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Chromophoric Water-Soluble Tetrakis(4-Carboxyphenylazo)-calix[4]arene: Binding of Arylammonium Ions and Benzene

J. CAMERON TYSON, DAVID M. COLLARD* and KENNETH D. HUGHES
School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia, 30332-0400, U.S.A.

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Abstract. The interaction of water-soluble tetrakis(4-carboxyphenylazo)calix[4]arene with N, N, N-trimethylarylammonium iodides and benzene was investigated in order to correlate the effect of anilinium salts on the binding properties of the cup-shaped hydrophobic cavity with the monolayer spreading behavior of a series of amphiphilic analogs. Whereas multiple binding is observed for N, N, N-trimethylanilinium iodide and benzene, NMR titration of tetrakis(4-carboxyphenylazo)calix[4]arene with N, N, N-trimethyl-N-(2-naphthyl)ammonium iodide indicates 2 : 1 complexation within the cavity of the calixarene with binding constants of K_1 = 8024 M^{-1} and K_2 = 3134 M^{-1}.

Key words: Water soluble chromophoric calixarene, arene binding.

1. Introduction

Photoswitchable host–guest interactions [1] and the use of molecular self-assembly to form ordered arrays of these molecular receptors for sensor applications have attracted considerable attention. Monolayers of molecular hosts on solid substrates are available by use of Langmuir–Blodgett transfer of monolayers of amphiphiles from the air–water interface [2] or by molecular self-assembly through chemisorption of functional adsorbates [3].

We recently reported the monolayer spreading of amphiphilic azo-substituted calixarenes to form densely packed monolayers at the air–water interface [4] (Figure 1). (4-Alkylphenylazo)-substituted calixarenes **1** and **2** were designed to have a *cis-trans* photoisomerizable azo linkage which might provide a mechanism for switching the cavity between binding and non-binding forms. The *para*-azo linkage renders the phenol acidic such that the tetraphenolic face of the molecule is hydrophilic, and 4′-substitution with an alkyl group provides a hydrophobic tail. The limiting areas and collapse pressures of monolayers of these amphiphilic calixarenes are sensitive to pH, electrolyte concentration, and the presence of anilinium salts in the subphase. The limiting area of tetrakis(4-octylphenylazo)calix[4]arene decreases in the presence of N, N, N-trimethylanilinium iodide in the subphase while no

* Author for correspondence.

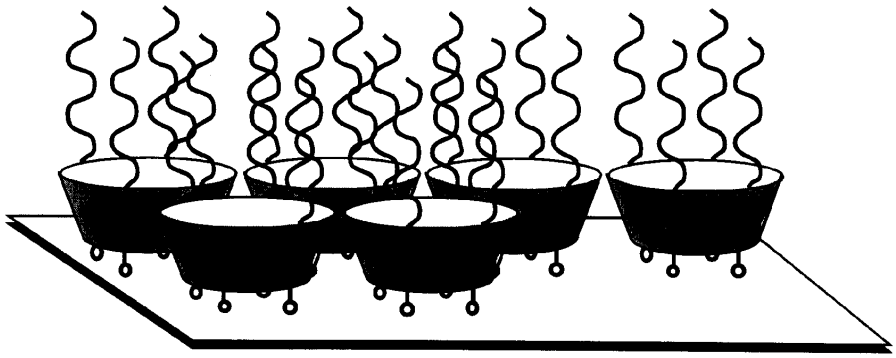
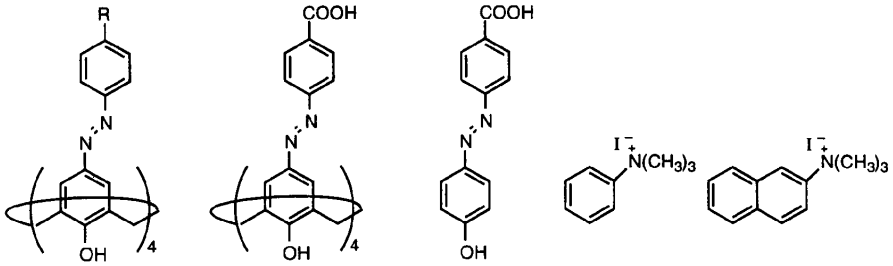


Figure 1. Sketch of amphiphilic cup-shaped calixarene in a close-packed monolayer at the air–water interface.

change is detected for tetramethylammonium iodide or N, N, N-trimethyl-N-(2-naphthyl)ammonium iodide [4]. Previous reports of (phenylazo)-substituted calixarenes include a description of the autocatalytic nature of azo bond formation [5], the selectivity for binding to metals [6], spectral properties of the azophenol–quinine–hydrazone tautomerization [7] and detection of amines with a chromophoric calix[8]arene [8].

The effects of anilinium salts on the spreading behavior of monolayers of the azo-substituted calixarenes **1** and **2** is consistent with binding interactions between the cavity of the calixarene and the arene. In order to investigate this effect in solution rather than at the interface we required a soluble derivative of the amphiphiles **1** and **2**. Calixarenes form complexes with a variety of organic molecules in the solid-state, but they form few complexes with neutral molecules in organic solution. Recent studies have focused on water-soluble calixarenes and their ability to bind molecules in aqueous solution [9]. Although binding of water-soluble calixarenes with charged aromatic guests through favorable electrostatic interactions has been demonstrated, complexation of calixarenes with neutral molecules in solution is still considered to be in its infancy.

In this report we focus on a water-soluble chromophoric 5,11,17,23-tetrakis[(4-carboxyphenyl)azo]-25,26,27,28-tetrahydroxycalix[4]arene, **3**, and its ability to bind both cationic and neutral aromatic molecules. Binding studies were performed by NMR titration of cup-shaped molecules with tetramethylammonium iodide, N, N, N-trimethyl-N-arylammonium iodides **5** and **6**, and benzene. 4-(4′-Carboxyphenylazo)phenol **4** was used as a non-cyclic analog of cup-shaped cyclic tetramer **3** to investigate the importance of the cavity on host–guest binding. The binding of aromatic compounds by **3** driven by the hydrophobic effect correlates with the observed effects on monolayer spreading of **1** and **2**.



1, R = ^{n}butyl
2, R = ^{n}octyl
3
4
5
6

Formulas **1–6**.

2. Experimental

2.1. Materials and methods

All reagents were obtained from commercial sources and used without further purification. Buffered solutions were prepared with sodium bicarbonate, sodium hydroxide, and D_2O. ^{1}H-NMR spectra were recorded on a Varian Gemini 300 MHz instrument. Chemical shifts are reported relative to tetramethylsilane for the organic solvents or sodium 3-(trimethylsilyl)-1-propanesulfonate for aqueous solutions. ^{13}C-NMR spectra were obtained at 75.5 MHz. IR analysis was performed on a Nicolet 520 FTIR spectrometer. Elemental analyses were performed by Atlantic Microlab (Atlanta, Georgia, U.S.A.).

For complexation, $\delta_{obs} = \delta_{free}$ ([Guest]$_0$ − [complex])/[Guest]$_0$) + $\delta_{complex}$ ([complex]/[Guest]$_0$) where [Guest]$_0$ is the initial concentration of guest. Through manipulation, $\delta_{obs} = \delta_{free} - (\delta_{free} - \delta_{complex}/(2[\text{Guest}]_0) \times (b - (b^2 - 4\ [\mathbf{3}]_0$ [Guest]$_0)^{1/2})$ where $b = 1/K_a +$ [**3**]$_0$ + [Guest]$_0$. Binding constants were determined by a computer-assisted nonlinear curve fitting program: HOSTEST 5.0.

2.2. Synthesis

2.2.1. *Calixarenes*

5,11,17,23-Tetra-*tert*-butyl-25,26,27,28-tetrahydroxycalix[4]arene was prepared by reaction of *tert*-butylphenol with formaldehyde according to the method of Gutsche [10]. Treatment of *tert*-butylcalixarene with aluminum chloride gave 25,26,27,28-tetrahydroxycalix[4]arene according to the method of Ungaro [11].

2.2.2. *5,11,17,23-Tetrakis[(4-carboxyphenyl)azo]25,26,27,28-tetrahydroxycalix[4]arene,* **3**

The diazonium salt of 4-aminobenzoic acid was prepared by adding a solution of sodium nitrite (109.6 mg, 1.588 mmol) in water (1 mL) dropwise to a solution

of 4-aminobenzoic acid (179.7 mg, 1.310 mmol) in 0.5 M aqueous HCl solution at 5 °C. A solution of calix[4]arene (91.8 mg, 216 μmol) in THF-pyridine (5 : 1 mL) was added dropwise to the solution of diazonium salt to form an orange solid. Filtration afforded 5,11,17,23-tetrakis[(4-carboxyphenyl)azo] 25,26,27,28-tetrahydroxycalix[4]arene as an orange crystalline solid (138.4 mg, 63%). m.p. = 356 °C. ^{1}H-NMR (DMSO-d_6) δ 3.8 (br. s, 4H, Ar—CH—Ar), 4.2 (br. s, 4H, Ar—CH—Ar), 7.82 (s, 8H, Ar—H), 7.86 (d, 8H, Ar′—H, J = 8.2 Hz), 8.02 (d, 8H, Ar′—H, J = 8.2 Hz). ^{13}C-NMR (DMSO-d_6) δ 32.2, 123.3, 125.9, 131.8, 132.0, 132.8, 146.3, 156.1, 160.1, 168.4. IR (KBr) 2500–3500 (OH stretch), 1697 (C=O stretch). UV-VIS (H_2O, pH = 11) λ_{max} 365 nm, $\varepsilon = 8 \times 10^4$ M^{-1} cm^{-1}. *Elemental analysis* for $C_{56}H_{40}N_8 \cdot 1.5\ H_2O$ *expected*: C, 64.42; H, 4.16; N, 10.73. *Found*: C, 64.51; H, 4.11; N, 10.66.

2.2.3. *4-(4′-Carboxyphenylazo)phenol* **4**

4-(4′-Carboxyphenylazo)phenol, **4**, was prepared from phenol and the diazonium salt prepared from 4-aminobenzoic acid according to the method of Fel'dman and Frankovskii [12]. ^{1}H-NMR ($CDCl_3$) δ 7.00 (d, 2H, Ar—H, J = 8.4 Hz), 7.82 (d, 2H, Ar′—H, J = 8.4 Hz), 7.83 (d, 2H, Ar—H, J= 8.4 Hz), 8.00 (d, 2H, Ar′—H, J = 8.4 Hz). ^{13}C-NMR (DMSO-d_6) δ 117.5, 123.4, 126.7, 132.0, 135.1, 146.8, 155.8, 163.4, 168.8. IR (KBr) 2500–3500 (OH stretch), 1683 (C=O stretch). UV-VIS (H_2O, pH = 11) λ_{max} 365 nm, $\epsilon = 2 \times 10^4$ M^{-1} cm^{-1}. m.p. = 265–266 °C ([12]: 264–266 °C).

3. Results and Discussion

5,11,17,23-Tetrakis[(4-carboxyphenyl)azo]-25,26,27,28-tetrahydroxycalix[4]-arene, **3**, was prepared by the reaction of the diazonium salt of 4-aminobenzoic acid with calix[4]arene. Preliminary determination of binding was performed by NMR titration whereby the chemical shift of a proton on the guest is monitored as a function of guest concentration [13]. Tetra-acid cup-shaped **3** is soluble in D_2O at a pD of 11 (bicarbonate buffer). Potential guests explored were benzene, N, N, N-trimethylanilinium iodide (**5**), N, N, N-trimethylammonium-N-(2-naphthyl) iodide (**6**), and sodium benzoate. Protons of aromatic guests experience an upfield shift in the presence of macrocyclic host **3** (1 mM) due to their location within the magnetic field generated by ring currents in the aryl units of the host. Figure 2 shows the chemical shift of the methyl protons of **5** in the presence of **3** in D_2O. The trimethylammonium protons of **5** shift upfield by more than 2.5 ppm upon complexation. These shifts are consistent with the effects of the ring currents on the protons within the cavity of the calixarene. The aryl protons of **5** are also subject to an upfield shift over the same concentration range (i.e., *ortho* protons shift from δ = 7.8 to 6.3 ppm). However, it is difficult to follow changes in chemical shift of these protons due to their coupling and low intensity in the NMR spectrum

relative to the trimethylammonium protons (owing to the low concentration used at this molar ratio). Upon titration of **3** with **5** in D_2O, the signals for the protons of **3** sharpen from broad absorptions to sharp peaks with the expected multiplicity. The peaks for the aromatic protons of **3** sharpen to a set of doublets for the *para*-disubstituted phenylene and a singlet for the tetra substituted ring, respectively. In addition, the signals for the methylene protons of **3** sharpen from broad peaks to a pair of doublets (Figure 3). This is consistent with the formation of a cone-shaped conformation with *exo* and *endo* bridging methylene protons. Similiar multiplicity is observed in DMSO-d_6 in the absence of **5** due to the ability of the phenols to hydrogen bond and hold the macrocycle in a rigid cone conformation. The signals for the protons of the bridging methylenes of **3** are broad in basic D_2O due to loss of hydrogen bonding and formation of a conformationally flexible macrocycle.

Figure 2B shows the chemical shift of benzene in the presence of **3** in D_2O. Due to a limited solubility in water a concentration range of only 0.1 mM to 10 mM benzene could be analyzed by NMR titration. The chemical shift of the protons of benzene moves upfield from 7.4 to 7.0 ppm. NMR titration of **3** with anionic aromatic salts, such as sodium benzoate, resulted in no change in the spectra (data not shown).

For comparison, the monomeric analog (i.e., **4**) of cyclic tetramer **3**, was prepared by the reaction of the diazonium salt of 4-aminobenzoic acid with phenol [12]. NMR titration of **4** with **5**, or with benzene, revealed no change in the chemical shift over similiar concentration ranges (Figure 2). The lack of an upfield shift upon titration of **4** or **5** with benzene illustrates the importance of the cavity of the macrocyclic host **3** for complexation.

Although favorable ionic interaction between the ammonium guest and tetracarboxylate might contribute to complexation, the upfield shift suggests that the guest is held within the cavity. The absence of electrostatic interactions for binding of a non-ionic, non-polar arene (benzene) to **3** further illustrates the importance of the hydrophobic cavity for the binding of arenes. Unfavorable ionic repulsion between **3** and sodium benzoate prevents complexation of the arene in the cavity.

Accurate determination of binding constants using the data obtained from these experiments proved to be unreliable owing to the concentration ranges used [14]. An important consideration in the choice of conditions for NMR titration is the ratio of the concentration of complex to the maximum possible concentration of complex, p. In general the value of p should be in the range of 0.20 to 0.80. In the above experiment and previous studies [13], the concentration ranges used gave p values of 0.9 or higher. Good fits to the data can afford binding constants under these conditions which vary by several orders of magnitude. Using more dilute solutions, determination of binding constants can be made by NMR titration whereby the chemical shift of the protons on the guest (at a constant concentration) are monitored as a function of host concentration. As expected, the chemical shift of the trimethyl protons of **5** moves upfield with increasing concentration of **3** (Figure 4). The peak of the trimethyl protons of the guest moves upfield from δ

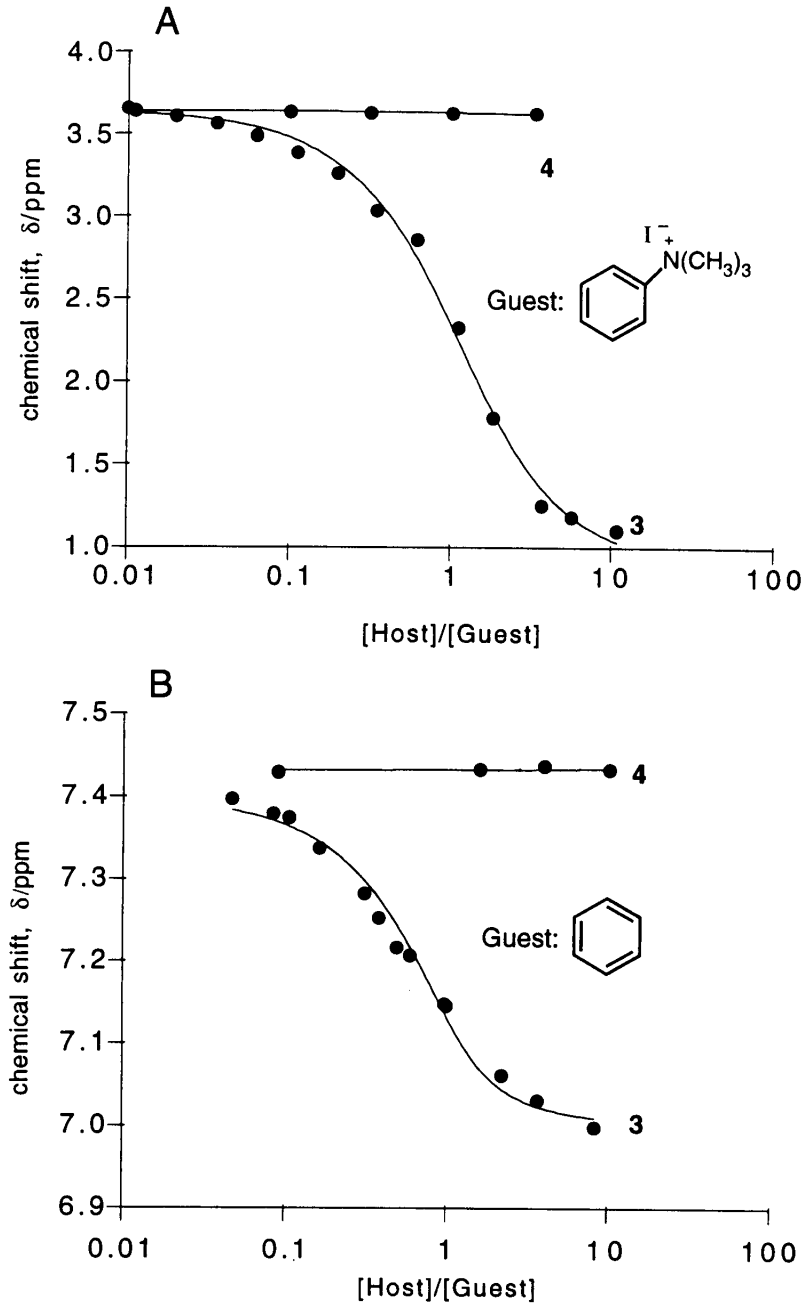


Figure 2. NMR titration curves of **3** and **4** with N, N, N-trimethylanilinium iodide (**5**) and benzene. (A) Plot of chemical shift of the methyl protons of N, N, N-trimethylanilinium iodide versus concentration ratio of "host" to guest (**3** or **4**); (B) plot of chemical shift of benzene protons versus concentration ratio of "host" to guest (**3** or **4**). The concentration of **3** and **4** was kept constant (1 mM) while that of N, N, N-trimethylanilinium iodide or benzene was varied (0.1 mM to 100 mM, or 0.1 mM to 10 mM, respectively), D_2O, 25 °C, pD = 11 (bicarbonate buffer).

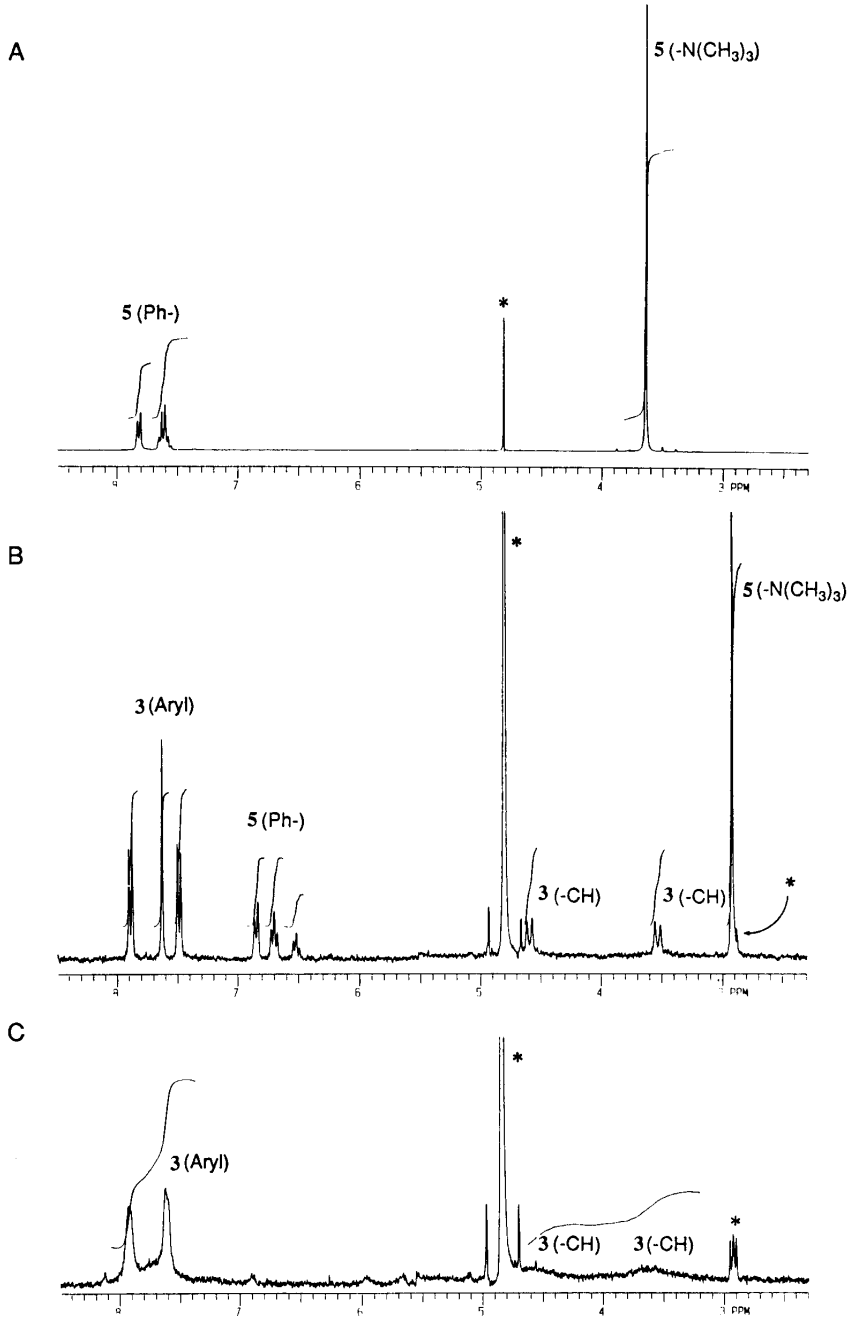


Figure 3. NMR spectra of mixtures of **3** and **5** (D_2O, 25 °C, pD = 11 bicarbonate buffer): Concentration ratios of *N*, *N*, *N*-trimethylanilinium iodide, **5**, to tetrakis(4′-carboxyphenylazo)calix[4]arene, **3**, are: (A) 100 mM/1 mM; (B) 1.8 mM/1 mM; (C) 0.3 mM/1 mM. Signals for D_2O (δ 4.8) and internal sodium 3-(trimethylsilyl)-1-propanesulfonate reference (t, δ 2.92) are starred.

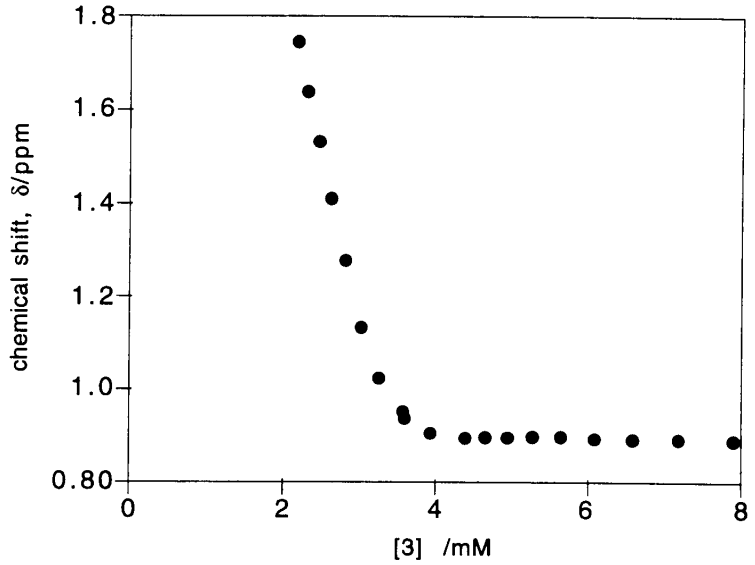


Figure 4. NMR titration curve of N, N, N-trimethylanilinium iodide (**5**) with **3**. Plot of chemical shift of the trimethylammonium protons of **5** versus concentration of **3**. The concentration of **5** was maintained constant (1.44 mM) while that of **3** was varied (0–8 mM): D_2O, 25 °C, pD = 11 (bicarbonate buffer).

3.65 ppm (unbound guest) to δ 0.9 ppm (complexed guest). The data presented in Figure 4 could not be fitted to 1 : 1 or 1 : 2 binding of the host **3** to N, N, N-trimethylanilinium iodide, implying a higher order binding of guest molecules to **3**. Although CPK models indicate that calix[4]arene alone is not large enough to bind multiple arenes, the phenylazo substituents of **3** provide a deeper and larger cavity suitable for binding.

The binding behavior of the tetrakis(4-carboxyphenylazo)calix[4]arene was further investigated by NMR titration using larger trimethylarylammonium salts in order to avoid multiple binding of guests. NMR titration of N, N, N-trimethyl-N-(2-naphthyl)ammonium iodide with **3** gives a chemical shift change for the trimethylammonium protons from δ3.6 to δ2.4 ppm (Figure 5). This data can be fitted for 2 : 1 binding of N, N, N-trimethyl-N-(2-naphthyl)ammonium iodide to macrocycle **3**, to give binding constants of K_1 = 8024 M^{-1} and K_2 = 3134 M^{-1}. It is clear from analysis of CPK models and data available from X-ray diffraction that the cavity of calix[4]arene is too small to accommodate two arenes. However, substitution of the upper rim of the calixarene with phenylazo substitutents provides for a deeper, wider, and more flexible hydrophobic pocket into which arenes can be sequestered.

In conclusion, a new water-soluble chromophoric calixarene **3** binds cationic and neutral arenes: N, N, N-trimethylaryl-N-ammonium iodides and benzene.

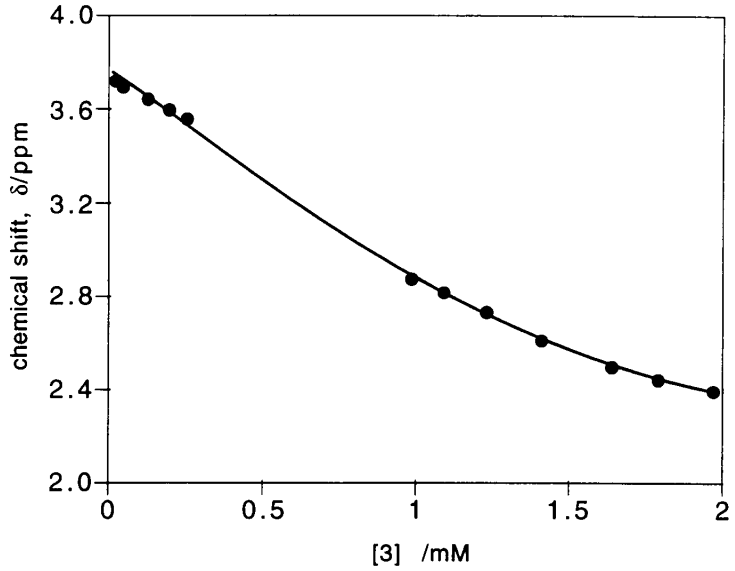


Figure 5. NMR titration curve of N, N, N-trimethyl-N-(2-naphthyl)ammonium iodide (**6**) with **3**. Plot of chemical shift of the trimethylammonium protons of **6** versus concentration of **3**. The concentration of **6** was kept constant (4.37 mM) while that of **3** was varied (0–2 mM): D_2O, 25 °C, pD = 11 (bicarbonate buffer). The curve is a fit corresponding to $K_1 = 8024\ M^{-1}$ and $K_2 = 3134\ M^{-1}$ with $R^2 = 0.9996$.

Binding of the larger N, N, N-trimethyl-N-naphthylammonium iodide guest indicates 2 : 1 complexation within the cavity.

The demonstration of binding of anilinium salts and benzene to the water soluble analog (**3**) correlates with the changes in spreading behavior of azo-functionalized amphiphiles **1** and **2** at the air–water interface. The chromophoric nature of **1–3**, their ability to bind neutral aromatic molecules, their assembly at the air–water interface, and the photochromism of the azo substituent suggest that they might be used in the development of sensors for detection of aromatic compounds. Studies of *cis-trans* photoisomerization and photoswitching of host–guest complexation of azo-substituted calixarenes in solution, and at the surface of a Langmuir trough and solid substrates, are in progress.

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