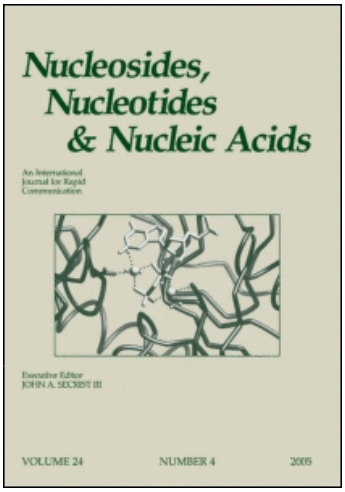

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Derivatives of Imidazole-4-carboxamide as Substrates for Various DNA Polymerases

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To cite this Article Bec, Christine Le , Roux, Pascal , Buc, Henri and Pochet, Sylvie(1997) 'Derivatives of Imidazole-4-carboxamide as Substrates for Various DNA Polymerases', Nucleosides, Nucleotides and Nucleic Acids, 16: 7, 1301 — 1302
To link to this Article: DOI: 10.1080/07328319708006175
URL: http://dx.doi.org/10.1080/07328319708006175

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1301-1302 (1997)

DERIVATIVES OF IMIDAZOLE-4-CARBOXAMIDE AS SUBSTRATES FOR VARIOUS DNA POLYMERASES

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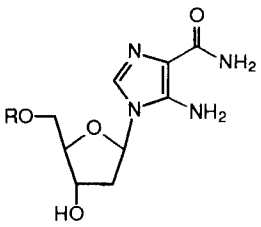
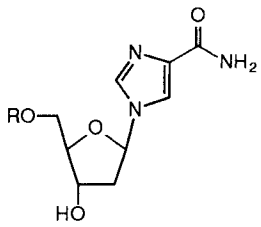
ABSTRACT: A series of nucleotide analogs bearing imidazole 4-carboxamides as bases were synthetized. The ability of Klenow fragment of pol I and HIV-1 reverse transcriptase to use these 5'-triphosphate derivatives as substrates in DNA elongation reactions was investigated.

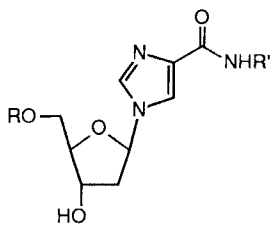
The synthesis of the 5-amino-1-(2-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide, trivially designated as dZ, and its 5'-triphosphate derivative dZTP has been reported.[1] Preliminary experiments showed that dZTP can be incorporated into DNA by the Klenow fragment of DNA pol I. However, this polymerase fails to further elongate the primed template after dZMP incorporation. We designed a new base dY in which the 5-amino group of dZ is deleted.[2] It is assumed that, due to the free rotations around the carboxamide moiety and the glycosidic bond, this nucleoside can form a base pair with the four canonical bases. The ability of the 5'-triphosphates dZTP and dYTP to be accepted as ambiguous substrates in DNA polymerization reactions was investigated. In order to evaluate the effect of hydrophobicity of the base in polymerization reactions, the carboxamide group was alkylated by aliphatic groups (methyl and propyl) to give the nucleosides dY^{Me} and dY^{Pr}.



dZ: R = H
dZTP: R = $P_3O_9H_4$



dY: R = H
dYTP: R = $P_3O_9H_4$



dY^{Me}: R = H, R' = Me
$dY^{Me}TP$: R = $P_3O_9H_4$, R' = Me
dY^{Pr}: R = H, R' = Pr
$dY^{Pr}TP$: R = $P_3O_9H_4$, R' = Pr

4-(5)-imidazolecarboxamide was prepared in four steps from imidazole-4,5-dicarboxylic acid.[2] N-methyl-4-(5)-imidazolecarboxamide and N-propyl-4-(5)-imidazolecarboxamide were obtained by condensation of N-succinimidyl-4-(5)-imidazolecarboxylate with the corresponding amines (n-propylamine, methylamine). The synthesis of nucleosides dY, dY^{Me} and dY^{Pr} was performed *via* enzymatic transglycosylation with a crude extract of N-deoxyribosyltransferase from *Lactobacillus leichmannii* according to a previously reported procedure.[2] The 5'-triphosphates of nucleosides dY, dY^{Me} and dY^{Pr} were prepared according to known synthetic protocols. Thus, the 3'-acetylated nucleosides were phosphorylated according to Tener's procedure. The resulting monophosphates were activated as morpholidates, and then condensed with tributylammonium pyrophosphate in DMF. The pure triphosphates (dYTP, $dY^{Me}TP$, $dY^{Pr}TP$) were further characterized by mass spectrometry and NMR spectroscopy.

The incorporation of standard dNTPs and deoxynucleotide analogs (dZTP, dYTP, $dY^{Me}TP$ and $dY^{Pr}TP$) has been studied opposite dA, dG, dT and dC at position +1 on a series of DNA hybrids using a Klenow fragment deficient in 3'-exonuclease activity (KFexo$^-$) and reverse transcriptase (RT) from human immunodeficiency virus 1 (HIV-1). The ability of these two polymerases to incorporate such analogs is compared to that of standard triphosphates (matched and mismatched nucleotides). As previously shown in the case of misincorporation performed with canonical nucleotides, the patterns of insertion depend strongly on the DNA sequence of the template as well as on the enzyme used.[3] These derivatives are therefore interesting complementary tools to understand the origin of this type of variability and the different requirements of the catalytic site of these two enzymes. On the other hand, it is clear that forward rates of incorporation cannot unambiguously define a base pairing and *a fortiori* a pairing scheme for these analogs. Relaxation of the enzyme specificity by changing the divalent cation as well as the quantitative assessment of misincorporation frequency at specific positions will be used to define an order of preference for canonical, non-canonical and ambiguous substrates.

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