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Citation for published version (APA):

Document license:
TAVERNE

DOI:
10.1021/jacs.9b07868

Document status and date:
Published: 16/10/2019

Document Version:
Publisher’s PDF, also known as Version of Record (includes final page, issue and volume numbers)

Please check the document version of this publication:
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Noncovalent Synthesis of Self-Assembled Nanotubes through Decoupled Hierarchical Cooperative Processes

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Supporting Information

ABSTRACT: Because of their wide number of biological functions and potential applications, self-assembled nanotubes constitute highly relevant targets in noncovalent synthesis. Herein, we introduce a novel approach to produce supramolecular nanotubes with defined inner and outer diameters from rigid rod-like monomers programmed with complementary nucleobases through two distinct, decoupled cooperative processes of different hierarchy and acting in orthogonal directions: chelate cooperativity, responsible for the formation of robust Watson–Crick H-bonded cyclic tetramers, and nucleation–growth cooperative polymerization.

INTRODUCTION

Noncovalent synthesis aims at the production of well-defined nanostructures, often mimicking those found in the natural world, and relies both on noncovalent interactions and on cooperative effects between chemically programmed molecules.1−5 One of the most appealing noncovalent synthetic targets are self-assembled nanotubes, due to their nanoscale dimensions, with inner cavities in the attoliter regime, their functions and potential applications, self-assembled nanotubes that is based on a novel molecular design, involving rigid rod-like monomers that interact through complementary nucleobases through two different hierarchy and acting in orthogonal directions: chelate cooperativity, responsible for the formation of robust Watson–Crick H-bonded cyclic tetramers, and nucleation–growth cooperative polymerization.

One of these approaches involves the combination of two hierarchical processes that are strongly coupled (Figure 1a; left). First, the association of dendron-shaped molecules with self-complementary H-bonding faces leads to rosette-like macrocycles, and, as soon as these transient intermediates are formed, a stacking process is triggered in the orthogonal direction.18−21 The most popular building blocks employed here contain heterocyclic head groups like guanine,22,23 cyanuric/barbituric acid,24,25 melamine/diaminotriazine,26 their combination,27,28 or pyrimido[4,5-d]pyrimidine derivatives.29,30 This strategy can be implemented in aqueous or organic media with molecules carrying diverse external functions. However, it affords little control on pore dimensions and function, and the resulting nanotubes typically have small pores, able in the best case to accommodate ions or very small molecules.

Here, we introduce a related strategy for the synthesis of self-assembled nanotubes that is based on a novel molecular design, involving rigid rod-like monomers that interact through their edges in a defined 90° geometry (Figure 1a; right). Specifically, our monomer structure (GC; Figure 1b) comprises complementary guanine (G) and cytosine (C) nucleobases linked by linear, π-conjugated spacers. The bases are also equipped with benzylic wedges substituted with long alkyl tails, so as to enhance solubility in apolar solvents, and with a peripheral amide group. This molecular design allowed one to control self-assembly through two orthogonal cooperative events of different hierarchy, as shown in Figure 1c, which can be now efficiently decoupled. First, chelate cooperativity is responsible for the formation of unstrained cyclic tetramers through G:C Watson–Crick H-bonding interactions,31 as demonstrated previously with related molecules bearing bulky riboses.32−38 After these planar macrocycles are generated quantitatively, a polymerization...
process, which occurs through a nucleation–growth mechanism and is guided by $\pi-\pi$ and H-bonding interactions along the stacking axis, can be triggered by a change in experimental conditions. Hence, both cooperative processes are independent, and self-assembly can be controlled either at the cycle stage or at the polymer stage. Because the "supramonomer" is cyclic in nature, the resulting polymer is tubular, with precisely defined inner and outer diameters.

**RESULTS AND DISCUSSION**

**Monomer–Cyclic Tetramer Equilibrium.** The association state of GC is, as expected, strongly dependent on the H-bonding competing ability of the solvent environment. Highly polar solvents like DMF or DMAC are able to produce full dissociation at concentrations below $10^{-3}$ M. Moving to solvents of intermediate polarity,32 like THF, allowed us to monitor the monomer (GC)—cyclic tetramer ($c$GC$_4$) equilibrium at concentrations between $10^{-2}$ and $10^{-5}$ M by $^1$H NMR, absorption, CD, and emission spectroscopies. Figure 2a shows the evolution of the GC aromatic $^1$H signals as a function of temperature at $10^{-4}$ M in THF-$d_8$ (see Figure S1A,B for additional $^1$H NMR spectra at different temperatures and concentrations). As the temperature decreases from 323 to 268 K, the GC monomer signals (rods) decrease in intensity at the expense of the $c$GC$_4$ signals (squares). H-bonding between G and C nucleobases was confirmed by the characteristic downfield shift of the G-amide and the C-amine proton signals to 13.2 and 10.3 ppm, respectively, as well as by NOESY cross-peaks between these two H-bonded protons (Figures 2b and S1C). It is interesting to note that both GC and $c$GC$_4$ species are in slow exchange at the NMR time scale and that the shape and position of the $^1$H signals do not change much with

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**Figure 1.** (a) Previous and current approaches to self-assembled nanotubes from cyclic species. (b) Structure of achiral GC and chiral R-GC/S-GC molecules. (c) Scheme of the two-step self-assembly of dinucleobase monomers into nanotubes. Side alkyl tails were shortened/removed in the model for the sake of clarity.

**Figure 2.** Cyclotetramerization. Changes observed in the (a) $^1$H NMR, (c) absorption, (d) CD, and (e) emission spectra as a function of temperature for $1.0 \times 10^{-4}$ M solutions of GC in THF-$d_8$ (a) or R-GC in THF (c–e). (b) NOESY NMR spectrum at $\tau_m = 0$ ms of GC ([GC] = $2.0 \times 10^{-3}$ M, 298 K) showing cross-peaks between H-bonded G and C protons. (fg) Representation of the molar fraction of GC molecules associated as $c$GC$_4$ cycles ($\chi_T$) calculated by $^1$H NMR ($\triangle$) or CD (□) as a function of (f) the temperature at different concentrations or (g) the total concentration at 298 K. In (g), the relative abundance of GC, $c$GC$_4$ and open oligomers (like GC$_2$) was simulated (solid lines) using $K_{G:C} = 1.1 \times 10^3$ M$^{-1}$ and $EM = 2$ M.
temperature or concentration,\textsuperscript{32} which underlines the slow dynamics and all-or-nothing behavior of this macrocyclization process.

The $^1\text{H}$ NMR data can be overlapped with optical spectroscopy techniques, and Figure 2c–e displays, respectively, the absorption, CD, and emission changes that occur at 10$^{-4}$ M in THF as a function of temperature. Upon cooling, GC cyclotetramerization is promoted, and a single, rigid conforma- tion, which maintains the two Watson–Crick edges pointing in the same direction, is fixed.\textsuperscript{32–35} This is typically monitored by (1) a red-shift in absorbance with a characteristic new maximum at 420 nm; (2) a decrease in emission intensity and a marked red-shift from 439 nm (GC) to 428 nm (cGC$_4$); and (3) a CD Cotton effect, positive for c-GC$_4$ and negative for c-R-GC$_4$ with zero-crossing at 434 nm. Figure S1D,E contains additional temperature- and concentration-dependent spectra in THF.\textsuperscript{32–35}

Such spectral changes can be used to determine the molar fraction of GC molecules associated as GC$_4$ ($X_T$) as a function of temperature (Figure 2f) or concentration (Figure 2g). The cyclization trends obtained from NMR and CD measurements at the same concentrations show an excellent agreement. Fitting the NMR data to a cyclotetramerization process afforded the corresponding equilibrium constants ($K_{GC}$; see the Supporting Information), from which the effective molarity (EM) values could be calculated as $EM = K_{GC}$ (Tables 1a and S1), where $K_{GC}$ is the reference association constant between nucleobases in THF.\textsuperscript{32}

Using these calculated EM and $K_{GC}$ values, we built speciation curves in which monomer, cycletetramer, and small open oligomers were included (Figure 2g). These curves, in which the relative distribution of species is represented as a function of overall concentration, illustrate graphically the all-or-nothing process observed in solution, where mostly GC monomer (green) and cGC$_4$ macrocycles (purple) are in equilibrium, and the participation of small open oligomers (like dimer GC$_3$; in red) is insignificant. Furthermore, these simulated curves overlap quite nicely the experimental dilution data measured by $^1\text{H}$ NMR and CD, which corroborates the high chelate cooperativity calculated for this system. On the other hand, a van't Hoff representation of the temperature-dependent data provided the enthalpic ($\Delta H_T$) and entropic ($\Delta S_T$) changes associated with the cyclization process in THF. These values are also displayed in Tables 1a and S1.

Analyses similar to those performed in THF were made in less-competing aromatic solvents like chlorobenzene or toluene (see section S2), where $K_{GC}$ increases considerably\textsuperscript{39,40} and the equilibrium is further shifted to the cycle side.

### Cyclic Tetramer–Polymer Equilibrium

We then proceeded to force further aggregation by employing highly apolar alkane media, like methycyclohexane (MCH), heptane, or dodecane. Unfortunately, GC is not sufficiently soluble in these solvents (see below and Figure S3A,B), even at low concentrations and/or high temperatures, which indicated that this compound forms large aggregates that prevented a proper analysis of the polymerization process. This could be solved, nonetheless, by analyzing good and bad solvent mixtures. Specifically, we performed a set of experiments in which the volume fraction of an alkane ($V_a$) is increased in mixtures with THF, CHCl$_3$, or toluene at constant concentration and temperature. Although a similar qualitative outcome is obtained in all of these experiments, no matter the good–bad solvent combination (see Figure S3B–D), the best results were obtained in THF–alkane mixtures.

As $V_a$ is increased, the spectroscopic features that characterize the cyclotetramerization process, as described above in THF and toluene, are first observed. For instance, upon monitoring this process by $^1\text{H}$ NMR at a 3.0 × 10$^{-4}$ M concentration (Figure 3a), the residual monomer signals first disappear and the cGC$_4$ macrocycle is formed quantitatively. When this process was recorded by CD, absorption, or emission spectroscopy at similar or lower concentrations (Figure 3b–d; green to purple spectra), we also saw the typical features of cGC$_4$ formation: a CD signal appears, a red-shifted absorption shoulder, and also a red shift and decrease in emission intensity as the heptane content is increased. Further increasing $V_a$ to 0.6–0.9 (depending on concentration; see below) produced no spectroscopic change, indicating that the cGC$_4$ species is quantitatively stabilized in solution.

However, at an even higher $V_a$ a distinct aggregation process is clearly detected (purple to pink spectra). In $^1\text{H}$ NMR, very minor chemical shifts ($\Delta$ppm < 0.05) are observed, and then the cGC$_4$ proton signals start to broaden and eventually disappear, which is characteristic of the formation of large aggregates. Unfortunately, as shown in Figure 3a, the moment the macrocycles start to aggregate, the proton signal of the peripheral amide also broadens and disappears, which prevented us from monitoring intramolecular H-bonding interactions. Turning to optical spectroscopy, an additional absorption red-shift to 425 nm is observed, while emission is further quenched and slightly blue-shifted. However, the most remarkable changes were detected in the CD measurements: at high $V_a$, the cGC$_4$ CD features evolve with clear isosbestic points to a new signal exhibiting a Cotton effect at 428 nm, that is, positive for S-GC and negative for R-GC (Figure S3E). Because of the similarity of these final spectroscopic features at
Figure 3. Supramolecular polymerization. (a–d) Changes observed in the (a) aromatic region of the \(^1\)H NMR spectra by increasing the volume fraction of cyclohexane-\(d_{12}\) (\(V_c\)) in mixtures with THF-\(d_4\) (\(\text{[GC]} = 3.0 \times 10^{-4} \text{ M}\)); (b) absorption, (c) CD, and (d) emission spectra as a function of the volume fraction of heptane (\(V_h\)) in mixtures with THF-\(d_4\) (\(\text{[S-GC]} = 3.0 \times 10^{-3} \text{ M}\)). (e–g) Changes in the S-GC CD signal at 429 nm at several concentrations as a function of (e) volume fraction of heptane (\(V_h\)) or (f) volume fraction of THF (\(V_{THF}\)) at 298 K or (g) temperature at \(V_h = 0.97\) (\(\alpha_f = \text{fraction of cyclotetramers}, \alpha_N = \text{fraction of nanotubes}, \alpha_{agg} = \text{fraction of aggregated species}\)).

high heptane contents to those measured for the initial dispersions in alkanes, this second sharp transition was attributed to a supramolecular polymerization process to yield \(\text{cGC}_{\text{f}_{\text{m}}}\) which would be driven by \(\pi-\pi\) stacking interactions between the large \(\pi\)-conjugated surface generated upon cyclization and by H-bonding interactions between the four peripheral amides (see Figure 1c). As a matter of fact, related G–C monomers lacking these amide groups at the C base did not undergo this second self-assembly process, and remained associated as \(\text{cGC}_{\text{f}}\) even in pure heptane (see Figure S3F).

Both self-assembly stages, cyclotetramerization and polymerization, are displayed in Figure 3e by monitoring the evolution of the GC CD features as a function of \(V_s\) at different concentrations (see also Figure S3G). It is clear that both processes are strongly dependent on the overall concentration. For instance, at \(3.0 \times 10^{-4} \text{ M}\) we mainly start with a GC–cGC\(_4\) equilibrium that is shifted to the cyclical species up to \(V_s = 0.3\), while the polymerization transition is detected above \(V_s = 0.65\). At \(1.0 \times 10^{-3} \text{ M}\) in contrast, we observe the whole two-step self-assembly process: from the monomer in pure THF, to the cGC\(_4\) cycle within the \(V_s = 0.5–0.9\) plateau, and then to the polymer above \(V_s = 0.95\).

Such \(\text{cGC}_{\text{f}}-\text{(cGC)}\) transitions as a function of the solvent composition were fitted to an extended nucleation–elongation model developed by de Greef, Meijer, and co-workers (Figures 3f and S3G, Tables 1b and S2),\(^{41}\) which allows the calculation of the Gibb's free energy gain upon monomer addition (\(\Delta G^\circ\)), the \(m\) parameter that characterizes the ability of the good solvent to associate with the monomer thereby destabilizing the supramolecular aggregated species, as well as the equilibrium nucleation (\(K_n\)) and elongation (\(K_e\)) constants, whose ratio defines the cooperative parameter (\(\sigma\)). A detailed explanation is provided in section S3 of the Supporting Information.

We then turned to temperature-dependent studies to obtain complementary thermodynamic parameters for this polymerization process. We fixed solvent composition at \(V_h = 0.97\) and analyzed the CD changes along cooling cycles within the 329–268 K range (Figures 3g and S3H). The nonsigmoidal curves obtained at three different concentrations could be fitted again to a cooperative nucleation–elongation model,\(^{42–44}\) in which the polymerization process can be divided into a nucleation and an elongation phase. The magnitudes \(T_e\) (elongation temperature), \(K_n\) and \(K_e\) (nucleation and elongation constants), \(\sigma\) (cooperativity factor), \(\Delta H^\circ\) and \(\Delta H^\circ_e\) (nucleation and elongation enthalpies), and \(\Delta S^\circ\) (polymerization entropy) can be obtained from a nonlinear least-squares analysis of the experimental melting curves (Tables 1c and S3; see also Supporting Information section S3).

The degrees of cooperativity calculated in these experiments are lower than those determined before as a function of solvent composition, but this is not surprising, because each kind of analysis and experimental conditions differ substantially.

Characterization of the Self-Assembled Nanotubes. We then proceeded to characterize the final GC aggregates obtained after the polymerization process, to confirm their dimensions and tubular nature. However, solution measurements, like dynamic light scattering (DLS) or small-angle X-ray scattering (SAXS), at high heptane contents faced a challenging scenario, because of the evolution of a third hierarchical assembly level that involves the formation of large bundled agglomerates. In fact, after the polymerization process is complete at high \(V_h\) and on a time scale that depends on concentration (ranging from a few hours to several days), we observed that the solution became turbid and a precipitate appeared. When monitoring this process spectroscopically over time, the shape of the absorption and CD spectra did not change, but a gradual loss in absorption intensity and a baseline rise, attributed to scattering, were noted. Once this final aggregation state is reached, we noted quite marked kinetic effects, and the \(\text{cGC}_{\text{f}}\) dispersions are rather inert to disaggregation, so solubilization by heating or dilution required unusually long times.
Despite these experimental difficulties, DLS measurements (Figure S4A) performed at several concentrations and THF:heptane ratios, thus targeting cycle ($V_a = 0.4$) and polymer ($V_a = 0.99$) states, were in agreement with the formation of large anisotropic aggregates at high alkane contents. On the other hand, SAXS experiments, obtained immediately after sample preparation at high $V_a$, provided an indication of cylindrical organization of large particles. As shown in Figure 4a (black curve), the position of the first peak (marked with an arrow) indicates a regular dimension of 3.8 nm, attributed to the distance between bundled cylinder centers, while according to the vague second peak the packing appears hexagonal. However, these SAXS measurements, which required relatively high concentrations, were particularly sensitive to the precipitation process, and the patterns recorded evolved with time (compare black and blue curves in Figure 4a), eventually providing a picture of the smaller particles that remained in solution. In any case, fitting of the data acquired at different time lapses to a cylindrical core−shell model was consistent with a cylinder diameter of $4 \pm 1$ nm and a core diameter of about 1 nm.

Finally, we analyzed (S−R)-GC samples, drop-casted from diluted solutions with high heptane contents ($V_a \geq 0.9$), by different microscopy techniques (SEM, AFM, and TEM; see section S4.3). SEM measurements (Figures 4b and S4C) confirmed the formation of networks of large fibrilar aggregates. A closer analysis by TEM (Figure 4c,d) revealed that these aggregates consist of heavily bundled longitudinal objects with a measured diameter of $3.9 \pm 0.7$ nm, which coincides with the hard aromatic section of the cyclic tetramers (Figure 4e). Organization into aligned nanotube bundles increases as the time in solution before deposition becomes longer (please compare Figures 4c/S4F and 4d/S4G), and is plausibly driven by van der Waals interactions between interdigitated peripheral chains.

**CONCLUSIONS**

In short, by means of a novel supramolecular design, which involves H-bonding motifs and cooperative effects of different hierarchy and acting in orthogonal directions, we demonstrated herein that we can guide the assembly of rod-like $\pi$-conjugated molecules into precisely defined nanotubular structures through a decoupled two-step process. A cyclo-tetramerization event, displaying remarkably high chelate cooperativities, takes place first through G:C Watson−Crick pairing. Subsequently, a nucleation−growth polymerization process can be triggered, where the cycle acts as a "supramolecular monomer". This unprecedented approach may allow access to diverse custom-tailored self-assembled nanotubes by performing small modifications in the monomer structure.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.9b07868.
Experimental details, compound synthesis and characterizations, additional spectroscopy and microscopy data, and analysis (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Funding from the European Research Council (ERC-Starting Grant 279534 PROGRAM-NANO) and MINECO (CTQ2014-57729-P and CTQ2017-84727-P) is gratefully acknowledged. V.V.-G. is grateful to MINECO for a FPI Grant. I.K.V. would like to acknowledge The Netherlands Organization for Scientific Research (NWO VIDI Grant 723.014.006) for financial support.

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