Toward artificial ion channels: self-assembled nanotubes from calix[4]arene–guanosine conjugates†

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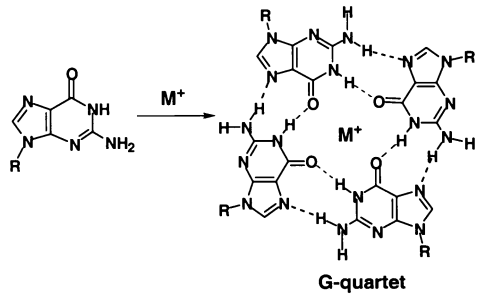
In the presence of Na^+, a 1,3-*alternate*-calix[4]arene bearing four guanosine units forms a self-assembled nanotube.

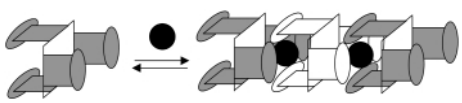
With the current interest in fabricating nanoscale structures for potential biomedical and materials applications much attention has focused on supramolecular tubes. Nanotubes have been made from cyclic peptides,[1] lipids,[2] oligocyclodextrins,[3] hydrogen-bonded rosettes[4] and coordination complexes.[5,6]

Predicting and controlling structure is essential for optimizing function. Herein, we report our initial efforts on building functional nanotubes, with an eye towards using them as artificial ion channels. Our design hinges on the metal cation-templated self-assembly of guanosine, a nucleobase that readily forms a hydrogen-bonded quartet in the presence of cations (Scheme 1).[7,8] The cyclic G-quartet, stabilized by Na^+ or K^+, can further organize by stacking. Telomeric DNA forms a G-quadruplex with a cation-filled channel;[9] and G-wires, over 1000 nm in length, have been imaged by atomic force microscopy.[10] We have also recently shown by X-ray crystallography that lipophilic G nucleosides self-associate to give extended, tube-like structures upon cation coordination.[11]



Scheme 1

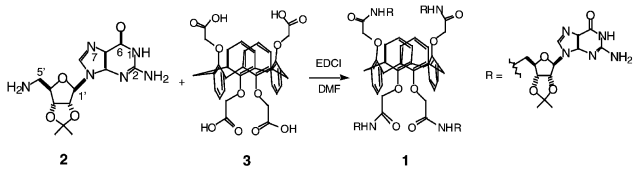
Our modular approach towards constructing nanotubes involves using sodium cations to trigger the one-dimensional polymerization of G_4-calix **1**, a compound with four G moieties attached to a calix[4]arene-1,3-*alternate* scaffold. The 1,3-*alt* core in monomer **1** orients two orthogonal pairs of self-complementary G nucleosides so that they are well positioned for intermolecular hydrogen bonding with neighboring monomers (Scheme 2). Hosseini and coworkers introduced the calix[4]arene-1,3-*alt* scaffold to organize groups in this alternating 'up-down-up-down' fashion[12] and demonstrated this scaffold's utility by using Ag^+–cyano coordination bonds to form a self-assembled 'metallatubulane.'[6]



Scheme 2 Representation of nanotube formation by G_4-calixarene **1** upon cation-templated self-assembly. Squares represent the 1,3-*alt*-calix[4]arene core, ovals represent the guanosine moieties, and solid spheres represent the Na^+ cations.

† Electronic supplementary information (ESI) available: experimental and synthetic details. See http://www.rsc.org/suppdata/cc/b0/b007332f/

Compound **1** was readily prepared in six steps from guanosine and the parent *tert*-butylcalix[4]arene.[13] The final step in the synthesis of G_4-calix **1** involved the EDCI-promoted coupling of 5′-amino-2′,3′-isopropylidene G **2**[14] with the calix[4]arene-1,3-*alt* tetraacid **3**[15] (Scheme 3). The G_4-calix **1** was isolated by precipitation from MeOH, purified on microcrystalline cellulose TLC plates, and characterized by FAB-MS and ^1H NMR spectroscopy. The FAB-MS indicated complete substitution of a G residue at each of the calixarene's four sidechains. The symmetrical ^1H NMR spectrum of G_4-calix **1** in DMSO-d_6 confirmed its 1,3-*alt* conformation, and also verified that nucleoside coupling to the calixarene had occurred at the more nucleophilic 5′-amine of G **2**, and not at the less reactive exocyclic N2 amino group.



Scheme 3

The G_4-calix **1** was soluble in a MeCN–H_2O (1:1) binary mixture. Addition of $NaBPh_4$ to a solution of G_4-calix **1** in this mixture resulted in the instantaneous and quantitative precipitation of a white solid. The ^1H NMR spectrum of this material in d_6-DMSO confirmed that it was a mixture of G_4-calix **1** and the BPh_4^- anion. Transmission electron microscopy (TEM) images (Fig. 1) show the precipitate to consist of micrometer-long

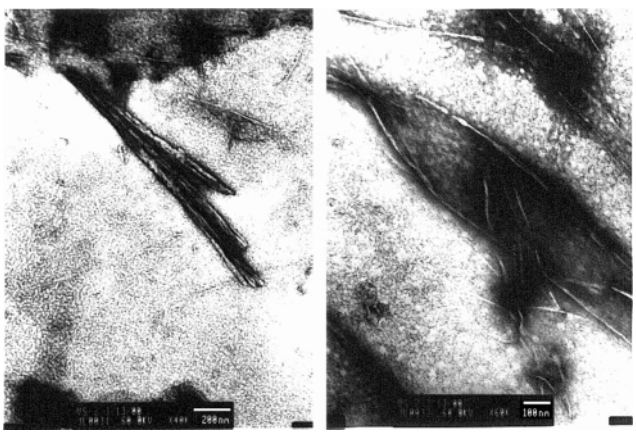


Fig. 1 Representative TEM images of the precipitate formed by G_4-calixarene **1** and $NaBPh_4$ in MeCN–H_2O (1:1) solution. Objects have thicknesses between *ca.* 3 nm (many of the objects in the right image) and *ca.* 50 nm (most objects in the left image), and lengths between 80 nm and 1.5 μm. The thinnest rods, with the approximate thickness of a G-quartet, may correspond to single self-assembled nanotubes. The thicker objects are likely bundles of nanotubes. The unit scale is 200 nm for the left image and 100 nm for the right image.

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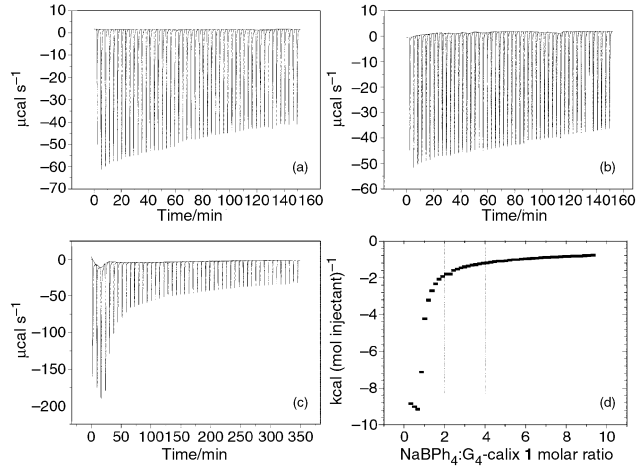

Fig. 2 Isothermal titration calorimetry. (a) Data for titration of $NaBPh_4$ into MeCN–H_2O (1 : 1) solution. (b) Data for titration of $NaBPh_4$ into a MeCN–H_2O (1 : 1) solution containing 2′,3′-isopropylidene guanosine. Note that there is essentially no difference between the results of experiments (a) and (b). (c) Data for titration of $NaBPh_4$ into a MeCN–H_2O (1 : 1) solution containing G_4-calix **1**. Note the difference in the scale of the *y*-axis (heat evolved) for experiment (c), as compared to experiments (a) and (b). (d) Integration curve for raw data from titration (c). The inflection point is consistent with formation of a complex having a 1 : 1 ratio for G_4-calix **1** : $NaBPh_4$. The total enthalpy of the interaction is −9 kcal mol^{-1}.

strands, visible as either single tubes or as bundles of tubes (ranging from *ca.* 3 nm to 50 nm in thickness). The strands are relatively straight, without much bending. The thickness of the individual tubes was near the microscope's limit of resolution, namely *ca.* 3 nm. Since our recent X-ray structure of a lipophilic G-quadruplex showed a tubular structure with a 2.65 nm diameter,[11] the individual rods formed from Na^+ templated aggregation of G_4-calix **1** have dimensions expected for a G-quartet. These electron micrographs of insoluble aggregates, showing such defined structure, are entirely consistent with nanotube formation by G_4-calix **1** upon Na^+ coordination.[16]

The stoichiometry of the insoluble aggregate formed between G_4-calix **1** and $NaBPh_4$ was quantitatively determined using isothermal titration calorimetry (ITC). Titration of a solution of G_4-calix **1** in MeCN–H_2O (1 : 1) with $NaBPh_4$ resulted in the rapid and significant generation of heat, until a 1 : 1 ratio of G_4-calix **1** and $NaBPh_4$ had been reached [Fig. 2(c) and 2(d)]. The total enthalpy of Na^+ binding by G_4-calix **1** was −9 kcal mol^{-1}; and this exothermic reaction coincided with precipitation of the $(\mathbf{1}\cdot Na^+)_n$ aggregate from solution as its BPh_4 salt. The absence of other inflection points in the ITC experiment, besides that for the prominent 1 : 1 G_4-calix **1**–$NaBPh_4$ stoichiometry, suggests that nanotube formation by G_4-calix **1** does not pass through shorter, intermediate structures. In other words, cation-templated nanotube formation of $(\mathbf{1}\cdot Na^+)_n$ is highly cooperative. Control ITC experiments showed that 2′,3′-isopropylidene guanosine, a compound lacking the calixarene framework, does not bind $NaBPh_4$ in this polar MeCN–H_2O (1 : 1) solvent mixture [Fig. 2(a) and (b)]. Thus, the heat evolved upon addition of $NaBPh_4$ to MeCN–H_2O (1 : 1) is the same whether or not 2′,3′-isopropylidene guanosine is present in solution. These ITC experiments clearly illustrate the entropic advantage of attaching the G moieties to the 1,3-*alt*-calixarene scaffold. Moreover, cation-templated aggregation of G_4-calix **1** is kinetically fast, and self-assembly of G_4 tetramers occurs even in this highly competitive hydrogen-bonding solvent mixture.

The assembly process that gives the $(G_4\text{-calix } \mathbf{1}\cdot Na^+)_n$ is completely reversible. Both temperature and pH can be used to control the cation-templated aggregation of G_4-calix **1**. For example, the precipitate formed from G_4-calix **1** and $NaBPh_4$ at 25 °C can be solubilized in MeCN–H_2O (1 : 1) simply by heating. DSC of a suspension of $(\mathbf{1}\cdot Na^+)_n$ in MeCN–H_2O (1 : 1) revealed a sharp transition temperature of 44.5 °C for dissolution. Cooling the sample back to 25 °C resulted, again, in precipitation of $(\mathbf{1}\cdot Na^+)_n(BPh_4^-)_n$. In addition to temperature, pH also had a dramatic effect on the solubility, and presumably the aggregation state, of $(\mathbf{1}\cdot Na^+)_n$. Protonation of the G nucleobase's exocyclic N2 amine and N7 should disrupt the structure of the hydrogen-bonded G-quartet. Indeed, this is consistent with the observation that the insoluble $(\mathbf{1}\cdot Na^+)_n(BPh_4^-)_n$ was completely redissolved in MeCN–H_2O upon changing from pH 7 to 2. This pH-dependent cycle was reversible, as addition of triethylamine base so as to change the solution's pH from 2 to 9 caused reprecipitation of $(\mathbf{1}\cdot Na^+)_n(BPh_4^-)_n$. The thermal lability and acid sensitivity of these hydrogen-bonded nanotubes formed from G_4-calix **1** is consistent with the reversible nature of self-assembly.

Three features of our nanotube design are worth emphasizing: (1) long-range structure can be controlled by cation templation, which triggers formation of an extensive hydrogen-bonded assembly based on the G-quartet; (2) aggregation is enabled by covalent attachment of guanosine units to the *alt*-1,3 calixarene scaffold, and (3) self-association of G_4-calix **1** occurs even in highly polar solvents. Future studies will focus on using temperature, pH, and other variables, to control the polymerization and depolymerization of G_4-calix **1**. The reversible formation of these structures in water at temperatures near 40 °C makes G_4-calix **1** and its analogs interesting as materials for biomedical applications. Finally, we are preparing lipophilic analogs of G_4-calix **1** in an effort to make artificial ion channels that self-assemble within a bilayer membrane.

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