramidates was planned with variation on the ester of the amino acid. The phosphorochloridate were resynthesised using the same procedure described in **Chapter Three, Chapter Four** and **Chapter Five**.

6.3.3 Synthesis of α-naphthyl β-2'-methyladenosine phosphoramidates

The synthesis of naphthyl phosphoramidates of β-2'-methyladenosine (**221**) was carried out using 2',3'-cyclopentylidene-β-2'-methyladenosine (**220**) using the Uchiyama procedure in the presence of an excess of tBuMgCl (**Figure 6.6**).[9]

The purification method required one column chromatography. The deprotection reaction was performed in the presence of a solution of 80% formic acid (**Figure 6.6**) and the purification required one column chromatography.
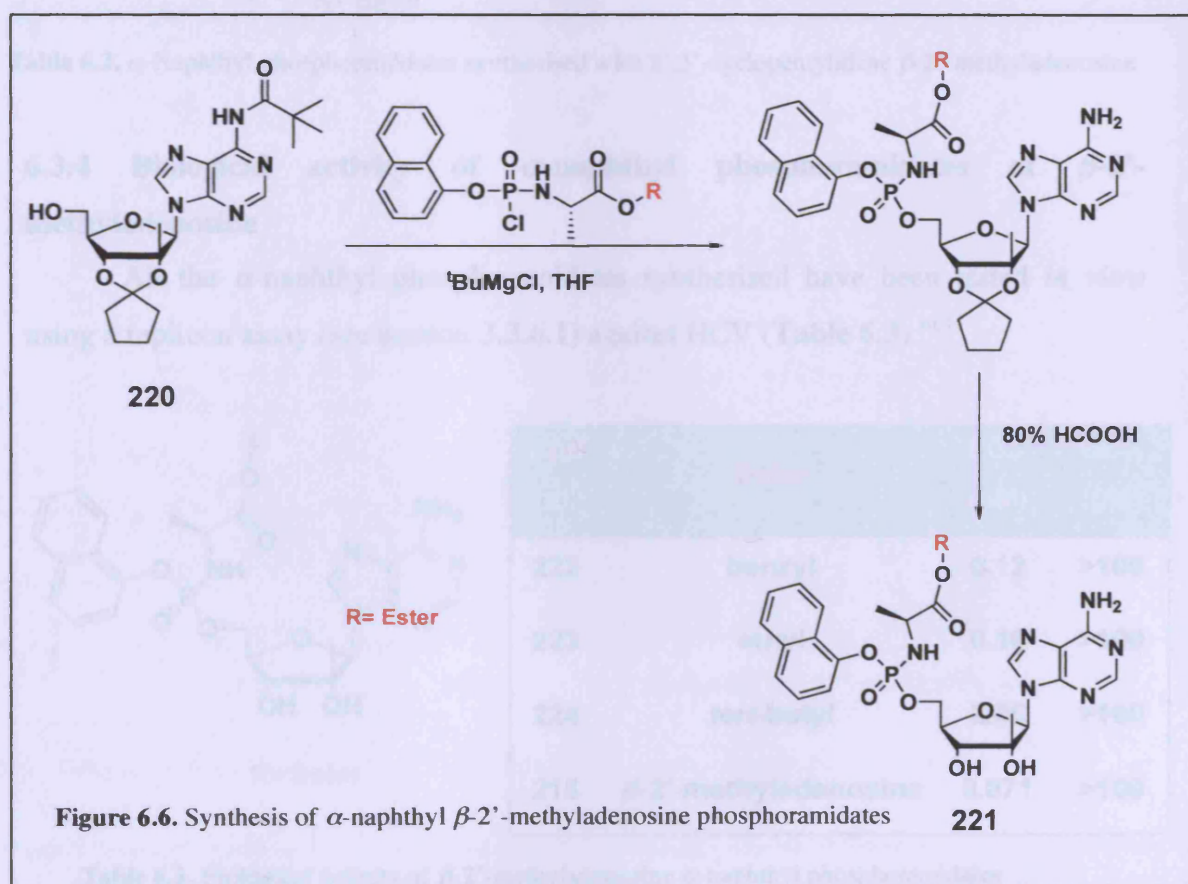
Figure 6.6. Synthesis of α-naphthyl β-2'-methyladenosine phosphoramidates **221**

The α-naphthyl phosphoramidates synthesised are reported in **Table 6.2.**
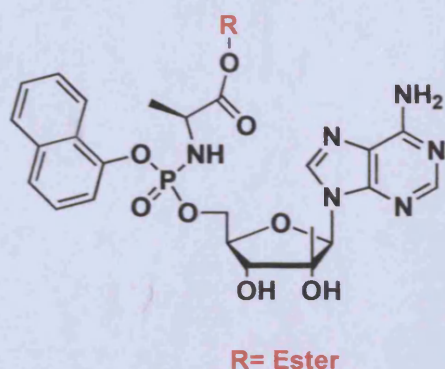
Cpd no.	Ester	^{31}P (ppm)
222	benzyl	4.25, 4.14
223	ethyl	4.23, 4.20
224	*tert*-butyl	4.20, 4.08

R= Ester

Table 6.2. α-Naphthyl phosphoramidates synthesised with 2',3'-cyclopentylidene β-2'-methyladenosine

6.3.4 Biological activity of α-naphthyl phosphoramidates of β-2'-methyladenosine

All the α-naphthyl phosphoramidates synthesised have been tested *in vitro* using a replicon assay (see section **3.3.6.1**) against HCV (**Table 6.3**).[10, 11]
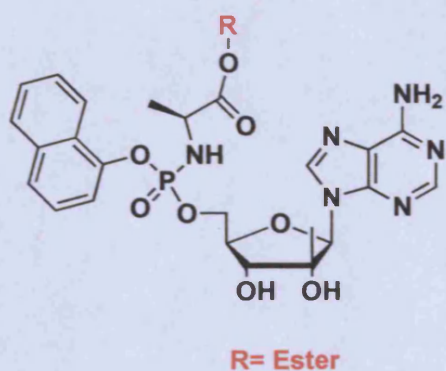
Cpd no.	Ester	EC_{50} (µM)	CC_{50} (µM)
222	benzyl	0.12	>100
223	ethyl	0.16	>100
224	*tert*-butyl	2.36	>100
215	β-2'-methyladenosine	0.071	>100

R= Ester

Table 6.3. Biological activity of β-2'-methyladenosine α-naphthyl phosphoramidates
EC_{50}= The concentration of phosphoramidate which produces the 50% of the maximum possible effect
CC_{50}= The concentration of phosphoramidate which produces the 50% of the maximum toxic effect

The nucleoside itself (**215**) was shown to be a potent inhibitor of HCV with an activity of 71 nM. The corresponding α-naphthyl phosphoramidates were found to be less active. In particular the *tert*-butyl ester phosphoramidate (**224**) showed a rather poor activity (2.36 µM), confirming that this kind of ester is a poor substrate for the enzyme involved in the cleavage of the amino acid ester.

The benzyl and ethyl ester, instead, were found to be active at sub-μM level, indicating that these two ester were well tolerated by the esterase. A possible explanation of the lower activity of the phosphoramidates (**222, 223, 224**) compared to the nucleoside (**215**) might be related to the enzymes involved in the phosphoramidate metabolism; in particular the enzyme with phosphoramidase activity might not tolerate the presence of a methyl group in the 2'-position and as a consequence was not able to efficiently cleave the P-N bond.
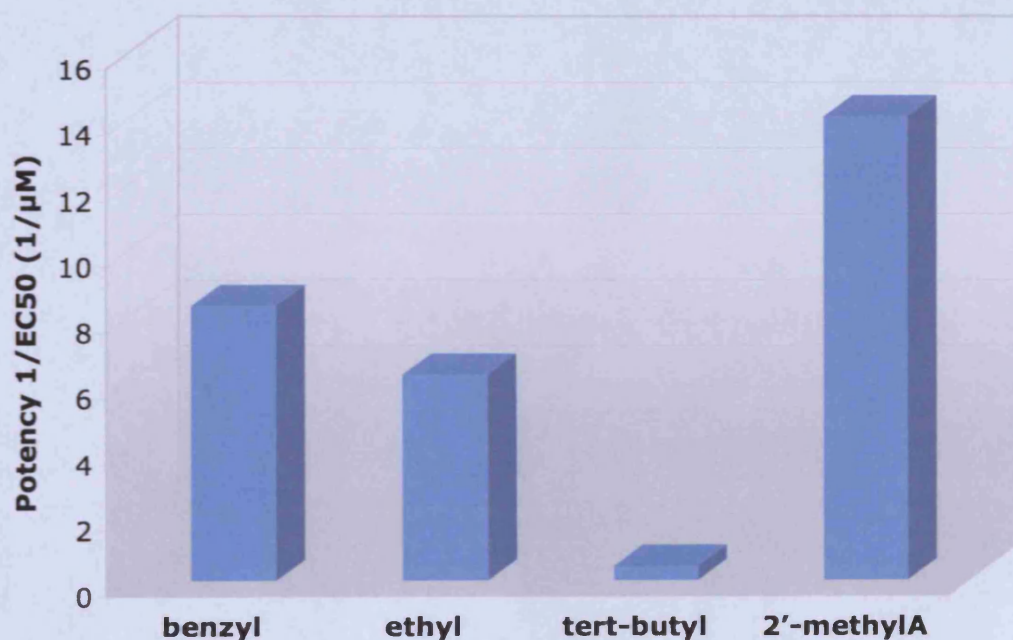
Graph 6.1. Biological activity of 2'-methyladenosine phosphoramidates synthesised

Moreover, the observed intracellular level of nucleoside monophosphate (Intracellular NTP/pmol per 10^6 cell= 105)[2] is reasonably high and it might be a consequence of an efficient first phosphorylation of β-2'-methyladenosine (**215**) by the first kinases. In this case, therefore, it might be possible that the second or third phosphorylations are the limiting step of the activation mechanism of the nucleoside (**215**). As a consequence the administration of the corresponding phosphoramidates does not overcome the limiting step in this process.

6.4 Synthesis of *β*-2'-methylguanosine

The synthesis of *β*-2'-methylguanosine (**226**) was subsequently planned, considering its high anti-HCV activity. The initial synthetic pathway started with the synthesis of *β*-2-methyl ribosugar (**213**) followed by a coupling reaction with guanine and finally deprotection to give the desired product (**Figure 6.7**).


a. Dess-Martin reagent, CH₂Cl₂ dry; b. MeTiCl₃; c. BzCl, DMAP, Et₃N. d.Guanine, DBU, TMS-triflate, CH₃CN; e. MeOH/NH₃

Figure 6.7. Synthesis of *β*-2'-methylguanosine
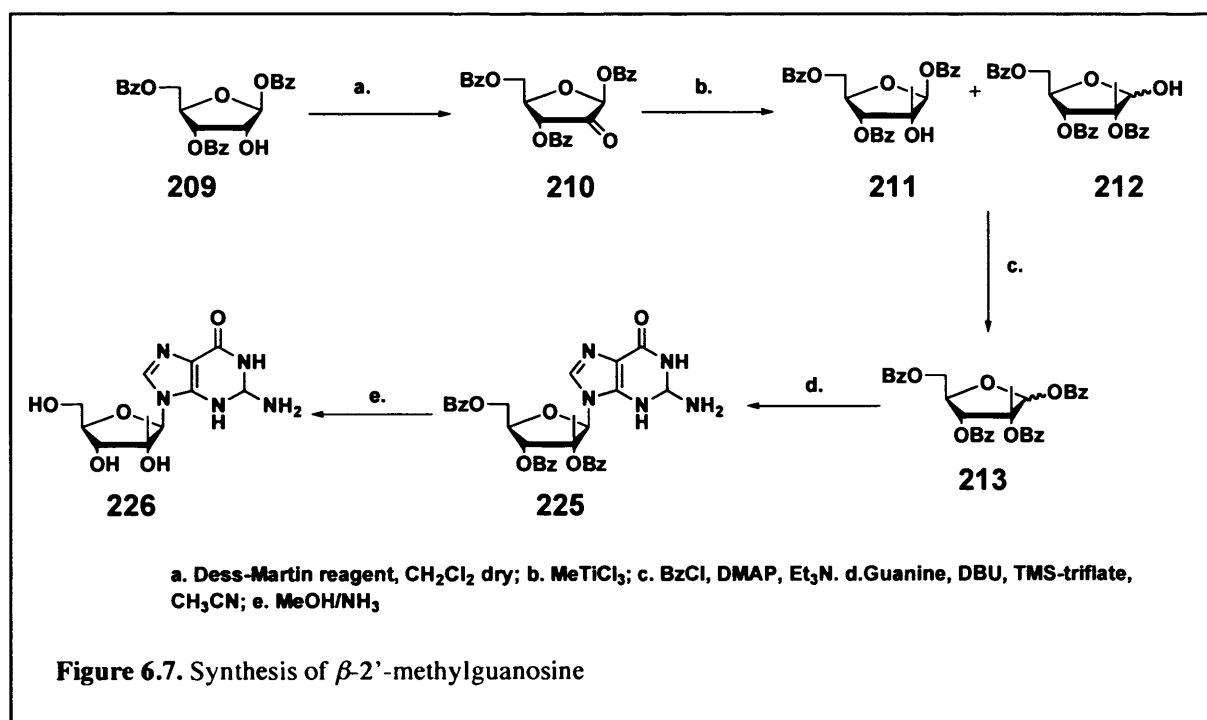
The synthesis of the *β*-2-methyl ribosugar (**213**) was performed with the same procedures and yields described for the synthesis of *β*-2'-methyladenosine (**215**). Considering what was observed during the synthesis of *β*-2'-methyladenosine (**215**), the free base (guanine) was used for the coupling reaction with *β*-2-methyl ribosugar with the same conditions previously described.[1,2]

The analysis of the HMBC and the NOESY confirmed that the synthesised nucleoside was *N*⁹- and *β*-structure. The ¹H-NMR of *β*-2'-methylguanosine (**226**) have been reported (**Figure 6.8**).

Figure 6.8. ^{1}H-NMR of the N^9-regioisomer
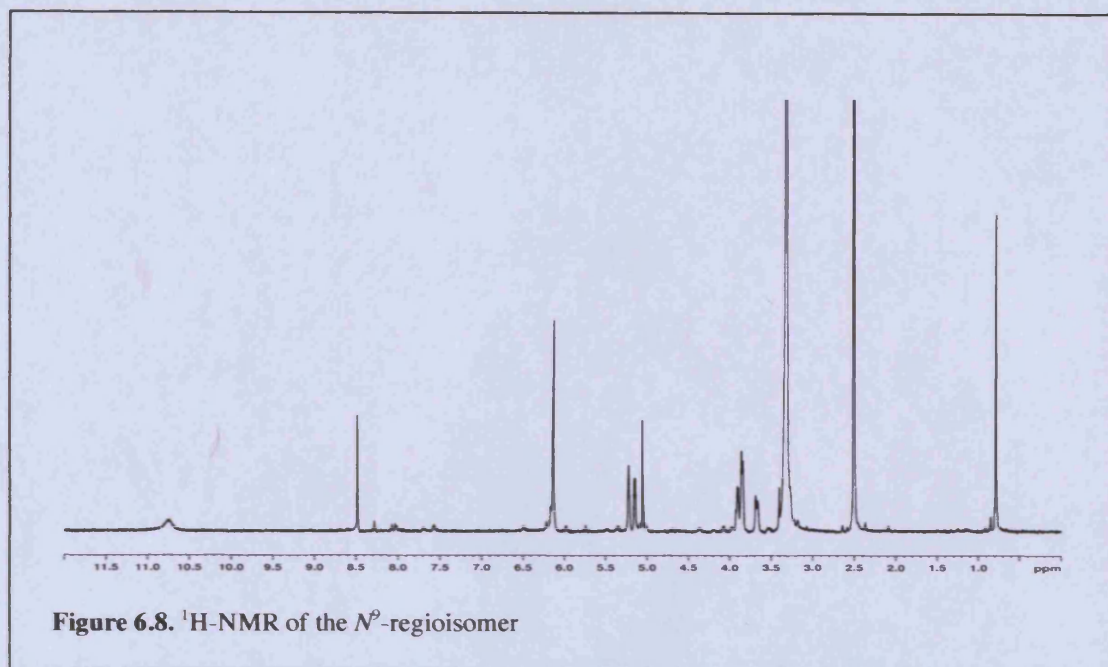
The reaction was repeated using the same conditions. Unfortunately in this case we isolated a fraction that from the ^{1}H-NMR showed the presence of at least two compounds, both of them with a signal compatible with a nucleoside structure suggesting the presence of a mixture of N^9- and N^7-regioisomers (**Figure 6.9**).

Figure 6.9. ^{1}H-NMR of the N^9- and N^7-regioisomers mixture
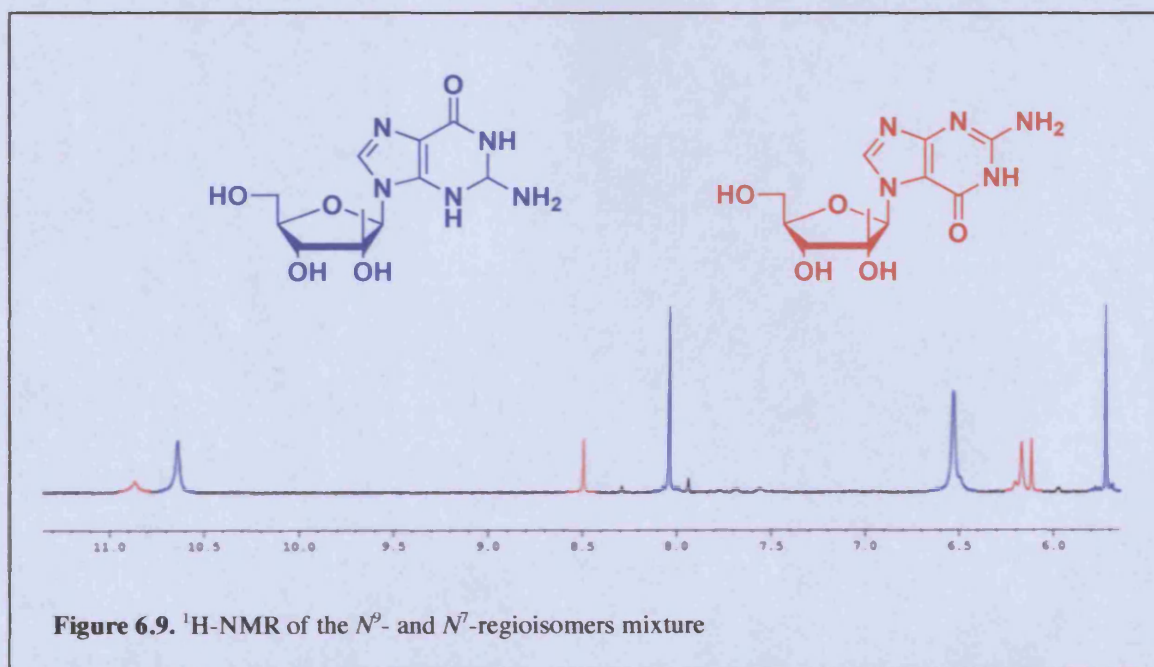
The reasons for the different product ratio on repeating the reaction are unclear. Further research in the literature brought to our attention a paper in which the

synthesis of β-2'-methylguanosine (**226**) was performed under regioselective conditions.[12]

In this case the reaction was performed in two steps: the first was the synthesis of the totally silylated acetylguanine followed by the coupling reaction in the presence of the previously synthesised 2-methyl ribosugar (**213**) and trimethylsilyl trifluoromethanesulfonate (TMS-triflate) using *para*-xylene as solvent (**Figure 6.10**).
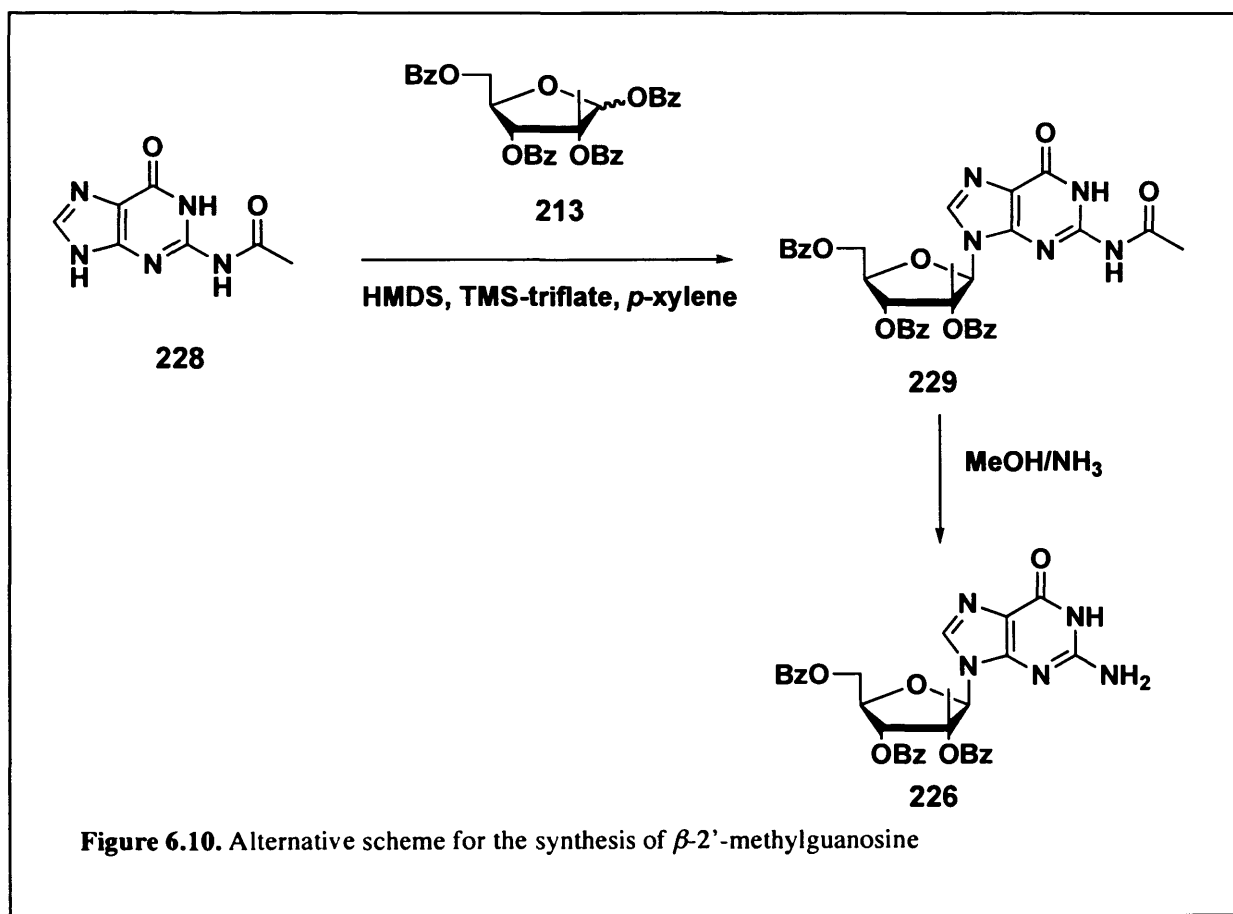
Figure 6.10. Alternative scheme for the synthesis of β-2'-methylguanosine

After purification by column chromatography, the N^9-regioisomer (**229**) in 63% yield was isolated without any traces of N^7-regioisomer. The final step was deprotection with methanolic ammonia to give the desired product (**226**) in quantitative yield.

6.5 α-naphthyl β-2'-methylguanosine phosphoramidates

Considering previously explored SAR (**Chapter Three** and **Chapter Four**) a series of L-alanine α-naphthyl phosphoramidates of β-2'-methylguanosine was planned.

6.5.1 Synthesis of 2',3'-isopropylidene β-2'-methyladenosine

In previous work (**Chapter Three, Chapter Four** and **Chapter Five**) the increase of solubility of the nucleoside in THF in the presence of a protecting group in 2'- and 3'-positions was noticed. For this reason the 2',3'-protected version of β-2'-methylguanosine was planned. The introduction of the cyclopentylidene group in the 2'- and 3'-positions was attempted. Unfortunately this attempt was unsuccessful and consequently the use isopropylidene as the protecting group was considered. The synthesis was performed in the presence of β-2'-methyladenosine (**226**) and a catalytic amount of perchloric acid in a solution of dry acetone (**Figure 6.11**).

Figure 6.11. Synthesis of 2',3'-isopropylidene β-2'-methylguanosine
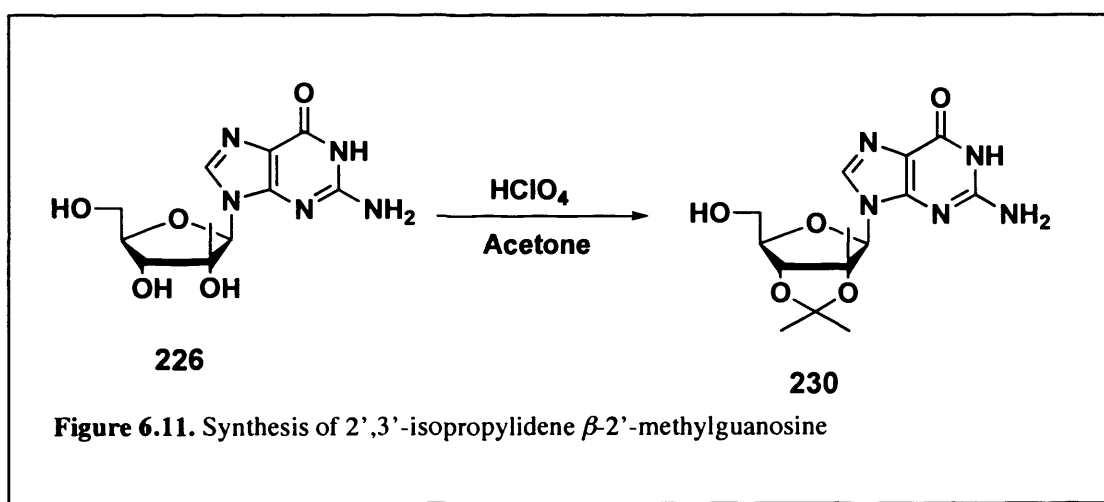
6.5.2 Synthesis of phosphorylating agents for coupling with β-2'-methylguanosine

The synthesis of phosphodichloridate and subsequently L-alanine phosphochloridate were repeated using the same conditions previously described (**Chapter Three, Chapter Four** and **Chapter Five**).

6.5.3 Synthesis of α-naphthyl β-2'-methylguanosine phosphoramidates

The synthesis of naphthyl phosphoramidates of β-2'-methylguanosine (**232**) was performed in the presence of 2',3'-isopropylidene β-2'-methylguanosine (**230**) and an excess of tBuMgCl (**Figure 6.13**). [9] The purification method required one column chromatography.

Figure 6.13. Synthesis of α-naphthyl β-2'-methylguanosine phosphoramidates
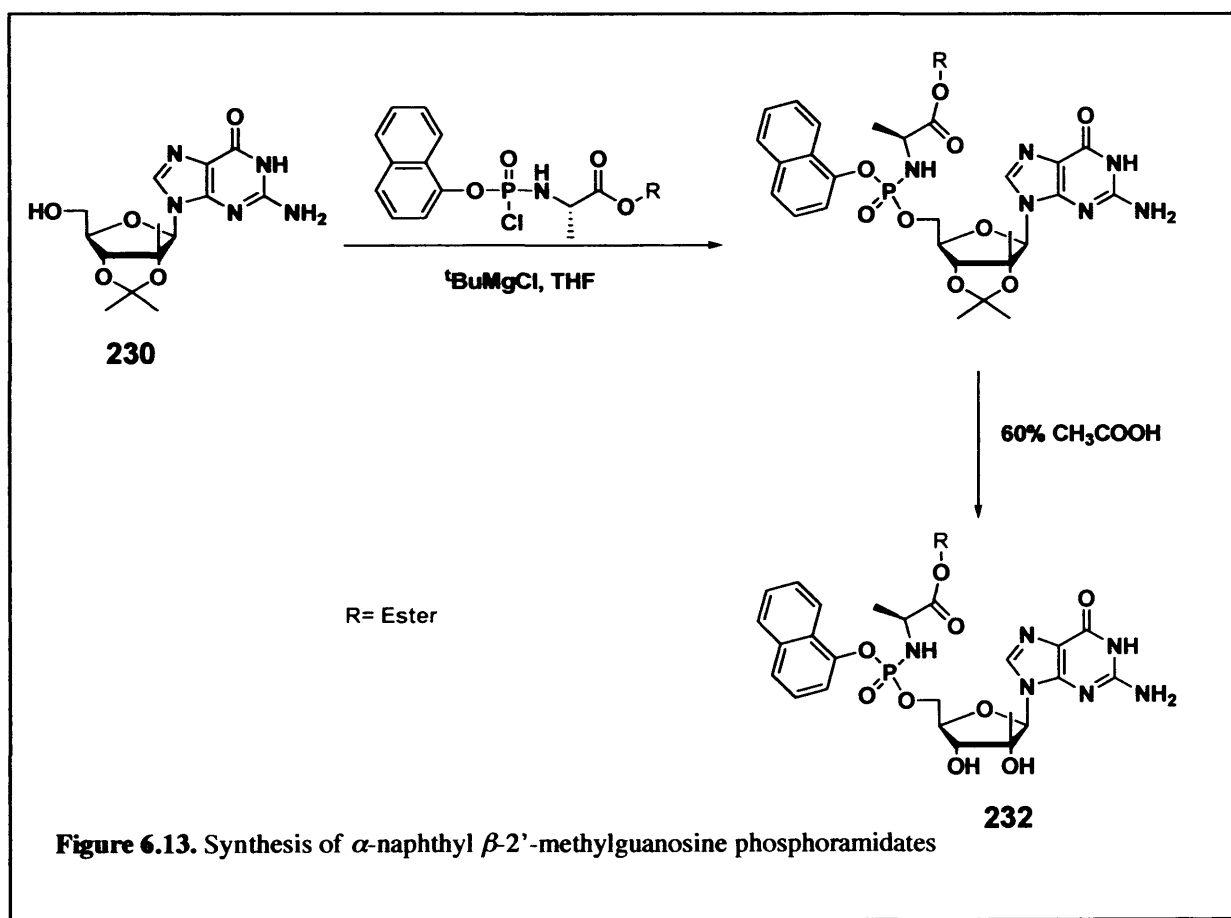
The deprotection was performed with 60% of acetic acid at 90° C overnight (**Figure 6.13**) and in most cases, the purification required one column

chromatography and one semi-preparative HPLC. The α-naphthyl phosphoramidates synthesised are reported in **Table 6.5**.
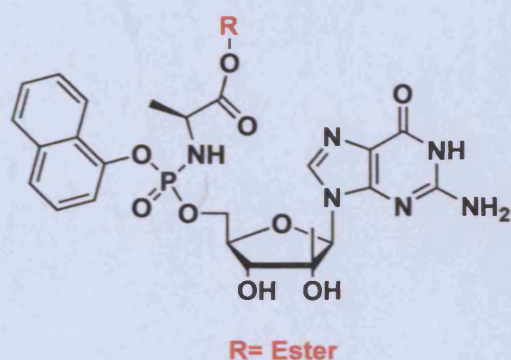
R= Ester

Cpd No.	Ester	^{31}P (ppm)
233	benzyl	4.25, 4.14
234	ethyl	4.25, 4.14
235	*tert*-butyl	4.23, 4.10
236	methyl	4.35, 4.26

Table 6.5. α-Naphthyl phosphoramidates synthesised with 2',3'-isopropylidene β-2'-methylguanosine

6.5.4 Biological activity of α-naphthyl phosphoramidates of β-2'-methylguanosine

All the α-naphthyl phosphoramidates synthesised have been tested *in vitro* using a replicon assay (see section **3.3.6.1**) against HCV (**Table 6.6**).[10, 11]
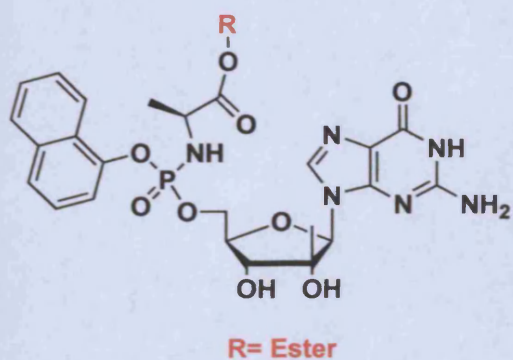
R= Ester

Cpd No.	Ester	EC$_{50}$ (μM)	CC$_{50}$ (μM)
233	benzyl	0.08	>100
234	ethyl	0.17	>100
235	*tert*-butyl	>50	>100
236	methyl	0.063	>100
226	β-2'-methylguanosine	4.0	>100

Table 6.6. Biological activity of β-2'-methylguanosine α-naphthyl phosphoramidates
EC$_{50}$= The concentration of phosphoramidate which produces the 50% of the maximum possible effect
CC$_{50}$= The concentration of phosphoramidate which produces the 50% of the maximum toxic effect

The most active phosphoramidates (**233** and **236**) showed a significant increase in activity (**Graph 6.2**).
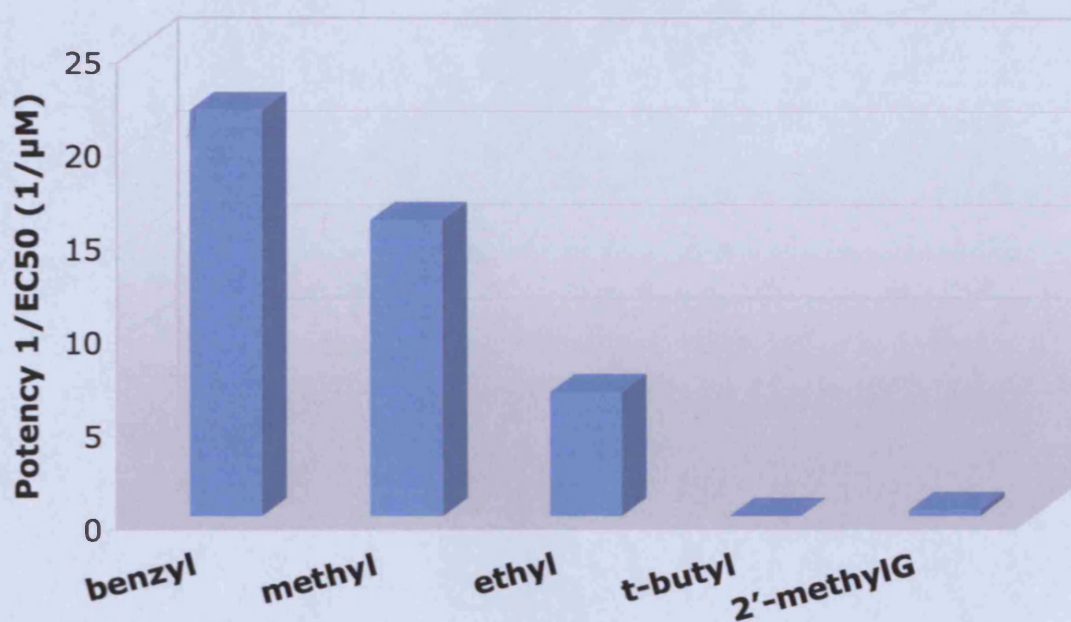
Graph 6.2. Biological activity of 2'-methylguanosine phosphoramidates synthesised

Considering the low intracellular level of nucleoside triphosphate observed (NTP/pmol per 10^6 cell= 0.2) and the boost of activity observed it is reasonable to suggest that the limiting step in the activation mechanism of β-2'-methylguanosine (**226**) is the first phosphorylation by the kinases. This problem has been overcome by synthesising the corresponding α-naphthyl phosphoramidates (**233, 234** and **236**).

The *tert*-butyl phosphoramidate (**235**) showed an activity comparable to its corresponding nucleoside (**226**) a direct consequence of poor activity of the enzyme ("esterase") responsible for the cleavage of this ester. The other esters are all roughly equivalent.

6.6 Phenyl β-2'-methylguanosine phosphoramidates

In 2006 Merck® published and patented a new series of β-2'-methylguanosine phenyl phosphoramidates. In order to compare the patented derivatives with our corresponding α-naphthyl compounds, a series of L-alanine phenyl phosphoramidates (methyl and benzyl) was planned.

6.6.1 Synthesis of phenyl β-2'-methylguanosine phosphoramidates

The phosphorylating agents (**205**) were resynthesised using the same procedure as previously described (**Chapter Three**, **Chapter Four** and **Chapter Five**). [6, 7, 8] The synthesis of phenyl phosphoramidates of β-2'-methylguanosine (**237**) was performed using the Uchiyama procedure in the presence of 2',3'-isopropylidene β-2'-methylguanosine (**230**) and an excess of tBuMgCl (**Figure 6.15**). [9] The purification method required one column chromatography.

Figure 6.13. Synthesis of phenyl β-2'-methylguanosine phosphoramidates

142
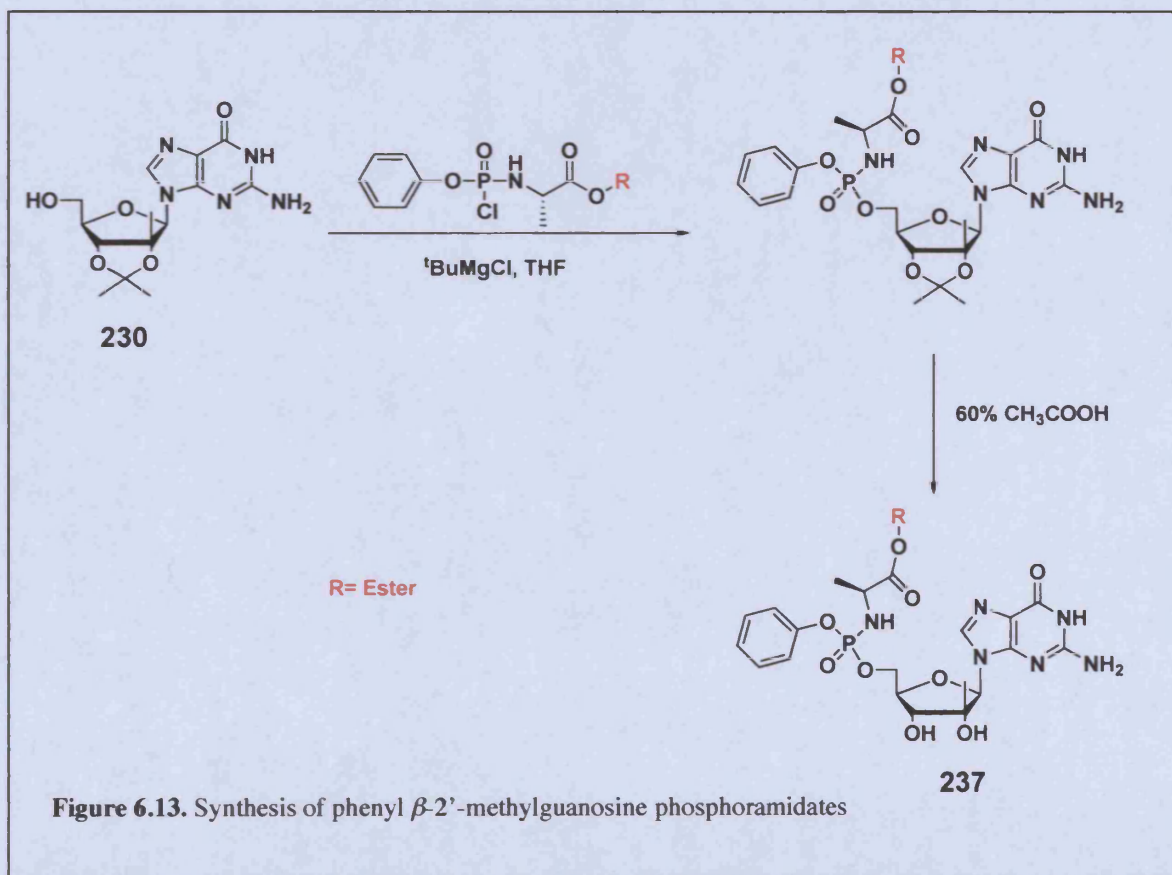
Also in this case, deprotection was carried out in presence of a solution 60% of acetic acid at 90° C overnight (**Figure 6.13**) and in most cases, the purification required one column chromatography and one semi-preparative HPLC. The phenyl phosphoramidates synthesised are reported in **Table 6.8**.
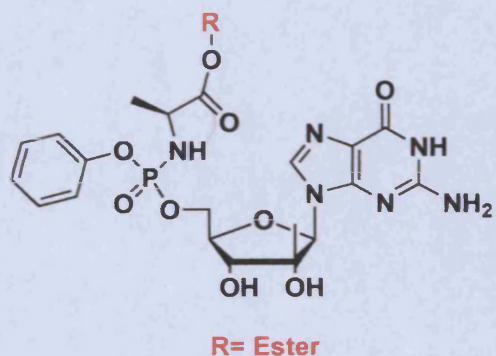
Cpd No.	Ester	^{31}P (ppm)
238	benzyl	4.23, 4.10
239	methyl	4.35, 4.26

R= Ester

Table 6.8. Phenyl phosphoramidates synthesised with 2',3'-isopropylidene β-2'-methylguanosine

6.6.2 Biological activity of phenyl phosphoramidates of β-2'-methylguanosine

All the phenyl phosphoramidates synthesised have been tested *in vitro* using a replicon assay (see section **3.3.6.1**) against HCV (**Table 6.9**).[10, 11]
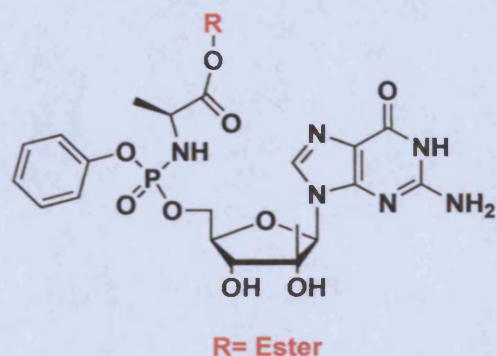
Cpd No.	Ester	EC_{50} (µM)	CC_{50} (µM)
238	Benzyl	36	>100
239	Methyl	0.88	>100
226	β-2'-methylguanosine	4.0	>100

R= Ester

Table 6.9. Biological activity of β-2'-methylguanosine phenyl phosphoramidates
EC_{50}= The concentration of phosphoramidate which produces the 50% of the maximum possible effect
CC_{50}= The concentration of phosphoramidate which produces the 50% of the maximum toxic effect

The obtained data were unclear; in fact the benzyl ester phosphoramidate (**238**) was shown to be poorly active and less active than the corresponding nucleoside (**226**), whilst, the corresponding methyl ester phosphoramidate (**239**) was found to be more active than the corresponding nucleoside (**226**) with a 4-fold increase of activity.

Although, compared to their corresponding α-naphthyl phosphoramidates (**233** and **236**), the two phenyl phosphoramidates synthesised were shown to be less active. The unclear behaviour observed for the benzyl and methyl ester cannot be easily explained. The phenyl phosphoramidate with the benzyl ester showed a poor activity that might be a consequence of the simultaneous presence of a phenyl group and a benzyl group that might drastically reduce the activity of one of the enzymes involved in the phosphoramidate metabolism. Another plausible explanation might be that compound **238** is actively removed from the cell because of its favourable interaction with Pgp (P-glycoprotein, present in the cell). In conclusion, we have increased the activity of the patented phenyl phosphoramidate (**239**) particularly in the benzyl series by synthesising the corresponding α-naphthyl phosphoramidates.

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Chapter Seven

Experimental Procedures

7.1 General Methods

Thin Layer Chromatography

Thin layer chromatography (TLC) was performed on commercially available Merck Kieselgel plates and separated components were visualised using ultraviolet light (245 and 366 nm).

Column Chromatography

Column chromatography was performed using Fluka silica (35-70 μm) as stationary phase. Glass columns were slurry packed in the appropriate eluent under gravity. Samples were applied as a concentrated solution in the same eluent, or pre-absorbed onto silica gel. Fractions containing the product were identified by TLC, pooled and the solvent removed *in vacuo*.

NMR Spectroscopy

^{1}H, ^{13}C, ^{31}P and ^{19}F were recorded on a Bruker Avance 500 spectrometer with operating frequencies of 500, 125, 202 and 470 MHz respectively. ^{31}P-NMR are reported in units of δ relative to 85% phosphoric acid as the external standard, positive shifts are downfield. The following abbreviations are used in the assignment of NMR signals: s (singlet), d (doublet), t (triplet), m (multiplet).

Mass Spectrometry

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

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Solvents and Reagents.

All solvents used were anhydrous and used as purchased from Aldrich. All reagents were used as received. All glassware was oven dried at 130 °C for several hours or overnight and allowed to cool under dry nitrogen.

7.2 Standard Procedures

For convenience, standard procedures have been given for reactions concerning the synthesis of precursors and derivatives of protides. Variations from these procedures and individual purification methods are given in the main text.

Standard procedure A1: *Preparation of amino acid ester hydrochloride salts*

Thionyl chloride (2.0 mol. equivalents) was added dropwise to a stirred solution of the appropriate alcohol (15.0 mol equivalent) at 0 °C under nitrogen. The mixture was stirred at 0°C for 30 minutes and then slowly allowed to warm to room temperature. The appropriate amino acid (1.0 mol. equivalent) was added and the mixture was heated at reflux overnight. The solvent was removed under reduced pressure (last traces of solvent removed by co-evaporation with increasingly volatile solvents) to give the crude product as the solid hydrochloride salt.

Standard procedure A2: *Preparation of L-amino acid ester sulfonate salts*

A mixture of the appropriate amino acid (1.0 mol. equivalent), the appropriate alcohol (15 mol. equivalent) and *para*-toluene sulfonic acid (*p*-TSA) monohydrate (1.1 mol equivalent) in toluene was heated at reflux overnight, using Dean-Stark apparatus. The solvent was removed under reduced pressure (last traces of solvent removed by co-evaporation with increasingly volatile solvents) to give the crude product as the solid *p*-toluene sulfonate salt.

Standard procedure B: Preparation of phosphorodichloridate species

Most of the phosphorochloridate species (exept for the D-alanine dodecyl ester and D-alanine octyl ester phosphorchloridates) were synthesised using the previously reported standard procedure.[1] Phenylphosphorodichloridate (1.0 mol. equivalent) and the appropriate amino ester (1.0 mol. equivalent) were suspended in anhydrous dichloromethane (DCM, 123 mol. equivalent). Anhydrous triethylamine (2.0 mol. equivalent) was added dropwise at -78°C and after 1 hour, the reaction was left to rise to room temperature and stirred overnight. The solvent was removed under reduced pressure and the crude residue was purified by rapid column chromatography (using

7:3 ethyl acetate/hexane as eluent). All phosphorochloridates were used as solutions in dry THF for subsequent reactions.

Standard procedure B1: *Preparation of Phosphorodichloridate species*

Phosphorus oxychloride (1.0 mol equivalent) and the appropriate substituted phenol (1.0 mol equivalent) were stirred in anhydrous diethyl ether (31 mol. equivalent). Anhydrous triethylamine was added (1.0 mol. equivalent) at -78°C and the solution was allowed to warm to room temperature after 25 minutes. The triethylamine hydrochloride salt was filtered off and the solvent removed under reduced pressure to give a clear liquid.

Standard procedure C: *Preparation of 4'-azidonucleoside phosphoramidates*

'BuMgCl (2.5 mol. equivalent) and 4'-azido-nucleoside (1.0 mol. equivalent) were dissolved in dry THF (31 mol. equivalent) and stirred for 15 minutes. Then a 1M solution of the appropriate phosphorochloridate (2.5 mol. equivalent) in dry THF was added dropwise, after 20 minutes, and then stirred overnight. A saturated solution of NH_4Cl was added and the solvent was removed under reduced pressure to give a yellow solid, which was subsequently purified.

Standard procedure C1: *Preparation of 4'-azido-2', 3'-dipentanoatecytidine phosphoramidates*

'BuMgCl (2.5 mol. equivalent) and 2',3'-dipentanoate-4'-azidocytidine (1.0 mol. equivalent) were dissolved in dry THF (31 mol. equivalent) and stirred for 15 minutes. Then a 1M solution of the appropriate phosphorochloridate (2.5 mol. equivalent) in dry THF was added dropwise, then stirred overnight. A saturated solution of NH_4Cl was added and the solvent was removed under reduced pressure to give a yellow solid, which was subsequently purified.

Standard procedure C2: *Preparation of 2', 3'-protected modified nucleoside phosphoramidates*

'BuMgCl (2.0 mol. equivalent) and 2',3'-protected modified nucleosides (1.0 mol. equivalent) were dissolved in dry THF (31 mol. equivalent) and stirred for 15 minutes. Then a 1M solution of the appropriate phosphorochloridate (2.0 mol.

equivalent) in dry THF was added dropwise, then stirred overnight. A saturated solution of NH_4Cl was added and the solvent was removed under reduced pressure to give a yellow solid, which was subsequently purified.

Standard procedure C3: *Preparation of phosphoramidates of modified nucleoside*

2', 3'-Cyclopentylidene modified nucleoside phosphoramidates were dissolved in a solution of 80% formic acid in water for 4 hours. The solvent was removed under reduced pressure to give a a white solid that was subsequently purified.

Standard procedure C4: *Preparation of phosphoramidates of modified nucleoside*

2', 3'-Isopropylidene modified nucleoside phosphoramidates were dissolved in a solution of 60% of acetic acid in water at 90 °C overnight. The solvent was removed under reduced pressure to give a white solid that was subsequently purified.

Standard procedure D: *Preparation of 4'-azidouridine phosphoramidates*

'BuMgCl (2.0 mol. equivalent) and 4'-azidouridine (1.0 mol. equivalent) were dissolved in dry THF (30 mol. equivalent) and stirred for 15 minutes. Then a 1M solution of the appropriate phosphorochloridate (2.0 mol. equivalent) in dry THF was added dropwise, over 20 minutes, and the mixture stirred overnight. A solution of NH_4Cl was added and the solvent was removed under reduced pressure to give a yellow solid, which was subsequently purified.

7.3 Synthesis of amino acid esters.

Synthesis of cyclopentylglycine benzyl ester *p*-toluene sulfonate salt (41)[2]
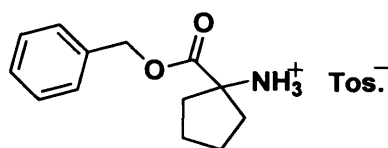
Prepared according to Standard procedure A2, from cyclopentylglycine (5.0 g, 39.0 mmol), *p*-TSA-monohydrate (8.159 g, 42.9 mmol), benzyl alcohol (20.4 mL, 194 mmol) and toluene (50 mL). The product was isolated as a white solid (9.15 g, 60%)

δ_H (d_4-CH$_3$OH): 8.56 (3H, s, NH$_3^+$-amino acid ester) (7.72 (2H, d, 2 CH-tosylate, $J=$ 9.0 Hz), 7.4 (2H, m, CH-phenyl), 7.35-7.30 (3H, m, CH-phenyl), 7.25 (2H, d, CH-tosylate, $J=$ 9.0 Hz), 5.28 (2H, s, CH$_2$-benzyl), 2.37 (3H, s, CH$_3$-tosylate), 2.33 (2H, m, CH$_2$-cyclopentyl), 1.93 (2H, m, CH$_2$-cyclopentyl), 1.89 (4H, m, CH$_2$-cyclopentyl).

Synthesis of cyclopentylglycine ethyl ester hydrochloride salt (42)[8]
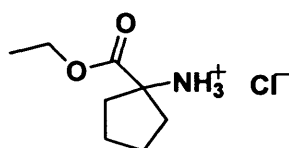
Prepared according to Standard Procedure A1, from cyclopentylglycine (5.0 g, 38.7 mmol), thionyl chloride (6.0 mL, 77.4 mmol) and ethanol (34.10 mL). The product was obtained as a white solid (2.25 g, 56%).

δ_H (d_6-DMSO): 8.82 (3H, s, NH$_3^+$-amino acid ester), 4.19 (2H, m, CH$_2$-ethyl, $J=$ 7.1 Hz), 2.08 (2H, m, CH$_2$-cyclopentyl), 1.96 (2H, m, CH$_2$-cyclopentyl), 1.89 (2H, m, CH$_2$-cyclopentyl), 1.72 (2H, m, CH$_2$-cyclopentyl), 1.23 (3H, t, CH$_3$-ethyl, $J=$ 7.1 Hz).

Synthesis of cyclopentylglycine isopropyl ester hydrochloride salt (43)[8]
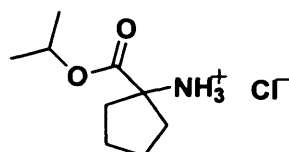
Prepared according to Standard Procedure A1, from cyclopentylglycine (2.5 g, 19.4 mmol), thionyl chloride (2.83 mL, 38.8 mmol) and isopropanol (50 mL). The product was obtained as a white solid (2.25 g, 56%).

δ_H (d_6-DMSO): 8.75 (3H, s, NH$_3^+$-amino acid ester), 4.97 (1H, m, CH-isopropyl, $J=$ 6.3 Hz), 2.51 (2H, m, CH$_2$-cyclopentyl), 2.09 (4H, m, CH$_2$-cyclopentyl), 1.79 (2H, m, CH$_2$-cyclopentyl), 1.25 (6H, d, CH$_3$ isopropyl, $J=$ 6.3 Hz).

Synthesis of L-isoleucine ethyl ester hydrochloride salt (44)
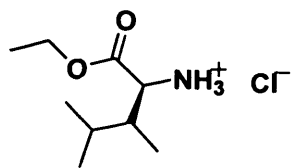
Prepared according to Standard Procedure A1, from L-isoleucine (5.0 g, 38.1 mmol), thionyl chloride (8.3 mL, 11.44 mmol) and ethanol (33.54 mL). The product was obtained as a clear oil (2.5 g, 39%).

δ_H (CDCl$_3$): 8.71 (3H, s, NH$_3^+$-amino acid ester), 4.21 (2H, m, CH$_2$-ethyl), 3.97 (1H, m, CHα), 2.15 (1H, m, CH-lateral chain), 1.48 (2H, m, CH$_2$-lateral chain) 1.25 (3H, s, CH$_3$-ethyl), 1.04 (3H, s, CH$_3$-lateral chain), 0.90 (3H, s, CH$_3$-lateral chain).

Synthesis of D-alanine -butyl ester *p*-toluene sulfonate salt (45)[3]
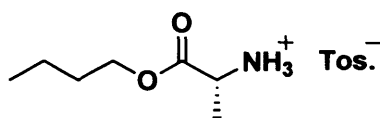
Prepared according to Standard procedure A2, from D-alanine (5.0 g, 56.1 mmol), *p*-TSA-monohydrate (11.74 g, 61.7 mmol), butanol (26 mL) and toluene (50 mL). The product was isolated as a white solid (11.0 g, 62%)

δ_H (d_4-CH$_3$OH): 8.18 (3H, s, NH$_3^+$-amino acid ester), 7.78 (2H, d, CH-tosylate, $J=$ 8.0 Hz), 7.16 (2H, d, CH-tosylate, $J=$ 8.0 Hz), 4.11-4.00 (3H, m, CHα, CH$_2$-butyl), 2.38 (3H, s, CH$_3$-tosylate), 1.56-1.51 (2H, m, CH$_2$-butyl), 1.47 (3H, d, CH$_3$-alanine, $J=$ 7.2 Hz), 1.39-1.25 (2H, m, CH$_2$-butyl), 0.89 (3H, m, CH$_3$-butyl).

Synthesis of D-alanine dodecyl ester *p*-toluene sulfonate salt (46)[4]
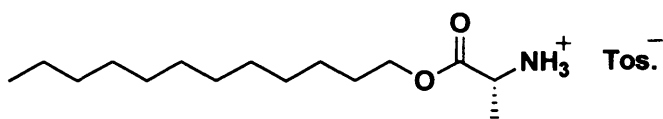
Prepared according to Standard procedure A2, from D-alanine (2.5 g, 28.0 mmol), *p*-TSA-monohydrate (5.87 g, 30.9 mmol), dodecyl alcohol (13.0 g, 280 mmol) and toluene (50 mL). The product was isolated as a white solid (7.92 g, 66%)

δ_H (d_4-CH$_3$OH): 8.45 (3H, s, NH$_3^+$-amino acid ester), 7.82 (2H, d, CH-tosylate, $J=$ 8.1 Hz), 7.19 (2H, d, CH-tosylate, $J=$ 8.1 Hz), 4.16-4.00 (3H, m, CHα, CH$_2$-dodecyl), 2.40 (3H, s, CH$_3$-tosylate), 1.49 (3H, d, CH$_3$-alanine, $J=$ 7.2 Hz), 1.30 (22H, m, CH$_2$-dodecyl), 0.94 (3H, t, CH$_3$-dodecyl, $J=$ 6.3 Hz).

Synthesis of L-alanine -butyl ester *p*-toluene sulfonate salt (48)[3]
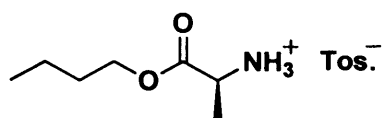
Prepared according to Standard procedure A2, from L-alanine (2.5 g, 28 mmol), *p*-TSA-monohydrate (5.86 g, 30.8 mmol), butanol (14 mL) and toluene (50 mL). The product was isolated as a white solid (5.70 g, 65%)

δ_H (d_4-CH$_3$OH): 8.42 (3H, s, NH$_3^+$-amino acid ester), 7.59 (2H, d, CH-tosylate, $J=$ 8.0 Hz), 7.18 (2H, d, CH-tosylate, $J=$ 8.0 Hz), 4.21-4.09 (3H, m, CHα, CH$_2$-butyl), 2.33 (3H, s, CH$_3$-tosylate), 1.65-1.56 (2H, m, CH$_2$-butyl), 1.44 (3H, d, CH$_3$-alanine, $J=$ 7.2 Hz), 1.42-1.28 (2H, m, CH$_2$-butyl), 0.92 (3H, m, CH$_3$-butyl).

Synthesis of D-alanine isopropyl ester hydrochloride salt (104)[6]
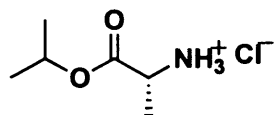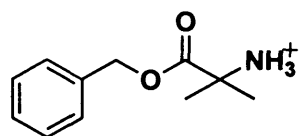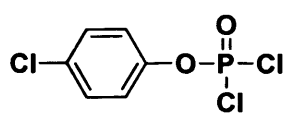
Prepared according to Standard Procedure A1, from D-alanine (7.0 g, 78.6 mmol), thionyl chloride (11.42 mL, 157.2 mmol) and isopropanol (90 mL). The product was obtained as a white solid (8.30 g, 64%).

δ_H (d_6-DMSO): 8.70 (3H, s, NH$_3^+$-amino acid ester), 4.87 (1H, m, CH-isopropyl, $J=$ 6.3 Hz), 3.95 (1H, d, CHα, $J=$ 6.7 Hz), 1.54 (3H, m, CH$_3$-alanine), 1.25 (6H, d, CH$_3$-isopropyl, $J=$ 6.3 Hz).

Synthesis of D-alanine 2-butyl ester *p*-toluene sulfonate salt (105)
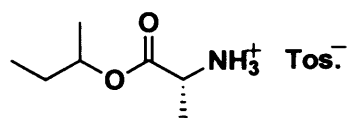
Prepared according to Standard procedure A2, from D-alanine (5.0 g, 56.1 mmol), *p*-TSA-monohydrate (11.74 g, 61.7 mmol), 2-butanol (26 mL, 280 mmol) and toluene (50 mL). The product was isolated as a white solid (12.44 g, 70%)

δ_H (d_4-CH$_3$OH): 8.49 (3H, s, NH$_3^+$-amino acid ester), 7.85 (2H, d, CH-tosylate, $J=$ 8.0 Hz), 7.15 (2H, d, CH-tosylate, $J=$ 8.0 Hz), 4.20-4.00 (2H, m, CHα, CH-2-butyl), 2.42 (3H, s, CH$_3$-tosylate), 1.49 (3H, d, CH$_3$-alanine, $J=$ 9.0 Hz), 1.24-1.20 (5H, m, CH$_2$-2-butyl, CH$_3$-2-butyl), 0.94 (3H, m, CH$_3$-2-butyl).

Synthesis of D-alanine -octyl ester *p*-toluene sulfonate salt (106)

Prepared according to Standard procedure A2, from D-alanine (2.5 g, 56.1 mmol), *p*-TSA-monohydrate (5.86 g, 30.8 mmol), butanol (24 mL, 154 mmol) and toluene (50 mL). The product was isolated as a white solid (7.90 g, 70%).

δ_H (d_4-CH$_3$OH): 8.40 (3H, s, NH$_3^+$-amino acid ester), 7.58 (2H, d, CH-tosylate, $J=$ 8.0 Hz), 7.16 (2H, d, CH-tosylate, $J=$ 8.0 Hz), 4.20-4.09 (3H, m, CHα, CH$_2$-octyl), 2.33 (3H, s, CH$_3$-tosylate), 1.65-1.58 (2H, m, CH$_2$-octyl), 1.44 (3H, d, CH$_3$-alanine, $J=$ 7.16 Hz), 1.29 (12H, m, CH$_2$-octyl), 0.90 (3H, m, CH$_3$-octyl).

Synthesis of L-alanine isopropyl ester hydrochloride salt (127)[6]

Prepared according to Standard Procedure A1, from L-alanine (5.0 g, 56.0 mmol), thionyl chloride (8.2 mL, 112.0 mmol) and isopropanol (65 mL,). The product was obtained as a white solid (8.0 g, 85%).

δ_H (d_6-DMSO): 8.73 (3H, s, NH$_3^+$-amino acid ester), 4.96 (1H, m, CH-isopropyl, $J=$ 5.0 Hz), 3.92 (1H, d, CHα, $J=$ 6.9 Hz), 1.40 (3H, d, CH$_3$-alanine, $J=$ 6.9 Hz), 1.20 (6H, d, CH$_3$-isopropyl, $J=$ 4.5 Hz).

Synthesis of α,α-dimethylglycine ethyl ester hydrochloride salt (123)[2]

Prepared according to Standard Procedure A1, from α,α-dimethylglycine (5.0 g, 48.0 mmol), thionyl chloride (7.0 mL, 96.5 mmol) and ethanol (42 mL). The product was obtained as a white solid (5.3 g, 73%).

δ_H (d_6-DMSO): 8.85 (3H, s, NH$_3^+$-amino acid ester), 4.18 (2H, d, CH$_2$-ethyl, $J=$ 7.1 Hz), 1.49 (6H, s, CH$_3$-α,α-dimethylglycine), 1.22 (3H, t, CH$_3$-ethyl, $J=$ 7.1 Hz).

Synthesis of α,α-dimethylglycine benzyl ester p-toluene sulfonate salt (122)[2]

Prepared according to Standard procedure A2, from α,α-dimethylglycine (5.0 g, 48.0 mmol), p-TSA-monohydrate (10 g, 53.1 mmol), benzyl alcohol (74 mL, 720 mmol) and toluene (60 mL). The product was isolated as a white solid (7.60 g, 42%).

δ_H (d_6-DMSO): 8.47 (3H, s, NH_3^+-amino acid ester), 7.51 (2H, d, CH-tosylate, $J=$ 7.7 Hz), 7.41-7.32 (5H, m, CH-benzyl), 7.13 (2H, d, CH- tosylate, $J=$ 7.7 Hz), 5.25 (2H, s, CH_2-benzyl), 2.30 (3H, s, CH_3-p-toluene sulfonate), 1.49 (6H, s, CH_3-α,α-dimethylglycine).

Synthesis of L-proline ethyl ester hydrochloride salt (124)[2]
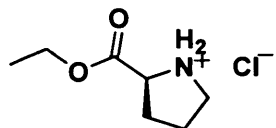
Prepared according to Standard Procedure A1, from L-proline (5.0 g, 43.0 mmol), thionyl chloride (6.5 mL, 86.0 mmol) and ethanol (38 mL). The product was obtained as yellow liquid (6.0 g, 77%).

δ_H (d_6-DMSO): 9.29 (2H, s, NH_2^+-amino acid ester), 4.31 (1H, t, $CH\alpha$, $J=$ 8.1 Hz), 4.20 (2H, q, CH_2-ethyl, $J=$ 7.0 Hz), 3.24-3.16 (1H, m, $CH\alpha$), 2.26-2.21 (2H, m, CH_2-proline), 1.99-1.93 (2H, m, CH_2-proline), 1.90 (2H, t, CH_2-proline, $J=$ 6.8 Hz), 1.23 (3H, t, CH_3-ethyl, $J=$ 7.0 Hz).

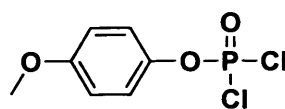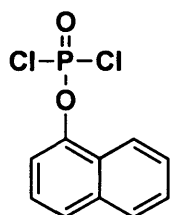## 7.4 Synthesis of aryl-dichlorophosphates.

Synthesis of *p*-chlorophenyl dichlorophosphate (78)[2]

Prepared according to Standard Procedure E, from *p*-chlorophenol (3.0 g, 23.0 mmol), phosphorus oxychloride (2.17 mL, 23.0 mmol) and triethylamine (3.25 mL, 23.0 mmol), dry diethylether (25 mL). The product was obtained as a yellow clear oil (4.15 g, 40%) used without further purification for the following reaction.

δ_P (CDCl$_3$): 5.00; δ_H (CDCl$_3$): 7.44 (2H, m, CH-phenyl), 7.30 (2H, m, CH-phenyl).

Synthesis of 3,4-dichlorophenyl dichlorophosphate (79)
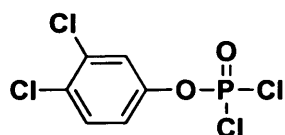
Prepared according to Standard Procedure E, from 3,4-dichlorophenol (3.79 g, 23.0 mmol), phosphorus oxychloride (2.17 mL, 23.0 mmol), triethylamine (3.25 mL, 23.0 mmol) and dry diethylether (25 mL). The product was obtained as a yellow clear oil (2.93 g, 45%) used without further purification for the following reaction.

δ_P (CDCl$_3$): 4.79; δ_H (CDCl$_3$): 7.41 (1H, m, CH-phenyl), 7.38 (1H, m, CH-phenyl), 7.33 (1H, m, CH-phenyl).

Synthesis of *p*-methylphenyl dichlorophosphate (80)
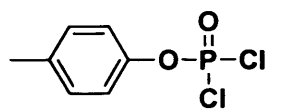
Prepared according to Standard Procedure E, from *p*-cresol (4.00 g, 37.0 mmol), phosphorus oxychloride (3.45 mL, 37.0 mmol), triethylamine (5.15 mL, 37.0 mmol) and dry diethylether (25 mL). The product was obtained as a yellow clear oil (7.91 g, 72%) used without further purification for the following reaction.

δ_p (CHCl$_3$): 4.98; δ_H (CHCl$_3$): 7.22 (4H, m, CH-phenyl), 2.39 (3H, s, CH$_3$-*p*-cresol).

Synthesis of *p*-methoxyphenyl dichlorophosphate (81)[5]

Prepared according to Standard Procedure E, from *p*-methoxyphenol (4.00 g, 32.0 mmol), phosphorus oxychloride (3.00 mL, 32.0 mmol), triethylamine (4.49 mL, 32.0 mmol) and dry diethylether (25 mL). The product was obtained as a yellow clear oil (5.16 g, 67%) used without further purification for the following reaction.

δ_p (CHCl$_3$): 5.45; δ_H (CHCl$_3$): 7.10 (2H, d, CH-phenyl, *J*= 9.1 Hz), 6.80 (2H, d, CH-phenyl, *J*= 9.1 Hz), 3.69 (3H, s, CH$_3$O-*p*-methoxyphenol).
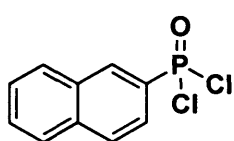
Synthesis of α-naphthyl dichloro phosphate (91)[6]

Prepared according to Standard Procedure E, from α-naphthol (5.00 g, 34.7 mmol), phosphorus oxychloride (3.23 mL, 34.7 mmol), triethylamine (4.84 mL, 34.7 mmol) and dry diethylether (25 mL). The product was obtained as a yellow clear oil (8.15 g, 90%) used without further purification for the following reaction.

δ_p (CHCl$_3$): 3.91; δ_H (CHCl$_3$): 7.92 (1H, d, CH-naphthyl, *J*= 8.4 Hz), 7.68 (1H, s, CH-naphthyl), 7.58 (1H, d, CH-naphthyl, *J*= 8.4 Hz), 7.40-7.35 (3H, m, CH-naphthyl), 7.23 (1H, t, CH-naphthyl, *J*= 7.9 Hz).

Synthesis of β-naphthyl dichloro phosphate (92)[6]

Prepared according to Standard Procedure E, from β-naphthol (5.00 g, 34.7 mmol), phosphorus oxychloride (3.23 mL, 34.7 mol) and triethylamine (4.84 mL, 34.7 mol) and dry diethylether (25 mL). The product was obtained as a yellow clear oil (7.85 g, 87%) used without further purification for the following reaction.

δ_p (CHCl$_3$): 3.60; δ_H (CHCl$_3$): 7.73-7.66 (3H, m, CH-naphthyl), 7.61 (1H, m, CH-naphthyl), 7.40-7.30 (2H, m, CH-naphthyl), 7.25-7.23 (1H, m, CH-naphthyl).

Synthesis of 8-quinolinyl dichloro phosphate (180)
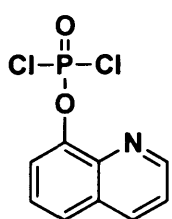
Prepared according to Standard Procedure E, from 8-quinoline (4.00 g, 27.5 mmol), phosphorus oxychloride (2.57 mL, 27.5 mmol) and triethylamine (3.83 mL, 27.5 mmol) and dry diethylether (35 mL). The product was obtained as a yellow clear oil (5.38 g, 75%) used without further purification for the following reaction.

δ_p (CHCl$_3$): 4.65; δ_H (CHCl$_3$): 8.91 (1H, m, CH-quinolinyl), 8.00 (1H, d, CH-quinolinyl, *J*= 8.3 Hz), 7.67 (1H, m, CH-quinolinyl), 7.61 (1H, m, CH-quinolinyl), 7.45-7.39 (2H, m, CH-quinolinyl).

7.5 Synthesis of aryl-phosphochloridates.

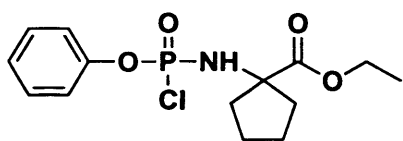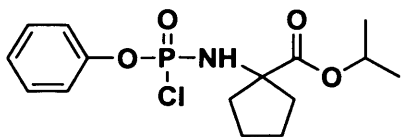### Synthesis of phenyl(benzyl-cyclopentylglycinyl) phosphorochloridate (50)[2]
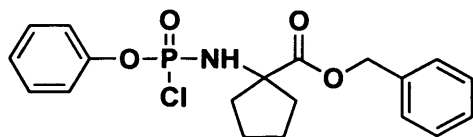
Prepared according to Standard Procedure B, from phenyl dichlorophosphate (1.56 mL, 7.67 mmol), cyclopentylglycine benzyl ester tosylate salt (41, 3.0 g, 7.67 mmol), dry triethylamine (2.13 mL, 15.34 mmol) and dry DCM (15 mL). The product was obtained as a clear yellow oil (2.57 g, 85%).

δ_P (CDCl$_3$): 7.90; δ_H (CDCl$_3$): 7.96 (2H, d, CH-phenyl, J= 8.4 Hz), 7.44 (2H, m, CH-benzyl), 7.39 (4H, m, CH-phenyl, CH-benzyl), 7.27 (2H, d, CH-benzyl, J= 8.4 Hz), 5.24 (2H, s, CH$_2$-benzyl), 4.65 (1H, s, NH), 2.43 (4H, m, CH$_2$-cyclopentyl), 1.98 (4H, m, CH$_2$-cyclopentyl).

Synthesis of phenyl(ethyl-cyclopentylglycinyl) phosphorochloridate (51)[2]

Prepared according to Standard Procedure B, from phenyl dichlorophosphate (2.64 mL, 12.9 mmol), cyclopentylglycine ethyl ester hydrochloride salt (42, 2.5 g, 12.9 mmol), dry triethylamine (3.60 mL, 25.8 mmol) and dry DCM (15 mL). The product was obtained as a clear white solid (3.37 g, 79%).

δ_P (CDCl$_3$): 8.09; δ_H (CDCl$_3$): 7.47 (1H, m, CH-phenyl), 7.34 (2H, m, CH-phenyl), 7.30 (2H, m, CH-phenyl), 4.74 (1H, s, NH), 4.27 (2H, m, CH$_2$-ethyl, J= 6.7 Hz), 2.30 (4H, m, CH$_2$-cyclopentyl), 1.92 (2H, m, CH$_2$-cyclopentyl), 1.87 (2H, m, CH$_2$-cyclopentyl), 1.35 (3H, t, CH$_3$-ethyl, J= 6.7 Hz).

Synthesis of phenyl(isopropyl-cyclopentylglycinyl) phosphorochloridate (52)[2]

Prepared according to Standard Procedure B, from phenyl dichlorophosphate (1.08 mL, 7.2 mmol), cyclopentylglycine isopropyl ester hydrochloride salt (43, 1.5 g, 7.2 mmol), dry triethylamine (2.00 mL, 14.4 mmol) and dry DCM 25 mL. The product was obtained as a clear yellow oil (2.11 g, 85 %).

δ_P (CDCl$_3$): 8.16; δ_H (CDCl$_3$): 7.45 (2H, m, CH-phenyl), 7.30 (1H, m, CH-phenyl), 6.85 (2H, m, CH-phenyl), 5.09 (1H, m, NH), 5.04 (1H, m, CH-isopropyl), 2.19 (2H,

m, CH$_2$-cyclopentyl), 2.18 (2H, m, CH$_2$-cyclopentyl), 1.89 (2H, m, CH$_2$-cyclopentyl), 1.81 (2H, m, CH$_2$-cyclopentyl), 1.27 (6H, m, CH$_3$-isopropyl).

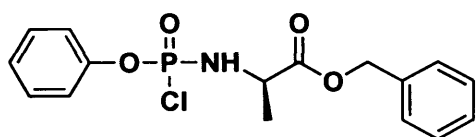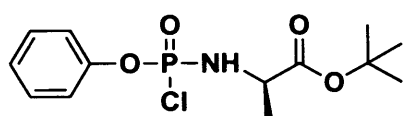## Synthesis of phenyl(ethyl-L-isoleucinyl) phosphorochloridate (53)
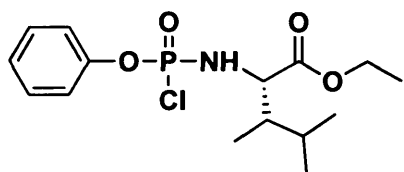
Prepared according to Standard Procedure B, from phenyl dichlorophosphate (1.00 mL, 6.76 mmol), L-isoleucine ethyl ester hydrochloride salt (44, 1.4 g, 6.76 mmol), dry triethylamine (1.88 mL, 13.52 mmol) and dry DCM (15 mL). The product was obtained as a clear white solid (1.6 g, 71%).

δ_p (CDCl$_3$): 10.64, 10.01; δ_H (CDCl$_3$): 7.26 (1H, m, CH-phenyl), 7.19 (2H, m, CH-phenyl), 7.16 (2H, m, CH-phenyl), 7.28 (1H, m, CH-phenyl), 4.76 (1H, m, NH), 4.15 (2H, m, CH$_2$-ethyl, J= 7.08 Hz), 3.89 (1H, m, CHα), 1.82 (1H, m, CH-lateral chain), 1.43 (2H, m, CH$_2$-lateral chain), 1.16 (3H, m, CH$_3$-ethyl), 0.87-0.86 (6H, CH$_3$-lateral chain).

Synthesis of phenyl(benzyl-D-alaninyl) phosphorochloridate (54)[2]

Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.64 mL, 4.27 mmol), D-alanine benzyl ester tosylate salt (0.9 g, 4.27 mmol), dry triethylamine (1.19 mL, 8.54 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (1.15 g, 76%).

δ_p (CDCl$_3$): 9.29, 9.05; δ_H (CDCl$_3$): 7.41 (6H, m, CH-phenyl, CH-benzyl), 7.32-7.30 (4H, m, CH-benzyl), 5.26 (2H, d, CH$_2$-benzyl), 4.66 (1H, m, NH), 4.34 (1H, m, CHα), 1.57 (3H, m, CH$_3$-alanine).

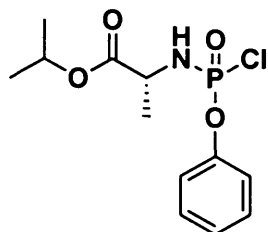## Synthesis of phenyl(tert-butyl-D-alaninyl) phosphorochloridate (55)

Prepared according to Standard Procedure B, from phenyl dichlorophosphate (1.64 mL, 11.00 mmol), D-alanine tert-butyl ester hydrochloride salt (2.0 g, 11.00 mmol), dry triethylamine (1.61 mL, 22.00 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (1.43 g, 41%).

δ_P (CDCl$_3$): 9.48, 9.30; δ_H (CDCl$_3$): 7.37-7.12 (5H, m, CH-phenyl), 4.84 (1H, m, NH), 4.80 (1H, m, CHα), 1.93 (9H, s, CH$_3$-*tert*-butyl), 1.39 (3H, d, CH$_3$-alanine, *J*= 4.2 Hz).
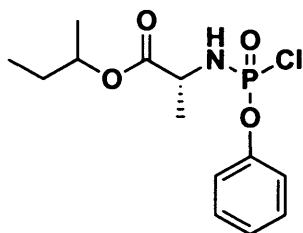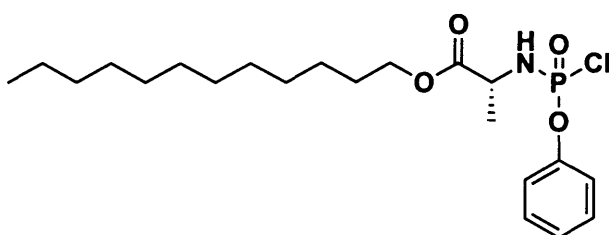
Synthesis of phenyl(isopropyl-D-alaninyl) phosphorochloridate (107)[6]

Prepared according to Standard Procedure B, from phenyl dichlorophosphate (1.79 mL, 12.0 mmol), D-alanine isopropyl ester hydrochloride salt (104, 2.0 g, 12.0 mmol), dry triethylamine (3.34 mL, 24.0 mmol) and dry DCM 25 mL. The product was obtained as a clear yellow oil (0.77 g, 21%).

δ_P (CDCl$_3$): 9.41, 9.09; δ_H (CDCl$_3$): 7.43 (2H, m, CH-phenyl), 7.25 (1H, m, CH-phenyl), 6.80 (2H, m, CH-phenyl), 5.00 (1H, m, NH), 4.97 (1H, m, CH-isopropyl, CH-α), 1.48 (3H, m, CH$_3$-alanine), 1.27 (6H, m, CH$_3$-isopropyl).

Synthesis of phenyl(2-butyl-D-alaninyl) phosphorochloridate (108)

Prepared according to Standard Procedure B, from phenyl dichlorophosphate (1.40 mL, 9.45 mmol), D-alanine 2-butyl ester tosylate salt (105, 3.0 g, 9.45 mmol), dry triethylamine (2.63 mL, 18.90 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (2.70 g, 72%).

δ_P (CDCl$_3$): 9.51, 9.32; δ_H (CDCl$_3$): 7.37-7.12 (5H, m, CH-phenyl), 4.96-4.76 (1H, m, NH, CHα), 4.05 (1H, m, CH-2-butyl), 1.41 (3H, d, CH$_3$-alanine), 1.24-1.19 (5H, m, CH$_2$-2-butyl, CH$_3$-alanine), 0.92 (3H, m, CH$_3$-2-butyl).

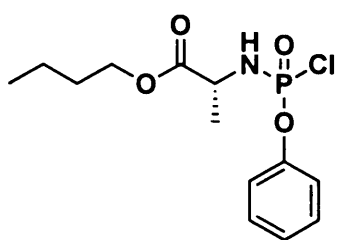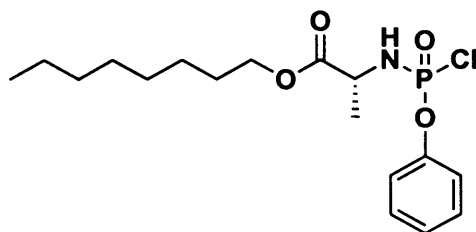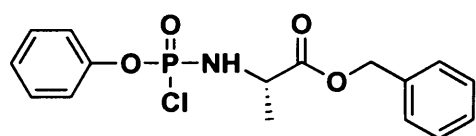## Synthesis of phenyl(dodecilyl-D-alaninyl) phosphorochloridate (57)

Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.52 mL, 3.49 mmol), D-alanine dodecyl ester tosylate salt (46, 1.5 g, 3.49 mmol), dry triethylamine (0.96 mL, 6.88 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (1.00 g, 67%).

δ_P (CDCl$_3$): 9.38, 9.15; δ_H (CDCl$_3$): 7.41-7.19 (5H, m, CH-phenyl), 4.78 (1H, m, NH), 4.25-4.00 (3H, m, CHα, CH$_2$-dodecyl), 1.54 (3H, m, CH$_3$-alanine), 1.28 (22H, m, CH$_2$-dodecyl), 0.91 (3H, t, CH$_3$-dodecyl, J= 6.3 Hz).

Synthesis of phenyl(butyl-D-alaninyl) phosphorochloridate (56)

Prepared according to Standard Procedure B, from phenyl dichlorophosphate (1.90 mL, 12.7 mmol), D-alanine butyl ester tosylate salt (45, 4.0 g, 12.7 mmol), dry triethylamine (3.50 mL, 25.40 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (2.37 g, 58%).

δ_P (CDCl$_3$): 9.45, 9.29; δ_H (CDCl$_3$): 7.35-7.15 (5H, m, CH-phenyl), 4.95-4.72 (1H, m, NH, CHα), 4.15 (2H, m, CH$_2$-butyl), 1.54-1.50 (2H, m, CH$_2$-butyl), 1.45 (3H, d, CH$_3$-alanine), 1.42-1.20 (2H, m, CH$_2$-butyl), 1.05-0.89 (3H, m, CH$_3$-butyl).

Synthesis of phenyl(octyl-D-alaninyl) phosphorochloridate (109)

Prepared according to Standard Procedure B, from phenyl dichlorophosphate (1.01 mL, 6.76 mmol), D-alanine octyl ester tosylate salt (106, 4.0 g, 12.7 mmol), dry triethylamine (1.86 mL, 13.52 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (2.54 g, 60%).

δ_P (CDCl$_3$): 9.43, 9.27; δ_H (CDCl$_3$): 7.38-7.17 (5H, m, CH-phenyl), 4.92-4.65 (1H, m, NH, CHα), 4.10 (2H, m, CH$_2$-octyl), 1.54-1.45 (2H, m, CH$_2$-octyl), 1.43 (3H, d, CH$_3$-alanine), 1.39-1.15 (10H, m, CH$_2$-octyl), 1.05-0.89 (3H, m, CH$_3$-octyl).

Synthesis of phenyl(benzyl-L-alaninyl) phosphorochloridate (132)[2]
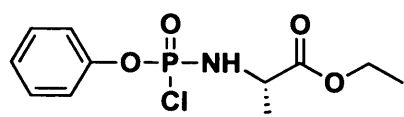

Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.70 mL, 4.63 mmol), L-alanine benzyl ester hydrochloride salt (1.0 g, 4.63 mmol), dry triethylamine (1.30 mL, 9.26 mmol) and dry DCM (20 mL). The product was obtained as a clear oil (1.21 g, 90%).

δ_P (CDCl$_3$): 7.79, 7.75; δ_H (CDCl$_3$): 7.25 (7H, m, CH-phenyl, CH-benzyl), 7.17-7.08 (3H, m, CH-benzyl), 5.11 (2H, d, CH$_2$-benzyl), 4.51 (1H, m, NH), 4.15 (1H, m, CHα), 1.42 (3H, m, CH$_3$-alanine).

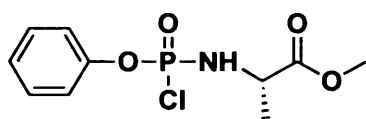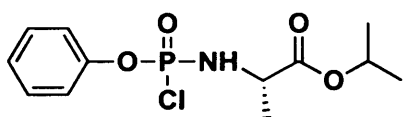## Synthesis of phenyl(ethyl-L-alaninyl) phosphorochloridate (129)[2]

Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.97 mL, 6.5 mmol), L-alanine ethyl ester hydrochloride salt (1.0 g, 6.5 mmol), dry triethylamine (1.80 mL, 13.0 mmol) and dry DCM (20 mL). The product was obtained as a clear oil (1.55 g, 82%).

δ_P (CDCl$_3$): 8.16, 7.94; δ_H (CDCl$_3$): 7.28-7.20 (2H, m, CH-phenyl), 7.18-7.14 (3H, m, CH-phenyl), 4.17 (1H, m, NH), 4.13 (1H, m, CHα), 3.39 (2H, m, CH$_2$-ethyl), 1.42 (3H, m, CH$_3$-alanine), 1.12 (3H, m, CH$_3$-ethyl).

Synthesis of phenyl(methyl-L-alaninyl) phosphorochloridate (128)[2]

Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.53 mL, 3.58 mmol), L-alanine methyl ester hydrochloride salt (0.50 g, 3.58 mmol), dry triethylamine (0.99 mL, 7.16 mmol) and dry DCM (20 mL). The product was obtained as a clear oil (0.85 g, 86%).

δ_P (CDCl$_3$): 8.01, 7.88; δ_H (CDCl$_3$): 7.30-7.27 (2H, m, CH-phenyl), 7.18-7.11 (3H, m, CH-phenyl), 4.63 (1H, m, NH), 4.11 (1H, m, CHα), 3.69 (3H, s, CH$_3$-methyl), 1.43 (3H, m, CH$_3$-alanine).

Synthesis of phenyl(isopropyl-L-alaninyl) phosphorochloridate (130)[6]
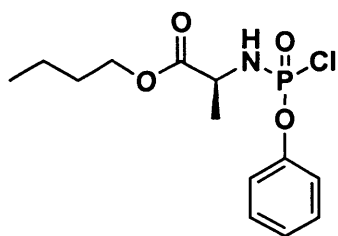
Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.45 mL, 2.98 mmol), L-alanine isopropyl ester hydrochloride salt (127, 0.50 g, 2.98 mmol), dry triethylamine (0.83 mL, 5.96 mmol) and dry DCM (20 mL). The product was obtained as a clear oil (0.58 g, 64%).

δ_P (CDCl$_3$): 8.36, 8.22; δ_H (CDCl$_3$): 7.23-7.20 (2H, m, CH-phenyl), 7.16-7.05 (3H, m, 3 CH-phenyl), 4.98-4.93 (2H, m, NH, CHα), 4.00 (1H, m, CH-isopropyl), 1.37 (3H, t, CH$_3$-alanine, J= 3.7 Hz), 1.15 (6H, m, CH$_3$-isopropyl).

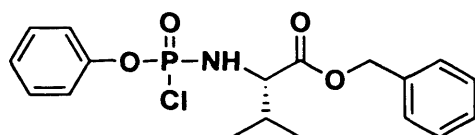## Synthesis of phenyl(*tert*-butyl-L-alaninyl) phosphorochloridate (131)[7]
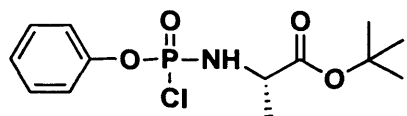
Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.83 mL, 5.5 mmol), L-alanine *tert*-butyl ester hydrochloride salt (1.0 g, 5.5 mmol), dry triethylamine (1.5 mL, 11.0 mmol) and dry DCM (20 mL). The product was obtained as a clear oil (1.71 g, 97%).

δ_P (CDCl$_3$): 8.27, 8.07; δ_H (CDCl$_3$): 7.28-7.26 (2H, m, CH-phenyl), 7.22-7.14 (3H, m, CH-phenyl), 4.47 (1H, m, NH), 3.96 (1H, m, CHα), 1.40 (12H, m, CH$_3$-alanine, CH$_3$-*tert*-butyl).

## Synthesis of phenyl(butyl-L-alaninyl) phosphorochloridate (58)

Prepared according to Standard Procedure B, from phenyl dichlorophosphate (1.90 mL, 12.7 mmol), L-alanine butyl ester tosylate salt (47, 4.00 g, 12.7 mmol), dry triethylamine (3.54 mL, 25.40 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (2.67 g, 65%).

δ_P (CDCl$_3$): 9.40, 9.19; δ_H (CDCl$_3$): 7.40-7.22 (5H, m, CH-phenyl), 4.82-4.66 (1H, m, NH, CHα), 4.38-4.30 (2H, m, CH$_2$-butyl), 1.69-1.61 (2H, m, CH$_2$-butyl), 1.42-1.39 (3H, m, CH$_3$-alanine), 1.37-1.32 (2H, m, CH$_2$-butyl), 1.00-0.90 (3H, m, CH$_3$-butyl).

Synthesis of phenyl(benzyl-L-valinyl) phosphorochloridate (134)[6]

Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.40 mL, 2.64 mmol), L-valine benzyl ester tosylate salt (1.00 g, 2.64 mmol), dry triethylamine (0.73 mL, 5.28 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (0.84 g, 99%).

δ_P (CDCl$_3$): 9.48, 8.95; δ_H (CDCl$_3$): 7.27 (6H, m, CH-phenyl, CH-benzyl), 7.24-7.14 (4H, m, CH-benzyl), 5.12 (2H, d, CH$_2$-benzyl), 4.25 (1H, m, NH), 3.91 (1H, m, CHα), 0.93 (3H, m, CH$_3$-lateral chain), 0.82 (3H, m, CH$_3$-lateral chain).

Synthesis of phenyl(ethyl-L-prolinyl) phosphorochloridate (140)[6]
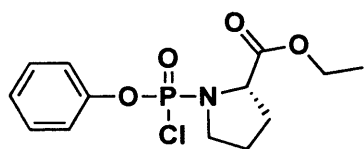
Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.41 mL, 2.78 mmol), L-proline ethyl ester hydrochloride salt (**124**, 0.50 g, 2.78 mmol), dry triethylamine (0.77 mL, 5.56 mmol) and dry DCM (20 mL). The product was obtained as a clear oil (0.65 g, 74%).

δ_P (CDCl$_3$): 7.83, 7.76; δ_H (CDCl$_3$): 7.29-7.21 (2H, m, CH-phenyl), 7.17-7.13 (3H, m, CH-phenyl), 4.39 (1H, m, CHα), 4.12 (2H, m, CH$_2$-ethyl), 3.49-3.44 (2H, m, CH$_2$-proline), 2.18-2.14 (1H, m, CH-proline), 2.08-2.05 (1H, m, CH-proline), 1.94 (2H, m, CH$_2$-proline), 1.19 (3H, m, CH$_3$-ethyl).

Synthesis of phenyl(ethyl-L-leucinyl) phosphorochloridate (139)[2]
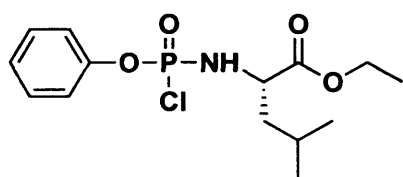
Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.38 mL, 2.55 mmol), L-leucine ethyl ester hydrochloride salt (0.50 g, 2.55 mmol), dry triethylamine (0.71 mL, 5.10 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (0.83 g, 82%).

δ_P (CDCl$_3$): 8.46, 8.22; δ_H (CDCl$_3$): 7.30-7.28 (2H, m, CH-phenyl), 7.19-7.14 (3H, m, CH-phenyl), 4.15 (1H, m, NH), 4.05 (2H, m, CH$_2$-ethyl), 3.39 (1H, m, CHα), 1.78-1.62 (1H, m, CH-lateral chain), 1.55 (2H, m, CH$_2$-lateral chain), 1.24-1.18 (3H, m, CH$_3$-ethyl), 0.88 (6H, t, CH$_3$-lateral chain, J= 3.6 Hz).

Synthesis of phenyl(ethyl-L-methioninyl) phosphorochloridate (141)
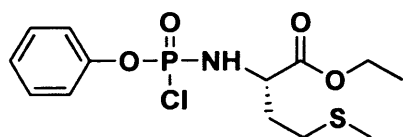
Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.35 mL, 2.34 mmol), L-methionine ethyl ester hydrochloride salt (0.50. g, 2.34 mmol), dry triethylamine (0.65 mL, 4.68 mmol) and dry DCM (20 mL). The product was obtained as a clear oil (0.81 g, 98%).

δ_P (CDCl$_3$): 8.70, 8.45; δ_H (CDCl$_3$): 7.27 (2H, m, CH-phenyl), 7.22-7.13 (3H, m, CH-phenyl), 4.94 (1H, m, NH), 4.19-4.12 (3H, m, CHα, CH$_2$-ethyl), 2.50 (2H, m, CH$_2$-lateral chain), 2.07-1.95 (5H, m, CH$_2$-lateral chain, CH$_3$-lateral chain), 1.42 (3H, m, CH$_3$-lateral chain), 1.11 (3H, m, CH$_3$-ethyl).

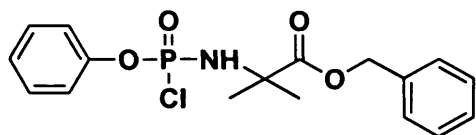## Synthesis of phenyl(benzyl-glycinyl) phosphorochloridate (133)[6]
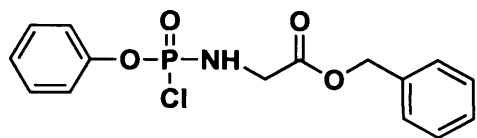
Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.44 mL, 2.96 mmol), glycine benzyl ester tosylate salt (1.00 g, 2.96 mmol), dry triethylamine (0.83 mL, 5.92 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (0.65 g, 79%).

δ_P (CDCl$_3$): 9.15; δ_H (CDCl$_3$): 7.25 (6H, m, CH-phenyl, CH-benzyl), 7.17-7.10 (4H, m, CH-benzyl), 5.11 (2H, d, CH$_2$-benzyl), 4.54 (1H, m, NH), 3.84 (2H, m, CH$_2$-glycine).

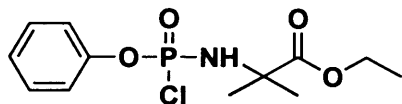## Synthesis of phenyl(ethyl-*N*-methyl-glycinyl) phosphorochloridate (143)[8]
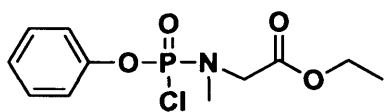
Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.48 mL, 3.25 mmol), *N*-methyl-glycine ethyl ester hydrochloride salt (126, 0.5 g, 3.25 mmol), dry triethylamine (0.90 mL, 6.50 mmol) and dry DCM (20 mL). The product was obtained as a clear oil (0.94 g, 99%).

δ_P (CDCl$_3$): 11.19; δ_H (CDCl$_3$): 6.55-6.42 (2H, m, CH-phenyl), 6.38-6.25 (3H, m, CH-phenyl), 3.33-3.30 (2H, m, CH$_2$-glycine), 3.16 (2H, m, CH$_2$-ethyl), 2.02 (3H, s, CH$_3$-N), 1.38 (3H, m, CH$_3$-ethyl).

Synthesis of phenyl(benzyl-*α,α*-dimethylglycinyl) phosphorochloridate (135)

Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.39 mL, 2.61 mmol), *α,α*-dimethylglycine benzyl ester tosylate salt (122, 1.00 g, 2.61 mmol), dry triethylamine (0.73 mL, 5.22 mmol) and dry DCM (20 mL). The product was obtained as a clear oil (0.54 g, 56%).

δ_P (CDCl$_3$): 5.53; δ_H (CDCl$_3$): 7.26 (6H, m, CH-phenyl, CH-benzyl), 7.23-7.12 (4H, m, CH-benzyl), 5.12 (2H, s, CH$_2$-benzyl), 4.67 (1H, b, NH), 1.61 (6H, d, CH$_3$-*α,α*-dimethylglycine).

Synthesis of phenyl(ethyl-α,α-dimethylglycinyl) phosphorochloridate (136)

Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.44 mL, 2.98 mmol), α,α-dimethylglycine ethyl ester hydrochloride salt (123, 0.50 g, 2.98 mmol), dry triethylamine (0.83 mL, 5.96 mmol) and dry DCM (20 mL). The product was obtained as a clear oil (0.84 g, 92%).

δ_P (CDCl$_3$): 5.58; δ_H (CDCl$_3$): 7.29 (1H, m, CH-phenyl, $J=$ 7.4 Hz), 7.20-7.14 (4H, m, CH-phenyl), 4.68 (1H, b, NH), 4.18 (2H, m, CH$_2$-ethyl), 1.61 (6H, d, CH$_3$-α,α-dimethylglycine), 1.13 (3H, m, CH$_3$-ethyl).

Synthesis of phenyl(diethyl-L-aspartyl) phosphorochloridate (59)
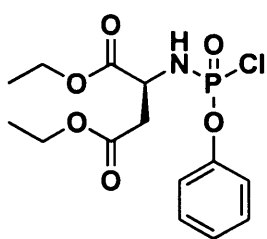
Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.67 mL, 4.49 mmol), L-ethylasparatate ethyl ester hydrochloride salt (1.0 g, 4.49 mmol), dry triethylamine (1.25 mL, 8.98 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (1.14 g, 70%).

δ_P (CDCl$_3$): 9.78, 9.54; δ_H (CDCl$_3$): 7.31-7.06 (5H, m, CH-phenyl), 4.86 (1H, m, NH), 4.25-4.00 (5H, m, CHα, CH$_2$-ethyl, CH$_2$-ethyl lateral chain), 2.78 (2H, m, CH$_2$-lateral chain), 1.23 (6H, m, CH$_3$-ethyl, CH$_3$-ethyl lateral chain).

Synthesis of phenyl(benzyl-L-phenylalaninyl) phosphorochloridate (137)
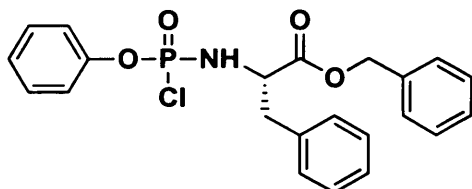
Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.26 mL, 1.71 mmol), L-phenylalanine benzyl ester hydrochloride salt (0.5 g, 1.71 mmol), dry triethylamine (0.48 mL, 3.42 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (0.68 g, 92%).

δ_P (CDCl$_3$): 7.91, 7.85; δ_H (CDCl$_3$): 7.37-7.21 (7H, m, CH-phenyl, CH-benzyl), 7.17-6.92 (8H, m, CH-benzyl, CH-lateral chain), 5.08 (2H, d, CH$_2$-benzyl), 4.38 (1H, m, NH), 4.13-4.08 (1H, m, CHα), 3.06 (2H, m, CH$_2$-lateral chain).

Synthesis of phenyl(ethyl-L-phenylalaninyl) phosphorochloridate (138)
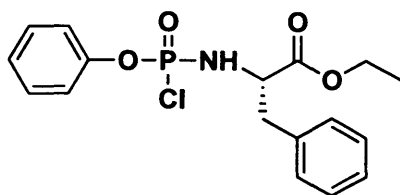
Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.32 mL, 2.18 mmol), L-phenylalanine ethyl ester hydrochloride salt (0.5 g, 2.18 mmol), dry triethylamine (0.60, 4.36 mmol) and dry DCM (20 mL). The product was obtained as a clear oil (0.72 g, 1.96 mmol, 90%).

δ_P (CDCl$_3$): 8.03, 7.95; δ_H (CDCl$_3$): 7.35-7.20 (2H, m, CH-phenyl), 7.15-6.91 (8H, m, CH-phenyl, CH-lateral chain), 4.36 (1H, m, CHα), 4.09 (1H, m, NH), 3.40 (2H, m, CH$_2$-ethyl), 3.08-3.02 (2H, m, CH$_2$-lateral chain), 1.12 (3H, t, CH$_3$-ethyl, J= 6.6 Hz).

Synthesis of phenyl(ethyl-β-alaninyl) phosphorochloridate (142)
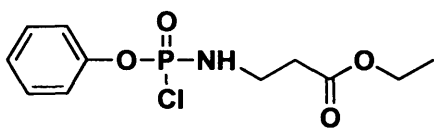
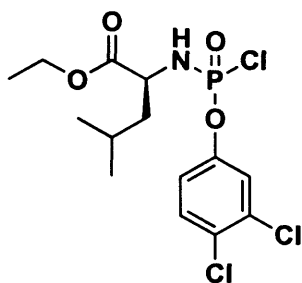Prepared according to Standard Procedure B, from phenyl dichlorophosphate (0.48 mL, 3.25 mmol), β-alanine ethyl ester hydrochloride salt (125, 0.5 g, 3.25 mmol), dry triethylamine (0.90 mL, 6.50 mmol) and dry DCM (20 mL). The product was obtained as a clear oil (0.91 g, 96%).

δ_P (CDCl$_3$): 10.00; δ_H (CDCl$_3$): 7.32-7.27 (2H, m, CH-phenyl), 7.19-7.10 (3H, m, CH-phenyl), 4.54 (1H, b, NH), 4.09 (2H, m, CH$_2$-ethyl), 3.33 (2H, m, CH$_2$-β-alanine), 2.54 (2H, t, CH$_2$-β-alanine, J= 5.9 Hz), 1.18 (3H, t, CH$_3$-ethyl, J= 7.0 Hz).

Synthesis of *p*-chloro-phenyl(ethyl-L-leucinyl) phosphorochloridate (82)
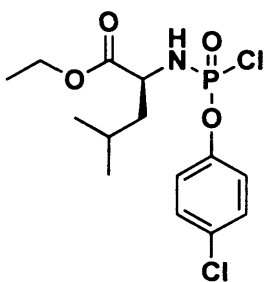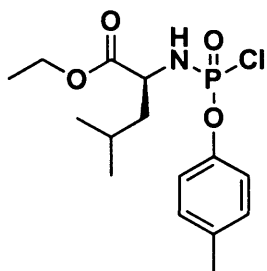
Prepared according to Standard Procedure B, from *p*-chloro-phenyl dichlorophosphate (78, 1.88 g, 7.66 mmol), L-leucine ethyl ester hydrochloride salt (1.5 g, 7.66 mmol), dry triethylamine (2.14 mL, 15.32 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (2.16 g, 77%).

δ_P (CDCl$_3$): 9.75, 9.73; δ_H (CDCl$_3$): 7.23 (2H, m, CH-phenyl), 7.12 (2H, m, CH-phenyl), 4.37 (1H, m, NH), 4.13 (2H, m, CH$_2$-ethyl), 3.99 (1H,m, CH-α), 1.78 (1H, m, CH-lateral chain), 1.53 (2H, m, CH$_2$-lateral chain), 1.16 (6H, m, CH$_3$-lateral chain), 0.82 (3H, m, CH$_3$-ethyl).

## Synthesis of 3,4-dichloro-phenyl(ethyl-L-leucinyl) phosphorochloridate (83)

Prepared according to Standard Procedure B, from a solution of 3,4-dichloro-phenyl dichlorophosphate (**79**, 2.14 g, 7.66 mmol), L-leucine ethyl ester hydrochloride salt (1.5 g, 7.66 mmol), dry triethylamine (2.14 mL, 15.32 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (2.28 g, 75%).

δ_P (CDCl$_3$): 9.84, 9.78; δ_H (CDCl$_3$): 7.41 (1H, m, CH-phenyl), 7.38 (1H, m, CH-phenyl), 7.33 (1H, m, CH-phenyl), 4.59 (1H, m, NH), 4.28 (1H, m, CHα), 4.09 (2H, m, CH$_2$-ethyl), 1.70 (1H, m, CH-lateral chain), 1.45 (2H, m, CH$_2$-lateral chain), 1.03 (6H, m, CH$_3$-lateral chain), 0.78 (3H, m, CH$_3$-ethyl).

Synthesis of *p*-methyl-phenyl(ethyl-L-leucinyl) phosphorochloridate (84)

Prepared according to Standard Procedure B, from *p*-methyl-phenyl dichloro phosphate (**80**, 1.72 g, 7.66 mmol), L-leucine ethyl ester hydrochloride salt (1.5 g, 7.66 mmol), dry triethylamine (2.14 mL, 15.32 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (2.28 g, 75%).

δ_H (CHCl$_3$): 10.06, 9.63; δ_H (CHCl$_3$): 7.05 (2H, d, CH-phenyl), 7.00 (2H, m, CH-phenyl), 4.67 (1H, m, NH), 4.26 (1H, m, CHα), 4.12 (2H, m, CH$_2$-ethyl), 1.87 (1H, m, CH-lateral chain), 1.69 (2H, m, CH$_2$-lateral chain), 1.45 (3H, m, CH$_3$-ethyl), 1.32 (3H, m, CH$_3$-lateral chain), 1.02 (3H, m, CH$_3$-lateral chain).

Synthesis of *p*-methoxy-phenyl(ethyl-L-leucinyl) phosphorochloridate (85)
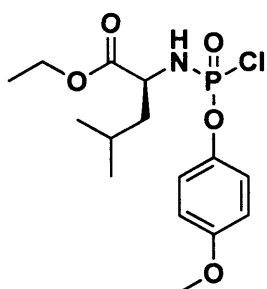
Prepared according to Standard Procedure B, from *p*-methoxy-phenyl dichlorophosphate (**81**, 1.85 g, 7.66 mmol), L-leucine ethyl ester hydrochloride salt (1.5 g, 7.66 mmol), dry triethylamine (2.14 mL, 15.32 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (1.71 g, 61%).

δ_P (CHCl$_3$): 10.54, 10.22; δ_H (CHCl$_3$): 7.12 (2H, d, CH-phenyl, $J=$ 9.0 Hz), 6.85 (2H, d, phenyl, $J=$ 9.0 Hz), 4.46 (1H, m, NH), 4.28 (1H, m, CHα),

3.85 (3H, s, CH_3O-*p*-phenyl), 1.86 (1H, m, CH-lateral chain),1.65 (2H, m, CH_2-lateral chain), 1.37 (3H, m, CH_3-ethyl), 1.32 (3H, m, CH_3-lateral chain), 0.96 (3H, m, CH_3-lateral chain).
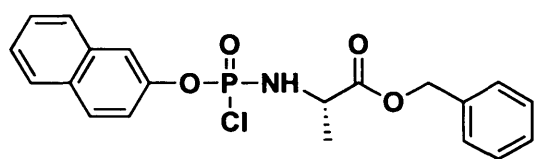
Synthesis of α-naphthyl(benzyl-L-alaninyl) phosphorochloridate (93)[6]
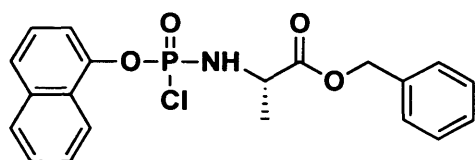
Prepared according to Standard Procedure B, from α-naphthyl dichlorophosphate (91, 2.22 g, 8.53 mmol), L-alanine benzyl ester hydrochloride salt (3.0 g, 8.53 mmol), dry triethylamine (2.38 mL, 17.07 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (1.62 g, 47%).

δ_P ($CDCl_3$): 8.25, 8.58; δ_H ($CDCl_3$): 7.97 (1H, m, CH-naphthyl), 7.73-7.09 (11H, m, CH-naphthyl, CH-benzyl), 5.09 (2H, s, CH_2-benzyl), 4.81 (1H, m, NH), 4.23 (1H, m, CHα), 1.42 (3H, m, CH_3-alanine).

Synthesis of β-naphthyl(benzyl-L-alaninyl) phosphorochloridate (94)[6]

Prepared according to Standard Procedure B, from β-naphthyl dichlorophosphate (92, 2.22 g, 8.53 mmol), L-alanine benzyl ester hydrochloride salt (3.0 g, 8.53 mmol), dry triethylamine (2.38 mL, 17.07 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (2.29g, 66%).

δ_P ($CDCl_3$): 8.32, 8.10; δ_H ($CDCl_3$): 7.85-7.62 (4H, m, 3 CH-naphthyl, CH-benzyl), 7.48-7.37 (3H, m, 3 CH-naphthyl), 7.27-7.18 (5H, m, CH-naphthyl, 4 CH-benzyl), 5.15 (2H, s, CH_2-benzyl), 5.10 (1H, m, NH), 4.20 (1H, m, CHα), 1.47 (3H, m, CH_3-alanine).

Synthesis of α-naphthyl(benzyl-cyclopentylglycinyl) phosphorochloridate (169)
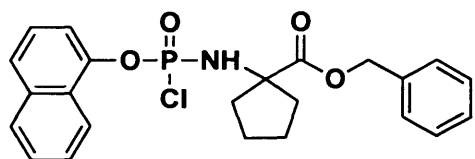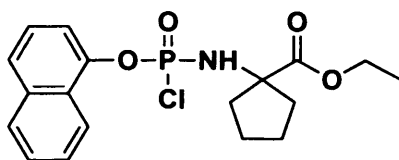
Prepared according to Standard Procedure B, from α-naphthyl dichlorophosphate (90, 1.33 g, 5.11 mmol), cyclopentylglycine benzyl ester tosylate salt (41, 2.0 g, 5.11 mmol), dry

triethylamine (1.42 mL, 10.22 mmol) and dry DCM (15 mL). The product was obtained as a clear yellow oil (1.41 g, 62%).

δ_P (CDCl$_3$): 7.05; δ_H (CDCl$_3$): 8.00 (1H, m, CH-naphthyl), 7.72 (1H, m, CH-naphthyl), 7.56 (1H, d, CH-naphthyl, J= 8.4 Hz), 7.47 (1H, d, CH-naphthyl, J= 8.4 Hz), 7.37 (1H, m, CH-naphthyl), 7.26 (1H, t, CH-naphthyl, J= 7.5 Hz), 7.24-7.13 (6H, m, CH-phenyl, CH-naphthyl), 5.07 (2H, s, CH$_2$-benzyl), 4.57 (1H, s, NH), 2.20-2.02 (4H, m, CH$_2$-cyclopentyl), 1.87-1.69 (4H, CH$_2$-cyclopentyl).

Synthesis of α-naphthyl(ethyl-cyclopentylglycinyl) phosphorochloridate (170)

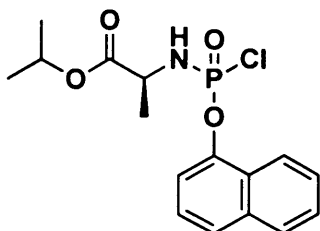
Prepared according to Standard Procedure B, from α-naphthyl dichlorophosphate (90, 2.7 g, 10.33 mmol), cyclopentylglycine ethyl ester hydrochloride salt (42, 2.0 g, 10.33 mmol), dry triethylamine (2.9 mL, 20.65 mmol) and dry DCM (15 mL). The product was obtained as a clear a white solid (3.40 g, 86%).

δ_P (CDCl$_3$): 6.90; δ_H (CDCl$_3$): 8.04 (1H, d, CH-naphthyl, J= 8.1 Hz), 7.79 (1H, d, CH-naphthyl, J= 7.4 Hz), 7.65 (1H, d, CH-naphthyl, J= 8.3 Hz), 7.54-7.44 (3H, m, CH-naphthyl), 7.36 (1H, t, CH-naphthyl, J= 8.1 Hz), 4.25 (1H, s, NH), 4.16 (2H, m, CH$_2$-ethyl, J= 7.0 Hz), 2.22-2.12 (4H, m, CH$_2$-cyclopentyl), 1.86-1.73 (4H, m, CH$_2$-cyclopentyl), 1.13 (3H, t, CH$_3$-ethyl, J= 7.0 Hz).
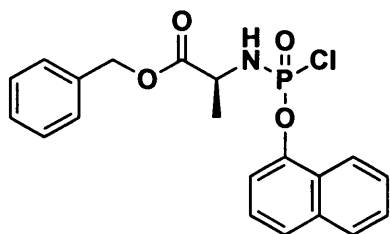
Synthesis of α-naphthyl(isopropyl-D-alaninyl) phosphorochloridate (171)[6]

Prepared according to Standard Procedure B, from α-naphthyl dichlorophosphate (90, 2.70 g, 10.33 mmol), D-alanine isopropyl ester hydrochloride salt (104, 1.4 g, 10.33 mmol), dry triethylamine (2.88 mL, 20.65 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (2.55 g, 68%).

δ_P (CDCl$_3$): 7.88, 7.37; δ_H (CDCl$_3$): 7.97 (1H, m, CH-naphthyl), 7.23 (1H, m, CH-naphthyl), 7.01 (1H, m, CH-naphthyl), 6.80-6.40 (4H, m, CH-naphthyl), 4.50 (1H, m, NH), 4.02 (1H, m, CHα), 3.79 (1H, m, CH-isopropyl), 1.48 (6H, d, CH$_3$-isopropyl, J= 6.2 Hz), 1.42 (3H, m, CH$_3$-alanine).

Synthesis of α-naphthyl(benzyl-D-alaninyl) phosphorochloridate (172)[6]

Prepared according to Standard Procedure B, from α-naphthyl dichlorophosphate (**90**, 2.70 g, 10.33 mmol), D-alanine benzyl ester hydrochloride salt (3.6 g, 10.33 mmol), dry triethylamine (2.88 mL, 20.65 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (1.23 g, 29%).
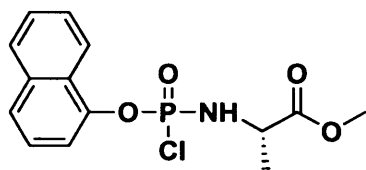
δ_P (CDCl$_3$): 7.40, 7.17; δ_H (CDCl$_3$): 7.17 (1H, m, CH-naphthyl), 6.99-6.28 (11H, m, CH-naphthyl, CH-benzyl), 4.30-4.21 (2H, m, CH$_2$-benzyl), 3.79 (1H, m, NH), 3.46 (1H, m, CHα), 1.45 (3H, m, CH$_3$-alanine).

Synthesis of phenyl(methyl-L-alaninyl) phosphorochloridate (231)[6]

Prepared according to Standard Procedure B, from α-naphthyl dichlorophosphate (**90**, 2.54 g, 9.76 mmol), L-alanine methyl ester hydrochloride salt (1.36 g, 9.76 mmol), dry triethylamine (2.70 mL, 19.52 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (2.49 g, 78%).

δ_P (CDCl$_3$): 8.25, 8.02; δ_H (CDCl$_3$): 8.10 (1H, t, CH-naphthyl), 7.88 (1H, d, CH-naphthyl, J= 7.6 Hz), 7.74 (1H, d, CH-naphthyl, J= 8.3 Hz), 7.62-7.55 (3H, m, CH-naphthyl), 7.45 (1H, t, CH-naphthyl, J= 7.9 Hz), 4.65 (1H, m, NH), 4.16-4.12 (1H, m, CHα), 3.82, 3.77 (3H, d, CH$_3$-methyl), 1.27 (3H, t, CH$_3$-alanine, J= 7.1 Hz).

Synthesis of α-naphthyl(ethyl-L-alaninyl) phosphorochloridate (207)[6]
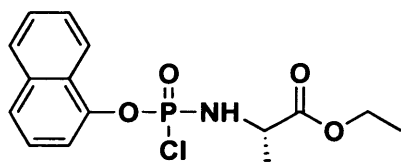
Prepared according to Standard Procedure B, from α-naphthyl dichlorophosphate (**90**, 2.54 g, 9.76 mmol), L-alanine ethyl ester hydrochloride salt (1.5 g, 9.76 mmol), dry triethylamine (2.70 mL, 19.52 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (2.87 g, 86%).
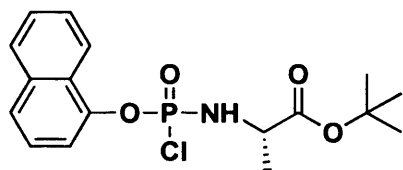
δ_P (CDCl$_3$): 8.42, 8.25; δ_H (CDCl$_3$): 8.00 (1H, m, CH-naphthyl), 7.75 (1H, m, CH-naphthyl), 7.62-7.28 (5H, m, CH-naphthyl), 4.83 (1H, m, NH), 4.21-3.98 (3H, m, CHα, CH$_2$-ethyl), 1.10 (3H, m, CH$_3$-ethyl), 1.22 (3H, d, CH$_3$-alanine, J= 4.2 Hz).

Synthesis of α-naphthyl(*tert*-butyl-L-alaninyl) phosphorochloridate (208)[6]

Prepared according to Standard Procedure B, from α-naphthyl dichloro phosphate (90, 2.15 g, 8.25 mmol), L-alanine *tert*-butyl ester hydrochloride salt (1.5 g, 8.25 mmol), dry triethylamine (2.30 mL, 16.50 mmol) and dry DCM (15 mL). The product was obtained as a clear oil (2.73 g, 89%).

δ_P (CDCl$_3$): 8.48, 8.19; δ_H (CDCl$_3$): 8.04 (1H, m, CH-naphthyl), 7.78 (1H, m, CH-naphthyl), 7.64-7.30 (5H, m, 5 CH-naphthyl), 4.50 (1H, m, NH), 4.08 (1H, m, CHα), 1.43 (9H, s, 3 CH$_3$-*tert*-butyl), 1.38 (3H, m, CH$_3$-alanine).

7.6 Synthesis of cytidine analogue phosphoramidates.

Synthesis of 4'-azidocytidine 5'-*O*-[phenyl(benzyl-cyclopentylglycinyl)]

phosphate (65)
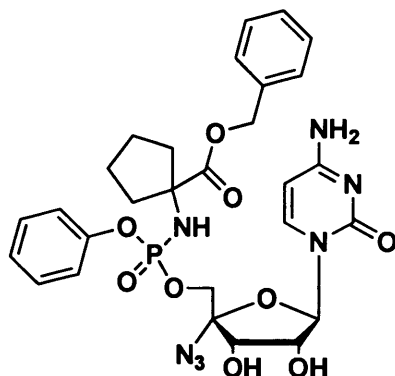
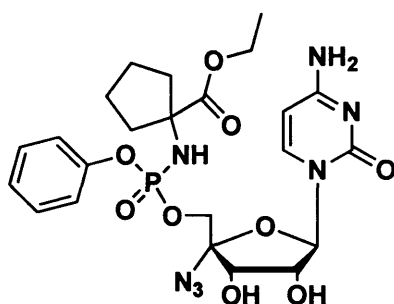Prepared according to Standard Procedure C, from 4'-azidocytidine (**23**, 300 mg, 0.986 mmol), ^{t}BuMgCl (2.46 mL, 1M solution in THF, 2.46 mmol) and phenyl(benzyl-cyclopentylglycinyl) phosphorochloridate (**50**, 2.46 mL of solution 1M in THF, 2.46 mmol). The crude product was purified twice by column chromatography, using as eluent for the first $CHCl_3$/MeOH (95:5) and for the second $CHCl_3$/MeOH (80:20) then a preparative TLC using as eluent $CHCl_3$/MeOH (9:1). The pure product was a white solid (30 mg, 5%).

δ_P (d_4-CH$_3$OH): 3.70, 3.67; δ_H (d_4-CH$_3$OH): 7.55 (1H, m, H6-cytidine, $J=$ 7.3 Hz), 7.24 (7H, m, CH-phenyl, CH-benzyl), 7.13 (3H, m, CH-phenyl, CH-benzyl), 6.07 (1H, m, H1'-cytidine), 5.75 (1H, m, H5-cytidine, $J=$ 7.3 Hz), 5.05 (2H, s, CH$_2$-benzyl), 4.23 (1H, m, H2'-cytidine), 4.15 (1H, m, H3'-cytidine), 4.10 (2H, m, H5'-cytidine), 2.00 (2H, m, CH$_2$-cyclopentyl), 1.92 (2H, m, CH$_2$-cyclopentyl), 1.63 (2H, m, CH$_2$-cyclopentyl), 1.55 (2H, m, CH$_2$-cyclopentyl); δ_C (d_4-CH$_3$OH): 176.82 (1C, C=O ester), 167.99 (1C, C4-cytidine), 158.56 (1C, C2-cytidine), 152.49, 152.42 (1C, C-phenyl), 143.48, 143.36 (1C, C6-cytidine), 137.74, 137.73 (1C, C-benzyl), 131.73, 131.55, 131.20 (2C, CH-phenyl, CH-benzyl), 130.20, 129.98 (1C, CH-phenyl), 129.73, 129.71, 129.71, 129.69 (2C, CH-phenyl, CH-benzyl), 129.59, 129.45 (1C, CH-benzyl), 127.37, 126.70 (2C, CH-phenyl, CH-benzyl), 122.05, 121.99, 121.92 (2C, CH-phenyl, CH-benzyl), 99.16, 99.04, 99.02 (1C, C5-cytidine), 97.24 (1C, C4'-cytidine), 93.87, 93.75 (1C, C1'-cytidine), 74.88, 74.84 (1C, C3'-cytidine), 73.79, 73.70 (1C, C2'-cytidine), 69.16, 69.07, 68.99, 68.76 (1C, C5'-cytidine), 68.72 (1C, C-cyclopentyl), 68.61 (1C, CH$_2$-benzyl), 40.34, 40.23, 40.16, 40.05 (1C, CH$_2$-

cyclopentyl), 39.62, 39.56, 39.50 (1C, CH$_2$-cyclopentyl), 25.10, 25.00 (1C, CH$_2$-cyclopentyl), 24.98 (1C, CH$_2$-cyclopentyl).

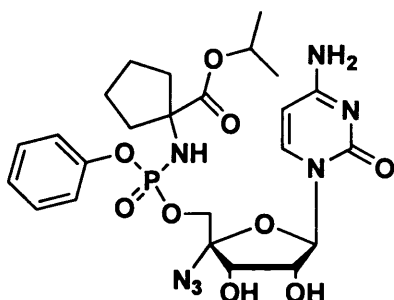**Synthesis of 4'-azidocytidine 5'-*O*-[phenyl(ethyl-cyclopentylglycinyl)] phosphate**

(66)

Prepared according to Standard Procedure C, from 4'-azidocytidine (**23**, 300 mg, 0.986 mmol), 'BuMgCl (2.46 mL 1M solution in THF, 2.46 mmol) and phenyl(ethyl-cyclopentylglycinyl) phosphorochloridate (**51**, 2.46 mL 1M solution of THF, 2.46 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (95:5) then a preparative TLC using as eluent CHCl$_3$/MeOH (85:15). The pure product was a white solid (100 mg, 18%).

δ_P (d_4-CH$_3$OH): 3.74; δ_H (d_4-CH$_3$OH): 7.70 (1H, m, H6-cytidine, $J=$ 7.6 Hz), 7.38 (2H, m, CH-phenyl), 7.36 (1H, m, CH-phenyl), 7.25 (2H, m, CH-phenyl), 6.19 (1H, m, H1'-cytidine), 5.87 (1H, m, H2-cytidine, $J=$ 7.6 Hz), 4.37 (2H, m, CH$_2$-ethyl), 4.28 (1H, m, H2'-cytidine), 4.26 (1H, m, H3'-cytidine), 4.15 (2H, m, H5'-cytidine), 2.00 (2H, m, CH$_2$-cyclopentyl), 1.92 (2H, m, CH$_2$-cyclopentyl), 1.63 (2H, m, CH$_2$-cyclopentyl), 1.55 (2H, m, CH$_2$-cyclopentyl), 1.25 (3H, m, CH$_3$-ethyl); δ_C (d_4-CH$_3$OH): 177.05 (1C, C=O ester), 168.05 (1C, C4-cytidine), 158.62 (1C, C2-cytidine), 152.52, 152.43 (1C, C-phenyl), 143.51, 143.43 (1C, C6-cytidine), 131.00 (2C, CH-phenyl), 126.70 (1C, CH-phenyl), 122.07, 122.00, 121.93 (2C, CH-phenyl), 99.22, 99.19, 99.09, 99.05 (1C, C5-cytidine), 97.21 (1C, C4'-cytidine), 93.86, 93.79 (1C, C1'-cytidine), 74.86 (1C, C3'-cytidine), 73.87, 73.79 (1C, C2'-cytidine), 69.20, 69.12, 69.05 (1C, C5'-cytidine), 68.72, 68.69 (1C, C-cyclopentyl), 62.93 (1C, CH$_2$-ethyl), 40.35, 40.23 (1C, CH$_2$-cyclopentyl), 40.10, 39.54 (1C, CH$_2$-cyclopentyl), 25.11, 25.00 (1C, CH$_2$-cyclopentyl), 24.98 (1C, CH$_2$-cyclopentyl), 14.86 (1C, CH$_3$-ethyl).

Synthesis of 4'-azidocytidine 5'-*O*-[phenyl(isopropyl-cyclopentylglycinyl)] phosphate (67)

Prepared according to Standard Procedure C, from 4'-azidocytidine (**23**, 300 mg, 0.986 mmol), 'BuMgCl (2.46 mL of solution 1M in THF, 2.46 mmol) and phenyl(isopropyl-cyclopentylglycinyl)phosphorochloridate (**52**, 2.46 mL, 1M solution in THF, 2.46 mmol). The crude product was purified twice by column chromatography, using as eluent for the first $CHCl_3$/MeOH (85:15) and for the second column $CHCl_3$/MeOH (80:20) then a preparative TLC using as eluent $CHCl_3$/MeOH (9:1). The pure product was a white solid (25 mg, 5%).

δ_P (d_4-CH_3OH): 3.77, 3.75; δ_H (d_4-CH_3OH): 7.69 (1H, m, H6-cytidine, $J=$ 10.59 Hz), 7.39 (2H, m, CH-phenyl), 7.27 (1H, m, CH-phenyl), 7.23 (2H, m, CH-phenyl), 6.20 (1H, t, H1'-cytidine), 5.89 (1H, m, H5-cytidine, $J=$ 10.59 Hz), 5.01 (1H, m, CH-isopropyl, $J=$ 3.3 Hz), 4.40 (1H, m, H2'-cytidine), 4.36 (1H, m, H3'-cytidine), 4.28 (2H, m, H5'-cytidine), 2.12 (2H, m, CH_2-cyclopentyl), 1.97 (2H, m, CH_2-cyclopentyl), 1.74 (2H, m, CH_2-cyclopentyl), 1.66 (2H, m, CH_2-cyclopentyl), 1.25 (6H, t, 2 CH_3-isopropyl, $J=$ 3.3 Hz).

Synthesis of 4'-azidocytidine 5'-*O*-[phenyl(ethyl-L-isoleucinyl)] phosphate (68)
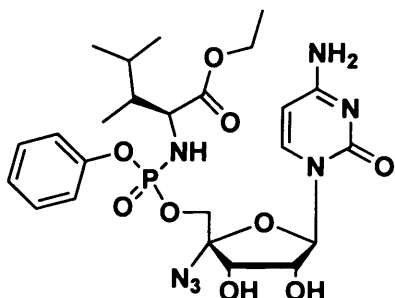
Prepared according to Standard Procedure C, from 4'-azidocytidine (**23**, 300 mg, 0.986 mmol), 'BuMgCl (2.46 mL 1M solution of THF, 2.46 mmol) and phenyl(ethyl-L-isoleucinyl) phosphorochloridate (**53**, 2.46 mL of solution 1M in THF, 2.46 mmol). The crude product was purified by column chromatography, using as eluent

CHCl₃/MeOH (95:5) then a preparative TLC using as eluent CHCl₃/MeOH (85:15). The pure product was a white solid (20 mg, 3%).

δ$_P$ (d_4-CH₃OH): 5.55, 5.30; δ$_H$ (d_4-CH₃OH): 7.62 (1H, m, H6-cytidine, J= 7.7 Hz), 7.34 (2H, m, CH-phenyl), 7.23 (1H, m, CH-phenyl), 7.17 (2H, m, CH-phenyl), 6.14 (1H, m, H1'-cytidine, J= 9.51 Hz), 5.83 (1H, m, H5-cytidine, J= 7.7 Hz), 4.31 (2H, m, CH₂-ethyl), 4.19 (1H, m, H2'-cytidine), 4.13 (1H, m, H3'-cytidine), 4.08 (2H, m, H5'-cytidine), 3.71 (1H, m, CHα), 1.74 (1H, m, CH-lateral chain), 1.45 (2H, m, CH₂-lateral chain) 1.15 (3H, m, CH₃-ethyl), 0.85-0.82 (6H, m, CH₃-lateral chain).

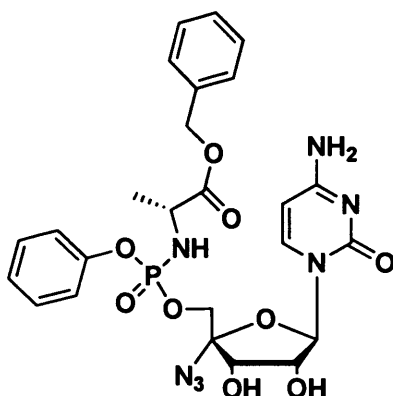## Synthesis of 4'-azidocytidine 5'-*O*-[phenyl(benzyl-D-alaninyl)] phosphate (69)

Prepared according to Standard Procedure C, from 4'-azidocytidine (23, 300 mg, 0.986 mmol), ʹBuMgCl (2.46 mL 1M solution of THF, 2.46 mmol) and phenyl(benzyl-D-alanine) phosphorochloridate (54, 2.46 mL of solution 1M in THF, 2.46 mmol). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (95:5), followed by a preparative TLC using as eluent CHCl₃/MeOH (85:15) and at the end it was purified using semi-preparative HPLC. The pure product was a white solid (20 mg, 3%).

δ$_P$ (d_4-CH₃OH): 4.80, 4.26; δ$_H$ (d_4-CH₃OH): 7.63 (1H, m, H6-cytidine, J= 7.6 Hz), 7.35 (6H, m, CH-phenyl, CH-benzyl), 7.23 (4H, m, CH-benzyl), 6.15 (1H, m, H1'-cytidine), 5.89 (1H, m, H5-cytidine, J= 7.6 Hz), 5.16 (2H, s, CH₂-benzyl), 4.35 (2H, m, H5'-cytidine), 4.26 (1H, m, H2'-cytidine), 4.23 (1H, m, H3'-cytidine), 4.16 (1H, m, CHα), 1.34 (3H, m, CH₃-alanine).

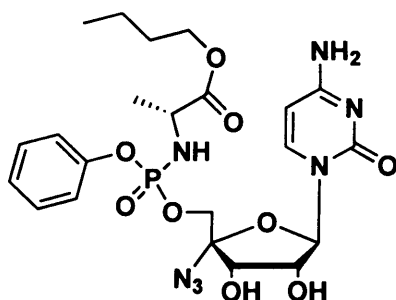## Synthesis of 4'-azidocytidine 5'-*O*-[phenyl(*tert*-butyl-D-alaninyl)] phosphate (70)
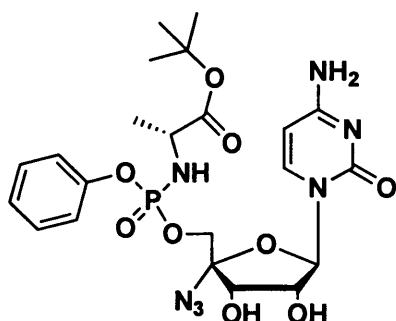
Prepared according to Standard Procedure C, from 4'-azidocytidine (23, 250 mg, 0.821 mmol), 'BuMgCl (2.0 mL 1M solution of THF, 2.054 mmol) and phenyl(*tert*-butyl-D-alaninyl) phosphorochloridate (55, 2.05 mL of solution 1M in THF, 2.054 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (95:5), followed by a preparative TLC using as eluent CHCl$_3$/MeOH (85:15). The pure product was a white solid (3.5 mg, 1%).

δ_P (d_4-CH$_3$OH): 4.91, 4.45; δ_H (d_4-CH$_3$OH): 7.67 (1H, m, H6-cytidine, $J=$ 7.5 Hz), 7.45 (5H, m, CH-phenyl), 6.17 (1H, m, H1'-cytidine), 5.92 (1H, m, H5-cytidine, $J=$ 7.5 Hz), 4.40-4.18 (2H, m, H2'-cytidine, H3'-cytidine, H5'-cytidine), 3.85 (1H, m, CHα), 1.45 (9, s, CH$_3$-*tert*-butyl), 1.37 (3H, m, CH$_3$-alanine).

MS (ES) m/e: 590 (MNa$^+$, 100%); Accurate mass: C$_{22}$H$_{30}$N$_7$O$_9$NaP required 590.1761, found 590.1740.

Synthesis of 4'-azidocytidine 5'-*O*-[phenyl(butyl-D-alaninyl)] phosphate (71)

Prepared according to Standard Procedure C, from 4'-azidocytidine (23, 300 mg, 0.924 mmol), 'BuMgCl (1.85 mL 1M solution of THF, 1.850 mmol) and phenyl(butyl-D-alaninyl) phosphorochloridate (56, 1.85 mL of solution 1M in THF, 1.850 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (95:5), followed by a preparative TLC using as eluent CHCl$_3$/MeOH (85:15). The pure product was a white solid (16 mg, 3%).

δ_P (d_4-CH$_3$OH): 4.73, 4.55; δ_H (d_4-CH$_3$OH): 7.65 (1H, m, H6-cytidine), 7.44-7.26 (5H, m, CH-phenyl), 6.20 (1H, m, H1'-cytidine, J= 5.0 Hz), 5.94 (1H, m, H5-cytidine), 4.42-4.22 (2H, m, H2'-cytidine, H3'-cytidine), 4.25-3.98 (5H, m, CHα, H5'-cytidine, CH$_2$-butyl), 1.73-1.59 (3H, m, CH$_3$-alanine), 1.43-1.18 (4H, m, CH$_2$-butyl), 0.96 (3H, m, CH$_3$-butyl).

MS (ES) m/e: 591.0 (MNa$^+$, 100%); Accurate mass: C$_{22}$H$_{29}$N$_6$O$_{10}$NaP required 590.1749, found 590.1740.

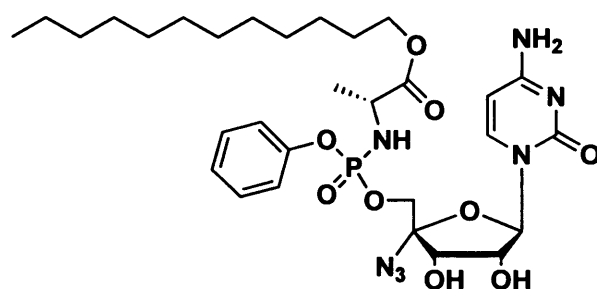## Synthesis of 4'-azidocytidine 5'-*O*-[phenyl(dodecyl-D-alaninyl)] phosphate (72)

Prepared according to Standard Procedure C, from 4'-azidocytidine (**23**, 200 mg, 0.657 mmol), ^{t}BuMgCl (1.64 mL 1M solution of THF, 1.640 mmol) and phenyl(dodecyl -D-alaninyl) phosphorochloridate (**57**, 1.64 mL of solution 1M in THF, 1.640 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (95:5), followed by a preparative TLC using as eluent CHCl$_3$/MeOH (85:15). The pure product was a white solid (23 mg, 5%).

δ_P (d_4-CH$_3$OH): 4.80, 4.33; δ_H (d_4-CH$_3$OH): 7.65 (1H, m, H6-cytidine, J= 9.0 Hz), 7.42-7.18 (5H, m, CH-phenyl), 6.18 (1H, m, H1'-cytidine), 5.90 (1H, m, H5-cytidine), 4.40-3.95 (7H, m, H2'-cytidine, H3'-cytidine, H5'-cytidine, CHα, CH$_2$-dodecyl), 1.54 (3H, m, CH$_3$-alanine), 1.30 (22H, m, CH$_2$-dodecyl), 0.92 (3H, t, CH$_3$-dodecyl, J= 6.5 Hz).

**Synthesis of 4'-azidocytidine 5'-*O*-[phenyl(ethyl-L-ethylaspartyl)] phosphate (73)**
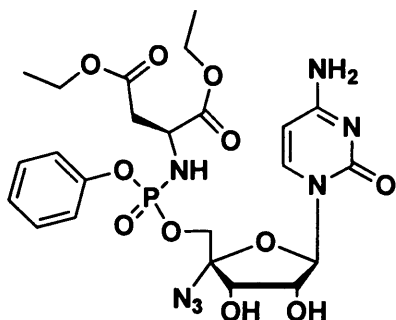
Prepared according to Standard Procedure C, from 4'-azido-cytidine (**23**, 300 mg, 0.986 mmol), 'BuMgCl (1.97 mL 1M solution of THF, 1.972 mmol) and phenyl(ethyl-L-ethylaspartyl) phosphorochloridate (**59**, 1.97 mL of solution 1M in THF, 1.972 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (95:5), followed by a preparative TLC using as eluent CHCl$_3$/MeOH (85:15). The pure product was a white solid (30 mg, 5%).

δ_P (d_4-CH$_3$OH): 4.67, 4.40; δ_H (d_4-CH$_3$OH): 7.70 (1H, m, H6-cytidine), 7.40-7.16 (5H, m, CH-phenyl), 6.16 (1H, m, H1'-cytidine), 5.85 (1H, m, H5-cytidine), 4.40-4.00 (9H, m, H2'-cytidine, H3'-cytidine, H5'-cytidine, CHα, CH$_2$-ethyl, CH$_2$-ethyl lateral chain), 2.78 (2H, m, CH$_2$-lateral chain), 1.23 (6H, m, CH$_3$-ethyl, CH$_3$-ethyl lateral chain).

MS (ES) m/e: 634.1 (MNa$^+$, 100%); Accurate mass: C$_{23}$H$_{30}$N$_7$O$_{11}$NaP required 634.1639, found 634.1624.

Synthesis of 4'-azidocytidine 5'-*O*-[phenyl(butyl-L-alaninyl)] phosphate (74)
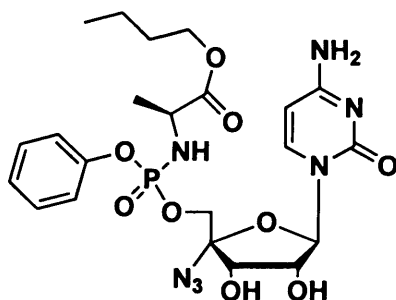
Prepared according to Standard Procedure C, from 4'-azidocytidine (**23**, 300 mg, 0.924 mmol), 'BuMgCl (1.8 mL 1M solution of THF, 1.849 mmol) and phenyl(butyl-L-alaninyl) phosphorochloridate (**58**, 1.8 mL of solution 1M in THF, 1.849 mmol). The crude product was purified by column chromatography, using as eluent

CHCl$_3$/MeOH (95:5), followed by a preparative TLC using as eluent CHCl$_3$/MeOH (85:15). The pure product was a white solid (11 mg, 2%).

δ_P (d_4-CH$_3$OH): 4.82, 4.32; δ_H (d_4-CH$_3$OH): 7.67 (1H, m, H6-cytidine, $J=$ 7.3 Hz), 7.41-7.21 (5H, m, CH-phenyl), 6.18 (1H, m, H1'-cytidine, $J=$ 4.0 Hz), 5.78 (1H, m, H5-cytidine, $J=$ 7.3 Hz), 4.39-4.27 (2H, m, H2'-cytidine, H3'-cytidine), 4.25-4.09 (5H, m, CHα, H5'-cytidine, CH$_2$-butyl), 1.63-1.58 (3H, m, CH$_3$-alanine), 1.41-1.23 (4H, m, CH$_2$-butyl), 0.95 (3H, m, CH$_3$-butyl).

MS (ES) m/e: 590.3 (MNa$^+$, 100%); Accurate mass: C$_{22}$H$_{29}$N$_6$O$_{10}$NaP required 590.1749, found 590.1740.

Synthesis of 4'-azidocytidine 5'-O-[p-chloro-phenyl(ethyl-L-leucinyl)] phosphate

(87)
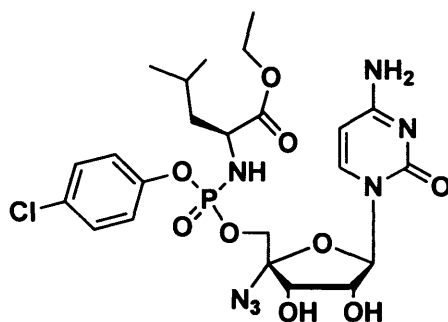
Prepared according to Standard Procedure C, from 4'-azidocytidine (**23**, 350 mg, 1.15 mmol), ^{t}BuMgCl (2.88 mL 1M solution of THF, 2.88 mmol) and p-chloro-phenyl(ethyl-L-leucinyl) phosphorochloridate (**82**, 2.88 mL of 1M solution in THF, 2.88 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (90:10). The pure product was a white solid (100 mg, 14%).

δ_P (d_4-CH$_3$OH): 5.19, 4.92; δ_H (d_4-CH$_3$OH): 7.74 (1H, m, H6-cytidine, $J=$ 7.4 Hz), 7.36 (2H, m, CH-phenyl), 7.24 (2H, m, CH-phenyl), 6.15 (1H, m, H1'cytidine), 5.96 (1H, m, H5-cytidine, $J=$ 7.4 Hz), 4.37 (1H, m, H2'-cytidine), 4.25 (1H, m, H3'-cytidine), 4.15 (2H, m, CH$_2$-ethyl), 4.13 (2H, m, H5'-cytidine), 3.88 (1H, m, CH-α), 1.73 (1H, m, CH-lateral chain), 1.55 (2H, m, CH$_2$-lateral chain), 1.25 (3H, m, CH$_3$-lateral chain), 1.19 (3H, m, CH$_3$-lateral chain), 0.90 (3H, m, CH$_3$-ethyl).

MS (ES) m/e: 638.1 (MNa$^+$, 100%); Accurate mass: C$_{23}$H$_{31}$N$_7$O$_9$NaPCl required 638.1495, found 638.1507.

Synthesis of 4'-azidocytidine 5'-*O*-[3,4-dichloro-phenyl(ethyl-L-leucinyl)] phosphate (88)

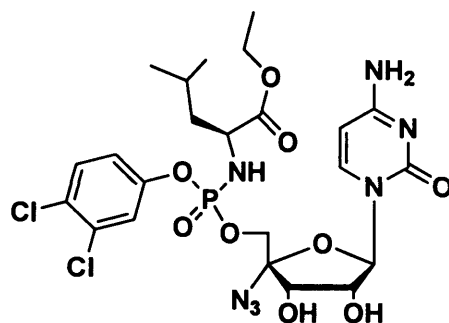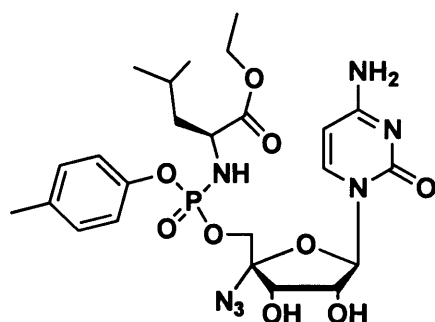Prepared according to Standard Procedure C, from 4'-azidocytidine (**23**, 350 mg, 1.15 mmol), 'BuMgCl (2.88 mL 1M solution of THF, 2.88 mmol) and 3,4-dichloro-phenyl(ethyl-L-leucinyl) phosphorochloridate (**83**, 2.88 mL of solution 1M in THF, 2.88 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (90:10) The pure product was a white solid (40 mg, 5%).

δ_P (d_4-CH$_3$OH): 5.27, 4.99; δ_H (d_4-CH$_3$OH): 7.70 (1H, m, H6-cytidine, J= 7.5 Hz), 7.54-7.48 (2H, m, CH-phenyl), 7.25, 7.18 (1H, m, CH-phenyl), 6.13 (1H, m, H1'cytidine), 5.93 (1H, t, H5-cytidine, J= 7.5 Hz), 4.39 (1H, m, H2'-cytidine), 4.28 (1H, m, H3'-cytidine), 4.13 (2H, m, CH$_2$-ethyl), 4.11 (2H, m, H5'-cytidine), 3.89 (1H, m, CH-α), 1.74 (1H, m, CH-lateral chain), 1.56 (2H, m, CH$_2$-lateral chain), 1.28 (3H, m, CH$_3$-lateral chain), 0.90 (3H, m, CH$_3$-ethyl); δ_C (d_4-CH$_3$OH): 175.19 (1C, C=O ester), 168.05 (1C, C4-cytidine), 156.62 (1C, C2-cytidine), 151.31 (1C, C-phenyl), 143.96, 143.49 (1C, C6-cytidine), 132.70 (2C, CH-phenyl), 131.85 (1C, C-phenyl), 124.17, 124.10 (1C, C-phenyl),122.32, 122.13, 122.06 (1C, CH-phenyl), 98.96, 98.83 (1C, C5-cytidine), 97.35, 97.23 (1C, C4'-cytidine), 94.14 (1C, C1'-cytidine), 74.84, 74.55 (1C, C3'-cytidine), 73.94, 73.81 (1C, C2'-cytidine), 69.65, 69.58 (1C, C5'-cytidine), 62.87, 62.78 (1C, CH$_2$-ethyl), 55.05, 54.98 (1C, CH-α), 44.38, 44.29 (1C, CH$_2$-lateral chain), 26.04, 25.91 (1C, CH-lateral chain), 23.68, 23.62, 23.52 (1C, CH$_3$-lateral chain), 22.23, 21.84 (1C, CH$_3$-lateral chain), 14.86 (1C, CH$_3$-ethyl).

MS (ES) m/e: 672.1 (MNa$^+$, 100%); Accurate mass: C$_{23}$H$_{30}$N$_7$O$_9$NaPCl$_2$ required 672.1101, found 672.1117.

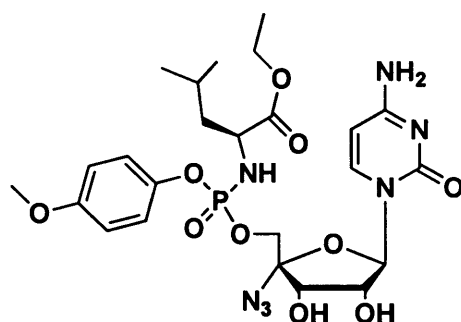## Synthesis of 4'-azidocytidine 5'-*O*-[*p*-methyl-phenyl(ethyl-L-leucinyl)] phosphate

(89)

Prepared according to Standard Procedure C, from 4'-azidocytidine (**23**, 350 mg, 1.15 mmol), 'BuMgCl (2.88 mL 1M solution of THF, 2.88 mmol) and *p*-methyl-phenyl(ethyl-L-leucinyl) phosphorochloridate (**84**, 2.88 mL of 1M solution in THF, 2.88 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (85:15) and a preparative TLC using as eluent $CHCl_3$/MeOH (85:15). The pure product was a white solid (14 mg, 4%).

δ_P (d_4-CH_3OH): 5.24, 4.90; δ_H (d_4-CH_3OH): 7.65 (1H, m, H6-cytidine, *J*= 7.5 Hz), 7.15 (2H, m, CH-phenyl), 7.06 (2H, m, CH-phenyl), 6.20 (1H, m, H1'cytidine), 5.89 (1H, t, H5-cytidine, *J*= 7.5 Hz), 4.32 (1H, m, H2'-cytidine), 4.23 (1H, m, H3'-cytidine), 4.17 (2H, m, CH_2-ethyl), 4.11 (2H, m, H5'-cytidine), 4.07 (1H, m, CH-α), 2.26 (3H, s, CH_3-*p*-methylphenyl), 1.72 (1H, m, CH-lateral chain), 1.55 (2H, m, CH_2-lateral chain), 1.25 (3H, m, CH_3-lateral chain), 1.18 (3H, m, CH_3-lateral chain), 0.91 (3H, m, CH_3-ethyl); δ_C (d_4-CH_3OH): 175.31 (1C, C=O ester), 167.97 (1C, C4-cytidine), 150.11 (1C, C2-cytidine), 143.31, 143.08 (1C, C6-cytidine), 131.65, 131.60 (1C, C-phenyl), 130.86 (2C, CH-phenyl), 121.65, 121.59 (1C, C-phenyl), 121.47, 121.41 (2C, CH-phenyl), 98.92 (1C, C5-cytidine), 97.35 (1C, C4'-cytidine), 93.89 (1C, C1'-cytidine), 75.03, 74.83 (1C, C3'-cytidine), 73.89 (1C, C2'-cytidine), 69.26, 69.07 (1C, C5'-cytidine), 62.78, 62.72 (1C, CH_2-ethyl), 54.96 (1C, CHα), 44.54 (1C, CH_2-lateral chain), 26.03, 25.81 (1C, CH-lateral chain), 23.65, 23.56, 23.44 (1C, CH_3, lateral chain), 23.18, 22.39 (1C, CH_3, lateral chain), 14.67, 14.45 (1C, CH_3-ethyl).

Synthesis of 4'-azidocytidine 5'-*O*-[*p*-methoxy-phenyl(ethyl-L-leucinyl)]

phosphate (90)

Prepared according to Standard Procedure C, from 4'-azidocytidine (**23**, 350 mg, 1.15 mmol), 'BuMgCl (2.88 mL 1M solution of THF, 2.88 mmol) and *p*-methoxy-phenyl(ethyl-L-leucinyl) phosphorochloridate (**85**, 2.88 mL of 1M solution in THF, 2.88 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (90:10) and then a preparative TLC using as eluent $CHCl_3$/MeOH (85:15). The pure product was a white solid (20 mg, 3%).

δ_P (d_4-CH_3OH): 5.59, 5.22; δ_H (d_4-CH_3OH): 7.71 (1H, m, H6-cytidine, J= 7.6 Hz), 7.17 (2H, m, CH-phenyl), 6.91 (2H, m, CH-phenyl), 6.22 (1H, m, H1'cytidine), 5.96 (1H, m, H2-cytidine, J= 7.6 Hz), 4.36 (1H, m, H2'-cytidine), 4.34 (1H, m, H3'-cytidine), 4.17 (2H, m, CH_2-ethyl), 4.10 (2H, m, H5'-cytidine), 3.91 (1H, m, CH-α), 3.79 (3H, s, CH_3O-phenyl), 1.74 (1H, m, CH-lateral chain), 1.56 (2H, m, CH_2-lateral chain), 1.27 (3H, m, CH_3-lateral chain), 1.21 (3H, m, CH_3-lateral chain), 0.92 (3H, m, CH_3-ethyl); δ_C (d_4-CH_3OH): 175.33 (1C, C=O ester), 167.50 (1C, C4-cytidine), 158.99 (1C, C2-cytidine), 145.85, 145.76 (1C, C-phenyl), 143.64, 143.42 (1C, C6-cytidine), 122.85, 122.79 (1C, C-phenyl), 122.67, 122.61 (2C, CH-phenyl), 157.11 (1C, C-phenyl), 99.30, 99.10, 98.97 (1C, C5-cytidine), 97.47, 97.39 (1C, C4'-cytidine), 93.90 (1C, C1'-cytidine), 74.99, 74.75, 74.05 (1C, C3'-cytidine), 73.90 (1C, C2'-cytidine), 69.22, 69.15 (1C, C5'-cytidine), 62.80, 62.73, 62.23 (1C, CH_2-ethyl), 56.53, 56.44 (1C, CH_3O-phenyl), 55.10, 54.96 (1C, CHα), 44.55, 44.45 (1C, CH_2-lateral chain), 26.04, 25.81 (1C, CH-lateral chain), 23.69, 23.59, 23.46, 23.23 (1C, CH_3, lateral chain), 22.41, 21.97 (1C, CH_3, lateral chain), 14.78, 14.56 (1C, CH_3-ethyl).

Synthesis   of   2',3'-*O,O*-cyclopentylidene-4'-azidocytidine   5'-*O*-[α-naphthyl(benzyl-L-alaninyl)] phosphate (97.a/97.b)
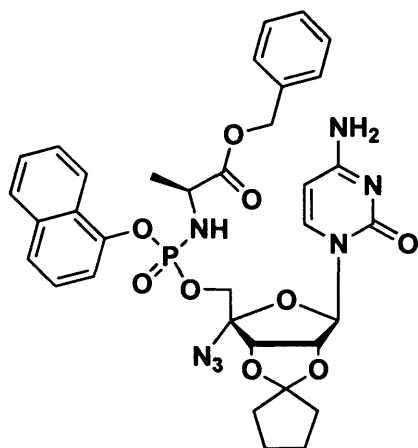
Prepared according to Standard Procedure C2, from 2',3'-*O,O*-cyclopentylidene-4'-azidocytidine (**95**, 200 mg, 0.570 mmol), ^tBuMgCl (1.1 mL of 1M solution in THF, 1.14 mmol) and α-naphthyl(benzyl-L-alaninyl) phosphorochloridate (**93**, 1.1 mL of solution 1M in THF, 1.14 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/Me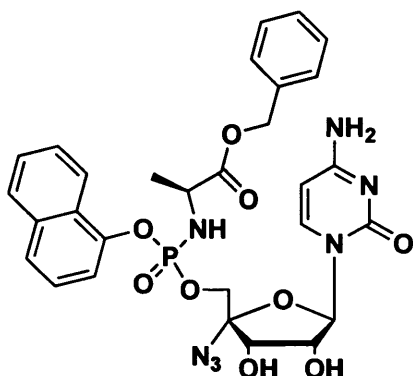OH (95:5) and preparative HPLC to separate the 2 diastereoisomers. The pure product was a white solid (50 mg of the more polar diastereoisomer **97.a**, 40 mg of the less polar diastereoisomer **97.b**, overall 347 mg of mixture, 85%).

More Polar Diasteroisomer: δ_P (d_4-CH_3OH): 3.45; δ_H (d_4-CH_3OH): 7.94 (1H, d, CH-naphthyl, *J*= 7.4 Hz), 7.67 (1H, m, CH-naphthyl), 7.49 (1H, d, H6-cytidine, *J*= 8.1 Hz), 7.34-7.30 (3H, m, CH-naphthyl, CH-phenyl), 7.27 (1H, d, CH-naphthyl, *J*= 7.7 Hz), 7.18 (1H, t, CH-naphthyl, *J*= 8.0 Hz), 7.08-7.05 (6H, CH-naphthyl, CH-phenyl) 5.69 (1H, d, H1'-cytidine, *J*= 1.7 Hz), 5.59 (1H, d, H5-cytidine, *J*= 7.4 Hz), 4.84 (2H, m, CH_2-benzyl), 4.76 (H, m, H3'-cytidine, *J*= 6.3 Hz), 4.69 (1H, d, H2'-cytidine, *J*= 1.7 Hz), 4.10 (2H, m, H5'-cytidine), 3.87 (1H, m, CHα), 1.96 (2H, m, CH_2-cyclopentyl), 1.53-1.49 (6H, m, CH_2-cyclopentyl), 1.14 (3H, d, CH_3-alanine, *J*= 0.9 Hz).

Less Polar Diasteroisomer: δ_P (d_4-CH_3OH): 3.41; δ_H (d_4-CH_3OH): 7.92 (1H, m, CH-naphthyl), 7.72 (1H, d, H6-cytidine, *J*= 7.3 Hz), 7.56-7.50 (4H, m, CH-naphthyl, CH-phenyl), 7.42 (1H, t, CH-naphthyl, *J*= 8.0 Hz), 7.35-7.31 (6H, CH-naphthyl, CH-phenyl) 5.87 (1H, d, H1'-cytidine, *J*= 1.6 Hz), 5.79 (1H, d, H5-cytidine, *J*= 7.3 Hz), 5.12 (2H, m, CH_2-benzyl), 5.00 (H, d, H3'-cytidine, *J*= 6.4 Hz), 4.97 (1H, d, H2'-cytidine, *J*= 1.6 Hz), 4.35 (2H, m, H5'-cytidine), 4.29 (1H, m, CHα), 2.25 (2H, m, CH_2-cyclopentyl), 1.76-1.72 (6H, m, CH_2-cyclopentyl), 1.37 (3H, d, CH_3-alanine, *J*= 0.6 Hz).

Synthesis of 4'-azidocytidine 5'-O-[α-naphthyl(benzyl-L-alaninyl) phosphate (98.a/98.b)

Prepared according to Standard Procedure C3, 2',3'-*O,O*-cyclopentylidene-4'-azidocytidine 5'-*O*-[α-naphthyl(benzyl-L-alaninyl)] phosphate (50 mg more polar **97.a**, 0.077 mmol, 40 mg less polar diastereoisomer **97.b**, 0.056 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (8:2). The pure product was a white solid (40 mg of the more polar diastereoisomer **98.a**, 80%, 30 mg of the less polar diastereoisomer **98.b**, 82%).

More Polar Diasteroisomer: δ_P (d_4-CH_3OH): 3.75; δ_H (d_4-CH_3OH): 8.18 (1H, m, CH-naphthyl), 7.90 (1H, m, CH-naphthyl), 7.72 (1H, d, H6-cytidine, $J=$ 8.2 Hz), 7.58-7.51 (4H, m, CH-naphthyl, CH-phenyl), 7.41 (1H, t, CH-naphthyl, $J=$ 7.8 Hz), 7.33-7.28 (5H, m, CH-naphthyl, CH-phenyl), 6.11 (1H, d, H1'-cytidine, $J=$ 4.5 Hz), 5.43 (1H, d, H5-cytidine, $J=$ 8.2 Hz), 5.09 (2H, m, CH_2-benzyl), 4.38 (1H, d, H3'-cytidine, $J=$ 5.9 Hz), 4.31-4.22 (3H, m, H2'-cytidine, H5'-cytidine), 4.10 (1H, m, CHα), 1.34 (3H, d, CH_3-alanine, $J=$ 7.1 Hz).

MS (ES) m/e: 674.2 (MNa$^+$, 100%); Accurate mass: $C_{29}H_{30}N_7O_9NaP$ required 674.1740, found 674.1751.

Less Polar Diasteroisomer: δ_P (d_4-CH_3OH): 3.87; δ_H (d_4-CH_3OH): 8.06 (1H, m, CH-naphthyl), 7.78 (1H, m, CH-naphthyl), 7.65 (1H, d, H6-cytidine, $J=$ 7.5 Hz), 7.45-7.35 (4H, m, CH-naphthyl, CH-phenyl), 7.25 (1H, t, CH-naphthyl, $J=$ 7.8 Hz), 7.21-7.12 (5H, m, CH-naphthyl, CH-phenyl), 6.03 (1H, d, H1'-cytidine, $J=$ 3.4 Hz), 5.65 (1H, d, H5-cytidine, $J=$ 7.5 Hz), 4.94 (2H, m, CH_2-benzyl), 4.27 (1H, d, H3'-cytidine, $J=$ 4.8 Hz), 4.20-4.12 (3H, m, H2'-cytidine, H5'-cytidine), 3.96 (1H, m, CHα), 1.23 (3H, d, CH_3-alanine, $J=$ 6.8 Hz).

MS (ES) m/e: 674.2 (MNa$^+$, 100%); Accurate mass: $C_{29}H_{30}N_7O_9NaP$ required 674.1740, found 674.1725.

Synthesis of 4'-azidocytidine 5'-*O*-[β-naphthyl(benzyl-L-alaninyl)] phosphate (99.a/99.b)
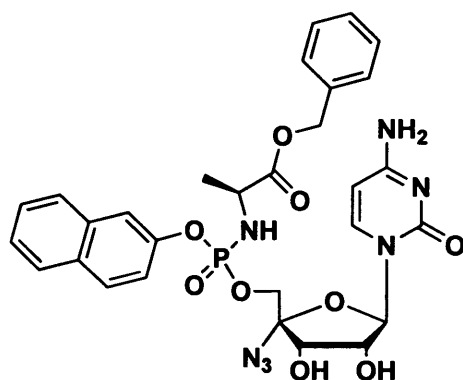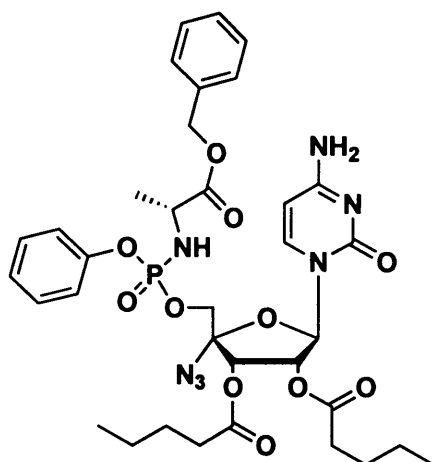

Prepared according to Standard Procedure C, from 4'-azidocytidine (**23**, 200 mg, 0.657 mmol), ^{t}BuMgCl (1.7 mL, 1M solution in THF, 1.64 mmol) and β-naphthyl(benzyl-L-alaninyl) phosphorochloridate (**94**, 1.7 mL of solution 1M in THF, 1.64 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5) and preparative HPLC to separate the 2 diastereoisomers. The pure product was a white solid (10 mg of the more polar diastereoisomer **99.a**, 15 mg of the less polar diastereoisomer **99.b**, with an overall yield of 5%)

More Polar Diasteroisomer: δ$_P$ (d_4-CH$_3$OH): 3.62; δ$_H$ (d_4-CH$_3$OH): 7.88-7.64 (5H, m, H6-cytidine, CH-naphthyl), 7.52-7.47 (2H, m, CH-naphthyl, CH-phenyl), 7.38-7.31 (6H, m, CH-naphthyl, CH-phenyl), 6.16 (1H, d, H1'-cytidine, *J*= 4.6 Hz), 5.83 (1H, d, H5-cytidine, *J*= 7.4 Hz), 5.13 (2H, m, CH$_2$-benzyl), 4.38 (1H, d, H3'-cytidine, *J*= 5.8 Hz), 4.31-4.26 (3H, m, H2'-cytidine, H5'-cytidine), 4.12 (1H, m, CHα), 1.37 (3H, d, CH$_3$-alanine, *J*= 7.1 Hz).

Less Polar Diasteroisomer: δ$_P$ (d_4-CH$_3$OH): 3.50; δ$_H$ (d_4-CH$_3$OH): 7.88 (2H, d, 2 CH-naphthyl, *J*= 8.7 Hz), 7.81 (1H, d, CH-naphthyl, *J*= 8.0 Hz), 7.62 (1H, d, H6-cytidine, *J*= 7.5 Hz), 7.52-7.48 (2H, m, CH-naphthyl, CH-phenyl), 7.37 (1H, m, CH-phenyl), 7.31 (6H, m, CH-naphthyl, CH-phenyl), 6.13 (1H, d, H1'-cytidine, *J*= 5.5 Hz), 5.78 (1H, d, H5-cytidine, *J*= 7.5 Hz), 5.09 (2H, m, CH$_2$-benzyl), 4.39 (1H, d, H3'-cytidine, *J*= 5.9 Hz), 4.32 (1H, d, H2'-cytidine), 4.27-4.22 (2H, m, H5'-cytidine), 4.12 (1H, m, CHα), 1.38 (3H, d, CH$_3$-alanine, *J*= 7.1 Hz).

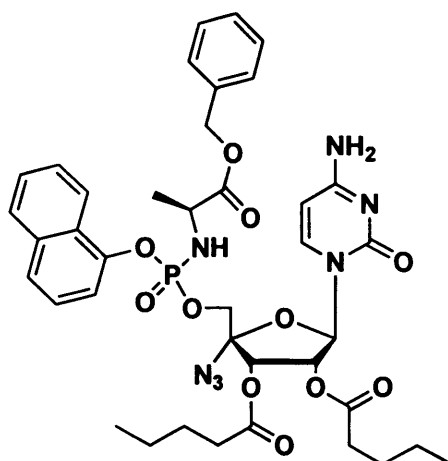**Synthesis of,4'-azido-2',3'-dipentanoatecytidine 5'-*O*-[phenyl(benzyl-D-alaninyl)] phosphate (101)**

Prepared according to Standard Procedure C1, from 4'-azido-2',3'-dipentanoatecytidine (**28**, 250 mg, 0.570 mmol), ^{t}BuMgCl (1.1 mL 1M solution of THF, 1.140 mmol) and phenyl(benzyl-D-alanine) phosphorochloridate (**54**, 1.1 mL of solution 1M in THF, 1.140 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (320 mg, 72%).

δ_P (d_4-CH_3OH): 4.60, 4.13; δ_H (d_4-CH_3OH): 7.59 (1H, m, H6-cytidine, *J*= 7.5 Hz), 7.34-7.18 (10H, m, CH-phenyl, CH-benzyl), 6.15 (1H, m, H1'-cytidine, *J*= 4.0 Hz), 5.89-5.81 (2H, m, H5-cytidine, H2'-cytidine), 5.63-5.59 (1H, m, H3'-cytidine), 5.14 (2H, s, CH_2-benzyl), 4.37-4.29 (2H, m, H5'-cytidine), 4.05 (1H, m, CHα), 2.40 (4H, t, CH_2-dipentanoate, *J*= 7.2 Hz), 1.61 (4H, m, CH_2-dipentanoate), 1.40-1.34 (7H, m, CH_3-alanine, CH_2-dipentanoate), 0.93 (6H, t, CH_3-dipentanoate, *J*= 7.3 Hz); δ_C (d_4-CH_3OH): 175.31, 175.26 (1C, C=O ester), 175.06, 174.99 (1C, C=O dipentanoate), 173.59, 173.56 (1C, C=O dipentanoate), 168.29 (1C, C4-cytidine), 158.00 (1C, C2-cytidine), 152.42, 152.37, 152.33, 152.28 (1C, C-phenyl), 144.61, 144.41 (1C, C6-cytidine), 137.61 (1C, C-phenyl), 131.27 (2C, 2 CH-phenyl), 130.14 (2C, 2 CH-phenyl), 129.75, 129.70 (2C, 2 CH-phenyl), 126.77, 126.69 (1C, CH-phenyl), 122.05, 121.99, 121.92 (1C, CH-phenyl), 98.06, 97.92 (1C, C5-cytidine), 97.41 (1C, C4'-cytidine), 94.23, 93.73 (1C, C1'-cytidine), 74.03 (1C, C3'-cytidine), 72.95, 72.62 (1C, C2'-cytidine), 69.34, 69.28, 68.86, 68.80 (1C, C5'-cytidine), 68.51, 68.45 (1C, CH_2-benzyl), 52.14, 52.90 (1C, CHα), 34.85, 34.70 (2C, 2 CH_2-dipentanoate), 28.41, 28.36

(2C, CH_2-dipentanoate), 23.64 (2C, CH_2-dipentanoate), 20.92, 20.84, 20.79, 20.69 (1C, CH_3-alanine), 14.55 (2C, CH_3-dipentanoate).

MS (ES) m/e: 792.2 (MNa$^+$, 100%); Accurate mass: $C_{35}H_{44}N_7O_{11}NaP$ required 792.2729, found 792.2734.

Synthesis of, 4'-azido-2',3'-dipentanoatecytidine 5'-*O*-[α-naphthyl(benzyl-L-alaninyl)] phosphate (102.a/102.b)
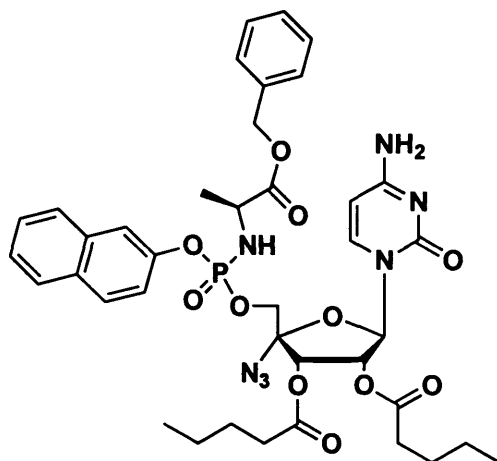

Prepared according to Standard Procedure C1, from 4'-azido-2',3'-dipentanoatecytidine (**28**, 150 mg, 0.342 mmol), 'BuMgCl (0.7 mL 1M solution of THF, 0.684 mmol) and α-naphthyl(benzyl-L-alanine) phosphorochloridate (**93**, 0.69 mL of solution 1M in THF, 0.684 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (224 mg, 80%).

δ_P (d_4-CH_3OH): 3.55, 3.54; δ_H (d_4-CH_3OH): 7.93 (1H, m, CH-naphthyl), 7.64 (1H, m, H6-cytidine), 7.46 (1H, m, CH-naphthyl), 7.30-7.03 (10H, CH-naphthyl, CH-benzyl), 5.84 (1H, m, H1'-cytidine, *J*= 3.8 Hz), 5.66 (1H, m, H5-cytidine, *J*= 6.6 Hz), 5.56 (1H, m H3'-cytidine), 5.39 (1H, m H2'-cytidine), 4.84 (2H, m, CH_2-benzyl), 4.17 (2H, m, H5'-cytidine), 3.86 (1H, m, CHα), 2.18-2.09 (4H, m, CH_2-dipentanoate), 1.63 (3H, m, CH_3-alanine), 1.38-1.32 (4H, m, CH_2-dipentanoate), 1.17-1.02 (4H, m, CH_2-dipenatanoate), 0.71-0.63 (6H, t, CH_3-dipentanoate).

MS (ES) m/e: 842.4 (MNa$^+$, 100%); Accurate mass: $C_{39}H_{46}N_7O_{11}NaP$ required 842.2891, found 842.2913.

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Synthesis of 4'-azido-2',3'-dipentanoatecytidine 5'-*O*-[β-naphthyl(benzyl-L-alaninyl)] phosphate (103)

Prepared according to Standard Procedure C1, from 4'-azido-2',3'-dipentanoatecytidine (**28**, 200 mg, 0.570 mmol), 'BuMgCl (1.14 mL 1M solution of THF, 1.140 mmol) and β-naphthyl(benzyl-L-alanine) phosphorochloridate (**94**, 1.14 mL of solution 1M in THF, 1.140 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (120 mg, 26%).

δ_P (d_4-CH_3OH): 3.32, 3.28; δ_H (d_4-CH_3OH): 7.87-7.29 (13H, m, CH-phenyl, CH-naphthyl, H6-cytidine), 6.11 (1H, d, H1'-cytidine, *J*= 4.0 Hz), 5.90-5.84 (2H, m, H5-cytidine, H2'-cytidine), 5.63-5.59 (1H, m, H3'-cytidine), 5.11 (2H, m, CH_2-benzyl), 4.40-4.35 (2H, m, H5'-cytidine), 4.12 (1H, m, CHα), 2.39 (4H, m, CH_2-dipentanoate), 1.61 (4H, m, CH_2-dipentanoate), 1.41-1.33 (7H, m, CH_3-alanine, CH_2-dipentanoate), 0.93 (6H, m, CH_3-dipentanoate); δ_C (d_4-CH_3OH): 174.57 (1C, C=O ester), 173.93, 173.88 (1C, C=O dipentanoate), 173.21, 173.16 (1C, C=O dipentanoate), 167.94, 167.86 (1C, C4-cytidine), 157.64, 157.60 (1C, C2-cytidine), 149.54 (1C, C-naphtyl), 144.20, 143.97 (1C, C6-cytidine), 137.24, 137.21 (1C, C-phenyl), 135.33 (2C, C-naphthyl), 132.52 (2C, CH-phenyl), 131.04, 130.98 (2C, CH-phenyl), 129.58, 129.30, 129.26 (3C, CH-naphthyl), 128.84, 128.82, 128.60, 128.55 (2C, CH-naphthyl), 127.93, 127.92 (1C, CH-phenyl), 126.74, 126.70 (1C, CH-naphthyl), 121.42, 121.38, 121.31 (1C, CH-phenyl), 117.93, 117.89, 117.84, 117.80 (2C, CH-naphthyl), 97.71, 97.65, 97.58 (1C, C5-cytidine), 96.97, 96.93 (1C, C4'-cytidine), 93.80, 93.54 (1C, C1'-cytidine), 73.66, 73.62 (1C, C3'-cytidine), 72.81, 72.62 (1C, C2'-cytidine), 69.05, 69.02 (1C, C5'-cytidine), 68.88, 68.08, 68.02 (1C, CH_2-benzyl), 51.79, 51.72 (1C,

CHα), 34.43, 34.27 (2C, CH$_2$-dipentanoate), 27.99, 27.95 (2C, CH$_2$-dipentanoate), 23.22, 23.20 (2C, CH$_2$-dipentanoate), 20.47, 20.42, 20.35, 20.29 (1C, CH$_3$-alanine), 14.06 (2C, CH$_3$-dipentanoate).

7.7 Synthesis of uridine analogue phosphoramidates.

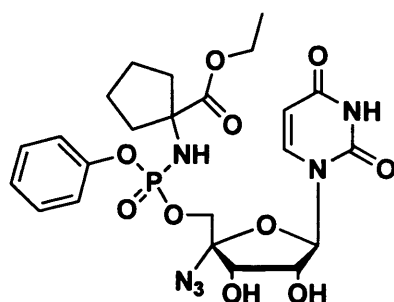**Synthesis of 4'-azidouridine 5'-*O*-[phenyl(benzyl-cyclopentylglycinyl)] phosphate**

(112)
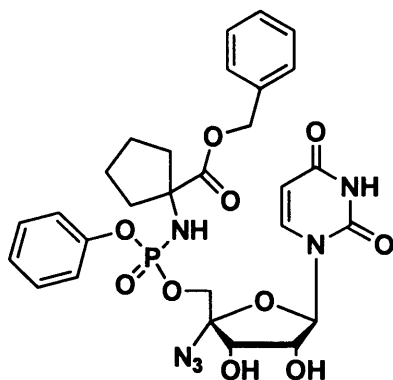
Prepared according to Standard Procedure D, from 4'-azidouridine (**24**, 300 mg, 1.052 mmol), 'BuMgCl (2.10 mL of solution 1M in THF, 2.10 mmol) and phenyl(benzyl-cyclopentylglycinyl) phosphorochloridate (**51**, 2.10 mL of solution 1M in THF, 2.10 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (95:5) then a preparative TLC using as eluent CHCl$_3$/MeOH (9:1). The pure product was a white solid (130 mg, 20%).

δ_P (d_4-CH$_3$OH): 3.77, 3.74; δ_H (d_4-CH$_3$OH): 7.58 (1H, m, H6-uridine, J= 8.13 Hz), 7.28 (7H, m, CH-phenyl, CH-benzyl), 7.15 (3H, m, CH-benzyl), 6.09 (1H, m, H1'-uridine), 5.55 (1H, m, H5-uridine, J= 8.13 Hz), 5.08 (2H, s, CH$_2$-phenyl), 4.29 (1H, m, H2'-uridine), 4.24 (1H, m, H3'-uridine), 4.09 (2H, m, H5'-uridine), 2.04 (2H, m, CH$_2$-cyclopentyl), 1.98 (2H, m, CH$_2$-cyclopentyl), 1.64 (2H, m, CH$_2$-cyclopentyl), 1.55 (2H, m, CH$_2$-cyclopentyl); δ_C (d_4-CH$_3$OH): 176.83 (1C, C=O ester), 166.20 (1C, C4-uridine), 152.62, 152.48, 152.39 (1C, C2-uridine), 143.00, 142.88 (1C, C6-uridine), 137.75, 137.73 (1C, C-phenyl), 131.22 (2C, CH-phenyl), 129.98 (1C, C-benzyl), 129.73 (2C, CH-phenyl), 129.69 (1C, CH-phenyl), 126.74 (2C, CH-benzyl), 122.00 (1C, CH-benzyl), 121.98, 121.93 (2C, CH-benzyl), 103.99, 103.96 (1C, C5-uridine), 99.23, 99.20, 99.06 (1C, C4'-uridine), 92.32, 92.13 (1C, C1'-uridine), 74.86 (1C, C3'-uridine), 69.32 (1C, C2'-uridine), 69.25, 68.79, (1C, C5'-urdine), 68.75, 68.62 (1C, CH$_2$-benzyl), 40.35, 40.24 (1C, CH$_2$-cyclopentyl), 40.07, 39.55 (1C, CH$_2$-cyclopentyl), 25.10 (1C, CH$_2$-cyclopentyl), 24.99 (1C, CH$_2$-cyclopentyl).

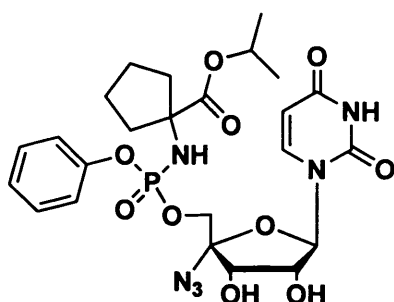## Synthesis of 4'-azidouridine 5'-*O*-[phenyl(ethyl-cyclopentylglycinyl)] phosphate

(113)

Prepared according to Standard Procedure D, from 4'-azidouridine (**24**, 300 mg, 1.052 mmol), ʹBuMgCl (2.10 mL of solution 1M in THF, 2.10 mmol) and phenyl(ethyl-cyclopentylglycinyl) phosphorochloridate (**52**, 2.10 mL of solution 1M in THF, 2.10 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5) then a preparative TLC using as eluent $CHCl_3$/MeOH (9:1). The pure product was a white solid (100 mg, 16%).

δ_P (d_4-CH_3OH): 3.85, 3.83; δ_H (d_4-CH_3OH): 7.69 (1H, m, H6-uridine, *J*= 8.1 Hz), 7.38 (2H, m, CH-phenyl), 7.27 (1H, m, CH-phenyl), 7.23 (2H, m, CH-phenyl), 6.18 (1H, m, H1'-uridine), 5.87 (1H, m, H5-uridine, *J*= 8.1 Hz), 4.40 (2H, m, CH_2-ethyl) 4.35 (1H, m, H2'-uridine), 4.22 (1H, m, H3'-uridine), 4.17 (2H, m, H5'-uridine), 2.11 (2H, m, CH_2-cyclopentyl), 1.97 (2H, m, CH_2-cyclopentyl), 1.73 (2H, m, CH_2-cyclopentyl), 1.64 (2H, m, CH_2-cyclopentyl), 1.25 (3H, m, CH_3-ethyl); δ_C (d_4-CH_3OH): 177.05 (1C, C=O ester), 166.22 (1C, C4-uridine), 152.63, 152.51, 152.42 (1C, C2-uridine), 143.06, 142.94 (1C, C6-uridine), 131.23 (2C, CH-phenyl), 126.75 (1C, CH-phenyl), 122.05, 121.99, 121.93 (2C, CH-phenyl), 103.94 (1C, C5-uridine), 99.29, 99.24, 99.15, 99.10 (1C, C4'-uridine), 92.23, 92.14 (1C, C1'-uridine), 74.30, 74.24 (1C, C3'-uridine), 69.34 (1C, C2'-uridine), 68.75 (1C, C5'-uridine), 62.95 (1C, C-cyclopentyl), 55.20 (1C, CH_2-ethyl), 40.37, 40.25 (1C, CH_2-cyclopentyl), 40.12, 39.52, 39.47 (1C, CH_2-cyclopentyl), 25.10, (1C, CH_2-cyclopentyl), 24.99 (1C, CH_2-cyclopentyl), 14.85 (1C, CH_3-ethyl).

Synthesis of 4'-azidouridine 5'-*O*-[phenyl(isopropyl-cyclopentylglycinyl)] phosphate (114)

Prepared according to Standard Procedure D, from 4'-azidouridine (24, 300 mg, 1.052 mmol), 'BuMgCl (2.10 mL of solution 1M in THF, 2.10 mmol) and phenyl(isopropyl-cyclopentylglycinyl) phosphorochloridate (53, 2.10 mL, 1M solution in THF, 2.10 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (95:5) then a preparative TLC using as eluent CHCl$_3$/MeOH (9:1). The pure product was a white solid (110 mg, 12%).

δ_P (d_4-CH$_3$OH): 3.87, 3.83; δ_H (d_4-CH$_3$OH): 7.69 (1H, m, H6-uridine, $J=$ 8.1 Hz), 7.37 (2H, m, CH-phenyl), 7.27 (1H, m, CH-phenyl), 7.24 (2H, m, CH-phenyl), 6.18 (1H, m, H1'-uridine), 5.66 (1H, m, H5-uridine, $J=$ 8.1 Hz), 5.01 (1H, m, CH-isopropyl, $J=$ 3.4 Hz), 4.40 (1H, m, H2'-uridine), 4.35 (1H, m, H3'-uridine), 4.24 (2H, m, H5'-uridine), 2.10 (2H, m, CH$_2$-cyclopentyl), 2.02 (2H, m, CH$_2$-cyclopentyl), 1.73 (2H, m, CH$_2$-cyclopentyl), 1.67 (2H, m, CH$_2$-cyclopentyl), 1.24 (6H, d, CH$_3$-isopropyl, $J=$ 3.4 Hz); δ_C (d_4-CH$_3$OH): 176.58 (1C, C=O ester), 166.20 (1C, C4-uridine), 152.64 (1C, C2-uridine), 152.52, 152.43 (1C, C4-uridine), 143.10, 142.95 (1C, C6-uridine), 131.26 (1C, CH-phenyl), 126.76 (1C, C-phenyl), 122.07, 122.08, 121.95 (1C, C-phenyl), 104.00, 103.95 (1C, C5-uridine), 99.26, 99.17, 99.12 (1C, C4'-uridine), 92.35, 92.15 (1C, C1'-uridine), 74.86 (1C, C3'-uridine), 70.65 (1C, C2'-uridine), 69.37, 69.30, 68.76 (1C, C5'-urdine), 67.31 (1C, C-cyclopentyl), 40.35, 40.24 (1C, CH$_2$-cyclopentyl), 39.51 (1C, CH$_2$-cyclopentyl), 25.19 (1C, CH$_2$-cyclopentyl), 25.10 (1C, CH$_2$-cyclopentyl), 22.35 (2C, CH$_3$-isopropyl).

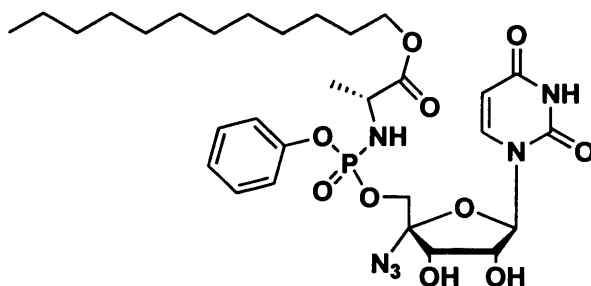**Synthesis of 4'-azidouridine 5'-*O*-[phenyl(benzyl-D-alaninyl)] phosphate (115)**
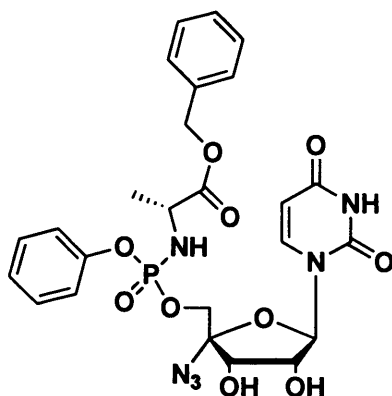
Prepared according to Standard Procedure D, from 4'-azidouridine (24, 200 mg, 0.701 mmol), 'BuMgCl (1.40 mL of solution 1M in THF, 1.40 mmol) and phenyl(benzyl-D-alaninyl) phosphorochloridate (55, 2.10 mL of solution 1M in THF, 2.10 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5) then a preparative TLC using as eluent $CHCl_3$/MeOH (9:1). The pure product was a white solid (100 mg, 16%).

δ_P (d_4-CH$_3$OH): 4.89, 4.29; δ_H (d_4-CH$_3$OH): 7.61 (1H, m, H6-uridine), 7.36 (7H, m, CH-phenyl, CH-benzyl), 7.25 (3H, m, CH-phenyl), 6.15 (1H, m, H1'-uridine), 5.68 (1H, m, H5-uridine), 5.17 (2H, s, CH_2-benzyl), 4.38 (1H, m, H3'-uridine), 4.32 (1H, m, H2'-uridine), 4.23 (2H, m, H5'-uridine), 4.05 (1H, m, CHα), 1.36 (3H, m, CH$_3$-alanine); δ_C (d_4-CH$_3$OH): 175.34, 175.29, 175.07, 175.01 (1C, C=O ester), 166.22 (1C, C4-uridine), 152.65, 152.56, 152.40, 152.36, 152.31, 152.27 (1C, C2-uridine), 142.94, 142.86 (1C, C6-uridine), 137.60, 137.54 (1C, C-phenyl), 131.31 (1C, C-benzyl), 130.00, 129.79 (2C, CH-phenyl), 129.76, 129.72 (2C, CH-benzyl), 126.79 (2C, CH-benzyl), 121.83, 121.77, 121.71, 121.64 (2C, CH-phenyl), 104.03,103.99 (1C, C5-uridine), 99.11, 98.98 (1C, C4'-uridine), 92.69, 92.43 (1C, C1'-uridine), 74.22, 74.16 (1C, C3'-uridine), 74.13, 73.93 (1C, C2'-uridine), 69.28, 69.21 (1C, CH_2-benzyl), 68.71, 68.65, 68.54, 68.48 (1C, C5'-uridine), 52.17, 51.92 (1C, CHα), 20.80, 20.70, 20.59 (1C, CH$_3$-lateral chain).

MS (ES) m/e: 625.1 (MNa$^+$, 100%); Accurate mass: $C_{25}H_{27}N_6O_{10}NaP$ required 625.1424, found 625.1424.

Anal. Calc. for $C_{25}H_{27}N_6O_{10}P$: C 49.84%, H 4.52%, N 13.95%. Found: C 50.30%, H 4.32%, N 14.03%.

Synthesis of 4'-azidouridine 5'-*O*-[phenyl(dodecyl-D-alaninyl)] phosphate (116)

Prepared according to Standard Procedure C, from 4'-azidouridine (**24**, 200 mg, 0.701 mmol), 'BuMgCl (1.40 mL 1M solution of THF, 1.402 mmol) and phenyl(dodecyl -D-alaninyl) phosphorochloridate (**58**, 1.40 mL of solution 1M in THF, 1.402 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5), followed by a preparative TLC using as eluent $CHCl_3$/MeOH (85:15). The pure product was a white solid (19 mg, 4%).

δ_P (d_4-CH_3OH): 4.92, 4.38; δ_H (d_4-CH_3OH): 7.67 (1H, m, H6-uridine, J= 8.7 Hz), 7.42-7.22 (5H, m, CH-phenyl), 6.15 (1H, m, H1'-uridine), 5.73 (1H, m, H5-uridine, J= 8.7 Hz), 4.40-3.95 (7H, m, H2'-uridine, H3'-uridine, H5'-uridine, CHα, CH_2-dodecyl), 1.64 (3H, m, CH_3-alanine), 1.30 (22H, m, CH_2-dodecyl), 0.92 (3H, t, CH_3-dodecyl).

Synthesis of 4'-azidouridine 5'-*O*-[phenyl(butyl-D-alaninyl)] phosphate (117)
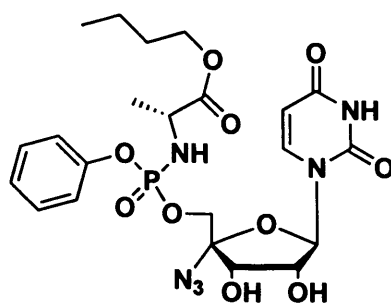
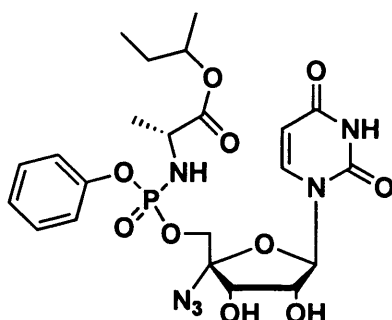Prepared according to Standard Procedure C, from 4'-azidouridine (**24**, 300 mg, 0.986 mmol), 'BuMgCl (2.46 mL 1M solution of THF, 2.46 mmol) and phenyl(butyl-D-alaninyl) phosphorochloridate (**57**, 2.46 mL of solution 1M in THF, 2.46 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5), followed by a preparative TLC using as eluent $CHCl_3$/MeOH (85:15). The pure product was a white solid (22 mg, 4%).

δ_P (d_4-CH_3OH): 4.81, 4.59; δ_H (d_4-CH_3OH): 7.60 (1H, m, H6-uridine), 7.38-7.18 (5H, m, CH-phenyl), 6.11 (1H, m, H1'-uridine), 5.66 (1H, m, H5-uridine), 4.33-4.30 (2H,

m, H2'-uridine, H3'-uridine), 4.17-4.04 (5H, m, CHα, H5'-uridine, CH₂-butyl), 1.59

(3H, m, CH₃-alanine), 1.55 (2H, m, CH₂-butyl), 1.36-1.25 (2H, m, CH₂butyl), 0.95

(3H, m, CH₃-butyl).

M:(3H, m, CH₃-alanine), 1.55 (2H, m, CH₂-butyl), 1.36-1.25 (2H, m, CH₂butyl), 0.95

591.1577, found 591.1580.

Synthesis of 4'-azidouridine 5'-*O*-[phenyl(2-butyl-D-alaninyl)] phosphate (118)

Prepared according to Standard Procedure C, from 4'-azidouridine (**24**, 200 mg, 0.701 mmol), ʰBuMgCl (1.40 mL 1M solution of THF, 1.40 mmol) and phenyl(2-butyl-D-alaninyl) phosphorochloridate (**108**, 1.40 mL of solution 1M in THF, 1.40 mmol). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (95:5), followed by a preparative TLC using as eluent CHCl₃/MeOH (85:15). The pure product was a white solid (18 mg, 5%).

δ$_P$ (d₄-CH₃OH): 4.97, 4.41; δ$_H$ (d₄-CH₃OH): 7.70 (1H, m, H6-uridine, *J*= 10.0 Hz), 7.42-7.21 (5H, m, CH-phenyl), 6.15 (1H, m, H1'-uridine), 5.72 (1H, m, H5-uridine, *J*= 10.0 Hz), 4.40-4.15 (4H, m, H2'-uridine, H3'-uridine, H5'-uridine), 3.91 (1H, m, CHα), 1.61 (3H, d, CH₃-alanine), 1.40-1.19 (5H, m, CH₂-2-butyl, CH₃-2-butyl), 0.91 (3H, m, CH₃-2-butyl); δ$_C$ dept (d₄-CH₃OH): 143.03, 142.94 (1C, C6-uridine), 131.32 (1C, CH-phenyl), 121.86, 121.79 (2C, CH-phenyl), 121.72, 121.66 (2C, CH-phenyl), 104.05 (1C, C5-cytidine), 92.60-92.43 (1C, C1'-uridine), 75.30, 74.84 (1C, C3'-uridine), 74.27, 74.15, 74.09, 74.04 (1C, C2'-uridine), 69.26 (1C, C5'-uridine), 68.86 (1C, CH-2-butyl), 52.01 (1C, CH-α), 30.20, 30.16 (1C, CH₂-2-butyl), 20.53, 20.47 (1C, CH₃-lateral chain), 20.18, 20.14 (1C, CH₃-2-butyl), 20.05, 14.45 (1C, CH₃-2-butyl).

Synthesis of 4'-azidouridine 5'-*O*-[phenyl(octyl-D-alaninyl)] phosphate (119)
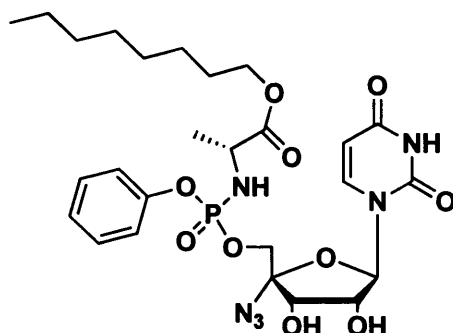
Prepared according to Standard Procedure C, from 4'-azidouridine (**24**, 300 mg, 0.986 mmol), 'BuMgCl (2.46 mL 1M solution of THF, 2.46 mmol) and phenyl(octyl-D-alaninyl) phosphorochloridate (**109**, 2.46 mL of solution 1M in THF, 2.46 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5), followed by a preparative TLC using as eluent $CHCl_3$/MeOH (85:15). The pure product was a white solid (15 mg, 3%).

δ_P (d_4-CH_3OH): 4.93, 4.65; δ_H (d_4-CH_3OH): 7.62 (1H, m, H6-uridine), 7.38-7.18 (5H, m, CH-phenyl), 6.15 (1H, m, H1'-uridine), 5.64 (1H, m, H5-uridine), 4.38-4.34 (2H, m, H2'-uridine, H3'-uridine), 4.17-4.00 (5H, m, CHα, H5'-uridine, CH_2-octyl), 1.55 (3H, m, CH_3-alanine), 1.52 (2H, m, CH_2-octyl), 1.36-1.20 (10H, m, CH_2-octyl), 0.95 (3H, m, CH_3-octyl).

**Synthesis of 4'-azidouridine 5'-*O*-[phenyl(isopropyl-D-alaninyl)] phosphate (121)**
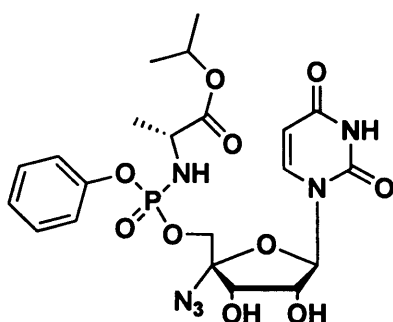
Prepared according to Standard Procedure D, from 4'-azidouridine (**24**, 200 mg, 0.701 mmol), 'BuMgCl (1.05 mL of solution 1M in THF, 1.05 mmol) and phenyl-(isopropyly-D-alaninyl) phosphorochloridate (**107**, 1.05 mL, 1M solution in THF, 1.05 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5) then a preparative TLC using as eluent $CHCl_3$/MeOH (9:1). The pure product was a white solid (20 mg, 4%).

δ$_P$ (d$_4$-CH$_3$OH): 4.97, 4.41; δ$_H$ (d$_4$-CH$_3$OH): 7.71 (1H, m, H6-uridine), 7.30 (2H, m, CH-phenyl), 7.27 (1H, m, CH-phenyl), 7.20 (2H, m, CH-phenyl), 6.18 (1H, m, H1'-uridine), 5.66 (1H, m, H5-uridine), 4.98 (1H, m, CH-isopropyl, J= 3.4 Hz), 4.42 (1H, m, H2'-uridine), 4.37 (1H, m, H3'-uridine), 4.24 (2H, m, H5'-uridine), 1.45 (3H, m, CH$_3$-alanine), 1.24 (6H, d, CH$_3$-isopropyl, J= 3.4 Hz).

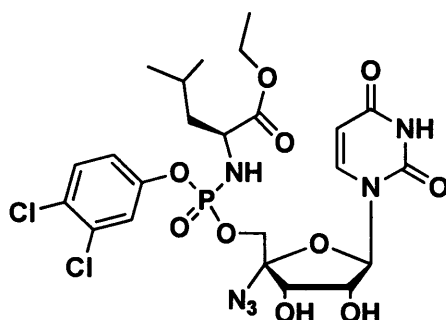## Synthesis of 4'-azidouridine 5'-*O*-[*p*-chloro-phenyl(ethyl-L-leucinyl)] phosphate

(165)
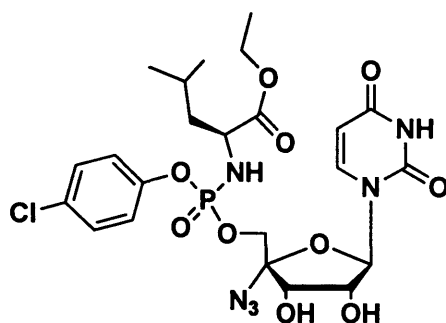
Prepared according to Standard Procedure C, from 4'-azidouridine (**24**, 250 mg, 0.876 mmol), 'BuMgCl (1.75 mL 1M solution of THF, 1.753 mmol) and *p*-chloro-phenyl(ethyl-L-leucinyl) phosphorochloridate (**82**, 1.75 mL of 1M solution in THF, 1.75 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (90:10) and a preparative TLC using the same eluent. The pure product was a white solid (100 mg, 14%).

δ$_P$ (d$_4$-CH$_3$OH): 3.76, 3.46; δ$_H$ (d$_4$-CH$_3$OH): 7.67 (1H, m, H6-uridine, J= 10.4 Hz), 7.38 (2H, d, CH-phenyl, J= 9.0 Hz), 7.26 (2H, d, CH-phenyl, J= 9.0 Hz), 6.15 (1H, m, H1'-uridine), 5.72 (1H, m, H5-uridine, J= 10.4 Hz), 4.38 (1H, m, H2'-uridine), 4.36 (1H, m, H3'-uridine), 4.17 (2H, m, CH$_2$-ethyl), 4.13 (2H, m, H5'-uridine), 3.89 (1H, m, CH-α), 1.73 (1H, m, CH-lateral chain), 1.55 (2H, m, CH$_2$-lateral chain), 1.26 (3H, m, CH$_3$-lateral chain), 1.23 (3H, m, CH$_3$-lateral chain), 0.91 (3H, m, CH$_3$-ethyl).

MS (ES) m/e: 639.1 (MNa$^+$, 100%); Accurate mass: C$_{23}$H$_{31}$N$_7$O$_9$NaPCl required 639.1348, found 639.1347.

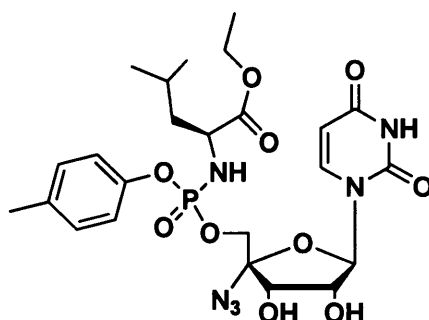**Synthesis of 4'-azidouridine 5'-*O*-[3,4-dichloro-phenyl(ethyl-L-leucinyl)] phosphate (166)**

Prepared according to Standard Procedure C, from 4'-azidouridine (**24**, 250 mg, 1.753 mmol), ^{t}BuMgCl (1.75 mL 1M solution of THF, 1.75 mmol) and 3,4-dichloro-phenyl(ethyl-L-leucinyl) phosphorochloridate (**83**, 1.75 mL of 1M solution in THF, 1.75 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (90:10) and then a preparative TLC using the same eluent. The pure product was a white solid (40 mg, 5%).

δ_P (d_4-CH$_3$OH): 3.84, 3.52; δ_H (d_4-CH$_3$OH): 7.67 (1H, m, H6-uridine, J= 12.0 Hz), 7.53 (1H, d, CH-phenyl, J= 9.0 Hz), 7.48 (1H, m, CH-phenyl), 7.22 (1H, d, CH-phenyl, J= 9.0 Hz), 6.12 (1H, m, H1'-uridine), 5.72 (1H, m, H5-uridine, J= 12.0 Hz), 4.39 (1H, m, H2'-uridine), 4.36 (1H, m, H3'-uridine), 4.22 (2H, m, CH_2-ethyl), 4.12 (2H, m, H5'-uridine), 3.88 (1H, m, CH-α), 1.72 (1H, m, CH-lateral chain), 1.55 (2H, m, CH_2-lateral chain), 1.30 (3H, m, CH_3-lateral chain), 1.25 (3H, m, CH_3-lateral chain), 0.92 (3H, m, CH_3-ethyl); δ_C *dept* (d_4-CH$_3$OH): 141.92 (1C, C6-uridine), 131.19 (1C, C5-uridine), 122.61, 122.55 (1C, CH-phenyl), 120.56, 120.49 (1C, CH-phenyl), 102.44 (1C, CH-phenyl), 98.65 (1C, C5-uridine), 92.03 (1C, C1'-uridine), 72.77, 72.66 (1C, C3'-uridine), 72.56, 72.41 (1C, C2'-uridine), 68.15 (1C, C5'-uridine), 61.26 (1C, CH_2-ethyl), 53.44 (1C, CH-α), 42.85, 42.76 (1C, CH_2-lateral chain), 24.53 (1C, CH-lateral chain), 22.15, 22.08 (1C, CH_3-lateral chain), 20.68, 20.28 (1C, CH_3-lateral chain), 13.32 (1C, CH_3-ethyl).

MS (ES) m/e: 673.1 (MNa$^+$, 100%); Accurate mass: $C_{23}H_{30}N_7O_9NaPCl_2$ required 673.0968, found 673.0958.

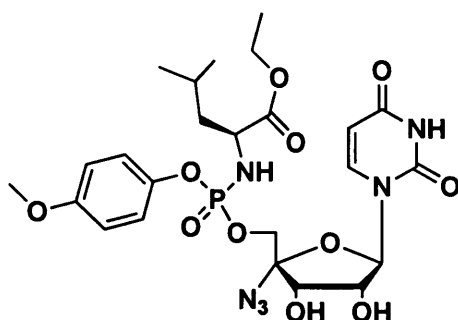**Synthesis of 4'-azidouridine 5'-*O*-[*p*-methyl-phenyl(ethyl-L-leucinyl) phosphate**

(167)

Prepared according to Standard Procedure C, from 4'-azidouridine (**24**, 250 mg, 1.753 mmol), 'BuMgCl (1.75 mL 1M solution of THF, 1.75 mmol) and *p*-methyl-phenyl(ethyl-L-leucinyl) phosphorochloridate (**84**, 1.75 mL of 1M solution in THF, 1.75 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (90:10). The pure product was a white solid (50 mg, 5%).

δ_P (d_4-CH$_3$OH): 5.63, 5.22; δ_H (d_4-CH$_3$OH): 7.55 (1H, m, H6-uridine), 7.08 (2H, m, CH-phenyl), 7.04 (2H, m, CH-phenyl), 6.08 (1H, m, H1'-uridine), 5.60 (1H, m, H5-uridine), 4.25 (1H, m, H2'-uridine), 4.23 (1H, m, H3'-uridine), 4.08 (2H, m, CH$_2$-ethyl), 4.04 (2H, m, H5'-uridine), 3.79 (1H, m, CH-α), 2.23 (3H, s, CH$_3$-*p*-methyl-phenyl), 1.64 (1H, m, CH-lateral chain), 1.47 (2H, m, CH$_2$-lateral chain), 1.15 (3H, m, CH$_3$-lateral chain), 1.13 (3H, m, CH$_3$-lateral chain), 0.80 (3H, m, CH$_3$-ethyl); δ_C (d_4-CH$_3$OH): 175.34 (1C, C=O ester), 166.19 (1C, C4-uridine), 152.71, 152.59 (1C, C2-uridine), 142.95, 142.66 (1C, C6-uridine), 131.68, 131.63 (2C, CH-phenyl), 121.65 (1C, C-phenyl), 121.59 (1C, C-phenyl), 121.43, 121.36 (2C, CH-phenyl), 99.29, 99.04, 98.91 (1C, C5-uridine), 92.52 (1C, C4'-uridine), 91.76 (1C, C1'-uridine), 74.45, 74.36 (1C, C3'-uridine), 74.19, 74.13 (1C, C2'-uridine), 69.33, 69.26 (1C, C5'-uridine), 62.81, 62.76, 62.66 (1C, CH$_2$-ethyl), 55.07, 54.93 (1C, CHα), 44.53, 44.44 (1C, CH$_2$-lateral chain), 44.10, 43.99 (1C, CH$_3$-*p*-methyl-phenyl), 26.22, 26.04, 25.82 (1C, CH$_2$-lateral chain), 23.67, 23.58, 23.46 (1C, CH$_3$, lateral chain), 22.87, 22.39, 21.99, 21.17 (1C, CH$_3$, lateral chain), 14.93, 14.87 (1C, CH$_3$-ethyl).

MS (ES) m/e: 619.1 (MNa$^-$, 100%); Accurate mass: C$_{24}$H$_{34}$N$_7$O$_9$NaP required 619.1891, found 619.1893.

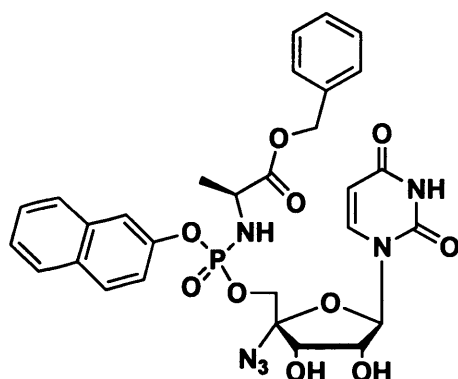**Synthesis of 4'-azidouridine 5'-*O*-[*p*-methoxy-phenyl(ethyl-L-leucinyl)] phosphate (168)**

Prepared according to Standard Procedure C, from 4'-azidouridine (**24**, 250 mg, 1.753 mmol), 'BuMgCl (1.75 mL 1M solution of THF, 1.75 mmol) and *p*-methoxy-phenyl(ethyl-L-leucinyl) phosphorochloridate (**85**, 1.75 mL of 1M solution in THF, 1.75 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (90:10). The pure product was a white solid (42 mg, 4%).

δ_P (d_4-CH$_3$OH): 5.64, 5.23; δ_H (d_4-CH$_3$OH): 7.65 (1H, m, H1-uridine, J= 16 Hz), 7.18 (2H, m, CH-phenyl), 6.91 (2H, m, CH-phenyl), 6.17 (1H, m, H1'-uridine), 5.71 (1H, m, H2-uridine), 4.36 (1H, m, H2'-uridine), 4.34 (1H, m, H3'-uridine), 4.18 (2H, m, CH$_2$-ethyl), 4.14 (2H, m, H5'-uridine), 3.89 (1H, m, CH-α), 3.78 (3H, s, CH$_3$O-phenyl), 1.75 (1H, m, CH-lateral chain), 1.57 (2H, m, CH$_2$-lateral chain), 1.26 (3H, m, CH$_3$-lateral chain), 0.97 (3H, m, CH$_3$-lateral chain), 0.90 (3H, m, CH$_3$-ethyl); δ_C *dept* (d_4-CH$_3$OH): 142.95, 142.67 (1C, C6-uridine), 122.83, 122.77, 122.63, 122.56 (2C, CH-phenyl), 116.16, 116.12 (2C, CH-phenyl), 104.18, 104.08 (1C, C5-uridine), 92.52 (1C, C4'-uridine), 91.78 (1C, C1'-uridine), 74.44, 74.37 (1C, C3'-uridine), 74.19, 74.12 (1C, C2'-uridine), 69.33, 69.26 (1C, C5'-uridine), 62.81, 62.76, 62.47 (1C, CH$_2$-ethyl), 56.52 (1C, CH$_3$O-phenyl), 55.08, 54.93 (1C, CHα), 45.16, 44.56 (1C, CH$_2$-lateral chain), 44.46, 43.99 (1C, CH$_3$-phenyl), 26.25, 26.05, 25.82 (1C, CH$_2$-lateral chain), 23.71, 23.61, 23.54 (1C, CH$_3$, lateral chain), 22.90, 22.41, 21.98 (1C, CH$_3$, lateral chain), 14.90 (1C, CH$_3$-ethyl).

MS (ES) m/e: 635.1 (MNa$^+$, 100%); Accurate mass: C$_{21}$H$_{34}$N$_7$O$_{10}$NaP required 635.1852, found 635.1843.

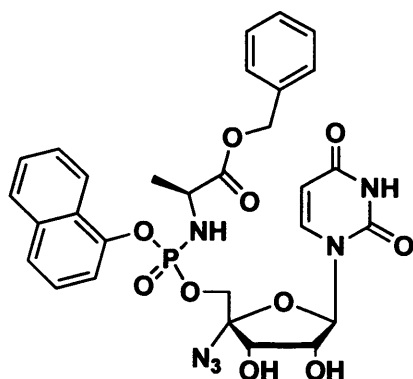**Synthesis of 4'-azidouridine 5'-*O*-[β-naphthyl(benzyl-L-alaninyl)] phosphate**

(174)

Prepared according to Standard Procedure D, from 4'-azidouridine (**24**, 200 mg, 0.701 mmol), 'BuMgCl (1.40 mL of solution 1M in THF, 1.40 mmol) and β-naphthyl(benzyl-L-alaninyl) phosphorochloridate (**94**, 1.40 mL of solution 1M in THF, 1.40 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5) then a preparative HPLC. The pure product was a white solid (20 mg, 5%).

$δ_P$ (d_4-CH_3OH): 3.78, 3.36; $δ_H$ (d_4-CH_3OH): 7.82 (1H, d, H6-uridine, *J*= 7.5 Hz), 7.50 (1H, m, CH-naphthyl), 7.38-7.20 (11H, m, CH-naphthyl, CH-phenyl), 6.03 (1H, m, H1'-uridine, *J*= 4.3 Hz), 5.81 (1H, d, H5-uridine, *J*= 7.5 Hz), 5.01 (2H, m, CH_2-benzyl), 4.27 (1H, m, H3'-cytidine), 4.24-4.17 (4H, m, H2'-cytidine, H5'-cytidine, CHα), 1.39 (3H, d, CH_3-alanine, *J*= 7.2 Hz).

MS (ES) m/e: 675.2 (MNa⁺, 100%); Accurate mass: $C_{29}H_{29}N_6O_{10}NaP$ required 675.1580, found 675.1583.

### Synthesis of 4'-azido-uridine 5'-*O*-[α-naphthyl(benzyl-L-alaninyl)] phosphate

(173)

Prepared according to Standard Procedure D, from 4'-azidouridine (24, 200 mg, 0.701 mmol), ^{t}BuMgCl (1.4 mL of solution 1M in THF, 1.402 mmol) and α-naphthyl(benzyl-L-alaninyl) phosphorochloridate (93, 1.4 mL of solution 1M in THF, 1.402 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5) then a preparative TLC using as eluent $CHCl_3$/MeOH (9:1). The pure product was a white solid (40 mg, 9%).

δ_P (d_4-CH$_3$OH): 3.94, 3.76; δ_H (d_4-CH$_3$OH): 8.18 (1H, m, CH-naphthyl), 7.90 (1H, m, CH-naphthyl), 7.72 (1H, m, H6-uridine), 7.57-7.30 (11H, m, CH-naphthyl, CH-phenyl), 6.11 (1H, m, H1'-uridine), 5.50 (1H, m, H5-uridine), 5.11 (2H, m, CH$_2$-benzyl), 4.37 (1H, m, H3'-uridine), 4.31-4.19 (3H, m, H2'-uridine, H5'-uridine), 4.11 (1H, m, CHα), 1.35 (3H, d, CH$_3$-alanine, J= 7.2 Hz).

MS (ES) m/e: 675.1 (MNa$^+$, 100%); Accurate mass: $C_{29}H_{29}N_6O_{10}P$ required 675.1575, found 675.1571.

Synthesis of 4'-azido-uridine 5'-*O*-[8-quinolinyl(benzyl-L-alaninyl)] phosphate
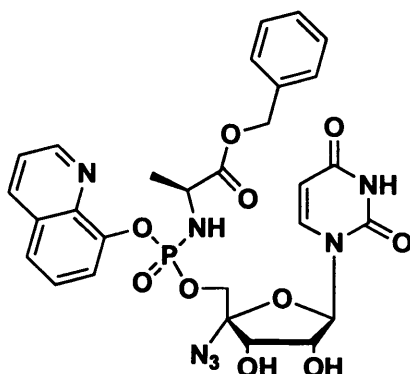
(179)

Prepared according to Standard Procedure C2, from 2',3'-*O*,*O*-cyclopentylidene-4'-azidouridine (**145**, 200 mg, 0.570 mmol), 'BuMgCl (1.42 mL, 1M solution in THF, 1.42 mmol) and the crude of the synthesis of 8-quinolinyl(benzyl-L-alaninyl) phosphorochloridate (**181**, 1.42 mL of solution 1M in THF, 1.42 mmol). The solvent was removed under reduced pressure and the obtained crude without further purification was dissolved in a solution 80% of HCOOH in water for 3 hours. The solvent was removed under reduced pressure and the obtained crude product was purified by column chromatography using as eluent CHCl₃/MeOH 85:15 and preparative HPLC. The pure product was a white solid (12 mg, 3%).

δ_P (d_4-CH₃OH): 4.01, 3.95; δ_H (d_4-CH₃OH): 8.93 (1H, m, CH-quinolinyl), 8.37 (1H, CH-quinolinyl), 7.80 (1H, m, H6-uridine), 7.76-7.69 (1H, m, CH-quinolinyl), 7.64-7.54 (3H, m, CH-quinolinyl, CH-phenyl), 7.43-7.31 (5H, 1 CH-quinolinyl, CH-phenyl), 6.15 (1H, m, H1'-uridine), 5.66-5.54 (1H, m, H5-uridine), 5.29 (2H, m, CH₂-benzyl), 4.42 (1H, m, H3'-uridine), 4.36-4.27 (3H, m, H2'-uridine, H5'-uridine), 4.11 (1H, m, CHα), 1.37 (3H, d, CH₃-alanine, *J*= 7.2 Hz).

Synthesis of 4'-azido-uridine 5'-*O*-[phenyl(butyl-L-alaninyl)] phosphate (121)
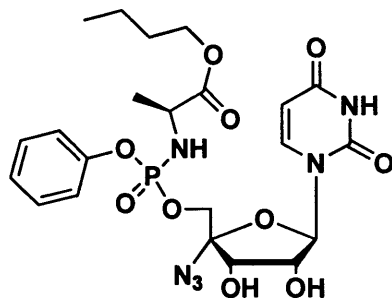
Prepared according to Standard Procedure C, from 4'-azidouridine (**24**, 300 mg, 0.986 mmol), ^{t}BuMgCl (2.46 mL 1M solution of THF, 2.46 mmol) and phenyl(butyl-L-alaninyl) phosphorochloridate (**59**, 2.46 mL of solution 1M in THF, 2.46 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (95:5), followed by a preparative TLC using as eluent CHCl$_3$/MeOH (85:15). The pure product was a white solid (17 mg, 3%).

δ_P (d_4-CH$_3$OH): 4.91, 4.35; δ_H (d_4-CH$_3$OH): 7.65 (1H, m, H6-cytidine, J= 8.1 Hz), 7.46-7.22 (5H, m, CH-phenyl), 6.16 (1H, m, H1'-cytidine, J= 3.6 Hz), 5.72 (1H, m, H5-cytidine, J= 8.1 Hz), 4.39-4.27 (2H, m, H2'-cytidine, H3'-cytidine), 4.24-4.09 (5H, m, CHα, H5'-cytidine, CH$_2$-butyl), 1.67-1.59 (3H, m, CH$_3$-alanine), 1.48-1.30 (4H, m, CH$_2$-butyl), 0.95 (3H, m, CH$_3$-butyl); δ_C *dept* (d_4-CH$_3$OH): 143.60, 142.98 (1C, C6-uridine), 131.57, 131.32 (2C, CH-phenyl), 121.72, 121.65 (2C, CH-phenyl), 104.04 (1C, C5-cytidine), 92.68-92.38 (1C, C1'-uridine), 74.25, 74.08 (1C, C3'-uridine), 73.95, 73.78 (1C, C2'-uridine), 71.77 (1C, CH$_2$-butyl), 69.30 (1C, C5'-uridine), 68.86 (1C, CH$_2$-butyl), 52.12, 51.90 (1C, CH-α), 32.15 (1C, CH$_2$-butyl), 20.92, 20.83 (1C, CH$_3$-lateral chain), 20.71 (1C, CH$_2$-butyl), 14.41 (1C, CH$_3$-butyl).

Synthesis of 2', 3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[α-naphthyl(benzyl-cyclopentylglycinyl)] phosphate
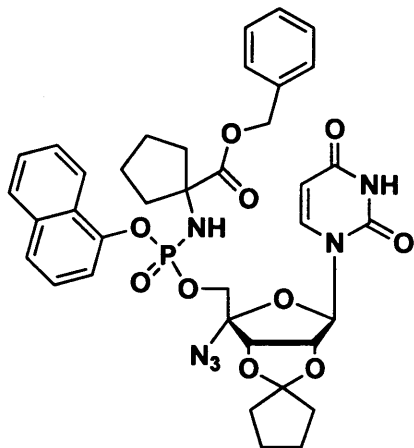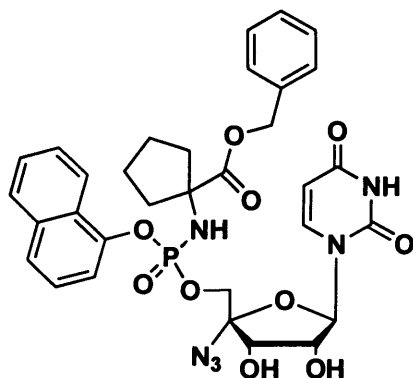
Prepared according to Standard Procedure C2, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (**145**, 200 mg, 0.569 mmol), 'BuMgCl (1.42 mL, 1M solution in THF, 1.42 mmol) and α-naphthyl(benzyl-cyclopentylglycinyl) phosphorochloridate (**169**, 1.4 mL of solution 1M in THF, 1.423 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (430 mg, quantitative).

δ_P (d_4-CH$_3$OH): 2.80; δ_H (d_4-CH$_3$OH): 8.32 (1H, d, H6-uridine, J= 7.8 Hz), 8.21 (1H, s, CH-naphthyl), 7.78 (1H, d, CH-naphthyl, J= 7.2 Hz), 7.66 (1H, m, CH-naphthyl), 7.51-7.27 (9H, m, CH-naphthyl, CH-phenyl), 5.93 (1H, d, H1'-uridine, J= 14.0 Hz), 5.56 (1H, m, H5-uridine, J= 7.8 Hz), 5.12 (2H, m, CH$_2$-benzyl), 5.04-4.88 (2H, m, H2'-uridine, H3'-uridine), 4.31-4.23 (2H, m, H5'-uridine), 2.22-2.03 (8H, m, CH$_2$-cyclopentyl), 1.68-1.18 (8H, m, CH$_2$-cyclopentyl).

Synthesis of 4'-azido-uridine 5'-*O*-[α-naphthyl(benzyl-cyclopentylglycinyl)] phosphate (175)
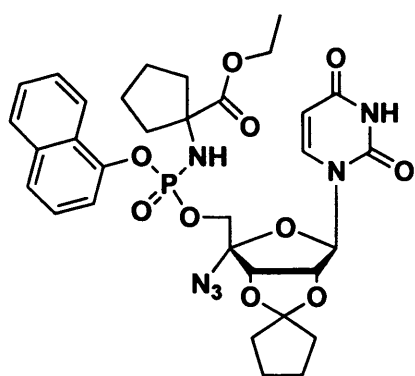
Prepared according to Standard Procedure C3, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (510 mg, 0.567 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (250 mg, 64%).

δ_P (d_4-CH$_3$OH): 3.10, 3.06; δ_H (d_4-CH$_3$OH): 8.21 (1H, m, H6-uridine), 7.89 (1H, m, CH-naphthyl), 7.72 (1H, t, CH-naphthyl, $J=$ 10.2 Hz), 7.66 (1H, m, CH-naphthyl), 7.57-7.27 (9H, m, CH-naphthyl, CH-phenyl), 6.12 (1H, d, H1'-uridine, $J=$ 6.7 Hz), 5.43 (1H, m, H5-uridine), 5.14 (2H, m, CH$_2$-benzyl), 4.37-4.21 (2H, m, H2'-uridine, H3'-uridine, H5'-uridine), 2.21-1.98 (4H, m, CH$_2$-cyclopentyl), 1.87-1.46 (8H, m, CH$_2$-cyclopentyl).

MS (ES) m/e: 715.1 (MNa$^+$, 100%); Accurate mass: C$_{32}$H$_{33}$N$_6$O$_{10}$NaP required 715.1893, found 715.1898.

Synthesis of 2', 3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[α-naphthyl(ethyl-cyclopentylglycinyl)] phosphate
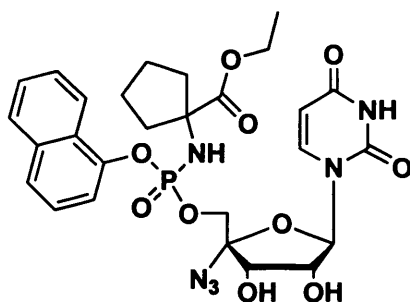

Prepared according to Standard Procedure C2, from 2', 3'-*O,O*-cyclopentylidene-4'-azidouridine (**145**, 200 mg, 0.986 mmol), 'BuMgCl (1.42 mL, 1M solution in THF, 1.42 mmol) and α-naphthyl(ethyl-cyclopentylglycinyl) phosphorochloridate (**170**, 1.42 mL of solution 1M in THF, 1.42 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (95:5). The pure product was a white solid (510 mg, quantitative).

δ_P (d_4-CH$_3$OH): 3.00; δ_H (d_4-CH$_3$OH): 8.22 (1H, t, H6-uridine, $J=$ 6.9 Hz), 7.85 (1H, m, CH-naphthyl), 7.70-7.39 (6H, m, CH-naphthyl), 5.96 (1H, m, H1'-uridine), 5.56 (1H, m, H5-uridine), 4.97-4.94 (2H, m, H2'-uridine, H3'-uridine), 4.36-4.32 (2H, m, H5'-uridine), 4.14 (2H, m, CH$_2$-ethyl), 2.17-2.00 (8H, m, CH$_2$-cyclopentyl), 1.77-1.59 (8H, m, 4 CH$_2$-cyclopentyl), 1.22 (3H, m, CH$_3$-ethyl).

Synthesis of 4'-azidouridine 5'-*O*-[α-naphthyl(ethyl-cyclopentylglycinyl)]

phosphate (170)

Prepared according to Standard Procedure C3, from 2', 3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[α-naphthyl(ethyl-cyclopentylglycinyl)] phosphate (510 mg, 0.732 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent for the first $CHCl_3$/MeOH (95:5). The pure product was a white solid (250 mg, 54%).

δ_P (d_4-CH$_3$OH): 3.23, 3.15; δ_H (d_4-CH$_3$OH): 8.21 (1H, d, H6-uridine, J= 7.5 Hz), 7.90 (1H, m, CH-naphthyl), 7.72 (1H, t, CH-naphthyl, J= 8.9 Hz), 7.60-7.41 (5H, m, CH-naphthyl), 6.14 (1H, m, H1'-uridine), 5.46 (1H, m, H5-uridine, J= 15 Hz), 4.97-4.24 (4H, m, H2'-uridine, H3'-uridine, H5'-uridine), 4.20-4.13 (2H, m, CH_2-ethyl), 2.20-1.96 (8H, m, CH_2-cyclopentyl), 1.24 (3H, CH_3-ethyl).

MS (ES) m/e: 653.1 (MNa$^+$, 100%); Accurate mass: $C_{27}H_{31}N_6O_{10}$NaP required 653.1737, found 653.1721.

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Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[α-naphthyl(benzyl-D-alaninyl)] phosphate
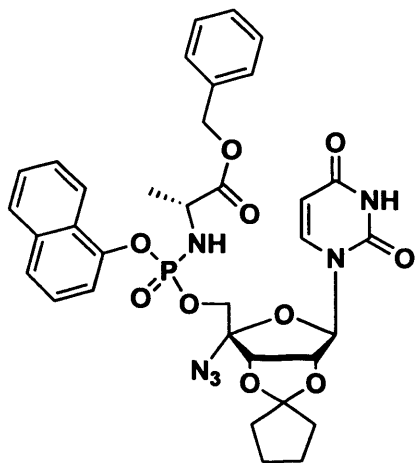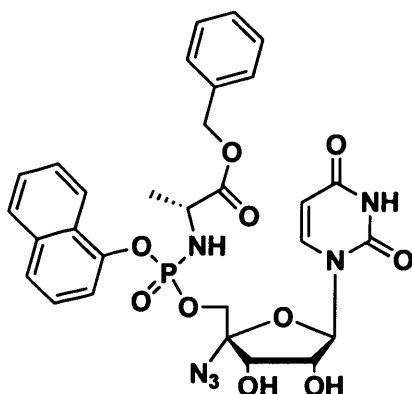
Prepared according to Standard Procedure C2, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (**145**, 200 mg, 0.569 mmol), 'BuMgCl (1.42 mL, 1M solution in THF, 1.42 mmol) and α-naphthyl(benzyl-D-alaninyl) phosphorochloridate (**178**, 1.42 mL of solution 1M in THF, 1.42 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (234 mg, 61%).

δ_P (d_4-CH_3OH): 3.67, 3.47; δ_H (d_4-CH_3OH): 8.04 (1H, m, CH-naphthyl), 7.76-7.73 (2H, m, CH-naphthyl, H6-uridine), 7.57-7.10 (10H, m, CH-naphthyl, CH-phenyl), 5.79 (1H, m, H1'-uridine), 5.45 (1H, m, H5-uridine), 4.99 (2H, m, CH_2-benzyl), 4.95 (1H, m, H2'-uridine), 4.91 (1H, m, H3'-uridine), 4.18-4.10 (1H, m, H5'-uridine), 4.02-3.96 (1H, m, CHα), 2.03-1.93 (2H, m, CH_2-cyclopentyl), 1.62-1.57 (6H, m, CH_2-cyclopentyl), 1.23 (3H, m, CH_3-alanine).

Synthesis of 4'-azidouridine 5'-*O*-[α-naphthyl(benzyl-D-alaninyl)] phosphate
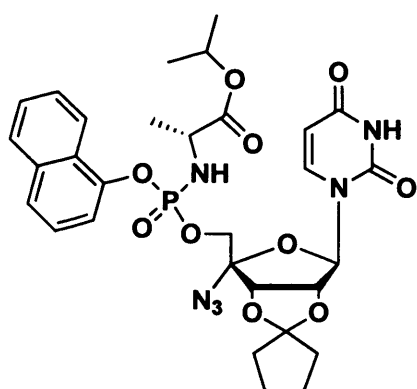
(172)

Prepared according to Standard Procedure C3, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[α-naphthyl(benzyl-D-alaninyl)] phosphate (234 mg, 0.347 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by

column chromatography, using as eluent $CHCl_3$/MeOH (8:2). The pure product was a white solid (80 mg, 35%).

δ_P (d_4-CH₃OH): 3.80, 3.60; δ_H (d_4-CH₃OH): 8.19 (1H, m, CH-naphthyl), 7.90 (1H, m, CH-naphthyl), 7.73 (1H, m, H6-uridine), 7.59-7.42 (10H, m, CH-naphthyl, CH-phenyl), 6.12 (1H, m, H1'-uridine), 5.51 (1H, m, H5-uridine), 4.97 (2H, m, CH₂-benzyl), 4.40-3.99 (5H, m, H2'-uridine, H3'-uridine, CHα, H5'-uridine), 1.22 (3H, m, CH₃-alanine); δ_C (d_4-CH₃OH): 174.62 (1C, C=O ester), 165.75, 165.69 (1C, C4-uridine), 152.10 (1C, C2-uridine), 147.76 (1C, C-naphthyl), 142.50, 142.17 (1C, C6-uridine), 137.11 (1C, C-phenyl), 136.33 (1C, C-naphthyl), 129.60, 129.58 (2C, CH-naphthyl), 129.37, 129.34, 129.28 (1C, CH-phenyl), 129.05, 128.98 (2C, CH-naphthyl), 127.98, 127.94 (2C, CH-naphthyl), 127.74, 127.67 (2C, CH-phenyl), 126.55 (1C, CH-naphthyl), 126.29, 126.23 (1C, CH-naphthyl), 122.66, 122.47 (2C, CH-phenyl), 103.60, 103.57 (1C, C5-uridine), 98.68, 98.60 (1C, C4'-uridine), 92.27, 92.22 (1C, C1'-uridine), 79.48 (1C, C3'-uridine), 73.74, 73.69, 73.54 (1C, C2'-uridine), 68.60, 68.56 (1C, CH₂-benzyl), 68.16, 68.09, 67.94 (1C, C5'-uridine), 51.81, 51.71 (1C, CHα), 20.39, 20.34, 20.25 (1C, CH₃-alanine).

MS (ES) m/e: 675.2 (MNa⁺, 100%); Accurate mass: $C_{29}H_{29}N_6O_{10}NaP$ required 675.1580, found 675.1594.

Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[α-naphthyl(isopropyl-D-alaninyl)] phosphate
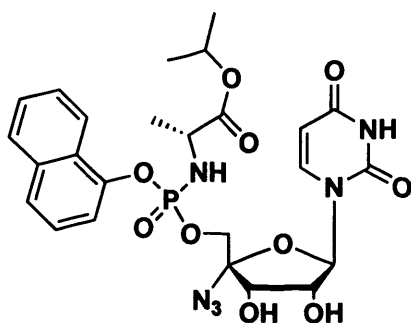

Prepared according to Standard Procedure C2, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (**145**, 200 mg, 0.569 mmol), ᵗBuMgCl (1.42 mL, 1M solution in THF, 1.42 mmol) and α-naphthyl(isopropoxy-D-alaninyl) phosphorochloridate (**171**, 1.42 mL of solution 1M in THF, 1.42 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (150 mg of, 39%).

δ_P (d_4-CH₃OH): 3.70, 3.61; δ_H (d_4-CH₃OH): 8.07 (1H, m, CH-naphthyl), 7.78-7.76 (2H, m, CH-naphthyl, H6-uridine), 7.61-7.29 (5H, m, CH-naphthyl), 5.83 (1H, m, H1'-uridine), 5.47 (1H, m, H5-uridine), 4.88 (1H, m, H2'-uridine), 4.85-4.77 (2H, m,

H3'-uridine, CH-isopropyl), 4.21-4.17 (1H, m, H5'-uridine), 3.92-3.87 (1H, m, CHα), 2.05-1.97 (2H, m, CH_2-cyclopentyl), 1.64-1.58 (6H, m, CH_2-cyclopentyl), 1.21 (3H, m, CH_3-alanine), 1.11-1.05 (6H, m , CH_3-isopropyl).

## Synthesis of 4'-azidouridine 5'-*O*-[α-naphthyl(isopropyl-D-alaninyl)] phosphate

Prepared according to Standard Procedure C3, from 2',3'-*O*,*O*-cyclopentylidene-4'-azidouridine 5'-*O*-[α-naphthyl(isopropyl-D-alaninyl)] phosphate (150 mg, 0.224 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (8:2). The pure product was a white solid (50 mg, 37 %).

$δ_P$ (d_4-CH_3OH): 3.73, 3.60; $δ_H$ (d_4-CH_3OH): 8.20 (1H, m, CH-naphthyl), 7.92 (1H, m, CH-naphthyl), 7.73 (1H, m, H6-uridine), 7.57-7.41 (6H, m, CH-naphthyl), 6.12 (1H, m, H1'-uridine), 5.51 (1H, m, H5-uridine), 4.97 (1H, m, CH-isopropyl), 4.37 (1H, m, H2'-uridine), 4.35-4.21 (3H, m, H3'-uridine, H5'-uridine), 3.99 (1H, m CHα), 1.38-1.33 (3H, m, CH_3-alanine), 1.30-1.19 (6H, CH_3-isopropyl); $δ_C$ (d_4-CH_3OH): 174.33 (1C, C=O ester), 165.71 (1C, C4-uridine), 152.21, 151.12 (1C, C2-uridine), 147.87, 147.82 (1C, C-naphthyl), 142.55, 142.27 (1C, C6-uridine), 136.37, 136.35 (2C, C-naphthyl), 129.05, 128.99 (1C, CH-naphthyl), 127.98, 127.96 (1C, CH-naphthyl), 127.71, 127.65 (1C, CH-naphthyl), 126.55 (1C, CH-naphthyl), 126.28, 126.22 (1C, CH-naphthyl), 122.67, 122.51 (1C, CH-naphthyl), 116.32, 116.30, 116.11, 116.09 (1H, CH-naphthyl), 103.59 (1C, C5-uridine), 98.69, 98.61 (1C, C4'-uridine), 92.29, 92.21 (1C, C1'-uridine), 79.48 (1C, C3'-uridine), 73.78, 73.69, 73.63 (1C, C2'-uridine), 70.38, 70.34 (1C, CH-isopropyl), 69.08, 68.77, 68.73 (1C, C5'-uridine), 51.93, 51.77 (1C, CHα), 21.99, 21.95, 21.91 (2C, CH_3-isopropyl) 20.53, 20.48, 20.39, 20.33 (1C, CH_3-alanine).

MS (ES) m/e: 627.2 (MNa^+, 100%); Accurate mass: $C_{25}H_{29}N_6O_{10}NaP$ required 627.1580, found 627.1584.
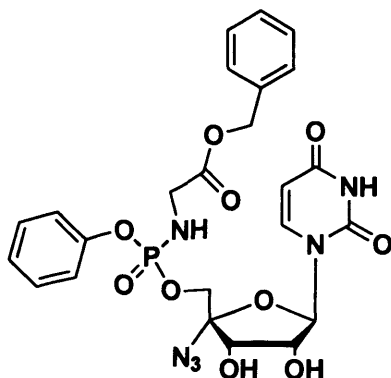
Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(benzyl-glycinyl)] phosphate
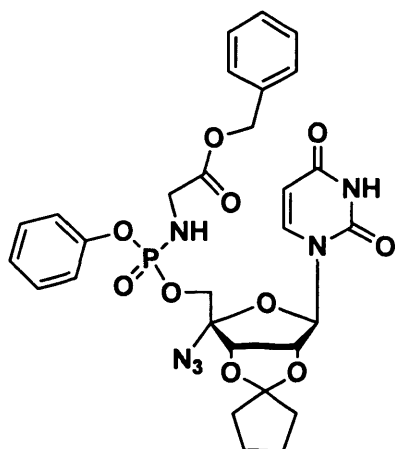
Prepared according to Standard Procedure C2, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (**145**, 150 mg, 0.427 mmol), 'BuMgCl (0.85 mL, 1M solution in THF, 0.85 mmol) and phenyl(benzyl-glycinyl) phosphorochloridate (**133**, 0.85 mL of solution 1M in THF, 0.85 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (173 mg, 62%).

δ_P (d_4-CH_3OH): 3.17, 3.02; δ_H (d_4-CH_3OH): 7.63 (1H, d, H6-uridine, *J*= 8.0 Hz), 7.37-7.34 (7H, m, CH-phenyl, CH-benzyl), 7.24-7.19 (3H, m, CH-phenyl), 5.98 (1H, m, H1'-uridine), 5.68 (1H, m, H5-uridine, *J*= 8.0 Hz), 5.18 (2H, s, CH_2-benzyl), 5.02 (1H, m, H2'-uridine), 4.97 (1H, m, H3'-uridine), 4.30-4.25 (2H, m, H5'-uridine), 3.83 (2H, d, CH_2-glycine), 2.20-2.11 (2H, m, CH_2-cyclopentyl), 1.80-1.73 (6H, m, CH_2-cyclopentyl).

Synthesis of 4'-azidouridine 5'-*O*-[phenyl(benzyl-glycinyl)] phosphate (153)

Prepared according to Standard Procedure C3, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(benzyl-glycinyl)] phosphate (173 mg, 0.264 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (8:2). The pure product was a white solid (70 mg, 43%).

δ_P (d_4-CH_3OH): 3.53, 3.28; δ_H (d_4-CH_3OH): 7.63 (1H, m, H6-uridine), 7.36 (6H, m, CH-phenyl, CH-benzyl), 7.34-7.19 (4H, m, CH-phenyl, CH-benzyl), 6.15 (1H, m,

H1'-uridine), 5.69 (1H, m, H5-uridine), 5.18 (2H, s, CH$_2$-benzyl), 4.39-418 (4H, m, H2'-uridine, H3'-uridine, H5'-uridine), 3.83 (2H, d, CH$_2$-glycine).

MS (ES) m/e: 611.0 (MNa$^+$, 100%); Accurate mass: C$_{24}$H$_{25}$N$_6$O$_{10}$NaP required 611.1271, found 611.1267.

Anal. Calc. for C$_{24}$H$_{25}$N$_6$O$_{10}$P: C 48.98%, H 4.28%, N 14.28%. Found: C 48.69%, H 3.91%, N 14.30%.
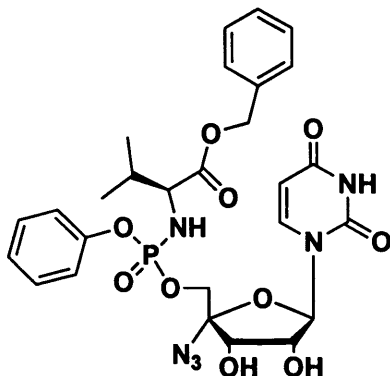
Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(benzyl-L-valinyl)] phosphate
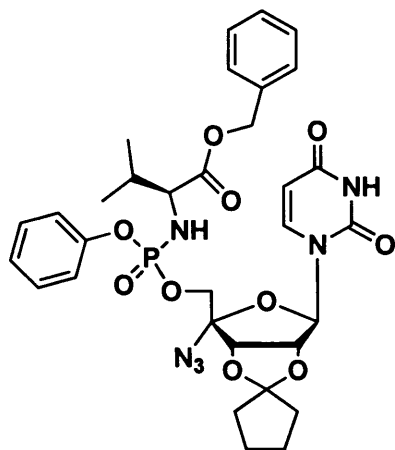
Prepared according to Standard Procedure C2, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (**145**, 150 mg, 0.427 mmol), 'BuMgCl (0.85 mL, 1M solution in THF, 0.85 mmol) and phenyl(benzyl-L-valinyl) phosphorochloridate (**134**, 0.85 mL of solution 1M in THF, 0.85 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (95:5). The pure product was a white solid (173 mg, 58%).

δ_P (*d$_4$*-CH$_3$OH): 3.96, 3.81; δ_H (*d$_4$*-CH$_3$OH): 7.62 (1H, d, H6-uridine, *J*= 6.1 Hz), 7.39-7.32 (7H, m, CH-phenyl, CH-benzyl), 7.24-7.18 (3H, m, CH-phenyl, CH-phenyl), 5.96 (1H, m, H1'-uridine), 5.68 (1H, m, H5-uridine, *J*= 6.1 Hz), 5.18-5.11 (3H, m, CH$_2$-benzyl, H2'-uridine), 4.98 (1H, m, H3'-uridine), 4.29-4.20 (2H, m, H5'-uridine), 3.76 (1H, m, CHα), 2.19-2.05 (3H, m, CH$_2$-cyclopenthyl, CH-lateral chain), 1.78-1.73 (6H, m, CH$_2$-cyclopentyl), 0.92-0.86 (6H, m, CH$_3$-lateral chain).

Synthesis of 4'-azidouridine 5'-*O*-[phenyl(benzyl-L-valinyl)] phosphate (154)
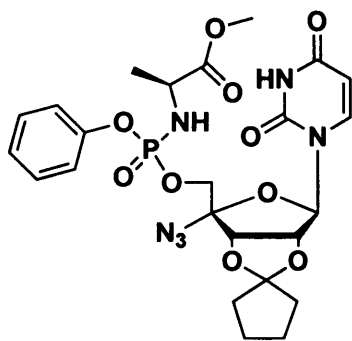
Prepared according to Standard Procedure C3, from 2',3'-*O*,*O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(benzyl-L-valinyl)] phosphate (173 mg, 0.248 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (8:2). The pure product was a white solid (70 mg, 55%).

δ_P (d_4-CH$_3$OH): 4.45, 4.14; δ_H (d_4-CH$_3$OH): 7.62 (1H, m, H6-uridine, J= 6.8 Hz), 7.39-7.32 (7H, m, CH-phenyl, CH-benzyl), 7.24-7.18 (3H, m, CH-phenyl, CH-phenyl), 6.14 (1H, m, H1'-uridine), 5.68 (1H, m, H5-uridine, J= 6.8 Hz), 5.19-5.10 (2H, m, CH$_2$-benzyl), 4.37-4.30 (2H, m, H2'-uridine, H3'-uridine), 4.22-4.14 (2H, m, H5'-uridine), 3.76 (1H, m, CHα), 2.07 (1H, m, CH-lateral chain), 0.90 (3H, t, CH$_3$-valine, J= 8.6 Hz), 0.84 (3H, t, 3 CH$_3$-lateral chain, J= 7.8 Hz).

MS (ES) m/e: 653.0 (MNa$^+$, 100%); Accurate mass: $C_{27}H_{31}N_6O_{10}$NaP required 653.1754, found 653.1737.

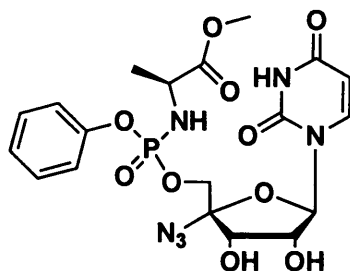**Synthesis of 2',3'-*O*,*O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(methyl-L-alaninyl)] phosphate**

Prepared according to the standard procedure 1, from 2',3'-*O*,*O*-cyclopentylidene-4'-azidouridine (**145**, 150 mg, 0.427 mmol), 'BuMgCl (0.85 mL, 1M solution in THF, 0.854 mmol) and phenyl(methyl-L-alaninyl) phosphorochloridate (**128**, 0.85 mL of solution 1M in THF, 0.854 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a a white solid (156 mg, 61%).

δ_P (d_4-CH$_3$OH): 3.14, 3.04; δ_H (d_4-CH$_3$OH): 7.66 (1H, t, H6-uridine), 7.35 (2H, t, CH-phenyl), 7.28-7.19 (3H, m, CH-phenyl), 5.97 (1H, m, H1'-uridine), 5.70 (1H, m, H5-uridine), 5.12-5.04 (2H, m, H2'-uridine, H3'-uridine), 4.31-4.27 (2H, m, H5'-uridine), 4.01 (1H, m, CHα), 3.70 (3H, d, CH$_3$-methyl), 2.21-2.11 (2H, m, CH$_2$-cyclopentyl), 1.79-1.73 (6H, m, CH$_2$-cyclopentyl), 1.37 (3H, t, CH$_3$-alanine, J= 9.5 Hz).

Synthesis of 4'-azidouridine 5'-*O*-[phenyl(methyl-L-alaninyl) phosphate (148)
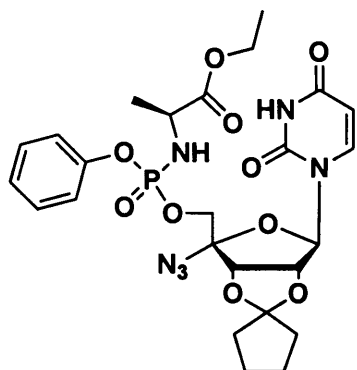

Prepared according to the standard procedure 2, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(methyl-L-alaninyl)] phosphate (135 mg, 0.222 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (8:2). The pure product was a white solid (65 mg, 54%).

δ_P (d_4-CH$_3$OH): 3.50, 3.31; δ_H (d_4-CH$_3$OH): 7.65 (1H, m, H6-uridine), 7.38 (2H, m, CH-phenyl), 7.28-7.20 (3H, m, CH-phenyl), 6.15 (1H, m, H1'-uridine), 5.72 (1H, m, H5-uridine), 4.42-4.36 (2H, m, H2'-uridine, H3'-uridine), 4.26-4.18 (2H, m, H5'-uridine), 4.00 (1H, q, CHα), 3.70 (3H, d, CH$_3$-methyl), 1.35 (3H, dd, CH$_3$-alanine).

MS (ES) m/e: 549.0 (MNa$^+$, 100%); Accurate mass: C$_{19}$H$_{23}$N$_6$O$_{10}$NaP required 549.1111, found 549.1124.

Anal. Calc. for C$_{19}$H$_{23}$N$_6$O$_{10}$P: C 43.35%, H 4.40%, N 15.97%. Found: C 43.22%, H 4.38%, N 15.45%.

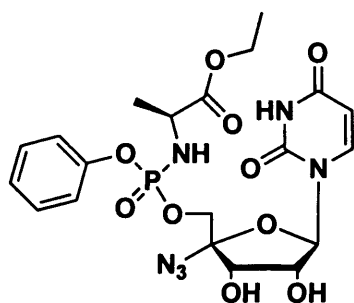## Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(ethyl-L-alaninyl)] phosphate

Prepared according to the standard procedure 1, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (**145**, 150 mg, 0.427 mmol), 'BuMgCl (0.85 mL, 1M solution in THF, 0.85 mmol) and phenyl(ethyl-L-alaninyl) phosphorochloridate (**129**, 0.85 mL of solution 1M in THF, 0.85 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (135 mg, 52%).

δ_P (d_4-CH_3OH): 3.18, 3.08; δ_H (d_4-CH_3OH): 7.65 (1H, d, H6-uridine, J= 7.9 Hz), 7.36 (2H, m, CH-phenyl), 7.28-7.19 (3H, m, CH-phenyl), 6.00 (1H, dd, H1'-uridine), 5.69 (1H, dd, H5-uridine, J= 7.9 Hz), 5.03 (2H, m, H2'-uridine, H3'-uridine), 4.30-4.26 (2H, m, H5'-uridine), 4.18-4.13 (1H, m, CH_2-ethyl), 3.98 (1H, m, CHα), 2.21-2.08 (2H, m, CH_2-cyclopentyl), 1.83-1.74 (6H, m, CH_2-cyclopentyl), 1.26 (3H, m, CH_3-ethyl), 1.25 (3H, m, CH_3-alanine).

Synthesis of 4'-azidouridine 5'-*O*-[phenyl(ethyl-L-alaninyl)] phosphate (149)

Prepared according to the standard procedure 2, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(ethyl-L-alaninyl)] phosphate (135 mg, 0.222 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (8:2). The pure product was a white solid (65 mg, 54%).

δ_P (d_4-CH_3OH): 3.56, 3.35; δ_H (d_4-CH_3OH): 7.65 (1H, m, H6-uridine), 7.37 (2H, m, CH-phenyl), 7.29-7.21 (3H, m, CH-phenyl), 6.17 (1H, m, H1'-uridine), 5.70 (1H, m, H5-uridine), 4.41-4.35 (2H, m, H2'-uridine, H3'-uridine), 4.26-4.15 (4H, m, H5'-

uridine, CH_2-ethyl), 3.97 (1H, m, CHα), 1.35 (3H, m, CH_3-ethyl), 1.26 (3H, m, CH_3-alanine).

MS (ES) m/e: 563.0 (MNa$^+$, 100%); Accurate mass: $C_{20}H_{25}N_6O_{10}NaP$ required 563.1257, found 563.1267.

Anal. Calc. for $C_{20}H_{25}N_6O_{10}P$: C 44.45%, H 4.66%, N 15.55%. Found: C 44.90%, H 4.07%, N 15.75%.

Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(isopropyl-L-alaninyl)] phosphate
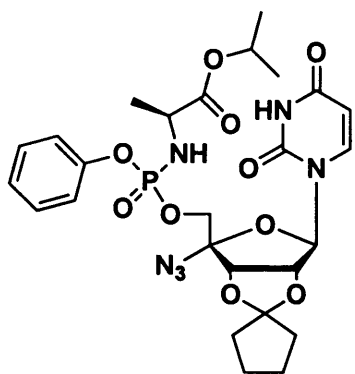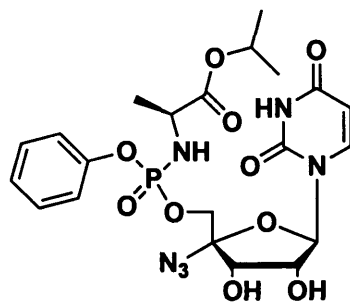
Prepared according to the standard procedure 1, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (**145**, 150 mg, 0.427 mmol), ^{t}BuMgCl (0.85 mL, 1M solution in THF, 0.85 mmol) and phenyl(isopropyl-L-alaninyl) phosphorochloridate (**130**, 0.85 mL of solution 1M in THF, 0.85 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (171 mg, 64%).

δ_P (d_4-CH_3OH): 3.22, 3.19; δ_H (d_4-CH_3OH): 7.65 (1H, d, H6-uridine, J= 7.3 Hz), 7.35 (2H, d, CH-phenyl), 7.28-7.19 (3H, m, CH-phenyl), 5.97 (1H, dd, H1'-uridine), 5.70 (1H, m, H5-uridine), 5.12-4.97 (3H, m, H2'-uridine, H3'-uridine, CH-isopropyl), 4.30-4.26 (2H, m, H5'-uridine), 3.95 (1H, m CHα), 2.21-2.10 (2H, m, CH_2-cyclopentyl), 1.79-1.73 (6H, m, CH_2-cyclopentyl), 1.36 (3H, d, CH_3-alanine, J= 7.2 Hz), 1.24 (6H, d, CH_3-isopropyl, J= 5.6 Hz).

Synthesis of 4'-azidouridine 5'-*O*-[phenyl(isopropyl-L-alaninyl)] phosphate (150)
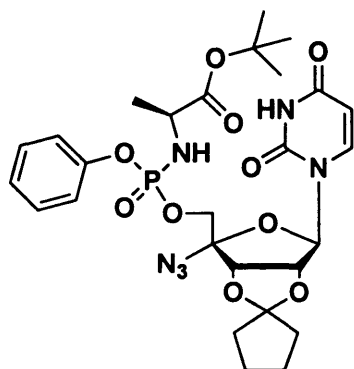

Prepared according to the standard procedure 2, from 2',3'-*O*,*O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(isopropyl-L-alaninyl)] phosphate (171 mg, 0.275 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (8:2). The pure product was a white solid (144 mg, 94%).

δ_P (d_4-CH_3OH): 3.59, 3.38; δ_H (d_4-CH_3OH): 7.64 (1H, dd, H6-uridine), 7.37 (2H, d, CH-phenyl), 7.28-7.22 (3H, m, CH-phenyl), 6.15 (1H, dd, H1'-uridine), 5.70 (1H, dd, H5-uridine), 5.00 (1H, q, CH-isopropyl), 4.40-4.35 (2H, m, H2'-uridine, H3'-uridine), 4.24-4.18 (2H, m, H5'-uridine), 3.94 (1H, q, CHα), 1.34 (3H, dd, CH_3-alanine), 1.24 (6H, m, CH_3-isopropyl).

MS (ES) m/e: 577.1 (MNa^+, 100%); Accurate mass: $C_{21}H_{27}N_6O_{10}NaP$ required 577.1424, found 577.1427.

Anal. Calc. for $C_{21}H_{27}N_6O_{10}P$: C 45.49%, H 4.91%, N 15.16%. Found: C 45.57%, H 4.91%, N 15.34%.

Synthesis of 2',3'-*O*,*O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(*tert*-butyl-L-alaninyl)] phosphate
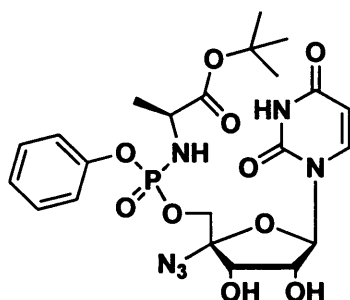

Prepared according to the standard procedure 1, from 2',3'-*O*,*O*-cyclopentylidene-4'-azidouridine (145, 150 mg, 0.427 mmol), 'BuMgCl (0.85 mL, 1M solution in THF, 0.85 mmol) and phenyl(*tert*-butyl-L-alaninyl) phosphorochloridate (131, 0.85 mL of solution 1M in THF, 0.85 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (180 mg, 67%).

δ_P (d_4-CH$_3$OH): 3.22, 3.19; δ_H (d_4-CH$_3$OH): 7.65 (1H, m, H6-uridine), 7.36 (2H, m, CH-phenyl), 7.27-7.18 (3H, m, CH-phenyl), 5.80 (1H, m, H1'-uridine), 5.67 (1H, m, H5-uridine), 5.03-5.00 (2H, m, H2'-uridine, H3'-uridine), 4.31-4.26 (2H, m, H5'-uridine), 3.86 (1H, m CHα), 2.21-2.10 (2H, m, CH$_2$-cyclopentyl), 1.79-1.46 (6H, m, CH$_2$-cyclopentyl), 1.46 (9H, s, CH$_3$-*tert*-butyl), 1.34 (3H, d, CH$_3$-alanine, J= 6.6 Hz).

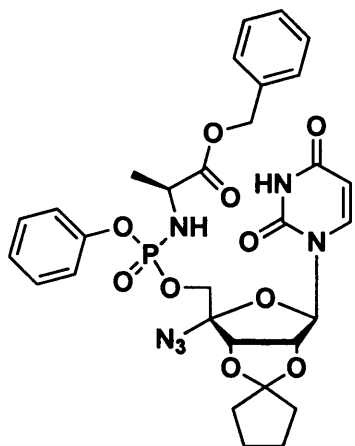### Synthesis of 4'-azidouridine 5'-*O*-[phenyl(*tert*-butyl-L-alaninyl)] phosphate

Prepared according to the standard procedure 2, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(*tert*-butyl-L-alaninyl)] phosphate (180 mg, 0.283 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (8:2). The pure product was a white solid (90 mg, 56%).

δ_P (d_4-CH$_3$OH): 3.63, 3.59; δ_H (d_4-CH$_3$OH): 7.65 (1H, m, H6-uridine), 7.37 (2H, m, CH-phenyl), 7.28-7.20 (3H, m, CH-phenyl), 6.14 (1H, m, H1'-uridine), 5.71 (1H, m, H5-uridine), 4.41-4.34 (2H, m, H2'-uridine, H3'-uridine), 4.24-4.19 (2H, m, H5'-uridine), 3.87-3.84 (1H, m, CHα), 1.46 (9H, s, CH$_3$-*tert*-butyl), 1.32 (3H, d, CH$_3$-alanine, J= 7.4 Hz).

MS (ES) m/e: 591.1 (MNa$^+$, 100%); Accurate mass: C$_{22}$H$_{29}$N$_6$O$_{10}$NaP required 591.1586, found 591.1580.

Anal. Calc. for C$_{20}$H$_{25}$N$_6$O$_{10}$P: C 46.48%, H 5.14%, N 14.78%. Found: C 46.70%, H 4.98%, N 14.53%.
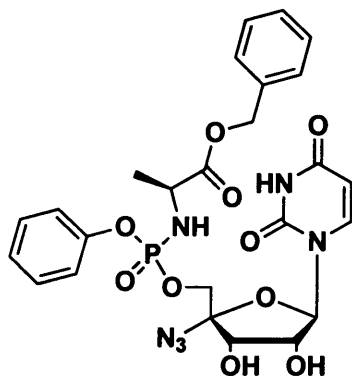
Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(benzyl-L-alaninyl)] phosphate

Prepared according to the standard procedure 1, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (**145**, 150 mg, 0.427 mmol), 'BuMgCl (0.85 mL, 1M solution in THF, 0.85 mmol) and phenyl(benzyl-L-alaninyl) phosphorochloridate (**132**, 0.85 mL of solution 1M in THF, 0.85 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (95:5). The pure product was a white solid (140 mg, 49%).

δ_P (d_4-CH$_3$OH): 3.17, 3.02; δ_H (d_4-CH$_3$OH): 7.49 (1H, d, H6-uridine, *J*= 7.9 Hz), 7.22 (6H, m, CH-phenyl, CH-benzyl), 7.13-7.02 (4H, m, CH-phenyl, CH-benzyl), 5.85 (1H, m, H1'-uridine), 5.54 (1H, m, H5-uridine, *J*= 7.9 Hz), 5.03 (3H, m, CH$_2$-benzyl, H2'-uridine), 4.87 (1H, m, H3'-uridine), 4.13-4.10 (2H, m, H5'-uridine), 3.94 (1H, m, CHα), 2.14-1.97 (2H, m, CH$_2$-cyclopentyl), 1.65-1.59 (6H, m, CH$_2$-cyclopentyl), 1.25 (3H, m, CH$_3$-alanine).

Synthesis of 4'-azidouridine 5'-*O*-[phenyl(benzyl-L-alaninyl)] phosphate (152)

Prepared according to the standard procedure 2, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(benzyl-L-alaninyl)] phosphate (140 mg, 0.209 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (8:2). The pure product was a white solid (70 mg, 55%).

δ_P (d_4-CH$_3$OH): 3.53, 3.28; δ_H (d_4-CH$_3$OH): 7.61 (1H, m, H6-uridine), 7.36 (6H, m, CH-phenyl, CH-benzyl), 7.31-7.19 (4H, m, CH-phenyl, CH-benzyl), 6.13 (1H, m,

H1'-uridine), 5.68 (1H, m, H5-uridine), 5.15 (2H, s, CH_2-benzyl), 4.36 (2H, m, H2'-uridine, H3'-uridine), 4.21-4.14 (2H, m, H5'-uridine), 4.05 (1H, m, CHα), 1.37 (3H, m, CH_3-alanine).

MS (ES) m/e: 625.0 (MNa^+, 100%); Accurate mass: $C_{25}H_{27}N_6O_{10}NaP$ required 625.1426, found 625.1424.

Anal. Calc. for $C_{25}H_{27}N_6O_{10}P$: C 49.84%, H 4.52%, N 13.95%. Found: C 49.91%, H 4.51%, N 13.66%.

Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(ethyl-α,α-dimethylglycinyl)] phosphate
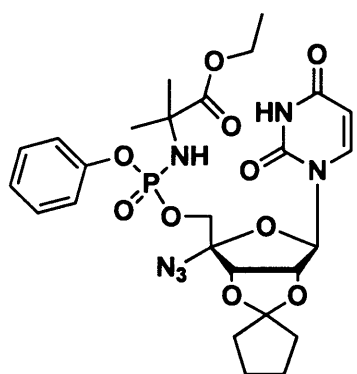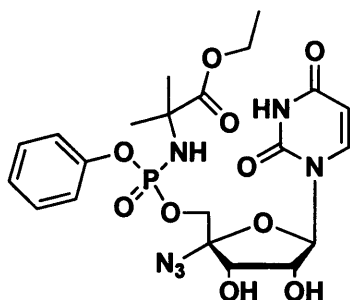

Prepared according to the standard procedure 1, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (**145**, 150 mg, 0.427 mmol), 'BuMgCl (0.85 mL, 1M solution in THF, 0.85 mmol) and phenyl(ethyl-α,α-dimethylglycinyl) phosphorochloridate (**136**, 0.85 mL of solution 1M in THF, 0.85 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (179 mg, 67%).

δ_P (d_4-CH_3OH): 1.68, 1.58; δ_H (d_4-CH_3OH): 7.66 (1H, dd, H6-uridine), 7.37-7.35 (2H, m, CH-phenyl), 7.27 (2H, b, CH-phenyl), 7.20 (1H, b, CH-phenyl), 5.98 (1H, b, H1'-uridine), 5.68 (1H, dd, H5-uridine), 5.14-5.01 (2H, m, H2'-uridine, H3'-uridine), 4.28 (2H, b, H5'-uridine), 4.17 (2H, q, CH_2-ethyl, J= 7.1 Hz), 2.21-2.12 (2H, m, CH_2-cyclopentyl), 1.79-1.73 (6H, m, CH_2-cyclopentyl), 1.50 (6H, d, CH_3-lateral chain), 1.26 (3H, t, CH_3-ethyl, J= 7.1 Hz).

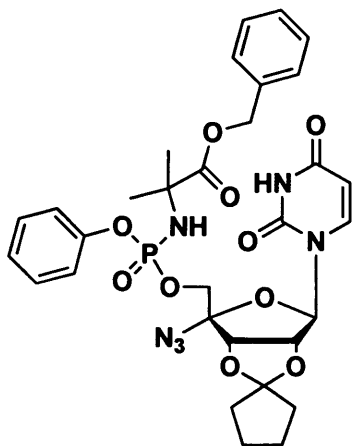## Synthesis of 4'-azidouridine 5'-*O*-[phenyl(ethyl-α,α-dimethylglycinyl)] phosphate (156)

Prepared according to the standard procedure 2, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(ethyl-α,α-dimethylglycinyl)] phosphate (179 mg, 0.288 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (8:2). The pure product was a white solid (144 mg, 90%).

δ_P (d_4-CH_3OH): 1.90, 1.87; δ_H (d_4-CH_3OH): 7.64 (1H, dd, H6-uridine), 7.39-7.35 (2H, m, CH-phenyl), 7.26 (2H, d, CH-phenyl), 7.20 (1H, d, CH-phenyl), 6.14 (1H, d, H1'-uridine, J= 2.6 Hz), 5.67 (1H, dd, H5-uridine), 4.40-4.37 (2H, m, H2'-uridine, H3'-uridine), 4.33 (2H, br, H5'-uridine), 4.23-4.15 (1H, m, CH_2-ethyl), 1.49 (6H, d, CH_3-lateral chain), 1.27 (3H, t, CH_3-ethyl).

MS (ES) m/e: 577.1 (MNa^+, 100%); Accurate mass: $C_{21}H_{27}N_6O_{10}NaP$ required 577.1424, found 577.1431.

Anal. Calc. for $C_{21}H_{27}N_6O_{10}P$: C 45.49, H 4.91%, N 15.16%. Found: C 45.45%, H 5.09%, N 14.74%.
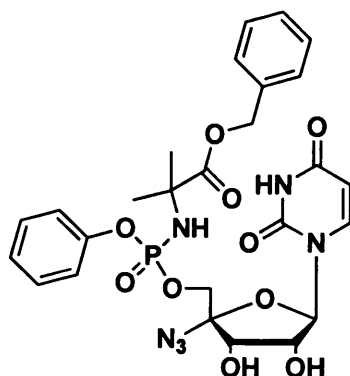
Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(benzyl-α,α-dimethylglycinyl)] phosphate

Prepared according to the standard procedure 1, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (**145**, 150 mg, 0.427 mmol), ᵗBuMgCl (0.85 mL, 1M solution in THF, 0.85 mmol) and phenyl(benzyl-α,α-dimethylglycinyl) phosphorochloridate (**155**, 0.85 mL of solution 1M in THF, 0.85 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (95:5). The pure product was a white solid (148 mg, 51%).

δ$_P$ (d_4-CH$_3$OH): 1.72, 1.63; δ$_H$ (d_4-CH$_3$OH): 7.62 (1H, dd, H6-uridine), 7.37-7.31 (7H, m, CH-phenyl, CH-benzyl), 7.23-7.18 (3H, m, CH-phenyl), 5.96 (1H, b, H1'-uridine), 5.67 (1H, dd, H5-uridine), 5.15 (2H, dd, CH$_2$-benzyl), 5.11-4.94 (2H, m, H2'-uridine, H3'-uridine), 4.22 (2H, dd, H5'-uridine), 2.27-2.11 (2H, m, CH$_2$-cyclopentyl), 1.77-1.72 (6H, m, CH$_2$-cyclopentyl), 1.52 (6H, s, CH$_3$-lateral chain).

Synthesis of 4'-azidouridine 5'-*O*-[phenyl(benzyl-α,α-dimethylglycinyl)] phosphate (135)

Prepared according to the standard procedure 2, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(benzyl-α,α-dimethylglycinyl)] phosphate (148 mg, 0.217 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (8:2). The pure product was a white solid (110 mg, 82%).

δ$_P$ (d_4-CH$_3$OH): 1.86, 1.83; δ$_H$ (d_4-CH$_3$OH): 7.60 (1H, dd, H6-uridine), 7.36-7.32 (7H, m, 2 CH-phenyl, 5 CH-benzyl), 7.24-7.17 (3H, m, 3 CH-phenyl), 6.12 (1H, dd, H1'-

uridine), 5.65 (1H, dd, H5-uridine), 5.15 (2H, dd, CH_2-benzyl), 4.38-4.29 (2H, m, H2'-uridine, H3'-uridine), 4.19-4.16 (2H, dd, H5'-uridine), 1.50 (6H, s, 2 CH_3-lateral chain).

MS (ES) m/e: 639.1 (MNa^+, 100%); Accurate mass: $C_{26}H_{29}N_6O_{10}NaP$ required 639.1580, found 639.1574.

Anal. Calc. for $C_{26}H_{29}N_6O_{10}P$: C 50.65, H 4.74%, N 13.63%. Found: C 50.64%, H 4.86%, N 13.12%.

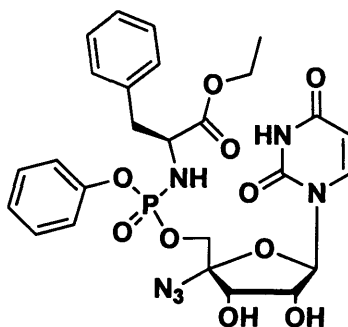## Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(ethyl-L-phenylalaninyl)] phosphate
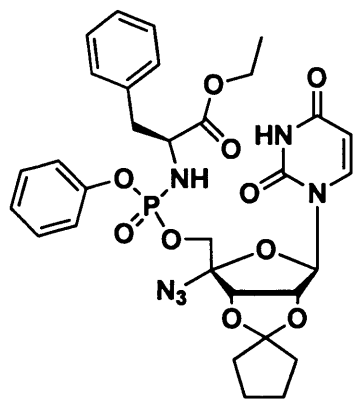
Prepared according to the standard procedure 1, from 2',3'-*O,O*-cyclopenthylidene-4'-azidouridine (**145**, 150 mg, 0.427 mmol), 'BuMgCl (0.85 mL, 1M solution in THF, 0.85 mmol) and phenyl(ethyl-L-phenylalaninyl) phosphorochloridate (**138**, 0.85 mL of solution 1M in THF, 0.85 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (190 mg, 65%).

δ_P (d_4-CH$_3$OH): 3.07, 2.72; δ_H (d_4-CH$_3$OH): 7.59 (1H, dd, H6-uridine), 7.32-7.09 (10H, m, CH-phenyl, CH-lateral chain), 5.95 (1H, dd, H1'-uridine), 5.68 (1H, dd, H5-uridine), 5.07-4.91 (2H, m, H2'-uridine, H3'-uridine), 4.15-4.08 (3H, m, H5'-uridine, CHα), 3.90-3.88 (2H, m, CH_2-ethyl), 3.07 (1H, q, CH_2-lateral chain), 2.94 (1H, q, CH_2-lateral chain), 2.20-2.10 (2H, m, CH_2-cyclopentyl), 1.78-1.73 (6H, m, CH_2-cyclopentyl), 1.18 (3H, m, CH_3-ethyl).

Synthesis of 4'-azidouridine 5'-*O*-[phenyl(ethyl-L-phenylalaninyl)] phosphate
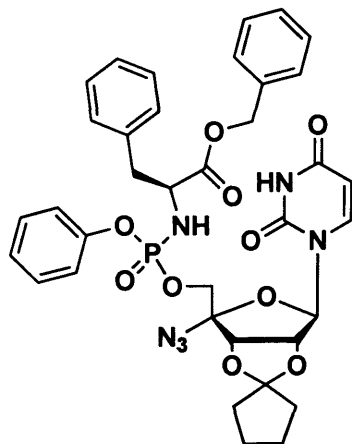
(158)

Prepared according to the standard procedure 2, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(ethyl-L-phenylalaninyl)] phosphate (190 mg, 0.278 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (8:2). The pure product was a white solid (152 mg, 88%).

δ_P (d_4-CH_3OH): 3.28, 3.06; δ_H (d_4-CH_3OH): 7.55 (1H, dd, H6-uridine), 7.34-7.07 (10H, m, CH-phenyl, CH-lateral chain), 6.14 (1H, dd, H1'-uridine), 5.70 (1H, dd, H5-uridine), 4.27-4.23 (2H, m, H2'-uridine, H3'-uridine), 4.15-4.00 (3H, m, H5'-uridine, CHα), 3.81-3.78 (2H, m, CH_2-ethyl), 3.10 (1H, q, CH_2-lateral chain), 2.89 (1H, q, CH_2-lateral chain), 1.20 (3H, m, CH_3-ethyl).

MS (ES) m/e: 639.0 (MNa$^+$, 100%); Accurate mass: $C_{26}H_{29}N_6O_{10}NaP$ required 639.1580, found 639.1594.

Anal. Calc. for $C_{26}H_{29}N_6O_{10}P$: C 50.65%, H 4.74%, N 13.63%. Found: C 50.61%, H 4.55%, N 13.51%.

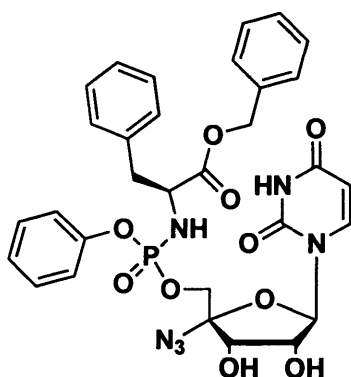## Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(benzyl-L-phenylalaninyl)] phosphate

Prepared according to the standard procedure 1, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (**145**, 150 mg, 0.427 mmol), ᵗBuMgCl (0.85 mL, 1M solution in THF, 0.85 mmol) and phenyl(benzyl-L-phenylalaninyl) phosphorochloridate (**137**, 0.85 mL of solution 1M in THF, 0.85 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (163 mg, 51%).

δ_P (d_4-CH_3OH): 2.98, 2.69; δ_H (d_4-CH_3OH): 7.53 (1H, dd, H6-uridine), 7.38-6.96 (15H, m, CH-phenyl, CH-lateral chain, CH-benzyl), 5.93 (1H, dd, H1'-uridine), 5.65 (1H, dd, H5-uridine), 5.10 (2H, m, CH_2benzyl), 5.04-4.87 (2H, m, H2'-uridine, H3'-uridine), 4.17-4.07 (3H, m, H5'-uridine, CHα), 3.07 (1H, m, CH_2-lateral chain), 2.85 (1H, m, CH_2-lateral chain), 2.22-2.07 (2H, m, CH_2-cyclopentyl), 1.88-1.71 (6H, m, CH_2-cyclopentyl).

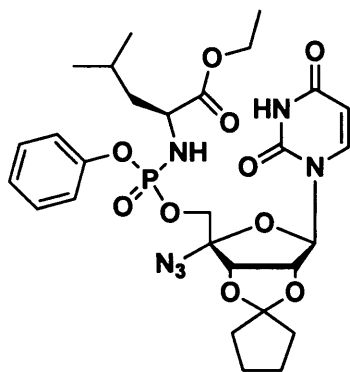## Synthesis of 4'-azidouridine 5'-*O*-[phenyl(benzyl-L-phenylalaninyl)] phosphate

(157)

Prepared according to the standard procedure 2, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(benzyl-L-phenylalaninyl)] phosphate (163 mg, 0.219 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (8:2). The pure product was a white solid (130 mg, 87%).

δ_P (d_4-CH$_3$OH): 3.21, 3.02; δ_H (d_4-CH$_3$OH): 7.51 (1H, dd, H6-uridine), 7.32-7.23 (6H, m, CH-phenyl, CH-lateral chain, CH-benzyl), 7.18-7.03 (9H, m, CH-phenyl, CH-lateral chain, CH-benzyl), 6.14 (1H, dd, H1'-uridine), 5.64 (1H, dd, H5-uridine), 5.16-5.09 (4H, m, H2'-uridine, H3'-uridine, CH$_2$benzyl), 4.24-4.12 (3H, m, H5'-uridine, CHα), 3.09 (1H, m, CH$_2$-lateral chain), 2.91-2.87 (1H, m, CH$_2$-lateral chain).

MS (ES) m/e: 701.1 (MNa$^+$, 100%); Accurate mass: C$_{31}$H$_{31}$N$_6$O$_{10}$NaP required 701.1737, found 701.1732.

Anal. Calc. for C$_{31}$H$_{31}$N$_6$O$_{10}$P: C 54.87%, H 4.60%, N 12.38%. Found: C 54.87%, H 4.60%, N 12.45%.

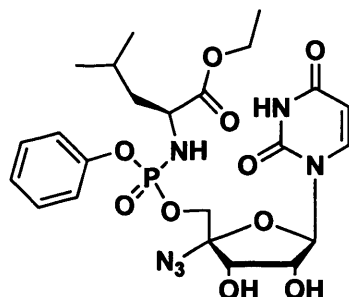## Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(ethyl-L-leucinyl)] phosphate

Prepared according to the standard procedure 1, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (**145**, 150 mg, 0.427 mmol), ^{t}BuMgCl (0.85 mL, 1M solution in THF, 0.85 mmol) and phenyl(ethoxy-L-leucinyl) phosphorochloridate (**139**, 0.85 mL of solution 1M in THF, 0.85 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (95:5). The pure product was a white solid (135 mg, 48%).

δ_P (d_4-CH$_3$OH): 3.44, 3.22; δ_H (d_4-CH$_3$OH): 7.65 (1H, dd, H6-uridine), 7.35 (2H, d, CH-phenyl), 7.27-7.19 (3H, m, CH-phenyl), 5.98 (1H, d, H1'-uridine), 5.69 (1H, dd, H5-uridine), 5.12-5.00 (2H, m, H2'-uridine, H3'-uridine), 4.30-4.22 (2H, m, H5'-uridine), 4.16-4.13 (2H, m, CH$_2$-ethyl), 3.90 (1H, m, CHα), 2.21-2.08 (2H, m, CH$_2$-cyclopentyl), 1.79-1.73 (6H, m, CH$_2$-cyclopentyl), 1.62-1.52 (3H, m CH-lateral chain, CH$_2$-lateral chain), 1.25 (3H, m, CH$_3$-ethyl), 0.89 (3H, d, CH$_3$-lateral chain, *J*= 6.6 Hz), 0.84 (3H, d, CH$_3$-lateral chain, *J*= 6.0 Hz).

Synthesis of 4'-azidouridine 5'-O-[phenyl(ethyl-L-leucinyl)] phosphate (159)
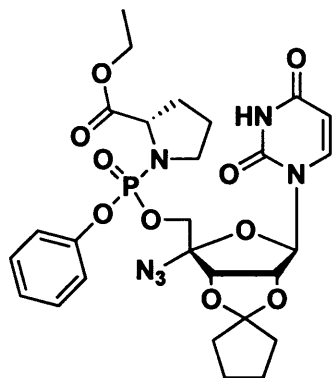

Prepared according to the standard procedure 2, from 2',3'-O,O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(ethyl-L-leucinyl)] phosphate (135 mg, 0.208 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (8:2). The pure product was a white solid (111mg, 91%).

δ_P (d_4-CH_3OH): 3.83, 3.47; δ_H (d_4-CH_3OH): 7.64 (1H, dd, H6-uridine), 7.36 (2H, t, 2 CH-phenyl), 7.25-7.20 (3H, m, CH-phenyl), 6.16 (1H, d, H1'-uridine), 5.72 (1H, dd, H5-uridine), 4.40-4.35 (2H, m, H2'-uridine, H3'-uridine), 4.22 (2H, b, H5'-uridine), 4.18-4.11 (2H, m, CH_2-ethyl), 3.90 (1H, b, CHα), 1.54 (3H, m CH-lateral chain, CH_2-lateral chain), 1.27-1.19 (3H, m, CH_3-ethyl), 0.86 (3H, t, CH_3-lateral chain), 0.80 (3H, t, CH_3-lateral chain).

MS (ES) m/e: 605.1 (MNa⁺, 100%); Accurate mass: $C_{23}H_{31}N_6O_{10}NaP$ required 605.1737, found 605.1733.

Anal. Calc. for $C_{23}H_{31}N_6O_{10}P$: C 47.42%, H 5.36%, N 14.43%. Found: C 47.43%, H 5.26%, N 14.40%.
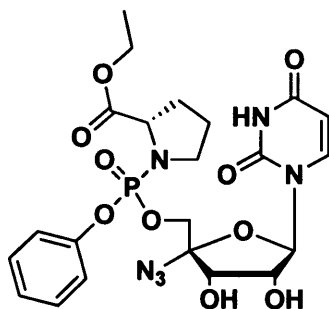
Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(ethyl-L-prolinyl)] phosphate

Prepared according to the standard procedure 1, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (**145**, 150 mg, 0.427 mmol), 'BuMgCl (0.85 mL, 1M solution in THF, 0.85 mmol) and phenyl(ethyl-L-prolinyl) phosphorochloridate (**140**, 0.85 mL of solution 1M in THF, 0.85 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (121 mg, 45%).

δ_P (d_4-CH_3OH): 1.58, 1.38; δ_H (d_4-CH_3OH): 77.66 (1H, dd, H6-uridine), 7.38 (2H, t, 2 CH-phenyl), 7.30-7.20 (3H, m, CH-phenyl), 5.95 (1H, dd, H1'-uridine), 5.69 (1H, dd, H5-uridine), 5.14 (2H, d, H2'-uridine, *J*= 6.2 Hz), 5.00 (1H, d, H3'-uridine, *J*= 5.3 Hz), 4.30 (2H, m, H5'-uridine), 4.19-4.13 (2H, m, CH_2-ethyl), 3.40 (1H, t, CHα), 2.26-2.17 (2H, m, CH_2-cyclopentyl), 2.12-1.99 (6H, m CH_2-proline), 1.79-1.73 (6H, m, CH_2-cyclopentyl), 1.26 (3H, m, CH_3-ethyl).

Synthesis of 4'-azidouridine 5'-*O*-[phenyl(ethyl-L-prolinyl) phosphate (160)

Prepared according to the standard procedure 2, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(ethyl-L-prolinyl)] phosphate (121 mg, 0.190 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (8:2). The pure product was a white solid (101 mg, 94%).

δ_P (d_4-CH_3OH): 1.60, 1.25; δ_H (d_4-CH_3OH): 7.65 (1H, dd, H6-uridine), 7.39 (2H, t, CH-phenyl), 7.30-7.23 (3H, m, CH-phenyl), 6.12 (1H, dd, H1'-uridine), 5.71 (1H, dd, H5-uridine), 4.39-4.29 (2H, m, H2'-uridine, H3'-uridine), 4.25-4.13 (4H, m, H5'-

uridine, CH_2-ethyl), 3.42 (1H, m, $CH\alpha$), 2.23-2.17 (2H, m, CH_2-proline), 2.02-1.84 (4H, m CH_2-proline), 1.28 (3H, m, CH_3-ethyl).

MS (ES) m/e: 589.1 (MNa^+, 100%); Accurate mass: $C_{22}H_{27}N_6O_{10}NaP$ required 589.1424, found 589.1416.

Anal. Calc. for $C_{22}H_{27}N_6O_{10}P$: C 46.65, H 4.80%, N 14.84%. Found: C 46.32%, H 4.86%, N 14.51%.
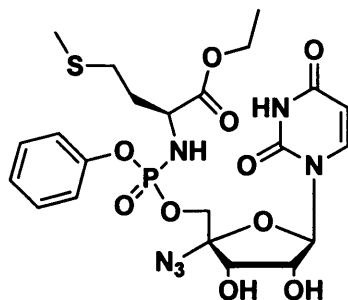
Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(ethyl-L-methioninyl)] phosphate
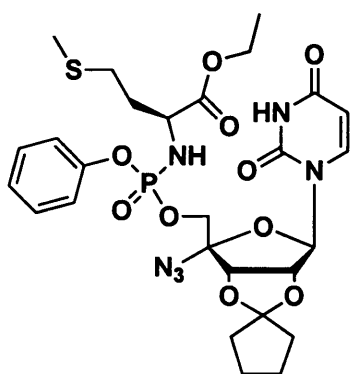
Prepared according to the standard procedure 1, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (**145**, 150 mg, 0.427 mmol), ^tBuMgCl (0.85 mL, 1M solution in THF, 0.85 mmol) and phenyl(ethyl-L-methioninyl) phosphorochloridate (**141**, 0.85 mL of solution 1M in THF, 0.85 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (187 mg, 65%).

δ_P (d_4-CH$_3$OH): 3.34, 3.21; δ_H (d_4-CH$_3$OH): 7.65 (1H, t, H6-uridine), 7.37 (2H, m, CH-phenyl), 7.29-7.19 (3H, m, CH-phenyl), 5.97 (1H, d, H1'-uridine), 5.70 (1H, dd, H5-uridine), 5.12-5.02 (2H, m, H2'-uridine, H3'-uridine), 4.30-4.26 (2H, m, H5'-uridine), 4.19-4.15 (2H, m, CH_2-ethyl), 4.07 (1H, m, $CH\alpha$), 2.60-2.46 (2H, m, CH_2-lateral chain), 2.21-2.07 (2H, m, CH_2-cyclopentyl), 2.03 (3H, d, CH_3-lateral chain, $J=$ 8.3 Hz), 1.94-1.85 (2H, m, CH_2-lateral chain), 1.79-1.74 (6H, m, CH_2-cyclopentyl), 1.26 (3H, m, CH_3-ethyl).

Synthesis of 4'-azidouridine 5'-O-[phenyl(ethyl-L- methioninyl)] phosphate (161)
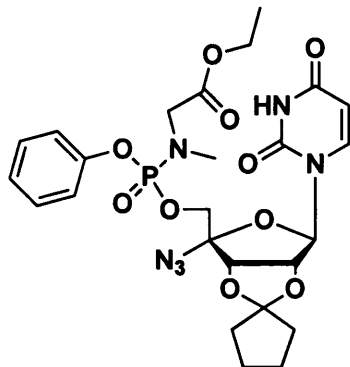

Prepared according to the standard procedure 2, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(ethyl-L-methioninyl)] phosphate (187 mg, 0.280 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8:2). The pure product was a white solid (133 mg, 79%).

δ_P (d_4-CH₃OH): 3.81, 3.48; δ_H (d_4-CH₃OH): 7.65 (1H, t, H6-uridine), 7.38 (2H, d, CH-phenyl), 7.30-7.21 (3H, m, CH-phenyl), 6.15 (1H, dd, H1'-uridine), 5.72 (1H, dd, H5-uridine), 4.41-4.35 (2H, m, H2'-uridine, H3'-uridine), 4.27-4.16 (4H, m, H5'-uridine, CH₂-ethyl), 4.08 (1H, t, CHα), 2.53 (2H, m, CH₂-lateral chain), 2.03 (3H, d, CH₃-lateral chain, *J*= 15.3 Hz), 1.88-1.84 (2H, m, CH₂-lateral chain), 1.31 (3H, m, CH₃-ethyl).

MS (ES) m/e: 563.0 (MNa⁺, 100%); Accurate mass: C₂₀H₂₅N₆O₁₀NaP required 563.1257, found 563.1267.

Anal. Calc. for C₂₀H₂₅N₆O₁₀P: C 44.45%, H 4.66%, N 15.55%. Found: C 44.90%, H 4.07%, N 15.75%.

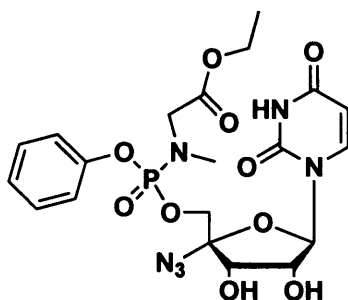## Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(ethyl-*N*-methyl-glycinyl)] phosphate

Prepared according to the standard procedure 1, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (**145**, 150 mg, 0.427 mmol), 'BuMgCl (0.85 mL, 1M solution in THF, 0.85 mmol) and phenyl(ethyl-L- *N*-methyl-glycinyl) phosphorochloridate (**143**, 0.85 mL of solution 1M in THF, 0.85 mmol). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (95:5). The pure product was a white solid (172 mg, 66%).

δ_P (d_4-CH₃OH): 3.36, 3.29; δ_H (d_4-CH₃OH): 7.74 (1H, dd, H6-uridine), 7.45-7.42 (2H, m, CH-phenyl), 7.34-7.28 (3H, m, CH-phenyl), 6.04 (1H, d, H1'-uridine, *J*= 1.1 Hz), 5.77 (1H, dd, H5-uridine), 5.20-5.07 (2H, m, H2'-uridine, H3'-uridine), 4.41 (1H, dd, H5'-uridine), 4.36 (1H, dd, H5'-uridine), 4.28-4.26 (2H, m, CH₂-ethyl), 3.88 (1H, m, CH₂-glycine), 3.42 (1H, m, CH₂-glycine), 2.90 (3H, s, CH₃-N), 2.27-2.18 (2H, m, CH₂-cyclopentyl), 1.86-1.80 (6H, m, CH₂-cyclopentyl), 1.34 (3H, m, CH₃-ethyl).

Synthesis of 4'-azidouridine 5'-*O*-[phenyl(ethyl-*N*-methyl-glycinyl)] phosphate (163)
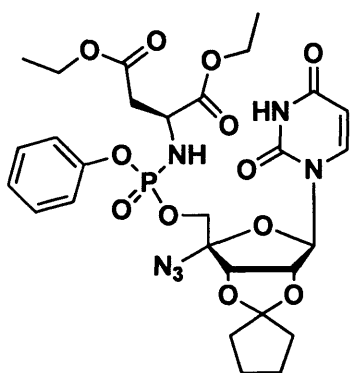
Prepared according to the standard procedure 2, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(ethyl-*N*-methyl-glycinyl)] phosphate (172 mg, 0.284 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8:2). The pure product was a white solid (135 mg, 88%).

δ_P (d_4-CH₃OH): 5.12, 4.93; δ_H (d_4-CH₃OH): 7.66 (1H, dd, H6-uridine), 7.42-7.38 (2H, m, CH-phenyl), 7.27-7.22 (3H, m, CH-phenyl), 6.15 (1H, d, H1'-uridine, *J*= 2.1 Hz),

5.70 (1H, dd, H5-uridine), 4.42-4.29 (3H, m, H2'-uridine, H3'-uridine, H5'-uridine), 4.26-4.17 (3H, m, H5'-uridine, CH_2-ethyl), 4.00 (1H, m, CH_2-glycine), 3.80 (1H, m, CH_2-glycine), 2.83 (3H, d, CH_3-N), 1.28 (3H, t, CH_3-ethyl, $J= 5.0$ Hz).

Anal. Calc. for $C_{20}H_{25}N_6O_{10}P$: C 44.45%, H 4.66%, N 15.55%. Found: C 44.60%, H 4.90%, N 15.54%.

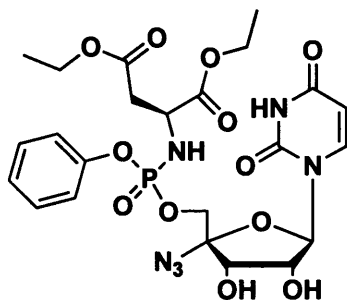## Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(diethyl-L-aspartyl)] phosphate

Prepared according to the standard procedure 1, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (**145**, 150 mg, 0.427 mmol), 'BuMgCl (0.85 mL, 1M solution in THF, 0.85 mmol) and phenyl(diethyl-L-aspartyl) phosphorochloridate (**144**, 0.85 mL of solution 1M in THF, 0.85 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (197 mg, 66%).

δ_P (d_4-CH_3OH): 3.24, 3.22; δ_H (d_4-CH_3OH): 7.65 (1H, d, H6-uridine, $J= 7.6$ Hz), 7.36 (2H, d, CH-phenyl), 7.27-7.19 (3H, m, CH-phenyl), 5.98 (1H, d, H1'-uridine), 5.70 (1H, dd, H5-uridine), 5.13-5.00 (2H, m, H2'-uridine, H3'-uridine), 4.29-4.25 (2H, m, H5'-uridine), 4.18-4.11 (4H, m, CH_2-ethyl, CH_2-ethyl lateral chain), 3.99-3.97 (1H, m, CHα), 2.48-2.35 (2H, m, CH_2-lateral chain), 2.21-2.10 (4H, m, CH_2-cyclopentyl, CH_2-lateral chain), 1.79-1.73 (6H, m, CH_2-cyclopentyl), 1.28-1.21 (6H, m, CH_3-ethyl lateral chain, CH_3-ethyl).

## Synthesis of 4'-azidouridine 5'-*O*-[phenyl(diethyl-L-aspartyl)] phosphate (164)

Prepared according to the standard procedure 2, from 2',3'-*O,O*-cyclopenthylidene-4'-azidouridine 5'-*O*-[phenyl(diethyl-L-aspartyl)] phosphate (197 mg, 0.284 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (8:2). The pure product was a white solid (168 mg, 94%).

δ_P (d_4-CH$_3$OH): 3.66, 3.39; δ_H (d_4-CH$_3$OH): 7.60 (1H, dd, H6-uridine), 7.35 (2H, m, CH-phenyl), 7.26-7.19 (3H, m, CH-phenyl), 6.12 (1H, d, H1'-uridine), 5.72 (1H, dd, H5-uridine), 4.40-4.32 (2H, m, H2'-uridine, H3'-uridine), 4.29-4.08 (6H, m, H5'-uridine, CH$_2$-ethyl, CH$_2$-ethyl lateral chain), 4.01-3.94 (1H, m, CHα), 2.42-2.16 (2H, m, CH$_2$-lateral chain), 2.10-1.82 (2H, m, CH$_2$-lateral chain), 1.28-1.22 (6H, m, CH$_3$-ethyl lateral chain, CH$_3$-ethyl).

Anal. Calc. for C$_{24}$H$_{31}$N$_6$O$_{10}$P: C 46.01, H 4.99%, N 13.41%. Found: C 46.15%, H 5.02%, N 13.62%.

Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(ethyl-β-alaninyl)] phosphate
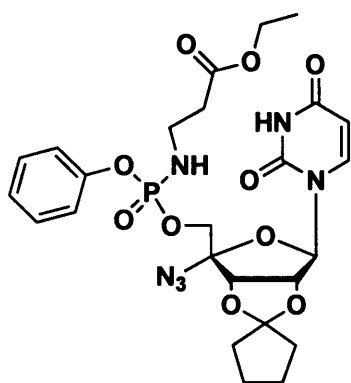
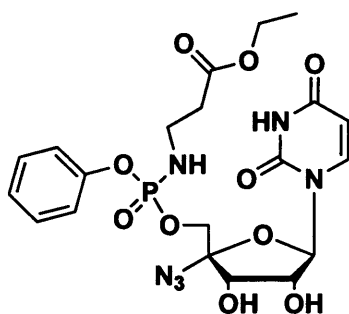Prepared according to the standard procedure 1, from 2',3'-*O,O*-cyclopentylidene-4'-azidouridine (145, 150 mg, 0.427 mmol), 'BuMgCl (0.85 mL, 1M solution in THF, 0.85 mmol) and phenyl(ethyl-β-alaninyl) phosphorochloridate (142, 0.85 mL of solution 1M in THF, 0.85 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (95:5). The pure product was a white solid (165 mg, 63%).

δ_P (d_4-CH$_3$OH): 3.50, 3.44; δ_H (d_4-CH$_3$OH): 7.70 (1H, dd, H6-uridine), 7.44-7.42 (2H, m, CH-phenyl), 7.33-7.26 (3H, m, CH-phenyl), 6.03 (1H, dd, H1'-uridine), 5.78 (1H, dd, H5-uridine), 5.11-5.09 (2H, m, H2'-uridine, H3'-uridine), 4.33 (2H, b, H5'-uridine), 4.20 (1H, q, CH$_2$-ethyl, J= 6.2 Hz), 3.35 (1H, t, CH$_2\alpha$), 2.60 (1H, b, CH$_2\beta$), 2.26-2.20 (2H, m, CH$_2$-cyclopentyl), 1.86-1.80 (6H, m, CH$_2$-cyclopentyl), 1.32 (3H, t, CH$_3$-ethyl, J= 6.2 Hz).

Synthesis of 4'-azidouridine 5'-O-[phenyl(ethyl-β-alaninyl)] phosphate (162)
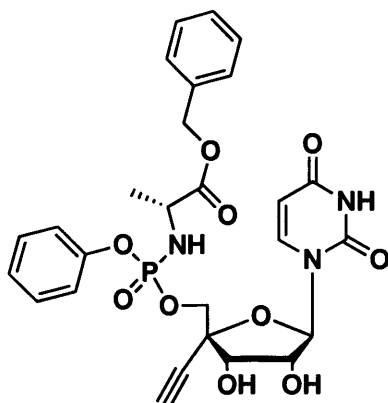

Prepared according to the standard procedure 2, from 2',3'-O,O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(ethyl-β-alaninyl)] phosphate (165 mg, 0.272 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (8:2). The pure product was a white solid (127 mg, 86%).

δ_P (d_4-CH$_3$OH): 3.33, 3.27; δ_H (d_4-CH$_3$OH): 7.62 (1H, dd, H6-uridine), 7.38 (2H, t, CH-phenyl), 7.26-7.20 (3H, m, CH-phenyl), 6.12 (1H, d, H1'-uridine, J= 3.2 Hz), 5.72 (1H, dd, H5-uridine), 4.39-4.34 (2H, m, H2'-uridine, H3'-uridine), 4.23-4.11 (4H, m, H5'-uridine, CH$_2$-ethyl), 3.27 (1H, m, CH$_2\alpha$), 2.54 (1H, br, CH$_2\beta$), 1.25 (3H, m, CH$_3$-ethyl).

MS (ES) m/e: 563.0 (MNa$^+$, 100%); Accurate mass: C$_{20}$H$_{25}$N$_6$O$_{10}$NaP required 563.1267, found 563.1279.

Anal. Calc. for C$_{20}$H$_{25}$N$_6$O$_{10}$P: C 44.45%, H 4.66%, N 15.55%. Found: C 44.24%, H 4.87%, N 15.58%.

## Synthesis of 4'-ethynyluridine 5'-*O*-[phenyl(benzyl-D-alaninyl)] phosphate (180)

Prepared according to Standard Procedure D, from 4'-ethynyluridine (25, 150 mg, 0.559 mmol), ^{t}BuMgCl (1.12 mL of solution 1M in THF, 1.12 mmol) and phenyl(benzyl-D-alaninyl) phosphorochloridate (115, 1.1 mL of solution 1M in THF, 1.12 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (90:10) then a preparative TLC using as eluent $CHCl_3$/MeOH (9:1). The pure product was a white solid (100 mg, 17%).

δ_P (d_4-CH_3OH): 4.80, 4.14; δ_H (d_4-CH_3OH): 7.55 (1H, m, H6-uridine), 7.35 (7H, m, CH-phenyl, CH-benzyl), 7.23 (3H, m, CH-phenyl, CH-benzyl), 6.00 (1H, m, H1'-uridine), 5.65 (1H, m, H2-uridine), 5.16 (2H, s, CH_2-benzyl), 4.32 (1H, m, H3'-cytidine), 4.28 (1H, m, H2'-cytidine), 4.15 (2H, m, H5'-cytidine), 4.05 (1H, m, $CH\alpha$), 3.18 (1H, CH-ethynyl), 1.36 (3H, m, CH_3-alanine); δ_C (d_4-CH_3OH): 175.33, 175.28, 175.08, 175.01 (1C, C=O ester), 166.37 (1C, C4-uridine), 152.68, 152.58, 152.39, 152.32 (1C, C2-uridine), 143.13, 143.06 (1C, C6-uridine), 137.62, 137.55 (1C, C-phenyl), 131.30, 131.27 (2C, CH-phenyl), 130.01 (2C, 2 CH-benzyl), 129.78, 129.76, 129.71 (2C, 2 CH-phenyl), 127.42 (1C, C-ethynyl), 126.77 (1C, C-benzyl), 124.24, 124.01 (2C, 2 CH-benzyl), 121.91, 121.85 (1C, CH-benzyl) 121.77, 121.70 (1C, CH-phenyl), 103.87, 103.82 (1C, C5-uridine), 99.11, 98.98 (1C, C4'-uridine), 91.97, 91.41 (1C, C1'-uridine), 74.55, 74.44 (1C, C3'-uridine), 72.40, 71.99 (1C, C2'-uridine), 70.32, 70.25 (1C, CH-ethynyl), 69.62, 69.56 (1C, CH_2-benzyl), 68.53, 68.45 (1C, C5'-uridine), 52.17, 51.89 (1C, $CH\alpha$), 20.87, 20.78, 20.68 (1C, CH_3-lateral chain).

MS (ES) m/e: 608.1 (MNa^+, 100%); Accurate mass: $C_{27}H_{28}N_3O_{10}NaP$ required 608.1410, found 608.14.

7.8 Synthesis of 4'-azidoadenosine.

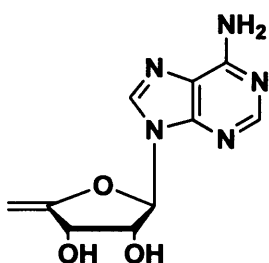**Synthesis of 5'-deoxy-5'-iodoadenosine (182)[9]**
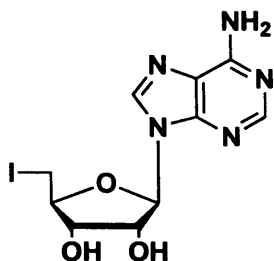
Iodine (35.43 g, 0.140 mol) and triphenylphosphine (36.80 g, 0.140 mol) were added to a solution of adenosine (**181**, 25 g, 0.093 mol) in pyridine (200 mL). After 2 hours a saturated solution of $Na_sS_2O_3$ was added; the solvent was removed under reduced pressure and the yellow solid was purified by column chromatography using as eluent $CHCl_3$/MeOH 9:1. The obtained product (60 g, >100 %) was pure enough for the following reaction.

δ_H (d_6-$(CH_3)_2SO$): 8.82 (1H, s, NH$_2$6-adenosine), 8.59 (1H, s, H2-adenosine), 8.36 (1H, s, H8-adenosine), 5.95 (1H, d, H1'-adenosine, $J=$ 5.6 Hz), 4.75 (1H, t, H2'-adenosine), 4.16 (1H, t, H3'-adenosine), 4.01 (1H, m, H4'-adenosine), 3.60 (1H, m, H5'-adenosine), 3.46 (1H, m, H5'-adenosine).

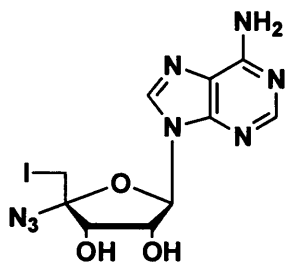**Synthesis of 1-(5-deoxy-β-D-glycero-pent-4-enofuranosyl)-adenine (183)**

tBuOK (47.0 g, 0.418 mol) was added to a solution of 5'-deoxy-5'-iodoadenosine (**182**, 35.0 g, 0.093 mol) in pyridine (200 mL), and the reaction stirred for 1 hour at 80 °C. The solvent was removed under reduced pressure and the black solid was purified by column chromatography using as eluent a mixture of $CHCl_3$/MeOH 9:1, then 8:2 and 7:3. The product was obtained as brown solid (18.5 g, 80%)

δ_H (d_6-$(CH_3)_2SO$): 8.37 (1H, s, H2-adenosine), 8.16 (1H, s, H8-adenosine), 7.32 (2H, s, NH$_2$6-adenosine), 6.16 (1H, d, H1'-adenosine, $J=$ 5.3 Hz), 5.73 (1H, s, OH2'-adenosine), 5.58 (1H, s, OH3'-adenosine), 4.83 (1H, t, H2'-adenosine), 4.73 (1H, t, H3'-adenosine), 4.31 (1H, s, H5'-adenosine), 4.21 (1H, s, H5'-adenosine).

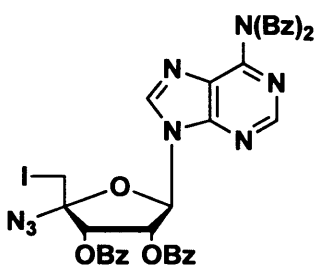## Synthesis of 4'-azido-5'-deoxy-5'iodoadenosine (184)

Sodium azide (11.73 g, 180.4 mmol) was added to a solution of ICl (14.65 g, 90.2 mol) in DMF (50 mL) and stirred at 30 °C for 20 minutes. Then a solution of 1-(5-deoxy-β-D-glycero-pent-4-enofuranosyl)-adenine (**183**, 9.0 g, 36.1 mmol) in DMF (200 mL) was added dropwise in 30 minutes. After 1 hour a saturated solution of $Na_sS_2O_3$ was added; the solvent was removed under reduced pressure. the solid was dissolved in MeOH, the precipitate was removed buy filtration. The solvent was removed under reduced pressure and the yellow solid was purified by column chromatography using as eluent $CHCl_3$/MeOH 9:1. The desire product was obtained as a yellow solid (19 g, >100).

δ_H (d_4-CH_3OH): 8.86 (1H, s, H2-adenosine), 8.78 (1H, s, H8-adenosine), 6.26 (1H, d, H1'-adenosine, J= 5.1 Hz), 5.28 (1H, m, H2'-adenosine), 4.73 (1H, t, H3'-adenosine), 3.71 (1H, s, H5'-adenosine), 3.68 (1H, s, H5'-adenosine); δ_C (d_4-CH_3OH): 157.39 (1C, C4-adenosine), 154.17 (1C, C2-adenosine), 153.80 (1C, C8-adenosine), 150.68 (1C, C6-adenosine), 120.70 (1C, C5-adenosine), 98.77 (1C, C4'-adenosine), 91.22 (1C, C1'-adenosine), 75.30 (1C, C3'adenosine), 73.98 (1C, C2'-adenosine), 9.61 (1C, C5'-adenosine).

Synthesis of N^4,N^4-dibenzoyl, 2',3'-O,O-dibenzoyl-4'-azido-5'-deoxy-5'-iodoadenosine (185)
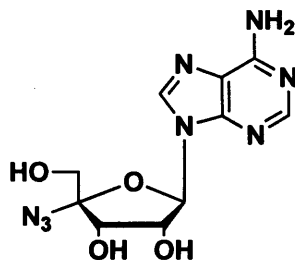
To a solution of 4'-azido-5'-deoxy-5'-iodoadenosine (**184**, 19.0 g, 45.0 mmol) in pyridine (200 mL), benzoyl chloride (10.5 mL, 180 mmol) was added. After 15 hours the solvent was removed under reduce pressure and the dark solid obtained was purified by column chromatography using as eluent EtOAc/Hexane 3:7. The desire product was obtained as a yellow solid (8.5 g, 35%).

δ_H (d_4-CH_3OH): 8.76 (1H, s, H2-adenosine), 8.67 (1H, s, H8-adenosine), 8.06-7.86 (10H, m, CH-benzoyl), 7.63-7.40 (10H, m, CH-benzoyl), 6.88 (1H, d, H3'-adenosine, J= 3.0 Hz), 6.60-6.59 (2H, m, H2'-adenosine, H1'-adenosine), 3.93 (1H, m, H5'-adenosine).

Synthesis of 4'-azidoadenosine (27)

To a solution of 4-N,N-dibenzoyl, 2',3'-*O,O*-dibenzoyl-4'-azido-5'-deoxy-5'-iodo-adenosine (**185**, 2.20 g, 2.64 mmol) in CH_2Cl_2 saturated with 1% of water (20 mL), 85% MCPBA (meta-chloro-perbenzoic acid, 3.63 g, 15.84 mmol) was added and the reaction stirred at 40°C for 1 hour. EtOAc was added and then washed with a saturated solution of $Na_sS_2O_3$. The organic layer was dried using $MgSO_4$ then the solvent was removed under reduced pressure. The yellow solid was dissolved in 6 mL of a solution 1N of MeONa in MeOH for 1 hour. The product (200 mg, 24%) was obtained after purification by column chromatography using as eluent a $CHCl_3$/MeOH 9:1 with 1% of $NH_4OH_{conc.}$.

δ_H (d_4-CH$_3$OH): 8.31 (1H, s, H2-adenosine), 8.19 (1H, s, H8-adenosine), 6.25 (1H, d, H1'-adenosine, *J*= 6.4 Hz), 5.00 (1H, t, H2'-adenosine), 4.53 (1H, d, H3'-adenosine), 3.77 (1H, d, H5'-adenosine, *J*= 12.2 Hz), 3.60 (1H, d, H5'-adenosine, *J*= 12.2 Hz).

MS (ES) m/e: 331.1 (MNa$^+$, 100%); Accurate mass: $C_{10}H_{12}N_8O_4Na$ required 331.0879, found 331.0886.

Synthesis of 2',3'-*tert*-butyldimethylsilyl-5'-deoxy-5'-iodoadenosine (189)
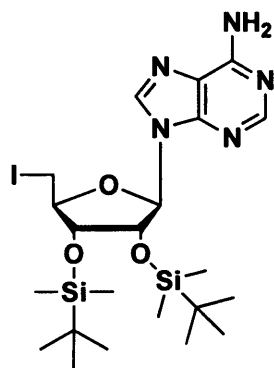

Iodine (14.22 g, 56.1 mmol) and triphenylphosphine (14.22 g, 56.1 mmol) were added to a solution of adenosine (**181**, 10.0 g, 37.4 mmol) in pyridine (45 mL). After 2 hours *tert*-butyldimethyl silyl chloride (24.54 g, 149.6 mmol) and imidazole (20.36 g, 299.2 mmol) were added at 0 °C. After 30 minutes the reaction was slowly warmed at room temperature and stirred for 15 hours. The solvent was removed under reduced pressure and the crude product was purified by column chromatography using as eluent a mixture of EtOAc/Hexane in gradient: 1:9, 8:2, 7:3, 65:35, 50:50 and 6:4. The pure product (10 g, 44%) was a white solid.

δ_H (CDCl$_3$): 8.38 (1H, s, H2-adenosine), 7.92 (1H, s, H8-adenosine), 5.87 (1H, d, H1'-adenosine, *J*= 5.2 Hz), 5.25 (2H, NH$_2$6-adenosine), 5.23 (1H, t, H2'-adenosine), 4.42 (1H, m, H3'-adenosine), 4.16 (1H, m, H4'-adenosine), 3.73 (1H, m, H5'-adenosine), 3.41 (1H, m, H5'-adenosine), 1.00 (9H, s, CH$_3$-*tert*-butyl), 0.81 (9H, s, CH$_3$-*tert*-butyl), 0.21 (3H, s, CH$_3$-silyl), 0.16 (3H, s, CH$_3$-silyl), -0.10 (3H, s, CH$_3$-silyl), -0.27 (3H, s, CH$_3$-silyl); δ_C (CDCl$_3$): 154.55 (1C, C4-adenosine), 151.91 (1C, CH2-adenosine), 148.68 (1C, C6-adenosine), 140.07 (1C, C8-adenosine), 119.91 (1C, C5-adenosine), 89.16 (1C, C1'-adeonsine), 83.67 (1C, C4'-adesonie), 74.22 (1C, C2'-adenosine), 72.33 (1C, C3'-adenosine), 61.60 (1C, C5'-adenosine), 25.11, 24.92, 24.73, 24.61 (6C, 6 CH$_3$-*tert*-butyl), 17.09 (1C, C-*tert*-butyl), 16.92 (1C, C-*tert*-butyl), -5.63 (2C, 2 CH$_3$-silyl), -6.10 (2C, 2 CH$_3$-silyl).
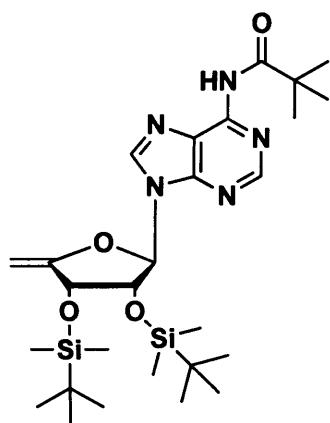
Synthesis of 2',3'-*tert*-butyldimethylsilyl-1-(5-deoxy-β-D-glycero-pent-4-enofuranosyl)-adenine (190)[10]
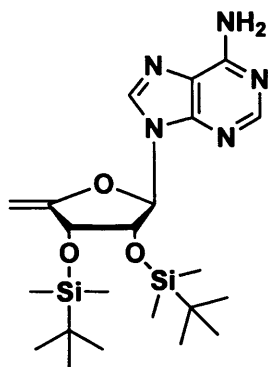
tBuOK (0.627 g, 4.95 mmol) was added to a solution of 2',3'-*tert*-butyldimethylsilyl-5'-deoxy-5'-iodoadenosine (189, 1.0 g, 1.65 mmol) in pyridine (15 mL), and the reaction stirred for 3 hour at room temperature. The solvent was removed under reduced pressure and the solid was purified by column chromatography using as eluent a mixture of EtOAc/Hexane in gradient: 7:3, 65:35, 50:50, then 8:2 and 7:3. The pure product was obtained as a white solid (700 mg, 89%)

δ_H (CDCl$_3$): 8.22 (1H, s, H2-adenosine), 7.72 (1H, s, H8-adenosine), 5.96 (1H, d, H1'-adenosine, *J*= 6.2 Hz), 5.52 (2H, s, NH$_2$6-adenosine), 4.92 (1H, m, H2'-adenosine), 4.44 (1H, d, H3'-adenosine, *J*= 4.2 Hz), 4.37 (1H, d, H5'-adenosine, *J*= 2.3 Hz), 4.13 (1H, d, H5'-adenosine, *J*= 2.3 Hz), 0.80 (9H, s, CH$_3$-*tert*-butyl), 0.61 (9H, s, CH$_3$-*tert*-butyl), 0.00 (3H, s, CH$_3$-silyl), -0.22 (3H, s, CH$_3$-silyl), -0.45 (3H, s, CH$_3$-silyl), -0.27 (3H, s, CH$_3$-silyl); δ_C (CDCl$_3$): 160.70 (1C, C4'-adenosine), 155.53 (1C, C4-adenosine), 153.36 (1C, C2-adenosine), 150.08 (1C, C6-adenosine), 140.05 (1C, C8-adenosine), 120.62 (1C, C5-adenosine), 89.27 (1C, C1'-adeonsine), 86.79 (2C, C5'-adesonie), 74.41 (1C, C2'-adenosine), 72.33 (1C, C3'-adenosine), 25.82, 25.71, 25.61 (6C, CH$_3$-*tert*-butyl), 18.23 (1C, C-*tert*-butyl), 17.88 (1C, C-*tert*-butyl), -4.65 (2C, CH$_3$-silyl), -5.37 (2C, CH$_3$-silyl).

Synthesis of *N⁶*-*tert*-butanoyl-2',3'-*tert*-butyldimethylsilyl-1-(5-deoxy-β-D-glycero-pent-4-enofuranosyl)-adenine (193)[10]
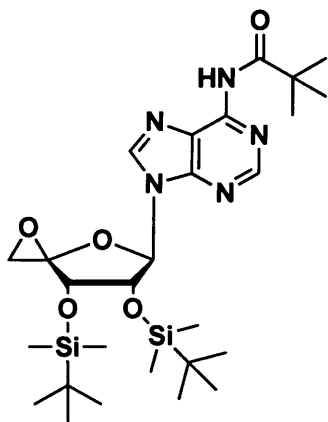
To a solution of 2',3'-*tert*-butyldimethylsilyl-1-(5-deoxy-β-D-glycero-pent-4-enofuranosyl)-adenine (**190**, 3.6 g, 7.53 mmol) in anhydrous DCM (145 mL), tBuOCl (*tert*-butanoyl chloride, 1.86 mL, 15.10 mmol) and diisopropylethylamine (2.63 mL, 15.10 mmol), were added and the reaction stirred for 3 hours at room temperature. The reaction was washed three times with a saturated solution of $NaHCO_3$. The organic layer was dried with $MgSO_4$ and the solvent was removed under reduced pressure. The cured was purified by column chromatography using as eluent a mixture of EtOAc and Hexane in gradient: 1:10, 1:5, 1:1. The pure compound was obtained as a white solid (4.0 g, 95%).

δ_H (CDCl₃): 8.62 (1H, s, H2-adenosine), 8.42 (1H, s, NH6-adenosine), 7.90 (1H, s, H8-adenosine), 6.02 (1H, d, H1'-adenosine, *J*= 6.2 Hz), 4.91 (1H, m, H2'-adenosine), 4.42 (1H, d, H3'-adenosine, *J*= 4.2 Hz), 4.38 (1H, d, H5'-adenosine, *J*= 2.3 Hz), 4.15 (1H, d, H5'-adenosine, *J*= 2.3 Hz), 1.26 (9H, s, CH₃-*tert*-butanoyl), 0.80 (9H, s, CH₃-*tert*-butyl), 0.60 (9H, s, CH₃-*tert*-butyl), 0.00 (3H, s, CH₃-silyl), -0.22 (3H, s, CH₃-silyl), -0.47 (3H, s, CH₃-silyl); δ_C (CDCl₃): 175.65 (1C, C=O-*tert*-butanoyl), 160.52 (1C, C4'-adenosine), 152.94 (1C, C2-adenosine), 151.64 (1C, C4-adenosine), 149.81 (1C, C6-adenosine), 142.02 (1C, C8-adenosine), 123.53 (1C, C5-adenosine), 89.31 (1C, C1'-adeonsine), 87.10 (2C, C5'-adesonie), 74.52 (1C, C2'-adenosine), 72.27 (1C, C3'-adenosine), 40.54 (1C, C-*tert*-butanoyl), 27.17 (3C, CH₃-*tert*-butyl), 25.79 (3C, CH₃-*tert*-butyl), 25.59 (3C, CH₃-*tert*-butanoyl), 18.21 (1C, C-*tert*-butyl), 17.84 (1C, C-*tert*-butyl), -4.63 (2C, CH₃-silyl), -5.35 (2C, CH₃-silyl).
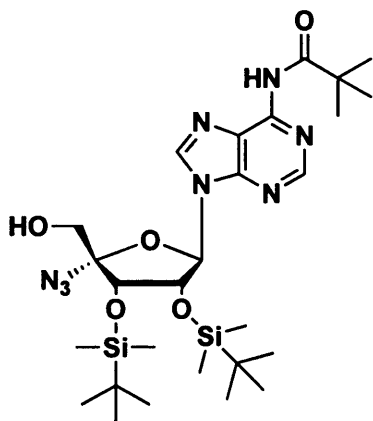
Synthesis of *N⁶*-*tert*-butanoyl-2',3'-*tert*-butyldimethylsilyl-4',5'epoxyadenosine

6N-*tert*-butanoyl-2',3'-*tert*-butyldimethylsilyl-1-(5-deoxy-β-D-glycero-pent-4-enofuranosyl)-adenine (**193**, 2.0 g, 3.56 mmol) was added to 110 mL of a 0.1 solution of DMDO (dimethoxydioxirane) in acetone. The reaction was stirred for 15 minutes at room temperature, then the solvent was removed under reduced pressure to give a white product (2 g, 100%).

δ_H (CDCl₃): 8.70 (1H, s, H2-adenosine), 8.07 (1H, s, NH6-adenosine), 8.07 (1H, s, H8-adenosine), 6.11 (1H, d, H1'-adenosine, *J*= 5.2 Hz), 4.94 (1H, m, H2'-adenosine), 4.12 (1H, d, H3'-adenosine, *J*= 3.8 Hz), 3.10 (1H, d, H5'-adenosine, *J*= 3.5 Hz), 2.94 (1H, d, H5'-adenosine, *J*= 3.5 Hz), 1.31 (9H, s, CH₃-*tert*-butanoyl), 0.85 (9H, s, CH₃-*tert*-butyl), 0.71 (9H, s, CH₃-*tert*-butyl), 0.03 (3H, s, CH₃-silyl), 0.00 (3H, s, CH₃-silyl), -0.14 (3H, s, CH₃-silyl), -0..Hz), 1.31 (9H, s, CH₃-*tert*-butanoyl), 0.85 (9H, s, CH₃-*tert*-butyl), 0.71 (9H, s, CH₃-*tert*-butyl), 0.03 (3H, s, CH₃-silyl), 0.00 (3H, s, CH₃-silyl), -0.14 (3H, s, CH₃-silyl), -

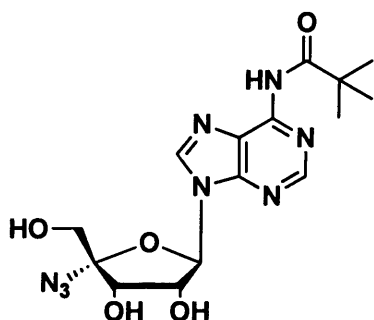## Synthesis of *N⁶*-*tert*-butanoyl-2',3'-*tert*-butyldimethylsilyl-4'-azidoadenosine (194)

To a solution of 6N-*tert*-butanoyl-2',3'-*tert*-butyldimethylsilyl-4',5'epoxyadenosine (3.09 g, 5.34 mmol) in anhydrous DCM (200 mL), TBSN₃ (*tert*-butyl azido silane, 2.10 mL, 16.02 mmol) and SnCl₄ (tin chloride, 3.4 mL, 16.02 mmol) were added at -78 °C. The reaction was stirred for 30 minutes then, it was slowly wormed at room temperature and stirred for other 30 minutes. A saturated solution of NaHCO₃ was added until the reaction reached pH 7. The a white emulsion formed was filtrated on celite pad and the solution was washed three times with a saturated solution of NaHCO₃. The organic layer was dried with MgSO₄. The solvent was removed under reduced pressure and the crude product was purified by column chromatography using as eluent a mixture of EtOAc/Hexane in gradient: 50:50 and 70:30. The pure product was obtained as a white solid (1.6 g, 52%).

δ_H (CDCl₃): 8.58 (1H, s, H2-adenosine), 8.50 (1H, s, NH6-adenosine), 7.91 (1H, s, H8-adenosine), 6.26 (1H, m, OH5'-adenosine), 5.84 (1H, d, H1'-adenosine, *J*= 7.9

Hz), 5.08 (1H, m, H2'-adenosine), 4.22 (1H, d, H3'-adenosine, J= 4.4 Hz), 3.64 (1H,

m, H5'-adenosine), 3.31 (1H, m, H5'-adenosine), 1.24 (9H, s, CH_3-$tert$-butanoyl),

0.83 (9H, s, CH_3-$tert$-butyl), 0.59 (9H, s, CH_3-$tert$-butyl), 0.03 (3H, s, CH_3-silyl), 0.00

(3H, s, CH_3-silyl), -0.32 (3H, s, CH_3-silyl), -0.88 (3H, s, CH_3-silyl).

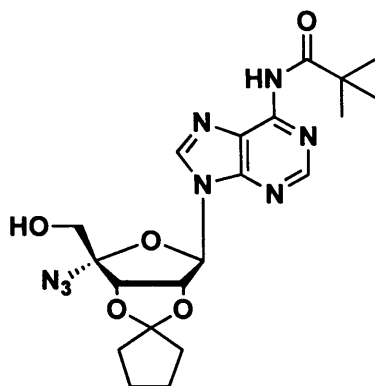## Synthesis of N^6-$tert$-butanoyl-4'-azidoadenosine (195)

To a solution of 6N-$tert$-butanoyl-2',3'-$tert$-
butyldimethylsilyl-4'-azidoadenosine (**194**, 2.8 g, 4.52
mmol) in THF (30 mL), a 1M solution of TBAF (*tetra-
butyl* ammonium fluoride, 9.0 mL, 9.0 mmol) was
added and the reaction stirred at room temperature for
one hour. The solvent was removed under reduced
pressure to give a yellow oil that was purified by
column chromatography using as eluent a mixture of $CHCl_3$/MeOH 85:15. The pure
product was obtained as a white solid (1.15 g, 76%).

δ_H (d_6-$(CH_3)_2SO$): 10.23 (1H, s, NH6-adenosine), 8.77 (1H, s, H2-adenosine), 8.74

(1H, s, H8-adenosine), 6.33 (1H, d, H1'-adenosine, J= 6.1 Hz), 5.97 (1H, d, OH2'-

adenosine, J= 4.7 Hz), 5.82 (1H, d, OH3'-adenosine, J= 5.96 Hz), 5.65 (1H, m, OH5'-

adenosine), 4.94 (1H, m, H2'-adenosine), 4.48 (1H, m, H3'-adenosine), 3.67 (1H, m,

H5'-adenosine), 3.51 (1H, m, H5'-adenosine), 1.33 (9H, s, CH_3-$tert$-butanoyl); δ_C (d_6-

$(CH_3)_2SO$): 176.29 (1C, C=O-$tert$-butanoyl), 152.09 (1C, C2-adenosine), 151.68 (1C,

C4-adenosine), 150.66 (1C, C6-adenosine), 142.96 (1C, C8-adenosine), 125.88 (1C,

C5-adenosine), 99.90 (1C, C4'-adenosine), 87.77 (1C, C1'-adeonsine), 72.56 (1C,

C2'-adenosine), 72.51 (1C, C3'-adenosine), 64.13 (1C, C5'-adenosine), 26.87 (3C,
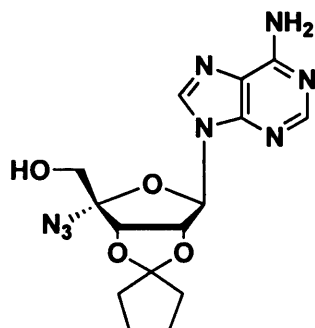
CH_3-$tert$-butanoyl).

Synthesis of *N⁶-tert*-butanoyl-2',3'-*O,O*-cyclopentylidene-4'-azidoadenosine (196)

A solution of 6N-*tert*-butanoyl-4'-azidoadenosine (**195**, 1.6 g, 4.08 mmol), and *p*-TSA (1.16 g, 6.12 mmol) in 1,1-dimethoxycyclopentane (15 mL) was stirred at room temperature for 4 hours. The solvent was removed and the cured was purified by column chromatography using as eluent a mixture of $CHCl_3$/MeOH 9:1. The pure product was obtained as a yellow solid (1.1 g, 52%).

δ_H (CDCl₃): 8.63 (1H, s, NH6-adenosine), 8.62 (1H, s, H2-adenosine), 8.14 (1H, s, H8-adenosine), 6.18 (1H, d, H1'-adenosine, *J*= 4.5 Hz), 5.23 (1H, m, H2'-adenosine), 5.02 (1H, d, H3'-adenosine, *J*= 5.8 Hz), 3.75 (1H, d, H5'-adenosine, *J*= 8.7 Hz), 3.57 (1H, m, H5'-adenosine, *J*= 8.7 Hz), 2.14-2.04 (2H, m, CH₂-cyclopentyl), 1.86-1.61 (6H, m, CH₂-cyclopentyl), 1.32 (9H, s, CH₃-*tert*-butyl); δ_C (d_6-(CH₃)₂SO): 175.78 (1C, C=O-*tert*-butanoyl), 152.60 (1C, C2-adenosine), 150.31 (1C, C4-adenosine), 150.25 (1C, C6-adenosine), 148.04 (1C, C8-adenosine), 124.88 (1C, C5-adenosine), 123.92 (1C, C-cyclopentyl), 103.75 (1C, C4'-adenosine), 92.84 (1C, C1'-adeonsine), 84.09 (1C, C2'-adenosine), 83.26 (1C, C3'-adenosine), 65.74 (1C, C5'-adenosine), 36.81 (1C, CH₂-cyclopentyl), 36.14 (1C, CH₂-cyclopentyl), 29.61 (1C, C-*tert*-butanoyl), 26.14 (1C, CH₂-cyclopentyl), 25.21 (1C, CH₂-cyclopentyl).

Synthesis of 2',3'-*O,O*-cyclopentylidene-4'-azidoadenosine (196)

N⁶-tert-butanoyl-2',3'-*O,O*-cyclopentylidene-4'-azidoadenosine (**195**, 1.12 g, 2.44 mmol) was solubelized in a solution of MeOH saturated with NH₃. The solution was sealed at room temperature for 15 hours. The solvent was removed under reduced pressure and the crude product was purified by column chromatography using as eluent a mixture of $CHCl_3$/MeOH in gradient: 100:0, 98:2 and 90:10. The pure product was obtained as a white solid (0.8 mg, 67%).

δ_H (CDCl₃): 8.44 (1H, s, H2-adenosine), 8.00 (1H, s, H8-adenosine), 6.23 (1H, d, H1'-adenosine, *J*= 5.1 Hz), 6.20 (2H, s, NH₂-adenosine), 5.46 (1H, m, H2'-adenosine), 5.21 (1H, d, H3'-adenosine, *J*= 5.6 Hz), 3.99 (1H, d, H5'-adenosine, *J*=

12.3 Hz), 3.75 (1H, d, H5'-adenosine, $J=$ 12.3 Hz), 2.39-2.22 (2H, m, CH_2-cyclopentyl), 2.00-1.85 (6H, m, CH_2-cyclopentyl); δ_C (d_6-$(CH_3)_2SO$): 156.19 (1C, C4-adenosine), 152.67 (1C, C2-adenosine), 148.14 (1C, C6-adenosine), 140.38 (1C, C8-adenosine), 124.76 (1C, C5-adenosine), 121.14 (1C, C-cyclopentyl), 100.29 (1C, C4'-adenosine), 93.37 (1C, C1'-adeonsine), 83.08 (1C, C2'-adenosine), 82.95 (1C, C3'-adenosine), 66.13 (1C, C5'-adenosine), 36.91 (1C, CH_2-cyclopentyl), 36.51 (1C, CH_2-cyclopentyl), 23.77 (1C, CH_2-cyclopentyl), 23.25 (1C, CH_2-cyclopentyl).

7.9 Synthesis of 4'-azidoadenosine phosphoramidates.

**Synthesis of 4'-azidoadenosine 5'-*O*-[α-naphthyl(benzyl-L-alaninyl)] phosphate (202)**
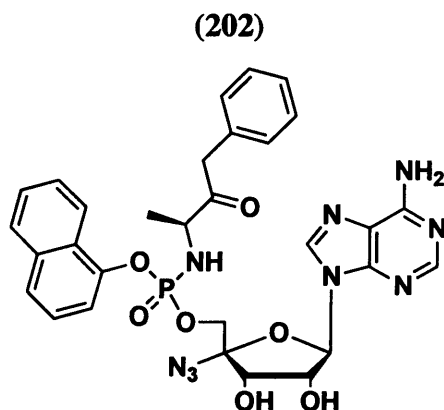
Prepared according to Standard Procedure C, from 4'-azidoadenosine (**27**, 165.6 mg, 0.054 mmol), ᵗBuMgCl (1.34 mL 1M solution of THF, 1.34 mmol) and α-naphthyl (benzyl-L-alaninyl) phosphorochloridate (**93**, 1.34 mL of solution 1M in THF, 1.34 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (85:15), a preparative HPLC. The pure product was a white solid (20 mg, 6%).

δ_P (d_4-CH_3OH): 3.71, 3.67; δ_H (d_4-CH_3OH): 8.26 (1H, d, H2-adenosine), 8.17 (1H, s, H8-adenosine), 8.17 (1H, s, CH-naphthyl), 7.88 (1H, d, CH-naphthyl, $J=$ 7.9 Hz), 7.69 (1H, m, CH-naphthyl), 7.53-7.43 (4H, m, CH-naphthyl, CH-phenyl), 7.38-7.25 (5H, CH-naphthyl, CH-phenyl), 6.28 (1H, d, H1'-adenosine, $J=$ 5.1 Hz), 5.05 (2H, m, CH_2-benzyl), 4.95 (1H, m, H2'-adenosine), 4.70 (1H, d, H3'-adenosine, $J=$ 5.4 Hz), 4.40 (2H, m, H5'-adenosine), 4.05 (1H, m, $CH\alpha$), 1.28 (3H, m ,CH_3-alanine).

MS (ES) m/e: 698.1 (MNa^+, 100%); Accurate mass: $C_{30}H_{30}N_9O_7Na$ required 698.1853, found 698.1852.

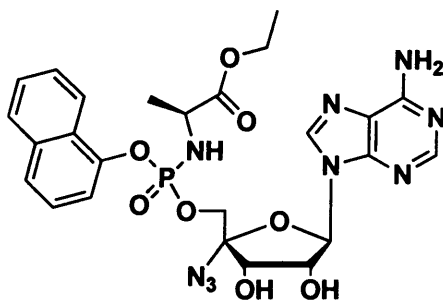## Synthesis of 2', 3'-*O,O*-cyclopentylidene-4'-azidoadenosine 5'-*O*-[α-naphthyl(ethyl-L-alaninyl)] phosphate
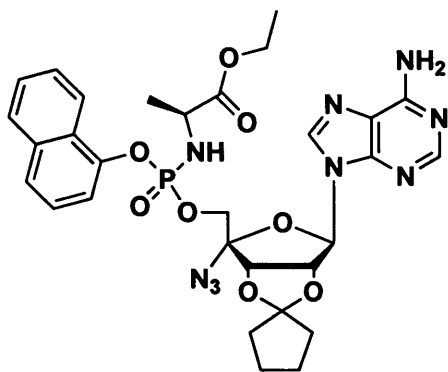
Prepared according to Standard Procedure C2, from 2', 3'-*O,O*-cyclopentylidene-4'-azidoadenosine (**196**, 150 mg, 0.40 mmol), 'BuMgCl (1.00 mL, 1M solution in THF, 1.00 mmol) and α-naphthyl(ethyl-L-alaninyl) phosphorochloridate (**200**, 1.00 mL of solution 1M in THF, 1.00 mmol). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (95:5). The pure product was a white solid (250 mg, 92%).

δ_P (CDCl₃): 2.74; δ_H (CDCl₃): 8.19 (1H, s, H2-adenosine), 7.91 (1H, m, CH-naphthyl), 7.83 (1H, s, H8-adenosine), 7.80 (1H, d, CH-naphthyl, *J*= 4.85 Hz), 7.52 (1H, d, CH-naphthyl, *J*= 8.2 Hz), 7.44-7.29 (3H, m, CH-naphthyl), 7.42-7.21 (1H, m, CH-naphthyl), 6.17 (1H, d, H1'-adenosine, *J*= 2.3 Hz), 6.03 (1H, s, NH₂6-adenosine), 5.06 (1H, m, H2'-adenosine), 4.96 (1H, d, H3'-adenosine, *J*= 6.5 Hz), 4.35, 4.25 (2H, m, NH-alanine, H5'-adenosine), 4.22 (1H, m, H5'-adenosine), 4.00-3.91 (3H, m, CHα, CH₂-ethyl), 2.18-2.03 (2H, m, CH₂-cyclopentyl), 1.67-1.60 (6H, m, CH₂-cyclopentyl), 1.23 (3H, d, CH₃-alanine), 1.08 (3H, CH₃-ethyl).

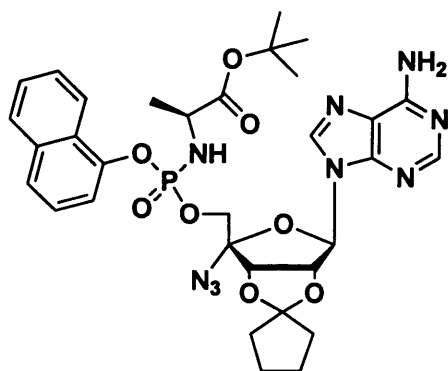## Synthesis of 4'-azidoadenosine 5'-*O*-[α-naphthyl(ethyl-L-alaninyl)] phosphate

(203)

Prepared according to Standard Procedure C3, from 2', 3'-*O,O*-cyclopentylidene-4'-azidoadenosine 5'-*O*-[α-naphthyl(ethyl-L-alaninyl)] phosphate (250 mg, 0.369 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent for the first CHCl₃/MeOH (95:5). The pure product was a white solid (100 mg, 51%).

δ_P (d_4-CH$_3$OH): 3.72, 3.68; δ_H (d_4-CH$_3$OH): 8.27 (1H, s, H2-adenosine), 8.13 (1H, s, H8-adenosine), 8.07 (1H, m, CH-naphthyl), 7.83 (1H, m, CH-naphthyl), 7.66 (1H, m, CH-naphthyl), 7.52-7.43 (3H, m, CH-naphthyl), 7.38-7.32 (1H, m, CH-naphthyl), 6.30 (1H, d, H1'-adenosine, J= 5.1 Hz), 4.97 (1H, m, H2'-adenosine), 4.75 (1H, d, H3'-adenosine, J= 5.5 Hz), 4.43 (1H, m, H5'-adenosine), 4.36 (1H, m, H5'-adenosine), 4.06-3.89 (2H, m, CH$_2$-ethyl), 3.73 (1H, m, CHα), 1.27 (3H, m, CH$_3$-alanine), 1.14 (3H, CH$_3$-ethyl); δ_C (d_4-CH$_3$OH): 175.03, 175.00 (1C, C=O-ethyl), 157.11 (1C, C4-adenosine), 153.74 (1C, C2-adenosine), 150.64 (1C, C6-adenosine), 147.80, 147.74 (1C, C-naphthyl), 141.73 (1C, C8-adenosine), 136.26, 136.21 (1C, C-naphthyl), 128.90, 128.83 (1C, CH-naphthyl), 127.82, 127.78 (1C, CH-naphthyl), 127.72, 127.67 (1C, C-naphthyl), 127.54, 127.47 (1C, CH-naphthyl), 126.44, 126.40 (1C, CH-naphthyl), 126.09, 126.03 (1C, CH-naphthyl), 122.61, 122.53 (1C, CH-naphthyl), 120.69 (1C, C5-adenosine), 116.11, 116.09 (1C, CH-naphthyl), 99.18, 99.10 (1C, C4'-adenosine), 91.14 (1C, C1'-adeonsine), 74.05, 74.00 (1C, C2'-adenosine), 73.96, 73.89 (1C, C3'-adenosine), 68.99, 68.80, 68.76 (1C, C5'-adenosine), 62.46, 62.40 (1C, CH$_2$-ethyl), 51.68 (1C, CHα), 20.56, 20.51, 20.37, 20.31 (1C, CH$_3$-alanine), 14.41 (1C, CH$_3$-ethyl).

MS (ES) m/e: 636.1 (MNa$^+$, 100%); Accurate mass: C$_{25}$H$_{28}$N$_9$O$_8$NaP required 636.1696, found 636.1682.

Synthesis of 2', 3'-*O,O*-cyclopentylidene-4'-azidoadenosine 5'-*O*-[α-naphthyl(*tert*-butyl-L-alaninyl)] phosphate
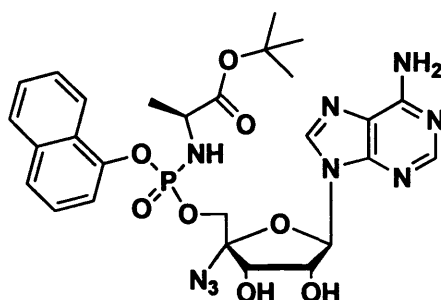

Prepared according to Standard Procedure C2, from 2', 3'-*O,O*-cyclopentylidene-4'-azidoadenosine (**196**, 150 mg, 0.40 mmol), 'BuMgCl (1.00 mL, 1M solution in THF, 1.00 mmol) and α-naphthyl(*tert*-butyl-L-alaninyl) phosphorochloridate (**201**, 1.00 mL of solution 1M in THF, 1.00 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (95:5). The pure product was a white solid (220 mg, 77%).

δ_P (CDCl$_3$): 2.96; δ_H (CDCl$_3$): 8.17 (1H, s, H2-adenosine), 8.05 (1H, m, CH-naphthyl), 7.82 (1H, s, H8-adenosine), 7.72-7.65 (2H, m, CH-naphthyl), 7.54-7.44

(2H, CH-naphthyl), 7.42-7.21 (2H, m, CH-naphthyl), 6.47 (1H, s, $NH_2$6-adenosine), 6.14 (1H, d, H1'-adenosine), 4.97 (2H, m, H2'-adenosine, H3'-adenosine), 4.61 (1H, m, NH-alanine), 4.30 (1H, m, H5'-adenosine), 4.19 (1H, m, H5'-adenosine), 3.94 (1H, m, $CH\alpha$), 2.13-2.01 (2H, m, CH_2-cyclopentyl), 1.65-1.56 (6H, m, CH_2-cyclopentyl), 1.36 (9H, CH_3-*tert*-butyl), 1.23 (3H, m, CH_3-alanine).

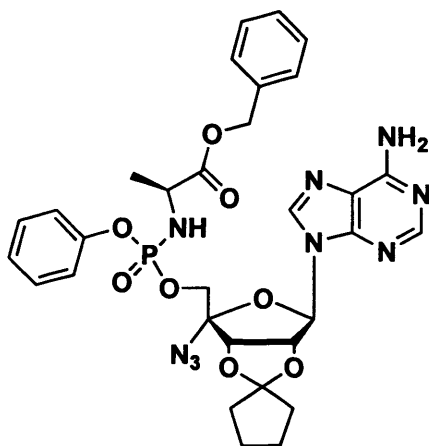## Synthesis of 4'-azidoadenosine 5'-*O*-[α-naphthyl(*tert*-butyl-L-alaninyl)] phosphate (204)

Prepared according to Standard Procedure C3, from 2', 3'-*O,O*-cyclopentylidene-4'-azidoadenosine 5'-*O*-[α-naphthyl(*tert*-butyl-L-alaninyl)] phosphate (220 mg, 0.308 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent for the first $CHCl_3$/MeOH (95:5). The pure product was a white solid (100 mg, 51%).

δ_P (d_4-CH_3OH): 3.85, 3.73; δ_H (d_4-CH_3OH): 8.25 (1H, s, H2-adenosine), 8.21 (1H, s, H8-adenosine), 8.10 (1H, m, CH-naphthyl), 7.84 (1H, m, CH-naphthyl), 7.64 (1H, m, CH-naphthyl), 7.51-7.48 (2H, m, CH-naphthyl), 7.45-7.32 (2H, m, CH-naphthyl), 6.29 (1H, d, H1'-adenosine, *J*= 5.1 Hz), 4.96 (1H, m, H2'-adenosine), 4.72 (1H, d, H3'-adenosine, *J*= 5.54 Hz), 4.42 (1H, m, H5'-adenosine), 4.36 (1H, m, H5'-adenosine), 3.85 (1H, m, $CH\alpha$), 1.35 (9H, s, CH_3-*tert*-butyl), 1.25 (3H, d, CH_3-alanine, *J*= 6.3 Hz); δ_C (d_4-CH_3OH): 174.30, 174.26 (1C, C=O-*tert*-butyl), 157.22 (1C, C4-adenosine), 153.92 (1C, C2-adenosine), 150.67 (1C, C6-adenosine), 147.82, 147.76 (1C, C-naphthyl), 141.64 (1C, C8-adenosine), 136.23 (1C, C-naphthyl), 128.90, 128.84 (1C, CH-naphthyl), 127.81, 127.77 (2C, CH-naphthyl), 127.72, 127.67 (1C, C-naphthyl), 127.53, 127.46 (2C, CH-naphthyl), 126.08, 126.02 (1C, CH-naphthyl), 122.67, 122.58 (1C, CH-naphthyl), 120.70 (1C, C5-adenosine), 99.18, 99.10 (1C, C4'-adenosine), 91.09, 90.91 (1C, C1'-adeonsine), 82.83, 82.71 (1C, C-*tert*-butyl), 74.04 (1C, C2'-adenosine), 73.97, 73.92 (1C, C3'-adenosine), 68.91,

68.87 (1C, C5'-adenosine), 52.29 (1C, CHα), 28.25, 28.21, 28.18 (3C, CH_3-*tert*-butyl), 20.78, 20.73, 20.55, 20.49 (1C, CH_3-alanine).

MS (ES) m/e: 664.4 (MNa^+, 100%); Accurate mass: $C_{27}H_{32}N_9O_8NaP$ required 664.2009, found 664.2015.
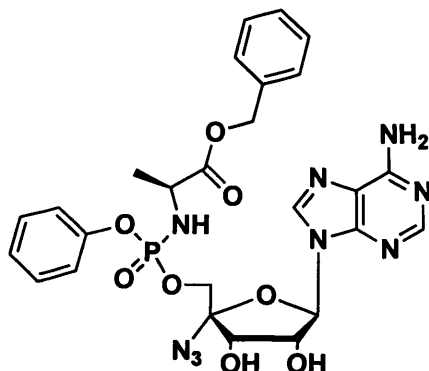
Synthesis of 2', 3'-*O,O*-cyclopentylidene-4'-azidoadenosine 5'-*O*-[phenyl(benzyl-L-alaninyl)] phosphate

Prepared according to Standard Procedure C2, from 2', 3'-*O,O*-cyclopentylidene-4'-azidoadenosine (**196**, 150 mg, 0.40 mmol), 'BuMgCl (1.00 mL, 1M solution in THF, 1.00 mmol) and phenyl(benzyl-L-alaninyl) phosphorochloridate (**132**, 1.00 mL of solution 1M in THF, 1.00 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (200 mg, 72%).

δ_P (CDCl$_3$): 2.28; δ_H (CDCl$_3$): 8.22 (1H, s, H2-adenosine), 7.90 (1H, s, H8-adenosine), 7.27-7.19 (6H, m, 1 CH-phenyl, CH-benzyl), 7.13-7.10 (2H, m, CH-phenyl), 7.97-6.98 (2H, m, 2 CH-phenyl), 6.33 (1H, s, H1'-adenosine), 6.20 (1H, s, NH$_2$6-adenosine), 5.12-4.98 (4H, CH$_2$-benzyl, H2'-adenosine, H3'-adenosine), 4.42 (2H, m, NH-alanine), 4.23 (1H, m, H5'-adenosine), 4.13 (1H, m, H5'-adenosine), 3.98 (1H, m, CHα), 2.16-2.03 (2H, m, CH$_2$-cyclopentyl), 1.68-1.62 (6H, m, CH$_2$-cyclopentyl), 1.25 (3H, d, CH$_3$-alanine, *J*= 6.9 Hz).
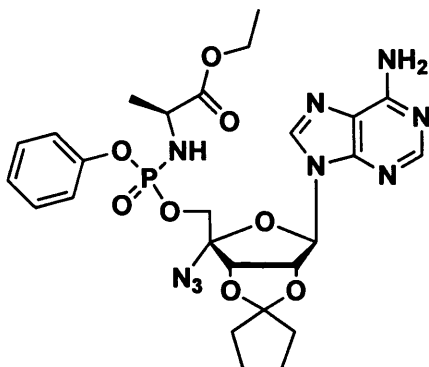
Synthesis of 4'-azidoadenosine 5'-*O*-[phenyl(benzyl-L-alaninyl)] phosphate (206)

Prepared according to Standard Procedure C3, from 2', 3'-*O,O*-cyclopentylidene-4'-azidoadenosine 5'-*O*-[phenyl(benzyl-L-alaninyl)] phosphate (200 mg, 0.290 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent for the first $CHCl_3$/MeOH (95:5). The pure product was a white solid (100 mg, 55%).

δ_P (d_4-CH$_3$OH): 3.38. 3.21; δ_H (d_4-CH$_3$OH): 8.30 (1H, s, H2-adenosine), 8.21 (1H, s, H8-adenosine), 7.44-7.33 (1H, m, CH-phenyl), 7.32-7.28 (7H, m, CH-phenyl, CH-benzyl), 7.25-7.15 (2H, m, CH-phenyl), 6.30 (1H, d, H1'-adenosine, *J*= 5.1 Hz), 5.12 (2H, m, CH$_2$-benzyl), 4.96 (1H, m, H2'-adenosine), 4.68 (1H, d, H3'-adenosine, *J*= 5.4 Hz), 4.32 (1H, m, H5'-adenosine), 4.23 (1H, m, H5'-adenosine), 3.92 (1H, m, CHα), 1.28 (3H, m, CH$_3$-alanine); δ_C (d_4-CH$_3$OH): 171.76 (1C, C=O-benzyl), 157.41 (1C, C4-adenosine), 154.14 (1C, C2-adenosine), 151.89 (1C, C6-adenosine), 150.70 (1C, C-phenyl), 141.47 (1C, C8-adenosine), 137.23 (1C, C-benzyl), 130.75 (2C, 2 CH-phenyl), 129.73 (2C, CH-benzyl), 129.63, 129.56 (2C, CH-benzyl), 129.29 (1C, CH-benzyl), 126.25 (1C, CH-phenyl), 121.39, 121.35 (2C, CH-phenyl), 120.56 (1C, C5-adenosine), 99.16 (1C, C4'-adenosine), 90.89 (1C, C1'-adeonsine), 74.19 (1C, C2'-adenosine), 73.95 (1C, C3'-adenosine), 68.54, 68.50 (1C, C5'-adenosine), 67.98 (1C, CH$_2$-benzyl), 51.69 (1C, CHα), 20.23, 20.17 (1C, CH$_3$-alanine).

MS (ES) m/e: 648.1 (MNa$^+$, 100%); Accurate mass: $C_{26}H_{28}N_9O_8NaP$ required 648.1696, found 648.1696.

Synthesis of 2', 3'-*O,O*-cyclopentylidene-4'-azidoadenosine 5'-*O*-[phenyl(ethyl-L-alaninyl)] phosphate
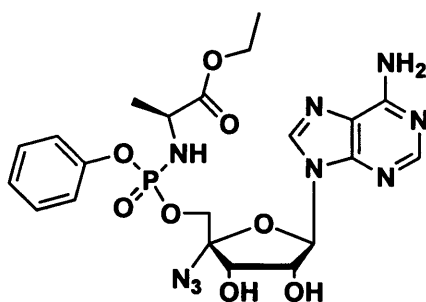
Prepared according to Standard Procedure C2, from 2', 3'-*O,O*-cyclopentylidene-4'-azidoadenosine (**196**, 150 mg, 0.40 mmol), ^tBuMgCl (1.00 mL, 1M solution in THF, 1.00 mmol) and phenyl(ethyl-L-alaninyl) phosphorochloridate (**129**, 1.00 mL of solution 1M in THF, 1.00 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (210 mg, 84%).

δ_P (CDCl₃): 2.34; δ_H (CDCl₃): 8.31 (1H, s, H2-adenosine), 7.97 (1H, s, H8-adenosine), 7.23-7.22 (2H, m, CH-phenyl), 7.12-7.09 (3H, m, CH-phenyl), 6.31 (1H, d, H1'-adenosine, *J*= 2.2 Hz), 6.23 (1H, s, NH₂6-adenosine), 5.24 (1H, m, H2'-adenosine), 5.14 (1H, d, H3'-adenosine, *J*= 6.3 Hz), 4.35-4.30 (2H, m, NH-alanine, H5'-adenosine), 4.28-4.22 (1H, m, H5'-adenosine), 4.15-4.11 (2H, m, CH₂-ethyl), 3.99 (1H, m, CHα), 2.26-2.14 (2H, m, CH₂-cyclopentyl), 1.76-1.69 (6H, m, CH₂-cyclopentyl), 1.33 (3H, CH₃-ethyl), 1.23 (3H, d, CH₃-alanine).

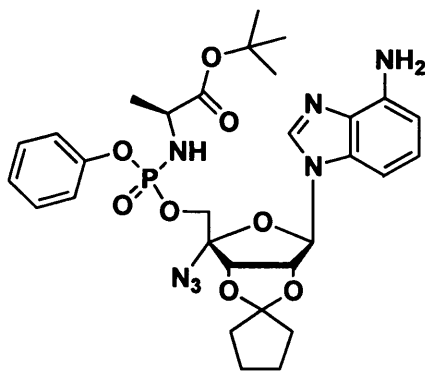# Synthesis of 4'-azido-adenosine 5'-*O*-[phenyl(ethyl-L-alaninyl)] phosphate (207)

Prepared according to Standard Procedure C3, from 2', 3'-*O,O*-cyclopentylidyn-4'-azido-adenosine 5'-*O*-[phenyl(ethyl-L-alaninyl)] phosphate (210 mg, 0.335 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent for the first $CHCl_3$/MeOH (95:5). The pure product was a white solid (100 mg, 48%).

δ_P (d_4-CH₃OH): 3.44. 3.28; δ_H (d_4-CH₃OH): 8.27 (1H, s, H2-adenosine), 8.18 (1H, s, H8-adenosine), 7.36-7.28 (2H, m, CH-phenyl), 7.16-7.11 (3H, m, CH-phenyl), 6.38

(1H, d, H1'-adenosine, $J=$ 5.0 Hz), 4.96 (1H, m, H2'-adenosine), 4.75 (1H, d, H3'-adenosine, $J=$ 5.3 Hz), 4.35 (1H, m, H5'-adenosine), 4.25 (1H, m, H5'-adenosine), 4.15 (2H, m, CH_2-ethyl), 3.76 (1H, m, CHα), 1.27 (3H, d, CH_3-ethyl, $J=$ 7.2 Hz), 1.22 (3H, m, CH_3-alanine); δ_C (d_4-CH_3OH): 172.78 (1C, C=O-ethyl), 157.38 (1C, C4-adenosine), 154.10 (1C, C2-adenosine), 151.99 (1C, C6-adenosine), 150.78 (1C, C-phenyl), 141.50 (1C, C8-adenosine), 130.76 (2C, CH-phenyl), 126.26 (1C, CH-phenyl), 121.36, 121.32 (2C, CH-phenyl), 120.62 (1C, C5-adenosine), 99.19, 99.11 (1C, C4'-adenosine), 90.89 (1C, C1'-adeonsine), 74.20 (1C, C2'-adenosine), 73.96 (1C, C3'-adenosine), 68.53, 68.49 (1C, C5'-adenosine), 62.42 (1C, CH_2-ethyl), 51.61 (1C, CHα), 20.30, 20.24 (1C, CH_3-alanine), 14.47 (1C, CH_3-alanine).

MS (ES) m/e: 586.4 (MNa^+, 100%); Accurate mass: $C_{21}H_{26}N_9O_8NaP$ required 586.1540, found 586.1544.

Synthesis of 2', 3'-*O,O*-cyclopentylidyn-4'-azidoadenosine 5'-*O*-[phenyl(*tert*-butyl-L-alaninyl)] phosphate (208)
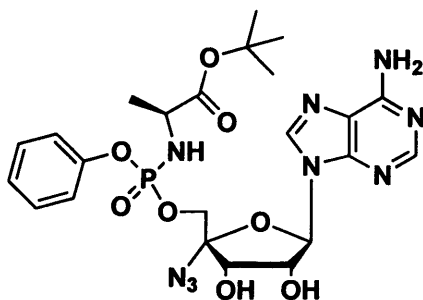

Prepared according to Standard Procedure C2, from 2', 3'-*O,O*-cyclopentylidene-4'-azidoadenosine (196, 150 mg, 0.40 mmol), 'BuMgCl (1.00 mL, 1M solution in THF, 1.00 mmol) and phenyl(*tert*-butyl-L-alaninyl) phosphorochloridate (131, 1.00 mL of solution 1M in THF, 1.00 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (210 mg, 71%).

δ_P ($CDCl_3$): 2.43; δ_H ($CDCl_3$): 8.28 (1H, s, H2-adenosine), 7.97 (1H, s, H8-adenosine), 7.27-7.00 (5H, m, CH-phenyl), 6.55 (1H, s, $NH_2$6-adenosine), 5.77 (1H, m, H1'-adenosine), 5.17 (1H, m, H2'-adenosine), 5.16 (1H, d, H3'-adenosine, $J=$ 2.1 Hz), 3.98 (1H, m, NH-alanine), 4.29 (1H, m, H5'-adenosine), 4.20 (1H, m, H5'-adenosine), 3.88 (1H, m, CHα), 2.21-2.09 (2H, m, CH_2-cyclopentyl), 1.76-1.63 (6H, m, CH_2-cyclopentyl), 1.37 (9H, CH_3-*tert*-buthyl), 1.26 (3H, d, CH_3-alanine, $J=$ 7.0 Hz).

Synthesis of 4'-azidoadenosine 5'-*O*-[phenyl(*tert*-butyl-L-alaninyl)] phosphate

(208)

Prepared according to Standard Procedure C3, from 2', 3'-*O,O*-cyclopentylidene-4'-azidoadenosine 5'-*O*-[phenyl(*tert*-butyl-L-alaninyl)] phosphate (210 mg, 0.356 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent for the first CHCl$_3$/MeOH (95:5). The pure product was a white solid (100 mg, 47%).

δ_P (d_4-CH$_3$OH): 3.42; δ_H (d_4-CH$_3$OH): 8.29 (1H, s, H2-adenosine), 8.21 (1H, s, H8-adenosine), 7.32-7.29 (2H, m, CH-phenyl), 7.19-7.14 (3H, m, CH-phenyl), 6.33 (1H, d, H1'-adenosine, *J*= 5.1 Hz), 4.97 (1H, m, H2'-adenosine), 4.72 (1H, d, H3'-adenosine, *J*= 5.45 Hz), 4.36 (1H, m, H5'-adenosine), 4.27 (1H, m, H5'-adenosine), 3.77 (1H, m, CHα), 1.42 (9H, s, CH$_3$-*tert*-butyl), 1.25 (3H, d, CH$_3$-alanine); δ_C (d_4-CH$_3$OH): 174.29, 174.25 (1C, C=O-*tert*-butyl), 157.39 (1C, C4-adenosine), 154.13 (1C, C2-adenosine), 152.00, 151.95 (1C, C6-adenosine), 150.77 (1C, C-phenyl), 141.62, 141.49 (1C, C8-adenosine), 130.81, 130.78 (2C, CH-phenyl), 126.24 (1C, CH-phenyl), 121.38, 121.34 (2C, CH-phenyl), 120.64 (1C, C5-adenosine), 99.21, 99.13 (1C, C4'-adenosine), 90.99, 90.91 (1C, C1'-adeonsine), 82.75 (1C, C-*tert*-butyl), 74.24 (1C, C2'-adenosine), 74.06, 74.01 (1C, C3'-adenosine), 68.84, 68.65, 68.61 (1C, C5'-adenosine), 52.24, 52.12 (1C, CHα), 28.27, 28.15 (3C, CH$_3$-*tert*-butyl), 20.77, 20.72, 20.53, 20.47 (1C, CH$_3$-alanine).

MS (ES) m/e: 614.4 (MNa$^+$, 100%); Accurate mass: C$_{23}$H$_{30}$N$_9$O$_8$NaP required 614.1853, found 614.1839.

7.10 Synthesis of 2'-methylnucleosides.

Synthesis of 1-hydroxy-1,2-benzoiodoxol-3 (1H)-one 1-oxide[11]
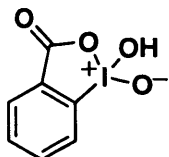
Potassium bromate (19g, 113.8 mmol) was added over 30 minutes to a suspension of 2-iodobenzoic acid (21.16 g, 85.32 mmol) in 0.73 M aqueous sulphuric acid (182 mL) at 55 °C stirred with an overhead stirrer. Once the addition was complete the reaction was then stirred at 68° C for 3.6 hours, then cooled in an ice-bath for 5 minutes and filtered. The resulting solid was washed with water (250 mL), ethanol (2 x 20 mL) and diethyl ether (3 x 20 mL). The desire product (17.7 g, 74%) was used without further purification in the next step.
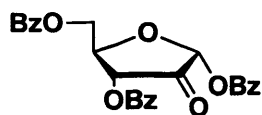
Synthesis of 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1H)-one[12]
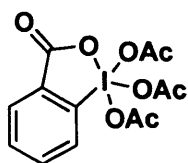
To a solution of crude 1-Hydroxy-1,2-benzoiodoxol-3 (1H)-one 1-oxide (17.7 g, 63 mmol) in acetic anhydride (88 mL, 897.4 mmol), was added *p*-TSA (0.11 g, 0.58 mmol) and the flask equipped with a drying tube. The reaction was stirred at 80 °C for 3 hours then cooled in an ice-water bath, filtered and washed with anhydrous diethyl ether (5 x 25 mL). The product was isolated as a white fine powder (12.0 g, 45%).

δ_H (CDCl$_3$): 8.32 (2H, m, CH-phenyl), 8.09 (1H, m, CH-phenyl), 7.28 (1H, m, CH-phenyl), 2.02 (9H, s, CH$_3$-acethyl).

Synthesis of α-D-1,3,5-tri-benzoyl-2-ketoribofuranose (210)[13]

To a solution of 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1H)-one (7.13 g, 16.8 mmol) in anhydrous DCM (50 mL), was added 1,3,5-tri-benzoyl-α-D-ribofuranose (5.0 g, 11.2 mmol) at 0 °C. The mixture was allowed to room temperature and stirred for 12 hours. The solvent was removed under reduced pressure and the residue triturated with diethyl ether (100 mL). The solid was filtrated trough MgSO$_4$ pad. The solvent was stirred with an equal volume of a solution of Na$_2$S$_2$O$_3$ (12.5 g) in 100 mL of a saturated solution of NaHCO$_3$.The organic layer was separated, washed with brine and

dried over MgSO$_4$. The solvent was removed under vacuum. The product was isolated as a white foam (4.9 g, 95%).

δ_H (CDCl$_3$): 8.37-7.92 (6H, m, CH-benzoyl), 7.56-7.45 (3H, m, CH-benzoyl), 7.41-7.29 (6H, m, CH-benzoyl), 6.13 (1H, s, H1-ribofuranose), 5.80 (1H, d, H3-ribofuranose, *J*= 8.8 Hz), 4.97 (1H, m, H4-ribofuranose), 4.75 (1H, m, H5-ribofuranose), 4.57 (1H, m, H5-ribofuranose); δ_C (CDCl$_3$): 201.31 (1C, C=O ketoribofuranose), 166.00 (1C, C=O benzoyl), 165.45 (1C, C=O benzoyl), 165.27 (1C, C=O benzoyl), 134.09 (1C, CH-benzoyl), 133.94 (1C, CH-benzoyl), 133.34 (1C, CH-benzoyl), 130.55 (3C, C-benzoyl), 130.34 (3C, CH-benzoyl), 129.84 (3C, CH-benzoyl), 128.67 (2C, C-benzoyl), 128.54 (1C, C-benzoyl), 128.30 (3C, CH-benzoyl), 91.44 (1C, C1-ribofuranose), 78.13 (1C, C3-ribofuranose), 72.09 (1C, C4-ribofuranose), 63.23 (1C, C5-ribofuranose).

Synthesis of *β*-2-methyl-1,3,5-tri-benzoyl-*α*-D-ribofuranose and *β*-2-methyl-2,3,5-tri-benzoyl-D-ribofuranose (211/212)[14]
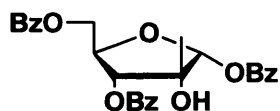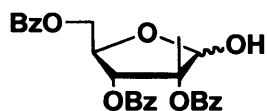

TiCl$_4$ (5.2 mL, 47.57 mmol) was added dropwise to anhydrous diethyl ester (140 mL) at -78 °C, followed by the addition of a solution 3.0 M of methyl magnesium bromide in diethyl ether (15.86 mL, 47.57 mmol). The reaction was allowed to -10° C and a solution of *α*-D-1,3,5-tri-benzoyl-2-ketoribofuranose (210, 5.28 g, 11.89 mmol) in diethyl ether (13 mL). After three hours the reaction was quenched with 60 mL of water. The water layer was separated and extracted with diethyl ether (3 x 50 mL). The combined organic layer was dried trough MgSO$_4$ and purified by column chromatography in gradient starting with a mixture of hexane/ethyl acetate 5:2 then 5:2.5 and 5:3.

The product was obtained as mixture of two compounds as a white solid (4.0 g, 70%).

Synthesis of *β*-2-methyl-1,2,3,5-tetra-benzoyl-*α*-D-ribofuranose (213)[14]

BzO⎯⎯⎯O⎯⎯OBz
           OBz OBz
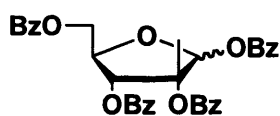
To a solution of dimethylaminopyridine (1.02 g, 8.40 mmol) and triethylamine (13 mL, 177 mmol) in anhydrous DCM (90 mL), was added benzoyl chloride (1.90 mL, 16.80 mmol) followed by the addition of a solution of *β*-2-methyl-1,3,5-tri-benzoyl-α-D-ribofuranose and *β*-2-methyl-2,3,5-tri-benzoyl-*α*-D-ribofuranose (211/212, 4.0 g, 8.40 mmol) in anhydours DCM (22 mL). After 4 hours at room temperature the reaction was poured into 250 mL of diethyl ether and washed with a solution 1 N of HCl (3 x 75 mL) followed by a saturated solution of NaHCO$_3$. The organic layer was dried trough MgSO$_4$, the solvent was removed under reduced pressure and the syrup obtained was purified by column chromatography using as eluent a mixture of hexane/ethyl acetate 4:1. The product was obtained as a white solid (2.0 g, 41%).

δ_H (CDCl$_3$): 8.12-8.02 (8H, m, CH-benzoyl), 7.61-7.58 (4H, m, CH-benzoyl), 7.49-7.39 (8H, m, CH-benzoyl), 5.97 (1H, d, H1-ribofuranose), 4.68-4.55 (2H, m, H3-ribofuranose, H4-ribofuranose), 4.62 (1H, m, H5-ribofuranose), 4.10 (1H, m, H5-ribofuranose), 1.96 (3H, s, CH$_3$-2-methyl-ribofuranose); δ_C (CDCl$_3$): 166.03 (1C, C=O benzoyl), 165.41(1C, C=O benzoyl), 164.77 (1C, C=O benzoyl), 164.51 (1C, C=O benzoyl), 134.50 (2C, CH-benzoyl), 133.67 (2C, CH-benzoyl), 133.62 (2C, CH-benzoyl), 133.25 (2C, CH-benzoyl), 130.53 (1C, C-benzoyl), 130.07 (4C, CH-benzoyl), 129.89 (4C, CH-benzoyl), 129.75 (1C, C-benzoyl), 129.70 (1C, C-benzoyl), 129.43 (1C, C-benzoyl), 128.54 (4C, CH-benzoyl), 93.85 (1C, C1-ribofuranose), 86.69 (1C, C2-ribofuranose), 77.39 (1C, C3-ribofuranose), 75.14 (1C, C4-ribofuranose), 63.83 (1C, C5-ribofuranose), 18.40 (1C, CH$_3$-2-methy-lribofuranose).
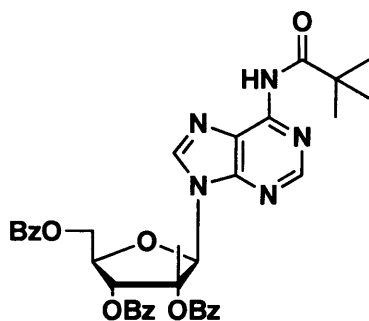
Synthesis of *N*⁶-*tert*-butanoyl adenine (217)
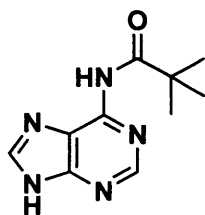
To a solution of adenine (216, 2.0 g, 14.80 mmol) in anhydrous DCM (200 mL), was added *tert*-butanoyl chloride (3.6 mL, 29.9 mmol), followed by the addition of diisopropyl-ethylamine (5.1 mL, 29.6 mmol). After 4 hours the reaction was washed with a saturated solution of NaHCO$_3$. The organic layer was dries trough MgSO$_4$. The solvent was removed under reduced pressure and the obtained solid was purified with column chromatography using as eluent a mixture of CHCl$_3$/MeOH 8.2. The pure produce was obtained as a white solid (2.6 g, 80%).

δ_H (d_6-DMSO): 12.20 (1H, NH7-adenine), 10.54 (1H, NH6-adenine), 8.66 (1H, s, H8-adenine), 8.44 (1H, s, H2-adenine), 1.32 (9H, s, CH_3-*tert*-butanoyl).
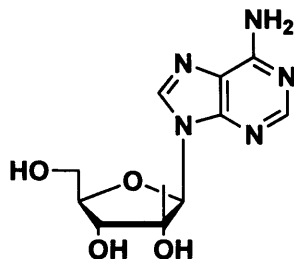
Synthesis of N^6-*tert*-butanoyl -β-2'-methyl-2',3',5'-tribenzoyl-adenosine (214)

To a solution of N6-*tert*-butanoyl adenine (217, 700 g, 1.206 mmol), β-2-methyl-1,2,3,5-tetra-benzoyl-α-D-ribofuranose (213, 291 mg, 1.327 mmol) and 7,11-diazabicyclo[5.4.0]undec-11-ene (0.55 mL, 3.618 mmol) in acetonitrile (14 mL) at 0 °C, trimethyl-silyl-triflate (0.88 mL, 4.824 mmol) was added dropwise then heated at 85 °C. After 5 hours the reaction the reaction was cooled at room temperature and a saturated solution of $NaHCO_3$ was added. The resulting solution was washed with $CHCl_3$ (3 x 50 mL). The organic layer was dried trough $MgSO_4$. The solvent was removed and the resulting solid was purified by column chromatography in gradient starting from a mixture of ethyl acetate/hexane 7:3 then only ethyl acetate. The product was obtained as a white solid (400 mg, 49%).

δ_H (CDCl$_3$): 8.99 (1H, s, H8-adenosine), 8.78 (1H, s, H2-adenosine), 8.05 (2H, d, CH-benzoyl, $J=$ 7.9 Hz), 7.98 (2H, d, CH-benzoyl, $J=$ 7.9 Hz), 7.91 (2H, d, CH-benzoyl, $J=$ 7.8 Hz), 7.51-7.44 (2H, m, CH-benzoyl), 7.38-7.24 (7H, m, CH-benzoyl), 6.77 (1H, s, H1'-adenosine), 6.16 (1H, d, H3'-adenosine, $J=$ 5.8 Hz), 4.88-4.84 (2H, m, H5'-adenosine), 4.68 (1H, m, H4'-adenosine), 1.53 (3H, s, CH_3-2'-adenosine), 1.33 (9H, s, CH_3-*tert*-butanoyl); δ_C (CDCl$_3$): 176.26 (1C, C=O-*tert*-butanoyl), 166.32 (1C, C=O benzoyl), 165.27(1C, C=O benzoyl), 165.15 (1C, C=O benzoyl), 152.93 (1C, C2-adenosine), 151.42 (1C, C6-adenosine), 150.04 (1C, C4-adenosine), 141.88 (1C, C8-adenosine), 133.83 (2C, CH-benzoyl), 133.52 (2C, CH-benzoyl), 133.35 (2C, CH-benzoyl), 130.22 (3C, CH-benzoyl), 129.89 (3C, CH-benzoyl), 129.43 (1C, C-benzoyl), 129.39 (1C, C-benzoyl), 128.62 (1C, C-benzoyl), 128.33 (3C, CH-benzoyl), 123.14 (1C, C5-adenosine), 88.85 (1C, C1'-adenosine), 84.85 (1C, C2'-adenosine), 80.12 (1C, C3'-adenosine), 75.80 (1C, C4'-adenosine), 63.50 (1C, C5'-adenosine), 18.10 (1C, CH_3-2-ribofuranose).

Synthesis of *β*-2'-methyl-adenosine (215)[15]

N6-*tert*-butanoyl-*β*-2'-methyl-2',3',5'-tribenzoyladenosine (**214**, 400 mg, 0.590 mmol) was added to a solution of MeOH saturated with ammonia, and stirred at room temperature. After 12 hours the solvent was removed and the obtained solid was purified by column chromatography in gradient starting with a mixture of $CHCl_3$/MeOH 9:1 then 8:2. The pure product was obtained as a white solid (120 mg, 72%).

δ_H (d_6-DMSO): 8.47 (1H, s, H8-adenosine), 8.15 (1H, s, H2-adenosine), 7.30 (1H, s, $NH_2$6-adenosine), 5.95 (1H, s, H1'-adenosine), 5.25-5.21 (3H, m, OH5'-adenosine, OH3'-adenosine, OH2'-adenosine), 4.12-4.05 (1H, d, H3'-adenosine, *J*= 8.6 Hz), 3.91 (1H, m, H4'-adenosine), 3.84 (1H, m, H5'-adenosine), 3.70 (1H, m, H5'-adenosine), 0.77 (3H, s, $CH_3$2'-adenosine); δ_C (d_6-DMSO): 156.02 (1C, C6-adenosine), 152.53 (1C, C2-adenosine), 149.01 (1C, C4-adenosine), 138.68 (1C, C8-adenosine), 118.67 (1C, C5-adenosine), 90.78 (1C, C1'-adenosine), 82.52 (1C, C4'-adenosine), 78.46 (1C, C2'-adenosine), 71.63 (1C, C3'-adenosine), 59.47 (1C, C5'-adenosine), 19.83 (1C, CH_3-2'-adenosine).

Anal. Calc. for $C_{11}H_{15}N_5O_4$: C 46.97%, H 5.38%, N 24.90%. Found: C 46.67%, H 5.22%, N 24.20%.

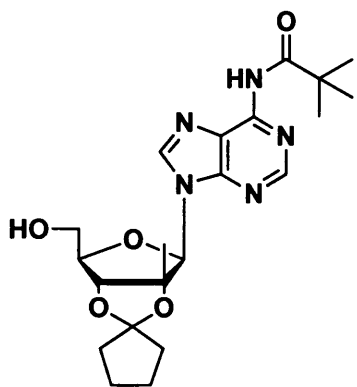## Synthesis of N^6-tert-butanoyl-β-2'-methyl-adenosine (218)
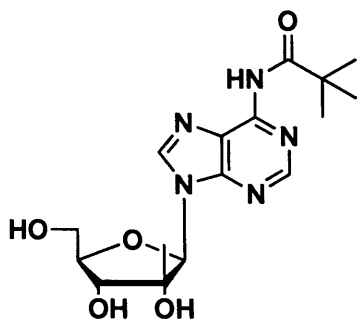
To a solution of N6-tert-butanoyl-β-2'-methyl-2',3',5'-tribenzoyl-adenosine (**214**, 740 mg, 1.093 mmol) in pyridine (6 mL) and EtOH (3 mL), a solution of NaOH 1 M was added dropwise. After 30 minutes at room temperature DOWEX resin was added to reach neutral pH. The resin was filtered and washed with a solution of pyridine/H_2O 4:1. The solvent was removed and the obtained brown oil was dissolved in diethyl ether and washed with H_2O. The water layer was dryness in vacuo to yield a yellow foam of the desire compound (320 mg, 80%).

δ_H (d_6-DMSO): 12.23 (1H, s, NH6-adenosine), 8.82 (1H, s, H8-adenosine), 8.71 (1H, s, H2-adenosine), 6.09 (1H, s, H1'-adenosine), 5.32-5.22 (3H, m, OH5'-adenosine, OH3'-adenosine, OH2'-adenosine), 3.92-3.71 (4H, d, H3'-adenosine, H4'-adenosine, 2 H5'-adenosine), 1.28 (9H, s, 3 CH_3-tert-butanoyll), 0.80 (3H, s, CH_3-2'-adenosine).

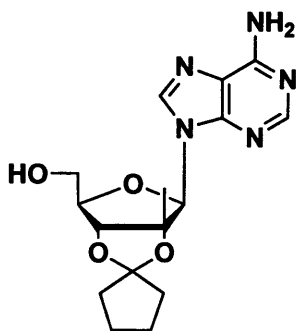## Synthesis of 2',3'-O,O-cyclopentyl- N^6-tert-butanoyl-β-2'-methyl-adenosine (219)

To a solution of N6-tert-butanoyl-β-2'-methyl-adenosine (**218**, 320 mg, 0.87 mmol) in 1,1-dimethoxypentane (5 mL), was added p-TSA (250 mg, 1,314 mmol) at 40° C. After three hours the reaction was cooled down at room temperature and a saturated solution of $NaHCO_3$ was added. The solvent was removed and the brown solid was purified by column chromatography in gradient starting from a mixture of $CHCl_3$/MeOH 9:1 then 85:15 and at the end 8:2. The product was isolated as a white solid (200 mg, 53%).

δ_H ($CDCl_3$): 11.71 (1H, s, NH6-adenosine), 8.31 (1H, s, H8-adenosine), 7.92 (1H, s, H2-adenosine), 6.17 (1H, s, H1'-adenosine), 4.75 (1H, s, H3'-adenosine), 4.42 (1H, s, H4'-adenosine), 4.07 (1H, d, H5'-adenosine, $J=$ 12.75), 3.84 (1H, d, H5'-adenosine, $J=$ 12.75 Hz), 2.05-2.00 (2H, m, CH_2-cyclopentyl), 1.74-1.63 (6H, 3 CH_2-cyclopentyl), 1.40 (9H, s, 3 CH_3-tert-butanoyl), 1.24 (3H, s, CH_3-2'-adenosine); δ_C ($CDCl_3$): 162.39 (1C, C=O tert-butanoyl), 156.12 (1C, C6-adenosine), 152.56 (1C, C2-adenosine), 148.83 (1C, C4-adenosine), 140.01 (1C, C8-adenosine), 123.12 (1C,

C5-adenosine), 120.72 (1C, C-cyclopentyl), 97.57 (1C, C1'-adenosine), 90.54 (1C, C2'-adenosine), 86.18 (1C, C3'-adenosine), 85.60 (1C, C4'-adenosine), 62.57 (1C, C5'-adenosine), 40.02 (1C, C-*tert*-butanoyl), 37.46 (2C, CH$_2$-cyclopentyl), 27.37 (3C, 3 CH$_3$-*tert*-butanoyl), 23.65 (1C, CH$_2$-cyclopentyl), 23.05 (1C, CH$_2$-cyclopentyl), 19.08 (1C, CH$_3$-2'-adenosine).

## Synthesis of 2',3'-*O,O*-cyclopentyl-*β*-2'-methyl-adenosine (220)

2',3'-*O,O*-cyclopentyl-N6-*tert*-butanoyl-*β*-2'-methyl-adenosine (**219**, 200 mg, 0.464 mmol) was added to a solution of MeOH saturated with ammonia, and stirred at room temperature. After 12 hours the solvent was removed and the obtained solid was purified by column chromatography in gradient starting with a mixture of CHCl$_3$/MeOH 98:2 then 9:1. The pure product was obtained as a white solid (120 mg, 75%).

δ_H (CDCl$_3$): 8.26 (1H, s, H8-adenosine), 7.92 (1H, s, H2-adenosine), 6.58 (2H, s, NH$_2$6-adenosine), 6.15 (1H, s, H1'-adenosine), 4.70 (1H, s, H3'-adenosine), 4.36 (1H, s, H4'-adenosine), 4.02 (1H, d, H5'-adenosine, J= 12.75), 3.81 (1H, d, H5'-adenosine, J= 12.75 Hz), 2.01-1.99 (2H, m, CH$_2$-cyclopentyl), 1.80-1.66 (6H, 3 CH$_2$-cyclopentyl), 1.07 (3H, s, CH$_3$-2'-adenosine); δ_C (CDCl$_3$): 156.25 (1C, C6-adenosine), 152.66 (1C, C2-adenosine), 148.82 (1C, C4-adenosine), 139.83 (1C, C8-adenosine), 123.06 (1C, C5-adenosine), 120.59 (1C, C-cyclopentyl), 96.95 (1C, C1'-adenosine), 90.50 (1C, C2'-adenosine), 85.44 (1C, C3'-adenosine), 84.44 (1C, C4'-adenosine), 62.41 (1C, C5'-adenosine), 37.48 (2C, CH$_2$-cyclopentyl), 23.60 (1C, CH$_2$-cyclopentyl), 23.04 (1C, CH$_2$-cyclopentyl), 19.03 (1C, CH$_3$-2'-adenosine).

Synthesis of 2',3',5'-tribenzoyl-β-2'-methyl- N^2-acetyl-guanosine (229)[16]
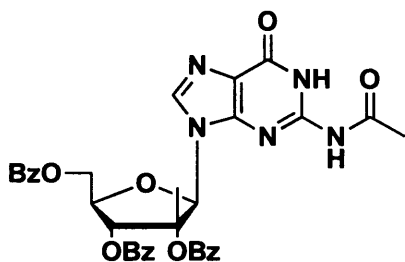
To a solution of N2-acetylguanine (228, 2.0 g, 10.34 mmol), in pyridine (10 mL), was added 1,1,1,3,3,3-hexamethyldisilazane (35 mL) and the reaction stirred for 45 minutes at 130° C. The solvent was removed with high vacuum under argon. The solid obtained was dissolved in *para*-xylene (100 mL), and β-2-methyl-1,2,3,5-tetra-benzoyl-α-D-ribofuranose (213, 2.0 g, 3.45 mmol) was added, followed by addition of TMS-triflate (1.87 mL, 10.34 mmol). The reaction was refluxed at 140° C for 5 hours. The reaction was washed with a saturated solution of NaHCO$_3$. The aqueous layer was extracted with CHCl$_3$ (3 x 100 mL). The combined organic layer was dried trough MgSO$_4$, the solvent was removed under reduced pressure and the resulting solid was purified by column chromatography using as eluent a mixture of CHCl$_3$/MeOH 98:2. The desire product was yielded as a white solid (1.4 g, 63%).

δ_H (d_6-DMSO): 12.37 (1H, s, NH1-guanosine), 11.96 (1H, s, NH2-guanosine), 8.50 (1H, s, H8-guanosine), 8.26-8.22 (4H, m, 4 CH-benzyol), 8.06 (2H, d, 2 CH-benzyol, *J*= 7.6 Hz), 7.98-7.82 (3H, m, 3 CH-benzyol), 7.73 (4H, m, CH-benzoyl), 7.60 (2H, m, 2 CH-benzoyl), 6.79 (1H, s, H1'-guanosine), 6.08 (1H, s, H3'-guanosine), 5.08-5.03 (3H, m, H4'-guanosine, H5'guanosine), 2.50 (3H, s, CH$_3$-acetyl), 1.83 (3H, CH$_3$2'-guanosine).

## Synthesis of β-2'-methylguanosine (226)[16]
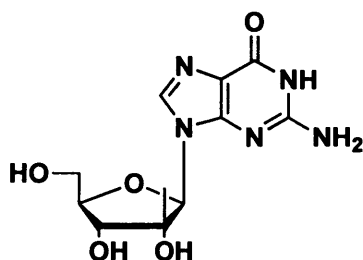
N2-acetyl-β-2'-methyl-2',3',5'-tribenzoyl-guanosine (229, 1.42 g, 2.18 mmol) was added to a solution of MeOH saturated with ammonia, and stirred at room temperature. After 12 hours the solvent was removed and the obtained solid was purified by column chromatography using as eluent a mixture of CHCl$_3$/MeOH 8:2. The pure product was obtained as a white solid (570 mg, 87%).

δ_H (d_6-DMSO): 10.52 (1H, s, NH1-guanosine), 8.48 (1H, s, H8-guanosine), 6.52 (2H, s, NH$_2$2-guanosine), 5.73 (1H, s, H1'-guanosine), 5.24 (1H, d, OH3'-guanosine, $J=$ 6.3 Hz), 5.11 (1H, m, OH5'-guanosine), 5.03 (1H, s, OH2'-guanosine), 3.97 (1H, m, H3'-guanosine), 3.85-3.79 (2H, m, H4', H5'-guanosine), 3.66 (1H, d, H5'-guanosine, $J=$ 12.2 Hz), 0.81 (3H, s, CH$_3$-2'-guanosine); δ_C (d_6-DMSO): 156.72 (1C, C6-guanosine), 153.68 (1C, C2-guanosine), 150.77 (1C, C4-guanosine), 135.07 (1C, C8-guanosine), 116.38 (1C, C5-guanosine), 90.10 (1C, C1'-guanosine), 82.30 (1C, C3'-guanosine), 78.52 (1C, C2'-guanosine), 71.63 (1C, C4'-guanosine), 59.40 (1C, C5'-guanosine), 19.96 (1C, CH$_3$-2'-guanosine).

MS (ES) m/e: 320.2 (MNa$^+$, 100%); Accurate mass: C$_{11}$H$_{15}$N$_5$O$_5$Na required 320.0968, found 320.0971.

Synthesis 2',3'-*O,O*-isopropyl-β-2'-methyl-guanosine (230)
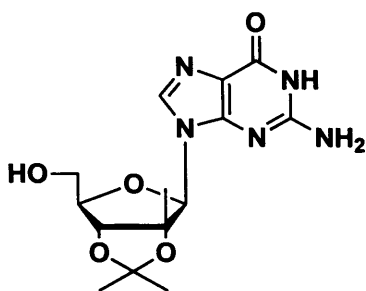
To a solution of β-2'-methyl-adenosine (**226.** 565 mg, 1,90 mmol) in anhydrous acetone (35 mL), perchloric acid (0.30 mL) was added dropwise at room temperature. After 2 hours a saturated solution of NH$_4$OH was added to reach neutral pH. The solvent was removed and the resulting solid was purified by column chromatography using as eluent a solution of CHCl$_3$/MeOH 92:8.

The desire product was obtained as a white solid (210 mg, 33%).

δ_H (d_6-DMSO): 10.63 (1H, s, NH1-guanosine), 7,92 (1H, s, H8-guanosine), 6.45 (2H, s, NH$_2$2-guanosine), 6.02 (1H, s, H1'-guanosine), 5.21 (1H, t, OH5'-guanosine, $J=$ 5.0 Hz), 4.59 (1H, s, H3'-guanosine), 4.17 (1H, s, H4'-guanosine), 3.71 (1H, d, H5'-guanosine, $J=$ 7.7 Hz), 3.66 (1H, d, H5'-guanosine, $J=$ 7.7 Hz), 1.54 (3H, s, CH$_3$-isopropylidine), 1.38 (3H, s, CH$_3$-isopropylidine), 1.16 (3H, s, CH$_3$-2'-guanosine).

7.11 Synthesis of 2'-methyladenosine phosphoramidates.

Synthesis of 2', 3'-*O,O*-cyclopentylidene-*β*-2'-methyladenosine 5'-*O*-[phenyl(benzyl-L-alaninyl)] phosphate
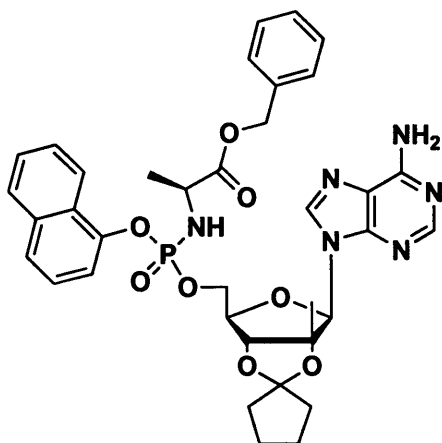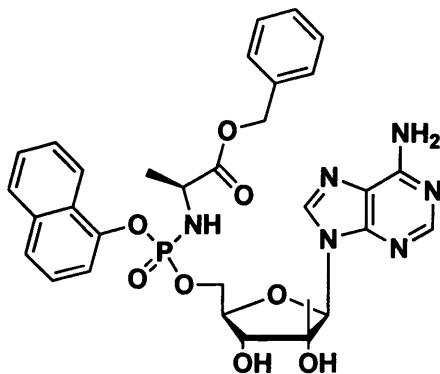
Prepared according to Standard Procedure C2, from 2', 3'-*O,O*-cyclopentylidene-*β*-2'-methyladenosine (**230**, 40 mg, 0.115 mmol), ^tBuMgCl (0.35 mL, 1M solution in THF, 0.345 mmol) and α-naphthyl(benzyl-L-alaninyl) phosphorochloridate (**93**, 0.35 mL of solution 1M in THF, 0.345 mmol). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (95:5). The pure product was a white solid (20 mg, 24%).

$δ_P$ (d_4-CH₃OH): 4.34, 4.18; $δ_H$ (d_4-CH₃OH): 8.50 (1H, s, H2-adenosine), 8.17 (1H, s, H8-adenosine), 7.90 (1H, m, CH-naphthyl), 7.71 (1H, m, CH-naphthyl), 7.69 (1H, CH-benzyl), 7.55-7.50 (3H, m, CH-naphthyl, CH-benzyl), 7.42-7.27 (6H, m, CH-naphthyl, CH-benzyl), 6.25 (1H, d, H1'-adenosine), 5.10 (2H, s, CH₂-benzyl), 4.61 (1H, m, H3'-adenosine), 4.41 (1H, m, H4'-adenosine), 4.15 (1H, m, CHα), 3.95 (1H, m, H5'-adenosine, *J*= 12.2 Hz), 3.85 (1H, m, H5'-adenosine, *J*= 12.2 Hz), 2.12-2.03 (2H, m, CH₂-cyclopentyl), 1.79-1.72 (6H, m, CH₂-cyclopentyl), 1.37 (3H, d, CH₃-alanine, *J*= 7.2 Hz), 0.89 (3H, s, CH₃-2'-adenosine).

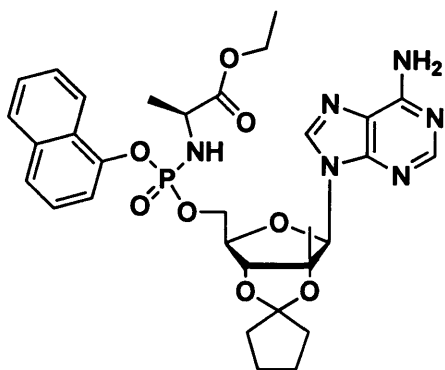**Synthesis of *β*-2'-methyladenosine 5'-*O*-[α-naphthyl(benzyl-L-alaninyl)] phosphate (222)**

Prepared according to Standard Procedure C3, from 2', 3'-*O,O*-cyclopentylidene-*β*-2'-methyladenosine 5'-*O*-[α-naphthyl(benzyl-L-alaninyl)] phosphate (30 mg, 0.036

266

mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent for the first $CHCl_3$/MeOH (95:5) followed by a semi-preparative HPLC. The pure product was a white solid (5 mg, 21%).

δ_P (d_4-CH_3OH): 4.25, 4.14; δ_H (d_4-CH_3OH): 8.04-7.95 (3H, m, H2-adenosine, H8-adenosine, CH-naphthyl), 7.68 (1H, m, CH-naphthyl), 7.48 (1H, m, CH-naphthyl), 7.32-7.23 (3H, m, CH-naphthyl, CH-benzyl), 7.16 (1H, m, CH-naphthyl), 7.05 (6H, m, CH-naphthyl, CH-benzyl), 5.88 (1H, d, H1'-adenosine, J= 2.9 Hz), 4.85-4.65 (2H, m, CH_2-benzyl), 4.37-4.35 (2H, d, H3'-adenosine, H4'-adenosine), 4.06 (2H, m, H5'-adenosine), 3.88-3.83 (1H, m, CHα), 1.35 (3H, m, CH_3-alanine), 0.88 (3H, s, CH_3-2'-adenosine).

MS (ES) m/e: 671.2 (MNa^+, 100%); Accurate mass: $C_{31}H_{33}N_6O_8NaP$ required 671.1990, found 671.1995.

Synthesis of 2', 3'-*O,O*-cyclopentylidene-*β*-2'-methyladenosine 5'-*O*-[phenyl(ethyl-L-alaninyl)] phosphate
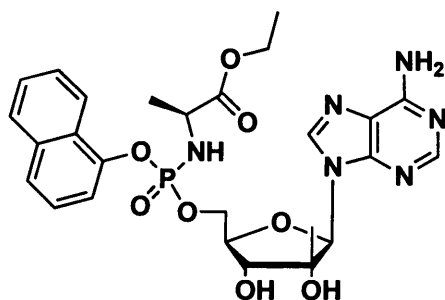

Prepared according to Standard Procedure C2, from 2', 3'-*O,O*-cyclopentylidene-*β*-2'-methyladenosine (**230**, 60 mg, 0.172 mmol), ^tBuMgCl (0.5 mL, 1M solution in THF, 0.519 mmol) and α-naphthyl(ethyl-L-alaninyl) phosphorochloridate (**200**, 0.5 mL of solution 1M in THF, 0.519 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (30 mg, 26%).

δ_P (d_4-CH_3OH): 4.31, 4.26; δ_H (d_4-CH_3OH): 8.19 (1H, s, H2-adenosine), 8.10 (1H, s, H8-adenosine), 7.88 (1H, m, CH-naphthyl), 7.73 (1H, m, CH-naphthyl), 7.57-7.52 (4H, m, CH-naphthyl), 7.45-7.43 (1H, m, CH-naphthyl), 6.26 (1H, m, H1'-adenosine), 4.56-4.42 (4H, m, H4'-adenosine, H3'-adenosine, 2 H5'-adenosine), 4.08 (3H, m, CHα, CH_2-ethyl), 2.21-2.09 (2H, m, CH_2-cyclopentyl), 1.76-1.71 (6H, m, CH_2-cyclopentyl), 1.35 (3H, d, CH_3-alanine, J= 6.9 Hz), 1.25 (3H, m, CH_3-ethyl), 0.95 (3H, s, $CH_3$2'-adenosine).

Synthesis of *β*-2'-methyladenosine 5'-*O*-[α-naphthyl(ethyl-L-alaninyl)] phosphate (223)
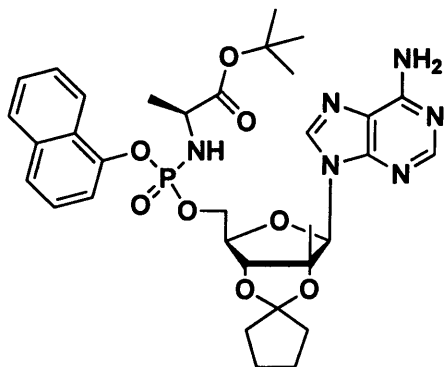

Prepared according to Standard Procedure C3, from 2', 3'-*O,O*-cyclopentylidene-*β*-2'-methyladenosine 5'-*O*-[α-naphthyl(ethyl-L-alaninyl)] phosphate (30 mg, 0.036 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent for the first $CHCl_3$/MeOH (95:5) followed by a semi-preparative HPLC. The pure product was a a white solid (4 mg, 19%).

δ_P (d_4-CH_3OH): 4.23, 4.20; δ_H (d_4-CH_3OH): 8.24-8.19 (3H, m, H2-adenosine, H8-adenosine, CH-naphthyl), 7.90 (1H, m, CH-naphthyl), 7.63 (1H, CH-naphthyl), 7.53 (4H, m, CH-naphthyl), 7.41 (1H, m, CH-naphthyl), 6.12 (1H, d, H1'-adenosine, *J*= 2.1 Hz), 4.61-4.59 (2H, d, H3'-adenosine, H4'-adenosine), 4.30 (1H, m, H5'-adenosine), 4.02-3.99 (3H, m, CHα, CH_2-ethyl), 1.37 (3H, m, CH_3-alanine), 1.27 (3H, m, CH_3-ethyl), 0.95 (3H, s, CH_3-2'-adenosine).

MS (ES) m/e: 609.2 (MNa^+, 100%); Accurate mass: $C_{26}H_{31}N_6O_8NaP$ required 609.1846, found 609.1839.

Synthesis of 2', 3'-*O,O*-cyclopentylidene-*β*-2'-methyladenosine 5'-*O*-[phenyl(*tert*-butyl-L-alaninyl)] phosphate
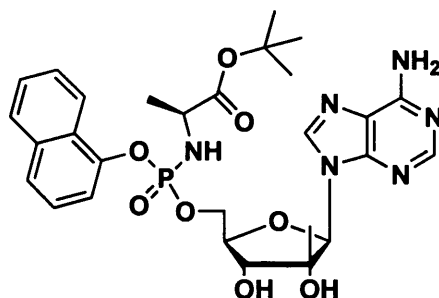
Prepared according to Standard Procedure C2, from 2', 3'-*O,O*-cyclopentylidene-*β*-2'-methyladenosine (**230**, 60 mg, 0.172 mmol), 'BuMgCl (0.51 mL, 1M solution in THF, 0.51 mmol) and α-naphthyl(*tert*-butyl-L-alaninyl) phosphorochloridate (**201**, 0.5 mL of solution 1M in THF, 0.519 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (95:5). The pure product was a white solid (27 mg, 22%).

δ_P (d_4-CH$_3$OH): 4.37, 4.28; δ_H (d_4-CH$_3$OH): 8.21 (1H, s, H2-adenosine), 8.13 (1H, s, H8-adenosine), 7.85 (1H, m, CH-naphthyl), 7.73 (1H, m, CH-naphthyl), 7.57-7.52 (4H, m, CH-naphthyl), 7.43-7.41 (1H, m, CH-naphthyl), 6.25 (1H, m, H1'-adenosine), 4.55-4.40 (4H, m, H4'-adenosine, H3'-adenosine, 2 H5'-adenosine), 4.05 (1H, m, CHα), 2.20-2.12 (2H, m, CH$_2$-cyclopentyl), 1.79-1.69 (6H, m, CH$_2$-cyclopentyl), 1.36 (9H, CH$_3$-*tert*-butyl), 1.25 (3H, d, CH$_3$-alanine, *J*= 6.9 Hz), 0.96 (3H, s, CH$_3$-2'-adenosine).

Synthesis of *β*-2'-methyladenosine 5'-*O*-[α-naphthyl(*tert*-butyl-L-alaninyl)] phosphate (224)
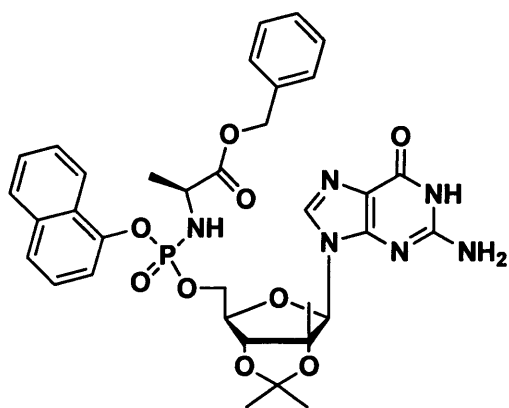
Prepared according to Standard Procedure C3, from 2', 3'-*O,O*-cyclopentylidene-*β*-2'-methyladenosine 5'-*O*-[α-naphthyl(*tert*-butyl-L-alaninyl)] phosphate (27 mg, 0.039 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent for the first CHCl$_3$/MeOH (95:5) followed by a semi-preparative HPLC. The pure product was a white solid (10 mg, 41%).

δ_P (d_4-CH$_3$OH): 4.20, 4.08; δ_H (d_4-CH$_3$OH): 8.20-8.15 (3H, m, H2-adenosine, H8-adenosine, CH-naphthyl), 7.81 (1H, m, CH-naphthyl), 7.60 (1H, CH-naphthyl), 7.54 (4H, m, CH-naphthyl), 7.39 (1H, m, CH-naphthyl), 6.15 (1H, d, H1'-adenosine), 4.63-4.57 (2H, d, H3'-adenosine, H4'-adenosine), 4.31 (1H, m, H5'-adenosine), 4.00-3.97 (1H, m, CHα), 1.39 (9H, CH$_3$-*tert*-butyl), 1.27 (3H, m, CH$_3$-alanine), 0.97 (3H, s, CH$_3$-2'-adenosine).

7.12 Synthesis of 2'-methylguanosine phosphoramidates.

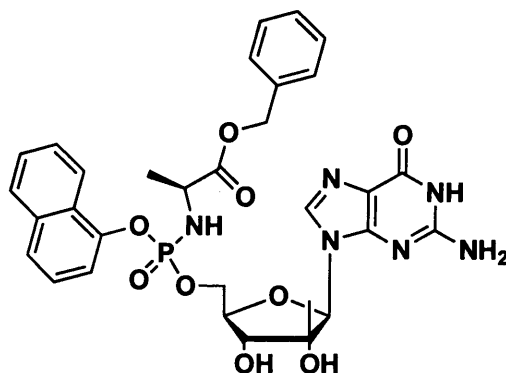**Synthesis of 2', 3'-*O,O*-isopropylidyn-*β*-2'-methylguanosine 5'-*O*-[α-naphthyl(benzyl-L-alaninyl)] phosphate**

Prepared according to Standard Procedure C2, from 2', 3'-*O,O*-isopropylidene-*β*-2'-methylguanosine (**230**, 170 mg, 0.503 mmol), 'BuMgCl (1.0 mL, 1M solution in THF, 1.006 mmol) and α-naphthyl(benzyl-L-alaninyl) phosphorochloridate (**93**, 1.0 mL of solution 1M in THF, 1.006 mmol). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (95:5). The pure product was a white solid (70 mg, 19%).

δ_P (d_4-CH₃OH): 4.53, 4.40; δ_H (d_4-CH₃OH): 8.28 (1H, s, H8-guanosine), 7.84 (1H, m, CH-naphthyl), 7.77-7.71 (1H, m, CH-benzyl), 7.55-7.49 (4H, m, CH-naphthyl, CH-benzyl), 7.44-7.29 (6H, m, CH-naphthyl, CH-benzyl), 6.06 (1H, d, H1'-guanosine), 5.10 (2H, s, CH₂-benzyl), 4.59 (1H, m, H3'-guanosine), 4.52-4.45 (1H, m, H4'-guanosine), 4.34 (2H, H5'guanosine), 4.14 (1H, m, CHα), 1.59 (3H, d, CH₃-isopropylidene, *J*= 10.4 Hz), 1.37 (6H, d, CH₃-alanine, CH₃-isopropylidene), 0.99 (3H, d, CH₃-2'-guanosine, *J*= 20.11 Hz).

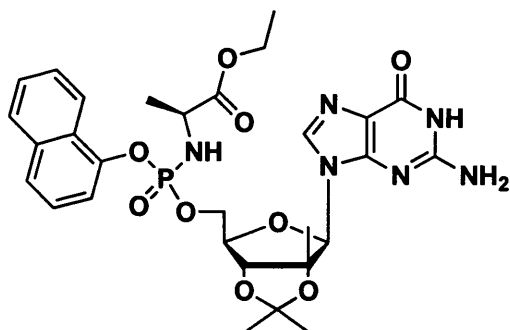**Synthesis of *β*-2'-methylguanosine 5'-*O*-[α-naphthyl(benzyl-L-alaninyl)] phosphate (233)**

Prepared according to Standard Procedure C4, from 2', 3'-*O,O*-isopropylidene-*β*-2'-methyl-guanosine 5'-*O*-[α-naphthyl(benzyl-L-alaninyl)] phosphate (70 mg, 0.098

mmol), and 10 mL of a solution 60% of CH_3COOH in water at 90 °C for 15 hours. The crude product was purified by column chromatography, using as eluent for the first $CHCl_3$/MeOH (85:5) followed by a semi-preparative HPLC. The pure product was a white solid (12 mg, 18%).

δ_P (d_4-CH_3OH): 4.25, 4.14; δ_H (d_4-CH_3OH): 8.17 (1H, m, H8-guanosine), 7.88 (1H, m, CH-naphthyl), 7.79 (1H, m, CH-naphthyl), 7.53 (2H, m, CH-naphthyl, CH-benzyl), 7.42-7.40 (1H, m, CH-naphthyl), 7.36-7.21 (7H, m, CH-naphthyl, CH-benzyl), 6.05 (1H, d, H1'-guanosine, J= 8.4 Hz), 5.15-4.90 (2H, m, CH_2-benzyl), 4.58-4.49 (2H, d, H3'-guanosine, H4'-guanosine), 4.44-4.34 (2H, m, H5'-guanosine), 4.17-4.11 (1H, m, CHα), 1.37 (3H, m, CH_3-alanine), 1.00 (3H, s, CH_3-2'-guanosine).

MS (ES) m/e: 687.2 (MNa^+, 100%); Accurate mass: $C_{31}H_{33}N_6O_9NaP$ required 687.1954, found 687.1944.

Synthesis of 2', 3'-*O,O*-isopropylidene-*β*-2'-methylguanosine 5'-*O*-[phenyl(ethyl-L-alaninyl)] phosphate

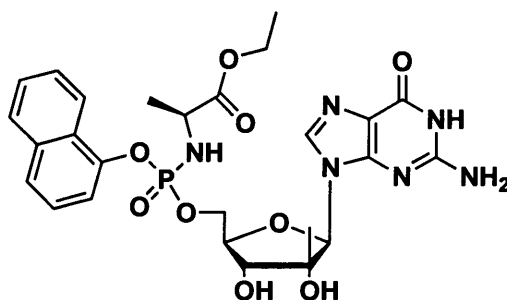

Prepared according to Standard Procedure C2, from 2', 3'-*O,O*-isopropylidene-*β*-2'-methylguanosine (**230**, 220 mg, 0.652 mmol), 'BuMgCl (1.3 mL, 1M solution in THF, 1.30 mmol) and α-naphthyl(ethyl-L-alaninyl) phosphorochloridate (**207**, 1.3 mL of solution 1M in THF, 1.30 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The pure product was a white solid (35 mg, 9%).

δ_P (d_4-CH_3OH): 4.41, 4.32; δ_H (d_4-CH_3OH): 8.18 (1H, s, H8-guanosine), 7.88 (1H, m, CH-naphthyl), 7.73 (1H, m, CH-naphthyl), 7.59-7.52 (4H, m, CH-naphthyl), 7.46-7.42 (1H, m, CH-naphthyl), 6.08 (1H, d, H1'-guanosine), 4.62-4.40 (4H, m, H3'-guanosine, H4'-guanosine, H5'guanosine), 4.11-4.09 (3H, m, CHα, CH_2-ethyl), 1.59 (3H, d, CH_3-isopropylidene, J= 13.2 Hz), 1.37 (6H, m, CH_3-alanine, CH_3-isopropylidene), 1.20 (3H, m, CH_3-ethyl), 1.00 (3H, m, CH_3-2'-guanosine).

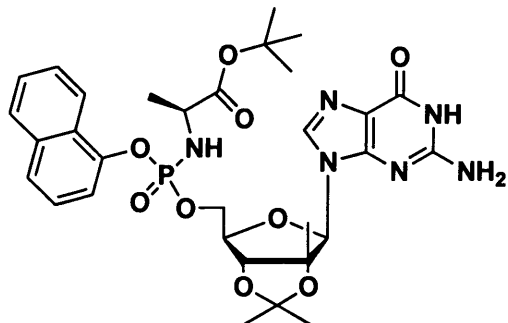**Synthesis of β-2'-methyl-guanosine 5'-*O*-[α-naphthyl(ethyl-L-alaninyl)] phosphate (234)**

Prepared according to Standard Procedure C4, from 2', 3'-*O,O*-isopropylidene-β-2'-methylguanosine 5'-*O*-[α-naphthyl(ethyl-L-alaninyl)] phosphate (35 mg, 0.054 mmol), and 10 mL of a solution 60% of CH_3COOH in water at 90 °C for 15 hours. The crude product was purified by column chromatography, using as eluent for the first $CHCl_3$/MeOH (85:5) followed by a semi-preparative HPLC. The pure product was a white solid (10 mg, 31%).

$δ_P$ (d_4-CH$_3$OH): 4.25, 4.14; $δ_H$ (d_4-CH$_3$OH): 8.18 (1H, m, H8-guanosine), 7.87 (1H, m, CH-naphthyl), 7.71 (1H, m, CH-naphthyl), 7.53 (4H, m, CH-naphthyl), 7.51-7.40 (1H, m, CH-naphthyl), 5.93 (1H, d, H1'-guanosine), 4.62-4.57 (2H, m, H3'-guanosine, H4'-guanosine), 4.24 (2H, m, H5'guanosine), 4.03-3.98 (3H, m, CHα, CH$_2$-ethyl), 1.31 (3H, d, CH$_3$-alanine, J= 7.9 Hz), 1.15 (3H, m, CH$_3$-ethyl), 1.00 (3H, m, CH$_3$-2'-guanosine).

MS (ES) m/e: 625.3 (MNa$^+$, 100%); Accurate mass: $C_{26}H_{31}N_6O_9NaP$ required 625.1795, found 6251788.

Anal. Calc. for $C_{26}H_{31}N_6O_9P$: C 51.83%, H 5.19%, N 13.95%. Found: C 51.86%, H 5.10%, N 12.04%.

Synthesis of 2', 3'-*O,O*-isopropylidene-*β*-2'-methylguanosine 5'-*O*-[phenyl(*tert*-butyl-L-alaninyl)] phosphate
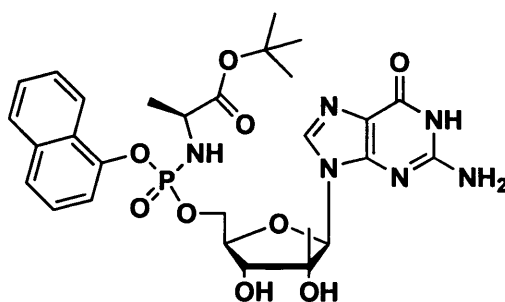

Prepared according to Standard Procedure C2, from 2', 3'-*O,O*-isopropylidene-*β*-2'-methylguanosine (**230**, 120 mg, 0.355 mmol), 'BuMgCl (0.70 mL, 1M solution in THF, 0.711 mmol) and α-naphthyl(*tert*-butyl-L-alaninyl) phosphorochloridate (**208**, 0.70 mL of solution 1M in THF,

0.711mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (95:5). The pure product was a white solid (24 mg, 10%).

δ_P (d_4-CH$_3$OH): 4.41, 4.32; δ_H (d_4-CH$_3$OH): 8.20 (1H, s, H8-guanosine), 7.89 (1H, m, CH-naphthyl), 7.73 (1H, m, CH-naphthyl), 7.59-7.54 (4H, m, CH-naphthyl), 7.49-7.42 (1H, m, CH-naphthyl), 6.07 (1H, d, H1'-guanosine), 4.62-4.40 (4H, m, H3'-guanosine, H4'-guanosine, 2 H5'guanosine), 3.99-3.86 (1H, m, CHα), 1.58 (3H, d, CH$_3$-isoprpylidene, *J*= 13.7 Hz), 1.44 (9H, s, CH$_3$-*tert*-butyl), 1.38-1.34 (6H, m, CH$_3$-alanine, CH$_3$-isopropylidene), 1.01 (3H, m, CH$_3$2'-guanosine).
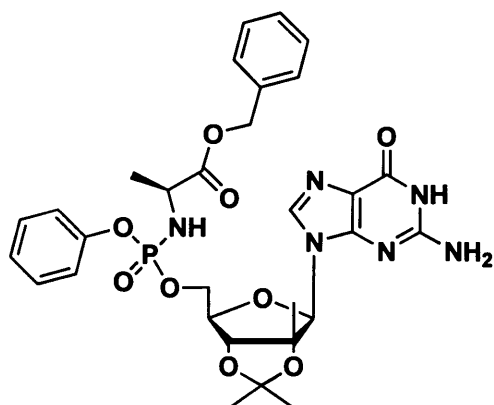
Synthesis of *β*-2'-methylguanosine 5'-*O*-[α-naphthyl(*tert*-butyl-L-alaninyl)] phosphate (235)

Prepared according to Standard Procedure C4, from 2', 3'-*O,O*-isopropylidene-*β*-2'-methylguanosine 5'-*O*-[α-naphthyl(*tert*-butyl-L-alaninyl)] phosphate (24 mg, 0.036 mmol), and 10 mL of a solution 60% of CH$_3$COOH in water. The crude product was purified by column chromatography, using as eluent for the first CHCl$_3$/MeOH (85:5) followed by a semi-preparative HPLC. The pure product was a white solid (4 mg, 17%).

δ_P (d_4-CH$_3$OH): 4.23, 4.10; δ_H (d_4-CH$_3$OH): 8.20 (1H, m, H8-guanosine), 7.85 (1H, m, CH-naphthyl), 7.67 (1H, m, CH-naphthyl), 7.57 (4H, m, CH-naphthyl), 7.53-7.43 (1H, m, CH-naphthyl), 6.00 (1H, d, H1'-guanosine), 4.61-4.55 (2H, m, H3'-guanosine, H4'-guanosine), 4.25 (2H, m, H5'guanosine), 4.00-3.97 (1H, m, CHα), 1.47 (9H, s, CH$_3$-*tert*-butyl), 1.36 (3H, m, CH$_3$-alanine), 1.00 (3H, m, CH$_3$2'-guanosine).

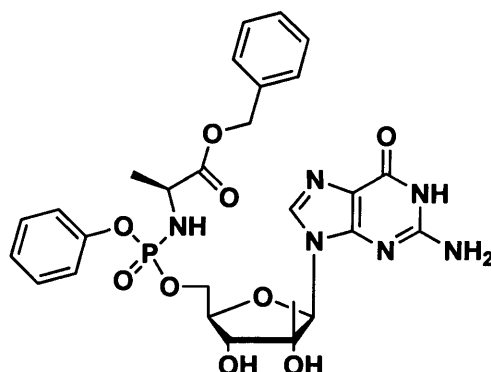## Synthesis of 2', 3'-*O,O*-isopropylidene-*β*-2'-methylguanosine 5'-*O*-[phenyl(benzyl-L-alaninyl)] phosphate

Prepared according to Standard Procedure C2, from 2', 3'-*O,O*-isopropylidene-*β*-2'-methylguanosine (**230**, 120 mg, 0.355 mmol), 'BuMgCl (1.0 mL, 1M solution in THF, 1.07 mmol) and phenyl(benzoxy-L-alaninyl) phosphorochloridate (**132**, 1.0 mL of solution 1M in THF, 1.07 mmol). The crude product was purified by column chromatography, using as eluent CHCl$_3$/MeOH (9:1) followed by semi-preparative HPLC. The pure product was a white solid (40 mg, 17%).

δ_P (d_4-CH$_3$OH): 4.63, 4.37; δ_H (d_4-CH$_3$OH): 7.85 (1H, d, H8-guanosine, *J*= 5.7 Hz), 7.36-7.34 (5H, m, CH-phenyl, CH-benzyl), 7.33-7.26 (5H, m, CH-benzyl, CH-phenyl), 6.02 (1H, d, H1'-guanosine, *J*= 11.4 Hz), 5.16 (2H, s, CH$_2$-benzyl), 4.67 (1H, d, H3'-guanosine, *J*= 1.1 Hz), 4.54-4.43 (1H, m, H4'-guanosine), 4.31 (2H, H5'guanosine), 4.10 (1H, m, CHα), 1.61 (3H, s, CH$_3$-isopropylidene), 1.53 (3H, s, CH$_3$-isopropylidene), 1.39 (3H, d, CH$_3$-alanine, *J*= 8.4 Hz), 1.00 (3H, s, CH$_3$-2'-guanosine).

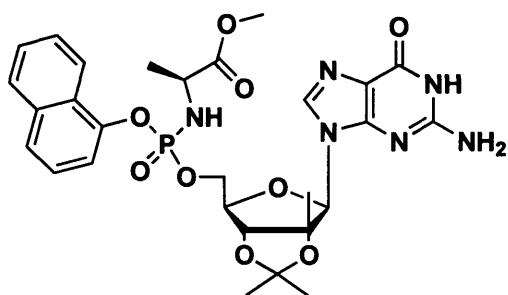## Synthesis of β-2'-methylguanosine 5'-*O*-[phenyl (benzyl-L-alaninyl)] phosphate

(238)

Prepared according to Standard Procedure C4, from 2', 3'-*O,O*-isopropylidene-β-2'-methylguanosine 5'-*O*-[phenyl(benzyl-L-alaninyl)] phosphate (40 mg, 0.061 mmol), and 10 mL of a solution 60% of CH_3COOH in water at 90° C for 15 hours. The crude product was purified by column chromatography, using as eluent for the first $CHCl_3$/MeOH (85:5) followed by a semi-preparative HPLC. The pure product was a white solid (15 mg, 40%).

δ_P (d_4-CH_3OH): 4.27, 4.10; δ_H (d_4-CH_3OH): 7.92 (1H, d, H8-guanosine, J= 8.3 Hz), 7.37-7.29 (5H, m, CH-phenyl, CH-benzyl), 7.25-7.18 (5H, m, CH-benzyl, CH-phenyl), 5.96 (1H, d, H1'-guanosine, J= 2.3 Hz), 5.15 (2H, s, CH_2-benzyl), 4.43-4.35 (2H, m, H3'-guanosine, H4'-guanosine), 4.33-4.28 (1H, m, H5'-guanosine), 4.24-4.19 (1H, m, H5'-guanosine), 4.08-3.93 (1H, m, CHα), 1.35 (3H, m, CH_3-alanine), 1.24 (3H, s, CH_3-2'-guanosine).
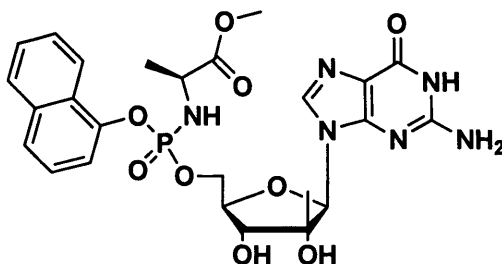
Synthesis of 2', 3'-*O,O*-isopropylidene-β-2'-methylguanosine 5'-*O*-[α-naphthyl (methyl-L-alaninyl)] phosphate

Prepared according to Standard Procedure C2, from 2', 3'-*O,O*-isopropylidene-β-2'-methyl-guanosine (**230**, 130 mg, 0.385 mmol), 'BuMgCl (0.96 mL, 1M solution in THF, 0.96 mmol) and α-naphthyl(methyl-L-alaninyl) phosphorochloridate (**231**, 0.96 mL of solution 1M in THF, 0.96 mmol). The crude product was purified by column chromatography, using as eluent $CHCl_3$/MeOH (97:3). The pure product was a white solid (26 mg, 11%).

δ_P (d_4-CH$_3$OH): 4.51, 4.45; δ_H (d_4-CH$_3$OH): 8.21 (1H, d, H8-guanosine, $J=$ 7.5 Hz), 7.91-7.89 (1H, m, CH-naphthyl), 7.73 (1H, m, CH-naphthyl), 7.58-7.53 (4H, m, CH-naphthyl), 7.48-7.45 (1H, m, CH-naphthyl), 6.09 (1H, d, H1'-guanosine, $J=$ 7.4 Hz), 4.63 (1H, d, H3'-guanosine, $J=$ 3.0 Hz), 4.57-4.53 (1H, m, H4'-guanosine), 4.43-4.41 (2H, m, H5'guanosine), 4.12-4.05 (1H, m, CHα), 3.62 (3H, d, CH$_3$-methyl, $J=$ 10.1 Hz), 1.59 (3H, d, CH$_3$-isopropylidene, $J=$ 7.9 Hz), 1.40 (3H, d, CH$_3$-alanine, $J=$ 3.4 Hz), 1.35 (3H, d, CH$_3$-isopropylidene, $J=$ 7.2 Hz), 1.05 (3H, d, CH$_3$-2'-guanosine, $J=$ 7.0 Hz).
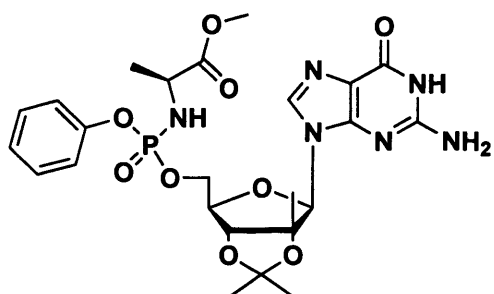
Synthesis of β-2'-methylguanosine 5'-O-[α-naphthyl(methyl-L-alaninyl)] phosphate (236)

Prepared according to Standard Procedure C4, from 2', 3'-O,O-isopropylidene-β-2'-methylguanosine 5'-O-[α-naphthyl(methyl-L-alaninyl)] phosphate (26 mg, 0.041 mmol), and 10 mL of a solution 60% of CH$_3$COOH in water at 90° C for 15 hours. The crude product was purified by column chromatography, using as eluent for the first CHCl$_3$/MeOH (92:8). The pure product was a white solid (4 mg, 17%).

δ_P (d_4-CH$_3$OH): 4.35, 4.26; δ_H (d_4-CH$_3$OH): 8.20 (1H, d, H8-guanosine, $J=$ 5.8 Hz), 7.91-7.87 (2H, m, CH-naphthyl), 7.70 (1H, m, CH-naphthyl), 7.58-7.52 (3H, m, CH-naphthyl), 7.50-7.41 (1H, m, CH-naphthyl), 5.93 (1H, s, H1'-guanosine), 4.58-4.56 (2H, m, H3'-guanosine, H4'-guanosine), 4.29-4.21 (2H, m, H5'guanosine), 4.06-4.03 (1H, m, CHα), 3.56 (3H, d, CH$_3$-methyl, $J=$ 1.7 Hz), 1.31 (3H, d, CH$_3$-alanine, $J=$ 7.4 Hz), 1.00 (3H, d, CH$_3$-2'-guanosine, $J=$ 12.4 Hz).

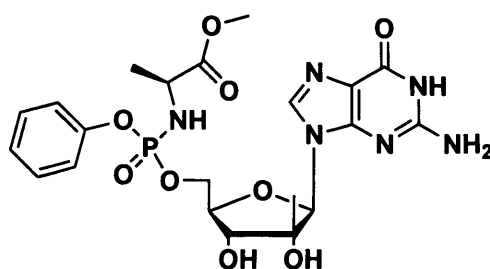## Synthesis of 2', 3'-*O,O*-isopropylidene-β-2'-methylguanosine 5'-*O*-[phenyl(methyl-L-alaninyl)] phosphate

Prepared according to Standard Procedure C2, from 2', 3'-*O,O*-isopropylidene-β-2'-methylguanosine (**230**, 140 mg, 0.415 mmol), 'BuMgCl (1.04 mL, 1M solution in THF, 1.04 mmol) and phenyl(methyl-L-alaninyl) phosphorochloridate (**128**, 1.04 mL of solution 1M in THF, 1.04 mmol). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (97:3). The pure product was a white solid (21 mg, 9%).

δ_P (d_4-CH₃OH): 4.09, 3.91; δ_H (d_4-CH₃OH): 7.88, 7.80 (1H, d, H8-guanosine), 7.41-7.35 (2H, m, CH-phenyl), 7.30-7.20 (3H, m, CH-phenyl), 6.14 (1H, d, H1'-guanosine, J= 11.8 Hz), 4.69 (1H, d, H3'-guanosine, J= 2.9 Hz), 4.49-4.39 (3H, m, H4'-guanosine, H5'guanosine), 4.04-3.99 (1H, m, CHα), 3.70 (3H, d, CH₃-methyl, J= 12.7 Hz), 1.63 (3H, d, CH₃-isopropylidene, J= 2.3 Hz), 1.44 (3H, d, CH₃-alanine, J= 3.1 Hz), 1.41 (3H, d, CH₃-isopropylidene, J= 6.8 Hz), 1.10 (3H, d, CH₃-2'-guanosine, J= 6.5 Hz).

Synthesis of β-2'-methylguanosine 5'-*O*-[phenyl(methyl-L-alaninyl)] phosphate

(239)

Prepared according to Standard Procedure C4, from 2', 3'-*O,O*-isopropylidene-β-2'-methylguanosine 5'-*O*-[phenyl(methyl-L-alaninyl)] phosphate (21 mg, 0.036 mmol), and 10 mL of a solution 60% of CH₃COOH in water at 90° C for 15 hours. The crude product was purified by column chromatography, using as eluent for the first CHCl₃/MeOH (92:8). The pure product was a white solid (7 mg, 36%).

δ_P (d_4-CH₃OH): 4.15, 3.90; δ_H (d_4-CH₃OH): 8.96 (1H, br, H8-guanosine), 7.40-7.35 (2H, m, CH-phenyl), 7.29-7.20 (3H, m, CH-phenyl), 6.08 (1H, d, H1'-guanosine, J=

8.5 Hz), 4.55-4.49 (2H, m, H3'-guanosine, H4'-guanosine), 4.26 (1H, m, H5'guanosine), 4.17-4.11 (1H, m, H5'-guanosine), 4.00-3.97 (1H, m, CHα), 3.73 (3H, d, CH_3-methyl, $J=$ 11.1 Hz), 1.36 (3H, d, CH_3-alanine, $J=$ 7.1 Hz), 1.14 (3H, d, CH_3-2'-guanosine, $J=$ 4.9 Hz).

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