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PAPER

Substituent effects on axle binding in amide pseudorotaxanes: comparison of NMR titration and ITC data with DFT calculations†‡

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The binding behaviour of differently substituted diamide axle molecules to Hunter/Vögtle tetralactam macrocycles was studied with a combination of NMR titration, isothermal titration calorimetry (ITC) experiments and calculations employing density functional theory (DFT), along with dispersion-corrected exchange-correlation functionals. Guests with alkyl or alkenyl chains attached to the diamide carbonyl groups have a significantly higher binding affinity to the macrocycle than guests with benzoyl amides and their substituted analogues. While the binding of the benzoyl and alkenyl substituted axles is enthalpically driven, the alkyl-substituted guest binds mainly because of a positive binding entropy. The electronic effects of *para*-substituents at the benzoyl moieties have an influence on the binding affinities. Electron donating substituents increase, while electron-withdrawing substituents decrease the binding energies. The binding affinities obtained from both NMR titration and ITC experiments correlate well with each other. The substituent effects observed in the experimental data are reflected in adiabatic interaction energies calculated with density functional methods. The calculated structures also agree well with pseudorotaxane crystal structures.

Introduction

Pseudorotaxanes are weakly bound host–guest complexes bearing a thread-like linear axle that penetrates the cavity of a macrocyclic host.[1] They are the precursors of their mechanically interlocked siblings, *i.e.* rotaxanes and catenanes, and therefore play a pivotal role in the construction of molecular machines based on interlocked molecules.[2] Understanding the binding properties of pseudorotaxanes has thus an important impact on the development of template effects for rotaxane and catenane synthesis which can be, for example, based on metal complexation,[3] charge transfer interactions,[4] or hydrogen bonding to neutral amides,[5] secondary ammonium ions,[6] or anions.[7] Multiply interlocked supramolecular architectures have been synthesized[8] and were analyzed with respect to multivalent binding.[9] Other, squaraine-based, fluorescent rotaxanes have been used as long-term stable dyes in biological research.[10]

Tetralactam macrocycles (TLM) of the Hunter/Vögtle-type are well-known as host molecules and as rotaxane or catenane wheels. They bear four converging amide groups, which can form hydrogen bonds to suitable axle molecules. This property has not only been utilized for rotaxane and catenane synthesis, but also for the construction of quartz microbalance sensors that can detect carbonyl compounds[11] and for STM investigations of pseudorotaxane formation in TLM monolayers on gold surfaces.[12] The processes that occur during pseudorotaxane and rotaxane formation are known to be quite sensitive to structure and electronic environment. Therefore, small changes can cause unexpectedly large effects as investigated earlier by kinetic studies on the reverse reaction, *i.e.* the deslipping of axles with stopper groups of intermediate sizes.[13]

Jeong and co-workers[14] have examined the binding of a dicarbonyl guest to TLMs similar to **1a** (Scheme 1). In this study, both hemispheres of the macrocycle were pyridine-2,6-dicarboxamides. Substituents at the position *para* to the pyridine nitrogen atom cause strong electronic effects on the binding behaviour of the TLM. This was rationalized by secondary effects[15] and perturbations of the intramolecular hydrogen bonds between the amide NH hydrogen atoms and the pyridine nitrogen. Therefore,

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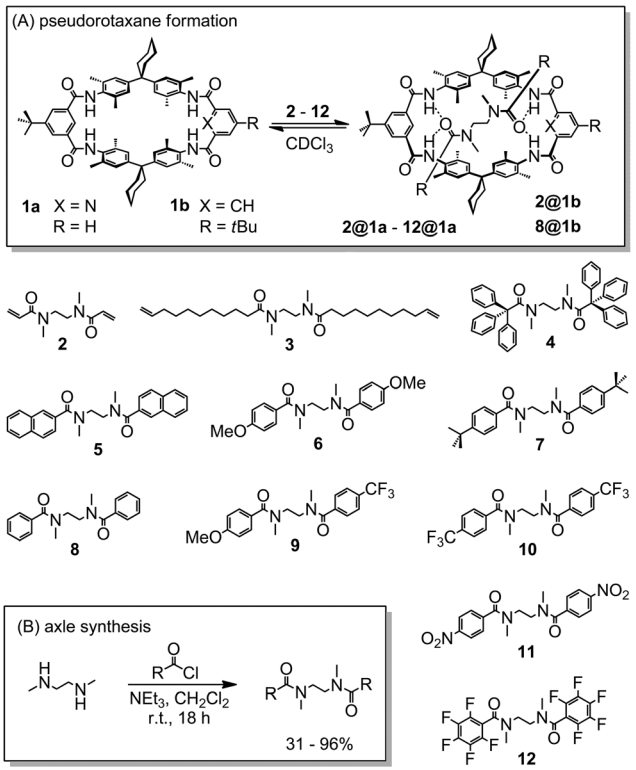

Scheme 1 (A) Binding motif of the pseudorotaxanes based on tetralactam macrocycles **1a** and **1b**; (B) synthesis of axles **2–12** with different R substituents and overview of diamide axles.

a nitro group attached to the pyridine ring increases the binding strength significantly, while electron-donating groups reduce it.

A recent theoretical study[16] systematically varied the substituents on a *mono*amide axle which is known to bind to Hunter/Vögtle TLMs by three hydrogen bonds. Two wheel amides donate one hydrogen bond each to the axle carbonyl group, which in turn donates its amide NH hydrogen to bind to a third amide carbonyl incorporated in the wheel. No significant substituent effects were observed, because the substituents alter the binding properties of the three hydrogen bonds in a counterbalancing way. When the (axle)C═O···H–N(wheel) hydrogen bonds are strengthened, the (axle)N–H···O═C(wheel) hydrogen bond is weakened and *vice versa*. Thus, the substituents affect the individual hydrogen bonding strengths, but overall the effects more or less cancel each other. In marked contrast, a second theoretical paper[17] on pseudorotaxanes with different diamide axles reported strong substituent effects on the total interactions energies. Here, the binding situation is different, since the TLM (in this case the smaller Leigh-type TLM)[18] binds with all four amide NH groups to the two carbonyl groups of the diamide axle and thus excludes a similar compensation effect. Although the individual hydrogen bonds of the diamide pseudorotaxanes are somewhat weaker than those of the monoamide pseudorotaxanes, the calculations predict differences in the total interaction energies of 20 kJ mol^{-1} between the strongest and the weakest axle investigated. The individual hydrogen bonding energies correlate nicely with N–H stretch vibrational frequencies. Good linear correlations between these frequencies and Hammett's substituent constants[19] were found. So far, however, no experimental data supporting these calculations have been reported.

This has encouraged us to investigate the structural and electronic effects of different substituents on the binding of diamide axles to the unsymmetrically substituted TLM **1a** (Scheme 1). In order to obtain reliable data, NMR titrations were combined with isothermal titration calorimetry (ITC) experiments. Sessler *et al.*[20] compared both methods for calix[4]pyrrole, which binds ammonium salts in an ion-pair binding motif. They obtained comparable K_a values as long as the binding constants are within the dynamic ranges of both methods. This combination of methods provides structural information on the binding motifs from NMR experiments with detailed thermodynamic data from ITC measurements and therefore is of great benefit for a complete characterization of the pseudorotaxanes under study.[21] The experimental data are again accompanied with theoretical calculations employing density functional theory (DFT), along with dispersion-corrected exchange-correlation functionals which reflect the substituent effects and show similar trends as experimentally observed.

Results and discussion

TLMs **1a,b** (Scheme 1) were synthesized by an established literature procedure.[22] Macrocycle **1a** contains a pyridine-2,6-dicarboxamide on one side whose N atom points into the cavity and forms two intramolecular (pyridine)N···HN(amide) hydrogen bonds. They force the amide NH atoms to converge into the cavity and thus provide preorganization for a better binding of the axle's carbonyl groups.[23] The opposite hemisphere of **1a** contains an isophthaloyl diamide substituted with a solubility-enhancing *t*-butyl group. On this side, one of the amide NH groups can rotate outwards so that one wheel carbonyl group can point into the cavity, if required by a guest. This conformational change neither has a significant barrier nor causes any substantial energy difference.[24]

The differently substituted diamide guest molecules **2–12** (Scheme 1) are available from simple one-step syntheses in good to excellent yields. Starting from *N*,*N*′-dimethyl ethylene diamine and the appropriately substituted acid chlorides, the desired products are obtained in separate reactions after stirring at room temperature in dichloromethane for 18 hours with NEt_3 as the base. An exception is guest **9**, which was synthesized in a step-by-step approach (see Experimental section). These diamide guests are known to bind non-covalently inside the cavity of **1a**—usually by forming four hydrogen bonds between the carbonyl groups of the guest and the amide hydrogen atoms of **1a,b**.[25] Consequently, a threaded geometry results. Because no bulky stopper groups terminate the axles, the association is reversible on a fast time scale. Since diamide axles are insoluble in organic solvents, when secondary amides are incorporated, their tertiary amide analogues were used for this study. The better solubility, however, comes at the price of higher conformational freedom, because the *cis*- and *trans*-amide isomers equilibrate. The *trans*, *trans*-isomer is the major isomer for the axles under study, but a significant contribution from the *trans*, *cis*- and a minor amount of the *cis*, *cis*-isomer is clearly visible in the axle NMR spectra.[26] Peak broadening at room temperature points to an

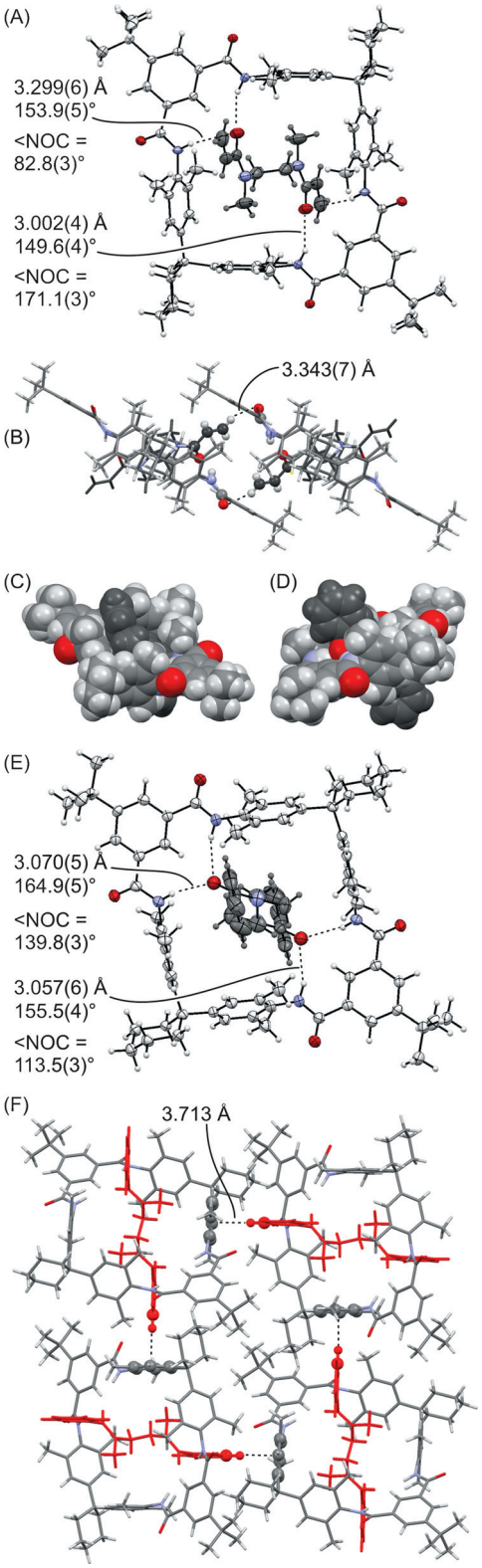

Fig. 1 (A) Ortep plot of the crystal structure of **2@1b** (50% probability level). (B) Packing of **2@1b** highlighting short C–H···O contacts between a vinyl H atom and a carbonyl O of the neighbor's wheel. (C, D) Space filling representations of **2@1b** and **8@1b**, respectively. (E) Ortep plot of the crystal structure of **8@1b** (50% probability level). (F) Crystal packing of **8@1b** highlighting C–H···π contacts between the axle phenyl group and one of the wheel aromatic rings of a neighbouring pseudorotaxane.

interconversion of these isomers slow on the NMR time scale, but close to the coalescence temperature. The binding constants determined here thus are apparent binding constants.

Single crystals of pseudorotaxanes **2@1b** and **8@1b** suitable for X-ray crystallography could be grown and their crystal structures were solved (Fig. 1 and ESI‡). In contrast to the other pseudorotaxanes under study here, these pseudorotaxanes bear the symmetrical wheel **1b** with two *t*-butyl-substituted isophthaloyl diamide moieties in the two hemispheres. Nevertheless, the two structures are quite instructive with respect to the hydrogen bonding connecting the wheel with the diamide stations of the two axles. For **8@1b**, four hydrogen bonds connect axle and wheel (Fig. 1E). The N–O distances of 3.057 and 3.070 Å are in a normal range as found earlier in crystal structures[25a,27] of rotaxanes and catenanes based on Hunter/Vögtle TLMs as well as in theoretical calculations.[24] Because of the zig-zag conformation of the ethylene diamide spacer connecting the two axle amide groups, the two axle carbonyl groups are unable to position themselves exactly in the mirror plane through the two isophthaloyl diamide groups of the macrocycle.

Consequently, the N–H···O hydrogen bond angles differ by about 10°. The deviation of the carbonyl groups from the mirror plane is also reflected in the (wheel)N–O═C(axle) angles that are 113.5° and 139.8°. Thus, the structure of **8@1b** is in good agreement with expectation from earlier studies. The structure of **2@1b** (Fig. 1A) exhibits some contrasting features: The two (wheel)N–H···O═C(axle) hydrogen bonds in each hemisphere are very different in length (3.002 *vs.* 3.299 Å). Consequently, the axle forms two stronger and two weaker hydrogen bonds with the wheel. While the N–H···O angles are not much smaller than those of **8@1b**, the two (wheel)N–O═C(axle) angles describing the tilt of the axle carbonyls out of the wheel's mirror plane are drastically different. The shorter hydrogen bond corresponds to an almost linear arrangement (<NOC = 171.1°), while the longer one relates to an NOC angle of only 82.8°. The tilt of the carbonyl group out of the plane is thus much larger in **2@1b** as compared to **8@1b**. The packing of the two pseudorotaxanes exhibits C–H···O and C–H···π interactions, respectively: one of the vinyl hydrogen atoms at the terminal carbon atom of the axle in **2@1b** directly points towards a carbonyl oxygen atom of the next neighbour's wheel (Fig. 1B). With a value of 3.343 Å, the C–O distance is quite short for a C–H···O hydrogen bond. Instead, each of the phenyl rings of the axle in **8@1b** has a C–H···π contact with one of the aromatic rings in the wheel of the next neighbour pseudorotaxane. The distance between the C–H carbon atom and the centroid of the wheel's aromatic ring is 3.713 Å (Fig. 1F).

Fig. 2 shows the results of a typical ^{1}H NMR titration experiment. Upon stepwise addition of axle **2** to **1a**, the two signals for the four wheel amide NH protons shift upfield by 1.46 ppm (py-NH) and 1.34 ppm (isoph-NH). Similarly, the proton at C(6) of the isophthaloyl moiety feels the presence of the axle inside the cavity of the wheel and shifts by 0.53 ppm to lower field.[28] The 1 : 1 stoichiometry of the axle–wheel complex is confirmed by the intersection of two lines fitted to the first and last four data points of the titration curve, respectively. They cross at a 1 : 1 host–guest ratio. In contrast, the signals for the diamide axle binding site, *i.e.* the axle NCH_3 and NCH_2 protons, shift to higher field, because they feel the anisotropy of the aromatic

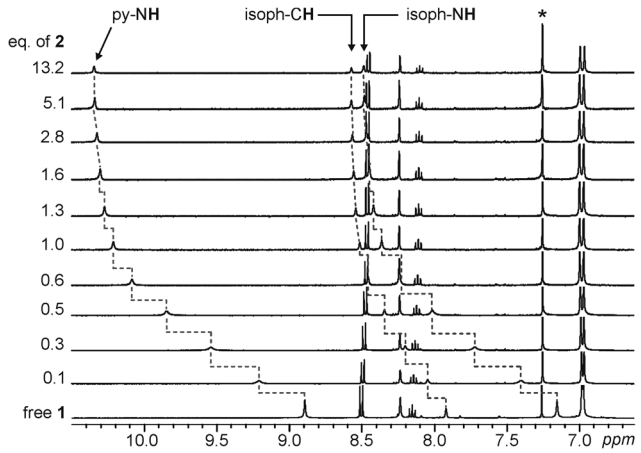

Fig. 2 ^{1}H NMR titration of **1a** with **2** ($CDCl_3$, 298 K). The wheel's amide NH signals undergo significant complexation-induced low-field shifts. Also, the inner isophthaloyl C–H experiences low-field shifts due to the presence of the axle in the wheel's cavity.

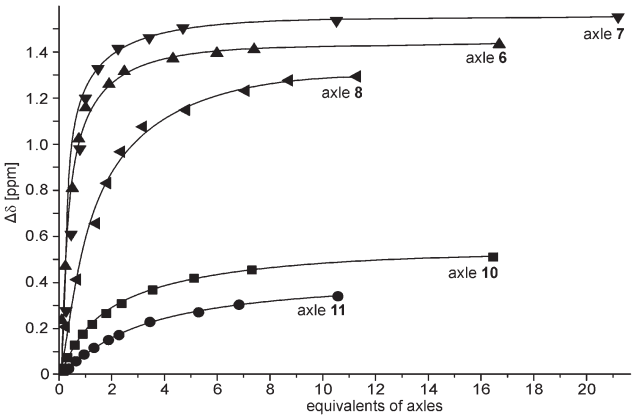

Fig. 3 Shifts of the pyridine dicarboxamide NH signal of **1a** during titrations with aryl-substituted axles **6**, **7**, **8**, **10** and **11** ($CDCl_3$, 298 K).

rings incorporated in the wheel's walls. These complexation-induced shifts clearly confirm that the axle binds inside the wheel cavity by hydrogen bond formation between the wheel's amide NH hydrogen atoms and the axle's carbonyl oxygen atoms. Axle **4** can be used as a control to confirm the threaded geometry of the pseudorotaxanes under study: the two bulky trityl groups prevent slipping of this axle into the cavity so that either no or very different NMR signal shifts are expected. For this axle, only very minor shifts ($\Delta\delta < 0.1$ ppm) of the wheel's amide NH hydrogen atoms are observed. Also, the axle NCH_2 and NCH_3 protons do not shift during the titration. These results thus indicate the axle to be only weakly bound—if at all—at the macrocycle periphery outside the cavity.

From the curvature of the titration curve (ESI‡), the binding constant was determined by non-linear curve fitting based on eqn (1) and a 1 : 1 binding model.[29] In this equation, δ_{obs} is the observed shift of the py-NH signals of the macrocycle at each titration step as a function of the initial concentrations of macrocycle $[M]_0$ and guest $[G]_0$. δ_0 is the chemical shift of the free macrocycle and $\Delta\delta_{max}$ is the difference between the theoretical signal shift at 100% complexation and δ_0.

$$\delta_{obs} = \delta_0 + \frac{\Delta\delta_{max}}{2[M]_0}\left[\frac{1}{K_a} + [M]_0 + [G]_0 - \sqrt{\left(\frac{1}{K_a} + [M]_0 + [G]_0\right)^2 - 4[M]_0[G]_0}\right] \quad (1)$$

$$c = nk_a[M]_0 \quad (2)$$

The same procedure was carried out for pseudorotaxanes **3@1a–12@1a** and the titration curves for the series of aryl-substituted axle derivatives **6–8**, **10** and **11** are summarized in Fig. 3. The resulting binding data are listed in Table 1. Two conclusions can be drawn from the NMR titration data. (i) Axles with alkyl (**3**) or alkenyl groups (**2**) attached to the diamide carbonyl groups have the highest binding constants among the axles under study. With about 4000 M^{-1}, they are roughly one order of magnitude higher than the phenyl substituted axle **8**. This results in the binding free energies of **2** and **3** being higher by *ca.* 6 kJ mol^{-1} than that of **8**. (ii) In the series of aryl-substituted diamides, the binding constants depend significantly on the electronic nature caused by the *para*-substituent. While electron-withdrawing substituents reduce the binding free energies, electron-donating substituents increase them. The axles with the strongest electron-withdrawing groups (*e.g.* NO_2) exhibit only vanishingly small binding constants; no binding was observed for the pentafluorophenyl-substituted axle **12**. Consequently, template effects for rotaxane synthesis based on similar diamide–TLM binding motifs can be modulated by substituent effects affecting the hydrogen bond acceptor qualities of the axle carbonyl groups.

Fig. 4 exemplarily shows the titration curves for the complexation of **2** and **8** to TLM **1a** as obtained from complementary ITC experiments. The determination of K_a, ΔH, ΔG, and ΔS is simultaneously possible in a single experiment so that all relevant thermodynamic binding parameters can be obtained.[30] An important parameter for ITC experiments of low affinity systems is the so-called Wiseman "*c*" value, which is the product of the total host concentration and the binding constant K_a (eqn (2)).[31] For $c < 10$, ITC experiments become challenging. In this equation, n is the number of binding sites in the receptor with $n = 1$ for the pseudorotaxanes under study here. One way to compensate for low affinity binding would be to increase the total concentration of the macrocycle. Unfortunately, the solubility of TLM **1a** limits this approach here. An earlier report[32] on alkali metal ion binding to crown ethers, however, confirmed that ITC measurements still provide reliable data even when c is small and the titration curves are not sigmoidal in shape provided that the following requirement is met: a sufficiently large part of the binding isotherm with sufficient signal-to-noise must be used for analysis. Also, the stoichiometry of the complex formed must be clear and the concentrations of both host and guest must be precisely known.

Making sure that these conditions are met, the binding free energies obtained from the ITC experiments (Table 1) agree well with those extracted from the NMR titrations within ±1.5 kJ mol^{-1} which corresponds to *ca.* 10% of the total binding energies. Consequently, the conclusions drawn from the NMR data above are

Table 1 Thermodynamic data obtained from the NMR titrations and ITC measurements with pseudorotaxanes formed from TLM **1a** and guests **2–12**. NMR titrations and ITC measurements were performed at 25 °C

		NMR titrations[a]		ITC experiments[a]			
Axle	Hammett constant σ	K_a [M^{-1}]	ΔG [kJ mol^{-1}][b]	K_a [M^{-1}]	ΔH [kJ mol^{-1}]	$-T\Delta S$ [kJ mol^{-1}]	ΔG [kJ mol^{-1}][b]
2		4000 ± 400	−20.6 ± 0.3	3500 ± 400	−38.2 ± 2.0	18.0 ± 2.3	−20.2 ± 0.3
3		3800 ± 400	−20.4 ± 0.3	2300 ± 300	−14.5 ± 0.8	−4.7 ± 1.1	−19.2 ± 0.3
4				No binding observed			
5		610 ± 70	−15.9 ± 0.3	490 ± 50	−24.9 ± 1.3	9.5 ± 1.6	−15.4 ± 0.3
6	−0.27	460 ± 50	−15.2 ± 0.3	510 ± 60	−18.9 ± 1.0	3.4 ± 1.3	−15.5 ± 0.3
7	−0.20	380 ± 40	−14.7 ± 0.3	640 ± 70	−29.6 ± 1.5	13.6 ± 1.8	−16.0 ± 0.3
8	0.00	370 ± 40	−14.7 ± 0.3	350 ± 40	−22.9 ± 1.2	8.4 ± 1.5	−14.5 ± 0.3
9		280 ± 30	−14.0 ± 0.3	190 ± 20	−32.5 ± 1.7	19.5 ± 2.0	−13.0 ± 0.3
10	0.43	<20	<−7.4	90 ± 10	−19.8 ± 1.0	8.6 ± 1.3	−11.2 ± 0.3
11	0.78	<10	<−5.7	No binding observed in ITC experiments			
12				No binding observed			

[a] Since NMR and ITC are different methods with respect to optimal concentration regimes and the primary information obtained, some deviations of the data can be expected as discussed earlier by Sessler *et al.*[20a] [b] Calculated with $\Delta G = -RT \ln K_a$.

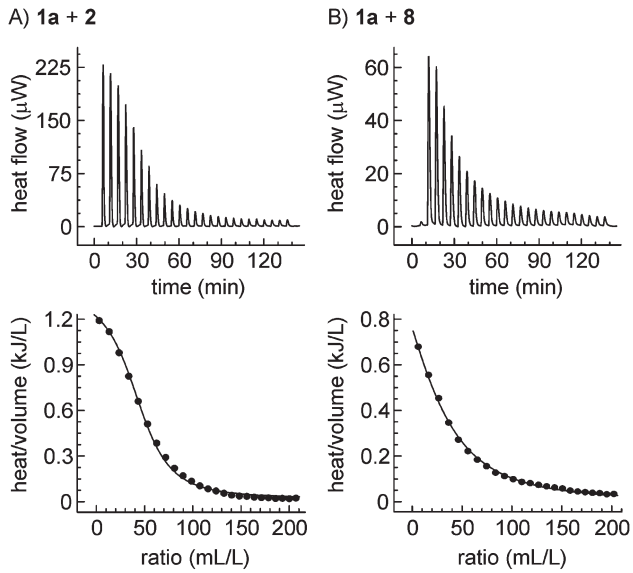

Fig. 4 ITC experiments (heat flow *versus* time and heat/volume *versus* guest/TLM ratio; $CHCl_3$, 298 K) of (A) axle **2** and (B) axle **8** with TLM **1a**.

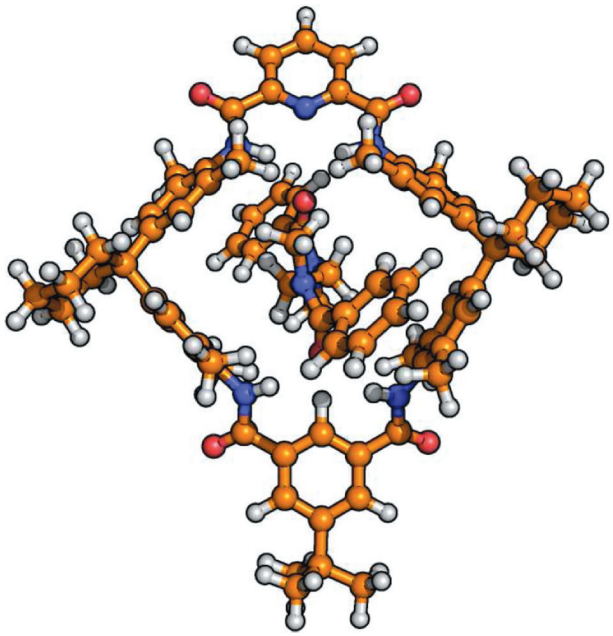

Fig. 5 Structure of **8@1a** optimized at the B97-D level of theory with the TZVP basis set. For the calculated structures of the other aryl-substituted pseudorotaxanes, see ESI.‡

confirmed by the ITC data. Since the ITC experiments also provide binding enthalpies and binding entropies, a more detailed picture is obtained here. Axle **3** with the two long, flexible alkyl chains directly attached to the amide carbonyl groups has quite a low binding enthalpy, but a favorable positive binding entropy thus indicating the complexation to be significantly supported by entropic effects. In marked contrast, all axles that carry phenyl derivatives attached to the amide carbonyls, as well as axle **2** with the short alkenyl substituent, reveal approximately two times larger negative binding enthalpies, while all binding entropies are negative. Binding is clearly driven by enthalpy in these cases. Similar data were obtained for monoamide binding in a related TLM.[16] We attribute the differences in binding entropies to solvent effects. The negative binding entropies for the aryl-substituted diamide axles are in line with the combination of two particles into one complex. The positive entropy for **3@1a** is then due to an overcompensation of this effect by differences in solvation.[33]

The substituent effects on the binding free energies have been investigated theoretically by density functional calculations at the B97-D/TZVP level. The structures of pseudorotaxanes with symmetrical aryl-substituted axles (**6@1a–8@1a** and **10@1a–12@1a**) were optimized starting from a geometry in which the diamide station was bound to the wheel by four (wheel)N–H···O═C(axle) hydrogen bonds. The optimized structure of **8@1a** is shown in Fig. 5 as a representative example. Hydrogen bond lengths and N–H···O angles are summarized in Table 2. The two hydrogen bonds formed to the pyridine dicarboxamide moiety are similar in length (3.01 and 3.02 Å) and somewhat shorter and likely somewhat stronger than the two hydrogen

Table 2 Calculated hydrogen bond lengths and angles, adiabatic interaction energies ΔE_{adia}, energies ΔE_{CF} calculated for the conformational change of wheel and axle upon binding, and shared-electron number SEN for the series of pseudorotaxanes with symmetrically aryl-substituted axles (for definitions, see theoretical section). The four hydrogen bonds between axle are denoted "**py1/2**" for those in the pyridine dicarboxamide hemisphere and "**iso1/2**" for those in the isophthaloyl diamide hemisphere. "**py1**" and "**iso1**" diametrically oppose each other in the calculated structures

	N–H···O hydrogen bond lengths [Å]				N–H···O hydrogen bond angles [°]						
Axle	py1	py2	iso1	iso2	py1	py2	iso1	iso2	ΔE_{adia} [kJ mol^{-1}]	ΔE_{CF} [kJ mol^{-1}]	SEN[a] σ_{total} [e]
6	2.92	3.12	2.91	2.95	145.5	148.7	158.3	155.0	−194.4	−40.6	0.1206
7	3.03	2.99	3.15	3.11	146.0	146.9	162.7	149.2	−193.5	−28.7	0.0803
8	3.02	3.01	3.15	3.10	144.6	146.6	162.1	147.8	−182.1	−25.9	0.0770
10	3.04	3.08	3.15	3.09	140.3	144.6	160.1	147.3	−180.5	−28.2	0.0688
11	3.03	3.09	3.25	3.11	141.7	145.6	161.2	145.4	−178.4	−25.9	0.0626
12	3.10	4.37[b]	3.16	3.81[b]	133.3	154.7	146.5	163.0	−131.3	−35.7	0.0235

[a] The shared-electron numbers for the individual hydrogen bonds are given in the ESI.‡ [b] These N–O distances are too long to count as hydrogen bonds and likely do not contribute significantly to the binding.

bonds formed with the isophthaloyldiamide group (3.15 and 3.10 Å). All four distances are nevertheless in the expected range (see above). Thus, the tilt of the axle carbonyl group is more pronounced in the isophthaloyldiamide hemisphere of the complex as indicated also by the larger differences in N–H···O hydrogen bond angles on that side of the pseudorotaxane. In addition, also the angles are within the expected range. The other calculated structures do not deviate much from these values with one exception. The most weakly bound pseudorotaxane, *i.e.* the pentafluorophenyl-substituted axle **12,** forms only two hydrogen bonds. For the **py2** and **iso2** hydrogen bonds (Table 2), the N–O distances are 3.81 and 4.37 Å so we can safely assume these do not contribute significantly to the binding.

In order to study the substituent effects on the binding energies theoretically, the adiabatic interaction energies ΔE_{adia} were calculated according to eqn (3), in which E_{total} is the total energy calculated for the complex, $E_{relaxed}$ the total energies of the two components in their conformationally relaxed geometries and ΔE_{BSSE} the basis set superposition error. The absolute values given in Table 2 are much higher than the experimental data. This, however, does not come as a surprise,[16] because the calculations do not involve competition of the axle with the solvent for binding inside the cavity of the TLM. More relevant are the substituent-dependent differences, which follow the same trend as the experimental values. More electron-donating axle substituents result in higher binding energies, because they increase the donor strengths of the axle carbonyl oxygen atoms and thus improve their hydrogen-bond acceptor qualities. Upon binding, both the wheel and the axle rearrange with respect to their conformations to some extent in order to mutually accommodate the binding partner. The ΔE_{CF} values in Table 1 (eqn (4)) provide an estimate of the rearrangement energy necessary to bring the wheel and axles from their relaxed conformations into the structure which they finally have in the complex. With values between −25 and −40 kJ mol^{-1}, these rearrangement energies are quite substantial.

$$\Delta E_{adia} = E_{total}^{complex} - E_{relaxed}^{wheel} - E_{relaxed}^{axle} - \Delta E_{BSSE} \quad (3)$$

$$\Delta E_{CF} = (E_{relaxed}^{wheel} - E_{relaxed}^{wheel}) + (E_{relaxed}^{axle} - E_{unrelaxed}^{axle}) \quad (4)$$

$$\Delta E_{SEN} = m \cdot \sigma_{HA} + b \quad (5)$$

Finally, the trend in substituent effects is also supported by the shared-electron numbers (SEN) for the hydrogen bonds between axles and wheels (also, see ESI‡). The SEN provides a semi-quantitative measure of the hydrogen bond strengths, because the linear relationship in eqn (5) exists, in which σ_{HA} represents the two-centre shared electron number of a hydrogen bond and ΔE_{SEN} its binding energy. Even if one does not determine the slope and intercept, which differ with acceptor atom and the combination of functional and basis set, trends can easily be determined from the SEN values. From the data given in Table 2, the trend to stronger binding with more electron-donating substituents is clearly visible.

Conclusions

In conclusion, the choice of the substituents at the diamide axle changes the binding properties in several ways and can thus be utilized to fine-tune the binding properties. First, the alkyl- and alkenyl-substituted diamides bind with binding constants higher than the aryl-substituted axles by an order of magnitude. Second, the binding is driven by enthalpic effects, if the axle carries substituents with π-systems attached to the amide carbonyl groups. Only the formation of pseudorotaxane **3@1a** with alkyl chains at the carbonyl carbon atoms is due to positive binding entropies. Third, within the series of differently substituted aryl diamides, the electronic substituent effects are important for the formation of pseudorotaxanes. Electron-withdrawing substituents diminish the binding constants as compared to electron-donating substituents. The trends of binding energies thus follow the Hammett substituent parameters.

The experimental and theoretical results nicely agree with respect to the electronic substituent effects and thus have an impact on the design of template effects for rotaxane synthesis. For example, it would be advantageous to elongate the axles for stopper attachment through ether linkages at the phenyl-C(4) rather than using esters or amides. Ethers would enhance the templating ability, while esters or amides would almost lead to the loss of the non-covalent connections between wheel and axle during rotaxane formation.

Our study complements an earlier study by Jeong *et al.*[14] who reported substituent effects on the ability of TLMs to bind the same diamide station. The changes found in our study are not as drastic as those reported by Jeong and coworkers, but they are still substantial. Furthermore, our binding data lends support to a recent theoretical study.[17] Even though a slightly different TLM was used for the calculated binding data, the trends are well confirmed in our study by experiment.

The present report is one of few which directly compare binding data from NMR titrations with ITC results and theoretical calculations. The pseudorotaxanes under study exhibit binding constants at the lower end of the ITC dynamic range, which is roughly between 10^2 M^{-1} < K_a < 10^7 M^{-1}, and thus belong to the low-affinity systems, which do not fulfil the broadly accepted requirement that Wiseman's *c* value should be larger than 10. Nevertheless, the data obtained from both methods reveals quite good agreement. In line with a previous report on alkali metal ion/crown ether binding,[32] we conclude that these low-affinity systems can be studied by isothermal titration calorimetry. On the one hand, the combination of both methods is advantageous, because they overlap in yielding binding free energies, which can be directly compared and—in the case of good agreement—provides more confidence in the data. On the other hand, both methods contribute their individual share to the understanding of complex formation. While structural information can be extracted from NMR experiments, ITC measurements directly provide binding enthalpies and binding entropies. Otherwise, the determination of these two values would require the much higher effort of temperature-dependent NMR titrations.

Experimental section

General methods

Reagents were purchased from Aldrich, ACROS or Fluka and used without further purification. TLMs **1a,b** were synthesized according to literature procedures.[22] The acid chloride for the synthesis of guest **4** was obtained from the corresponding triphenyl acetic acid by reaction with oxalyl chloride and a few drops of dry DMF in CH_2Cl_2. After a clear solution formed while stirring the reaction mixture at r.t., the solvents were evaporated under reduced pressure and the residue was used without further purification. All other acid chlorides were commercially available and used as purchased. Yields refer to chromatographically and spectroscopically homogeneous materials. Solvents were dried and distilled prior to use by usual laboratory methods. Thin-layer chromatography (TLC) was performed on precoated silica gel 60/F254 plates (Merck KGaA). Silica gel (0.04–0.063 mm; Merck) was used for column chromatography.

NMR spectroscopy and NMR titrations

^1H (400 MHz), ^{19}F (376 MHz) and ^{13}C (100 MHz) spectra were obtained on a Bruker ECX 400 instrument at 298 K. All chemical shifts are reported in ppm with signals of $CHCl_3$ (7.26 ppm (^1H) and 77.0 ppm (^{13}C)) or CCl_3F (0 ppm (^{19}F)) taken as internal standards; coupling constants are in Hz. The following abbreviations were used to indicate NMR multiplicities: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). Titration experiments were carried out in $CDCl_3$ at 25 °C on a Bruker ECX 400 instrument. Solutions of **1a** (*c* = 5 mM, 0.6 mL) were placed in NMR tubes and treated with various amounts of the guests **2–12** (*c* = 50 mM). After each injection, a ^1H NMR spectrum was recorded immediately. The true guest concentrations in the solution under study were determined by integration of the signals for the wheel *versus* the integration of the signals for guest protons. The binding constants were determined based on 1 : 1 binding model by fitting the experimental data with eqn (1). Nonlinear curve fitting was achieved with the tools implemented in *IgorPro* (Wavemetrics Inc., Lake Oswego, Oregon/USA).

Mass spectrometry

Samples were measured on an Agilent 6210 ESI-TOF, Agilent Technologies, Santa Clara, CA, USA, with methanol as the spray solvent. The solvent flow rate was adjusted to 4 μL min^{-1} and the spray voltage set to 4 kV. The drying gas flow rate was adjusted to 15 psi (1 bar). All other parameters were optimized for a maximum abundance of the $[M + Na]^+$ ions.

Isothermal titration calorimetry (ITC)

Titration experiments were carried out in $CHCl_3$ at 298 K on a TAM III microcalorimeter (Waters GmbH, TA Instruments, Eschborn, Germany). In a typical experiment, an 800 μL solution of **1a** was placed in the sample cell at a concentration of 2 mM, and 250 μL of one of the guests **2–12** were in the injection syringe at a concentration of 40 mM in the same solvent. The titration schedule consisted of 25 consecutive injections of 8 μL with a 5 min interval between injections. Heats of dilution, measured by titration of the guest into the sample cell with blank solvent, were subtracted from each data set. All solutions were degassed prior to titration. Titrations were repeated twice. The data were analyzed using the instrumental internal software package and fitted with a one-site binding model.

General procedure for the synthesis of the diamide guests (2–8, 10–12)

Under argon, *N*,*N*′-dimethylethylenediamine (300 mg, 3.4 mmol) was dissolved in 30 mL CH_2Cl_2 and treated with 1.5 mL NEt_3. A solution of acid chloride (7.0 mmol) in 20 mL CH_2Cl_2 was slowly added and the resulting solution was stirred at r.t. for 18 h. The solvent was then evaporated and the products were isolated by column chromatography (silica gel, pure CH_2Cl_2 to CH_2Cl_2–acetone 2 : 1 or pure EtOAc).

Synthesis of the diamide guest (9)

Under argon, *N*,*N*′-dimethylethylene diamine (300 mg, 3.4 mmol) was dissolved in 30 mL CH_2Cl_2 and treated with 1.5 mL NEt_3. A solution of 4-methoxybenzoyl chloride (2.72 mmol) in 10 mL CH_2Cl_2 was slowly added and the resulting solution was stirred at r.t. for 4 h. After that, a solution of 4-trifluoromethylbenzoyl chloride (2.72 mmol) in 10 mL CH_2Cl_2

was again slowly added and the resulting solution was left stirring at r.t. for another 14 h. The solvent was then evaporated and the product was isolated by column chromatography (preparative TLC, silica gel, CH_2Cl_2–EtOAc 1 : 1).

_N,N′_-(Ethane-1,2-diyl)bis(_N_-methylacrylamide) 2

Yellow oil (0.33 g, 50%); R_f = 0.34 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.03, 3.04, 3.12 (3 s, 6H; NCH_3), 3.51–3.62 (3 s, 4H; NCH_2), 5.63–5.68 (m, 2H; CH═CH_2), 6.26–6.35 (m, 2H; CH═CH_2), 6.46–6.65 ppm (m, 2H; CH═CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 34.2, 36.1, 37.4 (CH_3), 45.1, 47.3, 47.6 (CH_2), 127.0, 127.2, 127.8, 127.9, 128.4, 128.7 (CH_2, CH), 166.8 ppm (C═O); ESIMS: _m/z_ (%): 219.1 (100) [M + Na]$^+$; HRMS (ESI$^+$): _m/z_ calcd for $C_{26}H_{48}N_2O_2Na^+$: 219.1104 [M + Na]$^+$; found: 219.1107 (Δ = 1.4 ppm).

_N,N′_-(Ethane-1,2-diyl)bis(_N_-methylundec-10-enamide) 3

Yellow oil (0.95 g, 67%); R_f = 0.12 (CH_2Cl_2), 0.98 (CH_2Cl_2–acetone 2 : 1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.34 (br, 16H; CH_2), 1.39–1.44 (m, 4H; CH_2), 1.53–1.62 (m, 4H; CH_2), 2.06 (q, ^3J = 7.3 Hz, 4H; CH_2), 2.29 (m, 4H; CH_2), 2.96, 3.06, 3.08 (3 s, 6H; NCH_3), 3.54, 3.57, 3.60 (3 s, 4H; NCH_2), 4.91–5.02 (m, 4H; CH═CH_2), 5.77–5.87 ppm (m, 2H; CH═CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 25.1, 27.5, 29.0, 29.2, 29.4, 29.5, 29.6, 33.9, 35.7, 44.6 (CH_2, CH_3), 114.2 (CH_2), 139.3 (CH), 173.6 ppm (C═O); ESIMS: _m/z_ (%): 443.4 (100) [M + Na]$^+$; HRMS (ESI$^+$): _m/z_ calcd for $C_{26}H_{48}N_2O_2Na^+$: 443.3608 [M + Na]$^+$; found: 443.3605 (Δ = −0.7 ppm).

_N,N′_-(Ethane-1,2-diyl)bis(_N_-methyl-2,2,2-triphenylacetamide) 4

White powder (1.13 g, 53%); R_f = 0.10 (CH_2Cl_2), 0.95 (CH_2Cl_2–acetone 2 : 1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.42 (s, 6H; NCH_3), 3.62 (s, 4H; NCH_2), 7.19–7.31 ppm (m, 30H; ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 38.5 (CH_3), 53.4 (CH_2), 67.3 (C_q), 126.6, 127.7, 130.1 (Ar–CH), 142.8 (Ar–C_q), 173.0 ppm (C═O); ESIMS: _m/z_ (%): 651.3 (100) [M + Na]$^+$; HRMS (ESI$^+$): _m/z_ calcd for $C_{44}H_{40}N_2O_2Na^+$: 651.2982 [M + Na]$^+$; found: 651.2986 (Δ = 0.6 ppm).

_N,N′_-(Ethane-1,2-diyl)bis(_N_-methylnaphthamide) 5

White powder (0.62 g, 46%); R_f = 0.79 (CH_2Cl_2–acetone 2 : 1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.16 (br, 6H; NCH_3), 3.97 (br, 4H; NCH_2), 7.47–7.49 (m, 6H; ArH), 7.78–7.91 ppm (m, 8H; ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 37.8 (CH_3), 44.4 (CH_2), 67.3 (C_q), 112.1, 124.0, 126.4, 126.5, 126.8, 127.6, 128.0, 128.2, 132.5, 133.4 (Ar–CH, Ar–C_q), 171.8 ppm (C═O); ESIMS: _m/z_ (%): 419.2 (100) [M + Na]$^+$; HRMS (ESI$^+$): _m/z_ calcd for $C_{26}H_{24}N_2O_2Na^+$: 419.1730 [M + Na]$^+$; found: 419.1736 (Δ = 1.4 ppm).

_N,N′_-(Ethane-1,2-diyl)bis(4-methoxy-_N_-methylbenzamide) 6

White powder (1.16 g, 96%); R_f = 0.78 (CH_2Cl_2–acetone 2 : 1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.75–3.04 (br m, 6H; NCH_3), 3.39–3.80 (br m, 10H; NCH_2, OCH_3), 6.79 (d, ^3J = 6.3 Hz, 4H; ArH), 7.28–7.38 ppm (m, 4H; ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 37.8, 55.1 (CH_3), 44.3 (CH_2), 113.3, 128.6 (Ar–CH), 128.2, 160.3 (Ar–C_q), 171.6 ppm (C═O); ESIMS: _m/z_ (%): 379.2 (100) [M + Na]$^+$; HRMS (ESI$^+$): _m/z_ calcd for $C_{20}H_{24}N_2O_4Na^+$: 379.1634 [M + Na]$^+$; found: 379.1641 (Δ = 1.9 ppm).

_N,N′_-(Ethane-1,2-diyl)bis(4-(_t_-butyl)-_N_-methylbenzamide) 7

White powder (1.22 g, 88%); R_f = 0.81 (CH_2Cl_2–acetone 2 : 1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.29 (s, 18H; _t_Bu-H), 2.77–3.09 (br m, 6H; NCH_3), 3.60–3.87 (br m, 4H; NCH_2), 7.31–7.39 ppm (m, 10H; ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 31.1, 34.7 (CH_3), 37.9 (C_q), 44.4 (CH_2), 125.1, 126.7 (Ar–CH), 133.3, 152.6 (Ar–C_q), 172.0 ppm (C═O); ESIMS: _m/z_ (%): 431.3 (100) [M + Na]$^+$; HRMS (ESI$^+$): _m/z_ calcd for $C_{26}H_{36}N_2O_2Na^+$: 431.2675 [M + Na]$^+$; found: 431.2687 (Δ = 2.8 ppm).

_N,N′_-(Ethane-1,2-diyl)bis(_N_-methylbenzamide) 8

White powder (0.89 g, 88%); R_f = 0.73 (CH_2Cl_2–acetone 2 : 1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.58–3.08 (br m, 6H; NCH_3), 3.22–3.77 (br m, 4H; NCH_2), 7.06–7.26 ppm (m, 10H; ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 37.4 (CH_3), 44.0 (CH_2), 126.4, 127.9, 129.0 (Ar–CH), 135.9 (Ar–C_q), 171.5 ppm (C═O); ESIMS: _m/z_ (%): 319.1 (100) [M + Na]$^+$; HRMS (ESI$^+$): _m/z_ calcd for $C_{18}H_{20}N_2O_2Na^+$: 319.1417 [M + Na]$^+$; found: 319.1423 (Δ = 1.9 ppm).

4-Methoxy-_N_-methyl-_N_-(2-(_N_-methyl-4-(trifluoromethyl)-benzamido)ethyl)benzamide 9

White powder (0.41 g, 31%); R_f = 0.48 (CH_2Cl_2–EtOAc 1 : 1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.08–3.15 (br m, 6H; NCH_3), 3.80–3.92 (br m, 7H; NCH_2, OCH_3), 6.87 (d, ^3J = 7.8 Hz, 2H; ArH), 7.37 (d, ^3J = 7.8 Hz, 2H; ArH), 7.51 (d, ^3J = 8.1 Hz, 2H; ArH), 7.64 ppm (d, ^3J = 8.1 Hz, 2H; ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 37.8, 55.0 (CH_3), 44.4 (CH_2), 113.4, 125.3, 126.9, 128.7 (Ar–CH), 122.3, 128.1, 139.7, 141.1, 160.3 (C_q, Ar–C_q), 170.4, 171.2 ppm (C═O); ^{19}F NMR (376 MHz, $CDCl_3$): δ = −62.8 ppm (s, 6F; CF_3); ESIMS: _m/z_ (%): 417.1 (100) [M + Na]$^+$; HRMS (ESI$^+$): _m/z_ calcd for $C_{20}H_{21}F_3N_2O_3Na^+$: 417.1402 [M + Na]$^+$; found: 417.1413 ($\Delta$ = 2.6 ppm).

_N,N′_-(Ethane-1,2-diyl)bis(_N_-methyl-4-(trifluoromethyl)-benzamide) 10

White powder (1.37 g, 93%); R_f = 0.69 (CH_2Cl_2–acetone 2 : 1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.06 (br s, 6H; NCH_3), 3.90 (br s, 4H; NCH_2), 7.48 (d, ^3J = 8.2 Hz, 4H; ArH), 7.61 ppm (d, ^3J = 7.9 Hz, 4H; ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 37.7

(CH_3), 44.7 (CH_2), 125.5, 127.1 (Ar–CH), 122.9, 124.5, 139.8 (C_q, Ar–C_q), 170.7 ppm (C═O); ^{19}F NMR (376 MHz, $CDCl_3$): δ = −62.8 ppm (s, 6F; CF_3); ESIMS: *m/z* (%): 455.1 (100) [M + Na]$^+$; HRMS (ESI$^+$): *m/z* calcd for $C_{20}H_{18}F_6N_2O_2Na^+$: 455.1170 [M + Na]$^+$; found: 455.1153 ($\Delta$ = −3.7 ppm).

N,N′-(Ethane-1,2-diyl)bis(*N*-methyl-4-nitrobenzamide) 11

Yellow powder (1.16 g, 89%); R_f = 0.65 (CH_2Cl_2–acetone 2 : 1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.01 (br s, 6H; NCH_3), 3.89 (br s, 4H; NCH_2), 7.50 (d, ^3J = 8.9 Hz, 4H; ArH), 8.16 ppm (d, ^3J = 8.9 Hz, 4H; ArH); ^{13}C NMR (100 MHz, $CDCl_3$):δ = 37.6 (CH_3), 44.7 (CH_2), 123.7, 127.6 (Ar–CH), 142.3, 148.1 (Ar–C_q), 169.7 ppm (C═O); ESIMS: *m/z* (%): 409.1 (100) [M + Na]$^+$; HRMS (ESI$^+$): *m/z* calcd for $C_{18}H_{18}N_4O_6Na^+$: 409.1124 [M + Na]$^+$; found: 409.1133 (Δ = 2.2 ppm).

N,N′-(Ethane-1,2-diyl)bis(pentafluoro-*N*-methylbenzamide) 12

Orange powder (1.49 g, 92%); R_f = 0.14 (CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.04 (br s, 6H; NCH_3), 3.87 ppm (br s, 4H; NCH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 36.6 (CH_3), 44.8 (CH_2), 111.1, 136.4, 138.9, 140.7, 141.5, 144.0 (Ar–C_q), 159.2 ppm (C═O); ^{19}F NMR (376 MHz, $CDCl_3$): δ = −159.4 (m, 4F; ArF), −151.4 (m, 2F; ArF), −141.9 ppm (m, 4F; ArF); ESIMS: *m/z* (%): 499.1 (100) [M + Na]$^+$; HRMS (ESI$^+$): *m/z* calcd for $C_{18}H_{10}F_{10}N_2O_2Na^+$: 499.0475 [M + Na]$^+$; found: 499.0497 ($\Delta$ = 4.4 ppm).

Crystallographic section

Colorless crystals of **2@1b** and **8@1b** were obtained by diffusion of ether into a saturated chloroform solution of host and guest. The structural analysis was performed using Bruker-Nonius Kappa Apex II diffractometer with graphite-monochromatized Cu-Kα (λ = 1.54183 Å) radiation at 173 K for **2@1b** and Bruker-Nonius Kappa Apex II diffractometer with graphite-monochromatized Mo-Kα (λ = 0.71073 Å) radiation at 123 K for **8@1b**. Collect software[34] was used for the data measurement and DENZO-SMN[35] for the processing. The structures were solved by charge flipping method with SUPERFLIP[36] and refined by full-matrix least-squares methods using the WinGX-software,[37] which utilizes the SHELXL-97 module.[38] Multi-scan absorption correction was done by SADABS2008.[39] All C–H hydrogen positions were calculated using a riding atom model with U_H = 1.2 × U_C. All non-H atoms were refined anisotropically.

Crystal data for **2@1b** (CCDC-853879): colorless blocks, 0.15 × 0.20 × 0.25 mm, FW = 1691.08, $C_{82}H_{100}N_6O_6Cl_{12}$, triclinic, space group $P\bar{1}$, a = 13.087(3) Å, b = 13.192(2) Å, c = 14.620(3) Å, α = 103.23(2)°, β = 107.21(3)°, γ = 108.81(2)°, V = 2131(1) Å^3, Z = 1, D_c = 1.318 g cm^{-3}, F000 = 886, μ = 3.998 mm^{-1}, T = 173(2) K, $2\theta_{max}$ = 63.31°, 6843 independent reflections, 5265 with $I_o > 2\sigma(I_o)$, R_{int} = 0.1111, 485 parameters, 0 restraints, GoF = 1.038, R = 0.0758 [$I_o > 2\sigma(I_o)$], wR = 0.2160 (all reflections), 0.692 < $\Delta\rho$ < −0.501 eÅ^3.

Crystal data for **8@1b** (CCDC-853880): colorless blocks, 0.10 × 0.15 × 0.35 mm, FW = 1552.46, $C_{88}H_{102}N_6O_6Cl_6$, monoclinic, space group $C2/c$, a = 25.976(5) Å, b = 19.610(4) Å, c = 19.930(4) Å, β = 126.21(3)°, V = 8191(3) Å^3, Z = 4, D_c = 1.259 g cm^{-3}, $F000$ = 3288, μ = 0.266 mm^{-1}, T = 123(2) K, $2\theta_{max}$ = 25°, 7156 independent reflections, 4611 with $I_o > 2\sigma(I_o)$, R_{int} = 0.1102, 480 parameters, 0 restraints, GoF = 1.046, R = 0.0695 [$I_o > 2\sigma(I_o)$], wR = 0.1742 (all reflections), 0.380 < $\Delta\rho$ < −0.478 eÅ^3.

Theoretical section

The structure of all complexes were optimised using density functional theory (DFT) combined with the resolution of identity technique (RI).[40] The TZVP basis set was applied in combination with the general gradient approximated type functional B97-D with dispersion correction developed by Grimme.[41] All calculations were performed using the TURBOMOLE 6.0 program package.[42] The obtained complex interaction energies were counterpoise-corrected by the method introduced by Boys and Bernardi.[43] According to the supramolecular approach, the adiabatic complex interaction energies ΔE_{ad} can be calculated by subtracting the energies of the relaxed wheel E_{rel}(wheel) and axle E_{rel}(guest) as well as the BSSE contributions to the total cluster energy E_{tot}(complex) as given in eqn (3). Because of complex formation, the conformation of the molecules changes. The energy ΔE_{CF} related to this conformational change does not directly contribute to the hydrogen bond energies. Therefore, ΔE_{CF} was calculated according to eqn (4). We also carried out the computation of the two-center shared electron number (SEN).[42] The determination of the two-centre shared electron number is based on the population analysis by Davidson.[44] A linear relationship (eqn (5)) exists between the two-center shared-electron number of a hydrogen bond σ_{HA} and its binding energy which allows an estimation of the bond strength. Therefore, the slope m and the axis intercept b of eqn (5) need to be determined, because they depend on the acceptor atom of the hydrogen bonds and the combination of functional and basis set. It is well known that this procedure works for a broad variety of chemical applications concerning hydrogen bonds.[45] To get qualitative results on the bonding strength, a comparison of the two center shared electron number σ_{HA} is sufficient.

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