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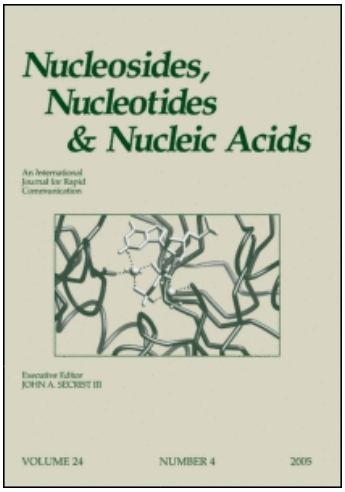

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2'-Deoxynucleoside 5'-Triphosphates with Reporter Groups Modified at α-, β,γ- or γ-Phosphates as Substrates for DNA Polymerases

L. A. Alexandrova[a]; A. Yu. Skoblov[a]; M. V. Jasko[a]; A. A. Murabuldayev[a]; L. S. Victorova[a]; A. A. Krayevsky[a]
[a] Engelhardt Institute of Molecular Biology, RAS, Moscow, Russia

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NUCLEOSIDES & NUCLEOTIDES, 18(4&5), 963–964 (1999)

2'-DEOXYNUCLEOSIDE 5'-TRIPHOSPHATES WITH REPORTER GROUPS MODIFIED AT α–, β,γ- OR γ- PHOSPHATES AS SUBSTRATES FOR DNA POLYMERASES

L. A. Alexandrova*, A. Yu. Skoblov, M. V. Jasko, A. A. Murabuldayev, L. S. Victorova and A.A. Krayevsky

Engelhardt Institute of Molecular Biology, RAS, 32 Vavilov Str., Moscow, 117984, Russia

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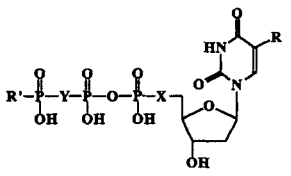
The presence of reporter or ligand groups in 2'-deoxynucleoside 5'-triphosphates (dNTP) makes it possible to monitor the diffusion of these compounds into cell and to observe their incorporation into DNA. Here we present the synthesis of new dNTPs modified at α–, β,γ- or γ-phosphates of types **I-IV**, and containing reporter or ligand groups at the C5 position of dUTP.



I X= CH_2O, Y= O, R'= OH, R= $CH{=}CHCH_2NHCO(CH_2)_5NHBio$ (**a**), $CH{=}CHCH_2NHFlu$ (**b**), CH_3 (**c**).

II X= O, Y= CBr_2, R'= OH, R= $CH_2O(CH_2)_6N_3$ (**a**), $CH_2O(CH_2)_6NH_2$ (**b**), $CH_2O(CH_2)_6NHCO(CH_2)_5NHBio$ (**c**), $CH_2O(CH_2)_6NHC(S)NHFlu$ (**d**), $CH_2O(CH_2)_6NHTMR$ (**e**).

III X=Y= O, R= CH_3 (**a-d**), R'= $CH_2CH_2N_3$ (**a**), $CH_2CH_2NH_2$ (**b**), CH_2CH_2NHDNP (**c**), $CH_2CH_2NHCO(CH_2)_5NHDNP$(**d**)

R'= C_6H_5 (**e-f**), R= $CH_2O(CH_2)_6N_3$ (**e**), $CH_2O(CH_2)_6NH_2$ (**f**)

IV X=Y= O, R'= OH, R= $CH_2O(CH_2)_6N_3$ (**a**), $CH_2(OCH_2CH_2)_3N_3$ (**b**), $CH_2O(CH_2)_6NH_2$ (**c**), $CH_2(OCH_2CH_2)_3NH_2$ (**d**), $CH_2O(CH_2)_6NHBio$ (**e**), $CH_2(OCH_2CH_2)_3NHCO(CH_2)_5NHBio$ (**f**), $CH{=}CHCH_2NHCO(CH_2)_5NHBio$ (**g**).

Bio = biotinyl, DNP = 2,4-dinitrophenyl, Flu = fluoresceinyl, TMR = tetramethylrhodaminyl.

The synthesis of **Ia,b** proceeded from 3'-O-tetrahydropyranyl-2'-deoxyuridine *via* its α-phosphonomethyl and β,γ-diphosphoryl-α-phosphonomethyl derivatives,

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respectively, and followed by a condensation with allylamine and then with N-succinidyl N-Bio-6-aminohexanoate or Flu isothiocyanate (FITS) [1]. Twice modified dNTPs **Ia,b** showed substrate properties close to those of **Ic**. Both compounds were effective and selective substrates of human DNA polymerases α and δ, but were not recognized by AMV and HIV reverse transcriptases (RTs), human DNA polymerase β and ε, and terminal deoxynucleotidyl transferase (TdT).

To obtain dNTPs **II-IV**, 5-bromomethyl-2'-deoxy-3',5'-diacetyluridine was coupled with either 1,6-hexanediol or triethylene glycol. After replacement of OH groups to azido one *via* methanesulfonyloxy group and deprotection, the resulting nucleosides were triphosphorylated into dNTPs **IIa**, **IIIe** and **IVa,b**. Their reduction gave dNTPs **IIb**, **IIIf**, and **IVc,d**. They were coupled with N-succinidyl N-Bio-6-aminohexanoate or FITS, or N-succinidyl TMR-carboxylate to give **IIc-e**, **IVe,f** [2]

The C5-substituted β,γ-dibromomethylenediphosphonates **IIa-e** demonstrated poor or none activity towards AMV RT. γ-Substituted dNTPs **IIIa-d** were substrates for AMV RT despite the large size of a substituent at the γ-phosphonate, but they were poorly utilized by DNA polymerase α. For twice modified dUTPs, **IIIe-f**, the affinity towards AMV RT was 1-2 orders of magnitude lower than that for their counterparts bearing substituents either at the γ-phosphonate or at the C5 position.

A strong effect of a linker in a 5-position of dNTPs **IVa-g** on their substrate properties was demonstrated. All the enzymes under investigation effectively catalyzed the primer extension in the presence of **IVa-g** by one nucletide residue, whereas **IVg** was incorporated into the DNA chain twice or more.

Thus, the substitution of α-, β,γ-, and γ-phosphate groups in dNTP by phosphonate ones significantly changes their substrate properties towards DNA polymerases and RTs, although most of the compounds under investigation keep substrate activity with respect to any of the enzymes and can extend the primer.

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