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Thermodynamic Analysis of Allosteric and Chelate Cooperativity in Di- and Trivalent Ammonium/Crown-Ether Pseudorotaxanes

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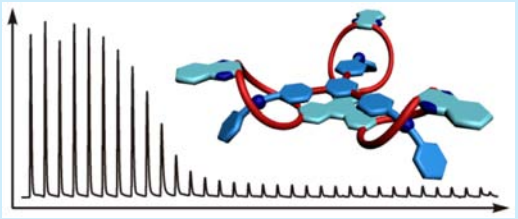
Supporting Information

ABSTRACT: A detailed thermodynamic analysis of the axle-wheel binding in di- and trivalent secondary ammonium/[24]crown-8 pseudorotaxanes is presented. Isothermal titration calorimetry (ITC) data and double mutant cycle analyses reveal an interesting interplay of positive as well as negative allosteric and positive chelate cooperativity thus providing profound insight into the effects governing multivalent binding in these pseudorotaxanes.



Multivalency[1] is fundamental for many biological processes such as virus-docking to cell surfaces. Strong, yet reversible Velcro-like binding is achieved through multiple interactions. Binding strength gains depend not only on the number of binding sites and their preorganized arrangement but also on allosteric and chelate cooperativity.[2] The number of binding sites needs to be known for an analysis of cooperativity. In biological systems, this is often not the case and precise thermodynamic analyses are challenging. The systematic variability of binding site number and spacer design is an advantage of synthetic complexes.[3] Intriguing examples are the "molecular elevators", triply threaded ammonium/crown-ether pseudorotaxanes which can be switched between two stations along their axles.[4] Here, multivalent binding not only causes higher binding strengths but also controls the relative positions of the subunits and thus function. While kinetic effects—a slow third threading step following two much faster ones—have been investigated,[5] a thorough thermodynamic analysis is not yet available.

Among the reports on di- and trivalent pseudorotaxanes,[4−6] only three analyze cooperativity effects in detail.[7] Attractive spacer−spacer interactions are an important factor for positive chelate cooperativity in divalent ammonium/crown-ether pseudorotaxanes **1@9**−**4@9** (Figure 1).[7a,c] In this work, we use isothermal titration calorimetry (ITC) to determine binding parameters of di- and trivalent ammonium/crown-ether pseudorotaxanes to assess allosteric and chelate cooperativity effects which govern multiple threading. We use the term "allosteric" in a broader sense. It comprises not only structural changes but also, for example, electronic effects that the first binding event has on the next as they occur in complexes that contain either monovalent axles and di- or trivalent wheels or, *vice versa*, monovalent wheels with di- or trivalent axles. Beyond this, the analysis of chelate cooperativity is performed by double mutant cycles that permit determination of effective molarities *EM*—a measure for chelate cooperativity—from only four titration experiments.[8]

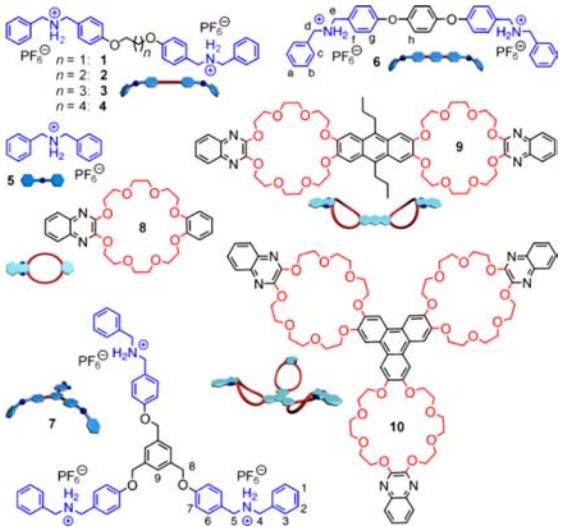


Figure 1. Pseudorotaxane axles and wheels.

The synthesis of the compounds under study is exemplarily shown in Scheme 1 for the two trivalent ones (for details, see Supporting Information). Briefly, the axles were prepared by ether synthesis followed by reductive amination. The free amines were then protonated with HCl followed by an anion exchange of Cl^- against PF_6^- to increase solubility and reduce competition of the crown and anion for the binding sites in organic solvents. Trivalent crown ether **10** was made by oxidative cyclotrimerization.

All pseudorotaxanes form spontaneously when axles and wheels are combined in $CHCl_3/CH_3CN$ mixtures. The solvent ratio can be adjusted to achieve sufficient solubility of both components. Typical complexation-induced ^1H NMR signal shifts demonstrate a quantitative formation of the pseudo-

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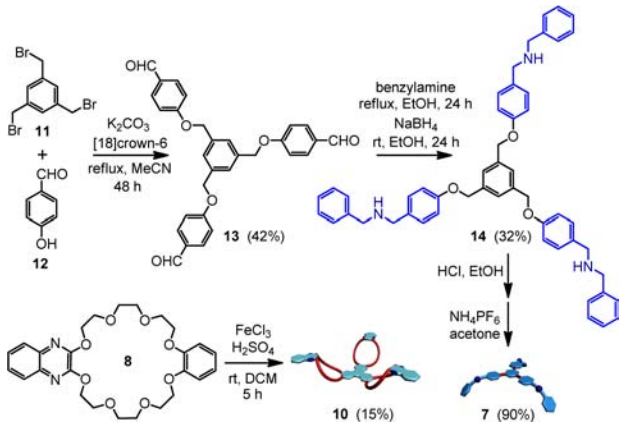
Scheme 1. Syntheses of Trivalent Axle 7 and Wheel 10



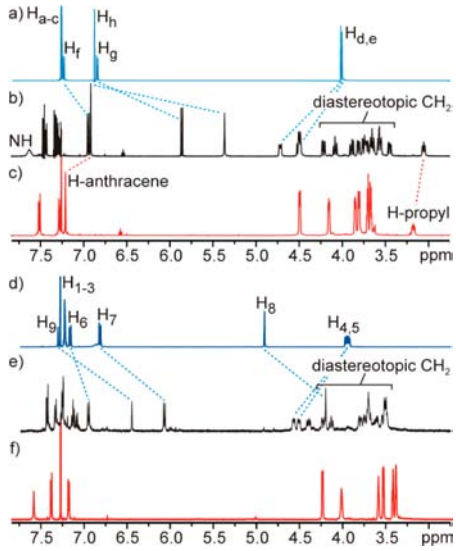


Figure 2. ^{1}H NMR spectra (500 MHz, 298 K, 2.5 mM) of (a) axle **6**, (b) pseudorotaxane **6@9**, and (c) crown ether dimer **9** in $CDCl_3$/CD_3CN 2:1 and (d) axle **7**, (e) pseudorotaxane **7@10**, and (f) crown ether trimer **10** in $CDCl_3$/CD_3CN 1:1.

rotaxanes (Figure 2). Particularly pronounced upfield shifts are observed for spacer protons H_g and H_h (**6@9**) as well as H_6, H_7, and H_9 (**7@10**), which experience the anisotropy of the anthracene spacers. In contrast, the benzylic protons H_d, H_e (**6@9**) and H_4, H_5 (**7@10**) significantly shift downfield caused by the formation of C−H···O hydrogen bonds between the polarized C−H bonds next to the charges and crown ether oxygen atoms. Threading of nonsymmetric axles renders the protons of all crown ether CH_2 groups diastereotopic and cause two sets of signals with complex coupling patterns for these protons in the pseudorotaxane, another clear piece of evidence for pseudorotaxane formation. Pseudorotaxane formation is also confirmed by ESI mass spectrometry (Supporting Information).

After establishing pseudorotaxane formation, isothermal titration calorimetry has been used to determine standard Gibbs binding energies, binding enthalpies and binding entropies. The data obtained for all mono-, di-, and trivalent pseudorotaxanes are summarized in Table 1. The monovalent pseudorotaxane was measured twice, as solubility required two different $CHCl_3$/CH_3CN solvent mixtures: 2.2:1 for the di- and 1:1 for the trivalent case.

Four titration experiments suffice for a detailed analysis of allosteric and chelate cooperativity. By determining the monovalent binding constant as a reference and by titrating the di- or trivalent axle with monovalent crown ethers and *vice versa* the di- or trivalent crown ether with monovalent axles, one can determine allosteric cooperativity, which describes any steric or electronic effect that one binding event has on the following ones and which are not related to the presence of both spacers. In the two latter titration experiments, the statistical factors[9] need to be taken into account for the two or three binding steps (Supporting Information). Normalizing the experimentally determined values K_1, K_2, and K_3 with these statistical factors provides the intrinsic binding constants K_n^{int} that equal K_{mono} in the absence of allosteric cooperativity. An allosteric cooperativity factor α^{allo} can thus easily be defined (eq 1) for the *n*-th binding step (divalent pseudorotaxane: $n = 2$, trivalent pseudorotaxane $n = 2, 3$). A value of $\alpha_n^{allo} > 1$ indicates positive allosteric cooperativity, and $\alpha_n^{allo} < 1$ indicates negative

Table 1. Thermodynamic Data Obtained for Mono-, Di-, and Trivalent Pseudorotaxanes from ITC Experiments

pseudorotaxane		K [M^{-1}]	ΔG [kJ mol^{-1}]	ΔH [kJ mol^{-1}]	$T\Delta S$ [kJ mol^{-1}]	K_σ	K_n^{int} [M^{-1}]	α^{allo}	EM [mM]	EM K_{mono}
6@9[a]	K	67,200 ± 6,800	−27.6 ± 0.3	−64.1	−36.5				779	164
6@8$_2$[a]	K_1	720 ± 80	−16.3 ± 0.4	−54.0	−37.7	4	180			
	K_2	310 ± 40	−14.2 ± 0.3	−15.0	−0.8	1	310	1.5		
5$_2$@9[a]	K_1	735 ± 90	−16.4 ± 0.3	−48.0	−31.6	4	185			
	K_2	145 ± 20	−12.3 ± 0.4	−75.2	−62.9	1	145	0.7		
5@8[a]	K[c]	420 ± 50	−7.5 ± 0.2	−30.7	−23.2	2[c]	210[c]			
7@10[b]	K	173,000 ± 18,000	−29.9 ± 0.3	−67.4	−37.5				47	12
7@8$_3$[b]	K_1	1,640 ± 170	−18.3 ± 0.2	−32.3	−14.0	6	275			
	K_2	600 ± 60	−15.9 ± 0.2	−32.0	−16.1	2	300	1.2		
	K_3	340 ± 40	−14.4 ± 0.2	−38.7	−24.3	2/3	510	2.0		
5$_3$@10[b]	K_1	1,510 ± 160	−18.2 ± 0.2	−24.6	−6.4	6	250			
	K_2	220 ± 30	−13.3 ± 0.2	−25.8	−12.5	2	110	0.4		
	K_3	70 ± 10	−10.6 ± 0.2	−22.1	−11.5	2/3	105	0.4		
5@8[b]	K[c]	520 ± 50	−7.8 ± 0.2	−15.5	−8.7	2[c]	260[c]			

[a]Titration in $CHCl_3$/CH_3CN 2.2:1. [b]Titration in $CHCl_3$/CH_3CN 1:1. [c]Our statistical factors take into account that the monovalent axle can be threaded into the monovalent crown ether in two different orientations and thus account for the symmetry of the monovalent axle that the other axles do not have. K_{mono} thus equals half the measured binding constant for the monovalent pseudorotaxanes (K_{mono} = 210 M^{-1} in $CHCl_3$/CH_3CN 2.2:1 and K_{mono} = 260 M^{-1} in $CHCl_3$/CH_3CN 1:1).

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allosteric cooperativity (K_σ is the corresponding symmetry factor).

$$\alpha_n^{\text{allo}} = \frac{\frac{1}{K_\sigma}K_n}{K_{\text{mono}}} = \frac{K_n^{\text{int}}}{K_{\text{mono}}} \quad (1)$$

Ammonium ion binding to crown ethers in low dielectric constant solvents is known to be strongly affected by ion pairing effects.[10] As the analysis of the concentration dependence of NMR shifts[10a,d] revealed, these effects affect the binding constants of the crown ether/ammonium complexes so strongly, because they are large for the free ammonium salts, while they are negligible for the crown ether complexes, in which the two charges are quite remote from each other as evidenced also by crystal structure analyses.[10d,11] Besides steric and electronic effects that a binding event has on the subsequent binding step, the allosteric cooperativity factor α_n^{allo} also comprises those (and only those) changes in ion pairing effects that depend on valence, while it excludes differences between ion pairing of free axle and pseudorotaxane that analogously occur for the monovalent pseudorotaxane.

A fourth titration, i.e. that of the di/trivalent crown ether with the di/trivalent axle, completes the data required for the evaluation of chelate cooperativity, which describes all cooperativity effects that result from tethering both binding partners. With the data of all four experiments available, the effective molarities *EM* for the di- and trivalent pseudorotaxanes can be determined from double mutant cycles as detailed in the Supporting Information. *EM* is a measure of the preference of intramolecular ring closure over the formation of supramolecular polymers. Note that a trivalent complex undergoes two cyclization steps, each one requiring an *EM* value. Without a divalent reference available, we use an apparent *EM* here as defined in the Supporting Information. The product *EM* K_{mono} is a dimensionless number, which provides a measure for chelate cooperativity: If *EM* $K_{\text{mono}} \gg 1$, binding occurs with positive chelate cooperativity and the formation of closed cyclic complexes is preferred. If *EM* $K_{\text{mono}} < 1$, negative chelate cooperativity is encountered resulting in open complexes that can oligomerize. The double mutant cycles are constructed in such a way that that effects that are not related to chelate cooperativity are warranted to cancel. This also includes the allosteric effects. Furthermore, the ion pairing effects hardly affect chelate cooperativity, as the double mutant cycle does not contain any free ammonium ions and is exclusively based on crown ether complexes, which are not affected by ion pairing effects significantly (also, see Supporting Information).

Binding in our ammonium/crown pseudorotaxanes is enthalpy-driven (Table 1). All binding entropies are negative, indicating higher order upon pseudorotaxane formation. The liberation of solvent during binding thus does not overcompensate for the entropic effects related to the particle number reduction and conformational fixation of spacers. These unfavorable entropic effects are nevertheless more than counterbalanced by the quite strongly negative binding enthalpies.

Positive allosteric cooperativity is found for pseudorotaxanes **6@8**$_2$ and **7@8**$_3$. In **7@8**$_3$, this effect is even more pronounced in the last binding step. We attribute this positive allosteric effect to the formation of ion pairs within the di- and trivalent axles in the rather unpolar solvents used. Taking **7@8**$_3$ as an example, free axle **7** can fold into a cyclic array of the three arms (Figure 3). The positive charges are electrostatically connected by three interdigitating PF_6^- counterions. After binding of the first crown ether, two such ion pairs have been broken up, so that ion pairing is partially destroyed and the competition of the second crown with the counterions is diminished. After binding of the second crown, also the last such interaction is lost and the crown/anion competition for the ammonium group is even lower, thus resulting in an even larger positive allosteric effect.

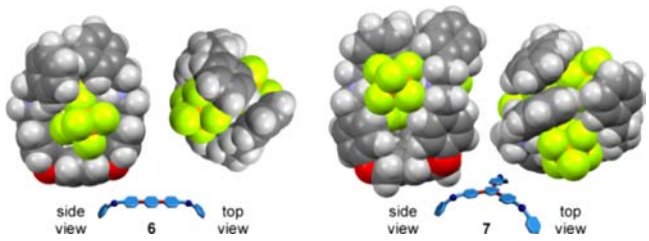

Figure 3. MM2 force-field-optimized structures[12] of the di- and trivalent axles **6** and **7** folded through ion-pairing effects.

In marked contrast, the two pseudorotaxanes **5**$_2$**@9** and **5**$_3$**@10** exhibit negative allosteric cooperativity that is more pronounced in the trivalent case. This effect can be rationalized by a polarization of the aromatic π-systems of the spacers after binding of a positively charged axle. The first binding event causes a polarization of the spacer toward the positive charge and thus reduces electron density at the remaining free binding sites and the following binding interaction. The finding that the second and third binding steps in **5**$_3$**@10** exhibit similar α^{allo} factors of 0.4 is well in agreement with this hypothesis.

In the di- and trivalent pseudorotaxanes **6@9** and **7@10**, both allosteric effects will at least in part counterbalance each other. For the divalent pseudorotaxane **6@9**, one would expect no significant overall effect, as the product of both allosteric effects is essentially one. For **7@10**, one would expect an overall negative allosteric effect. These arguments demonstrate how a detailed analysis of allosteric cooperativity can be very fruitful even in the absence of a substantial net effect, because the separation of these counterbalancing effects helps in analyzing which factors affect binding strongly and should thus be optimized in the design of supramolecular complexes.

A look at the effective molarities and the *EM* K_{mono} values for **6@9** and **7@10** clearly reveals positive chelate cooperativity effects for both pseudorotaxanes. With *EM* K_{mono} = 164, the divalent pseudorotaxane exhibits by far the larger effect compared to *EM* K_{mono} = 12 for the trivalent one. A direct comparison is, however, not easily possible, as the spacer structures are not directly related to each other and spacer−spacer interactions are thus not comparable. As the double mutant cycles waive all other effects except for those caused by the spacers incorporated in the pseudorotaxanes, we can conclude that a good geometric fit of axle and wheel and in addition favorable spacer−spacer interactions such as $\pi-\pi$ stacking between the two spacers are the origins of positive chelate cooperativity, which is important for the optimal design and high-yield syntheses of the above-mentioned "molecular elevators" and other multiply threaded, functional molecular machines. Furthermore, spacer−spacer interactions may alter the binding properties of different stations along the axles by favoring the closest one above the more remote one. An analysis of spacer−spacer interactions is therefore also very

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helpful in the design of the best spacers for a desired molecular machine.

In conclusion, this study analyzes in detail the thermodynamics of the formation of one di- and one trivalent ammonium/crown-ether pseudorotaxane. Based on data obtained by ITC, double mutant cycles were used to provide insight into chelate cooperativity. The same binding data also revealed interesting positive as well as negative allosteric cooperativity effects. They allow us to obtain insight into the details of multivalent binding and will thus help in designing and synthesizing other optimized pseudorotaxanes and multiply threaded molecular machines.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02592.

Experimental procedures, characterization data, original spectra, ITC titrations and double mutant cycle analyses (PDF)

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Notes

The authors declare no competing financial interest.

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