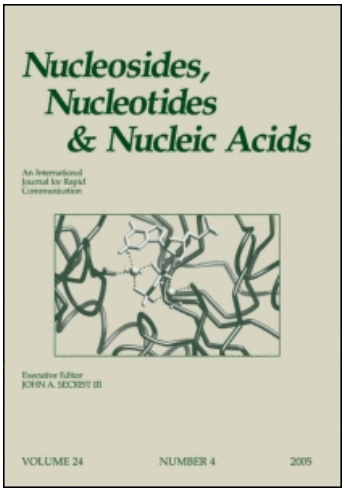

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Unexpected High Hybridization Properties of α-Anomeric Oligodeoxynucleoside Methylphosphonates

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UNEXPECTED HIGH HYBRIDIZATION PROPERTIES OF α-ANOMERIC OLIGODEOXYNUCLEOSIDE METHYLPHOSPHONATES

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ABSTRACT: The Watson-Crick base-pairing properties of oligodeoxynucleoside methylphosphonates containing α-nucleoside units have been evaluated and compared to those of others ionic and non-ionic α- or β-oligonucleotide analogs.

In order to improve cellular uptake and nuclease resistance of antisense oligonucleotides several sugar and phosphate backbone modified oligos have been synthesized and studied[1-3]. In particular, uncharged oligodeoxynucleoside methylphosphonates (P-CH_3) are resistant to nucleases, are readily taken up by cells and specifically inhibit expression of oncogenes or viral genes[4]. However their efficacy is limited by weak hybridization to their target.

Here, we have investigated a new class of non-ionic oligonucleotide analogs: α-methylphosphonates combining two different structural modifications, i.e. the inversion of the anomeric configuration in the sugar moieties and the substitution of anionic phosphate diester linkages by neutral methylphosphonates. Their hybridizing abilities to complementary DNA or RNA single strands were compared to those of the β-methylphosphonates, of the ionic α- or β- phosphodiester (PO^-) and phosphorothioate (PS^-) derivatives and of the non-ionic α- or β-phosphoramidate (P-NH_2) homologs[5]. Evaluation of the hybridization properties of α-methylphosphonate oligomers was performed with a dodecathymidine and a dodecamer directed against the splice acceptor site of HIV-1 *tat* RNA.

Oligodeoxyribonucleoside methylphosphonates α-dT_{12} and α-d(TCTTAACCCACA) were prepared on solid support (1μmole scale) using α-methylphosphonamidite synthons following the procedures used for the synthesis of β-(P-CH_3) oligos[6,7]. These oligomers

were deprotected by a treatment with ethylenediamine for 6h at room temperature and purified as described by Lin and al.[8].

Hybridization properties of the α-(P-CH_3) oligomers were evaluated by determining Tm values derived from melting curves recorded at 260nm. Regarding the binding to poly rA target, the non-ionic α-(P-NH_2) and α-(P-CH_3) dodecathymidines and the ionic α-(PO^-) dT_{12} exhibit the highest Tm values (ΔTm/modif.+1.23, +0.7, +1.23°C respectively) among all the studied analogs . Other modifications were destabilizing in comparison with the natural oligo β-dT_{12}. Except the α-(PO^-) dT_{12} (ΔTm/modif.-0.72°C), a similar situation was observed when duplex formation with poly dA was considered (ΔTm/modif.+2.32, +1.40°C for α-(P-NH_2) and α-(P-CH_3) respectively).The α-(P-CH_3) heteropolymer exibited the strongest hybridization to DNA (ΔTm/modif. +0.04°C) compared to α-(PS^-), β-(PS^-), α-(PO^-) and β-(P-CH_3) derivatives. The duplex formed between the α-(P-CH_3) dodecamer and its complementary RNA sequence, although less stable than the natural one (ΔTm/modif. -0.66°C), was much more stable than the duplex with the corresponding β-(P-CH_3) analog (ΔTm/modif. -1.7°C).

These results make this combination of two structural modifications, i.e. the inversion of the anomeric configuration in the sugar moieties and the change of the ionic backbone in neutral internucleotidic linkage, very attractive for antisense purpose.

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