Host–Guest Systems

Ditopic Complexation and Release of Neutral Guest Molecules by a Hydrogen-Bonded "*Endo–Exo*" Receptor

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The highest level of sophistication in noncovalent chemistry is found in living systems, where elegant supramolecular assemblies make up the machinery that enables and supports functions important for life. The understanding of the mechanism for (bio)molecular recognition is also crucial in the development of new drugs and for the mimicry of the activity of biosystems.[1–3] The principles of biomolecular recognition[4] have successfully been extended to synthetic receptors[5] that are able to bind guest species such as cations,[6] anions,[7] or small neutral molecules.[8] The study of different degrees of complexity in the molecular recognition process is also important for the understanding of different biomolecular processes. For example, at the active site of enzymes, strict recognition of the transition state by the enzyme is required (selective *endo* recognition), whereas the initial protein–protein recognition can be more loose and flexible (nonselective *exo* recognition). Nevertheless, and to the best of our knowledge, there are no synthetic examples in the literature that exploit the diverse levels (that is, *endo/exo*, selective/nonselective) of molecular recognition encountered in nature.

Further control over the complexity of the molecular recognition process can also be achieved through the formation of multiple interactions at different areas of the host molecule, as observed at antibody–antigen interfaces,[9] as well as by the interaction of one host with two or more different guest molecules.[10] There are only a few examples of synthetic receptors capable of complexing two different types of guest molecules, usually cations and anions.[11] However, the covalent synthesis of these receptors remains elusive and time consuming because of the complexity of the functionalities needed to complex the two different types of guest. Self-assembly provides a simpler and faster way to bring together the desired functionalities for the recognition of the guest,[12] but it is necessary to avoid interfering with the required functionalities during the self-assembly of the receptor. Thus, the complexation of two (or more) different neutral guest molecules with one synthetic receptor is rare and has only been achieved in the interior of capsulelike assemblies (*endo* receptors)[13] and other cavities by using the principles of crystal engineering.[14]

Herein, we report the first (noncovalent) receptor able to act simultaneously as an *endo* and *exo* receptor for neutral molecules (Figure 1). This hydrogen-bonded receptor is able to *selectively* encapsulate a neutral noncovalent trimer in the pocket situated in between two subdomains (floors) of the receptor (*endo* complexation) while simultaneously complexing different neutral guest molecules at the periphery of the assembly (*exo* complexation), thus resembling the different degrees of complexity encountered in biorecognition.[15] The receptor is formed by the self-assembly of nine components (three calix[4]arenes dimelamines and six barbiturate/cyanurate derivatives) through 36 cooperative hydrogen bonds. The periphery of the self-assembled receptor is decorated with six pyridyl groups that are able to complex a variety of dicarboxylic acids (nonselective process) in a 1:3 fashion through two-point hydrogen-bonding interactions. The receptor can simultaneously encapsulate a noncovalent trimer of alizarin through π–π stacking interactions. This self-assembly of the receptor and the recognition processes of the guests bring together 15 molecules with total specificity. Surprisingly, the order in which these guest molecules are added is very

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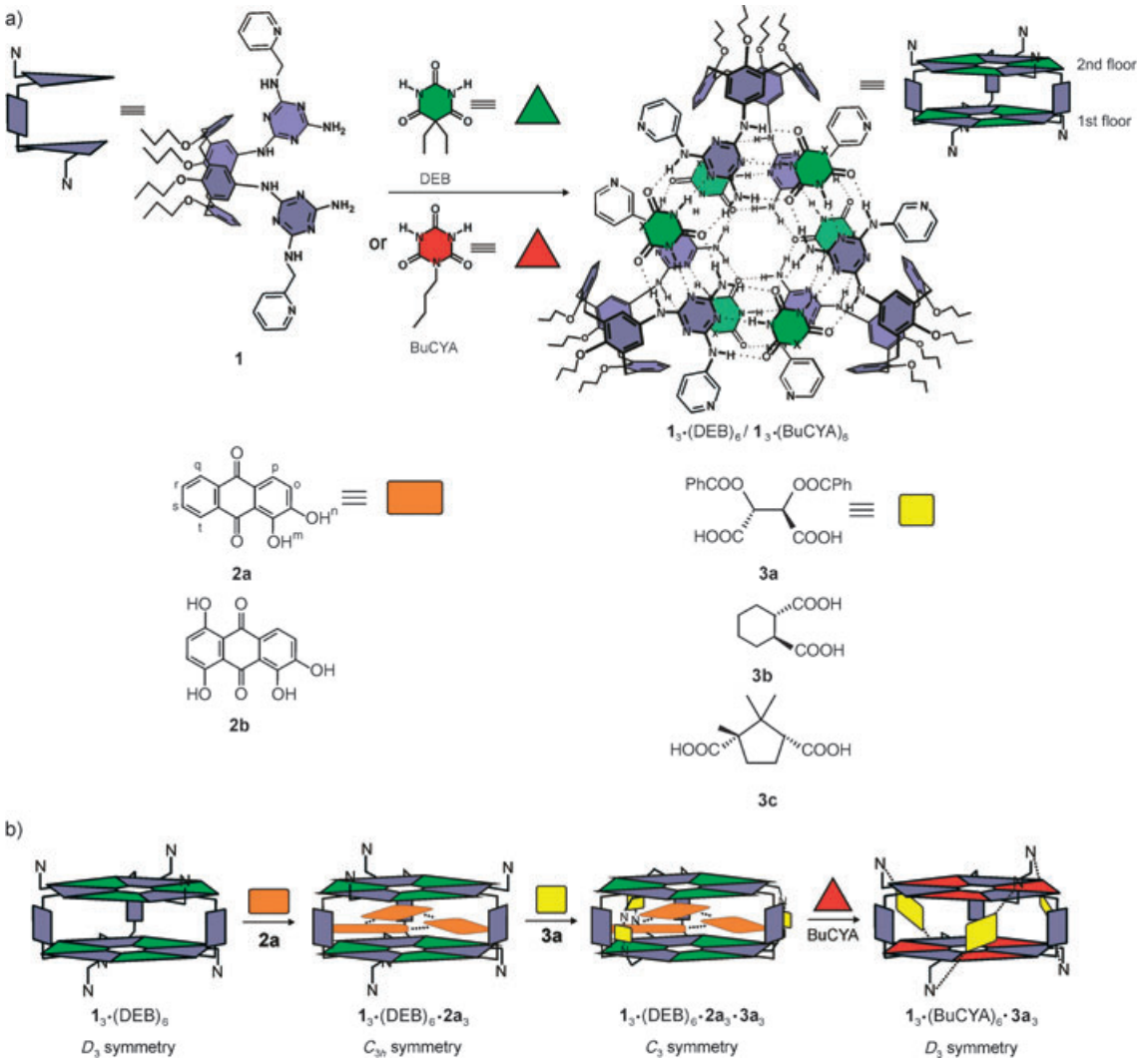

Figure 1. a) Molecular and schematic (side view) representations of the building blocks and the corresponding hydrogen-bonded assemblies $\mathbf{1}_3\cdot(DEB)_6/\mathbf{1}_3\cdot(BuCYA)_6$ and guest molecules **2a–b** and **3a–c**. b) Schematic representation of the recognition of guest **2a** and **3a** by $\mathbf{1}_3\cdot(DEB)_6/\mathbf{1}_3\cdot(BuCYA)_6$ receptors, and the release of **2a**. The corresponding symmetry of the assemblies is also given: assemblies with D_3 symmetry ($\mathbf{1}_3\cdot(DEB)_6$ and $\mathbf{1}_3\cdot(BuCYA)_6\cdot\mathbf{3a}_3$) have the two melamine rings in each calix[4]arene in a staggered orientation while these rings in assemblies with C_{3h} and C_3 symmetry ($\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3$ and $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3\cdot\mathbf{3a}_3$, respectively) have a parallel orientation.

important for the outcome of the recognition and encapsulation processes. Furthermore, the receptor has the ability to selectively release the guest molecules complexed in the internal cavity (when it receives the appropriate stimuli) while the guest molecules at the periphery remain complexed to the receptor.

Hydrogen-bonded receptors $\mathbf{1}_3\cdot(DEB)_6$ and $\mathbf{1}_3\cdot(BuCYA)_6$ are formed spontaneously upon mixing calix[4]arene dimelamine **1** and barbiturate (DEB) or cyanurate (BuCYA) derivatives, respectively, in a 1:2 ratio in apolar solvents (Figure 1).[16] The self-assembly process is driven by the cooperative formation of 36 hydrogen bonds, which leads to assemblies with a high thermodynamic stability, even at concentrations of 10^{-4} M. Despite the negative entropy arising from the assembly of nine components, the formation of the assembly is enthalpically driven ($\Delta H^{o} < 0$) as a result of the formation of the 36 hydrogen bonds between the complementary hydrogen-bonding arrays of **1** and DEB or BuCYA.

The first step in achieving the *endo–exo* complexation is the encapsulation of alizarin (**2a**) within the two rosette layers of the receptor. The ^{1}H NMR spectrum of a 1.0 mM solution of the hydrogen-bonded receptor $\mathbf{1}_3\cdot(DEB)_6$ in [D$_8$]toluene shows that nearly all the signals are shifted, both for the host assembly and for the guest molecules (Figure 2b), upon addition of three equivalents of alizarin (**2a**). For example, the signals of the NH$_{DEB}$ protons (H^a and H^b) involved in the hydrogen-bonding array shift upfield from $\delta = 14.85$ and 14.08 to 14.43 and 13.81 ppm, respectively. Similarly, proton H^i of the 2-methylpyridine substituents of the melamine rings undergoes an upfield shift of approximately 0.5 ppm upon addition of alizarin. Molecular simulation studies (Quanta 97, CHARMm 24.0) suggest that H^i is pointing towards the center of the receptor, thus resulting in the observed upfield shift in the ^{1}H NMR spectrum. Large upfield shifts were also observed for the aromatic protons of the guest molecules **2a** (> 3.0 ppm), which indicates their encapsulation in the

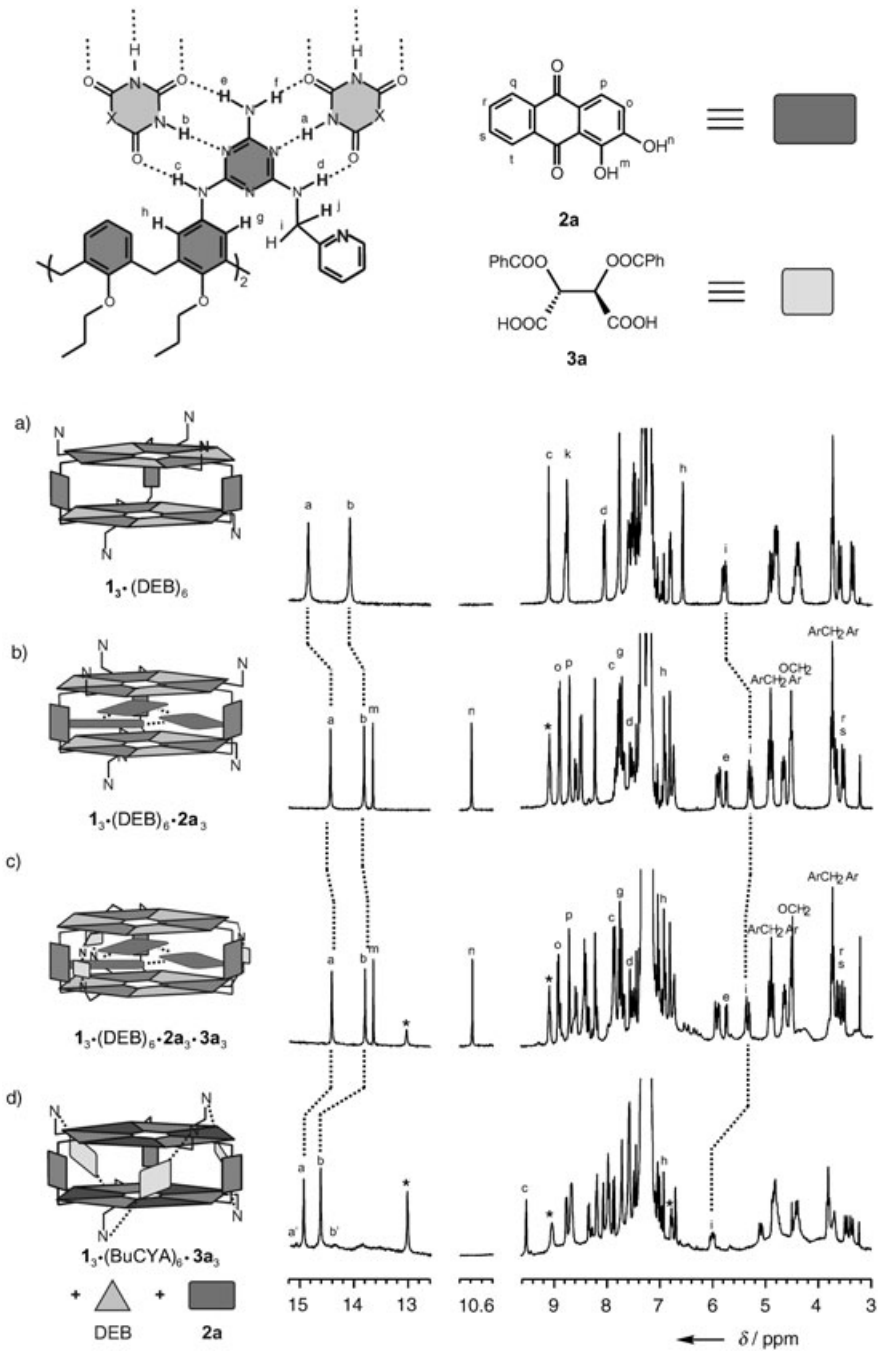

Figure 2. Parts of the ^{1}H NMR spectra (300 MHz) of a) assembly $\mathbf{1}_3$·(DEB)$_6$ (1 mM), b) $\mathbf{1}_3$·(DEB)$_6$ + 3 equiv **2a**, c) $\mathbf{1}_3$·(DEB)$_6$·$\mathbf{2a}_3$ + 3 equiv **3a**, and d) $\mathbf{1}_3$·(DEB)$_6$·$\mathbf{2a}_3$·$\mathbf{3a}_3$ + 6 equiv BuCYA. Signals marked with * belong to the free **2a**. All spectra were recorded at 298 K in [D$_8$]toluene.

interior of the assembly. Moreover, the downfield shift observed for alizarin hydroxyl OH^n proton ($\Delta\delta \approx 3.6$ ppm) indicates the formation of intermolecular hydrogen bonds between the carbonyl and hydroxy groups of adjacent guest molecules. These ^{1}H NMR studies shows clearly the encapsulation (*endo* complexation) of a noncovalent trimer of **2a** by receptor $\mathbf{1}_3$·(DEB)$_6$ to give the complex $\mathbf{1}_3$·(DEB)$_6$·$\mathbf{2a}_3$. The encapsulation of alizarin is very selective and sensitive to small structural changes. For example, no complexation was observed when protons H^q and H^t of alizarin **2a** were replaced by hydroxy groups (1,2,5,8-anthraquinone, **2b**, Figure 1).[17] The encapsulation is accompanied by a change in the symmetry of the host assembly, from D_3 to C_{3h} symmetry.[18,19]

After the encapsulation of $\mathbf{2a}_3$ in $\mathbf{1}_3$·(DEB)$_6$ had been achieved (*endo* complexation), the role of $\mathbf{1}_3$·(DEB)$_6$·$\mathbf{2a}_3$ as an *exo* receptor was studied. Addition of three equivalents of dibenzoyl-D-tartaric acid (**3a**) to the complex $\mathbf{1}_3$·(DEB)$_6$·$\mathbf{2a}_3$ resulted in upfield shifts of the signals H^a and H^b from $\delta = 14.43$ and 13.81 to 14.38 and 13.76 ppm, respectively (Figure 2c). The signal for proton H^i undergoes a downfield shift of approximately 0.1 ppm. The signal of H^i at $\delta = 5.40$ ppm is indicative of the formation of hydrogen bonds between the 2-methylpyridyl substituents of the calix[4]arene dimelamine and the diacid **3a**.[20] The stoichiometry of the complexation of **3a** by $\mathbf{1}_3$·(DEB)$_6$·$\mathbf{2a}_3$ is not clear from these ^{1}H NMR studies. However, circular dichroism (CD) studies (data not shown) on the complexation of **3a** by receptor $\mathbf{1}_3$·(BuCYA)$_6$ clearly showed the formation of a complex with 1:2 stoichiometry as the major species, although complexes with 1:1 and 1:3 stoichiometries were also present as minor species, probably because of steric effects or allosteric conformational changes.[21] The acids interact with the receptor through two-point interactions. These interactions are only possible "sideways", that is, each diacid interacts with both floors of the double rosette (Figure 1b), with one carboxylic acid of the guest hydrogen bonded to the pyridyl ring of the calix[4]arene dimelamine in the first floor while the other acid group of the same guest interacts with the other pyridyl group of the same calix[4]arene dimelamine (second floor).

The *exo* complexation is not very substrate selective: other diacids with different chemical structure, such as (1*R*,2*R*)-cyclohexane-1,2-dicarboxylic acid (**3b**) and (1*S*,3*R*)-camphoric acid (**3c**, Figure 1a), are also complexed in a similar fashion (data not shown).

Hence, the data show the complexation of six molecules of two different neutral guest molecules (alizarin **2a** and diacid **3a**). The hydrogen-bonded trimer $\mathbf{2a}_3$ is encapsulated in the internal cavity of the receptor $\mathbf{1}_3$·DEB$_6$ while three molecules of guest **3a** are complexed at the periphery of the host assembly. The receptor has two different degrees of selectivity in the molecular recognition process; that is, the *endo* recognition is very selective and sensitive to the structure of the guest while the *exo* recognition is not structurally very demanding. The relative

orientation of the melamine groups (eclipsed) is preserved during the complexation of **3a** and results in the complex $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3\cdot\mathbf{3a}_3$ with C_3 symmetry.

The controlled release of the guest molecules from the hydrogen-bonded complex $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3\cdot\mathbf{3a}_3$ was subsequently studied.[22] Cyanurates form stronger hydrogen bonds with melamines than do barbiturates, thus allowing the exchange of DEB for BuCYA.[23] The addition of the BuCYA to the complex $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3\cdot\mathbf{3a}_3$ results in the selective release of the hydrogen-bonded trimer $\mathbf{2a}_3$ to give the complex $\mathbf{1}_3\cdot(BuCYA)_6\cdot\mathbf{3a}_3$ in which the guest molecules at the periphery remain complexed. The release of the three guest molecules **2a** is achieved because cyanurate-based assemblies are not able to encapsulate **2a** as a result of geometrical differences between barbiturate- and cyanurate-based assemblies.[17] The release was proven by ^{1}H NMR and CD spectroscopy (Figure 2d and 3, respectively). Addition of six equivalents of BuCYA (with respect to complex $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3\cdot\mathbf{3a}_3$) to a solution of the complex $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3\cdot\mathbf{3a}_3$ in [D$_8$]toluene generated a ^{1}H NMR spectrum in which all the signals of $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3\cdot\mathbf{3a}_3$ had disappeared and only signals corresponding to complex $\mathbf{1}_3\cdot(BuCYA)_6\cdot\mathbf{3a}_3$ and free **2a** and DEB could be seen. For example, the signal at $\delta = 10.58$ ppm (which corresponds to OH^n when it forms intermolecular hydrogen bonds in the trimer $\mathbf{2a}_3$) is not present in the spectrum.[24] Moreover, the signals for protons H^a and H^b are split and shifted downfield from $\delta = 14.38$ and 13.76 ppm to $\delta = 14.95$ and 14.64 ppm, respectively (Figure 2d). The splitting of the signals arises from the transfer of chirality from the chiral guest (**3a**) to the assembly.[19] This induction of chirality is not complete, and therefore the two possible diastereomers (*P* and *M*) are formed.[25]

Additional proof for the complexation and selective release of guest molecules from $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3\cdot\mathbf{3a}_3$ was obtained from CD spectroscopy (Figure 3). Receptor $\mathbf{1}_3\cdot(DEB)_6$ with D_3 symmetry exists as a racemic mixture of *P* and *M* enantiomers[19] and is therefore CD inactive. As mentioned earlier, the formation of the complex $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3$ is accompanied by a change in the symmetry

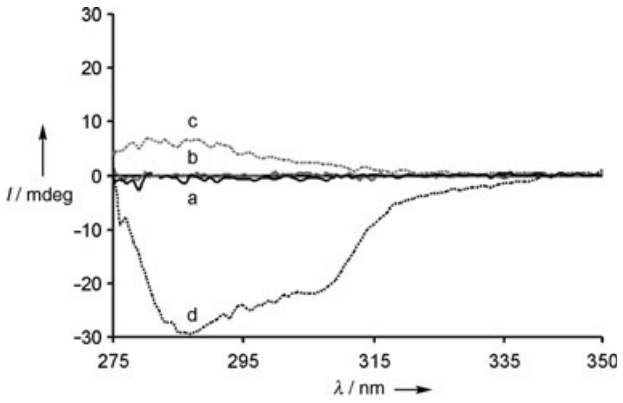

Figure 3. CD spectrum of a) $\mathbf{1}_3\cdot(DEB)_6$ (1.0 mM solution), b) $\mathbf{1}_3\cdot(DEB)_6$ + 3 equiv of **2a**, c) $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3$ + 3 equiv of **3a**, and d) $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3\cdot\mathbf{3a}_3$ + 6 equiv of BuCYA. The spectra were recorded at 298 K in [D$_8$]toluene.

of the hydrogen-bonded receptor from D_3 to C_{3h}. In the C_{3h} symmetry, the two melamine moieties adopt an eclipsed conformation, thus resulting in an achiral complex. Addition of **3a** to $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3$ results in the appearance of a small CD signal (Figure 3) arising from the complexation and transfer of the chirality from **3a** to $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3$ which causes a change in the symmetry from C_{3h} for the complex $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3$ to C_3 for $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3\cdot\mathbf{3a}_3$.[26] Moreover, addition of BuCYA to $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3\cdot\mathbf{3a}_3$ leads to a new signal in the CD spectrum. This signal is very similar to that observed in the CD spectrum of $\mathbf{1}_3\cdot(BuCYA)_6\cdot\mathbf{3a}_3$ formed by direct mixing of assembly $\mathbf{1}_3\cdot(BuCYA)_6$ and three equivalents of **3a**,[19] thus proving the release of the trimeric $\mathbf{2a}_3$ and the change of symmetry from C_3 back to D_3. In this case, the chirality of **3a** has been transferred to the complex $\mathbf{1}_3\cdot(BuCYA)_6\cdot\mathbf{3a}_3$ which results in the formation of one of the two possible diastereomeric assemblies ((P)-$\mathbf{1}_3\cdot(BuCYA)_6\cdot\mathbf{3a}_3$), and thus a signal is observed in the CD spectrum.[19]

Interestingly, changing the order in which the guest molecules **2a** and **3a** are added has a large influence on the self-assembling behavior of the system. Addition of three equivalents of **3a** directly to a solution of assembly $\mathbf{1}_3\cdot(DEB)_6$ in [D$_8$]toluene (1.0 mM) results in almost complete disassembly (only 8% remains intact) of the host assembly as can be seen by the disappearance of the signals for protons H^a and H^b in the ^{1}H NMR spectrum (Figure 4a,b). The higher acidity of the guest **3a** relative to DEB[27] possibly enables **3a** to form stronger hydrogen bonds with the calix[4]arene dimelamine building blocks **1**, thus leading to the destruction of the assembly. Nevertheless, the subsequent addition of three equivalents of **2a** to this solution results in a ^{1}H NMR spectrum that is identical to the one obtained previously when the guest molecules were added in reversed order (see Figures 2c and 4c). Thus, the host assembly is surprisingly reassembled by templation of the guest **2a** even in the presence of diacid **3a**. Moreover, the encapsulation of guest **2a** also allows the subsequent complexation of the guest **3a** in a similar fashion as described above, thus resulting in the quantitative formation $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3\cdot\mathbf{3a}_3$ (Figure 4c). The reassembling of the host receptor is probably a result of the stabilizing effect of the π–π interactions between the guest **2a** and the calix[4]arene and dimelamine rings of **1**.[28] Further addition of six equivalents of BuCYA to $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3\cdot\mathbf{3a}_3$ also leads to the exchange of DEB for BuCYA and release of the trimer $\mathbf{2a}_3$ (Figure 4d). The complexation of **3a** by $\mathbf{1}_3\cdot(BuCYA)_6$ after the exchange of DEB for BuCYA has also been proven by CD spectroscopy.[29]

Herein, we have demonstrated the complexation/encapsulation of two different kinds of neutral guest molecules in which perfect control is shown over the molecular recognition process at two different levels. The noncovalent host molecules $\mathbf{1}_3\cdot(DEB)_6$ display two different modes of complexation, that is, as an nonselective-*exo* receptor for three molecules of carboxylic acid **3a** at the periphery of the assembly and as an selective-*endo* receptor that templates the formation of a noncovalent hydrogen-bonded trimer of alizarin **2a** in the interior cavity of the assembly. The self-assembly of the receptor and the recognition processes bring together, using

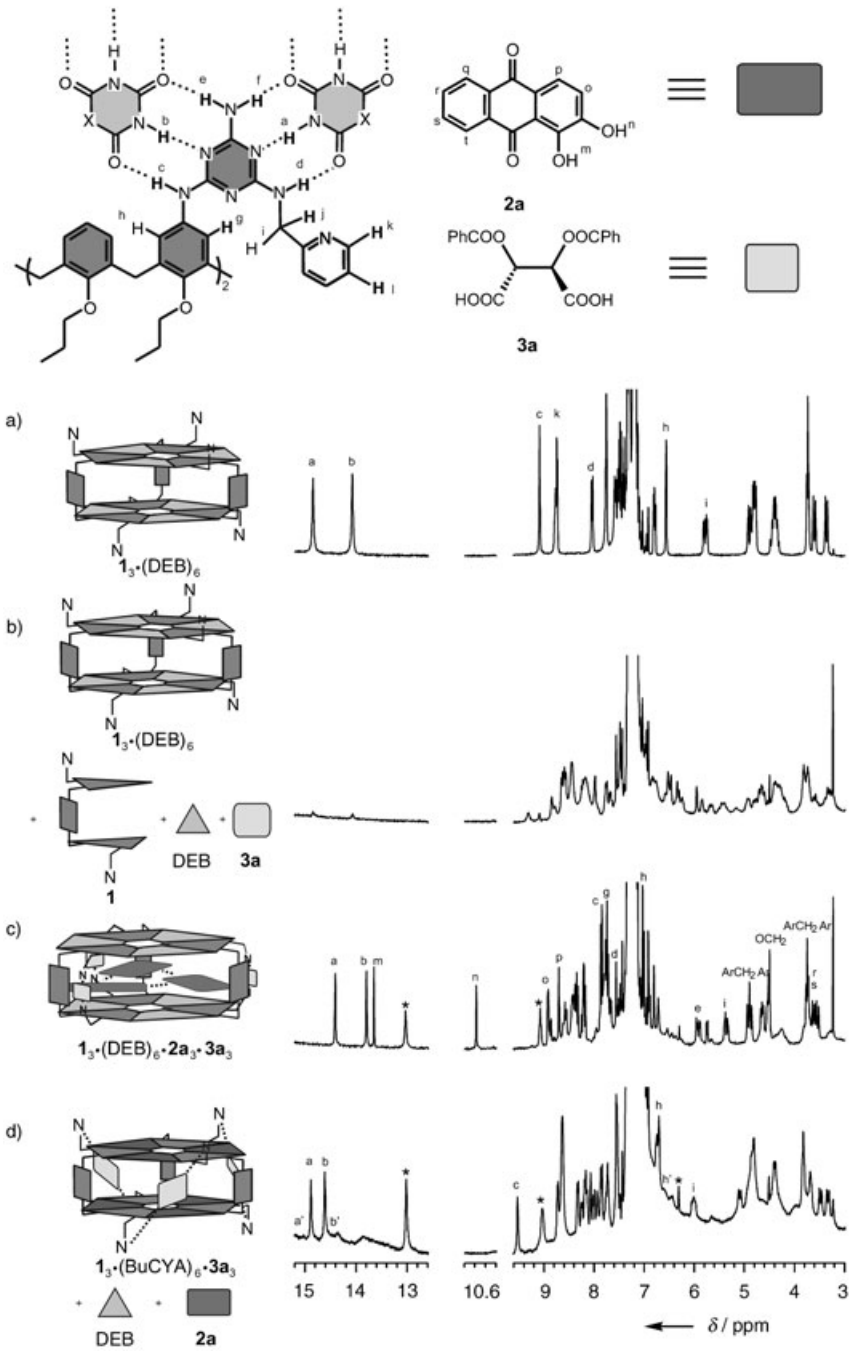

Figure 4. Parts of the ^{1}H NMR spectra (300 MHz) of a) assembly $\mathbf{1}_3\cdot(DEB)_6$ (1 mM), b) $\mathbf{1}_3\cdot(DEB)_6$ + 3 equiv **3a**, c) $\mathbf{1}_3\cdot(DEB)_6$ + 3 equiv **3a** + 3 equiv **2a**, and d) $\mathbf{1}_3\cdot(DEB)_6\cdot\mathbf{2a}_3\cdot\mathbf{3a}_3$ + 6 equiv BuCYA. Signals marked with * belong to the free guest **2a**. All spectra were recorded at 298 K in [D_8]toluene.

the same noncovalent interactions, nine building blocks of the receptor and six guest molecules with absolute control over their spatial disposition to form the complex $\mathbf{1a}_3\cdot(DEB)_6\cdot\mathbf{2a}_3\cdot\mathbf{3a}_3$. Moreover, the addition of BuCYA leads to the release of the noncovalent trimer, while the other guest molecules remain complexed. It has also been demonstrated that the order in which the different building blocks are added is very important for the outcome of the self-assembly process. This observation might have important consequences for noncovalent synthesis in general.

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[24] The ^{1}H NMR spectrum of the receptor complexing both $\mathbf{2a}_3$ and $\mathbf{3a}_3$ after the addition of BuCYA is similar to the ^{1}H NMR spectrum of $\mathbf{1}_3\cdot(\text{BuCYA})_6\cdot\mathbf{3a}_3$ formed by direct mixing of assembly $\mathbf{1}_3\cdot(\text{BuCYA})_6$ and three equivalents of **3a** (data not shown).

[25] In the absence of chiral auxiliary, the receptor is presented as a racemic mixture of *P* and *M* enantiomers and therefore only two signals (ca. 13–15 ppm) are expected for the NH protons of the barbiturate/cyanurate componets in the ^{1}H NMR spectrum. However, the addition of chiral diacids (D or L) leads to the formation of diastereomeric ((*P*)-D or (*M*)-L) assemblies (induction of chirality), and therefore four signals are expected, two signals for each diastereomeric receptor.

[26] The encapsulation of alizarin **2a** by assemblies bearing chiral building blocks leads to a change of symmetry from D_3 to C_3.[17] The CD spectrum of these assemblies resembles the CD spectrum obtained for complex $\mathbf{1}_3\cdot(\text{DEB})_6\cdot\mathbf{2a}_3\cdot\mathbf{3a}_3$.

[27] The pK_a value of DEB is 7.4 and of guest **3a** is 2.99 (see: a) R. M. C. Dawson, *Data for biochemichal research*, Clarendon Press, Oxford, **1959**; b) N. Chidambaram, D. J. Burgess, *AAPS PharmSciTech* **2000**, *2*, 1 – 11).

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[29] Addition of BuCYA to $\mathbf{1}_3\cdot(\text{DEB})_6\cdot\mathbf{2a}_3\cdot\mathbf{3a}_3$ results in the appearance of a negative CD signal similar to the signal obtained by direct mixing of assembly $\mathbf{1}_3\cdot(\text{BuCYA})_6$ and three equivalents of **3a**. Thus, addition of BuCYA results in the release of the trimeric species of **2a**.

