Supramolecular Chemistry

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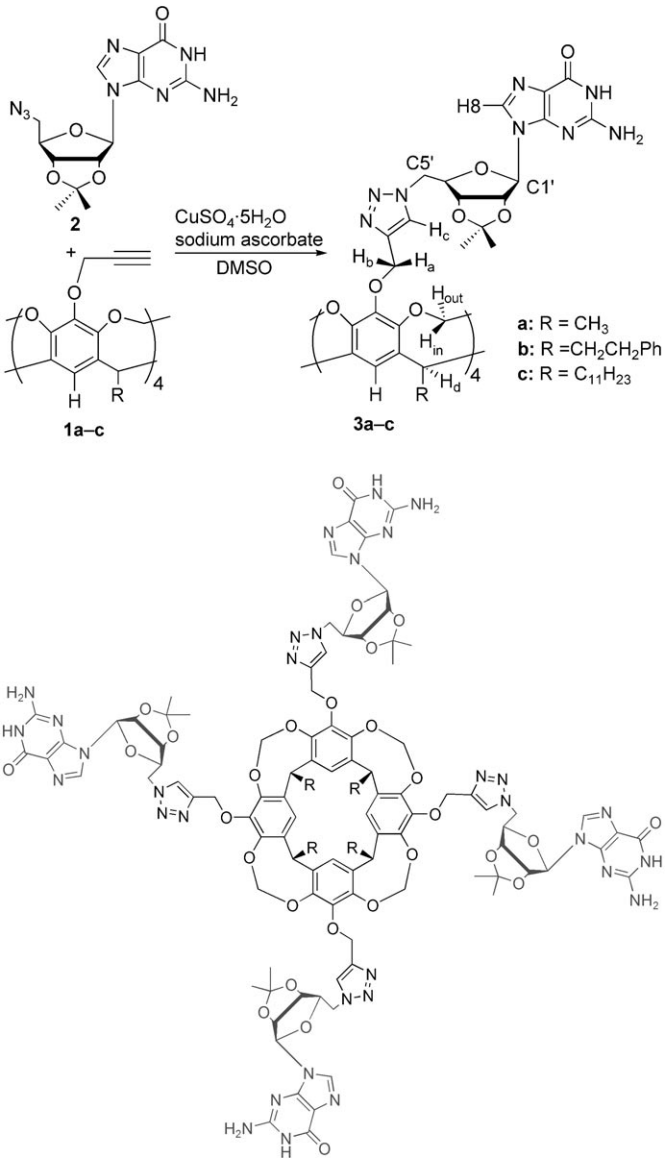
Template-Assembled Synthetic G-Quartets (TASQs)**

*Mehran Nikan and John C. Sherman**

Organization of small molecules into well-defined assemblies is one of the challenges of supramolecular chemistry.[1] A biologically relevant assembly that lends itself well to synthetic supramolecular study is the G-quartet, which is a H-bonded structure composed of four Hoogsteen-paired guanine bases.[2] Guanine-rich sequences are abundant in telomeric ends of chromosomes and promoter regions of DNA, and are capable of forming G-quartets in vitro.[3]

Guanine self-assembly in lipophilic systems has been the focus of much research in the past and has been reviewed in detail.[4] Guanines have been linked to calixarenes[5] for structural or recognition purposes, and synthetic hydrophilic unimolecular G-quartet assemblies have been reported.[6] G-quartets are typically templated and stabilized by cations,[7] whereas guanine aggregation in the absence of cations generally results in the formation of ribbonlike structures.[8] Cation-free G-quartets are rare due to the repulsion of the coplanar carbonyl groups and the high stability of less-ordered polymeric ribbons.[9] Herein, we introduce a new class of compounds, guanine-linked cavitands, and propose a general term for them, template-assembled synthetic G-quartets (TASQs), analogous to the term template-assembled synthetic proteins (TASPs) created by Mutter.[10] The lipophilic TASQs reported herein were synthesized by click chemistry,[11] and manifest unusual cation-independent stability. This stability is likely due to the preorganization afforded by the cavitand scaffold, thus exemplifying one of the hallmarks of supramolecular chemistry.[12] These TASQs link the chemistry of G-quartets to that of cavitands and offer potential opportunities, including the creation of singular G-quartet baskets that are stable at low concentrations and in the absence of cations.

Compounds **3a–c** were synthesized in 62–66% yield from cavitands **1a–c**[13] and 5′-azido-2′,3′-*O*-isopropylideneguanosine (**2**)[14] (Scheme 1). ^{1}H NMR spectroscopic data for **3c** in [D$_6$]DMSO and CDCl$_3$ are given in Figure 1 and Table 1 (see the Supporting Information for complete assignments). The sugar protons were identified by their correlations to adjacent protons starting from H1′. The diastereotopic H5′, H$_a$/H$_b$, and



Scheme 1. Synthesis of compounds **3a–c** and their two-dimensional representation. DMSO: dimethylsulfoxide.

H$_{in}$/H$_{out}$ protons were identified using HMQC spectra, and were distinguished from each other using NOESY spectra (see the Supporting Information). Assignments of H$_c$, H8, and the exchangeable protons were also obtained from NOESY spectra.

In the ^{1}H NMR spectrum of **3c** in DMSO the amino protons (NH$_2$) are equivalent and appear as a broad singlet at $\delta = 6.59$ ppm while the imino proton (NH) resonates at $\delta =$ 10.78 ppm (Figure 1b, Table 1). These chemical shift values suggest that the guanine bases are not bound in an assembly such as a G-quartet or a ribbon.[15]

[*] M. Nikan, Prof. Dr. J. C. Sherman
Department of Chemistry
The University of British Columbia
2036, Main Mall, Vancouver, BC V6T1Z1 (Canada)
Fax: (+1) 604-822-2847
E-mail: sherman@chem.ubc.ca

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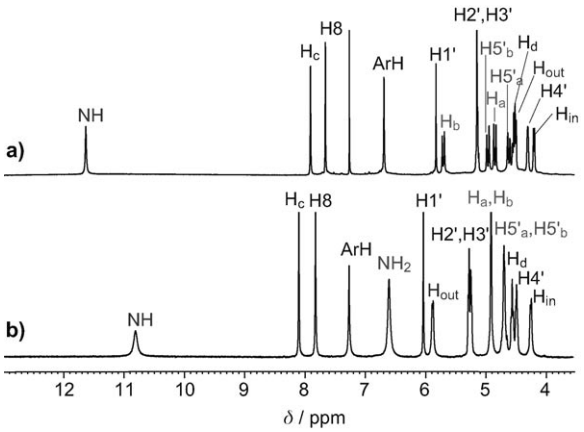

Figure 1. 400 MHz ^{1}H NMR spectra of **3c** at 25 °C a) in $CDCl_3$, b) in $[D_6]$DMSO.

Table 1: Spectral assignments of **3c** in $[D_6]$DMSO and $CDCl_3$ at ambient temperature.

Proton	δ_H([D$_6$]DMSO)[a]	δ_H($CDCl_3$)	^{1}H-^{1}H COSY[b,c]	^{1}H-^{13}C HMQC[b]
H1′	6.03	5.82	H2′*	89.17
H2′	5.27	5.14	H3′, H1′*	78.44
H3′	5.24	5.13	H2′, H4′	83.57
H4′	4.48	4.30	H3′, H5′$_a$*	82.90
H5′$_a$	4.69	4.61	H5′$_b$, H4′*	49.19
H5′$_b$	4.69	4.96	H5′$_a$	49.19
NH	10.78	11.63	–	–
NH_{2a}	6.59	broad	–	–
NH_{2b}	6.59	broad	–	–
H8	7.82	7.65	–	136.51
ArH	7.26	6.68	–	113.89
H_{in}	4.24	4.19	H_{out}	98.40
H_{out}	5.88	4.50	H_{in}	98.40
H_a	4.91	4.84	H_b	70.60
H_b	4.91	5.70	H_a	70.60
H_c	8.10	7.90	–	126.13
H_d	4.55	4.53	CH_2 (feet)	37.17

[a] The signals of diastereotopic protons overlap in $[D_6]$DMSO. [b] 2D data acquired for a 2×10^{-2} M solution of the sample in $CDCl_3$. [c] The asterisk indicates weak COSY cross-peaks.

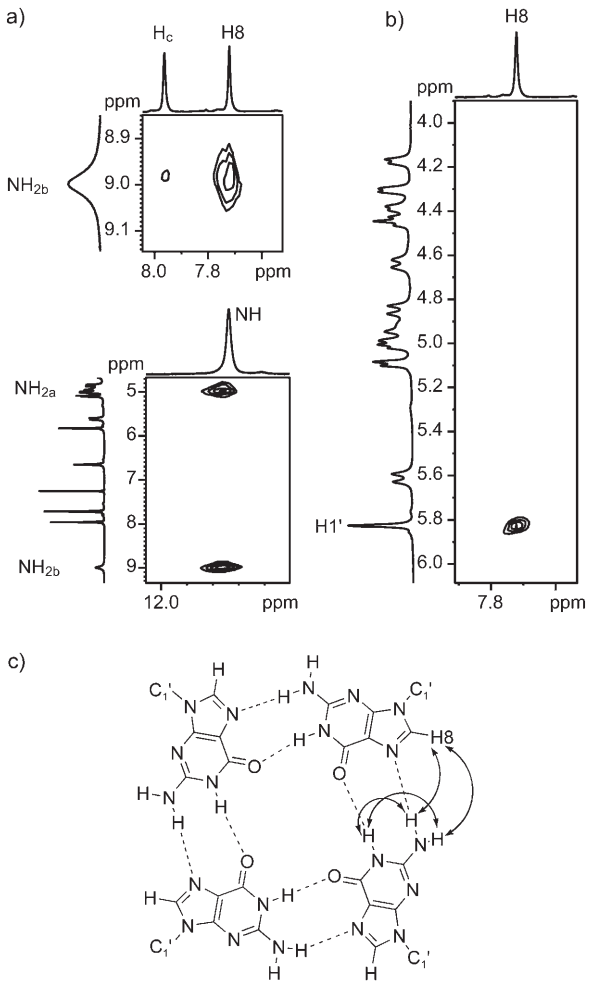

Figure 2. NOE effects indicative of a) the formation of a G-quartet, b) the *syn* conformation at 400 MHz in $CDCl_3$ at −40 °C. c) Inter- and intrabase NOE effects in a G-quartet.

A G-quartet appears to form when **3c** is dissolved in $CDCl_3$, even in the absence of cations. Such a species is highly unusual, and thus a detailed account is in order. The imino (NH) signal shifts downfield to 11.63 ppm, indicating a H-bonded system.[16] At low temperatures, the NH_2 signal appears as two distinct singlets, one at $\delta = 9$ and one at $\delta =$ 4.9 ppm (Figure 2a and Supporting Information), corresponding to H-bonded and non-H-bonded protons, respectively.[17] At −40 °C, a 2D NOESY spectrum yields a cross-peak between H8 and NH_{2b} (Figure 2a, top), a correlation that has been used to authenticate a G-quartet assembly.[18] Moreover, a strong (i.e., intraresidue) NOE between H8 and H1' was observed, which is indicative of a *syn* conformation along the glycosidic bond (Figure 2b).[19] *Syn* conformations are known to prevent the formation of G-ribbons.[9a] NOEs between amino and imino protons (Figure 2a, bottom) indicate Hoogsteen-paired guanine bases.[17,20] Taken together, these results suggest that **3c** spontaneously forms a G-quartet in $CDCl_3$.

The non-exchangeable protons also exhibit changes consistent with the formation of a G-quartet. The H_{out} proton of the cavitand undergoes a significant upfield shift in $CDCl_3$ relative to DMSO ($\Delta\delta = -1.38$ ppm; Figure 1), which suggests a crowding of the upper rim of the cavitand by the aromatic guanine residues in $CDCl_3$. Diastereotopic protons H_a and H_b, which appear as one broad signal in DMSO, give a set of doublets in $CDCl_3$, one of which exhibits a considerable downfield shift ($\Delta\delta = 0.79$). Examination of CPK molecular models suggests that this proton (H_b) is relegated to outside of the anisotropic current of the aromatic rings upon formation of a G-quartet.

As to kinetic stability, above 30 °C, the rate of rotation about the amino C–N bond is fast on the ^{1}H NMR time scale, as an average signal is apparent for the NH_2 protons (see the Supporting Information).[21] This kinetic stability for cation-free TASQ **3c** is comparable to that of some cation-bound structures.[16] As to thermodynamic stability, there is only a small change ($\Delta\delta = -0.2$ ppm) in the chemical shift of the imino (NH) signal over a 100 K temperature range (−50 to

+50 °C). This indicates that the H-bonding remains largely intact even at 50 °C in $CDCl_3$.

Cations contribute to the stability and polymorphism of G-quadruplex structures. They can induce structural changes or trigger conformational transitions.[22] Similar observations have been made in lipophilic systems.[23] Thus, we investigated the recognition of TASQ **3c** with different cations. Extraction of solid sodium picrate by a $CHCl_3$ solution of **3c**, for example, induced changes in the ^{1}H NMR spectrum of **3c**.[24] At low temperature the signal for the H-bonded amino group shifted from $\delta = 9$ ppm (Na^+-free) to $\delta = 10$ ppm in the presence of Na^+ (see the Supporting Information). This observation supports the notion that the former system is indeed cation-free and that **3c** recognizes common G-quartet stabilizing cations.[25]

In biological systems, cation templation is the key stabilizing element of a G-quadruplex. H-bonding, hydrophobic interactions and the phosphodiester backbone are other factors important for stabilizing a G-quadruplex. Likewise, in lipophilic systems, cation templation overcomes the repulsive interaction of the carbonyl oxygen atoms in the central core of a G-quartet. Little attention has been paid to the role of an external backbone or templating scaffold. This study provides a model system of how a lipophilic G-quartet can be designed and synthesized with the help of an external template. This is an unusually stable cation-free G-quartet whose scaffold-induced unimolecularity provides structural integrity even at low concentrations. These findings suggest potential applications for future TASQs, for example as G-quartet aptamers or as G-quartet recognizing protein screens. Current efforts include exploration of cation-bound morphologies of lipophilic TASQs, and creation of hydrophilic TASQs.

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[24] After analogous extraction of K^+, Sr^{2+}, and Cs^+ picrates, distinct signals (from **3c** alone) were observed.
[25] Addition of [2.2.2]cryptand to the solution of **3c**·Na^+ resulted in a ^{1}H NMR spectrum identical to the spectrum of the cation-free species. Addition of [2.2.2]cryptand to cation-free **3c** resulted in no change (see Figure S15). Atomic absorption experiments indicate that there are 250 ppm of Na^+ present in **3c**, which is less than 3 mol%. This agrees well with the NMR results (see Figure S2), and confirms cation-free **3c** as a different entity from the sodium-bound system (see the Supporting Information).

