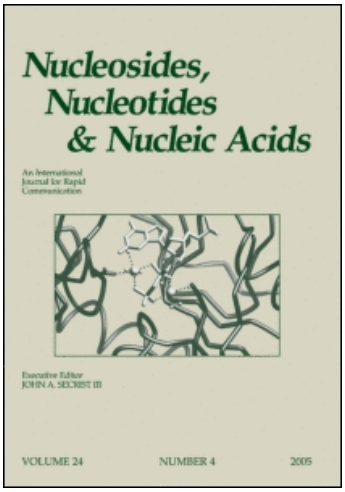

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Replacement of the Phosphodiester Backbone of Oligodeoxynucleotide Analogues by Non-Ionic Phosphoramidate (P-NH_2)

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REPLACEMENT OF THE PHOSPHODIESTER BACKBONE OF OLIGODEOXYNUCLEOTIDE ANALOGUES BY NON-IONIC PHOSPHORAMIDATE (P-NH_2)

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ABSTRACT: The synthesis and the biophysical properties of oligodeoxyribonucleotide analogues containing phosphoramidate (P-NH_2) internucleoside linkages and β or α deoxynucleoside units is reported.

The search for nuclease resistant oligodeoxynucleotides (ODNs) and ODN analogues with improved cellular uptake had resulted in the development of numerous modifications of the phosphodiester internucleoside link[1,2]. Of them, ODN phosphoramidates[3] represent a class of phosphate modified ODNs in which a non-bridged oxygen atom of the phosphodiester links is substituted by a primary or a secondary amino group. Up to now, substitution by a NH_2 group (P-NH_2) was limited to di- or trinucleotides[4,5] due to their instability under either basic or acidic conditions. However, in phosphoramidates (P-NH_2), the fact that the NH_2 is small, uncharged in aqueous solution and forms hydrogen bonds readily[6] gives them favourable solubility characteristics required for hydration with a minimum steric hindrance around the phosphorus atom and makes them attractive potential candidates for non-ionic analogues of ODNs.

Synthesis of dodecanucleoside phosphoramidates (P-NH_2) and mixed phosphoramidate-phosphodiester (PO^-) oligomers was achieved on a DNA synthesizer[7]

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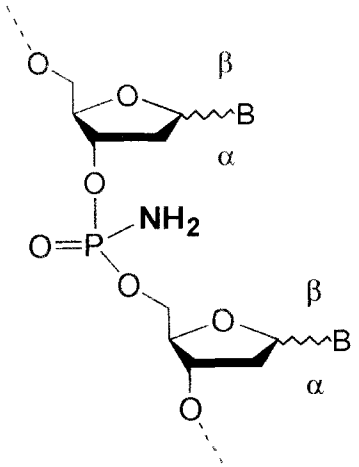
using both H-phosphonate and phosphoramidite chemistries, in combination with *tert*-butylphenoxyacetyl for N-protection of nucleobases, an oxalyl anchored solid support and a final treatment with methanolic ammonia (2 hours, 20°C). Oxydative amidation of the H-phosphonate diester linkages was carried out, after completion of the elongation steps , by treament of the support-bound oligomer with a saturated solution of ammonia in CCl_4-dioxan (4:1, v:v, 0°C, 30 min).



Modified Oligonucleotide Sequences

β-$(dTp)_5$dTpn$(dTp)_5$dT (1 modif)

β-$(dTpndTp)_5$dTpndT (6 modif)

β-$(dTpn)_3(dTp)_5(dTpn)_3$dT

β-$(dTpn)_{11}$dT (11 modif)

α-$(dTpn)_{11}$dT

β-d(ApnCpnApnCpnCpnCpnApnApnTpnTpnCpnT)

β-d(ApnCpnApnCpCpCpApApTpnTpnCpnT)

p is referring to a phosphodiester internucleoside link. and pn to a phosphoramidate ($P\text{-}NH_2$) linkage.

We found that phosphodiester internucleoside linkages were protected towards nuclease degradation by vicinal phosphoramidate ($P\text{-}NH_2$) links. β-$(dTpn)_{11}$dT does not elicit Rnase H activity. β-$(dTpn)_3(dTp)_5(dTpn)_3$dT with a central phosphodiester section is able to elicit RNase H hydrolysis of poly rA as well as β-$(dTp)_{11}$dT.

Tm values of β oligothymidylates (1 to 11 modif.), are almost a linear function of the number of modifications:

average ΔTm/ modif = **-1.4°C** with β-d($C_2A_{12}C_2$)

= **-1.1°C** with poly dA

= **-2.9°C** with poly rA

For β-d(ApnCpnApnCpCpCpApApTpnTpnCpnT), ΔTm/ modif = -2°C with DNA and RNA complementary strands. With β-d(ApnCpnApnCpnCpnCpnApnApnTpnTpnCpnT), only the upper portions of the melting transitions were observed with both DNA and RNA targets. Tm value of the hybrid involving β-$(dTpn)_{11}$dT and β-d($C_2A_{12}C_2$) is weakly modified by the ionic strength of the medium either at pH 7 or pH 5.45.

Table 1. Melting temperatures of duplexes formed between dodecathymidine analogues and poly dA or poly rA

ODNs	Versus poly dA		Versus poly rA	
	Tm (°C)	Δ Tm (°C)	Tm (°C)	Δ Tm (°C)
β-d(Tpn)$_{11}$T	20.5	-11.0	< 0	< -29.5
α-d(Tpn)$_{11}$T	57.1	+25.6	43.0	+13.5
β-d(Tp)$_{11}$T	31.5	/	29.5	/
α-d(Tp)$_{11}$T	23.6	-7.9	43.0	+13.5

Measurements were carried out at 60 μM nucleotide concentration for each strand, in 0.1 M NaCl, 10 mM sodium cacodylate (pH 7).

The inversion of the anomeric configuration (β to α) of the sugar moieties of a dodecathymidine combined with the replacement of the phosphodiester (PO^-) backbone by non ionic phosphoramidate (P-NH_2) produce an oligonucleotide exhibiting unexpected high affinity for DNA and RNA targets[8].

ΔTm/ modif = **+2.1°C** with β-d($C_2A_{12}C_2$)

= **+2.3°C** with poly dA

= **+1.2°C** with poly rA

With poly dA, no hypochromic effect was detected at 280 nm whereas it was observed at 260 nm indicating duplex formation. With poly rA, mixing curve titration indicated here again duplex formation.

Base pairing specificity was maintained as demonstrated by depressed Tm values observed in presence of a single central CT or GT mismatch in the duplex formed with β-d($C_2A_{12}C_2$). Extention of this work to α-oligonucleoside phosphoramidates involving the four common nucleobases is under way.

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