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Water-Mediated Association Provides an Ion Pair Receptor

Frank W. Kotch, Vladimir Sidorov, Yiu-Fai Lam, Katherine J. Kayser, Hojun Li, Mark S. Kaucher, and Jeffery T. Davis*

Contribution from the Department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland 20742

Received May 29, 2003; E-mail: jd140@umail.umd.edu

Abstract: In this paper, we report on the formation and properties of a water-stabilized dimer comprising calix[4]arene-guanosine conjugate cG **2**. The *1,3-alternate* calixarene cG **2** was poorly soluble in dry $CDCl_3$ and gave an ill-resolved NMR spectrum, consistent with its nonspecific aggregation. The compound was much more soluble in water-saturated $CDCl_3$. Two sets of well-resolved ^1H NMR signals for the guanosine residues in cG **2**, present in a 1:1 ratio, indicated that the compound's D_2 symmetry had been broken and provided the first hint that cG **2** dimerizes in water-saturated $CDCl_3$. The resulting dimer, (cG **2**)$_2$·(H$_2$O)$_n$, has a unique property: it extracts alkali halide salts from water into organic solution. This dimer is a rare example of a self-assembled ion pair receptor. The identity of the (cG **2**)$_2$·NaCl·(H$_2$O)$_n$ dimer was confirmed by comparing its self-diffusion coefficient in $CDCl_3$, determined by pulsed-field gradient NMR, with that of control compound cA **3**, an adenosine analogue. The dimer's stoichiometry was also confirmed by quantitative measurement of the cation and anion using ion chromatrography. Two-dimensional NMR and ion-induced NMR shifts indicated that the cation binding site is formed by an intermolecular G-quartet and the anion binding site is provided by the 5′-amide NH groups. Once bound, the salt increases the dimer's thermal stability. Both ^1H NMR and ion chromatography measurements indicated that the cG **2** dimer has a modest selectivity for extracting K^+ over Na^+ and Br^- over Cl^-. The anion's identity also influences the association process: NaCl gives a soluble, discrete dimer whereas addition of $NaBPh_4$ to (cG **2**)$_2$·(H$_2$O)$_n$ leads to extensive aggregation and precipitation. This study suggests a new direction for ion pair receptors, namely, the use of molecular self-assembly. The study also highlights water's ability to stabilize a functional noncovalent assembly.

Introduction

Water is crucial for the formation of biomolecular complexes.[1] Intermolecular association is often entropically driven, with release of each water molecule providing up to 2 kcal/mol in stabilization.[2] Alternatively, bound water can stabilize assemblies by forming hydrogen bond bridges, screening electrostatic repulsions, or filling holes at the intermolecular boundary. In this way, interfacial water helps control structure, stability, dynamics, and function.[3] Bound water contributes to the affinity and specificity of antibody−antigen complexes.[4] A hydration spine in the minor groove of B-form DNA stabilizes the duplex.[5,6] Interfacial water can also influence function. For example, a water layer between subunits allosterically regulates phosphofructokinase.[7]

Water's participation in synthetic assemblies has been, until recently, less appreciated. Water usually inhibits hydrogen-bonded complexes in organic solvents. Adrian and Wilcox showed, however, that "closed" receptors are less susceptible to such competition than are "open" analogues.[8] Bonar-Law and Sanders found that low concentrations of water can strengthen sugar−porphyrin binding,[9] most likely by filling gaps at the host−guest interface. Hydrogen bonding waters can fix the conformation of unimolecular receptors to enable productive binding.[10,11] Water also stabilizes hydrogen-bonded capsules. In the solid state, resorcinarenes form dimers sealed by solvent at their equators.[12,13] These bridging water molecules stabilize the dimers in solution, enabling guest encapsulation.[12b,13] Water is integral to even larger capsules. MacGillvary and Atwood

* Corresponding author: Telephone: 301-405-1845. Fax: 301-314-9121.

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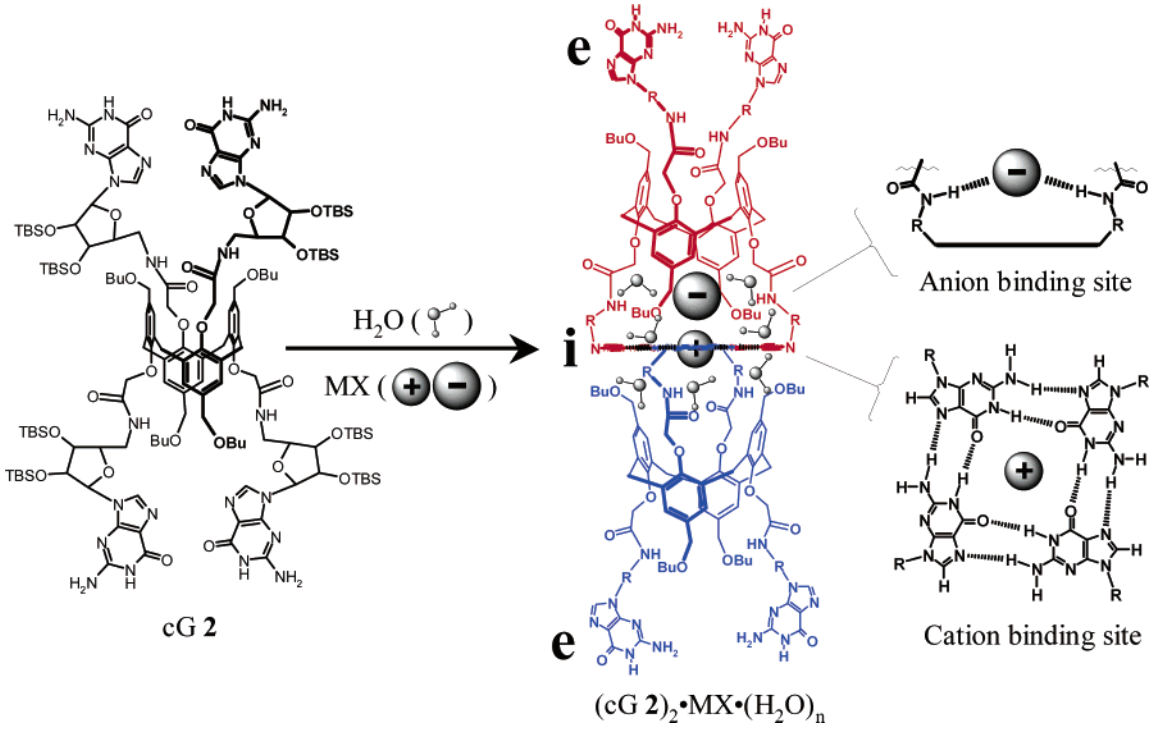

Figure 1. Schematic representation of (cG **2**)$_2$•MX•(H$_2$O)$_n$ formation from cG **2**, indicating the anion and self-assembled cation binding sites. Abbreviations: R = ribose; Bu = butyl; TBS = *tert*-butyldimethylsilyl. Internal (i) and external (e) regions of the dimer are labeled. Blue and red are used simply to distinguish between individual cG **2** molecules.

described the impressive solid-state structure of a hexameric resorcinarene with a water bound at each of its eight corners.[14] Rebek and Shivanyuk showed that these capsules bind large cationic guests in wet organic solvents.[15] Remarkably, NMR diffusion studies by Avram and Cohen revealed that these water-stabilized hexamers form spontaneously in $CDCl_3$, even in the guest's absence.[16] These diffusion NMR results, along with additional evidence from Rebek's group,[17] underscore water's key role in organizing large and functional assemblies in organic solution. In addition to stabilizing spherical containers, bridging water can promote formation of tubular structures.[18−20] One such assembly facilitated Na^+ transport across a lipid membrane.[18a] While developing synthetic anion channels, we described a calix[4]arene tetraamide that crystallizes to give channels held together by bridging water and chloride anions.[20]

Significant attention in supramolecular chemistry has recently turned toward ion pair recognition. One of the motivations for this work is to identify receptors that can cotransport inorganic salts across membranes, a significant challenge given the requirement to dehydrate the charge-dense ions. One ion pair recognition strategy uses a ditopic receptor containing a Lewis base for cation coordination and a Lewis acid for anion binding.[21] Among the growing number of ditopic receptors there are some that bind and transport hydrophilic salts such as NaCl quite efficiently.[22] Self-assembly of a ditopic receptor is another attractive strategy for simultaneous recognition of cations and anions.[23−25] Our interest in ion transporters has now led us to more fully appreciate water's ability to help organize structure and regulate function. We describe a water-stabilized dimer formed in water-saturated $CDCl_3$ by the calix[4]arene-guanosine conjugate cG **2** (Chart 1).[26] Water enhances the organic solubility and ionophoric properties of cG **2**, apparently by stabilizing an intermolecular hydrogen bonded G-quartet. The resulting (cG **2**)$_2$•(H$_2$O)$_n$ dimer extracts alkali halides from water into organic solution to give a salt-bound dimer, (cG **2**)$_2$•MX•(H$_2$O)$_n$, whose structure is schematically depicted in Figure 1.

Rationale. The G-quartet (Chart 1), a noncovalent macrocycle composed of four hydrogen-bonded guanines, is well-known in the context of mononucleotides and DNA. G-quartets, with

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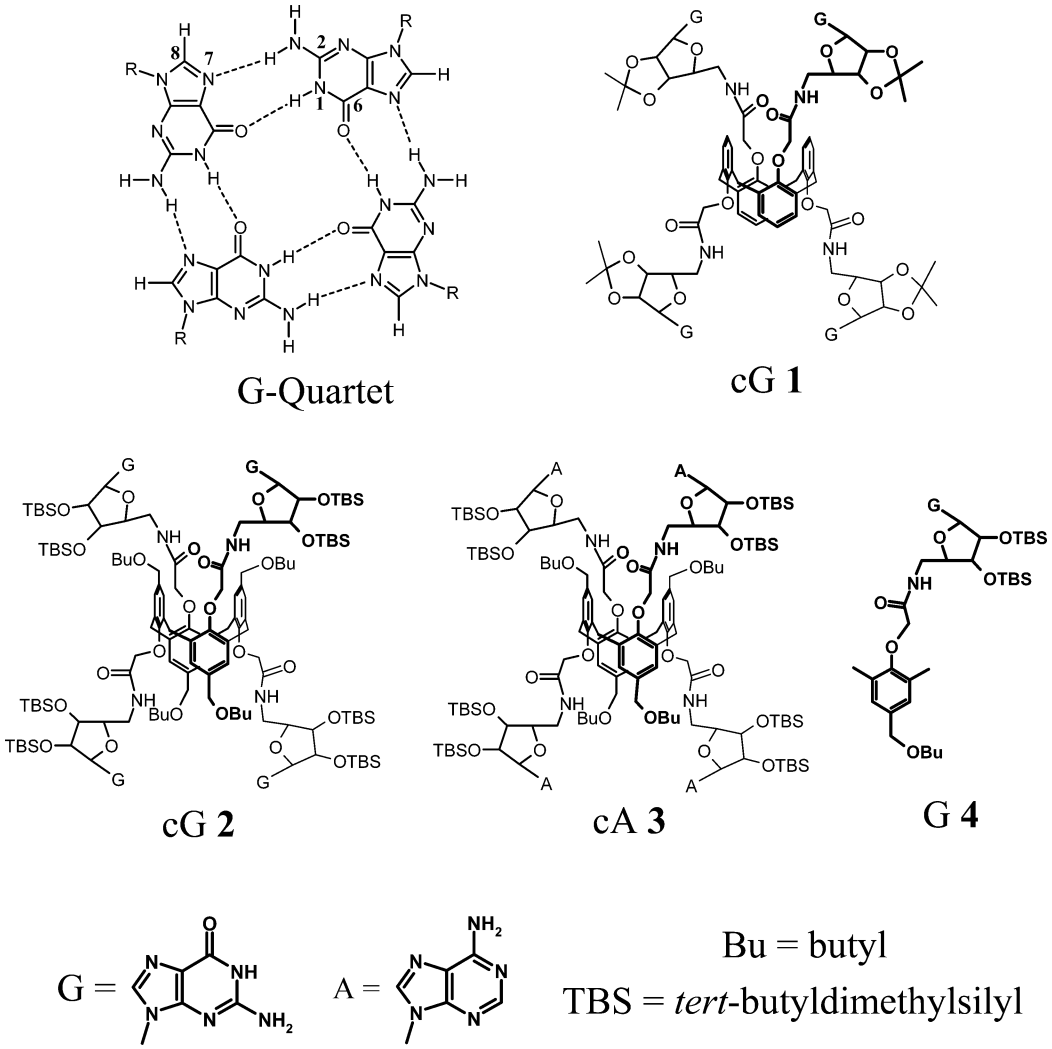
Chart 1



a strong affinity for alkali-metal cations, tend to stack to give structures known as G-quadruplexes.[27] In the mid-1990s, Gottarelli and co-workers showed that lipophilic guanosines formed G-quartets in organic solvents.[28] Later, we solved a crystal structure of a lipophilic guanosine quadruplex showing that four G-quartets stack to give a cation "channel" that is 30 Å long.[29] We reasoned that such a channel might be extended if guanosine bases were attached to a 1,3-*alternate* calix[4]-arene,[30] a platform that would orient orthogonal pairs of guanines so that tubular structures might be attained upon cation-templation of G-quartets. This approach has precedence in that other functionalized 1,3-alternate calixarenes form infinite tubes,[31] and 1,3-alternate calixarenes can function as ion channels in phospholipid membranes.[20,32] Our initial studies seemed promising,[33] as $NaBPh_4$ addition to cG **1** in 1:1 CH_3CN-H_2O gave quantitative precipitation of fibers with diameters consistent with the G-quartet's dimensions. Also, the 1:1 Na^+:cG **1** stoichiometry for this precipitate was that expected for a polymer composed of intermolecular G·Na^+ quartets.[33] However, the poor solubility of cG **1** made its purification tedious, precluded NMR investigation of self-assembly, and made it unlikely that this first-generation compound would function well as an ion transporter in membranes. Therefore, we prepared a more lipophilic derivative, cG **2** (Chart 1), with 2′,3′-TBDMS

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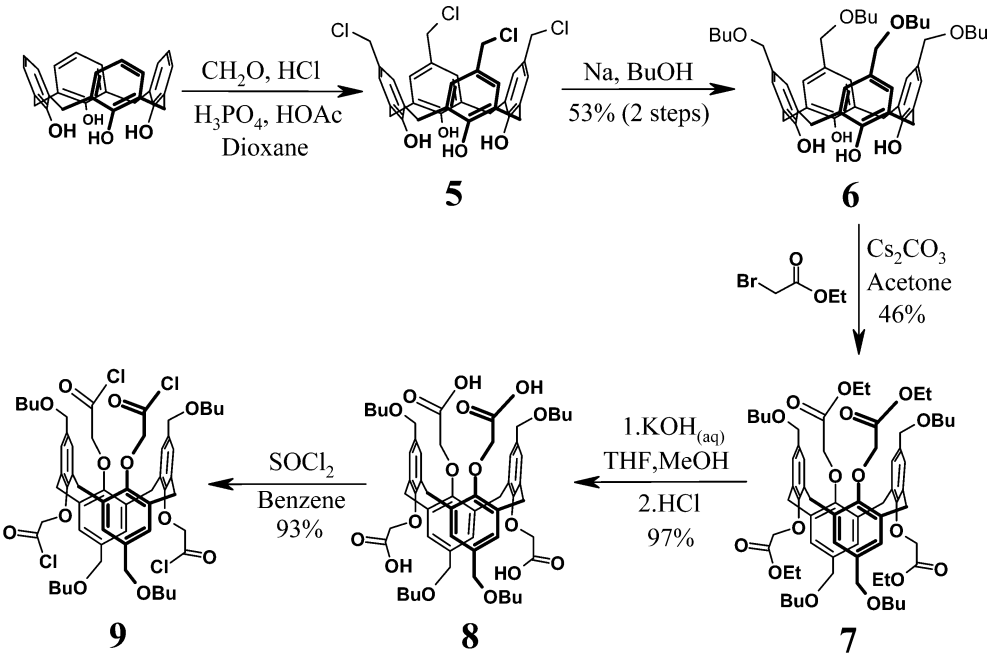
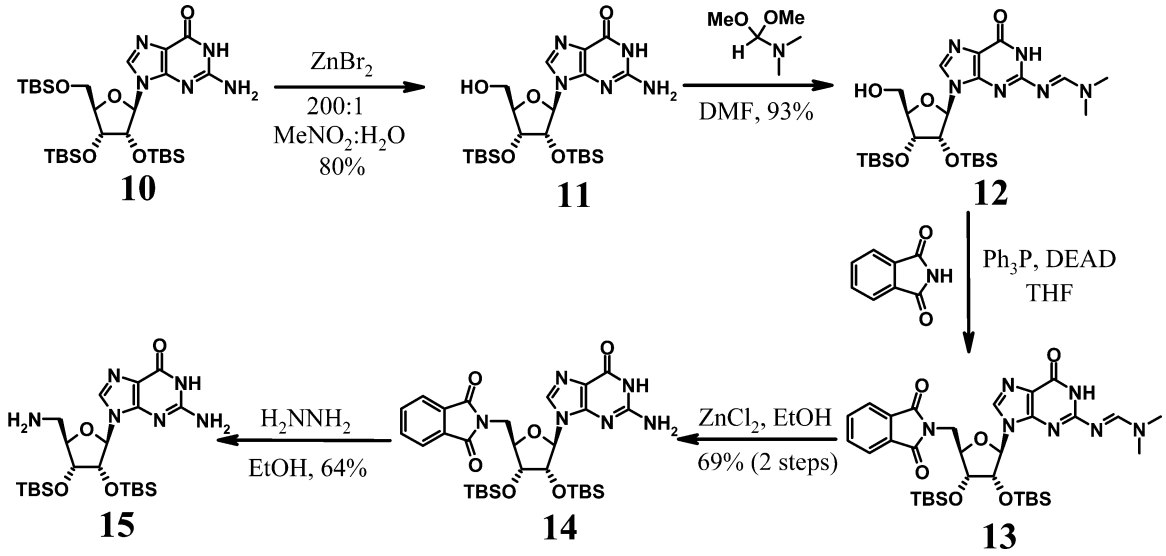
Scheme 1. Synthesis of Calix[4]arene-1,3-Alternate Acid Chloride **9**



Scheme 2. Synthesis of 5′-Amino-5′-deoxy-2′,3′-*O*-di-*tert*-butyldimethylsilylguanosine **15**



groups on the ribose rings and butoxymethylene chains on the calixarene's upper rim. We found that cG **2** can be synthesized and purified in gram quantities and that its solubility in water-saturated $CDCl_3$ enabled NMR investigation of its self-assembly and salt binding.

Results and Discussion

Synthesis of cG 2 and Control Compounds. Chart 1 shows the major compounds in this study. The 1,3-alternate calix[4]-arene cG **2** is the building block for the ion pair receptor. Calix-[4]arene cA **3** and nucleoside G **4** were controls for determining the roles of the nucleobase and calixarene core in self-assembly and salt binding. Adenosine does not form hydrogen-bonded quartets,[34] making the 1,3-alternate calixarene cA **3** ideal for determining guanine's involvement in self-association. Mono-nucleoside G **4** contains the same functional groups as cG **2**, but lacks the calix[4]arene's preorganization.

Calix[4]arene-1,3-*alt*-tetraacid chloride **9** was prepared as outlined in Scheme 1. Chloromethylation of calix[4]arene's upper rim,[35] followed by coupling with butoxide, gave **6**. Subsequent O-alkylation of the lower rim with ethyl bromo-acetate, using Cs_2CO_3 as base,[36] afforded a 46% yield of the conformationally fixed 1,3-alternate calixarene **7**. Base hydroly-sis of tetraester **7** gave tetraacid **8**, and subsequent reaction with thionyl chloride afforded the tetraacid chloride **9**.

The nucleoside component, 5′-amino-5′-deoxy-2′,3′-*O*-di-*tert*-butyldimethylsilylguanosine **15** (Scheme 2), was prepared starting with 2′,3′,5′-trisilyl guanosine **10.**[37] The 5′-silyl group

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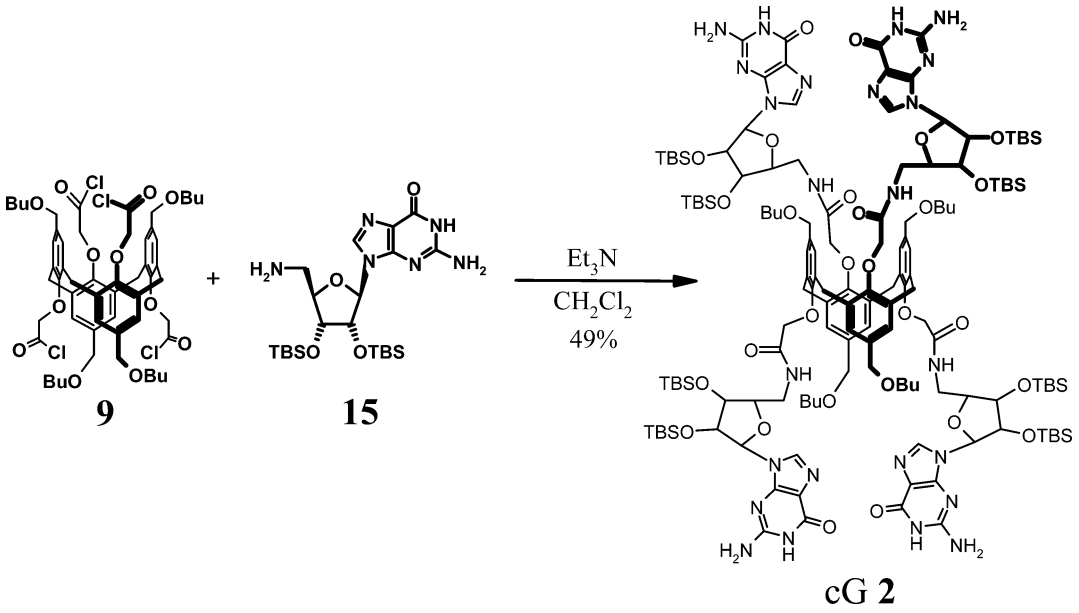
Scheme 3. Final Coupling to Give Calix[4]arene-guanosine cG **2**



of **10** was selectively removed using $ZnBr_2$ in aqueous nitromethane to give **11**.[38] Protection of the guanine N2 amine as an amidine, followed by Mitsunobu coupling of phthalimide to the 5′-carbon, afforded **13**. Amidine removal and subsequent hydrazinolysis gave 5′-amino G **15**.[39,40] The target cG **2** was obtained in 49% yield by coupling calix[4]arene acid chloride **9** and G **15** (Scheme 3). The ^{1}H NMR spectrum for cG **2** was consistent with a chiral 1,3-alternate calix[4]arene of D_2 symmetry. Achiral 1,3-alternate calixarenes with the same para substituents on each ring have D_{2d} symmetry and show a singlet for their homotopic bridging methylene protons and a singlet for their aromatic protons.[41] In cG **2**, the four homochiral guanosines attached to the lower rim make the methylene bridge protons and the aromatic protons diastereotopic.[41] In DMSO-d_6, cG **2** showed singlets at δ 7.00 and 6.93 for the aromatic protons and singlets at δ 3.71 and 3.70 for the methylene bridge protons.

Conjugate cA **3** was obtained by coupling 5′-amino-5′-deoxy-2′,3′-*O*-di-*tert*-butyldimethylsilyladenosine and acid chloride **9**. Mononucleoside G **4** was obtained in 66% yield upon coupling acid chloride **20** and G **15** (see supporting information).

cG 2 Forms a Discrete Species in Water-Saturated $CDCl_3$. NMR spectra in Figure 2 indicate that water enables discrete self-assembly of cG **2** in $CDCl_3$. In the competitive solvents DMSO-d_6 (Figure 2A) and CD_3OD (not shown) cG **2** gives a ^{1}H NMR spectrum that is consistent with a monomeric species having D_2 symmetry. Due to its D_2 symmetry, all four aryl groups in the 1,3-alternate calix[4]arene cG **2** are chemically equivalent. Therefore, a single set of ^{1}H NMR resonances is observed. In contrast, cG **2** was poorly soluble in commercial

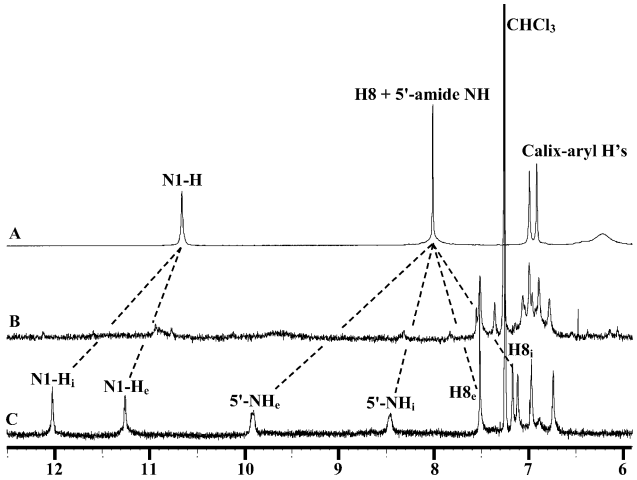


Figure 2. ^{1}H NMR spectra of (A) cG **2** in DMSO-d_6, (B) cG **2** in commercial $CDCl_3$, and (C) (cG **2**)$_2$·$(H_2O)_n$ in water-saturated $CDCl_3$. Dashed lines indicate the splitting of cG **2** signals.

$CDCl_3$, giving a NMR spectrum with broad or absent amide NH signals and multiple signals for carbon-bound protons (Figure 2B). We attributed this poor solubility and ill-resolved NMR spectrum to nonspecific aggregation of cG **2**. In addition, the quality of the spectra would vary with different batches of $CDCl_3$, suggesting that the solvent's water content was influencing the association state of cG **2**. Washing a $CDCl_3$ suspension of cG **2** with water completely dissolved the compound and greatly improved its NMR spectral resolution, indicating a water-mediated transformation from an aggregate to a discrete species. The same spectrum was also obtained by dissolving cG **2** in water-saturated $CDCl_3$.[42] Two sets of well-resolved ^{1}H NMR signals for the guanosine residues in cG **2**, present in a 1:1 ratio (Figure 2C), indicated that the compound's D_2 symmetry had been broken and provided the first hint that cG **2** dimerizes in water-saturated $CDCl_3$. Signals for the amide NH protons at G N1 ($\Delta\delta = +1.3$ and $+0.6$ ppm) and the 5′-NH position ($\Delta\delta = +1.9$ and $+0.5$ ppm) underwent significant downfield shifts in water-saturated $CDCl_3$ relative to DMSO-d_6, consistent with their participation in hydrogen bonds. This last feature is quite

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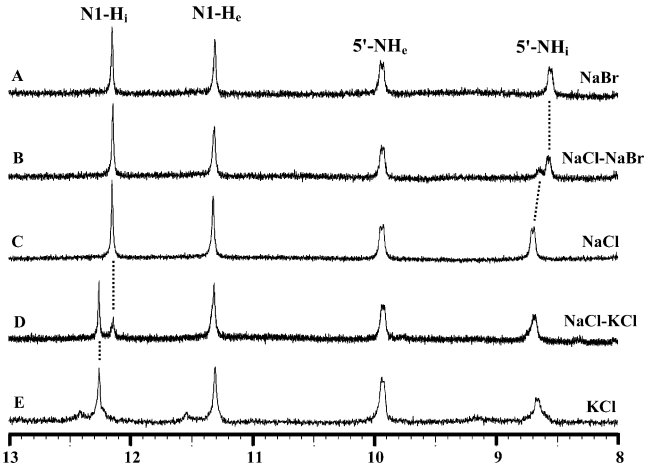

Figure 3. Low-field region of the ^{1}H NMR spectra of (cG **2**)$_2$·(H$_2$O)$_n$ in water-saturated CDCl$_3$ after washing with aqueous (A) 1.0 M NaBr, (B) 0.5 M NaBr/0.5 M NaCl, (C) 1.0 M NaCl, (D) 0.5 M NaCl/0.5 M KCl, and (E) 1.0 M KCl. Dashed lines highlight the individual complexes in the mixtures.

unusual for amide NH protons, as they are usually shifted farther upfield in CDCl$_3$ compared to DMSO-d_6 due to DMSO's stronger hydrogen bond acceptor properties. We tentatively attributed the two sets of NMR signals in water-saturated CDCl$_3$ to the "internal" (i) and "external" (e) guanosines in a noncovalent dimer of formula (cG **2**)$_2$·(H$_2$O)$_n$ (see Figure 1). As detailed below, we believe that this dimer arises from water-mediated formation of an intermolecular G-quartet.[43]

The Water Stabilized Dimer (cG 2)$_2$·(H$_2$O)$_n$ Is an Ion Pair Receptor. We used multinuclear NMR and ion chromatography to show that (cG **2**)$_2$·(H$_2$O)$_n$ extracts alkali halides from water into CDCl$_3$. The insolubility of alkali halides in CDCl$_3$ precluded the determination of stability constants, but the water-complex (cG **2**)$_2$·(H$_2$O)$_n$ clearly binds hydrophilic salts in this nonpolar organic solvent. Ion-induced changes in the ^{1}H NMR spectra first identified the cation and anion binding sites. Stirring a CDCl$_3$ solution of (cG **2**)$_2$·(H$_2$O)$_n$ for 12 h with either aqueous 1.0 M NaBr, 1.0 M NaCl, or 1.0 M KCl gave distinct spectra for the (cG **2**)$_2$·MX·(H$_2$O)$_n$ complexes (Figure 3A,C,E). The more upfield of the two 5′-NH amide protons (δ 8.6) showed different chemical shifts for the NaCl and NaBr complexes (Figure 3A,C), implicating this particular 5′-NH proton in anion binding. In contrast, the chemical shift of the other 5′-NH proton (δ 9.9 ppm) did not change after the salt washes. Comparative ^{1}H NMR spectra for the NaCl and KCl complexes showed that the amide N1−H near δ 12.2 is sensitive to the cation's identity, unlike the other N1−H signal at δ 11.3 (Figure 3C,E). Competition experiments revealed that the (cG **2**)$_2$•(H$_2$O)$_n$ has

Table 1. Ion Concentration from the Back Extraction of Salt from (cG **2**)$_2$·MX·(H$_2$O)$_n$ in CD$_2$Cl$_2$ into H$_2$O Measured by Ion Chromatography[a]

salt extracted	cation (concentration, mM)	anion (concentration, mM)
NaCl	Na^+ (0.72 ± 0.14)	Cl^- (0.60 ± 0.07)
NaBr	Na^+ (0.80 ± 0.06)	Br^- (0.65 ± 0.03)
KCl	K^+ (0.81 ± 0.08)	Cl^- (0.85 ± 0.08)
KBr	K^+ (0.95 ± 0.09)	Br^- (0.96 ± 0.04)
1:1:1:1 Na^+:K^+:Cl^-:Br^-	Na^+ (0.37 ± 0.07)	Cl^- (0.19 ± 0.02)
	K^+ (0.71 ± 0.06)	Br^- (0.69 ± 0.07)

[a] The concentration of cG 2 was 2 mM.

modest cation and anion extraction selectivities. A K^+:Na^+ selectivity of 2:1 was determined from integration of the separate N1−H signals near δ 12.2 after extraction of a 1:1 NaCl and KCl solution (Figure 3D). The K^+:Na^+ selectivity was the same whether using the Cl^- and Br^- salts, indicating little cooperativity in the extraction of alkali halide salts by cG **2**.[44] To measure anion extraction selectivity, an aqueous solution containing 1:1 NaCl:NaBr (0.5 M each) was extracted using cG **2**. Integration of the separate 5′-NH signals near δ 8.6 revealed a 2:1 Br^-:Cl^- extraction selectivity (Figure 3B). Again, no cooperativity in alkali halide extraction was observed, as the anion selectivity was the same for the Na^+ and K^+ salts.

Direct evidence for Na^+ extraction by cG **2** was obtained by ^{23}Na NMR spectroscopy. A 20 mM solution of (cG **2**)$_2$·NaCl·(H$_2$O)$_n$ in CDCl$_3$ gave a ^{23}Na NMR signal at δ −5.23 (Figure S1 in supporting information). Extraction of NaCl, NaBr, KCl, and KBr by cG **2** (2 mM) was quantified by ion chromatography (IC). Complexes of (cG **2**)$_2$·MX·(H$_2$O)$_n$ in CD$_2$Cl$_2$ were first generated by salt extraction from water. The receptor-bound salt was back-extracted into H$_2$O and the aqueous layer's ion content was measured using IC (Table 1). ^{1}H NMR spectra before and after salt back-extraction confirmed the complete conversion of the (cG **2**)$_2$·MX·(H$_2$O)$_n$ complexes to the water dimer (cG **2**)$_2$·(H$_2$O)$_n$. Back-extraction of KBr gave a cG **2**:salt ratio of ∼2:1, consistent with the stoichiometry for a calixarene dimer binding one ion pair, while ratios somewhat greater than 2:1 were obtained with NaCl, NaBr, and KCl. Importantly, the relative cation and anion concentrations measured by IC were always close to 1:1, consistent with ion pair binding by the cG **2** dimer.

We also directly determined the ion extraction selectivity of cG **2** by IC. An aqueous solution of a 1:1 mixture of NaBr (1.0 M) and KCl (1.0 M) was stirred over a solution of cG **2** in CD$_2$Cl$_2$ for 12 h, the organic solvent was back-extracted with water, and the ion content of the aqueous phase was measured. Extraction selectivities by cG **2** were approximately 2:1 for K^+:Na^+ and 3:1 for Br^-:Cl^- (Table 1), in good agreement with the ^{1}H NMR derived selectivities. One factor that certainly contributes to extraction selectivity is the lower desolvation energies for K^+ (70.5 kcal/mol) versus Na^+ (87.2 kcal/mol) and Br^- (75.3 kcal/mol) versus Cl^- (81.3 kcal/mol).[45] However, ion dehydration cannot be the only determinant. Otherwise, the K^+/Na^+ and Br^-/Cl^- selectivities would be much greater, given the large

(42) It is difficult to get cG **2** completely dry. Thus, ^{1}H NMR indicated that a 2 mM solution of cG **2** in CDCl$_3$ still contained 1−2 mM H$_2$O, even after the compound had been rotovaped from toluene 3 times followed by in vacuo drying over P$_2$O$_5$ for 24 h. In this paper we define "wet" CDCl$_3$ as having water concentrations between 0.5 and 10 mM H$_2$O. "Dry" CDCl$_3$ was prepared according to Bonar-Law and Sanders.[9] Thus, small amounts of powdered and activated 4-Å molecular sieves were added to CDCl$_3$ solutions of cG **2** until the residual H$_2$O peak at δ 1.5−1.7 had disappeared. "Water-saturated" CDCl$_3$ was prepared by stirring with an aqueous phase for 1 h; ^{1}H NMR showed it to contain 51 mM H$_2$O. For some other compounds with similar behavior in dry and wet CDCl$_3$, see refs 12b and 13.

(43) Titration with DMSO-d_6 in water-saturated CDCl$_3$ gave separate signals for the dimeric and monomeric forms of cG **2**. The monomer/dimer ratio increased with increasing DMSO-d_6 concentration due to denaturation of the hydrogen-bonded dimer.

(44) cG **2** does show cooperativity in the sense that it dimerizes to form a "salt" receptor for relatively small and coordinating ions. For example, according to ion-induced NMR shifts, cG **2** binds NaCl, NaBr, NaOAc, NaPF$_6$, and NaSCN. Salts with large, noncoordinating counterions do not interact with cG **2** to give discrete complexes in solution. As described below, NaBPh$_4$ and (cG **2**)$_2$·(H$_2$O)$_n$ gave a precipitate, and Bu$_4$NCl addition to a solution of cG **2** gave no evidence for Cl^- binding.

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difference in desolvation energies.[46] It is likely that the ions bound in the dimer are not completely dehydrated due to residence in a water-filled cavity at the dimer's core. There are clearly other factors that must enhance the Na^+ and Cl^- binding affinities of the dimer (cG **2**)$_2$.

Control compounds demonstrate that both guanine and the calix[4]arene are critical in cG **2** self-association and ion binding. In contrast to cG **2**, neither cA **3** nor G **4** demonstrated self-assembly or ionophore properties. Both cA **3** and G **4** were highly soluble in $CDCl_3$, gave single sets of ^{1}H NMR resonances, and showed only minor NMR spectral changes upon washing with water or aqueous salt solutions (see Figures S6 and S7 in supporting information). Both cA **3** and G **4** extracted little salt (<10% by IC) and showed no ^{23}Na NMR signal upon washing 20 mM solutions of the control compounds with 1.0 M $NaCl_{(aq)}$. These control experiments indicate that cG **2** requires both the guanine base and the calix[4]arene core for dimerization and ion pair extraction.

Confirmation of a Dimer by Pulsed-Field Gradient NMR. We used pulsed-field gradient NMR (PFG-NMR), a technique that provides self-diffusion coefficients (D_s),[47] to obtain supporting evidence for a solution-phase dimer of cG **2**.[48] Diffusion NMR, widely used for studying protein aggregation,[49] has only recently been applied to host−guest and hydrogen-bonded assemblies in organic solvents.[16,50] In particular, PFG-NMR has been used to confirm dimerization for a variety of noncovalent systems, from proteins to antibiotics to hydrogen-bonded complexes.[48,51,52] A number of theoretical studies have predicted that a dimer should have a self-diffusion coefficient (D_s) that is 72−75% that of its corresponding monomer.[49,53,54]

Hupp and Larive have stressed the importance of using appropriate standards for interpreting diffusion NMR measurements: "Excluding strictly spherical species, evaluation of molecular weights via diffusion parameters requires diffusion standards because diffusion coefficients are not directly related to molecular weight. Instead, they reflect effective hydrodynamic radii. Therefore, not only the size but also the shape of the molecule will determine the value of D_s." [55] Since reliable use of diffusion coefficients requires a standard with similar structure and molecular weight, we measured D_s values for samples containing both cG **2** (mw 2972) and cA **3** (mw 2908) in the same NMR tube. Monomeric cA **3** served as the internal standard for determining the association state of cG **2** in different solvents. NMR spectra indicated that there were no significant interactions between cG **2** and cA **3**. The D_s values for 1:1 mixtures of (cG **2**)$_2$·NaCl·$(H_2O)_n$ and cA **3** (both 6.7 mM in monomer) were determined in water-saturated $CDCl_3$ and DMSO-d_6 (Table 2). Values of D_s were determined based on the attenuation of the H1′ signal for cG **2** and cA **3**. Stejskal-Tanner plots ($\ln(I/I_o)$ versus $\gamma^2 g^2 \delta^2(\Delta - \delta/3)$) are shown in Figure 4.

Table 2. Self-Diffusion Coefficients (D_s) for (cG **2**)$_2$·NaCl·$(H_2O)_n$ and cA **3** Measured by PFG-NMR in Water-Saturated $CDCl_3$ and DMSO-d_6

solvent	D_s ((cG **2**)$_2$·NaCl·$(H_2O)_n$), 10^{-10} m^2/s	D_s (cA **3**), 10^{-10} m^2/s	ratio D_s(**2**):D_s(**3**)
$CDCl_3$	3.82 ± 0.10	5.37 ± 0.10	0.71 ± 0.01
DMSO-d_6	0.91 ± 0.02	0.94 ± 0.01	0.97 ± 0.01

Figure 4A and Table 2 show that the intact (cG **2**)$_2$·NaCl·$(H_2O)_n$ dimer, with a diffusion coefficient of 3.82×10^{-10} m^2/s, diffuses significantly slower than cA **3** (5.37×10^{-10} m^2/s) in water-saturated $CDCl_3$. The ratio of D_s(**2**):D_s(**3**) = 0.71 (Table 2) agrees well with the theoretical ratio of 0.72−0.75 expected for a dimer.[49,53,54] In DMSO-d_6, where (cG **2**)$_2$·NaCl·$(H_2O)_n$ is completely denatured to give a cG **2** monomer, both cG **2** and cA **3** diffuse at similar rates, as reflected by a D_s(**2**):D_s(**3**) ratio of 0.97 (Figure 4B and Table 2). These similar diffusion coefficients in DMSO are as expected for two molecules of similar size, shape, and molecular weight. This diffusion NMR study, confirming a dimer in $CDCl_3$, is entirely consistent with the two sets of ^{1}H NMR signals for (cG **2**)$_2$·NaCl·$(H_2O)_n$ and with the near 2:1 calixarene:salt stoichiometry determined by IC. We conclude that the dimer (cG **2**)$_2$·NaCl·$(H_2O)_n$ has 1 equiv of bound salt.

Variable Temperature NMR Shows that Salt Binding Stabilizes the Dimer. Both the water complex (cG **2**)$_2$·$(H_2O)_n$ and the salt complex (cG **2**)$_2$·NaCl·$(H_2O)_n$ have similar NMR spectra at room temperature, consistent with their similar structures. However, these 2 complexes have much different spectra at high and low temperatures. Above 35 °C the well-resolved signals for (cG **2**)$_2$·$(H_2O)_n$ degenerated into the ill-defined signals characteristic of aggregation (Figure 5A). This temperature-dependent aggregation of cG **2** is presumably entropically driven by the loss of bound water to the bulk solution. The process is reversible, as cooling the $CDCl_3$ solution of cG **2** back to 25 °C regenerated the spectrum for (cG **2**)$_2$·$(H_2O)_n$. In sharp contrast to the relative lability of the water complex at higher temperatures, the salt-bound dimer (cG **2**)$_2$·NaCl·$(H_2O)_n$ remained intact even up to 50 °C (Figure 5B). The dimer's enhanced stability in the presence of salt is likely due to Na^+ coordination by a G-quartet and Cl^- binding by the 5′-amide NH groups at the dimer's core (Figure 1).

The effects of low temperature on (cG **2**)$_2$·$(H_2O)_n$ and (cG **2**)$_2$·NaCl·$(H_2O)_n$ were also drastically different (see Figure S8 in supporting information). The (cG **2**)$_2$·$(H_2O)_n$ sample was "denatured" upon cooling to −60 °C. This denaturation was apparent upon warming the sample back to room temperature, as the sample displayed the ill-resolved ^{1}H NMR spectrum so

(46) In a DNA G-quadruplex study, Feigon and colleagues stated: "Na^+ actually binds the coordination sites of $[d(G_3T_4G_3)]_2$ with a more favorable free energy than K^+. However, Na^+ is displaced by K^+ because of the more negative hydration free energy of Na^+." Hud, N. V.; Smith, F. W.; Anet, F. A. L.; Feigon, J. *Biochemistry* **1996**, *35*, 15 383−15 390.
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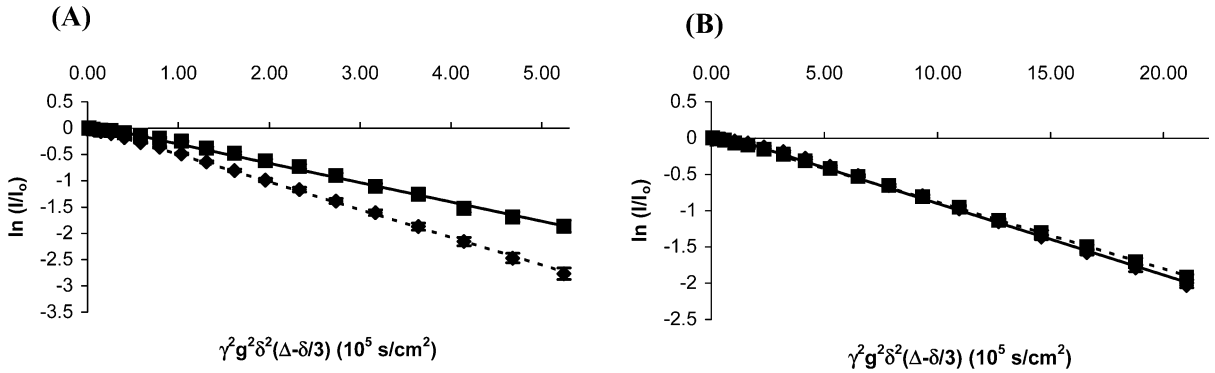

Figure 4. Stejskal-Tanner plots[47a] of the normalized signal intensity as a function of $\gamma^2g^2\delta^2(\Delta \angle \delta/3)$ for (A) dimeric (cG **2**)$_2$·NaCl·(H$_2$O)$_n$ (solid) and monomeric cA **3** (dashed) in water-saturated $CDCl_3$ and (B) cG **2** (solid) and cA **3** (dashed, overlaid) in DMSO-d_6. Data were recorded at 26 °C and are derived from the attenuation of H1′ signals of cG **2** and cA **3**. The slope of each line is equal to $-D_s$ for that species.

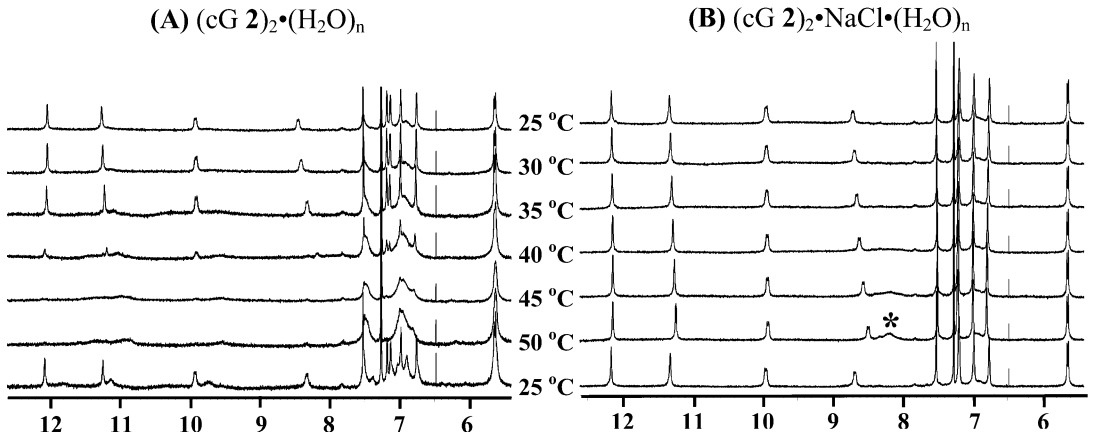

Figure 5. ^{1}H NMR spectra in water-saturated $CDCl_3$ from 25 °C up to 50 °C, then back to 25 °C for (A) (cG **2**)$_2$·(H$_2$O)$_n$ and (B) (cG **2**)$_2$·NaCl·(H$_2$O)$_n$. Samples were 2 mM in cG **2**. The asterisk in (B) indicates the N2−H$_i$ signal at 50 °C.

typical of nonspecific aggregation. Cooling to −60 °C must freeze out the bound water in (cG **2**)$_2$·(H$_2$O)$_n$, shifting the equilibrium from the dimer to an aggregate. In sharp contrast, the same experiment with (cG **2**)$_2$·NaCl·(H$_2$O)$_n$ resulted in no changes in the ^{1}H NMR spectra before and after this same cooling−warming cycle. These comparative experiments at high and low temperatures indicate that the bound salt stabilizes the calixarene dimer.

NMR Evidence for a G-Quartet in (cG 2)$_2$·NaCl·(H$_2$O)$_n$. Assignments for the two sets of NMR signals in (cG **2**)$_2$·NaCl·(H$_2$O)$_n$ in $CDCl_3$ were based on ^{1}H−^{1}H COSY, ^{1}H−^{1}H NOESY, and ^{1}H−^{13}C HMQC data (see Figures S3 and S4 in supporting information). The two sets of signals are referred to as internal (i) and external (e) guanosines, as depicted in Figure 1.

At low temperature (0 to −20 °C) in $CDCl_3$, two new signals appear for (cG **2**)$_2$·NaCl·(H$_2$O)$_n$ at δ 9.9 and 6.6 (Figure S2). These well-separated N2$_i$ amino signals, which belong to the internal G, arise due to slow C2−N2 bond rotation caused by the G-quartet's N2−H···N7 hydrogen bond.[56] For (cG **2**)$_2$·NaCl·(H$_2$O)$_n$ the hydrogen-bonded proton occurs at δ 9.9 and the "free" proton is at δ 6.6. At room temperature, these two signals broaden into the baseline. At 50 °C, faster C2−N2 bond rotation causes the two N2−H protons to become equivalent on the NMR time scale, giving a single resonance at δ 8.25 (marked with an asterisk in Figure 5B).

Diagnostic NOEs for the G-quartet were identified in (cG **2**)$_2$·NaCl·(H$_2$O)$_n$. As shown in Figure 6A,B, both N2−H$_i$ amino protons (δ 9.9 and 6.6) showed NOEs to G H8$_i$ at δ 7.2. These N2−H8 NOEs between hydrogen-bonded neighbors are hallmarks of the G-quartet structure;[56] N2−H and H8 within the same nucleobase are too far apart for an intramolecular NOE. Although the data does not distinguish between possible geometries, we are confident that (cG **2**)$_2$·NaCl·(H$_2$O)$_n$ is held together by an intermolecular G-quartet. Regardless of the exact morphology, it is the G-quartet's electronegative pocket that enables the (cG **2**)$_2$ dimer to bind hydrophilic cations.

Location of the Water in the (cG 2)$_2$·NaCl·(H$_2$O)$_n$ Complex by ^{1}H−^{1}H NOESY. NOEs are often used to locate bound water in biomolecules and in organic complexes.[57,58] For (cG **2**)$_2$·NaCl·(H$_2$O)$_n$, NOEs located the water to be bound near the G-quartet. At −20 °C, the downfield-shifted H$_2$O signal (δ 2.8) showed NOEs to N1−H$_i$ (δ 12.3), 5′-amido NH$_i$ (δ 8.6), both N2−H$_i$ amino protons (δ 9.9, 6.6), and, most importantly, the nonexchangeable H8$_i$ (δ 7.2). All of these NOEs to H$_2$O were from the "internal" guanosine that make up the G-quartet (Figure 6C). Water-nucleobase cross-peaks were not observed for the dimer's external set of signals. The G N1−H$_i$ and 5′-NH$_i$ amide protons that correlate with H$_2$O are also the same protons

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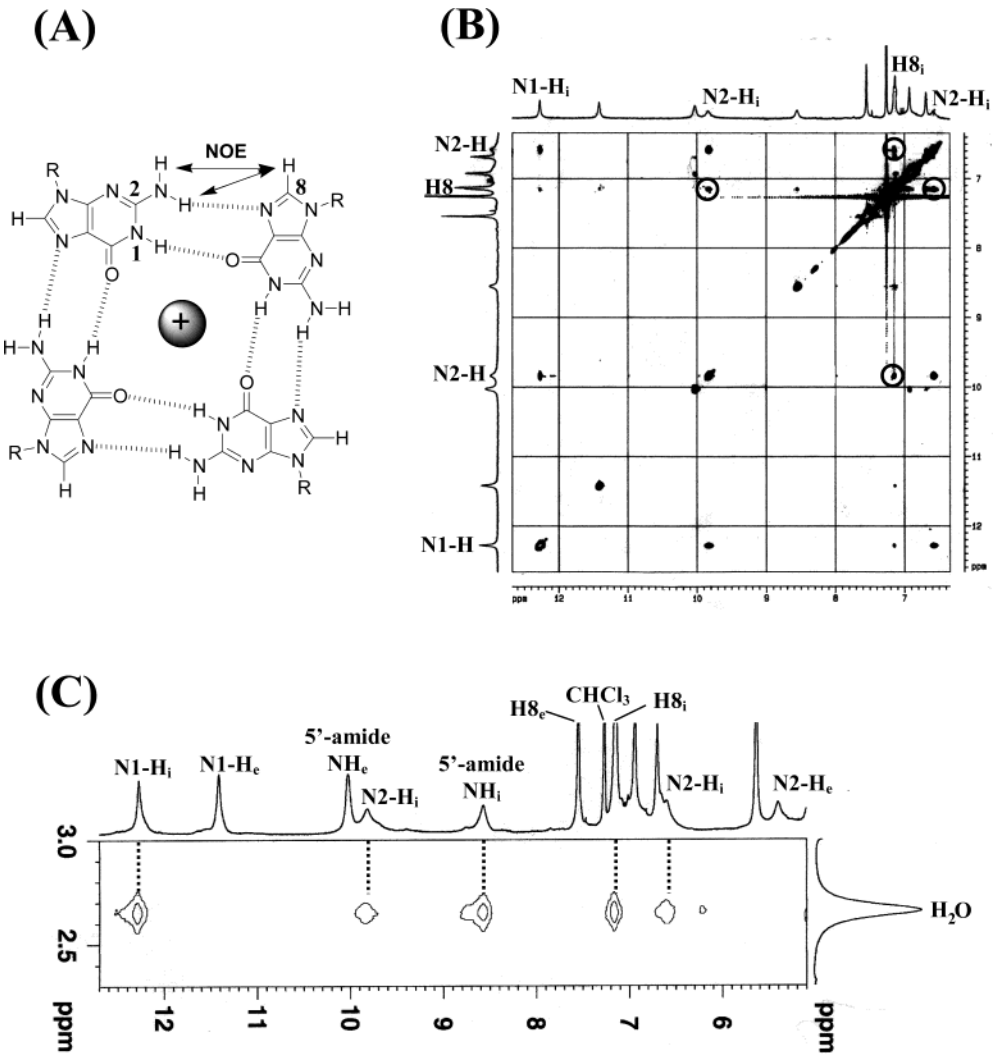

Figure 6. ^{1}H−^{1}H NOESY spectrum of (cG **2**)$_2$·NaCl·(H$_2$O)$_n$ in CDCl$_3$ at −20 °C: (A) G-quartet indicating characteristic intermolecular N2−H to H8 NOEs. (B) Low-field region of the spectrum showing correlations between N1H$_i$, N2H$_i$s, and H8$_i$ at the G-quartet bridge of (cG **2**)$_2$·NaCl·(H$_2$O)$_n$ (characteristic G-quartet N2H$_i$–H8$_i$ NOEs are circled). (C) Correlations to the H$_2$O signal. Only the internal (i) set of signals shows cross-peaks.

implicated to be in the dimer's cation and anion binding sites, indicating that the ion pair and water bind nearby in the dimer's core.

CD Spectra of cG 2 Depend on the Solvent and Conditions. Circular dichroism (CD) has previously been used to study the structure of chiral calixarenes[59,60] and of G-quartet based assemblies.[29,61] We found that CD spectroscopy also provided qualitative insight into self-association of cG **2**. The CD spectra of cG **2** (1.0 mM) were recorded using a 0.1-mm cell to ensure that the same concentrations were used for CD and NMR measurements. As shown in Figure 7, the spectra vary with solvent and ionic conditions, reflecting a significant conformational change by cG **2**. The CD spectrum in MeOD, a solvent in which cG **2** is monomeric, has a symmetrical exciton coupling, with a positive band centered at 282 nm and a negative band centered at 250 nm. The spectrum of (cG **2**)$_2$·(H$_2$O)$_n$ in wet CDCl$_3$ is quite different from the spectrum in MeOD, as it shows an asymmetric and positive Cotton band. This difference indicates a change in conformation and/or secondary structure

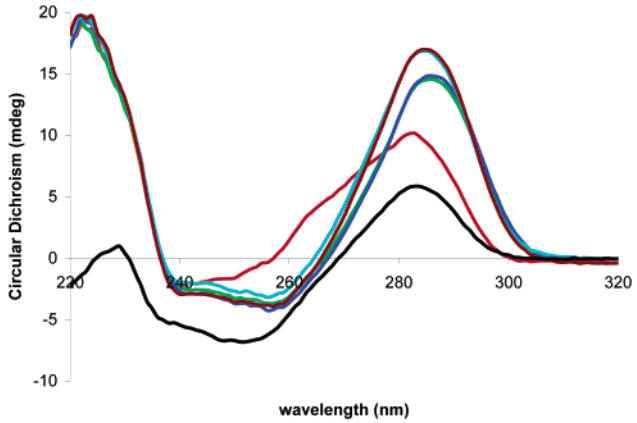

Figure 7. Circular dichroism spectra of cG **2** in MeOD (black line),(cG **2**)$_2$·(H$_2$O)$_n$ in water-saturated CDCl$_3$ (red line), and (cG **2**)$_2$·MX·(H$_2$O)$_n$ in water-saturated CDCl$_3$, where MX is NaCl (green line), NaBr (navy line), KCl (blue line), and KBr (maroon line). All samples were 1.0 mM in cG **2**.

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for cG **2** upon moving from MeOD to water-saturated $CDCl_3$. All of the salt complexes, (cG **2**)$_2$·MX·(H$_2$O)$_n$, give similar spectra, featuring an enhanced positive Cotton band at 284 nm for the K^+ salts and at 286 nm for the Na^+ salts. That the CD spectra of cG **2** change so markedly under conditions that favor self-association and salt binding is consistent with other studies on the association−dissociation of calixarene dimers.[60] We cannot yet unequivocally assign these CD bands to specific chromophores in cG **2**, since both the calixarene aromatic rings and the guanine base have UV absorption shoulders in the 270−280-nm region. Nonetheless, these preliminary experiments open the door for further and more detailed CD investigation of the structure and thermodynamics of these chiral assemblies.

The Anion Controls the Aggregation State of cG 2. In previous studies, we found that the first-generation compound, cG **1**, reversibly precipitated from solution in the presence of $NaBPh_4$, indicating formation of a noncovalent aggregate.[33] Sodium tetraphenylborate, with its noncoordinating anion, also triggers a similar aggregation of cG **2**.

Titration of (cG **2**)$_2$·(H$_2$O)$_n$ in water-saturated $CDCl_3$ with $NaBPh_4$ dissolved in 1:1 $CDCl_3$:CD_3CN resulted in the NMR signal broadening and precipitation that is characteristic for a noncovalent polymer (Figure S5).[33,62] Washing a $CDCl_3$ solution of (cG **2**)$_2$·(H$_2$O)$_n$ with 1.0 M $NaBPh_4$(aq) led to complete precipitation of (cG **2**·Na^+)$_n$·(BPh_4^-)$_n$. Semiquantitative EDX analysis of the precipitate gave a Si:Na ratio of 10.3:1 (±15%) in qualitative agreement with a noncovalent polymer comprising intermolecular Na^+-G-quartets. The expected Si:Na value for a polymer composed of a 1:1 cG **2**:Na ratio is 8:1, as cG **2** contains eight silicons.

These experiments demonstrate that the anion can modulate the supramolecular organization of cG **2** and provide another example of how supramolecular structures can be modulated by altering one component in a multicomponent assembly. With NaCl, cG **2** forms discrete dimers, whereas with $NaBPh_4$ a noncovalent polymer is obtained.

The (cG **2**·Na^+)$_n$(BPh_4^-)$_n$ precipitate could be converted to the soluble dimers (cG **2**)$_2$·(H$_2$O)$_n$ or (cG **2**)$_2$·NaCl·(H$_2$O)$_n$ by washing a $CDCl_3$ suspension of the precipitate with either H_2O or an aqueous salt solution, again demonstrating the assembly's reversibility and underscoring water's role in controlling the structure and properties of the cG **2** dimers (Figure 8). It remains an open question as to why (cG **2**)$_2$·(H$_2$O)$_n$ and (cG **2**)$_2$·NaCl·(H$_2$O)$_n$ form discrete dimers instead of polymerizing. One possible explanation for the lack of polymerization is that the water or salt that binds in the dimer's interior induces a conformational change disfavoring the external G residues from also forming intermolecular G-quartets. Such ion-induced conformational changes are well-known for 1,3-alternate calixarenes.[63]

Conclusions

This study underscores water's ability to stabilize a functional assembly. Calix[4]arene-guanosine cG **2** forms a dimer (cG **2**)$_2$·(H$_2$O)$_n$ in water-saturated $CDCl_3$. This dimer extracts alkali halides from aqueous solution into organic solvents. An intermolecular G-quartet formed by two molecules of cG **2** provides the cation binding site and the neighboring 5′-amide groups bind the halide anion. Formation of (cG **2**)$_2$·NaCl·(H$_2$O)$_n$ provides a prime example of the cooperative interactions of host, solvent, and guest. Salt binding by a cG **2** dimer is enabled by water, and the bound ion pair further stabilizes the dimer. These noncovalent structures based on self-assembly of cG **2** are tunable; changing the anion from a halide to the noncoordinating BPh_4^- switches the assembly from a discrete dimer to a noncovalent polymer.

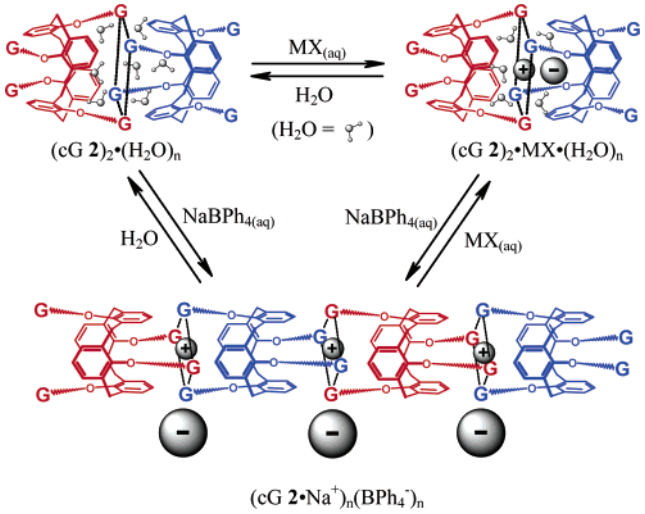

Figure 8. Schematic representation for the reversible polymerization of cG **2**. For individual molecules, the calix[4]arene-1,3-*alt* core is shown and "G" represents the guanosine nucleobase. All other atoms are removed for clarity. Blue and red are used simply to distinguish between individual molecules.

This study also provides evidence for an isolated G-quartet, a structure that typically occurs in stacks of neighboring quartets.[27,29] Although direct NMR evidence for the G-quartet came from (cG **2**)$_2$·NaCl·(H$_2$O)$_n$, it is likely that (cG **2**)$_2$·(H$_2$O)$_n$ is also held together by a G-quartet. Both (cG **2**)$_2$·(H$_2$O)$_n$ and (cG **2**)$_2$·NaCl·(H$_2$O)$_n$ have similar ^{1}H NMR and CD spectra in water-saturated $CDCl_3$, suggesting similar structure. A G-quartet formed by (cG **2**)$_2$·(H$_2$O)$_n$ could use its four O6 carbonyls to hydrogen bond to water. In this way, water would take the cation's usual place within the G-quartet.[64] Indeed, in the original paper describing 5′-GMP self-association, Gellert et al. wrote that a G-quartet "*...would contain a hole in the middle in which it might be possible to place one water molecule per tetramer*."[65] Bound water in (cG **2**)$_2$·(H$_2$O)$_n$ likely facilitates the recruitment and localization of ions by lowering the energy required for ion transfer from bulk water to the self-assembled ionophore's binding site. Finally, bound waters would also likely stabilize the salt complex (cG **2**)$_2$·NaCl·(H$_2$O)$_n$ by providing additional ligands to fill the cation's coordination sphere.[66]

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Experimental

Synthesis. Synthetic details are provided in the supporting information.

Complex Formation. Typically, a suspension of cG **2** (1−2 mM) in $CDCl_3$ (1 mL) was stirred with either H_2O or a 1 M aqueous salt solution (1 mL) for 12 h. The organic layer was separated, centrifuged to remove residual water, and analyzed.

^{23}Na NMR Measurements. ^{23}Na NMR spectra were recorded on a Bruker DRX-500 operating at 132.29 MHz and are reported in ppm relative to 25 mM NaCl in D_2O at 0 ppm (external standard). A 5-mm broad band probe was used. For all experiments, 95 000 transients were collected and a 90° pulse of 7.7 μs, acquisition time of 619 ms with a 1-s delay between pulses, and a sweep width of 13.2 kHz were used. The concentrations were 20 mM in cG **2**.

Ion Chromatography (IC). Ion chromatographs were run on a Dionex DX-120 ion chromatograph. Eluents were 4.8 mM Na_2CO_3/0.6 mM $NaHCO_3$ for anions and 20 mM methylsulfonic acid for cations. Ion concentrations are reported relative to a 1 mM standard for each ion. In all experiments, 1.2 mL of 2 mM L (L = cG **2**, cA **3**, G **4**) in CD_2Cl_2 was stirred with 1 mL of 1 M salt (salts were NaCl, NaBr, KCl, KBr, and 1:1:1:1 Na:K:Cl:Br) for 12 h. The aqueous salt solutions were removed and the organic layers were centrifuged to remove residual saltwater. Millipore H_2O (1 mL) was layered over the CD_2Cl_2 solution (1 mL) containing the ligand (and salt if extracted), and the biphasic mixture was stirred for 12 h to effect back-extraction of the salt into H_2O. Salt concentrations in the aqueous layer were measured by IC. Importantly, stirring the already back-extracted organic layers with Millipore H_2O for an additional 12 h resulted in no further back-extraction; i.e., all salt was removed during the first back-extraction.

Pulsed Field Gradient (PFG) NMR. Diffusion experiments were recorded on a Bruker Avance400 NMR spectrometer with a 5-mm broad-band inverse probe in Shigemi tubes (Shigemi, Inc., Allison Park, PA) at 26 °C using the pulse sequence developed by Tanner,[47b] with an added homo-spoil gradient (Bruker pulse sequence "stegp1s, v. 1.1.2.2"). The gradient strength was calibrated with water at 25 °C and the temperature was calibrated with a methanol sample. The pulsed gradients were incremented from 1.7 to 30.0 G/cm in 18 steps with a duration (δ) of 2 ms and 32 transients were collected with a pulse delay of 6 s in all cases. The pulse gradient separation (Δ) was 200 ms for experiments in $CDCl_3$ and 800 ms for experiments in DMSO-d_6. The sample height was maintained at 8 mm to minimize the effects of convection. Measurements of the (cG **2**)$_2$·NaCl·$(H_2O)_n$/cA **3** mixture in $CDCl_3$ and cG **2**/cA **3** mixture in DMSO-d_6 were repeated 16 times and 8 times, respectively, and the diffusion coefficients reported are the mean ± standard deviation of all experiments. Concentrations were 6.7−6.8 mM in cG **2** and cA **3**.

According to the pulse gradient spin−echo technique,[47a] the ratio between the echo intensity in the presence (I) and absence (I_o) of a pulsed gradient is given by

$$\ln(I/I_o) = -\gamma^2 g^2 \delta^2 (\Delta - \delta/3) D \quad (1)$$

where γ is the gyromagnetic ratio, g is the pulsed gradient strength (G/cm), δ and Δ are the duration and separation of the two gradient pulses, respectively, and D is the diffusion coefficient. All spectra were processed using XWINNMR 3.0 (Bruker) and data analyses were accomplished using the t1/t2 routine. Diffusion coefficients were obtained by fitting H1′ peak volumes to a single-exponential decay (eq 1) using the program Simfit (Bruker).

Energy Dispersive X-ray (EDX) Analysis. EDX was performed with an AMRAY 1820K scanning electron microscope with an acceleration potential of 20 kV. The sample was prepared by washing a solution of (cG **2**)$_2$·$(H_2O)_n$ (2 mM in cG **2**) in $CHCl_3$ with 1 M $NaBPh_4$(aq). The resulting precipitate (cG **2** polymer) was isolated by drawing off the liquid layers after centrifugation and purified by centrifugation with and removal of $CHCl_3$ (3×) and water (3×) followed by drying under high vacuum for 18 h. The data are the average and standard deviation of three runs at different spots on the sample.

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Supporting Information Available: Experimental and selected spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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