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G-Quartet Formation from an N^2-Modified Guanosine Derivative

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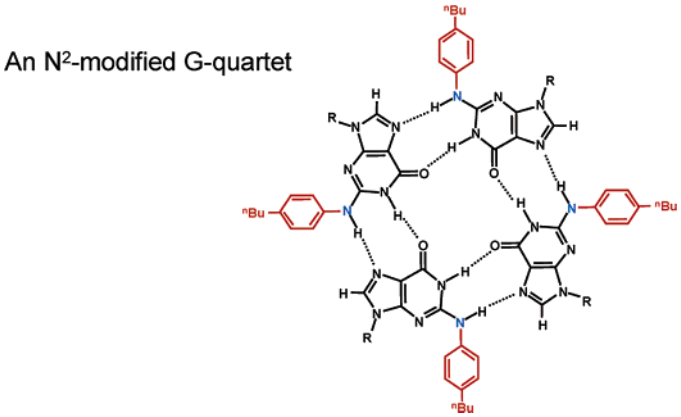
ABSTRACT



We report G-quartet formation from an N^2-modified lipophilic guanosine nucleoside, *N*^{2}-(4-*n*-butylphenyl)-2′,3′,5′-*O*-triacetylguanosine. We show that, in the presence of either K^+ or Na^+, this guanosine derivative self-assembles into a D_4-symmetric octamer consisting of two stacking all-*syn* G-quartets in a tail-to-tail (or head-to-head) fashion and a central ion.

G-Quartet formation is the fundamental driving force for a large number of guanosine derivatives including G-rich DNA and RNA oligomers to form highly ordered structures.[1−5] The G-quartet motif has not only been found in important regions in human genome such as telomeres,[6,7] but also shown potential usefulness in nanotechnology.[8,9] However, only a few cases are known to date where base-modified guanosine nucleosides are involved in G-quartet formation, and the modifications occur exclusively at the C_8 position of the guanine.[10−13] There are also two studies reporting improved G-quadruplex stability from N^2-modified DNA oligomers.[14,15] Here we report the first example of G-quartet formation from an N^2-modified lipophilic guanosine nucleoside: *N*^{2}-(4-*n*-butylphenyl)-2′,3′,5′-*O*-triacetylguanosine (G**1**); see Figure 1.[16]

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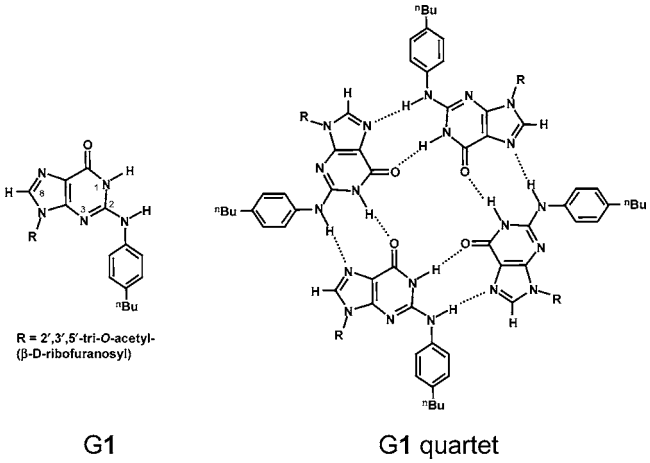

Figure 1. Molecular structure of an N^2-modified guanosine and the G-quartet formation.

Figure 2 shows the ^{1}H NMR spectra of G**1** in both monomeric and aggregate forms in $CDCl_3$.[17] The imino

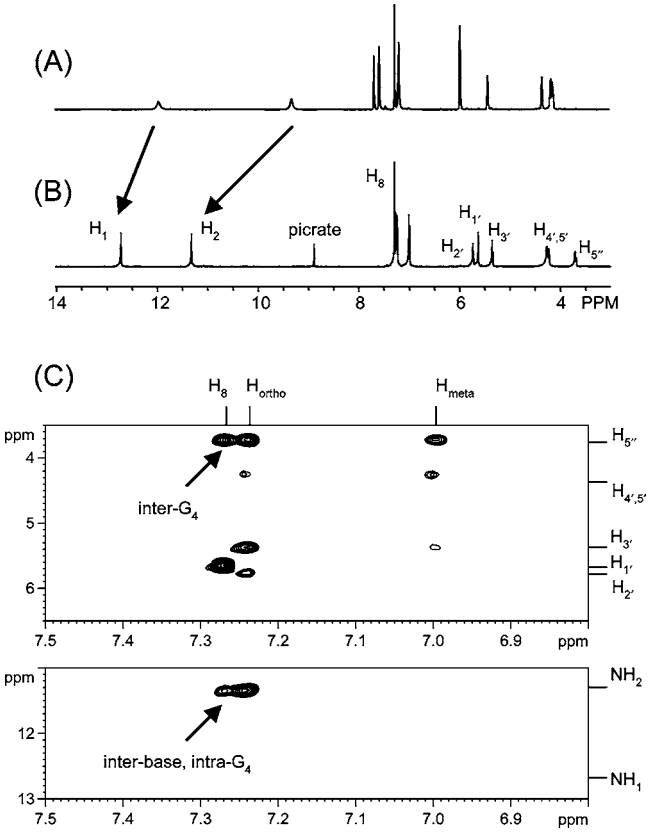

Figure 2. Portions of 1D ^{1}H NMR spectra for (A) G**1** monomer and (B) G**1** aggregate (in the presence of K^+ picrate) in $CDCl_3$ at 298 K. (C) A region of the 2D NOESY spectrum of G**1** aggregate at 298 K. A mixing time of 300 ms was used. A total of 64 transients were collected for each of the 256 t_1 increments with a recycle delay of 2 s. All ^{1}H NMR spectra were obtained on a Bruker Avance-600 spectrometer (B_0 = 14.1 T).

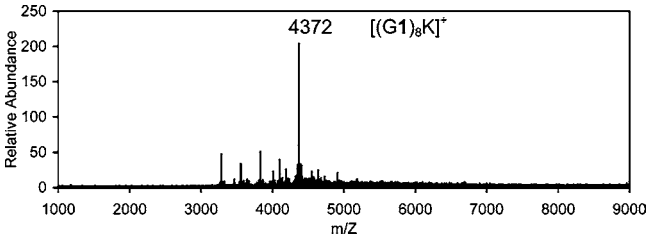

Figure 3. Positive-ion electrospray mass spectrum of G**1** aggregate prepared by liquid−liquid extraction of K^+ picrate into chloroform containing G**1**. The theoretical molecular weight for $[(G\mathbf{1})_8K]^+$ is 4364.5.

(N^1H_1) and amino (N^2H_2) signals for G**1** monomer (Figure 2A) are shifted considerably toward the high-frequency direction when interacting with K^+ picrate, characteristic of G-quartet formation (Figure 2B).[5] As seen in Figure 2C, an inter-base NOE cross-peak is observed between H_8 and NH_2 signals, which is also a spectral signature for G-quartet formation.[5] In addition, strong cross-peaks are observed between H_8 and H_1' and between phenyl and ribose protons. These are indicative of a *syn* conformation with respect to the N_9-C_1' glycosidic bond. The crystal structure of G**1** monomer (crystallized from CH_3CN in the absence of alkali metal ions) shows that G**1** adopts a *syn* conformation (χ_{CN} = 75.7°).[18]

Because only one set of ^{1}H NMR signals were observed for the G**1** aggregate, all G**1** molecules must adopt a *syn* conformation. The ^{1}H NMR signal integrations yield a G**1**/picrate ratio of 8:1, suggesting the formation of a G**1** octamer. To verify the size of the G**1** aggregate, we used a pulse-field-gradient diffusion NMR technique.[19] The translational diffusion coefficients determined for the G**1** monomer and aggregate in $CDCl_3$ at 298.2 K are $6.06 \pm 0.06 \times 10^{-10}$ and $3.09 \pm 0.06 \times 10^{-10}$ m^2 s^{-1}, respectively. This observed $D_{8mer}/D_{monomer}$ ratio of 0.51 is consistent with the formation of a G**1** octamer.[20,21] As shown in Figure 3, the positive-ion electrospray mass spectrum of the G**1** aggregate confirms that the octamer formation is predominant. Within the octamer, the two G-quartets are likely twisted by 30−45°

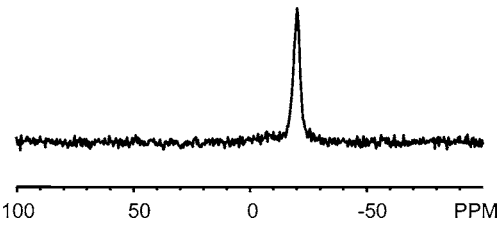

Figure 4. ^{23}Na magic-angle-spinning NMR spectrum of G**1** prepared in the presence of $NaClO_4$. Solid-state ^{23}Na NMR spectra were obtained on a Bruker Advance-500 spectrometer using a 4-mm double-resonance MAS probe. Experimental parameters: 9 kHz spinning, 7458 transients, 2 s recycle delay, and 70 kHz proton decoupling.

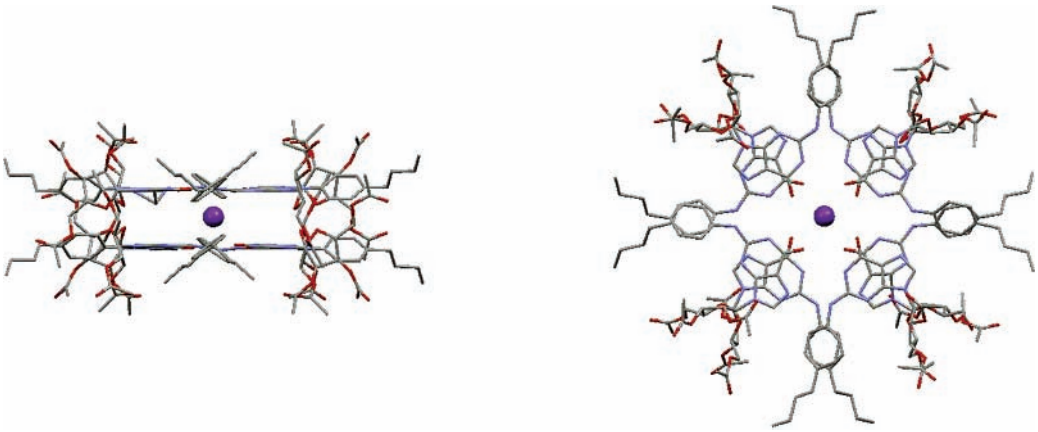

Figure 5. Molecular model (side and top views) for a D_4-symmetric G**1** octamer, $[(G\mathbf{1})_8K^+]$, where the two G-quartets are oriented in a tail-to-tail fashion. Hydrogen atoms are omitted for clarity.

with respect to each other, as supported by the fact that an inter-quartet NOE cross-peak is observed between H_8 and H_5'' signals.

To probe the alkali metal ion binding site, we obtained a solid-state ^{23}Na NMR spectrum for the G**1** aggregate prepared in the presence of Na^+ ions. As shown in Figure 4, a relatively narrow signal centered at −19 ppm was observed in the magic-angle spinning ^{23}Na NMR spectrum, suggesting that the Na^+ ion is sandwiched between two G-quartets, similar to the situations found in other G-quartet structures including DNA oligomers.[22−24]

Based on the experimental data, we can conclude that, in the presence of K^+ or Na^+, G**1** self-associates into two stacking all-*syn* G-quartets in a tail-to-tail (or head-to-head) arrangement with a central metal ion forming a D_4-symmetric G**1** octamer, as illustrated in Figure 5. This represents another example for this very rare type of (iso)guanine octamer.[21,25] The molecular model shown in Figure 5 also suggests a possible $\pi-\pi$ stacking between the phenyl rings from the two different G-quartets. The observed ^{1}H chemical shift changes for the ring protons between monomeric and aggregate forms ($\Delta\delta$ = 0.34 and 0.18 ppm for the *ortho*

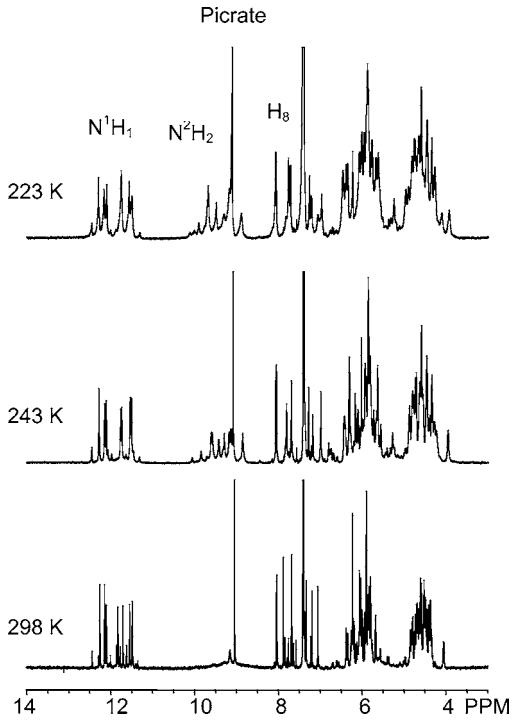

Figure 6. Portions of 1D ^{1}H NMR spectra for G**2** aggregate prepared by liquid−liquid extraction of K^+ picrate into chloroform solution of G**2**.

(16) G**1** was synthesized by following the literature procedure: Wright, G. E.; Dudycz, Z. W. *J. Med. Chem.* **1984**, *27*, 175−181.

(17) Preparation of G**1** aggregate was achieved by either liquid−liquid extraction or solid−liquid extraction. See: Forman, S. L.; Fettinger, J. C.; Pieraccini, S.; Gottarelli, G.; Davis, J. T. *J. Am. Chem. Soc.* **2000**, *122*, 4060−4067.

(18) Single crystals of G**1** monomer were obtained from slow evaporation of a CH_3CN solution in the absence of alkali salts. The crystal data were collected on a Bruker P4 single-crystal X-ray diffractometer with a Smart CCD-1000 detector, Mo Kα radiation, (50 kV and 30 mA) at 180 K. The structure was solved by using the direct method. Crystal data: formula $C_{26}H_{30}N_5O_8$, a = 9.019(2), b = 10.726(3), c = 14.449(4) Å, β = 105.111(4)°, V = 1349.5(6) Å^3, Z = 2, monoclinic space group $P2_1$. Convergence to R_1 = 0.055, wR_2 = 0.126 ($I > 2\sigma(I)$), GOF = 0.942 was achieved by using 4735 reflections and 353 parameters. All non-hydrogen atoms were refined anisotropically. Details of the crystal structure can be found in the supporting materials.

(19) All ^{1}H diffusion NMR spectra were recorded on a Bruker Avance-600 spectrometer using a pulse sequence of longitudinal-eddy-current delay (LED) with bipolar-gradient pulses. The pulse field gradient duration was varied from 4 to 15 ms, and the variable gradient strength (G) was changed from 6 to 350 mT/m. The diffusion period was varied from 50 to 90 ms. A total of 16 transients were collected for each of the 16 or 32 increment steps with a recycle delay of 20 s. The eddy-current delay was set to 5 μs.

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and *meta* ^{1}H resonances, respectively) are consistent with the formation of $\pi-\pi$ stacking.

To further examine the consequence of N^2-alkylation, we compared the G-quartet formation from unmodified 2′,3′,5′-*O*-triacetylguanosine (G**2**). As seen from Figure 6, the ^{1}H NMR spectra for G**2** aggregates in the presence of K^+ and Na^+ ions exhibit very complex features, indicating the formation of polymeric columnar aggregates. The 2D ^{1}H NOESY spectra confirmed the formation of G-quartet in these systems. The ESI-MS spectra for G**2**/K^+ aggregate show clearly the presence of octamer, dodecamer, and hexadecamer; see Supporting Information. Thus, the N^2-alkylation in G**1** changes the G-quadruplex structure from an extended polymer to a discrete ocatmer. This increased stability of the G**1** octamer formation is presumably due to the fact that the N^2-alkylation stabilizes the *syn* conformation. It is also possible that the $\pi-\pi$ stacking between the phenyl rings further favors the formation of a tail-to-tail (or head-to-head) stacking, resulting in a discrete D_4-symmetric octamer.

Although the present study is concerned with G-quartet formation from a lipophilic guansoine nucleoside in organic solvent, our finding that N^2-substitution does not hinder G-quartet formation may have implications in the design of new G-quadruplex structures formed by DNA and RNA. In this regard, there are several known cases where the exocyclic amino hydrogen atom that is not involved in the G-quartet formation participates in additional intermolecular hydrogen bonding in DNA and RNA G-quadruplexes.[26−30] These studies suggest that recognition of the N^3-N^2 edge of the G-quartet, which is located at the groove region of a G-quadruplex, may be important for DNA and RNA folding. Consequently, any modification at the N^2 site of the guanine base may interfere with nucleic acid folding and recognition. We anticipate that residue-specific N^2 modification can be exploited as a new handle for fine-tuning G-quadruplex structure and function.

Acknowledgment. This work was supported by NSERC of Canada. We thank Ramsey Ida for assistance in obtaining diffusion NMR data.

Note added in proof. After submission of this work, we have learned that Kaucher and Davis also observed G-quartet formation from an N^2, C_8-disubstituted guanosine derivative.[31]

Supporting Information Available: COSY and NOESY spectra for G**1** aggregates and a table containing ^{1}H chemical shifts; complete crystal data (in CIF format); diagrams showing the structure of G**1** and NOESY and ESI-MS spectra for G**2**/K^+ aggregates. This material is available free of charge via the Internet at http://pubs.acs.org.

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