A Selective Receptor for Arginine Derivatives in Aqueous Media. Energetic Consequences of Salt Bridges That Are Highly Exposed to Water

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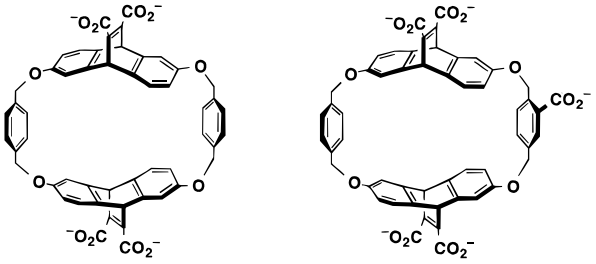
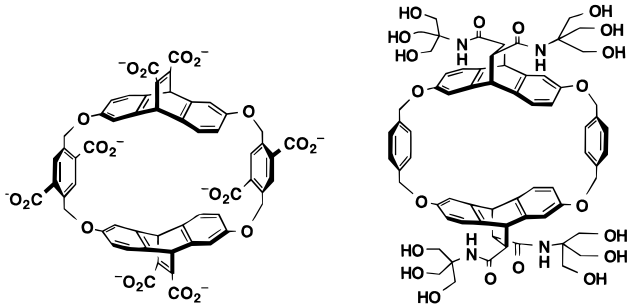
Abstract: Quantitative measures of salt-bridge-type interactions in a highly exposed aqueous environment have been obtained by modifying the well-studied cyclophane platform **1** to include carboxylates in close proximity to bound, cationic guests, producing hosts **2** and **3**. Many guests show significantly enhanced binding to **2** and **3**, but cations of the $RNMe_3^+$ type show little or no enhancement. We propose that the latter observations result from the fact that $RNMe_3^+$ compounds have very diffuse positive charges. Guests that show enhanced binding have focused regions of large, positive electrostatic potential. The highly charged **3** is able to bind very polar, very well-solvated guests, including a series of arginine-based dipeptides. Neutral, water-soluble host **4** was prepared and found to show a decreased affinity for cationic guests. We propose a novel induced dipole mechanism to rationalize these results.

Extensive previous studies of cyclophane host **1** establish that it presents a well-defined, hydrophobic binding site that can bind a wide range of guests with often very high affinities.[1] In addition to hydrocarbon-type guests, **1** binds cationic quaternary ammonium, iminium, guanidinium, and sulfonium compounds with high affinity and in relatively well-defined binding orientations. Central to these binding events are cation-π interactions,[2] in which positive charges are stabilized by interactions with electron-rich faces of aromatic rings. Such studies have suggested new roles for aromatic residues in a variety of biological receptors, especially those that bind the quaternary ammonium neurotransmitter, acetylcholine (ACh).[2,3]

Tetraalkylammonium ions are very good guests for **1**, but protonated amines, RNH_3^+, are not well bound. A similar result is seen with guanidinium ions—extensive alkylation is required for binding while simple systems such as arginine are not bound. Theoretical studies, gas-phase measurements, and evidence from a number of biological structures all indicate that cation-π interactions with RNH_3^+ compounds and simple guanidiniums are quite strong. We thus concluded that it was the greater hydration of protonated versus alkylated cations that made them poorer guests for cyclophane **1**. It is always true that binding is determined by a balance of two issues: the favorable attractions between host and guest and the solvation properties of the two. Tetraalkylammoniums are well solvated by water, but protonated amines are better solvated by as much as 30 kcal/mol.[3] Apparently, host **1** simply cannot overcome the extra solvation of RNH_3^+ guests. We naturally wondered whether we could improve **1** in some way to overcome the additional desolvation penalty of protonated amines. The obvious route would be to combine cation-π interactions with some additional binding interaction such as hydrogen bonding and/or ion pairing.



1 **2**



3 **4**

To that end, we report here the preparation and characterization of two new cyclophanes, **2** and **3**. In host **2** a "fifth" carboxylate is appended in close proximity to the binding site in hopes of providing stabilizing salt bridge interactions with

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(1) Petti, M. A.; Shepodd, T. J.; Barrans, J., R. E.; Dougherty, D. A. *J. Am. Chem. Soc.* **1988**, *110*, 6825−6840. Kearney, P. C.; Mizoue, L. S.; Kumpf, R. A.; Forman, J. E.; McCurdy, A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1993**, *115*, 9907−9919.

(2) Dougherty, D. A. *Science* **1996**, *271*, 163−168. Ma, J. C.; Dougherty, D. A. *Chem. Rev.* **1997**, *97*, 1303−1324.

(3) Dougherty, D. A.; Stauffer, D. A. *Science* **1990**, *250*, 1558−1560.

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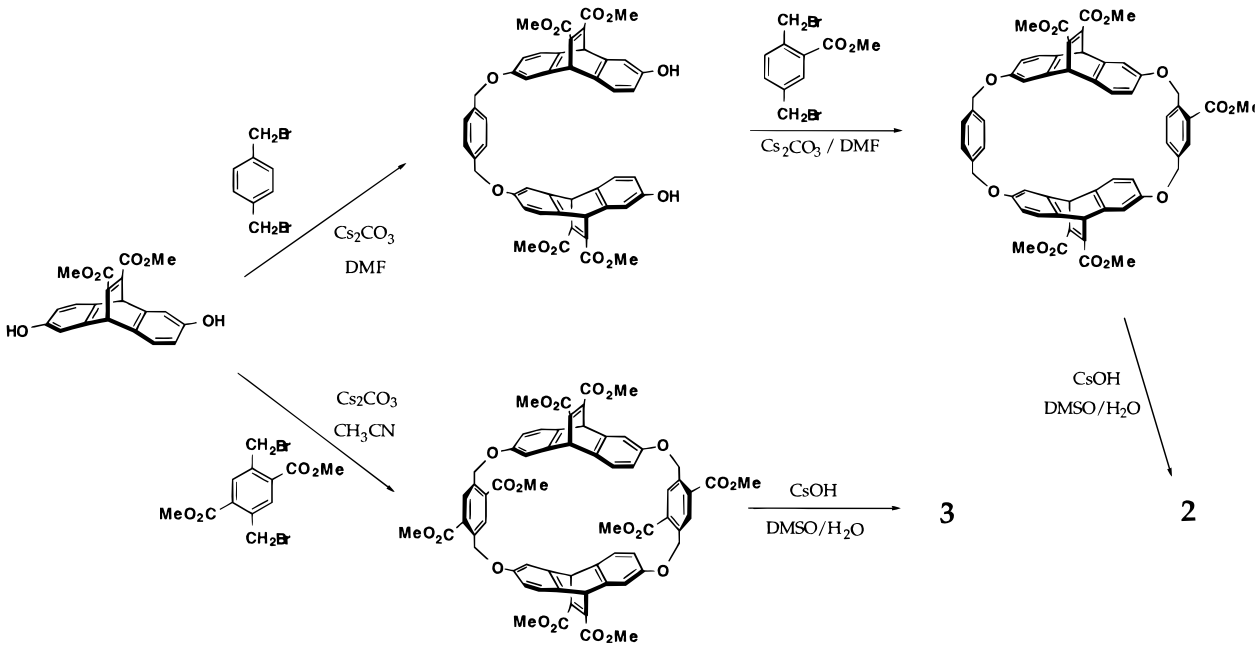


Figure 1. Synthesis of hosts **2** and **3**.

specific cationic guests. The highly charged octacarboxylate **3** could develop multiple salt bridges, as well as providing a very large overall negative charge that could attract cations generally.

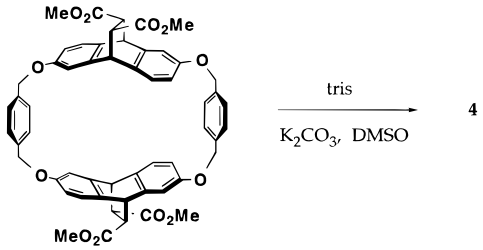
In our cyclophane work, a persistent question has been the role, if any, of the remote carboxylates of **1** in binding cations. Although they cannot come into close contact with guests, and they should be well solvated, there remains the possibility that they could aid cation binding. This issue became more pressing because of some of the results seen with **2** and **3** (see below), and because a number of biological binding sites for cations[4−7] seem to involve close contacts with aromatics but also possible interactions with more remote carboxylates. To probe potential long-range ionic interactions in aqueous media, we prepared the neutral but still water-soluble cyclophane **4**. While the amide groups of **4** are highly polar and well solvated like the remote carboxylates of **1**, they are not charged, and so comparisons between **1** and **4** should be informative on any possible long-range Coulombic interactions between cationic guests and the solvating groups.

Our results establish that even in aqueous media, exposed salt bridges can contribute significantly to binding, but the magnitude of the effect depends strongly on the nature of the charge in the guest. Host **3** is capable of binding highly solvated arginine dipeptides, making use of generic electrostatic attractions as well as specific salt bridges. Studies with **4** establish that the remote carboxylates of **1** can contribute to cation binding, perhaps via an effect that is transmitted by the aromatic rings of the cyclophane.

Synthesis and Characterization of Hosts

Pentacarboxylate 2 and Octacarboxylate 3. The syntheses of **2** and **3** required only minor modifications of the standard methodology we developed earlier.[1] The syntheses of **2** and **3** are summarized in Figure 1. Not surprisingly, both hosts are freely soluble in our aqueous buffer, and binding studies proceeded quite smoothly.

Neutral Host 4. The key to the synthesis of **4** is the efficient reaction of an ester with tris(hydroxymethyl)aminomethane (tris) to make the amide, as developed by Newkome in the context of preparing novel, water-soluble dendrimers.[8] This reaction, however, is not compatible with the ester related to **1**, perhaps because of reaction with the olefins or for steric reasons.



5

Removing the double bonds to produce ester **5** produces a good substrate for the tris reaction. Tetraester **5** is readily available in enantiomerically and diastereomerically pure form using our previously described asymmetric Diels−Alder route.[1] Control studies[9] with the analogue of **1** obtained by direct hydrolysis of **5** reveal that saturating the etheno bridge does not measurably alter the binding properties of such hosts.

The neutral, cyclophane, dodecaalcohol, tetraamide **4** is water-soluble at sub-millimolar concentrations. However, at concentrations necessary for binding studies, some aggregation does occur. As a result, most binding studies with **4** were done in a mixture of 90% (10 mM aqueous NaCl)/10% acetonitrile, in which no aggregation occurs. We have a large reference base

(4) Sussman, J. L.; Harel, M.; Frolow, F.; Oefner, C.; Goldman, A.; Toker, L.; Silman, I. *Science* **1991**, *253*, 872−879. Sussman, J. L.; Silman, I. *Curr. Opin. Struct. Biol.* **1992**, *2*, 721−729.

(5) Satow, Y.; Cohen, G. H.; Padlan, E. A.; Davies, D. R. *J. Mol. Biol.* **1986**, *190*, 593−604.

(6) Hibert, M. F.; Trumpp-Kallmeyer; Bruinvels, A.; Hoflack, J. *Mol. Pharmacol.* **1991**, *40*, 8−15. Trumpp-Kallmeyer, S.; Hoflack, J.; Bruinvels, A.; Hibert, M. *J. Med. Chem.* **1992**, *35*, 3448−3462.

(7) Zhong, W.; Gallivan, J. P.; Zhang, Y.; Li, L.; Lester, H. A.; Dougherty, D. A. *Proc. Natl. Acad. Sci.* **1998**, *95*, 12088−12093.

(8) Newkome, G. R.; Baker, G. R.; Arai, S.; Saunders, M. J.; Russo, P. S. *J. Am. Chem. Soc.* **1990**, *112*, 8458. Newkome, G. R.; Hu, Y. F.; Saunders, M. J.; Fronczek, F. R. *Tetrahedron Lett.* **1991**, *32*, 1133.

(9) Extensive further details on many aspects of this work can be found in the following: Mecozzi, S. Ph.D. Thesis, California Institute of Technology, 1997.

of binding constants for **1** in 10% acetonitrile medium.[1] The mixed solvent shows a remarkably consistent drop of 2.5 kcal/mol in binding for cationic guests of the type studied here to host **1**, presumably due to a diminution of the hydrophobic effect and possible changes in electrostatic/ionic interactions. Importantly, in no instances are any of the significant trends in binding data altered by changing from a strictly aqueous to a 10% acetonitrile medium. Note that in studies of **1**, the aqueous medium is a 10 mM borate buffer, to ensure deprotonation of all of the carboxylates. We therefore added 10 mM NaCl to the aqueous medium used in studies of **4,** in order to minimize ionic strength effects.

Binding Studies

The primary tool for evaluating binding constants in these systems has been NMR spectroscopy. This is now a quite standard tool in studies of molecular recognition. We simply note here that all data sets have been through the rigorous evaluation of statistical significance and precision described in detail elsewhere.[10] As in other studies, we have adopted the fairly conservative view that $-\Delta G°$ values are reliable to ± 0.2 kcal/mol.

Several binding constants have also been determined by circular dichroism (CD) methods. As discussed in detail elsewhere,[11] these cyclophane hosts are especially well suited to the CD method, and it provides an excellent independent check on the validity of the NMR method. In addition, CD allows measurement of some binding constants that are not accessible by NMR. This is especially true for relatively insoluble hosts such as **4**.

Pentacarboxylate Host 2. Figure 2 summarizes binding data for host **2**, with comparisons to analogous data for **1**. The equal affinities of the neutral guests (**6**−**8**) for **1** and **2** indicate that the additional carboxylate moiety of **2** has not had a major impact on the hydrophobic binding cavity. One could have imagined that the carboxylate would decrease the hydrophobicity of the cyclophane cavity or stabilize an unfavorable conformation of the host, either of which would have led to a general decrease in binding for all guests. Since this did not occur, any observed variations in the binding of cationic guests by **2** can be considered a direct result of the differing interactions of these guests with the additional carboxylate of that structure. For the neutral guests and for *all* other guests studied, the highly distinctive changes in ^{1}H NMR chemical shifts that are induced by binding are *identical* for hosts **1** and **2**.[12] Thus, the additional carboxylate of **2** does not alter the hydrophobic nature of the binding site, and it does not cause any guests to orient differently in **2** vs **1**.

Studies of cationic guests reveal considerable variability in the apparent interaction of the guest charge with the additional carboxylate of **2.** Iminium compounds such as **9** and **10** display an enhanced affinity for **2**. Within the class of guanidinium compounds examined, **11** and **12** exhibit significant increases in binding affinity, while **13** shows no enhancement in binding. In contrast, tetraalkylammonium compounds show smaller enhancements, with the extensively studied **14** showing no improvement at all.

The failure to see any enhancement in binding of a guest such as **14** could indicate that the cationic part of the molecule

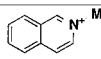
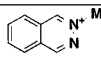
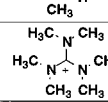
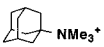
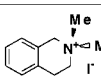
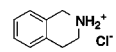
	Guest	-ΔG° (kcal/mol) 1	2	3
6		5.3	5.1	–
7		5.9 [3.5]	5.8 [3.6]	5.5
8		5.7	5.6	5.0
9		7.2 [4.9]	7.8 [5.4]	(9.0)[a,b]
10		6.5	6.9	–
11		5.0 [<3.5]	5.6 [3.8]	6.0
12		6.2	6.7	–
13		4.9	5.0	–
14		6.7 [4.1]	6.6 [4.6]	6.1
15		6.4	6.7	7.6[a]
16		5.9	6.2	6.6
17		5.0	5.3	6.1
18		5.7	–	5.2

Figure 2. Binding energies of guest molecules complexed with cyclophanes **1**−**3**. Value in brackets are in 10% CH_3CN; all others are in pure borate buffer. a. Determined by CD; all others by NMR. b. This value is a lower limit.

was simply unable to get into close contact with the additional carboxylate of **2**. However, modeling and chemical shift data (which are especially informative for guest **14**)[13] strongly indicated that guest **14** is ideally positioned to interact with the carboxylate, yet no effect is seen. Apparently, the strong solvating ability of water completely damps out any ion-pairing interactions in the **2**/**14** pair. If so, then switching to a less effective solvating medium might allow the interaction to be seen. Indeed, we find that simply adding 10% acetonitrile to the aqueous medium leads to a noticeable change (Figure 2). Now the **2**/**14** combination displays a significant ion pair component in its binding. These results clearly demonstrate that guest **14** *can* position itself to interact favorably with the additional carboxylate of **2**. In pure water, however, this interaction is apparently not energetically significant. Note that the acetonitrile effect is not constant for all of the guests studied with host **2**, unlike the observations for host **1**. Although we have only a few observations for host **2**, this is a further example of the context-sensitivity of ion pair interactions versus the relatively generic nature of the hydrophobic interaction.

Host **2** is unique compared to **1** and **3** in terms of conformational complexity—the single carboxylate of **2** completely desymmetrizes the system. Even considering only the rhomboid

(10) Barrans, R. E., Jr.; Dougherty, D. A. *Supramolec. Chem.* **1994**, *4*, 121−130.

(11) Forman, J. E.; Barrans, R. E., Jr.; Dougherty, D. A. *J. Am. Chem. Soc.* **1995**, *117*, 9213−9228.

(12) Extensive further details on many aspects of this work can be found in the following: Kearney, P. C. Ph.D. Thesis, California Institute of Technology, 1994.

(13) Shepodd, T. J.; Petti, M. A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1986**, *108*, 6085−6087.

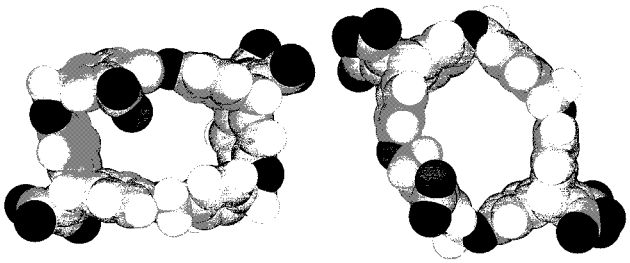


Figure 3. Limiting conformations of host **2**. The rhomboid form (left) is based on the X-ray structure[14] of the tetraester of **1**, with the fifth carboxylate added in an idealized position. The toroid form (right) is an idealized structure. Color code: white = H; gray = C; black = O.

structure, the fifth carboxylate can occupy four distinct positions, and these conformers interconvert via facile bond rotations. Detailed analysis of NMR spectra[12] provides some evidence that the guests that benefit from the extra carboxylate select particular conformations of the host. There would be an entropic cost to such behavior, which could impact all binding to host **2**.

Octacarboxylate Host 3. As the results in Figure 2 indicate, the more dramatic modifications on going from **1** to **3** have a broader influence on binding. Neutral guests (**7** and **8**) have a *decreased* affinity for **3** (relative to **1** and **2**). Again, one can imagine two possible reasons for this: a decrease in the hydrophobic nature of the cyclophane cavity or the development of an unfavorable conformation of the host.

To address the conformational issue, the host structures were examined in the presence of various guests. Cyclophanes such as **1**−**3** can adopt two general binding conformations, (Figure 3) depending on the type of guest bound. For flat aromatic guests such as **7** or **9**, the host adopts a C_2-symmetric rhomboid conformation. This maximizes both the hydrophobic and π-stacking interactions between the host and the guest. For large "spherical" guests such as **14**, the host adopts a D_2-symmetric toroid conformation. In this form, the host has a much more open cavity and can encapsulate the larger guest. The existence of these two conformers of **1** has been supported by ^{1}H NMR chemical shift data as well as circular dichroism (CD) data.[1,11] An X-ray structure of the host **1** tetramethyl ester revealed a rhomboid conformation,[14] in agreement with the favored structure predicted by modeling.

Extensive analysis of the binding conformations of host **3** by both NMR and CD reveal no major changes in the conformational analysis relative to **1** or **2**.[15] We thus conclude that the general diminution of binding for neutral guests exhibited by **3** results from a disruption of the hydrophobic binding site caused by the large number of polar groups.

Concerning cationic guests, relatively flat structures such as **9**, **11**, **15**, and **17** show substantial increases in affinity on going from **1** or **2** to **3**. The more spherical guest **14** shows a *decrease* in binding affinity; the other trimethylammonium compound, **16**, shows only a 0.7 kcal/mol increase (**1** → **3**) even though it is a dication designed to position charges to interact with carboxylates on both faces of the host cavity. A reasonable explanation for these results is that guests that fit well into the rhomboid conformation of these hosts can benefit substantially from the additional carboxylates of **3**. Those that prefer the more open, toroid-like conformation on the host apparently cannot benefit substantially from the additional carboxylates. Perhaps

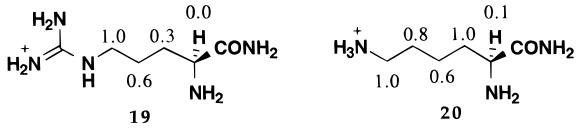


Figure 4. Structures of Arg-NH_2 (**19**) and Lys-NH_2 (**20**). Also shown are the *relative* upfield shifts induced by binding to **3**. The actual maximum shifts are 3.4 ppm for **19** and 3.0 ppm for **20**.

the toroid form is intrinsically less stable in **3** than in **1**, or perhaps in the toroid the carboxylates do not interact well with the cationic guest. Detailed analysis of NMR shift patterns and CD spectra[15] provide support for the notion that conformational factors contribute to the binding differences we see. An apparent exception is sulfonium guest **18**. Although it binds to the rhomboid form of the host, it does not benefit from the extra carboxylates. This could be because a great deal of the positive charge in such a system is found on the sulfur atom,[16] which is presumably buried in the cyclophane cavity and so not in close contact with the carboxylates.

Binding of Arginine Derivatives by Host 3. We were quite encouraged by the enhanced binding of **2** and especially **3** for cations that are not fully quaternized, such as **11** and **17**. Of course, the challenge with such guests is that relative to the fully alkylated structures such as **9** and **14**, ions such as **11** and **17** are very much better solvated by water. These initial results suggested that **3** may be able to bind well solvated cations. The cationic amino acids Arg and Lys seemed like good vehicles to evaluate this possibility.

Arg-NH_2 (**19**) was chosen as the initial ligand (Figure 4). Guest **19** has a 5.0 kcal/mol affinity for cyclophane **3**, which we felt was quite promising. Host **1** does not bind simple arginine derivatives at all. For comparison, we evaluated Lys-NH_2 (**20**), another amino acid with a cationic side chain. Although the hydration energies of **19** and **20** are essentially the same,[17] there are interesting differences between the two. One difference is that the cationic side chain of **19** has three sites for hydrogen-bonding/salt-bridge interactions, while **20** only has one. Also, while both the guanidinium of **19** and the ammonium of **20** can experience cation-π interactions, there is evidence that the former interaction is more favorable.[2]

Consistent with these expectations, **20** has a lesser affinity for cyclophane **3**—the $-\Delta G°$ for binding is 4.0 kcal/mol versus 5.0 kcal/mol for **19**. The NMR shift patterns for bound **19** and **20** (Figure 4) suggest different binding modes for the two. In **19**, the most upfield-shifted CH_2 (and thus the one most buried in the cavity) is directly adjacent to the guanidinium, as expected if a cation-π interaction with this group is important. With **20**, no such clear-cut pattern is evident; CH_2's adjacent to the NH_3^+ and adjacent to the α carbon are strongly upfield shifted. This suggests a coiled conformation that is not controlled by a cation-π interaction.

Encouraged by the binding of **19** to **3**, we decided to try to design a better guest. Since the guanidinium of **19** is presumably buried to some extent in the cavity of **3**, it may not be optimally positioned to make a tight salt bridge with a carboxylate of the host. So, we studied a series of dipeptides in which the side chain of the second residue could make such a contact. These results are summarized in Figure 5.

The NMR shifts of all of the dipeptides in the presence of cyclophane **3** support a uniform binding orientation with the guanidinium group buried in the host cavity and the second residue (X) remaining solvent-exposed.[15] In our initial design,

(14) Forman, J. E.; Marsh, R. E.; Schaefer, W. P.; Dougherty, D. A. *Acta Crystallogr.* **1993**, *B49*, 892−896.

(15) Extensive further details on many aspects of this work can be found in the following: Ngola, S. M. Ph.D. Thesis, California Institute of Technology, 1998.

(16) McCurdy, A. Ph.D. Thesis, California Institute of Technology, 1995.

(17) Eisenberg, D.; McLachlan, A. D. *Nature* **1986**, *319*, 199−203.

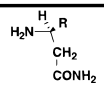
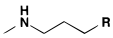
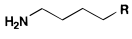
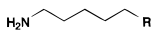
	X-Arg-NH_2	R =	-ΔG° (kcal/mol)	pK_a	%P
21	Asn		4.5	8.72	<50
22	Gly		5.1	9.78	83
23	Sar		4.9	10.20	94
24	β-Ala		5.5	10.24	94
25	γ-Abu		5.8	10.56	97
26	N-Me-γ-Abu		5.6	–	–
27	5-Ava		5.5	10.77	98
28	6-Ahx		5.4	10.80	98
29	Val_acid		5.2	N/A	N/A

Figure 5. Binding energies ($-\Delta G°$) of a set of dipeptides complexed with **3** and the pK_a values of the amines of the corresponding amino acids.[12] %P is the percentage of the amine that is protonated at the pH of the buffer used in the binding studies.

Asn was chosen as the second residue. A preliminary exercise with CPK models placed the amide side chain in a position to interact favorably with the carboxylates. However, the dipeptide (**21**) actually had a lower affinity than Arg-NH_2 (**19**) for cyclophane **3**. This is most likely the result of the increased hydration of the amide side chain.

In our second approach, we started with glycine as the second residue and varied it by increasing the number of methylene groups between the terminal ammonium and the amide bond (see structures in Figure 5). The amine is tethered to the arginine residue by different lengths which determine its proximity to the host carboxylates. Although the overall energy changes are not large, we believe that for a set of such closely related compounds, the trends across the series are meaningful, even though any single comparison may present differences that are only marginally outside our error bars.

The trend in the affinity for **3** of peptides terminating in primary amines is visible in the graph shown in Figure 6. There is clearly a peak when the alkyl chain is three units long, corresponding to γ-Abu-Arg-NH_2 (**25**). This implicates a specific interaction that depends on the precise position of the terminal amine relative to the host carboxylates, rather than just a generic electrostatic effect.

Figure 5 shows that the binding energies do not correlate with the amount of charge. There is an increase in pK_a with increasing number of methylene groups with the estimated degree of protonation ranging from 83 to 98%. When $n \geq 2$, >90% of the amine is protonated. If this were purely a general electrostatic effect, the binding energy should scale with the percentage of protonated amine. This is certainly not the case, although we cannot rule out the possibility that binding affects the pK_a values in a way that is not consistent across the series.

Hydrophobic interactions have a minimal role in binding the second residue. As seen in Figure 5, the trend in binding energy does not scale with hydrophobicity. Incorporation of a neutral

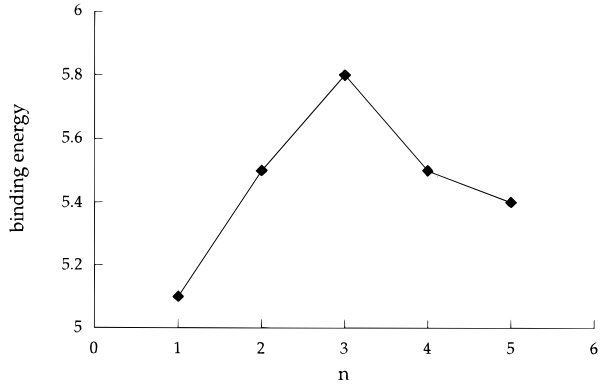
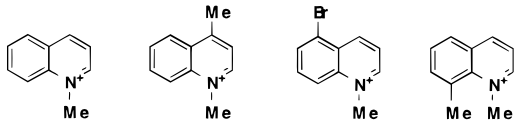


Figure 6. Graph of the trend in binding energy ($-\Delta G°$ in kcal/mol) to **3** with length of alkyl chain (n), where $n = 1$ corresponds to Gly-Arg-NH_2 (**22**). The guests included in this plot are (increasing n) **22**, **24**, **25**, **27**, and **28**. Data are from Figure 5.



30 **31** **32** **33**

4.4/5.8 **5.1/5.9** **4.7/6.1** **5.7/6.3**

[6.9/8.4]

Figure 7. Binding for hosts **4**/**1** to selected guests. Values are in 10% CH_3CN except the data in brackets, which is 100% aqueous (see text).

alkyl chain (**29**) caused a significant *reduction* in binding relative to its charged counterpart. Methylation of the terminal amine had little impact on the peptide's affinity for the host. To verify that the position of the amine in the second residue is most crucial, **23** and **24** were compared. These dipeptides are isomers, with virtually identical pK_a values for the amine. The difference in their affinity for **3** is 0.6 kcal/mol. This shows that the position of the amine in the chain is clearly important and can significantly affect the binding energy.

If a tight electrostatic interaction (i.e., a salt bridge) between the terminal amine and the carboxylate is important, then increasing the salt content of the buffer should cause the peptide's affinity for **3** to decrease. Indeed, we find that for guest **24**, increasing the salt concentration from 10 mM to 1 M drops the binding affinity from 5.5 to 4.6 kcal/mol, evidence of a substantial electrostatic component. Hydrophobic binding is usually increased by raising the salt concentration.

A brief study of the affinity of dipeptide guests for cyclophane **2** was also conducted. In general, we find a roughly 1 kcal/mol drop in affinity on going from **3** to **2**.

Neutral Host 4. Figure 7 summarizes binding data for host **4**. Also shown for comparison are corresponding data for tetraanionic host **1**. In all cases, NMR chemical shift changes seen in guests bound to host **4** are completely analogous to those seen for binding to **1**.[9] Thus, the basic binding geometries are the same in the two systems.

An important general observation concerning **4** should be emphasized. This completely neutral host still shows a substantial binding preference for cationic guests over neutral guests. Recall that in 10% CH_3CN, required for studies of **4**, neutral guests analogous to **30**−**33** do not bind to cyclophanes such as **1** (or **4**) within our detection limits (i.e., $-\Delta G° < 3.5$ kcal/mol).[1] Thus, the cation-π interaction is still quite obvious in this system, again establishing that the binding of cations by **1** was not primarily due to the carboxylates. We have previously studied a neutral analogue of **1** in chloroform, and seen the same trend.[18]

In general, however, **4** binds cations less tightly than **1**. The difference varies somewhat, but averages to about 1 kcal/mol in $-\Delta G°$. Importantly, this difference seems to be independent of the medium, in that it is essentially the same with **30** whether in the purely aqueous or the 10% acetonitrile system. It does appear, then, that the four remote, highly exposed, and thus highly water-solvated, carboxylates of **4** can contribute to the binding of a cation. Given a number of studies that would suggest that highly solvated ion pairs contribute little energetically, even when the ions are much closer than in the present system, this could be considered a surprising observation. These results will be discussed further below.

Discussion

A number of studies of protein and peptide systems have provided estimates for the importance of a salt bridge in stabilizing protein structure.[19] There is considerable variation, but such interactions are generally in the 0−3 kcal/mol range. The higher values are for more buried interactions—surface exposed interactions are typically worth ≤1 kcal/mol. In contrast, on the basis of a survey of a broad range of synthetic receptor types, Schneider has suggested a consistent contribution of 1.2 ± 0.2 kcal/mol for an ion pair in aqueous media.[20]

In the present work we have made several attempts to create well-defined electrostatic interactions and evaluate their energetic consequences for binding in a relatively exposed, aqueous environment. While there is some scatter in the data, several general conclusions emerge.

With host **2** the consequences of a single ion-pair interaction were evaluated. In some systems, a binding enhancement on the order of 0.5−0.6 kcal/mol is seen (**9**−**12**). Importantly, sterically similar neutral guests are unaffected by the fifth carboxylate of **2**, greatly simplifying the analysis. With other guests such as **14**−**17**, the effect is smaller: 0 to 0.3 kcal/mol. The results for the well-studied guest **14** were surprising. NMR and computer-modeling studies suggested the quaternary ammonium group should be able to achieve van der Waals contact with the fifth carboxylate of **2**, yet no binding enhancement is seen.

In previous studies, we have found that electrostatic potential surfaces (eps) can provide valuable insights into noncovalent interactions that have a significant electrostatic component.[1,2,21] Considering the guests of Figure 2,[12] the eps of tetraalkylammonium compounds such as **14** are relatively diffuse—there are no focal points of intense positive charge. In contrast, structures with N^+−H bonds, such as **11** and **12** and quinolinium compounds such as **9** and **10**, do have localized regions of relatively intense positive charge. For **11** and **12** these regions are focused on the N^+−H bonds, but, surprisingly, for **9** and **10** they are centered on the C−H hydrogens from C-1, adjacent to the N^+. We propose that in order for an electrostatic interaction with a carboxylate to be energetically significant in an exposed aqueous environment, the cation must have a region of intense positive electrostatic potential. For a guest such as **14**, it is not worth desolvating the carboxylate in order to establish a tight interaction with the diffuse positive charge of a $R-NMe_3^+$ group. Recall that studies with the less solvating 10% CH_3CN medium establish that guest **14** can bind within the cavity of host **2** in such a way that the positive charge is close to the fifth carboxylate. It is just that this interaction is energetically insignificant in pure water.

The large amount of negative charge presented by **3** strongly influences binding. Now, neutral guests such as **7** and **8** show diminished binding relative to hosts **1** and **2**. Presumably this is because a primarily hydrophobic binding interaction is incompatible with the very polar environment around the rim of the host. However, most cationic guests benefit substantially from the additional charges, with only **14** and **18** remaining as stubborn exceptions. The effect can be quite large, approaching 2 kcal/mol (relative to **1**) for guest **9**.

It is clear that host **3** can bind well-solvated guests through a combination of hydrophobic, cation-π, and ion-pairing interactions. This suggested that even more soluble structures such as the cationic amino acids Arg and Lys might be bound by **3**, and this is clearly true. Arg is a better guest than Lys (i.e., **19** is bound more tightly than **20** by 1 kcal/mol), although the water solubilities of the two are similar. We attribute this difference to the cation-π interaction. A number of arguments suggest that a guanidinium as in Arg is especially well suited to a cation-π interaction.[2] The flat delocalized π system stacks well on aromatics. In addition, there is some evidence[22] that the *face* of a guanidinium is hydrophobic, further encouraging stacking on aromatics. Finally, surveys of protein structures seem to indicate that cation-π interactions involving Arg are more common than those involving Lys, although both occur. The interaction of Arg stacking on the side chain of Trp seems especially favorable.

To probe for a tight, specific salt-bridge interaction with the carboxylates surrounding the rim of the host **3**, the dipeptide structures of Figure 5 were studied. The sum of the results from Figures 5 and 6 strongly suggests a specific and stabilizing salt bridge between an RNH_3^+ group of the guest and a carboxylate (or perhaps more than one) of host **3**. The effect peaks at a particular chain length (Figure 6). This indicates that a fairly precise geometrical arrangement is required, consistent with a salt bridge, but not with a global, electrostatic interaction. Ionic strength effects are consistent with a salt bridge. Hydrophobic effects are not controlling—adding a CH_2 can lead to a *decrease* in binding. The magnitude of the salt bridge interaction depends on the reference state. Taking the optimal case, **25**, the extra salt bridge could be worth as much as 1.3 kcal/mol (**21** as reference) or 0.7 kcal/mol (**22** as reference).

The binding of **25** by **3** represents a very rare example of using a highly polar interaction, such as a salt bridge or hydrogen bond, to enhance molecular recognition in an artificial receptor in fully aqueous media. The results indicate that in favorable cases a combination of binding interactions—cation-π, hydrophobic, and salt bridge—can lead to effective binding of very polar guests in water. They also suggest the potential for developing selective artificial receptors for Arg residues in peptides and proteins.

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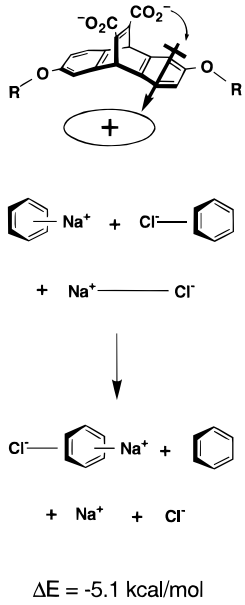

Figure 8. Induced-dipole model for the long-range interaction of the carboxylates of host **1** and a cationic guest. Top: schematic of the induced dipole. Bottom: the isodesmic reaction used to evaluate the phenomenon. Results are from ab initio, 6-31G** calculations. Note that on the "reactant" side, the Na^+-Cl^- structure has the ions 7.55 Å apart; the same separation as on the "product" side.

Given the findings for hosts **2** and **3**, the results from comparing hosts **1** and **4** suggest a surprisingly large long-range ionic interaction. The average increment by which **1** is a better host than **4** is about 1 kcal/mol, but since there are four carboxylates, $\leq$0.3 kcal/mol is implied for each interaction. However, given that in some situations probed here *contact* ion pairs appear to have interaction energies $<$0.3 kcal/mol, it is difficult to understand the stabilization seen due to the remote charges of **4**. It is not possible for the host to contort in such a way as to bring these carboxylates close to a charge of a guest. Also, the carboxylates are highly exposed to water and should be well solvated.

We have considered the possibility that the aromatic rings of the host provide an especially favorable intervening medium for transmitting long-range electrostatic effects, and we have attempted to model this computationally. On the basis of a crystal structure of the tetraester of **1**,[14] we estimate that the distance from the center of charge of a carboxylate in **1** to the center of the nearest benzene ring (from the ethenoanthracene) is 5.1 Å. The question is whether an anion at this distance can influence the binding of a cation to the opposite face of the benzene ring. A nearby anion could induce a dipole in the aromatic in such a way as to enhance binding of a cation to the opposite face of the aromatic (Figure 8). To evaluate this possibility we placed (computationally) a Cl^- 5.1 Å from an aromatic ring and evaluated its influence on the binding of a Na^+ to the opposite face of the ring.[9] With the Na^+ at its optimal distance from the benzene (2.45 Å), the Na^+ and the Cl^- are 7.55 Å apart. The induced dipole contribution to Na^+ binding can be considered to be ΔE for the isodesmic reaction of Figure 8. That is, the "reaction" of Figure 8 evaluates the extent to which it is favorable to have Cl^- and Na^+ bind simultaneously to opposite faces of a single benzene rather than have each bind to an individual benzene. The value of -5.1 kcal/mol for ΔE indicates that the intervening benzene ring does enhance the long-range electrostatic interaction by a significant amount—it is better to have an intervening benzene ring than to have a vacuum. Of course, the 5.1 kcal/mol value will be greatly attenuated by the hydration. The important point, though, is that the intervening medium between the carboxylates of **1** and a cationic guest is not bulk water, but rather a polarizable benzene ring. A magnitude of 0.3 kcal/mol for such an interaction seems quite plausible. The fact that changing the character of the solvent by adding 10% CH_3CN does not change the magnitude of this effect is consistent with this model. Note that this effect will not occur if just any aromatic system is positioned randomly between two charges. The host system locks the aromatic units into a specific alignment, such that the dipole induced by the anion is pointed toward the cationic guest. The situation is clearly different for the additional carboxylates of hosts **2** or **3**, which are closer to the cationic guests, yet in some instances provide no stabilizing interaction. Now the intervening medium is best though of as water, and it can substantially, or even completely, attenuate ionic interactions.

The picture that emerges from studies of **1** and **4** is reminiscent of a number of biological binding sites for organic cations. Two crystal structures of proteins that bind $R-NMe_3^+$ groups have appeared: the acetylcholine esterase[4] and the phosphocholine-binding antibody Fab McPC603.[5] In each case the quaternary ammonium group is in van der Waals contact with aromatic rings, while one or more carboxylate groups are further away, but still within likely interaction distance. A computation model of G protein-coupled receptors that bind acetylcholine and other amines suggests a similar arrangement,[6] and all available data on the nicotinic acetylcholine receptor[7] are also consistent with such a model. Thus, inadvertently, host **1** may have captured the essence of biological binding sites for acetylcholine and related structures—a belt of aromatics for cation-π interactions augmented by more remote carboxylates for ion-pair interactions.

Conclusions

A number of interesting conclusions emerge from these studies. For ion-pair/salt-bridge interactions that are substantially exposed to an aqueous medium, diffuse cations of the $RNMe_3^+$ type are unlikely to experience significant stabilization. However, ions with more focused electrostatic potential, such as those containing N^+-H groups, can substantially benefit from such interactions. When properly designed, as in the **3**/**25** pair, significant binding energy can develop. Through a combination of interactions—including cation-π, hydrophobic, and salt bridge—a receptor that shows a strong affinity and considerable selectivity for arginine derivatives can be developed. The results with host **4** suggest that fairly remote charges can have a small but nonnegligible effect on binding if the intervening medium is a properly positioned aromatic.

Experimental Section

General.[9,12,15] NMR spectra were recorded on Bruker AMX500, Bruker AM500, or General Electric QE-300 spectrometers. Routine spectra were referenced to the residual proton signals of the solvents and are reported in ppm downfield of 0.0 as δ values. All coupling constants, *J*, are in hertz. Spectra from aqueous binding studies were referenced to an internal standard of 3,3-dimethyl glutarate (DMG, δ 1.09). Spectra from mixed solvent (10% acetonitrile in aqueous 10 mM NaCl) binding studies were referenced to the individual proton signal of acetonitrile (δ 1.94). Preparative centrifugal chromatography was performed on a Harrison Research Chromatotron model 7924T using silica plates. HPLC was performed on a Waters dual 510 pump liquid chromatograph system equipped with a Waters 996 photodiode array detector and a Waters 490E wavelength detector. Mass spectral analyses were performed at the University of California, Riverside, the Nebraska Center for Mass Spectrometry, and Caltech. Tetrahydrofuran and diethyl

ether were distilled from sodium benzophenone ketyl, methylene chloride and acetonitrile were distilled from calcium hydride, and anhydrous *N*,*N*-dimethylformamide and anhydrous dimethyl sulfoxide were purchased from Aldrich and used without further treatment. All guests were either commercially available or were prepared by exhaustive methylation of the appropriate amines, with the exception of **11** and **18** which were prepared according to literature procedures. Commercially available Fmoc amino acids were purchased from Novabiochem. The peptide solid support resin and the reagents used in the peptide coupling and deprotection were obtained from Novabiochem, Fluka, and Aldrich. Arg-NH_2 (**19**) and Lys-NH_2 (**20**) were obtained from Sigma and also used without further purification.

Binding Constant Determinations. Studies with hosts **1** to **3** were carried out in a standard 10 mM deuterated cesium borate buffer at pD ≈9 (referred to as borate-d). Studies with host **4** were performed in 10% acetonitrile-d_3 in aqueous deuterated 10mM sodium chloride. Stock solutions of hosts **1** to **4** were obtained by dissolving the host in the appropriate solvent and quantified by integration against a primary standard solution of DMG in the same solvent. Guest solutions for NMR binding studies were prepared by dissolution of the compounds in the appropriate solvent. Guest solution concentrations were determined gravimetrically, by weight of solute, or through NMR integrations against DMG. NMR binding studies were performed by sequential addition of aliquots of guest solutions to an NMR tube containing a solution of cyclophane at an initial concentration of approximately 200 μM (hosts **1** to **3**) or 500 μM (host **4**). Binding data were fit to an appropriate association constant, using the MULTIFIT or EMUL programs.[10] Errors bars on the NMR binding constants measurements were calculated through the analysis packages PORTIA and LUCIUS.[10,23] All circular dichroism (CD) experiments were carried out using a JASCO J-600 spectropolarimeter with either 1.0 or 0.5 cm pathlength quartz cells. Solutions for CD studies were prepared in either borate buffer (pH = 9) or aqueous 10 mM sodium chloride using water passed through a Milli-Q purification system. The host concentration ranged from 1 to 5 μM. In a typical study, CD spectra of six solutions of equivalent host but varying guest concentrations were used. The spectra and $\Delta\epsilon$ values of the host were fit to an association constant using the CDFIT program.[9]

Methyl 2,5-Bis(bromomethyl)benzoate. 2,5-Dimethylbenzoic acid (3.95 g, 26.3 mmol, 1 equiv) was combined with thionyl chloride (4.0 mL, 55 mmol, 2.1 equiv) in a 50 mL round-bottomed flask fitted with a reflux condenser. A calcium chloride drying tube was attached to the condenser, and the thionyl chloride was brought to reflux. After 6 h the resulting clear solution was cooled, and the reflux condenser was replaced with a distillation apparatus. The excess thionyl chloride was removed under reduced pressure. The residue was then diluted with 20 mL of methanol and allowed to stir at room temperature overnight. The methanol was then removed via rotary evaporation. The resulting oil was dissolved in methylene chloride (50 mL) and extracted twice with a saturated solution of sodium bicarbonate (2 × 100 mL), followed by two further extractions with distilled water (2 × 100 mL). The organic layer was then dried over magnesium sulfate, filtered, and concentrated to yield the methyl ester (4.05 g, 24.6 mmol, 93%): ^{1}H NMR ($CDCl_3$) δ 7.71 (s, 1H), 7.16 (d, J = 3, 1H), 7.10 (d, J = 3, 1H),3.86 (s, 3H), 2.53 (s, 3H), 2.32 (s, 3H). This ester (2.46 g, 15.0 mmol, 1 equiv) was dissolved in 50 mL of methylene chloride in a 100 mL round-bottomed flask. *N*−Bromosuccinimide (5.61 g, 3.15 mmol, 2.1 equiv) was added to the flask. The reaction vessel was fitted with a reflux condenser, and its contents were heated to refluxing. The flask was irradiated for 7 min using a General-Electric model RSKB sunlamp with a 275 W, 110−125 V, ac bulb. The solution was allowed to reflux overnight. It was then cooled and filtered, to remove precipitated succinimide, and concentrated. The residue was chromatographed over silica gel, using CH_2Cl_2 as an eluent to separate the product from residual succinimide. The resulting material was twice recrystallized from cyclohexane to yield the desired dibromide in purified form (980 mg, 3.04 mmol, 20% yield): ^{1}H NMR ($CDCl_3$) δ 7.97 (d, J = 2, 1H), 7.50 (dd, J = 6, 2, 1H), 7.43 (d, J = 6, 1H), 4.92 (s, 2H), 4.46 (s, 2H), 3.93 (s, 3H).

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"Three-Quarters" Host. Into a 100 mL three-necked round-bottomed flask fitted with a stopper, a septum, and an Ar gas adapter were placed 1.46 g of cesium carbonate (4.50 mmol, 15 equiv) and 528 mg of (9*R*,10*R*)-2,6-dihydroxy-11,12-dicarbomethoxyethenoanthracene[1] (1.50 mmol, 5 equiv). The flask was then purged with argon, and 40 mL of anhydrous dimethyformamide was injected into the vessel. A solution of *p*-xylene dibromide (79 mg, 0.30 mmol, 1.0 equiv) in 10 mL anhydrous DMF was then delivered via syringe pump into the ethenoanthracene solution over 4 h. The reaction mixture was then allowed to stir at room temperature for 2 days. (Note: both the reaction vessel and the gastight syringe used in the reaction were covered with foil to shield the reactants and products from light.) The solution was then acidified using a few drops of concentrated aqueous HCl until the yellow color of the solution dissipated. The solution was then filtered to remove residual solids, and the solvent was stripped off in vacuo. Preparative centrifugal thin-layer chromatography was employed for further purification. Using 2-mm thick silica gel plates and a 3:1 (v/v) eluent solution of ether:petroleum ether, the excess ethenoanthracene (250 mg, 0.71 mmol) was first recovered. Altering the eluent solution to a 1:1 mixture of diethyl ether:chloroform yielded the desired compound (147 mg, 0.18 mmol, 61%): ^{1}H NMR (acetone-d_6) δ 8.26 (s, 2H), 7.41 (s, 4H), 7.28 (d, J = 8, 2H), 7.20 (d, J = 8, 2H), 7.13 (d, J = 2, 2H), 6.96 (d, J = 2, 2H), 6.60 (dd, J = 8, 2, 2H), 6.44 (dd, J = 8, 2, 2H), 5.43 (s, 2H), 5.41 (s, 2H), 5.04 (s, 4H), 3.71 (s, 12H).

Cyclophane 2, Pentamethyl Ester. Cesium carbonate (276 mg, 0.84 mmol, 4 equiv) was placed in a 250 mL three-necked round-bottomed flask fitted with a stopper, a septum, and an Ar gas adapter. The system was then purged with argon and charged with 50 mL of anhydrous DMF. In a separate flask, the above-prepared "three-quarters" host (171 mg, 0.21 mmol, 1 equiv) and methyl 2,5-bis(bromomethyl)benzoate (69 mg, 0.21 mmol, 1 equiv) were dissolved into 20 mL of anhydrous DMF. The contents of this flask were then drawn into a 25-mL gastight syringe. The solution was then injected into the cesium carbonate suspension over 48 h via a syringe pump. After the injection was complete, the solution was stirred for an additional 24 h. The syringe and the main reaction vessel were shielded from light throughout the course of the reaction. The DMF solution was then filtered to remove cesium salts. The collected solids were washed twice with approximately 10 mL of DMF. The filtrate and washings were then combined and the DMF removed in vacuo. The residue was then mixed with $CHCl_3$, filtered, and concentrated in vacuo. The crude material was then purified via flash chromatography over silica gel with an eluent mixture of 5% Et_2O in CH_2Cl_2 (v/v). Further purification using preparative centrifugal thin-layer chromatography (1-mm thick silica gel plate using an eluent gradient of CH_2Cl_2 to 5% Et_2O in CH_2Cl_2 (v/v)) afforded the pentamethyl ester of the host (53 mg, 0.05 mmol, 26% yield): ^{1}H NMR ($CDCl_3$) δ 7.95 (d, J = 2, 1H), 7.37 (d, J = 8, 1H), 7.37 (dd, J = 8,2, 1H), 7.20 (s, 4H), 7.09 (d, J = 8, 1H), 7.07 (d, J = 8, 2H), 7.05 (d, J = 8, 1H), 6.90 (d, J = 2, 1H), 6.89 (d, J = 2, 1H), 6.88 (d, J = 2, 1H), 6.84 (d, J = 2, 1H), 6.40 (dd, J = 8, 2, 1H), 6.39 (dd, J = 8, 2, 1H), 6.36 (dd, J = 8, 2, 1H), 6.35 (dd, J = 8, 2, 1H), 5.47 (s, 2H), 5.22 (s, 1H), 5.21 (s, 2H), 5.20 (s, 1H), 5.12−4.99 (m, 6H), 3.91 (s, 3H), 3.75 (s, 12H); FAB-MS *m*/*e* 967 (MH^+); HRMS 967.2986, calcd for $C_{58}H_{47}O_{14}$ (MH^+) 967.2965.

Cyclophane 2. The pentaester was dissolved in 2 mL of DMSO, 12.5 equiv of cesium hydroxide in a 1 M aqueous solution (2.5 equiv for each methyl ester) was added, and the solution allowed to stir overnight at room temperature. Water (1−2 mL) was then added, and the solution was allowed to stir for 24 h. The solution was frozen and lyophilized. The residue was dissolved in 25 mM aqueous ammonium acetate solution (1−2 mL) with acetic acid being added as necessary to bring the solution to pH ≈ 7. The pentaacid was then isolated by preparative HPLC. Using a Whatman Magnum 9 column (50 cm, Partisil 10, ODS-3), we found the following HPLC conditions suitable: flow rate of 4.5 mL/min; observation at 260 nm; eluent gradient of 25 mM aqueous ammonium acetate (t = 0 through t = 10 min), followed by a gradient eluent of 0 to 30% acetonitrile in 25 mM ammonium acetate over 50 min. The desired product eluted after approximately 42 min. The appropriate collected fractions were combined, frozen, and lyophilized. The material was then purified using

a cation exchange column (neutral pH, Dowex 50 × 4, NH_4^+ form). The fractions containing host were identified by their UV activity and then combined and lyophilized to give the acid compounds: ^{1}H NMR (10% CD_3CN/90% borate, referenced to internal DMG δ 1.09) δ 7.53 (s, 1H), 7.37 (d, J = 8, 2H), 7.36 (s, 4H), 7.28 (d, J = 8, 1H), 7.23 (d, J = 8, 1H), 7.21 (d, J = 8, 1H), 7.19 (d, J = 8, 1H), 7.17 (d, J = 8, 1H), 7.03 (bs, 4H), 6.62 (dd, J = 8, 2, 1H), 6.60 (dd, J = 8, 2, 1H), 6.59 (dd, J = 8, 2, 1H), 6.54 (dd, J = 8, 2, 1H), 5.32 (s, 2H), 5.19 (s, 3H), 5.18 (s, 1H), 5.13 (d, J = 4, 2H), 5.10 (bs, 4H).

2,5-Dicyano-*p*-xylene.[24] 2,5-Dibromo-*p*-xylene (7.92 g, 30 mmol, 1 equiv) and CuCN (8.1 g, 90 mmol, 3 equiv) were refluxed in DMF (100 mL) for 2 days under argon. The reaction mixture was poured into a solution of NH_4OH (250 mL) yielding a brown precipitate. The solid was filtered off, washed with NH_4OH (250 mL) and water (500 mL), and left open to air-dry. The solid was then hot-extracted with acetone in a Soxhlet apparatus (250 mL, 3 days) yielding a cloudy white solution with a white precipitate. The material was concentrated by removal of the solvent under vacuum to yield a white solid with a yellow tinge. The residual copper was removed by dissolving the material in $CHCl_3$ and running it down a silica gel plug to obtain white crystals (3.78 g, 24 mmol, 81% yield): ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 2H), 2.55 (s, 6H).

2,5-Dimethylterephthalic Acid, Dimethyl Ester.[25] A stirred mixture of the dicyano compound (3.48 g, 22 mmol, 1 equiv.), KOH (3.57 g, 89 mmol, 4 equiv), and diethylene glycol (50 mL) was refluxed overnight under argon. The reaction mixture was then diluted with water (100 mL) and acidified to pH 1 with 10% HCl. A brown solid was filtered off and left to air-dry overnight. The solid was then dissolved in 10% NaOH, and the resulting solution was decolorized with charcoal. The solution was then acidified and filtered to yield an off-white solid which was dried under vacuum. MeOH (100 mL) and $MeSO_3H$ (10 mL) were placed in a flask with the solid and brought up to reflux for 10 h. The reaction was poured into EtOAc (200 mL), 1 M KH_2PO_4 buffer (pH = 7, 400 mL), and brought up to pH 7 with a saturated aqueous solution of sodium bicarbonate. The organic layer was isolated, and the aqueous layer was washed with EtOAc. The EtOAc extracts were combined and dried over sodium sulfate. The solvent was removed using a rotary evaporator yielding an orange-tinged solid. The solid was dissolved in ether and run down a silica gel plug. After the solvent was removed using a rotary evaporator, the resulting material was washed with hexanes to obtain white crystals (3.91 g, 18 mmol, 80% yield): ^{1}H NMR ($CDCl_3$) δ 7.76 (s, 2H), 3.90 (s, 6H), 2.55 (s, 6H).

2,5-Bis(bromomethyl)terephthalic Acid, Dimethyl Ester. The above ester (1.00 g, 4.5 mmol, 1 equiv), *N*-bromosuccinimide (1.68 g, 9.5 mmol, 2.1 equiv), and benzoyl peroxide (~5 pellets) were placed in CCl_4 (15 mL) under argon. The reaction was initiated by bringing it to rapid reflux with a heat gun, and the reflux rate was maintained using a heating mantle. Additional benzoyl peroxide (~5 pellets) was added after 9 h because thin-layer chromatography (1:1 ether/hexanes) indicated that the reaction had not gone to completion. After a further 12 h, all solids were floating on the top of the solution which signaled the consumption of the *N*-bromosuccinimide to form succinimide. The solids were filtered off and recrystallized from CCl_4. The product was a white crystalline solid (0.72 g, 1.9 mmol, 42% yield): ^{1}H NMR ($CDCl_3$) δ 8.05 (s, 2H), 4.93 (s, 4H), 3.98 (s, 6H).

Cyclophane 3, Octamethyl Ester. Macrocyclization was performed in CH_3CN according to published procedures. After completion, the reaction mixture was filtered and the solvent was removed. The residue was flash-chromatographed over silica gel, eluting first with CH_2Cl_2 and then ether in order to separate the macrocyclic compounds from baseline impurities. The macrocycle was then isolated from oligomers using preparative centrifugal thin-layer chromatography (silica gel plates, 0−10% ether in CH_2Cl_2). The product was a white, translucent film (33 mg, 30 μmol, 8% yield): ^{1}H NMR ($CDCl_3$) δ 8.04 (s, 4H), 7.07 (d, J = 8, 4H), 6.92 (d, J = 2, 4H), 6.38 (dd, J = 2, 8, 4H), 5.39 (AB, J = 14, $\Delta\nu$ = 66 Hz, 8H), 5.23 (s, 4H), 3.78 (s, 12H), 3.75 (s, 12H); FAB-MS *m*/*e* 1141.3 (MH^+); HRMS (matrix, NBA) calcd for $C_{64}H_{52}O_{20}\cdot H^+$ 1141.3130, found 1141.3188, Δ = −5.1 ppm.

(24) Rehahn, M.; Schlüter, A.-D.; Feast, W. J. *Synthesis* **1988**, 386−388.

(25) Anzalone, L.; Hirsch, J. A. *J. Org. Chem.* **1985**, *50*, 2128−2133.

Cyclophane 3. Hydrolysis was performed by dissolving the octaester (11 mg, 10 μmol, 1 equiv) in DMSO (1 mL) and adding CsOH (1 M aqueous solution, 578 μL, 578 μmol, 60 equiv). After ~34 h, the reaction mixture was lyophilized. The resulting solid was redissolved in water and run down a freshly regenerated (20% aqueous HCl) Diaion (WK100) weakly acidic ion exchange column eluting with Millipored water. The UV-active fractions were collected and lyophilized to obtain a pale yellow solid. The colored impurities were removed by running the material through a Sephadex G-15 column (1 g) eluting with Millipored water. (Impurities can also be removed by running the material down a Sep-Pak C_{18} cartridge (6 cm^3, 1 g) and eluting with 0−20% CH_3CN/water). The product was lyophilized to obtain a white solid (5.3 mg): ^{1}H NMR (borate-d) δ 7.40 (s, 4H), 7.22 (d, J = 8, 4H), 7.03 (d, J = 2, 4H), 6.53 (dd, J = 2, 8, 4H), 5.21 (AB, J = 13, $\Delta\nu$ = 19 Hz, 8H), 5.22 (s, 4H).

Host 4. Into a 5 mL round-bottom flask were placed 25 mg (0.027 mmol) of host **5** (prepared according to standard procedures), 68 mg of tris (0.56 mmol), and 50 mg of potassium carbonate (dried overnight at 350 °C). The flask was fitted with a septum, and 0.25 mL of anhydrous DMSO was injected. The slurry was vigorously stirred at the temperature of 60 °C (the reaction vessel was covered with foil to shield the reactants and the products from light). After 60 h (TLC, $CHCl_3$/MeOH 7:3) the solution was cooled, 0.5 mL of DMSO was added, and the solid (mainly K_2CO_3) was filtered away. The DMSO solution was then frozen and lyophilized. The remaining residue was dissolved in 2 mL of water, frozen, and lyophilized again. This procedure was repeated twice in order to eliminate any trace of DMSO. The lyophilization products (white powder) were dissolved in 3 mL of MeOH, and undissolved solid, mainly tris, was filtered away. The methanolic solution was concentrated and depleted of the excess of tris by a fast column chromatography ($CHCl_3$/MeOH 7:3). Pure host **4** was recovered by a second column chromatography using a solvent gradient (95−75% chloroform in methanol) (21.5 mg 0.017 mmol, 62%): ^{1}H NMR (methanol-d_4) δ 7.21 (s, 8H), 7.02 (d, J = 8, 4H), 6.88 (d, J = 2, 4H), 6.54 (dd, J = 2,8, 4H), 5.07 (AB, 8H), 4.33 (s, 4H), 3.65 (AB, 24H), 3.19 (s, 4H); ^{13}C NMR (methanol-d_4) δ 174.30, 156.76, 144.56, 137.06, 132.51, 126.66, 125.06, 111.49, 111.36, 69.12, 62.13, 61.27; FAB-MS *m*/*e* 1291 (MNa^+).

***N*-(Fmoc), *N*-Methyl-γ-aminobutyric Acid.** *N*-Me-γ-Abu (1.53 g, 10 mmol, 1 equiv) was dissolved in 10% aqueous Na_2CO_3 (26.5 mL). Dioxane (30 mL) was added yielding a clear solution. Next, Fmoc-Cl (2.6 g, 10 mmol, 1 equiv) was added, and the solution was heated in a 70 °C oil bath. The solution gradually turned yellow, with bubbling and formation of a white fluffy precipitate. After 6 h, the bubbling ceased, and the reaction mixture was poured into water (400 mL) and extracted twice with ether (100 mL). Concentrated HCl was added to bring the aqueous layer to pH 3, resulting in a cloudy solution. The solution was left at 4 °C overnight and filtered to obtain a gummy yellow solid. This solid was washed with water and dissolved in CH_2Cl_2, and the solvent was removed under vacuum to yield a yellow oil, recrystallized from H_2O/CH_3CN to obtain white crystals (1.89 g, 5.6 mmol, 56% yield): ^{1}H NMR (CD_3OD) δ 7.79 (d, J = 7, 2H), 7.60 (d, J = 7, 2H), 7.33 (m, 4H), 4.53 (d, J = 5, 1H), 4.43 (d, J = 5, 1H), 4.23 (t, J = 5, 1H), 3.29 (t, J = 7, 1H), 2.96 (t, J = 7, 1H), 2.78 (s, 3H), 2.22 (t, J = 7, 1H), 1.95 (t, J = 7, 1H), 1.76 (m, 1H), 1.45 (m, 1H); FAB-MS *m*/*e* 340.2 (MH^+); HRMS (matrix, NBA) calcd for $C_{20}H_{21}NO_4\cdot H^+$ 340.1549, found 340.1545, Δ = 1.3 ppm.

Solid-Phase Peptide Synthesis. Rink Amide MBHA resin (0.5 mmol/g) was used to afford carboxyl terminus primary amides. Peptide synthesis was carried out using Fmoc-protected amino acids. Typical protocols for coupling a residue involved 50−90 min coupling cycles with 2 equiv of amino acid. Activated esters were formed in situ using benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP), 1-hydroxybenzotriazole (HOBt), and diisopropylethylamine (DIPEA). Deprotection of Fmoc-protected amine groups was performed using a 20-min cycle with 20% piperidine/dimethylformamide (DMF). The peptides were cleaved from the resin by treatment with trifluoroacetic acid (TFA)/*m*-cresol/ethanedithiol (92.5:1.3:6.2) for 90 min. The resin was filtered and washed with TFA. The combined filtrates were cooled to 0 °C, thioanisole/trimethylsilylbromide (TMSBr) (47:53) added and left under argon for 15 min. The peptide was

precipitated with ether and left at 4 °C overnight. The precipitate was trapped on a fine sintered funnel and washed with a large volume of ether. The peptide was then dissolved in H_2O and lyophilized. The resulting peptide was triturated with H_2O/ether and H_2O/acetonitrile several times sequentially. The peptides were then lyophilized, and if impurities were detected by NMR, reverse-phase C_{18} chromatography was used for further purification.

Asn-Arg-NH$_2$ (21): ^{1}H NMR (borate-d) δ 4.32 (dd, $J = 5, 8$, 1H), 3.77 (t, $J = 7$, 1H), 3.24 (t, $J = 7$, 2H), 2.65 (m, 2H), 1.92 (m, 1H), 1.79 (m, 1H), 1.69 (m, 2H); FAB-MS *m*/*e* 288.2 (MH^+); HRMS (matrix, NBA) calcd for $C_{10}H_{21}N_7O_3 \cdot H^+$ 288.1784, found 288.1786, $\Delta = -0.8$ ppm.

Gly-Arg-NH$_2$ (22): ^{1}H NMR (10% AcOD/D_2O) δ 4.27 (t, $J = 6$, 1H), 3.83 (s, 2H), 3.16 (t, $J = 7$, 2H), 1.76 (m, 2H), 1.61 (m, 2H); FAB-MS *m*/*e* 231.2 (MH^+); HRMS (matrix, NBA) calcd for $C_8H_{18}N_6O_2 \cdot H^+$ 231.1569, found 231.1579, $\Delta = -4.3$ ppm.

Sar-Arg-NH$_2$ (23): ^{1}H NMR (borate-d) δ 4.35 (dd, $J = 5, 8$, 1H), 3.39 (s, 2H), 3.23 (t, $J = 7$, 2H), 2.27 (s, 3H), 1.89 (m, 1H), 1.78 (m, 1H), 1.68 (m, 2H); FAB-MS *m*/*e* 245.2 (MH^+); HRMS (matrix, NBA) calcd for $C_9H_{20}N_6O_2 \cdot H^+$ 245.1726, found 245.1720, $\Delta = 2.3$ ppm.

***β*-Ala-Arg-NH$_2$ (24):** ^{1}H NMR (borate-*d*) δ 4.33 (dd, $J = 6, 8$, 1H), 3.23 (t, $J = 6$, 2H), 3.05 (t, $J = 7$, 2H), 2.59 (t, $J = 7$, 2H), 1.87 (m, 1H), 1.76 (m, 1H), 1.68 (m, 2H); FAB-MS *m*/*e* 245.2 (MH^+); HRMS (matrix, NBA) calcd for $C_9H_{20}N_6O_2 \cdot H^+$ 245.1726, found 245.1725, $\Delta = 0.2$ ppm.

***γ*-Abu-Arg-NH$_2$ (25):** ^{1}H NMR (borate-d) δ 4.28 (dd, $J = 5, 8$, 1H), 3.23 (t, $J = 7$, 2H), 2.95 (t, $J = 8$, 2H), 2.43 (t, $J = 7$, 2H), 1.92 (m, 2H), 1.86 (m, 1H), 1.75 (m, 1H), 1.68 (m, 2H); FAB-MS *m*/*e* 259.2 (MH^+); HRMS (matrix, NBA) calcd for $C_{10}H_{22}N_6O_2 \cdot H^+$ 259.1882, found 259.1873, $\Delta = 3.8$ ppm.

***N*-Me-*γ*-Abu-Arg-NH$_2$ (26):** ^{1}H NMR (D_2O) δ 4.18 (dd, $J = 5, 8$, 1H), 3.15 (t, $J = 7$, 2H), 2.99 (t, $J = 8$, 2H), 2.65 (s, 3H), 2.39 (t, $J = 7$, 2H), 1.90 (m, 2H), 1.68 (m, 2H), 1.62 (m, 2H); FAB-MS *m*/*e* 273.3 (MH^+); HRMS (matrix, NBA) calcd for $C_{11}H_{24}N_6O_2 \cdot H^+$ 273.2039, found 273.2039, $\Delta = 0.0$ ppm.

5-Ava-Arg-NH$_2$ (27): ^{1}H NMR (borate-*d*) δ 4.28 (dd, $J = 5, 8$, 1H), 3.23 (t, $J = 7$, 2H), 2.98 (t, $J = 7$, 2H), 2.38 (t, $J = 7$, 2H), 1.86 (m, 2H), 1.76 (m, 2H), 1.67 (m, 4H); FAB-MS *m*/*e* 273.3 (MH^+); HRMS (matrix, NBA) calcd for $C_{11}H_{24}N_6O_2 \cdot H^+$ 273.2039, found 273.2032, $\Delta = 2.5$ ppm.

6-Ahx-Arg-NH$_2$ (28): ^{1}H NMR (borate-*d*) δ 4.33 (dd, $J = 5, 8$, 1H), 3.23 (t, $J = 7$, 2H), 2.97 (t, $J = 8$, 2H), 2.34 (t, $J = 7$, 2H), 1.86 (m, 2H), 1.75 (m, 2H), 1.66 (m, 4H), 1.39 (m, 2H); FAB-MS *m*/*e* 287.2 (MH^+); HRMS (matrix, NBA) calcd for $C_{13}H_{26}N_6O_2 \cdot H^+$ 287.2195, found 287.2203, $\Delta = -2.7$ ppm.

Val_acid-Arg-NH$_2$ (29): ^{1}H NMR (D_2O) δ 4.17 (dd, $J = 5, 8$, 1H), 3.12 (t, $J = 7$, 2H), 2.22 (t, $J = 7$, 2H), 1.76 (m, 2H), 1.60 (m, 2H), 1.47 (m, 2H), 1.19 (m, 2H), 0.78 (t, $J = 7$, 2H); FAB-MS *m*/*e* 258.2 (MH^+); HRMS (matrix, NBA) calcd for $C_{11}H_{23}N_6O_2 \cdot H^+$ 258.1930, found 258.1929, $\Delta = 0.2$ ppm.

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