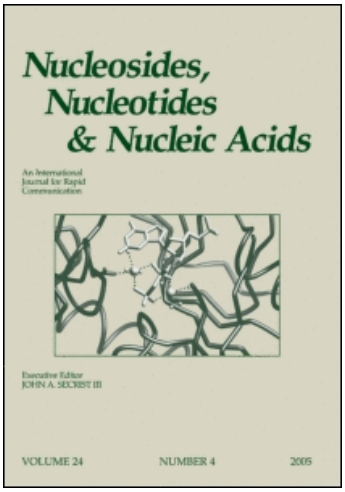

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Conjugates of Oligo(2′-O-Methylribonucleotides) with Minor Groove Binders as New Sequence-Specific Agents Recognizing Both Grooves of Double-Stranded DNA

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ABSTRACT

Design, synthesis and physico-chemical studies of new pyrimidine oligo(2′-O-methylribonucleotide) conjugates with one or two oligo(pyrrolecarboxamide) minor groove binders (MGB) are described.

Key Words: Oligo(Z′-methylribonucleotides); Conjugates; Minor groove binders; Triplex formation.

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Two classes of double-stranded DNA sequence-specific binders are known: oligo(pyrrolecarboxamide) minor groove binders (MGB) and synthetic triple helix forming oligonucleotides (TFO). Their covalent conjugates are able to bind simultaneously to the major and the minor grooves of the target DNA in a sequence-specific manner and thus potentially interfere with gene expression on the level of genomic DNA.[1] Oligo(2′-O-methylribonucleotides) as triple helix forming part of these conjugates have several advantages: the simple synthetic procedure, the high stability toward nuclease degradation and the efficient triple helix formation.

The solid phase H-phosphonate method[2] was adapted to the synthesis of a series of oligo(2′-O-methylribonucleotides) and their 3′-protected analogs bearing oligo(ethylene glycol) phosphate linker at the 5′-terminus (Fig. 1). ''Inverted'' (3′-3′-linked) thymidine was chosen as 3′-protecting group on the base of previously obtained results.[3] A synthetic 29-mer duplex containing a natural polypurine sequence of HIV proviral DNA of genes *nef* and *pol* was used as a target.

The ability of synthesized 2′-O-methyl oligomers to form triple helices with dsDNA was demonstrated by the native polyacrylamide gel electrophoresis and proved by the thermal denaturation assay. The 3′-modification of oligo(2′-O-methylribonucleotides) has no influence on the triple complex stability. The same temperature of triple helix denaturation (24°C) was observed for all species including control oligodeoxyribonucleotide, containing 5-methyldeoxycytidine.

For synthesis of covalent conjugates of oligo(2′-O-methylribonucleotides) and MGB we used direct coupling of amino-containing MGB residues to activated 5′-terminal phosphate group of the oligonucleotide according to.[1] One or two MGB residues were attached to the same terminal phosphate of the oligonucleotide (Table 1). The structures of the conjugates were confirmed by HPLC, gel electrophoresis, spectrophotometry and MALDI-TOF mass spectrometry. The dissociation constants of triple complexes formed by oligonucleotides-MGB conjugates and dsDNA were calculated from gel retardation experiments (Table 1). The results demonstrate that conjugates with two MGB residues form more stable complexes and hexa(ethylene glycol) linker is optimal.

The association and dissociation rate constants (k_1 and k_{-1}) for triplex formation with model dsDNA were calculated from gel shift assay (Table 2). These data were in agreement with kinetic constants of oligo(2′-O-methylribonucleotide) triplex formation obtained by Torigoe et al.[4] Introduction of MGB residue increases the association rate constant.

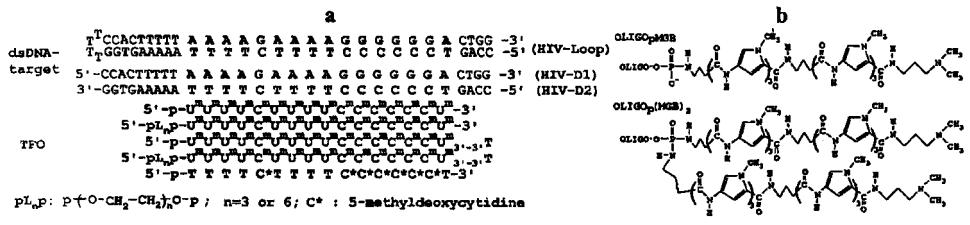

Figure 1. a – The NA-targets and synthesized oligonucleotide sequences; b – The structure of the synthesized conjugates.

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Table 1. Dissociation constants of triple complexes of dsDNA with triplex forming oligo(2′-O-methylribonucleotide)-MGB conjugates.[a]

Triplex forming constructions	$K_d \cdot 10^8$, mol
5′-p-T T T T C*T T T T C*C*C*C*C*C*T **d16**	6,6 ± 1,2
5′-p-$U^mU^mU^mU^mC^mU^mU^mU^mU^mC^mC^mC^mC^mC^mC^mU^m$ **16^m**	14,9 ± 3,6
5′-**MGB**p-$U^mU^mU^mU^mC^mU^mU^mU^mU^mC^mC^mC^mC^mC^mC^mU^m$ **16^m∼MGB**	6,8 ± 0,9
5′-(**MGB**)$_2$p-$U^mU^mU^mU^mC^mU^mU^mU^mU^mC^mC^mC^mC^mC^mC^mU^m$ **16^m∼(MGB)$_2$**	6,3 ± 0,4
5′-p-(**EG**)$_3$-p-$U^mU^mU^mU^mC^mU^mU^mU^mU^mC^mC^mC^mC^mC^mC^mU^m$ **16^m∼L$_3$**	40 ± 0,8
5′-**MGB**p-(**EG**)$_3$-p-$U^mU^mU^mU^mC^mU^mU^mU^mU^mC^mC^mC^mC^mC^mC^mU^m$ **16^m∼L$_3$MGB**	8,1 ± 0,6
5′-(**MGB**)$_2$p-(**EG**)$_3$-p-$U^mU^mU^mU^mC^mU^mU^mU^mU^mC^mC^mC^mC^mC^mC^mU^m$ **16^m∼L$_3$(MGB)$_2$**	1,4 ± 0,7
5′-p-(**EG**)$_6$-p-$U^mU^mU^mU^mC^mU^mU^mU^mU^mC^mC^mC^mC^mC^mC^mU^m$ **16^m∼L$_6$**	6,7 ± 0,1
5′-**MGB**p-(**EG**)$_6$-p-$U^mU^mU^mU^mC^mU^mU^mU^mU^mC^mC^mC^mC^mC^mC^mU^m$ **16^m∼L$_6$MGB**	0,7 ± 0,4
5′-(**MGB**)$_2$p-(**EG**)$_6$-p-$U^mU^mU^mU^mC^mU^mU^mU^mU^mC^mC^mC^mC^mC^mC^mU^m$ **16^m∼L$_6$(MGB)$_2$**	0,6 ± 0,3
5′-p-$U^mU^mU^mU^mC^mU^mU^mU^mU^mC^mC^mC^mC^mC^mC^mU^m{}_{3'\text{-}3'}$T **i$16^m$**	30,8 ± 5,2
5′-p-(**EG**)$_3$-p-$U^mU^mU^mU^mC^mU^mU^mU^mU^mC^mC^mC^mC^mC^mC^mU^m{}_{3'\text{-}3'}$T **i$16^m$∼L$_3$**	29,1 ± 3,5
5′-p-(**EG**)$_6$-p-$U^mU^mU^mU^mC^mU^mU^mU^mU^mC^mC^mC^mC^mC^mC^mU^m{}_{3'\text{-}3'}$T **i$16^m$∼L$_6$**	18,7 ± 3,0

[a]Obtained by gel shift assay at 13°C and pH 6.0 (50 mM MES, 50 mM NaCl, 5 mM $MgCl_2$). Duplex concentration was 60 nM, TFO concentration was between 0,5 and 50 μM. C* - 5-methyldeoxycytidine.

Table 2. Kinetic parameters for triplex formation.[a]

Oligomer	k_1, $M^{-1}s^{-1}$	k_{-1}, s^{-1}
16^m	$(1.1 \pm 0.4) \times 10^2$	$(1.0 \pm 0{,}4) \times 10^{-4}$
16^m∼MGB	$(1.5 \pm 0.5) \times 10^3$	$(5.0 \pm 2.3) \times 10^{-5}$
16^m∼(MGB)$_2$	$(2.7 \pm 1.0) \times 10^3$	$(1.0 \pm 0.5) \times 10^{-4}$

[a]Obtained by gel shift assay at 13°C and pH 6.0 (50 mM MES, 50 mM NaCl, 5 mM $MgCl_2$). Duplex concentration was 60 nM, TFO concentration was between 0,1 and 10 μM.

The constructions proposed in this work could be considered as molecular instruments for artificial sequence-specific regulation of gene expression on the level of double-stranded genomic DNA. Inhibition of the gene expression by these conjugates in the cellular test system containing polypurine sequence of proviral HIV-1 DNA integrated between promoter and reporter luciferase gene is currently under investigation.

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