

This paper is dedicated to Professor David Shugar on the occasion of his 80th birthday

Modified substrates of DNA polymerases and design of antivirals

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The results obtained in our laboratory on investigating of substrate properties of a large number of compounds towards different DNA polymerases have been summarized. On the basis of systematic analysis a directed synthesis of nucleotides with antiviral properties was performed.

Rational search for inhibitors of human immunodeficiency virus (HIV) reproduction in a series of nucleosides and nucleotides has been performed according to some empirical rules developed in several laboratories, including ours, during the last decade. The requirements concerning these compounds were chosen as follows. Deoxynucleosides or nucleotides must penetrate into HIV or other virus infected cells, be phosphorylated to corresponding modified deoxynucleoside 5'-triphosphates (dNTP) by cellular enzymes with subsequent effective and selective inhibition of DNA synthesis catalyzed by HIV reverse transcriptase on natural RNA and DNA templates, or by DNA polymerases of other human viruses. In this review we do not take into account some essential effects which such compounds exert on the rate of DNA metabolism in human organisms, the rate of formation of viral types resistant to the action of these compounds, etc. These aspects can be discussed elsewhere. A few deoxynucleosides

and nucleotides, however, are known to possess the above mentioned properties.

Our research dealt mainly, in a comparative aspect, with the behavior of human DNA polymerases and viral reverse transcriptases, including HIV reverse transcriptases, towards modified substrates as well as with evaluation of the contribution of sugar moiety (glycone) and phosphate modifications in nucleotides and nucleoside 5'-triphosphates to their substrate properties.

The results of comparative analysis of the role of glycone modifications in nucleoside 5'-triphosphates in the binding of the latter with DNA polymerases were described in previous publications [1-3]. This investigation was performed in collaboration with Dr R. Beabealashvili group (National Cardiology Research Center, Moscow), and Dr J. Gluzman group (American Cyanamid Company, Pearl River, U.S.A.). Herein we present only the main conclusions of this part of the research.

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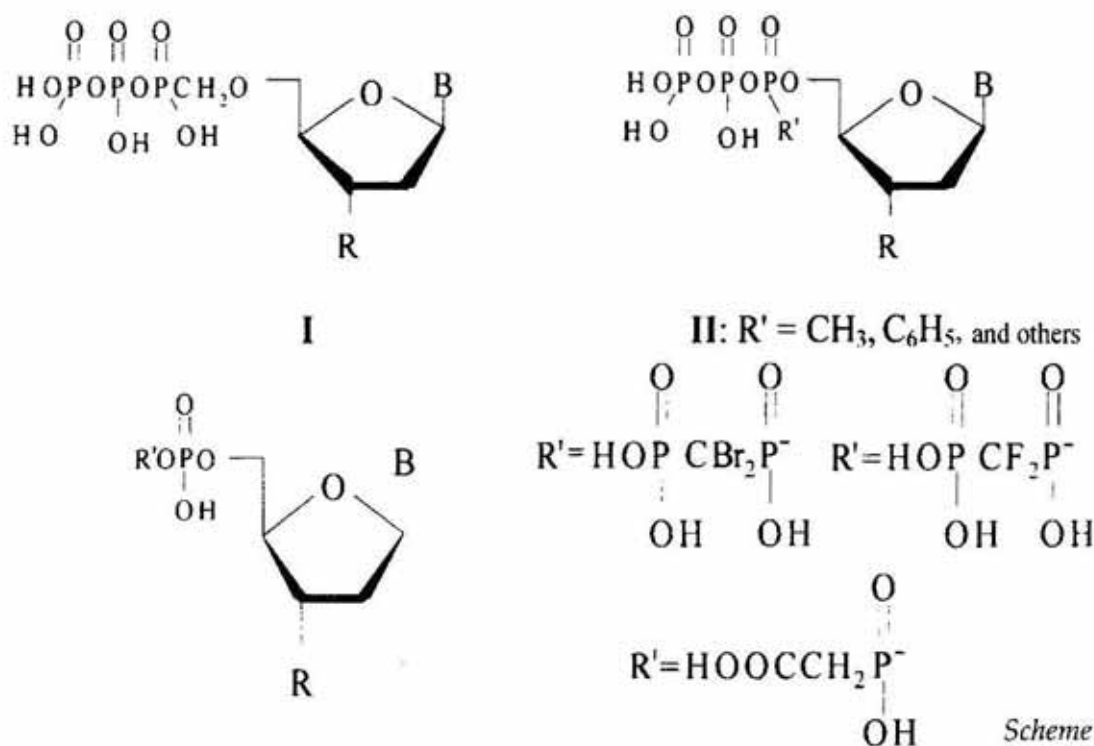
Abbreviations: CMV, cytomegalovirus; dNTP, deoxynucleoside 5'-triphosphates; HSV-1, herpes simplex virus type 1; TdT, terminal deoxynucleotidyl transferase.

- 1. Retroviral reverse transcriptases are less specific towards glycone modifications in β -D-nucleoside 5'-triphosphate series than are human DNA polymerases.
- 2. Modified 2'-deoxynucleoside 5'-triphosphates with bulky substituents at 3'-position (N_3 [4], NHR , NR_2 [5], SH [6] NO_2 [7]) selectively inhibit, by a terminating mechanism, DNA synthesis catalyzed by retroviral reverse transcriptases.
- 3. Retroviral reverse transcriptases are also less specific towards conformationally restricted modified nucleoside 5'-triphosphates [1, 8-12]. This was observed most clearly in the cases when the glycone structure could be made a planar one, with low energy consumption. We imply that the planar conformation of the glycone ring favors the transition state of a substrate in the DNA synthesizing complex.
- 4. Human replicative DNA polymerases α and ϵ reveal the highest substrate specificity; the repairing DNA polymerase β and terminal deoxynucleotidyl transferase show lower specificity.
- 5. Substrate specificity of the herpes simplex virus type 1 (HSV-1), cytomegalovirus (CMV), and adenovirus DNA polymerases is very close to that of human replicative DNA polymerases but it is slightly lower.

It should be mentioned that human DNA polymerases δ and γ were not at our disposal. Therefore we could not compare their substrate specificity with that of viral enzymes.

Studies on the influence of triphosphate modifications in nucleoside 5'-triphosphates on their substrate properties demonstrated that the introduction of additional methylene group at 5'-position of triphosphate (I, in Scheme 1) resulting in the hydrolytically stable P-C bond does not prevent the incorporation of the modified compound into the growing DNA chain in the process of DNA synthesis catalyzed by HIV reverse transcriptases [13]. If β,γ -diphosphate residue of nucleoside 5'-triphosphates is substituted by dibromomethylenephosphonic, difluoromethylenephosphonic or phosphonoacetate groups [13, 14] the affinity of such compounds towards DNA polymerases is strongly decreased whereas their affinity towards HIV reverse transcriptases is practically retained. Similar results were obtained for I towards HIV reverse transcriptases [15].

Hydroxyl substitution at α -phosphate residue by a methyl [16] or a phenyl group [17] (compounds II, in Scheme 1) also reduces substrate properties of nucleoside 5'-triphosphates towards retroviral reverse transcriptases but to a much lesser extent than towards human DNA polymerases.

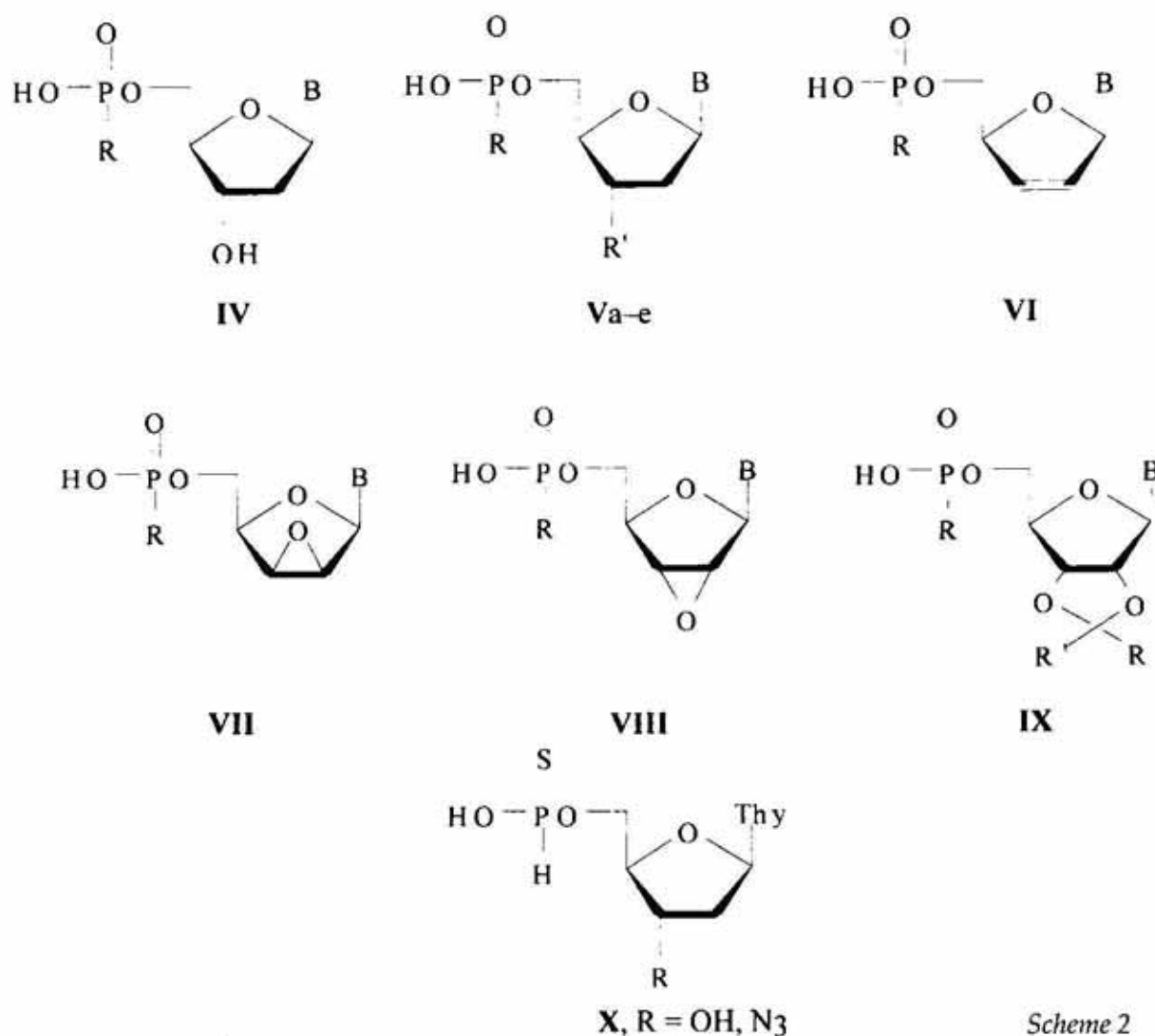


Scheme 1

We investigated the influence of β,γ -diphosphate replacement by other anionic residues. Compounds **III** (Scheme 1) were evaluated as terminating substrates towards retroviral reverse transcriptases. This part of the research was performed together with G. Galegov group (Institute of Virology, Moscow). The dNTP analogs in which β and γ phosphorous are bound through NH [18] or CH₂ [19] groups revealed only slightly reduced properties.

The results of these studies show that natural 2'-deoxynucleoside 5'-triphosphates even when modified at the glycone or at any phosphorus atom, can retain their substrate properties. The limits for such modifications and their combinations are under investigation. This research could be of use both for the investigation of the structure of DNA polymerases active centers and for designing of antiviral, antibacterial, antifungal, and anticancer drugs.

Then we evaluated a number of 2'-deoxynucleoside 5'-phosphonates (**IV**) as potential antiviral agents (Scheme 2). We expected that these compounds, if phosphorylated by cellular enzymes to corresponding modified triphosphates **III** (where R' = H, Me or an other radical), could selectively inhibit HIV replication. The tested compounds proved, however, to be inactive with the exception of **IV** (B = Gua, R = COOH) that inhibited HIV replication by 50% at a concentration of 5 μ M [20] in MT-4 cell culture. The lack of activity of the other compounds could have resulted from lack of recognition of these phosphonates by cellular phosphorylating enzymes. Other reasons are also possible, for instance, hydrolysis of compounds **IV** by dephosphorylating enzymes to natural deoxynucleosides. The latter, which undergo intensive phosphorylation thus compensating the inhibiting effect of the corre-



Scheme 2

sponding pyrophosphorylphosphonates that might be formed from IV. To check this metabolic pathway we tested the influence of IV (R = H) on inhibition of HIV replication by AZT. If IV (R = H) is hydrolyzed in the cells (even partially) it would compensate AZT action by the forming thymidine and in fact, H-phosphonate IV (R = H) completely compensated the inhibition effect of AZT that favors the enzymatic hydrolysis of IV (R = H) [20].

Then we evaluated nucleotides bearing two modifications — at glycone and at 5'-phosphate residues. Among them there were 3'-modified 2',3'-dideoxynucleotides (V) [21–27], 2',3'-dideoxy-2',3'-didehydronucleotides (VI) [24], 2',3'-anhydrocyclonucleotides of ribo- and lyxo-series (VII, VIII) [24], ribonucleotide acetals, ketals and orthoesters (IX) [28] (Scheme 2).

Several highly active inhibitors of HIV replication in some cell cultures were found, especially Va (B = Thy, R = H, F, CH₂OH, COOH, CH₂Cl, COOEt, R' = N₃), Vb (B = Thy, R = F, R' = F); Vc (B = Ade, R = H, R' = H); Vd (B = Ade, R = H, R' = H, F); Ve (B = Gua, R = R' = H); VI, (B = Thy, R = F); IX (B = Ade, R = H, R' = OMe) should be mentioned. Some other nucleoside 5'-phosphonates of V, VI, IX types showed moderate activity, which, as a rule, was lower than that of the parent nucleosides. Many compounds were inactive. Substances X revealed no activity [29] either. Antiviral researches were performed in Dr G. Galegov and Dr E. Karamov groups, Institute of Virology, Moscow, in Department of Prof. Dr E. De Clercq (Katholieke University, Leuven), in the groups of Dr K.A. Watanabe and Dr B.W. Polsky (Memorial Sloan Kettering Cancer Center, New York), and the group of Dr J. Gluzman (American Cyanamid Company, Pearl River). None of the compounds were reasonably active as inhibitors of human HSV-1 and 2 types, CMV or adenovirus.

The interpretation of the above data is difficult. One can mention that the activity values within the series of phosphonates of different 3'-modified nucleosides do not correlate. For example, antiviral activity values in the series of 3'-fluoro-3'-deoxythymidine 5'-phosphonates differ from those for the same phosphonates of 3'-azido-2',3'-dideoxythymidine.

The metabolism of nucleoside 5'-phosphonates is very poorly known. We examined stability of the most interesting compounds both

in the presence of several dephosphorylating enzymes and in human blood serum [30, 31]. Phosphonates IV and V (B = Thy, R = H) were shown to be resistant to dephosphorylation even after five days incubation with *Escherichia coli* alkaline phosphatase or phosphatases from human placenta and calf intestines. However, snake venom 5'-nucleotidase slowly dephosphorylated most of these compounds. The dephosphorylation rate for IV, V (R = H) was 5–7 and 0.3%, respectively, of that for thymidine 5'-phosphate. Under the same conditions, the dephosphorylation rate for V (B = Thy) depended on the structures of both R and R', the final effect being a combination of their separate effects. The hydrolysis of IV (B = Thy, R = H or F), and V (R' = F or N₃, R = H) in human serum was also reduced. For example, the dephosphorylation rate for IV and V with R = H, reached only 4–7% of the rate for the corresponding 5'-phosphates, whereas the dephosphorylation rate for IV and V with R = F decreased only by a half. The reaction ran in two stages, replacement of the fluorine by the hydroxyl followed by hydrolysis of the phosphate group. Compounds IV and V (B = Ade) with R = H, COOH, Me, R' = H, OH were shown to be stable in human serum even after a week at 37°C; neither deamination nor apurination were observed.

All these data demonstrate that the metabolism rate of nucleotides modified at the phosphate or both phosphate and glycone residues decreases as compared with that for nonmodified nucleotides and depends on the nature of these modifications and their combination. Probably slower metabolic transformations of nucleoside 5'-phosphonates in comparison with the corresponding nucleosides can account for the low toxicity of the former in cell cultures.

Taken together, these data demonstrate that nucleoside 5'-phosphonates are involved, due to the wide range of their properties both in inhibition of HIV replication and in cellular metabolism. The same picture is observed for different nucleosides modified at the glycone.

We come back now to modified nucleoside 5'-triphosphates. Comparative investigation on substrate specificity of different DNA polymerases towards various modified nucleoside 5'-triphosphates with terminating and other modes of action, resulted in preparation of several compounds specifically inhibiting

DNA synthesis catalyzed by different types of these enzymes.

As it was mentioned above, several highly specific terminating substrates of reverse transcriptases of retroviruses are known at present. Modified substrates **I** (R = OH) were shown to be specific for replicative DNA polymerase α [32]. The nucleotide residue of this compound is incorporated into 3'-terminus of DNA, sharply decreasing the rate of chain elongation and practically terminating the synthesis. Triphosphate **I** (R = OH) demonstrates the same properties towards DNA polymerases of HSV-1 and CMV [33, 34].

Arabino nucleoside 5'-triphosphates with a bulky 2'-substituent proved to be highly specific inhibitors of human HSV and CMV DNA polymerases [35]. They are not recognized by human DNA polymerases and retroviral reverse transcriptases. Nucleotide residues of these compounds are incorporated into DNA chain in the process of DNA biosynthesis catalyzed by viral DNA polymerases and stop further elongation. Arabino compounds with NH₂ and N₃ at the 2'-position reveal substrate properties towards both viral and human DNA polymerases and, after incorporation into growing DNA, terminate chain elongation [33-35].

So far, no highly selective inhibitors of terminal deoxynucleotidyl transferase (TdT) are known. This may be one of the reasons of the lack of data on its function in the cell. A high concentration of this enzyme was observed in premature leukocytes and in the cells of some forms of leukemia. We have found that modified nucleoside 5'-triphosphates with the *trans*-like position of the nucleic base towards 5'-CH₂-group are not recognized by template-dependent DNA polymerases but terminate DNA synthesis catalyzed by TdT [36]. Probably this rule is not a general one, but there are some other *trans*-like dNTP shown to be specific inhibitors of TdT [36].

Our results together with those from other laboratories allowed us to initiate the construction of DNA polymerase active center models. Two methods can be used for this purpose and within certain limits they are complementary. In the first one the necessity of functional groups in 2'-deoxynucleotide 5'-triphosphates (dNTP) substrates for their interaction with complexes (DNA polymerase + template-primer) is ana-

lyzed. The direct interaction of glycone functional groups in dNTP with the enzyme by formation of ionic or hydrogen bonds was shown not to be critical. The data available allow to conclude that practically no part of the glycone residue has any fatal influence on substrate properties of the dNTP modified in such a way towards different DNA polymerases including reverse transcriptases [37]. The glycone seems to play the role of a template only. It has to orientate properly the nucleic base and the triphosphate residue. However, these results should be interpreted with caution because of two reasons. The first is the wide diversity of conditions used by different researchers. This concerns especially the structure of template-primer complexes (monotonous poly(rA)-oligo(dT), poly(dA)-oligo(dT), and alternating poly[d(A-T)] and others). The scatter of the results obtained in such artificial systems is too wide. For example, Michaelis constants for the same modified dNTP differed in various systems up to two orders of magnitude. Secondly, for serious generalizations the quantitative measurements of affinity of modified substrates to DNA, synthesized complexes are required. In our opinion in such studies only template-primers with all four natural nucleotides should be used and only under standardized conditions.

In the second method, modified dNTP with bulky substituents at different positions of the molecule are used. The incorporation of such dNTP into DNA chain can mean that there are no topological restrictions for the site of this substituent. Several such positions in a substrate molecule are found. Some of them, namely 5-position of a pyrimidine and 7-position of a purine in dNTP are common for all DNA polymerases studied including *E. coli* DNA polymerase I [38, 39], T4 phage enzyme, α and β DNA polymerases of human origin, retroviral reverse transcriptases, DNA polymerases of human DNA containing viruses and other enzymes [40-42]. Some other substitutions are rather uncommon. As it was mentioned before CMV and HSV DNA polymerases are able to incorporate the nucleotide residue of arabino compound into DNA 3'-termini. One of the stereoisomers of compound **III** has been shown to be a substrate of some DNA polymerases. Some other dNTP with bulky substituents are also known to be substrates of different DNA

polymerases but their analysis is the subject of another paper (in preparation).

Analyzing these data one should keep in mind that the rule "All or nothing" does not apply to enzymology. Therefore a well justified conclusion on the role of any substituent in the substrate molecule for interaction with the enzyme center needs the quantitative evidence of its affinity and kinetic reaction constants. These parameters should be calculated under adequate conditions which so far have not been studied extensively enough in so complex multisubstrate systems as the DNA synthesizing complexes. A good illustration of the current state of this type of research is a wide dispersion of Michaelis constant and reaction rate values for the same pair of dNTP and the enzyme under different conditions. To help in developing adequate criteria for this investigation we would like to discuss here the key conditions.

In our opinion it is necessary:

- 1. to perform the experiments on DNA templates (RNA templates for reverse transcriptases) containing a full set of four natural nucleotide residues, but not monotonous or alternating templates;
- 2. to measure kinetic parameters either in steady-state or pre-steady-state (or in both of them). These procedures are well elaborated [43];
- 3. to use enzyme preparations standardized against several parameters with indication of their characteristics (properties);
- 4. to evaluate the minimum necessary set of reaction parameters, namely K_m , V_{max} in steady-state kinetics and pre-steady state kinetics.

Evaluation of properties of modified dNTPs in quantitative kinetic terms together with X-ray and physicochemical investigation of the structure of corresponding enzyme-substrate complexes may eventually result in development of adequate models of DNA polymerases active centers.

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REFERENCES

1. Krayevsky, A.A., Kukhanova, M.K., Atrazhev, A.M., Dyatkina, N.B., Papchikhin, A.V., Chidgeavadze, Z.G. & Beabealashvilli, R.Sh. (1988) Selective inhibitors of DNA chain elongation catalyzed by DNA polymerases. *Nucleosides & Nucleotides* **7**, 613-617.
2. Krayevsky, A.A. & Kukhanova, M.K. (1990) Physicochemical aspects of functioning of DNA polymerases. *Sov. Sci. Rev. D. Physicochem. Biol.* **9**, 179-242.
3. Krayevsky, A.A. (1992) Modified nucleosides and nucleotides, inhibiting HIV reproduction: analysis of situation and probable perspective. *Mol. Biol. Russian* **26**, 725-744.
4. Chidgeavadze, Z.G., Beabealashvilli, R.Sh., Krayevsky, A.A. & Kukhanova, M.K. (1986) Nucleoside 5'-triphosphates with modified sugars as substrates for DNA polymerases. *Biochim. Biophys. Acta* **868**, 145-152.
5. Jasko, M.V., Fedorov, I.I., Atrazhev, A.M., Mozzherin, D.G., Victorova, L.S., Bochkarev, A.V., Plavec, J., Gurskaya, G.V. & Krayevsky, A.A. (1995) Synthesis, molecular and crystal structure of 3'-N-alkylamino-3'-deoxythymidines and some biochemical properties of their phosphorus esters. *Nucleosides & Nucleotides* **14**, 23-38.
6. Juzhakov, A.A., Chidgeavadze, Z.G., Beabealashvilli, R.Sh., Krayevsky, A.A., Galegov, G.A., Korneyeva, M.A., Nosik, D.N. & Kileso, T.Ju. (1991) 3'-Mercapto-3'-deoxythymidine 5'-triphosphate as a terminator DNA synthesis catalyzed by RNA dependent DNA polymerases. *Bioorg. Chem. Russian* **17**, 504-509.
7. Kuznetsova, E.V., Kukhanova, M.K., Gurskaya, G.V., Fedorov, I.I., Jasko, M.V., Chattopadhyaya, J. & Krayevsky, A.A. (1995) Conformation of 3'-nitro-2',3'-dideoxythymidine in crystal and properties of its 5'-triphosphate as terminating substrate for DNA polymerases. *Mol. Biol. Russian* **29**, 407-414.
8. Dyatkina, N.B., Minassyan, Sh.Kh., Kukhanova, M.K., Krayevsky, A.A., von Janta-Lipinski, M., Chidgeavadze, Z.G. & Beabealashvilli, R.Sh. (1987) Properties of 2',3'-dideoxy-2',3'-dehydrothymidine 5'-triphosphate in terminating DNA synthesis catalyzed by several different DNA polymerases. *FEBS Lett.* **219**, 151-155.
9. Krayevsky, A.A. & Watanabe, K.A. (1993) Possibility for existence of a general conformational motif in active centers of enzymes which are involved in nucleic acids

- metabolism. *Nucleosides & Nucleotides* **12**, 649–670.
10. Semizarov, D.G., Victorova, L.S., Krayevsky, A.A. & Kukhanova, M.K. (1993) Modified nucleoside 5'-triphosphates containing 2',3'-fused three-membered rings as substrates for different DNA polymerases. *FEBS Lett.* **327**, 45–48.
 11. Krayevsky, A.A., Victorova, L.S., Mozzherin, D.Ju. & Kukhanova, M.K. (1993) Acyclic 2',3'-dideoxy-2',3'-didehydronucleoside 5'-triphosphates as termination substrates of broad set of DNA polymerases. *Nucleosides & Nucleotides* **12**, 83–93.
 12. Shirokova, E.A., Tarussova, N.B., Shipitsin, A.V., Semizarov, D.G. & Krayevsky, A.A. (1994) Novel acyclic nucleoside 5'-triphosphate analogs imitating 2',3'-dideoxy-2',3'-didehydronucleotides: synthesis and biological properties. *J. Med. Chem.* **37**, 3139–3148.
 13. Killeso, T.Ju., Tarussova, N.B., Atrazheva, E.D., Kukhanova, M.K., Shulenin, S.B., Bobkov, A.F., Garayev, M.M., Galegov, G.A. & Krayevsky, A.A. (1990) Inhibitory analysis of DNA biosynthesis catalyzed by retroviral reverse transcriptases. *Bioorg. Chem. Russian* **16**, 524–530.
 14. Rozovskaya, T.A., Mischenko, A.V., Tarussova, N.B., Kukhanova, M.K., Krayevsky, A.A. & Beabealashvilli, R.Sh. (1993) Reverse transcriptase of human immunodeficiency virus: isolation and substrate specificity. *Mol. Biol. Russian* **27**, 1051–1060.
 15. Jie, L., Van Aerschot, A., Balzarini, J., Janssen, G., Bueson, R., Hoogmartens, J., De Clercq, E. & Herdewijn, P. (1990) 5'-O-Phosphorylmethyl-2',3'-dideoxynucleosides: synthesis and anti-HIV activity. *J. Med. Chem.* **33**, 2481–2487.
 16. Victorova, L.S., Dyatkina, N.B., Mozzherin, D.Ju., Atrazhev, A.M., Krayevsky, A.A. & Kukhanova, M.K. (1992) Formation of phosphonoester bonds catalyzed by DNA polymerases. *Nucleic Acids Res.* **20**, 783–789.
 17. Dyatkina, N.B., Arzumanov, A.A., Victorova, L.S., Kukhanova, M.K. & Krayevsky, A.A. (1995) Synthesis and substrate properties of TTP analogs with large hydrophobic substituent groups at α -P atoms. *Nucleosides & Nucleotides* **14**, 91–104.
 18. Ma, Q.-F., Bathurst, I.C., Barr, F.J. & Kenyon, G.L. (1992) New thymidine triphosphate analogue inhibitors of human immunodeficiency virus type 1 reverse transcriptase. *J. Med. Chem.* **35**, 1938–1941.
 19. Balzarini, J., Herdewijn, P., Pauwels, R., Broder, S. & De Clercq, E. (1988) α,β - and β,γ -Methylene 5'-phosphonate derivatives of 3'-azido-2',3'-dideoxythymidine-5'-triphosphate. *Biochem. Pharmacol.* **37**, 2395–2403.
 20. Jasko, M., Shipitsin, A., Shirokova, E., Krayevsky, A., Polsky, B., Baron, P., MacLow, C., Ostrander, M. & O'Hara, B. (1993) 5'-Phosphonates of ribonucleosides and 2'-deoxyribonucleosides: synthesis and antiviral activity. *Nucleosides & Nucleotides* **12**, 879–892.
 21. Tarussova, N.B., Khorlin, A.A., Krayevsky, A.A., Korneyeva, M.N., Nosik, D.N., Kruglov, N.B., Galegov, G.A. & Beabealashvilli, R.Sh. (1989) Inhibition of human immunodeficiency virus reproduction in cell culture by 3'-azido-2',3'-dideoxynucleoside 5'-phosphonates. *Mol. Biol. Russian* **23**, 1716–1723.
 22. Karamov, E.V., Lukashov, V.V., Tarussova, N.B., Kornilayeva, G.V., Rodina, M.A., Kukhanova, M.K. & Krayevsky, A.A. (1990) Inhibition of immunodeficiency virus in cell cultures by 3'-azido-2',3'-dideoxynucleosides 5'-phosphites. *Mol. Biol. Russian* **24**, 1695–1701.
 23. Karamov, E.V., Lukashov, V.V., Gorbachova, A.L., Makarova, T.V., Kornilayeva, G.V., Tarussova, N.B. & Krayevsky, A.A. (1992) Inhibition of human immunodeficiency virus reproduction in cell cultures by 2',3'-dideoxynucleoside 5'-phosphites. *Mol. Biol. Russian* **26**, 201–207.
 24. Krayevsky, A.A., Tarussova, N.B., Zhu, Q.-Y., Vidal, P., Chou, T.-C., Baron, P., Polsky, B., Jiang, X.-Y., Matulic-Adamic, J., Rosenberg, I. & Watanabe, K.A. (1992) 5'-Hydrogen-phosphonates and 5'-methylphosphonates of sugar modified pyrimidine nucleosides as potential anti-HIV agents. *Nucleosides & Nucleotides* **11**, 177–196.
 25. Dyatkina, N., Arzumanov, A., Krayevsky, A., O'Hara, B., Gluzman, Ja., Baron, P., MacLow, C. & Polsky, B. (1994) Synthesis and antiviral activity of some fluorinated nucleotide derivatives. *Nucleosides & Nucleotides* **13**, 325–337.
 26. Matulic-Adamic, J., Rosenberg, I., Arzumanov, A.A., Dyatkina, N.B., Shirokova, E.A., Krayevsky, A.A. & Watanabe, K.A. (1993) A simple multigram synthesis of 5'-hydrogenphosphonates and 5'-phosphorofluoridates of sugar modified nucleosides. *Nucleosides & Nucleotides* **12**, 1085–1092.
 27. Krayevsky, A.A., Tarussova, N.B., Kukhanova, M.K., Balzarini, J., De Clercq, E., Karamov, E.V. & Lukashov, V.V. (1991) Investigation of DNA biosynthesis catalyzed by DNA polymerases: approach of synthesis of compounds with

- anti-HIV activity. *Nucleic Acids Res., Symp. Series*, **24**, 13–16.
28. Atrazheva, E.D., Lukin, M.A., Jasko, M.V., Shushkova, T.V., Tarussova, N.B., Krayevsky, A.A., Balzarini, J. & De Clercq, E. (1991) 2',3'-O-Cyclic derivatives of ribonucleosides and their 5'-phosphonates: synthesis and anti-HIV activity. *Med. Chem. Res.* **1**, 155–165.
29. Shirokova, E.A. (1992) Synthesis of new 5'-hydrogenthio phosphonates of pyrimidine nucleosides. *Bioorg. Chem.* **18**, 1255–1257.
30. Kukhanova, M.K., Tarussova, N.B., Jasko, M.V., Arzumanov, A.A., Gudima, S.O., Krayevsky, A.A., Chidgeavadze, Z.G. & Beabealashvili, R.Sh. (1992) Hydrolysis of nucleoside 5'-phosphonates and 5'-phosphates by phosphatases of different origin and by human and calf blood sera. *Mol. Biol. Russian* **26**, 1142–1154.
31. Kuznetsova, E.V., Kukhanova, M.K., Arzumanov, A.A. & Krayevsky, A.A. (1995) Reaction of 5'-hydrogenphosphonates, 5'-phosphorofluoridates and 5'-phosphates of modified nucleosides in blood serum. *Mol. Biol. Russian* **29**, in press.
32. Jasko, M.V., Semizarov, D.G., Victorova, L.S., Mozzherin, D.Ju., Krayevsky, A.A. & Kukhanova, M.K. (1995) New modified substrates for discriminating between human DNA polymerases α and ϵ . *FEBS Lett.* **357**, 23–26.
33. Kukhanova, M.K., Kuznetsova, E.V., Jasko, M.V., O'Hara, B., Bekker, J., Morin, J. & Gluzman, Ya. (1994) Inhibitory analysis of DNA polymerase of herpes simplex virus type 1. *Mol. Biol. Russian* **28**, 875–886.
34. Kukhanova, M.K., Kuznetsova, E.V., Krayevsky, A.A., O'Hara, B., Bekker, J., Morin, J. & Gluzman, Ya. (1994) Inhibitory analysis of human DNA polymerases by modified substrates. *Mol. Biol. Russian* **28**, 530–541.
35. Sharkin, J.A., Alexandrova, L.A., Victorova, L.S., Kuznetsova, E.V. & Krayevsky, A.A. (1995) Purine arabinonucleotide 5'-triphosphates with substituents at 2'-position as substrates for DNA polymerases. *Biochem. Mol. Biol. Int.* **35**, 1041–1048.
36. Semizarov, D.G., Victorova, L.S., Dyatkina, N.B., von Janta Lipinski, M. & Krayevsky, A.A. (1994) Selectivity of DNA polymerases toward α and β nucleotide substrates of D and L series. *FEBS Lett.* **354**, 187–190.
37. Krayevsky, A.A., Dyatkina, N.B. & Kukhanova, M.K. (1995) Does interaction of dNTP glycone with an active center of reverse transcriptases takes place? A model for a binding site. *Nucleosides & Nucleotides* **14**, in press.
38. Langen, P.R., Waldrop, A.A. & Ward, D.C. (1981) Enzymatic synthesis of biotin-labeled polynucleotides: novel nucleic acids affinity probes. *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6633–6637.
39. Alexandrova, L.A., Atrazhev, A.M., Kukhanova, M.K. & Krayevsky, A.A. (1987) Incorporation of fluorescent derivatives of 3'-deoxyuridine 5'-triphosphate into DNA chain with *E. coli* DNA polymerase catalysis. *Bioorg. Chem. Russian* **13**, 643–647.
40. Leary, J.J., Brigati, D.J. & Ward, D.C. (1983) Rapid and sensitive colorimetric method for visualizing biotin-labeled DNA probes hybridized to DNA or RNA immobilized on nitrocellulose: Bio-blots. *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4045–4049.
41. Alexandrova, L.A., Lukin, M.A., Rozovskaya, T.A., Atrazhev, A.M., Kukhanova, M.K. & Krayevsky, A.A. (1990) Fluorescent analogues of nucleoside 5'-phosphonates for investigation of nucleic acids by radioactive methods. *Mol. Biol. Russian* **24**, 1100–1108.
42. D'yachenko, L.V., Chenchik, A.A., Lukin, M.A., Alexandrova, L.A., Krayevsky, A.A. & Beabealashvili, R.Sh. (1994) ddUTP Derivatives modified in uridine 5-position as substrate terminators of reverse transcriptases; hydrolysis of oligonucleotides terminated by these analogues by phosphodiesterase I. *Mol. Biol. Russian* **28**, 102–112.
43. Reardon, J.E. (1992) Human immunodeficiency virus reverse transcriptase: steady-state and pre-steady-state kinetics of nucleotide incorporation. *Biochemistry* **31**, 4473–4479.