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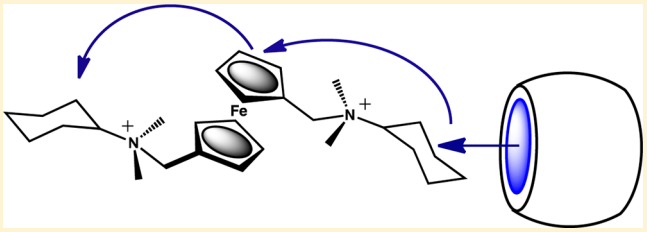
Binding Interactions between Cucurbit[*n*]uril Hosts and Tritopic, Dicationic Guests Containing a Central Ferrocenyl and Two Terminal Aminocyclohexyl Sites

Wei Li and Angel E. Kaifer*

Center for Supramolecular Science and Department of Chemistry, University of Miami, Coral Gables, Florida 33124-0431, United States

Supporting Information

ABSTRACT: The binding interactions between two cucurbit[*n*]uril hosts (*n* = 7, 8, CB7 and CB8) and the three new guests 1,1′-bis(cyclohexylammoniomethyl)ferrocene ($\mathbf{1}H_2^{2+}$), 1,1′-bis(cyclohexylmethylammoniomethyl)ferrocene ($\mathbf{2}H_2^{2+}$), and 1,1′-bis(cyclohexyldimethylammoniomethyl)ferrocene ($\mathbf{3}^{2+}$) were investigated in aqueous solution using ^{1}H NMR spectroscopy, voltammetry, and electrospray (ESI) mass spectrometry. The experimental data reveal that guests $\mathbf{1}H_2^{2+}$ and $\mathbf{2}H_2^{2+}$ behave similarly with both hosts, leading to the formation of pseudorotaxane complexes in which the host is located centrally around the ferrocenyl residue. A similar complex forms also between $\mathbf{3}^{2+}$ and CB7, but its formation is slower ($k = 2.5 \times 10^{-4}$ s^{-1}) and can be monitored by NMR spectroscopy. Finally, $\mathbf{3}^{2+}$ and CB8 give rise to a ternary 2:1 complex, in which CB8 receptors are bound to the terminal cyclohexyl groups, when the concentration of the host exceeds that of the guest.



INTRODUCTION

In aqueous solution the cucurbit[7]uril host (CB7) forms inclusion complexes of very high stability with suitable guests, such as ferrocenyl[1−3] and adamantyl[2,4] derivatives. In particular, the complex between CB7 and 1,1′-bis-(trimethylammoniomethyl)ferrocene exhibits an equilibrium association constant (*K*) of ca. 10^{15} M^{-1} in aqueous solution,[3] a value which is similar to that measured between avidin and biotin.[5] The fact that this guest is dicationic and the two positively charged ammonium nitrogens interact with the two rims of carbonyl oxygens lining the host cavity portals was initially thought to be an important factor contributing to the binding. However, thermodynamic studies[3] and more recent work[6] have shown that the high stability of the complex largely derives from hydrophobic forces and from the small entropic penalty associated with complex formation.

We have investigated in detail highly stable complexes formed between CB7 and various monocationic and neutral ferrocenyl derivatives.[7,8] In a related study, we investigated the binding interactions between cucurbit[8]uril (CB8) and monocationic, ditopic guests containing ferrocenyl and 2,2,6,6-tetramethylpiperidine-1-oxyl (Tempo) binding sites.[9] In this case, the degree of methylation of the central ammonium nitrogen has a pronounced effect on the binding preference of CB8 between the two sites on the guest. This was an unexpected result, which is still not well understood. More recently, we have reported on the complexation between CB7 and a monocationic guest containing adamantyl and ferrocenyl sites, in which the two possible microscopic complexes can be readily detected and the kinetics of interconversion between them monitored by NMR spectroscopy or electrochemical techniques.[10]

As another component of our work on highly stable cucurbituril complexes, we decided to prepare a new series of tritopic, dicationic guests containing a central ferrocenyl site and two terminal aminocyclohexyl sites (see Figure 1 for structures) and investigate their binding interactions with the CB7 and CB8 hosts. The three guests in this work differ in the level of methylation of the amine nitrogens separating the binding sites. Therefore, guest **1** does not bear any methyl groups on the amine nitrogens, while in guest **2** there is one methyl group directly attached to each nitrogen atom. Finally, guest $\mathbf{3}^{2+}$ is fully methylated and, thus, each of the nitrogen atoms is quaternized and positively charged. To increase the similarities between all guests, the first two compounds were used in their protonated, dicationic forms ($\mathbf{1}H_2^{2+}$ and $\mathbf{2}H_2^{2+}$). All guests possess a central ferrocenyl site flanked by two positive charges. This ferrocenyl binding site is similar to that in 1,1′-bis(trimethylammoniomethyl)ferrocene,[3] affording an excellent thermodynamic well for the CB7 host in the middle of these guest structures. On the other hand, the terminal aminocyclohexyl groups can also become effective binding sites for the hosts.

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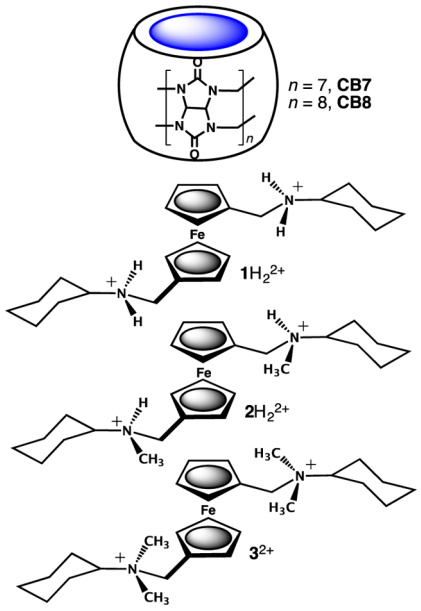

Figure 1. Structures of the hosts and guests used in this work.

RESULTS AND DISCUSSION

Synthesis. The preparation of the hosts CB7 and CB8 was done according to reported procedures.[11] Guest **1** was prepared by reaction of 1,1′-ferrocenedicarboxaldehyde with cyclohexylamine in methanolic solution, followed by reduction with $NaBH_4$. Guest **2** was similarly synthesized from the same ferrocene starting material and *N*-methylcyclohexylamine, but reduction was carried out with $NaBH_3CN$. Finally, dicationic $\mathbf{3}^{2+}$ was prepared by methylation of **2** with dimethyl sulfate in dichloromethane solution. All guests were characterized by ^1H and ^{13}C NMR spectroscopy, as well as high-resolution electrospray (ESI) mass spectrometry (see the Experimental Section for details). Compounds **1** and **2** were used in mildly acidic solutions (pH ∼4.5) to ensure that they would be present as their diprotonated, dicationic forms.

Binding with CB7. The binding interactions between the guests $\mathbf{1H_2}^{2+}$, $\mathbf{2H_2}^{2+}$, and $\mathbf{3}^{2+}$ and the CB7 host were investigated using ^1H NMR spectroscopy, cyclic voltammetry, and ESI mass spectrometry. Figure 2 shows the NMR data

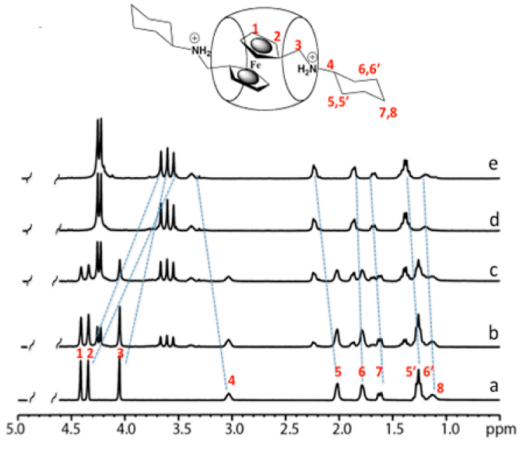

Figure 2. Partial ^1H NMR spectra (500 MHz, 0.1 M NaCl–D_2O, pD 4.5) of 1.0 mM $\mathbf{1H_2}^{2+}$ (a) in the absence and in the presence of (b) 0.25, (c) 0.5, (d) 1.0, and (e) 1.25 equiv of CB7.

obtained with guest $\mathbf{1H_2}^{2+}$ in the presence of various concentrations of CB7. An important observation from this series of spectra is that the ferrocenyl protons (labeled 1–3 in the figure) all shift upfield upon addition of CB7. This fact is a clear indication that the ferrocenyl group is engulfed inside the cavity of the host.[1,7] In contrast, the cyclohexyl protons (labeled 4–8) shift downfield in the presence of CB7, in good agreement with the expected behavior of protons that stay outside the host cavity but remain in the vicinity of the cavity portals. Therefore, the NMR spectroscopic data are consistent with the formation of an inclusion complex in which the host occupies the central ferrocenyl binding site, while the cyclohexyl side arms stay outside the cavity. Furthermore, the NMR spectroscopic data suggest that the formation of this complex is quantitative at the millimolar concentrations used in the NMR experiments, since the changes brought about by the presence of CB7 level off at 1.0 equiv of host, with excess beyond this point leading to no further spectral modifications (Figure 2d,e). Of course, this is consistent with the substantial stability anticipated for these complexes.

The anodic voltammetric behavior of the guests is highlighted by the reversible, one-electron oxidation of the ferrocenyl unit.[12] Indeed, Figure 3 shows the reversible

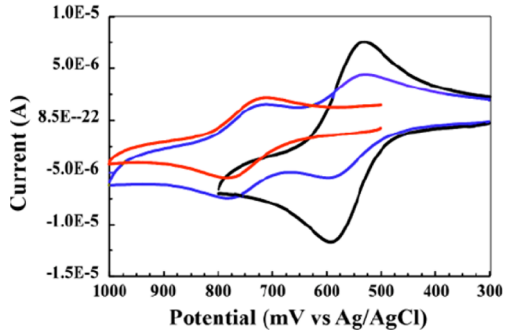

Figure 3. Cyclic voltammetric behavior on glassy carbon (0.071 cm^2) of 1.0 mM $\mathbf{1H_2}^{2+}$ in 0.1 M NaCl pH 4.5 in the absence (black) and in the presence of 0.5 equiv (blue) and 1.0 equiv (red) of CB7. Scan rate: 0.1 V s^{-1}.

oxidation process for guest $\mathbf{1H_2}^{2+}$ centered at a half-wave potential ($E_{1/2}$) of 0.56 V. Addition of 1.0 equiv of CB7 shifts the observed $E_{1/2}$ value to 0.75 V, which is entirely consistent with the inclusion of the ferrocenyl group inside the host cavity. We have observed, upon encapsulation with CB7, pronounced anodic $E_{1/2}$ shifts with a variety of cationic ferrocene derivatives.[1,7,8] These anodic potential shifts reflect the thermodynamic hindrance to oxidation associated with the poorer solvation of the oxidized, positively charged ferrocenium form inside the relatively hydrophobic cavity of CB7. In the presence of 0.5 equiv of CB7, the voltammetric behavior shows two sets of waves, corresponding to the free and bound guests. This "two-wave" behavior has been described in detail by our group and is fully expected when the host–guest complex exhibits considerable thermodynamic stability.[13]

Clearly, both ^1H NMR spectroscopic and voltammetric data led us to conclude that CB7 can easily reach the central binding site in the $\mathbf{1H_2}^{2+}$ guest, leading to the formation of a symmetric complex, CB7·$\mathbf{1H_2}^{2+}$, in which the ferrocenyl group is encapsulated and ion–dipole interactions develop between each of the positively charged ammonium groups and the carbonyl oxygens lining the portals of CB7. The resulting CB7·

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$\mathbf{1}H_2^{2+}$ complex has a pseudorotaxane structure, in which the CB7 "wheel" is centered between the two positive charges of the "thread" compound, the $\mathbf{1}H_2^{2+}$ guest, with the cyclohexyl end groups lacking the necessary bulk to keep the host trapped and prevent dissociation (see Figure 2, top). The formation and stoichiometry of this complex was also confirmed by ESI mass spectrometric data (see the Supporting Information, Figure S1).

The binding interactions between the guest $\mathbf{2}H_2^{2+}$ and CB7 follow exactly the same pattern, as evidenced by the NMR spectroscopic, voltammetric, and mass spectrometric data (Figures S2 and S3, Supporting Information) being similar to those obtained for guest $\mathbf{1}H_2^{2+}$. Therefore, we conclude that replacement of a proton by a methyl group on each of the ammonium nitrogens of the guest has no significant effect on the formation of pseudorotaxane complexes and, thus, the structures of the CB7·$\mathbf{1}H_2^{2+}$ and CB7·$\mathbf{2}H_2^{2+}$ complexes are basically the same.

Further methylation of the guest does change the observed interactions with the CB7 host. For instance, the voltammetric behavior of guest $\mathbf{3}^{2+}$ in the presence of 1.0 equiv of CB7 initially shows a pronounced cathodic shift in the observed $E_{1/2}$ value for the reversible oxidation of the central ferrocenyl residue, which shifts from 0.67 V in the free guest to 0.47 V in the CB7 complex (Figure 4). The negative shift in the half-wave

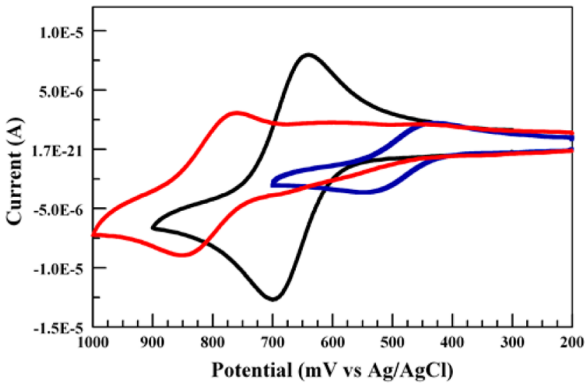

Figure 4. Cyclic voltammetric behavior on glassy carbon (0.071 cm^2) of 1.0 mM $\mathbf{3}^{2+}$ in 0.1 M NaCl pH 4.5 in the absence (black) and in the presence of 1.0 equiv of CB7 immediately after addition (blue) and at equilibrium (red) after ca. 4 h. Scan rate: 0.1 V s^{-1}.

potential strongly suggests that the ferrocenyl group is outside the CB7 cavity but in the vicinity of one of the host cavity portals. This finding is consistent with the inclusion of one of the terminal cyclohexyl groups inside the host cavity. However, this is not the most thermodynamically stable complex, because the voltammetric behavior of this solution changes as a function of time and, after ~4 h, we observe a single set of waves centered at 0.81 V, which corresponds to the ferrocenyl-included, symmetric pseudorotaxane.

Similar data were obtained in ^{1}H NMR spectroscopic experiments, indicating the initial formation of a complex in which one of the terminal cyclohexyl groups is included by CB7 followed by the gradual development of a set of peaks corresponding to the included ferrocenyl protons. In other words, the spectra clearly reveal the time evolution of the supramolecular system from an external complex (included cyclohexyl group) to an internal complex (pseudorotaxane), in which CB7 occupies the central binding site in the $\mathbf{3}^{2+}$ guest (Figure S5, Supporting Information). We used the ^{1}H NMR spectroscopic data to investigate the kinetics of the conversion between the two microscopic forms of the CB7·$\mathbf{3}^{2+}$ complex (Figure 5). The process was found to be first order, and the rate constant was determined to be 2.5×10^{-4} s^{-1} ($t_{1/2}$ = 46 min) at 25 °C.

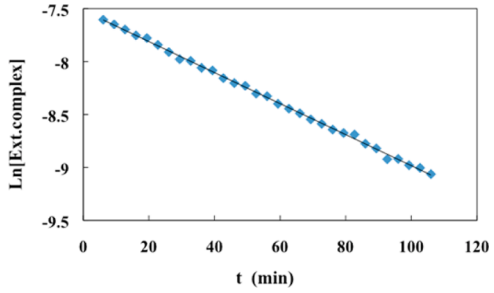

Figure 5. Concentration (as its natural log) of the external CB7·$\mathbf{3}^{2+}$ complex as a function of time in 0.1 M NaCl at 25 °C.

The 1:1 stoichiometry of the CB7·$\mathbf{3}^{2+}$ complex was also verified by ESI mass spectrometric data (Figure S4, Supporting Information). Ultimately, all three guests form the same pseudorotaxane, symmetric CB7 inclusion complex, with the host engulfing the central ferrocenyl residue. Remarkably, the level of methylation of the ammonium nitrogens affects the kinetics of formation of the complex. With guests $\mathbf{1}H_2^{2+}$ and $\mathbf{2}H_2^{2+}$ the kinetics of formation of the CB7 complex is too fast to be monitored by conventional NMR spectroscopic techniques and the system reaches equilibrium before any spectra can be recorded. However, with the fully methylated guest $\mathbf{3}^{2+}$, we can monitor the kinetics of formation of the pseudorotaxane using either NMR spectroscopy or voltammetric measurements (data not shown). We must conclude that full methylation of the two ammonium nitrogens on the guest slows down the threading process by which the CB7 host reaches the central binding site.

Several reasons can be proposed to explain the effects of ammonium methylation on the threading rate of CB7 along the $\mathbf{3}^{2+}$ guest. The first one is based on steric reasons, as two methyl groups attached to each ammonium nitrogen would create an obstacle when the host slides over, trying to reach the thermodynamic valley offered by the ferrocenyl binding site. The presence of protons attached to the ammonium nitrogen may facilitate its passage through the hydrophobic CB7 cavity through a deprotonation–protonation mechanism.[14] Alternatively, one could also argue that methylation may increase the binding affinity of the host for the terminal cyclohexyl residues, resulting in a higher energetic barrier that must be overcome for the host to reach the central binding site. As a quick way to assess the second possibility, we explored the relative stability of the external complexes by adding excess (over 1.0 equiv) CB7. If the external complexes are sufficiently stable, in the presence of 2.0 equiv of host, we may observe the formation of 2:1 complexes in preference to the already observed 1:1 pseudorotaxane. It is unlikely that a 2:1 complex will form with a CB7 host on the central ferrocenyl site, as binding of two CB7 hosts in close proximity has not been observed because of the resulting electrostatic repulsions between the rims of carbonyl oxygens lining the cavity entrances. For the same reasons, formation of a 3:1 complex has also not been observed with these guests. Therefore, the attempt to form 2:1

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complexes relies on the possibility that each of the terminal cyclohexyl groups will be included by a host, while the central ferrocenyl site remains unbound. Our attempts to observe any such 2:1 species were not successful. Once we observed the formation of the 1:1 pseudorotaxane complex, we failed to observe any convincing evidence for the formation of 2:1 CB7 complexes. This matter will be revisited later in the paper.

Binding with CB8. Since the rate of formation of the CB7 complexes appears to be affected by the size of the groups attached to the ammonium nitrogens on the guests, we decided to investigate the binding interactions with the next higher analogue, the cucurbit[8]uril (CB8) host. Our experimental approach relied on the same techniques. However, the lower aqueous solubility of CB8 forced us to carry out experiments with lower concentrations of guests in solution.

The ^{1}H NMR spectroscopic data for mixtures of guest $\mathbf{1}H_2^{2+}$ and CB8 are shown in Figure 6. Clearly, the addition of CB8

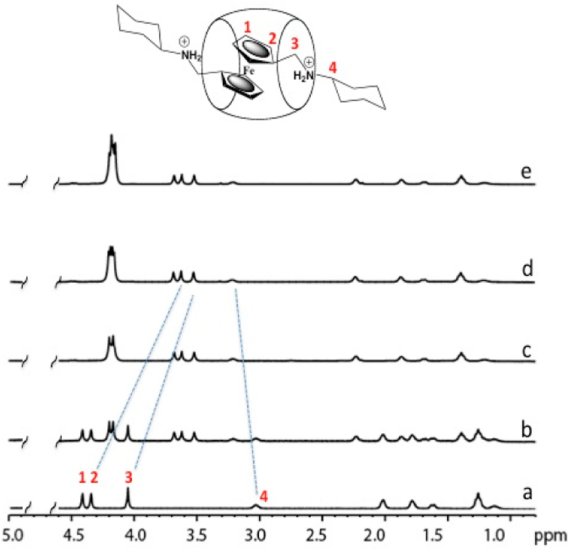

Figure 6. Partial ^{1}H NMR spectra (500 MHz, 0.1 M NaCl–D_2O pD 4.5) of 0.1 mM $\mathbf{1}H_2^{2+}$ (a) in the absence and in the presence of (b) 0.5, (c) 1.0, (d) 1.5, and (e) 2.0 equiv of CB8.

leads to an upfield shift for the protons (labeled 1–3) on the ferrocenyl residue. As in the case of CB7, this finding indicates the formation of a symmetric complex with the host engulfing the central ferrocenyl residue. Notice that addition of CB8 beyond 1.0 equiv does not lead to any changes in the structure of the complex. In other words, the formation of a 2:1 complex is not favored in this case and the CB8·$\mathbf{1}H_2^{2+}$ pseudorotaxane-type complex prevails, even under conditions of excess CB8 in the solution. Entirely similar NMR spectroscopic data were obtained for mixtures of guest $\mathbf{2}H_2^{2+}$ and CB8 (Figure S6, Supporting Information), revealing that the corresponding CB8·$\mathbf{2}H_2^{2+}$ complex has a similar structure.

In contrast to these findings, the binding interactions between $\mathbf{3}^{2+}$ and CB8 were different, as evidenced by the corresponding ^{1}H NMR spectroscopic data (Figure 7). In this case the addition of up to 1.0 equiv of CB8 leads to the formation of the pseudorotaxane complex, as revealed by the upfield shift of the ferrocenyl protons. However, addition of excess CB8 (more than 1.0 equiv) leads to the return of the ferrocenyl proton signals to chemical shifts close to those observed with the free guest. Furthermore, addition of 2.0 equiv

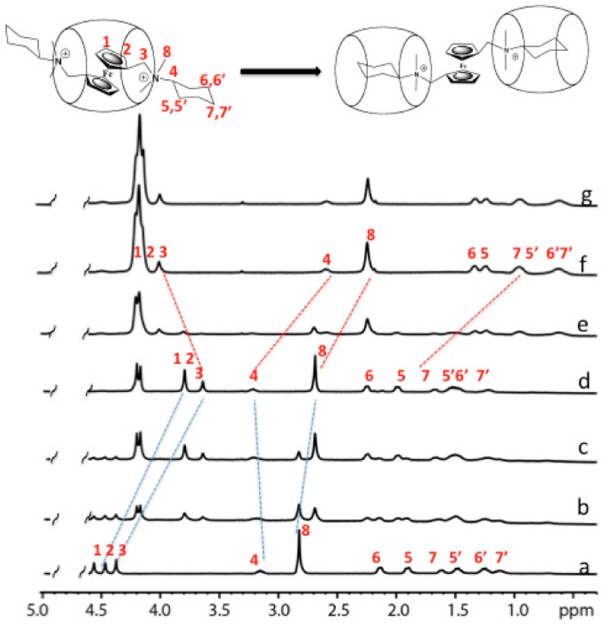

Figure 7. Partial ^{1}H NMR spectra (500 MHz, 0.1 M NaCl-D_2O pD 4.5) of 0.1 mM $\mathbf{3}^{2+}$ (a) in the absence and in the presence of (b) 0.44, (c) 0.88, (d) 1.0, (e) 1.8, (f) 2.6, and (g) 3.5 equiv of CB8.

of CB8 results in a clear upfield shift of the cyclohexyl protons, a finding which is consistent with the formation of a 2:1 complex in which each of the terminal cyclohexyl residues is bound by a CB8 host, leading to the formation of a dumbbell-type complex.

The differences in the structures and stoichiometries of the CB8 complexes formed with guests $\mathbf{1}H_2^{2+}$ and $\mathbf{2}H_2^{2+}$, on one side, and guest $\mathbf{3}^{2+}$, on the other side, are again triggered by the full methylation of the ammonium nitrogens on the guest. To confirm the conclusions derived from NMR spectroscopic data, we carried out square wave voltammetric (SWV) experiments. SWV was used instead of cyclic voltammetry because of its higher sensitivity, which is required in these experiments because of the low solubility of CB8. The reversible one-electron oxidation of free guest $\mathbf{1}H_2^{2+}$ is observed at a half-wave potential of 0.56 V vs Ag/AgCl, while upon addition of 1.0 equiv of CB8 the $E_{1/2}$ value shifts to 0.71 V. Addition of a second equivalent of CB8 does not result in any further potential changes. As mentioned before, the anodic shift of the $E_{1/2}$ value correlates well with the inclusion of the ferrocenyl group inside the cavity of the host. Therefore, the NMR spectroscopic and voltammetric data are fully consistent with the formation of a 1:1 pseudorotaxane CB8·$\mathbf{1}H_2^{2+}$ complex. The SWV data for the guest $\mathbf{2}H_2^{2+}$ are also very similar, with $E_{1/2}$ values of 0.62 and 0.72 V for the free and CB8-bound complex, respectively.

As expected from the NMR spectroscopic results, the voltammetric data obtained with guest $\mathbf{3}^{2+}$ are different. The free guest undergoes reversible oxidation at a half-wave potential of 0.68 V; upon addition of 2.0 equiv of CB8, the $E_{1/2}$ value shifts cathodically to 0.32 V! This considerable potential shift, equivalent to −0.36 V, is consistent with the formation of a dumbbell complex in which the ferrocenyl group is not included but suffers the electrostatic effects exerted by two CB8 hosts, which engulf the cyclohexyl residues at the ends of the guest. The formation of this 2:1 complex is, of course, consistent with the NMR spectroscopic data.

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ESI mass spectrometry was also used to investigate the CB8 complexes. While we could confirm again the formation and stoichiometry of the CB8·$\mathbf{1H_2}^{2+}$ and CB8·$\mathbf{2H_2}^{2+}$ complexes (Figures S7 and S8, Supporting Information), we could not observe any signals corresponding to the $(CB8)_2\cdot\mathbf{3}^{2+}$ 2:1 complex. However, we clearly detected the CB8·$\mathbf{3}^{2+}$ 1:1 complex (Figure S9, Supporting Information). The failed mass spectrometric detection of the 2:1 CB8 complex of guest $\mathbf{3}^{2+}$ cannot dispel the strong combination of evidence obtained in NMR spectroscopic and electrochemical experiments, which consistently support the formation of this ternary complex.

Binding of Model Compounds. Each of the guest compounds surveyed here is composed of three binding sites: two terminal cyclohexyl sites and a central ferrocenyl site. We decided to investigate a small series of model compounds resembling the individual binding sites in order to gain a better understanding of the key factors controlling the binding differences between the fully methylated $\mathbf{3}^{2+}$ and the other two guests. Therefore, we focused our effort on the three model compounds shown in Table 1, which were readily accessible to us. The cyclohexyl derivatives $\mathbf{4}^+$ and $\mathbf{5H}^+$ are intended to represent the terminal binding sites on the tritopic guests at either of the two extremes of full methylation ($\mathbf{4}^+$) and full protonation ($\mathbf{5H}^+$) of the ammonium group. The model guest $\mathbf{6}^{2+}$ represents the central ferrocenyl site on the tritopic guests.

Table 1. Equilibrium Association Constants (K) and Gibbs Free Energies ($\Delta G°$) for the Binding Interactions between Model Guest Compounds $\mathbf{4}^+$, $\mathbf{5H}^+$, and $\mathbf{6}^{2+}$ and the hosts CB7 and CB8 in 50 mM NaAc pH 4 solution at 25 °C

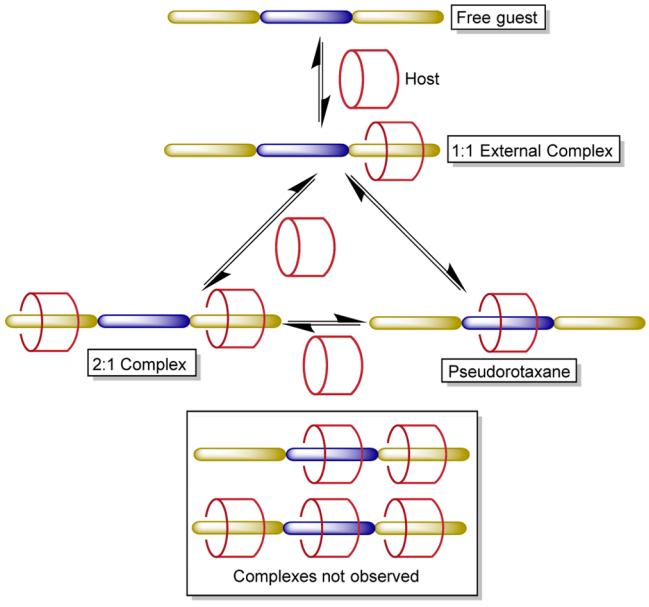
	Binding with CB7		Binding with CB8	
	K (M^{-1})	$\Delta G°$ (kJ/mol)	K (M^{-1})	$\Delta G°$ (kJ/mol)
$\mathbf{4}^+$	1.1×10^9	-52	1.4×10^9	-52
$\mathbf{5H}^+$	1.1×10^8	-46	1.4×10^6	-35
$\mathbf{6}^{2+}$	3.0×10^{15} (in pure water)	-88	5.4×10^9	-56

While it is tempting to try to estimate the $\Delta G°$ values of the various complexes between CB7 or CB8 and the tritopic guests on the basis of the thermodynamic parameters shown in Table 1, this exercise is likely to be marred by large errors. However, there are some trends in the data that are very useful to explain our experimental results with the tritopic guests. One important factor is that binding between $\mathbf{6}^{2+}$ and either host leads to more stable complexes than binding between either host and the other two cyclohexyl derivatives. Therefore, the general tendency to form symmetric, pseudorotaxane-type complexes with the ferrocenyl site engulfed by the host is supported by the data in Table 1. The slower formation of the pseudorotaxane complex, in the case of $\mathbf{3}^{2+}$ and CB7, can be rationalized by the more stable complex formed by CB7 with $\mathbf{4}^+$ in comparison to $\mathbf{5H}^+$. In other words, methylation of the ammonium nitrogen increases the stability of the complexes in which CB7 interacts with the cyclohexyl terminus of the tritopic guest. The larger relative stabilization of the host slows down the formation of the final symmetric complex, probably because the host sliding process over the ammonium group is associated with a higher energy barrier. The unavailability of a deprotonation–protonation mechanism[14] to assist the sliding of the CB7 over the central binding site may also be an important factor. However, an steric component must also play a role, because the kinetics of formation of the analogous pseudorotaxane complex between $\mathbf{3}^{2+}$ and CB8 is fast on the time scale of NMR spectroscopic and voltammetric experiments.

The binding events observed between CB8 and the tritopic guest $\mathbf{3}^{2+}$ suggest a better balance between the relative stabilization of the larger host at the two types of binding sites (terminal cyclohexyl or central ferrocenyl). In fact, the data in Table 1 show that the K value for the CB8 complexation of $\mathbf{4}^+$ is only slightly lower than that for the CB8·$\mathbf{6}^{2+}$ complex. Therefore, the binding interactions between $\mathbf{3}^{2+}$ and CB8 initially lead to the formation of the symmetric complex, but the terminal sites compete effectively when [CB8] > [$\mathbf{3}^{2+}$] and the 2:1 complex predominates under these conditions. This behavior is not observed with guests $\mathbf{1H_2}^{2+}$ and $\mathbf{2H_2}^{2+}$, because the protonated cyclohexyl ammonium site has a lower binding affinity for CB8, as reflected by the K value in Table 1 between CB8 and $\mathbf{5H}^+$. This finding is also supported by a previous report from our group[9] on the larger binding affinity with CB8 of methylated ammonium Tempo sites compared to similar sites with lower levels of methylation.

In conclusion, we have shown that the most common binding behavior between the tritopic, dicationic guests surveyed here and the hosts CB7 and CB8 is the rapid formation of a symmetric, pseudorotaxane 1:1 complex (see Scheme 1). The kinetic rate of formation of this complex is considerably slow between CB7 and $\mathbf{3}^{2+}$, probably because of the substantial stabilization of the 1:1 external complex. Also, the interactions between the same guest and CB8 are particularly interesting, as the predominant complex formed depends on the relative concentrations of both partners. When the host concentration is 1.0 equiv or less, the predominant supramolecular species is the symmetric pseudorotaxane.

Scheme 1. Possible Equilibria between the Tritopic Guests and the CB7 and CB8 Hosts



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However, as the concentration of host exceeds 1.0 equiv, a 2:1 complex, in which the two terminal cyclohexyl groups are bound by CB8 (Scheme 1), starts to appear and competes effectively with the pseudorotaxane. Once the CB8 concentration is 2.0 equiv or larger, this ternary "dumbbell" complex becomes the predominant supramolecular species. As shown in Scheme 1, we did not observe either the ternary complex, in which the two hosts occupy adjacent binding sites, or the quaternary complex, in which all three binding sites are occupied by host molecules. Presumably, these supramolecular species are destabilized by the electrostatic repulsions between nearby carbonyl oxygens on the host cavity portals.

EXPERIMENTAL SECTION

Materials. Cyclohexylamine, *N*,*N*-dimethylcyclohexylamine, 1,1′-ferrocenecarboxaldehyde, *N*-methylcyclohexylamine, MeI, $NaBH_4$, $NaBH_3CN$, and all other chemicals and solvents were commercially available. All amines were passed through basic alumina before use. The purity of the CB7 and CB8 hosts, prepared as described in the literature,[11] was assayed by the method previously reported by our group.[15]

*Synthesis of 1,1′-Bis(cyclohexylaminomethyl)ferrocene (**1**).* Cyclohexylamine (370 μL, 320 mg, 3.28 mmol) was dissolved in 20 mL of methanol. Aqueous HCl solution was used to adjust the solution pH value to around 5, followed by addition of 1,1′-ferrocenedicarboxaldehyde (132 mg, 0.54 mmol). Molecular sieves were used to absorb water in the reaction. The orange mixture was refluxed at 45 °C for 4 h under N_2. $NaBH_4$ (123 mg, 3.28 mmol) was then added to the solution stirred for 24 h at 45 °C and then cooled. After filtration through Celite, methanol was removed. The resulting orange oil was washed and extracted by ethyl ether/water, and the organic layers were collected for product purification. The mixture was separated through a neutral Al_2O_3 column with ethyl ether/methanol (1/9). The major product **1** was collected and dried, resulting in a yellow solid (132 mg, yield 60%). ^{1}H NMR (D_2O, 500 MHz): δ 1.13 (t, 2H, CH_2), 1.25 (m, 6H, CH_2), 1.60−1.63 (d, 2H, CH_2), 1.77 (s, 2H, CH_2), 2.01 (s, 2H, CH_2), 2.99 (s, H, CH), 4.01 (s, 2H, CH_2), 4.33 (s, 4H, C_5H_4), 4.40 (s, 4H, C_5H_4) ppm. ^{13}C NMR (D_2O, 500 MHz): δ 23.87 (1C, CH_2), 24.50 (2C, CH_2), 29.05 (2C, CH_2), 43.58 (C, CH), 56.06 (2C, CH_2), 70.55 (4C, C_5H_4), 70.70 (4C, C_5H_4), 77.7 (2C, C_5H_4) ppm. ESI-MS: found *m*/*z* 205.1174 $[M + 2H]^{2+}$, $C_{24}H_{38}FeN_2^{2+}$, calcd 205.1166.

*Synthesis of 1,1′-Bis(cyclohexylmethylaminomethyl)ferrocene (**2**).* *N*-Methylcyclohexylamine (390 μL, 335 mg, 2.94 mmol) was dissolved in 20 mL of methanol. An aqueous HCl solution was used to adjust the solution pH value to around 5, followed by addition of 1,1′-ferrocenedicarboxaldehyde (119 mg, 0.49 mmol). Molecular sieves were used to absorb water in the reaction. The orange mixture was refluxed at 45 °C for 4 h under N_2. The mild reducing agent $NaBH_3CN$ (186 mg, 2.96 mmol) was then added to the solution, which was stirred for 24 h at 45 °C and then cooled. After filtration through Celite, methanol was removed. The orange oil was washed and extracted by CH_2Cl_2/water, and the organic layers were collected for product purification. The mixture was separated on a neutral Al_2O_3 column with CH_2Cl_2/methanol (1/10). The product **2** was collected as a yellow solid (107 mg, yield 50%). ^{1}H NMR (D_2O, 500 MHz): δ 1.10−1.12 (m, 2H, CH_2), 1.24−1.27 (m, 4H, CH_2), 1.41−1.48 (m, 4H, CH_2), 1.61−1.63 (d, 2H, CH_2), 1.87 (m, 6H, CH_2), 1.97−1.98 (d, 2H, CH_2), 3.17 (t, 2H, CH), 4.08−4.25 (m, 4H, CH_2), 4.40 (s, 4H, C_5H_4), 4.47 (s, 4H, C_5H_4) ppm. ^{13}C NMR (D_2O, 500 MHz): δ 24.31 (10C, CH_2), 34.79 (2C, CH), 52.79 (2C, CH_2), 63.27 (2C, CH_3), 71.21 (4C, C_5H_4), 71.74 (4C, C_5H_4), 75.54 (2C, C_5H_4) ppm. ESI-MS: found *m*/*z* 2219.1377 $[M + 2H]^{2+}$, $C_{26}H_{42}FeN_2^{2+}$, calcd 219.1343.

*Synthesis of 1,1′-Bis(cyclohexyldimethylammoniomethyl)ferrocene (**3**$^{2+}$).* Compound **2** (66 mg, 0.15 mmol) was dissolved in 15 mL of CH_2Cl_2 under a N_2 atmosphere. Methyl sulfate (57 μL, 76 mg, 0.60 mmol) was added to the solution, and the mixture was stirred for 24 h. A brown precipitate was formed. The solid was collected by filtration and washed with CH_2Cl_2. The resulting light brown solid was characterized as the sulfate salt (36 mg, yield 43%). ^{1}H NMR (D_2O, 500 MHz): δ 1.10−1.12 (m, 2H, CH_2), 1.23−1.26 (m, 4H, CH_2), 1.46−1.48 (m, 4H, CH_2), 1.60−1.62 (d, 2H, CH_2), 1.88−1.91 (d, 4H, CH_2), 2.12−2.14 (d, 4H, CH_2), 2.81 (s, 12H, CH_3), 3.15 (t, 2H, CH), 4.37 (s, 4H, CH_2), 4.46 (s, 4H, C_5H_4), 4.55 (s, 4H, C_5H_4) ppm. ^{13}C NMR (D_2O, 500 MHz): δ 24.22 (2C, CH_2), 24.78 (C, CH_2), 25.62 (4C, CH_2), 46.60 (2C, CH), 62.24 (2C, CH_2), 71.18 (4C, CH_3), 71.73 (4C, C_5H_4), 73.06 (4C, C_5H_4), 73.64 (2C, C_5H_4) ppm. ESI-MS: found *m*/*z* 233.1503 $[M]^{2+}$, $C_{28}H_{46}FeN_2^{2+}$, calcd 233.1533.

*Synthesis of Cyclohexyltrimethylammonium (**4**$^+$).* *N*,*N*-Dimethylcyclohexylamine (500 mg, 589 μL, 3.98 mmol) was dissolved in 50 mL of CH_2Cl_2, followed by addition of MeI (837 mg, 367 μL, 5.89 mmol). The mixture was stirred under N_2 for 24 h at room temperature. A white precipitate was formed and washed with acetone. The white product was collected as the iodide salt (856 mg, yield: 80%). ESI-MS: found 142.1593 $[M]^+$, $C_9H_{20}N^+$, calcd 142.1596.

*Synthesis of 1,1′-Bis(trimethylammoniomethyl)ferrocene (**6**$^{2+}$).* 1,1′-Ferrocenedicarboxaldehyde (200 mg, 0.83 mmol) was dissolved in an ethanolic solution of methylamine (244 mg, 7.86 mmol in 30 mL) under a N_2 atmosphere. The red solution was refluxed for 1 h at 45 °C. After the mixture was cooled to 0 °C, $NaBH_4$ (125 mg, 0.3.30 mmol) was added in one portion. The mixture was stirred for 12 h at 45 °C. The ethanol was evaporated, and the residue was extracted between water and ethyl ether (10 mL/10 mL). The aqueous solution was further extracted by ethyl ether (3 × 10 mL). All the organic solutions were collected and dried over Na_2SO_4, and then the solvent was removed. The red oily residue was separated on a neutral Al_2O_3 column with ethyl ether/MeOH (1/5) as the eluent. A major yellow band was collected and characterized as the diamine (158 mg, 0.58 mmol, yield 70%). The diamine structure was confirmed by ^{1}H NMR spectroscopy and ready for the next step.

The diamine (158 mg, 0.58 mmol) was redissolved in CH_2Cl_2 (30 mL) under N_2 at room temperature. MeI (361 μL, 824 mg, 5.8 mmol) was added to the yellow solution. The reaction mixture was covered by aluminum foil and stirred in the dark for 24 h. A yellow precipitate was formed. After filtration and washing with CH_2Cl_2, the iodide salt of **6**$^{2+}$ was collected as a yellow solid (67 mg, yield: 20%). ^{1}H NMR (D_2O, 500 MHz): δ 2.94 (s, 18H, CH_3), 4.36 (s, 4H, CH_2), 4.47 (s, 4H, C_5H_4), 4.56 (s, 4H, C_5H_4) ppm. ESI-MS: found *m*/*z* 165.0881 $[M]^{2+}$, $C_{18}H_{30}FeN_2^{2+}$, calcd 165.0874.

Methods. Cyclic voltammetric and square wave voltammetric experiments were carried out with a BAS 100 W electrochemical workstation. A single-compartment cell fitted with a glassy-carbon working electrode, Pt counter electrode, and Ag/AgCl reference electrode was used. The surface of the working electrode was polished before each measurement on a felt surface with a slurry of 5 μm alumina powder and water.

^{1}H NMR spectra were recorded with a 500 MHz Bruker Avance spectrometer equipped with a cryoprobe. DCl and D_2O were purchased from Cambridge Isotopes. The pH measurements were done using a PHR-146 microcombination pH electrode on an Accumet model 50 pH/ion/conductivity meter, calibrated using standard buffers (pH 4, 7, and 10).

The equilibrium association constants between the hosts (CB7 and CB8) and the model guests (**4**$^+$, **5**H$^+$, and **6**$^{2+}$) were determined in competition binding experiments using cobaltocenium as the reference guest. The competition between the two guests under conditions in which the host concentration is not enough to bind both guests completely can be monitored by following the cobaltocenium absorbance at 261 nm. These experiments were all done in 50 mM sodium acetate (pH 4.5) solution, a medium in which the equilibrium association constants of cobaltocenium with CB7 and CB8 have been reported by us[15] as 5.7×10^9 and 1.9×10^8 M^{-1}, respectively. Details of similar competition binding experiments have been reported in previous publications from our group.[16,17]

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■ ASSOCIATED CONTENT

Supporting Information

Figures giving additional NMR and mass spectroscopic data as mentioned in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail for A.E.K.: akaifer@miami.edu.

Author Contributions

The manuscript was written through contributions of both authors. Both authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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