Highly Organized Spherical Hosts That Bind Organic Guests in Aqueous Solution with Micromolar Affinity: Microcalorimetry Studies

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Abstract: Two novel closed-shell hemicarcerand-like hosts with spherical cavities of 11 Å diameter that are soluble in aqueous solution were constructed. The binding of xylenes, aryl ethers, polyaromatic compounds, ferrocene derivatives, and bicyclic aliphatic compounds were examined by NMR spectroscopy and microcalorimetry. NMR binding studies indicated that binding depended upon guest hydrophobicity and shape. No binding was detected for guests in which a charge must be desolvated as part of inclusion or for guests that can not fit within the cavity of the host. Three complexes **2**·naphthalene, **2**·*p*-xylene, and **2**·ferrocene were isolated and found to be indefinitely stable in the solid phase and in aqueous solution. The binding constants for these complexes are estimated to be greater than $10^8\,\text{M}^{-1}$. Thirteen guests were examined by microcalorimetry with binding constants ranging between 10^7 and $10^3\,\text{M}^{-1}$. A comparison of results obtained here with those from previous work with β-cyclodextrin and cyclophane hosts, along with analysis of the entropy–enthalpy compensation data, indicate that there is a higher degree of guest desolvation with this host structure than with open-shell hosts. This accounts at least partially for the increase in affinity observed with these closed-shell hosts. Replacing a hydroxy group in the host portal with a hydrogen atom does not affect the binding constant, a finding consistent with the guest residing deeply buried within the host cavity. It was observed that aromatic guests are bound with higher affinity than aliphatic ones in agreement with results that point to the importance of London dispersion forces in the association of aromatic components in face-to-edge orientations. The correlation of changes in NMR chemical shift with microcalorimetry data supports a model in which increased CH-π interactions strengthen association between host and guest due to the dominant role of van der Waals dispersion forces. Remarkably, the binding constant for the 1,4 isomer of dimethoxybenzene is 32 times higher than for the 1,2 isomer, and even greater discrimination is observed between the xylene guests since the binding constant for *p*-xylene is 80 times greater than that for *o*-xylene. This discrimination between isomeric guests by a rigid host indicates that changes in specific hydrophobic interactions have substantial effects upon binding affinity.

Keywords: host–guest chemistry · supramolecular chemistry

Introduction

Of paramount importance to biology and chemistry is the readily observed tendency of water to reject nonpolar fatty materials and to be rejected by them. Water is the solvent found in nature, and the attractive forces that occur in aqueous solution are related to fundamental biological processes, such as the formation of cells and biomembranes, the catalytic efficiency of enzymes, and the folding of proteins. Complexation in an aqueous medium is a result of favorable changes in free energy due to rearrangements in solute–solute, solvent–solute, and solvent–solvent interactions involving van der Waals contacts, hydrogen bonds, and electrostatics.[1] The forces which govern the separation of organic and aqueous phases are often referred to collectively as the "hydrophobic effect". The hydrophobic effect has been a subject of intense interest and debate, although a general description of the process has been elusive. A large body of information has been obtained on protein–protein,[2] protein–peptide,[2] and protein–ligand[3] interactions in water in order to understand the importance of water in these crucially important associative processes. Concurrently, the association between structurally well-defined model systems such as

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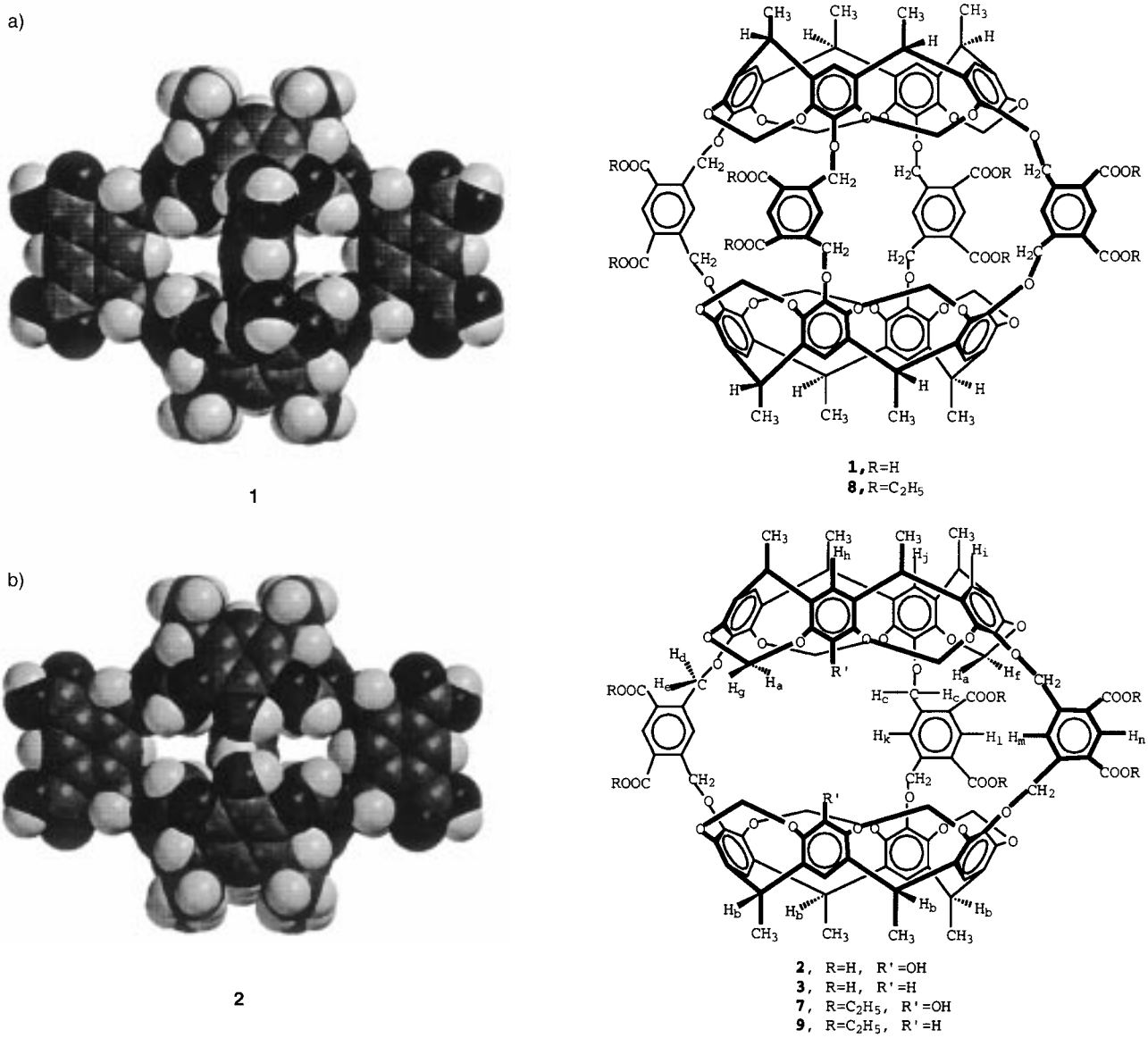

Figure 1. a) Structure of host **1** with corresponding ethyl ester analogue **8** (right); space-filling representation of **1** (left). b) Structure of hosts **2** and **3** and ethyl ester analogues **7** and **9** (right); space-filling representation of **2** (left).

cyclodextrins[4] and cyclophanes[5] and small organic guests in water has been studied to gain fundamental insight into molecular recognition and hydrophobic association in water.

It would be advantageous to our understanding of hydrophobic binding if a simple system could be studied in which a guest moves from water into a well-defined host environment that excludes water to maximize the guest desolvation. This ideal has been closely approximated by host **1**, introduced by Yoon and Cram (Figure 1).[6]

This water-soluble closed-shell molecule has an enforced spherical cavity with a diameter of 11 Å.[7] Several substances were added to NMR samples of **1** prepared in deuterated sodium borate buffer (pH 9). A total of 14 compounds formed complexes in which the chemical shift of the guest moved upfield by as much as 4 ppm. The change in magnetic environment of the guest indicates it is deeply held within the shielded interior of host **1**. This model is supported by CPK molecular models. No thermodynamic parameters have been reported for the association of host and guest for this system. The development of such a well-defined system yields an ideal opportunity for the study of the movement of small organic molecules from aqueous solution into an organized hydrophobic environment. An accurate quantitative investigation of thermodynamic parameters requires that the process be studied under equilibrium conditions. This requirement is orthogonal to traditional studies on hemicarceplexes which rely upon large activation barriers for the creation of stable complexes in organic solvent.[8] Diol host **2** and deoxy host **3** (Figure 1) were employed to ensure equilibrium conditions are maintained for a wide range of guests. The enlarged portal of hosts **2** and **3** is intended to allow free guest entrance and egress, thereby precluding kinetic barriers which make thermodynamic analysis of solute interactions with **1** nearly impossible.

Herein we describe our NMR and microcalorimetry investigation of the inclusion of neutral organic guests within these

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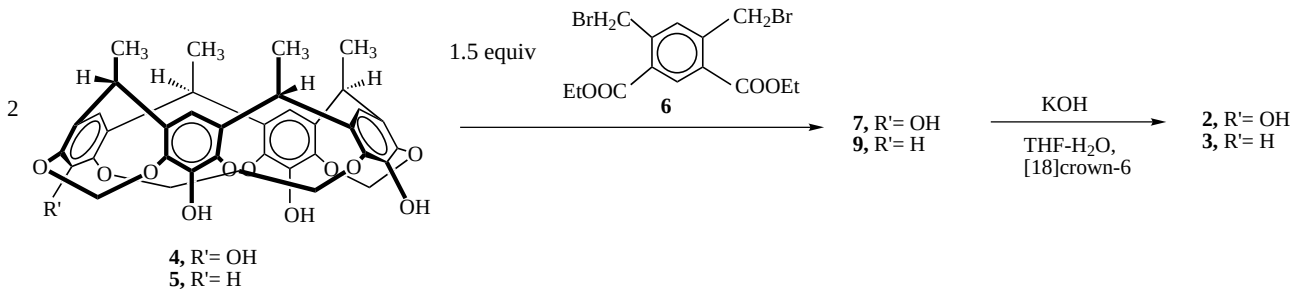

Scheme 1. Synthesis of hosts **2** and **3**.

novel hemicarcerand-like hosts. Our results are discussed in terms of recent results which suggest London dispersion forces are a predominant driving force in hydrophobic binding.

Results

Construction of hosts 2 and 3: The key step in the synthesis of hosts **2** and **3** is the connection of two phenol units **4** (prepared in four steps from resorcinol) with diester **6** (Scheme 1). Host **2** is prepared from tetrol **4**, while **3** is obtained from triol **5**, which is a by-product in the synthesis of **4.**

By adjusting the reaction conditions, it is possible to isolate hexaester **7** in 8% yield, while the octaester **8** (Figure 1) can be obtained in a 7% yield. Several attempts were made to optimize the reaction conditions, but in our hands the yield could not be improved. Hexaester **7** is hydrolyzed to give hexaacid **2**, which is purified by preparative HPLC. The NMR spectra of **2** and **3** are indicative of the C_{2V} symmetry of the host. Host **3** is constructed by connecting two triol units **5** to give hexaester **9**, which is subsequently hydrolyzed and purified. Solutions of **2** and **3** can be prepared up to concentrations of 5 mM with no evidence of micelle formation, as evidenced by NMR chemical shift studies. Since it is easier to synthesize **2**, the initial work was done with more abundant host and these experiments were repeated with **3**. The binding observed is unique to aqueous solution since **7** and **9** do not retain guests inside their cavities in organic solutions.

NMR studies: The initial experiments consisted of screening guests by NMR spectroscopy. Upfield changes in the chemical shifts of the guest protons indicated that the compounds were residing within the highly shielding environment of **2**. All **2**·guest complexes show slow exchange between the bound and free states on the NMR time scale, that is, both bound and free guests appear in the spectra along with the occupied and empty host. The appearance of guest peaks which shifted upfield upon the addition of host **2** was interpreted as a positive indication of binding. By manipulating the concentration of host and guest, a rough estimate of the binding constant could be obtained. Association constants that were too strong to be measured by NMR spectroscopy ($>10^3\,\text{M}^{-1}$) were observed for 18 highly hydrophobic guests. The list includes all possible trimethoxybenzene and dimethoxybenzene isomers, all xylene isomers, adamantane, norborenol, 2-adamantanone, ferrocene, and *trans*-4-[2-(1-ferrocenyl)vinyl]-1-methylpyridiniumiodide. Strong binding was also observed for naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, 2-methoxynaphthalene, and 1-bromonaphthalene. A second group of compounds containing camphor, nopinone, and norbornanemethanol show somewhat reduced affinity for **2**, $K_a \approx 10^3$, although at the accuracy of the experiment the magnitude of the difference between the two groups was not apparent. A third grouping of guests which displayed obvious but weaker association, $10^3 > K_a > 10^2$, included acetone, methanol, norcamphor, camphene, 5-fluorouracil, β-carotene, and ferrocenecarboxylic acid. A final group of compounds showed no sign of inclusion under the experimental conditions, $K_a < 10^2$, and included large hydrophobic molecules such as anthracene, pyrene, and 1,2-dimethylnaphthalene as well as compounds such as hexamethylenetetramine, 1-naphthoic acid, borylamine, and borylacetate that contained charged residues under the titration conditions.

Isolation of 2·guest complexes: It is possible to isolate selective guest complexes within **2** by adjusting the pH to >2 and by filtering the resulting precipitate. Subsequent trituration with $CHCl_3$ removes any residual uncomplexed guest. After the material is air dried, the solids can be redissolved in deuterium oxide borate buffer at pH 9. The NMR spectra of the isolated complexes show pure 1:1 complexes that are stable indefinitely. The **2**·ferrocene, **2**·naphthalene, and **2**·*p*-xylene complexes were stored in the solid state for several weeks and once redissolved in deuterium oxide at pH 9 give clean proton NMR spectra for the 1:1 complexes. The spectra of **2**·ferrocene and **2**·*p*-xylene are shown in Figure 2. This process was successful only for guests that have high binding constants. Attempts to isolate complexes between **2** and guests with $K_a \leq 10^3$ resulted in the recovery of empty **2**. The results described above indicate that **2** can be used to introduce insoluble substances into aqueous solution at greater than millimolar concentrations.

Microcalorimetry studies It is possible to obtain binding constants by ^{1}H NMR spectroscopy by using the relative integrated areas of the proton signals for free and bound guests for the guests. For example, analysis of the **2**·camphor spectra yielded a binding constant K_a of $3 \times 10^3\,\text{M}^{-1}$ ($\Delta G^\circ = -4.8\,\text{kcal mol}^{-1}$). Although a limited range of binding constants can be found using the NMR method, titrations using isothermal titration microcalorimetry can directly measure

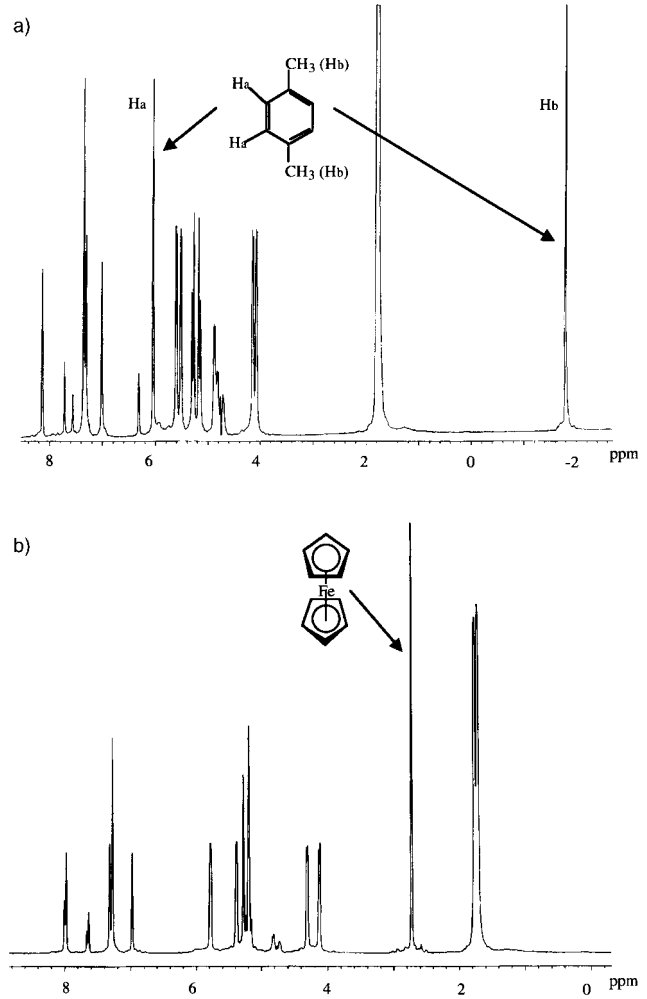

Figure 2. 400 MHz ^{1}H NMR spectra of the **2**·*p*-xylene (a) and **2**·ferrocene complexes (b) in D_2O at pH 9.

ΔH^o and K_a over a large range of K_a values; thus, this method was employed to acquire thermodynamic profiles for complexation. Titration of **2** with 1,2,4-trimethoxybenzene in the microcalorimeter produced the thermogram shown in Figure 3.

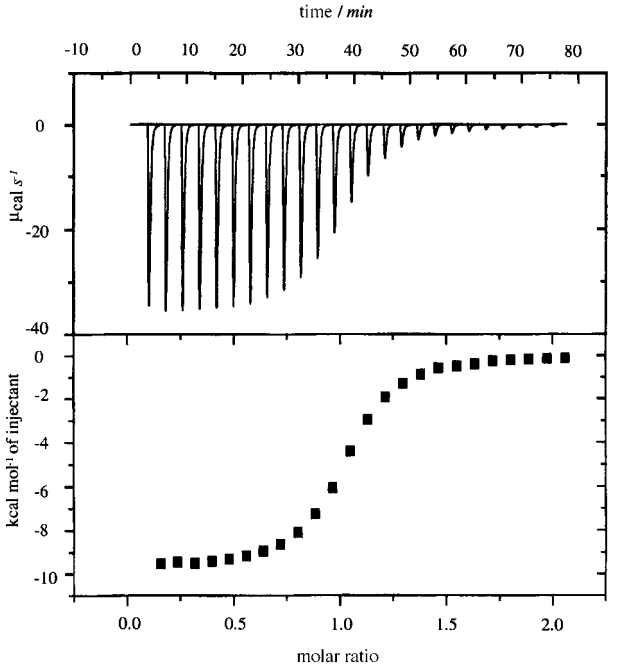

Figure 3. ITC thermogram (top) and isotherm (bottom) for the binding of 1,2,4-trimethoxybenzene to diol host **2**.

Integration of the heat data plotted versus the molar ratio of 1,2,4-trimethoxybenzene to **2**, produced the isotherm shown in Figure 3. A series of guests were studied calorimetrically. All host–guest combinations produced thermograms and isotherms of similar quality. The thermodynamic parameters K_a, ΔH^o, ΔG^o, and ΔS^o extracted from the calorimetric data for the interaction of all thirteen guests with host **2** and the results with four guests with host **3** are contained in Table 1. The excellent fit obtained for all thermograms obtained support our belief that the values reported here accurately describe thermodynamic equilibrium.

Unfortunately, the solubility of naphthalene and ferrocene in water are not sufficient to allow us to carry out calorimetric titrations with these guests. The binding constants for these

Table 1. Thermodynamic and selected chemical shift data for binding of organic guests by hosts **2** and **3**.

Guests	Host	ΔH^o [kcal mol^{-1}][a]	$T\Delta S^o$ [kcal mol^{-1}][b]	ΔG^o [kcal mol^{-1}]	K	$\Delta\delta$ [ppm][c]
p-xylene	**2**	−12.3	−2.6	−9.6	1.2×10^7	3.65
m-xylene	**2**	−12.9	−4.3	−8.6	4.9×10^6	3.75
o-xylene	**2**	−8.9	−1.8	−7.1	1.5×10^5	2.21
1,2,3-trimethoxybenzene	**2**	−15.6	−7.9	−7.6	4.1×10^5	
1,3,5-trimethoxybenzene	**2**	−12.5	−5.8	−6.9	1.1×10^5	
1,2,4-trimethoxybenzene	**2**	−9.7	−3.1	−6.6	7.1×10^4	
1,4-dimethoxybenzene	**2**	−10.9	−3.0	−7.9	6.4×10^5	4.05
1,3-dimethoxybenzene	**2**	−11.6	−4.0	−7.6	4.1×10^5	4.10
1,2-dimethoxybenzene	**2**	−8.1	−2.3	−5.8	1.9×10^4	1.95
camphor	**2**	−2.6	2.2	−4.8	3.2×10^3	
nopinone	**2**	−5.1	0.9	−4.2	1.2×10^3	
norbornanemethanol	**2**	−5.0	0.3	−5.2	6.9×10^3	
norborneol	**2**	−4.2	1.6	−5.8	1.8×10^4	
1,4-dimethoxybenzene	**3**	−11.3	−3.4	−7.9	6.0×10^5	
1,2,3-trimethoxybenzene	**3**	−14.7	−7.3	−7.4	2.9×10^5	
1,3,5-trimethoxybenzene	**3**	−12.9	−5.9	−5.9	1.4×10^5	
norborneol	**3**	−4.1	1.7	−5.8	1.8×10^4	

[a] The error of ΔH^o measurements is ±0.1 kcal mol^{-1}. [b] The error of K determination does not exceed 10%. [c] $\Delta\delta$ is the change of chemical shift of methyl group upon incarceration.

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highly hydrophobic molecules appear by rough competition experiments to be much greater than any of the guests that we measured and therefore a lower limit for the K_a of $10^8\,\text{M}^{-1}$ can be assigned for these guests. This value is a conservative estimate which indicates host **2** binds these guest with submicromolar affinity, one of the highest affinities recorded between a monomeric synthetic host and monomer guest.

Enthalpy–entropy compensation: Examination of the data in Table 1 reveals that the free energy of binding ranges from highly enthalpically driven in the case of 1,2,3-trimethoxybenzene to partially entropically driven for norborneol. A plot of $T\Delta S^o$ versus ΔH^o gives an excellent linear correlation ($R = 0.96$) (Figure 4) for a line described by Equation (1) over a wide range of ΔS^o and ΔH^o values where $\alpha = 0.75$ and $T\Delta S_o^o = 4.2$ kcal mol^{-1}.

$$T\Delta S^o = \alpha\Delta H^o + T\Delta S_o^o \quad (1)$$

In all cases the association is enthalpically driven with a systematic increase in ΔS^o as the enthalpic driving force for binding decreases. A compensation effect between enthalpy and entropy has been repeatedly observed for the association between receptor and ligand for several systems.[9] The origin and significance of entropy–enthalpy compensation have been reviewed in depth in several recent papers,[10] and therefore will not be discussed here. However, this data can be used to provide a qualitative comparison of binding between different guests.

Discussion

Guest selectivity: Size appears to be an important factor in determining how strongly a guest will bind within the interior of **2**. For example, although the naphthalene derivatives substituted at the 2- and/or 3-positions show strong association constants, when the substitution pattern is switched to the 1-position, no association is observed. A similar result is observed with anthracenene, which is too long to fit within the confines of **2**. In an earlier study, Quan and Cram[11] demonstrated that a kinetically stable complex could be formed between anthracene and a host with an enforced cavity similar to **1**. The contrast between the results from molecular incarceration and equilibrium binding studies points out that different factors determine kinetic and thermodynamic stability.

Other factors that are crucial to the formation of inclusion complexes are guest hydrophobicity and charge. No complexes are observed when a charge is required to be buried within the host interior, or when the guest has a high solubility in buffer (greater than approximately 0.05 M). This result is in agreement with that of Yoon and Cram[6] who observed no detectable binding of ammonium or carboxylate guests in **1**.[12] Interestingly, *trans*-4-[2-(1-ferrocenyl)vinyl]-1-methylpyridinium iodide shows strong complexation with **2**. Examination of CPK molecular models suggest that the ferrocene moiety can reside deeply buried within **2**, while the distal pyridinium center remains in contact with water. We interpret this result as indicating that if encapsulation of the hydrophilic region of the guest does not require desolvation of the charged portion of the molecule, the driving force for binding can be large. For example, cyclodextrin hosts can bind a charged species if a charged portion of the guest remains in contact with water, while a hydrophobic part will move into the host cavity so that these hosts can bind a charged guest effectively. An apparent contradiction is found with the work of Dougherty et al.,[13] which indicates that the cyclophane host they developed is effective at stabilizing charge through cation–π interactions. Although there is a strong interaction between the aromatic rings of the cyclophane host and the guest cation, the charge remains highly solvated. We attribute the absence of charge solvation for the lack of cation or anion binding by hosts **1** and **2**.

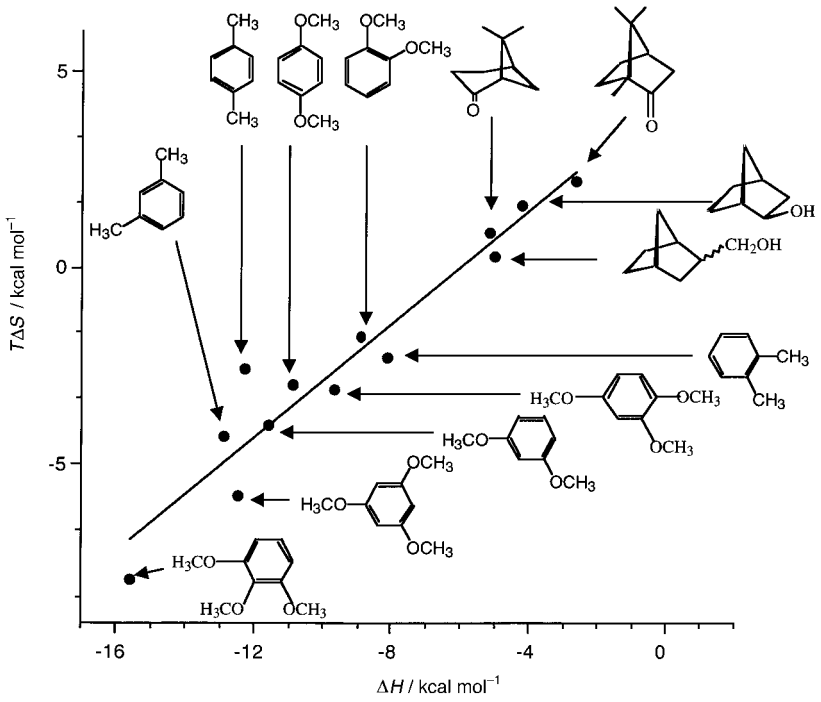


Figure 4. Enthalpy–entropy plot for host **2** and guests listed in Table 1.

Comparison with different hosts: It is also useful to compare the behavior of **2** with different hosts. A comparison of our *p*-xylene results with thermodynamic parameters obtained on Diederich's cyclophane host[14], $\Delta H^o = -7.4$ kcal mol^{-1}, $\Delta G^o = -5.3$ kcal mol^{-1} and $T\Delta S^o = -2.1$ kcal mol^{-1}, indicate that it is the 4.9 kcal mol^{-1} increase in enthalpic driving force which produces the larger value of K_a for host **2**. A comparison of association constants[3a] for *p*-

xylene between **2** (Table 1) and β-cyclodextrin ($K_a = 240\,\text{M}^{-1}$ and $\Delta G^o = -3.2$ kcal mol^{-1}), shows a difference in free energy of -6.4 kcal mol^{-1}. In contrast, larger guests such as anthracene and pyrene show significant binding constants to cyclophane hosts[14] and cyclodextrins.[3a] These hosts do not have as rigid requirements for size complementarity between host cavity and guest. While **2** shows higher affinity for guests which can fit inside the enforced cavity, larger guests such as anthracene are bound more effectively by cyclophane and β-cyclodextrin hosts.

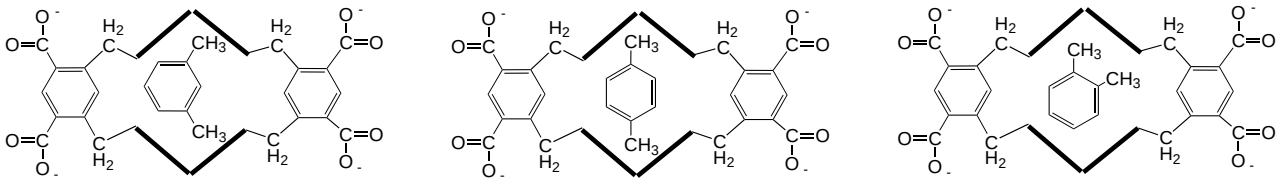
The results of the enthalpy–entropy compensation plot shown in Figure 4 can be used to help explain this increase in affinity for smaller guests. It has been suggested that the unitless slope (α) indicates to what extent the enthalpic gain is canceled by the entropic loss, and the value of this parameter reflects the amount of reorganization the host undergoes upon binding. For example, a flexible enzyme[16a] which undergoes substantial reorganization upon binding of substrate has an α value of 1, while β-cyclodextrin ($\alpha = 0.90$) undergoes less reorganization and cyclophane hosts ($\alpha = 0.78$) are quite rigid. The α value of 0.75 obtained over a similar range of ΔH and ΔS indicates host **2** is also inflexible. The $T\Delta S^o_o$ intercept represents the inherent free energy of complexation when $\Delta H^o = 0$ and can be used as a measure of guest desolvation upon binding.[16a] Interestingly, the $T\Delta S^o_o$ intercept for **2** (4.2 kcal mol^{-1}) is higher than either β-cyclodextrin (3.2 kcal mol^{-1}) or cyclophane (3.4 kcal mol^{-1}) hosts.[12a] Although the analysis presented above is qualitative, it is consistent with the model proposed by Diederich and Jorgensen[16] which shows that guests bound within the cyclophane remain partially solvated by water. The larger $T\Delta S^o_o$ intercept for **2** suggests that guest desolvation provides some of the driving force for complexation.

Comparison between hosts 2 and 3: If the model presented above is correct changes to the exterior structure of **2** should not have a detectable affect upon guest binding. Titrations that were carried out with host **3** used guests with representative structures from the binding study involving diol **2**. The results obtained with **3** were analogous to those obtained with diol host **2** in all cases (Table 1). The differences in portal structure appear to have no detectable effect upon binding, consistent with our model in which the guest has limited contact with the environment beyond the enforced cavity of the host. Our assertion that equilibrium has been reached in all cases is further supported by the comparison between hosts **2** and **3** since the change in the portal size between them has no detectable influence on the thermodynamic binding parameters of identical guests.

Interactions between host and guest: Examination of Figure 3 reveals a 3 kcal mol^{-1} gap in binding enthalpy between the aliphatic and aromatic guests. It is reasonable to suspect the presence, or absence, of polar functionality for the observed differences in binding enthalpy. However, there is no obvious trend in binding enthalpy observed between alcohols and ketones within the aliphatic compounds, or between methyl and methoxy compounds within the aromatic group. It is therefore inconsistent to invoke functional group polarity as an explanation for the observed gap in binding enthalpy. A possible clue to the underlying reason for this difference can be found in the work of Gellman et al.,[17] which indicates that the association between two aromatic components is energetically more favorable than a similar interaction between aliphatic and aromatic constituents. Further supporting evidence is provided by investigations of protein structure by Makhatadze and Privlalov[18] which indicates that the enthalpy of the van der Waals interactions is indeed higher for aromatic components (180 J mol^{-1} Å^{-2}) than for interactions between aliphatic components (130 J mol^{-1} Å^{-2}). These results are consistent with the possibility that we are observing an intrinsic affinity between aromatic groups, and suggests that London dispersion forces play a crucial role in the interactions being observed between the host and neutral guest.

It is important to note that no single parameter can take into account all the factors that lead to the observed selectivity, which must involve system desolvation as well as host–guest association. Our understanding of water–solute interaction is very limited at this time. Therefore, a quantitative analysis of solvation is not possible because the precise interactions between host and guest are not yet clear.[10b] However, the recent work of Wilcox et al.[19] has demonstrated through elegant experiments that London dispersion forces, and not electrostatics, are the predominant terms for the edge-to-face association of aromatic rings.[20] This model may help to explain the differences in binding enthalpy observed among the disubstituted aromatic guests for which there is a possibility of multiple CH–π interactions.

Results from CPK molecular model examination are illustrated in Figure 5 and show that when the guests are substituted with methyl or methoxy groups in either *para* or *meta* orientation, it is possible to establish CH–π interactions with both methyl groups. If our model is correct, additional CH–π interactions would increase the strength of the interaction between the host and the guest. In contrast, when guest methyl or methoxy groups have an *ortho* orientation, it is not possible to place both CH_3 groups within the aromatic regions of the host. If the strength of the host–guest interaction is related to the CH–π interactions, a difference



2•*m*-xylene **2**•*p*-xylene **2**•*o*-xylene

Figure 5. Illustration of interactions between different xylene isomers and host **2**.

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should be observed in binding enthalpy for the different disubstituted aromatic guests. Indeed, a trend is observed for ΔH where *meta* > *para* ≫ *ortho* for both the xylene and dimethoxybenzene guests (Table 1). Examination of CPK models can be used to conclude that the *meta* orientation is better than *para* orientation for maximizing CH–π interactions (Figure 5).

The proximity of the methyl group of the guest to the aromatic rings of the host should be proportional to the level of shielding observed in the proton NMR spectra as seen in the change in chemical shift between the free and bound guest. Importantly, the same trend observed for ΔH, *meta* > *para* ≫ *ortho*, is observed for the $\Delta\delta$ of the guest methyl group chemical shift of **2**·guest (Table 1). The large difference in the chemical shift between the *ortho* compounds and the other disubstituted aromatic guests point to a significant decrease in proximity of the methyl group to the aromatic rings of the host. It is reasonable to conclude that the correlation between the increase in $\Delta\delta$ to the increase in ΔH supports a model in which increased proximity between guest methyl group and host aromatic rings impart increased complex stability.

It is more difficult to interpret the binding enthalpy values for the trimethoxybenzene guests. Differences in guest shape can be used to create a model in which an increase in CH–π interactions corresponds with the values of ΔH. The analysis in this case is not as straightforward as in the disubstituted cases, and we feel a higher level of modeling is needed before a precise correlation can be proposed. The overall picture that results from this analysis is one in which edge-to-face interactions regulate the host–guest interface and London dispersion forces dominate this interaction.

Remarkable stereoselectivity has been observed with Rebek's self-assembled host system in which the guest acts as a template for host self-assembly,[21] showing that complementarity is important for organizing the assembly of the host. For complexes between host **2** and the xylene and dimethoxy benzene guest *selectivity is achieved without changes in hydrogen bonding either within the host or between host and guest.* A recent review by Davis and Teague[22] presents a compelling argument that hydrophobic interactions are underrated and hydrogen bonding correspondingly overemphasized in the analysis of complexes between biological receptors and drugs. Our results are consistent with their analysis. The binding constant for the 1,4 isomer of dimethoxy benzene is 32 times higher than for the 1,2 isomer. Even greater selectivity is observed between the xylene guests with the binding constant for *p*-xylene 80 times greater than for *o*-xylene. This discrimination between isomeric guests by a rigid host indicates that changes in specific hydrophobic interactions have substantial effects upon binding affinity.

Summary

We have investigated a new class of water-soluble hosts with an enforced cavity that tightly binds organic guests in aqueous solution with binding constants in some cases greater than $10^8\,\text{M}^{-1}$. It appears that by preorganizing a closed-shell hydrophobic cavity, the free energy of complexation is made more favorable for neutral guests that can reside inside the cavity. The closed-shell nature of these cages imparts a degree of selectivity to the system not observed with open-shell hosts. Association between host and guest appears to be driven by guest desolvation as well as specific CH-π interactions which ameliorate the London dispersion forces between host and guest. Our results also indicate that the hemicarcerand structure has a preference for aromatic guests over saturated ones, which can be accounted for by the increase in van der Waals interaction between aromatic components. Importantly, when binding constants greater than 10^7 are observed, it is possible to isolate 1:1 complexes at room temperature that are stable indefinitely both in the solid phase and in aqueous solution.

Work on modifying host structure in order to increase the range of potential guests is currently underway along with studies on the design of hosts which release guest upon irradiation into aqueous solution.[23]

Experimental Section

General methods: All chemicals used were reagent grade and all solvents were spectral grade, purchased from Aldrich Chemical Inc., and used without further purification. Tetrol **4** and diester **6** were prepared by methods described elsewhere.[24], [25] ^{1}H NMR spectra were obtained with a Varian Unity-plus NMR spectrometer. The ^{1}H NMR spectra obtained in D_2O where referenced against the residual water peak at $\delta = 4.75$. Mass spectrometry was carried out at the University of Illinois at Urbana-Champaign, School of Chemical Sciences.

Hexaester diol (7): To a solution of tetrol **4**[24] (0.5 g, 0.76 mmol) in dry 1-methyl-2-pyrrolidinone (NMP) (500 mL) was added anhydrous Cs_2CO_3 (4.0 g) and the solution was degassed. The degassed solution of diester **6**[25] (0.47 g, 1.1 mmol; 1.5 equivalents) was added dropwise over 16 h to the solution of tetrol **4**. After the mixture had been stirred under nitrogen for 24 h, it was poured into 1 L of a 1:1 mixture of distilled water and a saturated solution of NaCl. This mixture was stirred for 30 min and then allowed to sit until the precipitate settled out of the solution. The precipitate was filtered using an F-class filtering frit, dried on the frit, and then dissolved in CH_2Cl_2 (200 mL). The volume was reduced to 20 mL by rotary evaporation and loaded on 2 g of silica gel. The product was chromatographed first with CH_2Cl_2 and then with 1 % acetone in CH_2Cl_2 as the mobile phase. Isolated hexaester diol **7** was dissolved in a small amount of CH_2Cl_2 and diluted with methanol. The formed precipitate was filtered and dried under vacuum (10^{-2} Torr) at 100 °C to give pure **7** (62.5 mg, 8 %; m.p. > 300 °C (decomp); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.69$ (s, 2H), 8.64 (s, 2H), 8.62 (s, 1H), 8.19 (s, 1H), 7.05 (s, 2H), 6.98 (s, 2H), 6.96 (s, 4H), 6.12 (br. s, 2H; OH), 5.94 (d, 4H), 5.47 (d, 4H, $J = 7.2$ Hz), 5.45 (d, 4H, $J = 16.0$ Hz), 5.36 (s, 4H), 5.33 (d, 4H, $J = 16.0$ Hz), 4.90 (q, 8H, $J = 8.0$ Hz), 4.3 (m, 20H; 8 methine H and 6 CH_2 ester), 1.72 (m, 24H; CH_3 appendages), 1.39 (m, 18H; CH_3 ester); FAB$^+$-MS for [M^+]: calcd 2050.646, found 2050.647.

Hexaacid diol (2): To a solution of hexaester diol **7** (0.10 g, 0.049 mmol) in THF (2 mL) was added 1M KOH/[18]crown-6 solution (1.46 mL, 1.5 mmol; 30 equivalents) followed by 1M KOH solution (0.98 mL, 0.98 mmol; 20 equivalents). The reaction mixture was vigorously stirred at 70 °C for 6 h and then at 35 °C for 12 h. THF was removed under vacuum and an extra 20 mL of 1M KOH was added to dissolve precipitated hexaacid **2**. The solution was filtered using an F-class frit to eliminate undissolved impurities. The filtrate was acidified to pH 1 and allowed to sit until the precipitate settled out of the solution. The precipitated product was filtered and purified by preparative reverse-phase HPLC with acetonitrile/water as the mobile phase. The fraction containing the host was lyophilized to give free hexaacid diol **2.** After drying under vacuum at 130 °C, pure hexaacid diol **2** (78.4 mg; 85 %) was obtained: m.p. > 300 °C (decomp); ^{1}H NMR (400 MHz, D_20 pH = 9 (0.1M borate buffer), 25 °C): $\delta = 8.19$ (s, 2H), 7.71 (s,

1H), 7.62 (s, 1H), 7.28 (s, 4H), 7.27 (s, 2H), 7.22 (s, 2H), 6.86 (s, 2H), 5.96 (d, 4H, $J = 7.6$ Hz), 5.41 (d, 4H, $J = 7.6$ Hz), 5.30 (d, 4H, $J = 15.8$ Hz), 5.11 (d, 4H, $J = 15.8$ Hz), 5.00 (s, 4H), 4.90 (m, H, overlap with water, found in other solvents), 4.25 (m, 8H), 1.74 (m, 24H; CH_3 appendages); FAB$^+$-MS for [M^+]: calcd 1882.459, found 1882.459.

Deoxyhexaester (9): This synthesis was carried out according to a procedure that is analogous to the one for synthesis of compound **7**. As a starting material, methyl triol, which was isolated as a side product from the tetrol **4** reaction mixture, was used. The purification of the crude product involves at first a small silica gel precolumn to eliminate polymeric impurities and then a preparative silica gel plate. The top spot on the TLC plate is the desired product. Pure CH_2Cl_2 was used as an eluent for both precolumn and preparative silica gel plates. The overall yield of deoxyhexaester **9** was 5.4%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 8.69$ (s, 2H), 8.64 (s, 2H), 8.62 (s, 1H), 8.19 (s, 1H), 7.29 (s, 1H overlaps with the solvent peak), 7.05 (s, 2H), 6.96 (s, 4H), 6.35 (s, 2H), 6.12 (br. s, 2H; OH), 5.94 (d, 4H), 5.47 (d, 4H, $J = 7.2$ Hz), 5.45 (d, 4H, $J = 16.0$ Hz), 5.36 (s, 4H), 5.33 (d, 4H, $J = 16.0$ Hz), 4.90 (q, 8H, $J = 8.0$ Hz), 4.3 (m, 20H; 8 methine H and 6 CH_2 ester), 1.72 (m, 24H; CH_3 appendages), 1.39 (m, 18H; CH_3 ester); FAB$^+$-MS for [M^+]: calcd 2018.657, found 2018.653.

Deoxyhexaacid (3): The hydrolysis of deoxyhexaester **9** was carried out exactly like the hydrolysis of semicarceplex **7**. The yield of vacuum-dried deoxyhexaacid **3** was 92.0%. ^{1}H NMR (400 MHz, D_20 pH = 9 (0.1 M borate buffer), 25 °C): $\delta = 8.19$ (s, 2H), 7.71 (s, 1H), 7.62 (s, 1H), 7.57 (s, 2H), 7.28 (s, 4H), 7.27 (s, 2H), 7.22 (s, 2H), 5.96 (d, 4H, $J = 7.6$ Hz), 5.41 (d, 4H, $J = 7.6$ Hz), 5.30 (d, 4H, $J = 15.8$ Hz), 5.11 (d, 4H, $J = 15.8$ Hz), 5.00 (s, 4H), 4.90 (m, 8 methine H overlap with water, found in other solvents), 4.25 (m, 8H;), 1.74 (m, 24H; CH_3 appendages); FAB$^+$-MS for [M^+]: calcd 1850.469, found 1850.472.

Microcalorimetry: The binding parameters were determined by titrating 1 mM host solution with 21 mM guest solutions or 0.25 mM guest solutions with 5.25 mM host solution in an Omega isothermal titration calorimeter (MicroCal, Northampton, MA). Lower concentrations of host and guest were used in systems that exhibited very high binding affinities. All titrations were performed in borate buffer, pH 9. The cell was thermostatted to ±0.1 °C using a circulating bath. All of the experiments were performed at 25 °C. In all cases the concentration of the guest was determined by using measured absorbance and known extinction coefficients in buffer at pH 9. Host solutions were prepared by weighing out a known amount of compound and dissolving it in buffer at pH 9. The enthalpy of binding between host and guest was determined from heats of multiple single injections. Injection volumes were 5 μL, with 3 min of equilibration time allowed between injections. The heat of dilution of guest into buffer was determined and the host–guest titration heat was adjusted by this small contribution. In cases where the guest had low solubility in buffer, the solution of the host was added from the syringe into the guest solution contained in the sample cell.

The binding constants K and the number of binding sites n were extracted from the calorimetric data by employing the Origin™ data analysis software supplied with the Omega titration calorimeter. A complete description of the data analysis has been published by Brandts and coworkers.[26]

Acknowledgment

The work at BG was supported by a grant from the Petroleum Research Fund administered by the American Chemical Society. E. L. P. would like to thank the McMaster foundation for a fellowship. R. A. F. would like to thank the University of Toledo Office of Research for partial funding of this research.

[1] a) For a recent and extensive overview of work in this area see: *Comprehensive Supramolecular Chemistry* (Eds.: J. L. Atwood, J. E. D.Davies, D. D. MacNicol, F. Vögtle), Pergamon, Oxford, **1996**; b) a classic and still relevant discussion of the forces that control binding in water is found in: W. P. Jencks, *Catalysis in Chemistry and Enzymology*; McGraw-Hill, **1969**, pp. 393–436.
[2] W. E. Stites, *Chem. Rev.* **1997**, 97, 1233.
[3] R. E.; Babine, S. L. Bender, *Chem. Rev.* **1997**, 97, 1359.
[4] M. V. Rekharsky, Y. Inoue, *Chem. Rev.* **1998**, 98, 1875.
[5] a) F. N. Diederich in *Cyclophanes* (Ed.: J. F. Stoddart) Royal Society of Chemistry, Cambridge, UK, **1991**; b) D. B. Smithrud, T. B. Wyman, F. Diederich, *J. Am. Chem. Soc.* **1991**, 113, 5420.
[6] J. Yoon, D. J. Cram, *Chem. Commun.* **1997**, 497.
[7] The model was constructed with the Spartan 4.0 software package using the MM2 forcefield.
[8] D. J. Cram, J. M.Cram in *Container Molecules and Their Guests* (Ed. J. F. Stoddart), Royal Society of Chemistry, Cambridge, UK, **1994**.
[9] a) Y. Inoe, T. Hakushi, Y. Liu, L.-H. Tong, B.-J. Shen, D.-S. Jin, *J. Am. Chem. Soc.* **1993**, 115, 475; b) Y. Inoe, Y. Liu, L.-H. Tong, B.-J. Shen, D.-S. Jin, *J. Am. Chem. Soc.* **1993**, 115, 10637.
[10] a) Y. Inoe T. Wade *Supramol. Chem.* **1997**, 4, 55; b) J. D. Dunitz, *Chemistry and Biology*, **1995**, 2, 709; c) M. C. Searle, M. S. Westfall , D. H. Williams, *J. Chem. Soc. Perkin Trans. 2* **1995**, 141.
[11] M. L. Quan, D. J. Cram, *J. Am. Chem. Soc.* **1991**, 113, 2754.
[12] The ammonium compounds examined in ref. [6] are Me_4NBr, $PhNMe_3Br$, $BnNMe_3Br$ and the carboxylate is 3-$MeC_6H_4CO_2Na$.
[13] a) S. M. Ngola, P. C. Kearney, S. R. Mecozzi, D. A. Dougherty, *J. Am. Chem. Soc.* **1999**, *121*, 1192; b) P. C. Kearney, L. S. Mizoue, R. A. Kumpf, J. E. Forman, A. l. McCurdy, D. A. Dougherty, *J. Am. Chem. Soc.* **1993**, 115, 9907; c) D. A. Dougherty, C. B. Stauffer, *Science* **1990**, 250, 1558.
[14] S. B. Ferguson, E. M. Seward, F. Diederich, E. M. Sanford, A. Chou, P. Inocencio-Szweda, C. B. Knobler, *J. Org. Chem.* **1988**, 53, 5595.
[15] I. Sanemasa, Y. Akamine, *Bull. Chem. Soc. Jpn.* **1987**, 60, 2059.
[16] a) W. L. Jorgensen, T. B. Nguyen, E. M. Sanford, I. Chao, K. N. Houk, F. Diederich, *J. Am. Chem. Soc.* **1992**, 114, 4003; b) W. L. Jorgensen, T. B. Nguyen, *Proc. Natl. Acad. Sci. USA* **1993**, 90, 119.
[17] R. B. Gardner, L. A. Christianson, S. H. Gellman, *J. Am. Chem. Soc.* **1997**, 119, 5041.
[18] G. I. Makhatadze, P. L. Privalov, *Adv. Protein Chem.* **1995**, 47, 307.
[19] E. Kim, S. Paliwal, C. S. Wilcox, *J. Am. Chem. Soc.* **1998**, 120, 11192.
[20] W. L. Jorgensen, D. L. Severance, *J. Am. Chem. Soc.* **1990**, 112, 4768.
[21] T. Mártin, U. Olbst J. Rebek, Jr., *Science* **1998**, *218*, 1842.
[22] A. M. Davis, S. J. Teague, *Angew. Chem.* **1999**, *111*, 778; *Angew. Chem. Int. Ed.* **1999**, 38, 736.
[23] E. L. Piatnitski, K. D. Deshayes, *Angew. Chem.* **1998**, *110*, 1022; *Angew. Chem. Int. Ed.* **1998**, 37, 970.
[24] J. R. Fraser, B. Borecka, J. Trotter, J. C. Sherman, *J. Org. Chem.* **1995**, 60, 1207.
[25] A. Schroder, D. Karbach, R. Guther, F. Vögtle, *Chem. Ber.* **1992**, 125, 1881.
[26] T. Wiseman, S. Williston, J. F. Brandts, L.-N. Lin, *Anal. Biochem.* **1989**, 179, 131.

Received: July 12, 1999 [F1903]

  0947-6539/00/0606-1006 $ 17.50+.50/0