## Supramolecular Self-Sorting

# Pseudorotaxanes with Self-Sorted Sequence and Stereochemical Orientation**

*Carmen Talotta, Carmine Gaeta,* Zhenhui Qi, Christoph A. Schalley,* and Placido Neri**

Self-sorting is a fundamental process in living systems[1] which controls the spatiotemporal organization of complex mixtures of biomolecules,[1a] such as proteins, nucleic acids, oligosaccharides, and lipids, into large functional assemblies (e.g. the DNA double helix,[2a] multienzyme complexes,[1a] ribosomes,[2b] or membranes[1a]) and beyond these assemblies into cellular compartments essential for the development of life.[3] Stereochemical homogeneity[4] as well as precise building-block sequences—that is, accurate control of sequence isomerism—are pivotal for biomolecular function. For example, the sequence of only four DNA bases defines the genetic code.[2a,5] Control of the stereochemical[6] and sequence[7] information by self-sorting is thus of prime importance for life.

Inspired by nature, synthetic supramolecular self-sorting systems[8,9] have developed into a high-interest research area. Like their natural analogues, they form selectively a defined and small number of supramolecular assemblies out of a significantly larger pool of possible assemblies. The efficiency of a synthetic self-sorting system is related to its ability to discriminate between structurally similar individual subunits. A fundamental step towards mimicking the efficiency and complexity of natural systems is to design a synthetic system able to sort species that simultaneously differ at the sequence and stereochemical levels. Previously, one of us reported a heteropseudo[3]rotaxane[10,11] bearing two similar crown ethers (dibenzo[24]crown-8 and benzo-[21]crown-7) in a specific sequence along a constitutionally asymmetric dialkylammonium axle.[12] To the best of our knowledge, no examples of a system that sorts among species that differ in sequence and stereochemistry exist so far.

Recently, we synthesized[13] pseudo[3]rotaxanes with calix[6]arenes,[14] $\mathbf{1^H}$ or $\mathbf{1^{tBu}}$ (Figure 1), threaded on a bis(benzylalkylammonium) axle, $\mathbf{2^{2+}}$ or $\mathbf{3^{2+}}$,[15] by exploiting the weakly coordinating[15d,16] TFPB (tetrakis(3,5-

[*] Dr. C. Talotta, Dr. C. Gaeta, Prof. P. Neri
Dipartimento di Chimica e Biologia and
NANO_MATES Research Center, Università di Salerno
Via Giovanni Paolo II n. 132, 84084 Fisciano (Salerno) (Italy)
E-mail: cgaeta@unisa.it
        neri@unisa.it
Z. Qi, Prof. C. A. Schalley
Institut für Chemie und Biochemie, Freie Universität
Takustrasse 3, 14195 Berlin (Germany)
E-mail: christoph@schalley-lab.de

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201301570.



**Figure 1.** Structures of calix[6]arene wheels $\mathbf{1^H}$ and $\mathbf{1^{tBu}}$, dibenzyl- and alkylbenzylammonium axles $\mathbf{2^{2+}}$–$\mathbf{5^{2+}}$ (TFBP = tetrakis(3,5-bis(trifluoromethyl)phenyl)borate), and stereoselectively formed calix[6]arene-based pseudo[3]rotaxanes.
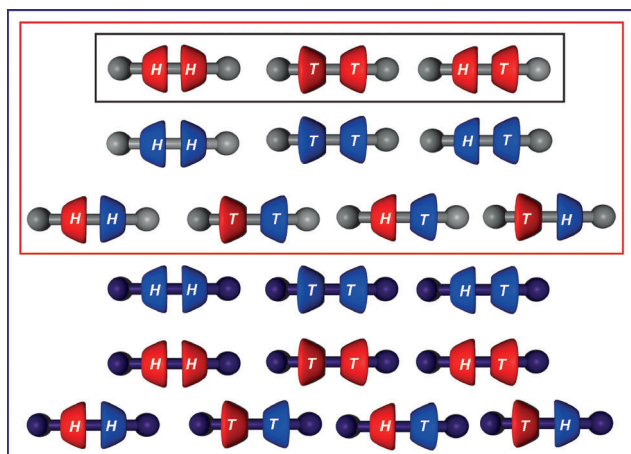
**Figure 2.** All species that can form in mixtures of one wheel and one axle (inner black box), mixtures of two different wheels and one axle (red box), and mixtures of two different wheels and two different axles (outer blue box).

wheel is combined with one axle, only the relative orientation of the two threaded wheels plays a role, and three stereoisomers (inner black box) can exist, as detailed above. Usually, one of the stereoisomers is clearly preferred (for example, the $(H,H)$-$1^H$·$2^{2+}$·$1^H$ and $(T,T)$-$1^{tBu}$·$3^{2+}$·$1^{tBu}$ pseudo[3]rotaxanes in Figure 1).[13] b) When two different wheels are used with only one axle, the axle may or may not have a preference for one of the wheels in addition to its stereochemical preference. Consequently, 10 different homo- and heteropseudo[3]rotaxanes can form (Figure 2, red box). c) Finally, it is highly interesting whether self-sorting also occurs for a combination of two different wheels with two different axles, in which case a total of 20 pseudo[3]rotaxanes can form (outer blue box). The latter two cases were investigated in this study as a step towards the goal to define a molecular code[9a] that would enable the implementation of a synthetic self-sorting system able to discriminate sequence and stereochemical isomers simultaneously.

We started with the simpler case of a [1+2] sorting system and mixed the benzyl-terminated symmetrical axle $2^{2+}$ with two equivalents of each of the wheels $1^H$ and $1^{tBu}$ in CDCl$_3$.[19] The two wheels differ only by the absence or presence of the *tert*-butyl groups at the upper rim and compete with each other for the axle. The absence of signals for $1^{tBu}$·$2^{2+}$·$1^{tBu}$ (Figure 3a) in the $^1$H NMR spectrum of the mixture (Figure 3b) rules out the presence of this pseudorotaxane as well as that of any heteropseudorotaxane,[20] for which very
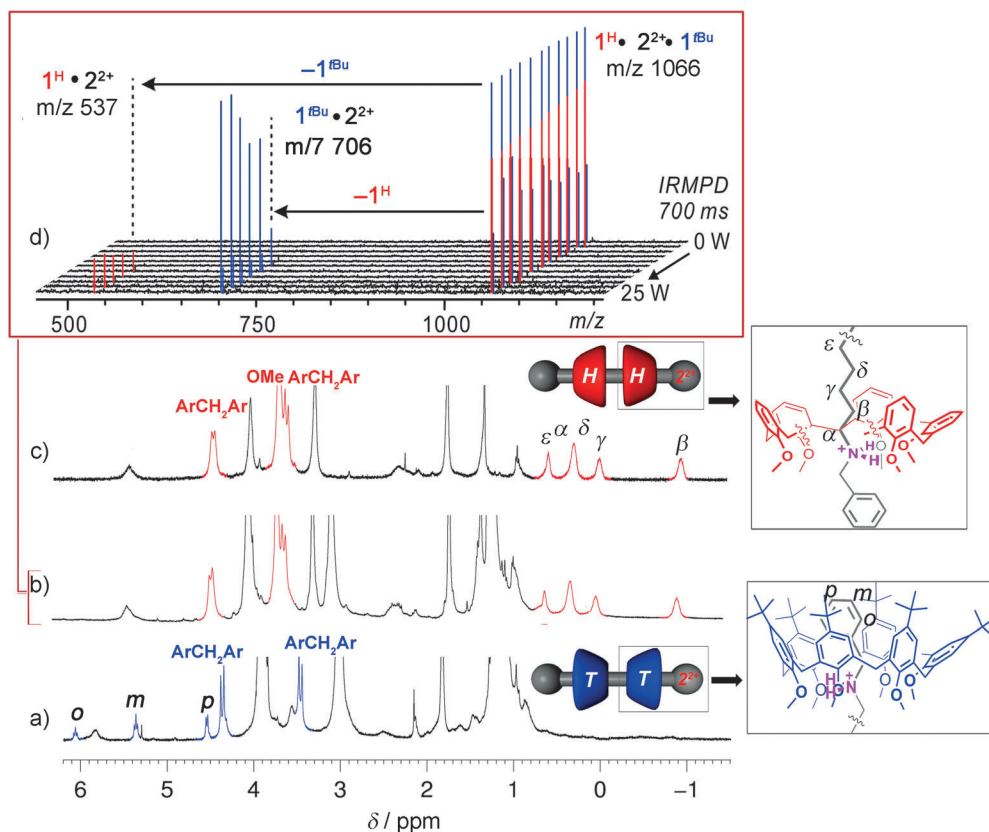
bis(trifluoromethyl)phenyl)borate or BARF) anion.[17] As the calix[6]arene wheels are directional because of the different upper and lower rims, three stereoisomers—head-to-head $(H,H)$, head-to-tail $(H,T)$, and tail-to-tail $(T,T)$ (Figure 1 and Figure 2, inner black box)—can form. A clear preference for *endo*-alkyl over *endo*-benzyl complexation is observed with $1^H$ wheels,[13,15d,18] whereas *endo*-benzyl inclusion is favored with $1^{tBu}$. Therefore, each stereoisomer can be formed in a rationally controlled way by choosing a suitable axle/wheel combination (Figure 1). Thus, a head-to-head orientation can be obtained by combining axle $2^{2+}$ with wheel $1^H$ (to give $(H,H)$-$1^H$·$2^{2+}$·$1^H$), whereas a tail-to-tail orientation can be obtained by the combination of the same wheel with axle $3^{2+}$ (to give $(T,T)$-$1^H$·$3^{2+}$·$1^H$).

On the basis of these stereochemical results, the question arises as to whether these pseudo[3]rotaxanes are also capable of social self-sorting:[8,9a] Do the axles choose a pair of identical or different wheels when the wheels compete for binding, and what is then the relative orientation of the two wheels? Figure 2 shows three different cases in terms of the choice of building blocks and the pseudorotaxanes that may form: a) When just one



**Figure 3.** Relevant portions of the $^1$H NMR spectra (CDCl$_3$, 400 MHz, 298 K) of solutions of $2^{2+}$ (3 mM) with a) $1^{tBu}$ (2 equiv), b) $1^H$ (2 equiv) and $1^{tBu}$ (2 equiv), and c) $1^H$ (2 equiv). d) Series of IRMPD spectra of the mass-selected heteropseudo[3]rotaxane ion $1^H$·$2^{2+}$·$1^{tBu}$ with increasing laser power (from 0 to 25 W).

similar, if not identical, signal shifts would be expected. In contrast, $1^H \cdot 2^{2+} \cdot 1^H$ (Figure 3c) is clearly present. From the 10 possible pseudorotaxane structures, the head-to-head homopseudo[3]rotaxane $(H,H)$-$1^H \cdot 2^{2+} \cdot 1^H$ (Figure 1) emerged as the only product (Figure 3). This result is in good agreement with the total binding constants $(K_{tot} = K_1 \cdot K_2)$[13] as evaluated by integration of the $^1$H NMR signals of the pseudorotaxanes in the [1+1] mixtures $((H,H)$-$1^H \cdot 2^{2+} \cdot 1^H$: $K_{tot} = 5.8 \pm 0.3 \times 10^6 \, \text{M}^{-2}$; $(T,T)$-$1^{tBu} \cdot 2^{2+} \cdot 1^{tBu}$: $K_{tot} = 2.5 \pm 0.4 \times 10^5 \, \text{M}^{-2})$. The 23-fold higher binding constant of $(H,H)$-$1^H \cdot 2^{2+} \cdot 1^H$ clearly renders it the dominant product.

ESI mass spectra obtained for a 1:2:2 mixture of $2^{2+}$, $1^H$, and $1^{tBu}$ indicate a 17:9:1 ratio for $1^H \cdot 2^{2+} \cdot 1^H$ ($m/z$ 898), $1^H \cdot 2^{2+} \cdot 1^{tBu}$ ($m/z$ 1066), and $1^{tBu} \cdot 2^{2+} \cdot 1^{tBu}$ ($m/z$ 1234; see Figure S26 in the Supporting Information). The deviation from the NMR spectroscopic results and with it the seemingly imperfect self-sorting may be attributed to the different conditions during the MS experiment.[19]

When the heteropseudo[3]rotaxane dication $1^H \cdot 2^{2+} \cdot 1^{tBu}$ was mass-selected and subjected to infrared multiphoton dissociation (IRMPD, Figure 3d), $2^{2+} \cdot 1^{tBu}$ ($m/z$ 706) was more prominent than $2^{2+} \cdot 1^H$ ($m/z$ 537). As the experiment probes the activation energies for the two possible dissociation processes, we can conclude that $1^{tBu}$ has a higher kinetic barrier to deslipping than $1^H$.[21]

The above results yielded the first rules of our molecular code (Figure 4). Rule 1 (social behavior): Benzylalkylammonium sites on diammonium axles (e.g. $2^{2+}$) select non-tert-
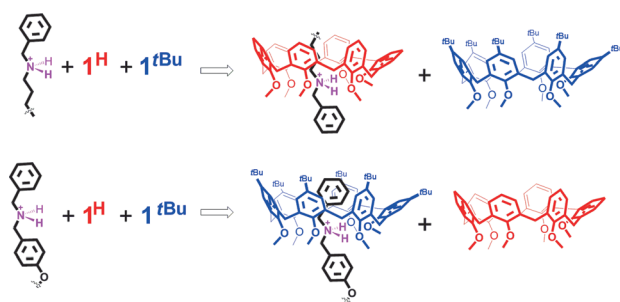


**Figure 4.** The "molecular code" with respect to both benzylalkylammonium and benzyl-p-alkoxybenzylammonium sites.

butylated calix[6]arene wheels (e.g. $1^H$) in preference to tert-butylated wheels (e.g. $1^{tBu}$), which thus remain uncomplexed. Rule 2 (stereochemistry): Threading of a directional alkylbenzylammonium station (e.g. in $2^{2+}$) through a non-tert-butylated hexaalkoxycalix[6]arene (e.g. $1^H$) occurs with preference for the endo-alkyl orientation.[13]

These rules were corroborated by a second [1+2] sorting system, in which the symmetrical bis(benzylalkylammonium) axle $3^{2+}$ (Figure 1), with terminal alkyl chains, was mixed with two equivalents of each of the wheels $1^H$ and $1^{tBu}$. As predicted by the rules, $1^H$ was selected also by axle $3^{2+}$, so that $1^H \cdot 3^{2+} \cdot 1^H$ formed exclusively out of the 10 possible isomers with the expected $T,T$ configuration (see Figure S27)—again in line with the total binding constants[13] $((T,T)$-$1^H \cdot 3^{2+} \cdot 1^H$: $K_{tot} = 7.4 \pm 0.2 \times 10^5 \, \text{M}^{-2}$; $(T,T)$-$1^{tBu} \cdot 3^{2+} \cdot 1^{tBu}$: $K_{tot} = 2.2 \pm 0.3 \times 10^3 \, \text{M}^{-2})$.

To expand the code, we synthesized axle $4^{2+}$ with two dibenzylammonium stations.[19] First, we tested the binding of the new axle to the tert-butylated calix[6]arene $1^{tBu}$. The $^1$H NMR spectrum of a 1:2 mixture of $4^{2+}$ and $1^{tBu}$ in CDCl$_3$ (see Figure S22) reveals shielded benzylic resonances in the 4–6 ppm region, whereas no signals for shielded p-alkoxybenzyl moieties were found. Thus, double endo-benzyl complexation of $4^{2+}$ to yield the pseudo[3]rotaxane $(T,T)$-$1^{tBu} \cdot 4^{2+} \cdot 1^{tBu}$ (Figure 1) was observed $(K_{tot} = 1.8 \pm 0.3 \times 10^4 \, \text{M}^{-2})$.[19] Twofold complexation of the wheel was confirmed by the presence of the $1^{tBu} \cdot 4^{2+} \cdot 1^{tBu}$ dication at $m/z$ 1298 in the ESI mass spectrum (see Figure S23).[19] Regarding the binding ability of $4^{2+}$ toward non-tert-butylated calix[6]arene $1^H$, the $^1$H NMR spectrum of a 1:2 mixture of $4^{2+}$ and $1^H$ (see Figure S28a) showed shielded p-alkoxybenzyl resonances (AX systems) in the 4–6 ppm region. This result was confirmed by a COSY spectrum, which indicated the presence of three pseudo[3]rotaxanes with a slight preference for endo-p-alkoxybenzyl complexation.

In a [1+2] self-sorting experiment, axle $4^{2+}$ and wheels $1^H$ and $1^{tBu}$ were used in a 1:2:2 ratio in CDCl$_3$. The corresponding $^1$H NMR spectrum (see Figure S28b) clearly shows endo-benzyl resonances corresponding to $(T,T)$-$1^{tBu} \cdot 4^{2+} \cdot 1^{tBu}$ in the 4–6 ppm region and rules out the presence of other homo- or heteropseudorotaxanes.

On the basis of this result, the molecular code could now be expanded with two new rules (Figure 4). Rule 3 (social behavior): Benzyl-p-alkoxybenzylammonium sites on a diammonium axle (e.g. $4^{2+}$) select tert-butylated calix[6]arenes (e.g. $1^{tBu}$) over non-tert-butylated calix[6]arenes (e.g. $1^H$), which remain uncomplexed. Rule 4 (stereochemistry): Threading of a benzyl-p-alkoxybenzylammonium site (e.g. in $4^{2+}$) through a tert-butylated hexaalkoxycalix[6]arene (e.g. $1^{tBu}$) occurs with preference for the endo-benzyl configuration.

On the basis of the complete set of all four rules,[22] we could now probe the validity of the code in more complex systems. Therefore, [2+2] self-sorting experiments with two different axles and two different wheels were performed. As shown in Figure 2 (blue box), a total of 20 possible homo- and heteropseudo[3]rotaxanes can form with symmetrical axles. In a [2+2] self-sorting experiment, axles $2^{2+}$ and $4^{2+}$ and wheels $1^H$ and $1^{tBu}$ were used in a 1:1:2:2 ratio in CDCl$_3$. The corresponding $^1$H NMR spectrum (see Figure S30b) was clearly the superposition of the spectra for the individual pseudo[3]rotaxanes $(H,H)$-$1^H \cdot 2^{2+} \cdot 1^H$ and $(T,T)$-$1^{tBu} \cdot 4^{2+} \cdot 1^{tBu}$ (see Figure S30a,c), whereas alternative combinations of axles and wheels were not found. The formation of these pseudo[3]rotaxanes was evident from the endo-benzyl resonances corresponding to $(T,T)$-$1^{tBu} \cdot 4^{2+} \cdot 1^{tBu}$ in the 4–6 ppm region and the characteristic endo-alkyl resonances for $(H,H)$-$1^H \cdot 2^{2+} \cdot 1^H$ at negative ppm values. As above, the ESI mass spectra did not show a perfect self-sorting picture, but the trend towards the situation expressed in the NMR spectra was clearly observed (see Figure S31). These results clearly confirm the validity of the molecular code even for the more complex [2+2] system, in which only two out of 20 possible pseudo[3]rotaxanes formed stereospecifically. Further experiments with different [2+2] systems corroborated a wider

scope for our molecular code. In fact, a [2+2] self-sorting experiment was performed in which axles $3^{2+}$, with terminal alkyl chains, and $4^{2+}$ were mixed with wheels $1^H$ and $1^{tBu}$ in a 1:1:2:2 ratio in CDCl$_3$. The corresponding $^1$H NMR spectrum (see Figure S32) was clearly the superposition of the spectra for the individual pseudo[3]rotaxanes $(T,T)$-$1^H\cdot3^{2+}\cdot1^H$ and $(T,T)$-$1^{tBu}\cdot4^{2+}\cdot1^{tBu}$, and alternative combinations of axles and wheels were not found.

As highlighted by Fuller et al.,[7] the minimal structural requirement for sequence isomerism in pseudorotaxanes is the use of two different wheels and a directional (i.e. constitutionally nonsymmetrical) thread. Our final aim to create self-sorting pseudorotaxanes selective for both sequence and stereochemical configuration thus required a new directional axle, $5^{2+}$, with two different ammonium binding sites. A [1+2] self-sorting system would potentially give rise to a total of 16 structures (Figure 5), 8 homo- and 8 heteropseudo[3]rotaxanes, which also comprise species simultaneously isomeric in sequence and configuration.
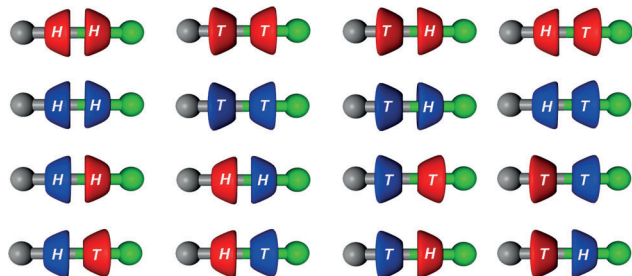


**Figure 5.** Survey of all possible species that can form in mixtures of two calix wheels (e.g. $1^H$, $1^{tBu}$) and a directional axle (e.g. $5^{2+}$).

On the basis of the molecular code (Figure 4), we encoded the sequence of alkylbenzylammonium and benzyl-*p*-alkoxybenzylammonium binding sites along the thread of $5^{2+}$ (Figure 1).[19] In the presence of $1^H$ and $1^{tBu}$, the first site was predicted to select *endo*-alkyl binding of $1^H$, whereas the second site should prefer *endo*-benzyl complexation of $1^{tBu}$ to yield the $(H,T)$-pseudo[3]rotaxane shown in Figure 6.

In line with this prediction, the ESI mass spectrum (see Figure S35a) of a 1:2:2 mixture of $5^{2+}$, $1^H$, and $1^{tBu}$ in CHCl$_3$ showed only one intense peak at $m/z$ 1091,[19] which corresponds to the $1^H\cdot5^{2+}\cdot1^{tBu}$ heteropseudo[3]rotaxane dication. Furthermore, $^1$H NMR (Figure 6) and 2D ROESY[19] spectra confirmed that wheels $1^H$ and $1^{tBu}$ were anchored at the alkylbenzyl- and benzyl-*p*-alkoxybenzylammonium sites, respectively, as evidenced by signals at negative chemical shifts for the *endo*-complexed alkyl chain of $5^{2+}$ and the shielded benzylic resonances in the 4–6 ppm region for the *endo*-complexed benzyl group of $5^{2+}$ (see Figures S33 and S34). The location of the benzyl moiety of $5^{2+}$ inside the aromatic cavity of $1^{tBu}$ was confirmed by diagnostic crosspeaks between the *tert*-butyl groups of $1^{tBu}$ and the shielded benzyl resonances of the axle in the 2D ROESY spectrum (see Figure S34).[19]

In analogy to $1^H\cdot2^{2+}\cdot1^{tBu}$ (Figure 3d), when the heteropseudo[3]rotaxane dication $1^H\cdot5^{2+}\cdot1^{tBu}$ was mass-selected and
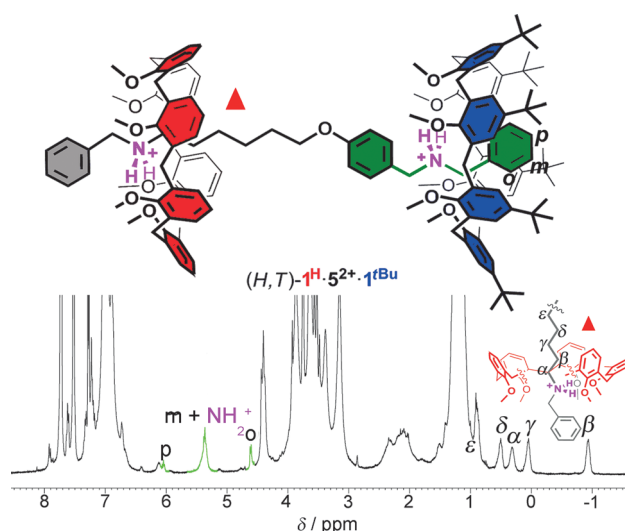


**Figure 6.** Relevant portion of the $^1$H NMR spectrum (CDCl$_3$, 400 MHz, 298 K) of a solution of $5^{2+}$ (3 mM) containing $1^{tBu}$ (2 equiv) and $1^H$ (2 equiv), and the corresponding self-sorting stereosequence (top).

subjected to infrared multiphoton dissociation (IRMPD; see Figure S35b), ion $5^{2+}\cdot1^{tBu}$ ($m/z$ 731) was more prominent than $5^{2+}\cdot1^H$ ($m/z$ 562). This result confirmed the higher kinetic barrier to deslipping of $1^{tBu}$ relative to that of $1^H$.[21]

In conclusion, we have successfully demonstrated that calix[6]arene-based pseudo[3]rotaxanes with bisammonium axles have intriguing self-sorting capabilities, even when structural differences are small and located remote from the binding sites. This is the first reported integrative self-sorting system that is able to discriminate simultaneously at the sequence and stereochemical level. All these aspects can be considered a further significant step toward mimicking the efficiency and complexity of natural systems.

[1] a) J. M. Berg, J. L. Tymoczko, L. Stryer, *Biochemistry*, 6th ed., W. H. Freeman, New York, **2007**; b) N. A. Campbell, J. B. Reece, M. R. Taylor, E. J. Simon, J. L. Dickey, *Biology: Concepts and Connections*, 6th ed., Benjamin/Cummings, San Francisco, **2008**.

[2] a) J. D. Watson, F. H. C. Crick, *Nature* **1953**, *171*, 737–738; b) A. Yonath, *Angew. Chem.* **2010**, *122*, 4438–4453; *Angew. Chem. Int. Ed.* **2010**, *49*, 4340–4354.

[3] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, *Molecular Biology of the Cell*, 4th ed., Garland Science, New York, **2002**.

[4] *Stereochemistry of Organic Compounds* (Eds.: E. L. Eliel, S. H. Wilen, L. N. Mander), Wiley, New York, **1994**.

[5] a) A. D. Hershey, M. Chase, *J. Gen. Physiol.* **1952**, *36*, 39–56; for the role of RNA sequence isomers as start and stop codons, see: C. T. Caskey, R. Tompkins, E. Scolnick, T. Caryk, M. Nirenburg, *Science* **1968**, *162*, 135–138.

[6] a) J. E. Hein, D. G. Blackmond, *Acc. Chem. Res.* **2012**, *45*, 2045–2054; b) M. Wu, S. I. Walker, P. G. Higgs, *Int. J. Astrobiol.* **2012**, *12*, 818–829; c) A. S. Burton, J. C. Stern, J. E. Elsila, D. P. Glavin, J. P. Dworkin, *Chem. Soc. Rev.* **2012**, *41*, 5459–5472; d) J. E. Hein, E. Tse, D. G. Blackmond, *Nat. Chem.* **2011**, *3*, 704–706.

[7] For a definition of sequence isomers, see: A.-M. L. Fuller, D. A. Leigh, P. J. Lusby, *J. Am. Chem. Soc.* **2010**, *132*, 4954–4959.

[8] A. X. Wu, L. Isaacs, *J. Am. Chem. Soc.* **2003**, *125*, 4831–4835.

[9] For a recent review, see: a) M. M. Safont-Sempere, G. Fernández, F. Würther, *Chem. Rev.* **2011**, *111*, 5784–5814; for some recent examples, see: b) M. L. Saha, S. Pramanik, M. Schmittel, *Chem. Commun.* **2012**, *48*, 9459–9461; c) E. Orentas, M. Lista, N.-T. Lin, N. Sakai, S. Matile, *Nat. Chem.* **2012**, *4*, 746–750; d) C. Li, X. Shu, J. Li, J. Fan, Z. Chen, L. Weng, X. Jia, *Org. Lett.* **2012**, *14*, 4126–4129; e) A. K. Mandal, P. Das, P. Mahato, S. Acharya, A. Das, *J. Org. Chem.* **2012**, *77*, 6789–6800; f) A. S. Singh, S.-S. Sun, *Chem. Commun.* **2012**, *48*, 7392–7394; g) R. C. Lirag, K. Osowska, O. Š. Miljanić, *Org. Biomol. Chem.* **2012**, *10*, 4847–4850; h) M. Lal Saha, M. Schmittel, *Org. Biomol. Chem.* **2012**, *10*, 4651–4684; i) M. Rancan, A. Dolmella, R. Seraglia, S. Orlandi, S. Quici, L. Armelao, *Chem. Commun.* **2012**, *48*, 3115–3117; j) H. Gan, B. C. Gibb, *Chem. Commun.* **2012**, *48*, 1656–1658; k) M. M. J. Smulders, A. Jiménez, J. R. Nitschke, *Angew. Chem.* **2012**, *124*, 6785–6789; *Angew. Chem. Int. Ed.* **2012**, *51*, 6681–6685; l) Y. Yamauchi, M. Yoshizawa, M. Akita, M. Fujita, *J. Am. Chem. Soc.* **2010**, *132*, 960–966; m) T. Murase, S. Horiuchi, M. Fujita, *J. Am. Chem. Soc.* **2010**, *132*, 7864–7865; n) Y. Rudzevich, V. Rudzevich, F. Klautzsch, C. A. Schalley, V. Böhmer *Angew. Chem.* **2009**, *121*, 3925–3929; *Angew. Chem. Int. Ed.* **2009**, 48, 3867–3871; *Angew. Chem. Int. Ed.* **2009**, *48*, 3867–3871; o) S. Ulrich, J.-M. Lehn, *Chem. Eur. J.* **2009**, *15*, 5640–5645; p) S. Ulrich, J.-M. Lehn, *J. Am. Chem. Soc.* **2009**, *131*, 5546–5549; q) Y. R. Zheng, H. B. Yang, K. Ghosh, L. Zhao, P. J. Stang, *Chem. Eur. J.* **2009**, *15*, 7203–7214; r) B. H. Northrop, Y. R. Zheng, K. W. Chi, P. J. Stang, *Acc. Chem. Res.* **2009**, *42*, 1554–1563; for a representative example of calixarene-based self-sorting, see: s) Y. Rudzevich, V. Rudzevich, F. Klautzsch, C. A. Schalley, V. Böhmer, *Angew. Chem.* **2009**, *121*, 3925–3929.

[10] W. Jiang, H. D. F. Winkler, C. A. Schalley, *J. Am. Chem. Soc.* **2008**, *130*, 13852–13853.

[11] The pseudorotaxane structural motif is prominent among self-sorting systems, in particular with crown ethers and cucurbiturils as the wheels; for recent reports, see: a) W. Jiang, D. Sattler, K. Rissanen, C. A. Schalley, *Org. Lett.* **2011**, *13*, 4502–4505; b) P.-N. Chen, C.-C. Lai, S.-H. Chiu, *Org. Lett.* **2011**, *13*, 4660–4663; c) W. Jiang, Q. Wang, I. Linder, F. Klautzsch, C. A. Schalley, *Chem. Eur. J.* **2011**, *17*, 2344–2348; d) W. Jiang, A. Schäfer, P. C. Mohr, C. A. Schalley, *J. Am. Chem. Soc.* **2010**, *132*, 2309–2320, and Ref. [9d].

[12] For the sequence-specific self-sorting of a hetero[4]pseudorotaxane, see: G. Celtek, M. Artar, O. A. Scherman, D. Tuncel, *Chem. Eur. J.* **2009**, *15*, 10360–10363.

[13] C. Talotta, C. Gaeta, T. Pierro, P. Neri, *Org. Lett.* **2011**, *13*, 2098–2101.

[14] C. D. Gutsche, *Calixarenes—An Introduction* in *Monographs in Supramolecular Chemistry* (Ed.: J. F. Stoddart), Royal Society of Chemistry, Cambridge, **2008**.

[15] For other examples of threaded architectures based on the calixarene/dialkylammonium motif, see: a) C. Gaeta, C. Talotta, S. Mirra, L. Margarucci, A. Casapullo, P. Neri, *Org. Lett.* **2013**, *15*, 116–119; b) C. Talotta, C. Gaeta, P. Neri, *Org. Lett.* **2012**, *14*, 3104–3107; c) T. Pierro, C. Gaeta, C. Talotta, A. Casapullo, P. Neri, *Org. Lett.* **2011**, *13*, 2650–2653; d) C. Gaeta, F. Troisi, P. Neri, *Org. Lett.* **2010**, *12*, 2092–2095; e) G. Gattuso, A. Notti, M. F. Parisi, I. Pisagatti, M. E. Amato, A. Pappalardo, S. Pappalardo, *Chem. Eur. J.* **2010**, *16*, 2381–2385.

[16] a) C. Gaeta, C. Talotta, F. Farina, M. Camalli, G. Campi, P. Neri, *Chem. Eur. J.* **2012**, *18*, 1219–1230; b) C. Gaeta, C. Talotta, F. Farina, F. A. Teixeira, P. M. Marcos, J. R. Ascenso, P. Neri, *J. Org. Chem.* **2012**, *77*, 10285–10293.

[17] a) N. A. Yakelis, R. G. Bergman, *Organometallics* **2005**, *24*, 3579–3581; b) S. H. Strauss, *Chem. Rev.* **1993**, *93*, 927–942; c) H. Nishida, N. Takada, M. Yoshimura, T. Sonoda, H. Kobayashi, *Bull. Chem. Soc. Jpn.* **1984**, *57*, 2600–2604.

[18] The preference for *endo*-alkyl over *endo*-benzyl complexation by **1**[H] wheels was recently confirmed[15a] by DFT calculations at the B3LYP/6-31G* level of theory, which indicated that the *endo*-alkyl stereoisomer was more stable than the *endo*-benzyl stereoisomer by 3.5 kcal mol$^{-1}$.

[19] See the Supporting Information for further details.

[20] The presence of **1**[H]·**2**$^{2+}$·**1**[tBu] heteropseudo[3]rotaxanes was excluded by means of analysis of the COSY-45 spectrum of the 1:2:2 mixture of **2**$^{2+}$, **1**[H], and **1**[tBu] (see Figure S26 in the Supporting Information). In particular, analysis of the upfield negative region of the COSY-45 spectrum revealed the presence of only one aliphatic chain (α–ε in Figure 3 and Figure S26) inside a calix cavity. These data, coupled with the signal integration, are in accordance with the presence of two equivalent calix wheels at the extremities of **2**$^{2+}$. The presence of only one type of calix wheel was confirmed by the observation of a single AX system for calixarene ArCH$_2$Ar groups in the 3–5 ppm region of the COSY-45 spectrum.[19] In fact, as detailed previously,[13,15] these ArCH$_2$Ar hydrogen atoms appear as a broad singlet for the conformationally mobile free wheel, whereas they give rise to two doublets (AX system) when the wheel is conformationally blocked by pseudorotaxane formation.

[21] The slower kinetics of threading/dethreading of a *tert*-butylated calix[6]arene with respect to a non-*tert*-butylated calix[6]arene was observed previously in equilibration experiments in CDCl$_3$.[15a]

[22] As suggested by one referee, an additional rule can be obtained from the 1:1:2 competition experiment with the two axles **2**$^{2+}$ and **3**$^{2+}$ and wheel **1**[H]. The corresponding $^1$H NMR spectrum (see Figure S36) clearly shows the formation of (*H,H*)-**1**[H]·**2**$^{2+}$·**1**[H] and thus indicates a clear preference for inner- over outer-alkyl complexation. Since this additional rule is not relevant to the development of integrative self-sorting system, it was not included in the main text.