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Chiral Recognition in Cucurbituril Cavities

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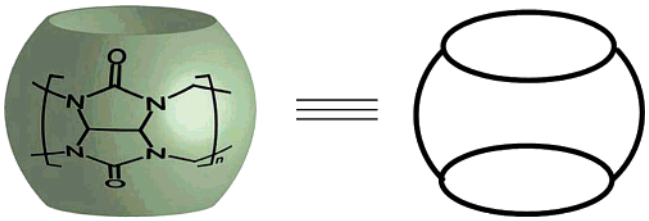
Abstract: For the first time, achiral cucurbiturils (CBs) were endowed with significant enantiomeric and distereomeric discrimination by incorporating a strong chiral binder. Calorimetric, nuclear magnetic, light-scattering, and mass spectral studies revealed that (*S*)-2-methylbutylamine (as a strong binder) can be discriminated by two enantiomeric supramolecular hosts, composed of CB[6] and (*R*)- or (*S*)-2-methylpiperazine, with an unprecedented 95% enantioselectivity in aqueous NaCl solution. This is the highest enantioselectivity ever reported for a supramolecular system derived from an achiral host. Similarly, CB[7], with a larger cavity, exhibited diastereoselectivities up to 8 times higher for diastereomeric dipeptides, as demonstrated for L-Phe-L-Leu-NH_3^+ versus L-Phe-D-Leu-NH_3^+.

Introduction

The pumpkin-shaped macrocycle cucurbit[6]uril (CB[6])[1] was first synthesized in 1905,[2] and its chemical structure (Chart 1) was established in 1981.[3] Nevertheless, fundamental and application studies on CB[6] have been rather limited until recently, mostly due to its low solubility in both water and common organic solvents. The use of CB[6], however, underwent an explosion in various fields of science after the discovery of the fact that it becomes readily soluble in aqueous solutions that contain alkali or other metal cations, as well as organic ammonium ions.[4] It was subsequently found that metal cations (M^+) coordinate to both portals of the barrel-shaped CB[6] to give a dicationic complex, $[CB[6]\cdot 2M]^{2+}$. We also reported the syntheses of smaller and larger CB homologues, cucurbit[*n*]-uril (CB[*n*], $n = 5-8$).[5] Since then, a wide variety of supramolecular architectures and devices based on CB macrocycles have been designed by us as well as by other groups,[6,7] as can be seen in a recent review.[8]

Chart 1. Chemical Structure of the Cucurbit[*n*]uril Macrocycle



Challenges associated with the chirality induction in an achiral host via noncovalent interactions with chiral ligands/auxiliaries were previously delineated by Aoyama et al.[9a] and discussed in further detail in our recent account.[9b] We now demonstrate, for the first time, that the intrinsically achiral CB[*n*] macrocycles,

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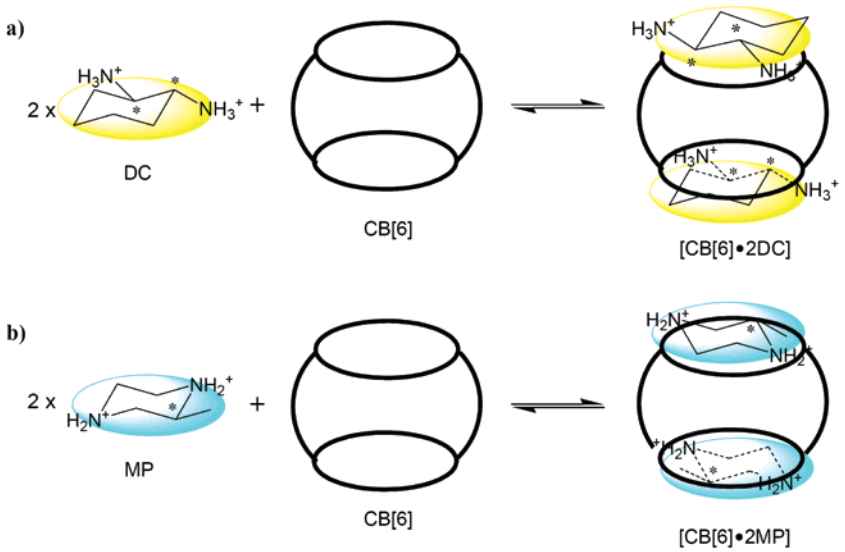

Figure 1. Formation of 1:2 complexes of CB[6] (a) with (*R*,*R*)- or (*S*,*S*)-*trans*-1,2-diaminocyclohexane (DC) and (b) with (*R*)- or (*S*)-2-methylpiperazine (MP).

particularly CB[6] and CB[7], can serve as effective chiral discriminators.

Experimental Section

Microcalorimetric experiments were performed using an isothermal titration calorimeter (VP-ITC, MicroCal, Northampton, MA). 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 separate experiments. All solutions were degassed prior to the 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.

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.

A Zetasizer Nano ZS instrument (Malvern Instruments, Worcestershire, UK) was used for light-scattering measurements at 25 °C and at a 173° angle. The Rayleigh equation was applied to estimate the molecular weight of nanoparticles in the solution.[10]

CB[6] and CB[7] were synthesized and purified by the method reported previously.[3−5] All other reagents, of the highest purity available, were obtained from Aldrich and Wako and were used without further purification.

Results and Discussion

Enantiodifferentiation in the Cucurbit[6]uril Cavity. Dissolving CB[6] in an aqueous solution of an enantiopure organic amine, such as (*R*)- or (*S*)-2-methylpiperazine (MP) or (*R*,*R*)- or (*S*,*S*)-*trans*-1,2-diaminocyclohexane (DC), leads to formation of the respective enantiopure complex, i.e., (*R*;*R*)- or (*S*;*S*)-[CB[6]·2MP]$^{4+}$ or (*R*,*R*;*R*,*R*)- or (*S*,*S*;*S*,*S*)-[CB[6]·2DC]$^{4+}$ (Figure 1). An appropriate chiral guest, added to this solution, can replace one of the originally bound enantiopure amines (MP or DC) at the CB portals, as illustrated in Figure 2. This idea is reminiscent of that proposed by Rebek et al.,[11] who attempted to achieve enantiodifferentiation by accommodating two different chiral guests inside a self-assembled achiral capsule; however, the chiral recognition ability was not very high, with a maximum difference in affinity of only 1.2 times. Strictly speaking, the molecular recognition event observed in Rebek's supramolecular system[11] as well as in the system described here (Figures 1 and 2) is not an enantioselection in a classical definition of this term, but rather a special kind of diastereoselectivity that arises from supramolecular assembling of chiral and achiral building blocks. More precisely, such a molecular recognition event may be called "assembled enantioselection" or "assembled enantiorecognition." Here, we report our results on the assembled enantiorecognition upon guest-exchange reactions with enantiopure CB[6]-based supramolecular complexes.

Aqueous solutions of 1:2 host−guest complexes (*R*,*R*;*R*,*R*)- and (*S*,*S*;*S*,*S*)-[CB[6]·2DC]$^{4+}$, formed in situ, were subjected to microcalorimetric (ITC) titration with aqueous (*R*)-leucineamide (Leu-NH_2) to reveal the formation of two 1:1:1 complexes, i.e., (*R*,*R*;*R*)- and (*S*,*S*;*R*)-[CB[6]·DC·Leu-NH_2]$^{3+}$. The thermodynamic parameters for these diastereomeric complexes were exactly the same, meaning no chiral recognition (Table 1). One of the most likely explanations for this result is the relatively large distance between the stereogenic centers of the two chiral guests in the termolecular complexes. Enantiomeric (*R*)- and (*S*)-*sec*-butylamines (BA) also gave practically the same affinities upon complexation with (*S*,*S*;*S*,*S*)-[CB[6]·2DC]$^{4+}$ (Table 1).

Interestingly, in the case of (*R*)- and (*S*)-BA guests, the differences between $\Delta H°$ and $\Delta S°$ values obtained for the diastereomeric (*S*,*S*;*R*)- and (*S*,*S*;*S*)-[CB[6]·DC·BA]$^{3+}$ complexes are clearly outside the experimental errors, despite their having practically the same complex stabilities (Table 1), for which the frequently observed enthalpy−entropy compensation effect[12,13] is responsible. The different $\Delta H°$ and $\Delta S°$ values

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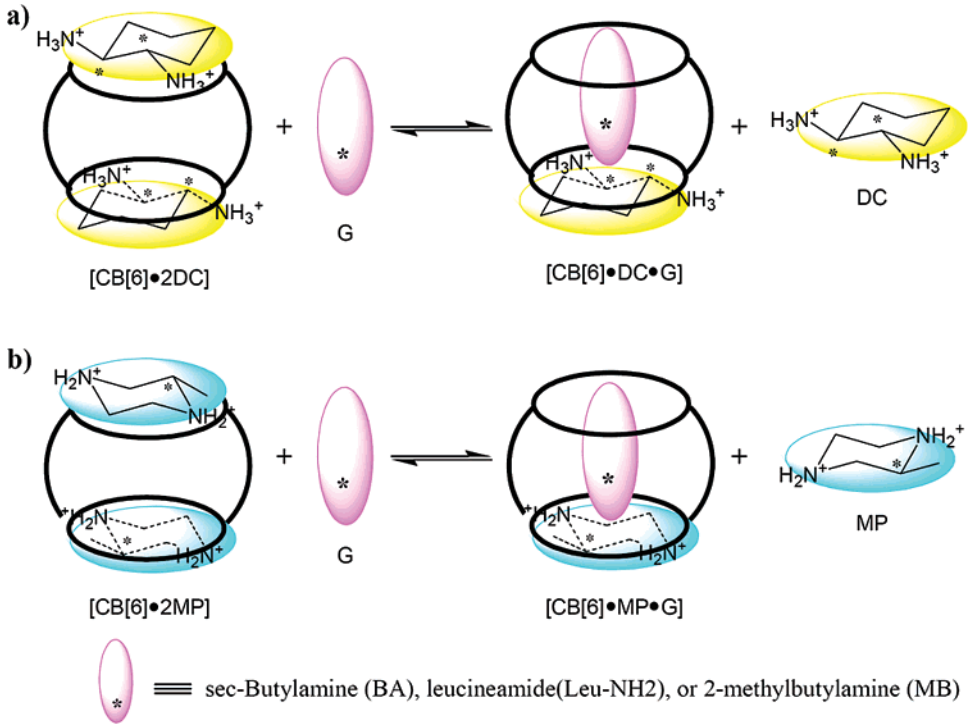

Figure 2. Replacement of (a) *trans*-1,2-diaminocyclohexane (DC) or (b) 2-methylpiperazine (MP) auxiliary by another chiral guest, such as *sec*-butylamine, leucineamide, or 2-methylbutylamine (G).

Table 1. Stability Constant (K) and Standard Enthalpy (ΔH°) and Entropy Changes ($T\Delta S^\circ$) for Complexation of Cucurbit[6]uril (CB[6]) with (*S*)-2-Methylbutylamine ((*S*)-MB), (*R*)- and (*S*)-*sec*-Butylamine ((*R*/*S*)-BA), and Related Guest Molecules in Aqueous Solutions of 0.025 M (*R*,*R*)- or (*S*,*S*)-1,2-Diaminocyclohexane ((*R*,*R*/*S*,*S*)-DC, soln 1), 0.025 M (*R*)- and (*S*)-2-Methylpiperazine ((*R*/*S*)-MP, soln 2), and 0.1 M NaCl (soln 3) at 298.15 K

complexation reaction	K/M^{-1}	ΔH°/kJ mol^{-1}	$T\Delta S^\circ$/kJ mol^{-1}
$[CB[6]\cdot 2((R,R)\text{-DC})]^{4+} + (R)\text{-Leu-NH}_2^+ \rightarrow$ $[CB[6]\cdot(R,R)\text{-DC}\cdot(R)\text{-Leu-NH}_2]^{3+} + (R,R)\text{-DC}^{2+}$ (soln 1)	315 ± 10	−19.5 ± 0.2	−5.2 ± 0.3
$[CB[6]\cdot 2((S,S)\text{-DC})]^{4+} + (R)\text{-Leu-NH}_2^+ \rightarrow$ $[CB[6]\cdot(S,S)\text{-DC}\cdot(R)\text{-Leu-NH}_2]^{3+} + (S,S)\text{-DC}^{2+}$ (soln 1)	320 ± 10	−19.3 ± 0.2	−5.0 ± 0.3
$[CB[6]\cdot 2((S,S)\text{-DC})]^{4+} + (R)\text{-BA}^+ \rightarrow$ $[CB[6]\cdot(S,S)\text{-DC}\cdot(R)\text{-BA}]^{3+} + (S,S)\text{-DC}^{2+}$ (soln 1)	1030 ± 50	−19.8 ± 0.3	−2.6 ± 0.3
$[CB[6]\cdot 2((S,S)\text{-DC})]^{4+} + (S)\text{-BA}^+ \rightarrow$ $[CB[6]\cdot(S,S)\text{-DC}\cdot(S)\text{-BA}]^{3+} + (S,S)\text{-DC}^{2+}$ (soln 1)	1070 ± 50	−18.6 ± 0.3	−1.3 ± 0.3
$[CB[6]\cdot 2((R)\text{-MP})]^{4+} + (S)\text{-MB}^+ \rightarrow$ $[CB[6]\cdot(R)\text{-MP}\cdot(S)\text{-MB}]^{3+} + (R)\text{-MP}^{2+}$ (soln 2)	15 000 ± 3000	−5.0 ± 0.5	18.8 ± 0.6
$[CB[6]\cdot 2((S)\text{-MP})]^{4+} + (S)\text{-MB}^+ \rightarrow$ $[CB[6]\cdot(S)\text{-MP}\cdot(S)\text{-MB}]^{3+} + (S)\text{-MP}^{2+}$ (soln 2)	800 ± 100	13.4 ± 0.8	30.0 ± 1.0
$[CB[6]\cdot 2Na]^{2+} + (S)\text{-MP}^{2+} \rightarrow$ $[CB[6]\cdot(S)\text{-MP}\cdot Na]^{3+} + Na^+$ (soln 3)	∼20	∼ −3	∼4
$[CB[6]\cdot(S)\text{-MP}\cdot Na]^{3+} + (S)\text{-MP}^{2+} \rightarrow$ $[CB[6]\cdot 2((S)\text{-MP})]^{4+} + Na^+$ (soln 3)	∼20	∼ −17	∼ −10
$[CB[6]\cdot 2Na]^{2+} + (S)\text{-MB}^+ \rightarrow$ $[CB[6]\cdot(S)\text{-MB}\cdot Na]^{3+} + Na^+$ (soln 3)	10 500 ± 500	−17.4 ± 0.2	5.6 ± 0.2

observed for (*R*)- and (*S*)-BA imply that the supramolecular enantiomeric complex of CB[6] can effectively recognize the guest's chirality when the right combination of chiral portal auxiliaries and replacing guest is chosen.

To optimize the stereospecific interactions in the CB[6] ternary complex, we changed the chiral auxiliary from (*R*,*R*)- and (*S*,*S*)-DC to (*R*)- and (*S*)-2-methylpiperazine (MP). The key idea is that the MP's hydrophobic methyl substituent is most likely included inside the CB cavity, so as to form a chiral nanospace for further inclusion of another guest. The guest's structure is also important, and hence we searched for a chiral amine that possesses a stereogenic center at a position remote from the amino group yet that is small enough to be accommodated in the same CB cavity together with MP. On the basis of such criteria, we selected chiral 2-methylbutylamine (MB) as the most promising guest in the following ITC experiments. MB has a crucial advantage over BA, since the stereogenic center is located α to the ammonium group in BA, but at the β position in MB. Consequently, the stereogenic center of MB should be forced to penetrate deeply into the chiral nanospace produced by the chiral auxiliary (MP) of the CB−MP complex.

Indeed, the choice of chiral MB as guest was very successful. As can be seen from the thermodynamic parameters shown in Table 1, the diastereomeric (*S*;*R*)- and (*S*;*S*)-$[CB[6]\cdot MP\cdot MB]^{3+}$ ternary complexes exhibited an unprecedented 19-fold difference in stability, or an enantioselectivity of 95%, which is the best "assembled enantioselectivity" reported for a supramolecular system incorporating an achiral host. To further elucidate the

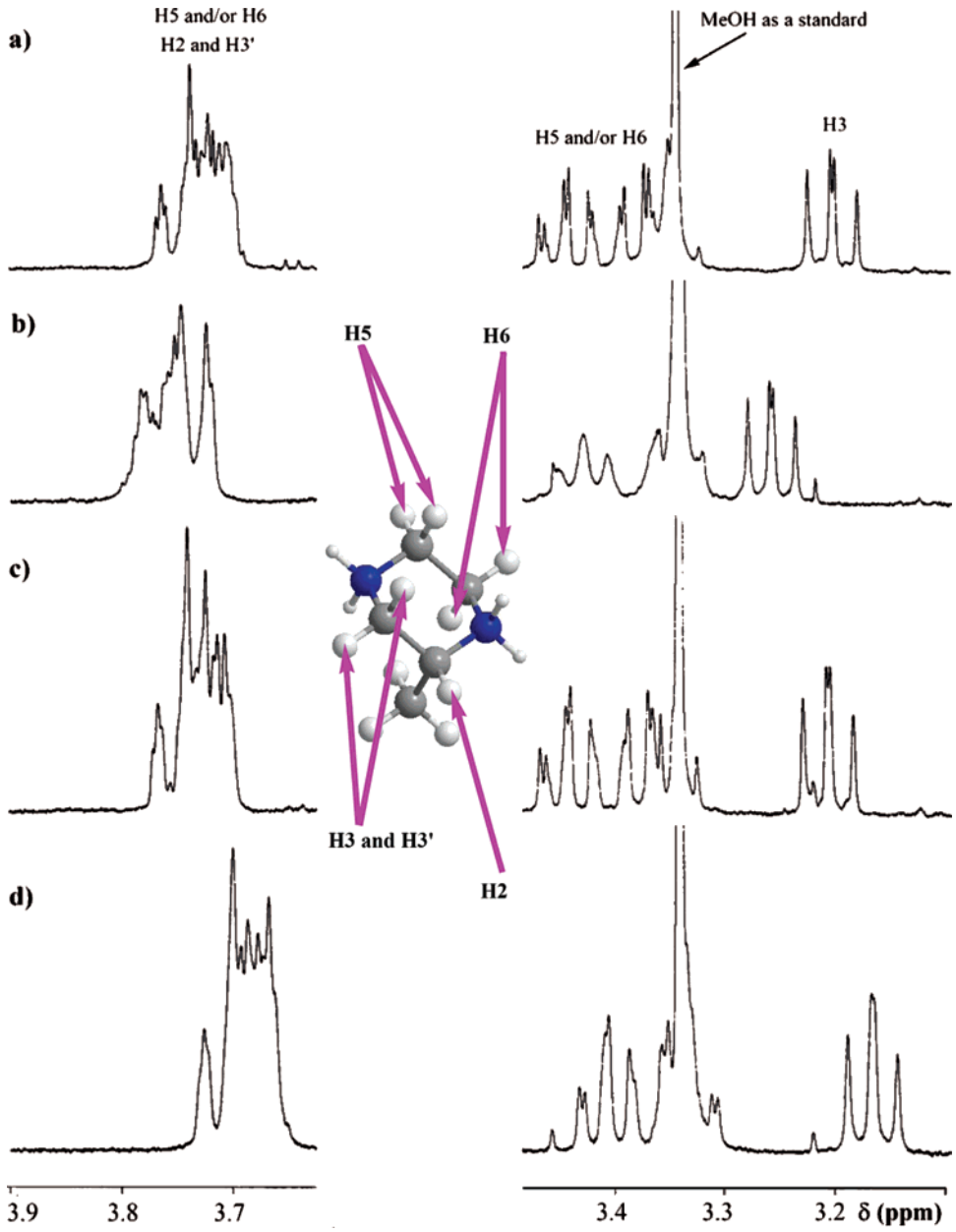

Figure 3. NMR spectra of supramolecular complexes composed of (*R*)/(*S*)-2-methylpiperazine, CB[6], and/or (*S*)-2-methylbutylamine in D_2O: (a) 5 mM (*R*)-2-methylpiperazine·2HCl, (b) 0.5 mM CB[6] + 5 mM (*R*)-2-methylpiperazine·2HCl, (c) 0.5 mM CB[6] + 0.5 mM (*S*)-2-methylbutylamine·HCl + 5 mM (*R*)-2-methylpiperazine·2HCl, and (d) 0.5 mM CB[6] + 0.5 mM (*S*)-2-methylbutylamine·HCl + 5 mM (*S*)-2-methylpiperazine·2HCl.

mechanism and the factors that lead to such a remarkable difference in complex stability, we performed additional ITC and NMR examinations.

The thermodynamic parameters for the complexation of chiral auxiliary (*S*)-MP with CB[6] in aqueous 0.1 M NaCl solution indicate that Na^+ and MP^{2+} are very comparable guests, competing for the CB[6] portals, since the replacement of Na^+ by MP^{2+} is associated with small free energy changes, as judged from the *K* values of ca. 20 M^{-1} for the successive complexation (Table 1). The favorable enthalpy changes observed for both steps strongly indicate that the MP^{2+} molecules occupy the optimal position at the CB portals, as is the case with Na^+, and therefore removal or severe displacement/dislocation of MP^{2+} from the optimal position leads to a large enthalpy loss.

The data presented in Table 1 allow us to elucidate the molecular events leading to the large assembled enantiorecognition upon formation of ternary $[CB[6]\cdot MP\cdot MB]^{3+}$ complexes. Formation of the (*S*;*S*)-$[CB[6]\cdot MP\cdot MB]^{3+}$ complex is exclusively entropy-driven and associated with a large unfavorable $\Delta H°$ of 13.4 kJ mol^{-1}. Such high endothermicity is unusual for a CB complex, and may be attributed to severe dislocation/displacement of the remaining (*S*)-MP in the CB cavity upon replacement of one of the initially bound two (*S*)-MP auxiliaries. In contrast, in the $[CB[6]\cdot 2((R)\text{-}MP)]^{4+}$ case, the remaining (*R*)-MP does not suffer such a large dislocation, probably as a result of smaller steric hindrance within the CB cavity, to afford the moderately exothermic $\Delta H°$ of −5.0 kJ mol^{-1} and the highly positive $T\Delta S°$ of 18.8 kJ mol^{-1}, which are jointly responsible for the contrasting behavior of the diastereomeric host−guest combination and the 19-fold difference in *K*.

Additional support for severe unfavorable dislocation/displacement of the remaining (*S*)-MP upon formation of the

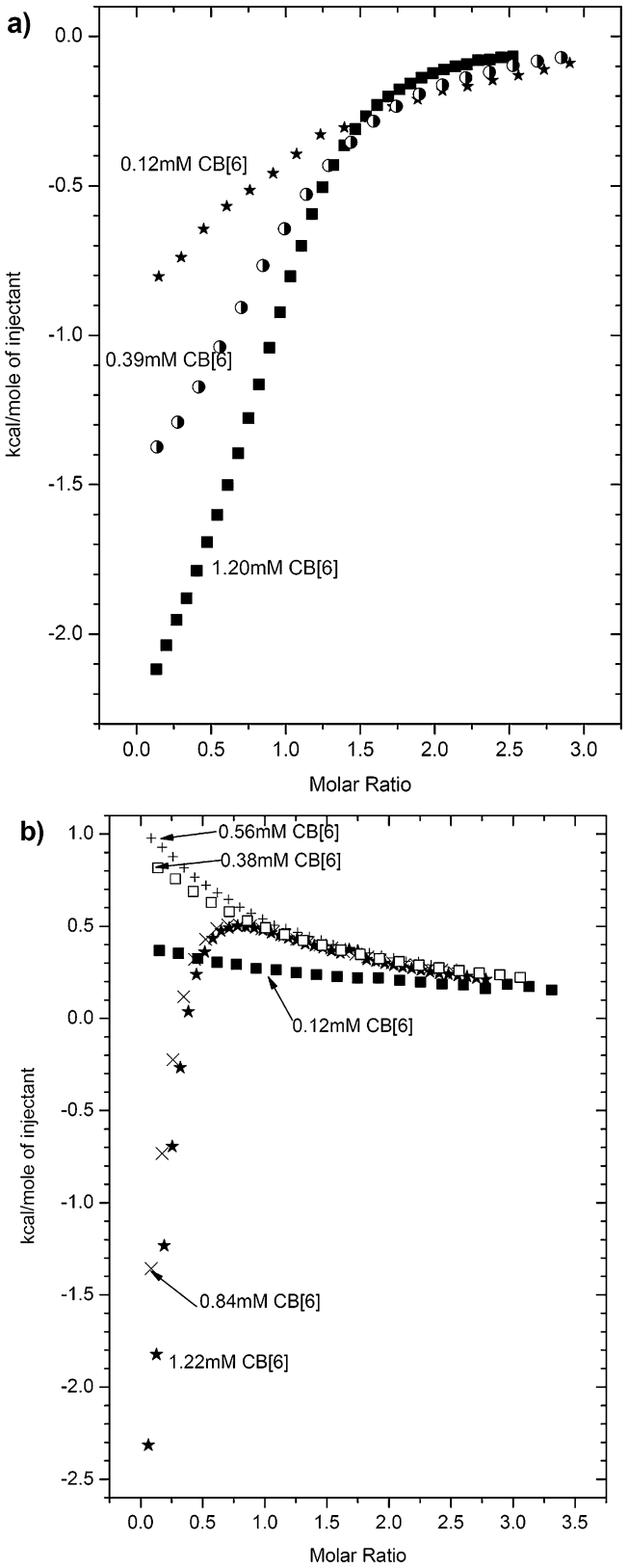

Figure 4. ITC titration curves upon addition of (*S*)-MB to solutions of CB[6] of various concentrations; experiments performed with (a) 0.025 M (*R*)-MP and (b) 0.025 M (*S*)-MP.

(*S*;*S*)-[CB[6]·MP·MB]$^{3+}$ complex can be derived from the thermodynamic parameters for the complexation of (*S*)-MB with CB[6] in 0.05 M NaCl (Table 1 and Supporting Information). Indeed, it is expected that, after insertion of the aliphatic chain of (*S*)-MB into the cavity, the small sodium cation coordinated at the opposite portal should not be significantly disturbed. Accordingly, we observed a large negative enthalpy of complexation, indicating optimization of host−guest van der Waals contacts inside the cavity and positive entropy arising from desolvation.

NMR studies provided us with strong evidence in support of the above discussion. The signals for MP's ring protons (Figure 3) are shifted downfield upon 1:2 host−guest complexation with CB[6], indicating partial insertion of the guest into the cavity. Replacement of one of the two (*R*)- or (*S*)-MP molecules by a stronger guest, i.e., (*S*)-MB, leads to the opposite upfield shifts (Figure 3), indicating displacement from the optimal position/location of the remaining (*R*)- or (*S*)-MP upon insertion of (*S*)-MB. This effect (upfield shift) is more pronounced for (*S*;*S*)-[CB[6]·MP·MB], in nice agreement with the highly endothermic $\Delta H°$ and the lower stability, suggesting larger dislocation/displacement of chiral MP auxiliary from its original optimal position/location. The MP's methyl protons also exhibit similar upfield/downfield shifts upon complexation, but the observed shifts are smaller (Supporting Information).

Interestingly, supramolecular interactions in a solution containing (*S*)-MB and (*S*;*S*)-[CB[6]·2MP]$^{4+}$ or (*R*;*R*)-[CB[6]·2MP]$^{4+}$ appear to be more complex than those of ternary (*S*;*S*)-[CB[6]·MP·MB]$^{3+}$ or (*S*;*R*)-[CB[6]·MP·MB]$^{3+}$ species only. These ternary complexes are the predominant species only at very low concentrations of CB[6] in the range of 0.1−0.3 mM. The "Single Set of Identical Sites" model was successful in simulating the experimental data obtained at a 0.12 mM concentration of (*S*;*S*)-[CB[6]·2MP]$^{4+}$ or (*R*;*R*)-[CB[6]·2MP]$^{4+}$ (Figure 4). Previously,[14,15] we discussed in more detail the gradual simplification of a reaction mixture upon dilution, eventually leading to the simplest possible species. In the present case, these simplest species are ternary (*S*;*S*)-[CB[6]·MP·MB]$^{3+}$ or (*S*;*R*)-[CB[6]·MP·MB]$^{3+}$ complexes. The thermodynamic parameters of the ternary complex formation are presented in Table 1; their formation is predominantly (for (*S*;*R*)-[CB[6]·MP·MB]$^{3+}$) or even exclusively (for (*S*;*S*)-[CB[6]·MP·MB]$^{3+}$) entropy-driven.

Higher CB[6] concentrations lead to spontaneous supramolecular assembling, as indicated by the results of ITC experiments (Figure 4). To reveal the molecular structure of supramolecular assemblies in the solution, we apply light-scattering and ESI-MS techniques. We estimated that the average molecular weight of supramolecular aggregates in 1.2 mM CB[6] solution (the same concentration used for ITC experiments; Table 1) is 2.2 kDa, which corresponds to a dimer composed of [MP·CB-[6]·MP·CB[6]·MP]. We succeeded in detecting the cation [MP·CB[6]·MP·CB[6]·MP]$^{3+}$ using ESI-MS techniques (Supporting Information). Certainly, the molecular weight of 2.2 kDa obtained from light-scattering experiments represents an average size of the nanoparticles, and in the real solution there must be a distribution among monomer, dimer, trimer, and probably some higher molecular aggregates. It is reasonable to expect that, at higher CB[6] concentrations, the distribution is shifted to the higher molecular weight side. Indeed, the average

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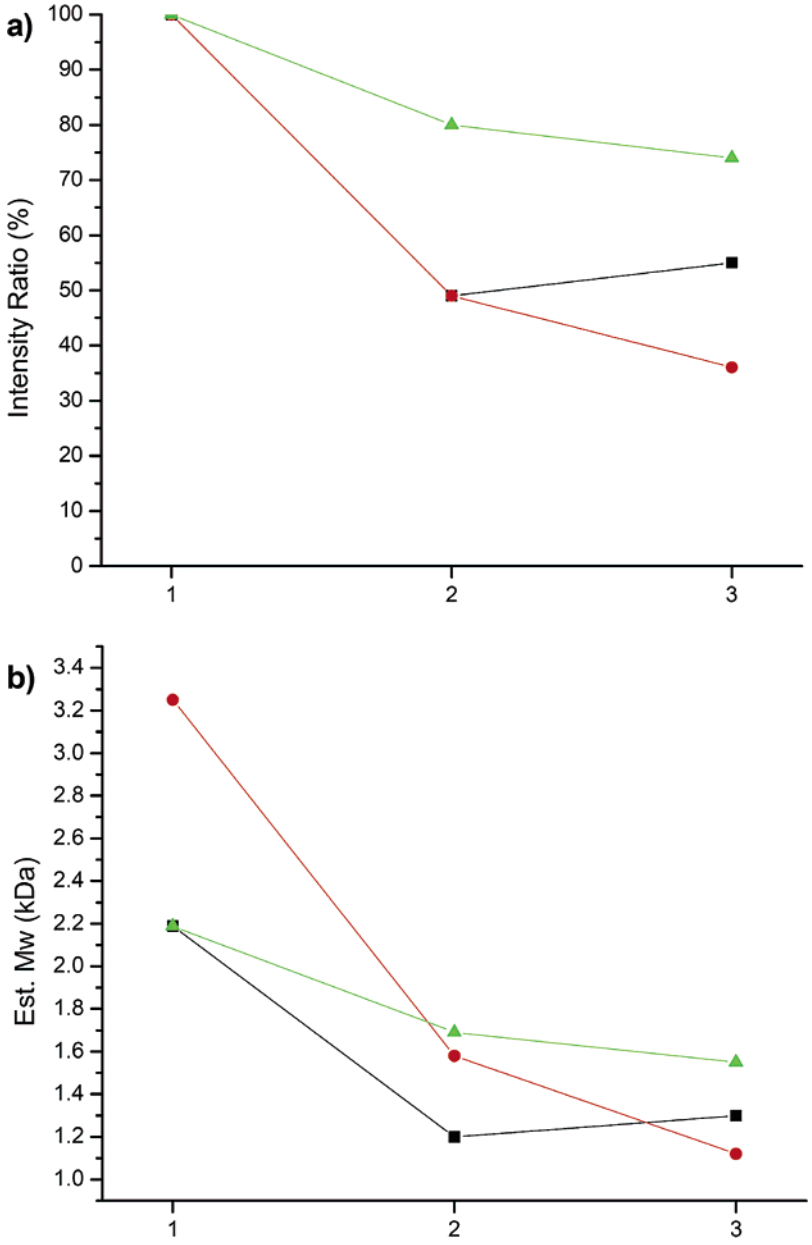

Figure 5. Results of light-scattering experiments: (a) intensity of scattered light and (b) estimated molecular weight of nanoparticles in solutions 1−3 (indicated in the horizontal scale) of varying MB concentrations, which contain (*R*)-MP (25 mM), CB[6] (4 mM), and (*S*)-MB at 0, 0.7, and 4 mM (red circles); (*R*)-MP (25 mM), CB[6] (1.2 mM), and (*S*)-MB at 0, 0.24, and 1.2 mM (black squares); and (*S*)-MP (25 mM), CB[6] (1.2 mM), and (*S*)-MB at 0, 0.24, and 1.2 mM (green triangles).

molecular weight of supramolecular aggregates in the presence of 4 mM CB[6] in the solution was determined as 3.2 kDa (Figure 5).

Addition of (*S*)-MB into the solution leads to gradual reduction of the size of the nanoparticles, and the solution of (*S*)-MB and CB[6] in equal concentrations contains predominantly the monomeric species, i.e., (*S*;*S*)-[CB[6]·MP·MB]$^{3+}$ or (*S*;*R*)-[CB[6]·MP·MB]$^{3+}$ (Figure 5). It should be emphasized that the molecular weight of the supramolecular aggregates in the solution of (*R*)-MP and CB[6] decreases faster than that in the solution of (*S*)-MP and CB[6] of the same concentration (Figure 5). This is consistent with the higher stability of (*S*;*R*)-[CB[6]·MP·MB]$^{3+}$ than that of (*S*;*S*)-[CB[6]·MP·MB]$^{3+}$ obtained in the ITC experiments (Table 1). Thus, a smaller concentration of (*S*)-MB is necessary to shift the equilibrium to the ternary monomeric complex, and consequently, it is easy to destroy higher molecular aggregates. The results of the NMR study are also consistent with the dominant formation of the monomeric ternary complex in the solution where (*S*)-MB is equal to CB[6] in concentration. Indeed, the observation of two doublets of equal intensity for each of Hx and Hy protons of CB[6] (Supporting Information) unequivocally reveals that the two portals of CB[6] are not identical to each other, and one portal is occupied by (*S*)-MB but another by (*R*)- or (*S*)-MP. Since the supramolecular complex formed from (*R*)-MP, (*S*)-MB, and CB[6] is diastereomeric to that from (*S*)-MP, (*S*)-MB, and CB[6], they may exhibit separate distinguishing patterns if the NMR spectra are measured in the presence of both (*R*)-MP and (*S*)-MP. However, in reality, it appears that exchange of (*R*)- and (*S*)-MP molecules between supramolecular species and bulk solution is very fast on the NMR time scale, and we

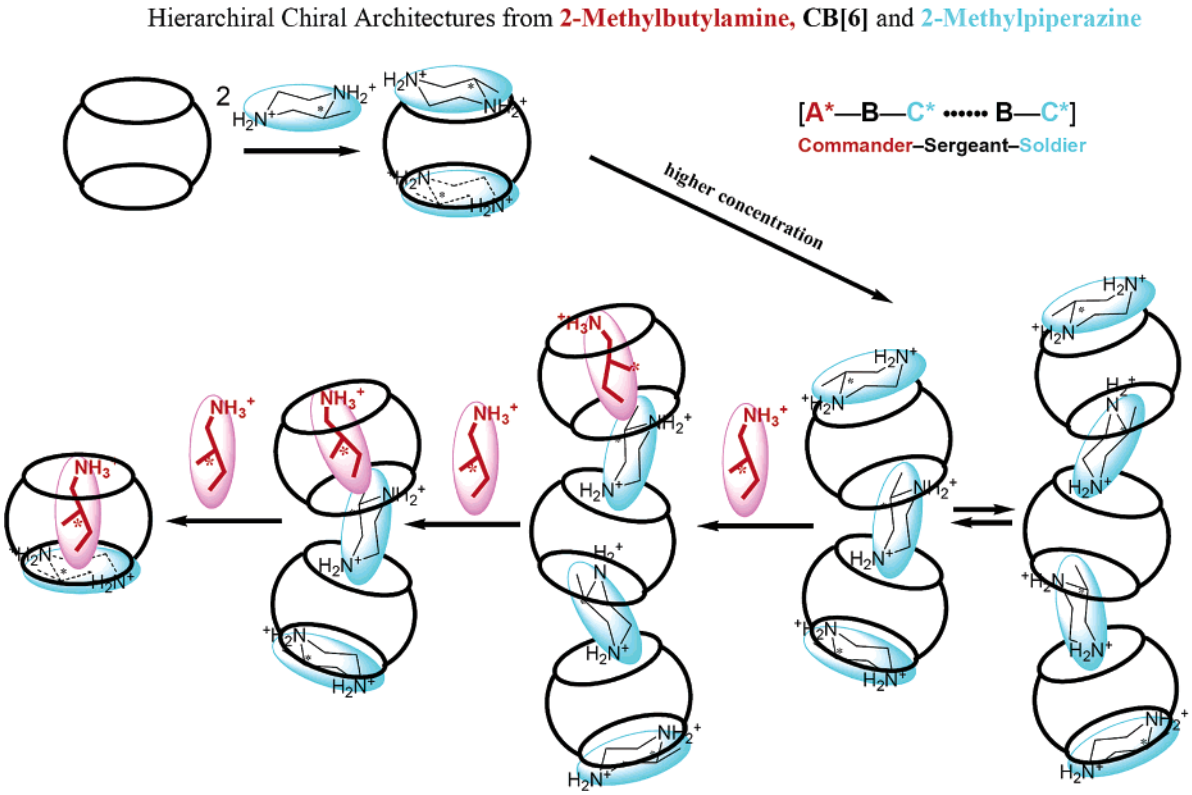

Figure 6. Hierarchical supramolecular assembling in the three-component "Commander−Sergeants−Soldiers" system, composed of (*S*)-2-methylbutylamine (A), CB[6] (B), and (*R*/*S*)-2-methylpiperazine (C).

Table 2. Stability Constant (K) and Standard Enthalpy ($\Delta H°$) and Entropy Changes ($T\Delta S°$) for Complexation of Cucurbit[7]uril (CB[7]) with Various Phenylalanine-Containing Dipeptides[a] in Aqueous 0.1 M NaCl Solutions at 298.15 K

complexation reaction	K/M^{-1}	K_{LL}/K_{DL} or K_{LL}/K_{LD}	$\Delta H°$/kJ mol^{-1}	$T\Delta S°$/kJ mol^{-1}
$[CB[7]\cdot 2Na]^{2+}$ + Phe-Ala^{+-} → $[CB[7]\cdot$Phe-Ala$\cdot Na]^{+(+-)}$ + Na^+	$(7.9 \pm 0.3) \times 10^5$	0.61	-30.6 ± 0.3	3.1 ± 0.4
$[CB[7]\cdot 2Na]^{2+}$ + D-Phe-Ala^{+} → $[CB[7]\cdot$D-Phe-Ala$\cdot Na]^{+(+-)}$ + Na^+	$(1.3 \pm 0.1) \times 10^6$		-32.5 ± 0.3	2.4 ± 0.4
$[CB[7]\cdot 2Na]^{2+}$ + Phe-Pro^{+-} → $[CB[7]\cdot$Phe-Pro$\cdot Na]^{+(+-)}$ + Na^+	$(2.9 \pm 0.2) \times 10^4$	0.58	-24.8 ± 0.3	0.7 ± 0.4
$[CB[7]\cdot 2Na]^{2+}$ + Phe-D-Pro^{+-} → $[CB[7]\cdot$Phe-D-Pro$\cdot Na]^{+(+-)}$ + Na^+	$(5.0 \pm 0.2) \times 10^4$		-27.1 ± 0.3	-0.3 ± 0.4
$[CB[7]\cdot 2Na]^{2+}$ + NH_3^+-Phe-Phe-$CONH_2$ → $[CB[7]\cdot NH_3$-Phe-Phe-$CONH_2\cdot Na]^{2+}$ + Na^+	$(5.3 \pm 0.2) \times 10^6$	4.1	-36.6 ± 0.4	1.8 ± 0.5
$[CB[7]\cdot 2Na]^{2+}$ + NH_3^+-D-Phe-Phe-$CONH_2$ → $[CB[7]\cdot NH_3$-D-Phe-Phe-$CONH_2\cdot Na]^{2+}$ + Na^+	$(1.3 \pm 0.1) \times 10^6$		-29.0 ± 0.3	5.9 ± 0.4
$[CB[7]\cdot 2Na]^{2+}$ + NH_3^+-Phe-Leu-$CONH_2$ → $[CB[7]\cdot NH_3$-Phe-Leu-$CONH_2\cdot Na]^{2+}$ + Na^+	$(1.4 \pm 0.2) \times 10^7$	8.2	-36.4 ± 0.4	4.4 ± 0.5
$[CB[7]\cdot 2Na]^{2+}$ + NH_3^+-Phe-D-Leu-$CONH_2$ → $[CB[7]\cdot NH_3$-Phe-D-Leu-$CONH_2\cdot Na]^{2+}$ + Na^+	$(1.7 \pm 0.1) \times 10^6$		-24.2 ± 0.3	11.4 ± 0.4

[a] L-Amino acid residue used unless stated otherwise.

observed only averaged signals for all species existing in the solution (Supporting Information).

By combining the results of light-scattering, ITC, NMR, and ESI-MS experiments, we can conclude that the molecular events occurring in the CB[6]−MP−MB system involve gradual reduction of the size/weight of oligomeric nanoparticles existing in the initial solution of CB[6] and (*R*)- or (*S*)-MP upon subsequent addition of (*S*)-MB, as illustrated in Figure 6. Addition of (*S*)-MB is considered as an interesting extension of the "Soldiers and Sergeants" principle, which has amply been observed in several supramolecular systems.[16−22] However, in our three-component system, only one enantiopure molecule (*S*)-MB controls the size/weight of oligomeric nanoparticles composed of enantiopure (*R*)-MP and CB[6] or enantiopure (*S*)-MP and CB[6]. Since nanoparticles consisting of (*R*)-MP/(*S*)-MB/CB[6] vs (*S*)-MP/(*S*)-MB/CB[6] are diastereomeric species, they possess distinctly different properties, as demonstrated in our ITC and light-scattering experiments. The properties of our three-component system are ruled by the new "Commander−Sergeants−Soldiers" principle. First, the strong binder, 2-methylbutylamine (MB), functioning as the Commander, replaces one 2-methylpiperazines (MP) under the control of CB[6] in the original oligomeric species (see the above discussion). Second, upon gradual increase of the MB concentration (too

many Commanders), the size/weight of oligomeric nanoparticles decreases, ultimately leading to monomeric ternary 1:1:1 CB-[6]−MP−MB complexes.

Diastereorecognition in the Cucurbit[7]uril Cavity. Above, we demonstrated that the right choice of two different chiral guests, which can be simultaneously accommodated in the same CB[6] cavity, forming a 1:1:1 complex, leads to an unprecedented assembled enantioselectivity. Indeed, the stability of (*S*;*R*)-[CB[6]·MP·MB]$^{3+}$ is 19 times higher than that of the diastereomeric (*S*;*S*)-[CB[6]·MP·MB]$^{3+}$. In this case, the chiral centers of the two guests are not connected by a covalent bond. However, it is reasonable to assume that an appropriate selection of a guest having two chiral centers connected covalently may also reveal high chiral recognition in the achiral CB cavity if the guest size, the distance between chiral centers, and the functional groups are carefully considered. Consequently, CB complexation reactions gave us a rare opportunity to compare diastereorecognition in a pair of two separate enantiomers, i.e., MP + MB, with the pair(s) of "classical" diastereomers, in which two asymmetric carbons are located in the same molecule. A large variety of dipeptides, which are readily available, are certainly among the best targets. CB[7] forms complexes with larger guest molecules that are not included in CB[6].[6,23] For example, CB[7] forms a 1:1 complex with a variety of positively charged aromatic compounds, including naphthalene, viologen, stilbene, and ferrocene derivatives.[5,23a−h] Recently, we also found that zwitterionic phenylalanine interacts strongly with CB-[7] in an aqueous 0.1 M NaCl solution ($K = 2.5 \times 10^4$ M^{-1}) and even more strongly in pure water ($K = 1.8 \times 10^6$ M^{-1}).[23h] This is an interesting finding, since it was believed that only positively charged and/or neutral compounds form strong complexes with CB[7].[6]

Various phenylalanine-containing dipeptide diastereomers were subjected to the ITC experiments employing CB[7] as host molecule. We selected two diastereomeric pairs of zwitterionic dipeptides, i.e., (1) L-Phe-L-Ala versus D-Phe-L-Ala and (2) L-Phe-L-Pro versus L-Phe-D-Pro, and two diastereomeric pairs of positively charged dipeptide amides, i.e., (1) NH_3^+-L-Phe-L-Phe-$CONH_2$ versus NH_3^+-D-Phe-L-Phe-$CONH_2$ and (2) NH_3^+-L-Phe-L-Leu-$CONH_2$ versus NH_3^+-L-Phe-D-Leu-$CONH_2$. The choice of a solution appropriate for the ITC experiment is also

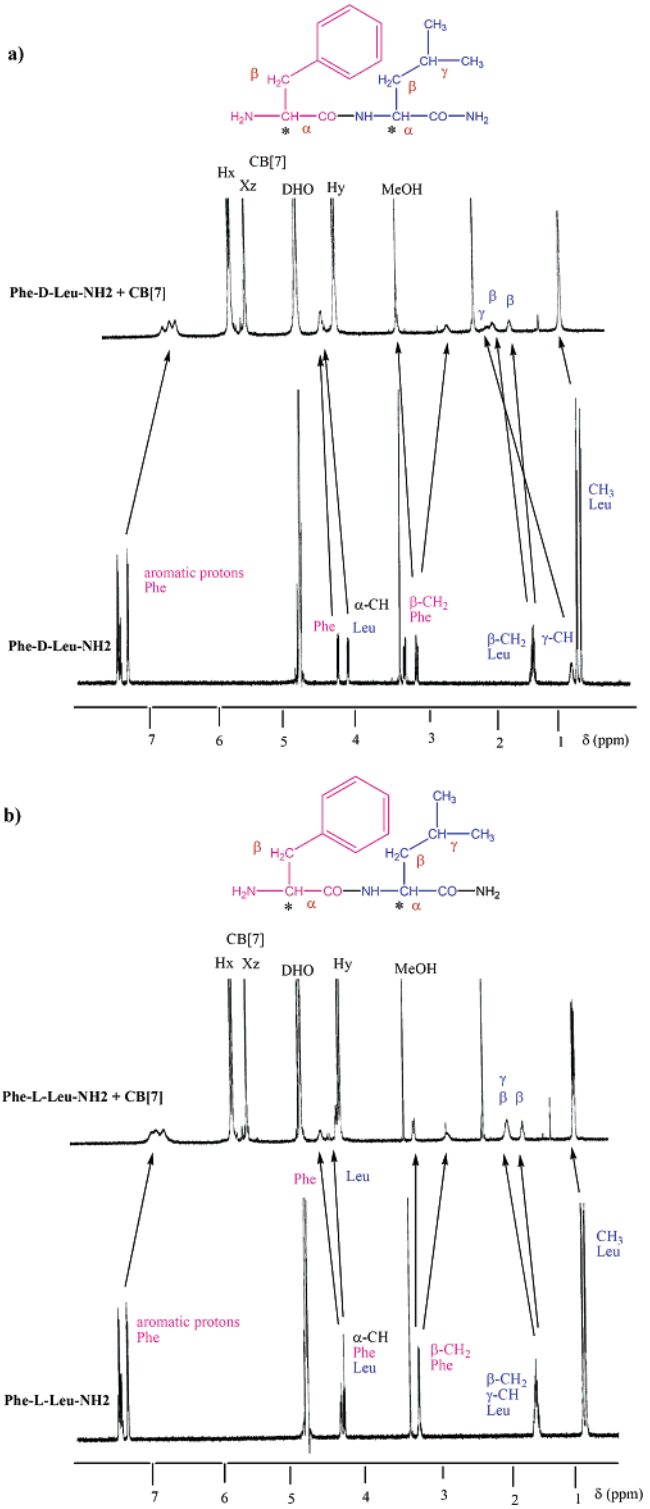

Figure 7. ^{1}H NMR spectra of (a) L-Phe-D-Leu-NH_2 (5 mM) and (b) L-Phe-L-Leu-NH_2 (5 mM) in the absence and in the presence of 1 equiv CB[7] in 0.1 M NaCl in D_2O.

an important issue. It is widely recognized that addition of hydrophobic residues and/or positive charges to a guest leads, in general, to a large enhancement of the affinity toward CB[7] and other CB homologues.[1a,6,24] Thus, we may expect much higher affinities for the selected dipeptides compared to zwitterionic phenylalanine. Taking into account the instrumental

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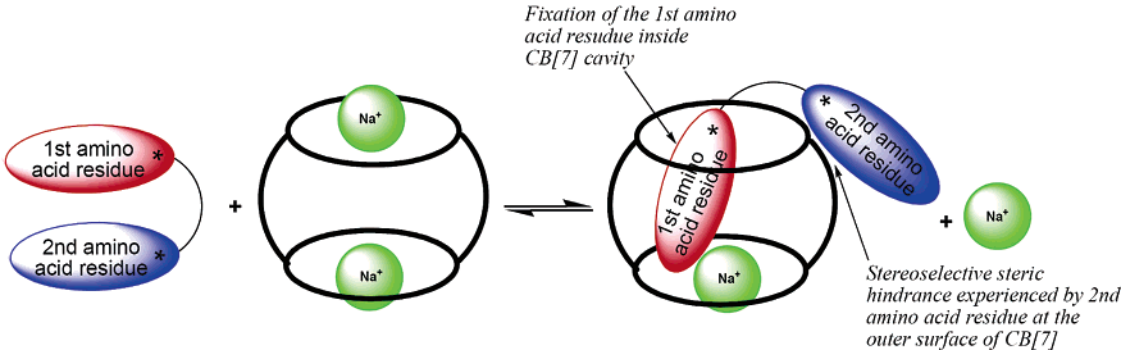

Figure 8. Molecular events leading to diastereodifferentiation upon interaction of Phe-containing dipeptides with CB[7].

upper limit of ITC in affinity determination of about 10^7-10^8 M^{-1}, we used aqueous 0.1 M NaCl solution rather than pure water. The results of our ITC experiments are given in Table 2.

Phe-Ala is the simplest diastereomeric dipeptide possessing a Phe residue at the N-terminus. The D,L-isomer reveals 1.65 times higher affinity for CB[7] than the L,L-isomer. This affinity enhancement is entirely enthalpic in origin. For a higher degree of diastereomeric recognition, we further examined Phe-Pro. The idea was to exploit the rigidity of the proline ring, with the expectation that one of the diastereomers would fit nicely into the CB[7] cavity, while the other causes large steric hindrance, leading to higher discrimination. However, in reality the affinities of the two Phe-Pro diastereomers are 25−30 times lower than those of the corresponding Phe-Ala, and the affinity enhancement for L-Phe-D-Pro versus L-Phe-L-Pro was merely 1.72 times, which is about the same as the value observed for Phe-Ala diastereomers.

Insertion of a zwitterionic guest into a CB[7] cavity is illustrated by three successive events: (a) inclusion of the hydrophobic moiety of the guest in the CB cavity, (b) disengagement of the ion-pairing interactions in the zwitterionic guest to liberate the ammonium ion (and also to expel the negatively charged carboxylate from the CB portal), and (c) coordination of the guest's ammonium ion by the carbonyl oxygens at the CB portal. From this picture, one may expect that zwitterionic and positively charged dipeptides display different chiral preferences toward the CB[7] cavity, simply because there is no second molecular event that disengages the ion-pairing interactions in the latter case. The lower affinities observed for the homochiral zwitterionic dipeptides than for their heterochiral analogues indicate that it is sterically more difficult in the former case to expel the negatively charged carboxylate from the CB portal upon complexation, with the remaining electrostatic repulsion between the carboxylate and portal oxygens resulting in the lowering of affinity. In contrast, in the positively charged dipeptide case, there is no electrostatic host−guest repulsion, and thus all dipeptide residues can participate in the formation of a strong complex. Consequently, upon complexation, the conformation of the homochiral dipeptide, which is unfavorable for optimal disengagement of the ion-pairing interactions, would be most likely favorable for optimizing the short-range van der Waals interactions inside the cavity, eventually leading to the enhanced affinity. This simple structural rationale is well compatible with the experimental data. Indeed, the complex stabilities of NH_3^+-L-Phe-L-Phe-$CONH_2$ and NH_3^+-L-Phe-L-Leu-$CONH_2$ with CB[7] are larger than those for the corresponding D,L- or L,D-diastereomers (Table 2).

A higher degree of diastereomeric recognition is expected to occur with positively charged dipeptides than with zwitterionic guests. Indeed, the major origin of the recognition of zwitterionic guests is the long-reaching electrostatic repulsion of the guest's carboxylate against the host's carbonyl oxygens. In contrast, with dipeptide amides, the origin of recognition is the short-range van der Waals interactions. In good agreement with the above discussion, both peptides reveal higher diastereomeric selectivities of 4.1 for NH_3^+-L-Phe-L-Phe-$CONH_2$ versus NH_3^+-D-Phe-L-Phe-$CONH_2$ and of 8.2 for NH_3^+-L-Phe-L-Leu-$CONH_2$ versus NH_3^+-L-Phe-D-Leu-$CONH_2$.

To gain a better understanding of the molecular origin of the high diastereomeric recognition, we subjected the diastereomeric complex pair of [CB[7]·NH_3^+-L-Phe-L-Leu-$CONH_2$] and [CB[7]·NH_3^+-L-Phe-D-Leu-NH_2] to NMR study. As shown in Figure 7, the signals for the aromatic protons of Phe are shifted upfield (strongly indicating the insertion of the aromatic ring into the cavity), while those for the protons in the Leu side group are shifted downfield, suggesting that this group is located outside the cavity. The ROESY spectra (Supporting Information) clearly reveal that the Leu residue is residing outside the cavity, since the NOE signals are seen between the aliphatic protons of Leu residue and the CB[7] protons located outside the cavity. Unusually, the large downfield shift of the γ-CH proton of the Leu residue in the [CB[7]·NH_3^+-L-Phe-L-Leu-$CONH_2$] complex and the large upfield shift of the β-CH proton of Phe in the [CB[7]·NH_3^+-L-Phe-D-Leu-$CONH_2$] complex underscore the differences in the structures of these two complexes. Missing NOE signals between one of β-CH protons of Leu and the outer surface protons of CB[7] in the [CB[7]·NH_3^+-L-Phe-L-Leu-$CONH_2$] complex (Supporting Information) suggest the deeper penetration of Phe compared with that in the diastereomeric [CB[7]·NH_3^+-L-Phe-D-Leu-NH_2]. Indeed, a deeper insertion of the Phe residue reduces the effective length of the peptide chain outside the cavity, which is necessary to place the β-CH proton in the nearest proximity of the outside host protons. As amply observed in host−guest chemistry, deeper insertion leads to improvement of the direct van der Waals contacts and is therefore associated with large favorable (negative) enthalpy changes (Table 2).

Figure 8 is a schematic illustration of molecular events leading to diastereodifferentiation upon interaction of Phe-containing dipeptides with CB[7], as elucidated from the NMR and ITC data. Insertion of the Phe residue into the cavity leads to

conformational restriction (fixation) of the guest, allowing a pronounced stereoselective interaction of the second amino acid residue at the outer surface of CB[7].

The observed affinity enhancement is clearly enthalpy-driven for all the diastereomeric recognition processes shown in Table 2, and thus we conclude that the optimization of van der Waals interactions is the major factor that determines the magnitude of diastereomeric recognition. It should be emphasized that the host's chirality (chiral β-CD or achiral CB[7]) plays practically no role in the recognition of Phe-containing dipeptides, since nearly the same magnitude of diastereomeric recognition was reported for inclusion of Phe-containing dipeptides with chiral β-CD.[25] Consequently, we may predict that any hydrophobic cavity of appropriate size and shape can serve as an effective selector to recognize, distinguish, or separate homochiral from heterochiral dipeptides (oligopeptides) with hydrophobic residues.

Interestingly, the magnitude of recognition of all the diastereomeric dipeptides listed in Table 2 by CB[7] is actually low as compared with that of (*S*)-MB + (*R*)-MP vs (*S*)-MB + (*S*)-MP by CB[6], underscoring the high potential of the supramolecular approach for the design of hosts for enantiorecognition. It should also be noted that both types of recognition reactions (dipeptides by CB[7] and MB + MP by CB[6]) reveal similarities in their thermodynamics. Indeed, in both cases, increasing affinity is exclusively enthalpy-driven, thus emphasizing the importance of optimization of intracavity host−guest interactions.

Conclusions

The main findings of this first comprehensive study on the chiral recognition of guests by achiral hosts (CBs) using calorimetric, light-scattering, nuclear magnetic, and mass spectral techniques can be summarized as follows:

(1) The assembled enantiorecognition of MB of up to 95% ee (or 19 times difference in affinity), achieved upon formation of diastereomeric (*S*;*R*)- and (*S*;*S*)-[CB[6]·MP·MB]$^{3+}$ ternary complexes, is the highest chiral recognition reported for an achiral host.

(2) An interesting expansion of the "Soldiers and Sergeants" principle was realized in the three-component system, where only one enantiopure molecule, (*S*)-MB, controls the degree of chiral supramolecular assembling of (*R*)-MP or (*S*)-MP with CB[6], which is strictly ruled by the novel hierarchical "Commander−Sergeants−Soldiers" principle.

(3) A diastereomeric pair of two separate enantiomers can exhibit similar or even higher selectivity toward an achiral host as compared with the pair of "classical" diastereomers in similar complexation reactions, as demonstrated by comparison of a MP + MB diastereomeric pair versus diastereomeric dipeptides.

These results further indicate that an inherently chiral host is not necessarily required for chiral recognition, but a combination of achiral host (CB) and chiral inductor can also serve as a supramolecular chiral host for such a purpose, where the chiral recognition ability may be tuned by choosing the chiral inductor. The "Commander−Sergeants−Soldiers" system will not be restricted to the combination of CB, strong binders, and weaker binders, but the basic concept could potentially be expanded to other supramolecular systems.

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Supporting Information Available: Figures showing interaction of (*S*)-MB cation with CB[6] in aqueous 0.1 M NaCl; methyl proton signals of MP in NMR spectra of D_2O solutions; ESI-MS spectra of an aqueous solution of CB[6], (*R*)/(*S*)-MP, and (*S*)-MB; NMR spectra of D_2O solutions of mixtures of CB[6], (*R*)-MP·2HCl, (*S*)-MB·2HCl, and (*S*)-MB·HCl; NMR spectrum of a mixture of diastereomeric complexes; and ROESY spectra of complexes of CB[7] with Phe-D-Leu-NH_2 and Phe-L-Leu-NH_2. This material is available free of charge via the Internet at http://pubs.acs.org.

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