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Solvent effects in molecular recognition

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Abstract - Synthetic cyclophane hosts form stable and highly structured inclusion complexes with organic molecules in aqueous solutions. The solution geometries of these complexes are determined in a conformational analysis using Monte Carlo methods. Solvation-desolvation processes are a central factor in determining the stability of apolar inclusion complexes. The tight binding of small aromatic solutes in water is entropically unfavorable and is predominantly enthalpy-driven. A large part of the favorable enthalpy term for strong complexation in water results from its specific contributions. Electron donor-acceptor interactions stabilize complexes between electron-rich cyclophane hosts and electron-deficient aromatic substrates; however, they may be masked by specific solvation effects. Computer liquid phase simulations are undertaken to evaluate at a microscopic level the origin of such solvation effects. The progress in the modeling studies is described. Apolar complexation also occurs in organic solvents. Solvents like 2,2,2-trifluoroethanol and ethylene glycol come close to water in their ability to promote apolar complexation. Binding strength decreases from water to polar protic to dipolar aprotic and to apolar solvents. Complexation strength in solvents of all polarity including water and in binary aqueous solvent mixtures is predictable according to a linear free energy relationship between the complexation free energy and the empirical solvent polarity parameter $E_T(30)$.

INTRODUCTION

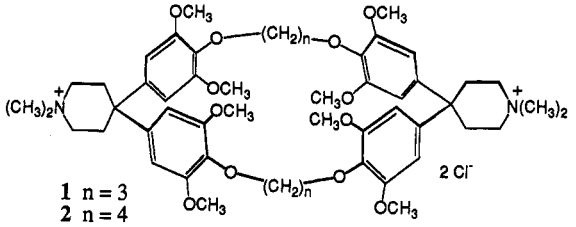
Over the past decade, we have prepared a variety of large cyclophanes with structurally defined cavities that provide binding sites of pronounced apolar character.[1,2] In water and organic solvents, these synthetic hosts form inclusion complexes with apolar organic guest molecules of complementary size and shape. In aqueous solution, the complexes are similar to enzyme-substrate complexes in their stability and specificity. The range of substrates extends from flat aromatic compounds to large aliphatic guests such as steroids. The great variety of cyclophane receptors now available permits the detailed analysis of the driving forces for molecular complexation in biological and chemical systems. Both experimental binding studies and computer liquid phase simulations are used for this analysis and ultimately should lead to an individual molecular level description of solvated host-guest systems.

This account describes how the nature of the solvent and specific solvation effects determine the stability of molecular complexes. Water is the essential biological fluid which promotes apolar aggregation and complexation processes necessary to sustain all functions of life. Therefore, complexation studies in water have been of special interest to us and are the focus of the first part of this report. In the following section, apolar binding strength and selectivity are analyzed as a function of the composition of binary aqueous solvent mixtures. Finally, comparative studies in water and organic solvents of a wide range of polarity dramatically demonstrate the central role solvent plays in the stability of apolar solution complexes.

ENTHALPICALLY CONTROLLED MOLECULAR COMPLEXATION IN WATER

Structure of cyclophanes and their complexes with aromatic guests To analyze the thermodynamic characteristics for the complexation of neutral benzene derivatives in water, cyclophanes **1** and **2** were prepared.[3] The X-ray crystal structure of **1** (Fig. 1) shows a deep apolar cavity shaped by the four aromatic rings of two diphenylmethane units. Two highly disordered water molecules, not shown in Fig. 1, are located inside the cavity. Two quaternary ammonium centers that provide water-solubility are attached to sites remote from the cavity. Therefore, the strong hydration of these ions does not perturb the hydrophobic character of the binding site.[1] The methoxy groups in **1** and **2**, oriented in the planes of the benzene rings to which they are attached, provide depth to the binding sites. In addition, they efficiently prevent the cyclophanes from forming aggregates in aqueous solution.[4] The formation of aggregates is highly undesirable in investigations of stoichiometric host-guest complexation.[2]

Cyclophanes **1** and **2** form stable 1:1 complexes with neutral *para*-disubstituted benzene derivatives (Table 1). We have undertaken a careful conformational analysis of the complexes of **1**.[5] The X-ray coordinates of the cyclophane provided the starting point in the determination of low energy complex conformations in the liquid phase.



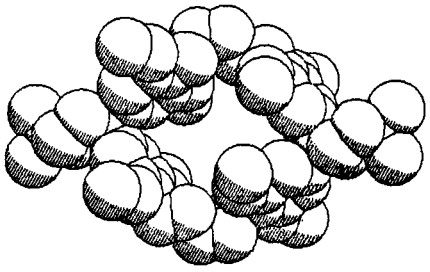


Fig. 1. Space-filling view of the X-ray crystal structure of **1**

A guest, such as *p*-xylene, is docked into the cavity prior to the conformational search in order to reduce the probability of generating nonproductive host conformations for binding. We have used the AMBER force field[6] and the Monte Carlo conformational search method in BATCHMIN,[7] both included in the MACROMODEL molecular modeling package,[8] to carry out our analysis. The low energy conformations of host-guest complexes located in the gas phase calculations were subjected to further solvation treatment to determine low energy conformers in the aqueous phase. The X-ray structure of **1** with two water molecules filling its cavity and the current lowest energy conformer of the **1**·*p*-xylene complex found after the surface area-based solvation treatment[9] are compared in Fig. 2. The similarity between the host geometry in the crystal and the calculated low-energy conformer is apparent. The conformations of the diphenylmethane units and the bridging dioxaalkane chains in both structures closely resemble each other.

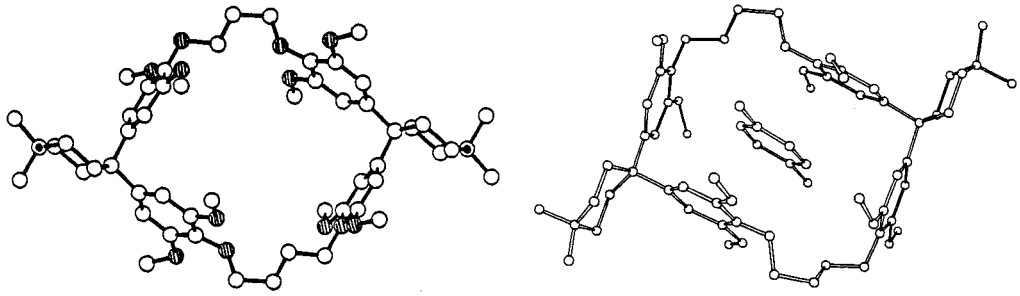


Fig. 2. Comparison of the X-ray crystal structure of cyclophane **1** and the current calculated lowest energy conformer of the **1**·*p*-xylene complex in aqueous solution. The two disordered water molecules in the cavity of the crystalline host are not shown.

The *p*-xylene complex in Fig. 2 reveals several general structural features of cyclophane-arene complexes.[10-16] The host adopts the geometry of a molecular box surrounded by four aromatic walls. Tight encapsulation of *p*-xylene generates a large number of contacts between the binding partners. The complex is stabilized by attractive π-π stacking interactions between parallel aromatic surfaces and by edge-to-face interactions between perpendicularly aligned aromatic rings of host and guest. Those same characteristic aromatic ring interactions are observed in biological systems, e.g., between aromatic amino acid side chains in proteins.[17]

Stability of cyclophane–benzene complexes in water Table 1 shows the stability data for the 1:1 inclusion complexes of *para*-disubstituted benzene derivatives with hosts **1** and **2** in water and methanol.[3] The thermodynamic characteristics ΔH° and $T\Delta S^{\circ}$ were obtained by van't Hoff analysis of ^{1}H NMR binding titrations at various temperatures. Over temperature ranges as large as 50 °C, the van't Hoff plots were linear indicating the absence of any significant heat capacity changes ΔC_p° accompanying the binding processes. The ΔH° values from the van't Hoff analysis are presently being confirmed by directly measuring the heats of complexation with a microcalorimeter.[18]

The results (Table 1) show that binding in water is entropically unfavorable (large negative $T\Delta S^{\circ}$) and is predominantly enthalpy-driven (large negative ΔH°). Obviously, a part of this favorable enthalpy results from attractive host-guest interactions in the tight complexes formed. However, a comparison of the thermodynamic data for complexation in water and methanol shows that water intrinsically provides a larger part of the enthalpic driving force. Complexation in methanol is much weaker, mainly as a result of a less favorable enthalpic term. The large difference in enthalpic driving force for complexation in water and methanol cannot be explained by differences in host-guest interactions. According to ^{1}H NMR analysis, the complexation geometries are similar in both solvents. Therefore, a large portion of the favorable enthalpic component observed in water must result from specific solvent contributions.

TABLE 1: Association constants K_a and enthalpic (ΔH^o) and entropic ($T\Delta S^o$) contributions to the free energies of complexation (ΔG^o) at 293.4 K for complexes of cyclophanes **1** and **2** with 1,4-disubstituted benzene guests in D_2O and methanol-d_4.

	K_a L mol^{-1}	ΔG^o kcal mol^{-1}	ΔH^o kcal mol^{-1}	$T\Delta S^o$ kcal mol^{-1}
Complexes of **2** in D_2O				
Dimethyl *p*-benzenedicarboxylate	1.2×10^5	- 6.81	- 10.7 ± 1.0	- 4.0 ± 1.0
p-Nitrotoluene	3.0×10^4	- 6.01	- 9.6 ± 3.0	- 3.6 ± 3.0
p-Dimethoxybenzene	1.0×10^4	- 5.38	- 10.2 ± 2.5	- 4.8 ± 2.5
p-Xylene	9.3×10^3	- 5.33	- 7.4 ± 1.0	- 2.1 ± 1.0
p-Benzodinitrile	7.8×10^3	- 5.23	- 9.5 ± 1.0	- 4.3 ± 1.0
Complexes of **1** in D_2O				
p-Nitrotoluene	2.1×10^3	- 4.47	- 8.5 ± 2.5	- 4.0 ± 2.5
Dimethyl *p*-benzenedicarboxylate	2.1×10^3	- 4.45	- 8.0 ± 1.0	- 3.5 ± 1.0
p-Xylene	1.3×10^3	- 4.18	- 6.4 ± 1.0	- 2.2 ± 1.0
p-Benzodinitrile	1.0×10^3	- 4.04	- 7.3 ± 1.0	- 3.3 ± 1.0
p-Dimethoxybenzene	3.7×10^2	- 3.45	- 5.7 ± 1.5	- 2.2 ± 1.5
Complexes of **2** in methanol-d_4				
p-Benzodinitrile	2.4×10^1	- 1.86	- 4.2 ± 1.5	- 2.4 ± 1.5
p-Dimethoxybenzene	8×10^0	- 1.20	- 4.4 ± 1.5	- 3.2 ± 1.5

Apolar complexation processes in water are characterized by large gains in solvent cohesive interactions. The water molecules that solvate the apolar surfaces of the free guests and especially the cavity of the free host have reduced cohesive interactions and are enthalpically higher in energy than the water molecules in the bulk solvent. Water molecules around apolar surfaces participate in fewer strong hydrogen bonds than bulk solvent molecules. Upon inclusion complexation, these water molecules are released into the bulk and become enthalpically lower in energy. Cohesive interactions in methanol are weaker than in water, and therefore less enthalpy is gained upon transfer of methanol molecules that solvate the complementary host-guest surfaces into the bulk.

A second contribution to the favorable enthalpic term, especially significant for complexation in water, results from the substitution of less favorable dispersion interactions between water molecules and the complementary host-guest surfaces by more favorable dispersion interactions between the hydrocarbon surfaces in the complex. The attractive B term in the Ar^{12} - Br^6 Lennard-Jones potential which describes London dispersion interactions is proportional to the polarizability α of the interacting atoms and groups. Dispersion energies are additive, and at constant distances between interacting atoms, the attractive forces increase with increasing atom polarizability. Oxygen atoms (α = 0.84 Å^3) and hydroxyl residues (α = 1.20 Å^3), the constituents of water, have low polarizability whereas the polarizabilities of hydrocarbon residues, e.g., a CH_2 group (α = 1.77 Å^3), a CH_3 group (α = 2.17 Å^3), or an aromatic CH group (α = 2.07 Å^3) are much larger.[19] Therefore, the dispersion forces between water molecules and a hydrocarbon surface are weaker than the forces between two hydrocarbon surfaces. Upon complexation by **1** or **2** with an aromatic guest, less favorable water-solute contacts are replaced by more favorable contacts between the surfaces of the two binding partners. Methanol, on the other hand, possesses a polarizable methyl group which interacts favorably with the host and guest. Therefore, the differences in dispersion interactions between the nonbinding and the binding states in methanol are not as large.

Cyclophanes represent only one class of receptors that complex apolar solutes in an enthalpy-driven way. Similar thermodynamic characteristics to those in Table 1 have been measured for tight binding of aromatic substrates in hydrophobic pockets of enzymes and antibodies,[20] in the cavity of cyclodextrins,[21] and in the narrow AT-rich region of the DNA minor groove.[22] These thermodynamic characteristics (large negative ΔH^o and negative $T\Delta S^o$) observed for a wide range of biotic and abiotic complexation processes of small molecules in water are in sharp contrast to those observed for the transfer of apolar solutes from water into organic solvents or the gas phase and for the formation of micelles and membranes.[23] These processes are characterized by $\Delta H^o \approx 0$, $T\Delta S^o > 0$, and $\Delta C_p^o < 0$ as measurable quantities. Processes leading to molecular complexes with a tight fit between the interacting components seem to be enthalpy-driven. High substrate selectivity in a binding event is normally characterized by a tight fit of the interacting molecules. A tight fit provides a large number of close van der Waals contacts but considerably reduces the degrees of freedom of the binding partners in the complex. Therefore, we and others measure large negative entropy components for tight complex formation in water. Desolvation of the complementary apolar surfaces of the guest should provide a favorable entropy component. However, this component is masked by the larger loss in entropy of the two binding partners. A more favorable overall entropy for tight complexation should be measured if larger surfaces are desolvated than in the binding of benzene derivatives by **1** and **2**.

In aggregation processes and phase-transfer processes for which a strong entropic driving force is measured, a major reduction in the degrees of freedom of the solutes involved does not occur. Hence, the entropically favorable desolvation processes lead to measurable positive entropy terms. The interactions between the components in micelles and membranes are less tight than in molecular complexes, and only small overall enthalpy terms are determined for the formation of these aggregates.[23]

Apolar binding in water is often referred to as "hydrophobic binding". The terms "hydrophobic effect", "hydrophobic interactions", and even "hydrophobic bond" are widely used to describe the phenomenon that the interactions between apolar surfaces of solutes and solvating water molecules are less favorable than the cohesive interactions of these water molecules in the bulk and the interactions between associated apolar surfaces. As shown above, binding and association processes in water may either be enthalpically or entropically driven. Unfortunately, in the past hydrophobic bonding has often been seen uniquely as an entropically driven process. The characterization of apolar binding processes in water as "hydrophobic" is acceptable as long as no specific thermodynamic quantities are implied.

APOLAR COMPLEXATION IN BINARY AQUEOUS SOLVENT MIXTURES

Linear free energy relationships predict complexation strength For a variety of reasons, biotic and abiotic complexation studies are often executed in aqueous solvent mixtures rather than in pure aqueous solution. (i) The addition of an organic co-solvent can enhance the solubility of a receptor, substrate, or complex. (ii) For very strong association processes in water, the binding strength might be too large and/or the complexation-decomplexation rate too slow for an evaluation of the thermodynamic quantities using ^{1}H NMR or other spectral binding titrations. In these cases, the addition of organic co-solvents can lower the binding strength, increase the rate of exchange, and meaningful ^{1}H NMR titrations in adequate concentration ranges become possible. (iii) Medium-induced conformational changes are well documented in peptide chemistry. For example, the addition of 2,2,2-trifluoroethanol to aqueous solutions is known to specifically stabilize peptide α-helices.[24] Therefore, it is desirable to understand how the composition of binary aqueous solvent mixtures affects apolar binding phenomena.[25]

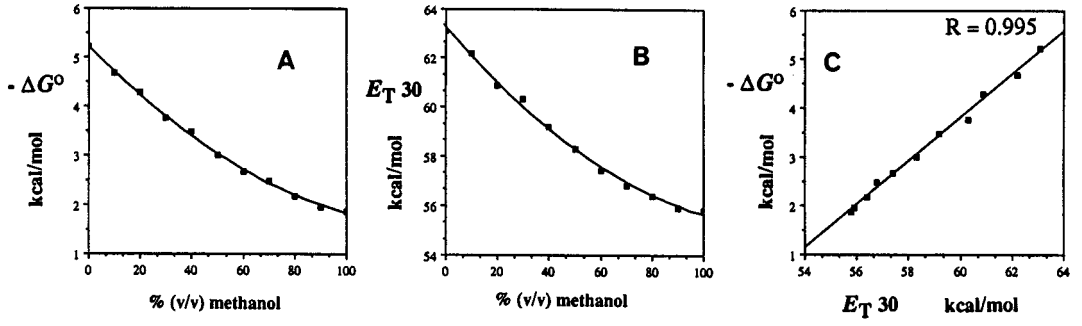


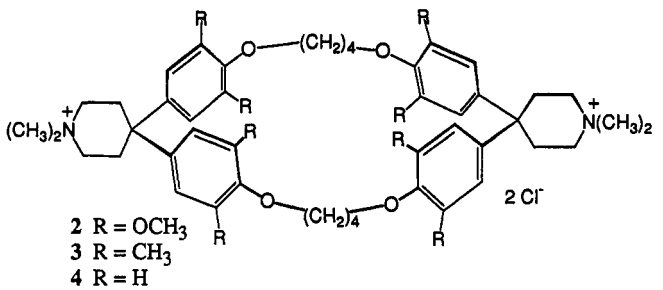
Fig. 3. (A) Free energy of formation of the **2**·*p*-benzodinitrile complex versus % (v/v) methanol, (B) solvent polarity parameter $E_T(30)$ versus % (v/v) methanol, and (C) LFER between the free energy of formation of the **2**·*p*-benzodinitrile complex and $E_T(30)$ in D_2O-CD_3OD mixtures.

We have investigated the complexation of *p*-benzodinitrile with host **2** as a function of the composition of water-methanol mixtures.[26] Fig. 3.A shows that the complexation free energy decreases in a nonlinear way with increasing methanol content of the solvent mixture. A similar correlation is obtained when the empirical solvent polarity parameter $E_T(30)$ is plotted against solvent composition (Fig. 3.B). Consequently, there exists an excellent linear free energy relationship (LFER) between the free energy of formation of the **2**·*p*-benzodinitrile complex and $E_T(30)$ (Fig. 3.C). Additional LFER's have been obtained for the **2**·*p*-dimethoxybenzene complex in D_2O-CD_3OD mixtures and for complexes of **2** in D_2O-$(CD_3)_2SO$ mixtures.[26] Establishing valid LFER's is important for rationalizing solvation effects and predicting complexation strength in binary solvent mixtures.

The role of substituents at the aromatic rings of cyclophanes In addition to the octamethoxy-cyclophanes **1** and **2**, we have prepared a variety of hosts bearing either methyl groups (in **3**) or hydrogen atoms (in **4**) on the aromatic rings *ortho* to the bridges.[2] The properties of the structurally similar macrorings **2**-**4** are substantially different; quantitative comparisons of their binding ability were performed in D_2O-CD_3OD (60:40, v/v).[26]

First, substituents in both positions *ortho* to the bridges as in **2** and **3** have a pronounced effect on the conformation of the macrocycles. In cyclophane **4** without *ortho*-substituents, the torsional angles Θ about the aryl ether C-O bonds are close to 0° placing the O-CH_2 bonds into the planes of the aromatic rings. In sharp contrast, these torsional angles in cyclophanes **2** and **3** are close to 90° for steric reasons. This geometry directs the first CH_2-unit of the bridge either in or out of the cavity. Models of anisole and 2,6-dimethylanisole (Fig. 4) illustrate this effect.

Secondly, the critical aggregation concentrations (cac's) of **2**-**4** differ dramatically in water.[2,4] As stated above, stoichiometric complexation studies in water are only meaningful in the absence of additional aggregation equilibria. Therefore, binding studies should be performed below the cac's of both the host and guest. The cac of **4** was determined as 1.6×10^{-4} mol L^{-1} (^{1}H NMR, light scattering). Upon introduction of methyl groups, aggregation becomes more favorable giving a low cac value of $< 2 \times 10^{-5}$ mol L^{-1} for **3** (estimated by ^{1}H NMR). In contrast, methoxy groups reduce the aggregation tendency resulting in a high cac value of $\approx 1 \times 10^{-2}$ mol L^{-1} for cyclophane **2**. The cac's of **3** and **4** are too low to perform meaningful binding studies in pure water. Therefore, to compare the binding properties of **2**-**4**, studies were performed in D_2O-CD_3OD (60:40, v/v) where aggregation of these macrocycles does not occur.



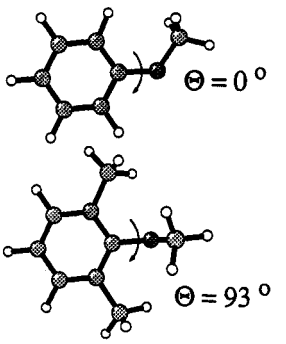


Fig. 4. Optimized geometries of anisole and 2,6-dimethylanisole illustrating the differences in the dihedral angles about the aryl ether linkages in hosts **2-4**.

TABLE 2: Association constants K_a and free energies of complexation ΔG^o at 293 K for complexes of cyclophanes **2-4** with 1,4-disubstituted benzene guests in D_2O-CD_3OD (60:40, v/v).

	Host	K_a L mol^{-1}	ΔG^o kcal mol^{-1}
Guest: *p*-Benzodinitrile			
	2	390	- 3.48
	3	1580	- 4.29
	4	140	- 2.89
Guest: *p*-Dimethoxybenzene			
	2	340	- 3.41
	3	580	- 3.72
	4	95	- 2.66

Table 2 shows that the octamethyl host **3** is the best binder, followed by the octamethoxy host **2** and unsubstituted cyclophane **4**.[26] The methoxy groups deepen the cavity making **2** a better binder than **4**. However, hydrogen-bonding between the methoxy groups and water molecules provides some favorable solvation to parts of the cavity and therefore, the solvation-dependent driving forces for apolar binding are reduced. A partial favorable cavity solvation does not occur in the octamethyl derivative **3** giving its deep cavity a pronounced apolar character resulting in strong complexation.

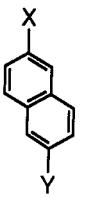
ELECTRON DONOR–ACCEPTOR INTERACTIONS IN CYCLOPHANE COMPLEXES

The formation of molecular complexes between electron-rich (donor) and electron-deficient (acceptor) π-systems has been the subject of intensive investigations since the 1950's.[27,28] Major components of the attractive interactions in intermolecular electron donor-acceptor (EDA) complexes are defined as electrostatic, polarization, and charge-transfer interactions as well as dispersion energy. EDA complexes showing a distinctive bathochromically shifted absorption band, not present in the spectra of each of the two associating partners, are described as charge-transfer complexes. However, most studies seem to agree that the charge-transfer term does not contribute significantly to the ground-state stabilization of EDA complexes but rather stabilizes their excited state.

To explore the contributions of EDA interactions on the stability of inclusion complexes formed by cyclophanes, the binding between host **3** and a series of 2,6-disubstituted naphthalene derivatives (**5a-i**) was investigated in CD_3OD and $(CD_3)_2SO$.[29] In the cavity of **3**, the naphthalene derivatives adopt a similar binding geometry to that of *p*-xylene in the cavity of host **1** (Fig. 2). This complex geometry favors π-π host-guest stacking interactions. ^1H NMR binding titrations reveal that electron-deficient guests **5g-i** having two acceptor substituents form more stable complexes than the electron-rich guests **5a-c**, while complexation of guests that have one donor and one acceptor substituent (**5d-f**) demonstrate an intermediate stability (Table 3). The difference in complex stability is as large as ≈ 1.5 kcal mol^{-1} resulting mainly from intermolecular EDA interactions. With its four trialkyl-substituted anisole units, compound **3** can be considered as a donor host. As expected for EDA interactions, the most stable complexes are formed with the acceptor guests **5g-i**. The same trends in complexation strength are observed in $(CD_3)_2SO$.[29]

TABLE 3: Association constants K_a and free enthalpies of complexation ΔG^o for the 1:1 complexes formed between cyclophanes **2** and **3** and the naphthalene derivatives **5a-i** in CD_3OD.[29]

Guest	X	Y	K_a L mol^{-1}	ΔG^o kcal mol^{-1}
	Host **3**, T = 303K			
5a	OH	OH	24	- 1.91
5b	NH_2	NH_2	33	- 2.11
5c	OCH_3	OCH_3	47	- 2.32
5d	NH_2	NO_2	102	- 2.78
5e	OCH_3	NO_2	109	- 2.82
5f	OCH_3	CN	117	- 2.87
5g	$COOCH_3$	$COOCH_3$	188	- 3.15
5h	NO_2	NO_2	213	- 3.23
5i	CN	CN	277	- 3.39
	Host **2**, T = 293 K			
5c	OCH_3	OCH_3	12	- 1.47
5f	OCH_3	CN	31	- 2.00
5i	CN	CN	78	- 2.54



5a-i

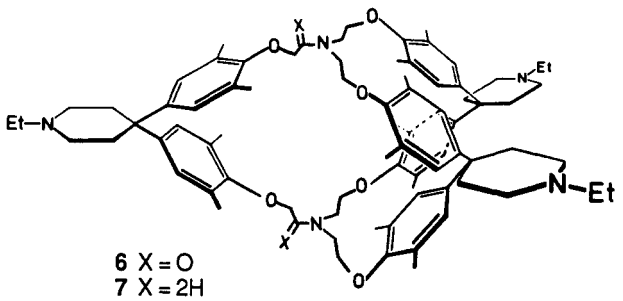
Cyclophane **2**, with its four trialkoxy-substituted benzene rings, was originally designed to be an even better donor host than **3**. In methanol, the stability of the complexes formed between **2** and naphthalene derivatives indeed correlates with the donor-acceptor properties of the guests (Table 3).[26] However, as shown in Table 1, the binding free energies measured for the complexes of benzene substrates and **2** in water do not correlate with the donor-acceptor potential of the guests. Apparently, in water EDA contributions to the stability of the benzene complexes are masked by specific solvation effects that are at present poorly understood. The substituents of the *p*-disubstituted benzene derivatives are located more inside the host cavity than those at the 2,6-positions in the naphthalene guests. Therefore, contributions to the observed binding from the solvation of the polar guest functionalities and from steric, dipolar, and polarization interactions between the various guest substituents and the host are more pronounced in the benzene than in the naphthalene complexes.

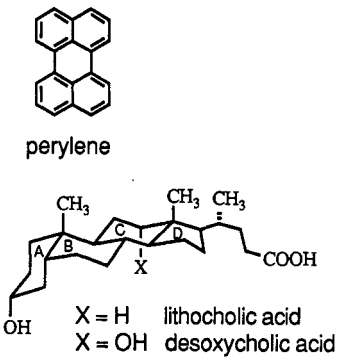
BINDING SELECTIVITY THROUGH COMPLEXATION-INDUCED CHANGES IN THE SOLVATION OF FUNCTIONAL GROUPS

The masking of EDA interactions in complexes of benzene derivatives and cyclophane **2** (Table 1) presumably results from specific solvation effects of the substituents. Here, two examples are given, where the changes in the solvation of functional groups in either host or guest during the complexation process dramatically influence complexation strength. These two examples illustrate a very general principle. If the energetically favorable solvation of a polar functional group of one of the binding partners is reduced in the complex as compared to the free component, and if no new binding interaction compensates for this loss in solvation energy, a considerable reduction in complexation strength is observed.[2]

Cryptand hosts **6** and **7** are structurally very similar and differ only by their nitrogen functionality.[2] The formation free energy of the **7**·perylene complex in methanol at 303 K is ΔG^o = - 7.0 kcal mol^{-1} (K_a = 1.1 x 10^5 L mol^{-1}) whereas the complexation of perylene by **6** is less favorable by 3.2 kcal mol^{-1} (ΔG^o = - 3.8 kcal mol^{-1}, K_a = 5.6 x 10^2 L mol^{-1}). According to CPK model examinations and ^{1}H NMR studies, the two perylene complexes have very similar geometries and closely resemble the **7**·pyrene complex shown below in Scheme 2. Therefore, we explain the large difference in binding strength by a complexation-induced reduction in the solvation of the two amide groups in the periphery of the cavity of **6**. Other examples also support that a reduction in the solvation of amide groups is particularly costly.

Cyclophane **8** with a large cavity shaped by two naphthylphenylmethane units forms inclusion complexes with a variety of steroid substrates in aqueous solutions.[30] According to CPK model examinations, the steroids are incorporated axially in the cavity of **8** (Fig. 5). In support of an axial inclusion geometry, the ^{1}H NMR resonances of the steroid methyl groups show characteristic upfield shifts in the complexes. The polar, strongly solvated groups at C(3) in ring A and in ring D of the steroids are oriented into the aqueous solution.





High binding selectivity was observed in the complexation of structurally similar bile acid derivatives. In D_2O-CD_3OD (1:1, v/v) at 293 K, the complex of lithocholic acid (K_a = 7075 L mol^{-1}, ΔG^o = - 5.18 kcal mol^{-1}) is much more stable than the complex of desoxycholic acid (K_a = 250 L mol^{-1}, ΔG^o = - 3.21 kcal mol^{-1}) which has an additional hydroxy group at C(12α). According to ^{1}H NMR analysis, the C and D rings of lithocholic acid are preferentially encapsulated by host **8** (Fig. 5). This generates a large number of favorable contacts between the apolar surfaces of the rings and the cavity walls. A similar orientation of desoxycholic acid in the cavity would require considerable desolvation of the hydroxy group at C(12α) since it would be located deep inside the apolar cavity. Apparently this is too costly, and inclusion occurs in a different orientation to minimize the energetically unfavorable desolvation of the hydroxy group. The characteristic complexation-induced upfield shifts of the steroid methyl resonances in the ^{1}H NMR spectrum indicate that desoxycholic acid is preferentially encapsulated with ring B which positions the hydroxy group more outside the cavity. The solvation-induced changes in inclusion geometry destabilize the complex of desoxycholic acid by ≈ 2 kcal mol^{-1} as compared to the complex of lithocholic acid.

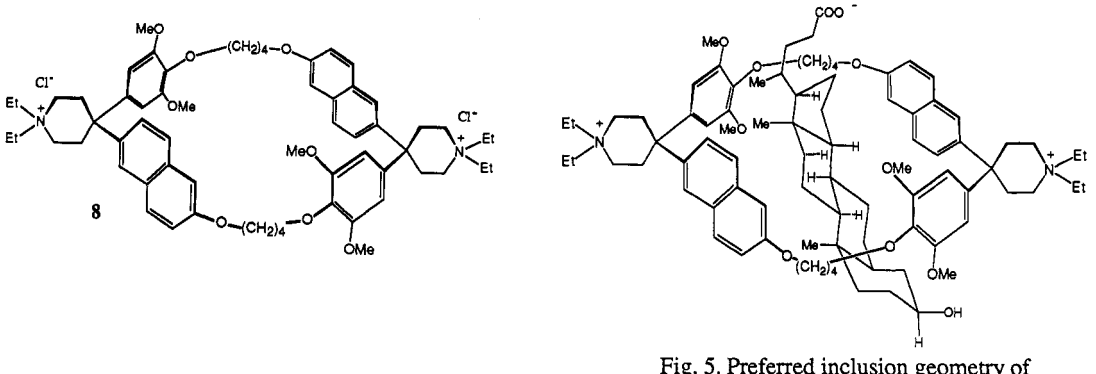


Fig. 5. Preferred inclusion geometry of lithocholic acid.

COMPUTER LIQUID PHASE SIMULATIONS

To fully evaluate the influence of solvation effects in aqueous solution, we decided to carry out liquid phase simulations on the complexes formed between host **1** and *p*-disubstituted benzenes.[5] Monte Carlo statistical mechanics and molecular dynamics simulations using perturbation theory techniques have been used by McCammon,[31] Kollman,[32] and Jorgensen[33] to obtain relative free energies of hydration and binding. Computational results compare favorably with available experimental thermodynamic data and have led to correct predictions.[34] We intend to calculate relative binding free energies for complexes of host **1** based on the thermodynamic cycle shown in Scheme 1. Our condensed phase simulations are based on the OPLS (optimized potential for liquid simulations) potential functions and the Monte Carlo statistical mechanics calculation program BOSS.[35] In order to reduce the complexity of the Monte Carlo calculations, the host will be kept rigid in low energy conformations throughout the simulations. Experimental observations and computer conformer searches justify this approach. Here, a brief account on our efforts to generate suitable OPLS parameters is given.

To correctly describe by OPLS potential functions the close interactions between aromatic residues in our complexes and the interactions between aromatic residues and surrounding solvent molecules, a careful parametrization is needed. For this purpose, we investigated by *ab initio* calculations the structures and energetics of monohydrates of various aromatic guests and of the aromatic rings that shape the binding site in **1**. Two interesting examples of monohydrate configurations, calculated at the RHF/6-31G* level,[36] are shown in Fig. 6. In the configurations having one O-H bond of water perpendicular to the aromatic π-system, the best hydrogen-bonding-type interaction expectedly occurs with the more electron-rich benzene derivatives (Fig. 6.A). A second significant interaction occurs

between the water oxygen atom and the aromatic C-H bonds, particularly those polarized by electron-withdrawing nitrile substituents (Fig. 6.B).

Scheme 1

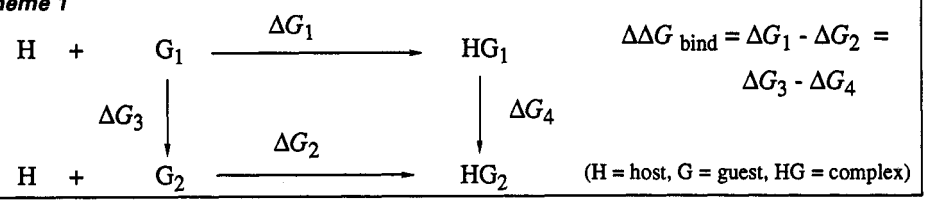
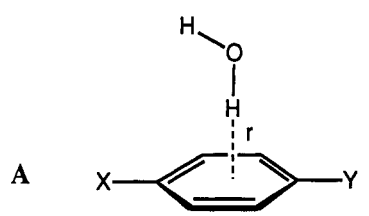
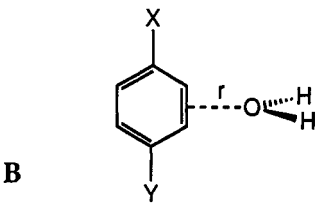


Fig. 6: Monohydrate configurations with water interacting (A) with the aromatic π-system and (B) with the aromatic C-H bonds. The results are calculated at the RHF/6-31G* level.





X	Y	r(Å)	ΔE (kcal mol^{-1})	X	Y	r(Å)	ΔE (kcal mol^{-1})
CN	CN	3.10	+ 0.07	CH_3	CH_3	3.60	- 1.2
CN	H	2.81	- 1.2	H	H	3.53	- 1.5
H	H	2.67	- 2.1	CN	H	3.40	- 2.8
CH_3	CH_3	2.64	- 2.3	CN	CN	3.28	- 4.2
OCH_3	H	2.64	- 2.3				
NH_2	NH_2	2.64	- 2.4				

The intermolecular solute-solvent and host-guest interactions (i.e. the interactions between two molecules a and b) are described by OPLS potential functions through Coulomb and Lennard-Jones terms, as given in eq. 1.[35]

$$\Delta E_{ab} = \sum_{i}^{\text{on a}} \sum_{j}^{\text{on b}} \left(\frac{q_i q_j e^2}{r_{ij}} + \frac{A_{ij}}{r_{ij}^{12}} - \frac{C_{ij}}{r_{ij}^{6}} \right) \qquad (1)$$

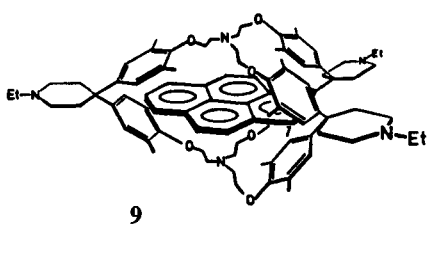
The host, guest, and solvent are represented by interaction sites located on the nuclei (except for water) that have charges, q_i, and Lennard-Jones parameters, σ_i and ε_i. The parameters σ_i and ε_i are related to A_{ij} and C_{ij} by $A_{ii} = 4\varepsilon_i\sigma_i^{12}$, $C_{ii} = 4\varepsilon_i\sigma_i^{6}$, $A_{ij} = (A_{ii}A_{jj})^{1/2}$, and $C_{ij} = (C_{ii}C_{jj})^{1/2}$. To ensure the quality of the generated OPLS parameters, Jorgensen's program TIPOPT is applied. The parameters are optimized to reproduce monohydrate structures and energetics consistent with *ab initio* results. Moreover, since dispersion interactions are important binding forces in the cyclophane-arene complexes, pure liquid simulations are performed to check whether the cohesive interactions are correctly described by the OPLS parameters. In a pure apolar liquid, e.g., benzene or *p*-xylene, the dispersion term is the major component of the cohesive interactions. Our OPLS parameters for *p*-xylene and other benzene derivatives reproduce accurately the monohydrate configurations and energies calculated by *ab initio* methods. Also, density and thermodynamic quantities of pure liquid *p*-xylene could be calculated with these parameters in excellent agreement with experimental values. A final check of the OPLS parameters was carried out in free energy perturbation calculations. We were able to reproduce the experimentally observed difference in free energy of hydration between benzene and *p*-xylene by using our OPLS parameters for *p*-xylene and those developed by Jorgensen et al. for benzene.[37]

Following the careful conformational analysis described in the beginning of this report and with high quality OPLS parameters for aromatic systems in hand, we will now continue to calculate the differences in free energy $\Delta\Delta G_{bind}$ between complexes formed by host **1** and *p*-disubstituted benzene guests using free energy perturbation cycles (Scheme 1), the results of which will be compared against experimental values. Subsequently, calculations are planned to predict the stability of new complexes of **1** for which no experimental data are available.

APOLAR COMPLEXATION IN ORGANIC SOLVENTS

To compare apolar binding strength in aqueous and organic solvents (Table 4), the pyrene complex **9** was ideal for the following reasons:[38] (i) Host **7** and complex **9** are soluble in solvents that span the entire polarity range. In some studies, dimethylsulfoxide (1 or 10 % v/v) was added as a co-solvent to increase the solubility of free pyrene. (ii) According to extensive ^{1}H NMR studies, complex **9** adopts a similar geometry in all solvents (Scheme 2)[39]. (iii) Even in apolar solvents, binding energies are large enough to be accurately obtained. (iv) All solvent molecules in Table 4 are small enough to easily enter and exit the large, highly preorganized host cavity without causing major conformational strain. Therefore, the host cavity is solvated before pyrene is bound, which is an important criterion for studies comparing solvent effects on complexation strength. Solvent comparisons would not be meaningful if differences in binding strength resulted from the fact that one solvent molecule solvates the binding site whereas a second, larger solvent molecule does not fit leaving a nonsolvated, empty cavity. Table 4 shows the free energies of formation ΔG^o (kcal mol^{-1}) of complex **9** in eighteen solvents at T = 303 K as well as the empirical solvent polarity parameter E_T (30) (kcal mol^{-1}) measured for these solvents. The linear free energy relationship between the free energies of formation of **9** and the solvent polarity parameter E_T(30) is shown in Fig. 7.[40]

Scheme 2



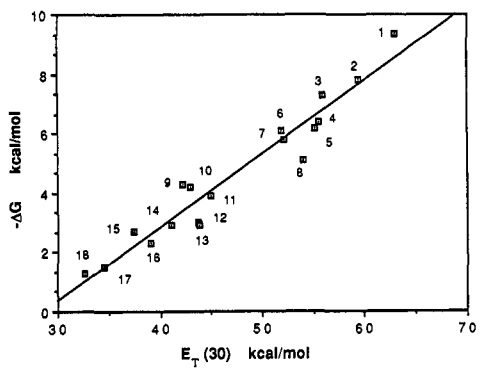


Fig. 7. Dependence of the free energy of formation -ΔG^o of complex **9** on the solvent polarity as expressed by E_T(30) values.

Table 4: Free energies of formation ΔG^o of complex **9** in eighteen solvents of different polarity as expressed by E_T(30) values, T = 303 K.

Run	Solvent	ΔG^o kcal mol^{-1}	E_T(30) kcal mol^{-1}
1	Water [a,b]	- 9.4	63.0
2	2,2,2-Trifluoroethanol [b]	- 7.8	59.4
3	Ethylene glycol [c]	- 7.3	55.9
4	Methanol	- 6.4	55.5
5	Formamide [c]	- 6.2	55.2
6	Ethanol	- 6.1	51.9
7	*N*-Methylacetamide [c]	- 5.8	52.1
8	*N*-Methylformamide [c]	- 5.1	54.0
9	Acetone	- 4.3	42.2
10	*N*,*N*-Dimethylacetamide [c]	- 4.2	43.0
11	Dimethylsulfoxide-d_6 [d]	- 3.9	45.0
12	*N*,*N*-Dimethylformamide-d_7 [c]	- 3.0	43.7
13	*N*,*N*-Dimethylformamide-d_7	- 2.9	43.8
14	Dichloromethane-d_2	- 2.9	41.4
15	Tetrahydrofuran-d_8	- 2.7	37.4
16	Chloroform-d_1	- 2.3	39.1
17	Benzene-d_6	- 1.5	34.5
18	Carbon disulfide	- 1.3	32.6

[a] Contains [Na_2CO_3] = 10^{-3} mol L^{-1}. [b] Contains 1% (v/v) Me_2SO. [c] Contains 10 % (v/v) Me_2SO. [d] H/D isotope effect below the error in ΔG^o.

The following conclusions are drawn from the data in Fig. 7 and Table 4:

(i) The empirical solvent polarity parameter E_T(30) is useful for predicting the strength of apolar host-guest complexation in solvents of all polarities. The correlation coefficient for the LFER in Fig. 7 is R = 0.934. This correlation allows to predict binding free energies of complex **9** in additional solvents according to the equation -ΔG^o = 0.25 E_T - 7.1 (kcal mol^{-1}).
(ii) The dependency of complexation strength on the nature of the solvent is impressive. Upon changing from the most polar solvent, water, to the least polar solvent considered in this study, carbon disulfide, complexation free energies decrease from ΔG^o = - 9.4 kcal mol^{-1} to ΔG^o = - 1.3 kcal mol^{-1} giving a difference in binding free energy of $\Delta(\Delta G^o)$ = 8.1 kcal mol^{-1}.
(iii) The linear free energy relationship also holds for water. Binding strength decreases regularly from water to polar protic solvents to dipolar aprotic solvents and to apolar solvents. Water does not promote apolar complexation beyond the level expected on the basis of its physical properties such as dielectric constant, polarizability, or dipole moment which are expressed in the solvent polarity parameter.
(iv) Of great interest is the finding that some organic solvents approach water (ΔG^o = - 9.4 kcal mol^{-1}) in their potential for promoting apolar complexation. Strong pyrene complexation is observed in 2,2,2-trifluoroethanol (ΔG^o = - 7.8 kcal mol^{-1}) and in ethylene glycol (ΔG^o = - 7.3 kcal mol^{-1}). This also supports that the magnitude of apolar binding in water does not need a special explanation.

In the first chapters of this account, we proposed that water is a better solvent than methanol for apolar complexation since water is composed of hydroxy groups of low polarizability and is characterized by larger solvent cohesive interactions. The analysis of the data in Table 4 shows that apolar binding is generally strongest in solvents with low

molecular polarizability and with high cohesive interactions.[38] Solvent molecules with high cohesive interactions interact more favorably with bulk solvent molecules than with the complementary apolar surfaces of free host and guest, and therefore, energy is gained upon the release of surface-solvating solvent molecules into the bulk during the complexation step. Upon complexation, the less favorable dispersion interactions between solvent molecules of low polarizability and highly polarizable hydrocarbon surfaces are replaced with more favorable dispersion interactions between the complementary surfaces of host and guest. We measured the heats of formation of complex **9** in a microcalorimeter and found that all binding events in Table 4 are exothermic[41]. The largest negative enthalpy values were seen in polar protic solvents, e.g., ΔH^{o} = - 20.0 kcal mol^{-1} in 2,2,2-trifluoroethanol, whereas the enthalpy term is the least favorable in apolar solvents, e.g., ΔH^{o} = - 0.8 kcal mol^{-1} in benzene. The complexation entropies also show a large variation and, except for complexation in *N*,*N*-dimethylacetamide, all values are negative. By plotting $T\Delta S^{o}$ versus ΔH^{o}, a strong linear correlation with a coefficient of R = 0.976 is obtained. This unique isoequilibrium relationship which spans a wide range of solvents is now the subject of further investigations.

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