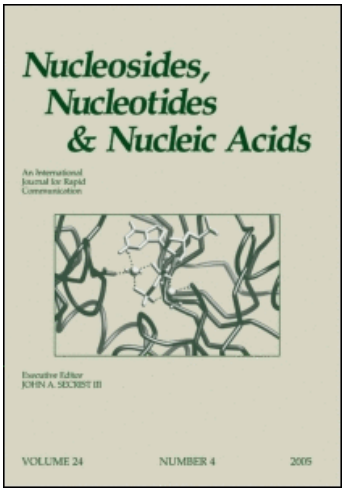

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Synthesis and Recognition by DNA Polymerases of a Reactive Nucleoside for DNA Diversification

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Synthesis and Recognition by DNA Polymerases of a Reactive Nucleoside for DNA Diversification

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ABSTRACT

The synthesis of 1-(2-deoxy-β-D-erythro-pentofuranosyl)imidazole-4-hydrazide having the features of an ambigous base is reported. The recognition of the analogue by DNA polymerases as an incoming triphosphate as well as a template base was investigated. The mutagenic properties was evaluated by PCR. The potential of this new monomer for DNA diversification is illustrated by the reactivity of the nucleobase towards various aldehydes.

Key Words: Aldehyde; Diversification; DNA polymerases; Hydrazide; Nucleoside triphosphate.

Several research groups have recently taken up the challenge of finding new nucleoside molecular designs that can dupe polymerases into accepting and replicating them. If polymerases accept some synthetic modifications either in the template strand or as incoming triphosphate, most of these modified base analogues suffer serious limitations with regard to enzyme recognition.

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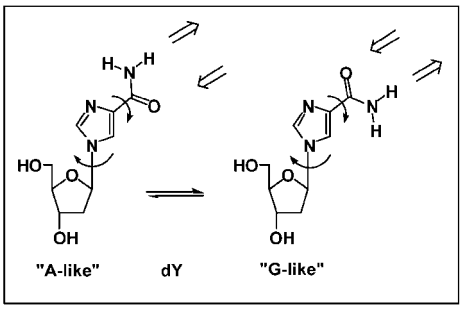

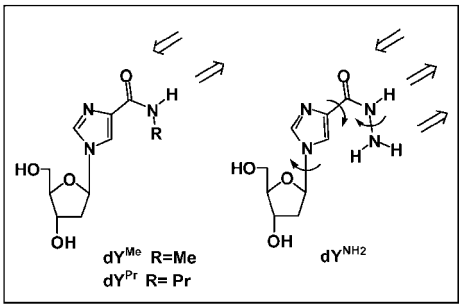

We have previously designed a nucleobase with imidazole moiety substituted at position 4 with a carboxamide group (Y) and demonstrated that this base can act as an ambiguous base in PCR.[1,2] Addition of an alkyl chain (methyl or propyl) to the carboxamide extremity yielded monomer (dY^{Me} or dY^{Pr}) still able to be incorporated and copied by DNA polymerases.[2] These results encouraged us to further explore the 4-carboxamide imidazole motif by anchoring variable side motifs to the carboxamide extremity.

We next considered the imidazole-4-hydrazide nucleobase (Y^{NH_2}). Rotations around the hydrazide function and glycosidic bond should allow ambivalent pairing schemes. Moreover, the presence of an hydrazine function on the imidazole moiety should permit to generate in a single step a large variety of new nucleobases by reaction with any aldehyde or ketone.

The nucleoside analogue was converted into the corresponding phosphoramidite and the triphosphate derivative ($dY^{NH_2}TP$). The ability of dY^{NH_2} to replace the canonical bases in DNA replication reactions both as an incoming triphosphate and as a template base was demonstrated in primer extension reactions.[3]

Due to its ambivalent pairing mode, the triphosphate derivative $dY^{NH_2}TP$ was able to induce mutations during PCR reactions when it substitutes for dATP or dGTP. The resulting substitution frequencies are comparable with those obtained with other random mutagenesis procedures, but the proportion of transversions are high and almost all possible mutations are present with no hot spot along the sequence.[3]

The presence of the hydrazide function on the base moiety allows the diversification of the imidazole motif by reaction with any aldehyde or ketone. Oligomers libraries could be generated by enzymatic incorporation as triphosphate derivative or chemical incorporation as phosphoramidite building block of the nucleoside followed by reaction of the hydrazide function. The reactivity of the base into DNA toward a first series of aldehydes of different size as well as fluorescamine was illustrated.

We are currently developing a parallel scheme that aims to generate libraries of DNA polymerase substrates and inhibitors.[4] Reaction of the triphosphate derivative $dY^{NH_2}TP$ and any aldehyde or ketone allows to produce in one step a large family of new analogues that could be tested in primer extension reactions.

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