VIP Affinity Pairs

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Cucurbit[7]uril·Guest Pair with an Attomolar Dissociation Constant**

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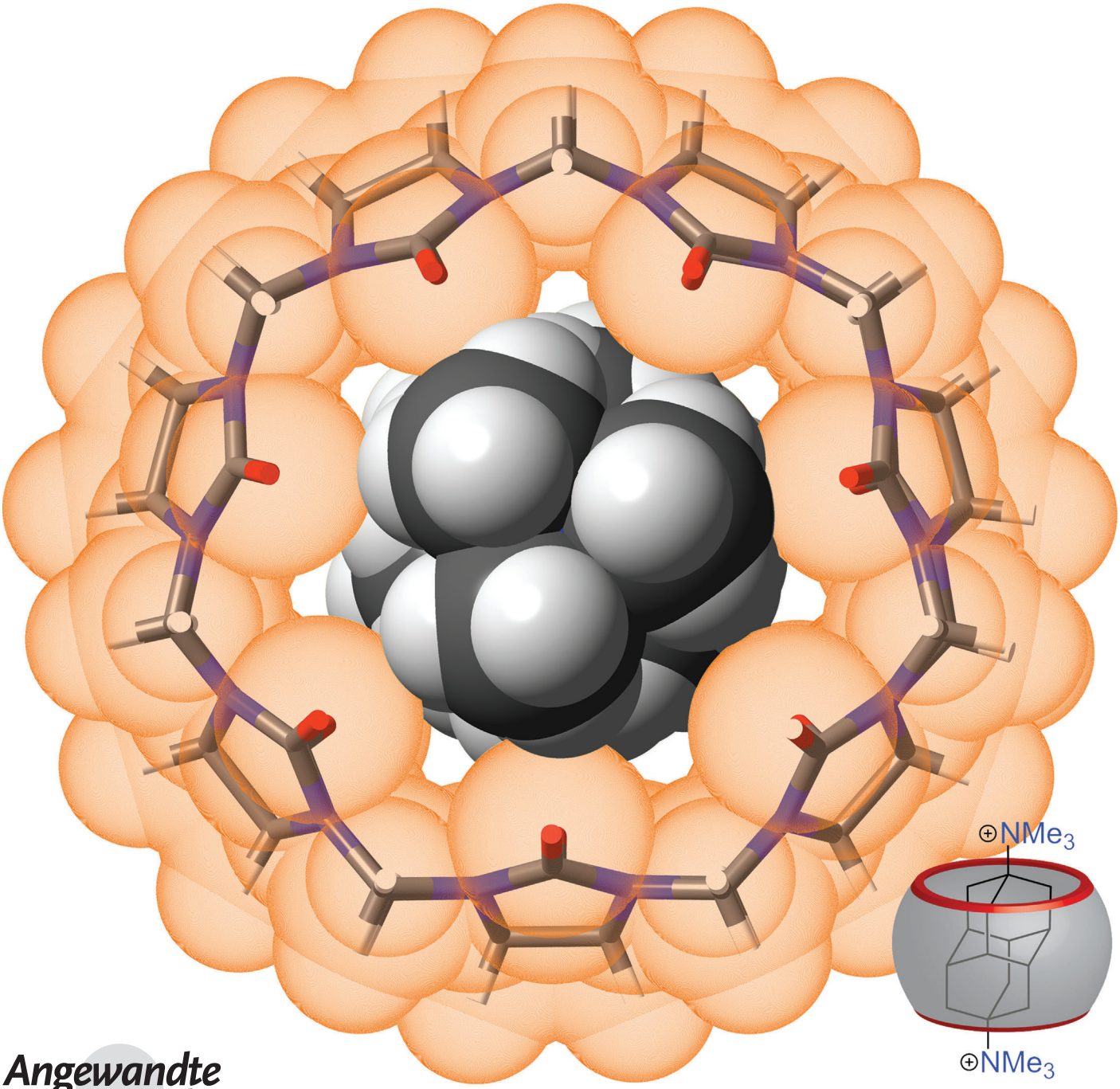

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Abstract: *Host·guest complexes between cucurbit[7] (CB[7]) or CB[8] and diamantane diammonium ion guests **3** or **6** were studied by ^{1}H NMR spectroscopy and X-ray crystallography. ^{1}H NMR competition experiments revealed that CB[7]·**6** is among the tightest monovalent non-covalent complexes ever reported in water with $K_a = 7.2 \times 10^{17}\,\text{M}^{-1}$ in pure D_2O and $1.9 \times 10^{15}\,\text{M}^{-1}$ in D_2O buffered with NaO_2CCD_3 (50 mM). The crystal structure of CB[7]·**6** allowed us to identify some of the structural features responsible for the ultratight binding, including the distance between the NMe_3^+ groups of **6** (7.78 Å), which allows it to establish 14 optimal ion-dipole interactions with CB[7], the complementarity of the convex van der Waals surface contours of **6** with the corresponding concave surfaces of CB[7], desolvation of the C=O portals within the CB[7]·**6** complex, and the co-linearity of the C_7 axis of CB[7] with the $N^+\cdots N^+$ line in **6**. This work further blurs the lines of distinction between natural and synthetic receptors.*

Pairs of receptors and ligands that exhibit extreme binding affinity are of tremendous value in both biotechnology and chemical applications. For example, the avidin·biotin affinity pair has been exploited in enzyme-linked immunosorbent assays, protein and nucleic acid detection and purification, and for immobilization chemistry on surfaces.[1,2] Aside from the technological applications, the development of new affinity pairs that demonstrate extreme affinity offers the opportunity to deepen our understanding of receptor–ligand interactions.[1,3] Over the past decade, the cucurbit[*n*]uril (CB[*n*], $n = 5, 6, 7, 8, 10$) family[4] of molecular containers (Figure 1) has emerged as a premiere platform for basic and applied studies of molecular recognition in water.[5] The CB[*n*] hosts feature two symmetry-equivalent ureidyl carbonyl portals, which are electrostatically negative and guard the entrance to a hydrophobic cavity. In pioneering work, Mock and Shih showed that guests which contain both cationic and hydrophobic regions bind with μM affinity and good selectivity toward CB[6] in acidic aqueous solution.[6] For example, CB[6] binds to spermine with $K_a = 1.3 \times 10^7\,\text{M}^{-1}$ by a combination of ion–dipole interactions and the hydrophobic effect.[6] In 2005, the Isaacs group discovered that CB[7] exhibits very high affinity toward adamantane and ferrocene derivatives, such as CB[7]·**1** ($K_a = 4.2 \times 10^{12}\,\text{M}^{-1}$) and CB[7]·**2** ($K_a = 3.31 \times 10^{11}\,\text{M}^{-1}$) in D_2O buffered with NaO_2CCD_3 (50 mM) at pH 4.74, by ^{1}H NMR competition experiments.[7,8] We immediately sought to reach even higher levels of CB[7]·guest affinity and prepared diamantane diammonium ion **3** in 2006, but only reported its binding toward bis(*ns*)CB[10].[9] In 2007, a multi-investigator team including the Isaacs group reported that the affinity of the CB[7]·**4** complex ($K_a = 3 \times 10^{15}\,\text{M}^{-1}$ in pure unbuffered H_2O; measured herein as $K_a = (1.9 \pm 0.4) \times 10^{13}\,\text{M}^{-1}$ in D_2O buffered with NaO_2CCD_3 (50 mM) at pH 4.74; see below) rivals that of avidin·biotin![10] The observed reduction in K_a for CB[7]·**4** in NaO_2CCD_3 (50 mM) is fully consistent with the known ability of Na^+ ions to bind at the C=O portals of CB[*n*], and thereby reduce the observed values of K_a by competition.[11] In the intervening years, some researchers have augmented known scaffolds,[12] explored new guest scaffolds (such as bicyclo-[2.2.2]octane),[13,14] whereas others have demonstrated the importance of the release of high-energy waters during the formation of CB[7]·guest complexes.[15] Despite this wide body of work, the tightest-binding CB[7]·guest complex known to date is the complex between CB[7] and adamantane derivative **5** (CB[7]·**5**: $K_a = 5 \times 10^{15}\,\text{M}^{-1}$ in pure water; measured herein as $K_a = 2.4 \times 10^{13}\,\text{M}^{-1}$ in D_2O buffered with NaO_2CCD_3 (50 mM) at pH 4.74; see below), which was reported in 2011.[14] This result was surprising to us because the two ammonium groups of the CB[7]·**5** complex reside at one ureidyl C=O portal, whereas the other C=O portal remains uncomplexed.[16] Accordingly, we decided to reconsider diamantane as a scaffold for the preparation of ultratight CB[7]·guest complexes where both C=O portals are complexed. In this paper, we report that CB[7] forms an ultratight complex with diamantane quaternary diammonium ion derivative **6** with $K_a = 7.2 \times 10^{17}\,\text{M}^{-1}$ in pure D_2O and $K_a = 1.9 \times 10^{15}\,\text{M}^{-1}$ in D_2O buffered with NaO_2CCD_3 (50 mM;

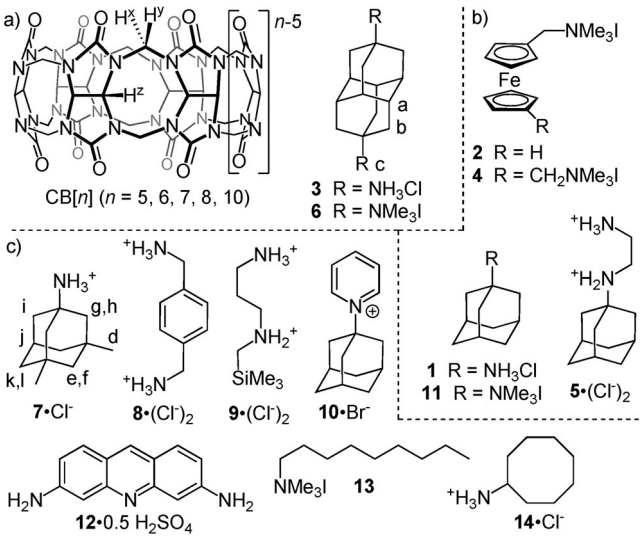

Figure 1. a) Chemical structures of CB[*n*] and diamantane guests (**3** and **6**), b) known tight binders for CB[7], and c) competitors used in this paper.

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pH 4.74), which are 143- (D_2O) and 79-fold (buffer) tighter than CB[7]·**5** under identical conditions!

Diamantane diammonium ion **6** was prepared by the alkylation of **3** with MeI in MeOH in 77% yield.[17] With our two target diamantanediammonium ions (**3** and **6**) in hand, we decided to investigate the formation of host guest complexes with CB[7] and CB[8]. Figure 2 shows the ^{1}H NMR spectra

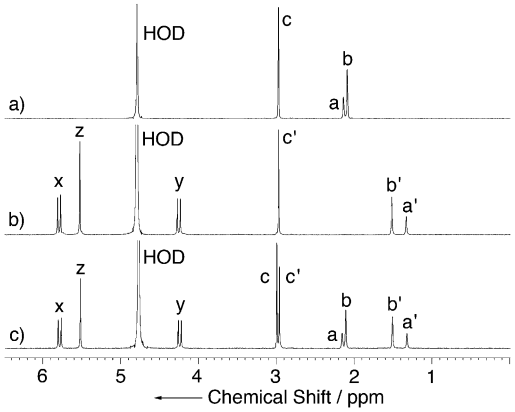

Figure 2. ^{1}H NMR spectra (400 MHz, D_2O, RT) recorded for: a) **6**, b) CB[7]·**6**, and c) a mixture of CB[7]·**6** and **6**.

recorded for guest **6** alone, as its CB[7]·**6** complex, and as a mixture of CB[7]·**6** with excess **6**. Figure 2b shows that H_a and H_b of the skeleton **6** undergo sizable upfield shifts upon complexation, which is consistent with their binding in the cavity of CB[7].[6] Figure 2c establishes that CB[7]·**6** undergoes slow exchange with free **6** on the NMR chemical shift timescale; integration of resonances for bound **6** versus CB[7] within CB[7]·**6** verify the 1:1 nature of the host·guest complex. The ^{1}H NMR spectra recorded for CB[8]·**6**, CB[7]·**3**, and CB[8]·**3** (see the Supporting Information) are completely analogous and establish a common mode of 1:1 cavity inclusion binding for all four CB[*n*]·guest complexes.

After having established the 1:1 nature of the CB[*n*]·guest complexes, we decided to measure their K_a values by means of ^{1}H NMR competition experiments using the method described by us previously.[7] In brief, we used competition experiments between a single host (CB[7] or CB[8]), a reference guest of known K_a (**7**–**10**) and a second guest of unknown K_a according to Equations (1) and (2). To determine K_{rel} we monitored these competition experiments by ^{1}H NMR spectroscopy, which takes advantage of the fact that many CB[*n*]·guest complexes display slow kinetics of exchange on the chemical shift timescale. This allows us to separately integrate peaks for the CB[*n*]·guest complex and unbound guest, which, when combined with the known total concentrations of CB[*n*], **G1**, and **G2**, and the mass balance

$$\begin{array}{ccc} \text{CB}[n]\cdot\mathbf{G1} & K_{rel} & \text{CB}[n]\cdot\mathbf{G2} \\ + & \rightleftharpoons & + \\ \mathbf{G2} & & \mathbf{G1} \end{array} \quad (1)$$

$$K_{rel} = \frac{[\text{CB}[n]\cdot\mathbf{G2}]\,[\mathbf{G1}]}{[\text{CB}[n]\cdot\mathbf{G1}]\,[\mathbf{G2}]} \quad (2)$$

expressions, allows us to determine the [CB[*n*]·**G2**], [CB[*n*]·**G1**], [**G1**], and [**G2**] values needed to calculate K_{rel} using Equation (2). To be concrete, we illustrate the process for the ^{1}H NMR competition experiment involving CB[7], guest **6**, and a large excess of competitor **10** of known K_a (Figure 3a).[7,18] After equilibrium has been established[19] we separately integrate the resonances for the 18 equivalent

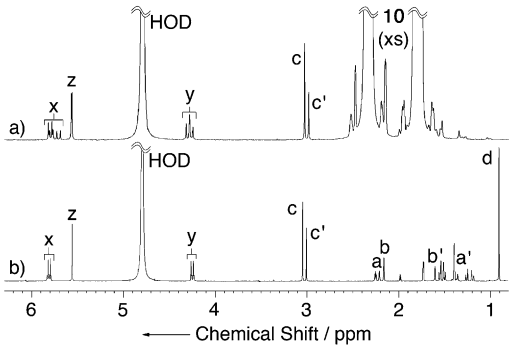

Figure 3. Portion of the ^{1}H NMR spectra (400 MHz, D_2O, RT) recorded during the competition experiments between: a) CB[7] (0.2539 mM), **6** (0.3638 mM), and **10** (209.8 mM), and b) CB[8] (0.2655 mM), **6** (0.3214 mM), and **7** (1.2156 mM).

$N(CH_3)_3$ protons of CB[7]·**6** and unbound **6** at ca. 3.0 ppm, which allows us to calculate [CB[7]·**6**] and [**6**] using the known total concentration of **6** and the mass balance expression. Subsequently, we use the known total concentrations of CB[7] and **10**, and the mass balance expressions to calculate [CB[7]·**10**] and [**10**], which allows a calculation of $K_{rel} = 958$ for CB[7]·**6** versus CB[7]·**10**. The Supporting Information shows that K_{rel} is equal to the ratio of the two individual binding constants ($K_{a,G2}/K_{a,G1}$). Accordingly, we multiply K_{rel} with the literature value of K_a for CB[7]·**10** ($K_a = (1.98 \pm 0.42) \times 10^{12}\,\text{M}^{-1}$)[7] to determine K_a for CB[7]·**6** as $K_a = (1.9 \pm 0.4) \times 10^{15}\,\text{M}^{-1}$ (Table 1) in D_2O buffered with NaO_2CCD_3 (50 mM) at pH 4.74.[20] Figure 3b shows the ^{1}H NMR competition experiment involving CB[8], **6**, and a slight excess of weaker-binding guest **7** used to calculate $K_a = (2.0 \pm 0.6) \times 10^{12}\,\text{M}^{-1}$ (Table 1). In an analogous way, we determined the K_a values given in Table 1 for CB[7]·**3** and CB[8]·**3** using competition experiments involving guest CB[7] or CB[8], **3** and competitive guests **7**–**9** of known K_a (see the Supporting Information). We also measured K_a values for the previous world record holders CB[7]·**4** and CB[7]·**5** in D_2O buffered with NaO_2CCD_3 (50 mM) for comparison purposes (Table 1).

Next, we sought to shed light on the reasons behind the record-breaking affinity of the CB[7]·**6** complex relative to CB[7]·**3**, and the previous champions CB[7]·**4** and CB[7]·**5**. Guest **3** forms a strong complex with CB[7] ($K_a = (1.3 \pm 0.3) \times 10^{11}\,\text{M}^{-1}$) and a slightly stronger complex with CB[8] ($K_a = (8.3 \pm 2.3) \times 10^{11}\,\text{M}^{-1}$). However, the complex between CB[7] and diamantane diammonium ion **3** is weaker than that measured for CB[7]·**1** ($K_a = (4.23 \pm 1.00) \times 10^{12}\,\text{M}^{-1}$), despite the potential for additional ion-dipole interactions and a larger hydrophobic core (such as diamantane vs. adamantane). Figure 4a,b shows the X-ray crystal structure for CB[8]·**3**.[21] The guest and the host occupy a special position

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Table 1: Binding constants (K_a, M^{-1}) measured for the host–guest complexes of guests **1**–**11** toward CB[7] and CB[8].[a]

Guest	CB[7]	CB[8]
1	$(4.23\pm1.00)\times10^{12}$ [b]	$(8.19\pm1.75)\times10^{8}$ [b]
2	$(3.31\pm0.62)\times10^{11}$ [b]	$(3.12\pm0.80)\times10^{9}$ [b]
3	$(1.3\pm0.3)\times10^{11}$ [c] $(1.4\pm0.3)\times10^{11}$ [d]	$(8.3\pm2.3)\times10^{11}$ [i]
4	$(1.9\pm0.4)\times10^{13}$ [c] $(3\pm1)\times10^{15}$ (H_2O) [g]	–
5	$(2.4\pm0.6)\times10^{13}$ [c] 5×10^{15} (H_2O) [h]	–
6	$(1.9\pm0.4)\times10^{15}$ [e] $(2.1\pm0.6)\times10^{15}$ [f] 7.2×10^{17} (D_2O) [e]	$(2.0\pm0.6)\times10^{12}$ [i]
7	$(2.50\pm0.39)\times10^{4}$ [b]	$(4.33\pm1.11)\times10^{11}$ [b]
8	$(1.84\pm0.34)\times10^{9}$ [b]	–
9	$(8.7\pm1.8)\times10^{9}$ [d]	–
10	$(1.98\pm0.42)\times10^{12}$ [b]	$(2.00\pm0.512)\times10^{9}$ [b]
11	$(1.71\pm0.40)\times10^{12}$ [b]	$(9.70\pm2.48)\times10^{10}$ [b]

[a] Conditions: NaO_2CCD_3 (50 mM) buffer, D_2O, pH = 4.74, RT; entries marked (H_2O) and (D_2O) were measured in unbuffered water. [b] Reference [7]; competition experiment used a limiting quantity of CB[7] and: [c] **9**, [d] **8**, [e] **10**, [f] **5**. [g] Reference [10]. [h] Reference [14]. [i] Competition experiment used a limiting quantity of CB[8] and **7**. – = not determined.

of inversion symmetry, which makes the two portals enantiotopic. As the 7.628 Å $N^+\cdots N^+$ distance in CB[8]·**3** is longer than the 6.06(2) Å C=O···O=C mean separation within a single glycoluril of CB[8], co-linear alignment of the $N^+\cdots N^+$ line of guest **3** with the pseudosymmetrical C_8 axis of CB[8] would place each N^+ atom 0.78 Å above the center of each portal. To form one $N^+\cdots$O=C H-bond (2.78 Å) at each portal, the $N^+\cdots N^+$ line of guest **3** must tilt towards the C=O rim. This tilt causes each N^+ atom to reside 0.16 Å inside the cavity below the least-squares plane of the eight C=O O atoms. The tilting of guest **3** within the CB[8]·**3** complex results in an energetically costly ellipsoidal deformation (long axis: 13.53 Å; short axis: 12.58 Å, measured at the equatorial methine C atoms). An MMFF minimized molecular model of the CB[7]·**3** complex (see the Supporting Information) shows similar structural features and deformations.

We were fortunate to obtain single crystals of CB[7]·**6** and to solve its X-ray crystal structure (Figure 4c and d). The crystalline CB[7]·**6** complex occupies a general position of symmetry in the unit cell, which means that it is asymmetric, although the diastereotopic top and bottom portals exhibit very similar geometries. Within the CB[7]·**6** complex, the CB[7] macrocycle assumes a nearly circular equatorial cross section (equatorial diameter: 11.70(5) Å), which is similar to that of uncomplexed CB[7][22] (equatorial diameter: 11.6(2)–11.7(9) Å). However, the C=O portal diameter of CB[7]·**6** (portal diameter: 8.62(3) Å) is larger and with higher precision than that of uncomplexed CB[7][22] (portal diameter: 8.4(4)–8.6(3) Å) which probably reflects the constraints of the $^+N(CH_3)_3\cdots$O=C close contacts at the relatively flexible portal O atoms (see below). Figure 4e shows that the convex van der Waals (vdW) surfaces of diamantane **6** nicely complement the concave vdW surfaces of the cavity of CB[7]. The pseudosymmetrical C_7 axis of CB[7] and the

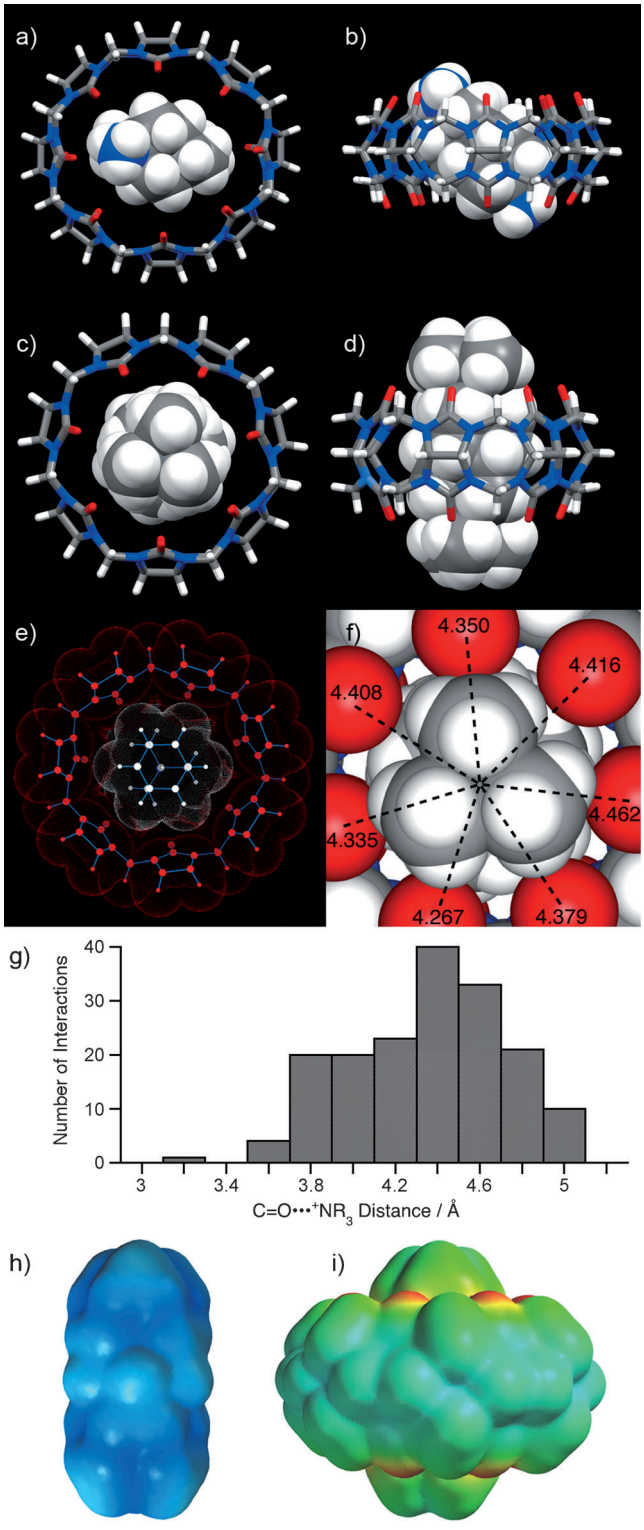

Figure 4. Representations of the X-ray crystal structures of: a) top view of CB[8]·**3**, b) side view of CB[8]·**3**, c) top view of CB[7]·**6**, d) side view of CB[7]·**6**. C gray, H white, N blue, O red. e) Van der Waals surfaces of CB[7] (red) and **6** (white) from the crystal structure of CB[7]·**6** sliced along the equator of CB[7]. f) $R_3N^+\cdots$O=C ion–dipole interaction distances in CB[7]·**6**. g) Histogram showing the distribution of intermolecular $R_3N^+\cdots$O=C distances from 89 structures retrieved from the Cambridge Structural Database. Side views of the electrostatic potential surfaces calculated using the MMFF minimized geometry of **6** and the X-ray crystal structure geometry of CB[7]·**6** by single point PM3 calculations for: h) **6** and i) CB[7]·**6**. The red to blue color range spans from +80 to +650 kJ mol^{-1}.

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$N^+\cdots N^+$ line of **6** are approximately co-linear (0.08(3) Å mean separation). As the $N^+\cdots N^+$ distance (7.783 Å) in CB[7]·**6** is longer than the C=O···O=C mean separation (6.19(3) Å) within a single glycoluril, the concentric alignment of the $N^+\cdots N^+$ line of **6** within the cavity of CB[7] places the N^+ atoms of the NMe_3^+ groups of **6** 0.80 Å above the center of each portal (Figure 4d). In this position, each NMe_3^+ group engages in seven $N^+\cdots O$=C ion–dipole interactions with each ureidyl C=O portal of CB[7] with distances that cluster around an average of 4.38(7) Å (Figure 4f). An analysis of 89 structures containing an acetylcholine-type $R_3N^+CCO(C=O)R$ unit from the Cambridge Structural Database revealed that the maximum of a histogram of the intermolecular $Me_3N^+\cdots O$=C distances was also ca. 4.4 Å (Figure 4g; see also the Supporting Information). This result, when coupled with the observed extreme affinity, suggests that the location of the $^+NMe_3$ groups at ca. 0.80 Å above the planes of the C=O portals and equidistant from the carbonyls represents an optimum geometry. Figure 4h,i shows side views of the electrostatic potential energy mapped onto the van der Waals surface of **6** and the CB[7]·**6** complex. Obviously, the formation of the CB[7]·**6** complex results in substantial reduction in the electrostatic potential at the N atoms and the adjacent CH_3 groups as a consequence of the $Me_3N^+\cdots O$=C ion-dipole interactions.

Although the previous section focused on direct interactions between CB[7] and **6**, the importance of both the high-energy H_2O molecules within the cavity of CB[7][15] and those solvating **6** that are released upon complex formation should not be neglected. Because the binding of compounds **4**, **5**, or **6** to CB[7] will release all the high energy H_2O molecules bound within CB[7] upon complex formation, we do not believe that these high-energy waters are responsible for the higher K_a value observed for CB[7]·**6** relative to CB[7]·**4** or CB[7]·**5**. In contrast, we suggest that the larger and more hydrophobic diamantane core (14 C atoms) of **6** relative to the adamantane core of **5** (10 C atoms) results in the release of additional solvating H_2O molecules upon complexation, which should provide an additional entropic driving force.

The measurements performed above in D_2O buffered with NaO_2CCD_3 (50 mM) at pH 4.74 establish that the affinity of the CB[7]·**6** ($K_a = (1.9 \pm 0.4) \times 10^{15}\,M^{-1}$) is 79-fold higher than that of CB[7]·**5** ($K_a = (2.4 \pm 0.6) \times 10^{13}\,M^{-1}$) under identical conditions. Previously, however, the affinity of the CB[7]·**5** complex was measured as $K_a = 5 \times 10^{15}\,M^{-1}$ in pure unbuffered water.[14] Because of the possibility of pH or buffer effects, we decided to allow **6** to compete against **5** for CB[7] in pure unbuffered D_2O to verify its superiority under identical conditions. Accordingly, we performed two complementary experiments using CB[7] (0.2615 mM), **6** (0.4340 mM), and a large excess of **5** (29.1470 mM) to ensure that equilibrium was reached: 1) CB[7] and **6** are mixed first to form CB[7]·**6** followed by addition of **5**, and 2) CB[7] and **5** are mixed first to form CB[7]·**5** followed by addition of **6**. Figure 5a shows a plot of the percent of CB[7]·**6** as a function of time at 70 °C. Both complementary experiments reach the same percentage of CB[7]·**6** and CB[7]·**5** after 22 h, which means that equilibrium has been achieved. We calculated $K_{rel} = 91.4$ for the competition between **6** and **5** for CB[7],

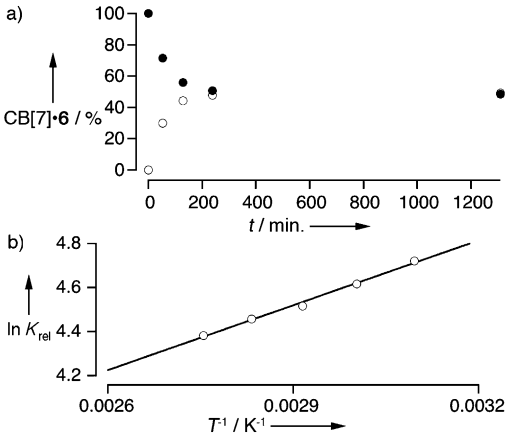

Figure 5. a) Plot of the percentage of CB[7]·**6** as a function of time at 70 °C in D_2O. ● = **5** added to a solution of CB[7]·**6**; ○ = **6** added to a solution of CB[7]·**5** plus excess **5**. b) Plot of $\ln(K_{rel})$ for the competition of **5** and **6** for CB[7] as a function of equilibration temperature.

which means that **6** is a 91.4-fold better guest for CB[7] in pure D_2O at 70 °C. These two complementary experiments were also performed at 90, 80, 60, and 50 °C to measure K_{rel} as a function of temperature. K_{rel} is only weakly dependent on temperature ($K_{rel,323} = 112$; $K_{rel,363} = 80$). We did not extend these experiments to room temperature because of very slow equilibration. Figure 5b shows a plot of $\ln K_{rel}$ versus $1/T$ for the competition between **6** and **5** for CB[7]. This van't Hoff type plot allows a determination of the difference in ΔH and ΔS between CB[7]·**6** and CB[7]·**5** ($\Delta\Delta H = -1.94$ kcal mol^{-1}; $\Delta\Delta S = 3.24$ cal mol^{-1} K^{-1}). Using standard analysis, we find that $-T\Delta\Delta S$ contributes -1.11 kcal mol^{-1} to the overall $\Delta\Delta G$ (-3.07 kcal mol^{-1}) at 70 °C. Complex CB[7]·**6** is both enthalpically and entropically more favorable than CB[7]·**5**. If we extrapolate the van't Hoff type plot shown in Figure 5b to 298 K, we obtain $K_{rel} = 143$, which, when combined with the previously published K_a value for CB[7]·**5** ($K_a = 5 \times 10^{15}\,M^{-1}$),[14] allows us to calculate the K_a for CB[7]·**6** at 25 °C in D_2O as $K_a = 7.2 \times 10^{17}\,M^{-1}$ ($K_d = 1.4 \times 10^{-18}$ M).

Because the K_a value determined above for CB[7]·**6** is so extreme and relies on the K_a values for CB[7]·**4** and CB[7]·**5** determined by a different technique (isothermal titration calorimetry) in another laboratory, we decided to determine the K_a for CB[7]·**6** by an independent series of competitions. For this purpose, we first measured the K_a for CB[7]·**12** as $(1.12 \pm 0.17) \times 10^7\,M^{-1}$ in water by direct fluorescence titration (see the Supporting Information).[23] Next, we performed ^{1}H NMR competition experiments (see the Supporting Information) with a series of guests of gradually increasing K_a (Table 2), which allowed us to determine K_a for CB[7]·**6** in D_2O as $(2.2 \pm 0.7) \times 10^{17}\,M^{-1}$ and $K_d = 4.5 \times 10^{-18}$ M! The agreement between the K_a values measured for CB[7]·**6** ($7.2 \times 10^{17}\,M^{-1}$ and $(2.2 \pm 0.7) \times 10^{17}\,M^{-1}$) by the two sets of competition experiments is reasonable given the multistep nature of the competition experiments and the possible influence of pH and solvent isotope effects.[24]

In summary, we have shown that the CB[7]·**6** is the strongest CB[7]·guest complex known by 79-fold with $K_a = (1.9 \pm 0.4) \times 10^{15}\,M^{-1}$ in D_2O buffered with NaO_2CCD_3

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Table 2: Binding constants (K_a, M^{-1}) measured for CB[7]·guest complexes for guests **12**, **13**, **8**, **14**, **10**, **5**, **6**.[a]

CB[7]·guest	Competitor	K_a
CB[7]·**12**	none	$(1.12\pm0.17)\times10^{7}$
CB[7]·**13**	**12**	$(4.6\pm0.8)\times10^{7}$
CB[7]·**8**	**13**	$(1.2\pm0.2)\times10^{10}$
CB[7]·**14**	**8**	$(3.2\pm0.7)\times10^{11}$
CB[7]·**10**	**14**	$(1.5\pm0.4)\times10^{14}$
CB[7]·**5**	**10**	$(1.5\pm0.4)\times10^{15}$
CB[7]·**6**	**5**	$(2.2\pm0.7)\times10^{17}$

[a] Conditions: D_2O, RT.

(50 mM) at pH 4.74 and by 143-fold with $K_a = 7.2\times10^{17}\,M^{-1}$ in D_2O. The X-ray crystal structure of CB[7]·**6** helps us to identify the reasons behind the extreme affinity including the complementarity of the van der Waals surface contours of the diamantane core of **6** and the cavity of CB[7], the spacing between the two Me_3N^+ groups of **6** (7.78 Å) allows it to establish 14 optimal ion–dipole interactions with CB[7], the desolvation of the ureidyl carbonyl portals within the CB[7]·**6** complex, the co-linearity of the C_7-axis of CB[7] with the $N^+\cdots N^+$ line in **6**, and the hydrophobic nature of the diamantane core relative to the adamantane and ferrocene cores of **4** and **5**. Within CB[*n*]·guest chemistry, the work establishes a set of principles that may be used to design high affinity pairs with other CB[*n*] homologues. The CB[7]·**6** complex provides a potent example of the principle of preorganization[25] in (bio)molecular recognition. Finally, the work blurs the line between designed synthetic and natural biomolecular receptors and presages the development of synthetic systems whose functions meet and exceed their biological counterparts.

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[16] Equally surprising was the fact that guest **5** was not a quaternary ammonium ion, which was previously posited to provide a potent entropic driving force for formation of CB[7]·**4** owing to desolvation of the ureidyl C=O portals within the complex.
[17] M. Šekutor, K. Molčanov, L. Cao, L. Isaacs, R. Glaser, K. Mlinarić-Majerski, unpublished results.
[18] In these competition experiments, CB[7] is the limiting reagent, tighter binding guest **6** is used in slight excess, and the concentration of weaker guest **10** is adjusted until comparable amounts of the two competing complexes CB[7]·**6** and CB[7]·**10** are present at equilibrium.
[19] To ensure we had reached equilibrium, we performed two complementary experiments: 1) CB[7] and **6** were mixed first to form CB[7]·**6** and then **10** was added, and 2) CB[7] and **10** were mixed first to form CB[7]·**10** and then **6** was added. The solutions were separately monitored as a function of time (several weeks, in some cases) until equilibrium was established.
[20] The details of the propagation of error calculations are given in the Supporting Information.
[21] CCDC 969768 (CB[8]·**3**) and CCDC 969769 (CB[7]·**6**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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