ORGANIC LETTERS

2006
Vol. 8, No. 16
3477−3480

Hybrid Cavitand–Resorcin[4]arene Receptor for the Selective Binding of Choline and Related Compounds in Protic Media

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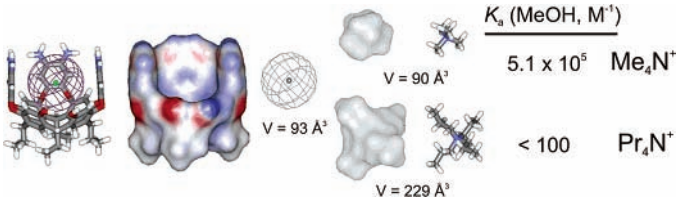
Received May 4, 2006

ABSTRACT



A hybrid cavitand–resorcin[4]arene receptor capable of displaying pH-modulated binding affinity toward trimethylalkylammonium ions is proposed as an alternative to the low selectivity exhibited by other receptors used in supramolecular fluorescent sensor systems for choline.

Calixarenes of the *p*-sulfonato type and phenolate resorcinares are efficient hosts for the molecular recognition of organic ammonium ions in protic solvents.[1,2] The affinity of the mentioned receptors for the ammonium ions is high (10^3−10^6 M^{-1}) and relies on the simultaneous charge−charge and cation−π interactions.[3] These receptors have been used in the development of promising binary systems for the signaling of biologically important ammonium cations (i.e., choline, acetylcholine, and carnitine).[4] The principle at work for the cation sensing is the so-called displacement indicator method.[5] The original signaling system made use of resorcin[4]arene **1** as the receptor and of the pyrene-appended pyridinium **2a** (Figure 1) as the indicator, and it operated in a strong alkaline−organic media.[4a] Schneider has reported an intriguing binding selectivity of receptor **1** toward tetramethylammonium cations in strong aqueous basic media.[1b,6] Regrettably, the harsh basic conditions in which the **1**/**2a**$^+$ detection sensor operates cause the decomposition of the indicator molecule with time, hampering any further studies on its selectivity. To solve this problem, calix[4]arene-*p*-sulfonates **3** were exploited instead.[4b] These receptors are higly efficient even in an aqueous neutral solution, and recently, Nau has shown that in combination with a fluorescent azoalkane, 2,3-diazabicyclo[2.2.2]oct-2-ene **4** (DBO), they form a truly water-soluble sensor system with enhanced fluorescence regeneration.[4d] In recent times, Chen and Kokube have reported that receptor **5** allows the use of

† ICREA and ICIQ.

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(1) (a) Schneider, H. J.; Kramer, R.; Simova, S.; Schneider, U. *J. Am. Chem. Soc.* **1988**, *110*, 6442−6448. (b) Schneider, H. J.; Guttes, D.; Schneider, U. *J. Am. Chem. Soc.* **1988**, *110*, 6449−6454.

(2) (a) Shinkai, S.; Araki, K.; Matsuda, T.; Nishiyama, N.; Ikeda, H.; Takasu, I.; Iwamoto, M. *J. Am. Chem. Soc.* **1990**, *112*, 9053−9058. (b) Shinkai, S.; Araki, K.; Manabe, O. *J. Am. Chem. Soc.* **1988**, *110*, 7214−7215.

(3) Ma, J. C.; Dougherty, D. A. *Chem. Rev.* **1997**, *97*, 1303−1324.

(4) (a) Inouye, M.; Hashimoto, K.-i.; Isagawa, K. *J. Am. Chem. Soc.* **1994**, *116*, 5517−5518. (b) Koh, K. N.; Araki, K.; Ikeda, A.; Otsuka, H.; Shinkai, S. *J. Am. Chem. Soc.* **1996**, *118*, 755−758. (c) Tan, S.-D.; Chen, W.-H.; Satake, A.; Wang, B.; Xu, Z.-L.; Kobuke, Y. *Org. Biomol. Chem.* **2004**, *2*, 2719−2721. (d) Bakirci, H.; Nau, W. M. *Adv. Funct. Mater.* **2006**, *16*, 237−242.

(5) Wiskur, S.; Ait-Haddou, H.; Anslyn, E.; Lavigne, J. *Acc. Chem. Res.* **2001**, *34*, 963−972.

(6) In MeOH or in water (pH 6.0 buffered with 10 mM phosphate), resorcine[4]arene **1** binds tetrabutylammonium chloride with higher affinity than tetramethylammonium chloride.

10.1021/ol061097i CCC: $33.50
Published on Web 07/01/2006

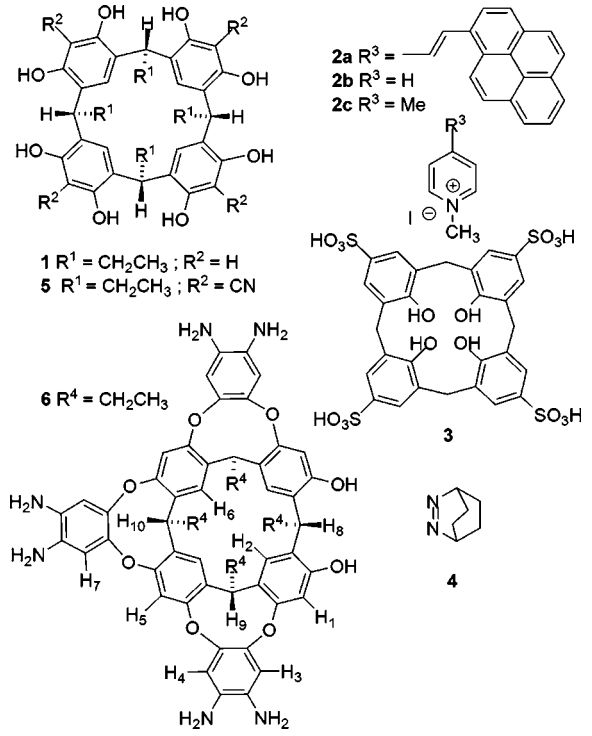

Figure 1. Molecular structures of the receptors **1**, **3**, **5**, and **6** and of the indicators **2a** and **4**.

the sensing ensemble **5**/**2a**$^+$ in neutral aqueous conditions.[7] Unfortunately, the main drawback of the receptors that are effective in protic media (**5** and **3**) is the low selectivity exhibited for the alkyltrimethylammonium motif, present in choline and carnitine derivatives, compared to bulkier tetraalkylammonium ions.[8] This is due to the modest concavity of these receptors that only surrounds a small fraction of the ammonium ion. In fact, deepening of the cavity has resulted in elaborated neutral and anionic cavitand hosts capable of much higher selectivity.[9]

We describe here the binding properties of the structurally simple hybrid receptor **6** for the molecular recognition of alkylammonium ions in protic solvents; it combines the selectivity of cavitands with the high affinity of the resorcin[4]arene.

The three diaminobenzene groups of **6** constitute the cavitand portion of the receptor. These groups can adopt an axial or an equatorial conformation. When the three groups are axial, the receptor contains an enforced scoop-shaped cavity that selectively binds alkylammonium ions complementary in size and shape through multiple cation$-\pi$ interactions (Figure 2). The two phenolic OH groups remain

(7) (a) Chen, W.-H.; Nishikawa, M.; Tan, S.-D.; Yamamura, M.; Satake, A.; Kobuke, Y. *Chem. Commun.* **2004**, 872−873. (b) Ref 4c.

(8) (a) Chen, W.-H.; Wei, Y.; Tan, S.-D.; Wang, B.; Xu, Z.-L. *Supramol. Chem.* **2005**, *17*, 469−473. log K_a(**5**·**7a**$^+$) = 5.8; log K_a(**5**·**7c**$^+$) = 6.0. (b) Morel, J. P.; Morel-Desrosiers, N. *J. Chem. Soc., Perkin Trans. 2* **2001**, 1075−1078. log K_a(**3**·**7a**$^+$) = 4.4; logK_a(**3**·**7c**$^+$) = 4.47.

(9) (a) Ballester, P.; Shivanyuk, A.; Far, A. R.; Rebek, J. *J. Am. Chem. Soc.* **2002**, *124*, 14014−14016. (b) Hof, F.; Trembleau, L.; Ullrich, E. C.; Rebek, J. *Angew. Chem., Int. Ed.* **2003**, *42*, 3150−3153.

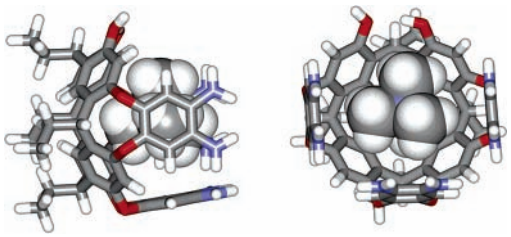

Figure 2. Front and side view of the optimized model of the **6**·**7a**$^+$ complex.[10]

on the resorcin[4]arene part of **6**. Consequently, deprotonation in alkaline aqueous media affords a negatively charged receptor which interacts even more strongly with quaternary ammonium salts by means of charge−charge interaction.

The synthesis of the hexaamine diol **6** has been previously reported by Rebek, but the compound was not isolated and characterized.[11] The ^{1}H NMR spectrum of **6** in DMSO-d_6 shows that the methine protons (H_9 and H_{10}) of the three nine-membered rings appear at 5.4 ppm indicating that the diaminobenzene groups occupy an "axial" orientation and that the system is in a vase-like conformation.[12] The methine proton (H_8) of the resorcinare moiety resonates at 4.1 ppm. Addition of incremental amounts of tetramethylammonium chloride **7a**$^+$Cl$^-$ (Figure 3) to a solution of **6** in DMSO-d_6

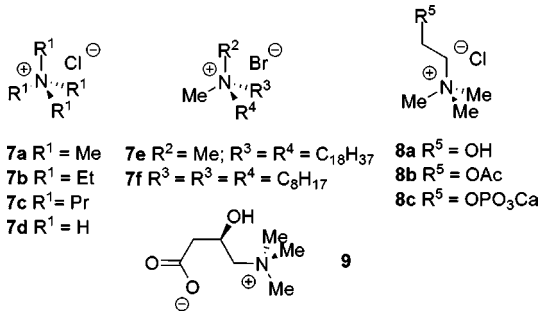

Figure 3. Structures of guests **7**−**9**.

produces a downfield shift of all aromatic signals. A broad signal also appears at 2.95 ppm corresponding to the average of the proton signals for the free and the bound tetramethylammonium cation (Figure 4). As the titration proceeds, this signal sharpens and shifts downfield, approaching the chemical shift value of the free tetramethylammonium ion. This behavior is indicative of fast chemical exchange on the NMR time scale between a free and bound ammonium ion. The titration data obtained for the chemical shift change of proton H_5 were fitted to a 1:1 binding model establishing the stability

(10) *CAChe WorkSystem*, version 6.1.12.33; Fujitsu Limited.

(11) Amrhein, P.; Shivanyuk, A.; Johnson, D. W.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2002**, *124*, 10349−10358.

(12) Moran, J. R.; Karbach, S.; Cram, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 5826−5828.

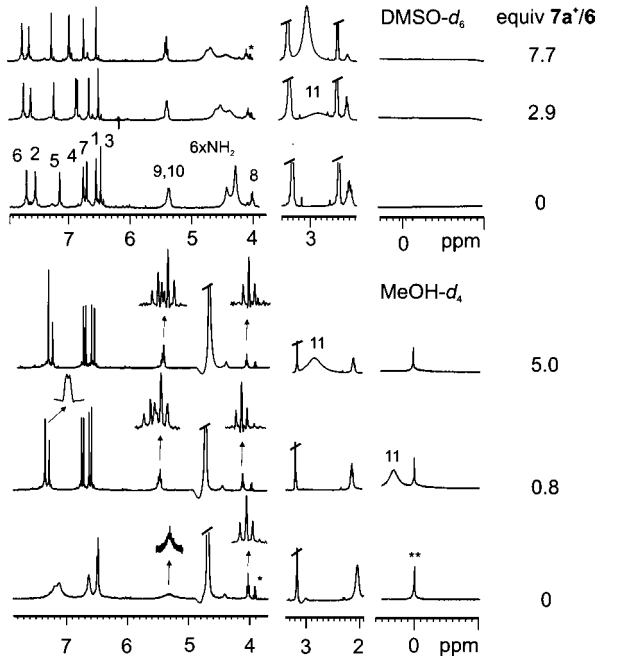

Figure 4. Changes in three regions of the ^{1}H NMR spectra acquired at 298 K during the titration of **6** with **7a**$^+$ in two different solvents. [**6**] = 10 mM in DMSO-d_6, and [**6**] = 5 mM in MeOH-d_4. See Figure 1 for proton assignments of **6**; 11 is the average signal for the methyl protons (free and bound) of **7a**$^+$. *Residual signal of ethyl acetate. **TMS. The arrow indicates the expansion of the signal.

constant for the **1**·**7a**$^+$Cl$^-$ complex as $1.2 \pm 0.1 \times 10^2$ M^{-1}.[13] The tetraethylammonium chloride **7b**$^+$Cl$^-$ is bound with a similar affinity, but the larger tetrapropylammonium chloride **7c**$^+$Cl$^-$ shows a sharp decrease in affinity (K_a < 5 M^{-1}). These preliminary results indicate that receptor **6** shows a size cutoff for binding tetraalkylammonium chlorides. Furthermore, receptor **6** also binds choline chloride **8a**$^+$Cl$^-$ in pure DMSO with a K_a of 80 M^{-1}, a value which is in line with those obtained above for **7a**$^+$Cl$^-$ and **7b**$^+$Cl$^-$ when statistically adjusted.[14]

The superior properties of receptor **6** for the molecular recognition of trimethylalkylammonium salts appear in alkaline media. The stability constants for the complexes **6**·**7a**$^+$ and **6**·**8a**$^+$ were determined in a 0.01 M KOH/DMSO-d_6 (1:1.5) solvent mixture as $1.5 \pm 0.2 \times 10^3$ and $1.2 \pm 0.1 \times 10^3$ M^{-1}, respectively. In both cases, ^{1}H NMR titrations revealed the appearance of a broad upfield signal, $\delta = -0.05$ ppm for **7a**$^+$ and $\delta = -0.32$ ppm for **8a**$^+$, corresponding to the methyl protons of the bound ammonium cation. The methyl protons of free **7a**$^+$ can be observed as a broad singlet at 2.98 ppm. This large upfield shift ($\Delta\delta = -3.03$ ppm) is in complete agreement with the estimated value for detaining the tetramethyl cation **7a**$^+$ in a cavity surrounded by three aromatic rings ($\Delta\delta = \sim 0.9$ ppm/ring).[9a]

In addition to an enhanced thermodynamic stability, the complexes formed with **6** and ammonium ions in alkaline media exhibit a higher kinetic stability (see ^{1}H NMR titration in the Supporting Information). No evidence of binding to tetrapropylammonium **7c**$^+$ is observed under identical conditions. In conclusion, it is possible to boost the affinity of **6** toward trimethylalkylammonium ions by combining cation−π and charge−charge interactions without compromising the receptor's size selectivity.

We also explored the binding properties of receptor **6** in neutral protic solvents.[15] The ^{1}H NMR spectrum of receptor **6** in a MeOH-d_4 solution shows broad signals for most of the aromatic protons, as well as for the methine protons. This is probably due to dynamic effects involving exchange equilibration between different conformations of **6**. When 0.8 equiv of **7a**$^+$ is added to solution of **6** in MeOH-d_4, the aromatic and methine signals experience a noticeable downfield shift and a clear narrowing effect. A new signal is also observed at $\delta = 0.46$ ppm. As more **7a**$^+$ is added, this signal gradually shifted downfield and broadened indicating a fast exchange equilibrium on the NMR time scale between free and bound tetramethylammonium cation **7a**$^+$.

When 1 equiv of **7a**$^+$ is present, the signals of the aromatic and methine protons of **6** are very sharp. The methine protons H_9 and H_{10} resonate close to $\delta = 5.5$ ppm as two distinguishable triplets. Taken together, these observations indicate that the binding of **7a**$^+$ in MeOH restricts the conformation of **6** to the one having a scoop-shaped aromatic cavity capable of nearly surrounding the cation. The fact that the chemical shift of the signals of receptor **6** remain invariable after the addition of more than 1 equiv of **7a**$^+$ points to a 1:1 stoichiometry for the **6**·**7a**$^+$ complex having a high stability constant.

The binding affinities of receptor **6** in MeOH to a series of quaternary tetraalkylammonium salts were established by means of the competitive displacement assay, using the pyrene-modified *N*-methylpyridinium cation **2a**$^+$ as the fluorescent indicator (Figure 5).[16] The addition of **6** (0−2.47 $\times 10^{-3}$ M) to a MeOH solution of **2a**$^+$ (1.67×10^{-4} M) results in an efficient fluorescence quenching (up to 96%, $\lambda_{exc} = 430$ nm, $\lambda_{em} = 580$ nm). The association constant for the **6**·**2a**$^+$ complex was calculated as $K_a = 1 \pm 0.2 \times 10^4$ M^{-1} from nonlinear curve fitting of the dependence of I/I_0 on [**6**] using a 1:1 binding model.[17,18] Successive addition of certain tetraalkylammonium salts to the complex formed in the presence of **6** (1.6×10^{-4} M) and **2a**$^+$ (1.8×10^{-4} M) led to the fluorescence recovery of **2a**$^+$ reaching a plateau

(13) In dipolar aprotic solvents such as DMSO, the ammonium salt is recognized as a close contact ion pair. Consequently, the chloride may also interact with the receptor.

(14) The reported association constants, K_a, are thermodynamic and reflect the multiple binding arrangements for the complex. For direct comparison, the statistically corrected (intrinsic) association constant K_i should be used. For N degenerate states of a complex, $K_a = NK_i$. The **6**·**7a**$^+$ complex has four degenerate states, and the **6**·**8a**$^+$ has two because the OH group can protrude through the top or the side.

(15) **6** is not soluble at millimolar concentrations in D_2O or mixtures of D_2O: MeOH-d_4 (1:1). In a solvent mixture D_2O/DMSO-d_6 (1:1.5), the affinity of receptor **6** for **7a**$^+$ is $K_a = 1.2 \pm 0.25 \times 10^2$ M^{-1}, a value very similar to that calculated in pure DMSO-d_6.

(16) See ref 4 for a detailed description of this methodology.

(17) The slope of the linear relationship dependence of I_0/I on [**6**] afforded a similar value. See Supporting Information.

(18) *SPECFIT*, version 3.0.36; Spectrum Software Associates.

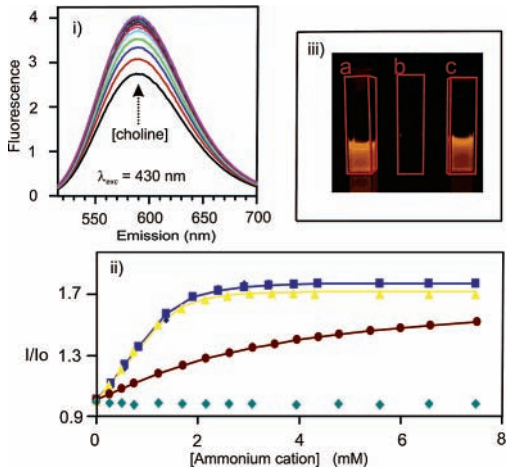

Figure 5. (i) Fluorescence spectra in MeOH of **2a**$^+$ (0.184 mM) in the presence of **6** (0.17 mM) upon addition of incremental amounts of **8a**$^+$ (0−2.5 mM). (ii) Plots of the increase of the relative fluorescence intensity of **2a**$^+$ in competitive binding experiments with **8c**$^+$ (circles), **9** (squares), **7e**$^+$ (triangles), and **7f**$^+$ (diamonds) fitted to a competitive binding scheme of two 1:1 complexes. (iii) Visual fluorescence regeneration of **2a**$^+$: (a) **2a**$^+$ (0.184 mM), (b) mixture of **2a**$^+$ (0.17 mM) and **6** (2.5 mM), and (c) mixture of **6** (0.15 mM), **2a**$^+$ (0.17 mM), and **8c**$^+$ (0.43 mM).

at high concentrations of the cation. The binding constants of **6** with the corresponding ammonium cation were obtained by SPECFIT[18] analyses of the I/I_0 growth of fluorescence as a function of the added ammonium salt using a competitive binding scheme (Table 1).

Additional conclusions can be drawn from the data presented in Table 1. Receptor **6** binds in MeOH solution tetramethyl **7a**$^+$, trimethylalkyl **8**$^+$, and tetraethyl **7b**$^+$ ammonium cations with association constants higher than 10^5 M^{-1}. Strong binding of methylpyridinium **2**$^+$ cations is also apparent. Size selectivity becomes evident during the binding of the tetrapropyl **7c**$^+$ and tetrabutylammonium cations. They show a weak affinity toward receptor **6**, some 5.4 kcal/mol less than their homologous tetramethyl **7a**$^+$ and tetraethyl **7b**$^+$ ammonium ions. Small changes in size have large consequences when a guest is highly surrounded by the receptor. The hexaamine **6** also recognizes dialkylated derivatives of the dimethylammonium ion-like **7e**$^+$ with high affinity due to the lateral and top openings of the receptor through which the long chains can protrude. In contrast, trialkylated methylammonium ion **7f**$^+$ is a bad fit for **6** and a dramatically weaker complexation is observed. Likewise, ammonium chloride **7d**$^+$ is not bound in the cavity of **6**.

Table 1. Binding Constants of Tetraalkylammonium Salts with **6**, Determined by Fluorescence Regeneration of **2a**$^+$ as a Competitive Guest[a]

ammonium salt	K_a [10^5 M^{-1}]	ammonium salt	K_a [10^5 M^{-1}]
7a$^+$	5.1 ± 0.6	**8a**$^+$	3.2 ± 0.9
7b$^+$	1.4 ± 0.4	**8b**$^+$	1.0 ± 0.1
7c$^+$	<0.001	**8c**$^+$	3.7 ± 0.2
7d$^+$	<0.001	**2b**$^+$	2.1 ± 0.1
7e$^+$	0.1± 0.01	**2c**$^+$	0.3 ± 0.01
7f$^+$	<0.001	**9**	3.3 ± 0.8

[a] In MeOH with [**6**] = 0.1−0.18 mM and [**2a**$^+$] = 0.15−0.21 mM.

In conclusion, we have demonstrated that hexaamine **6** represents an alternative to the low selectivity exhibited by other supramolecular fluorescent sensor systems for choline. Compound **6** being a *resorcinarene−cavitand hybrid* displays a pH-modulated binding affinity toward trimethylalkylammonium ions that features selectivity. Furthermore, in protic solvents such as MeOH, receptor **6** is a neutral species capable of forming remarkably thermodynamically stable complexes with ammonium cations complementary in size and shape exclusively through cation−π and CH−π interactions.

Acknowledgment. We thank Dirección General de Investigación, Ministerio de Ciencia y Tecnología (CTQ2005-08989-C02/BQU), for a generous grant. We also thank ICIQ Foundation and Generalitat de Catalunya, DURSI (2005SGR00108), for financial support.

Supporting Information Available: Preparation and characterization data for compound **6**, general procedures used for the titrations (^{1}H NMR and fluorescence) and for the fit of the titration data, and ^{1}H NMR titration spectra and NOESY of the **6**·**7a**$^+$ complex registered in 10 mM KOH-D_2O/DMSO-d_6. This material is available free of charge via the Internet at http://pubs.acs.org.

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