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Molecular Self-Assembly through Hydrogen Bonding: Aggregation of Five Molecules To Form a Discrete Supramolecular Structure

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Abstract: This article describes reactions of the trivalent melamine derivatives C_6H_3-1,3,5-[$CONHC_6H_4$-3-$N(CH_2C_6H_4$-4-$C(CH_3)_3)COC_6H_3$-2-$(NHC_3N_3(NH_2)(NHCH_2CH_2C(CH_3)_3))$-5-Br]$_3$ (hubM$_3$) and $(CH_3)_3COC(O)NHC[CH_2O(CH_2)_3$-$SCH_2C(O)NHC_6H_4$-2-$NHC_3N_3(NH_2)(NH(CH_2)_2C(CH_3)_3)]_3$ (trisM$_3$) with the bivalent isocyanurate derivatives C_6H_2-1,5-[$CH(CH_3)_2$]$_2$-2,4-[$CH_2NC(O)NHC(O)NHC(O)$]$_2$ (benzCA$_2$) and C_4O-2,5-[$CH(CH_3)_2$]$_2$-3,4-[$CH_2NC(O)NHC(O)$-$NHC(O)$]$_2$ (furanCA$_2$) in $CHCl_3$ to afford a series of supramolecular aggregates containing 2 equiv of the tris melamine and 3 equiv of the bis cyanurate (2 + 3 complexes). These complexes consist of two parallel hydrogen-bonded lattices that incorporate 36 hydrogen bonds. The structures have been characterized by ^{1}H NMR, ^{13}C NMR, and UV spectroscopies, gel permeation chromatography, and vapor pressure osmometry. These techniques demonstrate that the 2 + 3 aggregates in $CHCl_3$ solution are stable and structurally well-defined. hubM$_3$ is more rigid than trisM$_3$. This difference in rigidity is used to probe the relationship between the molecular structure of the trivalent melamine derivative and the geometry and stability of the resulting aggregate. (hubM$_3$)$_2$(benzCA$_2$)$_3$ and (hubM$_3$)$_2$(furanCA$_2$)$_3$ each seem to exist in one isomeric form; (trisM$_3$)$_2$(benzCA$_2$)$_3$ and (trisM$_3$)$_2$(furanCA$_2$)$_3$ are both mixtures of isomers (due, probably, to the relative flexibility of the arms of trisM$_3$).

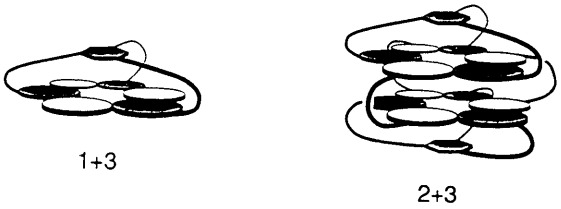
Introduction

We are developing molecular self-assembly as the basis of a program to design, synthesize, and characterize hydrogen-bonded supramolecular aggregates.[1-3] One impetus for this program is the precedent in biology for self-assembly and structural stabilization through networks of cooperative noncovalent interactions. These phenomena are illustrated by the folding of RNA chains into functioning tRNA molecules[4] and the stabilization of telomeres by stacked cyclic arrays of hydrogen bonds,[5] among many other examples.[6] A second impetus is the development of molecular self-assembly as a synthetic strategy for making nanostructures.[7]

To define rules for designing aggregates that are based on molecular self-assembly, we are preparing a series of hydrogen-bonded supramolecular aggregates. This series will (i) sharpen the criteria for design of self-assembled structures; (ii) explore the thermodynamic aspects of self-assembly, especially the interplay of enthalpic and entropic effects; and (iii) develop techniques for characterizing noncovalently bound species in organic solution.

The strategy that we have employed to build self-assembling aggregates has been to use fragments of the hydrogen-bonded lattice that exists in the 1:1 complex of cyanuric acid and melamine (CA·M) as the foundation for soluble structures.[8] We have described the preorganization of three melamine (M) units by covalently linking them together through a "hub" and "spoke" architecture to form the trivalent melamine derivative hubM$_3$.[2,9] This molecule complexes with 3 equiv of monomeric isocyanurates (CA) and forms highly structured 1 + 3 supramolecular aggregates (one hubM$_3$ + three CA) (Scheme I). In this article we describe reactions of the trivalent melamine derivatives hubM$_3$ and trisM$_3$ with the bivalent isocyanurates benzCA$_2$ and furanCA$_2$ that give 2 + 3 supramolecular aggregates (Scheme II). These systems are composed of two parallel, planar hydrogen-bonded lattices. Our objectives in the progression from single-layer structures (1 + 3) to double-layer structures (2 + 3) have been (i) to increase substantially the number of hydrogen bonds (from 18 to 36) that stabilize these aggregates while increasing the number of molecules involved (and thus the unfavorable entropy of association) relatively less; (ii) to generate a more stable structural motif; (iii) to increase the size and complexity of these structures; and (iv) to assess the role of preorganization (rigid hubM$_3$ vs flexible trisM$_3$) in the self-assembly of these complexes.

Scheme I. Schematic Representation of the 1 + 3 and 2 + 3 Supramolecular Aggregates



Design of Constituent Molecules. A. hubM$_3$. The molecular structure of the trivalent melamine derivative hubM$_3$ preorganizes this compound for complexation with isocyanurate derivatives. The crucial aspect of this organization seems to be a conformation determined by the *o*-anthranilate groups that facilitates the 180° turn necessary in the attachment of the melamine rings to the central "hub" to form aggregates based on the cyclic CA$_3$M$_3$ motif.[2]

B. trisM$_3$. trisM$_3$ is an analog of hubM$_3$ with more flexible spokes than hubM$_3$. We are interested in the interplay between the rigidity/flexibility of constituent molecules, their ease of synthesis, and their successful incorporation into self-assembled structures. trisM$_3$ has retained the ortho-substituted aromatic ring. The amino group of the central hub of trisM$_3$ will allow us eventually to link these self-assembling systems together to form large supramolecular arrays. Flexible analogs of hubM$_3$ lead to 1 + 3 aggregates with monomeric isocyanurates that are less stable than aggregates formed by hubM$_3$.[2] We wanted to determine whether the enthalpy of formation of the 2 + 3 complexes, which

(1) For a preliminary report of this work, see: Seto, C. T.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 712.
(2) Seto, C. T.; Whitesides, G. M. *J. Am. Chem. Soc.*, in press.
(3) For further examples of self-assembly, see: Lehn, J. M. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1304. Tecilla, P.; Dixon, R. P.; Slobodkin, G.; Alavi, D. S.; Waldbeck, D. H.; Hamilton, A. D. *J. Am. Chem. Soc.* **1990**, *112*, 9408. Stoddart, J. F.; et al. *J. Am. Chem. Soc.* **1992**, *114*, 193. Zimmerman, S. C.; Duerr, B. F. *J. Org. Chem.* **1992**, *57*, 2215.
(4) Draper, D. E. *Acc. Chem. Res.* **1992**, *25*, 201.
(5) Kang, C.; Zhang, X.; Ratliff, R.; Moyzis, R.; Rich, A. *Nature* **1992**, *350*, 126. Smith, F. W.; Feigon, J. *Nature* **1992**, *350*, 164.
(6) Lindsey, J. S. *New J. Chem.* **1991**, *15*, 153.
(7) Whitesides, G. M.; Mathias, J. P.; Seto, C. T. *Science* **1991**, *254*, 1312.
(8) The CA·M lattice is the putative two-dimensional hydrogen-bonded sheet structure that forms on mixing cyanuric acid and melamine.
(9) Seto, C. T.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, *112*, 6409.

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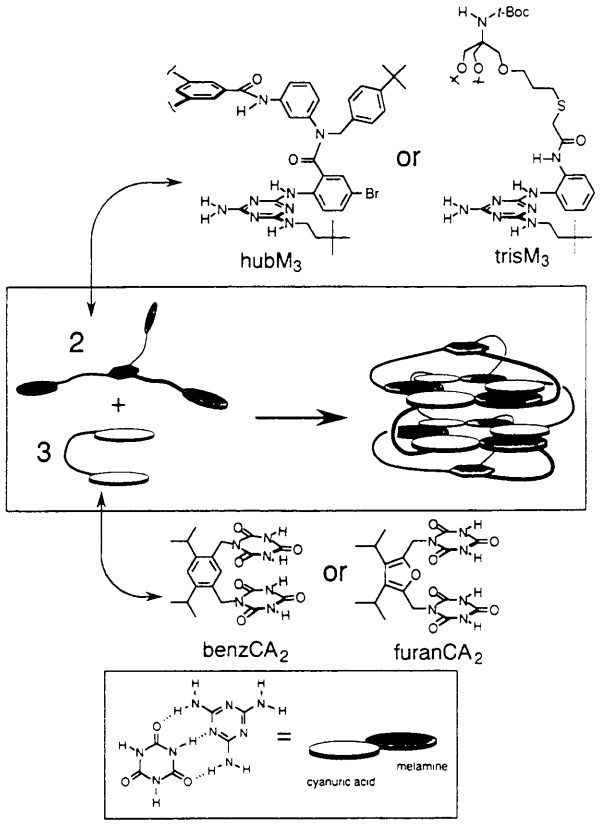
Scheme II. Self-Assembly of 2 equiv of $hubM_3$ or $trisM_3$ with 3 equiv of $benzCA_2$ or $furanCA_2$ To Give a 2 + 3 Supramolecular Aggregate



is twice the enthalpy of formation of the 1 + 3 complexes, is large enough to offset the unfavorable entropy of conformation associated with flexible spokes.

C. $benzCA_2$ and $furanCA_2$. For two $hubM_3$ or $trisM_3$ molecules to assemble face-to-face, the CA rings of the bis isocyanurate derivatives must be approximately parallel. Molecular models suggest that the CA rings in $furanCA_2$ are almost exactly parallel. In $benzCA_2$, they are tilted toward each other by approximately 20° when the phenyl ring and the CA groups are perpendicular (Scheme II); by skewing the phenyl ring it is possible to make the CA rings parallel. The similar stability of complexes based on $benzCA_2$ and $furanCA_2$ suggests that the difference in geometry between these molecules is not important. The isopropyl groups restrict the CA moieties to a conformation in which the CA rings are approximately parallel and pointed in the same direction. This preorganization minimizes the conformational entropy lost on forming the 2 + 3 aggregates.

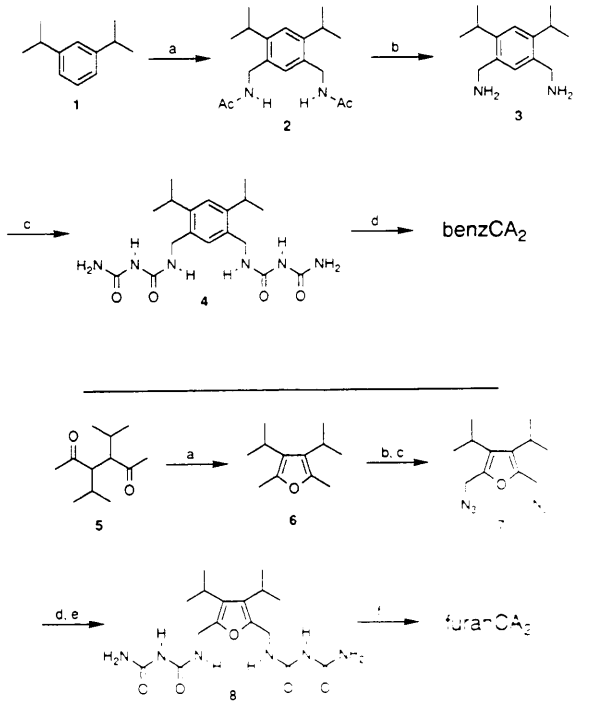
Results

Self-Assembly of 2 + 3 Supramolecular Aggregates. Scheme II outlines the self-assembly of the 2 + 3 complexes. The following discussions will focus on $(hubM_3)_2(benzCA_2)_3$ and $(trisM_3)_2(benzCA_2)_3$; the results obtained for $(hubM_3)_2(furanCA_2)_3$ and $(trisM_3)_2(furanCA_2)_3$ are analogous and are not outlined in detail.

Synthesis of Components. The synthesis of $hubM_3$ has been described previously.[2] The syntheses of $benzCA_2$, $furanCA_2$, and $trisM_3$ are outlined in Schemes III and IV.

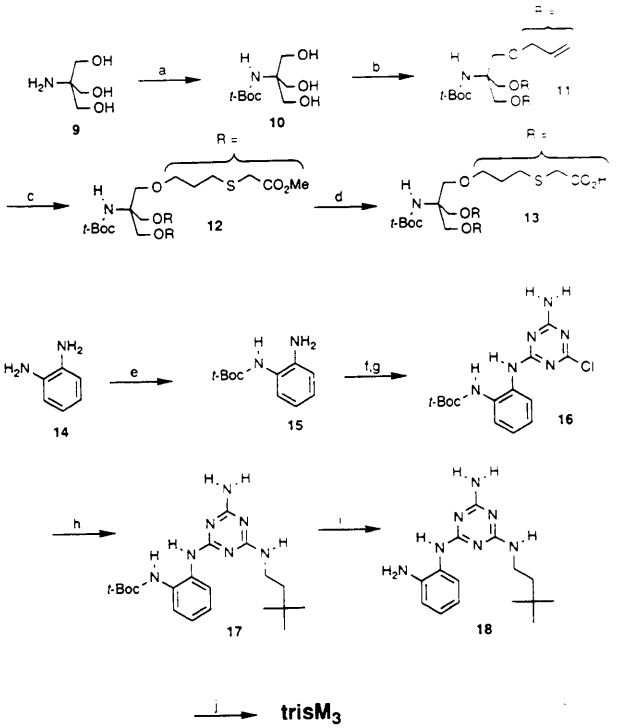
Preparation of Supramolecular Aggregates. $hubM_3$ and $trisM_3$ are readily soluble in $CHCl_3$ (>15 mM), but the solubilities of $benzCA_2$ and $furanCA_2$ in this solvent are low (<0.1 mM). Simply mixing 2 equiv of $hubM_3$ with 3 equiv of $benzCA_2$ in $CHCl_3$ at room temperature does not afford $(hubM_3)_2(benzCA_2)_3$, because the $benzCA_2$ does not dissolve. To prepare 2 + 3 complexes, we used three similar strategies (which we outline for the preparation of $(hubM_3)_2(benzCA_2)_3$): (1) $hubM_3$ and $benzCA_2$ were dissolved in a 1:1 mixture of MeOH and $CHCl_3$ to afford a homogeneous solution; (2) $hubM_3$ and $benzCA_2$ were suspended in $CHCl_3$ containing 5% MeOH and heated briefly (~10 s) until

Scheme III. Synthesis of $benzCA_2$[a] and $furanCA_2$[b]



[a] Reagents: (a) paraformaldehyde, CH_3CN, AcOH, H_2SO_4, 90 °C, 40%; (b) 3 N aqueous HCl, reflux, 94%; (c) $H_2NC(O)NHC(O)NH-NO_2$, H_2O, reflux, 85%; (d) $(EtO)_2CO$, NaOEt, EtOH, reflux, 48%. [b] Reagents: (a) *p*-toluenesulfonic acid, toluene, reflux, 58%; (b) *N*-bromosuccinimide, CCl_4, reflux; (c) NaN_3, DMF, 60 °C, 30% (2 steps); (d) $Pd(OH)_2$, H_2, MeOH; (e) nitrobiuret, H_2O, reflux, 69% (2 steps); (f) $(EtO)_2CO$, NaOEt, EtOH, reflux, 50%.

Scheme IV. Synthesis of $trisM_3$[a]



[a] Reagents: (a) $(CH_3)_3COCO_2N{=}C(C_6H_5)CN$ (BOC-ON), DMF, 50 °C, 78%; (b) allyl bromide, KOH, DMSO, 16 °C, 80%; (c) methyl 2-thioacetate, THF, *hν*, room temperature, 85%; (d) NaOH, THF, MeOH, room temperature, 90%; (e) BOC-ON, DMF, 55 °C, (66%); (f) cyanuric chloride, THF, 0 °C; (g) NH_3, THF, 0 °C, 94% (two steps); (h) neohexylamine, THF, reflux, 83%; (i) F_3CCO_2H, CH_2Cl_2, 25 °C, 94%; (j) 0.33 equiv of **13**, DCC, 1-hydroxybenzotriazole, THF, 25 °C, 88%.

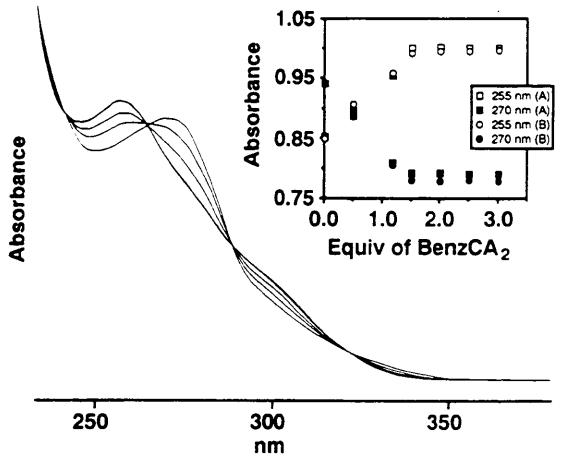


Figure 1. Titration of $hubM_3$ with $benzCA_2$ monitored by UV spectroscopy (0.1 mM in CH_2Cl_2). The inset graph shows plots of absorbance vs equivalents of $benzCA_2$ for two separate runs (A and B). Beyond the 2:3 stoichiometry (1.5 equiv of $benzCA_2$) the UV spectrum does not change.

the solution became homogeneous; (3) $hubM_3$ and $benzCA_2$ were suspended in $CHCl_3$ and heated at reflux until the solution became homogeneous (~5 min). In each case, concentration of the solution in vacuo to dryness afforded the 2 + 3 aggregate as a white solid that was readily soluble in $CHCl_3$. Analyses of complexes formed by each of these methods were indistinguishable.

Qualitative Evidence for the Formation of 2 + 3 Supramolecular Aggregates. If more than 1.5 equiv of $benzCA_2$ was present, the excess $benzCA_2$ did not dissolve in $CHCl_3$. This result suggests a 2:3 stoichiometry for the complex between $hubM_3$ and $benzCA_2$. Experiments using $trisM_3$ and $benzCA_2$ or $furanCA_2$ in $CHCl_3$ also indicated stoichiometries of 2:3.

$(hubM_3)_2(benzCA_2)_3$ and $(hubM_3)_2(furanCA_2)_3$. A. Characterization of $(hubM_3)_2(benzCA_2)_3$ by UV Spectroscopy. The change in λ_{max} from 270 nm in free $hubM_3$ to 255 nm in $(hubM_3)_2(benzCA_2)_3$ on titration of $hubM_3$ with $benzCA_2$ (Figure 1) is consistent with a 2:3 stoichiometry. Beyond this stoichiometry, no further changes occur because the added $benzCA_2$ does not dissolve.

B. Characterization of $(hubM_3)_2(benzCA_2)_3$ by ^1H NMR Spectroscopy. Titration of $hubM_3$ with $benzCA_2$. Figure 2 shows the titration of $hubM_3$ with $benzCA_2$ in $CDCl_3$ as monitored by ^1H NMR spectroscopy. The resonances in the spectrum of $hubM_3$ in $CDCl_3$ are broad because of self-association and hindered rotation around the amide and RNH triazine bonds. In the 2:1 mixture between $hubM_3$ and $benzCA_2$, sharp resonances appear that correspond to $(hubM_3)_2(benzCA_2)_3$ against a background of uncomplexed $hubM_3$. The hydrogen-bonded imide protons (y, y') at δ 14.8 and 15.5 ppm and resonances for free (broad) and bound (sharp) neohexyl groups and 4-*tert*-butylbenzyl groups in the region 2.0–0.8 ppm are clearly visible. In this mixture, one-third of the $hubM_3$ is present as fully formed $(hubM_3)_2(benzCA_2)_3$, and two-thirds of the $hubM_3$ remains uncomplexed. Exchange between free and bound species is slow on the NMR time scale. A solution of $hubM_3$ that has less that 1.5 equiv of $benzCA_2$ (i.e., below the 2:3 stoichiometry required for $hubM_3/benzCA_2$) contains only free $hubM_3$ and $(hubM_3)_2(benzCA_2)_3$ and does not contain partially formed complexes with stoichiometries other than 2:3. These observations suggest that formation of $(hubM_3)_2(benzCA_2)_3$ is a cooperative process. At the 2:3 ratio of $hubM_3$ to $benzCA_2$, the $hubM_3$ is fully complexed; further addition of $benzCA_2$ does not change the spectrum because it does not dissolve. The top spectrum of Figure 2 shows $hubM_3$ and $benzCA_2$ in a ratio of 2:"6"; the 3 equiv of $benzCA_2$ beyond that required for formation of the 2 + 3 complex remain insoluble. We believe that the minor resonances in the spectrum of $(hubM_3)_2(benzCA_2)_3$ are associated with geometrical isomers of $(hubM_3)_2(benzCA_2)_3$ (see the Discussion section). The spectra of $hubM_3$ and $benzCA_2$ in DMSO-d_6 illustrate the effect of

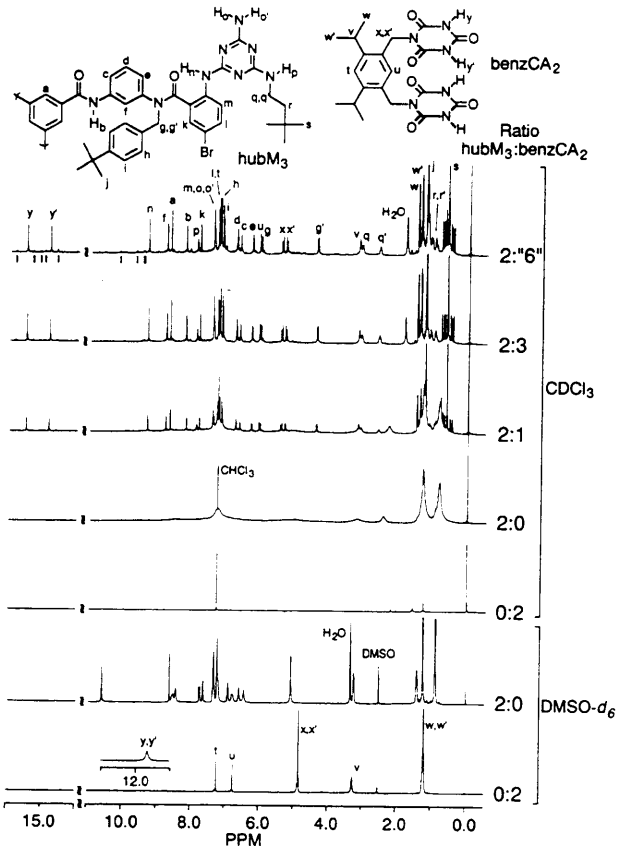


Figure 2. ^1H NMR spectra of mixtures of $hubM_3$ with $benzCA_2$ (500 MHz, $CDCl_3$). The peak assignments are shown at the top of the figure. The bottom two spectra show $hubM_3$ and $benzCA_2$ alone in DMSO-d_6 for reference. Several of the minor resonances that we believe correspond to other geometrical isomers of the complex have been marked below the base line of the top spectrum.

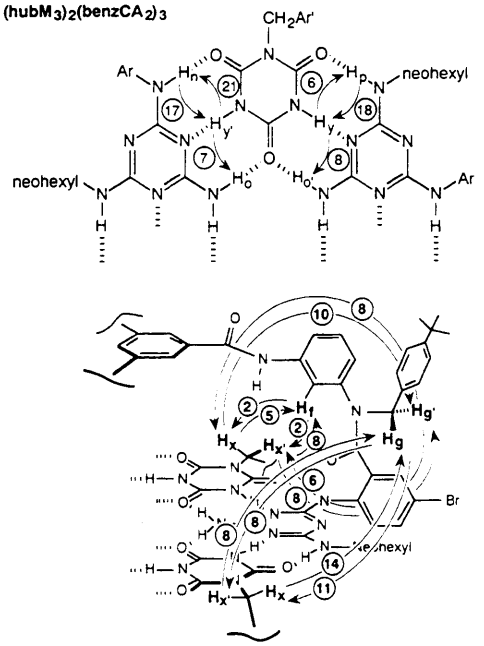


Figure 3. Intermolecular NOEs (%) among the protons in $(hubM_3)_2(benzCA_2)_3$. The NOEs among the hydrogen-bonded protons are shown in the upper structure, and the NOEs among other protons are shown in the lower structure. The NOEs that are not shown are either weak or the NOE signal is obscured by incomplete subtraction of other resonances in the difference spectrum.

breaking up self-association due to hydrogen bonding in these compounds.

C. Characteristic Features of the ^1H NMR Spectrum of $(hubM_3)_2(benzCA_2)_3$. 1. Assignment of Protons. $(hubM_3)_2$-

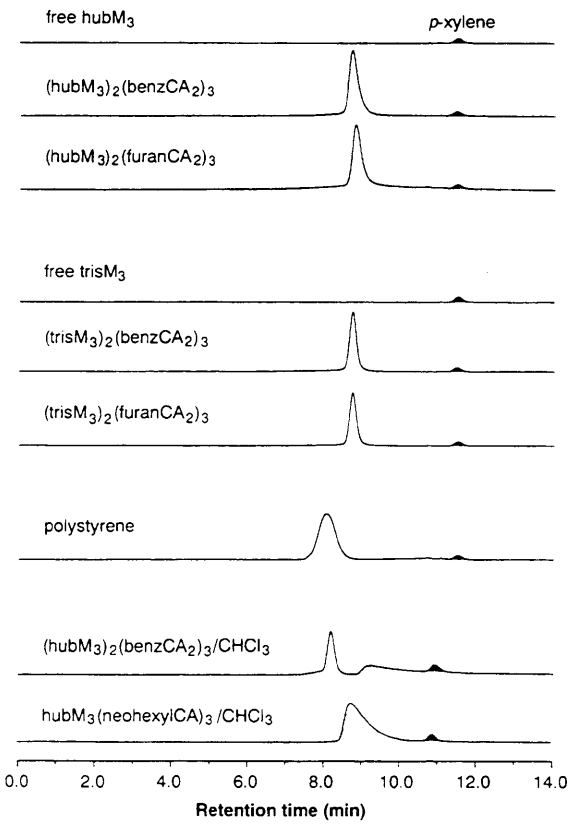


Figure 4. Gel permeation chromatograms of free and complexed $hubM_3$ and $trisM_3$. The small peak at 11.5 min is *p*-xylene used as an internal standard. The elution solvent was methylene chloride unless indicated otherwise. The bottom chromatogram shows polystyrene (av MW 5050; polydispersity = 1.05) for reference. $(hubM_3)_2(benzCA_2)_3$ and $hubM_3(neohexylCA)_3$ (a less stable complex that shows substantial tailing) eluted with chloroform are also shown for comparison. In $CHCl_3$, no peaks are detectable for $(trisM_3)_2(benzCA_2)_3$ or $(trisM_3)_2(furanCA_2)_3$.

$(benzCA_2)_3$ has been characterized using H → D exchange, COSY, and NOE experiments. The assignments of resonances are shown in Figure 2; the NOEs are summarized in Figure 3.

2. Nuclear Overhauser Effects. The proximity (~2.5 Å) of the hydrogen-bonded NH protons in $(hubM_3)_2(benzCA_2)_3$ results in strong intermolecular NOEs among these protons (Figure 3). Intermolecular NOEs between protons on the spokes of $hubM_3$ and the benzylic protons of $benzCA_2$ are consistent with the model proposed for the 2 + 3 complex. The large magnitudes of these NOEs indicate that the structure of $(hubM_3)_2(benzCA_2)_3$ is well-defined and stable in solution. We do not see NOEs between protons in adjacent layers of the complex. This observation is consistent with the spacing between these layers suggested by CPK models (~4.8 Å).

3. Hydrogen-Bonded Protons. The imide protons of $benzCA_2$ (y, y′) are equivalent in the uncomplexed molecule, appearing at δ 11.6 ppm in DMSO-d_6. In $(hubM_3)_2(benzCA_2)_3$ they occupy different hydrogen-bonding sites and thus appear as separate resonances at δ 14.8 and 15.5 ppm in $CDCl_3$. The downfield position of these protons indicates strong hydrogen bonds with the melamine ring nitrogen atoms.

4. Diastereotopic Protons. Several sets of protons that are equivalent in $hubM_3$ (g, g′; q, q′; and r, r′) and $benzCA_2$ (y, y′; w, w′; and x, x′) become diastereotopic in the 2 + 3 aggregate $(hubM_3)_2(benzCA_2)_3$. The proton g′ is shifted upfield by 1.6 ppm with respect to g. Molecular models suggest that this shift is caused by through-space shielding from the neighboring aromatic ring of the 1,3-diaminobenzene moiety.

D. Characterization of $(hubM_3)_2(benzCA_2)_3$ by Gel Permeation Chromatography (GPC). We used GPC to determine whether the self-assembled structures exist as a mixture of species or as single species with well-defined size. The shapes of peaks in GPC give a qualitative measure of the stability of the self-assembled

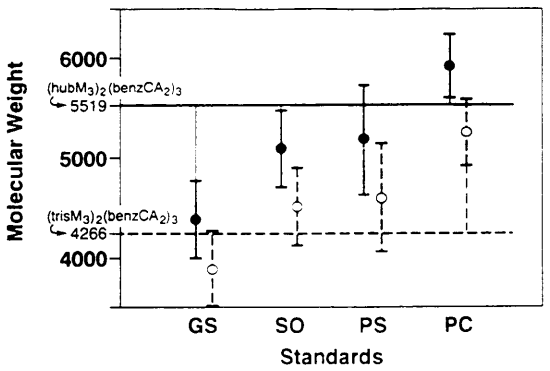


Figure 5. Estimation of the molecular weights of $(hubM_3)_2(benzCA_2)_3$ (●) and $(trisM_3)_2(benzCA_2)_3$ (○) by vapor pressure osmometry, using four different molecular weight standards. The solid and dashed horizontal lines correspond to the calculated MWs of $(hubM_3)_2(benzCA_2)_3$ and $(trisM_3)_2(benzCA_2)_3$, 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 β-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 $CHCl_3$ over the concentration range 2–16 mM complex. No special precautions were taken to ensure the dryness of the solvent.

aggregates under the times (10–15 min) and conditions of the run.[10] We used 3 mM solutions of *p*-xylene as an internal standard and CH_2Cl_2 or $CHCl_3$ as the eluent. The GPC column was made from cross-linked styrene/divinylbenzene and had a molecular weight cutoff of 30 kDa.

1. GPC of 2 + 3 Adducts with CH_2Cl_2 as the Eluent. Figure 4 shows the GPC traces of free and complexed $hubM_3$. $hubM_3$ alone in methylene chloride solution is self-associated. The peak due to $hubM_3$ is not detected because this peak is broadened into the base line and because free $hubM_3$ has a weaker absorbance at 254 nm (the wavelength to which the UV detector is set) than does complexed $hubM_3$. The uncomplexed molecule exists in a broad distribution of self-associated species with different apparent molecular weights in solution. In contrast, $(hubM_3)_2(benzCA_2)_3$ and $(hubM_3)_2(furanCA_2)_3$ give sharp peaks in their GPC traces at 8.7–8.8 min. These observations suggest that the complexes exist in solution as single species having well-defined molecular weights. Comparison of these retention times to that of the polystyrene standard (average MW 5050, polydispersity 1.05) confirms the similar sizes of these aggregates. The peak profiles of the complexes are sharper than that of the polystyrene: this observation reinforces the conclusion that the 2 + 3 aggregates are structurally homogeneous.

2. Partial Decomplexation in GPC with $CHCl_3$ as the Eluent. Decomplexation of $(hubM_3)_2(benzCA_2)_3$ that occurs during the GPC analysis is irreversible because free $hubM_3$ and $benzCA_2$ have different molecular weights (2093 and 445, respectively) and should separate (in principle) on the column.[11] The single, sharp peak in the GPC trace of $(hubM_3)_2(benzCA_2)_3$ with CH_2Cl_2 as the eluent shows that this aggregate does not decomplex during the analysis. In contrast, the GPC trace of $(hubM_3)_2(benzCA_2)_3$ with $CHCl_3$ as the eluent shows a sharp peak at 8.3 min corresponding to the 2 + 3 aggregate, along with a broad peak at 9.3 min corresponding to some form(s) of decomplexed material. This result indicates that $(hubM_3)_2(benzCA_2)_3$ does decomplex appreciably during GPC analysis in the more polar solvent. The trace of $hubM_3$ complexed with monomeric neohexylCA is shown in Figure 4 for comparison. This 1 + 3 complex [$hubM_3(neohexylCA)_3$] is less stable that the 2 + 3 aggregate and thus shows tailing.[12,13]

(10) Stable complexes give sharp, symmetrical peaks in GPC, while less stable complexes show tailing of their peaks caused by decomplexation during the analysis. Stevens, F. J. *Biochemistry* **1986**, *25*, 981. Stevens, F. J. *Biophys. J.* **1989**, *55*, 1155.

(11) The low solubility of $benzCA_2$ and the self-association of $hubM_3$ make their behavior difficult to predict.

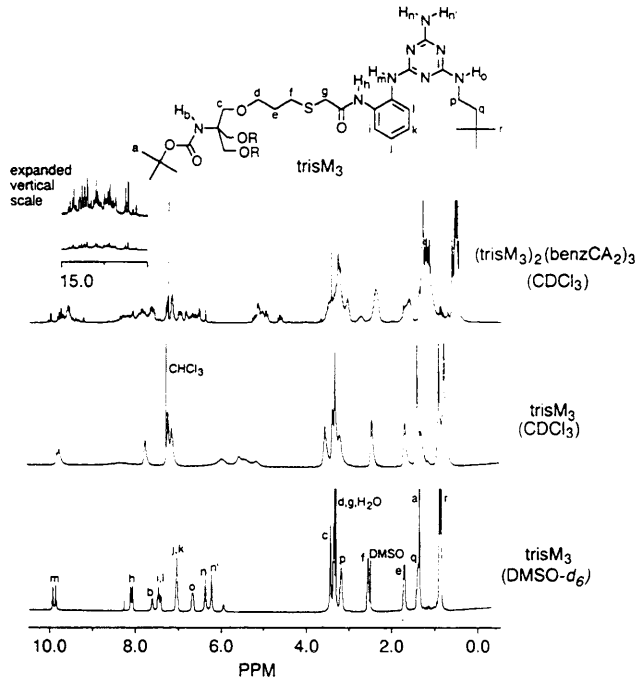


Figure 6. ^{1}H NMR spectra of trisM$_3$ in $CDCl_3$ and DMSO-d_6 and trisM$_3$ complexed with benzCA$_2$ in $CDCl_3$. The peak assignments for the spectrum in DMSO-d_6 are shown at the top of the figure. The region from 14 to 16 ppm (resonances for the cyanurate NH protons) is shown for (trisM$_3$)$_2$(benzCA$_2$)$_3$.

E. Molecular Weight of (hubM$_3$)$_2$(benzCA$_2$)$_3$ Determined by Vapor Pressure Osmometry (VPO). The calculated molecular weight of (hubM$_3$)$_2$(benzCA$_2$)$_3$ is 5.519 kDa. We determined the molecular weight of (hubM$_3$)$_2$(benzCA$_2$)$_3$ by VPO in $CHCl_3$ solution (Figure 5), using four different standards against which to calibrate the results. With sucrose octaacetate (MW = 679), polystyrene (MW 5050, polydispersity = 1.05), and perbenzoyl β-cyclodextrin (MW = 3321) as standards, the experimental molecular weights of (hubM$_3$)$_2$(benzCA$_2$)$_3$ were 5.1 ± 0.4, 5.2 ± 0.5, and 5.9 ± 0.3 kDa, respectively. These standards do not form strong hydrogen bonds and should not be self-associated in chloroform solution. Since these three standards gave experimental molecular weights for (hubM$_3$)$_2$(benzCA$_2$)$_3$ that were within ±10% of the calculated value, we infer that the 2 + 3 aggregate has a molecular weight in solution that corresponds to the calculated value and that the aggregate is also not strongly self-associated. With *N,N'*-bis(*t*-Boc)gramicidin S (MW = 1342) as a standard, the experimental molecular weight was ~20% low (4.4 ± 0.4 kDa). Since gramicidin S is a cyclic decapeptide with many hydrogen-bonding sites, this standard may itself be self-associated in chloroform solution.[14] The concentration dependence of the VPO data for this standard is consistent with self-association.[14] This result would cause the predicted molecular weight of an unknown to be low. These data show that the molecular weight of a complex obtained by VPO depends strongly on the choice of the molecular weight standard. We therefore believe, at the current state of understanding of nonidealities in these classes of complexes, that it is essential to use a number of internal standards and to obtain data over a range of concentrations in order to generate interpretable results by osmometry.

(trisM$_3$)$_2$(benzCA$_2$)$_3$ and (trisM$_3$)$_2$(furanCA$_2$)$_3$. A. Characterization of (trisM$_3$)$_2$(benzCA$_2$)$_3$ by ^{1}H NMR Spectroscopy. The ^{1}H NMR spectrum of (trisM$_3$)$_2$(benzCA$_2$)$_3$ indicates that this complex exists as a mixture of geometrical isomers (Figure 6). The resonances of (trisM$_3$)$_2$(benzCA$_2$)$_3$ are present in the regions expected by analogy with (hubM$_3$)$_2$(benzCA$_2$)$_3$, but are more complicated in multiplicity. For example, the region from 14 to 16 ppm shows a large number of peaks for the imide NH protons of benzCA$_2$ in (trisM$_3$)$_2$(benzCA$_2$)$_3$; in contrast, there are only two resonances in this region in the spectrum of (hubM$_3$)$_2$(benzCA$_2$)$_3$. The spectrum of (trisM$_3$)$_2$(furanCA$_2$)$_3$ is qualitatively similar to that of (trisM$_3$)$_2$(benzCA$_2$)$_3$, suggesting that this aggregate also exists as a mixture of isomers. Full structural characterization of (trisM$_3$)$_2$(benzCA$_2$)$_3$ and (trisM$_3$)$_2$(furanCA$_2$)$_3$ by ^{1}H NMR spectroscopy is made impossible by this complexity. This feature demonstrates the difficulty of using NMR spectroscopy to characterize supramolecular aggregates, unless they are highly and symmetrically structured.

B. Characterization of (trisM$_3$)$_2$(benzCA$_2$)$_3$ by GPC. Free trisM$_3$ is self-associated in CH_2Cl_2 and, similar to hubM$_3$, shows no discernible peaks in GPC (Figure 4). (trisM$_3$)$_2$(benzCA$_2$)$_3$ and (trisM$_3$)$_2$(furanCA$_2$)$_3$ each show a single sharp peak in their GPC trace. Although ^{1}H NMR spectroscopy suggests that these aggregates exist as a distribution of geometrical isomers, only species with a single molecular weight are present. The lack of tailing in these peaks suggests that these 2 + 3 aggregates have thermodynamic stability comparable to (hubM$_3$)$_2$(benzCA$_2$)$_3$. If the width of the peak in GPC is a measure, in fact, they may be slightly *more* stable than (hubM$_3$)$_2$(benzCA$_2$)$_3$. We do not observe any peaks for (trisM$_3$)$_2$(benzCA$_2$)$_3$ using $CHCl_3$ as the eluent. The absence of peaks may reflect either decomplexation of the aggregate during the analysis in the more polar and more hydrogen-bonding solvent and/or the low absorbance of the complex at wavelengths above the UV cutoff of $CHCl_3$ (~275 nm).

C. Characterization of (trisM$_3$)$_2$(benzCA$_2$)$_3$ by VPO. The calculated molecular weight of (trisM$_3$)$_2$(benzCA$_2$)$_3$ is 4.266 kDa. The results from the VPO analysis of this aggregate are shown in Figure 5. The trend with internal standard follows the same pattern as for (hubM$_3$)$_2$(benzCA$_2$)$_3$, although the deviation from the theoretical values is shifted to higher molecular weight. These results suggest that, in chloroform solution, (trisM$_3$)$_2$(benzCA$_2$)$_3$ is self-associated to a greater extent than is (hubM$_3$)$_2$(benzCA$_2$)$_3$, but they still confirm the assigned stoichiometry.

Discussion

Geometry of the 2 + 3 Aggregates. The melamine rings in hubM$_3$ and trisM$_3$ are not symmetrical; one exocyclic nitrogen is attached to a neohexyl group while another is attached to the spoke of hubM$_3$ or trisM$_3$. Each of the two hubM$_3$ or trisM$_3$ molecules in the 2 + 3 aggregate can exist in symmetrical or unsymmetrical forms with either a right- or left-handed twist. There are 16 possible geometrical isomers of the 2 + 3 aggregates (Figure 7). Two of these isomers are meso (AB and CD) and 14 exist as seven pairs of enantiomers.

The ^{1}H NMR spectrum of (hubM$_3$)$_2$(benzCA$_2$)$_3$ (Figure 2) demonstrates that most of this aggregate (>95%) is a single D_3 or C_3 symmetrical structure. There is one major set of resonances for the six spokes of (hubM$_3$)$_2$(benzCA$_2$)$_3$. The three geometrical isomers that have C_3 symmetry are AB and the pair of enantiomers AA and BB. Isomer AB is meso and should have a spectrum distinct from AA and BB. Since we observe a single major set of resonances for (hubM$_3$)$_2$(benzCA$_2$)$_3$, we believe that this aggregate exists as AB or AA + BB and is not a mixture of these isomers. The minor resonances in the spectrum of (hubM$_3$)$_2$(benzCA$_2$)$_3$ suggest that a small amount of this complex (<5%) is present in geometries other than AB or AA + BB.

The complexity of the ^{1}H NMR spectrum of (trisM$_3$)$_2$(benzCA$_2$)$_3$ (Figure 6) suggests that this complex exists in several isomeric forms. A mixture of isomers is favored entropically over a single isomer or pair of enantiomers. The enthalpies of formation of the 16 geometrical isomers of (trisM$_3$)$_2$(benzCA$_2$)$_3$ may be similar because the flexible spokes of trisM$_3$ may allow the isomers to be relatively free of strain.

Effects of Solvent on Self-Assembling Systems. A. *o*-Dichlorobenzene. The ^{1}H NMR spectra of (hubM$_3$)$_2$(benzCA$_2$)$_3$ in *o*-dichlorobenzene-d_4, methylene chloride-d_2, and chloroform-*d*

(12) Although the peak in the GPC trace for hubM$_3$(neohexylCA)$_3$ shows tailing, we believe that a majority of this complex remains in associated form during the analysis.

(13) In GPC, retention times vary with the eluent. *p*-Xylene appears at 11.6 min in CH_2Cl_2 and at 10.9 min in $CHCl_3$.

(14) Seto, C. T.; Whitesides, G. M. *J. Am. Chem. Soc.*, in press.

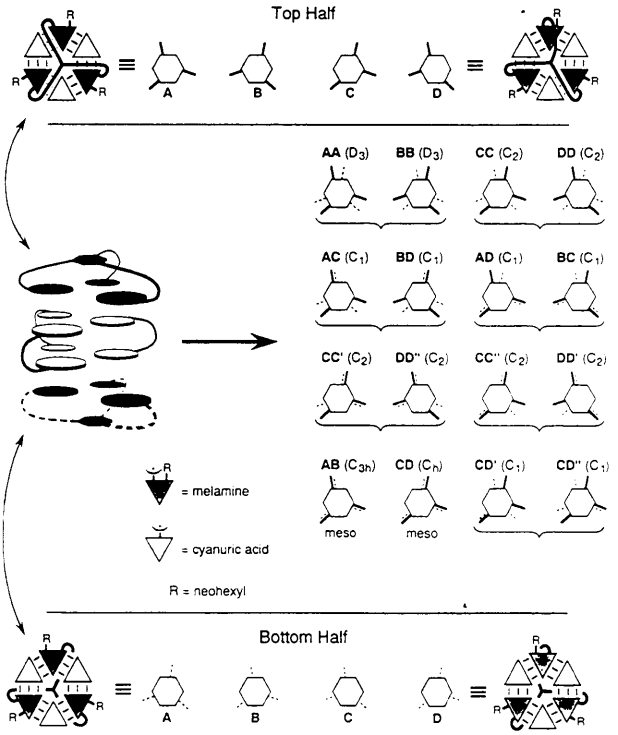


Figure 7. Schematic representation of the 16 geometrical isomers that are possible for the 2 + 3 molecular aggregates. The four conformations that a single tris melamine can adopt are structures A–D (shown at the top of the figure). This view is from the top of the aggregate, with the hydrogen-bonded lattice lying parallel to the plane of the page. At the bottom of the figure are shown the conformations of the bottom half of the aggregate. The geometries of the 2 + 3 aggregates are given a two-letter designation. The first letter corresponds to the tris melamine that is above the page and that is indicated by heavy lines, and the second letter corresponds to the tris melamine that is below the page and that is indicated by dashed lines. C′ and C″ indicate that conformation C has been rotated clockwise by 120° and 240°, respectively. The point group of each geometrical isomer is given in parentheses. The brackets indicate pairs of enantiomers.

are similar in general form, but differ in that many of the resonances appear farther downfield in the aromatic solvent.[15] The hydrogen bonds in the complex should be stronger in *o*-dichlorobenzene than in chloroform because *o*-dichlorobenzene is not a hydrogen-bond donor.[16] We do not know whether the downfield shift of the hydrogen-bonding protons of $(hubM_3)_2(benzCA_2)_3$ in *o*-dichlorobenzene compared to chloroform is caused exclusively by deshielding associated with the aromatic solvent or is partially caused by an increase in the strength of the hydrogen bonds.[16] The spectra of $(hubM_3)_2(benzCA_2)_3$ in methylene chloride-d_2 and chloroform-*d* are almost identical, even though the GPC results suggest that the aggregate is more stable in methylene chloride. In GPC, dissociation of the aggregate is irreversible because the free components are separated by the column, while in NMR spectroscopy, we observe the aggregate in equilibrium with its components. Because of this difference, it is easier to observe dissociation of the aggregate with GPC than with NMR spectroscopy, especially if the aggregate has a high association constant. Thus we observe the difference in stability of $(hubM_3)_2(benzCA_2)_3$ in methylene chloride and chloroform using GPC, while NMR spectroscopy is not sensitive enough to detect the difference. The ratio of C_3-symmetrical to non-C_3-symmetrical isomers of $(hubM_3)_2(benzCA_2)_3$ appears to be similar in all three solvents.

B. Chloroform/Methanol Mixtures. The network of hydrogen bonds in $(hubM_3)_2(benzCA_2)_3$ is robust. The complex is stable in mixtures of chloroform/methanol that contain up to 15% methanol. We titrated a solution of $(hubM_3)_2(benzCA_2)_3$ in chloroform-*d* with aliquots of methanol-d_4 and monitored the changes in the ^{1}H NMR spectrum.[17] The resonances remained sharp with only small changes in chemical shifts in solutions that contained up to 15% v/v methanol-d_4; we infer that the components remained aggregated in these solutions. At higher concentrations of methanol-d_4, the resonances became broad and the spectrum resembled an overlay of the individual spectra of $hubM_3$ and $benzCA_2$ in chloroform-*d*/methanol-d_4 mixtures. We infer that, in these solutions, the $(hubM_3)_2(benzCA_2)_3$ had mostly decomplexed into free $hubM_3$ and $benzCA_2$.[18]

The 1 + 3 complex $hubM_3(neohexylCA)_3$ is not stable in solutions of >5% methanol/chloroform. The stability of the 2 + 3 complexes is significantly the greater of the two, an effect we attribute to doubling the number of hydrogen bonds in the structure.

Conclusions

Characterization of Molecular Aggregates. The combination of NMR spectroscopy, gel permeation chromatography, and vapor pressure osmometry provides sufficient information to establish the structure and stability of hydrogen-bonded aggregates and to suggest their relative stabilities. At the start of the experiments, we knew the *molecular* structures of the components and were interested in how these components fit together in the self-assembled aggregate. NMR spectroscopy provided information about the geometry of the complexes and about cooperation in their formation. NOE measurements indicated the spatial relationships between components. GPC was a particularly useful technique for characterization, because it provided information simultaneously about the size, molecular weight distribution, and stability of molecular aggregates. VPO provided information about the stoichiometry of components in the aggregates; interpretation of results from VPO was complicated by their dependence on the MW standard chosen and by interactions between aggregates. We believe that our analysis of these assemblies provides good evidence for the structures we have proposed, although all of the methods require inference.[19]

Flexible Spokes. The geometries of the 2 + 3 aggregates are influenced by the rigidity/flexibility of the spokes. The rigid spokes of $hubM_3$ cause $(hubM_3)_2(benzCA_2)_3$ to exist as C_3-symmetrical isomers that are straightforward for characterization by ^{1}H NMR spectroscopy. The flexible spokes of $trisM_3$ allow $(trisM_3)_2(benzCA_2)_3$ to exist as a mixture of geometrical isomers that can only be analyzed qualitatively by ^{1}H NMR spectroscopy.

The thermodynamic stabilities of 1 + 3 aggregates are influenced by the conformational flexibility of the spokes.[2] Aggregates based on $flexM_3$, a molecule with flexible spokes, are less stable than aggregates based on $hubM_3$.[2] This observation suggests an important contribution from conformational entropy to the stability of these aggregates. In the 2 + 3 series, we cannot detect a difference in stability between complexes of $hubM_3$ and $trisM_3$, even though we believe that aggregates based on $hubM_3$ should be more stable. The loss of conformational entropy associated with the flexible spokes of $trisM_3$ may be counterbalanced by an increase in the enthalpy of formation of the network of 36 hydrogen bonds. Aggregates based on $trisM_3$ also exist as mixtures of geometrical isomers. A mixture of isomers is entropically favored over a single isomer, and this factor further contributes to the stability of the $trisM_3$ aggregates. As far as we can infer from GPC measurements, 2 + 3 aggregates based on $hubM_3$ and $trisM_3$ have similar stabilities.

(15) Sanders, J. K. M.; Hunter, B. K. *Modern NMR Spectroscopy*; Oxford University Press: New York, 1987.
(16) Williams, L. D.; Chawla, B.; Shaw, B. R. *Biopolymers* **1987**, *26*, 591.
(17) During these experiments, we did not observe resonances for the NH protons of $hubM_3$ or $benzCA_2$ because these protons had exchanged completely with the methanol-d_4.
(18) There is no significant difference between the ^{1}H NMR spectrum of $(hubM_3)_2(benzCA_2)_3$ in dry $CDCl_3$ and $CDCl_3$ that has been saturated with water. Adrian, J. C.; Wilcox, C. S. *J. Am. Chem. Soc.* **1991**, *113*, 678.
(19) X-ray crystallography may be the only way to prove their structure. We have observed a structure in the solid state that is similar to the 2 + 3 aggregates. The solid-state structure is derived from two parallel stacked $CA_3{\cdot}M_3$ cyclic hexamers. Zerkowski, J. A.; Seto, C. T.; Whitesides, G. M. *J. Am. Chem. Soc.* **1992**, *114*, 5473.

benzCA$_2$ and furanCA$_2$. Aggregates that incorporate benzCA$_2$ and furanCA$_2$ have similar stabilities and similar distributions of geometrical isomers. The phenyl ring of benzCA$_2$ may be skewed in the 2 + 3 aggregates so that the CA groups become parallel, or the hydrogen-bonded network in these aggregates may be flexible enough to tolerate deviations from planarity.

Thermodynamics of Self-Assembly. The 2 + 3 structures described here are significantly more stable thermodynamically than 1 + 3 aggregates assembled from similar components. The difference in stability reflects the doubling of the enthalpy of formation (by doubling the number of hydrogen bonds formed) at a relatively small additional price in entropy (the marginal difference in assembling aggregates from five and four particles). Aggregation and orientation of five molecules in dilute solution is entropically unfavorable; the aggregate only forms because of the large enthalpy of formation provided by the 36 hydrogen bonds.[20]

Experimental Section

General Methods. NOE experiments were performed with a Bruker AM 500 instrument with $CDCl_3$ as the solvent. Elemental analyses were performed by Spang Microanalytical Laboratory. THF was distilled from sodium benzophenone ketyl. Methylene chloride and triethylamine were distilled from calcium hydride. Dimethylformamide was dried and stored over 4-Å molecular sieves. The compounds that have a triazine unit in their chemical structures show doubling of several resonances in their ^{1}H and ^{13}C NMR spectra due to slow exchange of conformers around the NHR triazine bonds.

***N,N'*-Diacetyl-1,3-bis(aminomethyl)-4,6-diisopropylbenzene (2).** This compound was synthesized following the procedure of Parris and Christenson[21] for the synthesis of *N,N'*-diacetyl-1,3-bis(aminomethyl)-4,6-dimethylbenzene. A 1-L 3-necked round-bottomed flask equipped with a pressure-equalizing addition funnel and a reflux condenser was charged with 350 mL of glacial acetic acid, 30 mL of concentrated sulfuric acid, and 14.4 g (480 mmol) of powdered 95% paraformaldehyde. The mixture was heated at 50 °C in an oil bath until all of the solid was dissolved. Acetonitrile (27 mL) was slowly added to the mixture in small portions. This addition must be performed slowly in order to avoid a vigorous exotherm. After the spontaneous reaction was completed, 32.5 g of 1,3-diisopropylbenzene (200 mmol) was added to the mixture and the reaction was heated at 90 °C for 15 h. The solution was cooled to room temperature, and the solvent was removed by rotary evaporation at aspirator pressure. The residue was diluted with 500 mL of water and cooled in an ice bath. The solution was made basic (pH ~12) with solid NaOH, and the precipitate was collected by vacuum filtration and washed with 1 L of water. The solid was suspended in 1 L of water, and the solution was stirred for 1 h. The solid was recollected, washed with 1 L of water, and air-dried on the filtration funnel. The product was recrystallized from MeOH (2 crops), giving 24.4 g (80.3 mmol, 40%) of the title compound as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.12 (t, J = 5.1 Hz, 2 H), 7.19 (s, 1 H), 7.04 (s, 1 H), 4.22 (d, J = 5.4 Hz, 4 H), 3.08 (m, 2 H), 1.83 (s, 6 H), 1.16 (d, J = 6.8 Hz, 12 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.65, 145.72, 132.71, 129.78, 121.92, 39.86, 27.95, 23.81, 22.45; HRMS-FAB (M + H$^+$) calcd for $C_{18}H_{29}N_2O_2$ 305.2229, found 305.2212.

1,3-Bis(aminomethyl)-4,6-diisopropylbenzene (3). Compound **2** (18.6 g, 61.2 mmol) was suspended in 100 mL of 3 N aqueous HCl solution, and the mixture was heated at reflux for 16 h. The reaction was cooled in an ice bath, and the solution was made basic (pH ~ 13) with solid NaOH. The solution was extracted four times with 150-mL portions of CH_2Cl_2. The organic layer was dried over KOH, and the solvent was removed by rotary evaporation at aspirator pressure. The crude product was taken on to the next reaction without further purification. The crude yield was 12.6 g (57.2 mmol, 94%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.22 (s, 1 H), 7.11 (s, 1 H), 3.70 (s, 4 H), 3.19 (m, 2 H), 1.57 (br s, 4 H), 1.18 (d, J = 6.7 Hz, 12 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 144.01, 137.27, 127.93, 121.05, 42.79, 27.66, 23.99; HRMS-CI (M + H$^+$) calcd for $C_{14}H_{25}N_2$ 221.2018, found 221.1988.

Bisbiuret 4. Crude **3** (2.91 g, 13.2 mmol) from the previous reaction and nitrobiuret[2] (3.91 g, 26.4 mmol) were combined with 50 mL of water and the mixture was heated at reflux for 2 h. The solution was cooled to room temperature and diluted with 100 mL of water, and the precipitate was collected by vacuum filtration and washed with 300 mL of water. The product was dried in an oven at 115 °C, giving 4.41 g (11.2 mmol, 85%) of the product as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.54 (s, 2 H), 7.71 (s, 2 H), 7.24 (s, 1 H), 7.07 (s, 1 H), 6.76 (s, 4 H), 4.28 (d, J = 5.2 Hz, 4 H), 3.09 (m, 2 H), 1.18 (d, J = 6.7 Hz, 12 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.49, 154.03, 146.21, 132.62, 129.37, 122.49, 40.29, 28.13, 23.85; HRMS-FAB (M + H$^+$) calcd for $C_{18}H_{29}N_6O_4$ 393.2250, found 393.2257.

benzCA$_2$. A 250-mL round-bottomed flask was charged with 75 mL of absolute ethanol and cooled in an ice bath under a nitrogen atmosphere, and 1.98 g (86.2 mg-atom) of sodium was added. The solution was stirred at 0 °C until all of the sodium had dissolved, and then 4.23 g (10.8 mmol) of **4** and 5.09 g (5.22 mL, 43.1 mmol) of diethyl carbonate were added. The reaction was heated at reflux for 24 h and cooled in an ice bath, and the precipitate was collected by vacuum filtration and washed with 50 mL of ethanol. The solid was dissolved in 100 mL of water, and the solution was filtered to remove any solid that did not dissolve. The filtrate was acidified to pH 2 with concentrated aqueous HCl solution, and the precipitated product was collected by vacuum filtration and washed with 300 mL of water. The solid was suspended in 100 mL of water, and 1 N aqueous NaOH solution was added until all of the solid had dissolved. The pH was then adjusted to 8.5 with 1 N aqueous HCl solution, and the mixture was filtered to remove any precipitate. The filtrate was then acidified to pH 2 with 1 N aqueous HCl solution, and the precipitated product was collected by vacuum filtration and washed with 300 mL of water. The solid was suspended in 200 mL of water, stirred for 1 h at room temperature, and then recollected by vacuum filtration and washed with 300 mL of water. The solid was dried in an oven at 110 °C to give 2.27 g (5.11 mmol, 48%) of the product as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.57 (s, 4 H), 7.20 (s, 1 H), 6.71 (s, 1 H), 4.81 (s, 4 H), 3.25 (m, 2 H), 1.19 (d, J = 6.8 Hz, 12 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.94, 148.46, 144.79, 130.53, 124.21, 121.70, 40.55, 27.80, 23.48; HRMS-FAB (M + Na$^+$) calcd 467.1654, found 467.1655. Anal. Calcd for $C_{20}H_{24}N_6O_6$: C, 54.05; H, 5.44; N, 18.91. Found: C, 54.01; H, 5.61; N, 18.65.

2,5-Dimethyl-3,4-diisopropylfuran (6). A 500-mL round-bottomed flask equipped with a Dean–Stark trap and a stirring bar was charged with 3,4-diisopropyl-2,5-hexanedione (17.6 g, 88.8 mmol) (mixture of isomers, prepared according to the method of Szakal-Quin et al.[22]), *p*-toluenesulfonic acid (1.69 g, 8.9 mmol), and 150 mL of toluene, and the mixture was heated at reflux for 12 h. The solvent was removed by rotary evaporation at aspirator pressure, and the crude material was purified by vacuum distillation at aspirator pressure. The fraction that distilled between 95 and 100 °C was collected to give 9.27 g (54.4 mmol, 58%) of the product as a clear, colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.81 (m, 2 H), 2.21 (s, 6 H), 1.23 (d, J = 7.1 Hz, 12 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.06, 124.64, 24.29, 22.63, 13.05; HRMS-EI (M$^+$) calcd for $C_{12}H_{20}O$ 180.1514, found 180.1518.

2,5-Bis(azidomethyl)-3,4-diisopropylfuran (7). Compound **6** (5.0 g, 27.7 mmol), *N*-bromosuccinimide (9.86 g, 55.4 mmol), and benzoyl peroxide (20 mg) were combined in 150 mL of CCl_4, and the solution was heated at reflux under a nitrogen atmosphere for 3 h. The solution was cooled, and the precipitated succinimide was removed by filtration. The solvent was removed by rotary evaporation at aspirator pressure, and the residue was dissolved in 50 mL of DMF. To the solution was added 10.8 g (166 mmol) of sodium azide and the mixture was heated at 60 °C under a nitrogen atmosphere for 2 h. The solution was cooled to room temperature, diluted with 400 mL of hexanes, washed six times with 400-mL portions of water, and dried over $MgSO_4$, and the solvent was removed by rotary evaporation at aspirator pressure. The crude material was purified by flash chromatography (eluted with 1:99 ethyl acetate/hexanes) to give 2.15 g (8.18 mmol, 30%) of the product as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.31 (s, 4 H), 2.93 (m, 2 H), 1.27 (d, J = 7.2 Hz, 12 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.79, 130.23, 46.33, 24.45, 23.19; HRMS-FAB (M$^+$) calcd for $C_{12}H_{18}N_6O$ 262.1542, found 262.1532.

Bisbiuret 8. Compound **7** (2.15 g, 8.18 mmol) and 20% Pd(OH)$_2$/C were combined in 100 mL of methanol, and the solution was stirred under a hydrogen atmosphere for 2 h. The catalyst was removed by filtration and the solvent was removed by rotary evaporation at aspirator pressure. The crude diamine was combined with 2.42 g (16.4 mmol) of nitrobiuret and 50 mL of water, and the solution was heated at reflux for 2 h. The

(20) Semiquantitative estimates of the thermodynamic contributions to self-assembly suggest the conclusion that the entropy of conformation has a large influence on $\Delta G_{formation}$; this conclusion is useful in molecular design. In designing self-assembling systems, the molecules should be constrained to have similar conformations in the free and bound states in order to minimize this term. Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 1039. Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1009. Rebek, J., Jr. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 245. Rebek, J., Jr. *Acc. Chem. Res.* **1990**, *23*, 399.

(21) Parris, C. L.; Christenson, R. M. *J. Org. Chem.* **1960**, *25*, 1888.

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mixture was cooled to room temperature and diluted with 100 mL of water, and the precipitated product was collected by vacuum filtration. The solid was washed with 300 mL of water and dried in an oven at 115 °C to give 2.15 g (5.62 mmol, 69%) of the product as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.57 (s, 2 H), 7.74 (br s, 2 H), 6.78 (s, 4 H), 4.28 (d, J = 5.3 Hz, 4 H), 2.91 (m, 2 H), 1.19 (d, J = 7.2 Hz, 12 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.46, 154.08, 144.84, 127.25, 34.94, 23.81, 22.75; HRMS-FAB (M + H$^+$) calcd for $C_{16}H_{27}N_6O_5$ 383.2043, found 383.2046.

furanCA$_2$. A 250-mL round-bottomed flask was charged with 50 mL of absolute ethanol and cooled in an ice bath under a nitrogen atmosphere, and 0.97 g (42.0 mg-atom) of sodium was added. The solution was stirred at 0 °C until all of the sodium had dissolved, and then 2.01 g (5.25 mmol) of **8** and 2.48 g (2.54 mL, 21.0 mmol) of diethyl carbonate were added. The reaction was heated at reflux for 24 h and cooled in an ice bath, and the precipitate was collected by vacuum filtration and washed with 50 mL of ethanol. The solid was dissolved in 150 mL of water, and the solution was filtered to remove any solid that did not dissolve. The pH was adjusted to 8.5 with 1 N aqueous HCl, and the solution was filtered to remove any precipitate. The filtrate was acidified to pH 2 with 1 N aqueous HCl solution, and the precipitated product was collected by vacuum filtration and washed with 100 mL of water. The solid was suspended in 100 mL of water, stirred for 1 h at room temperature, recollected by vacuum filtration, and washed with 50 mL of water. The solid was recrystallized from methanol and dried in an oven at 115 °C to give 1.13 g (2.60 mmol, 50%) of the product as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.48 (s, 4 H), 4.76 (s, 4 H), 2.99 (m, 2 H), 1.20 (d, J = 7.2 Hz, 12 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.50, 148.47, 142.13, 126.68, 36.49, 23.82, 22.27; HRMS-FAB (M + H$^+$) calcd 435.1628, found 435.1619. Anal. Calcd for $C_{18}H_{22}N_6O_7$: C, 49.77; H, 5.10; N, 19.35. Found: C, 49.74; H, 5.12; N, 19.31.

***N*-(*tert*-Butyloxycarbonyl)tris(hydroxymethyl)aminomethane (10).** A 250-mL round-bottomed flask was charged with tris(hydroxymethyl)-aminomethane (7.5 g, 60 mmol), 2-[(*tert*-butyloxycarbonyloxy)imino]-2-phenylacetonitrile (BOC-ON) (15 g, 60 mmol), Et_3N (8.25 mL, 60 mmol), and DMF (200 mL). The solution was heated at 50 °C under a nitrogen atmosphere for 2 h. The reaction was cooled, the solvent was removed by rotary evaporation at aspirator pressure, and the crude material was purified by titration with cold EtOAc (55 mL). Vacuum filtration gave 10.4 g (47 mmol, 78%) of the product as a white solid after drying under high vacuum: ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.68 (br s, 1 H), 4.46 (t, J = 7.5 Hz, 3 H), 3.52 (d, J = 7.5 Hz, 6 H), 1.38 (s, 9 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.78, 81.58, 67.07, 64.13, 31.96; HRMS-FAB (M + Na$^+$) calcd for $C_9H_{19}NO_5Na$ 244.1161, found 244.1172.

***N*-(*tert*-Butyloxycarbonyl)tris[(allyloxy)methyl]aminomethane (11).** A 250-mL round-bottomed flask was charged with **10** (5 g, 21 mmol) and DMSO (200 mL). The solution was cooled to 16–18 °C, and then allyl bromide (10.25 mL, 117 mmol) was added, followed by finely ground KOH (6.5 g, 117 mmol) over 15 min. The solution was stirred at room temperature for 2 h before the solvent was removed by rotary evaporation at aspirator pressure. The residue was partitioned between hexanes (250 mL) and water (200 mL), the organic extract was washed with brine (2 × 75 mL), dried over $MgSO_4$, and filtered, and the solvent was removed by rotary evaporation at aspirator pressure. The crude material was purified by flash chromatography (eluted with 5:95 ethyl acetate/hexanes) to give 5.75 g (16.8 mmol, 80%) of the product as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.95–5.80 (m, 3 H), 5.30–5.12 (m, 6 H), 4.96 (br s, 1 H), 4.02–3.96 (m, 6 H), 3.70 (s, 6 H), 1.43 (s, 9 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.93, 138.94, 119.77, 82.37, 75.07, 71.65, 62.26, 31.84; HRMS-CI (M + H$^+$) calcd for $C_{18}H_{31}NO_5$ 341.2280, found 341.2202.

9-[[(1,1-Dimethylethoxy)carbonyl]amino]-9-[[3-[(2-methoxy-2-oxoethyl)thio]propoxy]methyl]-7,11-dioxa-3,15-dithiaheptadecanedioic Acid Dimethyl Ester (12). A 100-mL round-bottomed flask was charged with **11** (1 g, 3 mmol), methyl 2-thioacetate (1.07 mL, 12 mmol), azobis-(isobutyronitrile) (AIBN) (~10 mg), and THF (35 mL). The solution was photolyzed for 4 h with a medium-pressure mercury lamp. The solvent was removed by rotary evaporation at aspirator pressure, and the residue was purified by flash chromatography (eluted with 1:4 ethyl acetate/hexanes) to give 1.67 g (2.54 mmol, 85%) of the product as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.96 (br s, 1 H), 3.73 (s, 9 H), 3.62 (s, 6 H), 3.51 (t, J = 7.1 Hz, 6 H), 3.22 (s, 6 H), 2.7 (t, J = 7.1 Hz, 6 H), 1.84 (quin, J = 7.1 Hz, 6 H), 1.43 (s, 9 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.36, 157.92, 82.61, 72.67, 71.93, 62.25, 55.65, 36.30, 32.35, 32.29, 31.86; HRMS-FAB (M + Na$^+$) calcd for $C_{27}H_{49}NO_{11}S_3Na$ 682.2365, found 682.2386.

9-[[3-[(Carboxymethyl)thio]propoxy]methyl]-9-[[(1,1-dimethylethoxy)carbonyl]amino]-7,11-dioxa-3,15-dithiaheptadecanedioic Acid (13). A 50-mL round-bottomed flask was charged with **12** (1 g, 1.5 mmol), NaOH (0.4 g, 10 mmol), and a 1:1 mixture of THF/MeOH (20 mL). The homogeneous solution was stirred at 25 °C for 3 h. The solvent was removed by rotary evaporation at aspirator pressure, and the residue was dissolved in water (50 mL). The solution was overlaid with EtOAc (50 mL) and acidified (pH ~3) with 1 N aqueous HCl solution. The organic extract was removed and the aqueous layer was washed with EtOAc (2 × 50 mL). The combined organic residues were dried over $MgSO_4$ and filtered, and the solvent was removed by rotary evaporation at aspirator pressure to give 0.82 g (1.28 mmol, 90%) of the product as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.50 (br s, 3 H), 5.07 (br s, 1 H), 3.62 (s, 6 H), 3.52 (t, J = 7.1 Hz, 6 H), 3.26 (s, 6 H), 2.75 (t, J = 7.1 Hz, 6 H), 1.84 (quin, J = 7.1 Hz, 6 H), 1.44 (s, 9 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.23, 157.48, 82.68, 78.54, 71.94, 62.88, 36.84, 32.39, 32.28, 31.90; HRMS-FAB (M + Na$^+$) calcd for $C_{24}H_{43}NO_{11}S_3Na$ 640.1896, found 640.1893.

***N*-(*tert*-Butyloxycarbonyl)-1,2-diaminobenzene (15).** A 250-mL round-bottomed flask was charged with 1,2-diaminobenzene (5 g, 46 mmol), BOC-ON (12.3 g, 50 mmol), Et_3N (7 mL, 50 mmol), and DMF (100 mL). The solution was heated at 55 °C under an atmosphere of nitrogen for 3 h, before the solvent was removed by rotary evaporation at aspirator pressure. The residue was partitioned between toluene (120 mL) and brine (100 mL), the organic extract was washed with 1 N aqueous NaOH solution (2 × 75 mL), brine (2 × 100 mL), dried over $MgSO_4$, and filtered, and the solvent was removed by rotary evaporation at aspirator pressure. The crude material was recrystallized from $CHCl_3$/hexanes (1:1 v/v) and filtered to give 6.32 g (30 mmol, 66%) of the product as a white crystalline solid after drying under high vacuum: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.25 (br s, 1 H), 7.15 (d, J = 7.5 Hz, 1 H), 6.79 (t, J = 7.5 Hz, 1 H), 6.64 (t, J = 7.5 Hz, 1 H), 6.47 (d, J = 7.5 Hz, 1 H), 4.75 (br s, 2 H), 1.39 (s, 9 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.32, 134.59, 133.04, 129.23, 128.62, 119.96, 119.37, 82.61, 31.87; HRMS-FAB (M$^+$) calcd for $C_{11}H_{16}N_2O_2$ 208.1211, found 208.1214.

2-[[2-[*N*-(*tert*-Butyloxycarbonyl)amino]phenyl]amino]-4-amino-6-chloro-1,3,5-triazine (16). A 250-mL round-bottomed flask was charged with cyanuric chloride (2.88 g, 15.6 mmol), *N*,*N*-diisopropylethylamine (DIPEA) (2.4 mL, 15.6 mmol), and THF (130 mL). The solution was cooled to 0 °C under an atmosphere of nitrogen, and 3 g of **15** (15.4 mmol) was added in portions over 25 min. After an additional 40 min, gaseous NH_3 was bubbled through the solution for 90 min to afford a heavy white suspension. The solvent was removed by rotary evaporation at aspirator pressure, and the residue was partitioned between EtOAc (200 mL) and brine (100 mL). The organic extract was washed with brine (2 × 100 mL), dried over $MgSO_4$, and filtered, and the solvent was removed by rotary evaporation at aspirator pressure. The crude material was purified by flash chromatography (eluted with 2:3 ethyl acetate/hexanes) to give 4.4 g (13.6 mmol, 94%) of the product as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (br s, 1 H), 8.49 (br s, 1 H), 7.53 (d, J = 7.4 Hz, 1 H), 7.47 (br s, 1 H), 7.42 (br s, 2 H), 7.11 (t, J = 7.4 Hz, 1 H), 7.04 (t, J = 7.4 Hz, 1 H), 1.44 (s, 9 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.65, 168.71, 156.80, 135.95, 132.78, 130.44, 129.16, 127.39, 126.99, 83.08, 31.75; HRMS-FAB (M + H$^+$) calcd for $C_{14}H_{18}ClN_6O_2$ 337.1178, found 337.1189.

2-[[2-[*N*-(*tert*-Butyloxycarbonyl)amino]phenyl]amino]-4-amino-6-(neohexylamino)-1,3,5-triazine (17). A 250-mL round-bottomed flask was charged with **16** (3.5 g, 10.5 mmol), DIPEA (5 mL, 30 mmol), neohexylamine (4 mL, 30 mmol), and THF (100 mL). The solution was heated at reflux under an atmosphere of nitrogen for 5 h. The solvent was removed by rotary evaporation at aspirator pressure, and the residue was partitioned between EtOAc (150 mL) and brine (100 mL). The organic extract was washed with brine (2 × 75 mL), dried over $MgSO_4$, and filtered, and the solvent was removed by rotary evaporation at aspirator pressure. The crude material was purified by flash chromatography (eluted with 1:1 ethyl acetate/hexanes, loaded in $CHCl_3$) to give 3.5 g (8.75 mmol, 83%) of the product as a white crystalline solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78, 8.61 (two conformers, br s, 1 H), 8.19, 8.03 (two conformers, br s, 1 H), 7.57–7.34 (two conformers, br m, 1 H), 6.97 (m, 1 H), 6.73–6.58 (two conformers, br m, 2 H), 6.33 (br s, 1 H), 6.21 (br s, 1 H), 3.13 (m, 2 H), 1.44 (s, 9 H), 1.38 (m, 2 H), 0.84, 0.82 (two conformers, s, 9 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.53, 170.43, 169.59, 168.54, 168.42, 135.49, 134.32, 128.52, 127.61, 127.35, 82.88, 46.72, 46.49, 40.21, 33.09, 32.97, 31.76; HRMS-FAB (M + H$^+$) calcd for $C_{20}H_{32}N_7O_2$ 402.2617, found 402.2614.

2-[(2-Aminophenyl)amino]-4-amino-6-(neohexylamino)-1,3,5-triazine (18). A 250-mL round-bottomed flask was charged with **17** (2.5 g, 6.2 mmol) and CH_2Cl_2 (80 mL). The solution was cooled to 0 °C under an atmosphere of nitrogen, and trifluoroacetic acid (15 mL) was added dropwise over 20 min. The reaction mixture was stirred at 25 °C for 2 h before it was diluted with toluene (35 mL), and the solvent was removed by rotary evaporation at aspirator pressure. The residue was

partitioned between EtOAc (150 mL) and 5% aqueous Na_2CO_3 solution (75 mL), washed with brine (2 × 70 mL), dried over $MgSO_4$, and filtered, and the solvent was removed by rotary evaporation at aspirator pressure to give 1.76 g (5.8 mmol, 94%) of the product as a white crystalline solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81, 7.71 (two conformers, br s, 1 H), 7.34, 7.18 (two conformers, br s, 1 H), 6.76 (br s, 1 H), 6.65 (d, J = 7 Hz, 1 H), 6.51–6.46 (br m, 2 H), 6.19 (br s, 1 H), 6.05 (br s, 1 H), 4.77 (br s, 2 H), 3.15 (m, 2 H), 1.34 (m, 2 H), 0.82 (s, 9 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.73, 168.82, 129.91, 129.02, 128.54, 128.10, 120.03, 119.68, 48.11, 47.91, 40.64, 33.18; HRMS-FAB (M + H$^+$) calcd for $C_{15}H_{24}N_7$ 302.2093, found 302.2090.

[1,2-Bis[3-[[2-[[2-[[4-amino-6-[(3,3-dimethylbutyl)amino]-1,3,5-triazin-2-yl]amino]phenyl]amino]-2-oxoethyl]thio]propoxy]-1-[[3-[[2-[[2-[[4-amino-6-[(3,3-dimethylbutyl)amino]-1,3,5-triazin-2-yl]amino]phenyl]amino]-2-oxoethyl]thio]propoxy]methyl]ethyl]carbamic Acid 1,1-Dimethylethyl Ester (trisM$_3$). A solution of the tris acid **13** (800 mg, 1.3 mmol), triazine derivative **18** (1.2 g, 4 mmol), and 1-hydroxybenzotriazole (675 mg, 5 mmol) in THF (40 mL) was cooled under an atmosphere of nitrogen to 0 °C. Addition of dicyclohexylcarbodiimide (DCC) (1.04 g, 5 mmol) was performed over 15 min and the mixture was stirred at 25 °C for 24 h. The resulting suspension was filtered to remove dicyclohexylurea (DCU), and the solvent was removed by rotary evaporation at aspirator pressure. The crude material was purified by flash chromatography (eluted with a 2.5% solution of MeOH in $CHCl_3$) to give 1.68 g (1.14 mmol, 88%) of the product as a white crystalline solid, after drying under high vacuum: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.0–9.90 (br s, 3 H), 8.11 (m, 3 H), 7.63–7.40 (m, 7 H), 7.12–7.05 (m, 6 H), 6.78 (m, 3 H), 6.40, 6.20 (two conformers, br s, 6 H), 3.42 (br s, 6 H), 3.33 (m, 6 H), 3.28 (br s, 6 H), 3.13 (m, 6 H), 2.58 (m, 6 H), 1.72 (m, 6 H), 1.40 (m, 6 H), 1.38 (s, 9 H), 0.82 (br s, 27 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.69, 170.71, 170.36, 169.53, 168.40, 168.21, 136.11, 135.91, 134.47, 133.80, 128.89, 128.63, 128.40, 127.44, 127.24, 81.33, 72.79, 71.98, 62.20, 46.75, 46.44, 40.21, 40.11, 39.31, 39.20, 33.08, 33.01, 32.38, 32.33, 31.89; LRMS-FAB (M + Na$^+$) calcd 1489, found 1489. Anal. Calcd for $C_{69}H_{106}N_{22}O_8S_3$: C, 56.46; H, 7.28; N, 20.99; S, 6.55. Found: C, 56.66; H, 7.33; N, 20.68; S, 6.39.

Preparation of (hubM$_3$)$_2$(benzCA$_2$)$_3$. The (hubM$_3$)$_2$(benzCA$_2$)$_3$ complex was prepared by dissolving 523.3 mg (0.25 mmol) of hubM$_3$ and 166.7 mg (0.375 mmol) of benzCA$_2$ in a minimum amount of 1:1 CH_2Cl_2/MeOH. The solvent was removed by rotary evaporation at aspirator pressure, and the resulting solid was dried in an oven at 85 °C for 12 h.

Titration of hubM$_3$ with benzCA$_2$ Monitored by ^{1}H NMR Spectroscopy. Five 5.0-mL vials were charged with the following amounts of hubM$_3$ and benzCA$_2$ (vial, amount of hubM$_3$, amount of benzCA$_2$): A, 20.9 mg (10 μmol), 0 mg (0 μmol); B, 20.9 mg (10 μmol), 2.2 mg (5 μmol); C, 20.9 mg (10 μmol), 4.4 mg (10 μmol); D, 20.9 mg (10 μmol), 6.7 mg (15 μmol); E, 20.9 mg (10 μmol), 13.3 mg (30 μmol). The solid in each vial was dissolved in a minimum amount of 1:1 CH_2Cl_2/MeOH, and then the solvent was removed by evaporation and the solid was dried in an oven at 85 °C for several hours. The solid in each vial was dissolved in 1.0 mL of $CDCl_3$, the solution was transferred to an NMR tube, and the spectrum was recorded at ambient temperature. For vial E, all of the solid did not dissolve in the $CDCl_3$.

NOE Spectra of (hubM$_3$)$_2$(benzCA$_2$)$_3$. The NOE spectra of (hubM$_3$)$_2$(benzCA$_2$)$_3$ were recorded at 25 °C. The complex (5.0 μmol) was dissolved in 0.5 mL of $CDCl_3$, 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 and Waters analytical gel permeation column (Ultrastyragel, 1000 Å pore size). Elutions were performed at room temperature using HPLC-grade CH_2Cl_2 as the solvent at a flow rate of 1.0 mL/min. The samples were prepared at concentrations of 0.125 mM for the complexes and 0.25 mM for free hubM$_3$ and free trisM$_3$ in CH_2Cl_2 that contained p-xylene (3.0 mM) as an internal reference. The injection volume was 20 μL.

Molecular Weight Determinations of (hubM$_3$)$_2$(benzCA$_2$)$_3$ 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 approximately 2, 4, 8, and 16 mM. At each concentration 3–4 measurements were taken. Calibration curves were generated using sucrose octaacetate (Aldrich), perbenzoyl β-cyclodextrin,[23] polystyrene (MW 5050, polydispersity = 1.05) (Polymer Laboratories), and a gramicidin S derivative in which the ornithine amino groups had been converted to the *tert*-butylcarbamates (MW 1342)[2] as molecular weight standards.

Titration of hubM$_3$ with benzCA$_2$ Monitored by UV Spectroscopy. UV spectra were recorded on a Perkin-Elmer Model 551 spectrophotometer in CH_2Cl_2 solution. Two stock solutions were prepared. Stock solution A was prepared by dissolving 20.9 mg (10 μmol) of hubM$_3$ in 1.0 mL of HPLC-grade CH_2Cl_2. Stock solution B was prepared by dissolving 27.6 mg (5 μmol) of (hubM$_3$)$_2$(benzCA$_2$)$_3$ in 10 mL of CH_2Cl_2.

Four samples were prepared (sample number, amount of solution A, amount of solution B, amount of CH_2Cl_2): **1**, 100 μL, 0 μL, 9.9 mL; **2**, 66 μL, 33 μL, 9.9 mL; **3**, 33 μL, 66 μL, 9.9 mL; **4**, 0 μL, 100 μL, 9.9 mL. A 0.30-mL aliquot of each of the samples was transferred to a 1.0-mm quartz cuvette, and the UV spectrum was recorded from 390 to 190 nm. The quartz cuvette was rinsed thoroughly with CH_2Cl_2 and dried in a stream of nitrogen between each measurement. Excess benzCA$_2$ that was added to sample solution **4** did not appreciably dissolve and did not change the UV spectrum of sample **4**.

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