Evidence for the Importance of Polarizability in Biomimetic Catalysis Involving Cyclophane Receptors

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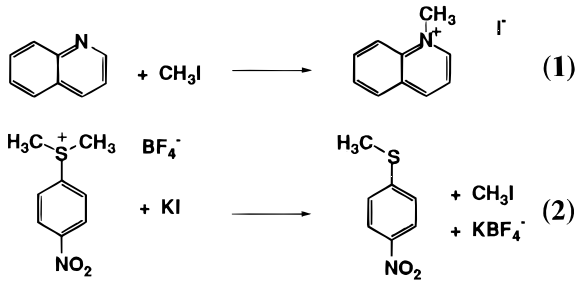
Cyclophanes **1**−**6** catalyze the nucleophilic dealkylation of a simple sulfonium compound by potassium iodide. The cation−π interaction is important in substrate binding, but the primarily electrostatic nature of this effect does not explain all observations concerning catalysis. As a series of substituents are placed on the cyclophane framework, a systematic variation in catalyst effectiveness is seen, such that more polarizable substituents produce more potent catalysts. This provides support for the notion that transition states are especially polarizable, and catalysis can be enhanced by maximizing London dispersion forces. The reactions studied here are very similar to the broad class of biological methylations mediated by *S*-adenosylmethionine, and the biological catalysts may use forces similar to those described here.

Introduction

Cyclophane **1** is a general receptor for organic cations such as quaternary ammonium, imminium, and dialkylarylsulfonium compounds.[1] Complexation arises in part from the cation−π interaction, in which the positive charge of the guest is stabilized through its interaction with the π face of simple aromatic systems.[2] We have shown that, to a considerable extent, binding through the cation−π interaction can be thought of as primarily electrostatic in nature, involving the quadrupole moment of the aromatic and the charge of the ion.[2−4] Of course, a full, quantitative model of the cation−π interaction would require consideration of additional terms, such as the interaction of the ion with induced dipoles in the aromatics.

Given the general cation-binding ability of host **1**, it was not surprising that it is also a catalyst for S_N2 reactions in which the transition state has a partial positive charge (eqs 1 and 2).[5,6] This catalysis by the host, carried out in aqueous media, is biomimetic, in that it involves prior binding of a substrate followed by reaction in the binding cavity. In contrast to most biomimetic systems, however, there is no contribution from proximity effects. Both the catalyzed and uncatalyzed reactions are bimolecular reactions, and there is no evidence for interactions between the host and the nucleophile to form a ternary complex with the substrate. All rate acceleration is achieved solely through transition-state stabilization due to interactions with the host. While this may not be the best way to maximize the rate enhancements seen, it does allow an evaluation of any special stabilization afforded the transition state by the microenvironment of the host.



Both an alkylation reaction involving formal creation of positive charge in the transition state (eq 1) and a dealkylation reaction in which positive charge is formally destroyed (eq 2) are accelerated by the host. This establishes that a transition state with only a partial positive charge can be stabilized more effectively than either fully charged or completely neutral substrates.[5,6] The first-order electrostatic analysis of the cation−π interaction cannot account for such behavior. Instead, in our initial analysis we proposed a prominent role for London dispersion forces in the catalysis by host **1**.[5,6] London dispersion forces arise from induced-dipole−induced-dipole interactions between molecules, and the strength of an induced-dipole is dependent on the polarizability of the molecule. Since transition states have long, weak bonds, they are expected to be more polarizable than ground-state substrates or products.[7] The role of the host is to surround the transition state with an electron-rich π-system that is polarizable and in a relatively fixed orientation, so that induced dipoles in both the host and the transition state are suitably aligned. Water is much less polarizable than benzene (Table 1), and so the host provides a more suitable environment for London dispersion interactions than an aqueous medium.[8] Note that in this model, it is not sufficient that polarizability contributes to binding. Polarizability must be *more* important for binding transition states than for binding ground states. Only in this way can it enhance catalysis.

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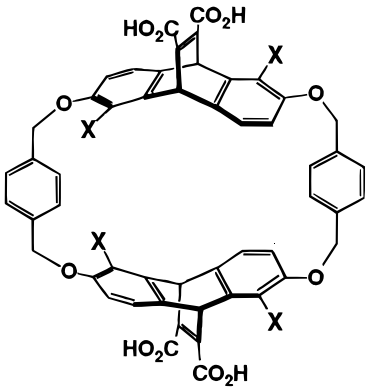
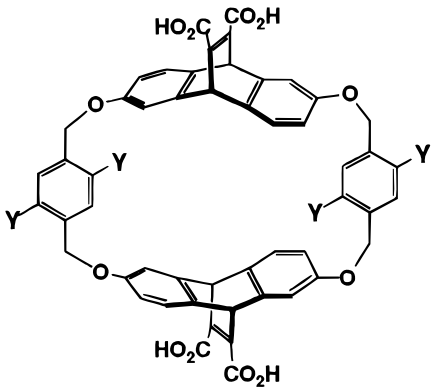
Chart 1



X	
1	H
2	Br



Y	
3	Me
4	OMe
5	Cl
6	Br

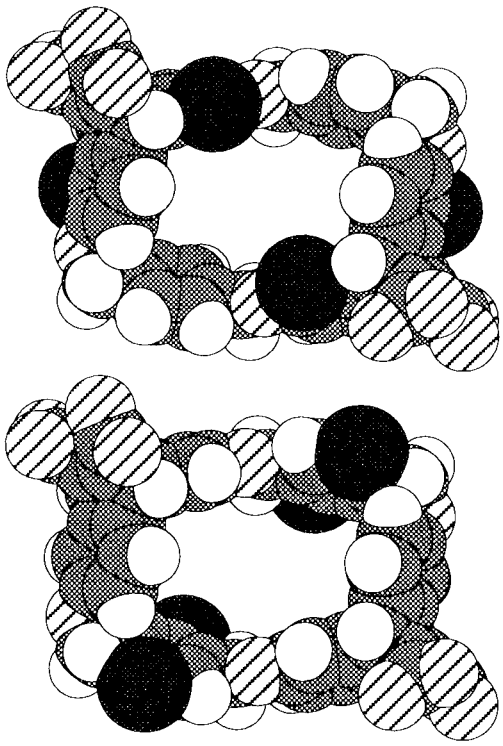


Figure 1. CPK representations of the *S,S,S,S* enantiomer of host **2** (top) and **6** (bottom) in rhomboid binding conformations. The host structure was obtained from the X-ray structure of the tetraester of host **1**[9] with the methyl groups deleted for clarity. Bromine atoms were then placed in standard positions. The oxygen atoms of the molecule are cross-hatched and the bromine atoms are shaded.

In order to seek support for this model, we have studied hosts **2**−**6** to evaluate potential substituent effects. Special emphasis was placed on the extent to which the substituents might alter the polarizability of the host environment. Table 1 shows the experimental polarizabilities of several reference compounds, which we will use to order the polarizabilities of the host substituents. In host **2**, the bromines are in fixed positions on the ethenoanthracene units of the host, while in **3**−**6** the substituents are attached to the more flexible "linker" region of the host. As shown in Figure 1, both substitution patterns appear to position the substituents in favorable locations for interacting with the transition state. We find that the substituents exert small but significant effects on the catalytic effectiveness of host **1**. Intriguingly, the trend in substituent effects on catalysis does indeed parallel the expected changes in polarizability.

Table 1. Polarizabilities[a] of Selected Compounds

compound	polarizability (10^{-24} cm^{-3})
water	1.45
benzene	10.32
toluene	12.3
anisole	13.1
chlorobenzene	14.1
bromobenzene	14.7

[a] *CRC Handbook of Chemistry and Physics*, 71st ed.; CRC Press: Boca Raton, FL, 1990; pp 10-198−10-207.

Results and Discussion

Hosts **1**−**6** were used to catalyze the dealkylation of sulfonium salt **7** with iodide ion acting as the nucleophile (eq 2). Significant rate enhancements are seen, and different hosts produce measurably different rate enhancements, as shown in Figure 2.

The kinetics of the host-catalyzed dealkylation can be described by a modified Michaelis−Menten scheme (Figure 3).[5,6] K_s and K_p are known quantities that can be obtained by NMR[1] or CD[10] binding studies. The uncatalyzed rate constant k_{un} is obtained under pseudo first-order conditions by using a large excess of the nucleophile. Therefore, the only unknown is k_{cat}, which can be obtained by recording the change in substrate concentration over time in the presence of host and using this data to numerically solve the rate equations for the value of k_{cat}. A typical fit is shown in Figure 4. As in previous work,[5,6] 5-nitroquinoline (**8**) was a competitive inhibitor

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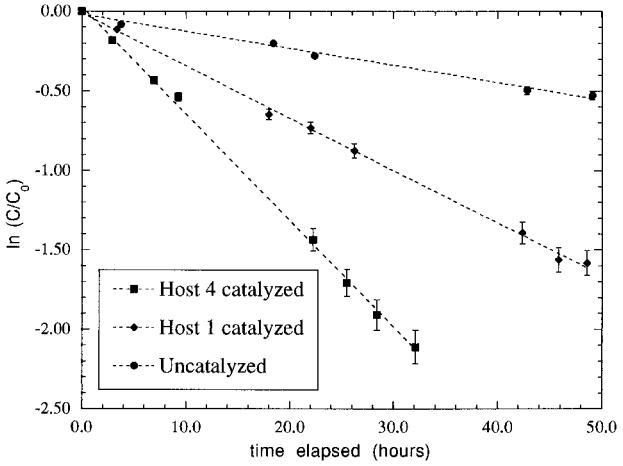


Figure 2. Disappearance of substrate with time for selected reactions.

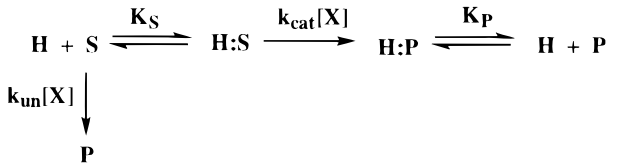
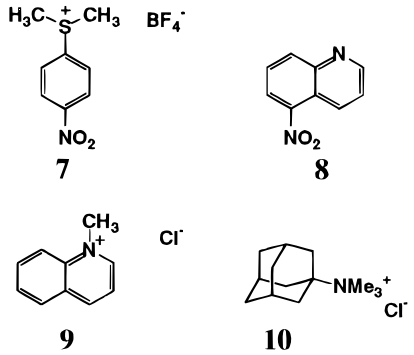


Figure 3. Kinetic scheme for the reactions considered here (H = host, S = substrate, P = product, X = nucleophile).



of the host-catalyzed dealkylation, blocking the host binding site and effectively preventing catalysis from taking place. This rules out catalysis by association of the guest with a host aggregate rather than actual binding of the guest in the host cavity.

The results from the study of the host-catalyzed dealkylation reactions are summarized in Table 2. As discussed above, the rate enhancements (k_{cat}/k_{un}) in this system are not large. Still, we believe that not only the rate enhancements but also the *differences* in rate enhancements are meaningful. Figure 2 clearly shows that there are significant differences between the rates of the uncatalyzed reaction and the two host-catalyzed reactions indicated. A valid comparison between the two host-catalyzed reactions can be made since the initial percentage of the guest bound is the same in both cases. This is a well-behaved kinetic system with a simple rate law. The value of k_{un} is the same for all systems, so the only variables are the binding affinity of the substrate, which can be determined with good accuracy, and the observed rate of disappearance of substrate, which is easily monitored by HPLC and found to be reproducible. The quantity $\Delta\Delta G^{\ddagger}$ ($= RT \ln(k_{cat}/k_{un})$) represents the extent to which the transition state is *preferentially*

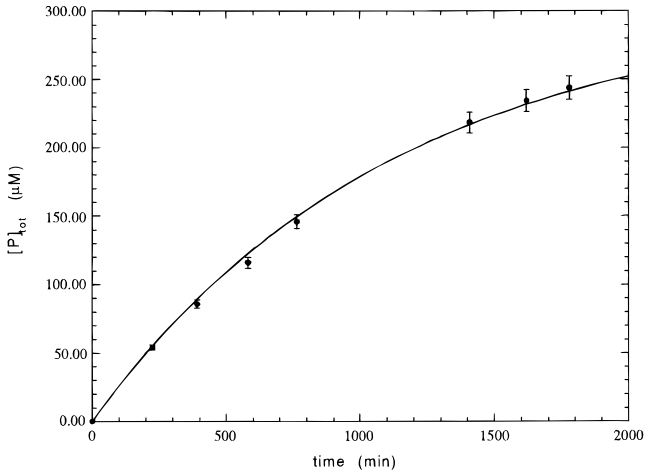


Figure 4. The experimental rise in product with host **3** as catalyst plotted with the simulated curve obtained from the numerical solution of the kinetic scheme of Figure 3.

Table 2. Binding and Catalysis Data

host	$-\Delta G_s$ (kcal/mol)[a]	k_{cat}/k_{un}[b]	$-\Delta\Delta G^{\ddagger}$ (kcal/mol)[b]
1	5.7	4.9	1.0
2	6.1	5.9	1.1
3	5.2	6.4	1.2
4	4.6	9.7	1.4
5	5.8	9.8	1.4
6	5.6	12.0	1.6

[a] At 298 K. [b] At 319 K.

stabilized relative to substrate upon moving from water into the host cavity.

It should be appreciated that, in one sense, it is surprising that adding substituents should increase the reaction rate. Since S_N2 transition states are more crowded than the substrate alone, it might have been expected that adding steric bulk in the region where the nucleophile must approach would slow the reaction. It is possible that such adverse interactions do influence the rate, but that they are overwhelmed by the electronic influence of the substituents.

It is also important to emphasize that all the hosts appear to be binding the guest in a similar orientation. A method for determining binding constants using circular dichroism (CD) has been developed recently in our group,[10] and certain features of the CD spectrum have implications for the conformation of the host and the binding orientations for the guest. Previous studies using cyclophane **1** indicate that there are two major binding orientations of the host: rhomboid and toroid. The C_2-symmetric rhomboid form binds flat aromatic guests such as **9**, while the D_2-symmetric toroid form binds more nearly spherical guests such as **10**. This information was used to design a study to establish the host binding orientation in the presence of the sulfonium guest **7**. CD spectra of each host were taken in the presence of **7**, **9**, and **10** as guests. These spectra were then compared, to infer the adopted binding conformation of each host with each of three guests. For example, with host **3** in the presence of **10**, there is a peak shift from 220 to 235 nm which is not observed in the presence of either **7** or **9** (Figure 5), and this appears to be characteristic of a toroid binding conformation. The CD spectra of this host in the presence of both **7** and **9** are very similar and support a rhomboid binding conformation (as shown in

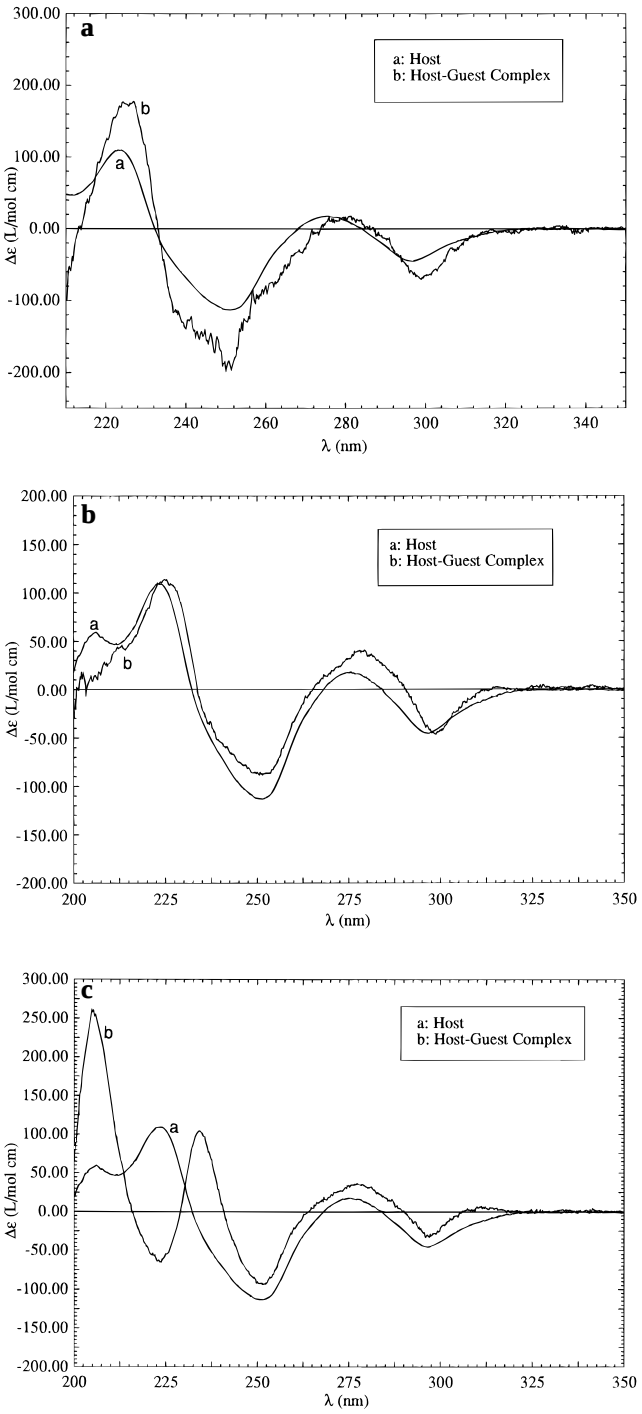


Figure 5. (a) Best fit CD spectra of host (*R*,*R*,*R*,*R*)-**4** and its complex with guest **7** in aqueous buffer (pH 9). (b) Best fit CD spectra of host (*R*,*R*,*R*,*R*)-**4** and its complex with guest **9** in aqueous borate buffer (pH 9). (c) Best fit CD spectra of host (*R*,*R*,*R*,*R*)-**4** and its complex with guest **10** in aqueous borate buffer (pH 9).

Figure 1). The changes in the CD spectrum of host **3** upon binding each of the three guests are representative of the whole series of hosts. In addition, there is induced CD with the binding of **7** which occurs at 254 nm (Figure 5). In each host, the induced CD is of the same sign, supporting similar binding conformations of the hosts in the presence of **7**.

The observation of induced CD in a guest allowed the calculation of binding orientations using the coupled oscillator approach to calculating induced CD developed previously.[10] This calculated induced CD was then

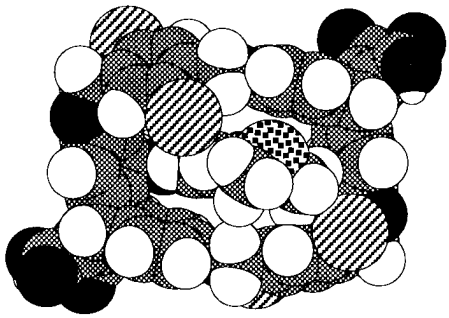


Figure 6. Host **5** with guest **7** aligned in the cavity at the midpoint of the angle range that is calculated to produce induced CD of the same sign as that observed experimentally.

Table 3. Values of the Upfield ^{1}H NMR Shifts (ppm) of the Protons of Guest 7 Scaled to the Highest Shift

host	methyl protons	aromatic protons
1	0.58, 0.72	0.83, 1.00
3	0.56, 0.80	0.68, 1.00
4	0.47, 0.76	0.98, 1.00
5	0.33, 0.52	0.90, 1.00
6	0.30, 0.45	0.91, 1.00

compared to the induced CD observed experimentally to infer the binding orientation of the guest as a range of possible angles of rotation of the guest about a set axis in the host coordinate system.[10] In Figure 6, the guest **7** is shown positioned at an angle midway between the range predicted by the coupled oscillator calculations. The positively-charged sulfur atom is positioned for maximal interaction with one of the aromatic rings of the host, while one of the methyl groups is placed in close proximity to one of the chlorine atoms.

The binding orientation of the guest can also be partially inferred from ^{1}H NMR shifts seen on binding. The upfield ^{1}H NMR shifts of the guest protons reflect how close a proton is to the shielding face of the aromatic rings of the host cavity. The overall pattern of shifts seen for guest **7** is similar for all the hosts (Table 3), with only slight variations.

We thus conclude that host **1** has provided a useful platform for evaluating substituent effects on catalysis. All spectroscopic evidence suggests that the structural perturbations of substitution are minimal. A large amount of structural information places the substituent very near the reacting center. As in any study of substituent effects, one can never be certain that unanticipated, and otherwise undetectable, structural changes are occurring with substitution, and that these are responsible for the rate variation. At present, however, we feel all available evidence argues against this view.

For purposes of comparison, the series of hosts **3**−**6**, along with the parent **1**, is preferable. In host **2** the substantially different steric environment makes comparison more difficult. We presume that the much larger rate enhancement seen with tetrabromo host **6** compared to isomeric tetrabromo host **2** is due to a more favorable positioning of the bromine atoms in **6**, and so the series **3**−**6** is preferable for comparative studies.

The data of Table 2 lead immediately to one important conclusion. Clearly, there is no correlation between binding energies ($-\Delta G_s$) and catalysis ($\Delta\Delta G^{\ddagger}$). That is, the substituent effects seen in catalysis are not just the result of making the host a generically better binding site. In fact, no typical substituent parameter (electro-

negativity, hydrophobicity, steric size, π donor/acceptor ability ...) correlates with the k_{cat}/k_{un} data.

In contrast, the correlation of k_{cat}/k_{un} with the polarizability data of Table 1 is very good. Adding substituents near a reacting center leads to a consistent rate effect that correlates with the extent to which the substituent is polarizable. As such, we consider the current findings to support the model that increasing the polarizability of the microenvironment in which a reaction is occurring leads to a rate increase. That is, London dispersion forces, working in concert with electrostatics (the cation$-\pi$ interaction), hydrophobic interactions, and other forces can be an important contributor to catalysis.

We have previously noted the similarity of the dealkylation reaction studied here to the important class of biological methylation reactions involving the sulfonium compound *S*-adenosylmethionine (SAM).[11] We also speculated that perhaps cation$-\pi$ interactions would be prominent in the active sites of enzymes that mediate such transformations. Recently, this speculation has received some support from an X-ray structure of a DNA methylase enzyme.[12] The sulfonium of SAM is positioned in van der Waals contact with a tryptophan from the enzyme,[2] in an ideal arrangement for cation$-\pi$ interactions.

In a more general context, it will always be true that any organic structure will be more polarizable than water. Thus, it seems safe to say that any enzyme active site will be more polarizable than water and so better able to employ London dispersion forces for catalysis. Of course, there is considerable variability in the polarizabilities of amino acid side chains, and it will be interesting to consider which reaction types are best suited to strong influence by London dispersion interactions.

Conclusions

The current studies establish that precisely positioning substituents within putative van der Waals contact with a transition state can enhance catalysis. Interestingly, the effectiveness of a given substituent correlates well with the polarizability of the substituent, suggesting a special role for London dispersion interactions in transition state stabilization. This is a potentially general effect that should have significant implications for a number of biological catalysts.

Experimental Section

General Methods. Instrumental and analytical methods were as in previous work from these laboratories.[1,10,13]

Hosts **1**, **2**, and **4** were synthesized according to literature procedures.[1] Compound **8** was commercially available. Guests **9** and **10** were prepared by exhaustive alkylation of the appropriate quinoline and amine with iodomethane. Guest **7** was prepared by refluxing trimethyloxonium tetrafluoroborate with the corresponding sulfide.

Macrocyclizations. The tetramethyl ester of hosts **3**, **5**, and **6** were prepared by a condensation of the appropriate ethenoanthracene with the corresponding bis(halomethyl)-benzene in a suspension of cesium carbonate in anhydrous CH_3CN or DMF following the procedure developed for host **1**.[1] In all macrocyclizations, enantiomerically pure ethenoanthracenes were coupled, although both *R*,*R* and *S*,*S* forms were used. Workup of the macrocyclic products differed slightly from that previously reported for host **1**. After the macrocyclizations were complete, the reactions were filtered and the DMF or CH_3CN removed. The residues were then flash chromatographed over silica eluting first with methylene chloride and then ether in order to separate the macrocyclic compounds from baseline impurities. The macrocycles were then isolated from oligomers using preparative centrifugal thin-layer chromatography (silica plates, 0−5% ether in CH_2Cl_2).

Host 3, Tetramethyl Ester. Yield 6%; ^{1}H NMR ($CDCl_3$) δ 7.08 (d, J = 8, 4H), 6.98 (s, 4H), 6.90 (d, J = 2, 4H), 6.38 (dd, J = 2, 8, 4H), 5.23 (s, 4H), 4.98 (s, 8H), 3.78 (s, 12H), 2.14 (s, 12H); FAB-MS m/e 964 (M^+); HRMS of M − Li^+ 971.3621, calcd for $C_{60}H_{52}O_{12}Li$: 971.3619.

Host 5, Tetramethyl Ester. Yield 8%; ^{1}H NMR ($CDCl_3$) δ 7.40 (s, 4H), 7.13 (d, J = 8, 4H), 6.93 (d, J = 2, 4H), 6.43 (dd, J = 2, 8, 4H), 5.25 (s, 4H), 5.04 (AB, J = 14, $\Delta\upsilon$ = 52 Hz, 8H), 3.78 (s, 12H); FAB-MS m/e M − Li^+ cluster 1050−1060 (1053 100 integral % within cluster); HRMS of M − Li^+ 1051.1422, calcd for $C_{56}H_{40}O_{12}{}^{35}Cl_4Li$: 1051.1434.

Host 6, Tetramethyl Ester. Yield 10%; ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 4H), 7.13 (d, J = 8, 4H), 6.94 (d, J = 2, 4H), 6.44 (dd, J = 2, 8, 4H), 5.26 (s, 4H), 4.99 (AB, J = 14, $\Delta\upsilon$ = 39 Hz, 8H), 3.78 (s, 12H); FAB-MS m/e M^+ cluster 1220−1230 (1224 100 integral % within cluster); HRMS of M − Li^+ 1226.9399, calcd for $C_{56}H_{40}O_{12}{}^{79}Br_4Li$: 1226.9413.

Ester Hydrolysis. All tetraacid macrocycles were prepared from the corresponding tetramethyl esters using the following hydrolytic procedure. The tetraesters were dissolved in 1−2 mL of DMSO. Thirty equivalents of aqueous CsOH (1.0 M solution) were added, which caused a white precipitate to form. Water (1−2 mL) was then added, and the solution was allowed to stir for 24 h. The solution was then frozen and lyophilized. The resulting residue was dissolved in a minimum amount of water and loaded onto a cation exchange column (neutral pH, Dowex 50 × 4, NH_4^+ form). The material was eluted with water that had been passed through a Milli-Q purification system. The fractions containing host were identified by their UV activity on TLC silica gel plates. The appropriate fractions were then combined and lyophilized to give the acid compounds. Standard aqueous solutions of these host compounds were prepared by dissolving them in borate-*d* buffer (50−70% yields).

Host 3, Tetraacid. ^{1}H NMR (10% CD_3CN/90% borate, referenced to internal DMG δ 1.09) δ 7.21 (d, J = 8, 4H), 7.17 (s, 4H), 7.07 (bs, 4H), 6.63 (d, J = 8, 4H), 5.19 (s, 4H), 5.05 (AB, J = 13, $\Delta\upsilon$ = 37 Hz, 8H), 2.20 (s, 12H).

Host 5, Tetraacid. ^{1}H NMR (10% CD_3CN/90% borate, referenced to internal DMG δ 1.09) δ 7.57 (s, 4H), 7.24 (d, J = 8, 4H), 7.09 (bs, 4H), 6.65 (d, J = 8, 4H), 5.22 (s, 4H), 5.13 (AB, J = 14, $\Delta\upsilon$ = 48 Hz, 8H).

Host 6, Tetraacid. ^{1}H NMR (10% CD_3CN/90% borate, referenced to internal DMG δ 1.09) δ 7.71 (s, 4H), 7.25 (d, J = 8, 4H), 7.10 (bs, 4H), 6.64 (d, J = 8, 4H), 5.22 (s, 4H), 5.09 (AB, J = 14, $\Delta\upsilon$ = 52 Hz, 8H).

Kinetics of Sulfonium Salt Dealkylation. Stock solutions of hosts **1**−**6** were prepared in borate-*d* buffer. Stock solutions of KI, KSCN, KHP (internal integration standard, potassium hydrogen phthalate), **7**, and **8** for the HPLC studies were made by weighing each solid and dissolving it in the nondeuterated borate buffer. The reaction rates were monitored by integration of substrate and internal standard peak areas from an HPLC trace using a Waters Baseline 810 software package. Each kinetic run was performed at least twice. A representative reaction mixture consisted of 316 μM host **1**, 338 μM guest **7**, 0.01160 M KI, and 513 μM KHP for a total volume of 500 μL in borate buffer. The ratio of host to guest was varied with the binding constant to keep the percentage of guest bound in the range of 60 to 80%.

For each experiment, the reaction solution is prepared in an Eppendorf tube without the nucleophile. The solution of nucleophile is then added, and the tube is shaken vigourously just prior to the first injection of sample for the first time point.

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The tube is then placed in an oil bath maintained at 46 °C by a Thermo Watch. At each time point, a 20 μL aliquot is removed from the sample which is then promptly returned to the oil bath. This 20 μL aliquot is neutralized with 20 μL of phosphate buffer (pH = 7) and the 40 μL sample injected onto the column. A gradient elution was used to separate the reaction solution components. Solvent A was 0.1% TFA/H_2O (v/v) and solvent B was 0.1% TFA/CH_3CN (v/v). Elution was performed at 1.8 mL/min with 80% A from 0 to 8 min, a linear gradient to 100% B from 8 to 13 min, maintained at 100% B from 13 to 19 min, brought back linearly to 80% A from 19 to 25 min, and allowed to equilibrate at 80% A from 25 to 42 min. Eluting compounds were detected at 254 and 230 nm. A calibration curve consisting of measuring relative peak areas of five samples of various sulfonium salt concentrations and a fixed KHP concentration was used to convert peak areas to concentrations.

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