Hydrogen-bond self-assembly of DNA-analogues into hexameric rosettes†

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The self-assembly of a DNA-analogue hexameric rosette from triaminopyrimidine and cyanuric acid–based nucleosides, and its subsequent aggregation into rod-like morphologies is reported.

The association of DNA beyond the regular double helix has been the subject of tremendous research interest.[1–4] In particular, DNA triple helices[1] and guanine (G)-quadruplexes[2] are attractive because of their well-defined morphologies, their biological properties and therapeutic implications.[3,4] Expanding the molecularity of DNA beyond these motifs can result in new functions for this already versatile molecule, including artificial ion channels and novel templates for nanotechnology. [5] A DNA *iso*-guanosine pentaplex has been generated using metal-assisted hydrogen-bonding.[6] Artificial bases have been incorporated into DNA[7] to develop new hydrogen-bonded,[7a,b] π-stacked,[7c] or metal-coordinated[7d–f] DNA base pairs or duplexes, many of which can be enzymatically replicated to possibly perform new biological tasks. DNA-inspired hydrogen-bond self-assembly has been used to create supramolecular cages,[8] helical,[9] linear,[10] and macrocyclic structures.[11,12] We here report the synthesis of new DNA-based artificial nucleosides, and their self-assembly into the first example of a *DNA-analogue hexameric rosette*. This study provides the basis to potentially expand the molecularity of DNA to a hexaplex.

The strategy developed here uses DNA base analogues **1** and **3** to generate a new family of hydrogen-bonded supramolecular structures (Chart 1). While natural DNA bases normally use one hydrogen-bonding "face", and as such, associate into a double helix, nucleoside analogue **1** contains a triaminopyrimidine unit attached to a protected deoxyribofuranose, providing two donor–acceptor–donor (DAD) hydrogen-bonding faces. A 120°-angle spatial orientation is expected between these faces. Thus, instead of dimeric complexes, compound **1** is expected to associate with diethylbarbituric acid **2** and cyanuric acid molecule **3** to give hexameric rosettes,[11e,13] or alternatively linear tapes structures (Chart 1).

The feasibility of the proposed self-assembly was first examined using semi-empirical (PM3) molecular orbital calculations, as well as molecular mechanics (MM+) calculations. The geometries of the diethylbarbituric acid, triaminopyrimidine **1** and cyanuric acid **3** monomers were first investigated by PM3 calculations.[14] These monomers showed 120°-orientations between the triple hydrogen-bonding sites. Using these optimized structures, MM^+ calculations were carried out to evaluate the geometry of the hydrogen-bonded supramolecular structures arising from the self-assembly of **1** and **2**, as well as **1** and **3**. (Chart 1) These calculations suggest that the self-assembly of nucleosides **1** and **3** to form linear tapes presents a significant steric cost, because of the resulting close packing distances of the silyl protecting groups on the sugar units of **1** and **3**.†[15] On the other hand, the self-assembly of **1** and **3** into discrete hexameric macrocycles would relieve these steric interactions. The MM+ calculations also show that the steric cost in the formation of linear tapes from nucleoside **1** and diethylbarbituric acid **2** is lower, both because of the smaller size of **2**, and because the tetrahedral carbon atom in **2** allows these linear tapes to deviate from planarity, thus reducing the steric repulsions between the silyl groups. Thus, these calculations predict that the formation of discrete rosettes from nucleosides **1** and **3** is sterically favored.†[13,15]

The hydrogen-bonded self-assembly of nucleoside **1** with diethylbarbituric acid **2** was examined by ^{1}H NMR spectroscopy and electrospray ionisation mass spectrometry (ESI-MS). ^{1}H NMR titration of nucleoside **1** by diethylbarbituric acid **2** in CD_2Cl_2 showed a 1.8 ppm downfield shift of the amino protons, characteristic of hydrogen-bond formation. However, the NMR peak widths of **1**, which were broad in $CDCl_3$ as a result of self-association of the triaminopyrimidine units, were not improved upon addition of 1 equiv. **2**, even after ultrasonication for

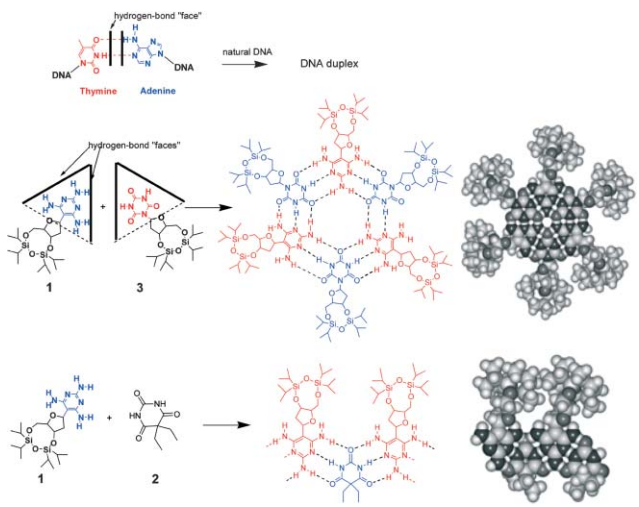

Chart 1

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2 days.†[15] This result suggests the formation of polydisperse, oligomeric structures from **1** and **2** in solution. ESI-MS analysis of equimolar mixtures of **1** and **2** showed two major peaks for the individual monomers **1** and **2**. Peaks of much lower intensity could be attributed to dimers, trimers, tetramers, pentamers and hexamers, bound to solvent molecules or ions, consistent with the association of triaminopyrimidine **1** and diethylbarbituric acid **2** into oligomeric species.

The self-assembly of nucleosides **1** and **3** was also investigated by ^{1}H NMR and by ESI-MS. Titration of **1** with a solution of **3** caused a downfield shift of the amino protons (1.75 ppm), due to hydrogen-bonding of the two DNA-base analogues. Sharper ^{1}H NMR peaks were observed for the complementary molecules upon adding 1 equivalent of cyanuric acid **3**, suggesting the creation of a discrete structure from DNA base analogues **1** and **3** in solution.†[15] In addition to peaks corresponding to monomeric species, ESI-MS analysis of an equimolar mixture of **1** and **3** showed the presence of aggregates corresponding to the triply charged hexameric rosettes containing chloride ions in the 900–1000 Da mass range, as well as the doubly charged rosette with Cl^- ions in the 1500 Da range. Vapor pressure osmometry (VPO) studies were carried out on equimolar solutions of **1** and **3** in CH_2Cl_2. Two molecular weight standards were used for these experiments (benzil and polystyrene). The plots of VPO molecular weight *versus* concentration show clear *y*-intercepts corresponding to M_n = 2844 using benzil (Δ = 2.1%) and M_n = 3060 using polystyrene (Δ = 5.3%), consistent with the formation of a hexameric rosette (M_n = 2907) in solution. As a result, ^{1}H NMR, ESI-MS and VPO studies, as well as molecular modeling calculations are all consistent with the hydrogen-bonded self-assembly of nucleosides **1** and **3** into discrete cyclic hexamers. This is in contrast with the oligomeric linear tapes obtained from triaminopyrimidine nucleoside **1** and diethylbarbituric acid **2**.

Substituted pyrimidines, including 2,4,6-triaminopyrimidine derivatives have been shown to exhibit fluorescence.[16] We were thus interested in examining the photophysical properties of triaminopyrimidine nucleoside **1**, and in determining whether these properties are affected when **1** undergoes self-assembly with the cyanuric acid building block **3**. The UV-Vis absorbance spectra of chloroform solutions of **1** showed a principal absorbance at λ_{max} = 254 nm, corresponding to a $\pi \rightarrow \pi^*$ transition of the triaminopyrimidine **1**, along with a shoulder around 350 nm. Emission spectra were obtained upon excitation of chlororoform solutions of **1** at 350 nm, and showed a principal peak at 410 nm.[17] Addition of one equivalent of **3** to a solution of **1** in chloroform induced a 1.4-fold fluorescence decrease, along with a 3 nm-bathochromic shift.[15] This behavior is consistent with possible π-stacking of the triaminopyridine unit, as a result of the hydrogen-bond assembly of **1** and **3** (see below, and Fig. 1c), which can result in fluorescence quenching and shifts to lower emission frequencies.[18] Thus, **1** can act as a fluorescence probe of its local environment, and can detect the self-assembly process through fluorescence quenching.

Transmission electron microscopy (TEM) studies of triaminopyrimidine **1**–diethylbarbituric acid **2** and triaminopyrimidine **1**–cyanuric acid **3** mixtures allowed direct visualisation of their different self-assembly behaviour. These studies also suggested a second level of self-organization of these molecules. Fig. 1 shows TEM of carbon-coated grids prepared from equimolar mixtures of

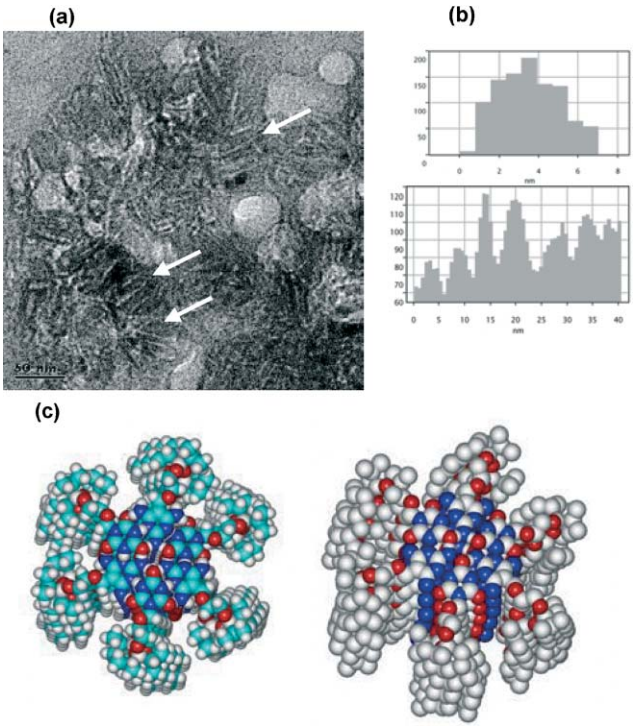

Fig. 1 (a) Transmission electron micrographs prepared from solutions of **1** and **3** using uranyl acetate staining. (b) Bottom: Profile analysis of the TEM images. Top: magnified profile analysis of a single rod-like structure. (c) Proposed model for the stacking geometry of the nucleoside hexameric rosette, generated from the MM$^+$ optimised macrocycles, top and side view.

1 and **3** in dichloromethane.†[15] Large, sheet-like aggregates were observed for **1** and diethylbarbituric acid **2**.†[15] On the other hand, the TEM of mixtures of nucleosides **1** and **3** show the formation of elongated aggregates, with length as great as 170 nm (Fig. 1a). The outer hydrodynamic diameter of these aggregates was determined from the intensity profile of the electron micrograph (Figs. 1b) as *ca.* 4.2 $\pm$ 1.0 nm, in agreement with the calculated diameter of the DNA analogue hexamer (3.2 nm). This diameter was observed on freshly purified samples of **1** and **3** mixtures, and for various direct deposition conditions (with and without staining). As suggested by the fluorescence measurements, this result is consistent with further organization of the supramolecular cycles from triaminopyrimidine **1** and cyanuric acid **3** equimolar mixtures through π–π stacking and/or alkyl–alkyl interactions (Fig. 1c).

Dynamic light scattering (DLS) studies were carried out on equimolar mixtures of triaminopyrimidine **1** and cyanuric acid **3** in dichloromethane at scattering angles of 45°, 90°, and 135°, and confirmed the presence of these rod-like aggregates in solution. The hydrodynamic diameters obtained for these angles were significantly different from one angle to the other, *e.g.*, 165.1 nm at 45° and 93.8 nm at 135°, demonstrating the presence of elongated, rather than spherical aggregates in solution.

Previous studies of hydrogen-bonded rosettes have shown that cylindrical structures can arise as a result of stacking interactions along the normal to the hexameric rosette plane.[11e,12d] Fig. 1c shows the proposed model for π-stacked hexameric rosettes from **1** and **3**, generated from the MM$^+$ optimised macrocycle geometry. In this model, favourable π–π stacking of these supramolecular

cycles can lead to helical structures with high aspect ratio, where the hexameric rosettes are slightly twisted relative to the normal of the macrocycle plane at interplanar distances ranging between *ca.* 4.5 Å and 6 Å.

In conclusion, we have shown the synthesis of a new triaminopyridine nucleoside, and its self-assembly with a cyanuric acid nucleoside into the first example of a DNA-based hexameric rosette. DLS and TEM studies suggest further self-organization of these rosettes into long rod-like aggregates, and emission studies show that the triaminopyridine DNA analogue can act as a fluorescence probe of this self-assembly process. While natural DNA is prone to form higher-order aggregates, such as triplexes and quadruplexes, there are no previous examples of the formation of hexameric complexes from this molecule. Our studies suggest that one can modify the interior of the DNA molecule, and use the principles of supramolecular chemistry, in order to expand its information content, and induce it to code for higher-order structures. Further studies are underway to generate oligonucleotides using **1** and **3**, and to study their self-assembly into expanded structures.

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