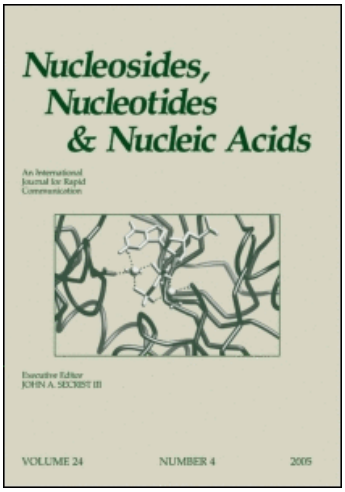

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MODIFIED DINUCLEOSIDE TETRAPHOSPHONATES, NEW POTENTIAL INHIBITORS OF HIV REVERSE TRANSCRIPTASE

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ABSTRACT

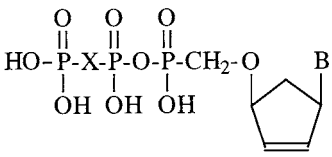
New γ-substituted analogues of dNTP were synthesized and their enzymatic stability and antiviral properties were evaluated.

INTRODUCTION

To inhibit HIV reproduction, nucleoside-based anti-HIV drugs must pass through the intracellular triphosphorylation cascade. The effectiveness of the triphosphate formation is very low (for AZT it is only about 0.1%), and it would be desirable to design phosphorylation-independent agents, which could directly inhibit the target HIV reverse transcriptase.

We recently reported the synthesis and some properties of nucleoside triphosphate mimetics bearing a cyclopentenyl residue as a glycone and a triphosphonate chain in place of a triphosphate one (**I**, **II**) [1]. They showed high stability in human blood serum, and some of them were selective inhibitors of DNA synthesis catalyzed by HIV reverse transcriptase. However, they are highly hydrophilic and may poorly penetrate through cell walls.

Dinucleoside 5′,5′-tetraphosphates were demonstrated to be good substrates of some human and bacterial DNA polymerases and HIV reverse transcriptase. In



I (B = Ade) **II** (B = Gua)
Ia X = CF_2 **IIa** X = CF_2
Ib X = CBr_2 **IIb** X = CBr_2
Ic X = O **IIc** X = O

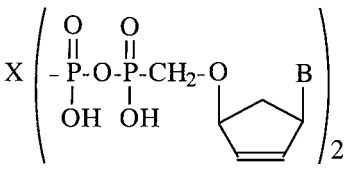
the latter case, the efficiency of incorporation into the DNA chain was close to that of the corresponding dNTPs [2]. It is worth mentioning that according to the TLC and HPLC data these dinucleotide analogues were much more lipophilic if compared with the corresponding triphosphates.

With this in mind, we synthesized, and evaluated enzymatic resistance of dinucleotide polyphosphate analogues (**IIIa–b**, **IVa–c**), which can be regarded as modified nucleoside triphosphates substituted at the γ-phosphate by a nucleotide residue. We also studied the inhibition by these compounds of pSG1 virus replication in rat Rat1 fibroblasts.

CHEMISTRY

The synthesis of target dinucleoside polyphosphonates **III** and **IV** as racemic mixtures was carried out using the corresponding monophosphonates **V** as key intermediates (Chart). Previously, for the preparation of diadenosine tetraphosphate analogues bearing β,β'-methylene or -dihalomethylene bridges, various procedures were used [3,4]. We carried out the coupling reaction with CDI as an activating agent. The formation of the side dimer (the corresponding dinucleoside α,α'-diphosphonate) was minimal when the molar excess of CDI was more than eight. Better yields of the target dinucleoside tetraphosphonates **III** and **IV** were obtained when the corresponding monophosphonates **V**, rather than diphosphonic acids, were activated. The target **III** and **IV** were also synthesized by coupling of triphosphonates **I** or **II** with the imidazolide prepared from the corresponding **V**. For difluoromethylene analogues **IIIa** and **IVa**, the yields of the target products in both procedures were comparable (20–30% relative to the monophosphonate **V**), whereas dibromomethylene derivatives **IIIb** and **IVb** were only obtained from triphosphonates **Ib** and **IIb**.

We also synthesized dinucleoside triphosphate analogues (**VI**) and (**VII**) using a reaction of imidazolide prepared from **V** and phosphoric acid under similar conditions.



III, IV

	B	X
IIIa	**Ade**	**CF_2**
IIIb	**Ade**	**CBr_2**
IVa	**Gua**	**CF_2**
IVb	**Gua**	**CBr_2**
IVc	**Gua**	**O**

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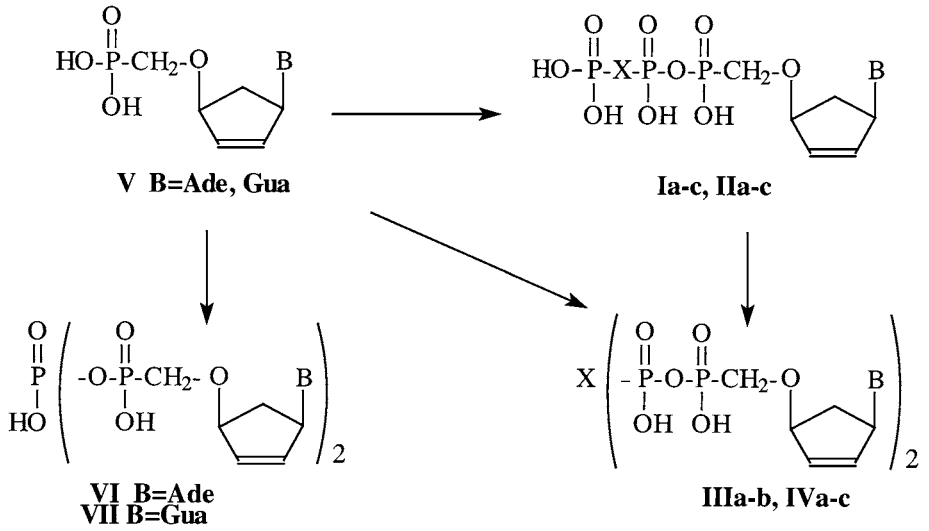

Chart.

The structures of the compounds synthesized were confirmed by UV, ^{1}H and ^{31}P NMR spectroscopy and mass-spectrometry data [5].

ENZYMATIC STABILITY

The enzymatic stability of the guanine derivatives **IVa**,**c** was evaluated in human blood serum used as a model of a biologically active medium containing various types of hydrolyzing enzymes. The product composition was analysed by reverse-phase HPLC.

These dinucleotide derivatives demonstrated close enzymatic resistance, which was by one order of magnitude higher than that of natural dGTP, although twice lower than that of the corresponding difluoromethylene triphosphonate **IIa** (Table).

ANTIVIRAL ACTIVITY

Polyphosphonates **IIIa–b** and **IVa–b** were evaluated as antiviral agents in rat fibroblast Rat1 cell culture with the artificial virus containing Moloney murine

Table

Compounds	Half-Life Time, h
IVa	3
IVc	3
IIa	7
dGTP	0.3

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leukemia virus reverse transcriptase. Their activity did not exceed 3–7 μM versus 7 nM for AZT. The activity of these polyphosphonates in HIV-infected cells is under investigation.

ACKNOWLEDGMENTS

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REFERENCES

1. Shipitsyn, A. V.; Victorova L. S.; Shirokova, E. A.; Dyatkina, N. B.; Goryunova, L. Ye.; Beabealashvilli, R. Sh.; Hamilton, C. J., Roberts, S. M.; Krayevsky, A. A. *J. Chem. Soc. Perkin Trans 1*, **1999**, 1039–1050.
2. Victorova, L.; Sosunov, V.; Skoblov, A.; Shipitsyn, A.; Krayevsky, A. *FEBS Lett.,* **1999**, *453*, 6–10.
3. Tarussova, N. B.; Osipova, T. I.; Purygin, P. P.; Yakimova, I. A. *Bioorg. Chem. (Russian)*, **1986**, *12*, 404–407.
4. Guranovsky, A.; Starzynska, E.; Taylor, G. E.; Blackburn, G. M. *Biochem. J.*, **1989**, *262*, 241–244.
5. ^{31}P NMR (if not stated otherwise, 163 MHz, D_2O, δ, proton decoupling): **IIIa**: 9.7 (m, $J_{P\alpha,P\beta}$ 33 Hz, P-α), –6.1 (m, $J_{P,F}$ 81 Hz, P-β); MS (m/e): 798.13 [M^+]; **IIIb**: ^{31}P NMR: 9.7 (d, J 23 Hz, P-α), −0.8 (d, P-β); MS (m/e) 920.23 [M^+]; **IVa**:^{31}P NMR: 9.45 (d, J 20 Hz, P-α), −6.36 (m, $J_{P,F}$ 83 Hz, P-β); MS (m/e): 830.35 [M^+]; **IVb**: 9.7 (d, J 26 Hz, P-α), −0.8 (d, P-β); MS (m/e): 952.21 [M^+]; **IVc**: 8.8 (d, J 22 Hz, P-α), −22.7 (d, P-β); MS (m/e): 796.13 [M^+]; **VI**: 9.1 (d, J 24 Hz, P-α), −22.4 (t, P-β); MS (m/e): 684.17 [M^+]; **VII**: 9.1 (d, J 26 Hz, P-α), −22.3 (t, P-β); MS (m/e): 716.14 [M^+].

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