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Chelating Rotaxane Ligands as Fluorescent Sensors for Metal Ions

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Abstract: Although metal-ion-binding interlocked molecules have been under intense investigation for over three decades, their application as scaffolds for the development of sensors for metal ions remains underexplored. In this work, we demonstrate the potential of simple rotaxanes as metal-ion-responsive ligand scaffolds through the development of a proof-of-concept selective sensor for Zn^{2+} .

Small-molecule fluorescent probes are powerful tools for visualizing metal ions in living systems due to their rapid response times and potential for non-invasive, high resolution, and quantitative imaging.^[1] In particular, the development of small-molecule sensors^[2,3] for the detection and quantification of Zn^{2+} in vivo has attracted considerable recent attention due to the spectroscopically silent nature of the d^{10} Zn^{2+} ion, combined with the recognition that changes in zinc homeostasis are associated with high-morbidity diseases such as Alzheimer's disease,^[4] Type II diabetes,^[5] and age-related macular degeneration.^[6]

Such small-molecule probes are generally composed of a multidentate chelating ligand linked to a fluorophore whose output is modulated by the metal binding event. Mechanically interlocked molecules,^[7] particularly those synthesized using metal-mediated approaches,^[8] often possess a well-defined binding pocket containing multiple donor atoms for metal ions.^[9] Such multidentate “mechanically chelating” ligands^[10] seem ideal for the development of metal-selective ligands and related metal-ion sensors by exploiting the size and shape of the three dimensional cavity formed by the mechanical bond. However, almost all interlocked molecules that display a fluorescent response^[11] upon metal binding rely on large-amplitude motion in relatively structurally complex molecular shuttles.^[12–14] Furthermore, in most cases, selectivity between competing analytes is not reported. Indeed, to our knowledge, only one example has been reported in which the

mechanical bond is used to generate a metal binding pocket to report the binding of competing analytes; in 2004 Hiratani and co-workers disclosed a [1]rotaxane that selectively binds Li^+ over Na^+ and K^+ and reports metal binding through a “switch on” fluorescence response.^[15,16]

Given that the synthesis of mechanically chelating ligands is now relatively simple, it is perhaps surprising that these scaffolds have been overlooked in the development of cation sensors, particularly since a related strategy for the sensing of anions has been developed by Beer and co-workers.^[17] We thus set out to demonstrate the potential of the mechanical bond as a structural motif in the development of selective metal-ion sensors through the demonstration of a proof-of-concept selective sensor for Zn^{2+} . Herein, we report not only that this approach is successful, but that relatively small structural changes in the axle component lead to large changes in the photophysical response to divalent metal ions.

We synthesized rotaxane **4**^[18] in excellent yield (88%) using the active-template^[19] Cu-mediated alkyne–azide cycloaddition (AT-CuAAC) reaction^[20,21] between azido fluorophore **3** and acetylene **2** in the presence of readily available bipyridine macrocycle **1**^[22] and $[Cu(MeCN)_4]PF_6$. Addition of one equivalent of $Zn(ClO_4)_2 \cdot 6H_2O$ to a solution of **4** in CD_3CN resulted in large changes in the 1H NMR spectrum (see Figure S39 in the Supporting Information), which is consistent with the binding of the metal ion into the cavity of the macrocycle, confirming that **4** is capable of acting as a ligand. The behavior of rotaxane **4** as a metal-responsive sensor for Zn^{2+} was investigated by fluorescence titration with $Zn(ClO_4)_2 \cdot 6H_2O$. Portion-wise addition of Zn^{2+} to rotaxane **4** in MeCN led to monotonic quenching of the emission at 560 nm^[23] that plateaued once a full equivalent had been added (Figure 1a). In contrast, titration of the non-interlocked axle with Zn^{2+} revealed no change by UV/Vis or fluorescence spectroscopy (Figure S61).^[24]

Rotaxane **4** is a candidate “switch off” fluorescent sensor for Zn^{2+} . However, examination of the selectivity of this response revealed a complete lack of discrimination between

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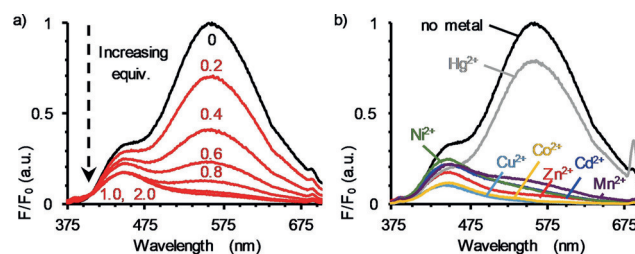


Figure 1. Emission profile of rotaxane **4** (MeCN, 100 μ M, λ_{ex} = 343 nm) in the presence of varying amounts of $Zn(ClO_4)_2 \cdot 6H_2O$ (a), and in the presence of 5 equiv $M(ClO_4)_2$ (b). No emission was observed with $Fe(ClO_4)_2$.

Zn²⁺ and selected divalent metal cations; addition of M(ClO₄)₂ (M = Mn²⁺, Fe²⁺, Co²⁺, Ni²⁺, Cu²⁺, Cd²⁺, Hg²⁺) to a solution of **4** led to quenching of the emission to a greater or lesser extent than that observed with Zn²⁺ (Figure 1b).^[23] Thus, **4** cannot be classed as a metal-ion sensor since, although it responds to metal-ion binding, it cannot discriminate between competing analytes.

Having confirmed that the binding of metal ions within the cavity of the rotaxane can, in principle, lead to an optical response, we extended our investigation to heteroatom-substituted naphthalimide rotaxanes **5–7** (Figure 2), which

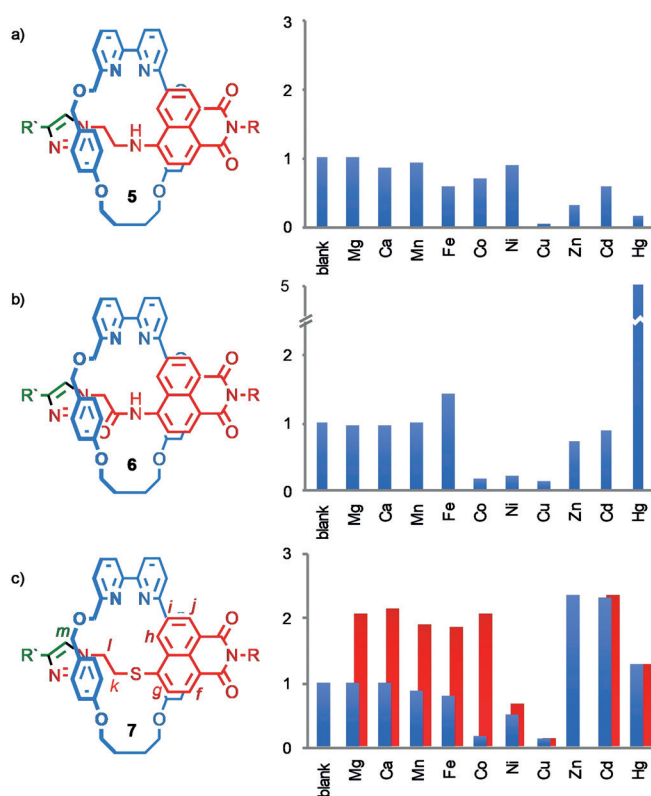


Figure 2. Rotaxanes a) **5** ($\lambda_{\text{ex}} = 435$ nm), b) **6** ($\lambda_{\text{ex}} = 380$ nm), and c) **7** ($\lambda_{\text{ex}} = 379$ nm) and their fluorescence response to 5 equiv M(ClO₄)₂ (MeCN, 100 μM ; blue bars). Red bars in (c) refer to the fluorescence response upon sequential addition of M²⁺ followed by Zn²⁺ (5 equiv each). R = CH₂C(H)Ph₂, R' = 3,5-di-^tBu-C₆H₃.

were readily synthesized in good to excellent yield (62%, 72% and 86% respectively, see the Supporting Information). These were selected because heteroatoms can potentially interact directly with the metal ion and are known to significantly alter the photophysical properties of naphthalimide fluorophores.^[25] ¹H NMR spectroscopy (Figures S39–41) confirmed that rotaxanes **5–7** act as ligands for Zn²⁺, and UV/Vis titration revealed excellent goodness of fit to a 1:1 binding isotherm; addition of Zn(ClO₄)₂·6H₂O resulted in the appearance of absorbance bands at 312 and 322 nm corresponding to the metal-bound bipyridine moiety (Figures S46–48).^[26]

The fluorescence response of rotaxanes **5–7** to the binding of selected divalent metal ions revealed very different

behavior. Similar to rotaxane **4**, rotaxane **5** displays a simple switch-off response to Zn²⁺ and the majority of other metal ions investigated (Figure 2a), although the extent of quenching varied considerably depending on the metal ion. In contrast, rotaxane **6** (Figure 2b) displays little or no response to Mg²⁺, Ca²⁺, or Mn²⁺, a switch-off response of varying degree to Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, and Cd²⁺ and a weak switch-on response to Fe²⁺. Strikingly, Hg²⁺ produced a significant switch-on response, suggesting that rotaxane **6** is a good starting point for the development of a selective sensor for Hg²⁺. Pleasingly, addition of Zn²⁺ to rotaxane **7** triggers a switch-on response with a concomitant blue shift in the emission of 15 nm. All other metal ions, with the exception of Cd²⁺, which produces a switch-on response and a blue shift of 8 nm, produce a switch-off or no response (Figure 2c). Competition experiments demonstrate that in many cases, Zn²⁺ is also able to displace metal ions from ligand **7**; addition of M²⁺ followed by Zn²⁺ to **7** resulted in recovery of fluorescence in the case of Mg²⁺, Ca²⁺, Mn²⁺, Fe²⁺, and Co²⁺.

Having identified rotaxane **7** as a switch-on sensor for Zn²⁺, albeit with Cd²⁺ as a confounding analyte,^[27] we investigated its behavior in more detail. The ¹H NMR spectrum of **7** (Figure 3b and Scheme 1) displays a number of differences to the corresponding non-interlocked axle (Figure 3a). In particular triazole proton H_m appears significantly deshielded in the interlocked structure by $\Delta\delta = 1.36$ ppm, which is consistent with an expected C–H⋯N hydrogen bond with the bipyridine moiety,^[28] and alkyl

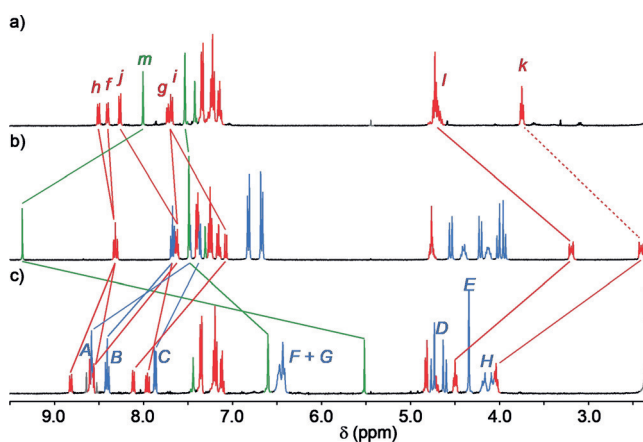
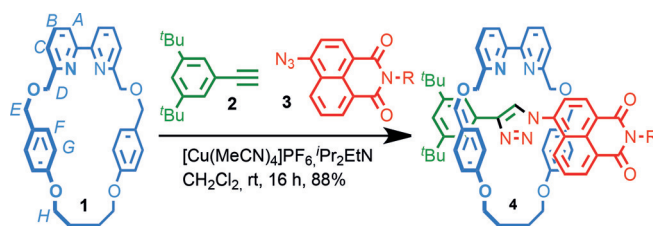


Figure 3. Partial ¹H NMR spectra (CD₃CN, 400 MHz, 298 K) of a) the non-interlocked axle of rotaxane **7**, b) rotaxane **7**, and c) rotaxane **7** + Zn(ClO₄)₂·6H₂O. For labelling scheme, see Scheme 1 (macrocycle) and Figure 2 (axle).



Scheme 1. Synthesis of fluorescent rotaxane **4** using the AT-CuAAC reaction. R = CH₂C(H)Ph₂.

protons H_k and H_l appear at lower ppm, suggesting that they engage in $C-H\cdots\pi$ contacts with the aromatic rings of the macrocycle. Crystals of **7** suitable for X-ray analysis were grown by slow evaporation from MeCN. The solid-state structure is largely consistent with the interactions proposed to be present in solution (Figure 4a); triazole proton H_m is engaged in a $C-H\cdots N$ hydrogen bond with the bipyridine nitrogens, as is one of H_k , and one each of protons H_k and H_l are in close contact with the phenyl rings of the macrocycle.

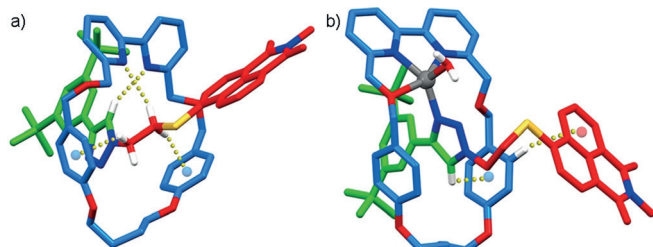


Figure 4. a) Solid-state structure of rotaxane **7** (selected distances in Å: $C-H_m\cdots N=2.46$, $C-H_k\cdots N=2.71$, $C-H_k\cdots\pi=2.66$, $C-H_l\cdots\pi=2.79$; dihedral angle $C_k-S-C-C_{ipso}=8.2^\circ$). b) Solid-state structure of $[Zn(\mathbf{7})]^{2+}$ (selected distances: $C-O\cdots Zn=2.28$, $C-H_m\cdots\pi=2.74$, $C-H_l\cdots\pi=2.86$; dihedral angle $C_k-S-C-C_{ipso}=29.9^\circ$).

Portion-wise addition of $Zn(ClO_4)_2\cdot 6H_2O$ to **7** in CD_3CN led to broadening of the 1H NMR resonances corresponding to **7** and the appearance of a new set of resonances assigned to $[Zn(\mathbf{7})]^{2+}$ (Figure S42). Once one equivalent of Zn^{2+} had been added (Figure 3c), no further change was observed. Strikingly, in addition to the expected deshielding of the bipyridine resonances H_A , H_B , and H_C , the triazole and alkyl resonances shift significantly upon Zn^{2+} binding. H_m is observed at 5.52 ppm in the metal complex ($\Delta\delta = -3.84$ ppm), suggesting it is considerably shielded relative to the non-interlocked axle, while H_k and H_l shift to higher ppm, suggesting that the shielding $C-H\cdots\pi$ interactions are interrupted.

The solid-state structure of $[Zn(\mathbf{7})](OTf)_2$ obtained by slow evaporation of a MeCN solution confirms coordination of Zn^{2+} within the macrocycle cavity and exhibits a number of features consistent with the solution-state 1H NMR data. The Zn^{2+} ion is coordinated by the bipyridine and triazole N-donors, alongside one of the aliphatic ether O-donors and a water molecule.^[29] This coordination interrupts the triazole $C-H\cdots N$ hydrogen bond and this proton is now engaged in a shielding $C-H\cdots\pi$ interaction with one of the macrocycle phenyl rings. The shielding $C-H\cdots\pi$ interactions of H_k and H_l

are also interrupted. Interestingly, in the solid state, coordination leads to the appearance of a $C-H\cdots\pi$ interaction between one of the aromatic phenyl ring protons and the naphthalene rings and a large change in the dihedral angle about the S-naphthyl bond. With the obvious caveat that the solid-state structure of $[Zn(\mathbf{7})]^{2+}$ is not necessarily representative of the solution-state (co)conformation, the observed changes in the interactions between the macrocycle and fluorophore component, along with the changes in the conjugation between the S-donor and the naphthalene core and the altered dihedral angle, suggest that the changes in emission properties of **7** on metal binding may be due to changes in the (co)conformation of the ensemble.

The binding of rotaxane **7** with Zn^{2+} was determined to be extremely strong ($K_d < 10^{-8} M^{-1}$) by UV/Vis titration^[30] with non-linear regression analysis (Figure 5a), stronger than that of macrocycle **1** alone ($K_d = 8.9 \times 10^{-8} M^{-1}$; Figure S57), suggesting that the mechanically chelating triazole ligand significantly enhances Zn^{2+} binding in **7**.^[31] Conversely, the binding of rotaxane **7** to Cd^{2+} ($K_d = 3.6 \times 10^{-5} M^{-1}$; Figure 5b) is about three orders of magnitude weaker than that of macrocycle **1** alone ($7.5 \times 10^{-8} M^{-1}$; Figure S59). The difference in binding strength for **7** with Zn^{2+} and Cd^{2+} was further corroborated by 1H NMR titration. In the presence of 1 equiv of both Zn^{2+} and Cd^{2+} , $[Zn(\mathbf{7})]^{2+}$ is observed to form selectively (Figure S43), whereas the same experiment with macrocycle **1** produced a 4:1 mixture of Zn^{2+} and Cd^{2+} complexes (Figure S45). These results demonstrate that the mechanical bond imparts a significant degree of selectivity to the binding of otherwise similar metal ions, perhaps due to the different sizes of the Zn^{2+} and Cd^{2+} ions (88 pm vs. 109 pm, respectively)^[32] or by sterically excluding additional ligands from the coordination sphere of the metal ion.

The stronger binding of Zn^{2+} compared with Cd^{2+} allowed us to use a more competitive solvent mixture (2% water in MeCN) to impart selectivity to sensor **7**. In the presence of H_2O (Figures 5c,d), the binding of both Zn^{2+} and Cd^{2+} to **7** was diminished ($K_d = 7.8 \times 10^{-5}$ and $1.8 \times 10^{-3} M^{-1}$ respectively). As a result, whereas 1 equiv of Zn^{2+} achieved 50% of F_{max} and saturation was achieved at approximately 12 equiv, Cd^{2+} required around 20 equiv to achieve 50% switch on and around 100 equiv to achieve saturation, demonstrating that under these conditions, Cd^{2+} is not bound by **7** to a significant extent. Thus, whereas addition of 5 equivalents of Cd^{2+} to **7** in MeCN/ H_2O leads to a weak response, when Zn^{2+} is added to the same solution, the expected switching on of luminescence is observed, thus demonstrating selectivity for Zn^{2+} over Cd^{2+} (Figure 5e).

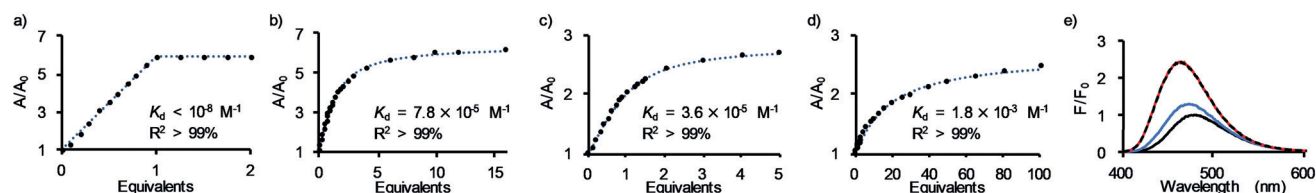


Figure 5. UV/Vis ($\lambda = 322$ nm) titrations of **7** (100 μM) with $M(ClO_4)_2$ as a function of a) Zn^{2+} , b) Zn^{2+} (2% $H_2O/MeCN$), c) Cd^{2+} (MeCN), and d) Cd^{2+} (2% $H_2O/MeCN$). e) Emission spectra ($\lambda_{ex} = 380$ nm) of **7** (black), **7** + 5 equiv Cd^{2+} (blue), **7** + 5 equiv Zn^{2+} (red), **7** + 5 equiv each Zn^{2+} and Cd^{2+} (black dashed) in 2% $H_2O/MeCN$.

In conclusion, we have demonstrated that relatively simple interlocked molecules can provide an excellent scaffold for the design of metal ion sensors. Importantly we show that the binding pocket provided by the mechanical bond can impart not only an optical response but also a degree of binding selectivity, as in the case of rotaxane **7**. It is also noteworthy that, in addition to sensor **7**, which shows the desired Zn²⁺-selective response in MeCN/H₂O, rotaxane **6** also appears to show a selective switch-on response, in this case to Hg²⁺.^[33] The mode of switching, at least in the case of **7**, appears to be reorientation of the components upon metal binding, thereby altering the relative positions of the fluorophore and the macrocycle and leading to an enhancement of fluorescence, but this requires more detailed investigation. The origin of the different behaviors of **4–6** also requires further investigation; since it appears that the different linker units are not directly involved in the binding event, it seems likely that the specific photophysical properties of the fluorophore unit are important.^[25] From a practical viewpoint, the next step in the development of interlocked hosts for the detection of metal ions in biological systems is to render them water soluble, a task that is ongoing in our laboratories and is greatly facilitated by the synthetic flexibility of the AT-CuAAC reaction. More generally, the results presented here suggest that, although interlocked molecular machines remain an exciting and important direction for the field, the use of the mechanical bond as a structural feature, for instance in the design of mechanically chelating ligands, has the potential to lead to new developments in a range of areas.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: fluorescent probes · rotaxanes · sensors · supramolecular chemistry · zinc

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