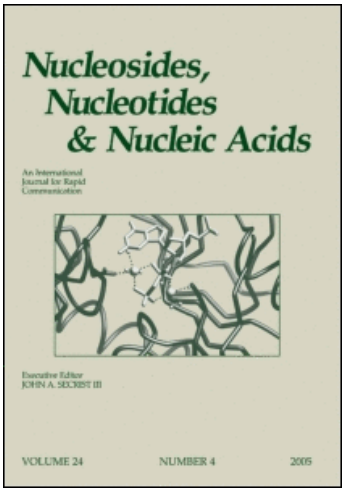

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SYNTHESIS AND STRUCTURAL STUDY OF QUADRUPLEX STRUCTURES CONTAINING 2′-DEOXY-8-METHYLADENOSINE

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▫ *The synthesis and preliminary structural studies of ODNs $A^{8Me}GGGT$ and $TA^{8Me}GGGT$, where A^{8Me} represents 2′-deoxy-8-methyladenosine, are reported.*

Keywords Oligonucleotide, Quadruplex, 2′-Deoxy-8-Methyladenosine

INTRODUCTION

G-quadruplex structures are characterized by stacked tetrads in which four guanines are arranged in a square-planar array with each of them serving as both hydrogen bond acceptor and donor in a reverse Hoogsteen base pair.[1] G-quadruplexes show a surprising structural variability. In the last decade, the number of sequences able to form quadruplexes has been further increased by the discovery of other types of tetrads such A-tetrad,[2–4] T-tetrad,[5] and C-tetrad.[6] Particularly, A-tetrads are present in parallel quadruplex structures formed by the oligonucleotides AGGGT,[2] TTAGGGT,[3] and TGGAGGC,[4] in which both A *syn*- and A *anti*-tetrads have been found. In view of the above considerations, we have undertaken the synthesis of 2′-deoxy-8-methyladenosine containing ODNs (A^{8Me}-ODNs) potentially able to form stable quadruplex structures in order to find a possible connection among relative strand orientation, glycosidic conformation in A-tetrads and thermal stability.

The synthetic strategy of the fully protected A^{8Me} monomer to be used for A^{8Me}-ODNs assembly is outlined in Scheme 1. 2′-Deoxyadenosine (**1**) was brominated by treatment with bromine in CH_3COOH/CH_3COONa aqueous

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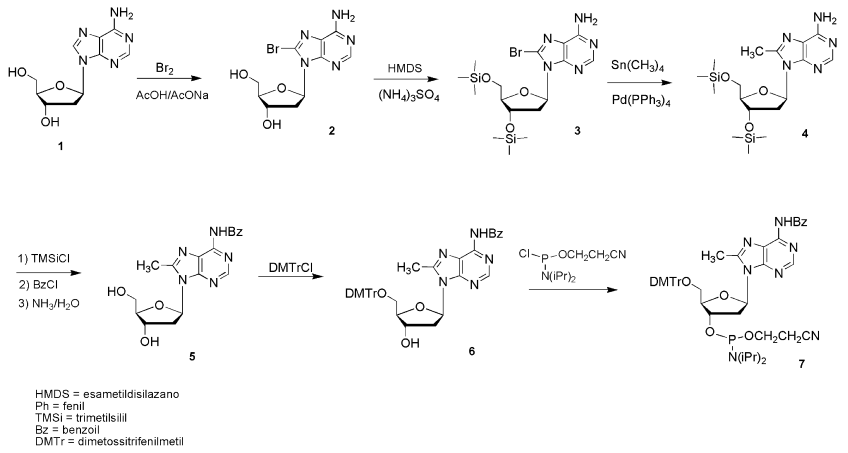

SCHEME 1 (a) Br_2 in acetate buffer; (b) hexamethyldisilazane, $(NH_4)_2SO_4$ in dry dioxane; (c) $Pd(PPh)_4$, $(CH_3)_4Sn$ in *N*-methylpirrolidinone; (d) trimethylsilyl chloride in dry pyridine; (e) benzoyl chloride in dry pyridine; (f) H_2O/NH_3; (g) dimethoxytriphenylmethyl chloride in dry pyridine; (h) 2-cyanoethyl-N,N-diisopropyl chlorophosphoramidite in dry CH_2Cl_2.

buffer,[7] thus affording 8-bromo-2′-deoxyadenosine (**2**). Compound **2** was protected at 3′ and 5′ hydroxyl functions by HMDS to yield **3,** and subsequently transformed into the intermediate **4**.[8] Since a partial detachment of protecting groups at 3′ and 5′ functions occurred during the chromatographic purification of **4**, the successive reaction was the well-known transient protection[9] carried out on the partial deprotected intermediates collected from the column. The so obtained N-benzoyl-2′-deoxy-8-methyladenosine (**5**) was treated with 5,5′-dimethoxytritylchloride in dry pyridine for the final protection of the 5′-OH group[9] and the derived compound (**6**) was, in turn, transformed into the corresponding phosphoramidite monomer (**7**) by 2-cyanoethyl-N,N-diisopropyl chlorophosphoramidite[10] and used for the preparation of $A^{8Me}GGGT$ (**I**), $TA^{8Me}GGGT$ (**II**).

The ^{1}H-NMR spectra of **I** and **II** (Figures 1 and 2, respectively) are relatively simple (some weaker resonances attributable to minor forms are present). For **I**, resonances corresponding to three G-H8 and to T-H6 and A^{8Me}-H2 protons in the aromatic region and two methyl resonances around 1.6 ppm for T-CH_3 and 2.3 ppm for A^{8Me}-CH_3 are observed. Analogously, in the case of proton spectrum of **II**, there are only six signals in the aromatic region, whereas, as expected, three methyl resonances are present in the region between 1.5 and 2.5 ppm, where the chemical shift of the methyl group of A^{8Me} is *ca.* 0.7 ppm further downfield shifted than in thymine. Since resonances from only one strand are observed for both molecules, should the ODNs be structured in multistranded complexes, these must be symmetric. Furthermore, proton NMR spectra of both **I** and **II** in K^+ containing aqueous solution at a temperature of 30°C also show three signals in the region 10.5–12 ppm attributable to three exchangeable guanine N1 imino protons. Thus, the whole of data suggest that the guanines from each strand form three G-tetrads

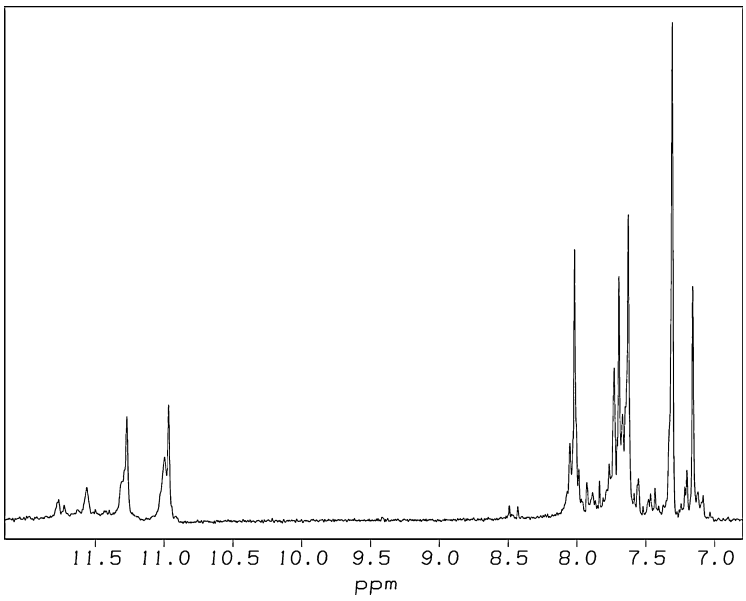

FIGURE 1 ^{1}H NMR spectrum of **I**.

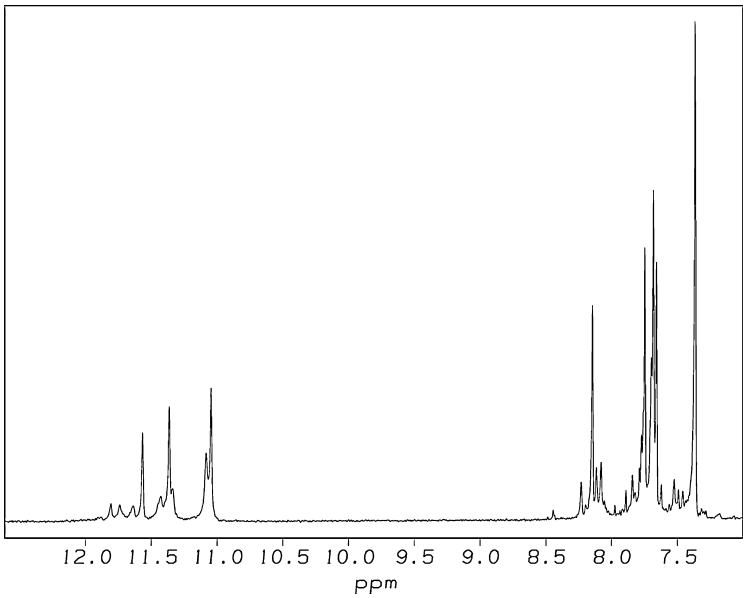

FIGURE 2 ^{1}H NMR spectrum of **II**.

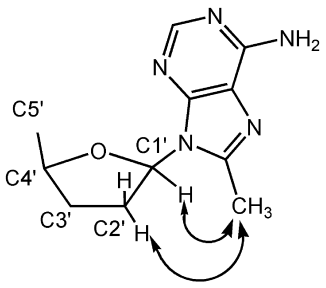

FIGURE 3 Syn glicosidic conformation in A^{8Me} residues. Arrows indicate observed Noe.

and the observed number of resonances is consistent with symmetrical four-stranded quadruplexes with all strands equivalent to each other. Circular dichroism data further confirm the formation of parallel quadruplexes. In fact, the presence of a maximum and minimum Cotton effects at 262–264 nm and 243 nm, respectively, are typical of quadruplexes involving four parallel strands.[11] NOESY and TOCSY spectra, obtained at 500 MHz (T = 30°C) for both **I** and **II**, showed well-dispersed cross-peaks, so both exchangeable and non-exchangeable protons could be nearly completely assigned following the standard procedures.[12,13] The observed NOEs among G H8/T H6 and their own H1′, H2′, and H2″ sugar protons and the H1′, H2′, and H2″ protons of the preceding residue suggested that both quadruplexes adopt a right-handed helical winding. As for the glycosidic torsion angles, useful information could be obtained comparing the relative intensities of NOEs between H8/H6 and H2′ and H8/H6 and H1′ protons of the same residue. All Gs and Ts resulted to possess an *anti* glycosidic conformation, while the modified adenosines (A^{8Me})

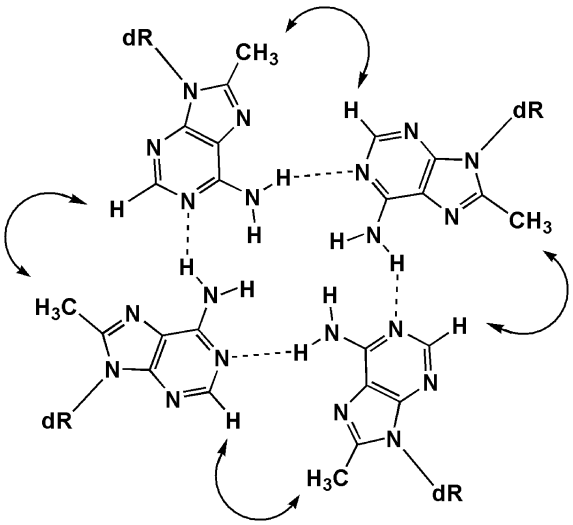

FIGURE 4 N61 H-bond patterns for an A-tetrad. H-bonds are indicated by dashed lines and observed Noe are indicated by arrows.

adopt a *syn* conformation, showing intense NOEs between methyl group in 8 position and H1′ sugar proton and more weak cross-peaks between methyl and H2′ (Figure 3). Furthermore, NMR data of both **I** and **II** show interstrand NOEs between the methyl group of an A^{8Me} residue and the H2 proton of the modified base on the adjacent strand (Figure 4), and intrastrand NOEs between A^{8Me} protons nucleotides and the protons of the adjacent Gs on the same strand suggest that A^{8Me} residues are not randomly oriented and are in mutual close proximity arranging in a symmetrical fashion and stacking on the top of G-tetrads in both $[d(A^{8Me}GGGT)]_4$ and $[d(TA^{8Me}GGGT)]_4$. In order to estimate the effects of the substitution of a regular A residue with an A^{8Me} one on the thermal stability of the resulting quadruplex, structures were further analyzed by CD thermal denaturation experiments. The melting temperatures of **I** (61°C), **II** (68°C), and their natural counterparts (63°C and 67°C, respectively) recorded at 264 nm show that thermal stability of both **I** and **II** are quite similar to that observed for the reference structures, thus suggesting that the incorporation of a methyl group does not affect the quadruplex stability.

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