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N2, C8-disubstituted guanosine derivatives can form G-quartets

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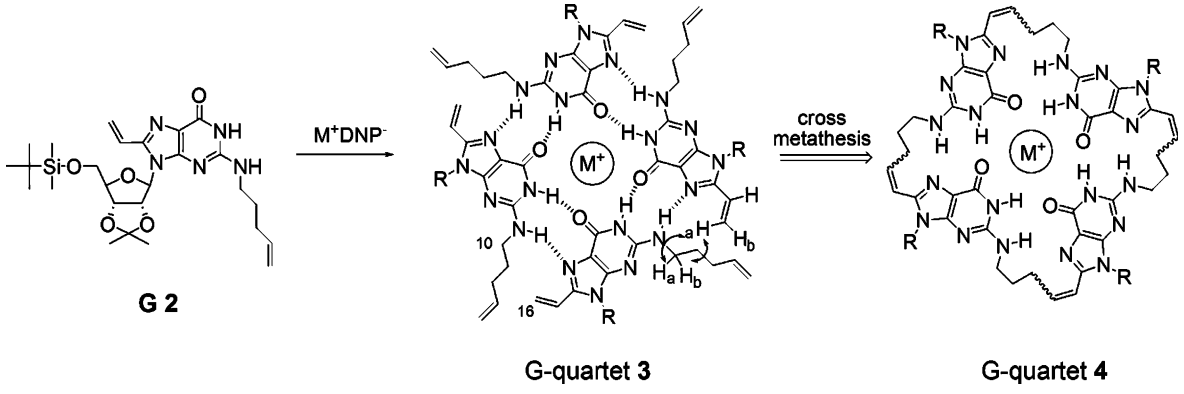
Abstract—A lipophilic guanosine with alkene groups attached to the nucleobase's N2 and C8 positions was synthesized with the intent of using olefin cross-metathesis to covalently capture an individual G-quartet. Modification of the N2 and C8 positions gave a derivative that formed a stable G-quartet structure in the presence of a cation template. This is the first example of a N2, C8-disubstituted guanosine forming a G-quartet.

Guanosine (G) derivatives have been used as building blocks for gelators,[1] nanowires,[2] nanomachines,[3] biosensors,[4] selective cation and anion extractants,[5,6] and transmembrane ion transporters.[7,8] The key feature in these structures is a hydrogen-bonded G-quartet stabilized by an alkali(ine) cation.[9] Modification of the G building block has typically been on the sugar unit or on the nucleobase's N9 position. Recent studies have described G-quadruplexes made from G derivatives substituted at their N2 or C8 positions.[10,11] In some cases, the N2 or C8 substituents stabilized the G-quadruplex relative to the unmodified derivative.[10c,11] As outlined in Scheme 1, we reasoned that modification at *both* N2 and C8 would lead to a G-quartet that might then be cross-linked to give a 'covalent' macrocycle.[12]

We have previously shown that 5′-*t*-BDMS-2′,3′-isopropylidene G **1** self-assembles in the presence of potassium picrate to form a G-quadruplex with formula [G **1**]$_{16}$·4K^{+}·4pic^{-}.[13] The crystal structure of this G-quadruplex showed four stacked G-quartets with four cations aligned along a central channel. This hexadecameric G-quadruplex is kinetically and thermodynamically stable in non-polar solvents such as CD_2Cl_2, but it is in equilibrium with 'free' G **1** in CD_3CN.[14] To make a more stable assembly, we used a covalent capture strategy to form a unimolecular G-quadruplex.[8] Thus,



Scheme 1. Design scheme for covalent capture of G **2** through formation of G-quartet **3** and cross-metathesis to yield G-quartet **4**. NOEs between H16a and H10 for G-quartet **3** are shown, see text for details.

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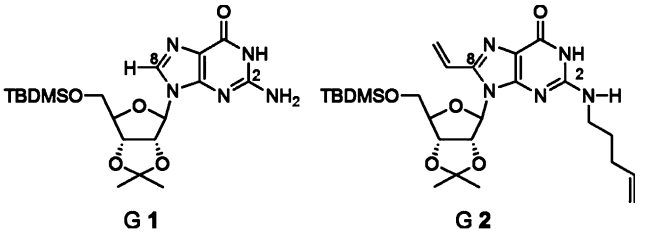
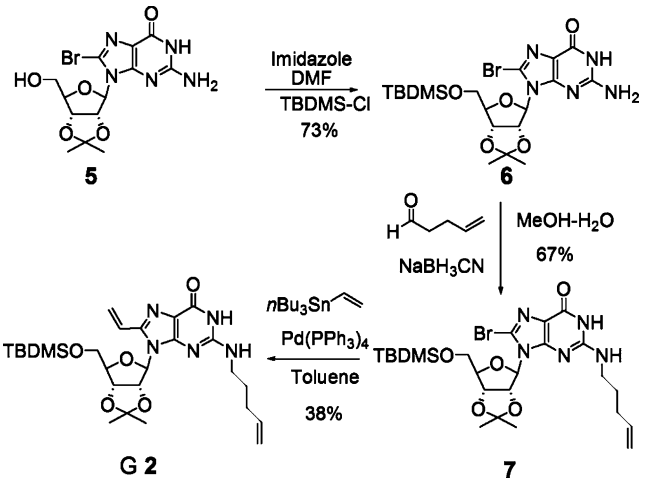
cation-templated self-assembly of a G derivative with 5′-*O*-allyl groups, followed by olefin cross-metathesis, provided a unimolecular G-quadruplex able to transport Na^+ across phospholipid membranes.

As outlined in Scheme 1, our aim was to use olefin metathesis to cross-link N2, C8-disubstituted G units within an individual G-quartet **3**. A 'covalent' G-quartet such as **4** should be stable and functional in water. We would also like to evaluate the cation binding properties of a 'covalent' G-quartet such as **4**, relative to its hydrogen-bonded precursor **3**. In this letter, we describe two key steps in achieving our goal of preparing 'covalent' G-quartet **4**; (1) synthesis of N2, C8-disubstituted G **2** and (2) cation-templated formation of an assembly, $[G\ \mathbf{2}]_8{\cdot}Ba^{+2}{\cdot}2DNP^-$ containing hydrogen-bonded G-quartets. This is the first example, to our knowledge, of a G-quartet functionalized at its N2 and C8 positions.



The necessary precursor G **2** was synthesized in four steps from 8-bromo-2′,3′-isopropylidene G **5** (Scheme 2).[15] The 5′-OH of **5** was first protected with a *t*-BDMS group.[16] Reductive amination of 4-pentenal with the N2 amine of G **6** gave **7**, and introduced the first alkene unit into the nucleobase.[17] The second alkene group was added to C8 via Stille coupling; cross-coupling of **7** with vinyl-tri-*n*-butyltin afforded the target compound G **2**.[18]

Next, we confirmed that G **2** formed stable G-quartets. The N2, C8-disubstituted G **2** extracted $Ba(DNP)_2$ and K(DNP) salts from water into $CDCl_3$, as determined by NMR, ESI-MS, and UV–vis data. Integration of



Scheme 2. Synthesis of N2, C8-disubstituted G **2**.

^1H NMR signals indicated formation of an octamer with formula $[G\ \mathbf{2}]_8{\cdot}Ba^{+2}{\cdot}2DNP^-$.[19] NMR spectra also showed that the N2 amino proton in $[G\ \mathbf{2}]_8{\cdot}Ba^{+2}{\cdot}2DNP^-$ moved far downfield relative to uncomplexed G **2** ($\Delta\delta = 3.40$ ppm), consistent with its involvement in a hydrogen bond (Fig. 1b). Stronger support for a G-quartet came from NOEs between the C10 hydrogens and the C16a olefinic hydrogen (Fig. 1c). These C10–C16a NOEs are a firm indication of a G-quartet since the C10 substituent (on the N2 side chain) and C16 (attached to C8) are too far apart for intramolecular NOEs. These NOEs can only arise from intermolecular interactions; models show that H10 and H16 are within 3–5 Å in a G-quartet. NOEs between N2H and a C16 hydrogen were also consistent with G-quartet formation by G **2**.[6]

The size of the complex formed by G **2** and Ba^{+2} was assessed by diffusion NMR.[20] Pulse field gradient (PFG) experiments revealed that the complex generated by salt extraction with G **2** had a diffusion coefficient (D_s) of 6.36×10^{-10} m^2/s, a value much lower than that for the internal standard, monomeric 5′-*t*-BDMS-2′,3′-isopropylidene adenosine **A** ($D_s = 13.3 \times 10^{-10}$ m^2/s).[21] The ratio of these two diffusion coefficients confirmed the formation of octamer $[G\ \mathbf{2}]_8{\cdot}Ba^{+2}$.[14] Mass spectrometric analysis of the complex showed a major peak ($m/z = 2195.6$) for $[(G\ \mathbf{2})_8{\cdot}Ba]^{+2}$ (Fig. 2). The signal for $[(G\ \mathbf{2})_4{\cdot}Na]^+$ ($m/z = 2149.2$) is likely due to cation exchange during sample preparation and analysis. In marked contrast, ESI-MS analysis of uncomplexed G

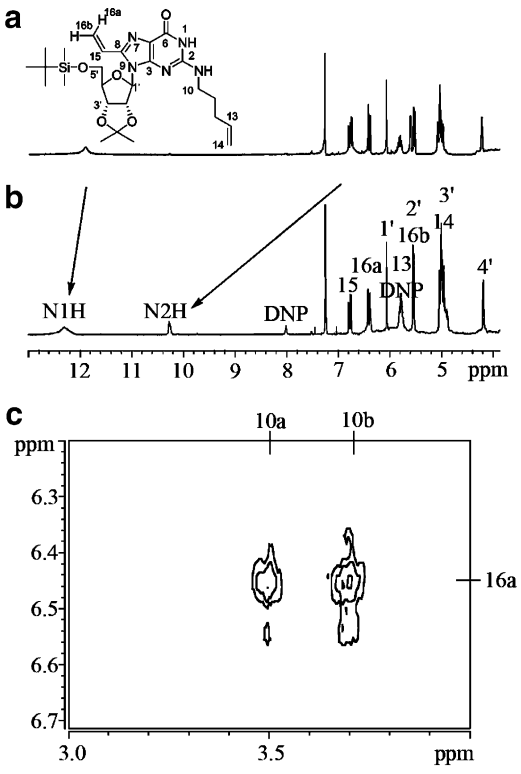


Figure 1. (a) ^1H NMR spectrum of G **2** in $CDCl_3$, (b) after extraction of $Ba(DNP)_2$, from water into $CDCl_3$ and (c) selected region of 2D NOESY spectrum of sample from (b) showing NOEs between H10 and H16a.

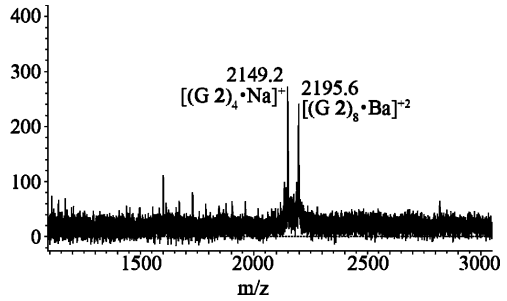


Figure 2. ESI-MS of $[G\ \mathbf{2}]_8{\cdot}Ba^{+2}{\cdot}2DNP^-$ formed by liquid–solid extraction of $Ba(DNP)_2$ with G **2** in $CHCl_3$.

2 showed only peaks for the molecular ion $[G\ \mathbf{2}{+}H]^+$ and for a dimer $[(G\ \mathbf{2})_2{+}H]^+$. The NMR and MS data all indicate that the N2, C8-disubstituted G **2** can form stable G-quartets in the presence of a cation template.

Having demonstrated the formation of octamer $[(G\ \mathbf{2})_8{\cdot}Ba]^{+2}$, we turned our attention to covalently linking neighbors within an individual G-quartet. Attempts to effect olefin cross-metathesis on $[(G\ \mathbf{2})_8{\cdot}Ba]^{+2}$, using either Grubb's first or second generation catalysts, have so far been unsuccessful.[22] This lack of cross-metathesis for $[(G\ \mathbf{2})_8{\cdot}Ba]^{+2}$ may be due to (a) an improper relative orientation for the N2 and C8 alkene groups within G-quartet **3** or (b) the fact that the C8 vinyl group on the purine may be too electron deficient.[23] Another issue that we must consider, even when metathesis conditions are defined for this system, is that these G_8 octamers are formed by π-stacked quartets, which creates the potential to generate cross-links between G-quartet layers. Such cross-links would preclude our goal to generate an isolated covalently linked G-quartet. We are currently using the chemistry described in this letter to prepare other N2, C8-disubstituted G monomers that may overcome these potential geometric and electronic problems.

In conclusion, we have described a synthetic route to prepare G analogs substituted at both N2 and C8 positions. Importantly, the N2, C8-disubstituted G **2** forms stable G-quartets in the presence of templating cations. The next challenge will be to identify G analogs and cross-linking strategy (olefin metathesis, disulfide, or hydrazone bonds) that will enable covalent capture of an individual G-quartet.

Note Added in Proof. We recently learned of a relevant study about G-quartet formation from an N2-modified guanosine, see: Ref. 24.

Acknowledgment

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16. 8-Bromo-2′,3′-*O*-isopropylidene-5′-*t*-BDMS-guanosine **6**. To a solution of **5** (2.4 g, 6.0 mmol) and imidazole (0.61 g, 8.0 mmol) in DMF (100 mL) was added *t*-butyldimethylsilylchloride (1.4 g, 9.0 mmol). The reaction mixture was stirred at rt under N_2 for 8 h. The mixture was poured into water (1 L) and filtered to yield 0.57 mg (73%) of a white precipitate. ^1H NMR (400 MHz, DMSO-d_6) δ 10.86 (s, 1H, N1H), 6.65 (br s, 2H, N2H2), 5.89 (d, 1H, H1′, $J = 0.59$ Hz), 5.47 (dd, 1H, H2′, $J = 6.26$, 0.59 Hz), 5.13 (dd, 1H, H3′, $J = 6.26$, 3.52 Hz), 4.07 (ddd, 1H, H4′, $J = 5.48$, 3.52, 2.54 Hz), 3.69 (d, 1H, H5′a, $J = 5.48$ Hz), 3.65 (d, 1H, H5′b, $J = 2.54$ Hz), 1.49 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 0.75 (s, 9H, Si–*t*-butyl), −0.14 (s, 3H, Si–CH_3), −0.16 (s, 3H, Si–CH_3). ESI-MS, calcd for $C_{19}H_{31}BrN_5O_5Si$ $[M+H]^+$: 516.1278, found: 516.1273.
17. *N*2-(4-Pentenyl)-8-bromo-2′,3′-*O*-isopropylidene-5′-*t*-BDMS-G **7**. This reaction was conducted as described by Sako, M.; Kawada, H.; Hirota, K. *J. Org. Chem.* **1999**, *64*, 5719. To a solution of **6** (4 g, 7.8 mmol) and 4-pentanal (2.5 mL, 31 mmol) in 1:1 H_2O–MeOH (300 mL) was added sodium cyanoborohydride (2.9 g, 46.5 mmol). The reaction mixture was stirred at 50 °C under N_2 for 8 h (reaction beyond this period gave N2 dialkylation). After removal of the solvent, 200 mL of H_2O was added and pH was adjusted to 7.0. After extraction with CH_2Cl_2, the organic layer was washed with satd $NaHCO_3$, 0.1 N HCl, and

water. Following evaporation of the solvent, the resulting brown solid was purified by flash chromatography (2% MeOH in CH_2Cl_2) to give 3.0 g (67%) of a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ 10.77 (s, 1H, N1H), 6.65 (br s, 1H, N2H), 5.91 (d, 1H, H1′, $J = 1.17$ Hz), 5.81 (ddt, 1H, H13, $J = 17.22$, 10.37, 6.65 Hz), 5.68 (dd, 1H, H2′, $J = 6.26$, 1.17 Hz), 5.03 (dq, 1H, H14a, $J = 17.22$, 1.96 Hz), 4.96 (dq, 1H, H14b, $J = 10.33$, 2.15 Hz), 4.96 (dd, 1H, H3′, $J = 6.65$, 3.33 Hz), 4.07 (dt, 1H, H4′, $J = 6.65$, 3.33 Hz), 3.62 (d, 2H, H5′, $J = 6.65$ Hz), 3.19 (m, 2H, H10), 2.07 (m, 2H, H12), 1.64 (p, 2H, H11, $J = 7.24$ Hz) 1.51 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 0.76 (s, 9H, Si–*t*-butyl), −0.11 (s, 3H, Si–CH_3), −0.14 (s, 3H, Si–CH_3). ESI-MS, calcd for $C_{24}H_{39}BrN_5O_5Si$ $[M+H]^+$: 584.1904, found: 584.2024.

18. *N*2-(4-pentenyl)-8-vinyl-2′,3′-*O*-isopropylidene-5′-*t*-BDMS G **2**. This reaction was conducted as described by Sessler et al.[10b] To a solution of **7** (1 g, 7.8 mmol) and vinyltributyltin (1.25 mL, 4.3 mmol) in freshly distilled toluene (30 mL, three freeze–pump thaw cycles) was added tetrakis (triphenyl-phosphine)palladium(0) (0.32 g, 0.27 mmol). The mixture was stirred at reflux under N_2 for 24 h. The solvent was removed and the residue redissolved in CH_2Cl_2. The organic layer was washed with satd $NaHCO_3$, 0.1 N HCl, and water. Following evaporation of the solvent, the resulting solid was purified by flash chromatography (1:2:98 NEt_3–MeOH–CH_2Cl_2) to give 0.35 g (38%) of a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ 10.85 (br s, 1H, N1H), 6.87 (dd, 1H, H15, $J = 17.02$, 11.15 Hz), 6.76 (br s, 1H, N2H), 6.14 (dd, 1H, H16a, $J = 17.02$, 1.76), 6.08 (d, 1H, H1′, $J = 1.96$ Hz), 5.80 (ddt, 1H, H13, $J = 17.22$, 10.37, 6.65 Hz), 5.60 (dd, 1H, H2′, $J = 6.26$, 1.96 Hz), 5.46 (dd, 1H, H16b, $J = 11.15$, 1.76 Hz), 5.02 (dq, 2H, H14a, $J = 17.22$, 1.76 Hz), 4.96 (m, 1H, H14b, H3′), 4.08 (dt, 1H, H4′, $J = 6.06$, 3.52 Hz), 3.62 (d, 2H, H5′, $J = 6.06$ Hz), 3.21 (m, 2H, H10), 2.06 (m, 2H, H12), 1.62 (p, 2H, H11, $J = 7.24$ Hz) 1.51 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 0.78 (s, 9H, Si–*t*-butyl), −0.09 (s, 3H, Si–CH_3), −0.11 (s, 3H, Si–CH_3). ESI-MS, calcd for $C_{26}H_{42}N_5O_5Si$ $[M+H]^+$: 532.2955, found: 532.3411.
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