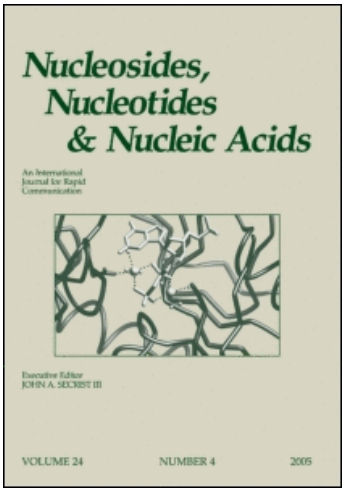

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Human DNA Polymerases and Retroviral Reverse Transcriptases: Selectivity in Respect to dNTPs Modified at Triphosphate Residues

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To cite this Article Victorova, L. S. , Semizarov, D. G. , Shirokova, E. A. , Alexandrova, L. A. , Arzumanov, A. A. , Jasko, M. V. and Krayevsky, A. A.(1999) 'Human DNA Polymerases and Retroviral Reverse Transcriptases: Selectivity in Respect to dNTPs Modified at Triphosphate Residues', Nucleosides, Nucleotides and Nucleic Acids, 18: 4, 1031 — 1032
To link to this Article: DOI: 10.1080/15257779908041641
URL: http://dx.doi.org/10.1080/15257779908041641

NUCLEOSIDES & NUCLEOTIDES, 18(4&5), 1031–1032 (1999)

HUMAN DNA POLYMERASES AND RETROVIRAL REVERSE TRANSCRIPTASES: SELECTIVITY IN RESPECT TO dNTPs MODIFIED AT TRIPHOSPHATE RESIDUES

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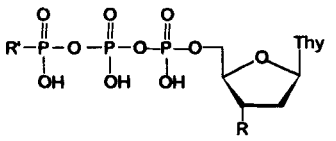
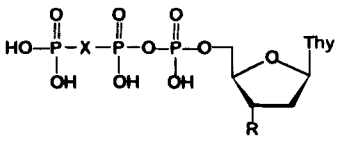
A series of dTTP and ddTTP(3'N_3) γ-phosphonates and β,γ-diphosphonates are studied as substrates or terminating substrates towards different human DNA polymerases and retroviral reverse transcriptases.

The aim of our research was the design of dNTP analogs with high inhibitory properties towards HIV replication which do not require cellular phosphorylation.

A comparative study on the *in vitro* activity of nucleotide analogs modified at triphosphate and sugar moieties has been carried out with human DNA polymerases α and β, calf thymus terminal deoxynucleotidyl transferase (TDT), HIV and avian myeloblastosis virus (AMV) reverse transcriptases (RTs). We present here the results of the study of four groups of compounds, namely γ-phosphonate analogs of dTTP (**I**), ddTTP(3'N_3) (**II**), β,γ-diphosphonate analogs of dTTP (**III**), and ddTTP(3'N_3) (**IV**) [1,2].





Ia-b, R=OH ; **IIa-b**, R=N_3 ; **IIIa-d**, R=OH ; **IVa-d**, R=N_3
a: R`= $CH_3$ , **b**: R`= C_6H_5 **a**: X=CF_2; **b**: X=CFH; **c**: X=$CFCH_3$; **d**: X=CBr_2

The replacement of the γ-phosphate by γ-phosphonyl groups in **I** did not significantly affect the substrate properties in respect to HIV and AMV RTs, whereas the affinity of **Ia-b** towards human DNA polymerases was essentially decreased. The kinetic data for **I-II** in the reaction catalyzed by AMV RT are shown in Table. These data demonstrate that

a decrease in affinity to the RTs of the compounds with a rather bulky substituent at the γ-phosphate was insignificant. It implies that there are no steric hindrances for binding the modified substrate to the RT active center. It is also noteworthy that the substitution of the γ-phosphate by phenylphosphonate in **I** and **II** considerably increased their hydrophobicity and stability in human serum.

Table. Kinetic parameters of the AMV RT catalyzed addition of **I** and **II** to the primer

Compound	K_m, μM	V_{max}/V_{max}dTTP	Compound	K_m, μM	V_{max}/V_{max}dTTP
Ia	4.4 ± 0.3	0.74	**IIa**	20 ± 0.6	0.68.
Ib	3.0 ± 0.2	0.78	**IIb**	10 ± 0.4	0.68
dTTP	1.3 ± 0.1	1	ddTTP(3'N_3)	0.96± 0.08	0.92

The substitution of the β and γ phosphate groups by the diphosphonate one in **III** and **IV** allows one to modulate their substrate activity and selectivity towards different types of DNA polymerases. RTs proved to be the enzymes the least specific to these modifications. The substrate activity of compounds **III** (and terminating substrate activity of **IV**) was decreased in the following order of **X**: CF_2 = CFH > CBr_2 > $CFCH_3$. For the other enzymes the substrate activity of compounds **III** differed in another manner: CF_2 = CBr_2 = CFH > $CFCH_3$ (for TDT), CF_2 = CFH > $CFCH_3$ >> CBr_2 (for DNA polymerase α), and CFH > CF_2 > $CFCH_3$ (for DNA polymerase β); the compound **IIId** (X = CBr_2) was neither a substrate nor an inhibitor of this enzyme.

Thus , we demonstrated that the direct interpolation of the data on the substrate activity of differently modified dNTPs towards DNA polymerases is not correct. This effect can be resulted from the summary of the contribution of each modification as well as the contribution of their combination.

These data allow us to consider phosphate modified dNTPs as potential anti-HIV drugs which can inhibit virus replication in intercellular medium before virus penetrates into cells.

ACKNOWLEDGEMENTS. The work was supported by the Russian Foundation for Basis Research, Projects 96-04-48277 and 96-04-48278; Program Russian National Priorities in Medicine, AIDS, Projects sp1 and sp2; the National Institute of Health (Career Development Award Grant).

1. Arzumanov, A.A., Semizarov, D.G.., Victorova, L.S., Dyatkina, N.B, and Krayevsky, A.A. *J.Biol.Chem.* **1996,** *271*, 24389-24394.
2. Martynov, B.I., Shirokova, E.A., Jasko, M.V., Victorova, L.S., Krayevsky, A.A. *FEBS Lett.*, **1997**, *410*, 423-427.