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Thermodynamic and Kinetic Stabilities of G-Quadruplexes in Apolar Solvents

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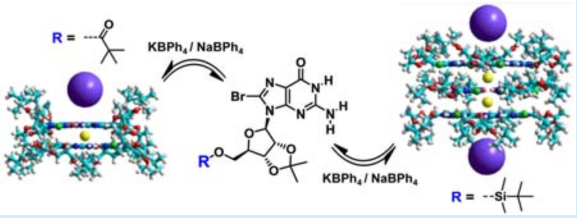
Supporting Information

ABSTRACT: In contrast to more polar media, G-quadruplex assembly reveals remarkably high thermodynamic and kinetic stability in toluene solutions. Depending on the substituents installed at the lipophilic guanosine, either a dodecamer or an octamer complex is formed in the presence of K$^+$ or Na$^+$ salts that resist conditions of high dilution and elevated temperatures without exhibiting significant dissociation. Moreover, kinetic exchange between complexed and uncomplexed G is slow enough in NMR to monitor G-quadruplex formation along a day time scale.



Molecular self-assembly[1] offers an excellent tool to easily and cheaply construct nanostructured objects in which the morphology and the relative organization of individual molecules can be controlled by rational selection of different supramolecular motifs.[2] The use of noncovalent interactions often fails, however, in providing nanoarchitectures that are monodisperse and persistent in size and shape in different conditions. On one hand, most intermolecular interactions ($\pi-\pi$ stacking, H-bonding, solvophobic interactions) promote supramolecular polymerization processes[3] and size control is generally complex to achieve. On the other hand, the weak nature of the noncovalent interactions that hold together these assemblies make them very sensitive to concentration and temperature changes, which affects considerably their homogeneity. The supramolecular synthesis of uniform stable nano-objects[4] is hence a challenging objective that needs to be addressed so that physical properties can be related to well-defined assemblies and the molecular organization is not altered with the surrounding environment.[5]

In this context, a remarkable example of self-assembled, discrete nano objects is represented by G-quadruplexes.[6] In organic solvents, guanine (G) or guanosine derivatives typically associate into loosely bound, rapidly exchanging hydrogen (H)-bonded oligomeric species. However, in the presence of alkaline salts (typically Na$^+$ or K$^+$), multiple noncovalent forces (H-bonding, $\pi-\pi$ stacking, cation-dipole coordination) work cooperatively to supply well-defined G-quadruplexes.[7] These assemblies are constituted by H-bonded cyclic tetramers (G-quartets) that can incorporate size-matching metal cations by coordination to eight internal carbonyl groups between $\pi-\pi$ stacked quartets. Three main types of quadruplexes are most commonly obtained in organic solvents: octamer (8mer), dodecamer (12mer), and hexadecamer (16mer), in which 8, 12, or 16 G molecules are complexing one, two, or three cations, respectively (Figure 1a).[8]

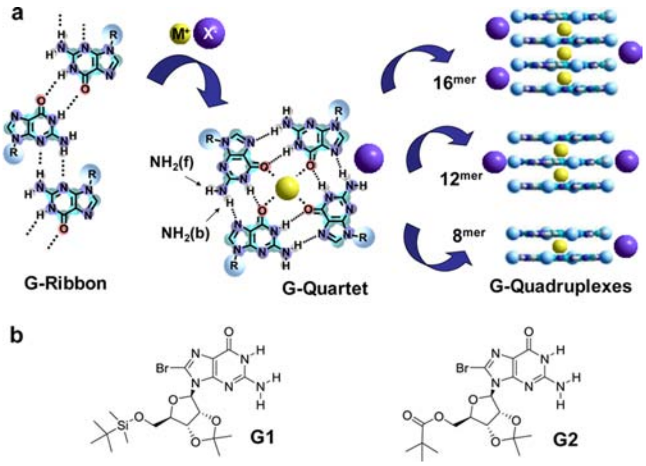


Figure 1. (a) G-quadruplex self-assembly in the presence of cations (M$^+$X$^-$) to yield octamer (8mer), dodecamer (12mer), and hexadecamer (16mer) complexes. (b) Structures of **G1** and **G2**.

We and other research groups have considered G-quadruplexes as ideal scaffolds to which functional organic molecules can be attached to build assemblies that are endowed with physical properties markedly different from their molecular constituents.[6c,9] So far, π-conjugated photoactive units like porphyrins,[9a] pyrene,[9b] oligo(thiophene),[9c] oligo(phenylenevinylene),[9d] perylenes,[9e,f] or phthalocyanines[9g] have been installed within discrete quadruplex frameworks. For instance, as demonstrated in the group of Wasielewski, the resulting dye

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nanoclusters may benefit from internal charge delocalization upon photoexcitation, which implies possible applications for organic photovoltaics.[9e,f] In order to process these noncovalent assemblies into thin-film device architectures and maintain intact their supramolecular structure, apolar solvents like CCl_4, toluene, or cyclohexane are preferred as transfer media because stability is supposed to increase.[9d,g] However, a detailed study of the self-assembly of simple guanine reference molecules in apolar solvents, so as to compare thermodynamic and kinetic features in environments of diverse polarity, has not been performed so far. Here, we investigate the kind of complexes formed by compounds **G1**[8c] and **G2**[10] (Figure 1b) in toluene solutions and demonstrate that their thermodynamic and kinetic stability is substantially enhanced,[11] which can guarantee the structural integrity of these nanoclusters when processed into devices.

First, the kind of assemblies formed by compounds **G1** and **G2** in toluene, in the presence of a small excess (1 equiv) of K^+ ($KBPh_4$ or KPF_6) or Na^+ ($NaBPh_4$ or $NaPF_6$) salts, was evaluated. Typically, a **G1**/**G2** toluene solution is stirred overnight with the corresponding salt. The G extracts the amount it needs to form the most stable complex, while the rest of the salt remains undissolved and it is removed afterward by centrifugation. Figure 2 shows the changes in the N−H^1 amide (13−10 ppm) and *tert*-butyl (1.5−0.5 ppm) regions of the ^1H NMR spectra of **G1** and **G2** after solid−liquid extraction with different salts. The use of $KBPh_4$ or $NaBPh_4$ produced similar complexes, characterized by three sets of signals for each proton for **G1** and one set of signals for **G2**.

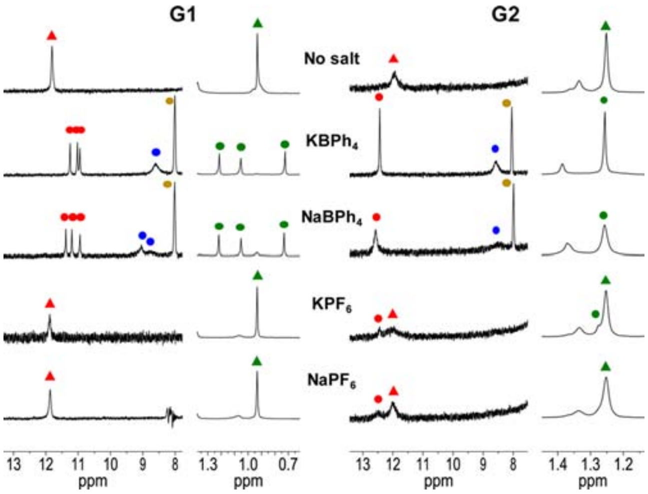

Figure 2. Amide and *tert*-butyl regions of the ^1H NMR spectra (10^{-2} M; 298 K, toluene-d_8) of **G1** and **G2** in the presence of different salts. Color code: red, amide NH protons; blue, amine (NH_2(b)) protons; brown, BPh_4^- protons; green, *tert*-butyl protons. Shape code: circles, complexed G; triangles, uncomplexed G.

Given the multiplicity observed for the **G1** and **G2** ^1H signals upon complexation, and by comparison with previously published data, we can anticipate the kind of complex formed in the presence of $KBPh_4$ or $NaBPh_4$ salts: a C_4-symmetric dodecamer (C_4-12mer) for **G1** and a D_4-symmetric octamer (D_4-8mer) for **G2**. In both cases, the aromatic signals of the extracted BPh_4^- anion can be observed as well. The shape and position of the N−H^1 amide signals are very informative in G-quadruplex chemistry. These are found as a broad signal at 11.8 and 11.9 ppm for 10^{-2} M toluene solutions of **G1** and **G2**, respectively. However, in the K^+ or Na^+ **G2** D_4-8mers this proton signal sharpens and shifts downfield to 12.45 and 12.55 ppm, respectively, which is indicative for the formation of octameric species with D_4-symmetry.[8c,d] On the other hand, in the **G1** C_4-12mers the N−H^1 amide proton is split into three signals, due to the nonequivalent nature of each quartet in the complex, that are found at 11.5, 11.25, and 11.2 ppm and at 11.6, 11.45, 11.2 ppm for the K^+ or Na^+ complexes, respectively. NOESY NMR (Figure S1) allowed us to determine which ^1H signals corresponded to the outer quartets and which to the inner quartet in the C_4-12mer complex. Moreover, although they need lower temperatures for higher resolution, the NH_2 amino protons split into H-bound (NH_2(b); found between 8.0 and 9.5 ppm) and solvent-exposed (NH_2(f); found in the 5.0−6.5 ppm region) signals on complexation in toluene.

In order to confirm G-quadruplex size, DOSY experiments (Figure S2) were performed in toluene comparing **G1** and **G2** with their corresponding complexes. The analysis of the diffusion coefficients[11b] provided the experimental hydrodynamic radii for each complex (R^e, see Figure S2), which are somewhat higher than the theoretical values (R^t) derived from computational models.[8c] This might be ascribed to the bulkiness of the surrounding BPh_4^- anion(s), which were not included in the theoretical hydrodynamic radii calculations and exhibit diffusion coefficients close to those of the complex. This finding, together with the intermolecular NOE cross-peaks observed between the anion and some ribose proton signals (see Figure S1), suggest an intimate union of the BPh_4^- anion to the complex. However, from our data we cannot conclude an exact position of the anions around the quadruplexes.

Remarkably, a single complex, either 12mer or 8mer, is obtained upon cation complexation by **G1** or **G2**, respectively. This underlines on one hand the sensibility and on the other the extraordinary fidelity of G-quadruplex self-assembly in toluene. Why these particular complexes are formed with **G1** and **G2** is something we do not fully understand yet. In this context, it is also interesting to note the strong influence of the anion. The use of KPF_6 or $NaPF_6$ resulted in very minor changes in the ^1H NMR spectra compared to uncomplexed **G1** or **G2** solutions, and only traces of a D_4-symmetric octamer were detected in toluene. The use of other salts, like KI, KCl, or KBF_4 in solid−liquid extraction processes, produced similar results, and a very inefficient complexation was noted. It looks that the chemical nature of BPh_4^-, being a low-coordinating anion that is shielded by four phenyl residues, is crucial to achieve quantitative complexation in apolar solutions.

Next, we subjected each of the four complexes (**G1**−$KBPh_4$−C_4-12mer, **G1**−$NaBPh_4$−C_4-12mer, **G2**−$KBPh_4$−D_4-8mer, and **G2**−$NaBPh_4$−D_4-8mer) to a series of experiments in which concentration or temperature was varied (Figure 3 and Figures S3 and S4). The results are quite revealing: the complexes in toluene are unusually stable and much less sensitive to concentration or temperature changes than those obtained in more polar solvents. It is in the concentration-dependent experiments where the fragility of the G-quadruplex in polar vs apolar solvents is better compared. For instance, as can be observed in Figure 3a and Figure S3, dilution of related C_4-12mer or D_4-8mer in acetone or THF results in total G-quadruplex dissociation below a 3×10^{-3} M concentration. In sharp contrast, the same potassium complexes remain stable in toluene until the NMR detection limits at 10^{-5} M.

These experiments also indicate that K^+ complexes are significantly more stable than Na^+ complexes, presumably due to

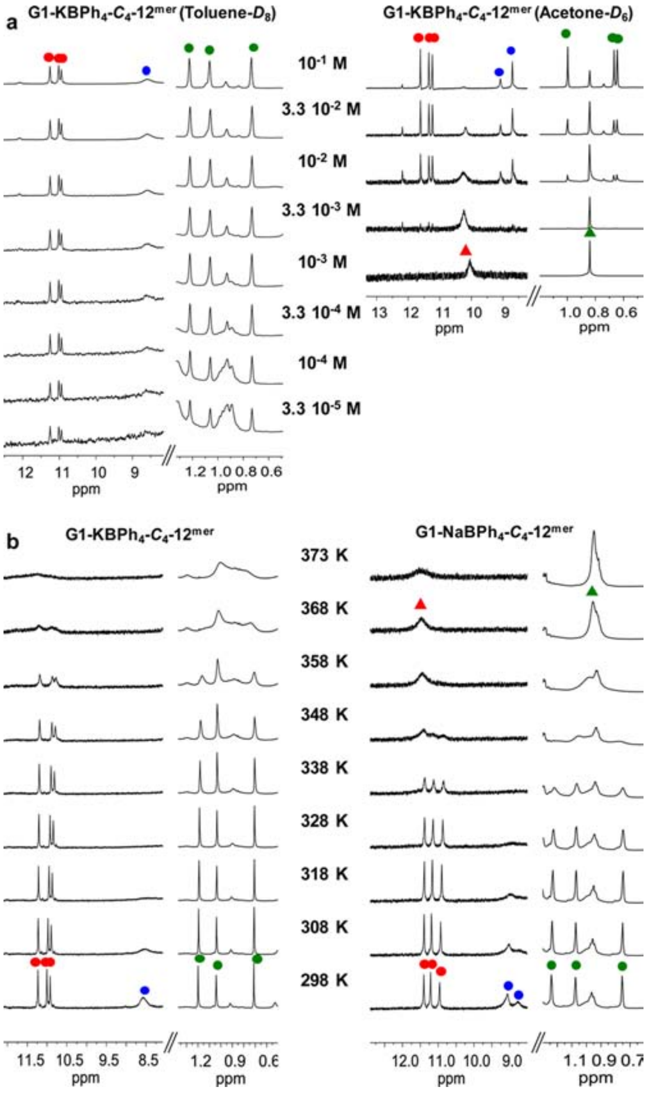

Figure 3. Evolution of the amide and *tert*-butyl regions of the ^{1}H NMR spectra of **G1** in the presence of $KBPh_4$ or $NaBPh_4$ as a function of (a) temperature (10^{-2} M in toluene-d_8) or (b) concentration in an apolar (toluene-d_8) or a polar (acetone-d_6) solvent (298 K). Color and shape code same as Figure 2.

a better fit in the cavity between stacked quartets. For instance, the three sets of sharp amide signals that are characteristic of C_4-12mer assemblies broaden considerably and merge into a single signal above 348 K for the Na^+ complex, whereas this phenomenon is not observed until 373 K for the related $KBPh_4$−C_4-12mer (Figure 3b). A similar trend was observed for the corresponding $NaBPh_4$− and $KBPh_4$−D_4-8mer **G2** complexes (Figure S4). In addition, the **G1**−C_4-12mer exhibits a higher stability than the **G2**−D_4-8mer under the same conditions. As shown in Figure S4, the **G1**−$KBPh_4$−C_4-12mer can maintain relatively sharp signals up to 358 K, while the **G2**−$KBPh_4$−D_4-8mer signals broaden considerably above 328 K, indicating rapid molecular exchange.

We then turned our attention to the kinetic characteristics of our complexes in toluene. A series of experiments were performed in which we evaluated the evolution of the NMR spectra with time just after producing different mixtures and until reaching new equilibrium conditions (i.e., a stationary state):

(1) A 1:1 mixture of complexed and the same uncomplexed G (i.e., **G1**−12mer + **G1**− and **G2**−8mer + **G2**, Figure S5).

(2) A 1:1 mixture of complexed and the other uncomplexed G (i.e., **G1**−12mer + **G2**− and **G2**−8mer + **G1**, Figure S6).

(3) A 1:1 mixture of the two complexes (i.e., **G1**−12mer + **G2**−8mer, Figure S7).

(4) A 1:1 mixture of the Na^+ or K^+ complex and the other $KBPh_4$ or $NaBPh_4$ salt (i.e., **G1**−$NaBPh_4$−12mer + $KBPh_4$ and **G1**−$KBPh_4$−12mer + $NaBPh_4$, Figure S8).

(5) A mixture of uncomplexed G and an excess of Na^+ or K^+ salt (i.e., **G1** + $NaBPh_4$, **G1** + $KBPh_4$, **G2** + $NaBPh_4$ and **G2** + $KBPh_4$; Figure 4 and Figure S9).

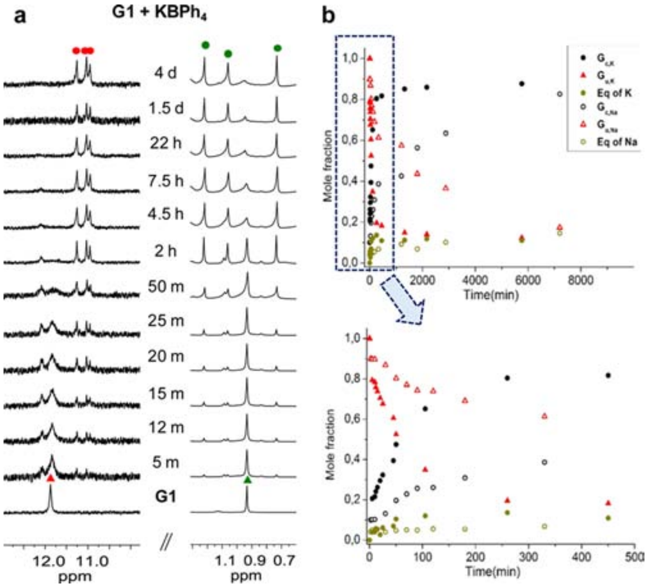

Figure 4. (a) Evolution of the amide and *tert*-butyl regions of the ^{1}H NMR spectra of **G1** after mixing with a small excess of $KBPh_4$ in toluene-d_8 (298 K; 10^{-2} M). m = minutes; h = hours; d = days. Color and shape code same as Figure 2. (b) Evolution with time of the relative molar fraction of (1) complexed G (black circles), (2) uncomplexed G (red triangles), (3) solubilized salt (brown circles). Filled circles: in the presence of $KBPh_4$. Open circles: in the presence of $NaBPh_4$. Bottom: magnification of the initial evolution.

Obviously, mixtures 1 should not display any evolution with time and result in an equilibrium where complexed (G_C) and uncomplexed G (G_U) are exchanged in solution. We have to note, however, that we did observe some changes with time. Specifically, a small amount of complex was further produced when adding free G, which indicates that there was some extra salt available in the pristine quadruplex solutions. This excess of salt may come from incomplete separation after centrifugation or to partial, weak complexation of the salt by the outer quartet faces of the complex. In any case, we increased the amount of G added to reach a G_C/G_U 1:1 equilibrium and then analyzed the mixtures by EXSY NMR to determine the exchange rates, since complex and free G are clearly in slow exchange in the NMR time scale (see Figure S5, for example). Unfortunately, although several attempts were made, we were not successful in reliably integrating cross-peaks between the relevant exchanging species, and no quantitative data could be derived.

In mixtures 2−4 we combined different components that should logically lead to a complex mixture of G-quadruplexes with time. We were nonetheless interested to see how fast those equilibria were reached and if any complex was particularly stabilized in such mixtures. As a general rule (see Figures S6−S8), equilibrium was reached very slowly, on the scale of several hours, which is ascribed to a slow subcomponent exchange. Products and intermediates were always complex mixtures of quadruplexes where diverse components are mixed (i.e., **G1** and **G2**, Na^+ and K^+).

More interesting was the evolution of mixtures 5. Here, we are actually monitoring complex formation, which, to the best of our knowledge, has never been performed before in G-quadruplex chemistry, probably due to its rapid formation in the usual solvents. In toluene, in contrast, the evolution of the different species can be followed along an entire day, which is probably due to the scarce solubility of the Na^+ and K^+ salts. Figure 4a shows, as a selected example extracted from Figure S9, the change in the ^1H NMR spectra as the C_4-12mer complex is formed from **G1** and $KBPh_4$ during the course of 4 days. It should be remarked that along these experiments we could even identify key intermediates. For instance, the presence of small amounts of a **G1**−$KBPh_4$−D_4-8mer, having a characteristic amide signal above 12.0 ppm is clearly noted during formation of the corresponding **G1**−$KBPh_4$−C_4-12mer (Figure 4a).

These experiments also allowed us to compare the formation kinetics of K^+ vs Na^+ complexes as well as 8mer vs 12mer assemblies (Figure S9). While no difference was noted regarding the size of the complex, it appears that **G1**−$KBPh_4$−C_4-12mer and **G2**−$KBPh_4$−D_4-8mer are formed significantly faster that their $NaBPh_4$ analogues (Figure 4b). We mainly ascribe these trends to the different solubilization rate of the salt in toluene. In addition, in analogy to a covalent reaction, the formation of each K^+ or Na^+ complex can be stopped at any given time (by removing the excess of salt via centrifugation) so as to control G-quadruplex yield.

Our results shed light on the remarkably high fidelity and thermodynamic stability and on the slow kinetic characteristics of G-quadruplexes in apolar aromatic solvents like toluene. When combined with suitable functional units, these valuable attributes may be used for the noncovalent synthesis of self-assembled functional nanoclusters whose physical properties could be related to well-defined structures that are not altered significantly by changing environment conditions.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b03606.

> Experimental details and Figures S1 (NOESY NMR), S2 (DOSY NMR), S3, S4 (concentration and temperature-dependent NMR experiments), and S5−S9 (time evolution of NMR spectra in different mixtures) (PDF)

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Notes

The authors declare no competing financial interest.

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