Toward Artificial Ion Channels: A Lipophilic G-Quadruplex

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Abstract: Single crystals of a lipophilic G-quadruplex formed by 5′-*tert*-butyl-dimethylsilyl-2′,3′,-di-O-isopropylidene G **2** were obtained from a CH_3CN solution containing potassium picrate and cesium picrate. The X-ray structure showed that 16 units of G **2** and 4 equiv of alkali picrate form the lipophilic G-quadruplex. The quadruplex has a filled cation channel, with three K^+ ions and one Cs^+ ion located along its central axis. The quadruplex is formed by a pair of head-to-tail (G **2**)$_8$-K^+ octamers. Both octamers use eight carbonyl oxygens to coordinate K^+. The two (G **2**)$_8$-K^+ octamers are of opposite polarity, being coaxially stacked in a head-to-head orientation. A Cs^+ cation, with an unusual coordination geometry, caps the cation channel. The Cs^+ is coordinated to four acetonitrile solvent molecules in an η^2-fashion. Within an octamer the two tetramers are stacked so that they are 3.3 Å apart and twisted by 30°. A second stacking interaction is defined by the head-to-head arrangement between the two (G **2**)$_8$-K^+ octamers. This stacking, with a 90° twist, positions the exocyclic amines of the central two quartets so that both exocyclic $NH2_B$ protons can hydrogen bond to the picrate anions that rim the quadruplex equator. The four picrates form an anionic belt that wraps around the cation channel. The sugars are well ordered in the structure. Circular dichroism spectra indicate that the G-quadruplex retains its helical structure in chlorinated solvents.

Some diverse compounds have been proposed to form ion channels. These include magainin, cecropin, and gramicidin[1] and synthetic peptides that form bundles and nanotubes.[2,3] Various organic compounds also conduct ions across membranes.[4] Lipophilic ion pairs, modified phospholipids, bouquet molecules, unusual macrocycles, sterols, bolaamphiles, rigid rods, and crown-peptides all may form ion channels.[5−12] For many of these compounds, self-assembly in the membrane presumably gives channels with hydrophobic exteriors and hydrophilic interiors.

Nucleobases self-associate via hydrogen bonding and base stacking. Thus, artificial ion channels are conceivable from lipophilic nucleobases.[13] Guanosine (G) is notorious for its propensity to aggregate. In a cation-templated process, G derivatives self-associate in water to give the G-quartet (Figure 1).[14,15] The planar G-quartet is stabilized by hydrogen bonds between the NH1 amide and NH2 amino donors on one purine and the O6 and N7 acceptor atoms on a neighboring base. The G-quartet, with four oxygens surrounding a cavity, binds alkali cations with a selectivity of $K^+ > Na^+$, $Rb^+ \gg Cs^+$, Li^+.[16] For example, K^+ forms a sandwich with two G-quartets.[17] These

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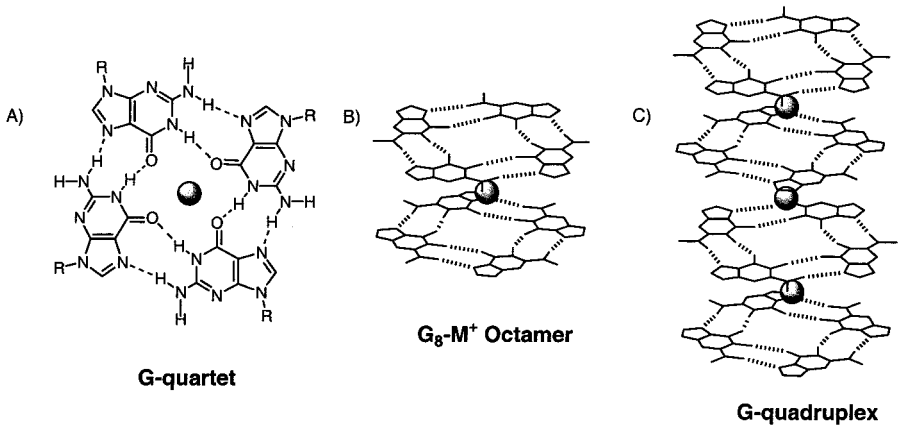
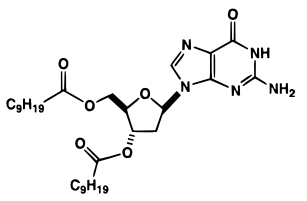


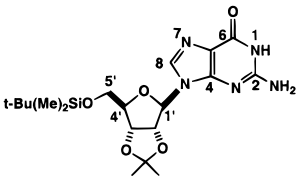
Figure 1. (A) The G-quartet, (B) an octamer, G_8-M^+, (C) a G-quadruplex formed by stacking of G-quartets around a column of cations.

G_8-K^+ octamers can then stack to give columnar aggregates known as G-quadruplexes. Fiber diffraction has shown that both mono- and dinucleotides form G-quadruplexes in the presence of alkali cations.[18,19]

Polyguanylic acid,[20] and G-rich oligonucleotides also form these higher-ordered structures.[21] Due to the potential relevance of DNA G-quadruplexes, there has been much activity in determining the three-dimensional structures of oligonucleotide G-quadruplexes through the use of NMR spectroscopy and X-ray crystallography.[22−24] An X-ray structure of a G-quadruplex formed from d($G_4T_4G_4$) identified a dimer containing four contiguous G-quartets connected by T_4 loops.[23] Poorly resolved electron density between two of the stacked G-quartets was tentatively assigned to be a K^+ ion. Later, the X-ray structure of [d(TG_4T)]$_4$ revealed a four-stranded helix with discrete Na^+ cations located within or between G-quartet planes.[24] Recently, $^1H-^{15}N$ NMR techniques were used to locate NH_4^+ between G-quartets in [d($G_4T_4G_4$)]$_2$.[25] The mobility of NH_4^+ in and out of the complex led Feigon to note that the G-quadruplex resembles an ion channel. G-wires, obtained from cation-templated polymerization of G-rich oligonucleotides,[26] have been imaged using atomic force microscopy.[27] These G-wires, 10−1000 nm in length, have been proposed as potential electronic devices.

We have been studying lipophilic nucleosides that form hydrogen-bonded aggregates in organic solvents.[28−31] These aggregates may function as self-assembled ionophores or ion channels in low dielectric media.[32] The derivative, 3′,5′-didecanoyl-2′deoxyguanosine, dG **1**, extracts alkali salts from water into chlorinated solvents.[29] We recently showed by NMR spectroscopy that this phase transfer of salt involves formation of the "octamer", (dG **1**)$_8$-K^+, in $CDCl_3$.[31] The octamer has two unique G-quartets sandwiching a K^+ cation. Inter-tetramer NOEs were consistent with a stereospecific "head-to-tail" stacking of the quartets.[33] This K^+-bound octamer represents the first observable stage in the cation-templated aggregation of dG **1** in organic solvents.





The analogue, 5′-*tert*-butyl-dimethylsilyl-2′,3′,-di-O-isopropylidene G **2**,[28] also self-associates to give hydrogen bonded

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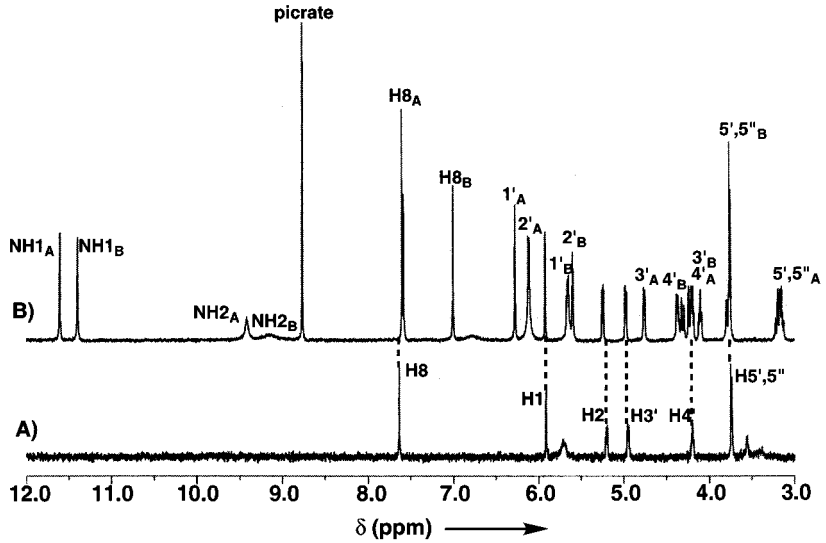

Figure 2. A region of the 500 MHz ^{1}H NMR spectra of (A) G **2** in CD_3CN at 25 °C and (B) after extraction of G **2** by K^+ picrate in CD_3CN. Coordination of K^+ picrate by G **2** gave two new sets of ^{1}H NMR resonances, labeled as group A and group B. Assignments were made from 2D NMR experiments.

structures. Below, we describe the crystal structure for a G-quadruplex formed from G **2** and a 1:1 mixture of K^+ and Cs^+ picrate. The lipophilic G-quadruplex is composed of four stacked G-quartets. Three coaxial K^+ cations and a Cs^+ cation are located inside the G-quadruplex, while hydrophobic picrate counteranions bind to the exterior of the lipophilic G-quadruplex. For many artificial ion channels the function is clearly evident, but the channel's structure is not as well defined.[5−12] Here, we describe the converse. While we do not yet know about function, self-assembly of the lipophilic nucleoside G **2** certainly gives the suggestive appearance of an ion channel.

In addition, the present study provides the first structure where bound K^+ ions have been definitively located within a G-quadruplex.[23] As such, this structure should be valuable to reseachers carrying out ab initio calculations on the structure, energetics and dynamics of DNA G-quadruplexes.[34−37]

Results and Discussion

The K^+ Cation Templates Specific Aggregation of G 2 in Solution. Proton NMR experiments indicated little defined self-association of the poorly soluble G **2** (1.8 mM) in CD_3CN (Figure 2A). First, the NMR signal for the exchangeable NH1 amide proton was broadened into the baseline, probably due to exchange with solvent. Second, the two exchangeable NH2 amino protons gave a single, broad resonance at δ 5.70 ppm. Third, the chemical shifts of the non-exchangeable protons were similar in CD_3CN and in the competitive solvent, d_6-DMSO. These data were consistent with G **2** being "monomeric" in the absence of K^+.

Nucleoside G **2** was much more soluble in CD_3CN containing K^+. Thus, 5 mM K^+ picrate (λ_{max} = 354 nm, ϵ =17 000 cm^{-1} M^{-1})[38] in CD_3CN extracted solid G **2** (λ_{max} = 251 nm, ϵ = 12 500 cm^{-1} M^{-1}) to give a solution that was approximately 20 mM in the nucleoside. The 4:1 ratio of G **2** to K^+ picrate was consistent with an oligomer of stacked G-quartets.

After extraction of G **2** by K^+ picrate, ^{1}H NMR spectroscopy indicated a discrete aggregate. We observed three sets of ^{1}H NMR resonances, all in slow exchange on the chemical shift time-scale (Figure 2B). One set of signals was due to monomeric G **2**, while the other two sets of NMR resonances, present in a 1:1 ratio, arose from K^+ picrate coordination. The NMR spectrum contained sharp resonances for the NH1 amide protons at δ 11.64 and 11.47 ppm, indicating hydrogen bond formation upon K^+ binding. On the basis of 2D NMR correlations we assigned the aggregated species to be a head-to-tail octamer, (G **1**)$_8$-K^+.[39]

Crystal Formation and General Description. An X-ray structure unambiguously showed that G **2** forms a lipophilic G-quadruplex. Although single crystals could be obtained from solutions containing G **2** and K^+ picrate, diffraction was enhanced by the Cs^+ ion. A solution of K^+ picrate (2.5 mM) and Cs^+ picrate (2.5 mM) in CH_3CN was stirred with a suspension of G **2**. After filtration of insoluble material, evaporation gave single crystals.[40] Table 1 lists key data from the X-ray structure determination.

The crystals of the G **2** complex were tetragonal, of space group I4, with unit cell dimensions of a = 30.572(1) Å and c = 25.713(1) Å. The asymmetric unit contained four independent molecules of G **2**. The structure, solved and refined to give a final R value of 7.6%, revealed that 16 units of G **2** and 4 equiv of alkali picrate form the G-quadruplex. Figure 3A shows a side view of this complex, including the numbering of the four stacked G-quartets (G_4 1−G_4 4).

Four cations occupy the central channel, while the modified sugars provide the G-quadruplex with a lipophilic exterior. The nucleobases of an individual G-quartet, G_4 1, are shown in Figure 3B. Each quartet is symmetric, as demonstrated by the identical O6−O6 diagonal distances. These diagonal O6−O6 distances, which range between 4.50 and 4.69 Å, are similar to

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(39) Details about the NMR analysis of the G-quadruplex formed by G **2** will be presented separately. See ref 31 for NMR studies that show that the lipophilic nucleoside, dG **1**, forms a head-to-tail (dG **1**)$_8$-K^+ octamer.
(40) The picrate anion was important for obtaining crystals. Other lipophilic counterions, I^-, Ph_4B^-, SCN^-, and PF_6^-, have so far failed to give quality crystals.

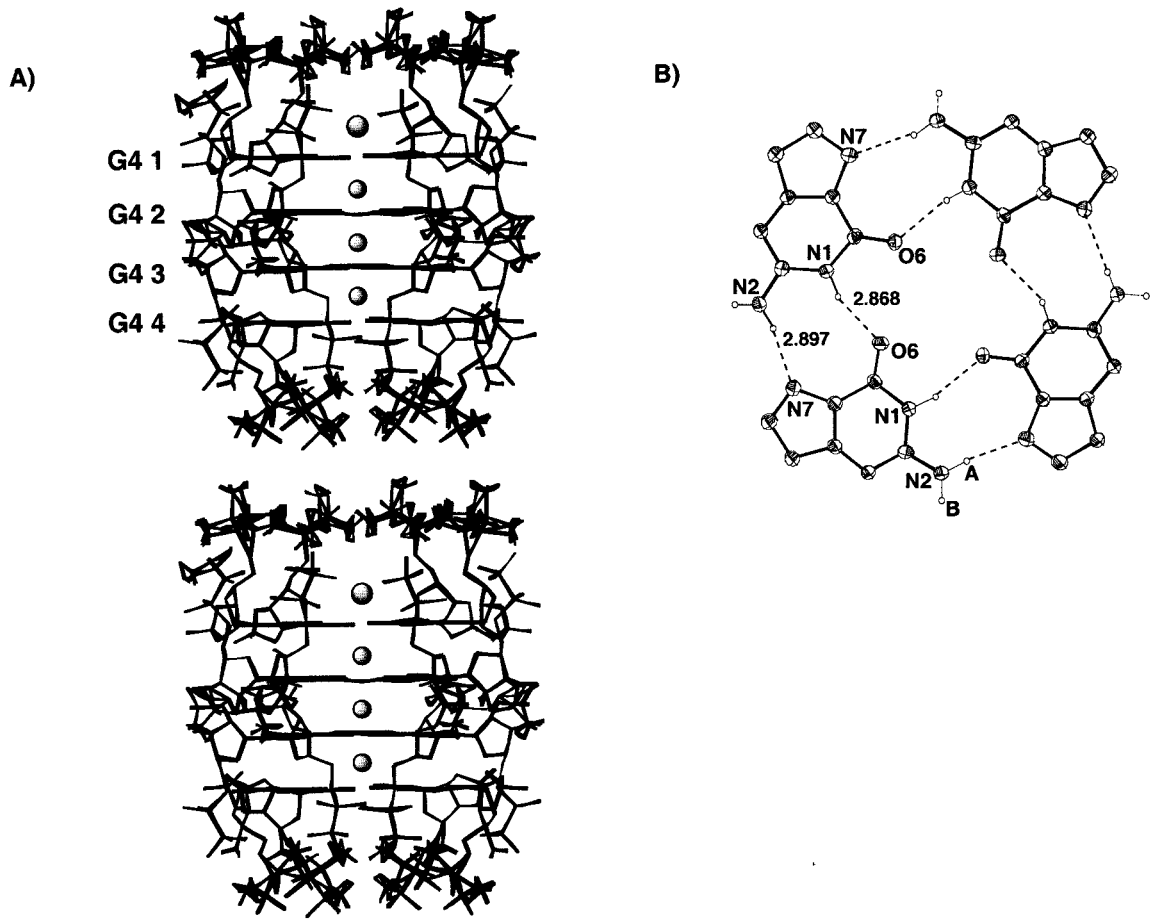

Figure 3. (A) A side view of the crystal structure of the G-quadruplex formed from G **2** and K^+/Cs^+ picrate. The picrate counterions have been deleted. The four individual G-quartets, G_4 1−G_4 4, that make up the G-quadruplex are labeled. In this view, two G-quadruplex unit cells stack on top of each other along the *c* axis. (B) An ORTEP diagram showing the four bases in the G_4 1 quartet. The drawing shows 40% probability ellipsoids for non-hydrogen atoms. Hydrogen atoms are depicted as spheres of arbitrary diameter. The hydrogen bond distances between heteroatoms are indicated.

Table 1. Crystal Data and Structure Refinement for G-quadruplex Formed from G **2** and Cs^+/K^+ Picrate

empirical formula	$C_{357.5}\ H_{545}\ Cs_{1.0}\ K_{3.0}\ N_{106}\ O_{108}\ Si_{16}$
formula weight	8755.0
temperature	293(2) K
crystal system	tetragonal
space group	*I*4
unit cell dimensions	
	$a = 30.572(10)$ Å, $\alpha = 90.0°$
	$b = 30.572(10)$ Å, $\beta = 90.0°$
	$c = 25.713(10)$ Å, $\gamma = 90.0°$
volume	24 033(15) Å^3
Z	2
density (calculated)	1.210 mg/m^3
absorption coefficient	0.226 mm^{-1}
data/restraints/parameters	21296/276/1438
goodness-of-fit on F_2	0.978
final *R* indices [$I > 2\sigma(I)$]	R1 = 0.0495, wR2 = 0.1241
R indices (all data)	R1 = 0.0763, wR2 = 0.1381

O6−O6 distances calculated for K^+-containing G-quadruplexes.[35,37] The planar G-quartet has eight hydrogen bonds. Table 2 lists the hydrogen bond distances for the separate G-quartets, G_4 1−G_4 4. Hydrogen bond distances (mean d_{N1-O6} = 2.88 Å and mean d_{N2-N7} = 2.90 Å for the four G-quartets) are within 0.1 Å of the mean hydrogen bond distances found in $[d(TG_4T)]_4$.[24] Each quartet also has four amino protons ($NH2_B$) that are not involved in intra-quartet hydrogen bonds. As discussed below, these $NH2_B$ amino protons are important for organization of the G-quadruplex.

Table 2. Hydrogen Bond Lengths (Å) and Angles (deg) for the Four G-quartets from the X-ray Structure of the Lipophilic G-quadruplex

G-quartet	D−H···A	*d*(D···A)	∠(DH···A)
G_4 1	N(1)−H···O(6)	2.868(4)	161.0
	N(2)−HA···N(7)	2.897(4)	170.2
G_4 2	N(1)−H···O(6)	2.916(4)	160.3
	N(2)−HA···N(7)	2.909(4)	169.8
G_4 3	N(1)−H···O(6)	2.864(4)	159.7
	N(2)−HA···N(7)	2.884(4)	170.0
G_4 4	N(1)−H···O(6)	2.860(4)	165.0
	N(2)−HA···N(7)	2.905(4)	166.2

Cation Coordination in the Channel. The G-quadruplex has a fully occupied cation channel, with three collinear K^+ ions and one Cs^+ ion along the central axis (Figure 3A). Each K^+ ion is located between G-quartet planes. The lipophilic quadruplex can be viewed as a pair of head-to-tail (G **1**)$_8$-K^+ octamers.[33] Both octamers, the one formed from quartets G_4 1 and G_4 2, and the octamer made from G_4 3 and G_4 4, use eight carbonyl oxygens to coordinate a K^+ cation. This octahedral geometry confirms earlier models for K^+ binding by G-rich DNA.[41,42] The two (G **1**)$_8$-K^+ octamers within the G-quadruplex are of opposite polarity, being coaxially stacked in a head-to-

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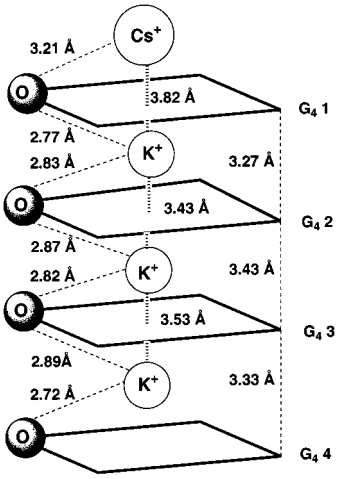

Figure 4. This representation of the G-quadruplex formed by G **2** shows the cation−oxygen distances, the cation−cation distances, and the G quartet−G quartet distances within the crystal structure.

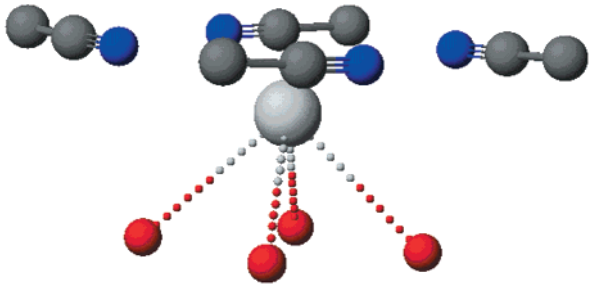

Figure 5. The four acetonitrile solvent molecules coordinated to Cs^+ via their π-bonds. The four oxygens (in red) from the upper G_4 1 quartet are also shown.

head orientation. A third octacoordinate K^+, bound between quartets G_4 2 and G_4 3, stabilizes the interface between the individual (G **1**)$_8$-K^+ octamers. The K^+−O bond lengths vary between 2.72 and 2.89 Å, well within the range expected for octacoordinate K^+ (Figure 4).[43]

A Cs^+ cation caps the ion channel. The Cs^+ cation, too large for the cavity between stacked G-quartets,[34] can fit above the G_4 1 quartet. This capping Cs^+ has a unique coordination geometry (Figure 5). First, the Cs^+ is bound to the four oxygens of G_4 1. The Cs−O distance of 3.21 Å is within the 3.0−3.3 Å range for a Cs−O bond in cesium-crown ether complexes.[44,45] Second, the Cs^+ is bound "side-on" to the triple bond (mean $d_{Cs-N} = 3.32$ Å and $d_{Cs-C} = 3.68$ Å) of four CH_3CN molecules. Dihapto coordination of an alkali cation by CH_3CN is unusual, having been reported only recently for a crown−Cs^+ complex.[45] Usually, CH_3CN coordinates alkali metal ions via the nitrogen's lone-pair. As discussed by Bryan,[45] η^2-nitrile coordination to Cs^+ is due to electronic and steric factors. First, Cs^+ has a small charge density, and it can coordinate soft π-donors. Second, linear η^2-coordination of CH_3CN to Cs^+ fills space within the hydrophobic pocket atop the G-quadruplex, while simultaneously completing the ion's coordination sphere.

Figure 4 depicts key dimensions within the complex. The individual K^+ cations are close together (d_{KK} 3.43−3.53 Å), although the separation is longer than the sum of their ionic radii.[43] As noted by Phillips et al.,[24b] the C6−O6 carbonyl groups of the G ligand must dissipate charge density to overcome the electrostatic repulsion between proximate cations. Recent ab initio calculations conclude that the G-quartet's center has a large negative electrostatic potential that helps neutralize the cation's charge.[36] The present crystal structure supports the idea that the G-quartets can provide the ionic stabilization necessary to allow a filled ion channel.

Base Stacking. The structure of the lipophilic G-quadruplex shows extensive quartet stacking. Within an individual (G **1**$_8$)-K^+ octamer the two tetramers are 3.3 Å apart and twisted by 30°. The 3.3 Å spacing is characteristic of optimal nucleobase stacking.[46] The vertical association of G-tetramers is different within and between the two (G **1**$_8$)-K^+ octamers. Therefore, a second stacking interaction is defined by the head-to-head arrangement between the two (G **1**$_8$)-K^+ octamers. This geometry, with a 3.4 Å spacing between layers and a 90° twist between G_4 2 and G_4 3, positions the five-membered rings of the two interfacial tetramers so that they are maximally overlapped. In this arrangement four of the five stacking atoms are of opposite polarity. This type of inter-tetramer stacking, also present in oligonucleotide crystal structures,[24] positions the carbonyl oxygens of the interfacial tetramers directly over each other. These oxygens then coordinate to the K^+ sandwiched between the G_4 2 and G_4 3 layers.

Calculations conclude that base-stacking provides a large driving force for G-quadruplex self-assembly in DNA.[37] Apparently, the attractive van der Waals and cation−π interactions between G-quartets overcome any electrostatic repulsion associated with stacking. The present crystal structure shows that extensive base-stacking is also possible for lipophilic G mononucleosides, even in an organic solvent. This crystal structure bodes well for the formation of stacked structures in a nonpolar lipid membrane.

The Anion Belt. The head-to-head stacking of the two octamers, with a 90° rotation between the interfacial quartets, also places the exocyclic amines of quartets G_4 2 and G_4 3 directly over each other. These accessible $NH2_B$ protons on G_4 2 and G_4 3 form bifurcated hydrogen bonds with the hydrophobic picrate counteranions. The picrates pack into grooves on the exterior of the lipophilic G-quadruplex (Figure 6). Consequently, four picrates rim the quadruplex equator, helping to clamp together quartets G_4 2 and G_4 3. The picrate's phenoxide hydrogen bonds with the amino groups from the G_4 2 (d_{N-O} 3.00 Å) and G_4 3 (d_{N-O} 2.99 Å) tetrads. The picrate's two *o*-nitro groups also interact with these $N2H_B$ protons, further strengthening the hydrogen bond network. In essence, the picrate anions serve as molecular clips, helping to hold together the stacked G-octamers.[40] The resulting G-quadruplex structure resembles an anionic belt wrapped around the central cation channel (Figure 6B).

Sugar Conformation. The sugars in this lipophilic G-quadruplex are well ordered.[47] The glycosyl torsion angle χ_{CN} (O4′−C1′−N9−C4) defines the relative orientation of the sugar and base.[48] In *anti* nucleosides ($\chi = -180° \pm 90$), the base is oriented away from the sugar, while *syn* nucleosides ($\chi = 0° \pm 90$) have the base located over the sugar. The *anti* conformer

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(47) The ribose rings are nearly planar, with some minor twisting out of the plane by either C4′ or C1′. The C(4′)−C(5′) side-chain conformation for all four G-quartets is gauche, trans with Φ_{OC} [O5′−C5′−C4′−C3′] = 178°. The peripheral 5′-*O*-silyl groups in the G_4 1 and G_4 4 quartets are disordered. The dioxalane ring in the G4 2 quartet was modeled as a 50:50 mixture of two major conformations.
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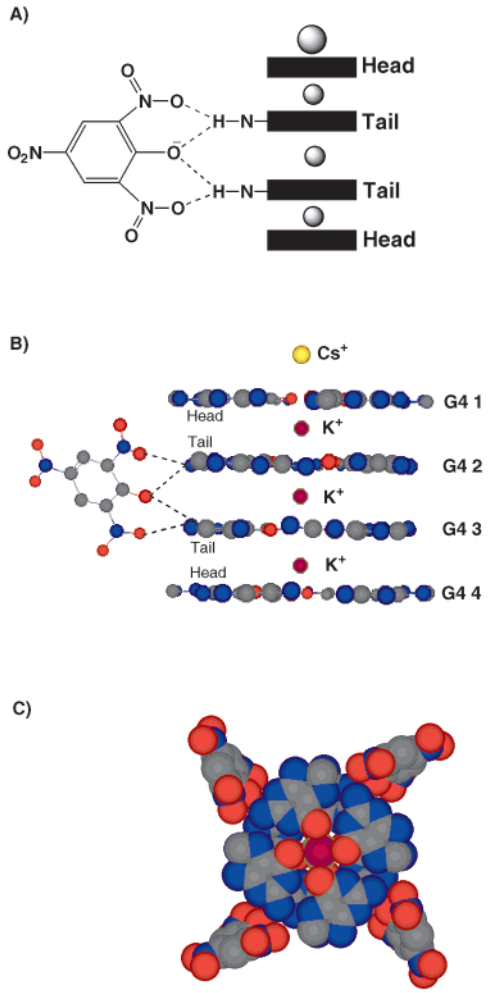

Figure 6. (A) Picrate hydrogen bond interactions with the amino $NH6_B$ proton on the G_4 2 and G_4 3 quartets. (B) This side view shows the aromatic rings of the G-quadruplex and the alkali metal cations coordinated along the central axis of the quadruplex. One of the picrate anions is depicted with its hydrogen bond network to the N2 amino groups on the G_4 2 and G_4 3 quartets. (C) A top view showing the four picrate anions that encircle the interface between the G_4 2 and G_4 3 quartets. The ribose units have been removed for clarity.

usually predominates for nucleosides in the solid state.[49] Two of the four tetramers (G_4 2 and G_4 3) have nucleosides in a *syn* conformation, $\chi = 68.3°$, while G_4 1 and G_4 4 are in the "high *syn*" region with $\chi = 96.0$ and 92.7°. One interaction that may stabilize the "all-*syn*" quartets is a hydrogen bond between the $NH2_B$ proton of the "head" tetramer (G_4 1 and G_4 4) and the 5′-silyl ether oxygen of the "tail" tetramer (G_4 2 and G_4 3).[50] This base-sugar hydrogen bond (mean d_{N-O} 2.99 Å) is only possible if the G_4 2 and G_4 3 nucleosides adopt a *syn* orientation about the glycosyl bond. An "all-*syn*" G-quartet is unusual, different from all of the structures described for G-rich oligonucleotides.[21−24] Recently, we showed by NMR that (dG **1**)$_8$-K^+ had one tetramer with all of the dG **1** components in a *syn* conformation, while the other tetramer had an "all-*anti*" conformation.[31] Apparently, the electrostatic and base-stacking interactions that drive G-quadruplex assembly force some of the subunits to adopt the *syn* glycosidic bond conformation.

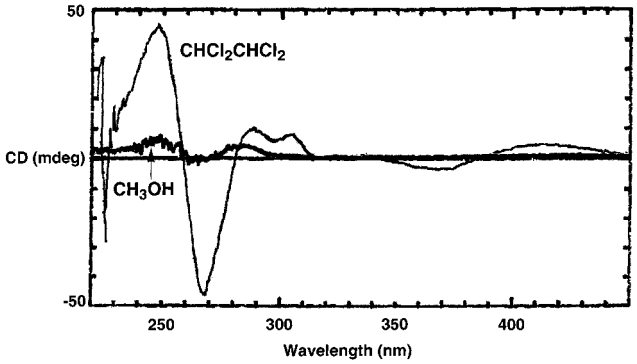

Figure 7. Circular dichroism spectra for the G-quadruplex crystals dissolved in $CHCl_2CHCl_2$ and CH_3OH at 25 °C. The strong CD bands centered at 258 nm correspond to the G chromophore, while the induced bands at 365 and 413 nm correspond to the picrate's chromophore.

Molecular Packing. The G-quadruplex formed by stacking two (G **1**)$_8$-K^+ octamers gives a cylinder that is 26 Å along the *c* axis. Because of the head-to-head orientation of the two octamers, a cluster of four hydrophobic *tert*-butyl-dimethylsilyl ethers project from both ends (G_4 1 and G_4 4) of the G-quadruplex. End-to-end packing of these hydrophobic side chains helps orient the G-quadruplexes in the solid state (Figure 3A). Thus, interdigitation of the *tert*-butyl-dimethylsilyl groups from neighboring G-quadruplexes align these units so that they form infinite columns along the *c*-axis. This stacking of G-quadruplexes, while perhaps due to crystal packing, enhances the impression of a lipophilic ion channel.

CD Solution Data. Circular dichroism (CD) spectroscopy provides insight into the chirality of this supramolecular assembly in solution. As shown below, the G-aggregate was ordered in non-competitive organic solvents, but not in hydrogen-bonding solvents. Thus, CD spectra of the (G **2**)-K^+ quadruplex crystals dissolved in methanol showed a weak guanine absorption near 250 nm (Figure 7), indicating that the hydrogen bonded aggregate decomposes in this competitive solvent. The CD spectra of the G **2**·K^+ aggregate, obtained by dissolving crystals in tetrachloroethane (TCE), were much different (Figure 7). The dominant feature of the CD spectra in TCE was the degenerate negative exciton couplet centered at 258 nm. This CD signature corresponds to the long-axis polarized transition of the G chromophore.[52] This couplet is diagnostic of an aggregate composed of at least two G-quartets chirally rotated with respect to each other. The spectrum in TCE is compatible with either an octamer or a stack of three or more G-quartets. The negative-to-positive sequence of the CD bands in TCE indicates that there is a counterclockwise rotation around the C_4 symmetry axis from one G-quartet to the next,[51] in agreement with the X-ray result, where 30° twist angles were observed within a G_8-K^+ octamer. The two CD bands at ∼365 and 413 nm correspond to absorption maxima for the picrate anion. These picrate signals are not of the degenerate exciton type.[52] Since picrate is achiral, the CD bands at 365 and 413 nm must be induced by interaction of the counteranion with the chiral G-aggregate. The intensity of the picrate's CD bands was high for an induced effect,[53] indicating that this hydrophobic anion remains closely associated with the chiral aggregate in solution.

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This CD data is significant in that this lipophilic G-quadruplex, as opposed to DNA helices, is much more stable in nonpolar solvents than in polar solvents. This stability is undoubtly due to cooperativity between ion coordination, hydrogen bonding, and base stacking. The CD study demonstrates that the lipophilic G-quadruplex can exist in a nonpolar solvent, suggesting that such a structure is reasonable in a lipid membrane environment. It is possible, of course, that G **2** will prefer to form an ion carrier rather than a true ion channel. Future transport experiments using G **2** and related analogues should allow us to distinguish between ion carriers and ion channels.

Conclusions

Guanosine analogues are well-known to self-associate in water. In a 1990 review, Guschlbauer commented that "*(w)ater appears to be an indispensable solvent for the auto-association of guanosine... organic solvents give rise to poorly organized aggregates*".[15] Recently, we presented NMR evidence that lipophilic G analogues do self-associate in organic solvents to give discrete and stable aggregates, namely octamers such as (G **1**)$_8$-K$^+$.[31] These octamers can further assemble into higher-ordered structures. In this paper, we have described a detailed structure of a lipophilic G-quadruplex formed by self-assembly of 16 units of G **2** and 4 equiv of alkali picrate. The G-quadruplex consists of four stacked G-quartets that stabilize a column of K$^+$ cations in a central channel. This lipophilic G-quadruplex structure is the first atomic resolution structure that clearly locates the bound K$^+$ cations.[23] The exterior surface of the G-quadruplex, with its modified sugars, is hydrophobic, and a belt of four lipophilic picrate anions encircles the filled ion channel.

It is striking that this G-quadruplex, formed from 16 equivalents of mononucleoside **2**, is so similar in structure to that of the oligonucleotide G-quadruplex [d(TG$_4$T)]$_4$,[24] particularly with regard to hydrogen bond and base stacking geometries. Of course, the G-quadruplexes from G **2** and d(TG$_4$T)$_4$ have a major difference: there is no backbone holding the G **2** nucleobases together. Without the entropic constraints of a covalent backbone, there are far more stereoisomers possible for a G-quadruplex formed from a mononucleoside. Yet, G **2** self-associates to give a highly ordered supramolecular complex. That a backbone is not necessary emphasizes the potential for using self-assembly to build functional structures from seemingly simple subunits.[54] In addition to its aesthetic appeal, this G-quadruplex structure suggests that lipophilic nucleosides may be able to form artificial ion channels. Our next step is to determine if lipophilic nucleosides function as ion channels, or as ion carriers, in lipid membranes.

Experimental Section

Materials, Methods, and Instrumentation. Reagents and solvents were obtained from commercial sources and used after purification. NMR data were collected on a Bruker DRX-500 spectrometer. The 1-D ^{1}H NMR spectra were obtained with a 10 s relaxation delay to ensure accurate integration. The probe temperature was controlled to + 0.1 °C. NMR solvents were from Cambridge Isotope Laboratories, Inc. and were 99.0−99.9% deuterium-enriched. Circular dichroism spectra were collected on a JASCO J-710 spectropolarimeter equipped with a NesLab RTE 111 temperature control unit. The UV/vis absorption spectroscopy was performed on a Gilford Response spectrophotometer at 25 °C, using 1-cm path length quartz cuvettes. Analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, New York.

5′-*tert*-Butyl-dimethylsilyl-2′,3′-*O*-isopropylidene Guanosine 2. To a suspension of 2′,3′-isopropylidene guanosine (Sigma, 4.54 g, 14.1 mmol) and imidazole (2.01 g, 29 mmol) in methylene chloride (140 mL) was added *tert*-butyl dimethylsilyl chloride (4.45 g, 29 mmol). The reaction mixture was stirred for 20 h, after which time TLC analysis indicated the reaction was complete. The reaction mixture was washed with 0.01 N HCl, saturated $NaHCO_3$, and saturated NaCl and concentrated in vacuo. Flash chromatography (10:1 CH_2Cl_2:MeOH) gave G **2** as a white solid (4.99 g, 81.2%). The chromatographed G **2** was crystallized from 2-propanol prior to use in the G-quadruplex experiments. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.72 (s, 1 H, NH1), 7.79 (s, 1 H, H8), 6.51 (bs, 2 H, NH2), 5.95 (d, 1 H, J = 2.4 Hz, H1′), 5.19 (dd, 1 H, J = 2.4, 6.0 Hz, H2′), 4.95 (dd, 1 H, J = 2.4, 6.0 Hz, H3′), 4.07 (m, 1 H, J = 2.4, 10.0 Hz, H4′), 3.67 (m, 2 H, H5′, H5″), 1.47 (s, 3 H, CH_3), 1.28 (s, 3 H, CH_3), 0.90 (s, 9 H, t-Bu), 0.01 (s, 3 H, Si(CH_3)), 0.00 (s, 3 H, Si(CH_3)). ^{13}C NMR (125.77 MHz, DMSO-d_6) δ 161.0, 157.7, 156.4, 154.4, 139.3, 113.1, 86.3, 84.3, 73.2, 69.9, 34.3, 25.8, 19.3, 5.5; UV_{max} = 254 nm (ϵ = 12 500 cm^{-1} M^{-1});

Anal. Calcd for $C_{19}H_{29}N_5O_5Si$: C, 52.15; H, 7.14; N, 16.01. Found: C, 52.37; H, 7.42; N, 16.08.

G-Quadruplex Crystals. A suspension of G **2** (37.0 mg, 87.5 umol) was stirred overnight in 5 mL of CH_3CN containing K$^+$ picrate[38] (3.3 mg, 12.5 umol) and Cs$^+$ picrate[38] (4.5 mg, 12.5 umol). The suspension was filtered, and the mother liquor was allowed to evaporate slowly at room temperature in a N_2-filled desiccator. Yellow, cubelike crystals formed after 3−4 days.

Crystallography on the G-Quadruplex. A canary-yellow block with dimensions 0.175 × 0.165 × 0.15 mm^3 was optically centered on a Bruker SMART1000 single-crystal CCD-diffractometer. The crystal's initial unit cell parameters and crystal orientation matrix were determined from a least-squares analysis of a random set of reflections collected via three sets (30 frames/set) of 0.3° wide-scans that were well-distributed in reciprocal space. The intensity data were collected with 0.3°-wide scans (60 s/frame) and a crystal-to-detector distance of 4.97 cm, thus providing a complete sphere of data to 55° in 2θ. The unit cell was optimized using all 606 frames from the initial series. Data were corrected for Lorentzian and polarization effects using the SAINT+ data reduction program.[55] An empirical absorption correction was applied to the data, based upon equivalent reflection measurements using Blessing's method in the program SADABS.[56] The program XPREP was used to check the cell symmetry,[57] confirming the possible space groups as *I*4 (no. 79), *I*4 (no. 82), or *I*4/*m* (no. 87). Initial SHELX files were created for all three possibilities. The structure was determined by direct methods using the program XS,[58] and resulted in the successful location of the central heavy atom core and several additional atoms comprising the four independent G **2** molecules within the complex. The remaining non-hydrogen atoms were located via countless full-matrix least-squares difference Fourier map cycles. The structure was refined using the program XL.[59] A majority of the non-hydrogen atoms were refined anisotropically and only those partially occupied were refined isotropically. All of the hydrogen atoms were placed in calculated positions and the structure was refined to convergence with $R(F)$ = 7.63%, $wR(F^2)$ = 13.81% and GOF = 0.978 for all 21 296 unique reflections [$R(F)$ = 4.95%, $wR(F^2)$ = 12.41% for those 15 512 data with $F_o > 4\sigma(F_o)$]. A final difference Fourier map was featureless with $|\Delta\rho| \leq 0.88$ eÅ^{-3}. The absolute structure parameter was also refined, giving a Flack parameter of (x) = −0.023-(12).[60]

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Supporting Information Available: Tables of crystallographic data including diffractometer and refinement data, final coordinates, bond lengths and bond angles, hydrogen bond lengths and angles, and anisotropic displacement values (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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