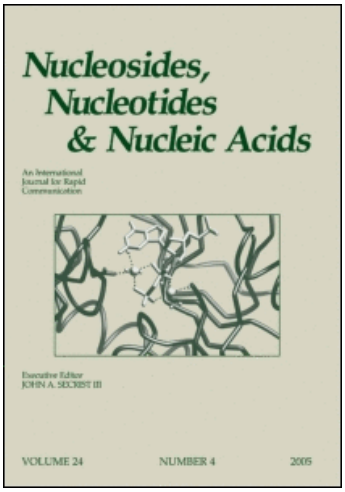

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Synthesis of Oligonucleotide-Intercalator Conjugates Capable to Inhibit HIV-1 DNA Integration

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SYNTHESIS OF OLIGONUCLEOTIDE-INTERCALATOR CONJUGATES CAPABLE TO INHIBIT HIV-1 DNA INTEGRATION

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ABSTRACT: This investigation is devoted to design of short "switch" oligonucleotides mono- or bi-functionnalized with intercalating agents capable to form a stable triplex with HIV integrase-cognate sequences and inhibit selectively HIV integration. Methods of intercalator incorporation at 5'- and/or 3'-terminal positions or one of the pyrimidine heterocyclic bases are developed.

The aim of the present investigation is the search of new inhibitors of the human immunodeficiency virus (HIV) DNA integration into the host genome which is one of the key step in the retrovirus replication cycle. Integration is mediated by the integrase (IN), a viral protein which binds to specific sequences located on both extremities of the DNA long terminal repeats (LTRs). IN binding site located in U5 LTR sequence of HIV DNA (5'-GGAAAATCTCT / 3'-CCTTTTAGAGA) was chosen as a target for the triplex-forming oligonucleotides. A 11-member oligonucleotide composed from G and T and containing 5'-end linked oxazolopyridocarbazole residue (OPC) was shown to form a triplex with this DNA fragment and inhibit HIV DNA integration[1]. In order to increase the inhibition efficiency it was decided to vary the third strand oligonucleotide sequences and intercalator structures. Three oligonucleotide sequences were selected in order to prepare oligonucleotide-intercalator conjugates capable of binding this site: 5'-GGTTTTTGTGT-3'; 5'-GGTTTTAGAGA-3' and 5'-GGAAAAC*AGAGA-5'. The latter is of especial interest. In accordance with a recent molecular modeling study this branched oligonucleotide can form a thermodynamically stable triple helix with unusual

parallel orientation of the third strand[2]. In order to synthesize this oligonucleotide a special original polymer support permitting to perform sequential synthesis of two oligonucleotide fragments was prepared. The first support bound monomer represents 5'-O-monomethoxytrithyl-4-N-[(6-levulynyl)hexamethylene]cytidine (C*).

All third strand oligonucleotides were functionnalized by different intercalators at 5'- and/or 3'- terminal positions or one of the pyrimidine heterocyclic bases. Amino-containing OPC (OPC-NH_2) was bound to the 5'-hydroxyl group of fully protected assembled solid support linked oligonucleotides in the mixture of pyridine/DMSO/H_2O=2/2/1 in the presence of 5% triethylamine. The hydroxyl group was preliminary activated by N,N-carbonyldiimidazole in anhydrous dioxane. 5'-Acridine containing conjugates were prepared by acylation of an amino group preliminary incorporated at the 5'-end of oligonucleotides using 5'-Amino-Modifier C6 (Glen Research) by N-hydroxysuccinimide ester of 9-acridinecarboxylic acid. In order to bind daunomycin and ethidium bromide residues an additional acylation of this amino group by succinic anhydride was performed. The intercalator coupling was carried out in the presence of O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU). 3'-Acridine containing oligonucleotides were synthesized using commercial polymer support Acridine CPG 500 (Glen Research). In order to prepare oligonucleotides containing intercalator residues at one of the pyrimidine bases, oligonucleotides with 4-triazolide derivative of 5-methylpyrimidinone-2 were synthesized. The oligonucleotide-intercalator conjugates were obtained via transamination (OPC-NH_2) or transamination and subsequent acylation (acridine) of assembled solid support bound oligonucleotides (DMF, 18h). Crude reaction mixtures were separated using reverse phase HPLC. Changing of the retention time of the modified oligonucleotides in comparison with non-modified ones was observed by ion-pair HPLC analysis in accordance with hydrophobic properties of intercalator residues.

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