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Construction of a cross-layer linked G-octamer via conformational control: a stable G-quadruplex in H-bond competitive solvents†

Ying He,^{‡a} Yanbin Zhang,^{‡b} Lukasz Wojtas,^a Novruz G. Akhmedov,^c David Thai,^a Heng Wang,^a Xiaopeng Li,^a Hao Guo^{ID}*^b and Xiaodong Shi^{ID}*^a

Methanol soluble and stable guanosine octamers were successfully achieved via H-bond self-assembly. Through structural conformational design, we developed a new class of guanosine derivatives with modification on guanine (8-aryl) and ribose (2',3'-isopropylidene). This unique design led to the formation of the first discrete G₈-octamer with its structure characterized by single crystal X-ray diffraction, MS and NMR spectroscopy. The G₈-octamer showed unique cation recognition properties, including the formation of a stable Rb⁺ templated G-quadruplex. Based on this observation, further modification on the 8-aryl moiety was performed to incorporate a cross-layer H-bond or covalent linkage. Similar G-octamers were obtained in both cases with structures confirmed by single crystal X-ray diffraction. Furthermore, the covalently linked G-quadruplex exhibited excellent stability even in MeOH and DMSO, suggesting a promising future for this new H-bond self-assembly system in biological and material applications.

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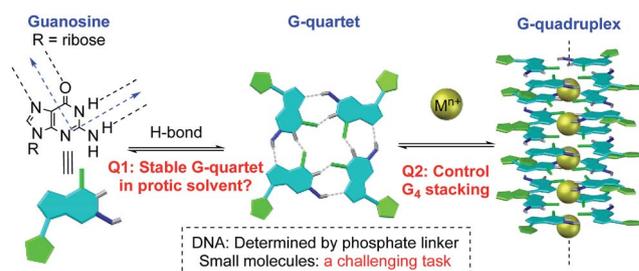
Introduction

Non-covalent interactions enable construction of large structural motifs from small molecules.^{1–3} Molecular self-assembly, an important process typically driven by non-covalent interactions, is often dynamic and generally under thermodynamic control.^{4,5} With interest in the application of the self-assembly process to biomedical research, there is a growing demand with respect to preparing stable non-covalent assemblies in a biocompatible environment.^{6–8} However, supramolecular structures built through H-bonds are often studied in less polar aprotic solvents (such as CH₂Cl₂) to avoid the competition of H-bond interactions between substrates and solvents.^{9,10} Thus, the development of novel supramolecular systems which are stable in a polar protic solvent is highly desirable, though very challenging.^{11,12}

A G-quartet is an interesting supramolecular scaffold formed by H-bonds.^{13,14} As shown in Scheme 1, with an approximately 90-degree angle between the H-bond donor and acceptor, four

guanine units are held together to form a G-quartet. Through ion–dipole interactions, alkali and alkaline earth metal cations can enhance the process by serving as the template to coordinate with central oxygen atoms.^{15–17} Stacking of G-quartets gives G-quadruplexes as bioactive building blocks found in DNA and RNA folding.^{18,19} In this case, the extent of G-quartet stacking in a G-quadruplex will be determined by the phosphate backbone, which is often associated with the formation of a counter folding subunit, such as the i-motif.²⁰

Inspired by this unique H-bond assembly, researchers have been devoted to developing guanine derivatives to achieve controllable G-quadruplex formation from small molecules.^{21–23} Some interesting applications have been identified with various G-quartet assemblies, including lipophilic ion channels,^{24–26} supramolecular hydrogels,^{27–29} nanomaterials,^{30,31} potential targets for cancer therapy,^{32,33} and more.^{34–37} Although many examples of G-quartet formation through various modified



Scheme 1 G-quadruplex formation: equilibrium and stability.

^aDepartment of Chemistry, University of South Florida, 4202 E. Fowler Avenue, Tampa, Florida 33620, USA. E-mail: xmshi@usf.edu

^bDepartment of Chemistry, Fudan University, 2005 Songhu Road, Shanghai, 200438, People's Republic of China

^cDepartment of Chemistry, West Virginia University, Morgantown, WV 26505, USA

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‡ Equal contribution.



guanine derivatives have been reported, studies on controlling G-quartet stacking molecularly are relatively rare.^{38,39} Factors to be taken into consideration include the cation concentration,³⁹ the solvent,⁴⁰ the anion,⁴¹ and so on.⁴² In many cases, mixtures of various “stacking isomers” (G_8 , G_{12} or G_{16}) were observed, which highlights the significant challenges associated with controlling the vertical stacking.^{43–46} Moreover, the assembly of a specific and stable G-quadruplex in H-bond competitive solvents remains a challenging task.⁴⁸ Herein, we report the construction of the first G-octamer with structures characterized by single crystal X-ray diffraction through monomer conformational design. Moreover, with this new system, stable G-quadruplexes were formed with significantly improved stability. Through the design of cross-layer H-bonds and covalent linkage, G-octamers were prepared with excellent stability in MeOH (no dissociation) and even in 50% DMSO, which offers a potential opportunity to extend the H-bond assembly system into biosystems for future applications.

Results and discussion

Design, synthesis and characterization of G-octamers

Ideally, a G_4 -tetramer would be the most concise target towards the construction of a simple and stable assembled structure. However, with a metal template in solution, further stacking of G_4 -tetramers leads to the mixture of G-quadruplex species.⁴⁹ Thus, G_4 -tetramers are unfavorable for the formation of well-defined supermolecules.

The simplest plausible G-quadruplex would be a G-octamer which is likely to adopt either top-to-top (T-T) or bottom-to-bottom (B-B) stacking patterns (Scheme 2A).⁵⁰ In previous

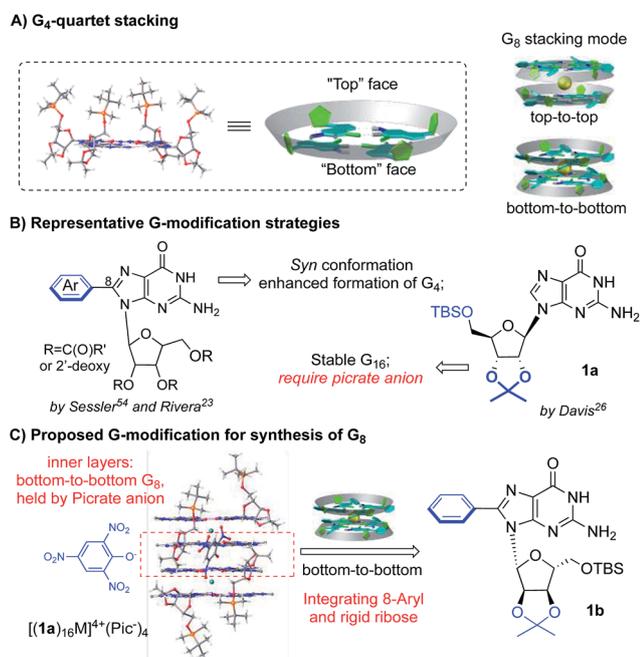
studies, Meijer *et al.* employed different concentrations of a guanosine derivative resulting in the formation of tunable G-octamers.⁴¹ Spada has reported G-octamer formation upon exposure to UV light through alkene isomerization.⁵¹ Wu presented several excellent examples of G-octamer formation through π - π interactions using NMR and MS studies.⁵² Hirao and coworkers applied Au(I)-Au(I) interaction between two G-quartet layers to achieve a G-octamer confirmed by NMR and CD spectra.⁵³ However, to the best of our knowledge, no single crystal structure of a G-octamer has ever been reported, implying the challenging nature of preparing a stable and discrete G-octamer in a dynamic equilibrium.

To tackle this problem, we set out to design a G-derivative where the structure is rigid and predisposed for the conformation of a potential octamer structure so as to minimize the entropy cost involved in the self-assembly process. As shown in Scheme 2B, modification of guanosine often occurs on two positions: C-8 of purine and the hydroxyl of ribose. The Sessler group first reported on 8-aryl substituted guanosine in the formation of a G-quartet without templating cation in both solution and solid state.⁵⁴

This seminal work initiated the concept of conformational control for G-quartet formation: the steric effect between the aryl substituent and protected ribose helped guanosine to adopt a *syn* conformation, preventing the ribbon formation and giving a tetramer as the dominant conformation. On the other hand, Davis's group developed lipophilic guanosine with bulky ribose to form a G-hexadecamer (Scheme 2B) both in solution and solid state ($M^{n+} = K^+, Ba^{2+}, Sr^{2+},$ and Pb^{2+}).⁵⁵ Notably, a picrate anion bridge played a crucial role: as revealed by single crystal X-ray diffraction (Scheme 2C), four picrate anions linked two G-octamers through H-bonds between anion and two inner G-quartets. The two G-octamers (from adjacent inner and outer G_4) gave top-to-bottom stacking with ribose interdigitated between the adjacent layers. Interestingly, the two inner layers adopted bottom-to-bottom (B-B) stacking, which is more favorable than the T-T mode with the cation binding on the more “naked” convex face between the two layers. This result aroused our interest in developing a G-octamer through similar B-B stacking.

Considering the steric interaction between the C-8 substituent and ribose, we postulated that incorporation of C-8 aryl and the rigid ribose ring might provide a new system with steric hindrance between the G-quartet to force the G_4 bowls to stack in a bottom-to-bottom manner, while obstructing ribose interdigitation at the top-face (Scheme 2C). To confirm this idea, compound **1b** was designed, prepared and applied to assemble with various alkali and alkaline earth metal cations. ¹H NMR spectra were obtained and selected regions of the ¹H NMR spectra of these G-quadruplexes were compared with the G-hexadecamer from **1a** as shown in Fig. 1.

As previously reported, treating **1a** with alkali and alkaline earth metal salts (K^+, Ba^{2+}, Sr^{2+} and so on) gave two sets of signals in the ¹H NMR spectra, corresponding to the inner and outer G-quartet.⁵⁶ Conducting similar cation binding experiments with **1b** in $CDCl_3$ gave a single set of ¹H NMR signals in all cases ($M = K^+, Ba^{2+},$ and Sr^{2+} ; $A^- = Picrate^-$ or PF_6^-). Furthermore, ESI-MS demonstrated a clear doubly charged



Scheme 2 Achieving a stable G-octamer by controlling G-quartet stacking.



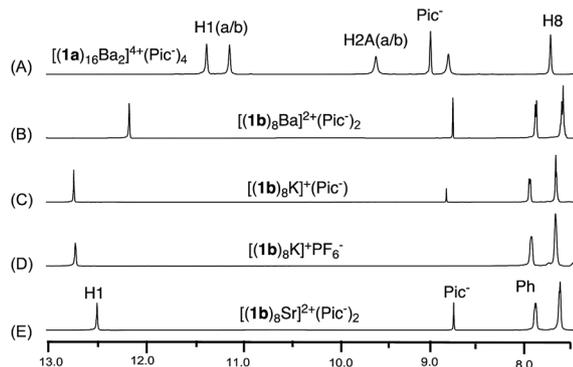


Fig. 1 ^1H NMR spectra of G-quadruplexes (A) $[(\mathbf{1a})_{16}\text{Ba}_2]^{4+} \cdot (\text{Pic}^-)_4$; (B) $[(\mathbf{1b})_8\text{Ba}]^{2+} \cdot (\text{Pic}^-)_2$; (C) $[(\mathbf{1b})_8\text{K}]^+ \cdot (\text{Pic}^-)$; (D) $[(\mathbf{1b})_8\text{K}]^+ \cdot (\text{PF}_6^-)$; (E) $[(\mathbf{1b})_8\text{Sr}]^{2+} \cdot (\text{Pic}^-)_2$ in CDCl_3 .

peak at $m/z = 2123.01$, corresponding to a mol. wt of 4246.68 for $[(\mathbf{1b})_8\text{Ba}]^{2+}$. The experimental and calculated isotope patterns further suggested an octameric composition. In addition, traveling wave ion mobility-mass spectrometry (TWIM-MS)⁵⁷ confirmed no formation of stacking isomers, which excluded the formation of random aggregates in gas phase (see ESI† for details).

Finally, single crystal structures were obtained and unambiguously verified the G_8 -octamer formation with the proposed bottom-to-bottom stacking (Fig. 2A). The top view of the crystal structure (Fig. 2B) shows the G-quartet self-assembly in the tail to tail orientation. The five crystal structures obtained with ligand $\mathbf{1b}$ include monovalent cations (K^+ and Rb^+) and divalent cations (Ba^{2+} and Sr^{2+}). Picrate anion showed no clear binding with the G-quartet, consistent with what was observed in the ^1H NMR spectra (see Fig. S2†). A complex with non-coordinated PF_6^- anion was also successfully obtained, $[(\mathbf{1b})_8\text{K}]^+ \cdot (\text{PF}_6^-)$, confirming the “anion-free” binding mode of this new type of G_8 -quadruplex. The distances of the H-bond within the G_4 -quartet and between the G_4 layers are compared in Table 1.

According to these crystal structures, all G_8 -octamers from $\mathbf{1b}$ gave G_4 -quartets with an (N1...O6) and (N2...N7) H-bond distance of around 2.9 Å, similar to that of the inner and outer layer in the G_{16} -hexadecamer formed from $\mathbf{1a}$.⁴⁶ These results indicated that both cations and the C-8 phenyl substituent had little influence on the H-bond in the G_4 -quartet.

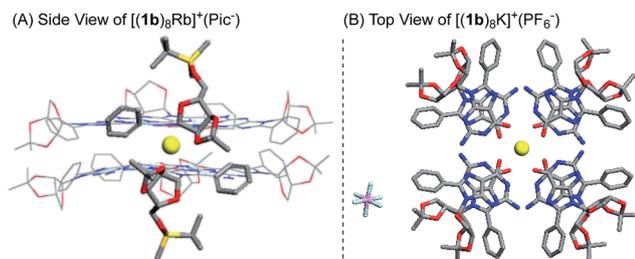


Fig. 2 X-ray single crystal structure of the G_8 -octamer (A) $[(\mathbf{1b})_8\text{Rb}]^+ \cdot (\text{Pic}^-)$; (B) $[(\mathbf{1b})_8\text{K}]^+ \cdot (\text{PF}_6^-)$. Similar structures for $[(\mathbf{1b})_8\text{K}]^+ \cdot (\text{Pic}^-)$, $[(\mathbf{1b})_8\text{Ba}]^{2+} \cdot (\text{Pic}^-)_2$ and $[(\mathbf{1b})_8\text{Sr}]^{2+} \cdot (\text{Pic}^-)_2$ were also obtained.†

However, the size of the G_8 was influenced by the average O–M distances of all these complexes from $\mathbf{1b}$, which might follow the trend where higher ionic potential (Z/r) resulted in shorter O–M distance (2.75–2.81 Å). Interestingly, in comparison with the G_{16} -hexadecamer $[(\mathbf{1a})_{16}\text{Ba}_2]^{4+} \cdot (\text{Pic}^-)_4$, the G_8 -octamers $[(\mathbf{1b})_8\text{Ba}]^{2+} \cdot (\text{Pic}^-)_2$ gave a slightly shorter distance between the two G_4 layers (2.89 Å vs. 3.06 Å and 3.58 Å). This result implied the stronger cation interaction of the $(\mathbf{1b})_4$ -quartet than the $(\mathbf{1a})_4$ -quartet. This improved cation interaction has been supported by G_4 -binding studies with Rb^+ using the $\mathbf{1b}$ ligand and led to the first crystal structures of Rb^+ coordinated G -quadruplexes. Among all the G_8 crystals, $[(\mathbf{1b})_8\text{Rb}]^+ \cdot (\text{Pic}^-)$ gave the longest O–M and G_4 – G_4 distance due to its large radii⁵⁸ and low valency. Very few examples of Rb coordinated G -quartets have been reported so far, indicating how challenging it is for guanosine to bind with Rb to form a discrete G -quadruplex.⁵⁹ To the best of our knowledge, this is the first single crystal structure of a G -quadruplex containing Rb^+ , clearly suggesting the promising cation binding ability of guanosine derivative $\mathbf{1b}$.

Having successfully confirmed a new concise G_8 -quadruplex structure in solution (NMR), solid state (XRD) and gas phase (ESI-MS and TWIM-MS), we evaluated its stability in MeOH. Dissolving octamer $[(\mathbf{1b})_8\text{K}]^+ \cdot (\text{Pic}^-)$ in CD_3OD gave a mixture of two sets of signals in NMR spectra, suggesting partial decomposition of this G_8 -octamer (*vide infra*). To obtain MeOH stable G -quadruplexes, further modification is still needed.

Cross-layer H-bonded G_8 -octamers

To further improve the stability of the G -quadruplex, we sought to establish the interactions between the G -quartet layers. As highlighted in Fig. 2A, the 8-phenyl group in $\mathbf{1b}$ adopted a tilted conformation and reached out from the G -quartet. This geometry provided an opportunity to further enhance the supramolecular structure by introducing new interactions between the two G_4 -quartets.

Notably, the Rivera group have reported the formation of an intralayer H-bond between carbonyl oxygen with N(2)H within the same G -quartet by using 2'-deoxy guanosine derivatives without rigid ribose functionalization.^{34,36–38} This work suggested the possibility of forming extra H-bonds by using both hydrogens of the N(2)– NH_2 group. Inspired by this work, a carbonyl group was introduced at the *meta*-position of the 8-aryl position of $\mathbf{1b}$ as illustrated in Fig. 3A. Based on this design, we hypothesized that N(2)– H_A would form a H-bond with neighboring guanosine within the G -quartet, while the N(2)– H_B could interact with the carbonyl group by forming a cross-layer H-bond.

To confirm this idea, compound $\mathbf{1c}$ was synthesized and applied to G -quadruplex construction upon interacting with metal cations. According to the ^1H NMR spectra, treating $\mathbf{1c}$ with $\text{Ba}(\text{Pic})_2$ led to the formation of a new G -quadruplex with one set of signals, similar to the G_8 -octamer obtained from $\mathbf{1b}$. Analysis of the NMR sample (in CDCl_3) by ESI-MS gave a dominant, doubly charged peak with m/z at 2291.34, corresponding to $[(\mathbf{1c})_8\text{Ba}]^{2+}$ (mw = 4582.68).



Table 1 G-quadruplex structural comparison (Å)

G-quadruplex		$d(N1\cdots O6)$	$d(N2\cdots N7)$	$d(O-M)$	$d(G_4-G_4)$
[(1a) ₁₆ Ba ₂] ⁴⁺ ·(Pic [−]) ₄ ^a	Inner	2.92 ± 0.01	2.91 ± 0.07	2.75 ± 0.02	3.06 (i-o)
	Outer	2.86 ± 0.01	2.89 ± 0.04	2.79 ± 0.03	3.58 (i-i)
[(1b) ₈ K] ⁺ ·(Pic [−])		2.82 ± 0.08	2.87 ± 0.04	2.72 ± 0.17	2.85
[(1b) ₈ K] ⁺ ·(PF ₆ [−])		2.82 ± 0.04	2.89 ± 0.05	2.77 ± 0.03	2.96
[(1b) ₈ Ba] ²⁺ ·(Pic [−]) ₂		2.89 ± 0.04	2.89 ± 0.04	2.72 ± 0.05	2.89
[(1b) ₈ Sr] ²⁺ ·(Pic [−]) ₂		2.83 ± 0.03	2.86 ± 0.04	2.62 ± 0.05	2.75
[(1b) ₈ Rb] ⁺ ·(Pic [−])		2.85 ± 0.03	2.89 ± 0.03	2.81 ± 0.05	3.04

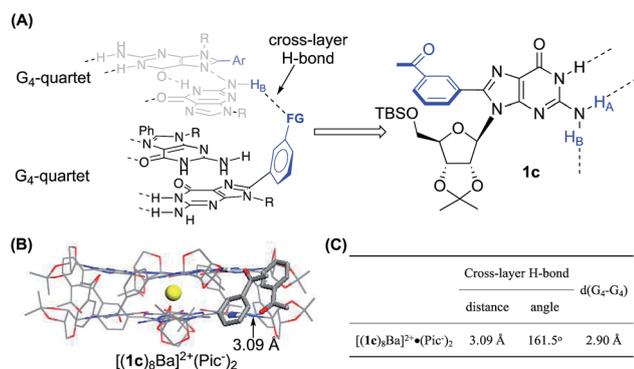
^a See ref. 46.

Fig. 3 (A) General design of establishing a cross-layer H-bond; (B) single crystal structure and (C) H-bond information of [(**1c**)₈Ba]²⁺·(Pic[−])₂.

Having confirmed the G₈-octamer [(**1b**)₈Ba]²⁺·(Pic[−])₂ formation, our next goal was to determine if there was a cross-layer H-bond as designed above. The ¹H NMR spectra of [(**1b**)₈Ba]²⁺·(Pic[−])₂ and [(**1c**)₈Ba]²⁺·(Pic[−])₂ did not show peaks corresponding to N(2)H at room temperature. This is likely due to the rapid exchange between the two NH₂ protons, even with the formation of a H-bond. Thus, the exchange rate of the two protons provides a direct indication of the H-bond strength in the G₄-quartet. To explore the dynamic structure, variable temperature (VT) NMR experiments with [(**1b**)₈Ba]²⁺·(Pic[−])₂ and [(**1c**)₈Ba]²⁺·(Pic[−])₂ were performed and are summarized in Fig. 4.

For complex [(**1c**)₈Ba]²⁺·(Pic[−])₂, the NH₂ protons started appearing as broad peaks at 0 °C with the chemical shift at 10.28 ppm (H_A) and 7.25 ppm (H_B). In contrast, the VT NMR

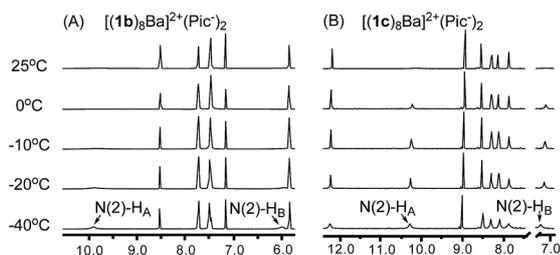


Fig. 4 (A) VT NMR spectra of [(**1b**)₈Ba]²⁺·(Pic[−])₂ and (B) VT NMR spectra of [(**1c**)₈Ba]²⁺·(Pic[−])₂ confirmed the cross-layer H-bond design in establishing a cross-layer H-bond.

spectra of [(**1b**)₈Ba]²⁺·(Pic[−])₂ did not show apparent peaks of the N(2)–H signals until further cooling the sample to −40 °C. The results indicated that there might be an extra H-bond in the **1c** complex to lock the N(2)–NH₂ from rapid exchange. Furthermore, a significantly downfield shifted chemical shift (7.25 ppm) was ascribed to the N(2)–H_B proton in the (**1c**)₈-octamer compared with the (**1b**)₈-octamer (5.98 ppm). These observations provide clear evidence of the formation of a cross-layer H-bond in the (**1c**)₈-octamer as designed.

Finally, the G₈-octamer was verified by X-ray crystallography as shown in Fig. 3B. The crystal structure also confirmed the presence of the cross-layer interactions with a mean H-bond distance of 3.09 Å and a bond angle of 161.5°, suggesting a weak cross-layer H-bond present in solid state (Fig. 3C). This makes the structure a “self-assembled molecular-cuboid” purely constructed by H-bond linkage with all eight guanosine units. On the other hand, the distance of the two G-quartet layers (2.90 Å) in [(**1c**)₈Ba]²⁺·(Pic[−])₂ remained similar to the **1b** complex. Considering the specific “cage” size in the G₈-octamer shown in Table 1, the cross-layer H-bond was not strong enough to generate extra enthalpy gain to balance the entropy cost caused by holding the two layers tighter.

With the confirmed cross-layer H-bonds, we evaluated the complex stability of [(**1c**)₈K]⁺·(Pic[−]) in methanol. The results showed a similar stability to the complex formed with **1b** (*vide infra*). Although the cross-layer H-bond approach could not boost G₈-quadruplex stability in MeOH as anticipated, it provided an effective and novel approach to enhance G-quadruplex stability from monomer conformational design. Structural amendment was required to further improve the stability of the G₈-octamer.

Cross-layer linkage through a covalent bond

To increase the stability to a new level, we turned to establishing a potential covalent linkage between the two G-quartets. By scrutinizing the crystal structure of [(**1b**)₈Ba]²⁺·(Pic[−])₂, we found that the distance between the two *meta* position of the phenyl ring from each tetramer was 4.0 Å, a distance similar to three single bond lengths.⁵⁸ According to the observation, the *meta* position of the two phenyl groups could serve as a reference site for constructing cross-layer covalent linkers. The guanosine dimer **1d** and **1d'** were then prepared using the synthetic route summarized in Fig. 5A.



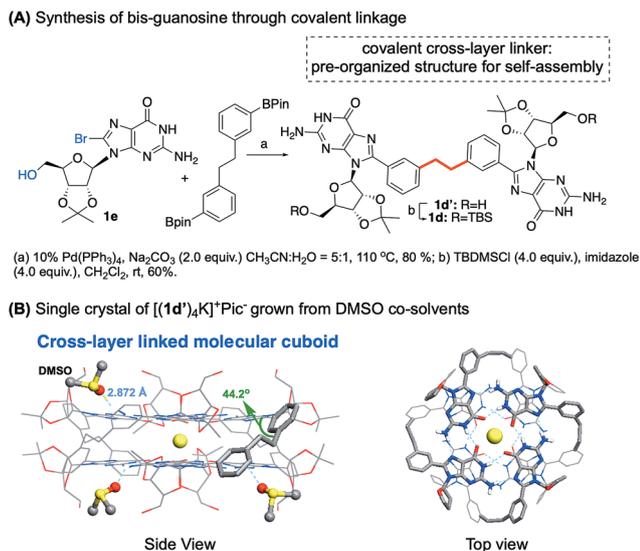


Fig. 5 (A) Synthesis of bis-guanosine derivatives. (B) Single crystal structure of [(1d')₄K]⁺·(Pic⁻)₂.

Self-assembly of **1d** with K⁺ and Ba²⁺ cations in CDCl₃ gave a similar one-set of signals in the ¹H NMR spectra, consistent with the formation of a G₈-octamer. ESI-MS of complexes from **1d** and Ba²⁺ gave a doubly charged peak at *m/z* = 2175.55 as the dominant signal, indicating a mol. wt of 4351.1 for the supermolecule as [(1d)₄Ba]²⁺. An attempt to obtain a single crystal of the **1d** complex failed initially, resulting in a rather thin, film-like solid formation. Fortunately, the single crystal structure was successfully obtained by switching the monomer to **1d'** using DMSO as a co-solvent, confirming the cross-layer covalent linked structure as proposed (Fig. 5B). Notably, for complex [(1d')₄Ba]²⁺·(Pic⁻)₂, the dihedral angle between 8-aryl and guanine is 40.7 degrees, similar to the dihedral angles in complex [(1b)₈Ba]²⁺·(Pic⁻)₂ (42.5 degrees). Overall, through G-monomer conformational analysis, a series of G₈-octamers was successfully prepared with functionalization at 8-phenyl (**1b**), a cross-layer H-bond linker (**1c**) and a covalent linker (**1d**).

G-quadruplex H-bond stability in MeOH

As discussed above, our intrinsic motivation in exploring these different G-quadruplexes was to develop H-bonded guanosine self-assembly that could survive in protic solvents (H-bond competitive). With all these different G₁₆ and G₈ quadruplexes prepared, we dissolved them in CD₃OD to compare the ¹H NMR spectra. As shown in Fig. 6A, N(1)–H and N(2)–H protons did not appear in ¹H NMR spectra with CD₃OD as the solvent due to the H/D exchange. Thus, evaluation of the ¹H NMR spectra will mainly be focused on the non-exchangeable aromatic protons and ribose protons. Dissolving the G₁₆-hexadecamer [(1a)₁₆-K₄]⁴⁺·(Pic⁻)₄ in CD₃OD gave only one set of signals, identical to the **1a** monomer in CD₃OD. The result suggested complete dissociation of the G₁₆-hexadecamer to **1a** monomer in MeOH. Interestingly, the G₈-octamer [(1b)₈K]⁺·(Pic⁻) and [(1c)₈-K]⁺·(Pic⁻) in CD₃OD gave two sets of signals, indicating the existence of dissociated monomer and possible oligomers or

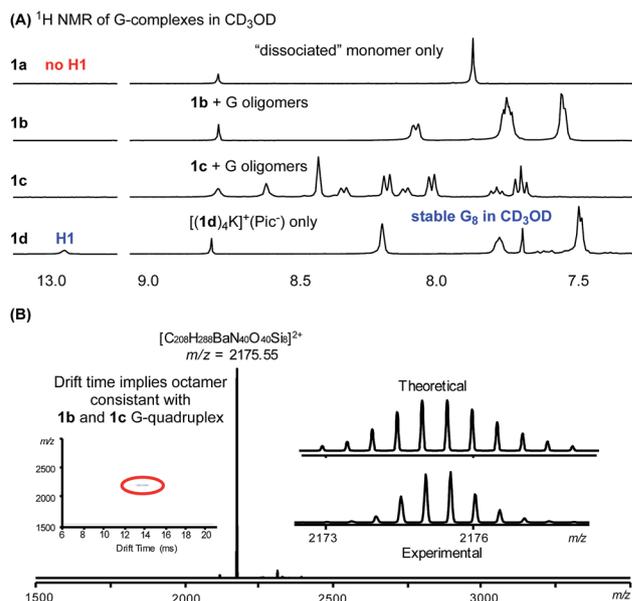


Fig. 6 (A) ¹H NMR spectra showing G-quadruplex stability in CD₃OD. (B) MS of [(1d)₄Ba]²⁺·(Pic⁻)₂ in MeOH.

a G-quadruplex in the solution phase. Although the exact structures of the guanosine species in these two cases are not determined at this moment, the fact that H-bonded guanosine complexes were formed with **1b** and **1c** clearly suggests the improved H-binding ability of these two monomers over **1a**. Surprisingly, when dissolving the G₈-octamer [(1d)₄K]⁺·(Pic⁻) in CD₃OD, only one set of signals was observed. Notably, in this case, N(1)–H gave a broad signal at 12.95 ppm, clearly suggesting the formation of a G-quadruplex through a H-bond. NMR solvent signal suppression was applied for the G-quadruplex [(1d)₄-K]⁺·(Pic⁻) in CD₃OH. The peak at 12.9 ppm clearly showed up and was confirmed to be H1 of G in the G-quadruplex (see detailed NMR spectra in Fig. S7†). Thus, with monomer **1d**, a G-quadruplex remains intact in protic solvent CD₃OD. Impressively, this G-quadruplex did not dissociate even at elevated temperature in CD₃OD, with the N(1)H peak remaining at 60 °C (see Fig. S6† for VT NMR spectra). This observation indicated that there was a high kinetic barrier to break the **1d** G-quartet for H/D exchange, which highlighted the stability of [(1d)₄K]⁺·(Pic⁻) in a H-bond competitive system. Injecting an MeOH solution of [(1d)₄-Ba]²⁺·(Pic⁻)₂ complex into ESI-MS gave a dominant double charged peak with *m/z* = 2175.55, corresponding to [(1d)₄Ba]²⁺ (Fig. 6B). It is noteworthy that TWIM-MS of this G-quadruplex in methanol solution was recorded as a single band (*m/z* = 2175.55) with drift time at 14.33 ms, which is in agreement with the size of the G₈-octamer (see detailed discussion in Table S1†). To the best of our knowledge, this is one of the few stable G-quadruplex systems from small molecule self-assembly to survive in a H-bond competitive environment.

Evaluating G-quadruplex stability

With the success in maintaining G-quadruplex stability in protic solvent MeOH, we sought to evaluate whether a similar



stability trend exists with polar aprotic solvents. DMSO is a strong polar solvent, which can disrupt the H-bond in G-quartets and cause the decomposition of G-quadruplexes. To evaluate how the incorporation of the phenyl group and cross-layer interaction impact on thermodynamic stability, G-quadruplexes were treated with $\text{CDCl}_3/\text{DMSO-d}_6$ solvent mixture. A summary of ^1H NMR spectra from these experiments is shown in Fig. 7.

As shown in the ^1H NMR spectra, G-quadruplexes from **1a**, **1b** and **1c** started to dissociate in mixed solvents containing 20% DMSO-d_6 . Compared with the reported G_{16} -hexadecamer from **1a**,⁴⁶ the G_8 -octamer formed by **1b** and **1c** showed comparable stability in 20% DMSO-d_6 . Eventually, all three G-quadruplexes gave complete dissociation in 50% DMSO-d_6 solution with only one set of signals corresponding to the monomer. In contrast, $[(\mathbf{1d})_4\text{K}]^+(\text{Pic}^-)$ showed significantly improved stability, with only 2% complex dissociation in 50% DMSO-d_6 . This result demonstrates the significantly enhanced stability of G-quadruplexes constructed by **1d**.

To quantify the thermodynamic stability of G-quadruplexes,⁴⁷ VT NMR experiments were carried out for pure complexes of $[(\mathbf{1a})_{16}\text{K}_4]^{4+} \cdot (\text{Pic}^-)_4$, $[(\mathbf{1b})_8\text{K}]^+(\text{Pic}^-)$, $[(\mathbf{1c})_8\text{K}]^+(\text{Pic}^-)$, and $[(\mathbf{1d})_4\text{K}]^+(\text{Pic}^-)$ in $\text{CDCl}_3/\text{DMSO-d}_6$ with a fraction of 20% DMSO-d_6 . The values of complex dissociation enthalpy and entropy for each G-quadruplex were calculated from van't Hoff plots and are compared in Table S6 (see detailed discussion in the ESI[†]). For complex $[(\mathbf{1d})_4\text{K}]^+(\text{Pic}^-)$, no significant increase in monomer concentration was observed with the increase in temperature. This might be attributed to the high kinetic barrier for G-quadruplex $[(\mathbf{1d})_4\text{K}]^+(\text{Pic}^-)$ dissociation. To confirm this hypothesis, a NOESY experiment at 50 °C was performed for $[(\mathbf{1b})_8\text{K}]^+(\text{Pic}^-)$, $[(\mathbf{1c})_8\text{K}]^+(\text{Pic}^-)$ and $[(\mathbf{1d})_4\text{K}]^+(\text{Pic}^-)$ (Fig. S14–S16[†]). The results suggested that kinetic exchange between the complex and monomer for $[(\mathbf{1d})_4\text{K}]^+(\text{Pic}^-)$ was too slow to be recorded by NMR spectroscopy.

In addition to DMSO titration, the stability of G_8 and G_{16} complexes could also be evaluated using tandem-MS by

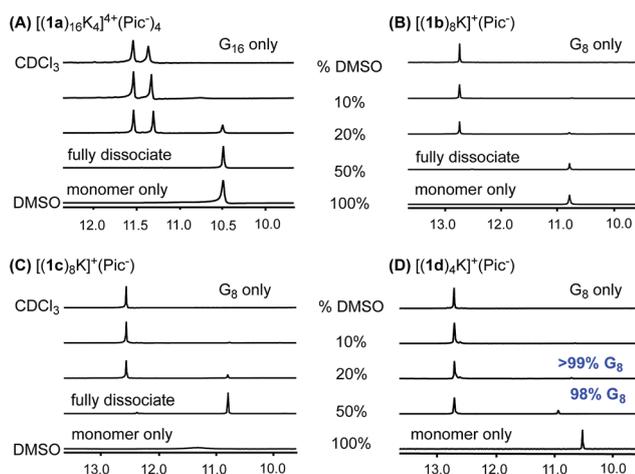


Fig. 7 DMSO- d_6 titration: ^1H NMR spectra of (A) $[(\mathbf{1a})_{16}\text{K}_4]^{4+} \cdot (\text{Pic}^-)_4$, (B) $[(\mathbf{1b})_8\text{K}]^+(\text{Pic}^-)$, (C) $[(\mathbf{1c})_8\text{K}]^+(\text{Pic}^-)$, (D) $[(\mathbf{1d})_4\text{K}]^+(\text{Pic}^-)$ in CDCl_3 - DMSO-d_6 with DMSO-d_6 fractions of 10%, 20% and 50%.

Table 2 Decomposition voltage

	$[(\mathbf{1a})_8\text{Ba}]^{2+}$	$[(\mathbf{1b})_8\text{Ba}]^{2+}$	$[(\mathbf{1c})_8\text{Ba}]^{2+}$	$[(\mathbf{1d})_4\text{Ba}]^{2+}$
Start ^a	30 V	40 V	50 V	70 V
End ^a	40 V	45 V	65 V	80 V

^a Operating voltage (V).

increasing the operating voltage. The cation fragments of G-quadruplexes were separated and treated with increasing voltage. The operating voltage for G-quadruplex cation fragment decomposition are summarized in Table 2.

It is noteworthy that $[(\mathbf{1a})_{16}\text{Ba}_2]^{4+} \cdot (\text{Pic}^-)_4$ only showed a doubly charged peak at $m/z = 2123.31$ corresponding to $[(\mathbf{1a})_8\text{Ba}]^{2+}$, indicating that the picrate bridge dissociated under the MS conditions. Further comparison of all the cation fragments of the G-quadruplexes revealed a clear stability trend as $[(\mathbf{1d})_4\text{Ba}]^{2+} > [(\mathbf{1c})_8\text{Ba}]^{2+} > [(\mathbf{1b})_8\text{Ba}]^{2+} > [(\mathbf{1a})_8\text{Ba}]^{2+}$. Overall, the covalent linking strategy significantly helped to stabilize the G_8 -octamer, both in H-bond competitive solvents and gas phase.

Conclusions

In summary, with modification on both guanine (8-aryl) and ribose (sterically hindered 2',3' position), a stable G_8 -octamer was formed with its structure characterized by single crystal X-ray diffraction for the first time. Through the analysis of cross-layer interactions, a covalently tethered 8-aryl guanosine dimer was designed and prepared for supramolecular assembly. The expected G_8 -octamer was confirmed by X-ray, MS and NMR spectroscopy with significantly improved stability in MeOH and 1 : 1 $\text{DMSO}/\text{CDCl}_3$ mixture. To the best of our knowledge, this is the first example of discrete G-quadruplexes formed from small molecules with enhanced stability in a protic solvent (MeOH) and a polar aprotic solvent (DMSO). Meanwhile, formation of the stable G_8 -octamer with a concise and well-defined bottom-to-bottom stacking mode provides a novel supramolecular platform. Incorporation of this new system into material and biological applications is expected and currently undergoing in our group.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

§ All the structures reported in this article have been deposited with the Cambridge Crystallographic Data Centre. The accession numbers for $[(\mathbf{1b})_8\text{Ba}]^{2+} \cdot (\text{Pic}^-)_2$; $[(\mathbf{1b})_8\text{K}]^+(\text{PF}_6^-)$; $[(\mathbf{1b})_8\text{K}]^+(\text{Pic}^-)$; $[(\mathbf{1b})_8\text{Sr}]^{2+} \cdot (\text{Pic}^-)_2$; $[(\mathbf{1b})_8\text{Rb}]^+(\text{Pic}^-)$; $[(\mathbf{1c})_8\text{Ba}]^{2+} \cdot (\text{Pic}^-)_2$; $[(\mathbf{1d})_4\text{K}]^+(\text{Pic}^-)$ reported in this paper are:



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