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A BUNCH-OLIGONUCLEOTIDE FORMING STABLE MONOMOLECULAR QUADRUPLEX CONTAINING A T-TETRAD

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□ *The chemical synthesis of bunch-ODN I and II prone to form quadruplex structures containing G- and T-tetrads has been reported. Structural studies were performed by ¹H-NMR and CD melting experiments.*

Keywords DNA, T-Tetrad, Oligonucleotide, Quadruplex, Bunch-ODN

INTRODUCTION

Guanine rich sequences in DNA can form four stranded quadruplex structures stabilized by G-tetrads. Quadruple helices based on G-tetrads may play important roles in a number of biological processes, such as the regulation of the apoptotic event of the cells and several human diseases.^[1–6] Extensive NMR, X-ray, and CD structural studies are centered on oligodeoxyribonucleotides (ODN) models forming G-quadruplex structures aiming at characterizing all the possible conformational varieties that are sequence and molecularity dependent. Quadruplex structures having in the tetrads other bases than G have been recently discovered. Particularly, the tetrads G:C:G:C,^[7] G:C:A:T,^[8] and T:T:T:T^[9] have been identified and studied by ¹H NMR and molecular dynamics. However, the low stability of these tetrads deeply limits the conformational investigation of the quadruplex in which they are contained. Recently, we proposed a new class of ODNs called bunch-ODNs, which are capable of forming very stable monomolecular quadruplexes that show pseudo-symmetry and their ¹H NMR profiles are similar to those of tetramolecular

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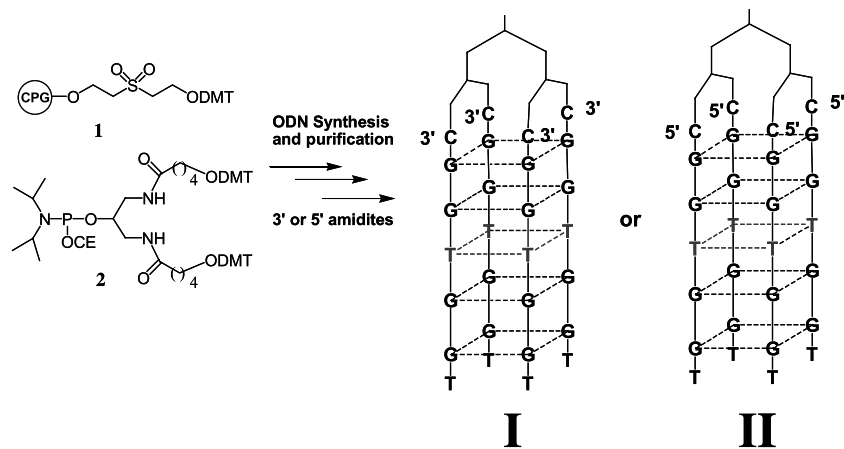


FIGURE 1 Synthetic approach scheme for **I** and **II**.

counterparts and are characterized by more favorable thermodynamic parameters.^[10] Therefore, a bunch-quadruplex model could be an useful entry to study ODN quadruplex with less stable tetrads. We report here the synthesis and the preliminary ¹H NMR and CD structural studies on the bunch-[d(CGGTGGT)]₄ quadruplex containing a T-tetrad. To assess the effect of ODN's polarity on quadruplex folding and stability, we synthesized the bunch-[d(CGGTGGT)]₄ **I** and **II** using 3' or 5'-phosphoramidite nucleotide monomers, respectively (Figure 1).

The synthesis has been performed using the recently reported solid-phase procedure that uses the CPG support (**1**) and the branching bifunctional linker (**2**) to prepare the bunch-spacer linking the ODN strands.^[10]

In order to evaluate whether **I** and **II** adopt T-tetrad containing quadruplex structures and, in case, to estimate their stabilities, we performed ¹H NMR studies at 25, 45, 65, and 80°C using pulse field gradient WATERGATE sequence for H₂O

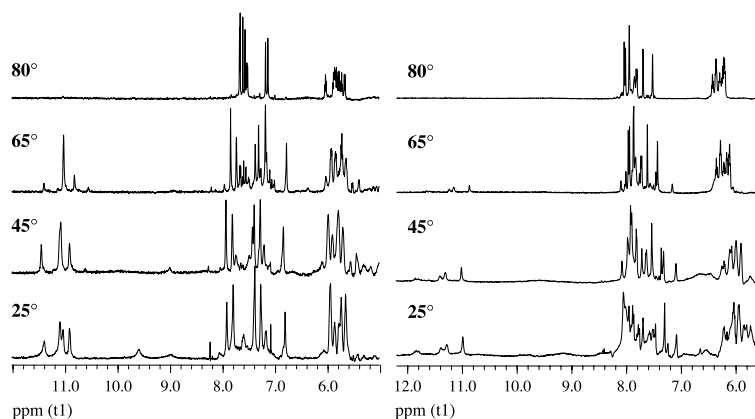


FIGURE 2 ¹H NMR spectra of **I** and **II** in 100 mM K⁺ buffer (500 MHz, H₂O/D₂O 9:1, v/v).

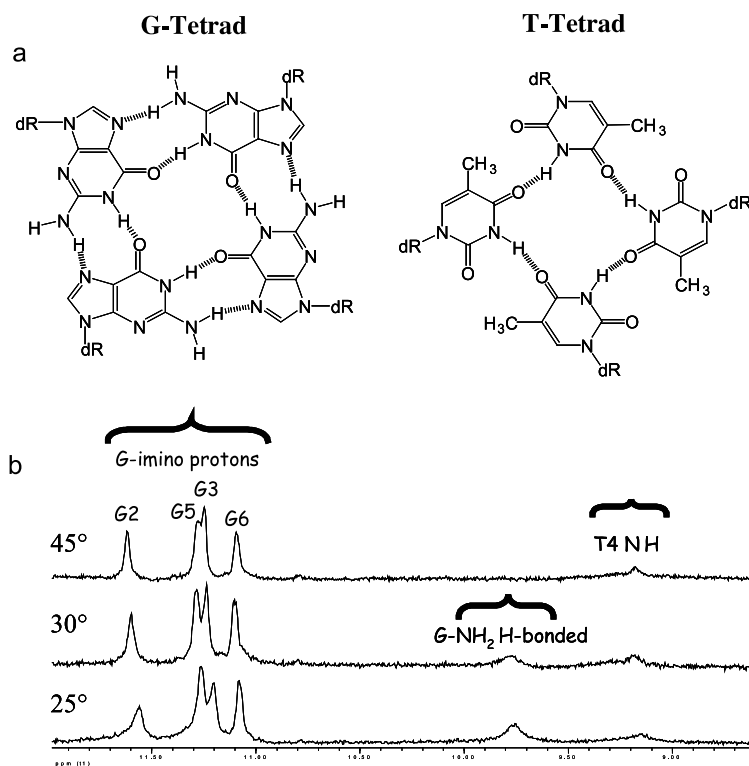


FIGURE 3 a) Scheme of G and T tetrads; b) low field region of ¹H NMR spectra of I in 100 mM K⁺ buffer.

suppression. Stacked-plot spectra of **I** and **II** show that both adopt quadruplex structures in presence of K⁺ ions with marked differences concerning thermal stability and T-tetrad observation (Figure 2).

The quenching of imino and amino proton signals revealed that **I** is more stable than its 5'-3' counterpart (**II**) at each tested temperature. Furthermore, in **II** neither the T-tetrad characteristic signal (T₄NH) nor any G-NH₂ exchangeable protons (Figure 3) were observed. These data are in agreement with CD melting profiles that showed a well-defined sigmoidal curve with a derivatizable T_m value of 72°C for complex **II**, while for complex **I** no well-defined change in convexity was observed up to 90°C.

REFERENCES

1. Shafer, R.H.; Smirnov, I. Biological aspects of DNA/RNA quadruplexes. *Biopolymers* **2001**, *56*(3), 209–227.
2. Arthanari, H.; Bolton, P.H. Functional and dysfunctional roles of quadruplex DNA in cells. *Chem. Biol.* **2001**, *8*(3), 221–230.
3. Jing, N.; Marchand, C.; Guan, Y.; Liu, J.; Pallansch, L.; Lackman-Smith, C.; De Clercq, E.; Pommier, Y. Structure-activity of inhibition of HIV-1 integrase and virus replication by G-quartet oligonucleotides. *DNA Cell Biol.* **2001**, *20*, 499–508.

4. Smirnov, I.; Shafer, R.H. Effect of loop sequence and size on DNA aptamer stability. *Biochemistry* **2000**, *39*(6), 1462–1468.
5. Pileur, F.; Andreola, M.-L.; Dausse, E.; Michel, J.; Moreau, S.; Yamada, H.; Gaidamakov, S.A.; Crouch, R.J.; Toulmé, J.-J.; Cazenave, C. Selective inhibitory DNA aptamers of the human RNase H1. *Nucleic Acids Res.* **2003**, *31*(19), 5776–5788.
6. Chinnappen, D.J.F.; Sen, D. Hemin-stimulated docking of cytochrome c to a hemin-DNA aptamer complex. *Biochemistry* **2002**, *41*, 5202–5212.
7. Davis, J.T. G-quartets 40 years later: from 5-GMP to molecular biology and supramolecular chemistry. *Angew. Chem., Int. Ed.* **2004**, *43*(6), 668–698.
8. Escaja, N.; Gelpi, J.L.; Orozco, M.; Rico, M.; Pedroso, E.; Gonzalez, C.J. Four-stranded DNA structure stabilized by a novel G:C:A:T Tetrad. *J. Am. Chem. Soc.* **2003**, *125*(19), 5654–5662.
9. Patel, P.K.; Hosur, R.V. NMR observation of T-tetrads in a parallel stranded DNA quadruplex formed by *Saccharomyces cerevisiae* telomere repeats. *Nucleic Acids Res.* **1999**, *27*(12), 2457–2464.
10. Oliviero, G.; Borbone, N.; Galeone, A.; Varra, M.; Piccialli, G.; Mayol, L. Synthesis and characterization of a bunched oligonucleotide forming a monomolecular parallel quadruplex structure in solution. *Tetrahedron Lett.* **2004**, *45*, 4869–4872.