VOLUME 119, NUMBER 12
MARCH 26, 1997

JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Self-Assembled Ionophores. An Isoguanosine-K^+ Octamer

Sampath Tirumala and Jeffery T. Davis*

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

Received September 12, 1996[⊗]

Abstract: The mononucleoside, 5′-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylidene isoguanosine (isoG) **2**, has a strong affinity for alkali metal cations. Previously, it was shown that isoG **2** self-assembles via hydrogen bonds to give a stable tetramer, $(isoG)_4$ **4**, in organic solvents (Davis et al. *J. Org. Chem.* **1995,** *60*, 4167−4176). The isoG tetramer **4** can then coordinate metal cations. In this present study, vapor phase osmometry and further ^{1}H NMR experiments confirmed that isoG **2** self-assembles via complementary hydrogen bonds to form tetramer **4**. Molecular models obtained from molecular dynamics and MM2 energy minimization indicate that the isoG tetramer **4** is bowl-shaped, with four C2 oxygens located on the tetramer's convex surface. It is likely that these four oxygens on the tetramer's convex face coordinate cations. Potassium picrate was used to determine the stoichiometry of the isoG-K^+ complex and its K^+ binding affinity. Both ^{1}H NMR and UV−vis spectroscopic analysis demonstrated that isoG **2** forms an octamer, $(isoG)_8$-K^+ (**5**), in the presence of potassium picrate in $CDCl_3$ and CD_3CN. The octamer $(isoG)_8$-K^+ (**5**) has a single set of ^{1}H NMR resonances, even at −90 °C, consistent with a D_4-symmetric head-to-head stacking of two tetramers around the central K^+ cation. Analysis of the picrate's optical spectra indicated that the picrate salt of $(isoG)_8$-K^+ (**5**) is a separated ion pair in $CDCl_3$, consistent with the K^+ being sandwiched between two isoG tetramers. Picrate extraction experiments revealed that $(isoG)_8$ **5** is an impressive ionophore, with a K^+ association constant ($\log K_a = 8.2$ M^{-1}) approaching that of 18-crown-6 ether derivatives. Indeed, NMR competition experiments for K^+ binding between dicyclohexano-18-crown-6 and isoG **2** confirm that the K^+ binding constants in $CDCl_3$ for the crown ether and for the self-assembled ionophore are of the same magnitude ($K_a = 10^8$ M^{-1}).

Introduction

Ion complexation and transport are fundamental to many chemical and biological processes, and the metal-binding properties of many natural and synthetic ionophores have been studied.[1] Almost all of the known ionophores are constrained by covalent bonds into a preorganized conformation that enables metal ion binding. The use of noncovalent interactions to construct self-assembled structures has attracted recent attention.[2,3] An alternative ionophore design, therefore, involves the use of noncovalent interactions to build molecular self-assemblies that bind metal cations.[4,5]

Self-assembled ionophores may be particularly valuable in

* Corresponding author.
[⊗] Abstract published in *Advance ACS Abstracts*, March 1, 1997.

(1) (a) Izatt, R. M.; Pawlak, K.; Bradshaw, J. S.; Bruening, R. L. *Chem. Rev.* **1995**, *95*, 543. (b) Izatt, R. M.; Bradshaw, J. S.; Pawlak, K.; Bruening, R. L.; Tarbet, B. *Chem. Rev.* **1992**, *92*, 1261. (c) Izatt, R. M.; Pawlak, K.; Bradshaw, J. S.; Bruening, R. L. *Chem. Rev.* **1991**, *91*, 1721.

(2) For a review on molecular self-assembly, see: Lawrence, D. S.; Jiang, T.; Levett, M. *Chem. Rev.* **1995**, *95*, 2229.

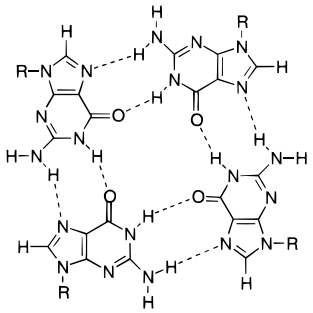
(3) For some interesting self-assembled structures, see: (a) Persico, F.; Wuest, J. D. *J. Org. Chem.* **1993**, *58*, 95. (b) Branda, N.; Wyler, R.; Rebek, J., Jr. *Science* **1994**, *263*, 1267. (c) Hartgerink, J. D.; Granja, J. R.; Milligan, R. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1996**, *118*, 43. (d) Zimmerman, S. C.; Zeng, F.; Reichert, D. E. C.; Kolutchin, S. *Science* **1996**, *271*, 1095.

(4) For examples of self-assembled ionophores, see: (a) Schepartz, A.; McDevitt, J. P. *J. Am. Chem. Soc.* **1989**, *111*, 5976. (b) Schall, O. F.; Robinson, K.; Atwood, J. L.; Gokel, G. W. *J. Am. Chem. Soc.* **1991**, *113*, 7434. (c) Schall, O. F.; Robinson, K.; Atwood, J. L.; Gokel, G. W. *J. Am. Chem. Soc.* **1993**, *115*, 5962. (d) Bell, T. W.; Jousselin, H. *Nature* **1994**, *367*, 441.

S0002-7863(96)03213-1 CCC: $14.00

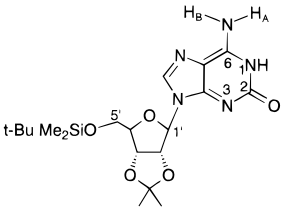
the development of ion-selective sensors[6] and in the recovery and purification of rare earth metals and radioactive isotopes.[7] It is often challenging to recover a metal ion after its complexation. Because of the high association constants, harsh conditions are required to remove a metal ion from its ionophore complex.[8] An ionophore that is held together by hydrogen bonds, however, should release the cation after a protic solvent is added to a metal ion complex that is dissolved in a nonprotic organic solvent.

Nature uses weak intermolecular forces to form self-assembled structures. One example is the G-quartet (**1**), formed from four hydrogen bonded guanosines.[9,10] The G-quartet has the properties of an ionophore, since it is able to coordinate metal cations in its central cavity. The G-quartets have modest K^+ and Na^+ selectivities, and cation ligation further stabilizes coaxial stacking of individual G-quartets.[11] The G-quartet structure has recently become the basis for the design of self-assembled ionophores. Gottarelli and co-workers showed that

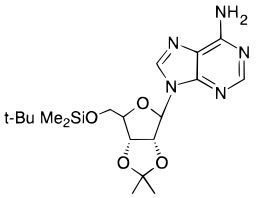


G Quartet **1**

an organic-soluble nucleoside, 3′,5′-didecanoyl-2′-deoxy-G, can extract alkali metal picrate salts from water to form M^+-bound octamers.[12]



IsoG **2**

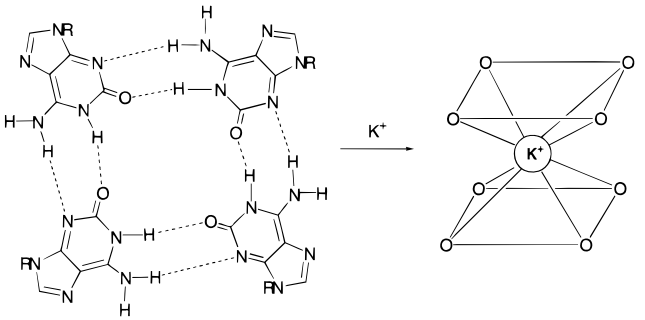


A **3**

Intrigued by the G-quartet's ability to complex metal cations, we have been investigating the remarkable ionophore properties of a related nucleoside, the 5′-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylidene isoguanosine (isoG) **2**. IsoG **2**, with an oxygen at the purine C2 position, is an oxidized analog of adenosine **3**.

IsoG self-association has precedent, as poly(isoguanlyic acid) forms aggregates and the oligonucleotide d(T_4isoG_4T_4) also forms tetraplex structures, both in the presence of Na^+.[13,14] Recently, we demonstrated that the monoribonucleoside isoG **2** forms an extremely stable self-assembly via intermolecular hydrogen bonds in $CDCl_3$, CD_3CN, and acetone-d_6.[5] On the basis of multinuclear NMR data, we proposed that a C_4-symmetric tetramer **4** is the fundamental unit in the isoG self-assembly (Scheme 1). IsoG differs from G in the transposition of the purine's C2 carbonyl and C6 amino group. This simple change in hydrogen bond donor and acceptor groups significantly stabilized the isoG tetramer **4** relative to the corresponding G quartet **1**. We proposed that the structural basis for the stabilization of the isoG tetramer **4** was due to intermolecular base−sugar hydrogen bonds that are not possible for the G quartet.

Scheme 1



$(isoG)_4$ **4** $(isoG)_8$-K^+ **5**

The self-assembled isoG tetramer **4** is also a potent ionophore. Each C2 oxygen in the isoG tetramer has a nonbonded electron pair that points into the tetramer's central cavity, enabling cation coordination. For example, addition of KI to isoG **2** in acetone-d_6 resulted in formation of the octamer, $(isoG)_8$-K^+ (**5**) (Scheme 1).[5] IsoG octamer **5** is likely a sandwich complex stabilized by potassium's coordination of two isoG tetramers.

In this work, use of potassium picrate has made possible an accurate determination of the stoichiometry and K^+ association constant of the isoG-K^+ complex, since the picrate anion can be analyzed by both ^{1}H NMR and UV spectroscopy. Impor-

(5) Davis, J. T.; Tirumala, S.; Jenssen, J. R; Radler, E.; Fabris, D. *J. Org. Chem.* **1995**, *60*, 4167.

(6) (a) *Supramolecular Chemistry I-Directed Synthesis and Molecular Recognition*; Weber, E., Ed.; Springer-Verlag: New York, 1993. (b) Marsella, M. J.; Swager, T. M. *J. Am. Chem. Soc.* **1993**, *115*, 12214. (c) Marsella, M. J.; Newland, R. J.; Carroll, P. J.; Swager, T. M. *J. Am. Chem. Soc.* **1995**, *117*, 9842.

(7) (a) Nakagawa, K.; Inoue, Y.; Hakushi, T. *J. Chem. Soc., Chem. Commun.* **1991**, 1683. (b) McDowell, W. J.; Case, G. N.; McDonough, J. A.; Bartsch, R. A. *Anal. Chem.* **1992**, *64*, 3013. (c) Zhangyu, Y.; Ranqi, K.; Men, Q.; Binghan, W.; Bin, Z. *J. Chem. Soc., Chem. Commun.* **1994**, 1753. (d) Casnati, A.; Pochini, A.; Ungaro, R.; Ugozzoli, F.; Arnaud, F.; Fanni, S.; Shwing, M.; Egberink, R. J. M.; De Jong, F.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1995**, *117*, 2767.

(8) (a) Pedersen, C. J. *J. Am. Chem. Soc.* **1967**, *89*, 7017. (b) Du, H. S.; Wood, D. J.; Elshani, S.; Wai, C. M. *Talanta* **1993**, *40*, 173.

(9) For G-quartets formed from nucleotides, see: (a) Gellert, M.; Lipsett, M. N.; Davies, D. R. *Proc. Natl. Acad. Sci. U.S.A.* **1962**, *48*, 2013. (b) Pinnavaia, T. J.; Miles, F. T.; Becker, E. D. *J. Am. Chem. Soc.* **1975**, *97*, 7198. (c) Zimmerman, S. B. *J. Mol. Biol.* **1976**, *106*, 663. (d) Borzo, M.; Petellier, C.; Laszlo, P.; Paris, A. *J. Am. Chem. Soc.* **1980**, *102*, 1124. (e) Fisk, C. L.; Becker,E. D.; Miles, H. T; Pinnavaia, T. J. *J. Am. Chem. Soc.* **1982**, *104*, 3307. (f) Bouhoutos-Brown, E.; Marshall, C. L.; Pinnavaia, T. J. *J. Am. Chem. Soc.* **1982**, *104*, 6576. (g) Walmsley, J. A.; Barr, R. G.; Bouhoutsos-Brown, E.; Pinnavaia, T. J. *J. Phys. Chem.* **1984**, *88*, 2599. (h) West, R. T.; Garza, L. A.; Winchester, W. R.; Walmsley, J. A. *Nucleic Acids Res.* **1994**, *22*, 5128. (i) Jiang, F. S.; Makinen, M. W. *Inorg. Chem.* **1995**, *34*, 1736.

(10) For leading references regarding G-quartets in oligonucleotides, see: (a) Sen, D.; Gilbert, W. *Nature* **1988**, *334*, 364. (b) Williamson, J. R., Raghuraman, M. K.; Cech, T. R. *Cell* **1989**, 871. (c) Sundquist, W. I.; Klug, A. *Nature* **1989**, *342*, 825. (d) Wang, Y.; Patel, D. J. *Biochemistry* **1992**, *31*, 8112. (e) Smith, F. W.; Feigon, J. *Nature* **1992**, *356*, 164. (f) Cheong, C.; Moore, P. B. *Biochemistry* **1992**, *31*, 8406. (g) Aboul-ela, F.; Murchie, A. I. H.; Lilley, D. M. J. *Nature* **1992**, *360*, 280. (h) Kang, C.; Zhang, X.; Ratliff, R.; Moyzis, R.; Rich, A. *Nature* **1992**, *356*, 126. (i) Wang, K.; McCurdy, S.; Shae, R. G.; Swaminathan, S.; Bolton, P. H. *Biochemistry* **1993**, *32*, 1899. (j) Laughlan, G.; Murchie, A. I. H.; Norman, D. G.; Moore, M. H.; Moody, P. C. E.; Lilley, D.; Luisi, B. *Science* **1994**, *265*, 520.

(11) (a) Pinnavaia, T. J.; Marshall, C. L.; Mettler, C. M.; Fisk, C. L.; Miles, F. T.; Becker, E. D. *J. Am. Chem. Soc.* **1978**, *100*, 3625. (b) Detellier, C.; Lazlo, P. *J. Am. Chem. Soc.* **1980**, *102*, 1135. (c) Eimer, W.; Dorfmüller *J. Phys. Chem.* **1992**, *96*, 6790. (d) Xu, Q.; Braunlin, W. H. *Biochemistry* **1993**, *32*, 13130.

(12) Gottarelli,G.; Masiero, S.; Spada, G. P. *J. Chem. Soc., Chem. Commun.* **1995**, 2555.

(13) Golas, T.; Fikus, M.; Kazmierczuk, Z.; Shugar, D. *Eur. J. Biochem.* **1976**, *65*, 183.

(14) Seela, F; Wei, C; Melenewski, A. *Nucleic Acids Res.* **1996**, *24*, 4940.

tantly, the isoG mononucleoside **2** is able to extract potassium picrate from water into $CDCl_3$. Both ^1H NMR and optical spectroscopic analysis demonstrate that isoG **2** forms $(isoG)_8$-K^+ **5** in the presence of potassium picrate in both $CDCl_3$ and CD_3CN. In addition, molecular modeling and variable temperature ^1H NMR data support the hypothesis of head-to-head coaxial stacking of two tetramers to give $(isoG)_8$-K^+ **5**. Picrate extraction experiments have also provided isoG's K^+ association constant in $CDCl_3$. Both picrate extraction experiments and competition experiments with dicyclohexano-18-crown-6 reveal that $(isoG)_8$-K^+ **5**, held together by noncovalent interactions, has a K^+ binding strength approaching that of the covalent crown ether.

Results and Discussion

Characterization of isoG Self-Association by Vapor Phase Osmometry. IsoG **2** was prepared, with minor modifications, as previously described.[5] Our first paper relied on extensive NMR studies to demonstrate isoG self-association. The NMR data were consistent with the tetramer, $(isoG)_4$ **4**, being the basic unit in isoG self-association in organic solvents. In this present study, we have characterized hydrogen-bonded association by a technique other than NMR spectroscopy, namely vapor phase osmometry (vpo).[15] Molecular weights for isoG **2** and the analogous 5′-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylidene adenosine (A) **3** were determined in dichloromethane solution at 37 °C with concentrations of 30, 60, and 90 mM. In both cases, vpo measurements were made against benzil (mw 210) as a standard. The experimental molecular weight of 1710 ± 25 for isoG **2** was close to the expected molecular weight of 1753 for the self-assembled tetramer, $(isoG)_4$ **4**. As a control, the experimental molecular weight of A **3** was determined to be 355 under similar conditions. This value is close to the molecular weight of 422 for monomeric A **3**. Unlike isoG **2**, A **3** should not self-associate to any significant degree in organic solvents. These vpo measurements clearly indicate that isoG **2** self-associates in a non-hydrogen bonding solvent to form a tetramer. Detailed information about the self-assembled tetramer's structure were then determined using ^1H NMR spectroscopy.

^1H NMR Data Indicates That isoG 2 Self-Associates in Organic Solvents To Give a Bowl-Shaped Tetramer. Both the isoG self-assembly and K^+ binding processes are readily monitored by ^1H NMR spectroscopy. In aprotic organic solvents, isoG monomer **2** and $(isoG)_4$ **4** have distinct groups of ^1H NMR resonances that are in slow exchange at room temperature. The isoG tetramer's extraordinary kinetic stability makes analysis of the monomer−tetramer equilibrium straightforward. Thus, we previously used variable temperature ^1H NMR to show that isoG tetramer **4** is extremely stable even in the modestly competitive acetone-d_6 solution, with $K_a = 10^9-10^{10}$ M^{-3}, $\Delta H = -18$ kcal/mol, and $\Delta S_{298} = -19$ eu.[5] As pointed out by a reviewer, but prior to our vpo results showing a tetramer, K_a values are model dependent. While our NMR data are most consistent with a tetramer structure, K_a would necessarily be lower for a dimer, while K_a would be much higher for an octamer. The above vpo results, however, strongly support the thermodynamic parameters that were previously determined assuming a tetramer model.

(15) Vapor phase osmometry measurements of isoG **2** and A **3** in CH_2Cl_2 at 37 °C were done by Schwarzkopf Microanalytical Laboratory, Woodside, NY using a Wescan 233 vpo instrument. For a discussion of the use of vpo to characterize the molecular weights of other hydrogen bonded aggregates see the series of papers by Seto and Whitesides: (a) Seto, C. T.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 905. (b) Seto, C. T.; Mathias, J. P.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 1321. (c) Seto, C. T.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 1330.

Table 1. ^1H NMR Chemical Shifts (ppm) for IsoG **2** in $CDCl_3$ and CD_3CN in the Absence and Presence of K^+ Picrate[a]

resonance	$CDCl_3$	$CDCl_3$ with K^+Pic	CD_3CN	CD_3CN with K^+Pic
NH1	13.03	13.93	13.03	14.01
NH6A	10.70	10.84	10.70	10.78
NH6B	6.98	6.75	7.09	6.90
H8	7.48	7.79	7.58	7.83
H1′	5.76	5.62	5.79	5.64
H2′	4.98	4.83	5.00	4.83
H3′	4.76	4.83	4.81	4.83
H4′	4.51	4.31	4.52	4.37
H5′	3.84	3.95	3.91	3.98
H5″	3.71	3.79	3.76	3.81
CH3	1.79	1.66	1.78	1.66
CH3	1.41	1.38	1.40	1.33
t-Bu	0.75	0.86	0.76	0.84
SiMe	−0.03	0.09	−0.01	0.08
SiMe	−0.06	0.09	−0.03	0.06

[a] IsoG **2** self-assembles to give $(isoG)_4$ **4** in the absence of potassium picrate. IsoG **2** forms $(isoG)_8$-K^+ **5** in the presence of potassium picrate.

The solvents used in the present ^1H NMR study were $CDCl_3$ and CD_3CN. Prior to performing K^+ binding experiments, we confirmed that the tetramer **4** was the predominant species in these solvents. For example, a 5 mM solution of isoG **2** in either CD_3CN or $CDCl_3$ showed a single set of ^1H resonances at room temperature (Table 1). The individual resonance assignments were made from a series of 2D homonuclear and heteronuclear NMR experiments. The chemical shifts for both the exchangeable and nonexchangeable hydrogens corresponded to those previously assigned to the isoG tetramer **4**.

The ^1H NMR resonances for the exchangeable protons are particularly diagnostic for monitoring self-assembly, hydrogen bonding, and cation binding. In polar solvents such as DMSO-d_6 or CD_3OD, the isoG monomer's NH1 imino and NH6 exocyclic amino protons are not observed at room temperature because of exchange with solvent. In less competitive solvents such as $CDCl_3$, acetone-d_6, and CD_3CN, however, the ^1H NMR resonances for isoG's exchangeable protons are observed as sharp lines, even at temperatures as high as 45 °C. For example, in CD_3CN at 25 °C the signal at 13.03 ppm corresponds to the isoG tetramer's imino NH1, while the resonances at 10.70 and 7.09 ppm are due to the exocyclic amino protons, $NH6_A$ and $NH6_B$. Importantly, the chemical shifts for the NH1 imino resonance and the $NH6_A$ resonance are the same in $CDCl_3$ and in the more polar CD_3CN, while the chemical shift of $NH6_B$ is affected only slightly by the change in solvent (Table 1). The solvent independence of the NH1 and $NH6_A$ chemical shifts, and the small solvent dependance of $NH6_B$, provides strong evidence that all three of isoG's exchangeable protons are shielded from solvent and involved in intermolecular hydrogen bonds.

Previously, $^1H-^1H$ NOESY experiments in acetone-d_6 containing excess KI provided evidence for isoG self-association in the presence of K^+. In this present study, we also used NOE experiments to demonstrate that isoG **2** self-assembles in CD_3CN and $CDCl_3$ even in the absence of potassium salts. Thus, unique, long-range NOEs confirmed isoG self-association in both CD_3CN and $CDCl_3$. For example, a 2D ROESY experiment in CD_3CN showed dipolar interactions between both the exocyclic amino NH_2 protons and the ribose H1′ and H2′ protons (Figure 1).[16] These NH_2-H1′,H2′ NOEs can only arise from intermolecular interactions between different isoG monomers, since the intramolecular NH_2-H1′,H2′ distances are much too

(16) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811.

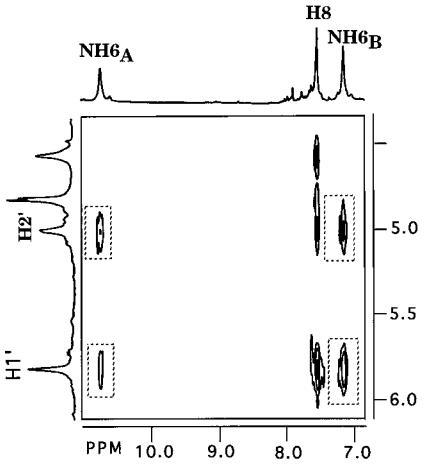


Figure 1. A region of the 2D ROESY NMR spectrum of isoG **2** (39 mM) in CD_3CN at 0 °C. The horizontal axis, from 11.0−6.9 ppm, shows the resonances for $NH6_A$, H8, and $NH6_B$. The vertical axis, from 6.3−4.3 ppm, shows the resonances for the ribose H1′, H2′, H3′, and H4′ protons. The NOEs between the exocyclic NH_A and NH_B amino protons and the ribose H1′ and H2′ protons are boxed for emphasis.

great for $^1H-^1H$ dipolar interactions.[17] Molecular models of isoG dimer and trimer structures are inconsistent with these NOEs. These intermolecular NH_2-H1′,H2′ NOEs are consistent, however, with a hydrogen-bonded tetramer.

Our model for the structure of isoG tetramer **4** is depicted in Figure 2.[18] The isoG tetramer **4** is stabilized by hydrogen bonds between the imino NH1 of one monomer and the C2 carbonyl oxygen of an adjacent purine base as well as by hydrogen bonds between an $NH6_A$ donor in one base and an N3 acceptor in the adjacent isoG monomer (Figure 2A). These two base−base hydrogen bonds would be further strengthened by secondary electrostatic attractions between NH1 and N3 and between $NH6_A$ and O2.[19] The isoG tetramer's structure is consistent with the observed long-range C6NH_2-H1′,H2′ NOEs in Figure 1. In tetramer **4**, the exocyclic amino protons are within 3.0−3.6 Å of the adjacent monomer's H1′ and H2′ protons.

Another interaction that may be crucial for the isoG tetramer's extraordinary stability is an intermolecular base−sugar hydrogen bond between adjacent isoG monomers. Molecular models of the tetramer **4** show that the exocyclic amino $NH6_B$ of one isoG monomer is within hydrogen bonding distance of an adjacent ribose O2′ atom (*d* N6H_B−O2′ 2.10 Å, <N6--H_B--O2′ 146°). Such intermolecular base−sugar hydrogen bonds are not possible in the G-quartet. As proposed in our earlier paper,[5] the presence of these additional hydrogen bonds would stabilize the isoG tetramer relative to the G-quartet. Again, the solvent

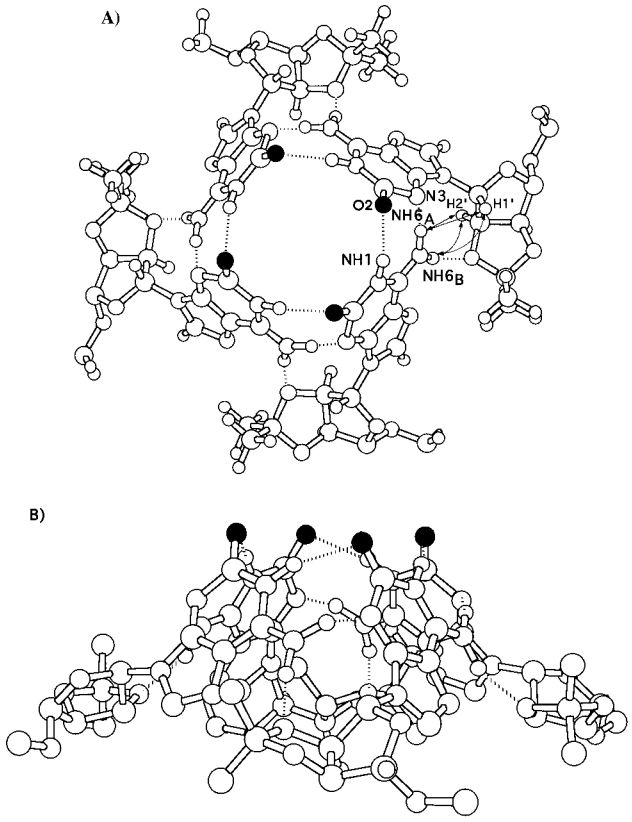


Figure 2. A ball and stick model of the isoG tetramer **4**. The structure was obtained after molecular dynamics and energy minimization using the CaChe molecular modeling program (ref 18). Some atoms are omitted for clarity. (A) Top view looking into the isoG tetramer's central cavity. The dotted lines represent hydrogen bonds. The tetramer's four O2 atoms are filled for emphasis. Double-headed arrows represent the C6NH_2-H1′,H2′ NOEs highlighted in Figure 1. (B) Side view of the bowl-shaped tetramer **4**.

independence of the ^1H chemical shift suggests that $NH6_B$ is shielded from solvent and involved in an intermolecular hydrogen bond. The chemical shift of 7.09 ppm for $NH6_B$ in CD_3CN (Table 1), while upfield of the unusually downfield shifted $NH6_A$ (σ 10.50 ppm), is still relatively deshielded for a nucleobase amino proton. For example, Williams and Shaw found that the non-hydrogen bonded G amino proton in the $(C:G)_2$ tetramer has a resonance at 4.94 ppm in $CDCl_3$ at −57 °C.[20] Also, the non-hydrogen bonded amino protons in the d(TG_4T) G-quadraplex are observed between 5.85 and 6.05 ppm at 6 °C in H_2O.[21]

The 2′,3′-isopropylidene protecting group on the isoG's ribose appears to be crucial for the base−sugar interactions and for the tetramer's structural stability. By limiting the sugar's conformational mobility and orienting O2′ for an intermolecular hydrogen bond with $NH6_B$ in an adjacent monomer, the bicyclic isopropylidene preorganizes isoG monomer **2** for self-assembly into the isoG tetramer **4**.[22] These four intermolecular $NH6_B$-O2′ base−sugar hydrogen bonds help enforce a distinct curvature to isoG tetramer **4**. Molecular models show that isoG tetramer **4** is bowl-shaped, with the four C2 oxygens located on the bowl's convex surface (Figure 2B). Analysis of the low-energy structure indicate that the $NH6_B$−O2′ hydrogen bonds cause the tetramer's four bases to be nonplanar, with average values for hydrogen bond angles of <N1--H--O2 154°, <N6-

(17) Neuhaus, D.; Williamson, M. P. *The Nuclear Overhauser Effect in Structural and Conformational Analysis;* VCH Publishers: New York; 1989; pp 63−103.

(18) Using the CaChe Molecular Editor program (Version 3.5), a model of $(isoG)_4$ **4** was built using 12 intermolecular hydrogen bonds (NH1 to O2, $NH6_A$ to N3, and $NH6_B$ to O2′). A low-energy structure was obtained by first performing molecular mechanics (MM2) calculations. The 12 explicit hydrogen bonds were removed and a molecular dynamics simulation on this structure for $(isoG)_4$ **4** was then performed to give a family of tetramer structures. The dynamics simulation was performed at 300 K, with a 1 ps pre-equilibration time and time steps of 1 fs. The dynamics simulation was sampled every 20 steps, and the structures were saved. Ten isoG tetramer structures were randomly selected from the dynamics simulation. Energy minimization (MM2) gave 10 new structures, all with energies that were within 2.5 kcal/mol of each other. The low energy isoG tetramer structure is depicted in Figure 2.

(19) (a) Jorgensen, W. L.; Pranata, J. *J. Am. Chem. Soc.* **1990**, *112*, 2008. (b) Pranata, J.; Wierschke, S. G.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1991**, *113*, 2810. (c) Murray, T. J., Zimmerman, S. C. *J. Am. Chem. Soc.* **1992**, *114*, 4010. (d) Fan, E; van Arman, S A., Kincaid, S., Hamilton, A. D. *J. Am. Chem. Soc.* **1993**, *115*, 369.

(20) (a) Williams, N. G.; Williams, L. D.; Shaw, B. R. *J. Am. Chem. Soc.* **1989**, *111*, 7205. (b) Williams, N. G.; Williams, L. D.; Shaw, B. R. *J. Am. Chem. Soc.* **1990**, *112*, 829.

(21) Aboul-ela, F.; Murchie, A. I. H.; Norman, D. G.; Lilley, D. M. J. *J. Mol. Biol.* **1994**, *243*, 458.

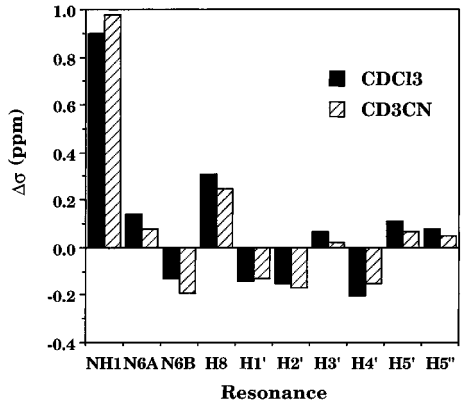


Figure 3. Bar graph of the ^{1}H chemical shift changes caused by addition of excess potassium picrate to a 5 mM solution of isoG **2** in $CDCl_3$ (solid bars) and CD_3CN (hatched bars).

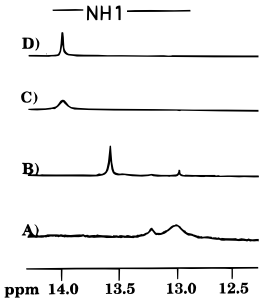


Figure 4. A region of the 500 MHz ^{1}H NMR spectra of isoG **2** (2.9 mM) in the presence of varying amounts of potassium picrate in CD_3CN at 25 °C. The signals correspond to the isoG imino NH1 proton (13.0 −14.0 ppm): (A) without potassium picrate, (B) with 1 equiv of potassium picrate per 12 isoG monomers, (C) with 1 equiv of potassium picrate per eight isoG monomers, and (D) with 1 equiv of potassium picrate per six isoG monomers.

-HA--N3 128°, and <N6--HB--O2′ 146°. While these hydrogen bonds are not linear, their angles are within the range of hydrogen bond angles typically observed in the crystal structures of nucleosides and nucleotides.[23,24] Also, the bowl-shaped structure would help relieve unfavorable electrostatic repulsion between the lone pair electrons on the four oxygen atoms that are directed into the tetramer's central cavity.[25] It is these four oxygens on the tetramer's convex face that are well oriented for coordination of metal cations.

Stoichiometry and Structure of the IsoG-K$^+$ Complex. A Head-to-Head Octamer. The isoG isopropylidene **2** has a high affinity for various metal cations, particularly Na^+ and K^+, in organic solvents. Our previous NMR binding studies used NaI and KI as titrants.[5] In this study, potassium picrate and sodium picrate, which can be analyzed by ^{1}H NMR and UV spectroscopy,[26] were used to obtain more accurate values for the stoichiometry and binding affinity of the isoG-K$^+$ complex.

Cation binding by the isoG tetramer **4** was readily monitored by ^{1}H NMR spectroscopy. The isoG tetramer's ^{1}H NMR spectrum in both CD_3CN and $CDCl_3$ changes significantly upon potassium picrate addition (Table 1 and Figure 3). For example, in the presence of excess K^+, the NH1 imino proton resonance shifted downfield by approximately 1.0 ppm, and the H8 resonance shifted downfield by 0.25 ppm. Other resonances, including $NH6_A$, $NH6_B$, H1′, H2′, and H4′, underwent more modest, but significant, chemical shift changes after K^+ addition. Importantly, Figure 3 also shows that the ^{1}H chemical shift changes upon formation of the isoG-K$^+$ complex are similar in CD_3CN and $CDCl_3$. Moreover, ^{1}H chemical shifts for the isoG-K$^+$ complex do not change with temperature over the range of −30 °C to 25 °C in CD_3CN and over the range of −67 °C to 25 °C in $CDCl_3$. The solvent and temperature independence of these ^{1}H NMR spectra, particularly for the hydrogen bonded protons NH1, $NH6_A$, and $NH6_B$, clearly indicate formation of a stable isoG-K$^+$ complex in solution.

Using ^{1}H NMR spectroscopy, a potassium picrate titration in CD_3CN revealed the stoichiometry of the isoG-K$^+$ complex. As depicted in Figure 3, the isoG NH1 resonance is particularly sensitive to K^+ binding, being shifted downfield by 0.99 ppm in the presence of excess potassium picrate. The NH1 resonance in tetramer **4** occurs at 13.04 ppm in CD_3CN at room temperature (Figure 4A). Addition of substoichiometric potassium picrate caused the isoG NH1 signal to shift downfield (Figure 4B). In CD_3CN at room temperature, the ^{1}H NMR signals for $(isoG)_4$ **4** and the K^+ bound species are in fast exchange, giving rise to a single set of averaged NMR resonances. The NH1 resonance reached a limiting value of 14.03 ppm after addition of 1 equiv of potassium picrate per 8 equiv of isoG monomer **2** (Figure 4C). Titration of excess potassium picrate, beyond this 1/8 ratio, resulted in narrowing of line widths but caused no further chemical shift changes for the isoG resonances (Figure 4D). This titration experiment clearly indicates formation of the octamer $(isoG)_8$-K$^+$ **5** in CD_3CN.

Both solid and aqueous picrate extractions confirmed that $(isoG)_8$-K$^+$ **5** is also formed in $CDCl_3$. First, the insoluble potassium picrate was quantitatively extracted into $CDCl_3$ when a suspension of the salt was stirred in the presence of a 10 mM solution of isoG derivative **2**. Integration of the ^{1}H NMR spectra after potassium picrate extraction revealed an 8:1 ratio of isoG **2** to picrate anion.

For a self-assembled ionophore to be practical it should extract metal salts from water into an organic solution. Importantly, isoG nucleoside **2** quantitatively extracted potassium picrate from water into $CDCl_3$. Thus, a 10 mM solution of isoG **2** in $CDCl_3$ was shaken with a 25 mM solution of potassium picrate in water. Both ^{1}H NMR and UV measurements revealed that isoG derivative **2** extracted potassium picrate into $CDCl_3$ to give $(isoG)_8$-K$^+$ **5**. Figure 5 shows isoG's UV−vis spectra in $CDCl_3$ before and after potassium picrate extraction. The N1-keto tautomer of isoG derivatives has an

(22) Marlow, A.; Davis, J. T., unpublished results. Detailed 500 MHz ^{1}H NMR experiments show that 5′-*t*-BDMS-2′,3′-OMe isoG and 5′-*t*-BDMS-2′,3′-OAc isoG, compounds that lack the bicyclic ring that exists in isopropylidene **2**, are completely monomeric in $CDCl_3$ and CD_3CN at room temperature. Nonspecific aggregation of 5′-*t*-BDMS-2′,3′-OMe isoG and 5′-*t*-BDMS-2′,3′-OAc isoG is only observed below 0 °C. Significantly, both of these "acyclic" isoG derivatives, like isoG **2**, form octamers in the presence of potassium picrate. The 2′,3′-OMe-K$^+$ octamer, unlike isopropylidene octamer **5**, has an $NH6_B$ proton whose chemical shift is very temperature sensitive. For example, the chemical shift for $NH6_B$ in the 2′,3′-OMe-isoG K$^+$ octamer is 6.38 ppm at 20 °C in $CDCl_3$. At −40 °C this resonance is downfield shifted to 6.85 ppm. The $NH6_B$ resonance in the K$^+$ octamer **5**, on the other hand, occurs at 6.78 ppm over the same temperature range. That isoG's $NH6_B$ chemical shift is sensitive to the 2′ and 3′ sugar substituents clearly indicates that sugar-base interactions are important in the isoG self-assembly process. Details of the synthesis, structural characterization, and metal cation binding properties of these and other isoG analogs will be the subject of a future report.

(23) Jeffrey, G. A.; Malosynzska, H.; Mitra, J. *Int. J. Biol. Macromol.* **1985**, *7*, 336.

(24) The crystal structure of an intramolecular G-quadraplex formed from d(GGGGTTTTGGGG) shows one of the hydrogen bonded G residues to be buckled by 25° from the plane containing the other three G bases, see: Kang, C.; Zhang, X.; Ratliff, R.; Moyzis, R; Rich, A. *Nature* **1992**, *356*, 1126.

(25) For a similar argument in discussing the cyclic structures of polyinosinic acid and polyguanylic acid, see: (a) Rich, A. *Biochim. Biophys. Acta* **1958**, *29*, 503. (b) Zimmerman, S. B.; Cohen, G. H.; Davies, D. R. *J. Mol. Biol.* **1975**, *92*, 181.

(26) Coplan, M. A.; Fuoss, R. M. *J. Phy. Chem.* **1964**, *68*, 1177.

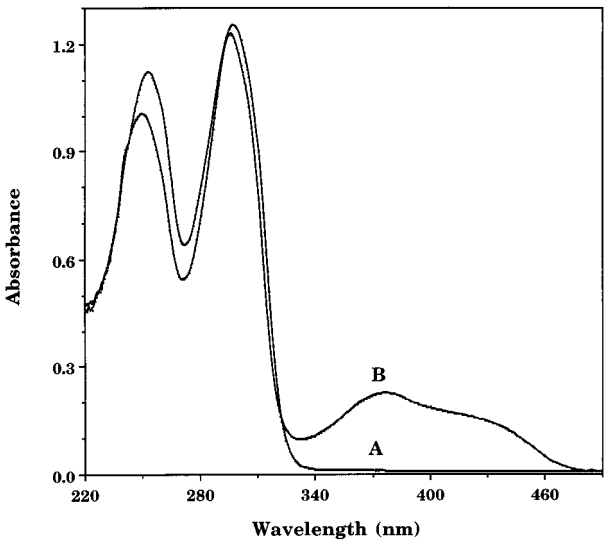


Figure 5. Optical spectra of a $CDCl_3$ solution of isoG **2** (0.11 mM): (A) before potassium picrate extraction and (B) after extraction of an aqueous potassium picrate (3.0 mM) solution. Integration of the isoG absorption at 295 nm and the picrate absorption at 378 nm indicates formation of $(isoG)_8$-K^+ **5**.

absorbance maximum at 295 nm (ϵ = 11 100 M^{-1}),[27] while the band at 378 nm (ϵ = 16 900 M^{-1}) corresponds to the picrate anion's absorption maximum. After picrate extraction, the relative absorbance values for isoG and picrate in the $CDCl_3$ layer indicated the presence of 8 isoG monomers per equiv of picrate, consistent with the formation of $(isoG)_8$-K^+ **5**. Similar NMR and UV−vis analysis of titration and extraction experiments with sodium picrate showed that isoG **2** binds Na^+ as a tetramer in both $CDCl_3$ and in CD_3CN (data not shown).

The $(isoG)_8$-K^+ **5** most likely forms by coaxial stacking of two isoG tetramers around a central K^+ cation (Scheme 1). Coaxial stacking of tetramers around K^+ is well-known for the related G-quartets.[10,11] It is reasonable that such a sandwich complex, with the K^+ coordinated to eight isoG O2 atoms, would exist as a separated ion pair in organic solvents. As outlined below, analysis of the picrate's optical spectra provided strong evidence that $(isoG)_8$-K^+ **5** is, indeed, a separated ion pair in $CDCl_3$. Smid first demonstrated that potassium picrate's absorption band undergoes a significant bathochromic shift in the presence of macrocyclic ionophores that can encapsulate K^+ and stabilize separated ion pairs in organic solvents.[28] This bathochromic shift was found to be diagnostic of separated ion pairs formed after potassium picrate extraction by macrocycles from water into CH_2Cl_2. Thus, Inoue showed that the 18-crown-6:potassium picrate complex absorbed at 369 nm, but that the picrate's absorbance was shifted to 375 nm when extracted by [2.2.2]cryptand.[29] Complexation by [2.2.2]cryptand would result in a more separated ion pair than would coordination by 18-crown-6. The picrate's electronic spectra, therefore, can provide important information about cation-ligand geometry in solution.

In our experiments, depicted in Figure 6, potassium picrate was extracted from water into $CDCl_3$ by three different ionophores, 18-crown-6, [2.2.2]cryptand, and isoG isopropylidene **2**, to give the respective ionophore-K^+ picrate complexes. The UV−vis spectra of the organic phase after potassium picrate extraction demonstrated that the $(isoG)_8$-K^+ picrate **5** is a separated ion pair in $CDCl_3$. In the 1:1 crown-ether K^+ complex, which exists as a contact ion pair in nonpolar solvents,[28] the picrate anion absorption maximum was observed at 365 nm. The picrate's absorption band moved to 378 nm in the [2.2.2]cryptate-K^+ complex. The [2.2.2]cryptand, unlike 18-c-6, encapsulates K^+ to give a separated ion pair. Significantly, the picrate's absorption band in $(isoG)_8$-K^+ **5** was similar in bandshape and wavelength maximum (378 nm) to that observed for the [2.2.2]cryptate-K^+ complex. This spectroscopic analysis demonstrates that a similar environment exists around the picrate ion in $(isoG)_8$-K^+ **5** and in the [2.2.2]cryptate complex. The structure of $(isoG)_8$ K^+ **5** in $CDCl_3$ is, then, consistent with a separated ion pair, with the K^+ sandwiched between two tetramers and the picrate anion removed from contact with its K^+ counterion.

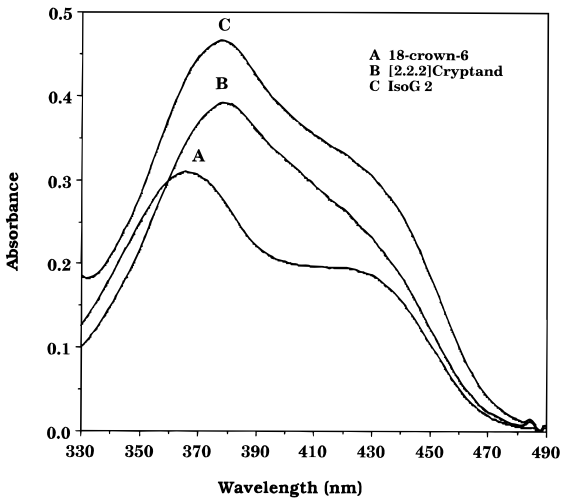


Figure 6. Optical spectra of $CDCl_3$ solutions of various ionophore-K^+ complexes after potassium picrate extraction from water. The region from 330−490 nm shows the picrate anion's absorbance: (A) 18-crown-6 (17.7 μM), (B) [2.2.2]cryptate (22.4 μM), and (C) IsoG **2** (0.215 mM).

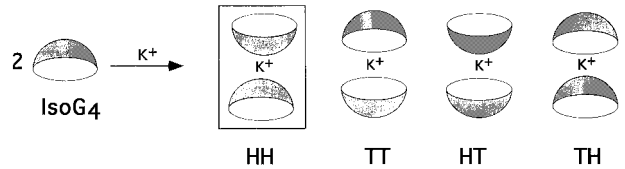


Figure 7. Illustration of possible stacking modes to form $(isoG)_8$-K^+ **5** from the bowl-shaped tetramer **4**: HH, head-to-head; HT, head-to-tail; TH, tail-to-head; TT, tail-to-tail. The proposed HH stacking for $(isoG)_8$-K^+ **5** is boxed.

As depicted in Figure 7, two tetramers can stack in four possible orientations: either head-to-tail, tail-to-head, tail-to-tail, or head-to-head. For example, both X-ray diffraction and solution NMR data have indicated that G-quartets formed from 5′-GMP stack predominantly in a head-to-tail orientation in the presence of K^+.[9c−f] In the absence of fast chemical exchange between the two tetramers, a head-to-tail or tail-to-head octamer should give two ^1H NMR signals for each monomeric hydrogen, since positions in the top and bottom tetramer are different by symmetry. Consistent with these arguments, the 5′-GMP head-to-tail octamer gave two sets of ^1H NMR signals in water.[9f] Similarly, Gottarelli also interpreted the doubling of ^1H NMR signals to indicate that K^+ complexes of 3′,5′-didecanoyl-2′-deoxy-G formed head-to-tail octamers in $CDCl_3$.[12]

(27) (a) Sepiol, J.; Kazmierczuk, Z.; Shugar, D. *Z. Naturforsch.* **1976**, 361. (b) Seela, F.; Wei, C.; Kazimierczuk, Z. *Helv. Chim. Acta* **1995**, *78*, 1843.

(28) (a) Wong, K. H.; Bourgoin, M.; Smid, J. *J. Chem. Soc., Chem. Commun.* **1974,** 715. (b) Bourgoin, M.; Wong, K. H.; Hui, J. Y.; Smid, J. *J. Am. Chem. Soc.* **1975**, *97*, 3462.

(29) Inoue, Y.; Fujiwara, C.; Wada, K.; Tai, A.; Hakushi, T. *J. Chem. Soc., Chem. Commun.* **1987**, 393.

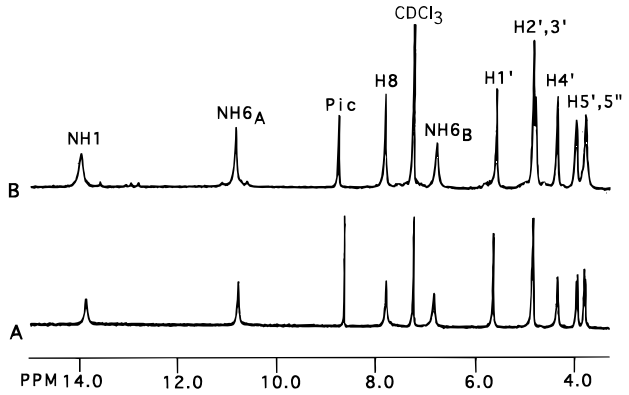


Figure 8. 500 MHz ^{1}H NMR spectra of a $CDCl_3$ solution of $(isoG)_8$-K^+ **5** (2.5 mM). The solution was formed by extraction of potassium picrate from water into $CDCl_3$: (A) At 25 °C and (B) At −67 °C.

Table 2. K^+ Associates Constants (K_a) for Various Ionophores

entry	ionophore	*R* value[a]	log K_a (M^{-1})	$-\Delta G$ (kcal/mol)	ref
1	(isoG **2**)$_8$	0.88	8.2	11.3	this work
2	A **3**	0.05	<3.0	<5.5	this work
3	dicyclohexano-18-c-6	0.81	8.2	11.3	31

[a] *R* value is the molar ratio of picrate ion to host in the organic layer at equilibrium.[30]

Variable temperature ^{1}H NMR spectroscopy indicated that $(isoG)_8$-K^+ **5**, unlike 5′-GMP and 3′,5′-didecanoyl-2′-deoxy-G, does not stack in a head-to-tail orientation. Figure 8 shows the ^{1}H NMR spectrum of a $CDCl_3$ solution of $(isoG)_8$-K^+ **5** at two different temperatures, 25 and −67 °C. At room temperature, there is a single set of ^{1}H NMR resonances for the octamer **5**. It could be argued that $(isoG)_8$-K^+ **5** exists as a head-to-tail octamer and that fast exchange of the tetramers might give rise to time-averaged ^{1}H NMR signals at 25 °C. Lowering the temperature by 90 °C, however, did not change the appearance of the isoG octamer's ^{1}H NMR spectrum (Figure 8B). Even at −67 °C in $CDCl_3$, $(isoG)_8$ K^+ **5** displayed only a single set of resonances, with the same chemical shifts as were observed at room temperature. Since $(isoG)_8$-K^+ **5** has a single set of ^{1}H NMR resonances even at low temperature, we conclude that the octamer does not form by head-to-tail stacking of tetramers. In contrast to head-to-tail stacking, either head-to-head or tail-to-tail stacking of isoG tetramers would give a D_4-symmetric octamer. Due to its D_4-symmetry, which makes the two component tetramers identical, head-to-head and tail-to-tail octamers would exhibit a single set of ^{1}H resonances. Based on structural considerations, a head-to-head orientation of two isoG tetramers is more likely than tail-to-tail stacking. The molecular model in Figure 2 shows that the bowl-shaped isoG tetramer **4** has four C2 oxygens located on its convex surface. Two isoG tetramers would prefer to stack in a head-to-head geometry, rather than tail-to-tail, to best coordinate the K^+ cation (Figure 7).

The isoG Octamer 5 Is a Potent K^+ Ionophore. We next determined the association constant (K_a) for K^+ binding by $(isoG)_8$-K^+ **5** in order to compare it with the covalent ionophore, dicyclohexano-18-crown-6.

The K^+ association constants in $CDCl_3$ were determined using Cram's picrate extraction method.[30] Association constants for $(isoG)_8$ **5** and dicyclohexano-18-crown-6 are presented in Table 2. A log K_a value of 8.2 M^{-1} indicates that the self-assembled isoG octamer **5** is a potent K^+ ionophore. IsoG's K^+ binding affinity in $CDCl_3$, as determined by picrate extraction, is the same as that for dicyclohexano-18-crown-6.[31] Cram has noted that K_a values calculated from the picrate extraction method can have large errors when *R* values are >0.8, where *R* values refer to the molar ratios of picrate ion to host in the organic layer at equilibrium.[30] Since the *R* value for the isoG octamer **5** was $R = 0.88$, as determined by UV spectroscopy, we were concerned about the accuracy of comparing picrate extraction K_a values for $(isoG)_8$ **5** and dicyclohexano-18-crown-6. Thus, a direct competition experiment for K^+ binding in $CDCl_3$ between isoG **2** and dicyclohexano-18-crown-6 was carried out. A solution of the picrate salt of the octamer, $(isoG)_8$-K^+ **5**, in $CDCl_3$ was prepared by extraction of potassium picrate from water. Addition of 1 equiv of dicyclohexano-18-crown-6 to the $(isoG)_8$-K^+ **5** solution resulted in formation of two sets of isoG ^{1}H resonances in a 2.7:1 ratio (Figure 9). Separate NMR resonances for $(isoG)_4$ **4** and $(isoG)_8$-K^+ **5** indicate that the tetramer and the K^+-bound octamer are in slow exchange in $CDCl_3$ at room temperature. The major NMR resonances corresponds to those for a cation-free tetramer, $(isoG)_4$ **4**, while the minor set of NMR signals corresponds to resonances for $(isoG)_8$-K^+ **5**. This NMR competition experiment indicates that isoG has a K^+ association constant of the same magnitude as that of dicyclohexano-18-crown-6. Indeed, K_a for dicyclohexano-18-crown-6 is only three times greater than that for the self-assembled ionophore, $(isoG)_8$-K^+ **5**. It is remarkable that the isoG octamer **5**, a noncovalent assembly, has a K^+ binding constant approaching that of an 18-c-6 crown ether. As mentioned in the Introduction, self-assembled ionophores may have applications in the selective extraction and recovery of metal cations. Future experiments will focus on characterizing the metal cation selectivity of this self-assembled ionophore.

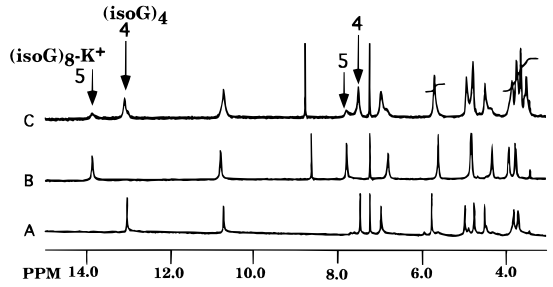


Figure 9. A region of 500 MHz ^{1}H NMR spectra in $CDCl_3$ at 23 °C: (A) $(IsoG)_4$ **4**, (B) $(IsoG)_8$-K^+ **5** formed by extraction of potassium picrate from water into $CDCl_3$, and (C) a 1:1 mixture of $(isoG)_8$-K^+ **5** and dicyclohexano-18-crown-6.

Summary. We have demonstrated that the organic-soluble isoG derivative **2** is a potent K^+ ionophore, as it quantitatively extracts potassium picrate from water into $CDCl_3$ to give $(isoG)_8$-K^+ **5**. Molecular modeling and NMR and UV spectroscopic data indicate that the octamer **5** is a head-to-head stack of two bowl-shaped isoG tetramers. The self-assembled ionophore's K^+ association constant in $CDCl_3$ ($K_a = 10^8$ M^{-1}) approaches that of covalent 18-crown-6 derivatives. The stability of the $(isoG)_8$-K^+ octamer **5** is remarkable, and these studies provide an excellent foundation from which to develop even better self-assembled ionophores.

Experimental Section

General Methods. All solvents were reagent grade and were distilled before use. Solvents used for NMR spectroscopy were 99.0−99.9% deuterium enriched. The $CDCl_3$ was distilled from P_2O_5 under

(30) Helgeson, R. C.; Weisman, G. R.; Toner, J. L.; Tarnowski, T. L.; Chao, Y; Mayer, J. M.; Cram, D. J. *J. Am. Chem. Soc.* **1979**, *101*, 4928.
(31) Koening, K. E.; Lein, G,M.; Stuckler, P.; Kaneda, T.; Cram, D. J. *J. Am. Chem. Soc.* **1979**, *101*, 3553.
(32) Redfield, A.; Kunz, S. D. *J. Magn. Reson.* **1975**, *19*, 250.
(33) Burke, S. D.; O'Donnell, C. J.; Porter, W. J.; Song, Y. *J. Am. Chem. Soc.* **1995**, *117*, 12649.

nitrogen. Elemental analysis was done by Schwarzkopf Microanalytical Laboratory, Inc. (Woodside, NY).

Sample Preparation. Synthesis of the 5′-*t*-BDMS-isoguanosine (isoG) isopropylidene **2** and 5′-*t*-BDMS-adenosine (A) isopropylidene **3** was as described.[5] Prior to the ^{1}H NMR experiments, isoG derivative **2** was purified by flash chromatography on silica gel using 10:1 CH_2Cl_2:EtOH as eluant.

Preparation of Potassium Picrate. Potassium picrate was prepared by neutralizing picric acid with an equal molar amount of potassium hydroxide in EtOH. Solid impurities were removed by filtration. The resulting potassium picrate, which precipitated from solution, was recrystallized twice from water and dried in a vacuum desiccator for 2 days. Characterization: ^{1}H NMR (500 MHz, CD_3CN): σ 8.60 (s, 2H); UV (THF) λ_{max} 357 nm (16 900). Anal. Calcd for $C_6H_2N_3O_7K$: C, 27.95; N, 15.73; K, 14.63. Found: C, 27.35; N, 15.75; K, 13.91.

NMR Experiments. The ^{1}H NMR spectra were recorded on a Bruker AMX-500 MHz spectrometer, and chemical shifts are reported in ppm relative to the nondeuterated solvent peak. The temperature was controlled to ±0.1 °C. The spectral window was 16 ppm for ^{1}H. Typical ^{1}H 90° pulse widths were 11 μs. Data processing was done with Triad (Tripos) software on a SUN sparc station. The 1D spectra were processed by zero-filling to 32 K and multiplied by a Lorentzian function (0.3−1 Hz) prior to Fourier transformation. The ROESY experiment on isoG **2** (39 mM) in CD_3CN at 0 °C was obtained using TPPI quadrature detection.[32] The data were collected with an F2 spectral window of 8064 Hz and 2048 data points for each FID, with 64 transients for each of the 512 separate experiments. A 200 ms spin-locking time was used with a 4 s relaxation delay. The 2D matrix was zero-filled to 1024 (t1) × 4096 (t2) data points and multiplied by a sine bell window function in each dimension. Both zero and first order phase corrections were applied.

Potassium Picrate ^{1}H NMR Titrations. A solution of potassium picrate in CD_3CN (7.5 mM) was titrated in 5 μL portions into a solution of isoG **2** (5.0 mM) in CD_3CN. The stoichiometry of the isoG-potassium picrate solution could be verified by comparing the integration of the picrate's ^{1}H resonance at 8.66 ppm with the integration of the isoG resonances. The solution was vortexed for 2 min in the NMR tube, and then ^{1}H NMR spectra were recorded at 25 °C. A relaxation delay of 10 s between scans was used to ensure accurate integration of the picrate and isoG ^{1}H resonances.

Potassium Picrate Extraction ^{1}H NMR Experiments. Both aqueous and solid extraction experiments were performed to determine the stoichiometry of $(isoG)_8$-K^+ **5**. (A) Aqueous extractions: To a centrifuge tube containing 1 mL of a 5.0 mM solution of isoG **2** in $CDCl_3$ was added 1 mL of a 7.5 mM solution of potassium picrate in distilled water. The mixture was covered and vortexed for 10 min at room temperature. The biphasic solution was centrifuged to force the organic layers to the bottom of the tube. An aliquot (400 uL) of the $CDCl_3$ layer was carefully removed, and its ^{1}H NMR spectrum were recorded at 500 MHz. The stoichiometry for $(isoG)_8$-K^+ **5** was determined by comparing the integration of the picrate's ^{1}H resonance at 8.66 ppm with the integration of the isoG resonances. (B) Solid phase extractions of potassium picrate into $CDCl_3$: Excess potassium picrate was added to a 5 mM solution of isoG **2** in $CDCl_3$. The suspension was stirred for 20 min at room temperature. After filtration of the excess potassium picrate the ^{1}H NMR spectrum of the $CDCl_3$ layer was recorded, and the isoG:picrate stoichiometry was determined by integration of the appropriate resonances.

Determination of isoG's K^+ Association Constants (K_a). All optical measurements were made at 25 °C on a Milton Roy 2000 Diode Array spectrometer. The water used in the preparation of all solutions was first passed through a Nanopure ion exchange purification system. All glassware used in K_a determinations was cleaned by first washing thoroughly with water and acetone, washing again with distilled water, and submerging in concentrated H_2SO_4 for 24 h. The glassware was rinsed with distilled water, immersed in saturated $NaHCO_3$ for 5 h, washed thoroughly with water, and oven-dried at 140 °C for 15 h.

Association constants were determined using Cram's picrate extraction method.[30,33] Picrate salts were vacuum dried before use. Aqueous solutions of potassium picrate (7.5 mM) were prepared using nanopure water. Into each of four 3 mL centrifuge tubes was transferred 0.50 mL of the aqueous picrate solution. Aliquots (0.50 mL) of a $CDCl_3$ solution of isoG **2** (7.5 mM) were added to each centrifuge tube. To ensure adequate mixing of the layers, the samples were vortexed for 5 min at high speed. The tubes were centrifuged for 1 min to drive the $CDCl_3$ layer to the bottom. Aliquots (0.0050 mL) of the organic layer were carefully taken from each tube with a Hamilton Gastight syringe, transferred to 5 mL volumetric flasks, and filled to the mark with CH_3CN. For each aliquot, a blank was also made by measuring the same volume (0.0050 mL) from the $CDCl_3$ layer of a H_2O blank and diluting to the mark with CH_3CN in a 5 mL volumetric flask. The absorbance of each sample was measured against the blank solution at 378 nm.

Equation 1 was used for the calculation of the R value, the molar ratio of picrate to isoG **2** in the $CHCl_3$ layer after extraction, where c is the measured picrate concentration determined by UV spectroscopy after dilution in CD_3CN, D is the dilution factor, and H_i is the initial concentration of isoG **2** in $CHCl_3$.

$$R = cD/H_i \tag{1}$$

Association constants were calculated from eq 2, where K_d is the distribution constant of potassium picrate between water and $CHCl_3$, G_i is the initial potassium picrate concentration in water, V is the volume of water, and V^* is the volume of the $CHCl_3$ layer. The k_d value for potassium picrate was obtained from the literature.[30]

$$K_a = R/K_d(1 - R)\{[G_i - R(H_i)](V^*/V)\}^2 \tag{2}$$

The following assumptions were made while using eq 2: (a) potassium picrate is dissociated in water; (b) potassium picrate is monomeric in $CHCl_3$; (c) isoG **2** is insoluble in water; and (d) only 1 equiv of K^+ binds to the isoG octamer complex.

Acknowledgment. We thank the American Cancer Society, Maryland Division and the Donors of the Petroleum Research Fund, administered by the American Chemical society, for financial support. We thank Matt Disney and Allison Marlow for their assistance in the lab and Yui-Fai Lam for assistance with NMR studies. We thank Neil Blough, Steve Rokita, and Dan Fabris for helpful discussions. We also thank the reviewers for critical suggestions.

JA963213Q