KOH/H$_2$O (40%, 10.0 mL) was added slowly to *N*-nitroso-*N*-methyl urea (1.0 g, 9.6 mmol). The evolving gas was then purged through a column filled with KOH pellets into a Pyrex vessel containing 2 mL of a 1:1 mixture of perdeuteriobenzene and benzene. After all the diazomethane dissolved, the vessel was cooled and irradiated until the color of the solution disappeared (30 min). Control experiments indicate that prolonged irradiation does not alter the product distribution.

Calculations were performed on an ALLIANT FX/40 computer using the GAUSSIAN86[19] and GAUSSIAN90[20] packages of programs.

Supplementary Material Available: Tables of calculated Cartesian coordinates and point groups for **1a–f** (5 pages). Ordering information is given on any current masthead page.

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

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Contribution from the Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138. Received June 8, 1992. Revised Manuscript Received October 7, 1992

Abstract: Reaction of the tris(melamine) derivatives hubM$_3$ (C$_6$H$_3$-1,3,5-[CONHC$_6$H$_4$-3-N(CH$_2$C$_6$H$_4$-4-C(CH$_3$)$_3$)COC$_6$-H$_3$-2-NHC$_3$N$_3$(NH$_2$)(NHCH$_2$CH$_2$C(CH$_3$)$_3$)-5-Br]$_3$) and flexM$_3$ (C$_6$H$_3$-1,3,5-[CO$_2$(CH$_2$)$_3$OCOC$_6$H$_4$-2-NHC$_3$N$_3$(NH$_2$)(N-HCH$_2$CH$_2$C(CH$_3$)$_3$)]$_3$) with R'CA (neohexyl isocyanurate) and R''CA (3,3,3-triphenylpropyl isocyanurate) in CHCl$_3$, respectively, yields structurally well-defined supramolecular aggregates hubM$_3$(R'CA)$_3$ and flexM$_3$(R''CA)$_3$. These structures were characterized using ^{1}H NMR, ^{13}C NMR, and UV spectroscopy, gel permeation chromatography, and vapor pressure osmometry. flexM$_3$ is a conformationally flexible analog of hubM$_3$. The greater degree of preorganization that is built into the molecular structure of hubM$_3$ compared to flexM$_3$ makes hubM$_3$(R'CA)$_3$ a more stable aggregate than flexM$_3$(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 rest on 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] Lehn,[9] Hamilton,[10] and

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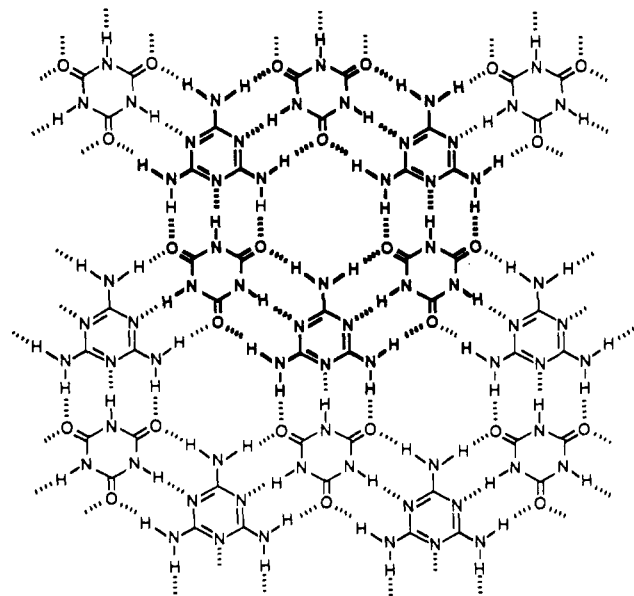(8) Rebek, J., Jr. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 245.
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Scheme I. Self-Assembly of hubM₃ with R′CA and flexM₃ with R″CA To Give Supramolecular Aggregates

hubM₃(R′CA)₃

R′ = CH₂CH₂C(CH₃)₃

R″ = CH₂CH₂C(C₆H₅)₃

flexM₃(R″CA)₃
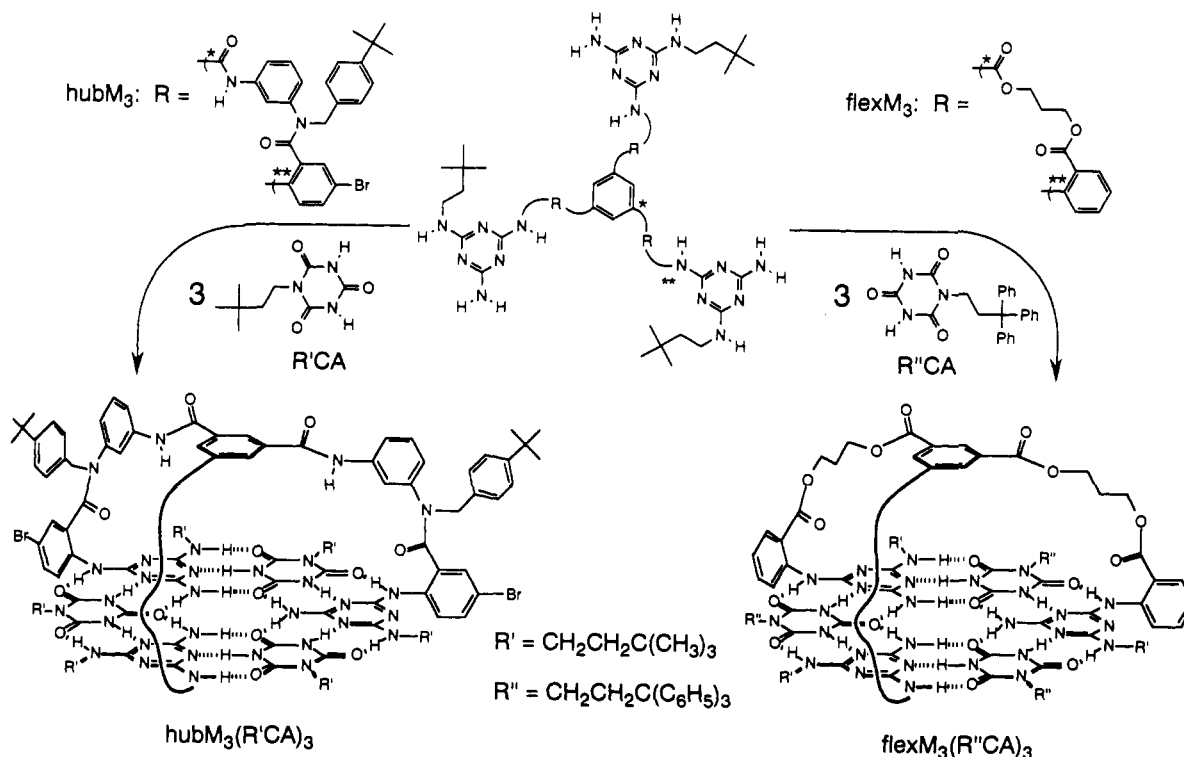
others[11] have studied hydrogen bonding in organic media and provided a basis for understanding molecular recognition via hydrogen bonds.

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).[12] 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.[13]

CA·M

(10) Chang, S.-K.; Hamilton, A. D. *J. Am. Chem. Soc.* **1988**, *110*, 1318.
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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, CA₃·M₃ (see the boldfaced section of CA·M). A crystal structure of the 1:1 complex between N,N′-bis(4-tert-butylphenyl)melamine and barbital shows that this hydrogen-bonded complex adopts the cyclic geometry in the solid state.[4] We also wished to develop analytical methods that could be used to characterize the solution structure of supramolecular aggregates.

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 CA₃·M₃ (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 CA₃·M₃ 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.
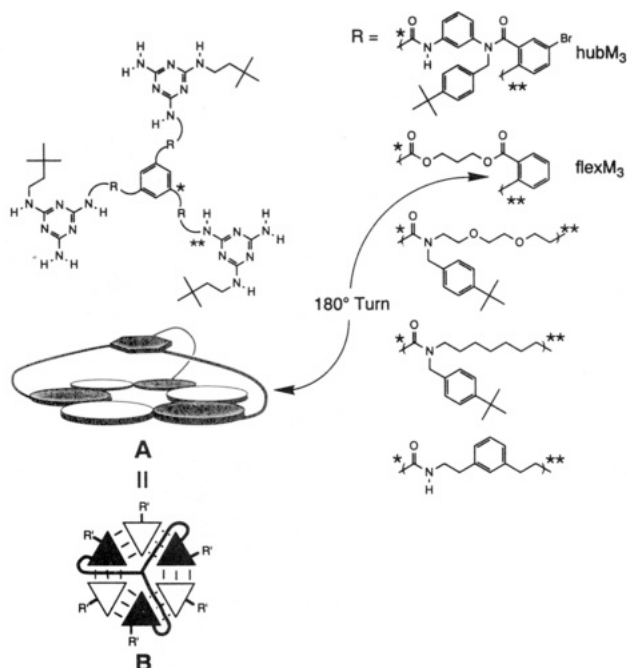
Design of Self-Assembling Structures. (a) hubM₃. We constructed the hub and spokes of hubM₃ with aromatic spacers connected through amide linkages (see the structure of hubM₃

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Cyanuric Acid·Melamine Lattice Based Aggregates

J. Am. Chem. Soc., Vol. 115, No. 3, 1993 907

Scheme II. Four Derivatives of hubM$_3$ That Have Varying Degrees of Flexibility in the Spokes^a

^{a} The asterisks indicate the orientation of the spokes in the structure. Schematic diagram A shows a side view and schematic B shows a top view of hubM$_3$(R'CA)$_3$.

in Scheme I). Molecular models suggested that this type of construction would preorganize hubM$_3$ 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 substituents of hubM$_3$ are necessary to increase the solubility of the molecule in organic solvents (an earlier version of hubM$_3$ that lacked these groups was not soluble in chloroform). The choice of neohexyl isocyanurate (R'CA) as a component of the aggregate was also based on the solubility conferred by the neohexyl groups. The bromine atoms on hubM$_3$ were added as heavy atom markers to facilitate X-ray crystallographic analysis of hubM$_3$(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 hubM$_3$: Preorganization of the Spokes. We wanted to investigate how much preorganization is needed in the hub and spokes of hubM$_3$ 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 hubM$_3$ 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 hubM$_3$ 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 flexM$_3$ 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). hubM$_3$ and flexM$_3$ 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 the 180° turn, the other three compounds must restrict several conformationally unrestrained bonds to gauche or eclipsed con-

formations. 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.

(c) hubM$_3$ vs flexM$_3$: Rigid vs Flexible Spokes. While hubM$_3$ and flexM$_3$ have similar structural components, the spoke of hubM$_3$ is much more rigid than that of flexM$_3$. The more rigid spoke of hubM$_3$ makes hubM$_3$(R'CA)$_3$ a more stable complex than flexM$_3$(R''CA)$_3$ because hubM$_3$ loses less conformational freedom than flexM$_3$ upon complexation. The greater stability of the hubM$_3$ complex, compared to the flexM$_3$ complex, is demonstrated in a competition experiment in which 1 equiv of hubM$_3$ and 1 equiv of flexM$_3$ are mixed with 3 equiv of R''CA. The R'CA complexes only with hubM$_3$: by ^{1}H NMR spectroscopy we cannot detect any flexM$_3$(R''CA)$_3$ in this mixture.

(d) Cooperativity. We believe that complexation of hubM$_3$ with 3 equiv of R'CA is a cooperative process. ^{1}H NMR experiments monitoring the titration of hubM$_3$ with R'CA show that, in a solution of hubM$_3$ that has less than 3 equiv of R'CA, the hubM$_3$ is present only as free hubM$_3$ and fully formed hubM$_3$(R'CA)$_3$ complex and is not present as partially formed complexes with stoichiometries of hubM$_3$(R'CA)$_1$ or hubM$_3$(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. ^{1}H 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

hubM$_3$(R'CA)$_3$. **(a) Synthesis of hubM$_3$.** The synthesis of hubM$_3$ is outlined in Scheme III.

(b) Qualitative Evidence for Formation of hubM$_3$(R'CA)$_3$. The complex between hubM$_3$ and R'CA is formed by combining the two components in chloroform and stirring the mixture for approximately 5 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 hubM$_3$(R'CA)$_3$ is very soluble (>20 mM). Adding hubM$_3$ 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 hubM$_3$ and R'CA is 1:3.

Reversed-phase TLC (eluted with 5% 2-propanol in CH$_2$Cl$_2$) gives two spots that are visible under a UV lamp: a major spot for hubM$_3$(R'CA)$_3$ (R_f 0.35–0.45) and a minor spot for uncomplexed hubM$_3$ (R_f 0.15–0.30). R'CA is not visible by UV irradiation.

(c) Titration of hubM$_3$ with R'CA Monitored by ^{1}H NMR Spectroscopy. We monitored the titration of hubM$_3$ with R'CA by ^{1}H NMR spectroscopy in CDCl$_3$ (Figure 1). The only distinguishing features that appear in the spectrum of uncomplexed hubM$_3$ in CDCl$_3$ 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. hubM$_3$ has a broad spectrum due to self-association and restricted rotation around the amide and RNH–triazine bonds. In more polar solvents such

Scheme III. Synthesis of hubM₃[a]
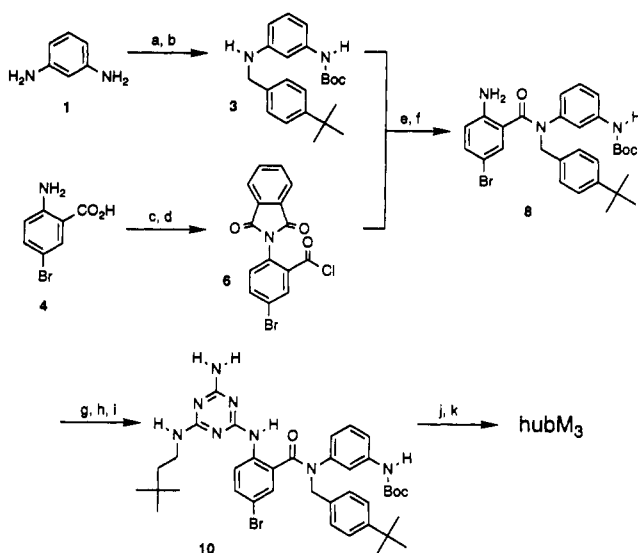
[a]Reagents: (a) (CH₃)₃COCO₂N=C(C₆H₅)CN (BOC-ON), DMF, 50 °C, 64% (2); (b) 4-*tert*-butyl benzyl bromide, THF, reflux, 68%; (c) *o*-phthaloyl dichloride, CH₂Cl₂, reflux, 73% (5); (d) SOCl₂, reflux; (e) Et₃N, CH₂Cl₂, 0 °C, 90% (2 steps) (7); (f) H₂NNH₂, MeOH, reflux, 95%; (g) cyanuric chloride, THF, 0 °C; (h) NH₃, THF, 0 °C, 85% (2 steps) (9); (i) H₂N(CH₂)₂C(CH₃)₃, THF, reflux, 86%; (j) F₃CCO₂H, CH₂Cl₂, 25 °C, 90% (11); (k) 1,3,5-C₆H₃(COCl)₃ (0.33 equiv), CH₂-Cl₂, 25 °C, 98%.
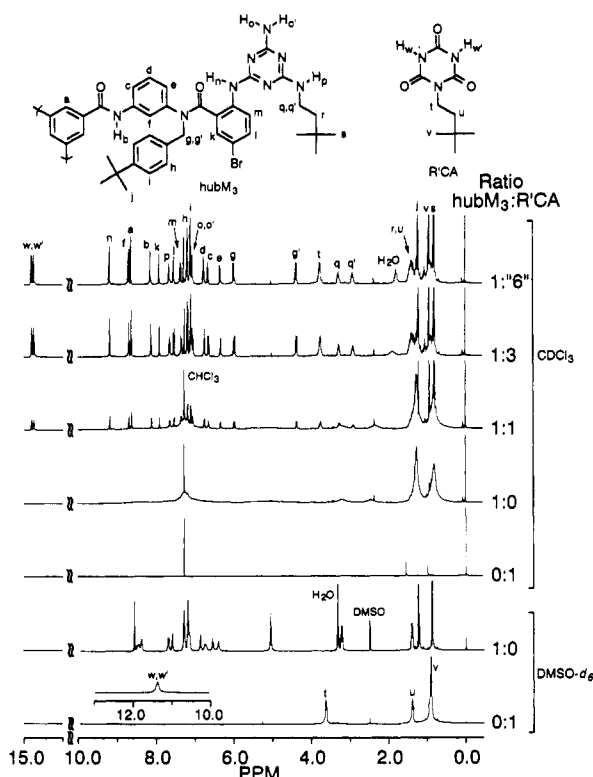

Figure 1. Spectra of the ¹H NMR titration of hubM₃ (500 MHz, 10 mM in CDCl₃) with R′CA. The peak assignments are shown at the top of the figure. The bottom two spectra show hubM₃ and R′CA alone in DMSO-d_6 for reference.

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)₃ 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)₃. This ob-
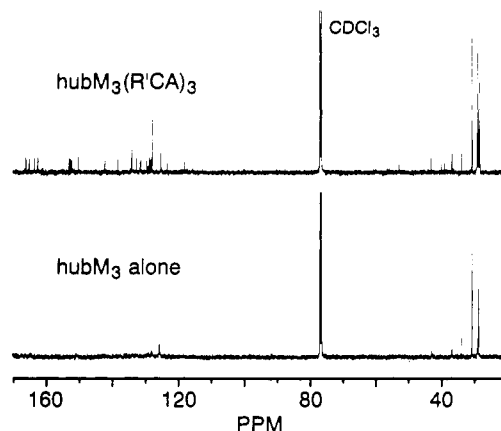

Figure 2. ¹³C NMR spectra of hubM₃ alone and the hubM₃(R′CA)₃ complex (125 MHz, 19.4 mM in CDCl₃).

servation 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 1 shows hubM₃/R′CA in a ratio of 1:"6". The sample contains 3 equiv of unsolubilized R′CA.

(d) Characteristic Features of the ¹H NMR Spectrum of hubM₃(R′CA)₃. (*i*) **Diastereotopic Protons.** Two sets of methylene protons (g,g′ and q,q′) of hubM₃ become diastereotopic in hubM₃(R′CA)₃ 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′ upfield 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 (w 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-d_6), 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.[14]

(e) ¹³C NMR Spectra of Free hubM₃ and hubM₃(R′CA)₃. The ¹³C and ¹H 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 (~30 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 ¹H 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

(14) 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-d_6 the imide protons of uncomplexed R′CA appear at 11.4 ppm.
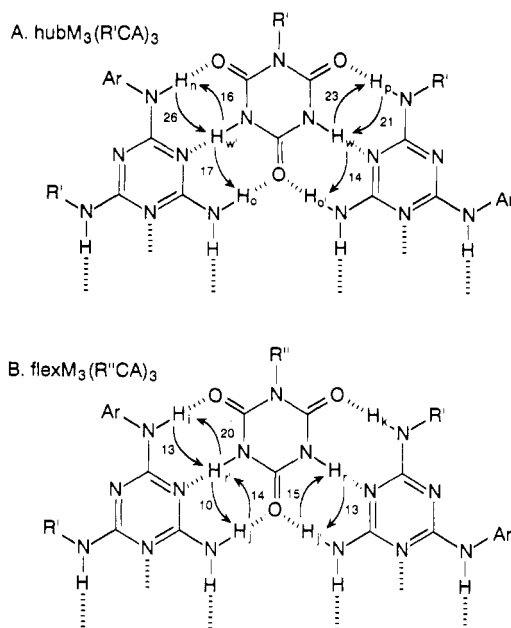
A. hubM₃(R'CA)₃

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.
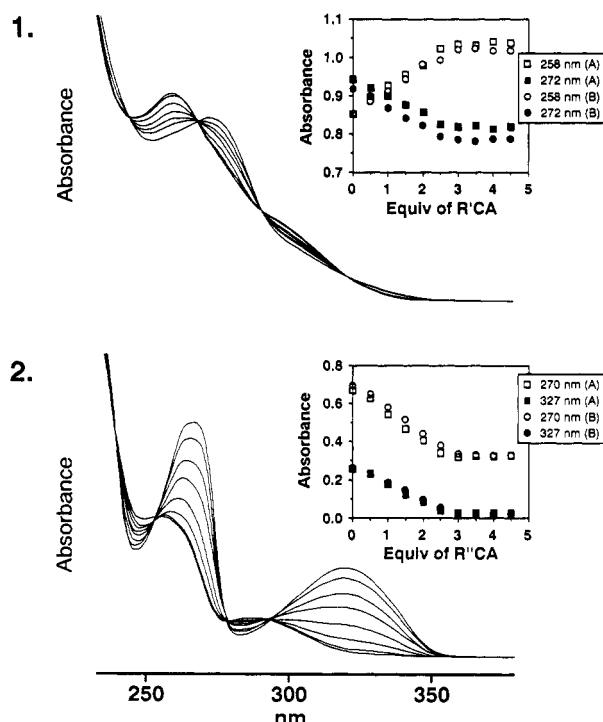

1.

2.

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).

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.[15] This technique is useful for estimating both the
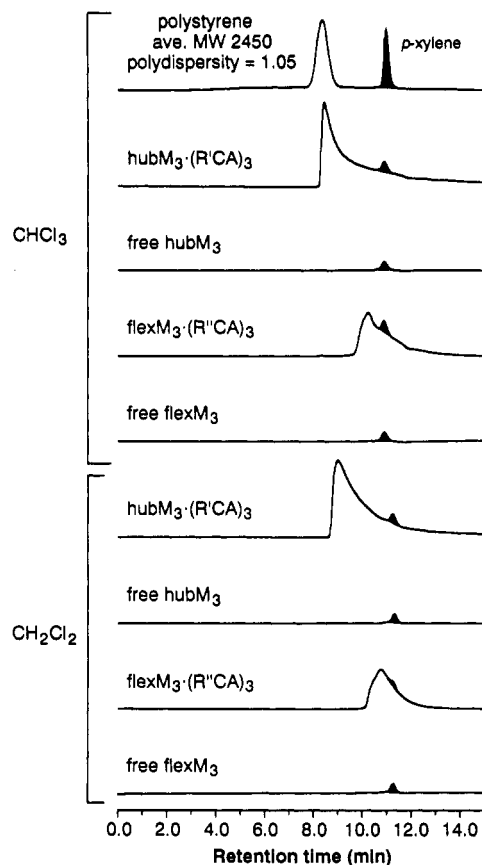

Figure 5. Gel permeation chromatograms of complexed and free hubM₃ and flexM₃. Free R'CA and R''CA are too insoluble to examine separately. The eluents are indicated to the left of the plots. The peaks at 11.0 (in CHCl₃) and 11.3 min (in CH₂Cl₂) are *p*-xylene used as an internal standard. The top chromatogram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

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 uncomplexed 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.[16]

(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).[17] 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

$$MW_{standard} \lim_{c \to 0} \left[\frac{\Delta V}{c_{standard}} \right] = MW_{unknown} \lim_{c \to 0} \left[\frac{\Delta V}{c_{unknown}} \right] \quad (1)$$

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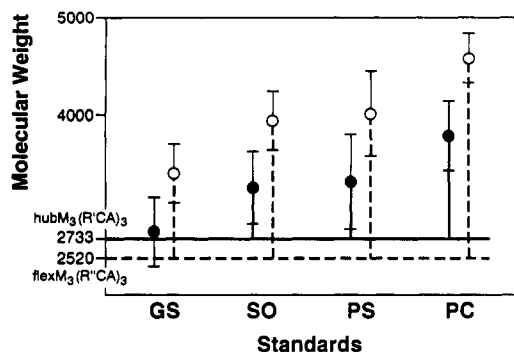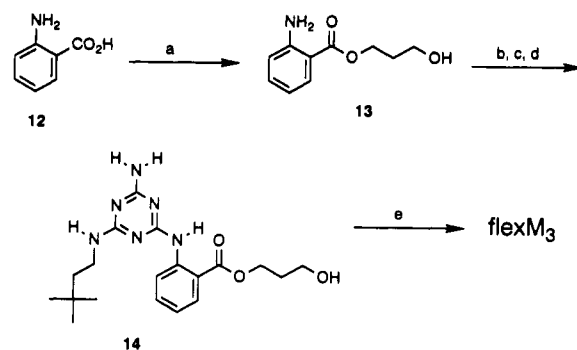
Figure 6. Experimental molecular weights of $hubM_3(R'CA)_3$ (●) and $flexM_3(R''CA)_3$ (○) determined by vapor pressure osmometry using four different molecular weight standards. The solid and dashed horizontal lines correspond to the calculated MWs of $hubM_3(R'CA)_3$ and $flexM_3$-$(R''CA)_3$, respectively. 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 of complex.

where $\Delta V/c$ (in the limit that $c \to 0$) for the standard and unknown are values that are determined by VPO.[18] With bis-t-Boc-derivatized gramicidin S as a standard,[19] the experimental molecular weight of the complex is close to the expected value for the structure $hubM_3(R'CA)_3$, while the sucrose octaacetate, polystyrene, and perbenzoyl β-cyclodextrin as standards the molecular weight of the complex inferred from VPO is 15–35% high. We hypothesize that $hubM_3(R'CA)_3$ 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 (gramicidin S is a cyclic decapeptide with many hydrogen-bonding sites), and the similar aggregation of the two would produce compensating errors in VPO. The values of molecular weight measured relative to sucrose octaacetate, polystyrene, and perbenzoyl β-cyclodextrin are all high. These molecules are all hydrophobic, have no sites for hydrogen bonding, and might be expected to be less associated in $CHCl_3$ solution than the gramicidin standard. Using these standards, the experimental molecular weight for the [1 + 3] complex would be high.

N,N'-bis(t-Boc)gramicidin S
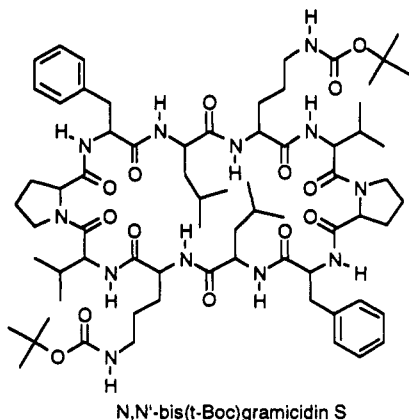
$flexM_3(R''CA)_3$. (a) Synthesis of $flexM_3$. $flexM_3$ is based on the same hub-and-spoke architecture as $hubM_3$ although the spokes of $flexM_3$ are derived from a flexible 1,3-propanediol spacer.

(18) In eq 1, c = concentration, and ΔV 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.

Scheme IV. Synthesis of $flexM_3$[a]

[a]Reagents: (a) $HO(CH_2)_3OH$, DCC, ether/THF, 25 °C, 32%; (b) cyanuric chloride, THF, 0 °C; (c) NH_3, THF, 0 °C; (d) $H_2N(CH_2)_2$-$C(CH_3)_3$, THF, reflux, 75% (3 steps); (e) $1,3,5$-$C_6H_3(COCl)_3$ (0.33 equiv), THF, 25 °C, 67%.
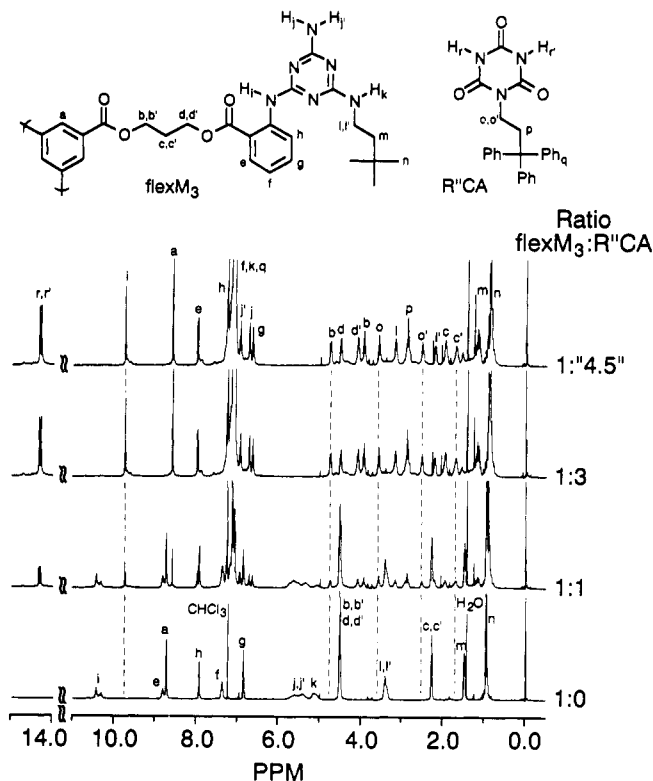

Figure 7. Spectra of the ¹H NMR titration of $flexM_3$ (500 MHz, 10 mM in $CDCl_3$) with R''CA. The peak assignments are shown at the top of the figure. The dashed lines are provided as a guide for the eye.

The synthesis of $flexM_3$ is outlined in Scheme IV.[20]

(b) Characterization of $flexM_3(R''CA)_3$ by ¹H NMR Spectroscopy. The ¹H NMR titration of $flexM_3$ with R''CA is shown in Figure 7. Unlike $hubM_3$, the spectrum of uncomplexed $flexM_3$ in $CDCl_3$ has sharp resonances: this molecule has fewer conformationally restrained bonds and fewer sites for self-association through intermolecular hydrogen bonding than $hubM_3$. These sharp resonances allow us to observe both the disappearance of peaks for uncomplexed $flexM_3$ and the appearance of those corresponding to $flexM_3(R''CA)_3$ during the titration. These spectra clearly demonstrate that exchange between free and complexed $flexM_3$ is slow on the NMR time scale. The absence of resonances for partially formed complexes emphasizes the cooperative nature of this self-assembly process.

(20) We complexed $flexM_3$ with 3,3,3-triphenylpropyl isocyanurate (R''CA) because R''CA is more soluble in organic solvents than is neohexyl cyanurate. Kelly, T. R.; Maguire, M. P. *J. Am. Chem. Soc.* **1987**, *109*, 6549.

Scheme V. Schematic Representation of the Four Possible Geometrical Isomers of These Supramolecular Aggregates

Chart I. Complexation of hubM$_3$ with Various Isocyanuric and Barbituric Acid Derivatives

R		% Unsymmetrical isomers in hubM$_3$(CA)$_3$
N(CH$_2$)$_2$C(CH$_3$)$_3$	15	
N(CH$_2$)$_7$CH$_3$	16	<1%
NCH$_2$C$_6$H$_5$	17	
CH$_2$	18	
C(C$_6$H$_5$)$_2$	19	
C(CH$_2$-4-BrC$_6$H$_5$)$_2$	20	1 - 5%
C(CH$_2$CH$_3$)$_2$	21	
CCl$_2$	22	
CBr$_2$	23	
NC$_6$H$_5$	24	5 - 10%
N-4-CH$_3$C$_6$H$_5$	25	
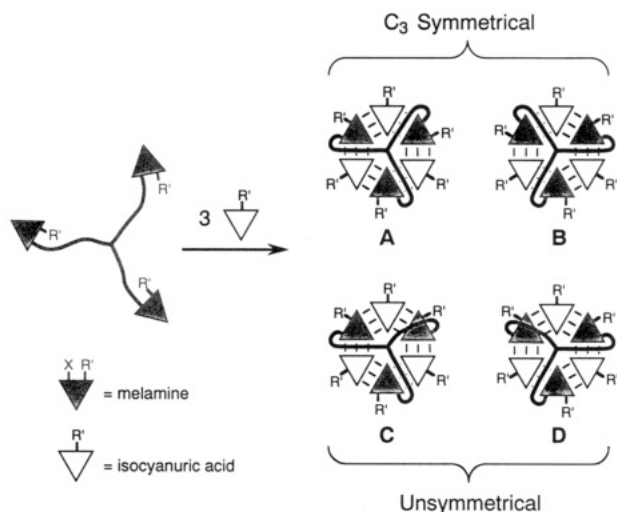
The methylene protons of flexM$_3$ and protons o and o′ of R″CA become diastereotopic in the complex. The imide protons of R″CA appear as separate resonances in the flexM$_3$(R″CA)$_3$ complex because they occupy different hydrogen-bonding environments. The melamine protons j, j′, and k shift downfield 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 flexM$_3$ is disrupted. NOE studies of flexM$_3$(R″CA)$_3$ (Figure 3B) show intermolecular NOEs among the melamine protons of flexM$_3$ and the imide protons of R″CA, in analogy with those seen in the hubM$_3$(R′CA)$_3$ complex.

,(c) **UV Spectroscopy.** Titration of flexM$_3$ with R″CA monitored by UV spectroscopy (Figure 4) confirms that this complex also has a 1:3 stoichiometry between flexM$_3$ and R″CA.

(d) **Gel Permeation Chromatography.** Figure 5 shows the GPC traces of free flexM$_3$ and flexM$_3$(R″CA)$_3$ eluted with CH$_2$Cl$_2$ and CHCl$_3$. The traces of free flexM$_3$ 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 flexM$_3$(R″CA)$_3$ shows peaks at 10.2 min (CHCl$_3$) and 10.8 min (CH$_2$Cl$_2$) for the complex. These peaks show tailing similar to that of hubM$_3$(R′CA)$_3$ caused by decomplexation of the aggregate during the analysis. The leading edge of these peaks is broader than the leading edge of the hubM$_3$(R′CA)$_3$ peaks, suggesting that flexM$_3$(R″CA)$_3$ may have a broader molecular weight distribution in solution than hubM$_3$(R′CA)$_3$.

(e) **Vapor Pressure Osmometry.** The VPO analysis of flexM$_3$(R″CA)$_3$ has the same basic trends as seen for the hubM$_3$(R′CA)$_3$ complex (Figure 6). In this case, however, all of the molecular weight standards give an experimental molecular weight for flexM$_3$(R″CA)$_3$ 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 hubM$_3$ with Derivatives of R′CA.** There are four possible geometrical isomers of hubM$_3$(R′CA)$_3$ (Scheme VA–D) because the spokes of hubM$_3$ 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 CA$_3$·M$_3$ cyclic hexamer. Enantiomers A and B are C_3 symmetrical

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(21) Most of the self-assembling structures based on the CA·M lattice that we have synthesized give molecular weights, as determined by VPO, that are higher than the calculated values.

structures. Enantiomers C and D have only the identity element (C_1).

Complexation of hubM$_3$ 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 C_3 symmetrical species A and B by ¹H NMR spectroscopy (we observe only one set of resonances) for the three spokes of hubM$_3$. We infer that the absence of unsymmetrical structures indicates that the C_3 enantiomers A and B are enthalpically favored over the unsymmetrical enantiomers and that the C_3 symmetry is not caused by kinetic averaging of the structures in the NMR spectrometer (an average of A–D would also have effective C_3 symmetry). We estimate that the symmetrical isomers of hubM$_3$-(R′CA)$_3$ 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 hubM$_3$(R′CA)$_3$; we should be able to detect 1% of these species.

Complexation of hubM$_3$ with aryl isocyanurates or barbituric acid derivatives rather than what we believe to be a mixtures of isomers A–D, although in all cases the C_3 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 hubM$_3$ 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) **hubM$_3$(barbital)$_3$.** 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 hubM$_3$(barbital)$_3$. The minor resonances of the unsymmetrical isomers are clearly resolved from the major resonances of the C_3 symmetrical isomers. We assigned the resonances of symmetrical hubM$_3$(barbital)$_3$ in analogy to those of hubM$_3$(R′CA)$_3$, 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 hubM$_3$(R′CA)$_3$ rather than unsymmetrical isomers (see the Discussion section).

Discussion

Preorganization. The molecular aggregates hubM$_3$(R′CA)$_3$ and flexM$_3$(R″CA)$_3$ establish the hydrogen-bonded CA·M lattice
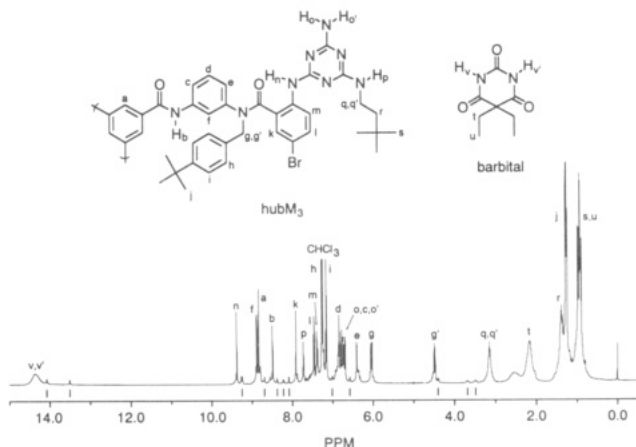
Figure 8. ^{1}H NMR spectrum of hubM$_3$(barbital)$_3$ (500 MHz, CDCl$_3$). The major resonances in the spectrum correspond to the C_3 symmetrical isomers and are assigned. The minor resonances, some of which are indicated with lines above the scale (~5% of the material), may correspond to the unsymmetrical isomers. These resonances are not caused by impurities in the sample.

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-diethylbenzene spokes (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 flexM$_3$) is necessary to make complexation energetically more favorable than polymerization. The [1 + 3] aggregates are further stabilized by using the semirigid spokes of hubM$_3$: 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. hubM$_3$ and R'CA self-assemble into the same, structurally well-defined molecular aggregate hubM$_3$(R'CA)$_3$ in all three of these solvents. Qualitative ^{1}H 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 ^{1}H NMR spectrum of hubM$_3$(R'CA)$_3$ is similar in CDCl$_3$ and o-dichlorobenzene-d_4 except that the hydrogen-bonded protons appear approximately 0.4 ppm farther downfield in o-dichlorobenzene-d_4 than in CDCl$_3$. These molecular aggregates are not stable in polar solvent mixtures such as CHCl$_3$/MeOH and CHCl$_3$/DMSO that compete for hydrogen-bonding sites.

Ambiguities in the Structures of the [1 + 3] Complexes. The solubility studies, and the UV and ^{1}H NMR titration data, demonstrate that the stoichiometry in the molecular aggregates composed of hubM$_3$/R'CA and flexM$_3$/R''CA is 1:3. The VPO

Chart II. Possible Structure for a Dimer of the [1 + 3] Complexes (Left) and a Stable Hydrogen-Bonded [1 + 1] Complex between a Tris Melamine Derivative and a Tris Isocyanuric Acid Derivative (Right)

[hubM$_3$(R'CA)$_3$]$_2$ hubM$_3$·hubCA$_3$

data indicate that the MWs of these aggregates are higher than the theoretical MWs expected for hubM$_3$(R'CA)$_3$ and flexM$_3$-(R''CA)$_3$. 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 [hubM$_3$(R'CA)$_3$]$_2$. (Chart II shows one possible structure for a dimer.) The ^{1}H 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 hubM$_3$ molecule are hydrogen-bonded in one CA$_3$·M$_3$ unit, while the third spoke is extended out in order to hydrogen bond in the other CA$_3$·M$_3$ unit. The ^{1}H NMR spectrum of the dimer should reflect the different chemical environments of these spokes; our experimental observation shows that all three spokes of hubM$_3$ are in indistinguishable environments. Given the large error that is associated with our osmometry measurements and the dependence of the measurement on the choice of molecular weight standard, we do not consider our VPO results as conclusive evidence for or against the existence of such dimers or higher oligomers.

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 hubM$_3$ and hubCA$_3$. hubCA$_3$ consists of three CA units that are covalently linked through the hub-and-spoke architecture.[24] The VPO-determined MW of hubM$_3$·hubCA$_3$ is also 35–80% higher than the expected theoretical MW. This result is similar to that obtained with flexM$_3$(R''CA)$_3$ 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 [hubM$_3$(R'CA)$_3$]$_2$.

Other Methods for Characterizing Self-Assembling Structures. The methods that we have used to characterize hubM$_3$(R'CA)$_3$ and flexM$_3$(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.[4]

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 hubM$_3$(R'CA)$_3$ or flexM$_3$(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 hubM$_3$ with R'CA will be presented in a future publication.

Experimental Section

General Methods. NOE experiments were performed with a Bruker AM 500 instrument. Elemental analyses were performed by Galbraith Laboratories, Inc. THF was distilled from sodium benzophenone ketyl.

(22) Variable-temperature ^{1}H NMR studies on a mixture of *N,N'*-bis(4-*tert*-butylphenyl)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 corresponds to a BA$_3$·M$_3$ cyclic hexamer, although we believe that the cyclic hexamer is the most plausible 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. Williams, L. D.; Chawla, B.; Shaw, B. R. *Biopolymers* **1987**, *26*, 591.

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

Cyanuric Acid·Melamine Lattice Based Aggregates

J. Am. Chem. Soc., Vol. 115, No. 3, 1993 913

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-[(1,1-Dimethylethoxy)carbonyl]-1,3-diaminobenzene (2). A 250-mL round-bottomed flask was charged with 9.05 (50.0 mmol) of 1,3-diaminobenzene dihydrochloride, 25.3 g (34.9 mL, 250 mmol) of triethylamine, 13.6 g (55.0 mmol) of 2-[[[(tert-butyloxy)carbonyl]oxy]imino]-2-phenylacetonitrile (BOC-ON), and 60 mL of DMF. The solution was heated to 50 °C in an oil bath under a nitrogen atmosphere for 3 h and cooled to room temperature, and the solvent was removed by rotary evaporation at 0.1 Torr. The residue was partitioned between 200 mL of 1 N aqueous sodium hydroxide and 250 mL toluene. The organic layer was separated, washed twice with 150-mL portions of 1 N aqueous sodium hydroxide, three times with 200-mL portions of water, and once with 50 mL of brine, and dried over MgSO$_4$, and the solvent was removed by rotary evaporation at aspirator pressure. The clear reddish oil was purified by flash chromatography (eluted with 20:80 ethyl acetate/hexanes), giving 6.67 g (32.0 mmol, 64%) of the product as a white solid: R_f 0.37 (50:50 ethyl acetate/hexanes); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.01 (s, 1 H), 6.87–6.81 (m, 2 H), 6.53 (d, J = 7.9 Hz, 1 H), 6.16 (d, J = 8.0 Hz, 1 H), 4.97 (s, 2 H), 1.45 (s, 9 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 152.66, 148.89, 140.02, 128.71, 108.21, 106.33, 103.93, 78.47, 28.14; HRMS-CI (M + H$^+$) calcd 209.1289, found 209.1308. Anal. Calcd for C$_{11}$H$_{16}$N$_2$O$_2$: C, 63.44; H, 7.74. Found: C, 63.61; H, 7.81.

N-[(1,1-Dimethylethoxy)carbonyl]-N'-[4-(1,1-dimethylethyl)benzyl]-1,3-diaminobenzene (3). 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-butylbenzyl bromide, 5.06 g (6.97 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 and residual triethylamine were removed by rotary evaporation at aspirator pressure. The residue was partitioned between 300 mL of ether and 300 mL of water. The organic layer was separated, washed three times with 300-mL portions of water and once with 150 mL of brine, and dried over MgSO$_4$, and the solvent was removed by rotary evaporation at aspirator pressure. The product was purified by flash chromatography (eluted with 5:95 ethyl acetate/hexanes), giving 6.00 g (16.9 mmol, 68%) of 3 as a white solid: R_f 0.50 (25:75 ethyl acetate/hexanes); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.02 (s, 1 H), 7.32–7.24 (m, 4 H), 6.48 (t, J = 8.0 Hz, 1 H), 6.81 (s, 1 H), 6.60 (d, J = 8.0 Hz, 1 H), 6.16 (t, J = 6.8 Hz, 2 H), 4.15 (d, J = 5.5 Hz, 2 H), 1.44 (s, 9 H), 1.25 (s, 9 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.67, 149.06, 148.83, 140.12, 137.28, 128.79, 126.99, 124.93, 106.47, 106.27, 102.29, 78.53, 46.08, 34.12, 31.19, 28.16; HRMS-CI (M + H$^+$) calcd for C$_{22}$H$_{31}$N$_2$O$_2$ 355.2384, found 355.2398.

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 1.08 g (5.0 mmol) of 2-amino-5-bromobenzoic acid, 1.01 g (1.39 mL, 10.0 mmol) of triethylamine, and 50 mL of dry methylene chloride. The solution was cooled in an ice bath under a nitrogen atmosphere, and 1.02 g (0.72 mL, 5.0 mmol) of phthaloyl dichloride was added dropwise. After the addition was complete, the reaction was heated at reflux for 1 h, diluted with 100 mL of methylene chloride, washed three times with 100-mL portions of 1.0 N aqueous hydrochloric acid, twice with 100-mL portions of water, and once with 50 mL of brine, and dried over MgSO$_4$. The solvent was removed by rotary evaporation at aspirator pressure, and the product was purified by flash chromatography (eluted with 50:50 ethyl acetate/hexanes followed by ethyl acetate, loaded on column preadsorbed to silica), giving 1.26 g (3.63 mmol, 73%) of the product as an off-white crystalline solid (as an alternate workup, the crude product may be recrystallized from toluene): R_f 0.16 (1:1 ethyl acetate/hexanes); ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.7–13.3 (br s, 1 H), 8.15 (d, J = 2.3 Hz, 1 H), 8.02–7.98 (m, 3 H), 7.94–7.92 (m, 2 H), 7.54 (d, J = 8.3 Hz, 1 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.81, 164.90, 135.77, 134.89, 133.47, 132.65, 131.68, 131.30, 130.66, 128.18, 123.60, 122.08; HRMS-FAB (M + H$^+$) calcd for C$_{15}$H$_9$BrNO$_4$ 345.9714, found 345.9684.

[3-[N-[2-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2-yl)-5-bromobenzoyl]-N-[[4-(1,1-dimethylethyl)phenyl]methyl]amino]phenyl]carbamic Acid 1,1-Dimethylethyl Ester (7). A 250-mL round-bottomed flask equipped with a stirring bar was charged with 5.61 g (16.2 mmol) of 5 and 10.0 mL of thionyl chloride. The solution was heated at reflux under a nitrogen atmosphere for 2 h, cooled to room temperature, and diluted with 75 mL of toluene, and the solvent containing excess thionyl chloride was removed by rotary evaporation at aspirator pressure. The residue was again dissolved in 75 mL of toluene, and the solvent was removed by rotary evaporation at aspirator pressure to ensure that all of the residual

thionyl chloride and hydrogen chloride was removed. The crude acid chloride was dried briefly at 0.1 Torr and then dissolved in 20 mL of methylene chloride and 150 mL of toluene. The solution was cooled in an ice bath, and 6.58 g (9.06 mL, 65.0 mmol) of triethylamine was added followed by 4.31 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 250 mL of water, 200 mL of saturated aqueous sodium carbonate, twice with 250-mL portions of water, and 100 mL of brine. The solution was dried over MgSO$_4$ and the solvent removed by rotary evaporation at aspirator pressure. The crude product was purified by flash chromatography (eluted with 25:75 ethyl acetate/hexanes), giving 7.49 g (11.0 mmol, 90%) of the product as a white foam: R_f 0.23 (33:66 ethyl acetate/hexanes); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.37 (s, 1 H), 8.01–7.92 (m, 4 H), 7.71 (d, J = 8.5 Hz, 1 H), 7.52 (s, 1 H), 7.38 (m, 2 H), 7.22 (d, 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 (t, J = 8.0 Hz, 1 H), 6.82 (d, J = 7.7 Hz, 1 H), 4.88 (s, 2 H), 1.46 (s, 9 H), 1.23 (s, 9 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.50, 164.86, 152.43, 149.13, 142.02, 140.28, 138.49, 135.06, 134.86, 133.78, 133.03, 132.44, 131.75, 131.48, 129.61, 128.72, 126.93, 124.88, 123.54, 121.37, 120.68, 116.68, 116.57, 79.17, 51.95, 34.05, 31.07, 28.06; HRMS-FAB (M + H$^+$) calcd for C$_{37}$H$_{37}$BrN$_3$O$_5$ 682.1914, found 682.1903.

[3-[N-(2-Amino-5-bromobenzoyl)-N-[[4-(1,1-dimethylethyl)phenyl]methyl]amino]phenyl]carbamic Acid 1,1-Dimethylethyl Ester (8). A 500-mL round-bottomed flask equipped with a stirring bar was charged with 5.83 g (8.54 mmol) of 7, 0.41 g (0.41 mL, 12.8 mmol) of hydrazine, and 150 mL of methanol. The solution was heated and refluxed gently for 4 h under a nitrogen atmosphere. The solution was then cooled to room temperature, diluted with 250 mL of toluene and 250 mL of ethyl acetate, and stirred for 12 h. The precipitated phthalhydrazide was filtered off and washed with 50 mL of ethyl acetate, and the combined filtrates were washed three times with 300-mL portions of water and once with 200 mL of brine and dried over MgSO$_4$, and the solvent was removed by rotary evaporation at aspirator pressure. The product was purified by flash chromatography (eluted with 25:75 ethyl acetate/hexanes) to give 4.51 g (18.6 mmol, 95%) of 8 as a white foam: R_f 0.33 (33:66 ethyl acetate/hexanes); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.34 (s, 1 H), 7.32 (app d, J = 8.3 Hz, 3 H), 7.21 (app d, J = 8.3 Hz, 3 H), 7.07–7.01 (m, 2 H), 6.91 (d, J = 2.3 Hz, 1 H), 6.64 (d, J = 7.9 Hz, 1 H), 6.59 (d, J = 8.8 Hz, 1 H), 5.61 (s, 2 H), 4.98 (s, 2 H), 1.44 (s, 9 H), 1.25 (s, 9 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.22, 168.48, 152.42, 149.26, 146.42, 143.11, 140.08, 134.34, 132.49, 130.87, 128.75, 127.26, 125.04, 120.64, 120.57, 117.60, 116.29, 116.09, 79.11, 52.33, 34.10, 31.07, 28.03; HRMS-FAB (M + H$^+$) calcd for C$_{29}$H$_{34}$BrN$_3$O$_3$ 551.1784, found 551.1752.

[3-[N-[2-[(4-Amino-6-chloro-1,3,5-triazin-2-yl)amino]-5-bromobenzoyl]-N-[[4-(1,1-dimethylethyl)phenyl]methyl]amino]phenyl]carbamic Acid 1,1-Dimethylethyl Ester (9). A 250-mL round-bottomed flask equipped with a stirring bar was charged with 2.77 g (15.0 mmol) of cyanuric chloride, 3.88 g (5.23 mL, 30.0 mmol) of diisopropylethylamine, and 100 mL of THF. The solution was cooled in an ice bath under a nitrogen atmosphere, and 5.53 g (10.0 mmol) of 8 was added in several portions. The solution was stirred in the ice bath for 1 h and then gaseous ammonia was passed over the solution in a gentle stream for an additional 1 h. The solution was then warmed to room temperature, and the solvent was removed by rotary evaporation at aspirator pressure. The residue was taken up to 300 mL of toluene, washed three times with 150-mL portions of water and once with 50 mL of brine, and dried over MgSO$_4$, and the solvent was removed by rotary evaporation at aspirator pressure. The product was purified by flash chromatography (eluted with 33:66 ethyl acetate/hexanes) to give 5.79 g (8.5 mmol, 85%) of the product as a white foam: R_f 0.14 (33:66 ethyl acetate/hexanes); ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.32 (s, 1 H), 9.26 (s, 1 H), 7.67 (d, J = 8.4 Hz, 1 H), 7.61 (s, 1 H), 7.53–7.40 (m, 2 H), 7.36–7.24 (m, 4 H), 7.21–7.13 (m, 3 H), 7.01 (t, J = 7.8 Hz, 1 H), 6.74 (d, J = 6.6 Hz, 1 H), 4.97 (s, 2 H), 1.43 (s, 9 H), 1.25 (s, 9 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.62, 166.84, 166.79, 164.36, 152.38, 149.34, 142.35, 140.04, 135.22, 134.06, 132.36, 131.26, 130.38, 128.79, 127.18, 126.57, 125.07, 120.88, 116.44, 116.28, 115.30, 79.19, 52.41, 34.11, 31.10, 28.05; HRMS-FAB (M + H$^+$) calcd 680.1749, found 680.1712. Anal. Calcd for C$_{32}$H$_{35}$BrClN$_7$O$_3$: C, 56.44; H, 5.18; Br, 11.73; Cl, 5.21. Found: C, 56.40; H, 5.24; Br, 11.66; Cl, 5.27.

[3-[N-[2-[[4-Amino-6-[(3,3-dimethylbutyl)amino]-1,3,5-triazin-2-yl]amino]-5-bromobenzoyl]-N-[[4-(1,1-dimethylethyl)phenyl]methyl]amino]phenyl]carbamic Acid 1,1-Dimethylethyl Ester (10). A 250-mL round-bottomed flask equipped with a stirring bar was charged with 2.72 g (4.0 mmol) of 9, 1.62 g (2.15 mL, 16.0 mmol) of neohexylamine, 0.78 g (1.05 mL, 6.0 mmol) of diisopropylethylamine, and 50 mL of dry 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 200 mL of toluene, washed three times with 150-mL portions of water and once with 50 mL of brine, and dried over MgSO$_4$, 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, loaded on column as a solution in toluene) to give 2.55 g (3.42 mmol, 86%) of **10** as a white foam: R_f 0.28 (50:50 ethyl acetate/hexanes); ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.33 (s, 1 H), 8.50–8.35 (m, 2 H), 7.36–7.28 (m, 3 H), 7.28–7.03 (m, 6 H), 6.92 (t, J = 5.5 Hz, 1 H), 6.66 (d, J = 5.9 Hz, 1 H), 6.59 (s, 1 H), 6.44 (s, 1 H), 5.02 (s, 2 H), 3.32–3.20 (m, 2 H), 1.48–1.38 (m, 11 H), 1.25 (s, 9 H), 0.92 (s, 9 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.87, 167.06, 166.70, 165.86, 164.08, 163.73, 152.35, 149.44, 142.61, 140.23, 138.28, 133.91, 132.22, 131.00, 129.04, 127.29, 125.12, 122.94, 120.46, 116.30, 111.40, 79.20, 52.56, 43.09, 42.68, 36.53, 34.10, 31.04, 29.30, 27.98; HRMS-FAB (M + H$^+$) calcd for C$_{38}$H$_{50}$BrN$_8$O$_3$ 745.3190, found 745.3192.

[3-[*N*-[2-[[4-Amino-6-[(3,3-dimethylbutyl)amino]-1,3,5-triazin-2-yl]-amino]-5-bromobenzoyl]-*N*-[[4-(1,1-dimethylethyl)phenyl]methyl]amino]-phenyl]amine (11). A 250-mL round-bottomed flask was charged with 6.12 g (8.2 mmol) of **10** and 100 mL of methylene chloride. The solution was cooled in an ice bath, and 20 mL of trifluoroacetic acid was added dropwise. After the addition was complete, the solution was stirred for 2 h at room temperature and diluted with 100 mL of toluene, and the solvent and excess trifluoroacetic acid were removed by rotary evaporation at aspirator pressure. The residue was taken up in 400 mL of toluene, washed twice with 300-mL portions of 1:1 water/saturated aqueous sodium carbonate, twice with 300-mL portions of water, and once with 100 mL of brine, and dried over MgSO$_4$, and the solvent was removed by rotary evaporation at aspirator pressure. The product was purified by flash chromatography (eluted with 66:33 ethyl acetate/hexanes, loaded on column as a solution in toluene) to give 4.75 g (7.36 mmol, 90%) of the product as a white foam: R_f 0.13 (50:50 ethyl acetate/hexanes); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.58–8.46 (m, 2 H), 7.33 (s, 2 H), 7.27 (d, J = 8.7 Hz, 1 H), 7.20 (d, J = 7.9 Hz, 2 H), 7.17 (s, 1 H), 6.98–6.92 (m, 1 H), 6.85 (m, 1 H), 6.61 (s, 1 H), 6.47 (s, 1 H), 6.33 (dd, J = 8.0, 1.5 Hz, 1 H), 6.26 (d, J = 5.1 Hz, 1 H), 6.19 (d, J = 7.3 Hz, 1 H), 5.14 (s, 2 H), 4.99 (s, 2 H), 3.34–3.24 (m, 2 H), 1.43 (t, J = 7.8 Hz, 2 H), 1.25 (s, 9 H), 0.92 (s, 9 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.74, 167.08, 166.72, 165.87, 164.07, 163.73, 149.36, 143.23, 138.34, 134.18, 132.13, 130.90, 129.30, 127.16, 125.09, 122.79, 113.98, 112.64, 111.75, 111.42, 52.61, 43.10, 42.68, 36.51, 34.11, 31.09, 29.32; HRMS-FAB (M + H$^+$) calcd for C$_{33}$H$_{42}$BrN$_8$O 645.2665, found 645.2626.

N,*N*′,*N*″-Tris[3-[*N*-[2-[[4-amino-6-[(3,3-dimethylbutyl)amino]-1,3,5-triazin-2-yl]amino]-5-bromobenzoyl]-*N*-[[4-(1,1-dimethylethyl)phenyl]-methyl]amino]phenyl]-1,3,5-benzenetricarboxamide (hubM$_3$). A 250-mL round-bottomed flask was charged with 1.94 g (4.0 mmol) of **11**, 0.78 g (1.05 mL, 6.0 mmol) of diisopropylethylamine, and 100 mL of methylene chloride. The solution was cooled in an ice bath under a nitrogen atmosphere, and 0.27 g (1.0 mmol) of 1,3,5-benzenetricarboxylic acid chloride was added and the solution stirred for 1 h at room temperature. The solvent was removed by rotary evaporation at aspirator pressure, and the residue was taken up in 250 mL of ethyl acetate. The organic layer was washed with 125 mL of water, 125 mL of saturated aqueous sodium carbonate solution, twice with 125-mL portions of water, and once with 75 mL of brine and dried over MgSO$_4$. The solvent was removed by rotary evaporation at aspirator pressure, and the product was purified by flash chromatography (eluted with 5:95 methanol/chloroform) to give 2.05 g (0.98 mmol, 98%) of hubM$_3$ as an off-white foam: R_f 0.11 (5:95 methanol/chloroform); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.57 (s, 3 H), 8.62 (s, 3 H), 8.53 (br d, J = 18.8 Hz, 3 H), 8.44 (s, 3 H), 7.74 (br d, J = 10.4 Hz, 3 H), 7.64 (br d, J = 7.3 Hz, 3 H), 7.37–7.28 (br m, 9 H), 7.28–7.16 (br m, 12 H), 6.91 (br s, 3 H), 6.78 (br d, J = 14.7 Hz, 3 H), 6.60 (br s, 3 H), 6.45 (br s, 3 H), 5.09 (s, 6 H), 3.25 (br s, 6 H), 1.41 (br s, 6 H), 1.24 (s, 27 H), 0.89 (s, 27 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.92, 167.12, 166.75, 165.95, 165.85, 164.41, 164.19, 163.83, 149.56, 142.83, 142.69, 139.73, 138.53, 138.31, 135.17, 133.95, 132.37, 131.31, 129.99, 129.21, 127.27, 125.25, 122.89, 118.71, 118.37, 111.73, 52.85, 43.13, 42.69, 36.65, 34.18, 31.14, 29.53, 29.36; HRMS-FAB (M + H$^+$) calcd 2089.7678, found 2089.7637. Anal. Calcd for C$_{108}$H$_{123}$Br$_3$N$_{24}$O$_6$: C, 61.98; H, 5.92; Br, 11.45. Found: C, 61.91; H, 6.15; Br, 11.23.

1-Nitrobiuret.[25] A 100-mL beaker equipped with a stirring bar was charged with 12.5 mL of 96% sulfuric acid and 3.0 mL of 70% nitric acid, and the mixture was cooled in an ice bath. (Caution: *N*-Nitro compounds are potentially explosive. Although we have not had difficulties, this procedure was carried out behind a safety shield.) To this mixture

was added 5.15 g (50.0 mmol) of biuret in small portions over 0.5 h. After the addition was complete, the beaker was removed from the ice bath and the mixture allowed to stir for 1.5 h 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 and washed on the filter with 100 mL of water. The solid was then suspended in 100 mL of water (cooled in an ice bath), and 1 N aqueous sodium hydroxide was added until all of the solid dissolved and the pH was 8.5. The cold solution was filtered and the filtrate acidified with 37% hydrochloric acid to a pH of 2.0. The precipitated product was collected by vacuum filtration, washed on the filter with 100 mL of water, and dried at 0.1 Torr to give 3.48 g (23.5 mmol, 47%) of the product as a white solid: ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.53 (br s, 1 H), 9.45 (s, 1 H), 7.42 (br s, 1 H), 7.09 (br s, 1 H); mp 161–162 °C dec (lit. mp 165 °C); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.26, 148.54; HRMS-CI (M$^+$) calcd for C$_2$H$_4$N$_4$O$_4$ 148.0232, found 148.0225.

1-(3,3-Dimethylbutyl)biuret. A 100-mL round-bottomed flask equipped with a stirring bar was charged with 1.48 g (10.0 mmol) of nitrobiuret, 1.01 g (1.35 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 1.21 g (6.5 mmol, 65%) of the product as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.53 (s, 1 H), 7.41 (br s, 1 H), 6.70 (s, 2 H), 3.10 (m, 2 H), 1.33 (m, 2 H), 0.88 (s, 9 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.54, 154.26, 43.14, 35.33, 29.53, 29.21; HRMS-EI (M + H$^+$) calcd for C$_8$H$_{18}$N$_3$O$_2$ 188.1399, found 188.1378.

N-(3,3-Dimethylbutyl)isocyanuric Acid (R′CA). 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 was added. The solution was stirred at 0 °C until all of the sodium had dissolved, and then 1.12 g (6.0 mmol) of neohexylbiuret and 1.42 g (1.45 mL, 12.0 mmol) of diethyl carbonate were added. The reaction was heated at 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 toluene. 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 R′CA as a white solid: mp 266–268 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.39 (s, 2 H), 3.65 (m, 2 H), 1.40 (m, 2 H), 0.91 (s, 9 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 149.63, 148.56, 40.50, 37.22, 29.49, 28.96; HRMS-FAB (M + H$^+$) calcd 214.1191, found 214.1201. Anal. Calcd for C$_9$H$_{15}$N$_3$O$_3$: C, 50.69; H, 7.09. Found: 50.94; H, 7.11.

3-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 anthranilic 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 precipitated 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 MgSO$_4$, 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), giving 3.12 g (16.0 mmol, 32%) of **13** as a yellow solid: R_f 0.21 (50:50 ethyl acetate/hexanes); ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.71 (dd, J = 7.9, 1.2 Hz, 1 H), 7.24 (dt, J = 7.7, 1.4 Hz, 1 H), 6.76 (d, J = 8.5 Hz, 1 H), 6.64 (s, 2 H), 6.53 (app t, J = 7.5 Hz, 1 H), 4.59 (t, J = 5.1 Hz, 1 H), 4.27 (t, J = 6.4 Hz, 2 H), 3.55 (dt, J = 6.0, 5.5 Hz, 2 H), 1.84 (tt, J = 6.3, 6.3 Hz, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.59, 151.43, 134.00, 130.73, 116.60, 114.81, 109.13, 61.29, 57.55, 31.71; HRMS-FAB (M + Na$^+$) calcd for C$_{10}$H$_{13}$NO$_3$Na 218.0792, found 218.1798.

2-[[4-Amino-6-[(3,3-dimethylbutyl)amino]-1,3,5-triazin-2-yl]amino]-benzoic Acid 3-Hydroxypropyl Ester (14). A 250-mL round-bottomed flask equipped with a stirring bar was charged with 2.02 g (9.65 mmol) of **13**, 1.37 g (1.85 mL, 10.6 mmol) of diisopropylethylamine, and 75 mL of THF. The solution was cooled in an ice bath under a nitrogen atmosphere, and 1.96 g (10.6 mmol) of cyanuric chloride was added in one portion. The solution was stirred in the ice bath for 1 h, and then gaseous ammonia was passed over the solution in a gentle stream for an additional

(25) Davis, T. L.; Blanchard, K. C. *J. Am. Chem. Soc.* **1929**, *51*, 1801 and references therein.

1 h. 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 1500 mL of ethyl acetate(the product is not very soluble), washed three times with 400-mL portions of water and once with 300 mL of brine, and dried over $MgSO_4$. 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, 2.50 g (3.36 mL, 19.3 mmol) of diisopropylethylamine, 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 1000 mL of ethyl acetate (the product is not very soluble), washed four times with 500 mL portions of water and once with 200 mL of brine, and dried over $MgSO_4$, 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, 75%) of **14** as a white solid: R_f 0.13 (ethyl acetate): ^1H NMR (400 MHz, DMSO-d_6) δ 10.31, 10.25 (two s, two conformers, 1 H), 9.00, 8.96 (two d, two conformers, $J = 8.5$ Hz, 1 H), 7.96 (t, $J = 6.2$ Hz, 1 H), 7.53, 7.48 (two t, two conformers, $J = 7.5$ Hz, 1 H), 7.00 (m, 2 H), 6.64 (br s, 1 H), 6.48 (br s, 1 H), 4.62 (t, $J = 5.1$ Hz, 1 H), 4.35 (t, $J = 6.3$ Hz, 2 H), 3.56 (dt, $J = 5.9, 5.7$ Hz, 2 H), 3.29 (m, 2 H), 1.87 (tt, $J = 6.2, 6.2$ Hz, 2 H), 1.44 (m, 2 H), 0.93 (d, $J = 9.8$ Hz, 9 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.63, 167.04, 166.69, 165.95, 165.74, 164.28, 163.95, 143.02, 142.85, 134.01, 133.77, 130.62, 120.19, 119.98, 114.03, 113.66, 62.31, 57.27, 43.13, 42.65, 36.56, 31.40, 29.34; HRMS-FAB (M + H$^+$) calcd for $C_{19}H_{29}N_6O_3$ 389.2299, found 389.2325.

1,3,5-Benzenetricarboxylic Acid Tris[3-[[2-[[4-amino-6-[(3,3-dimethylbutyl)amino]-1,3,5-triazin-2-yl]amino]benzoyl]oxy]propyl] Ester (flexM$_3$). A 100-mL round-bottomed flask equipped with a stirring bar was charged with 1.17 g (3.0 mmol) of **14**, 0.45 g (0.61 mL, 3.5 mmol) of diisopropylethylamine, and 40 mL of THF. The solution was cooled in an ice bath under a nitrogen atmosphere, and 0.27 g (1.0 mmol) of 1,3,5-benzenetricarboxylic acid chloride was added, and the solution was stirred at 0 °C for 2 h and room temperature for 10 h. The solvent was removed by rotary evaporation at aspirator pressure, and the residue was taken up in 300 mL of ethyl acetate. The organic layer was washed twice with 150-mL portions of water, twice with 100-mL portions of saturated aqueous sodium carbonate, twice with 150-mL portions of water, and once with 100 mL of brine and dried over $MgSO_4$, and the solvent was removed by rotary evaporation at aspirator pressure. The crude product was purified by flash chromatography (eluted with ethyl acetate followed by 97:3 ethyl acetate/methanol, loaded on column preadsorbed to silica) to give 0.88 g (0.67 mmol, 67%) of flexM$_3$ as a white foam: R_f 0.13 (97:3 ethyl acetate/methanol); ^1H NMR (400 MHz, DMSO-d_6) δ 10.19 (d, $J = 7.2$ Hz, 3 H), 8.93, 8.85 (two d, two conformers, $J = 8.5$ Hz, 3 H), 8.42 (t, $J = 10.2$ Hz, 3 H), 7.89 (d, $J = 7.8$ Hz, 3 H), 7.43, 7.34 (two t, two conformers, $J = 7.7$ Hz, 3 H), 6.95 (t, $J = 5.3$ Hz, 3 H), 6.85 (m, 3 H), 6.62 (br s, 3 H), 6.45 (br s, 3 H), 4.46 (m, 12 H), 3.25 (m, 6 H), 2.22 (m, 6 H), 1.43 (m, 6 H), 0.90 (s, 27 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.49, 167.00, 166.64, 165.88, 165.71, 164.13, 163.85, 143.05, 142.88, 133.96, 133.67, 133.28, 130.59, 130.40, 119.81, 119.69, 113.48, 113.24, 63.10, 62.29, 43.08, 42.62, 36.53, 29.27, 27.44; HRMS-FAB (M + H$^+$) calcd 1321.6589, found 1321.6583. Anal. Calcd for $C_{66}H_{84}N_{18}O_{12}$: C, 55.99; H, 6.41; N, 19.08. Found: C, 60.07; H, 6.28; N, 19.17.

3,3,3-Triphenylpropionamide. A 100-mL round-bottomed flask equipped with a stirring bar was charged with 1.00 g (3.31 mmol) of 3,3,3-triphenylpropanoic acid and 5 mL of thionyl chloride. The mixture was heated at reflux under a nitrogen atmosphere for 2 h, cooled to room temperature, and diluted with 50 mL of toluene. The solvent and excess thionyl chloride were removed by rotary evaporation at aspirator pressure. The residue was again diluted with 50 mL of toluene, and the solvent was removed by rotary evaporation. The resulting acid chloride was taken up in 50 mL of toluene and cooled in an ice bath, and a gentle stream of gaseous ammonia was bubbled into the solution for 15 min. The solvent was removed by rotary evaporation at aspirator pressure, and the residue was taken up in 150 mL of ethyl acetate, washed once with 75 mL of water, twice with 50-mL portions of saturated aqueous sodium carbonate, twice with 75-mL portions of water, and once with 50 mL of brine and dried over $MgSO_4$. 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, 89%) of the product as a white solid: R_f 0.27 (66:33 ethyl acetate/hexanes); ^1H NMR (400 MHz, DMSO-d_6) δ 7.26–7.14 (m, 15 H), 7.05 (s, 1 H), 6.54 (s, 1 H), 3.58 (s, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.76, 147.41, 129.21, 127.43, 125.70, 55.47, 46.19; HRMS-EI (M$^+$) calcd for $C_{21}H_{19}NO$ 301.1466, found 301.1487.

(3,3,3-Triphenylpropyl)amine. A 200-mL round-bottomed flask equipped with a stirring bar was charged with 60 mL of THF and 0.76 g (35.0 mmol) of lithium borohydride. To this solution was added 7.60 g (8.88 mL, 70.0 mmol) of trimethylchlorosilane via syringe followed by 4.69 g (15.5 mmol) of 3,3,3-triphenylpropionamide, which was added in several portions. The reaction was heated at reflux under a nitrogen atmosphere for 12 h, cooled in an ice bath, and quenched by the dropwise addition of 15 mL of methanol. The solvents were removed by rotary evaporation at aspirator pressure, and the residue was taken up in 150 mL of 1 N aqueous sodium hydroxide. The aqueous phase was extracted three times with 150-mL portions of methylene chloride, the combined organic phases were dried over $MgSO_4$, and the solvents were removed by rotary evaporation at aspirator pressure. The crude product was purified by flash chromatography (eluted with 97:3 methylene chloride/triethylamine) to give 3.61 g (12.6 mmol, 81%) of the product as a slightly yellow glass: R_f 0.20 (97:3 methylene chloride/triethylamine); ^1H NMR (400 MHz, DMSO-d_6) δ 7.30–7.16 (m, 15 H), 4.22 (br s, 2 H), 2.65 (m, 2 H), 2.17 (m, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.38, 128.56, 128.07, 126.08, 55.03, 38.72, 37.21; HRMS-EI (M$^+$) calcd for $C_{21}H_{21}N$ 287.1673, found 287.1666.

1-(3,3,3-Triphenylpropyl)biuret. A 250-mL round-bottomed flask equipped with a stirring bar was charged with 1.94 g (6.8 mmol) of (3,3,3-triphenylpropyl)amine dissolved in 40 mL of dioxane. To this solution was added 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 purified by flash chromatography (eluted with ethyl acetate followed by 50:50 ethyl acetate/THF followed by THF) to give 2.20 g (5.87 mmol, 87%) of the product as a white solid: R_f 0.45 (90:10 methylene chloride/methanol); ^1H NMR (400 MHz, DMSO-d_6) δ 8.56 (s, 1 H), 7.52 (s, 1 H), 7.32–7.18 (m, 15 H), 6.72 (s, 2 H), 2.74 (s, 4 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.47, 154.50, 146.68, 128.67, 127.98, 125.96, 55.00, 39.38, 36.64; HRMS-FAB (M + H$^+$) calcd 374.1868, found 374.1866. Anal. Calcd for $C_{23}H_{23}N_3O_2$: C, 73.97; H, 6.21. Found: C, 74.12; H, 6.32.

N-(3,3,3-Triphenylpropyl)isocyanuric Acid (R″CA). A 250-mL round-bottomed flask equipped with a stirring bar was charged with 100 mL of ethanol and cooled in an ice bath under a nitrogen atmosphere, and 0.45 g (19.5 mmol) of sodium was added. The solution was stirred at 0 °C until all of the sodium had dissolved, and then 2.08 g (5.58 mmol) of (triphenylpropyl)biuret and 1.32 g (1.35 mL, 11.2 mmol) of diethyl carbonate were added. The reaction was heated at reflux for 12 h and cooled to room temperature, and the solvent was removed by rotary evaporation at aspirator pressure. The residue was taken up in 300 mL of ethyl acetate and 150 mL of 1 N aqueous hydrochloric acid, and the organic phase was washed three times with 150-mL portions of water and once with 100 mL of brine and dried over $MgSO_4$. The solvent was removed by rotary evaporation at aspirator pressure. The crude product was purified by flash chromatography (eluted with 25:75 ethyl acetate/methylene chloride, loaded on column preadsorbed to silica) to give 1.55 g (3.88 mmol, 70%) of R″CA as a white solid: R_f 0.51 (90:10 methylene chloride/methanol); ^1H NMR (300 MHz, DMSO-d_6) δ 11.38 (s, 2 H), 7.35–7.15 (m, 15 H), 3.33 (m, 2 H), 2.28 (m, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.82, 148.80, 146.57, 128.67, 127.99, 126.08, 54.85, 38.43, 36.85; HRMS-FAB (M + H$^+$) calcd 400.1660, found 400.1670. Anal. Calcd for $C_{24}H_{21}N_3O_3$: C, 72.16; H, 5.30. Found: C, 72.56; H, 5.37.

N,N′-Bis(*tert*-butyloxycarbonyl)gramicidin S. Gramicidin S·2HCl (Sigma) (0.50 g, 0.41 mmol) and triethylamine (0.20 g, 0.28 mL, 2.0 mmol) were dissolved in 10 mL of dioxane and 10 mL of water. To the solution was added 2-[[(*tert*-butyloxycarbonyl)oxy]imino]-2-phenylacetonitrile (BOC-ON) (0.49 g, 2.0 mmol), and the mixture was stirred for 12 h at room temperature. The solvents were removed by rotary evaporation at aspirator pressure, and the residue was partitioned between 150 mL of ethyl acetate and 75 mL of water. The organic layer was washed twice with 75-mL portions of 1 N aqueous NaOH solution, twice with 75-mL portions of water, and 50 mL of brine 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 ethyl acetate), giving the product as a white solid: ^1H NMR (500 MHz, DMSO-d_6) δ 8.95 (s, 2 H), 8.57 (d, $J = 9.0$ Hz, 4 H), 8.38 (s, $J = 9.0$ Hz, 4 H), 7.25 (s, 16 H), 7.21 (m, 2 H), 6.91 (s, 2 H), 4.74 (m, 2 H), 4.58 (m, 2 H), 4.50 (d, $J = 7.6$ Hz, 2 H), 4.43 (m, 2 H), 4.34 (m, 2 H), 3.52 (br s, 2 H), 2.95 (m, 2 H), 2.89 (m, 10 H), 2.42 (m, 2 H), 2.00 (m, 4 H), 1.67 (m, 2 H), 1.48 (br s, 4 H), 1.38 (m, 18 H), 0.81 (m, 24 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.72, 170.74, 170.61, 170.33, 169.55, 155.35, 136.39, 129.36, 128.13, 126.89, 77.14, 59.61, 56.58, 53.79, 51.38, 49.55, 45.66, 41.10, 35.82, 31.36, 29.96, 29.01, 28.23,

25.26, 24.03, 23.02, 22.79, 22.49, 19.05, 17.95; HRMS-FAB (M + H⁺) calcd for $C_{70}H_{109}N_{12}O_{14}$ 1363.8010, found 1363.8000.

Titration of hubM₃ with R′CA or flexM₃ with R″CA Monitored by ¹H NMR Spectroscopy. An NMR tube was charged with hubM₃ (0.0196 g, 0.0094 mmol) or flexM₃ (0.0132 g, 0.0100 mmol) and CDCl₃ (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 hubM₃(R′CA)₃ and flexM₃(R″CA)₃ 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 CDCl₃, 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 (Ultrastyragel, 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 hubM₃ samples and 2.0 mM for the flexM₃ 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 hubM₃(R′CA)₃ and flexM₃(R″CA)₃ 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 gramacidin S derivative in which the ornithine amino groups had been converted to the tert-butyl carbamates (MW 1342) as molecular weight standards.

Titration of hubM₃ with R′CA and flexM₃ 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 hubM₃ (0.0209 g, 0.0100 mmol) or flexM₃ (0.0132 g, 0.0100 mmol) and 100 mL of CH₂Cl₂. 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.

Supplementary Material Available: Details of the synthesis of compounds other than hubM₃ and flexM₃ that are shown in Scheme II (9 pages). Ordering information is given on any current masthead page.

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¹³C Kinetic Isotope Effects in the Reactions of Grignard Reagents with Benzophenone

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Abstract: The rate-determining step for the reaction of benzophenone with Grignard reagents is an inner sphere electron transfer (ET) with a simultaneous transfer of magnesium to produce the magnesium benzophenone ketyl and the alkyl radical. For tertiary and secondary reagents the alkyl radical is free to escape from the cage, while for primary reagents and methyl partial bond formation between the radicals stabilizes the transition state and prevents escape. Substitution in the carbonyl group with isotopic carbon gives rise to a kinetic isotope effect (KIE), which for ¹⁴C has been reported to be near unity for the reaction of tert-butylmagnesium chloride. In the present investigation, the KIE when ¹³C was used was found to be high and of the same magnitude when either tert-butylmagnesium chloride or methylmagnesium bromide was used. The reported and confirmed very low KIE for allylmagnesium bromide is not evidence for, but rather evidence against, the formation of ketyl during the reaction.

Introduction

The reaction of benzophenone with Grignard reagents has been studied for several decades, and the list of probes and mechanistic tools which have been used is long.[1–5] The more recent publications on the subject,[6–8] however, leave the impression that the

Scheme I

A

B
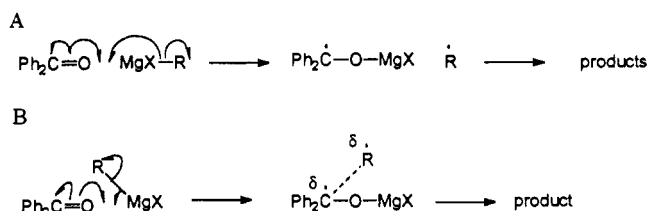
problem is totally unsettled and that results obtained more than 10–15 years ago are obsolete and should be revised. This author holds almost the opposite view, and the intention of the present article is to examine the logical and experimental foundation of

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