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Supramolecular Chemistry

Functional G-Quartet Macroscopic Membrane Films**

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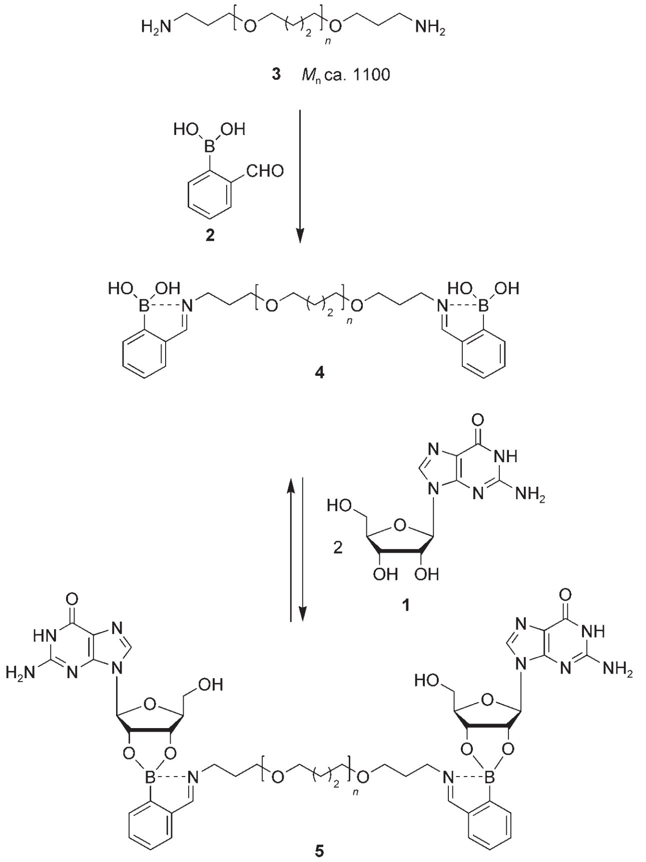
G-quartets, formed by the hydrogen-bonding self-assembly of four guanosine (G) residues and stabilized by alkali-metal cations, play an important role in biology, in particular in nucleic acid telomers of potential interest to cancer therapy.[1] The G-quartet architecture represents a nice example of a dynamic supramolecular system that has been used as a building block for gelators,[2] columnar polymeric aggregates,[3] self-organized surfaces,[4] and prototypes of chemical dynamic devices.[2,5]. In the last few decades, G-quartets[1,6] and the similar folic acid quartet[7] have also been proposed as powerful scaffolds for building synthetic ion channels. Although stable in organic solvents, they do not seem to have defined transport functions in hydrophobic membranes. Barrel-stave,[6a] lipophilic,[6b,g] calix[4]arene,[6c] and 8-aromatic–guanosine[6d] conjugates have been used to stabilize the formation of G-quartets. Recently, a new strategy based on reversible metathesis was successfully used by Davis et al.[6e] to generate a rich array of interconverting ion-channel conductance states of a unimolecular G-quartet in a phospholipid membrane.

Despite such impressive progress, considerable challenges still lie ahead and the more significant one is to improve the stability of G-quartet dynamic aggregates in polymeric devices, such as films or membranes, to extend (address) the transport studies to the macroscopic level. Several earlier studies reported the preparation of discrete supramolecular assemblies of nucleobases embedded in synthetic polymers[8] and hybrid materials.[9] However, the "dynamic communication" between the supramolecular self-assembly of nucleobases and the polymerization processes, which kinetically and stereochemically might communicate, is not so trivial.



Scheme 1. Synthesis of bisiminoboronic **4** and bisiminoboronate-guanosine **5** macromonomers, and the self-assembly of **5** into G-quartet-type superstructures.

For all these reasons, in this study building blocks of guanosine (**1**; Scheme 1), 2-formylphenylboronic acid (**2**), and bis(3-aminopropyl)polytetrahydrofuran (PTHF, **3**; number-average molecular weight M_n ca. 1100 g mol^{-1}) are used as molecular precursors to conceive G-quartet polymeric membrane materials at the macroscopic scale. Our efforts involve the successive synthesis of the ditopic bisiminoboronic **4** and bisiminoboronate-guanosine **5** macromonomers, and then the self-assembly of **5** into G-quartet-type supramolecular superstructures (Scheme 1).

The main strategy consists of generating (amplifying) dynamic supramolecular G-quartets by K^+ ion templating, from a dynamic pool of oligomeric ribbon-type or cyclic supramolecular architectures. Then, the G-quadruplex architectures are fixed in self-supporting polymeric membrane films. A standard sample without potassium chloride, which resulted in the formation of an H-bond ribbon-type superstructure[6f] of the guanine moieties, was prepared as reference under the same conditions as described above (Figure 1).

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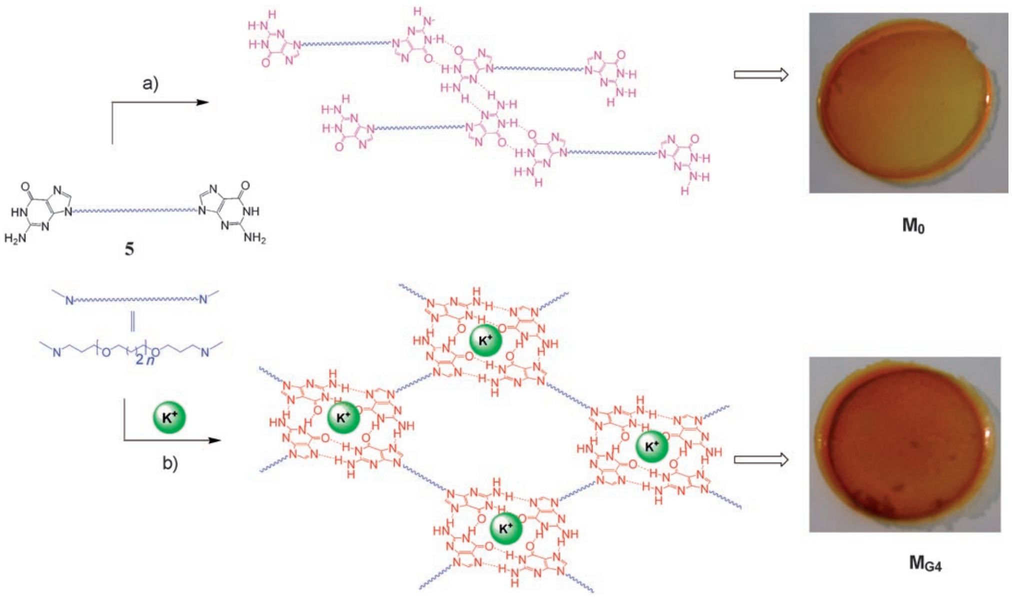

Figure 1. The cation-templated hierarchical self-assembly of bisiminoboronate-guanosine **5** macromonomer gives G-quartet networks in solid, self-supporting, polymeric membrane films in the a) absence (**M$_0$**) and b) presence of templating K$^+$ ions (**M$_{G4}$**).

Compound **5** generates G-quartet-encoded polymeric films by K$^+$ cation templating, based on three structural features:

1) The polymeric network might be considered to contain various superstructures resulting from the combination of cross-linked arrays of G-quartets interconnected by PTHF linkers, reminiscent of the polymeric guanosine hydrogels recently reported by Lehn et al.[2b]
2) The linkage between the guanosine moiety and the PTHF macromonomer is based on a double reversible covalent iminoboronate connection. The imino moiety contributes to the stabilization of the reversible boronate–guanosine ester bond by the formation of a dative N–B bond.[10] Then this contributes to implementing a dynamic adaptative character on the molecular and supramolecular level in a double "dynamer"-type system,[11,12,16] which combines reversible supramolecular polyassociation (G-quartets) with molecular polycondensation (iminoboronate bonds) of components. This might contribute to the high level of adaptability and correlativity of the self-organization of the dynamic supramolecular and rigid polymeric systems.
3) As a result of the hydrophobic nature of PTHF linkers, G-quartets might self-organize in a lipophilic environment, thus maximizing the transport of ionic species along the G-quartet pathways and avoiding bulk-transport phenomena across such a polymeric matrix.

The 2-formylphenylboronic acid (**2**) was treated with PTHF (**3**; M_n ca. 1100 g mol^{-1}, CHCl$_3$, reflux) to afford the bisiminoboronic macromonomer **4** as a yellow oil. Then polymeric membrane films **M$_{G4}$** and **M$_0$** can be achieved by mixing derivative **4** with guanosine, thus leading to bisiminoboronate-guanosine macromonomer **5** in the presence or absence of potassium chloride, respectively.

^{1}H NMR analysis of **4** in CD$_3$OD and of membrane materials **M$_{G4}$** and **M$_0$**, which are soluble only in [D$_6$]DMSO, allows easy identification of peaks corresponding to bisimino-substituted compounds. ^{1}H NMR experiments indicate low defined self-association of **M$_0$** in [D$_6$]DMSO. The NMR signal of exchangeable NH^1 protons is broadened into the baseline around $\delta = 9.7$ ppm, probably because of exchange with the solvent (Figure 2a). This result is consistent with **M$_0$** being "monomeric" in the absence of K$^+$ cations in [D$_6$]DMSO. After equilibration with K$^+$ ions, the ^{1}H NMR spectrum of **M$_{G4}$** presents sharp resonances for NH^1 amide protons ($\delta = 10.6$ and 10.7 ppm) and for NH^2 amine protons ($\delta = 8.8$ ppm) in [D$_6$]DMSO (Figure 2b). Unfortunately, the NOESY spectrum of **M$_{G4}$** does not show clear NOEs between NH^1 and the H^8 protons of the guanosine, which confirms that intermolecular H-bonding interactions are minimized in [D$_6$]DMSO, as previously reported.[6]

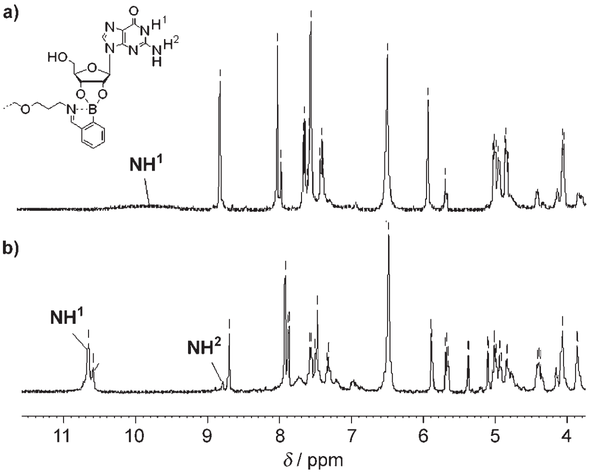

Figure 2. Portions of ^{1}H NMR spectra in [D$_6$]DMSO at 25 °C of a) **M$_0$** and b) **M$_{G4}$**.

Further evidence for H-bonding and K$^+$ complexation was obtained from the FTIR analysis of solids **M$_{G4}$** and **M$_0$**. The free $\nu_{C=O} = 1725$ cm^{-1} band was downshifted to 1689 or 1691 cm^{-1}, which indicates the formation of G-quartets (Supporting Information). The formation of iminoboronate esters stabilized by a donor N→B dative bond is supported by the vibrational shifts of the N=C bonds ($\nu_{C=N} = 1640$ to $1605\,cm^{-1}$) and the presence of the $\nu_{B-N} = 814\,cm^{-1}$ band, most predominant in the FTIR spectrum of $\mathbf{M_{G4}}$. Additionally, $\mathbf{M_{G4}}$ and $\mathbf{M_0}$ show an absorption band at around $1014\,cm^{-1}(\nu_{B-OC})$ instead of the vibration at $1300\,cm^{-1}$ (ν_{B-OH}) initially observed for compound **4**.

^{11}B magic-angle-spinning (MAS) NMR spectroscopic experiments confirm the FTIR analysis and are in agreement with the presence of an N→B dative bond, which stabilizes the formation of a dynamic boronate ester with a guanosine sugar moiety (Supporting Information).[10] The ^{11}B MAS NMR spectrum of **4** is consistent with an equilibrium between N→B coordinated tetrahedral ($\delta = 3.8$ ppm) and N–B uncoordinated trigonal planar ($\delta = 0.2$ ppm) configurations. Next, after guanosine complexation into the $\mathbf{M_0}$ material, the signal is shifted downfield ($\delta = 6.2$ ppm) from the resonance of free boronic acid in compound **4** to the resonance of the boronate compound, where the boron center adopts a mainly uncoordinated trigonal configuration in equilibrium with the coordinated tetrahedral form (a shoulder may be identified at $\delta \approx 0$–4 ppm). The formation of G-quartets by K$^+$ templating in $\mathbf{M_{G4}}$ is consistent with the appearance of a sharp peak at $\delta = 4.7$ ppm, which indicates that the boron atom adopts completely a tetrahedral configuration upon associating with guanosine to form G-quartets.

Further insights into the structure and morphology of the membrane materials $\mathbf{M_{G4}}$ and $\mathbf{M_0}$ were obtained by X-ray powder diffraction (XRPD) studies. Figure 3 shows the XRPD patterns of the reference nontemplated $\mathbf{M_0}$, the K$^+$-templated $\mathbf{M_{G4/KCl}}$, and water-washed $\mathbf{M_{G4}}$ materials. All these samples display a broad Bragg diffraction peak at $2\theta = 19.7°$ ($d = 4.5$ Å) and the $\mathbf{M_{G4}}$ XRPD pattern presents a supplementary peak at $2\theta = 13.3°$ ($d = 6.65$ Å). The KCl-templated material $\mathbf{M_{G4/KCl}}$ presents three additional peaks at $2\theta = 28.35$ ($d = 3.14$ Å) and 40.42° ($d = 2.23$ Å; data not shown in Figure 3), which correspond to crystalline KCl, and at $2\theta = 26.8°$ ($d = 3.31$ Å), representative of the π–π stacking distance between two planar G-quartets (Figure 3).[6f,9] This last peak, which is missing in the XRPD pattern of $\mathbf{M_0}$, is amazingly amplified after washing with water, even in the absence of the templating KCl in the membrane (the representative peaks of KCl disappeared after this step).

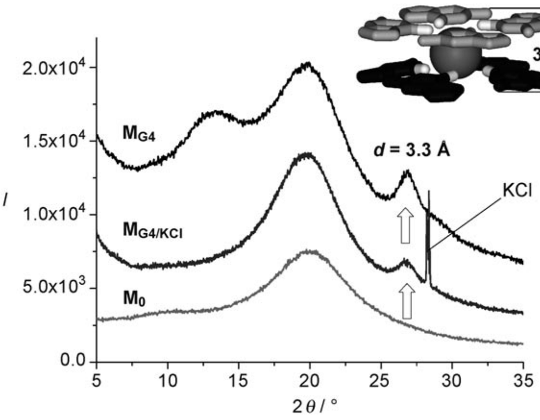

Figure 3. XRD patterns of nontemplated ($\mathbf{M_0}$) and K$^+$-templated ($\mathbf{M_{G4/KCl}}$ and water-washed $\mathbf{M_{G4}}$) membrane materials. Inset: the π–π stacking between planar G-quartets in stick representation from crystallographic data[6h] (the K$^+$ ion is shown as a sphere).

Scanning electron microscopy (SEM) reveals that the membrane films $\mathbf{M_{G4}}$ and $\mathbf{M_0}$ (thickness of about 300 µm) were dense and without pinholes (Figure 4a). Figure 4b depicts a typical SEM image of the surface of an $\mathbf{M_{G4}}$ film, and shows well-resolved and highly ordered longitudinal rows with several orientations for the bulk crystalline areas at the micrometer level.

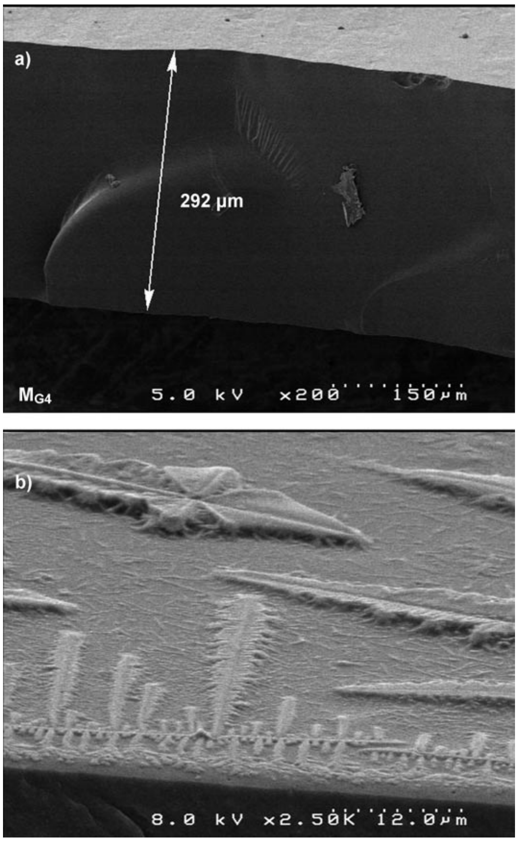

Figure 4. SEM images of a) the cross section and b) the surface of an $\mathbf{M_{G4}}$ membrane film.

To study the transport properties of the synthesized membrane films, ac impedance spectroscopy studies were performed on the $\mathbf{M_0}$ and $\mathbf{M_{G4}}$ materials. The $\mathbf{M_{G4}}$ shows a conductivity of $1.2 \times 10^{-8}\,S\,cm^{-1}$ (resistance $R = 3.3 \times 10^6\,\Omega$), although the $\mathbf{M_0}$ membrane showed a conductivity of $1.1 \times 10^{-11}\,S\,cm^{-1}$ ($R = 3.1 \times 10^9\,\Omega$) at 25 °C. Stacked G-quartet superstructures stabilized by K$^+$ templating ions in $\mathbf{M_{G4}}$ membrane films induce an important increase of the conductivity by a factor of 1000 compared to the nontemplated $\mathbf{M_0}$ membrane, which presents a very low conductivity. These measured conductivities probably originate from synergetic proton/electron transfer along the guanosine chains formed through the membrane films. It was demonstrated that H-bonding does not support electronic band transport in planar ribbons or isolated quartets.[13] Instead, base stacking accompanied by π–π interactions may induce coherent charge mobility[13a] or polarizability[13b] in stable G-quartet materials. Similarly, it is known that the proton conductivity of an ordered arrangement is higher than that of a randomly arranged one.[13c–e] The stacked G-quartets in $\mathbf{M}_{\mathbf{G4}}$ certainly induce directional transportation pathways for protons through a proton-wire mechanism.

In a second experiment, the competitive transport of $\mathrm{Na^+}$ and $\mathrm{K^+}$ cations across membrane $\mathbf{M}_{\mathbf{G4}}$,[14] according to the solution–diffusion mechanism[15] and against its thermodynamic gradient, was evaluated under passive transport conditions. The transport rate is thus governed by diffusion coefficients and by the interaction equilibrium of the hydrated ions, which lose part of the hydration shell in exchange with the specific G-quartet groups within the membrane. Figure 5 shows the concentration versus time transport profiles of $\mathrm{Na^+}$ and $\mathrm{K^+}$ ions. The feed phase was filled with an equimolar solution of NaCl/KCl, while the strip phase was distilled water. The G-quartet membrane $\mathbf{M}_{\mathbf{G4}}$ presents a nonlinear saturation behavior of the transport profile of $\mathrm{Na^+}$ and $\mathrm{K^+}$ ions, which indicates a strong affinity of the membrane towards the solutes.[15]

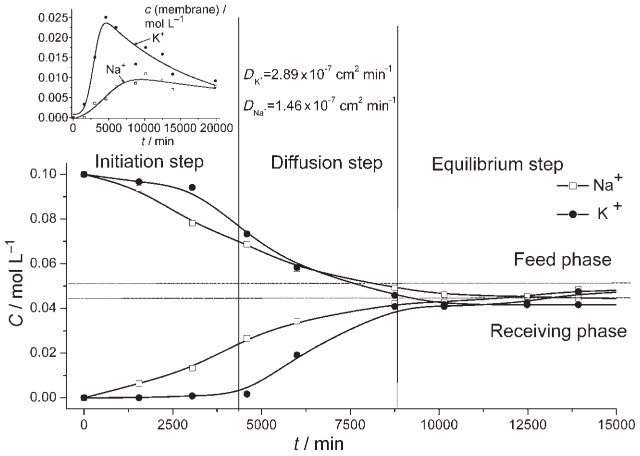

Figure 5. Transport profiles of $\mathrm{Na^+}$ and $\mathrm{K^+}$ ions through the $\mathbf{M}_{\mathbf{G4}}$ membrane, shown as concentration in the feed, the membrane, and the receiving phase versus time.

We first noted an initiation step: the membrane functions like a "sponge" for the $\mathrm{K^+}$ ions, while the smallest $\mathrm{Na^+}$ ions are transported faster through the membrane. Mixed cationic $\mathrm{Na^+/K^+}$ G-quadruplexes are probably formed along ion-exchange pathways.[6g] Certainly, a substantial contribution to this phenomenon arises from the high affinity of G-quartets for $\mathrm{K^+}$ ions, which may stay within the hydrophilic G-quadruplex pathways. After this initiation step, in a second diffusion step the $\mathrm{K^+}$ ions are transported twice as fast as $\mathrm{Na^+}$ ions ($J_{\mathrm{K^+}}/J_{\mathrm{Na^+}} = 2$, J = flux of ions; Figure 5). This apparent selectivity is consistent with the development of $\mathrm{K^+}$-conducting pathways along membrane-spanning $\mathrm{K^+}$-filled oligomers. Finally, the system reaches the equilibrium step.

The dynamic assembly behavior of the G-quartet[1] and the transcription of its structural information into a functional unimolecular G-quartet[6e] or into reversible polymeric gels[2a] have been described. Recently, we reported the long-range amplification of the G-quadruplex supramolecular chirality into hybrid organic–inorganic twisted nanorods, followed by transcription into inorganic silica microsprings by using the sol–gel process.[9] The present results give an example of the long-range amplification of G-quadruplex self-organization into macroscopic polymeric functional films.

The proposed strategy for stabilizing the G-quadruplexes in the double dynamer system is novel. It involves a double dynamic connection between a dynamic supramolecular system (G-quartet/G-quadruplex superstructures) and a rigid polymeric network. This contributes to making functional G-quadruplexes by correlating the reversible supramolecular system (G-quartets) with polymeric self-assembly through reversible molecular connections (iminoboronate G-macromonomer bonds) between the components. The fixed ("frozen") G-quadruplexes self-correlate with a directional order, as proved by XPRD and transport experiments, to generate anisotropic mesophases interconnected by condensed macromonomeric hydrophobic bridges. The G-quadruplex ordered membrane films contribute to the fast electron/proton transfer by the formation of directional conduction pathways. Mixed cationic $\mathrm{Na^+/K^+}$ or selective $\mathrm{K^+}$ transport enabled us to better understand the diffusional ion exchanges along "fixed" G-quadruplex polymeric pathways.

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