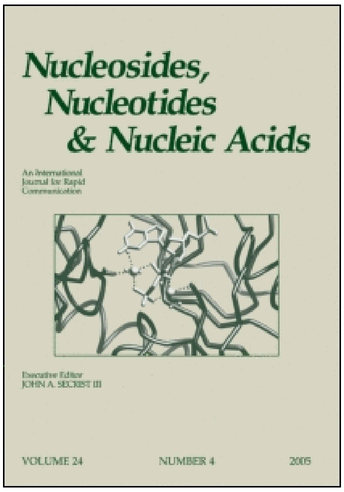

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SYNTHESIS AND CHARACTERIZATION OF TETRA-END LINKED OLIGONUCLEOTIDES CAPABLE OF FORMING MONOMOLECULAR G-QUADRUPLEXES

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□ *The chemical synthesis of two new G-rich Tetra-End-Linked-oligodeoxyribonucleotides (TEL-ODNs) as well as ^{1}H-NMR and CD spectra of the corresponding monomolecular quadruplexes (***IVa*** and* **IVb***) has been reported. The new TEL-ODNs, characterized by the presence of short branches in the linker moiety, could be very useful for the achievement of monomolecular quadruplexes with predetermined strand orientation.*

Keywords TEL-ODN; G-quadruplex; NMR

INTRODUCTION

DNA quadruple helices (quadruplexes) based on guanine (G) tetrads (Figure 1a) have recently emerged as biologically important structures.[1] G-rich sequences, which are found primarily in telomeric region at the 3′-end of chromosomes, are able to fold into G-quadruplex structures. It has been suggested that quadruplex formation could inhibit the telomere maintenance provided by telomerase activity, thus affecting the lifespan of the cells of a number of cancer types characterized by a high level of telomerase expression.[2] Furthermore, G-quadruplex structures are present in the scaffold of several aptamers provided with useful biological properties.[3] G quadruplexes can be classified on the basis of the number and the polarity of the strands (Figure 1b–d), as well as for loop composition and conformation. Several studies have investigated the effect of loops on quadruplex stability and typology.[4] We recently proposed the synthesis of a new class of modified tetra-end-linked G-rich ODNs (TEL-ODNs) capable of forming

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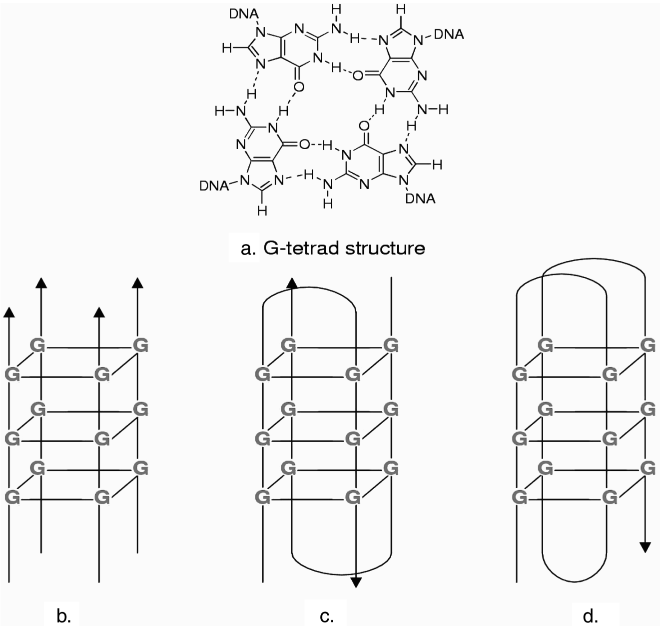
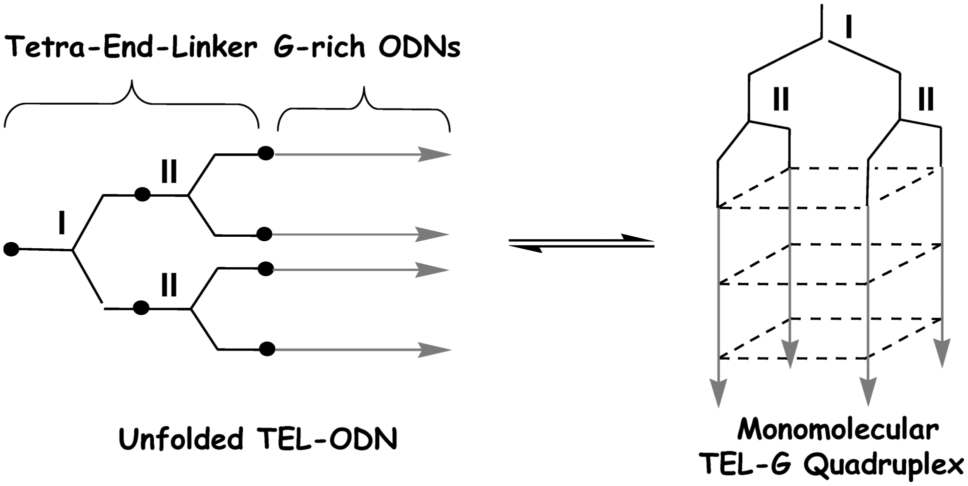


a. G-tetrad structure

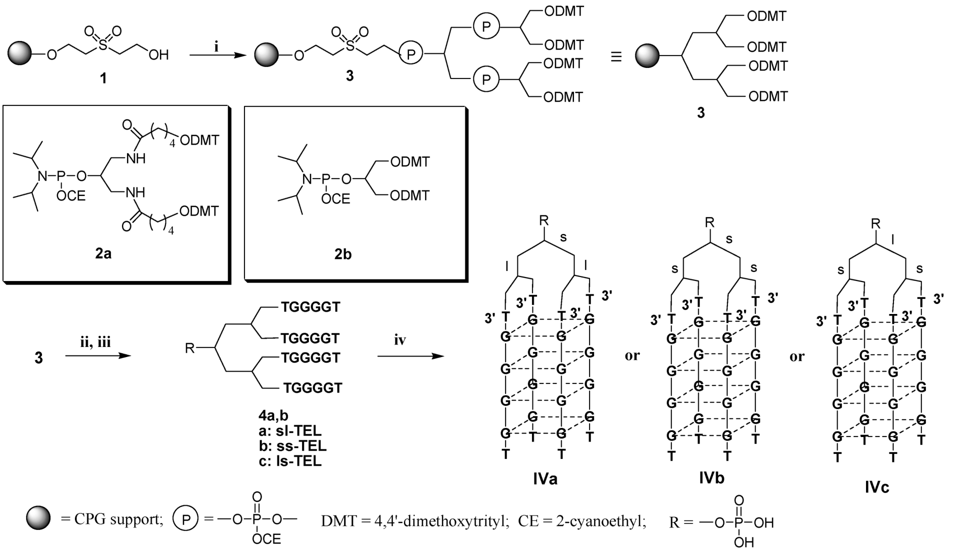
b. c. d.

FIGURE 1 a) G-tetrad structure; b) tetramolecular parallel quadruplex; c) bimolecular antiparallel quadruplex; d) monomolecular antiparallel quadruplex.

monomolecular parallel G-quadruplexes characterized by faster kinetics and higher thermal stability than the correspondent tetramolecular counterparts (Scheme 1).[5,6] Moreover, in the attempt to obtain monomolecular quadruplex structures characterized by predetermined strands stoichiometry and polarity, to be used as simple and stable models for structural studies



SCHEME 1 Schematic representation of unfolded (left) and folded (right) TEL-ODNs.



SCHEME 2 i) Two coupling procedures with **2a,b**; ii) ODN synthesis with 3′-phosphoramidites; iii) detachment and deprotection by NH_4OH conc. 32% (7 hours, 55°C); iv) HPLC purification and annealing.

on the quadruplex-ligand interaction, we recently synthesized a set of TEL-d(TGGGGT)$_4$ in which the ODN strands were linked with different orientations to the tetra-end linker. Structural studies performed on such compounds indicated that the TEL-d(TGGGGT)$_4$ assembled with two pairs of antiparallel ODN strands lead to parallel G-quadruplexes thanks to the considerable folding of the tetra-branched-linker around the whole quadruplex scaffold.[7]

In this article, we report the syntheses of two new TEL-d(TGGGGT)$_4$ molecules (Scheme 2, **4a** and **4b**) characterized by the presence of one or three shorter branches in the TEL moieties (sl-TEL and ss-TEL, respectively). These structural features were chosen in the attempt to hinder the folding of the linker around the whole quadruplex structure thus forcing the predetermination of quadruplex strands polarity upon the basis of the relative orientation of the ODN strands into the TEL-ODN molecules.

RESULTS AND DISCUSSION

In the attempt to obtain monomolecular quadruplexes having predetermined strands polarity we designed the synthesis of two new tetra-branchedlinker moieties characterized by the shortening of the primary or both primary and secondary TEL branches (**sl-TEL** or **ss-TEL**, Scheme 2). To test if the sl-TEL and ss-TEL moieties were still able to allow the formation of stable quadruplex structures, we synthesized **4a** and **4b** via the

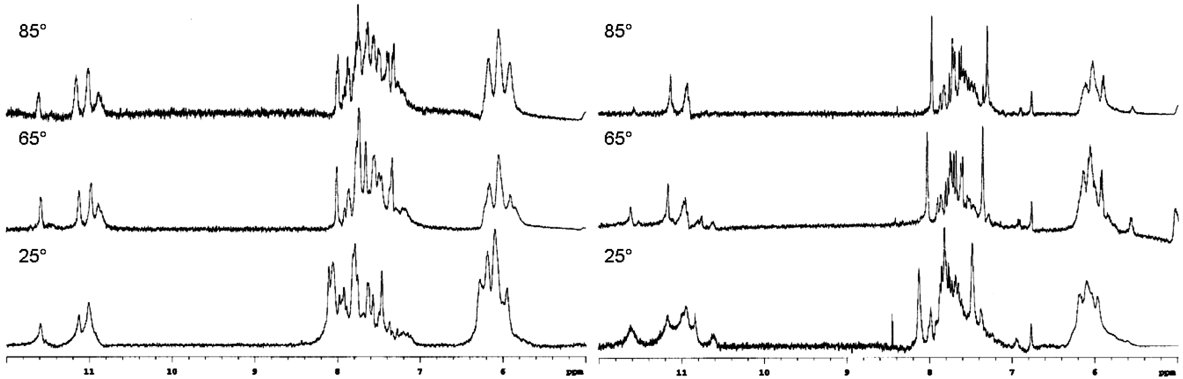


FIGURE 2 ^{1}H NMR spectra of **IVa** and **IVb** in 100 mM K$^+$ buffer (500 MHz, H_2O/D_2O 9:1, v/v).

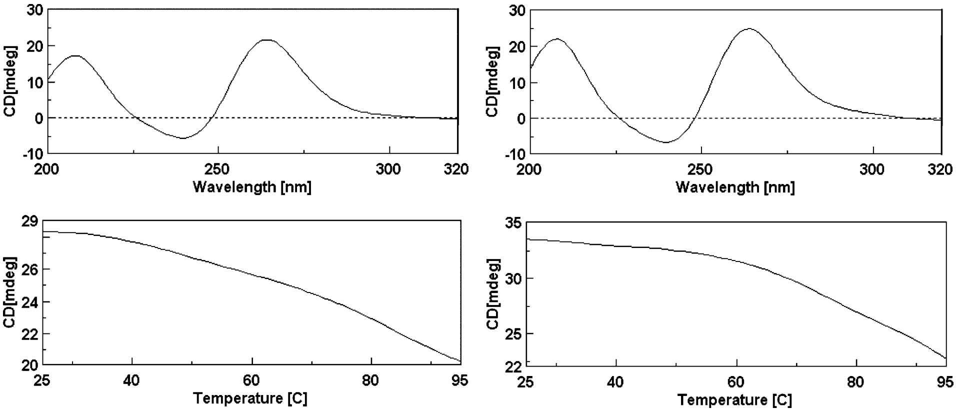


FIGURE 3 CD spectra (top; 25°C) and CD melting profiles (bottom; 264 nm, 1°C/minute) of **IVa** (left) and **IVb** (right) in 100 mM K^+ buffer.

recently reported solid-phase procedure[5] using the CPG support and the branching bi-functional linkers **2a** and **2b** as described in Scheme 2. We decided to not synthesize the ls-TEL-d(TGGGGT)$_4$ (**4c** in Scheme 2) because its primary branch is long enough to span the distance between the quadruplex edges, thus allowing the loss of strands polarity predetermination.

In order to assess if **4a** and **4b** could still fold into the quadruplex structures **IVa** and **IVb**, respectively, and if such were the case, to estimate their stabilities, we performed ^{1}H-NMR (at 25, 65, and 85°C using pulse field gradient WATERGATE[8] sequences for H_2O suppression) and CD studies. Comparison of ^{1}H-NMR stacked spectra of **IVa** and **IVb** (Figure 2) showed that both adopted parallel quadruplex structures in the presence of K^+ ions. However, it appeared that the presence of the ss-TEL moiety in **4b** lead to the lowering of the thermal stability of **IVb**, and to the partial loss of the magnetic equivalence between the imino protons in each G tetrad. On the other hand, **IVa**, characterized by the presence of the sl-TEL moiety, appeared to be very stable up to 85°C. Indeed, in the case of **IVa** no loss of imino protons magnetic equivalence was observed. Finally, the CD spectra and the CD melting curves were well in agreement with the NMR data (Figure 3). Upon the basis of these findings, the sl-TEL seems to be very promising for the achieving of monomolecular multistranded quadruplex structures characterized by both high thermal stability and predeterminable strands orientation. The synthesis of the antiparallel sl-TEL-d(TGGGGT)$_4$ is currently in progress in our laboratories.

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