Molecular Self-Assembly through Hydrogen Bonding: Supramolecular Aggregates Based on the Cyanuric Acid-Melamine Lattice

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Abstract: Reaction of the tris(melamine) derivatives hubMr (CnHr-1,3,5-[CONHC6H4-3-NH(CHr)2COC6H2-2-NHCTN(9Hr)r(NH2)(NHCHTCHT(Cr)r)]-5-Br.) and flexM, (CnHr-1,3,5[CO:(CHr),OCOC6H2-2-NHCTN](NH2)(NHCHTCHT(Cr)r)] with R,CA (neohexyl isocyanurate) and R"CA (3,3,3-triphenylpropyl isocyanurate) in CHCl, respectively, yields structurally well-defined supramolecular aggregates hubMr(R'CA)3 and flexM3(R"CA)3. These structures were characterized using 1H NMR, 13C NMR, and UV spectroscopy, gel permeation chromatography, and vapor pressure osmometry. flexM3 is a conformationally flexible analog of hubMr. The greater degree of preorganization that is built into the molecular structure of hubMr compared to flexM, took hubMr(R'CA)3 a more stable aggregate than flexMr(R"CA)3. These self-assembling structures are the first step in a program to design, synthesize, and develop methods to characterize supramolecular complexes that are held together by networks of noncovalent interactions.

Introduction

Molecular self-assembly is the spontaneous association of molecules under equilibrium conditions into stable aggregates, joined by noncovalent bonds, with well-defined composition and structure.1,2 We are developing a program whose objective is to design, synthesize, and characterize macromolecular aggregates that are the products of molecular self-assembly, using networks of hydrogen bonds to form these aggregates.3,4 Molecular self-assembly is a principle demonstrated in many biological systems: the hierarchy of interactions in nucleic acids and proteins provides examples.5 The backbone of tRNA is composed of covalent bonds; secondary structure (the arms of the cloverleaf structure of tRNA), tertiary structure, and interactions between tRNA and proteins are held together by networks of noncovalent interactions (hydrogen bonds and van der Waals, hydrophobic, and Coulombic interactions). A feature common to many self-assembled biological structures is cooperativity. An initiation event is followed by subsequent steps that lead to the completed assembly without accumulation of intermediates.6

A strategy for forming structure through molecular self-assembly differs fundamentally from that most highly developed in organic synthesis—the formation of covalent bonds—in several important respects. In self-assembly, enthalpy and entropy are approximately balanced and structures are at equilibrium; in covalent synthesis, enthalpy dominates and the structures are formed in irreversible processes.7

We limited our initial studies to structures based on hydrogen bonds because these interactions have a strong directional component that should simplify the design of complementary subunits for recognition and binding. Rebek,8 Leh,9 Hamilton,10 and

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Scheme I. Self-Assembly of hubM₃ with R'CA and flexM₃ with R''CA To Give Supramolecular Aggregates

Objective. Our first objective was to design molecules that would spontaneously assemble into an aggregate comprising three M units and three CA units arranged in the cyclic hexamer geometry, CA₃M₃ (see the boldfaced section of CA⋅M). The template on which we are building supramolecular aggregates is the 1:1 complex (CA⋅M) between cyanuric acid (CA) and melamine (M). This complex has a high density of hydrogen bonds, so that any structure based on CA⋅M will have a large enthalpic driving force for self-assembly. The crystal structure of CA⋅M⋅3HCl has been reported.

Strategy. Our strategy to synthesize an assembly based on the cyclic hexamer was to link covalently three M units to a central "hub" using "spokes" that are compatible with the geometry necessary for forming molecular aggregates based on CA⋅M (Scheme I). Linking the three M units together increased the formation constant by reducing the loss of translational entropy on assembly of the desired hydrogen-bonded network (one hubM₃ unit and three CA units assemble into one particle rather than three M units and three CA units); proper design of the spokes reduces the loss of conformational entropy associated with assembly.

Scope of This Article. This article describes two molecules with the hub-and-spoke architecture: hubM₃ and flexM₃. Complexation of these molecules with 3 equiv of alkyl isocyanurates gives the molecular aggregates-hubM₃(R'CA)₃ and flexM₃(R''CA)₃—that are CHCl₃-soluble derivatives of the CA₃M₃ cyclic hexamer (Scheme I). The aggregate based on hubM₃ is more stable than that based on flexM₃ because (we presume) the rigid spokes of hubM₃ preorganize this molecule for complexation to a greater extent than do the flexible spokes of flexM₃.

In this article, we (1) examine the design and synthesis of these complexes, (2) detail the structural features that correlate with stability in them, and (3) discuss the techniques that are used to characterize these types of molecular aggregates. Chloroform, methylene chloride, and o-dichlorobenzene have been the only solvents used for the aggregates.

Design of Self-Assembling Structures. (a) hubM₃. We constructed the hub and spokes of hubM₃ with aromatic spacers connected through amide linkages (see the structure of hubM₃).

in Scheme 1). Molecular models suggested that this type of construction would preorganize hubM₁ into the conformation necessary for hydrogen bonding with 3 equiv of a cyanuric acid derivative, and yet provide enough flexibility for the complex, once formed, to settle into the geometry that maximized the strength of the hydrogen-bonding interactions. The neohexyl and tert-butylbenzyl substitutants of hubM₁ are necessary to increase the solubility of the molecule in organic solvents (an earlier version of hubM₁ that lacked these groups was not soluble in chloroform). The choice of neohexyl isocyanurate (R'CA) as a component of the aggregate was based on the solubility conferred by the neohexyl groups. The bromine atoms on hubM₁ were added as heavy atom markers to facilitate X-ray crystallographic analysis of hubM₁(R'CA)₃, in the event that we could obtain diffraction quality single crystals. To date, we have not been able to grow crystals of the complex.

(b) Analogs of hubM₁: Preorganization of the Spokes. We wanted to investigate how much preorganization is needed in the hub and spokes of hubM₁ to maintain the [1 + 3] complex as the most stable, thermodynamically favored structure. Put another way, we wished to determine the unfavorable conformational entropy we could tolerate during the assembly process and still maintain the [1 + 3] complex (1 equiv of hubM₁ or an analog thereof and 3 equiv of an alkyl isocyanurate) as the most favored species, as opposed to hydrogen-bonded oligomers or polymers. To investigate this issue we synthesized four analogs of hubM₁ that had varying degrees of conformational freedom in the spoke regions (Scheme II). The three compounds with spokes based on triethylene glycol, heptamethylene, and 1,3-diethylbenzene moieties each formed what we believe are insoluble hydrogen-bonded polymers when complexed with alkyl isocyanurates, rather than discrete [1 + 3] complexes. Only flexM₁ formed a soluble, well-defined complex. We have rationalized this result as follows. In order for a [1 + 3] complex to form, the spoke must make a 180° turn between the hub and the melamine units (indicated by the arrow in Scheme II). hubM₁ and flexM₁ both have this turn already built into their spokes by the ortho-substituted benzene ring; the other three molecules do not. In order to accommodate this 180° turn, the other three compounds must restrict several conformationally unrestrained bonds to gauche or eclipsed conformations. The combination of the entropic price (due to restriction of bond rotations) and the enthalpic price (due to introduction of unfavorable steric interactions associated with the gauche and eclipsed conformations) paid for this 180° turn makes the [1 + 3] complex less stable than hydrogen-bonded polymeric structures.

In Scheme II, Four Derivatives of hubM₁ That Have Varying Degrees of Flexibility in the Spokes

(c) hubM₁ vs flexM₁: Rigid vs Flexible Spokes. While hubM₁ and flexM₁ have similar structural components, the spoke of hubM₁ is much more rigid than that of flexM₁. The more rigid spoke of hubM₁ makes hubM₁(R'CA)₃ a more stable complex than flexM₁(R'CA)₃, because hubM₁ loses less conformational freedom than flexM₁ upon complexation. The greater stability of the hubM₁ complex, compared to the flexM₁ complex, is demonstrated in a competition experiment in which 1 equiv of hubM₁ and 1 equiv of flexM₁ are mixed with 3 equiv of R'CA. The R'CA complexes only with hubM₁; by ¹H NMR spectroscopy we cannot detect any flexM₁(R'CA)₃ in this mixture.

(d) Cooperativity. We believe that complexation of hubM₁ with 3 equiv of R'CA is a cooperative process. ¹H NMR experiments monitoring the titration of hubM₁ with R'CA show that, in a solution of hubM₁ that has less than 3 equiv of R'CA, the hubM₁ is present only as free hubM₁ and fully formed hubM₁(R'CA)₃ complex and is not present as partially formed complexes with stoichiometries of hubM₁(R'CA)₁ or hubM₁(R'CA)₂.

Characterization of Hydrogen-Bonded Molecular Aggregates. Characterization of molecular aggregates that are held together by noncovalent interactions requires a different approach than characterization of fully covalent organic compounds. We have already established the molecular structure of the individual components of the aggregate, and our main interest lies in understanding how these components fit together in the overall three-dimensional structure. ¹H NMR spectroscopy is useful to gain information about the structure and conformation of self-assembled aggregates. Intermolecular NOE measurements provide information about the spacial relationships between components in these complexes. Gel permeation chromatography (GPC) assesses the monodispersity of the complexes and nonspecific interactions between complexes. Tailing of peaks in the GPC traces gives a qualitative measure of the stability of the aggregates. Molecular weight measurements made by vapor pressure osmometry (VPO) reflect the stoichiometry of the constituent subunits that are present in the self-assembled aggregates.

Results

hubM₁(R'CA)₃. (a) Synthesis of hubM₁. The synthesis of hubM₁ is outlined in Scheme III.

(b) Qualitative Evidence for Formation of hubM₁(R'CA)₃. The complex between hubM₁ and R'CA is formed by combining the two components in chloroform and stirring the mixture for approximately 3 min. During this time the solution, which initially contains a suspension of R'CA, becomes homogeneous. R'CA alone has a low solubility in chloroform (<0.1 mM), but hubM₁(R'CA)₃ is very soluble (≥20 mM). Adding hubM₁ to a suspension of R'CA in chloroform solubilizes up to, but not more than, 3 equiv of the suspended R'CA. This observation provides qualitative evidence that the stoichiometry of the complex between hubM₁ and R'CA is 1:3.

Reversed-phase TLC (eluted with 5% 2-propanol in CH₂Cl₂) gives two spots that are visible under a UV lamp: a major spot for hubM₁(R'CA)₃ (R = 0.35–0.45) and a minor spot for an uncomplexed hubM₁ (R = 0.15–0.30). R'CA is not visible by UV irradiation.

(c) Titration of hubM₁ with R'CA Monitored by ¹H NMR Spectroscopy. We monitored the titration of hubM₁ with R'CA by ¹H NMR spectroscopy in CDCl₃ (Figure 1). The only distinguishing features that appear in the spectrum of uncomplexed hubM₁ in CDCl₃ are the broad resonances at 0.8 and 1.4 ppm corresponding to the tert-butyl protons of the neohexyl and tert-butylbenzyl groups and the broad peak centered at 7.2 ppm corresponding to the aromatic protons. hubM₁ has a broad spectrum due to self-association and restricted rotation around the amide and RNH–triazine bonds. In more polar solvents such
Figure 1. Spectra of the 'H NMR titration of hubM₃ (500 MHz, 10 mM in CDCl₃) with R'CA. The peak assignments are shown at the top of the figure. The bottom two spectra show hubM₃ and R'CA alone in DMSO-d₆, for reference.

Figure 2. ¹³C NMR spectra of hubM₃ alone and the hubM₃(R'CA)₃ complex (125 MHz, 19.4 mM in CDCl₃).

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as DMSO that break up association due to hydrogen bonding, the spectrum has sharper resonances. In the spectrum of hubM₃ and R'CA at the 1:1 stoichiometry, there are many sharp resonances that correspond to hubM₃(R'CA), that appear against a broad background of uncomplexed hubM₃. At the 1:1 stoichiometry, two-thirds of the hubM₃ remains uncomplexed while one-third is present as fully formed hubM₃(R'CA). This observation emphasizes that the formation of this aggregate is a cooperative process. Exchange between these species is slow on the NMR time scale; we observe distinct resonances for complexed and uncomplexed hubM₃ rather than a spectrum that is an average of the two.

Beyond the 1:3 ratio of hubM₃/R'CA the spectrum does not change, and further aliquots of R'CA do not dissolve in the solution. This observation confirms the 1:3 stoichiometry of the complex. The top spectrum of Figure 2 shows hubM₃/R'CA in a ratio of 1:3.6. The sample contains 3 equiv of unsolubilized R'CA.

(d) Characteristic Features of the 'H NMR Spectrum of hubM₃(R'CA)₃. (i) Diastereotopic Protons. Two sets of methylene protons (g and q) of hubM₃ become diastereotopic in hubM₃(R'CA), and thus appear as separate resonances. The benzylic protons (g and g') are of special note because they are separated by almost 1.7 ppm, even though they are bonded to the same carbon. Molecular models of the complex suggest that one of these protons (g') is positioned in the shielding cone of the aromatic ring that is derived from 1,3-diaminobenzene. The shielding effect of this aromatic ring shifts g' unfield with respect to g. The magnitude of this shielding effect suggests that the hubM₃ component of the hubM₃(R'CA)₃ complex is confined to a single, well-defined geometry.

(ii) Hydrogen-Bonded Protons. The imide NH protons (u and w) are equivalent by symmetry in uncomplexed R'CA (note the single resonance for these protons in the spectrum of uncomplexed R'CA in DMSO-d₆), but appear as separate resonances in the complex because they occupy different hydrogen-bonding sites on the triazine ring. The downfield position of these resonances (~14.8 ppm) indicates that the protons participate in strong hydrogen bonds.

(e) ¹³C NMR Spectra of Free hubM₃ and hubM₃(R'CA)₃. The ¹³C and 'H NMR spectra of free hubM₃ and hubM₃(R'CA)₃ in CDCl₃ have similar characteristics (Figure 2). The ¹³C spectrum of free hubM₃ has only a few distinct resonances in the alkane (~10 ppm) and aromatic (~130 ppm) regions (Figure 2). The majority of the resonances are broad and are difficult to distinguish from the base line. The ¹³C spectrum of hubM₃(R'CA)₃, has many sharp resonances that indicate that the complex has adopted a well-defined geometry. The changes that occur in the ¹³C NMR spectrum of hubM₃ upon complexation with R'CA mirror the behavior in the corresponding 'H NMR spectrum.

(f) Nuclear Overhauser Effects. The triad pattern of hydrogen bonds between M and CA places the NH protons of melamine in close proximity to the imide NH protons of cyanuric acid (~2.5 Å), and we expected strong intermolecular NOEs among these protons. A better indication of hydrogen bond strength than the absolute value of the chemical shift is the change in chemical shift that occurs upon hydrogen bonding. In this system, the chemical shift of uncomplexed R'CA cannot be determined because of the low solubility of R'CA in CDCl₃. In DMSO-d₆, the imide protons of uncomplexed R'CA appear at 11.4 ppm.
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A hubM₃(R'CA)₃

B. flexM₃(R'CA)₃

Figure 3. Intermolecular NOEs (%) among the hydrogen-bonding protons in hubM₃(R'CA)₃ and flexM₃(R'CA)₃. The NOEs that are not shown are either weak or the NOE signal is obscured by incomplete subtraction of other resonances in the difference spectrum.

1.

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Figure 4. Titration of (1) hubM₃ and R'CA and (2) flexM₃ with R'CA monitored by UV spectroscopy (0.1 mM in CH₂Cl₂). The inset graphs show plots of absorbance vs equivalents of cyanurate for two separate runs (A and B).

Figure 5. Gel permeation chromatograms of complexed and free hubM₃ and flexM₃. Free R'CA and R''CA are too insoluble to examine separately. The eluents are indicated to the left of the plots. The peaks at 11.0 (in CHCl₃) and 11.3 min (in CH₂Cl₂) are p-xylene used as an internal standard. The top chromatogram shows polystyrene for reference.

Figure 6. Titration of (1) hubM₃ and R'CA and (2) flexM₃ with R'CA monitored by UV spectroscopy (0.1 mM in CH₂Cl₂). The inset graphs show plots of absorbance vs equivalents of cyanurate for two separate runs (A and B).

protons. Figure 3A summarizes the observed NOEs in hubM₃-(R'CA)₃. These NOEs confirm the geometry of the hydrogen-bonding network and support our proposed structure of hubM₃-(R'CA)₃.

(g) UV Spectroscopy. We also followed the formation of the hubM₃(R'CA)₃ complex by UV spectroscopy. Figure 4 shows the changes in the UV spectrum of hubM₃ as aliquots of R'CA are added. Beyond the 1:3 stoichiometry of hubM₃·R'CA the spectrum does not change with added aliquots of R'CA.

(g) Gel Permeation Chromatography. Gel permeation chromatography (GPC) separates molecules according to hydrodynamic radii. This technique is useful for estimating both the molecular weight of a solute (by using a suitable reference) and its distribution of molecular weights. We examined hubM₃-(R'CA)₃ by GPC in CHCl₃ and CH₂Cl₂; Figure 5 shows representative results. We infer that hubM₃ alone in solution is highly self-associated: its GPC chromatogram shows a broad distribution of molecular weights with no well-defined peaks. Uncomplexed hubM₃ also has a weaker absorbance at 254 nm than complexed hubM₃ (Figure 4), and thus the UV detector is less sensitive to the complexed species. In contrast, hubM₃-(R'CA)₃ shows a sharp peak (8.5 min in CHCl₃, 9.0 min in CH₂Cl₂), suggesting that it is a discrete structure with little self-association. This peak shows tailing that is, we believe, due to slow dissociation of the complex on the GPC column. The top trace in Figure 5 shows polystyrene (no tailing) for comparison.

(i) Vapor Pressure Osmometry. We also determined the molecular weight of hubM₃(R'CA)₃ by vapor pressure osmometry (VPO) in CHCl₃ solution (Figure 6). This technique requires calibration with a molecular weight standard; the experimentally determined molecular weight of the unknown is dependent on the choice of standard. The standard and the unknown are related by

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\text{MW}_{\text{unknown}} \approx \text{MW}_{\text{standard}} \left( \frac{\Delta V}{c_{\text{standard}}} \right) = \text{MW}_{\text{unknown}} \left( \frac{\Delta V}{c_{\text{unknown}}} \right) \tag{1}\]


(16) Other more stable hydrogen-bonded complexes show no tailing in their GPC traces: their peaks are symmetrical with a peak width at a half-height of 0.25 min. Seo, C. T.; Whitesides, G. M. J. Am. Chem. Soc. in press.

where $\Delta V/c$ (in the limit that $c \to 0$) for the standard and unknown are values that are determined by VPO. With bis-$t$-Boc-derivatized gramicidin S as a standard, the experimental molecular weight of the complex is close to the expected value for the structure hubM1(R'CA), while the sucrose octaacetate, polystyrene, and perbenzoyl $\beta$-cyclodextrin as standards the molecular weight of the complex inferred from VPO is 15–35% high. We hypothesize that hubM1(R'CA), may experience weak intercomplex hydrogen-bonding interactions that are not evident by NMR spectroscopy or GPC and that these interactions increase the effective molecular weight sensed in VPO. This weak aggregation would be mimicked by the gramicidin standard. Using these standards, the experimental molecular weight for the [1 + 3] complex would be high.

The synthesis of flexM1 is outlined in Scheme IV. The H NMR titration of flexM1 with R'CA is shown in Figure 7. Unlike hubM1, the spectrum of uncomplexed flexM1 in CDCl3 has sharp resonances: this molecule has fewer conformationally restrained bonds and fewer sites for self-association through intermolecular hydrogen bonding than hubM1. These sharp resonances allow us to observe both the disappearance of peaks for uncomplexed flexM1 and the appearance of those corresponding to flexM1(R'CA) during the titration. These spectra clearly demonstrate that exchange between free and complexed flexM1 is slow on the NMR time scale. The absence of resonances for partially formed complexes emphasizes the cooperative nature of this self-assembly process.

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**Scheme IV. Synthesis of flexM1**

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12 \[\text{NH}_2 \quad \text{CO}_2\text{H} \] 
13 \[\text{NH}_2 \quad \text{O} \quad \text{OH} \] 

(a) Synthesis of flexM1. flexM1 is based on the same hub-and-spoke architecture as hubM1 although the spokes of flexM1 are derived from a flexible 1,3-propanediol spacer.

(b) Characterization of flexM1(R'CA) by 1H NMR Spectroscopy. The H NMR titration of flexM1 with R'CA is shown in Figure 7. Unlike hubM1, the spectrum of uncomplexed flexM1 in CDCl3 has sharp resonances: this molecule has fewer conformationally restrained bonds and fewer sites for self-association through intermolecular hydrogen bonding than hubM1. These sharp resonances allow us to observe both the disappearance of peaks for uncomplexed flexM1 and the appearance of those corresponding to flexM1(R'CA) during the titration. These spectra clearly demonstrate that exchange between free and complexed flexM1 is slow on the NMR time scale. The absence of resonances for partially formed complexes emphasizes the cooperative nature of this self-assembly process.

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(18) In eq 1, $c$ = concentration, and $\Delta V$ is proportional to the temperature change that is measured in VPO.

(19) We converted the free amino groups of gramicidin S to $N$-$t$-Boc groups in order to decrease the amount of hydrogen bonding associated with the ornithine residues.

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**Figure 6.** Experimental molecular weights of hubM1(R'CA), (•) and flexM1(R'CA), (○) determined by vapor pressure osmometry using four different molecular weight standards. The solid and dashed horizontal lines correspond to the calculated MWs of hubM1(R'CA), and flexM1(R'CA), respectively. The four MW standards were GS = N,N'-bis($t$-Boc)gramicidin S (MW 1342), SO = sucrose octaacetate (MW 679), PS = polystyrene (average MW 5050, polydispersity = 1.05), and PC = perbenzoyl $\beta$-cyclodextrin (MW 3321). The error bars correspond to the sum of the standard deviations of the VPO measurements of the standard and unknown. These experiments were performed at 37 °C in CHCl3 over the concentration range 2–16 mM of complex.

**Figure 7.** Spectra of the $^1$H NMR titration of flexM1 (500 MHz, 10 mM in CDCl3) with R'CA. The peak assignments are shown at the top of the figure. The dashed lines are provided as a guide for the eye.

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(20) We complexed flexM1 with 3,3,3-triphenylpropyl isocyanurate (R'CA) because R'CA is more soluble in organic solvents than is neohexyl cyanurate. Kelly, T. R.; Maguirc, M. P. J. Am. Chem. Soc. 1987, 109, 6549.
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Scheme V. Schematic Representation of the Four Possible Geometrical Isomers of These Supramolecular Aggregates

The methylene protons of flexM₁ and protons α and α' of R''CA become diastereotopic in the complex. The imide protons of R''CA appear as separate resonances in the flexM₁(R''CA), complex because they occupy different hydrogen-bonding environments. The melamine protons j, j', and k shift downfield upon complexation, as expected for hydrogen bonding. Proton i is shifted upfield on complexation because the intramolecular hydrogen bond that this proton forms with the benzoate carbonyl group in uncomplexed flexM₁ is disrupted. NOE studies of flexM₁(R''CA): suggest that this proton forms with the benzoate carbonyl group in uncomplexed flexM₁(R''CA). Studies of flexM₁(R''CA)₃ (Figure 3B) show intermolecular NOEs among the melamine protons of flexM₁ and the imide protons of R''CA, in analogy with those seen in the hubM₁(R''CA), complex.

(c) UV Spectroscopy. Titration of flexM₁ with R''CA monitored by UV spectroscopy (Figure 4) confirms that this complex also has a 1:3 stoichiometry between flexM₁ and R''CA.

(d) Gel Permeation Chromatography. Figure 5 shows the GPC traces of free flexM₁ and flexM₁(R''CA), eluted with CH₂Cl₂ and CHCl₃. The traces of free flexM₁ show no well-defined peaks, reflecting the fact that this compound is self-associated and has a broad distribution of molecular weights in these solvents. The trace of flexM₁(R''CA), shows peaks at 10.2 min (CHCl₃) and 10.8 min (CH₂Cl₂) for the complex. These peaks show tailing similar to that of hubM₁(R''CA), caused by decomposition of the aggregate during the analysis. The leading edge of these peaks is broader than the leading edge of the hubM₁(R''CA) peaks, suggesting that flexM₁(R''CA), may have a broader molecular weight distribution in solution than hubM₁(R''CA).

(e) Vapor Pressure Osmometry. The VPO analysis of flexM₁(R''CA), has the same basic trends as seen for the hubM₁(R''CA), complex (Figure 6). In this case, however, all of the molecular weight standards give an experimental molecular weight for flexM₁(R''CA), that is 35–80% too high. These results suggest a degree of intercomplex association not anticipated from our molecular design and that is not evident by our other analytical methods. One possible explanation is that there is an interaction between the carbonyl groups of the benzene 1,3,5-triester hub of one complex with the plane of the hydrogen-bonding network of a second complex.²¹

(f) Unsymmetrical Structures Obtained by Complexing hubM₁ with Derivatives of R''CA. There are four possible geometrical isomers of hubM₁(R''CA), (Scheme VA–D) because the spokes of hubM₁ are not symmetrically attached to the M units. These geometrical isomers correspond to structures in which one or more of the M units has flipped 180° with respect to the plane of the CA₃M₃ cyclic hexamer. Enantiomers A and B are C₃ symmetrical structures. Enantiomers C and D have only the identity element (C₃).

Complexation of hubM₁ with R''CA might, in principle, yield a mixture of isomers A–D: a mixture is entropically favorable compared to a single isomer. In practice, we observe only the C₃ symmetrical species A and B by ¹H NMR spectroscopy (we observe only one set of resonances) for the three spokes of hubM₁). We infer that the absence of unsymmetrical structures indicates that the C₃ enantiomers A and B are enthalpically favored over the unsymmetrical enantiomers and that the C₃ symmetry is not caused by kinetic averaging of the structures in the NMR spectrometer (an average of A–D would also have effective C₃ symmetry). We estimate that the symmetrical isomers of hubM₁(R''CA), are at least 2.7 kcal/mol more stable than the unsymmetrical isomers. This estimate is based on the fact that we do not observe any unsymmetrical isomers in the ¹H NMR spectrum of hubM₁(R''CA), we should be able to detect 1% of these species.

Complexation of hubM₁ with aryl isocyanurates or barbituric acid derivatives rather than alkyl isocyanurates does lead to what we believe to be a mixture of isomers A–D, although in all cases the C₃ symmetrical structures are the major components (≥90%) of the mixtures. Chart I shows the percent of unsymmetrical isomers that are present in these mixtures (as determined by ¹H NMR spectroscopy). The alkyl isocyanurates give complexes with hubM₁, that are free of unsymmetrical isomers; the aryl isocyanurates and the barbiturates have up to 10% of these unsymmetrical species. We do not understand the details of the interactions that cause some complexes to be mixtures of isomers while others are not.

(g) hubM₁(barbital). Since isomers C and D (Scheme V) have a dissymmetric arrangement of their spokes, the ¹H NMR spectrum of these isomers should have separate resonances for each of the three spokes because each spoke is in a slightly different chemical environment. Figure 8 shows the spectrum of hubM₁(barbital). The minor resonances of the unsymmetrical isomers are clearly resolved from the major resonances of the C₃ symmetrical isomers. We assigned the resonances of symmetrical hubM₁(barbital), in analogy to those of hubM₁(R''CA), but we have not assigned the resonances of the unsymmetrical isomers. We cannot completely rule out the possibility that the minor resonances correspond to dimers of hubM₁(R''CA), rather than unsymmetrical isomers (see the Discussion section).

Discussion

Preorganization. The molecular aggregates hubM₁(R''CA)₃ and flexM₁(R''CA), establish the hydrogen-bonded CA-M lattice
as a template for building supramolecular assemblies. Substantial preorganization of the molecules is necessary in order to form discrete assemblies rather than hydrogen-bonded oligomers or polymers. Without the preorganizing hub-and-spoke scaffold, CA and M derivatives associate in solution to form hydrogen-bonded oligomers that exchange rapidly at room temperature.22

Covalently linking the three M units together via the hub-and-spoke architecture using flexible triethylene glycol, heptamethylene, or 1,3-diolethylene benzene spacers (Scheme II) does not provide enough preorganization to make the [1 + 3] complexes stable. A higher level of preorganization (the ortho-substituted benzene ring in flexM3) is necessary to make oligomerization energetically more favorable than polymerization. The [1 + 3] aggregates are further stabilized by using the semirigid spokes of hubM3: the aromatic rings and amide linkages in these spokes minimize the loss of conformational entropy that is lost upon complexation.

Solvent Effects. In these initial studies, we limited our investigation of solvent effects to the chlorinated hydrocarbons chloroform, methylene chloride, and o-dichlorobenzene. hubM3 and R'CA self-assemble into the same, structurally well-defined molecular aggregate hubM3(R'CA)3 in all three of these solvents. Qualitative 1H NMR data suggest that the hydrogen bonds that hold the aggregate together are stronger in o-dichlorobenzene than in chloroform as a result of less competition from the solvent in hydrogen-bonding.23 The 1H NMR spectrum of hubM3(R'CA)3 is similar in CDCl3 and o-dichlorobenzene, except that the hydrogen-bonded protons appear approximately 0.4 ppm farther downfield in o-dichlorobenzene than in CDCl3. These molecular aggregates are not stable in polar solvent mixtures such as CHCl3/Methanol and CHCl3/DMSO that compete for hydrogen-bonding sites.

Ambiguities in the Structures of the [1 + 3] Complexes. The solubility studies, and the UV and 1H NMR titration data, demonstrate that the stoichiometry in the molecular aggregates composed of hubM3/R'CA and flexM3/R'CA is 1:3. The VPO data indicate that the MWs of these aggregates are higher than the theoretical MWs expected for hubM3(R'CA)3 and flexM3(R'CA). Although we believe that this result is caused by weak and nonspecific association between aggregates, we have not entirely ruled out the possibility that some fraction of these aggregates exist as dimers or higher oligomers of complexes with stoichiometries such as [hubM3(R'CA)]2. (Chart II shows one possible structure for a dimer.) The 1H NMR data do not support the existence of these discrete dimers as significant (>10%) components of the population of aggregates. In the structure shown in Chart II, two of the spokes of one hubM3 molecule are hydrogen-bonded in one CA-M3 unit, while the third spoke is extended out in order to hydrogen bond in the other CA-M3 unit. The 1H NMR spectrum of the dimer should reflect the different chemical environments of these spokes; our experimental observation shows that all three spokes of hubM3 are hydrogen-bonded in one CA-M3 unit. The 1H NMR spectrum of the dimer should reflect the different chemical environments of these spokes; our experimental observation shows that all three spokes of hubM3 are hydrogen-bonded in one CA-M3 unit. The 1H NMR spectrum of the dimer should reflect the different chemical environments of these spokes; our experimental observation shows that all three spokes of hubM3 are hydrogen-bonded in one CA-M3 unit.

The most stable molecular aggregate that we have made to date is shown schematically in the right side of Chart II. This stable structure is a 1:1 complex between an analog of hubM3 and hubCA. hubCA consists of three CA units that are covalently linked through the hub-and-spoke architecture.24 The VPO-determined MW of hubM3-hubCA is also 35–50% higher than the expected theoretical MW. This result is similar to that obtained with flexM3(R'CA), and suggests that molecular weights by VPO that are higher than expected reflect some experimental artifact (plausibly interaggregate hydrogen bonding) and do not require the formation of aggregates such as [hubM3(R'CA)]2. Other Methods for Characterizing Self-Assembling Structures. The methods that we have used to characterize hubM3(R'CA)3, and flexM3(R'CA)3 (with the exception of VPO) support the stoichiometries and structures that we proposed for these complexes in Scheme I, although all of these methods require inference. Unequivocal proof of the these structures may not be attainable until we grow single crystals of the complexes that are suitable for X-ray diffraction studies. Even then it is not certain that the solid-state structures would accurately reflect the solution structures of these complexes. As noted earlier, we have obtained a crystal structure of monomeric melamine and monomeric barbituric acid derivatives that do form the cyclic hexamer in the solid state.

We have not been able to obtain mass spectrometry evidence for the [1 + 3] complexes. Fast atom bombardment mass spectrometry (FAB-MS) gives ions corresponding to the individual components, but none corresponding to either hubM3(R'CA)3 or flexM3(R'CA)3, from a variety of FAB matrices.

The thermodynamics of self-assembly in these systems is an important topic that we have not addressed in this paper. A thermodynamic analysis of the complexation of hubM3 with R'CA will be presented in a future publication.

**Experimental Section**

**General Methods.** NOE experiments were performed with a Bruker AM 300 instrument. Elemental analyses were performed by Galbraith Laboratories, Inc. THF was distilled from sodium benzophenone ketyl.

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22. Variable-temperature 1H NMR studies on a mixture of N,N'-bis(4-tert-butylyphenyl)melamine and barbital (BA) showed that, as the temperature is lowered, the molecules reach a slow exchange regime at ~60 °C. The resonances for the hydrogen-bonded NH protons that are broad at room temperature shift downfield and become sharp at ~60 °C. These observations are consistent with the interpretation that the molecules are present in one predominant geometry at ~60 °C. We do not know whether this geometry is a crystal structure or a true solid-state structure. Seto, C. T.; Whitesides, G. M. Unpublished results.

23. Shaw has performed calorimetric studies that show that the enthalpy of a hydrogen bond is more favorable in o-dichlorobenzene than in chloroform. Willams, L. D.; Chawla, B.; Shaw, B. R. *Biopolymers* 1987, 26, 59.

Methylene chloride and triethylamine were distilled from calcium hydride. Dimethylformamide was dried and stored over 4 Å molecular sieve that has a different point property. The chemical structures show doubling of several resonances in their 1H and 13C NMR spectra due to slow exchange of conformers around the NHR-triazine bonds.

5-Bromo-2-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)benzoic acid (5). A 250-mL round-bottomed flask was charged with 5.21 g (25.0 mmol) of 2,8.83 g (7.15 mL, 35.0 mmol) of 4-tert-butylbutyl benzyl bromide, 5.06 g (67.7 mL, 50.0 mmol) of triethylamine, and 50 mL of dry THF. The solution was heated at reflux under a nitrogen atmosphere for 12 h and cooled to room temperature, and the solvent was removed by rotary evaporation at aspirator pressure. The clear reddish oil was purified by flash chromatography (eluited with 20:80 ethyl acetate/hexanes), giving 6.67 g (32.0 mmol, 64%) of the product as a white solid: R, 0.37 (50:50 ethyl acetate/hexanes). 1H NMR (300 MHz, DMSO-d6) 6 13.7-13.1 (br s, 1 H), 8.15 (d, J = 2.3 Hz, 2 H), 7.54 (d, J = 8.3 Hz, 2 H), 6.81 (s, 1 H), 6.50 (d, J = 8.0 Hz, 1 H). 6.16 (d, J = 8.0 Hz, 1 H), 6.16 (s, 1 H), 6.60 (d, J = 8.0 Hz, 1 H). 6.14 (s, 1 H), 1.44 (s, 9 H), 1.25 (s, 9 H); 13C NMR (125 MHz, DMSO-d6) 6 166.81, 166.49, 152.43, 149.26, 149.91, 138.49, 135.06, 134.83, 133.78, 132.10, 128.72, 128.16, 127.65, 126.60, 125.28, 124.79, 124.65, 124.46, 116.40, 116.29, 116.00, 115.90, 52.41, 34.10, 31.07, 28.03; HRMS-FAB (M + H+) calcd for C21H19NO3: 327.1288, found 327.1280.

5-Bromo-2-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)-5-bromobenzoyl-1,3-diaminobenzene (3). A 250-mL round-bottomed flask was charged with 2.77 g (50.0 mmol) of 1,5.0 mL of diisopropylethylamine, and 50 mL of dry THF. The solution was heated at reflux under a nitrogen atmosphere for 12 h and cooled to room temperature, and the solvent was removed by rotary evaporation at aspirator pressure. The crude product was purified by flash chromatography (eluited with 75:25 ethyl acetate/hexanes), giving 7.49 g (11.0 mmol, 90%) of the product as a white solid: 1H NMR (300 MHz, DMSO-d6) 6 9.36 (s, 1 H), 8.01-7.92 (m, 4 H), 7.59-7.51 (m, 3 H), 5.47 (d, J = 8.0 Hz, 1 H). 6.43 (s, 1 H), 4.29 (app d, J = 8.0 Hz, 1 H), 3.29 (app d, J = 8.0 Hz, 1 H); 13C NMR (125 MHz, DMSO-d6) 6 166.50, 164.86, 152.43, 149.13, 142.02, 138.28, 134.98, 135.06, 134.81, 133.78, 132.14, 131.75, 131.48, 126.91, 126.72, 126.93, 124.88, 123.54, 121.37, 120.68, 116.68, 115.76, 57.19, 51.95, 34.01, 31.07, 28.06; HRMS-FAB (M + H+) calcd for C21H19NO3: 327.1288, found 327.1280.

5-Bromo-2-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)-5-chlorobenzoyl-1,3-diaminobenzene (4). A 250-mL round-bottomed flask was equipped with a stirring bar and was charged with 7.49 g (25.0 mmol) of sodium bicarbonate, 5 g (52.3 mL, 30.0 mmol) of disopropylamine, and 100 mL of THF. The solution was cooled in an ice bath under a nitrogen atmosphere, and 5.58 g (90.6 mL, 55.0 mmol) of triethylamine was added followed by 4.11 g (12.2 mmol) of 3. The solution was stirred at room temperature for 1 h, diluted with 150 mL of toluene, and washed with 200 mL of water, 200 mL of 1 N aqueous sodium hydroxide, and 150 mL of 1 N aqueous hydrochloric acid. The combined aqueous phases were extracted with 250-mL portions of water, and 100 mL of brine. The solution was dried over MgSO4 and the solvent was removed by rotary evaporation at aspirator pressure. The crude product was purified by flash chromatography (eluited with 25:75 ethyl acetate/hexanes), giving 7.49 g (11.0 mmol, 90%) of the product as a white solid: R, 0.50 (25:75 ethyl acetate/hexanes). 1H NMR (300 MHz, DMSO-d6) 6 9.37 (s, 1 H), 8.01-7.92 (m, 4 H), 7.57 (d, J = 8.5 Hz, 1 H), 7.52 (s, 1 H), 7.38 (m, 2 H), 7.22 (m, J = 8.1 Hz, 2 H), 7.15 (d, J = 8.0 Hz, 1 H), 7.06 (d, J = 8.1 Hz, 2 H). 6.97 (m, J = 8.0 Hz, 1 H), 6.82 (m, J = 8.1 Hz, 1 H), 4.07 (app d, J = 8.0 Hz, 1 H), 3.29 (app d, J = 8.0 Hz, 1 H), 3.26 (app d, J = 8.0 Hz, 1 H), 3.23 (app d, J = 8.0 Hz, 1 H), 3.19 (app d, J = 8.0 Hz, 1 H), 3.16 (app d, J = 8.0 Hz, 1 H); 13C NMR (125 MHz, DMSO-d6) 6 170.20, 168.46, 152.43, 149.13, 139.02, 128.48, 127.26, 126.93, 126.88, 125.40, 123.54, 120.68, 116.88, 115.76, 57.19, 51.95, 34.05, 31.07, 28.06; HRMS-FAB (M + H+) calcd for C21H19ClNO3: 353.1584, found 353.1572.
evaporation at aspirator pressure. The residue was taken up in 200 mL of toluene, washed three times with 150-ML portions of water and once with 50 mL of brine, and dried under MgSO₄, and the solvent was removed by rotary evaporation at aspirator pressure. The product was purified by flash chromatography eluted with 45:55 ethyl acetate/hexanes, loading 20 mL column volume in toluene and eluting with 2.55 g of toluene. The solid was then suspended in 100 mL of water cooled in an ice bath, and excess sodium hydroxide was added until the pH was 8.7. The product was filtered and the filtrate acidified with 37% hydrochloric acid to a pH of 2.0. The precipitated product was collected by vacuum filtration and washed with 50 mL of water and dried at 0.1 Torr, giving 0.626 g (41.5 mmol, 60%) of RCI as a white solid.

1-(3,3-Dimethylbutyl)acetanilide (3A). A 100-ML round-bottomed flask equipped with a stirring bar was charged with 1.45 g (10.0 mmol) of 3A, 1.01 g (3.5 mL, 10.0 mmol) of (3,3-dimethylbutyl)amine, and 20 mL of water. The reaction was heated to reflux under a nitrogen atmosphere for 1.5 h and cooled in an ice bath, and the precipitated product was collected by vacuum filtration and washed with 75 mL of water. The product was dried at 0.1 Torr, giving 0.556 g (6.6 mmol, 60%) of RCI as a white solid.

1-(3,3-Dimethylbutyl)isocyanuric Acid (RCA). A 100-ML round-bottomed flask equipped with a stirring bar was charged with 15 mL of ethanol and cooled in an ice bath under a nitrogen atmosphere, and 0.41 g (18.0 mmol) of sodium carbonate was added. The solution was cooled to 0 °C under a nitrogen atmosphere, and then 1.80 g (10.5 mL, 250 mmol) of 1,3-propanediol, 12.38 g (12.0 mmol) of diethyl carbonate, and excess triethylamine were added. The reaction was heated to reflux for 12 h, cooled to room temperature, and diluted with 75 mL of toluene. The precipitated sodium salt of the product was collected by vacuum filtration and washed with 50 mL of water. The white solid was dissolved in 30 mL of water, filtered, cooled in an ice bath, and acidified to pH 2 with 37% hydrochloric acid. The precipitated product was collected by vacuum filtration, washed with 100 mL of water, and dried at 0.1 Torr, giving 0.77 g (3.6 mmol, 60%) of RCA as a white solid.

1-Hydroxypropyl 2-Aminobenzoate (13). A 1-L round-bottomed flask equipped with a stirring bar was charged with 6.86 g (50.0 mmol) of anilinic acid, 19.03 g (18.07 mL, 250 mmol) of 1,3-propanediol, 12.38 g (60.0 mmol) of dicyclohexylcarbodiimide, 0.61 g (5.0 mmol) of 4-(dimethylamino)pyridine, 250 mL of ether, and 150 mL of THF. The solution was stirred in an ice bath for 1 h and at room temperature for 12 h under a nitrogen atmosphere, and then filtered to remove dicyclohexylurea. After the solvent was removed by rotary evaporation at aspirator pressure, the residue was taken up in 400 mL of ethyl acetate, and the solution was filtered again to remove precipitated dicyclohexylurea. The filtrate was washed twice with 300-ML portions of water, three times with 300-ML portions of 1:1 water/saturated aqueous sodium carbonate, three times with 300-ML portions of water, and once with 150 mL of brine and dried over MgSO₄, and the solvent was removed by rotary evaporation at aspirator pressure. The crude product was purified by flash chromatography eluted with 50:50 ethyl acetate/hexanes, loading 20 mL column volume in toluene and eluting with 2.55 g of toluene. The solid was then suspended in 100 mL of water cooled in an ice bath, and the mixture was cooled to 0 °C until all of the solid had dissolved. The reaction was poured over 100 g of ice and stirred until the ice had melted, and the precipitated product was collected by vacuum filtration, washed with a 1:1 water/saturated aqueous sodium carbonate, and dried at 0.1 Torr.
The solution was then warmed to room temperature, and the solvent was removed by rotary evaporation at aspirator pressure. The residue was taken up in 1,500 mL of ethyl acetate and was washed three times with 400-mL portions of water and once with 300 mL of brine and dried over MgSO₄. The solvent was removed by rotary evaporation at aspirator pressure, giving the intermediate chlorotriazine as a white solid. This crude chlorotriazine was combined with 1.95 g (2.60 mL, 19.3 mmol) of neohexylamine, 1.63 g (3.36 mL, 19.3 mmol) of diisopropylamine, and 100 mL of THF. The solution was heated at reflux for 4 h under a nitrogen atmosphere and cooled to room temperature, and the solvent was removed by rotary evaporation at aspirator pressure. The residue was taken up in 1,000 mL of ethyl acetate (the product is not very soluble), washed four times with 500 mL of brine and dried over MgSO₄, and the solvent was removed by rotary evaporation. The product was purified by flash chromatography (eluted with ethyl acetate, loaded on column preadsorbed to silica) to give 2.80 g (7.21 mmol, 757%) of 14 as a white solid: Rf 0.13 (ethyl acetate). 


The crude product was purified by flash chromatography (eluted with ethyl acetate followed by 9:3 ethyl acetate/methanol, loaded on column preadsorbed to silica) to give 2.38 g (6.65 mmol, 79.7%) of N-(3,3,3-triphenylpropyl)isocyanuric Acid (RCA). A 100-mL round-bottomed flask equipped with a stirring bar was charged with 1.94 g (6.65 mmol) of RCA and cooled in an ice bath under a nitrogen atmosphere, The solution was added to 1.0 g (6.8 mmol) of nitrobiuret followed by 40 mL of water. The reaction was heated at reflux under a nitrogen atmosphere for 2 h and cooled to room temperature, and the solvents were removed by rotary evaporation at aspirator pressure. The residue was dissolved in 250 mL of warm THF, adsorbed to silica, and washed four times with 500 mL of ethyl acetate (THF followed by THF) to give 2.20 g (5.87 mmol, 87%) of the product as a white solid: Rf 0.45 (90:10 methylene chloride/methanol). 

HRMS (M⁺) calcd for C₄₃H₃₀N₄O₈: 819.5546, found 819.5547. Anal. Caled for C₄₀H₃₀N₄O₈: C 74.12; H, 5.29; N, 10.5. Found: C 73.9; H, 5.4; N, 10.5.

The solvent was removed by rotary evaporation at aspirator pressure, and the residue was purified by flash chromatography (eluted with ethyl acetate/hexanes, loaded on column preadsorbed to silica) to give 0.89 g (3.0 mmol, 79.7%) of 15 as a white solid: Rf 0.27 (66:33 ethyl acetate/hexanes). 

HRMS (M⁺) calcd for C₅₀H₄₁N₄O₈: 897.2894, found 897.2898. Anal. Caled for C₄₇H₃₅N₄O₈: C 71.77; H, 5.22; N, 11.0. Found: C 71.3; H, 5.1; N, 11.3.

The solvent was removed by rotary evaporation at aspirator pressure, and the crude product was purified by flash chromatography (eluted with 66:33 ethyl acetate/hexanes, loaded on column preadsorbed to silica) to give 0.89 g (3.0 mmol, 79.7%) of 15 as a white solid: Rf 0.27 (66:33 ethyl acetate/hexanes). 

HRMS (M⁺) calcd for C₅₀H₄₁N₄O₈: 897.2894, found 897.2898. Anal. Caled for C₄₇H₃₅N₄O₈: C 71.77; H, 5.22; N, 11.0. Found: C 71.3; H, 5.1; N, 11.3.

The solvent was removed by rotary evaporation at aspirator pressure, and the crude product was purified by flash chromatography (eluted with 66:33 ethyl acetate/hexanes, loaded on column preadsorbed to silica) to give 0.89 g (3.0 mmol, 79.7%) of 15 as a white solid: Rf 0.27 (66:33 ethyl acetate/hexanes). 

HRMS (M⁺) calcd for C₅₀H₄₁N₄O₈: 897.2894, found 897.2898. Anal. Caled for C₄₇H₃₅N₄O₈: C 71.77; H, 5.22; N, 11.0. Found: C 71.3; H, 5.1; N, 11.3.

The solvent was removed by rotary evaporation at aspirator pressure, and the crude product was purified by flash chromatography (eluted with 66:33 ethyl acetate/hexanes, loaded on column preadsorbed to silica) to give 0.89 g (3.0 mmol, 79.7%) of 15 as a white solid: Rf 0.27 (66:33 ethyl acetate/hexanes). 

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The solvent was removed by rotary evaporation at aspirator pressure, and the crude product was purified by flash chromatography (eluted with 66:33 ethyl acetate/hexanes, loaded on column preadsorbed to silica) to give 0.89 g (3.0 mmol, 79.7%) of 15 as a white solid: Rf 0.27 (66:33 ethyl acetate/hexanes). 

HRMS (M⁺) calcd for C₅₀H₄₁N₄O₈: 897.2894, found 897.2898. Anal. Caled for C₄₇H₃₅N₄O₈: C 71.77; H, 5.22; N, 11.0. Found: C 71.3; H, 5.1; N, 11.3.
Titration of hubM, with R'CA or flexM, with R"CA Monitored by 1H NMR Spectroscopy. An NMR tube was charged with hubM, (0.0196 g, 0.0094 mmol) or flexM, (0.0132 g, 0.0100 mmol) and CDCl, (1.0 mL). Solid aliquots of neohexyl cyanurate (R'CA) (0.0010 g, 0.0047 mmol) or triphenylpropyl cyanurate (R"CA) (0.0020 g, 0.0050 mmol) were added to the NMR tube, and the tube was shaken until all of the solid had dissolved. The 1H NMR spectrum was recorded after each aliquot was added. After the sixth aliquot was added there was no further change in the spectrum. Additional aliquots of R'CA or R"CA did not go into solution.

NOE Spectra of the hubM,(R'CA), and flexM,(R"CA), Complexes. The NOE spectra of the 1:3 complexes were recorded at 25 °C. The procedures for both complexes were identical. The complex (0.0100 mmol) was dissolved in 1.0 mL of CDCl, and the sample was degassed with five freeze-pump-thaw cycles. The NOE spectra were collected with an evolution period of 3.0 s and a relaxation delay of 6.0 s.

Gel Permeation Chromatography. Gel permeation chromatography was performed using a Waters 600E HPLC with a Waters 484 UV detector (set at 254 nm) and Waters analytical gel permeation column (Ultra-styragel, 1000 Å pore size). Elutions were performed at room temperature using HPLC grade chloroform and methylene chloride as the solvents at a flow rate of 1.0 mL/min. The samples were prepared at concentrations of 1.0 mM for hubM, samples and 2.0 mM for the flexM, samples in solvent that contained p-xylene (3.0 mM) as an internal reference. The injection volume was 20 μL.

Molecular Weight Determinations of the hubM,(R'CA), and flexM,(R"CA), Complexes by Vapor Pressure Osmometry. Molecular weight determinations were made with a Wescan Model 233 vapor pressure osmometer operated at 35 °C. The molecular weights of the complexes were measured in HPLC grade glass-distilled chloroform at concentrations of 2, 4, 8, and 16 mM. At each concentration, 3-4 measurements were taken. Calibration curves were generated using sucrose octaacetate, perbenzoyl β-cyclodextrin, polystyrene (MW 5050, polydispersity = 1.05), and a gramicidin S derivative in which the ornithine amino groups had been converted to the tert-butyl carbamates (MW 1342) as molecular weight standards.

Titration of hubM, with R'CA and flexM, with R"CA Monitored by UV Spectroscopy. UV spectra were recorded on a Perkin-Elmer Model 551 spectrophotometer. A 125-mL Erlenmeyer flask equipped with a stirring bar was charged with hubM, (0.0209 g, 0.0100 mmol) or flexM, (0.0132 g, 0.0100 mmol) and 100 mL of CHCl,. Solid aliquots of neohexyl cyanurate (0.0011 g, 0.0050 mmol) or triphenylpropyl cyanurate (0.0020 g, 0.0050 mmol) were added to the flask, and the solution was stirred until all of the solid had dissolved. After each aliquot was added, a 0.30-mL sample of the solution was transferred to a 1.0-mm quartz cuvette and the UV spectrum was recorded from 390 to 190 nm. The sample was transferred back to the Erlenmeyer flask and the next aliquot was added. After the sixth aliquot was added there was no further change in the spectrum. The quartz cuvette was rinsed thoroughly with THF and dried in a stream of nitrogen between each measurement.

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Supplementary Material Available: Details of the synthesis of compounds other than hubM, and flexM, that are shown in Scheme II (9 pages). Ordering information is given on any current masthead page.