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# Cucurbit[8]uril Forms Tight Inclusion Complexes with Cationic Triamantanes

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**We report the synthesis of quaternary (di)cationic triamantane derivatives G1 and G3 by the permethylation of the corresponding primary ammonium ions G2 and G4. The complexation behaviors of G1 – G4 toward CB[7] and CB[8] were examined by $^1$H NMR spectroscopy, which reveals that CB[8] is capable of fully encapsulating G1 – G4 whereas CB[7] forms inclusions complexes with G1, G2, and G4 but cannot fully encapsulate the central hydrophobic core of bis-quaternary ammonium ion G3. The geometry of the CB[n]•guest complexes were determined by analyzing the complexation induced changes in chemical shifts and were further confirmed by molecular modelling using the Conformer-Rotamer Ensemble Sampling Tool (CREST) based on the GFN methods. Finally, the complexation thermodynamics were determined by a combination of $^1$H NMR competition experiments, direct isothermal titration calorimetry (ITC) measurements, and competitive ITC titrations using a tight binding ternary complex as competitor.**

## Introduction

The synthesis and molecular recognition properties of the cucurbit[n]uril (CB[n]) family of molecular container compounds has undergone rapid development since the turn of the millennium.[1] Figure 1 shows the molecular structure of CB[n] which is composed of *n* glycoluril units connected by *2n* methylene bridges that form a barrel shaped macrocycle with two electron rich ureidyl carbonyl fringed portals and a central hydrophobic cavity. Accordingly, CB[n] hosts bind strongly to guests that feature a central hydrophobic moiety that is flanked by two cationic groups. For example, Mock and co-workers showed that CB[6] binds strongly to alkanediammonium ions in aqueous formic acid solution with selectivity for pentane- and hexanediammonium ions (**1** and **2**);[2] the CB[6]•spermine (**3**) complex achieved $K_a$ = 1.3 × 10$^7$ M$^{-1}$ ($K_d$ = 76 nM). Later studies by Kim, Inoue, and co-workers demonstrated even higher binding affinity could be achieved by working in the less competitive environment of pure water.[3]

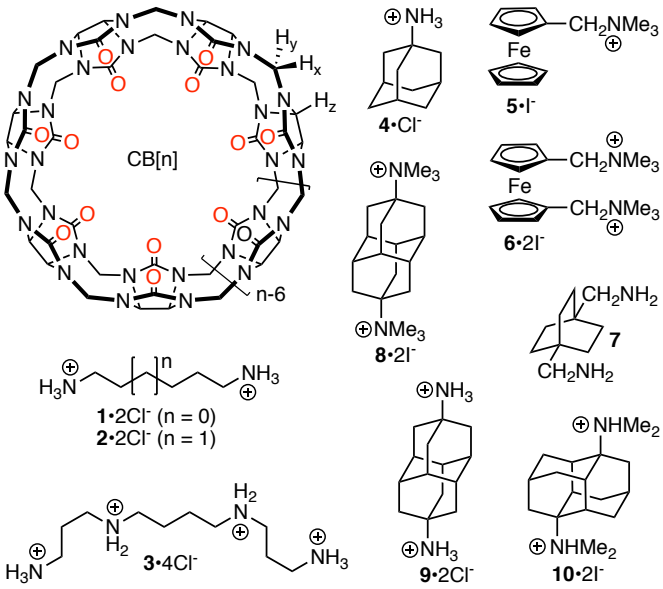


**Figure 1** Structure of ultratight binding hosts CB[n] (n = 6, 7, 8) and selected guests.

Clues from CB[n] derived self-sorting systems[4] led us to measure the binding constants of CB[n] (n = 6, 7, 8) toward a panel of ammonium ions in pH 4.74 acetate buffered water and discover the ultratight binding affinity of the CB[7]•adamantane ammonium (**4**) ion ($K_a$ = 4.2 × 10$^{12}$ M$^{-1}$) using $^1$H NMR competition experiments.[5] The hydrophobic adamantane skeleton contains ten carbon atoms. Contemporaneously, Kim, Inoue, and Kaifer published the binding affinity of the CB[7]•trimethylaminomethyl ferrocene (**5**; hydrophobic core: ten C-atoms + Fe) complex ($K_a$ = 4 × 10$^{12}$ M$^{-1}$) in pure water.[6] In follow up work, the Kim, Kaifer, Isaacs,

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Gilson and Inoue groups collaboratively explored the CB[7]•(bis)trimethylaminomethyl ferrocene (**6**) complex and determined $K_a$ = 2.9 × $10^{15}$ $M^{-1}$ in pure water by competitive ITC titrations.[7] The potential of [2.2.2]bicyclooctane as a hydrophobic core (eight C-atoms) (e.g., **7**) to construct ultratight binding complexes was subsequently reported by the Kim, Inoue, and Gilson team.[8] The high affinity of CB[n]•guest complexes has been traced, in part, to the presence of intracavity "high energy" water molecules that lack a full complement of H-bonds and that are released upon complexation as shown by DeSimone, Scherman, and Nau.[9] The $K_a$ values for CB[n]•guest complexes have been featured prominently in a series of blinded challenges (SAMPL and Hydrophobe) that aim to improve computational approaches to free energy computations in water.[10] As illustrated in Figure 2, the changes in aqueous solvation of both the CB[n] host and the hydrophobic guest contribute to the thermodynamics of complexation.[9b]

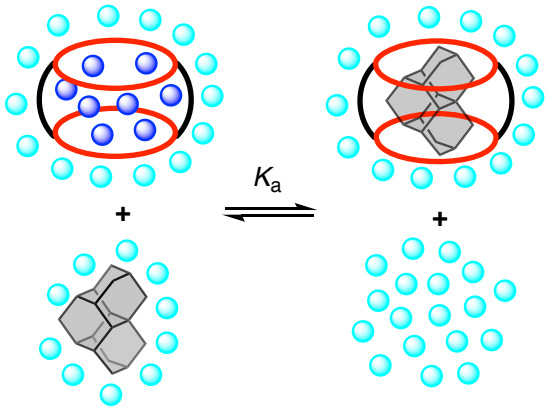


**Figure 2** Illustration of the changes in solvation of host and guest that occur during formation of CB[n]•guest complexes. Aqua spheres, bulk water; Blue spheres, intracavity "high energy" water.

More recently, in collaboration with Glaser and Mlinarić-Majerski, we have explored various cationic guests featuring diamantane (14 C-atoms) as the hydrophobic core and demonstrated attomolar binding affinity of the CB[7]•diamantane-bis(trimethylammonium) ion (**8**) in pure water ($K_a$ = 7.2 × $10^{17}$ $M^{-1}$).[11] The 10,000-fold weaker binding affinity of the CB[7]•**9** complex illustrates that the nature of the ammonium (1˚ versus 4˚) can be a very important factor in some but not all situations.[12, 11] In the CB[8] series, the CB[8]•**10** complex achieved $K_a$ = 5.7 × $10^{14}$ $M^{-1}$ in 50 mM acetate buffered water (pH = 4.74).[13] CB[n]•guest complexes have also been shown to be highly responsive to suitable stimuli (e.g., photochemical, electrochemical, chemical, pH).[14] These high affinity, highly selective, and stimuli responsive binding events render CB[n]•guest complexes useful as a supramolecular latching and switching element in a variety of complex systems. Accordingly, macrocyclic CB[n] have found numerous uses including as a component of (bio)sensing and imaging ensembles,[15] for drug formulation, delivery and sequestration,[16] creating supramolecular organic frameworks,[17] and performing supramolecular catalysis.[9d] In this paper, we further develop our line of inquiry into cationic CB[n]•diamondoid complexation events by progressing from the C14 diamantane to the larger and more hydrophobic C18 triamantane skeleton. Very recently, Biedermann and co-workers have studied the binding of CB[n] toward diamondoid (adamantane, diamantane, and triamantane) alcohols by a combination of calorimetry and chemical computations.[18] Among other results, Biedermann and co-workers found that CB[8] bound 3,9-dihydroxytriamantane with log $K_a$ = 7.0 in deionized water which represented the first example of triamantane complexation with CB[n]. Overall, Biedermann's work showed that peculiar host solvation – rather than London dispersion interactions, electronic energies, or entropic factors – is largely responsible for the ultratight binding exhibited by CB[n] hosts.

**Results and Discussion**

This results and discussion section is organized as follows. First, we describe the selection, synthesis, and characterization of guests **G1** – **G4**. Next, we investigate the complexation of **G1** – **G4** by complexation induced changes in $^1$H NMR chemical shifts along with molecular modelling to glean information about the geometry of the CB[n]•**G** complexes. Subsequently, we measure the binding constants for the CB[n]•**G** complexes by direct isothermal titration calorimetry (ITC), $^1$H NMR competition experiments, and competitive ITC titrations as appropriate. Finally, we discuss the data and offer conclusions.

***Selection, Synthesis, and Characterization of G1 – G4.*** As described above, the Isaacs group has a longstanding interest in the design and discovery of tight binding host•guest complexes with an emphasis on CB[n]•cationic diamondoid systems. Previous investigations focused on (di)cationic adamantane (C10) and diamantane (C14) derived guests and showed that both ion-dipole (C=O•••ammonium) interactions and the hydrophobicity of the diamondoid skeleton play significant roles in determining host•guest binding affinity.[5, 19, 13] As the next logical step toward the creation of even tighter binding guests for CB[n], we decided to investigate cationic derivatives of triamantane (C18) which is the next larger diamondoid homologue. Accordingly, we synthesized hydrochloride salts **G2** and **G4** (Figure 3) from triamantane by three step procedures (hydroxylation, modified Ritter reaction with chloroacetonitrile, and cleavage of the formed chloroacetamide to the corresponding amine) described in the literature.[20] The separate permethylation reactions of **G2** and **G4** with an excess of MeI (15 equiv.) and $NaHCO_3$ (10 equiv.) were conducted in hot (60 ˚C) MeOH for 48 h which delivered quaternary ammonium salts **G1** and **G3** in 47 and 62% yields, respectively. High resolution mass spectrometry showed ions for **G1** at 298.2536 (calc. for $C_{21}H_{32}N$: 298.2535) and **G3** at 379.3100 (calc. for $C_{24}H_{40}N_2Na$: 379.8089) which are in accord with the depicted molecular formulas. Please note that **G1** and **G3** are prepared and used as iodide salts whereas **G2** and **G4** are hydrochlorides; we do not consider the influence of counterion in this paper. $C_s$-symmetric guests **G1** and **G2** feature a single mirror plane whereas guests **G3** and **G4**

possesses two mirror planes and are therefore $C_{2v}$-symmetric. In accord with symmetry considerations, the $^{13}C$ NMR spectrum of **G1** and **G3** recorded in $CDCl_3/CD_3OD$ consist of 14 and 9 resonances, respectively (Supporting Information, Figures S2 and S4). Whereas the $^1H$ NMR spectrum of **G1** suffers from spectral overlap, the spectrum for $C_s$-symmetric **G3** (Supporting Information, Figure S3) is more diagnostic and displays seven resonances in an 18:4:4:4:6:2:2 ratio; the resonance at 1.79 pm with an integral of six is caused by accidental overlap of two resonances (4H and 2H).

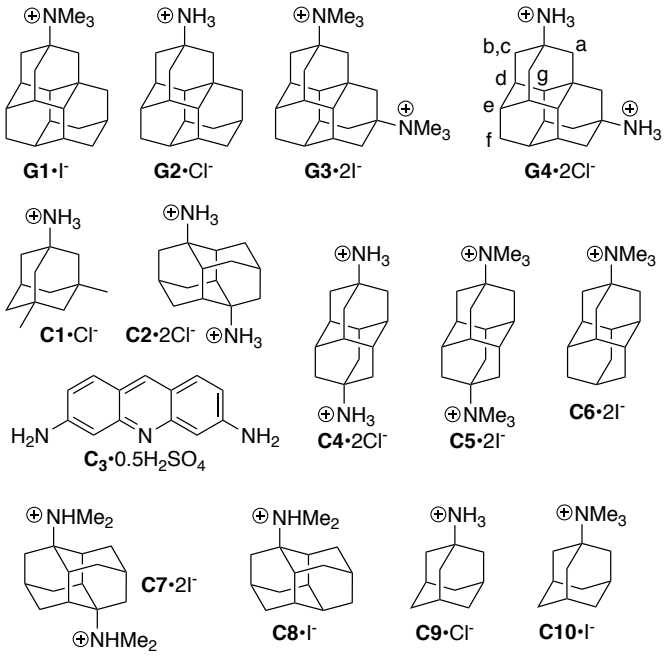


**Figure 3** Structures of cationic guests **G1** – **G4**, competitors **C1** – **C3**, and comparison compounds **C4 – C10** used in this study.

***Investigation of Host•Guest Complexation by $^1H$ NMR Spectroscopy.*** After having synthesized and fully characterized guests **G1** – **G4**, we decided to perform a qualitative investigation of the host•guest binding of CB[7] and CB[8] toward guests **G1** – **G4** by $^1H$ NMR spectroscopy (Supporting Information, Figure S5-S16). For example, Figure 4c shows the $^1H$ NMR spectra recorded for **G4** along with the assignments of the resonances. Because $H_b$ and $H_c$ are diastereotopic they appear as a pair of coupled doublets. The resonances for $H_a$, $H_b$, and $H_c$ appear downfield of the other resonances due to the electron withdrawing effect of the adjacent $NH_3^+$ group. The $^1H$ NMR spectra separately recorded for 1:1 mixtures of **G4** with CB[8] and CB[7] are shown in Figures 4b and 4d, respectively. As expected, all of the resonances for guest **G4** shift upfield upon formation of the CB[7]•**G4** and CB[8]•**G4** complex indicating that guest **G4** is bound within the magnetically shielding environment of the CB[n] cavity.[2, 1a] At 1:2 CB[n]:**G4** ratio (Figure 4a,e), we observe separate resonances for free **G4** and CB[n]•**G4** complex which establishes slow kinetics of guest exchange on the $^1H$ NMR timescale which is typical for ultratight CB[n] guest complexes.[5] The $^1H$ NMR spectrum for $D_{nh}$-symmetric CB[n] hosts show one set of diastereotopic resonances ($H_x$ and $H_y$) for the methylene bridges. In the CB[n]•**G4** complex we still observe one set of doublets for $H_x$ and $H_y$ which indicates that its time averaged geometry has a mirror plane passing through the equator of the complex. The magnitude of the complexation induced changes in chemical shift are presented in Figure 4b,d. Protons $H_a$, $H_d$, and $H_g$ undergo substantial upfield shifts ($\Delta\delta$ from –0.68 to –0.86 ppm) whereas $H_b$, $H_c$, and $H_e$ undergo smaller shifts ($\Delta\delta$ from –0.16 to –0.51 ppm) which reflects their position with respect to the magnetically shielding CB[n] cavity (*vide infra*). To the best of our knowledge, the inclusion of the 18 carbon triamantane skeleton inside the CB[7] cavity is the largest number of heavy (non-hydrogen) atoms incorporated to date. Recently, Biedermann et al. studied the binding of CB[7] toward 3,9-dihydroxytriamantane and 9,15-dihydroxytriamantane and concluded that "the experimental evidence ruled out the positioning of the guest in the hosts' cavity".[18] Accordingly, we conclude that the presence of the cationic groups on **G4** provide sufficient ion-dipole interactions to drive formation of the otherwise unfavorable inclusion of the triamantane framework inside CB[7]. Similar $^1H$ NMR measurements were performed for the CB[7]•**G1**, CB[8]•**G1**, CB[7]•**G2**, CB[8]•**G2**, and CB[8]•**G3** complexes which indicate inclusion of the triamantane skeleton in the CB[n] cavity (Supporting Information, Figures S5–S12). In contrast, the $^1H$ NMR spectra recorded for mixtures of CB[7] and **G3** show small upfield shifts for the $NMe_3^+$, $H_b$, and $H_c$ resonances (Supporting Information, Figures S9–S10) which suggests that CB[7]•**G3** forms an exclusion complex where only one $NMe_3^+$ group enters the CB[7] cavity and the other $NMe_3^+$ group is outside the cavity (Supporting Information, Figure S57). Such exclusion complexes are typically weak. In contrast, $^1H$ NMR results for CB[7]•**G1**, CB[8]•**G1**, CB[7]•**G2**, CB[8]•**G2**, CB[8]•**G3** (Supporting Information, Figures S5–S12) show that the resonances for the triamantane frameworks of **G1**, **G2**, and **G3** undergo complexation induced upfield changes in chemical shift which is indicative of cavity binding. In addition, separate $^1H$ NMR resonances for free guest and complexed guest are present at a 1:2 CB[n]•guest stoichiometry for CB[7]•**G1**, CB[8]•**G1**, CB[7]•**G2**, CB[8]•**G2**, CB[8]•**G3** which indicates that the kinetics of guest exchange are slow on the chemical shift timescale. For the $C_s$-symmetric guest **G1** we observe a slight downfield shift of the $NMe_3^+$ resonance which indicates that the $NMe_3^+$ group is located in the deshielding region just outside the C=O portals.[2, 1a] In addition, upon formation of the CB[7]•**G1**, CB[7]•**G2**, and CB[8]•**G2** complexes we observe two sets of resonances for the diastereotopic methylenes of CB[n] ($H_x$, $H_y$) which is due to the top-bottom C=O portal dissymmetry induced by the $C_s$-symmetric guests.

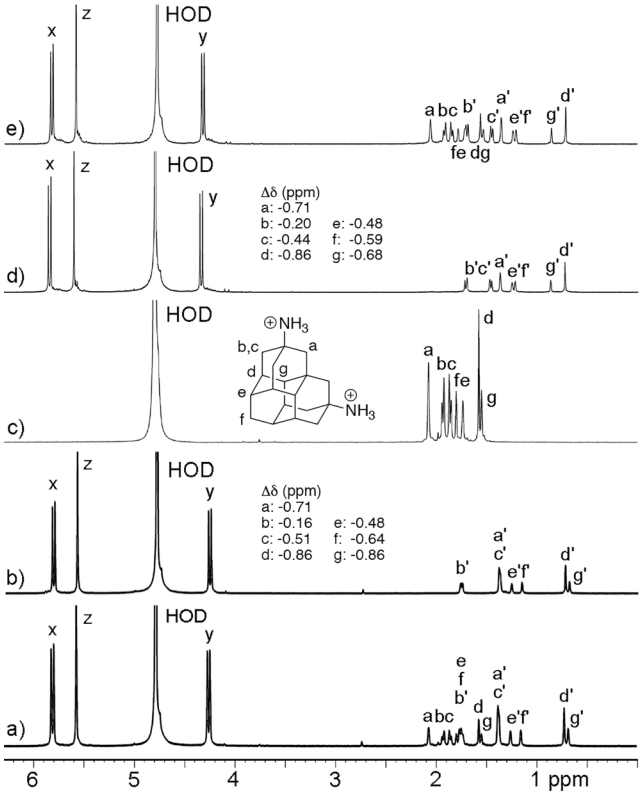


**Figure 4** $^1$H NMR spectra recorded (600 MHz, $D_2O$) for: a) a mixture of **G4** (2 mM) and CB[8] (1 mM), b) a mixture of **G4** (1 mM) and CB[8] (1 mM), c) **G4** (1 mM), d) a mixture of **G4** (1 mM) and CB[7] (1 mM), and e) a mixture of **G4** (2 mM) and CB[7] (1 mM). Resonances marked with primes (') arise from the host•**G4** complex.

***Molecular Modelling.*** To gain further insight into the geometry characteristics of the CB[n]•**G4** complexes we performed molecular modelling. The search for favorable complex geometries was done using the Conformer-Rotamer Ensemble Sampling Tool (CREST) based on the GFN methods[21] by applying the iterative meta-dynamic sampling for non-covalently bound complexes, clusters or aggregates (NCI-iMTD mode). The analytical linearized Poisson-Boltzmann (ALPB) solvation model was used to account for the implicit influence of water in the xTB computations. Figure 5 shows top and side of the found geometries of the CB[7]•**G4** and CB[8]•**G4** complexes. Minimized molecular models for the CB[7]•**G1** – CB[7]•**G3** and CB[8]•**G1** – CB[8]•**G3** complexes are shown in the Supporting Information (Figures S55–S57). In accord with the analysis of the complexation induced changes in chemical shifts described above, the molecular models show the encapsulation of the hydrophobic triamantane skeleton in the center of the CB[n] cavity. The average distances of cage H-atoms from the mean equatorial plane defined by the glycoluril methine C-atoms are as follows for CB[7]•**G4**: $H_a$, 1.26; $H_b$, 3.37; $H_c$, 2.55; $H_d$, 1.26; $H_e$, 2.13; $H_f$, 0.07; $H_g$ 0.19 Å and for CB[8]•**G4**: $H_a$, 1.20; $H_b$, 3.25; $H_c$, 2.41; $H_d$, 1.22; $H_e$, 2.05; $H_f$, 0.50; $H_g$, 0.61 Å. As shown in Figure 5b,d for CB[7]•**G4** and CB[8]•**G4**, $H_a$, $H_d$, $H_f$, $H_g$ which undergo substantial upfield shifts in the NMR spectrum reside closer to the equatorial plane running through the center of the CB[n] cavity. In contrast, the diastereotopic methylene resonance for $H_b$ – which shows the smallest upfield shift for both CB[7]•**G4** and CB[8]•**G4** – is the farthest from the equator. The average distance between the O-atoms on a single glycoluril ranges from 5.95 to 6.20 Å for CB[7]•**G4** and 5.77 to 5.95 Å for CB[8]•**G4** with averages of 6.05 and 5.87 Å, respectively. This is consistent with the expected buttressing effect of the sterically demanding **G3** guest against the C=O portals more significantly for CB[7] than CB[8]. Each $NH_3^+$ group in CB[7]•**G4** forms two H-bonds to the ureidyl C=O groups of CB[7] with the following NH•••O=C distances (2.00 Å; 1.90 Å), N•••O=C distances (2.89 Å; 2.76 Å) and NH•••O=C angles (157.9˚; 127.7˚). The guests' N-atoms reside slightly outside the cavity (0.68 Å) in CB[7]•**G4** as defined by the distance to the mean plane of the ureidyl O-atoms. The H-bonding metrics for CB[8]•**G4** are: NH•••O=C distances (1.77 and 1.80 Å; 1.98 and 2.02 Å), N•••O=C distances (2.80 and 2.83 Å; 2.87 and 2.91 Å) and NH•••O=C angles (168.3˚ and 168.4˚; 143.4˚ and 142.4˚). The guests' N-atoms reside slightly outside the cavity (0.48 Å; 0.76 Å) in CB[8]•**G4** as defined by the distance to the mean plane of the ureidyl O-atoms. The average distance for CB[7]•**G4** (CB[8]•**G4**) from the centroid of the equatorial methine C-atoms to those methine C-atoms averages 5.84 Å (6.58 Å) whereas the distance from the centroid of the ureidyl O-atoms back to the ureidyl O-atoms averages 4.22 Å (4.80 Å) which defines the width of the cavity and portals, respectively. Note that our modelling results also point towards preferential formation of the CB[7]•**G3** exclusion complex since the geometry where only one $NMe_3^+$ group is inside the host cavity (Supporting Information, Figure S25) is energetically much more favorable than the hypothetical structure where full inclusion is realized (Supporting Information, Table S1).

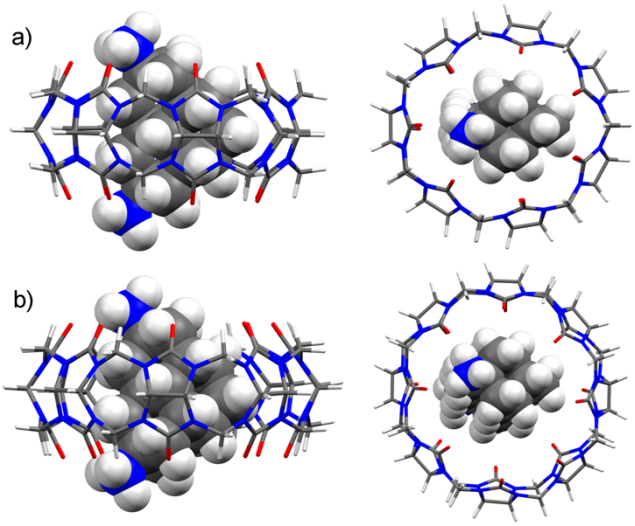


**Figure 5** Side and top views of the energy-minimized geometries of: a) CB[7]•**G4**, and b) CB[8]•**G4**. Color coding: C, gray; H, white; O, red; N, blue.

***Measurement and Discussion of the Thermodynamic Parameters of Complex Formation.*** The measurement of all binding constants in this paper were performed in 50 mM NaOAc buffered water at pH = 4.74 to allow comparison with binding constants for cationic adamantane and diamantane

derivatives measured previously.[5, 19a, 22, 13] Given the bulkiness of guests **G1** – **G4** which feature the C18 triamantane skeleton and the fast kinetics of guest exchange observed for CB[7]•**G3** we suspected the CB[7] complexes with these guests would be weak. Accordingly, we performed direct isothermal titration calorimetric titrations for CB[7]•**G1**, CB[7]•**G2**, and CB[7]•**G4** (Supporting Information, Figures S17–S19). Figure 6a shows the thermogram recorded when a solution of CB[7] (145 μM) in the cell was titrated with a solution of **G1** in the syringe. The direct titration data were processed and analyzed with the PEAQ ITC data analysis software. Figure 6b shows a plot of the integrated heat versus CB[7]:**G1** molar ratio fitted to a 1:1 binding model that was used to determine the $K_a$ = (1.6 ± 0.1) × $10^5$ $M^{-1}$ and $\Delta H$ = –10.4 ± 0.076 kcal $mol^{-1}$ values (Table 1). The $K_a$ and $\Delta H$ values for CB[7]•**G2** and CB[7]•**G4** were determined similarly and are presented in Table 1 along with data for selected comparison compounds **C4** – **C10** drawn from the literature.[5, 19a, 13] We performed $^1$H NMR competition experiments using the protocols described previously[5, 23, 19a, 19b, 13] to measure the $K_a$ value for CB[7]•**G3** ($K_a$ = (3.0 ± 0.5) × $10^5$ $M^{-1}$) using **C1** ($K_a$ = (2.5 ± 0.4) × $10^4$ $M^{-1}$) as a competitor of known affinity (Supporting Information, Figure S21).[5]

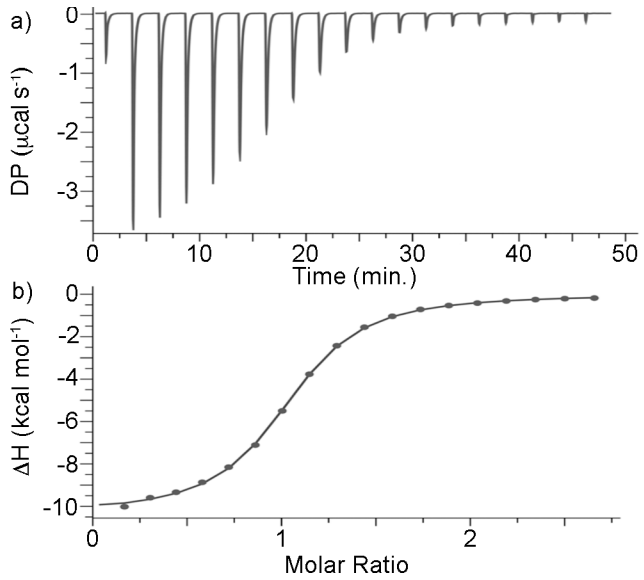


**Figure 6** a) ITC thermogram recorded during the titration of CB[7] (145 μM) in the cell with guest **G1** in the syringe, b) Fitting of the data to a 1:1 binding model with $K_a$ = (1.6 ± 0.1) × $10^5$ $M^{-1}$ and $\Delta H$ = –10.4 ± 0.076 kcal $mol^{-1}$.

**Table 1** Binding constants ($K_a$, $M^{-1}$) and binding enthalpies ($\Delta H$, kcal $mol^{-1}$) measured for the complexes between hosts CB[7] or CB[8] with guests **G1** – **G4** and **C1** – **C10**. Conditions: 50 mM NaOAc buffered $H_2O$ or $D_2O$, 298 K, pH 4.74).

| G | CB[7] | CB[8] |
|---|---|---|
| **G1** | (1.6 ± 0.1) × $10^5$ [a]<br>–10.4 ± 0.076 | (2.1 ± 0.1) × $10^{14}$ [c] |
| **G2** | (7.5 ± 0.2) × $10^4$ [a]<br>–4.98 ± 0.034 | n.d. [d] |
| **G3** | (3.0 ± 0.5) × $10^5$ [c] | (1.15 ± 0.17) × $10^{13}$ [f]<br>–10.1 ± 0.0 |
| **G4** | (6.73 ± 1.41) × $10^5$ [a]<br>–3.79 ± 0.10 | (1.1 ± 0.3) × $10^{14}$ [e]<br>(1.14 ± 0.21) × $10^{14}$ [f]<br>–11.5 ± 0.1 |
| **C1** | (2.5 ± 0.4) × $10^4$ [b] | (4.3 ± 1.1) × $10^{11}$ [b] |
| **C2** | 2030 [b] | (3.3 ± 0.8) × $10^{13}$ [b] |
| **C3** | – | (2.67 ± 0.32) × $10^7$ (1:1)<br>–9.23 ± 0.04<br>(7.47 ± 1.75) × $10^6$ (1:2)<br>–8.28 ± 0.06 |
| **C4** | (1.3 ± 0.3) × $10^{11}$ [b] | (8.3 ± 2.3) × $10^{11}$ [b] |
| **C5** | (1.9 ± 0.4) × $10^{15}$ [b] | (2.0 ± 0.6) × $10^{12}$ [b] |
| **C6** | (8.0 ± 1.9) × $10^{11}$ [b] | (2.7 ± 0.7) × $10^{12}$ [b] |
| **C7** | 686 [b] | (5.7 ± 1.5) × $10^{14}$ [b] |
| **C8** | 643 [b] | (7.8 ± 0.8) × $10^{13}$ [b] |
| **C9** | (4.2 ± 1.0) × $10^{12}$ [b] | (8.2 ± 1.8) × $10^8$ [b] |

[a]Measured by direct ITC titration. [b]Literature values.[5, 19a, 13] [c]Measured by $^1$H NMR competition experiments with **C1** as competitor. [d]CB[8]•**G2** complex is insoluble at room temperature. [e]Measured by $^1$H NMR competition experiments with **C2** as competitor. [f]Measured by ITC competition experiments using **C3** as competitor

We expected the binding constants for the cationic triamantanes toward CB[8] to far exceed the range that can be measured by direct titrations, so we elected to perform competitive titrations monitored by $^1$H NMR or ITC. The literature $K_a$ values for CB[8]•**C1** and CB[8]•**C2** are given in Table 1. Initially, we performed $^1$H NMR competition studies for CB[8]•**G1** using **C1** ($K_a$ = 4.3 × $10^{11}$ $M^{-1}$) as competitor. Experimentally, we prepared a solution of CB[8] (0.100 mM) and **C1** (16.5 mM) and then added **G1** (0.110 mM) and monitored the equilibration process by $^1$H NMR spectroscopy (Supporting Information, Figure S20). Specifically, we monitor the two separate $H_z$ resonances for CB[8]•**C1** and CB[8]•**G1** at ≈ 5.5 ppm until equilibrium is reached. Integration of the resonances by spectral deconvolution, followed by application of the equilibrium and mass balance equations as described previously[5, 23, 19a, 19b, 13] allowed calculation of $K_a$ = (2.12 ± 0.1) × $10^{14}$ $M^{-1}$ for CB[8]•**G1**. Separate experiments that approached equilibrium from the other direction (e.g., starting with CB[8]•**G1** and adding **C1**) gave identical results. The binding constant for CB[8]•**G4** (($K_a$ = (1.1 ± 0.3) × $10^{14}$ $M^{-1}$), Supporting Information, Figure S22) was similarly measured by competitive $^1$H NMR assays using **C2** as competitor. Unfortunately, we were not able to measure the binding constant for CB[8]•**G3** by $^1$H NMR competition assays because equilibration was extraordinarily slow and complicated by extraneous resonances due to unknown guest decomposition products.

Given the difficulties in measuring $K_a$ for CB[8]•**G3** by $^1$H NMR competition assays, we turned to ITC competition experiments.[24] Biedermann et al. have previously suggested a cationic cyclophane as a tight binding competitor for CB[8],[25] but because this compound was not commercially available we were unable to test this approach. To avoid problems of slow kinetics which plagued the $^1$H NMR competition assays, after much experimentation, we selected the tight binding

CB[8]•**C3**$_2$ ternary complex as the competitive complex.[26] Figure 7a depicts the equilibrium binding model governing this system. Initially, we performed a concatenated series of three direct ITC titrations of a solution of CB[8] (5 μM) in the cell with a solution of **C3** (40 μM) from the syringe (Figure 7b). Figure 7c shows a plot of the integrated heat data versus molar ratio fitted to the stepwise binding model shown (Figure 7a) using the Affinimeter™ software package to deliver the thermodynamic parameters for the formation of the CB[8]•**C3** and CB[8]•**C3**$_2$ complexes. Affinimeter™ was used because the PEAQ ITC data analysis software cannot implement the model shown in Figure 7a. Subsequently, we performed the competitive ITC titration of a solution of CB[8] (30 μM) and **C3** (175 μM) in the cell with a solution of **G3** (100 μM) from the syringe (Figure 7d). The DP versus time data were exported to Affinimeter™ then integrated to create the plot of $\Delta H$ versus molar ratio shown in Figure 6e. The solid line represents the best global fit of the data to the binding model given in Figure 7a to calculate the $K_a$ value for CB[8]•**G3** ($K_a$ = (1.15 ± 0.17) × $10^{13}$ $M^{-1}$). The complete Affinimeter™ reports are given in the Supporting Information (Figures S23-S54). Given that this strategy of using a tight CB[8]•**C3**$_2$ ternary complex as competitor is new and uses a new analysis package (Affinimeter™) we decided to further validate our results by performing related competitive titration of CB[8]•**C3**$_2$ with **G4** which was measured above by $^1$H NMR competition experiments. Gratifyingly, the $K_a$ value measured for CB[8]•**G4** by competitive ITC titration ($K_a$ = (1.1 ± 0.3) × $10^{14}$ $M^{-1}$) is the same as that measured by $^1$H NMR competition experiments ($K_a$ = (1.14 ± 0.21) × $10^{14}$ $M^{-1}$).

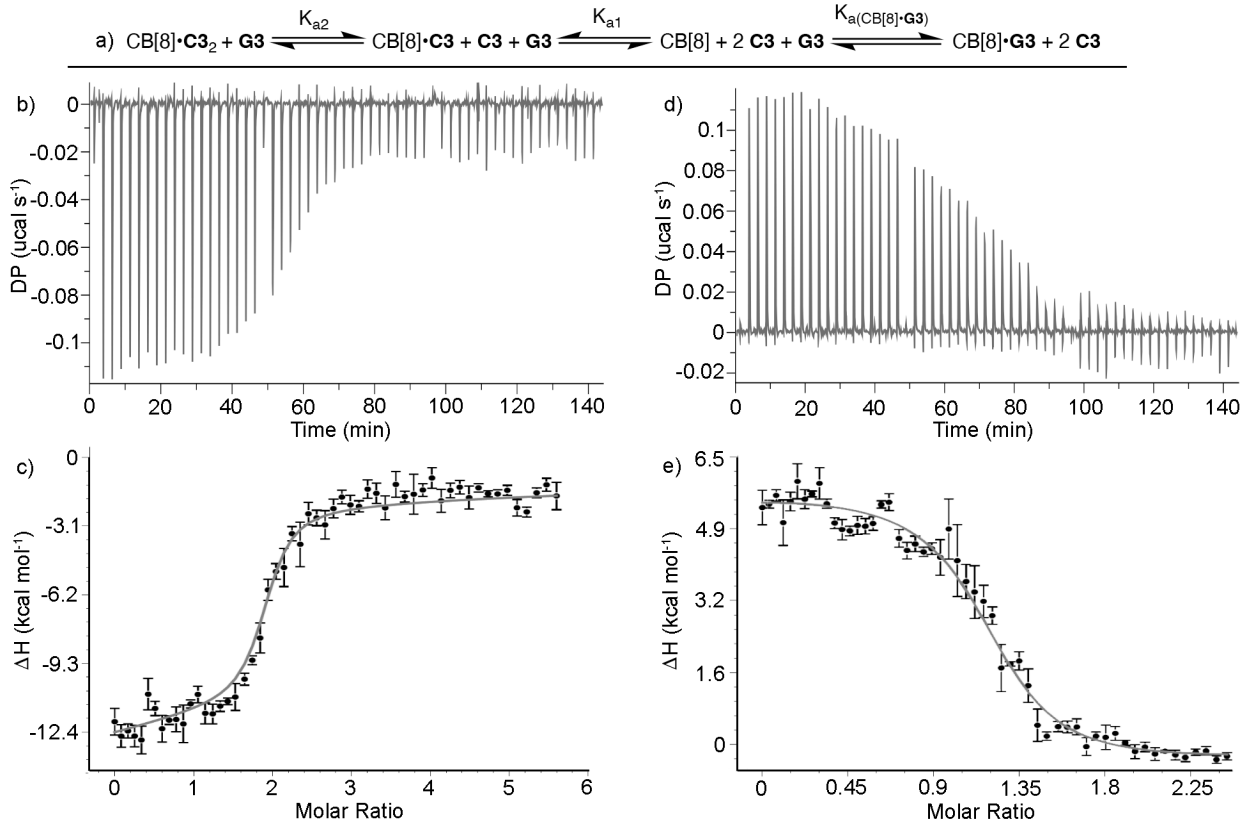


**Figure 7** a) Schematic representation of binding models implemented in Affinimeter™ to determine the $K_a$ values for formation of CB[8]•**C3** and CB[8]•**C3**$_2$ in the direct titration of CB[8] with **C3** and the competition binding model used to determine the $K_a$ for CB[8]•**G3** during the titration of a mixture of CB[8] and **C3** with **G3**. b) Thermogram from the direct titration of CB[8] (5 μM) with **C3** (40 μM) in the syringe. Three successive titrations were concatenated. c) Plot of $\Delta H$ versus molar ratio. The solid line represents the best fit of the data to the stepwise binding model performed using Affinimeter™. d) Thermogram from the competitive ITC titration of a solution of CB[8] (30 μM) and **C3** (175 μM) in the cell with a solution of **G3** (100 μM) in the syringe. e) Plot of $\Delta H$ versus molar ratio. The solid line represents the best fit of the data to the stepwise binding model performed using Affinimeter™.

With a complete dataset of CB[n]•**G** thermodynamic parameters in hand, some discussion of the trends in the data is warranted. The magnitude of the binding constants of **G1** – **G4** toward CB[7] (7.5 × $10^4$ to 6.7 × $10^5$ $M^{-1}$) are dramatically different than those toward CB[8] (1.15 × $10^{13}$ to 2.1 × $10^{14}$ $M^{-1}$). Related effects were seen previously in the binding constants of **C1**, **C7**, and **C8**) toward CB[7] and CB[8] which differ dramatically (**C1**: $10^7$, **C7**: $10^{12}$, **C8**: $10^{11}$).[5] We attribute this effect to the fact that triamantane skeleton (254 Å$^3$, PM3 calculation) is too voluminous to be comfortably encapsulated inside CB[7] (volumes: expanded, 272; inner, 242; truncated 158 Å$^3$)[9a] with packing coefficient over 100% of the inner cavity whereas triamantane can be easily encapsulated inside CB[8] (volumes: expanded, 479; inner, 367; truncated 263 Å$^3$) with a

 

packing coefficient of 69% which is in line with other tight binding CB[n]•diamondoid complexes.[18] For comparison, the calculated volume of diamantane is 206 Å$^3$ (PM3) which is known to display high affinity toward both CB[7] (packing coefficient 85%) and CB[8].[19a, 13, 18] The observation of the inclusion complexes for CB[7]•**G1**, CB[7]•**G2**, and CB[7]•**G4** demonstrates that the binding free energy of **G1**, **G2**, and **G4** are sufficiently large to pay the energetic cost to overstuff the cavity of CB[7]. This, coupled with the observation that CB[7]•**G3** forms an exclusion complex explains the overall modest binding affinities of CB[7] toward **G1 – G4**. In the CB[7] complexes there is little difference in binding affinity of the primary ammonium **G2** relative to the quaternary ammonium **G1**. Somewhat surprisingly, amongst the CB[8] complexes, the quaternary ammonium guest **G1** binds 18-fold stronger than the quaternary diammonium guest **G3** and two-fold more strongly than primary diammonium guest **G4**. Unfortunately, the strongest binding achieved among **G1** – **G4** toward CB[8] was for CB[8]•**G1** ($K_a$ = 2.21 × 10$^{14}$ M$^{-1}$) which is lower than the diamantane diammonium compounds (e.g., **C7**) measured previously.[13] Informative comparisons can also be made across homologous series of guests and comparators (e.g., adamantane vs. diamantane vs. triamantane) to tease out the effect of enlarging the hydrophobic framework. For example, the binding of mono trimethylammonium ions **C10**, **C6**, and **G1** toward CB[7] decrease in magnitude as the size of the hydrophobic skeleton increases due to the overstuffing of the CB[7] cavity as described above. Conversely, the binding constants of **C10**, **C6**, and **G1** toward CB[8] increase by three orders of magnitude as the hydrophobic skeleton is increased from 10 to 14 to 18 C-atoms, which reflects the enhanced hydrophobic effect associated with desolvation of the larger hydrophobic residue. In a similar way, CB[8] prefers to bind primary diammonium triamantane **G4** over diamantane **C4** by a factor of 137-fold which once again reflects the influence of the larger hydrophobic residue. Conversely, CB[7] prefers **C4** over **G4** by over five orders of magnitude because the packing coefficient of triamantane derivative **G4** is too high for CB[7]. Related trends are seen when comparing the binding constants for quaternary triamantane and diamantane diammonium ions **G3** and **C5** toward CB[n]. CB[7] prefers the smaller diamantane **C5** by nearly ten orders of magnitude whereas the larger CB[8] binds six-fold more strongly to the larger triamantane derivative **G3**.

## Conclusions

We report the preparation and characterization of cationic triamantane derivatives **G1** – **G4** which differ in overall charge (mono- and dication) and in the degree of nitrogen substitution (primary and quaternary). The binding behavior of **G1** – **G4** toward CB[7] and CB[8] was studied by a combination of $^1$H NMR spectroscopy, analysis of complexation induced changes in chemical shift, and molecular modelling. Remarkably, CB[7] forms inclusion complexes with triamantanes **G1**, **G2**, and **G4** which exhibit slow kinetics of exchange on the $^1$H NMR timescale. To the best of our knowledge, the encapsulation of 18 heavy (non-hydrogen) atoms inside CB[7] is the highest number observed to date. The binding constants of CB[7] and CB[8] toward triamantane guests **G1** – **G4** were determined by $^1$H NMR competition experiments, direct ITC titrations, and competitive ITC titrations as appropriate based on the magnitude of the binding constants and the kinetics of guest exchange. The use of an ultratight binding ternary complex (CB[8]•**C3**$_2$) with fast kinetics of guest exchange represents a new method to measure ultratight CB[8]•guest complexes. This new method capitalized on the ability of Affinimeter™ to implement this complex binding model and perform global fits of the binding data. Comparisons of the binding data of the homologous series of guests (e.g., adamantane to diamantane to triamantane) showed that the larger C-18 triamantane skeleton delivered enhanced binding affinity toward CB[8] whereas the smaller CB[7] cavity could not accommodate the triamantane framework without incurring substantial energetic penalties due to over-packing. Overall, this work extends our knowledge of the importance of the hydrophobic residue on the binding affinity of cationic diamondoids toward CB[n] and delivers a new competition ITC method via ultratight but fast exchanging ternary complex CB[8]•**C3**$_2$ to measure ultratight CB[8]•guest complex affinity.

## Experimental.

***General Experimental.*** $^1$H and $^{13}$C NMR spectra were recorded with Bruker AV-300, AV-400 or AV-600 NMR spectrometers and the NMR spectra were referenced to tetramethylsilane as an internal standard. The spectral reference for spectra measured in $D_2O$ was one drop of dioxane-$d_8$ added after recording the original spectrum. IR spectra were recorded with a FT-IR ABB Bomem MB 102 or FT IR-ATR PerkinElmer UATR Two spectrometers. MALDI-TOF MS spectra were obtained in reflectron mode with an Applied Biosystems Voyager DE STR instrument (Foster City, CA). GC-MS analyses were performed on an Agilent 7890B/5977B GC/MSD instrument equipped with a HP-5ms column. Melting points were obtained by using an Original Kofler Mikroheitztisch apparatus (Reichert, Wien). All solvents were obtained from commercial sources and used without further purification. Aminotriamantanes **G2** and **G4** were prepared according to the previously published procedures[20] and their permethylation afforded salts **G1**•I$^-$ and **G3**•2I$^-$, respectively.

***General procedure for the permethylation reactions.*** A mixture of the respective amine (1 equivalent), excess methyl iodide (15 equivalents) and $NaHCO_3$ (10 equivalents) in methanol (10 mL) was heated in a sealed tube for 48 h at 60 °C.[11] The mixture was cooled, the solvent evaporated, and the crude product washed with a suitable solvent mixture to afford the corresponding permethylated salt.

***N,N,N*-Trimethyltriamantane-9-aminium iodide (G1•I$^-$):** Permethylation of 9-aminotriamantane hydrochloride (**G2**•Cl$^-$) (146 mg, 0.5 mmol) afforded a solid that was washed with $CH_2Cl_2$ (20 mL). Evaporation of $CH_2Cl_2$ gave the crude product which was dissolved in a minimal amount of MeOH and then

an excess of $Et_2O$ (20 mL) was added. The solvent was decanted and the washing was repeated two more times, in the end yielding quaternary ammonium salt **G1**•$I^-$ as a white solid (100 mg, 47%). M.p. 296-297 ˚C. IR (KBr, $cm^{-1}$): 3473 (br), 3006 (w), 2905 (s), 2874 (s), 2854 (s), 1635 (w), 1480 (m), 1441 (m), 1418 (m), 1341 (w), 1233 (w), 1136 (w), 945 (w), 847 (m). $^1$H NMR ($CDCl_3$ + few drops of $CD_3OD$, 400 MHz): 1.47 (br. s, 2H), 1.52 (br. s, 2H), 1.64 (s, 2H), 1.67-1.83 (m, 10H), 1.90 (br. s, 1H), 1.99-2.05 (m, 2H), 2.06-2.14 (m, 4H), 3.04 (s, 9H, Me). $^{13}$C NMR ($CDCl_3$ + few drops of $CD_3OD$, 100 MHz): 26.3 (CH, 1C), 32.2 (CH, 1C), 33.5 (CH, 1C), 34.2 ($CH_2$, 2C), 34.8 (C, 1C), 35.8 ($CH_2$, 1C), 36.1 (CH, 2C), 36.5 ($CH_2$, 2C), 38.8 (CH, 2C), 40.9 ($CH_2$, 1C), 43.7 ($CH_2$, 1C), 43.8 (CH, 2C), 47.0 ($CH_3$, 3C, Me), 71.8 (C, 1C, C-N). HR-MS: calcd. for $[C_{21}H_{32}N]^+$ 298.2535; found 298.2536.

***N*,*N*,*N*,*N*',*N*',*N*'-Hexamethyltriamantane-9,15-diaminium diiodide (G3•2I$^-$):** Permethylation of 9,15-diaminotriamantane dihydrochloride (**G4**•$2Cl^-$) (137 mg, 0.40 mmol) afforded a solid that was washed with $CH_2Cl_2$ (20 mL). Evaporation of $CH_2Cl_2$ gave the crude product which was washed with a MeOH/$CH_2Cl_2$/ether (0.1:1.9:8 v:v:v ratio, 100 mL) mixture, yielding quaternary ammonium salt **G3**•$2I^-$ as a white solid (152 mg, 62%). M.p. >350 °C. IR (neat, $cm^{-1}$): 3421 (br), 3210 (m), 1621 (m), 1604 (s), 1045 (w), 560 (w). $^1$H NMR (600 MHz, $D_2O$): 1.55 (s, 2H), 1.73 (s, 2H), 1.79 (s, 6H), 2.02-2.07 (m, 4H), 2.10-2.16 (m, 4H), 2.21 (s, 4H), 3.03 (s, Me, 18H) ppm. $^{13}$C NMR (75 MHz, $CD_3OD$), δ: 33.5 (CH, 2C), 35.8 ($CH_2$, 4C), 36.2 ($CH_2$, 1C), 39.5 (C, 1C), 39.6 (CH, 4C), 42.0 ($CH_2$, 2C), 43.4 (CH, 2C), 49.4 ($CH_3$, Me, 6C), 73.3 (C-N, 2C) ppm. HR-MS: calcd. for $[C_{24}H_{40}N_2+Na]^+$ 379.3089; found 379.3100.

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## Conflicts of interest

The authors have no competing interests to declare.

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