Structure and Selectivity in Host-Guest Complexes of Cucurbituril[1]

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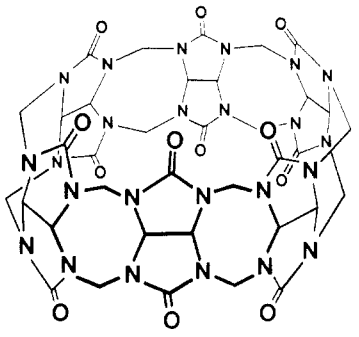
Evidence for inclusion complexation in solution between cucurbituril ($C_{36}H_{36}N_{24}O_{12}$) and various alkyl- and aryl-substituted ammonium ions is elaborated. Induced NMR chemical shifts and UV spectral perturbations of guests complexed within cucurbituril are noted and may be used to quantitate binding. Dissociation constants (K_d) for over 50 guests are recorded, and the kinetics of complexation have also been investigated. On the basis of selectivity among bound species, a detailed model for the structure of the host-guest complexes is deduced. The cavity within cucurbituril has dimensions equivalent to the size of a para-disubstituted benzene ring. Successful inclusion is attributable to hydrophobic interactions (freeing of solvent molecules upon complexation) and to a charge-dipole attraction between ammonium cations and the electronegative oxygens of the urea moieties in cucurbituril.

The subject of host-guest complexation, with its implications for biomimetic organic chemistry (i.e., ligand-receptor interactions), has become a topic of strong current interest.[2] It has been known for some time that the phenomenon of biochemical specificity depends upon noncovalent attractions between large (e.g., enzyme or receptor = host) and small (e.g., substrate or ligand = guest) organic moieties. These interactions appear largely to be hydrophobic in nature, although they are modulated by electrostatic and/or specific hydrogen-bonding relationships. What has heretofore been lacking is detailed knowledge of noncovalent bonding, which would be sufficient to allow its constructive incorporation into, for example, synthetic organic chemistry. Because intermolecular forces are intrinsically weaker than covalent bonds, they can only quantitatively be studied when their effects are cumulative. Hence, the recent availability of macrocyclic organic molecules having potential or actual cavities within them has greatly stimulated research in this area.

Cucurbituril (**1**, $C_{36}H_{36}N_{24}O_{12}$), the host species considered in this article, is a recently rediscovered nonadecacyclic cage structure of hexagonal symmetry, which is



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readily assembled by acid-catalyzed condensations between urea, glyoxal, and formaldehyde [$2H_2NCONH_2 + CHOCHO \rightarrow C_4H_6N_4O_2$ (glycoluril), $6C_4H_6N_4O_2 + 12CH_2O \rightarrow$ **1**].[3,4] The structure of **1** is relatively rigid, and it contains a hollow core of several angstroms diameter, which is accessible from the exterior by two carbonyl-fringed portals. Details of a crystallographic examination of **1** are available,[5] and pertinent features will be pointed out subsequently. Commentary upon its synthesis and on its catalytic capacity[6] will be provided elsewhere. The purpose of this article is a survey of the binding of small alkyl- and aryl-substituted ammonium ions within the cavity of cucurbituril.[7] We have been able to secure an extensive set of quantitative data on the affinity of such ions for **1**, and an analysis of the results provides a detailed structural model for complexation.

Results

Evidence for Internal Complexation. The ^1H NMR spectrum of 1,5-diaminopentane in HCO_2H-D_2O solution (the preferred solvent for **1**) exhibits two CH resonance multiplets, at δ 3.17 and 1.77. These correspond respectively to the methylene units adjacent to ammonium ions (4 H) and to the remaining, or central, methylene groups (6 H). Successive addition of small portions of **1** to such a solution causes a diminution of these resonances, with a concurrent appearance of two *new* multiplets at δ 2.73 and 0.77. Eventually the former signals are completely replaced by the new, upon addition of a sufficient amount of **1**. As subsequently described, this indicates formation of an inclusion complex, wherein the cationic termini of the alkanediammonium ion are associated with the negative ends of the carbonyl dipoles of **1** and in which the hydrocarbon chain at the guest extends through the core of the cage structure. By NMR integration of appropriate proton resonances of host and guest, stoichiometric complexation (1:1 mole ratio) was demonstrated. Apparently any exchange between bound and free pentanediammonium ions is slow on the NMR time scale, since signals from both species can be seen when an excess of guest is present. For some complexes this is not the case; for example with *n*-propylammonium ion as guest only an averaged NMR spectrum is observed throughout, although the total proton-induced shift is comparable upon addition of an excess of **1**. This NMR technique was applied to other alkanediammonium ions, and the results (induced shifts of proton resonances) are summarized in Figure 1. It may be seen that the shielding region extends for approximately 4.5 methylene units, or 6 Å, which coincidentally is the interatomic distance between carbonyl oxygens axially spanning the cavity of **1**. Evidently the interior of cucurbituril comprises a proton-shielding region

(1) Taken from the Ph.D. Thesis of N.-Y. Shih, University of Illinois at Chicago, 1981: *Diss. Abstr. Int. B* **1982**, *42*, 4071.
(2) For an overview, see *Inclusion Compounds*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Eds.; Academic: Orlando, FL 1984; Vol. 2. For other ureido-hosts, see: Nolte, R. J. M.; Cram, D. J. *J. Am. Chem. Soc.* **1984**, *106*, 1416.
(3) Behrend, R.; Meyer, E.; Rusche, F. *Justus Liebigs Ann. Chem.* **1905**, *339*, 1.
(4) Freeman, W. A.; Mock, W. L.; Shih, N.-Y. *J. Am. Chem. Soc.* **1981**, *103*, 7367.
(5) Freeman, W. A. *Acta Crystallogr., Sect. B* **1984**, *B40*, 382.
(6) Mock, W. L.; Irra, T. A.; Wepsiec, J. P.; Manimaran, T. L. *J. Org. Chem.* **1983**, *48*, 3619.
(7) Preliminary communication: Mock, W. L.; Shih, N.-Y. *J. Org. Chem.* **1983**, *48*, 3618.

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Table I. Rate Constants for Displacement of One Guest within 1 by Another

primary (complexed) guest	secondary (displacing) guest	mole ratio (primary/secondary)	$10^3 k_{obsd}$,[a] s^{-1}
$(CH_3)_2CHCH_2CH_2NH_2$	$(2\text{-}C_4H_3O)CH_2NH_2$[b]	1:1	0.37 (±0.03)
		1:10	0.24 (±0.01)
$H_2N(CH_2)_5NH_2$	$(H_2NCH_2CH_2)_2S$	13:1	0.95 (±0.10)
		13:10	1.16 (±0.05)
$(2\text{-}C_4H_3O)CH_2NH_2$[b]	$H_2N(CH_2)_5NH_2$	1:1	6.3 (±0.4)
		1:10	3.9 (±0.3)
$(H_2NCH_2CH_2)_2S$	$H_2N(CH_2)_5NH_2$	1.1:1	0.194 (±0.016)
		1.1:10	0.25 (±0.02)

[a] Temperature 40 °C, HCO_2H–D_2O solution. [b] 2-Furanmethylamine.

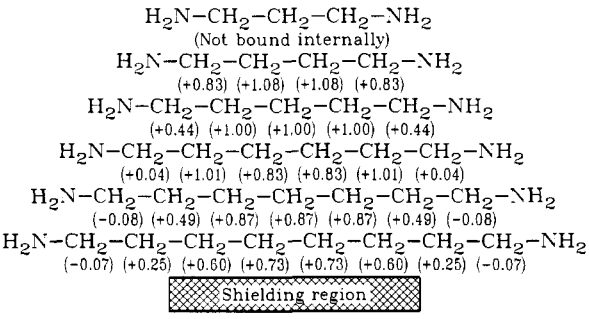

Figure 1. ^1H NMR induced shifts (ppm) of methylene groups of alkanediammonium ions upon complexation with 1 (D_2O–HCO_2H solution).

relative to the acidic aqueous medium employed for solvating the host species. The induced shift probably arises from a cumulative effect of the 12 urea residues of cucurbituril, each of which presents a face to the interior of the cavity. Similar induced chemical shift effects are noted in ^{13}C NMR spectra.

Selective perturbations of NMR peak positions may be observed for 1 as well as for its guests. A host resonance at δ 4.4, part of an AB coupled system attributed to the 12 methylene (formaminal) groups which bridge between glycoluril moieties of 1, becomes slightly split upon complexation of 1 with isobutylammonium ion. This guest is one which exchanges slowly on the NMR time scale.[7] The splitting within the complex arises because the cation coordinated to a set of six urea carbonyls surrounding a portal of 1 creates a different magnetic environment for nearby methylene residues, as compared with that provided for the remaining half of the host molecule. In other words, reflection symmetry perpendicular to the six-fold axis of 1 is destroyed upon complexation with a single ammonium ion.

Inclusion complex formation may cause a change in the electronic absorption spectrum of a guest species.[8] The acid-solution UV spectrum of 4-methylbenzylamine shows a substantial perturbation in the presence of cucurbituril. In particular, fine structure appears in the aryl absorbance at 270 nm (an enhancement of the $0 \rightarrow 0$ transition of the ^1L_b band) upon addition of an excess of 1 to an HCO_2H–H_2O solution of the aromatic amine. Since NMR evidence also indicates complex formation with this substrate, the phenomenon must be associated with encapsulation of the aryl ring. The same perturbation was also observed for *p*-toluidine but not in the cases of benzylamine or aniline, although all of these guests form complexes with 1 according to NMR or other data. The significantly perturbed spectrum produced upon complexation of (4-methylbenzyl)ammonium ion with 1 is quite similar to that obtained from the aromatic amine dissolved in cyclohexane. The monomeric moiety glycoluril ($C_4H_6N_4O_2$), which has no cavity, showed no significant effect upon the UV spectrum of the aromatic amine in aqueous acid, even when present at six times the molar concentration of 1. The similarity of the spectrum of the cucurbituril–aryl complex (in aqueous solution) to the cyclohexane solution spectrum of the aromatic ring is probably more than coincidental. It suggests that in solution the benzene chromophore is incorporated into the host cavity, which provides a nonpolar environment like that of a hydrocarbon solvent.

Conclusive evidence for internal complexation has been obtained by crystallography. A complete structure for the *p*-xylylenediammonium chloride adduct with 1 ($C_{36}H_{36}N_{24}O_{12}\cdot NH_2CH_2C_6H_4CH_2NH_2\cdot 2HCl\cdot 10H_2O$) clearly shows the postulated mode of binding.[5]

Guest Exchange Mechanism. Different alkylammonium ions have been found to bind to cucurbituril with differing affinities. Consequently, one guest can be displaced by another, provided that the second guest either is intrinsically stronger binding or is present in large excess. The ^1H NMR resonances of a number of free and complexed alkylammonium ions are sufficiently separated that the change in intensity of the signal from a complex may be used to monitor the displacement process. Experiments were designed based on this observation in order to study the mechanism of displacement. The change of signal intensity upon addition of a displacing alkylammonium ion to solutions of a preexisting complex of 1 were found to follow an exponential decay. Procedural details for such relaxation measurements are provided in the Experimental Section; results are summarized in Table I.

Were the displacement to proceed associatively (second order), the reaction velocity should accelerate proportionately upon an increase in the concentration of displacing guest. However, in the diverse examples cited in Table I the rates were only slightly affected when the concentration of the second ammonium ion was increased tenfold. On the basis of these observations, we conclude that the rate is independent of the concentration of the displacing agent; that is, the initial guest dissociates from the cavity (or is displaced by solvent) in a slow step, with a subsequent competition between the secondary and the primary guests for the free cage molecule.

There is another feature of guest dissociation from 1 which is of interest, particularly in the case of alkanediammonium ions. Obviously one of the nitrogens of a bound alkanediammonium ion must be drawn through the core of the host molecule. With the nitrogen protonated, desolvation of the charged moiety as it passes into an apparently "hydrocarbon-like" environment should contribute substantially to the activation energy of the process, but transient deprotonation of one nitrogen could obviate this problem. Therefore, it is relevant to question whether the nitrogen remains protonated during the dissociation process. Since rate measurements are obtained in buffered medium (HCO_2H/HCO_2^-), this uncertainty may be resolved by a study of the pH dependence of the velocity of a displacement. Pertinent results are shown in Table II. Upon a pH increase of one unit, there would be a tenfold increase in the small amount of (unprotonated) alkylamine

(8) VanEtten, R. L.; Sebastian, J. F.; Clowes, G. A.; Bender, M. L. *J. Am. Chem. Soc.* **1967**, *89*, 3242.

Table II. pH Dependence for Velocity of Dissociation of Guests from 1

primary (complexed) guest[a]	secondary (displacing) guest[a]	pH[b]	$10^4 k_{obsd}$, s^{-1}
$(H_2NCH_2CH_2)_2S$	$H_2N(CH_2)_5NH_2$	3.23	2.3 (±0.2)
		4.15	2.3 (±0.2)
$(CH_3)_2CHCH_2CH_2NH_2$	$(2\text{-}C_4H_3O)CH_2NH_2$[c]	0.96	4.4 (±0.3)
		1.84	3.7 (±0.3)
		2.15	2.8 (±0.2)

[a] Mole ratio (primary/secondary) 1:1, temperature 40 °C, D_2O–HCO_2H–HCO_2Na solution. [b] pH meter reading at completion of displacement (glass electrode, 24 °C). [c] 2-Furanmethylamine.

in acidic solution. Should deprotonation of an ammonium ion be necessary for passage through the core of the cage, then the observed reaction velocity should accelerate tenfold for each incremental pH unit. However, the experimental observation is no significant velocity dependence upon pH, for either an alkanediammonium ion or for an alkyl(mono)ammonium ion. This suggests that deprotonation is not required for dissociation.

Binding Parameters for 2. For quantitative measurement of dissociation constants involving 1, we have adopted as our primary reference guest 4-methylbenzylamine ($p\text{-}CH_3C_6H_4CH_2NH_2$, **2**). As previously noted, **2** undergoes a characteristic change in electronic spectrum upon inclusion complex formation with **1**. This makes feasible spectrophotometric examination of the process of complex formation.

$$1 + 2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} 1\cdot 2$$

A net rate of inclusion was determined by measuring the absorbance at 270.7 nm as a function of time, upon addition of **2** to a solution of an excess of **1** in HCO_2H–H_2O. A slow increase in optical density was noted, which could be satisfactorily fitted to an exponential decay function, yielding the pseudo-first-order rate constant $k_{obsd} = k_1[1] + k_{-1}$ (because complex formation was incomplete under the experimental conditions). After equilibrium had been attained, an excess of a second, much more strongly (and instantaneously) binding substrate, 1,6-hexanediamine, was added to displace **2** from **1**. Since the mechanism of substitution is dissociative and independent of the concentration of the displacing agent, an exponential decrease in the absorbance of **1·2** was noted, which could be fitted to yield k_{-1}. By difference, the true value of $k_1 = (k_{obsd} - k_{-1})/[1]$ is obtained. Accurate knowledge of both k_1 and k_{-1} allows calculation of the dissociation constant for **1·2**, $K_d = k_{-1}/k_1$. This procedure was carried out at six different temperatures, and the results are compiled in Table III. The generally slow rates of exchange presumably are attributable to the necessity of squeezing a 6 Å diameter arene through a small portal of **1** (nominal diameter 4 Å). Values for the dissociation constant were extrapolated to 40 °C, to give $K_d = 3.075 \times 10^{-3}$ M, which number was subsequently used as the standard reference value for measurement of dissociation constants of other guests, by competition methods to be described. It might be noted that K_d values, as given in Table III and subsequently, are the *inverse* of formation constants, which are sometimes employed, for example, in studies of the extent of chelation. (*Smaller* values of K_d mean *stronger* binding.)

Appropriate plots of the reduced kinetic data as a function of temperature ($\Delta G^{\ddagger}/T$ vs. $1/T$) were satisfactorily linear. The least-squares slope ($\Delta H^{\ddagger}$) and intercept ($\Delta S^{\ddagger}$) were determined both for dissociation and for formation of the complex **1·2** (standard state = 1 M). The derived parameters are as follows: for dissociation $\Delta H^{\ddagger}$ = 18.8 (±0.3) kcal/mol, $\Delta S^{\ddagger}$ = −8.5 (±1.1) eu; for association $\Delta H^{\ddagger}$ = 13.4 (±0.4) kcal/mol, $\Delta S^{\ddagger}$ = −14.0 (±1.3) eu; overall $\Delta H°$ = −5.4 (±0.2) kcal/mol, $\Delta S°$ = −5.5 (±0.5) eu. The values of $\Delta H°$ and $\Delta S°$ for formation of **1·2** show that the complex is enthalpy stabilized, but entropy destabilized, as would be expected for a bimolecular process. Such compensation between $\Delta H°$ and $\Delta S°$ has also been observed in other host–guest systems, for example, inclusion complex formation of α-cyclodextrin with *p*-nitrophenol, *p*-nitrophenolate anion, and 1-adamantanecarboxylate anion ($\Delta H°$ = −4.2, −7.2, and −3.4 kcal/mol, while $\Delta S°$ = −2.8, −8.7, and −1.3 eu, respectively[9]), or β-cyclodextrin with benzoylacetic acid and (*p*-methylbenzoyl)acetic acid ($\Delta H°$ = −5.7 and −6.6 kcal/mol, while $\Delta S°$ = −8.6 and −9.8 eu, respectively[10]). These values presumably reflect not only interactions between host and guest, but also changes in the ordering of solvent molecules in the strongly hydrogen-bonding medium employed.[11] We suggest that $\Delta S°$ for this bimolecular addition reaction is only slightly negative because water structure sheathing the aromatic ring (and cationic substituent) of **2** and occupying the cavity of **1** is disrupted in the course of complex formation, according to the usual understanding of hydrophobic bonding.[12]

Table III. Dissociation Parameters for 1 + 2 ⇌ 1·2[a]

temp,[b] °C	k_1, s^{-1} M^{-1}	$10^3 k_{-1}$, s^{-1}	$10^3 K_d$, M
6.00	0.151 (±0.003)	0.162 (±0.0015)	1.075 (±0.03)
9.29	0.216 (±0.006)	0.256 (±0.0025)	1.18 (±0.05)
14.09	0.311 (±0.004)	0.446 (±0.003)	1.43 (±0.03)
21.70	0.594 (±0.008)	1.06 (±0.01)	1.78 (±0.04)
25.29	0.755 (±0.015)	1.55 (±0.02)	2.05 (±0.07)
29.74	1.15 (±0.03)	2.58 (±0.08)	2.24 (±0.13)

[a] [1] = 6.956×10^{-3} M, [2] = 7.85×10^{-4} M, $[H_2N(CH_2)_6NH_2]$ (added displacing agent) = 7.44×10^{-3} M, solvent HCO_2H–H_2O (1:1). [b] Temperature determined by calibrated thermocouple (Cary Model 210 spectrophotometer).

Competitive Complexation. In an effort to define practically the limits to the dimensions of the host cavity, we have surveyed the complexing capability of **1** toward a variety of potential guests. The primary technique for quantitative determination is ^1H NMR. For example, the relative affinities of a pair of alkylammonium ions for **1** may be ascertained by allowing an excess of a mixture of the two cations to form an equilibrium combination of complexes with a limited amount of **1**. The composition of the resulting mixture is then determined by accurate NMR integration. By use of this method, with appropriate relays between ammonium ions with strongly dissimilar affinities for **1**, quantitative binding efficiencies of a large number of alkylammonium ions have been determined, with ultimate correlation to 4-methylbenzylamine (**2**) for which the absolute K_d value is known.

Certain guests were not amenable to such direct measurement. This includes (i) those having a complicated NMR spectrum (without clearly separated peaks for free and complexed alkylammonium ions or with reasonances obscured by solvent), (ii) those for which the exchange rate was so fast that only an averaged peak between free and complexed alkylammonium ion was observed (e.g., $CH_3CH_2NH_3^+$), and (iii) those for which chemical shift evidence suggests noninternal binding [e.g.,

(9) Cramer, F.; Saenger, W.; Spatz, H.-Ch. *J. Am. Chem. Soc.* **1967**, *89*, 14. Gelb, R. I.; Schwartz, L. M.; Laufer, D. A. *Bioorg. Chem.* **1980**, *9*, 450.
(10) Straub, T. S.; Bender, M. L. *J. Am. Chem. Soc.* **1972**, *94*, 8881.
(11) Lewis, E. A.; Hansen, L. D. *J. Chem. Soc., Perkin Trans. 2* **1973**, 2081.
(12) Tanford, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd ed.; Wiley: New York, 1980.

$(CH_3)_3CCH_2CH_2NH_3^+$]. Indirect evidence was obtained for these. As an example, introduction of such a potential guest into a solution containing a weak inclusion complex leads to the observation of the NMR spectrum of the weak addend changing from "complexed" to "free". Although the newly formed complex may not be seen, its concentration may be estimated by difference, or in cases of rapid exchange on the NMR time scale, by the extent of induced shift for the averaged guest resonance. Frequently such a technique was applied with **2** as the reference guest, for which UV spectrophotometry provided quantitative data regarding the status of binding. In the latter instance the net absorbance change of **2** (at 270.7 nm) generally followed a sigmoidal curve, fitted by a least-squares iterative procedure, when plotted against the logarithm of the concentration of the displacing alkylammonium ion. The competitive affinity for **1** could thereby be obtained analytically. In most instances the apparent displacement was cleanly unimolecular in newly added guest, indicating a stoichiometric process. A notable exception was neopentylammonium ion [$(CH_3)_3CCH_2NH_3^+$] for which a *second-order* equilibrium dependence upon displacing agent was found ($\log \beta_2 = 3.65$). In this case (and only in this case) it appears that for dislodgement of **2** from **2**·**1**, an ammonium ion must become coordinated to each portal of **1**. Evidence to be adduced suggests that the neopentyl residues remain outside of the cavity of **1** in this case.

In summary, quantitative determinations of host–guest binding have been obtained from NMR (integration and induced shift) and UV absorption intensity measurements of mixtures of **1** with various alkyl- and arylammonium ions. Potential guests which show no such evidence are concluded not to bind significantly to **1**. In Table IV may be found a compilation of a large number of K_d values obtained in this way. These provide a rather complete picture of the mode of binding of guests within **1**, as will be developed subsequently.

Discussion

We now draw certain comparisons between various guests and their affinities for **1**, as revealed by K_d values (Table IV). Where quantitative differences are to be contrasted, we provide in parentheses a number which is the affinity of the guest relative to **2** (1.0); i.e., a formation constant ratio adjusted to the standard reference substance 4-methylbenzylamine. Although we list guests as the neutral amines, it is to be understood that the protonated form is that which is actually bound to **1**.

Maximum Guest Size. Although the nominal opening within the portals of **1** is only 4 Å, the internal cavity of the host calculates to be somewhat larger than 5 Å across, suggesting that groups of greater diameter than a polymethylene chain could be successfully incorporated. This is the case; we have observed complexation with isobutyl-, isopentyl-, and isohexylamines, as well as with cyclopropane-, cyclobutane-, and cyclopentane-substituted amines. This indicates that the cavity can accommodate an isobutyl group and an aliphatic ring size up to five. On the other hand, neopentylamine ($Me_3CCH_2NH_2$) and neohexylamine ($Me_3CCH_2CH_2NH_2$), ethyl-substituted and dimethyl-substituted *n*-alkylamines ($CH_3CH_2CH_2CH_2CHEtCH_2NH_2$, $CH_3CH_2CHEtCH_2NH_2$, $CH_3CHMeCHMeCH_2NH_2$), and cyclohexylmethylamine [c-$(CH_2)_5CHCH_2NH_2$] all showed no perturbation of NMR peak positions in the presence of **1**. Apparently they are too bulky to form an internal complex. Monomethyl branched alkylamines do bind ($CH_3CHMeCH_2CH_2NH_2$, $CH_3CH_2CHMeCH_2NH_2$, $CH_3CH_2CHMeCH_2CH_2NH_2$, and $CH_3CH_2CH_2CH_2CHMeNH_2$), except for 2-methylpentylamine ($CH_3CH_2CH_2CHMeCH_2NH_2$). Interestingly, the benzene ring, which has van der Waals dimensions (6 Å diameter × 4 Å thick) larger than the estimated internal cavity of **1**, incorporates readily (i.e., **2**). Binding of arenes will be considered separately.

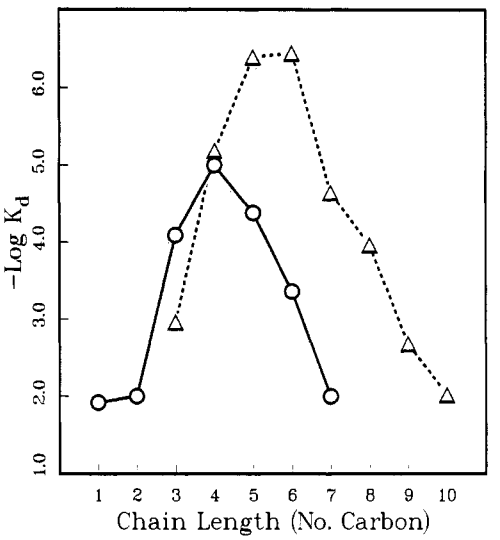

Figure 2. Dependence of strength of binding to **1** upon chain length for *n*-alkylammonium ions (○—○) and *n*-alkanediammonium ions (△---△). Vertical axis proportional to free energy of binding ($\log K_d$).

Chain Length Effect. Within homologous series, the general pattern is one of a gradual enhancement in host affinity as chain length of guest is increased until a maximum is reached, followed by diminished affinity upon further extension. This is conveniently seen graphically in Figure 2 for the *n*-alkylamines, $H(CH_2)_nNH_2$. *n*-Butylamine (307, relative to **2**) forms the most stable complex ($n = 4$) and the order of complex stability follows the trend $n = 1 < 2 < 3 < 4 > 5 > 6 > 7$. In Figure 3a we depict a *cross-sectional schematic* of the butylammonium ion adduct of **1**, drawn so as to display the maximum van der Waals radii of both host and guest. According to this model the pattern graphed in Figure 2 (solid line) is comprehensible. Nearly complete occupancy of the cavity is achieved with butylammonium ion; longer guests presumably must extend out through the second portal, where interference with solvation of the polar carbonyl groups of **1** may occur. A similar trend is observed with the alkanediamines, for which a hydrocarbon chain length of 5 or 6 is optimal (Figure 2, dashed line). In this case longer guests apparently encounter difficulty in simultaneously coordinating both ammonium ions to a portal as implied in Figure 3a. A rapid shuttling of **1** along the hydrocarbon chain between nitrogens is suggested by a "smearing out" of the NMR shielding zone, as may be seen particularly for octanediamine from the data in Figure 1. Propanediamine seems to be exceptional; although it appears to bind to **1** by the displacement criterion, no induced shift is seen for its methylene groups. We suspect that it is too short to fit the cavity of **1** and that it binds externally to but a single portal (see later). As is evident from Figure 2, the best alkanediamines [$H_2N(CH_2)_6NH_2$, 8500 relative to **2**] form somewhat more stable complexes than does butylamine. However, the range of stabilities overlap; it seems that a suitable hydrocarbon moiety makes a contribution to complex stabilization which may be comparable to that of a second ammonium ion.

Cyclic Hydrocarbon Residues. It might be anticipated that a better fit of guest to the cavity of **1** would produce a more tightly bound complex. We observe that the binding strength increases as the ring size of cycloalkyl-substituted amines is increased up to five carbon

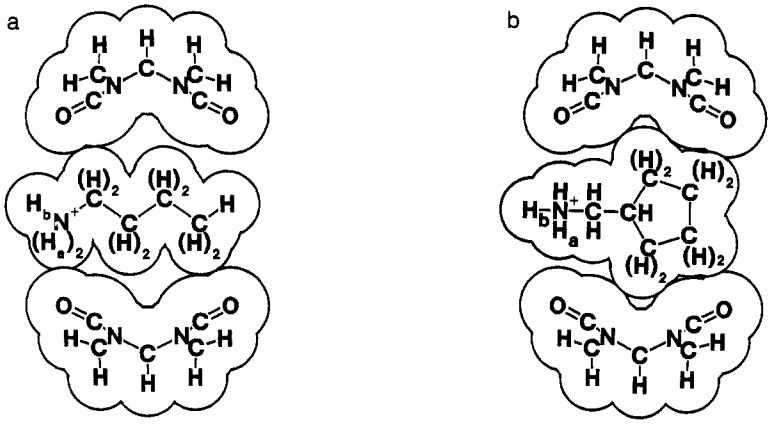

Figure 3. Conjectured cross-sectional representation of host–guest complex for (a) 1·$CH_3CH_2CH_2CH_2NH_3^+$ and (b) 1·c-$(CH_2)_4CHCH_2NH_3^+$. Outlines drawn to van der Waals radii (maximum projection for all atoms upon axial rotation of 1, crystallographically determined interatomic distances for 1).

atoms: c-$(CH_2)_2CHCH_2NH_2$ (45) < c-$(CH_2)_3CHCH_2NH_2$ (1130) ~ c-$(CH_2)_4CHCH_2NH_2$ (1040) ≫ c-$(CH_2)_5CHCH_2NH_2$ (<0.06) and c-$(CH_2)_2CHNH_2$ (1.2) < c-$(CH_2)_3CHNH_2$ (9.2) < c-$(CH_2)_4CHNH_2$ (19.5) > c-$(CH_2)_5CHNH_2$ (not bound, insoluble). Figure 3b depicts the nearly optimal complexation of cyclopentanemethylammonium ion. This adduct (and its four-membered ring congener) are approximately 3.5-fold more stable toward dissociation than is the *n*-butylammonium ion complex. It may be noted from the preceding data that when the ammonium ion of the guest is brought closer to the aliphatic ring, affinity for 1 decreases greatly (i.e., compare cycloalkylamines with cycloalkylmethylamines). This is probably a consequence of a displacement of the cation toward the center of the cavity of 1 and away from optimal engagement with the polar carbonyl groups. When the nitrogen atom is incorporated within the ring of the guest, as in pyrrolidine [c-$(CH_2)_4NH$], there is no indication of inclusion.

The Ammonium Ion Binding Site. The preceding evidence suggests that proper alignment of the cationic moieties of the guest with the host carbonyl groups is critical. Since there are six of the latter surrounding each portal of 1, it would seem that a tripodal hydrogen-bonding scheme is possible, employing the oxygen atoms of alternate carbonyls as H-bond receptors from the alkylammonium ion. Indeed, this should be the case for very small guests (e.g., CH_3NH_2). However, closer examination of structural models such as Figure 3a suggests that this may not be valid for guests which fill the cavity of 1. The difficulty is that such a symmetrical H-bond network requires a N–C bond within the guest which is *colinear* with the central axis of the host. For the *n*-butyl group in the staggered conformation (Figure 3a), the N–C bond must be tilted *off-axis* in order to avoid unacceptable van der Waals contacts between the rest of the guest and the interior of the cavity of 1. As a consequence only *two* of the protons on nitrogen (H_A in Figure 3a) may contact carbonyl oxygens, and the third (H_B) projects away from the portal. While this may seem a recondite point, it is susceptible to experimental test. Comparison of the affinities toward 1 of $CH_3CH_2CH_2CH_2NH_2$ (307), $CH_3CH_2CH_2CH_2NHCH_3$ (342), and $CH_3CH_2CH_2CH_2N(CH_3)_2$ (2.3) reveals that *one* of the H–N linkages of *n*-butylamine may be replaced by an alkyl residue without detriment to binding (i.e., CH_3 may occupy the H_B site), but substitution of *two* H–N bonds by methyls strongly perturbs binding. The same trend is noted for hexanediamine: $H_2N(CH_2)_6NH_2$ (8500), $CH_3NH(CH_2)_6NHCH_3$ (5340), $(CH_3)_2N(CH_2)_6N(CH_3)_2$ (28). Therefore, we suggest that Figure 3a contains a true representation of the structure of the complex.[13]

The binding affinity toward 1 of the biological bases spermidine and spermine was also examined. Spermidine [$H_2N(CH_2)_4NH(CH_2)_3NH_2$] (4170) has a potentially internally residing tetramethylenediamine moiety with an appended 3-aminopropyl substituent. It binds 9 times more tightly than does butanediamine [$H_2N(CH_2)_4NH_2$] (474). The higher affinity must be conferred by the aminopropyl substituent, which in its cationic form may fold back externally to 1 and interact additionally with the carbonyl groups of the portal from which it emerges. Spermine [$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$] (40400), which has an additional coordinating group at both ends of the central butanediamine moiety, binds 85 times more avidly than does the parent, indicating that the aminopropyl substituent effect is cumulative energywise. Propanediamine (2.8) shows no indication of inclusion (by NMR), yet by the displacement test it does bind with 1 and more tightly by an order of magnitude than does methylamine (0.24) or ammonia itself (0.25). The postulated external binding mode explains this difference. The extremely high affinity between 1 and these polyamine bases (spermine, $K_d = 7.8 \times 10^{-8}$ M) suggests potential biochemical applications. (Lysine has also been observed to bind to 1.)

It is pertinent to inquire as to whether the pair of hydrogen bonds in Figure 3 is truly significant energywise. The relative affinities for 1 of $H_2N(CH_2)_6H$ (7), $H_2N(CH_2)_6NH_2$ (8500), and $H_2N(CH_2)_6OH$ (3.6) provide an answer to this question. Formal replacement of the terminal hydrogen of *n*-hexylamine with another amino group enhances binding 1200-fold, reflecting the importance of a properly aligned ammonium ion to binding. However, replacement of this hydrogen by a *hydroxyl* group instead contributes *nothing* to stabilization of the complex, in spite of the fact that the alcohol is also a good hydrogen-bonding functionality. This is explicable. The important consideration is the *difference* in stabilization of free and complexed guests. While the alcohol (and ammonium ions) may be hydrogen bonded in the complex, in the absence of 1 they would also be fully hydrogen bonded within the polar aqueous medium employed in our investigations. The consequential feature of ammonium ions is that they are *charged*. The portals of 1 represent a region of negative charge accumulation, since the anionic ends of the dipoles of six urea carbonyl groups are focused there. Hence, it is our understanding that the high specificity for ammonium ions is largely an electrostatic *ion–dipole attraction*.

(13) See also ref 5 for additional evidence for this mode of coordination.

Table IV. Dissociation Constants for Host–Guest Complexes of Substituted Ammonium Ions with 1[a]

guest	K_d, M
NH_3	1.2×10^{-2}
CH_3NH_3	1.2×10^{-2}
$CH_3CH_2NH_2$	1.0×10^{-2}
$CH_3(CH_2)_2NH_2$	8.2×10^{-5}
$CH_3(CH_2)_3NH_2$	1.0×10^{-5}
$CH_3(CH_2)_4NH_2$	4.2×10^{-5}
$CH_3(CH_2)_5NH_2$	4.4×10^{-4}
$CH_3(CH_2)_6NH_2$	9.9×10^{-3}
$H_2N(CH_2)_3NH_2$	1.1×10^{-3}
$H_2N(CH_2)_4NH_2$	6.5×10^{-6}
$H_2N(CH_2)_5NH_2$	4.1×10^{-7}
$H_2N(CH_2)_6NH_2$	3.6×10^{-7}
$H_2N(CH_2)_7NH_2$	2.3×10^{-5}
$H_2N(CH_2)_8NH_2$	1.1×10^{-4}
$H_2N(CH_2)_9NH_2$	2.1×10^{-3}
$H_2N(CH_2)_{10}NH_2$	9.6×10^{-3}
$(CH_3)_2CHCH_2NH_2$	4.6×10^{-5}
$(CH_3)_2CH(CH_2)_2NH_2$	2.8×10^{-5}
$(CH_3)_2CH(CH_2)_3NH_2$	2.4×10^{-4}
$CH_2CH_2CH(CH_3)CH_2NH_2$	5.1×10^{-4}
$CH_3CH_2CH(CH_3)(CH_2)_2NH_2$	8.8×10^{-4}
$CH_3(CH_2)_2CH(CH_3)NH_2$	5.6×10^{-2}
$CH_3(CH_2)_3CH(CH_3)NH_2$	1.3×10^{-3}
$CH_3(CH_2)_4CH(CH_3)NH_2$	3.7×10^{-3}
$CH_3(CH_2)_5CH(CH_3)NH_2$	2.6×10^{-2}
$(CH_3)_3C(CH_2)_2NH_2$	5.7×10^{-2}
c-$(CH_2)_2CHNH_2$	2.6×10^{-3}
c-$(CH_2)_3CHNH_2$	3.3×10^{-4}
c-$(CH_2)_4CHNH_2$	1.6×10^{-4}
c-$(CH_2)_2CHCH_2NH_2$	6.8×10^{-5}
c-$(CH_2)_3CHCH_2NH_2$	2.7×10^{-6}
c-$(CH_2)_4CHCH_2NH_2$	3.0×10^{-6}
$CH_3(CH_2)_3NHCH_3$	9.0×10^{-6}
$CH_3(CH_2)_3N(CH_3)_2$	1.3×10^{-3}
$CH_3NH(CH_2)_6NHCH_3$	5.8×10^{-7}
$(CH_3)_2N(CH_2)_6N(CH_3)_2$	1.1×10^{-4}
$H_2N(CH_2)_4NH(CH_2)_3NH_2$	7.4×10^{-7}
$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$	7.6×10^{-8}
$C_6H_5NH_2$	1.9×10^{-4}
p-$CH_3C_6H_4NH_2$	7.9×10^{-4}
$C_6H_5CH_2NH_2$	3.7×10^{-3}
p-$CH_3C_6H_4CH_2NH_2$	3.1×10^{-3}
(2-C_4H_3S)CH_2NH_2	4.3×10^{-6}
(2-C_4H_3S)$(CH_2)_2NH_2$	1.5×10^{-2}
(2-C_4H_3O)CH_2NH_2	8.8×10^{-6}
$CH_3S(CH_2)_2NH_2$	6.0×10^{-5}
$CH_3CH_2S(CH_2)_2NH_2$	2.9×10^{-5}
$CH_3S(CH_2)_3NH_2$	1.1×10^{-4}
$CH_3CH_2S(CH_2)_3NH_2$	1.3×10^{-3}
$H_2N(CH_2)_2S(CH_2)_2NH_2$	2.4×10^{-6}
c-$(CH_2S)_2CHCH_2NH_2$	1.7×10^{-6}
$CH_3O(CH_2)_2NH_2$	5.7×10^{-4}
$CH_3CH_2O(CH_2)_3NH_2$	1.8×10^{-2}
$HO(CH_2)_6NH_2$	8.5×10^{-4}
$H_2N(CH_2)_2O(CH_2)_2NH_2$	1.9×10^{-4}
c-[$(CH_2)_3O$]$CHCH_2NH_2$[b]	1.0×10^{-5}

[a] H_2O–85% HCO_2H (1:1, v/v), 40 °C. Thereshold for detectability is $K_d < 5.0 \times 10^{-2}$ M by techniques used. Amines mentioned in text as not binding have greater values. [b] 2-Tetrahydrofuranmethylamine.

Hydrogen bonding may occur, but this is incidental; it merely allows the closest contact between cation and dipole. This generalization is extendable to biochemical systems.

Arylamines. It is known that a benzene ring may be ensconced within 1, even though it has a van der Waals diameter larger than the calculated internal cavity of the host. Complexes with most arylamines are weak (typically, p-$CH_3C_6H_4CH_2NH_2$, 1.0 by definition). Exceptions are (2-C_4H_3S)CH_2NH_2 (thiophenemethylamine, 708) and (2-C_4H_3O)CH_2NH_2 (furanmethylamine, 351), which have dimensions closer to that of the internal diameter of host. This suggests to us that the benzene ring does indeed exceed the strain-free binding capacity of 1. The latter idea finds confirmation in the crystal structure of the *p*-xylylenediamine·2HCl complex of 1.[5] The cage structure of the host is clearly distorted into an ellipsoid shape in this adduct, with more than a 0.4 Å *decrease* in diameter (compared with uncomplexed 1) in a direction *perpendicular* to the guest benzene ring and with a compensating increase within the plane of the aromatic ring. Hence, the relatively low affinities of benzenoid guests reflects a balanced compensation between favorable noncovalent binding forces and a stress–strain relationship involving host and guest. If the difference between the stability of complexes of benzylamine (0.84) and of thiophenemethylamine (708) is taken as a measure of misfit, then there is a ΔG of 4.2 kcal/mol which should be partitioned as 2.1 kcal/mol distortion energy in 1 and 2.1 kcal/mol of compression energy within a bound benzene ring. In any event, a practical limit to the inclusion capacity of cucurbituril is the volume of a benzene ring.

The specificity of benzenoid binding is also informative. Although 4-methylbenzylamine forms a complex, the 3-methyl and 2-methyl isomers show no indication of inclusion. The same behavior occurs in the toluidines ($CH_3C_6H_4NH_2$), in which the para isomer binds, but not the ortho and meta isomers. It is apparent that when the core of 1 is occupied by a benzene ring, there is no extra room to accommodate an additional methyl group within the cavity. For para isomers both substituents are able to extend toward a portal of host. However, 4-ethylbenzylamine showed no evidence for inclusion, and the same is true for *p*-ethyl- and *p*-isopropylaniline. Possibly an ethyl group interferes more than does a methyl group with solvation of carbonyl moieties surrounding the second portal. These specificities stand in contrast to cyclodextrin complexes, in which the arene substituent pattern characteristically has only a minor effect upon strength of binding.[8,10] The advantages in selectivity attendant to working with a rigid host, which is unable to adjust itself freely to the shape of guests, are evident in comparison of 1 to the examples of cyclodextrins[11] and many flexible cryptands.[2]

The consequences of insertion of methylene groups between arene and ammonium ion was systematically examined: $C_6H_5NH_2$ (16) > $C_6H_5CH_2NH_2$ (0.84) > $C_6H_5CH_2CH_2NH_2$ (qualitatively estimated in HCl solution for solubility reasons) > $C_6H_5(CH_2)_3NH_2$, $C_6H_5(CH_2)_4NH_2$ (the latter two substances showed no evidence for inclusion); p-$CH_3C_6H_4NH_2$ (3.9) > p-$CH_3C_6H_4CH_2NH_2$ (1.0); (2-C_4H_3S)CH_2NH_2 (2-thiophenemethylamine, 708) ≫ (2-C_4H_3S)$CH_2CH_2NH_2$ (2-thiopheneethylamine, 0.2). Since the aryl rings of these guests fully occupy the internal cavity of 1, an extension of the alkyl chain moves the ammonium ion away from the portal of host, apparently diminishing the ion–dipole attraction. Pyridines (C_5H_5N), for which the cationic center would reside within the cavity, also showed no evidence for inclusion.

Heteroatom-Containing Guests. A generalization derivable from our data is that a thioether-containing guest binds *more* strongly than an (oxo)ether-containing guest, but *less* strongly than the corresponding alkylamine: $H_2N(CH_2)_5NH_2$ (7500) > $H_2N(CH_2)_2S(CH_2)_2NH_2$ (1270) > $H_2N(CH_2)_2O(CH_2)_2NH_2$ (16), $CH_3(CH_2)_3NH_2$ (307) > $CH_3S(CH_2)_2NH_2$ (52) > $CH_3O(CH_2)_2NH_2$ (5.4), $CH_3(CH_2)_5NH_2$ (7) > $CH_3CH_2S(CH_2)_3NH_2$ (2.3) > $CH_3CH_2O(CH_2)_3NH_2$ (0.18), and c-$(CH_2)_4CHCH_2NH_2$ (1040) > c-[$(CH_2)_3O$]$CHCH_2NH_2$ (2-tetrahydrofuranmethylamine, 298). This is attributable to a solvation effect operating primarily on the uncomplexed guest; oxygen has greater intrinsic *hydrophilicity* than does sulfur, and a methylene group is more *hydrophobic* than is a thioether linkage.[14]

There are some observed exceptions: $CH_3CH_2SCH_2CH_2NH_2$ (105) > $CH_3(CH_2)_4NH_2$ (74), and c-$(CH_2S)_2CHCH_2NH_2$ (ethane dithioacetal of aminoacetaldehyde, 1810) > c-$(CH_2)_4CHCH_2NH_2$ (1040). The heterocyclic five-membered ring containing two sulfur atoms in the latter comparison represents an attempt to match exactly the dimensions of guest to that of host. Because of the greater bond length of C–S compared to C–C, the effective diameter of this heterocycle should be intermediate between that of a cyclopentane and a benzene ring. This guest is indeed the most tightly bound of the monoamines; whether the optimum has been reached is not known.

Because of the favored inclusion of five-membered rings, the binding affinities of such species without a substituent ammonium ion were qualitatively checked. In the presence of 1, cyclopentane [c-$(CH_2)_5$], tetrahydrothiophene [c-$(CH_2)_4S$], tetrahydrofuran [c-$(CH_2)_4O$], and methylcyclopentane [c-$(CH_2)_4CHCH_3$] all showed induced NMR shifts of the magnitude expected (with separate resonances for free and complexed guests). The relative affinities for 1 in aqueous formic acid follow the trend C_5H_{10} > C_4H_8S > C_4H_8O (the NMR of $C_5H_9CH_3$ is too diffuse to include in this comparison). It has not proven feasible to obtain quantitative K_d values.

Anomalous Binding of α-Methyl-*n*-alkylammonium Ions. For the branched alkylamine guests of 1, a methyl-substituent at the β- or γ-position [that is, $CH_3CH_2CH\mathit{Me}CH_2NH_2$ (6) and $CH_3CH\mathit{Me}CH_2CH_2NH_2$ (109)] is shifted significantly *upfield* in the ^1H NMR spectrum upon complexation as previously noted (by 0.76 and 0.70 ppm, respectively). However, for the α-isomer [$CH_3CH_2CH_2CH\mathit{Me}NH_2$ (0.55)] the methyl exhibits only a slight downfield shift in the presence of 1. The same behavior was noted for homologues [$CH_3(CH_2)_3CH\mathit{Me}NH_2$ (2.3), $CH_3(CH_2)_4CH\mathit{Me}NH_2$ (0.83), and $CH_3(CH_2)_5CH\mathit{Me}NH_2$ (0.12)], although β-, γ-, and δ-methylene groups of these alkylamines all suffer the usual upfield shift. This indicates to us that these α-Me-substituted *n*-alkylamines bind with the substituent and adjacent methine carbon *outside* of the cavity of 1, for steric reasons having to do with the narrowness of the portals of 1. It is noteworthy that the tightest complex in this generally weakly bound series occurs with α-methyl-*n*-pentylamine rather than with *n*-butylamine as shown in Figure 2. Consequently, some latitude in the geometry of binding is indicated, and Figure 3 does not represent the exclusive mode of complexation.

Conclusion. The following characteristics of host–guest complexation involving cucurbituril (1) have been established: (i) alkyl- and aryl-substituted ammonium ions bind internally with diagnostic upfield ^1H NMR induced chemical shifts and UV spectral perturbations. (ii) Exchange of one guest for another follows a dissociative mechanism in acid solution, and does not involve transient deprotonation of an ammonium ion. (iii) Quantitative measurements of dissociation constants may be obtained by competition experiments. Values range from 1.2×10^{-2} M (ammonia) to 7.6×10^{-8} M (spermine). The free energy difference encompassed by this span amounts to 7.4 kcal/mol. (iv) Structure–selectivity relationships among various guests allow formulation of an explicit model for optimal complexation. A primary interaction is charge–dipole attraction between the ammonium cation and the electronegative oxygens of the urea carbonyls which surround the portals of 1. Generally, this involves only bipodal hydrogen bonding. Hydrocarbon substituents on the ammonium ion having dimensions no greater than a para-disubstituted benzene ring will enter the cavity of 1 and participate in the noncovalent binding, to an extent which may be comparable energetically to the ion–dipole attraction in favorable cases. (v) We believe that this latter contribution is largely a hydrophobic effect;[11] i.e., the freeing of polar solvent molecules which otherwise would be ordered within a solvent shell about the nonpolar surface of the guest and within the cavity of 1 (as detected by crystallography[4,5]). We intend in a subsequent publication to attempt to quantitate this latter phenomenon, by appropriate analysis of structure–selectivity relationships.

(14) Hine, J.; Mookerjee, P. K. *J. Org. Chem.* **1975**, *40*, 292.

Experimental Section

Cucurbituril (1) was prepared by a modification of the procedure of Behrend.[3] It may be recrystallized by dissolution in warm concentrated hydrochloric or sulfuric acid with subsequent dilution by cold water. Material so obtained has the approximate composition of a tetradecahydrate. Drying leads to variable dehydration, depending on temperature. Amine guests used in this study were either obtained from commercial sources or were prepared by standard procedures (i.e., Gabriel synthesis); for details, see ref 1.

Competitive Binding. As an example of measurement of relative affinities by quantitative ^1H NMR, 60.4 μmol of $(CH_3)_2CHCH_2NH_2$, 314.2 μmol of *p*-$CH_3C_6H_4CH_2NH_2$, and 56.0 μmol of 1 were allowed to equilibrate in D_2O–85% HCO_2H (1:1, v/v) for 20 h. The composition of the resulting mixture was then determined at 40 °C by NMR integration (Varian T60) of methyl resonances for free and complexed species, enabling calculation of a *relative* binding constant of 67. Competition between isobutylamine and another, more strongly bound guest then allowed extension of the scale, etc. As a cross check, several guest affinities were measured by using more than one comparison (i.e., cycles were constructed wherein independent estimates of relative binding constants were acquired). Averaged reproducibility in K_d by such tests was ±15% (most likely error in NMR integration). This suggests the possibility of accumulation of considerable inaccuracy across the scale of Table IV, although the qualitative relative affinities of individual pairs of guests is probably correctly represented throughout.

As an example of kinetic technique, a solution of 45 μmol of $H_2N(CH_2)_2S(CH_2)_2NH_2$ and 49 μmol of 1 in 0.34 mL of D_2O–HCO_2H containing 2 μL of *t*-BuOH (internal standard) was temperature equilibrated (40 °C) for 10 min. Then 43 μmol (or more) of $H_2N(CH_2)_5NH_2$ in 10 μL of D_2O–HCO_2H (40 °C) was added by syringe. Integrals of both *t*-BuOH (δ 1.5) and $H_2N(CH_2)_5NH_2$ (δ 0.53–1.37) were taken at measured time intervals until no more variation was noted. Normalized integral values as a function of time were fitted by iterative nonlinear least-squares to an exponential decay function, yielding a pseudo-first-order rate constant.

For determination of the K_d value of 2 (our absolute reference), sufficient quantities of stock solutions of 1 and 2 were mixed in a cuvette so as to yield a reaction mixture initially 7.0 mM in 1 and 0.8 mM in 2. A subsequent exponential decay (increase in absorbance at 270.7 nm), attributable to formation of 1·2, was obtained and fitted as before. The solution was then made 8.0 mM in $H_2N(CH_2)_6NH_2$ (by addition of a concentrated stock solution in H_2O–HCO_2H), and the decrease in absorbance due to decay of 1·2 was similarly recorded. Derived rate constants at various temperatures are given in the Results section (Table III). Tolerances given in this article are standard errors from least-squares analysis.

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