JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Article

pubs.acs.org/JACS

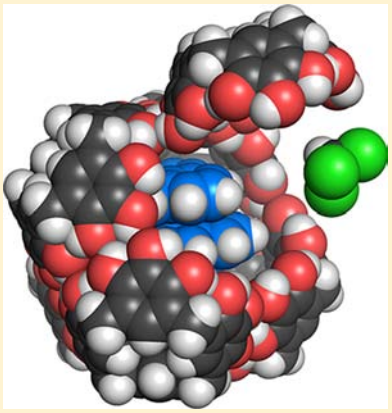
Molecular Encapsulation in Pyrogallolarene Hexamers Under Nonequilibrium Conditions

Jennifer C. Chapin,[†] Miroslav Kvasnica,[‡] and Byron W. Purse*[,†]

[†]Department of Chemistry and Biochemistry, University of Denver, 2199 S. University Blvd., Denver, Colorado 80208, United States
[‡]IOCB AS CR, Flemingovo nam. 2, 166 10 Praha 6, Czech Republic

Supporting Information

ABSTRACT: Pyrogallol[4]arene is a macrocycle with a concave surface and 12 peripheral hydroxyl groups that mediate its self-assembly to form hexamers of octahedral symmetry in the solid state, in solution, and in the gas phase. These hexamers enclose approximately 1300 Å^3 of space, which is filled with small molecules. In this study, we show that solvent-free conditions for guest entrapment in these hexamers, using molten guest molecules as solvent and allowing the capsules to assemble during cooling, results in exceptionally kinetically stable encapsulation complexes that are not formed in the presence of solvent and are not thermodynamically stable. The capsules' kinetic stabilities are strongly dependent on the size and shape of both guest and solvent molecules, with larger or nonplanar molecules with rigid geometries providing enhanced stability. The greatest observed barrier to guest exchange, $\Delta G^{\ddagger} = 32 \pm 0.7$ kcal mol^{-1} for encapsulated CCl_4 → encapsulated pyrene, is, to the best of our knowledge, indicative of the most powerful kinetic trap ever observed for a synthetic, hydrogen-bonded encapsulation complex. Detailed NMR studies of the structures of the assemblies and the kinetics and mechanisms for guest exchange reveal that subtle differences in guest and solvent structure can impart profound effects on the behavior of the systems. Kinetic and thermodynamic stability, capsule symmetry and structure, guest tumbling rates, susceptibility to disruption by polar solvents, and even the mechanism for equilibration—the presence or absence of supramolecular intermediates—are all greatly influenced. The strongest observed kinetic traps provide encapsulation complexes that are not at equilibrium but are nonetheless indefinitely persistent at ambient temperatures, a property that invites future applications of supramolecular chemistry in open systems where equilibrium is not possible.



INTRODUCTION

Supramolecular chemistry is founded on the synthesis of complex but well-defined assemblies of molecules, largely under conditions of thermodynamic control, programmed by the structural features of the building blocks.[1,2] The vast majority of reported supramolecular assemblies have short lifetimes once they are removed from the equilibrium conditions of their formation, which provides useful, intrinsic environmental responsiveness, but also limits applications. Accordingly, profound new opportunities for supramolecular chemistry exist when complex assemblies can be trapped in a thermodynamic local minimum after synthesis using carefully chosen conditions.[3−7] Such assemblies can then be used for applications in environments that do not provide the kinetic energy needed for disruption, but nonetheless thermodynamically favor disassembly. Particularly attractive are the prospects for applications of supramolecular assemblies in nonequilibrium systems, such as those with continual flow or in living organisms.[8] We now report on the properties of a molecular encapsulation system using hydrogen-bonded pyrogallol[4]arene hexamers that provides sufficiently powerful kinetic traps so as to invite these types of future applications.

Pyrogallol[4]arene (e.g., Figure 1) (and the related resorcin[4]arene)[9−21] hexamers have been studied in great detail since they were first observed in the solid state and later found to predominate when dissolved in non-hydrogen bonding solvents.[13,15,20,22−30] Both form roughly spherical assemblies encapsulating some 1300 Å^3 of space, which is filled with guest molecules that may be solvent.[29] The interiors tend to be disordered in crystal structures, although exceptions have been claimed,[25,27,31] and encapsulated molecules are clearly observable in solution using ^{1}H NMR spectroscopy, with guest signals typically shifted 2−3 ppm upfield.[10,16,26,30,32] Exchange of capsule occupants is slow on the NMR time scale,[32] and the exchange of pyrogallolarenes between capsules has been measured using diffusion NMR and fluorescent labels, showing half-times on the order of hours under ambient conditions.[20] The hexamers are also known to persist in the gas phase,[17] where the equilibrium population often includes a significant proportion of the smaller pyrogallolarene dimers.[33] Applications of these assemblies include gas sequestration and the control of catalytic activity.[34,35] Related pyrogallol[4]arene and

Received: June 4, 2012
Published: August 31, 2012

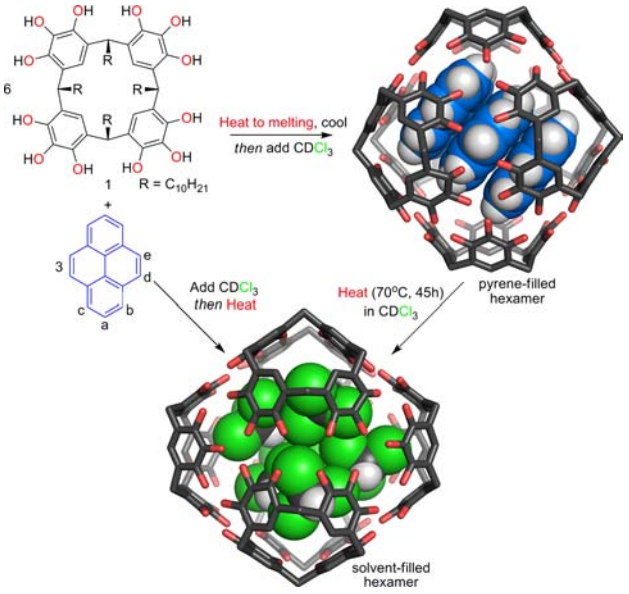
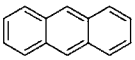
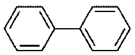
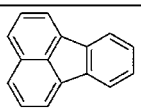
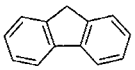
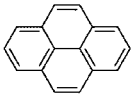


Figure 1. When heated to melting and then cooled, six pyrogallol[4]-arene molecules (**1**) and three pyrene molecules assemble to form a pyrene-filled capsule. When the components are first dissolved in solvent and then heated, only solvent-filled capsules are seen. Further heating of solutions of the pyrene-filled capsules is required to reach equilibrium, yielding the solvent-filled capsule. R groups were truncated in molecular models for clarity.

resorcin[4]arene capsules of exceptional stability have been prepared by the addition of metals, which coordinate to the pyrogallolarene hydroxyl groups, replacing the hydrogen bonds that stabilize these otherwise purely organic assemblies.[36] Similar metal-seemed pyrogallolarene dimers have also appeared in polymers.[37]

Our interest in these capsules began with a search for the optimal conditions for efficiently loading the capsules' interiors with the broadest possible range of guest molecules. Prior NMR studies of guest binding in solution had focused almost exclusively on guests that are liquids under ambient conditions—solvents and a few tertiary amines—with the one exception of an encapsulated solid being cobaltocenium.[16,24,26,30] Fluorescence measurements have been used as evidence for the encapsulation of large polycyclic aromatic hydrocarbons (PAHs) in solution, up to and including pentacene, but the reported NMR spectra for these samples show the PAHs present at concentrations below 10 mol % and with chemical shifts identical to those of the nonencapsulated species.[25,27,31,38] The percent capsule occupancy by the PAHs in these solutions must therefore be low. We hypothesized that competition from solvent molecules was limiting the efficiency of capsule loading, and devised a solvent-free protocol whereby pyrogallolarene and a prospective guest molecule were melted at temperatures near 200 °C, allowed to cool, dissolved in NMR solvents, and characterized. The result of this procedure was the observation of near-complete loading of the capsules with a variety of hydrocarbon guests, giving kinetically trapped complexes that are not formed when a solvent is present.[32] We now report on a detailed study of these kinetically trapped complexes under nonequilibrium conditions, assessing the nuances of their structures and the effects of solvent, guest, and added polar species on the kinetics and thermodynamics of guest exchange. The results show exceptionally large influences on kinetics and thermodynamics by relatively small differences in guest and solvent structure, and reveal what is, to the best of our knowledge, by far the highest level of kinetic stability yet

Table 1. Summary of Guest→Solvent Exchange Process and Symmetry Assignments[a]

Entry	Guest	Structure	Molecules Encapsulated	Calculated VdW Volume /Å^3	Guest→Solvent Exchange Process	Symmetry Point Group	Packing Coefficient[a]
1	Anthracene		3	188	Multiple Intermediates[b]	*O*	0.43
2	Biphenyl		4[c]	173	Multiple Intermediates	*O*	0.53
3	Fluoranthene		3	202	Direct Replacement	*O*	0.47
4	Fluorene		4	180	Single Intermediate	*O*	0.55
5	Naphthalene		6[c]	145	Multiple Intermediates	*O*	0.67
6	Norbornene		4	115	Direct Replacement	*O*	0.35
7	Pyrene		3	198	Single Intermediate[e]	$O_h \rightarrow D_3$	0.46

[a]Molecular models for all tabulated encapsulation complexes are shown in the Supporting Information, Figures S10–S16 [b]Calculated using a capsular cavity volume of 1300 Å^3. [c]Seen only in chloroform, direct replacement seen in carbon tetrachloride. [d]Estimated. [e]Rearrangement of capsule without release of guests; Seen only in chlorinated solvents and benzene, direct replacement seen in other solvents tested.

observed for any synthetic, hydrogen-bonded molecular capsule in solution.[3−7]

RESULTS

Capsule Assembly, Guest Loading, and Kinetic Trapping. Molecular encapsulation in pyrogallol[4]arene (**1**) hexamers was investigated using self-assembly under solvent-free conditions, as previously reported by our lab (Figure 1).[32] Briefly, a 1:1 mixture (w/w) of pyrogallolarene and a prospective guest molecule was heated to the melting point of the guest using a heat gun, resulting in a molten mixture, which was immediately allowed to cool. During the cooling step, the pyrogallolarene molecules self-assemble to give hexamers, entrapping the guest. If the prospective guest is not suitable for occupying the cavity (e.g., it is too large), then the pyrogallolarene molecules are presumed to solidify as disordered aggregates. After cooling, the solidified mixtures were dissolved in nonpolar solvents and characterized by ^{1}H NMR spectroscopy. An alternative to this procedure is also available for situations in which the melting point of the guest may be prohibitively high. Paraffin wax may be used as a "solvent" that can be melted and cooled in combination with pyrogallolarene and the guest; the wax molecules are too large to fill the cavity and so do not compete with the desired guests during assembly.

Using the first method (i.e., without paraffin wax), a variety of nonpolar guest molecules were found to be very suitable for encapsulation, some (those in Table 1) yielding near complete occupancy of all capsule molecules in solution (Tables 1 and S1, Supporting Information). Packing coefficients suggest that the number of guest molecules encapsulated within each hexamer is typical for self-assembled capsules in solution, near to an average optimal space filling of 55%.[39,40] When these experiments were repeated without melting the mixtures, instead by simply dissolving the pyrogallolarene and guest in solvent, no encapsulation of the guests was seen in any experiment—instead solvent-filled pyrogallolarene hexamers were observed (Figure 1). The guest-filled capsules, assembled by melting and cooling, are kinetically trapped species that exist only prior to the establishment of equilibrium, at which point the solvent-filled assemblies predominate. Moreover, the stability of the kinetic traps, the rates of guest release, and the observation of "supramolecular intermediates" on the pathways to equilibrium depend strongly on the nature of the guest and solvent, as described below.

Structure and Symmetry. ^{1}H NMR measurements of the guest-filled assemblies revealed that they exist in one of three apparent symmetries at 296 K (Table 1). The predominant case is hexamers that are members of the point group *O*, characterized by a chiral arrangement of hydrogen-bonded hydroxyl groups with slow exchange on the NMR time scale.[26] NMR spectroscopy of these assemblies shows four peaks in the region from 6.3−9.5 ppm belonging to the pyrogallolarene hexamer; one corresponding to the aryl hydrogen and three which represent the hydroxyl hydrogens (Figure S1, Supporting Information). The chiral nature of the assembly differentiates the two hydroxyl groups *ortho* to the bridging methines of each benzene ring of pyrogallolarene (Figure 2a and S3). Similarly, all six carbons of each benzene ring are differentiated by this chirality in the ^{13}C NMR spectrum (Figure S2). For hexamers of *O* symmetry, guest tumbling inside the capsule is rapid on the NMR time scale. All solvent-filled capsules (DCM, $CDCl_3$, CCl_4, C_6D_6, toluene-d_8, xylenes, and mesitylene) were observed to have *O* symmetry.

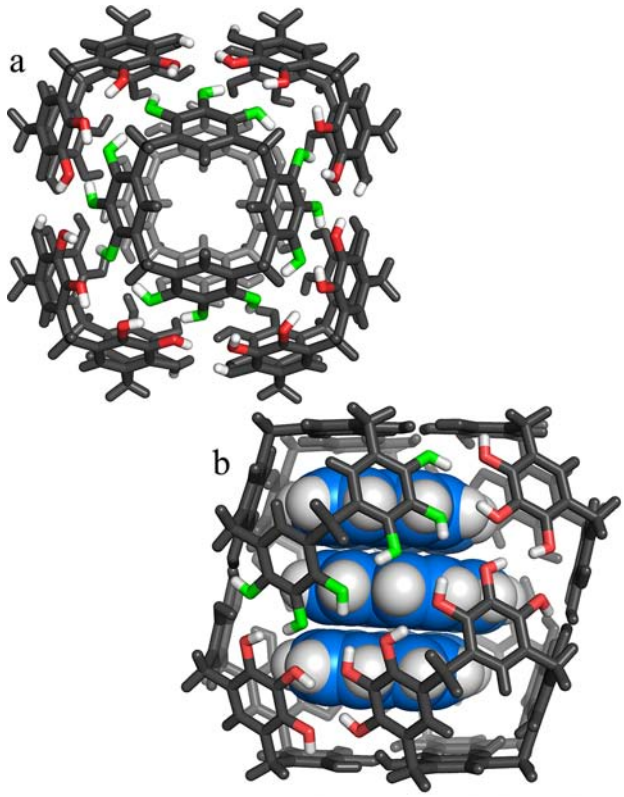


Figure 2. (a) Molecular model of the pyrogallolarene hexamer illustrating the chiral arrangement of the hydroxyls, giving three unique hydroxyls by symmetry. (b) Molecular model of the D_3-symmetric capsule containing pyrene. Both the chirality of the hexamer and the slow tumbling of guest pyrenes contribute to the symmetry.

A special exception to the typical, apparent *O* symmetry was observed when three molecules of pyrene were encapsulated within the hexamer. The initially formed complex following melting and cooling has a disordered seam of hydrogen bonds with rapid shuffling and three pyrene guests, which also tumble rapidly. The ^{1}H NMR signals for the hydroxyl hydrogens are broadened along the baseline (spanning 6.5−9.5 ppm and proven by an H−D exchange experiment)[32] and the signals for the encapsulated guest molecules show moderate broadening (Figure 3). This complex has achiral octahedral symmetry, and is a member of the point group O_h.

Over the course of six hours at 296 K in $CDCl_3$, the complex reorganizes following first-order kinetics and without dissociation[41] to form a second configuration of reduced symmetry. NMR signal integration and DOSY measurements[32] show the complexes to have the same stoichiometry and diffusion rate: they are both hexamers encapsulating three pyrenes. In this second configuration, splitting of the ^{1}H NMR signals of both host and guest indicate a loss of symmetry, corresponding to the point group D_3 (Figures 2b and 3). Three pyrene guest signals (a triplet at 5.67 ppm and two doublets at 5.75 and 5.83 ppm, correspond to hydrogens at positions **a**, **b**, and **c**, Figure 1; their coupling is confirmed by COSY; Figure S5, Supporting Information) show that hydrogens at **b** and **c** are diastereotopic, indicating the chiral nature of the assembly and the slow guest tumbling. The remaining pyrene guest signals at 6.2 ppm correspond to hydrogens at positions **d** and **e**. The complexity

dx.doi.org/10.1021/ja305252t | J. Am. Chem. Soc. 2012, 134, 15000−15009

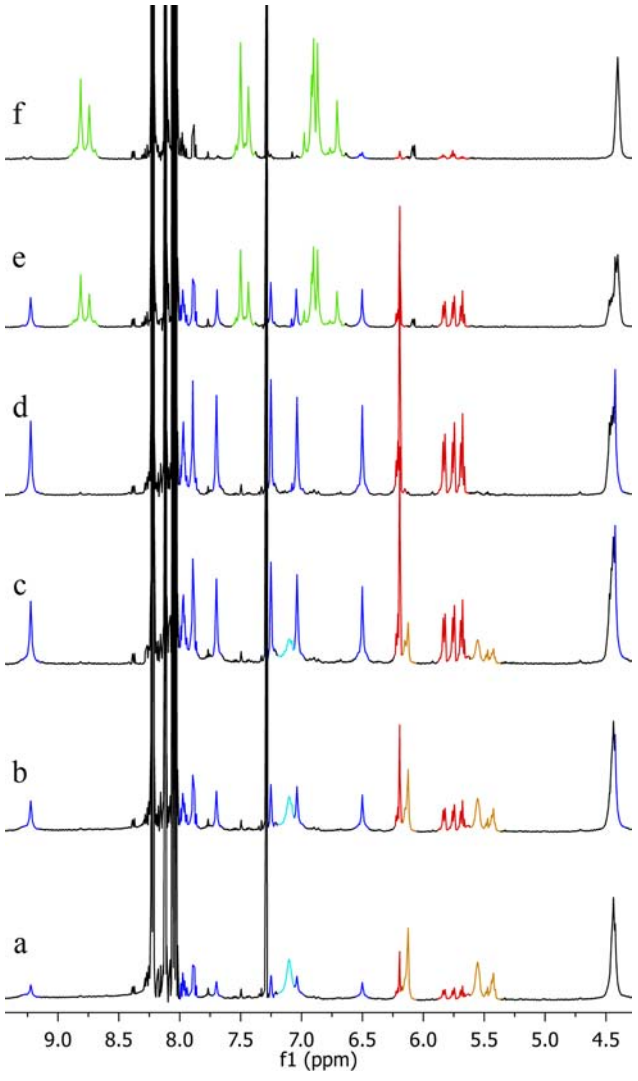


Figure 3. ^{1}H NMR spectra tracking the change in symmetry of the pyrene-filled capsule in $CDCl_3$. O_h configuration guest peaks are shown in yellow and aryl hydrogen peaks shown in cyan. Hydroxyl hydrogen peaks are broadened along the baseline. D_3 configuration guest peaks are shown in red, aryl and hydroxyl peaks are shown in blue. Solvent-filled hexamer peaks are shown in green; (a) 20 min, (b) 40 min, (c) 1.5 h, (d) 5.5 h, all at 296 K. (e) Ten hours at 343 K, (f) 43 h at 343 K.

of this part of spectrum is likely due to the stacked arrangement of the three pyrenes, which differentiates the sandwiched pyrene, and it appears that the diastereotopicity of **d** and **e** is not manifest in all three. The hydrogen bonds of the capsule have the same chiral arrangement as described for the *O*-symmetric capsules and exchange slowly on the NMR time scale. The guest pyrene molecules spin rapidly on an axis perpendicular to their planes, but do not readily tumble in the capsule or swap positions in their stacked arrangement. The appearance of the ^{1}H NMR for this complex is almost identical in CD_2Cl_2 and is similar but with a greater number of pyrogallolarene signals in CCl_4 (Figure S6). The difference in number of signals is likely due to increased signal overlap in $CDCl_3$ and CD_2Cl_2, which may result from small structural differences attributable to changes in capsule solvation. In all three solvents, this highly complementary host–guest pair shows slow exchange of guest positions within the capsule and maximizes the intercommunication between host and guest: each reduces the apparent symmetry of the other.

Kinetics Studies. The guest-filled capsules are kinetically trapped species that exist, under the reported conditions, only prior to the establishment of equilibrium, at which point the solvent-filled assemblies predominate. We used periodic NMR measurements to monitor directly the kinetics of equilibration, including both the formation and disappearance of intermediates and the establishment of solvent-filled capsules at equilibrium. (Tables 2 and 3 and NMR stacked plots in the Supporting Information).

For fluoranthene and norbornene (entries 3 and 6, Table 1), the NMR peaks for the initially encapsulated guest molecules simply decreased over time indicating direct replacement by solvent with the absence of significant populations of capsules with mixed occupancy. The same direct replacement was seen with pyrene in larger aromatic solvents and with anthracene in CCl_4 (Table 1). In all other cases, NMR signals corresponding to intermediates—capsules with mixed occupants or a different hydrogen bonding configuration or both—were observed during the course of the guest→solvent exchange. For fluorene and pyrene (entries 4 and 7, Table 1) a single, predominant intermediate state was observed and was almost completely populated as the initial state was depopulated. Guest release from these intermediates eventually led to the presence of only solvent-filled capsules. For anthracene, biphenyl, and naphthalene (entries 1, 2, and 5, Table 1); however, the relatively large number of intermediate capsular species made it prohibitively difficult to definitively assign the NMR spectra

Table 2. Guest-Dependent Hexamer Stabilities

	chloroform guest→solvent exchange				carbon tetrachloride guest→solvent exchange			
guest	$t_{1/2}$/h	total time[a]/h	$\Delta G^{\ddagger}$/kcal mol^{-1}	temperature/K	$t_{1/2}$/h	total time[a]/h	$\Delta G^{\ddagger}$/kcal mol^{-1}	temperature/K
Anthracene	nd	50		296	140	300	25.3 ± 0.3	296
Biphenyl	nd	20		296	nd	100		296
Fluoranthene	55	150	24.7 ± 0.3	296	10	50	27.6 ± 0.3	323
Fluorene[b]	10	60	23.7 ± 0.3	296	1010	1400	26.5 ± 0.6	296
Naphthalene	nd	8		296	nd	200		296
Norbornene	17	80	24.1 ± 0.4	296	850	1500	26.3 ± 0.4	296
Pyrene[c]	8.1	45	27.5 ± 0.2	343	1.0	N/A[d]	30 ± 0.7	393

[a]Time needed for 95% completion. [b]Kinetics for solvent exchange from the second configuration. The first configuration has a half-life of 0.5 h and activation energy of 21.9 ± 0.6 kcal/mol in $CDCl_3$ and 150 h and 25.4 ± 0.4 kcal/mol in CCl_4. [c]Kinetics for solvent exchange from D_3 configuration. [d]Solvent exchange was not complete, rather an equilibrium was established after heating for 10 h at 393K. Using KinTekSim the activation energy for the reverse process of solvent→pyrene exchange was determined to be 32 ± 0.7 kcal/mol. nd = not determined due to multiple intermediate states.

dx.doi.org/10.1021/ja305252t | J. Am. Chem. Soc. 2012, 134, 15000–15009

Table 3. Effects of Solvent on the Stability of the Pyrene-Filled Hexamer

	configuration change				guest→solvent exchange			
solvent	$t_{1/2}$/h	total time[a] /h	$\Delta G^{\ddagger}$/kcal mol^{-1}	temperature/K	$t_{1/2}$/h	total time[a]/h	$\Delta G^{\ddagger}$/kcal mol^{-1}	temperature/K
CD_2Cl_2	nd	0.3		296	2.2	9	25.0 ± 0.3	323
$CDCl_3$	1.3	6	22.5 ± 0.2	296	8.1	45	27.5 ± 0.2	343
CCl_4	2.0	8	26.5 ± 0.4	343	1.0	N/A	30[b] ± 0.7	393
C_6D_6	8	30	21.2[b] ± 0.4	296	16	80	21.6[b] ± 0.4	296
Toluene-d_8	N/A				23	80	24.2 ± 0.3	296
Xylenes[c,d]	N/A				38	140	24.5 ± 0.4	296
Mesitylene[c]	N/A				16	30	26 ± 0.5	323

[a]Time needed for 95% completion. [b]KinTek Sim was used to determine rate constants (see Supporting Information). [c]Proteo solvent used. [d]Mixture of xylenes, 1:1:1 ortho, meta, para was used. nd = not determined due to fast exchange. N/A = Configuration change or solvent exchange did not occur or was not complete.

sufficiently well so as to determine the number of distinct capsular species present (Tables 1 and 2).

All of the observed guest release processes and configuration changes follow kinetics that are first-order in capsule (Equations S1 and S2, Supporting Information). In most cases, the NMR peaks corresponding to the guest-filled capsule were integrated, normalized, and then used in nonlinear curve fitting by regression to determine the rate constant, k, from which the half-life, $t_{1/2}$, was calculated (Equation S3). The activation energy needed for guest release to occur, $\Delta G^{\ddagger}$, was determined using the Eyring-Polanyi equation (Equation S4).

Results of these calculations are tabulated (Tables 2 and 3). Exceptions to this kinetic analysis are as follows. Kinetics calculations for the configuration change of the pyrene complex in C_6D_6 were performed using the KinTekSim software package,[42] which performs regression-based curve fitting for complex rate laws that cannot be integrated analytically. The concentration of each distinct capsular species was tracked using ^{1}H NMR, and a pyrene-filled (O_h)→pyrene-filled (D_3)→solvent-filled pathway proved to be an adequate but imperfect model for the guest exchange mechanism. The imperfection of the model is indicated by the nonideal fit (Figure S7, Supporting Information), but reasonable approximations for the first-order rate constants for each step could be calculated (Table 3). The fluorene complex is kinetically analogous to that of the pyrene complex in C_6D_6 as there is a single intermediate state that is populated prior to guest→solvent exchange, yet neither state is stable at room temperature. As a result the kinetics calculations for the fluorene complexes were fit to the same model using KinTekSim. Last, because pyrene is not completely exchanged for solvent in CCl_4 (10% pyrene-filled capsule persists at equilibrium), KinTekSim was used with regression analysis to simultaneously fit the rate constants for the forward and reverse guest exchange processes and, implicitly, the equilibrium position (see Supporting Information).

Choosing norbornene as a test case (because of its direct substitution by solvent), we measured $\Delta G^{\ddagger}$ for guest exchange in CCl_4 at 296, 310, 323, 343, and 363 K. Eyring analysis using a plot of ln(k/T) vs 1/T (Equation S5, Figure S8, Supporting Information) gave $\Delta H^{\ddagger}$ = 25.5 ± 1.5 kcal mol^{-1}, but we were unable to obtain sufficiently precise data to determine whether $\Delta S^{\ddagger}$ is positive or negative ($\Delta S^{\ddagger}$ = −2.9 ± 4.5 cal mol^{-1} K^{-1}). Clearly, however, $\Delta S^{\ddagger}$ is small and the vast majority of $\Delta G^{\ddagger}$ is of enthalpic origin (c.f. Table 2).

Because of the pyrene-filled hexamer's high level of stability, we chose it as a representative case for an investigation of solvent effects on both the configuration change and the guest→solvent exchange processes (measured as half-life and activation energy), examining a set of chlorinated and aromatic solvents (Table 3). For those solvents that were not deuterated, the experiments were performed using coaxial NMR tubes with an external solvent lock on C_6D_6. The high dynamic range of the instrument allowed for reproducible quantification of encapsulation complexes in spite of the presence of intense solvent signals. The initial structure of the pyrene complex in all solvents tested is that with a disordered network of hydrogen bonds, belonging to the point group O_h. When a configuration change is observed, the symmetry always decreases to D_3 (Table 3).

Last, we examined the effects of polar solvent additives on the rate of configuration change and on the stability of the pyrene-filled capsules. These tests were conducted using CCl_4 as solvent because the configuration change from O_h→D_3 is slow at room temperature. Both methanol and ethyl acetate were tested as polar additives in separate experiments. First, it was found that the addition of a large amount of CD_3OD (e.g., 50% v/v) completely disrupts the assembly, resulting in a ^{1}H NMR spectrum that shows only free pyrogallolarene, the unencapsulated guest, and solvent peaks. In a second experiment, we titrated in CH_3OH and recorded ^{1}H NMR spectra following each addition (Figure 4). With only 1% methanol added to the solution the encapsulated guest peaks of the initial configuration nearly disappeared, and were replaced by a new set of broad signals. At 5% methanol there were distinct ^{1}H signals corresponding to the second, D_3 configuration of pyrene within the hexamer, although these signals were not as intense as those for the initial, O_h configuration. The D_3 configuration was persistent up to 20% methanol addition, at which point there was no further evidence for the presence of assembled hexamer.

An ethyl acetate titration showed a different result, with the guest-filled capsule more resistant to ethyl acetate addition than it had been to methanol (Figure S9, Supporting Information). The initial, O_h configuration capsule peaks were clearly seen with up to 10% addition of ethyl acetate, and signals corresponding to the second, D_3 configuration were not observed. New signals seen in the 5.4−6.5 ppm window are seen, likely attributable to mixed encapsulation complexes involving the partial substitution of solvent for encapsulated pyrene. At 40% ethyl acetate addition, the capsules were completely disrupted.

DISCUSSION

The melting−cooling cycle for pyrogallolarene capsule assembly described in this work has resulted in the efficient

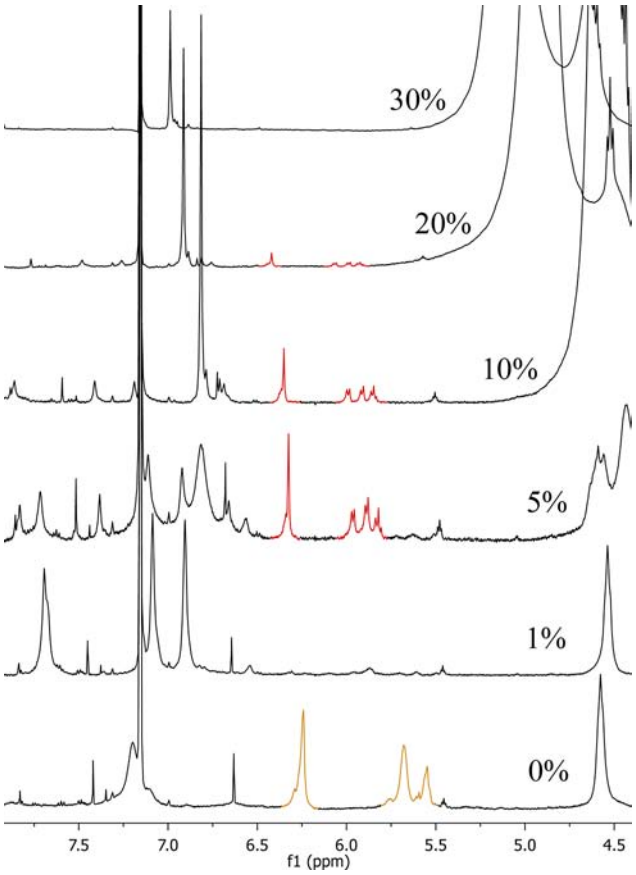


Figure 4. Titration of methanol into a solution of the pyrene-filled capsule in CCl_4. Initial encapsulated guest peaks for O_h configuration (yellow) disappear after 1% methanol addition, leaving broad signals indicative of disordered assembly. Further addition of methanol yields encapsulated guest peaks corresponding to the D_3 configuration (red), which persists with up to 20% methanol addition.

loading of hydrocarbon guests to produce host−guest complexes of exceptional kinetic stability. Under the conditions studied, these capsules will eventually (sometimes only with heating) exchange their occupants for solvent molecules in processes that do not involve complete dissociation of the capsule. We have observed that the nature of the guest and solvent produce large effects not only on kinetic stability, but also on the thermodynamic preference for guest or solvent binding, the mechanistic pathways to equilibrium, and on the apparent structures and symmetries of the observed assemblies.

Guest Effects. Our studies of the influence of the guests on hexamer properties have revealed trends linking guest size and shape to kinetic stability and the dynamics of guest movement within the capsules. Other observations such as the population of intermediate states during the guest→solvent exchange are determined by the nature of the guest, but general trends are not readily apparent.

In general, guest molecules that are larger in size or have rigid nonplanar geometries result in more kinetically stable capsules (Table 2). Pyrene encapsulation results in by far the most long-lived assemblies under these nonequilibrium conditions in all solvents tested, and fluoranthene, its structural isomer, provides the second-most kinetically stable complexes. Shape effects, in addition to size, are clearly important. Pyrene and fluoranthene are both flat aromatics with VdW volumes of 198 and 202 $Å^3$ respectively, the main difference between them is geometry. Pyrene has the widest part of the molecule in the middle, which is a close shape-match to the interior of the hexamer, while fluoranthene has the widest part of the molecule at one end. The additional splitting of the hexamer and guest ^1H NMR peaks in the pyrene sample (D_3 symmetry) indicates that pyrene tumbles slowly in the interior and is consistent with the tightest fit between host and guest observed in our study, explaining the unique stability of the complex. The more loosely fitting (and seemingly less shape-complementary) fluoranthene tumbles rapidly in the capsule on the NMR time scale (overall O symmetry), and is exchanged for $CDCl_3$ with an activation energy 2.8 kcal/mol less than that for pyrene (~500-fold faster). In CCl_4, the fluoranthene and pyrene complexes are again the most stable of the guests tested. Fluoranthene requires heat to induce the guest→solvent exchange while the pyrene complex is never fully replaced with a solvent-filled complex, even at 150 °C.

Anthracene, biphenyl and fluorene are the next smaller guests that we examined. All are of similar size; they have 14, 12, and 13 carbons, respectively, and VdW volumes of 188, 173, and 180 $Å^3$. Anthracene is a rigid, planar, aromatic molecule, biphenyl is also aromatic but has partial freedom of rotation about the single bond, and fluorene has an sp^3-hybridized carbon between the aromatic rings making it rigid but nonplanar. All three guest molecules show encapsulated guest peaks between 4.2−6.0 ppm in the ^1H NMR spectrum and rapid guest tumbling within a complex of apparent O symmetry. Once again, the geometry of the guests has a large effect on the capsules' kinetic stabilities. Biphenyl, the smallest of the three guests, has the shortest exchange time in both $CDCl_3$ and CCl_4. Anthracene and fluorene have similar exchange times in $CDCl_3$, but the fluorene→CCl_4 guest exchange is an order of magnitude slower than the anthracene→CCl_4 guest exchange.

The smallest guest molecules that we tested were naphthalene (VdW volume is 145 $Å^3$) and norbornene (VdW volume is 115 $Å^3$). Both guests again tumble rapidly inside each respective hexamer, giving O symmetry in both cases. While norbornene is smaller than naphthalene, it creates a much more kinetically stable guest-filled hexamer. In $CDCl_3$, the norbornene encapsulation complex exchanges with solvent ten times slower than the naphthalene complex. In CCl_4 the naphthalene complex again has one of the fastest guest→solvent exchange rates. Norbornene, however, despite being the smallest guest molecule tested, has the most stable guest encapsulated complex with the exception of pyrene and fluoranthene in both $CDCl_3$ and CCl_4.

The trends described here can be explained in relation to a structural model and proposed mechanism for guest exchange involving the partial dissociation of one (or possibly more than one) pyrogallolarene from the hexamer to create a minimally sized opening. This opening must be large enough for guest molecules to escape and solvent molecules to take their places. Pyrogallolarene is intrinsically quite rigid and there is an absence of good hydrogen bonding partners in solution under the studied conditions (there is effectively no concentration of "free" pyrogallolarene that is not a part of a hexamer). Consequently, in order for the hexamer to partially open, a relatively large number of hydrogen bonds must be simultaneously interrupted. If a single pyrogallolarene molecule were to completely dissociate from the hexamer up to 24 hydrogen bonds would need to be broken, however, the number needed to create an opening for guest exchange is less and must depend on the size of the opening needed to release

the guest molecule. Multiple simultaneous hydrogen bond interruption coupled to the transient loss of van der Waals, CH−π, π−π, and other weak interactions can reasonably account for the observed activation energies for guest exchange. This interpretation implies that the principle component of $\Delta G^{\ddagger}$ should be enthalpic, which agrees with the result of the Eyring analysis for norbornene exchange. Noting that a difference in $\Delta G^{\ddagger}$ of 1.3 kcal/mol (approximately equal to a single hydrogen bond or van der Waals interactions involving a single methyl group) accounts for approximately a 10-fold difference in the rates of first-order chemical processes, we can explain the apparently large effects of structural differences on the kinetic stability of the hexamers. Guest molecules that require larger openings to exchange with solvent if, for example, that difference is as small as a single hydrogen bond, can be expected to exchange 10× more slowly. We propose that both increasing the size of planar aromatic guests and the introduction of rigid deviations from planarity (e.g., in fluorene and norbornene) increase the size of the opening that is needed for exchange, thereby slowing the processes. Moreover, this model predicts that sterically larger solvents will similarly decrease exchange rates, which is seen in the data and is described in the next section.

Solvent Effects. As a general trend, larger solvent molecules increase the kinetic stability of the capsules irrespective of which guests occupy the interior. For the range of guests studied, we measured the rate of guest→solvent exchange both in $CDCl_3$ and CCl_4, and found that the exchange is roughly an average of 100-times slower in CCl_4 (Table 2). No exceptions were observed whereby exchange is faster in CCl_4.

To make a more detailed analysis, we measured the rate of pyrene→solvent exchange in CD_2Cl_2, $CDCl_3$, CCl_4, C_6D_6, toluene-d_8, xylenes, and mesitylene. The general trend in exchange rate holds true—exchange is slower in sterically larger solvents—but there is a clear difference between the aromatic and nonaromatic solvents. Although they have smaller VdW volumes, the chlorinated solvents generally undergo exchange at significantly slower rates than the aromatics, with CCl_4 providing the greatest kinetic stability observed in this study. Increasing the size of the chlorinated solvents also slows the rate of exchange with CD_2Cl_2 having the fastest rate and CCl_4 being the slowest. The upper limit for slow exchange was found to be for encapsulated CCl_4→pyrene exchange, with $\Delta G^{\ddagger}$ = 32 ± 0.7 kcal/mol. A similar trend is seen for the aromatic solvents, with the fastest guest exchange for C_6D_6 and the slowest for mesitylene. All of the exchange processes with aromatic solvents are faster than those for the chlorinated solvents, with the exception that the mesitylene exchange is slower than the CD_2Cl_2 exchange. The guest studies show that larger guests with greater deviations from planarity exchange more slowly, which we explain by proposing that their exchange requires a greater extent of opening of the pyrogallolarene hexamers. Likewise, because guest exchange is intrinsically tied to solvent entering the capsule to take the place of the former guests, our model supports an interpretation that solvents that are larger will in the same way hinder these processes. This interpretation is a direct reflection of the guest effects, following the principle of microscopic reversibility.

Our studies of pyrene encapsulation within the hexamer in CCl_4 revealed the only observed case in which the solvent-filled assembly is not the only species observed at equilibrium. Tests were done with two samples, one of the melted mixture of pyrogallolarene and pyrene and one of the individual components without prior melting, each dissolved together in CCl_4. After heating (1−2 h at 393 K) both samples showed small encapsulated pyrene peaks corresponding to a minor amount of guest-filled hexamer (Figure 5). After 24 h at 423 K

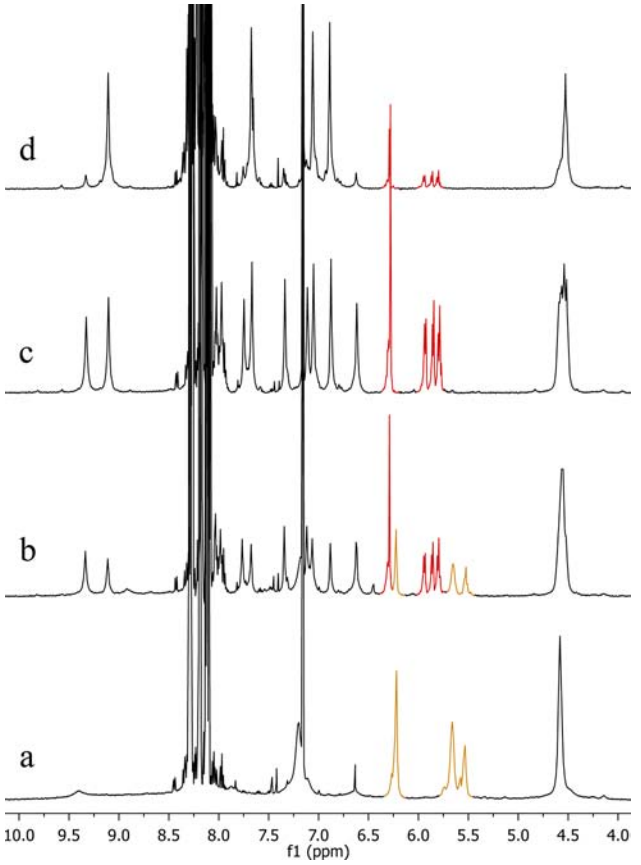


Figure 5. ^{1}H NMR spectra tracking the pyrene-filled (O_h)→pyrene-filled (D_3)→CCl_4-filled hexamer process. O_h configuration guest peaks (yellow) are replaced by the lower symmetry D_3 configuration guest peaks (red) upon heating at 343 K. Upon heating at 393 K most of the encapsulated pyrene exchanges with solvent but there is a small portion of the encapsualted guest that persists. (a) Prior to heating; (b) 2 h at 343 K; (c) 24 h at 343 K; (d) 4 h at 393 K. Equilibrium established after 4 h.

there was a measurable population of approximately 10% guest-filled hexamer as determined by NMR integration. The encapsulated guest peaks correspond to the lower symmetry configuration of pyrene, D_3.

A second aspect of our solvent studies was the titration of solutions of the pyrene-encapsulation complexes with the hydrogen-bonding solvents methanol and ethyl acetate. The addition of these solvents disrupts the assemblies, and methanol addition has a stronger effect because of its better hydrogen bonding ability. The addition of small amounts of methanol resulted in the complete and immediate disappearance of the initial O_h-symmetric assembly, which has disordered hydrogen bonds in rapid exchange on the NMR time scale. Clearly this less-stable hydrogen bonding arrangement is more sensitive to disruption. The appearance of new, broadened signals suggests that the newly added solvent participates in hydrogen bonding to the capsules. With further solvent addition, we observe only the D_3-symmetric configuration,

which has the ordered, chiral arrangement of hydrogen bonds, and some of the capsules are now solvent-filled or dissociated (Figure 4). Polar solvent addition can thus catalyze the conversion of the O_h-symmetric assembly to the D_3-symmetric configuration and induce some disassembly and guest exchange. The addition of small amounts of ethyl acetate did not have as strong an effect on the disappearance of the O_h-symmetric, pyrene-filled capsule, as it was still visible with up to 10% addition of ethyl acetate. Ethyl acetate addition also did not catalyze the transition to the lower symmetry D_3 capsule as was seen with methanol addition (Figure S9, Supporting Information). Further addition of methanol or ethyl acetate eventually leads to the complete dissociation of the assemblies.

These solvent effects are easily reconciled with our model for the guest exchange processes. The addition of hydrogen bonding solvents catalyzes the configuration change (i.e., the rearrangement of the hydrogen bonds of the capsule) by providing temporary hydrogen-bonding partners. Moreover, it accelerates guest exchange by satisfying hydrogen bond donors and acceptors in a partially opened capsule, which is the proposed transition state. Last, sufficient amounts of hydrogen-bonding solvents completely disrupted the assemblies, as is typical for hydrogen-bonded assemblies. It is noteworthy that some pyrene-filled capsules are still observed at up to 20% CH_3OH addition, which is a rare tolerance for a hydrogen bond-driven assembly and results from the highly cooperative nature of the capsules' seams.

Configuration Changes and Intermediates on the Pathways to Guest Release. In several observed cases (fluoranthene, norbornene, and under some conditions, pyrene, and anthracene), hexamers loaded with guests using the melting−cooling procedure undergo gradual one-to-one conversion to the solvent-filled hexamers over time, when dissolved in chlorinated or aromatic organic solvents (Table 1). Two distinct exceptions were observed.

The first, observed only when the capsules were initially loaded with pyrene (and seen only in CD_2Cl_2, $CDCl_3$, CCl_4, and C_6D_6), is the change in the structure of the capsules from the initially observed O_h symmetry to the more stable D_3 configuration, with its organized seam of hydrogen bonds and slow guest tumbling. After the completion of this non-dissociative rearrangement (which requires heating in CCl_4), direct one-to-one conversion to the solvent-filled hexamers is observed and follows first order kinetics.

The second exception is the appearance of a multitude of "supramolecular intermediates" on the pathway for solvent exchange (seen with anthracene [only in $CDCl_3$], biphenyl, and naphthalene; Table 1). All of these capsules, when initially loaded with guest, have an observed symmetry of O, characterized by the ordered, chiral arrangement of capsule hydrogen bonds and rapid guest tumbling in the interior. On the pathway to guest exchange for solvent, a number of additional capsular species are seen as characterized by encapsulated guest signals in the ^{1}H NMR within the 4.0−6.5 ppm window (Figures S17, S19, S20, S25, and S26 in the Supporting Information). All of these intermediates (at least those with populations large enough for clear resolution of the ^{1}H NMR signals) appear to have O symmetry and rapid guest tumbling. Exact structural determination is elusive, but we have observed an important clue in the case of anthracene. By comparing changes in the NMR spectra over time for a sample in $CDCl_3$ against one in $CHCl_3$, we observed encapsulated $CHCl_3$ signals growing in and later disappearing at 4.2 ppm. These signals seem to track an increase in the intensity of an encapsulated anthracene signal at 6.2 ppm and the eventual total disappearance of that signal (Figure 6).[43] Accordingly, we

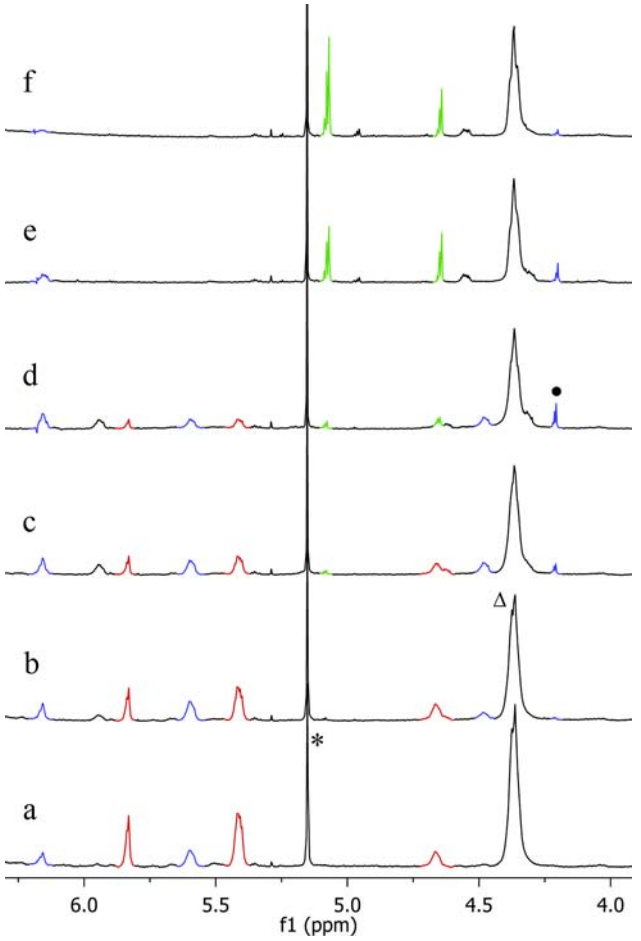


Figure 6. NMR spectra for anthracene encapsulated within the pyrogallolarene hexamer measured in $CHCl_3$ at 296K over time. The initial guest encapsulated peaks (red) are replaced with intermediate guest encapsulated peaks (blue), one of which (6.2 ppm) changes in shape and then disappears, tracking the growth and disappearance of an encapsulated guest peak for $CHCl_3$ (•). These intermediate peaks are eventually replaced by encapsulated $CHCl_3$ (green); (a) 8 min, (b) 30 min, (c) 1.5 h, (d) 3 h, (e) 27 h, (f) 48 h. (*) indicates a solvent impurity and (Δ) is the signal for the pyrogallolarene methine.

assign this intermediate to be a species of mixed occupancy, which is a capsule containing both $CHCl_3$ and anthracene. Clear indications for this mixed occupancy were difficult to discern for other species when comparing directly the guest→solvent exchange processes for protio- and deuterio- solvents using ^{1}H NMR. It may be that similar signals for encapsulated $CHCl_3$ in other mixed-occupancy intermediates (i.e., those formed during the exchange of other guests) are buried under the host methine signal (nearby at 4.35 ppm). Moreover, solvent impurities may be encapsulated in mix-occupancy capsules, and these can be hard to detect.[7] We propose that this interpretation of mixed occupancy (with solvent or solvent impurities or both) is the most likely structural explanation for all of the observed intermediates, except for the symmetry change of the pyrene-filled capsules. Referring specifically to the case of fluorene (Figures S23 and S24), we observed a one-to-one change of capsule species prior to complete guest→solvent

dx.doi.org/10.1021/ja305252t | J. Am. Chem. Soc. 2012, 134, 15000−15009

exchange, both species having apparent *O* symmetry. The most likely stucture for the second species is one that is coencapsulating a solvent impurity. The guests tumble rapidly in the capsule and there is no apparent change in the hydrogen bonding of the capsule (as seen when pyrene is encapsulated), as such this interpretation seems to be the most plausible.

CONCLUSIONS

The results of these studies show that the highly cooperative nature of hydrogen bond-mediated pyrogallolarene assembly leads to kinetically trapped encapsulation complexes of exceptional stability. By using solvent-free conditions and a melting−cooling cycle, a series of hydrocarbon guests was loaded into the cavities of pyrogallolarene hexamers, resulting in encapsulation complexes that do not form when solvent is present. These complexes, when dissolved in a series of solvents, are typified by *O* symmetry and a chiral arrangement of hydrogen bonds stabilizing the capsules. Over time, the capsules equilibrate to give hexamers containing only solvent molecules, except in the case of pyrene-filled hexamers dissolved in carbon tetrachloride, which persist as a minor species at equilibrium. Studies of solvent and guest effects on the rate of equilibration revealed large differences in kinetic stability that depend on the size and shape both of solvent and of guest. Larger guest and solvent molecules or those that deviate from a planar geometry dramatically increase the kinetic stability of the complexes, likely because they require the rupture of more hydrogen bonds to create a capsule opening of sufficient size for occupant exchange. With large guests (pyrene, fluoranthene) in sterically larger solvents ($CDCl_3$, CCl_4, mesitylene), the guest→solvent exchange was not observed at room temperature, and instead required heating, indicating the presence of a powerful kinetic trap. The specific example of the CCl_4→pyrene guest exchange process is exceptionally slow, with an activation energy $\Delta G^{\ddagger} = 32 \pm 0.7$ kcal/mol. To the best of our knowledge, this example has the greatest barrier to guest exchange ever observed for a synthetic, hydrogen−bonded host−guest complex. The addition of hydrogen bonding cosolvents provides a lower−energy pathway for capsule configuration change and occupant exchange, catalyzing both processes, and eventually leading to disassembly when a sufficient amount of polar solvent is added.

Hydrogen bond-mediated assemblies are known for their environmental responsiveness. Moreover, self-assembly provides an innate ability for error correction, which derives from the continual exchange of components at equilibrium. In this study, we have identified conditions that raise the activation energy for component exchange of pyrogallolarene hexamers beyond that which is available under ambient conditions, resulting in molecular encapsulation complexes that are not at equilibrium but are nonetheless indefinitely persistent. It is possible to "pre-load" the capsules under one set of conditions, and then study them in another set of conditions under which the same capsules could not have been formed. We expect that further efforts to control self-assembly with kinetics traps will lead to new applications of supramolecular chemistry, both using hydrogen bonds and using metal−ligand coordination, in open systems where an equilibrium cannot exist.

EXPERIMENTAL SECTION

General. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer. Molecular modeling was performed using HyperChem 8.0.6 and Spartan '04 and visualized using PyMol 1.2r1. Geometry minimizations were performed by fixing the atoms of the hexamer as determined from an X-ray crystal structure[44] and minimizing the guests on the interior at the AM1 level of theory. Kinetics calculations were performed using Origin 8.1 and KinTekSim.[42] Cavity and VdW volumes were calculated using Swiss-PdbViewer. Pyrogallolarene was synthesized according to a previously published procedure.[32]

Encapsulation Studies. A 1:1 (by weight) mixture of decylpyrogallolarene and guest was melted with a heat gun under ambient air and allowed to cool. The resulting solid was dissolved in the chosen solvent (deuterio or proteo) and characterized by NMR. All spectra were measured at 296 K. Samples that were monitored at 296 K were maintained at that temperature throughout the course of the exchange and were measured periodically during that time; measurement frequency was dependent on the stability of the capsule. Samples that were stable at room temperature were measured initially at ambient temperature and then heated in a constant temperature oil bath for a specified amount of time. Samples were removed from the oil bath and measurements were taken periodically during heating. Coaxial NMR tubes filled with deuterated benzene were inserted into the standard NMR tubes with nondeuterated samples to allow for an external deuterium lock.

ASSOCIATED CONTENT

Supporting Information

Additional NMR spectra and molecular models. Data tables and kinetics plots for kinetics analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

bpurse@du.edu

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by funding provided by the University of Denver. We thank Prof. Kari Rissanen for helpful discussions.

REFERENCES

(1) Steed, J. W.; Atwood, J. L. *Supramolecular Chemistry*, Second ed.; Wiley: West Sussex, 2009.
(2) Prins, L. J.; Reinhoudt, D. N.; Timmerman, P. *Angew. Chem., Int. Ed.* **2001**, *40*, 2382−2426.
(3) Davis, A. V.; Raymond, K. N. *J. Am. Chem. Soc.* **2005**, *127*, 7912−7919.
(4) Chen, J.; Körner, S.; Craig, S. L.; Rudkevich, D. M.; Rebek, J. *Nature* **2002**, *415*, 385−386.
(5) Mal, P.; Breiner, B.; Rissanen, K.; Nitschke, J. R. *Science* **2009**, *324*, 1697−1699.
(6) Meng, W.; Breiner, B.; Rissanen, K.; Thoburn, J. D.; Clegg, J. K.; Nitschke, J. R. *Angew. Chem., Int. Ed.* **2011**, *50*, 3479−3483.
(7) Jiang, W.; Ajami, D.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2012**, *134*, 8070−8073.
(8) Matson, J. B.; Stupp, S. I. *Chem. Commun.* **2012**, *48*, 26−33.
(9) MacGillivray, L.; Atwood, J. *Nature* **1997**, *389*, 469−472.
(10) Shivanyuk, A.; Rebek, J. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 7662−7665.
(11) Avram, L.; Cohen, Y. *J. Am. Chem. Soc.* **2002**, *124*, 15148−15149.
(12) Shivanyuk, A.; Rebek, J. *J. Am. Chem. Soc.* **2003**, *125*, 3432−3433.
(13) Avram, L.; Cohen, Y. *Org. Lett.* **2003**, *5*, 3329−3332.
(14) Yamanaka, M.; Shivanyuk, A.; Rebek, J. *J. Am. Chem. Soc.* **2004**, *126*, 2939−2943.

(15) Avram, L.; Cohen, Y. *J. Am. Chem. Soc.* **2004**, *126*, 11556−11563.
(16) Philip, I.; Kaifer, A. E. *J. Org. Chem.* **2005**, *70*, 1558−1564.
(17) Beyeh, N. K.; Kogej, M.; Åhman, A.; Rissanen, K.; Schalley, C. A. *Angew. Chem., Int. Ed.* **2006**, *45*, 5214−5218.
(18) Evan-Salem, T.; Cohen, Y. *Chem.—Eur. J.* **2007**, *13*, 7659−7663.
(19) Shimizu, S.; Kiuchi, T.; Pan, N. *Angew. Chem., Int. Ed.* **2007**, *46*, 6442−6445.
(20) Barrett, E. S.; Dale, T. J.; Rebek, J. *J. Am. Chem. Soc.* **2008**, *130*, 2344−2350.
(21) Mileo, E.; Yi, S.; Bhattacharya, P.; Kaifer, A. E. *Angew. Chem., Int. Ed.* **2009**, *48*, 5337−5340.
(22) Gerkensmeier, T.; Iwanek, W.; Agena, C.; Fröhlich, R.; Kotila, S.; Näther, C.; Mattay, J. *Eur. J. Org. Chem.* **1999**, 2257−2262.
(23) Atwood, J. L.; Barbour, L. J.; Jerga, A. *J. Supramol. Chem.* **2001**, *1*, 131−134.
(24) Avram, L.; Cohen, Y. *J. Am. Chem. Soc.* **2003**, *125*, 16180−16181.
(25) Dalgarno, S. J.; Tucker, S. A.; Bassil, D. B.; Atwood, J. L. *Science* **2005**, *309*, 2037−2039.
(26) Palmer, L. C.; Rebek, J. *Org. Lett.* **2005**, *7*, 787−789.
(27) Dalgarno, S. J.; Bassil, D. B.; Tucker, S. A.; Atwood, J. L. *Angew. Chem., Int. Ed.* **2006**, *45*, 7019−7022.
(28) Kulikov, O. V.; Rath, N. P.; Zhou, D.; Carasel, I. A.; Gokel, G. W. *New J. Chem.* **2009**, *33*, 1563.
(29) Avram, L.; Cohen, Y.; Rebek, J., Jr. *Chem. Commun.* **2011**, *47*, 5368−5375.
(30) Avram, L.; Cohen, Y. *Org. Lett.* **2006**, *8*, 219−222.
(31) Dalgarno, S. J.; Szabo, T.; Siavosh-Haghighi, A.; Deakyne, C. A.; Adams, J. E.; Atwood, J. L. *Chem. Commun.* **2009**, 1339.
(32) Kvasnica, M.; Chapin, J. C.; Purse, B. W. *Angew. Chem., Int. Ed.* **2011**, *50*, 2244−2248.
(33) Winkler, H. D. F.; Dzyuba, E. V.; Sklorz, J. A. W.; Beyeh, N. K.; Rissanen, K.; Schalley, C. A. *Chem. Sci.* **2011**, *2*, 615.
(34) Tian, J.; Thallapally, P. K.; Dalgarno, S. J.; McGrail, P. B.; Atwood, J. L. *Angew. Chem., Int. Ed.* **2009**, *48*, 5492−5495.
(35) Cavarzan, A.; Scarso, A.; Sgarbossa, P.; Strukul, G.; Reek, J. N. H. *J. Am. Chem. Soc.* **2011**, *133*, 2848−2851.
(36) Dalgarno, S. J.; Power, N. P.; Atwood, J. L. *Coord. Chem. Rev.* **2008**, *252*, 825−841.
(37) Fowler, D. A.; Mossine, A. V.; Beavers, C. M.; Teat, S. J.; Dalgarno, S. J.; Atwood, J. L. *J. Am. Chem. Soc.* **2011**, *133*, 11069−11071.
(38) Whetstine, J. L.; Kline, K. K.; Fowler, D. A.; Ragan, C. M.; Barnes, C. L.; Atwood, J. L.; Tucker, S. A. *New J. Chem.* **2010**, *34*, 2587−2591.
(39) Ajami, D.; Rebek, J. *Angew. Chem., Int. Ed.* **2008**, *47*, 6059−6061.
(40) Kitaigorodsky, A. I. In *Molecular Crystals and Molecules*; Academic Press: New York, 1973; p 18.
(41) We presume that the reorganization takes place without dissociation based on our observation that mixtures of host and guest were found to encapsulate only solvent in $CDCl_3$ when those mixtures had not melted and cooled to induce solvent-free assembly. Complete dissociation of the components must therefore lead to the formation of only solvent-filled capsules.
(42) Zimmerle, C. T.; Frieden, C. *Biochem. J.* **1989**, *258*, 1−7.
(43) The appearance of encapsulated chloroform signals as apparent multiplets and at more than one chemical shift position likely reflects coencapsulation with solvent impurities or the presence of multiple capsular species occupied by different numbers of $CHCl_3$ molecules, or both. Similar spectra were previously reported by Cohen, who determined that the apparent multiplicity is not a manifestation of *J* coupling.[30]
(44) Dalgarno, S. J.; Antesberger, J.; McKinlay, R. M.; Atwood, J. L. *Chem.—Eur. J.* **2007**, *13*, 8248−8255.