The Supramolecular Organization of Guanosine Derivatives

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Abstract: 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. This mini-review describes some recent advances in the form and function provided by self-assembly of guanine (G) based systems. Although the large variety of supramolecular networks originated by guanosine derivatives has been investigated for a couple of decades, only in recent years several research groups focused on their applications in supramolecular chemistry, material science and nanotechnology. Our attempt here is 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 finally focuses on examples of how G analogs have been used as building blocks for functional applications.

Key Words: Guanosine, self-assembly, G-quartet, supramolecular chemistry, scaffolding, liquid crystals.

1. A BRIEF HISTORY AND PERSPECTIVE

Guanosine analogs, with their self-complementary hydrogen-bonding edges and aromatic surfaces, are programmed to self-associate. Guanine has hydrogen bond acceptors (N7, N3 and O6) and hydrogen bond donors (N1 amide and N2 amino) (Fig. **1**). Depending on the conditions, guanosine derivatives can self-associate into dimers, ribbons, macrocycles or layers. These hydrogen-bonded structures can stack in solution due to their polarizable aromatic surfaces.

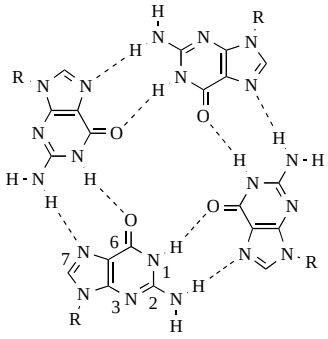

Fig. (1). The G-quartet.

The G-quartet was identified in 1962 as the structural unit behind hydrogels formed by 5'-guanosine monophosphate (5'-GMP) [1]. Many nucleosides, oligonucleotides and synthetic derivatives form G-quartets and related structures [2].

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 [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 [3]. Recently, Wu 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**) [4]. For 5'-GMP at concentrations between 18-34 wt %, the columns are 8-30 nm long; the impressive length of these stacks underlines the cooperation of hydrogen bonding, π–π stacking and cation-dipole interactions inherent to G-quartet assemblies.

The G-quartet-based self-assembly can be conveniently followed by circular dichroism (CD) spectroscopy, as a diagnostic bisignate CD signal originates from exciton coupling between two stacked G-quartets [5]. Recently, K-templated G-quartets of 5'-GMP have been

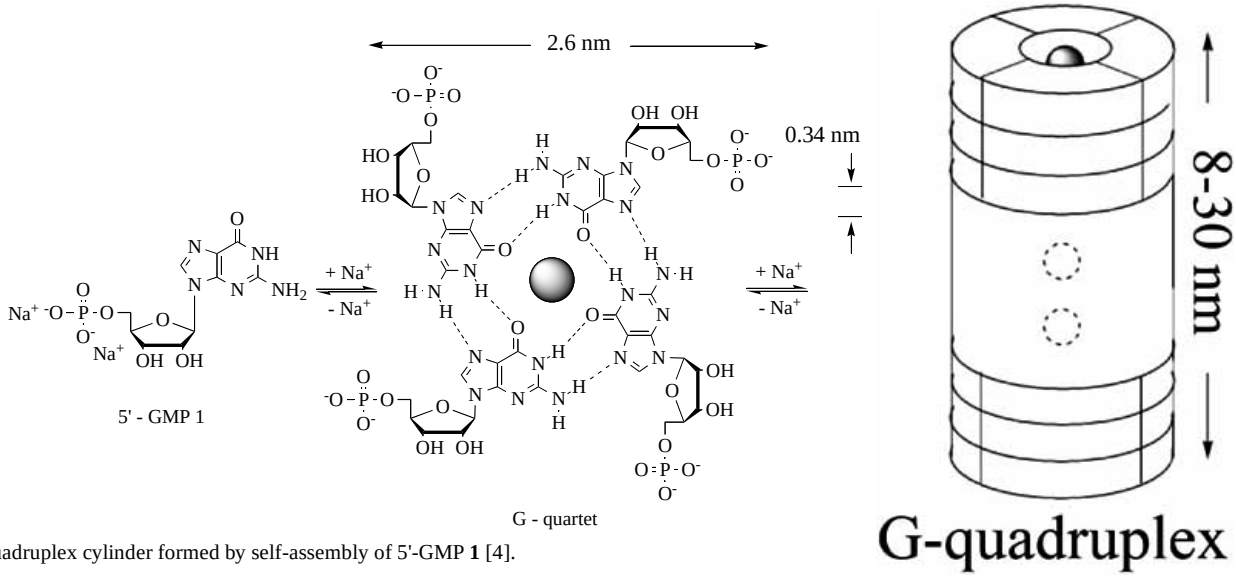

Fig. (2). G-quadruplex cylinder formed by self-assembly of 5'-GMP **1** [4].

used by Matile and colleagues as CD probes for detecting osmotic stress, and the activity of ion channels and pores within chirogenic vesicles [6]. In 1995, we reported that the lipophilic guanosine analogue 3',5'-didecanoyl-2'-dG **2** extracts K^+ picrate from water into

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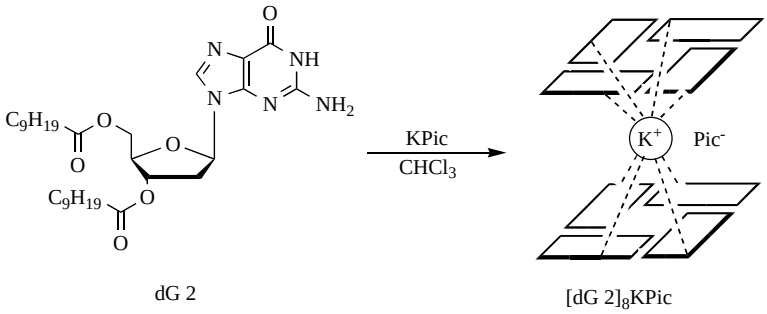


Fig. (3). Lipophilic $[dG\ \mathbf{2}]_8 \bullet K^+$ octamer formed by extraction of K^+ picrate from water.

$CDCl_3$ to give a discrete octamer $[dG\ \mathbf{2}]_8 \bullet K^+\ Pic^-$ [7]. The K^+ cation was essential for the formation of this lipophilic octamer (Fig. **3**).

Without templating cations, dG **2** organized into two different hydrogen-bonded ribbons [8]. A change either in the solvent or the sugar substituents produced a change of the ribbon's hydrogen-bonding pattern (giving ribbon A or B as in Fig. **4**) [9].

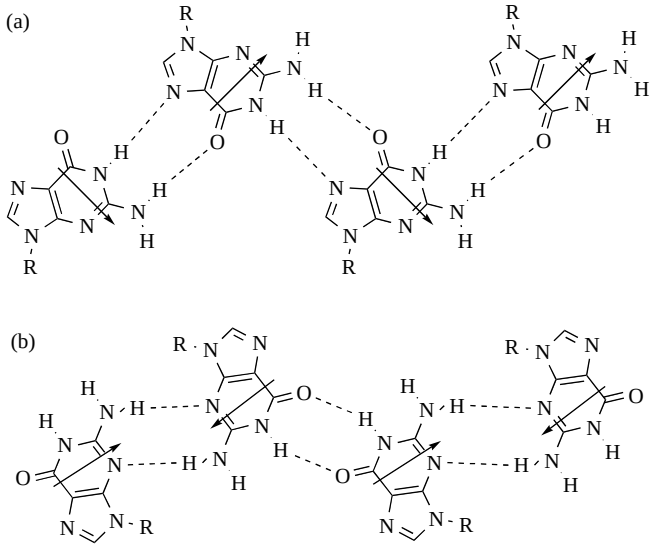


Fig. (4). Two different H-bonded ribbons formed by self-assembly of lipophilic guanosines in absence of cations. Ribbon A has a net dipole, whereas ribbon B contains no dipole.

Recently, we described another unique structure obtained upon self-assembly of 8-oxo lipophilic nucleosides [10]. Thus, both 8-oxoG **3** and the corresponding 2-deamino analog (8-oxoI) form hydrogen-bonded helices in organic solvents (Fig. **5**). This self-assembly pattern was quite different from the hydrogen-bonded ribbons formed by dG **2**.

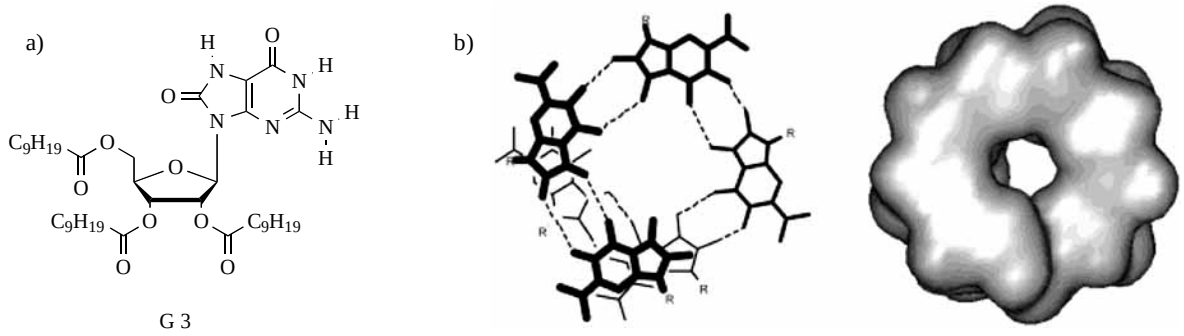


Fig. (5). a) 8-oxoG **3** and b) its helical supramolecular structure [10].

2. SUPRAMOLECULAR STRUCTURE OF G-QUARTET BASED ASSEMBLIES

To better understand how individual G-quartets organize within G-quadruplexes, our and Davis groups solved the NMR structure of $[dG\ \mathbf{2}]_8 \bullet KI$ in $CDCl_3$ [11]. This study showed that the octamer $[dG\ \mathbf{2}]_8 \bullet KI$ exists 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 (Fig. **6**) [12]. The lipophilic G-quadruplex $[G\ \mathbf{4}]_{16} \bullet 3K^+ \bullet Cs^+ \bullet 4pic^-$ consists of 4 stacked G-quartets. This G-quadruplex can be described as a pair of head-to-tail $[G\ \mathbf{4}]_8$ octamers with each G_8-octamer using its 8 carbonyl oxygens to coordinate a K^+ ion. A third K^+ ion holds the two $[G\ \mathbf{4}]_8$ octamers together and a Cs^+ cation caps the structure. The G-quartets within $[G\ \mathbf{4}]_{16} \bullet 3K^+ \bullet Cs^+ \bullet 4pic^-$ 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 (Fig. **7**). The lipophilic G-quadruplex looks like a cation channel with an anionic belt wrapped around its middle. These lipophilic G-quadruplexes can be used as models for DNA G-quadruplexes and for the development of functional nanostructures, as described below.

The identity of the anions bound to the surface controls the solution properties of these lipophilic G-quadruplexes. The rate of supramolecular isomerization of G_8 octamers in solution depends on the identity of the bound anions: both the pK_a and the structure of the phenolates influence the exchange rates for the bound cations and for the G subunits. Anions that hydrogen bond strongly to the central two G-quartets stop subunit exchange in CD_2Cl_2, presumably by increasing the kinetic stability of the complex and making subunit dissociation difficult [13]. Like the anions, also the specific cations bound in the central channel influence the solution properties of these assemblies. 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^+ [13]. Davis and coll. attributed this enhanced stability in the presence of divalent cations to stronger ion-dipole interactions between the cations and the nucleobase oxygen, as well as to a strengthening of the G-quartet's

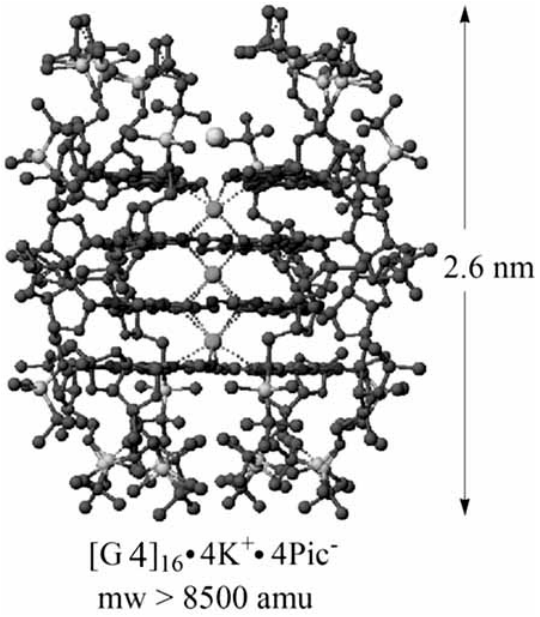


Fig. (6). The crystal structure of the lipophilic G-quadruplex [G **4**]$_{16}$•3K$^+$/Cs$^+$•4Pic$^-$ obtained from cation-templated self-assembly of G **4** [12].

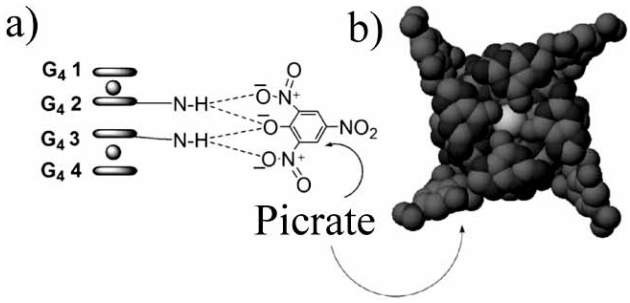
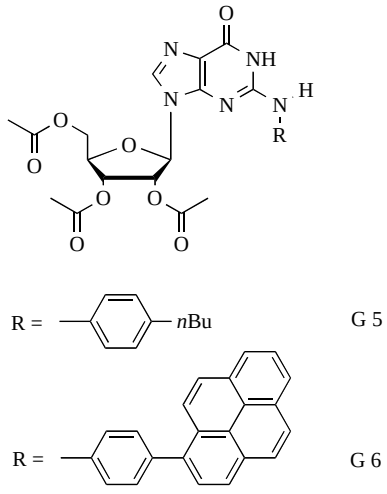


Fig. (7). a) A schematic showing the nucleobase-picrate hydrogen bonds in the hexadecamer [G **4**]$_{16}$•2Sr^{2+}•4Pic$^-$. b) Top view of the X-ray structure of the G-quadruplex with the sugars removed [13].

hydrogen bonds. Thus, the studies showed that both the cation and the anion influence the stability of these lipophilic G-quadruplexes.



With the proper combination of cations and anions to stabilize these structures, post-assembly modifications could be directed selectively to only the outer quartets [14].

Specific cations in G-quadruplexes can also be directly studied by NMR spectroscopy. Wu and coll. used solid state ^{23}Na and ^{39}K as NMR probes [15], as well as solution ^{43}Ca NMR for characterizing the Ca^{2+} templated G-quartet formation from 2',3',5'-triacetylguanosine (TAG) [16]. Recently, they showed that also trivalent lanthanide metal ions (La^{3+}, Eu^{3+}, Tb^{3+}, Dy^{3+}, Tm^{3+}) can promote formation of stacking G-quartets [17]. The relative stability of the octameric $[TAG]_8 \cdot M^{3+}$ and dodecameric $[TAG]_{12} \cdot M^{3+}$ species observed by ESI-MS/MS experiments depends on the ionic radius of the lanthanide ion. In this context, a new mode of metal ion binding in a G-quartet structure has been proposed, a triple-decker G dodecamer containing a single metal ion in the central G-quartet.

In the last years, diffusion NMR spectroscopy has become a valuable tool for the characterization of these supramolecular systems. Davis and Cohen groups used diffusion measurements to study the cation-templated self-assembly of different guanosine and isoguanosine derivatives [18]. They provided information on the structural size of complexes, the effect of the solvent, the role of the cation and anion, demonstrating the utility of combining diffusion NMR techniques with conventional NMR methods, especially in analyzing symmetrical species.

N2–arylation on 2',3',5'-triacetylguanosine does not hinder G-quartet formation. Wu, Wang *et al.* showed that G **5** and G **6,** in the presence of either sodium or potassium, self-assemble into D_4-symmetric octamers consisting of two stacking all-*syn* G-quartets, most likely in a tail-to-tail fashion, sandwiching a central ion [19]. NMR spectroscopic results suggest the formation of π-π stacking between the phenyl or pyrenyl groups, that may stabilize the G **5** and G **6** octamers, respectively (Fig. **8**).

3. ENANTIOMERIC SELF-ASSOCIATION OF LIPOPHILIC GUANOSINES

Cation control over self-assembly was also illustrated by the expression of supramolecular stereochemistry by these chiral nucleosides. Cation identity (Ba^{2+} vs. K^+) had a significant influence on the diastereoselectivity in self-association of G **4** [20]. When K^+ was added to a solution of racemic (D,L)-G **4** the resulting G-quadruplexes were a mixture of heterochiral diastereomers. The divalent cation Ba^{2+}, however, directed enantiomeric self-recognition of (D,L)-G **4**, giving homochiral G-quadruplexes. To explain this cation-dependent diastereoselectivity, Davis and coll. proposed that the increased en-

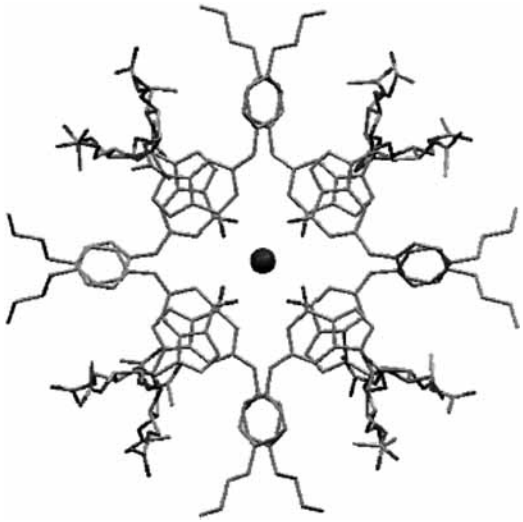


Fig. (8). Molecular model (top view) for a D_4–symmetric G **5** octamer, [(G **5**)$_8$K$^+$], where the two G-quartets are oriented in a tail-to-tail fashion [19].

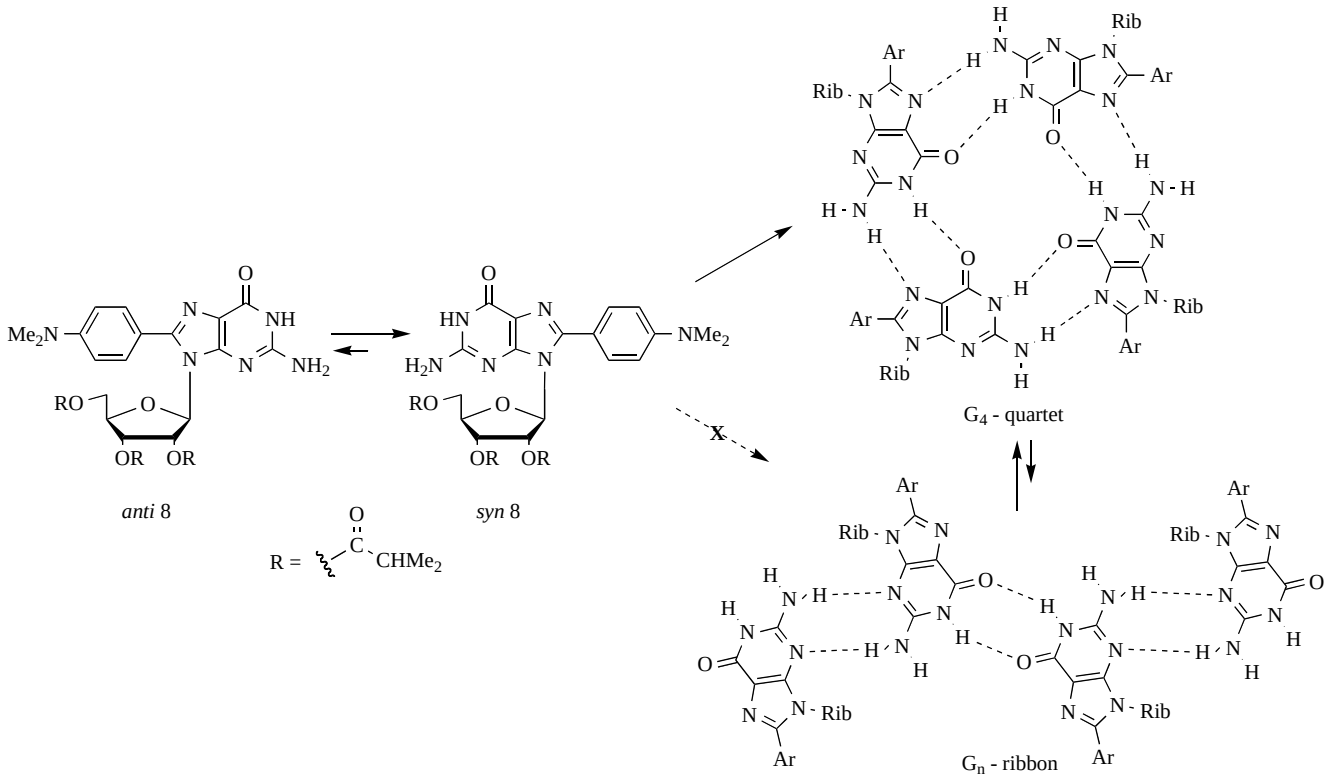


Fig. (9). Conformationally constrained G **8** forms a G-quartet in the absence of cations.

thalpy inherent to the divalent cation-oxygen interaction must help overcome the unfavorable entropy associated with enantiomeric self-sorting.

Lipophilic G-quartets might potentially be useful as chiral resolving agents. For instance, we showed that G-quartets formed from dG **7** are enantioselective in their ability to extract chiral anions from water into 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 the existence of significant interactions between these anions and the chiral G-quadruplex [21].

4. "EMPTY" G-QUARTETS

In the absence of the appropriate templating cation, guanosine analogs usually, but not always, form hydrogen-bonded dimers or ribbons. Sessler and coll. synthesized a G analog **8** that self-associates into an "empty" G-quartet without the assistance of cation templation [22]. Attachment of a dimethylaniline moiety to the guanine C8 position gives a conformationally constrained nucleoside, which adopts a *syn* glycosidic bond conformation both 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. **9**). This study showed how synthetic chemistry could be used to produce unnatural nucleobases for the non-covalent synthesis of stable supramolecular assemblies.

Otero *et al.* showed that guanine is able to form kinetically stable "empty" G-quartets, when placed on a gold surface [23]. STM measurements showed that these empty G-quartets were not the thermodynamic minimum, as annealing the deposited G-quartet network led to rearrangement into a hydrogen-bonded ribbon. In the case of guanine, 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.

5. "EXPANDED" HYDROGEN BONDED ASSEMBLIES

Rivera and coworkers have demonstrated another approach toward stabilizing G-quartets by using 8-aryl-dG analogs such as dG 9 [24]. By introducing at the C8 position a residue with a properly positioned hydrogen bond acceptor, they succeeded in involving the exocyclic N2 amino hydrogen, which does not normally participate in G-quartet hydrogen bonding (Fig. **10**).

Variable temperature and dilution NMR experiments on the G-quadruplex $[dG\ \mathbf{9}]_{16}\bullet 3K^+$ 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 was due to three factors. First, C8 substitution forces dG **9** into the *syn* glycosidic conformation, inhibiting the formation of hydrogen bonded ribbons. Second, the additional aromatic rings attached to C8 provide a larger surface for stronger π-π interactions between stacked G-quartets. Finally, the C8 substituent in dG **9** enables four additional hydrogen bonds per G-quartet, as illustrated in Fig. (**10**).

6. DITOPIC GUANOSINE DERIVATIVES FOR FUNCTIONAL G-QUARTET NANOSHEETS

An example of the long-range amplification of G-quadruplex self-organization into macroscopic polymeric functional films has been recently reported by Barboiu and coworkers [25]. The reversible synthesis of bisiminoboronate-guanosine macromonomers G-G **10**, followed by the self-assembly of G-G **10** into G-quartet type structures allowed to conceive G-quartet polymeric membrane materials at the macroscopic scale (Fig. **11**). Structure and morphology of these solid materials were determined, and the cation transport properties were investigated (mixed cationic Na^+/K^+ or selective K^+ transport).

All results, together with the higher conductivity measured on these membranes compared to the nontemplated ones, indicate that the G-quadruplexes self-correlate with a directional order that allows the formation of directional transport pathways.

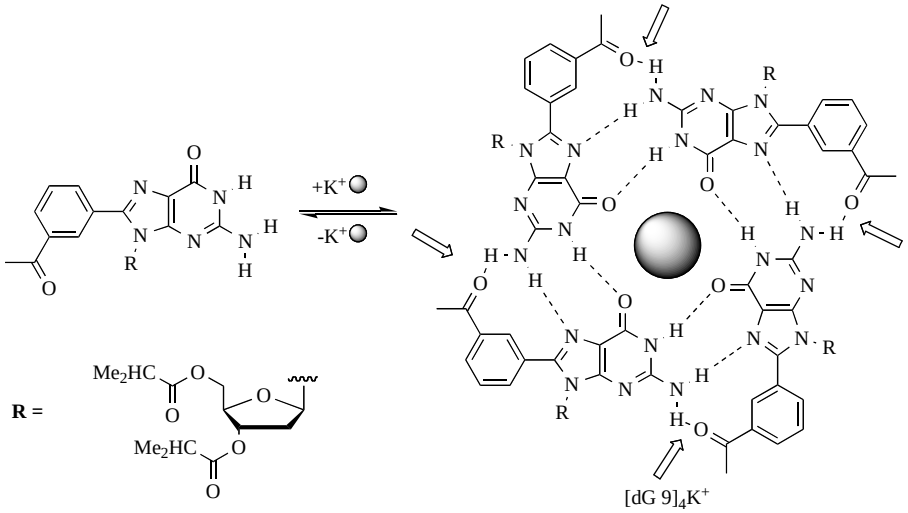


Fig. (10). The "expanded" hydrogen bonded G-quartet obtained from the modified nucleobase dG **9**.

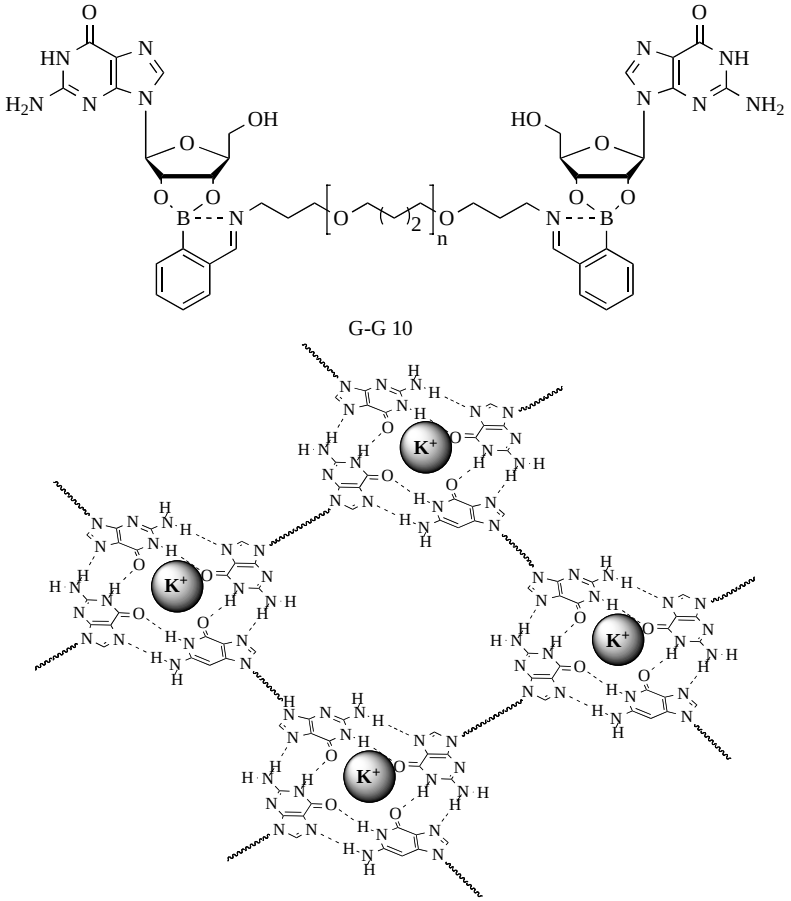


Fig. (11). The cation-templated hierarchical self-assembly of G-G **10** gives G-quartet networks in solid, self-supporting, polymeric membrane films in the presence of templating K^+ ions [25].

Davis and coworkers investigated a different ditopic guanosine derivative forming discrete channels in phospholipid bilayer [26]. Membrane insertion of ditopic G-sterol G-G **11**, followed by formation of G-quartets, provided functional pores whose conductance is typically in the range of 1-5 nS. The magnitude and lifetime of ion conductance supported by G-G **11** varied during a single experiment and were higher than those observed for most synthetic channels. The pattern of "open" and "closed" conductance observed is consistent with the dynamic formation and disgregation of self-assembled channels formed by G-G **11**.

Shi and colleagues demonstrated how the anion binding sites in G-quadruplexes can serve as a new synthetic handle to extend the ion-mediated self-assembly of guanosines. They proposed a novel supramolecular architecture by using the covalently-linked dianion 2,2',6,6'-tetranitrobiphenolate ($TNBP^{2-}$) to tether individual G-hexadecamers ($[G\ \mathbf{4}]_{16}\bullet Na_4^+$) (Fig. **12**) [27]. A highly ordered nanoscale structure was produced at the solid-state upon cross-linking the lipophilic G-quadruplexes: the non-covalent polymeric nanosheets extend along the horizontal direction without changing the vertical dimension of the complex (3.0 nm).

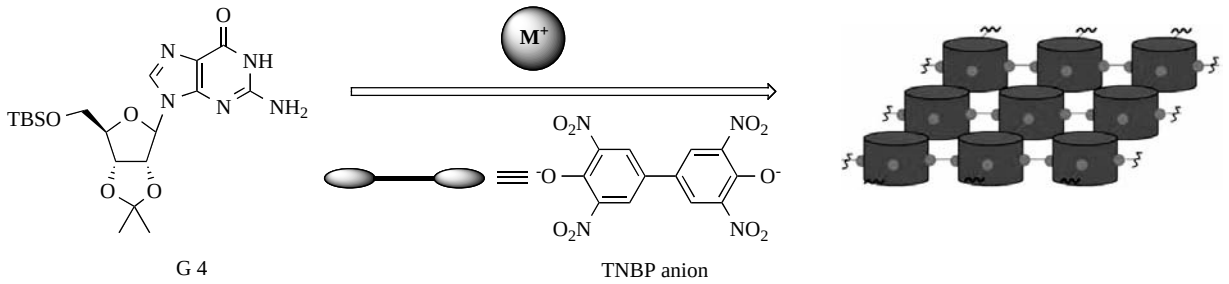


Fig. (12). Schematic illustration of the nanosheet obtained by the use of the dianion TNPB [27].

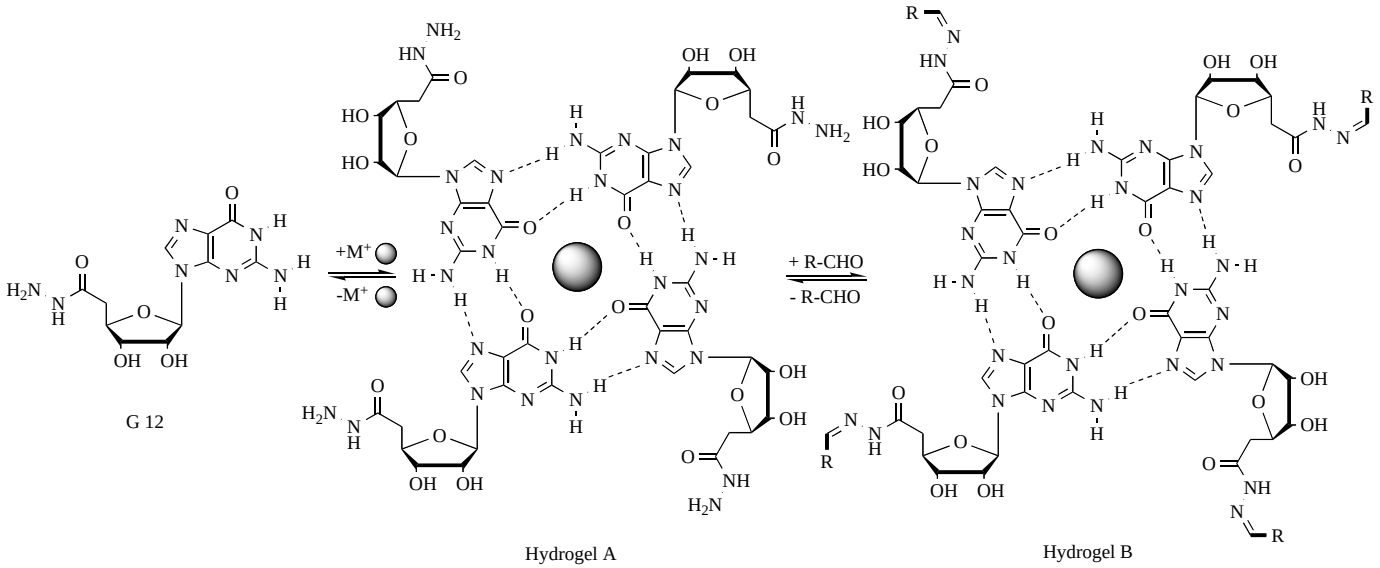


Fig. (13). Dynamic hydrogels using a G-quartet scaffold.

7. THE G-QUARTET 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 [28,29]. 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 coll. 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 [30].

In addition to the discovery of new ligands that interact with tetraplex structures, the DCC concept has also been used to form new G-quadruplexes, each with its own unique properties. The same authors 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 [31]. They demonstrated that formation of the non-covalent PNA G-quadruplex preceded covalent bond formation.

Sreenivasachary and Lehn described a G-quartet system wherein component selection from a DCL is driven by the physical properties of the product [28]. They described dynamic hydrogels formed by covalent modification of the sugar side chains that extend from stacked G-quartets. Reaction of hydrogel A, formed from 5'-hydrazido G **12** [28,32] with a mixture of aldehydes, produced a family of acylhydrazones (Fig. **13**). 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.

Hydrogels offer also a promising medium for controlled release of bioactive substances, and they are of interest because of their hydrophilic character and potential biocompatibility. Lehn *et al.* demonstrated that hydrogelators G **12** can incorporate analogs compounds, containing a guanine moiety, forming mixed G-quartets: ^{1}H NMR spectrosopy was used to study the inclusion of various guanine derivatives into the gel and their releasing rates [33].

Recently Ghoussoub and Lehn 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 **13** were converted to soluble $(G\text{-}G)_n$ polymers upon addition of [2.2.2]-cryptand **14**, an ionophore that extracts K^+ from the G-quartet hydrogel. The gel was regenerated upon expelling K^+ from the $[K^+ \subset 2.2.2]$-cryptate by protonation of the cryptand's bridgehead nitrogen to give $[2H^+ \subset 2.2.2]$ **14**. 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. **14**).

In a related system, we 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, we could stabilize either hydrogen-bonded ribbons or a discrete K^+ G-octamer. The combination of CD spectroscopy and ^{1}H NMR spectroscopy was particularly compelling in establishing equilibrium shifting between the distinct species.

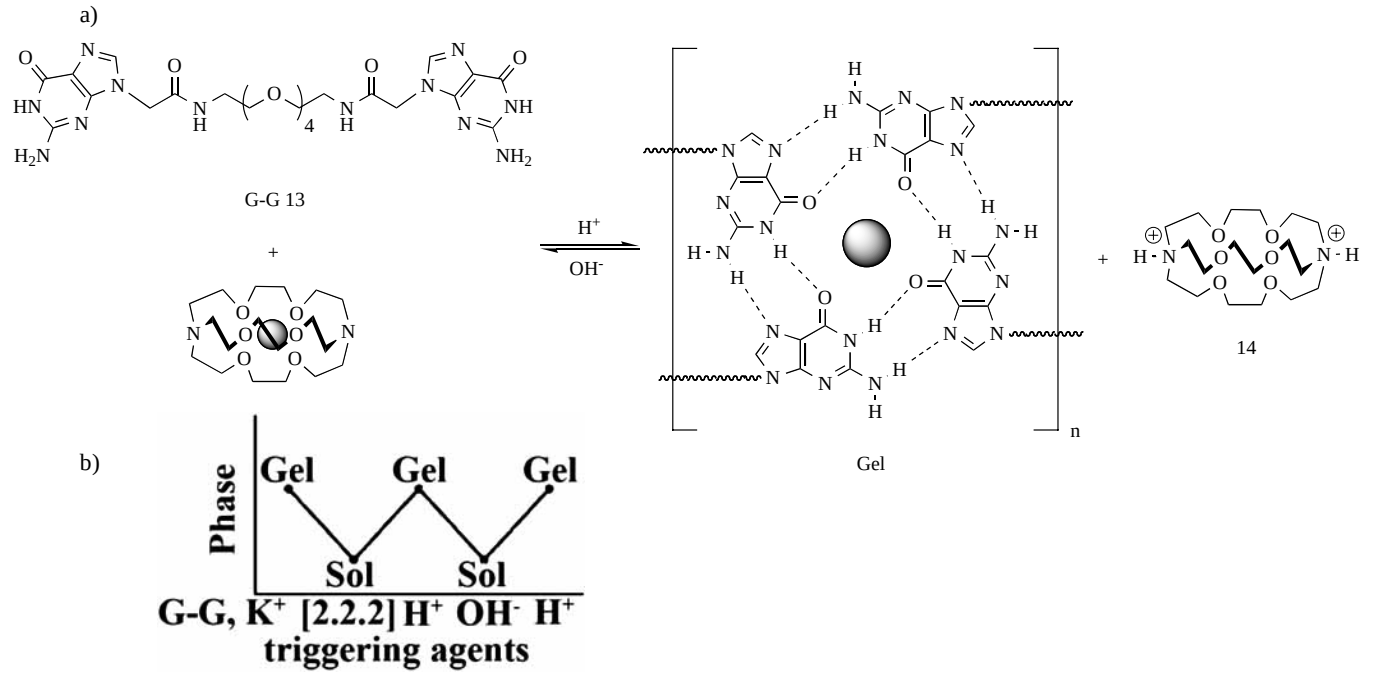


Fig. (14). a) Structure of G-G **13** and schematic of the reversible formation of polymeric G-quartet based hydrogels. Changing pH in the presence of [2.2.2] cryptand modulated the sol-gel equilibrium. b) Modulation of the gel–sol status induced by the sequence of triggering agents [34].

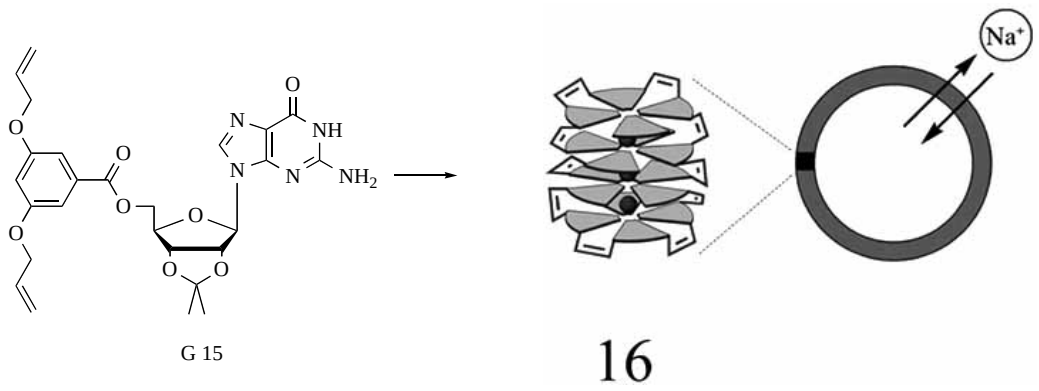


Fig. (15). The unimolecular G-quadruplex **16** obtained from olefin metathesis of G **15** transports Na^+ ions across phospholipid bilayer membranes [36].

In another example of the power of the DCC approach, Davis and coll. described a unimolecular G-quadruplex **16** 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. Reversible olefin metathesis was used to cross-link subunits that had been preorganized within a G-quadruplex (Fig. **15**).

The precursor, 5'-(3,5-bis(allyloxy)benzoyl)-2',3'-isopropylidene G **15** carried two 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 **15** formed a hexadecameric G-quadruplex $[G\ \mathbf{15}]_{16}\bullet 4K^+\bullet 4DNP^-$. Olefin metathesis of this non-covalent G-quadruplex resulted in a high yield of metathesis product **16**. This unimolecular G-quadruplex apparently folds into a conformation that allows transport of Na^+ cations across phospholipid bilayer membranes. Evidence for the ability of the lipophilic G-quadruplex **16** 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, structures closely related to the G-quartet, also function as synthetic ion channels [37].

8. GUANINE DERIVATIVES AS VERSATILE SCAFFOLDS TO CONTROL SELF-ORGANIZATION OF MATERIALS

Recently Rowan and co-workers [38] initiated a program aimed at investigating the potential of assembling supramolecular polymers, derived from low-molecular weight nucleobase-endcapped monomers, on a surface as a way to organize functional groups at the nanoscale and as such act as molecular-scale surface scaffolds (Fig. **16**).

The monomer G-G **17** comprises three components, (1) a hydrocarbon core to enhance adsorption onto a hydrophobic surface in the presence of an aqueous medium, (2) the guanine end groups, to facilitate adsorbate-adsorbate interactions through hydrogen bonding, and (3) peptide nucleic acid (PNA) chains primarily used to link the hydrocarbon cores and the guanine moieties. The results suggested that the hydrogen-bonding ability of the guanine was important to the formation of molecular–sized bands, whose widths could be systematically varied by simply changing the length of the core hydrocarbon unit. Furthermore, this concept has been extended into using these assemblies as scaffolds to supramolecularly graft groups (TEG) on to HOPG. These grafted assemblies showed the ability to influence biological processes, namely static platelet adhesion.

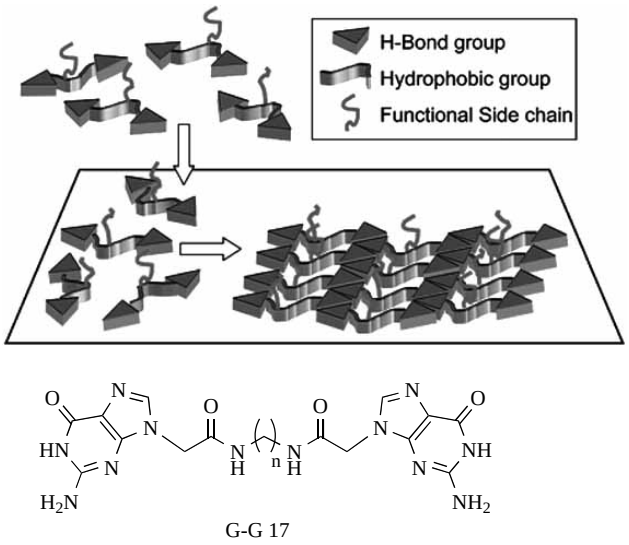


Fig. (16). Schematic representation of the concept of surface-aided supramolecular polymerization [38].

Our group started investigating the potential of self-assembled guanine architectures as scaffold for functional hybrid materials some years ago by reporting the behavior of a porphyrin-functionalized guanosine [39]. Along this line, supramolecular assemblies arising from conjugates between guanosine and either paramagnetic or organic semiconducting residues have been studied [40].

Other research groups have demonstrated that functional self-organization can be readily transcribed into hybrid nanostructures by using sol-gel process. In particular Barboiu and co-workers have reported a synthetic route for preparing self-organized ion-channel systems that have been "frozen" in a polymeric matrix, as a straightforward approach for the design of a novel class of solid hybrid membranes [41]. In this study, the guanine alkoxysilane building block G **18** is used as a molecular precursor to conceive hybrid chiral materials at the nanometric and micrometric scales. The main strategy consists of generating (amplifying) dynamic supramolecular G-quartets and G-quadruplex by K^+ ion templating, from a dynamic pool of supramolecular dimeric, oligomeric ribbon-type, or cyclic supramolecular architectures (Fig. **17**). The G-quadruplex architectures are then fixed in a hybrid organic-inorganic material by using a sol-gel transcription process, followed by a second inorganic transcription in silica, that is, calcination.

9. LYOTROPIC LIQUID CRYSTAL FORMATION FROM G-QUARTET BASED SYSTEMS

While the ability of guanosine derivatives to form gel-like structures has been known since the early twentieth century [2], 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 [42] during the investigation of the sodium salt of 2'-deoxyguanylyl-(3'-5')-2'-deoxyguanosine (dG_2 **19**). This compound exhibits in water 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 **20** and 3'-dGMP dG **21**) and G-rich oligonucleotides was described [43]. Typically, cholesteric (chiral nematic) and hexagonal phases are formed upon self-assembly of these G derivatives.

It was known that several biopolymers, including DNA [44], 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

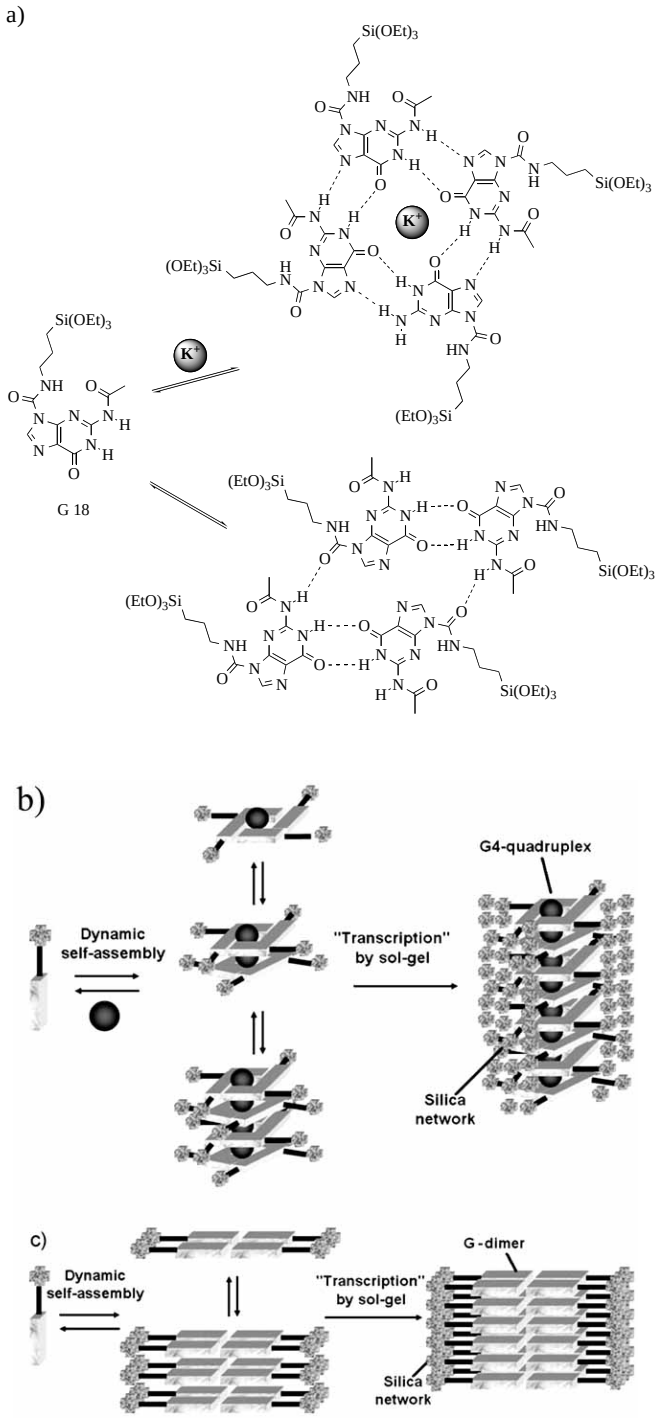


Fig. (17). The cation-templated hierarchic self-assembly of guanine alkoxysilane and the transcription of the G-quadruplex into solid hybrid materials by sol-gel process [41].

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.

However, guanosines dG_2 **19**, dG **20** or dG **21** are neither polymers nor long anisometric molecules like DNA. The lyotropic mesomorphism is a consequence of the formation of a self-assembled structure, wherein the basic unit is a chiral columnar aggregate based on G-quartets held together by non-covalent interactions. The G-

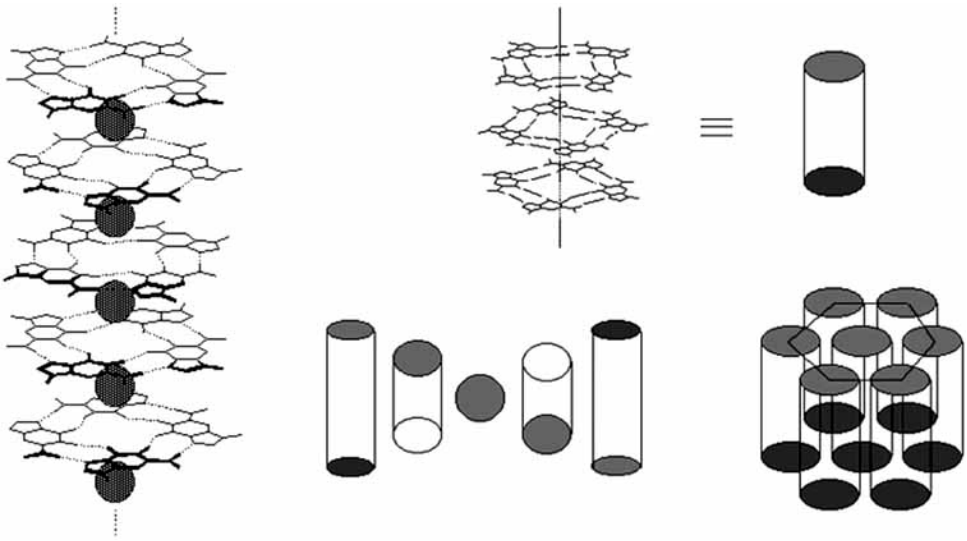


Fig. (18). Lyotropic liquid crystals from self-assembled guanosines.

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 **18**). 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, each G-quartet is rotated with respect to the adjacent G-quartet layers.

Depending on concentration, temperature, and amount of added salts, these aggregates self-correlate to generate a 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 texture (under polarizing optical microscopic, POM, observation) without unwinding the cholesteric helix that is oriented parallel to the applied field. 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 [43].

Low angle X-ray diffraction work confirmed the assignment of the phases detected by 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 [43].

The self-assembly process for guanosine derivatives, including mesophase formation, can easily and conveniently be followed by circular dichroism spectroscopy (CD) [5]. Spectra of isolated species are usually drastically different from those of the assembled species and of the cholesteric phases. In Fig. (**19**) the case of dG **21**, whose assembly process is driven by temperature, is presented as an example [45]. 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.

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 [46]. 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 as well give liquid crystalline phases under the appropriate conditions [47].

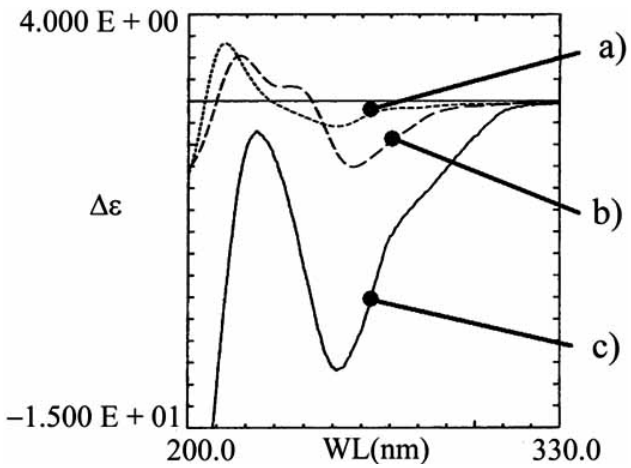


Fig. (19). CD spectra of an aqueous solution of dG **21** (ammonium salt) in different self-organized states: unassembled (a), isotropic G-quartet based columnar state (b), and cholesteric (c) [45].

10. G-RIBBONS AND THEIR APPLICATIONS IN MOLECULAR ELECTRONICS

As anticipated, lipophilic guanosine derivatives self-assemble into linear ribbon-like motifs in the absence of alkali cation templates (Fig. **4**). These ribbon structures in solution were identified mainly by NMR [48] and, in the solid state, by single crystal X-ray diffraction [8,49]. Lipophilic guanosines are able to form lyotropic mesophases in several solvents. For example, dG **2** in hexadecane gives, above a critical concentration, a viscous birefringent (LC) phase. X-ray dif-

fraction 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. **20**) [8].

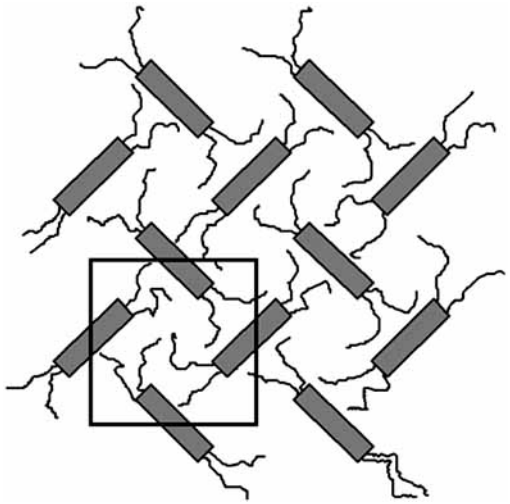


Fig. (20). A model for the square LC phase of dG **2** in hydrocarbon solvents [8].

Araki and coworkers introduced non-polar and flexible alkylsilyl groups into 2-deoxyguanosine, obtaining dG **22** as an efficient organogelator for alkanes [50].

ated by the formation of self-complementary G-G base pairs through double N2-H---N3 hydrogen bonds which further develop into a 2D supramolecular assembly (Fig. **22**).

(Bio)molecular electronics is gaining an increasing attention 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. (**23**). The width of the ribbon, around 6.2 nm, is consistent with its proposed supramolecular structure [48].

As depicted in Fig. (**24**), these nanoribbons interdigitate. The unit cell dimension b is estimated to be 1.2 nm and perfectly matches that of the type A ribbon found by single crystal X-ray analysis [48].

Rinaldi *et al.* have proposed the use of nanoribbons formed from dG **2** guanine units in the design of molecular electronic nanodevices [52]. Self-assembled nanoribbons obtained by drop casting were used to interconnect gold nanoelectrodes fabricated by electron beam lithography (see Fig. **25**).

Given that the typical length of the oriented arrays of ribbons (a "nanocrystal") is approximately 100 nm, for a contact gap of 60 nm or less only one nanocrystal of the dG **2** assembly is probed. Under these conditions the plot of current intensity vs voltage (I-V) shows a clear diode-like behavior, with currents on the order of μA for positive bias and nA for negative bias. This rectifying feature points out the existence, in each nanocrystal, of a strong dipole that originates

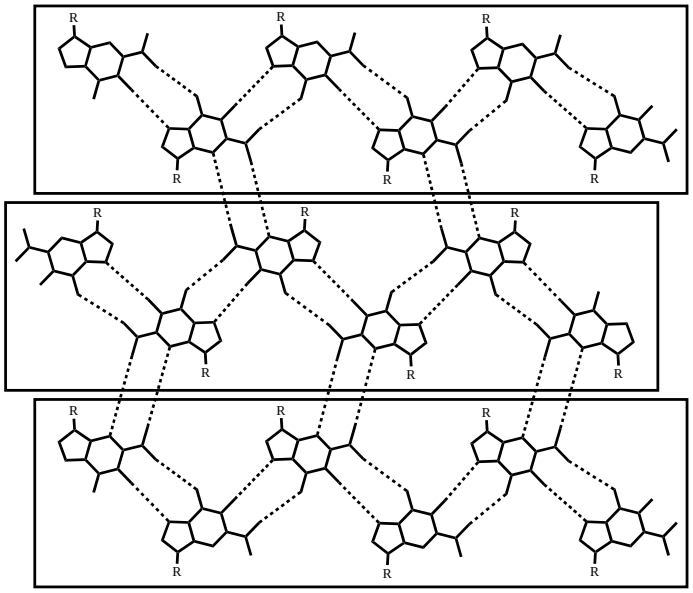


Fig. (21). Two-dimensional H-bonded sheet of guanine moieties. The boxes highlight the guanine ribbons A (see Fig. **4**) connected by H-bonds.

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. (**21**), is composed of anti-parallel G-ribbons of type A (Fig. **4**) with additional double inter-tape hydrogen bonds between N2-H and N3 of two guanine units located in adjacent ribbons. The gel-to-liquid crystal phase transition for these organogels from dG **22**, 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. (**21**).

More recently the same author proposed a different supramolecular structure for 2',3'-*O*-isopropylideneguanosine organogelators having bulky alkylsilyl moieties in *O*-5' [51]. The gelation is medi-

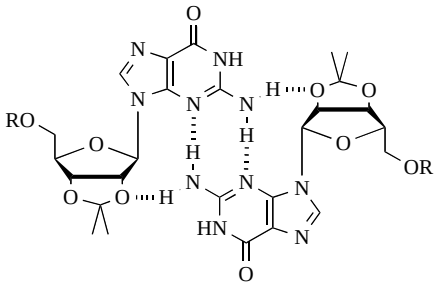


Fig. (22). G-G base pair as propobed by Araki *et al.* [51].

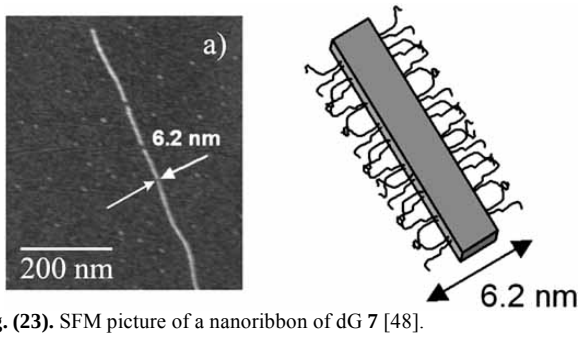


Fig. (23). SFM picture of a nanoribbon of dG **7** [48].

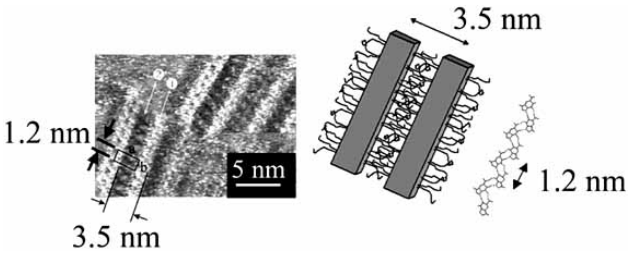


Fig. (24). A quasi-molecular resolution Scanning Tunneling Microscopy (STM) image (at the graphite/solution interface) of closely packed arrays of H-bonded ribbons formed by self-assembly of dG **7** [48].

from the molecular dipole of the guanine units ordered in the ribbon-like structure of type A (see Fig. **4**). 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 [53].

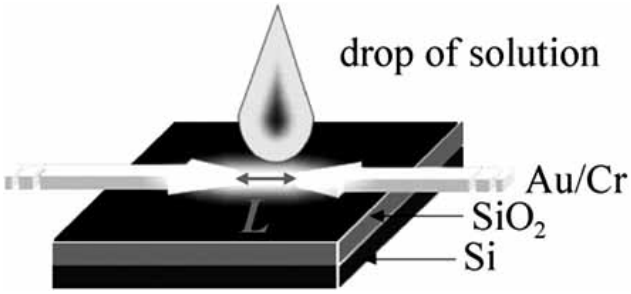


Fig. (25). Schematic preparation of a G-based electronic nanodevice [52].

The situation changes dramatically in the 120 nm-gap device. In this case, a few nanocrystals of self-assembled dG **2** 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 [54].

Recently, self-assembled dG **2** has been used to fabricate two-terminal diode on GaN semiconductor substrate. Due to polarity induced along the direction of the guanosine nanocrystal wires during the self-assembly process, the output current of the GaN based photodiodes is significantly higher than hybrid Si/self-assembled guanosine based photodiodes at similar input voltage [55].

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 [56]. 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).

CONCLUSIONS

Modified nucleosides are of great interest due to their remarkable biological activity and wide range of applicability. Among these, substituted guanosines are of greatest importance, specially because of its properties of forming important supramolecules, a variety of self-assembled structures including G-ribbons, and tetrameric complexes.

In this mini-review some recent advances in the form and function provided by self-assembly of guanine (G) based systems have been described. After a brief historical context of G self-assembly, the article focused on connections between the structures of the assemblies and their properties and on examples of how G analogs have been used as building blocks for functional applications in supramolecular chemistry, material science and nanotechnology.

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