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Supramolecular Complexation of *N*-Alkyl- and *N*,*N*′-Dialkylpiperazines with Cucurbit[6]uril in Aqueous Solution and in the Solid State

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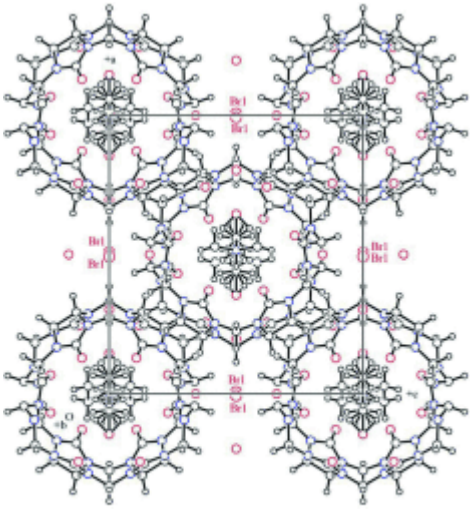
Abstract

Water seeds: Complex stoichiometry/composition and degree of oligomerization (oligomeric supramolecular complex formation) of cucurbit[6]uril (CB[6]) with *N*-alkyl- and *N*,*N*′-dialkylpiperazine were investigated in aqueous solutions by means of isothermal titration calorimetry (ITC), ESI-MS, NMR and light scattering measurements.



CB[6] –1-propylpiperazine

Complex stoichiometry/composition and degree of oligomerization (oligomeric supramolecular complex formation) of cucurbit[6]uril (CB[6]) with *N*-alkyl- and *N*,*N*′-dialkylpiperazine were

investigated in aqueous solutions by means of isothermal titration calorimetry (ITC), ESI-MS, NMR and light scattering measurements. It was found that the complex stability and the degree of oligomerization increase with elongating the alkyl chain attached to the piperazine core. X-ray crystallographic studies revealed a clear correlation between the structure of CB[6]–alkylpiperazine crystals obtained from aqueous solutions and the molecular weight/properties of host–guest oligomers existed in the solution as supramolecular “seeds” of crystal formation.

Introduction

Cucurbit[6]uril (CB[6]), the oldest and most popular member of the cucurbit[*n*]uril (*n*=5–10) family,[1–4] consists of six glycoluril units to form a relatively slim hydrophobic cavity with two identical carbonyl-decorated portals (Figure 1). CB[6] is practically insoluble in water or any other conventional organic solvents, but, as it was found about a decade ago,[5, 6a] becomes highly soluble in aqueous solutions containing inorganic salts, such as NaCl, through coordination of the cationic species to the portal carbonyls to form a 1:2 complex, for example, $[CB[6]\cdot 2\,Na]^{2+}$. Since this discovery,[5, 6a] a wide variety of supramolecular architectures and devices based on CB[6] as well as the other members of the CB family have been designed and synthesized,[3, 7] as can described in a recent review.[4]

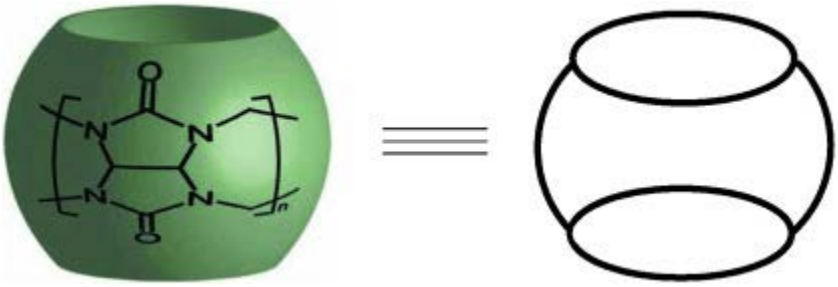


Figure 1. Chemical structure of the cucurbit[*n*]uril (*n*=6) macrocycle.

As it possesses electronegative portals and a hydrophobic cavity, CB[6] strongly binds aliphatic mono-, di-, and polyammonium guests of appropriate alkyl chain lengths through the electrostatic and van der Waals interactions.[3, 4, 8] Recently,[9] we have shown that tetracationic spermine, $NH_3^+(CH_2)_3NH_2^+(CH_2)_4NH_2^+(CH_2)_3NH_3^+$, forms an extraordinarily stable complex with CB[6] in 0.2 M LiCl with a binding constant of $K=5.4\times10^{10}$ M^{-1}, which is the highest affinity ever reported for a CB[6] complex. In this complex, the central tetramethylene moiety of spermine threads through the CB[6] cavity, while the two dicationic side arms coordinate to the both portals. Larger cavities in CB[7]–CB[10] can accommodate more sizable guests[10] and some of these complexes have exhibited extraordinarily high stabilities of up to $K=10^{12}$ M^{-1}[11] or even $K=10^{15}$ M^{-1}.[12]

Similar to tetracationic spermine, dicationic 1,4-butanediammonium, a model of the spermine's "core" part, also forms a very strong complex with CB[6] at K=2.0×10^{7} M^{-1} in 0.05 M NaCl.[9] In sharp contrast, dicationic 1,3-propanediammonium, a side-arm mimic of spermine, forms an extremely weak complex with CB[6], exhibiting a 60 000-fold smaller binding constant than that of 1,4-butanediammonium guest under the same conditions. This is the largest per-methylene increment in K ever reported for a supramolecular complex, and may be attributed to a switching of the complexation mode from cavity-binding to rim-binding due to the critical change in inter-ammonium distance between the two guests, which hinders the smooth threading through the CB cavity and the simultaneous coordination of the 1,3-propanediammonium cations to the opposite portals.[9]

Six-membered alicyclic diammonium guests derived from piperazine and 1,2-diaminocyclohexane, which have an inter-ammonium distance even shorter than that of 1,3-propanediammonium, are obviously incapable of forming threading inclusion complexes, but instead yield 1:2 complexes with CB[6]. This is made possible by binding the dicationic guest to each portal of CB[6], as is the case with 1:2 CB[6]–metal ion complex such as [CB[6]·2 Na]$^{2+}$.[8] However, different from metal ions, organic piperazine or 1,2-diaminocyclohexane dications can be readily modified by introducing substituent(s) to the nitrogen/carbon atom(s). Utilizing this advantage, we can prepare a highly symmetrical ditopic guest molecule which is capable of interacting with two CB[6] macrocycles to give a mixture of supramolecular oligomers containing multiple CB[6] units in aqueous solutions.[8]Comparison of X-ray crystallographic structures of CB[6] complexes of structurally related guests would provide a basis for rationally designing supramolecular crystals.

CB[6] crystals obtained from an aqueous salt solution are known to bind a metal ion between two CBs through coordination of selected carbonyl oxygens of two facing CB portals.[6] We may anticipate a similar architecture in CB[6] crystals obtained from an aqueous solution of piperazine derivatives, where one guest is shared by two CB[6]s. These CB[6] salt crystals exhibit wide structural diversity depending on the size and charge of inorganic cations.[6] However, the effects on the binding behavior by systematic modification of dicationic organic guests, such as piperazine, have never been examined so far. In contrast to a solution of inorganic salt and CB[6], where only a monomeric 1:2 complex exists as a thermodynamically stable species, several oligomers are expected to coexist in an aqueous solution of *N*-alkylpiperazine and CB[6]. It is likely that, in such oligomers, one piperazine guest is shared by two CB[6]s thorough the electrostatic interaction with the ammonium ion and the van der Waals contacts with the alkyl chain, and the composition and size of these oligomers determine the resulting crystal structure acting as seeds.

In this combined ITC, X-ray, NMR, ESI-MS, and light scattering study, we performed the systematic investigations of molecular events occurring in aqueous solutions containing CB[6] and protonated *N*-

alkyl- and *N*,*N*′-dialkylpiperazine and also compared the crystal structures of CB[6] complexes with these dicationic guests.

Results and Discussion

Molecular model examinations of a 1:2 complex of CB[6] with *N*-ethylpiperazine reveal that two ethyl units inserted into CB[6] cavity from opposite portals would probably be simultaneously accommodated (although in a close contact with each other) inside the cavity. However, upon simultaneous insertion of two longer alkyl chains, such as propyl, and butyl, the severe clashes between terminal methyls of the two alkyl chains penetrating from the opposite portals may most likely disturb stable complex formation. Consequently, we first examined the complexation behavior of *N*-ethyl-, *N*-propyl-, and *N*-butylpiperazine with CB[6] by using the ITC technique. We also subjected *N*,*N*′-diethylpiperazine to the ITC experiment to evaluate the effect of a second *N*-alkyl group introduced to a piperazine guest on complexation thermodynamics. For a comparison, we used unsubstituted piperazine as a guest, although a very low affinity was anticipated. Since the solubility of CB[6] in pure water is very limited, all ITC experiments with CB[6] were performed in the presence of 0.05 M NaCl.

Complexation thermodynamics of CB[6] with dicationic *N*-alkylpiperazines: As shown in Figure 2, the ITC thermograms obtained for *N*-ethylpiperazine and *N*,*N*′-diethylpiperazine clearly indicate the stepwise formation of 1:1 and then 1:2 host–guest complexes upon gradual addition of the guest into an aqueous 0.05 MNaCl solution of CB[6]. However, unsubstituted piperazine produced only a small amount of heat upon addition to CB[6] solution under the conditions employed (Figure 2a). These contrasting results confirm the importance of the *N*-alkyl chain for strong inclusion complexation with CB[6]. Computer simulations (curve fitting) of the experimental ITC data obtained for *N*-ethylpiperazine and *N*,*N*′-diethylpiperazine were performed using the ORIGIN 7.0 software adapted for ITC data analysis. The Sequential Binding Sites model was applied in both cases. A typical example of the curve fitting analysis for the stepwise interaction of *N*-ethylpiperazine with CB[6] is shown in Figure 2b, and the thermodynamic parameter obtained are summarized in Table 1.

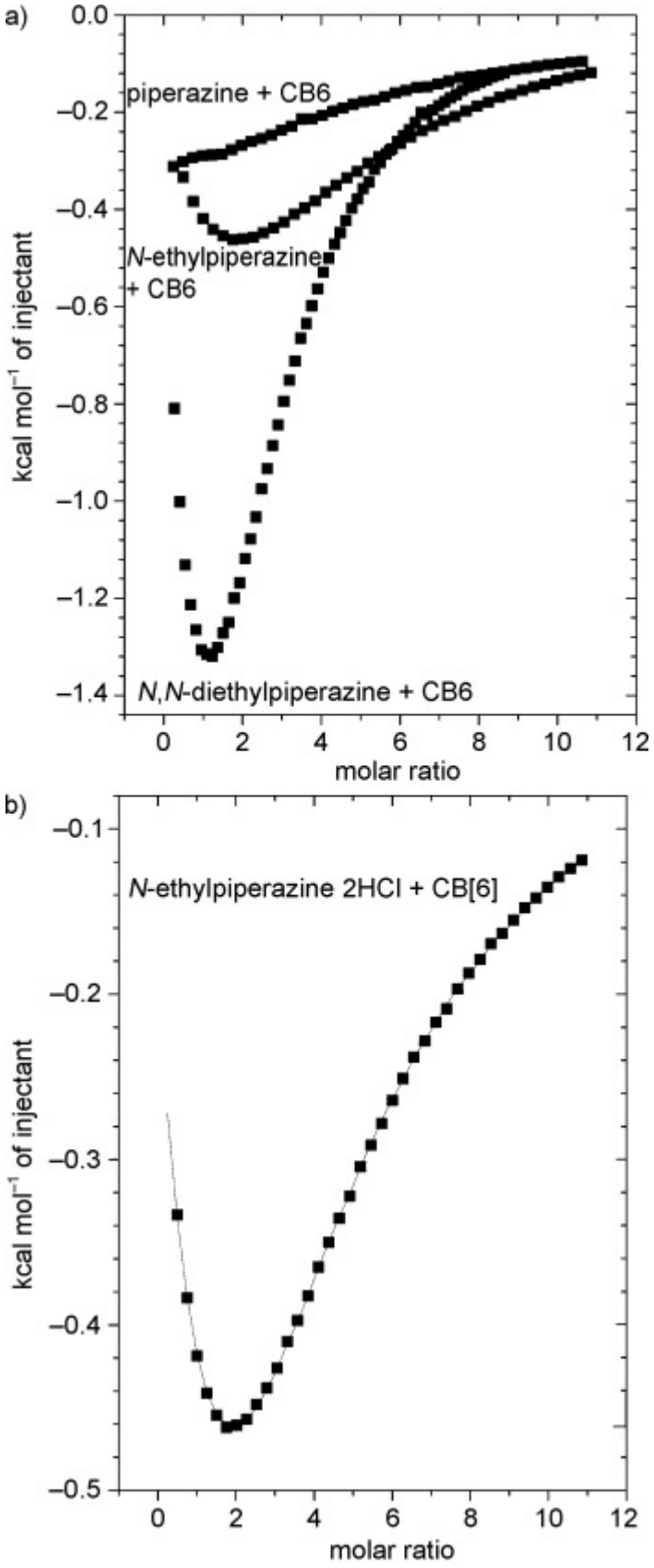

a)
piperazine + CB6
N-ethylpiperazine + CB6
N,N-diethylpiperazine + CB6
kcal mol−1 of injectant
molar ratio
b)
N-ethylpiperazine 2HCl + CB[6]
kcal mol−1 of injectant
molar ratio

Figure 2. a) Results of ITC experiments upon interaction of CB[6] with piperazine·2 HCl, *N*-ethylpiperazine·2 HCl, and *N*,*N*′-diethylpiperazine·2 HCl in aqueous 0.05 M NaCl solutions. b) Curve fitting of the experimental results for the stepwise 1:1 and 1:2 complexation of *N*-ethylpiperazine with CB[6].

As can be seen from Table 1, the 1:1 complexation of *N*-ethylpiperazine with CB[6] with accompanying insertion of the ethyl group into the CB cavity (Figure 3a) is facilitated primarily by a large entropic gain ($T\Delta S°$=11.4 kJ mol^{-1}) with a small enthalpic assistance ($\Delta H°$=−2.5 kJ mol^{-1}). This pattern of thermodynamic parameters is consistent with the shallow insertion of the ethyl group, which does not allow strong van der Waals contacts inside the cavity (thus giving only a small enthalpic gain), and also with the profound desolvation from the CB[6] portal and the dicationic guest (thus affording a large positive entropic gain).

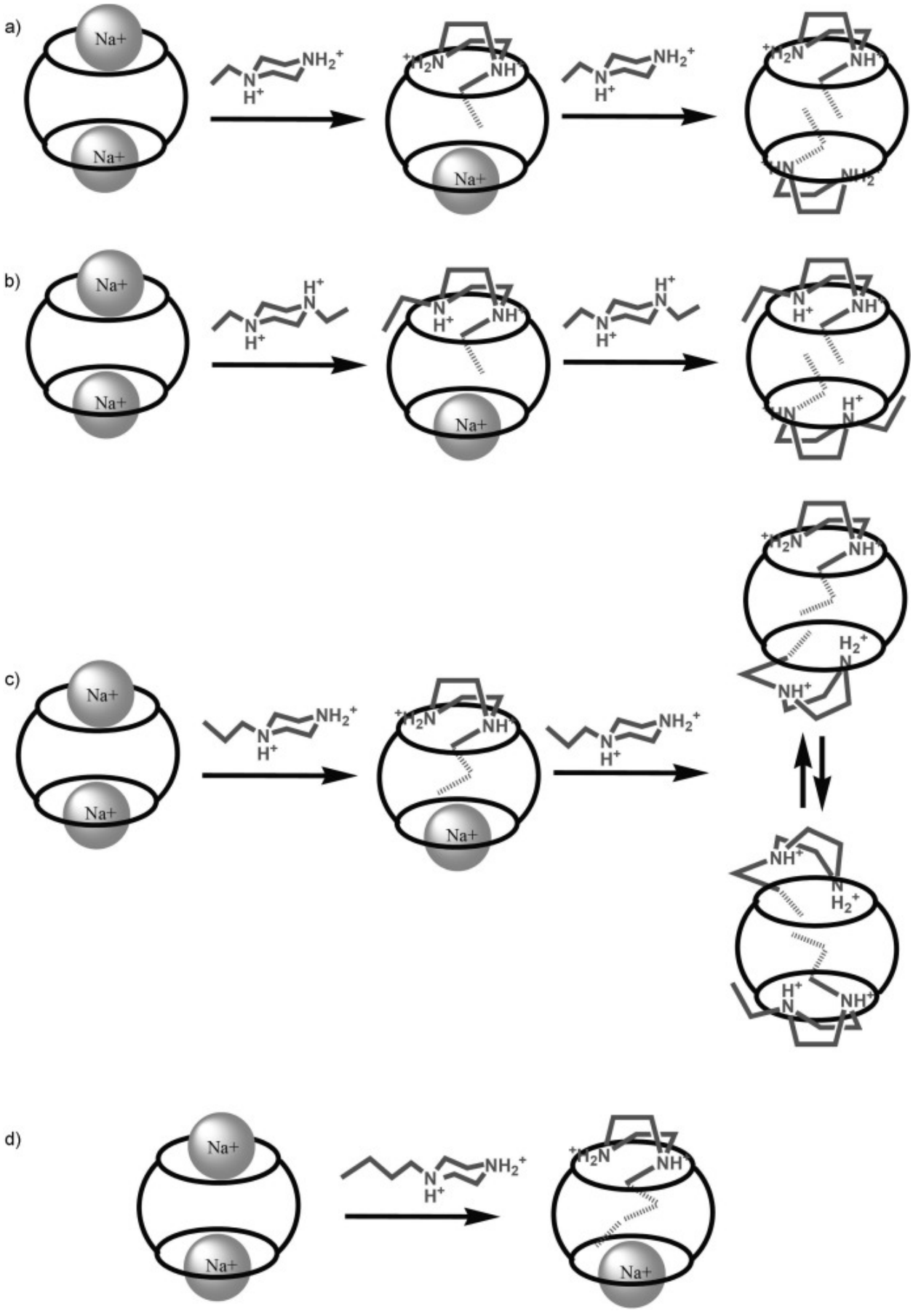

a)
Na+
Na+
Na+
b)
Na+
Na+
Na+
c)
Na+
Na+
Na+
d)
Na+
Na+
Na+

Figure 3. Schematic drawings of molecular events occurring upon interaction of a series of *N*-alkylpiperazines with CB[6] in aqueous 0.05 M NaCl solution: a) *N*-ethylpiperazine + CB[6], b) *N*,*N*′-diethylpiperazine + CB[6], c) *N*-propylpiperazine + CB[6], and d) *N*-butylpiperazine + CB[6].

Interaction of a second *N*-ethylpiperazine with 1:1 *N*-ethylpiperazine–CB[6] complex, illustrated in Figure 3a (second step), is accompanied by a large enthalpic gain (−19.1 kJ mol^{-1}) and a moderate entropic loss (−8.1 kJ mol^{-1}) (Table 1). Judging from the geometrical dimensions of small CB[6] cavity, we deduce that the insertion of a second ethyl group into the same cavity to form a 1:2 host–guest complex leads to enhanced van der Waals contacts with the cavity walls as well as significant conformational restriction of the two ethyl groups inside the cavity, which are the major sources of the enthalpic gain and the entropic loss, respectively.

Table 1. Stability constant (*K*), standard enthalpy (Δ*H*°), and entropy changes (*T*Δ*S*°) for complexation of CB[6] with protonated *N*-ethylpiperazine (EP), *N*,*N*′-diethylpiperazine (DEP), *N*-propylpiperazine (PP), *N*-butylpiperazine (BP), and a series of relevant alkylammonium (Et-, Pr-, and $BuNH_3^+$) ions in aqueous 0.05 M NaCl solutions at *T*=298.15 K.

Complexation reaction		K [M^{-1}]	K_1/K_2	$\Delta H°$ [kJ mol^{-1}]	$T\Delta S°$ [kJ mol^{-1}]
$[CB[6]\cdot 2\,Na]^{2+} + EP^{2+} \rightleftarrows [CB[6]\cdot EP\cdot Na]^{3+} + Na^+$	K_1	270±20	3.2	−2.5±0.2	11.4±0.3
$[CB[6]\cdot EP\cdot Na]^{3+} + EP^{2+} \rightleftarrows [CB[6]\cdot 2(EP)]^{4+} + Na^+$	K_2	85±5		−19.1±0.3	−8.1±0.4
$[CB[6]\cdot 2Na]^{2+} + DEP^{2+} \rightleftarrows [CB[6]\cdot DEP\cdot Na]^{3+} + Na^+$	K_1	1200±100	4.4	−4.8±0.3	12.8±0.4
$[CB[6]\cdot DEP\cdot Na]^{3+} + DEP^{2+} \rightleftarrows [CB[6]\cdot 2(DEP)]^{4+} + Na^+$	K_2	270±20		−21.5±0.4	−7.6±0.4
$[CB[6]\cdot 2Na]^{2+} + PP^{2+} \rightleftarrows [CB[6]\cdot PP\cdot Na]^{3+} + Na^+$	K_1	16 300±300	52.6	−16.3±0.3	7.7±0.4
$[CB[6]\cdot PP\cdot Na]^{3+} + PP^{2+} \rightleftarrows [CB[6]\cdot 2(PP)]^{4+} + Na^+$	K_2	310±30		−10.3±0.4	3.9±0.4
$[CB[6]\cdot 2Na]^{2+} + BP^{2+} \rightleftarrows [CB[6]\cdot BP\cdot Na]^{3+} + Na^+$	K_1	$(9.0\pm0.5)\times10^5$		−26.5±0.3	7.5±0.4

Complexation reaction		K [M^{-1}]	K_1/K_2	$\Delta H°$ [kJ mol^{-1}]	$T\Delta S°$ [kJ mol^{-1}]
$[CB[6]\cdot 2Na]^{2+} + EtNH_3^+ \rightleftarrows [CB[6]\cdot EtNH_3\cdot Na]^{2+} + Na^+$	K_1	990±30		−9.3±0.2	7.8±0.2
$[CB[6]\cdot 2Na]^{2+} + PrNH_3^+ \rightleftarrows [CB[6]\cdot PrNH_3\cdot Na]^{2+} + Na^+$	K_1	(1.55±0.08)×10^5		−19.1±0.3	10.6±0.3
$[CB[6]\cdot 2Na]^{2+} + BuNH_3^+ \rightleftarrows [CB[6]\cdot BuNH_3\cdot Na]^{2+} + Na^+$	K_1	(3.1±0.2)×10^6		−28.7±0.3	8.4±0.3

The thermodynamic parameters obtained for the 1:1 and 1:2 complexation of *N*,*N*′-diethylpiperazine exhibit practically the same trends. Thus, the first step (1:1 complexation) is associated with a large entropic gain (12.8 kJ mol^{-1}) and a small enthalpic gain (−4.8 kJ mol^{-1}), while the second step (1:2 complexation) leads to a large enthalpic gain (−21.5 kJ mol^{-1}) and a moderate entropic loss (−7.6 kJ mol^{-1}) (Table 1). This resemblance in thermodynamic trend may indicate similar complex structures in both cases and the insertion of only one ethyl of each *N*,*N*′- diethylpiperazine into the cavity. This mode of insertion is consistent with the geometrical dimensions of CB[6] cavity, which do not allow penetration of a second ethyl of the same guest from the same portal. It should be noted also that the enthalpic gains obtained upon 1:1 and 1:2 complexation are consistently larger (by 2.3 and 2.4 kJ mol^{-1}, respectively) for *N*,*N*′-diethylpiperazine than for *N*-ethylpiperazine, which would be attributed to the hydrophobic interactions of the outside ethyl group with the outer surface of CB[6].

ITC experiments with *N*-propylpiperazine and CB[6] turned out to be a difficult task. It was anticipated that the 1:1 complexation of *N*-propylpiperazine with CB[6] is much stronger than that of *N*-ethylpiperazine, since the propyl group can penetrate much deeper into the cavity. Molecular model examinations indicated that even after the penetration of the guest's propyl, some hydrophobic space remains in the CB[6] cavity, which may allow shallow insertion of another propyl group from the opposite portal, as schematically illustrated in Figure 3c. Due to this steric hindrance upon second guest inclusion, we anticipated that the K_2value is much smaller than the K_1 value, which may deter reliable determination of the thermodynamic parameters by computer simulation of raw ITC experimental data. Nevertheless we succeeded in determination of the thermodynamic parameters as it is described in details in Supporting Information.

As anticipated, *N*-propylpiperazine gives a much more stable 1:1 complex with CB[6] than *N*-ethylpiperazine and *N*,*N*′-diethylpiperazine (Table 1). In contrast to the *N*-ethylpiperazine–CB[6]

and *N*,*N*′-diethylpiperazine–CB[6] systems where $\Delta H_2$is more negative than ΔH_1, the *N*-propylpiperazine–CB[6] system shows an opposite trend with ΔH_1 (−16.3 kJ mol^{-1}) being more negative than ΔH_2 (−10.3 kJ mol^{-1}). This is consistent with the schematic illustration of molecular events occurring in alkylpiperazine–CB[6] systems (Figure 3c). Due to the small cavity dimensions of CB[6], two propyl groups cannot be fully accommodated simultaneously in the same cavity. After insertion of one propyl, the remaining free space inside the cavity is probably enough only for one additional methyl/methylene unit. Consequently, insertion of a second propyl cannot enjoy full van der Waals contacts with the cavity walls, giving a significantly smaller ΔH_2. Interestingly, the corresponding entropy change ($T\Delta S_2$=3.9 kJ mol^{-1}) is in sharp contrast to the negative $T\Delta S_2$ values for *N*-ethylpiperazine (−8.1 kJ mol^{-1}) and *N*,*N*′-diethylpiperazine complexes (−7.6 kJ mol^{-1}) (Table 1). This observation strongly indicates that the 1:2 complex [CB[6]·(*N*-propylpiperazine)$_2$] is conformationally more flexible and diverse, as a result of the partial inclusion of the second propyl chain and the dynamic equilibrium between the two conformers illustrated in Figure 3c.

It should be noted that the combined enthalpic gain ($\Delta H_1 + \Delta H_2$) for 1:1 and 1:2 complexation is not very different, or even comparable (−21.6, −26.3, and −26.6 kJ mol^{-1}), for *N*-ethyl, *N*,*N*′-diethyl, and *N*-propylpiperazine, despite the difference in alkyl chain length. This is compatible with the idea that only four methyl/methylene units, which are comfortably accommodated in the CB[6] cavity, can fully contribute to the enthalpic stabilization and the extra methylene groups make marginal contributions. It is also interesting to note that the combined entropic gain ($T\Delta S_1 + T\Delta S_2$) steadily increases from +3.3 to +5.2 kJ mol^{-1}and then to +11.6 kJ mol^{-1} on going from *N*-ethyl to *N*,*N*′-diethyl, and then to *N*-propylpiperazine, indicating more extensive dehydration upon complexation and formation of progressively flexible 1:2 complex in this order.

In good agreement with the above discussion, *N*-butylpiperazine, the alkyl chain of which is expected to fill out the inner space of CB[6], did not show any indication of 1:2 complex formation with CB[6] in the ITC experiment, and the titration experimental data were successfully fitted by the simple Single Set of Identical Sites model with the 1:1 guest–host stoichiometry to give the data shown in Table 1 (and Supporting Information). Quite interestingly, the enthalpic gain for 1:1 complexation of *N*-butylpiperazine (ΔH_1=−26.5 kJ mol^{-1}) is very close to the combined enthalpic gains for the lower homologues ($\Delta H_1 + \Delta H_2$=−21.6 to −26.6 kJ mol^{-1}).

Interaction of CB[6] with alkylpiperazines in H_2O: Since CB[6] is practically insoluble in water in the absence of a salt, we cannot perform direct ITC measurements and determine the thermodynamic parameters for complexation of CB[6] in pure water. However, we can estimate the stability of CB[6] complex in H_2O based on the data presented in Table 1, by using the results obtained for water-soluble host CB[7] in H_2O with and without added NaCl (see Supporting Information). Due to the competition

with sodium ions for the same CB[7] portal, the affinities of various guests toward CB[7] in aqueous 0.1 M NaCl solutions are about two orders of magnitude lower than those in H_2O (Supporting Information). The effect of added salts to reduce the stability of CB[7] complexes was also reported by Kaifer et al.[7a] and discussed in further detail for various CBs in a recent review.[4] Since sodium ions are better fitted to the smaller CB[6] portals, they are expected to behave as stronger competitors than the CB[7] case. Probably, it is a fair estimation that the affinities of piperazines should be at least 100 times higher in pure water than in aqueous 0.05 M NaCl solutions (Table 1). Consequently, we may anticipate affinities (K_1) in the order of 10^4, 10^5, 10^6, and 10^8 for *N*-ethyl-, *N*,*N*'-diethyl-, *N*-propyl-, and *N*-butylpiperazine, respectively. The affinities for *N*,*N*'-diethyl-, *N*-propyl-, and *N*-butylpiperazine are high enough to form the corresponding 1:1 complexes with CB[6] at micromolar and even sub-micromolar concentrations.

One of us recently reported a water-soluble CB[6] derivative, CB*[6], containing six cyclohexano-glycoluril units, which has the same cavity and portal sizes as CB[6].[13] The solubility of CB*[6] in water allowed us to measure the guest binding constants of the host in water (pH ≈7) by ITC. As expected, the affinity of butylammonium toward CB*[6] in H_2O (K=7.6×10^8 M^{-1}) was found to be about 1100-fold higher than that in aqueous 0.05 M sodium acetate buffer solution (K=6.8×10^5 M^{-1}).[14]

However, our major concern is the concentration of these complexes in the solution sufficient to start an interaction with each other to form oligomeric *n:n*supramolecular complexes as illustrated in Figure 4. To promote the formation of oligomeric *n:n* species, we should keep the CB[6]/alkylpiperazine ratio as close as unity and hence we eventually added an excess amount of CB[6] to the solution. For that purpose, we prepared a solution of *N*-propylpiperazine·2 HCl in D_2O and monitored the changes in ^{1}H NMR spectrum upon gradual addition of CB[6] to the solution (Figure 5). At host/guest ratios close to or slightly exceeding 0.5, we observed the formation of new peaks in the ^{1}H NMR spectra. Similar new peaks were also observed for the solutions of *N*,*N*'-diethylpiperazine·2 HCl and *N*-butylpiperazine·2 HCl saturated with CB[6] (see Supporting Information). These new peaks may correspond to simple monomeric 1:1 host/guest complexes as well as to an oligomeric species.

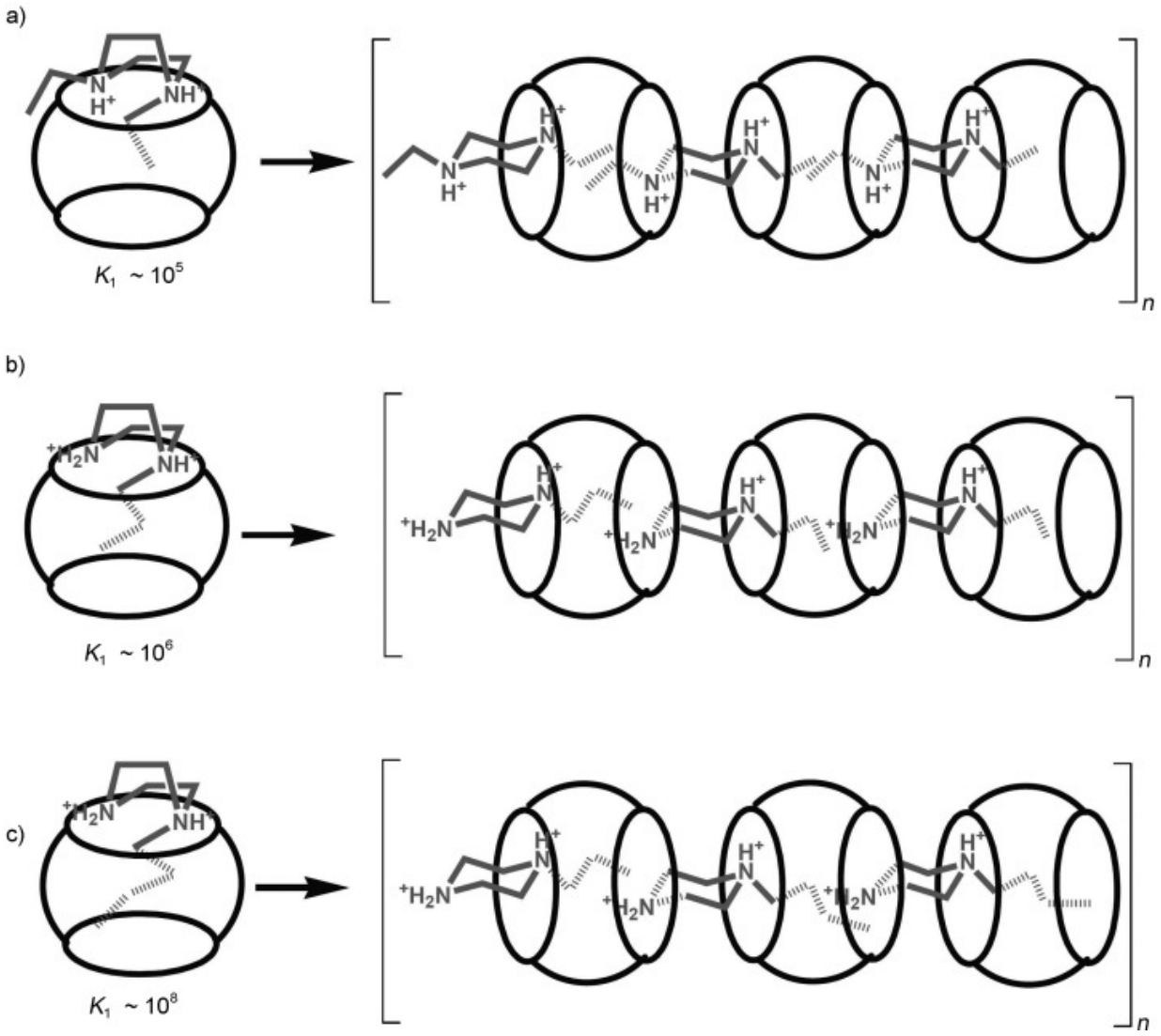


Figure 4. Schematic representation of oligomerization in the solution containing sufficient concentrations of the simplest complexes of CB[6] with a) *N*,*N*'-diethylpiperazine, b) *N*-propylpiperazine, and c) *N*-butylpiperazine.

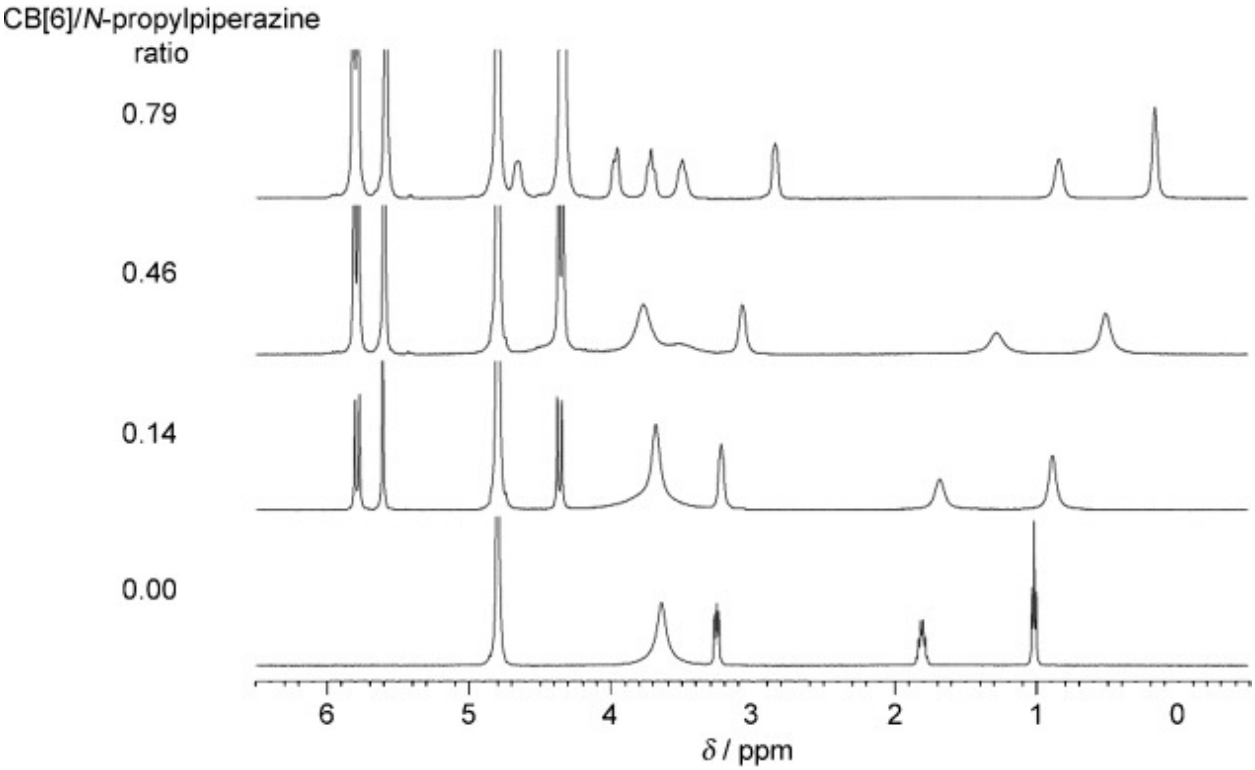


Figure 5. Changes in ^{1}H NMR spectra of a CB[6] + *N*-propylpiperazine·2 HCl solution in D_2O at various host/guest ratios.

The degree of oligomerization and composition of oligomers in aqueous solutions containing alkylpiperazines and CB[6] were examined by ESI-MS and dynamic light scattering experiments. An aqueous solution containing 2 mM *N*,*N*′-diethyl-, *N*-propyl-, or *N*-butylpiperazine·2 HCl was saturated with CB[6], filtrated, and then subjected the ESI-MS measurements. In all three cases, the predominant species detected in the spectra were 1:1 host–guest complexes (see Supporting Information). However, not only the univalent 1:1 host–guest complexes with one positive charge but also divalent 2:2^{2+}, trivalent 3:3^{3+} and multivalent *n*:*n*$^{n+}$oligomers give the same mass/charge ratio and thus appear at the same *m*/*z*position in the ESI-MS spectrum. For this reason, we performed the light scattering experiments as described previously[8] and estimated that the average molecular weights of the complexes existing in the 2 mM solutions of all three *N*-alkylpiperazines saturated with CB[6] are close to 1 kDa, which are very close to the molecular weights of these 1:1 host–guest complexes. Although the 1:1 host–guest complexes are obviously the major species in these solutions, we cannot rule out the possibility that the 1:1 complex peak contains some fragments of *n*:*n*oligomers originally existing in the solution. Indeed, appreciable amounts of various oligomers were detected in the ESI-MS spectra (see Supporting Information).

X-ray crystallographic structure of CB[6]-*N*-alkylpiperazine complexes: Aqueous solutions of *N*,*N*′-diethyl-, *N*-propyl-, and *N*-butylpiperazine·2 HCl saturated by CB[6] were subjected to slow evaporation for a 1–2 month period. In the case of *N*,*N*′-diethyl- and *N*-propylpiperazine·2 HCl, we obtained several good single crystals suitable for X-ray crystallographic analysis. However, *N*-butylpiperazine·2 HCl solutions saturated with CB[6] did not give any good single crystals after several attempts under a variety of conditions of slow evaporation. One possible explanation would be the coexistence of several large oligomers in the solution (see discussion above). Upon evaporation these oligomers spontaneously coagulate preventing formation of good crystals with a regular structure.

Results of the X-ray crystallographic analyses reveal that the crystals obtained from *N*-propyl- and *N*,*N*′-diethylpiperazine·2 HCl have different structures; see Figures 6 and 7, respectively. Crystals of the CB[6]–*N*-propylpiperazine complex consist of long chains where two adjacent CB[6] units are connected to each other by one *N*-propylpiperazine molecules. Since only four methyl/methylene units can be comfortably accommodated inside the CB[6] cavity, two propyl groups cannot be included in one cavity. Consequently, we obtained highly ordered crystals, in which all *N*-propylpiperazine molecules in a single chain are oriented in the same direction (Figure 6). The propyl group is inserted deeply into the CB[6] cavity and thus hosts and guests are interlocked by strong van der Waals interactions and conformationally restricted. The second nitrogen of the *N*-propylpiperazine molecule is coordinated to the portal of the neighboring CB[6]. This nitrogen is not deeply included into the cavity and thus the chain is rather flexible at that point. The chain flexibility allows optimization and fine adjustment of the location/position of one chain relative to the other, eventually leading to a very symmetrical crystal structure with close packing/interaction.

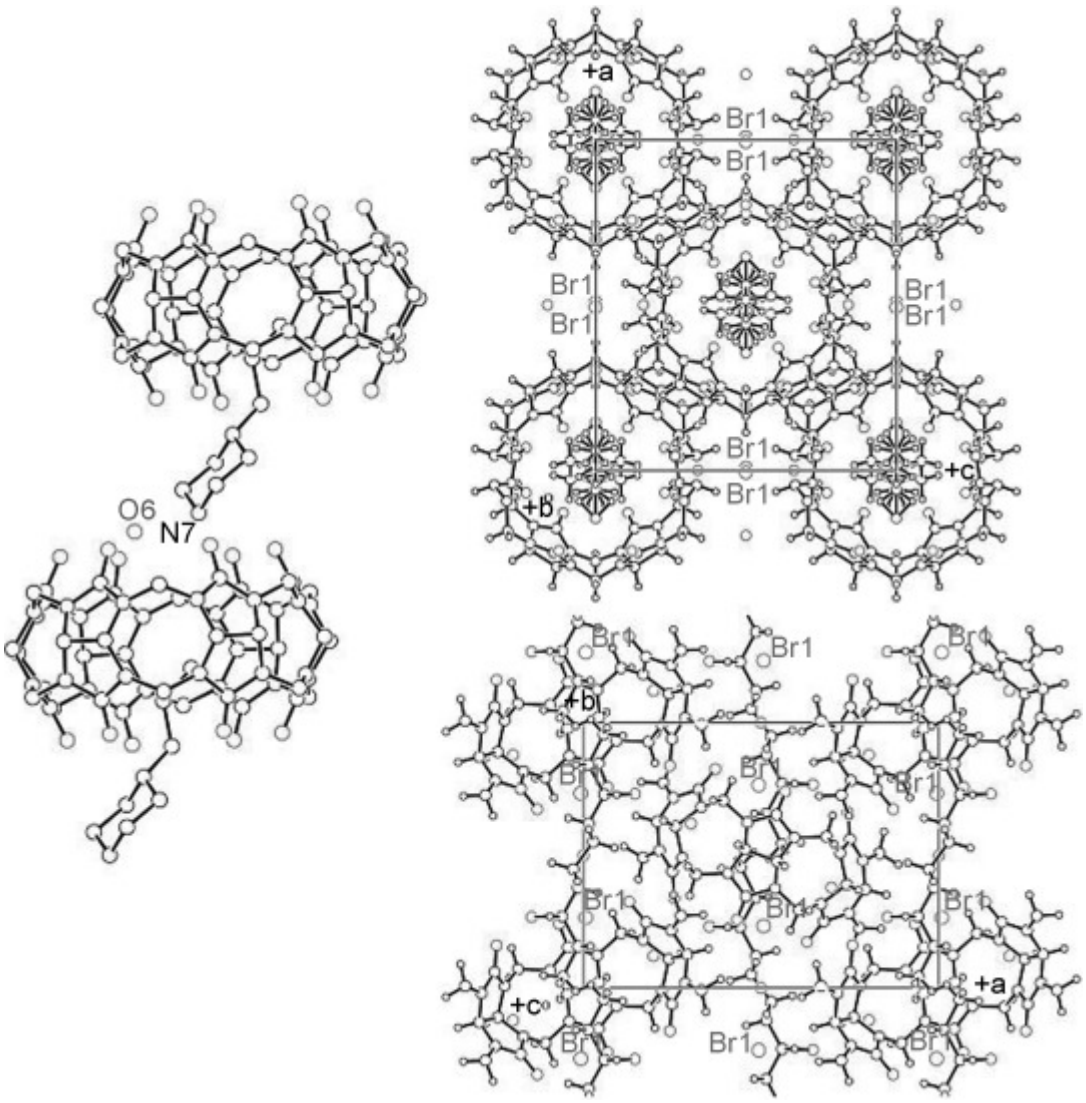


Figure 6. X-ray crystal structure of CB[6] complex with *N*-propylpiperazine·2 HCl

In the crystal of the CB[6] complex with *N*,*N*′-diethylpiperazine·2 HCl shown in Figure 7, two ethyl groups of neighboring *N*,*N*′-diethylpiperazine molecules are included in one CB[6] cavity and occupy practically all accessible space. Such a tight intracavity packing inevitably stiffens the chains by reducing their flexibility. Naturally, the optimization and fine tuning of the location/position of one chain relative to the other is impossible. By contrast, the chains only loosely interact to each other, allowing penetration of a large amount of solvent molecules in the crystal.

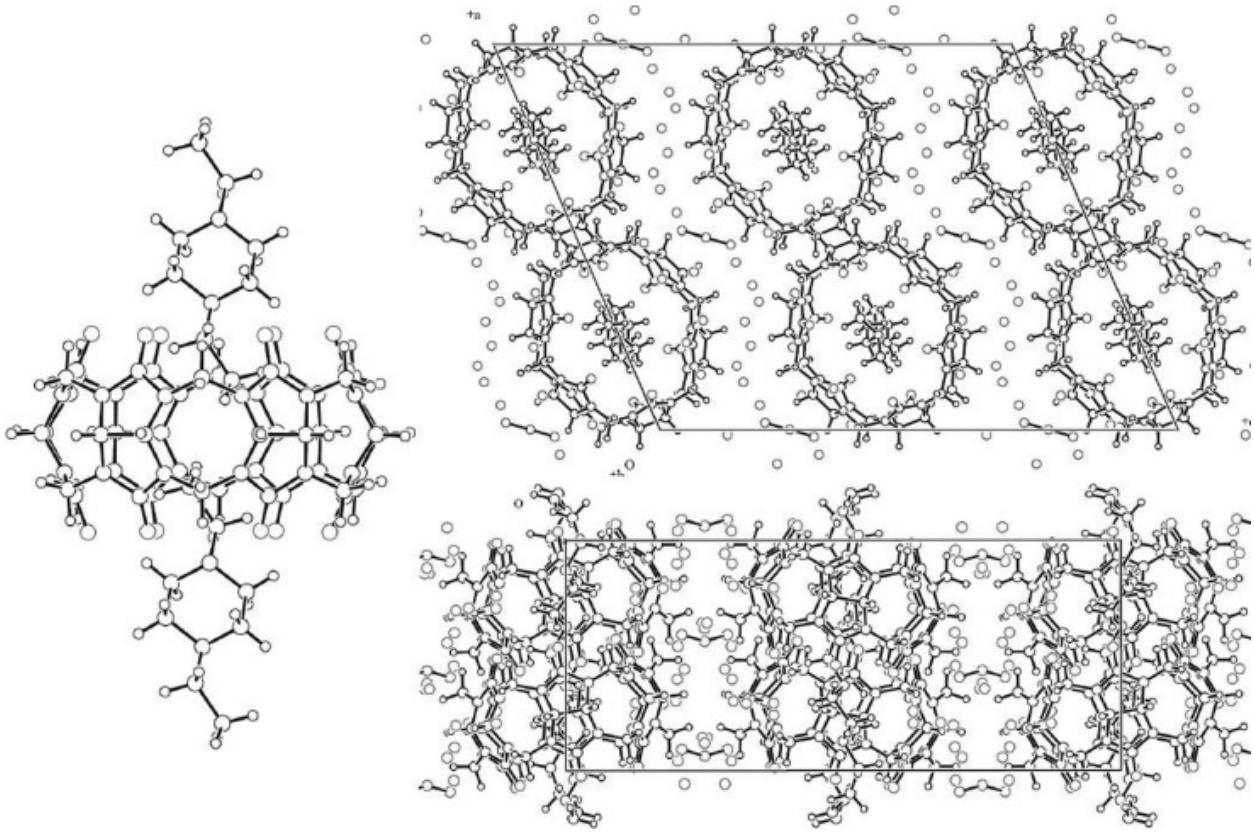


Figure 7. X-ray crystal structure of CB[6] complex with *N*,*N*′-diethylpiperazine·2 HCl.

We may therefore conclude that the degree of fixation of the guest's ammonium at the CB[6] portal is a crucial factor that determines the crystal structure of a CB[6] complex. Indeed, CB[6] complexes of *N*-propylpiperazine (fixed at one portal) and of *N*,*N*′-diethylpiperazine (fixed at both portals) give very different crystal structures. This contrasting behavior is compatible with our claim that the degrees of fixation in the cavity and the stiffness of the supramolecular polymer chain formed play the key role in determining crystal structure. If this is a correct explanation and the stiffness of the chain is indeed the major factor that determines the crystal structure, we may engineer the crystal structure and switch from one architecture (Figure 6) to another (Figure 7) by adjusting the length of aliphatic groups introduced to the piperazine core. For instance, if we take *N*,*N*′-dimethylpiperazine instead of *N*,*N*′-diethylpiperazine, we may expect that the stiffness of the chains be greatly reduced and the degree of fine tuning of the location/position of one chain relative to another may resemble to the *N*-propylpiperazine rather than the *N*,*N*′-diethylpiperazine case.

Hence, an aqueous solution of *N*,*N*′-dimethylpiperazine·2 HCl saturated with CB[6] was subjected to slow evaporation for 1–2 months and we thus obtained several well organized single crystals suitable for X-ray analysis. As anticipated above, the crystal structure of the CB[6] complex with *N*,*N*′-

dimethylpiperazine·2 HCl (Figure 8) was almost identical to that of the CB[6] complex with *N*-propylpiperazine·2 HCl (Figure 6).

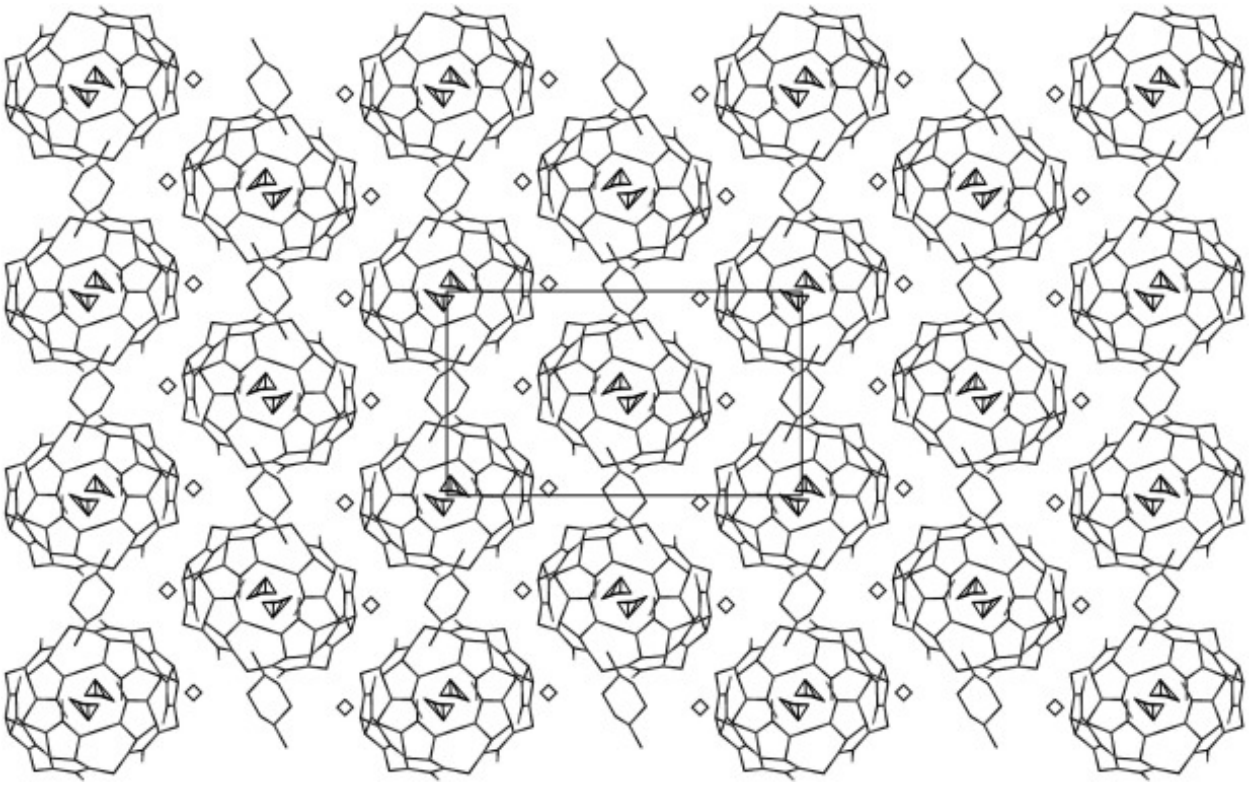


Figure 8. X-ray crystal structure of CB[6] complex with *N*,*N*′-dimethylpiperazine·2 HCl

Conclusion

Understanding the molecular architecture and the stability of supramolecular complexes as well as the nature of intramolecular weak interactions involved in the aggregation/association in solution would provide a theoretical basis for rational design of supramolecular crystals. Indeed, the results obtained in the present work using various experimental methods and approaches (ITC, NMR, ESI-MS, light scattering and X-ray crystallography) indicate that detailed knowledge of molecular events occurring in the solution can be used to rationally alter/optimized supramolecular structure of alkylpiperazine–CB[6] crystals. In the case of alkylpiperazine–CB[6] crystals, an important factor is the occupancy of CB[6] cavity, in which four methyl/methylene units can be comfortably accommodated. Occupation of the whole cavity by four methyl/methylene groups, which is effectively achieved upon formation of *N*,*N*′-diethylpiperazine–CB[6] crystal, rigidifies supramolecular chains and consequently leads to a crystal structure different from those for *N*,*N*′-dimethylpiperazine–CB[6] and propylpiperazine–CB[6] crystals with only partial cavity occupation. We believe that the rigidity of supramolecular chains and

degree of occupancy of cavity/cage/pore upon supramolecular complex formation are the most important factors to be seriously considered in designing supramolecular crystals.

Experimental Section

Isothermal titration calorimetry: Microcalorimetric experiments were performed using an isothermal titration calorimeter VP-ITC (MicroCal, USA). Each experiment consisted of 25–35 consecutive injections (5–10 µL) of a guest solution into the microcalorimetric reaction cell (1.4 mL) charged with a solution of CB[6] or CB[7]. The heat of reaction was corrected for the heat of dilution of the guest solution determined in the separate experiments. All solutions were degassed prior to titration experiment according to the method provided by MicroCal. Computer simulations (curve fitting) were performed using the ORIGIN 7.0 software adapted for ITC data analysis. The "Single Set of Identical Sites" model was applied in all cases.

NMR measurements: 1D and 2D NMR spectra, including ROESY, COSY, and HOHAHA, were obtained at 600 MHz in D_2O at 25 °C on a Bruker Avance 600 instrument. HOHAHA experiments were performed by using the MLEV-17 pulse sequence with a mixing time of 120 ms, while ROESY spectra were recorded with a mixing time of 200 ms.

Light scattering measurements: Zetasizer Nano ZS instrument (Malvern Instruments) was used for light scattering measurements at 25 °C and at 173 degree angle. Rayleigh equation was applied to estimate molecular weight of nano-particles in the solution.[15]

Materials: CB[6] and CB[7] were synthesized and purified by the method reported previously.[2, 5, 6a, 16] All other reagents of highest purity available were obtained from Aldrich and Wako and were used without further purification.

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