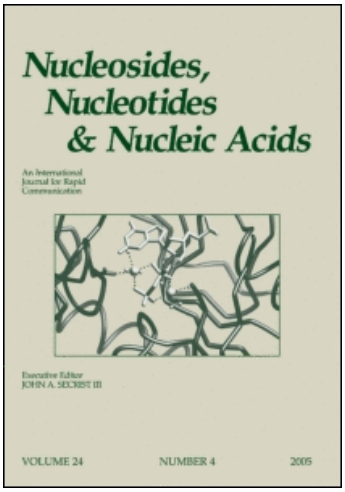


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

DNA Triple Helix Formation by N-Alkylphosphoramidate α-Oligonucleosides

A. Laurent[a]; M. Naval[a]; F. Debart[a]; J. J. Vasseur[a]; B. Rayner[a]
[a] Laboratoire de Chimie Bio-organique, Montpellier Cedex, France

To cite this Article Laurent, A. , Naval, M. , Debart, F. , Vasseur, J. J. and Rayner, B.(1999) 'DNA Triple Helix Formation by N-Alkylphosphoramidate α-Oligonucleosides', Nucleosides, Nucleotides and Nucleic Acids, 18: 6, 1629 — 1630
To link to this Article: DOI: 10.1080/07328319908044804
URL: http://dx.doi.org/10.1080/07328319908044804

NUCLEOSIDES & NUCLEOTIDES, 18(6&7), 1629–1630 (1999)

DNA TRIPLE HELIX FORMATION BY N-ALKYLPHOSPHORAMIDATE α-OLIGONUCLEOSIDES

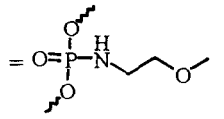
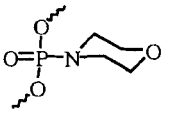
A. Laurent, M. Naval, F. Debart*, J.-J. Vasseur and B. Rayner

Laboratoire de Chimie Bio-organique, UMR 5625 CNRS-UM II, CC008, Université Montpellier II, Place Eugène Bataillon, 34095 Montpellier Cedex 05, France

ABSTRACT: Triplex formation of pyrimidine N-alkylphosphoramidate α-oligonucleosides (12-mer) containing either dC or 5-Me-dC with their phosphodiester oligonucleoside homopurine target was evaluated by UV melting experiments.

Recently, we have demonstrated that oligodeoxynucleotides (ODNs) combining two structural modifications, i.e. the inversion of the anomeric configuration in the sugar moieties and the substitution of phosphate diester by non-ionic N-alkylphosphoramidate linkages, form stable double helices with complementary RNA and DNA single strands[1]. Among these backbone-modifications, N-(2-methoxyethyl)phosphoramidate α-ODNs hybridized more tightly to their targets than the corresponding natural phosphodiester β-oligonucleoside.

These results prompted us to investigate the ability of the phosphoramidate α-ODNs to form triple helices with double-stranded DNA targets. For this purpose, pyrimidine N-alkyl-phosphoramidate α-ODNs (12-mer) containing either cytosine or 5-methylcytosine were synthesized. Their thermal stability with their phosphodiester oligonucleoside homopurine target was evaluated at various pH (5.5, 6.2 and 7.0) by UV melting experiments. Data are presented in Table 1.

$\alpha^{3'}$-(T*p*T*p*T*p*C*p*T*p*T*p*C*p*C*p*T*p*C*p*T*p*T)$^{5'}$ $p =$  or 

$\beta^{5'}$-(**GpCp**ApApApGpApApGpGpApGpApApC
$^{3'}$(**C**pGpTpTpTpCpTpTpCpCpTpCpTpTp**G**) $\frown$ $\mathbf{T_4}$ ds DNA target phosphodiester

TABLE 1. Tm (°C) data of triple helices at several pH

Oligomer	Phosphodiester β-ODN	N-(2-methoxyethyl)-phosphoramidate α-ODN		Phosphoromorpholidate α-ODN	
	cytosine	cytosine	5-Me-dC	cytosine	5-Me-dC
pH 5.5	29.0	55.0	61.0	46.0	53.5
pH 6.2	19.0	39.5	44.0	30.0	36.5
pH 7.0	<5	23.0	28.5	13.5	22.0

Experimental conditions: 3 μM oligonucleotide concentration in 10 mM sodium cacodylate, 100 mM NaCl at pH 5.5, 6.2 and 7.0. Heating rate 20°C/min.

We have shown that N-alkylphosphoramidate α-ODNs formed anti-parallel (relative to purine strand of the ds DNA target) triple helices much more stable than natural phosphodiester β-ODNs did (ΔTm +20°C for N-(2-methoxyethyl)phosphoramidate and ΔTm +10°C for phosphoromorpholidate α-ODNs at pH 7). Stability of triple helices depends on pH conditions whatever the third strand is (phosphoramidate or phosphodiester). Furthermore the replacement of cytosine residues by 5-methyl-cytosine in N-alkylphosphoramidate α-ODN enhanced the thermal stability of the triple helices (ΔTm +25.5°C for N-(2-methoxyethyl)phosphoramidate and ΔTm +18.5°C for phosphoromorpholidate α-oligonucleosides at pH 7).

In conclusion, as already observed in the case of methylphosphonate α-ODN[2], the combination of modifications (up to three) in the phosphodiester backbone, sugar and nucleobase produces new non-ionic analogues exhibiting high affinity for single- or double-stranded nucleic acid targets. These properties make these new oligonucleotides attractive for antisense and antigene applications.

REFERENCES

1. Laurent, A.; Debart, F.; Bologna, J.-C.; Vasseur, J.-J.; Rayner, B. *Nucleosides, Nucleotides* **1998,** *17*, 1645-1649.

2. Debart, F.; Meyer, A.; Vasseur, J.-J.; Rayner, B. *Nucleic Acids Res.* **1998,** *26*, 4551-4556.