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Theoretical Study of Anion Binding to Calix[4]pyrrole: the Effects of Solvent, Fluorine Substitution, Cosolute, and Water Traces

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Abstract: The binding of different anions to calix[4]pyrrole has been studied by means of molecular dynamics coupled to thermodynamic integration calculations. The effect of different apolar solvents, octafluoro substitution, and the change in binding free energy derived from the presence of cosolute and water traces (the hydrated salt used to introduce the anion in the solution) were examined. Calculations allow us to rationalize the differential binding of ions to calix[4]pyrrole and octafluorocalix[4]pyrrole as well as to predict the behavior in new solvents for which experimental data are not available yet. It is found that both calix-[4]pyrrole and octafluorocalix[4]pyrrole have a dramatic preference for F^- in the gas phase and pure aprotic solvents, but the situation can change dramatically in protic solvents or in the presence of the hydrated cation which is used as cosolute of the anion. Overall, our results provide interesting clues for a better understanding of the process detected experimentally as "binding".

Introduction

Ion binding is a key process in many chemical and biochemical phenomena.[1−9] The design of molecules that bind ions specifically is the subject of intense chemical research,[1−9] partly because of the potential biotechnological application of these *host* molecules as chemosensors.[2−11] Calix[4]pyrrole (Figure 1) and its derivatives are among the most promising molecules currently being explored for the purpose of anion binding.[12−24] Although calix[4]pyrrole itself was originally synthesized in the

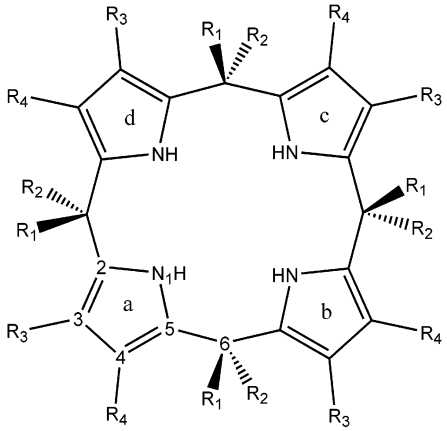

Figure 1. Chemical representation of calix[4]pyrrole.

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(1) Lange, L. G., III.; Riordan, J. F.; Vallèe, B. L. *Biochemistry* **1974**, *13*, 4361.
(2) Lehn, J. M. *Supramolecular Chemistry*; VCH Press: Weinheim, Germany, 1995.
(3) Desvergne, J. P.; Czarnik, A. W. *Chemosensors of Ion and Molecular Recognition*; NATO ASI Series C; Kluwer: Dordrecht, The Netherlands, 1997.
(4) de Silva, A. P.; Guanaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515.
(5) Bianchi, A.; Bowman-James, K.; García-España, E. *Supramolecular Chemistry of Anions*; Willey: New York, 1997.
(6) Schmidtchen, F. P. *Nachr. Chem., Tech. Lab.* **1988**, *36*, 10.
(7) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609.
(8) Jorgensen, W. L. *Chemtracts: Org. Chem.* **1991**, *4*, 91.
(9) Sessler, J. L.; Cyr, M.; Furata, H.; Kral, V.; Tody, T.; Morishima, T.; Shionoya, M.; Weghorn, S. *Pure Appl. Chem.* **1993**, *65*, 393.
(10) Beer, P. D. *Chem. Commun.* **1996**, 689.
(11) Lavigne, J. J.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1999**, *38*, 3666.
(12) Baeyer, A. *Ber. Dtsch. Chem. Ges.* **1866**, *19*, 214.
(13) Gale, P. A.; Sessler, J. L.; Král, V.; Lynch, V. *J. Am. Chem. Soc.* **1996**, *118*, 5140.
(14) Gale, P. A.; Sessler, J. L.; Allen, W. E.; Tvermoes, N. A.; Lynch, V. *Chem. Commun.* **1997**, 665.
(15) Gale, P. A.; Sessler, J. L.; Král, V. *Chem. Commun.* **1998**, 1.
(16) Sessler, J. L.; Gale, P. A.; Genge, J. W. *Chem—Eur. J.* **1998**, *4*, 1095.
(17) Anzenbacher, P.; Jursíková, K.; Sessler, J. L. *J. Am. Chem. Soc.* **2000**, *122*, 9350.

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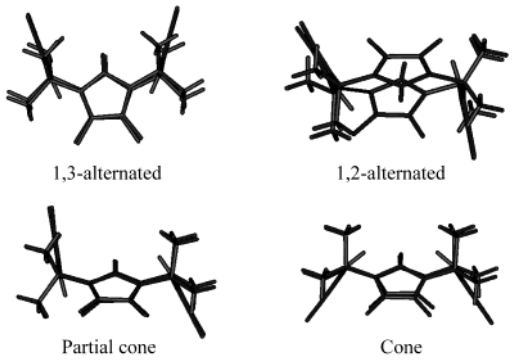


Figure 2. Structures of the major conformations of calix[4]pyrrole.

nineteenth century,[12] it is only in recent years that it has been subjected to detailed study and synthetic modification. Specifically, substituents on both the pyrrole meso-like bridges have been introduced, and extra pyrrole units have been added.[12−24] More recently, calixpyrroles have been modified to yield cryptand-like structures having interesting ligand binding properties.[25]

Calix[4]pyrroles bind a variety of small anions.[12−25] The binding properties of calix[4]pyrroles can be modulated by various structural modifications[14,20] or by the change of solvent.[26] Among the known modified systems, octafluorinated calix[4]pyrrole stands as being particularly interesting. This system shows an increased affinity for small anions, as inferred from ^{1}H NMR, ^{19}F NMR, and fluorescence emission spectroscopic analyses.[19,20] The increase in affinity for the phosphate anion is especially noticeable, since it opens up the possibility of using these compounds to develop specific sensors for this biologically important anion.[20]

Calix[4]pyrroles, like related calix[4]arenes, are very flexible because of rotations around the interpyrrole bonds. The 1,3-alternate conformation (Figure 2) is preferred in vacuum,[27] in the solid state[13,28,29], and in several apolar solvents.[30] Density functional calculations by Wu et al.[27] suggest that the partial cone conformation (Figure 2) is the second preferred form in the gas phase (4.8 kcal/mol less stable than the 1,3-alternate form), but this latter partial cone conformation has not been observed experimentally yet. The 1,2-alternate conformation (Figure 2) is disfavored for the unbound form of the host, but it becomes the main species in the presence of some polar neutral ligands.[28] All experimental data show that the cone conformation (Figure 2) is the most populated form when calix[4]pyrroles form 1:1 complexes with small anions.[13,15]

Despite the impressive amount of recent experimental work on calix[4]pyrroles, many issues remain unclear. For instance, the influences of solvent, traces of water, countercation, cosolutes, and the shape of the conformational space of the unbound form are not well understood. Also unclear are the reasons for the unique binding properties of the octafluorinated derivative or the large specificity for F^- apparently displayed by calix[4]pyrroles under some conditions but not others.[26] These and other unanswered questions have stimulated theoretical studies of these compounds. Thus, Wu et al.[27] have analyzed ion binding to calix[4]pyrrole and made an extensive conformational study of the molecule in the gas phase using density functional theory. They also simulated the influence of apolar solvents by using an electrostatic continuum model.[27] In another study, van Hoorn and Jorgensen analyzed halide anion binding to calix[4]pyrrole in dichloromethane[31] using Monte Carlo and free energy perturbation simulations. More recent work is due to Essex and Gale groups, who analyzed the binding of anions to a modified calix[4]pyrrole in DMSO using Monte Carlo simulations and WHAM analysis.[32]

In an effort to complement and extend these previous theoretical studies,[27,31] we present here a systematic theoretical study focused on anion binding to calix[4]pyrrole and its octafluorinated derivative in different solvents. Also examined is the effect of what we are calling the cosolute, namely the countercation, and traces of water (that could come from the solvent or a hydrated salt used to introduce the anion into solution). Calculations show that both hosts have a great intrinsic preference for F^- in the gas phase and pure aprotic solvents, but the situation changes in the presence of the cosolute trihydrated tetrabutylammonium ($TBA(H_2O)_3^+$), which strongly stabilizes the "unbound" form of F^-, leading to an apparent decrease in the relative affinity of calix[4]pyrrole derivatives for this small anion. Our calculations, which combine quantum mechanics, molecular dynamics, and free energy calculations, provide an unexpected alternative view to the process of binding in real experimental conditions.

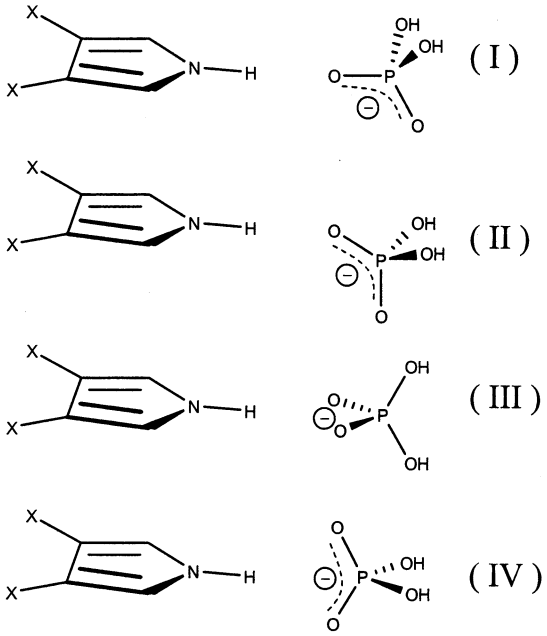
Methods

Quantum Mechanical Calculations. Ab initio calculations were used to estimate the interaction energies of the four anions (F^-, Cl^-, Br^-, and $H_2PO_4^-$) with calix[4]pyrrole (R1 = R2 = Me, R3 = R4 = H; Figure 1) and octafluorocalix[4]pyrrole (R1 = R2 = Me, R3 = R4 = F; Figure 1). Calculations were performed at the MP2/6-31+G(d,p) level using MP2/6-31G(d) optimized geometries on model systems containing one anion and pyrrole or 3,4-difluoropyrrole. For the phosphate anion, four different orientations of the anion were considered (Scheme 1). In all cases, the total interaction energy was corrected from basis set superposition error.[33]

Generalized Molecular Interaction Potential calculations including polarization correction (GMIPp)[34,35] were performed to estimate the relative magnitude of dispersion−repulsion, electrostatic, and polarization energy components of the interaction energies of ions with calix[4]pyrrole and octafluorocalix[4]pyrrole. Calculations were performed using a negative charge as a probe, MP2/6-31+G(d,p) geometries, HF/6-31G(d) wave functions, and the reduced models for calix[4]pyrrole and octafluorocalix[4]pyrrole noted above.

(18) Sessler, J. L.; Anzenbacher, P.; Miyaji, H.; Jursíková, K.; Bleasdale, E. R.; Gale, P. A. *Ind. Eng. Chem. Res.* **2000**, *39*, 3471.
(19) Sessler, J. L.; Anzenbacher, P.; Shriver, J. A.; Jursíková, K.; Lynch, V. M.; Marquez, M. *J. Am. Chem. Soc.* **2000**, *122*, 12061.
(20) Anzenbacher, P.; Try, A. C.; Miyaji, H.; Jursíková, K.; Marquez, M.; Sessler, J. L. *J. Am. Chem. Soc.* **2000**, *122*, 10268.
(21) Miyaji, H.; Sato, W.; Sessler, J. L. *Angew. Chem., Intl. Ed.* **2000**, *39*, 1777.
(22) Miyaji, H.; Anzenbancher, P.; Sessler, J. L.; Blesdale, E. R.; Gale, P. A. *Chem. Commun.* **1999**, 1723.
(23) Turner, B.; Shterenberg, A.; Kapon, M.; Suwinska, K.; Eichen, Y. *Chem. Commun.* **2001**, 13.
(24) Anzenbacher, P.; Jursíková, K.; Lynch, V. M.; Gale, P. A.; Sessler, J. L. *J. Am. Chem. Soc.* **1999**, *121*, 11020.
(25) Bucher, C.; Zimmerman, R. S.; Lynch, V.; Sessler, J. L. *J. Am. Chem. Soc.* **2001**, *123*, 9716.
(26) Schmidtchen, F. P. *Org. Lett.* **2002**, *4*, 431.
(27) Wu, Y. D.; Wang, D. F.; Sessler, J. L. *J. Org. Chem.* **2001**, *66*, 3739.
(28) Allen, W. E.; Gale, P. A.; Brown, C. T.; Lynch, V. M.; Sessler, J. L. *J. Am. Chem. Soc.* **1996**, *118*, 12471.
(29) Jacoby, D.; Floriani, C.; Chiesi-Villa, A.; Rizzoli, C. *J. Chem. Soc., Chem. Commun.* **1991**, 790.
(30) Blas, J. R.; Luque, F. J.; Márquez, M.; Orozco, M. To be published.
(31) van Hoorn, W. P.; Jorgensen, W. L. *J. Org. Chem.* **1999**, *64*, 7439.
(32) Woods, C. J.; Camiolo, S.; Light, M. E.; Coles, S. J.; Hursthouse, M. B.; King, M. A.; Gale, P. A.; Essex, J. W. *J. Am. Chem. Soc.* **2002**, *124*, 8644.
(33) Boys, S. F.; Bernardi, F. *Mol. Phys.* **1989**, *214*, 15.
(34) Luque, F. J.; Orozco, M. *J. Comput. Chem.* **1998**, *19*, 866.
(35) Cubero, E.; Luque, F. J.; Orozco, M. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 5976.

Scheme 1



Force Field Parametrization. The cone conformations of calix[4]-pyrrole and octafluorocalix[4]pyrrole were optimized at the B3LYP/6-31G(d) level.[36] The final conformations were used to derive atomic electrostatic charges for both molecules at the HF/6-31G(d) level using the RESP procedure.[37] Bonded and van der Waals parameters for calix-[4]pyrrole and octafluorocalix[4]pyrrole were transferred from similar groups in the AMBER-99 force field.[38] Force field parameters for dichloromethane, methanol, and DMSO were taken from the OPLS force field.[39] Force field parameters for acetonitrile were taken from a previous parametrization work.[40] van der Waals hardness values (ϵ) for halogens were taken from the OPLS force field,[39] but van der Waals radii had to be refined to reproduce ab initio interaction geometries and energies (see Figure 3) for the different halogen−pyrrole complexes. Finally, charges for H_2PO4^- were obtained using the RESP strategy and HF/6-31G(d) wave functions, and van der Waals parameters were transferred from the AMBER-99 force field.[38] No extra refinement of the van der Waals parameters of the phosphate was necessary to reproduce ab initio data.[41]

System Setup. As a first step, we defined 16 "unbound" systems corresponding to the four anions in four solvents: CH_2Cl_2, CH_3CN, DMSO, and CH_3OH. These "unbound" systems were defined by placing a single anion in a Monte Carlo preequilibrated cubic box of solvent.[42] The simulated system included from 230 (CH_3CN) to 837 (CH_3OH) solvent molecules, which correspond to cubic boxes from 43 000 to 90 000 Å^3. The systems were optimized for 4000 cycles (2000 cycles of steepest descent + 2000 cycles of conjugated gradient), thermalized (298 K), and equilibrated for 500 ps of MD simulations at constant temperature (T = 298 K), and pressure (P = 1 atm). All simulations were monitored showing good convergence in terms of temperature, potential energy, and densities, among other properties. The equilibrated systems were used as starting geometries for free energy calculations described below.

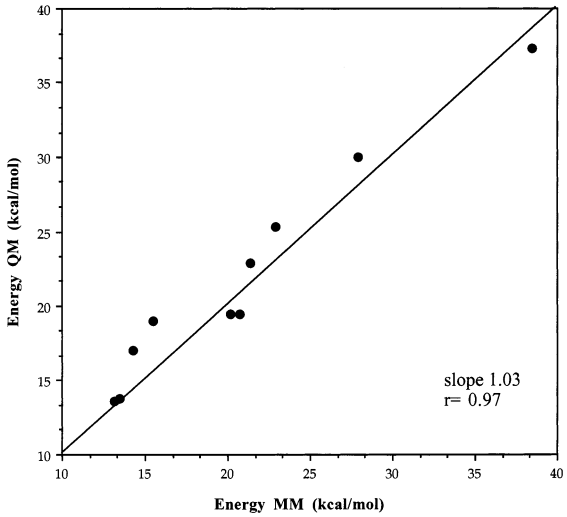


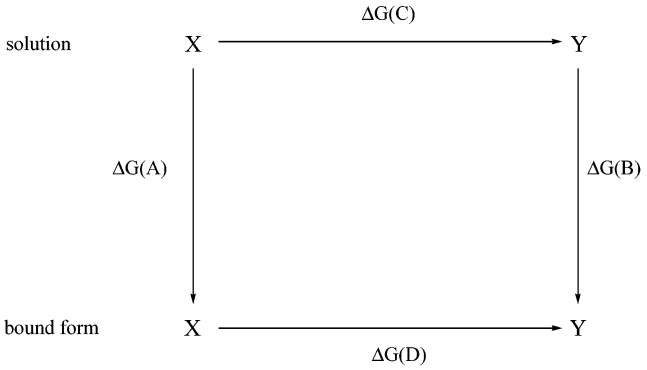
Figure 3. Representation of MM vs QM interaction energies for different complexes of F^-, Cl^-, Br^-, and $H_2PO_4^-$ with pyrrole and 4,5-difluoropyrrole after refinement of the van der Waals radii of the three halides (see ref 41). All values are in kcal/mol.

To analyze the effect of $TBA(H_2O)_3^+$, the cation used to introduce the different anions, on the anion binding, additional "unbound" systems containing each of the four anions together with $TBA(H_2O)_3^+$ were considered. Systems were solvated, optimized, thermalized, and equilibrated for 500 ps of MD simulations (200 ps with harmonic restrains keeping the salt complex bound and 300 ps without restrains). At the end of the MD simulations, direct anion−cation contacts were found only for the F^- anion, which suggests that the effect of the cosolute on anion binding should be moderate for the other systems. As noted before, the final systems were used as starting points for free energy calculations.

To further dissect the effects of the salt and the hydration water in dimerization, calculations for the $F^- \rightarrow Cl^-$ mutation were repeated in aprotic solvents considering $TBA(H_2O)_3^+$ by zero to six water molecules, as well as for a system consisting of just one to six hydration water molecules (without the cation).

Next, "bound" systems corresponding to calix[4]pyrrole or octafluoro-calix[4]pyrrole bound to each anion in each solvent were defined, as were 8 control systems defined for the four ions and the two hosts in the gas phase. The cone conformations found in B3LYP/6-31G(d) optimizations were used as starting conformations of the hosts, and the anions were placed at the optimum positions and orientations ($H_2PO_4^-$) predicted by Poisson−Boltzmann calculations as implemented

(36) Lee, C., Yang, W.; Parr, R. G. *Phys. Rev. B* **1998**, *37*, 785.
(37) Bayly, C. I.; Cieplak, P.; Cornell, W. D.; Kollman, P. A. *J. Phys. Chem.* **1993**, *97*, 10269.
(38) Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 5179.
(39) Jorgensen, W. L.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1988**, *110*, 1657.
(40) Guàrdia, E.; Pinzón, R.; Casulleras, J.; Orozco, M.; Luque, F. J. *Mol. Simul.* **2001**, *26*, 287.
(41) Optimized van der Waals parameters (R^* in Å, ϵ in kcal/mol) for halogens are the following: F^- (1.33, 0.72), Cl^- (2.18, 0.12), Br^- (2.32, 0.09). The radii used here are ∼0.2−0.3 Å shorter than those typically used to simulate halogens in aqueous solution (like the OPLS parameters). Energy calculations using standard OPLS parameters lead to a sizable underestimation of pyrrol−halogen interaction and to an overestimation of the halogen−pyrrol distance. Thus, classical calculations using the OPLS force field for halogens lead to a systematic underestimation of ∼4−6 kcal/mol in the stabilization energy of the halogen−pyrrol (or 3,4-difluoropyrrole) complex and to an overestimation of ∼0.3 Å of the equilibrium distance. Since the deviation between QM and OPLS values is similar for all halogens, the change of van der Waals radii does not have an impact in the relative free energy of binding of halogens but can be important for the description of the preferential binding of halogens relative to other ions such as the phosphate, which are described with van der Waals parameters which lead to an accurate description of the ion−pyrrole interaction (0.5 (pyrrole) and 0.9 (3,4-difluoropyrrole) kcal/mol difference between classical and QM calculations).
(42) Different authors have suggested that phosphate exists mostly as a dimer in solution (Chu, F.; Flatt, L. S.; Anslyn, E. V. *J. Am. Chem. Soc.* **1994**, *116*, 4194), but this implies the existence of a monoanionic complex, with one phosphate and one phosphoric acid. The protonation of the phosphate anion is not likely to occur in the aprotic systems simulated here, and molecular dynamics simulations have shown that the phosphate−phosphate dimer is unstable in all the solvents studied here. Accordingly, the unbound form of the phosphate considered here is that corresponding to the isolated anionic compound.



$\Delta\Delta G(\text{binding}) = \Delta G(A)-\Delta G(B)= \Delta G(C)-\Delta G(D)$

$\Delta\Delta G(\text{sol}) = \Delta G(C)$

$\Delta\Delta G(\text{int}) = \Delta G(D)$

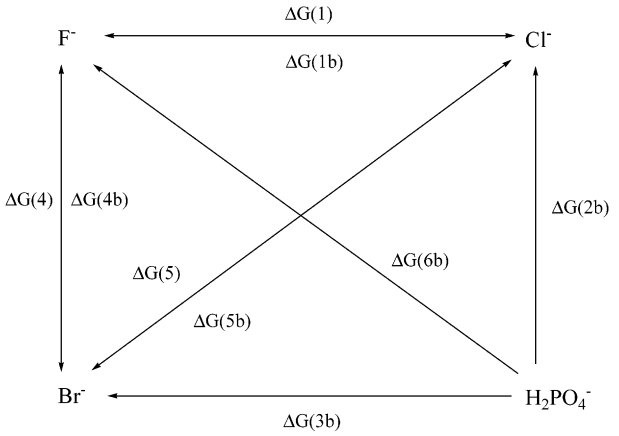
Figure 4. Thermodynamic cycle used to compute differences in the binding free energy between two ions.

in the cMIP program.[43] These complexes were immersed when necessary in Monte Carlo preequilibrated cubic boxes of solvent containing from 230 (CH_3CN) to 837 (CH_3OH) solvent molecules. These starting systems were optimized for 4000 cycles (2000 cycles of steepest descent + 2000 cycles of conjugated gradient), thermalized (298 K), and preequilibrated for 200 ps using harmonic restrains to ensure the stability of the anion−host complex. Finally, all restrictions were removed, and the systems were fully relaxed through 500 ps of MD simulations at constant pressure and temperature ($P = 1$ atm, $T =$ 298 K). In all the cases, the anion remained bound in the central position of the host, which in all instances retained the cone conformation.

Free Energy Calculations. The relative anion binding affinities of calix[4]pyrrole and octafluorocalix[4]pyrrole were determined by using thermodynamic integration (TI) coupled to molecular dynamics simulations (MD-TI) and standard thermodynamic cycles (Figure 4). For this purpose, the anions in question (F^-, Cl^-, Br^- and $H_2PO_4^-$) were mutated by coupling their Hamiltonians to a mutation variable λ which is changed from 0 to 1 along the mutation X → Y (see eq 1) in both unbound and bound states for the two hosts in the different solvents. All possible mutations between the four ions (in the bound and unbound states) were performed in both "forward" and "reverse" directions (Figure 5), with the exception of mutations involving $H_2PO_4^-$ (calculations always started from this ion, since it is easy to annihilate a branched structure but very difficult to create one from a sphere because of technical problems with the holonomic constrains). The combination of the individual free energies for all the mutations provided us with 11−13 independent estimates of the difference in the free energies of solvation or free energies of interaction for each pair of ions (see one example in Figure 5). This lengthy and tedious strategy allowed us the following: (i) to estimate the difference in the binding free energies between ions with a very good statistical quality and (ii) to verify the statistical goodness of the individual mutations by closing many futile cycles (see Figure 5), where *X* and *Y* are the two ions which are mutated.

$$V_\lambda = (1 - \lambda)V_X + \lambda V_Y \qquad (1)$$

Mutations in solution in the presence of the cosolute $TBA(H_2O)_3^+$ were performed always starting from the F^- anion, which is bound more strongly to the cosolute. All the mutations from $H_2PO_4^-$ were performed, considering also nonbonded intramolecular terms, which allowed us to include free energy contributions to binding arising from geometrical distortions. Accordingly, mutations from $H_2PO_4^-$ to another




```
ΔG(1)                    =
-ΔG(1b)                  =
ΔG(4)+ΔG(5)              =
ΔG(4)-ΔG(5b)             =
-ΔG(4)+ΔG(5)             =
-ΔG(4)-DG(5b)            =
-ΔG(6b)+ΔG(2b)           =
ΔG(4)-ΔG(3b)+ΔG(2b)      =
-ΔG(4b)-ΔG(3b)+ΔG(2b)    =
-ΔG(6b)+ΔG(3b)+ΔG(5)     =
-ΔG(6b)+ΔG(3b)-ΔG(5b)    = ΔG(F⁻->Cl⁻)
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Figure 5. All possible mutation pathways between the four anions. The different ways to compute an individual free energy difference ($\Delta G(1)$) are displayed.

anion in either bound or unbound systems were corrected using the corresponding mutation for the unbound state in the gas phase.

Mutations in both bound (with and without cosolute) and unbound states were performed from the previously equilibrated MD systems (see earlier) in 21 windows consisting of 10 ps of equilibration and 10 ps of averaging for a total of 420 ps of MD simulation at constant pressure and temperature ($P = 1$ atm, $T = 298$ K). This means that the differences in solvation/interaction free energies were obtained from a total of 4.6−5.5 ns of 11−13 different MD trajectories. Simulations for the complexes in vacuo were performed following the same protocol noted above.

All the MD and MD/TI trajectories were performed using periodic boundary conditions and a residue-based cutoff of 12 Å. All bond lengths were kept fixed at their equilibrium distance using SHAKE,[44] which allowed us to use an integration step of 2 fs.

Computational Details. Preequilibration of solvent boxes (see earlier) was performed using the BOSS4.2 computer program.[45] Solvent boxes were equilibrated for 10 million configurations at constant pressure and temperature (1 atm, 298 K) using a residue-based cutoff of 10 Å. Molecular dynamics and thermodynamic integration calculations were carried out using the AMBER-5.1 computer program.[46] Poisson−Boltzmann calculations were performed using the cMIP program.[43] Quantum mechanical computations were carried out using the Gaussian-98 computer program.[47] All calculations were performed on the supercomputers of the CESCA as well as on SGI workstations.

Results and Discussion

Molecular Dynamics Simulations. The structures of the complexes between the anion and calix[4]pyrrole or octafluoro-

(43) Gelpí, J. L.; Kalko, S.; de la Cruz, X.; Barril, X.; Cirera, J.; Luque, F. J.; Orozco, M. *Proteins: Struct., Funct., Genet.* **2001**, *45*, 428.

(44) Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C. *J. Comput. Phys.* **1977**, *23*, 327.

(45) Jorgensen, W. L. BOSS 4.2 computer program; Yale University: New Haven, CT, 2000.

(46) Case, D. A.; Pearlman, D. A.; Caldwell, J. W.; Cheatham, T. E.; Ross, W. S.; Simmerling, C. L.; Darden, T. A.; Merz, K. M.; Stanton, R. V.; Cheng, A. L.; Vincent, J. J.; Crowley, M.; Ferguson, D. M.; Radmer, R. J.; Seibel, G. L.; Singh, U. C.; Weiner, P. K.; Kollman, P. A. *AMBER-5*; University of California: San Francisco, CA, 1997.

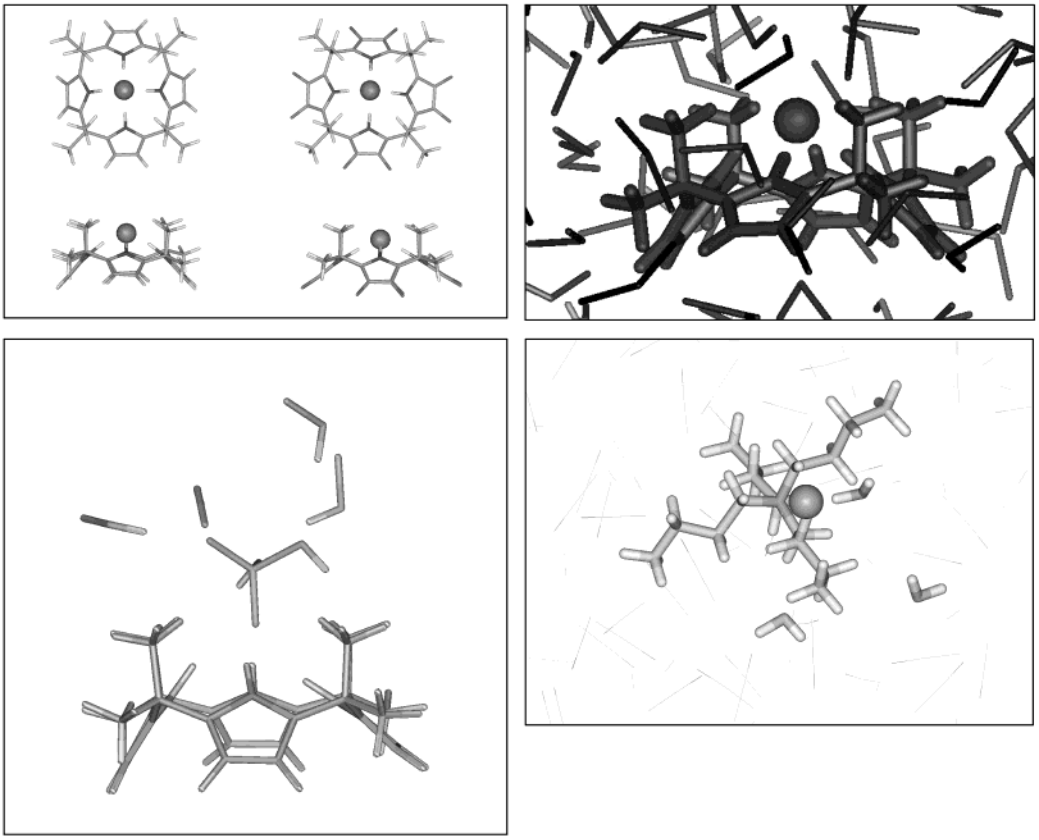

Figure 6. Selected snapshots showing representative configurations of the chloride−octafluorocalix[4]pyrrole complex in the gas phase (top left), fluoride−calix[4]pyrrole complex in CH_2Cl_2 (top right), phosphate−calix[4]pyrrole complex in MeOH (bottom left), and fluoride−$TBA(H_2O)_3^+$ system in acetonitrile (bottom right).

calix[4]pyrrole remained quite stable during all the simulations, sampling regions of the configurational space typical of the cone conformation (see Figure 6 for examples). In particular, no transitions to partial cone conformations were detected, as noted in the fact that the four dihedral angles N1x−C2x−C6y−C5y (for x = a,b,c,d and y = d,a,b,c; see Figure 1) in the range 70−80 degrees in 95−99% of the trajectory. No large differences in terms of flexibility are found, depending on the nature of the host, the solvent, or the bound ion, as noted in the results of principal component analysis of the structures collected along the simulations (data not shown).

As expected from our gas phase calculations and previous quantum mechanical calculations,[48] the distance between the pyrrole nitrogens and the anions depends on the size of the anion (from 2.6 Å for F^- to 3.4 Å for Br^-). The ion−host distance is not much influenced by the solvent and changes very slightly depending on the host, it being ∼0.05−0.1 Å shorter for octafluorocalix[4]pyrrole. Analysis of the pyrrole−pyrrole distance (measured as the average N−N distance) shows no major differences, depending on the host, solvent, or anion, though complexes with F^- have slightly smaller (∼0.1 Å) 1,3-interpyrrole distances than those of the others. The distances between 1,3-pyrroles and 1,2-pyrroles are in the range 4.6−4.9 Å and 3.3−3.4 Å, respectively, in agreement with X-ray values.[20] Finally, the dipole moment of the cone structure is 1.3−1.5 D for calix[4]pyrrole and 2.9−3.3 D for octafluorocalix-[4]pyrrole, irrespective of the solvent or anion. The larger dipole of the latter host accounts for its better binding properties (see later). Finally, it is worth noting that the standard errors in anion−host and interpyrrole distances are typically 0.1 Å and in the dipoles are generally 0.2D, irrespective of the host, anion, or solvent. These findings reveal the rigidity of the bound complexes, something that was already inferred from the dihedral analysis given above.

Free Energy and QM Calculations in the Gas Phase. The free energy profiles for the different mutations $X \leftrightarrow Y$ were smooth without any discontinuity. Further, there was generally good agreement between the "forward" ($X \rightarrow Y$) and "reverse" ($Y \rightarrow X$) estimates, indicating the lack of important hysteresis effects. Individual mutation free energy values computed from 11 to 13 independent estimates (see Methods and Figure 5) are similar, as noted in the standard error of the averages (the standard error varies between 0.1 and 0.6 kcal/mol, i.e., ∼1% of the free energy estimate). Finally, as noted in Figure 5, it is possible to define five futile cycles for each environment (gas phase and four solvents) and host (calix[4]pyrrole and octafluorocalix[4]pyrrole). The 50 futile cycles are closed with an average root-mean-square deviation (rmsd) of only 0.4 kcal/

(47) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.7; Gaussian, Inc.: Pittsburgh, PA, 1998.
(48) Pichierri, F. *THEOCHEM* **2002**, *581*, 117.

Table 1. Differences of the Free Energies of Binding (note that in the gas phase this is equivalent to the difference in free energy of interaction) of Different Ions to Calix[4]pyrrole (Cal) and Octafluorocalix[4]pyrrole in the Gas Phase

mutation[a]	$\Delta\Delta G_{binding}$(Cal)[b,c]	$\Delta\Delta G_{binding}$(8FCal)[b,c]
$Cl^- \rightarrow F^-$	-32.4 ± 0.4	-41.8 ± 0.5
	(-38.6 ± 4.8)	(-53.5 ± 4.5)
$Cl^- \rightarrow Br^-$	3.8 ± 0.4	5.7 ± 0.7
	(5.3 ± 4.3)	(6.7 ± 4.9)
$Cl^- \rightarrow H_2PO_4^-$	-1.3 ± 0.4	-3.7 ± 0.6
	(-1.1 ± 4.1)	(-0.3 ± 5.4)

[a] Cl^- is used as reference in all the cases. [b] A positive value means that the binding of Cl^- is preferred. Standard errors obtained after averaging over 11−13 individual estimates for each change are displayed. The differences in energies of binding (ion−host interaction energy) computed from the analysis of anion−host interactions in the MD trajectories are displayed in parentheses with their standard errors. [c] As a reference to obtain absolute values of interaction energies, the interaction energies for Cl^- are -45.3 ± 2.0 (calix[4]pyrrole) and -70.0 ± 2.7 (octafluorocalix[4]pyrrole). All the values are in kcal/mol.

Table 2. Interaction Energies between the Four Ions and Pyrrole or 3,4-Difluoropyrrole

ion	pyrrole[b]	3,4-difluoropyrrole[b]
F^-	−30.0	−37.3
Cl^-	−19.0	−25.3
Br^-	−17.0	−22.9
$H_2PO_4^-$ (I)[a]	−13.8	−19.5
$H_2PO_4^-$ (II)[a]	−13.6	−19.5
$H_2PO_4^-$ (III)[a]	−3.1	−9.1
$H_2PO_4^-$ (IV)[a]	−0.5	−6.6

[a]Four orientations of the phosphate ion were considered (see Scheme 1). [b] Calculations were performed at the MP2/6-31+G(d,p)//MP2/6-31G(d) level. All values are in kcal/mol.

mol, and the largest rmsd found was 0.8 kcal/mol (i.e., ∼2% of the magnitude of the individual mutation free energy). When taken together, these findings give us confidence in the high statistical quality of the average free energies used to support the later discussion.

Table 1 shows the relative interaction free energies of the anions to calix[4]pyrrole and octafluorocalix[4]pyrrole in the gas phase (which, for the gas phase, correspond directly to binding free energies). Relative to the case of the chlorine anion, the two hosts clearly prefer to bind F^-, followed by $H_2PO_4^-$, while Br^- binding is slightly less favorable. This result leads to the conclusion that as the size of the anion increases, the strength of the anion−receptor interaction decreases, at least in the absence of other effects (e.g., solvent, cosolutes, etc.). Further, it suggests that the increase in the size of the anion reduces the anion−host interaction. Binding free energy differences agree qualitatively with differences in anion−host interaction energies (including correction for distortion contributions when necessary) obtained by averaging along the MD trajectories (the last 200 ps of the 0.5 ns trajectories were used) of the anion−host systems. This suggests that the differences in binding (interaction) free energies in the gas phase are dominated by the differences in interaction energies.

Ab initio calculations (see Table 2) show that the interaction energy of pyrrole and 3,4-difluoropyrrole is very favorable from an enthalpic point of view. GMIPp calculations show that the electrostatic interaction is the leading term, even the sizable magnitude of polarization effects (data not shown). The analysis of the optimum geometries of the anion−host complexes shows that the shorter the distance between the anion and the pyrrolic hydrogen, the stronger the interaction in the gas phase. Thus, the small size of F^- justifies the strength of the host−F^- interaction (see Tables 1 and 2), and the large size of Br^- or the phosphate group explains its poorer interaction in the gas phase.

Absolute anion−host MD interaction energies (easily derived from results in Table 1) can be compared with the ab initio values for pyrrole and 3,4-difluropyrrole model systems (Table 2). For the halide anions, the interaction energy with the host is ∼2−3-fold larger than the interaction energy with a single pyrrole, which might be due to the existence of geometrical restrictions of the host backbone that hinder the formation of optimum pyrrole−anion interactions. This finding explains why receptors such as dipyrrolquinoxalines, with only two pyrrole groups, are almost as good binders as calix[4]pyrroles.[20,22] The situation differs for $H_2PO_4^-$, since the interaction energy with the host is more than 3 times larger than that with pyrrole (or 3,4-difluoropyrrole). While not conclusive, this finding suggests that the branched structure of $H_2PO_4^-$ fits nicely into the calixpyrrole framework. Presumably, as a consequence of this effect, the interaction between calix[4]pyrrole and $H_2PO_4^-$ in the gas phase is better than that between calix[4]pyrrole and Cl^- (see Table 1), when the opposite was expected from data on isolated monomers (see Table 2).

In summary, ab initio and classical calculations demonstrate that the binding of anions to calyx[4]pyrrole derivatives is a very favorable process driven by electrostatic interactions. The relative binding preference between ions can be in general rationalized in terms of the magnitude of pyrrole−anion electrostatic interactions. Clearly, the situation might change when solvent effects are taken into account, as it is discussed in the following subsection.

Free Energy Calculations in Pure Solvents. Solvation, especially in the most polar solvents, is expected to largely influence the binding free energy, since the "unbound" anion is stabilized by anion−solvent interactions. Solvent effect is expected to have a specific role in favoring the ion specificity of calix[4]pyrroles. Thus, the marked preference of calix[4]-pyrrole for the binding of F^- in CH_2Cl_2[13−15,18,20,22] is apparently absent in DMSO[49] and CH_3CN.[26] To explore the solvent effect on the relative binding free energies of the four anions, we repeated the calculations by using MD-equilibrated systems in different solvents (CH_2Cl_2, CH_3CN, DMSO, and CH_3OH; see Methods). Results are summarized in Tables 3−6.

The preference for solvation is always $F^- > H_2PO_4^- > Cl^- > Br^-$. For halides, the solvation decreases as the size of the ion increases, as expected from Born theory.[50,51] The relatively good solvation energy of $H_2PO_4^-$ should be explained by its electronic distribution which favors interaction with solvent molecules despite its large size. It is worth noting that the same ordering of solvation free energies is obtained from Poisson−Boltzmann calculations and by SCRF computations (data not shown). Interestingly, the difference in solvation free energies between the four anions (i.e., F^-, $H_2PO_4^-$, Cl^-, and Br^-) remains quite constant for three of the solvents analyzed, namely CH_2Cl_2, CH_3CN, and DMSO. This proved true, although these solvents have quite different dielectric constants. On the other hand, in methanol, the preferential solvation of F^- is maximized

(49) Camiolo, S; Gale, P. A. *Chem. Commun.* **2000**, 1129.
(50) Born, M. *Z. Phys.* **1920**, *1*, 45.
(51) Orozco, M.; Luque, F. J. *Chem. Rev.* **2000**, *100*, 4187.

Table 3. Differences in Free Energies of Solvation ($\Delta\Delta G_{sol}$), Free Energies of Interaction ($\Delta\Delta G_{int}$), and Free Energies of Binding ($\Delta\Delta G_{binding}$) to Calix[4]pyrrole (Cal) and Octafluorocalix[4]pyrrole (8FCal) between Cl^- and the Rest of the Ions in CH_2Cl_2

mutation	$\Delta\Delta G_{sol}$[a,b]	$\Delta\Delta G_{int}$(Cal)[a,b]	$\Delta\Delta G_{int}$(8FCal)[a,b]	$\Delta\Delta G_{binding}$(Cal)[a,b]	$\Delta\Delta G_{binding}$(8FCal)[a,b]
$Cl^- \rightarrow F^-$	-18.7 ± 0.1 (-31.1)	-34.5 ± 0.1	-43.6 ± 0.1	-15.8 ± 0.1 (-3.4)	-24.0 ± 0.1 (-7.4)
$Cl^- \rightarrow Br^-$	2.6 ± 0.1 (2.3)	3.9 ± 0.1	5.7 ± 0.2	1.3 ± 0.1 (1.6)	1.6 ± 0.2 (1.1)
$Cl^- \rightarrow H_2PO_4^-$	-0.3 ± 0.1 (-0.4)	0.3 ± 0.1	1.8 ± 0.1	0.5 ± 0.1 (0.7)	2.1 ± 0.2 (2.2)

[a] A positive value means that the solvation, interaction, or binding of Cl^- is preferred. Standard errors obtained after averaging over 11−13 individual estimates for each change are displayed. All the values are in kcal/mol. [b]Values in parentheses are those obtained when the trihydrated tetrabutylammonium cation was present in the "unbound" simulations.

Table 4. Differences in Free Energies of Solvation ($\Delta\Delta G_{sol}$), Free Energies of Interaction ($\Delta\Delta G_{int}$), and Free Energies of Binding ($\Delta\Delta G_{binding}$) to Calix[4]pyrrole (Cal) and Octafluorocalix[4]pyrrole (8FCal) between Cl^- and the Rest of the Ions in CH_3CN

mutation	$\Delta\Delta G_{sol}$[a,b]	$\Delta\Delta G_{int}$(Cal)[a,b]	$\Delta\Delta G_{int}$(8FCal)[a,b]	$\Delta\Delta G_{binding}$(Cal)[a,b]	$\Delta\Delta G_{binding}$(8FCal)[a,b]
$Cl^- \rightarrow F^-$	-12.9 ± 0.1 (-33.4)	-33.0 ± 0.3	-43.6 ± 0.1	-20.1 ± 0.2 (-0.4)	-30.7 ± 0.1 (-10.2)
$Cl^- \rightarrow Br^-$	2.1 ± 0.1 (1.9)	4.4 ± 0.2	5.2 ± 0.2	2.3 ± 0.2 (2.5)	3.1 ± 0.2 (3.3)
$Cl^- \rightarrow H_2PO_4^-$	-0.8 ± 0.1 (-0.5)	0.2 ± 0.2	1.0 ± 0.1	1.0 ± 0.2 (0.7)	1.9 ± 0.2 (1.5)

[a]A positive value means that the solvation, interaction, or binding of Cl^- is preferred. Standard errors obtained after averaging over 11−13 individual estimates for each change are displayed. All the values are in kcal/mol. [b]Values in parenthese are those obtained when the trihydrated tetrabutylammonium cation was present in the "unbound" simulations.

Table 5. Differences in Free Energies of Solvation ($\Delta\Delta G_{sol}$), Free Energies of Interaction ($\Delta\Delta G_{int}$) and Free Energies of Binding ($\Delta\Delta G_{binding}$) to Calix[4]pyrrole (Cal) and Octafluorocalix[4]pyrrole (8FCal) between Cl^- and the Rest of Ions in DMSO

mutation	$\Delta\Delta G_{sol}$[a,b]	$\Delta\Delta G_{int}$(Cal)[a,b]	$\Delta\Delta G_{int}$(8FCal)[a,b]	$\Delta\Delta G_{binding}$(Cal)[a,b]	$\Delta\Delta G_{binding}$(8FCal)[a,b]
$Cl^- \rightarrow F^-$	-12.5 ± 0.1 (-33.7)	-34.1 ± 0.3	-42.8 ± 0.1	-21.7 ± 0.2 (-0.47)	-30.4 ± 0.3 (-9.2)
$Cl^- \rightarrow Br^-$	2.3 ± 0.1 (2.3)	4.5 ± 0.3	4.9 ± 0.3	2.2 ± 0.4 (2.2)	2.6 ± 0.3 (2.6)
$Cl^- \rightarrow H_2PO_4^-$	-3.0 ± 0.2 (-3.4)	-1.7 ± 0.3	-0.1 ± 0.3	1.2 ± 0.4 (1.7)	2.9 ± 0.2 (3.3)

[a] A positive value means that the solvation, interaction, or binding of Cl^- is preferred. Standard errors obtained after averaging over 11−13 individual estimates for each change are displayed. All the values are in kcal/mol. [b]Values in parenthese are those obtained when the trihydrated tetrabutylammonium cation was present in the "unbound" simulations.

Table 6. Differences in Free Energies of Solvation ($\Delta\Delta G_{sol}$), Free Energies of Interaction ($\Delta\Delta G_{int}$) and Free Energies of Binding ($\Delta\Delta G_{binding}$) to Calix[4]pyrrole (Cal) and Octafluorocalix[4]pyrrole (8FCal) between Cl^- and the Rest of the Ions in CH_3OH

mutation	$\Delta\Delta G_{sol}$[a,b]	$\Delta\Delta G_{int}$(Cal)[a,b]	$\Delta\Delta G_{int}$(8FCal)[a,b]	$\Delta\Delta G_{binding}$(Cal)[a,b]	$\Delta\Delta G_{binding}$(8FCal)[a,b]
$Cl^- \rightarrow F^-$	-49.3 ± 0.4 (-49.8)	-39.9 ± 0.2	-47.6 ± 0.1	9.4 ± 0.4 (9.88)	1.7 ± 0.4 (2.2)
$Cl^- \rightarrow Br^-$	4.4 ± 0.4 (3.0)	4.5 ± 0.3	8.0 ± 0.3	0.1 ± 0.5 (1.48)	3.6 ± 0.3 (5.0)
$Cl^- \rightarrow H_2PO_4^-$	-0.6 ± 0.3 (-0.8)	-1.2 ± 0.1	-3.9 ± 0.2	-0.5 ± 0.4 (-0.4)	-3.3 ± 0.2 (-3.1)

[a] A positive value means that the solvation, interaction, or binding of Cl^- is preferred. Standard errors obtained after averaging over 11−13 individual estimates for each change are displayed. All the values are in kcal/mol. [b]Values in parenthese are those obtained when the trihydrated tetrabutylammonium cation was present in the "unbound" simulations.

relative to the other ions, a result that is easily interpreted in terms of the protic nature of this solvent. In any event, when taken together, these findings highlight the fact that not only the dielectric constant of the solvent but also its hydrogen-bond donor/acceptor capabilities have to be taken into consideration when trying to understand the solvation free energies of anions.

As expected from gas phase calculations, the F^- anion give rise to the highest interaction free energy (see Figure 4 for definition of this magnitude) when allowed to react with the two calix[4]pyrrole receptors considered in this study (cf. $\Delta\Delta G_{int}$ values in Tables 3−6), followed by Cl^- and $H_2PO_4^-$, which show similar ΔG_{int} values. The anions with the most negative free energy of solvation are also those that display the most negative anion−host free energy of interaction (see Figure 4). Interestingly, the differences in interaction free energies are generally similar for all the solvents and not far from the gas phase values (see Table 1). This suggests that, in general, solvent interactions with the bound ion are not crucial for the relative stabilization of the complex. The only clear exception to this trend is methanol, since there is a specific stabilization of the complexes with F^- and $H_2PO_4^-$ with respect to those with Cl^- and Br^-. Specific hydrogen bond interactions of methanol molecules with the bound F^- anion or with the oxygen atoms of the bound $H_2PO_4^-$ anion can explain this finding (see Figure 6).

The combination of solvation and interaction free energies allows us (see Methods) to estimate the difference in binding free energies (see Tables 3−6). Previous discussion has pointed

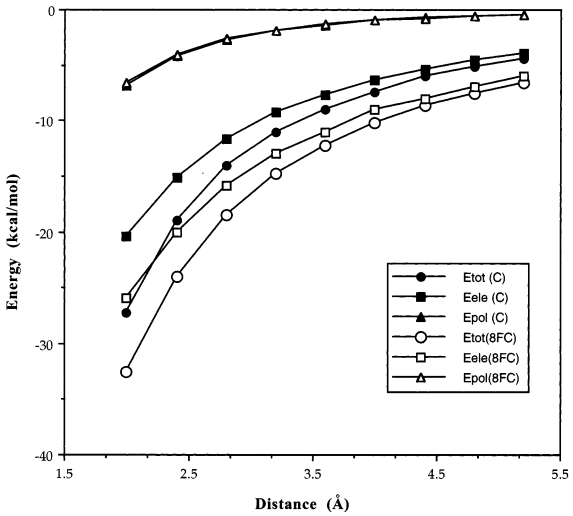

Figure 7. Total GMIPp interaction energies (E_{tot}) and their components (E_{ele} (electrostatic) and E_{pol} (polarization)) for the interaction of a negative charge to models of calix[4]pyrrole (C) and octafluorocalix[4]pyrrole (8FC). See text for details.

out that binding is a competition between the favorable anion−host interaction and the free energy penalty related to desolvation of the interacting molecules, and that both terms are very large and highly correlated (see above). It is worth noting that the desolvation term is large even for very apolar solvents such as CH_2Cl_2, which argues against neglecting the effect of apolar solvents in the binding of charged species.

For all aprotic solvents and for the two hosts, the affinities of the anions follow typically the order $F^- \gg Cl^- \geq H_2PO_4^- > Br^-$. When compared to the gas phase results, the preferential binding of F^- in aprotic solvents is reduced by ∼16 kcal/mol (taken Cl^- as reference) because of the better solvation of the fluorine ion. The preference for $H_2PO_4^-$ versus Cl^- binding in aprotic solvents is reversed with respect to the situation in the gas phase, because of the better solvation of the phosphate ion, which leads to a reduction of the differential binding energy of $H_2PO_4^-$ versus Cl^-. The order of stabilities in methanol is $H_2PO_4^- \geq Cl^- \geq Br^- > F^-$ for calix[4]pyrrole and $H_2PO_4^- > Cl^- > F^- > Br^-$ for octafluorocalix[4]pyrrole. This means that the binding of $H_2PO_4^-$ is largely favored and that of F^- is largely disfavored compared to those of the other anions in a protic solvent like methanol. Analysis of Table 6 and the comparison with Tables 1 and 3−5 show that the predicted shift in the $F^-/H_2PO_4^-$ preference mostly arises from the differential desolvation term of the two anions in methanol.

Though the general qualitative trends of the preferential binding of anions to calix[4]pyrrole and octafluorocalix[4]pyrrole are similar, there are quantitative differences. Thus, ab initio gas phase calculations (Table 2) suggest that the binding of ions to octafluorocalix[4]pyrrole is energetically favored with respect to that to calix[4]pyrrole. The partition of interaction profiles into components (see Figure 7) shows that the electrostatic energy (because of the greater acidity of the pyrrolic hydrogens) is responsible for the preferential interaction of anions with octafluorocalix[4]pyrrole, while other contributions to the interaction energy, like polarization, are the same for the two hosts (see Figure 7). In general, replacement of calix[4]pyrrole by octafluorocalix[4]pyrrole enhances the difference in interaction energies between the ions and the hosts, which leads to an increase in the differences of binding free energies between the ions. This, in turn, leads to the theoretical suggestion that highly fluorinated calix[4]pyrroles would generally represent better platforms than calix[4]pyrrole upon which to build highly specific fluoride anion receptors, sensors, and carriers.

Quantitative comparison of theoretical data with available experimental information is difficult because of the lack of extensive experimental analysis on anion binding in different solvents and the intrinsic errors in free energy calculations involving charged species. Furthermore, the comparison of experimental data is hindered by the fact that a large part is available for derivatives of calix[4]pyrroles modified in the interpyrrole units (i.e., meso-like linkers) by the addition of large groups, including polar moieties which can interact specifically with some ions.[17] Finally, the presence of water as the result of using wet solvents or hydrated anion salts could complicate the interpretation (and perhaps even the reliability) of the experimental values, making comparisons to theory even more difficult[15,17,23]. Nonetheless, comparisons between theory and experiments are valuable and can help to set the stage for future receptor design. In the present instance, they could also help to resolve the contradictory claims of Sessler, who on the basis of studies in dichloromethane has suggested that calix[4]pyrroles are fluoride anion selective anion receptors, and Schmidtchen, who on the basis of studies in acetonitrile claims it is not.

Present theoretical calculations provide unequivocal support for the notion that F^- is the preferred anionic substrate of calix[4]pyrrole in dichloromethane, as determined by Sessler and colleagues[13−15,18,20,22]. It is also clear from experimental measures that Br^- binds less than Cl^- to modified and unmodified calix[4]pyrroles and dipyrrolequinoxalines in dichloromethane,[13,20,22] in good agreement with our theoretical results. On a different level, experimental studies have shown that octafluorocalix[4]pyrrole is a far better anion binding receptor than simple calix[4]pyrrole,[19] as it is suggested here by theoretical calculations.

The present theoretical simulations suggest that Cl^- will be bound by calix[4]pyrroles somewhat more favorably than $H_2PO_4^-$ in aprotic solvent (see earlier). This prediction is more specific than any inference that can be safely drawn from the available experimental data. These data, taken in concert, lead to the inference that these two anions are bound by calix[4]pyrrole receptors with similar affinities, although there may be a slight hint that chloride is in fact bound slightly more strongly (for calix[4]pyrrole, the K_a for Cl^- is ∼350 M^{-1}, whereas that for $H_2PO_4^-$ is ∼100 M^{-1}, as recorded in CD_2Cl_2 using the corresponding tetrabutylammonium salts; see refs 13 and 15). On the other hand, our calculations do show that the difference in the binding free energies of Cl^- and $H_2PO_4^-$ (when interacting with either calix[4]pyrrole) is small, underscoring the fact that changes in the structure (i.e., adding or modifying substituents) or other external effects (such as those involving cosolute; vide infra) could cause the intrinsic chloride-over-dihydrogen phosphate selectivity to be reversed, as has indeed been observed experimentally in dichloromethane and wet acetonitrile.[17,18,20,22−24]

Unfortunately, at present, to our knowledge, no experimental data exists that relates to the binding of F^-, Cl^-, Br^-, and $H_2PO_4^-$ to either calix[4]pyrrole or octafluorocalix[4]pyrrole in methanol. On the other hand, experimental K_a values for F^- and Cl^- binding to calix[4]pyrrole have been measured in

DMSO solution by Gale and co-workers and found to be nearly identical to one another,[49] and similar results are obtained by Scmidtchen and co-workers in CH_3CN.[26] However, in this case, the effect of cosolute (countercation and hydration water) complicates the comparison of experimental and theoretical data (see below).

In summary, theoretical calculations provide a general picture of ligand binding to calix[4]pyrrole and octafluorocalix[4]pyrrole not too different to that obtained experimentally. However, intriguing quantitative discrepancies between some of the affinities predicted by theory and those actually measured experimentally are noted. Specifically, while theoretical calculations are able to predict well the Cl^- versus Br^- preference of calix[4]pyrrole in dichloromethane and acetonitrile (from 0.8 to 2.1 kcal/mol experimentally[13,20,22,23] and from 1.3 to 2.3 kcal/mol theoretically), they fail to reproduce quantitatively the preference for F^- over Cl^- seen in dichloromethane. Thus, experimental measures in dry CD_2Cl_2 imply a preference of only 1−3 kcal/mol[13−15,17,18,20,22−24] in favor of F^- relative to Cl^-, while the theoretical results suggest a much larger difference (see Table 3). The quantitative discrepancy is even larger in the case of DMSO and CH_3CN, where, in marked contrast to the current theoretical predictions (which favor fluoride binding), the available experimental data reveal little (or none) preference for F^- over Cl^-.[26,49] The possible reasons for this discrepancy will be analyzed in detail in the following subsection.

Calculations Including Cosolute Effects. A naive explanation of the discrepancies between theory and calculation is to assume that errors in force field or simulation conditions are responsible for the inability of theoretical methods to reproduce experimental evidence. However, even though this cannot be completely ruled out, we believe this is not likely to be the major reason here, since the simulation protocol seems to be correct (as noted in the statistical quality of the results) and the force field seems accurate, reproducing high level (MP2/6-31+G(d,p)) quantum mechanical calculations (see Figure 3). Furthermore, Jorgensen and co-workers performed accurate theoretical studies of halide binding to calix[4]pyrrole and calix-[4]arenes[31,52] using a different simulation strategy and force field and found the same discrepancies between experiment and theory, namely a higher predicted difference in the F^- over Cl^- preference than measured in CD_2Cl_2 using tetrabutylammonium fluoride and chloride salts. We, thus, feel that the discrepancies between theory and experiment are too large and systematic to rationalize in terms of computational errors. Rather, we think they have their basis in physical phenomena that are not reflected in standard simulations of simple anion−receptor−solvent interactions.

Analysis of the experimental protocol for the measure of ion binding shows that ions are added as $TBA(H_2O)_3^+$ salts, although recently Schmidtchen has used $\{K.[2.2.2]\}^+$ cryptand as a countercation when studying fluoride binding in acetonitrile.[26] Often, such salts are obtained and used in their hydrated forms or after being subject to a drying procedure that is often imprecise in terms of defining final water content. These issues are particularly problematic in the case of tetrabutylammonium fluoride (TBAF). This material, as purchased from commercial sources, is nominally the trihydrate but in at least one instance, the sample denoted as such was found to contain a much larger number of equivalents of water per F^- anion (Anzenbacher et al., unpublished data). In practical terms, this means that, for each molar equivalent of anion introduced into apolar solution during studies of anion affinities, a countercation gets added as do a significant number of water molecules (at least three in the case of fluoride), unless great care is taken. As noted by Jorgensen and co-workers,[31,52] the presence of a small amount of water should hinder the binding of the ion to the hosts, since the ion−water interaction is very strong in apolar media. Furthermore, in apolar media such as those considered here, the tetrabutylammonium countercation can interact with the anions, leading to an apparent (and ion-specific) decrease in the apparent binding free energy of the host. The effects of the countercation and trace water seem also a reasonable explanation to the discrepancy between theoretical and experimental estimates of F^- versus Cl^- binding in DMSO and acetonitrile, as well for the apparent discrepancy between accurate experimental measures of the relative binding of F^- and Cl^- to calix[4]pyrrole in acetonitrile.[20,26]

To get a rough estimate of the effect of the $TBA(H_2O)_3^+$ cosolute in the relative binding of ions, we recomputed the relative solvation free energies of the four ions in the different solvents, introducing three water molecules and the tetrabutylammonium cation in the simulation system. As noted in Methods, we always forced the anion−cation complexation at the beginning of the simulation, but after equilibration, the system was completely free, meaning that the putative cation−anion (ion pair) complex is either formed or does not depend on the entropic/enthalpic characteristics of the process. The resulting relative free energies of solvation (in the presence of the cosolute consisting of three water molecules and one tetrabutylammonium countercation per anion) and the corresponding relative binding free energies are displayed in parentheses in Tables 3−6.[53]

Clearly, the effect of the cosolute in the specificity of the binding of ions to calix[4]pyrroles is very large, since the ability of the ions to interact with the hydrated cation in solution is very different. Thus, in aprotic solvents, F^- is directly bound to its tetrabutylammonium countercation, while the other ions are not typically bound to at least this particular cation. In methanol, no complexes are found for any ion, a finding that is consistent with the protic nature of this solvent.

Since no direct complex with the cation is found for Cl^-, Br^-, and $H_2PO_4^-$, their relative solvation free energies are not altered by the presence of the cosolute, as seen in Tables 3−6. On the contrary, the relative solvation free energy of F^- with respect to the other ions increases (in absolute value) dramatically in the presence of $TBA(H_2O)_3^+$, at least for apolar−aprotic solvents (see Tables 3−6). This leads to a parallel decrease in the binding affinity of F^- with respect to the other ions in aprotic, apolar solvents (see Tables 3−6). In the polar, protic solvent methanol, the introduction of the cosolute does not alter

(52) McDonald, N. A.; Duffy, E. M.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1998**, *120*, 5014.

(53) Molecular dynamics or Monte Carlo simulations in heterogeneous solvents (like those considered in this part of the paper) display an important equilibration process, which limit the accuracy of the calculations. Furthermore, free energy changes associated with mutations of $F^- \rightarrow Cl^-$ are subjected to non-negligible statistical errors related to the dissociation of the ion pair along the mutation. Accordingly, all the free energy calculations reported in this part of the work should be taken with caution, since they might not be as accurate as simulations in pure solvents.

(54) Sessler, J. Unpublished results.

the estimates of the binding free energy, as expected from the lack of direct cation−anion interactions in this solvent.

It is worth noting that the relative F^- versus Cl^- preference for binding in the presence of $TBA(H_2O)_3^+$ is dramatically reduced, and the difference in free energies of binding (see Tables 3−6) agree well now with the range of experimental values. Thus, for CH_2Cl_2, we are predicting that F^- binds ∼3 kcal/mol better to calix[4]pyrrole than Cl^- (see Table 3), a value which agrees with experimental measures[13−15,17,18,20,22−24]. Furthermore, our simulations suggest that the preference for F^- versus Cl^- of calix[4]pyrrole in acetonitrile and DMSO is dramatically reduced in the presence of cosolute ($\Delta\Delta G_{binding} \approx -0.4$ kcal from Tables 4 and 5), in agreement with the experimental evidence in these solvents[20,26,49]. In other words, the apparent quantitative discrepancy between high level theoretical calculations and experimental measures disappears when the effect of the cosolute is roughly taken into account in theoretical calculations. This strongly suggests that, in fact, the process detected experimentally as binding F^- to calix[4]pyrrole is really an exchange reaction, where F^- moves from $TBA(H_2O)_3^+$ to the calix[4]pyrrole derivative. Binding constants, at least for F^-, detected experimentally in aprotic solvents should be then considered as "apparent" binding constants, which would depend on the traces of water and on the cation used to introduce the ion in the apolar solvent. These systems are then paradigmatic models of the importance of cosolute interactions in binding.

Our theoretical calculations provide also a neat explanation of the controversy between Sessler's and Schmidtchen's groups[20,26] on the relative binding of F^- and Cl^- in acetonitrile. Clearly, $\Delta\Delta G_{binding}$ differences in Table 4 strongly suggest that the binding of F^- is preferred in dry acetonitrile if no cosolute is present. However, as noted above, such an ideal system is never studied experimentally, and not only the cation but also traces of water are present in the measures. Theoretical results in Tables 2−6 show that the interfering effect of the cosolute on the predicted binding behavior is not only large but also ion-specific because of the different tendencies of small and large anions to interact with countercations. It is, thus, clear that by changing the nature of the salt or the amount of water, even at trace levels, large changes can be engendered in the experimentally determined anion binding affinities and inferred F^- versus Cl^- preferences. The results of the theoretical calculations summarized in Table 4 lead to the conclusion that when $TBA(H_2O)_3^+$ is considered in the calculations, the preference for F^- over Cl^- is reduced to less than 1 kcal/mol in acetonitrile. Accordingly, it is possible that, by employing different states of hydration or by employing different countercations (e.g., $\{K\bullet[2.2.2]\}^+$ cryptand), this selectivity could be reduced further or even reversed. In other words, on the basis of our theoretical findings, we propose that the differences between Schmidtchen's and Sessler's conclusions are ascribable as much to changes in cosolutes as they are to differences in solvents.

Previous calculations have shown the large impact of hydrated salt in the relative binding free energies of F^- and Cl^-. However, two questions need to be answered: (i) does the situation change when the total number of hydration molecules changes and (ii) which is the major factor responsible for the specific solvation of the F^- anion, the "nude" cation or the hydration waters? To obtain rough, preliminary insights on these two questions, the $F^- \rightarrow Cl^-$ mutations in CH_2Cl_2, acetonitrile, and DMSO were repeated for different definitions of the cosolute: with or without cation and with a number of water molecules ranging from 0 to 6. These types of calculations must be taken with caution, since these calculations are very noisy for the determination of both $\Delta\Delta G_{sol}$ and $\Delta\Delta G_{int}$ (data is shown as Supporting Information). The calculations suggest that the countercation alone has a poor specificity for fluoride, but the introduction of the three or more water molecules leads to a dramatic increase in the ability to bind fluoride compared with other anions. When TBA^+ is not present, there is a continuous stabilization of the fluoride anion when the number of water molecules is above three, suggesting that trace water can have a subtle and complex effect in the relative stabilization of different anions.

Overall, our theoretical results underscore the importance of considering all relevant factors, including solvent, hydration, countercation, concentration, binding stoichiometry, and so forth when making comparisons between different experimental results and drawing global conclusions about the properties of a receptor, the utility of a measurement technique, or, as highlighted by the results presented here, the predictive validity of a given type of theoretical method.

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Supporting Information Available: Figure S1 (charges and AMBER-99 atom type for calix[4]pyrrole, octafluorocalix[4]-pyrrole, tetrabutylammonium, and $H_2PO_4^-$), Figure S2 (free energy of solvation data effected by solvent, TBA^+, and water), Table S1 (nonstandard force field parameters). This material is available free of charge via the Internet at http://pubs.acs.org.

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