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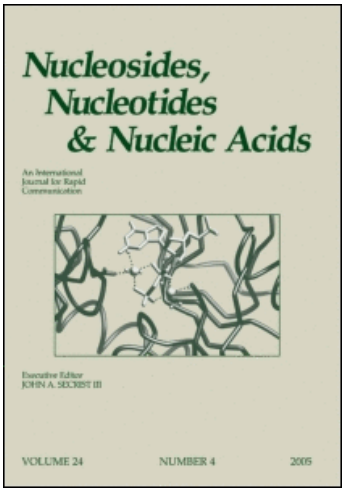


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NUCLEOSIDES & NUCLEOTIDES, 17(9-11), 1577-1581 (1998)

# DIVERS STEREOISOMERS OF *N*-ACETYLHYDROXYPROLINOL AS SUGAR SUBSTITUTE IN OLIGONUCLEOTIDES

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**ABSTRACT.** A detailed comparison of the hybridisation characteristics of oligonucleotides with 4-hydroxy-*N*-acetylprolinol or 3-hydroxy-*N*-acetylprolinol as sugar substitute, reveals dramatic differences. The 4-HO oligonucleotides are able to form stable complexes which are in general duplexes when natural nucleic acids are used as the complement, and the system has a strong preference for isochiral interaction. For the 3-HO oligonucleotides on the other hand complexes are generally weak, triple stranded, and often isochiral and heterochiral hybrids have a similar stability.

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## INTRODUCTION.

We have reported before on the synthesis and some hybridisation properties of *trans*-4-HO-*N*-acetylprolinol oligonucleotides[1] (4-HO-NAP) (**FIG. 1**) We have since elaborated our investigations[2] and also concluded the synthesis of a second series of related oligomers, using 3-hydroxy analogues[3] (3-HO-NAP) (**FIG. 1**).
Considering the availability of several stereoisomers, a detailed comparison regarding the hybridisation properties of these molecules is possible.

Specifically the chiral preferences seemed interesting to investigate, because recent reports also deal with the interaction selectivity of enantiomerically unnatural nucleic acids[4-9]. Of these, L-ribonucleic acid seems to be capable of interacting strongly and selectively with its D-RNA complement. Interactions with its D-DNA complement are only of low stability. It seemed worthwhile to investigate whether RNA's indifference towards chirality can also be found in systems other than the RNA-homocomplexes.

This report also highlights the exact nature of the complexes formed with our unnatural constructs, being triple or double stranded, considering our interest in antisense therapeutics.

## RESULTS AND DISCUSSION.

Oligonucleotides composed of 4-HO-NAP moieties form more stable complexes with DNA as well as RNA than their 3-HO congeners[2,3]. However, regrouping of the results as outlined in TABLE 1 and TABLE 2, reveals a marked indifference of the 3-HO-oligomer regarding the chirality of its natural complement, which is not encountered for



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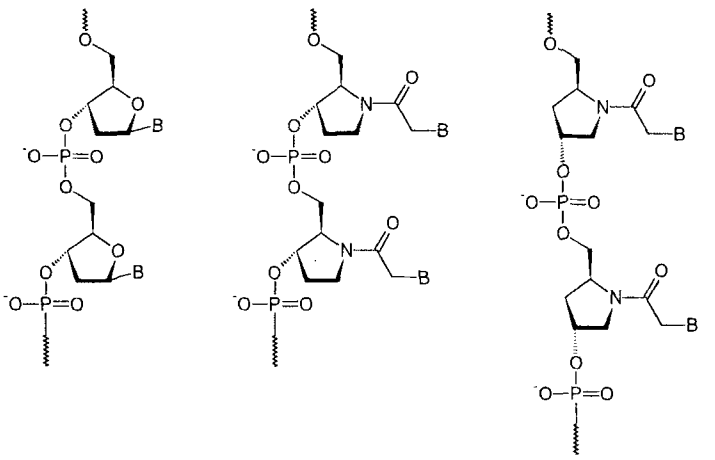


**FIG. 1: Structural comparison between DNA, 3-HO-NAP and 4-HO NAP oligonucleotides**

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**TABLE 1: Homochiral hybridisation.**

*Tm were measured in a buffer containing 0.02M $KH_2PO_4$, pH 7.5, 0.1mM EDTA with 1M NaCl and 4μM of each oligonucleotide. When two numbers are given, the first one applies to the Tm obtained upon heating, the second one to the cooling experiment.*

| | 4-HO-L | Tm | 3-HO-L | Tm |
|---|---|---|---|---|
| | | Complement NAP | | |
| 1 | $T^*_{13}/A^*_{13}$ | 28.5 | $T^*_{13}/A^*_{13}$ | 17/14 |
| 2 | 3-HO-$A^*_{13}/T^*_{13}$ | 25 | 4-HO-$A^*_{13}/T^*_{13}$ | 30/25 |
| | | Complement DNA | | |
| 3 | $A_{13}/T^*_{13}$ | / | $A_{13}/T^*_{13}$ | / |
| 4 | $T_{13}/A^*_{13}$ | 48 | $T_3/A^*_{13}$ | 21 |
| | | Complement RNA | | |
| 5 | polyA/$T^*_{13}$ | 30 | polyA/$T^*_{13}$ | / |
| 6 | polyU/$A^*_{13}$ | 57 | polyU/$A^*_{13}$ | 40 |

**TABLE 2: Heterochiral hybridisation**

*Conditions were as described for hoochiral hybridisation.*

| | 4-HO-D | Tm | 3-HO-D | Tm |
|---|---|---|---|---|
| | | Complement NAP | | |
| 1 | $T^*_{13}/A^*_{13}$ | 28.5 | $T^*_{13}/A^*_{13}$ | / |
| 2 | 3-HO-$LA^*_{13}/T^*_{13}$ | / | 4-HO-$LA^*_{13}/T^*_{13}$ | / |
| | | Complement DNA | | |
| 3 | $A_{13}/T^*_{13}$ | / | $A_{13}/T^*_{13}$ | / |
| 4 | $T_{13}/A^*_{13}$ | / | $T_{13}/A^*_{13}$ | 21 |
| | | Complement RNA | | |
| 5 | polyA/$T^*_{13}$ | / | polyA/$T^*_{13}$ | / |
| 6 | polyU/$A^*_{13}$ | 27/23 | polyU/$A^*_{13}$ | 39 |

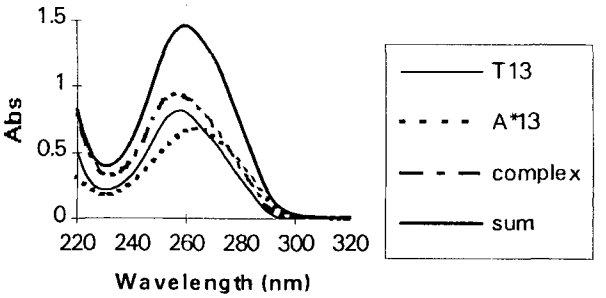


**Figure 2: Wavelength scan in the *trans*-4HO-L-NAP-$A_{13}/T_{13}$ system.**

*The wavelength scan of both single strands (T13 and A*13) are depicted as well as their calculated sum (sum). The absorption of the complex is at all times equal to or lower than the calculated absorption sum of the single strands, which explains the observation of melting curves at divers wavelengths.*

the 4-HO structure. The hybrids formed with natural nucleic acids are of equal stability for both optical isomers of the 3-HO oligonucleotide (compare lines 3 and 6 left of both tables). On the other hand, the 4-HO-**D**-$A^*_{13}$ oligomer is only capable to interact weakly with its RNA complement, while all other interactions are abolished.

The 3-HO-NAP oligomers are thus much less discriminatory in their interactions.

Furthermore, microcalorimetry proves that most of the complexes formed with 3-HO oligonucleotides are triple stranded, whereas for 4-HO constructs, the more important hybrids (with natural nucleic acids) are shown to be double stranded only. Contrary to our expectations[1] therefore, for the 4-HO-oligomers, the homocomplexes (triplex, **TABLE 3**) are of a different nature than the hybrids with DNA or RNA (duplex). The hypochromicity at 284nm seems to be a general characteristic of complexes incorporating

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**TABLE 3: Classification as double and triple stranded complexes and hypochromicity at 260nm.**

*The hypochromicity data are displayed in two columns: D (duplex), T (triplex). Duplex and triplex assignements were made based on microcalorimetry titrations; sometimes UV mixing curves were used.*
*[a]Complex where the amount of strands was not determined.*

| 4-HO-L | D | T | 3-HO-L | D | T |
|---|---|---|---|---|---|
| | | | Complement NAP | | |
| $T^*_{13}/A^*_{13}$ | | 28% | $T^*_{13}/A^*_{13}$ | | 24% |
| $DT^*_{13}/A^*_{13}$ | | 28% | $DT^*_{13}/A^*_{13}$ | | 28% |
| 3-HO-$A^*_{13}/T^*_{13}$ | 18% | | 4-HO-$A^*_{13}/T^*_{13}$ | | 27% |
| | | | Complement DNA | | |
| $T_{13}/A^*_{13}$ | 31% | | $T_{13}/A^*_{13}$ | | 24% |
| $T_{13}/DA^*_{13}$ | / | | $T_{13}/DA^*_{13}$ | | 24% |
| | | | Complement RNA | | |
| $polyU/A^*_{13}$ | 29% | | $polyU/A^*_{13}$ | 28%[a] | |
| $polyU/DA^*_{13}$ | 33%[a] | | $polyU/DA^*_{13}$ | 33%[a] | |

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these modified structures, since it could be identified for many interactions, irrespective of their duplex or triplex nature (**FIG. 2**). Obviously, due to the large variation in Tm of the different complexes, the percentage of hypochromicity at 260nm does not provide reliable information regarding the amount of strands in the complex either (**TABLE 3**).

Although the all-adenine *trans*-4-HO-L-NAP oligomer hybridises with natural nucleic acids forming particularly stable double stranded complexes and also the all-thymine analogue is able to form a duplex with complementary RNA, the antisense potential of *trans*-4-HO-L-NAP constructs is jeopardised by the inability of mixed sequences to interact with their DNA and RNA complement[2].

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