Optimization of a Synthetic Arginine Receptor. Systematic Tuning of Noncovalent Interactions

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The simple arginine binder **1** could be optimized by strengthening π-cation as well as electrostatic interactions. Electron-donating or -withdrawing substituents in the 5-position provide experimental evidence for π-cation interactions, because binding energies increase by up to 0.6 kcal/mol due to a single benzene−guanidinium interaction. Even more effective is the introduction of a third phosphonate functionality at the correct distance, so that the guanidinium cation is recognized by optimal electrostatic and hydrogen bond interactions. Monte Carlo simulations and NOESY experiments confirm the expected complex geometries. The optimized host molecule **8** binds arginine half an order of magnitude more efficiently than the parent molecule.

Introduction

The molecular recognition of arginine is a key process in many vital biological control mechanisms. Thrombine and trypsin cleave peptides directly after an arginine residue.[1] The highly efficient self-organization of the extremely long DNA double helix in nucleosomes strongly relies on electrostatic contacts between the negatively charged phosphate moieties and positively charged basic amino acids, when it winds around the histone proteins.[2] Arginine-rich regions in regulatory proteins often bind sequence-specifically to the phosphodiester backbone of RNA and DNA molecules.[3]

Despite their fundamental importance in Nature, these arginine recognition events have only recently been imitated by chemists with artificial receptor molecules. Dougherty and Bell have recently presented two powerful arginine receptor molecules.[4] However, these host molecules are relatively large and only accessible by a multistep synthesis. Back in 1998, we introduced the small, readily available benzylic bisphosphonate **1** as a new binding site for the guanidinium cation.[5] Molecular tweezers with a sulfonate hinge group **2** are even capable of rotating their phosphonate anions into the same plane as the guanidinium cation.[6]

This favorable arrangement makes them highly selective for guanidinium ions over ammonium ions and especially well suited for the molecular recognition of arginine derivatives in a peptidic environment (Figure 1). However, the respective binding constants are only high in dipolar aprotic solvents such as DMSO.[5−7] We asked ourselves, which structural changes could be made in order to optimize the recognition of arginine derivatives by these small tweezer molecules. To achieve this goal, we strived to better understand the nature and force of noncovalent interactions involved in the binding process. In most cases, it is very difficult to quantify the contribution of single noncovalent interactions to a host−guest complex with multiple binding sites.[8] Since host molecule **1** is very small, it represents a good candidate for the systematic probing of specific noncovalent attractive forces. To this end, we introduced an additional substituent in the 5-position of the phosphonate-carrying benzene ring and systematically varied its electronic and stereochemical properties to enforce and complement the existing noncovalent interactions.

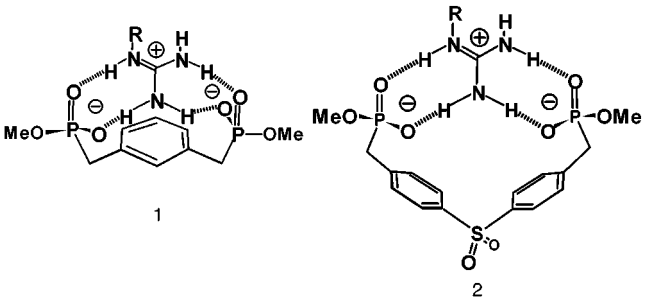

Figure 1. Benzylic bisphosphonate host molecules **1** and **2** for strong guanidinium recognition.

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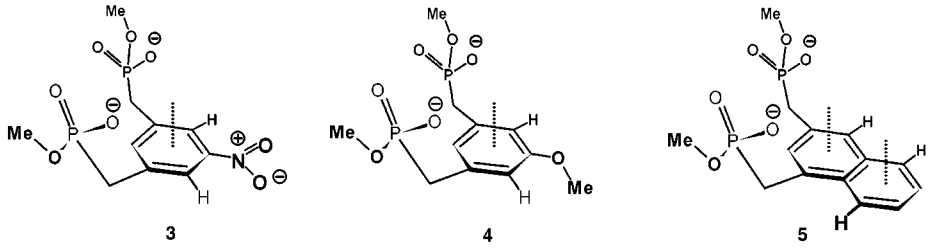


Figure 2. Modified *m*-xylylene bisphosphonate host molecules **3**−**5** for optimized guanidinium recognition: probing the π-cation interaction.

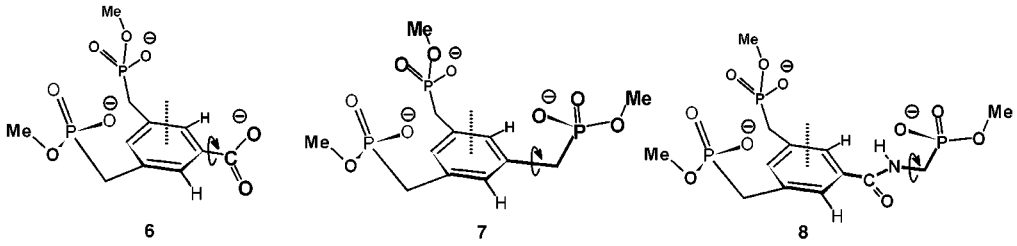


Figure 3. Modified *m*-xylylene bisphosphonate host molecules **6**−**8** for optimized arginine recognition: introducing an additional negative charge.

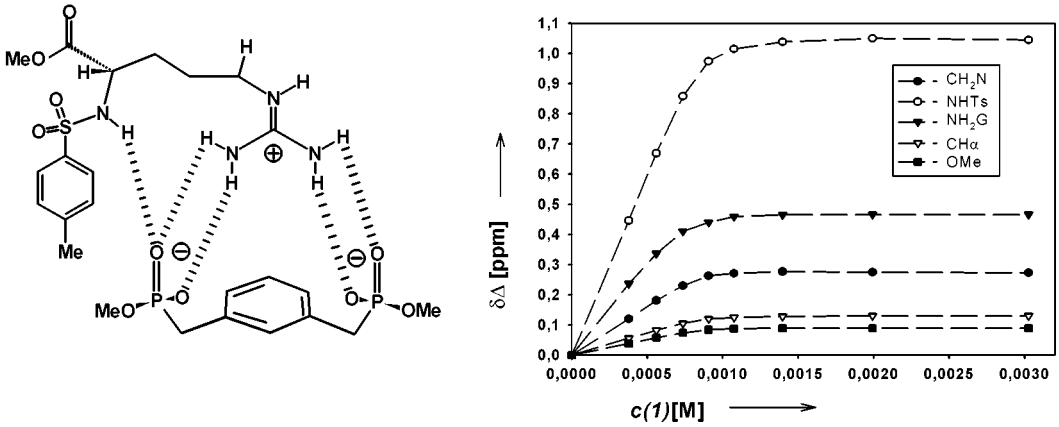


Figure 4. Dependence of the change in chemical shift of selected N*H*- and C*H*-protons of *N*-tosylarginine methyl ester hydrochloride (in bold) on addition of the basic bisphosphonate derivative **1** in DMSO at 20 °C.

This protocol produced indeed much more powerful host systems in several cases.

We assumed that binding of the guanidinium ion occurs mainly through three kinds of noncovalent interactions, i.e., electrostatic attraction, hydrogen bonding, and π-cation interaction. From preliminary experiments, we knew that in general for the simple xylylene bisphosphonates a 100-fold drop was observed in the association constants if the solvent was changed from DMSO to methanol. This is a strong indication for the dominating influence of electrostatic attractions and the absence of significant hydrophobic forces. However, in DMSO strong ionic hydrogen bonds are observed as the large downfield shifts of all guanidinium N*H*-protons demonstrate (Figure 4). Furthermore, a seizable downfield shift of the amidic NH proton in arginine derivatives indicates an additional intermolecular hydrogen bond to one of the phosphonate anions. It correlates well with a marked increase in K_a of up to 1 order of magnitude.

Results and Discussion

The contribution of π-cation interactions in complexes of **1** with guanidinium cations has to date only been postulated.[1,2] For a direct proof, small electron-withdrawing as well as -donating substituents were placed in the 5-position of the central benzene ring (compounds **3** and **4**, Figure 2). Furthermore, we enlarged the available π-face (host molecule **5**, Figure 2). With these modifications, only π-cation interactions would furnish significantly altered binding constants in a predictable manner.[9] A carboxylate group in the 5-position was intended to increase the negative charge density at this place and possibly create an additional hydrogen bond if a slight rotation out of the aromatic plane would be energetically tolerable (receptor molecule **6**, Figure 3). Even better would be the placement of a phosphonate moiety in this region, connected to the aromatic core unit by way of a fairly rigid spacer. Thus, according to molecular modeling simulations[10] the fifth guanidinium N*H*-proton could be reached for maximum hydrogen bond contact. Simulta-

(9) One could argue, that in methoxy-substituted host **4** an increase in negative charge density close to the benzene ring would result in additional electrostatic attraction of the guanidinium cation. This is indeed the case, as MEP calculations show (vide infra).

(10) Molecular Modeling Program: CERIUS[2] from Molecular Simulations Inc., Force field: Dreiding 2.21. Force-field minimizations were initially carried out in the gas phase.

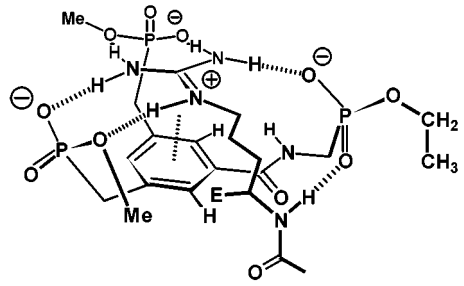
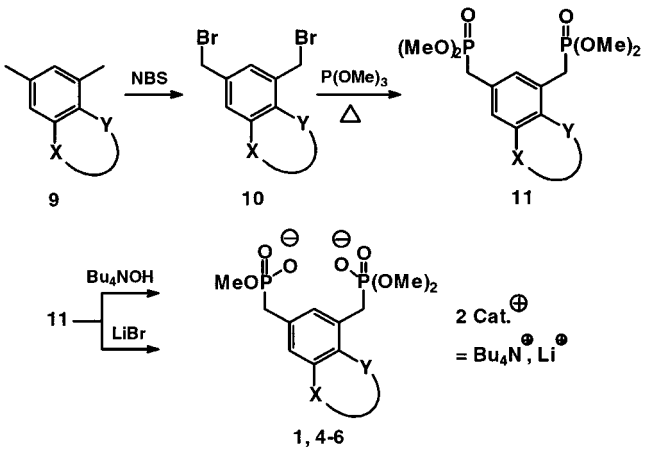


Figure 5. Design of the new arginine receptor molecule **8**. The introduction of the third phosphonate anion at a certain distance of the *m*-xylylene bisphosphonate moiety should facilitate formation of two additional ionic hydrogen bonds with the fifth guanidinium proton as well as the amidic NH proton (E = ester group).

Scheme 1. Synthesis of Model Host Compounds 1 and 3−5 via Dibromination and Arbuzov Reaction

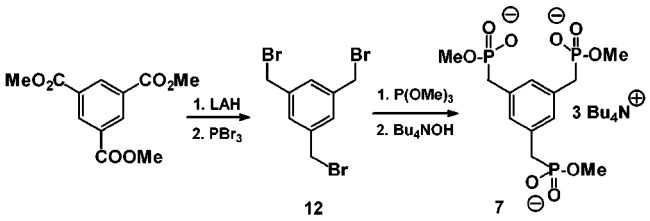


Nr	a	b	X	Y
1	Bu_4N^+	Li^+	H	H
3	Bu_4N^+	Li^+	NO_2	H
4	Bu_4N^+	Li^+	OMe	H
5	Bu_4N^+	Li^+	$(CH)_2$-	-$(CH)_2$
6	Bu_4N^+	Li^+	CO_2^-	H

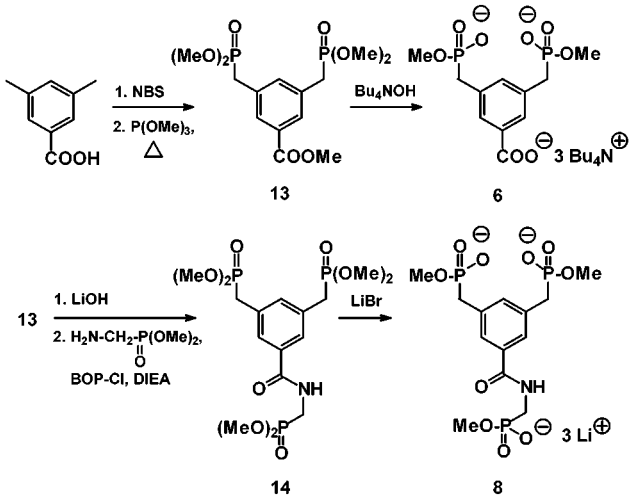
neously the third phosphonate moiety could interact with amidic N*H*-protons in the peptide backbone, without the necessity of an unfavorable folding of the arginine side-chain. We prepared trisphosphonates **7** and **8** for this purpose (Figure 3). With them we hoped to achieve a much more effective and selective molecular recognition of arginine derivatives in a peptidic environment. In earlier experiments, a sensitive ^{1}H NMR probe was discovered which sheds light on the mutual arrangement of both complex partners: a strong downfield-shift of the arginine amide N*H* indicates the additional and often cooperative interaction of a host phosphonate anion with the peptide backbone. Usually it is accompanied by a significant increase in association energy compared with simple methyl guanidinium chloride (Figure 4).

In all syntheses of the above-mentioned 2nd generation model compounds (Figure 5) the key-step consists of an Arbuzov-reaction of *m*-(bisbromomethyl)arenes, similar to the preparation of the basic xylylene bisphosphonates. These are obtained by NBS-bromination from the respective *m*-dimethylarenes.[11−14] The final hydrolysis is achieved by refluxing the dialkyl esters with equimolar amounts

Scheme 2. Synthesis of Model Host Compound 7 from Benzene Tricarboxylic Acid



Scheme 3. Synthesis of Model Host Compounds 6 and 8 Involving Selective Cleavage of the Methyl Ester, Followed by Amide Coupling with BOP-Cl



of aqueous tetrabutylammonium hydroxide;[15] sensitive derivatives can also be cleaved with LiBr in dipolar aprotic solvents (Scheme 1).[16]

Trisphosphonate **7** can be conveniently prepared from benzene tricarboxylic acid via reduction of the respective methyl ester to the benzylic alcohol, bromination, e.g., by PBr_3, followed by the Arbuzov-step and subsequent dealkylation, either with aqueous tetrabutylammonium hydroxide or with lithium bromide in a dipolar aprotic solvent (Scheme 2).[17]

A similar sequence starts from 3,5-dimethylbenzoic acid and furnishes, after bromination, Arbuzov reaction, and hydrolysis, model compound **6** in high yield (Scheme 3).[18] We found, that the first two steps can be performed in one pot, so that key intermediate **13** is now accessible in a very economical and fast route (the published route requires five steps instead of two). After selective hydrolysis of the methyl ester in **13**, the corresponding

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Table 1. Association Constants K_a [M^{-1}] Measured in DMSO for the 1:1 Complexation of Alkyl Guanidinium Derivatives with Receptor Molecules 1−7 by Means of NMR Titration Experiments

guest	**1**[b]	**2**	**3**	**4**	**5**	**6**	**7**
methylguanidine HCl	23000 ± 26%	9000 ± 49%[c]	14000 ± 38%	40000 ± 57%[c]	45000 ± 30%	3000 ± 19%	5500 ± 25%
N-benzoylarginine ethyl ester HCl	86000 ± 28%	56000 ± 36%	50000 ± 29%	107000 ± 13%	120000 ± 42%[c]	5000 ± 18%	31000 ± 18%
stoichiometry[a]	1:1	1:1	1:1	1:1	1:1	1.6:1	1:1

[a] Determined with Job plots. [b] Errors are standard deviations from the nonlinear regressions. [c] The large standard deviation in this case can be explained with small chemical shift differences, which give especially large errors with high binding constants.

benzoic acid derivative can be coupled with aliphatic and aromatic amines. In our case, reaction with aminomethylphosphonic acid dimethyl ester leads to intermediate **14**, which is finally cleaved mildly to the trisphosphonate lithium salt **8**.[19] The coupling reaction is not trivial: Initial attempts to activate the benzoic acid derivative with thionyl chloride produced the corresponding acid chloride, but no coupling occurred after addition of primary amines and triethylamine. DCC gave no product either, and even the highly efficient peptide coupling reagent PyClop (trispyrrolidinochlorophosphonium hexafluorophosphate) did not allow us to prepare pure coupling products.[20] In this case, the reaction proceeded up to ∼50% conversion, but the crude product complexed the byproduct trispyrrolidinophosphinoxide strongly, so that both substances eluted together from any chromatographic column, irrespective of the coupling partner. Finally, we were successful with BOPCl, another peptide coupling reagent, which is normally not recommended for aromatic acids.[21] In this case, however, the amide was formed smoothly in quantitative yield, and the highly polar ionic byproduct bis(2-oxo-3-oxazolidinyl) phosphate could be separated from the desired end products to give analytically pure compounds in high yields (Scheme 3).

With these new receptor molecules at hand, we carried out numerous binding experiments in polar organic solvents. For direct comparison, we titrated methyl guanidine hydrochloride as well as *N*-benzoylarginine ethyl ester hydrochloride with hosts **1**−**7** as tetrabutylammonium salts in DMSO. On the other hand, the association constants between the highly polar lithium salts of **1**, **2**, and **8** and both above-mentioned guanidinium derivatives were each checked in methanol. The results of these NMR titrations are shown in Tables 1−4. From the binding curves, association constants were calculated by standard nonlinear regression methods (Table 1).[22] Job plots confirmed a 1:1 stoichiometry in almost every case.[23]

Entries **1** and **2** show the binding affinities of the two basic receptor molecule structures: although in **2** the phosphonate moieties can be directed perfectly coplanar to the chelated guanidinium ion, its recognition by the *m*-xylylene bisphosphonate is much more effective. We attribute this superiority to the additional π−cation interaction operating only in **1**. Here the guanidinium cation and the receptor's benzene ring are stacked on top of each other with an ideal distance of ∼3.5 Å.

Table 2. Free Binding Enthalpies ΔG [kcal/mol] Measured in DMSO for the 1:1 Complexation of Alkyl Guanidinium Derivatives with Receptor Molecules 1−7 by Means of NMR Titration Experiments

guest	**1**	**2**	**3**	**4**	**5**	**6**	**7**
methylguanidine HCl	5.8	5.3	5.6	6.2	6.2	4.7	5.0
N-benzoylarginine ethyl ester HCl	6.6	6.4	6.3	6.8	6.8	5.0	6.0

The additional introduction of electron-withdrawing or -donating substituents into the core unit of **1** leads to distinct changes in binding affinity: whereas the electron-withdrawing nitro group in **3** lowers K_a, the electron-donating methoxy functionality in **4** rises the binding constant both by a factor of roughly 2 compared to **1**. It is important to note, that the phosphonate moieties are electronically separated from the receptor's benzene ring by the methylene group, and both the nitro as well as the methoxy group are geometrically locked in the aromatic plane of the receptor molecule, so that they cannot form hydrogen bonds to the guanidinium cation. Hence the observed changes in binding energy must reflect the attenuated or enlarged interactions between the guanidinium cation and the aromatic system. According to the electrostatic model, the variation in ion binding energies across a series of similar aromatics is almost completely reflected in the electrostatic term.[24] Two good qualitative indicators for the influence of substituents on the electrostatics are found in the σ_{meta} Hammett parameter and the electrostatic potential above the center of the aromatic ring.[25] In our case, the strongly positive σ_{meta} value of 0.71 for the nitro group correlates well with the marked decrease in binding energy between receptor molecule **1** and **3** of 0.2−0.3 kcal mol^{-1} (Table 2).[26] Accordingly, the calculated MEP (molecular electrostatic potential, AM1) shifts from −20 kcal/mol in benzene to +2 kcal/mol in nitrobenzene. Contrary to Dougherty's results from the Na^+/benzene system as well as to its slightly negative σ_{meta} value of −0.1, we find an activating effect of the methoxy group on the cation−aromatic interaction, corresponding to an increase in binding energy of 0.2−0.4 kcal mol^{-1} from **1** to **4** (Table 2). A careful inspection of anisole's potential surface, however, offers a plausible explanation. Although the electrostatic potential above anisole is essentially the same as above benzene (−19 kcal/mol), a high negative charge density resides in the neighboring O atom (MEP: −56 kcal/mol) at the opposite side of its methyl substitu-

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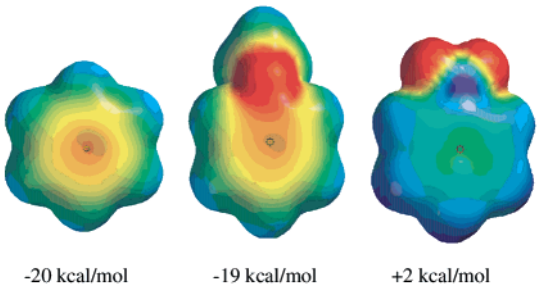

Figure 6. Electrostatic surface potentials (ESP) of benzene, anisole, and nitrobenzene (AM1 calculations).[27] Local MEP's have been calculated at the positions indicated by asterisks and are given below the structures.

ent. This (and not the cation−π interaction) is responsible for an additional electrostatic attraction leading to enhanced binding of **4** (Figure 6).[27]

The association constant also rises if the π-face of the receptor is enlarged as in **5**: it is well-known that π−cation as well as π-stacking interactions depend linearly on the overall size of the interacting π-faces. In our case, an additional term may be provided by the higher polarizability of naphthalene's larger surface area. The energy gain compared to benzene is again 0.2−0.4 kcal from **1** to **5** (Table 2). We conclude, that by systematically altering the electrostatic potential above the receptor's π-face, the existence and important contribution of π−cation interactions to the overall binding energy were proven experimentally. The difference in complexation free enthalpies between guanidinium/**3** and guanidinium/**4** amounts to 0.5−0.6 kcal mol^{-1} (Table 2). If this can be totally attributed to the cation−π interaction, we have at least reached the order of magnitude found by Schneider in systematic experiments with an increasing number of phenyl rings: He determined that in aqueous solution the cation−π interaction contributes ∼0.5 kcal mol^{-1} per phenyl group.[28] The difference in binding energies between complexes of guanidinium ions with **1** and **2** is also in the range of 0.2−0.5 kcal mol^{-1} (Table 2). Contrary to **2**, only flat **1** can accommodate guanidinum ions stacked on top of its benzene ring. Although the association constants are almost doubled, the relative change in free binding energy ΔG for the whole complex is not dramatic, as can be seen from Table 2: a doubled binding constant refers to an increase in free binding enthalpy of only ∼7−8%. This reflects the dominating importance of salt bridges and (in organic solvents) strong hydrogen bonds in our guanidinium binders.

In recent years the π−cation interaction has been established as one of the fundamental noncovalent forces used by Nature in determining protein structures and protein−ligand interactions.[29] Extensive statistical analyses of hundreds of high-resolution protein structures proved that especially arginine is often involved in stacking interactions with phenyl rings of aromatic amino acid side chains.[30] We would like to point out that our simple binding motif shown in Figures 1−3 exactly mimicks the preferred natural arrangement in two ways. First, the guanidinium moiety of arginine is placed directly on top of the receptor molecule's benzene ring for optimal π-cation as well as hydrophobic interactions. Second, salt bridges and hydrogen bonds are simultaneously formed between the guanidinium NH-groups and anionic binding sites which lead to enhanced lateral recognition. The effective combination of these two interactions is beautifully illustrated in the crystal structure of the human growth hormone receptor, where interdigitating protein strands are locked relative to each other by multiple cation−π interactions and salt bridges.[31]

Was the introduction of negatively charged substituents in the 5-position of **1** more effective with respect to an increased binding effectivity of the guanidinium receptor? Entries **6** and **7** prove the opposite: In both cases the obtained values for K_a remain disappointingly low, much lower than even the parent compound **1**. How can this be explained? In the case of **6**, σ_{meta} Hammett parameters and the electrostatic potential surfaces indicate that no increase in cation−π attraction can be expected when compared to the benzene case (**1**). Furthermore, rotation of the carboxylate group out of the aromatic plane for a potential (kinked) hydrogen bond with the guanidinium cation is restricted because the delocalization energy is lost. Another effect adds to this: A careful analysis of the complex stoichiometry reveals an average of 1.6:1. Obviously, instead of intramolecular stabilization, the carboxylate group attracts another guanidinium molecule, leading in part to a 2:1 complex. Therefore, the evaluation of the obtained binding curve according to a simple 1:1 or even 2:1 fit-procedure produces poor results. For **7**, however, we found a perfect 1:1 stoichiometry. The remarkably low binding constants must hence result from unproductive conformations in the trisfunctionalized host molecule. Only if each one of the three phosphonate-carrying arms point upward, can the host molecule seize the guanidinium cation from all sides (Figure 7). This is certainly entropically, but also thermodynamically, costly. Molecular modeling reveals a strong repulsion between the phosphonate anions in this conformation.[32] We also found only a small increase in binding energy when in DMSO the simple unsubstituted guanidinium cation was first titrated with bisphosphonate **1** (5.8 kcal mol^{-1}) and then with trisphosphonate **7** (6.1 kcal mol^{-1}).[33]

(27) Semiempirical calculation with AM1; color coding reaches from red (−25 kcal mol^{-1}) to blue (+25 kcal mol^{-1}). We thank Prof. Dr. G. Klärner (Essen) for these calculations. Contrary to the extended cationic system of the guanidinium cation, the small Na^+-cation probe used by Dougherty et al. does obviously not reach far enough to experience any additional electrostatic attraction by the phenolic oxygen.

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(31) de Vos, A. M.; Ultshc, M.; Kossiakoff, A. A. *Science* **1992**, *255*, 306−312.

(32) Modeling Program: MacroModel 7.0 (Schrödinger Inc.), 2000. Energy-minimizations and Monte Carlo-Simulations were carried out in chloroform with the AMBER*-force-field. The best structure from the conformational searches was subsequently subjected to a molecular dynamics calculation at ambient temperature for 10 ps.

(33) Grawe, T.; Schrader, T. Unpublished results. The unsubstituted guanidinium cation $CN_3H_6^+$ can form four hydrogen bonds with **1**, but six hydrogen bonds with **7**. Although a highly symmetrical energetically favorable complex structure is calculated for the 1:1 complex between the guanidinium ion and **7**, it is obviously not much stronger than the related complex with **1**. In the first case, the three anionic phosphonate groups in **7** come close to each other in order to surround the guanidinium cation. Mutual charge repulsion and unfavorable entropy losses could also be applicable here and explain the rather disappointing results of the binding experiments with **7**.

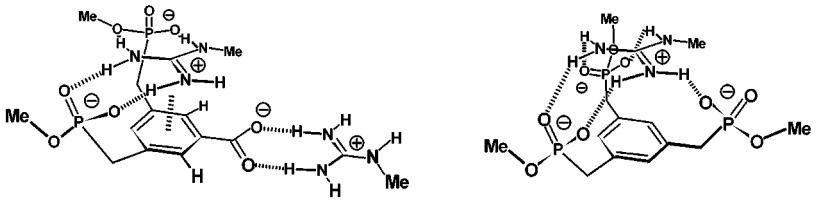

Figure 7. Proposed binding mode of hosts **6** and **7** with guanidium guests.

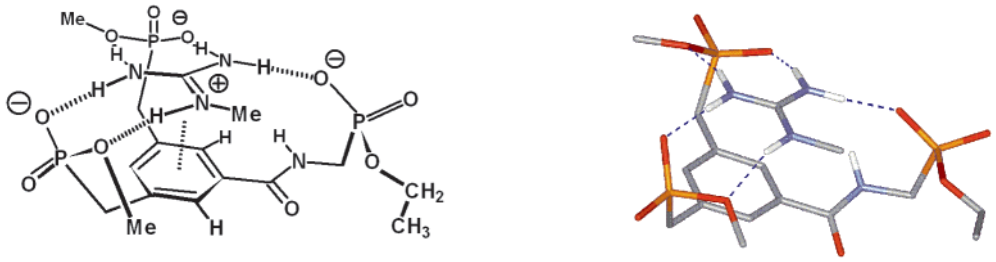

Figure 8. Proposed binding mode of host **8** with methyl guanidine hydrochloride. Left: Lewis structure; right: Monte Carlo simulation in water.

Table 3. Association Constants K_a [M^{-1}] Measured in MeOD for the 1:1 Complexation of Alkyl Guanidinium Derivatives with Receptor Molecules 1, 2, and 8 by Means of NMR Titration Experiments

guest	**1**	**2**	**8**
methylguanidine HCl	800 ± 6%	490 ± 15%	3500 ± 8%
N-benzoylarginine ethyl ester HCl	800 ± 19%	570 ± 17%	3500 ± 16%
stoichiometry	1:1	1:1	1:1

Table 4. Free Binding Enthalpies ΔG [kcal/mol] Measured in MeOD for the 1:1 Complexation of Alkyl Guanidinium Derivatives with Receptor Molecules 1, 2, and 8 by Means of NMR Titration Experiments

guest	**1**	**2**	**8**
methylguanidine hydrochloride	4.0	3.6	4.8
N-benzoylarginine ethyl ester hydrochloride	4.0	3.7	4.8

We would like to emphasize that in DMSO the arginine derivative was always bound three to six times more strongly by hosts **1**−**7** than the simple methylguanidine, which was always indicated by a seizable downfield shift of the amidic arginine-NH proton of 1−2 ppm. Thus, receptor molecules **1**−**7** are all arginine-selective.

The less symmetrical trisphosphonate **8** could only be synthesized as the trilithium salt and was completely insoluble in DMSO. Therefore, we prepared the respective lithium salts of **1** and **2** and compared their binding properties with those of **8** in pure methanol. As Tables 3 and 4 show, **1** is again more effective than **2**, and we explain this difference once more with the additional π−cation interaction. However, to our great pleasure, host molecule **8** is half an order of magnitude more efficient in guanidine binding than **1** and **2**. This agrees with comparable results from conformational searches by Monte Carlo simulations:[34] The distance between the three converging phosphonate anions of **8** in the complex is tailored for optimal electrostatic attraction as well as hydrogen bonding with all five guanidinium NH-protons. The counterproductive mutual electrostatic repulsion of the anionic groups (as in **7**!) is minimized by introduction of the amide spacer when going from **7** to **8**. During the Monte Carlo simulations, several energetically favorable structures were found which mainly differ in the number of oxygen atoms per phosphonate unit, determining the hydrogen bond pattern in the complex. These structures differ distinctly from those obtained in chloroform in one respect: Only one of the negatively charged oxygen atoms on the phosphonate anions points to the guanidinium moiety; the other one rotates outward to gain solvation energy with water (Figure 8). Nevertheless, five strong hydrogen bonds are found in the minium energy structures: In addition to the oxygen anion, the ester oxygen also participates in guanidinium binding. Here the atomic charge is still calculated at −0.35 as opposed to −0.65 in the anionic oxygen. This unusual binding motif has been repeatedly found in Monte Carlo simulations in water if multiple phosphonate anions were used to recognize one positively charged cation (amidinium and ammonium binding).

To confirm the contribution of all three phosphonate arms in guanidinium binding, we performed a NOESY experiment of the complex between methylguanidinium chloride and **8** in methanol. In addition to relatively weak intermolecular NOE's between guanidium's methyl group and the two methyl phosphonates, both the methyl and methylene signal of the ethyl phosphonate produced relatively strong NOE contacts. This is in accord with the above postulated complex structure, depicted in Figure 9. Since both methyl phosphonates are involved in two hydrogen bonds, the alkyl substituent of the guanidine must occupy a position close to the third receptor arm with its ethyl phosphonate functionality.

For a closer inspection of potential contacts between the receptor arms and the guanidinium NH protons, we also performed a NOESY experiment in a mixture of deuterated and nondeuterated methanol (4:1) with suppression of the solvent signal. At room temperature, only one significant NOE contact could be identified, i.e., between the guanidinium NH_2 signals and the two aromatic receptor protons close to the benzamide group. This shows that the guanidinium moiety lies on top of the receptor aromatic, which is necessary for π−cation

(34) Modeling Program: MacroModel 7.0 (Schrödinger Inc.), 2000. Energy-minimizations and Monte Carlo Simulations were carried out in water with the AMBER* force-field. The best structure from the conformational searches was subsequently subjected to a molecular dynamics calculation at ambient temperature for 10 ps.

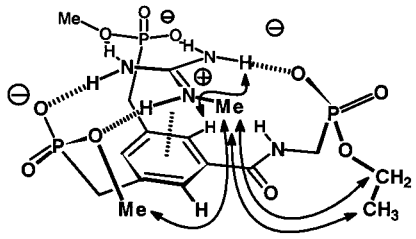

Figure 9. Summarized NOE contacts between **8** and the methylguanidinium cation in methanol.

interactions. Since all the guanidinium NH signals are broad and not separated from each other, only very strong intermolecular NOE's could be detected. In addition, a dynamic equilibrium must be assumed at this temperature with fast interconversion of different complex geometries and hydrogen bond patterns. Therefore, the above-mentioned experiment can only give indications for the complex geometry in averaged structures. In the future we intend to use well soluble complexation partners to carry out these NOESY experiments at low temperatures (<−50 °C). In a related example Gschwind and co-workers were able to generate five different NH signals for the guanidinium cation in arginine involved in complex formation with a bisphosphonate tweezer by cooling the NMR tube down to −73 °C.[35]

Only one drawback remains: In methanol, the receptor molecules cannot distinguish between methylguanidine and amide-protected arginine ester. The additional cooperative hydrogen bond formed between the receptor's phosphonate and one of the peptidic NH-groups in DMSO is absent in methanol. This may be different in the hydrophobic active sites of enzymes.

Conclusions

In conclusion, the binding strength of a simple arginine receptor molecule could be considerably increased by strengthening π−cation as well as electrostatic interactions. The selective introduction of electron-donating or -withdrawing substituents in the 5-position of the parent bisphosphonate revealed the presence of π−cation interactions, which contribute at least 0.5 kcal/mol for a single benzene−guanidinium stack. Much more effective, however, was the introduction of a third phosphonate functionality at the correct distance, so that five hydrogen bonds can be formed to all five guanidinium NH protons, surrounded by three phosphonate arms. Monte Carlo simulations and NOESY experiments confirm these complex geometries. The optimized host molecule binds arginine half an order of magnitude more efficiently than the parent molecule. With the new guanidinium host **8**, we are currently developing an artificial receptor molecule for the RGD sequence, which is essential for many cell surface recognition processes.[36]

Experimental Section

Synthesis of α,α′-Dibromoxylylene Derivates. General Dibromination Procedure. A 5 g amount of the xylylene derivative, 2 equiv of *N*-bromosuccinimide, and a catalytic amount of AIBN are dissolved in 200 mL of tetrachloromethane. After refluxing the reaction mixture for 3 h, complete conversion is reached, and the reaction mixture is allowed to cool to ambient temperature. The solvent is distilled off, and products **10a** and **10b** are purified over silica gel (KG60, hexanes/dichloromethane 3:2), whereas methyl benzoate **10c** and naphthalene derivative **10d** are used without further purification. **3,5-Bis(bromomethyl)nitrobenzene 10a**: Yield: 18%; mp: 103−104 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.46 (s, 4H), 7.68 (s, 1H), 8.13 (s, 2H); ^{13}C NMR(50 MHz, $CDCl_3$) δ 30.3 (s), 123.3 (s), 134.8 (s), 139.9 (s), 148.1 (s). Anal. Calcd for $C_8H_7Br_2NO_2$: C, 31.10; H, 2.28; N, 4.53. Found: C, 32.01; H, 2.96; N, 5.00. **3,5-Bis(bromomethyl)methoxybenzene 10b**: Yield: 27%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.72 (s, 3H), 4.37 (s), 6.82 (s, 2H), 7.06 (s, 2H); ^{13}C NMR(50 MHz, $CDCl_3$) δ 32.8 (s), 55.4 (s), 114.7 (s), 121.8 (s), 136.6 (s), 160.0 (s). Anal. Calcd for $C_9H_{10}Br_2O$: C, 36.77; H, 3.43. Found: C, 36.32; H, 3.26.

Synthesis of *m*-Xylylene Bisphosphonates. General Procedure. The dibromoderivative **10** is dissolved in 3 equiv of trimethyl phosphite and refluxed for 3−4 h. Remaining trimethyl phosphite and methylphosphonic acid dimethyl ester are removed by vacuum distillation at 80 °C/0.1 mbar. The product is purified over silica gel (KG 60, ethyl acetate/methanol 10:1). Recrystallization from ethyl actetate/hexanes affords colorless crystals. **5-Nitro-*m*-xylylene bisphosphonic acid tetramethyl ester 11a**: Yield: 98%; mp: 103−104 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.18 (d, 4H, $^2J_{HP}$ = 21.9 Hz), 3.66 (d, 12H, $^3J_{HP}$ = 11.0 Hz); 7.53 (s, 1H), 7.98 (q, 2H, $^4J_{HP}$ = 1.7 Hz); ^{13}C NMR(50 MHz, $CDCl_3$) δ 32.9 (d, $^1J_{CP}$ = 139.0 Hz), 53.5 (d, $^2J_{CP}$ = 6.8 Hz), 123.7 (m), 134.5 (s), 137.4 (m).^{31}P NMR(80 MHz, $CDCl_3$) δ 27.4. Anal. Calcd for $C_{12}H_{19}NO_8P_2$: C, 44.33; H, 6.30. Found: C, 43.74; H, 6.33. **5-Methoxy-*m*-xylylene bisphosphonic acid tetramethyl ester 11b**: Yield: 98%; ^{1}H NMR (200 MHz, D_2O) δ 3.11 (d, 4H, $^2J_{HP}$ = 22.0 Hz), 3.47 (d, 12H, $^3J_{HP}$ = 13.0 Hz), 3.61 (s), 6.55−6.75 (m, 3H); ^{13}C NMR (50 MHz, D_2O) δ 31.1 (d, $^1J_{CP}$ = 135.0 Hz), 53.7 (d, $^2J_{CP}$ = 8 Hz), 55.6 (s), 114.6 (m), 124.0 (s), 133.2 (m).^{31}P NMR(80 MHz, D_2O) δ 34.0. Anal. Calcd for $C_{13}H_{22}O_7P_2$: C, 44.33; H, 6.30. Found: C, 43.74; H, 6.33. **5-Methoxycarbonyl-*m*-xylylene bisphosphonic acid tetramethyl ester 11c:** Yield: 35% (both steps); mp: 110−111 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.14 (d, 4H, $^2J_{HP}$ = 21.9 Hz), 3.63 (d, 12H, $^3J_{HP}$ = 11,0 Hz), 3.83 (s, 3H), 7.39 (s, 1H), 7.79 (s, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 32.9 (d, $^1J_{CP}$ = 138.5 Hz), 52.6 (s), 53.3 (d, $^2J_{CP}$ = 6.8 Hz), 129.9 (m), 131.3 (t, $^4J_{CP}$ = 2.8 Hz), 132.7 (t, $^3J_{CP}$ = 6.2 Hz), 135.8 (t, $^3J_{CP}$ = 6.2 Hz); 166.8 (s); ^{31}P NMR(80 MHz, $CDCl_3$) δ 28.5. Anal. Calcd for $C_{14}H_{22}O_8P_2$: C, 44.22; H, 5.83. Found: C, 44.64; H, 5.57. **1,3-Bis(dimethoxyphosphorylmethyl)naphthalene 11d**: Yield: 32% (both steps); mp.: 118 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.56 (d, 2H, $^2J_{HP}$ = 20.2 Hz), 3.24 (d, 2H, $^2J_{HP}$ = 20.2 Hz), 3.54 (d, 6H, $^3J_{HP}$ = 10.1 Hz), 3.61 (d, 6H, $^3J_{HP}$ = 10.1 Hz), 7.94−7.21 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 30.3 (d, $^1J_{CP}$ = 139.0 Hz), 33.2 (d, $^1J_{CP}$ = 139.0 Hz), 53.3 (d, $^2J_{CP}$ = 6.8 Hz), 53.4 (d, $^2J_{CP}$ = 6.8 Hz), 124.4 (s), 126.6 (s), 128.5−130.0 (m), 130.6 (m), 131.3 (m), 134.4 (s). ^{31}P NMR (80 MHz, $CDCl_3$) δ 29.8; 29.2. Anal. Calcd for $C_{16}H_{22}O_6P_2$: C, 51.62; H, 5.96. Found: C, 49.91; H, 6.16.

Synthesis of Tetrabutylammonium Salts of *m*-Xylylene Bisphosphonates. General Procedure. A 1 g amount of the respective *m*-xylylene bisphosphonate is dissolved in 50 mL of water and treated with 2 equiv of 1 N aqueous tetrabutylammonium hydroxide. The solution is then refluxed for 7 days. Evaporation of the solvent affords a colorless oil. **Bis(tetrabutylammonium)-5-nitro-*m*-xylylene dimethyl bisphosphonate 3a**: Yield: quantitative; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, 36H, $^3J_{HH}$ = 7.31 Hz), 1.31 (m, 24H), 1.51 (m, 24H), 2.87 (d, 4H, $^2J_{HP}$ = 20.2 Hz), 3.13 (m, 24H), 3.43 (d, 6H, $^3J_{HP}$ = 11.0 Hz), 7.23 (s, 1H), 7.67 (s, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.6 (s), 19.6 (s), 23.9 (s), 34.8 (d, $^1J_{CP}$ = 125.2 Hz), 51.6 (d, $^2J_{CP}$ = 6.2 Hz), 58.5 (s), 121.3 (m), 130.5 (m), 137.8 (m), 138.8 (m,); 147.7 (s); ^{31}P NMR(80 MHz, $CDCl_3$): 18.0 (s). MS (ESIneg): *m*/*z* 337.16, found 168 (M^{2-}), 337 ((M + 1)$^-$), 579 ((M + NBu_4^+)$^-$). Anal. Calcd for $C_{58}H_{121}N_3O_8P_2 \cdot 4H_2O$: C 56.42, H 10.48, N 4.70. Found: C 56.42, H 10.83, N 4.53. **Bis(tetrabutylammonium)-5-methoxy-*m*-xylylene dimethyl bisphosphonate 4a**: Yield: quantitative; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.90 (t, 36H, $^3J_{HH}$ = 7.21 Hz), 1.30 (m, 24H), 1.52

(35) Gschwind, R.; Armbrüster, M. diploma thesis, Marburg, 2001.
(36) Haubner, R.; Finsinger, D.; Kessler, H. *Angew. Chem.* **1997**, *109*, 1440−1456.

(m, 24H), 2.95 (d, 4H, $^2J_{HP}$ = 20.1 Hz), 3.18 (m, 24H), 3.45 (d, 12H, $^3J_{HP}$ = 10.2 Hz), 7.58 (s, 1H), 7.97 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1 (s), 20.0 (s), 24.3 (s), 31.1 (d, $^1J_{CP}$ = 135.0 Hz), 53.7 (d, $^2J_{CP}$ = 8 Hz), 55.6 (s), 59.0 (s), 114.6 (m), 124.0 (s), 133.2 (m). ^{31}P NMR (80 MHz, $CDCl_3$) δ 20.1(s). **Tris-(tetrabutylammonium)-5-carboxylato-*m*-xylylene dimethyl bisphosphonate 6a**: Yield: quantitative; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, 36H, $^3J_{HH}$ = 7.21 Hz), 1.35 (m, 24H), 1.55 (m, 24H), 2.97 (d, 4H, $^2J_{HP}$ = 20.1 Hz), 3.20 (m, 24H), 3.46 (d, 6H, $^3J_{HP}$ = 10.2 Hz), 7.45 (s, 1H), 7.70 (s, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.5 (s), 19.5 (s), 23.8 (s), 34.4 (d, $^1J_{CP}$ = 128.9 Hz), 51.7 (d, $^2J_{CP}$ = 5.7 Hz), 58.6 (s), 128.3 (m), 130.4 (m), 131.6 (m), 135.2 (m); 169.3 (s); ^{31}P NMR (80 MHz, $CDCl_3$): 20.0 (s). MS (ESIneg): *m*/*z* 335.16, found 335 ((M + 1)$^-$), 577 ((M + NBu_4^+)$^-$). Anal. Calcd for $C_{59}H_{121}N_3O_8P_2 \cdot 11H_2O$: C 56.21, H 11.43, N 3.33. Found: C 56.36, H 11.08, N 3.62.

Synthesis of Lithium Salts of *m*-Xylylene Bisphosphonates. General Procedure. A 1 g amount of the respective *m*-xylylene bisphosphonate tetraester is dissolved in a small amount of dry acetonitrile and treated with 2.5 equiv of lithium bromide. The solution is refluxed overnight. After cooling to room temperature, the precipitate is filtered off, washed two times with a small amount of hot acetonitrile, and dried in vacuo. **Dilithium-*m*-xylylene dimethyl bisphosphonate 1b:** Yield: 79%; mp >350 °C (dec); ^{1}H NMR (200 MHz, D_2O) δ 2.86 (d, 4H, $^2J_{HP}$ = 20.6 Hz), 3.35 (d, 6H, $^3J_{HP}$ = 10.2 Hz), 7.01 (m, 3H).^{13}C NMR (50 MHz, D_2O) δ 33.6 (d, $^1J_{CP}$ = 129.5 Hz), 52.0 (d, $^2J_{CP}$ = 6.4 Hz), 127.5 (m), 128.8 (m), 130.8 (m), 135.0 (m).^{31}P NMR (80 MHz, D_2O) δ 27.3 (s). Anal. Calcd for $C_{10}H_{13}O_6P_2Li_2 \cdot 3H_2O$: C, 35.11; H, 5.30. Found: C, 34,86; H, 5,32. **Dilithium 1,3-bis(methylphosphonatomethyl)-naphthalene 5b:** Yield: 89%; mp > 350 °C (dec); ^{1}H NMR (200 MHz, D_2O) δ 3.13 (d, 2H, $^2J_{HP}$ = 20.5 Hz), 3.36 (d, 3H, $^3J_{HP}$ = 10.3 Hz), 3.45 (d, 2H, $^2J_{HP}$ = 20.5 Hz), 3.48 (d, 3H, $^3J_{HP}$ = 10.3 Hz), 7.32 (m, 1H), 7.49 (m, 2H), 7.61 (m, 1H), 7.81 (m, 1H), 8.06 (m, 1H). ^{13}C NMR (50 MHz, D_2O) δ 31.1 (d, $^1J_{CP}$ = 143.1 Hz), 33.7 (d, $^1J_{CP}$ = 141.0 Hz), 52.2 (d, $^2J_{CP}$ = 6.2 Hz), 124.8 (m), 125.9 (m), 126.4 (m), 126.9 (m), 128.4 (m), 130.2 (m), 130.5 (m), 131.5 (m), 132.2 (m), 134.0 (m). ^{31}P NMR (80 MHz, D_2O) δ 29.7 (s), 29.5 (s). Anal. Calcd for $C_{14}H_{16}O_6P_2Li_2 \cdot 3H_2O$: C 41.00; H, 5.41. Found: C 40.36; H 5.44.

Synthesis of Tetrabutylammonium Salt 5a. A 1 g amount of dilithium salt **5b** is dissolved in a small amount of water and acidified with 3 equiv of 1 N hydrochlorid acid. The precipitate is filtered off, washed two times with cold water, and dried in vacuo. Yield: 99%; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.27 (d, 2H, $^2J_{HP}$ = 21.7 Hz), 3.57 (d, 2H, $^2J_{HP}$ = 21.7 Hz), 3.56 (d, 3H, $^3J_{HP}$ = 10.8 Hz), 3.59 (d, 3H, $^3J_{HP}$ = 10.8 Hz), 7.35 (m, 2H), 7.62 (m, 2H), 7.70 (m, 1H), 8.35 (m, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) δ 30.7(d, $^1J_{CP}$ = 144.3 Hz), 33.3 (d, $^1J_{CP}$ = 143.1 Hz), 52.0 (d, $^2J_{CP}$ = 6.2 Hz), 51.9 (d, $^2J_{CP}$ = 6.2 Hz), 125.3 (m), 125.6 (m), 126.1 (m), 128.1 (m), 130.7 (m), 133.8 (m), 140.1 (m), 140.5 (m); ^{31}P NMR(80 MHz, DMSO-d_6) δ 29.7 (s), 29.5 (s). The colorless product is triturated with a small amount of water and then treated with 2 equiv of tetrabutylammonium hydroxide. The mixture is stirred until the acid is completely dissolved; then the solution is stirred for another 5 min. The solvent is removed to afford a colorless oil. **Bis(tetrabutylammonium)-1,3-bis-(methylphosphonatomethyl)naphthalene 5a**: Yield: quantitative; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.94 (t, 36H, *J* = 7.34 Hz), 1.31 (m, 24H), 1.45 (m, 24H), 3.02 (m,24H), 3.16 (d, 2H, $^2J_{HP}$ = 20.2 Hz), 3.44 (d, 2H, $^2J_{HP}$ = 20.2 Hz), 3.51 (d, 3H, $^3J_{HP}$ = 10.1 Hz), 3.58 (d, 3H, $^3J_{HP}$ = 10.1 Hz), 7.32−7.35 (m), 7.62 (m), 7.70 (m), 8.35 (m); ^{13}C NMR (80 MHz, $CDCl_3$) δ 30.3 (d, $^1J_{CP}$ = 139.0 Hz), 33.2 (d, $^1J_{CP}$ = 139.0 Hz), 53.3 (d, $^2J_{CP}$ = 6.8 Hz), 53.4 (d, $^2J_{CP}$ = 6.8 Hz), 124.4 (s), 126.6 (s), 128.5−130.0 (m), 130.6 (m), 131.3 (m), 134.4 (s). ^{31}P NMR (80 MHz, $CDCl_3$) δ 18.8 (s); 18.5 (s). MS (ESIneg): *m*/*z* 342.16, found 171(M^{2-}), 342 ((M + 1)$^-$), 584 ((M + NBu_4^+)$^-$).

Synthesis of Trilithium Trisphosphonate 8. The methyl benzoate **13** is dissolved in 30 mL of methanol/water (2:1), cooled to −15 °C, and treated with 1.5 equiv of lithium hydroxide. The solution is stirred at −10 °C for 6 h and subsequently overnight at room temperature. The solvent is removed to afford the product as a colorless powder. **Lithium bis(dimethoxyphosphorylmethyl)benzoate**: Yield: 89%; ^{1}H NMR (200 MHz, D_2O) δ 3.24 (d, 4H, $^1J_{HP}$ = 21.5 Hz); 3.60 (d, 12H, $^2J_{HP}$ = 10.8 Hz); 7.15 (s, 1H);7.70 (m, 2H); ^{13}C NMR (80 MHz, D_2O) δ 32.9 (d, $^1J_{CP}$ = 138.5 Hz), 52.6 (s), 53.3 (d, $^2J_{CP}$ = 6.8 Hz), 129.9 (m), 131.3 (t, $^4J_{CP}$ = 2.8 Hz), 132.7 (t, $^3J_{CP}$ = 6.2 Hz), 135.8 (t, $^3J_{CP}$ = 6.2 Hz); 169.1 (s); ^{31}P NMR (80 MHz, D_2O) δ 34.5. A solution of 500 mg of lithium bis-(dimethoxyphosphorylmethyl)benzoate in ethanol/dichloromethane (1:2) is treated with 1 equiv of aminomethylphosphonic acid diethyl ester, 1 equiv of diisopropylethylamine, and 1 equiv of bis(2-oxo-3-oxazolidinyl)phosphinic acid chloride and stirred for 3 days at room temperature. After filtration, the solvent is removed in vacuo, and the product (**14**) is purified over silica gel (KG 60, ethanol). **3,5-Bis-(dimethoxyphosphorylmethyl)benzoic acid diethoxyphosphorylmethyl amide 14:** Yield: 75%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.15 (dt, 6H, $^3J_{HH}$ = 7.0 Hz, $^4J_{HP}$ = 1.0 Hz), 3.12 (d, 4H, $^2J_{HP}$ = 21.8 Hz), 3.62 (d, 12H, $^3J_{HP}$ = 10.1 Hz), 3.84 (dd, 2H,$^3J_{HH}$ = 6.4 Hz, $^2J_{HP}$ = 10.8 Hz), 4.11 (m, 4H), 7.30 (s, 1H), 7.59 (m, 2H); ^{13}C NMR (80 MHz, $CDCl_3$) δ 16.3 (d, $^2J_{CP}$ = 5.9 Hz), 32.3 (d, $^1J_{CP}$ = 138.5 Hz), 35.2 (d, $^1J_{CP}$ = 156.9 Hz), 52.4 (d, $^2J_{CP}$ = 6.8 Hz), 62.6 (d, $^2J_{CP}$ = 6.5 Hz), 126.9 (m), 127.3 (m), 131.5 (m), 132.1 (m),134.0 (m), 134.9 (m), 167.2 (dd, $^2J_{CP}$ = 34.0 Hz, $^5J_{CP}$ = 4.4 Hz). ^{31}P NMR (80 MHz, $CDCl_3$) δ 28.7 (s); 23.5 (s). Anal. Calcd for $C_{18}H_{32}NO_{10}P_3$: C, 41.95; H, 6.26, N, 2,72. Found: C, 41.62; H, 6.16; N 2,70. A 1 g amount of intermediate **14** is dissolved in a small amount of acetonitrile and treated with 2.5 equiv of lithium bromide. The solution is refluxed overnight. After cooling to room temperature, the precipitate (**8**) is filtered off, washed two times with a small amount of hot acetonitrile, and dried in vacuo. **Trilithium 3,5-bis(methylphosphonatomethyl)benzoic acid ethylphosphonato methyl amide 8:** Yield: quantitative; mp > 350 °C (dec); ^{1}H NMR (200 MHz, D_2O) δ 1.33 (t, 3H, $^2J_{HP}$ = 7.2 Hz), 3.20 (d, 4H, $^2J_{HP}$ = 20.2 Hz), 3.63 (d, 6H, $^2J_{HP}$ = 12.0 Hz), 3.74(d, 2H, 20.0 Hz), 4.07 (m, 2H), 7.48 (s, 1H), 7.62 (s, 1H); ^{13}C NMR (80 MHz, D_2O) δ 17.4 (d, $^2J_{CP}$ = 5.8 Hz), 30.3 (d, $^1J_{CP}$ = 139.0 Hz), 33.2 (d, $^1J_{CP}$ = 139.0 Hz), 53.3 (d, $^2J_{CP}$ = 6.8 Hz), 53.4 (d, $^2J_{CP}$ = 6.8 Hz), 124.4 (s), 126.6 (s), 128.5−130.0 (m), 130.6 (m), 131.3 (m), 134.4 (s) 169.1 (d, $^2J_{CP}$ = 34.0 Hz). ^{31}P NMR(80 MHz, D_2O) δ 26.6 (s); 20.9 (s). MS (ESIneg): *m*/*z* 456.24, found 456 (M^-), 564 ((M + Li^+)$^-$), 470 ((M + 2Li^+)$^-$). Anal. Calcd for $C_{14}H_{21}Li_3NO_{10}P_3 \cdot 3H_2O$: C, 31.66; H, 5.12, N, 2.64. Found: C, 31.65; H, 4.95, N, 2.60.

NMR Titrations. Ten NMR tubes were filled each with 0.8 mL of a solution of the guest compound (c_{guest} = 0.5−4 mM) in a deuterated solvent (DMSO-d_6, or methanol-d_4). The host compound (1.525 equiv corresponding to the guest) was dissolved in 0.61 mL of the same solvent, and the resulting solution was added with increasing volumes from 0 to 5 equiv to the guest solution in 10 NMR tubes. In some cases, guest solutions were added with increasing volumes from 0 to 5 equiv to the host solution in 10 NMR tubes.

Volume and concentration changes were taken into account during analysis. The association constants were calculated by nonlinear regression methods.

Job Plots. Equimolar solutions (10 mmol/10 mL, approximately 1 mM) of guanidinium compound and bis- or trisphosphonate host were prepared and mixed in various amounts. ^{1}H NMR spectra of the mixtures were recorded, and the chemical shifts were analyzed by Job's method modified for NMR results.

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Supporting Information Available: NMR titration data, titration curves, and Job plots for all new hosts with methylguanidinium hydrochloride and *N*-benzoylarginine ethyl ester hydrochloride. This material is available free of charge via the Internet at http://pubs.acs.org.

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