Supplementary Material for Chemical Communications

Hydrogen-Bond Self-Assembly of DNA-Analogues into Hexameric Rosettes

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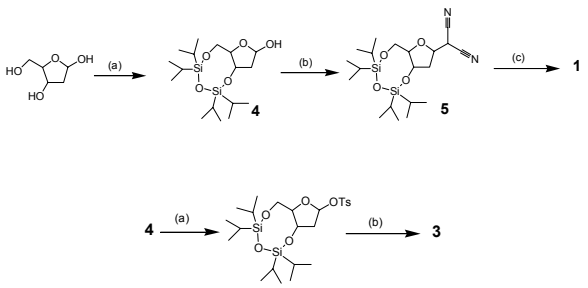
Supporting Information

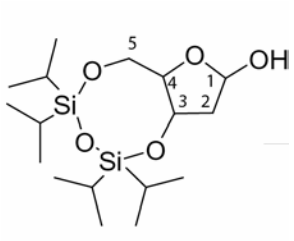
Materials. All reagents and compound **2** were purchased from the Aldrich Chemical Co. The structures of the intermediates and the final products were confirmed by NMR spectroscopy and mass spectrometry.

Equipment. ^{1}H and ^{13}C-NMR spectra were acquired on a Varian Mercury 400 and Varian Unity 500 spectrometers, 2D-COSY NMR spectra were acquired on a Varian Unity 500 spectrometer. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) mass spectra were acquired by direct infusion into a Quattro II triple quadrupole (Micromass, Manchester, UK) with the spray voltage set to 3.5 kV, the source temperature 150-200ºC, sample infusion rate 5 μl/min using a syringe pump (Harvard Apparatus). Vapor pressure osmometry (VPO) measurements were carried out in toluene using a Knauer K-7000 osmometer. UV-vis spectra were measured on a Varian CARY 1 UV-vis spectrophotometer equipped with a Peltier temperature controller. Transmission electron microscopy (TEM) was conducted on a JEOL-2000 FX instrument by using the direct method with and without staining.

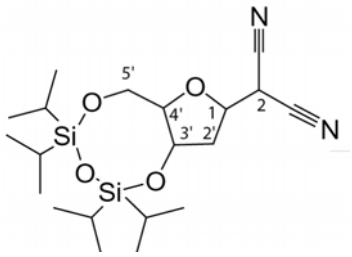
Synthesis



Scheme 1. Synthesis of protected nucleosides **1** and **3**. (a) 1,1',3,3'-tetraisopropyldisiloxane, pyridine, 40 °C, 2 hrs, 70%. (b) Malononitrile (3 eq.), NaH (3 eq.), THF, 0 °C → r.t., 2 hrs, 40%. (c) Guanidine hydrochloride, Na, EtOH, reflux, 5 hrs. (d) *p*-toluenesulfonyl chloride, Et_3N, CH_2Cl_2, 35%. (e) Potassium nonaflurobutanesulfonate, hexamethyldisilazane, $(CH_3)_3SiCl$, reflux, 3 hrs, 70%.

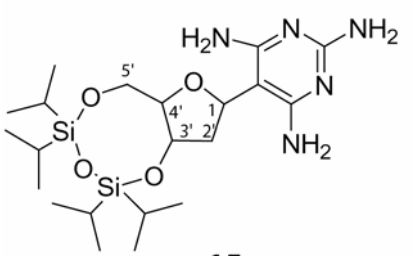


2-deoxy-3,5-*bis*-O-(tetraisopropylsiloxyl)-D-ribofuranose (4). 2-deoxy-D-ribose (1 g, 7.41 mmol) in freshly distilled pyridine (9.26 mL) was stirred under N_2 atmosphere at 40°C in presence of 1,3-dichloro-1,1',3,3'-tetraisopropyldisiloxane (2.6 mL, 8.89 mmol). After 2 hrs, the reaction mixture was neutralized using 1M HCl (3 mL) and diluted with diethyl ether (50 mL). The organic layer was washed with saturated $NaHCO_3$ (50 mL) and water (2 x 50 mL), then dried over $MgSO_4$. The solvent was evaporated under reduced pressure to give a colorless oil. The desired compound **4** was isolated by column chromatography on silica gel using an ethylacetate/hexanes (20:90) as the eluent. **4** (1.922 g, 69 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.46 (dd, H_1, 4.8 Hz), 4.45 (m, H_4), 4.06 (dd, H_3, 7.6 Hz), 3.85 (d, H_5, 7.2 Hz), 3.68 (dd, H_5, 8.4 Hz, 15.8), 2.31 (m, H_2), 2.02-2.12 (m, H_2), 1.07 (s, 28 H). ESI-MS (neg): 376.



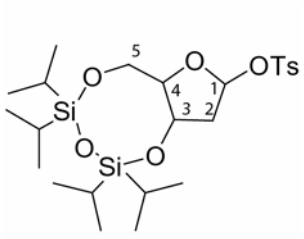
1-(2'-deoxy-5',3'- *bis*-O-(tetraisopropylsiloxyl)- ribofuranosyl) propanedinitrile (5).

Malononitrile (52.7 mg, 0.80 mmol) in freshly distilled THF (0.5 mL) was added dropwise to a suspension of 95% NaH (19.2 mg, 0.80 mmol) in THF (0.25 mL) at 0°C. Protected sugar **4** (100 mg, 0.26 mmol) in THF (0.5 mL) was added dropwise, and the reaction mixture was stirred at room temperature under N_2 atmosphere. The reaction was monitored by TLC until disappearance of the starting material (1 hr), then quenched with saturated NH_4Cl (6 mL). The reaction mixture was diluted with dichloromethane (25 mL) and washed with water (2 x 25 mL) and brine (25 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and the solvents were evaporated under reduced pressure. Compound **22** (37.7 mg, 33 %) was obtained as an off-white oil after silica gel chromatography using ether/hexanes (60:40) as eluent. $R_f \sim 0.25$. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.38 (dd, H_4', 8.0 Hz, 2.4 Hz), 4.13-3.98 (m, H_1', H_3', H_5'), 3.77 (dd, H_5', 6.0Hz, 9.6 Hz), 3.13-3.18 (m, H_2'), 2.55-2.22 (m, H_2'), 1.06 (s, 28 H). APCI-MS: m/z: Calc. 425.0 (M+1), 849.0 (2M+1); found: 425.4 (M+1), 849.1 (2M+1) .



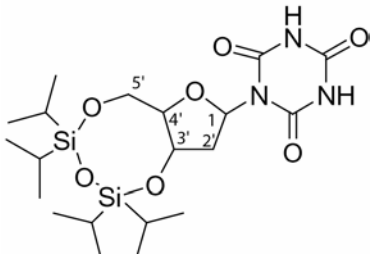
1-(2'-deoxy-3',5'-*bis*-O-(tetraisopropylsiloxyl)-ribofuranosyl)pyrimidine-2,4,6-triamine (1). Sodium (15.05 mg, 0.65 mmol) and guanidine hydrochloride (62.5 mg 0.65 mmol) were

stirred in freshly distilled EtOH (1.5 mL) under N_2 atmosphere until a solution was obtained. A solution of **22** (185.0 mg, 0.44 mmol) in freshly distilled EtOH was added dropwise to the mixture which was heated to reflux for 5 hrs. The reaction mixture was cooled to room temperature and the solvents were evaporated under reduced pressure. The desired compound (60.9 mg, 29%) was isolated by column chromatography on silica gel using dichloromethane/ethanol (0:100 to 40:60 gradient). $R_f \sim 0.1$ (10:90 dichloromethane/ethanol). ^{1}H-NMR (400 MHz, CD_3OD) δ 6.98 (s, NH_2), 4.25 (m, H_1'), 3.82-4.01 (dd, H_3', H_4'), 3.61 (m, H_5'), 2.63 (m, H_5'), 2.01-1.71 (m, $2H_2$'), 1.06 (s, 28 H). ^{13}C-NMR (100 MHz, CD_3OD) δ 159.34, 158.65, 118.69, 77.52, 77.16, 76.41, 69.27, 68.85, 44.85, 38.29, 36.06, 24.43, 23.82, 16.98, 13.00. ESI-MS: m/z : Calc. 484 (M+1), 507 (M+Na+1), found 484.3 (M+1), 507.3 (M+Na+1).



1-O-(*p*-toluenesulfonyl)-2-deoxy-3,5- *bis*-O-(tetraisopropylsiloxyl)- ribofuranose (6). A solution of compound **4** (250 mg, 0.66 mmol) in dry CH_2Cl_2 (5 mL) was stirred with *p*-toluenesulfonyl chloride (150.0 mg, 0.66 mmol) under N_2 atmosphere in presence of Et_3N (0.1 μL) for 2 hrs. The reaction was quenched by adding CH_2Cl_2 (10 mL) and water (10 mL). The organic layer was washed with saturated $NaHCO_3$ (2 x 10 mL) and water (10 mL), then dried over $MgSO_4$. The solvents are evaporated under reduced pressure. The formation of the *p*-toluenesulfonyl derivative **6** was verified by ^{1}H-NMR in $CDCl_3$ which showed 2 sets of doublets of doublets in the aromatic region and 2 singlets corresponding to the methyl protons, consistent with the presence of 2 anomers α and β. (341.7 mg, 97 %) ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.92 (d, Ar-H_1, 8.0 Hz), 7.78 (d, Ar-H_1', 8.0 Hz), 7.40 (d, Ar-H_2, 8.0 Hz),

7.12 (d, Ar-H_2', 8.0 Hz), 5.56 (t, H_1, 7.2 Hz), 4.57 (dd, H_4, 7.2 Hz), 4.12-3.84 (m, H_3, $2H_5$), 3.17 (dd, H_2, 6.8 Hz, 11.6 Hz), 3.00 (m, H_2), 2.50 (s, $1CH_3$), 2.50 (s, $1CH_3$), 2.33 (s, $1CH_3$), 1.04 (s, 28 H).



1-(2-deoxy-3,5-*bis*-O-(tetraisopropylsiloxyl)- ribofuranosyl)cyanuric acid (3). A mixture of the tosyl derivative **6** (353 mg, 0.67 mmol), cyanuric acid (85.2 mg, 0.67 mmol), potassium nonafluorobutanesulfonate (539.6 mg, 1.75 mmol), hexamethyldisilazane (98 μL, 0.54 mmol), and trimethylsilyl chloride (261 μL, 2.08 mmol) was heated to reflux in freshly distilled acetonitrile (6.66 mL) under N_2 atmophere. The reaction was proceeded for 16 hrs, then allowed to cool to room temperature. The solvents were evaporated under reduced pressure and the residue was dissolved with $CHCl_3$ (25 mL), washed with saturated $NaHCO_3$ (25 mL) and water (25 mL), then dried over $MgSO_4$. The solvents were evaporated on the rotovap. The desired compound (119.2 mg, 37%) was isolated by two consecutive column chromatographies on silica gel using dichloromethane then ethylacetate/hexanes 1:1 as the eluent. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.44 (m, H_1'), 4.96 (dd, H_4'), 4.55-4.28 (m, H_3'), 3.98 (dd, H_5'), 3.78 (dd, H_5'), 2.75 (m, H_2'), 2.40 (m, H_2'), 1.07 (s, 28 H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 148.53, 148.16, 147.91, 85.79, 83.60, 81.75, 72.74, 70.42, 63.58, 38.87, 17.86, 13.69. ESI-MS: m/z : Calc. 487 (M+1), 510 (M+Na+1), found 487.3 (M+1), 510.3 (M+Na+1).

Electrospray Ionisation Mass Spectrometry (ESI) measurements. An equimolar solution of triaminopyrimidine **1** with diethylbarbituric acid **2** or cyanuric acid **3** in a 10 % $MeOH/CH_2Cl_2$ mixture was directly infused into a Quattro II triple quadrupole (Micromass, Manchester, UK) configured for positive or negative product ion analysis and used with the spray voltage set to 3.5 kV, source temperature 100-200ºC, sample infusion rate 5 µl/min using a syringe pump (Harvard Apparatus). For molecular weight determination, the spectrometer was programmed to scan for the product ions from *m/z* 150 to *m/z* 2000 in multichannel acquisition mode (MCA) showing m/z for the monomers and the hexamer.

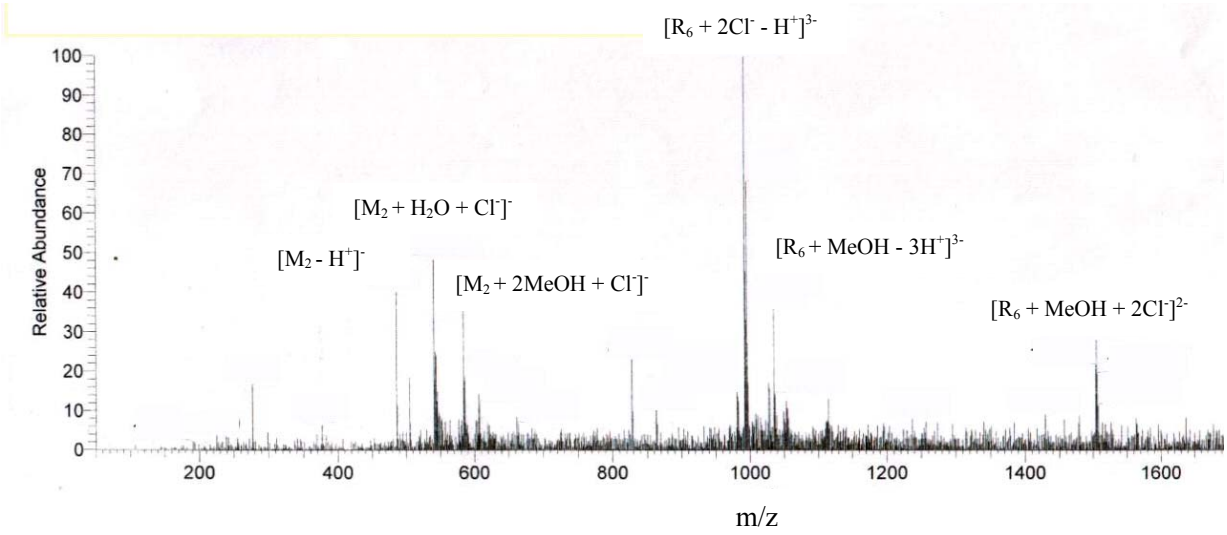


Figure S 1. Electrospray ionisation mass spectrum of equimolar triaminopyrimidine 1 and cyanuric acid 3 mixture in 10% $CHCl_3$/MeOH, negative ion mode. M_1 and M_2 are the monomer molecular weights (M_1 = 483, M_2 = 486), R_6 is the hexameric rosette expected molecular weight (R_6 = 2907)

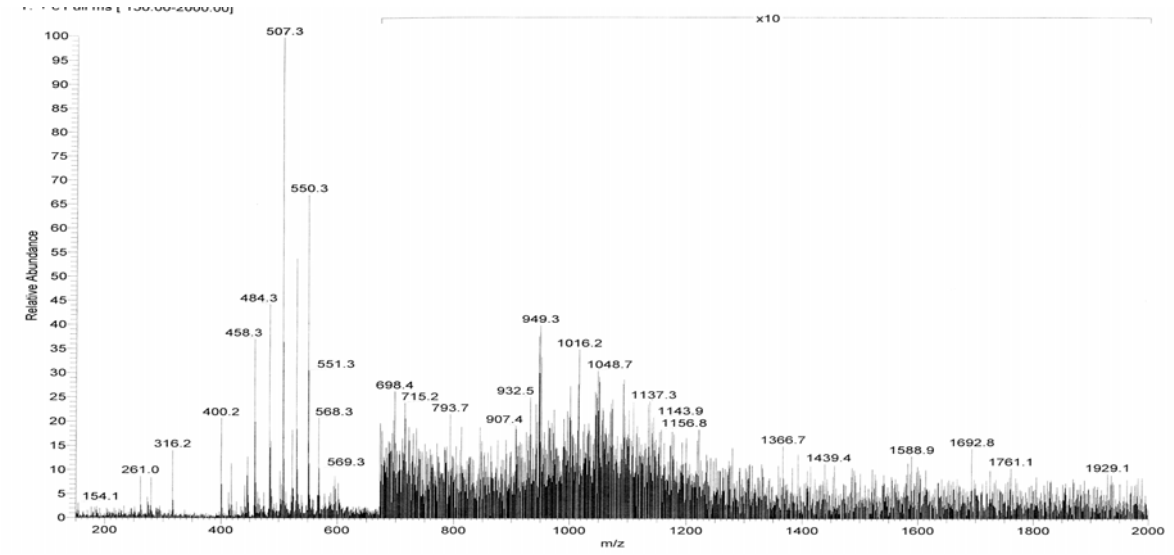


Figure S 2. Electrospray ionisation mass spectrum of equimolar triaminopyrimidine nucleoside **1** and diethylbarbituric acid **2** mixture

^{1}H NMR titration.

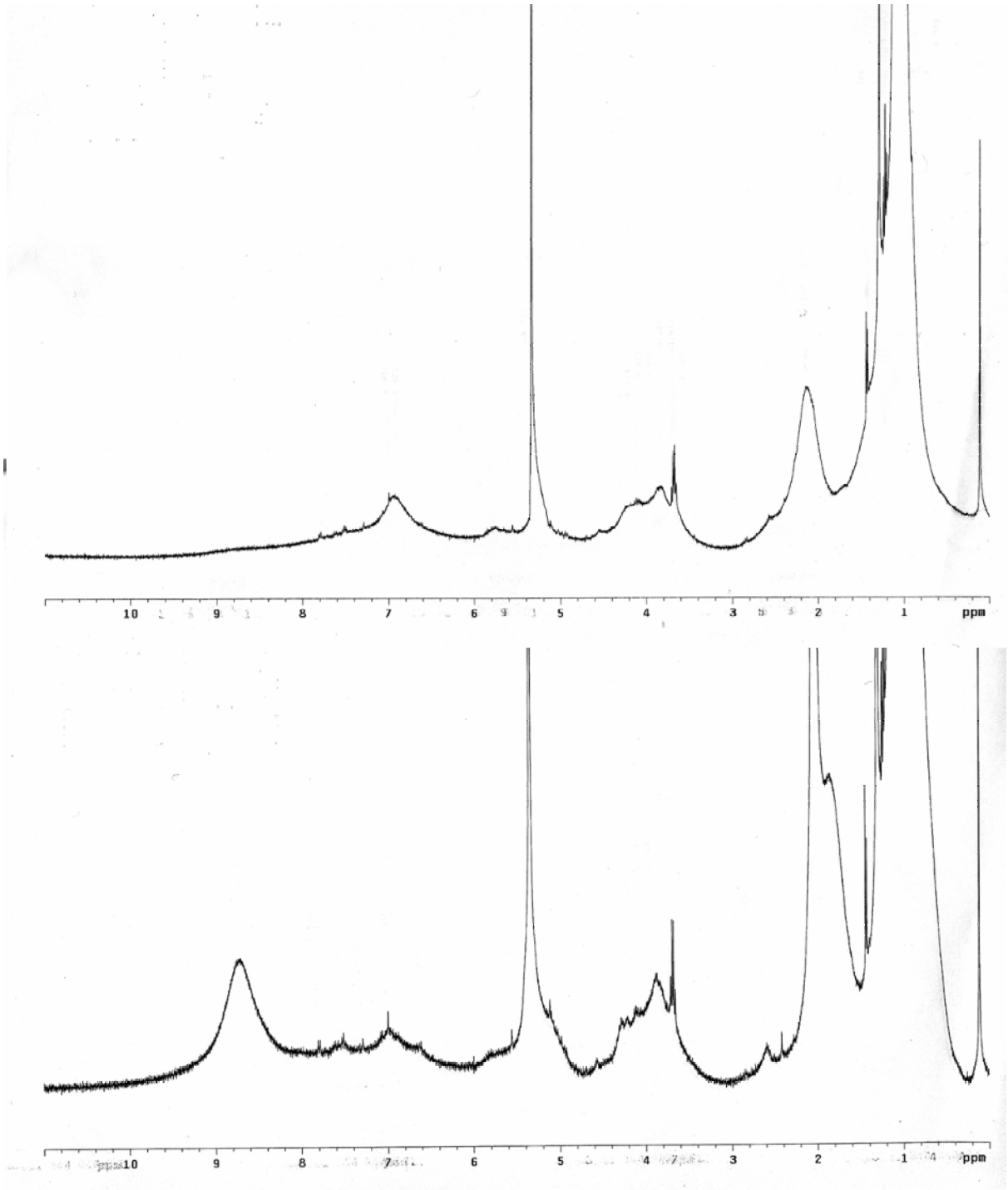


Figure S 3. (a) ^{1}H NMR spectrum of triaminopyrimidine nucleoside **1** alone in $CDCl_3$. (b) ^{1}H NMR spectrum of triaminopyrimidine nucleoside **1** and diethylbarbituric acid **2** in an equimolar mixture in $CDCl_3$. The presence of broad peaks is consistent with the formation of oligomeric aggregates in solution.

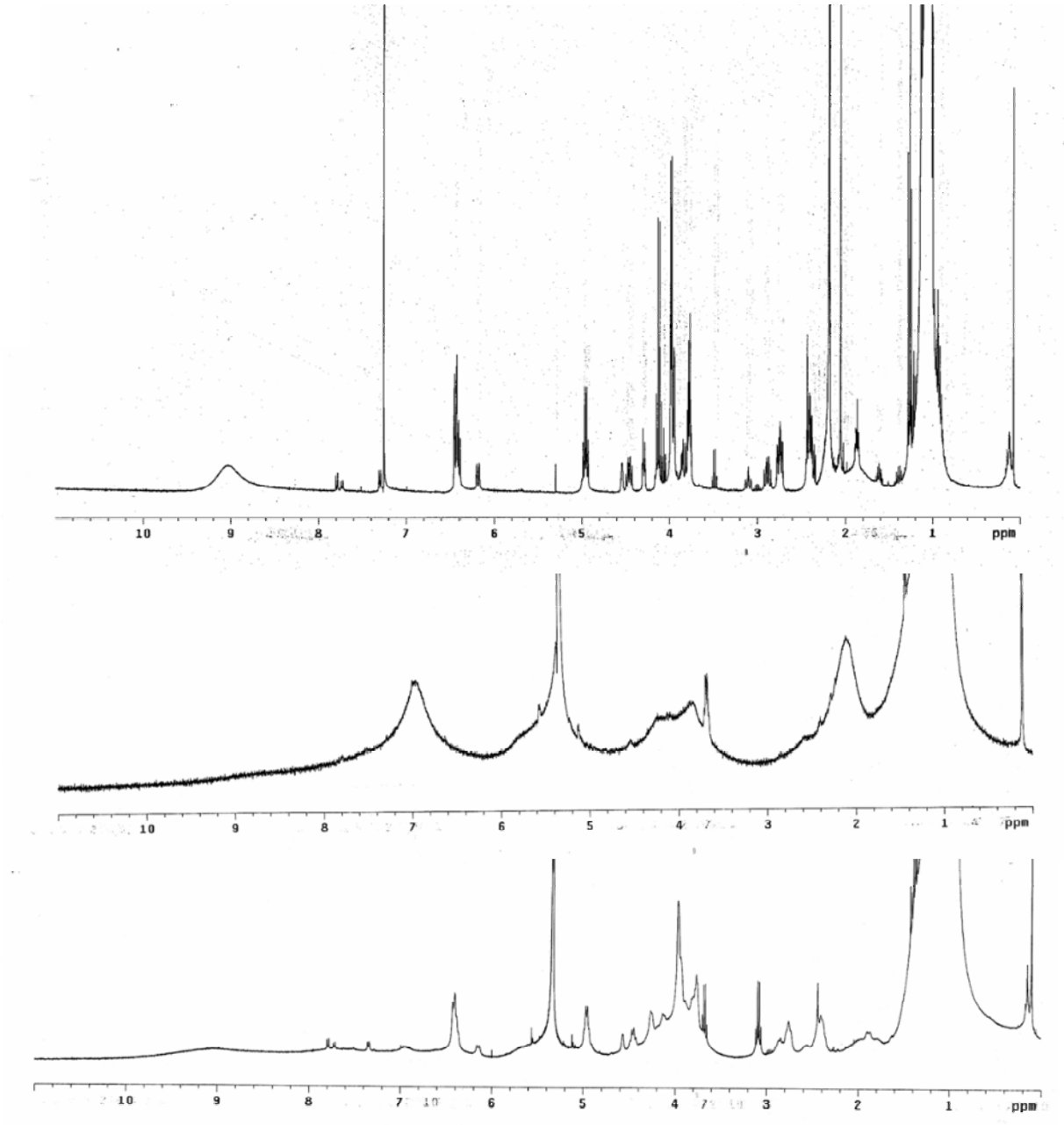


Figure S 4. (a) ^{1}H NMR spectrum of cyanuric acid 3 alone in $CDCl_3$. (b) ^{1}H NMR spectrum of triaminopyrimidine nucleoside 1 alone in $CDCl_3$, showing broad peaks consistent with the presence of oligomeric aggregates in solution. (c) ^{1}H NMR spectrum of triaminopyrimidine nucleoside 1 and cyanuric acid 3 in an equimolar mixture in $CDCl_3$. The significantly narrower peaks suggest the formation of discrete hydrogen-bonded aggregates in solution. Despite a significant gain in peak resolution, a slight peak broadening could be still be observed, likely as a result of the presence of two anomers from both triaminopyrimidine 1 and cyanuric acid 3 nucleosides. (^{1}H NMR spectra for nucleosides 1 and 3 are consistent with the presence of both α- and β-anomeric sugars in solution).

Fluorescence properties.

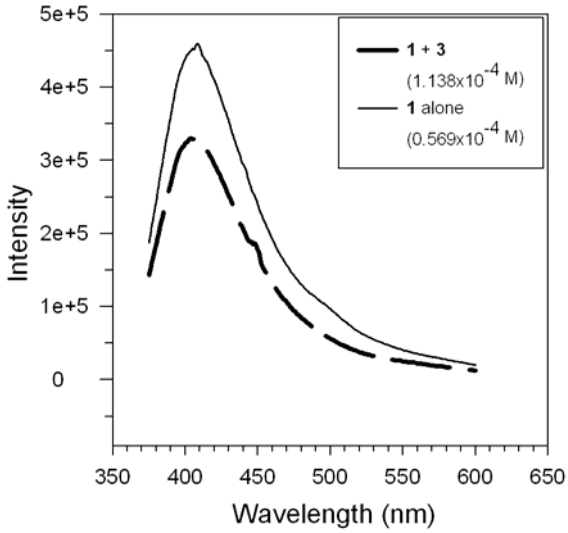


Figure S 5. Steady-state emission fluorescence spectra of triaminopyrimidine **1** alone and in equimolar mixtures of cyanuric acid **3** at λ_{exc} = 350 nm.

Sample preparation for TEM measurements by staining. A solution of cyanuric acid and 2,4,6-triaminopyrimidine (1:1) was prepared by dissolving the sample in dichloromethane at room temperature. The solution (10 μL) was deposited on a carbon-coated 400 mesh copper grid, prewashed with chloroform for 12 hrs, then blotted. A drop of aqueous 2 % uranyl acetate was deposited on the air-dried copper grid, blotted, rinsed with a drop of water after 30 s, then dried on paper filter.

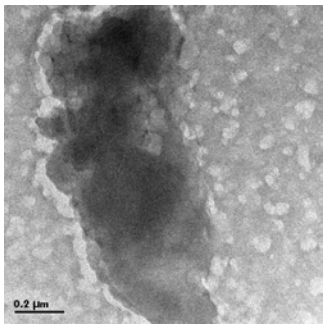


Figure S 6. Transmission electron micrographs from solutions of triaminopyrimidine **1**/diethylbarbituric acid **2**. On the micrographs, the uranyl acetate appears dark gray and the carbon light gray. The error on the measurements is estimated to be ±1 nm.

Dynamic light scattering (DLS) measurements. Dynamic light scattering measurements were carried out using a 532 nm laser equipped photo-multiplier detector. The autocorrelation functions were acquired for θ values of 45°, 90° and 135° for prefiltered equimolar solutions of triaminopyrimidine **1** and cyanuric acid **3** in dichloromethane. Data acquisition was carried out at 20°C and within a few hours to get a better particle size distribution. Inverse Laplace transforms of the data were performed using Provencher's FORTRAN program CONTIN,[1] and diffusion coefficients were determined from the slope of the relaxation frequencies (Γ) vs q^2. Assuming a spherical behaviour for the particles and using the translational coefficient D_{Tr} and the Stokes-Einstein equation, the hydrodynamic diameters are calculated for each scattering angle.

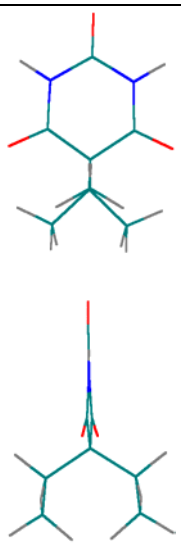
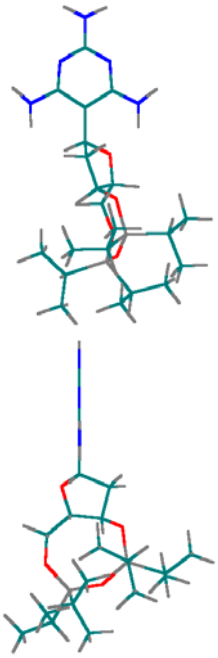
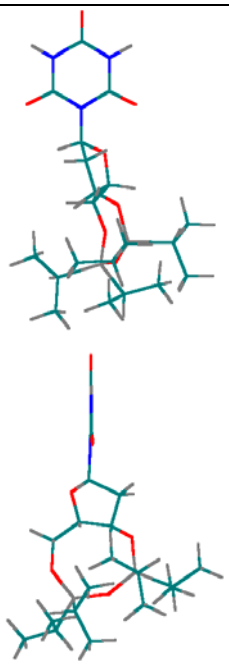
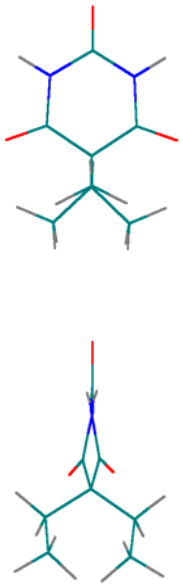
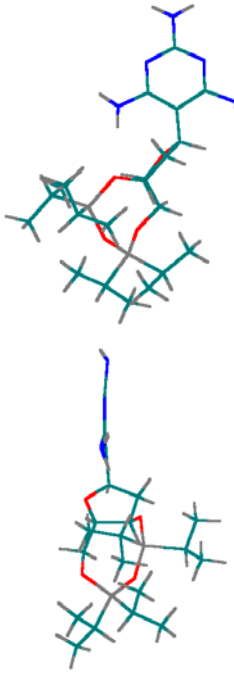
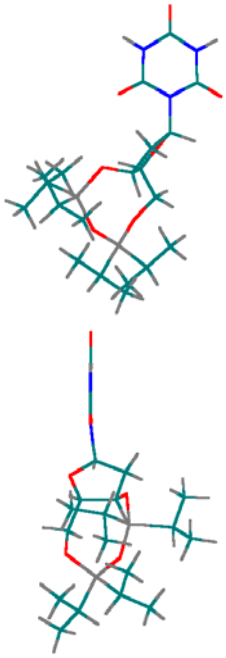
Theoretical Calculations

The geometries of the triaminopyrimidine, cyanuric acid, and barbituric acid monomers were optimized using PM3 until an RMS gradient of 0.01 was reached. Optimisation of the medium-size supramolecular structures was performed using the PM3 semi-empirical molecular orbital theory. After this optimization, another monomer was added and the hydrogen-bonded structure energy was minimized. This monomer addition-optimization sequence was repeated up to the hexameric compound. The hydrogen-bonded geometry and the total heat of formation were extracted for each optimized species. Geometry optimizations used the PM3 method contained in the Gaussian98W[2] program package.

Optimisation of the large-size supramolecular structures were performed using the MM^+ molecular mechanics method. The hydrogen-bonded geometry and the heats of formation

were extracted for each MM^+ optimized species by performing a single point calculation using the semi-empirical PM3 method.

Table 1. Theoretical monomer structures determined using MM^+ and PM3 geometry optimisations.

	Diethylbarbituric acid **2**	Triaminopyrimidine **1**	Cyanuric acid **3**
MM^+			
PM3			

Molecular Modelling of Hydrogen-bonded Aggregates

The MM^+ optimised hydrogen-bonded structures obtained from triaminopyrimidine **1** and diethylbarbituric acid **2,** as well as triaminopyrimidine **1** and cyanuric acid **3** were analysed to determine the most favourable supramolecular geometry. The influence of the sterically encumbered nucleoside on the self-assembly behaviour was also determined from the computational results (Table 2).

In all optimized DNA-analogue aggregates,[3] the barbituric acid and cyanuric acid C=O bond lengthens slightly from 1.21 Å to 1.23 Å, the N–H bond from 0.99 Å to 1.02 Å when bound. The triaminopyrimidine N-H bond also lengthens from 1.00 Å to 1.02 Å, leading to the formation of strong, ionic N···H and O···H hydrogen-bonds (N···H = 1.00 Å, O···H = 0.95 Å). PM3 calculations performed on similar triaminopyrimidine/barbituric acid complexes resulted in lengths of 1.66 Å and 1.78 Å for the N···H and O···H hydrogen-bonds, along with significant N-H and C=O bond lengthening in both monomers.

Table 2. Theoretical hydrogen-bonded **2** + **1** complexes determined using MM^+ geometry optimisations

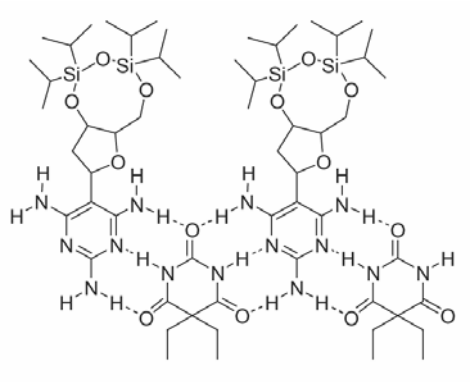
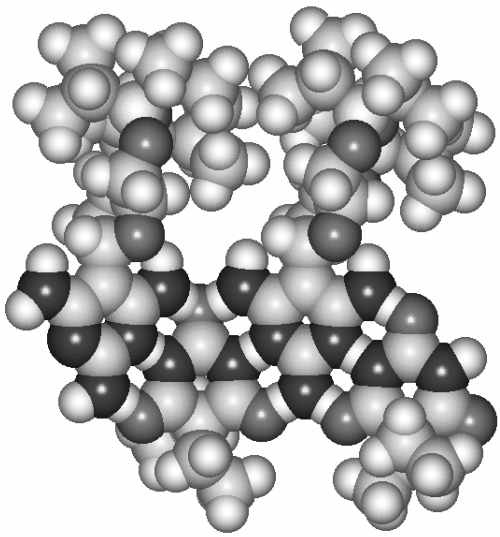
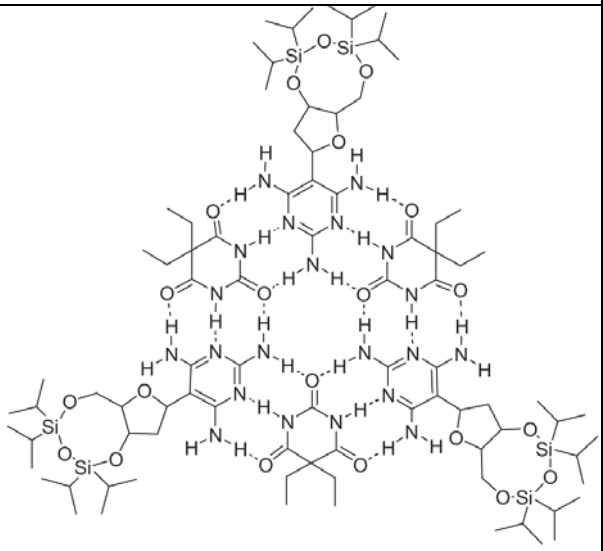
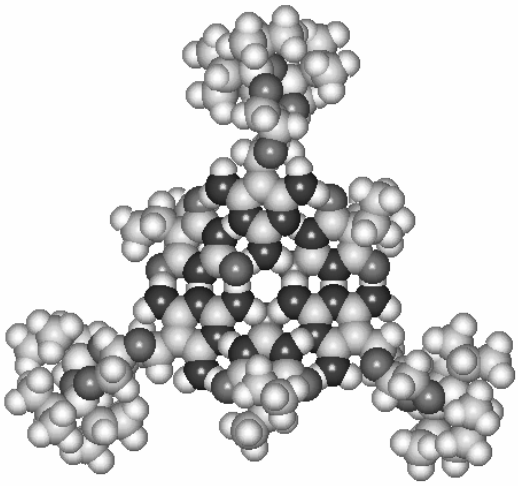
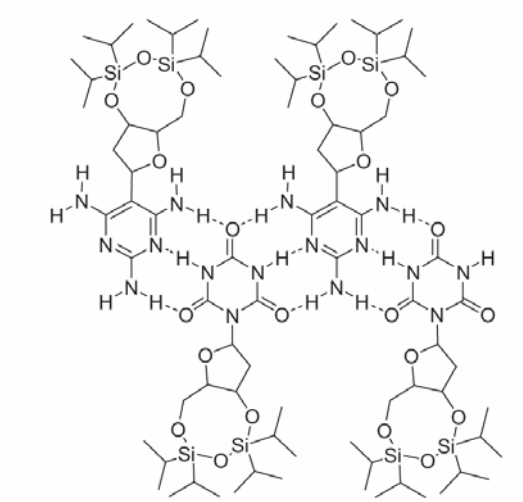
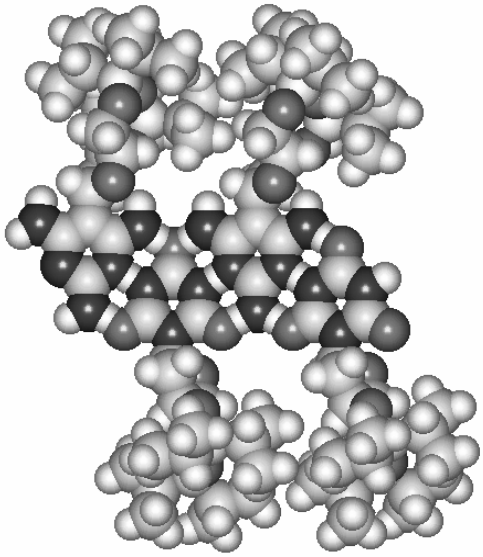
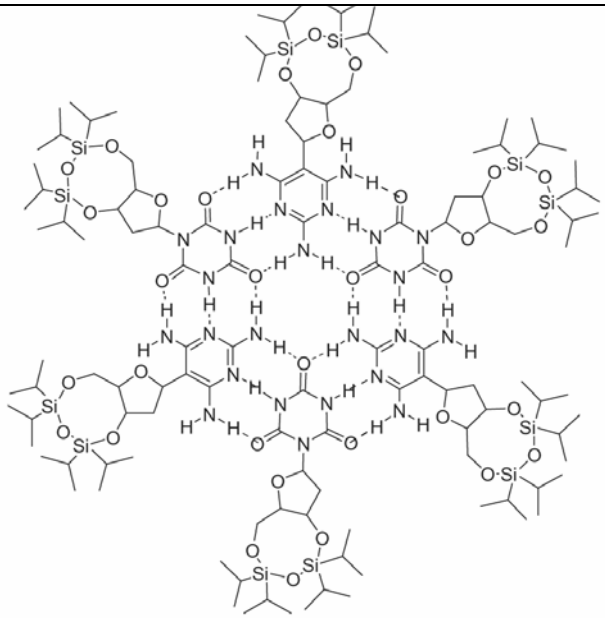
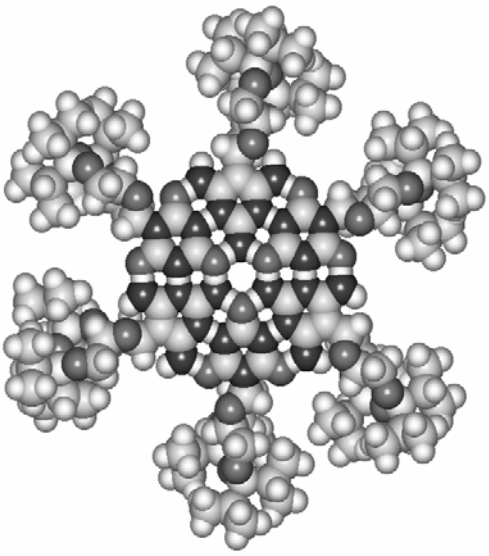
2 + **1** complexes	MM^+ optimised structures
	 MM^+ energy = 81.5 kcal/mol
	 MM^+ energy = 177.1 kcal/mol

Table 3. Theoretical hydrogen-bonded **1** + **3** complexes determined using MM^+ geometry optimisations

1 + **3** complexes	MM^+ optimised structures
	 MM^+ energy = 107.9 kcal/mol
	 MM^+ energy = 195.5 kcal/mol

These preliminary molecular mechanics MM^+ calculations allowed a direct visualisation of steric interactions between the substituents in these hydrogen-bonded structures. Both hydrogen-bonded linear tapes formed with triaminopyrimidine **1** and diethylbarbituric acid **2,** and with triaminopyrimidine **1** and cyanuric acid **3** showed the protected sugar groups at close alkyl-alkyl packing distances of 3.7 Å.[4] The overlaid structures in Figure S12 show that, while the cyanuric acid nitrogen atom enables the triaminopyrimidine **1**/cyanuric acid **3** tape to remain within the same plane, the barbituric acid tetrahedral carbon atom allows the triaminopyrimidine **1**/ diethylbarbituric acid **2** tape to deviate from planarity. This absence of planarity tape suggested that the triaminopyrimidine **1**/ diethylbarbituric acid **2** tape should be able to compensate for unfavourable steric repulsions between bulky disiloxane substituents by adopting a distorted geometry. In constrast, steric hindrance can only be overcome through the formation of hexameric rosettes in the case of the triaminopyrimidine **1**/cyanuric acid **3** pair.

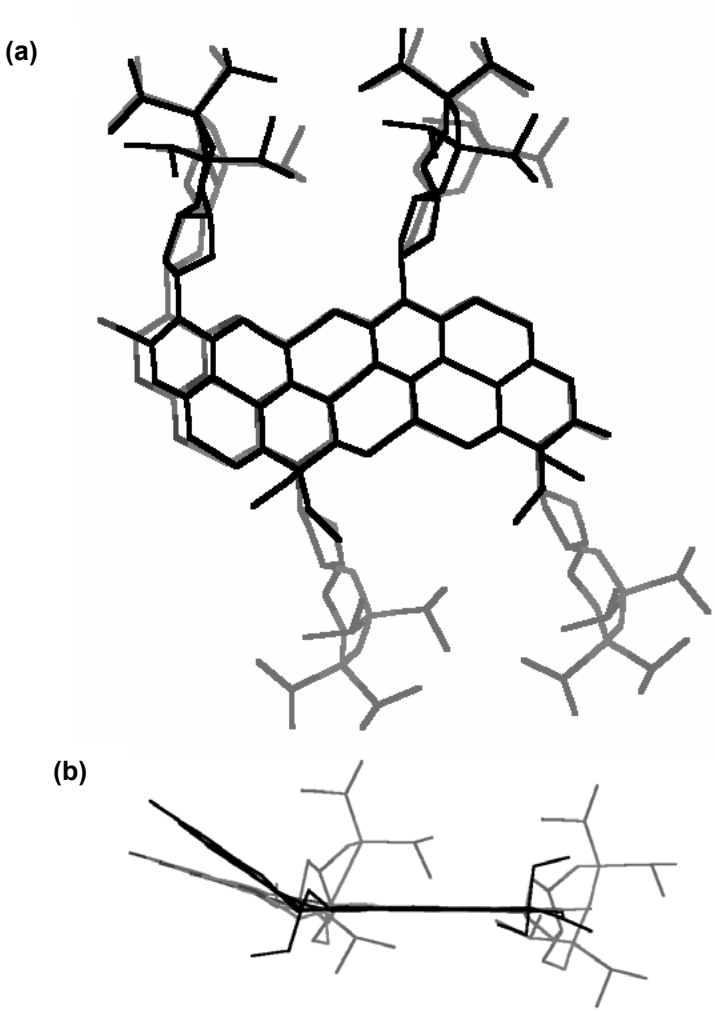


Figure S 7 . Overlay of triaminopyrimidine **1**/ diethylbarbituric acid **2** (black) and triaminopyrimidine **1**/ and cyanuric acid **3** linear tapes. (a) Top view. (b) Side view.

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[1] (a) S. W. Provencher, *Comput. Phys.* **1982**, *27*, 213. (b) S. W. Provencher, *Comput. Phys.* **1982**, *27*, 229. (c) K. S. Schmitz, *An Introduction to Dynamic Light Scattering by Macromolecules*, Academic Press Inc., San Diego, CA, **1990**.

[2] Gaussian 98W (Revision A.7), M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck,

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[3] The aggregates were designed by deliberately adding hydrogen-bonds to obtain strong, directional ionic hydrogen-bond to the calculated MM^+ hydrogen-bonds.

[4] A. J. Dickie, A. K. Kakkar, M. A. Whitehead, *Langmuir*, **2002**, *18*, 5657.