Supramolecular architectures generated by self-assembly of guanosine derivatives†

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Nature's use of a simple genetic code to enable life's complex functions is an inspiration for supramolecular chemistry. DNA nucleobases carry the key information utilizing a variety of cooperative and non-covalent interactions such as hydrophobic, van der Waals, π–π stacking, ion–dipole and hydrogen bonding. This *tutorial review* describes some recent advances in the form and function provided by self-assembly of guanine (G) based systems. We attempt to make connections between the structures of the assemblies and their properties. The review begins with a brief historical context of G self-assembly in water and then describes studies on lipophilic guanosine analogs in organic solvents. The article also focuses on examples of how G analogs have been used as building blocks for functional applications in supramolecular chemistry, material science and nanotechnology.

1 A brief history and perspective

The G-quartet was identified in 1962 as the basis for hydrogels formed by 5′-guanosine monophosphate (5′-GMP).[1] Many nucleosides, oligonucleotides and synthetic derivatives form G-quartets and related structures.[2–4] This section reviews guanosine self-assembly in supramolecular chemistry, with a focus on structure and function.

Guanosine analogs, with their self-complementary hydrogen-bonding edges and aromatic surfaces, are programmed to self-associate. Guanine has two hydrogen bond acceptors (N7 and O6) on its Hoogsteen face and two hydrogen bond donors (N1 amide and N2 amino) on its Watson–Crick face (Fig. 1a). Depending on the conditions, guanosine derivatives can self-associate into dimers, ribbons, or macrocycles. These hydrogen-bonded structures can stack in solution due to their polarized aromatic surfaces.

Based on fiber diffraction data of the 5′-GMP hydrogels, Gellert and colleagues proposed that the G-quartet was formed by 8 intermolecular hydrogen bonds between complementary Watson–Crick and Hoogsteen edges of neighboring guanines

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† This paper is dedicated to Prof. Giovanni Gottarelli on the occasion of his retirement.

Jeffery Davis

Jeffery Davis, born and raised in Western Massachusetts, received his BA degree in 1981 from Colby College in Waterville, Maine. He earned his PhD from MIT in 1987 under the guidance of Professor Satoru Masamune. After 3 years as Group Leader of Bioorganic Chemistry at Genzyme Co. in Boston, he continued his training with Professor Brian Reid at the University of Washington in Seattle, where he was a NIH post-doctoral fellow. In 1993 he joined the faculty in the Department of Chemistry and Biochemistry at the University of Maryland in College Park. He and his research group work on problems in molecular self-assembly, molecular recognition and transmembrane ion transport.

Gian Piero Spada

Gian Piero Spada was born in Faenza (Italy) in 1956 and graduated cum laude in Pharmaceutical Chemistry in Bologna in 1980. After a post-Lauream in Organic Chemistry under the guidance of Prof. Giovanni Gottarelli, he joined the Faculty of Pharmacy of the University of Bologna in 1983 as Research Associate. He became Associate Professor in 1992 and since 2000 he has been full Professor of Organic Chemistry. His research activity is in the fields of stereochemistry and supramolecular chemistry; in particular, his current interests focus on the chiroptical properties, self-assembly and liquid crystal formation of organic molecules and on the use of liquid crystals in configurational and conformational investigations. He is member of the International Liquid Crystal Society and has been member of the Board of Directors of the Italian Liquid Crystal Society.

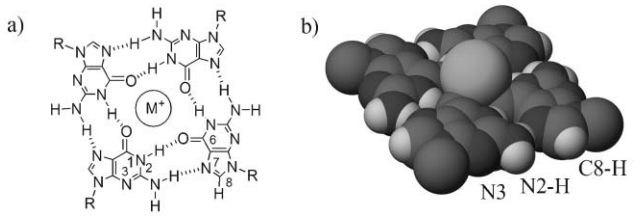


Fig. 1 a) The G-quartet and b) a space-filling model showing a G-quartet with a K^+ bound above the plane of the G-quartet. (Adapted from reference 4.)

(Fig. 1a).[1] A decade later, Pinnavaia and colleagues reported that G-quartets are stabilized by Na^+ and K^+. These cations coordinate to the four carbonyl oxygens in each G-quartet (Fig. 1b). They showed that 5′-GMP **1** formed diastereomeric G_8-K^+ octamers by sandwiching two G-quartets around each cation.[5] Recently, Wu, Spindler and colleagues used diffusion NMR and dynamic light scattering to calculate the dimensions of stacks formed by 5′-GMP **1** at pH 8 (Fig. 2).[6] They identified two dominant species: stacked 5′-GMP monomers and stacked G-quartets. For 5′-GMP concentrations between 18–34 wt%, the columns were 8–30 nm long, corresponding to a cylinder composed of 24–87 stacked G-quartets. The impressive length of these stacks underscores the cooperation of hydrogen bonding, π–π stacking and cation–dipole interactions inherent to G-quartet assemblies.

G-quartet assemblies may be used for the synthesis of nanostructures. Sreenivasachary and Lehn described dynamic hydrogels formed by covalent modification of the sugar sidechains that extend from stacked G-quartets.[7] Reaction of hydrogel A formed from 5′-hydrazido G **2** with a mixture of aldehydes produced a family of acylhydrazones (Fig. 3). This dynamic combinatorial library of G-quartet acylhydrazones gave the most stable hydrogel, showing that G-quartets can be used as scaffolds to control self-organization of materials.

2 Recent studies on the molecular self-assembly of lipophilic guanosine analogs: Form and function

In 1995, Gottarelli, Spada and colleagues reported that 3′,5′-didecanoyl-2′-dG **3** extracts K^+ picrate from water into $CDCl_3$ to give a discrete octamer $[dG\ \mathbf{3}]_8 \cdot K^+\ Pic^-$.[8] The K^+ cation was essential for formation of this lipophilic octamer (Fig. 4).

Without templating cations, dG **3** organized into two different hydrogen-bonded ribbons.[9] Changing the sugar

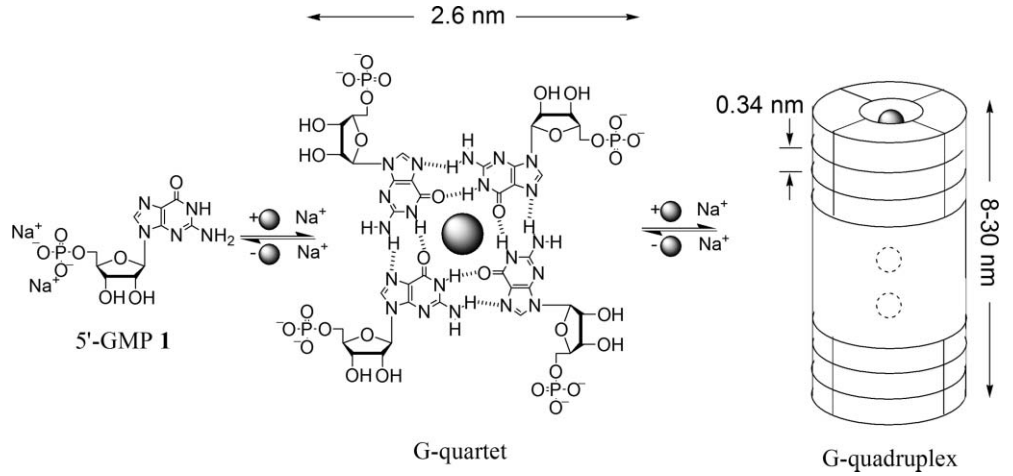


Fig. 2 G-quadruplex cylinder formed by self-assembly of 5′-GMP **1**. (See reference 6.)

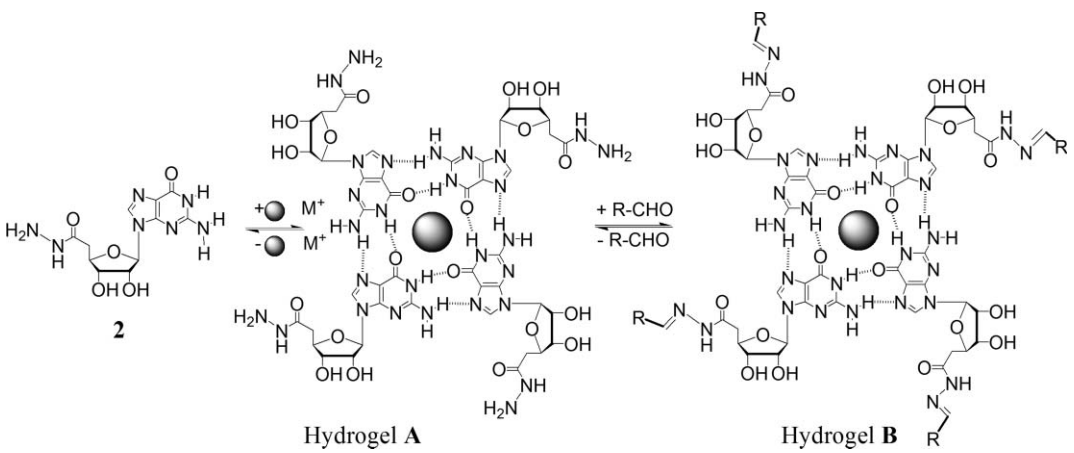


Fig. 3 Dynamic hydrogels using a G-quartet scaffold. (Adapted from reference 7.)

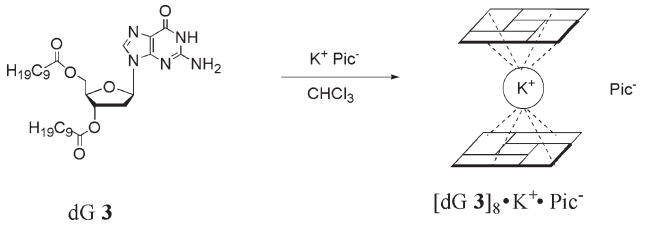


Fig. 4 Lipophilic [dG **3**]$_8$·K^+ octamer formed by extraction of K^+ picrate from water.

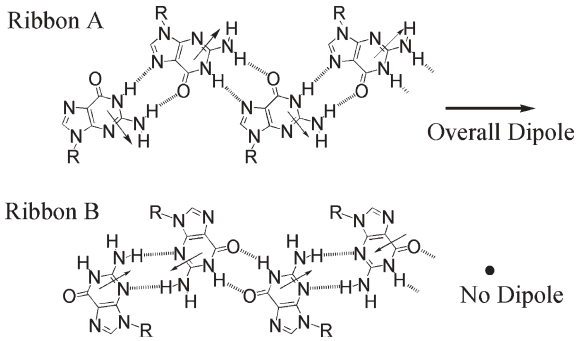


Fig. 5 Two different H-bonded ribbons formed by self-assembly of lipophilic dG **3** in absence of cations. Ribbon A has a net dipole, whereas ribbon B contains no dipole.

substituents or the solvent modulated the ribbon's hydrogen-bonding pattern (giving ribbon A or B as in Fig. 5). As described below in Section 3, these ribbons have applications in the molecular electronics field.[10]

Recently, Gottarelli, Spada and colleagues described another unique structure obtained upon self-assembly of a lipophilic nucleoside.[11] Thus, 8-oxoG **4** formed a hydrogen-bonded helix in organic solvents (Fig. 6). This self-assembly pattern for 8-oxoG **4** was much different from the hydrogen-bonded ribbons formed by dG **3**.

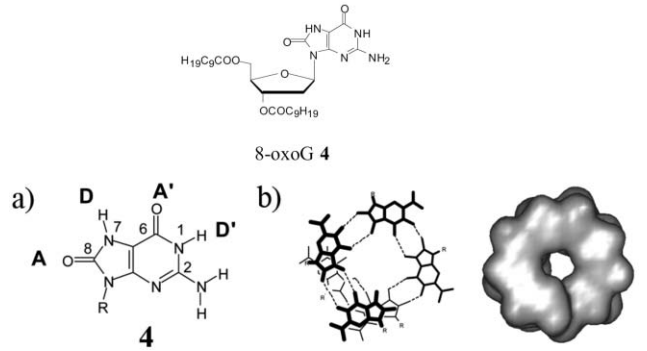


Fig. 6 a) 8-Oxoguanine and b) 8-oxoG-helical structure. (Adapted from reference 11.)

To better understand how individual G-quartets organize within G-quadruplexes, the Gottarelli and Davis groups solved the NMR structure of [dG **3**]$_8$·KI in $CDCl_3$.[12] This study showed that the octamer [dG **3**]$_8$·KI existed as a single diastereomer with the templating K^+ sandwiched between an all-*anti* G-quartet and an all-*syn* G-quartet. In 2000, an X-ray structure illustrated that lipophilic G-quadruplexes are formed in high diastereoselectivity in organic solvents.[13] The lipophilic G-quadruplex [G **5**]$_{16}$·3K^+·Cs^+·4pic^- consists of 4 stacked G-quartets. The complex was generated when 5′-silyl-2′, 3′-isopropylidene G **5** was used to extract K^+ picrate from water into CH_2Cl_2 (Fig. 7). Diffraction-quality crystals of the lipophilic G-quadruplex were grown from acetonitrile. This G-quadruplex can be described as a pair of head-to-tail [G **5**]$_8$ octamers with each G_8-octamer using its 8 carbonyl oxygens to coordinate a K^+ ion. A third K^+ ion holds the two [G **5**]$_8$ octamers together and a Cs^+ cation caps the structure. The G-quartets within [G **5**]$_{16}$·3K^+·Cs^+·4pic^- showed π–π stacking separations of 3.3–3.4 Å. In addition to stabilization by cations, four picrate anions form hydrogen bonds to N2 amino groups that extend from the two "central" G-quartets. The lipophilic G-quadruplex looks like a cation channel with an

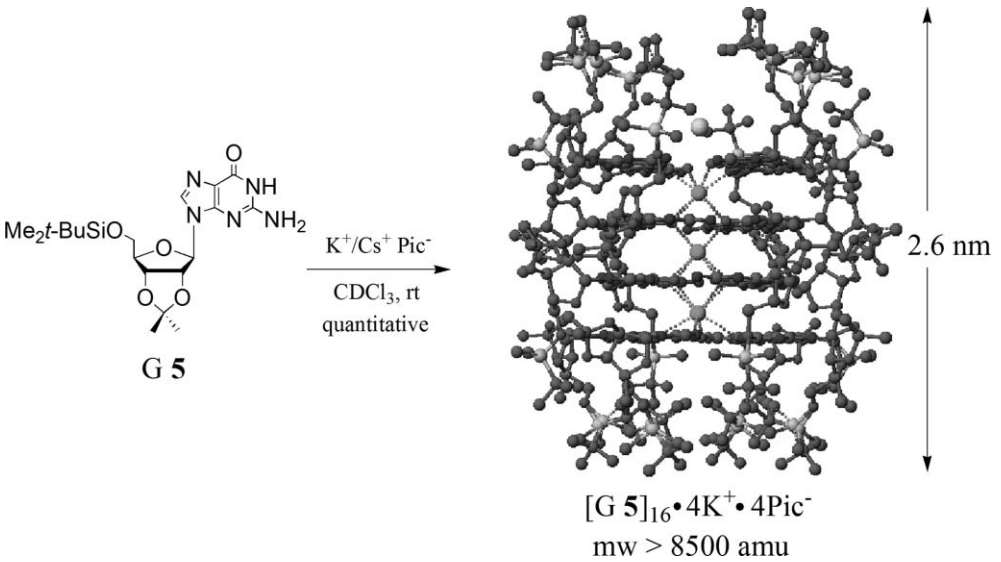


Fig. 7 Crystal structure shows that cation-templated self-assembly of 16 equiv. of G **5** gives a lipophilic G-quadruplex [G **5**]$_{16}$·3K^+/Cs^+·4Pic^-. (Adapted from reference 13.)

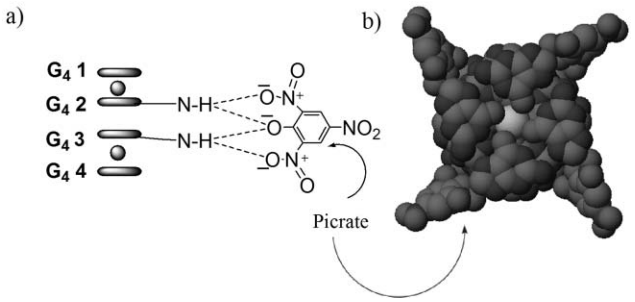


Fig. 8 a) A schematic showing the nucleobase–picrate hydrogen bonds in the hexadecamer [G **5**]$_{16}$·2Sr^{2+}·4Pic^{-}. b) Top view of the X-ray structure of the G-quadruplex with the sugars removed. Four picrate anions form an anionic belt around the G-quadruplex between G-quartet layers 2 and 3. (Adapted from reference 17.)

anionic belt wrapped around its middle (Fig. 8). Kaucher and colleagues used diffusion NMR to show that the hexadecamer [G **5**]$_{16}$·4K^{+}·4pic^{-} observed in the solid-state was also the major species in solution.[14] As described below, these lipophilic G-quadruplexes can be used as models for DNA G-quadruplexes and for the development of functional nanostructures.

In addition to the crystal structures, bound cations in these lipophilic G-quadruplexes have also been observed by solid-state NMR spectroscopy. Wu and colleagues used ^{23}Na and ^{39}K NMR to identify specific cations within the channels of these lipophilic G-quadruplexes.[15,16] This solid-state NMR work was an important development, as these lipophilic G-quadruplexes are reliable models that can clarify ambiguous issues about how Na^{+} and K^{+} bind to DNA G-quadruplexes. Furthermore, the identity of the bound cations also controls the solution properties of these lipophilic G-quadruplexes. For example, G-quadruplexes containing divalent cations such as Ba^{2+} or Sr^{2+} are thermodynamically and kinetically more stable than G-quadruplexes that contain monovalent Na^{+} or K^{+}.[17] Davis and colleagues attributed this enhanced stability in the presence of divalent cations to stronger ion–dipole interactions between the cations and the nucleobase oxygens, as well as to a strengthening of the G-quartet's hydrogen bonds.

Like the cations bound in the central channel of [G **5**]$_{16}$·4K^{+}, the phenolate anions bound to the surface of the lipophilic G-quadruplex also control the solution properties of these assemblies (Fig. 8). Both the pK_a and the structure of the phenolates influence the exchange rates for the bound cations and for the G subunits that make up the hexadecameric G-quadruplex. The rate of supramolecular isomerization of G$_8$ octamers in solution depends on the identity of the bound anions. Anions that hydrogen bond strongly to the central two G-quartets stopped subunit exchange in CD_2Cl_2, presumably by increasing the kinetic stability of the complex and making subunit dissociation difficult.[17] The studies showed that both the cation and anion influence the stability of these lipophilic G-quadruplexes. With the proper combination of cations and anions to stabilize these structures, Davis and colleagues showed that they could direct post-assembly modifications to only the outer quartet in these G-quadruplexes.[18]

2.1 Supramolecular structure with monomer building blocks

Guanosine is not the only nucleobase that can self-associate into discrete assemblies. IsoG **6** is an isomer of guanosine, differing by transposition of the nitrogen and oxygen atoms at the C2 and C6 positions. This minor positional change causes these G and isoG isomers to self-assemble into different arrangements. Whereas G derivatives form hydrogen-bonded quartets, cation templated self-assembly of isoG **6** leads to a decamer composed of two hydrogen-bonded isoG$_5$ pentamers sandwiching a Cs^{+} cation.[19] This difference in self-assembly pattern, and the resulting size of the hydrogen-bonded macrocycle, has been rationalized by considering the optimal hydrogen bonding geometries for the two nucleobases (Fig. 9).[4,19] For G, the donor and acceptor sites are located 90° relative to each other, an orientation that is optimal for formation of a cyclic tetramer. For isoG **6**, the 67° angle between the donor and acceptor edges is best for formation of a planar pentamer. The larger size of the isoG$_5$ pentamer, relative to the G$_4$ quartet, also explains the different ion binding selectivity shown by these derivatives. IsoG **6** is selective for coordinating the largest alkali cation, Cs^{+} (r = 1.67 Å), whereas G-quartets are K^{+} selective (r = 1.33 Å).[19]

Davis and colleagues conducted a "self-sorting" study in $CDCl_3$ to illustrate how the cation dictates the self-assembly patterns for G **5** and isoG **6**.[20] An equimolar mixture of the two isomers in $CDCl_3$, in the absence of cations, formed a mix of hydrogen-bonded species. Addition of Ba^{2+} to this mixture gave quantitative formation of two discrete hydrogen-bonded complexes, the G-quadruplex [G **5**]$_{16}$·2Ba^{2+} and the decamer [isoG **6**]$_{10}$·Ba^{2+} (Fig. 10). This self-sorting illustrated that a cation is needed to template formation of distinct assemblies in solution from this mixture of nucleosides. This experiment was a prime example of the equilibrium shifting that characterizes dynamic non-covalent chemistry.

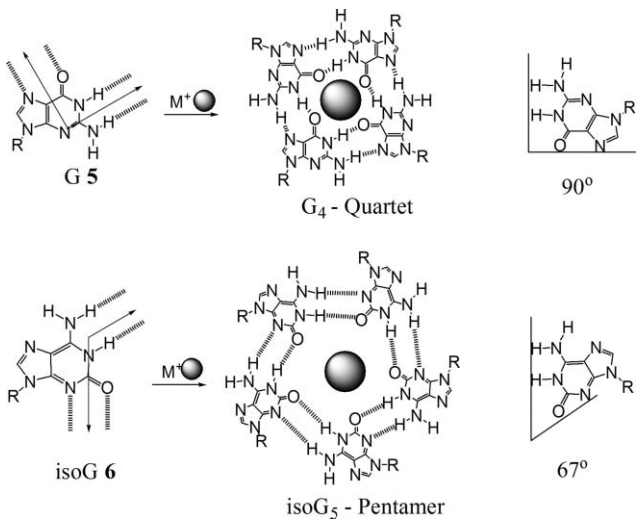


Fig. 9 Lipophilic nucleosides G **5** and isoG **6** self-associate in the presence of cations to give G$_4$-quartets or isoG$_5$-pentamers. The orientation of the nucleoside's hydrogen bond donor and acceptor groups determines assembly size. (Adapted from reference 4.)

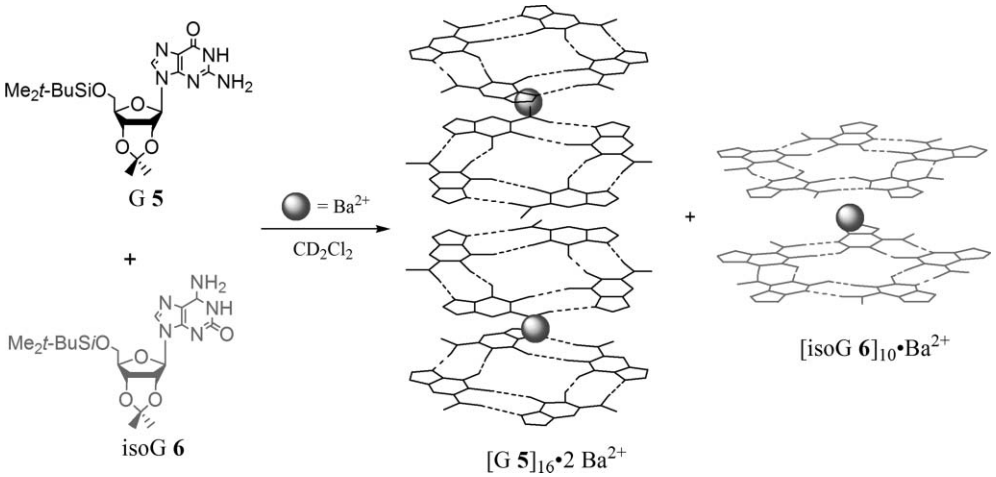


Fig. 10 G **5** and isoG **6** "self-sort" in the presence of cation to give discrete complexes, $[G\ \mathbf{5}]_{16}{\cdot}2Ba^{2+}{\cdot}4Pic^{-}$ and $[isoG\ \mathbf{6}]_{10}{\cdot}Ba^{2+}{\cdot}2Pic^{-}$. (Adapted from reference 20.)

2.2 Enantiomeric self-association of lipophilic nucleosides

The cation's control over self-assembly was also illustrated by the expression of supramolecular stereochemistry by these chiral nucleosides. The cation's identity (Ba^{2+} *vs.* K^{+}) had a significant influence on the diastereoselectivity in self-association of G **5**.[21] When K^{+} was added to a solution of racemic (D,L)-G **5** the resulting G-quadruplexes were a mixture of heterochiral diastereomers. The divalent cation Ba^{2+}, however, directed enantiomeric self-recognition of (D,L)-G (**5**), giving homochiral G-quadruplexes (Fig. 11). To explain this cation-dependent diastereoselectivity, Davis and colleagues proposed that the increased enthalpy inherent to the divalent cation–oxygen interaction must help overcome the unfavorable entropy associated with enantiomeric self-sorting.

Cation-templated self-association of isoG **6** is also highly diastereoselective. A crystal structure showed that (D,L)-isoG **6** undergoes enantiomeric self-recognition in the presence of Cs^{+}. The X-ray structure revealed that the "meso" decamer, $[(D)\text{-isoG}\ \mathbf{6}]_5{\cdot}Cs^{+}{\cdot}[(L)\text{-isoG}\ \mathbf{6}]_5{\cdot}Ph_4B^{-}$ had one $isoG_5$ pentamer unit composed of (D)-isoG **6** and the other pentamer contained only (L)-isoG **6** (Fig. 12).[22] This "meso" diastereomer was also the major species in solution. The X-ray structure showed that, within a hydrogen-bonded pentamer, each ribose formed sugar–base hydrogen bonds with its neighboring nucleoside. Davis and colleagues suggested that these intermolecular sugar–base hydrogen bonds efficiently transmit stereochemical information from one chiral sugar to its base-paired neighbor, thus leading to formation of a homochiral pentamer.

Lipophilic G-quartets might potentially be useful as chiral resolving agents. For instance, Gottarelli, Spada and colleagues showed that G-quartets formed from dG **7** are enantioselective in their ability to extract chiral anions from water into

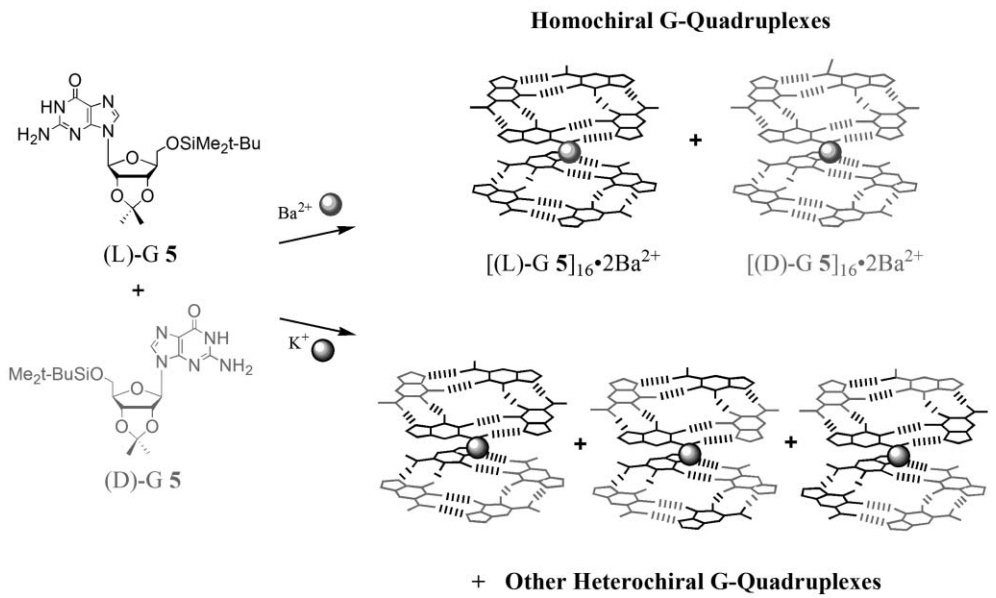


Fig. 11 G **5** undergoes enantiomeric self-association. Racemic (D,L)-G **5** self-assembles in the presence of Ba^{2+} to give homochiral G-quadruplexes $[(D)\text{-G}\ \mathbf{5}]_{16}{\cdot}2Ba^{2+}{\cdot}4Pic^{-}$ and $[(L)\text{-G}\ \mathbf{1}]_{16}{\cdot}2Ba^{2+}{\cdot}4Pic^{-}$. Addition of K^{+} to G **5** gave heterochiral assemblies. (Adapted from reference 21.)

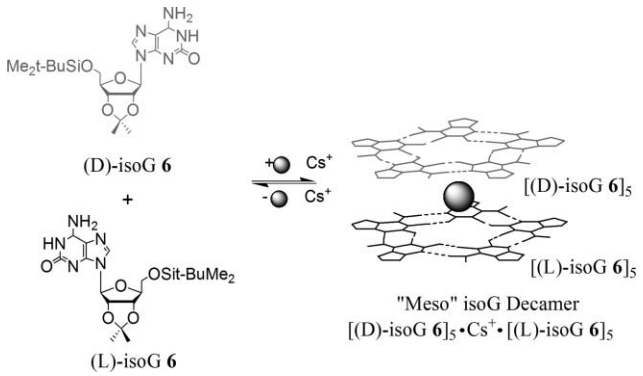


Fig. 12 Addition of Cs^+ to racemic (D,L)-isoG **6** resulted in formation of homochiral hydrogen-bonded pentamers through enantiomeric self-association. The major species is a "meso" decamer, [(D)-isoG **6**]$_5$·Cs^+·[(L)-isoG **6**]$_5$. (Adapted from reference 22.)

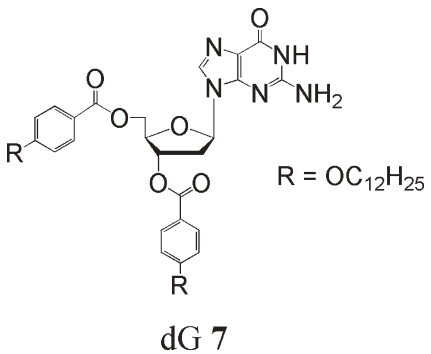


Chart 1

organic solution. Thus, dG **7** extracted a K^+ *N*-dinitrophenyl-(L)-tryptophan salt from water into $CDCl_3$ with a 3 : 1 enantioselectivity over the (D)-Trp enantiomer, indicating significant interactions occur between these anions and the chiral G-quadruplex.[23]

2.3 Self-assembled ionophores as selective metal ion extractants

These lipophilic G and isoG assemblies also show promise for effecting separations of environmentally important cations. A major challenge in nuclear waste remediation involves separating radioactive $^{137}Cs^+$ from the large excess of Na^+ and K^+ in solution. Davis and colleagues have shown that isoG **6** is highly selective for binding and transporting Cs^+.[19] Although [isoG **6**]$_{10}$·Cs^+ is thermodynamically stable, it readily exchanges its bound cation with free cations in solution, a key point for developing a practical extractant that can be used in waste remediation. Thus, the Cs^+ guest in [isoG **6**]$_{10}$·Cs^+ exchanges with "free" Cs^+ in solution approximately 40,000 times faster than isoG **6** ligand exchange with the complex (Fig. 13).[24] These different exchange rates indicate that the isoG decamer does not dissociate during the cation exchange process.

Another radioactive ion of environmental concern is the cancer causing $^{226}Ra^{2+}$, a naturally occurring species. The Reinhoudt and Davis groups recently reported that the self-assembled decamer [isoG **6**]$_{10}$ extracts $^{226}Ra^{2+}$ with high

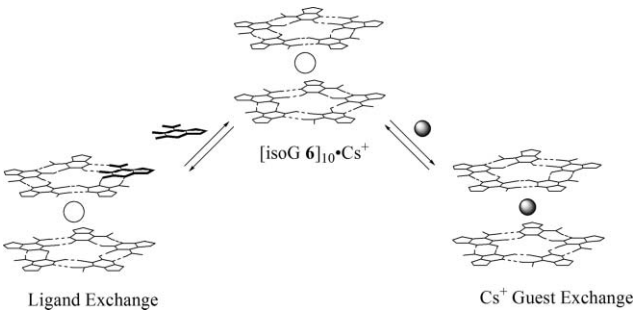


Fig. 13 Dynamic exchange in [isoG **6**]$_{10}$·Cs^+. Both the isoG ligand **6** and the cationic guest Cs^+ exchange with free species in solution. NMR data shows that Cs^+ exchange is over 40,000 faster than ligand exchange of isoG **6**. (Adapted from reference 24.)

selectivity and affinity from both simulated and real wastewater.[25] Even in the presence of higher concentrations of other alkaline earth metals, isoG **6** showed a remarkable selectivity for binding radium ion (>10,000 to 1 for $^{226}Ra^{2+}$ *vs.* Ba^{2+}).

2.4 "Empty" G-quartets

In the absence of the appropriate templating cation, guanosine analogs usually form hydrogen-bonded dimers or ribbons. But, not always. Sessler and colleagues synthesized a G analog **8** that self-associates into an "empty" G-quartet without the assistance of a cation template.[26] Attachment of a dimethylaniline moiety to the guanine C8 position gave a conformationally constrained nucleoside that adopts a *syn* glycosidic bond conformer in the solid state and in solution. This *syn* conformation prevents the nucleoside from forming a hydrogen-bonded ribbon and ensures G-quartet formation (Fig. 14). This study showed how synthetic chemistry could be used to produce unnatural nucleobases for the non-covalent synthesis of stable supramolecular assemblies. The use of designer bases to build discrete assemblies is clearly important in supramolecular chemistry and nanoscience.

Kotch *et al*. also showed that a calixarene–guanosine analog forms a hydrogen-bonded dimer (cG **9**)$_2$·$(H_2O)_n$ in wet $CDCl_3$, with water filling the G-quartet's central cavity. This water-filled dimer serves as a ditopic salt receptor, able to bind a cation with the G-quartet and an anion with the nearby amide NH groups (Fig. 15).[27] This observation was entirely consistent with a prediction, made by Gellert in his original 1962 paper, that a G-quartet "*...would contain a hole in the middle in which it might be possible to place one water molecule*".[1]

Recently, Besenbacher, Otero and colleagues showed that guanine (G **10**) is able to adopt a kinetically stable "empty" G-quartet when placed on a gold surface (Fig. 16).[28] STM measurements showed that this empty G-quartet was not the thermodynamic minimum, as annealing the deposited G-quartet network led to rearrangement into a hydrogen-bonded ribbon. In the case of G **10**, the available N9-H and the neighboring N3 positions may be crucial for stabilizing the network of connected G-quartets. This paper is, to our knowledge, the first demonstration that guanine itself forms cyclic quartets, as other G-quartets have always involved N9-substituted G nucleobases.

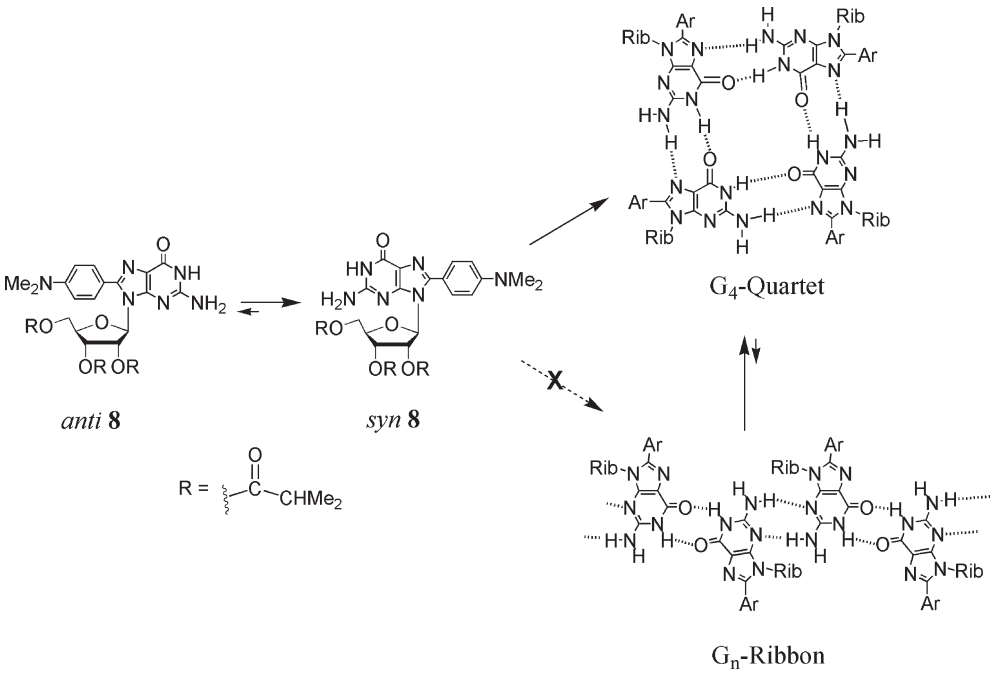


Fig. 14 Conformationally constrained G **8** forms a G-quartet without a cation.

2.5 New hydrogen-bonded assemblies from other nucleoside analogs

Rivera and coworkers have demonstrated another approach toward stabilizing G-quartets by using 8-aryl-dG analogs such as dG **11**.[29] By adding a hydrogen-bond acceptor to the C8 position, they succeeded in involving the exocyclic N2 amino hydrogen that does not normally participate in G-quartet hydrogen bonding (Fig. 17).

Variable temperature and dilution NMR experiments on the G-quadruplex $[dG\ \mathbf{11}]_{16}\cdot 3K^+$ showed increased stability when compared with assemblies formed from unsubstituted G derivatives. Rivera proposed that the stability of G-quartets formed from this 8-aryl-dG analog **11** was due to three factors. First, C8 substitution forces dG **11** into the *syn* glycosidic conformation, prohibiting formation of hydrogen bonded ribbons. Second, the additional aromatic rings attached to C8 provide a larger surface for stronger π–π interactions

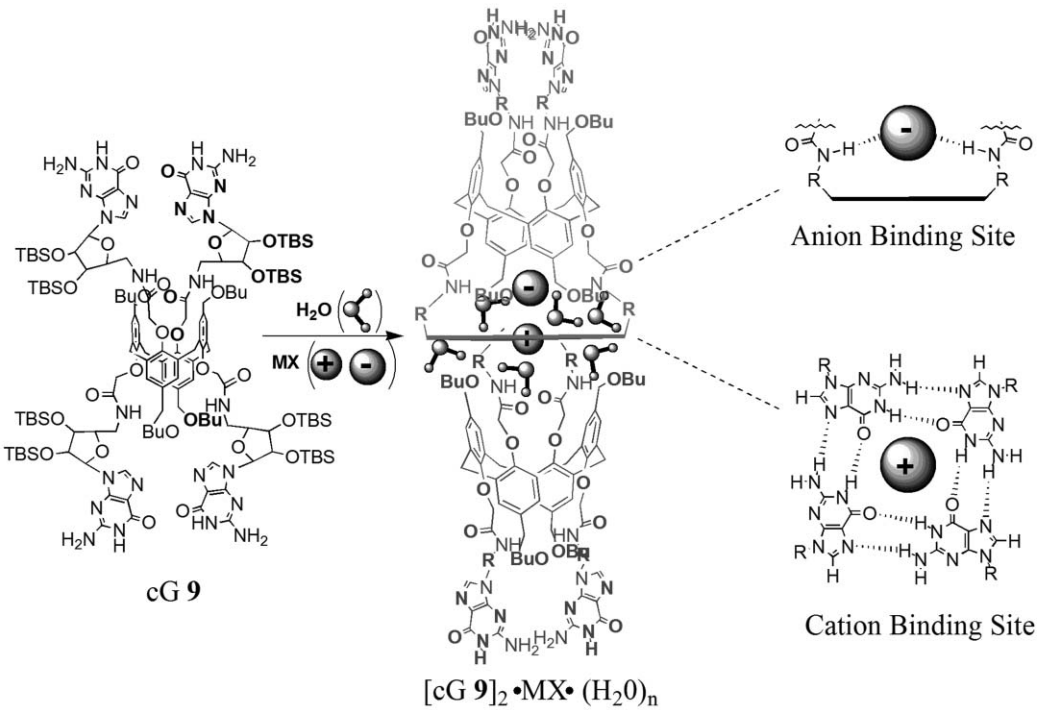


Fig. 15 A schematic of $[cG\ \mathbf{9}]_2\cdot MX\cdot(H_2O)_n$ showing anion and cation binding sites. (Adapted from reference 27.)

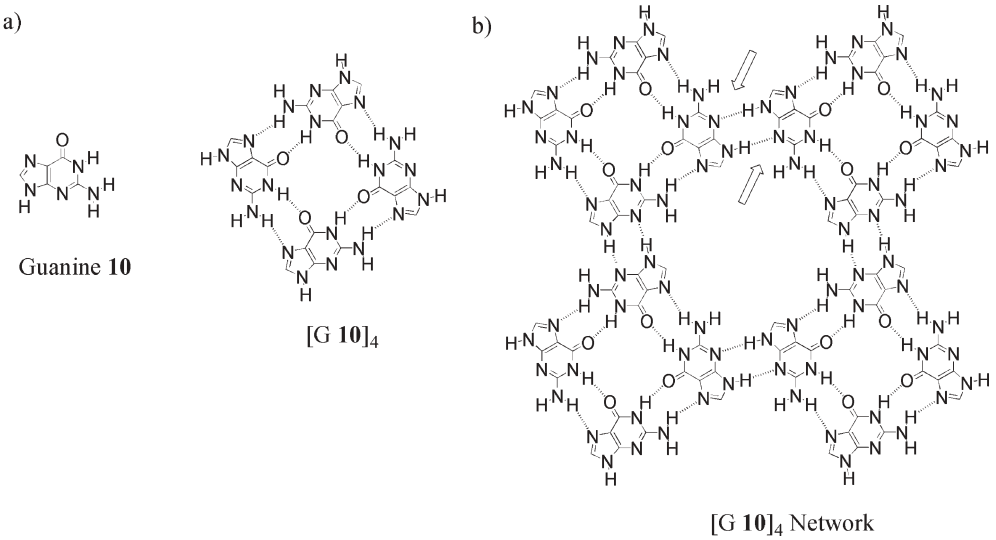


Fig. 16 (a) An empty G-quartet formed by guanine **10**. (b) A hydrogen bound network of empty G-quartets. Each G-quartet can form up to eight additional hydrogen bonds with neighboring G-quartets (arrows). (See reference 28.)

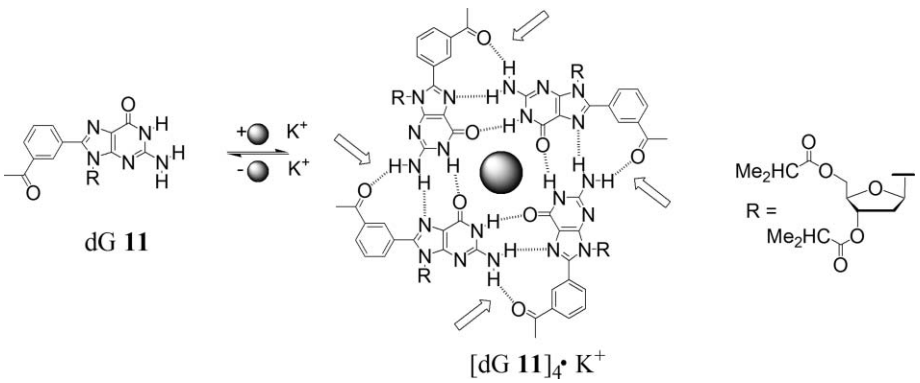


Fig. 17 A G-quartet formed from dG **11**, a modified nucleobase with an expanded Hoogsteen hydrogen bonding face. (See reference 29.)

between stacked G-quartets. Finally, the C8 substituent in dG **11** enables four additional hydrogen bonds per G-quartet, as illustrated in Fig. 17.

Sessler and coworkers have synthesized a guanosine–cytidine dinucleoside **12** that self-associates into a cyclic trimer in organic solvents (Fig. 18).[30] They used the potent GC hydrogen-bonding motif to direct assembly formation. An ethylene bridge separates the guanosine and cytidine moieties in **12** and preorganizes these groups for formation of the macrocycle *via* three GC basepairs. This well-defined supramolecular structure may find use in the construction of self-assembled dendrimers and other nanostructures.

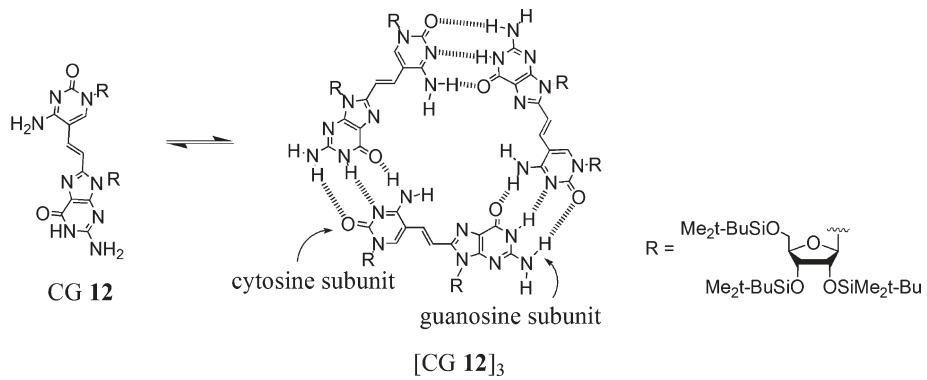


Fig. 18 Self-assembly of lipophilic dinucleoside CG **12** into cyclotrimer [CG **12**]$_3$.

2.6 The G-quartet and dynamic covalent chemistry

Dynamic covalent chemistry (DCC) is a major strategy in supramolecular chemistry, enabling amplification of selected compounds from a dynamic combinatorial library (DCL) of equilibrating compounds.[7,31] In the DCC approach, building blocks that form reversible covalent bonds are used to build a DCL. Stabilization of a particular library member upon addition of a template shifts the equilibrium, amplifying any stabilized products in the mixture.

The DCC strategy has been used to produce small molecule ligands that bind to DNA G-quadruplexes. Previous studies have shown that (i) acridone ligands (A) stack on the terminal G-quartet of a G-quadruplex and that (ii) various peptides (P) interact with the grooves formed by the tetraplex backbone. Balasubramanian and colleagues used a disulfide exchange reaction, with glutathione disulfide and a G-quadruplex template, to identify novel G-quadruplex binders that combine both the acridone and peptide recognition units.[32] Disulfide exchange can be carried out in water under reversible conditions at moderate pH, but the reaction is quenched with acid to determine the composition of products. Using an oligonucleotide of sequence 5′-biotin$(GTTAGG)_5$, that contains the human telomere sequence, as a template, Balasubramanian showed a 400% increase in formation of a heterodimeric disulfide AssP, a compound containing the acridone (A **15**) and peptide (P **16**) domains (Fig. 19). In addition, the authors discovered that a peptide dimer PssP was formed in 5-fold greater amount in the presence of the G-quadruplex. Surface plasmon resonance measurements showed that the complexes formed by these ligands and the human telomere G-quadruplex had dissociation constants of K_d = 30 and 22.5 μM for AssP and PssP. These K_d values are much lower than the dissociation constant for the AssA0–DNA complex ($K_d > 2.5$ mM). This study established that the DCC approach could identify new G-quadruplex ligands, a potentially important endeavor in the search for potent telomerase inhibitors.

In addition to the discovery of new ligands that interact with tetraplex structures, the DCC concept has also been used by the Balasubramanian, Lehn, Spada and Davis groups to form new G-quadruplexes, each with its own unique properties.[7,33–36] Balasubramanian and colleagues reported that a G-rich PNA, modified so as to allow for covalent bond formation between individual strands, underwent a ''self-templation'' process to form a bimolecular G-quadruplex.[33] They demonstrated that formation of the non-covalent PNA G-quadruplex preceded covalent bond formation. They first showed that an equimolar mixture of Lys-TGGG-GlyGlyCys-SH (G_S) and Lys-TTTT-GlyGlyCys-SH (T_S) gave a 1 : 2 : 1 statistical mixture of the 3 possible disulfides $G_{SS}G$, $G_{SS}T$ and $T_{SS}T$ when oxidized with sodium perborate (Fig. 20). In contrast, air oxidation of a mixture of the same 2 PNA strands gave a 2 : 1 : 2 ratio of $G_{SS}G$, $G_{SS}T$ and $T_{SS}T$ indicating that $G_{SS}G$ was stabilized under these particular conditions. Both mass spectrometry and UV melting experiments indicated that the $G_{SS}G$ dimer formed a bimolecular G-quadruplex $(G_{SS}G)_2$, presumably a bimolecular hairpin wherein the Gly-Cys-Cys-Gly tetrapeptide forms the loops. Other measurements indicated that the G_S PNA strands were preorganized into a G-quadruplex prior to formation of the disulfide bond that gave the $G_{SS}G$ product. Formation of the $G_{SS}G$ disulfide

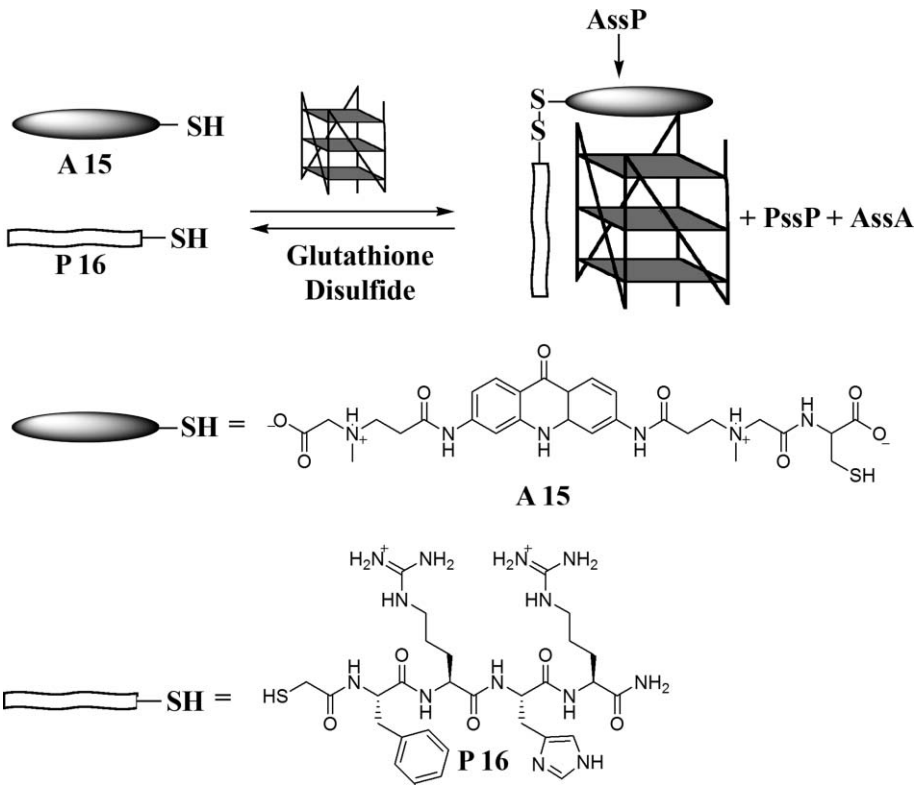


Fig. 19 The AssP disulfide product is amplified in the presence of a G-quadruplex template. (See reference 32.)

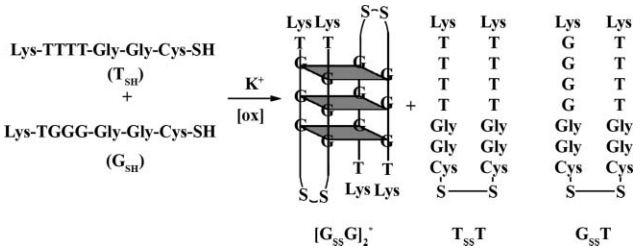


Fig. 20 Oxidation of the PNA strands T_{SH} and G_{SH} provides disulfides. In the presence of K^+, $G_{SS}G$ is amplified. (Adapted from reference 33.)

depended strongly on the template, being most effective with K^+, the cation that can best stabilize a G-quadruplex.

Lehn and Sreenivasachary described a G-quartet system wherein component selection from a DCL is driven by the physical properties of the product.[7] They showed that guanosine hydrazide **2** formed thermally reversible gels at moderate pH in the presence of both Na^+ and K^+. These gels presumably are formed by the stacking and crosslinking of G-quartets. The 5′-hydrazide in the G-quartet gels was reacted with a library of aldehydes to form acylhydrazone bonds, allowing the authors to study the effects of sidechain modification on gel properties. While addition of some aldehydes destroyed the hydrogels, other aldehydes (including **17**) formed acylhydrazone gels that were stronger than the parent gel formed from hydrazide G **2**. These findings prompted Lehn and Sreenivasachary to determine whether the thermodynamic stability of the gel phase might actually drive the component selection in their DCL (Fig. 21). Thus, a mixture composed of 4 acylhydrazones, formed from reaction of aldehydes **17** and **18** with hydrazides G **2** and serine **19**, was generated under conditions where the 5′-acylhydrazones could equilibrate by undergoing reversible bond cleavage and reformation. The product mixture, measured by ^1H NMR, was sensitive to temperature. At 80 °C, above the gel transition temperature, the distribution of products was statistical, indicating that the 4 acylhydrazones (A–D) were of similar stability. Between 25–55 °C, acylhydrazone B, in its gel-state, and C in solution were favored over acylhydrazones A and D. In this case, self-assembly of G hydrazide **2** was driven by selection of the components that gave the most stable hydrogels. The stability of the G-quartet hydrogel altered the dynamic equilibrium of acylhydrazones and directed reaction of the G hydrazide **2** with aldehyde **18**. Lehn explained that *"…(t)he process amounts to gelation-driven self-organization with component selection and amplification…based on G-quartet formation and reversible covalent connections."* This DCC approach may well have broad applications in medicinal chemistry and material science.

Ghoussoub and Lehn also recently described another dynamic sol–gel interconversion process, triggered by the reversible binding and release of K^+ by a G-quartet hydrogel.[34] Hydrogels formed by the ditopic monomer G-G **20** were converted to soluble $(G\text{-}G)_n$ polymers upon addition of [2.2.2]-cryptand **21**, an ionophore that extracts K^+ from the G-quartet hydrogel. The gel was regenerated upon expelling K^+ from the [K^+ 2.2.2]-cryptate by protonation of the cryptand's bridgehead nitrogen to give [$2H^+$ 2.2.2] **21**. In this way, gel–sol interconversion was triggered over multiple cycles by controlling the equilibrium of the bound K^+ between the G-quartet and the [2.2.2] cryptand (Fig. 22). In a related system, Spada and colleagues demonstrated a strategy for switching between two distinct supramolecular motifs in organic solvents.[35] By modulating the protonation state of the K^+ ionophore, 2.2.2 cryptand, they also showed that they could stabilize either a hydrogen-bonded ribbon or a discrete K^+ G_8-octamer. The combination of CD spectroscopy and ^1H NMR spectroscopy was particularly compelling in establishing equilibrium shifting between the distinct species.

In another example of the power of the DCC approach, Davis and colleagues described a unimolecular G-quadruplex **23** that functions as a transmembrane Na^+ transporter.[36] The particular strategy combined non-covalent synthesis and post-assembly modification of a non-covalent G-quadruplex.

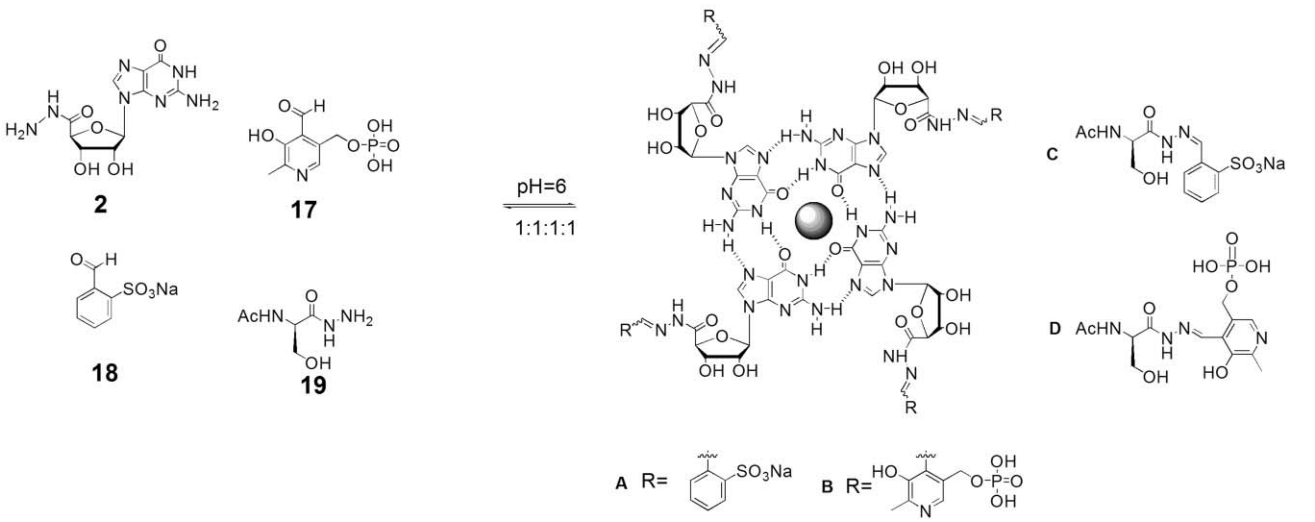


Fig. 21 Stability of G-quartet hydrogel B alters equilibrium of acylhydrazones and directed reaction of G hydrazide **2** with aldehyde **18**. (Adapted from reference 7.)

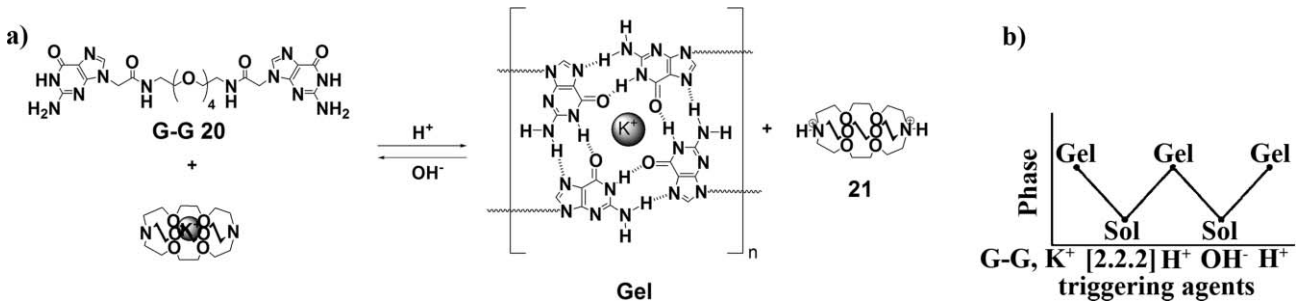


Fig. 22 (a) Structure of G-G **20** and schematic of the reversible formation of polymeric G-quartet based hydrogels. Changing pH in the presence of [2.2.2 cryptand] **21** modulated the sol–gel equilibrium. (b) Modulation of the gel–sol status induced by the sequence of triggering agents. (Adapted from reference 34.)

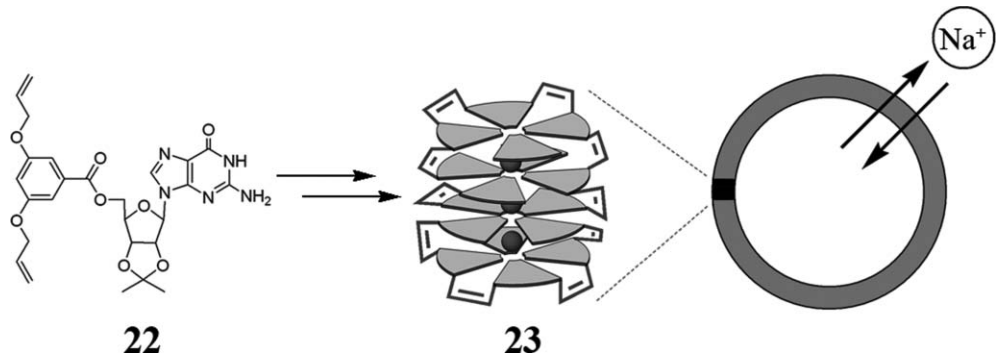


Fig. 23 Olefin metathesis was used to cross-link sub-units in the lipophilic guanosine **22**. The resulting unimolecular G-quadruplex **23** was shown to transport Na^+ ions across phospholipid bilayer membranes. (Adapted from reference 36.)

Reversible olefin metathesis was used to cross-link subunits that had been preorganized within a G-quadruplex (Fig. 23).

The precursor, 5′-(3,5-bis(allyloxy)benzoyl)-2′,3′-isopropylidene G **22** was substituted with two meta-allyl ethers to enable olefin metathesis to be carried out within an individual G-quartet and between G-quartet layers. Mass spectrometry, NMR and CD spectroscopy, confirmed that G **22** formed a hexadecameric G-quadruplex $[G\ \mathbf{22}]_{16}{\cdot}4K^+{\cdot}4DNP^-$. Olefin metathesis of this non-covalent G-quadruplex (8 mM), using Grubb's second-generation catalyst, resulted in a high yield of metathesis product **23**. This unimolecular G-quadruplex **23** apparently folds into a conformation that allows transport of Na^+ cations across phospholipid bilayer membranes. Evidence for the ability of the lipophilic G-quadruplex **23** to transport Na^+ across phospholipid liposomes was obtained using ^{23}Na NMR spectroscopy. The Matile and Kato groups have also shown that rosettes prepared from lipophilic folate **24**, structures closely related to the G-quartet, also function as synthetic ion channels (Fig. 24).[37]

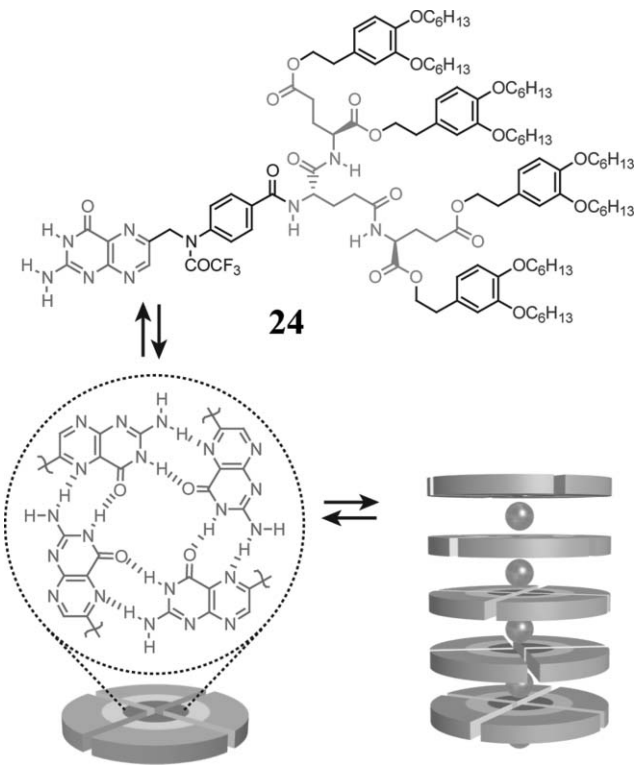
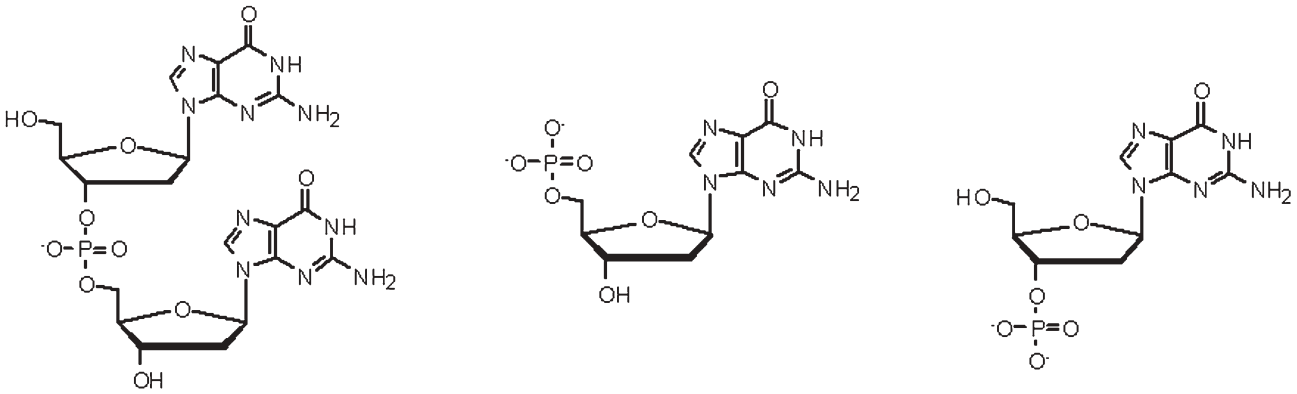


Fig. 24 A synthetic ion channel formed by self-association of folate derivative **24**. The folic acid tetramer is similar to a G-quartet. (Reproduced with permission from reference 37. Copyright 2006 American Chemical Society.)

3 Lyotropic liquid crystal formation from guanosine derivatives

3.1 Alkali metal salts of guanylates in water

While the ability of guanosine derivatives to form gel-like structures has been known since the early twentieth century,[2–4] evidence for liquid crystalline (LC) phases in solution were reported only at the end of the 1980's by Gottarelli and Spada as a consequence of a fortuitous observation.[3,38] They found



dG2 **25** dG **26** dG **27**

Chart 2

that a 5% w/w aqueous solution of the sodium salt of 2′-deoxyguanylyl-(3′-5′)-2′-deoxyguanosine (dG_2 **25**), prepared for a routine ^{1}H NMR experiment was highly viscous and liquid crystalline. The NMR spectrum was, in fact, dominated by the resonance corresponding to water (if the laboratory had been equipped with a better performing NMR spectrometer, the experiment would have been done at much lower concentration, in isotropic solution, and the lyotropic behavior of dG_2 **25** would not have been noticed!). This compound exhibits a cholesteric and a hexagonal phase with the following transition concentrations (w/w at room temperature): isotropic – 2.5% – cholesteric – 18% – hexagonal. In the following years, the lyomesomorphism of many other guanylic nucleotides (including 5′-dGMP dG **26** and 3′-dGMP dG **27**) and G-rich oligonucleotides was described.[39] Typically, cholesteric (chiral nematic) and hexagonal phases are formed upon self-assembly of these G derivatives.

How can formation of aqueous lyotropic mesophases from small molecules such as **25–27** be explained? It was known that several biopolymers, including DNA,[40] show LC phases in water. The formation of DNA LC has been interpreted as follows: the DNA double helix can be assimilated into a rod with a hydrophilic surface and a lipophilic core. These elongated objects are chiral and can self-correlate with a cholesteric or a hexagonal order, depending on the water content (see Fig. 25).

The texture of guanosine mesophases obtained in polarizing optical microscopy (POM) is reminiscent of the mesophases observed for DNA. However, guanosines dG_2 **25**, dG **26** or dG **27** are neither polymers nor long anisometric molecules like

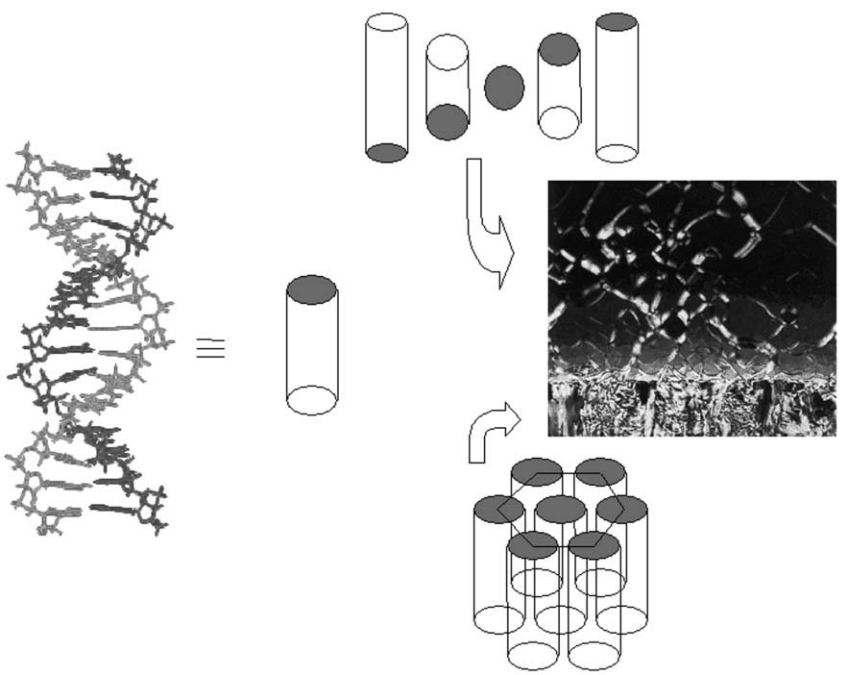


Fig. 25 Lyotropic liquid crystals from DNA fragments.

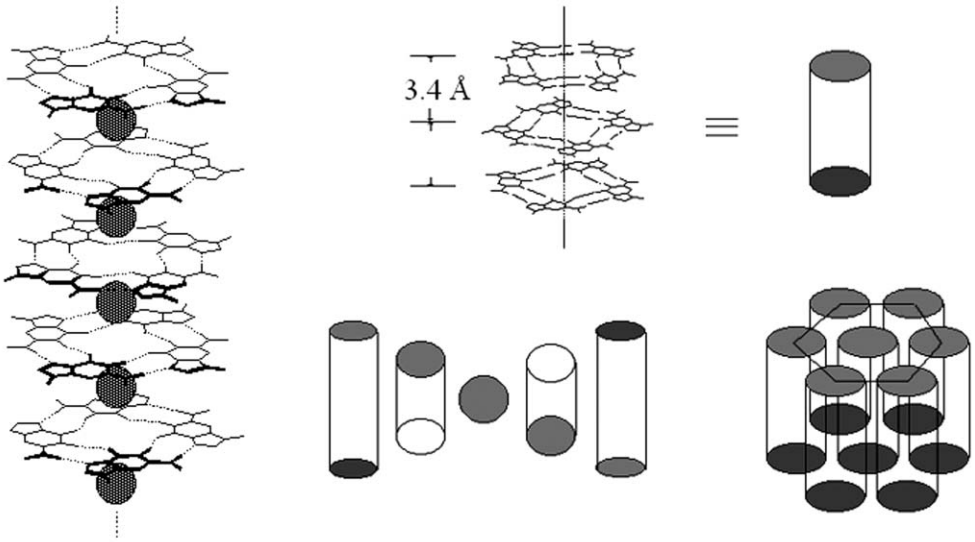


Fig. 26 Lyotropic liquid crystals from self-assembled guanosines.

DNA. The lyotropic mesomorphism is a consequence of the formation of a self-assembled structure, wherein the basic structure is a chiral columnar aggregate based on G-quartets held together by non-covalent interactions. The G-quartets are piled up one on top of the other at the van der Waals distance and the cations are sandwiched between them (see Figs. 2 and 26). Stacking and ion–dipole interactions stabilize these supramolecular structures, which have a hydrophilic surface and a lipophilic core, even without any covalent bridges between the adjacent G-quartets. As a consequence of the intrinsic chirality of the nucleotide compounds, the stacking is not in register. Instead, each G-quartet is rotated with respect to the adjacent G-quartet layers.

Depending on the concentration, temperature, and amount of salts added, these aggregates self-correlate to generate the mesophases of either the cholesteric or hexagonal type. The cholesteric phase can be easily aligned with a magnetic field to give a fingerprint or planar POM texture without unwinding the cholesteric helix that is oriented parallel to the applied field (Fig. 27). This magnetic behavior indicates that the objects composing the phase have negative diamagnetic anisotropy, as expected for rod-like aggregates with their aromatic planes perpendicular to the long axis.[39]

Low angle X-ray diffraction work confirmed the assignment of the phases detected from the optical microscopy. In particular, in the high angle region of the XRD pattern a sharp peak corresponding to the periodicity of 3.4 Å, typical of stacked aromatic systems, is present. Electron density maps have been calculated and they support the existence of a G-quartet based system.[39]

The self-assembly process for guanosine derivatives, including mesophase formation, can easily and conveniently be followed by circular dichroism spectroscopy (CD). Spectra of isolated species are usually drastically different from those of

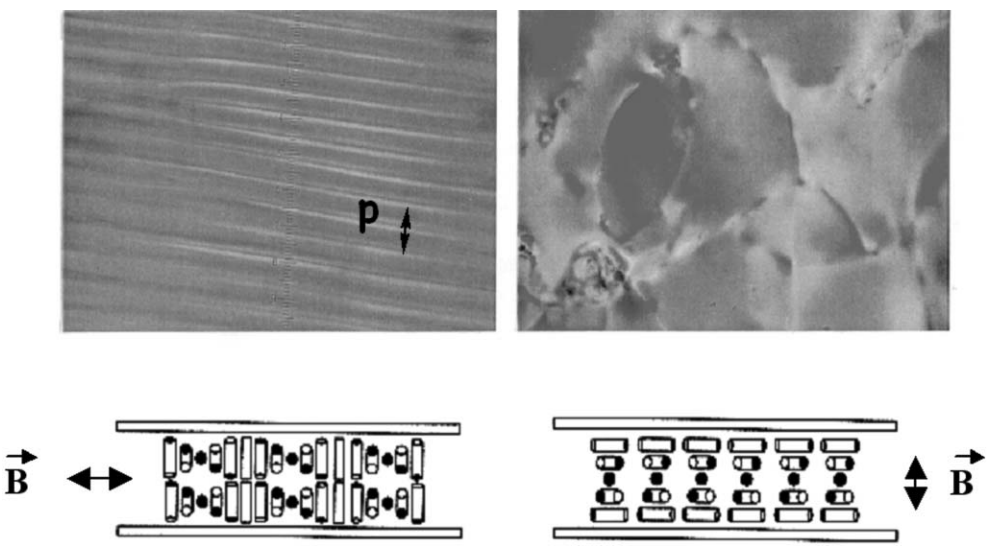


Fig. 27 Fingerprint (left) and planar (right) textures from a cholesteric aqueous solution of dG2 **25** (ammonium salt). (Adapted from reference 39.)

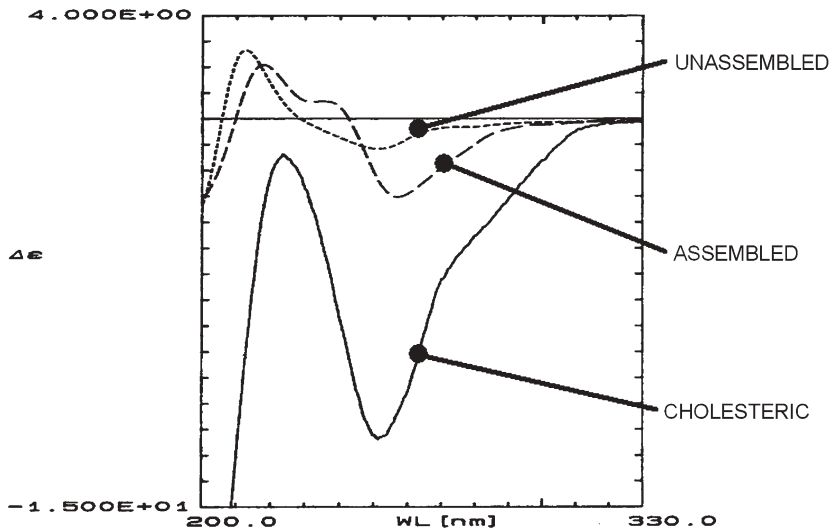


Fig. 28 CD spectra of an aqueous solution of dG **27** (ammonium salt) in different self-organized states: unassembled (a), isotropic G-quartet based columnar state (b), cholesteric (c). (Adapted from reference 42.)

the assembled species and of the cholesteric phases.[41] In Fig. 28 the case of dG **27**, whose assembly process is driven by temperature, is presented as an example.[42] At 30 °C the spectrum of the unassembled molecule dominates. At lower temperature (5 °C) an exciton couplet is observed corresponding to guanine's main absorption band. The particular sequence of the oppositely signed bands (negative–positive) can be related to a left-handed stacking of adjacent G-quartets. At 1° C the solution is cholesteric and an intense signal appears; this signal's negative sign is indicative of a left-handed phase. Therefore, from CD spectroscopy, we can determine the handedness of the chiral columnar aggregate and of the cholesteric phase.

3.2 Lipophilic guanosine assemblies templated by ions

Lipophilic columnar G-quadruplex structures give liquid-crystalline phases in organic solvents just as the ordinary G-rich oligonucleotides do in water, as confirmed by both POM and X-ray measurements. In particular, X-ray diffraction confirms the columnar nature of both phases with a characteristic stacking repetition of 3.4 Å. The cholesteric phase may be aligned with magnetic fields (Fig. 29 c,d) and its magnetic behavior is analogous to that observed for the hydrophilic guanosines.[43] This result may seem obvious, but is instead surprising considering the subtle contributions of different intermolecular forces in the formation of lyotropic phases. Subsequently, Kato and coworkers found that lipophilic folic acid derivatives, which also form hydrogen-bonded tetrads, could also give liquid crystalline phases under the appropriate conditions.[44]

3.3 Lipophilic guanosine assemblies not templated by ions

As anticipated from the discussion in Section 2 of this tutorial review, lipophilic guanosine derivatives self-assemble into linear ribbon-like motifs in the absence of alkali cation templates (Fig. 5). These ribbon structures in solution were identified mainly by NMR[45] and, in the solid state, by single crystal X-ray diffraction.[9] Lipophilic guanosines are able to form lyotropic mesophases in several solvents. For example, dG **3** in hexadecane gives, above a critical concentration, a viscous birefringent (LC) phase. X-ray diffraction measurements gave narrow Bragg reflections whose reciprocal spacing is indicative of a two-dimensional square packing of extended hydrogen-bonded elements with the alkyl chains and solvent molecules filling the lateral gap between the tapes (Fig. 30).[9]

Araki and coworkers recently introduced non-polar and flexible alkylsilyl groups into 2-deoxyguanosine, obtaining dG

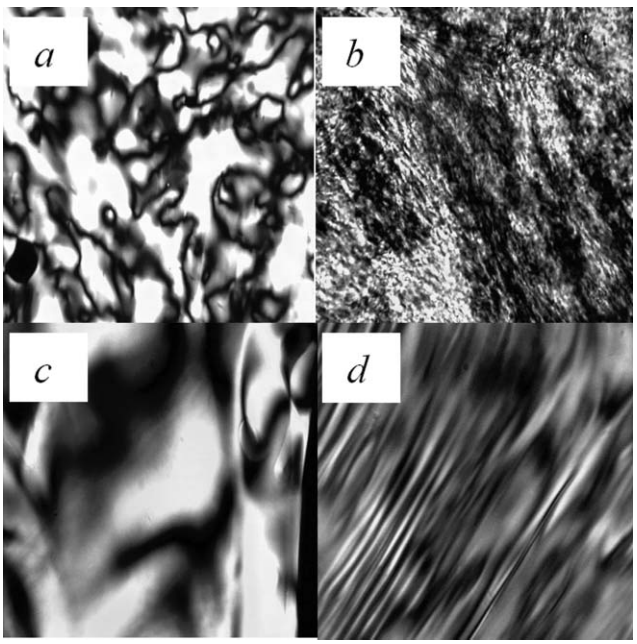


Fig. 29 POM textures of a cholesteric (10% w/w) (a, not aligned; c, magnetic field perpendicular to the cell wall; d, magnetic field parallel to the cell wall) and (b) of a hexagonal solution (20% w/w) of (dG **3**)·4KPic in heptane. (Adapted from reference 43.)

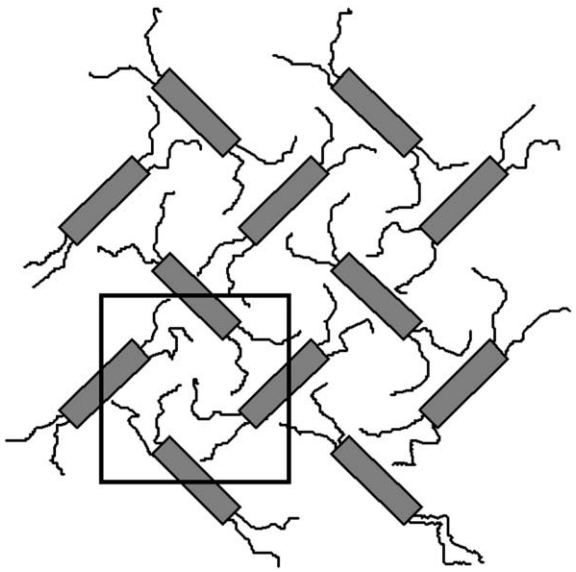
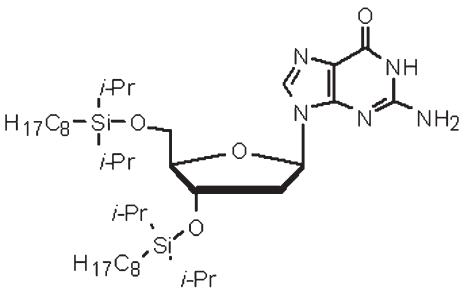


Fig. 30 A model for the square LC phase of dG **3** in hydrocarbon solvents. (From reference 9.)



dG **28**

Chart 3

28 as an efficient organogelator for alkanes.[46] From an in-depth structural analysis, Araki concluded that the basic structure of these gels is a sheet-like assembly. This supramolecular structure, as sketched in Fig. 31, is composed of anti-parallel G-ribbons of type A (Fig. 5) with additional double inter-tape hydrogen bonds between NH2 and N3 of two guanine units located in adjacent ribbons. The gel-to-liquid crystal phase transition for these organogels from dG **28**, triggered by heating, has been observed and this transition was shown to be due to the selective cleavage of the inter-tape hydrogen bonds pictured in Fig. 31.

4 Self-assembled guanosine derivatives in molecular electronics

(Bio)molecular electronics is gaining an increased interest worldwide due to the appealing possibility of realizing cheap and easy-to-fabricate devices that exploit the self-assembly, self-recognition and self-repairing capability of engineered organic or bio-inspired molecules. Self-assembling guanosines are, therefore, promising candidates for fabrication of electronic nanodevices. A Scanning Force Microscopy (SFM) image of a dried nanoribbon formed from self-assembly of dG **7**, located on the basal plane of a mica substrate, is shown in Fig. 32. The width of the ribbon, around 6.2 nm, is consistent with its proposed supramolecular structure.[45]

Fig. 33 shows, at a quasi-molecular resolution, a Scanning Tunneling Microscopy (STM) image (at the graphite/solution interface) of closely packed arrays of H-bonded ribbons formed by self-assembly of dG **7**. As depicted, these

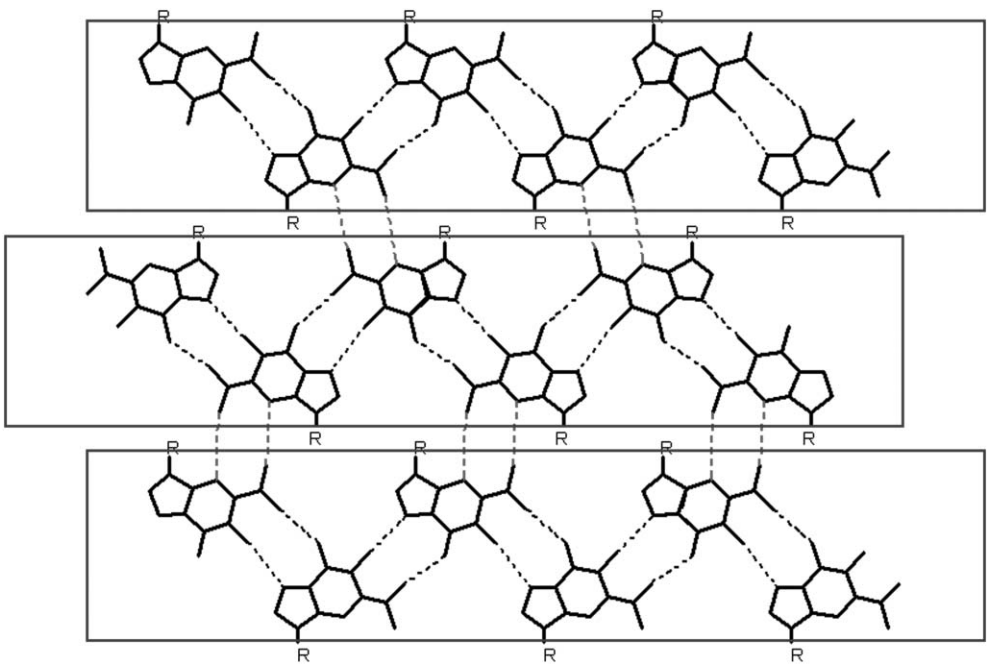


Fig. 31 Two-dimensional H-bonded sheet of guanine moieties. The boxes highlight the guanine ribbons A (see Fig. 5) connected by H-bonds.

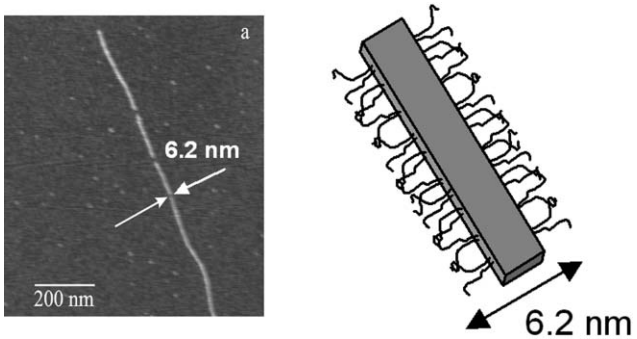


Fig. 32 SFM picture of a nanoribbon of dG **7**. (Adapted from reference 45.)

nanoribbons interdigitate. The unit cell dimension b perfectly matches that of the type A ribbon found in single crystal X-ray analysis.[45]

Cingolani and Rinaldi have proposed the use of nanoribbons formed from dG **7** guanine units in the design of molecular electronic nanodevices.[10] Self-assembled nanoribbons obtained from drop casting were used to interconnect gold nanoelectrodes fabricated by electron beam lithography (Fig. 34). The typical length of the oriented arrays of ribbons (a "nanocrystal") was approximately 100 nm.

For a contact gap of 60 nm or less only one nanocrystal of the dG **7** assembly is probed. Under these conditions the plot of current intensity *vs.* voltage (*I–V*) shows a clear diode-like

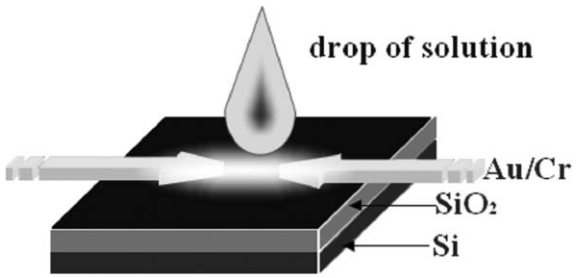


Fig. 34 The preparation of the nanodevice. (Adapted from reference 10.)

behavior (Fig. 35 a), with currents on the order of μA for positive bias and nA for negative bias. This rectifying feature points out the existence of the strong dipole in each nanocrystal that originates from the dipole of the guanine units ordered in the ribbon-like structure of type A (see Fig. 5). If a three-terminal device is prepared, the system behaves as a "Field Effect Transistor" when the guanosine nanoribbons are used to interconnect the drain and source terminals.[47]

A major challenge is to orient this material between the electrodes. In fact, with the drop casting procedure, there is no control on the orientation of the nanocrystals with respect to the nanocontacts. Some devices rectify in one direction, others in the opposite direction, and other devices do not rectify at all. The situation changes dramatically in the 120 nm device (Fig. 35 b). In this case, a few nanocrystals of self-assembled

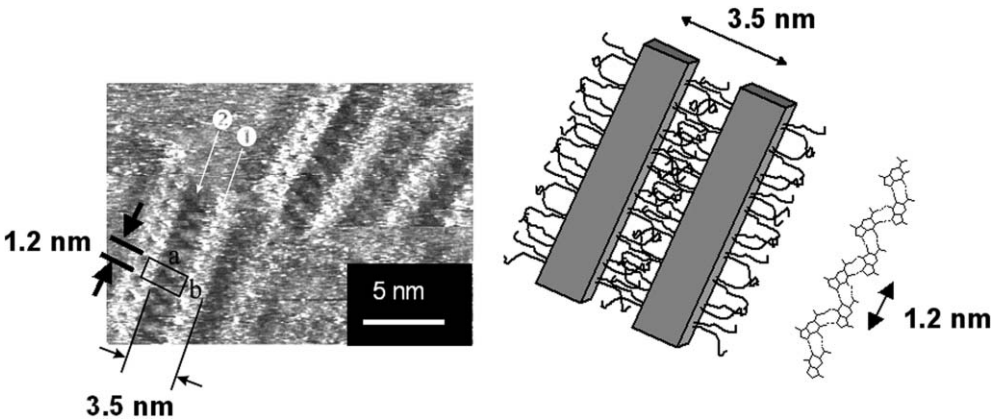


Fig. 33 STM picture of an array of nanoribbons of dG **7**. (Adapted from reference 45.)

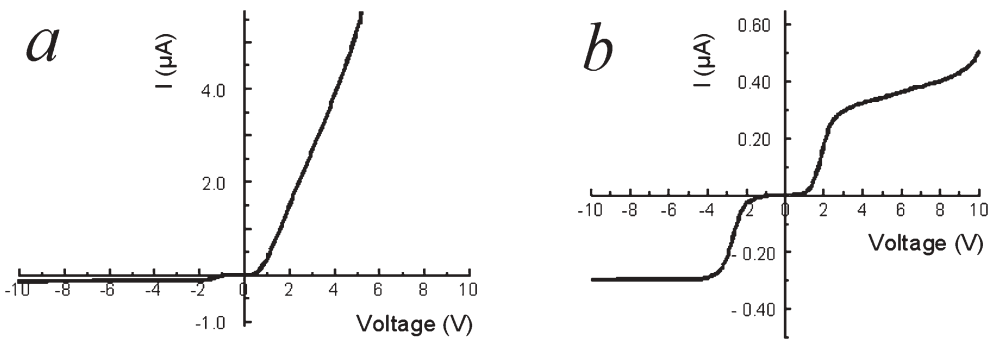


Fig. 35 Current intensity *vs.* voltage (*I–V*) plot for nanoribbons of dG **7** in 60 (*a*) and 120 nm (*b*) contact gap devices. (Adapted from references 10 and 48.)

dG **7** are probed by the electrodes and the total dipole of the sample between the electrodes averages to zero because the nanocrystals are randomly oriented. The *I–V* plot is non-linear and symmetric with a zero-current region between −2 V and +2 V. At higher bias, the current increase at sub-μA levels is typical of a metal–semiconductor–metal device. An interesting property of this 120 nm device is its high photo-responsivity, as the current increases from sub-μA level in the dark to sub-mA levels under illumination of a few mW of power.[48]

Semiconductor quantum dots have the potential to become fluorescent bioprobes for many biological applications. Self-assembled guanosines conjugated to luminescent quantum dots have been recently proposed for biophotonic applications.[49] A significant enhancement of photoluminescent emission is observed when the G-ribbons are conjugated to GaN quantum dots. This novel material system could allow the development of biocompatible nanophotonic sensors sensitive to UV wavelength (as most of biological agents absorb or emit in this regime).

5 Summary

In this paper, we have described just some of the supramolecular structures that have been built using guanine self-assembly. The synthetic G-quartet systems, in addition to providing models for understanding assembly in DNA and RNA, also have potential impact on sensor development, materials science, and nanoscience. Combining this inspiration from Nature with the fertile imagination of supramolecular chemists will undoubtedly provide more discoveries and advances in the use of nucleobase assembly to make functional architectures. The use of nucleobase self-assembly to form self-assembled ionophores, synthetic ion channels, dynamic gel, liquid crystals, hydrogels, noncovalent polymers, nanomachines, molecular electronic devices, biosensors, therapeutic aptamer and catalysts highlight the many functions that can arise from these interesting supramolecular assemblies.

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