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Thermodynamic insights on a bistable acid-base switchable molecular shuttle with strongly shifted co-conformational equilibria

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Dedicated to Professor Vincenzo Balzani, pioneer of molecular machines, on his 80th birthday

Abstract: Bistable [2]rotaxanes in which the affinities of the two stations can be reversed form the basis of molecular shuttles. Gaining quantitative information on such rotaxanes in which the ring distribution between the two stations is largely nonsymmetric has proven to be very challenging. Here we report on two independent experimental methodologies - based on luminescence lifetime measurement and acid-base titrations - to determine the relative populations of the two co-conformations of a [2]rotaxane. The assays yield convergent results and are sensitive enough to measure an equilibrium constant (K ~ 4000) out of reach for NMR spectroscopy. We also estimate the ring distribution constant in the switched (deprotonated) state ($K' < 10^{-4}$), and report the highest positional efficiency for stimuli-induced shuttling to date (>99.92%). Finally, our results show that the pK_a of the pH-responsive station depends on the ring affinity of the pH-insensitive station, an observation that paves the way for the design of new artificial allosteric systems.

Introduction

Rotaxanes and related interlocked molecules are intriguing chemical architectures with high relevance for the development of artificial nanoscale machines and switches as well as functional materials.[1] The tremendous progresses for their synthesis have broadened the scope of their use.[2] In particular bistable [2]rotaxanes, consisting of a macrocycle threaded on a molecular axle with two potential sites of interaction (the 'stations') and stoppered at both ends, can offer a wide range of functions including molecular transport,[3] ion sensing,[4] information storage[5] and catalysis.[6]

The operation of a bistable rotaxane as a switch relies on the ability of an external stimulus (chemical, electrochemical or photonic) to reversibly modify the relative affinity of the ring for the two stations, leading to a drastic structural change in the molecule. A simplified view of the process pictures the ring moving from one station to the other one after the application of the stimulus (molecular shuttling). This view, however, does not represent the behavior of a statistically significant population of rotaxanes, because an equilibrium exists between the two translational isomers (or co-conformations) corresponding to the positioning of the ring on either site on the axle.[7] In line with proposed definitions,[8] the most abundant translational isomer is referred to as the stable co-conformation (SCC) while the second isomer is called the metastable co-conformation (MCC).

Hence, a full characterization of the starting state of a bistable rotaxane - which in turn is important for understanding successive stimuli-induced switching processes - involves the measurement of the equilibrium constant $K = [\mathrm{SCC}]_{eq}/[\mathrm{MCC}]_{eq}$. This is a nontrivial task because K is an intramolecular quantity which reflects relative binding strengths within an individual molecular entity. Indeed, the free energy difference between the SCC and MCC can be accurately calculated from the value of K, thereby enabling correlations with the noncovalent intercomponent interactions.

^{1}H NMR Spectroscopy can be used to measure $[\mathrm{SCC}]_{eq}$ and $[\mathrm{MCC}]_{eq}$ but, because of its inherent sensitivity, it provides reliable results only when K does not exceed ca. 20.[9] Rotaxanes characterized by high values of K, however, are required to make efficient molecular shuttles. Thus, new assays that can yield information on bistable rotaxanes with highly shifted co-conformational equilibria need to be developed.

Paolucci and co-workers used cyclic voltammetric (CV) experiments to evaluate K.[10] Stoddart and co-workers also reported a method based on CV whose sensitivity was comparable with that of ^{1}H NMR.[11a] Later, by using variable scan-rate CV, the sensivity was dramatically increased,[11b,11c] enabling the measurement of K values up to 10^4. These methods, however, can only be applied to rotaxanes having a well behaved electroactive unit (e.g. a redox-switchable ring recognition site). For example such a sensitive assay is still missing for pH-triggered bistable rotaxanes, another important and widely studied class of switches.[1f]

Here we report on an alternative and sensitive methodology for the quantitative investigation of the co-conformational equilibria of [2]rotaxanes, and we apply it on an acid-base switchable molecular shuttle designed to exhibit a largely nonsymmetric ring distribution between the two stations. As the only structural requirement of this approach is the presence of a luminescent moiety in the rotaxane,[12] it can exhibit a general applicability and is complementary to the strategies discussed

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above.

The results are confirmed by another independent experimental approach based on acid-base titrations of the rotaxane and appropriate model compounds. This method also enabled us to estimate the ring distribution constant after the application of the switching signal (K'), and to determine the highest efficiency for reversible (forward and backward) stimuli-induced shuttling reported to date. Another significant outcome of this investigation is that the acidity of the pH-sensitive site depends on the affinity of the ring for the pH-insensitive station, and that the interaction between the two sites is mediated by the movement of the ring. This kind of behaviour can contribute to the understanding of allosteric phenomena in biomolecules and has intriguing implications for the design of artificial molecular devices.

Results and Discussion

Design

Recently, the 1,2,3-triazolium unit (tria$^+$) has been identified as a convenient secondary station for a dibenzo[24]crown-8 ring (**DB24C8**) in rotaxanes in which a dialkylammonium unit (amm$^+$) plays the role of the primary station.[13,14] While the intermolecular apparent association constant between a dialkylammonium guest and **DB24C8**[15] is ca. 10^4–10^5 M^{-1} in CH_2Cl_2,[16] this value has not yet been reported in the case of a triazolium axle, presumably because of the very inefficient complexation. Indeed, it was found that the triazolium moiety cannot be used as a template for rotaxane formation.[13] Consequently, the triazolium unit is considered to be a weak station within a rotaxane. Such a difference in the behavior of the two guests should ensure a largely unbalanced co-conformational distribution, and prompted us to synthesize and investigate a [2]rotaxane containing ammonium and triazolium stations in the axle for the development of a sensitive method for measuring large values of *K*.

Figure 1a shows the bistable rotaxane studied in this work, **Rot$_{2s}$**, and the equilibrium linking its SCC and MCC. A few model compounds necessary for the experiments are represented in Figure 1b. The details on the synthesis and characterization of the compounds are reported in the Supplementary Information. In particular, **Rot$_{1s}$** is structurally identical to **Rot$_{2s}$** except for the absence of the secondary triazolium station; thus, the only translational co-conformation of **Rot$_{1s}$** is that shown in the figure. The axle in both **Rot$_{2s}$** and **Rot$_{1s}$** possesses a fluorescent naphthalimide (NI) stopper placed in the vicinity of the ammonium station. Encouraged by the results obtained for many previously reported fluorescent rotaxanes,[1f,17,18] we anticipated that the fluorescence properties of the naphthalimide group would be sensitive to the position of the ring on the axle and could be used to quantitatively report on its distribution.

Intermolecular association of the ring with individual stations

As in a rotaxane the two stations compete intramolecularly for the ring, the stability of the 1:1 complexes of the latter with individual guests representing each station can offer a prediction of the co-conformational distribution in the rotaxane.[11c] We therefore measured the intermolecular association constant of **DB24C8** with compounds **NI-amm$^+$** and **NI-tria$^+$** (Figure 1b). Since we anticipated a low association constant with the triazolium axle, the titrations were performed in CH_2Cl_2, a solvent expected to enhance the electrostatic and solvophobic interactions that stabilize the supramolecular complex.[19]

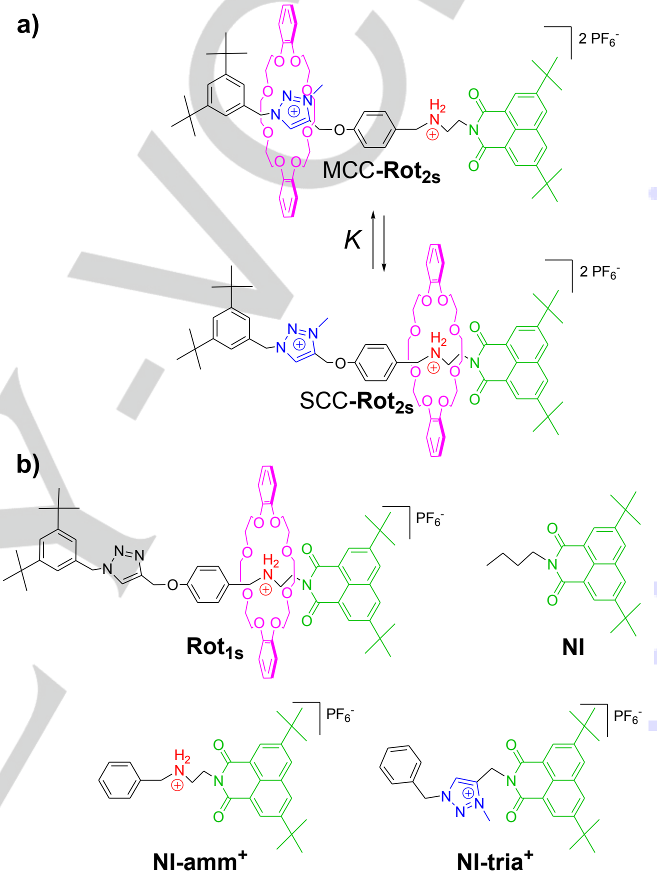

Figure 1. Structural formulas of (a) **Rot$_{2s}$** and the equilibrium between its two translational isomers, SCC and MCC, and (b) model compounds studied in this work.

The association between the model axle **NI-amm$^+$** and **DB24C8** was monitored by UV-absorption and fluorescence spectroscopies. Upon addition of an increasing amount of **DB24C8** to a solution of **NI-amm$^+$** (ca. 50 μM), the band centered at 355 nm underwent a hypsochromic shift (Figure S38). This blue shift is consistent with the loss of an intramolecular hydrogen bond between the ammonium unit and the carbonyl groups of NI when the interaction with the ring is established (*vide infra*). The addition of **DB24C8** also caused a strong decrease of the fluorescence intensity (Figure 2a). The quenching of the fluorescence is ascribed to a photoinduced electron transfer (PET) from the crown ether to the singlet excited state of the naphthalimide unit.[20] From the analysis of the spectroscopic titration profiles an apparent association constant[16] of log($K_{\mathbf{amm}}$+) = 5.61 ± 0.15 was determined for the [**NI-amm⊂DB24C8**]$^+$ complex.[21] The ^{1}H NMR spectrum of a 1:1 mixture of **NI-amm$^+$** and **DB24C8** (Figure S39) attests the formation of the pseudorotaxane complex. The value of the association constant is in line with those found for similar ammonium/**DB24C8** complexes under similar conditions.[16,22]

The association constant between a triazolium axle and **DB24C8** has never been reported, most likely because of the low stability of the corresponding complex. Indeed, ^{1}H NMR experiments performed on ring/axle mixtures at mM concentrations did not show any appreciable complexation. It is likely that the formation of ion pairs between the positively charged axle and its PF_6^- counteranions competes with threading and, as for the ammonium guest, significantly lowers the apparent binding constant at relatively high concentrations.[16]

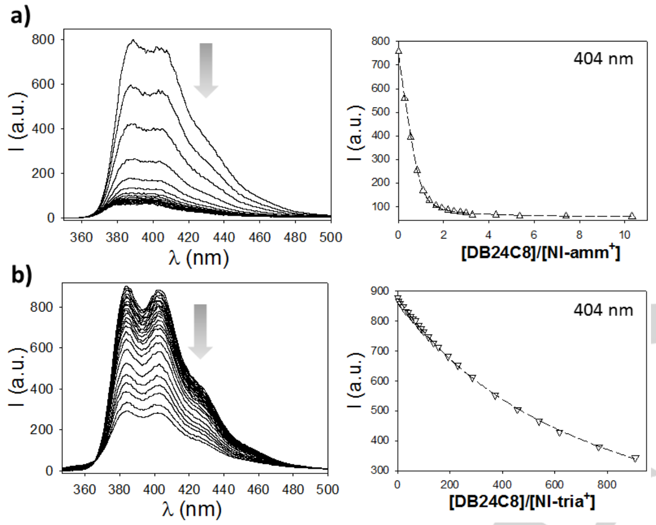

Figure 2. Fluorescence spectral changes (left) and corresponding titration curves (right) in CH_2Cl_2 at room temperature of (a) **NI-amm$^+$** (54 μM, λ_{exc} = 342 nm) and (b) **NI-tria$^+$** (53 μM, λ_{exc} = 340 nm) by **DB24C8**. The white triangles are the experimental points and the dashed line is the curve fitting obtained according to a 1:1 association model.

The design of the axle **NI-tria$^+$** takes advantage of the efficient quenching of the naphthalimide fluorescence by **DB24C8** mentioned above. Fluorescence titrations performed at low concentration (ca. 50 μM) in CH_2Cl_2 enabled us to measure for the first time the intermolecular association constant between a triazolium axle and **DB24C8**. No absorption spectral changes were noticed upon adding the ring to a solution of **NI-tria$^+$**. On the contrary, the NI fluorescence was substantially quenched (Figure 2b). Fluorescence lifetime measurements on a mixture of **NI-tria$^+$** (100 μM) and **DB24C8** (10 mM) ruled out a dynamic quenching. Two different lifetimes (τ_1 = 1.78 ns and τ_2 = 637 ps) were obtained, the longer one being close to that of **NI-tria$^+$** alone (τ = 1.64 ns) (Figures S41-S42). The data could be fitted according to the formation of a 1:1 complex with an apparent association constant of log($K_{\mathbf{tria}}$+) = 1.38 ± 0.03. From the ratio of the two intermolecular association constants, a value of log*K* = 4.23 ± 0.18 can be predicted. This high figure reflects indeed the great difference of affinity for the crown ether between the ammonium and triazolium stations acknowledged in the literature.[13]

Determination of the ring distribution from luminescence measurements

The UV-visible absorption and emission spectra of **NI-amm$^+$**, **Rot$_{1s}$** and **Rot$_{2s}$** in CH_3CN are similar and are dominated by the bands of the NI chromophore (λ_{max} = 354 nm, ε = 14000 M^{-1} cm^{-1}). In the case of **Rot$_{1s}$** and **Rot$_{2s}$** the absorption band of the **DB24C8** component (λ_{max} = 275 nm) is also present. All compounds show a luminescence band centered at ca. 390 nm, assigned to the NI fluorescence (Figures S49-S50). The relevant fluorescence data are given in Table 1.

Table 1. Fluorescence data (quantum yields Φ, fluorescence lifetimes τ_i and pre-exponential factors β_i) for **NI**, **NI-amm$^+$**, **Rot$_{1s}$** and **Rot$_{2s}$**.

		τ_i / ps (β_i / a.u.) [a]		
	Φ / % [a]	τ_1	τ_2	τ_3
NI	18.4 ± 1	1530	–	–
NI-amm$^+$	20.2 ± 1.2	2660	–	–
Rot$_{1s}$	1.0 ± 0.1	–	621 (5)	186 (20)
Rot$_{2s}$	1.8 ± 0.2	2620 (0.11)	763 (6)	220 (12.3)

[a] In CH_3CN at room temperature. All the fluorescence intensity decays are shown in the SI.

The emission quantum yield of **NI-amm$^+$** is very close to that of the bare naphthalimide model **NI**. The presence of the ammonium is nevertheless testified by a longer lifetime (2.66 ns *vs* 1.53 ns), a 2 nm bathochromic shift of the absorption band, and a fluorescence maximum shifted to longer wavelengths (Figure S49). These observations are consistent with the presence of an intramolecular hydrogen bond between the ammonium unit and a carbonyl group of the naphthalimide both in the ground and excited states.[23] The emission quantum yields in the two rotaxanes are significantly smaller because of PET quenching.[20]

Insightful information about the co-conformational distribution of **Rot$_{2s}$** can be obtained from a careful analysis of the time-correlated fluorescence decays. While **NI-amm$^+$** exhibits a mono-exponential decay (τ = 2.66 ns), the fluorescence decay of **Rot$_{1s}$** is biexponential with τ_2 = 621 ps and τ_3 = 186 ps. In analogy with previous studies on the time-resolved luminescence properties of crown-ether ligands,[24] these two short lifetimes are tentatively ascribed to the decay of the NI excited singlet in two different conformations of the ring placed on the ammonium station.[25] Interestingly, in **Rot$_{2s}$** a third longer lifetime (τ_1 = 2.62 ns) similar to the lifetime determined for the ammonium model **NI-amm$^+$** is present, while the two shorter lifetimes τ_2 = 763 ps and τ_3 = 220 ps) have values comparable to those found in **Rot$_{1s}$** (Table 1 and Figure 3).[26]

Since the emission decay (~ns) is much faster than the ring shuttling (~μs-ms),[27,28] the NI fluorophores in the two co-

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conformations can be treated as two isolated fluorophores placed in different environments. By comparison with the behavior of the models, the lifetime τ_1 for **Rot$_{2s}$** was therefore attributed to the MCC, in which the ring is far enough from the fluorophore to avoid any interaction with the NI excited state. Consequently we associated τ_2 and τ_3 with the SCC, in which the ring surrounds the amm$^+$ station. The remarkable correspondence between the lifetimes of the studied models and those found in **Rot$_{2s}$** confirms that the two stations can be considered as spectroscopically independent and are appropriately described by the reference compounds. With this requirement satisfied, the pre-exponential factors β_i represent the fractions of SCC (α_{SCC}) and MCC (α_{MCC}), weighted for a factor *F* which takes into account the inherent photophysical differences (e.g. fast static quenching, radiative rates, absorption and emission spectra) of the NI fluorophore in the two situations.[29] The *F* factor can be measured with time-correlated fluorescence decay experiments by comparing the initial photon count accumulated during the same time under identical instrumental conditions for two solutions of **Rot$_{1s}$** and **NI-amm$^+$** having the same concentration (Figure S47). From the ratio between the initial photon counts for **NI-amm$^+$** and **Rot$_{1s}$** a value $F = 9.82 \pm 0.04$ was determined. Hence, *K* can be obtained from equation (1) where $\beta_{SCC} = \beta_2 + \beta_3$ and $\beta_{MCC} = \beta_1$:

$$K = \alpha_{SCC}/\alpha_{MCC} = F \times (\beta_{SCC}/\beta_{MCC}) \qquad (1)$$

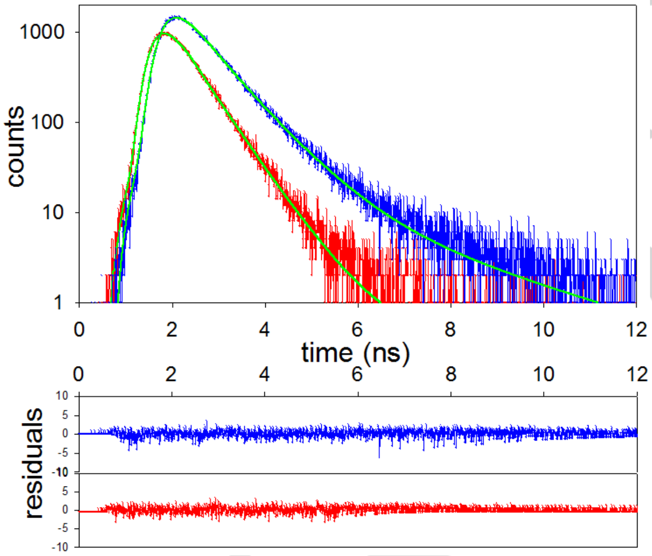


Figure 3. Fluorescence intensity decays in CH_3CN at room temperature for **Rot$_{1s}$** (red) and **Rot$_{2s}$** (blue) and their respective residuals. The green curves are the two best fit for both decays and correspond to the parameters listed in Table 1. Conditions: λ_{exc} = 340 nm, λ_{em} = 415 nm, c = 10 μM.

By introducing the experimental values in equation (1) an equilibrium constant $\log K = 3.2 \pm 0.2$ can be determined. This value is independent on the wavelength of observation.

The *K* value measured with this method is one order of magnitude lower than that calculated from the ratio of the intermolecular association constants. This discrepancy can arise from the fact that **NI-tria$^+$** is not a perfectly accurate model for the triazolium station in **Rot$_{2s}$**. Another explanation is related to the different solvents employed in the two methods. The choice of CH_2Cl_2 for the intermolecular association experiments was dictated by the need of enhancing the stability of the **[NI-tria⊂DB24C8]$^+$** complex. On the other hand the luminescence behavior observed in CH_3CN was quite rich and, as discussed above, contained interesting structural information.

Time-resolved fluorescent measurements were also performed in CH_2Cl_2, where the emission of **Rot$_{2s}$** exhibited a biexponential decay with τ_2 = 781 ps and τ_3 = 219 ps (Figure S48). In line with the previous discussion, these two components are assigned to two different arrangements of the ring in the SCC. The absence of a third decay component with longer lifetime (τ_1), attributable to the metastable translational isomer, indicates that in this particular case the fluorescence-based method is not sensitive enough to detect the MCC in CH_2Cl_2. Such an observation points to a significantly higher value for *K*, in agreement with the prediction based on intermolecular association data obtained in the same solvent. Presumably, in CH_3CN the interaction of the ring with the amm$^+$ station is affected to a higher extent than with the tria$^+$ one.

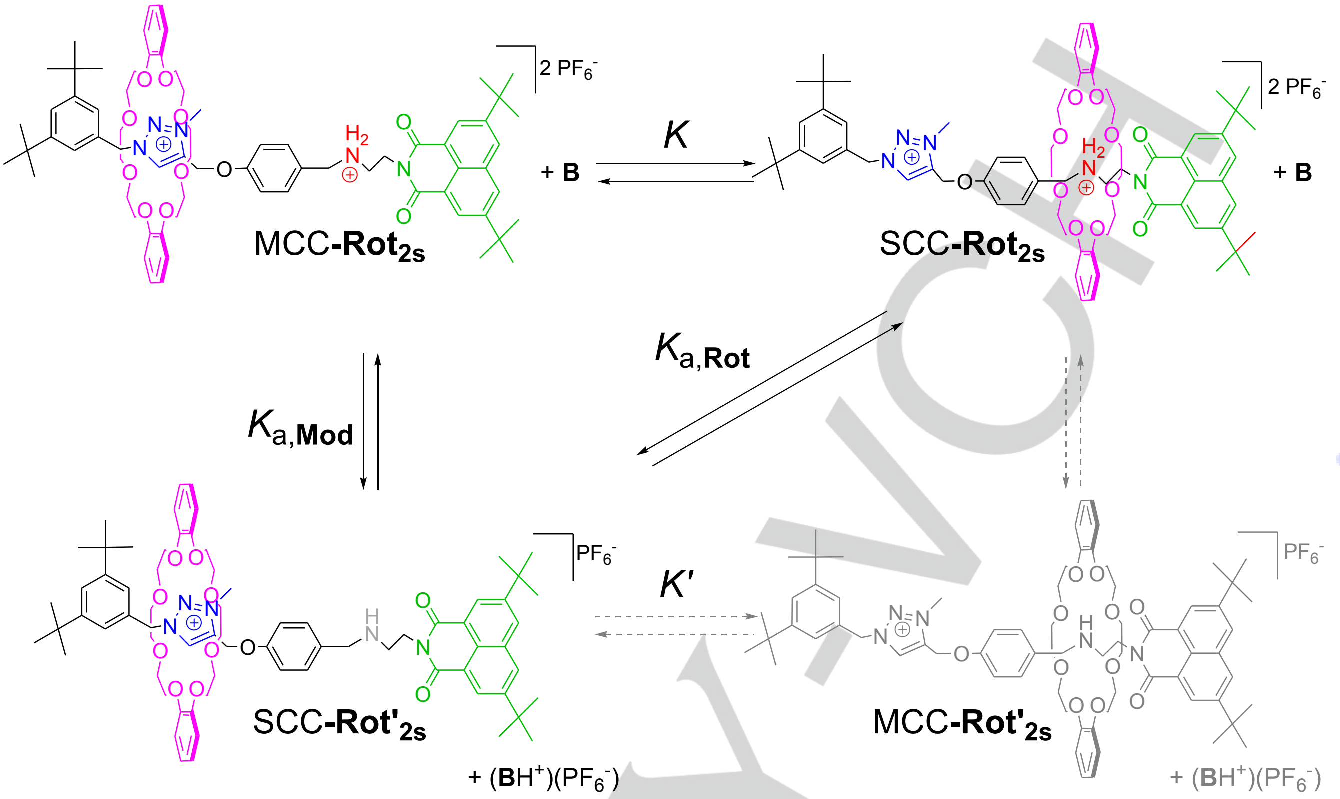
Acid-base titrations

As discussed above, the quantification of the co-conformational equilibrium with the luminescence-based method requires the knowledge of the fluorophore photophysics and a careful analysis of its luminescence decay. We therefore set up a more practical assay, which takes advantage of the acid-base properties of **Rot$_{2s}$** and is based on two titration experiments.

The operation of **Rot$_{2s}$** as a pH-controlled molecular shuttle is shown in Scheme 1, wherein co-conformational and acid-base equilibria are represented as the horizontal and vertical processes, respectively. The reversible stimuli-induced shuttling is based on the fact that the SCC and MCC for **Rot$_{2s}$** and its deprotonated counterpart **Rot'$_{2s}$** correspond to opposite translational isomers. In fact, for **Rot'$_{2s}$** only the isomer in which the ring encircles the tria$^+$ station was observed by ^{1}H NMR spectroscopy, in keeping with the fact that the amm$^+$ site is switched off upon deprotonation (Figure S40).[13,14]

Considering that deprotonation of SCC-**Rot$_{2s}$** affords immediately and quantitatively SCC-**Rot'$_{2s}$**, the titration of **Rot$_{2s}$** with a base enables the determination of an apparent acidity constant $K_{a,\mathbf{Rot}}$ linking these two forms (Scheme 1). On the other hand, it is reasonable to assume that **NI-amm$^+$** is an appropriate model for measuring the acidity constant of the ammonium station of **Rot$_{2s}$** when it is not surrounded by the ring ($K_{a,\mathbf{Mod}}$). A thermodynamic cycle can thus be established between the two co-conformations of the protonated rotaxane (**Rot$_{2s}$**) and the deprotonated rotaxane (**Rot'$_{2s}$**), as shown by the full arrows in Scheme 1. The translational equilibrium constant *K* is then straightforwardly calculated from equation (2):

$$K = K_{a,\mathbf{Mod}}/K_{a,\mathbf{Rot}} \qquad (2)$$



Scheme 1. Square scheme describing the switching between different co-conformations (horizontal processes) and protonated/deprotonated forms (vertical processes) for rotaxane **Rot$_{2s}$**. The base employed in the titration and its conjugated acid are labelled respectively as **B** and **BH**$^+$. The thermodynamic cycle linking K and the acidity constants $K_{a,Rot}$ and $K_{a,Mod}$ is identified by the full arrows (valid for **B** = CH_3CN). The translational isomer shown in grey is not observed experimentally. See the text for more details.

In order to perform an accurate assay, the pK_a of the bases used for the titration of the rotaxane and the axle model should be as close as possible to the pK_a of the molecules under investigation. A search for bases compatible with our experiments (that is, operating in CH_3CN and spectroscopically silent) and known pK_a was thus performed. The analysis of the absorption spectral changes observed upon titration of **Rot$_{2s}$** by Et_3N (pK_a = 18.82)[30] yielded to a value of p$K_{a,\mathbf{Rot}}$ = 19.9 ± 0.1 (Figure 4a). When the titration was performed on **NI-amm**$^+$ using a weaker base ($PhCH_2NH_2$, pK_a = 16.91)[30], a value of p$K_{a,\mathbf{Mod}}$ = 16.3 ± 0.1 was obtained (Figure 4b). By introducing these values in equation (2), it was calculated that log$K$ = 3.6 ± 0.2. Within errors, this number is the same as that determined with the luminescence-based method.

The titration of **Rot$_{1s}$** with a base can provide information on the acid-base properties of the amm$^+$ site when it is surrounded by **DB24C8** (vertical process in the right hand part of Scheme 1). Indeed, no spectroscopic modification could be observed when **Rot$_{1s}$** was treated with a huge excess of 1,4-diazabicyclo[2.2.2]octane (DABCO, pK_a = 18.2).[31] This observation indicates that the pK_a of the amm$^+$ unit encircled by **DB24C8** is larger than 23.9.[32]

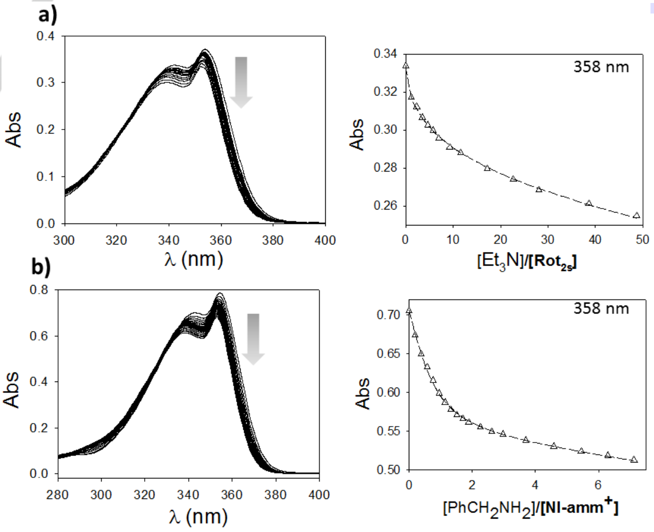


Figure 4. UV-absorption spectral changes (left) and corresponding titration curves (right) in CH_3CN at room temperature of (a) **Rot$_{2s}$** (29 μM) by Et_3N and (b) **NI-amm**$^+$ (56 μM) by $PhCH_2NH_2$. The white triangles are the experimental points and the dashed line is the curve fitting according to a proton exchange model.

The free energy values associated with the processes displayed in Scheme 1 can be calculated from the corresponding equilibrium constants ($\Delta G° = -RT \ln K$) and an energy-level diagram describing the acid-base induced co-conformational switching of **Rot**$_{2s}$ can be constructed (Figure 5). The levels shown in Figure 5 correspond to absolute (SCC) or relative (MCC) energy minima; the energy maxima (i.e., the barriers) that separate the two minima in each curve are not known in the present case. However, these values could be measured from kinetic experiments performed by, e.g., stopped-flow spectrometry, as it was demonstrated for a related pH-responsive bistable rotaxane.[27] Indeed, an accurate knowledge of such an energy diagram implies that the switching properties of the device are fully characterized in terms of both thermodynamics and kinetics.

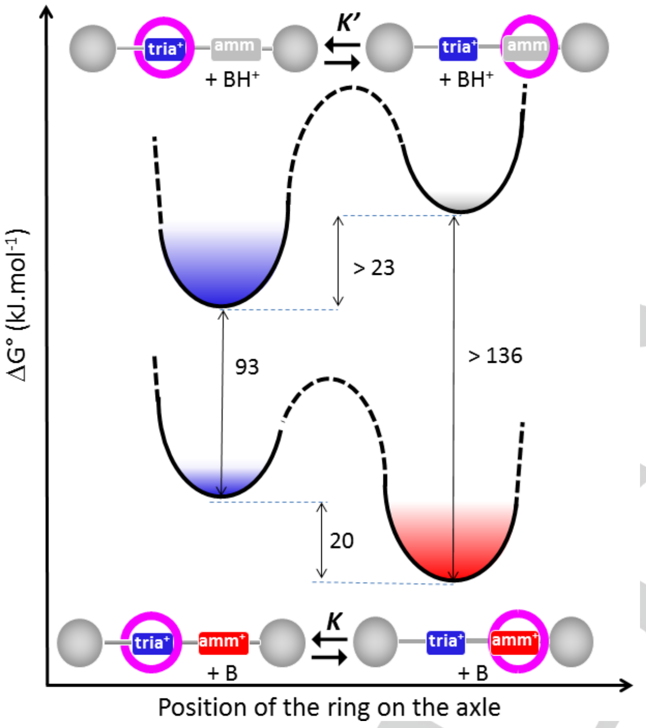


Figure 5. Simplified energy-level diagram as a function of the relative ring-axle position for the rotaxane in its protonated (bottom) and deprotonated (top) forms in CH_3CN (the energies are given in kJ/mol for **B** = CH_3CN).

On the basis of the energy levels shown in Figure 5 we can evaluate the distribution constant in the deprotonated state to be K' = [MCC-**Rot'**$_{2s}$]$_{eq}$/[SCC-**Rot'**$_{2s}$]$_{eq}$ $< 10^{-4}$. The knowledge of the values of K and K' enables us to make quantitative considerations about the shuttling efficiency of the rotaxane. A constant K = 4000 means that 99.97% of the rings in a population of rotaxanes are located on the primary station (amm$^+$). On the other hand, with a value of K' lower than 10^{-4}, more than 99.99% of the rings reside on the tria$^+$ station after deprotonation of the ammonium site. Hence, in the hypothesis that the acid-base switching reactions are performed quantitatively (which can be done by adding a sufficiently strong base or acid in an appropriate excess), the overall yield of the device in a full shuttling cycle (base-induced forward and acid-induced backward movements) amounts to 99.92% (see the SI). This is indeed the highest yield reported to date for a stimuli-controlled molecular shuttle. It is worthwhile to note that a rotaxane capable of performing efficient shuttling cycles requires that the co-conformational equilibria in both states (in the present case, the protonated and deprotonated ones) are strongly shifted towards opposite co-conformations (Scheme 1); in other words, the system should be designed such that K is very large while K' is very small.

The energy-level diagram shown in Figure 5 is also interesting because it clearly shows that the acid strength of the pH-switchable site is affected by the position of the ring. Previous experiments indicate that deprotonation of the ammonium unit is extremely difficult to achieve in the absence of an alternative station for the crown ether macrocycle.[9b, 14l, 33-35] The data collected on the present rotaxane show that the acidity of its ammonium unit changes by at least 7.6 pK_a units upon switching the ring position.

As a matter of fact, by adjusting the relative stabilities of the SCC and MCC in the protonated and deprotonated forms, one can modulate the acid-base properties of the pH-sensitive station. In principle, such a result could even be achieved by tuning the ring affinity of the sole pH-insensitive station. This observation is conceptually very important because it enables the possibility of designing acid or basic sites with made-to-order pK_a using the very same functional group (e.g. a secondary amine as in the present case). In other words, the acid-base properties of the site are determined by its dynamic molecular environment – a behavior which resembles that exhibited by many enzymes.[36]

Conclusions

The distribution between the two translational isomers (co-conformations) in a bistable [2]rotaxane containing **DB24C8** as the ring, and ammonium and triazolium recognition sites in its axle, has been quantified with two novel and sensitive methodologies. The first one relies on time-correlated fluorescence measurements and the second one is based on appropriately designed acid-base titrations. The great difference of affinity of the ring for the two stations could be measured in the interlocked compound (log$K$ = 3.6 in CH_3CN). So far, K could be determined by NMR spectroscopy experiments in case of low values (up to ca. 20) or with more sensitive CV-based methods but only for redox active switches.[10,11]

The K values measured from the acid-base and the time-correlated fluorescence assays are the same within errors. While the lifetime-based assay requires a fluorescent rotaxane in which the emissive excited state of the fluorophore is influenced by to the position of the ring, the acid-base approach seems more general because in principle it can be applied to any pH-switchable rotaxane. The sensitivity of the fluorescence assay strongly depends on the spectroscopic differences of the fluorophore in the two different co-conformations. For instance, the higher the quantum yield ratio in favor of the minor (metastable) co-conformation, the more sensitive the measurement. Also, the deconvolution of the emission decay is facilitated if the difference between the lifetimes attributed to the two co-conformations is significant. For the case reported here, K values of ~10^3 with an upper limit estimated at ~10^4 could be measured. Last but not least, to gain information on the co-conformational equilibrium distribution the emission decay should be faster than the ring shuttling. This requirement is easily fulfilled with organic fluorophores, whose fluorescence lifetimes (~ns) are much shorter than typical ring shuttling times (~μs-ms).[27,28]

On the contrary, the acid-base assay does not rely on the photophysical properties of the system (as long as there is an analytical signal to be monitored in the titrations) and its sensitivity is limited only by experimental considerations – for instance, the availability of the suitable bases whose pK_a values in the solvent of choice are reported. The results of these experiments, summarized in Figure 5, not only show that the investigated rotaxane is a highly efficient molecular shuttle, but also indicate that the affinity of the (pH-insensitive) tria$^+$ station for the **DB24C8** ring influences the acid-base behavior of the amm$^+$ station. This observation is highly interesting because, in principle, it enables the use of programmed allosteric effects to bring about predetermined acid-base properties in artificial multicomponent systems.

The two methods presented in this work expand the toolbox for the characterization of the thermodynamic behavior of bistable interlocked compounds and related species exhibiting a pH-dependent behavior. The information that can be accessed with these experimental approaches is essential for a detailed understanding of the switching mechanism and for assisting the design of new molecular devices and machines.

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Keywords: molecular device • molecular machine • rotaxane • fluorescence • acidity • supramolecular chemistry • nanoscience

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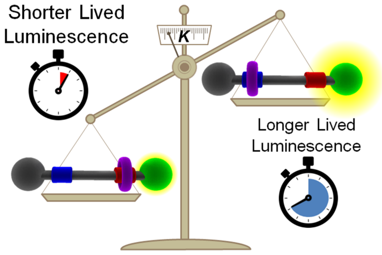
Entry for the Table of Contents

FULL PAPER

In a lifetime: The co-conformational equilibria of a pH-switchable bistable [2]rotaxane have been investigated with two independent and sensitive assays based on its time-resolved fluorescence and acid-base properties. The results show that this rotaxane exhibits the highest positional efficiency reported so far for a molecular shuttle.



Giulio Ragazzon, Alberto Credi, Benoit Colasson**

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Thermodynamic insights on a bistable acid-base switchable molecular shuttle with strongly shifted co-conformational equilibria