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Binding of Acetylcholine and Tetramethylammonium to Flexible Cyclophane Receptors: Improving on Binding Ability by Optimizing Host's Geometry

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Received January 16, 2004

The structure of a cyclophanic tetraester (**1**), previously employed for investigations on the cation−π interaction, has been optimized to better accommodate acetylcholine (ACh) and tetramethylammonium (TMA) guests. Following indications from molecular modeling calculations, a flexible cyclophane receptor of significantly improved binding properties has been obtained by removing the four carbonyl groups of the parent host. 2,11,20,29-Tetraoxa[3.3.3.3]paracyclophane (**2**) was prepared by an improved procedure, which was conveniently devised to avoid the formation of contiguous cyclooligomers that caused serious separation issues. Association of **2** with TMA picrate was measured in $CDCl_3$ at $T = 296$ K by ^1H NMR titrations and compared to binding data obtained for a set of reference hosts, including the parent tetraester **1**, the corresponding cyclophanic tetraamine, the open-chain counterpart of **2**, and its cyclooligomers from pentamer to octamer. Binding enhancements ranging from 15-fold (with respect to the tetraester and the tetraamine) to over 80-fold (with respect to the open-chain tetraether) were achieved by geometry optimization of the host. Binding of **2** to ACh and TMA was investigated for a variety of counterions. A constant binding free energy increment of nearly 8 kJ mol^{-1} with respect to **1** was observed, independent from the anion and irrespective of the different structure of the cationic guests. Results showed that the electrostatic inhibiting contribution of the counterion to the cation's binding is a characteristic constant of each anion. The value of $-\Delta G° = 44.9$ kJ mol^{-1} extrapolated for TMA in the absence of a counterion indicates that 28−34 kJ mol^{-1} of binding free energy are lost in ion pairing.

Introduction

The interaction of alkylammonium guests with synthetic receptors possessing aromatic binding sites has been central in the investigation of the cation-π interaction, mainly because of its biological implications.[1] Since binding of quaternary ammonium cations to aromatics is weak, a substantial effort has been dedicated to the design and the synthesis of rigid hosts possessing preorganized cavities, which could exploit the advantage of preorganization to increase their binding strength. Typical examples are capsules[2] and rigidified arenes,[3] cyclophanes,[4] and calixarenes,[5] of which some cases exhibiting outstanding binding properties have recently been reported.[6] Significant binding enhancements have also been achieved with hosts capable of complexing the whole ion pair,[7] or by tayloring the host around the guest through the innovative dynamic combinatorial approach.[8] On the other hand, flexibility is not always an enemy; indeed, nonpreorganized receptors have been useful in gaining an insight into the basic attractive force underlying the cation−π interaction: by making use of flexible cyclophanic and open-chain phanic hosts, that is, receptors possessing little or no preorganization at all, we were able to gather a body of information on the intrinsic affinity of quaternary ammonium cations for aromatics

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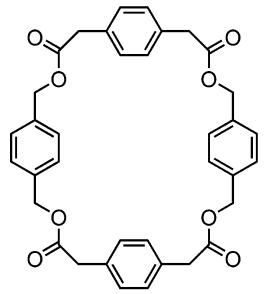
10.1021/jo049899j CCC: $27.50
Published on Web 04/29/2004

and on the energetics of the cation−π interaction.[9] In these studies, focused on tetramethylammonium (TMA) and acetylcholine (ACh), we have extensively employed the cyclophanic tetraester **1**, mainly because of the conveniently large upfield shifts induced upon binding on the guests' signals in the ^{1}H NMR spectra, which allowed for accurate measurements of binding constants.



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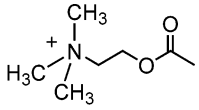
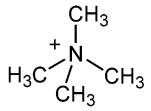


ACh

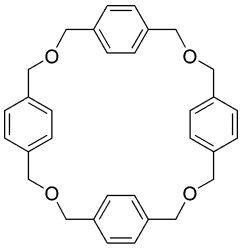


TMA

Despite the useful shift variations, the observed association constants were indeed very modest. Some obvious questions are the following: Can the binding ability of the cyclophanic tetraester **1** be improved through structural modifications, still preserving the flexibility of the host and the large induced shifts? How much can be gained in binding energy with a flexible cyclophane just by geometry optimization? Since the interaction is electrostatic in nature,[1d,9b,e] how critical are ring size and functional groups nature? In an effort to answer these questions, we thought that even flexible nonpreorganized cyclophanes might show significantly enhanced binding properties based on pure cation−π interactions, provided that the steric, geometric, and coordinative requirements of the guest could be finely matched by a correctly designed host. In the present paper we report that a significant improvement of binding ability with respect to the parent cyclophane has indeed been achieved just by appropriate modifications of the geometry and the constitution of the cyclophane, without resorting to a more rigid structure or to additional attractive contributions.

Results and Discussion

Design and Synthesis. Molecular modeling calculations showed that **1** needs to wrap around TMA and the cationic head of ACh to establish binding interactions;[9e] such a conformational reorganization suggests that the cyclophane cannot accommodate the guest inside the ring cavity, even though the ring size is large enough for the task. This feature is presumably due to the conformational restraints imposed by the four ester groups in the backbone, which hamper the cyclophane from reaching the proper binding conformation. From computational[10] and crystallographic[11] literature data we gathered that optimal binding of TMA by a cyclophane receptor is achieved when the cation is located in the center of a region delimited by four aromatic rings, at a distance of 4.2−4.4 (computational) or 4.5−4.6 Å (crystallographic) from the aromatics' centroids. Such an arrangement could be achieved by removing the ester carbonyl groups from the structure of **1** to give a paracyclophanic tetraether, in which four aromatic rings are bridged by three-atom spacers; the central ether oxygen atom in the bridge would avoid the steric hindrance of a full-carbon spacer, caused by hydrogen atoms perching into the ring cavity; removal of constraints and correct geometry could thus be simultaneously achieved from **1** through this transformation. Indeed, molecular modeling calculations indicated that the tetraoxa[3.3.3.3]paracyclophane **2** can assume a binding conformation capable of accommodating TMA in the center of the ring's cavity at appropriate distances from the aromatics' centroids (4.2−4.7 Å), and corresponding space-filling models showed that the cavity is correctly filled by the guest (Figure 1).



2

The synthesis of **2** was accomplished by the method of Komatsu,[12] by reacting terephthalaldehyde and trieth-

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(11) From a search of the Cambridge Crystallographic database (CSD), the distance between the quaternary nitrogen and the centroid of the aromatic ring for all the available structures containing quaternary ammonium−aromatic ring contacts below 5 Å shows a maximum at 4.56 Å (116 structures), with 126 structures showing a distance in the 4.2−4.4 Å range.

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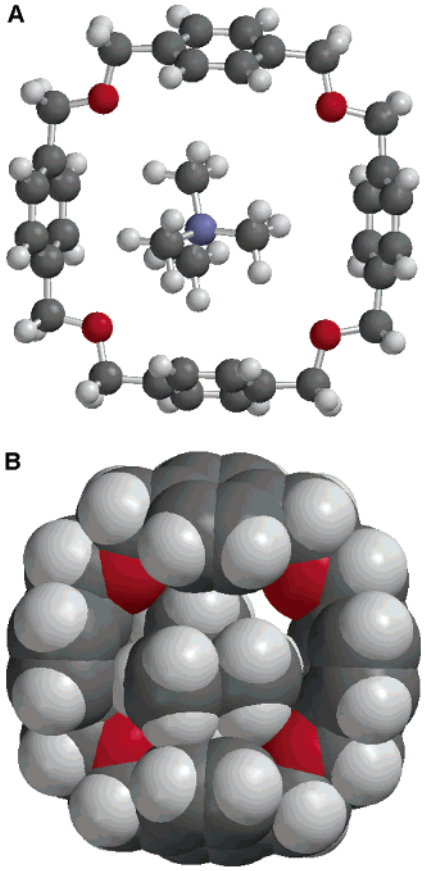
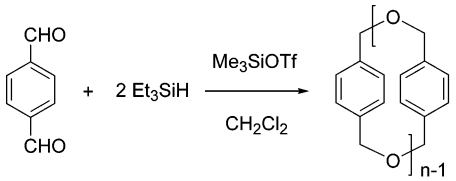


FIGURE 1. PM3-computed model of the complex between **2** and TMA: (A) ball-and-stick representation and (B) space-filling representation. PM3 optimization was performed on a complex's geometry obtained through a molecular mechanics (MMFF94) conformational search.

SCHEME 1. Synthesis of Oligomeric Oxa[3.*n*]paracyclophanes



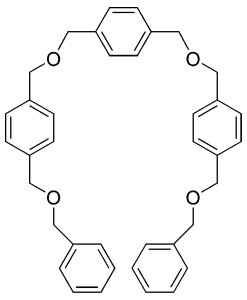
2, n = 4
3, n = 5
4, n = 6
5, n = 7
6, n = 8

ylsilane in the presence of catalytic amounts of trimethylsilyl triflate (Scheme 1).

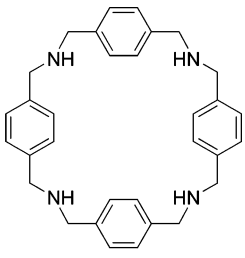
This reductive cyclooligomerization reaction afforded a whole family of oxa[3.*n*]paracyclophanes, from which the oligomers from the tetramer to the octamer (**2**−**6**) could be isolated as pure compounds. Besides the target cyclophanic tetraether, a set of cyclooligomeric homologues were thus available to ascertain the effect of ring size on binding properties. Although Komatsu's reaction is a practical entry to cyclophanic polyethers, it suffers from two major drawbacks: (a) optimized (analytical) yields of tetrameric **2** never exceeded 5% on a 10 mmol scale at 0.02 M substrate concentration and (b) separation of cyclic oligomers is extremely tedious, requiring repeated chromatographic and crystallization steps and eventually leading to very poor isolated yields of pure compounds.[13] To overcome these limitations, we devised a modified procedure that conveniently avoids the formation of contiguous oligomeric products (Scheme 2). The key feature of this procedure consists of reacting a preformed "trimeric" dialdehyde instead of terephthalaldehyde with the bis-silyl ether of 1,4-benzenedimethanol in a 3 + 1 cyclization reaction, which ties up the cyclooligomerization to proceed in steps multiple of 4 units, thus excluding the formation of those cyclooligomers that caused separation issues. Reaction mixtures obtained this way could be efficiently separated by a single chromatographic step to give **2** in pure form.

A second major improvement was obtained by changing the order of addition of the reactants and taking advantage of the influx technique in the cyclization step, that is, by slow addition through a syringe pump of a 1:1 mixture of the two reactants to a solution of the reducing agent and the catalyst. Analytical yields of **2** in the reaction mixture were above 10%, giving 8−9% of isolated pure product by flash column chromatography. The longer synthesis compared to the original method, required for the preparation of the dialdehyde **9** from commercial terephthalaldehyde mono-diethylacetal, was compensated by the limited number of high-yielding steps that did not require the isolation of intermediates **7** and **8**.

The phanic tetraether **10**, the open-chain counterpart of **2**, was prepared as a reference compound by a similar procedure, i.e., by reacting the dialdehyde **9** with the trimethylsilyl ether of benzyl alcohol in the presence of catalytic amounts of trimethylsilyl triflate. For comparison reasons, the cyclophanic tetraamine **11** was also prepared by literature methods,[14] to ascertain whether binding properties may be affected by the nature of the heteroatom in the spacers or by slight variations in the size of the ring.



10



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Binding Studies. To evaluate the binding properties of the described hosts, the association constants (K_a) for the formation of the 1:1 complexes with tetramethylammonium picrate (TMAPic) were measured in $CDCl_3$ at T = 296 K by ^1H NMR titrations, in analogy to previous studies,[9] monitoring the time-averaged signal of the free and complexed TMA with increasing host concentration. The results are reported in Table 1, together with data

(13) In the original work, the authors stated that separation of oligomers was accomplished by GPC chromatography. See ref 12, p 1532.

(14) Shinmyozu, T.; Shibakawa, N.; Sugimoto, K.; Sakane, H.; Takemura, H.; Sako, K.; Inazu, T. *Synthesis* **1993**, 1257−1260.

SCHEME 2. Synthesis of Tetraoxa[3.3.3.3]paracyclophane 2

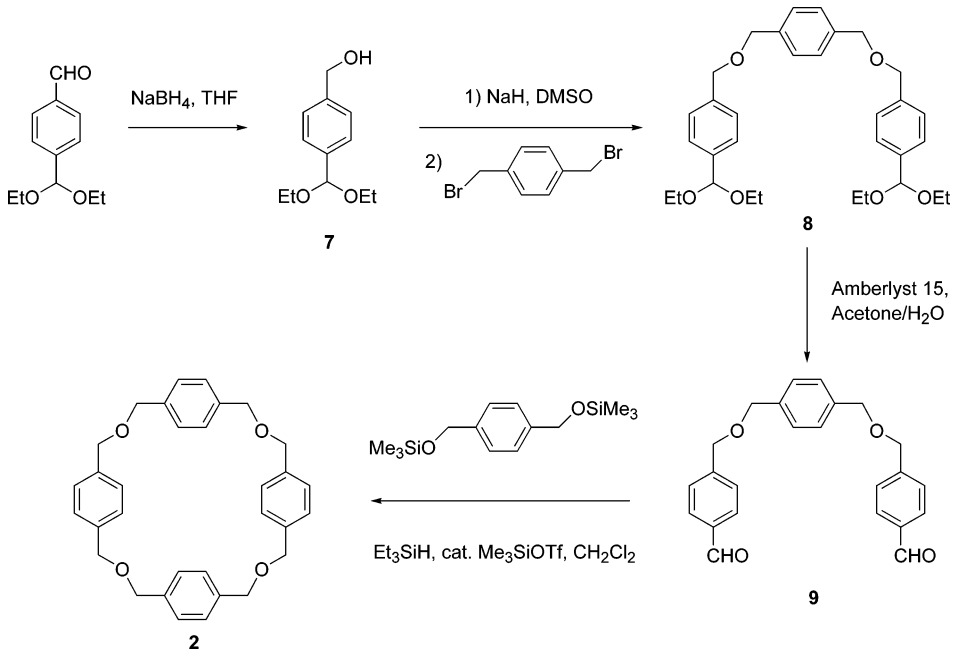

TABLE 1. Association Constants K_a (M^{-1}), Standard Free Energies of Binding $-\Delta G°$ (kJ mol^{-1}), and Guest Limiting Shift Values $\Delta\delta_\infty$ (ppm) for the Formation of 1:1 Complexes of Tetramethylammonium Picrate (TMAPic) with Phanic Hosts[a]

host	K_a (SE)[b]	$-\Delta\delta_\infty$	$-\Delta G°$
1[c]	29.7 (0.4)	1.479	8.35(3)
1[c,d]	6.6 (0.1)	1.150	4.64(4)
2	458 (6)	2.090	15.08(3)
3	14.2 (0.1)	1.225	6.53(2)
4	11.3 (0.1)	0.781	5.97(2)
5	n.d.[e]	n.d.[e]	n.d.[e]
6	16.4 (0.5)	0.798	6.88(8)
10	5.5 (0.1)	0.909	4.20(4)
11	29 (1)	0.755	8.29(8)
11[d]	9.6 (0.1)	0.910	5.57(2)

[a] Measured by ^1H NMR (200/300 MHz) at $T = 296$ K in $CDCl_3$ on 0.1 mM solutions of TMAP, using host concentrations up to 60 mg/mL. [b] Standard error of the nonlinear least-squares fit. [c] Data from ref 9b. [d] Data for tetramethylammonium chloride. [e] Non detectable; for $K_a \leq 2$ titration curves become indistinguishable from each other in the investigated concentration range.

previously obtained for **1** under the same conditions. It can immediately be noted that the cyclophanic tetraether **2** is by far the best receptor of the whole set: the observed binding constant is over 15 times larger that that of the parent cyclophane **1**, with an increase in binding free energy of nearly 7 kJ mol^{-1}, while the larger induced shift upon complexation indicates a closer contact of the guest with the aromatic π faces. The improvement achieved through the devised transformation supports the hypotheses made about the optimal geometry of a cyclophanic receptor for TMA. A further support is provided by the structure of **2** in the solid state that, to avoid empty space, adopts a flattened conformation in which opposite pairs of aromatic rings are parallel but staggered (Figure 2); extensive reorganization is consequently required to reach the correct binding conformation but, despite the energetic cost of such a reorganization, the binding improvement obtained with respect to **1** is in fact substantial. Comparison with higher cyclooligomers (Table 1) indicates to what extent the correct size of the cyclophane is significant to its binding capabilities: except for the heptamer **5**, whose association is undetectable, all the other oligomers (**3**−**6**) show binding free energies measurable but distinctly smaller than **1**, suggesting a reduced adaptivity to the guest with respect to the latter, despite the larger number of binding sites and the lack of conformational restraints.

Some degree of organization is however evident from comparison with the open-chain tetraether **10**, which can be viewed as the nonpreorganized reference; **3**, **4**, and **6** bind TMAPic stronger than **10** by 1.8−2.7 kJ mol^{-1}, demonstrating little binding energy gain, with respect to an open-chain counterpart, for cyclophanic hosts of

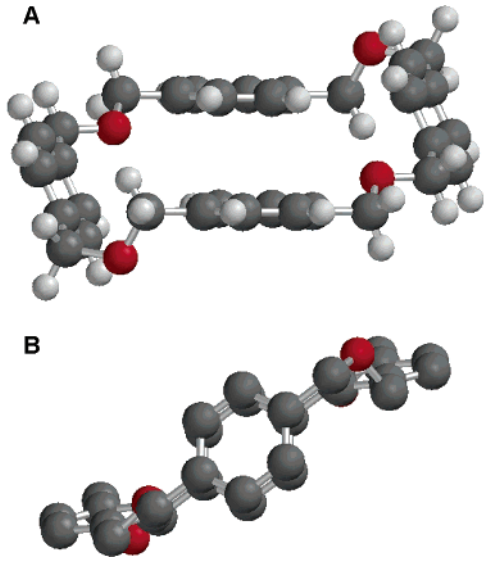

FIGURE 2. Ball-and-stick representation of the X-ray structure of **2**: (A) view down two of the four aromatic ring planes and (B) view down the other two aromatic ring planes. Hydrogen atoms are omitted for clarity in part B. Opposite pairs of aromatic rings are parallel but staggered.

TABLE 2. Association Constants K_a (M^{-1}), Guest Limiting Shift Values $\Delta\delta_\infty$ (ppm), Standard Free Energies of Binding $-\Delta G°$ (kJ mol^{-1}), Increments of Standard Free Energy of Binding $-\Delta\Delta G°$ (kJ mol^{-1}) with Respect to 1, and Calculated Electrostatic Potential EP (kcal mol^{-1}) for 1:1 Complexes of 2 with Acetylcholine (ACh) and Tetramethylammonium (TMA) Salts[a]

guest	anion	K_a (SE)[b]	$-\Delta\delta_\infty$	$-\Delta G°$	$-\Delta\Delta G°$ [c]	EP[d]
ACh[e]	Cl	92.5 (0.4)	2.012 (CH_3N) 1.522 (CH_2N) 2.702 (CH_2O)	11.14(1)	7.93	36.715
	Me_2SnCl_3[f]	443 (6)	2.351 (CH_3N) 1.647 (CH_2N) 2.857 (CH_2O)	15.00(3)	7.45	
	Pic	362 (2)	2.078 (CH_3N) 1.440 (CH_2N) 2.531 (CH_2O)	14.50(1)	8.17	47.815
TMA	Cl	165 (2)	2.025	12.57(3)	7.93	41.590
	Me_2SnCl_3[g]	1004 (2)	2.169	17.01(1)	8.20	
	AcO	75 (2)	1.659	10.63(6)	8.03	37.511
	$Me_2Sn(OAc)Cl_2$[h]	1068 (3)	2.147	17.16(1)		
	TFA	215 (2)	2.038	13.22(2)	7.17	44.708
	TfO	556 (4)	2.050	15.56(1)	7.88	47.920
	PFF	303 (5)	2.069	14.06(4)	7.48	45.253
	Pic	458 (6)	2.090	15.08(3)	6.73	49.362

[a] Measured by ^1H NMR (200/300 MHz) at $T = 296$ K in $CDCl_3$ on 0.1−1 mM solutions of salt, using host concentrations up to 0.04 M. Pic: picrate; AcO: acetate; TFA: trifluoroacetate; TfO: triflate; PFF: pentafluorophenate. [b] Standard error of the nonlinear least-squares fit. [c] Increments calculated from data reported in ref 9b. [d] Data from ref 9b. [e] Simultaneous fit of the three most shifting signals. [f] [AChCl] $= 1.1 \times 10^{-3}$ M; [DMTC] $= 4.3 \times 10^{-2}$ M. [g] [TMACl] $= 5.0 \times 10^{-4}$ M; [DMTC] $= 4.2 \times 10^{-2}$ M. [h] [TMAOAc] $= 7.5 \times 10^{-4}$ M; [DMTC] $= 4.2 \times 10^{-2}$ M.

grossly mismatched size for the guest. On the contrary, the association constant ratio between **2** and **10** sets to over 80-fold the binding advantage shown by the cyclic over the open-chain structure, i.e., the macrocyclic effect exhibited by this phanic receptor. That a correct host−guest fit is critical is demonstrated even more clearly by the results for tetraamine **11**. Although the ring size is very similar to that of **2**, the observed binding constant is nearly identical with that of the parent cyclophane **1**, that is, 15-fold smaller than **2**, a much too large drop to be explained on the basis of pure size arguments. In addition, it has been demonstrated that the contribution from ether oxygen donors to the binding of TMA is markedly smaller than that of aromatic rings;[9a] thus, a significant contribution from ether oxygen donors in **2** can be ruled out and, likewise, it can hardly be expected from amine nitrogen donors in **11**. We believe that the marked drop of binding ability, compared to **2**, observed for **11** should most reasonably be ascribed to the hydrogen atoms on nitrogen, likely pointing toward the cavity and therefore preventing the guest from fitting in or, alternatively, hampering the cyclophane from reaching the correct binding conformation. Tetraamine **11** may therefore suffer from a binding inhibition that may be steric or conformational in origin, although possessing the correct size. Eventually, in **11** the hydrogen atoms on nitrogen could be involved in hydrogen bonding to the anion, leading to enhanced association with the host due to synergistic binding of the ion pair.[7] Occurrence of hydrogen bonding may not be evident with picrate, but it would emerge with chloride, a counterion much more effective than picrate as H-bonding acceptor. Comparison of results for the association of TMA chloride with **1** and **11** clearly shows that the chloride anion exerts a very similar inhibiting effect on binding for both hosts, excluding any synergistic contribution from hydrogen bonding. In conclusion, the improved binding capabilities of **2** appear to rely essentially on correct geometry of the binding site.

Contribution of the Anion. It has been shown that the counterion exerts an inhibiting effect on the interaction of ACh and TMA with **1**, which is due to the electrostatic attraction exerted by the anion on the cation and is stronger for anions of larger charge density.[9b,c] It has also been shown that converting a strongly inhibiting anion, such as chloride, into a more charge-dispersed species, such as dimethyltrichlorostannate, induces a marked binding enhancement of the corresponding TMA and ACh salts to **1**.[9b,c] Since we expected these effects to be general, we tested the contribution of several counterions to the association of ACh and TMA salts with **2**. The results obtained for a set of anions selected among those employed in binding studies with **1** are reported in Table 2.[15] Significantly enhanced binding constants with respect to **1** were obtained with both guests for all the salts investigated; corresponding $-\Delta G°$ values were all larger than 10 kJ mol^{-1}, spanning a range of 7 kJ mol^{-1}, whereas for **1** $-\Delta G°$ values, although covering the same range, did not exceed the limit of 9 kJ mol^{-1}. Surprisingly, conversion of chloride into dimethyltrichlorostannate induced for both guests the same binding enhancement of 4 kJ mol^{-1} observed for **1**, despite the different structures of the cations and independently of the markedly different binding abilities of the two hosts. While confirming the beneficial effect of charge dispersion on cation binding, which raised the association constant to the order of 10^3 M^{-1}, the unexpectedly constant increment suggested that participation of anions with a constant contribution may be a general feature. The effect of charge dispersion was even more evident in the conversion of acetate into the corresponding dimethylacetoxydichlorostannate anion, which raised the binding free energy of TMA to **2** by 6.5 kJ mol^{-1}, with a 14-fold increment of the association constant. In the latter case, the combined contributions of the host and anion set to

(15) Stannate salts are generated in situ by addition of excess dimethyltin dichloride to a solution of the chloride or acetate salt in deuteriochloroform as previously described. See ref 9b,c.

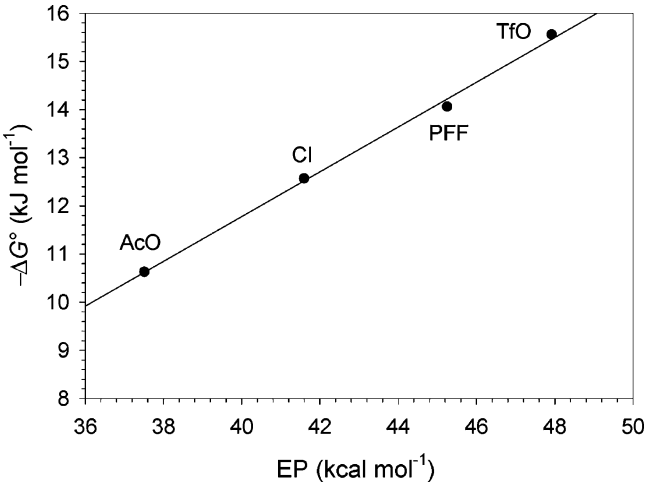

FIGURE 3. Plot of ion pair electrostatic potential vs standard free energy of binding to **2** for TMA salts. Data points are experimental $-\Delta G°$ values vs calculated EP values from Table 2; the solid line is the best fit line calculated by linear regression: slope = 0.465; intercept = −6.822; $r^2 = 0.997$.

over 400-fold the binding increase achieved with respect to TMA acetate and **1**, corresponding to nearly 15 kJ mol^{-1} in terms of binding free energy.

A general feature of data in Table 2 was that binding constant values followed the same trend observed for host **1**, that is, they were ranked according to the previously observed anion's inhibiting power. Even more interestingly, the binding enhancement with respect to **1** appeared to be constant throughout the series. This feature can be better appreciated by looking at the increments in binding free energy $-\Delta\Delta G°$ with respect to **1** that, with only one exception, cluster within 1 kJ mol^{-1} around the value of 8 kJ mol^{-1} (7.7 ± 0.5 kJ mol^{-1}). This remarkable evidence shows that (a) **2** binds nearly 8 kJ mol^{-1} stronger than **1** to both guests *regardless of the counterion*,[16] (b) the anion's inhibiting effect on cation's binding is a constant contribution for each anion independent from the host, as anticipated on the basis of the results for anion conversion, and (c) the observed trend appears to be generally valid for different hosts. To validate these conclusions, since for host **1** the inhibiting power of anions was found to correlate with the electrostatic potential EP of the ion pair as long as purely electrostatic factors are involved,[9b] $-\Delta G°$ values obtained for **2** were plotted vs the calculated ion pair EP values reported in Table 2.[17] A good linear correlation was found for all data ($r^2 = 0.945$) that became excellent ($r^2 = 0.997$) when excluding ACh salts, which were shown to be affected by factors other than pure electrostatics,[9b] with the value slightly out of range for picrate (Figure 3).

The slope of the linear regression (slope = 0.465) is very close to the value obtained in the case of **1** (slope = 0.483), while the intercept (intercept = − 6.82) indicates that the correlation line is shifted up by 8.6 kJ mol^{-1} with respect to that of **1**, in close agreement with the 7.7 ± 0.5 kJ mol^{-1} average increment value of $-\Delta G°$ found between **1** and **2**. The increment 1 kJ mol^{-1} smaller than average for picrate may be explained on the basis of steric effects. The larger shifts induced on all guests upon binding (Table 2) compared to **1** indicate a closer contact between the guest and the aromatic cavity of the host for **2**, that is, a deeper fit into the host's cavity; under these conditions, binding of tight ion pairs[18] may suffer from steric hindrance for large anions possessing bulky substituents, such as picrate, to a greater extent than in the case of **1**, which relies on wrapping around the guest for binding.

Although based on a limited number of points, the linear correlation running parallel to that obtained for **1** inequivocally confirmed (a) the extent of the binding increase from the intercept difference, (b) that the enhancement is independent of the counterion, and (c) that the sensitivity to the electrostatic inhibition from the anion is the same for both hosts. Eventually, as in the case of **1**, the free energy of binding for the "free" TMA cation, i.e., in the absence of the counterion, may be extrapolated for **2**, giving a value of $-\Delta G° = 44.9$ kJ mol^{-1}, which consistently is 6.3 kJ mol^{-1} larger than that found for **1** and which shows that, for the anions scrutinized, 28−34 kJ mol^{-1} of binding free energy are lost in ion pairing because of the unavoidable presence of a counterion.[19] The most relevant conclusion is, however, that a characteristic constant can be assigned to each anion to describe the electrostatic inhibition factor: calculated EP values may be used as a quantitative measure of these constants, which can be generally employed for predicting binding strengths as long as factors other than pure electrostatics are not involved.

Conclusion. We have redisigned the structure of a cyclophanic tetraester (**1**) that has been previously used to investigate the cation−π interaction to improve its binding ability. Molecular modeling calculations indicated that the cyclophanic tetraether resulting from removal of the four carbonyl groups would better accommodate the tetramethylammonium (TMA) cation inside the ring cavity. Cyclophanic tetraether **2** has thus been prepared, together with a set of appropriate reference compounds, and its binding properties toward TMA and acetylcholine (ACh) were measured by using a variety of counterions. From a comparative analysis of binding data, the following conclusions have been drawn: (a) the binding ability of the flexible cyclophane **1** was significantly improved by a correct redesign of the host's structure, without resorting to a more preorganized architecture; (b) a marked binding enhancement was achieved with **2** not only with respect to the parent tetraester, but also compared to its open-chain counterpart, to its oligomeric higher homologues, and to the corresponding cyclophanic teraamine of the same ring

(16) The independence of the intrinsic host's binding ability from the counterion in cation−π interactions has also been observed in other systems: Hunter, C. A.; Low, C. M. R.; Rotger, C.; Vinter, J. G.; Zonta, C. *Chem. Commun.* **2003**, 834−835.

(17) For a discussion on calculated EP values and computational methods, see ref 9b.

(18) The investigated salts have been shown to be present as tight ion pairs in $CDCl_3$ solution by spectroscopic evidence and conductivity measurements. See ref 9b,c,e. Binding of quaternary ammonium salts as tight ion pairs has also been demonstrated in the formation of complexes between aromatic crown ethers and paraquats and viologens: Huang, F.; Jones, J. W.; Slebodnick, C.; Gibson, H. W. *J. Am. Chem. Soc.* **2003**, *125*, 14458−14464.

(19) The large amount of binding free energy lost in ion pairing, corresponding to ion pair association constants $K_{ip} = 10^5-10^6$ M^{-1}, confirmed that in chloroform ion pair dissociation is negligible and, therefore, considering the whole ion pair as guest in the host−guest association is a correct approximation.

size; (c) geometry is as crucial as ring size, but the functional group's nature is not, as long as it does not impose conformational constraints; (d) the binding enhancement is independent from the counterion and from the structure of the cationic guest, as may be expected for electrostatic interactions; (e) the counterion's inhibiting contribution to binding appears to be a characteristic constant of each anion, the value of which can be expressed by the calculated electrostatic potential of the ion pair; (f) with the appropriate choice of the anion, i.e., with a poorly inhibiting counterion, the association constant for TMA is raised to the order of 10^3 M^{-1}, with a binding increase of over 400-fold with respect to **1**, corresponding to nearly 15 kJ mol^{-1} in terms of binding free energy. In summary, a rational optimization of the structure of a cyclophanic host has led to a synthetic receptor of significantly improved binding properties and to a deeper understanding of the factors and the contributions involved in the cation$-\pi$ interaction, such as the predominant role of ion pairing.

Experimental Section

Synthesis of Oligomeric Oxa[3.*n*]paracyclophanes, 2−6. To a solution of terephthalaldehyde (1.6 g, 11.93 mmol) in dry dichloromethane (480 mL) was added trimethylsilyl triflate (0.22 mL, 1.2 mmol) at 0 °C under stirring. A solution of triethylsilane (3.05 g, 26.23 mmol) in dry dichloromethane (120 mL) was added dropwise in 30 min at 0 °C and the mixture was then stirred at 0 °C for a further 3 h. The solution was washed with $NaHCO_3$ (2 × 200 mL) and water (2 × 200 mL) and dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure to give a white solid (4.76 g) that was purified by flash column chromatography (silica gel 60, dichloromethane/diethyl ether 95:5) to give the cyclic oligomers **2**−**6** (493 mg, 34% yield), of which **5** and **6** were obtained as pure compounds. The residual mixture [analytical yields (NMR): 4% (**2**), 14% (**3**), 7% (**4**)] was repeatedly chromatographed (dichloromethane/diethyl ether, from 95:5 to 85:15) until **2** and **3** could be obtained as pure compounds. The residual solid was then crystallized from acetonitrile to give pure **4**.

2,11,20,29-Tetraoxa[3.3.3.3]paracyclophane, 2. White solid (21 mg, 1.5%). Mp 139.5−140 °C (lit.[12] mp 145−147 °C). ^{1}H NMR (200 MHz, 0.1 F in $CDCl_3$) δ 7.19 (s, 16 H), 4.50 (s, 16 H). ^{13}C NMR (50 MHz, 0.1 F in $CDCl_3$) δ 137.9, 128.1, 71.8. ESI-M: 498.2 (M + NH_4^+), 503.2 (M + Na^+), 519.3 (M + K^+). Anal. Calcd for $C_{32}H_{32}O_4$: C 79.97, H 6.71. Found: C 79.71, H 6.62.

2,11,20,29,38-Pentaoxa[3.3.3.3.3]paracyclophane, 3. White solid (145 mg, 10%). Mp 138−139 °C (lit.[12] mp 143−145 °C). ^{1}H NMR (200 MHz, 0.1 F in $CDCl_3$) δ 7.35 (s, 20 H), 4.56 (s, 20 H). ^{13}C NMR (50 MHz, 0.1 F in $CDCl_3$) δ 137.6, 127.8, 71.5. ESI-MS 618.3 (M + NH_4^+), 623.3 (M + Na^+), 639.4 (M + K^+), 646.3 (M + 2Na^+). Anal. Calcd for $C_{40}H_{40}O_5$: C 79.97, H 6.71. Found: C 80.21, H 6.60.

2,11,20,29,38,47-Hexaoxa[3.3.3.3.3.3]paracyclophane, 4. White solid (65 mg, 4.5%). Mp 141−142 °C (lit.[12] mp 155−156 °C). ^{1}H NMR (200 MHz, 0.1 F in $CDCl_3$) δ 7.36 (s, 24 H), 4.55 (s, 24 H). ^{13}C NMR (50 MHz, 0.1 F in $CDCl_3$) δ 137.6, 127.9, 71.7. ESI-MS 738.3 (M + NH_4^+), 743.3 (M + Na^+). Anal. Calcd for $C_{48}H_{48}O_6$: C 79.97, H 6.71. Found: C 80.07, H 6.56.

2,11,20,29,38,47,56-Heptaoxa[3.3.3.3.3.3.3]paracyclophane, 5. White solid (26 mg, 1.8%). Mp 89−90 °C (lit.[12] mp 97−100 °C). ^{1}H NMR (200 MHz, 0.1 F in $CDCl_3$) δ 7.36 (s, 28 H), 4.54 (s, 28 H). ^{13}C NMR (50 MHz, 0.1 F in $CDCl_3$) δ 137.6, 127.9, 71.7. ESI-MS 858.4 (M + NH_4^+), 863.4 (M + Na^+), 879.4 (M + K^+). Anal. Calcd for $C_{56}H_{56}O_7$: C 79.97, H 6.71. Found: C 79.76, H 6.52.

2,11,20,29,38,47,56,65-Octaoxa[3.3.3.3.3.3.3.3]paracyclophane, 6. White solid (35 mg, 2.4%). Mp 116.5−117 °C (lit.[12] mp 122−124 °C). ^{1}H NMR (200 MHz, 0.1 F in $CDCl_3$) δ 7.35 (s, 32 H), 4.54 (s, 32 H). ^{13}C NMR (50 MHz, 0.1 F in $CDCl_3$) δ 137.7, 127.9, 71.8. Anal. Calcd for $C_{64}H_{64}O_8$: C 79.97, H 6.71. Found: C 79.75, H 6.72.

Synthesis of 2,11,20,29-Tetraoxa[3.3.3.3]paracyclophane, 2. To a solution of terephthalaldehyde mono-diethylacetal (5.03 g, 24.16 mmol) in THF (50 mL) was added sodium borohydride (1.41 g, 37.24 mmol) portionwise under stirring at 0 °C. The mixture was stirred at room temperature for a further 1.5 h, then poured into water and extracted with 4 portions of dichloromethane, washed with water, and dried over anhydrous Na_2SO_4. The hydroxymethyl derivative **7** (5.07 g, quantitative) was obtained by removing the solvent under reduced pressure as a colorless oil, which was used as such for the next step. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.48−7.32 (m, 4 H), 5.49 (s, 1 H), 4.66 (br s, 2 H), 3.69−3.44 (m, 4 H), 2.07 (br s, 1 H), 1.23 (t, J = 7.4 Hz, 6 H). **7** (4.73 g, 22.50 mmol) was dissolved in DMSO (60 mL) and the solution was degassed and saturated with Ar. NaH (60% dispersion in oil, 0.9 g, 22.50 mmol) was added and the suspension was heated under stirring in an Ar atmosphere at 80 °C, until hydrogen evolution had ceased leaving a clear solution. 1,4-Bis-bromomethylbenzene (2.97 g, 11.25 mmol) was quickly added, stirring the mixture for a further 0.5 h at 80 °C. The solution was then poured into water (150 mL), extracted with dichloromethane (4 × 50 mL), washed with water (4 × 50 mL), and dried over anhydrous Na_2SO_4. By removing the solvent under reduced pressure, the bis-benzyl ether **8** was obtained as a yellow oil (5.37 g, 91%), which was used for the subsequent step without further purification. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.49−7.36 (m, 12 H), 5.50 (s, 2 H), 4.55 (s, 8 H), 3.70−3.46 (m, 8 H), 1.24 (t, J = 7.1 Hz, 12 H). A solution of **8** (5.28 g, 10.10 mmol) in acetone (150 mL) and water (1.5 mL) was left to stand overnight at room temperature over Amberlyst 15 (1.2 g). The resin was filtered off and the solvent was removed under reduced pressure to give 3.97 g of a yellow oil that solidified on standing. The crude dialdehyde **9** was purified by flash column chromatography (silica gel 60, petroleum ether/ethyl acetate 3:2) to give **9** as a pure compound. White solid (2.76 g, 66% overall yield). Mp 84−86 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 10.00 (s, 2 H), 7.89−7.51 (m, 8 H), 7.39 (s, 4 H), 4.64 (s, 4 H), 4.61 (s, 4 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 191.9, 145.4, 137.5, 129.9, 127.9, 127.7, 72.4, 71.4. Trimethylsilyl triflate (0.035 mL, 0.2 mmol) was added at 0 °C to a solution of triethylsilane (308 mg, 2.65 mmol) in dry dichloromethane (42 mL). A solution of **9** (296 mg, 0.79 mmol) and 1,4-bis-trimethylsilyloxymethylbenzene (223 mg, 0.79 mmol) in dry dichloromethane (21 mL) was added in 7 h at 0 °C by means of an infusion pump. The clear solution was washed with water (4 × 20 mL) and dried over anhydrous Na_2SO_4 to give, after solvent removal, 531 mg of a white solid that was washed twice with petroleum ether to remove siloxanes, affording a crude oligomeric mixture (330 mg) as a white powder. The mixture showed the presence of **2** (10.5% by NMR) and no evidence of oligomers **3**−**5** by gel permeation chromatography (GPC). Separation by flash column chromatography (silica gel 60, petroleum ether/acetone 8:2) gave pure **2** (32 mg, 8.4%) as a white powder. A sample of **2** was crystallized from acetonitrile by slow evaporation of the solvent to obtain X-ray quality crystals. The identity and the structure assignment of **2** was confirmed by an X-ray crystallographic structure determination that, however, could not be refined to an R factor lower than 14%. Details of structure determination and refinement, together with an ORTEP plot with atom numbering, are reported in the Supporting Information.

Synthesis of Open-Chain Phane 10. Trimethylsilyl triflate (0.030 mL, 0.17 mmol) and triethylsilane (362 mg, 3.11 mmol) in dry dichloromethane (15 mL) were reacted with **9** (500 mg, 1.33 mmol) and trimethylsilyloxymethylbenzene (480 mg, 2.66 mmol) in dry dichloromethane (15 mL) according to

the previous procedure, performing the addition in 15 min. A white solid (562 mg) was obtained after workup and washing with petroleum ether, which was chromatographed (silica gel 60, dichloromethane/diethyl ether 96:4) to give 183 mg (25% yield) of pure **10** as a white solid. Mp 94−95 °C. ^{1}H NMR (200 MHz, 0.025 M in $CDCl_3$) δ 7.37−7.29 (m, 22 H), 4.56 (s, 16 H). ^{13}C NMR (50 MHz, 0.025 M in $CDCl_3$) δ 138.2, 137.7, 128.4, 127.9, 127.8, 127.6, 72.8, 72.1. Anal. Calcd for $C_{38}H_{38}O_4$: C 81.69, H 6.86. Found: C 81.37, H 6.81.

Acknowledgment. The X-ray crystallographic structure determination was performed at the Centro di Cristallografia Strutturale (CRIST) of the University of Florence. We would like to thank Dr. S. Ciattini for the structural analysis.

Supporting Information Available: ORTEP plot of the structure of **2** with atom numbering and X-ray crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

JO049899J