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Self-Association of Facially Amphiphilic Methylene Bridged Glycoluril Dimers

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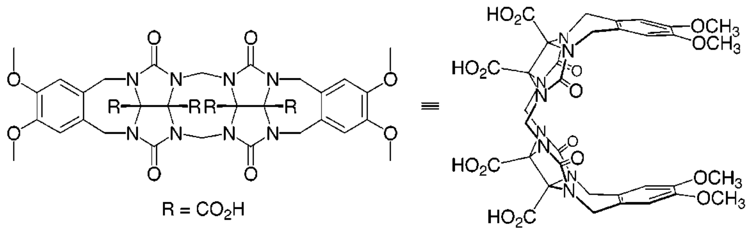
ABSTRACT



Facially amphiphilic derivatives of methylene bridged glycoluril dimers are a versatile model system for systematic studies of self-assembly in water. Thorough physical organic characterization, including analytical ultracentrifugation, a technique rarely used in synthetic self-assembly studies, allows us to conclude that this class of molecules undergoes hydrophobically driven self-association to yield tightly associated discrete dimeric assemblies.

The hydrophobic effect is widely regarded as a major driving force in a variety of molecular recognition processes including the folding of proteins into their native states, protein−protein interactions, and the formation of lipid bilayers.[1] The past decade has witnessed the increasingly sophisticated use of hydrogen bonds, $\pi-\pi$ interactions, and metal−ligand interactions to form highly structured aggregates.[2] Despite these advances, the use of the hydrophobic effect as a driving force in the self-association of nonnatural molecules into tightly associated aggregates that are well defined in terms of structure and degree of association remains a challenge.[3,4] The reason is simple: the hydrophobic effect typically involves the association in water between molecules with large apolar regions. These molecules do not typically have structural features that lend themselves to forming directional, specific intermolecular contacts that lead to stable structured aggregates in a predictable manner. We report that facially amphiphilic methylene bridged glycoluril dimers **1a** and **2a**

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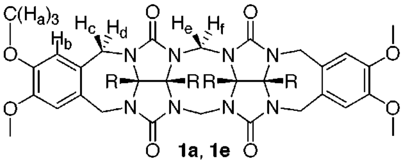
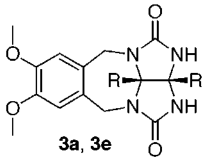
form tightly associated, discrete dimeric aggregates **1a•1a** and **2a•2a** in water by hydrophobically driven self-association.

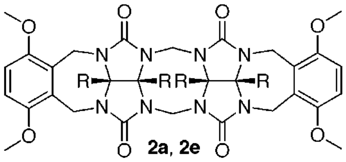
Nolte has pioneered the use of glycoluril-based molecular clips in molecular recognition, self-assembly, and catalysis.[3e,5,6] They have observed dimerization of molecular clips in the solid state,[5a−f] thin lamellar films,[5e,f] organic solvents,[5c,f,g] and in aqueous solution.[5h,i] We recently described a method for the synthesis of methylene bridged glycoluril dimers exemplified by **1e** and **2e**.[7] On the basis of the precedent of Nolte[5] we hypothesized that facially amphiphilic[8] compounds **1a** and **2a** that bear strictly hydrophilic carboxylate solubilizing groups on their convex face and whose concave face is defined by two roughly parallel aromatic rings might display interesting self-association behavior in water.[9] This model system combines four features that makes it well suited for systematic physical organic studies of hydrophobic self-assembly in water: (1) strong self-association, which is (2) hydrophobically driven, yielding (3) discrete dimers that (4) display slow exchange processes that can allow for structural elucidation of the aggregates.



1a, 1e



3a, 3e



2a, 2e

a: R = CO_2H
e: R = CO_2Et

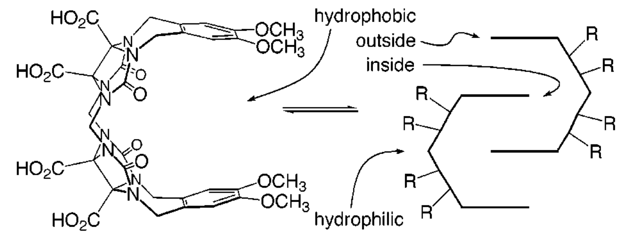


Figure 1. Three-dimensional shape of **1a** and a schematic illustration of **1a•1a** that highlights the reduction in symmetry that results upon dimerization.

Compounds **1a**−**3a** were prepared by the alkaline hydrolysis of the corresponding ethyl ester derivatives **1e**−**3e**.[7] We used isothermal titration calorimetry (ITC) dilution experiments[10] to measure association constants and thermodynamic parameters for the formation of **1a•1a** and **2a•2a**. Aggregates **1a•1a** and **2a•2a** are tightly associated at 298 K (100 mM sodium phosphate buffered D_2O, pD 7.4), and their formation is enthalpically and entropically driven (**1a•1a**, $K_d = 39\ \mu M$, $\Delta H = -3.5$ kcal mol^{-1}, $\Delta G = -6.0$ kcal mol^{-1}, $\Delta S = 8.5$ eu; **2a•2a**, $K_d = 24\ \mu M$, $\Delta H = -4.75$ kcal mol^{-1}, $\Delta G = -6.3$ kcal mol^{-1}, $\Delta S = 5.2$ eu).[11] In contrast to **1a** and **2a**, ^1H NMR dilution experiments indicate that **3a** (0.2−50 mM), which lacks a well-defined hydrophobic cleft, undergoes very weak self-association at room temperature ($K_a < 5\ M^{-1}$).[12] To unambiguously establish that the dimerization process was driven by the hydrophobic effect, we determined the change in heat capacity (ΔC_p) for the formation of **1a•1a** by performing ITC measurements from 288 to 328 K (Figure 2) and calculating the slope of a plot of ΔH versus T. The observed negative value of ΔC_p ($\Delta C_p = -185 \pm 6$ cal mol^{-1} K^{-1}) allows us to conclude that the formation of **1a•1a** is a hydrophobically driven event.[13]

In studies of self-association it can be challenging to unambiguously establish the degree of association. For

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(5) (a) Reek, J. N. H.; Rowan, A. E.; de Gelder, R.; Beurskens, P. T.; Crossley, M. J.; De Feyter, S.; de Schryver, F.; Nolte, R. J. M. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 361−363. (b) Jansen, R. J.; Rowan, A. E.; de Gelder, R.; Scheeren, H. W.; Nolte, R. J. M. *Chem. Commun.* **1998**, 121−122. (c) Reek, J. N. H.; Rowan, A. E.; Crossley, M. J.; Nolte, R. J. M. *J. Org. Chem.* **1999**, *64*, 6653−6663. (d) Jansen, R. J.; de Gelder, R.; Rowan, A. E.; Scheeren, H. W.; Nolte, R. J. M. *J. Org. Chem.* **2001**, *66*, 2643−2653. (e) Holder, S. J.; Elemans, J. A. A. W.; Barberá, J.; Rowan, A. E.; Nolte, R. J. M. *Chem. Commun.* **2000**, 355−356. (f) Holder, S. J.; Elemans, J. A. A. W.; Donners, J. J. J. M.; Boerakker, M. J.; de Gelder, R.; Barberá, J.; Rowan, A. E.; Nolte, R. J. M. *J. Org. Chem.* **2001**, *66*, 391−399. (g) Reek, J. N. H.; Priem, A. H.; Engelkamp, H.; Rowan, A. E.; Elemans, J. A. A. W.; Nolte, R. J. M. *J. Am. Chem. Soc.* **1997**, *119*, 9956−9964. (h) Reek, J. N. H.; Kros, A.; Nolte, R. J. M. *Chem. Commun.* **1996**, 245−247. (i) Elemans, J. A. A. W.; de Gelder, R.; Rowen, A. E.; Nolte, R. J. M. *Chem. Commun.* **1998**, 1553−1554.

(6) Compounds **1a** and **2a** are members of the class of molecules known as molecular clips and molecular tweezers. We refer to them as facial amphiphiles since this terminology fully describes their topological features and provides a rationale for the observed self-association behavior. For examples of molecular tweezers, see: (a) Chen, C.-W.; Whitlock, H. W. *J. Am. Chem. Soc.* **1978**, *100*, 4921−4922. (b) Zimmerman, S. C.; VanZyl, C. M. *J. Am. Chem. Soc.* **1987**, *109*, 7894−7896. (c) Zimmerman, S. C. *Top. Curr. Chem.* **1993**, *165*, 71−102. (d) Harmata, M.; Barnes, C. L.; Karra, S. R.; Elahmad, S. *J. Am. Chem. Soc.* **1994**, *116*, 8392−8393. (e) Maitra, U.; Potluri, V. K. *J. Org. Chem.* **2000**, *65*, 7764−7769. (f) Brown, S. P.; Schaller, T.; Seelbach, U. P.; Koziol, F.; Ochsenfeld, C.; Klärner, F.-G.; Spiess, H. W. *Angew. Chem., Int. Ed.* **2001**, *40*, 717−720. (g) Pardo, C.; Sesmilo, E.; Gutiérrez-Puebla, E.; Monge, A.; Elguero, J.; Fruchier, A. *J. Org. Chem.* **2001**, *66*, 1607−1611.

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(9) In the crystal structure of a related C-shaped molecule[7] the distance between the tips of the aromatic rings is 7.04 Å and the angle between the aromatic rings is 18°.

(10) Burrows, S. D.; Doyle, M. L.; Murphy, K. P.; Franklin, S. G.; White, J. R.; Brooks, I.; McNulty, D. E.; Scott, M. O.; Knutson, J. R.; Porter, D.; Young, P. R.; Hensley, P. *Biochemistry* **1994**, *33*, 12741−12745.

(11) We estimate the error in the ITC derived values of ΔH and K_d to be 0.2 kcal mol^{-1} and 10%, respectively.

(12) We would have preferred to use the S-shaped diastereomer[7] of **1a** for this control experiment but it is not soluble in aqueous solution.

(13) (a) Sturtevant, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 2236−2240. (b) Murphy, K. P.; Privalov, P. L.; Gill, S. J. *Science* **1990**, *247*, 559−561. (c) Spolar, R. S.; Ha, J.-H.; Record, M. T. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 8382−8385.

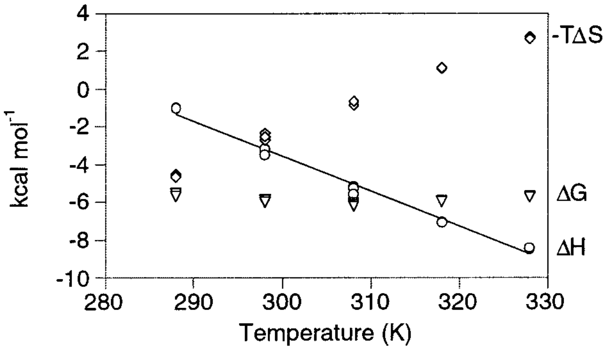

Figure 2. A plot of the ITC derived thermodynamic parameters for the formation of **1a•1a** versus temperature. ΔH (○), ΔG (▽), $-T\Delta S$ (◇).

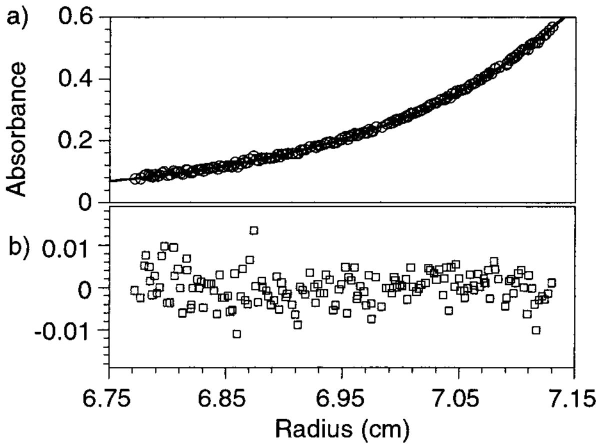

Figure 3. (a) A plot of absorbance (○) versus radius obtained for **1a** at sedimentation equilibrium. The best fit of the data to a model comprising a single homogeneous species is overlaid (—). Conditions: [**1a**] = 400 μM; 55,000 rpm; λ = 259 nm. (b) A plot of the residuals (□) versus radius for the data shown in part a.

example, ^{1}H NMR dilution experiments performed with **1a** at 55 °C in water fit well to both 2-fold and 3-fold self-association equilibrium models. To differentiate between these possibilities and provide strong evidence that **1a** undergoes controlled self-association to yield a discrete dimeric assembly in water, we turned to analytical ultracentrifugation (AUC). Sedimentation equilibrium measurements allowed a determination of the molecular weight of **1a•1a** (Figure 3) under solution-based equilibrium conditions.[14] The data fit well to a model comprising a single species with a molecular weight of 1915 ± 115 (calcd MW (**1a•1a**) = 1616) with random residuals.[15] We also performed the more standard vapor pressure osmometry (VPO), gel permeation chromatography (GPC), and electrospray mass spectrometry (ES-MS) measurements. The molality of solutions of **1a** and **2a** were about half of the prepared concentrations, suggesting that **1a** and **2a** exist exclusively as the dimers **1a•1a** and **2a•2a**. In contrast, VPO showed that **3a**, which lacks a hydrophobic cleft, is monomeric in water. The ES-MS spectrum of **2a** showed the presence of [**2a•2a** − H]$^-$ and [**2a** − H]$^-$; peaks corresponding to singly or multiply charged ions of higher order aggregates were not observed. GPC measurements performed with **1a•1a** and **2a•2a** (4.1 mM) gave estimated molecular weights of 1605 and 1465, respectively. Chromatograms recorded at lower concentrations (41 μM) showed longer migration times and significant peak tailing that indicated that **1a•1a** and **2a•2a** are in equilibrium with **1a** and **2a** on the time scale of the GPC measurement. The combined inference of these four techniques provides a validation of our design hypothesis and indicates that **1a** and **2a** undergo discrete self-association processes to yield dimers.

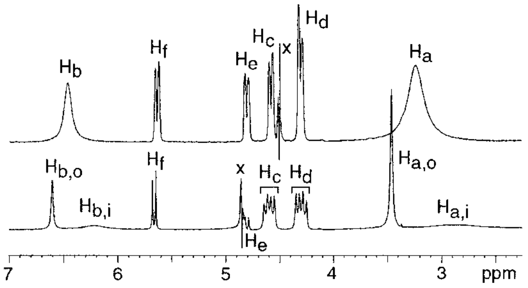

Figure 4. ^{1}H NMR spectrum of **1a** (500 MHz, 5 mM, D_2O buffer) at 324 K (top) and 294 K (bottom). The resonance marked (×) is due to incomplete suppression of residual HOD.

Hydrogen bonds and metal−ligand interactions are extremely useful in self-assembly studies because they are strong and have well-defined directional preferences. Our goal is to determine similar directional preferences governing the self-association of **1a** and derivatives. Figure 4 shows the ^{1}H NMR spectrum obtained for **1a•1a** at 324 and 294 K. As the temperature is decreased from 324 to 294 K, the time-averaged C_{2v} symmetry observed at higher temperature is reduced and two resonances are observed for the methoxy (H_a) and aromatic protons (H_b). We suggest that coalescence at higher temperatures results from an exchange process between protons on the inside of the aggregate ($H_{a,i}$ and $H_{b,i}$), which are upfield shifted as a result of the anisotropic effect of two aromatic rings, with those on the outside ($H_{a,o}$ and $H_{b,o}$). Although we are currently unable to determine the precise structural details of **1a•1a** because of exchange processes that are in the intermediate exchange regime on the NMR time scale, we believe that structural modifications

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[15] We determined the density of the buffer (100 mM phosphate buffered D_2O, pD = 7.4, ρ = 1.1155 g mL^{-1}) and the partial specific volume for **1a** ($\bar{v}$ = 0.6137 mL g^{-1}) by making high precision density measurements on solutions of **1a** ([**1a**] = 0−10 mg mL^{-1}).

will slow the exchange processes and allow structural determinations of related dimeric aggregates.

We have presented facially amphiphilic derivatives of methylene bridged glycoluril dimers as a model system to study self-assembly in aqueous solution. This model system combines several advantageous features, namely, hydrophobically driven self-association to form tightly associated, discrete dimeric assemblies in water and sufficiently slow dynamic exchange processes that should allow for structural elucidation. We are currently studying facial amphiphiles bearing different functional groups and substitution patterns on their aromatic rings with the goal of determining the structural details and thermodynamic parameters for a series of related dimeric aggregates. By identifying trends in the structural and thermodynamic properties of this series of aggregates we plan to deduce some of the rules governing their self-association, which hopefully will be broadly applicable to other hydrophobically driven self-assembly processes.

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Supporting Information Available: Experimental procedures and spectral data for **1a**−**3a** and representative data from the VPO, ES-MS, GPC, ITC, ^{1}H NMR, and AUC experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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