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Anion Recognition by a Macrobicycle Based on a Tetraoxadiaza Macrocycle and an Isophthalamide Head Unit

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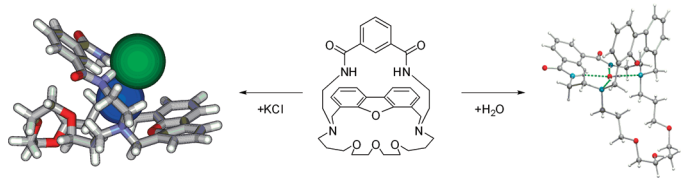

A macrobicycle formed by a tetraoxadiaza macrocycle containing a dibenzofuran (DBF) spacer and an isophthalamide head unit, named DBF-bz, was used as receptor for anion recognition. The molecular structure of DBF-bz was established in solution by NMR and ESI-MS spectroscopies and in single crystal by X-ray diffraction analysis. The X-ray structure showed a water molecule encapsulated into the macrobicyclic cavity by four hydrogen bonds, two of them involving the two N−H amide binding sites and the oxygen of the water molecule (N−H···O hydrogen bonds) and the other two (O−H···N) involving the amine groups as hydrogen bonding acceptors. ^{1}H NMR temperature dependence studies demonstrated that the same structure exists in solution. The ability of this ditopic receptor to recognize alkali halide salts was evaluated by extraction studies followed by ^{1}H NMR and ESI-MS spectroscopies. The macrobicycle showed a capacity to extract halide salts from aqueous solutions into organic phases. The binding ability of this macrobicycle for halides was also quantitatively investigated using ^{1}H NMR titrations in $CDCl_3$ (and DMSO-d_6) solution, and in acidic D_2O solution. The largest binding association constant was found for the chloride anion and the completely protonated receptor. The results suggest that the diammonium-diamide unit of the receptor strongly bind the anionic substrate via multiple N−H···Cl^- hydrogen bonds and electrostatic interactions. The binding trend follows the order $Cl^- > Br^- > I^- \approx F^-$ established from the best fit between the size of the anion and the cavity size of the protonated macrobicycle. Molecular dynamics (MD) simulations of the DBF-bz in $CHCl_3$ solution allowed a detailed insight into the structural and binding properties of the receptor.

Introduction

Recognition of anionic species is a vigorous research field of supramolecular chemistry due to the important role of these charged molecules in biological, industrial, and environmental fields.[1−3] Recent developments focused on the use of artificial receptors as membrane transport agents for chloride in biological systems, ion-pair receptors, sensors for the detection of biologically important anionic species, anion template reactions, etc.[4] In this area, the design and synthesis of three-dimensional molecular receptors for selective recognition of anions play a crucial role.

Electron-deficient, positively charged, or neutral groups can be used as binding sites for the recognition of anionic species. A large number of anionic associations using amine-[5] and amide-based[6] receptors have been structurally characterized. They generally bind the substrate by multiple electrostatic interactions and/or hydrogen bonds. Selectivity is generally

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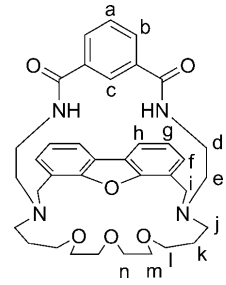
achieved by cooperative effects of geometric fit and strong interactions between partners.[2c,5g]

Macrobicycles (also named cryptands) are particularly interesting artificial receptors, which offer a wide range of potential useful molecular recognition properties for anions, cations, or both.[2,5g,7] These systems may consist of two different compartments, one of them designed for the appropriate bind of an anion (in general formed by N−H fragments from amine, amide, or urea groups) and the other one for the binding of a cation (in general containing a crown ether moiety). For instance, Esteban-Gómez et al.[8] studied macrobicyclic receptors exhibiting high binding selectivity to Cl^- over the other halides and sulfate over nitrate. Moreover, the three-dimensional preorganized structure of macrobicycles may offer the possibility of mutual location of an ion pair into the two compartments of the receptor producing, in certain cases, a positive cooperative effect leading to an increase in the binding affinity. For instance, the macrobicycle containing oxa-crown and amide binding sites presented by Smith et al.[9] is capable to accommodate both the cation (e.g., K^+) and the anion (e.g., Cl^-) in close contact: in the presence of K^+, the receptor affinity toward Cl^- was 13-fold enhanced, due to a cooperative electrostatic effect.

CHART 1. Structure of the Macrobicycle DBF-bz



In spite of some attractive examples in the literature, the recognition phenomena based on macrobicyclic receptors remain a research area to explore. In this context, we report here the design and the synthesis of a macrobicycle, DBF-bz (Chart 1), formed by an isophthalamide bridge linked through two ethylenic chains to a tetraoxadiaza macrocycle. The macrocyclic moiety, mainly composed by a rigid dibenzofuran unit and a flexible trioxa fragment, is expected to present enough space for inclusion of cationic species such as alkali cations or ammonium substrates.[10] On the other hand, the isophthalamide unit can strongly interact with anionic substrates such as chloride[6d,e] or carboxylate.[6a,b] Thus, the recognition phenomena between BDF-bz and different substrates was studied in the present work in order to obtain information on the binding process. Results will be discussed in terms of binding strength and geometrical requirements.

Results and Discussion

Synthesis and Characterization of the Macrobicyclic Compound. The synthetic approach (Chart 2) is modular allowing the two building blocks of the molecule, the isophthalamide head unit and the tetraoxadiaza macrocycle, to be modified leading to a wide variety of receptors. The target compound, DBF-bz, was prepared by reaction of the *N*,*N*′-bis(2-aminoethyl) derivative of the macrocycle [22](DBF)N_2O_3[10] with *m*-xylyldicarbonyl dichloride in high dilution at 0 °C, with Et_3N as base and in dry CH_2Cl_2/THF mixture of solvents. The compound was obtained as a crystalline powder upon silica gel column chromatography purification in 37% yield. Single crystals suitable for X-ray diffraction determination were grown from slow evaporation of CH_2Cl_2/CH_3OH solution of the compound at low temperature; see below.

^1H and ^{13}C NMR spectra of the macrobicycle are consistent with a symmetric species, and cross-peak correlations of COSY, HMQC and HMBC spectra provided the complete assignment of all magnetically different nuclei. The ^1H spectrum, in $CDCl_3$ solution, shows the H_i proton resonances as an AB system at 4.66/3.37 ppm ($J_{ii'}$ = 12.2 Hz), and the two triplet resonances of H_e and H_j protons as two AA′BB′ systems; see Chart 1 for labeling. One of these systems involves the H_k/H_e at 2.13/2.98 ppm (J = 12.0 Hz) and the other H_j/H_d at 2.83/3.27 ppm (J = 12.5 Hz). The H_i, H_e, and H_j protons are thus diastereotopic in pairs, attesting the rigidity of the molecule around the tertiary amines. In contrast, the DBF protons (H_f, H_g, and H_h) as well as those of the isophthalamide group (H_a, H_b, and H_c) and the

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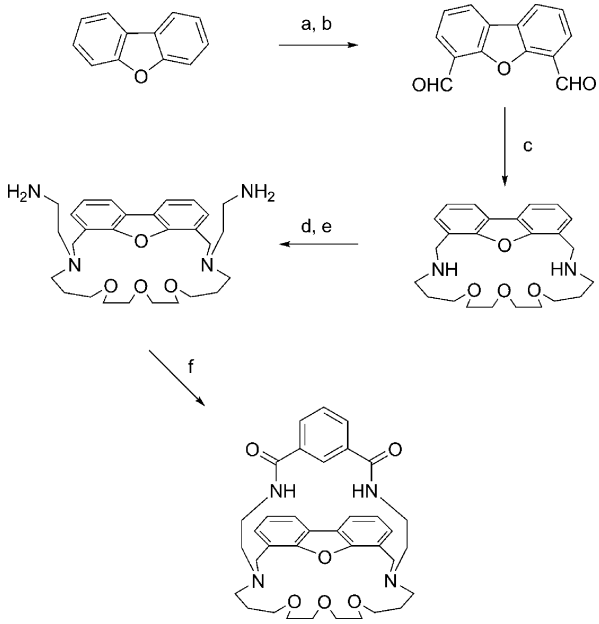
CHART 2. Preparation of DBF-bz[a]



[a] Reagents and Conditions: (a) Et_2O, TMEDA, *n*-BuLi, reflux; (b) DMF, 0 °C then rt, 24 h; (c) 4,7,10-trioxa-1,13-tridecanediamine, EtOH, 0 °C overnight, then $NaBH_4$, rt (91%); (d) bromoacetonitrile, K_2CO_3, CH_3CN, reflux (89%); (e) BH_3 THF (92%); (f) Et_3N, CH_2Cl_2/THF (37%).

polyether chain (H_l, H_m, and H_n) are all isochronous. This spectrum confirms the dual composition of the DBF-bz macrobicycle, the two rigid aromatic frameworks, and the flexible aliphatic ether linkage.

Furthermore, ROESY experiments performed in $CDCl_3$ at rt revealed the folded conformation of the macrobicyclic structure through the axis connecting the two amine nitrogen atoms leading to face both aromatic units. As expected, signals between H_i, H_e, and H_j are clearly seen (see the Supporting Information). Additionally, long-range coupling between H_c and H_f protons of the two different aromatic units was observed, showing that these protons are located at a short distance from each other. Contacts between H_l, H_m, and H_n with either H_i or H_e were not observed, indicating that the ether linkage is located far away from the DBF and the benzene units.

The X-ray determination (see below) and the elemental analysis revealed the presence of one H_2O molecule encapsulated into the cavity of DBF-bz interacting by N−H···O and O−H···N hydrogen bonds. An ^1H NMR temperature dependence experiment was used to follow the behavior of the N_{amide}−H···O−H_{water} proton resonance in $CDCl_3$ and at 298 K. It was assumed that fast exchange occurs between the protons of the water molecule and the protons of the diamide functions in the NMR time scale when the water molecule is inside the diamide−diamine cavity. The chemical shift of this diamide/water proton signal observed at 2.79 ppm can be a consequence of the downfield shift of the water proton signal from 1.56 ppm (free water molecule in $CDCl_3$) and the upfield shift of the NH amide proton signal (expected at about 8 ppm). Experimental evidence of the involvement of NH amide protons in intramolecular hydrogen bonds can be obtained from the temperature coefficients of the amide protons ($\Delta\delta/\Delta T$).[11] These measurements were widely used to study conformational changes

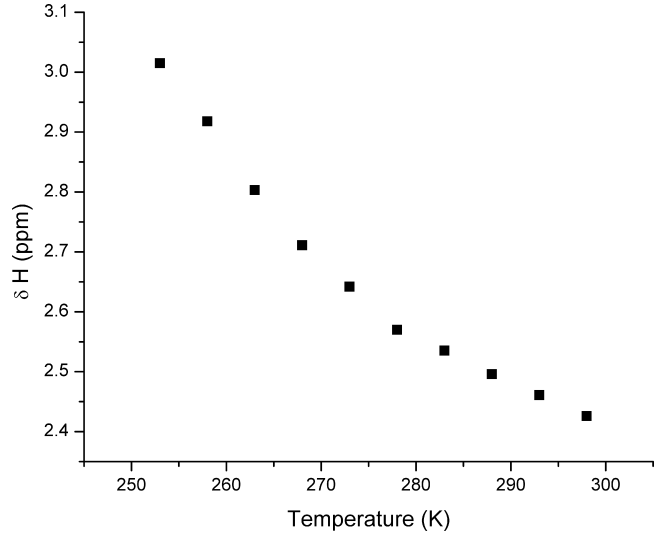


FIGURE 1. Amide−water proton NMR chemical shifts of DBF-bz (δ H) as a function of temperature (5 mM solution in $CDCl_3$).

induced by intramolecular hydrogen bonds in peptides and protein.[12,13] In fact, the NH group sheltered from its environment at low temperature, will become exposed to the solvent when the temperature increase.[14] Strong variations ($\Delta\delta/\Delta T \geq -4.6$ ppb K^{-1}) indicate low solvent accessibility of the amide protons and, hence, of their involvement in intramolecular hydrogen bonds,[12] although other factors have to be taken into account.[13] In DBF-bz, the amide proton resonance presents a remarkable temperature dependence of −20.3 ppb K^{-1} (in the range 253−268 K) and −7.2 ppb K^{-1} (278−298 K); see Figure 1. The behavior of other protons are significantly less dependent on temperature. Thus, these results support the presence of hydrogen bonds between the water molecule and the diamide/diamine unit of the receptor. In addition, the linear relationship δ vs T showed two ranges of temperature (253−268 and 278−298 K), which must be related to the hydrogen bond established in the DBF-bz(H_2O)-associated compound and the interference of free water molecule in $CDCl_3$.

X-ray Analysis of Solid-State Structure. The single-crystal X-ray structure of DBF-bz **1** is presented in Figure 2 and shows a water molecule encapsulated into the macrobicycle cavity with the oxygen barely out 0.05(1) Å from the plane determined by the four nitrogen donor atoms. The two N−H amide binding sites and the water molecule establish two N−H···O hydrogen bonds with H···O distances of 2.18 and 2.28 Å and corresponding angles of 170 and 171°, respectively. In addition, the two lone pairs of tertiary amines are also directed toward the center of the cavity with an N···O intermolecular distance of 2.931(2) and 2.894(2) Å, leading to two additional O−H···N hydrogen bonds with H···O distances of 2.13(2) and 2.07(2) Å and angles of 160(2) and 167(2)°, respectively. Therefore, the X-ray shows the formation of a supramolecular entity between the water molecule and DBF-bz. The macrobicycle adopts a cleft topology with the planes of the isophthalamide group and the dibenzofuran moiety making a dihedral angle of 65.10(4)°. This conformation is asymmetric and confers to the

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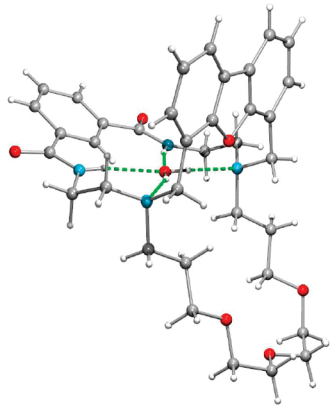

FIGURE 2. X-ray crystal structure of DBF-bz **1**.

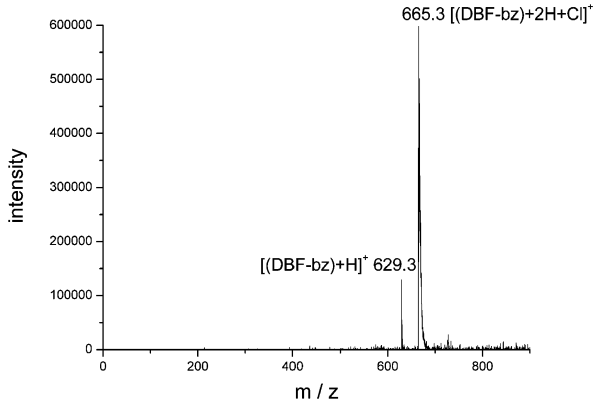

FIGURE 3. ESI-MS spectra of the receptor DBF-bz in the presence of chloride anion in $CHCl_3$.

macrocycle an apparent rigid structure with the isophthalamide group intersecting the plane determined by the three ether oxygen donors and the two tertiary amine groups at 46.45(7)°. The DBF ring makes with this plane an angle of 18.66(9)°, with two short intermolecular contacts of 2.056 and 2.218 Å between H_i and H_k protons from different CH_2 groups. Therefore, the X-ray determination revealed a second conformation of the macrobicyclic structure where the two aromatic fragments are nearly perpendicular to each other.

Binding Studies. The macrobicycle DBF-bz displays two binding units with different donor atoms. The first one, composed of two amide and two tertiary amine nitrogen donors, is designed to accommodate anionic substrates particularly upon protonation of both amines. The second one, defined by the three oxygen atoms of the ether linkage provides a binding environment for hard metal ions or ammonium cations. In fact, the distance between the mass centers defined by the ether oxygen donors and the nitrogen donors is 6.19 Å, suggesting that it provides a cage with enough room to assemble simultaneously one cation and one anionic substrate.

The studies in solution were limited by the low solubility of DBF-bz and the supramolecular entities formed. $CHCl_3$ or DMSO were the only possible solvents for the neutral receptor and H_2O for the diprotonated receptor.

ESI-MS Studies. The DBF-bz macrobicycle has a strong tendency to uptake one chloride anion, as shown by the ESI-MS spectrum of the compound; see Figure 3. The peak at 665.3 $[(DBF\text{-}bz) + 2H + Cl]^+$ is clearly detected, and its intensity relative to the peak at 629.3 $[(DBF\text{-}bz) + H]^+$ expresses the pronounced affinity of the macrobicycle toward Cl^- anion. This result led us to study the binding properties of the macrobicycle with halides, in particular.

TABLE 1. ^1H NMR Chemical Shift Variation $\Delta\delta$ (ppm) of the Extracted Entity in Comparison to the Free Ligand in $CDCl_3$[a]

resonance	KF	KCl	KBr	KI
c	−0.028	−0.034	−0.048	−0.050
f	−0.015	−0.019	−0.029	−0.029
j			−0.010	−0.010

[a] Variations lower than 0.010 ppm were not taken into account. The experiments were carried out at concentrations of 2.5 mM (1.0 mL each phase).

Extraction Studies. The extraction capacity of the DBF-bz receptor for the uptake of substrates, such as ammonium salts ($MeNH_3Cl$), alkali halides (KF, KCl, KBr, KI), and amino acids, $H_3N^+\text{-}(CH_2)_n\text{-}CO_2^-$ (with n = 1, 2, and 3, glycine, β-alanine, and 4-aminobutyric acid, respectively), was investigated.

The cryptand DBF-bz is able to extract halide salts (KF, KCl, KBr, and KI) to chloroform solution from a neutral aqueous phase. Liquid−liquid extraction studies were performed by allowing a solution of DBF-bz in $CHCl_3$ exposed to an aqueous solution of the desired salt prepared in a 1:1 receptor/substrate molar ratio. The chemical shift variations of H_c, H_f, and H_j proton resonances correspond to the formation of supramolecular entities with the substrate located in the rigid part of the molecule, between the two aromatic subunits; see Table 1. The resonances of these aromatic protons undergo a downfield shift in relation to the free receptor, which indicates that the isophthalamide group is involved in the association process via two $X^-\cdots H\text{-}NCO$ hydrogen bonds. However, the shifts of the NMR signals are downfield, which may be explained by conformational restrictions of the receptor around the two aromatic subunits, and the consequent structural modifications induced by the binding of the halides in the same location.[15] These shifts can also be explained by formation of the ion pair K^+Cl^-, with, in this case, the K^+ coordinated by the two amine nitrogens and the oxygen of DBF unit, as found in the Molecular Modeling Studies (see below). Although the coordination of the K^+ was not expected at this position but at the ether moiety instead, the fact that no variation of the chemical shift of H_l, H_m, or H_n proton signals was observed supports the model proposed by the theoretical studies. Mass spectral analyses of the same solutions confirmed the presence of both potassium and halide anions forming supramolecular entities $[DBF\text{-}bz(X)]^-$ and $[DBF\text{-}bz(K)]^+$ (see the Supporting Information). Unhappily, the entity [(DBF-bzK)X] cannot be detected by this technique. In fact, the ion species observed by the ESI process, such as $[M + H]^+$, $[M + 2H + Cl]^+$, and $[M + K]^+$, resulted from proton transfer or removal of weakly bound substrates. Consequently, the proton transfer needed to induce the ionizable species leads to the demetalation of the potassium cation.

On the other hand, the chemical shift variations increase from fluoride to iodide, according to the sequence $I^- > Br^- > Cl^- > F^-$, which was confirmed by mass spectral analysis.[16] This observed order may be ascribed to the solvent-induced Hofmeister tendency favoring large charge-diffuse anions and therefore the degree of aqueous solvation or to the selective fit of the

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(16) The relative intensity of the peaks of 663.1 $[(DBF\text{-}bz) + Cl]^-$, 707.1 $[(DBF\text{-}bz) + Br]^-$, and 755.1 $[(DBF\text{-}bz) + I]^-$ indicate that chloride is completely substituted by iodide inside the cavity, only partially substituted by bromide, and not substituted by fluoride.

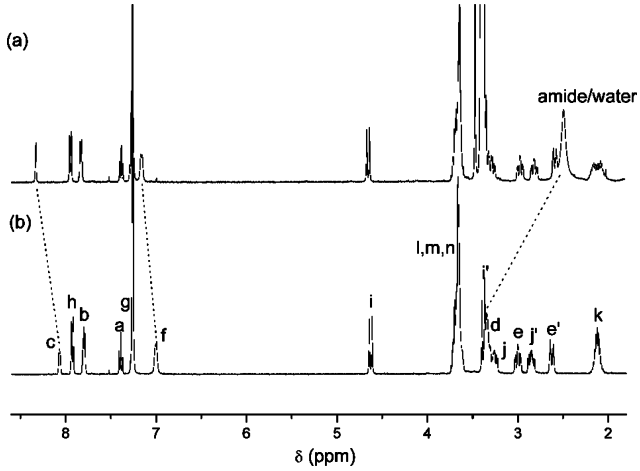

FIGURE 4. ^{1}H NMR spectra of DBF-bz (a) and the associated entity formed upon addition of ~4 equiv of *n*-Bu_4NCl (b). [DBF-bz] = 5 mM in $CDCl_3$ solution at 298 K and 400 MHz.

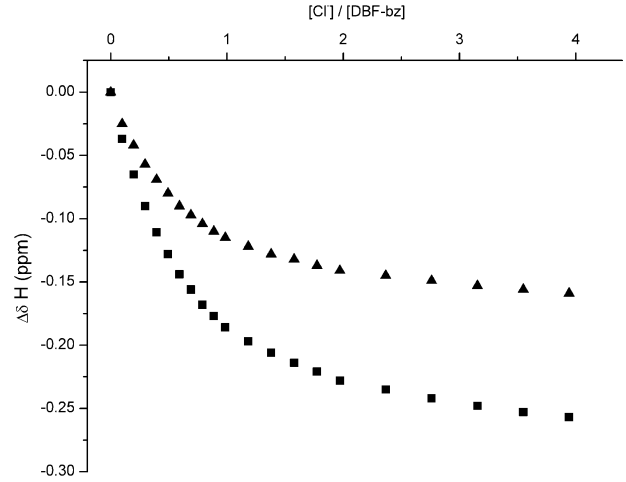

FIGURE 5. ^{1}H NMR chemical shift variations ($\Delta\delta$ H) in function of the amount of added anion observed in the course of the titration of DBF-bz with Cl^-, following H_c (■) and H_f (▲), in $CDCl_3$.

larger anions into the macrobicyclic cavity.[17] In general, it is difficult to overcome the Hofmeister tendency in liquid−liquid systems,[1b] and examples of true deviations from this expected order by hydrogen bond donor receptors are rare.[18] However, the selective fit exists in this case as revealed by ^{1}H NMR titration experiments, see below.

Furthermore, solid−liquid and liquid−liquid extraction experiments of methylammonium chloride and some amino acids (glycine, β-alanine, and 4-aminobutyric acid) by DBF-bz, from neutral or acidic aqueous solutions, failed. ^{1}H NMR spectra (changes in the chemical shifts of H_c, H_f, and H_j proton resonances) and ESI-MS (peak at m/z = 665.3 [(DBF-bz) + 2H + Cl]$^+$ indicated only the inclusion of chloride anion. These results indicate that the trioxa moiety of the macrocycle cannot adopt an appropriate conformation for the binding of these cationic species, and emphasizes the importance of K^+ in the extraction process. This result can be explained by the small number of donor atoms of the trioxa framework together with the restricted flexibility imposed by the geometry of the macrobicycle, and the particular position occupied by K^+ in the complexation (see the Molecular Modeling Studies section).[9,10]

^{1}H NMR Titration. The binding affinity process between the neutral receptor DBF-bz and the series of halide anions was quantitatively evaluated by ^{1}H NMR titration experiments in $CDCl_3$ (and in DMSO-d^6 for some of them) at rt. The binding affinity of the halide anions for the receptor was determined by titration experiments followed by ^{1}H NMR spectroscopy.

No chemical shift variation of the H_l, H_m, and H_n proton resonances was observed. Thus, the macrobicycle has negligible affinity for the tetraalkylammonium cation, and the binding constants observed only reflect the receptor-anion affinity. Changes in chemical shift of H_c and H_f proton resonances are induced by the formation of the associated entity, see Figure 4, which is consistent with $X^-\cdots H-NCO-$ hydrogen bonds.

The association constants were determined by fitting the data of titration curves to a 1:1 binding model using the HypNMR program;[19] see Figure 5 and Table 2. As expected, the receptor DBF-bz presents weak affinity for halide in polar solvent like DMSO-d_6, which is also the case for other macrobicycles.[9a,20] Significantly higher binding constants are obtained by using $CDCl_3$. For instance, the association constant between DBF-bz and chloride increase from K = 57 in DMSO-d_6 to K = 1202 in $CDCl_3$, i.e., 21 times. It is well-known that the nature of the solvent has a profound influence on the binding constants, especially when hydrogen bonds are involved in the recognition process.[1]

TABLE 2. Association Constants (log *K*) of DBF-bz with Halide Anions[a]

anion	log K^b	log K^c
F^-	2.51(4)	
Cl^-	3.08(5)	1.76(4)
Br^-	3.20(4)	1.78(6)
I^-	3.27(3)	

[a] Values in parentheses are standard deviations in the last significant figure given directly by the HypNMR program.[19] [b] In $CDCl_3$ at 300 K, with *n*-Bu_4N^+ as "spectator ion". [c] In DMSO-d_6 at 300 K, with *n*-Bu_4N^+ as "spectator ion".

Addition of F^-, Br^- or I^- induces similar spectral changes. The values of association constants (Table 2) are low, and small differences of affinity are perceived among the various halide anions. The trend followed, $I^- > Br^- > Cl^- > F^-$, is identical to that found in the extraction studies. Therefore, this sequence is not only due to the Hofmeister tendency of selectivity toward large charge-diffuse anions but also, in a significant part, to the binding properties of the macrobicyclic receptor itself. Additionally, these results suggest that all the studied anions interact with the receptor in the same sites and by the same pattern of interactions. As the potassium salts are not soluble in $CDCl_3$, the effect of the potassium binding could not be quantitatively evaluated.

In order to use the total ability of the receptor toward anions, the binding properties of its diprotonated form, $(H_2DBF\text{-}bz)^{2+}$, were also studied by using the ^{1}H NMR spectroscopic titration technique. The diprotonated receptor is soluble in water;

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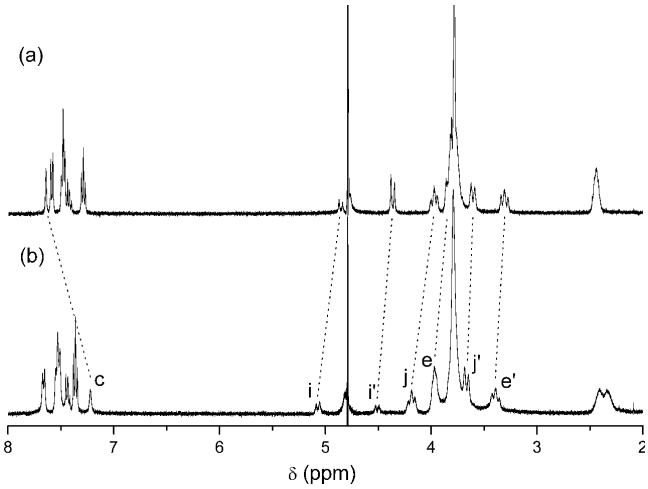

FIGURE 6. ^{1}H NMR spectra of $(H_2DBF\text{-}bz)^{2+}$ (a) and the associated entity formed upon addition of ~4 equiv of KCl (b). [DBF-bz] = 5 mM in D_2O solution at 298 K and 400 MHz.

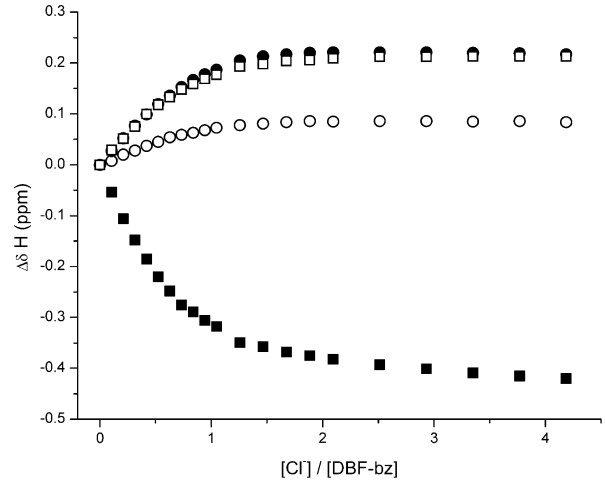

FIGURE 7. ^{1}H NMR chemical shift variations ($\Delta\delta$ H) in function of the amount of added anion observed in the course of the titration of DBF-bz with Cl^-, following H_c (■), H_i (●), H_j (□), and H_e (○), in D_2O and in the presence of 11 equiv of CF_3COOD.

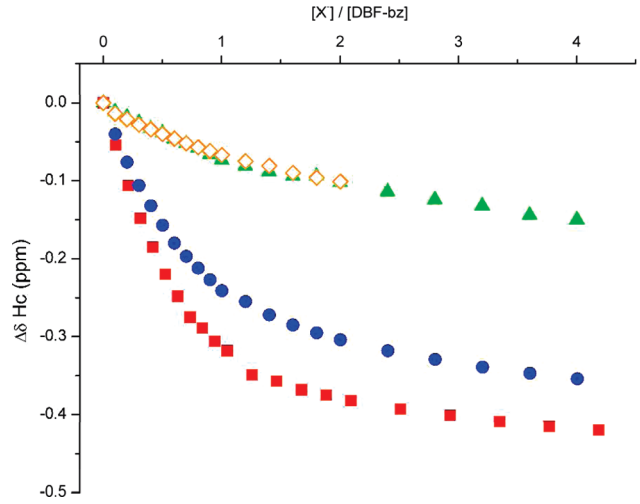

FIGURE 8. ^{1}H NMR chemical shift variations in function of the amount of anion added observed during the course of the titration of DBF-bz with F^- (▲), Cl^- (■), Br^- (●), and I^- (◇), following H_c in D_2O and in the presence of 11 equiv of CF_3COOD. X = anion.

therefore, the studies were performed in this medium. The two tertiary amine of the macrobicycle DBF-bz, upon protonation, can be involved in anion recognition together with the amide functions. Even in highly polar solvent media, the charged receptor should bind anions forming up to four hydrogen bonds.

The receptor was fully protonated by addition of 11 equiv of trifluoroacetic acid (pD = 1.40) in order to improve the solubility of all species.[8] Some advantages were found in using this acid: first, this acid does not present visible nucleous in ^{1}H NMR spectra that eventually may disturb the interpretation by overlapping of signals (which is the case of TosOH, for instance); second, the counterion CF_3COO^- is weakly bound to the macrobicycle and can be easily displaced by the other anionic species.

The ^{1}H NMR spectra of $(H_2DBF\text{-}bz)^{2+}$ display dramatic changes in the chemical shift values of the ammonium/amide moiety upon addition of the halide anions, Figure 6. For instance, for Cl^- the signal of the H_c proton moved downfield, $\Delta\delta$ (H_c) = −0.420 ppm, whereas those around the ammonium groups, H_e, H_i or H_j, shifted upfield, $\Delta\delta$ (H_e) = +0.123 ppm, $\Delta\delta$ (H_i) = +0.217 ppm, $\Delta\delta$ (H_j) = +0.213 ppm. Addition of F^-, Br^-, or I^- induces similar spectral changes as shown in Figure 7. These observations suggest that the halide anions displace the trifluoroacetate counteranion by binding the receptor in the same place and by similar pattern of interactions *via* multiple N−H···Cl^- hydrogen bonds in aqueous solution.

Quantitative assessment of the anion binding affinities of the receptor $(H_2DBF\text{-}bz)^{2+}$ in acidic D_2O revealed that the macrobicycle only forms species of 1:1 stoichiometry with the halide anions (see Figure 8 and Table 3). Among the different anions investigated, the largest affinity for the $(H_2DBF\text{-}bz)^{2+}$ receptor is observed for chloride. The association constants for the interaction of halide anions with the protonated form of the macrobicycle follows the order $Cl^- > Br^- > I^- \approx F^-$ with an increase in the *K* values from 135 with I^- to 4900 with Cl^-. The observed discrimination between halide anions, monocharged and spherical by nature, must be based on size-match between the cavity of the macrobicyclic receptor and the anionic substrate, which display the best fit for chloride. Similar pattern was also found by Esteban-Gómez et al.[8]

It is interesting to notice that the association constants and the selectivity increase in the case of the chloride anion from the neutral receptor, in aprotic solvents, to the double charged receptor in the highly polar solvent D_2O, while the inverse effect was observed for the other halide substrates.

TABLE 3. Association Constants (log *K*) of $(H_2DBF\text{-}bz)^{2+}$ with Halide Anions in D_2O (pD = 1.40 and *T* = 298.2 K)

anion	log K^a
F^-	2.28(2)
Cl^-	3.69(4)
Br^-	3.11(1)
I^-	2.13(5)

[a] Values in parentheses are standard deviations in the last significant figure given directly by the HypNMR program.[19]

Molecular Modeling Studies. The structural preferences and the binding ability of DBF-bz toward K^+Cl^- ion pair and water molecule were investigated theoretically by means of molecular mechanics (MM) and molecular dynamic (MD) simulations with the Amber10[21] software, using the GAFF[22] force field as described in the Experimental Section. Our studies started with conformational analysis of free DBF-bz by molecular quenching

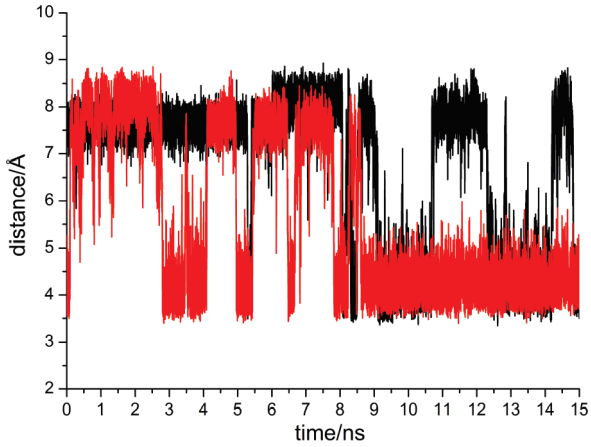

FIGURE 9. Evaluation of the distances between the centroids of the aromatic ring units during the course of the two independent 15 ns simulations. The red and black lines correspond to simulations 1 and 2, respectively.

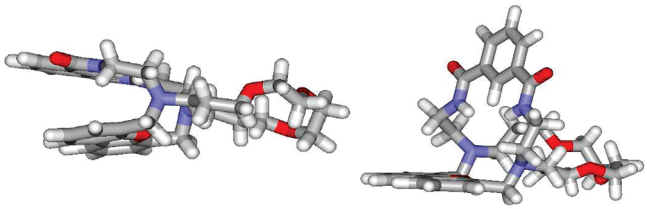

FIGURE 10. Average structures of the arrangements A (left) and B (right) during the simulation period. The snapshots were taken from 9 to 15 ns (simulation 1) and 0−5 ns (simulation 2). The solvent molecules were omitted.

dynamics. Two different conformations separated in energy by only 2.2 kcal mol^{-1} and having different relative spatial dispositions of the DBF and isophthalamide frameworks were found. In the lowest energy conformation (arrangement A), the two aromatic ring units are approximately parallel at an interplanar distance of 3.84 Å while in a higher energy, arrangement B, the two aromatic fragments are nearly perpendicular to each other making a dihedral angle of 66.7°, without the possibility of establishing $\pi-\pi$ stacking interactions.

The dynamic flexibility of DBF-bz was evaluated by two independent MD simulations carried out with arrangement A (simulation 1) and B (simulation 2) in $CHCl_3$ explicit solvent at 300 K for 15 ns. The variation of the distance between the centroids of the aromatic rings observed during the entire simulations period (Figure 9) shows that both structures are converted to each other. In solution the arrangement A is slightly favored in 1.03 kcal mol^{-1} by molecular mechanics energy, probably due to the $\pi-\pi$ stacking interactions. Figure 10 shows the average structures for the A and B arrangements obtained from the simulations 1 and 2, respectively.

The extraction and mass spectra studies revealed the capability of the neutral receptor DBF-bz to extract halide salts from aqueous solutions into the organic phase. In order to obtain

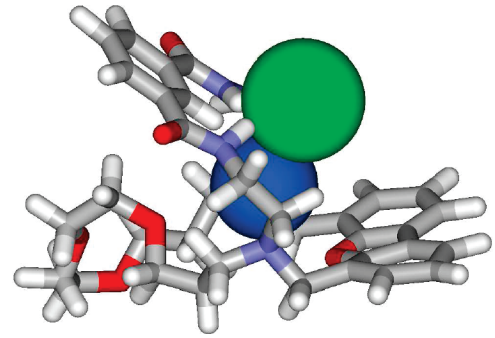

FIGURE 11. Snapshot taken at 8 ns of MD simulation. The receptor is represented in the sticks model and the potassium (blue) and chloride (green) in the space-filling model. The $CHCl_3$ molecules were omitted for clarity.

further insight into the binding process, namely the hypothetical ion pair recognition, MD simulations in $CHCl_3$ were also carried out with chloride and potassium in close contact and located into the macrobicyclic cavity, between the isophthalamide cleft and ether linkage, with anion hydrogen bond to N−H binding sites. Two different binding scenarios were analyzed as described in the Experimental Section. In the first binding arrangement, the receptor has the conformation A and, in the second, a conformation B. The first binding scenario is not stable with the anion leaving the cavity even during the heating stage of the equilibration process. By contrast, the second binding scenario, equivalent to the crystal structure of DBF-bz·H_2O, the supramolecular association is maintained during the entire course of the simulation as shown by the evolution of intramolecular $N_{DBF\text{-}bz}\cdots Cl^-$ and $O_{ether}\cdots K^+$ distances (Figures S17 and S18 in the Supporting Information). Two $N-H\cdots Cl^-$ hydrogen bonding interactions occur simultaneously during almost the entire simulation leading to average $H\cdots Cl^-$ distances of 2.41 and 3.09 Å, respectively. The position of the potassium relatively to the oxygen atoms of DBF-bz was also monitored showing that potassium ion is weakly bound only to one oxygen from the ether linkage and to the oxygen of the DBF ring, with an average distances of 2.97 and 2.71 Å, respectively. In addition, the average distances between the potassium and the two tertiary amines are of 2.93 and 2.99 Å, suggesting that the cation is also bonded to these donor atoms. The average distance between the potassium and the chloride along the simulation period is 2.73 Å. Therefore, the structural findings of this simulation suggest the simultaneous recognition of the chloride and potassium in a binding arrangement illustrated in Figure 11 with a snapshot of MD simulation. The potassium is inside of the receptor cavity while the chloride is located outside of cavity bonded to two amide groups of isophthalamide fragment by $N-H\cdots Cl^-$ hydrogen bonds and to potassium by electrostatic interactions.

The DBF-bz is capable of binding a water molecule as revealed by the X-ray structure and the NMR studies described above. Although in the literature other compounds containing isophthalamide fragments (or pyridine instead of benzene) presenting the same behavior[9a,23−25] do not exist studies of this binding interaction. The dynamic behavior of the DBF-bz·H_2O binding association was also evaluated by solvating the X-ray

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structure with $CHCl_3$ and performing a 15 ns long MD simulation at 300 K. The water molecule remains encapsulated during the first ca. 11 ns, and then it leaves the receptor during ca. 2 ns and after that returns again to the cavity until the end of the simulation. When the water molecule is inside the cavity, two intermolecular N−H···O hydrogen bonding interactions are formed simultaneously with the amide binding groups, with average distances of 1.94 to 2.64 Å, respectively. This dynamic behavior is consistent with the X-ray and ^{1}H NMR findings.

It is interesting to emphasize that the experimental results pointed out that some structural modifications will be induced into the receptor by the substrates upon the formation of the association, which was confirmed theoretically.

Conclusions

A straightforward synthetic route to prepare the DBF-bz macrobicycle is reported. The folded conformation of the macrobicycle DBF-bz, having rigid aromatic frameworks and a flexible aliphatic linkage, was confirmed in solution by NMR studies, crystal X-ray diffraction analysis and MD simulations. In aprotic solvents the electroneutral macrobicycle DBF-bz behaves as an effective anion receptor that not only displays high binding constants for halide ions, but also is capable to extract potassium halide salts from aqueous solutions into organic phases. This finding may afford new opportunities for chloride ion transport studies.

On the other hand, in acidic aqueous solution, the diprotonated macrobicycle uptakes spherical halide anions *via* multiple hydrogen bonds and electrochemical interactions inducing discrimination between them favoring the chloride anion.

These findings led us to consider to redesign the macrobicyclic receptor in order to increase the number of well-located hydrogen bond donor groups around the anionic substrate. The increase of the number of oxygen atoms in the ether moiety of the DBF-bz will also contribute to enhance ion-pairs affinity. This work is under progress.

Experimental Section

Reagents. The reagents were obtained from commercial suppliers and used as received unless noted otherwise. *N*,*N*′-Bis(2-aminoethyl)-[22](DBF)N_2O_3 was prepared by reported methods.[10] KF (>99%), KCl (>99,5%), KBr (>99.5%), KI (>99,5%), tetramethylammonium bromide (>99%), tetramethylammonium chloride (>98%), tetrabutylammonium fluoride (>97%), tetrabutylammonium bromide (>99%), and tetrabutylammonium iodide (98%) were of reagent grade and dried under vacuum before use. The tetraalkylammonium salts were previously recrystallized from isopropanol.

Synthesis of DBF-bz. *N*,*N*′-Bis(2-aminoethyl)-[22](DBF)N_2O_3 (0.430 g, 0.86×10^{-3} mol) was dissolved in dry THF/CH_2Cl_2 1:1 (200 mL), and isophthaloyl dichloride (0.174 g, 0.86 mmol) was also dissolved in the same volume of the mixture solvents. A 1000 mL three-neck round-bottom flask was charged with 300 mL of CH_2Cl_2 and triethylamine (0.191 g, 1.89 mmol). Via a two pressure-equalizing dropping funnels, both reactants were added simultaneously dropwise to the flask over a period of 4 h with vigorous stirring and under nitrogen at 0 °C. The reaction mixture was then stirred for further 14 h, and the solvent was removed under vacuum, leaving a yellow powder. The crude material was dissolved in CH_2Cl_2 and washed successively with saturated $NaHCO_3$ and saturated brine. Evaporation of the organic layer and purification on a silica gel column (50:1 CH_2Cl_2/MeOH) yielded pure DBF-bz: yield 37%; ^{1}H NMR (400 MHz, $CDCl_3$, δ/ppm, *J*/Hz) 2.13 (m, $J = 12.0$, 4 H), 2.60 (dt, $J = 13.1$, 2 H), 2.83 (dt, $J = 12.5$, 2 H), 2.98 (dt, $J = 12.1$, 2 H), 3.27 (m, $J = 12.5$, 6 H), 3.37 (d, $J = 12.2$, 2 H), 4.66 (d, $J = 12.2$, 2 H), 7.15 (d, 2 H), 7.28 (t, 2 H), 7.39 (t, 1 H), 7.83 (d, 2 H), 7.95 (d, 1 H), 8.32 (s, 1 H); ^{13}C NMR (400 MHz, $CDCl_3$) 25.4, 36.9, 51.1, 53.0, 55.3, 69.7, 69.8, 70.9, 120.3, 122.4 (×2), 124.6, 128.9, 129.5, 131.2 (×2), 133.8, 154.9, 165.8 (see Figure S19 of the Supporting Information). Anal. Calcd for $C_{36}H_{44}N_4O_6 \cdot 1.5H_2O$: C, 65.93; H, 7.22; N, 8.54. Found: C, 65.84; H, 7.56; N, 8.48. Mp (dec): 206−7 °C.

Preparation of Tetrabutylammonium Chloride Salt. A solution of tetrabutylammonium hydroxide (18 mmol) in methanol (180 mL) was stirred with a concentrated solution of hydrochloric acid (4 M, ∼4 mL) until pH∼8. The solvent was removed under vacuum, and the residue was dissolved in water (50 mL). Then, the pH was carefully adjusted to 7.00 by adding small aliquots of hydrochloric acid (0.1 M). Evaporation of the solvent and purification of the salt by recrystallization from 2-propanol yielded white crystals: yield 57%. Anal. Calcd for $C_{16}H_{36}NCl \cdot 0.1i$-PrOH: C, 68.95; H, 13.06; N, 4.93. Found: C, 68.87; H, 13.19; N, 4.63.

Crystallography. Crystallographic Data. Crystal data for DBF-bz: $C_{36}H_{45}N_4O_7$, $M = 645.76$; triclinic $P\bar{1}$, $a = 10.9758(4)$ Å, $b = 12.22418(4)$ Å, $c = 14.2488(5)$ Å, $\alpha = 95.135(2)°$, $\beta = 106.852(2)°$, $\gamma = 113.930(2)°$, $V = 1627.19(10)$ Å^3, $Z = 2$, $D_c = 1.320$ Mg/m^3, $\mu = 0.092$ mm^{-1}. CCD from Bruker Axs Mo Kα radiation, $\lambda = 0.71073$ Å, $T = 150(2)$ K. 8392 reflections were collected and used in solution and refinement of structure. The refinement of 432 parameters converged to *R* indices $R_1 = 0.0928$, w$R_2 = 0.1356$ for all data and $R_1 = 0.0475$, w$R_2 = 0.1107$ for 5390 reflections with $I > 2\sigma(I)$.

The structure was solved by direct methods and subsequently refined by full-matrix least-squares methods on F^2 using the SHELX-97 suite.[26] Anisotropic thermal parameters were used for all non hydrogen atoms. The C−H and N−H hydrogen atoms were placed at calculated positions with $U_{iso} = 1.2U_{eq}$ of the parent atom refined with individual isotropic thermal parameters. The atomic positions of the hydrogen atoms of the water solvent were obtained from final difference Fourier maps and they were included in the refinement of the structure with individual isotropic temperature factors. Molecular diagrams were drawn with PLATON[27] and PyMOL.[28]

Liquid−liquid Extraction Studies. A solution of BDF-bz in $CHCl_3$ (1 mL, 5.0 mM) and solutions of the salt (KF, KCl, KBr, KI) or the substrate ($MeNH_3Cl$, glycine, β-alanine, 4-aminobutyric acid) in water (1 mL, 5 mM) were prepared in a 1:1 molar ratio. When acidic aqueous phase was used, 2 equiv of HCl was first added to the substrate. Extractions were performed in a 2 mL glass tube. The organic phases collected (3 × $CHCl_3$) were dried on Na_2SO_4 and evaporated under vacuum. The residue was dissolved in $CDCl_3$, and ^{1}H NMR spectra were performed for the DBF-bz and the associated entity formed. The chemical shift variations were calculated as $\Delta\delta = \delta_{initial} - \delta_{final}$.

Titrations Followed by ^{1}H NMR Spectroscopy in Acidic D_2O Solution, in $CDCl_3$ or in DMSO-d_6. The acidic solutions of the receptor $(H_2DBF\text{-}bz)^{2+}$ (5.00 mM) was prepared at pD = 1.4 in D_2O by addition of 11 equiv of CF_3COOD. The substrate solutions (50.0−52.0 mM) were prepared from the potassium salts in D_2O. In deuterated solvents ($CDCl_3$ or DMSO-d_6), solutions of the receptor DBF-bz (5.00 mM, 500 μL), and solutions of the envisaged substrates (50.0 mM, 1000 μL) in the tetramethyl- or tetrabutylammonium form were prepared just before measurement.

Typically for the solution of DBF-bz (500 μL), small aliquots of anion salt solutions were added using a micropipet of 25 μL directly into the NMR tube, and an ^{1}H NMR spectrum was acquired after each addition. A 2 min free period was carefully respected

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(27) Spek, L., *PLATON a Multipurpose Crystallographic Tool*, Utrecht University, Utrecht, The Netherlands, 1999.
(28) Delano, W. L. *The Pymol Molecular Graphics System*, 2002, http://www.pymol.org.

before measurement. About 20 additions were necessary for each titration until no significant change in the chemical shift was observed. All measurements were done at 300 K. No effort was made to maintain constant the ionic strength in order to avoid competition of other anions.

The association constants of the various species formed in solution were determined from the experimental titration data using HypNMR,[19] which requires as input the concentration of each component and the observed chemical shift (proton resonances which exhibit significant induced shifts upon the formation of the associated entity were chosen). The fitting included the binding constants and the chemical shift of each species. Initial calculations provided overall stability constants, $\beta[(H_nL)A_a]$ values, being $\beta[(H_nL)A_a] = [(H_nL)A_a]/[H_nL] \times [A]^a$, L = DBF-bz and A = anion. The errors quoted are the standard deviations of the overall stability constants given directly by the program from the input data, including the experimental points for all resonances of the titration curves.

Molecular Modeling Simulations. Molecular mechanics (MM) and molecular dynamics (MD) simulations were carried out using Amber10[21] suites of program, with parameters for DBF-bz taken from the GAFF[22] force field. The chloride and potassium ions were described with nonbonded parameters developed for the TIP3P water model obtained from ref 29. The $CHCl_3$ solvent molecules were described using an explicit full atoms solvent model with atomic charges and force field parameters taken from ref 30. The chloride and potassium charges were assigned to -1 and $+1$, respectively. The atomic charges for the receptor were calculated at the HF/6-31G(d) level using RESP methodology with Gaussian03.[31] The starting model for receptor was generated from the X-ray structure removing the water molecule. The conformational analysis of the receptor was carried out by MD using the following procedure: the MM minimized structure was subjected to a MD quenching run in gas phase at 2000 K using a time step of 1 fs. Twenty thousand conformations were collected over 2 ns simulation time, and then they were minimized by MM using an in-house script. Two different conformations were found: the lowest energy (arrangement A) and an equivalent to the X-ray structure (arrangement B) (see above). They were both used as starting geometries for the simulations carried out with free receptor in $CHCl_3$. The simulations of KCl binding recognition were carried out in both conformational arrangements with chloride and potassium inserted into the macrobicyclic cavity positioned in close contact and pointing to the N−H biding sites and to the oxygen atoms of isophthalamide fragment and ether oxygen atoms respectively. In addition, the binding arrangement A was build using the lowest energy conformation of the free receptor while the binding arrangement B was derived directly from BDF-bz X-ray structure by replacement of water by chloride. This structure was also used for the simulation with supramolecular entity formed with TIP3P water molecule.

The DBF-bz and the supramolecular entities were solvated with the $CHCl_3$ model giving cubic boxes containing between 381 and 438 solvent molecules. All the MD simulations started with equilibration of the system under periodic boundary conditions using a multistage protocol composed of two successive MM minimizations, to eliminate undesired short potential contacts. In the first MM minimization 10000 steps of steepest descent method followed by 10000 steps of conjugate gradients methods was done keeping a positional restrain of 500 kcal mol^{-1} $Å^{-2}$ on the receptor or receptor/substrate. In the second MM minimization the positional restrain was removed and 1000 steps by the steepest descent method followed by 10000 steps of conjugate gradient methods was made. Next, a MD-NVT simulation with weak positional restrain of 10 kcal mol^{-1} $Å^{-2}$ on the receptor or receptor/substrate was run for 50 ps to bring the temperature to 300 K. Then, the positional restrain was removed and the equilibration process proceed with a MD-NPT simulation run of 500 ps to adjust the solvent density given rise to a cubic boxes with final sizes between 37.47 and 39.11 Å. At this stage, the average value of the density remained constant at least during the last 100 ps of the NPT simulation. The density of the periodic box was in agreement with the experimental density for liquid $CHCl_3$ at room temperature. Finally, MD data collection run was produced at 300 K and 1 atm for 15 ns using a NPT assemble. All the bonds involving hydrogen atoms were constrained using SHAKE,[32] which allowed the use of a time step of 2 fs. The particle mesh Ewald method[33] was used to describe long-range electrostatic interactions and the nonbonded van der Waals interactions were subjected to a 12 Å cutoff.

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Supporting Information Available: ROESY NMR spectrum of DBF-bz in $CDCl_3$; ESI-MS spectra of liquid−liquid extraction experiments of KF, KCl, KBr, KI, and $MeNH_3^+Cl^-$ in positive- and negative-ion mode by DBF-bz in $CHCl_3$ solutions; ^1H NMR titration curves in $CDCl_3$ (with F^-, Cl^-, Br^-, and I^-) or in DMSO-d_6 (with Cl^- and Br^-) and in acidic D_2O solution (with F^-, Cl^-, Br^-, and I^-). Molecular modeling studies: evaluation of the NDBF-bz···Cl^- and the O_{ether}···K^+ distances during time. This material is available free of charge via the Internet at http://pubs.acs.org.

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