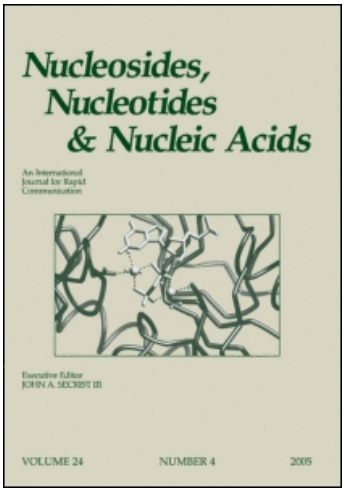

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PROPERTIES OF OLIGONUCLEOTIDES WITH SIX MEMBERED CARBOHYDRATE MIMICS AND A 1,4-RELATIONSHIP BETWEEN THE BASE MOIETY AND THE HYDROXYMETHYL GROUP

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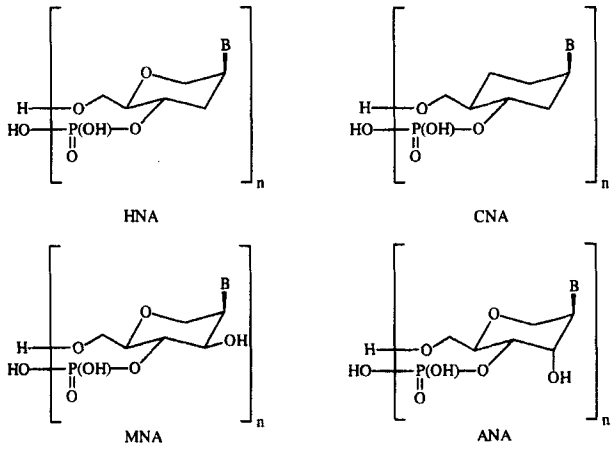
ABSTRACT: The properties of oligonucleotides with a six-membered carbohydrate mimic in the backbone structure and a 1,4-relationship between the base moiety and the hydroxymethyl group are summarized. The different six-membered rings that were studied are: 1,5-anhydro-2,3-dideoxy-D-arabino-hexitol, 1,5-anhydro-2-deoxy-D-mannitol, 1,5-anhydro-2-deoxy-D-altritol and 3-hydroxy-4-hydroxymethyl-cyclohexane.

Hexitol nucleic acids (HNA) differ from natural nucleic acids by replacement of the (deoxy)ribofuranose moiety with a 1',5'-anhydrohexitol ring (Scheme 1). They distinguish themselves from 2',3'-dideoxyhexopyranosyl oligomers by displacement of the base moiety from the anomeric position to the 2'-position. HNA can be considered as a hybrid structure between RNA and homo-DNA, showing the structural characteristics of the former, due to the axial orientation of the base moieties on the 2'-carbon atoms of the hexitol rings[1]. HNA hybridize sequence-selective with natural nucleic acids with a clear preference for RNA: duplexes formed between a HNA and a RNA strand are much more stable than the corresponding HNA-DNA duplexes. In addition, HNA are stable against enzymatic degradation, but a HNA-RNA duplex is a poor inducer of RNase H activity. HNA display sequence-selective activities when evaluated in different biological assays (ras, ICAM-1, malaria)[2]. Their biological activity, however, is lower than that of phosphorothioates, most probably due to both a lower cellular uptake and the absence of an RNase H mediated mechanism. As well CD experiments[1] as molecular



SCHEME 1

modelling[3] and NMR analysis[4] demonstrated that associations between HNA and natural nucleic acids have an A-type geometry. The increased stability of HNA containing complexes may be due to a favourable entropy factor upon hybridization as a result of single strand preorganization.

The structural characteristics of HNA can be attributed to the C1 conformation of the six-membered sugar moiety. This is also the preferred conformation in solution (NMR) and in solid phase (X-ray) for the monomer[5]. However, lowest energy conformations calculated for hA (the hexitol nucleoside with an adenine base moiety) showed[6] that the C1 conformer is favoured by 1.7 $kJmol^{-1}$. The same calculations performed for hT revealed a preference for the 1C conformer and an energy difference between both chair conformers of 5.9 $kJmol^{-1}$. These low energy differences indicate the possibility of the existence of an equilibrium between both conformers in biological media. It was demonstrated that, although the C1 conformer is the favoured one in solution, hU^{I} (the iodouracil congener) co-crystallizes in the active site of herpes virus thymidine kinase in the 1C form[7]. This compound (hU^{I}) demonstrates selective antiherpes virus activity. In order to be able to study more profoundly the influence of this conformational equilibrium on the hybridization properties of oligonucleotides, we synthesized the carbocyclic congeners of the hexitol nucleosides (Scheme 1)[6]. The monomers were obtained starting from ethyl 1,3-cyclohexadiene-1-carboxylate. The racemic carbocyclic nucleosides were separated as their diasteromeric esters. Due to

absence of the ring-oxygen atom, both cA (cyclohexanyl adenine nucleoside) and cT adopt a conformation with an equatorial base moiety and the energy differences between the two low energy conformers are 2.7 $kJmol^{-1}$ and 17.8 $kJmol^{-1}$, respectively[6]. Due to this shift in equilibrium between both conformers we did not expect stable hybridization between carbocyclic oligomers (CNA) and natural nucleic acids. However, CNA hybridize sequence-selectively with both RNA and DNA[6] (at least the isomer corresponding to the D-hexitol series, see Scheme 1). This can only be explained by a chair inversion of the cyclohexane ring upon oligomerization of these carbocyclic nucleosides and hybridization with complementary sequences (RNA, DNA). These investigations prove that the conformation of a nucleoside may be clearly different when evaluated as monomer or when analysed after incorporation in an oligomer. This example, likewise, allows us to demonstrate a possible influence of conformational stress of a nucleoside on duplex stability. Indeed, more energy is required for chair inversion of the pyrimidine nucleosides compared to chair inversion of the purine nucleosides. As chair inversion is a prerequisite for duplex formation of CNA oligomers, pyrimidine rich CNA containing duplexes may be expected to be less stable than purine rich CNA associations. Concerning mixed AT sequences, the CNA oligomer clearly prefers RNA as a complementary partner over DNA, as in the HNA series (Table 1). The CNA-RNA duplex formed likewise is more stable than the respective DNA-DNA or DNA-RNA counterparts, in analogy with HNA sequences.

Another striking property of HNA is its selectivity for pairing with RNA[1]. One of the reasons for the higher stability of HNA-RNA duplexes over HNA-DNA duplexes is the more efficient hydration of the minor groove of the former[3] complex and this stabilizing factor is of enthalpic origin. Indeed, when evaluating the minor groove hydration using molecular modelling, the 2'-hydroxyl group of the RNA strand is involved in well-defined hydrogen bonding interactions with a first hydration layer. This interaction is missing in the HNA-DNA duplex. This finding let us to hypothesize that a further increase in duplex stability might be obtained, when we succeed in increasing also the polarity of the hexitol-flank of the minor groove of the HNA-RNA duplex. In a first effort we synthesized and oligomerized D-mannitol nucleosides (Scheme 1)[8] However, the stability of duplexes formed between these oligomers (MNA) and natural nucleic acids proved to be very poor. Upon evaluation of the polarity of the solvent

TABLE 1: Melting point (°C) of HNA, MNA, ANA, CNA with their RNA or DNA complement as determined in 0.1 M NaCl.[a]

	complement: RNA	DNA
Sequence 6'(5')-AGG AGA-4'(3')		
HNA	45	31[b]
MNA	14[b]	-
ANA	49	39[b]
DNA	-	10[b]
Sequence 6'(5')- $A_2TATA_3T_4$-4'(3')		
CNA	42	30
DNA	22	27
RNA[c]	37[d]	15

[a] with data taken from references 6, 8 and 9; [b] determined at 1 M NaCl; [c] T replaced by U; [d] Tm of a dsRNA sixteen mer 5'-r($UA_2UA_2UAUA_3U_4$)-3':3'-r($AU_2AU_2AUAU_3A_4$)-5'.

TABLE 2: Melting point (°C) of a mixed RNA octamer sequence in complex with its HNA, ANA or RNA complement respectively, as determined in 0.1 M NaCl[9].

Sequence	RNA complement
HNA (6'-GCGTAGCG-4')	54.4
ANA (6'-GCGUAGCG-4')	59.6
RNA (5'-GCGUAGCG-3')	47.6

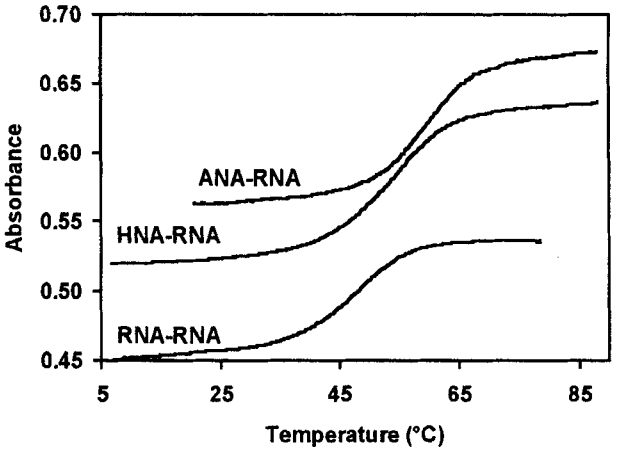


FIGURE 1: Melting curves (260 nm) of the duplexes mentioned in Table 2.

accessible surface of modelled MNA-RNA duplexes, no differences are observed with the polarity of the surface of the HNA-RNA duplex. The low stability of MNA-RNA duplexes may be attributed to a preorganization of single stranded MNA in a conformation different from typical A and B helical conformations of RNA or DNA. This preorganization is due to formation of an intramolecular hydrogen bond between the 3'-hydroxyl group of the D-mannitol sugar and the 6'-*O* in the phosphate backbone of the following nucleotide[8]. This research demonstrated that introduction of a new substituent into an oligomer may influence the conformation of the oligomer profoundly and, thus, also its hybridization properties. The previously mentioned hydrogen bond between 3'-OH and 6'-O of MNA cannot be formed when the configuration at the 3'-carbon atom is inverted. Therefore, we started synthesis of D-altritol nucleic acids (ANA) (Scheme 1). In case of a D-altritol sugar, the hydroxyl group in the 3'-position points into the direction of the minor groove and both flanks of the minor groove of ANA-RNA duplex might be well hydrated. As a result, an increase in thermal stability of this duplex (as compared with HNA-RNA) might be expected (although influence of the hydroxyl group on single strand preorganization cannot be excluded, yet). This was indeed borne out. D-altritol nucleic acids hybridize in a sequence-selective manner with RNA and DNA, with both complexes (ANA-RNA and ANA-DNA) being more stable than the respective HNA complexes[9] (Tables 1 and 2). Evaluating Table 2, and taking into account the stacking effect of an extra methyl group for the HNA sequence in comparison with the ANA sequence (replacement of T for U), an increase of almost 1 °C per extra hydroxyl group is noticed for the ANA sequence.

Together with hydrogen bonding and stacking interactions, efficient hydration of a nucleic acid duplex may be considered as an important enthalpic factor influencing hybridization in a favourable way.

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