Langmuir

Article

pubs.acs.org/Langmuir

Insights into the Structure of the Supramolecular Amphiphile Formed by a Sulfonated Calix[6]arene and Alkyltrimethylammonium Surfactants

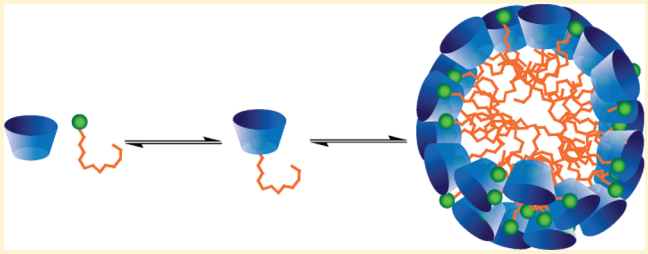
Nuno Basilio,[†] M. Martín-Pastor,[‡] and Luis García-Río*[,†]

[†]Departamento de Química Física y Centro Singular de Investigación en Química Biológica y Materiales Moleculares, Universidad de Santiago, 15782 Santiago, Spain

[‡]Unidade de Resonancia Magnética, RIAIDT, Universidad Santiago de Compsostela, 15706 Santiago, Spain

Supporting Information

ABSTRACT: In this work, we have studied the interactions between the water-soluble *p*-sulfonatocalix[6]arene and cationic surfactants octyltrimethylammonium bromide below the cmc and dodecyltrimethylammonium bromide above the cmc, by saturation transfer difference (STD) NMR spectroscopy. From the STD build-up curves, we have obtained the T1 independent cross relaxation rates, and the results show that the interactions established between the cationic headgroup of the surfactant and the OMe group of the macrocycle play an important role in the stabilization of the complex, both below and above the cmc.



INTRODUCTION

Calixarenes[1] are cyclic oligomers made of phenol units linked by methylene bridges at the ortho-position. Because of their relatively facile chemical modification,[2] calixarene derivatives are often chosen as noncovalent building blocks for the construction of supramolecular assemblies, such as self-assembling capsules,[3] rotaxanes and catenanes,[4] micelles and vesicles.[5] Among the several assemblies that can be constructed from calixarene building blocks, we are especially interested in the study of micelles and vesicles formed from amphiphilic calixarenes and related systems.[6] Like other amphiphiles, those constructed from the calixarene basic structure are obtained by attaching hydrophilic and hydrophobic groups to selected positions.[7] Generally, hydrophobic or hydrophilic groups are linked by means of covalent bonds to the calixarene framework to form amphiphilic calixarenes. However, due to the recognition properties of calixarenes, these macrocycles can be used to construct supramolecular amphiphiles. In this case, instead of covalent bonds, the functional segments required to provide or improve the amphiphilic properties of the molecular species are linked by means of noncovalent interactions.[8] Using this approach, we and others have been able to construct vesicles and micelles based on supramolecular amphiphiles formed from *p*-sulfonato-calix[*n*]arenes and cationic guests.[9]

The present work focuses on the structural characterization of the complex formed from 5,11,17,23,29,35-hexasulfonato-37,38,39,40,41,42-hexamethoxycalix[6]arene (SC6HM) and alkyltrimethylammonium bromide surfactants (C*n*TAB). We have recently reported that SC6HM promotes the formation of micelles at concentrations below the critical micelle concentration (cmc) of pure C*n*TAB.[9a] For instance, it was found that the addition of 5 mM of SC6HM reduces the cmc of dodecyltrimethylammonium bromide (C12TAB) from 14 to 0.2 mM. It was suggested that SC6HM forms a complex with the ammonium surfactant that self-aggregates at lower concentration than that of pure tensioactive. On the other hand, in the course of our studies, we found that the most common and less flexible *p*-sulfonatocalix[4]arene, bearing OH groups at the lower rim, leads to the formation of vesicles in the presence of cationic surfactants.[9b]

As a further step to understand at the molecular level the structural effects that govern the micellization in this mixture of compounds, in this Article we have employed saturation transfer difference NMR spectroscopy (STD NMR) experiments to determine the type of interactions involved and the structural properties of the complexes formed between SC6HM and C*n*TAB in the range of concentrations below and above the cmc. STD NMR, together with transfer NOE, is one of the most widespread NMR methods for the study of the interactions between small ligands and macromolecular receptors.[10] The STD is aimed to map the interaction epitope by determining the ligand regions in contact with the receptor.

EXPERIMENTAL SECTION

All commercial reagents were of the highest purity available and were used as received. *p*-Sulfonatocalix[6]arene (SC6) was prepared by

Received: February 19, 2012
Revised: March 21, 2012
Published: March 26, 2012

dx.doi.org/10.1021/la3006794 | *Langmuir* 2012, 28, 6561−6568

ipso-sulfonation of the correspondent *p-tert*-butylcalix[6]arene in H_2SO_4 at 80 °C and alkylated with CH_3I in basic media following literature procedures.[5a] NMR experiments were performed on a 17.6 T Varian INOVA NMR spectrometer operating at a ^1H frequency of 750 MHz. The spectrometer control software was VNMR/VNMRJ 2.2D. The spectra were referenced with an external standard reference of TSP (3-(trimethylsilil)-propionic-d_4 acid) dissolved in D_2O that was inserted in the NMR tube. The NMR studies were conducted at 25 °C. The temperature was calibrated with a standard reference of 4% methanol-d_4 in methanol sample. The spectra were processed and analyzed with MestRe-C v3.9 software (Mestrelab Inc.). Plots and nonlinear data fitting of the data were performed with Origin v7.0 (Originlab Inc.).

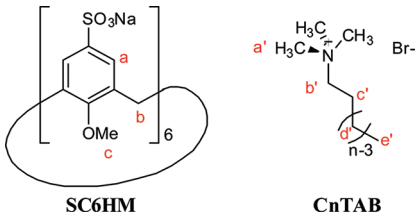


Figure 1. Molecules studied in this work: *p*-sulfonatocalix[6]arene hexamethyl ether (SC6HM) and alkyltrimethylammonium surfactants (C*n*TAB). The labeling of the proton signals that were studied by NMR is indicated.

NMR Sample Preparation. Mixtures of SC6HM and surfactant C*n*TAB (Figure 1) were prepared in 5 mm standard NMR tubes. Four samples were prepared with concentrations of SC6HM:C*n*TAB of 1:0, 1:1, 10:10, and 10:30. These concentrations are expressed in millimolar concentration. In the sample 1:1, we used the cationic surfactant octyltrimethylammonium bromide (n = 8), which allowed us the use of higher concentration below the cmc (25 mM in the presence of SC6HM[9a]). In all other samples, we used dodecyltrimethylammonium bromide (n = 12).

Saturation Transferred-Difference (STD) Experiments. Signals corresponding to protons Ha, Hb, and Hc of SC6HM and Ha′, Hc′, or He′ of C*n*TAB (see Figure 1) were saturated in independent STD experiments.[10a] STD experiments were acquired by selective saturation of the correspondingly proton signal with a train of 50 ms Gaussian 90° pulses separated by 1 ms.[10] For each signal saturated, the STD experiment was repeated with the following values of the saturation time: 0.05, 0.1, 0.3, 0.5, 0.7, 0.9, 1.0, 1.1, 1.3, 1.5, 2, 2.5, 3, and 3.5 s. The interscan relaxation delay (d_1) and the acquisition time were set to 6 and 1 s, respectively, to achieve complete relaxation prior to any scan. The STD spectra were processed, and the cross relaxation rates $\sigma_{I\{S\}}^{obs}$ were determined as described in the theory section (see the Supporting Information).

RESULTS AND DISCUSSION

Despite our efforts to apply methodology based on ROESY experiments to determine the binding mode between SC6HM and C*n*TAB, these experiments were unsuccessful. The explanation for this result has to be found in the fact that for concentrations above the cmc, the system calixarene−surfactant is in the negative NOE regime typical of relative large size molecular aggregates. Under this regime, NOE-based experiments are always preferable over ROE experiments[11] because the former provide stronger measurable effects and are not affected by the efficient transversal relaxation of large aggregates that cause important sensitivity losses in ROESY.

Among the different methods relying on NOE, the STD is now a convenient and a well-established tool to study association equilibria between molecules. In favorable situations, the experiment permits one to determine the binding epitope of a small ligand that is attached to a target macromolecule.[10] However, it has been found that when the T_1 relaxation time of individual protons is significantly different, there are possibilities for misinterpretation of the STD responses, and, consequently, the determination of the binding epitope with this method[12] may be erroneous, although some solutions have been recently proposed.[13]

In this article, the association equilibrium between SC6HM and C*n*TAB (Figure 1) is studied with STD NMR at 25 °C in D_2O at several concentrations (1:0, 1:1, 10:10, and 10:30 in mM) covering the range of sample states corresponding to free calixarene, complex, and micelle. In each case, a sample was prepared at the required concentration of SC6HM and C*n*TAB that was described in a previous study in our lab.[9a] While sample 1:0 corresponds to free calixarene, in sample 1:1 both free SC6HM and C*n*TAB exist in dynamic equilibrium with the complex formed from the association of these two species. It is worth noting that in our previous work we confirmed the existence of a complex with a 1:1 stoichiometry. Samples 10:10 and 10:30 are above the cmc, and apart from the complex and the free species, micellar aggregates are present in solution. Indeed, judging by the cmc of this system (0.2 mM), it is likely that a high fraction of SC6HM and C*n*TAB are in the micellized state.

Figure 2 shows ^1H NMR reference spectrum (a) of a sample containing 1 mM of calixarene SC6HM and 1 mM C_8TAB (sample 1:1) along with the STD NMR appearing in the STD spectra when the signals of C_8TAB are saturated and vice versa. Figure 2 proves the binding interaction between SC6HM and C_8TAB. In addition, the differential STD signals permit a qualitative characterization of the binding epitope of the ligand. As can be observed, when the C_8TAB signals are saturated, the STD is more intense for the protons corresponding to the OMe group of SC6HM. In addition, the STD intensity of the calixarene protons is higher when the protons of the trimethylammonium group are saturated (Figure 2d). This set of observations seems to suggest that the ammonium headgroup of the surfactant is closer to the OMe group of SC6HM than to other protons. When the SC6HM signals are saturated, the results point in the same direction: saturation of the aromatic (Figure 2g) and bridge CH_2 protons (Figure 2f) results mainly in intramolecular STD effects, while when the OMe protons are saturated (Figure 2e), apart from the intramolecular STD, the saturation is also transferred to the protons of the $N(CH_3)_3$ group (STD effect is also observed for the b′ protons, but this result is not conclusive because the signal is not sufficiently displaced from the signal that is being saturated). This is quite a surprising result, because SC6HM lacks well-defined binding pockets due to its high flexibility,[14] and in consequence one might expect that its complexation properties toward oppositely charged guest are dominated by coulombic forces[15] between the sulfonate groups and the ammonium group. However, our results suggest that the oxygen atoms in the OMe groups play an important role in the complexation of cationic guests, presumably by charge−dipole interactions between the electron lone pairs of the oxygen and positive charge of the ammonium group. The formation of complexes between calix[6]arenes derivatives and trimethylammonium cations driven by cation−dipole interactions had been previously reported.[16]

p-Sulfonatocalix[*n*]arenes (SC*n*) have aromatic cavities that can induce large upfield chemical shifts ($\Delta\delta = \delta_{complex} - \delta_{guest}$)

dx.doi.org/10.1021/la3006794 | *Langmuir* 2012, 28, 6561−6568

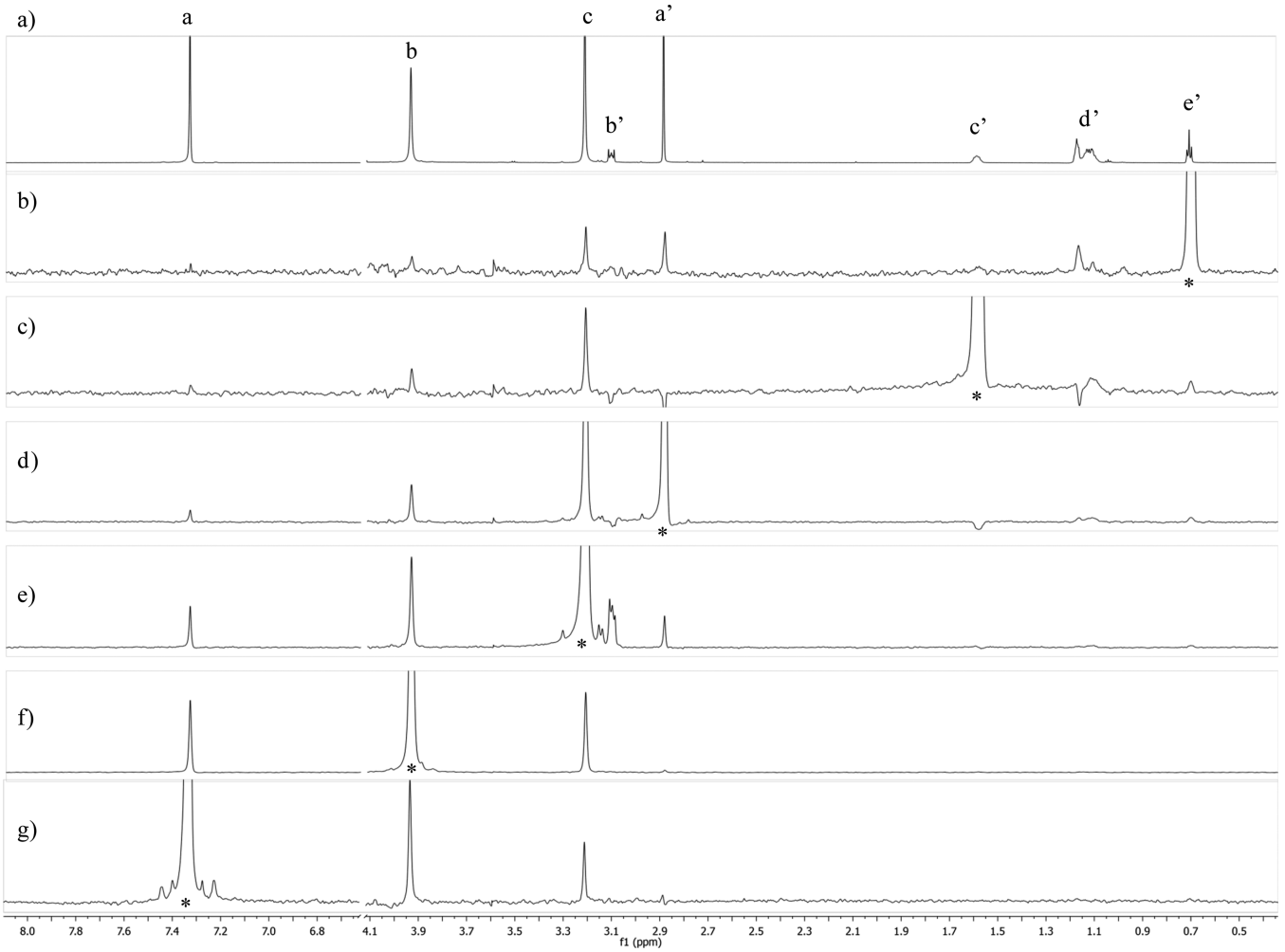


Figure 2. 1D STD spectra (STDon − STDoff) of the mixture SC6HM:C_8TAB in a ratio 1 mM:1 mM at 25 °C. (a) 1H reference spectrum; (b) 1D STD with saturation of e′ signal of surfactant (0.7 ppm); (c) 1D STD with saturation of c′ signal of surfactant (1.58 ppm); (d) 1D STD with saturation of the a′ signal of surfactant (2.89 ppm); (e) 1D STD with saturation of c signal of calixarene (3.21 ppm); (f) 1D STD with saturation of b signal of calixarene (3.94 ppm); and (g) 1D STD with saturation of a signal of calixarene (7.33 ppm). The STD saturation time was 3 s, and the signal saturated in each case is indicated with an asterisk below.

on the ^{1}H NMR signals of their included guests molecules. Carefully analysis of the magnitude of the $\Delta\delta$ for the different protons of the guests has been used to elucidate the structure of several inclusion complexes.[17] Moreover, in the study[18] of the complexation of bicyclic azoalkanes with *p*-sulfonatocalix[4]-arene (SC4), it was found that the structural assignments based on the complexation induced $\Delta\delta$ were fully in line with 2D ROESY NMR experiments. The low $\Delta\delta$ obtained for the trimethylammonium protons of C*n*TAB in the presence of SC6HM (~ -0.13 ppm)[9a] is in agreement with results presented here, suggesting that surfactant guest is not included in the pseudocavity of SC6HM. On the other hand, in a recent study, we observed values of $\Delta\delta \approx -1.86$ ppm for the ammonium headgroup of tetradecyltrimethylammonium bromide (TTABr) in the presence of SC4,[7] indicating that, in this case, the ammonium group is incorporated into the aromatic cavity of the host, probably with cation−π interactions contributing to the stabilization of the complex. In the case of the present study, the results indicate that the most important interaction between SC6HM and C*n*TAB is probably of the ion−dipole type.

Values of the binding constants of organic ammonium cations with SC*n*[19] are in the range of 10^4−10^5 M^{-1} due principally to the cooperation of π-stacking, CH−π, and cation−π interactions inside the cavity of the host and additional coulombic interactions provided by the presence of the sulfonate groups at the portal of the macrocycle. In the case of the complex formed between SC6HM and C12TAB, we obtained an association constant[9a] of approximately 1500 M^{-1}, which is 1 or 2 orders of magnitude lower than the majority of the reported binding constants for the formation of complexes between SC*n* and ammonium cations.[20] This decrease in binding ability could be due to the lack of cooperative interactions in the case of SC6HM, which could be explained by the flexibility of host that leads to a nondefined cavity, which is not able to promote, for example, cation−π interactions. Alkylation of the phenolic oxygens seems to play an important role in the ability of the SC*n* to form complexes with organic cations. It had been shown that the 5,11,17,23-tetrasulfonato-25,26,27,28-tetrakis(*n*-butyl)-calix[4]arene (SC4TB) derivative blocked in the cone conformation presents weaker binding affinities (2−3 orders of magnitude) for organic cations, with respect to SC4.[21] This observation had been attributed to the

dx.doi.org/10.1021/la3006794 | *Langmuir* 2012, 28, 6561−6568

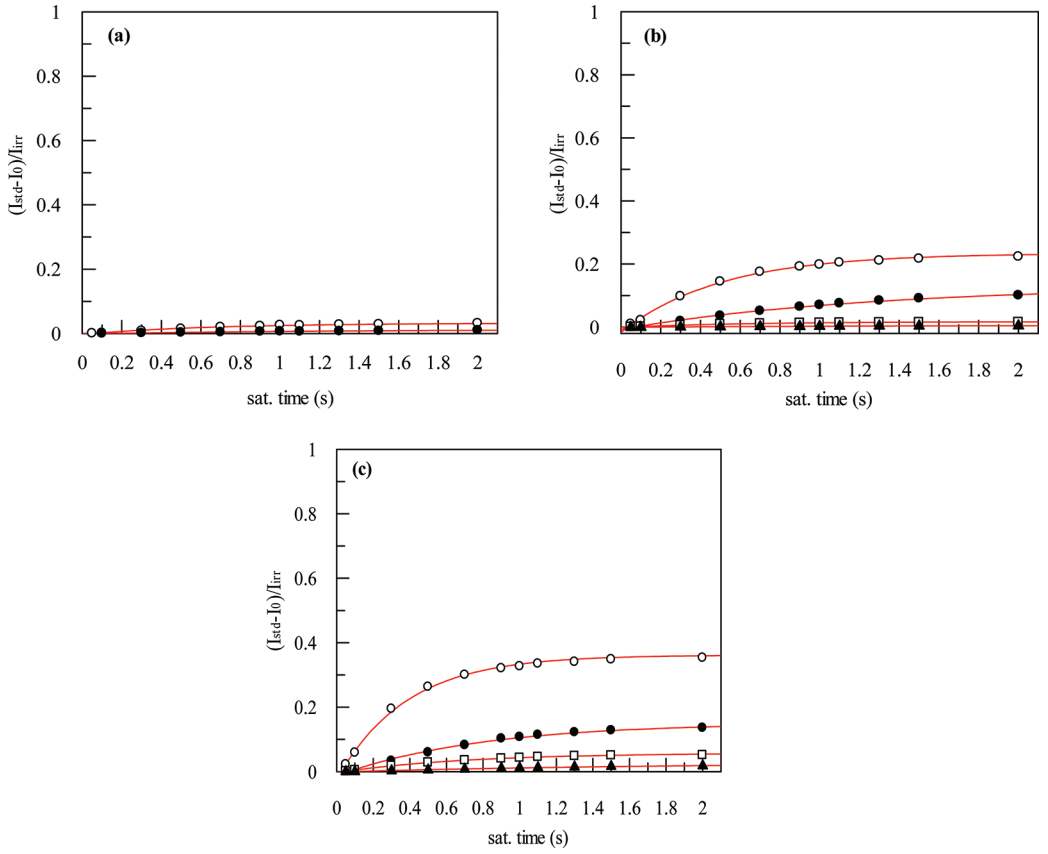


Figure 3. Normalized STD build-up curves of the mixture of SC6HM and C*n*TAB, with saturation of signal a of SC6HM. The concentrations used are: (a) 1 mM:1 mM (below the cmc), (b) 10 mM:10 mM, and (c) 10 mM:30 mM (with b and c above the cmc). (○) $STD_{b(a)}$, (●) $STD_{c(a)}$, (□) $STD_{a'(a)}$, (▲) $STD_{e'(a)}$.

fact that SC4TB presents a weaker π-electron density (due to the alkylation of the phenolic oxygens, which, in contrary to SC4, are no longer ionizable), the absence of complexed high-energy water molecules, and its tighter cavity. Because of this combination of factors, it had been proposed that the organic cations interact predominantly with the oppositely charged sulfonate groups of SC4TB rather than with the aryl rings using π-interactions. Conversely, the organic cations are placed outside the aromatic cavity at the level of the sulfonate groups when complexed with SC4TB, while in the case of SC4 this kind of cationic guests penetrates more deeply inside the aromatic cavity.

To validate the results presented here, 1D STD experiments were acquired for samples made of mixtures of SC4 with tetramethylammonium chloride (TMA), SC4TB with TMA, and also SC6HM with TMA (see the Supporting Information). In the case of SC4 and SC4TB, saturation of the CH_3 protons of TMA leads to a STD response almost exclusively on the ArH groups of the corresponding calixarene. This indicates that in both cases the TMA cation is located inside the aromatic cavity (SC4) or in the upper rim interacting with the sulfonate groups (SC4TB), and the magnetization is, as expected, mostly transferred to the ArH protons. On the other hand, in the case of SC6HM (Figure S3), the only appreciable STD response is observed for the OMe groups when the signal of the TMA cation is saturated. This result suggests that in the complex formed between SC6HM and TMA, the binding mode should be similar to that observed for C*n*TAB and ion−dipole interactions between oxygen atoms in the OMe group, and the positive charge of TMA should play an important role. In addition, the absence of aliphatic chain in TMA seems to not alter the binding mode significantly.

The differences in the binding modes of SC6HM and SC4 in the complexes formed between trimethylammonium surfactants and these two calixarenes could help to explain the differences in the type of aggregates formed (micelles in the case of SC6HM and vesicles for SC4),[9] but the high conformational flexibility of SC6HM in comparison with SC4 could also play a significant role in the aggregation process.

STD Build-Up Curves. As was commented on above, the size of the observed STD signal is not only dependent on the distance between the ligand and the macromolecule. Saturation of a signal in the bound state is counteracted by their longitudinal relaxation times T1 in the free state.[10] To prevent possible misinterpretations due to T1 effects, the construction of STD build-up curves was proposed,[12b] and the slope of the STD build-up curves at 0 saturation time can be used to eliminate T1 bias at long saturation times.

Figure 3 shows some of the STD build-up curves obtained when the aromatic protons of the calixarene (a) are saturated, and, in the same way, Figure 4 shows selected STD build-up curves obtained from saturation of the methyl protons in the ammonium headgroup of the surfactant. In these plots, the STD normalized intensity of a given proton signal is represented against the saturation time. In what follows, we will use the notation $STD_{x\{y\}}$ to refer to the STD curve obtained for proton x upon saturation of proton y.

dx.doi.org/10.1021/la3006794 | *Langmuir* 2012, 28, 6561−6568

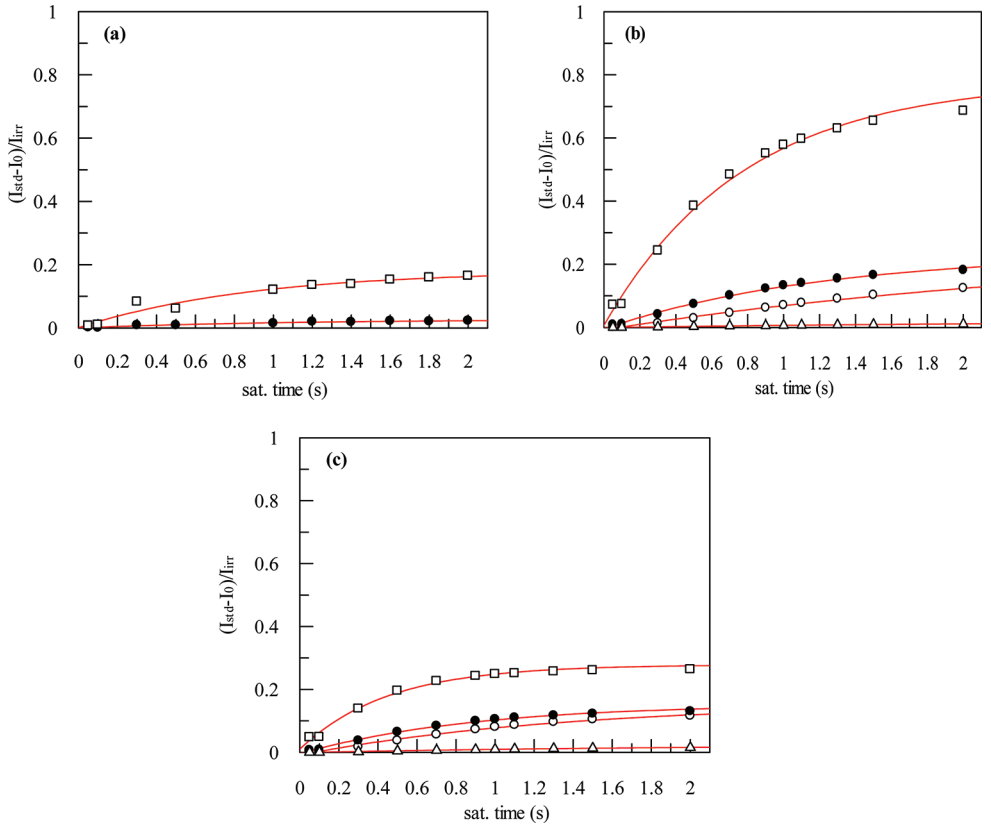


Figure 4. Normalized STD build-up curves of the mixture of **1** and **2**, with saturation of signal a′ of surfactant **2**. The concentrations used are: (a) 1 mM:1 mM (below the cmc), (b) 10 mM:10 mM, and (c) 10 mM:30 mM (with b and c above the cmc). (□) $STD_{a(a')}$, (●) $STD_{b(a')}$, (○) $STD_{c(a')}$, (△) $STD_{e'(a')}$.

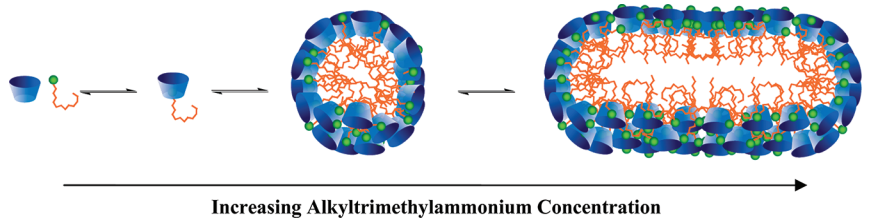


Figure 5. Aggregate evolution on increasing the concentration of C*n*TAB. Below the cmc, the complex exists in dynamic equilibrium with free SC6HM and C*n*TAB. When the concentration of C*n*TAB is increased to values above the cmc, micellar aggregates composed of SC6HM and C*n*TAB are formed. When the concentration of C*n*TAB is further increased from 10 to 30 mM, most of the added surfactant is incorporated into the micelles, leading to micellar growth due to surface charge neutralization and, eventually, to complexation of free SC6HM.

The plots of Figure 3 show the curves obtained for the saturation of proton a of SC6HM, corresponding to the mixtures of SC6HM and C*n*TAB below and above the cmc. In each plot, the intramolecular $STD_{b\{a\}}$ and $STD_{c\{a\}}$ have the strongest STD effect. In the three plots, the shortest saturation time for which the curves $STD_{b\{a\}}$ and $STD_{c\{a\}}$ reach their maximum follows the order 10 mM:30 mM ≅ 10 mM:10 mM ≪ 1 mM:1 mM. On the other hand, the maximum normalized intensity of $STD_{b\{a\}}$ in the three curves follows the order 10 mM:30 mM > 10 mM:10 mM > 1 mM:1 mM. The fact that the intramolecular $STD_{b\{a\}}$ is not very dependent on the conformation adopted by the calixarene (molecular modeling calculations performed by us showed that the intramolecular NOE distances of proton a to the two methylene protons b are 2.7 ± 0.3 and 3.6 ± 0.2 Å; the possibility of these NOEs corresponding to short intermolecular distances calixarene−calixarene can be safely ruled out) suggests that the intensity of $STD_{b\{a\}}$ primarily reflects the average molecular tumbling correlation time that SC6HM feels for the different states considered (i.e., association complex and micellar aggregates). The maximum intensity reached by $STD_{b\{a\}}$ in each of the plots of Figure 3 suggests that the correlation time follows the order 10 mM:30 mM > 10 mM:10 mM > 1 mM:1 mM. Utterly, the molecular tumbling correlation time of the calixarene is consistent with the expected larger average size of the micellar aggregates, in which SC6HM is forming part, in comparison with the association complex. The fact that the correlation time for the sample 10 mM:30 mM is higher than for the sample 10 mM:10 mM can indicate that the micelles grown when the concentration of C*n*TAB increases with respect to that of SC6HM or that a higher fraction of SC6HM is incorporated in the micelles or both (Figure 5). This is in line with the conclusions presented in our previous study of this system.[9a]

The plots of Figure 4 show the curves correspondingly to the 1 mM:1 mM, 10 mM:10 mM, and 10 mM:30 mM samples upon saturation of proton a′ of the surfactant. In each plot, the

dx.doi.org/10.1021/la3006794 | *Langmuir* 2012, 28, 6561−6568

intermolecular curve $STD_{c\{a'\}}$ has the strongest STD effect as compared to the intermolecular $STD_{b\{a'\}}$ and $STD_{a\{a'\}}$. These results strongly suggest that in the three samples considered for the mixture of SC6HM and C12TAB, the molecules form a complex in which the NMe_3^+ group of the surfactant is in close proximity to the OMe pendant group of SC6HM (Figure 6). In

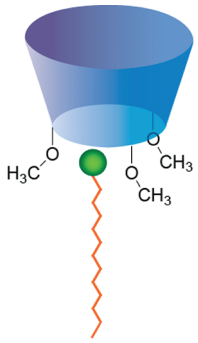


Figure 6. Cartoon illustration of a possible structure of the complex formed between SC6HM and C12TAB, with the ammonium headgroup of the surfactant interacting with the oxygen atoms of the calixarene. Because of the inherent flexibility, it should not be assumed that SC6HM is in one fixed conformation.

these three plots, the shortest saturation time for which the curve $STD_{c\{a'\}}$ reaches its maximum follows the order 10 mM:30 mM > 10 mM:10 mM > 1 mM:1 mM. However, the intensity of $STD_{c\{a'\}}$ is much stronger in 10 mM:10 mM sample (Figure 4b) than in the 1 mM:1 mM (Figure 4a) and 10 mM:30 mM (Figure 4c) samples. These sets of observations could reflect a combination of factors that lead to a closer distance between protons a′−c in the micelles formed in sample 10 mM:10 mM with respect to that of sample 10 mM:30 mM and for the association complex (sample 1 mM:1 mM), such as changes in the composition of the micelles that might lead to different packing, geometry, aggregation number, etc., and/or different conformational rearrangement of the complex in the three states.

Cross Relaxation Rates (CRR). The STD normalized intensities and the CRR are both sensitive to the r^{-6} (or $\langle r^{-3}\rangle^2$) average distances of the protons involved. However, the direct interpretation of the STD can be misleading due to the pernicious effects that arise from the differences in T_1 longitudinal relaxation times,[12] NOE spin diffusion thru dipolar networks, and rebinding contributions.[22] Those effects are negligible in the determined values of CRR under the extrapolation to zero saturation time used to determine them (see Supporting Information, eqs 3−5).

The analysis of a $STD^{obs}_{I\{S\}}$ curve such as those represented in Figures 3 and 4 with the initial build-up approach permits one to obtain the CRR $\sigma^{obs}_{I\{S\}}$ of the proton pair I−S. In the association equilibrium established between SC6HM and C*n*TAB, eq 6 (see the Supporting Information) applies, and the determined values of $\sigma^{obs}_{I\{S\}}$ represent a combination of the individual CRR in the free and bound states, $\sigma^{free}_{I\{S\}}$ and $\sigma^{bound}_{I\{S\}}$, weighted by their respective molar fractions, χ^{free} and χ^{bound}.

The results of $\sigma^{obs}_{I\{S\}}$ obtained for selected proton pairs of SC6HM and C*n*TAB in the three samples considered are given in Tables S1−S3 (see the Supporting Information), respectively. For comparison, the $\sigma^{obs}_{I\{S\}}$ values obtained for a sample of SC6HM in the absence of surfactant are also given in Table S4 (Supporting Information). From Tables S1−S4, the plots of Figure 7 were built. In these figures, $\sigma^{obs}_{I\{S\}}$ is represented as a function of the concentration of SC6HM and C*n*TAB in the mixtures for selected intramolecular proton pairs.

As the concentration of SC6HM and/or C*n*TAB is raised and the sample passes from free calixarene (1:0), complex (1:1), to micelles (10:10) and micelles (10:30), the curves of $\sigma^{obs}_{I\{S\}}$ in Figure 7 notoriously increase their magnitude. Such enhancement in CRR has to be primarily related to the molecular tumbling correlation time, or in other words with the average molecular size of the environment felt by molecules SC6HM and C12TAB. The molecular environment is considerably larger in the micellar states than in the complex or free calixarene, as was commented on above for Figures 3 and 4.

In Figure 8, we show the intermolecular $\sigma^{obs}_{I\{S\}}$ obtained from the irradiation of selected surfactant signals in sample 10:10. As can be observed, the large $\sigma^{obs}_{I\{S\}}$ value obtained corresponds to the c protons of the calixarene (OMe group) when the signal a′ of the surfactant ($N(CH_3)_3$ group) is irradiated. These results clearly show that the ammonium headgroup of the surfactant is closer to the OMe group than to the other protons of the calixarene (aromatic and bridge), supporting the results previously described in this Article. It must be pointed that the same trend was observed in all other samples (1:1 and 10:30), suggesting that the binding mode between SC6HM and C12TAB does not change substantially upon aggregation.

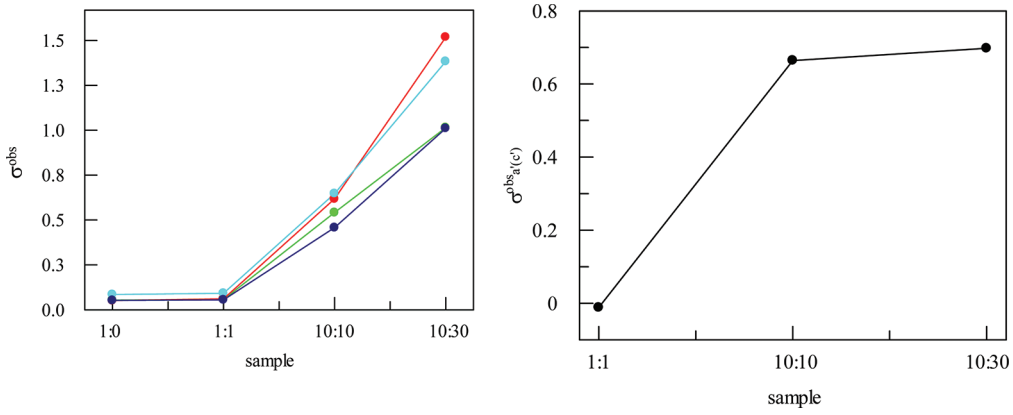


Figure 7. CCR $\sigma^{obs}_{I\{S\}}$ of selected proton pairs as a function of the concentration of SC6HM and C*n*TAB expressed in millimilar. Left: Intramolecular or intraspecie $\sigma^{obs}_{I\{S\}}$ of SC6HM, (black) $\sigma^{obs}_{b\{a\}}$; (red) $\sigma^{obs}_{a\{b\}}$; (green) $\sigma^{obs}_{c\{b\}}$; and (blue) $\sigma^{obs}_{b\{c\}}$. Right: Intramolecular or intraspecie $\sigma^{obs}_{a'\{c'\}}$ of C*n*TAB.

dx.doi.org/10.1021/la3006794 | *Langmuir* 2012, 28, 6561−6568

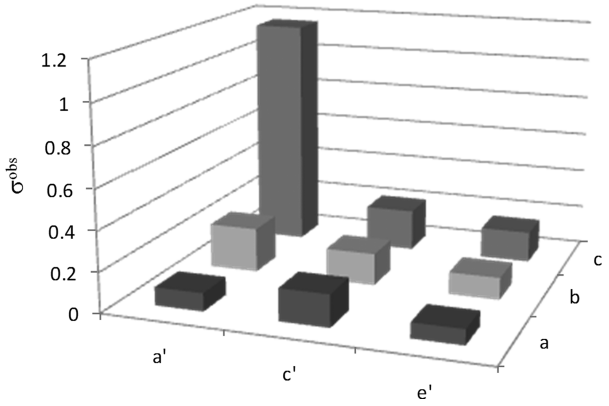


Figure 8. Intermolecular CCR σ^{obs} for SC6HM protons (a, b, c) obtained from the STD build-up curve, corresponding to saturation of the C*n*TAB signals (a′, b′, c′) in sample 10 mM:10 mM.

The results in Figure 9 show the influence of the sample composition on the intermolecular CCR for most relevant

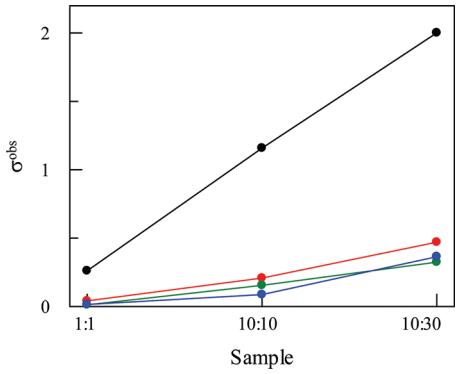


Figure 9. Intermolecular CCR $\sigma^{obs}_{I\{S\}}$ of selected proton pairs as a function of the concentration of SC6HM and C12TAB expressed in millimolar. (black) $\sigma^{obs}_{c\{a'\}}$; (red) $\sigma^{obs}_{c\{c'\}}$; (green) $\sigma^{obs}_{c\{e'\}}$; and (blue) $\sigma^{obs}_{a'\{c\}}$. The values obtained for sample 10:30 upon irradiation of the surfactant signals, $\sigma^{obs}_{c\{a'\}}$, $\sigma^{obs}_{c\{c'\}}$, and $\sigma^{obs}_{c\{e'\}}$, were multiplied by 3 to account for a higher fraction of free C12TAB.

proton pairs. When the surfactant protons are irradiated, it can be observed that the CRR for the c protons of SC6HM decreases in the order $\sigma^{obs}_{c\{a'\}} > \sigma^{obs}_{c\{c'\}} > \sigma^{obs}_{c\{e'\}}$ for the three samples studied. This in agreement with the existence of major interactions between the calixarene OMe group with the ammonium headgroup of the surfactant, as discussed above.

All CRR show the same dependence of the sample concentration independently of the surfactant proton that is irradiated. In all cases, the CRR increases with the concentration of surfactant on the sample. This is probably related to the fact that when the micellization process takes place, a higher fraction of complex is present in solution and with the different composition of the micelles in the samples 10:10 and 10:30. When the concentration of surfactant is increased, it is expected to observe an increase in hydrodynamic radius of the aggregates,[9a] and the molecular tumbling correlation time should play an important role in these values.

CONCLUSIONS

We have applied saturation transfer NMR techniques to characterize a complex formed by the C*n*TAB and water-soluble calixarene SC6HM. The results permitted us to conclude that complex is formed by interaction between the oxygen atoms in the OMe groups and the cationic head of the surfactant. This is a surprising result because the interaction was supposed to be mainly between the oppositely charged SO_3^- groups of SC6HM. Moreover, we found that the binding mode does not change upon the micellization of the complex. The results obtained here also support the dynamic behavior of this system, in particular, the variation in the micellar morphology imposed by the changes in the SC6HM:C*n*TAB molar ratio.

ASSOCIATED CONTENT

Supporting Information

Theory, tables of cross relaxation rates, and CRR $\sigma^{obs}_{I\{S\}}$ determined for selected proton pairs in the different mixtures of surfactants and calixarenes are reported. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Fax: +34 981 595012. E-mail: luis.garcia@usc.es.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Financial support from Ministerio de Ciencia y Tecnología (Project CTQ2011-22436) and Xunta de Galicia (PGIDIT10-PXIB209113PR and 2007/085) is gratefully acknowledged. N.B. acknowledges FCT for Ph.D. Grant SFRH/BD/29218/2006.

ABBREVIATIONS

CRR = cross relaxation rates

$\sigma^{obs}_{I\{S\}}$ = observed cross relaxation rate for the pair of protons I−S upon saturation of proton S

$\sigma^{free}_{I\{S\}}$ = cross relaxation rate for the pair of protons I−S in the free state upon saturation of proton S

$\sigma^{bound}_{I\{S\}}$ = cross relaxation rate for the pair of protons I−S in the bound state upon saturation of proton S

$STD_{I\{S\}}$ = normalized saturation transfer difference intensity for the pair of protons I−S upon saturation of proton S

REFERENCES

(1) Asfari, Z., Bohmer, V., Harrowfield, J., Vicens, J., Eds. *Calixarenes 2001*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2001.

(2) Böhmer, V. Calixarenes, macrocycles with (almost) unlimited possibilities. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 713−745.

(3) Rebek, J. Host−guest chemistry of calixarene capsules. *Chem. Commun.* **2000**, 637−643.

(4) Bogdan, A.; Rudzevich, Y.; Vysotsky, M. O.; Böhmer, V. Topologically novel multiple rotaxanes and catenanes based on tetraurea calix[4]arenes. *Chem. Commun.* **2006**, 2941−2952.

(5) For some examples, see: (a) Shinkai, S.; Mori, S.; Koreishi, H.; Tsubaki, T.; Manabe, O. Hexasulfonated calix[6]arene derivatives: a new class of catalysts, surfactants, and host molecules. *J. Am. Chem. Soc.* **1986**, *108*, 2409−2416. (b) Strobel, M.; Kita-Tokarczyk, K.; Taubert, A.; Vebert, C.; Heiney, P. A.; Chami, M.; Meier, W. Self-assembly of amphiphilic calix[4]arenes in aqueous solution. *Adv. Funct. Mater.* **2006**, *16*, 252−259. (c) Kellermann, M.; Bauer, W.; Hirsch, A.; Schade, B.; Ludwig, K.; Bottcher, C. The first account of a structurally persistent micelle. *Angew. Chem., Int. Ed.* **2004**, *43*, 2959−2962. (d) Lee, M.; Lee, S. J.; Jiang, L. H. Stimuli-responsive supramolecular nanocapsules from amphiphilic calixarene assembly. *J. Am. Chem. Soc.* **2004**, *126*, 12724−12725. (e) Houmadi, S.; Coquière, D.; Legrand, L.;

Fauré, M. C.; Goldmann, M.; Reinaud, O.; Rémita, S. Architecture-controlled "SMART" calix[6]arene self-assemblies in aqueous solution. *Langmuir* **2007**, *23*, 4849−4855.
(6) (a) Basílio, N.; García-Río, L.; Martín-Pastor, M. NMR evidence of slow monomer−micelle exchange in a calixarene-based surfactant. *J. Phys. Chem. B* **2010**, *114*, 4816−4820. (b) Basílio, N.; García-Río, L.; Martín-Pastor, M. Calixarene-based surfactants: Evidence of structural reorganization upon micellization. *Langmuir* **2012**, *28*, 2404−2414.
(7) Helttunena, K.; Shahgaldian, P. Self-assembly of amphiphilic calixarenes and resorcinarenes in water. *New J. Chem.* **2010**, *34*, 2704−2714.
(8) Zhang, X.; Wang, C. Supramolecular amphiphiles. *Chem. Soc. Rev.* **2011**, *40*, 94−101.
(9) (a) Basílio, N.; García-Río, L. Sulfonated calix[6]arene host−guest complexes induce surfactant self-assembly. *Chem.-Eur. J.* **2009**, *15*, 9315−9319. (b) Francisco, V.; Basílio, N.; Garcia-Rio, L.; Leis, J. R.; Marques, E. F.; Vázquez-Vázquez, C. Novel catanionic vesicles from calixarene and single-chain surfactant. *Chem. Commun.* **2010**, *46*, 6551−6553. (c) Wang, K.; Guo, D.-S.; Liu, Y. Temperature-controlled supramolecular vesicles modulated by p-sulfonatocalix[5]arene with pyrene. *Chem.-Eur. J.* **2010**, *16*, 8006−8011. (d) Wang, K.; Guo, D.-S.; Wang, X.; Liu, Y. Multistimuli responsive supramolecular vesicles based on the recognition of p-sulfonatocalixarene and its controllable release of doxorubicin. *ACS Nano* **2011**, *5*, 2880−2894.
(10) (a) Mayer, M.; Meyer, B. Characterization of ligand binding by saturation transfer difference NMR spectroscopy. *Angew. Chem., Int. Ed.* **1999**, *38*, 1784−1788. (b) Meyer, B.; Peters, T. NMR spectroscopy techniques for screening and identifying ligand binding to protein receptors. *Angew. Chem., Int. Ed.* **2003**, *42*, 864−890.
(11) (a) Neuhaus, D.; Williamson, M. *The Nuclear Overhauser Effect in Structural and Conformational Analysis*; VCH: New York, 2000. (b) Hu, H.; Krishnamurthy, K. Revisiting the initial rate approximation in kinetic NOE measurements. *J. Magn. Reson.* **2006**, *182*, 173−177.
(12) (a) Yan, J.; Kline, A. D.; Mo, H.; Shapiro, M. J.; Zartler, E. R. The effect of relaxation on the epitope mapping by saturation transfer difference NMR. *J. Magn. Reson.* **2003**, *163*, 270−276. (b) Mayer, M.; James, T. L. NMR-based characterization of phenothiazines as a RNA binding scaffold. *J. Am. Chem. Soc.* **2004**, *126*, 4453−4460.
(13) Fritzinger, B.; Moreels, I.; Lommens, P.; Koole, R.; Hens, Z.; Martins, J. C. In situ observation of rapid ligand exchange in colloidal nanocrystal suspensions using transfer NOE nuclear magnetic resonance spectroscopy. *J. Am. Chem. Soc.* **2009**, *131*, 3024−3032.
(14) (a) Castro, R.; Godinez, L. A.; Crissm, C. M.; Bott, S. G.; Kaifer, A. E. Solid-state and solution complexation of cobalt(iii) sepulchrate by a water-soluble calix[6]arene host. *Chem. Commun.* **1997**, 935−936. (b) Alvarez, J.; Wang, Y.; Gómez-Kaifer, M.; Kaifer, A. E. Importance of intramolecular hydrogen bonding for preorganization and binding of molecular guests by water-soluble calix[6]arene hosts. *Chem. Commun.* **1998**, 1455−1456.
(15) Castro, R.; Godínez, L. A.; Criss, C. M.; Kaifer, A. E. Host properties of α-cyclodextrin and a water-soluble calix[6]arene probed with dimeric bipyridinium guests. *J. Org. Chem.* **1997**, *62*, 4928−4935.
(16) Casnati, A.; Jacopozzi, P.; Pochini, A.; Ugozzoli, F.; Cacciapaglia, R.; Mandolini, L.; Ungaro, R. Bridged calix[6]arenes in the cone conformation: New receptors for quaternary ammonium cations. *Tetrahedron* **1995**, *51*, 591−598.
(17) See, for example: (a) Shinkai, S.; Araki, K.; Matsuda, T.; Nishiyama, N.; Ikeda, H.; Takasu, L.; Iwamoto, M. NMR and crystallographic studies of a p-sulfonatocalix[4]arene-guest complex. *J. Am. Chem. Soc.* **1990**, *112*, 9053−9058. (b) Arena, G.; Casnati, A.; Contino, A.; Lombardo, G. G.; Sciotto, D.; Ungaro, R. Water-soluble calixarene hosts that specifically recognize the trimethylammonium group or the benzene ring of aromatic ammonium cations: A combined 1H NMR, Ccalorimetric, and molecular mechanics investigation. *Chem.-Eur. J.* **1999**, *5*, 738−744. (c) Arena, G.; Gentile, S.; Gulino, F. G.; Sciotto, D.; Sgarlata, C. Water-soluble pentasulfonatocalix[5]arene: selective recognition of ditopic trimethylammonium cations by a triple non-covalent interaction. *Tetrahedron Lett.* **2004**, *45*, 7091−7094. (d) Guo, D.-S.; Wang, L.-H.; Liu, Y. Highly effective binding of methyl viologen dication and its radical cation by p-sulfonatocalix[4,5]arenes. *J. Org. Chem.* **2007**, *72*, 7775−7778. (e) Beshara, C. S.; Jones, C. E.; Daze, K. D.; Lilgert, B. J.; Hof, F. A simple calixarene recognizes post-translationally methylated lysine. *ChemBioChem* **2010**, *11*, 63−66.
(18) Bakirci, H.; Koner, A. L.; Nau, W. M. Spherical shape complementarity as an overriding motif in the molecular recognition of noncharged organic guests by p-sulfonatocalix[4]arene: Complexation of bicyclic azoalkanes. *J. Org. Chem.* **2005**, *70*, 9960−9966.
(19) Guo, D.-G.; Wang, K.; Liu, Y. Selective binding behaviors of p-sulfonatocalixarenes in aqueous solution. *J. Inclusion Phenom. Macrocyclic Chem.* **2008**, *62*, 1−21.
(20) It should be noted that is possible to find association constants of the order of 10^3 M^{-1} in the bibliography, but care should be taken when comparisons are made, because some of them were obtained in the presence of an appreciable concentration of buffer, and sodium cations are known to compete with the guest for the complexation with SC*n*, resulting in observed binding constant significantly lower than that measured in the absence of added cations in solution. For detailed information, please see: Bakirci, H.; Koner, A. L.; Nau, W. M. Binding of inorganic cations by p-sulfonatocalix[4]arene monitored through competitive fluorophore displacement in aqueous solution. *Chem. Commun.* **2005**, 5411−5413.
(21) Cui, J.; Uzunova, V. D.; Guo, D.-S.; Wang, K.; Nau, W. M.; Liu, Y. Effect of lower-rim alkylation of p-sulfonatocalix[4]arene on the thermodynamics of host−guest complexation. *Eur. J. Org. Chem.* **2010**, 1704−1710.
(22) Angulo, J.; Díaz, I.; Reina, J. J.; Tabarani, G.; Fieschi, F.; Rojo, J.; Nieto, P. M. Saturation transfer difference (STD) NMR spectroscopy characterization of dual binding mode of a mannose disaccharide to DC-SIGN. *ChemBioChem* **2008**, *9*, 2225−2227.