Synthesis of Prolinol-Based Phosphonate Nucleotide Analogues

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Ph.D. Thesis

Prague 2008
Acknowledgement

I would like to express my gratitude to my supervisor dr. Ivan Rosenberg for his support and valuable discussions and especially for teaching me the practice of work in the field of organic chemistry. I owe my special thanks to dr. Miloš Buděšinský for the NMR measurements and spectra interpretation. I also want to thank the staff of the Mass Spectrometry department for providing the analysis.

Last but not least, I would like to thank all the colleagues and friends from the Department of Oligonucleotides, Institute of Organic Chemistry and Biochemistry for great social background, and all those who have supported me over the past few years and contributed towards shaping this thesis.

The financial support by MŠMT Research Centers LC 06061 and LC 06077 is gratefully acknowledged.
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<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>A</td>
<td>Adenin-9-yl</td>
</tr>
<tr>
<td>A&lt;sup&gt;Bz&lt;/sup&gt;</td>
<td>6-N-Benzoyladenin-9-yl</td>
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<td>Ac</td>
<td>Acetyl</td>
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<tr>
<td>APT</td>
<td>Attached proton test</td>
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<tr>
<td>AZT</td>
<td>3’-Azido-3’-deoxythymidine</td>
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<td>B</td>
<td>Nucleobase</td>
</tr>
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<td>Benzyl</td>
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<td>tert-Butyloxy carbonyl</td>
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<td>Bz</td>
<td>Benzyoyl</td>
</tr>
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<td>C</td>
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<tr>
<td>C&lt;sup&gt;O&lt;/sup&gt;</td>
<td>Cytosin-2-O-yl</td>
</tr>
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<td>COSY</td>
<td>2D NMR correlation spectroscopy</td>
</tr>
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<td>CPG</td>
<td>Controlled pore glass</td>
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<td>DAP</td>
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<td>DCC</td>
<td>Dicyclohexylcarbodiimide</td>
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<td>Diisopropyl azodicarboxylate</td>
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<td>Double-stranded DNA</td>
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<td>Fast-atom bombardment</td>
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<tr>
<td>G</td>
<td>Guanin-9-yl</td>
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<tr>
<td>HIV-1, HIV-2</td>
<td>Human immunodeficiency virus type 1 and 2</td>
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<tr>
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</tr>
<tr>
<td>HVB</td>
<td>Hepatitis virus B</td>
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<tr>
<td>iPr</td>
<td>Isopropyl</td>
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<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
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<tr>
<td>$J$</td>
<td>Coupling constant</td>
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<td>Matrix-Assisted Laser Desorption/Ionization Time-of-Flight</td>
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<td>Me</td>
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<td>MCPBA</td>
<td><em>meta</em>-Chloroperbenzoic acid (3-Chloroperoxybenzoic acid)</td>
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</tr>
<tr>
<td>NOE</td>
<td>Nuclear Overhauser effect</td>
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<tr>
<td>NPA</td>
<td>Nucleoside phosphonic acid</td>
</tr>
<tr>
<td>Ph</td>
<td>Phenyl</td>
</tr>
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<td>PMEA</td>
<td>9-(2-Phosphonylmethoxyethyl)-adenine</td>
</tr>
<tr>
<td>PMPA</td>
<td>(1R)-9-(2-Phosphonylmethoxypropyl)-adenine</td>
</tr>
<tr>
<td>PNA</td>
<td>Peptide nucleic acid</td>
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<tr>
<td>PNBA</td>
<td><em>para</em>-Nitrobenzoic acid (4-Nitrobenzoic acid)</td>
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<td>Pyridine</td>
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<td>Alkyl</td>
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<td>Ribonucleic acid</td>
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<tr>
<td>TEAB</td>
<td>Triethylammonium bicarbonate</td>
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<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
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<td>Tetrahydrofurane</td>
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<td>2,4,6-Triisopropylbenzenesulfonyl chloride</td>
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<td>TLC</td>
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<td>Trimethylsilyl</td>
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<tr>
<td>TP</td>
<td>Thymidine phosphorylase</td>
</tr>
<tr>
<td>U</td>
<td>Uracil-1-yl</td>
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<tr>
<td>Z</td>
<td>Benzyloxy carbonyl</td>
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1. Introduction

Nucleic acids represent an essential part of biological systems. They are fundamental to the storage, transmission, and translation of genetic information. No wonder that such compounds and their analogues have attracted growing interest of scientists from many different fields in the last few decades. Today, several nucleic acids derivatives (e.g. nucleotide analogues and oligonucleotides) can be found among the most potent antivirotics, cytostatics, and antimicrobiotics.

Unlike classical drugs, oligonucleotides can be used directly in the sequence specific control of gene expression and have therefore immense potential as therapeutical agents.\textsuperscript{1}

The main principle mode of action of these oligonucleotides is through the binding, \textit{via} Watson and Crick base pairing, to a specific mRNA sequence associated with a diseased state and the subsequent inhibition the translational event leading to a detrimental protein (\textit{antisense}).\textsuperscript{2-4} Once bound to the target RNA, the antisense agent either sterically blocks the synthesis of ribosomal proteins or induces RNase H-mediated degradation of the target mRNA.\textsuperscript{5,6}

In addition, transcription can be inhibited by the binding of an oligonucleotide to duplex DNA, principally through Hoogsteen base pairing and the formation of a triple helix, or strand displacement (\textit{antigene}) (Figure 1).\textsuperscript{7}

However, unmodified oligodeoxynucleotides are rapidly degraded in a biological environment and, therefore, of limited use. Chemical modification leads to resistance to degradation by nucleases and, hence, increased biological activity of antisense compounds. Beside enhanced nucleolytic stability, the chemical modification of choice should possess a number of other features, such as good hybridisation properties, good synthetic accessibility, cellular uptake and induction of RNase H. This finding resulted in the generation of a large number of oligonucleotide analogs modified at (a) base, (b) sugar or (c) backbone.\textsuperscript{5}
Modified nucleobases are mostly utilized for connecting of reporters and additional functionalities. Modifications of nucleic acid backbone are used mainly in antisense therapeutics for enhancing oligonucleotide stability and binding affinity while sugar modifications in RNA and DNA conformation and stability studies.

1.1. Backbone Modification of Nucleic Acids

All therapeutic oligonucleotides must be stable to enzymatic degradation and pass efficiently through the cell membrane. Native oligonucleotides are rapidly degraded by extra and intracellular nucleases; oligodeoxynucleotides mainly by exonucleases, with 3’–exonuclease degradation occurring most rapidly. For this reason backbone modification strategies were first introduced.

Increased stability toward nuclease digestion has been reached with every modification of the backbone (which involves replacing the phosphodiester linkage with an alternative moiety) investigated so far. Even those modifications that most closely resemble the native structures are stabilised significantly, whereas more radical backbone modifications result in complete resistance to degradation.⁷

The native phosphodiester linkage can be replaced by alternative anionic, neutral, and cationic groups.
1.1.1. Anionic Analogues

Anionic analogues were the very first modifications investigated since they resemble the native linkage most closely. The earliest example of backbone modification is the replacement of one of the non-bridging oxygen atoms in the phosphodiester group of oligodeoxyribonucleotides with a sulfur atom. This gives rise to phosphorothioate oligonucleotides (PS-oligonucleotides, Figure 2). Phosphorothioate dinucleotides and oligonucleotides have a chirality center at the phosphorus, with the negative charge being distributed unsymmetrically and located mainly on sulfur.

\[ \text{Figure 2. Phosphorothioates (1), N3'→P5' phosphoramidates (2), N5'→P3' phosphoramidates (3), boranophosphates (4), and 3'-methylenephosphonates (5).} \]

PS-oligonucleotides, which are most widely used in antisense technology, are easily synthesized and exhibit high nuclease resistance. In addition their complex with RNA target sequence (hybrid duplex) is capable to elicit RNase H cleavage activity. This has led to the extensive testing of fully modified antisense PS-oligonucleotides in various clinical trials against numerous targets and culminated in a PS-oligonucleotide called Vitravene (used in the treatment of cytomegalovirus induced retinitis in AIDS patients; the first antisense drug approved for marketing). However, PS-oligonucleotides have a number of limitations, such as unwanted physiological side effects, toxicity, cleavage of non-target mRNAs, that are only partially complementary, due to the activation of RNase H, and low affinity to RNA. In addition, each PS-oligonucleotide is theoretically a mixture of \(2^n\) diastereoisomers (\(n\) is number of PS internucleotide linkages) due to a chirality on the phosphorus atom.

The phosphoramidates are, like the thiophosphates, an easily obtainable group of nucleotide analogues with anionic backbone. Apart from neutral and cationic
phosphoramidates (see later), two different groups of anionic phosphoramidates can be distinguished; the N3’→P5’ type\(^{12,13}\) 2 and the oppositely orientated N5’→P3’ type\(^{14}\) 3, both of them achiral.

In particular, the N3’→P5’ phosphoramidates appear to be the most promising with superior qualities for binding to single and double stranded nucleic acid targets, together with an exceptional resistance toward nucleases and hydrolysis in human plasma.\(^{15}\) Moreover, the increased affinity of triple helix forming antisense oligonucleotides for double stranded DNA (dsDNA) could be exploited in antigene strategies.\(^{16}\) However, in contrast to PS-oligonucleotides, the N3’→P5’ phosphoramidate oligonucleotides fail to activate RNase H when bound to complementary RNA.\(^{17}\)

Boranophosphate oligonucleotides\(^{18-21}\) 4 isoelectronic with phosphodiester oligonucleotides seem to be therapeutically promising since in general they are more stable to various nucleases than phosphorothioate mimics\(^{22}\) and are able to activate RNase H.\(^{23,24}\) But also in this case, the chirality on the phosphorus atom gives rise to large number of diastereoisomers.

Methylene bridged phosphonates (e.g. 5) will be discussed in more detail in Chapter 1.1.4.

1.1.2. Neutral Backbone Modifications

Neutral backbone modifications were designed with intent to decrease the electrostatic repulsion forces between negatively charged strands and therefore they should increase affinity to the target nucleic acids; they represent the largest group among all internucleotide linkage-modified oligonucleotide analogues synthesized so far.\(^{7}\)

Methylphosphonates (MP-oligonucleotides, 6, Figure 3)\(^{1,3,4,11}\) are one of the earliest examples of neutral backbone analogues. In methylyphosphonates, the phosphorus hydroxy group of the phosphodiester moiety is replaced by sterically undemanding methyl group; this change creates (like in phosphorothioates and boranophosphates) a chiral center at a phosphorus atom. The methylphosphonates are highly resistant toward nucleolytic enzyme degradation and are easily available. However, they suffer from several disadvantages, especially poor solubility in aqueous solutions, inability to activate RNase H, and low affinity for native nucleic acids in comparison with native oligonucleotides. MP-oligonucleotides were amongst the first modified oligonucleotides tested as antisense
agents in vitro and in vivo against targets such as herpes simplex virus (HSV) and vasicular stromatitits virus (VSV).25,26

![Chemical structures](image)

6 $X = O; Y = O; Z = CH_3$

7 $X = O; Y = O; Z = NH_2$

**Figure 3.** Methylphosphonates (6), neutral phosphoramidates (7), methylene(methylimino) (8), carbonate (9) and carboxymethyl ester (10) linkages.

Among numerous neutral phosphodiester replacements reported to date, some other examples will be briefly mentioned:

Neutral phosphoramidates$^{27,28}$ (7), with a chiral center at phosphorus, are of somewhat less importance than acidic phosphoramidates referred to earlier, as well as the phosphate triester linkers$^{29}$, e.g. bearing the OMe group attached to the phosphorus atom; the latter are similar to methylphosphonates.

Methylene(methylimino) linkages$^{30}$ (8) showed several promising results in antisense technology close to phosphorothioates.

Compounds with carbonate$^{31}$ (9), carboxymethyl esters$^{32,33}$ (10), siloxane$^{34,35}$ (11, Figure 4) and formacetal$^{36,37}$ (12) or thioformacetal$^{38,39}$ (13) bridges although often bearing interesting properties are mostly limited by their poorer acidobasic stability, shorter half-life *in vivo* and/or solubility problems.$^{7,11}$
A lot of other neutral and achiral backbone replacements differing more distinctively from the native phosphodiester were investigated, especially in order to avoid the phosphorus chirality problem. Amide linkages\textsuperscript{40,41} (14, 15) showed hybridisation properties and duplex stability comparable with native structures but, due to the synthetic difficulties during oligomer synthesis, not much biological effect have been reported yet.

Acetamidate bridges\textsuperscript{42,43} (16, Figure 5) can be derived from carboxymethyl esters by replacing the bridge ester oxygen by amide bond. They possess greater pH stability but their stability in water and hybridization characteristics were found insufficient.
From many replacements of phosphodiester group with neutral, isosteric and isoelectronic sulfonyl-based (-SO\(_2\)-) groups, only sulfamates (3’-N-sulfamates\(^{45,46}\) in particular, 18) were reported in binding studies to have approximately the same affinity as native DNA.\(^7\)

1.1.3. Positively Charged Backbones

Positively charged backbones were supposed to reduce the electrostatic repulsion between nucleic acid strands and therefore improve the formation of duplexes and triplexes with complementary sequences. Indeed, most of the cationic backbones prepared so far proved extremely strong binding properties. However, formation and strength of the complexes often depends strongly on the concentration of salts which suggests that binding is mainly contributed by electrostatic attraction between the oppositely charged backbones. This non-specific mode of binding (via salt bridges, including the direct contact between backbones) could be deleterious for antigen and antisense applications. In contrary, studies of the complex stability revealed that base pair mismatches decrease the complex dissociation temperature (\(T_m\)) significantly. The contribution of the both binding principles and the sequence specificity of the particular backbone cationic replacements requires further investigation.\(^7\)

![Figure 6. Aminoethylphosphonates (19), N,N-diethylethylenediamine phosphoramidates (20), and dimethylaminopropylene phosphoramidates (21).](image)

Cationic phosphoramidates bearing pendant amino side chains were probably the first cationic backbone replacements protonated at physiological pH. Typical examples of these positively charged backbones with chirality at phosphorus atom are aminoethylphosphonates\(^{47}\) (19, Figure 6), N,N-diethylethylenediamine (DEED) phosphoramidates\(^{48,49}\) (20), or dimethylaminopropylene phosphoramidates\(^{50}\) (21).
Interestingly, only a few *de novo* modified types of cationic backbones have been published to date. Besides several tertiary amine backbones\(^i\) (22, 23, Figure 7), replacement of the phosphodiester group by a positively charged guanidinium group led to the remarkable deoxyribonucleic guanidine (DNG) oligomers\(^52-54\) (24).

![Figure 7. Tertiary amine backbone (22 and 23), guanidine (24), and methylthiourea (25) linkages.](image)

DNG oligomers form duplexes and triplexes with both DNA and RNA whereas the duplexes, at low salt concentration, are stable even at temperatures reaching the boiling point of water. The solid-phase synthesis of DNG oligonucleotides was successfully worked up\(^55\) and recent findings suggested a possible utilization of DNG chimeras in anticancer therapy\(^56\).

In addition, several interesting cationic backbones containing methylated thiourea linkage were developed (25). These methylthiourea oligonucleotides\(^57,58\) (DNmt) form, similarly to DNG, duplexes and triplexes with DNA or RNA which are only slightly less stable than DNG, and much more stable than native structures; the DNmt oligomers are also more hydrophobic than DNG\(^7\).

### 1.1.4. Methylene Bridged Phosphonates

Methylene bridged phosphonates are derived from phosphodiester linkage by replacing one bridging oxygen atom with a methylene group to form a -P-CH\(_2\)- bond. They belong to the group of anionic backbones mentioned earlier and are isopolar with natural phosphodiester linkage. Figure 8 gives an overview of the main phosphonate backbone types.
<table>
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<tr>
<th></th>
<th>5'-phosphonate analogues</th>
<th>3'-phosphonate analogues</th>
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<td><img src="image2.png" alt="Image" /> <strong>27</strong></td>
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<td><strong>non-isosteric analogues</strong></td>
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<td><img src="image4.png" alt="Image" /> <strong>29</strong></td>
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<tr>
<td><strong>non-isosteric analogues</strong></td>
<td><img src="image5.png" alt="Image" /> <strong>30</strong></td>
<td><img src="image6.png" alt="Image" /> <strong>31</strong></td>
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</table>

**Figure 8.** Anionic phosphonate backbones.

The analogues 26 and 27 are isosteric (of the same size and shape) with the natural phosphodiester linkage\textsuperscript{59}, while linkages in compounds 28-31 differ in one atom in length. They generally exhibit good to excellent stability toward hydrolysis and enzymatic cleavage.
The isosteric oligomers of the general formula 26 (5’-phosphonates) and 27 (3’-phosphonates) were first obtained by the group of Moffatt as dimers (32-33, Figure 9).

![Figure 9](image)

32a B = uracil  
32b B = adenine  
33a B = uracil  
33b B = adenine

However, synthesis of such analogues did not receive much attention for the next 20 years, probably due to some inconveniences in their preparation.

Breaker et al. incorporated 5’-phosphonated adenosine into adenosine oligomers by enzyme catalyzed polymerization and investigated their properties with respect to the potential utilization in antisense technologies. Caruthers and Böhringer prepared 5’-deoxy-5’-methylphosphonate linked thymidine dinucleotide; its 5’-phosphoramidite was used to synthesize oligonucleotides. Oivanen et al. studied kinetics of mutual isomerization of the 5’-phosphonate analogues of dinucleoside 2,5- and 3,5-monophosphates in aqueous solution and, finally, Stawinski and Szabo developed an efficient synthesis of 5’-deoxy-5’-methylphosphonate linked thymidine dimer and carried out a conformational analysis of the sugar units.

Backbone analogues containing the isosteric 3’-methylphosphonate moiety were given even less attention than the 5’-methylphosphonates. Again, the reason could have been, at least to some extent, the lack of an efficient synthesis methodology.

Besides the dimers 33a and 33b reported in the early work by Moffatt et al., a synthesis of 3’-methylphosphonate analogue of UpUpU was published in 1984. Later, Collingwood reported the preparation of monomers for solid phase synthesis of modified DNA. Finally, in 2002, Winqvist and Strömberg developed an efficient route to 3’-methylphosphonate oligomers by oxidation of the corresponding protected methylenephosphinates.
The nonisosteric “short” analogues (linkage in structures 28 and 29) differ from the natural molecules simply by noninclusion of the phosphate ester oxygen (despite the linkage type 29 does not contain the -P-CH₂- moiety, it should be logically considered a member of this group).

The first compound with internucleotide linkage containing 5’-phosphonate moiety was a UpU analogue synthesized by Holý et al.⁶⁸; it was incorporated into trimers and used in biological study. Rammler (1972)⁶⁹ investigated the capacity of 5’-deoxythymidine-5’-phosphonate trimers to inhibit the hydrolysis of DNA by Micrococcal nuclease. Despite the promising results, there were no works published concerning 5’-phosphonate analogues till 2006, when Lönnberg et al.⁷⁰ reported a study of isomerization of the internucleosidic 3’-O-P-CH₂-5’ phosphonate linkages to their 2’,5’ counterparts over a wide pH-range.

The nucleoside 3’-phosphonic acid derivatives have been prepared only as monomers⁷¹,⁷² so far; no oligomers of this type (29) have been reported to date.

Phosphodiester bond analogues with methylene group inserted between the phosphorus atom and ester oxygen atom (30 and 31) were first reported in 1987 by Rosenberg and co-workers⁷³ as dimers in ribo-series and their stability toward nucleolytic cleavage was investigated. Later, structural and conformational properties of these dimers (diribonucleoside monophosphates) were studied by Raman spectroscopy⁷⁴ and by NMR techniques.⁷⁵ Pressová⁷⁶ prepared ApA analogues containing both modifications and tested their ability to form complexes with polyU.

 Whereas the number of works concerning oligonucleotide phosphonate mimics, despite their promising properties, is still quite low, the research in the field of phosphonate nucleotide analogues (nucleoside phosphonic acids) has been very intensive during the last decades (see the next section).
1.2. Phosphonate nucleotide analogues

Modified nucleosides are widely used in medical practice as antivirotics (AIDS therapy) and as anticancer drugs. To become active, these nucleosides must be successively phosphorylated by cellular kinases to give the corresponding 5’-mono-, -di-, and –triphosphates; and, therefore, may be considered as prodrugs. The phosphorylation suffers from extremely low efficiency (e.g., 0.3 % for AZT). Thus, many efforts have been made to improve the therapeutic properties by shortening the cascade and bypassing at least the first phosphorylation step. This approach led to the synthesis of numerous nucleotide analogues.77-79

Phosphonate analogues which mimic nucleoside monophosphates (nucleoside phosphonic acids, NPAs) do not require the first intracellular phosphorylation step necessary for activation of nucleosides. Moreover, they are not cleaved by nucleases and have therefore an advantage of being metabolically stable; furthermore, the increase of lipophilicity enhances the cellular uptake.

The amount of phosphonate nucleotide analogues developed so far and their structural diversity is so large that it is beyond the scope of this work to review the entire group. The reader is referred to several other detailed works and reviews.79-81

Herein, only examples of the the most important nucleotide phosphonate antivirotics will be given, and a review of the azacyclic phosphonate analogues.

1.2.1 Nucleoside Phosphonate Antivirotics

The 3’-azido-3’-deoxythymidine 5’-H-phosphonate was the first phosphonated cyclic nucleoside analogue for treatment of HIV-infected patients (Nikavir™, approved in Russia in 1999; see Figure 10).

![Figure 10](image-url)
However, much greater attention has been given to acyclic nucleotide analogues. Several acyclic nucleoside phosphonate acids, e.g. HPMPC (cidofovir), PMEA (adefovir), PMPA (tenofovir), PMEG, or PMEDAP, were found to be very potent antivirus in the treatment of HIV, hepatitis B and CMV-induced retinitis (Figure 11). Recently GILEAD reported highly promising preclinical results of $N^6$-cyclopropyl-PMEDAP derivative GS-9219 (a PMEG prodrug) against leukaemia and non-Hodgkin’s lymphoma.

![Structures of some significant acyclic NPAs.](image)

**Figure 11.** Structures of some significant acyclic NPAs.

### 1.2.2. Phosphonate Nucleotide Analogue Containing Aza-Sugar Ring

Up to date, only few aza-sugar nucleotide phosphonates were reported. Harnden et al. prepared a series of phosphonomethoxy derivatives (Figure 12) containing a pyrrolidine ring linked to the base via the heteroatom, which exerted weak antiviral properties. Isoxazolidine phosphonates reported by Adams et al., bearing phosphonomethyl moiety attached to the ring nitrogen atom, showed no anti-HIV1 activity. In contrast, similar isoxazolidine nucleotide analogues (PCOANs, phosphonated carbocyclic 2'-oxa-3'-azanucleosides) developed by Chiacchio et al. with phosphonomethyl moiety joined to the 4'-carbon atom exhibited significant inhibition of
reverse transcriptase comparable to AZT in efficiency, as well as low levels of cytotoxicity. However, an attempt to obtain more potent inhibitors 37a-c by inserting a second methylene group into a 4'-carbon and phosphonate group linker (to form an isoster of natural 5’-phosphate) led to the complete loss of antiviral activity. Recently, extending the former series brought compounds 34d-g which gave accordingly good bioactivity results.

Even fewer aza-sugar phosphonate analogues containing other than five-membered ring is known to date. Sheikha et al. synthesized a series of aziridine N-methylphosphonate nucleotides 38a-d, which were proposed as conformationally constrained analogues of well-known phosphonate antivirals of PMPA type; none of these compounds showed antiviral or antimicrobial activity. Finally, Chakhmakhcheva et al. prepared morpholine-based monomers 39a-b suitable for solid-phase synthesis of oligonucleotides.

\[ \text{Figure 12. Pyrrolidine, isoxazolidine, aziridine, and morpholine nucleoside phosphonate analogues.} \]
1.3. Pyrrolidine-Based Analogues

1.3.1. Pyrrolidine-Based Nucleoside Analogues

The first reported nucleoside analogues bearing nucleobase attached to the pyrrolidine ring were purine derivatives $40a$-$b$ prepared by Temple and co-workers\(^9\) in 1972, which were found inactive toward L1210 leukemic cells implanted in mice (Figure 13).

![Figure 13](image.png)

<table>
<thead>
<tr>
<th>R</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>$40a$</td>
<td>$CH_3$</td>
</tr>
<tr>
<td>$40b$</td>
<td>Ph$_2$CH</td>
</tr>
<tr>
<td>$41a$</td>
<td>H 6-Dimethylaminopurin-9-yl</td>
</tr>
<tr>
<td>$41b$</td>
<td>Ac 6-Dimethylaminopurin-9-yl</td>
</tr>
<tr>
<td>$42a$</td>
<td>H Adenin-9-yl</td>
</tr>
<tr>
<td>$42b$</td>
<td>H Cytosin-1-yl</td>
</tr>
<tr>
<td>$42c$</td>
<td>H Uracil-1-yl</td>
</tr>
<tr>
<td>$43$</td>
<td>H Guanin-9-yl</td>
</tr>
<tr>
<td>$45$</td>
<td>H 5-Ethyluracil-1-yl</td>
</tr>
<tr>
<td>$43a$</td>
<td>H Thymin-1-yl</td>
</tr>
<tr>
<td>$43b$</td>
<td>OH Thymin-1-yl</td>
</tr>
<tr>
<td>$44a$</td>
<td>H Adenin-9-yl</td>
</tr>
<tr>
<td>$44b$</td>
<td>H Inosin-9-yl</td>
</tr>
<tr>
<td>$44c$</td>
<td>H 2,6-Diaminopurin-9-yl</td>
</tr>
<tr>
<td>$44d$</td>
<td>H Guanin-9-yl</td>
</tr>
<tr>
<td>$46a$</td>
<td>Uracil-1-yl</td>
</tr>
<tr>
<td>$46b$</td>
<td>Thymin-1-yl</td>
</tr>
<tr>
<td>$46c$</td>
<td>Cytosin-1-yl</td>
</tr>
</tbody>
</table>

The L-prolinol derivatives $41a$-$b$ prepared in 1974 by Kaspersen \textit{et al.}\(^9\) as precursors in the synthesis of puromycine analogues represent the first prolinol-based nucleoside analogues. Later, the same group synthesized several similar pyrimidine\(^5\) and purine\(^6\) derivatives $42a$-$c$. Ng and Orgel\(^7\) synthesized $43a$-$b$ from D-prolinol; these compounds inhibited the growth of breast carcinoma (MCF-7M), colon carcinoma (HT-29), and SK-MES-1 lung carcinoma cell lines.


The Harnden group reported\(^9\) unusual nucleoside analogues $46a$-$c$ bearing nucleobase connected to the nitrogen atom of a pyrrolidine ring.

Westwood\(^10\) published the synthesis of L-prolinol-based nucleoside $47$ and its several derivatives.
Pyrrolidine analogues of oxetanocin A 48a-b prepared by Oohashi et al.\textsuperscript{101} as possible antivirotics against HSV-1, HSV-2, and HIV-1 were found completely inactive. From 5'-fluorouracil pyrrolidine derivatives 49a-d screened for antitumor properties, only 49b showed significant activity (Figure 14).\textsuperscript{102} Altman\textsuperscript{103} reported synthesis of compounds 50a-b, and Varaprasad\textsuperscript{104} 51a-e starting from L-lyxose. Rassu\textsuperscript{105} synthesized nucleoside analogues 52a-c; they showed no biological activity against HIV and HVB replication in vitro. Costenaro\textsuperscript{106} reported the synthesis of analogues of antiviriotics stavudine and dideoxyuridine: aza-stavudine (aza-D4T) and aza-2',3'-didehydro-3'-deoxyuridine (aza-D4U) 53a-b (Figure 15). Richichi et al.\textsuperscript{107} utilized Mitsunobu reaction to obtain 3',4'-pyrrolidine nucleosides 54a-b and Miyabe\textsuperscript{108} prepared similar compounds 55a-c.

Recently, Kočalka\textsuperscript{109} and Rejman\textsuperscript{110} reported the synthesis of both racemic and optically active pyrrolidine nucleoside analogues 56a-f and 57a-b. All compounds were tested for cytostatic and antiviral properties but no significant activity was found.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure14.png}
\caption{Figure 14.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure15.png}
\caption{Figure 15.}
\end{figure}
1.3.2. Nucleotide Analogues Containing Pyrrolidine Ring

The only pyrrolidine-based phosphonate nucleotide analogues reported so far are derivatives prepared by Harnden et al.\textsuperscript{86} 34a-e (Figure 12) mentioned earlier in Chapter 1.2.2.

1.3.3. Pyrrolidine-Based Modified Oligonucleotides

Pyrrolidine ring was first introduced into the modified oligonucleotides within the context of peptide nucleic acids research.

Peptide nucleic acids (PNA, Figure 16) are DNA mimics with an achiral pseudopeptide (polyamide) backbone. PNA is an extremely good structural mimic of DNA (or RNA), and PNA oligomers are able to form very stable duplex structures with Watson-Crick complementary DNA, RNA (or PNA) oligomers, and they can also bind to targets in duplex DNA by helix invasion; PNAs bind with higher affinity to complementary nucleic acids than their natural counterparts. Unique is the ability of PNAs to displace one strand of a DNA double-helix, an inefficient process with natural oligonucleotides.\textsuperscript{111-113}

![Figure 16. Peptide nucleic acid.](image)

In search for PNAs with improved water solubility and some other properties, introduction of the pyrrolidine ring has proved advantageous. Pyrrolidine containing PNAs are, in comparison, more rigid and can be obtained with fixed chirality.

Pyrrolidine-based homo-pyrimidine oligomer \textsuperscript{58} (Figure 17) was found to hybridize with complementary RNA sequences only.\textsuperscript{114} Positively charged pyrrolidine monomers \textsuperscript{59} were incorporated into PNA in order to reduce its flexibility.\textsuperscript{115} Oligomers containing modified type \textsuperscript{60} monomers showed good water solubility but destabilized the complexes with RNA,\textsuperscript{116} as well as type \textsuperscript{61} monomers; in addition, no binding to the complementary
DNA targets was detected. On the contrary, pyrrolidine-based pentamers 62-64 exerted higher affinity toward unmodified RNA and DNA oligomers than native structures. 118-120

![Chemical structures](image)

**Figure 17.**

The DNA repair enzyme DNA glycosylase II (AlkA) was strongly inhibited by short pyrrolidine-containing oligonucleotide (65, Figure 18) developed by Schärer; later, specific inhibitors of a variety of additional DNA glycosylases were prepared using the same motif. Proline nucleoside 66 was used in the synthesis of oligopeptide sequence-specific dsDNA binding ligands with pyrimidine specificity.

Pyrrolidine monomer bearing pyrene moiety 67 prepared by Prokhorenko et al. was used in the synthesis of oligomers in order to investigate the properties of oligonucleotide conjugates via fluorescent emission characteristics. Similar phosphoramidite hydroxyproline-based monomers were prepared for the automated synthesis of oligonucleotides type 68. A series of closely related phosphonate oligomers was prepared by the Efimov group; several other examples of hydroxyproline-based DNA mimics are discussed in a recently published review.
Figure 18.
2. Specific Aims and Synthetic Strategies

The specific aims of the Thesis are as follows:

1. Development of synthesis of nucleotide analogues related to α-L- and β-L-2′-deoxynucleoside 3′-phosphate, containing prolinol moiety instead of the pentofuranosyl sugar residue, in which the 4′-oxygen atom is replaced by methylene group and the pyrrolidine nitrogen atom is located in place of the 3′-sugar carbon atom.

2. Development of universal synthetic route to all diastereoisomeric O-protected 4′-mesyloxyprolinol-N-phosphonate precursors of nucleotide analogues mentioned above.

3. Examination of constraining the conformational flexibility of the N-phosphonomethyl moiety by N-oxidation or N-methylation of the ring nitrogen atom.


5. Development of synthesis of suitably protected nucleotide monomers for solid phase synthesis of oligonucleotides thereof.
3. Results and Discussion

3.1. Synthesis of 1,4-Pyrrolidine Nucleotide Phosphonates

Herein, the synthesis of nucleotide analogues related to α-L- and β-L-2′-deoxynucleoside 3′-phosphate, containing prolinol moiety instead of the pentofuranosyl sugar residue, in which the 4′-oxygen atom is replaced by methylene group and the pyrrolidine nitrogen atom is located in place of the 3′-sugar carbon atom is reported (Figure 19). Looking these compounds as aza-pentofuranosyl derivatives, it can be also considered the pair of pyrrolidine nucleotides differing in the chirality on C4′ as the α- and β-anomers. The presence of nitrogen atom at the 3′-position leads to the loss of unambiguously defined configuration at this centre and thus, the N-phosphonomethyl moiety could adopt cis or trans orientation to any of both stereogenic centres. It is obvious that the 3′-nitrogen moiety, as a tertiary amine, will be protonated in a large extent of pH values due to the presence of the acidic phosphorus moiety. In this case the formed chiral protonated form may be stabilized via intramolecular hydrogen bridge (Figure 19).

![Diagram of α-L and β-L nucleoside 3'-phosphate structures](Figure 19)
For the synthesis of all prolinol-based nucleoside phosphonates the commercially available trans-4-hydroxy-L-proline (L-69) served as a convenient starting material (Scheme 1). The initial steps essentially follow methods previously described by Ceulemans et al. \(^{129}\) Inversion of configuration at C(2) of L-69 afforded cis-4-hydroxy-D-proline D-69 in high yield. The epimerisation of L-69 with acetic anhydride (first described by Robinson et al., \(^{130}\) total yield 42 %, later improved by Baker et al., \(^{131}\) total yield 57 %) was carried out as one-pot reaction according to Verheijen et al. \(^{132}\) (yield only 27 %); slightly modified method of final crystallisation gave pure D-69 in 70% yield which is close to results of detailed study published recently. \(^{133}\) Both L- and D-hydroxyprolines were protected on nitrogen atom with benzyloxy carbonyl group providing carbamates L-70 (L-) and D-70 (D-), and these compounds were then converted to their respective methyl esters L-71 and D-71 in quantitative yield by direct introduction of gaseous diazomethane into the ethanolic solution of L-70 or D-70 at low temperature. Reduction of methyl ester to alcohol proceeded smoothly by treatment with NaBH\(_4\) in ethanol, giving 4-hydroxyprolinol derivatives L-72 \(^{134}\) and D-72. The use of NaBH\(_4\)/EtOH (yields around 90%) instead of LiBH\(_4\)/THF (reported yields 90-97\% \(^{129,134,135}\)) caused only small decrease of yield of the reduction but lowered the cost essentially. Removal of the Z protecting group by catalytic hydrogenolysis afforded the 4-hydroxyprolinols L-73 and D-73. The N-phosphonomethyl moiety was introduced into the compounds via Kabachnik-Fields reaction, following the general method described by Fields. \(^{136}\) Prolinols L-73 and D-73 were treated with aqueous formaldehyde followed by adding the diisopropyl phosphonate. At first, the reaction was performed in different solvents (acetonitrile, methanol, isopropanol) but it demanded prolonged heating and the yields of these reactions were low and irregular in most cases. Exclusion of the solvent (in fact its replacement with excess of diisopropyl H-phosphonate) led to the exothermic reaction providing significantly increased yields of phosphonates L-74 and D-74. Primary hydroxyl of these phosphonates was protected with dimethoxytrityl group to form L-75 and D-75, and the key synthons L-76 (α-L-) and D-76 (β-D-) were prepared by the reaction with methanesulfonyl chloride in CH\(_2\)Cl\(_2\)-pyridine mixture at 0 °C. Since there was an aspiration to obtain a complete set of synthons suitable for the alkylation of nucleobases, it was necessary to prepare also the two remaining enantiomeric synthons L-79 (β-L-) and D-79 (α-D-), which demanded inversion of the configuration at C4 of the pyrrolidine ring. It was accomplished by a simple nucleophilic substitution of mesyl group in L-76 and D-76 by acetate ion followed by methanolysis of acetates L-77
and D-77, which afforded enantiomeric alcohols L-78 and D-78, respectively. Finally, mesylation of L-78 and D-78 gave the desired synthons L-79 and D-79.

Scheme 1. Preparation of the key synthons L-76, L-79 and D-76 and D-79.

Reagents and conditions: (i) (a) Ac₂O, AcOH, refl.; (b) HCl, refl.; (ii) BnOCOCl, NaHCO₃, H₂O-dioxane, rt.; (iii) CH₂N₂, Et₂O, 0 °C; (iv) NaBH₄, EtOH, 0-20 °C; (v) H₂, Pd/C, MeOH-EtOAc, rt; (vi) HP(O)(OiPr)₂, CH₂O aq., 60 °C; (vii) DMTrCl, Py, rt; (viii) MsCl, CH₂Cl₂, Py, 0 °C; (ix) NaOAc, DMF, 120 °C; (x) MeONa, MeOH, rt.
Protected nucleotide analogues were obtained by direct alkylation of nucleobases with mesylates (previous attempts to use alcohols L-75 and L-78 for Mitsunobu reaction failed). Only the preparation of the α-L- and β-L- series was attempted so far to prove the feasibility of the synthetic pathway; the L-series was chosen in preference because, according to the results of Ceulemans et al.\textsuperscript{128,129}, the similar N-acetyl-L-prolinol oligomers hybridized with natural nucleic acids preferably over the D-prolinol type.

The alkylation of nucleobases was performed in DMF in the presence of Cs\textsubscript{2}CO\textsubscript{3} at 100-120 °C (Schemes 2 and 3). In general, higher yields were usually achieved with higher temperature and shorter reaction time. In case of cytosine, a mixture of O-alkylated and N-alkylated nucleobase was obtained while using mesylate L-76 under conditions described above; with mesylate L-79, no N-alkylated product was isolated at all. This problem was successfully solved after changing the solvent and base from DMF/Cs\textsubscript{2}CO\textsubscript{3} to DMSO/NaH.\textsuperscript{109} Reaction of 2-amino-6-chloropurine with the mesylates L-76 and L-79 afforded intermediates 80e and 85e. The 6-chloro atom was substituted by an azido group on treatment with NaN\textsubscript{3} in DMF at 110 °C, giving the 6-azido derivatives 81 and 86, which were hydrogenated to yield the 2,6-diaminopurine compounds 82 and 87. Hydrolysis of the 6-chloropurine derivatives 80e and 85e with 80% formic acid furnished the respective guanine analogues 83 and 88.

Cleavage of the phosphonate diesters and/or dimethoxytrityl group by bromotrimethylsilane in anhydrous acetonitrile finally afforded the free nucleotide analogues 84a-89e, which were purified by reverse-phase chromatography, converted to the sodium salts via Dowex 50 (Na\textsuperscript{+}), and lyophilized from water.
Scheme 2. Reagents and conditions: (i) DMF or DMSO, Cs$_2$CO$_3$ or NaH, base, 120 °C; (ii) DMF, Cs$_2$CO$_3$, 2-amino-6-chloropurine, 110 °C; (iii) 80% HCOOH, 80 °C; (iv) BrSiMe$_3$, AcCN, rt; (v) NaN$_3$, DMF, 110 °C; (vi) Pd/C, H$_2$, MeOH/HCl, rt.
Scheme 3. Reagents and conditions: (i) DMF or DMSO, Cs$_2$CO$_3$ or NaH, base, 120 °C; (ii) DMF, Cs$_2$CO$_3$, 2-amino-6-chloropurine, 110 °C; (iii) 80% HCOOH, 80 °C; (iv) BrSiMe$_3$, AcCN, rt; (v) NaN$_3$, DMF, 110 °C; (vi) Pd/C, H$_2$, MeOH/HCl, rt.
3.2. Reactions on Tertiary Amine of Pyrrolidine Ring

In order to constrain the conformational flexibility of the \( N \)-phosphonomethyl moiety, several pyrrolidine derivatives were subjected to the \( N \)-oxidation and \( N \)-methylation resulting in \( N \)-oxides and quaternary ammonium salts, respectively.

The \( N \)-oxidation was carried out under various conditions employing most of the reasonable methods published, i.e. \( \text{H}_2\text{O}_2/\text{H}_2\text{O}, \text{H}_2\text{O}_2/\text{MeOH}, \text{H}_2\text{O}_2/\text{AcCN}, \text{H}_2\text{O}_2/\text{AcOH}, \text{SO}_3^{2-}/\text{H}_2\text{O}, \text{tBuOOH}/\text{V}_2\text{O}_5 \) \(^{137} \), \( \text{WO}_4^{2-}/\text{H}_2\text{O}_2 \) \(^{138} \), and \( \text{MCPBA}/\text{CH}_2\text{Cl}_2 \) \(^{139,140} \).

Attempted oxidation of the free nucleoside phosphonic acids 89a-89d (bearing adenine, cytosine or thymine as nucleobase) resulted in their fast decomposition, as well as the oxidation of the respective protected precursors 85a, 85b and 85d. Partial deprotection of these precursors (acidic removal of the DMTr group) afforded alcohols which proved sufficiently stable toward oxidative decomposition at rt. The reaction pathways for cytosine derivative are depicted in Scheme 4.

In general, only the reaction conditions involving oxidation by aqueous \( \text{H}_2\text{O}_2 \) and with exclusion of catalyst appeared mild enough to prevent decomposition of the starting material. Oxidation with the solution of \( \text{H}_2\text{O}_2 \) in \( \text{MeOH}/\text{H}_2\text{O} \) was chosen as the most convenient one of all the methods tested. At rt and with the concentration of \( \text{H}_2\text{O}_2 \) under 10 \% the reactions proceeded very slowly, giving only a trace of the desired product after 5 days. Increase in hydrogen peroxide concentration and/or reaction temperature led to the formation of a small peak of putative \( N \)-oxide (reaction monitored by HPLC); however, intense decomposition of the reaction mixture allways occurred allmost simultaneously not allowing the \( N \)-oxide to be isolated.

Only in the case of cytosine derivative 90, the desired product was isolated in 5\% yield after careful quenching the reaction by adding a catalytic amount of Pd/C, followed by resolution of the resulting mixture by preparative HPLC. Structure of the \( N \)-oxide 91 obtained was confirmed by MS and NMR techniques. The configuration at the nitrogen atom was determined by ROESY NMR spectra which showed \( cis \) configuration of the methylene group of the \( N \)-phosphonomethyl and \(-\text{CH}_2\text{-OH} \) moiety.

However, an attempt to convert the \( N \)-oxide to free phosphonic acid \textit{via} the standard TMSBr/AcCN deprotection led to the complete decomposition of the compound.
Scheme 4.

Quaternization of the pyrrolidine ring nitrogen was first tested using compounds L-75 (α-L-configuration) and L-78 (β-L-configuration) lacking nucleobase (Scheme 5). The reaction was accomplished by adding methylene iodide in large excess to a solution of the appropriate amine in diethyl ether. After standing overnight at rt, the products were isolated by simple evaporation of the solvent and unreacted CH₃I, and the crude methylpyrrolidinium quaternary salts were purified by column chromatography on silica.
While the β-L- derivative L-78 gave only a single product 93 with N-CH₃ cis- to CH₂-ODMTr group, the α-L- derivative L-75 furnished compound 92 which consisted, according to NMR spectra, of two epimers in the ratio 75 : 25 differing in a configuration at nitrogen atom; the configuration was determined by ROESY NMR for the major isomer as N-CH₃ cis- to CH₂-ODMTr group. The preferred orientation of the phosphonomethyl moiety can probably be explained by the strong steric hindrance of the bulky dimethoxytrityl group.

Finally, nucleotide analogue 94 bearing thymine was subjected to methylation by CH₃I under the same conditions (Scheme 6). In this case, a mixture of two epimers was obtained, with 65% majority of cis-N-CH₃ to thymine base, indicating lesser steric requirements of thymine compared to DMTr group.
An example of the ROESY NMR analysis of the compound 95 is shown on the next page (Figure 20).
Figure 20. Selected observed NOE contacts (shown with red arrows) in the mixture of isomers of compound 95.
3.3. Carboxamide-Type Phosphonates

The route towards the synthesis of amidic phosphonates was based on reaction of suitable phosphonofomrates, phosphonodithioformates, or phosphonoacetic acid with secondary amine group of pyrrolidine nucleosides. Feasibility of this approach was examined in α-D-series using thymine derivative.

At first, the 5’-OH of diol D-72 (Chapter 3.1.) was protected by DMTTr group followed by mesylation of the 1’-hydroxyl (Scheme 7). Mesylate 97 was then used for alkylation of thymine under standard conditions to give the fully protected derivative 98 in 34% yield. This result is comparable to the yield of thymine alkylation by mesylate L-79 (see Chapter 3.1.) bearing phosphonomethyl instead of benzoyloxycarbonyl group. Finally, the desired amine 99 was obtained by hydrogenolysis of 98 with Pd/C in methanol containing hydrochloric acid, with subsequent purification on Dowex 50 (H⁺) column.

![Scheme 7.](image)

The phosphonoformate (carbamoylphosphonate) was synthesized by direct amide formation from secondary amine 99 and phosphonoformic acid triester according to the method of Reetz et al.141 (Scheme 8). The appropriate phenyl-diisopropylphosphonoformate 100 was prepared following the literature procedure,142 via the Arbuzov reaction, from phenyl chloroformate and triisopropylphosphite. Whereas the Reetz group condensed the ester with only low-molecular liquid amines under solvent-free conditions which led to a strongly exothermic reaction, the pyrrolidine derivative required
heating to 80 °C in DMF. The desired amide 103 was isolated in 74% yield; two rotamers around >N-CO bond were identified in NMR spectra in the ratio approx. 79 : 21.

Thiocarbonyl analogue of the former compound was synthesized according to the work of Bulpin et al.143 by the reaction between methyl-diisopropylphosphonodithioformate and amine 99. The necessary thioester 101 was prepared using published procedure144 from diisopropyl phosphite and carbon disulfide, followed by treatment with methyl iodide. Formation of the amide 104 proceeded smoothly at 60 °C, giving the product in 55 % yield. Rotamers around >N-CS bond again were observed in NMR spectra in the ratio 60 : 40. Existence of distinct rotamers in NMR spectra of thiocarbamoylphosphonates is in accordance with published findings.143

Finally, preparation of the phosphonoacetate 105 was accomplished following procedure of Patel and co-workers.145 Direct coupling of diisopropylphosphonoacetic acid 102 with amine in DMF mediated by N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDC) and 4-dimethylaminopyridine (DMAP) proceeded at rt with an excellent yield (92 %) of the desired amide 105. NMR spectra showed two isomers around >N-CO in the ratio 77 : 23.

Despite the observation of distinct rotamers in NMR spectra of compounds 103-105, there was no indication of possible isomer resolution by TLC or column chromatography. However, the only work146 reports examples of proline-type amide rotamers which do not detectably interconvert at temperatures up to 100 °C.

The protected compounds 103-105 were converted to free phosphonic acids by TMSBr in acetonitrile. However, in contrast to deprotection of methylphosphonates (Chapter 3.1.), complete dealkylation of the -PO(OiPr)₂ group required heating to 50 °C for 48 hours. The crude phosphonic acids were purified by reversed-phase chromatography, the aqueous solution of the product was then passed through a column of Dowex 50 (Na⁺-form), and the sodium salts obtained were lyophilised from water to give the pure target compounds 106-108.
3.4. Synthesis of Dinucleotides and Oligonucleotides

To study the complexation properties of oligonucleotides containing pyrrolidine analogues, there was a need to make such oligomers available first. Two synthetic routes leading to the incorporation of the available analogues were explored: (1) preparation of simple dimers by direct coupling of suitably protected nucleotides and (2) preparation of monomers for automated oligonucleotide synthesis.

3.4.1. Preparation of Dimers

The route towards nucleotide dimers via direct condensation of suitably protected nucleotides was examined with adenine analogue from β-D-series. It was proposed to
react with natural (β-D-) deoxyadenosine which would lead to a homo-A chimeric dinucleotide.

Due to the well-known instability of deoxyribonucleosides in acids caused by facile N-glycosyl bond hydrolysis\textsuperscript{147}, it was necessary to avoid the use of acid-labile protecting groups. Thus, tert-butylphenylsilyl (TBDPS) group, which is easily cleaved by fluorides, was chosen as an alternative to DMTr for hydroxyl group protection.

The synthesis started from mesylate D-79, which was used for alkylation of adenine under standard conditions described in Chapter 3.1. DMTrO group of the resulting adenine derivative 109 was then cleaved by diluted hydrochloric acid in methanol, giving alcohol 110. Its hydroxyl function was TBDPS-protected by treatment with tert-butylphenylsilyl chloride in anhydrous pyridine. Finally, the phosphonate diester moiety was converted to free phosphonic acid 112 by action of TMSBr in acetonitrile, and the crude product was purified by flash chromatography on silica (Scheme 9).

For the preparation of dimer, a standard “diester” condensation method was employed, using DCC in anhydrous pyridine: To a solution of nucleotide analogue 112 in pyridine was added 6-N-benzoyl-3′-O-tert-butylphenylsilyl-2′-deoxyadenosine 113\textsuperscript{148} and DCC. The reaction was allowed to proceed for 5 days at rt, giving protected dimer 114 in 47%
yield. Standard basic debenzylation followed by desilylation with tetrabutylammonium fluoride afforded crude dimer 115 which was purified by reverse phase chromatography, and lyophilised from water (Scheme 10).

\[
\begin{align*}
&\text{TBDPSO} & \text{A} & \text{P(OH)}_2 & \text{O} \\
&\text{N} & \text{A} & \text{O} & \text{OTBDPS} \\
\text{112} & \text{DCC} & \text{Py, rt} & \\
&\text{HO} & \text{A}^{\text{Bz}} & \text{O} & \text{OH} \\
\text{113} & 1. \text{NH}_3, \text{EtOH, rt} & 2. \text{Bu}_4\text{NF, Py/THF, rt} & \\
&\text{TBDPSO} & \text{A} & \text{P} & \text{O} \\
&\text{N} & \text{A} & \text{O} & \text{OH} \\
\text{114} & \text{115} &
\end{align*}
\]

Scheme 10.

Hybridisation properties of the prepared ApA analogue with polyU have been studied in neutral and slightly acidic conditions. Stability of complex formed from 115 with polyU (1:2) in solution was examined under neutral (50 mM Tris-HCl, pH 7.6) and acidic (50 mM NaH$_2$PO$_4$, pH 4.15) conditions, in the presence of 10 mM MgCl$_2$ (Figure 21). It showed, in general, a low complex stability but under acidic conditions, the T$_m$ value$^a$ was remarkably higher. The value did not surpass T$_m$ of natural ApA but it is comparable to T$_m$ of d(ApA).

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$^a$ T$_m$ - The temperature at which 50% of the single strands are in duplex.
3.4.2. Preparation of Monomers for Oligonucleotide Synthesis

Preparation of monomers for automated oligonucleotide synthesis was examined with α-D- and β-D- thymine nucleoside analogues. In general, monomers for oligonucleotide synthesis must be enantiomerically pure and should possess suitable protecting groups. The most widely used protection for primary hydroxyl is dimethoxytrityl group which is cleaved under mild acidic conditions and, due to the orange colour of the DMTr cation, allows optical determination of the synthetic yields. Phosphonate is usually esterified with 4-methoxy-1-N-oxido-2-pyridylmethyl (MOP) group, which also acts as an intramolecular O-nucleophilic catalyst improving significantly the condensation yields;\textsuperscript{149} it is almost exclusively cleaved by thiophenol in Et\textsubscript{3}N/dioxane (Figure 22).

Figure 21. Melting curves of the dimer 115 at different pH.
Monomers bearing phosphonomethyl moiety (122 and 123) were synthesized starting from mesylates D-76 and D-79 which were used for thymine alkylation following the standard procedure (see Chapter 3.3.). The resulting α-D- (116) and β-D- (117) thymine derivatives were subjected to partial deprotection: Treatment with TMSBr in the presence of excessive 2,6-lutidine led to phosphonate dealkylation without a loss of DMTr group. The free phosphonic acids were esterified with 4-methoxy-1-N-oxido-2-pyridylmethanol (MOPOH) in pyridine by action of DCC, and the diesters were then partially dealkylated by treatment with a mixture of PhSH and Et3N in dioxane to afford monoesters 122 and 123. Finally, the product was converted to triethylammonium salt by partitioning between CHCl3 and aqueous TEAB (1 M), and lyophilised from dioxane (Scheme 11).
Scheme 11.

For the synthesis of phosphonoformate-type monomers 137 and 138, a route from nucleoside analogues 99 and 129 (Figure 23) was examined. The synthesis of α-D-intermediate 99 has been previously described (Chapter 3.3., Scheme 7); thus, it was necessary to prepare the epimeric β-D-derivative 129.

Figure 23.
Starting from β-D- derivative 96 (see Chapter 3.3., Scheme 7), an alternative reaction pathway to the α-D- series via direct inversion of OH at C4 by Mitsunobu reaction was tested (Scheme 12). Reaction of alcohol 96 with Ph3P, 4-nitrobenzoic acid (PNBA), 2,6-lutidine and diisopropyl azodicarboxylate (DIAD) proceeded smoothly, affording ester 125. The presence of 2,6-lutidine in this reaction protected DMTr group from the cleavage by PNBA. Methanolysis of ester 125 gave the desired alcohol 126 (59% overall yield from 96). However, the reaction mixture work-up (Ph3PO removal in particular) in larger scale was very time-consuming and tedious. Therefore, the approach was changed to simple nucleophilic substitution of mesyl group in mesylate 97 by acetate ion followed by methanolation of acetate 124, which afforded the same alcohol 126 (51% overall yield from 96).

In conclusion, the three-step route via acetate was found, despite somewhat lower yield, more convenient for large-scale synthesis than the two-step Mitsunobu inversion.

Scheme 12.

Synthetic route from 126 to the intermediate 129 basically repeated the steps used in the preparation of the epimeric amine 99 (Chapter 3.3.). Alcohol 126 was converted to mesylate 127 which was then employed in the alkylation of thymine followed by hydrogenolysis to give the desired compound 129 (Scheme 13).
The intermediates 99 and 129 were coupled with dimethyl-phenylcarbonylphosphonate in DMF at 60 °C to give crude phosphonofomates 130 and 132 (Scheme 14). Treatment with DMTrCl in pyridine did not afford the expected compounds with protected primary hydroxyl group but also caused selective demethylation of the phosphonate diester, leading directly to monomethyl esters 133 and 135. Whilst the overall yield from 129 to 135 reached 44 %, the yield from 99 to 133 was only 14 %. Thus, an alternative approach was examined for the synthesis of 133: The crude 130 was first demethylated by treatment with PhSH/Et3N in dioxane, which gave the monomethyl ester 131 in high yield. Its further reaction with DMTrCl in pyridine furnished the compound 133 in reasonable overall yield 39 %.

Esterification of 133 and 135 with 4-methoxy-1-N-oxido-2-pyridylmethanol (MOPOH) in the presence of 1-methylimidazole and 1,3,5-trisopropylbenzensulfonylchloride (TIPSCI) in acetonitrile afforded esters 134 and 136 which, after partial hydrolysis with 60% aqueous pyridine, gave the desired monomers 137 and 138. The selectivity of the hydrolysis was, however, quite low, recovering significant amount of monomethyl esters 133 or 135 besides the main product.

To sum up, the set of target monomers 122, 123, 137 and 138 was successfully prepared from commercial trans-4-hydroxy-L-proline in 12-15 steps.
Scheme 14.
3.4.3. Synthesis of Oligonucleotides

The prepared monomers 122, 123, 137 and 138 (see previous section) were utilized in the synthesis of modified oligonucleotides connected by phosphonate internucleotide bond.

The oligomers were prepared on solid support by method “trityl on” employing automated synthesizer “GENESYN”, in 0.4-1 µmol scale. The solid support was provided by long chain alkylamine (LCAA) controlled pore glass (CPG) carrying 3’-O-dimethoxytrityl-5’-O-hemisuccinyl-2-N—isobutyrylguanine linker (Figure 23) and the deoxynucleotide units were incorporated using standard amidite monomers (Figure 24).

![Figure 23.](image)

![Figure 24.](image)

The oligonucleotides were synthesized from the 5’ to 3’ end by a combination of “triester” and “amidite” method, according to the following synthetic protocol:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Reagent</th>
<th>Vol (µl)</th>
<th>Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Detritylation</td>
<td>3% CCl₃COOH in 1,2-dichloroethane</td>
<td>3000</td>
<td>120</td>
</tr>
<tr>
<td>2. Coupling</td>
<td>0.05M phosphoramidite in CH₃CN</td>
<td>75</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>0.5M tetrazol in CH₃CN</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>3. Capping</td>
<td>20% acetic anhydride in CH₃CN</td>
<td>150</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>DMAP/2,4,6-collidine/CH₃CN (6/30/70; w/v/v)</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>4. Oxidation</td>
<td>1.1 M t-BuOOH in DCM</td>
<td>250</td>
<td>90</td>
</tr>
</tbody>
</table>
**Introduction of phosphonate unit**

<table>
<thead>
<tr>
<th>Step</th>
<th>Reaction Details</th>
<th>Temperature</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Detritylation 3% CCl₃COOH in 1,2-dichloroethane</td>
<td></td>
<td>3000</td>
</tr>
<tr>
<td>2.</td>
<td>Coupling 0.05M monomer in pyridine 0.15M TIPSCl in CH₃CN 20% acetic anhydride in CH₃CN</td>
<td></td>
<td>75</td>
</tr>
<tr>
<td>3.</td>
<td>Capping DMAP/2,4,6-collidine/CH₃CN (6/30/70; w/v/v)</td>
<td></td>
<td>150</td>
</tr>
</tbody>
</table>

After the synthesis of oligonucleotide was finished, the MOP-protecting groups were removed by treatment with PhSH/Et₃N/dioxane mixture at rt for 16 h. Then was the CPG carrying oligonucleotide washed with acetonitrile, dried in a stream of argon, and heated at 55 °C for 16 h in 2 mL of 37% aqueous ammonia to remove the dibutylaminomethylene, isobutyryl, benzoyl and cyanoethyl protecting groups and to release the oligonucleotide from the CPG. Solution containing the 3'-O-DMTr protected oligonucleotide was then evaporated in the presence of TEAB (200 µl); the final cleavage of DMTr- group by aqueous TFA and purification was carried out on the reversed phase column (Luna C18 5 µm 10x50 mm), using gradient of 0.1 M TEAA in acetonitrile. Solution of the product was then evaporated in vacuo, and the remaining salts were removed by passing the solution through a column of Sephadex G-25 in 1% aqueous ammonia. The pure target oligonucleotides were lyophilized from water and stored at -20 °C.

Molecular weight of the prepared oligonucleotides **139-142** (Figure 25) was confirmed by MALDI-TOF MS. The typical yield of the condensation steps ranged between 97.1-97.9 %.

Concerning the hybridization properties of prepared oligonucleotide chimeras this study was not completed yet, and therefore, no results are given in this work.
139: 5'-d(GXG AXA XGC)-3'
140: 5'-d(GYG AYA YGC)-3'
141: 5'-d(GZG AZA ZGC)-3'
142: 5'-d(GQG AQA QGC)-3'

Figure 25. Modified oligonucleotides 139-142; compound 139 is shown in detail.
3.5. Conformational Study of 1,4-Pyrrolidine Nucleotide Phosphonates\textsuperscript{b}

NMR spectra of selected nucleosides 84a, 84c, 89a and 89c in buffered water solutions at pH 3.6, 6.6 and 11.6 showed only small changes of chemical shifts and coupling constants with pH and the preferred $N$-protonated form. Significant changes in NMR spectra were observed in still more alkaline solutions (pH ~ 12.5) obviously due to deprotonation at nitrogen atom.

The configurational assignment of methylene protons of pyrrolidine ring was determined from 2D-H,H-ROESY spectra using the observed NOE contacts with protons and substituents at positions 1 and 4 with known configuration. The preferred configuration at protonated nitrogen atom with NH proton \textit{cis}-oriented to neighbouring CH$_2$OH group was derived from NOE contacts of CH$_2$-P(O)(OH)$_2$ group and supported by the theoretical calculations.

For the conformation analysis of pyrrolidine ring in these compounds have been used a concept of pseudorotation pathway, two-state model and generalized Karplus equation for vicinal proton coupling constants (see Experimental). Similarly like it is common in proline containing compounds the greek letters are used for description of carbon atoms in pyrrolidine ring and conformation type (Figure 27).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure26.png}
\caption{Figure 26.}
\end{figure}

\textsuperscript{b} All the measurements, calculations and interpretations were kindly provided by dr. Miloš Buděšínský, to whom the autor is indebted.
Results of calculation with modified version of PSEUROT program for each nucleoside at strongly acidic and alkaline pH are summarized in table below.

<table>
<thead>
<tr>
<th>Compound</th>
<th>pH</th>
<th>Major conformer</th>
<th>Minor conformer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Phase angle P</td>
<td>Max. pucker Φmax</td>
</tr>
<tr>
<td>89a (B = A)</td>
<td>3.60</td>
<td>270 (αE)</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>12.65</td>
<td>246 (γTδ)</td>
<td>42</td>
</tr>
<tr>
<td>89c (B = T)</td>
<td>3.60</td>
<td>282 (αTδ)</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>12.45</td>
<td>279 (αE ⇔ βTδ)</td>
<td>39</td>
</tr>
<tr>
<td>84a (B = A)</td>
<td>3.60</td>
<td>261 (γTδ) ⇔ γE</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>12.66</td>
<td>249 (γTδ)</td>
<td>42</td>
</tr>
<tr>
<td>84c (B = T)</td>
<td>3.60</td>
<td>258 (γTδ)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>12.47</td>
<td>252 (γTδ)</td>
<td>39</td>
</tr>
</tbody>
</table>

The major calculated conformers for nucleotides 84a, 84c, 89a and 89c appear in a small range of 36° (P = 246-282°) that cover the conformation types NTδ, NE and αTδ. For β-L type nucleotides 89a and 89c only conformers of this type are present in water solution while in α-L type nucleotides 84a and 84c a smaller amount (5-35 %) of minor conformer of the type γE seems to be present in the fast conformation equilibrium. Selected representative types of calculated conformers are shown in Figure 28.
The results show clearly remarkable conformational differences between $\alpha$-L- and $\beta$-L-prolinol nucleoside phosphonic acids. In case of $\beta$-L-nucleotides the mutual position of nucleobase and phosphonomethyl moiety is trans, whereas the $\alpha$-L-nucleotides occurs in cis conformation. It suggests some similarity of $\alpha$-L- and $\beta$-L-prolinol nucleoside phosphonic acids to $\text{D}$-nucleoside 5'-phosphates and $\text{L}$-nucleoside 3'-phosphates, respectively.

Figure 28. Schematic representation of selected calculated types of preferred conformers in nucleotides $84\text{a}$, $84\text{c}$, $89\text{a}$ and $89\text{c}$. 
Figure 29. Calculated dependence (Hyperchem MM+) of potential energy on the phase angle of pyrrolidine ring (for max. pucker 40°).
3.6. Biological Activity Screenings\textsuperscript{c}

The cytostatic activity of analogues \textsuperscript{84a-e} and \textsuperscript{89a-e} was examined on L1210, L929, and HeLa S3 cell lines. The antiviral activity against DNA viruses was evaluated using infected E6SM, HeLa, and Vero cell cultures. No significant activity was found.

Thymine derivatives \textsuperscript{84c, 89c, 99, 106-108}, and \textsuperscript{129} were tested as potential inhibitors of SD-lymphoma thymidine phosphorylase (TP) and human recombinant TP (purchased from Fluca); the compounds \textsuperscript{89c} and \textsuperscript{106} showed very promising results (Figure \textsuperscript{30 and 31}). The biological activity of the compounds will be further investigated.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{inhibition.png}
\caption{Inhibition of thymidine phosphorylase by compounds \textsuperscript{106} and \textsuperscript{89c}.}
\end{figure}

\textsuperscript{c} The author is grateful to dr. Ivan Votruba and dr. Natalya Panova for providing the biological activity screenings.
Figure 31. Competitive inhibition of human recombinant TP by 89e.
4. Conclusion

A series of novel isosteric 3’-nucleotide analogues (84a-e and 89a-e) was synthesized, \(\alpha\)-L- and \(\beta\)-L-prolinol nucleoside \(N\)-methylphosphonic acids distinguished for the loss of unambiguously defined configuration at the nitrogen atom in 3’-position of prolinol ring. Remarkable conformational differences between \(\alpha\)-L- and \(\beta\)-L-prolinol nucleotides determined by NMR study suggest some similarity with the natural 5’-D-nucleotide. The same conformational changes in D-series of prolinol nucleotides fit even better the 3’- and 5’-D-nucleotides.

In addition, a series of four diastereoisomeric synthons L-76, D-76, L-79 and D-79 was prepared from the commercially available \textit{trans}-4-hydroxy-L-proline, giving access to a complete set of prolinol-derived nucleotide analogues bearing \(\alpha\)-L-, \(\beta\)-L-, \(\alpha\)-D- and \(\beta\)-D-configuration.

In order to constrain the conformational flexibility of the \(N\)-phosphonomethyl moiety, several protected compounds were subjected to \(N\)-oxidation or \(N\)-methylation which gave chiral \(N\)-oxides or quaternary ammonium salts. However, the synthesis of the respective unprotected phosphonic acids was unsuccessful, probably due to their fast decomposition.

Acylation of the pyrrolidine ring nitrogen atom by several acylphosphonic acid derivatives led to the novel \(N\)-phosphonoformyl, \(N\)-phosphonoacetyl and \(N\)-phosphonothioformyl nucleotide analogues 106-108 with interesting physical properties and biological activity.

The homo-A chimeric dimer 115 was prepared by direct synthesis in solution; thermal stability of its complex with polyU was found to be identical to that of natural one. Therefore, the synthetic routes to the thymine-containing \(N\)-phosphonomethyl and \(N\)-phosphonoformyl monomers 122, 123, 137 and 138 were developed for the synthesis of longer oligonucleotides. The monomers were successfully incorporated using phosphotriester method on solid support into short DNAs (9-mers) giving rise to oligomers 139-142, the hybridization properties of which are being studied.

Most of the target compounds were tested for cytostatic activity; the compounds 89c and 106 showed very promising results in inhibiton of thymidine phosphorylase. The biological activity of these compounds will be further investigated.
5. List of Publications of the Author Related to the Thesis


6. Experimental Section

General
The solvents were evaporated at 40 °C and 2 kPa, and the products were dried over phosphorus pentoxide at r. t. and 13 Pa. The course of the reactions was checked on TLC cards (Fluka, Merck) whereby the products were detected by UV monitoring, by ninhydrine spraying (dark blue colour of amines) and by spraying with 1% ethanolic solution of 4-(4-nitrobenzyl)pyridine followed by heating and treating with gaseous ammonia (blue colour of diesters of phosphonic acids). For flash column chromatography, silica gel 40-60 µm (Fluka) was used. The TLC and the preparative silica gel chromatography were carried out in the following solvent systems (v/v): chloroform-ethanol 9:1 (C1); chloroform-ethanol 19:1 (C2); ethyl acetate-acetone-ethanol-water 4:1:1:1 (H1); ethyl acetate-acetone-ethanol-water 6:1:1:0.5 (H3); 2-propanol-conc. aqueous ammonia-water 7:1:2 (I); 50% EtOAc-toluene (T1), 20% ethyl acetate-toluene (T2). Analytical HPLC was performed on Nucleosil 100-5 C18 (4.6 x 150 mm; Macharey-Nagel) using a linear gradient of methanol in 0.1M TEAA. Preparative reversed-phase chromatography was carried out on an octadecyl silica column (25x250 mm, 20 µm, IOCB Prague); compounds were eluted with a linear gradient of methanol in water at 15 ml/min. UV spectra and thermal characteristics were taken on a Cary Bio 100 (Varian) spectrophotometer. High resolution FAB mass spectra were recorded on a ZAB-EQ (VG Analytical) instrument with glycerol and thioglycerol as matrices. NMR spectra were measured on a Varian UNITY-500, Bruker AVANCE-500 and AVANCE-600 instrument (\(^1\)H at 500 or 600 MHz; \(^13\)C at 125.7 or 150.9 MHz frequency) in d\(_6\)-DMSO and/or D\(_2\)O at room temperature. The chemical shifts were referenced either to solvent signal (converted to \(\delta\) scale using relations \(\delta_{\text{H}}(\text{DMSO}) = 2.50\) and \(\delta_{\text{C}}(\text{DMSO}) = 39.7\) ppm) or to DSS (in D\(_2\)O). The 2D-H,\(H\)-COSY spectra were used for the structural assignment of coupled protons and 2D-H,\(H\)-ROESY spectra for the configuration determination. Carbon-13 chemical shifts and coupling constants \(J(C, P)\) were obtained from broad band proton-decoupled spectra using APT pulse sequence. The 2D-correlated H,\(C\)-HSQC and H,\(C\)-HMBC spectra were used for structural assignment of carbon signals.
cis-4-Hydroxy-d-proline (D-69)
A solution of trans-4-hydroxy-L-proline L-69 (100 g, 763 mmol) in acetic anhydride (500 mL) and acetic acid (1000 mL) was heated to reflux for 7 h. The solution was then concentrated in vacuo, the residue was diluted with 2 M aqueous HCl (1000 mL), and the mixture was heated under reflux for 4 h. Then activated charcoal (5 g) was added, the hot mixture was filtered immediately through a Celite layer and the cake was washed with hot water. The colourless solution was neutralised with triethylamine, and evaporated to dryness. The crude product was heated with ethanol (2500 mL) under reflux, and water was added carefully to the boiling mixture until the solid disappeared (but the solution remained still a little turbid). The solution was then left to stand overnight at –20 °C to afford white crystals, which were filtered off, washed with cold ethanol, and dried in vacuo to yield 70 g (70 %) of compound D-69.

HRMS (FAB) calcd for C5H10NO3 [M+H]+ 132,0661; found: 132,0663.

\[ \alpha \]D\textsuperscript{20} +57.2

\textsuperscript{1}H and \textsuperscript{13}C NMR spectra recorded were in accordance with lit.\textsuperscript{132,133}

N-Benzoylcarbonyl-trans-4-hydroxy-L-proline (L-70)
A solution of benzyl chloroformate (65 mL, 457 mmol, 1.2 eq.) in dioxane (150 mL) was added dropwise to the vigorously stirred solution of trans-4-hydroxy-L-proline L-69 (50 g, 381 mmol) and sodium hydrogen carbonate (84 g, 1.0 mol, 2.6 eq.) in water (1000 mL) at rt, and the reaction was stirred overnight. After the reaction was complete (TLC in I), the excess of benzyl chloroformate was extracted with diethyl ether (2x100 mL), and the aqueous layer was acidified with hydrochloric acid to pH 2. The product was then extracted with ethyl acetate (3x150 mL), combined extracts were dried over MgSO\textsubscript{4}, and evaporated in vacuo to give a viscous colourless oil (86 g, 85 %). The crude was used directly in the next step without further purification.

HRMS (FAB) calcd for C\textsubscript{13}H\textsubscript{16}NO\textsubscript{5} [M+H]+ 266,1029; found: 266,1025.

\textsuperscript{1}H and \textsuperscript{13}C NMR spectra recorded were in accordance with lit.\textsuperscript{150,151}

N-Benzoylcarbonyl-cis-4-hydroxy-d-proline (D-70)
Using conditions and amounts described above, crude D-70 was prepared from cis-4-hydroxy-d-proline D-69 as a viscous oil (84 g, 83 %).

HRMS (FAB) calcd for C\textsubscript{13}H\textsubscript{16}NO\textsubscript{5} [M+H]+ 266,1029; found: 266,1020.

\textsuperscript{1}H and \textsuperscript{13}C NMR spectra recorded were in accordance with lit.\textsuperscript{152}
**N-Benzylxycarbonyl-trans-4-hydroxy-L-proline methyl ester (L-71)**

Stirred solution of potassium hydroxide (28 g, 500 mmol) in an Et₂O-EtOH mixture (780 mL, 2:1) was treated with Diazald (21.4 g, 100 mmol). The diazomethane generated at 50 °C was blown via a stream of argon into an ice-cooled flask containing L-70 (20.05 g, 75.6 mmol) in Et₂O (250 mL) at 0 °C until the reaction mixture turned yellow. The solution was concentrated and the crude product was obtained as a colourless oil (22 g, 100%), which was used directly in the next step without further purification.

HRMS (FAB) calcd for C₁₄H₁₈NO₅ [M+H]+ 280.1185; found: 280.1189.

¹H and ¹³C NMR spectra recorded were in accordance with lit.134,153

**N-Benzylxycarbonyl-cis-4-hydroxy-D-proline methyl ester (D-71)**

Using the procedure outlined for L-71, compound D-71 was prepared from D-70 (12.8 g, 48.7 mmol) as a colourless oil (13.6 g, 100%).

HRMS (FAB) calcd for C₁₄H₁₈NO₅ [M+H]+ 280.1185; found: 280,1190.

¹H and ¹³C NMR spectra recorded were in accordance with lit.154

**N-Benzylxycarbonyl-trans-4-hydroxy-L-prolinol (L-72)**

Sodium borohydride (17.16 g, 453.5 mmol, 6 eq.) was added in one portion to a stirred solution of methyl ester L-71 (22 g, 75.6 mmol) in EtOH (600 mL) at 0 °C. Temperature slowly increased, and after being stirred for 12 h at rt (TLC in C2), the reaction was cooled to 0 °C, and carefully quenched by dropwise adding of AcOH (32 mL). The mixture was then evaporated in vacuo, the residue was dissolved in water (1000 mL), the product was extracted with EtOAc (3x100 mL), organic layer was dried over MgSO₄, and evaporated in vacuo. Pale yellow thick oil (17.20 g, 91%).

HRMS (FAB) calcd for C₁₃H₁₈NO₄ [M+H]+ 252,1236; found: 252,1226.

¹H and ¹³C NMR spectra recorded were in accordance with lit.134

**N-Benzylxycarbonyl-cis-4-hydroxy-D-prolinol (D-72)**

Sodium borohydride (11.06 g, 292.2 mmol, 6 eq.) was added in one portion to a stirred solution of methyl ester D-71 (13.60 g, 48.7 mmol) in EtOH (600 mL) at 0 °C. Temperature slowly increased, and after being stirred for 12 h at rt (TLC in C2), the reaction was cooled to 0 °C, and carefully quenched by dropwise adding of AcOH (20 mL). The mixture was then evaporated in vacuo, the residue was dissolved in water (1000 mL), the product was extracted
with EtOAc (3x100 mL), organic layer was dried over MgSO₄, and evaporated in vacuo. Diol **D-72** was obtained as a pale yellow thick oil (11.01 g, 90 %).

HRMS (FAB) calcd for C₁₃H₁₈NO₄ [M+H]+ 252.1236; found: 252.1246.

^1^H NMR (d₆DMSO):
- two sets of signals belonging to two isomers around N-CO bond (ratio ca 3 : 2) are observed only for some hydrogen atoms but nearly for all carbon atoms
- heavy overlap of does not allow to determine most of J(H,H)s

7.29 – 7.39 m, 5 H (C₆H₅ (Z)); 5.16 d, 1 H, J(OH,1) = 4.7 (1-OH); 4.955 dd, 1 H, J(OH,5A) = 5.8, J(OH,5B) = 5.0 (5-OH); 5.07 d, 1 H and 5.04 d, 1 H, J(gem) = 12.7 + 5.10 d, 1 H and 5.04 d, 1 H, J(gem) = 12.7 (CH₂(Z)); 4.195 m, 1 H (H-1); 3.81 m, 1 H (H-4); 3.58 m, 2 H + 3.55 m, 2 H (H-5A, H-5B); 3.52 m, 1 H (H-2A); 3.13 m, 1 H + 3.13 m, 1 H (H-2B); 2.09 m, 1 H + 2.07 m, 1 H (H-4aA); 1.89 m, 1 H + 1.84 m, 1 H (H-4aB).

^1^3^C NMR (d₆DMSO):
154.57 + 154.42 (C=O); 137.27, 128.60(2), 127.96 and 127.68(2) (C₆H₅ (Z)); 69.02 + 68.26 (C-1); 65.90 + 65.03 (CH₂(Z)); 61.86 + 62.52 (C-5); 58.88 + 58.15 (C-4); 55.43 + 55.88 (C-2); 35.89 + 36.66 (C-4a).

**trans-4-Hydroxy-L-prolinol (L-73)**

To a solution of N-Z-hydroxyprolinol **L-72** (17.19 g, 68 mmol) in deoxygenated MeOH-EtOAc mixture (2:1, 500 mL) was added 10% Pd on charcoal (0.2 g) under argon atmosphere, and the vigorously stirred reaction mixture was left to react under an atmosphere of hydrogen (10 psi) at rt for 12 h (TLC in C1). The catalyst was filtered off, and the solution was passed through a column of Dowex 50 (H⁺), which was then washed with MeOH. The product was liberated from Dowex with diluted aqueous ammonia (2.5 %), the solution was evaporated, the residue was co-distilled with ethanol, and dried in vacuo, giving **L-73** as a pale yellow thick oil (7.87 g, 98 %).

HRMS (FAB) calcd for C₅H₁₂NO₂ [M+H]^+ 118.0868; found: 118.0865.

^1^H NMR (d₆DMSO):
4.18 m, 1 H, J(1,2A) = 4.7, J(1,2B) = 2.7, J(1,4aA) = 2.3, J(1,4aB) = 5.6 (H-1); 3.32 m, 3 H (H-4, H-5A, H-5B); 2.93 dd, 1 H, J(2A,1) = 4.7, J(2A,2B) = 11.4 (H-2A); 2.67 dd, 1 H, J(2B,1) = 2.7, J(2B,2A) = 11.4, J(2B,4aA) = 1.3 (H-2B); 1.65 dddd, 1 H, J(4aA,1) = 2.3, J(4aA,4aB) = 13.1, J(4aA,4) = 6.8, J(4aA,2B) = 1.3 (H-4aA); 1.51 dddd, 1 H, J(4aB,1) = 5.6, J(4aB,4aA) = 13.1, J(4aB,4) = 8.1 (H-4aB).

^1^3^C NMR (d₆DMSO):
cis-4-Hydroxy-D-prolinol (D-73)

Using the procedure outlined for L-73, compound D-73 was prepared from D-72 (11.01 g, 43.8 mmol) as a pale yellow thick oil (5.03 g, 98%).

HRMS (FAB) calcd for C₅H₁₂NO₂ [M+H]+ 118.0868; found: 118.0872.

1H NMR (d₆DMSO):
4.16 m, 1 H, J(1,2A) = 5.4, J(1,2B) = 3.6, J(1,4aA) = 6.5, J(1,4aB) = 4.2 (H-1); 3.41 dd, 1 H, J(5A,4) = 6.3, J(5A,5B) = 10.9 (H-5A); 3.39 dd, 1 H, J(5B,4) = 5.1, J(5B,5A) = 10.9 (H-5B); 3.13 m, 1 H, J(4,4aA) = 8.2, J(4,4aB) = 6.8, J(4,5A) = 6.3, J(4,5B) = 5.1 (H-4); 2.84 dd, 1 H, J(2A,1) = 5.4, J(2A,2B) = 11.1 (H-2A); 2.71 ddd, 1 H, J(2B,1) = 3.6, J(2B,2A) = 11.1, J(2B,4aB) = 1.0 (H-2B); 1.98 ddd, 1 H, J(4aA,1) = 6.5, J(4aA,4aB) = 13.2, J(4aA,4) = 8.2 (H-4aA); 1.34 dddd, 1 H, J(4aB,1) = 4.2, J(4aB,4aA) = 13.2, J(4aB,4) = 6.8, J(4aB,2B) = 1.0 (H-4aB).

13C NMR (d₆DMSO):
70.66 (C-1); 63.76 (C-5); 59.62 (C-4); 54.32 (C-2); 37.32 (C-4a).

Diisopropyl-(2,3-dideoxy-3-aza-4a-carba-α-L-glycero-pentofuranosyl)-3-N-methylphosphonate (L-74)

Aqueous formaldehyde (14.5 M solution; 7.9 mL, 114 mmol, 1.7 eq.) was added to a stirred solution of 4-hydroxyprolinol L-73 (7.87 g, 67 mmol) in diisopropyl phosphonate (15.7 mL, 94 mmol, 1.4 eq.), and the mixture was heated to 60 °C for 3 h (TLC in H1). The reaction was evaporated, dissolved in water, and the solution was passed through a column of Dowex 50 (H⁺-form), which was then washed with a mixture of H₂O-MeOH (1:1, 1 L). The product was liberated from Dowex with diluted (approx. 2.5 %) aqueous ammonia, and evaporated in vacuo to obtain L-74 as a slightly brown thick oil (17.00 g, 86%).

HRMS (FAB) calcd for C₁₂H₂₇NO₅P [M+H]+ 296.1627; found: 296.1616.

1H NMR (d₆DMSO):
4.72 bs, 1 H and 4.44 bs, 1 H (2x OH); 4.07 m, 1 H, J(1,2A) = 5.7, J(1,2B) = 5.3, J(1,4aA) = 4.3, J(1,4aB) = 6.9 (H-1); 4.56 m, 2 H, 1.237 d, 3 H, J = 6.2, 1.235 d, 6 H, J = 6.2 and 1.233 d, 3 H, J = 6.2 (P(OiPr)₂); 3.32 dd, 1 H, J(5A,4) = 5.0, J(5A,5B) = 11.0 (H-5A); 3.28 dd, 1 H, J(5B,4) = 5.2, J(5B,5A) = 11.0 (H-5B); 3.28 dd, 1 H, J(2A,1) = 5.7, J(2A,2B) = 9.8, (H-2A); 3.27 dd, 1 H, 1 H, J(gem) = 15.2, J(H,P) = 16.3 and 2.70 dd, 1 H, J(gem) = 15.2, J(H,P) = 5.5 (N-CH₂-P); 2.76 m, 1 H, J(4,4aA) = 7.6, J(4,4aB) = 7.8, J(4,5A) = 5.0, J(4,5B) = 5.2 (H-4);
2.29 dd, 1 H, J(2B,1) = 5.3, J(2B,2A) = 9.8 (H-2B); 1.63 ddd, 1 H, J(4aA,1) = 4.3, J(4aA,4aB) = 12.8, J(4aA,4) = 7.6 (H-4aA); 1.58 ddd, 1 H, J(4aB,1) = 6.9, J(4aB,4aA) = 12.8, J(4aB,4) = 7.8 (H-4aB).

$^1$C NMR (d$_6$DMSO):
69.88 d, J(C,P) = 6.8, 69.65 d, J(C,P) = 6.8, 24.04 d, J(C,P) = 4.9, 23.98 d, J(C,P) = 4.9 and 23.88(2) d, J(C,P) = 4.9 (P(OiPr)$_2$); 68.65 (C-1); 65.57 d, J(4,P) = 16.1 (C-4); 64.49 (C-5); 64.31 d, J(C,P) = 2.0 (C-2); 51.50 d, J(C,P) = 164.1 (N-CH$_2$-P); 37.98 (C-4a).

Diisopropyl-(2,3-dideoxy-3-aza-4a-carba-$\beta$-D-glycero-pentofuranosyl)-3-N-methylphosphonate (D-74)

Using the procedure outlined for L-74, phosphonate D-74 was prepared from D-73 (5.03 g, 42.8 mmol), aqueous formaldehyde (5.0 mL, 72.8 mmol), and diisopropyl phosphonate (10.0 mL, 60.0 mmol) as a slightly brown thick oil (9.01 g, 71 %).

HRMS (FAB) calcd for C$_{12}$H$_{27}$NO$_5$P [M+H]$^+$ 296.1627; found: 296.1633.

$^1$H NMR (d$_6$DMSO):
4.11 m, 1 H, J(1,2A) = 2.0, J(1,2B) = 5.6, J(1,4aA) = 6.8, J(1,4aB) = 3.7 (H-1); 4.59 m, 2 H, 1.246 d, 3 H, J = 6.2, 1.242 d, 3 H, J = 6.2, 1.238 d, 3 H, J = 6.2 and 1.235 d, 3 H, J = 6.2 (P(OiPr)$_2$); 3.42 dd, 1 H, J(5A,4) = 4.8, J(5A,5B) = 10.8 (H-5A); 3.37 dd, 1 H, J(5B,4) = 5.2, J(5B,5A) = 10.8 (H-5B); 3.26 dd, 1 H, 1 H, J(gem) = 15.2, J(H,P) = 16.0 and 2.61 dd, 1 H, J(gem) = 15.2, J(H,P) = 5.8 (N-CH$_2$-P); 3.10 ddd, 1 H, J(2A,1) = 2.0, J(2A,2B) = 10.0, J(2A,4aB) = 1.0 (H-2A); 2.55 m, 1 H, J(4,4aA) = 8.0, J(4,4aB) = 7.2, J(4,5A) = 4.8, J(4,5B) = 5.2 (H-4); 2.47 ddd, 1 H, J(2B,1) = 5.6, J(2B,2A) = 10.0, J(2B,4aB) = 0.7 (H-2B); 2.06 ddd, 1 H, J(4aA,1) = 6.8, J(4aA,4aB) = 13.0, J(4aA,4) = 7.2 (H-4aA); 1.34 bddd, 1 H, J(4aB,1) = 3.7, J(4aB,4aA) = 13.0, J(4aB,4) = 7.2, J(4aB,2A) = 1.0, J(4aB,2B) = 0.7 (H-4aB).

$^{13}$C NMR (d$_6$DMSO):
70.03 d, J(C,P) = 6.8, 69.70 d, J(C,P) = 6.7, 24.18 d, J(C,P) = 3.6, 24.10 d, J(C,P) = 3.5, 24.01 d, J(C,P) = 4.8 and 23.99 d, J(C,P) = 4.6 (P(OiPr)$_2$); 68.77 (C-1); 66.30 d, J(4,P) = 15.8 (C-4); 64.62 d, J(C,P) = 1.6 (C-2); 64.34 (C-5); 51.12 d, J(C,P) = 163.2 (N-CH$_2$-P); 37.98 (C-4a).

Diisopropyl-(2,3-dideoxy-5-$\delta$-dimethoxytrityl-3-aza-4a-carba-$\alpha$-1-glycero-pentofuranosyl)-3-N-methylphosphonate (L-75)

65
Phosphonate L-74 (16.98 g, 57.5 mmol) was co-evaporated repeatedly with pyridine to remove traces of water, dissolved in dry pyridine (300 mL), and treated with dimethoxytrityl chloride (29.22 g, 86.2 mmol, 1.5 eq.) at rt (TLC in C2). After 1 h, the reaction was quenched by adding mixture of methanol, water, and triethylamine (3:1:1, 50 mL), and concentrated under reduced pressure. The residue was dissolved in chloroform, and the solution was washed with water. The organic layer was dried over Na₂SO₄, and evaporated. Crude product was purified by flash chromatography on silica (elution with a linear gradient of EtOH in CHCl₃), and dried in vacuo to give L-75 as a pale yellow thick oil (30.52 g, 90 %).

HRMS (FAB) calcd for C₃₃H₄₃NO₇P [M-H]- 596.2777; found: 596.2757.

**1H NMR (CDCl₃):**
7.43 m, 2 H, 7.33 m, 4 H, 7.27 m, 2 H, 7.20 m, 1 H and 6.81 m, 4 H (2x C₆H₄ and C₆H₅ (ODMTr)); 4.25 m, 1 H, $J(1,2A) = 5.0$, $J(1,2B) = 2.8$, $J(1,4aA) = 2.3$, $J(1,4aB) = 5.7$ (H-1); 4.68 m, 2 H, 1.307 d, 3 H, $J = 6.1$, 1.294 d, 3 H, $J = 6.2$, 1.287 d, 3 H, $J = 6.1$ and 1.25 d, 3 H, $J = 6.2$ (P(OiPr)₂); 3.78 s, 6 H (2x OMe (ODMTr)); 3.56 dd, 1 H, $J(2A,1) = 5.0$, $J(2A,2B) = 11.1$, $J(2A,P) = 2.4$ (H-2A); 3.40 m, 1 H, $J(4,4aA) = 6.5$, $J(4,4aB) = 9.6$, $J(4,5A) = 6.0$, $J(4,5B) = 5.0$, $J(4,2B) = 1.5$ (H-4); 3.29 dd, 1 H, 1 H, $J$ (gem) = 15.6, $J(H,P) = 12.0$ and 3.20 dd, 1 H, $J$ (gem) = 15.6, $J(H,P) = 6.0$ (N-CH₂-P); 3.08 dd, 1 H, $J(5A,4) = 6.0$, $J(5A,5B) = 9.2$ (H-5A); 3.04 dd, 1 H, $J(5B,4) = 5.0$, $J(5B,5A) = 9.2$ (H-5B); 2.77 m, 1 H, $J(2B,1) = 2.8$, $J(2B,2A) = 11.1$, $J(2B,4) = 1.5$, $J(2B,4aA) = 1.5$ (H-2B); 1.95 m, 1 H, $J(4aA,1) = 2.3$, $J(4aA,4aB) = 13.2$, $J(4aA,4) = 6.5$, $J(4aA,2B) = 1.5$ (H-4aA); 1.60 ddd, 1 H, $J(4aB,1) = 5.7$, $J(4aB,4aA) = 13.2$, $J(4aB,4) = 9.6$ (H-4aB).

**13C NMR (CDCl₃):**
158.38(2), 145.05, 136.28, 136.25, 130.07(4), 128.22(2), 127.69(2), 126.63 and 113.02(4) (2x C₆H₄ and C₆H₅ (ODMTr)); 86.24 (>C< (ODMTr)); 70.86 (C-1); 70.64 d, $J(C,P) = 6.3$, 70.28 d, $J(C,P) = 6.3$, 24.16 dd, $J(C,P) = 3.8$, 24.13 d, $J(C,P) = 3.8$, 24.08 d, $J(C,P) = 3.8$ and 24.00 d, $J(C,P) = 3.8$ (P(OiPr)₂); 67.35 (C-5); 63.18 (C-2); 62.34 d, $J(4,P) = 11.3$ (C-4); 55.15 (2x OMe (ODMTr)); 50.73 d, $J(C,P) = 148.4$ (N-CH₂-P); 39.48 (C-4a).

**Diisopropyl-(2,3-dideoxy-5-O-dimethoxytrityl-3-aza-4a-carba-β-D-glycero-pentofuranosyl)-3-N-methylphosphonate (D-75)**
Using the procedure outlined for L-75, compound D-75 was prepared from D-74 (8.73 g, 29.6 mmol) and dimethoxytrityl chloride (15.03 g, 44.4 mmol) as a pale yellow thick oil (16.24 g, 92 %).

HRMS (FAB) calcd for C₃₃H₄₅NO₇P [M+H]+ 598.2934; found: 598.2949.
$^1$H NMR (CDCl$_3$):
7.44 m, 2 H, 7.33 m, 4 H, 7.28 m, 2 H, 7.20 m, 1H and 6.83 m, 4 H (2x C$_6$H$_4$ and C$_6$H$_5$
(ODMTr); 4.16 m, 1 H, J(1,2A) = 1.5, J(1,2B) = 3.7, J(1,4aA) = 6.0, J(1,4aB) = 3.0 (H-1);
4.70 m, 2 H, 1.31 d, 6 H, J = 6.2, 1.28 d, 3 H, J = 6.2 and 1.26 d, 3 H, J = 6.2 (P(OiPr)$_2$);
3.782 s, 3 H and 3.780 s, 3 H (2x OMe (ODMTr)); 3.36 dd, 1 H, J(gem) = 15.0, J(H,P) = 16.5 and
2.73 dd, 1 H, J(gem) = 15.0, J(H,P) = 6.0 (N-CH$_2$-P); 3.28 dd, 1 H, J(5A,4) = 3.8, J(5A,5B) =
9.8 (H-5A); 3.08 dd, 1 H, J(5B,4) = 4.5, J(5B,5A) = 9.8 (H-5B); 2.80 m, 1 H, J(4,4aA) = 10.0, J(4,4aB) =
5.0, J(4,5A) = 3.8, J(4,5B) = 4.5 (H-4); 2.62 ddd, 1 H, J(2B,1) = 3.7, J(2B,2A) = 9.8, J(2B,4aB) =
2.0 (H-2B); 2.20 ddd, 1 H, J(4aA,1) = 6.0, J(4aA,4aB) = 14.0, J(4aA,4) = 10.0 (H-4aA); 1.60 m, 1 H,
J(4aB,1) = 3.0, J(4aB,4aA) = 14.0, J(4aB,4) = 5.0, J(4aB,2B) = 2.0 (H-4aB).

$^{13}$C NMR (CDCl$_3$):
158.42(2), 144.70, 136.06, 135.93, 130.18(2), 130.11(2), 128.30(2), 127.76(2), 126.74 and
113.04(4) (2x C$_6$H$_4$ and C$_6$H$_5$ (ODMTr)); 86.66 (>C< (ODMTr)); 70.59 (C-1); 70.54 d, J(C,P) =
7.4, 70.02 d, J(C,P) = 6.8, 24.04-24.20 4x d, J(C,P) = 3.8 (OiPr)$_2$; 65.50 (C-5); 63.47 (C-
2); 63.15 d, J(4,P) = 15.1 (C-4); 55.14 (2x OMe (ODMTr)); 50.03 d, J(C,P) = 158.1 (N-CH$_2$-
P); 37.95 (C-4a).

Diisopropyl-(2,3-dideoxy-5-O-dimethoxytrityl-1-O-methanesulfonyl-3-aza-4a-carba-α-L-
glycero-pentofuranosyl)-3-N-methylphosphonate (L-76)
Dimethoxytrityl derivative L-75 (29.25 g, 49.0 mmol) was co-evaporated with toluene to
remove traces of water, dissolved in dichloromethane (400 mL) with pyridine (39.6 mL, 489.5
mmol, 10 eq.), and cooled to 0 °C. Mesyl chloride (18.9 mL, 244.7 mmol, 5 eq.) was added to
the stirred mixture in one portion. After 30 min (TLC in C2), the reaction was quenched by
careful dropwise adding water to the mixture intensively stirred and cooled to 0 °C in an ice
bath. The solution was diluted with chloroform, washed subsequently with water, 10%
aqueous citric acid, saturated sodium hydrogen carbonate solution, and finally with water. The
organic layer was dried over Na$_2$SO$_4$, and concentrated in vacuo. Product was isolated by
flash chromatography on silica (elution with a linear gradient of EtOAc in toluene) and dried
in vacuo, giving yellow thick oil (30.62 g, 93 %).

HRMS (FAB) calcd for C$_{34}$H$_{47}$NO$_9$PS [M+H]$^+$ 676.2709; found: 676.2739.

$^1$H NMR (CDCl$_3$):
7.40 m, 2 H, 7.29 m, 4 H, 7.28 m, 2 H, 7.22 m, 1H and 6.82 m, 4 H (2x C$_6$H$_4$ and C$_6$H$_5$
(ODMTr); 5.10 m, 1 H, J(1,2A) = 4.0, J(1,2B) = 6.0, J(1,4aA) = 3.0, J(1,4aB) = 7.0 (H-1);
4.65 m, 2 H, 1.29 d, 3 H, \( J = 6.2 \), 1.27 d, 3 H, \( J = 6.2 \) and 1.20 d, 3 H, \( J = 6.2 \) (P(OiPr)\( _2 \)); 3.78 s, 6 H (2x OMe (ODMTr)); 3.76 dd, 1 H, \( J(2A,1) = 4.0 \), \( J(2A,2B) \sim 11 \) (H-2A); 3.30 dd, 1 H, 1 H, \( J(\text{gem}) = 15.2 \), \( J(H,P) = 17.4 \) and 2.81 dd, 1 H, \( J(\text{gem}) = 15.2 \), \( J(H,P) = 5.5 \) (N-CH\( _2 \)-P); 3.18 dd, 1 H, \( J(5A,4) = 4.0 \), \( J(5A,5B) = 9.0 \) (H-5A); 3.10 m, 1 H, \( J(4aA) = 6.4 \), \( J(4aA,4aB) = 7.0 \), \( J(4aA,5) = 4.0 \), \( J(4aA,5B) = 5.0 \) (H-4); 3.07 dd, 1 H, \( J(5B,4) = 5.0 \), \( J(5B,5A) = 9.0 \) (H-5B); 2.99 s, 3 H (OMs); 2.85 bdd, 1 H, \( J(2B,1) = 6.0 \), \( J(2B,2A) \sim 11 \) (H-2B); 2.22 m, 1 H, \( J(4aA,1) = 3.0 \), \( J(4aA,4aB) = 14.0 \), \( J(4aA,4) = 6.4 \) (H-4aA); 1.90 m, 1 H, \( J(4aB,1) = 7.0 \), \( J(4aB,4aA) = 14.0 \), \( J(4aB,4) = 7.0 \) (H-4aB).

\(^{13}\)C NMR (CDCl\( _3 \)):

158.38(2), 144.72, 135.88, 135.85, 129.94(4), 128.02(2), 127.74(2), 126.70 and 113.03(4) (2x C\( _6 \)H\( _4 \) and C\( _6 \)H\( _5 \) (ODMTr)); 86.32 (>C< (ODMTr)); 79.68 (C-1); 70.72 d, \( J(C,P) = 6.3 \), 70.23 d, \( J(C,P) = 6.3 \), 24.08 d, \( J(C,P) = 3.8 \), 23.98(2) d, \( J(C,P) = 3.8 \) and 23.92 d, \( J(C,P) = 3.8 \) (P(OiPr)\( _2 \)); 65.98 (C-5); 62.32 d, \( J(4,P) = 16.4 \) (C-4); 60.61 (C-2); 55.10 (2x OMe (ODMTr)); 50.88 d, \( J(C,P) = 166.0 \) (N-CH\( _2 \)-P); 38.31 (OMs); 36.45 (C-4a).

**Diisopropyl-[2,3-dideoxy-5-O-dimethoxytrityl-1-O-methanesulfonyl-3-aza-4a-carba-\( \beta \)-D-glycero-pentofuranosyl]-3-N-methylphosphonate (D-76)**

Using the procedure outlined for L-76, compound D-76 was prepared from D-75 (16.24 g, 27.2 mmol), pyridine (22.0 mL, 271.7 mmol), and mesyl chloride (10.5 mL, 135.8 mmol) as a yellow thick oil (14.95 g, 81 %).

HRMS (FAB) calcd for C\(_{34}\)H\(_{46}\)NO\(_9\)NaPS \([\text{M+Na}]^+\) 698.2529; found: 698.2547.

\(^1\)H NMR (CDCl\( _3 \)):

7.42 m, 2 H, 7.31 m, 4 H, 7.28 m, 2 H, 7.21 m, 1H and 6.82 m, 4 H (2x C\( _6 \)H\( _4 \) and C\( _6 \)H\( _5 \) (ODMTr)); 5.12 m, 1 H, \( J(1,2A) \sim 1.0 \), \( J(1,2B) = 4.8 \), \( J(1,4aA) = 7.1 \), \( J(1,4aB) = 2.2 \) (H-1); 4.67 m, 2 H, 1.29 d, 6 H, \( J = 6.2 \), 1.285 d, 3 H, \( J = 6.2 \), 1.27 d, 3 H, \( J = 6.2 \) and 1.22 d, 3 H, \( J = 6.2 \) (P(OiPr)\( _2 \)); 3.79 s, 6 H (2x OMe (ODMTr)); 3.71 dd, 1 H, \( J(2A,1) \sim 1.0 \), \( J(2A,2B) = 11.8 \) (H-2A); 3.39 dd, 1 H, 1 H, \( J(\text{gem}) = 15.3 \), \( J(H,P) = 18.1 \) and 2.68 dd, 1 H, \( J(\text{gem}) = 15.3 \), \( J(H,P) = 5.3 \) (N-CH\( _2 \)-P); 3.26 dd, 1 H, \( J(5A,4) = 6.0 \), \( J(5A,5B) = 9.6 \) (H-5A); 3.11 dd, 1 H, \( J(5B,4) = 8.6 \), \( J(5B,5A) = 9.6 \) (H-5B); 2.94 s, 3 H (OMs); 2.78 m, 1 H, \( J(4aA) = 8.6 \), \( J(4,5A) = 6.0 \), \( J(4,5B) = 5.5 \) (H-4); 2.69 bdd, 1 H, \( J(2B,1) = 4.8 \), \( J(2B,2A) = 11.8 \) (H-2B); 2.39 ddd, 1 H, \( J(4aA,1) = 7.1 \), \( J(4aA,4aB) = 15.0 \), \( J(4aA,4) = 8.6 \) (H-4aA); 1.86 m, 1 H, \( J(4aB,1) = 1.6 \), \( J(4aB,4aA) = 15.0 \), \( J(4aB,4) = 2.2 \) (H-4aB).

\(^{13}\)C NMR (CDCl\( _3 \)):
Diisopropyl-(2,3-dideoxy-5-O-dimethoxytrityl-1-O-acetyl-3-aza-4a-carba-ß-L-glycero-pentofuranosyl)-3-N-methylphosphonate (L-77)

Anhydrous sodium acetate (26.00 g, 316.5 mmol, 10 eq.) was suspended in a solution of mesylate L-76 (21.38 g, 31.6 mmol) in dry dimethyl formamide (200 mL). The flask was equipped with a calcium dichloride tube, and the stirred mixture was heated to 120 °C for 6 h (TLC in C2). The reaction was then cooled, DMF was evaporated in vacuo, the residue was dissolved in ethyl acetate, and inorganic salts were filtered off. The organic layer was washed with water and dried over Na₂SO₄, and evaporated in vacuo. The crude was purified by flash chromatography on silica (elution with a linear gradient of EtOAc in toluene), and dried in vacuo. Pale yellow thick oil (10.62 g, 52 %).

HRMS (FAB) calcd for C₃₅H₄₅NO₈P [M-H]- 638.2883; found: 638.2904.

¹H NMR (CDCl₃):

7.41 m, 2 H, 7.30 m, 4 H, 7.27 m, 2 H, 7.20 m, 1H and 6.82 m, 4 H (2x C₆H₄ and C₆H₅ (ODMTr)); 5.12 m, 1 H, J(1,2A) = 6.1, J(1,2B) = 4.2, J(1,4aA) = 2.6, J(1,4aB) = 7.5 (H-1); 4.65 m, 2 H, 1.28 d, 3 H, J = 6.2, 1.27 d, 3 H, J = 6.2, 1.24 d, 3 H, J = 6.2 and 1.20 d, 3 H, J = 6.2 (P(OiPr)₂); 3.79 s, 6 H (2x OMe (ODMTr)); 3.69 dd, 1 H, J(2A,1) = 6.1, J(2A,2B) = 11.2 (H-2A); 3.30 dd, 1 H, 1 H, J(gem) = 15.0, J(H,P) = 17.9 and 2.77 dd, 1 H, J(gem) = 15.0, J(H,P) = 5.6 (N-CH₂-P); 3.18 dd, 1 H, J(5A,4) = 4.8, J(5A,5B) = 8.8 (H-5A); 3.03 dd, 1 H, J(5B,4) = 5.6, J(5B,5A) = 8.8 (H-5B); 3.01 m, 1 H, J(4aA,4A) = 6.5, J(4aA,4B) = 8.6, J(4,5A) = 4.8, J(4,5B) = 5.6, J(4,2B) = 0.8 (H-4); 2.60 ddt, 1 H, J(2B,1) = 4.2, J(2B,2A) = 11.2, J(2B,4) = 0.8, J(2B,4A) = 0.8 (H-2B); 2.04 s, 3 H (OAc); 1.98 dddd, 1 H, J(4aA,1) = 2.6, J(4aA,4B) = 13.8, J(4aA,4) = 6.5, J(4aA,2B) = 0.8 (H-4aA); 1.82 dddd, 1 H, J(4aB,1) = 7.5, J(4aB,4aA) = 13.8, J(4aB,4) = 8.6 (H-4aB).

¹³C NMR (CDCl₃):

170.66 and 21.20 (OAc); 158.40(2), 144.90, 136.12, 136.10, 130.03(4), 128.77(2), 128.15(2), 126.71 and 113.03(4) (2x C₆H₄ and C₆H₅ (ODMTr)); 86.27 (>C< (ODMTr)); 73.16 (C-1); 70.67 d, J(C,P) = 6.8, 70.15 d, J(C,P) = 6.9, 24.18 d, J(C,P) = 3.9, 24.06 d, J(C,P) = 3.9, 24.04 d, J(C,P) = 4.8 and 23.98 d, J(C,P) = 4.8 (P(OiPr)₂); 66.49 (C-5); 63.76 d, J(4,5) = 17.5
Diisopropyl-(2,3-dideoxy-5-O-dimethoxytrityl-1-O-acetyl-3-aza-4a-carba-α-D-glycero-pentofuranosyl)-3-N-methylphosphonate (D-77)

Using the procedure outlined for L-77, compound D-77 was prepared from D-76 (7.82 g, 11.6 mmol), and NaOAc (9.49 g, 115.7 mmol) as a pale yellow thick oil (4.30 g, 58%). HRMS (FAB) calcd for C_{35}H_{46}NO_{8}NaP [M+Na]^+ 662.2859; found: 662.2832.

\[ ^1H \text{ NMR (CDCl}_3\text{):} \]
7.41 m, 2 H, 7.30 m, 4 H, 7.27 m, 2 H, 7.20 m, 1H and 6.82 m, 4 H (2x C\textsubscript{6}H\textsubscript{4} and C\textsubscript{6}H\textsubscript{5} (ODMTr)); 5.12 m, 1 H, J(1,2A) = 6.2, J(1,2B) = 4.2, J(1,4aA) = 2.8, J(1,4aB) = 7.1 (H-1);
4.65 m, 2 H, 1.280 d, 3 H, J = 6.1, 1.274 d, 3 H, J = 6.2, 1.242 d, 3 H, J = 6.2 and 1.198 d, 3 H, J = 6.2 (P(OiPr\textsubscript{2})); 3.78 s, 6 H (2x OMe (ODMTr)); 3.70 dd, 1 H, J(2A,1) = 6.2, J(2A,2B) = 11.3 (H-2A); 3.30 dd, 1 H, J(gem) = 15.0, J(H,P) = 17.9 and 2.77 dd, 1 H, J(gem) = 15.0, J(H,P) = 5.6 (N-CH\textsubscript{2}-P);
3.18 dd, 1 H, J(5A,4) = 4.8, J(5A,5B) = 8.8 (H-5A); 3.03 dd, 1 H, J(5B,4) = 5.6, J(5B,5A) = 8.8 (H-5B); 3.01 m, 1 H, J(4,4aA) = 6.5, J(4,4aB) = 8.5, J(4,5A) = 4.8, J(4,5B) = 5.6, J(4,2B) < 1.0 (H-4); 2.60 ddt, 1 H, J(2B,1) = 4.2, J(2B,2A) = 11.3, J(2B,4) < 1.0, J(2B,4aA) < 1.0 (H-2B); 2.04 s, 3 H (OAc); 1.98 bddd, 1 H, J(4aA,1) = 2.8, J(4aA,4B) = 13.8, J(4aA,4) = 6.5, J(4aA,2B) < 1.0 (H-4aA); 1.82 m, 1 H, J(4aB,1) = 7.1, J(4aB,4aA) = 13.8, J(4aB,4) = 8.5 (H-4aB).

\[ ^13C \text{ NMR (CDCl}_3\text{):} \]
170.56 and 21.13 (OAc); 158.41(2), 144.90, 136.12(2), 130.02(4), 128.15(2), 127.72(2), 126.67 and 113.02(4) (2x C\textsubscript{6}H\textsubscript{4} and C\textsubscript{6}H\textsubscript{5} (ODMTr)); 86.27 (>C< (ODMTr)); 73.13 (C-1);
70.59 d, J(C,P) = 7.5, 70.08 d, J(C,P) = 6.3, 24.14 d, J(C,P) = 3.8, 24.03 d, J(C,P) = 3.8, 24.01 d, J(C,P) = 5.0 and 23.95 d, J(C,P) = 5.0 (P(OiPr\textsubscript{2})); 66.47 (C-5); 63.72 d, J(4,P) = 17.6 (C-4); 60.89 (C-2); 55.14 (2x OMe (ODMTr)); 51.30 d, J(C,P) = 164.8 (N-CH\textsubscript{2}-P); 36.17 (C-4a).

Diisopropyl-(2,3-dideoxy-5-O-dimethoxytrityl-3-aza-4a-carba-β-L-glycero-pentofuranosyl)-3-N-methylphosphonate (L-78)

2 M solution of sodium methoxide in methanol (1 mL) was added to a solution of L-77 (10.62 g, 16.6 mmol) in anhydrous methanol (100 mL). After 30 min (TLC in C2) the reaction was worked up by adding Dowex 50 (Et\textsubscript{3}N\textsuperscript{+}-form), which was then filtered off, and the solution was concentrated in vacuo, giving pale yellow thick oil (9.92 g, ca. 100%).
HRMS (FAB) calcd for C\textsubscript{33}H\textsubscript{45}NO\textsubscript{7}P [M+H]\textsuperscript{+} 598.2934; found: 598.2920.  
\textsuperscript{1}H and \textsuperscript{13}C NMR spectra were identical to those recorded for enantiomeric D-75.

**Diisopropyl-(2,3-dideoxy-5-O-dimethoxytrityl-3-aza-4a-carba-\textalpha-D-glycero-pentofuranosyl)-3-N-methylphosphonate (D-78)**

Using the procedure outlined for L-78, compound D-78 was prepared from D-77 (4.30 g, 6.72 mmol) as a pale yellow thick oil (4.02 g, ca. 100 %).

HRMS (FAB) calcd for C\textsubscript{33}H\textsubscript{45}NO\textsubscript{7}P [M+H]\textsuperscript{+} 598.2934; found: 598.2940.

\textsuperscript{1}H and \textsuperscript{13}C NMR spectra were identical to those recorded for enantiomeric L-75.

**Diisopropyl-(2,3-dideoxy-5-O-dimethoxytrityl-1-O-methanesulfonyl-3-aza-4a-carba-\textbeta-L-glycero-pentofuranosyl)-3-N-methylphosphonate (L-79)**

Using the procedure outlined for L-76, compound L-79 was prepared from L-78 (9.92 g, 16.6 mmol), pyridine (13.4 mL, 166 mmol), and mesyl chloride (6.42 mL, 83 mmol) as a yellow thick oil (9.22 g, 82 %).

HRMS (FAB) calcd for C\textsubscript{34}H\textsubscript{47}NO\textsubscript{9}PS [M+H]\textsuperscript{+} 676.2709; found: 676.2701.

\textsuperscript{1}H and \textsuperscript{13}C NMR spectra were identical to those recorded for enantiomeric D-76.

**Diisopropyl-(2,3-dideoxy-5-O-dimethoxytrityl-1-O-methanesulfonyl-3-aza-4a-carba-\textalpha-D-glycero-pentofuranosyl)-3-N-methylphosphonate (D-79)**

Using the procedure outlined for L-76, compound D-79 was prepared from D-78 (4.02 g, 6.72 mmol), pyridine (5.44 mL, 67.2 mmol), and mesyl chloride (2.60 mL, 33.6 mmol) as a yellow thick oil (4.14 g, 91 %).

HRMS (FAB) calcd for C\textsubscript{34}H\textsubscript{46}NO\textsubscript{9}NaPS [M+Na]\textsuperscript{+} 698.2529; found: 698.2516.

\textsuperscript{1}H and \textsuperscript{13}C NMR spectra were identical to those recorded for enantiomeric L-76.

**Alkylation of nucleobases (General procedure A)**

To a suspension of nucleobase (1.5 eq.) in dry DMF (6 mL/mmol) was added anhydrous cesium carbonate (1.2 eq.), and the reaction mixture was vigorously stirred under anhydrous conditions at 120 °C for 20 min. Then solution of mesylate (1 eq.) in DMF (3 mL/mmol) was added and the reaction was stirred at 110 °C. After the reaction was complete (TLC in C2), DMF was evaporated, the residue was dissolved in CHCl\textsubscript{3}, and inorganic precipitate was
filtered off. Solvent was removed under reduced pressure, and the crude product was purified by flash chromatography on silica (elution with a linear gradient of EtOH in CHCl₃).

**Diisopropyl-[9-(2′,3′-dideoxy-5′-O-dimethoxytrityl-3′-aza-4a′-carba-α-L-glycero-pentofuranosyl)adenine]-3′-N-methylphosphonate (80a)**

The general procedure A was followed using mesylate L-79 (1.60 g, 2.36 mmol), Cs₂CO₃ (0.923 g, 2.83 mmol) and adenine (0.479 g, 3.54 mmol). The reaction was complete after 3 h. White foam (0.709 g, 42 %).


1H NMR (d₆DMSO):
8.23 s, 1 H (H-8); 8.12 s, 1 H (H-2); 7.40 m, 2 H, 7.32 m, 2 H, 7.27 m, 4 H, 7.23 m, 1H and 6.90 m, 4 H (2x C₆H₄ and C₆H₅ (ODMTr)); 7.20 bs, 2 H (NH₂); 4.95 m, 1 H, J(1′,2′A) = 6.7, J(1′,2′B) = 8.0, J(1′,4′aA) = 6.8, J(1′,4′aB) = 8.8 (H-1′); 4.51 m, 2 H, 1.20 d, 3 H, J = 6.1, 1.185 d, 3 H, J = 6.1, 1.17 d, 3 H, J = 6.1 and 1.12 d, 3 H, J = 6.1 (P(OiPr)₂); 3.74 s, 6 H (2x OMe (ODMTr)); 3.66 dd, 1 H, J(2′A,1′) = 6.7, J(2′A,2′B) = 9.2, (H-2′A); 3.28 m, 1 H, J(4′,4′aA) = 8.8, J(4′,4′aB) = 5.8, J(4′,5′A) = 5.4, J(4′,5′B) = 5.2 (H-4′); 3.26 dd, 1 H, J(gem) = 15.0, J(H,P) = 16.4 and 2.83 dd, 1 H, J(gem) = 15.0, J(H,P) = 6.1 (N-CH₂-P); 3.10 dd, 1 H, J(5′A,4′) = 5.4, J(5′A,5′B) = 9.7 (H-5′A); 3.06 dd, 1 H, J(5′B,4′) = 5.2, J(5′B,5′A) = 9.7 (H-5′B); 2.93 dd, 1 H, J(2′B,1′) = 8.0, J(2′B,2′A) = 9.2 (H-2′B); 2.40 ddd, 1 H, J(4′aA,1′) = 6.8, J(4′aA,4′aB) = 13.4, J(4′aA,4′) = 8.8 (H-4′aA); 2.07 ddd, 1 H, J(4′aB,1′) = 8.8, J(4′aB,4′aA) = 13.4, J(4′aB,4′) = 5.8 (H-4′aB).

13C NMR (d₆DMSO):
158.28(2), 145.17, 135.85, 135.82, 129.95(4), 128.07(2), 127.91(2), 126.90 and 113.40(4) (2x C₆H₄ and C₆H₅ (ODMTr)); 156.12 (C-4); 152.48 (C-2); 149.57 (C-6); 139.68 (C-8); 119.28 (C-5); 86.00 (>C< (ODMTr)); 70.12 d, J(C,P) = 7.5, 69.70 d, J(C,P) = 6.3, 24.08 d, J(C,P) = 5.0, 24.00(2) d, J(C,P) = 5.0 and 23.88 d, J(C,P) = 5.0 (P(OiPr)₂); 66.09 (C-5′); 62.99 d, J(4′,P) = 17.6 (C-4′); 59.62 (C-2′); 55.23 (2x OMe (ODMTr)); 52.15 (C-1′); 49.90 d, J(C,P) = 174.6 (N-CH₂-P); 33.81 (C-4′a).

**Diisopropyl-[1-(2′,3′-dideoxy-5′-O-dimethoxytrityl-3′-aza-4a′-carba-α-L-glycero-pentofuranosyl)cytosine]-3′-N-methylphosphonate (80b)**

The general procedure A was followed using mesylate L-79 (1.51 g, 2.23 mmol) and cytosine (0.371 g, 3.34 mmol). NaH (60% suspension in oil, 0.107 g, 2.67 mmol, 1.2 eq.) and DMSO
were used instead of Cs$_2$CO$_3$/DMF. The reaction was complete after 3 h. White foam (0.400 g, 26 %).

HRMS (FAB) calcd for C$_{37}$H$_{48}$N$_4$O$_7$P [M+H]$^+$ 691.3261; found: 691.3241.

$^1$H NMR (d$_6$DMSO):

7.64 d, 1 H, $J$(6,5) = 7.5 (H-6); 7.38 m, 2 H, 7.31 m, 2 H, 7.25 m, 4 H, 7.23 m, 1H and 6.89 m, 4 H (2x C$_6$H$_4$ and C$_6$H$_5$ (ODMTr); 7.04 bs, 1 H and 6.99 bs, 1 H (NH$_2$); 5.68 d, 1 H, $J$(5,6) = 7.5 (H-5); 4.89 m, 1 H, J(1',2'A) = 6.8, J(1',2'B) = 7.8, J(1',4'aA) = 6.5, J(1',4'aB) = 9.5 (H-1'); 4.49 m, 2 H, 1.20 d, 3 H, J = 6.2, 1.18 d, 3 H, J = 6.2, 1.17 d, 3 H, J = 6.2 and 1.12 d, 3 H, J = 6.2 (P(OiPr)$_2$); 3.74 s, 6 H (2x OMe (ODMTr)); 3.39 dd, 1 H, $J$(2'A,1') = 6.8, $J$(2'A,2'B) = 9.0 (H-2'A); 3.16 dd, 1 H, 1 H, J(gem) = 15.0, J(H,P) = 16.0 and 2.76 dd, 1 H, J(gem) = 15.0, J(H,P) = 6.8 (N-CH$_2$-P); 3.15 m, 1 H, J(4',4'aA) = 9.5, J(4',4'aB) = 6.0, J(4',5'A) = 5.2, J(4',5'B) = 5.0 (H-4'); 3.03 dd, 1 H, J(5'A,4') = 5.2, J(5'A,5'B) = 9.8 (H-5'A); 3.01 dd, 1 H, J(5'B,4') = 5.0, J(5'B,5'A) = 9.8 (H-5'B); 2.58 dd, 1 H, J(2'B,1') = 7.8, J(2'B,2'A) = 9.0 (H-2'B); 1.96 ddd, 1 H, J(4'aA,1') = 6.5, J(4'aA,4'aB) = 13.0, J(4'aA,4') = 9.5 (H-4'aA); 1.86 ddd, 1 H, J(4'aB,1') = 9.5, J(4'aB,4'aA) = 13.0, J(4'aB,4') = 6.0 (H-4'aB).

$^{13}$C NMR (d$_6$DMSO):

165.42 (C-4); 158.24(2), 145.12, 135.80, 135.76, 129.89(4), 128.03(2), 127.85(2), 126.86 and 113.36(4) (2x C$_6$H$_4$ and C$_6$H$_5$ (ODMTr)); 155.84 (C-2); 142.99 (C-6); 93.98 (C-5); 85.94 (>C< (ODMTr)); 70.02 d, J(C,P) = 6.5, 69.62 d, J(C,P) = 6.2, 24.04 d, J(C,P) = 5.0, 23.94(2) d, J(C,P) = 4.9 and 23.85 d, J(C,P) = 4.3 (P(OiPr)$_2$); 65.72 (C-5'); 63.07 d, J(4',P) = 15.6 (C-4'); 59.04 (C-2'); 55.20 (2x OMe (ODMTr)); 53.61 (C-1'); 49.62 d, J(C,P) = 163.0 (N-CH$_2$-P); 33.42 (C-4'a).

Diisopropyl-[2-O-(2',3'-dideoxy-5'-O-dimethoxytrityl-3'-aza-4a'-carba-α-L-glycero-pentofuranosyl)cytosine]-3'-N-methylphosphonate (80c)

The general procedure A was followed using mesylate L-79 (1.59 g, 2.37 mmol), Cs$_2$CO$_3$ (0.922 g, 2.83 mmol) and cytosine (0.393 g, 3.54 mmol). The reaction was complete after 3 h. White foam (0.584 g, 36 %).

HRMS (FAB) calcd for C$_{37}$H$_{48}$N$_4$O$_7$P [M+H]$^+$ 691.3261; found: 691.3291.

$^1$H NMR (d$_6$DMSO):

7.84 d, 1 H, J(6,5) = 5.7 (H-6); 7.38 m, 2 H, 7.31 m, 2 H, 7.24 m, 4 H, 7.22 m, 1H and 6.89 m, 4 H (2x C$_6$H$_4$ and C$_6$H$_5$ (ODMTr); 6.79 bs, 2 H (NH$_2$); 6.06 d, 1 H, J(6,5) = 5.7 (H-6); 5.13 m, 1 H, J(1',2'A) = 5.9, J(1',2'B) = 4.2, J(1',4'aA) = 3.1, J(1',4'aB) = 7.2 (H-1'); 4.48 m, 2
H, 1.19 d, 3 H, J = 6.1, 1.17 d, 3 H, J = 6.2, 1.16 d, 3 H, J = 6.2 and 1.09 d, 3 H, J = 6.2 (P(OiPr)2); 3.73 s, 6 H (2x OMe (ODMTr)); 3.63 dd, 1 H, J(2′A,1′) = 5.9, J(2′A,2′B) = 11.0, (H-2′A); 3.28 dd, 1 H, 1 H, J(gem) = 15.0, J(H,P) = 16.4 and 2.70 dd, 1 H, J(gem) = 15.0, J(H,P) = 5.2 (N-CH2-P); 3.03 m, 1 H, J(4′,4′aA) = 6.6, J(4′,4′aB) = 8.2 (H-4′); 3.03 m, 1 H (H-5′A); 2.97 m, 1 H (H-5′B); 2.51 dd, 1 H, J(2′B,1′) = 4.2, J(2′B,2′A) = 11.0 (H-2′B); 1.92 ddd, 1 H, J(4′aA,1′) = 3.1, J(4′aA,4′aB) = 13.6, J(4′aA,4′) = 6.6 (H-4′aA); 1.74 ddd, 1 H, J(4′aB,1′) = 7.2, J(4′aB,4′aA) = 13.6, J(4′aB,4′) = 8.2 (H-4′aB).

13C NMR (d6DMSO):

166.56 (C-4); 164.50 (C-2); 158.25(2), 145.17, 135.88, 135.85, 129.90(4), 128.05(2), 127.88(2), 126.87 and 113.39(4) (2x C6H4 and C6H5 (ODMTr)); 156.38 (C-6); 99.63 (C-5); 85.91 (>C< (ODMTr)); 73.93 (C-1’); 70.04 d, J(C,P) = 7.5, 69.58 d, J(C,P) = 6.3, 24.09 d, J(C,P) = 3.8, 24.00 d, J(C,P) = 3.8, 23.96 d, J(C,P) = 5.0 and 23.83 d, J(C,P) = 5.0 (P(OiPr)2); 66.65 (C-5’); 63.52 d, J(4′,P) = 17.6 (C-4’); 61.53 (C-2’); 55.23 (2x OMe (ODMTr)); 51.13 d, J(C,P) = 163.4 (N-CH2-P); 35.88 (C-4’a).

Diisopropyl-[1-(2′,3′-dideoxy-5′-O-dimethoxytrityl-3′-aza-4′-carba-α-L-glycero-pentofuranosyl)thymine]-3′-N-methylphosphonate (80d)

The general procedure A was followed using mesylate L-79 (1.31 g, 1.94 mmol), Cs2CO3 (0.757 g, 2.32 mmol) and thymine (0.366 g, 2.90 mmol). The reaction was complete after 2 h. White foam (0.515 g, 38 %).


1H NMR (d6DMSO):

11.23 s, 1 H (NH); 7.585 q, 1 H, J(6,Me) = 1.2 (H-6); 7.38 m, 2 H, 7.32 m, 2 H, 7.25 m, 4 H, 7.23 m, 1H and 6.89 m, 4 H (2x C6H4 and C6H5 (ODMTr)); 4.87 m, 1 H, J(1′,2′A) = 7.2, J(1′,2′B) = 7.8, J(1′,4′aA) = 6.6, J(1′,4′aB) = 9.5 (H-1’); 4.50 m, 2 H, 1.202 d, 3 H, J = 6.2, 1.186 d, 3 H, J = 6.2, 1.179 d, 3 H, J = 6.2 and 1.129 d, 3 H, J = 6.2 (P(OiPr)2); 3.73 s, 3 H and 3.735 s, 3 H (2x OMe (ODMTr)); 3.39 dd, 1 H, J(2′A,1′) = 7.2, J(2′A,2′B) = 9.2 (H-2′A); 3.17 m, 1 H (H-4’); 3.15 dd, 1 H, 1 H, J(gem) = 15.2, J(H,P) = 15.8 and 2.79 dd, 1 H, J(gem) = 15.2, J(H,P) = 7.2 (N-CH2-P); 3.04 m, 2 H (H-5′A + H-5′B); 2.64 dd, 1 H, J(2′B,1′) = 7.8, J(2′B,2′A) = 9.2 (H-2′B); 2.01 ddd, 1 H, J(4′aA,1′) = 6.6, J(4′aA,4′aB) = 13.5, J(4′aA,4′) = 8.6 (H-4′aA); 1.88 ddd, 1 H, J(4′aB,1′) = 9.5, J(4′aB,4′aA) = 13.5, J(4′aB,4′) = 5.8 (H-4′aB); 1.78 d, 3 H, J(Me,6) = 1.2 (5-Me).

13C NMR (d6DMSO):
Diisopropyl-[9-(2',3'-dideoxy-5'-O-dimethoxytrityl-3'-aza-4a'-carba-α-L-glycero-pentofuranosyl)-2-amino-6-chloropurine]-3’-N-methylphosphonate (80e)

The general procedure A was followed using mesylate L-79 (1.278 g, 1.89 mmol), Cs₂CO₃ (0.740 g, 2.28 mmol) and 2-amino-6-chloropurine (0.481 g, 2.84 mmol). The reaction was complete after 3 h. White foam (0.581 g, 41%).

HRMS (FAB) calcd for C₃₈H₄₇N₆O₆PCl [M+H]+ 749.2983; found: 749.3002.

¹H NMR (d₆DMSO):
8.25 s, 1 H (H-8); 7.41 m, 2 H, 7.32 m, 2 H, 7.26 m, 4 H, 7.23 m, 1H and 6.90 m, 4 H (2x C₆H₄ and C₆H₅ (ODMTr)); ~6.90 bs, 2 H (NH₂); 4.83 m, 1 H, J(1’,2’A) = 6.6, J(1’,2’B) ≤ 2.0, J(1’,4’aA) = 8.5, J(1’,4’aB) = 6.0 (H-1’); 4.50 m, 2 H, 1.195 d, 3 H, J ≤ 2.0, J(1’A) = 1.17 d, 3 H, J = 6.0 and 1.115 d, 3 H, J = 6.1, 1.17 d, 3 H, J = 6.0 and 1.115 d, 3 H, J = 6.1 (P(OiPr)₂); 3.86 bd, 1 H, J(2’A,1’) ≤ 2.0, J(2’A,2’B) = 9.4, (H-2’A); 3.74 s, 6 H (2x OMe (ODMTr)); 3.26 m, 1 H, J(4’,4’aA) = 6.5, J(4’,4’aB) = 8.6, J(4’,5’A) = 5.5, J(4’,5’B) = 5.0 (H-4’); 3.24 dd, 1 H, 1 H, J(gem) = 15.2, J(H,P) = 16.4 and 2.84 dd, 1 H, J(H,P) = 16.4; 3.09 dd, 1 H, J(5’A,4’) = 5.5, J(5’A,5’B) = 9.7 (H-5’A); 3.04 dd, 1 H, J(5’B,4’) = 5.0, J(5’B,5’A) = 9.7 (H-5’B); 2.86 dd, 1 H, J(2’B,1’) = 6.6, J(2’B,2’A) = 9.4 (H-2’B); 2.36 ddd, 1 H, J(4’aA,1’) = 8.5, J(4’aA,4’aB) = 13.6, J(4’aA,4’A) = 6.5 (H-4’aA); 2.04 ddd, 1 H, J(4’aB,1’) = 6.0, J(4’aB,4’aA) = 13.6, J(4’aB,4’) = 8.6 (H-4’aB).

¹³C NMR (d₆DMSO):
159.78 (C-2); 158.28(2), 145.12, 135.80, 135.79, 129.95(4), 128.07(2), 127.90(2), 126.91 and 113.40(4) (2x C₆H₄ and C₆H₅ (ODMTr)); 154.13 (C-4); 149.58 (C-6); 141.74 (C-8); 123.75 (C-5); 86.01 (>C< (ODMTr)); 70.12 d, J(C,P) = 6.3, 69.72 d, J(C,P) = 7.5, 24.06 d, J(C,P) = 3.8, 23.98(2) d, J(C,P) = 3.8 and 23.87 d, J(C,P) = 5.0 (P(OiPr)₂); 66.02 (C-5’); 62.82 d, J(4’,P) = 16.4 (C-4’); 59.33 (C-2’); 55.24 (2x OMe (ODMTr)); 51.99 (C-1’); 49.80 d, J(C,P) = 162.2 (N-CH₂-P); 33.65 (C-4’a).
Diisopropyl-[9-(2',3'-dideoxy-5'-O-dimethoxytrityl]-3'-aza-4a'-carba-α-L-glycero-pentofuranosyl)-2-amino-6-azidopurine]-3'-N-methylphosphonate (81)

To a solution of 80e (0.342 g, 0.46 mmol) in anhydrous DMF (10 mL) was added NaN₃ (0.297 g, 4.57 mmol, 10 eq.). The reaction mixture was stirred for 3 h at 110 °C (TLC in C1), after which DMF was evaporated, the residue was suspended in CHCl₃ and the precipitate removed by filtration. Removal of solvent in vacuo afforded the crude product, which was purified by flash chromatography on silica (elution with a linear gradient of EtOH in CHCl₃) to yield 81 as a colourless glassy solid (0.205 g, 59 %).


¹H NMR (d₆DMSO):
8.36 bs, 2 H (NH₂); 8.30 s, 1 H (H-8); 7.41 m, 2 H, 7.33 m, 2 H, 7.27 m, 4 H, 7.24 m, 1H and 6.90 m, 4 H (2x C₆H₄ and C₆H₅ (ODMTr)); 4.98 m, 1 H, J(1’,2’A) = 6.7, J(1’,2’B) 8.0, J(1’,4’aA) = 8.8, J(1’,4’aB) = 6.3 (H-1’); 4.51 m, 2 H, 1.20 d, 3 H, J = 6.2, 1.19 d, 3 H, J = 6.2, 1.17 d, 3 H, J = 6.2 and 1.13 d, 3 H, J = 6.2 (P(OiPr)₂); 3.74 s, 6 H (2x OMe (ODMTr)); 3.69 dd, 1 H, J(2’A,1’) 6.7, J(2’A,2’B) = 9.2 (H-2’A); 3.30 m, 1 H, J(4’,4’aA) = 6.5, J(4’,4’aB) = 9.2, J(4’,5’A) = 5.5, J(4’,5’B) = 5.2 (H-4’); 3.26 dd, 1 H, J(gem) = 15.0, J(H,P) = 16.0 and 2.86 dd, 1 H, J(gem) = 15.0, J(H,P) = 6.2 (N-CH₂-P); 3.12 dd, 1 H, J(5’A,4’) = 5.5, J(5’A,5’B) = 9.7 (H-5’A); 3.07 dd, 1 H, J(5’B,4’) = 5.2, J(5’B,5’A) = 9.7 (H-5’B); 2.91 dd, 1 H, J(2’B,1’) = 8.0, J(2’B,2’A) = 9.2 (H-2’B); 2.39 ddd, 1 H, J(4’aA,1’) = 8.8, J(4’aA,4’aB) = 13.4, J(4’aA,4’a’) = 6.5 (H-4’aA); 2.11 ddd, 1 H, J(4’aB,1’) = 6.3, J(4’aB,4’aA) = 13.4, J(4’aB,4’a’) = 9.2 (H-4’aB).

¹³C NMR (d₆DMSO):
158.28(2), 145.12, 135.80, 135.79, 129.95(4), 128.07(2), 127.90(2), 126.91 and 113.40(4) (2x C₆H₄ and C₆H₅ (ODMTr)); 146.23 (C-2); 144.81 (C-4); 143.67 (C-6); 138.54 (C-8); 112.14 (C-5); 86.01 (>C< (ODMTr)); 70.09 d, J(C,P) = 6.8, 69.70 d, J(C,P) = 6.7, ~24.06 4x d, J(C,P) = 3.8 (P(OiPr)₂); 66.04 (C-5’); 62.91 d, J(4’,P) = 16.2 (C-4’); 59.84 (C-2’); 55.21 (2x OMe (ODMTr)); 52.34 (C-1’); 49.44 d, J(C,P) = 149.1 (N-CH₂-P); 34.10 (C-4’a).

Diisopropyl-[9-(2’,3’-dideoxy-5’-O-dimethoxytrityl]-3’-aza-4a’-carba-α-L-glycero-pentofuranosyl)-2,6-diaminopurine]-3’-N-methylphosphonate (82)

To a solution of 81 (0.205 g, 0.27 mmol) in MeOH (50 mL) and conc. HCl (1 mL) was added 10% Pd on charcoal (0.1 g), and the vigorously stirred reaction mixture was left to react under an atmosphere of hydrogen at rt for 5 h (TLC in H1). The catalyst was filtered off and the solvent removed under reduced pressure. The crude residue was purified by flash...
chromatography on silica (elution with a linear gradient of H1 in EtOAc) to afford the desired product 82 as a colourless glassy solid (0.072 g, 62 %).

HRMS (FAB) calcd for C17H31N7O4P [M+H]+ 428.2175; found: 428.2188.

1H NMR (d6DMSO):

7.83 s, 1 H (H-8); 6.62 bs, 2 H and 5.73 bs, 2H (2x NH2); 4.78 m, 1 H, J(1’,2’A) = 6.7, J(1’,2’B) 8.0, J(1’,4’aA) = 7.0, J(1’,4’aB) = 8.7 (H-1’); 4.59 m, 2 H, 1.251 d, 3 H, J = 6.2, 1.247 d, 3 H, J = 6.2, 1.243 d, 3 H, J = 6.2 and 1.240 d, 3 H, J = 6.2 (P(OiPr)2); 3.56 dd, 1 H, J(2’A,1’) 6.7, J(2’A,2’B) = 9.0 (H-2’A); 3.47 dd, 1 H, J(5’A,4’) = 5.2, J(5’A,5’B) = 10.8 (H-5’A); 3.40 dd, 1 H, J(5’B,4’) = 5.3, J(5’B,5’A) = 10.8 (H-5’B); 3.06 m, 1 H, J(4’,4’aA) = 8.7, J(4’,4’aB) = 5.8, J(4’,5’A) = 5.2, J(4’,5’B) = 5.3 (H-4’); 3.29 t, 1 H, J(gem) = 15.3, J(H,P) = 15.3 and 2.91 dd, 1 H, J(gem) = 15.3, J(H,P) = 7.0 (N-CH2-P); 2.86 dd, 1 H, J(2’B,1’) = 8.0, J(2’B,2’A) = 9.0 (H-2’B); 2.23 ddd, 1 H, J(4’aA,1’) = 7.0, J(4’aA,4’aB) = 13.2, J(4’aA,4’) = 8.7 (H-4’aA); 2.07 ddd, 1 H, J(4’aB,1’) = 8.7, J(4’aB,4’aA) = 13.2, J(4’aB,4’) = 5.8 (H-4’aB).

13C NMR (d6DMSO):

160.21 (C-2); 156.24 (C-6); 151.84 (C-4); 135.70 (C-8); 113.53 (C-5); 70.01 d, J(C,P) = 6.8, 69.79 d, J(C,P) = 6.4, ~24.00 4x d, J(C,P) = 3.9 (P(OiPr)2); 64.76 d, J(4’,P) = 14.2 (C-4’); 63.55 (C-5’); 59.85 (C-2’); 51.15 (C-1’); 49.26 d, J(C,P) = 164.1 (N-CH2-P); 33.81 (C-4’a).

Diisopropyl-[9-(2’,3’-dideoxy-5’-O-dimethoxytrityl-3’-aza-4a’-carba-β-L-glycero-pentofuranosyl)adenine]-3’-N-methylphosphonate (85a)

The general procedure A was followed using mesylate L-76 (1.34 g, 1.98 mmol), Cs2CO3 (0.78 g, 2.38 mmol) and adenine (0.40 g, 2.98 mmol). The reaction was complete after 4 h. White foam (0.530 g, 37 %).


1H NMR (d6DMSO):

8.26 s, 1 H (H-8); 8.12 s, 1 H (H-2); 7.30 m, 2 H, 7.26 m, 2 H, 7.19 m, 1 H, 7.17 m, 2 H, 7.16 m, 2 H and 6.83 m, 4 H (2x C6H4 and C6H5 (ODMTr); 7.22 bs, 2 H (NH2); 4.97 m, 1 H, J(1’,2’A) ~ 1.0, J(1’,2’B) = 5.7, J(1’,4’aA) = 8.4, J(1’,4’aB) = 2.4 (H-1’); 4.56 m, 2 H, 1.24 d, 3 H, J = 6.1, 1.20 d, 3 H, J = 6.1, 1.195 d, 3 H, J = 6.1 and 1.16 d, 3 H, J = 6.1 (P(OiPr)2); 3.72 s, 6 H (2x OMe (ODMTr)); 3.63 bd, 1 H, J(2’A,1’) ~ 1.0, J(2’A,2’B) = 10.8 (H-2’A); 3.41 dd, 1 H, J(gem) = 14.8, J(H,P) = 18.8 and 2.66 dd, 1 H, J(gem) = 14.8, J(H,P) = 4.6 (N-CH2-P); 3.03 d, 2 H, J(5’A,4’) ~ J(5’B,4’) ~ 5.2 (H-5’A +H-5’B); 2.84 dd, 1 H, J(2’B,1’) = 5.7, J(2’B,2’A) = 10.8 (H-2’B); 2.81 m, 1 H, J(4’,4’aA) = 8.8, J(4’,4’aB) = 7.0, J(4’,5’A) ~
Diisopropyl-[2-(2',3'-dideoxy-5'-O-dimethoxytrityl-3'-aza-4a'-carba-β-L-glycero-pentofuranosyl)cytosine]-3’-N-methylphosphonate (85b)

The general procedure A was followed using mesylate L-76 (1.41 g, 2.09 mmol), Cs$_2$CO$_3$ (0.82 g, 2.51 mmol) and cytosine (0.35 g, 3.13 mmol). The reaction was complete after 4 h. Purification by flash chromatography afforded N-alkylated 85b (white foam, 0.388 g, 27 %) and O-alkylated diisopropyl-[2-O-(2',3'-dideoxy-5'-O-dimethoxytrityl-3'-aza-4a'-carba-β-L-glycero-pentofuranosyl)cytosine]-3'-N-methylphosphonate 85c (white foam, 0.475 g, 33 %).

85b: HRMS (FAB) calcd for C$_{37}$H$_{48}$N$_4$O$_7$P [M+H]$^+$ 691.3261; found: 691.3259.

1H NMR (d$_6$DMSO):
7.99 d, 1 H, $J$(6,5) = 7.4 (H-6); 7.36 m, 2 H, 7.30 m, 2 H, 7.23 m, 2 H, 7.22 m, 3H and 6.87 m, 4 H (2x C$_6$H$_4$ and C$_6$H$_5$ (ODMTr)); 7.04 bs, 1 H and 6.96 bs, 1 H (NH); 5.62 d, 1 H, $J$(5,6) = 7.4 (H-5); 4.89 m, 1 H, $J$ (1',2'A) ~ 0, $J$(1',2'B) = 6.7, $J$(1',4'aA) = 8.8, $J$(1',4'aB) = 2.8 (H-1'); 4.56 m, 2 H, 1.23 d, 3 H, $J$ = 6.3, 1.22 d, 3 H, $J$ = 6.3, 1.20 d, 3 H, $J$ = 6.3 and 1.16 d, 3 H, $J$ = 6.3 (P(OiPr)$_2$); 3.73 s, 6 H (2x OMe (ODMTr)); 3.30 dd, 1 H, $J$(gem) = 14.9, $J$(H,P) = 19.3 and 2.47 dd, 1 H, $J$(gem) = 14.8, $J$(H,P) = 4.8 (N-CH$_2$-P); 3.09 dd, 1 H, $J$(5’,A,4’) = 3.9, $J$(5’,A,5’) = 10.2 (H-5’A); 2.95 dd, 1 H, $J$(5’B,4’) = 4.8, $J$(5’B,5’A) = 10.2 (H-5’B); 2.63 m, 1 H, $J$(4’,4’aA) = 9.0, $J$(4’,4’aB) = 3.9, $J$(4’,5’A) = 3.9, $J$(4’,5’B) = 4.8 (H-4’); 2.62 dd, 1 H, $J$(2’B,1’) = 6.7, $J$(2’B,2’A) = 11.0 (H-2’B); 2.36 ddd, 1 H, $J$(4’aA,1’) = 8.8, $J$(4’aA,4’aB) = 14.0, $J$(4’aA,4’) = 9.0 (H-4’aA); 1.47 ddd, 1 H, $J$(4’aB,1’) = 2.8, $J$(4’aB,4’aA) = 14.0, $J$(4’aB,4’) = 7.8 (H-4’aB).

13C NMR (d$_6$DMSO):
165.49 (C-4); 158.25, 158.24, 145.10, 135.86, 135.79, 129.90(2), 129.87(2), 128.00(2), 127.9(2), 126.86, 113.35(2) and 113.33(2) (2x C$_6$H$_4$ and C$_6$H$_5$ (ODMTr)); 155.83 (C-2); 142.62 (C-6); 93.55 (C-5); 85.79 (>C< (ODMTr)); 70.04 d, $J$(C,P) = 6.3, 69.82 d, $J$(C,P) = 6.3.
7.5, 24.06 d, J(C,P) = 5.0, 24.01 d J(C,P) = 5.0, 24.00 d J(C,P) = 5.0 and 23.90 d, J(C,P) = 5.0
(P(OiPr)_2); 64.50 d, J(4',P) = 18.9 (C-4'); 63.73 (C-5'); 60.03 (C-2'); 55.22 (2x OMe
(ODMTr)); 52.26 (C-1'); 49.50 d, J(C,P) = 170.5 (N-CH2-P); 35.43 (C-4'a).

85c: HRMS (FAB) calcd for C_{37}H_{48}N_{4}O_{7}P [M+H]^+ 691.3261; found: 691.3270.

^1H NMR (d_6DMSO):
7.81 d, 1 H, J(6,5) = 5.7 (H-6); 7.37 m, 2 H, 7.30 m, 2 H, 7.24 m, 4 H, 7.22 m, 1 H and 6.88 m, 4 H (2x C_6H_4 and C_6H_5 (ODMTr)); 6.87 bs, 1 H and 6.76 bs, 1 H (NH_2); 6.04 d, 1 H, J(5,6) = 5.7 (H-5); 5.15 m, 1 H, J(1',2'A) = 1.6, J(1',2'B) = 5.5, J(1',4'aA) = 7.7, J(1',4'aB) = 3.6 (H-1'); 4.48 m, 2 H, 1.185 d, 3 H, J = 6.2, 1.15 d, 6 H, J = 6.2 and 1.105 d, 3 H, J = 6.2 (P(OiPr)_2); 3.73 s, 6 H (2x OMe (ODMTr)); 3.35 dd, 1 H, J(2'A,1') = 1.6, J(2'A,2'B) = 11.2 (H-2'A); 3.33 dd, 1 H, 1 H, J(gem) = 15.5 and 2.57 dd, 1 H, J(gem) = 15.5, J(H,P) = 4.8 (N-
CH_2-P); 3.09 dd, 1 H, J(5'S,A,4') = 6.2, J(5'S,A,5'B) = 9.6 (H-5'S); 3.04 dd, 1 H, J(5'B,4') = 5.0, J(5'B,5'A) = 9.6 (H-5'B); 2.72 m, 1 H, J(4',4'aA) = 7.7, J(4',4'aB) = 8.2, J(4',5'S) = 6.2, J(4',5'B) = 5.0 (H-4'); 2.61 dd, 1 H, J(2'B,1') = 5.5, J(2'B,2'A) = 11.2 (H-2'B); 2.26 dt, 1 H, J(4'aA,1') = 7.7, J(4'aA,4'aB) = 13.8, J(4'aA,4') = 7.7 (H-4'aA); 1.46 ddd, 1 H, J(4'aB,1') = 3.6, J(4'aB,4'aA) = 13.8, J(4'aB,4') = 8.2 (H-4'aB).

^13C NMR (d_6DMSO):
165.51 (C-4); 164.65 (C-2); 158.24(2), 135.88, 135.87, 129.88(4), 128.03(2),
127.87(2), 126.85, 113.37(2) and 113.36(2) (2x C_6H_4 and C_6H_5 (ODMTr)); 156.30 (C-6); 99.45 (C-5); 85.92 (>C< (ODMTr)); 73.96 (C-1'); 70.16 d, J(C,P) = 6.3, 69.54 d, J(C,P) =
6.3, 24.08 d, J(C,P) = 3.8, 24.00 d J(C,P) = 3.8, 23.94 d J(C,P) = 5.0 and 23.85 d, J(C,P) = 5.0 (P(OiPr)_2); 66.96 (C-5'); 64.18 d, J(4',P) = 17.6 (C-4'); 61.81 (C-2'); 55.21 (2x OMe
(ODMTr)); 50.62 d, J(C,P) = 163.4 (N-CH_2-P); 35.81 (C-4'a).

Diisopropyl-[1-(2',3'-dideoxy-5'-O-dimethoxytrityl-3'-aza-4a'-carba-β-L-glycero-
-pentofuranosyl)thymine]-3'-N-methylphosphonate (85d)
The general procedure A was followed using mesylate L-76 (1.35 g, 2.00 mmol), Cs_2CO_3 (0.78 g, 2.40 mmol) and thymine (0.38 g, 3.00 mmol). The reaction was complete after 3 h.
White foam (0.554 g, 39 %).

HRMS (FAB) calcd for C_{38}H_{49}N_{3}O_{7}P [M+H]^+ 706,3257; found: 706,3269.

^1H NMR (d_6DMSO):
11.20 bs, 1 H (NH); 7.76 q, 1 H, J(6,Me) = 1.2 (H-6); 7.35 m, 2 H, 7.28 m, 2 H, 7.24 m, 2 H, 7.22 m, 4 H, 6.87 m, 2 H and 6.86 m, 2 H (2x C_6H_4 and C_6H_5 (ODMTr)); 4.83 m, 1 H,
J(1',2'\text{A}) \leq 1.0, J(1',2'\text{B}) = 6.6, J(1',4'\text{aA}) = 9.2, J(1',4'\text{aB}) = 3.0 (H-1'); 4.55 m, 2 H, 1.22 d, 3 H, J = 6.1, 1.21 d, 3 H, J = 6.2, 1.19 d, 3 H, J = 6.2 and 1.14 d, 3 H, J = 6.2 (P(OiPr)_2);
3.72 s, 6 H (2x OMe (ODMTr)); 3.36 dd, 1 H, 1 H, J(gem) = 14.8, J(H,P) = 10.0 and 2.52 dd, 1 H, J(gem) = 14.8, J(H,P) = 5.0 (N-CH_2-P); 3.33 bd, 1 H, J(2'\text{A},1') \leq 1.0, J(2'\text{A},2'\text{B}) = 11.2 (H-2'\text{A}); 3.05 d, 2 H, J(5'\text{A},4') \sim J(5'\text{B},4') \sim 4.8 (H-5'\text{A} + H-5'\text{B}); 2.70 m, 1 H, J(4',4'\text{aA}) = 8.2, J(4',4'\text{aB}) = 7.5, J(4',5'\text{A}) \sim J(4',5'\text{A}) \sim 4.8 (H-4'); 2.65 dd, 1 H, J(2'\text{B},1') = 6.6, J(2'\text{B},2'\text{A}) = 11.2 (H-2'\text{B}); 2.39 ddd, 1 H, J(4'\text{aA},1') = 9.2, J(4'\text{aA},4'\text{aB}) = 14.0, J(4'\text{aA},4') = 8.2 (H-4'\text{aA}); 1.50 ddd, 1 H, J(4'\text{aB},1') = 3.0, J(4'\text{aB},4'\text{aA}) = 14.0, J(4'\text{aB},4') = 7.5 (H-4'\text{aB}); 1.62 d, 3 H, J(Me,6) = 1.2 (5-Me).

\textsuperscript{13}C NMR (d_6DMSO):
163.95 (C-2); 158.26, 158.22, 145.08, 135.76(2), 129.90(2), 129.87(2), 128.00(2), 127.89(2), 126.89, 113.35(2) and 113.32 (2x C_6H_4 and C_6H_5 (ODMTr)); 151.06 (C-4); 137.94 (C-6); 108.95 (C-5); 85.85 (>C< (ODMTr)); 70.06 d, J(C,P) = 6.3, 69.84 d, J(C,P) = 6.3, 24.02 d, 3 H, J(C,P) = 3.8, 23.96 d, 6 H, J(C,P) = 3.8, 23.92 d, 3 H, J(C,P) = 3.8 (P(OiPr)_2); 64.55 (C-5'); 64.39 d, J(4',P) = 17.6 (C-4'); 59.69 (C-2'); 55.20 (2x OMe (ODMTr)); 52.22 (C-1'); 49.83 d, J(C,P) = 163.5 (N-CH_2-P); 35.19 (C-4'a); 12.57 (5-Me).

Diisopropyl-[9-(2',3'-dideoxy-5'-O-dimethoxytrityl-3'-aza-4a'-carba-\beta-L-glycero-pentofuranosyl)-2-amino-6-chloropurine]-3'-N-methylphosphonate (85e)
The general procedure \textbf{A} was followed using mesylate \textbf{L-76} (2.95 g, 4.37 mmol), Cs_2CO_3 (1.71 g, 5.24 mmol) and 2-amino-6-chloropurine (1.11 g, 6.55 mmol). The reaction was complete after 4 h. White foam (1.317 g, 40 %).

HRMS (FAB) calcd for C_{38}H_{45}N_6O_6PCl [M-H]^- 747.2827; found: 747.2843.

\textsuperscript{1}H NMR (d_6DMSO):
8.25 s, 1 H (H-8); 7.28 m, 2 H, 7.25 m, 2 H, 7.19 m, 1 H, 7.16 m, 2 H, 7.15 m, 2 H and 6.82 m, 4 H (2x C_6H_4 and C_6H_5 (ODMTr)); 6.90 bs, 2 H (NH_2); 4.81 m, 1 H, J(1',2'\text{A}) \sim 1.0, J(1',2'\text{B}) = 5.2, J(1',4'\text{aA}) = 8.0, J(1',4'\text{aB}) \sim 2.0 (H-1'); 4.56 m, 2 H, 1.24 d, 3 H, J = 6.1, 1.21 d, 3 H, J = 6.0, 1.20 d, 3 H, J = 6.0 and 1.16 d, 3 H, J = 6.1 (P(OiPr)_2); 3.72 s, 6 H (2x OMe (ODMTr)); 3.62 bd, 1 H, J(2'\text{A},1') \sim 1.0, J(2'\text{A},2'\text{B}) = 10.7 (H-2'\text{A}); 3.40 dd, 1 H, J(gem) = 15.0, J(H,P) = 18.8 and 2.66 dd, 1 H, J(gem) = 15.0, J(H,P) = 4.5 (N-CH_2-P); 3.02 dd, 1 H, J(5'\text{A},4') = 5.2; J(5'\text{A},5'B) = 10.0 (H-5'\text{A}); 2.99 dd, 1 H, J(5'\text{B},4') = 4.7; J(5'\text{B},5'\text{A}) = 10.0 (H-5'\text{B}); 2.81 dd, 1 H, J(2'\text{B},1') = 5.2, J(2'\text{B},2'\text{A}) = 10.7 (H-2'\text{B}); 2.80 m, 1 H, J(4',4'\text{aA}) = 9.0, J(4',4'\text{aB}) = 5.2, J(4',5'\text{A}) = 5.2, J(4',5'\text{B}) = 4.7 (H-4'); 2.47 ddd, 1 H,
\[ J(4'aA,1') = 8.0, \quad J(4'aA,4'aB) = 14.0, \quad J(4'aA,4') = 9.0 \quad \text{(H-4'aA)}; \quad 1.70 \text{ ddd}, \quad 1 H, \quad J(4'aB,1') = \sim 2.0, \quad J(4'aB,4'aA) = 14.0, \quad J(4'aB,4') = 6.5 \quad \text{(H-4'aB)}. \]

\[ 1^3\text{C NMR (d}_6\text{DMSO)}: \]

159.88 (C-2); 158.20, 158.19, 144.95, 135.82, 135.71, 129.83(2), 129.80(2), 127.93(2), 127.88(2), 126.83, 113.29(2) and 113.27(2) (2x C\textsubscript{6}H\textsubscript{4} and C\textsubscript{6}H\textsubscript{5} (ODMTr)); 153.90 (C-4); 149.40 (C-6); 140.90 (C-8); 123.54 (C-5); 85.81 (>C< (ODMTr)); 70.12 \text{ d}, \quad J(C,P) = 6.3, \quad 69.94 \text{ d}, \quad J(C,P) = 6.3, \quad 24.04 \text{ d}, \quad J(C,P) = 5.0, \quad 24.01(2) \text{ d}, \quad J(C,P) = 5.0 \quad \text{and} \quad 23.93 \text{ d}, \quad J(C,P) = 5.0 \quad \text{(P(OiPr)}_2); \quad 64.78 \quad (C-5'); \quad 63.84 \text{ d}, \quad J(4',P) = 18.9 \quad (C-4'); \quad 59.82 \quad (C-2'); \quad 55.20 \quad (2x \text{ OMe (ODMTr)}); \quad 52.07 \quad (C-1'); \quad 49.73 \text{ d}, \quad J(C,P) = 163.5 \quad (N-CH}_2\text{-P); \quad 35.14 \quad (C-4'a). 

\[ \text{Diisopropyl-[9-(2',3'-dideoxy-5'-O-dimethoxytrityl-3'-aza-4a'-carba-\beta-1-glycero-pentofuranosyl]-2-amino-6-azidopurine-3'-N-methylphosphonate (86)} \]

Using the procedure outlined for \textbf{81}, compound \textbf{86} was prepared from \textbf{85e} (0.767 g, 1.02 mmol) and NaN\textsubscript{3} (0.666 g, 10.24 mmol) as a colourless glassy solid (0.503 g, 65 %).

\[ \text{HRMS (FAB) calcd for C}_{38}\text{H}_{45}\text{N}_{9}\text{O}_{6}\text{P} \quad [\text{M-H}]- \quad 754.3230; \quad \text{found:} \quad 754.3244. \]

\[ \text{1H NMR (d}_6\text{DMSO):} \]

8.37 bs, 2 H (NH\textsubscript{2}); 8.28 s, 1 H (H-8); 7.30 m, 2 H, 7.24 m, 2 H, 7.16 m, 5 H and 6.80 m, 4 H (2x C\textsubscript{6}H\textsubscript{4} and C\textsubscript{6}H\textsubscript{5} (ODMTr)); 4.97 m, 1 H, \( J(1',2'A) \leq 2.0, \quad J(1',2'B) = 5.6, \quad J(1',4'aA) = 8.2, \quad J(1',4'aB) \sim 2.0 \quad \text{(H-1')}; \quad 4.57 m, 2 H, 1.25 d, 3 H, \quad J = 6.2, \quad 1.21 d, \quad 3 H, \quad J = 6.2, \quad 1.205 d, \quad 3 H, \quad J = 6.2 \quad \text{and} \quad 1.16 d, \quad 3 H, \quad J = 6.2 \quad \text{(P(OiPr)}_2); \quad 3.79 s, \quad 3 H \quad \text{and} \quad 3.685 s, \quad 3 H \quad \text{(2x OMe (ODMTr));} \quad 3.66 \text{ bd}, \quad 1 H, \quad J(2'A,1') \leq 2.0, \quad J(2'A,2'B) = 11.0 \quad \text{(H-2'A);} \quad 3.44 dd, \quad 1 H, \quad J(\text{gem}) = 15.3, \quad J(H,P) = 18.8 \quad \text{and} \quad 2.69 dd, \quad 1 H, \quad J(\text{gem}) = 15.3, \quad J(H,P) = 4.5 \quad \text{(N-CH}_2\text{-P);} \quad 3.12 dd, \quad 1 H, \quad J(5'S,4'A') = 5.4; \quad J(5',5'B) = 10.0 \quad \text{(H-5'S);} \quad 2.98 dd, \quad 1 H, \quad J(5'B,4') = 5.2; \quad J(5'B,5'A) = 10.0 \quad \text{(H-5'B);} \quad 2.88 dd, \quad 1 H, \quad J(2'B,1') = 5.6, \quad J(2'B,2'A) = 11.0 \quad \text{(H-2'B);} \quad 2.84 m, \quad 1 H, \quad J(4',4'aA) \leq 2.0, \quad J(4',4'aB) = 7.0, \quad J(4',5'A) = 5.4, \quad J(4',5'B) = 5.2 \quad \text{(H-4');} \quad 2.55 ddd, \quad 1 H, \quad J(4'aA,1') = 8.2, \quad J(4'aA,4'aB) = 14.0, \quad J(4'aA,4') \leq 2.0 \quad \text{(H-4'aA);} \quad 1.66 ddd, \quad 1 H, \quad J(4'aB,1') = \sim 2.0, \quad J(4'aB,4'aA) = 14.0, \quad J(4'aB,4') = 7.0 \quad \text{(H-4'aB).} \]

\[ \text{13C NMR (d}_6\text{DMSO):} \]

158.13(2), 144.87, 135.69, 135.62, 129.74(4), 127.82(2), 127.79(2), 126.69 and 113.19(4) (2x C\textsubscript{6}H\textsubscript{4} and C\textsubscript{6}H\textsubscript{5} (ODMTr)); C-2 not observed; 146.16 (C-6); 143.66 (C-4); 138.02 (C-8); 111.68 (C-5); 85.86 (>C< (ODMTr)); 70.02 d, \quad J(C,P) = 6.8, \quad 69.82 d, \quad J(C,P) = 6.8, \quad 23.95-23.79 4x d, \quad J(C,P) = 4.0 \quad \text{(P(OiPr)}_2); \quad 65.20 \quad (C-5'); \quad 63.78 d, \quad J(4',P) = 17.6 \quad (C-4'); \quad 60.14 \quad (C-2'); \quad 52.37 \quad (C-1'); \quad 55.12 \quad (2x \text{ OMe (ODMTr));} \quad 49.79 d, \quad J(C,P) = 161.7 \quad \text{(N-CH}_2\text{-P);} \quad 35.68 \quad (C-4'a).
Diisopropyl-[9-(2',3'-dideoxy-5'-O-dimethoxytrityl-3'-aza-4a'-carba-β-L-glycero-pentofuranosyl)-2,6-diaminopurine]-3'-N-methylphosphonate (87)

Using the procedure outlined for 82, compound 87 was prepared from 86 (0.503 g, 0.67 mmol) as a colourless glassy solid (0.188 g, 66 %).

HRMS (FAB) calcd for C_{17}H_{31}N_{7}O_{4}P [M+H]^+ 428.2175; found: 428.2167.

$^1$H NMR (d$_6$DMSO):
7.94 s, 1 H (H-8); 6.62 bs, 2 H (NH$_2$); 5.72 bs, 2 H (NH$_2$); 4.77 m, 1 H, $J$(1',2'A) ~ 1.0, $J$(1',2'B) = 5.8, $J$(1',4'aA) = 8.7, $J$(1',4'aB) = 2.6 (H-1'); 4.71 bs, 1 H (5'-OH); 4.59 m, 2 H, 1.26 d, 3 H, $J$ = 6.0, 1.245 d, 3 H, $J$ = 6.0, 1.24 d, 3 H, $J$ = 6.0 and 1.21 d, 3 H, $J$ = 6.0 (P(OiPr)$_2$); 3.49 dd, 1 H, $J$(5'A,4') = 4.8; $J$(5'A,5'B) =11.0 (H-5'A); 3.46 bd, 1 H, $J$(2'A,1') ~ 1.0, $J$(2'A,2'B) = 10.5; $J$(2'A,4'aB) ≤ 2 (H-2'A); 3.44 dd, 1 H, $J$(gem) = 15.2, $J$(H,P) = 17.7 and 2.66 dd, 1 H, $J$(gem) = 15.2, $J$(H,P) = 5.3 (N-CH$_2$-P); 3.41 dd, 1 H, $J$(5'B,4') = 4.8; $J$(5'B,5'A) =11.0 (H-5'B); 2.76 dd, 1 H, $J$(2'B,1') = 5.8, $J$(2'B,2'A) = 10.5 (H-2'B); 2.64 m, 1 H, $J$(4',4'aA) = 8.7, $J$(4',4'aB) = 7.0, $J$(4',5'A) = 4.8, $J$(4',5'B) = 4.8 (H-4'); 2.48 ddd, 1 H, $J$(4'aA,1') = 8.7, $J$(4'aA,4'aB) = 14.0, $J$(4'aA,4') = 8.7 (H-4'aA); 1.68 ddd, 1 H, $J$(4'aB,1') = 2.6, $J$(4'aB,2'A) ≤ 2, $J$(4'aB,4'aA) = 14.0, $J$(4'aB,4') = 7.0 (H-4'aB).

$^{13}$C NMR (d$_6$DMSO):
160.29 (C-2); 156.19 (C-6); 151.57 (C-4); 135.44 (C-8); 113.08 (C-5); 70.12 d, $J$(C,P) = 6.7, 69.85 d, $J$(C,P) = 6.7, 24.06 d, $J$(C,P) = 5.5, 24.03 d, $J$(C,P) = 5.8, 23.93(2) d, $J$(C,P) = 4.9 (P(OiPr)$_2$); 65.98 d, $J$(4',P) = 16.5 (C-4'); 63.35 (C-5'); 61.22 (C-2'); 50.76 (C-1'); 49.68 d, $J$(C,P) = 164.1 (N-CH$_2$-P); 35.55 (C-4'a).

Phosphonate group dealkylation (General procedure B)

To a solution of protected nucleotide in anhydrous acetonitrile (10 mL/mmol) was added bromotrimethylsilane (8 eq.), and the reaction was allowed to proceed at rt for 48 h under exclusion of moisture (TLC in I). The reaction was then concentrated in vacuo, residue dissolved in small amount of water, and the solution was passed through a column of Dowex 50 (H$^+$-form), which was then washed successively with MeOH, and a mixture of H$_2$O-MeOH (1:1). The product was liberated from Dowex with diluted (approx. 2.5 %) aqueous ammonia, evaporated in vacuo and purified by reverse phase chromatography. Finally, the nucleotide was passed through a column of Dowex 50 (Na$^+$-form) and the sodium salt obtained was lyophilised from water.
9-(2',3'-Dideoxy-3'-aza-4a'-carba-α-L-glycero-pentofuranosyl)adenine-3'-N-methylphosphonic acid (84a)

The general procedure B was followed using phosphonate 80a (0.709 g, 0.99 mmol), and Me$_3$SiBr (1.05 mL, 7.96 mmol). White solid (0.177 g, 48 %).

HRMS (FAB) calcd for C$_{11}$H$_{18}$N$_6$O$_4$P [M+H]$^+$ 329.1127; found: 329.1121.

IR $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3435 (vs, br) (OH, H$_2$O, NH$_2$); 1641 (s), 1605 (m, sh), 1574 (w), 1416 (w), 1332 (w), 1303 (w), 975 (w), 647 (w), 798 (w), 728 (w) (adenine); 1067 (w, br), 903 (w, br), 545 (m, br), 465 (w, br) (PO$_3$$^2$)

$^1$H NMR (D$_2$O):
8.24 s, 1 H (H-8); 8.21 s, 1 H (H-2); 5.31 m, 1 H, $J$(1',2'A) = 7.6, $J$(1',2'B) = 7.3, $J$(1',4'aA) = 9.2, $J$(1',4'aB) = 5.8 (H-1'); 4.23 dd, 1 H, $J$(2'A,1') = 7.6, $J$(2'A,2'B) = 12.0 (H-2'A); 4.12 m, 1 H, $J$(4',4'aA) = 7.3, $J$(4',4'aB) = 8.7, $J$(4',5'A) = 3.5, $J$(4',5'B) = 3.6 (H-4'); 4.07 dd, 1 H, $J$(5'A,4') = 3.5, $J$(5'A,5'B) = 12.8 (H-5'A); 3.84 dd, 1 H, $J$(5'B,4') = 3.6, $J$(5'B,5'A) = 12.8 (H-5'B); 3.78 dd, 1 H, $J$(2'B,1') = 7.3, $J$(2'B,2'A) = 12.0 (H-2'B); 3.34 dd, 1 H, 1 H, $J$(gem) = 14.4, $J$(H,P) = 13.2 and 3.17 dd, 1 H, $J$(gem) = 14.4, $J$(H,P) = 10.5 (N-CH$_2$-P); 2.66 ddd, 1 H, $J$(4'aA,1') = 9.2, $J$(4'aA,4'aB) = 14.5, $J$(4'aA,4') = 7.3 (H-4'aA); 2.61 ddd, 1 H, $J$(4'aB,1') = 5.8, $J$(4'aB,4'aA) = 14.5, $J$(4'aB,4') = 8.7 (H-4'aB).

$^{13}$C NMR (D$_2$O):
156.12 (C-4); 153.03 (C-2); 149.48 (C-6); 141.55 (C-8); 119.48 (C-5); 67.82 d, $J$(4',P) = 7.0 (C-4'); 59.63 (C-2'); 59.42 (C-5'); 52.56 (C-1'); 51.63 d, $J$(C,P) = 174.6 (N-CH$_2$-P); 33.03 (C-4'a).

1-(2',3'-Dideoxy-3'-aza-4a'-carba-α-L-glycero-pentofuranosyl)cytosine-3'-N-methylphosphonic acid (84b)

The general procedure B was followed using phosphonate 80b (0.400 g, 0.58 mmol), and Me$_3$SiBr (0.61 mL, 4.63 mmol). White solid (0.075 g, 37 %).

HRMS (FAB) calcd for C$_{10}$H$_{18}$N$_4$O$_5$P [M+H]$^+$ 305.1015; found: 305.1016.

IR $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3431 (s, br), 3201 (w, br) (OH, H$_2$O, NH$_2$); 1647 (vs), 1608 (m, sh), 1577 (m, sh), 1528 (m), 1492 (m), 1406 (w), 1294 (w), 980 (w), 787 (w) (cytosine); 1063 (w, br), 903 (w, br), 886 (w, br), 549 (m), 475 (w, br) (PO$_3$$^2$)

$^1$H NMR (D$_2$O):
7.64 d, 1 H, J(6,5) = 7.5 (H-6); 5.99 d, 1 H, J(5,6) = 7.5 (H-5); 4.90 m, 1 H (H-1'); 4.00 m, 2 H (H-4' + H-5'A); 3.78 m, 2 H (H-2'A + H-2'B); 3.62 m, 1 H (H-5'B); 3.25 dd, 1 H, 1 H, J(gem) = 14.0, J(H,P) = 12.8 and 3.05 dd, 1 H, J(gem) = 14.0, J(H,P) = 10.7 (N-CH2-P); 2.45 m, 2 H (H-4'aA + H-4'aB).

$^{13}$C NMR (D$_2$O):
166.85 (C-4); 158.52 (C-2); 146.44 (C-6); 96.54 (C-5); 68.34 d, J(4',P) = 6.5 (C-4'); 59.23 (C-2'); 58.78 (C-5'); 58.04 (C-1'); 51.29 d, J(C,P) = 128.5 (N-CH2-P); 31.75 (C-4'a).

1-(2',3'-Dideoxy-3'-aza-4a'-carba-α-L-glycero-pentofuranosyl)thymine-3'-N-methylphosphonic acid (84c)
The general procedure B was followed using phosphonate 80d (0.515 g, 0.73 mmol), and Me$_3$SiBr (0.77 mL, 5.84 mmol). White solid (0.094 g, 35 %).

HRMS (FAB) calcd for C$_{11}$H$_{18}$N$_3$O$_6$NaP [M+Na]$^+$ 342.0831; found: 342.0841.

IR $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3425 (s, br), 3260 (m, br, sh) (OH, H$_2$O, NH$_2$); 3062 (m), 1695 (vs, br), 1472 (m), 1438 (m), 938 (m), 768 (w), 2973 (m), 1373 (w) (thymine); 1064 (s, br), 905 (m, br), 548 (m), 476 (w, br) (PO$_3^{2-}$)

$^1$H NMR (D$_2$O):
7.51 q, 1 H, J(6,Me) = 1.2 (H-6); 4.94 m, 1 H, J(1',2'A) = 8.4, J(1',2'B) = 7.6, J(1',4'aA) ~ J(1',4'aB) ~ 7.8 (H-1'); 4.09 dd, 1 H, J(2'A,1') = 8.4, J(2'A,2'B) = 12.1 (H-2'A); 4.06 m, 1 H (H-4'); 4.02 m, 1 H and 3.80 m, 1 H (H-5'A + H-5'B); 3.65 dd, 1 H, J(2'B,1') = 7.6, J(2'B,2'A) = 12.1 (H-2'B); 3.32 dd, 1 H, 1 H, J(gem) = 14.4, J(H,P) = 12.6 and 3.09 dd, 1 H, J(gem) = 14.4, J(H,P) = 10.6 (N-CH2-P); 2.49 m, 2 H (H-4'aA + H-4'aB); 1.87 d, 3 H, J(Me,6) = 1.2 (5-Me);

$^{13}$C NMR (D$_2$O):
167.30 (C-4); 152.60 (C-2); 142.33 (C-6); 111.66 (C-5); 68.88 d, J(4',P) = 6.9 (C-4'); 58.64 (C-5'); 58.10 (C-2'); 56.74 (C-1'); 51.52 d, J(C,P) = 130.1 (N-CH2-P); 31.02 (C-4’a); 11.96 (5-Me (Thy)).

9-(2',3'-Dideoxy-3'-aza-4a'-carba-α-L-glycero-pentofuranosyl)guanine-3'-N-methylphosphonic acid (84d)
A solution of 80e (0.220 g, 0.29 mmol) in formic acid (80%, 15 mL) was heated at 90 °C for 6 h. The reaction mixture was then evaporated in vacuo, co-evaporated successively with
ethanol and anhydrous acetonitrile, and treated with bromotrimethylsilane (0.31 mL, 2.36 mmol) according to the general procedure. White solid (0.039 g, 34 %).

HRMS (FAB) calcd for C_{11}H_{18}N_{6}O_{5}P [M+H]^+ 345.1076; found: 345.1065.

IR ν_{max}/cm^{-1} (KBr): 3428 (vs, br), 3122 (m, br, sh), 2800 (m, br, sh) (OH, H2O, NH2); 1680 (s, br), 1634 (s, br), 1574 (m), 1535 (w), 1485 (w), 1393 (w, br), 1120 (m, br, sh), 1079 (m), 979 (w), 782 (w) (guanine); 1079 (m), 907 (w, br), 553 (w, br), 484 (w, br) (PO_{3}^{2-})

^1H NMR (D_{2}O):
7.77 s, 1 H (H-8); 5.22 m, 1 H, J(1',2'A) = 7.6, J(1',2'B) = 3.9, J(1',4'aA) = 9.8, J(1',4'aB) = 4.3 (H-1'); 4.18 m, 1 H, J(4',4'aA) = 4.9, J(4',4'aB) = 9.1, J(4',5'A) = 3.2, J(4',5'B) = 3.6 (H-4'); 4.12 dd, 1 H, J(5'A,4') = 3.2, J(5'A,5'B) = 13.3 (H-5'A); 4.00 dd, 1 H, J(2'A,1') = 7.6, J(2'A,2'B) = 12.3 (H-2'A); 3.88 dd, 1 H, J(2'B,1') = 3.9, J(2'B,2'A) = 12.3 (H-2'B); 3.81 dd, 1 H, J(5'B,4') = 3.6, J(5'B,5'A) = 13.3 (H-5'B); 3.26 m, 2 H (N-CH_{2}-P); 2.72 ddd, 1 H, J(4'aA,1') = 9.8, J(4'aA,4'aB) = 14.9, J(4'aA,4') = 4.9 (H-4'aA); 2.45 ddd, 1 H, J(4'aB,1') = 4.3, J(4'aB,4'aA) = 14.9, J(4'aB,4') = 9.1 (H-4'aB).

^13C NMR (D_{2}O):
161.80 (C-6); 156.80 (C-2); 153.04 (C-4); 141.46 (C-8); 118.99 (C-5); 67.92 d, J(4',P) = 4.7 (C-4'); 62.53 d, J(2',P) = 4.2 (C-2'); 61.94 (C-5'); 55.31 (C-1'); 51.92 d, J(C,P) = 130.4 (N-CH_{2}-P); 36.40 (C-4'a).

9-(2',3'-Dideoxy-3'-aza-4a'-carba-α-L-glycero-pentofuranosyl)-2,6-diaminopurine-3'-N-methylphosphonic acid (84e)

The general procedure B was followed using phosphonate 82 (0.072 g, 0.17 mmol), and Me_{3}SiBr (0.18 mL, 1.35 mmol). White solid (0.064 g, 98 %).

HRMS (FAB) calcd for C_{11}H_{19}N_{7}O_{4}P [M+H]^+ 344.1236; found: 344.1247.

IR ν_{max}/cm^{-1} (KBr): 3424 (vs, br), 3205 (s, br) (OH, H2O, NH2); 1639 (s), 1627 (s), 1600 (s), 1513 (w), 1474 (m), 1408 (m), 1348 (w), 1282 (w), 1225 (w, br), 977 (w), 638 (w), 790 (w) (diaminopurine); 1064 (m), 907 (w, br), 549 (m), 470 (w, br) (PO_{3}^{2-})

^1H NMR (D_{2}O):
7.86 s, 1 H (H-8); 5.18 m, 1 H, J(1',2'A) = 7.4, J(1',2'B) = 4.0, J(1',4'aA) = 9.6, J(1',4'aB) = 4.4 (H-1'); 4.13 m, 1 H, J(4',4'aA) = 5.2, J(4',4'aB) = 8.8, J(4',5'A) = 3.1, J(4',5'B) = 3.5 (H-4'); 4.10 dd, 1 H, J(5'A,4') = 3.1, J(5'A,5'B) = 12.7 (H-5'A); 4.01 dd, 1 H, J(2'A,1') = 7.4, J(2'A,2'B) = 12.0 (H-2'A); 3.80 dd, 1 H, J(5'B,4') = 3.5, J(5'B,5'A) = 12.7 (H-5'B); 3.71 dd, 1 H, J(2'B,1') = 4.0, J(2'B,2'A) = 12.0 (H-2'B); 3.25 dd, 1 H, J(gem) = 14.2, J(H,P)
= 14.4 and 3.19 dd, 1 H, J(gem) = 14.2, J(H,P) = 10.2 (N-CH₂-P); 2.68 ddd, 1 H, J(4’aA,1’) = 9.6, J(4’aA,4’aB) = 14.6, J(4’aA,4’) = 5.2 (H-4’aA); 2.44 ddd, 1 H, J(4’aB,1’) = 4.4, J(4’aB,4’aA) = 14.6, J(4’aB,4’) = 8.8 (H-4’aB).

13C NMR (D₂O):
162.60 (C-2); 159.00 (C-6); 153.04 (C-4); 141.39 (C-8); 115.93 (C-5); 67.70 d, J(4’,P) = 4.9 (C-4’); 62.58 d, J(2’,P) = 4.4 (C-2’); 62.12 (C-5’); 54.85 (C-1’); 52.26 d, J(C,P) = 131.4 (N-CH₂-P); 36.37 (C-4’a).

9-(2’,3’-Dideoxy-3’-aza-4a’-carba-β-L-glycero-pentofuranosyl)adenine-3’-N-methylphosphonic acid (89a)

The general procedure B was followed using phosphonate 85a (0.530 g, 0.74 mmol), and Me₃SiBr (0.78 mL, 5.93 mmol). White solid (0.169 g, 61%).


IR νmax/cm⁻¹ (KBr): 3427 (vs, br) (OH, H₂O, NH₂); 1642 (s), 1604 (m), 1574 (m), 1477 (w), 1416 (w), 1333 (w), 1302 (w), 978 (w), 798 (w), 728 (w) (adenine); 1062 (m, br), 903 (w, br), 542 (m), 467 (m, br) (PO₃²⁻)

1H NMR (D₂O):
8.25 s, 1 H (H-2); 8.18 s, 1 H (H-8); 5.43 m, 1 H, J(1’,2’A) = 1.6, J(1’,2’B) = 8.0, J(1’,4’aA) = 9.4, J(1’,4’aB) = 4.6 (H-1’); 4.14 dd, 1 H, J(5’,A,4’) = 3.2, J(5’,A,5’B) = 13.6 (H-5’A); 4.33 dd, 1 H, J(2’,A,1’) = 1.6, J(2’,A,2’B) = 13.2 (H-2’A); 3.89 dd, 1 H, J(5’,B,4’) = 3.3, J(5’,B,5’A) = 13.6 (H-5’B); 3.79 dd, 1 H, J(2’,B,1’) = 8.0, J(2’,B,2’A) = 13.2 (H-2’B); 3.71 m, 1 H, J(4’,4’aA) = 8.8, J(4’,4’aB) = 10.8, J(4’,5’A) = 3.2, J(4’,5’B) = 3.3 (H-4’); 3.45 dd, 1 H, J(gem) = 14.3, J(H,P) = 13.3 and 3.01 dd, 1 H, J(gem) = 14.3, J(H,P) = 10.3 (N-CH₂-P); 2.96 ddd, 1 H, J(4’aA,1’) = 9.4, J(4’aA,4’aB) = 14.8, J(4’aA,4’) = 8.8 (H-4’aA); 2.23 ddd, 1 H, J(4’aB,1’) = 4.6, J(4’aB,4’aA) = 14.8, J(4’aB,4’) = 10.8 (H-4’aB).

13C NMR (D₂O):
156.06 (C-4); 152.78 (C-2); 148.48 (C-6); 142.07 (C-8); 119.51 (C-5); 70.40 d, J(4’,P) = 7.3 (C-4’); 60.90 (C-2’); 57.85 (C-5’); 52.73 (C-1’); 51.74 d, J(C,P) = 128.4 (N-CH₂-P); 33.67 (C-4’a).

1-(2’,3’-Dideoxy-3’-aza-4a’-carba-β-L-glycero-pentofuranosyl)cytosine-3’-N-methylphosphonic acid (89b)
The general procedure B was followed using phosphonate 85b (0.388 g, 0.56 mmol), and Me₃SiBr (0.59 mL, 4.49 mmol). White solid (0.084 g, 43 %).
HRMS (FAB) calcd for C₁₀H₁₈N₄O₅P [M+H]+ 305.1015; found: 305.1005.
IR ν_max/cm⁻¹ (KBr): 3431 (vs, br), 3205 (m, br, sh) (OH, H₂O, NH₂); 1635 (m, br), 1602 (m), 1558 (m), 1495 (w), 1409 (m), 1296 (w, br), 980 (w), 789 (w) (cytosine); 1068 (m, br), 909 (w, br), 548 (m, br), 465 (w, br) (PO₃²⁻)
¹H NMR (D₂O):
7.89 d, 1 H, J(6,5) = 6.0 (H-6); 6.29 d, 1 H, J(5,6) = 6.0 (H-5); 5.49 m, 1 H, J(1’,2’A) = 1.0, J(1’,2’B) = 5.4, J(1’,4’aA) = 7.3, J(1’,4’aB) = 2.6 (H-1’); 4.12 bd, 1 H, J(2’A,1’) = 1.0, J(2’A,2’) = 13.0, J(2’A,4’aB) ≤ 2 (H-2’A); 4.00 m, 1 H (H-5’A); 3.89 m, 2 H (H-4’ + H-5’B); 3.72 dd, 1 H, J(2’B,1’) = 5.4, J(2’B,2’) = 13.0 (H-2’B); 3.38 dd, 1 H, J(gem) = 14.5, J(H,P) = 12.5 and 3.10 dd, 1 H, J(gem) = 14.5, J(H,P) = 10.8 (N-CH₂-P); 2.77 ddd, 1 H, J(4’aA,1’) = 7.3, J(4’aA,4’) = 8.8, J(4’aA,4’aB) = 15.0 (H-4’aA); 2.12 ddd, 1 H, J(4’aB,1’) = 2.6, J(4’aB,2’) = ≤ 2, J(4’aB,4’) = 7.2, J(4’aB,4’aA) = 15.0 (H-4’aB).
¹³C NMR (D₂O):
166.29 (C-4); 163.56 (C-2); 156.60 (C-6); 101.31 (C-5); 74.12 (C-1’); 69.42 d, J(4’,P) = 5.8 (C-4’); 61.18 (C-2’); 59.34 (C-5’); 52.32 d, J(C,P) = 126.3 (N-CH₂-P); 33.10 (C-4’a).

1-(2’,3’-Dideoxy-3’-aza-4’a’-carba-β-L-glycero-pentofuranosyl)thymine-3’-N-methylphosphonic acid (89c)
The general procedure B was followed using phosphonate 85d (0.554 g, 0.78 mmol), and Me₃SiBr (0.83 mL, 6.28 mmol). White solid (0.089 g, 31 %).
IR ν_max/cm⁻¹ (KBr): 3426 (vs, br), 3260 (m, br, sh) (OH, H₂O, NH₂); 3056 (m), 1690 (vs, br), 1474 (w), 1438 (w), 1285 (m), 1226 (w), 978 (w), 767 (w), 1375 (w) (thymine); 1065 (m, br), 903 (w, br), 549 (m, br), 479 (w, br) (PO₃²⁻)
¹H NMR (D₂O):
7.59 d, 1 H, J(6,Me) = 1.2 (H-6); 6.29 d, 1 H, J(5,6) = 6.0 (H-5); 4.85 m, 1 H, J(1’,2’A) = 2.1, J(1’,2’B) = 9.0, J(1’,4’aA) = 9.7, J(1’,4’aB) = 5.8 (H-1’); 4.14 dd, 1 H, J(2’A,1’) = 2.1, J(2’A,2’) = 13.0 (H-2’A); 4.06 dd, 1 H, J(5’A,4’) = 3.3, J(5’A,5’B) = 13.0 (H-5’A); 3.81 dd, 1 H, J(5’B,4’) = 2.8, J(5’B,5’A) = 13.0 (H-5’B); 3.55 dd, 1 H, J(2’B,1’) = 9.0, J(2’B,2’) = 13.0 (H-2’B); 3.49 m, 1 H, J(4’,4’aA) = 7.7, J(4’,4’aB) = 11.2, J(4’,5’A) = 3.3, J(4’,5’B) = 2.8 (H-4’); 3.34 dd, 1 H, J(gem) = 13.3, J(H,P) = 14.7 and 2.83 dd, 1 H, J(gem) = 13.3,
\( J(H,P) = 10.0 \) (N-CH\(_2\)-P); 2.69 ddd, 1 H, \( J(4'aA,1') = 9.7 \), \( J(4'aA,4') = 7.7 \), \( J(4'aA,4'aB) = 14.2 \) (H-4'aA); 2.25 ddd, 1 H, \( J(4'aB,1') = 5.8 \), \( J(4'aB,4') = 11.2 \), \( J(4'aB,4'aA) = 14.2 \) (H-4'aB); 1.92 d, 3 H, \( J(\text{Me},6) = 1.2 \) (5-Me).

\(^{13}\)C NMR (D\(_2\)O):

167.60 (C-4); 152.82 (C-2); 143.44 (C-6); 111.22 (C-5); 69.87 d, \( J(4',P) = 7.8 \) (C-4'); 59.92 (C-2'); 57.79 (C-1'); 57.54 (C-5'); 51.41 d, \( J(C,P) = 129.4 \) (N-CH\(_2\)-P); 31.79 (C-4'a); 11.96 (5-Me).

9-(2',3'-Dideoxy-3'-aza-4a'-carba-\( \beta \)-L-glycero-pentofuranosyl)guanine-3'-N-tert-methylphosphonic acid (89d)

Using the procedure outlined for 84d, compound 89d was prepared from 85e (0.545 g, 0.73 mmol) and bromotrimethylsilane (0.77 mL, 5.82 mmol). White solid (0.086 g, 31 %).

HRMS (FAB) calcd for C\(_{11}\)H\(_{18}\)N\(_6\)O\(_5\)P [M+H]\(^{\dagger}\) 345.1076; found: 345.1087.

IR \( \nu_{\max}/\text{cm}^{-1} \) (KBr): 3386 (s, br), 3315 (s , br), 3114 (s, vbr) (OH, H\(_2\)O, NH\(_2\)); 1680 (s, br), 1696 (vs), 1654 (s), 1535 (w), 1609 (s), 1484 (m), 1398 (m), 1364 (m), 1358 (m), 1169 (s), 973 (m), 780 (m), 641 w (guanine); 1075 (s), 922 (m, br), 537 (m), 456 (w, br) (PO\(_3\)\(^2-\))

\(^1\)H NMR (D\(_2\)O+NaOD):

8.21 s, 1 H (H-8); 4.81 m, 1 H, \( J(1',2'A) \sim 2.0 \), \( J(1',2'B) = 6.8 \), \( J(1',4'aA) = 8.5 \), \( J(1',4'aB) = 4.0 \) (H-1'); 3.72 dd, 1 H, \( J(5'A,4') = 3.7 \), \( J(5'A,5'B) =12.2 \) (H-5'A); 3.67 dd, 1 H, \( J(2'A,1') \sim 2.0 \), \( J(2'A,2'B) = 11.2 \) (H-2'A); 3.52 dd, 1 H, \( J(5'B,4') = 3.5 \); \( J(5'B,5'A) =12.2 \) (H-5'B); 2.985 dd, 1 H, \( J(2'B,1') = 6.8 \), \( J(2'B,2'A) = 11.2 \) (H-2'B); 2.92 dd, 1 H, \( J(\text{gem}) = 14.4 \), \( J(H,P) = 15.7 \) and 2.39 dd, 1 H, \( J(\text{gem}) = 14.4 \), \( J(H,P) = 8.4 \) (N-CH\(_2\)-P); 2.75 m, 1 H, \( J(4',4'aA) = 8.5 \); \( J(4',4'aB) = 8.0 \), \( J(4',5'A) = 3.7 \), \( J(4',5'B) = 3.5 \) (H-4'); 2.60 dt, 1 H, \( J(4'aA,4'aB) = 13.7 \), \( J(4'aA,1') = 8.5 \), \( J(4'aA,4') = 8.5 \) (H-4'aA); 1.79 ddd, 1 H, \( J(4'aB,1') = 4.0 \), \( J(4'aB,4'aA) = 13.7 \), \( J(4'aB,4') = 8.0 \) (H-4'aB).

\(^{13}\)C NMR (D\(_2\)O+NaOD):

170.93 (C-6); 163.70 (C-2); 153.84 (C-4); 139.94 (C-8); 120.06 (C-5); 69.05 d, \( J(C,P) = 13.6 \) (C-4'); 63.88 (C-5'); 63.17 (C-2'); 55.23 d, \( J(C,P) = 145.8 \) (N-CH\(_2\)-P); 54.24 (C-1'); 37.87 (C-4'a).

9-(2',3'-Dideoxy-3'-aza-4a'-carba-\( \beta \)-L-glycero-pentofuranosyl)-2,6-diaminopurine-3'-N-tert-methylphosphonic acid (89e)
The general procedure B was followed using phosphonate 87 (0.188 g, 0.44 mmol), and Me₃SiBr (0.47 mL, 3.52 mmol). White solid (0.168 g, 98 %).

HRMS (FAB) calcd for C₁₁H₁₉N₇O₄P [M+H]⁺ 344.1236; found: 344.1230.

IR νmax/cm⁻¹ (KBr): 3383 (s, br), 3334 (s, br), 3192 (s, br), 3110 (s, br, sh) (OH, H₂O, NH₂); 1641 (vs), 1608 (vs), 1600 (s), 1518 (w), 1475 (m), 1419 (m), 1334 (w), 1273 (w), 1179 (m, br), 972 (w), 638 (w), 790 (w) (diaminopurine); 1073 (m, br), 912 (w, br), 548 (m), 461 (w, br) (PO₃²⁻)

¹H NMR (D₂O + NaOD):
8.36 s, 1 H (H-8); 4.81 m, 1 H, J(1’,2’A) ≤ 2.0, J(1’,4’aA) = 6.5, J(1’,4’aB) = 8.7, J(1’,4’aB) = 3.2 (H-1’); 3.74 dd, 1 H, J(5’A,4’) = 3.7, J(5’A,5’B) = 12.2 (H-5’A); 3.73 dd, 1 H, J(2’A,1’) ≤ 2.0, J(2’A,2’B) = 11.0 (H-2’A); 3.49 dd, 1 H, J(5’B,4’) = 3.2, J(5’B,5’A) = 12.2 (H-5’B); 2.98 dd, 1 H, J(gem) = 14.2, J(H,P) = 16.2 and 2.37 dd, 1 H, J(gem) = 14.2, J(H,P) = 8.2 (N-CH₂-P); 2.95 dd, 1 H, J(2’B,1’) = 6.5, J(2’B,2’A) = 11.0 (H-2’B); 2.72 m, 1 H, J(4’,4’aA) = 8.7, J(4’,4’aB) = 7.5, J(4’,5’A) = 3.7, J(4’,5’B) = 3.2 (H-4’); 2.59 dt, 1 H, J(4’aA,1’) = 8.7, J(4’aA,4’aB) = 13.7, J(4’aA,4’) = 8.7 (H-4’aA); 1.80 ddd, 1 H, J(4’aB,1’) = 3.2, J(4’aB,4’aA) = 13.7, J(4’aB,4’) = 7.5 (H-4’aB).

¹³C NMR (D₂O + NaOD):
162.52 (C-2); 158.71 (C-6); 153.52 (C-4); 142.03 (C-8); 115.60 (C-5); 69.05 d, J(4’,P) = 14.0 (C-4’); 63.60 (C-2’); 62.97 (C-5’); 35.11 d, J(C,P) = 146.1 (N-CH₂-P); 54.56 (C-1’); 37.59 (C-4’a).

**Diisopropyl-[1-(2’,3’-dideoxy-3’-aza-4a’-carba-β-L-glycero-pentofuranosyl)cytosine]-3’-N-methylphosphonate (90)**

Protected nucleotide 85b (0.100 g, 0.14 mmol) was treated with Dowex 50 (H⁺-form) in MeOH-H₂O (9:1 v/v, 10 mL) solution. After 1 h at rt, the solution was filtered off, the Dowex layer was then washed successively with MeOH, and a mixture of H₂O-MeOH (1:1). The product was then liberated from Dowex with diluted (approx. 2.5 %) ammonia in MeOH-H₂O (1:1), and the solution was evaporated in vacuo to give 90 (0.051 g, 90 %).


¹H NMR (DMSO):
7.95 d, 1 H, J(6,5) = 7.4 (H-6); 6.94 b, 2 H (NH₂); 5.64 d, 1 H, J(5,6) = 7.4 (H-5); 4.91 ddd, 1 H, J(1’,2’a) < 1, J(1’,2’b) = 6.7, J(1’,4’aA) = 9.2 and J(1’,4’aB) = 3.2 (H-1’); 4.67 b, 1 H (5’-OH); 4.60 m, 1 H and 4.60 m, 1 H (2x O-CH< (2x iPr)); 3.44 m, 1 H and 3.34 m, 1 H,
overlapped with HDO (H-5’a and H-5’b); 3.42 dd, 1 H, J(NCHaNCHb) = 15.2 and J(NCHa,P) = 2.5 (N-CHa); 3.26 bd, 1 H, J(2’a,2’b) = 11.0 and J(2’a,1’) < 1 (H-2’a); 2.64 dd, 1 H, J(2’b,2’a) = 11.0 and J(2’b,1’) = 6.7 (H-2’b); 2.55 dd, 1 H, J(NCHb,NCHa) = 15.2 and J(NCHb,P) = 5.4 (N-CHb); 2.53 m, 1 H (H-4’); 2.38 ddd, 1 H, J(2’a,1’) = 9.2 and J(4’aA,4’a’) = 8.4 (H-4’aA); 1.41 ddd, 1 H, J(4’aB,4’aA) = 14.0, J(4’aA,1’) = 3.2 and J(4’aB,4’a’) = 7.5 (H-4’aB); 1.258 d, 3 H, J(CH3,CH) = 5.9 and 1.232 d, 3 H, J(CH3,CH) = 6.1 (4x CH3 (2x iPr)).

Diisopropyl-[1-(2’,3’-dideoxy-3’-N-oxido-3’-aza-4a’-carba-β-L-glycero- -pentofuranosyl)cytosine]-3’-N-methylphosphonate (91)

To a solution of 90 (0.051 g, 0.13 mmol) in MeOH (3 mL) was added 30% aqueous hydrogen peroxide (1.5 mL), and the reaction was stirred at 50 °C for 2 h. The reaction was then quenched by adding 10% Pd on charcoal (0.01 g), and the suspension was vigorously stirred at rt until the excess of hydrogen peroxide decomposed (1 h). The catalyst was filtered off and the solvent was removed in vacuo to afford a white residue, which was purified by HPLC to give the desired product as a colourless glassy solid (2.6 mg, 5 %).

HRMS (FAB) calcd for C16H30N4O6P [M+H]+ 405.1903; found: 405.1912.

1H NMR (DMSO):
8.78 d, 1 H, J(6,5) = 7.4 (H-6); 5.74 d, 1 H, J(5,6) = 7.4 (H-5); 5.46 bm, 1 H, J(1’,2’a) = 2.6, J(1’,2’b) = 10.0, J(1’,4’aA) = 10.0, J(1’,4’aB) = 7.5 (H-1’); 4.90 m, 1H and 4.63 m, 1 H (2x – CH< (iPr)); 4.25 dd, 1 H, J(N-CHaN-N-CHb) = 15.0, J(N-CHa,P) = 16.3 (N-CHa); 4.11 dd, 1 H, J(2’a,1’) = 10.0, J(2’a,2’b) = 13.0 (H-2’a); 4.02 dd, 1 H, J(5’a,4’) = 2.0, J(5’a,5’b) = 13.0 (H-5’a); 3.88 dd, 1 H, J(2’b,1’) = 2.6, J(2’b,2’a) = 13.0 (H-2’b); 3.78 dd, 1 H, J(5’b,4’) = 4.0, J(5’b,5’a) = 13.0 (H-5’b); 3.78 dd, 1 H, J(N-CHb,N-CHa) = 15.0, J(N-CHa,P) = 10.5 (N-CHb);
3.61 m, 1 H, J(4’,4’aA) = 6.5, J(4’,4’aB) = 12.7, J(4’,5’a) = 2.0, J(4’,5’b) = 4.0 (H-4’); 2.40 m, 1 H, J(4’aA,1’) = 10.0, J(4’aA,4’) = 6.5, J(4’aA,4’aB) = 12.7 (H-4’aA); 2.17 dt, 1 H, J(4’aB,1’) = 7.5, J(4’aB,4’) = 12.7, J(4’aB,4’aA) = 12.7 (H-4’aA); ~1.26 4x d, 12 H, J(CH3,CH) = 6.6 (4x CH3 (iPr)).

(2S,4R)-N-Diisopropylphosphonomethyl-N-methyl-2-dimethoxytrityloxymethyl-4- -hydroxypyrrolidinium iodide (92)
To a solution of **L-75** (0.100 g, 0.17 mmol) in Et₂O (5 mL) was added methyl iodide (1 mL, 100 eq.) and the solution was left to react at rt overnight (TLC in C1). The reaction mixture was then evaporated in vacuo, and the crude product was purified by flash chromatography on silica (elution with a linear gradient of HI in EtOAc) to yield **92** as a yellow glassy solid (0.090 g, 73 %).

HRMS (FAB) calcd for C₃₄H₄₇NO₇P⁺ [M]+ 612.3085; found: 612.3072.

NMR: two epimers (different in a configuration at nitrogen atom) in the ratio 75 : 25 are observed in NMR spectra

**Major isomer (N-CH₃ cis- to CH₂-ODMTr group):**

¹H NMR (DMSO):

7.39 m, 2 H, 7.35 m, 2 H, 7.29 m, 2 H, 7.27 m, 3 H, 6.935 m, 2 H and 6.925 m, 2 H (2x C₆H₄ and C₆H₅ (DMTr)); 5.65 bd, 1 H, J(OH,1) ~ 4.0 (OH); ~4.73 m, 2 H (2x –CH< (iPr)); 4.56 m, 1 H, J(4,4aA) = 11.8, J(4,4aB) = 7.3, J(4,5a) = 3.2, J(4,5b) = 8.7 (H-4); 4.45 m, 1 H, J(1,2a) = 5.8, J(1,2b) = 4.5, J(1,OH) ~4.0, J(1,4aA) = 6.8, J(1,4aB) ≤ 2 (H-1); 4.26 dd, 1 H, J(N-CH₃,N-CHb) = 13.6, J(N-CH₃,P) = 15.5 (N-CH₃-P); 4.15 dd, 1 H, J(N-CHb,N-CHb) = 13.6, J(N-CHb,P) = 15.5 (N-CHb-P); 3.92 dd, 1 H, J(2a,1) = 5.8, J(2a,2b) = 12.6 (H-2a); 3.80 dd, 1 H, J(2b,1) = 4.5, J(2b,2a) = 12.6 (H-2b); 3.75 s, 6 H (2x OCH₃ (DMTr)); 3.59 dd, 1 H, J(5a,5b) = 3.2, J(5a,5b) = 12.6 (H-5a); 3.38 dd, 1 H, J(5b,4) = 8.7, J(5b,5a) = 12.6 (H-5b); 2.89 s, 3 H (N-CH₃); 2.20 ddd, 1 H, J(4aA,4) = 11.8, J(4aA,4aB) = 14.3, J(4aA,1) = 6.8 (H-4aA); 1.96 ddd, 1 H, J(4aB,4) = 7.3, J(4aB,4aA) = 14.3, J(4aB,1) ≤ 2 (H-4aB); 1.32 d, 3 H, 1.315 d, 3H, 1.295 d, 3 H and 1.25 d, 3 H, J(CH₃,CH) = 6.2 (4x CH₃ (iPr)).

¹³C NMR (DMSO):

158.45(2), 144.13, 134.78, 134.43, 129.81(2), 129.79(2), 128.21(2), 127.58(2), 127.13, 113.63(2) and 113.58(2) (2x C₆H₄ and C₆H₅ (ODMTr)); 87.19 (>C< (ODMTr)); 79.07 d, J(4,P) = 7.2 (C-4); 73.51 (C-2); 72.70 d, J(C,P) = 7.5, 72.60 d, J(C,P) = 7.5, 23.82 d, J(C,P) = 6.3, 23.78 d, J(C,P) = 6.3, 23.64 d, J(C,P) = 5.0 and 23.62 d, J(C,P) = 5.0 (P(OiPr)₂); 67.18 (C-1); 60.32 (C-5); 55.45 (N-CH₃); 55.26(2) (2x OMe (ODMTr)); 53.28 d, J(C,P) = 148.3 (N-CH₂-P); 35.32 (C-4a).

**Minor isomer (N-CH₃ trans- to CH₂-ODMTr group):**

¹H NMR (DMSO):

7.39 m, 2 H, 7.35 m, 2 H, 7.29 m, 2 H, 7.27 m, 3 H, 6.935 m, 2 H and 6.925 m, 2 H (2x C₆H₄ and C₆H₅ (DMTr)); 5.66 bd, 1 H, J(OH,1) ~ 4.0 (OH); ~4.73 m, 2 H (2x –CH< (iPr)); 4.52 m, 1 H, J(1,2a) = 5.2, J(1,2b) = 3.0, J(1,OH) ~4.0, J(1,4aA) = 6.3, J(1,4aB) ≤ 2 (H-1); 4.45 m, 1
H, J(4,4aA) = 10.0, J(4,4aB) = 7.2, J(4,5a) = 3.2, J(4,5b) = 8.7 (H-4); 4.01 dd, 1 H, J(2a,1) = 5.2, J(2a,2b) = 12.7 (H-2a); 3.75 s, 6 H (2x OCH₃ (DMTr)); 3.69 t, 1 H, J(N-CHa,N-CHb) < J(N-CHA,P) = 15.0 (N-CHA-P); 3.585 dd, 1 H, J(5a,4) = 3.2, J(5a,5b) = 12.6 (H-5a); 3.56 dd, 1 H, J(2b,1) = 3.0, J(2b,2a) = 12.7 (H-2b); 3.51 s, 3 H (N-CH₃); 3.39 overlapped (N-CHb-P); 3.35 dd, 1 H, J(5b,4) = 8.7, J(5b,5a) = 12.6 (H-5b); 2.32 ddd, 1 H, J(4aA,4) = 10.0, J(4aA,4aB) = 14.3, J(4aA,1) = 6.3 (H-4aA); 2.08 ddd, 1 H, J(4aB,4) = 7.2, J(4aB,4aA) = 14.3, J(4aB,1) ≤ 2 (H-4aB); 1.27 d, 3 H, 1.26 d, 3H and 1.23 d, 6 H, J(CH₃,CH) = 6.2 (4x CH₃ (iPr)).

13C NMR (DMSO):
158.45(2), 144.13, 134.78, 134.43, 129.81(2), 129.79(2), 128.21(2), 127.58(2), 127.13, 113.63(2) and 113.58(2) (2x C₆H₄ a C₆H₅ (ODMTr)); 87.19 (>C< (ODMTr)); 76.22 d, J(4,P) = 7.8 (C-4); 73.25 (C-2); 72.70 d, J(C,P) = 7.5, 72.60 d, J(C,P) = 7.5, 23.82 d, J(C,P) = 6.3, 23.78 d, J(C,P) = 6.3, 23.64 d, J(C,P) = 5.0 and 23.62 d, J(C,P) = 5.0 (POiPr₂); 66.16 (C-1); 60.56 d, J(C,P) = 147.2 (N-CH₂-P); 60.13 (C-5); 55.26(2) (2x OMe (ODMTr)); 46.46 (N-CH₃); 34.24 (C-4a).

(2S,4S)-N-Diisopropylphosphonomethyl-N-methyl-2-dimethoxytrityloxyethyl-4-hydroxypyrrolidinium iodide (93)
Using the procedure outlined for 92, compound 93 was prepared from L-78 (0.100 g, 0.17 mmol) as a yellow glassy solid (0.091 g, 74 %).
HRMS (FAB) calcd for C₃₄H₄₇NO₇P⁺ [M⁺] 612,3085; found: 612,3094.

1H NMR (DMSO):
7.37 m, 2 H, 7.35 m, 2 H, 7.28 m, 1 H, 7.27 m, 2 H, 7.26 m, 2 H, 6.93 m, 2 H and 6.92 m, 2 H (2x C₆H₄ and C₆H₅ (DMTr)); 5.62 d, 1 H, J(OH,1) = 3.4 (OH); 4.72 m, 2 H (2x –CH< (iPr)); 4.52 m, 1 H, J(1,2a) = 6.2, J(1,2b) ≤ 2, J(1,OH) = 3.4, J(1,4aA) = 7.3, J(1,4aB) = 3.6 (H-1); 4.20 m, 1 H, J(4,4aA) = 7.3, J(4,4aB) = 11.5, J(4,5a) = 3.3, J(4,5b) = 8.3 (H-4); 4.11 d, 2 H, J(N-CH₂,P) = 13.5 (N-CH₂-P); 3.90 bdd, 1 H, J(2a,1) = 6.2, J(2a,2b) = 12.7 (H-2a); 3.755 s, 6 H (2x OCH₃ (DMTr)); 3.68 bd, 1 H, J(2b,1) ≤ 2, J(2b,2a) = 12.7 (H-2b); 3.62 dd, 1 H, J(5a,4) = 3.3, J(5a,5b) = 12.7 (H-5a); 3.49 dd, 1 H, J(5b,4) = 8.3, J(5b,5a) = 12.7 (H-5b); 2.99 s, 3 H (N-CH₃); 2.58 dt, 1 H, J(4aA,4) = 7.3, J(4aA,4aB) = 14.5, J(4aA,1) = 7.3 (H-4aA); 1.69 ddd, 1 H, J(4aB,4) = 11.5, J(4aB,4aA) = 14.5, J(4aB,1) = 3.6 (H-4aB); 1.315 d, 6H, 1.29 d, 3 H and 1.25 d, 3 H, J(CH₃,CH) = 6.2 (4x CH₃ (iPr)).

13C NMR (DMSO):
158.55(2), 144.20, 134.84, 134.49, 129.88(4), 128.34(2), 127.66(2), 127.27, 113.72(2) and 113.67(2) (2x C6H4 a C6H5 (ODMTr)); 87.30 (>C< (ODMTr)); 76.73 d, \(J_{(4,P)} = 7.8\) (C-4); 73.52 (C-2); 72.62(2) d, \(J(C,P) = 6.8, 23.90\) d, \(J(C,P) = 3.4, 23.88\) d, \(J(C,P) = 3.9, 23.73\) d, \(J(C,P) = 5.4\) and 23.69 d, \(J(C,P) = 5.4\) (P(OiPr)2); 66.03 (C-1); 60.13 (C-5); 59.53 d, \(J(C,P) = 150.8\) (N-CH2-P); 55.32(2) (2x OMe (ODMTr)); 46.21 (N-CH3); 34.52 (C-4a).

**Diisopropyl-[1-(2’,3’-dideoxy-3’-aza-4a’-carba-\(\alpha\)-D-glycero-pentofuranosyl)thymine]-3’-N-phosphonate (94)**

Aqueous formaldehyde (14.5 M solution; 0.26 mL, 3.75 mmol, 1.7 eq.) was added to a stirred solution of **129** (0.494 g, 2.19 mmol) in diisopropyl phosphonate (0.54 mL, 3.20 mmol, 1.4 eq.), and the mixture was heated to 90 °C for 5 h (TLC in H3). The reaction was evaporated, residue dissolved in EtOAc, and purified by flash chromatography on silica (elution with a linear gradient of HI in EtOAc) to yield **94** as a colourless oil (0.70 g, 79%).


**\(^1\)H NMR (CDCl3):**

8.95 bs, 1 H (NH); 8.03 q, 1 H, \(J_{(6,CH3)} = 1.2\) (H-6); 5.16 m, 1 H, \(J(1’,2’a) = 1.0, J(1’,2’b) = 7.0, J(1’,4’aA) = 9.4\) and \(J(1’,4’aB) = 3.6\) (H-1’); 4.73 m, 2 H (2x O-CH< (2x OiPr)); 3.85 dd, 1 H, \(J(5’a,5’b) = 12.9\) and \(J(5’a,4’) = 2.65\) (H-5’a); 3.55 dd, 1 H, \(J(5’b,5’a) = 12.9\) and \(J(5’b,4’) = 2.3\) (H-5’a); 3.26 dd, 1 H, \(J(2’a,2’b) = 10.7\) and \(J(2’a,1’) = 1.0\) (H-2’a); 3.00 dd, 1 H, \(J(NCha,NChb) = 15.9\) and \(J(NCha,P) = 11.9\) (N-CHa); 2.92 dd, 1 H, \(J(2’b,2’a) = 10.7\) and \(J(2’b,1’) = 7.0\) (H-2’b); 2.81 dd, 1 H, \(J(NChb,NCha) = 15.9\) and \(J(NChb,P) = 10.3\) (N-CHb); 2.62 tdd, 1 H, \(J(4’,4’aA) = 8.5, J(4’,4’aB) = 8.5, J(4’,5’a) = 2.65\) and \(J(4’,5’b) = 2.3\) (H-4’); 2.48 ddd, 1 H, \(J(4’aA,4’aB) = 14.6, J(4’aA,4’) = 8.5\) and \(J(4’aA,1’) = 9.4\) (H-4’aA); 1.96 ddd, 1 H, \(J(4’aB,4’aA) = 14.6, J(4’aB,4’) = 8.5\) and \(J(4’aB,1’) = 3.6\) (H-4’aB); 1.92 d, 3 H, \(J(CH3,6) = 1.2\) (5-CH3); 1.34 d, 6 H, \(J(CH3,CH) = 6.3, 1.34\) d, 3 H, \(J(CH3,CH) = 6.3\) and 1.26 d, 3 H, \(J(CH3,CH) = 6.3\) (4x CH3 (2x OiPr).

**\(^13\)C NMR (CDCl3):**

163.77 (C-4); 151.10 (C-2); 138.40 (C-6); 110.75 (C-5); 71.32 d, \(J(C,P) = 7.4\) and 71.26 d, \(J(C,P) = 7.1\) (2x O-CH< (2x OiPr)); 66.90 d, \(J(C,P) = 10.3\) (C-4’); 62.43 d, \(J(C,P) = 6.5\) (C-2’); 60.64 (C-5’); 51.57 (C-1’); 48.37 d, \(J(C,P) = 176.0\) (N-CH2-P); 33.81 (C-4’a); 24.12 d, \(J(C,P) = 5.0\), 24.09 d, \(J(C,P) = 5.0, 23.99\) d, \(J(C,P) = 5.0\) and 23.96 d, \(J(C,P) = 5.0\) (4x CH3 (2x OiPr)); 12.62 (5-CH3).
(2R,4S)-N-Diisopropylphosphonomethyl-N-methyl-2-hydroxymethyl-4-(thymin-1-yl)pyrrolidinium iodide (95)

Phosphonate 94 (0.020 g, 0.05 mmol) was dissolved in methyl iodide (0.250 mL, 4.0 mmol, 80 eq.) and heated to 80 °C for 3 h in a sealed ampule. After 3 h the reaction was cooled to rt, and the excess of CH₃I was evaporated under vacuo. Pale yellow glassy solid (0.0267 mg, 99%).

HRMS (FAB) calcd for C₁₈H₃₃N₃O₆P⁺ [M]⁺ 418.2102; found: 418.2112.

NMR:
Mixture of epimers at nitrogen: 65% β-CH₃ + 35% α-CH₃

¹H NMR (CDCl₃):

65% β-CH₃: 9.77 b, 1 H (NH); 7.35 q, 1 H, J(6,CH₃) = 1.2 (H-6); 5.57 m, 1 H (H-1'); 4.85 m, 2 H (2x O-CH< (2x OiPr)); 4.82 m, 1 H (H-4'); 4.73 m, 1 H (H-2'a); 4.63 dd, 1 H, J(NCHa,NCHb) = 15.0 and J(NCHa,P) = 12.5 (N-CHa); 4.45 dd, 1 H, J(NCHb,NCHa) = 15.0 and J(NCHb,P) = 14.0 (N-CHb); 4.23 m, 1 H (H-5'a); 4.14 dd, 1 H, J(2'b,2’a) = 13.2 and J(2'b,1') = 7.5 (H-2'b); 4.02 dt, 1 H, J(5’b,5’a) = 14.0, J(5’b,4’) = 8.8 and J(5’b,OH) = 8.8 (H-5'b); 3.60 s, 3 H (N-CH₃); 2.80 m, 1 H (H-4'aA); 2.49 m, 1 H (H-4'aB); 1.91 d, 3 H, J(CH₃,6) = 1.2 (5-CH₃); 1.73 – 1.35 (4x d, J ~ 6, 4x CH₃ (2x OiPr)).

35% α-CH₃: 9.40 b, 1 H (NH); 7.51 q, 1 H, J(6,CH₃) = 1.2 (H-6); 5.60 m, 1 H (H-1'); 4.93 m, 2 H (2x O-CH< (2x OiPr)); 4.84 m, 1 H (H-2'a); 4.73 m, 1 H (H-4'); 4.55 dd, 1 H, J(NCHa,NCHb) = 15.0 and J(NCHa,P) = 14.0 (N-CHa); 4.27 m, 1 H (H-5'a); 4.23 m, 1 H (H-5'b); 4.02 m, 1 H (H-5'b); 3.90 bt, 1 H, J(NCHb,NCHa) = 15.0 and J(NCHb,P) = 15.0 (N-CHb); 3.71 s, 3 H (N-CH₃); 2.77 m, 1 H (H-4’aA); 2.64 m, 1 H (H-4’aB); 1.895 d, 3 H, J(CH₃,6) = 1.2 (5-CH₃); 1.73 – 1.35 (4x d, J ~ 6, 4x CH₃ (2x OiPr)).

¹³C NMR (CDCl₃):

65% β-CH₃: 164.05 (C-4); 150.71 (C-2); 141.51 (C-6); 111.71 (C-5); 78.18 d, J(C,P) = 8.4 (C-4’); 74.16 d, J(C,P) = 6.2 and 73.20 d, J(C,P) = 6.9 (2x O-CH< (2x OiPr)); 67.86 (C-2’); 62.52 d, J(C,P) = 147.9 (N-CH₂-P); 58.97 (C-5’); 58.06 (C-1’); 48.60 (N-CH₃); 27.38 (C-4’a); 24.35 – 23.75 (4x d, 4x CH₃ (2x OiPr)); 12.06 (5-CH₃).

35% α-CH₃: 163.96 (C-4); 150.59 (C-2); 141.12 (C-6); 111.79 (C-5); 79.88 d, J(C,P) = 8.4 (C-4’); 74.14 d, J(C,P) = 6.0 and 73.15 d, J(C,P) = 7.2 (2x O-CH< (2x OiPr)); 65.92 (C-2’); 58.94 (C-5’); 56.84 (C-1’); 55.18 (N-CH₃); 53.58 d, J(C,P) = 150.2 (N-CH₂-P); 28.43 (C-4’a); 24.35 – 23.75 (4x d, 4x CH₃ (2x OiPr)); 12.54 (5-CH₃).
\textbf{N-Benzylxocarbonyl-cis-4-hydroxy-6-O-dimethoxytrityl-d-prolinol (96)}

Using the procedure outlined for \textbf{L-75}, compound 96 was prepared from \textbf{D-72} (30.0 g, 119.4 mmol) and dimethoxytrityl chloride (60.67 g, 179.1 mmol) as a pale yellow thick gum (61.5 g, 93 %).

HRMS (FAB) calcd for C_{34}H_{35}NO_{6}Na [M+Na]^+ 576,2362; found: 576,2379.

NMR:
- two isomers around N-C(=O) bond observed (ratio 58 : 42)
- signal of major isomer is given first if signals of both isomers are resolved
- \( J(H,H) \) values could not be determined due to strong line broadening

\(^1\)H NMR (d\textsubscript{6}DMSO)

\begin{align*}
7.14-7.37 & \text{ m} + 7.08 \text{ m} + 6.84 \text{ m}, 18 \text{ H (C}_6\text{H}_5 \text{ (Bn) + C}_6\text{H}_5 \text{ and 2x C}_6\text{H}_4 \text{ (ODMTr)); 4.95 d, 1 H, } \text{J(OH,1')} = 3.5 \text{ (OH); 4.92 bs and 5.03 bs, 1 H (O-CH}_2\text{-C}_6\text{H}_5); 4.21 \text{ m, 1 H, (H-1'); 3.94 m and 3.98 m, 1 H (H-4'); 3.70-3.73, 6 H (2x OMe (ODMTr)); 3.54 bdd and 3.61 bdd, 1 H (H-2'a); 3.12 m and 3.25 m, 2 H (H-5'a + H-5'b); 3.11 bdd and 3.04 bdd, 1 H (H-2'b); 2.12 m, 1 H (H-4'aA); 1.99 bdt and 1.93 bdt, 1 H (H-4'aB);}
\end{align*}

\(^{13}\)C NMR (d\textsubscript{6}DMSO):

\begin{align*}
158.17(2), 145.39, 136.10(2), 129.85(4), 127.95(4), 126.80, 113.26(4) \text{ (2x C}_6\text{H}_4 \text{ and C}_6\text{H}_5 \text{ (ODMTr)); 154.20 and 154.25 (N-CO-O); 136.94 and 137.28, 127.60-128.60 (C}_6\text{H}_5 \text{ (Bn)); 85.51 and 85.41 (>C< (ODMTr)); 68.26 and 68.95 (C-1'); 66.07 and 65.90 (O-CH}_2\text{ (Bn)); 65.40 and 64.00 (C-5'); 56.25 and 56.72 (C-4'); 55.20 (2x OCH}_3\text{); 55.04 and 54.60 (C-2'); 36.74 and 35.60 (C-4'a).}
\end{align*}

\textbf{N-Benzylxocarbonyl-cis-4-methanesulfonyl-6-O-dimethoxytrityl-d-prolinol (97)}

Using the procedure outlined for \textbf{L-76}, compound 97 was prepared from 96 (15.17 g, 27.4 mmol) and mesyl chloride (10.6 mL, 137.0 mmol) as a white foam (16.76 g, 97 %).

HRMS (FAB) calcd for C_{35}H_{37}NO_{8}S [M+H]^+ 631,2240; found: 631,2252.

NMR provided broad signals which could not be fully analyzed.

\textbf{1-([N-Benzylxocarbonyl-trans-6-O-dimethoxytrityl-d-prolinol-4-yl]thymine (98)}

The general procedure \textbf{A} was followed using mesylate 97 (8.80 g, 13.93 mmol), Cs\textsubscript{2}CO\textsubscript{3} (5.45 g, 16.72 mmol) and thymine (2.64 g, 20.89 mmol); DMF was replaced by DMSO. The reaction was complete after 5 h. White foam (3.14 g, 34 %).

HRMS (FAB) calcd for C_{39}H_{40}N_{3}O_{7} [M+H]^+ 662,2866; found: 662,2883.

\(^1\)H NMR (d\textsubscript{6}DMSO; T = 80°C):
11.00 bs, 1 H (NH); 7.46 q, 1 H, J(6,CH₃) = 1.2 (H-6); 7.38-7.15 m, 10 H, 7.24 m, 4 H and 6.86 m, 4 H (2x C₆H₄ and C₆H₅ (ODMTr)) + C₆H₅ (Bn)); 5.30 m, 1 H, J(1’,2’a) ~ J(1’,4’aA) ~ 7.5, J(1’,2’b) = 7.2, J(1’,4’aB) = 8.9 (H-1’); 5.10 bd, 1 H and 5.01 bd, 1 H, J(gem) ~ 12 (CH₂ (Bn)); 4.14 m, 1 H (H-4’); 3.74 s, 6 H (2x OMe (ODMTr)); 3.73 um, 1 H (H-2’a); 3.50 dd, 1 H, J(2’b,2’a) = 11.0, J(2’b,1’) = 7.2 (H-2’b); 3.29 um, 1 H (H-5’a); 3.10 dd, 1 H, J(5’b,4’) = 3.5, J(5’b,5’a) = 9.5 (H-5’b); 2.37 td, 1 H, J(4’aA,1’) ~ J(4’aA,4’) = 8.9, J(4’aA,4’aB) = 12.7 (H-4’aA); 2.19 um, 1 H (H-4’aB); 1.78 d, 3 H, J(CH₃,6) = 1.2 (5-CH₃).

1-(trans-D-Prolinol-4-yl)thymine (99)

To a solution of 98 (2.80 g, 4.23 mmol) in MeOH/EtOAc (9:1, 200 mL) and conc. HCl (3 mL) was added 10% Pd on charcoal (0.1 g), and the vigorously stirred reaction mixture was left to react under an atmosphere of hydrogen at rt overnight (TLC in C1). The catalyst was filtered off and the solvent removed under reduced pressure. The reaction was then concentrated in vacuo, residue dissolved in small amount of H₂O-MeOH (1:1), and the solution was passed through a column of Dowex 50 (H⁺-form), which was then washed successively with MeOH, and a mixture of H₂O-MeOH (1:1). The product was liberated from Dowex with diluted (approx. 2.5 %) aqueous ammonia, and evaporated in vacuo to afford the desired product 99 as a pale yellow solid (0.893 g, 94 %).

HRMS (FAB) calcd for C₁₀H₁₆N₃O₃ [M+H]+ 226.1192; found: 226.1191.

1H NMR (d₆DMSO):
11.16 bs, 1 H (NH); 7.54 q, 1 H, J(6,CH₃) = 1.2 (H-6); 4.84 m, 1 H, J(1’,2’a) = 7.0, J(1’,2’b) = 5.2, J(1’,4’aA) = 5.2, J(1’,4’aB) = 8.8 (H-1’); 3.35-3.25 m, 3 H (H-4’, H-5’a and H-5’b); 3.14 dd, 1 H, J(2’a,1’) = 7.0, J(2’a,2’b) = 11.3 (H-2’a); 2.76 dd, 1 H, J(2’b,1’) = 5.2, J(2’b,2’a) = 11.3 (H-2’b); 1.88-1.78 m, 2 H (H-4’aA and H-4’aB); 1.77 d, 3 H, J(CH₃,6) = 1.1 (5-CH₃).

13C NMR (d₆DMSO):
163.95 (C-4); 151.11 (C-2); 138.24 (C-6); 109.17 (C-5); 63.93 (C-5’); 59.07 (C-4’); 55.27 (C-1’); 50.88 (C-2’); 33.42 (C-4’a); 12.32 (5-CH₃).

Diiisopropyl-[1-(2’,3’-dideoxy-3’-aza-4a’-carba-α-D-glycero-pentofuranosyl)thymin]-3’-N-carbonylphosphonate (103)

Phenyl-diisopropylphosphonoformate (0.4 mL, 1.40 mmol, 1.5 eq.) was added to a stirred solution of amine 99 (0.209 g, 0.93 mmol) in DMF (6 mL), and the mixture was heated to 80 °C for 2 h (TLC in H1). The reaction was evaporated, and the residue was purified by flash
chromatography on silica (elution with a linear gradient of EtOH in CHCl₃) to yield 103 as a colourless glassy solid (0.287 g, 74%).

HRMS (FAB) calcd for C₁₇H₂₉N₃O₇P [M+H]⁺ 418,1743; found: 418,1754.

NMR:
- mixture of isomers around >N-CO bond in the ratio ~ 71 : 29
- some signals are doubled, some others are heavily overlapped
- the complete structural assignment of signals is not possible

¹H NMR (DMSO):
11.32 bs and 11.27 bs (NH); 7.48 q and 7.54 q, J(6,CH₃) = 1.2 (H-6); 5.12 m and 5.23 m (H-1'); 4.99 t and 5.08 t (5’-OH); 4.61 m and 4.68 m (2x O-CH< (2x OiPr)); 4.34 m (H-4’); 3.65 – 3.45 m (H-5’a + H-5’b); 3.59 m (H-2’a); 3.47 m (H-2’b); 2.33 m and 2.24 m (H-4’aA + H-4’aB); 1.756 d and 1.759 d, J(CH₃,6) = 1.2 (5-CH₃); 1.31 – 1.24 (doublets, 4x CH₃ (2x OiPr)).

¹³C NMR (DMSO):
163.89 (C-4); 151.15 (C-2); 137.58 and 138.27 (C-6); 109.41 and 109.59 (C-5); 72.48 d, J(C,P) = 6.8 (2x O-CH< (2x OiPr)); 64.03 and 66.54 (C-5’); 60.66 and 60.48 (C-2’); 58.43 d, J(C,P) = 5.9 (C-4’); 54.15 and 52.30 (C-1’); 29.89 and 32.13 (C-4’a); 23.93 – 23.50 doblets, J(C,P) ~ 5.0 (4x CH₃ (2x OiPr)); 12.30 and 12.24 (5-CH₃).

Diisopropyl-[1-(2’,3’-dideoxy-3’-aza-4a’-carba-α-D-glycero-pentofuranosyl)thymine]-3’-N-thiocarbonylphosphonate (104)

Methyl-diisopropylphosphonodithioformate⁴⁴ (0.15 mL, 0.80 mmol, 2.0 eq.) was added to a stirred solution of amine 99 (0.090 g, 0.40 mmol) in DMF (2 mL), and the mixture was heated to 60 °C for 2 h (TLC in C1). The reaction was evaporated, and the residue was purified by flash chromatography on silica (elution with a linear gradient of EtOH in CHCl₃) to yield 104 as a yellow glassy solid (0.096 g, 55%).

HRMS (FAB) calcd for C₁₇H₂₉N₃O₇PS [M+H]⁺ 434,1515; found: 434,1505.

NMR:
- two isomers around N-C(=S) bond observed - ratio 60 : 40

¹H NMR (d₆DMSO):
Major isomer:
11.34 bs, 1 H (NH); 7.49 q, 1 H, J(6,CH₃) = 1.2 (H-6); 5.26 m, 1 H, J(1’,2’a) = 5.8, J(1’,2’b) ~ J(1’,4’aB) = 7.8, J(1’,4’aA) ~ 8.0 (H-1’); 5.12 t, 1 H, J(OH,5’a) ~ J(OH,5’b) = 5.1 (5’-OH); 5.00 m, 1 H, J(4’,4’aA) ~ 8.0, J(4’,4’aB) = 3.7, J(4’,5’a) = 5.7, J(4’,5’b) = 3.0 (H-4’); 4.65 m, 2H and ~1.28 d(4x), 12 H (2x CH(CH₃)₂); 4.24 dd, 1 H, J(2’a,1’) = 5.8, J(2’a,2’b) = 13.3 (H-
2’a); 4.16 dd, 1 H, J(2’b,1’) = 7.8, J(2’b,2’a) = 13.3 (H-2’b); 3.92 ddd, 1 H, J(5’a,4’) = 5.7, J(5’a,5’b) = 11.2, J(5’a,OH) = 5.1 (H-5’a); 3.56 ddd, 1 H, J(5’b,4’) = 3.0, J(5’b,5’a) = 11.2, J(5’b,OH) = 5.1 (H-5’b); 2.47 m, 1 H, J(4’aA,1’) ~ J(4’aA,4’) ~ 8.0, J(4’aA,4’aB) = 13.3 (H-4’aA); 2.41 m, 1 H, J(4’aB,1’) = 7.8, J(4’aB,4’) = 3.7, J(4’aB,4’aA) = 13.3 (H-4’aB); 1.75 d, 3 H, J(CH3,6) = 1.2 (5-CH3).

Minor isomer:
11.28 bs, 1 H (NH); 7.61 q, 1 H, J(6,CH3) = 1.2 (H-6); 5.33 m, 1 H (H-1’); 5.22 t, 1 H, J(OH,5’a) ~ J(OH,5’b) = 5.2 (5’-OH); 5.20 m, 1 H (H-4’); 4.75 m, 2H and ~1.31 d(4x), 12 H (2x CH(CH3)2); 4.02 m, 1 H (H-2’a); 3.94 m, 1 H (H-2’b); 3.74 dt, 1 H, J(5’a,4’) ~ J(5’a,OH) = 5.2, J(5’a,5’b) = 11.2, (H-5’a); 3.64 ddd, 1 H, J(5’b,4’) = 2.3, J(5’b,5’a) = 11.2, J(5’b,OH) = 5.2 (H-5’b); 2.55 m, 1 H (H-4’aA); 2.36 m, 1 H (H-4’aB); 1.76 d, 3 H, J(CH3,6) = 1.2 (5-CH3).

13C NMR (d6DMSO):

Major isomer:
190.75 d, J(C,P) = 176.0 (C=S); 163.89 (C-4); 151.09 (C-2); 137.82 (C-6); 109.49 (C-5); 73.5-73.0 and 23.9-23.4 (2x CH(CH3)2); 64.38 (C-4’); 58.62 (C-5’); 57.28 (C-2’); 54.31 (C-1’); 29.88 (C-4’a); 12.30 (5-CH3).

Minor isomer:
190.20 d, J(C,P) = 157.2 (C=S); 163.89 (C-4); 151.03 (C-2); 137.75 (C-6); 109.58 (C-5); 73.5-73.0 and 23.9-23.4 (2x CH(CH3)2); 63.85 (C-5’); 52.39 (C-1’); 58.18 (C-2’); 32.29 (C-4’a); 12.23 (5-CH3).

Diisopropyl-[1-(2’,3’-dideoxy-3’-aza-4a’-carba-α-D-glycero-pentofuranosyl)thymine]-3’-N-carbonylmethylphosphonate (105)
A mixture of amine 99 (0.263 g, 1.17 mmol) and DMAP (0.64 g, 5.27 mmol, 4.5 eq.) was co-evaporated repeatedly with toluene to remove traces of water, dissolved in dry DMF (8 mL), and treated with diisopropylphosphonoacetic acid145 (0.58 g, 2.57 mmol, 2.2 eq.) and N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDC) (0.67 g, 3.51 mmol, 3.0 eq.) at rt (TLC in H1). After 12 h, the reaction was concentrated under reduced pressure. The residue was diluted with chloroform, washed subsequently with water, 10% aqueous citric acid, saturated sodium hydrogen carbonate solution, and finally with water. The organic layer was dried over Na2SO4, and concentrated under reduced pressure. Product was isolated by flash chromatography on silica (elution with a linear gradient of EtOH in CHCl3) and dried in vacuo, giving pale yellow thick oil (0.465 g, 92%).
HRMS (FAB) calcd for $\text{C}_{18}\text{H}_{31}\text{N}_3\text{O}_7\text{P}$ [M+H]$^+$ 432.1900; found: 432.1887.

NMR (d$_6$DMSO) – two isomers around N-C(=O) bond observed; ratio 77 : 23; only the complete data for major isomer could be obtained

**Major isomer:**

$^1$H NMR (d$_6$DMSO):

- 11.31 bs, 1 H (NH); 7.56 q, 1 H, $J$(6,CH$_3$) = 1.2 (H-6); 5.18 m, 1 H, $J$(1’,2’a) = 7.8, $J$(1’,2’b) = 7.1, $J$(1’,4’aA) = 8.8, $J$(1’,4’aB) = 7.4 (H-1’); 4.60 m, 2H and ~1.24 d(4x), 12 H (2x CH(CH$_3$)$_2$); 4.39 m, 1 H, $J$(4’,4’aA) = 8.8, $J$(4’,4’aB) = 2.8, $J$(4’,5’a) = 5.8, $J$(4’,5’b) = 3.7 (H-4’); 4.15 dd, 1 H, $J$(5’a,4’) = 5.8, $J$(5’a,5’b) = 10.7 (H-5’a); 4.12 dd, 1 H, $J$(5’b,4’) = 3.7, $J$(5’b,5’a) = 10.7 (H-5’b); 3.99 dd, 1 H, $J$(2’a,1’) = 7.8, $J$(2’a,2’b) = 10.6 (H-2’a); 3.72 dd, 1 H, $J$(2’b,1’) = 7.1, $J$(2’b,2’a) = 10.6 (H-2’b); 3.09 d, 2 H, $J$(CH$_2$,P) = 21.3 (CO-CH$_2$-P); 2.38 dt, 1 H, $J$(4’aA,1’) ~ $J$(4’aA,4’) = 8.8, $J$(4’aA,4’aB) = 13.3 (H-4’aA); 2.13 ddd, 1 H, $J$(4’aB,1’) = 7.4, $J$(4’aB,4’) = 2.8, $J$(4’aB,4’aA) = 13.3 (H-4’aB); 1.77 d, 3 H, $J$(CH$_3$,6) = 1.2 (5-CH$_3$).

$^{13}$C NMR (d$_6$DMSO):

- 165.85 d, $J$(C,P) = 5.9 (N-C=O); 163.89 (C-4); 151.11 (C-2); 137.88 (C-6); 109.50 (C-5); ~70.80 and 24.0-23.7 (2x CH(CH$_3$)$_2$); 63.94 (C-5’); 54.40 (C-4’); 53.21 (C-1’); 50.44 (C-2’); 30.83 (C-4’a); 12.32 (5-CH$_3$).

1-(2’,3’-Dideoxy-3’-aza-4a’-carba-α-D-glycero-pentofuranosyl)thymine-3’-N-carbonylphosphonic acid (106)

To a solution of 103 (0.280 g, 0.67 mmol) in anhydrous acetonitrile (7 mL) was added bromotrimethylsilane (0.71 mL, 5.36 mmol, 8 eq.), and the reaction was allowed to proceed at 50 °C for 48 h under exclusion of moisture (TLC in H1). The reaction was then concentrated in vacuo, co-evaporated with MeOH (2x5 mL), the residue was dissolved in small amount of water, and washed twice with Et$_2$O. The crude product was purified by reversed-phase chromatography and the aqueous solution of the product was then passed through a column of Dowex 50 (Na$^+$-form), and the sodium salt obtained was lyophilised from water. White solid (0.101 g, 45 %).

HRMS (FAB) calcd for $\text{C}_{11}\text{H}_{17}\text{N}_3\text{O}_7\text{P}$ [M+H]$^+$ 334.0804; found: 334.0810.

NMR:

- mixture of isomers around $>$N-CO bond in the ratio ~ 71 : 29
- some signals are doubled, some others are heavily overlapped
- the complete structural assignment of signals is not possible
$^1$H NMR (DMSO):
11.32 bs and 11.27 bs (NH); 7.48 q and 7.54 q, $J$(6,CH$_3$) = 1.2 (H-6); 5.12 m and 5.23 m (H-1’); 4.99 t and 5.08 t (5’-OH); 4.61 m and 4.68 m (2x O-CH< (2x OiPr)); 4.34 m (H-4’); 3.65 – 3.45 m (H-5’a + H-5’b); 3.59 m (H-2’a); 3.47 m (H-2’b); 2.33 m and 2.24 m (H-4’aA + H-4’aB); 1.756 d and 1.759 d, $J$(CH$_3$,6) = 1.2 (5-CH$_3$); 1.31 – 1.24 (doublets, 4x CH$_3$ (2x OiPr)).

$^{13}$C NMR (DMSO):
163.89 (C-4); 151.15 (C-2); 137.58 and 138.27 (C-6); 109.41 and 109.59 (C-5); 72.48 d, $J$(C,P) = 6.8 (2x O-CH< (2x OiPr)); 64.03 and 66.54 (C-5’); 60.66 and 60.48 (C-2’); 58.43 d, $J$(C,P) = 5.9 (C-4’); 54.15 and 52.30 (C-1’); 29.89 and 32.13 (C-4’a); 23.93 – 23.50 doblets, $J$(C,P) ~ 5.0 (4x CH$_3$ (2x OiPr)); 12.30 and 12.24 (5-CH$_3$).

1-(2’,3’-Dideoxy-3’-aza-4a’-carba-α-D-glycero-pentofuranosyl)thymine-3’-N-thiocarbonylphosphonic acid (107)

Using the procedure outlined for 106, compound 107 was prepared from 104 (0.096 g, 0.22 mmol) and bromotrimethylsilane (0.71 mL, 0.23 mmol) as a white solid (0.040 g, 52 %).

HRMS (FAB) calcd for C$_{11}$H$_{17}$N$_3$O$_6$PS [M+H]+ 350.0576; found: 350.0569.

NMR:
- two isomers around N-C(=S) bond observed - ratio 60 : 40

$^1$H NMR (d$_6$DMSO):

**Major isomer:**
11.34 bs, 1 H (NH); 7.49 q, 1 H, $J$(6,CH$_3$) = 1.2 (H-6); 5.26 m, 1 H, $J$(1’,2’a) = 5.8, $J$(1’,2’b) ~ $J$(1’,4’aB) = 7.8, $J$(1’,4’aA) ~ 8.0 (H-1’); 5.12 t, 1 H, $J$(OH,5’a) ~ $J$(OH,5’b) = 5.1 (5’-OH); 5.00 m, 1 H, $J$(4’,4’aA) ~ 8.0, $J$(4’,4’aB) = 3.7, $J$(4’,5’a) = 5.7, $J$(4’,5’b) = 3.0 (H-4’); 4.65 m, 2H and ~1.28 d(4x), 12 H (2x CH(CH$_3$)$_2$); 4.24 dd, 1 H, $J$(2’a,1’) = 5.8, $J$(2’a,2’b) = 13.3 (H-2’a); 4.16 dd, 1 H, $J$(2’b,1’) = 7.8, $J$(2’b,2’a) = 13.3 (H-2’b); 3.92 ddd, 1 H, $J$(5’a,4’) = 5.7, $J$(5’a,5’b) = 11.2, $J$(5’a,OH) = 5.1 (H-5’a); 3.56 ddd, 1 H, $J$(5’b,4’) = 3.0, $J$(5’b,5’a) = 11.2, $J$(5’b,OH) = 5.1 (H-5’b); 2.47 m, 1 H, $J$(4’aA,1’) ~ $J$(4’aA,4’) ~ 8.0, $J$(4’aA,4’aB) = 13.3 (H-4’aA); 2.41 m, 1 H, $J$(4’aB,1’) = 7.8, $J$(4’aB,4’) = 3.7, $J$(4’aB,4’aA) = 13.3 (H-4’aB); 1.75 d, 3 H, $J$(CH$_3$,6) = 1.2 (5-CH$_3$).

**Minor isomer:**
11.28 bs, 1 H (NH); 7.61 q, 1 H, $J$(6,CH$_3$) = 1.2 (H-6); 5.33 m, 1 H (H-1’); 5.22 t, 1 H, $J$(OH,5’a) ~ $J$(OH,5’b) = 5.2 (5’-OH); 5.20 m, 1 H (H-4’); 4.75 m, 2H and ~1.31 d(4x), 12 H (2x CH(CH$_3$)$_2$); 4.02 m, 1 H (H-2’a); 3.94 m, 1 H (H-2’b); 3.74 dt, 1 H, $J$(5’a,4’) ~ $J$(5’a,OH) = 5.2, $J$(5’a,5’b) = 11.2, (H-5’a); 3.64 ddd, 1 H, $J$(5’b,4’) = 2.3, $J$(5’b,5’a) = 11.2, $J$(5’b,OH)
= 5.2 (H-5’b); 2.55 m, 1 H (H-4’aA); 2.36 m, 1 H (H-4’aB); 1.76 d, 3 H, J(CH₃,6) = 1.2 (5-CH₃).

¹³C NMR (d₆DMSO):

**Major isomer:**

190.75 d, J(C,P) = 176.0 (C=S); 163.89 (C-4); 151.09 (C-2); 137.82 (C-6); 109.49 (C-5);
73.5-73.0 and 23.9-23.4 (2x CH(CH₃)₂); 64.38 (C-4’); 58.62 (C-5’); 57.28 (C-2’); 54.31 (C-1’);
29.88 (C-4’a); 12.30 (5-CH₃).

**Minor isomer:**

190.20 d, J(C,P) = 157.2 (C=S); 163.89 (C-4); 151.03 (C-2); 137.75 (C-6); 109.58 (C-5);
73.5-73.0 and 23.9-23.4 (2x CH(CH₃)₂); 63.85 (C-5’); 64.43 (C-4’); 52.39 (C-1’); 58.18 (C-2’);
32.29 (C-4’a); 12.23 (5-CH₃).

1-(2’,3’-Dideoxy-3’-aza-4a’-carba-α-D-glycero-pentofuranosyl)thymine-3’-N-carbonylmethylphosphonic acid (108)

Using the procedure outlined for 106, compound 108 was prepared from 105 (0.460 g, 1.07 mmol) and bromotrimethylsilane (1.13 mL, 8.53 mmol) as a white solid (0.107 g, 29 %).

HRMS (FAB) calcd for C₁₂H₁₉N₃O₇P [M+H]+ 348.0961; found: 348.0962.

NMR (d₆DMSO) – two isomers around N-C(=S) bond observed; ratio 77 : 23; only the complete data for major isomer could be obtained

**Major isomer:**

¹H NMR (d₆DMSO):

11.31 bs, 1 H (NH); 7.56 q, 1 H, J(6,CH₃) = 1.2 (H-6); 5.18 m, 1 H, J(1’,2’a) = 7.8, J(1’,2’b) = 7.1, J(1’,4’aA) = 8.8, J(1’,4’aB) = 7.4 (H-1’); 4.60 m, 2H and ~1.24 d(4x), 12 H (2x CH(CH₃)₂); 4.39 m, 1 H, J(4’,4’aA) = 8.8, J(4’,4’aB) = 2.8, J(4’,5’a) = 5.8, J(4’,5’b) = 3.7 (H-4’); 4.15 dd, 1 H, J(5’a,4’) = 5.8, J(5’a,5’b) = 10.7 (H-5’a); 4.12 dd, 1 H, J(5’b,4’) = 3.7, J(5’b,5’b) = 10.7 (H-5’b); 3.99 dd, 1 H, J(2’a,1’) = 7.8, J(2’a,2’b) = 10.6 (H-2’a); 3.72 dd, 1 H, J(2’b,1’) = 7.1, J(2’b,2’a) = 10.6 (H-2’b); 3.09 d, 2 H, J(CH₂,P) = 21.3 (CO-CH₂-P); 2.38 dt, 1 H, J(4’aA,1’) ~ J(4’aA,4’) = 8.8, J(4’aA,4’aB) = 13.3 (H-4’aA); 2.13 ddd, 1 H, J(4’aB,1’) = 7.4, J(4’aB,4’) = 2.8, J(4’aB,4’aA) = 13.3 (H-4’aB); 1.77 d, 3 H, J(CH₃,6) = 1.2 (5-CH₃).

¹³C NMR (d₆DMSO):

165.85 d, J(C,P) = 5.9 (N-C=O); 163.89 (C-4); 151.11 (C-2); 137.88 (C-6); 109.50 (C-5);
~70.80 and 24.0-23.7 (2x CH(CH₃)₂); 63.94 (C-5’); 54.40 (C-4’); 53.21 (C-1’); 50.44 (C-2’);
30.83 (C-4’a); 12.32 (5-CH₃).
Diisopropyl-[9-(2’,3’-dideoxy-5’-O-dimethoxytrityl-3’-aza-4a’-carba-α-D-glycero-pentofuranosyl)adenine]-3’-N-methylphosphonate (109)

The general procedure A was followed using mesylate D-79 (0.800 g, 1.19 mmol), Cs₂CO₃ (0.465 g, 1.43 mmol) and adenine (0.241 g, 1.78 mmol). The reaction was complete after 3 h. White foam (0.342 g, 40 %). HRMS (FAB) calcd for C₃₈H₄₈N₆O₆P [M+H]+ 715.3373; found: 715.3339. ¹H and ¹³C NMR spectra were identical to those recorded for enantiomeric 80a.

9-(2’,3’-Dideoxy-5’-O-tert-butyldiphenylsilyl-3’-aza-4a’-carba-α-D-glycero-pentofuranosyl)adenine-3’-N-methylphosphonic acid (112)

To a solution of 109 (0.342 g, 0.48 mmol) in MeOH (10 mL) was added 1 mL of 5 M HCl and the reaction mixture was stirred at rt for 10 min. The solution was passed through a column of Dowex 50 (H⁺), which was then washed with MeOH. The product was liberated from Dowex with diluted aqueous ammonia (2.5 %), the solution was evaporated, the residue was co-distilled with ethanol, and dried in vacuo, giving diisopropyl-[9-(2’,3’-dideoxy-3’-aza-4a’-carba-α-D-glycero-pentofuranosyl)adenine]-3’-N-methylphosphonate 110 as a pale yellow thick oil (0.187 g, 95 %).

Phosphonate 110 (0.187 g, 0.45 mmol) was co-evaporated twice with pyridine before being dissolved in anhydrous pyridine (5 mL). TBDPSCI (0.130 mL, 0.50 mmol, 1.1 eq.) was added and the reaction mixture was stirred at rt for 24 h (TLC in C1). The reaction was then concentrated, the residue was dissolved in CHCl₃ and washed with water. The organic layer was then dried over Na₂SO₄, evaporated and purified by flash chromatography on silica (elution with a linear gradient of EtOH in CHCl₃) to yield diisopropyl-[9-(2’,3’-dideoxy-5’-O-tert-butyldiphenylsilyl-3’-aza-4a’-carba-α-D-glycero-pentofuranosyl)adenine]-3’-N-methylphosphonate 111 as a pale yellow glassy solid (0.268 g, 91 %).

To a solution of protected nucleotide 111 (0.268 g, 0.41 mmol) in anhydrous acetonitrile (5 mL) was added bromotrimethylsilane (0.435 mL, 3.30 mmol, 8 eq.), and the reaction was allowed to proceed at rt for 48 h under exclusion of moisture (TLC in C1). The reaction was then concentrated in vacuo, and the residue was partitioned between CHCl₃ and aqueous citric acid (10 %). Organic layer was then dried over Na₂SO₄, evaporated, and the product was purified by flash chromatography on silica (elution with a linear gradient of EtOH in CHCl₃) to yield 112 as a white glassy solid (0.206 g, 88 %).
HRMS (FAB) calcd for C_{27}H_{36}N_{6}O_{4}PSi [M+H]^+ 567.2305; found: 567.2322.
NMR provided broad signals which could not be fully analyzed.

**Dimer 115**
To a solution of 112 (0.206 g, 0.36 mmol) and 6-N-benzoyl-3’-O-tert-butylidiphenylsilyl-2’-deoxyadenosine 113 (0.320 g, 0.54 mmol, 1.5 eq.) in anhydrous pyridine (8 mL) was added DCC (0.371 g, 1.80 mmol, 5 eq.) and the mixture was allowed to react at rt for 5 d (TLC in C1). After quenching with 0.2 mL H_2O, the reaction was then evaporated, and the residue was purified by flash chromatography on silica (elution with a linear gradient of EtOH in CHCl_3) to yield 114 as a pale yellow glassy solid (0.197 g, 47 %).

**Deprotection**
Protected dimer 114 was dissolved in anhydrous EtOH (6 mL), and the solution was saturated with gaseous NH_3 at 0 °C. The reaction was allowed to proceed at rt for 1 d. The solution was then concentrated in vacuo, co-evaporated with pyridine and dissolved in anhydrous pyridine (5 mL). A solution of tetrabutylammonium fluoride in THF (0.5 M, 5 mL) was added, and the mixture was allowed to react at rt overnight. The reaction was evaporated, the residue dissolved in water (10 mL) and washed twice with Et_2O (10 mL). The aqueous layer was purified by reverse phase chromatography and the product was lyophilised from water to yield 115 as a white solid (0.026 g, 27 %).

HRMS (FAB) calcd for C_{21}H_{29}N_{11}O_{6}P [M+H]^+ 562.2040; found: 562.2024.

^{1}H NMR (D_{2}O):
8.13 s, 1 H, 8.01 s, 1 H, 7.99 s, 1 H and 7.97 s, 1 H (2x H-2 and 2x H-8 (2x Ade));
5.07 b, 1 H (H-1’ (upper)); 3.92 dd, 1 H and 3.51 b, 1 H (H-2’a and H-2’b (upper));
3.43 b, 1 H (H-4’ (upper));
3.56 bt, 1 H and 3.14 um, 1 H (H-5’a and H-5’b (upper));
2.78 dt, 1 H and 1.98 ddd, 1 H (H-4’aA and H-4’aB (upper));
3.90 dd, 1 H, J(NCHa,NChb) = 14.1, J(NCHa,P)=3.3 (NCha);
3.70 dd, 1 H, J(NChb,NCha) = 14.1, J(NChb,P)=3.5 (NChb);
6.22 dd, 1 H, J(1’,2’’) = 6.7, J(1’,2’’) = 6.1 (H-1’ (lower));
2.54 ddd, 1 H, J(2’,1’) = 6.7, J(2’,2’’) = 14.0, J(2’,3’) = 5.2 (H-2’ (lower));
2.67 ddd, 1 H, J(2’,1’) = 6.1, J(2’’,2’’) = 14.0, J(2’’,3’) = 6.7 (H-2’’ (lower));
4.62 ddd, 1 H, J(3’,2’’) = 5.2, J(3’,2’’) = 6.7, J(3’,4’) = 4.4 (H-3’ (lower));
4.20 m, 1 H (H-4’ (lower));
~4.20 m, 2 H (H-5’a and H-5’b (lower)).
**Diisopropyl-[1-(2’,3’-dideoxy-5’-O-dimethoxytrityl-3’-aza-4a’-carba-α-D-glycero-pentofuranosyl)thymine]-3’-N-methylphosphonate (116)**

The general procedure A was followed using mesylate D-76 (1.52 g, 2.25 mmol), Cs₂CO₃ (0.88 g, 2.70 mmol) and thymine (0.43 g, 3.38 mmol). The reaction was complete after 2 h. White foam (0.560 g, 35%).

HRMS (FAB) calcd for C₃₈H₄₉N₃O₈P [M+H]⁺ 706,3257; found: 706,3243.

¹H and ¹³C NMR spectra were identical to those recorded for enantiomeric 80d.

**Diisopropyl-[1-(2’,3’-dideoxy-5’-O-dimethoxytrityl-3’-aza-4a’-carba-β-D-glycero-pentofuranosyl)thymine]-3’-N-methylphosphonate (117)**

The general procedure A was followed using mesylate D-79 (1.69 g, 2.50 mmol), Cs₂CO₃ (0.98 g, 3.00 mmol) and thymine (0.47 g, 3.75 mmol). The reaction was complete after 3 h. White foam (0.650 g, 37%).

HRMS (FAB) calcd for C₃₈H₄₉N₃O₈P [M+H]⁺ 706,3257; found: 706,3267.

¹H and ¹³C NMR spectra were identical to those recorded for enantiomeric 85d.

**1-(2’,3’-Dideoxy-5’-O-dimethoxytrityl-3’-aza-4a’-carba-α-D-glycero-pentofuranosyl)thymine-3’-N-methylphosphonic acid, 4-methoxy-1-oxido-2-pyridylmethyl ester (122)**

To a solution of 116 (0.560 g, 0.79 mmol) in anhydrous acetonitrile (9 mL) was added 2,6-lutidine (1.83 mL, 15.8 mmol, 20 eq.) and bromotrimethylsilane (0.83 mL, 6.32 mmol, 8 eq.), and the reaction was allowed to proceed for 48 h at rt under exclusion of moisture (TLC in H1). The reaction was then concentrated in vacuo, dissolved in EtOAc, and precipitated salts were filtered off. Removal of solvent under reduced pressure afforded the crude product, which was purified by flash chromatography on silica (elution with a linear gradient of EtOAc in toluene) to yield 3-(2’,3’-Dideoxy-5’-O-dimethoxytrityl-3’-aza-4a’-carba-α-D-glycero-pentofuranosyl)thymine-3’-N-methylphosphonic acid 118 as pale yellow solid (0.414 g, 84%).

118 (0.414 g, 0.67 mmol) and 4-methoxy-1-oxido-2-pyridylmethanol (MOPOH) (0.26 g, 1.67 mmol, 2.5 eq.) were co-evaporated twice with toluene before being dissolved in anhydrous pyridine (10 mL), then DCC (0.69 g, 3.33 mmol, 5 eq.) was added and the solution was allowed to react at rt for 48 h. The reaction then was quenched by adding water (5 mL).
After 30 min, the solution was concentrated under reduced pressure and co-evaporated three times with EtOH to give crude diester 119 which was converted to monoester by dissolving in a mixture of PhSH, Et$_3$N and dioxane (1:1:2 v/v, 5 mL) and stirring at rt for 2 h. The reaction mixture was diluted with CH$_2$Cl$_2$ and directly purified by flash chromatography on silica (elution with a linear gradient of HI in acetone). Finally, the product was converted to triethylammonium salt by partitioning between CHCl$_3$ and aqueous TEAB (1 M), the organic layer was dried over Na$_2$SO$_4$, evaporated, and lyophilised from dioxane to afford the desired product as a white solid (0.130 g, 23 %).

HRMS (FAB) calcd for C$_{39}$H$_{43}$N$_4$O$_{10}$NaP [M+Na]$^+$ 781.2615; found: 781.2643. 

NMR provided broad signals which could not be fully analyzed.

1-(2’,3’-Dideoxy-5’-O-dimethoxytrityl-3’-aza-4a’-carba-β-D-glycero-pentofuranosyl)thymine-3’-N-methylphosphonic acid, 4-methoxy-1-oxido-2-pyridylmethyl ester (123)

Using the procedure outlined for 118, 3-(2’,3’-Dideoxy-5’-O-dimethoxytrityl-3’-aza-4a’-carba-β-D-glycero-pentofuranosyl)thymine-3’-N-methylphosphonic acid 120 was prepared from 117 (0.650 g, 0.92 mmol), 2,6-lutidine (2.14 mL, 18.4 mmol) and bromotrimethylsilane (0.97 mL, 7.36 mmol) as a pale yellow solid (0.544 g, 95 %).

Then, using the procedure outlined for 122, compound 123 was prepared from 120 (0.544 g, 0.88 mmol), MOPOH (0.34 g, 2.20 mmol) and DCC (0.91 g, 4.40 mmol) as a white solid (0.266 g, 35 %).

HRMS (FAB) calcd for C$_{39}$H$_{44}$N$_4$O$_{10}$P [M+H]$^+$ 759.2795; found: 759.2758.

NMR provided broad signals which could not be fully analyzed.

N-Benzylxycarbonyl-trans-4-acetyloxy-6-O-dimethoxytrityl-D-prolinol (124)

Using the procedure outlined for L-77, compound 124 was prepared from 97 (19.7 g, 31.2 mmol) and NaOAc (25.6 g, 312 mmol) as a pale yellow glassy solid (10.40 g, 56 %).

HRMS (FAB) calcd for C$_{36}$H$_{37}$NO$_7$ [M+H]$^+$ 595.2570; found: 595.2599.

NMR:
- two isomers around N-C(=O) bond observed (ratio 52 : 48)
- signal of major isomer is given first (if signals of both isomers are resolved)
- $J$(H,H) values could not be determined due to strong line broadening

$^1$H NMR (d$_6$DMSO):
7.18-7.35 m + 7.05 m + 6.85 m, 18 H (C₆H₅ (Bn) + C₆H₅ and 2x C₆H₄ (ODMTr)); 5.28 m and 5.265 m, 1 H (H-1’); 5.08 m and 4.94 d + 4.92 d (O-CH₂-C₆H₅); 4.04 m and 4.02 m, 1 H (H-4’); 3.71 s, 6 H (2x OMe (ODMTr)); 3.67 bdd and 3.59 bdd, 1 H (H-2’a); 3.60 bdd and 3.59 bdd, 1 H (H-2’b); 3.25 bdd and 3.035 bdd (H-5’b); 3.14 bdd and 3.255 bdd (H-5’a); 2.23 m and 2.21 m, 1 H (H-4’aA); 2.15 m and 2.12 m, 1 H (H-4’aB); 1.99 s, 3 H (OAc);

13C NMR (d₆DMSO):
170.43 (C=O (OAc)); 158.27(2), 145.16, 135.77(2), 129.82(4), 127.49-128.66(10), 126.93, 113.38(4) (C₆H₅ (Bn) + 2x C₆H₄ and C₆H₅ (ODMTr)); 154.18 and 154.00 (N-CO-O); 85.62 and 85.34 (>C< (ODMTr)); 72.93 and 72.40 (C-1’); 66.43 and 666.06 (O-CH₂ (Bn)); 64.51 and 63.50 (C-5’); 56.07 and 55.49 (C-4’); 55.23 (2x OCH₃); 52.63 and 52.26 (C-2’); 34.94 and 33.84 (C-4’a); 21.10 (CH₃ (OAc)).

N-Benzylxycarbonyl-trans-4-(4-nitrobenzoyloxy)-6-O-dimethoxytrityl-D-prolinol (125)
To a solution of 96 (115.7 g, 209 mmol) in toluene (500 mL) was added Ph₃P (109.6 g, 0.418 mmol, 2 eq.), 4-nitrobenzoic acid (PNBA) (52.4 g, 313.5 mmol, 1.5 eq.) and 2,6-lutidine (36.4 mL, 313.5 mmol, 1.5 eq.), and the suspension was co-evaporated twice with toluene to remove traces of water, and finally co-evaporated with anhydrous THF. Then the mixture was dissolved in anhydrous THF (1 L), cooled to 0 °C, and diisopropyl azodicarboxylate (DIAD) (61.7 mL, 313.5 eq., 1.5 eq.) was added dropwise over ca. 10 min. The reaction was allowed to proceed for 1 h (TLC in T2). The solvent was evaporated under reduced pressure, and the residue was co-distilled twice with toluene to remove rest of THF. The solid was then dissolved in toluene-hexane mixture (1:1 v/v, 1 L), and cooled to 0 °C for 1 h. The precipitated Ph₃PO was filtered off, and the filtrate was concentrated in vacuo to afford the crude product, which was purified by flash chromatography on silica (elution with a linear gradient of EtOAc in toluene) to yield 125 as a white foam (94.3 g, 64 %).

HRMS (FAB) calcd for C₄₁H₃₉N₂O₉ [M+H]+ 703.2656; found: 703,2643.

1H NMR (d₆DMSO; T = 80°C):
8.31 m, 2 H and 8.15 m, 2 H (CO-C₆H₄-NO₂); 7.36 m, 2 H, 7.30 m, 2 H, 7.23 m, 5 H and 6.86 m, 4 H (2x C₆H₄ and C₆H₅ (ODMTr)); 7.28-7.22 m, 5 H (C₆H₅ (Bn)); 5.59 dq, 1 H, J(1’,2’a) = 2.4, J(1’,2’b) ~ J(1’,4’aA) ~ J(1’,4’aB) = 4.5 (H-1’); 5.04 bs, 2 H (CH₂ (Bn)); 4.17 m, 1 H (H-4’); 3.82 dd, 1 H, J(2’a,2’b) = 12.0, J(2’a,1’) = 2.4 (H-2’a); 3.75 s, 6 H (2x OMe (ODMTr)); ~3.75 m, 1 H (H-2’b); 3.30 dd, 1 H, J(5’a,4’) = 3.3, J(5’a,5’b) = 9.3 (H-5’a); 3.22 dd, 1 H, J(5’b,4’) = 5.0, J(5’b,5’a) = 9.3 (H-5’b); 2.36 m, 2 H (H-4’aA + H-4’aB).
**N-Benzylxocarbonyl-trans-4-hydroxy-6-O-dimethoxytrityl-D-prolinol (126)**

**Route A:** Using the procedure outlined for L-78, compound 126 was prepared from 124 (10.40 g, 17.46 mmol) as a pale yellow thick oil (9.00 g, 93%).

**Route B:** To a solution of 125 (94.3 g, 134.2 mmol) in a mixture of toluene (500 mL) and THF (500 mL) was added MeOH (500 mL) followed by 1 M methanolic solution of sodium methoxide (5 mL), and the mixture was left to react at room temperature overnight (TLC in T2). The reaction was worked up by adding Dowex 50 (Et3N+-form, ca. 10 mL), which was then filtered off, and the solution was concentrated in vacuo. The residue was dissolved in toluene (500 mL) and left to stand at –20 °C overnight. Precipitated crystals of 4-nitrobenzoic acid methyl ester were quickly filtered off, and washed with a small amount of cold toluene. The crystallization was then repeated to afford a second crop of the ester. Filtrate was evaporated and crude product was purified by flash chromatography on silica (elution with a linear gradient of EtOAc in toluene) to yield 126 as a pale yellow thick oil (67.3 g, 92%).

**HRMS (FAB) calcd for C34H35NO6Na [M+Na]+ 576.2362; found: 576.2334.**

**NMR:**
- some proton and most of carbon signals are doubled due to cis-/trans- isomerism on the >N-CO- bond
- many \(J(H,H)\) could not be determined due to heavy overlap of multiplets and line broadening of signals

**1H NMR (DMSO):**
7.37 – 6.85 m, 18 H (2x C6H4 and C6H5 (DMTr) + C6H5 (Bn)); 5.06 s, 4.93 d, \(J(\text{gem}) = 12.5\) and 4.90 d, \(J(\text{gem}) = 12.5\) (CH2 (Bn)); 4.98 d, \(J(\text{OH,1}) = 3.6\) and 4.96 d, \(J(\text{OH,1}) = 3.6\) (OH-1); 4.34 m and 4.30 m (H-1); 4.015 m and 4.00 m (H-4); 3.72 - 3.71 s, 6 H (2x OCH3 (DMTr)); 3.47 – 3.37 m (H-2a + H-2b); 3.21 dd, d, \(J(5a,5b) = 9.2\), d, \(J(5a,4) = 5.2 + 3.02\) dd, \(J(5a,5b) = 9.2\), d, \(J(5a,4) = 3.0\) and 3.09 dd, d, \(J(5a,5b) = 9.0\), d, \(J(5a,4) = 5.6 + 3.05\) dd, \(J(5a,5b) = 9.0\), d, \(J(5a,4) = 3.2\) (H-5a + h-5b); 2.05 – 1.90 m (H-4aA + H-4aB).

**13C NMR (DMSO):**
158.20, 154.50, 154.43, 145.25, 136.03, 135.95, 135.88, 129.80, 129.74, 129.12, 128.62, 128.47, 128.42, 128.02, 127.95, 127.76, 127.51 and 113.34 (2x C6H4 and C6H5 (ODMTr) + C6H5 (Bn)); 85.46 and 85.34 (>C< (ODMTr)); 68.66 and 68.04 (C-1); 66.18 and 65.86 (CH2 (Bn)); 64.60 and 63.61 (C-5); 56.27 and 55.68 (C-4); 55.47 and 55.12 (C-2); 55.21 (2x OMe (ODMTr)); 37.89 and 36.88 (C-4a).

**N-Benzylxocarbonyl-trans-4-methanesulfonyl-6-O-dimethoxytrityl-D-prolinol (127)**
Using the procedure outlined for L-76, compound 127 was prepared from 126 (47.0 g, 84.9 mmol) and mesyl chloride (32.9 mL, 424.4 mmol) as a pale yellow glassy solid (48.4 g, 90 %).

HRMS (FAB) calcd for C_{35}H_{38}NO_{8}S \ [M+H]^+ 632,2318; found: 632,2341.

{\textsuperscript{1}}H NMR (CDCl\textsubscript{3}):
7.38 – 7.25 m, 10 H (2x C\textsubscript{6}H\textsubscript{5}); 7.17 m, 4 H and 6.83 m, 4 H (2x C\textsubscript{6}H\textsubscript{4} (DMTr)); 5.22 m, 1 H (H-1); 5.16 s, 2 H (CH\textsubscript{2} (Bn)); 4.20 m, 1 H-4); 3.96 m, 1 H (H-2a); 3.86 m, 1 H (H-5a); 3.64 m, 1 H (H-2b); 3.62 m, 1 H (H-5b); 3.01 s, 3 H (SO\textsubscript{2}-CH\textsubscript{3}); 2.39 ddt, 1 H, J(4aA,4aB) = 14.2, J(4aA,4) = 7.1, J(4aA,1) = 2.1, J(4aA,2b) = 2.1 (H-4Aa); 1.97 ddd, 1 H, J(4aB,1) = 4.7, J(4aB,4aA) = 14.2, J(4aB,4) = 9.3 (H-4AB).

{\textsuperscript{13}}C NMR (CDCl\textsubscript{3}):
158.61(2), 147.30, 139.44(2), 129.96, 129.94, 129.11(4), 128.60, 128.31, 128.08, 128.00, 127.83(2), 127.74(2), 127.06 and 113.14(4) (2x C\textsubscript{6}H\textsubscript{4} and C\textsubscript{6}H\textsubscript{5} (ODMTr) + C\textsubscript{6}H\textsubscript{5} (Bn)); 81.00 (>C< (ODMTr)); 77.99 (C-1); 67.65 (CH\textsubscript{2} (Bn)); 65.42 (C-5); 59.10 (C-4); 55.24(2) (2x OMe (ODMTr)); 53.43 (C-2); 38.75 (SO\textsubscript{2}CH\textsubscript{3}); 35.14 (C-4a).

1-(N-Benzylxycarbonyl-cis-6-O-dimethoxytrityl-d-prolinol-4-yl)thymine (128)
The general procedure A was followed using mesylate 127 (11.70 g, 18.52 mmol), Cs\textsubscript{2}CO\textsubscript{3} (7.24 g, 22.2 mmol) and thymine (3.50 g, 27.78 mmol); DMF was replaced by DMSO and the temperature was kept at 120 °C. The reaction was complete after 5 d. White foam (3.71 g, 30 %).

HRMS (FAB) calcd for C_{39}H_{40}N_{3}O_{7} \ [M+H]^+ 662,2866; found: 662,2895.

{\textsuperscript{1}}H NMR (d\textsubscript{6}DMSO; T = 50\textdegree C):
11.16 bs, 1 H (NH); 7.35 q, 1 H, J(6,CH\textsubscript{3}) = 1.1 (H-6); 7.35-7.20 m, 10 H, 7.21 m, 4H and 6.84 m, 4H (2x C\textsubscript{6}H\textsubscript{4} and C\textsubscript{6}H\textsubscript{5} (ODMTr)) + C\textsubscript{6}H\textsubscript{5} (Bn)); 5.10 bs, 2 H (CH\textsubscript{2} (Bn)); 4.84 m, 1 H, J(1',2'a) ~ J(1',4'aA) ~ 7.7, J(1',2'b) ~ 9.0, J(1',4'aB) = 9.8 (H-1'); 4.02 m, 1 H, J(4',4'aA) ~ J(4',4'aB) ~ 7.5, J(4',5'a) = 3.4, J(4',5'b) ~ 7.0 (H-4'); 3.92 dd, 1 H, J(2'a,1') ~ 7.7, J(2'a,2'b) = 11.0 (H-2'a); 3.73 s, 6 H (2x OMe (ODMTr)); 3.43 bt, 1 H, J(2'b,1') ~ 9.0, J(2'b,2'a) = 11.0 (H-2'b); 3.32 dd, 1 H, J(5'a,4') = 3.4, J(5'a,5'b) = 8.8 (H-5'a); 3.21 dd, 1 H, J(5'b,4') ~ 7.0, J(5'b,5'a) = 8.8 (H-5'b); 2.43 dt, 1 H, J(4'aA,1') ~ 7.7, J(4'aA,4') ~ 7.5, J(4'aA,4'aB) = 12.8 (H-4'aA); 2.11 ddd, 1 H, J(4'aB,1') = 9.8, J(4'aB,4') ~ 7.5, J(4'aB,4'aA) = 12.8 (H-4'aB);
1.68 d, 3 H, J(CH\textsubscript{3},6) = 1.1 (5-CH\textsubscript{3}).
1-(cis-D-Prolinol-4’-yl)thymine (129)

Using the procedure outlined for 99, compound 129 was prepared from 128 (3.71 g, 5.61 mmol) as a pale yellow solid (1.20 g, 95%).

HRMS (FAB) calcd for C₁₀H₁₆N₃O₃ [M+H]+ 226.1192; found: 226.1194.

¹H NMR (DMSO):
7.82 q, 1 H, J(6,CH₃) = 1.2 (H-6); 4.89 m, 1 H, J(1’,2’a) = 7.6; J(1’,2’b) = 4.0; J(1’,4’aA) = 8.8; J(1’,4’aB) = 6.4 (H-1’); 3.42 d, 2 H, J(5’,4’) = 5.4 (H-5’a + H-5’b); 3.05 ddt, 1 H, J(4’,4’aA) = 7.5; J(4’,4’aB) = 8.8; J(4’,5’a) = 5.4 and J(4’,5’b) = 5.4 (H-4’); 3.01 dd, 1 H, J(2’a,1’) = 7.6 and J(2’a,2’b) = 11.2 (H-2’a); 2.85 dd, 1 H, J(2’b,1’) = 4.0 and J(2’b,2’a) = 11.2 (H-2’b); 2.20 ddd, 1 H, J(4’aA,4’aB) = 13.3; J(4’aA,1’) = 8.8 and J(4’aA,4’) = 7.5 (H-4’aA); 1.76 d, 3 H, J(CH₃,6) = 1.2 (CH₃-5); 1.40 ddd, 1 H, J(4’aB,4’aA) = 13.3; J(4’aB,1’) = 6.4 and J(4’aB,4’) = 8.8 (H-4’aB).

¹³C NMR (DMSO):
163.98 (C-4); 151.17 (C-2); 138.22 (C-6); 109.23 (C-5); 64.46 (C-5’); 59.74 (C-4’); 54.44 (C-1’); 51.40 (C-2’); 34.45 (C-4’a); 12.48 (CH₃-5).

Methyl-[3-(2’,3’-dideoxy-5’-O-dimethoxytrityl-3’-aza-4a’-carba-α-D-glycero-pentofuranosyl)thymine]-3’-N-carbonylphosphonate (133)

**Route A:** Dimethyl-phenylcarbonylphosphonate (0.552 mL, 3.00 mmol, 1.5 eq.) was added to a stirred solution of amine 99 (0.450 g, 2.00 mmol) in DMF (2 mL), and the mixture was heated to 60 °C for 2 h (TLC in H1). The reaction was evaporated and co-distilled with toluene to afford crude dimethyl-[3-(2’,3’-dideoxy-3’-aza-4a’-carba-α-D-glycero-pentofuranosyl)thymine]-3’-N-carbonylphosphonate (130).

To a solution of the crude product in anhydrous pyridine (5 mL) was added DMTrCl (0.813 g, approx. 1.2 eq.), and the mixture was left to react for 2 h at rt (TLC in C1). After quenching with MeOH (2 mL), the solution was evaporated and the residue was purified by flash chromatography on silica (elution with a linear gradient of HI in EtOAc) to yield 133 as a pale yellow glassy solid (0.180 g, 14%).

**Route B:** Dimethyl-phenylcarbonylphosphonate (0.362 mL, 2.00 mmol, 1.5 eq.) was added to a stirred solution of amine 99 (0.295 g, 1.31 mmol) in DMF (1.3 mL), and the mixture was heated to 60 °C for 2 h (TLC in H1) to afford crude (130).

A mixture of PhSH, Et₃N and dioxane (1:1.4:2 v/v, 2 mL) was then added and the reaction was allowed to react for 1 h at rt. The solution was diluted with EtOAc and purified by flash chromatography on silica (elution with a linear gradient of MeOH in EtOAc) to yield methyl-
-[3-(2',3'-dideoxy-3'-aza-4a'-carba-α-β-D-glycero-pentofuranosyl)thymine]-3'-N-carboxyphosphonate 131 as a white solid (0.447 g, 98 %).

To a solution of 131 in anhydrous pyridine (5 mL) was added DMTrCl (0.523 g, 1.2 eq.), and the mixture was left to react for 2 h at rt (TLC in C1). After quenching with MeOH (2 mL), the solution was evaporated and the residue was purified by flash chromatography on silica (elution with a linear gradient of HI in EtOAc) to yield 133 as a colourless glassy solid (0.336 g, 40 %). The overall yield from 99 to 133 is 39 %.


H NMR (d6DMSO):
11.25 bs, 1 H (NH); 9.87 bs (P-OH); 7.65 q, 1 H, J(6,CH3) = 1.1 (H-6); 7.36 m, 2 H, 7.31 m, 2 H, 7.23 m, 4 H, 7.21 m, 1 H and 6.89 m, 4 H (2x C6H4 and C6H5 (ODMTr)); 5.14 m, 1 H, J(1',2'a) = 7.0, J(1',2'b) = 5.0, J(1',4'aA) = 6.5, J(1',4'aB) = 7.1 (H-1'); 4.30 dd, 1 H, J(2'a,1') = 5.0, J(2'a,2'b) = 12.4 (H-2'a); 4.29 m, 1 H, J(4',4'aA) = 8.4, J(4',4'aB) = 4.7, J(4',5'a) = 4.9, J(4',5'b) = 3.2 (H-4'); 4.02 dd, 1 H, J(2'b,1') = 7.0, J(2'b,2'a) = 12.4 (H-2'b); 3.73 s, 6 H (2x OMe (ODMTr)); 3.42 d, 1 H, J(OCH3,P) = 10.5 (P-OCH3); 3.22 dd, 1 H, J(5'a,4') = 4.9, J(5'a,5'b) = 9.2 (H-5'a); 2.95 dd, 1 H, J(5'b,4') = 3.2, J(5'b,5'a) = 9.2 (H-5'b); 2.23 ddd, 1 H, J(4'aA,1') = 6.5, J(4'aA,4') = 8.4, J(4'aA,4'aB) = 13.5 (H-4'aA); 2.17 ddd, 1 H, J(4'aB,1') = 7.1, J(4'aB,4') = 4.7, J(4'aB,4'aA) = 13.5 (H-4'aB); 1.75 d, 3 H, J(CH3,6) = 1.1 (5-CH3).

13C NMR (d6DMSO):
163.89 (C-4); 158.13, 158.14, 145.12, 135.87, 135.78, 129.89(2), 129.83(2), 128.02(2), 127.85(2), 126.69, 113.42(2) and 113.37(2) (2x C6H4 and C6H5 (ODMTr)); 151.26 (C-2); 137.57 (C-6); 109.08 (C-5); 85.51 (>C< (ODMTr)); 63.57 (C-5'); 55.15 and 55.13 (2x OCH3); 54.50 (C-4'); 53.94 (C-1'); 52.28 d, J(C,P) = 6.3 (P-OCH3); 50.53 (C-2'); 31.70 (C-4'a); 12.22 (5-CH3).

Methyl-[3-(2',3'-dideoxy-5'-O-dimethoxytrityl-3'-aza-4a'-carba-β-β-D-glycero-pentofuranosyl)thymine]-3'-N-carboxyphosphonate (135)

The title compound was synthesized from amine 129 (0.308 g, 1.37 mmol) according to the procedure described above for 133 (Route A via dimethyl-[3-(2',3'-dideoxy-3'-aza-4a'-carba-β-β-D-glycero-pentofuranosyl)thymine]-3'-N-carboxyphosphonate 132) to yield 135 as a colourless glassy solid (0.392 g, 44 %).

HRMS (FAB) calcd for C33H35N3O10P [M-H]- 648,2111; found: 648,2090.
$^1$H NMR (d$_6$DMSO):
11.27 bs, 1 H (NH); 9.66 bs (P-OH); 7.40 q, 1 H, $J$(6,CH$_3$) = 1.2 (H-6); 7.36 m, 2 H, 7.30 m, 2 H, 7.22 m, 4 H, 7.21 m, 1 H and 6.88 m, 4 H (2x C$_6$H$_4$ and C$_6$H$_5$ (ODMTr)); 4.76 m, 1 H, $J$(1',2'a) = 7.1, $J$(1',2'b) ~ 9.0, $J$(1',4'aA) ~ 8.2, $J$(1',4'aB) = 6.9 (H-1'); 4.57 dd, 1 H, $J$(2'a,1') = 7.1, $J$(2'a,2'b) = 11.3 (H-2'a); 4.18 m, 1 H, $J$(4',4'aA) ~ 8.2, $J$(4',4'aB) = 9.3, $J$(4',5'a) = 3.4, $J$(4',5'b) = 3.2 (H-4'); 3.73 s, 6 H (2x OMe (ODMTr)); 3.72 m, 1 H, $J$(2'b,1') ~ 9.0, $J$(2'b,2'a) = 11.3 (H-2'b); 3.43 d, 1 H, $J$(OCH$_3$,P) = 10.4 (P-OCH$_3$); 3.25 dd, 1 H, $J$(5'a,4') = 3.4, $J$(5'a,5'b) = 8.6 (H-5'a); 3.06 dd, 1 H, $J$(5'b,4') = 3.2, $J$(5'b,5'a) = 8.6 (H-5'b); 2.34 td, 1 H, $J$(4'aA,1') ~ $J$(4'aA,4') ~ 8.2, $J$(4'aA,4'aB) = 12.6 (H-4'aA); 2.03 ddd, 1 H, $J$(4'aB,1') = 6.9, $J$(4'aB,4') = 9.3, $J$(4'aB,4'aA) = 12.6 (H-4'aB); 1.61 d, 3 H, $J$(CH$_3$,6) = 1.2 (5-CH$_3$).

$^{13}$C NMR (d$_6$DMSO):
163.64 (C-4); 158.17, 158.14, 145.11, 135.94, 135.80, 129.73(4), 127.95(2), 127.78(2), 127.61 and 113.34(4) (2x C$_6$H$_4$ and C$_6$H$_5$ (ODMTr)); 151.05 (C-2); 137.01 (C-6); 109.46 (C-5); 85.58 (>C< (ODMTr)); 63.75 (C-5'); 55.12 (2x OCH$_3$); 54.38 (C-4'); 52.92 (C-1'); 52.19 d, $J$(C,P) = 5.9 (P-OCH$_3$); 49.42 (C-2'); 31.16 (C-4'a); 12.06 (5-CH$_3$).

4-Methoxy-1-oxido-2-pyridylmethyl-[3-(2',3'-dideoxy-5'-O-dimethoxytrityl-3'-aza-4a'-carba-α-D-glycero-pentofuranosyl)thymine]-3'-N-carbonylphosphonate (137)

133 (0.330 g, 0.51 mmol) and (MOPOH) (0.118 g, 0.76 mmol, 1.5 eq.) were co-evaporated twice with toluene before being dissolved in anhydrous acetonitrile (2 mL), then 1-methylimidazole (0.242 mL, 3.05 mmol, 6 eq.) followed by 1,3,5-triisopropylbenzenesulfonyl chloride (TIPSCl) (0.462 g, 1.52 mmol, 3 eq.) was added and the solution was allowed to react at rt for 0.5 h. The reaction mixture was diluted with toluene and directly purified by flash chromatography on silica (elution with a linear gradient of HI in EtOAc) to yield impure diester 134 as a pale yellow glassy solid.

134 was converted to monoester by dissolving in a mixture of Py and H$_2$O (3:2 v/v, 5 mL) and stirring at rt overnight. The solution was then evaporated in vacuo, and the residue purified by flash chromatography on silica (elution with a linear gradient of HI in EtOAc). Finally, the product was converted to triethylammonium salt by partitioning between CHCl$_3$ and aqueous TEAB (1 M), the organic layer was dried over Na$_2$SO$_4$, evaporated, and purified by reversed-phase chromatography affording two major products of a similar polarity: monomethyl ester 133 (0.080 g, 21 %) and the desired monopicolyl ester 137 which was then lyophilised from dioxane. White solid (0.056 g, 13 %).

¹H NMR (d₆DMSO):
11.25 bs, 1 H (NH); 9.49 bs (P-OH); 8.13 d, 1 H, J(6,5) = 7.3 (H-6 (Pic)); 7.67 q, 1 H, J(6,CH₃) = 1.2 (H-6); 7.36 m, 2 H, 7.31 m, 2 H, 7.23 m, 2 H, 7.22 m, 2 H, 7.19 m, 1 H and 6.89 m, 4 H (2x C₆H₄ and C₆H₅ (ODMTr)); 7.23 d, 1 H, J(3,5) = 3.4 (H-3 (Pic)); 6.96 dd, 1 H, J(5,3) = 3.4, J(5,6) = 7.3 (H-5 (Pic)); 5.21 m, 1 H, J(1’,2’a) = 5.0, J(1’,2’b) = 7.2, J(1’,4’aA) = 6.4, J(1’,4’aB) = 7.3 (H-1’); 4.935 d, 2 H, J(CH₂P) = 7.4 (P-CH₂); 4.32 dd, 1 H, J(2’a,1’) = 5.0, J(2’a,2’b) = 12.3 (H-2’a); 4.30 m, 1 H, J(4’,4’aA) = 8.6, J(4’,4’aB) = 4.6, J(4’,5’a) = 4.4, J(4’,5’b) = 3.0 (H-4’); 4.10 dd, 1 H, J(2’b,1’) = 7.2, J(2’b,2’a) = 12.3 (H-2’b); 3.79 s, 3 H (OMe (Pic)); 3.725 s, 3 H and 3.72 s, 3 H (2x OMe (ODMTr)); 3.28 dd, 1 H, J(5’a,4’) = 4.4, J(5’a,5’b) = 9.0 (H-5’a); 2.89 dd, 1 H, J(5’b,4’) = 3.0, J(5’b,5’a) = 9.0 (H-5’b); 2.23 ddd, 1 H, J(4’aA,1’) = 6.4, J(4’aA,4’) = 8.6, J(4’aA,4’aB) = 12.5 (H-4’aA); 2.17 ddd, 1 H, J(4’aB,1’) = 7.3, J(4’aB,4’) = 4.6, J(4’aB,4’aA) = 12.5 (H-4’aB); 1.75 d, 3 H, J(CH₃,6) = 1.1 (5-CH₃).

¹³C NMR (d₆DMSO):
163.80 (C-4); 158.14, 158.10, 145.07, 135.77(2), 129.77(2), 129.70(2), 127.95(2), 127.82(2), 127.63, 113.38(2) and 113.33(2) (2x C₆H₄ and C₆H₅ (ODMTr)); 151.21 (C-2); 139.36, 110.54 and 109.12 (3x –CH= (Pic)); 137.41 (C-6); 109.12 (C-5); 85.50 (>C< (ODMTr)); 63.53 (C-5’); 61.76 d, J(C,P) ~ 5.0 (O-CH₂-Pic); 56.12 (OCH₃ (Pic)); 55.09 and 55.06 (2x OCH₃ (ODMTr)); 54.56 (C-4’); 53.83 (C-1’); 50.55 (C-2’); 31.20 (C-4’a); 12.14 (5-CH₃).

4-Methoxy-1-oxido-2-pyridylmethyl-[3-(2’,3’-dideoxy-5’-O-dimethoxytrityl-3’-aza-4a’-carba-β-D-glycero-pentofuranosyl)thymine]-3’-N-carbonylphosphonate (138)
Using the procedure outlined for 137, compound 138 was prepared from 135 (0.392 g, 0.60 mmol) as a white solid (0.087 g, 17 %); besides the desired product, monoester 135 was also isolated (white solid, 0.093 g, 21 %).

HRMS (FAB) calcd for C₃₉H₄₀N₄O₁₁P [M+H]⁺ 771.2431; found: 771.2440.

¹H NMR (d₆DMSO):
11.29 bs, 1 H (NH); 9.37 bs (P-OH); 8.14 d, 1 H, J(6,5) = 7.3 (H-6 (Pic)); 7.41 q, 1 H, J(6,CH₃) = 1.2 (H-6); 7.36 m, 2 H, 7.30 m, 2 H, 7.23 m, 4 H, 7.19 m, 1 H, and 6.89 m, 4 H (2x C₆H₄ and C₆H₅ (ODMTr)); 7.23 d, 1 H, J(3,5) = 3.5 (H-3 (Pic)); 6.97 dd, 1 H, J(5,3) = 3.4, J(5,6) = 7.3 (H-5 (Pic)); 4.94 d, 2 H, J(CH₂P) = 7.3 (O-CH₂ (Pic)); 4.81 m, 1 H, J(1’,2’a) = 7.2, J(1’,2’b) = 9.5, J(1’,4’aA) = 8.3, J(1’,4’aB) = 7.0 (H-1’); 4.64 dd, 1 H, J(2’a,1’) = 7.2, J(2’a,2’b) = 11.2 (H-2’a); 4.19 m, 1 H, J(4’,4’aA) = 8.3, J(4’,4’aB) = 9.3, J(4’,5’a) = 3.5, J(4’,5’b) = 6.2 (H-4’); 3.79 s, 3 H (OMe (Pic)); 3.76 dd, 1 H, J(2’b,1’) = 9.5, J(2’b,2’a) =
11.2 (H-2’b); 3.73 s, 3 H and 3.725 s, 3 H (2x OMe (ODMTr)); 3.18 dd, 1 H, $J(5’a,4’)=3.5$,
$J(5’a,5’b)=8.8$ (H-5’a); 3.14 dd, 1 H, $J(5’b,4’)=6.2$, $J(5’b,5’a)=8.8$ (H-5’b); 2.35 td, 1 H,
$J(4’aA,1’)=J(4’aA,4’)=8.3$, $J(4’aA,4’aB)=12.8$ (H-4’aA); 2.03 ddd, 1 H, $J(4’aB,1’)=7.0$,
$J(4’aB,4’)=9.3$, $J(4’aB,4’aA)=12.8$ (H-4’aB); 1.60 d, 3 H, $J(CH_3,6)=1.2$ (5-CH$_3$).

$^{13}$C NMR (d$_6$DMSO):
163.63 (C-4); 158.18, 158.12, 145.12, 135.84, 135.78, 129.74(2), 129.66(2), 127.96(2),
127.77(2), 126.70, 113.36(2) and 113.35(2) (2x C$_6$H$_4$ and C$_6$H$_5$ (ODMTr)); 156.90, 150.71 d,
$J(C,P)=6.8$, 139.40, 110.58 and 108.80 (C$_3$H$_7$N (Pic)); 151.05 (C-2); 136.92 (C-6); 109.53
(C-5); 85.60 (>C< (ODMTr)); 63.63 (C-5’); 61.79 d, $J(C,P)=4.9$ (O-CH$_2$-Pic); 56.14 (OCH$_3$
(Pic)); 55.11 and 55.08 (2x OCH$_3$ (ODMTr)); 54.41 d, $J(C,P)=4.9$ (C-4’); 52.78 (C-1’);
49.50 (C-2’); 31.01 (C-4’a); 12.06 (5-CH$_3$).
7. References


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I hereby declare that this thesis is my own work and effort and that it has not been submitted anywhere for any award. Where other sources of information have been used, they have been acknowledged.

22. 5. 2008

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