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Chelate cooperativity effects on the formation of di- and trivalent pseudo[2]rotaxanes with diketopiperazine threads and tetralactam wheels†

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The formation of singly, doubly and triply threaded pseudo[2]rotaxanes with diketopiperazine threads and tetralactam wheels is investigated with respect to chelate cooperativity effects on multivalent binding. Two series of guest molecules are prepared which differ with respect to their spacers, one with pre-organised centrepieces with di- or tripodal roof-like structures, one with more flexible spacers. The thermodynamics of pseudorotaxane formation is examined using isothermal titration calorimetry and ^{1}H NMR spectroscopy. Force-field calculations provide more detailed structural insight and help rationalizing the thermodynamic data. All di- and trivalent pseudorotaxanes exhibit positive chelate cooperativity presumably arising from spacer–spacer interactions. Higher cooperativity factors are observed for the more preorganised threads.

Introduction

In nature, multivalency is often present in systems in which strong, yet reversible interactions are required. One of the most prominent examples is the influenza virus which attaches during infection to the cell surface with its multiple trivalent hemagglutinin binding sites.[1] In order to study the phenomena related to such polyvalent binding, model systems need to be established. Supramolecular chemistry has the great advantage to reduce the complexity of natural systems to the most essential segments. Taking advantage of the broad toolbox of modern organic synthesis, supramolecular complexes can be adjusted to suit the requirements of a systematic investigation. This can help to obtain a more profound understanding of synthetic as well as natural multivalent binding.[2–4] The number of binding sites, the spacer length and flexibility,[5,6] as well as the nature of the interaction can be changed. Thus, a growing number of studies is focused on multivalency due to the broad range of prospective applications especially in materials science and medicine.[1,7–11] Nevertheless, fundamental aspects such as allosteric and chelate cooperativity are still not fully understood and are currently under intense investigation.[1–4,12,13] The formation as well as the geometry of a multivalent complex can be improved by changing several parameters. In this context, we recently demonstrated that elongating the spacer unit of divalent crown/ammonium pseudorotaxanes strongly influences chelate cooperativity.[14] Also, strong chelate cooperativity effects caused gated photochromism in divalent crown/ammonium pseudorotaxanes with azobenzene spacers in their threads.[15]

Here, we report the formation of two series of pseudorotaxanes based on mono-, di- and trivalent Hunter/Vögtle-type tetralactam macrocycles (TLM) **H1**–**H3** and novel covalently preorganised as well as flexible mono-, di- and trivalent diketopiperazine (DKP) thread molecules (Fig. 1).[16,17] TLMs have been widely used for the synthesis of amide rotaxanes and pseudorotaxanes,[18–25] as they exhibit four converging amide groups which can form hydrogen bonds to suitable guest molecules like dicarbonyl compounds.[17,26–28] DKP fits nicely into the cavity of the TLM where it can form up to four strong hydrogen bonds, leading to extraordinarily stable pseudorotaxanes.[17] Two series of guest molecules with different spacer units are investigated which converge with the corresponding TLM hosts. The first series contains flexible spacers based on a planar centrepiece, *i.e.* 1,3-dihydroxy- and 1,3,5-trihydroxybenzene. It consists of two or three alkyl chains linked by 1,3-dihydroxy- or 1,3,5-trihydroxybenzene (**dG1**, **dG2**, **tG1**, **tG2**). The centrepieces of the second series have a preorganised roof-like structure. Therefore, the somewhat shorter alkyl chains are linked by 4-[2-(4-hydroxyphenyl)propan-2-yl]phenol (**dG3**, **dG4**) or 4-[1,1-di-(4-hydroxyphenyl)ethyl]phenol (**tG3**, **tG4**). A detailed thermodynamic study utilizing isothermal

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† Electronic supplementary information (ESI) available: Experimental procedures and analytical data for new compounds, ITC experiments, force-field modelling, original ^{1}H-, ^{13}C-NMR and mass spectra. See DOI: 10.1039/c5ob01687h

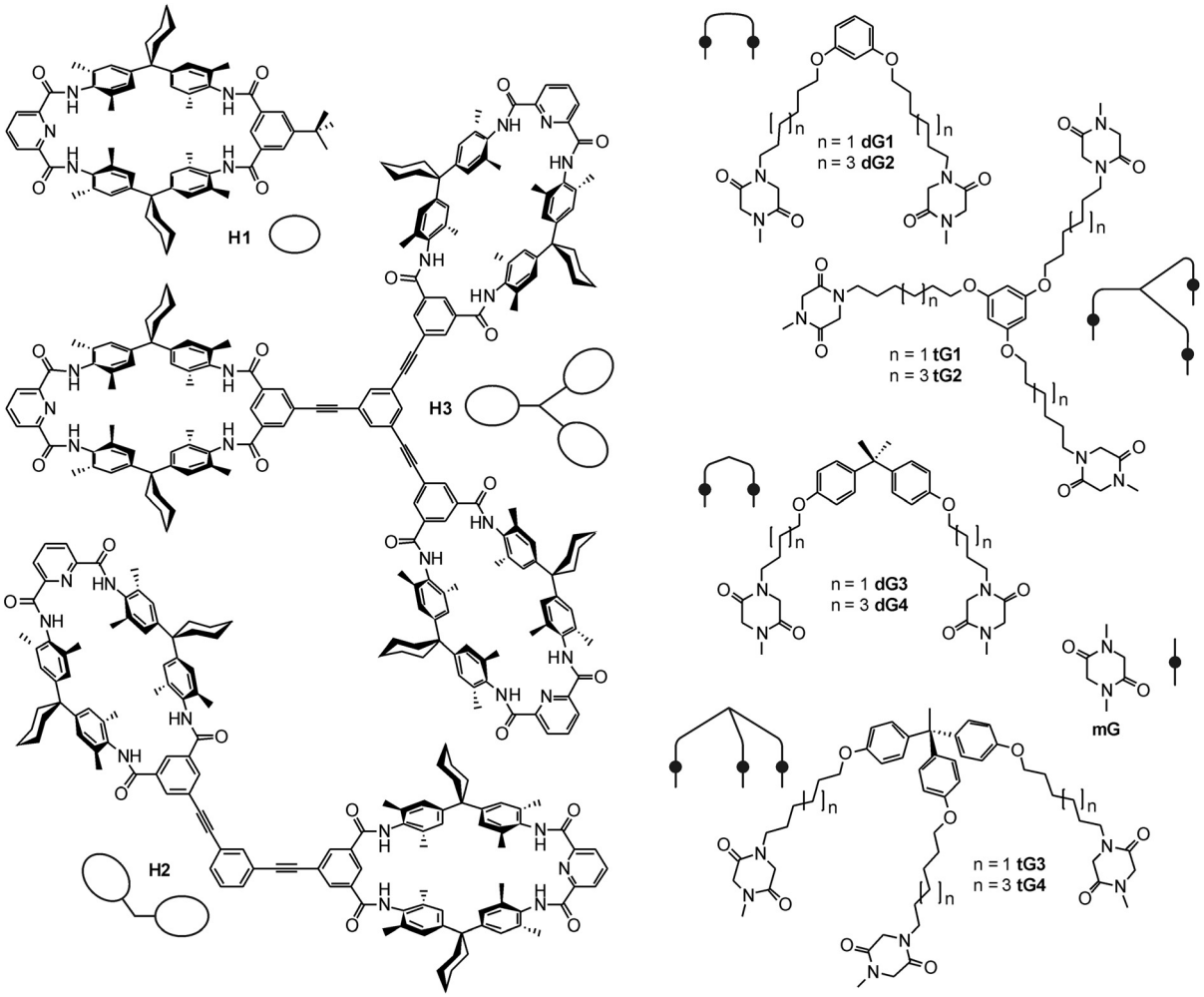

Fig. 1 Chemical structures of the wheels and threads under study and the cartoons identifying them.

titration calorimetry (ITC) is supported by structural evidence from ^{1}H NMR spectroscopy. Double mutant cycle analyses provide insight into the binding behaviour and the cooperativity of the multivalent systems.[29,30]

Results and discussion

Design and synthesis

Based on former studies, the well-established Hunter/Vögtle-type tetralactam macrocycle was used as the host molecule.[24] As the solubility of these TLM macrocycles in nonpolar organic solvents like chloroform is significantly higher, when one isophthaloyl diamide unit is replaced by a pyridine diamide, the modified version of the TLM bearing a pyridine-2,6-dicarboxamide unit was used for the present study. Nonpolar solvents are required for pseudorotaxane formation, as they do not compete strongly with hydrogen bond formation between thread and wheel.[24–26] Diketopiperazine was selected as the thread binding site because it offers rather strong interactions with the TLM wheels.[31,32] These strong binding interactions of diketopiperazine itself were rationalised by a combination of amide-NH···O=C hydrogen bonds and NH···π interactions.[31,32] To improve thread solubility, free NH groups in the diketopiperazine binding motif were methylated. This of course waives the NH···π interactions. Nevertheless, even the substituted diketopiperazines remain excellent binding sites (K = 15 700 M^{-1}; see below). One of the diketopiperazine amide groups also serves as the connection point to the spacer. The length of the spacer unit is varied to fine-tune the binding interactions.

The syntheses of the mono-, di- and trivalent wheels have been described earlier.[16] Briefly, brominated TLM macrocycles were coupled to di- and triethynylbenzene by Sonogashira cross coupling reactions. The threads were synthesised by first connecting the alkyl chains to the spacer centrepieces by nucleophilic substitution. Afterwards, the methylated diketopiperazine, which was synthesised by intramolecular peptide bond formation of glycylsarcosine,[33] was introduced to the other ends of the alkyl chains to connect the binding sites

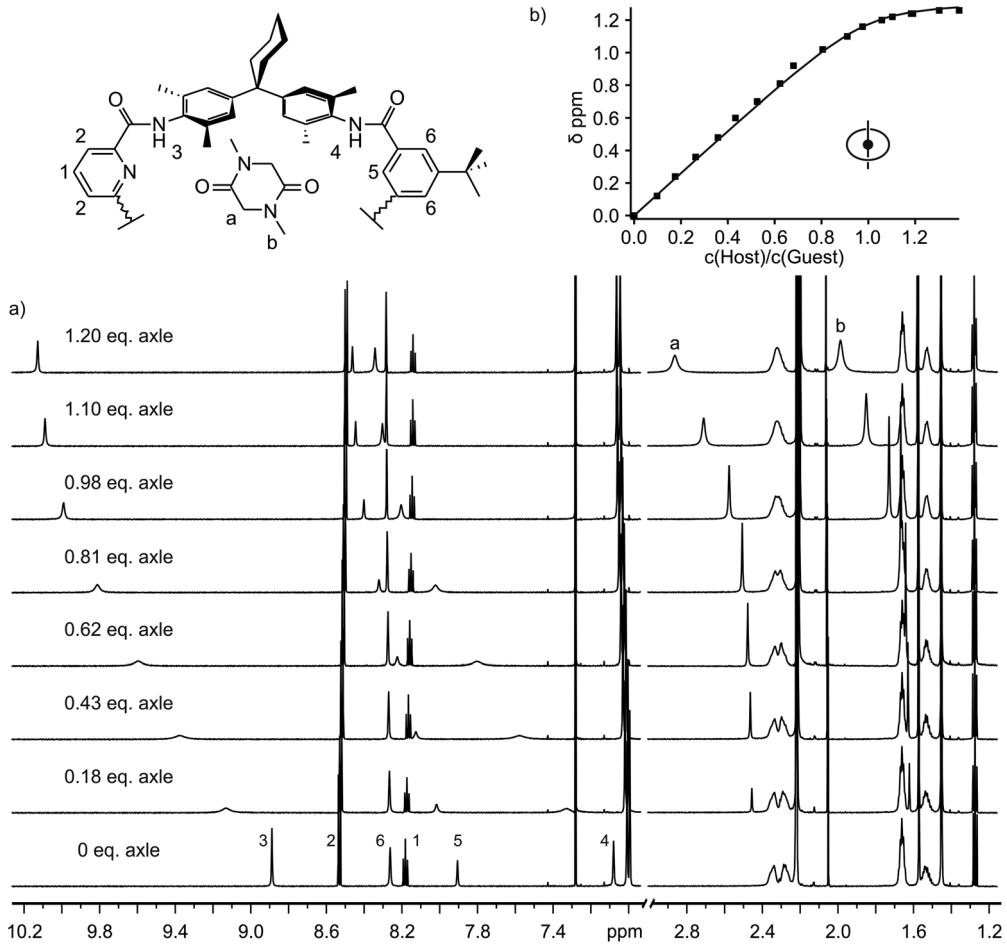

Fig. 2 ^{1}H NMR titration of **H1** with **mG** (500 MHz, 298 K, $CDCl_3$). Top left: Assignment of protons. Top right: Titration curve and curve fitting for binding constant determination.

with the spacers. The only exceptions are **dG1** and **tG1**, for which the sequence was reversed. Detailed procedures for the compounds under study are available in the ESI.†

^{1}H NMR analysis

Pseudorotaxane formation can be followed by ^{1}H NMR titrations (Fig. 2; for titrations of di- and trivalent complexes, see ESI†). Typical complexation-induced shifts not only indicate a guest exchange fast on the NMR time scale, but are also consistent with **mG** binding inside the cavity of **H1**. The **H1** amide protons (H-3, H-4) shift downfield by 1.26 and 1.24 ppm, respectively, due to hydrogen bond formation to the DKP carbonyl oxygen atoms. The inner isophtaloyl diamide proton H-5 is affected by the presence of the thread and shifts downfield by 0.56 ppm. The thread protons a and b experience the anisotropy of the aromatic rings surrounding the cavity and are shifted upfield by 1.51 and 0.92 ppm, respectively. Similar shifts are detected for all multivalent pseudorotaxanes discussed. Using the amide signals H-3 for titration curve fitting,[34] the binding constants for the mono-, di- and trivalent pseudorotaxanes **mG@H1** (16 000 M^{-1}) **dG1@H2** (130 000 M^{-1}) and **tG1@H3** (380 000 M^{-1}) were exemplarily determined. Although the latter values are beyond the limits of NMR titration experiments (K_a < 100 000 M^{-1} is usually accepted)[35] and therefore prone to large experimental errors, all three values compare well to those determined by the ITC experiments summarised in Table 1.

Thermodynamic analysis

Thermodynamic binding data of mono- and multivalent pseudorotaxanes has been obtained by isothermal titration calorimetry,[36] a very sensitive method that provides the complete set of parameters in one single measurement.[37] In a typical ITC experiment, a solution of the host (**H1**, **H2**, or **H3**) is placed in the sample cell and is treated stepwise with small amounts of a solution of the guest (**mG**, **dG1–dG4** or **tG1–tG4**). The titrations were performed at 298 K in dry chloroform. The obtained raw data were analysed with the instrument's internal software package and fitted with a 1 : 1, 2 : 1 and 3 : 1 binding model, respectively. Fig. 3 shows the ITC fitting curves of (a) the divalent **dG1@H2–dG4@H2** and (b) the trivalent pseudorotaxanes

Table 1 Thermodynamic data and double mutant cycle analysis of the divalent and trivalent pseudorotaxanes **dG1@H2–dG4@H2** and **tG1@H3–tG4@H3** investigated by isothermal titration calorimetry (298 K, chloroform). Errors in binding constants are in the range of ±10%. Errors in ΔG amount to ±0.4 kJ mol^{-1}, while errors in ΔH and $T\Delta S$ are higher due to uncertainties in the fitting procedure

Pseudo-rotaxane		K [M^{-1}]	ΔG [kJ mol^{-1}]	ΔH [kJ mol^{-1}]	$T\Delta S$ [kJ mol^{-1}]	EM[a] [mM]	EM·K_{mono}
dG1@H2		129 000	−29.2	−41.7	−12.5	EM = 1.03	8.1
dG2@H2		203 000	−30.3	−61.4	−31.1	EM = 1.93	15.2
dG3@H2		400 000	−32.0	−62.5	−30.5	EM = 2.72	21.4
dG4@H2		669 000	−33.3	−68.1	−34.8	EM = 4.31	33.8
tG1@H3		387 000	−31.9	−74.9	−43.0	EM = 0.27	2.1
tG2@H3		370 000	−31.8	−83.4	−51.6	EM = 0.25	1.9
tG3@H3		466 000	−32.4	−65.5	−33.1	EM = 0.30	2.3
tG4@H3		690 000	−33.3	−86.7	−53.4	EM = 0.36	2.8
dG1@H1$_2$	K_1	29 400	−25.5	−36.8	−11.3		
	K_2	5630	−21.4	−11.3	+10.1		
dG2@H1$_2$	K_1	29 100	−25.5	−28.5	−3.0		
	K_2	4790	−21.0	−37.5	−16.5		
dG3@H1$_2$	K_1	30 300	−25.6	−32.6	−7.0		
	K_2	6430	−21.7	−35.8	−14.1		
dG4@H1$_2$	K_1	30 200	−25.6	−27.3	−1.7		
	K_2	6800	−21.9	−45.8	−23.9		
mG$_2$@H2	K_1	30 700	−25.6	−40.5	−14.9		
	K_2	6070	−21.6	−4.3	+17.3		
tG1@H1$_3$	K_1	47 300	−25.8	−32.5	−5.7		
	K_2	15 100	−24.1	−28.8	−4.9		
	K_3	4980	−20.5	−36.3	−15.2		
tG2@H1$_3$	K_1	47 800	−26.7	−35.9	−9.2		
	K_2	15 400	−23.9	−36.6	−12.7		
	K_3	5370	−21.3	−30.4	−9.1		
tG3@H1$_3$	K_1	47 200	−26.7	−31.8	−5.1		
	K_2	14 600	−23.8	−40.9	−17.1		
	K_3	5040	−21.1	−20.7	+0.4		
tG4@H1$_3$	K_1	47 100	−26.7	−35.3	−8.6		
	K_2	15 100	−23.6	−35.7	−11.9		
	K_3	5020	−21.1	−30.5	−9.4		
mG$_3$@H3	K_1	47 600	−26.7	−40.2	−13.5		
	K_2	15 900	−24.0	−44.0	−20.0		
	K_3	5020	−21.1	−6.5	+14.6		
mG@H1	$2K_{mono}$ [a]	15 700	−24.0	−42.6	−18.6		

[a] Effective molarities EM were determined by double mutant cycle analyses. The cooperativity factor defined by the product EM·K_{mono} is based on $K_{mono} = \frac{1}{2}K^D = 7850$ M^{-1} (for details, see ESI).

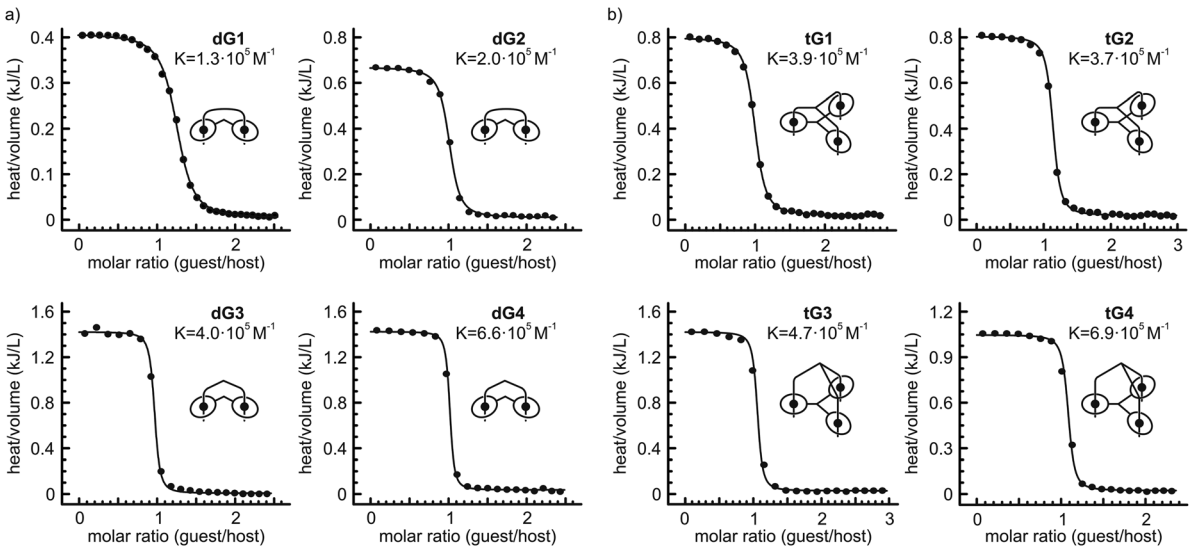

Fig. 3 ITC titration plots (heat/volume over molar ratio guest/host) of (a) divalent tetralactam macrocycle **H2** with divalent diketopiperazine threads (**dG1–dG4**) and (b) trivalent tetralactam macrocycle **H3** with trivalent diketopiperazine threads (**tG1–tG4**). All titrations were performed at 298 K in dry $CHCl_3$.

tG1@H3–tG4@H3. The data of all titrations are summarised in Table 1.

Free binding energies ΔG of the flexible divalent pseudorotaxanes decrease slightly with increasing spacer length ($\Delta G_{\mathbf{dG1@H2}}$ = −29.2 kJ mol^{-1}, $\Delta G_{\mathbf{dG2@H2}}$ = −30.3 kJ mol^{-1}). Similar values are obtained for the more preorganised divalent pseudorotaxanes ($\Delta G_{\mathbf{dG1@H2}}$ = −32.0 kJ mol^{-1}, $\Delta G_{\mathbf{dG4@H2}}$ = −33.2 kJ mol^{-1}). The trivalent pseudorotaxanes reveal free binding energies in a similar range for **tG1@H3**, **tG2@H3**, **tG3@H3** and **tG4@H3** ($\Delta G_{\mathbf{tG1@H3}}$ = −31.8 kJ mol^{-1}, $\Delta G_{\mathbf{tG2@H3}}$ = −31.8 kJ mol^{-1}, $\Delta G_{\mathbf{tG3@H3}}$ = −31.9 kJ mol^{-1}, $\Delta G_{\mathbf{tG4@H3}}$ = −33.3 kJ mol^{-1}). This indicates that the last binding step does not contribute significantly to the overall binding energy, likely due to some strain in the trivalent pseudorotaxanes. The values for diketopiperazine pseudorotaxanes are up to 10 kJ mol^{-1} higher compared to previously published di- and trivalent TLM pseudorotaxanes with diamide threads.[16] Thus, diketopiperazines have a significantly higher binding affinity to TLMs than other dicarbonyl compounds.

The entropy changes ΔS are negative for all di- and trivalent pseudorotaxanes in line with the conformational fixation of the spacers. The negative $T\Delta S$ values are nevertheless overcompensated by high enthalpy contributions.[16] The enthalpies ΔH of the divalent flexible pseudorotaxanes decrease with increasing spacer length from −41.7 kJ mol^{-1} (**dG1@H2**) to −61.4 kJ mol^{-1} (**dG2@H2**), whereas the preorganised pseudorotaxanes exhibit only slight changes from −62.5 kJ mol^{-1} (**dG3@H2**) to −68.1 kJ mol^{-1} (**dG4@H2**). With one exception, a comparable trend is observed for the flexible as well as preorganised trivalent pseudorotaxanes ($\Delta H_{\mathbf{tG1@H3}}$ = −74.9, $\Delta H_{\mathbf{tG2@H3}}$ = −83.4 kJ mol^{-1}, $\Delta H_{\mathbf{tG3@H3}}$ = −65.5 kJ mol^{-1}, $\Delta H_{\mathbf{tG4@H3}}$ = −86.7 kJ mol^{-1}).

Double mutant cycle analysis

To obtain more profound insight into the binding situation of the multivalent pseudorotaxanes, the double mutant cycle (DMC) approach was used (for details, see ESI†).[13,29,30,38–43] Effective molarities EM for the cyclisation steps in di- and trivalent supramolecular complexes can be determined from such a DMC analysis which represent the critical concentrations above which oligomerisation is preferred over the formation of the closed multivalent complex.[44] The product of the monovalent binding constant K_{mono} and the effective molarity EM provides a measure of chelate cooperativity. When EM·$K_{mono} \gg 1$, the system exhibits positive chelate cooperativity. A value of EM·K_{mono} = 1 indicates a non-cooperative and EM·$K_{mono} < 1$ a negatively cooperative system.

An advantage of the DMC analysis is the exclusion of all effects that are not due to chelate cooperativity.[29,40] The DMCs for the di- and trivalent pseudorotaxanes including the determination of the statistical factors involved are detailed in the ESI.† The resulting EM and EM·K_{mono} values are listed in Table 1. All pseudorotaxanes exhibit positive chelate cooperativity. While the divalent pseudorotaxanes exhibit significant positive chelate cooperativity, the EM·K_{mono} values of the trivalent analogues are surprisingly small and indicate only a rather minor positive cooperativity effect. Furthermore, the values differ with spacer structure for the divalent complexes, while they are quite constant for the trivalent analogues. We therefore continue with a detailed discussion of the divalent pseudorotaxanes here.

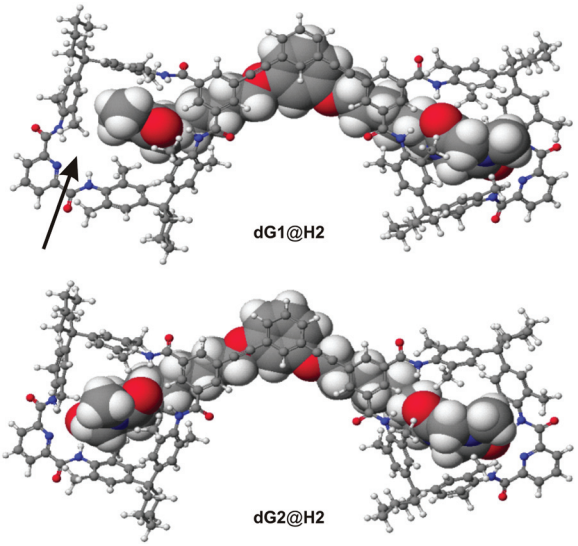

Fig. 4 MM2 force-field-optimised structures (CACHE 5.0 program package, Fujitsu, Krakow/Poland) of divalent pseudorotaxanes **dG1@H2** (top, C_6 alkyl chains) and **dG2@H2** (bottom, C_8 alkyl chains). The arrow indicates imperfect binding of the second diketopiperazine in **dG1@H2**.

In order to correlate the differences in cooperativity observed for the divalent pseudorotaxanes with their spacer structures, MM2 force-field-optimised structures were calculated (Fig. 4 and ESI†).

Comparing **dG1@H2** (containing C_6 alkyl groups between the binding sites and the spacer centrepiece) with **dG2@H2** (C_8 alkyl chains), the calculated structures (Fig. 4) clearly show a mismatch in length for the shorter spacer. While one diketopiperazine is bound to the wheel in a more or less optimal arrangement with four hydrogen bonds, the second diketopiperazine is unable to form all four hydrogen bonds. The longer spacer in **dG2@H2** instead nicely spans between two unstrained binding sites. Both diketopiperazines are bound by four H-bonds here. This interpretation is in agreement with the thermodynamic data. For **dG1@H2**, the binding enthalpy ΔH is in the same range as that of the monovalent complex indicating that significant strain exists in this pseudorotaxane. In contrast, ΔH is significantly larger for **dG2@H2** in turn indicating that this pseudorotaxane is not significantly suffering from strain. Instead, the longer alkyl chains cause a higher degree of conformational fixation and thus result in larger negative entropy for **dG2@H2** as compared to **dG1@H1**.

A similar conclusion emerges from a comparison of **dG3@H2** and **dG4@H2**. Again, a shorter spacer leads to higher strain as expressed in a somewhat smaller, but still clearly observable difference in the two binding enthalpies.

When one instead compares **dG1@H2** with its more flexible thread spacer to the more preorganised pseudorotaxane **dG@H2**, a clear-cut increase in chelate cooperativity is found for the more preorganised spacer. The same trend is observed for the second pair **dG2@H2** and **dG4@H2**.

Conclusions

Four di- and four trivalent diketopiperazine/tetralactam macrocycle pseudorotaxanes were prepared, which differ in the spacers connecting the binding sites with respect to two parameters: two different spacer centrepieces are employed, one of which preorganises the binding sites to a higher extent than the other. In addition, the binding sites are connected to these centrepieces by alkyl chains of two different lengths.

A detailed thermodynamic analysis based on ITC binding data and double mutant analyses results in four major conclusions:

(i) All pseudorotaxanes exhibit positive chelate cooperativity, which can be attributed to an increase in the local concentration of hosts and guests, favourable rebinding effects and attractive spacer–spacer interactions.

(ii) The positive cooperativity effects on the formation of divalent pseudorotaxanes are significantly larger than those observed for the trivalent pseudorotaxanes. We attribute this to unfavourable strain in the trivalent complexes, which renders at least the last threading step non-cooperative. In contrast, the divalent pseudorotaxanes are more flexible and thus, the spacers can adopt favourable geometries relative to each other.

(iii) The spacers chosen show that an exact match of the lengths of thread and wheel components is favourable as too short spacers deform the binding sites and thus cause unfavourable strain.

(iv) Even in spacers, in which the binding sites are connected with the central unit through quite flexible alkyl chains, favourable effects of preorganisation through the structure of the centrepiece are observed.

Our study thus helps understanding the effects of subtle details of the spacer structure on chelate cooperativity. Positive cooperativity is important for the synthesis of (pseudo)rotaxanes and other multiply interlocked molecules, because yields of the fully threaded structure should be as high as possible in order to avoid tedious separation procedures. A more profound knowledge on the parameters that affect chelate cooperativity will therefore in future help to predict, which spacer structures will be favourable.

Acknowledgements

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