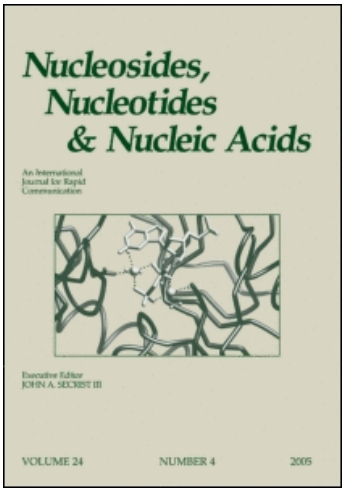


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

α-OLIGONUCLEOTIDES WITH ANIONIC PHOSPHODIESTER AND CATIONIC PHOSPHORAMIDATE LINKAGES ENHANCED STABILITY OF DNA TRIPLE HELIX

F. Ehrenmann[a]; J. -J. Vasseur[a]; F. Debart[a]
[a] Université Montpellier II, Montpellier, France

Online publication date: 31 March 2001

To cite this Article Ehrenmann, F. , Vasseur, J. -J. and Debart, F.(2001) 'α-OLIGONUCLEOTIDES WITH ANIONIC PHOSPHODIESTER AND CATIONIC PHOSPHORAMIDATE LINKAGES ENHANCED STABILITY OF DNA TRIPLE HELIX', Nucleosides, Nucleotides and Nucleic Acids, 20: 4, 797 — 799
To link to this Article: DOI: 10.1081/NCN-100002432
URL: http://dx.doi.org/10.1081/NCN-100002432

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 797–799 (2001)

α-OLIGONUCLEOTIDES WITH ANIONIC PHOSPHODIESTER AND CATIONIC PHOSPHORAMIDATE LINKAGES ENHANCED STABILITY OF DNA TRIPLE HELIX

F. Ehrenmann, J.-J. Vasseur, and F. Debart*

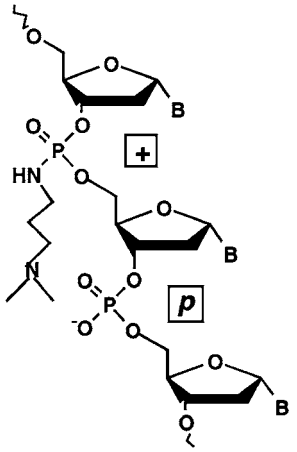
Laboratoire de Chimie Organique Biomoléculaire de Synthèse,
UMR 5625 CNRS-UM II, CC 008, Université Montpellier II,
Place Eugène Bataillon, 34095 Montpellier, France

ABSTRACT

Synthesis of several α-oligonucleotides containing both anionic phosphodiester and cationic *N*-(dimethylaminopropyl)phosphoramidate internucleosidic linkages is described. Their ability to form triple helix with dsDNA was evaluated at various pH by UV melting experiments.

Some years ago, Letsinger and al (1). showed that pyrimidine oligonucleotides (ODNs) containing alternating phosphodiester and stereo-uniform cationic *N*-(dimethylaminopropyl)phosphoramidate linkages formed triple-stranded complexes with double-stranded DNA. One stereoisomer bound with higher affinity, and the other stereoisomer with lower affinity, than the corresponding all-phosphodiester (PO) ODN. Furthermore, Dagle and Weeks (2,3) concluded that replacement of PO bonds with positively-charged *N*,*N*-diethylethylenediamine phosphoramidate linkages results in more efficient triplex formation. More recently, we have demonstrated (4) that pyrimidine α-ODNs containing fully modified internucleosidic linkages with non-ionic *N*-(2-methoxyethyl)phosphoramidate or phosphoromorpholidate backbones as diastereoisomeric mixtures formed triple helices much more stable than natural PO β-ODN.

*Corresponding author.



β5'-(GpCp**ApApApGpApApGpGpApGpApA**pC
3'(CpGp**TpTpTpCpTpTpCpCpTpCpTpT**pG T_4

ds DNA target phosphodiester

α5'-(T+T*p*C*p*T*p*C*p*C*p*T+T*p*C*p*T+T*p*T) 3' ODN **1**
α5'-(T*p*T+C*p*T+C*p*C*p*T*p*T+C*p*T+T*p*T) 3' ODN **2**
α5'-(T*p*T+C*p*T+C*p*C+T*p*T+C*p*T+T*p*T) 3' ODN **3**
α5'-(T*p*T*p*C*p*T+C+C+T+T+C*p*T*p*T*p*T) 3' ODN **4**
α5'-(T+T+C*p*T*p*C*p*C*p*C*p*T*p*T*p*C+T+T+T) 3' ODN **5**

β5'-(T*p*T*p*T*p*C*p*T*p*T*p*C*p*C*p*C*p*T*p*C*p*T*p*T) 3'

Figure 1. Oligonucleotides synthesized.

These results (1–4) prompted us to investigate on the ability of pyrimidine α-ODNs containing mixed anionic PO and cationic *N*-(dimethylaminopropyl)phosphoramidate (PNHDMAP) linkages to form triple helices with dsDNA targets. For this purpose, we have synthesized several pyrimidine α-ODNs (12-mers) where three (ODN **1**), four (ODN **2**) or five (ODN **3**, **4** and **5**) cationic PNHDMAP linkages were incorporated into PO α-ODNs (Fig. 1).

The phosphoramidate linkages were introduced in PO α-ODNs **1**, **2** and **3** through 3′-phosphoramidite dinucleoside building blocks containing a PNHDMAP linkage, which were prepared according to a procedure described in the literature for the synthesis of β-analogs (5). α-ODNs **4** and **5** containing five PNHDMAP linkages either in the central window or in the wings were synthesized in blocks. PO bonds were generated through standard phosphoramidite chemistry except for oxidation step which was performed with t-butyl hydroperoxide rather than iodine. PNHDMAP bonds were obtained by amidative oxidation of hydrogen phosphonate diesters with CCl_4/N-dimethylaminopropylamine. Oxidation was performed manually at the end of the elongation of the ODNs before standard ammonia deprotection.

The hybridization properties of these α-ODNs with their complementary homopurine DNA target were evaluated at various pH by UV melting experiments and Tm values are compiled in Table 1. Pyrimidine α-dodecamers containing three, four or five cationic PNHDMAP linkages formed triple helices much more stable than the corresponding natural PO ODN did in the same pH conditions. At pH 7, ΔTm/mod. was between 4 and 5°C whereas PO β-ODN did not form a triple helix. Stability of triple helices depended on pH conditions whatever the third strand was (all-PO or mixed anionic and cationic linkages). The lower the pH, the higher the Tm of all triple helices. Tm of triplex increased with the number of cationic phosphoramidate linkages. At pH 7, Tm of ODN **3** with five cationic linkages was 24.9°C whereas Tm of ODN **1** and ODN **2** were 13.4°C and 16°C respectively.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 1. Thermal Stability (Tm°C) of Triplex Formed Between PO β-ODN and PO α-ODNs Containing Three, Four or Five Cationic PNHDMAP Linkages and their dsDNA Target

	Tm (°C)		
	pH 7.0	pH 6.2	pH 5.5
β PO	<5.0	18.6	30.8
ODN **1**	13.4	29.6	43.6
ODN **2**	16.0	35.6	52.0
ODN **3**	24.9	45.2	69.8
ODN **4**	17.5	41.1	73.3
ODN **5**	20.5	44.0	72.4

Experiments were carried out at 3 μM oligonucleotide concentration in 10 mM sodium cacodylate, 100 mM NaCl. Heating rate 20°C/hour.

To evaluate the effect of cationic charges distribution on triple helix formation, we designed three PO α-12-mers with five PNHDMAP linkages incorporated either alternating with PO bonds (ODN **3**) or in the central window (ODN **4**) or in the wings of the chimeric oligos (ODN **5**). Thermal stability of triple helices formed with these ODNs **3**, **4** and **5** were determined (Table 1). At pH 7, Tm of triplex with fully-alternated PO/PNHDMAP ODN **3** was higher than Tm of triplexes formed with ODN **4** ($\Delta Tm + 7.2°C$) or with ODN **5** ($\Delta Tm + 4.4°C$). At pH 6.2 and 5.5, the stability of triple helices formed with ODNs **3**, **4** and **5** were nearly similar.

Compared to natural phosphodiester β-ODNs, the higher affinity of pyrimidine α-ODNs combining alternating PO and PNHDMAP internucleosidic linkages for dsDNA target makes them attractive for an antigene purpose. The stabilizing effect of these chimeric oligonucleotides was more pronounced than the one previously obtained with fully non-ionic phosphoramidate linkages (4). The synthesis of α-ODNs containing a higher ratio PNHDMAP/PO linkages is currently in progress in order to obtain stable triple helices under physiological conditions. Furthermore, the hybridization properties of these new cationic/anionic oligonucleotide chimeras with ssDNA and RNA targets is underway.

REFERENCES

1. Chaturvedi, S.; Horn, T.; Letsinger, R. L. *Nucleic Acids Res*. **1996**, *24*, 2318–2323.
2. Dagle, J. M.; Weeks, D. L. *Nucleic Acids Res*. **1996**, *24*, 2143–2149.
3. Bailey, C. P.; Dagle, J. M.; Weeks, D. L. *Nucleic Acids Res*. **1998**, *26*, 4860–4867.
4. Laurent, A.; Naval, M.; Debart, F.; Vasseur, J.-J.; Rayner, B. *Nucleosides, Nucleotides* **1999**, *18*, 1629–1630.
5. Horn, T.; Chaturvedi, S.; Balasubramaniam, T. N.; Letsinger, R. L. *Tetrahedron Lett*. **1996**, *37*, 743–746.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Request Permission or Order Reprints Instantly!