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Calix[4]pyrrole as a Chloride Anion Receptor: Solvent and Countercation Effects

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Abstract: The interaction of calixpyrrole with several chloride salts has been studied in the solid state by X-ray crystallography as well as in solution by isothermal titration calorimetry (ITC) and ^{1}H NMR spectroscopic titrations. The titration results in dimethylsulfoxide, acetonitrile, nitromethane, 1,2-dichloroethane, and dichloromethane, carried out using various chloride salts, specifically tetraethylammonium (TEA), tetrapropylammonium (TPA), tetrabutylammonium (TBA), tetraethylphosphonium (TEP), tetrabutylphosphonium (TBP), and tetraphenylphosphonium (TPhP), showed no dependence on method of measurement. The resulting affinity constants (K_a), on the other hand, were found to be highly dependent on the choice of solvent with K_a's ranging from 10^2-10^5 M^{-1} being recorded in the test solvents used for this study. In dichloromethane, a strong dependence on the countercation was also seen, with the K_a's for the interaction with chloride ranging from 10^2-10^4 M^{-1}. In the case of TPA, TBA, and TBP, the ITC data could not be fit to a 1:1 binding profile.

Introduction

meso-Octamethylcalix[4]pyrrole (CP; e.g., **1**) along with its many derivatives are a well recognized class of anion receptor. This easy to make tetrapyrrolic macrocycle has been known for over a century,[1] yet its anion binding potential has only recently been discovered.[2] This discovery has prompted various structural modifications[3,4] in hopes of fine-tuning the affinity and selectivity for a variety of anions. Despite the considerable amount of experimental[5,6] and theoretical[7] work devoted to understanding the anion binding behavior of calixpyrroles, there remain a number of issues that are not fully resolved, including those associated with solute, solvent, and countercation effects.

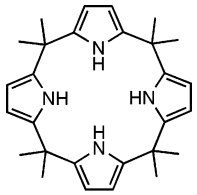

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Figure 1. *meso*-Octamethylcalix[4]pyrrole.

Much of the problem reflects the fact that, to date, many of the anion binding studies involving calix[4]pyrrole have relied on the use of anions in the form of their tetrabutylammonium (TBA) salts[2,4−6] and have been predicated on the tacit assumption that the anion (A^-) behaves as a free species (cf. eq 1). Recently, however, we have discovered that calixpyrrole can act as an ion-pair receptor, particularly, in the solid state, as the result of π-stacking or π-cation interactions involving large diffuse cations and the "walls" of the electron rich calix[4]-pyrrole cavity.[8] Such findings, coupled with apparent disparities in solution phase anion binding data (see below),[5] have led us

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to consider that solvation and ion-pairing effects, illustrated in eq 2, may be more important than hitherto appreciated. In this paper, we report the results of a detailed study of the effect of solvent and countercation (represented by R^+ in eq 2) on the binding of chloride anion to calix[4]pyrrole, as well as further solid-state structural analyses of the resulting complexes.

$$CP + Cl^- \rightarrow [CP\text{-}Cl]^- \quad (1)$$

$$[CP]_{solv} + [Cl^-R^+]_{solv} \rightarrow \{[CP-Cl]^-R^+\}_{solv} \quad (2)$$

The apparent disparities in solution phase anion binding data alluded to above reflect the fact that different groups, in particular, those of the authors at The University of Texas at Austin (UT) and the Technische Universität München (TU), have obtained seemingly different K_a values when studying the anion binding behavior of calix[4]pyrrole. In initial investigations involving ^{1}H NMR spectroscopic titrations (hereafter "NMR") carried out in dichloromethane-d_2, the UT group reported[2] K_a's of 1.7×10^4 and 3.5×10^2 M^{-1} for the binding of fluoride and chloride anion (TBA salts) to calix[4]pyrrole **1**, respectively. In a subsequent report,[6] the Southampton group found that when dimethylsulfoxide-d_6 was used as the solvent selectivity for fluoride over chloride was diminished significantly (K_a's, determined by NMR, were found to be 1.1×10^3 and 1.0×10^3 M^{-1} for fluoride and chloride, respectively, added again as the corresponding TBA salts). More recently, while studying the interaction of **1** with various anions in acetonitrile by isothermal titration calorimetry (ITC), the TU group determined[5] K_a's of 1.5×10^5 and 1.9×10^5 M^{-1} for the binding of potassium⊂cryptand[222] salts of fluoride and chloride, respectively. In separate work, de Namor reported K_a values of 5.0×10^4 and 5.5×10^4 M^{-1} for the binding of TBA−Cl and tetramethylammonium chloride (TMA−Cl) to calixpyrrole **1**, as derived from calorimetric measurements carried out in acetonitrile.[9] This same group reported affinity constants for fluoride, derived by competition studies, in this same solvent that were ca. 1.5 orders of magnitude higher. Eichen and coworkers also reported a K_a of 6.8×10^3 M^{-1} when studying the interaction of **1** with chloride in acetonitrile/chloroform (1:9 v/v) by NMR.[4c] The problem of calix[4]pyrrole anion binding has also been addressed by several theoretical studies, as noted above.[7] Nonetheless, no satisfactory explanation for the apparent disparity between the results obtained by the UT and TU groups has so far been forthcoming. Examination of the findings from these two groups and others leads to the conclusion that there may be a strong dependence on the solvent and that both this and the choice of countercation could play important roles in regulating the selectivity and strength of the binding interaction of **1** with anionic guests. However, because the various studies involved the use of (often) different solvents, chloride anion sources, receptor concentrations, and analysis methods, this conclusion is far from established. Moreover, the work in question was carried out by different investigators, and this could be a source of potential error or bias. Thus, first and foremost, it was felt necessary to corroborate the measurement methods and to eliminate any concerns associated with the choice of experimental venue. Toward this end, a collaborative study involving the UT, TU, and Southampton groups was launched. Because of the greater complexity associated with working with fluoride salts,[10] the focus of this first report arising from these joint efforts is on the association of **1** with various chloride salts and in several representative solvents. As detailed further below, we have chosen solvents with a range of polarities and dielectric constants, namely, acetonitrile, dimethylsulfoxide, nitromethane, 1,2-dichloroethane, and dichloromethane, and have explored anion binding in solution using both ITC and NMR titrations, in addition to X-ray crystallography in the solid state. The net result of these investigations is that the choice of countercation can be significant, even though in many cases the effects of solvation and ion pairing can be largely ignored, as long as bona fide comparisons are being made within a similar solvent system. Another key finding is that the K_a's determined by ^{1}H NMR spectroscopic titrations and ITC are generally comparable, as long as the values in question fall within the range that can be reliably measured by the method in question (ca. $10 < K_a < 10^4$ M^{-1} for NMR; $10^2 < K_a < 10^7$ M^{-1} for ITC).

Results and Discussion

The first key question to be addressed in this study was whether K_a values determined by NMR and ITC could be compared and, if so, under what conditions. Such studies were viewed as being of critical, predicative importance since the absence of such comparisons and the apparent disparity between the UT and TU findings could easily be rationalized in terms of differences in the measurement methods. While under ideal conditions, including those where eq 1 provides a reasonable approximation of the binding equilibrium, these two methods should give rise to the same K_a value, it is to be appreciated that they sample different aspects of the processes occurring in solution. As generally utilized, ^{1}H NMR spectroscopic titrations monitor the effect that an added substrate ("guest"), a salt of an anion in the present instance, has on the chemical shift of one or more proton signals present in the spectrum of the receptor ("host") being subject to titration. Under conditions of fast exchange and for a simple 1:1 binding equilibrium, the chemical shift of the signal in question is directly proportional to the mole fraction of the host−guest complex. Thus, by monitoring the change in the chemical shift as a function of added substrate, it is possible to construct binding profiles, from which apparent K_a values may be derived. Related methods, in particular, the construction of so-called Job plots, allow the stoichiometry of the reaction to be determined, again in the case of clean, well-behaved equilibrium situations. In practice,

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because of instrument limitations and those imposed by sample solubility, the effective K_a range for which NMR titrations can provide reliable values for 1:1 binding equilibria is ca. $10 < K_a < 10^4$ M^{-1}. When care is exercised and suitably high signal-to-noise ratios are obtained, this range can be extended by an order of magnitude on either side.

In contrast to NMR, ITC analyses provide a measure of the change in the heat of the whole sample. They thus provide direct access to the energetics in an interacting system without retreat to a structural probe (e.g., a NMR signal) that may or may not reflect the entirety of the associative process. In other words, calorimetry accounts for the individual contributions of all simultaneous processes in solution. However, since the observable output comprises the sum of these processes, the deconvolution of ITC data in terms of an interpretable binding model can present a major challenge, especially if the complexation process under study depends on a number of variables, such as solvation, ion pairing, pH, etc.

Because the interpretation of ITC data depends on the model chosen (vide infra), it is necessary to establish the binding stoichiometry. In NMR studies, this is often accomplished conveniently by use of Job plots, at least in well-behaved situations, as noted above. In the case of ITC, a particularly powerful approach to determining the stoichiometry of a complex is to invert the concentration relations of the host and guest species. Since an idealized 1:1 binding relationship is symmetrical, it should furnish identical molar heats irrespective of the sequence of addition of the binding partner. Thus experiments where the guest is titrated into the host solution and those where the host is added to the guest solution (the latter is named an "inverse titration") should give rise to identical K_a values. Deviations from this idealized result reveal changes in the nominal host−guest interaction energetics and generally indicate the involvement of additional complicating processes, such as oligomerization, ion pairing, acid−base reactions, and the involvement of higher order binding events (i.e., complete or partial formation of complexes of non-1:1 stoichiometry).

The characterization or exclusion of such perturbations depends on the acquisition of high quality data, a process that has been aided by the introduction of completely computer-operated calorimeters. A benefit of the modern computer-controlled instruments is that the statistical error is in general small. Moreover, its origin is necessarily analyzed rigorously[11] with respect to the size of the heat output, the number of titration steps, the titrant volume, and the c value.[12] Nonetheless, systematic errors can be present, and the fact that the ITC experiments are automated can make it difficult to spot such errors. Thus, a critical inspection of the data set is required in order to avoid misinterpretation of the energetic parameters delivered by the evaluation software.[13] When appropriate caveats are met, the effective K_a range for which ITC can provide reliable values for 1:1 binding equilibria is ca. $10^2 < K_a < 10^7$ M^{-1}. One reason (among many) for why ITC permits access to a higher K_a range than NMR is that the measurements are generally carried out at a ≥10-fold lower concentration.

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Given the above differences in measurement methods, a first goal of this study was to determine if conditions could be found where both NMR and ITC gave concordant K_a values for the binding of an anion (as an appropriately chosen salt) to calix[4]pyrrole **1**. In the context of this objective, it was felt necessary to establish first that the measurements themselves, be they NMR- or ITC-based, were fully reproducible. Toward this end, key NMR analyses were carried out in Austin (UT) and Southampton, whereas nearly every ITC-derived K_a value was reproduced in UT and TU. Under conditions where solvents of similar purity and dryness were employed and identical salts, again of analogous dryness and purity, were used, good reproducibility was obtained, provided also that the temperature of the analysis was kept constant. Thus, the "null solution", namely, that the discrepancies in values obtained in the lead authors various laboratories reflected simple operator error (i.e., a non-reliability in the measured values), could be discounted. The more interesting question of whether these discrepancies reflected differences in measurement method, NMR versus ITC, could thus be pursued.

Initial effort was devoted to finding conditions where NMR and ITC values could be reasonably compared. This required finding a set of solvent(s) and salt(s), where the K_a value(s) fell within the ca. 10^2-10^4 M^{-1} range accessible to both NMR and ITC analyses. After some preliminary screening experiments, it was found that such conditions are met when calix-

(13) A frequent cause of systematic error are influences that do not derive from the supramolecular interaction under investigation. Some of the unspecific heat effects can be corrected by subtraction of blank titrations that omit one or the other host−guest binding partner. Yet, there may be synergistic effects from within the solution[18] or from the instrument itself that may not be taken account of by running a blank titration. In simple 1:1 host−guest systems, such errors may cause an offset from zero after background subtraction (i.e., after correcting for the blank titration) in the region of the titration where host−guest complexation approaches saturation. In these cases, the calculated fit function shows systematic deviations, and while error minimization may finally converge, the energetic parameters deduced can nonetheless be characterized by low accuracy and precision. Fortunately, a dramatic improvement in terms of convergence and parameter reliability can be achieved by translating the entire data set in the *y*-direction to overlay the data points recorded at the higher molar ratios with those derived from the initially calculated fit. Reminimization now results in a new fit function with an improved fit and a reduced variance in the fit parameters. Reiteration of this procedure gives, in due course, an optimal fit that constitutes the best representation of the data set by the model chosen (1:1 binding in the case of the present study). In essence, this optimization is equivalent to the inclusion of an adjustable additive constant to the model function and can also be treated as another fit parameter by the minimization software after suitable modification. Such an adaptation of the minimization procedure is legitimate since the desired energetic state functions $\Delta H°$ and $\Delta G°$ depend entirely on the shape of the sigmoidal response curve, but not on the absolute position of the data points along the *Y*-axis. This is because $\Delta H°$ is correlated with the extrapolated step height, while $\Delta G°$ is derived from the slope in the inflection point. The quality of fit (χ^2), in contrast, reflects all nominal differences of the data coordinates to the ideal model function that is implemented in the data evaluation software generally provided with the calorimeter. Since this ideal model excludes all experimentally observed contributions not related to the core supramolecular process, it can lead to massive systematic errors if applied incorrectly. An ideal model, by definition, does not pay attention to any non-ideality inherent in the experimental data. That is why one needs to correct for non-ideality in the data before applying a proposed binding model. Frequently, blank titrations accomplish this only in part. Hence, there is a requirement for, and benefit of, the above adjustments. Another key point to stress is that, in contrast to NMR, calorimetry gives immediate access to the energetics of the system but does so without regard to the structures involved in the supramolecular interaction. Structural information can only be deduced from trend analyses involving the change in energetics that arise from making incremental and well-defined modifications in the host−guest binding system. Thus, in the case of ITC, structural information is inferred far less directly than in the case of NMR, where specific chemical shifts can be monitored, at least in favorable cases. Nonetheless, by using closely related binding partners or varying the solvent while studying the same host−guest combination, ITC can provide a correlation between binding energetics and structure that—at least in theory—complements, rather than rivals, the insights obtained from structure-based NMR spectroscopic analyses.

Table 1. Results of Titrations of Calixpyrrole **1** Carried Out in Acetonitrile and Nitromethane at 298 K (calorimetry) or 295 K (NMR)

	guest	N^a	ΔH (kcal/mol)	$T\Delta S$ (kcal/mol)	ΔG (kcal/mol)	K_a(ITC) (M^{-1})	K_a(NMR) (M^{-1})
acetonitrile	TEA−Cl	5	-10.10 ± 0.94	-3.07 ± 0.73	-7.19 ± 0.12	$1.9 \pm 0.4 \times 10^5$	2.2×10^5
	TBA−Cl	5	-10.16 ± 0.20	-2.91 ± 0.26	-7.29 ± 0.06	$2.2 \pm 0.2 \times 10^5$	2.5×10^5
nitromethane	TEA−Cl	6	-7.54 ± 1.05	-1.83 ± 0.88	-5.84 ± 0.22	$2.0 \pm 0.7 \times 10^4$	1.9×10^4
	TBA−Cl	4	-7.49 ± 0.99	-1.80 ± 0.80	-5.68 ± 0.20	$1.6 \pm 0.5 \times 10^4$	2.4×10^4

[a] N = number of independent calorimetric experiments.

Table 2. Results of Titrations of Calixpyrrole **1** Carried Out in Dimethylsulfoxide at 298 K (calorimetry) or 295 K (NMR)

	N^a	guest	ΔH (kcal/mol)	$T\Delta S$ (kcal/mol)	ΔG (kcal/mol)	K_a(ITC) (M^{-1})	K_a(NMR) (M^{-1})
dimethylsulfoxide	2	TEA−Cl	−1.93	2.26	−4.19	1.2×10^3	2.3×10^3
	2	TBA−Cl	−1.87	2.30	−4.17	1.1×10^3	2.2×10^3

[a] N = number of independent calorimetric experiments.

Table 3. Results of Titrations of Calixpyrrole **1** Carried Out in 1,2-Dichloroethane and Dichloromethane

	guest	N^a	ΔH (kcal/mol)	$T\Delta S$ (kcal/mol)	ΔG (kcal/mol)	K_a(ITC) (M^{-1})	K_a(NMR) (M^{-1})
1,2-dichloroethane	TEA−Cl	5	-9.87 ± 0.29	-3.28 ± 0.17	-6.59 ± 0.27	$7.5 \pm 3.2 \times 10^4$	4.2×10^4
	TBA−Cl	6	-10.39 ± 0.37	-4.32 ± 0.26	-6.06 ± 0.17	$2.8 \pm 0.7 \times 10^4$	1.5×10^4
dichloromethane	TEA−Cl[b]	4	-9.32 ± 0.71	-3.19 ± 0.78	-6.14 ± 0.08	$3.2 \pm 0.4 \times 10^4$	3.7×10^4
	TEA−Cl[c]	2	−9.57	−3.56	−6.12	3.4×10^4	3.6×10^4
	TEA−Cl[d]	2	−10.96	−4.63	−6.33	4.9×10^4	N.D.
	TBA−Cl		no reliable fit to a 1:1 binding isotherm possible				4.3×10^2

[a] N = number of independent experiments. [b] At 298 K. [c] At 295 K. [d] At 293 K; N.D. = not determined.

[4]pyrrole **1** is studied in nitromethane and either tetraethylammonium chloride (TEA−Cl) or tetrabutylammonium chloride (TBA−Cl) are used as the anion source. Here, as can be seen by an inspection of the K_a values listed in Table 1, there is no appreciable difference in the results obtained using NMR and ITC. The data in these cases fit a 1:1 stoichiometry model very cleanly, with no difference in affinity being observed for the TEA and TBA salts. Further support for the proposed 1:1 stoichiometry was obtained by Job plot analyses in the case of the NMR studies (cf. Supporting Information).

A clean fit to a 1:1 model was also seen when analogous measurements were carried out in acetonitrile, a conclusion that was again supported by Job plot analyses in the case of the NMR studies. While in this case the high K_a values in question required extra care in carrying out the NMR titrations, to the extent that the resulting values may be considered reliable, a good concordance is again seen between NMR and ITC (Table 1). Further, these studies revealed that the choice of cation again has little, if any, effect of the derived K_a value. On the other hand, an appreciable difference is observed between the solvents, with the K_a values determined in nitromethane being ca. 10-fold lower than those recorded in acetonitrile. On the basis of the ITC analyses, this order of magnitude change in overall affinity is ascribed almost exclusively to differences in the enthalpy of binding.

With the basic correspondence between NMR and ITC established under (at least) appropriately favorable conditions, a survey of solvent effects was undertaken. This was motivated by the fact that another simple explanation for the initial discrepancy between the UT and TU data could reflect the fact that different solvents (dichloromethane vs acetonitrile and DMSO) were being used. Because NMR analyses in DMSO had been carried out by the Southampton group, this solvent was selected as the next point of focus. Again, two salts (TEA and TBA) were chosen for study, and both NMR and ITC measurements were carried out. The resulting K_a values, summarized in Table 2, were found to be consistent with those reported earlier and, again, revealed a concordance between ITC and NMR. No effect of countercation was seen in this solvent. Interestingly, however, in DMSO, the formation of a calix[4]pyrrole−chloride anion complex is highly driven by entropy, with nearly 50% of the total energy being due to entropic factors. This behavior diverges from what is seen in acetonitrile and nitromethane (vide supra) and, indeed, all other solvents in this study, wherein the entropic term typically has a value less than half that of the enthalpic term and has an opposing sign.

The small enthalpic contribution to chloride anion binding seen in the case of DMSO also provides support for the notion that **1** interacts with neutral species, such as dimethylsulfoxide, as previously reported.[14] This latter interaction likely causes the calixpyrrole to be fully or partially preorganized, reducing the entropic penalty associated with anion complexation. The release of strongly bound DMSO molecules to the bulk solution surfaces as a positive entropy component on the one hand, while serving to diminish the enthalpic gain associated with guest binding (smaller exothermicity) on the other. To the extent this rationalization is correct, it provides an explanation for what would be otherwise a highly anomalous solvent effect.

The results of chloride anion binding titrations carried out in 1,2-dichloroethane were also found to be independent of titration method (Table 3). However, in this case, a slight dependence on countercation is seen. In particular, when switching from TEA−Cl to TBA−Cl, a small difference (a factor of 2−3) is noted in the association constant. This small deviation could stem from an ion-pairing effect in this nonpolar solvent.

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Table 4. Titrations of Calixpyrrole **1** in Dichloromethane with Various Chloride Salts at 298 K (calorimetry) or 295 K (NMR)

	ΔH (kcal/mol)	$T\Delta S$ (kcal/mol)	ΔG (kcal/mol)	K_a (ITC) (M^{-1})	K_a (NMR) (M^{-1})
TEA−Cl	−9.91	−3.80	−6.11	3.1×10^4	3.7×10^4
TPA−Cl	*a*	*a*	*a*	*a*	6.6×10^2
TBA−Cl	*a*	*a*	*a*	*a*	4.3×10^2
TEP−Cl	−9.59	−4.68	−4.91	3.9×10^3	3.6×10^3
TBP−Cl	*a*	*a*	*a*	*a*	*a*
TPhP−Cl	−6.8	−2.2	−4.6	2.8×10^3	N.D.

[a] No reliable fit to a 1:1 binding isotherm could be made. N.D. - not determined.

Such putative effects are enhanced in the case of dichloromethane (DCM), which has a similar dielectric constant. In this case, NMR titrations reveal a 100-fold decrease in affinity when switching from TEA−Cl to TBA−Cl. This is a striking result because the thermodynamic data for the interaction of TEA−Cl with calixpyrrole in both solvents are virtually identical. This divergence in behavior prompted a more detailed look into the thermodynamics of chloride anion salt binding when dichloromethane is used as the solvent. The results of these analyses, carried out using ITC, are detailed below.

To probe the putative effect of countercation in DCM, a number of organic chloride salts were studied, namely, TMA−Cl, TEA−Cl, tetrapropylammonium chloride (TPA−Cl), TBA−Cl, tetraethylphosphonium chloride (TEP−Cl), tetrabutylphosphonium chloride (TBP−Cl), and tetraphenylphosphonium chloride (TPhP−Cl) (Table 4). The latter salts are expected to be dissociated into the corresponding "free" cations and anions to a greater extent than TBA−Cl, which has been estimated to be as much as 80% ion paired as a 1 mM solution in DCM.[15]

In the case of the studies involving TPA−Cl, TBA−Cl, and TBP−Cl in DCM, the resulting titration isotherm could not be fit to a 1:1 stoichiometric model, with the data in the case of TBA−Cl fitting nicely to a two-site sequential model. This was true even though Job plot analyses of the corresponding NMR data were consistent with a 1:1 stoichiometry. This leads us to suggest that there is a second process that is significant under the conditions of the ITC analysis. Unfortunately, due to the lack of inflection point seen in the binding profile and its sigmoidal character, a reliable energetic characterization of this putative process could not be made. Nonetheless, it is apparent that the two putative events are characterized by opposing enthalpies and entropies. Conversely, when TEA−Cl, TEP−Cl, and TPhP−Cl were used as the source of chloride anion, the data could be fit cleanly to a 1:1 model, resulting in calculated K_a's of 3.1×10^4 versus 3.9×10^3 and 2.8×10^3 M^{-1} for the latter two salts, respectively. Interestingly, in the case of TEP−Cl and TPhP−Cl, the K_a is an order of magnitude less than that obtained using TEA−Cl. The discrepancy in the effective chloride affinities seen for these different chloride salts supports the notion that ion-pairing effects play an important role in regulating the anion binding behavior of calixpyrrole in dichloromethane. Such ion pairing may interfere with the host−guest binding process at the starting state (by reducing the amount of free anion prior to complexation) and after initial anion binding alike. It is also conceivable that the latter process may occur in a stepwise fashion depending on the actual microscopic binding constants of the anionic and cationic guests. Alternatively, one can also envisage a highly cooperative association of both salt constituents[16] that would minimize the free concentration of an anion−host complex. These variants cannot be distinguished at present, nor can they be excluded. The experimental observations, however, point to a considerably more complex binding process than is represented by the simple anion−calixpyrrole binding model given in eq 1. Presumably, in the DCM solvent, the charge density and countercation size have a substantial effect on the stability of the ion pair that would form as the result of the cation interacting with the electron-rich walls of the calix[4]pyrrole "bowl". Such effects would be masked to a greater extent in a more highly solvating solvent and hence would not necessarily be reflected in observable changes in the calculated affinity constant for anion binding, K_a.

Support for the proposal that cations of different size can interact with the calixpyrrole bowl differently comes from solid-state structural analyses of the complexes formed from a range of ostensibly similar chloride anion salts. The crystal structure of the TBA−Cl complex of compound **1** was reported by us in 1996[2] and first revealed the anion-induced cone conformation of the calix[4]pyrrole in the solid state. Crystals of the TMA−Cl and TEA−Cl were prepared by slow evaporation of dichloromethane solutions of the macrocycle in the presence of excess alkylammonium chloride salt. Crystals of the TPA−Cl complex grew as colorless prisms by vapor diffusion of hexanes into a dichloromethane solution containing the complex. The crystal structures were elucidated and are shown in Figure 2 (together with the original TBA−Cl complex). Interestingly, the structures show two distinct modes of cation inclusion in the solid state. In the cases of cations containing an odd number of atoms in each alkyl chain (TMA and TPA), one methyl group from the cation is directed into the calixpyrrole cavity. However, in the cases of the TEA−Cl and TBA−Cl complexes, two of the methylene CH_2 groups from the cation are oriented into the cavity. The space filling view shown in Figure 3 illustrates the fit of the TEA cation in the cavity of the macrocycle. The degree of encapsulation of the cation into the macrocycle can be quantified to some degree by measuring the distance between the centroid defined by the four nitrogen atoms in the macrocycle and the nitrogen atom in the cation. In the cases of the TMA, TEA, TPA, and TBA−Cl salts, respectively, these distances were found to be 3.906, 4.361, 6.214, and 4.445 Å.

Crystals of the TEP−Cl and TBP−Cl complexes of compound **1** were obtained by vapor diffusion of hexanes in a dichloromethane solution of the complex (Figure 4). Both complexes crystallized as the dichloromethane solvate and are isostructural with their analogous tetraalkylammonium chloride complexes described above and in a previous report.[2] In these cases, the distances between the centroid defined by the four nitrogen atoms of the macrocycle and the phosphorus atom in the cation were found to be 4.524 and 4.546 Å for **1**−TEP−Cl and **1**−TBP−Cl, respectively.

Taken in concert, the various X-ray crystal structures presented here clearly show differing degrees of encapsulation

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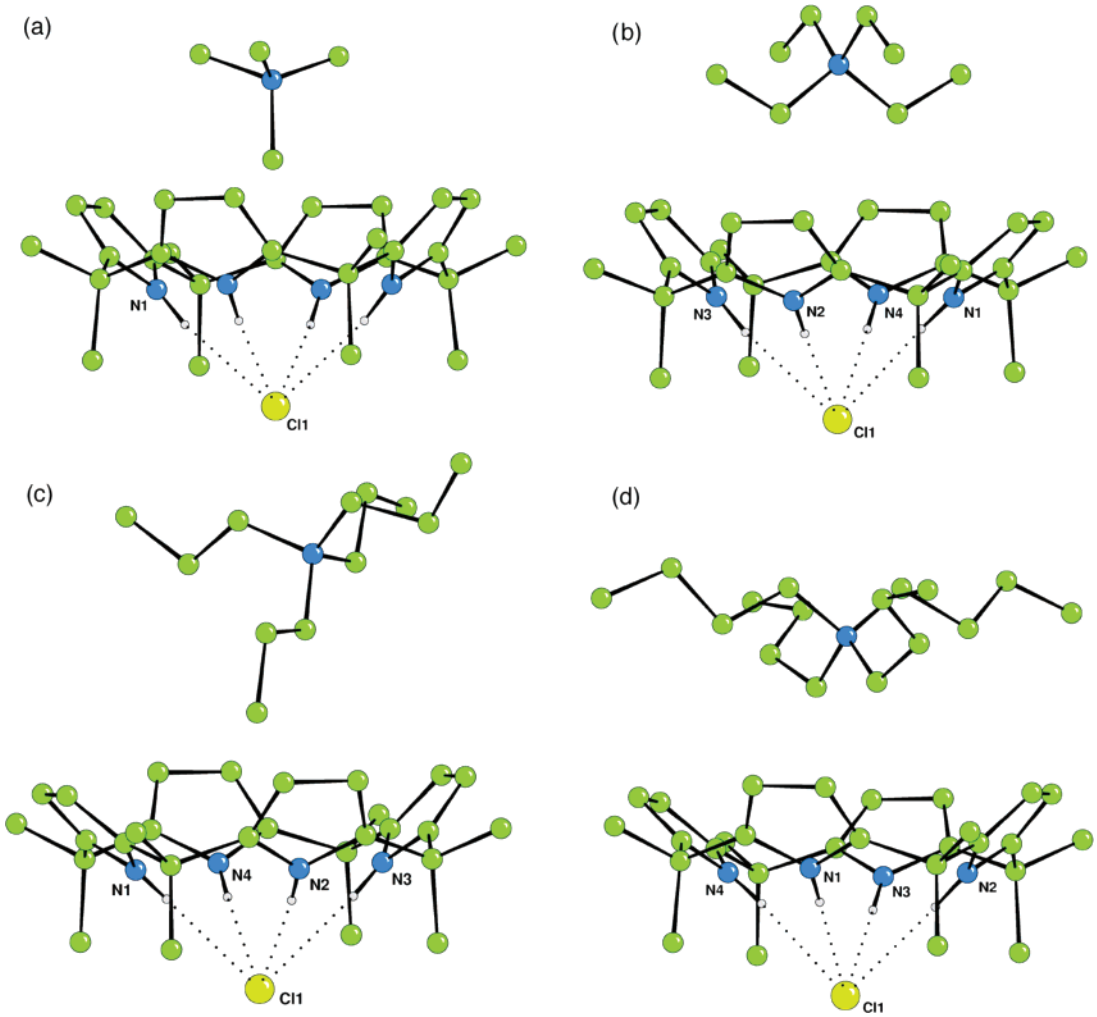

Figure 2. The X-ray crystal structures of complexes of *meso*-octamethylcalix[4]pyrrole with (a) tetramethylammonium chloride, (b) tetraethylammonium chloride, (c) tetrapropylammonium chloride, and (d) tetrabutylammonium chloride. Nonacidic hydrogen atoms (and in the cases of (b), (c), and (d) dichloromethane) have been omitted for clarity.

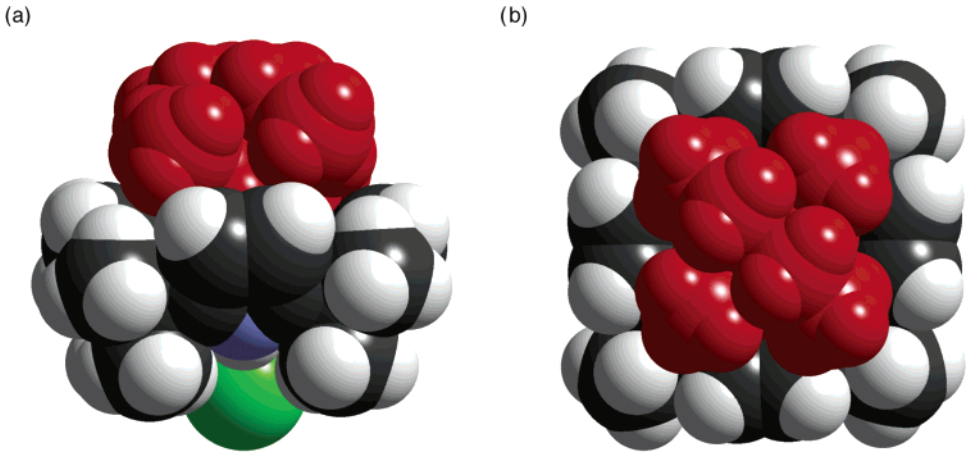

Figure 3. (a) Side and (b) top views of a space filling representation of the X-ray crystal structure of **1**−TEA−Cl illustrating inclusion of the cation in the anion-induced calixpyrrole cup. Cation is rendered in red.

of the cation into the anion-induced calixpyrrole cup-shaped cavity in the solid state. As such, they provide support for the notion that ion pairing, and hence countercation-derived effects, could be important under at least some solution-phase conditions. To the extent this is true, it is important to view calix[4]pyrrole **1**, not as a pure anion binding agent, but as a potential ion-pair receptor. Under these conditions, eq 2 with all its attendant complexities must be used when considering what is ostensibly a clean 1:1 binding equilibrium.

Conclusion

The initial findings of the variation of the anion stability constants of *meso*-octamethylcalix[4]pyrrole with solvent presented something of a mystery. The increase in stability

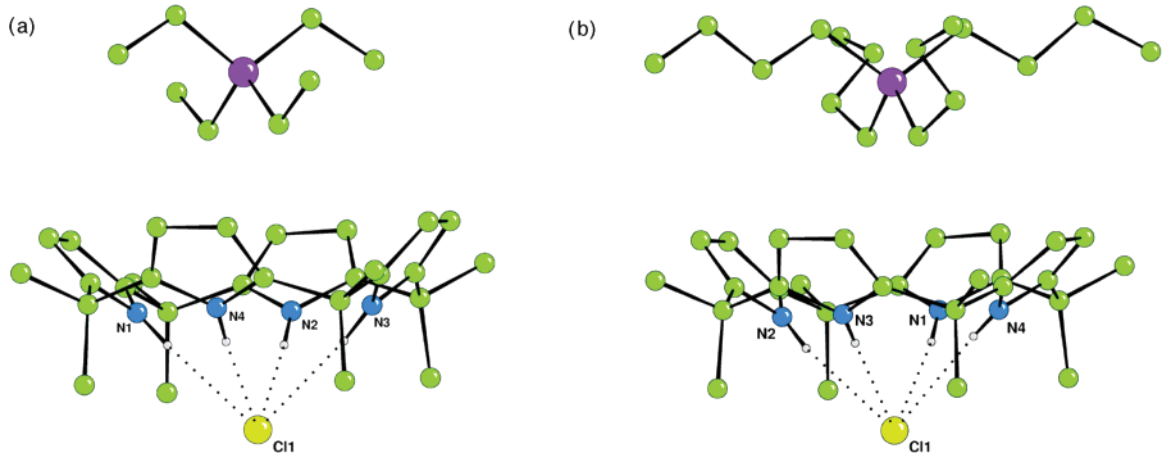

Figure 4. The X-ray crystal structures of (a) TEP−Cl and (b) TBP−Cl complexes of compound **1**. Nonacidic hydrogen atoms and solvent have been omitted for clarity.

constants when going from dichloromethane-d_2 to acetonitrile-d_3 solution was unexpected and prompted us to study, in more detail, the anion complexation processes occurring in solution. We have demonstrated through the use of NMR and ITC analyses that the interactions of calix[4]pyrrole **1** with a variety of chloride salts are dependent on solvent. While substantive differences are seen in terms of the binding affinities, there is no apparent correlation between the observed binding behavior and the permittivity, refractive index (polarizability), dielectric constant, donicity, or acceptor strength of the solvent. On the other hand, the effects of countercation, while generally small, can in certain instances be large, with this being especially true in the case of dichloromethane. Thus, the seeming disparity between the original K_a values reported by the UT group using TBA−Cl in DCM and the subsequent studies involving the use of a cryptand salt in acetonitrile can be rationalized in terms of both a change in the solvent used for analysis and the choice of a countercation, TBA, that is not well behaved in DCM in terms of providing for a clean 1:1, anion + receptor binding process in accord with the simplified binding equilibrium of eq 1. In light of this conclusion, it is recommended that anion binding studies involving new receptors be carried out in several different solvents and with several different countercations before a detailed understanding of the anion binding properties or receptor-based selectivities is claimed. Another important conclusion to emerge from this study is that, at least within the appropriate sensitivity range where reliable data can be collected (ca. $10 < K_a < 10^4$ M^{-1} for NMR; $10^2 < K_a < 10^7$ M^{-1} for ITC), the results of NMR and ITC analyses are generally concordant, even though these two methods probe different aspects of the binding interaction (magnetic effect on selected proton signals and changes in overall system energetics, respectively). Such agreement provides reassurance that much of the data reported in the anion receptor literature can be considered with confidence, at least subject to the caveats that the effects of solvent, countercation, and other non-first-order effects are not introducing unexpected biases.

Experimental Section

Extensive efforts were made to exclude moisture from the prepared solutions because the presence of water could serve to reduce the strength of the presumed receptor−anion interactions, thus reducing the value of calculated binding affinities. Therefore, all salts were either recrystallized and/or dried in a vacuum oven at 40 °C overnight. TEA−Cl, TPA−Cl, TBA−Cl, TBP−Cl, and TPhP−Cl (Fluka) and TEP−Cl (Aldrich) were all greater than 98% purity. The solutions used for ITC experiments were made from freshly opened, dry solvents (<50 ppm H_2O) packaged in sure seal bottles over molecular sieves (Fluka). All solutions for use in ^{1}H NMR spectroscopic titrations were prepared from freshly opened ampoules of deuterated solvent (Cambridge Isotopes). In general, the ITC titrations were carried out at 25 °C, but a representative number were carried out 22 °C so as to permit direct comparisons with studies carried out by NMR.

Microcalorimetric Titrations. Both VP-ITC and MCS-ITC instruments made by MicroCal were used to determine the molar enthalpy (ΔH) of complexation. Subsequent fitting of the data to a 1:1 binding profile using Origin software provided access to the K_a and thus Gibbs free energy (ΔG) which could be used along with ΔH to determine the entropy (ΔS). The actual experiment consisted of filling the sample cell with calixpyrrole solution (Method A) or with chloride solution (Method B), filling the syringe with the matching binding partner and titrating via computer-automated injector. In view of the instability of calixpyrrole solutions, they were prepared fresh each day. Furthermore, the interaction stoichiometry, n, determined experimentally from the fit procedure, was considered a correction factor of the host concentration. This is equivalent to assuming a strict 1:1 binding interaction. On this basis, the experimentally observed enthalpies in Method B were multiplied by n (a value usually in the range of 0.9−1.06) to relate this observable to the concentration of the chloride salt, an input parameter that was considered stable and reliable. This conversion enabled the direct comparison of the enthalpy values derived from the different titration modes, "normal" and "reversed". Blank titrations into plain solvent were also performed and subtracted from the corresponding titration to remove any effect from the heats of dilution from the titrant. The titration experiments were repeated multiple times as noted in the tables, and in all cases, researchers in the separate laboratories (UT and TU) obtained concordant results. In the cases where three or more independent calorimetric titrations were performed, a statistical analysis was performed to determine the random error; these values are reported where relevant. Most titrations were run at 25 °C; however, a few were run at 22 °C to allow direct comparison to NMR results. In general, the differences in K_a values determined at 22 and 25 °C were within experimental error.

^{1}H NMR Spectroscopic Titrations. A Varian Mercury 400 MHz NMR spectrometer was used to measure the ^{1}H NMR shifts of the NH proton of the pyrrole. Solutions of ~1 mM **1** were titrated with ~10 mM chloride salt in a ~1 mM solution of **1** at 22 °C. The titration data were plotted Δppm versus concentration of guest and fit to a 1:1 binding

equation developed by Wilcox[17] using the nonlinear curve-fitting procedure in Origin software.

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Supporting Information Available: Exemplary titration plots for ITC and NMR titrations and crystallographic experimental data. This material is available free of charge via the Internet at http://pubs.acs.org.

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