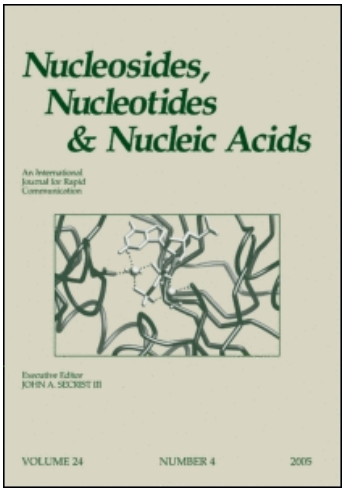

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Biophysical Properties of Quadruplexes Containing Two or Three 8-Bromodeoxyguanosine Residues

Luigi Petraccone[a]; Ida Duro[a]; Antonio Randazzo[b]; Ada Virno[b]; Luciano Mayol[b]; Concetta Giancola[a]
[a] Dipartimento di Chimica, Università degli Studi di Napoli "Federico II", Napoli, Italy [b] Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II", Napoli, Italy

To cite this Article Petraccone, Luigi , Duro, Ida , Randazzo, Antonio , Virno, Ada , Mayol, Luciano and Giancola, Concetta(2007) 'Biophysical Properties of Quadruplexes Containing Two or Three 8-Bromodeoxyguanosine Residues', Nucleosides, Nucleotides and Nucleic Acids, 26: 6, 669 — 674
To link to this Article: DOI: 10.1080/15257770701490589
URL: http://dx.doi.org/10.1080/15257770701490589

Nucleosides, Nucleotides, and Nucleic Acids, 26:669–674, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701490589

Taylor & Francis
Taylor & Francis Group

BIOPHYSICAL PROPERTIES OF QUADRUPLEXES CONTAINING TWO OR THREE 8-BROMODEOXYGUANOSINE RESIDUES

Luigi Petraccone and Ida Duro □ *Dipartimento di Chimica, Università degli Studi di Napoli "Federico II", Napoli, Italy*

Antonio Randazzo, Ada Virno, and Luciano Mayol □ *Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II", Napoli, Italy*

Concetta Giancola □ *Dipartimento di Chimica, Università degli Studi di Napoli "Federico II", Napoli, Italy*

□ *A physico-chemical characterization, based on NMR and CD spectroscopy, of quadruplexes formed by the oligonucleotide d(TGGGT), where two or three Gs are substituted by 8-bromo-2′-deoxyguanosine residues (dG^{Br}), is reported. The oligonucleotidic sequences $d(TG^{Br}G^{Br}GT)$, $d(TG^{Br}GG^{Br}T)$, $d(TGG^{Br}G^{Br}T)$, and $d(TG^{Br}G^{Br}G^{Br}T)$ have been synthesized. Only sequences $d(TG^{Br}GG^{Br}T)$ and $d(TG^{Br}G^{Br}GT)$ were able to fold into a well defined quadruplex structure, and their CD profiles and thermal stabilities turned out to be very different from those observed for the natural counterpart, indicating that the 8-Br-dG residues dramatically affect the structure of the quadruplex.*

Keywords G-quadruplex; modified guanine; NMR; CD

Telomeric DNA contains runs of guanine bases that can adopt an important structural motif based on G-quartets, cyclic hydrogen-bonded arrays of four coplanar guanine bases, stacking on each other to form G-quadruplexes. G-quadruplexes can be classified in terms of the number of strands that associate to form the structure (i.e., one, two, or four strands), orientation of the strands (i.e., parallel or antiparallel), and the disposal of the loops.[1] Usually, short oligonucleotides, like d(TGGGT), form tetramolecular quadruplexes with four parallel strands and with all guanine bases in the *anti*-glycosidic conformation. However, the substitution of a single atom in a guanine may produce remarkable changes in the physical and biological properties of the resulting nucleic acid fragments. For example, the

This work was supported by a PRIN-MURST grant from the Italian "Ministero dell'Università e della Ricerca Scientifica e Tecnologica," Rome.

Address correspondence to Concetta Giancola, Dipartimento di Chimica, Università di Napoli Federico II, Via Cintia, 80126, Napoli, Italy. E-mail: giancola@unina.it

presence of a bulky substituent such as the bromine atom at the C8 position of guanosine could destabilize the normal *anti* orientation of the base, sterically constraining the glycosidic bond to the *syn* conformation.

We have already explored the effects of the incorporation of a single 8-bromodeoxyguanosine (8-Br-dG) on the structure and thermal stability of $[d(TGGGT)]_4$.[2] In this frame, here we report the incorporation of two or three 8-Br-dG on the same sequence. Commercially available 5′-O-DMT-N-isobutyrroyl-8-bromodeoxyguanosine-3′-phosphoramidite was used to prepare the modified oligonucleotides. The pentamers $d(TG^{Br}G^{Br}GT)$, $d(TG^{Br}GG^{Br}T)$, $d(TGG^{Br}G^{Br}T)$, and $d(TG^{Br}G^{Br}G^{Br}T)$ were assembled using the standard solid phase β-cyanoethylphosphoramidite chemistry. The crude oligomers were purified by HPLC and desalted. The NMR samples were prepared at a concentration of approx. 1.0 mM (0.5 ml, 90% H_2O/10% D_2O), having 10 mM potassium phosphate, 70 mM KCl, 0.2 mM EDTA (pH 7.0) buffer. The samples were annealed for 5–10 minutes at 80°C and slowly cooled down to room temperature, then the ^{1}H-NMR spectra of them were recorded by using pulsed-field gradient WATERGATE[3] for H_2O suppression. Interestingly, only $d(TG^{Br}GG^{Br}T)$ and $d(TG^{Br}G^{Br}GT)$ were able to fold into a well-defined quadruplex structure. In fact, three well defined singlets were present in the region between 10.5 and 12 ppm of their spectra. These signals were attributed to imino protons involved in the Hoogsteen hydrogen bonds of G quartets. Moreover, a total of three signals, belonging to one guanine H8 and two thymine H6 protons, were present in the region between 7 and 8.5 ppm, and this is in perfect agreement with the presence of two 8-Br-dG residues in the oligonucleotides (Figure 1). Thus, these data are consistent with the formation of quadruplex structures characterized by three G-tetrads and possessing a fourfold symmetry with all strands parallel to each other. NOESY experiments (500 MHz, T = 300 K, mixing time = 50, 100, and 200 ms) acquired for both molecules further corroborated this observation. Moreover, as reported for other parallel quadruplex structures,[4] the observed polarity NOE connectivities (G H8 to ribose protons on the 5′ side only) suggested the right handed nature of the helices of the quadruplexes. As for the glycosidic torsion angles of the G residues, useful information could be obtained analyzing the relative intensities of NOEs between G H8 and ribose H2′ compared with the NOEs observed between G H8 and H1′. Thus, as for the same sequence containing the unmodified G residue, weak NOE between G H8 and H1′ and strong NOEs between G H8 and ribose H2′ indicates it is in an *anti* conformation. Unfortunately, the lack of the H8 proton in the 8-Br-dG residues prevented us from determining the conformation of the glycosidic torsion angle for these residues.

To obtain further information on the conformational features of the modified quadruplexes, we performed circular dichroism (CD) experiments in 10 mM KH_2PO_4 pH 7.0, 200 mM KCl, 0.1 mM EDTA

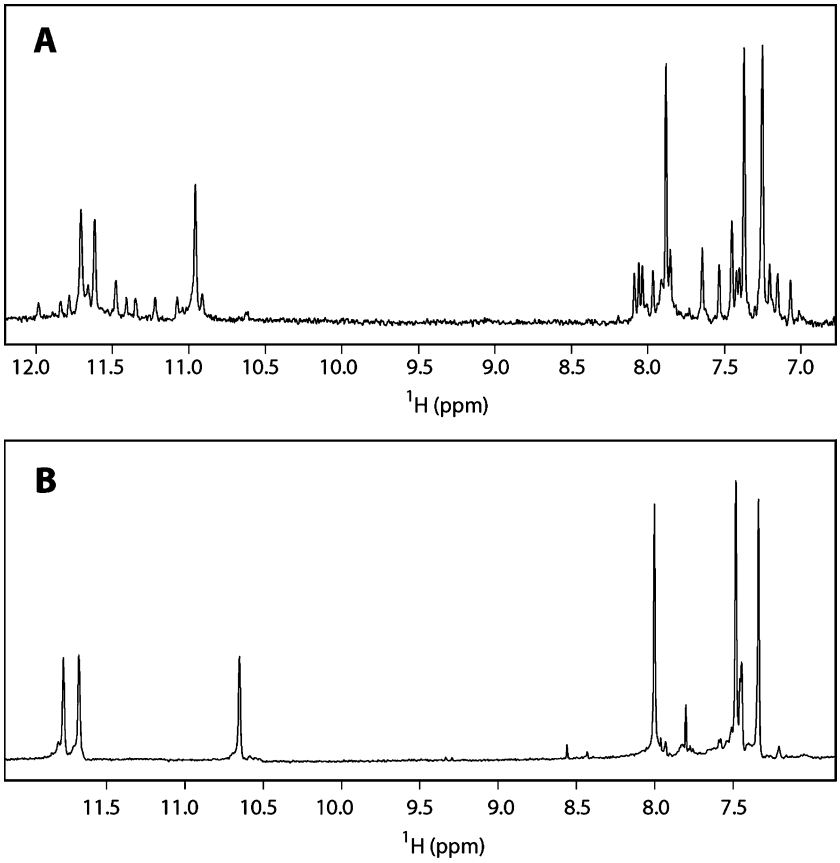

FIGURE 1 Expanded region of the proton NMR spectra of $[d(TG^{Br}G^{Br}GT)]_4$ (A) and $[d(TG^{Br}GG^{Br}T)]_4$ (B) (500 MHz, T = 25°C).

at single strand concentration of 2×10^{-4} M. CD spectra of the d(TGGGT), d(TG^{Br}G^{Br}GT), d(TG^{Br}GG^{Br}T) were acquired at 10°C (Figure 2). The spectra of the unmodified sequence show the typical bands for quadruplexes involving four parallel strands, a positive band at 263 nm, and a negative band at 243 nm[5] whereas the introduction of the 8-Br-dG residues causes significant changes in the CD spectra. Particularly, the CD spectrum of d(TG^{Br}GG^{Br}T) exhibited a negative band at 263 nm and a positive band at 295 nm whereas the spectrum of d(TG^{Br}G^{Br}GT) has two positive bands at about 263 and 295 nm. These data suggest that the 8-Br-dG residues affect the structure of the quadruplex and that the 8-Br-dG residues actually adopt a *syn* glycosidic conformation.

In order to evaluate the thermodynamic stability of the modified quadruplexes, CD thermal experiments were performed. Taking into account that the rates of quadruplex formation/dissociation are extremely

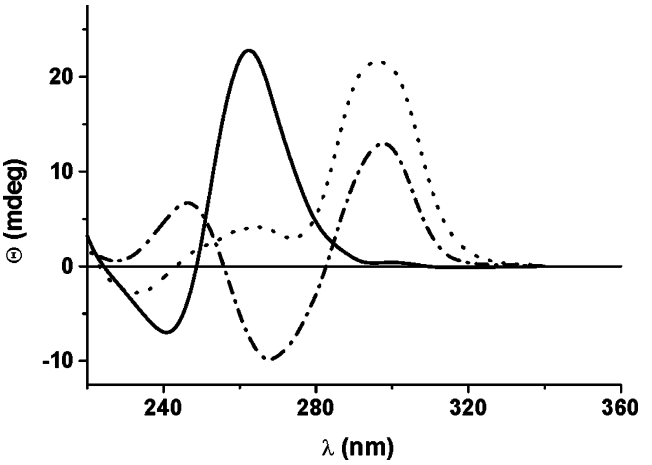

FIGURE 2 CD spectra of quadruplexes at 10°C, (—) $[d(TGGGT)]_4$, (· · ··) $[d(TG^{Br}G^{Br}GT)]_4$, (-·-·-·-·-) $[d(TG^{Br}GG^{Br}T)]_4$.

slow, to avoid a kinetic influence on the collected data, we allowed thermodynamic equilibrium to be reached at each temperature by following a previously reported procedure.[6] All the examined quadruplexes showed sharp transitions and well-shaped sigmoidal curves (Figure 3). The thermodynamic parameters obtained from the CD melting analysis are reported in Table 1. Interestingly, the introduction of two 8-Br-dG residues in different positions of the same sequence d(TGGGT) affects in the opposite way the thermodynamic stability of the canonical $[d(TGGGT)]_4$ quadruplex. Indeed, the modification of two adjacent dG residues near the dT at the 5' position to give $d(TG^{Br}G^{Br}GT)$ results in a more stable quadruplex in comparison with the unmodified one, while modification of two, non-adjacent dG residues to give $[d(TG^{Br}GG^{Br}T)]_4$ results in a decreased thermodynamic stability. Indeed, the melting temperatures were 21°C and 37°C for $[d(TG^{Br}GG^{Br}T)]_4$ and $[d(TG^{Br}G^{Br}GT)]_4$, respectively, whereas a melting temperature of 30°C was observed for the unmodified quadruplex. In addition, the enthalpy change value for the $[d(TG^{Br}G^{Br}GT)]_4$ melting is 50 kJ mol^{-1} higher than the value obtained for the canonical $[d(TGGGT)]_4$ quadruplex, indicating that the highest thermodynamic stability observed for this quadruplex is enthalpic in origin.

TABLE 1 Thermodynamic parameters for the dissociation of the quadruplexes

Quadruplex	T_m (°C) ± 1	ΔH^ov.H. (KJ mol^{-1})	ΔS^o (KJ mol^{-1} K^{-1})	ΔG^o 298 (KJ mol^{-1}) ± 1
$[d(TGGGT)]_4$	30	220 ± 11	0.51 ± 0.03	68
$[d(TG^{Br}GG^{Br}T)]_4$	21	200 ± 9	0.47 ± 0.02	60
$[d(TG^{Br}G^{Br}GT)]_4$	37	270 ± 12	0.65 ± 0.04	76

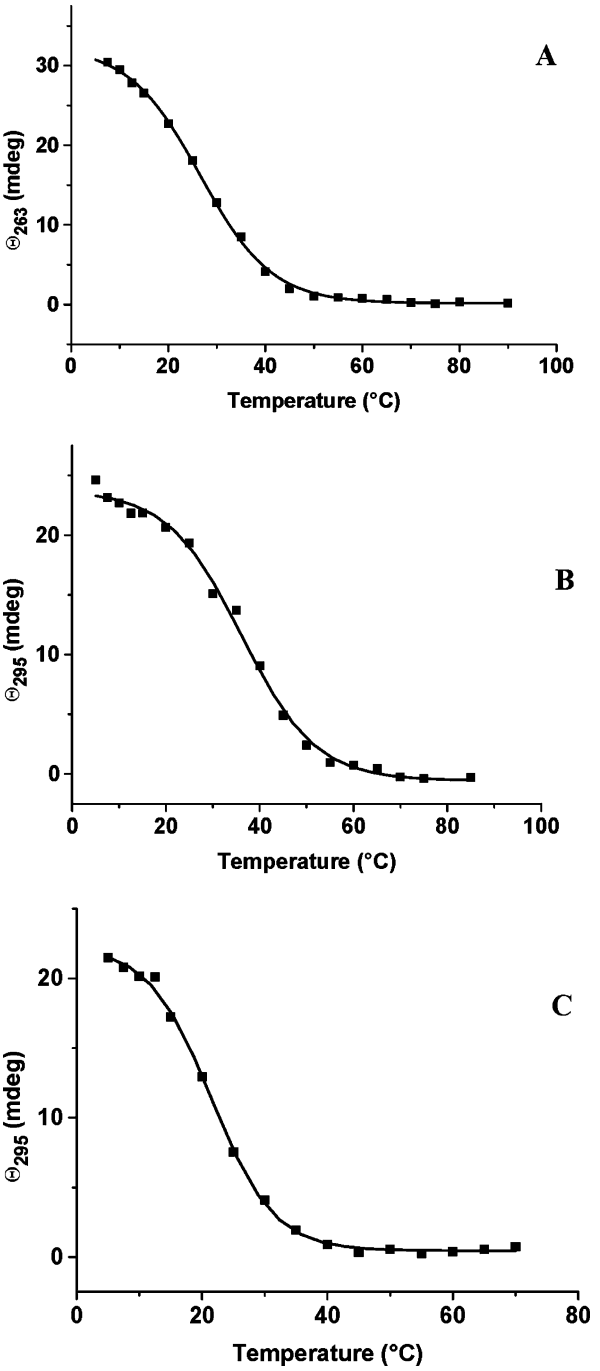

FIGURE 3 CD melting curves of the $[d(TGGGT)]_4$ (A), $[d(TG^{Br}G^{Br}GT)]_4$ (B), and $[d(TG^{Br}GG^{Br}T)]_4$ (C).

These data demonstrate that the substitution of a single atom, such as a bromine atom at the C8 position of guanines, significantly affect the structure and stability of the resulting quadruplex.

REFERENCES

1. Keniry, M.A. Quadruplex structures in nucleic acids. *Biopolymers* **2001,** 56, 123–146.
2. Esposito, V.; Randazzo, A.; Piccialli, G.; Petraccone, L.; Giancola, C.; Mayol, L. Effects on an 8-bromodeoxyguanosine incorporation on the parallel quadruplex structure $[d(TGGGT)]_4$. *Org. Biomol. Chem.* **2004**, 2, 313–318.
3. Piotto, M.; Saudek, V.; Sklenar, V.J. Gradient-tailored excitation for single-quantum NMR spectroscopy of aqueous solutions. *J. Biomol. NMR* **1992**, 2, 661–665.
4. Wang, Y.; Patel, D.J. Solution structure of a parallel-stranded G-quadruplex DNA. *J. Mol. Biol.* **1993**, 234, 1171–1183.
5. Petraccone, L.; Erra, E.; Esposito, V.; Randazzo, A.; Mayol, L.; Nasti, L.; Barone, G.; Giancola, C. Stability and structure of telomeric DNA sequences forming quadruplexes containing four G-tetrads with different topological arrangements. *Biochem.* **2004**, 43, 4877–4884.
6. Petraccone, L.; Erra, E.; Esposito, V.; Randazzo, A.; Galeone, A.; Barone, G.; Giancola, C. Biophysical properties of quadruple helices of modified human telomeric DNA. *Biopolymers* **2005**, 77, 75–85.