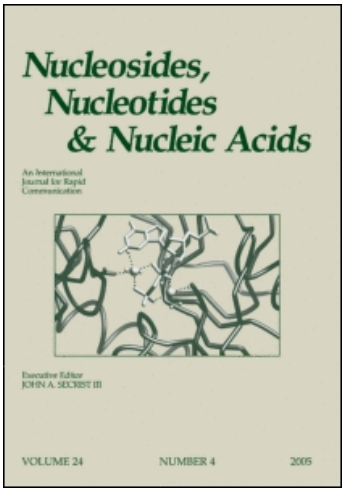


**Nucleosides, Nucleotides and Nucleic Acids**
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# G-Octamer Formation from N$^9$-Modified Guanine Derivatives

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Online publication date: 01 December 2010

**To cite this Article** McCallum, Jeremy , Amare, Solomon and Nolan, Rebecca(2010) 'G-Octamer Formation from N$^9$-Modified Guanine Derivatives', Nucleosides, Nucleotides and Nucleic Acids, 29: 11, 801 — 808
**To link to this Article: DOI:** 10.1080/15257770.2010.528113
**URL:** http://dx.doi.org/10.1080/15257770.2010.528113

*Nucleosides, Nucleotides and Nucleic Acids*, 29:801–808, 2010

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770.2010.528113

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# G-OCTAMER FORMATION FROM $N^9$-MODIFIED GUANINE DERIVATIVES

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□ *The properties of the self-assembly of two lipophilic guanine derivatives, 2′,3′,5′-O-tris*(tert-*butyldimethylsilyl)-guanosine and* $N^9$*-(3,5-bis*(tert-*butyldimethylsilyloxy)-benzyl)-guanine, are described. In the presence of* $K^+$*, both guanine derivatives self-associate into* $D_4$*-symmetric octamers consisting of two G-quartets stacked around a central ion.*

**Keywords** Guanine derivative; G-quartet; octamer; self-assembly



## INTRODUCTION

With the recent interest in the anticancer potential of G-quadruplexes, the need exists for understanding the self-assembly of G-quadruplexes and G-quartets. As such, recent studies have focused on the formation of G-quartet structures from guanosine derivatives.[1–4] Substituted guanosine and 2′-deoxyguanosine derivatives have been found to form a variety of quartet structures, including octamers, dodecamers, hexadecamers, and helices. The type of guanine modification and choice of protecting groups and templating ions play a critical role in which G-quartet structure predominates.[5–18]

Herein, we present discrete G-quartet octamer formation from a guanosine derivative and from an $N^9$-modified guanine derivative.

## RESULTS AND DISCUSSION

Lipophilic guanine derivatives, 2′,3′,5′-O-tris(*tert*-butyldimethylsilyl)-guanosine (**G1**) and $N^9$-(3,5-bis(*tert*-butyldimethylsilyloxy)-benzyl)-guanine (**G2**) (as shown in Figure 1), were synthesized as previously described.[19,20]

Received 21 July 2010; accepted 27 September 2010.
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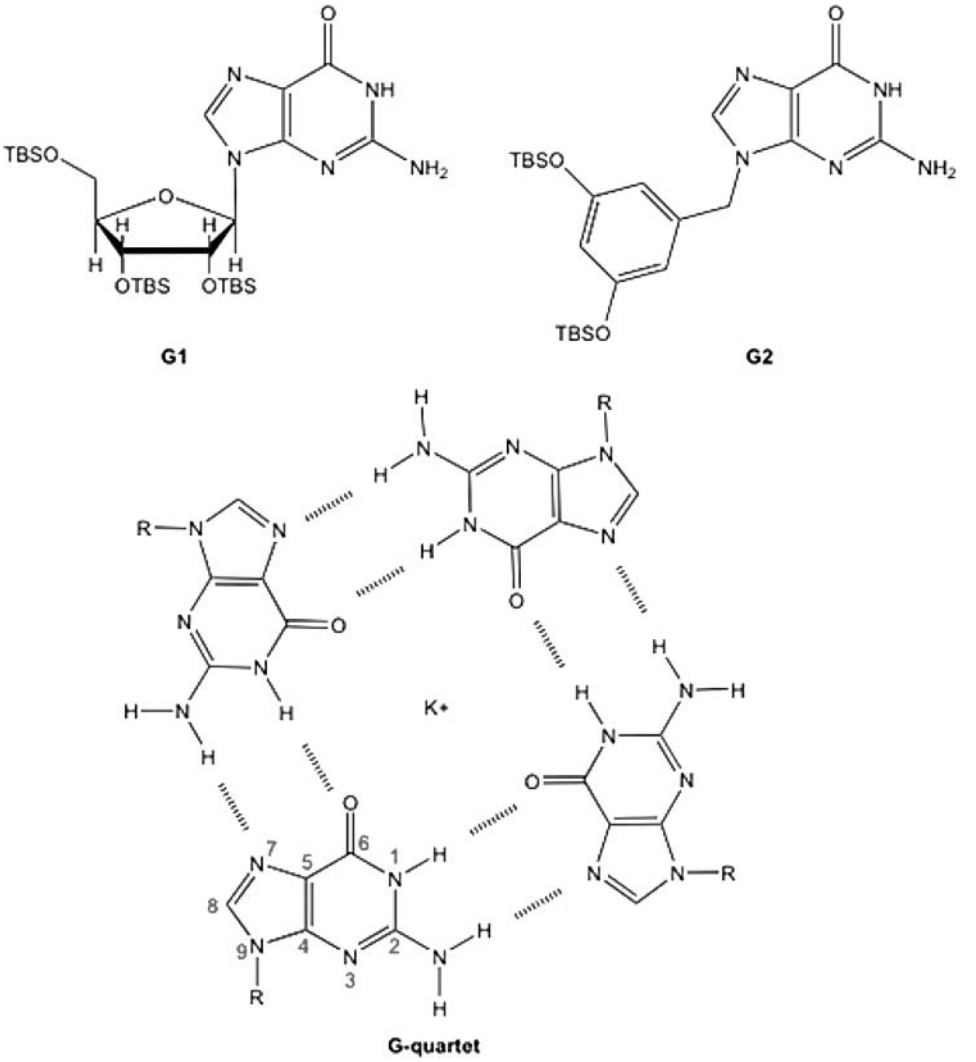


**FIGURE 1** Molecular structure of lipophilic guanine derivatives **G1** and **G2** and the G-quartet structure.

The $^1$H NMR spectrum of **G1** in DMSO, as shown in Figure 2a, demonstrates sharp peaks, indicative of the monomeric species predominating. A similar spectrum is observed for **G1** in $CDCl_3$ in the presence of [2,2,2]cryptand. The cryptand sequesters any residual salts that may template aggregate formation, resulting in sharp peaks, with the exception of the N1H signals, signifying the monomeric species. Upon the addition of KI to **G1**, a single sharp imino (N1H) signal appeared near $\delta = 12.4$ ppm, indicative of the involvement of the imino protons in strong intermolecular hydrogen bonds, typical in G-quartet formation.[21] Two new peaks appeared in the $^1$H NMR spectra at low temperature, consistent with amino

protons $N2H_A$ and $N2H_B$, which are exchange-broadened into the baseline above 0°C. Two-dimensional NOESY experiments confirmed quartet formation, as indicated by the NOE cross-peak shown in Figure 2g. NOE cross-peaks are observed for H8-$N2H_A$ and H8-$N2H_B$, demonstrating G-quartet formation.[18]

Circular dichroism (CD) spectroscopy has been widely used to probe self-assembly processes of guanosine quartets. The CD spectrum of **G1** in the presence of $K^+$ is shown in Figure 3 and is consistent with G-quartet self-assembly.[22] No spectrum was observed for **G1** in $CDCl_3$ in the presence of [2,2,2]cryptand.

When potassium picrate was added to **G1** in $CDCl_3$, identical $^1H$ NMR spectra were observed with one additional peak in the spectrum corresponding to the picrate signal at $\delta_{pic} = 8.83$ ppm. Peak integration of this signal allows for the determination of the stoichiometry of $K^+$ to be determined and indicated an 8:1 **G1** to $K^+$ ratio. In addition, the chemical shift of the picrate signal ($\delta_{pic} = 8.83$ ppm) is consistent with formation of a $[G1]_8$-$K^+$ octamer. Davis et al. reported a value of $\delta_{pic} = 8.85$ for an octamer bound to one equivalent of sodium picrate ($[G]_8 \cdot Na^+ \cdot pic^-$) and found an upfield shift for the picrate signal when the octamer was bound to two equivalents of sodium picrate ($\delta_{pic} = 8.58$ for $[G]_8 \cdot 2Na^+ \cdot 2\ pic^-$).[23]

As only one set of signals is observed for the octamer, all molecules must adopt a *syn* or *anti* conformation. The H8 signal at $\delta = 7.99$ ppm is consistent with an *anti* conformation; *syn* conformation typically exhibit H8 signals at $\delta \approx 7.3$ ppm. Additionally, NOE cross-peaks in the two-dimensional NOESY spectrum supports the all-*anti* conformation of the octamer. In Figure 2f, a medium strength H8-H2′ NOE cross-peak is observed with a weaker H8-H1′ cross-peak, consistent with results obtained by Davis et al. for the *anti* conformation of a mixed *syn-anti* octamer.[18] Two tetramers can stack in four possible orientations: head-to-head, tail-to-tail, head-to-tail, or tail-to head. Head-to-tail or tail-to-head octamers should exhibit two $^1H$ NMR signals for each monomeric hydrogen due to differences in symmetry; this is not observed for the **G1** quartet.[24]

Based on this evidence, we conclude that **G1** self-associates into two all-*anti* G-quartets stacked in a head-to-head (or tail-to-tail) fashion around $K^+$. We believe this is one of the first examples of a guanosine derivative forming an all-*anti*, head-to-head (or tail-to-tail) $D_4$-symmetric octamer. Indeed, examples of exclusive octamer formation are rare. Wu et al. demonstrated formation of an all-*syn*-$D_4$-symmetric octamer from an $N^2$-modified guanosine derivative, while Davis et al. reported head-to-head octamer formation from an isoguanosine derivative and octomer formation composed of one all-*anti* quartet and one all-*syn* quartet for a lipophilic guanosine derivative.[6,7,18,23,25] As guanine structure, cation, and anion choice all play a crucial role in quartet formation, there are numerous examples of hexadecamer quadruplex structures and larger aggregates formed from a variety

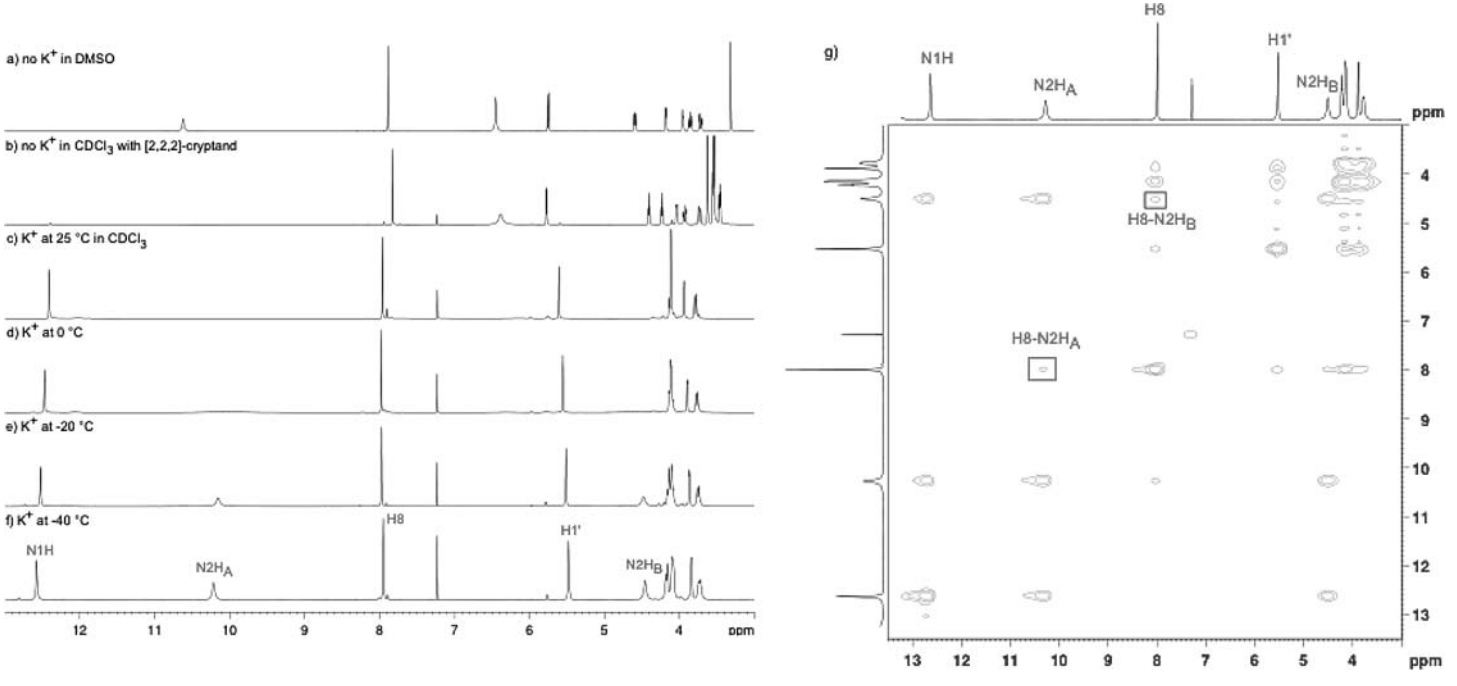


**FIGURE 2** Portions of the $^1$H NMR spectra for (a) **G1** in DMSO in the absence of $K^+$; (b) **G1** in $CDCl_3$ in the presence of [2,2,2]cryptand; (c) **G1** in the presence of $K^+$ in $CDCl_3$ at 25°C; (d) **G1** with $K^+$ in $CDCl_3$ at 0°C; (e) at −20°C; and (f) at −40°C. (g) A portion of the two-dimensional NOESY spectrum for derivative **G1** with $K^+$ in $CDCl_3$ at −40°C.

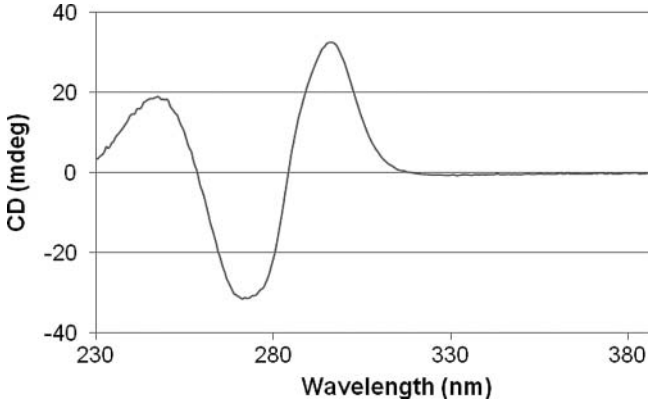


**FIGURE 3** Circular dichroism spectrum of **G1** (0.12 mM) with KI in $CHCl_3$ at 25°C.



of guanosine derivatives. We saw no evidence of larger aggregate formation with >1/8 equivalents of $K^+$.

Guanine derivative, $N^9$-(3,5-bis(*tert*-butyldimethylsilyloxy)-benzyl)-guanine (**G2**) proved to be an interesting model for investigating G-quartet formation. We were curious what role, if any, the sugar moiety had on quartet formation; there are limited examples of quartet formation from guanine derivatives not possessing a sugar moiety.[26–28] As shown in Figure 4, the $^1H$ NMR of **G2** in DMSO demonstrated well-defined signals indicating the presence of mostly monomeric species. This compound was slightly soluble in $CDCl_3$ and upon addition of potassium picrate to **G2**, the spectra changed significantly. One sharp imino (N1H) signal appeared near $\delta =$ 12.3 ppm, indicative of strong hydrogen bonding within the G-quartet and, as previously demonstrated with **G1**, two amino proton ($N2H_A$ and $N2H_B$) signals appeared below 0°C. Additionally, the methylene protons became diastereotopic, exhibiting a typical geminal coupling constant (J = 14 Hz), indicative of a highly ordered structure in which rotation around the methylene carbon is not observed on the NMR time scale. Interestingly, the H8 signal was significantly shifted upfield ($\Delta\partial = 3$ ppm). This dramatic shift can only be attributed to a shielding effect from the aromatic ring. Indeed, two-dimensional NOESY experiments confirm the proximity of the aromatic ring to H8 as indicated by the Ar2H-H8 NOE cross-peak, shown in Figure 4g. NOE cross-peaks are also observed between H8-$N2H_A$ and H8-$N2H_B$, consistent with G-quartet formation. As was the case for **G1,** the stoichiometry of the picrate signal and its chemical shift value indicated formation of a $[G2]_8$-$K^+$ octamer. We propose **G2** self-associates into an octamer consisting of two quartets surrounding $K^+$ in a head-to-head (or tail-to-tail) fashion.

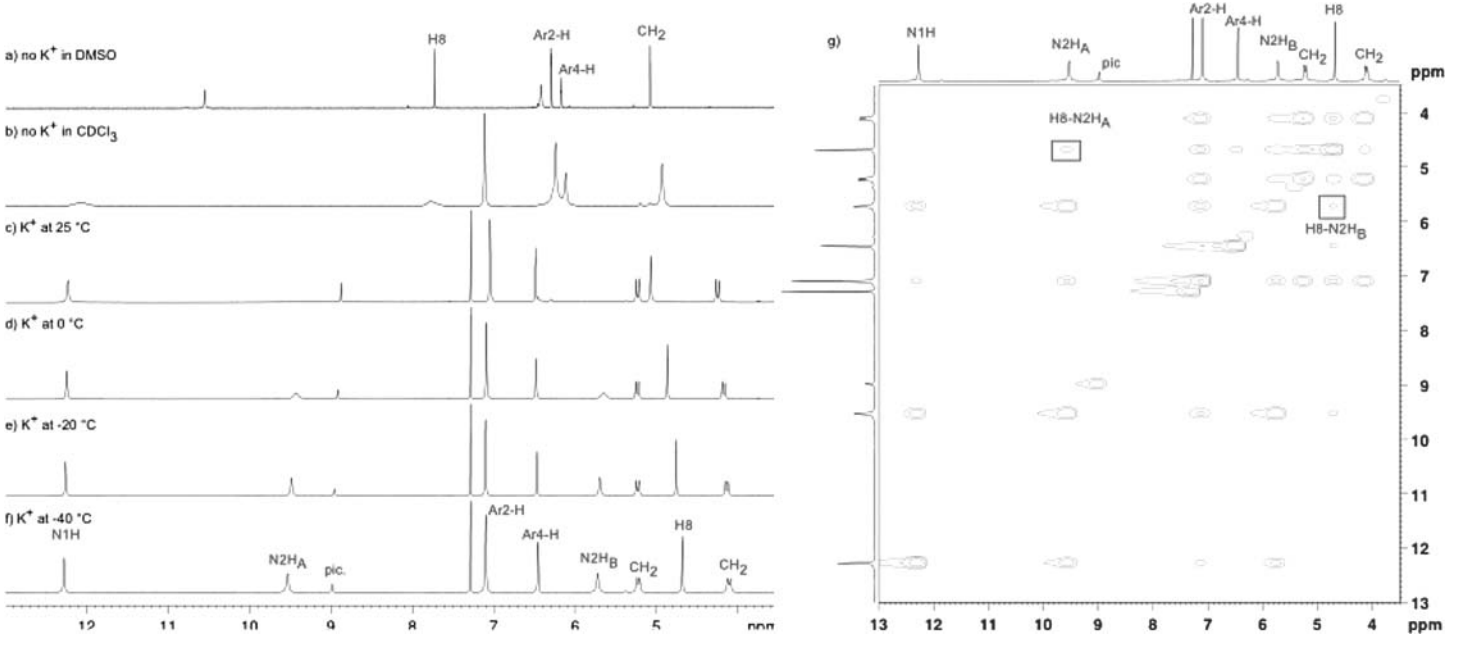


**FIGURE 4** Portions of the $^1$H NMR spectra for (a) **G2** in DMSO; (b) **G2** in $CDCl_3$ in the absence of $K^+$; (c) **G2** in the presence of $K^+$ in $CDCl_3$ at 25°C; (d) **G2** with $K^+$ in $CDCl_3$ at 0°C; (e) at −20°C; and (f) at −40°C. (g) A portion of the two-dimensional NOESY spectrum for derivative **G2** with $K^+$ in $CDCl_3$ at −40°C.

## CONCLUSION

In summary, we have demonstrated the self-assembly of a lipophilic guanosine derivative, 2′,3′,5′-O-Tris(*tert*-butyldimethylsilyl)-guanosine (**G1**), into two all-*anti* G-quartets stacked in a head-to-head fashion around a central K+ ion. Additionally, we have demonstrated the first example of octamer formation of two G-quartets from an achiral guanine derivative, $N^9$-(3,5-bis(*tert*-butyldimethylsilyloxy)-benzyl)-guanine (**G2**). Both derivatives formed octamer structures exclusively, presumably due to the steric effect of the bulky TBS protecting groups.

## EXPERIMENTAL

All reagents were purchased from Aldrich Chemical Company (St. Louis, MO, USA) and used without further purification. 2′,3′,5′-O-Tris(*tert*-butyldimethylsilyl)-guanosine (**G1**) and $N^9$-(3,5-bis(*tert*-butyldimethylsilyloxy)-benzyl)-guanine (**G2**) were prepared as reported.[19,20] $(\mathbf{G1})_8$-$K^+$ and $(\mathbf{G2})_8$-$K^+$ were prepared by adding solid KI ($\geq$1/8 eq.) or potassium picrate ($\geq$1/8 eq.) to **G1** (30 mg, 50 mM) or **G2** (15 mg, 30 mM) in $CDCl_3$ (1 mL). 1 eq. of cryptand [2,2,2] was added to **G1** in $CDCl_3$ to sequester residual cations. NMR spectra were recorded on a Bruker Avance-400 Spectrometer. All NOESY spectra were acquired at 258 K using the noesygpphpp pulse sequence with a mixing time of 0.3 seconds and a total of 256 scans collected for each time increment. Circular dichroism spectra were collected on a JASCO J-715 circular dichroism spectrophotometer using 0.1-cm path length quartz cuvettes.



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