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Self-Assembled Ionophores from Isoguanosine: Diffusion NMR Spectroscopy Clarifies Cation's and Anion's Influence on Supramolecular Structure

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Abstract: Cation-templated self-assembly of the lipophilic isoguanosine (isoG**1**) with different monovalent cations ($M^+ = Li^+$, Na^+, K^+, NH_4^+, and Cs^+) was studied in solvents of different polarity by using diffusion NMR spectroscopy. Previous studies that did not use diffusion NMR techniques concluded that isoG**1** forms both pentamers $(isoG\mathbf{1})_5{\cdot}M^+$ and decamers $(isoG\mathbf{1})_{10}{\cdot}M^+$ in the presence of alkali-metal cations. The present diffusion NMR studies demonstrate, however, that isoG**1** does not form $(isoG\mathbf{1})_5{\cdot}M^+$ pentamers. In fact, the diffusion NMR data indicates that both doubly charged decamers of formula $(isoG\mathbf{1})_{10}{\cdot}2M^+$ and singly charged decamers, $(isoG\mathbf{1})_{10}{\cdot}M^+$, are formed with lithium, sodium, potassium, and ammonium tetraphenylborate salts ($LiB(Ph)_4$, $KB(Ph)_4$, $NaB(Ph)_4$ and $NH_4B(Ph)_4$), depending on the isoG**1**:salt stoichiometry of the solution. In the presence of $CsB(Ph)_4$, isoG**1** affords only the singly charged decamers $(isoG\mathbf{1})_{10}{\cdot}Cs^+$. By monitoring the diffusion coefficient of the $B(Ph)_4^-$ ion in the different mixtures of solvents, we also concluded that the anion is more strongly associated to the doubly charged decamers $(isoG\mathbf{1})_{10}{\cdot}2M^+$ than to the singly charged decamers $(isoG\mathbf{1})_{10}{\cdot}M^+$. The $(isoG\mathbf{1})_{10}{\cdot}2M^+$ species can, however, exist in solution without the mediation of the anion. This last conclusion was supported by the finding that the doubly charged decamers $(isoG\mathbf{1})_{10}{\cdot}2M^+$ also prevail in 1:1 CD_3CN:$CDCl_3$, a solvent mixture in which the $B(Ph)_4^-$ ion does not interact significantly with the self-assembled complex. These diffusion measurements, which have provided new and improved structural information about these decameric isoG**1** assemblies, demonstrate the utility of combining diffusion NMR techniques with conventional NMR methods in seeking to characterize labile, multicomponent, supramolecular systems in solution, especially those with high symmetry.

Keywords: anions · cations · diffusion NMR spectroscopy · isoguanosine · self-assembly · supramolecular chemistry

Introduction

Self-assembly entails organization of molecules into discrete supramolecular systems held together by intermolecular interactions. These intermolecular interactions may include, inter alia, hydrogen or coordination bonds, as well as electrostatic, hydrophobic, ion–dipole, and π–π interactions.[1] The determination of the structures of many noncovalent assemblies is, however, not always an easy task. Single crystals of labile supramolecular systems are, in many cases, difficult to obtain. In addition, these solid-state structures may frequently fail to represent the distribution of all of the species in solution. Furthermore, conventional NMR methods may have limited success in probing the difference between supramolecular structures with high symmetry. This is even more problematic for self-assembled systems that have sym-

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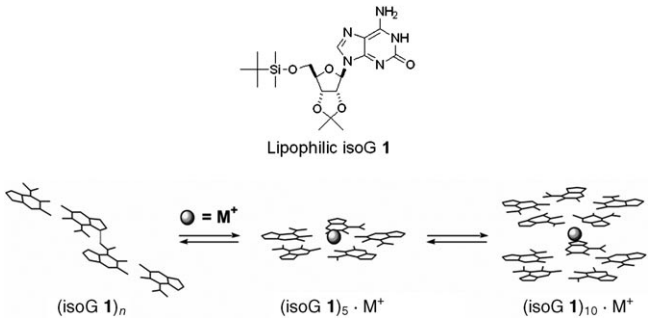
metry and NMR properties similar to their monomeric and/or oligomeric building blocks. However, diffusion NMR, as measured by pulsed-field gradient techniques, can indeed help bridge this gap by enabling the accurate determination of the size and shape of molecules in solution.

Diffusion NMR techniques have been particularly useful in assisting with the determination of supramolecular systems in solution.[2] These techniques were very useful in probing encapsulation[3] and provided, for example, conclusive evidence of the spontaneous formation of hexameric capsules of resorcin[4]arenes and pyrogallol[4]arenes in chloroform.[4] Diffusion NMR spectroscopy was also used to identify the size and shape of different supramolecular systems.[5] In addition, it was used to probe the structure of a handful of organometallic complexes.[6] More recently, diffusion NMR techniques have been used to probe the structures of some of the intriguing systems formed by the cation-templated self-assembly of guanosine compounds.[7] As described below, this present study uses diffusion NMR analysis to identify different species formed in the cation-templated self-assembly of the lipophilic nucleoside 5′-*tert*-butyl-dimethylsilyl-2′,3′,-di-*O*-isopropylidene isoguanosine (isoG**1**) (Scheme 1).[8]



Scheme 1. Structure of lipophilic isoguanosine (isoG**1**) and a cartoon showing a hydrogen-bonded pentamer (isoG**1**)$_5$·M$^+$ and decamer (isoG**1**)$_{10}$·M$^+$ of isoG**1**.

Much like guanosine, which forms higher-ordered structures based on the well-known G-quartet,[9] the isoguanosine nucleobase also self-associates into discrete hydrogen-bonded assemblies in the presence of cations. For example, the affinity and selectivity of the lipophilic analogue isoG**1** for binding the Cs^+ ion is relatively high with respect to the other alkali-metal cations.[10,11] Various techniques, including X-ray crystallography, multinuclear ^1H and ^{133}Cs NMR spectroscopy, electrospray mass spectrometry, and extraction studies with radioactive $^{137}Cs^+$ tracer have all unequivocally shown that isoG**1** forms a decamer (isoG**1**)$_{10}$·Cs^+ in organic solvents.[11] The crystal structure of (isoG**1**)$_{10}$·Cs^+ revealed that this decamer is composed of two hydrogen-bonded isoG**1** pentamers that sandwich a central Cs^+ ion.[11,12] In accord with these studies on nucleosides in organic solvents, DNA oligonucleotides with contiguous d(isoG) units also form 5-stranded structures in the presence of Cs^+.[13] Computational studies have supported the experimental findings that isoG forms stable pentameric/decameric assemblies with alkali cations and have provided insight into the basis for isoG's outstanding Cs^+ selectivity.[13a,14] Recently, isoG**1** has been used to develop methods for the selective extraction and membrane transport of radioactive waste products, $^{137}Cs^+$ and $^{226}Ra^{2+}$.[11,15,16] To better understand the structural factors that help govern this extraordinary ion-binding selectivity for isoG**1** we undertook the present diffusion NMR study.

Formation of the decamer (isoG**1**)$_{10}$·Cs^+ was previously shown to be highly cooperative, as ^1H and ^{133}Cs NMR titration experiments indicated only the presence of isoG**1** "monomer" and decamer.[11] The situation was quite different, however, for self-assembly of isoG**1** in the presence of other alkali-metal ions. Thus, NMR titrations in 50% $CDCl_3$/50% CD_3CN with isoG**1** and $M^+B(Ph)_4^-$ salts ($M^+ = Li^+$, Na^+, K^+, Rb^+) clearly indicated two distinct species in solution.[17,18] Moreover, solid–liquid extractions of these $M^+B(Ph)_4^-$ salts by isoG**1** gave complexes with a 5:1 stoichiometry, as determined by integration of chemical shifts for the isoG**1** ligand and $B(Ph)_4^-$ ion. These NMR data were interpreted to indicate that isoG**1** formed two different hydrogen-bonded complexes with $M^+B(Ph)_4^-$, namely, a discrete pentamer (isoG**1**)$_5$·M$^+$ and the decamer (isoG**1**)$_{10}$·M$^+$ (Scheme 1).[17,18] As described below, results of the present diffusion NMR studies reveal that this original interpretation is wrong. Thus, the species originally identified as a pentamer is instead a doubly charged decamer (isoG**1**)$_{10}$·2M$^+$. These new findings, uncovered by diffusion NMR experiments that probed the nature of the cation, the role of the anion, and the effect of solvents on the self-assembly of isoG**1**, are significant in the identification of stable intermediates is critical for learning how to construct and manipulate these self-assembled ionophores that show such highly selective binding of $^{137}Cs^+$ and $^{226}Ra^{2+}$.

Results and Discussion

Figure 1 shows the stack plot as a function of the gradient strength (G) for one representative peak (ribose H1′) of solutions of isoG**1** in $CDCl_3$, in which the isoG**1**:$LiB(Ph)_4$ stoichiometries are 5:1 and 10:1. As described above, these peaks were previously assigned to represent the pentamer (isoG**1**)$_5$·Li^+ and the decamer (isoG**1**)$_{10}$·Li^+, respectively, upon self-assembly of isoG**1** with $LiB(Ph)_4$ (Scheme 1).[15,16] Surprisingly, this figure shows that the observed signal decays for both representative peaks are similar.

Figure 2 depicts the normalized signal decay of one representative peak (ribose H1′) of the alleged pentamer (isoG**1**)$_5$·Li^+ and decamer (isoG**1**)$_{10}$·Li^+ in $CDCl_3$, along with the normalized signal decay of the $B(Ph)_4^-$ ion in these solutions as a function of the diffusion weighting. The signal-decay peaks representing the alleged pentamer and decamer of isoG**1** are similar, whereas the signal decays of the corresponding $B(Ph)_4^-$ ion in each stoichiometry behave differently. Clearly, the signal decay of $B(Ph)_4^-$ ion is faster than that of the two aggregates of isoG**1**, which indicates

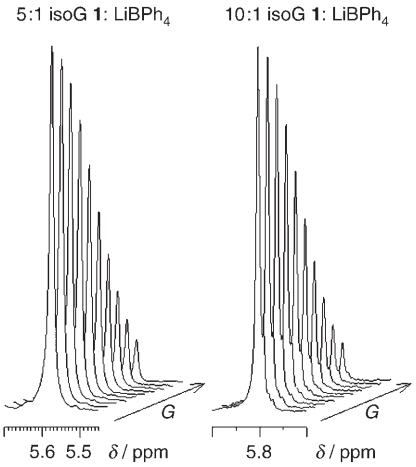


Figure 1. Stack plots showing the signal decay in a diffusion experiment as a function of the gradient strength (G) of one representative peak (ribose H1′) for each of the alleged pentamer (isoG**1**)$_5$·Li$^+$ and decamer (isoG**1**)$_{10}$·Li$^+$ of **1** with LiB(Ph)$_4$ in $CDCl_3$.

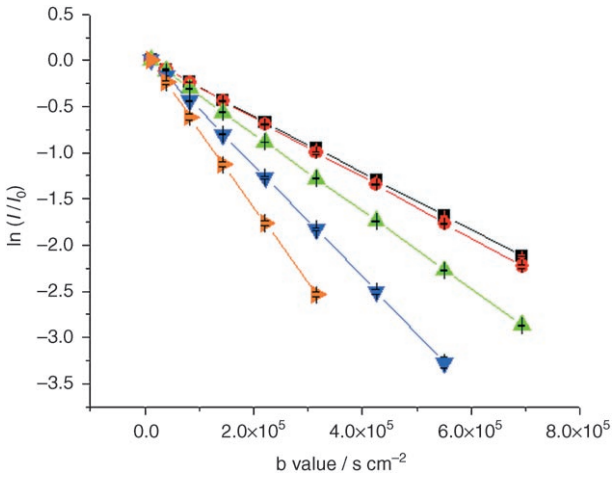


Figure 2. Normalized signal decay ($\ln(I/I_0)$) versus the diffusion weighing (b value) for one representative peak of the alleged pentamer (isoG**1**)$_5$·Li$^+$ (■), the decamer (isoG**1**)$_{10}$·Li$^+$ (●), the corresponding B(Ph)$_4^-$ ions (▲ = BPh$_4^-$ in the alleged "pentamer", ▼ = BPh$_4^-$ in the decamer), and of the free B(Ph)$_4^-$ ion (▶) in $CDCl_3$.

the higher diffusion coefficients of the anion in both of these solutions.

Figure 3 shows sections of the ^{1}H NMR spectra of solutions of isoG**1** in $CDCl_3$ with and without lithium tetraphenylborate (LiB(Ph)$_4$) in different stoichiometries, along with the diffusion coefficients extracted for isoG**1** in these solutions. From these spectra it is clear that isoG**1** forms discrete assemblies with LiB(Ph)$_4$ at different stoichiometries. The ^{1}H NMR spectrum of the 7.5:1 solution of isoG**1**:LiB(Ph)$_4$ (Figure 3c) is a superposition of the spectra shown in Figures 3b and 3d, which represent the 5:1 and 10:1 solutions of isoG**1**:LiB(Ph)$_4$, respectively. Indeed, already the few diffusion coefficients depicted in this figure provide the first unexpected result in this present study. We consistently found similar diffusion coefficients for what was originally believed to be the (isoG**1**)$_5$·Li$^+$ pentamer and the (isoG**1**)$_{10}$·Li$^+$ decamer. In fact, we found the diffusion coefficient of the alleged pentamer ($0.313 \pm 0.001 \times 10^{-5}$ cm^{2}s^{-1}) to be slightly lower than that assigned to the decamer ($0.329 \pm 0.002 \times 10^{-5}$ cm^{2}s^{-1}), a result that would be counterintuitive if the species with the 5:1 isoG**1**:LiB(Ph)$_4$ ratio was an isolated (isoG**1**)$_5$·Li$^+$ pentamer. These results were also obtained when the two species, which are in slow exchange on the NMR chemical-shift timescale, were measured simultaneously in the same $CDCl_3$ sample, in which all external conditions for both systems are similar. These results brought us to the conclusion that the two species have similar molecular weights. The most plausible explanation for

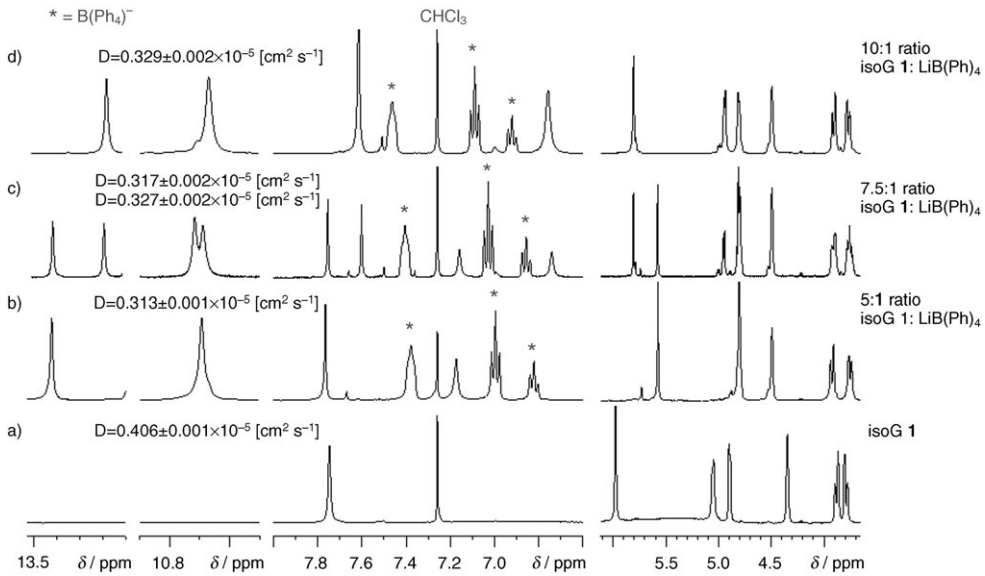


Figure 3. Sections of ^{1}H NMR spectra (400 MHz, 298 K, $CDCl_3$) of **1** alone or with different stoichiometries of LiB(Ph)$_4$, along with the diffusion coefficients extracted for **1** in each solution. The * symbols represent the peaks of the B(Ph)$_4^-$ ion.

these similar diffusion coefficients is that the alleged (isoG**1**)$_5$·Li$^+$ pentamer is, in fact, a doubly charged decamer with formula (isoG**1**)$_{10}$·2Li$^+$, a species that would have a 5:1 ligand:LiB(Ph)$_4$ ratio, but also a molecular weight similar to that of a (isoG**1**)$_{10}$·Li$^+$ decamer.

Because our explanation about formation of (isoG**1**)$_{10}$·2Li$^+$ entails the effective formation of a dimer of (isoG**1**)$_5$·Li$^+$ pentamers, one can question whether this dimerization is mediated through the anion. Such mediation by the anion was observed in the solid state and in solution for a few lipophilic G-quartets that are held together by metal-picrate salts.[19] To answer this question, we examined the diffusion coefficients of the anion, which proved an easy task in this case because the anion is an organic anion that can be followed simultaneously in the ^{1}H NMR diffusion experiment. We found that the diffusion coefficients of the tetraphenylborate anion in both of the isoG**1**·Li$^+$ complexes are significantly lower than those of "free" B(Ph)$_4^-$. The diffusion coefficients of B(Ph)$_4^-$ were $0.429\pm0.001\times10^{-5}$ and $0.590\pm0.010\times10^{-5}$ cm^{2}s^{-1} for (isoG**1**)$_{10}$·2Li$^+$ and (isoG**1**)$_{10}$·Li$^+$, respectively, whereas the diffusion coefficient of the B(Ph)$_4^-$ ion in the solution LiB(Ph)$_4$ in chloroform was found to be $0.838\pm0.009\times10^{-5}$ cm^{2}s^{-1}. These results demonstrate that the B(Ph)$_4^-$ ion is, on average, more tightly bound to the doubly charged decamer (isoG**1**)$_{10}$·2Li$^+$ than it is to the singly charged decamer (isoG**1**)$_{10}$·Li$^+$. This suggests a more significant involvement of the anion in stabilizing the (isoG**1**)$_{10}$·2Li$^+$ complex, as might be expected for a supramolecular assembly that contains two bound Li$^+$ ions. Note, however, that even in this doubly charged decamer (isoG**1**)$_{10}$·2Li$^+$, the diffusion coefficient of the B(Ph)$_4^-$ ion is still significantly higher than that of the discrete (isoG**1**)$_{10}$·2Li$^+$ assembly. In addition, in a solution in which two sets of peaks representing the two discrete assemblies of isoG**1** were observed, only one set of peaks was found for the B(Ph)$_4^-$ ion. Thus, we concluded that the different anion pools are in fast exchange on the NMR timescale. Therefore, the diffusion coefficient measured for the anion is a weighted average of the diffusion coefficients of the B(Ph)$_4^-$ ion in the different pools.

Because we questioned whether the formation of the doubly charged decamer (isoG**1**)$_{10}$·2Li$^+$ might be mediated by the anion, we explored the diffusion characteristics of the 5:1 and 10:1 solutions of isoG**1**:LiB(Ph)$_4$ in solvent mixtures of different polarities. The diffusion characteristics of the two systems were, therefore, recorded in the less-polar 10:1 C_6D_6:$CDCl_3$ mixture and in the more-polar 1:1 CD_3CN:$CDCl_3$ mixture. The extracted diffusion coefficients of the aggregates of isoG**1** and the anion in the different mixtures are shown graphically in Figure 4 and the numerical values are given in Table 1.

Figure 4 clearly shows that, in all three solutions, the diffusion coefficient of the system, now identified as

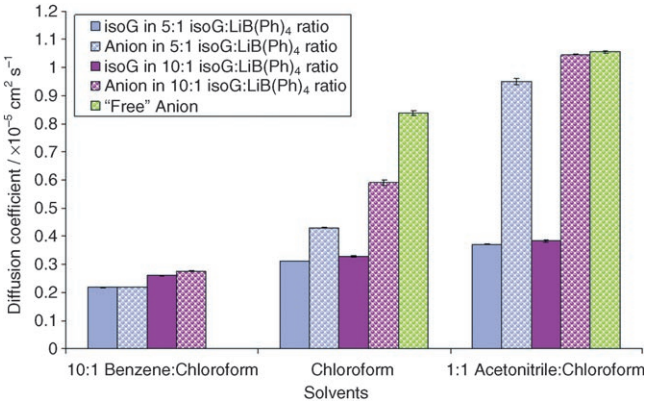


Figure 4. Extracted diffusion coefficients of isoG**1** and B(Ph)$_4^-$ ion for isoG**1**:LiB(Ph)$_4$ stoichiometries of 5:1 and 10:1, and of "free" B(Ph)$_4^-$ ion in the different solvent mixtures used in this study.

Table 1. Diffusion coefficients measured (400 MHz, 298 K) for **1** and LiB(Ph)$_4$ in the three different solvents used in this study, as single substances in solution and in two different stoichiometries (5:1 and 10:1). All diffusion coefficients [cm^{2}sec^{-1}] have been multiplied by 10^5.

Solvent	Sample	Chloroform	BPh_4^- ion	"Monomer"	Doubly charged decamer	Singly charged decamer	$^7Li^+$ ion
10:1 C_6D_6:$CDCl_3$[a]	22.7 mM:4.5 mM (5:1) isoG**1**:$LiBPh_4$	2.16±0.01	0.220±0.001	–	0.219±0.002	–	0.210±0.001
	22.7 mM:2.3 mM (10:1) isoG**1**:$LiBPh_4$	2.15±0.01	0.275±0.001	–	–	0.260±0.001	0.257±0.003
$CDCl_3$	$LiBPh_4$[b]	2.42±0.05	0.838±0.009	–	–	–	–
	25 mM isoG**1**	2.47±0.02	–	0.406±0.001	–	–	–
	25 mM:5 mM (5:1) isoG**1**:$LiBPh_4$	2.42±0.03	0.429±0.001	–	0.313±0.001	–	0.329±0.002
	25 mM:3.3 mM (7.5:1) isoG**1**:$LiBPh_4$	2.40±0.03	0.482±0.002	–	0.317±0.002	0.327±0.002	0.301±0.004 0.310±0.001
	25 mM:2.5 mM (10:1) isoG**1**:$LiBPh_4$	2.37±0.04	0.590±0.010	–	–	0.329±0.002	0.341±0.007
1:1 CD_3CN:$CDCl_3$	2.5 mM $LiBPh_4$	2.68±0.03	1.056±0.004	–	–	–	1.326±0.023
	25 mM isoG**1**	[c]	–	0.426±0.001	–	–	–
	25 mM:5 mM (5:1) isoG**1**:$LiBPh_4$	2.55±0.04	0.955±0.010	–	0.372±0.001	–	0.375±0.002
	25 mM:2.5 mM (10:1) isoG**1**:$LiBPh_4$	2.63±0.04	1.044±0.001	–	–	0.384±0.004	0.381±0.001

[a] $LiBPh_4$ and isoG are insoluble in 10:1 C_6D_6:$CDCl_3$. [b] Only a small fraction of the salt dissolved. Thus, the ^{7}Li diffusion NMR spectrum of $LiBPh_4$ in $CDCl_3$ could not be measured. [c] The chloroform peak could not be measured due to overlap with an isoG peak.

(isoG**1**)$_{10}$·2Li$^+$, is slightly lower than that of the decamer (isoG**1**)$_{10}$·Li$^+$. In the low-polarity 10:1 C_6D_6:$CDCl_3$ mixture, the diffusion coefficients of the anion were similar to that of the two discrete isoG assemblies. Even in the more-polar CD_3CN:$CDCl_3$ mixture, in which the diffusion coefficient of the B(Ph)$_4^-$ ion is nearly equal to that of the free anion in solution, the diffusion coefficient of (isoG**1**)$_{10}$·2Li$^+$ remained slightly smaller than that of the (isoG**1**)$_{10}$·Li$^+$ decamer. These results clearly show that the formation of the doubly charged decamer (isoG**1**)$_{10}$·2Li$^+$, under the experimental conditions used, is not mediated by the B(Ph)$_4^-$ ion in the more-polar 1:1 CD_3CN:$CDCl_3$ solvent mixture.

Diffusion NMR analysis performed by monitoring the ^{7}Li NMR signals in the two systems indicates that the lithium cation diffuses at rates similar to those of both discrete isoG assemblies (see Table 1). This observation is true for all solvent mixtures used and demonstrates that the isoG aggregate and the Li$^+$ ion diffuse as a single molecular entity. Interestingly, for the 7.5:1 isoG**1**:LiB(Ph)$_4$ solution, two signals were observed in the ^{7}Li NMR spectrum, indicating slow exchange of the lithium cation in the singly and doubly charged decamers, as shown in Figure 5. This figure shows the stack plot and the normalized signal decay of the ^{7}Li NMR signals as a function of the diffusion weighting for the 7.5:1 isoG**1**:LiB(Ph)$_4$ in chloroform and demonstrates that the two lithium signals have similar diffusion coefficients. Clearly, one signal has a slightly slower diffusion coefficient than the other, results that are consistent with those obtained from the ^{1}H diffusion NMR experiments. Thus, we conclude that in the case of the doubly charged decamer (isoG**1**)$_{10}$·2Li$^+$, the repulsion that might be expected between the two bound Li$^+$ ions is overcome by the attractive ion–dipole forces between the cations and the isoG's carbonyl oxygen atoms, as well as the attractive forces that arise due to interactions between the hydrogen-bond donors and acceptors and between the stacked aromatic rings.[3b] A summary of the intermolecular interactions that prevail in the different solutions, as obtained from diffusion NMR analysis, is depicted in Scheme 2. In the three solutions studied, we found that the previously assigned (isoG**1**)$_5$·Li$^+$ pentamer formed by isoG**1** after addition of $LiBPh_4$ is, in fact, a doubly charged decamer (isoG**1**)$_{10}$·2Li$^+$ that is not very different from the decamer (isoG**1**)$_{10}$·Li$^+$ in terms of molecular weight. The formation of (isoG**1**)$_{10}$·2Li$^+$ appears not to be significantly mediated by the anion. Both the anions and cations are in fast exchange on the NMR timescale, as only one lithium signal and one set of B(Ph)$_4^-$ signals are observed for each of the species.

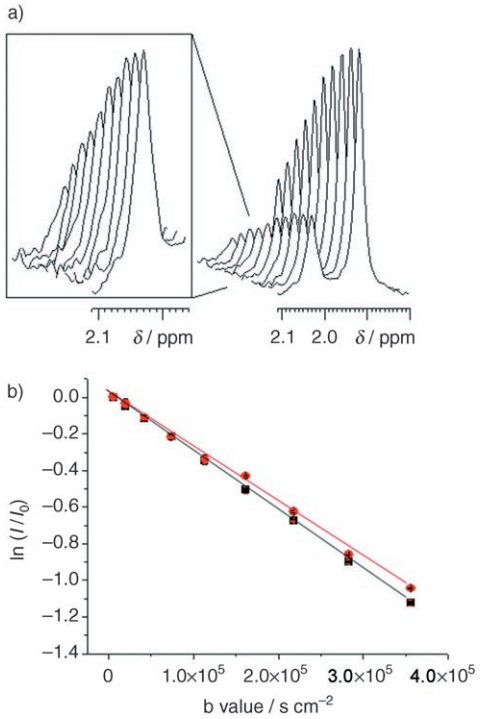
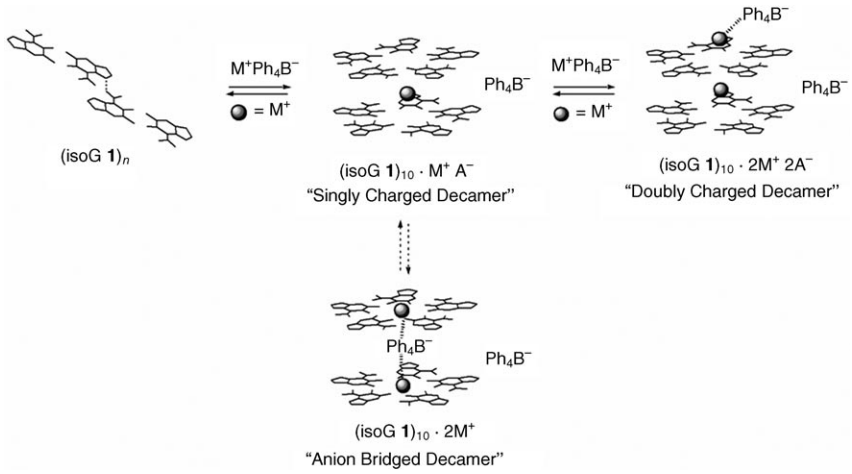


Figure 5. a) A stack plot showing the signal decay in a diffusion experiment as a function of the gradient strength (G) of ^{7}Li NMR spectra (400 MHz, 298 K, $CDCl_3$) of 7.5:1 stoichiometry of a isoG**1**:LiB(Ph)$_4$ solution, with insert. b) The normalized signal decay (ln(I/I_0)) versus the diffusion weighing (b value) for these peaks in the same stoichiometries (■ = Li$^+$ in alleged pentamer, ● = Li$^+$ in decamer).



Scheme 2. Likely structures for the isoG assemblies based on diffusion NMR results.

To verify and evaluate the significance and generality of our findings, we studied the diffusion characteristics of the supramolecular systems formed by the addition of isoG**1** to several different salts (MB(Ph)$_4$, in which M$^+$ = Na$^+$, K$^+$, Cs$^+$, and NH_4^+). The systems were studied in two stoichio-

metric ratios (5:1 and 10:1 of isoG**1**:$MB(Ph)_4$) in $CDCl_3$ and in a 1:1 CD_3CN:$CDCl_3$ mixture. Sections of the ^1H NMR spectra of the discrete complexes of isoG**1** with the different tetraphenylborate salts, along with their diffusion coefficients in the $CDCl_3$, are depicted in Figures 6 and 7. The same sections of the ^1H NMR spectra of the different salts at the two stoichiometric ratios (5:1 and 10:1 of isoG**1**:$MB(Ph)_4$) in the 1:1 CD_3CN:$CDCl_3$ mixture, along with the corresponding diffusion coefficients, are depicted in Figures S1 and S2 in the Supporting Information. The stack plot and the normalized signal decay of the ^7Li NMR signals as a function of the diffusion weighting for the 5:1 and 10:1 stoichiometries of isoG**1**:$LiB(Ph)_4$ in $CDCl_3$ and in 1:1 CD_3CN:$CDCl_3$ are given in Figures S3 and S4, respectively.

All the salts, except for $CsB(Ph)_4$, afforded different and discrete complexes if the isoG**1**:salt ratio was 5:1 and 10:1, respectively. As previously reported,[11,17] isoG**1** interacts with $CsB(Ph)_4$ to afford only the $(isoG\mathbf{1})_{10}{\cdot}Cs^+$ decamer in both solutions. The numerical values of the calculated diffusion coefficients for all the different supramolecular systems formed in $CDCl_3$ and in the 1:1 CD_3CN:$CDCl_3$ mixture are summarized in Table 2 and in Table S1 of the Supporting Information, respectively. This diffusion data shows in all cases (cations except Cs^+ and solvents) that the discrete species formed in the 5:1 ligand:salt ratio are, indeed, doubly charged decamers of formula $(isoG\mathbf{1})_{10}{\cdot}2M^+$, having slightly lower diffusion coefficients than their respective $(isoG\mathbf{1})_{10}{\cdot}M^+$ decamers. Once again, formation of $(isoG\mathbf{1})_{10}{\cdot}2M^+$ appears not to be mediated by the $B(Ph)_4^-$ ion. This conclusion was lent further support by the fact that the doubly charged decamers $(isoG\mathbf{1})_{10}{\cdot}2M^+$ were also observed in the 1:1 CD_3CN:$CDCl_3$ solvent mixture, in which there is practically no interaction of the $B(Ph)_4^-$ ion with the discrete isoG complexes. Again, for the assemblies containing these cations ($M^+ = Na^+$, K^+, and NH_4^+), the $B(Ph)_4^-$ ion is more strongly associated with the doubly charged decamers $(isoG\mathbf{1})_{10}{\cdot}2M^+$ than with the singly charged decamers $(isoG\mathbf{1})_{10}{\cdot}M^+$ in the $CDCl_3$.

Scheme 2 depicts a reasonable structural model, consistent with these diffusion NMR results, for formation of cation-stabilized assemblies by isoG**1**. Thus, addition of alkali-metal cations to a solution of isoG**1** triggers formation of a discrete hydrogen-bonded decamer $(isoG\mathbf{1})_{10}{\cdot}M^+$. In the singly charged decamer $(isoG\mathbf{1})_{10}{\cdot}M^+$

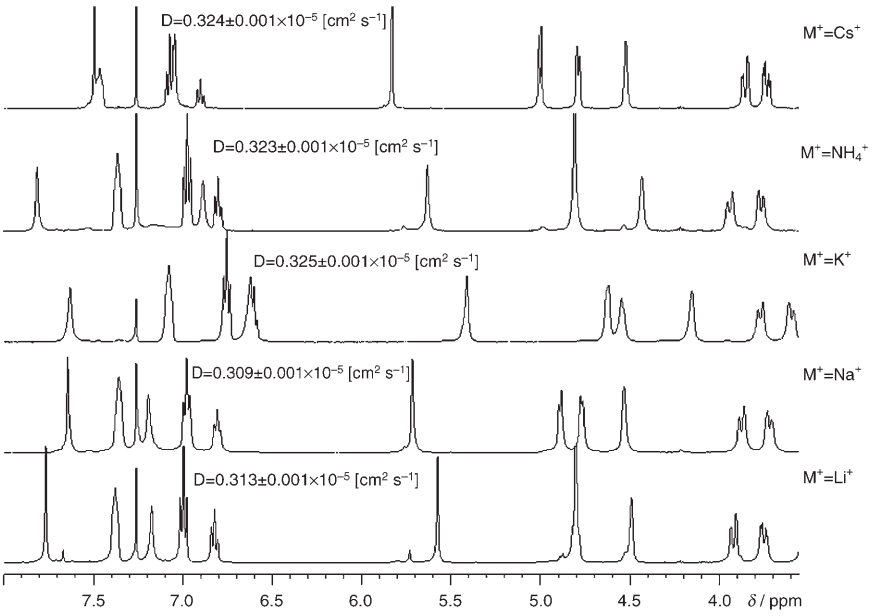


Figure 6. Sections of the ^1H NMR spectra (400 MHz, 298 K, $CDCl_3$) of the 5:1 **1**:$MB(Ph)_4$ solutions ($M^+ = Na^+$, K^+, Cs^+, and NH_4^+) along with the diffusion coefficients extracted for **1**.

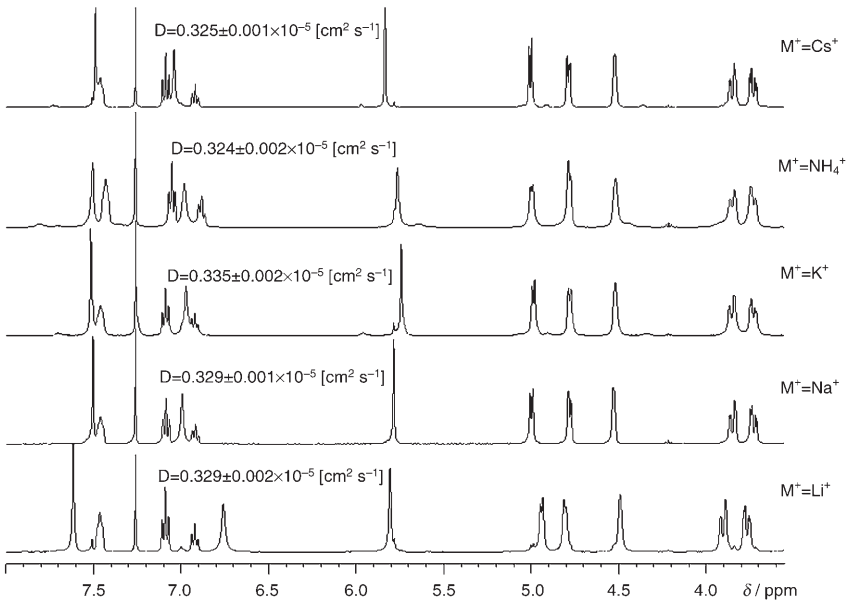


Figure 7. Sections of the ^1H NMR spectra (400 MHz, 298 K, $CDCl_3$) of the 10:1 **1**:$MB(Ph)_4$ solutions ($M^+ = Na^+$, K^+, Cs^+, and NH_4^+) along with the diffusion coefficients extracted for **1**.

Table 2. Diffusion coefficients measured (400 MHz, 298 K, $CDCl_3$) for 5:1 and 10:1 stoichiometries of **1**:$MB(Ph)_4$ ($M^+ = Na^+$, K^+, Cs^+, and NH_4^+). All diffusion coefficients [cm^2sec^{-1}] have been multiplied by 10^{-5}.

Sample	Chloroform	BPh_4^- ion	Doubly charged decamer	Singly charged decamer
25 mM:5 mM (5:1) isoG**1**:$NaBPh_4$	2.35 ± 0.01	0.416 ± 0.003	0.309 ± 0.001	–
25 mM:2.5 mM (10:1) isoG**1**:$NaBPh_4$	2.48 ± 0.02	0.590 ± 0.006	–	0.329 ± 0.001
25 mM:5 mM (5:1) isoG**1**:$KBPh_4$	2.48 ± 0.03	0.453 ± 0.001	0.325 ± 0.001	–
25 mM:2.5 mM (10:1) isoG**1**:$KBPh_4$	2.46 ± 0.02	0.594 ± 0.001	–	0.335 ± 0.002
25 mM:5 mM (5:1) isoG**1**:$CsBPh_4$	2.43 ± 0.04	0.556 ± 0.027	–	0.324 ± 0.001
25 mM:2.5 mM (10:1) isoG**1**:$CsBPh_4$	2.47 ± 0.01	0.595 ± 0.011	–	0.325 ± 0.001
25 mM:5 mM (5:1) isoG**1**:NH_4BPh_4	2.43 ± 0.03	0.436 ± 0.002	0.323 ± 0.001	–
25 mM:2.5 mM (10:1) isoG**1**:NH_4BPh_4	2.47 ± 0.01	0.546 ± 0.011	–	0.324 ± 0.002

, the single cation is likely nested between two pentameric units in a structure reminiscent of ferrocene. Therefore, the cation is shielded from the anion by ten molecules of isoG**1**. This is certainly the case for the Cs^+ decamer, as was shown by X-ray crystallography results.[11,12] Upon addition of more than 1/10 of an equivalent of cation, a new species, namely $(isoG\mathbf{1})_{10}{\cdot}2M^+$, with two bound cations is formed. In the doubly charged decamers $(isoG\mathbf{1})_{10}{\cdot}2M^+$, it is likely that one metal cation remains sandwiched between two isoG**1** pentamer units, while the second, more weakly bound cation, is located above the plane of one of the isoG**1** decamer's two pentameric units, from which the anions can approach this cation more closely. In addition, the doubly charged decamers $(isoG\mathbf{1})_{10}{\cdot}2M^+$ have two positive charges and should, therefore, attract the anion more strongly than the singly charged decamer $(isoG\mathbf{1})_{10}{\cdot}M^+$ can, due to stronger electrostatic attractions. Therefore, in $(isoG\mathbf{1})_{10}{\cdot}2M^+$, the interactions that prevail between the cations and the anions are stronger than those of the $(isoG\mathbf{1})_{10}{\cdot}M^+$ decamers. Scheme 2 also shows an alternative structure for $(isoG\mathbf{1})_{10}{\cdot}2M^+$, wherein an anion bridges two $(isoG\mathbf{1})_5{\cdot}M^+$ units whose cations are coplanar with the pentamer's carbonyl binding site. This structure is unlikely, as diffusion NMR analysis in the polar 1:1 CD_3CN:$CDCl_3$ solvent mixture showed that the $B(Ph)_4^-$ ion does not interact at all with the intact doubly charged decamers.

In conclusion, results of diffusion NMR studies clearly show that the lipophilic isoG**1** self-assembles into pentameric/decameric assemblies after the addition of cations in organic solvents. However, we found that the species previously assigned to be a discrete pentamer with formula $(isoG\mathbf{1})_5{\cdot}Li^+$ is, in fact, a doubly charged decamer $(isoG\mathbf{1})_{10}{\cdot}2Li^+$. Although the $B(Ph)_4^-$ ion is more strongly associated with the $(isoG\mathbf{1})_{10}{\cdot}2M^+$ species, the formation of these doubly charged decamers is not mediated by the anion. This can be deduced from the fact that the species also prevail in the 1:1 CD_3CN:$CDCl_3$ mixture, in which the interaction of the anion with the supramolecular system formed is negligible. Interestingly, we found the same behavior in solution for the different $MB(Ph)_4$ salts ($M^+ = Na^+$, K^+, and NH_4^+). In the case of $CsB(Ph)_4$, only discrete, singly charged decamers $(isoG\mathbf{1})_{10}{\cdot}Cs^+$ were observed. These results are also consistent with the ESI-MS data collected for solutions of isoG**1** with excess of CsI and KI in acetone. As shown in Figure 8, a molecular peak of a singly charged decamer was observed for the solution containing isoG**1** and CsI, whereas only a doubly charged molecular peak was observed for the solution containing isoG**1** and KI.

These results clearly demonstrate that diffusion NMR analysis is a valuable tool for the characterization of symmetrical supramolecular systems. This is even more apparent if one wishes to analyze self-assembled systems in which the

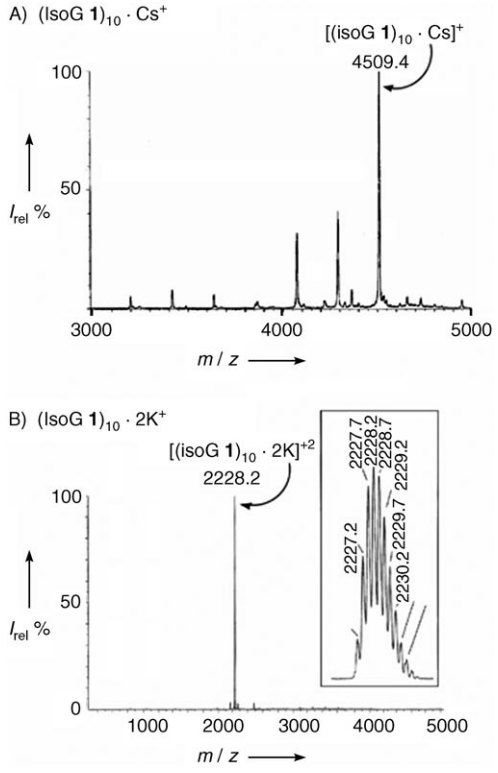


Figure 8. ESI mass spectra of A) an acetone solution of isoG**1** (0.5 mM) and excess CsI 15 min after mixing. There were no major peaks within the *m*/*z* range of 0–3000; B) an acetone solution of isoG**1** (0.5 mM) and excess KI 15 min after mixing, the inset shows the experimental isotope pattern.

symmetry of the complex is similar to the symmetry of the building blocks that make up the assembly. In these cases, conventional NMR techniques cannot, for example, distinguish between these symmetrical pentamers and decamers. In this present study of isoG self-association, the diffusion NMR results clearly show that $(\text{isoG}\,\mathbf{1})_{10}$ decamers are not just capable of binding a single cation, but they can coordinate to two alkali-metal cations if the ligand:salt ratios are less than 10:1. These insights gained from diffusion NMR analysis will hopefully help us develop new self-assembled ionophores that can bind metal cations with high affinity and selectivity.

Experimental Section

Materials: All starting materials, guest molecules, reagents, and deuterated solvents ($CDCl_3$, C_6D_6, CD_3CN) were purchased from Aldrich (Milwaukee, WI) and were used as supplied. The isoG **1** system was prepared as described previously.[8]

Sample preparation: The $CDCl_3$ samples were prepared by dissolving the isoG **1** in $CDCl_3$, followed by the addition of the desired $MB(Ph)_4$ salt ($M^+ = Li^+$, Na^+, K^+, Cs^+, and NH_4^+) at concentration ratios of isoG **1**:$MB(Ph)_4$ of 25 mM:2.5 mM or 25 mM:5 mM for the 10:1 or 5:1 stoichiometries, respectively, with a total volume of 0.3 mL. In the sample of $LiB(Ph)_4$ in $CDCl_3$, only a small fraction of the added salt dissolved. This allowed the ^{1}H diffusion NMR of the sample to be measured, but not the ^{7}Li diffusion NMR.

The 1:1 CD_3CN:$CDCl_3$ samples were prepared by dissolving the isoG in 0.15 mL $CDCl_3$, followed by the addition of 0.15 mL CD_3CN. The salts were added in a similar manner to that described above for the chloroform samples.

The isoG **1**:$MB(Ph)_4$ samples in the 10:1 C_6D_6:$CDCl_3$ mixture were prepared by dissolving the isoG **1** and the $LiB(Ph)_4$ salt in 0.3 mL C_6D_6, followed by the addition of 0.03 mL of $CDCl_3$, resulting in concentration ratios of isoG **1**:$LiB(Ph)_4$ of 22.7 mM:2.27 mM or 22.7 mM:4.54 mM for the 10:1 or 5:1 stoichiometries, respectively. The isoG **1** and the $LiB(Ph)_4$ salt did not dissolve in this solvent mixture.

NMR methods: NMR-diffusion measurements were performed by using a 400 MHz Avance Bruker NMR spectrometer equipped with a Great1 gradient system capable of producing magnetic-field-pulse gradients in the z-direction of about 50 G cm^{-1}. All experiments were carried out by using a 5-mm inverse probe. All diffusion measurements were performed in a 4-mm NMR tube inserted into a 5-mm NMR tube. This acts as a thermal insulating system and increases the accuracy and reproducibility of the diffusion measurements by reducing the possibility of convections in the sample. This precaution is more important if diffusion NMR experiments are performed in nonviscous solvents with low boiling points and heat capacities. All measurements were performed at 298 K. Diffusion measurements were preformed by using a LED sequence.[20]

^{1}H NMR diffusion measurements were performed at least three times and the reported diffusion coefficients are the mean ± standard deviation of at least three experiments. Only data with correlation coefficients of $\ln(I/I_0)$ versus $\gamma^2\delta^2G^2(2/\pi)^2(\Delta-\delta/4)$ (in which γ is the gyromagnetic ratio, G is the pulsed-gradient strength, and Δ and δ are the time separation between the pulsed gradients and their duration, respectively), generally termed the "diffusion weighting" and denoted as the b values, higher than 0.999 are reported.

^{7}Li NMR diffusion measurements were performed at least twice and the reported diffusion coefficients are the mean ± standard deviation of at least two experiments. Only data with correlation coefficients of $\ln(I/I_0)$ versus $\gamma^2\delta^2G^2(2/\pi)^2(\Delta-\delta/4)$ higher than 0.995 are reported.

The diffusion experiments were performed by using the LED pulse sequence with the following parameters: For ^{1}H NMR diffusion measurements, the sine-shape pulsed gradients, of 4-ms duration, were incremented from 0 to 36 G cm^{-1} in ten steps, and the pulse-gradient separation Δ was 60 ms. The echo time, the mixing time, and eddy current delay (te) were 28, 46, and 50 ms, respectively. For ^{7}Li NMR diffusion measurements, the sine-shape pulsed gradients, the pulse-gradient separation, the echo time, and the mixing time, were 11, 30, 26, 17 ms, respectively.

The diffusion measurements were performed in three different solvents or solvent mixtures: $CDCl_3$, 1:1 CD_3CN:$CDCl_3$, and 10:1 C_6D_6:$CDCl_3$.

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