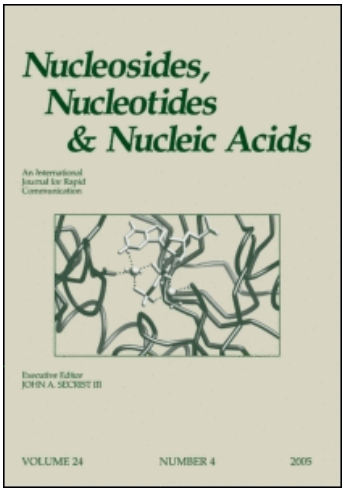

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High Affinity Of Backbone-Modified α-Anomeric Oligodeoxynucleottoes For Dna And Rna Targets

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HIGH AFFINITY OF BACKBONE-MODIFIED α-ANOMERIC OLIGODEOXYNUCLEOTIDES FOR DNA AND RNA TARGETS

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ABSTRACT: Methylphosphonate and various substituted phosphoramidate α-anomeric oligonucleotides were synthesized. We have investigated the chirality effect of the internucleoside linkage and the influence of steric hindrance around phosphorus on hybridization properties of these new α-analogs.

Phosphate-modified oligonucleotides, in particular ionic phosphorothioate (PS^-) and non-ionic methylphosphonate ($P\text{-}CH_3$) oligos, have been investigated to improve the cellular uptake and nuclease resistance of antisense oligonucleotides[1]. Phosphoramidate oligos in which a non-bridged oxygen is substituted by primary or secondary amino groups have also been studied[2]. These backbone modifications decrease the affinity of oligos for their complementary nucleic acids and especially for their RNA targets. This decrease has been assigned to the chirality of the internucleotidic linkage, and the steric hindrance of the P-substituent has a detrimental impact on the overall stability of the duplex[3].

Some years ago, our group developed nuclease-resistant α-anomeric oligonucleotides which form stable parallel-stranded duplexes with complementary natural β-DNA or β-RNA strands[4]. Interestingly we found that phosphorothioate (PS^-) α-oligos hybridized to their DNA and RNA targets more strongly than their (PS^-) β-homologs did[5]. More recently the same behavior was observed with α- and β-dodecathymidylates containing non-ionic primary phosphoramidate ($P\text{-}NH_2$) internucleoside linkages. When hybridized

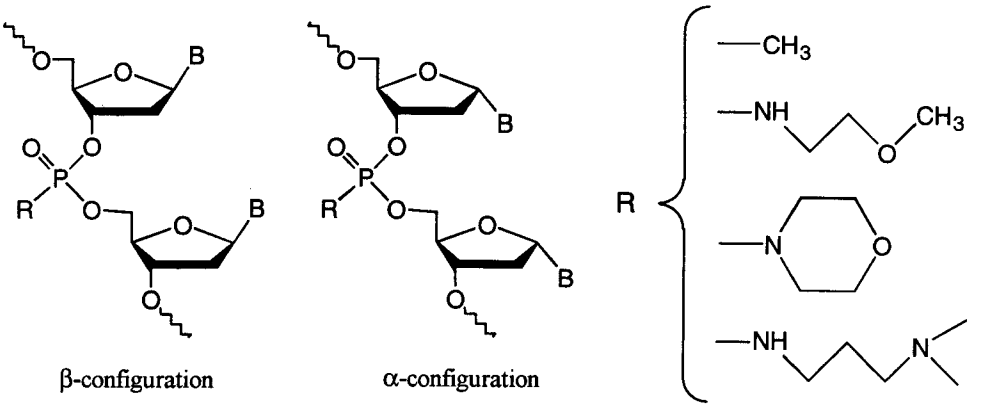
with poly dA and poly rA, the (P-NH_2) α-oligomer bound to their nucleic acid targets more tightly than the β-homolog did. Surprisingly the non-ionic α-analog hybridized to its targets much better than the parent phosphodiester β-oligonucleotide[6].

These data obtained with (PS^-) and (P-NH_2) α-oligos prompted us to study new phosphate-modified α-oligonucleotide analogs with bulkier substituents on the phosphorus atoms. We report here hybridization properties of four classes of α-oligos with non-ionic or cationic internucleotidic linkages as methylphosphonate, methoxyethylphosphoramidate, morpholinophosphoramidate and dimethylaminopropyl-phosphoramidate (Scheme 1). In this paper we focus on the effect of the chirality of internucleotidic linkage and the effect of the steric hindrance around phosphorus on hybridization properties of these new α-analogs.

First to evaluate the effect of the chirality, we prepared chirally pure phosphorus substituted oligos with one methylphosphonate or phosphoramidate modification in the middle of the sequence β-3'-d(TCTTAA*****CCCACA)5' or α-5'-d(TCTTAA*****CCCACA)3' complementary to the splice acceptor site of HIV-1 *tat* RNA. This modification was introduced in phosphodiester α- or β-dodecamers with backbone-modified dimers in α- or β-configuration as 3'-phosphoramidite synthons. Chirally pure oligonucleotides were obtained after separation of diastereoisomers either at the dimer stage or at the oligo stage. For each backbone-modification, two oligonucleotides with α-configuration and two oligos with β-configuration were obtained and hybridized with their complementary DNA (AGAATTGGGTGT) and RNA (AGAAUUGGGUGU) targets.

For each phosphate-modified analog we determined the change of Tm (ΔTm) relative to phosphodiester duplexes. β-analogs were compared to phosphodiester (PO^-) β-oligo and α-analogs were compared to (PO^-) α-oligomer.

Versus DNA target: Concerning β-oligos, whatever the backbone-modification was, the two isomers formed duplexes with DNA less stable than the phosphodiester parent β-oligo. As already known, one isomer (the "good" one) bound to its DNA target less tightly than the other one (the "bad" one) did. With α-analogs the same difference of behavior between isomers was observed and this difference was even more pronounced than in β-series. But in α-configuration, the "good" isomer induced a stabilizing effect



Scheme 1

whereas the "bad" isomer induced a slightly destabilizing effect. This "bad" isomer was even better than the "good" one in β-configuration.

Versus RNA target: For β-analogs, the backbone-modification destabilized duplexes with RNA target more dramatically than with DNA target whereas for α-analogs, the backbone-modification destabilized duplexes very slightly. For each phosphate-modified analog, the difference between Tm values of the two α-isomers was very weak and this difference was more pronounced in β-series.

These data show a chirality effect on hybridization properties of phosphate-modified α- and β-analogs but this effect is not detrimental for duplex stability in α-configuration.

To study the effect of the steric hindrance around phosphorus on hybridization properties of these new α-analogs, we synthesized fully modified phosphorus-substituted dodecamers β-3'- and α-5'-(TCTTAACCCACA). Methylphosphonate β-and α-oligonucleotides were prepared *via* phosphonamidite chemistry following literature procedures[7]. Phosphoramidate oligomers were synthesized *via* H-phosphonate chemistry followed by oxydation with CCl_4 in the presence of amine[8]. All the oligos were obtained as diastereoisomeric mixtures and were hybridized with their complementary DNA and RNA targets.

As expected, the modified β-dodecamers hybridized weakly with their complementary DNA strand and even more so with RNA target, and as already known, the bulkier the

phosphorus substituent, the lower the Tm. In contrast to the β-series, we showed that fully backbone-modified α-oligos exhibited high affinity for DNA as well as for RNA targets. For example, the replacement of all the phosphodiester linkages by methoxyethylphosphoramidate groups into the β-oligo decreased Tm value by 15°C versus DNA and 30°C versus RNA and inversely increased the Tm of α-oligo (+10°C) with DNA and (+1°C) RNA. The stability of duplexes formed between phosphate-modified α-oligos and their complementary DNA or RNA strands was not considerably affected by the bulk of the P-substituent. Even in the case of the bulky morpholidate α-oligo, the Tm was very closed to the Tm of phosphodiester β or α-oligos.

In conclusion, we demonstrated that the detrimental effects on affinity towards nucleic acid targets of the bulk and the orientation of phosphorus substituent are much less pronounced in α-configuration than in β-configuration. In this regard, the combination of two modifications, i. e. inversion of the anomeric configuration (β to α) and change of the ionic linkages in non-ionic backbones produce α-oligonucleotides exhibiting high affinity for single DNA or RNA strands.

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