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Amplification of Chirality in Hydrogen-Bonded Tetrarosette Helices

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Abstract: The amplification of chirality in hydrogen-bonded tetrarosette assemblies under thermodynamic equilibrium is described. The extent of the chiral amplification obtained by means of "sergeants-and-soldiers" experiments depends only on the structure of the assembly and it is independent of the methodology used for the formation of the tetrarosette assemblies. The difference in free energy ($\Delta G^{o}_{M/P}$) between the *M*- and *P*-diastereomeric helices is up to 40 times higher than for double rosette assemblies.

Keywords: chiral amplification · hydrogen bonds · self-assembly · supramolecular chirality

Introduction

Amplification of chirality, describing the inductive effect of a small population of chiral components on the chiroptical outcome of macromolecular systems,[1] has repeatedly been tackled by chemists[2,3] to understand how organic compounds with a very high enantiomeric enrichment are obtained from starting materials with very low levels of enantiomeric or diastereomeric excess (*ee* or *de*, respectively).[4]

The terms "sergeants-and-soldiers" and "majority rule" were introduced by Green et al. in their studies on the chirality in stiff helical polymers such as polyisocyanates.[5,6] The "sergeants-and-soldiers" principle is characterized by a (strong) nonlinear response of the optical properties of the (achiral) systems to the addition of a small amount of homochiral material,[5,7] whereas the "majority rule" represents a similar effect for chains consisting of both enantiomeric forms, one of which is present in (small) excess.[8,9] These phenomena have been reported for polymeric structures, both covalent and noncovalent,[10,11] as well as in other supramolecular systems, such as the recognition of chiral amines or acids by hydrogen-bonded assemblies or polymers.[12]

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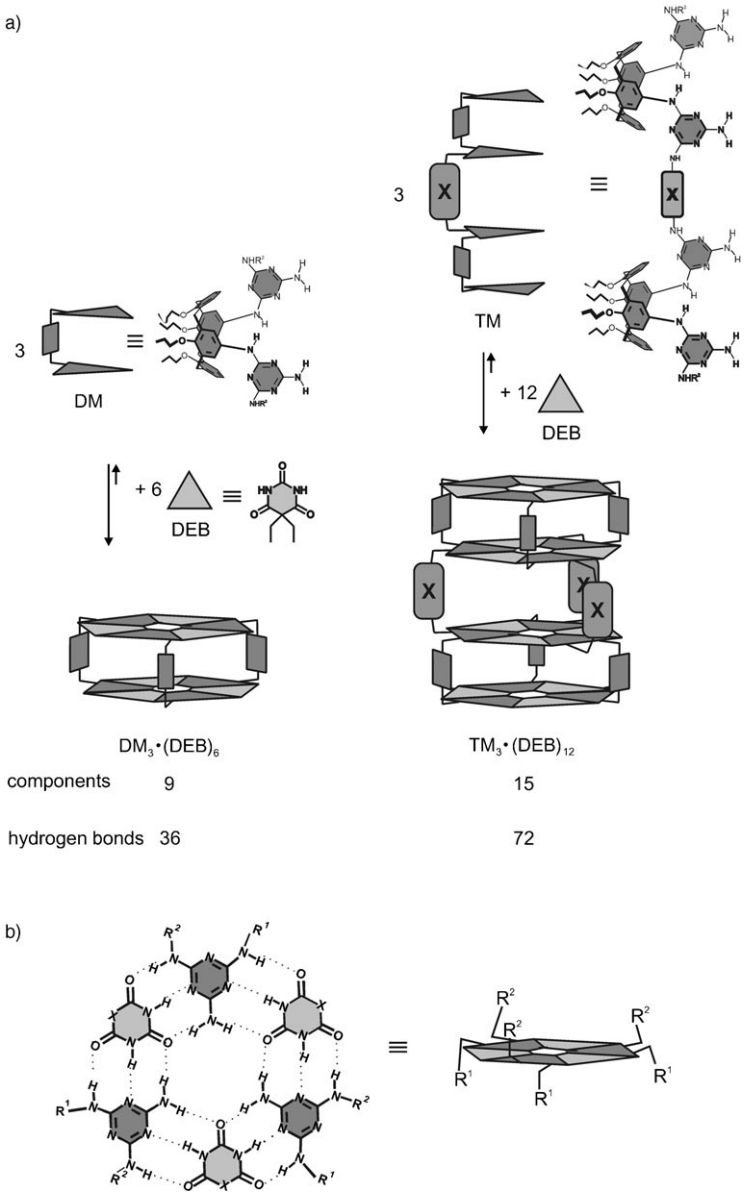
Previous studies in our group investigated the amplification of chirality in self-assembled double rosettes,[13,14] and the parameters that govern this phenomenon. It was found that an important factor for the amplification of chirality in these hydrogen-bonded assemblies is the dissociation rate of the dimelamine building blocks (DM, Scheme 1). A lower dissociation rate constant should lead to a higher degree of amplification of chirality, that it is to say, a larger optical activity with lower amounts of chiral component. It is theoretically possible to achieve a high degree of chiral amplification even when only 0.1% of the components are chiral.[13] Moreover, it was shown that the structure of the different building blocks (dimelamine and cyanurate) has a strong influence on the degree of chiral amplification.[14]

In this article the amplification of chirality of tetrarosette assemblies,[15,16] under thermodynamically controlled conditions is studied. These larger assemblies can be considered as two covalently linked double rosette assemblies (Scheme 1). The dissociation of one tetramelamine building block (TM) requires the disruption of 24 hydrogen bonds and therefore a smaller dissociation rate constant with respect to double rosette assemblies (in which only 12 hydrogen bonds have to be disrupted to dissociate one DM moiety) is predicted. Thus, according to the model previously developed,[13] a much higher amplification of chirality is expected for the system formed with tetrarosette assemblies. Therefore, to verify our theoretical model,[13] the chiral amplification of cyanurate-based tetrarosette assemblies was studied in detail. In particular the effect of the procedure followed for the formation of the assemblies and of the flexibility of the spacer (X; Scheme 2) used to covalently link the two calix[4]arene dimelamines was investigated.



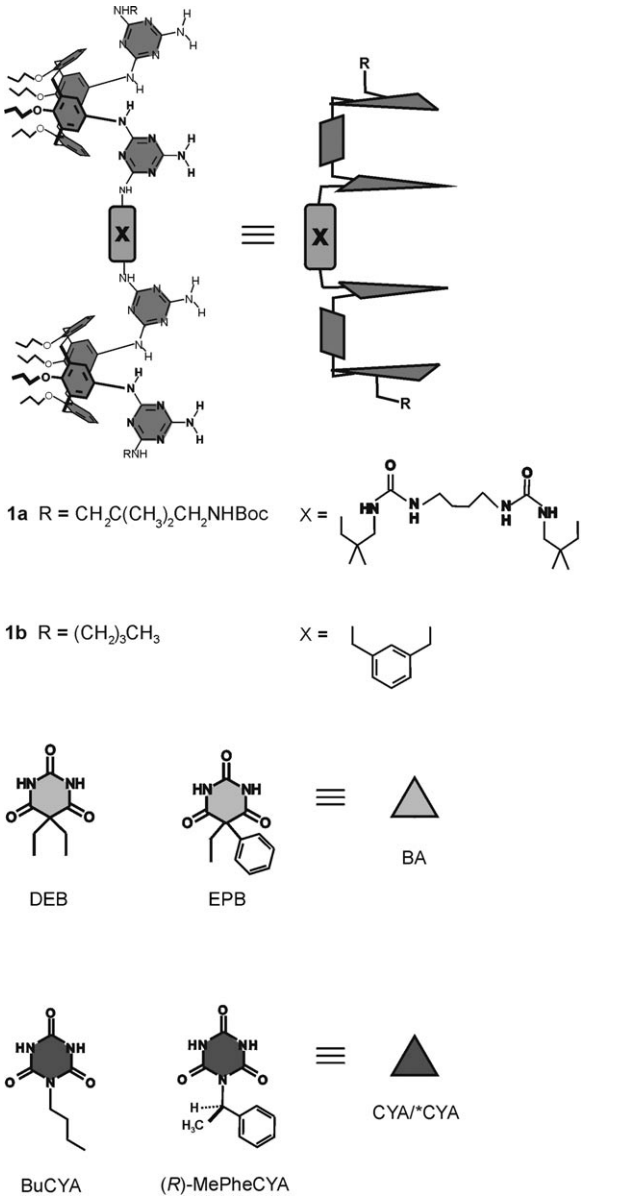
Scheme 1. a) General schematic representation of the formation of double and tetrarosette assemblies $DM_3 \cdot (DEB)_6$ and $TM_3 \cdot (DEB)_{12}$, (DM = calix[4]arene dimelamine; TM = calix[4]arene tetramelamine, DEB = 5,5-diethylbarbituric acid). The number of components and hydrogen bonds that held the assemblies together is shown. b) Molecular structure of one rosette motif/"floor" showing the formation of 18 hydrogen bonds between the melamine and barbiturate/cyanurate derivatives.

Results and Discussion

Synthesis and characterization: Tetramelamines **1a** and **1b** (Scheme 2) were synthesized following methods previously described.[15,16]

The tetrarosette assemblies can conveniently be characterized by ^{1}H NMR spectroscopy.[15] Upon formation of the assembly $(\mathbf{1a})_3/(\mathbf{1b})_3 \cdot (DEB)_{12}$ (see below; DEB = 5,5-diethylbarbiturate), four singlets of equal intensity are observed in the region $\delta = 13$–16 ppm, characteristic of the hydrogen-bonded imide NH protons of DEB. Integration of the appropriate signals in the ^{1}H NMR spectrum confirmed the expected 1:4 stoichiometry for assemblies $(\mathbf{1a})_3/(\mathbf{1b})_3 \cdot (DEB)_{12}$. Tetrarosette assemblies can adopt a large variety of different isomeric structures as result of the staggered (S) or eclipsed (E) orientation of the melamine rings in each rosette layer. Nevertheless, ^{1}H NMR spectroscopy (four signals for the NH protons of DEB) and gas-phase MM calculations have shown the formation of only the SSS isomer.[17]

In the absence of any source of chirality, the SSS isomer exists as a mixture of enantiomers (*P* and *M* enantiomers) due to the staggered orientation of the different melamine rings. However, the introduction of chiral centers in one of the building blocks of the assembly leads to the formation of only one of the two possible diastereomers.[18] Therefore, these assemblies are highly circular dichroism (CD) active.



Scheme 2. Chemical and schematic representation of tetramelamines **1a**,**b** and barbiturate (BA)/cyanurate (CYA/*CYA) building components.

Tetrarosette formation: Barbiturate-based tetrarosette assemblies $\mathbf{1}_3\cdot(DEB)_{12}$ are spontaneously and selectively formed in apolar solvents, such as chloroform, benzene, or toluene, when three equivalents of tetramelamine (**1**) are mixed with twelve equivalents of DEB at room temperature (Scheme 1). The driving force for the self-assembly of these nanostructures is the formation of 72 hydrogen bonds (18 hydrogen bonds for each rosette "floor").[15] The self-assembling process brings together fifteen components leading to the formation of assemblies with high kinetic stability, with a dissociation rate constant for one tetramelamine of $2.8\times10^{-5}\,s^{-1}$ (in $CHCl_3$ at 25 °C), and an activation energy of 98.7 kJ mol^{-1},[16] indicating the enormous enthalpy price that it must be paid in order to dissociate one tetramelamine from the tetrarosette assembly, a process that involves the disruption of 24 hydrogen bonds.

However, similar assembly experiments with **1a**,**b** and cyanuric acid derivatives (BuCYA and (*R*)-MePheCYA) instead of DEB gave completely different results.[19] For example, mixing tetramelamine **1a** with BuCYA (1:4 ratio) in chloroform at room temperature did not show the expected formation of the corresponding assembly $\mathbf{1a}_3\cdot(BuCYA)_{12}$. This mixing process leads to the formation of nondefined structures, which display an extremely high kinetic stability.

This problem in the noncovalent synthesis of tetrarosette assemblies with CYA can be overcome using two different approaches: 1) Mixing the two building blocks (tetramelamine and cyanurate derivatives (1:4 ratio)) in toluene and heating the resulting solution at 100 °C for one week (direct method, Scheme 3a), and 2) formation of the assembly using DEB and subsequent exchange by cyanuric acid derivatives (1:1 DEB/cyanuric acid; exchange method, Scheme 3b).[19] This method is based on the strategy previously exploited for the enantioselective formation of double rosette assemblies.[20]

The formation of the tetrarosette assemblies by the two different methods was studied by ^{1}H NMR spectroscopy.

The ^{1}H NMR spectrum of a mixture of tetramelamine **1a** and a cyanurate derivative in [D_8]toluene at room temperature shows a complicated set of signals (Figure 1a), suggesting the formation of a mixture of ill-defined assemblies. However, formation of tetrarosette assembly $\mathbf{1a}_3\cdot(BuCYA)_{12}$ is observed upon heating at 100 °C for one week in toluene as it can be judge from the appearance of four signals of equal intensity in the region $\delta=15$–13 ppm in the ^{1}H NMR spectrum (1.0 mM in [D_8]toluene, direct method, Figure 1b).

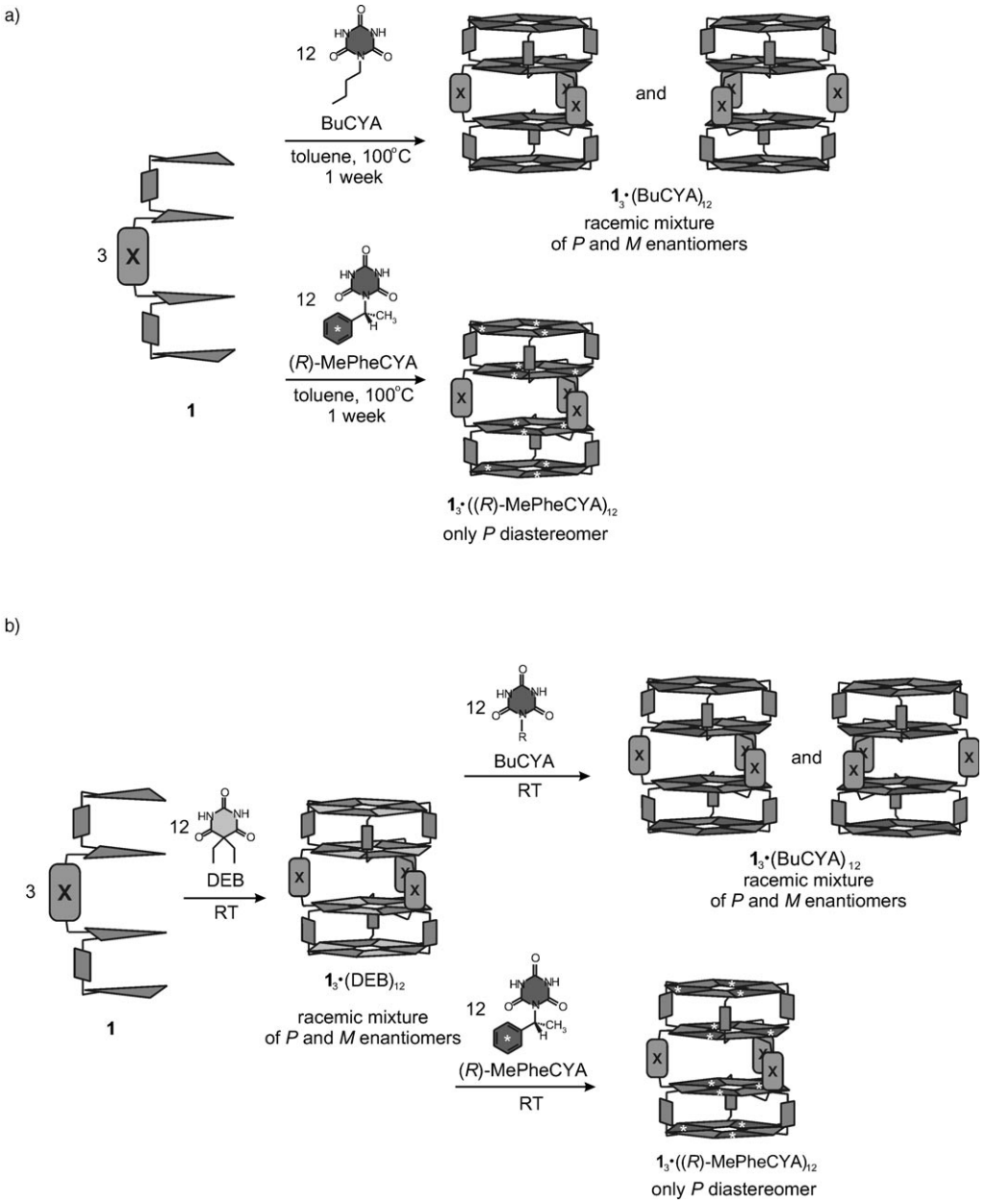
The first step of tetrarosette formation using the second method (exchange method) is the noncovalent synthesis of the barbiturate-based assembly $\mathbf{1a}_3\cdot(DEB)_{12}$ (Figure 2). The ^{1}H NMR spectrum of this assembly (1.0 mM in [D_8]toluene) shows four signals in the region between $\delta=15$–13 ppm, characteristic of the formation of tetrarosette assemblies (Figure 2a). The second step is the exchange of DEB by cyanurate derivatives (BuCYA or (*R*)-MePheCYA), resulting in the exclusive formation of $\mathbf{1a}_3\cdot(BuCYA)_{12}$ or $\mathbf{1a}_3\cdot((R)\text{-MePheCYA})_{12}$. The ^{1}H NMR spectra of these assemblies (1.0 mM in [D_8]toluene) show the disappearance of the signals of assembly $\mathbf{1a}_3\cdot(DEB)_{12}$ and the appearance of four new signals at lower magnetic field ($\delta=15$–14 ppm) and a new broad signal at ~8.4 ppm corresponding to free DEB (Figure 2b and c). Thus, the ^{1}H NMR spectroscopy clearly indicates that the exchange of DEB by the cyanurate derivatives has taken place.[19] The replacement of the barbiturate by cyanurate derivatives is due to the formation of stronger hydrogen bonds between the melamine–cyanurate than between melamine–barbiturate, because of the higher acidity of the cyanurate derivative.[21]

Amplification of chirality in tetrarosette assemblies $\mathbf{1}_3\cdot(CYA/*CYA)_{12}$: Solutions of the assembly (*P*)-$\mathbf{1a}_3\cdot((R)\text{-MePheCYA})_{12}$ and racemic $\mathbf{1a}_3\cdot(BuCYA)_{12}$ in toluene (1 mM) (formed using the direct method) were mixed in ratios ranging from 90:10 to 10:90 and introduced in a thermostated bath at 100 °C.[22] Aliquots of these solutions were taken and the CD spectra of these mixtures were measured every 24 h over a period of two days (Figure 3). After this time the thermodynamic equilibrium is reached.

The maximum CD intensity of the mixtures of (*P*)-$\mathbf{1a}_3\cdot((R)\text{-MePheCYA})_{12}$ and $\mathbf{1a}_3\cdot(BuCYA)_{12}$ immediately after mixing corresponds to the CD intensity of the chiral assembly component; for example, the mixture with 50 % of chiral assembly displays a CD intensity corresponding to the 50 % of chiral assembly component (Figure 3a). Thus, immediately after mixing the "sergeants-and-soldiers" phenomenon has not taken place. However, after 24 h at 100 °C, the CD intensity no longer corresponds to the percentage of chiral assembly in the mixture, showing the typical "sergeants-and-soldiers" behavior (Figure 3b). After 48 h at 100 °C, the CD intensity of the different mixtures of chiral/achiral assemblies does not increase anymore, indicating that the thermodynamic equilibrium has been reached (Figure 3c).

From these measurements, the relative CD intensities were related to a calculated value based on the ratio (*P*)-$\mathbf{1a}_3\cdot((R)\text{-MePheCYA})_{12}$/(*P*)-$\mathbf{1a}_3\cdot(BuCYA)_{12}$ and plotted as a function of the molecular fraction of the chiral component.[23]

The plot of the relative CD intensity (measured at 286 nm) as a function of the molar ratio of chiral rosette assembly immediately after mixing (Figure 4a) shows clearly that the CD intensities of the mixtures do not increase with the percentage of chiral assembly present, indicating that chiral amplification has not taken place. However, the plots after 24 and 48 h at 100 °C show a nonlinear increase of the CD intensities at different molar ratios of chiral assembly (Figure 4b and c, respectively), that is, the typical nonlinear behavior resulting from the "sergeants-and-soldiers" principle shows clearly the amplification of chirality. For example, when a 90:10 mixture of $\mathbf{1a}_3\cdot(BuCYA)_{12}$ and (*P*)-$\mathbf{1a}_3\cdot((R)\text{-MePheCYA})_{12}$ reaches the thermodynamic equilibrium, the relative CD intensity has increased from 10 % (expected in the case where there is no chiral amplification) to 52 %. The nonlinear increase of the CD intensity is due to the exchange between the chiral and nonchiral cyanurates within



Scheme 3. Schematic representation of a) direct method and b) exchange method for the formation of cyanurate-based tetrarosette assemblies. In the absence of chiral centers, the assemblies are present as a racemic mixture. However, upon introduction of chiral centers ((*R*)-MePheCYA) only the *P* diastereomer is formed.

the assemblies, that is to say, to the presence of the heteromeric assemblies $\mathbf{1a}_3\cdot(\mathrm{BuCYA})_n((R)\text{-MePheCYA})_{12-n}$ ($n=$ 1–12), in which the presence of only 1–11 chiral centers ((*R*)-MePhe) in these assemblies still leads to the preferential formation of the *P* diastereomer.

The curve of the amplification of chirality for the system formed by mixing $\mathbf{1a}_3\cdot(\mathrm{BuCYA})_{12}$ and (P)-$\mathbf{1a}_3\cdot((R)\text{-MePheCYA})_{12}$ after 48 h at 100 °C (Figure 4) was fitted to a thermodynamic model based on the difference in free energy between the *P* and *M* diastereomers of the homo/heteromeric assemblies $\mathbf{1a}_3\cdot(\mathrm{BuCYA})_n((R)\text{-MePheCYA})_{12-n}$ ($n=0$–12) at the thermodynamic equilibrium. All the equilibria accounted in the model are depicted in Figure 5. The formation of the tetrarosette assemblies is considered to take place in one step from the different building blocks. Cooperativity is not included in the model and therefore, ΔG° is assumed to increase linearly with the number of chiral components. Each chiral substituent present in the unfavorable *M* diastereomer[24] induces a free-energy difference $\Delta G^{\circ}_{M/P}$, which results in a decrease of the equilibrium constant (K) between the *M* isomer and the free components with a factor of f_m [see Eq. (1)].

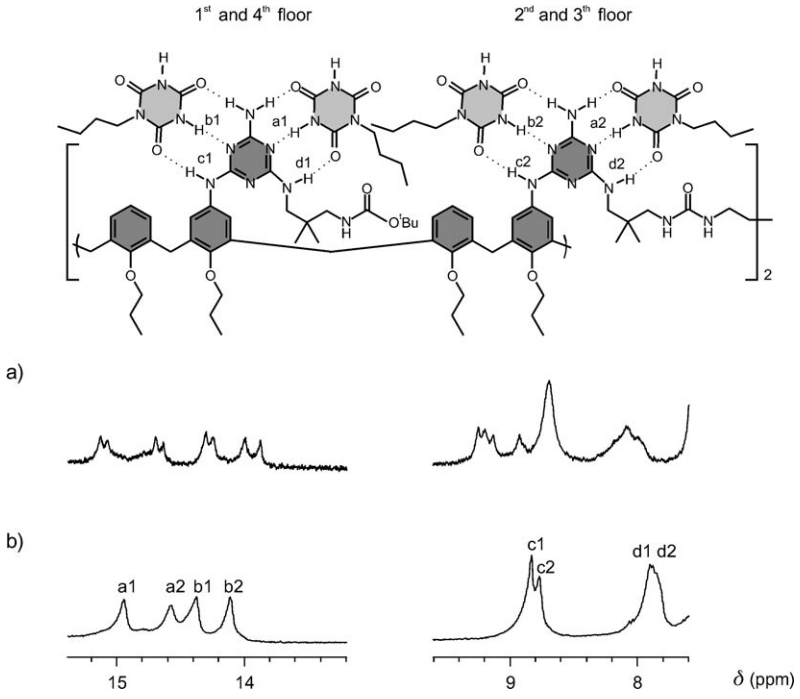


Figure 1. Parts of the ^{1}H NMR spectra of a 1:4 mixture of **1a** and BuCYA: a) immediately after mixing, and b) after one week at 100 °C in toluene (**1a**$_3$·(BuCYA)$_{12}$. Both spectra were recorded in [D$_8$]toluene at room temperature.

$$f_m = e^{\frac{-\Delta G^o_{M/P}}{RT}} \quad (1)$$

For example, for an assembly containing six chiral centers, the equilibrium constant is decreased by a factor of f_m^6. Statistical factors are included to account for the statistically different possibilities of formation of each assembly. Thus, least-squares fit of the CD data for the system formed from **1a**$_3$·(BuCYA)$_{12}$ and (*P*)-**1a**$_3$·((*R*)-MePheCYA)$_{12}$ (Figure 4c) using the model described above resulted in $\Delta G^o_{M/P} = 119.4 \pm 1.7$ kJ mol^{-1} per chiral substituent. This corresponds to a total free-energy difference $\Delta G^o_{tot} = 1432.8 \pm 20.4$ kJ mol^{-1} between the favored *P* and the disfavored *M* diastereomers of assembly (*P*)-**1a**$_3$·((*R*)-MePheCYA)$_{12}$ containing twelve chiral centers.

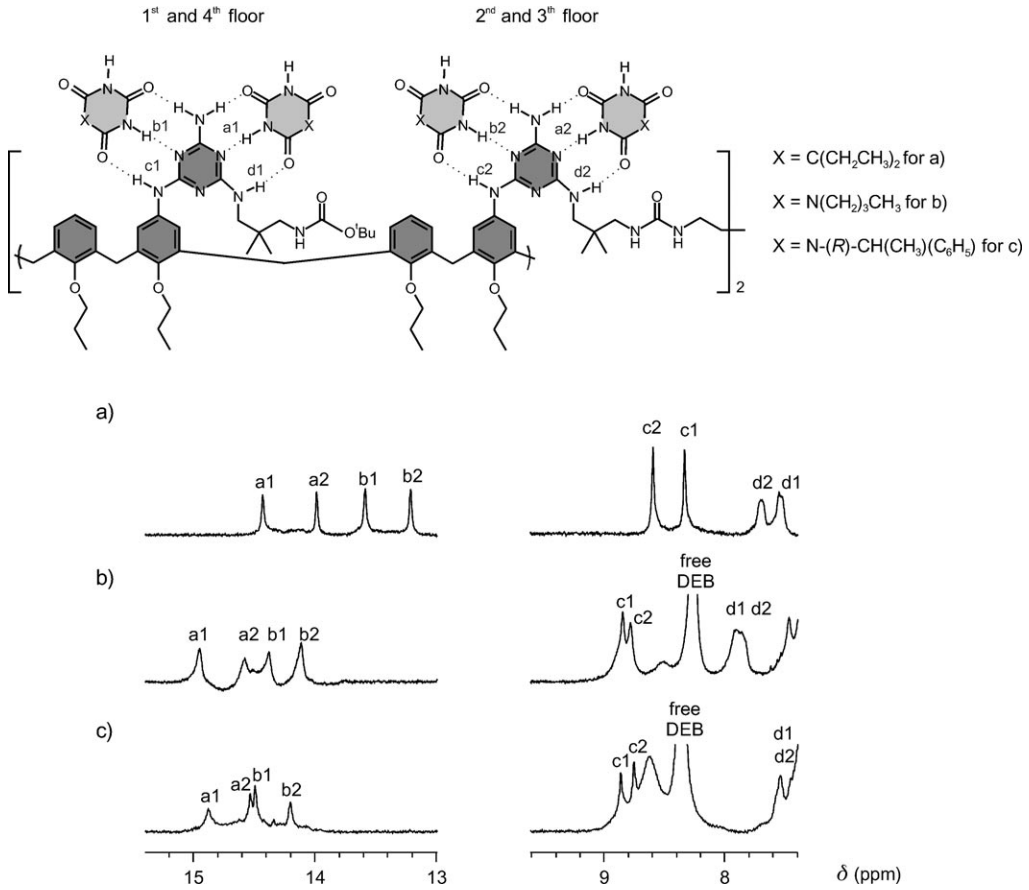


Figure 2. Parts of the ^{1}H NMR spectra of **1a**$_3$·(DEB)$_{12}$ before (a) and after the addition of 12 equivalents (1:1 ratio DEB:cyanurate) of BuCYA (b) and (*R*)-MePheCYA (c). All ^{1}H NMR spectra were recorded in [D$_8$]toluene (1.0 mM) at room temperature.

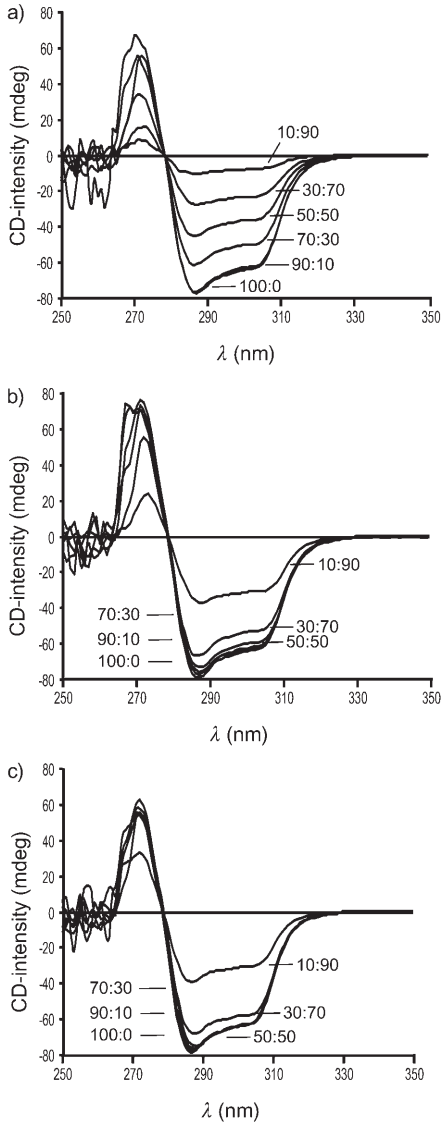


Figure 3. CD spectra of mixtures of (*P*)-$\mathbf{1a}_3$·((*R*)-MePheCYA)$_{12}$ and $\mathbf{1a}_3$·(BuCYA)$_{12}$: a) immediately after mixing, b) after 24 h at 100 °C, and c) after 48 h at 100 °C. Both tetrarosette assemblies were formed using the direct method.

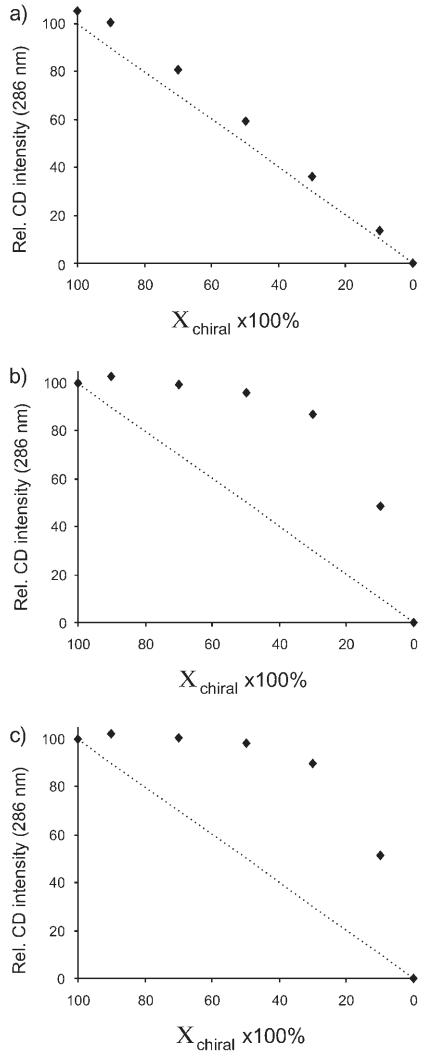


Figure 4. Plots of the relative CD intensity measured at 286 nm for the molar ratio of chiral assembly (*P*)-$\mathbf{1a}_3$·((*R*)-MePheCYA)$_{12}$: a) immediately after mixing, b) after 24 h at 100 °C, and c) after 48 h at 100 °C. The assemblies were formed using the direct method. The dotted lines represent the expected CD intensity in absence of chiral amplification.

Influence of the noncovalent synthetic procedure on the amplification of chirality: The extent of the chiral amplification by using the thermodynamic model mentioned before was studied for $\mathbf{1a}_3$·(BuCYA)$_{12}$ and (*P*)-$\mathbf{1a}_3$·((*R*)-MePheCYA)$_{12}$ prepared by using the exchange method to assess the influence of the synthetic path followed in the formation of the tetrarosettes assemblies in the amplification of chirality.

Thus, similar "sergeants-and-soldiers" experiments were carried out with mixtures of assemblies $\mathbf{1a}_3$·(BuCYA)$_{12}$ and (*P*)-$\mathbf{1a}_3$·((*R*)-MePheCYA)$_{12}$ formed using the exchange method. The calculated difference in free energy between the favored *P* and disfavored *M* diastereomers of (*P*)-$\mathbf{1a}_3$·((*R*)-MePheCYA)$_{12}$ was also $\Delta G^{\circ}_{M/P} = 119.4 \pm 2.1$ kJ mol^{-1}!

Comparison of the results obtained in the amplification of chirality for the system $\mathbf{1a}_3$·(BuCYA)$_{12}$ and (*P*)-$\mathbf{1a}_3$·((*R*)-MePheCYA)$_{12}$ formed using the direct and the exchange method showed no dependence of the method of formation of the assemblies in the extent of the chiral amplification under thermodynamically controlled conditions (Figure 6 and Table 1).

There is a large difference in free energy of the *P* and *M* diastereomers introduced in the assemblies per chiral center between double and tetrarosettes. For the best case of chiral amplification in double rosette assemblies a $\Delta G^{\circ}_{M/P}$ of 4.3 kJ mol^{-1} was obtained.[14] This difference in free energy for tetrarosette assemblies is $\Delta G^{\circ}_{M/P} = 119.4$ kJ mol^{-1}. Kinetic studies on the amplification of chirality for double rosettes have shown the important role of the dissociation rate constant of the dimelamine components to obtain a high chiral amplification at the thermodynamic equilibrium.[13] Thus, a possible explanation for the difference in amplification of

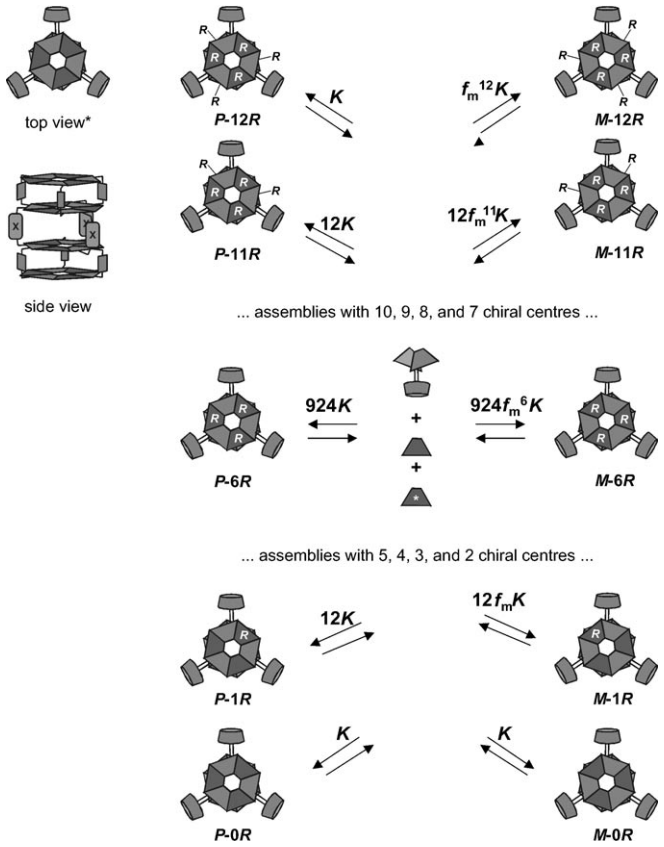


Figure 5. Thermodynamic model. Each chiral substituent in assemblies $\mathbf{1a}_3$·(BuCYA)$_n$((*R*)-MePheCYA)$_{12-n}$ (n=0–12) lowers the equilibrium constant of the disfavored *M* isomer by a factor f_m [Eq. (1)]. *R* denotes the chiral components of the assembly. *: For clarity only one of the two double rosette submotifs of the tetrarosette assembly is depicted.

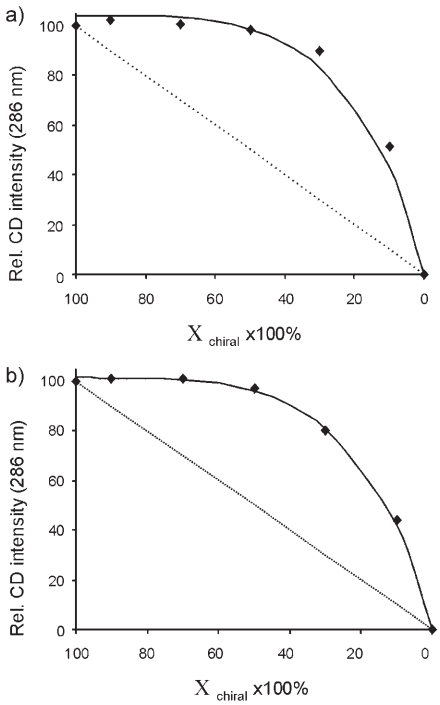


Figure 6. Plots of the relative CD intensity measured at 286 nm against the molar ratio of chiral assembly (*P*)-$\mathbf{1a}_3$·((*R*)-MePheCYA)$_{12}$ formed using a) the direct method, and b) exchange method.

Table 1. Difference in free energy between the (*P*)- and (*M*)-diastereomers of assemblies $\mathbf{1x}_3$·((*R*)-MePheCYA)$_{12}$ as a result of the presence of chiral centers in the assembly calculated using the thermodynamic model.[a]

	Method	$\Delta G^{o}_{M/P}$ [kJ mol^{-1}][b]	ΔG^{o}_{tot} [kJ mol^{-1}][c]
1a	direct	119.4	1432.8
1a	exchange	119.4	1432.8
1b	direct	2.8	33.6
1b	exchange	2.7	32.4

[a] [(*P*)-$\mathbf{1x}_3$·((*R*)-MePheCYA)$_{12}$]=[$\mathbf{1x}_3$·(BuCYA)$_{12}$]=1.0 mM, 343 K, benzene. [b] Difference of free energy per chiral center. [c] Total free-energy difference between *P* and *M* diastereomers of assemblies $\mathbf{1x}_3$·((*R*)-MePheCYA)$_{12}$.

chirality between double and tetrarosettes assemblies might come from the decrease of the dissociation rate constant of the tetramelamine building blocks. This decrease is due to the increase in the number of hydrogen bonds that held the melamine together (12 and 24 hydrogen bonds for double and tetrarosette assemblies, respectively).

To investigate the role of the dissociation rate constant in the amplification of chirality in tetrarosette assemblies, kinetic experiments were carried out with the system comprising assemblies $\mathbf{1a}_3$·(BuCYA)$_{12}$ and (*P*)-$\mathbf{1a}_3$·((*R*)-MePheCYA)$_{12}$, formed by using the direct method. The CD intensity at 286 nm of mixtures $\mathbf{1a}_3$·(BuCYA)$_{12}$ and (*P*)-$\mathbf{1a}_3$·((*R*)-MePheCYA)$_{12}$ in benzene (1.0 mM) at 70 °C was measured as a function of time (Figure 7).

Figure 7 shows that the relative CD intensities at t=0 corresponds to the initial mole fraction of (*P*)-$\mathbf{1a}_3$·((*R*)-MePheCYA)$_{12}$. However, the relative CD intensity starts to change (t_{init}) after about 30 minutes and 15 minutes for mixtures of $\mathbf{1a}_3$·(BuCYA)$_{12}$ and (*P*)-$\mathbf{1a}_3$·((*R*)-MePheCYA)$_{12}$ of ratios 90:10 and 70:30, respectively. However, for double rosette assemblies, the CD intensities increase more rapidly in time.[14] Thus, from this difference in t_{init} for double and tetrarosettes is possible to conclude that the dissociation rate of the tetramelamine moieties is indeed smaller than the one of the dimelamine moieties and, therefore, a larger amplification of chirality is expected for the systems formed with tetrarosette assemblies. Moreover, simulations using the kinetic model developed for double rosettes showed that lowering the dissociation rate of the melamines results in an increase of t_{init} and the degree of chiral amplification.[13] Unfortunately, due to the complexity of the system (twelve chiral centers), the development of a kinetic model to fit the data and obtain an accurate value for the dissociation rate constant for tetrarosette assemblies was not successful.[25]

Influence of the spacer (X) of the tetramelamine on the amplification of chirality: The extent of the chiral amplification

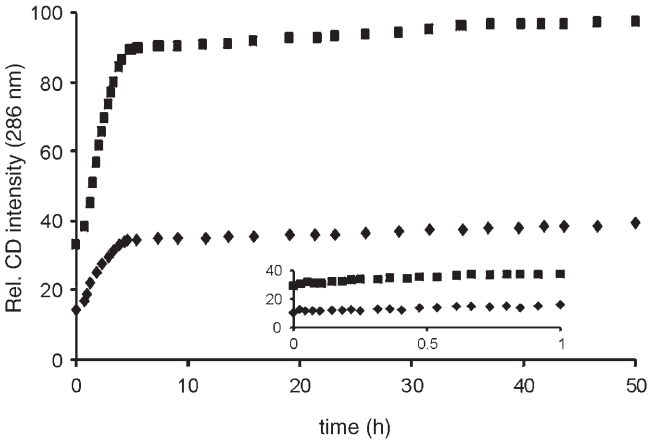


Figure 7. Increase of the relative CD intensity (at 286 nm) in time for mixtures of $\mathbf{1a}_3$·(BuCYA)$_{12}$ and (*P*)-$\mathbf{1a}_3$·((*R*)-MePheCYA)$_{12}$ with different initial mole fractions of $\mathbf{1a}_3$·(BuCYA)$_{12}$ (◆: 10%; ■: 30%). The inset shows the increase of the relative CD intensity (at 286 nm) during the first hour. All spectra were recorded in benzene at 70°C.

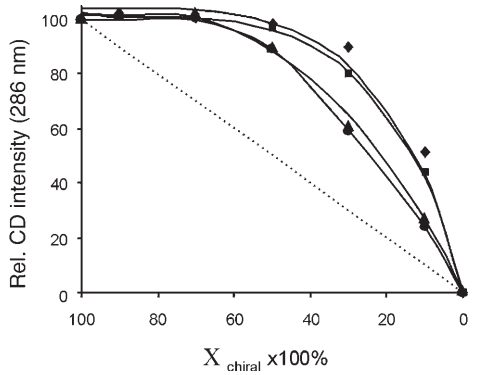


Figure 8. Plot of the relative CD intensities at the thermodynamic equilibrium for different mole ratios of chiral component (**1a**: exchange method: ■, direct method: ◆; **1b**: direct method: ▲, exchange method: ●). The solid lines represent the calculated best fit using the thermodynamic model previously described. The dotted line represents the expected CD intensity in absence of amplification of chirality.

by using the thermodynamic model mentioned earlier was also studied for systems in which the spacer X of the tetramelamine becomes less flexible (Scheme 2). For this purpose, the amplification of chirality was studied in systems in which the bisureido spacer of tetramelamine **1a** was replaced by the rigid *m*-xylene spacer in **1b**.

Thus, mixtures of assemblies (*P*)-$\mathbf{1b}_3$·((*R*)-MePheCYA)$_{12}$ and $\mathbf{1b}_3$·(BuCYA)$_{12}$ in ratios varying between 90:10 and 10:90 prepared by using both the direct and exchange methods were introduced in a thermostated bath at 100°C for two days. Aliquots of these solutions were taken and their CD spectra were measured (every 24 h) over a period of two days, a period after which the thermodynamic equilibrium is reached. The plot of the relative CD intensity (at 286 nm) at the thermodynamic equilibrium shows, in all cases, that the CD intensities are significantly higher than the sum of the CD intensities of the individual assemblies ("sergeants-and-soldiers" behavior). Similar to assemblies formed with tetramelamine **1a**, the method of assembly formation with tetramelamine **1b** does not influence the amplification of chirality in these systems. In Figure 8 and Table 1 the results obtained for the systems formed with the flexible tetramelamine **1a** and the rigid tetramelamine **1b** are summarized.

The analysis of the data obtained using the thermodynamic model clearly shows that the introduction of a rigid spacer (*m*-xylene) in the tetramelamine building block decreases drastically the amplification of chirality in tetrarosette assemblies. The decrease in $\Delta G^{o}_{M/P}$ is probably due to the introduction of geometrical constrains in the assemblies bearing the rigid *m*-xylene spacer. Previous studies have shown that the formation of assemblies with the tetramelamine **1b** (rigid spacer) displays negative cooperativity[26] between the two double rosette layers,[15] while assemblies with tetramelamine **1a** display positive cooperativity between the two double rosette layers. Therefore, to obtain high degrees of chiral amplification, the tetrarosette assemblies should be formed with tetramelamine building blocks connected through a flexible spacer allowing the cooperative formation of the assembly.

Conclusion

In this article, the amplification of chirality in tetrarosette assemblies under thermodynamically controlled conditions has been described. The degree of chiral amplification is not influenced by the method of formation of the system, that is, direct or exchange method. The difference in free energy between the *M* and *P* diastereomers of the tetrarosettes introduced by a chiral center is 40 times higher than in the case of double rosette assemblies. This difference in the extent of chiral amplification is due to a decrease in the dissociation rate constant of the tetramelamine building blocks. Yet, the substitution of the bisureido spacer by the more rigid *m*-xylene spacer results in a considerable decrease in the amplification of chirality, probably due to geometric/steric constrains introduced in the system by the rigid spacer.

Thus, it is possible to control the amplification of chirality in these self-assembled systems increasing the number of layers in the assemblies obtaining extremely high chiral amplification, similarly to the chiral amplification obtained with covalent polymeric structures. The amplification of the chirality in self-assembled systems is very relevant in the bottom-up (chemical) assembly of nanostructures.

Experimental Section

Synthesis: The synthesis of tetramelamines **1a**[16] and **1b**,[15] and cyanurate derivatives BuCYA and (*R*)-MePheCYA have been reported previously.[27]

Assembly formation

Direct method: Assemblies were formed by dissolving the calix[4]arene tetramelamines **1x** (**x**=**a**,**b**) and the corresponding cyanurate (BuCYA/(*R*)-MePheCYA) in a 1:4 molar ratio in toluene, after which the solution was heated for one week at 100 °C. After being dried under high vacuum, the assemblies were ready for use. In a standard example, tetramelamine **1a** (7.08 mg, 0.003 mmol) and BuCYA (2.22 mg, 0.012 mmol) were dissolved in toluene (5 mL) and the resultant solution was heated at 100 °C for one week. After evaporation of the solvent, the assemblies were ready to use.

Exchange method: Assemblies $\mathbf{1x}_3$·(BuCYA/(*R*)-MePheCYA)$_{12}$ (**x**=**a**, **b**) were prepared from $\mathbf{1x}_3$·(DEB)$_{12}$ by exchange of DEB with BuCYA/(*R*)-MePheCYA. In a typical example, assembly $\mathbf{1a}_3$·(DEB)$_{12}$ (9.30 mg, 0.001 mmol) was dissolved in $CDCl_3$ (1 mL), and one equivalent (with respect to DEB) of BuCYA (2.22 mg, 0.012 mmol) was added. The solution was stirred for 30 minutes. After this time, the assembly $\mathbf{2}_3$·(BuCYA)$_{12}$ was ready to use.

CD titration studies: Assembly solutions (1.0 mM) of the homomeric assemblies were mixed in ratios 90:10 to 10:90 at room temperature and introduced in a thermostated bath at 100 °C immediately after mixing. The CD intensities were monitored in time at constant temperature. The resulting plots were treated as described in the text.

Thermodynamic model: The model was implemented in MicroMath® Scientist® for Windows, Version 2.01. The text file of the model is provided in the Supporting Information.

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[17] The isomers are coded with three letters (S for staggered; E for eclipsed), which represent the relative orientation of the melamine fragments between the rosette layers 1st and 2nd, 2nd and 3rd, and 3rd and 4th floors, respectively. Staggered means that the two melamine rings on a calix[4]arene adopt an antiparallel orientation, and eclipsed means that the two melamine rings adopt a parallel orientation. The presence of only four signals for the NH_{DEB} protons in the ^{1}H NMR spectrum indicates that the assembly can only exist as SSS, SES, and EEE isomer. For a graphical representation see Figure 4 of reference [16]. Previous studies have demonstrated that only the SSS isomer is present.[15]
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[22] A mixture of assemblies (*P*)-$\mathbf{1a}_3$·((*R*)-MePheCYA)$_{12}$ and racemic $\mathbf{1a}_3$·(BuCYA)$_{12}$ were investigated once the corresponding tetrarosettes were fully formed (see text for direct method synthesis).
[23] Assembly (*P*)-$\mathbf{1a}_3$·(BuCYA)$_{12}$, which is formed by using an enantioselective process (substitution of a chiral barbiturate derivative for the nonchiral BuCYA)[16,20] has a slightly lower $\Delta\varepsilon$ than assembly (*P*)-$\mathbf{1a}_3$·((*R*)-MePheCYA)$_{12}$. For this reason, the relative CD intensities were related to the ratio (*P*)-$\mathbf{1a}_3$·((*R*)-MePheCYA)$_{12}$/(*P*)-$\mathbf{1a}_3$·(BuCYA)$_{12}$.
[24] When the chiral centers are in the cyanurate building blocks, a center with *R* stereochemistry generally induces the formation of assemblies with *P* helicity. Thus, when (*R*)-MePheCYA is present the formation of the *M* diastereomer is unfavorable.
[25] The kinetic model was implemented in MicroMath® Scientist® for Windows, version 2.01. However, an internal error of the software precluded the possibility to use this software and therefore the model.
[26] Negative cooperativity in assemblies formed with tetramelamine **1b** means that formation of the first double rosette disfavors the formation of the second double rosette, while positive cooperativity in assemblies formed with tetramelamine **1a** means that formation of the first double rosette favors the formation of the whole assembly.
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