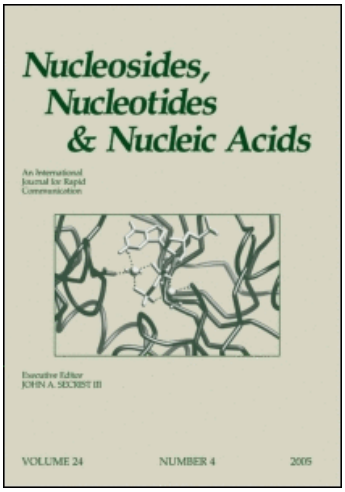


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# Novel Nucleotide Analogues as Potential Substrates for TMPK, a Key Enzyme in the Metabolism of AZT

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# Novel Nucleotide Analogues as Potential Substrates for TMPK, a Key Enzyme in the Metabolism of AZT

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## ABSTRACT

Novel cyclic and acyclic analogues of dTMP and AZTMP were synthesized from the corresponding *cyclo*Sal-phosphotriesters. This method yielded the nucleotides in good yields with a simple work-up. Investigation of the substrate properties of the modified nucleotides towards TmpK showed, that they are very poor substrates for this key enzyme in the bioactivation of AZT.

## INTRODUCTION

Nucleoside-based inhibitors of reverse transcriptase were the first drugs to be used in the chemotherapy of AIDS. After entering the cell, these substances have to be activated to their triphosphates by cellular kinases. Thus, for the most extensively used drug AZT, the product of the initial phosphorylation is a very poor

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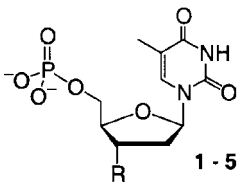
substrate for the second kinase, thymidylate kinase (TmpK). The steric demand of the azido-group in the 3'-position of AZTMP causes the so-called P-loop repulsion and therefore a 200-fold decrease in the rate of phosphorylation.[1] Here, we present the syntheses of novel analogues of dTMP and AZTMP as potential substrates of TmpK by hydrolysis of the corresponding *cyclo*Sal-phosphotriesters[2] on a preparative scale.

## RESULTS

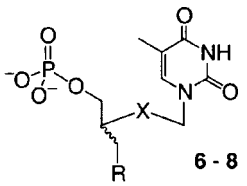
Since *cyclo*Sal-phosphotriesters have been designed to selectively deliver nucleoside monophosphates (NMPs) by means of chemical hydrolysis, these compounds can be used as synthons in the synthesis of NMPs. The *cyclo*Sal-nucleoside monophosphates were synthesized directly from the corresponding nucleoside analogues following either the chlorophosphite- or the phosphoramidite-approach.[2] The phosphotriesters were then hydrolysed by treatment with triethylamine in acetonitrile/water. The work up consisted of reversed-phase chromatography followed by ion exchange. The work up was very simple since no polar side-products are formed. This method yields the monophosphates in high yields (up to 74%).

The evaluation of the substrate properties of the NMPs towards TmpK showed, that the cyclic nucleotides **3**–**5** are hardly phosphorylated by TmpK at all, although the substituents were very similar or even isoster to the azido-group in AZTMP **2**. This might be explained by the fact, that these substituents lack the zwitter-ionic structure of the azido-group. In contrast to suggestions from molecular modelling experiments,[1] the acyclic nucleotides **6**–**8** were also poor substrates for TmpK,

***Table 1.*** Steady-state kinetics of nucleotides **1**–**8** with human TmpK.




**1 - 5**




**6 - 8**

| NMP | R | X | $k_{cat}[s^{-1}]$[a] | $K_M[\mu M]$[b] | $k_{cat}/K_M[M^{-1}s^{-1}10^{-3}]$ |
|---|---|---|---|---|---|
| **1** | OH | – | ≈0.73 | 6 ± 1 | 122 ± 22 |
| **2** | $N_3$ | – | ≈0.0122 | 12 ± 3 | 1.0 ± 0.2 |
| **3** | SCN | – | < 0.02 | Not detectable | Not detectable |
| **4** | $CH_2CHCH_2$ | – | < 0.02 | Not detectable | Not detectable |
| **5** | $CH_2CCH$ | – | < 0.02 | Not detectable | Not detectable |
| **6** | OH | O | ≈0.008 | ≈1300 | ≈0.006 |
| **7** | OH | $CH_2$ | ≈0.0014 | ≈20 | ≈0.07 |
| **8** | $N_3$ | $CH_2$ | Not detectable | Not detectable | Not detectable |

[a]Catalytic rate at substrate saturation.
[b]Michaelis constant.





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caused possibly by entropic effects. However, compound **6**, which has both the aminal oxygen and the free hydroxy-group of the natural substrate dTMP **1**, shows a higher rate of phosphorylation than compound **7**, which lacks the aminal oxygen. Compound **8**, having no aminal oxygen and the azido-group instead of the hydroxy-group is hardly phosphorylated at all. Table 1 summarizes the enzyme kinetical data.

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