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# 2′-Deoxy-8-(propyn-1-yl)adenosine-Containing Oligonucleotides: Effects on Stability of Duplex and Quadruplex Structures

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**Abstract**—-2′-Deoxy-8-(propyn-1-yl)adenosine has been incorporated in synthetic oligodeoxyribonucleotides and its influence on thermal stability of duplex and quadruplex structures investigated by UV, CD and $^1$H NMR. The obtained results seem to indicate that the presence of the modified base negatively affects the stability of double stranded DNA whereas remarkably increases the stability of parallel quadruplex structures. 

## Introduction

There is a growing interest in synthetic modified oligonucleotides containing nucleoside-analogues. The incorporation of modified bases into oligonucleotides may indeed produce useful changes in physical and biological properties of the resulting DNA fragments, which can be used as effective tools for investigation of nucleic acid structures as well as protein–nucleic acid interaction. As far as the former research theme is concerned, non-canonical DNA structures (hairpins, three- and four-way junctions, triplexes, etc.)[1,2] are currently the subject of extensive investigations because of their important biological implications. Particularly, quadruplex structures are an attractive topic of a large number of researches ranging from chemistry to molecular biology and pharmacology, since they can be present in the single-stranded G-rich telomere sequence[3,4] and it has been demonstrated that G-quadruplex formation inhibits initiation of telomerase activity in *Oxytricha*.[5] Furthermore, a number of quadruplex forming oligonucleotides have resulted to be potent inhibitors of thrombin[6] as well as HIV-1 integrase,[7] the enzyme responsible for the insertion of viral DNA into the host genome.

It is well known that G-rich oligonucleotides may adopt a quadruplex structure at physiological concentration of monovalent ions forming stacked G-tetrads. Several types of quadruplex structures with G-tetrads have been reported: parallel strands, alternating antiparallel strands etc.[8] Each of these structures is characterized by particular conformational requirements for G-tetrads; the four parallel-stranded quadruplex demands all dG in an *anti* conformation, whereas in antiparallel quadruplex structures a *syn* conformation for deoxyguanosine in given positions is required.[8] For example, 15-mer 5′-dGGTTGGTGTGGTTGG-3′ (a thrombin activity inhibitor) adopts a chairlike structure, with alternating antiparallel strands and a *syn* conformation for dG1, dG5, dG10 and dG14.[9–11] As a matter of fact, it has already been shown that the introduction of several substituents in 8 position of dG1, dG5, dG10 and dG14 determines an increased activity, probably due to the stabilizing effect on *syn* conformation of dG.[12]

Recently, several non-G-tetrads[13,14] have been described as well. Particularly the presence of an A-tetrad[14] (with all dA in a *syn* conformation)(Fig. 1) in parallel stranded quadruplex 5′-d(AGGGT)$_4$-3′ (**I**) have been evidenced. However, authors were not able to observe A-tetrads in the parallel stranded quadruplex 5′-d(TAGGGT)$_4$-3′ (**II**), probably due to the lack of H bond between 5′-OH and N-3 of A2 promoting *syn* conformation.

In this frame we have synthesized 2′-deoxy-8-(propyn-1-yl)-adenosine-containing oligonucleotides (dA$^{pr}$-ODNs) and investigated their behavior in formation and stability of both duplex and quadruplex structures by UV melting, CD and $^1$H NMR spectra measurements.

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## Chemistry

The key step in the synthesis of dA[pr]-ODNs is the preparation of the suitably protected dA[pr] phosphoramidite monomer to allow its site-specific incorporation into DNA oligomers. The synthesis of the fully protected dA[pr] monomer was carried out through the reactions outlined in Scheme 1.

2′-Deoxyadenosine (**1**) was brominated by bromine in $CH_3COOH/CH_3COONa$ aqueous buffer according to Eason et al.[15] affording 8-bromo-2′-deoxyadenosine (**2**). Compound **2** was coupled with propyne following the De Clercq procedure[16] with minor modifications [in particular $Pd^0(PPh_3)_4$ was used instead of $(PPh_3)_2PdCl_2$], to give 2′-deoxy-8-(propyn-1-yl)adenosine (**3**). The following step consists in the protection of the exocyclic amino-group. We adopted the one-pot procedure known as transient protection, developed by Gait,[17] that provides: **i**) temporary protection of 3′- and 5′-hydroxyl groups by trimethylchlorosilane in dry pyridine; **ii**) protection of the exocyclic amino-group by benzoyl chloride in dry pyridine; **iii**) removal of the 3′- and 5′-OH protecting groups by aqueous ammonia. The so-obtained *N*-benzoyl-2′-deoxy-8-(propyn-1-yl)adenosine (**4**) was treated with 5,5′-dimethoxytritylchloride in dry pyridine[18] for the final protection of the 5′-OH group and the derived compound (**5**) was, in turn, transformed into the corresponding phosphoramidite monomer (**6**) by 2-cyano-ethyl-*N*,*N*-diisopropyl chlorophosphoramidite.[19] Compound **6** was used for the preparation of dA[pr]-ODNs following the usual protocols. During the automated syntheses, **6** exhibited similar coupling efficiencies as the common, commercially available phosphoramidite derivatives.

## Results and Discussion

### Duplex stability of 5′-d(CGCGAA[pr]TTCGCG)$_2$-3′ (III)

The effect of the insertion of dA[pr] was checked by comparison of the well-studied Dickerson dodecamer

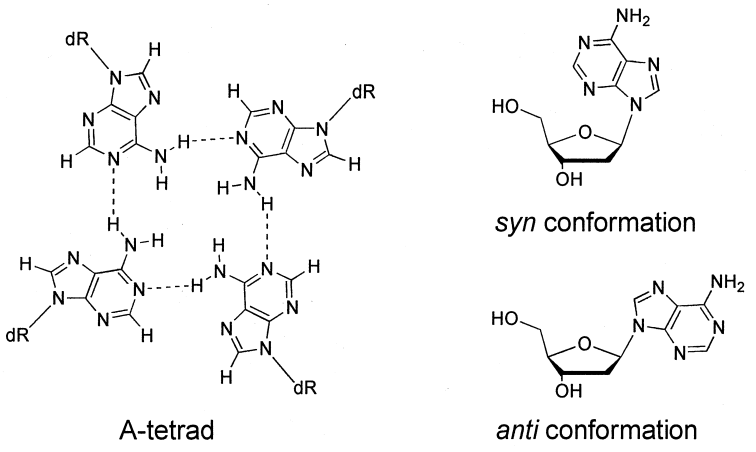
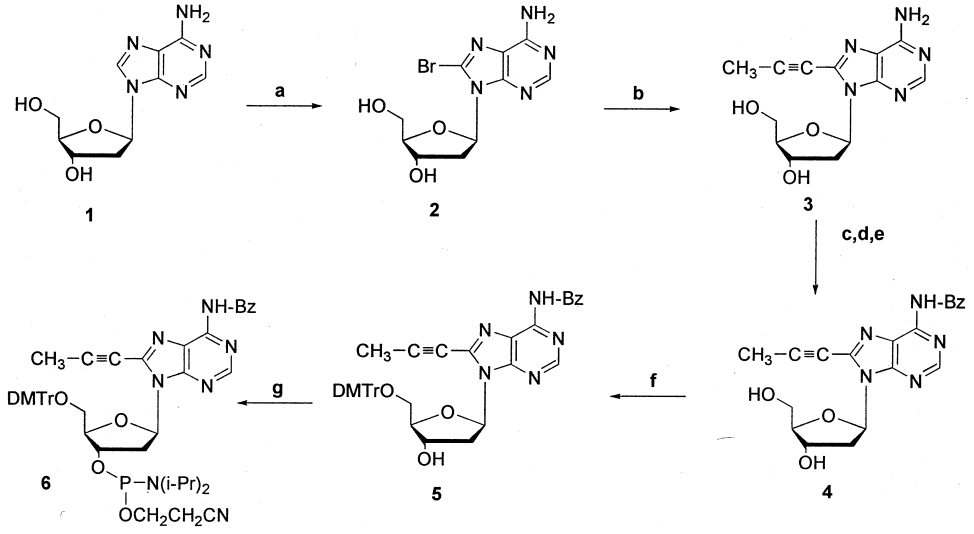



**Figure 1.** Scheme of an A-tetrad and *syn* and *anti* conformations. The H-bonds are indicated by dotted lines.




**Scheme 1.** Synthesis of fosforoamidite derivative **6**. (a) $CH_3COOH/CH_3COONa$ pH 4.0; $Br_2$: 12 h; room temp; (b) triethylamine, CuI, $PD^0(PPh_3)_4$], propyne gas in dry deoxygenated DMF at 50 °C, 6 h; (c) $(CH_3)_3SiCl$ in dry Py, 0 °C 30 min; (d) BzCl, 30 °C, 2 h; (e) $NH_4OH$, 30 min; (f) 4,4′-dimetoxytrytil chloride, triethylamine, 4-(dimethylamino)pyrridine in dry Py, 6 h room temp; (g) *N*,*N*-diisopropylethylamine, 2-cyanoethyl-*N*,*N*-diisopropyl chlorophosphoramidite in dry $CH_2Cl_2$, room temp. 80 min.

5′-d(CGCGAATTCGCG)$_2$-3′[20] versus the modified counterpart 5′-d(CGCGAA$^{pr}$TTCGCG)$_2$-3′ (**III**). Table 1 lists the NMR chemical shifts of non-exchangeable protons of **III** obtained by standard methods.[21] NMR properties of **III** suggested that this oligonucleotide adopts B-DNA-like global folding, although minor local deviation may exist, especially in proximity of the modified nucleotide. The CD spectrum of **III** confirms the B-DNA-like structure (data not shown).

In Fig. 2 (right) the immino-proton resonances of **III**, taken at several temperatures are shown (assignments were obtained by standard methods[21]). At 300 K five resonances occur between 12.5 and 14.0 ppm, suggesting that all the base residues are involved in hydrogen bonding with exception of the frying end bases. At 310 K a reduction of the intensity of signals at 13.45 ppm (T7N1H of A$^{pr}$6:T7 base pair) and at 13.30 ppm (G2N1H of G2:C11 base pair) is evident. At 315 K the resonance at 13.45 ppm is broader and just visible showing an increased exchange rate with the solvent due to the A$^{pr}$:T base pair opening. At 320 K the signal at 13.20 ppm also disappears indicating a further advance of the melting process.

In Fig. 2 (left) the same experiment for the Dickerson dodecamer is shown. A different pattern of melting is evident. At 300 K five resonances of the internal base pairs are present. At 310 K the signal at 13.50 ppm (G2N1H of G2:C11 base pair) shows a lower intensity and completely disappears at 320 K. At 325 K the residue signals are broadened and reduced in intensity. Finally, at 330 K the melting process is complete.

**Table 1.** $^1$H NMR chemical shift values (ppm) of 5′-d(CGCGA A$^{pr}$TTCGCG)$_2$-3′ (**III**)

| | H8/H6 | H2/H5/CH$_3$ | H1′ | H2′ | H2″ |
|---|---|---|---|---|---|
| C1 | 7.62 | 5.89 | 5.73 | 1.94 | 2.38 |
| G2 | 7.93 | | 5.85 | 2.61 | 2.66 |
| C3 | 7.26 | 5.34 | 5.67 | 1.82 | 2.23 |
| G4 | 7.84 | | 5.28 | 2.61 | 2.69 |
| A5 | 8.05 | | 6.22 | 2.62 | — |
| A$^{pr}$6 | — | /2.22 | 5.92 | 2.87 | 2.88 |
| T7 | 7.13 | 1.36 | 5.86 | 1.94 | 1.94 |
| T8 | 7.33 | 1.46 | 6.08 | 2.09 | 2.09 |
| C9 | 7.49 | 5.63 | 5.55 | 2.12 | 2.12 |
| G10 | 7.89 | | 5.83 | 2.39 | 2.39 |
| C11 | 7.31 | 5.40 | 5.72 | 1.85 | 2.29 |
| G12 | 7.92 | | 6.12 | 2.33 | 2.59 |

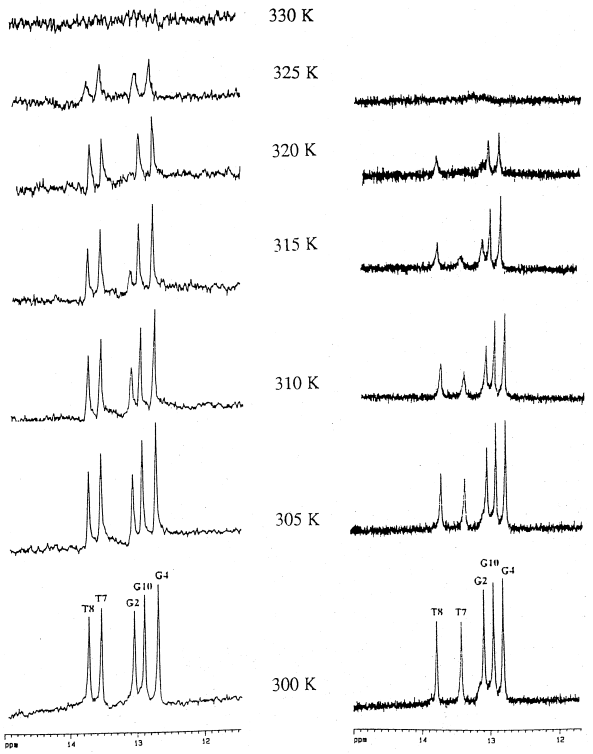



**Figure 2.** Comparison of imino region of $^1$H NMR spectra at different temperatures of **III** (right) and Dickerson dodecamer (left).

Thermal NMR experiments clearly point out to a minor stability of the A$^{pr}$:T base pair when compared to a canonical A:T base pair. This could be tentatively explained by the steric hindrance between the bulky C8-substituent and the sugar moiety which forces, as observed in similar derivatives,[15] the nucleoside to adopt a *syn* glycosidic conformation in contrast with the *anti* conformation of normal nucleoside residues in B-DNA duplex.

By the way, the reduced stability of A$^{pr}$:T base pair might also result in a local variation of DNA flexibility thus pointing to dA$^{pr}$-ODNs as tools for studying DNA/DNA-bending protein interactions. In fact, the target selection by DNA-bending proteins depends on local, sequence dependent variation in DNA flexure, as indicated by recent studies on 5-hydroxymethyl-2′-deoxy-uridine containing DNA fragments.[22–24]

## Quadruplex stability of 5′-d(A$^{pr}$GGGT)$_4$-3′ (IV) and 5′-d(TA$^{pr}$GGGT)$_4$-3′ (V)

A preliminary study of the effect of dA$^{pr}$ in quadruplex structures was carried out by UV melting profiles. The melting temperature of quadruplex structures can be estimated following the thermal denaturation at the λ

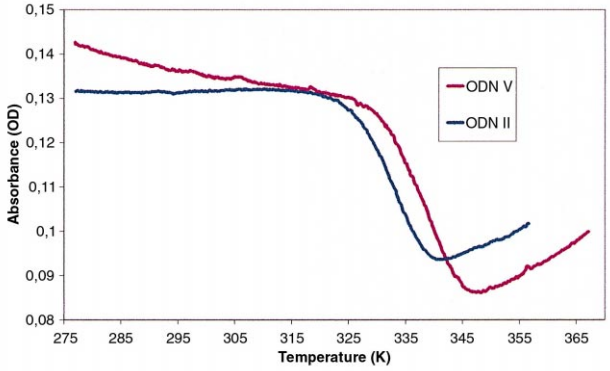



**Figure 3.** Melting UV profiles at pH 7.0 for the ODNs **II** at 295 nm (blue) and **V** at 315 nm (red).

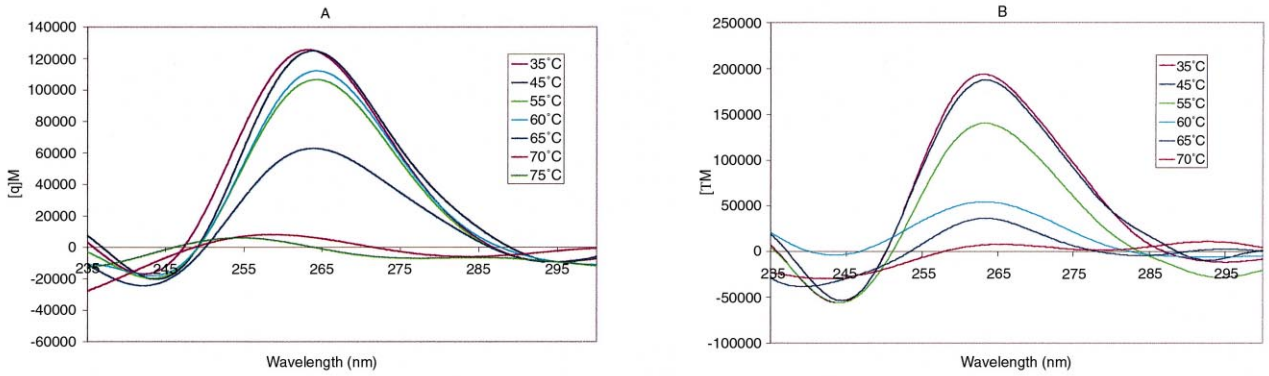



**Figure 4.** CD experiments at several temperatures of the modified 6-mer **V** (A) and the 6-mer **II** (B).

value corresponding to the maximum hyperchromism effect upon G-quartet formation. This can be determined from the difference spectrum of each oligonucleotide at 4 °C and 90 °C.[25,26] As previously noted, natural ODNs show an isosbestic point around 280 nm, and the λ value of the maximum hyperchromism effect upon G-quartet formation is found to be 295 nm. Modified ODNs behave in a different fashion: the 6-mer **V** is characterized by a bathochromic shift of the isosbestic point to 305 nm, while the UV profiles of the modified 5-mer **IV** didn't show any isosbestic point. Consequently, the melting profile of **V** (Fig. 3) was recorded at 315 nm, giving a well-shaped sigmoid curve, whereas no suitable melting curve could be obtained for the 5-mer **IV** at any λ value.

Further information on the structure was obtained recording a series of CD experiments at variable temperature (Fig. 4). At room temperature, **IV** and **V** showed a positive Cotton effect at 263 nm and a less strong negative effect at 246 nm. These spectra were similar to those described for parallel-stranded guanine quadruplexes, such as the CD spectra of the sequences $dT_4G_4$, $dT_4G_4T$ etc.[27]

The molar ellipticity at 264 nm for the different samples has been indicated as a measure of quadruplex formation[28] and could be used for monitoring transition of an ODN from a quadruplex to a single strand. Single strand CD spectra generally present a null point at this wavelength and a positive band at 250 nm ca. This means that the contribution to the band at 264 nm is only due to a quadruplex structure.

**Table 2.** Melting temperature of quadruplex forming ODNs

| ODN | Tm (K)[a] |
|---|---|
| 5′d$(AGGGT)_4$-3′ (I) | 328.4 |
| 5′d$(TAGGGT)_4$-3′ (II) | 333.5 |
| 5′d$(A^{pr}GGGT)_4$-3′ (IV) | 332.3* |
| 5′d$(TA^{pr}GGGT)_4$-3′ (V) | 339.6 |

[a]Obtained by UV melting experiments, except (*) obtained by CD measurements.

The results are summarized in Table 2. Analysis of melting temperature (Tm), obtained by UV and CD measurement, proves that in the 5-mer, as well as in the 6-mer the introduction of 2′-deoxy-8-(propyn-1-yl)adenosine in quadruplex forming oligonucleotides, strongly enhances the stability of the structure in both cases. A more detailed structural investigation is in order to clarify the molecular bases of the observed increase in stability and particularly to establish if, in the case of the 6-mer, this is due to the presence of a non-5′-end A-tetrad.

## Conclusions

The above data indicate that the modified base $A^{pr}$ if, on the one hand, destabilizes B-DNA duplexes, on the other hand may increase the stability of particular quadruplexes, most likely assuming a prevalent glycosidic *syn* conformation. This observation indicates that the substitution of natural bases with modified ones might improve the performance of synthetic ODNs as potential drugs since biological activity seems to be directly correlated to stability in quadruplex aptamers.[12,29]

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