Investigation of Synthetic Hosts That Model Cation-π Sites Found at Protein Binding Domains

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Small cyclophanes containing aromatic groups and dialkyl ammonium ions were created as model systems of the cation-π complexes found at some protein binding domains. The hosts had different shapes in order to investigate the effect the arrangement of ammonium ions to aromatic surfaces has on their reactivity. pK_a values of the hosts were substantially different in DMSO or (95/5) DMSO/D_2O solutions, which showed that the ions existed in different environments of the hosts. Electrostatic charges, as determined by density functional calculations, revealed that the magnitude of a cationic charge depends on its position relative to an aromatic ring. Association constants of the hosts bound to the sodium salt of *N*-acetyl phenylalanine in d_6-DMSO and in (95/5) d_6-DMSO/D_2O solutions were inversely proportional to the magnitude of the hosts' acidity constants. These results suggest that the magnitude of the positive charge for cationic groups of cation-π complexes is reduced by being associated with electron-rich faces of aromatic rings. The aromatic rings, however, lessen the desolvation penalty that must be overcome for ligand binding, giving an overall more favorable association.

Introduction

Since the time it was first noted that amino groups were predominantly associated with aromatic rings in X-ray crystallographic structures of proteins,[1] the cation-π interaction has been postulated to be an important component of protein stabilization,[2] ligand recognition,[3] and catalysis.[4] Because protein interactions involve a complex mixture of noncovalent forces, important insights into the cation-π interaction have been obtained by investigating model systems. Gas-phase studies of small ions with benzene rings,[5] high-level calculations,[6,7] and model peptides[8] have shown that cations bind aromatic rings with a substantial amount of free energy. For example, the interaction between K^+ and benzene in the gas phase produces 19 kcal/mol of energy, which is actually 1 kcal/mol more favorable than the binding energy observed for K^+ interacting with H_2O.[5c] Artificial receptors, based on cyclophanes, bind tetraalkylammonium salts tighter than neutral guests in water and organic solvents,[9] which strongly supports the hypothesis that the cation-π interaction is important source of free energy for the binding of quaternary ammonium ligands by proteins.[10] Lariet ether based receptors interact with Na^+ and K^+ in the solid and solution phases.[11] Dougherty's studies, along with theoretical calculations, have revealed that the free energy is obtained through favorable interactions between the cation and the electron-rich face of an aromatic ring, which is a consequence of the ring's permanent quadrupole moment.[6f,9] Polarization and dispersion interactions may also contribute to complex stability.[6b,7c,12]

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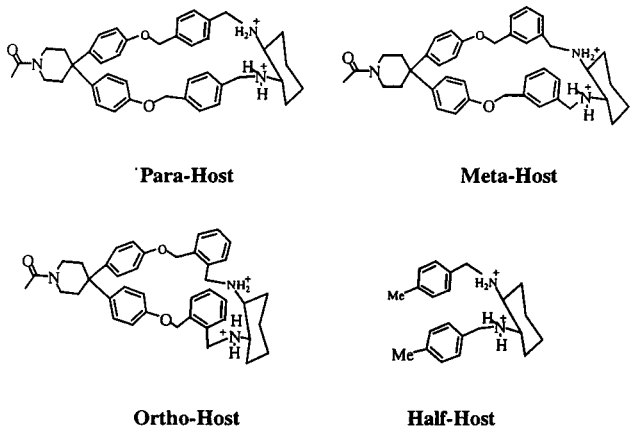
The studies mentioned above examine the interaction energies between a cation and a π-system. They do not explore the interaction energies that occur between a cation-π site, displayed at some protein binding domains, and polar or negatively charged ligands. For example, Arg43 of the human growth hormone receptor (hGH_R) is nestled between Trp104 and Trp169, and this cation-π site contacts Thr175 and Asp171 of the human growth hormone binding protein.[13] An even more pronounced cation-π stacked domain is seen in the extracellular domain of hGH_R.[14] Lys-Tyr-Arg-Phe-Arg-Trp-Lys exists in an almost continuous stacked arrangement, but the biological function, if any, of this domain has not been determined.[9] At first glance, it seems reasonable that a cation-π site would interact favorably with negatively charged or polar ligands. Ostensively, the role of aromatic rings is to partially desolvate the cation, which lowers the desolvation penalty for salt bridge formation or to align the positive charge, giving more structure to the binding domain. On the other hand, favorable interactions between the positively charged side chain and the aromatic surface of a cation-π site must detract from the free energy available for salt bridge formation.

To investigate the structure−function relationship of cation-π sites exposed at binding domains, we have created a series of artificial receptors that contain hydrophobic patches and dialkyl ammonium ions. The shapes of the hosts were altered to determine what effect the spatial arrangement of ammonium ions and aromatic rings has on a host's activity. Activity of the cation-π domain was ascertained by comparing the acidity constants of the hosts and their association constants for complexes of the sodium salt of *N*-acetyl phenylalanine (Phe(**I**)). Phe(**I**) was chosen as a representative ligand because it contains a carboxylate and a phenyl ring that are likely to interact favorably with a cation-π site. We specifically wanted to determine whether the spatial arrangement of ammonium ions to aromatic rings affects the magnitude of the cationic charge, whether the shape of cation-π sites affects their ability to form a salt bridge, and what affect solvent has on these interactions.

Results and Discussion

Host Design. Three hosts, referred to as para, meta, and ortho, were designed to bind Phe(**I**) in DMSO and DMSO−water mixtures through mainly electrostatic interactions. The pockets of the hosts were made too small to fully encapsulate an aromatic guest. We wanted to mimic the narrow grooves found at protein surfaces and not their deep binding pockets. Thus, van der Waals interactions and the hydrophobic effect, for complexes formed in a DMSO−water mixture, should be minor contributors to the overall binding energy. Changes in the position of the hydrophobic pocket to the dialkyl ammonium ions were accomplished by changing the substitution pattern of the central aromatic ring, which we refer to as the hinge. The cyclohexyl ring also gives the hosts complex shapes similar to the clefts and bulges observed at protein binding domains. The physical properties of a half host were investigated as well to determine whether the more rigid alignment of cationic charges and aromatic rings in preformed pockets enhances Phe(**I**) association.

Chart 1



Synthesis of the Hosts. The hosts were designed to have a rigid aromatic domain, defined by a diphenylmethane unit,[15] and cationic domain through a *trans*-cyclohexanediamine ring in which the amino groups are in a fixed *gauche* orientation. Alkylation of bisphenol **1** with *para*, *meta*, *ortho* bromides **2a**−**c** in the presence of sodium hydride gave the corresponding diesters **3a**−**c** in good yields (Scheme 1). Reduction of the esters to alcohols using either DIBALH or LAH also resulted in the undesirable reduction of the tertiary amide to the amine. Selective ester reduction was obtained by using lithium borohydride generated in situ from sodium borohydride and lithium chloride. Alcohols **4a**−**c** were readily converted into aldehydes **5a**−**c** via Swern oxidation. Host formation was completed upon the addition of 1,2-diaminocyclohexane **6** through a reductive amination reaction. Disappearance of the aldehyde signal in ^{1}H NMR spectra and the concomitant emergence of the imine proton provided an easy means to monitor the reaction progression. Once the imine was formed, sodium borohydride in methanol was added to give essentially pure hosts. Half host **7** was prepared via a condensation reaction between *trans*-cyclohexanediamine **6** and *para*-tolualdehyde followed by reduction with sodium borohydride. The hosts were exposed to HCl(g) and studied as the dichloride salts.

Hosts' geometries were obtained by analyzing two-dimensional ^{1}H NMR spectra and performing molecular modeling. Proton resonances were readily assigned from one-dimensional ^{1}H NMR, COSY, and NOESY spectra.[16] There were no long range NOEs observed in the para host, which is consistent with it having a flat shape. The meta host appears to be slightly bent because a weak NOE was observed between a β-H of the cyclohexyl

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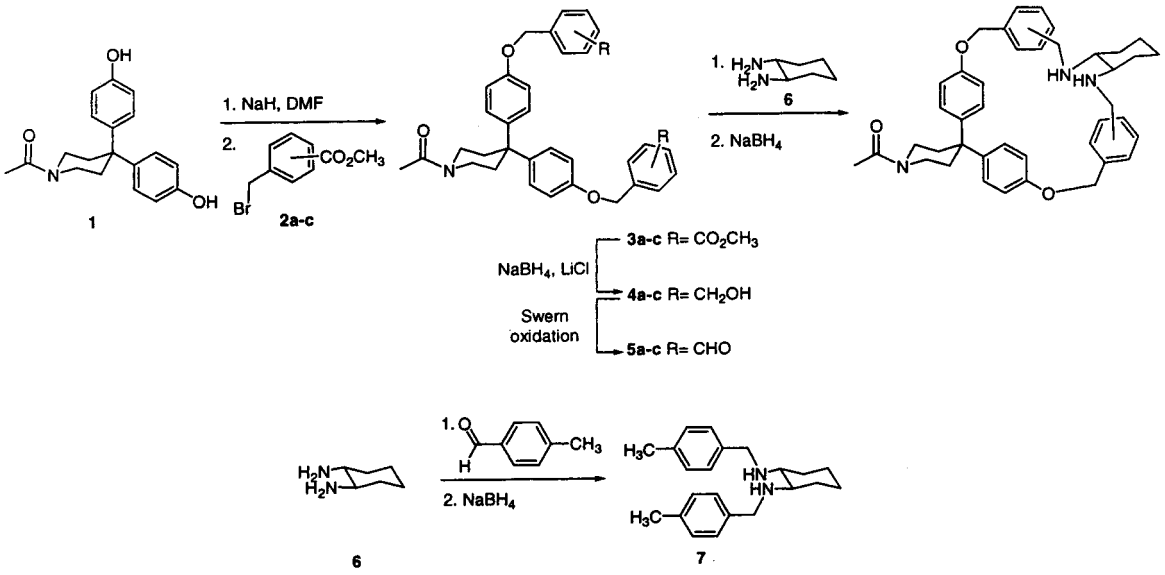
Scheme 1



diammonium ring and a meta proton of a nonhinge-aromatic ring. A more intense NOE was observed between these two protons in the ortho host, showing that it has a very compacted shape. Energy minimized structures (Monte Carlo search procedure, MMFF94 force field as presented by SpartanPro,[17] Figure 1) were consistent with the observed NOEs. The calculated structures showed that the size of the hydrophobic pockets decrease from the para to the ortho host and the N^+-H's are held in different environments. The four N^+-*H*'s (italics are used to indicate that the H-atom is being discussed) of the para host are held within an aromatic pocket, pointing inside. For the meta and ortho hosts, two N^+-*H*'s are held within and two are held without their hosts' pockets. All N^+-*H*'s are held at the face of an aromatic ring, except for the two N^+-*H*'s of the meta host that are positioned outside its cleft nearer to an aromatic edge (H3 and H4, Figure 1).

Whether an N^+-*H* is positioned at the face or edge of an aromatic ring should be reflected in its pK_a. Increasing the electronic density around a N^+-H should stabilize its positive charge and raise its pK_a, whereas decreasing the electronic density should have the opposite effect. Lund-Katz observed that the pK_a's of Lys residues are raised when they reside near aromatic surfaces and lowered when they are held close together because of electrostatic repulsion.[18] The electron density of Thr13 OH proton is increased by being placed in the center of the aromatic ring of Tyr6, according to density functional theory (DFT) calculations performed on this complex found in μ-glutathione S-transferase (μ-GST).[7b]

In our studies, a large drop in pK_{a1} is observed when one compares the para to the meta and to the ortho host in DMSO (Table 1). This lowering across the series is consistent with the energy minimized structures of the free hosts (Figure 1) and the cation-π theory. Ammonium ions held at the electron-rich face of aromatic rings, such as the ones buried within the para host, are stabilized through the cation-π interaction, and thus their pK_a's are raised. Similarly large pK_a's are observed for the half host, which has two N^+-*H*'s (H1 and H2, Figure 1D) held in the face of aromatic rings. Although the pK_a's of the meta and ortho hosts are more similar, their energy minimized structures are dissimilar. H1 and H2 of the meta host are held within an aromatic cleft, which is smaller than the pocket of the para host. The other two N^+-*H*'s of the meta host are positioned outside the pocket (H3 and H4). The N^+-H's of the ortho host are not held within an aromatic cleft, but instead they are arranged in a more elongated groove. Each N^+-H group is associated with a sole aromatic ring. The calculated partial charges (vide infra) of the N^+-H's positioned outside the pocket of the meta host are substantially larger (especially H3) than for the ortho hosts. Because the pK_a's are similar, the charges of the dialkyl ammonium ions of the meta and ortho hosts should be more similar. To account for this inconsistency, the exposed N^+-*H*'s of the meta host could interact strongly with solvent molecules, which reduces the magnitude of their charge. The meta host has a slightly greater drop in pK_{a1} and pK_{a2} (ΔpK_a's $\cong$ 0.7) than the other hosts (ΔpK_a's $\cong$ 0.3) when comparing the values obtained in DMSO to DMSO/water solutions. This larger solvent effect on pK_{a1} and pK_{a2} is consistent with the meta host's N^+-H proton being more exposed to solvent. A smaller drop is observed across the host series for pK_{a2}. This smaller change may be caused by the stabilization of the remaining single N^+-H through interactions with the electron pair of its neighboring amine.

Table 1. Acidity Constants for Hosts at 298 K

host	pK_{a1}[a] (DMSO)	pK_{a2} (DMSO)	pK_{a1} (DMSO/H_2O, 95/5)	pK_{a2} (DMSO/H_2O, 95/5)
para	6.2	9.8	5.8	9.6
meta	4.5	9.2	3.8	8.4
ortho	3.6	8.2	3.3	8.4
half	5.4	9.5	5.1	9.9

[a] Uncertainties for the pK_a's < 4%.

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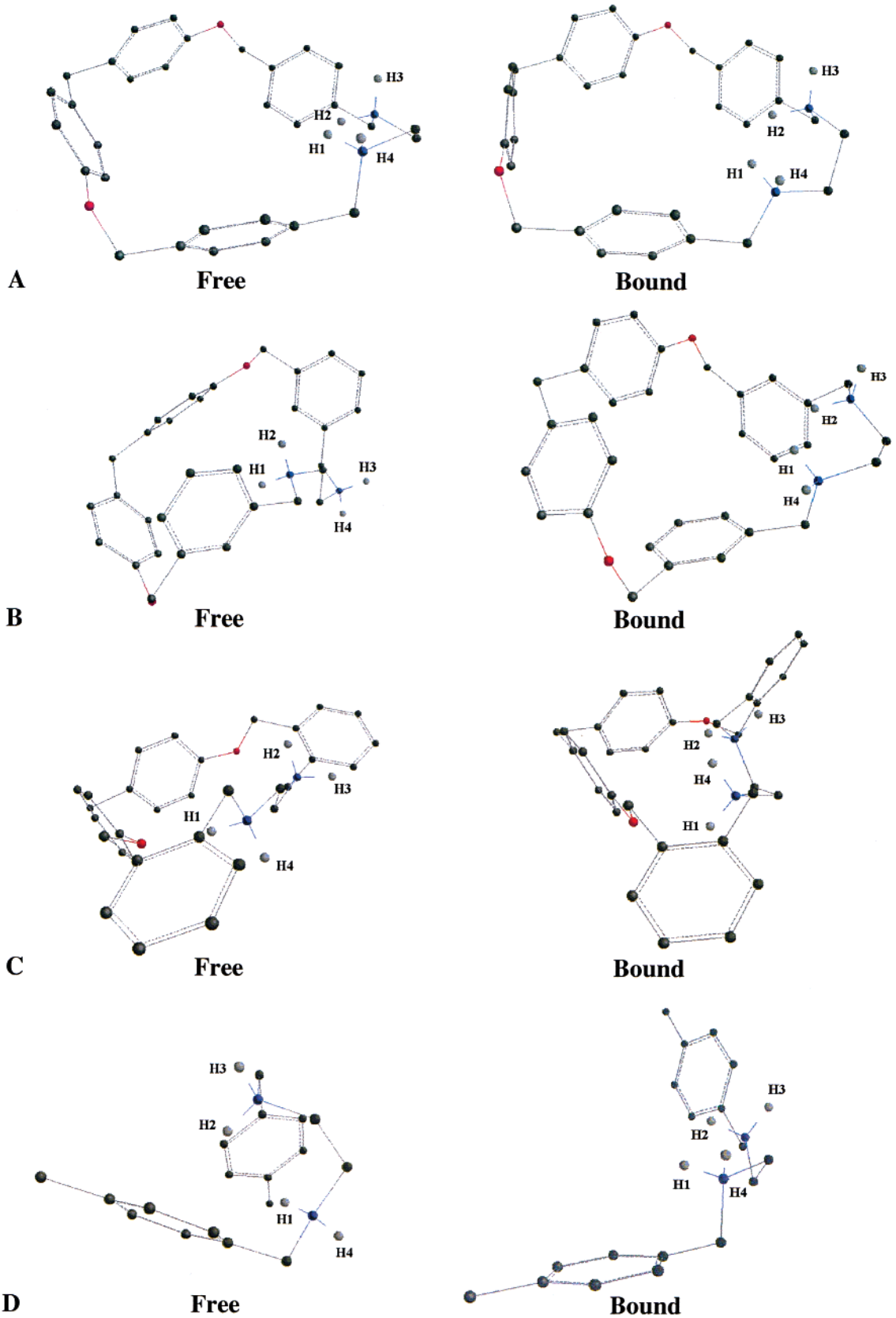

Figure 1. Structures of the (A) para, (B) meta, (C) ortho, and (D) half hosts without (Free) and with (Bound) Phe(**I**). Free conformations were obtained through Monte Carlo simulations, whereas the bound structures were solved using a combination of Monte Carlo docking simulations combined with conjugate gradient energy minimizations. Phe(**I**), C-H, *N*-acetylpiperidine, and cyclohexyl rings are removed for clarity. These structures were used to calculate the electrostatic charges of the N-H (H1-H4) hydrogens (DFT calculations), and the values are given in Table 2.

The combination of experimental and calculated results show that larger pK_a's are obtained for ammonium ions that interact favorably with the electron-rich face of aromatic rings. Stronger cation-π interactions occur for

Table 2. Electrostatic Charges[a] for Hosts and Their Diaminoethyl Portions Held in Energy Minimized Structures in Free and Bound to Phe(I) Conformations

host	structure[b]	H1[c]	H2	H3	H4
para	free	0.171	0.210	0.256	0.257
	diamine	0.283	0.317	0.336	0.342
	bound	0.160	0.186	0.289	0.267
meta	free	0.187	0.239	0.330	0.298
	diamine	0.338	0.321	0.310	0.326
	bound	0.203	0.167	0.252	0.246
ortho	free	0.121[d]	0.258	0.205	0.229
	diamine	0.285	0.301	0.315	0.313
	bound	0.205	0.181	0.213	0.301
half	free	0.156	0.156	0.273	0.273
	diamine	0.290	0.290	0.318	0.318
	bound	0.238	0.198	0.270	0.287

[a] Calculated from density functional theory pBP/DN*.[17] [b] Structures are defined as free means hosts without guest, diamine means only the diamine portion of the hosts, and bound means that the host were bound to Phe(**I**). [c] See Figure 1 for atom labeling and host geometries. [d] H1 of the ortho host makes contact with an ether-oxygen atom and is unavailable for binding.

ammonium ions placed within narrow aromatic pockets than in broader clefts or near a single aromatic ring. Stronger interactions also occur for cations positioned at an aromatic face than their edge, which is consistent with previous mp2 calculations on ammonia−benzene complexes.[6b]

Differences in the hosts' pK_a's may result from the juxtaposition of their dialkyl ammonium ions to each other and not from the positions of N^+-H's to the aromatic rings. To address this possibility, electrostatic charges of the ammonium ions were derived for the energy minimized structures of the hosts and half host using DFT calculations (Table 2; pBP/DN* model as presented by SpartanPro).[17] Semiempirical calculations using the AM1 force field did not produce significant differences in the magnitude of the charges. DFT calculations have been used successfully to predict and understand the properties of cation-π complexes.[7] The *N*-acetylpiperidine and cyclohexyl portions of the hosts were replaced with H-atoms after the hosts were minimized to lessen the computational time (Figure 1). The electrostatic charges of the hosts' N^+-*H*'s were compared to ones calculated for only the diaminoethyl domain of the hosts, which had the pockets, cyclohexanecarboxamidyl, and cyclohexyl rings of the minimized structures replaced by H-atoms. Electrostatic charges of the diaminoethyl domains are similar throughout the host series (Table 2). Only in the presence of the host's pockets are the charges of the N^+-*H*'s substantially different. In these latter calculations, smaller partial charges were obtained for N^+-*H*'s positioned within electron-rich pockets (H1 and H2, Table 2) and larger charges occurred for N^+-*H*'s held at aromatic edges or at exposed regions (H3 and H4, Table 2). These results are consistent with the measured pK_a's, and they are in accordance with the cation-π theory.

Another possibility is that the properties of the conjugate bases of the hosts are responsible for the differences observed in the pK_a's. Molecular modeling results of the various conjugate bases showed that the structure of these host were not significantly different than the fully protonated hosts except there was a slight flattening of the aromatic rings of the para host in its doubly deprotonated state. Preference for this conformation appears to be caused by an H-bond existing between the amines. Electrostatic charges of the deprotonated amines throughout the host series were not substantially different. Both results support our conclusion that the magnitude of a host's pK_a is controlled by the juxtaposition of its N^+-*H* to an aromatic surface.

Table 3. Association Constants for Host−Phe(I) Complexes at 298 K[a]

host	K_A (M^{-1}) (d_6-DMSO)	K_A (M^{-1}) (d_6-DMSO/D_2O) (95/5)
para	81 ± 5	81 ± 7
meta	59 ± 6	65 ± 13
ortho	51 ± 7	18 ± 5
half	47 ± 5	<5

[a] Obtained from ^{1}H NMR titrations; Phe(**I**) was the sodium salt.

To more accurately represent the environment of protein binding domains, we added 5% H_2O to the DMSO−host solutions. A higher percentage of water precipitated the hosts. The addition of water lowered the hosts' pK_{a1}'s about 10% (Table 1), which can be explained by the higher basicity of water as compared to DMSO. The similar drop in pK_{a1}'s throughout the host-series suggests that their structures are not altered in the presence of 5% H_2O. Furthermore, similar 2D NMR spectra were observed for each host in both solvent systems. More variable changes in pK_{a2}'s are observed with the addition of water except for the meta hosts, which is once again consistent with its N^+-*H* being more exposed to solvent.

Having different cation-π properties makes these hosts a good model system of protein receptors, allowing us to test whether cation-π sites bind negatively charged ligands and determine what factors promote this association. All hosts bound Phe(**I**) in d_6-DMSO (Table 3), which shows that the dialkyl ammonium ions are accessible for guest association. 2-D ^{1}H NMR analysis of host−guest complexes did not reveal NOEs between the binding components. Thus, it appears that association forms mainly through salt bridges. We were surprised to find that the para host binds Phe(**I**) more favorably in d_6-DMSO than the ortho and meta hosts, which had similar binding energies. According to their large pK_a's, N^+-*H*'s of the para host interact the strongest with the aromatic rings, and thus they should interact the weakest with the guest. One possible advantage afforded the para host is that its ammonium ions are both positioned within an aromatic cleft and partially desolvated, which should lower the desolvation penalty for salt bridge formation.

A greater solvent effect for association is observed with the addition of 5% D_2O to the solutions (Table 3). The affinities of the para and meta hosts for Phe(**I**) are approximately unchanged. A new NOE was observed between an aromatic proton of the phenyl ring of Phe(**I**) and the β-H of the cyclohexyl diammonium ring of the para host. This interaction is consistent with the energy minimized structure of the para host-Phe(**I**) complex. A large drop in binding energy is observed for the ortho and half hosts. Most likely, the more exposed N^+-H's of the ortho host and the half host, after it undergoes a rearrangement, vide infra, interact favorably with water molecules, increasing the desolvation penalty for salt bridge formation. Another interesting feature is that with the addition of water, the smallest drops in pK_{a1}'s are observed for the ortho and half hosts, but their ability to bind Phe(**I**) is greatly reduced. The converse phenomenon is observed for the para and meta hosts. It is possible

that N^+-*H*'s held within rigid pockets or clefts are protected from solvent (H1 and H2 for the para and meta hosts), and thus they are not deprotonated with added base. These protons instead interact with a ligand such as Phe(**I**) because their weaker association with solvent molecules gives them a smaller desolvation penalty for complex formation. The lack of a preformed cleft in the ortho host or a rigid structure of the half host keeps their ammonium ions from being discriminated into binding and acidic N^+-*H*'s.

To relate the measured pK_a's with K_A's, energy minimized host−guest complexes (molecular mechanics calculations using the MM+ force field as presented by HyperChem) were compared to the conformations of the free hosts. The structure of the para host free and bound to Phe(**I**) is basically unchanged (Figure 1). Minimized structures of the other hosts bound to Phe(**I**), however, were different than their unbound states. These compounds had both sets of N^+-H's aligned for carboxylate binding instead of having two N^+-H's inside and two outside the host's pocket. Similar binding-conformers for the meta and ortho hosts were observed in the set of conformers obtained from the Monte Carlo simulations of the free hosts. These conformers were approximately 2 and 3 kcal/mol higher in energy, respectively, than their global minima. If these are the binding conformers, the smaller association constants for the ortho and meta hosts in d_6-DMSO, as compared to the para host, could be caused by their conversion to less stable conformers during association. Additional loss in entropic energy upon association will occur for flexible hosts that bind through a single conformer. If the hosts do not rearrange upon guest binding, the larger association constants obtained for the para host could be due to it having a greater number of N^+-H's held in the binding domain than the meta and ortho hosts. One consistent feature for the computed structures of the free and bound hosts is that the alignment of the aromatic rings provides for a pocket, cleft, and groove for the para, meta, and ortho hosts, respectively. More importantly electrostatic charges calculated for N^+-*H*'s of the hosts in the binding-conformers have values similar to the ones obtained for free hosts (Table 2).

Conclusion

These studies demonstrated that dialkyl ammonium ions interact with aromatic rings in a manner consistent with the cation-π theory. According to the theoretical results and experimental pK_a's, the cationic charge of N^+-*H*'s positioned at electron-rich aromatic domains are reduced compared to ones residing at the edges of aromatic rings or exposed to solvent. Domains displaying cation-π sites can form stable salt bridges in DMSO and in DMSO−water mixtures. The binding energy depends strongly on the desolvation penalty that occurs for ammonium ions exposed to solvent, especially water. Therefore, it appears that the activity of positively charged side chains involved in cation-π sites is reduced, but this reduction in potential energy for ligand binding is compensated for by a decrease in the desolvation penalty for complex formation. Slight differences in the position of the cation with the aromatic surface produced large differences in the properties of the hosts. DFT calculations provided electrostatic charges that were consistent with the observed properties of the hosts. Our results show that protein binding domains need to be finely tuned; the arrangement of side chains in cation-π sites needs to provide both structural stability, desolvation, and potential energy for ligand binding.

Experimental Section

General Methods. All syntheses were carried out under positive argon pressure, solvents were freshly distilled prior to use, and other reagents were used as purchased. NMR spectra used for compound identification and deriving association constants were measured at 400.14 or 249.99 MHz for proton nuclei and 62.9 MHz for carbon nuclei. Chemical shifts are referenced using an internal TMS standard for proton spectra and $CDCl_3$ for carbon spectra. COSY and NOESY spectra were obtained at 298 K, and NOESY experiments had mixing times of 150, 300, and 500 ms.

Methyl *m*-Bromomethylbenzoate (2b). Methyl *m*-toluate (5.00 g, 33.3 mmol) was dissolved in carbon tetrachloride (85 mL), and *N*-bromosuccinimide (5.93 g, 33.3 mmol) was added, followed by benzoyl peroxide (0.16 g, 0.67 mmol). The mixture was gently refluxed for 4 h, and the white succinimide residue was filtered off. The solvent was evaporated under reduced pressure to give a yellow liquid that consisted of the product and starting methyl *m*-toluate in a 90:10 ratio (^{1}H NMR). After the mixture was dissolved in ether and cooled to −78 °C (CO_2/acetone), the product precipitated and liquid was decanted. Repeating this procedure two more times gave product of sufficient purity (> 95%) as a yellow liquid at room temperature (6.48 g, 85% yield). The product's ^{1}H NMR spectrum matched literature data.[19]

Methyl *o*-bromomethylbenzoate **(2c)** was prepared by the same procedure from methyl *o*-methyltoluate in a 63% yield. The product's ^{1}H NMR spectrum matched literature data.[20]

1-{4,4-Bis[4-(4-methoxycarbonylbenzyloxy)-phenyl]-piperidin-1-yl}-ethanone (3a). Bisphenol **1**[21] (4.00 g, 12.9 mmol) was dissolved in 50 mL of DMF, and NaH suspension in mineral oil (0.68 g, 28 mmol) was added in small portions. The mixture was stirred for 30 min until evolution of hydrogen ceased, and then methyl *p*-bromomethylbenzoate **2a** (6.48 g, 28.3 mmol) in 30 mL of DMF was added dropwise. The reaction mixture was stirred for 2 h. After removal of the solvent under reduced pressure, the residue was taken up in CH_2Cl_2 and the inorganic salts were filtered off. Filtrate was concentrated under reduced pressure and purified by column chromatography in CH_2Cl_2/EtOH (95:5) to give a white foam (7.33 g, 94% yield): mp 80−82 °C. ^{1}H NMR ($CDCl_3$): 2.08 (3H, s), 2.33 (4H, m), 3.47 (2H, m), 3.64 (2H, m), 3.92 (6H, s), 5.08 (4H, s), 6.89 (4H, d, J = 9.0 Hz), 7.14 (4H, d, J = 9.0 Hz), 7.48 (4H, d, J = 8.0 Hz), 8.05 (4H, d, J = 7.8 Hz). ^{13}C NMR: 21.5, 36.0, 36.9, 38.6, 52.2, 69.3, 114.9, 127.0, 128.1, 129.7, 129.9, 139.2, 142.3, 156.7, 166.9, 168.9. HR-MS(ES) calcd for $C_{37}H_{37}NO_7$ [M^+] 607.2570, found 607.2538.

Meta and ortho diesters **3b,c** were prepared by this procedure from methyl *m*-bromomethylbenzoate **2b** and methyl *o*-bromomethylbenzoate **2c** respectively.

1-{4,4-Bis[4-(3-methoxycarbonylbenzyloxy)-phenyl]-piperidin-1-yl}-ethanone (3b). White foam, yield 65%, mp 62−64 °C. ^{1}H NMR ($CDCl_3$): 2.08 (3H, s), 2.33 (4H, m), 3.48 (m, 2H), 3.65 (2H, m), 3.92 (6H, s), 5.06 (4H, s), 6.90 (4H, d, J = 8.8 Hz), 7.45 (2H, t, J = 7.5 Hz), 7.62 (2H, d, J = 7.5 Hz), 8.00 (2H, d, J = 8.0 Hz), 8.09 (2H, s), ^{13}C NMR: 21.1, 35.7, 36.6, 38.3, 51.9, 69.1, 114.5, 127.7, 128.2, 128.4, 128.9, 130.2, 131.6, 137.2, 138.8, 156.4, 166.5, 168.6. HR-MS(ES) calcd for $C_{37}H_{38}NO_7$ [M + 1] 608.2648, found 608.2697.

1-{4,4-Bis[4-(2-methoxycarbonylbenzyloxy)-phenyl]-piperidin-1-yl}-ethanone (3c). White foam, yield 64%, mp 68−70 °C. ^{1}H NMR ($CDCl_3$): 2.09 (3H, s), 2.34 (4H, m), 3.48

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(2H, m), 3.65 (2H, m), 3.89 (6H, s), 5.46 (4H, s), 6.92 (4H, d, $J = 8.5$ Hz), 7.14 (4H, d, $J = 9.0$ Hz), 7.37 (2H, t, $J = 7.8$ Hz), 7.55 (2H, t, $J = 7.5$ Hz), 7.74 (2H, d, $J = 7.5$ Hz), 8.02 (2H, d, $J = 8.3$ Hz). ^{13}C NMR: 21.2, 35.5, 36.7, 38.6, 51.9, 68.0, 114.8, 127.1, 127.3, 127.5, 127.8, 130.5, 132.4, 138.7, 139.4, 156.7, 167.2, 169.0. HR-MS(ES) calcd for $C_{37}H_{38}NO_7$ [M + 1] 608.2648, found 608.2625.

1-{4,4-Bis[4-(2-hydroxymethylbenzyloxy)-phenyl]-piperidin-1-yl}-ethanone (4c). Diester **3c** (0.43 g, 0.71 mmol) was dissolved in 10 mL of EtOH, and $NaBH_4$ (0.43 g, 11 mmol), dissolved in 10 mL THF, was added in portions. Anhydrous LiCl (0.48 g, 11 mmol) was added very slowly over a period of 15 min (exothermic!). The thick mixture was stirred for 24 h, and the solvent was evaporated under reduced pressure. After partitioning the residue between methylene chloride and water, the water layer was extracted with methylene chloride (2 × 20 mL). The combined extracts were dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by column chromatography in CH_2Cl_2/EtOH (95:5) to give a white solid (0.24 g, 61% yield), mp 68−70 °C. ^{1}H NMR ($CDCl_3$): 2.08 (3H, s), 2.33 (4H, m), 3.48 (2H, m), 3.65 (2H, m), 4.73 (4H, s), 5.02 (4H, s), 6.92 (4H, d, $J = 8.5$ Hz), 7.15 (4H, d, $J = 8.5$ Hz), 7.3−7.5 (8H, m). ^{13}C NMR: 21.1, 35.7, 36.6, 38.5, 60.3, 68.2, 114.7, 127.3, 127.8, 128.4, 128.6, 128.9, 134.5, 138.9, 139.4, 156.5, 169.0. HR-MS(ES) calcd for $C_{35}H_{37}NO_5Na^+$ [M + Na^+] 574.2569, found 574.2529.

Para and meta dialcohols **4b,c** were prepared by the same procedure from diesters **3a** and **3b**, respectively.

1-{4,4-Bis[4-(4-hydroxymethylbenzyloxy)-phenyl]-piperidin-1-yl}-ethanone (4a). White solid, yield 40%, mp 75−77 °C. ^{1}H NMR ($CDCl_3$): 1.76 (1H, br s), 2.08 (3H, s), 2.33 (4H, m), 3.48 (2H, m), 3.63 (2H, m), 4.71 (4H, d, $J = 3.8$ Hz), 5.02 (4H, s), 6.89 (4H, d, $J = 9.1$ Hz), 7.13 (4H, d, $J = 8.4$ Hz), 7.40 (8H, m). ^{13}C NMR: 21.2, 35.8, 36.7, 38.6, 43.6, 64.6, 69.6, 114.7, 127.0, 127.5, 127.8, 136.0, 138.7, 140.9, 156.7, 169.0. HR-MS(ES) calcd for $C_{35}H_{37}NO_5$ [M^+] 551.2672, found 551.2658.

1-{4,4-Bis[4-(3-hydroxymethylbenzyloxy)-phenyl]-piperidin-1-yl}-ethanone (4b). White solid, yield 30%, mp 58−60 °C. ^{1}H NMR ($CDCl_3$): 1.79 (1H, br s), 2.07 (3H, s), 2.33 (4H, m), 3.48 (2H, m), 3.64 (2H, m), 4.71 (4H, s), 5.02 (4H, s), 6.90 (4H, d, $J = 9.0$ Hz), 7.13 (4H, d, $J = 8.5$ Hz), 7.3−7.5 (8H, m). ^{13}C NMR: 20.8, 35.3, 36.1, 38.2, 63.8, 69.4, 114.3, 125.4, 125.8, 125.9, 127.4, 128.1, 136.5, 138.4, 141.5, 156.4, 168.9. HR-MS(ES) calcd for $C_{35}H_{37}NO_5Na^+$ [M + Na^+] 574.2569, found 574.2529.

1-{4,4-Bis[4-(4-formylbenzyloxy)-phenyl]-piperidin-1-yl}-ethanone (5a). A 1.30 mL (2.59 mmol) portion of a 2 M solution of oxalyl chloride was diluted in 10 mL of CH_2Cl_2 and cooled to −65 °C. DMSO (0.40 mL, 5.59 mmol) in 2.4 mL of CH_2Cl_2 was added dropwise during a period of 5 min. Stirring was continued for 10 min, and alcohol **4a** (0.24 g, 0.44 mmol) in 4.8 mL of methylene chloride was added during a period of 5 min. Stirring was continued for an additional 15 min, and triethylamine (1.65 mL, 11.9 mmol) was added over 5 min. The reaction mixture was brought to room temperature, and 3 mL of water were added. The organic layer was extracted with 1 M HCl, 1 M Na_2CO_3, and then dried over Na_2SO_4. Solvent was evaporated under reduced pressure, and the residue was purified by column chromatography in CH_2Cl_2/EtOH (95:5) to give a yellow amorphous solid (0.21 g, 87% yield), mp 135−137 °C. ^{1}H NMR ($CDCl_3$): 2.08 (3H, s), 2.33 (4H, m), 3.48 (2H, m), 3.64 (2H, m), 5.11 (4H, s), 6.89 (4H, d, $J = 8.5$ Hz), 7.15 (4H, d, $J = 8.5$ Hz), 7.58 (4H, d, $J = 7.8$ Hz), 7.90 (4H, d, $J = 7.6$ Hz), 10.02 (2H, s). ^{13}C NMR: 21.1, 35.7, 36.6, 38.3, 43.6, 68.9, 114.5, 127.2, 127.7, 129.7, 135.7, 139.0, 143.7, 156.2, 168.5, 191.5. HR-MS(ES) calcd for $C_{35}H_{33}NO_5$ [M^+] 547.2359, found 547.2332.

The meta and ortho dialdehydes **5b,c** were prepared by the same procedure from dialcohols **4b** and **4c**, respectively.

1-{4,4-Bis[4-(3-formylbenzyloxy)-phenyl]-piperidin-1-yl}-ethanone (5b). Off-white solid, yield 99%, mp 137−139 °C. ^{1}H NMR ($CDCl_3$): 2.08 (3H, s), 2.35 (4H, m), 3.49 (2H, m), 3.65 (2H, m), 5.10 (4H, s), 6.91 (4H, d, $J = 8.8$ Hz), 7.15 (4H, d, $J = 8.5$ Hz), 7.56 (2H, t, $J = 7.5$ Hz), 7.70 (2H, d, $J = 7.5$ Hz), 7.94 (2H, s), 10.04 (2H, s). ^{13}C NMR: 21.1, 35.7, 36.6, 38.3, 68.9, 114.6, 127.6, 127.8, 128.0, 129.0, 132.9, 136.4, 138.1, 139.0, 156.3, 168.6, 191.8. HR-MS(ES) calcd for $C_{35}H_{34}NO_5$ [M + 1] 548.2437, found 548.2432.

1-{4,4-Bis[4-(2-formylbenzyloxy)-phenyl]-piperidin-1-yl}-ethanone (5c). Off-white solid, yield 94%, mp 83−85 °C. ^{1}H NMR ($CDCl_3$): 2.09 (3H, s), 2.35 (4H, m), 3.49 (2H, m), 3.65 (2H, m), 5.48 (4H, s), 6.94 (4H, d, $J = 8.5$ Hz), 7.16 (4H, d, $J = 8.5$ Hz), 7.52 (2H, t, $J = 7.5$ Hz), 7.64 (2H, t, $J = 7.5$ Hz), 7.78 (2H, d, $J = 7.5$ Hz), 7.90 (2H, d, $J = 7.5$ Hz), 10.18 (2H, s). ^{13}C NMR: 21.3, 35.9, 36.8, 38.6, 67.4, 114.8, 127.7, 127.9, 127.9, 133.0, 133.7, 134.0, 139.1, 139.4, 156.5, 168.9, 193.0. HR-MS(ES) calcd for $C_{35}H_{34}NO_5$ [M + 1] 548.2437, found 548.2432.

8,11-Diaza-4,14-dioxa-2(4,4)-*N*-acetylpiperidina-1,3,6,-12(1,4)-tetrabenzena-9(1,2)-*trans*-cyclohexanacyclotetradecaphane (Para Host). Cyclohexane-*trans*-1,2-diamine **6** (0.044 g, 0.38 mmol) was dissolved in 20 mL of acetonitrile. Dialdehyde **5a** (0.21 g, 0.38 mmol) in 20 mL of acetonitrile was added slowly, and the reaction mixture was stirred for 24 h. After removing the solvent under reduced pressure, the residue was dissolved in 10 mL of methanol, and 7 mL of $CHCl_3$ and $NaBH_4$ (0.10 g, 2.7 mmol) were added. The reaction mixture was stirred for 2 h. Solvents were evaporated under reduced pressure, and the residue was dissolved in CH_2Cl_2 and extracted with water (2 × 10 mL). Organic extracts were collected and dried over Na_2SO_4, and the solvents were removed in vacuo. The resulting product (0.24 g, 99%) was of sufficient purity to be used in the studies (>95%, by ^{1}H NMR), mp 118−120 °C. ^{1}H NMR ($CDCl_3$): 0.88 (2H, m), 1.04 (2H, m), 1.24 (2H, m), 1.6−1.9 (4H, m), 2.06 (3H, s), 2.2−2.4 (6H, m), 3.45 (2H, m), 3.58 (2H, m), 3.55 (2H, d, 13.4 Hz), 3.97 (2H, d, 13.4 Hz), 5.17 (4H, s), 6.74 (4H, d, 9.2 Hz), 6.99 (4H, d, 7.5 Hz), 7.18 (8H, m). ^{13}C NMR: 21.3, 25.0, 31.3, 35.4, 36.3, 38.5, 50.6, 61.6, 68.8, 115.1, 126.2, 127.5, 127.8, 135.9, 138.6, 139.9, 156.0, 168.7. HR-MS(ES) calcd for $C_{41}H_{48}N_3O_3^+$ [M + 1] 630.3696, found 630.3719.

Meta and ortho cyclophanes were prepared by the same procedure from dialdehydes **5b** and **5c**, respectively.

8,11-Diaza-4,14-dioxa-2(4,4)-*N*-acetylpiperidina-1,3(1,4),-6,12(1,3)-tetrabenzena-9(1,2)-*trans*-cyclohexanacyclotetradecaphane (Meta Host). White foam, yield 79%, mp 95−97 °C. ^{1}H NMR ($CDCl_3$): 0.7−1.0 (4H, m), 1.0−1.3 (2H, m), 1.4−1.9 (4H, m), 2.06 (3H, s), 2.14 (2H, m), 2.25 (4H, m), 3.45 (2H, m), 3.5−3.7 (4H, m), 3.83 (2H, d, $J = 13.3$ Hz), 5.13 (4H, m), 6.72 (4H, d, $J = 8.5$ Hz), 6.93 (4H, d, $J = 8.8$ Hz), 7.15−7.30 (8H, m). ^{13}C NMR: 21.3, 24.8, 31.4, 36.8, 38.6, 51.0, 60.9, 69.8, 114.7, 125.3, 126.6, 127.4, 127.8, 128.6, 137.5, 138.8, 141.2, 156.1, 168.8. HR-MS(ES) calcd for $C_{41}H_{48}N_3O_3^+$ [M + 1] 630.3696, found 630.3691.

8,11-Diaza-4,14-dioxa-2(4,4)-*N*-acetylpiperidina-1,3(1,4),-6,12(1,2)-tetrabenzena-9(1,2)-*trans*-cyclohexanacyclotetradecaphane (Ortho Host). White foam, yield 88%, mp 103−105 °C. ^{1}H NMR ($CDCl_3$): 0.7−1.0 (4H, m), 1.0−1.3 (2H, m), 1.5−1.9 (4H, m), 2.0−2.4 (9H, m), 3.42 (2H, m), 3.5−3.7 (4H, m), 3.92 (2H, d, $J = 13.3$ Hz), 5.02 (4H, s), 6.80 (4H, d, $J = 8.5$ Hz), 7.06 (4H, d, $J = 8.8$ Hz), 7.2−7.5 (8H, m). ^{13}C NMR: 21.4, 24.9, 31.5, 36.0, 36.9, 38.6, 53.4, 61.4, 67.8, 114.6, 127.2, 127.4, 127.9, 128.2, 129.3, 135.0, 138.8, 139.1, 156.9, 168.8. HR-MS-(ES) calcd for $C_{41}H_{48}N_3O_3^+$ [M + 1] 630.3696, found 630.3732.

***N,N′*-Bis(4-methylbenzyl)-cyclohexane-1,2-*trans*-diamine (7).** *trans*-1,2-Cyclohexanediamine **6** (0.50 g, 4.4 mmol) was dissolved in 5 mL of MeOH, and *p*-tolualdehyde (1.1 mL, 9.0 mmol), also dissolved in 5 mL of methanol, was added. A white precipitate was produced, and the mixture was stirred for 1 h. After the liquid phase was decanted off, the precipitate was dissolved in 10 mL of an EtOH/$CHCl_3$ (50:50) solution. $NaBH_4$ (0.32 g, 9.0 mmol) was added, and the reaction mixture was stirred for 1 h. After the solvents were evaporated under reduced pressure, the residue was taken up in 50 mL of ether and extracted with 1 M NaOH (2 × 50 mL). The ether layer was dried over pellets of NaOH and evaporated to give a yellow solid (1.16 g, 82% yield), mp 62−64 °C. ^{1}H NMR ($CDCl_3$): 1.04 (2H, m), 1.22 (2H, m), 1.71 (2H, m), 1.80 (2H, m), 2.1−2.3 (4H, m), 2.33 (6H, s), 3.60 (2H, d, $J = 13.0$ Hz), 3.86 (2H, d, $J = 13.3$ Hz), 7.10 (4H, d, $J = 7.8$ Hz), 7.20 (4H, d, $J = 8.0$ Hz).

^{13}C NMR: 20.9, 24.9, 31.4, 50.4, 60.7, 127.9, 128.8, 136.0, 137.9. HR-MS(ES) calcd for $C_{22}H_{31}N_2$ [M + 1] 323.2487, found 323.2497.

Preparation of Host's Diamine Dihydrochlorides. Host diamines (1 mmol) were dissolved in 10 mL of chloroform, and 1 mL of 4 M HCl in dioxane was added. The solvents were evaporated under reduced pressure to give the hosts as white solids.

Monitoring Association. Association of hosts with the sodium salt of *N*-acetyl L-phenylalanine was detected by monitoring changes in the chemical shifts of the guest's α-H that occurred with the increasing concentration of a host. Association constants were derived by performing a nonlinear least-squares fitting procedure of plots that correlated the changes in the α-H of Phe(**I**) against the changes in host concentrations.[22]

Molecular Modeling. Global minimum structures of the hosts were determined through a Monte Carlo search method using the MMFF94 force field (package procedure of SpartanPro, Wavefunction, Inc. Irvine, CA). Generally, over 1500 structures were created and analyzed for potential energy through a simulated annealing process starting at 5000 K and ending at 300 K. The lowest energy conformers were greater than 1 kcal/mol lower in energy than the next stable structure. To obtain electrostatic charges of the hosts in a reasonable amount of time, the cyclohexyl and *N*-acetylpiperidine rings were replaced by hydrogen atoms (see Figure 1 for the structures used in these calculations). These structures were not altered before being subjected to single point energy calculations using the density functional method (pBP/DN* as presented by SpartanPro). Similar calculations were performed on the diaminoethyl portion of the hosts.

Docking interactions were determined by first obtaining the energy minimized structure of Phe(**I**) using a conjugate gradient method (Polak-Ribiere) to a rms gradient of 0.001 kcal/(mol·Å) (molecular mechanics calculations, MM+ force field as presented by HyperChem, Hypercube, Inc. Gainesville, FA). Phe(**I**) was combined with hosts in their energy minimized structures, obtained from the Monte Carlo simulations, at various positions and distances from the host's combining site. The energy of the complexes was minimized using a conjugate gradient method (Polak-Ribiere) to a rms gradient of 0.01 kcal/(mol·Å) (molecular mechanics calculations, MM^+ force field). The structure of the para host did not change significantly, whereas new binding conformations were obtained for the meta and ortho hosts. Monte Carlo docking simulations were performed starting from these energy minimized complexes (molecular mechanics calculations, MM+ force field, 1000 structures were analyzed). Low energy structures were chosen and a final round of energy minimized calculations were performed to give the final energy minimized complexes.

Potentiometric Titrations. For titrations in DMSO solutions, the electrode was calibrated using standard solutions of 2,6-dinitrophenol and 4-chloro-2,6-dinitrophenol, according to literature procedures.[23] Solutions (5 mM) of the templates were prepared in freshly distilled DMSO (over CaH_2) and titrated under Ar with a 0.10 M solution of Bu_4NOH in DMSO. Similar titrations were performed for solutions containing templates, DMSO, and boiled water (95:5 v/v). The pK_a values of the templates were calculated using the BEST program.[24]

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Supporting Information Available: 2D COSY and NOESY spectra of the hosts, energy minimized host-Phe(**I**) complexes, and potentiometric plots. This material is available free of charge via the Internet at http://pubs.acs.org.

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