Homochiral G-Quadruplexes with Ba^{2+} but Not with K^+: The Cation Programs Enantiomeric Self-Recognition

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In biological and synthetic systems, noncovalent interactions often control formation of assemblies from multiple components, and the thermodynamically favored structure can vary with changes in the building blocks, template, or environmental conditions.[1] Some intriguing examples of this dynamic equilibrium shifting[2] involve formation of homochiral assemblies from racemic ligands.[3,4] Enantiomeric self-recognition of nucleosides is particularly interesting,[5] since the genetic material is homochiral.[6] We report that cations of similar size, but of different charge, promote formation of stereoisomeric assemblies from the same racemic nucleoside. Specifically, (D,L)-5′-silyl-2′,3′-*O*-isopropylidene guanosine (G **1**) forms homochiral aggregates in the presence of Ba^{2+} picrate but gives heterochiral diastereomers when K^+ is the guest (Scheme 1).

Guanosine derivatives self-associate in the presence of cations to form hydrogen-bonded G-quartets.[7] Individual G-quartets stack to give G_8-M^+ sandwiches and higher-ordered G-quadruplexes. Besides K^+ and Na^+, G-quadruplexes also bind divalent cations.[8−10] Since octacoordinate K^+ (r = 1.51 Å) and Ba^{2+} (r = 1.42 Å) have similar ionic radii,[11] we reasoned that comparing the K^+ and Ba^{2+} complexes formed from G **1** would reveal how the cation's charge affects G-quadruplex structure and dynamics.[12] While studying ligand exchange, we discovered that Ba^{2+} picrate directs enantiomeric self-recognition of (D,L)-G **1** (Scheme 1). In this case, the divalent cation and the picrate anion cooperate to enable chiral resolution of (D,L)-G **1** on the supramolecular level.

First, we confirmed that Ba^{2+} picrate templates G-quadruplex formation. NMR integration showed that (D)-G **1** extracted Ba^{2+} picrate from water into CD_2Cl_2 to give a hydrogen-bonded complex with 8 equiv of nucleoside bound to each Ba^{2+} picrate. A crystal structure of [(D)-G **1**]$_{16}$·2[$BaPic_2$] indicated that 16 units of G **1** associate around two Ba^{2+} cations to give a complex with four G-quartet layers.[13] This lipophilic G-quadruplex is composed of two coaxially stacked C_4-symmetric octamers, [(D)-G **1**]$_8$·Ba^{2+}. Within an octamer, sugar−nucleobase hydrogen bonds connect the 5′-ribose oxygen of the "inner" G-quartet with the N_2 amino group of the "outer" G-quartet. The [(D)-G **1**]$_8$·Ba^{2+} octamers are linked by four picrate anions that hydrogen bond to the "inner" G-quartets (Figure 1). NMR mixing experiments using [(D)-G **1**]$_{16}$·2[$BaPic_2$] and the isomorphous [(D)-G **1**]$_{16}$·2[$SrPic_2$] indicated that the M^{2+} G-quadruplexes retain the hexadecamer structure in CD_2Cl_2 and that the picrate anions stabilize this hexadecamer in solution.[14] The only significant difference between the X-ray structures of the Ba^{2+} and K^+ G-quadruplexes[15] is the cation occupancy. For the K^+ G-quadruplex, a cation is present between each G-quartet, raising the possibility that charge−charge repulsion between adjacent cations might destabilize the K^+ G-quadruplex relative to the Ba^{2+} G-quadruplex. For the Ba^{2+} complex, the divalent cations are sandwiched within individual octamers, and there is no cation located between the "inner" two G-quartets.

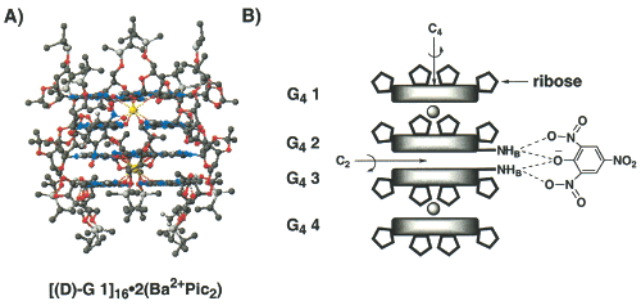
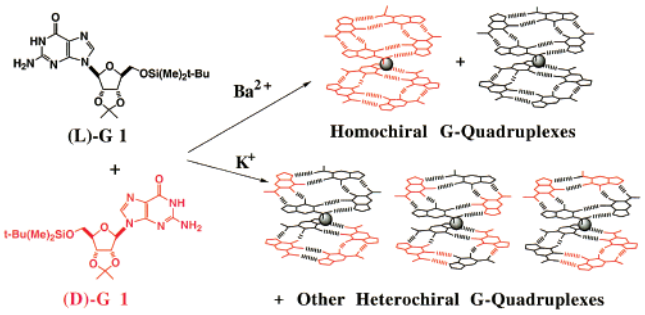


Figure 1. (A) A side view of the crystal structure of the G-quadruplex formed from (D)-G **1** and Ba^{2+} picrate. The picrate counterions are deleted for clarity. (B) Four G-quartets, G_4 **1**−G_4 **4**, make up the G-quadruplex. The two Ba^{2+} cations are indicated, as are the picrate hydrogen bonds with the amino N2 HB proton of G_4 **2** and G_4 **3**.

Scheme 1



Although structurally similar, the Ba^{2+} G-quadruplex is thermodynamically and kinetically more stable than the K^+ G-quadruplex. For example, the picrate anion binds more strongly to the G-quadruplex filled with divalent cations. For the Ba^{2+} system, separate picrate ^{1}H NMR signals were observed for an equimolar mixture of [(D)-G **1**]$_{16}$·2[$BaPic_2$] (δ = 8.99 ppm) and "free" anion (δ = 8.71 ppm) in CD_2Cl_2 (Supporting Information Figure 4).[16] In contrast, a time-averaged NMR signal (δ = 8.85 ppm) for a 1:1 mixture of [(D)-G **1**]$_{16}$·4[Kpic] and solvated picrate indicated faster anion exchange between the K^+ G-quadruplex

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(13) Crystal data for [(D)-G **1**)]$_{16}$·2Ba^{2+}(picrate)$_4$·$(H_2O)_{11}$·$(CH_3CN)_6$: $C_{352.76}H_{562}Ba_2N_{104}O_{119}Si_{16}$; M_r = 8888.18, crystal dimensions 0.515 × 0.255 × 0.161 mm^3, tetragonal, space group $I4$, a = 30.780 Å, b = 30.780 Å, c = 25.831(5) Å, α = 90°, β = 90°, γ = 90°, V = 24 472(6) Å^3, Z = 2, D_x = 1.206 mg/m^3, u(Mo Kα) = 0.283 mm^{-1}. Data were collected on a Bruker SMART 1000 CCD diffractometer at 193(2) K. Structure determination was done by direct methods using the program XS.[19] Refinement, using the XL program,[20] was done to convergence on F^2 with $R(F)$ = 9.94% and $wR(F^2)$ = 22.03% for all 15 958 independent reflections.

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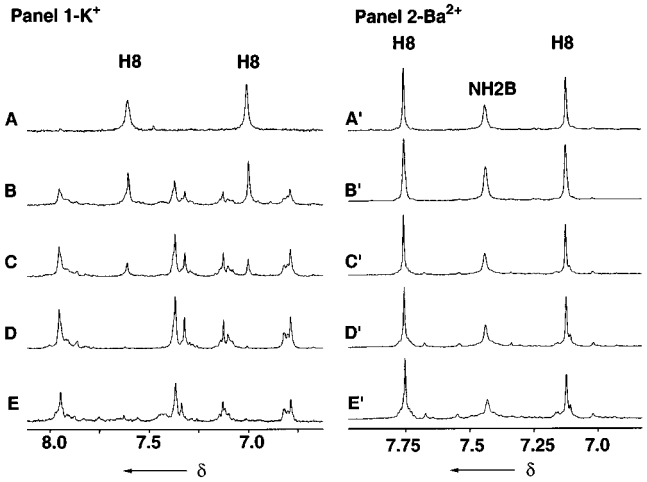

Figure 2. A series of ^{1}H NMR spectra (CD_2Cl_2 at room temperature) showing the H8 region for the K^+ G-quadruplex and the Ba^{2+} G-quadruplex during enantiomeric mixing experiments. Due to the "head-to-tail" stacking of two G_4-quartets an enantiomerically pure G-quadruplex has two signals for each proton in G **1**. Panel 1: (A) enantiomerically pure [(D)-G **1**]$_{16}$·4[Kpic]; (B) an equimolar mixture of [(D)-G **1**]$_{16}$·4[Kpic] (2 mM) and [(L)-G **1**]$_{16}$·4[Kpic] (2 mM) 1 day after mixing; (C) 2 days after mixing; (D) 5 days after mixing; (E) a control experiment generated by extracting K^+ picrate from water into CD_2Cl_2 using (D,L)-G **1**. Panel 2: (A′) enantiomerically pure [(D)-G **1**]$_{16}$·2Ba^{2+}(pic)$_4$; (B′) an equimolar mixture of [(D)-G **1**]$_{16}$·2Ba^{2+}(pic)$_4$ (2 mM) and [(L)-G **1**]$_{16}$·2Ba^{2+}(pic)$_4$ (2 mM) 1 day after mixing; (C′) 3 days after mixing; (D′) 9 days after mixing; (E′) a control experiment generated by extracting Ba^{2+} picrate from water into CD_2Cl_2 using (D,L)-G **1**.

and the unbound state. Picrate is held tighter to the Ba^{2+} G-quadruplex than to the K^+ G-quadruplex, probably due to the greater charge density of Ba^{2+}.

NMR mixing experiments illustrated the difference between Ba^{2+} picrate and K^+ picrate in promoting enantiomeric self-recognition of (D,L)-G **1** in CD_2Cl_2. First, we grew separate crystals of [(D)-G **1**]$_{16}$·M^{n+} picrate and [(L)-G **1**]$_{16}$·M^{n+} picrate for the K^+ and Ba^{2+} complexes. We then monitored the dynamic equilibration of stereoisomers by ^{1}H NMR spectroscopy.

The racemic mixture of [(D)-G **1**]$_{16}$·4K^+ picrate and [(L)-G **1**]$_{16}$·4K^+ picrate slowly equilibrates in CD_2Cl_2 (Figure 2-Panel 1) to give a new set of NMR signals, distinct from those for the enantiomeric G-quadruplexes. These new NMR signals indicate formation of heterochiral diastereomers of lower symmetry. The diasteromeric mixture was not statistical, as the simple ^{1}H NMR spectrum in Figure 2D indicates a bias toward a few of the many possible heterochiral diastereomers for a [(D,L)-G **1**]$_{16}$·4K^+ hexadecamer. In these mixing experiments, we made sure that the system reached equilibrium. Thus, the NMR spectrum 5 days after enantiomer mixing (Figure 2D) was similar to that of a control experiment (Figure 2E) obtained by using racemic (D,L)-G **1** to extract K^+ picrate from water into CD_2Cl_2.

In contrast, significant enantiomeric recognition occurred when the templating cation was the divalent Ba^{2+} (Figure 2-Panel 2). The NMR spectrum in Figure 2E′ indicates that the homochiral pair, [(D)-G **1**]$_{16}$·2Ba^{2+} and [(L)-G **1**]$_{16}$·2Ba^{2+}, account for at least 90% of the diastereomers observed at equilibrium. Furthermore, CD spectra of mixtures of [(D)-G **1**]$_{16}$·2Ba^{2+} and [(L)-G **1**]$_{16}$·2Ba^{2+}in CH_2Cl_2 showed a linear relationship between the chiroptical activity at 262 nm and the enantiomeric composition of the solution, consistent with homochiral self-sorting (Supporting Information, Figure 5).[4a] Most importantly, treatment of the diasteromeric K^+ G-quadruplexes with Ba^{2+} illustrated the reversible nature of these nucleoside assemblies. NMR data

(16) The solvated picrate anion was generated in CD_2Cl_2 by encapsulating the Ba^{2+} or K^+ cations with [2.2.2]-cryptand: Lehn, J.-M., J. P. Sauvage, J. P. *J. Am. Chem. Soc.* **1975**, *97*, 6700−6707.

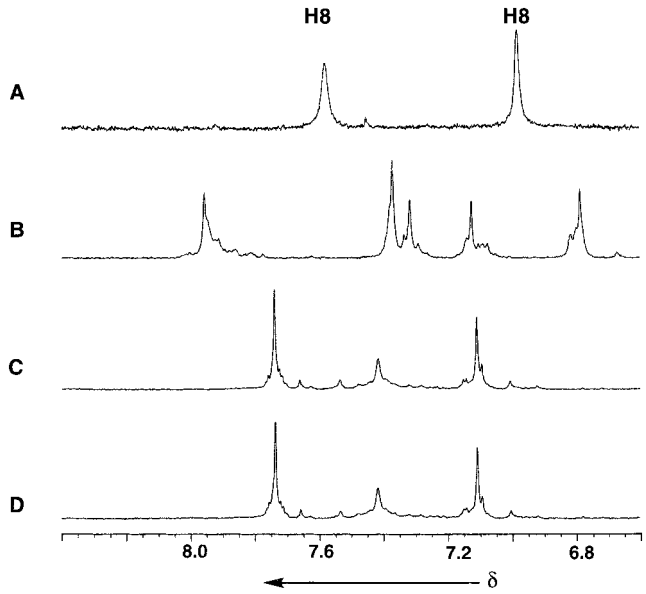

Figure 3. A series of ^{1}H NMR spectra in CD_2Cl_2 showing the H8 region during dynamic equilibration. (A) [(D)-G **1**]$_{16}$·4K^+(pic)$_4$. (B) after extraction of K^+ picrate from water by (D,L)-G **1**. (C) after stirring the solution from (B) overnight with a water solution of Ba^{2+}picrate; (D) after extraction of Ba^{2+} picrate from water into CD_2Cl_2 using (D,L)-G **1**.

showed that stirring a CD_2Cl_2 solution of the heterochiral K^+ G-quadruplexes with water containing Ba^{2+} picrate established an equilibrium favoring the homochiral Ba^{2+} G-quadruplexes (Figure 3).

Simply switching from a monovalent to a divalent cation changed the expression of stereochemical information embedded in (D,L)-G **1**. Barium picrate directs significant enantiomeric self-recognition of (D,L)-G **1**, while K^+ picrate leads to heterochiral G-quadruplexes. With Ba^{2+}picrate as template, there is efficient stereocontrol within and between individual G-quartets, resulting in a homochiral hexadecamer. Since $Ba^{2+}$and K^+ are similar in size, the cation's charge density is the major factor controlling enantiomeric self-association of (D,L)-G **1**.[17] By strengthening cation−dipole interactions, G-quartet hydrogen bonds, and G-quadruplex−picrate interactions the divalent cation may provide the enthalpic stabilization needed to overcome the unfavorable entropy associated with enantiomeric self-recognition. In this system, bridging picrate anions play a supporting role in the enantiomeric self-association of G **1** by enhancing stereochemical communication between the two G_8-Ba^{2+} octamers.[18] Finally, since only (D)-nucleosides are found in DNA and RNA it is tempting to suggest that enantiomeric self-association of nucleosides might be relevant to biomolecular homochirality.[6] Certainly, we have shown that homochiral self-association of nucleosides can occur under the appropriate conditions.

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Supporting Information Available: Crystallographic tables, final coordinates and thermal parameters, selected bond lengths and angles, and selected ^{1}H NMR spectra (PDF). X-ray crystallographic files (CIF). This material is available free of charge at http://pubs.acs.org.

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(17) The divalent cation is essential for homochiral G-quadruplexes. We observed similar enantiomeric self-association of G **1** with Sr^{2+} picrate and Pb^{2+} picrate.

(18) While the G-quadruplex is stabilized by picrate, relative to a less coordinating anion like thiocyanate (ref 14), mixing experiments with [(D)-G **1**)]$_{16}$·2Ba^{2+}(SCN)$_4$ and [(L)-G **1**)]$_{16}$·2Ba^{2+}(SCN)$_4$ also showed significant enantiomeric self-association of G **1**.

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