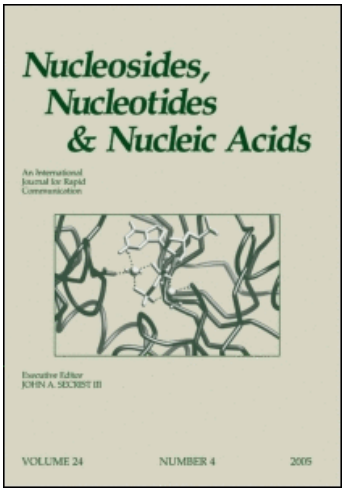

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Debart, F. , Tosquellas, G. , Rayner, B. and Imbach, J. -L.(1995) 'Optimization of Antisense Chimeric Oligonucleotides Containing α- and β-Anomeric Deoxynucleotides', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 1015 — 1018
To link to this Article: DOI: 10.1080/15257779508012523
URL: http://dx.doi.org/10.1080/15257779508012523

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 1015-1018 (1995)

OPTIMIZATION OF ANTISENSE CHIMERIC OLIGONUCLEOTIDES CONTAINING α- AND β-ANOMERIC DEOXYNUCLEOTIDES

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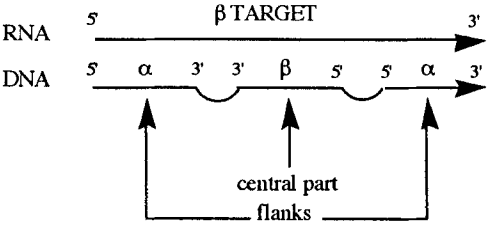
Abstract: Various chimeric oligonucleotides containing α- and β-anomeric deoxynucleotide units and directed against the splice acceptor site of the HIV-1 *tat* RNA have been synthesized. Their hybridizing abilities to complementary DNA or RNA single strands, and their stability in cell culture medium or cell extracts were studied.

Various chimeric oligonucleotides consisting of a central part with phosphodiester or phosphorothioate able to elicit RNase H-mediated hydrolysis of the RNA moiety in the resulting RNA-DNA duplex and of nuclease-resistant flanks[1,2] have proven to be efficient antisense agents. Here, we report the synthesis and the properties of a new class of "sandwich" oligonucleotides consisting of a stretch of natural deoxy-β-nucleotide units with several deoxy-α-nucleotide units at both 5'- and 3'-ends.



"sandwich" oligomers

Opposite mode of binding of α- and β-anomeric oligonucleotides[3] requires the introduction of 3'→ 3' or 5'→ 5' linkages at the junctions between two nucleosides of opposite anomeric configuration.

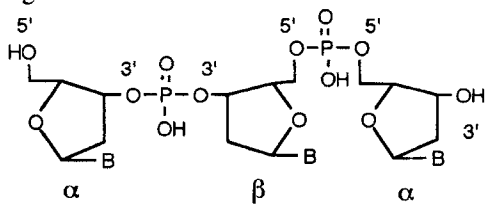


TABLE 1. Sequences of HIV-1 *tat* target and oligonucleotide analogues complementary to the target

Oligos	Sequences	Comments
1	βA βG βA βA βT βT βG βG βG βT βG βT	single-stranded DNA target
2	βA βG βA βA βU βU βG βG βG βU βG βU	single-stranded RNA target
3	βT βC βT βT βA βA βC βC βC βA βC βA	unmodified β-strand antiparallel to the target
4	αT αC αT αT αA αA αC αC αC αA αC αA	all-α-strand parallel to the target
5	αT αC αT αT βA βA βC βC αC αA αC αA	α,β,α stretches parallel to the target
6	αT αC αT αT βA βA βC βC αC αA αC αA	α,β,α stretches antiparallel to the target
7	αT αC αT αT βA βA βC βC αC αA αC αA	central β-stretch with external α-stretches
8	βT αC βT αT βA βA βC βC αC βA αC βA	central β-stretch with external alternating α,β-stretches
9	βT αC βT αT βA αA βC αC βC αA βC αA	fully alternating α,β strand with two 3'-OH termini
10	αT βC αT βT αA βA αC βC αC βA αC βA	fully alternating α,β strand with two 5'-OH termini

Greek type letter preceeding each upper case letter refers to the anomeric configuration of the corresponding individual nucleotide. Arrows indicate the 5'→ 3' orientation. A single arrow covering several upper case letters indicates a homogeneous orientation of the corresponding nucleotides. Head to head arrows and tail to tail arrows depict the presence of unnatural 3'→ 3'- and 5'→ 5'- phosphodiester links respectively

Chimeric α,β-oligonucleotides complementary to the splice acceptor site of HIV-1 *tat* RNA and corresponding to different combinations were prepared (TABLE 1) on an ABI 381 A DNA synthesizer via the phosphoramidite route following the standard procedure and using deoxy α- and β-nucleosides 3'- or 5'-phosphoramidites.

Deoxy-α-nucleoside 5'-phosphoramidites were obtained with an overall yield ranging from 25% to 68% starting from corresponding base-protected α-nucleosides. First, the starting compound was sequentially protected in 5'-position with a *tert*-butyldimethylsilyl (TBDMS) group and in 3'-position with a 4,4'-dimethoxytrityl (DMTr) group. 5'-hydroxyl of the resulting nucleoside was then deprotected by treatment with

TABLE 2. Thermal stability of hybrids formed between antisense oligomers and DNA or RNA targets. **n**: number of unnatural phosphodiesters links; **r**: ratio of purine-α-nucleotides to total α-nucleotides

			vs DNA		vs RNA	
Oligos	n	r	Tm °(C)	ΔTm/n	Tm °(C)	ΔTm/n
3	0		47.6*		46.1*	
4	0	0.33	42.8*		43.1*	
5			13.2		ND	
6			<0		ND	
7	2	0.25	44	-1.8	41.2	-2.4
8	8	0	41.5	-0.6	36.8	-1.2
9	11	0.5	25.4	-2	19.6	-2.4
10	11	0.17	36	-1	27.3	-1.7

Melting temperature experiments were performed with equimolar mixtures of complementary oligonucleotides each at a concentration of 3 μM in 0.1 M NaCl, 10 mM sodium cacodylate, pH 7. An Uvikon 810 spectrophotometer (Kontron) fitted with a thermostated cell holder and interfaced with an IBM PC compatible was used. Heating rate was 0.5°C min^{-1}. * from reference 4.

tetrabutylammonium fluoride and subsequently phosphitylated to give the corresponding α-nucleoside 5'-cyanoethyl-phosphoramidite.

Hybridization properties of oligos **5-10** to the DNA and RNA targets were evaluated by determining Tm values derived from melting curves recorded at 260 nm (TABLE 2).

Comparison of the data obtained with the oligos **5-7** confirmed that the orientation found previously[3] with α-oligonucleotides for duplex formation with DNA or RNA complementary strands was also valid for chimeric oligos consisting of α- and β-stretches. Chimeric mixed-sequence oligonucleotides **7-8** consisting of a stretch of β-nucleotides with several α-nucleotides at both 5'- and 3'-ends are able to form stable and fully base-paired duplexes with complementary DNA or RNA strands. Thermal stability of these heteroduplexes moderately decreases with the number of unnatural junctions between α and β units and with the ratio r of purine-α-nucleotides to total α-nucleotides within the chimeric oligonucleotide.

Optimal stability against exonucleases (TABLE 3) present in sera and cellular extracts can be attained by introduction of terminally inverted polarity whatever the

TABLE 3. **A:** Half-life of chimeric oligonucleotides in RPMI 1640 medium supplemented with 10% inactivated fetal calf serum incubated at 37°C[4]; **B:** Percentage of degradation of oligomers in CEM-SS cell extracts at 37°C[5]. Analyses were performed by HPLC[6].

	A RPMI 1640 medium + 10% FCS	**B** CEM-SS cell extracts
Oligos	$t_{1/2}$ of 12-mer	% degradation of 12-mer at t=24 hours
3	11 min.	100% ($t_{1/2}$=24 min.)
4	25 min.*	34%
7	32 min.*	23%
8	16 min.*	13%
9	15 min.**	4%
10	5.5 days	ND

* Formation of a stable shorter fragment was observed which was tentatively attributed to a 11-mer.
** Formation of a stable fragment was observed which was tentatively attributed to a 10-mer.

anomeric configuration of the terminal nucleotide. Stability against endonuclease hydrolysis is obtained by introducing, as much as possible, unnatural 3'→ 3' and 5'→ 5' phosphodiester linkages by alternating α- and β-nucleotide units.

These results provide guide lines for optimization in the design of antisense chimeric oligodeoxynucleotides.

Acknowledgment. This work was supported by grants from the "Association pour la Recherche contre le Cancer" (A.R.C.) and from the "Agence Nationale de Recherche sur le Sida" (A.N.R.S.).

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