JOC Article

Buried Solvent Determines Both Anion-Binding Selectivity and Binding Stoichiometry with Hydrogen-Bonding Receptors

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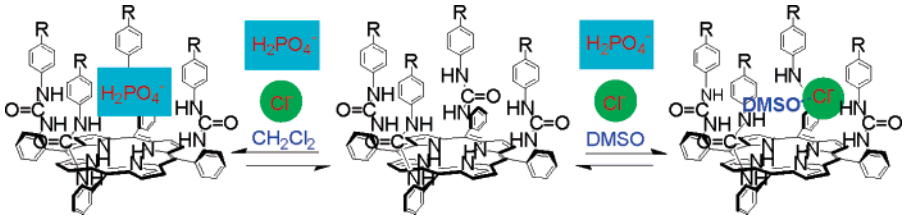

The crystal structure of a tetraurea picket porphyrin−chloride anion complex has previously shown the anion to be situated between two adjacent ureas and hydrogen bonded via four NH protons (*J. Am. Chem. Soc.* **1998**, *120*, 11684−11692). The porphyrin receptor also binds a DMSO molecule and utilizes it as a participant in its anion recognition unit, in a manner similar to enzymes that bind water for use as part of their substrate recognition unit. The bound solvent molecule determines the anion-binding affinity, selectivity, and stoichiometry of binding. With a bound DMSO molecule, the tetraurea picket porphyrin is a highly selective receptor for chloride anion and binds all anions with a 1:1 binding stoichiometry. Absent the buried DMSO molecule, the receptor is selective for phosphate anion and binds chloride and phosphate anions with both 1:1 and 1:2 receptor−anion stoichiometries. Additionally, a remarkable reversal in the selectivity of anion complexation between various picket porphyrin receptors is observed, wherein the binding constant ratios change over 3 orders of magnitude as the receptor's number of urea pickets change from four to two. The latter receptor has no urea pickets available to bind to solvent after complexation with an anion. The results demonstrate that anion complexation with hydrogen-bonding receptors in a competitive solvent is enhanced when a ubiquitous solvent molecule is incorporated into the binding motif. In this way, competitive solvent adds to the overall complexation energy and thereby strengthens binding rather than weakens it, as commonly believed. The results are pertinent to drug design, for they suggest that pharmaceuticals need not be completely desolvated to selectively bind to their biological target when water can be included in the binding motif.

Introduction

Water plays many essential roles in biological systems, including structural roles. Buried water (water bound within a biopolymer) takes part in substrate recognition in carbohydrate-binding enzymes,[1] in protein kinase A,[2] in amionacyl-tRNA synthetase,[3] and in lipid-binding proteins.[4] Buried water is believed to play an important role in control of the specific conformation of HIV-1 protease for the initiation of enzyme reaction[5] and in the structure and function of aspartic proteinases,[6] MHC class-I proteins,[7] and the phospholipase OMPLA.[8] Clearly, water plays a vital role in the molecular recognition between biological host and guest, and as such, it could prove useful in drug design to engineer water-binding sites into the interface between a drug and its biological target.[9] However, studies which systematically probe and

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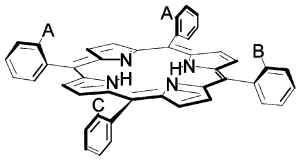
CHART 1. Urea Picket Porphyrins



1a-d: A,B,C = $NHCONHC_6H_4R$
R = H(**1a**), Cl(**1b**), F(**1c**), NO_2(**1d**)
2a: A,B = NH_2, C = H
2b: A,B = $NHCONHC_6H_4F$, C = H
3a: A = NH_2, B,C = H
3b: A = $NHCONHC_6H_4F$, B,C = H

quantify these types of solvent-recognition effects with model receptors are lacking.[10]

Our group's involvement in the design of recognition elements for hydrated anionic phospholipids in vivo has led to a study examining the effects on anion complexation when a ubiquitous solvent molecule is incorporated into an anion-receptor binding motif. We recently reported the anion-binding behavior of a series of tetraurea picket porphyrins[11] which we believe are relevant to the issue of solvent-recognition effects. The (α,α,α,α-5,10,-15,20-tetrakis(2-(arylurea)phenyl))porphyrins **1a**−**d** (Chart 1) bind strongly to chloride anion in the highly competitive solvent DMSO-d_6 (K (M^{-1}) > 10^5) and solvent system 12% D_2O/DMSO-d_6 (K (M^{-1}) > 10^3). The porphyrins **1a**−**d** exhibit significant binding selectivity,[12] forming a 1:1 anion−receptor complex with association constant ratios of $Cl^-/H_2PO_4^-$ > 200:1, and of $Cl^-/CH_3CO_2^-$, HSO_4^-, NO_3^- > 1000:1. The X-ray crystal structure of chloride anion−porphyrin **1b** (R = Cl) complex shows the anion to be situated between two adjacent ureas and hydrogen bonded via the four NH protons. A DMSO molecule, hydrogen bonded to a third urea, is positioned in the center of the pocket. The electron-deficient sulfur is in near van der Waals contact with the chloride anion, suggesting a Coulombic interaction. We hypothesized that this stabilizing "ordered solvent shell" enhanced the binding constant for halides, thus increasing the receptor's selectivity for chloride anion, reminiscent of buried water's role in enzyme recognition of substrates. The same binding motif with trigonal or tetrahedral anions would necessitate removal of the pocket-bound DMSO due to their size and shape.

To examine this hypothesis, it was decided to probe the effects of bound DMSO on the anion binding of urea picket porphyrin receptors by two complementary methods. The first approach was to prepare porphyrin receptors with reduced numbers of urea-pickets, thereby removing the ability of the receptor to bind solvent when utilizing two adjacent urea groups in its anion binding motif. The second method was to examine the binding of a tetraurea porphyrin receptor in a solvent unable to hydrogen bond to the urea pickets. We now report on the results of this two-pronged approach in the modeling of buried solvent's effects on anion complexation with hydrogen-bonding receptors.

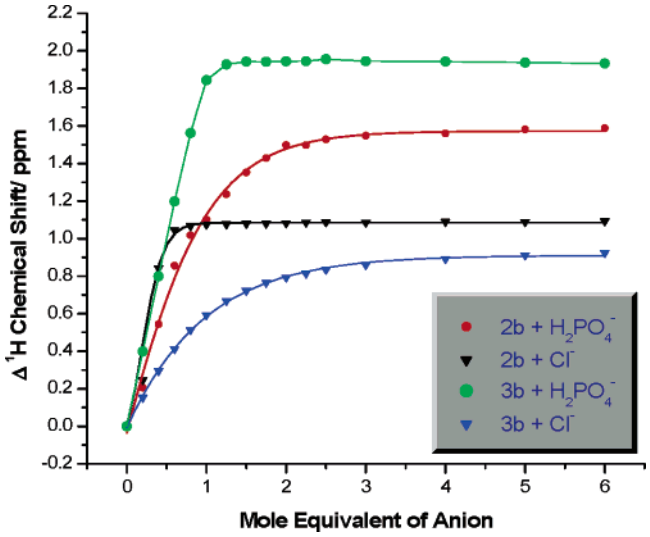


FIGURE 1. Representative NMR titration curves for **2b** and **3b** plus anions.

Results and Discussion

Anion Coordination Studies in DMSO-d_6 with Receptors That Contain Reduced Numbers of Urea Pickets. In the first approach to test our "ordered solvent shell" hypothesis, tri- and diurea picket porphyrin receptors **2b**−**3b** were prepared. The triurea picket porphyrin provides a more open pocket, and the diurea picket porphyrin contains no extra urea picket to bind solvent when binding an anion. If receptor-bound solvent was really a determinant of anion-binding selectivity, then these changes in receptor pocket should certainly mirror a change in anion selectivity.[13]

The urea picket porphyrins **2b**−**3b** were synthesized by the addition of 4-flourophenyl isocyanate to the all-α atropisomer of the aminophenyl porphyrins **2a**−**3a**.[14] Association constants were determined for porphyrins **2b**−**3b** by following their titrations with tetrabutylammonium chloride or dihydrogen phosphate salts in DMSO-d_6 using ^{1}H NMR (Figure 1).[15] Binding constants for **2b**−**3b**, along with those of tetra-rea picket porphyrin **1c** (R = F) for comparison, are shown in Table 1.

As the number of urea pickets on the receptors is reduced from 4 to 3 to 2, there is a corresponding dramatic change observed in anion-binding selectivity of the three receptors. With porphyrin **1c**, the previously determined association constant ratio was $Cl^-/H_2PO_4^-$ > 200:1. With porphyrin **2b**, the association constant ratio is $Cl^-/H_2PO_4^-$ = 6:1, while with porphyrin **3b** the

(10) Bonar-Law and Sanders have reported an increase in the sugar binding strength of a steroid- capped porphyrin receptor with the addition of methanol or water. Bonar-Law, R. P.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1995**, *117*, 259−271.

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(13) The tetraurea picket porphyrin receptor has four identical anion-binding units (i.e., each binding unit is two urea groups that donate four hydrogen bonds), the triurea picket porphyrin receptor has two identical anion binding units, and the diurea picket porphyrin receptor has one binding unit. For any given anion the binding constants will differ among the receptors **1**−**3** since their k_{on} (and k_{off}) will change due to statistical factors. However, the ratio of the binding constants among different anions, and thus complexation selectivity, would not be expected to vary greatly unless solvent was a determinant in anion binding selectivity.

(14) The syntheses of the urea picket porphyrins will be reported elsewhere. All new compounds gave satisfactory spectroscopic and elemental analyses.

(15) Binding constants were determined by nonlinear regression analysis using EQNMR for Windows: Hynes, M. J. *J. Chem. Soc., Dalton Trans.* **1993**, 311−312.

TABLE 1. Association Constants[a] (M^{-1}) for Porphyrins 1c, 2b, and 3b in DMSO (errors range from 10 to 15%)

	Cl^-	$H_2PO_4^-$
1c	$>10^5$	1.4×10^3
2b	1×10^3	1.0×10^3
3b	2.7×10^2	1.5×10^4

[a] Each K was determined from an average of three titrations and an average of several different proton shifts.

association constant ratio is $Cl^-/H_2PO_4^- = 1{:}55$. *This is a remarkable reversal in the selectivity of anion complexation between the receptors, wherein the binding constant ratios change over 3 orders of magnitude as the receptor's number of urea pickets change from four to two.*

The change in anion-selectivity patterns observed with these modified urea-picket receptors indicates the importance of solvent to binding selectivity. Two urea groups complex to the anion, while any additional urea groups bind solvent DMSO. The following facts provide validity to our assumption that receptors **2b**−**3b** have the same anion binding motif seen in the X-ray crystal structure of tetraurea **1b**: (i) Job plot analysis shows all three receptors have the same stoichiometry of anion binding in DMSO; (ii) all three receptors utilize identical urea binding units, only differing in their number; (iii) urea proton shifts observed for any given anion titration among the three receptors are essentially the same. Thus it is that receptor **3b**, which can only bind anion and not solvent, exhibits a complete reversal of binding selectivity from that of the tetraurea picket porphyrin receptor which can bind anion + solvent.

NMR Anion-Binding Studies with 1c in CD_2Cl_2 and CD_2Cl_2−DMSO-d_6. The above results support the idea that the presence of a buried DMSO solvent molecule within urea picket porphyrin receptors plays a pivotal role in its anion binding. To evaluate the effect an absence of buried DMSO molecule has on receptor binding, ^1H NMR anion titration studies were undertaken in CD_2Cl_2 with tetra-urea picket porphyrin **1c**. In CD_2Cl_2, the resonances of the receptor's fluorophenyl protons are somewhat broadened compared to those observed when the receptor is in DMSO-d_6. This is most likely due to intramolecular hydrogen bonding between the urea groups. With the addition of one equivalent of anion during the titration experiment, the peaks sharpen and look similar to those observed in DMSO-d_6. The other receptor proton resonances are very similar to each other in either solvent (although the doublet and triplet resonances of meso-phenyl protons centered about 8.0 ppm are reversed in the two solvents).

The anion titration experiments done in CD_2Cl_2 produced measurable differences in the receptor's binding properties as compared with those titrations done in DMSO-d_6. Representative binding curves for Cl^-, $H_2PO_4^-$, and NO_3^- anions are shown in Figure 2, and their association constants[15] (K (M^{-1})) in CD_2Cl_2 and DMSO-d_6 for comparison are shown in Table 2. As with the titrations carried out in DMSO-d_6, proton shifts were observed for the receptor porphyrin *meso*-phenyl and β-pyrrole protons, porphyrin NH, and urea NH. In both solvent systems significant downfield shifts were observed for the urea NH protons, indicative of their essential role in the anion recognition process via hydrogen bonding.[11] In both solvent systems the β-pyrrolic protons remain a sharp singlet upon anion complexation, showing the anion is bound in the urea pocket of the porphyrin. Unlike titrations done in DMSO-d_6, however, several protons exhibit shifts in CD_2Cl_2 which result in binding curves for Cl^- and $H_2PO_4^-$ anions that are not well behaved, suggesting a different stoichiometry of binding then the simple 1:1 receptor:anion complex seen in DMSO-d_6. This is confirmed by Job plot analysis, which shows there exists a mixture of 1:1 and 1:2 receptor/Cl^- anion complexes in CD_2Cl_2. The titration curves for NO_3^- anion appeared well behaved, and the mole ratio method[11] shows that NO_3^- anion binds with a 1:1 stoichiometry. While it is not clear why NO_3^- anion's stoichiometry of binding is different than the other anions, it is interesting to note that receptor **1c** appears to favor the binding of trigonal shaped anions when no competitive solvent is present, as it exhibits preference for binding acetate anion when it is embedded in the polymer matrix of an ion selective electrode.[16]

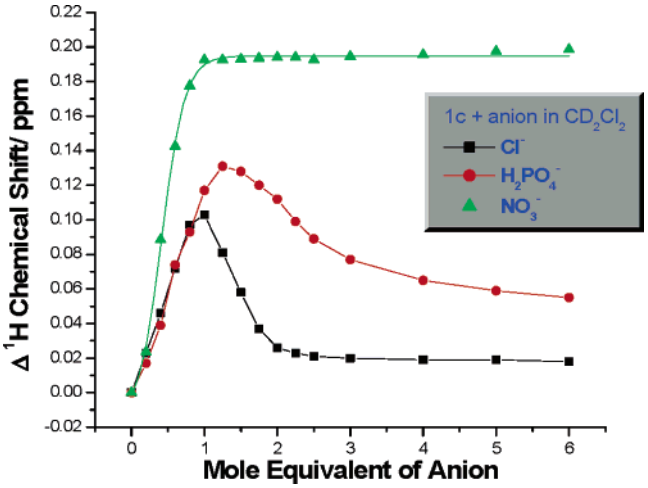

FIGURE 2. Representative NMR titration curves for **1c** plus anions in CD_2Cl_2.

TABLE 2. Association Constants[a] (M^{-1}) for Porphyrin 1c in CD_2Cl_2 (errors range from 10 to 20%)

	1c (K_1)	**1c** (K_2)
Cl^-	1.5×10^3	8.5×10^3
$H_2PO_4^-$	1.8×10^4	4.8×10^2
NO_3^-	3.3×10^4	

[a] Each K was determined from an average of three titrations and an average of several different proton shifts.

Analysis[15] of the titration curves for either Cl^- or $H_2PO_4^-$ anion using both a 1:1 and 1:2 binding model results in a set of K_1 values that are the same, e.g., the K_1 in both models for Cl^- anion is 10^3, and in both models for $H_2PO_4^-$ anion it is 10^4. In the case of Cl^- anion the K_2 is larger than K_1, whereas K_2 is smaller than K_1 in the case of $H_2PO_4^-$ anion. The association constants determined for the anions in the noncompetitive solvent CD_2Cl_2 show that $H_2PO_4^-$ (comparing K_1 values) and NO_3^- anions bind to **1c** 10 and 600 times stronger, respectively, than they bind to **1c** in DMSO-d_6.

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The question can be asked, is it possible to distinguish between the effect of bulk solvent and the effect of buried solvent on the observed anion binding selectivity for the porphyrin receptor? One must be careful in interpretation of bulk solvent effects in anion binding, which are due not only to the solvation of the anion, but due to the solvation of the counterion, the interaction of anion and counterion in the given solvent, and the solvation of the receptor's binding groups. While information from the literature regarding the affect bulk solvent has on anion binding is sparse,[17] the following published data does support our hypothesis that buried solvent is responsible for the observed binding behaviors of the urea porphyrins. Theoretical results by Luque and Orozco[17] show that the relative free energies of solvation for chloride and dihydrogenphosphate anion are not altered by the tetrabutylammonium counterion in aprotic solvents. Thus, it would appear as if the counterion is not responsible for the selectivity between chloride and dihydrogenphosphate anions observed for the porphyrin urea receptors in DMSO-d_6 or CD_2Cl_2. Experimental results show that chloride anion is solvated by −3 kcal over that of acetate anion in DMSO,[18] yet our tetraurea porphyrin receptor binds more strongly to chloride anion by 3 orders of magnitude.[11] The solvation enthalpies of tetrabutylammonium chloride and tetrabutylammonium nitrate in DMSO are within 0.5 kcal of each other,[19] yet the tetraureaporphyrin receptor binds chloride anion 3 orders of magnitude greater than it does nitrate anion in DMSO.[11] The Gibbs free energy of transfer of chloride and nitrate anion from water to DMSO and to chlorinated solvents demonstrates that the ability to solvate the inorganic anion is lower in the less polar chlorinated solvent.[20] Thus, that the neutral hydrogen bonding receptor **1c** would be more effective at binding anions in the non competitive solvent CD_2Cl_2 is not surprising. *What is surprising, however, is that Cl⁻ anion (comparing K_1 values) binds more than 2 orders of magnitude less strongly in CD_2Cl_2 than it binds to* ***1c*** *in DMSO-d_6.* Calculating the difference in the free energies for the anion-receptor complexes in the two solvents shows that a buried DMSO molecule affords between 2 and 3 kcal per mole stability to the Cl⁻ anion-receptor **1c** complex.

To further support the hypothesis that the switch in solvent from DMSO-d_6 to CD_2Cl_2 with concomitant loss of buried solvent was the cause of the change in the receptor's association constants and stoichiometry of binding, anion titrations were carried out with **1c** in dual solvent systems of 5% or 10% DMSO-d_6 in CD_2Cl_2. These experiments were designed to determine if the addition of DMSO would reverse the changes, observed in the receptor's binding profiles of anion titrations acquired in 100% CD_2Cl_2, back to the binding profiles that were observed in 100% DMSO-d_6 (Supporting Information Figure S-12 shows that receptor **1c** is not fully solvated with DMSO molecules at even 10% DMSO-d_6, as the urea NH protons hydrogen bonded to the DMSO-d_6 are not as far downfield as in the ^{1}H NMR spectrum taken in 100% DMSO-d_6). Titration curves and association constants[15] for **1c** and Cl⁻ anion in CD_2Cl_2 containing 0%, 5%, and 10% DMSO-d_6 are illustrated in Figure 3 and Table 3, respectively. With the addition of increasing amounts of DMSO-d_6, the titration curves appear more and more well behaved, and at 10% DMSO support a 1:1 stoichiometry of anion/receptor binding (mole ratio method). The data clearly shows an increase in association constant with the increase in concentration of DMSO-d_6, even though the urea groups on the receptor are more strongly solvated by the addition of cosolute DMSO-d_6 due to hydrogen bonding stabilization (vide supra). Thus, all of the anion titration results with **1c** in CD_2Cl_2 and CD_2Cl_2−DMSO-d_6 support our contention that buried solvent determines receptor **1c**'s anion selectivity, affinity, and stoichiometry of anion binding.

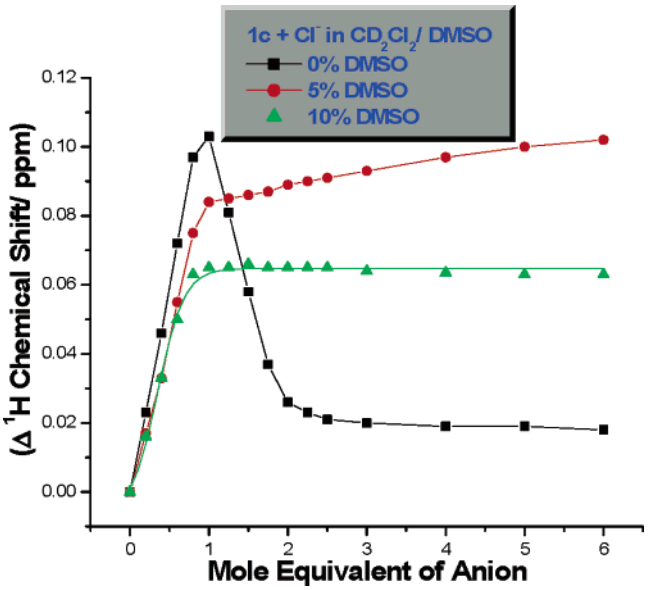

FIGURE 3. Representative NMR titration curves for **1c** plus chloride anion in *x*% DMSO-d_6 + CD_2Cl_2 (black: 0% DMSO; red: 5% DMSO; green: 10% DMSO).

TABLE 3. Association Constants[a] (M^{-1}) for Porphyrin 1c in CD_2Cl_2 + *x*% DMSO-d_6 (Errors Range from 10 to 20%)

	0% DMSO	5% DMSO	10% DMSO
1c + Cl⁻	1.5×10^3	9.4×10^3	2.2×10^4

[a] Each *K* was determined from an average of two titrations and an average of several different proton shifts.

ESMS Studies. Electrospray mass spectrometry (ESMS) studies were undertaken to see if the anion−solvent−porphyrin complex would form in the gas phase, as it did in solution and the solid state. ESMS was taken in negative mode, so the observed species contain the anion. Desolvation energies precluded an anion−solvent−porphyrin complex from being observed with receptors **1c**, **2b**, or **3b**. High enough energy was provided in desolvation to separate the tetrabutylammonium counterion from the anion−receptor complex and in so doing separated the DMSO solvates as well. However, ESMS did corroborate the Job plot stoichiometry, where a 1:1 anion−porphyrin complex was observed with **1c**, **2b**, and **3b** in DMSO.

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(20) Marcus, Y. *Pure Appl. Chem.* **1983**, *55*, 977−1021.

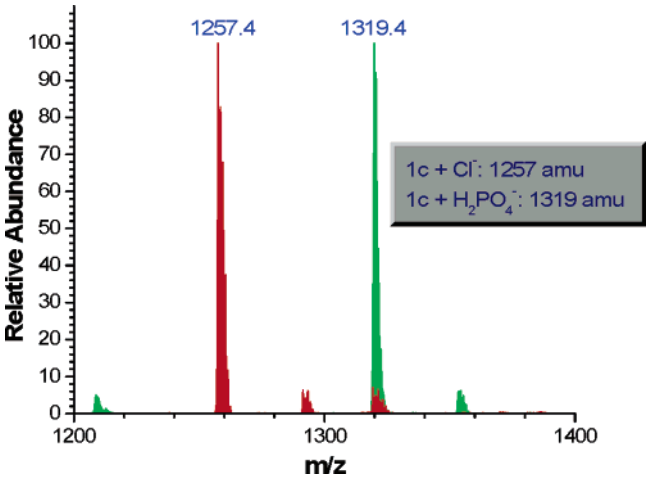

FIGURE 4. Combined ESMS spectra of competition study with porphyrin **1c** + Cl^- and $H_2PO_4^-$ (red: spectrum from DMSO solution; green: spectrum from CH_2Cl_2 solution).

While ESMS was not amenable to the observation of a gas-phase ternary complex, it still provided interesting results from a series of competition studies undertaken with picket porphyrins that contain an extra picket for solvent complexation. Equal amounts of chloride and dihydrogen phosphate anion in either DMSO or CH_2Cl_2 were allowed to equilibrate with either receptors **1c** or **2b**, and ES mass spectra were then acquired from the solutions of porphyrin−anion complexes (Figure 4). The spectra obtained from the competition studies show that little $H_2PO_4^-$−**1c** or $H_2PO_4^-$−**2b** exists in the gas phase when the complex is formed in DMSO solution but that little Cl^-−**1c** or Cl^-−**2b** exists in the gas phase when the complex is formed in CH_2Cl_2 solution. No $H_2PO_4^-$ dianion−receptor complexes were observed in the gas phase. Very little $Cl^-/H_2PO_4^-$ mixed dianion−receptor complex (1354 amu) was observed in samples taken from the CH_2Cl_2 solution, and very little Cl^- dianion−receptor complex (1292 amu) was seen in samples taken from the DMSO solution. If the ESMS spectra are a semiquantitative reflection of the amounts of porphyrin−monoanion complex formed in solution, then the phosphate anion would seem to bind stronger than chloride anion to the porphyrin receptor in non-hydrogen-bonding dichloromethane. If dianion complexes are less easily volatilized than monoanion complexes, however, the observations may reflect the fact that there are more $H_2PO_4^-$ monoanion−receptor complexes in solution then dianion complexes ($K_1 > K_2$), while there are more Cl^- dianion−receptor complexes in solution then monoanion complexes ($K_1 < K_2$).[21] Whether either is true, one observes with ESMS a corresponding reversal in anion-binding selectivity with the change from a noncompetitive to competitive (and buried) solvent. Whether in the solid, liquid, or gas phase, when a DMSO molecule and chloride anion is readily available to hydrogen bond within the receptor pocket, the most stable complex is formed as the monochloride anion.

Conclusions

In summary, a tetraurea picket porphyrin can bind a DMSO molecule and utilize it as a participant in its anion recognition unit in a manner similar to enzymes that bind water for use as part of their substrate recognition unit. This work results in two discoveries detailing solvent's affect on anion binding with hydrogen-bonding receptors: (1) anion complexation with hydrogen-bonding receptors in a competitive solvent *is enhanced* when a ubiquitous solvent molecule is incorporated into the binding motif, i.e., competitive solvent adds to the overall complexation energy and thereby strengthens binding via hydrogen bonding rather than weakens it, as commonly believed, and (2) solvent incorporated into the binding motif of a (bi-substrate) receptor can determine its selectivity and stoichiometry of anion binding. Put a different way, a receptor's binding pocket that is complementary for one anion can be modified and made complementary for a different anion with the binding and appropriate positioning of a solvent molecule! The results are pertinent to drug design, for they suggest that pharmaceuticals need not be completely desolvated to selectively bind to their biological target when water can be included in the binding motif.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the ACS, for support of this research. We thank Professor Mike Van Stipdonk and Dr. Vincent Storhaug for acquiring the ESMS data

Supporting Information Available: ^{1}H NMR spectra of porphyrins **2b** and **3b** in DMSO-d_6 and **1c** in CH_2Cl_2; ^{1}H NMR stacked plot of **2b** + 0, 1, and 6 equiv of dihydrogenphosphate anion and ^{1}H NMR stacked plot of **3b** + 0, 1, and 6 equiv of chloride anion both in DMSO-d_6; ^{1}H NMR stacked plot of **1c** + 0, 1, 1.75, and 6 equiv of chloride anion in CD_2Cl_2; ^{1}H NMR stacked plot of **1c** in 100% CD_2Cl_2, 5% DMSO-d_6/95% CD_2Cl_2, 10% DMSO-d_6/90% CD_2Cl_2, and 100% DMSO-d_6; Job plots of **2b** + chloride anion and **3b** + chloride anion in DMSO-d_6 and Job plot of **1c** + chloride anion in CD_2Cl_2; and ESMS spectra from competition study of **2b** + dihydrogenphosphate and chloride anion in both DMSO and CH_2Cl_2. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) It could be argued that the reversal in binding selectivity observed in the two solvents with ESMS could be an artifact of a change in the receptor's conformation when in different milieus. Since ^{1}H NMR shows that the receptor's urea groups are engaged in intramolecular hydrogen bonding in CH_2Cl_2, the anions would have to compete for binding sites. Phosphate anion forms strong hydrogen bonds and may be better able to compete for the urea sites, which in turn, could result in the higher selectivity of the receptors that is observed with ESMS for the phosphate anion in CH_2Cl_2. However, the fact that the weakly hydrogen bonding NO_3^- anion complexes as strongly to **1c** in CD_2Cl_2 as $H_2PO_4^-$ anion does not give credence to this argument.