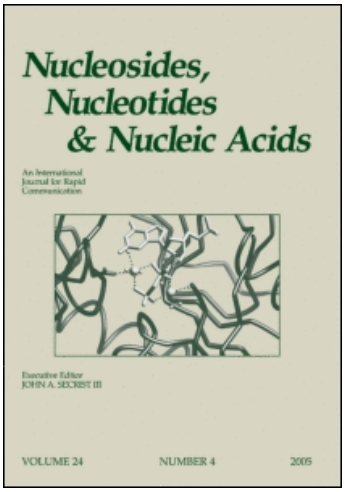


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Reasons and Limits of Substrate Activity of Modified L-dNTP in DNA Biosynthesis

Alexander A. Krayevsky[a]; Natalya B. Dyatkina[a]; Dmitry G. Semizarov[a]; Liubov S. Victorova[a]; Elena A. Shirokova[a]; Fritz Theil[b]; Martin Von Janta Lipinski[c]; Gilles Gosselin[d]; Jean-Louis Imbach[d]
[a] Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia [b] Centre of Selective Organic Synthesis, Berlin-Adlershof, Germany [c] Max-Delbruck-Center of Molecular Medicine, Berlin-Buch, Germany [d] Universite de Montpellier II, Montpellier Cedex 5, France

To cite this Article Krayevsky, Alexander A. , Dyatkina, Natalya B. , Semizarov, Dmitry G. , Victorova, Liubov S. , Shirokova, Elena A. , Theil, Fritz , Lipinski, Martin Von Janta , Gosselin, Gilles and Imbach, Jean-Louis(1999) 'Reasons and Limits of Substrate Activity of Modified L-dNTP in DNA Biosynthesis', Nucleosides, Nucleotides and Nucleic Acids, 18: 4, 863 — 864
To link to this Article: DOI: 10.1080/15257779908041583
URL: http://dx.doi.org/10.1080/15257779908041583

NUCLEOSIDES & NUCLEOTIDES, 18(4&5), 863–864 (1999)

REASONS AND LIMITS OF SUBSTRATE ACTIVITY OF MODIFIED L-dNTP IN DNA BIOSYNTHESIS

Alexander A.Krayevsky*, Natalya B.Dyatkina, Dmitry G.Semizarov, Liubov S.Victorova, Elena A. Shirokova, Fritz Theil[1], Martin von Janta Lipinski[2], Gilles Gosselin[3], Jean-Louis Imbach[3]

Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 32 Vavilov str., Moscow, 117984, Russia; [1]Centre of Selective Organic Synthesis, Rudower Chaussee 5, D-12484 Berlin-Adlershof, Germany; [2]Max-Delbruck-Center of Molecular Medicine, Rossle-Strasse 10, D-13125, Berlin-Buch, Germany; [3]Universite de Montpellier II, Place Eugene Bataillon, 34095, Montpellier Cedex 5, France

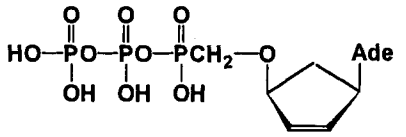
ABSTRACT: Theoretical and experimental analysis of interaction of modified D- and L- dNTP as substrates for template-dependent and template-independent DNA polymerases was performed. It is shown that if the modified nucleoside 5′-triphosphates do not contain a substituent in position 3′ DNA chains can be extended by both strereoisomeric series with the same kinetic parameters. But the presence of even a 3′- hydroxy group in L-dNTP prevents their incorporation into the DNA chain.

During the last years a number of papers were published on the similar activity of both D- and L- modified dNTP as terminating substrates of some DNA polymerases. All these modified dNTP do not contain a bulky substituent at the 3'-position. The theoretical analysis of similar terminating activity of both enantiomers was made. By computing the lowest energy conformation, we showed that, if to superimpose both nucleic bases and phosphate residues, respectively, glycons are located in different positions. We can imply that in these modified dNTP a glycon is not bound specifically and has no sterical hindrances in the active center of retroviral reverse transcriptases[1].

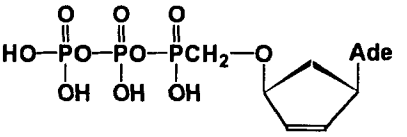
However, some contradictions for the 3'-substituted dNTP were published, especially for L-enantiomers of natural dNTP; these contradictions dealt with the properties of the used artificial test systems. We reexamined the properties of L-dNTP

towards several DNA polymerases in cell-free systems. It was shown that none of tested template-dependent human enzymes (DNA polymerases α, β and ε), as well as AMV and HIV reverse transcriptases were able to incorporate the L-deoxynucleotide residue in the 3'-position of DNA primers[2]. Only calf thymus terminal deoxynucleotidyl transferase utilized one L-deoxynucleotide residue. However, the effectivity of this process was about 1% if to compare with the incorporation of D-deoxynucleotides. We can explain this fact by the sterical hindrance of the 3'-hydroxyl of L-dNTP in the active center.

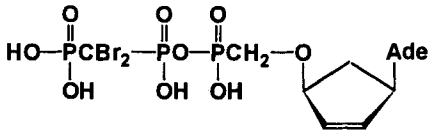
The effect of the modification in the triphosphate residue of glycon-modified L-dNTPs on their substrate properties has also been studied. It was shown that in the series of carbocyclic *iso*-d_4NTP the substitution of one (structures **I**)[3] or all the three phosphate residues (structures **II**)[4] by different phosphonates similarly affects the DNA primer extension both for L- and D-enantiomers. It was also demonstrated that the



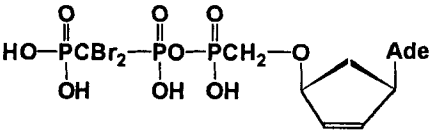
D-I



L-I



D-II



L-II

modification of triphosphates in carbocyclic *iso*-d_4NTP does not dramatically change their substrate properties in both series towards several DNA polymerases.

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