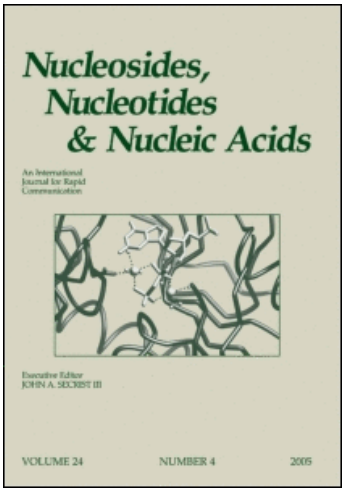

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A Single Carbocyclic Nucleotide Substitution in a 12mer DNA Gives a Hoogsteen Basepaired Duplex (Till 38°C) and a Hairpin (Till 65°C). A 600 MHz NMR Spectroscopic Study

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A SINGLE CARBOCYCLIC NUCLEOTIDE SUBSTITUTION IN A 12MER DNA GIVES A HOOGSTEEN BASEPAIRED DUPLEX (TILL 38°C) AND A HAIRPIN (TILL 65°C). A 600 MHZ NMR SPECTROSCOPIC STUDY.

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ABSTRACT: The impact of intramolecular stereoelectronic effects has been examined by comparison of the solution structures of natural oligo-DNA duplex, $5'(^1C^2G^3C^4G^5A^6A^7T^8T^9C^{10}G^{11}C^{12}G)_2{}^{3'}$, and its carbocyclic-nucleotide analogues in which the pentose sugar in 2'-dA residue is replaced with its carbocyclic counterpart (*i.e.* 2'-deoxyaristeromycin). Based on the NMR evidences, it has been shown, that 2'-deoxyaristeromycin analog exists in a dynamic equilibrium between the two forms of duplexes, one with W-C bp and the second with Hoogsteen bp in ca 1:1 ratio at lower temperature (below 35°C) and as hairpin at higher temperature (from ~40° - 60°C).

We have earlier shown that the interplay of stereoelectronic anomeric and gauche effects are energetically important to drive the sugar-phosphate backbone in nucleosides and nucleotides[1]. In the present work, we show the consequences of the absence of stereoelectronic effects in an oligonucleotide by incorporating a carbocyclic analog of adenosine (*i.e.* 2'-deoxyaristeromycin: **^{6}A**) into $5'(^1C^2G^3C^4G^5A^{\mathbf{6}}A^7T^8T^9C^{10}G^{11}C^{12}G)_2{}^{3'}$ duplex (II). We have therefore determined the solution structures of natural oligo-DNA, $5'(^1C^2G^3C^4G^5A^6A^7T^8T^9C^{10}G^{11}C^{12}G)_2{}^{3'}$ duplex (I), its aristeromycin incorporated analog, duplex (II), its C7' methyl carbocyclic analog (at **^{7}T**, **^{8}T**), duplex (III) and its C7'-hydroxy carbocyclic analog (at **^{5}A**, **^{6}A**, **^{7}T**, **^{8}T**), duplex (IV) to examine the importance of steric versus stereoelectronic effects in the carbocyclic-modified oligo-DNA vis-a-vis natural counterpart (Table 1).

TABLE 1. Comparison of the melting temperatures (T_m) of the natural 12mer and its modified analogs obtained by UV measurement with 1M NaCl + 0.2M phosphate, pH 7.0.

	Duplex	T_m
(I)	5'(^{1}C^{2}G^{3}C^{4}G^{5}A^{6}A^{7}T^{8}T^{9}C^{10}G^{11}C^{12}G)$_2$3'	67.6
(II)	5'(^{1}C^{2}G^{3}C^{4}G^{5}A^6**A**^{7}T^{8}T^{9}C^{10}G^{11}C^{12}G)$_2$3'	65.2[a]
(III)	5'(^{1}C^{2}G^{3}C^{4}G^{5}A^{6}A^7**T**^{8}**T**^{9}C^{10}G^{11}C^{12}G)$_2$3'	60.4[b]
(IV)	5'(^{1}C^{2}G^{3}C^{4}G^{5}A^6**A**^{7}**T**^{8}**T**^{9}C^{10}G^{11}C^{12}G)$_2$3'	65.3[c]

[a] -with single modification in the pentose sugar in ^{5}A residue (marked by bold font) is 2'-deoxyaristeromycin;[b] -with double modifications in duplex of ^{7}T and ^{8}T residues with C7' methyl carbocyclic analog of pentose sugar[3]; [c] - with four modifications in duplex of^{5}A, ^{6}A, ^{7}T and ^{8}T residues with C7' methyl carbocyclic analog of pentose sugar[3].

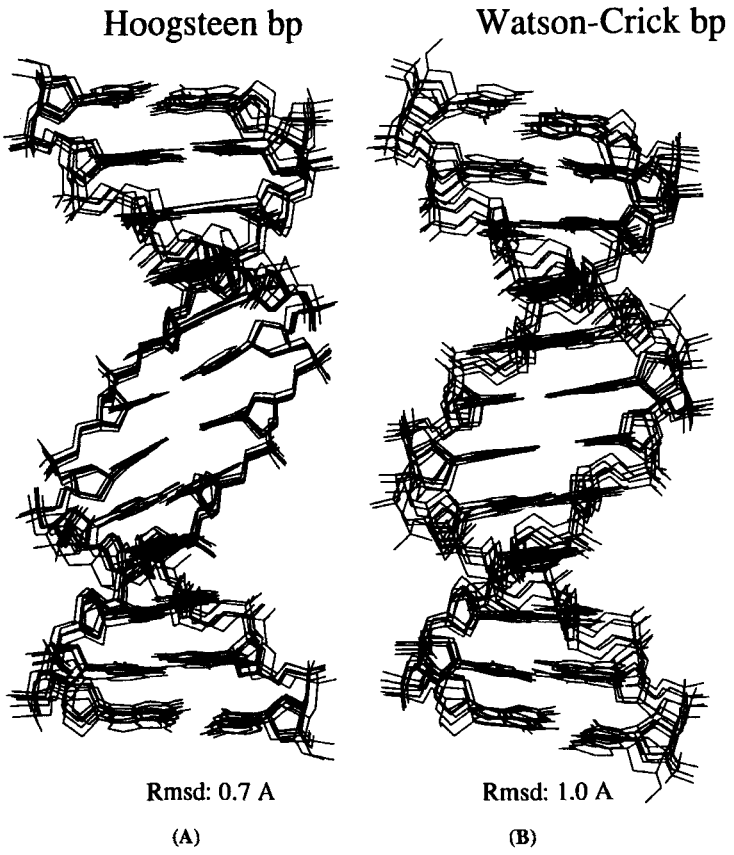

FIG. 1 Superposition of 6 structures with zero nOe and dihedral violations of the 2'-deoxyaristeromycin-modified 12mer, duplex (II) for Hoogsteen bp type duplex (A) and Watson-Crick bp type duplex (B)

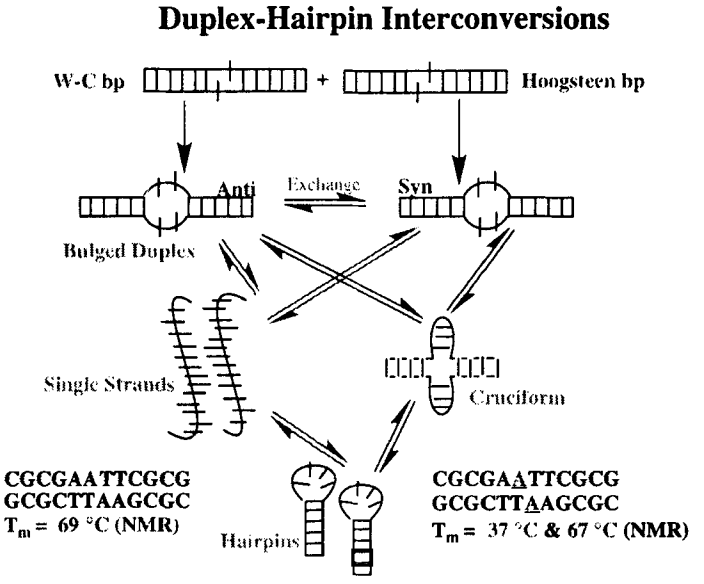

FIG. 2 Two mechanisms of Duplex-Hairpin Interconversions: (a) through single strands and (b) through cruciform

(A) Solution Structure of *Duplex (II)*

Based on the 600 MHz NMR data, it has been shown that below 35°C the 2'-deoxyaristeromycin analog of the natural 12mer oligo-DNA (II) exists in the dynamic equilibrium between the two forms of duplexes: one with standard W-C bp and the second with Hoogsteen A-T bp in ca 1:1 ratio (2 mM oligo in 100 mM NaCl and 10 mM phosphate) compared with the natural duplex (I) which exist entirely in a B-type DNA form[2]. The superposition of 6 structures with zero nOe and dihedral violation [XPLOR calculation based on 258 nOes/strand (22 nOes/residue) for W-C bp duplex and on 236 nOes/strand (20nOes/residue) for Hoogsteen A-T bp duplex and torsion constraints obtained from J-coupling constants] presented in Fig. 1. The rmsd for these 6 structures for W-C bp duplex is 1.0 Å and for Hoogsteen A-T bp duplex is 0.7 Å.

It is however noteworthy that at a higher temperature (from ~40° - 60°C) the 2'-deoxyaristeromycin analog (duplex II) exists as a hairpin compared to the natural counterpart in which no hairpin formation is found at an NMR concentration under our condition.

The mechanism of transition of aristeromycin-modified 12mer (duplex II) at different temperatures are presented in Fig. 2, showing the intermediacy of the cruciform structure below 37°C, which melts to hairpin giving subsequently the single strand structure above 60°C .

(B) Solution Structures of *Duplex (III) and Duplex (IV)*

The data obtained on the structure of duplex (II) is in strong contrast with the conformation of an identical oligo with C7'-methylcarbocyclic-T (duplex III) or C7'-hydroxycarbocyclic-A/T (duplex (IV) analog which can successfully take up the B-DNA form as the natural counterpart (I), and their rmsd is less than 1.0Å[2].

The above finding highlights on three different intrinsic structural properties of natural nucleoside in a native oligo-DNA that make it unique compared to 2'-deoxyaristeromycin-modified 12mer analogue: the Anomeric effect, the Gauche effect and the ability of the aglycone to take up restricted orientation across the glycosidic bond due to O4' in the pentose (compared to 2'-deoxyaristeromycin in which the glycosidic bond can act as a free propeller because of substitution of O4' with 6'-CH_2, which also makes it deprive of the ubiquitous anomeric and gauche effects).

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