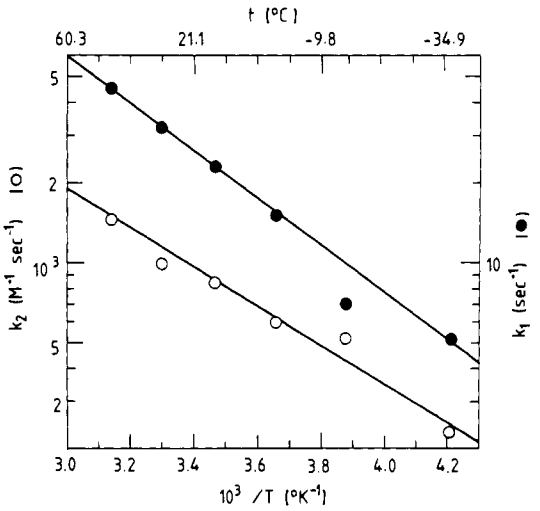

Figure 5. Arrhenius plots for the monomolecular k_1 and bimolecular k_2 rate constants derived from the analysis of the results in Figure 4.

The kinetic behavior suggests that there are two mechanisms involved in the transformation between the tautomers; one is first order with respect to the MDHP concentration while the other is second order. The first-order reaction involves the solvent in the protolytic reaction

$$\text{MDHP} + \text{Sol} \xrightarrow{k_1} \text{Sol H}^+ + \text{MDHP}^- \qquad (3)$$

where in the reverse reaction the protonation may occur at the second nitrogen. Actually this reaction may also proceed with remnant impurities still left in the solution that may serve as conjugate bases to the MDHP molecules. Therefore, the observed k_1 must be considered as an upper limit for the reaction with $CDCl_3$.

The second-order reaction can be considered as a proton exchange between two MDHP molecules as a result of a bimolecular collision. Alternatively, the reaction may involve proton transfer between MDHP molecules and disproportionation products of the self-ionization reaction

$$2\text{MDHP} \rightleftharpoons \text{MDHP}^+ + \text{MDHP}^- \qquad (4)$$

$$\text{MDHP}^{\pm} + (\text{MDHP}) \rightleftharpoons \text{MDHP} + (\text{MDHP}^{\pm}) \qquad (5)$$

where the + and − superscript refer to protonated or deprotonated species.

In concentrated solutions (above 20 mM MDHP) the bimolecular proton-transfer reaction dominates, while in dilute solutions, the exchange process is dominated by the monomolecular protolysis reaction.

There are no other published quantitative kinetic data on similar amidinic systems. However, preliminary results in our laboratory suggest that both structural and substitution effects are important in determining the tautomeric rate. In particular, it appears that the presence of phenyls instead of methyls in positions 2 and 6 of dihydropyrimidine considerably reduces the tautomeric rate, while reduction of the C=C double bond increases it. Similar substitution effects were also observed in dihydro-1,3,5-triazine derivatives.

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A New Water-Soluble Macrocyclic Host of the Cyclophane Type: Host–Guest Complexation with Aromatic Guests in Aqueous Solution and Acceleration of the Transport of Arenes through an Aqueous Phase

François Diederich* and Klaus Dick

Contribution from the Max-Planck-Institut für medizinische Forschung, Abteilung Organische Chemie, D-6900 Heidelberg, Federal Republic of Germany. *Received March 5, 1984*

Abstract: A concept is presented for the design of water-soluble macrocyclic hosts of the cyclophane type which possess a cavity of very pronounced hydrophobic character as binding site for apolar guests in aqueous solution. In host **8** four spiro piperidinium rings attached to the 1,7,21,27-tetraoxa[7.1.7.1]paracyclophane framework locate the water-solubility-providing quaternary ammonium nitrogens remote from the cavity. The geometry of **8** is discussed in terms of CPK molecular models. The aggregation behavior of **8** in aqueous solution was studied by ^{1}H NMR spectroscopy. Aqueous solutions of host–guest complexes of **8** with neutral arenes were prepared by solid-liquid and liquid-liquid extraction. Evidence for the formation of host–guest complexes with exclusive 1:1 stoichiometry was obtained, and host–guest association constants were determined by the two methods of extraction. Hydrophobic and van der Waals interactions are shown to be the major driving forces for the strong complexation of **8** with neutral arenes. The association constants of the complexes of **8** with fluorescing aromatic guests bearing polar or anionic residues were determined either directly from fluorescence titration curves or by a Benesi–Hildebrand treatment of the fluorescence data. Host–guest association constants were also estimated from competitive inhibition experiments. Compound **8** not only binds neutral arenes but also is a good host for aromatic guests bearing anionic (sulfonate) residues. Complexes of the latter type make use of attractive forces of both apolar and electrostatic nature. The transport of neutral arenes through an aqueous phase along a concentration gradient mediated by **8** as molecular carrier was studied in a U-type cell.

There exists a close relationship between the field of synthetic host–guest chemistry[1] and molecular recognition in biological systems. Artificial host–guest systems have been designed to mimic the binding of substrates by enzymes, antibodies, and receptors and the transport of ions across biological membranes mediated by ionophores as natural carriers.[2]

(1) (a) Cram, D. J.; Cram, J. M. *Science (Washington, DC)* **1974**, *183*, 803. (b) Cram, D. J. In "Applications of Biochemical Systems in Organic Chemistry"; Jones, J. B., Sih, C. J., Perlman, D., Eds. "Techniques in Chemistry"; Weissberger, A., Ed.; Wiley: New York, 1976; Vol. 10, Part II, pp 815–873. (c) Cram, D. J.; Cram, J. M. *Acc. Chem. Res.* **1978**, *11*, 8.

(2) (a) "Host Guest Complex Chemistry I"; Vögtle, F., Ed.; "Topics in Current Chemistry"; Springer: Berlin, 1981; Vol. 98. (b) "Host Guest Complex Chemistry II"; Vögtle, F., Ed.; "Topics in Current Chemistry"; Springer: Berlin, 1982; Vol. 101.

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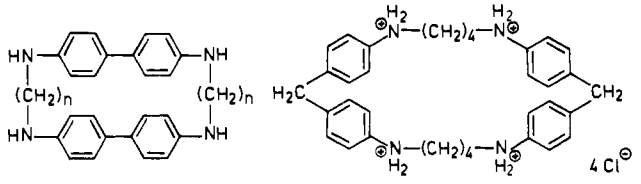
During the last decade, water-soluble macrocycles of the cyclophane type have been introduced as artificial hosts with hydrophobic cavities as selective binding sites for apolar guests, and complexation in aqueous solution has been studied.[3-8] The interest in water-soluble artificial hosts with apolar cavities was largely derived from the fact that hydrophobic intereactions are a major driving force for the binding of substrates in biological systems[9] and especially from the increasing insight into the mode of binding of enzymatic systems, provided by X-ray crystallographic studies. In several enzymes like α-chymotrypsin,[10] carboxypeptidase A,[11] and thermolysin,[12] hydrophobic pockets were found to be located at the active site. Selective binding of apolar substrate moieties in these pockets and, as a result, regioselectivity of the reactions catalyzed by these enzymes are observed. A well-known example is the regiospecific hydrolysis of proteins by α-chymotrypsin on the carboxyl side of aromatic amino acids as a consequence of the specific binding of aromatic rings in such a hydrophobic pocket located at the active site of the enzyme.[10]

Additional interest in this kind of artificial host was stimulated by the intriguing properties of the inclusion complexes of native and modified cyclodextrins with a wide range of guests in aqueous solution as well as in the solid state.[13-16]

The design of water-soluble macrocyclic hosts of the cyclophane type during the last decade was certainly influenced by observations of Stetter and Roos,[17] who in 1955 described the potential ability of cyclophanes **1a–c** having two benzidine units to form



1a n = 2
1b n = 3
1c n = 4

2

host–guest complexes. They reported the formation of stable 1:1 complexes with benzene and dioxane when the macrocycles **1b** and **1c** with a suitably sized cavity were recrystallized from these solvents. These complexes were thought for a long time to be of the cavity inclusion type until recently Hilgenfeld and Saenger[18] showed by X-ray analysis that in the complex of the largest host **1c** with benzene the benzene is located not in a molecular cavity but between molecules in the crystal lattice. As the crystalline complex was not formed in aqueous solution, the absence of hydrophobic interactions during the formation of the complex might well be the possible explanation for a cavity inclusion complex not forming.

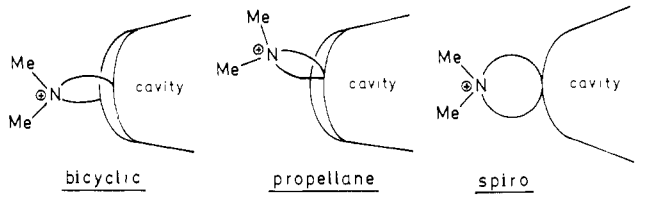
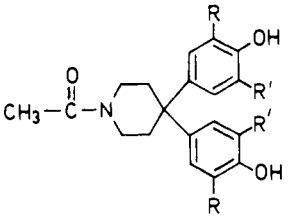


Figure 1. Possible arrangements which locate quaternary ammonium nitrogens remote from the cavity.

By replacing the benzidine units as aromatic walls surrounding the cavity by 4,4′-diaminodiphenylmethane units, Koga et al.[6] obtained a new series of macrocyclic tetraazacyclophanes. These hosts are water-soluble in their protonated form, and in acidic solution host–guest complexation with apolar guests being enclosed in the cavity was evidenced by NMR and fluorescence spectroscopy. A crystalline 1:1 complex of host **2** with durene as guest was obtained from aqueous solution, and molecular cavity inclusion was demonstrated by a crystal structure analysis.[6a]

We wanted to design and construct macrocyclic hosts of the cyclophane type which are water soluble at room temperature and neutral pH. These hosts should have a cavity of well-defined size and shape and of very pronounced hydrophobicity. Strong inclusion complex formation with 1:1 host–guest stoichiometry should occur in aqueous solution with apolar guests, especially with aromatic hydrocarbons, and hydrophobic interactions should be the major driving force for complexation. Our hosts should be good models for the hydrophobic pockets located at the active site of certain enzymes.[10-12] Diphenylmethane units which had been sucessfully introduced by Koga[6] should function as rigid aromatic spacers and cavity walls in the macrocyclic skeleton. Water solubility at neutral pH should be achieved by the introduction of quaternary ammonium residues. The hydrophobicity of the binding site should, however, not be perturbed by strongly hydrated ionic centers built into the macrocyclic skeleton surrounding the cavity. Therefore the quaternary ammonium residues should be located remote from the cavity. Three arrangements seemed predestinated to achieve this goal. The quaternary ammonium ions could either be introduced by bicyclic, propellane, or spiro-type arrangements (Figure 1).

We have chosen spiro piperidinium rings since **3–5** provide



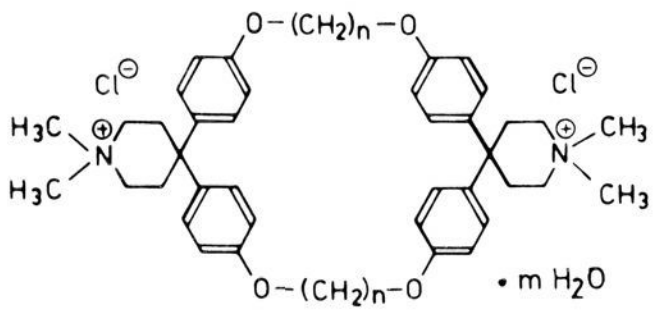
3 R = R′ = H
4 R = CH_3; R′ = H
5 R = R′ = CH_3

building blocks easily available in large amounts which combine the spiro system with the diphenylmethane unit in a desired spatial arrangement. Such units also provide for their introduction into our host compounds.

The diphenylmethane units in our hosts are bridged by α,ω-dioxaalkane chains. Variation of the chain length allows the design of cavities of variable size, depending on the guest to be complexed.

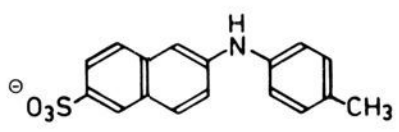
(3) Murakami, Y. In "Cyclophanes II"; Vögtle, F., Ed.; "Topics in Current Chemistry"; Springer: Berlin, 1983; Vol. 115, pp 107–155.
(4) Tabushi, I.; Yamamura, K. In "Cyclophanes I"; Vögtle, F., Ed.; "Topics in Current Chemistry"; Springer: Berlin, 1983; Vol. 113, pp 145–182.
(5) (a) Jarvi, E. T.; Whitlock, H. W., Jr. *J. Am. Chem. Soc.* **1980**, *102*, 657. (b) Adams, S. P.; Whitlock, H. W. *J. Am. Chem. Soc.* **1982**, *104*, 1602. (c) Jarvi, E. T.; Whitlock, H. W. *J. Am. Chem. Soc.* **1982**, *104*, 7196.
(6) (a) Odashima, K.; Itai, A.; Iitaka, Y.; Koga, K. *J. Am. Chem. Soc.* **1980**, *102*, 2504. (b) Odashima, K.; Itai, A.; Iitaka, Y.; Arata, Y.; Koga, K. *Tetrahedron Lett.* **1980**, *21*, 4347. (c) Soga, T.; Odashima, K.; Koga, K. *Ibid.* **1980**, *21*, 4351. (d) Odashima, K.; Soga, T.; Koga, K. *Ibid.* **1981**, *22*, 5311. (e) Odashima, K.; Koga, K. *Heterocycles* **1981**, *15*, 1151.
(7) (a) Diederich, F.; Dick, K. *Tetrahedron Lett.* **1982**, *23*, 3167. (b) For a preliminary report of a part of this work, see: Diederich, F.; Dick, K. *Angew. Chem.* **1983**, *95*, 730; *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 715; *Angew. Chem. Suppl.* **1983**, 957–972.
(8) Winkler, J.; Coutouli-Argyropoulou, E.; Leppkes, R.; Breslow, R. *J. Am. Chem. Soc.* **1983**, *105*, 7198.
(9) Tanford, C. "The Hydrophobic Effect: Formation of Micelles and Biological Membranes", 2nd ed.; Wiley: New York, 1980.
(10) Blow, D. M. *Acc. Chem. Res.* **1976**, *9*, 145.
(11) Ludwig, M. L.; Lipscomp, W. N. In "Inorganic Biochemistry"; Eichhorn, G. I. Ed.; Elsevier: Amsterdam, 1973; Vol. 1, pp 438–487.
(12) (a) Colman, P. M.; Jansonius, J. N.; Matthews, B. W. *J. Mol. Biol.* **1972**, *70*, 701. (b) Kester, W. R.; Matthews, B. W. *Biochemistry* **1977**, *16*, 2506.
(13) Bender, M. L.; Komiyama, M. "Cyclodextrin Chemistry"; Springer: Berlin, 1978.
(14) Saenger, W. *Angew Chem.* **1980**, *92*, 343; *Angew Chem., Int. Ed. Engl.* **1980**, *19*, 344.
(15) Tabushi, I. *Acc. Chem. Res.* **1982**, *15*, 66.
(16) Breslow, R. *Chem. Br.* **1983**, *19*, 126.
(17) Stetter, H.; Roos, E.-E. *Chem. Ber.* **1955**, *88*, 1390.
(18) Hilgenfeld, R.; Saenger, W. *Angew Chem.* **1982**, *94*, 788; *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 781; *Angew. Chem. Suppl.* **1982**, 1690–1701.

This is shown for the first series of hosts **6a–c** which we prepared.[7a,19]



6a n = 2 m = 2.5
6b n = 3 m = 2
6c n = 4 m = 4

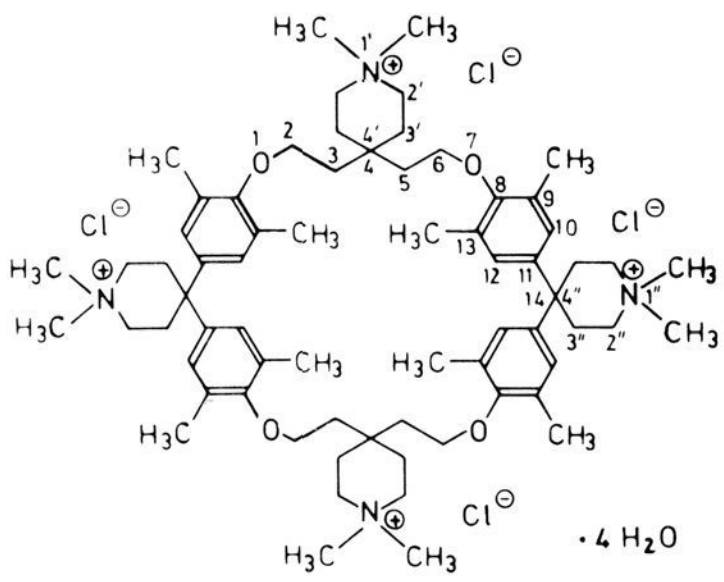
In aqueous solution the critical micelle concentration (cmc) of **6a–c**, below which 1:1 host–guest complexation has to be studied, was found to be rather low. For **6c**, a cmc of 1.6×10^{-4} mol·L^{-1} was determined by ^{1}H NMR spectroscopy. The investigations of 1:1 host–guest complexation were therefore limited to very dilute solutions, and ^{1}H NMR spectroscopy could not be applied in these studies. By fluorescence spectroscopy, evidence for the formation of an inclusion complex of host **6c** with 6-[(4-methylphenyl)-amino]-2-naphthalenesulfonate (TNS, **7**) was obtained. We



7

presume the naphthalene moiety of the guest is incorporated in the molecular cavity of **6c** in the complex. The association constant for the 1:1 complex between **6c** and TNS, $K_a = 4.3 \times 10^3$ L·mol^{-1} (293 K), is in the same range as the association constant for the 1:1 complex between TNS and β-cyclodextrin.[20]

As a consequence of these studies, two improvements of our hosts were desirable: a higher critical micelle concentration and a stronger binding of apolar guests. We therefore designed and synthesized the new host, **8**.[7b,21]



8

Retaining the concept of the quaternary ammonium residues remote from the cavity, two more of these groups were introduced via spiro piperidinium rings attached to the aliphatic chains

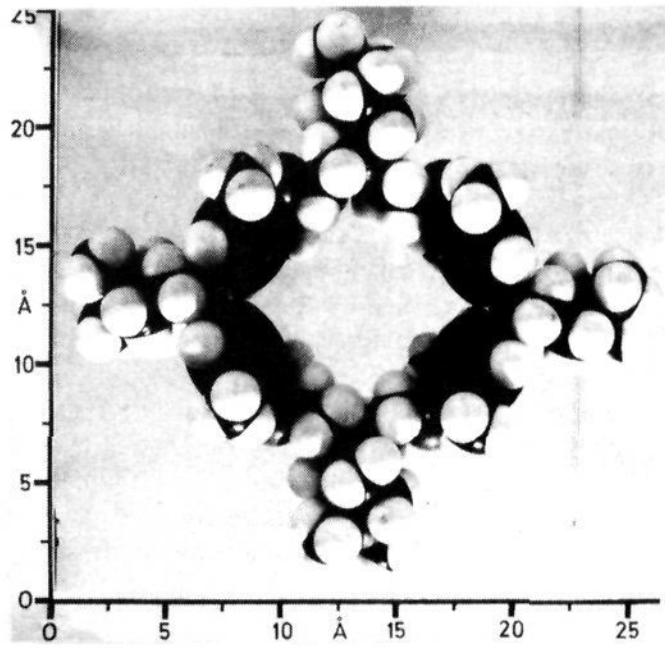


Figure 2. CPK molecular model of host **8**.

bridging the diphenylmethane units in order to obtain a higher critical micelle concentration. The introduction of eight methyl groups at the diphenylmethane units should enhance the hydrophobic character of the cavity and lead to increased binding of apolar guests. This could be expected from previous experiments with hosts related to **6c** bearing additional methyl groups. The stepwise introduction of two, four, six, and eight methyl groups at the aromatic carbon atoms ortho to the ether oxygens of host **6c** in other work led incrementally to much stronger binding of apolar guests.[22]

In this paper the geometry of **8** and its aggregation behavior in aqueous solution will be discussed. The methods of formation of inclusion complexes of **8** with apolar, predominantly aromatic guests in aqueous solution will be presented. The various methods applied for the determination of the association constants of the host–guest complexes will be discussed. The ability of **8** to accelerate via host–guest complexation the transport of aromatic hydrocarbons through an aqueous phase along a concentration gradient will be demonstrated.

In the following paper[23] extensive ^{1}H NMR studies of host–guest complexation of **8** with aromatic guests in aqueous solution will provide ample evidence for the formation of highly structured cavity inclusion complexes with exclusive 1:1 stoichiometry. The high discrimination of host **8** between various apolar guests will be discussed in terms of the structures of the host–guest complexes in aqueous solution.

Results and Discussion

Geometry of Host 8. Figure 2 is a picture of a CPK molecular model of host **8**. Except for the orientation of the spiro piperidinium rings attached to the aliphatic bridges the picture shows the binding conformation of **8** strongly supported by the ^{1}H NMR studies of the inclusion complexes of **8** in aqueous solution.[23] The four benzene rings in the "face-to-face" conformation of **8**^{4} are orientated perpendicular to the mean molecular plane of **8**. The dimensions of the cavity as estimated from this model are about 9 Å between the spiro carbon atoms of the diphenylmethane units and about 6.6 Å between parallel benzene rings. The extension of the cavity between the aliphatic chains bridging the diphenylmethane units is about 5.4–6 Å. The spiro piperidinium rings fixed in these chains can be located within the mean molecular plane of **8** as depicted in Figure 2. As a consequence of the conformational flexibility of the aliphatic chains the two piperidinium rings can also turn out of this plane on one or on both sides of the cavity. The cavity deepens as the two piperidinium rings of the aliphatic bridges approach a position in which their least planes are perpendicular to the mean molecular plane

(19) A water-insoluble dispiro cyclophane comparable to **6b** ($n = 3$) with spiro cyclohexane rings instead of spiro piperidinium rings connected to the diphenylmethane units has been previously reported: Smolinski, S.; Deja, I.; Nowicka, J. *Tetrahedron* **1975**, *31*, 1527; *Zesz. Nauk. Uniw. Jagiellon., Pr. Chem.* **1975**, *20*, 71.

(20) Kondo, H.; Nakatani, H.; Hiromi, K. *J. Biochem.* **1976**, *79*, 393.

(21) For the synthesis of **8**, see: Diederich, F.; Dick, K. *Chem. Ber.*, submitted for publication.

(22) Diederich, F.; Dick, K.; Griebel, D. *Chem. Ber.*, submitted for publication.

(23) Diederich, F.; Griebel, D. *J. Am. Chem. Soc.*, following paper in this issue.

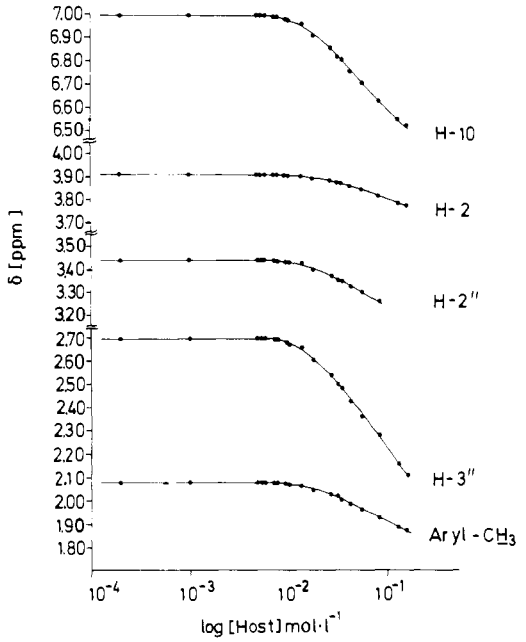


Figure 3. Chemical shifts of selected protons of **8** as a function of the concentration of **8** in D_2O (303 K, TSP_{ext} in D_2O).

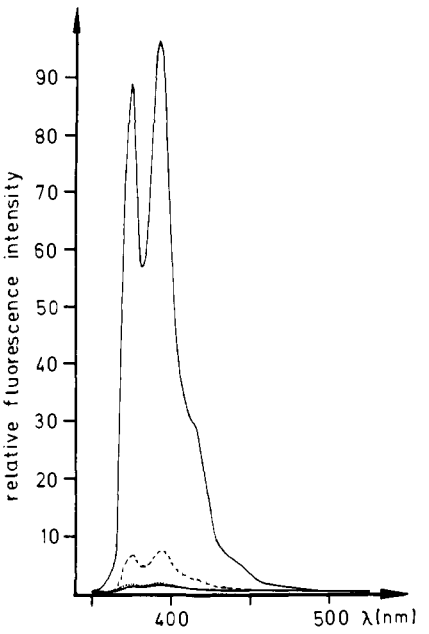


Figure 4. Emission spectra (excitation at 343 nm) of solutions obtained at 293–295 K by solid-liquid extraction of excess solid pyrene with a 2 × 10^{-5} M aqueous solution of **8** (—), **9** (- - -), and γ-cyclodextrin (···). The curve of weakest intensity was recorded from solutions which were obtained by extraction of pyrene with doubly distilled water or with a 2 × 10^{-4} M solution of **10**.

of **8**. The latter orientations of the piperidinium rings according to ^1H NMR[23] are of great importance in the inclusion complexes of **8**. The depth of the cavity of **8** varies between ≈5.4 and ≈7.8 Å. On the basis of CPK molecular models, the cavity of **8** was expected to be large enough to allow the inclusion of one polycyclic aromatic hydrocarbon of the size of pyrene or even perylene. The four oxygens of the macrocyclic skeleton in the binding conformation are turned outward away from the cavity thus enhancing the hydrophobic character of the binding site. This is supported by the ^1H NMR studies[23] as well as by X-ray analysis of a host–guest complex of the macrocyclic bis(*N*-methylpiperidine) precursor to **6c**.[24]

Aggregation Behavior of 8 in Aqueous Solution. Since the formation of stoichiometric 1:1 host–guest complexes in aqueous solution has to be studied below the critical micelle concentration of the host in order to avoid the interference of aggregation effects, the aggregation behavior of **8** in aqueous solution was investigated. As already found for the hosts **6a–c**,[7a] this could be easily done by ^1H NMR spectroscopy. The ^1H NMR spectra in D_2O in the concentration range 2 × 10^{-4}–7 × 10^{-3} mol·L^{-1} (360 MHz, T = 303 K, sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate (TSP) as external standard)[25] gave for all protons of **8** highly resolved signals with chemical shifts independent of the concentration of **8**: δ 1.90 (m, 8 H, H-3′), 1.99 ("t", J = 7 Hz, 8 H, H-3), 2.08 (s, 24 H, aryl–CH_3), 2.70 (m, 8 H, H-3″), 3.16 (s, 24 H, N(1′)–CH_3 and N(1″)–CH_3), 3.43 (m, 16 H, H-2′ and H-2″), 3.91 ("t", J = 7 Hz, 8 H, H-2), 6.99 (s, 8 H, H-10).

At higher concentrations strong line broadening of all signals starts appearing and the chemical shifts of selected protons shown in Figure 3 become strongly dependent on concentration, whereas for all other protons within ±0.03 ppm the same chemical shifts are observed up to concentrations of **8** higher than 0.1 mol·L^{-1}. In all the plots of Figure 3 there is a relatively well-defined discontinuity at approximately the same concentration (≈ 7.5 × 10^{-3} mol·L^{-1}) of **8**. This concentration can be considered as the ^1H NMR critical micelle concentration (cmc) of **8**.[26] This value is considerably higher than the cmc of 1.6 × 10^{-4} mol·L^{-1} determined by ^1H NMR for **6c** with only two ionic centers remote from the cavity. Thus with **8**, one of the desired improvements of our host has been achieved and, as we shall see, easy ^1H NMR monitoring of host–guest complexation is possible below this relatively high cmc.[23]

A considerable difference between the ^1H NMR spectra of **6c** and **8** in aqueous solution above the cmc is noticeable. All signals of **6c** are found to be dependent on concentration.[7a] Especially the protons in the neighborhood of the quaternary ammonium nitrogens of **6c** are considerably shifted to higher field at higher concentrations. This is not the case with **8** where the three groups of protons of the spiro piperidinium rings fixed in the aliphatic chains are not shifted at all with increasing concentration. Similarly the chemical shifts of the protons of the methyl group at N(1″) are not affected. However, all protons of the diphenylmethane units of **8** and H-2, being located close to these units, move markedly upfield with increasing concentration. The upfield shifts of these protons suggest that upon aggregation the diphenylmethane units of one molecule of **8** are coming into close proximity to the diphenylmethane units of two other molecules of **8** approaching from both sides of the cavity. We tentatively propose the stacking of **8** molecules with the geometry depicted in Figure 2 as the favored aggregation form, which could explain the observed ^1H NMR behavior. Further investigations are necessary in order to obtain more insight into the aggregation behavior of **8** and other related hosts like **6a–c**.

Formation of Host–Guest Complexes of 8 with Apolar Guests in Aqueous Solution and Determination of the Association Constants. The methods of forming host–guest complexes of **8** with various apolar guests and the determination of the attendant association constants will be presented first. The association constants for all complexes will then be discussed together. Strong

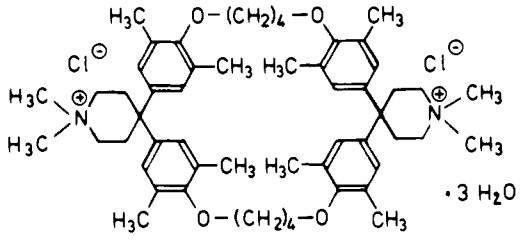
(24) Upon recrystallization of the macrocyclic bis(*N*-methylpiperidine) precursor of **6c** from benzene or *p*-xylene, stoichiometric host–guest complexes suitable for X-ray crystallography were obtained. In the 1:2 host–guest complex with benzene, one benzene ring is perfectly enclosed within the intramolecular cavity of the host. In the 1:1 complex of *p*-xylene, the host molecules form stacks and *p*-xylene molecules are sandwiched by two adjacent host molecules in the stack. The conformation of the host in both complexes is similar to the one shown in Figure 2 for **8**. All four benzene rings are oriented about perpendicular to the mean molecular plane of the host, the four ether oxygens are turned outward of the cavity, and the *N*-methylpiperidine rings have almost ideal chair conformations. Krieger, C.; Diederich, F. *Chem. Ber.*, submitted for publication.

(25) In all ^1H NMR studies in D_2O, TSP in D_2O was used as the external standard.[7b] All multiplets in the spectrum of **8** have symmetrical shape and are characterized by their centers for better comparison.

(26) For the determination of critical micelle concentrations by ^1H NMR see, for example: Fendler, E. J.; Constien, U. G.; Fendler, J. H. *J. Phys. Chem.* **1975**, *79*, 917.

evidence for the exclusive formation of cavity-inclusion complexes with 1:1 stoichiometry in aqueous solution is obtained by ^{1}H NMR spectroscopy for all host–guest complexes which we have studied.[23] Additional evidence will be provided in the following sections. In all binding studies only concentrations of host **8** below the cmc were used.

A. Formation of Host–Guest Complexes by Solid-Liquid Extraction. Solutions of host–guest complexes of **8** with polycyclic aromatic hydrocarbons which are extremely insoluble in water could be prepared by extracting the solid guests with aqueous solutions of **8**. When a suspension of excess solid pyrene in a 2×10^{-5} M aqueous solution of host **8** is shaken and exposed to ultrasound sonification for about 20 min, the solution, after centrifugation[27] and filtration, exhibits upon excitation at 343 nm the intense monomeric fluorescence of pyrene shown in Figure 4. This was the first evidence we obtained that during solid-liquid extraction strong host–guest complexation occurs by pyrene being incorporated in the hydrophobic cavity of **8**. A 2×10^{-5} M aqueous solution of the macrocyclic host **9**[7b,21] with a comparable



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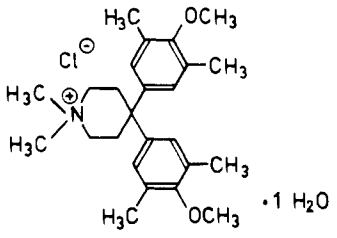

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but smaller hydrophobic binding site gives a much weaker effect. The smaller cavity allows only incomplete inclusion of pyrene. Therefore weaker binding and less extraction of solid guest are observed. With a 2×10^{-5} M aqueous solution of γ-cyclodextrin which possesses a suitably sized cavity with a less-pronounced hydrophobic character, extraction through complexation is lower.[28] The fluorescence intensity of pyrene obtained with a 2×10^{-4} M solution of **10**[21] being a model compound for half of the cavity of **8** is the same as that obtained by extraction with doubly distilled water (Figure 4). The solution of **10** giving the same effect as water clearly proves the importance of a hydrophobic cavity for the host–guest complexation. By the same solid-liquid extraction procedure solutions of host–guest complexes of **8** with perylene, fluoranthene, naphthalene, 1,5- and 2,6-dimethylnaphthalene, and durene were obtained.

By multiple extraction with *n*-hexane of the aqueous solutions of complexes obtained by solid-liquid extraction, the aromatic guests are quantitatively transferred into the organic phase. By monitoring the decrease of fluorescence intensity of pyrene, perylene, and fluoranthene in the aqueous phase after each extraction, the quantitative removal of the guest from the aqueous solution was easily determined. In the remaining aqueous phase, within experimental error, the initial concentration H_0 of **8** in the aqueous solution used for extraction was measured by electronic absorption spectroscopy. Clear evidence was obtained that no precipitation of complex from the aqueous solution occurred during the extraction. This could be demonstrated for all experiments involving different guests and aqueous solutions with various concentrations of **8**. In the combined *n*-hexane phases the total concentration of the guest, $G_{H_2O}(tot)$, which was present in the aqueous solution of complex, was determined by electronic absorption spectroscopy.

Table I. Association Constants K_a (L·mol^{-1}) (T = 293–295 K) for 1:1 Host–Guest Complexes Formed between **8** and Neutral Aromatic Guests in Aqueous Solution, Determined by Solid-Liquid Extraction (Method A) and Liquid-Liquid Extraction (Method B)[a]

guest	K_a L·mol^{-1}	method of determination	G_{H_2O}(max), mol·L^{-1}	K_d
perylene	1.6×10^7	A	$<2 \times 10^{-9}$ [b]	
fluoranthene	1.2×10^6	A	1.2×10^{-6}	
	1.8×10^6	B		1.1×10^{-5}
pyrene	1.1×10^6	A	8.0×10^{-7}	
	1.8×10^6	B		8.3×10^{-6}
1,5-dimethylnaphthalene	3.3×10^4	A	1.6×10^{-5}	
2,6-dimethylnaphthalene	2.6×10^4	A	8.2×10^{-6}	
biphenyl	2.2×10^4	B		8.1×10^{-5}
azulene	2.1×10^4	B		6.0×10^{-4}
naphthalene	1.5×10^4	A	1.8×10^{-4}	
	1.2×10^4	B		3.8×10^{-4}
1-(dimethylamino)naphthalene	9.3×10^3	B		4.0×10^{-4}
durene	2.0×10^3	A	4.7×10^{-5}	
	1.9×10^3	B		3.8×10^{-5}

[a] For guests studied by solid–liquid extraction the maximum solubility in water G_{H_2O}(max) (T = 293–295 K) is given. For guests studied by liquid-liquid extraction the equilibrium constant for the distribution between water and *n*-hexane K_d is given (T = 293–295 K). [b] Taken from ref 30.

In all solid-liquid extraction experiments with one aromatic hydrocarbon, the amount of uncomplexed guest G_{H_2O} in the aqueous solution of complex can be considered to be constant and equal to the maximum amount of this hydrocarbon, G_{H_2O}(max), which is soluble in water.[29] By subtracting this value from the total concentration of guest in the solution of complex, G_{H_2O}(tot), the amount of complexed guest, G_{compl}, is obtained which in the case of exclusive 1:1 complexation is equal to the concentration of complex, HG_{H_2O}, and the concentration of complexed host, H_{compl}. If the maximum solubility of the guest in water, G_{H_2O}(max), is known, all concentrations of eq 1 which describes the formation of a 1:1 host–guest complex in the aqueous phase can be obtained from one solid-liquid extraction experiment and the association constant K_a can be calculated (eq 2).

$$H_{H_2O} + G_{H_2O} \rightleftharpoons HG_{H_2O} \quad (1)$$

$$K_a\ (\text{L·mol}^{-1}) = \frac{HG_{H_2O}}{H_{H_2O}G_{H_2O}} \quad (2)$$

The maximum solubility of the hydrocarbons in water, G_{H_2O}, was determined by extraction of solid guest with pure water and analysis of the resulting aqueous solution as previously described. Our values for the solubilities of polycyclic aromatic hydrocarbons in water (Table I) are in good agreement with the values reported.[30,31]

(27) Upon sonification, crystals of the guest are divided into small particles that pass through all common filter papers. This was indicated by the observation of the excimer fluorescence of crystalline pyrene at longer wavelength (see, for example: Schweitzer, D.; Hausser, K. H.; Kirrstetter, R. G. H.; Staab, H. A. *Z. Naturforsch., A* **1976**, *31A*, 1189) beside the monomer emission of complexed pyrene. After centrifugation at 6000 rpm for about 30 min, this excimer emission had disappeared completely even at highest spectrometer sensitivity.

(28) Kobayashi, N.; Saito, R.; Hino, H.; Hino, Y.; Ueno, A.; Osa, T. *J. Chem. Soc., Perkin Trans. 2* **1983**, 1031. Kano, K.; Takenoshita, I.; Ogawa, T. *Chem. Lett.* **1982**, 321. Yorozu, T.; Hoshino, M.; Imamura, M. *J. Phys. Chem.* **1982**, *86*, 4426.

(29) Schlenck, H.; Sand, D. M. *J. Am. Chem. Soc.* **1961**, *83*, 2312.

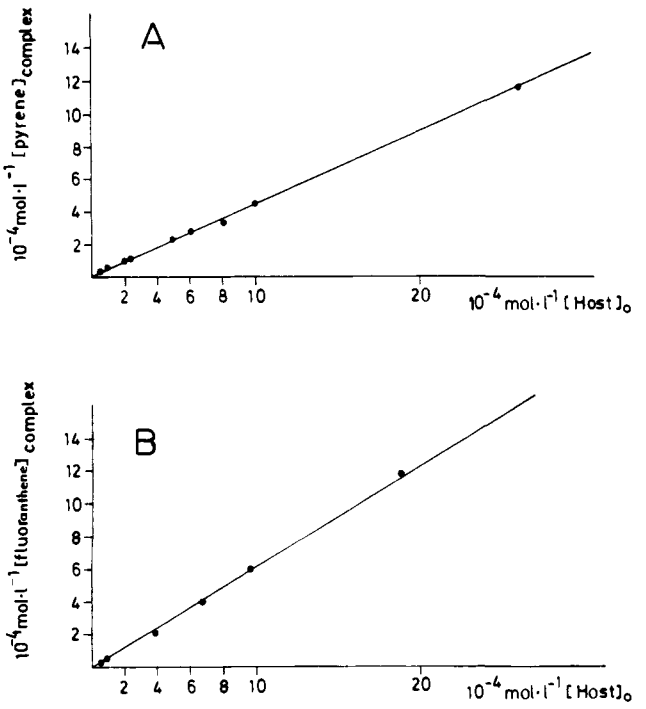


Figure 5. The amount of pyrene (A) and of fluoranthene (B) solubilized during solid-liquid extraction by host–guest complexation G_{compl} is plotted as a function of the total concentration of **8** in the aqueous solution used for the extraction (293–295 K).

The determination of the association constant K_a from eq 1 and 2 is only correct if beside 1:1 host–guest complexation no host–guest complexation with other stoichiometry occurs in the aqueous solution. We therefore looked for more evidence for the exclusive formation of cavity inclusion complexes with 1:1 stoichiometry. In order to learn whether external binding of guest to **8** outside of the cavity provides an additional amount of guest transferred into the aqueous phase during the solid-liquid extraction, solid pyrene was extracted with a 2×10^{-4} solution of **10**. The same pyrene concentration (8×10^{-7} mol·L^{-1}) in the aqueous phase as in the extraction with pure water was obtained within experimental error. This result indicates that all guest which in an aqueous solution of **8** is solubilized in addition to G_{H_2O}(max) is bound in the cavity of **8**. Solid-liquid extractions were performed by using aqueous solutions with a wide range of concentrations of **8**. Except for perylene as guest the extraction equilibrium was always reached by ultrasound sonification of the suspension of the guest in the solution of **8** for 20 min. Extraction of perylene, due to its extreme insolubility needed more time. Hence, ultrasound sonification was prolonged to 3 h. A strong argument for the same host–guest complexes being formed by using various concentrations of host H_0 in the range 5.5×10^{-3}–4×10^{-5} mol·L^{-1} was obtained with pyrene and fluoranthene as guests. The amount of guest G_{compl} solubilized by complexation increases linearly with the total concentration of host, H_0 (Figure 5). A constant ratio, H_0/G_{compl}, over a wide concentration range cannot be expected if several equilibria exist in the aqueous solution leading to complexes with different stoichiometry. The constant ratio H_0/G_{compl} is a strong argument for the exclusive formation of host:guest complexes with 1:1 stoichiometry in the aqueous solution. By solid-liquid extraction we obtained the following ratios H_0/G_{compl}, which were reproducible at all considered H_0: $H_0/G_{compl} = 2$ for pyrene, 1.7 for fluoranthene, 31 for perylene, and 1.4 for naphthalene.

In the electronic absorption spectra of the aqueous solutions of the complexes of **8** with pyrene, fluoranthene, and perylene, all bands at $\lambda > 260$ nm can be assigned to the complexed aromatic guest. Significant reduction in extinction and slight bathochromic shifts of these bands as compared to the corresponding bands in organic solvents are observed as is shown for pyrene in Figure 6. These changes result from interactions in the complex between host and guest rather than from changes of the medium polarity, since almost the same maxima and very similar extinction coefficients are obtained for the bands of pyrene in solvents of different polarity like *n*-hexane, dioxane, and methanol. The spectra of solutions of the complexes of pyrene and fluoranthene above λ 260 nm exhibit bands of identical shape and position in

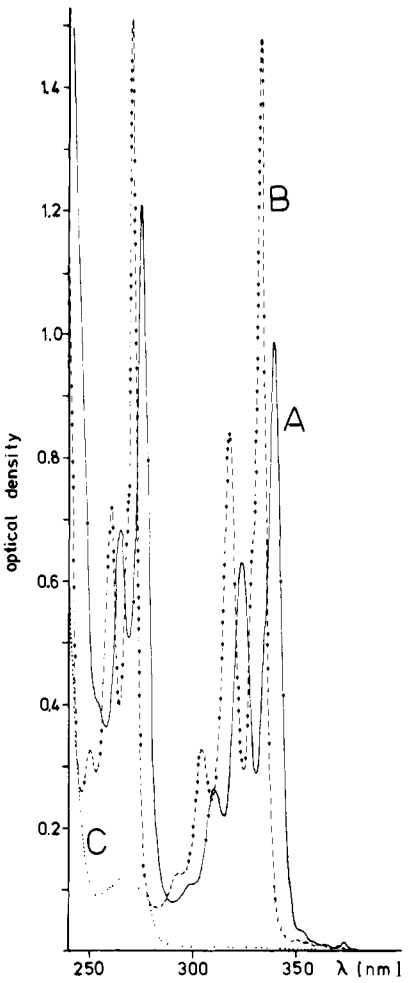


Figure 6. Electronic absorption spectra (A) of an aqueous solution of the **8**–pyrene complex prepared by solid-liquid extraction of excess pyrene with a 2.75×10^{-4} M aqueous solution of **8** (the concentration of the complex is 1.45×10^{-4} mol·L^{-1}), $d = 0.2$ cm (—), (B) of a 1.45×10^{-4} M solution of pyrene in methanol, $d = 0.2$ cm (- - -), and (C) of a 2.75 M aqueous solution of **8**, $d = 0.2$ cm (···).

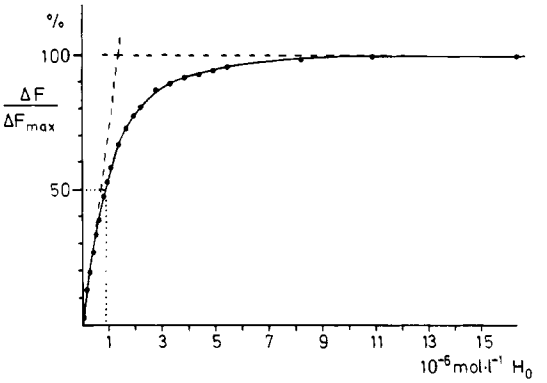


Figure 7. Fluorescence intensity increase $\Delta F/\Delta F_{max}$ (%) of ANS ($c = 1.13 \times 10^{-6}$ mol·L^{-1}) as a function of the total concentration of **8**, H_0, at 292.5 K, λ_{exc} 365 nm, λ_{em} 483 nm.

(30) Davis, W. W.; Krahl, M. E.; Clowes, G. H. A. *J. Am. Chem. Soc.* **1942**, *64*, 108.
(31) Klevens, H. B. *J. Phys. Chem.* **1950**, *54*, 283.

Table II. Association Constants from Fluorescence Data for 1:1 Host–Guest Complexes Formed with Host **8** in Aqueous Solution (T = 292.5 K)[a]

guest (method A + B) inhibitor (method C)	K_a, L·mol^{-1}	method	typical experimental conditions		
			G_0, mol·L^{-1}	H_0, mol·L^{-1}	I_0, mol·L^{-1}
TNS[b]	5.0×10^6	A	2.0×10^{-7}	2.5×10^{-8}–5.0×10^{-5}	
ANS[c]	3.2×10^6	A	8.9×10^{-7}	1.0×10^{-7}–5.0×10^{-5}	
DNS[d]	1.4×10^5	B	5.0×10^{-7}	5.1×10^{-6}–5.1×10^{-5}	
1,3-dihydroxynaphthalene	9.8×10^3	B	4.9×10^{-6}	5.2×10^{-5}–5.2×10^{-4}	
1-(dimethylamino)naphthalene	9.4×10^3	B	1.2×10^{-6}	1.25×10^{-5}–1.25×10^{-4}	
2,6-naphthalenedisulfonate[d]	$>10^6$	C	2.2×10^{-7}	2.1×10^{-6}–2.1×10^{-5}	2×10^{-4}
1,5-naphthalenedisulfonate[d]	4.4×10^5	C	2.2×10^{-7}	2.1×10^{-6}–2.1×10^{-5}	2.1×10^{-4}
2-naphthalenesulfonate[d]	4.0×10^5	C	2.3×10^{-7}	2.9×10^{-6}–2.9×10^{-5}	3.0×10^{-4}
1-naphthalenesulfonate[d]	3.5×10^5	C	2.5×10^{-7}	2.6×10^{-6}–2.6×10^{-5}	3.0×10^{-4}
2,7-dihydroxynaphthalene	1.9×10^4	C	2.0×10^{-7}	2.0×10^{-6}–2.0×10^{-5}	7.5×10^{-4}
naphthalene	1.6×10^4	C	1.0×10^{-7}	1.0×10^{-6}–1.0×10^{-5}	8.5×10^{-5}
p-toluenesulfonate[d]	7.6×10^3	C	2.2×10^{-7}	2.6×10^{-6}–2.6×10^{-5}	2×10^{-3}
1-(trimethylammonio)naphthalene fluorosulfonate	1.7×10^3	C	2.3×10^{-7}	2.5×10^{-6}–2.5×10^{-5}	3.9×10^{-3}
1-adamantanol	1.6×10^2	C	2.3×10^{-7}	2.5×10^{-6}–2.5×10^{-5}	3.9×10^{-3}

[a] The methods applied are (A) direct evaluation of a titration curve at half-saturation binding of the guest, (B) Benesi–Hildebrand treatment of the fluorescence data, and (C) competitive inhibition of the binding of TNS. Typical experimental conditions are given. [b] Potassium salt. [c] Ammonium salt. [d] Sodium salt.

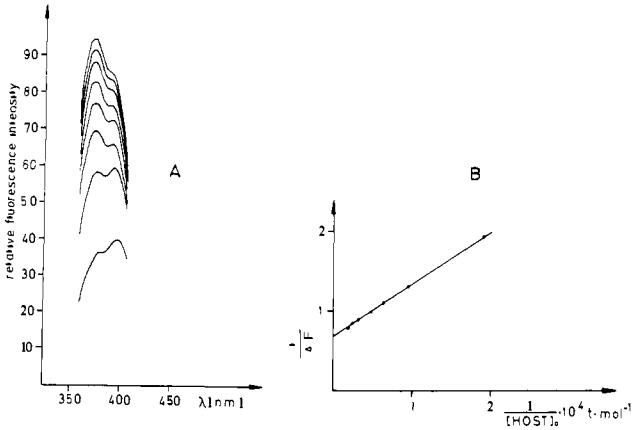


Figure 8. (A) Fluorescence spectra of 1,3-dihydroxynaphthalene in aqueous solution in the presence of host **8** (λ_{exc} 338 nm, T = 292.5 K). $G_0 = 4.86 \times 10^{-6}$ mol·L^{-1}; H_0 from top to bottom spectrum: 5.23, 4.19, 3.14, 2.09, 1.57, 1.05, and 0.52×10^{-4} mol·L^{-1}. The lowest curve shows the fluorescence spectrum of free 1,3-dihydroxynaphthalene in aqueous solution. (B) Benesi–Hildebrand plot of the fluorescence intensity of 1,3-dihydroxynaphthalene in the presence of **8**; λ_{exc} 338 nm, λ_{em} 375 nm; the experimental conditions are identical with those of Figure 8A.

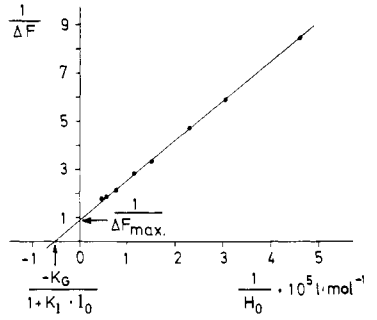


Figure 9. Graphical evaluation of K_I according to eq 9. The fluorescence intensity increase ΔF upon addition of host **8** ($H_0 = 2.1 \times 10^{-6}$–2.1×10^{-5} mol·L^{-1}) to an aqueous solution of TNS ($G_0 = 2.2 \times 10^{-7}$ mol·L^{-1}) and inhibitor 2-naphthalenesulfonate ($I_0 = 2.15 \times 10^{-4}$ mol·L^{-1}) is monitored at 292.5 K (λ_{exc} 360 nm, λ_{em} 445 nm).

the concentration range $H_0 = 5 \times 10^{-3}$–5×10^{-5} mol·L^{-1}. In this range the bands arising from free guest are so weak as compared to those of complexed guest, since $G_{compl} \gg G_{H_2O}$, that they are not detected. In lower concentration ranges the bands of free guest start appearing and partially overlap the otherwise unchanged bands of complexed guest.

The electronic emission spectra of the aqueous solutions of the complexes of **8** with perylene, pyrene (Figure 4), and fluoranthene also exhibit over the whole concentration range measured similar shape and position of the emission bands. In the fluorescence spectrum of an aqueous solution of the **8**–pyrene complex even at a total concentration of pyrene of 2.8×10^{-3} mol·L^{-1}, no excimer emission could be observed at highest spectrometer sensitivity. We consider this fact to provide a strong argument against the presence of host–guest complexes with 1:2 and 2:2 stoichiometry.[28]

With ample evidence for exclusive 1:1 host–guest stoichiometry, we calculated with eq 1 and 2 the association constants K_a at 293–295 K (Table I) for the complexes formed by solid-liquid extraction.

B. Formation of Host–Guest Complexes by Liquid-Liquid Extraction. Aqueous solutions of host–guest complexes of **8** with polycyclic aromatic hydrocarbons were also prepared by liquid-liquid extraction.[32] In an extraction experiment a $\approx 10^{-2}$ M solution of guest in *n*-hexane was shaken with a $\approx 2 \times 10^{-4}$ M solution of **8**. After careful phase separation, the aqueous solution was analyzed as described for the solution of complex obtained by solid-liquid extraction. In a distribution experiment the $\approx 10^{-2}$ M solution of guest in *n*-hexane was shaken with pure water and the resulting aqueous layer was analyzed in the same way. From these experiments the association constants, K_a for 1:1 host–guest complex formation, the distribution constants K_d, and the extraction constants K_e (which will be needed for the discussion of the transport experiments presented below) were determined by using eq 1–6.

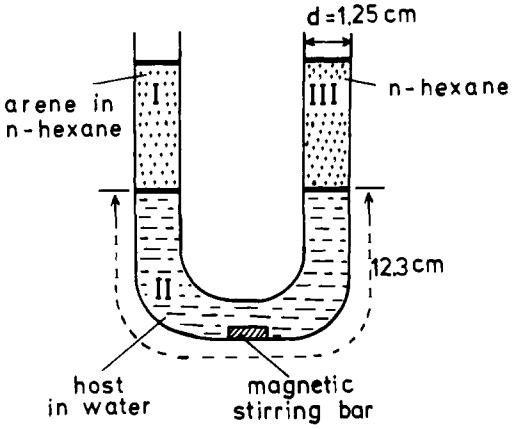


Figure 10. U-type cell used to study the transport of arenes through an aqueous phase mediated by **8** as molecular carrier.

$$G_{hexane} \overset{K_d}{\rightleftharpoons} G_{H_2O} \qquad (3)$$

(32) Koenig, K. E.; Lein, G. M.; Stuckler, P.; Kaneda, T.; Cram, D. J. *J. Am. Chem. Soc.* **1979**, *101*, 3553.

$$G_{hexane} + H_{H_2O} \overset{K_e}{\rightleftharpoons} HG_{H_2O} \quad (4)$$

$$K_d = \frac{G_{H_2O}}{G_{hexane}} \quad (5)$$

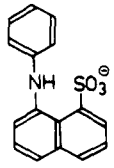
$$K_e = K_a K_d \quad (6)$$

In eq 1–6, G_{H_2O} and G_{hexane} are the concentrations of free guest in the aqueous phase respectively in the *n*-hexane layer. H_{H_2O} is the concentration of free host and HG_{H_2O} the concentration of the host–guest complex in the water layer. The quantitative evaluation of our liquid-liquid extractions is simplified by the complete insolubility of our host **8** and the complexes in the *n*-hexane layer. The total concentrations of **8**, H_0, and the complex are therefore always present in the aqueous layer.

In our experiments the initial concentration of guest in the *n*-hexane layer is the same in both extraction and distribution experiments. Thus the concentration of the free guest in the aqueous layer of the extraction experiment can be considered identical with the concentration of free guest, G_{H_2O}, in the distribution experiment from which it is determined. For this purpose the aqueous layer from the distribution experiment is extracted exhaustively with *n*-hexane, and the amount of guest in *n*-hexane is measured by electronic absorption spectroscopy. The total amount of guest in the aqueous layer of the extraction experiment $G_{H_2O}(tot)$ is then determined in the same way. By subtracting $G_{H_2O}(tot) - G_{H_2O}$ the concentration of complexed guest equal to HG_{H_2O} in the aqueous layer of the extraction experiment is obtained. Finally the subtraction $H_0 - HG_{H_2O}$ gives H_{H_2O}, and all concentrations of eq 1 are now known.

The host–guest association constants K_a obtained by liquid-liquid extraction with various guests as well as the distribution constants K_d of the guests are shown in Table I. The concentrations of complexes in the aqueous layer obtained by liquid-liquid extraction of a 10^{-2} M solution of the guest in *n*-hexane with a 2×10^{-4} M aqueous solution of **8** are $HG_{H_2O} \approx 3.4 \times 10^{-5}$ mol·L^{-1} with fluoranthene as guest, $\approx 2.8 \times 10^{-5}$ mol·L^{-1} with pyrene, and $\approx 1 \times 10^{-5}$ mol·L^{-1} with naphthalene.

C. Host–Guest Association Constants K_a from Fluorescence Data If Saturation Binding Occurs. Substituted aminonaphthalenesulfonates like 8-(phenylamino)-1-naphthalenesulfonate (ANS)[33] (**11**), 6-[(4-methylphenyl)amino]-2-



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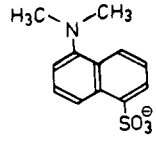

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naphthalenesulfonate (TNS)[34] (**7**) or 5-(dimethylamino)-1-naphthalenesulfonate (DNS)[35] (**12**) are well-known for the remarkable sensitivity of their fluorescence emission to the polarity of the environment. These compounds have been widely used as fluorescence probes in proteins and other macromolecules.[36,37] In water they exhibit a very weak fluorescence intensity, whereas in less polar environments a blue shift and a strong increase of the fluorescence intensity are obtained. This emission behavior makes these molecules good hydrophobic guests whose host–guest complexation can be easily monitored. If in aqueous solution their apolar naphthalene moiety is incorporated into a suitably sized hydrophobic cavity of a macrocyclic host, their fluorescence spectra exhibit a blue shift and an intensity increase. The evaluation of the fluorescence intensity as a function of the concentration of host by the Benesi–Hildebrand treatment has been applied to the determination of the association constants of host–guest complexes of cyclodextrins with ANS,[38] TNS,[20] and DNS.[39] This same method has been used for studies of host–guest complexation of water-soluble cyclophanes as hosts with ANS[3,4,6d] and TNS[6d,7a] as guests.

Large blue shifts are observed when **8** is added to aqueous solutions of all three fluorescing guests. The emission maximum of TNS is shifted from ≈500 nm in water to 445 nm (λ_{exc} 360 nm), λ_{max} of ANS from ≈526 to 483 nm (λ_{exc} 365 nm), and λ_{max} of DNS from 497 to 465 nm (λ_{exc} 365 nm). Together with the blue shift a strong increase of the fluorescence intensity is observed: with $H_0 = G_0 = 10^{-6}$ mol·L^{-1} the relative fluorescence intensity of TNS at λ_{max} 445 nm is about 80 times higher as the intensity at λ_{max} 500 nm of a 1×10^{-6} M aqueous solution of TNS. These large blue shifts and strong fluorescence intensity increases can only be expected if the guests are incorporated in the apolar cavity of **8**. The binding of host **8** to TNS or ANS was so strong that the association constant for the 1:1 inclusion complexes had no longer to be evaluated by a Benesi–Hildebrand treatment. For a given low concentration of guest ($G_0 \approx 10^{-7}$–10^{-6} mol·L^{-1}) saturation binding was reached with only a small excess of host **8** ($H_0 \approx 10^{-5}$ mol·L^{-1}). This allowed a direct determination of the association constants.[40] Host **8** exhibits no emission in aqueous solution. The fluorescence intensity increase of the guest (ΔF) upon addition of **8** was monitored at 292.5 K at the fluorescence maximum. ΔF_{max} is the fluorescence intensity at saturation binding of the guest. The percent fluorescence intensity increase ($\Delta F/\Delta F_{max}$) was plotted as a function of the total concentration of host H_0. This fluorescence titration curve shows a clear knee at 1:1 molar ratio of host and guest which in titrations of TNS and ANS with **8** in the higher concentration ranges becomes very sharp (Figure 7). The curve is easily evaluated at half-saturation binding of the guest. If 50% of the guest is complexed, then in eq 1 and 2 $G_{H_2O} = HG_{H_2O}$, and eq 2 simplifies to eq 7.

$$K_a = \frac{1}{H_{H_2O}} \quad (7)$$

From the titration curve, the total concentration of host H_0 at 50% saturation binding of the guest was determined. By subtraction from H_0 (50%) of the concentration HG_{H_2O}, which at 50% saturation is equal to half of the total concentration of guest ($G_0/2$), the concentration of free host H_{H_2O} was obtained. Association constants were obtained by this method for TNS of $K_a = (5.0 \pm 0.1) \times 10^6$ L·mol^{-1} and for ANS of $K_a = (3.2 \pm 0.2) \times 10^6$ L·mol^{-1}.

With their naphthalene and benzene rings ANS and TNS possess two potential binding sites. In the presence of large excess of host a 2:1 complex of β-cyclodextrin with TNS was indeed observed.[20] We found if complexation of TNS beyond 1:1 stoichiometry in the presence of a very large excess of host **6c** and related hosts bearing two, four, six, or eight methyl groups attached to the aromatic rings.[22] In these solutions of complexes, no knee in the titration curve at 1:1 host–guest ratio was observed. No saturation binding of the guest was obtained even with a very large excess of host, although strong host–guest complexation occurred in each case. The emission maximum of TNS changed considerably with the concentration of the hosts. With host **8** and TNS or ANS a clear knee at 1:1 molar ratio of host and guest was observed. Even with a 1000-fold excess of **8** the position of λ_{max} of the emission of the complexed guest remained unchanged and the fluorescence intensity was equal to the one obtained at the concentration of **8** needed for saturation binding of the guest. This exclusive 1:1 complexation was expected from CPK molecular model examination. Host **8** provides a binding site for both the naphthalene and benzene moieties of ANS and TNS at the same time.

(33) Stryer, L. *J. Mol. Biol.* **1965**, *13*, 482.
(34) McClure, W. O.; Edelman, G. M. *Biochemistry* **1966**, *5*, 1908.
(35) Li, Y.-H.; Chan, L.-M.; Tyer, L.; Moody, R. T.; Himel, C. M.; Hercules, D. M. *J. Am. Chem. Soc.* **1975**, *97*, 3118.
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(38) Cramer, F.; Saenger, W.; Spatz, H.-Ch. *J. Am. Chem. Soc.* **1967**, *89*, 14.
(39) Kinoshita, T.; Iinuma, F.; Tsuji, A. *Chem. Pharm. Bull.* **1974**, *22*, 2413.
(40) Schimerlik, M.; Quast, U.; Raftery, M. A. *Biochemistry* **1979**, *18*, 1884.

D. Host–Guest Association Constants K_a from Fluorescence Data Evaluated by Benesi–Hildebrand Treatment. The association constants of complexes of **8** with the fluorescing guests DNS (**12**), 1-(dimethylamino)naphthalene, and 1,3-dihydroxynaphthalene (Table II) could not be determined directly from a titration curve. Saturation binding of these guests was not obtained in the very dilute solutions that are necessary for a reliable quantitative evaluation of fluorescence data. Therefore the Benesi–Hildebrand treatment of the fluorescence data (eq 8)[41-43] was applied to obtain the association constants of the complexes of **8** with these guests.[44]

$$\frac{1}{\Delta F} = \frac{1}{K_a \Delta F_{max}} \frac{1}{H_0} + \frac{1}{\Delta F_{max}} \tag{8}$$

H_0 in eq 8 is the total concentration of host. ΔF is the fluorescence intensity increase of the guest upon addition of host. ΔF_{max} is the fluorescence intensity increase if all guest is complexed. The values of K_a and ΔF_{max} were obtained with good agreement either by graphic evaluation of the data or by a least-square-fit treatment with constant absolute error.

The considerable sensitivity to the solvent polarity of the emission of the nonionic fluorescing guest 1-(dimethylamino)-naphthalene (DAN)[35] is less familiar than the solvent dependency of the fluorescence of ANS, TNS, and DNS. We could successfully use it for the determination of the association constant of the complex of **8** with DAN. For DAN (λ_{exc} 320 nm) in water a weak fluorescence with λ_{max} 441 nm was measured; the emission intensity increased and the maximum shifted to higher energy when changing to less polar solvents. The emission maxima of DAN appeared at 411 nm in methanol, 405 nm in ethanol, 401 nm in dioxane, and 391 nm in *n*-hexane. The emission maximum of DAN (λ_{exc} 340 nm) complexed by **8** appears at 410 nm. With $H_0 = 10^{-4}$ mol·L^{-1} and $G_0 = 1.25 \times 10^{-6}$ mol·L^{-1} about 50% of the guest is complexed and the fluorescence intensity measured at 410 nm is about 8.5 times higher than the fluorescence at this wavelength of pure DAN in aqueous solution.

Since the inclusion complexes of **8** with various dihydroxynaphthalenes are studied by ^{1}H NMR spectroscopy[23] it was of interest to determine association constants for these complexes. A preliminary screening of the fluorescence emission in aqueous solution of the various dihydroxynaphthalenes showed that the fluorescence especially of 1,3-dihydroxynaphthalene changed upon addition of **8**. In aqueous solution this guest shows fluorescence bands at 380 and 397 nm (λ_{exc} 338 nm). Upon addition of host **8**, the total fluorescence intensity increases and the intensities of the two bands relative to each other change (Figure 8A). Both bands show small hypsochromic shifts to 375 and 388 nm. The changes of the fluorescence intensity upon addition of **8** were large enough for a determination of K_a from a double-reciprocal Benesi–Hildebrand plot as shown in Figure 8B.

E. Estimation of Association Constants of Complexes of 8 with Apolar Guests from Competitive Inhibition of the Binding of TNS and Evaluation of the Fluorescence of TNS by a Benesi–Hildebrand Type Treatment. There was the need for the estimation of association constants of host–guest complexes of **8** which were studied by ^{1}H NMR spectroscopy[23] and where the properties of the guest did not allow the application of the methods described in the previous sections. Either the guest did not exhibit any emission at all or no significant changes of the fluorescence occurred upon complexation. In the concentration ranges where changes in the absorption spectra of the guest upon complexation could have been observed and evaluated by a Benesi–Hildebrand treatment, with most of the guests, quantitative complexation occurred. A competitive inhibition experiment[45] was therefore applied to estimate the binding of guests like 2,7-dihydroxynaphthalene or naphthalenemonosulfonates and -disulfonates. These molecules as inhibitors compete for the cavity binding site of **8** with the guest TNS. At a constant concentration of inhibitor, the fluorescence intensity ΔF of TNS as a function of the total concentration of host is evaluated according to eq 9. Equation

$$\frac{1}{\Delta F} = \frac{1}{\Delta F_{max}} + \frac{1}{\Delta F_{max} H_0 K_G}(1 + K_I I_0) \tag{9}$$

9 can be derived under the assumption that $I_0 >> H_0 >> G_0$ (I_0, H_0, and G_0 are the total concentrations of inhibitor, host, and guest). In eq 9, K_G is the association constant for the 1:1 complex of **8** with the guest TNS and K_I is the association constant for the complex of **8** with inhibitor. ΔF is the fluorescence intensity increase of TNS in the aqueous solution of inhibitor upon addition of host, and ΔF_{max} is the fluorescence intensity increase if all TNS is complexed. All inhibitors used in our experiments do not exhibit fluorescence emission at λ_{exc} 360 nm of TNS. The Benesi–Hildebrand type plot of the fluorescence intensity is shown in Figure 9. From the intersect with the abscissa, the association constant K_I is obtained. Alternatively, the fluorescence data can be treated according to the Benesi–Hildebrand eq 6, and an apparent association constant K_{app} for the guest TNS is obtained with $K_{app} < K_G$. K_I can now be calculated from eq 10 derived from eq 9.

$$K_I = \frac{K_G - K_{app}}{K_{app} I_0} \tag{10}$$

The association constants so obtained for complexes of **8** with various guests are included in Table II together with typical experimental conditions. In selected experiments with various concentrations of the inhibitor ($I_0 = 10H_0(max)$, $20H_0(max)$, $30H_0(max)$, and $50H_0(max)$) essentially identical values of K_I were realized. The same association constants within the experimental limits were obtained by using instead of the sodium salts of 1- and 2-naphthalenesulfonate the corresponding sulfonic acids in aqueous solution or by using the sodium salts in a phosphate buffer (pH 6.86, ion strength 0.050 mol·L^{-1}).

The values of K_I are as expected independent of the fluorescing guest whose binding is inhibited. Thus for the complex of **8** with 2,7-dihydroxynaphthalene an association constant of 2.5×10^4 L·mol^{-1} was determined with ANS as the guest whose binding was inhibited. The reliability of the K_a values obtained by competitive inhibition was tested with naphthalene as inhibitor. The association constant from competitive inhibition was the same within experimental error as the association constant obtained from solid-liquid or liquid-liquid extraction. The solution of inhibitor for this experiment was prepared by extraction of solid naphthalene with water. For the successful application of the competitive inhibition method it was of great importance that the K_a values of the guests ANS and TNS had been determined with great accuracy, since all of the error in K_G adds to the error of K_I (eq 9 and 10). We found that with TNS as guest and under our experimental conditions association constants of the inhibitors in the range between $K_I \approx 10^2$ and 6×10^5 L·mol^{-1} could be determined. Below $K_I \approx 10^2$ L·mol^{-1} no significant inhibition of TNS occurs even when concentrations of inhibitor are used that are 200 times larger than the highest concentrations of host. Above $K_I \approx 6 \times 10^5$ L·mol^{-1} the binding of TNS is inhibited so efficiently that no significant fluorescence emission of TNS is observed. The association constant for the complex of **8** with 2,6-naphthalenedisulfonate, a very strong inhibitor, could therefore not be exactly determined and is estimated to be higher than 10^6 L·mol^{-1}. An association constant in this high range is supported by ^{1}H NMR experiments.[23]

F. Discussion of the Association Constants Determined for 1:1 Host–Guest Complexes of Host 8 in Aqueous Solution. All association constants were determined under the assumption of exclusive 1:1 host–guest complexation in aqueous solution. Extensive evidence for the correctness of this assumption was provided in the solid-liquid extraction experiments, by the fluorescence titration curves and by ^{1}H NMR investigations of host–guest complexation.[23] If association constants determined by different

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methods are compared, the question has to be answered how justified are such comparisons. We therefore chose guests which allowed the determination of the association constants of their complexes with **8** by two or more independent methods. Table I shows that similar values of K_a for the complexes of 8 with naphthalene, pyrene, fluoranthene, and durene were obtained from either solid-liquid or liquid-liquid extraction, so that the values of K_a from both methods can be compared with confidence. The K_a values obtained for ANS and TNS in very dilute solution by direct evaluation of the fluorescence titration curves (Figure 7) are considered as very accurate. This accuracy is a prerequisite for the use of TNS and ANS as guest in competitive inhibition experiments (eq 9 and 10). With naphthalene as inhibitor it could be shown that the K_a values obtained from competitive inhibition experiments are not only relevant relative to each other but also seem to be good absolute estimations. The value of K_a obtained for the complex with naphthalene by competitive inhibition (Table II) is identical within experimental error with the values obtained from the two extraction methods (Table I). 1-(Dimethylamino)naphthalene as substrate interrelates the association constants obtained by liquid-liquid extraction and by the Benesi–Hildebrand treatment of fluorescence data, since the K_a values determined by the two independent methods are in very good agreement. We can therefore conclude that the association constants of Tables I and II, which are obtained from five different methods, can be discussed in comparison.

The cavity of host **8** was designed as an apolar binding site with high affinity especially for neutral aromatic hydrocarbons as guests. CPK molecular models indicate that the highest complementarity exists between the cavity of **8** and large aromatic guests like perylene, pyrene, or fluoranthene. In agreement with these observations, the strongest complex with a very high association constant $K_a = 1.6 \times 10^7$ mol·L^{-1} is formed by perylene which is the best fitting guest with the largest apolar surface for hydrophobic and van der Waals interactions. With smaller sized guests the complementarity between host and guest is less ideal, and the association constants of the complexes decrease. Perylene binds considerably stronger than pyrene and fluoranthene, and a strong decrease of the stability of complex is obtained with the smaller guests biphenyl, azulene, and naphthalene (Table I). The association constants of the complexes with naphthalene and azulene are almost a magnitude higher than the association constant of the complex of **1** with durene, a guest of very similar size and shape. These facts indicate that an important contribution to the complex stability results from the polarizability of the guest, which is larger for naphthalene and azulene than for durene. The importance of van der Waals interactions has been previously recognized also for the inclusion complexes formed by cyclodextrins.[46,47] Hydrophobic interactions, however, are the most important driving force for the complexation of **8** with aromatic hydrocarbons. Water as solvent is essential, since according to ^{1}H NMR studies[23] strong complexation of **8** with pyrene occurs only in aqueous solution. Only weak complexation is found in methanol, and no complexation can be detected in dimethyl sulfoxide. The exclusivity of water in providing strong host–guest binding is attributed to the considerable reduction upon complexation of the unfavorable contacts between water molecules and the apolar surfaces of the guest as well as of the cavity of host **8**.

There is no significant difference between the association constants for complexes with naphthalene or methyl-substituted naphthalenes and those determined for complexes of naphthalene derivatives bearing one or two polar nonionic substituents like hydroxy- or dimethylamino groups (Table II). No important change in hydrophobic and van der Waals interaction can be expected if in the complexes the polar groups of the guests reach out of the cavity and the apolar naphthalene moiety of the guests is incorporated in the cavity of **8**. Host **8** seems to be specific for aromatic guests. With 1-adamantanol, an aliphatic guest with a large apolar surface, only weak complexation is found, although this guest according to CPK molecular models seems to fit into the cavity of **8**. The higher polarizability of aromatic guests might be one contribution to the stronger binding of these guests. As we shall see in detail in the ^{1}H NMR studies,[23] a specially favored location of aromatic guests in the cavity of **8** is presumably the major reason for the stronger complexation of these guests. Before, however, speaking more generally of a binding specificity of **8** for aromatic guests, the complexation of other apolar aliphatic guests with different structures has to be studied.

Unexpected at first view were the strong association constants for complexes with aromatic guests bearing anionic sulfonate residues. The high association constants for the complexes with ANS and TNS (Table II) were first thought to be a consequence of the binding of both the naphthalene and the benzene moiety to one host molecule. No such explanation was possible when we looked at the following series of comparable guests: the association constant for naphthalene as guest is 1.5×10^4 L·mol^{-1}. For 1-(dimethylamino)naphthalene, we found a comparable K_a value of 9.4×10^3 L·mol^{-1}. For 5-(dimethylamino)-1-naphthalenesulfonate (DNS), however, the association constant was found to be considerably higher with 1.4×10^5 L·mol^{-1}. This increase of the binding constant by a power of 10 must be ascribed to additional electrostatic stabilization of the complex. Also the very large binding constants for the complexes with ANS and TNS have to be explained by this additional stabilization. Again this explanation was evidenced by ^{1}H NMR studies of the complexes of **8** with naphthalenedisulfonates, naphthalenemonosulfonates, and tosylate[23] whose high association constants are shown in Table II. As a consequence of the conformational flexibility of the aliphatic bridges of **8** the piperidinium rings fixed in these bridges can turn out of the mean molecular plane of **8**. The quaternary nitrogens of these rings now can come in close proximity to the anionic centers of the guests which by their apolar part are enclosed in the cavity of **8** and both hydrophobic and electrostatic interactions (ion pairing) lead to very strong complexation. This will be discussed in detail in the following paper. The most striking difference is seen when comparing the association constant for the complexes with sodium 1-naphthalenesulfonate ($K_a = 3.5 \times 10^5$ L·mol^{-1}) and 1-(trimethylammonio)naphthalene fluorosulfonate ($K_a = 1.7 \times 10^3$ L·mol^{-1}). In both complexes the same apolar naphthalene core can be enclosed in the cavity of **8**. With the anionic guest additional electrostatic stabilization occurs, whereas with the cationic guest repulsive interactions with the quaternary nitrogens of the spiro piperidinium rings seem to be effective since the association constant for this cationic guest is even lower than the K_a value for the complex with 1-(dimethylamino)naphthalene.

In conclusion, besides being the expected powerful host for neutral arenes, **8** was also found to be a good host for aromatic guests bearing anionic residues. The K_a values for the complexes of **8** with neutral arenes are the highest reported for 1:1 complexes formed by artificial molecular hosts. The K_a values for the complexes of **8** with aromatic guests bearing sulfonate residues are even higher than those reported for complexes with hosts like **2** where cationic centers for strong electrostatic interactions are directly built into the macrocyclic framework surrounding the cavity.[6d]

Acceleration by Host–Guest Complexation of the Transport of Aromatic Hydrocarbons through an Aqueous Phase. The transport of cations through lipophilic natural or artificial membranes accelerated by natural[48-50] or synthetic ionophores[51,52] is a well-

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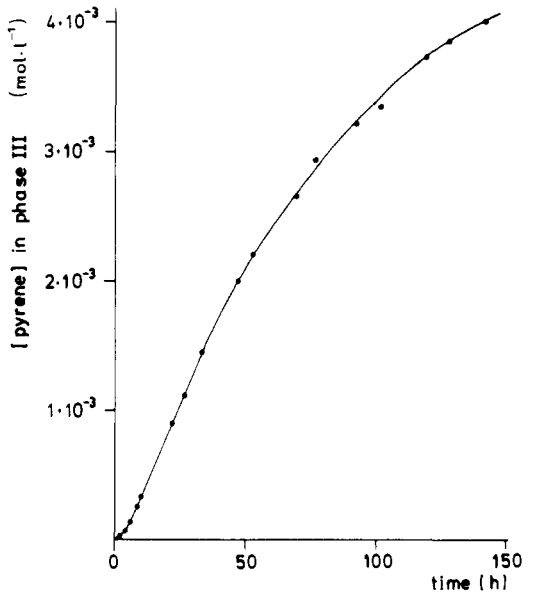

Figure 11. Transport of pyrene through a 5×10^{-3} M aqueous solution of host **8** as phase II; the initial concentration of pyrene in the *n*-hexane source phase I is 10^{-2} mol·L^{-1}; T = 293–295 K.

known process. There is, however, only little information available about the transport of lipophilic substances through an aqueous solution mediated by artificial carriers. Recently, the transport of neutral arenes through an aqueous medium mediated by oil-in-water microemulsion globules has been reported.[53] We want to present an example of the acceleration of the transport of neutral arenes through an aqueous phase along a concentration gradient mediated by **8** as a molecular carrier.

All experiments with the various arenes were performed in the U-type cell shown in Figure 10. The source phase I is a solution of the arene in *n*-hexane, the initial concentration of arene in the source phase is always 10^{-2} mol·L^{-1}. Phase II is a magnetically stirred solution of host **8** in water and the receiving phase III is pure hexane. In the receiving phase the concentration of delivered arene which is a measure for the relative rate of transport of the guest across the aqueous phase II is determined by electronic absorption spectroscopy. The plot of Figure 11 shows the concentration of pyrene delivered in phase III as a function of time when phase II is a 5×10^{-3} M solution of host **8**. After an induction period, in which the equilibrium between phase I and phase II is established, the transport rate is constant. When the concentration gradient between phase I and phase III decreases, the transport rate slows. The relative transport rate is obtained from the slope of the linear part of the plot as the increase of the concentration of pyrene in phase III per hour.

The relative rates of transport of pyrene, fluoranthene, azulene, naphthalene, and durene across a 5×10^{-4} M aqueous solution of **8** were determined from the plots shown in Figure 12. The relative rates of passage of these arenes across phase II as pure water were determined similarly. We found that the rates of transport of pyrene and fluoranthene through a 5×10^{-4} M aqueous solution of **8** are 430 and 395 times higher, respectively, than the rates of passage through pure water. A considerably smaller but still significant acceleration in the presence of **8** was obtained for azulene, naphthalene, and durene with acceleration factors of 3.6, 3.7, and 1.8, respectively.

In very dilute solutions the acceleration was found to be proportional to the amount of **8** in phase II. A relative transport rate of 1.1×10^{-6} mol·L^{-1}·h^{-1} corresponding to a 47-fold acceleration was obtained for pyrene when phase II was a 5×10^{-5} M aqueous solution of **8**. At higher concentrations of **8** in phase II the relationship between the rate of transport and the amount of **8** in phase II was no longer linear. The rate of transport of pyrene through a 5×10^{-3} M solution of **8** (Figure 11) was determined to be 4.9×10^{-5} mol·L^{-1}·h^{-1} corresponding to a 2100-fold acceleration. All given rate values were reproducible within 10% at constant experimental conditions using the same cell, the same magnetic stirring bar, and a constant stirring speed of ≈1250 rpm at which very clean interfaces exist between the three clear phases.

That host–guest complexation in the aqueous phase II between **8** and the arene was essential for the acceleration of transport was evidenced by a competitive inhibition experiment. 2,6-Naphthalenedisulfonate with its high association constant (Table II) is an efficient competitive inhibitor of the complexation of **8** with pyrene. If phase II is a 5×10^{-4} M aqueous solution of host **8** containing also a 10^{-2} M concentration of 2,6-naphthalenedisulfonate, the relative rate of transport of pyrene is only 3.6×10^{-8} mol·L^{-1}·h^{-1} and is almost reduced to the rate through pure water.

After establishing the importance of complexation to transport acceleration, we wanted to know if there is a simple relationship between the relative rates of transport in the presence of carrier or the acceleration factors shown in Table III and thermodynamic parameters of the host–guest complexation. The transport of cations through lipophilic liquid membranes mediated by artificial

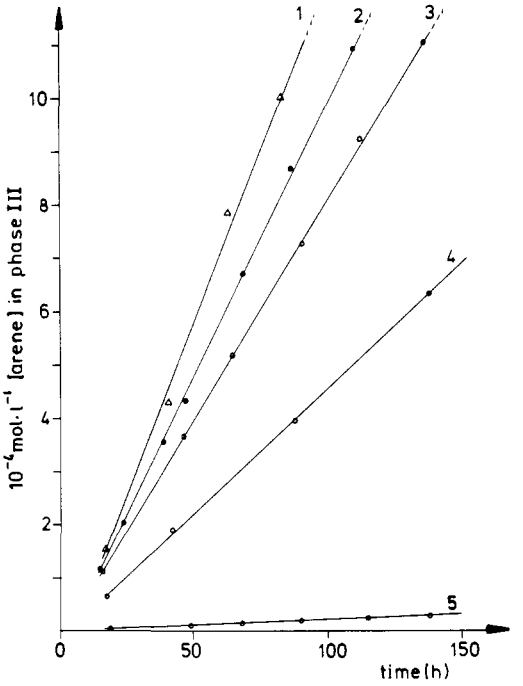

Figure 12. Transport of fluoranthene (1), pyrene (2), azulene (3), naphthalene (4), and durene (5) across a 5×10^{-4} M solution of host **8** as phase II. The initial concentration of arene in source phase I is 10^{-2} mol·L^{-1}; T = 293–295 K. Plots 1 and 2 are drawn by considering additional data points which are out of the scale of Figure 12.

Table III. Relative Rates of Transport of Arene through the Aqueous Phase II of the U-Type Cell Shown in Figure 10, T = 293–295 K[a]

arene	relative rate, mol·L^{-1}·h^{-1} through a 5×10^{-4} M solution of **8**	relative rate, mol·L^{-1}·h^{-1} through water	acceleration factor	K_e, L·mol^{-1}
fluoranthene	1.3×10^{-5}	3.3×10^{-8}	395	19.8
pyrene	1.0×10^{-5}	2.3×10^{-8}	430	14.9
azulene	8.6×10^{-6}	2.4×10^{-6}	3.6	12.6
naphthalene	4.4×10^{-6}	1.2×10^{-6}	3.7	4.6
durene	2.2×10^{-7}	1.2×10^{-7}	1.8	0.07

[a] The initial concentration of arene in source phase I (*n*-hexane) is 10^{-2} mol·L^{-1}. The extraction constants are calculated from eq 6 by using the values of K_a and K_d (Table I) which were determined by liquid-liquid extraction.

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macrocyclic ionophores as carriers has been studied with regard to this question.[52,54,55] No simple relationship between the rates of cation transport and thermodynamic parameters like the stability of the host–guest complex formed in the liquid membrane between cation and carrier or the extractability of the cation from the aqueous phase into the membrane was found. In our experiments we found an approximate proportionality between the relative rates of transport through phase II in the presence of carrier **8** and the extractability of the arene from *n*-hexane into the aqueous phase as expressed by the extraction constant K_e (Table III). As the rates of passage through pure water were found to be approximately proportional to the distribution constants K_d (Table I), all rates are proportional to the amount of arene present in the aqueous phase II, no matter whether a carrier is present or not.

The various acceleration factors for different arenes in our experiments can now be better understood. Pyrene and fluoranthene have low distribution constants. Accordingly, there is only a very small amount of these guests in the pure aqueous phase II and the passage of the arene through pure water is slow. However, by strong host–guest complexation in phase II a significantly larger amount of these two arenes is extracted in this phase and a faster transport is observed. Therefore, for arenes with low distribution constants and high association constants, a strong acceleration is obtained. Since naphthalene and azulene have higher valued distribution constants than pyrene and fluoranthene, there is a higher amount of the former arenes in the pure aqueous phase II and the rates of passage are already rather large. The amount of naphthalene and azulene which upon the weaker host–guest complexation with **8** is additionally extracted into phase II is not so much larger and therefore a smaller acceleration is obtained. Although host–guest complexation with durene is even 1 order of magnitude weaker, a small but significant acceleration is measured because of its low distribution constant.

Experimental Section

General. ^{1}H NMR spectra were recorded on a Bruker HX-360 MHz spectrometer. For spectra in deuterium oxide a capillary tube with TSP as external standard in D_2O was placed inside the NMR tube. Electronic absorption spectra were recorded with a Cary 17 spectrometer. Uncorrected fluorescence spectra were measured at (292.5 ± 0.1) K with a SLM 8000 spectrofluorometer. The solvents for optical spectroscopy were either doubly distilled water or spectral grade *n*-hexane (Merck, Uvasol). Aqueous solutions in all experiments were prepared with doubly distilled water from a Heraeus-Schott Bi 4 double distillation apparatus; in selected experiments an aqueous standard phosphate buffer solution (Riedel de Haën), pH 6.86 (25 °C), ion strength 0.05 mol·L^{-1} was used. Ultrasonifications were performed in a Bandelin Sonorex RK 255H ultrasonification bath whose water temperature was maintained at 293–295 K.

Host 8. Crystals of **8**[7b,23] from methanol–ether are hygroscopic and take up 4 equiv of water from the atmosphere. Water uptake beyond this stoichiometric amount is very slow, and the elemental analysis (C, H, N, Cl) gives correct values (within 0.20%) for **8**·4H_2O ($C_{68}H_{104}N_4O_4Cl_4$·4H_2O, M_r 1255.5, mp 270 °C dec) even after 1 week of exposure to laboratory air. After standing for 2 months in a glass bottle at ordinary laboratory conditions, the UV spectroscopically determined purity of **8**·4H_2O was still 97%. Host **8** was used in all experiments within 4 weeks after its preparation and all concentrations were calculated from **8**·4H_2O. The absorption spectrum of **8** in water: λ_{max} (log ϵ) 302 (1.66 sh), 274 (3.22, sh), 268 (3.30), 232 (4.24, sh), 196 (5.05).

Guests. Reagent grade perylene, 2,6-dimethylnaphthalene, azulene (EGA), naphthalene, biphenyl, pyrene (Merck), durene (Roth), and fluoranthene (Fluka) were purified by recrystallization,[56] followed by sublimation. 1,5-Dimethylnaphthalene[57] and 1-(trimethylammonio)-naphthalene fluorosulfonate[58] were prepared and characterized according to the literature procedures. 1-Adamantanol and 1,3- and 2,7-dihydroxynaphthalenes (EGA) were recrystallized from water. 1-(Dimethylamino)naphthalene (EGA) was distilled prior to use. Potassium 6-[(4-methylphenyl)amino]-2-naphthalenesulfonate (Serva), ammonium 8-(phenylamino)-1-naphthalenesulfonate (Fluka), sodium *p*-toluenesulfonate, sodium 2-napthalenesulfonate, disodium 1,5-naphthalenesulfonate (Merck), and disodium 2,6-naphthalenesulfonate (Fluka) were used without further purification. Aqueous solutions of the sodium salts of 5-(dimethylamino)naphthalene-1-sulfonic acid (Fluka) and 1-naphthalenesulfonic acid (Merck) were prepared by addition of the stoichiometric amount of 0.1 N aqueous NaOH to the solutions of the sulfonic acids. All commercial sulfonates and sulfonic acids were of very high isomeric purity (^{1}H NMR) except 1-naphthalenesulfonic acid which contained about 10% of 2-naphthalenesulfonic acid. The electronic absorption spectra of aromatic guests used in solid-liquid extraction, liquid-liquid extraction and the transport experiments are of importance for the quantitative evaluation of these experiments. The bands which have been used for the determination of concentrations are listed. Pyrene (*n*-hexane): λ_{max} (ϵ) 351 (510), 334 (56 200), 318 (30 550), 304 (11 700), 293 (4300), 272 (54 050), 261 (25 950), 251 (11 600), 239 (88 000). Perylene (*n*-heptane):[59] λ_{max} (ϵ) 435 (39 500), 408 (29 000), 386 (13 400). Fluoranthene (*n*-hexane): λ_{max} (ϵ) 358 (8630), 341 (8000), 323 (6240), 308 (3620), 287 (50 540). Azulene (*n*-heptane):[59] λ_{max} (ϵ) 353 (1200), 337 (3950), 296 (3800), 280 (49 000), 275 (52 000), 271 (52 000). Naphthalene (*n*-hexane):[59] λ_{max} (ϵ) 286 (3900), 275 (5600), 266 (5000), 221 (117 000). Durene (*n*-heptane): λ_{max} (ϵ) 217 (9500), 196 (56 000). Biphenyl (light petroleum, bp 100–120 °C:[59] λ_{max} (ϵ) 247 (17 000), 201 (46 500). 1,5-Dimethylnaphthalene (*n*-hexane): λ_{max} (ϵ) 297 (6410), 285 (9200), 274 (7170), 227 (114 750). 2,6-Dimethylnaphthalene (*n*-hexane): λ_{max} (ϵ) 274 (4870), 226 (129 550). 1-(Dimethylamino)naphthalene: λ_{max} (ϵ) 303 (5320), 239 (13 610), 214 (57 180).

Formation of Host–Guest Complexes of 8 with Neutral Arenes by Solid-Liquid Extraction and Determination of Association Constants (K_a). All experiments were conducted at 293–295 K. A suspension of solid guest in an aqueous solution of host **8** was exposed to ultrasonification for 20 min except for perylene as guest where 3 h was used. With fluoranthene and pyrene the concentration of **8** (H_0) in the aqueous solution was chosen in the range 5.5×10^{-3}–5×10^{-5} mol·L^{-1}, with perylene H_0 was 10^{-3}–10^{-4} mol·L^{-1}, with naphthalene and durene H_0 was 5.5×10^{-3}–1×10^{-3} mol·L^{-1}, and with 1,5- and 2,6-dimethylnaphthalene H_0 was 5.5×10^{-3} mol·L^{-1}. At $H_0 \approx 5 \times 10^{-5}$ mol·L^{-1} about 30 mg of solid guest was extracted with 50 mL of aqueous solution of **8** and at $H_0 \approx 5 \times 10^{-3}$ mol·L^{-1} about 150 mg of solid guest was extracted with 10 mL of aqueous solution of **8**. After ultrasonification and vigorous shaking for 5 min, the suspension was centrifugated at 6000 rpm for 30 min and afterward the decanted aqueous solution of complex was filtered. Electronic absorption and emission and, when a D_2O solution of **8** was used, ^{1}H NMR spectra[23] were recorded from the so obtained solution of complex. The major bands of complexed guest in the electronic absorption spectra which are not overlapped by bands of host **8** are in the aqueous solution of **8**–perylene: λ_{max}(nm) 441, 415, 392, of **8**–fluoranthene: λ_{max}(nm) 365, 348, 325, 310, 288 and of **8**–pyrene: λ_{max}(nm) 339, 323, 310, 297, 276, 264. The bands of complexed guest in the electronic emission spectra of the aqueous solution of **8**–pyrene (λ_{exc} 340 nm) appear at λ_{em}(nm) 377, 394, 413 (sh), and 438 (sh); of **8**–fluoranthene (λ_{exc} 368 nm) at λ_{em}(nm) 446 and 457 (sh); and of **8**–perylene (λ_{exc} 402 nm) at λ_{em}(nm) 445, 477, and 507.

An aliquot of the aqueous solution was extracted in a 75-mL separatory funnel six times with 30 mL of *n*-hexane, and the combined *n*-hexane phases in a 200-mL volumetric flask were brought to the mark with *n*-hexane. From a concentrated aqueous solution of complex, an aliquot of 3 mL was used which for the extraction was diluted to 30 mL with water. From an aqueous solution with low concentration of complex, an aliquot of 30 mL was used for the extraction with *n*-hexane. The amount of guest in the 200 mL of *n*-hexane was determined by electronic absorption spectroscopy and from this value the total concentration of guest in the aqueous solution of complex, G_{H_2O}(tot), was calculated. From the absorbance of an aliquot of the aqueous phase after the extraction with *n*-hexane, the total concentration of the host in the aqueous solution of complex was determined and found in all experiments to be equal to the initial concentration of host, H_0, within experimental error.

For the determination of the maximum solubility of the guest in water, G_{H_2O}(max), 50 mg of solid guest was suspended in 50 mL of doubly distilled water and were extracted by the procedure described above. An aliquot of 40 mL of the aqueous solution obtained after sonification, centrifugation, and filtration was extracted twice with 24 mL of *n*-hexane. In the combined *n*-hexane extracts, which in a 50-mL volumetric flask were brought to the mark with the same solvent, the amount of the guest

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was determined by electronic absorption spectroscopy (a path length of 100 mm was used for the determination of pyrene and fluoranthene) and the concentration of guest in the aqueous solution equal to $G_{H_2O}(max)$ was calculated. By subtracting $G_{H_2O}(tot) - G_{H_2O}(max)$, the concentration of the complex HG_{H_2O} was obtained, and $H_0 - HG_{H_2O}$ gave the concentration of free host H_{H_2O} in the aqueous solution of complex. All concentrations in eq 1 and 2 being known, the association constants K_a, listed together with the values of $G_{H_2O}(max)$ in Table I, were determined.

Formation of Host–Guest Complexes of 8 with Aromatic Hydrocarbons by Liquid-Liquid Extraction and Determination of Association Constants (K_a). All operations were conducted at 293–295 K. An aqueous solution of host **8** (30 mL) and a solution of guest (30 mL) in *n*-hexane in a 75-mL separatory funnel were vigorously shaken for 10 min. With all guests except durene the initial concentration of the guest in the *n*-hexane solution was 10^{-2} mol·L^{-1} and the concentration of the host in the aqueous solution H_0 was 2×10^{-4} mol·L^{-1}. For durene, the initial concentration in *n*-hexane was 5×10^{-1} mol·L^{-1} and H_0 was 10^{-3} mol·L^{-1}. Upon standing overnight, the interface between the two clear phases was very clean and most of the aqueous phase was carefully let out of the separatory funnel. An aliquot of 28 mL of the aqueous phase was extracted with three portions of 30 mL of *n*-hexane, and the combined *n*-hexane extracts in a 100-mL volumetric flask were brought to the mark with *n*-hexane. The amount of guest in the 100 mL of *n*-hexane was determined by electronic absorption spectroscopy and from this value the total concentration of guest in the aqueous phase, $G_{H_2O}(tot)$, was calculated. Similarly, 30 mL of the described solutions of guest in *n*-hexane was shaken with 30 mL of doubly distilled water. After clean phase separation overnight most of the aqueous phase was removed carefully from the separatory funnel and an aliquot of 28 mL was extracted with two portions of 24 mL of *n*-hexane. The combined *n*-hexane extracts in a 50-mL volumetric flask were brought to the mark with additional *n*-hexane. The amount of guest in the 50 mL of *n*-hexane was determined by electronic absorption spectroscopy, and the concentration of guest in the aqueous phase G_{H_2O} was calculated. Subtracting $G_{H_2O}(tot) - G_{H_2O}$ gave the concentration of the complex in the aqueous phase HG_{H_2O}, and $H_0 - HG_{H_2O}$ gave the concentration of free host in the aqueous phase H_{H_2O}. All concentrations of eq 1 and 2 being known, the association constants (K_a) listed in Table I were calculated.

Association Constants (K_a) from Fluorescence Data. The solution for each data point of a fluorescence titration was prepared by the addition of the calculated amounts of freshly prepared stock solutions of host **8** and guest in water or aqueous phosphate buffer (pH 6.86) into light-protected 10- or 20-mL volumetric flasks using Brand macrotransferpettors. With additional doubly distilled water or buffer solution the solutions were brought to the mark. In the competitive inhibition experiments, a stock solution of **8** and a stock solution containing both guest and inhibitor were used. All data were collected at the fluorescence maximum of the guest at (292.5 ± 0.1) K after 10 min of thermostatization of the 1-cm cuvette. The excitation and emission wavelengths and typical concentrations of host, guest, and the inhibitor in the competitive inhibition experiments are given in Results and Discussion. Inner-filter effects were neglegible in all experiments; with 5-cm cuvettes all solutions used for fluorescence spectroscopy showed no optical density at either λ_{exc} or λ_{em}.

Transport Experiments. All experiments were conducted at 293–295 K by using the U-type cell of Figure 11. The cell was held in exactly the same position to a magnetic stirring motor in all experiments. A total of 15 mL of pure double distilled water or of an aqueous solution of host **8** (concentrations of **8**: 5×10^{-5}, 5×10^{-4}, and 5×10^{-3} mol·L^{-1}) was located at the bottom of the cell as phase II. Atop phase II in one arm of the cell 8 mL of a 10^{-2} M solution of arene in *n*-hexane was placed as source phase I. In the other arm 8 mL of *n*-hexane was placed atop phase II as receiving phase III. The aqueous phase II was agitated with an approximately cylindrical magnetic stirring bar 10 mm in length and 7 mm in diameter at a constant stirring rate of ≈1250 rpm. At this rate clean interfaces were obtained between the clear phases. The two arms of the cell were closed with removable stopcocks during the experiment. Samples taken from the receiving phase III for the determination of the amount of delivered guest by electronic absorption were added to phase III after the recording of the spectra.

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Registry No. 8, 86765-98-2; **8·7**, 93254-53-6; **8·11**, 93254-54-7; **8·12**, 93279-98-2; **8**–perylene, 93254-43-4; **8**–fluoranthrene, 93254-44-5; **8**–pyrene, 93254-45-6; **8**–1,5-dimethylnaphthalene, 93254-46-7; **8**–2,6-dimethylnaphthalene, 93254-47-8; **8**–biphenyl, 93254-48-9; **8**–azulene, 93254-49-0; **8**–naphthalene, 93254-50-3; **8**–1-(dimethylamino)-naphthalene, 93254-51-4; **8**–durene, 93254-52-5; **8**–1,3-dihydroxy-naphthalene, 93254-55-8; **8**–2,6-naphthalenedisulfonate, 93254-56-9; **8**–1,5-naphthalenedisulfonate, 93254-57-0; **8**–2-naphthalenesulfonate, 93254-58-1; **8**–1-naphthalenesulfonate, 93254-59-2; **8**–2,7-dihydroxy-naphthalene, 93254-60-5; **8**–*p*-toluenesulfonate, 93254-61-6; **8**–1-(trimethylammonio)naphthalene fluorosulfonate, 93254-62-7; **8**–1-adamantanol, 93254-63-8; TNS^-K^+, 32752-10-6; $ANS^-NH_4^+$, 28836-03-5; DNS^-Na^+, 21263-87-6; $p\text{-}MeC_6H_4SO_3^-Na^+$, 657-84-1; perylene, 198-55-0; floroanthrene, 206-44-0; pyrene, 129-00-0; 1,5-dimethylnaphthalene, 571-61-9; 2,6-dimethylnaphthalene, 581-42-0; biphenyl, 92-52-4; azulene, 275-51-4; naphthalene, 91-20-3; 1-(dimethylamino)-naphthalene, 86-56-6; durene, 95-93-2; 1,3-dihydroxynaphthalene, 132-86-5; disodium 2,6-naphthalenedisulfonate, 1655-45-4; disodium 1,5-naphthalenedisulfonate, 1655-29-4; sodium 2-naphthalenesulfonate, 532-02-5; sodium 1-naphthalenesulfonate, 130-14-3; 2,7-dihydroxy-naphthalene, 581-43-1; 1-(trimethylammonio)naphthalene fluoro-sulfonate, 93254-42-3; 1-adamantanol, 768-95-6.