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Thinking Outside the *Blue Box*: Induced Fit Within a Unique Self-assembled Polycationic Cyclophane †

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Supporting Information Placeholder

ABSTRACT: We present herein the development of a new polycationic molecular receptor, inspired by the ubiquitous cyclobis(paraquat-*p*-phenylene)cyclophane (*blue box*). Our analogue, the *white box*, has been easily self-assembled on a preparative scale in water, using a template-assisted process by acyl hydrazone bonding of complementary *bis*(pyridinium)-xylylene tweezers, followed by kinetic trapping of the empty receptor. The obtained macrocycle was found to display a marked pH-responsiveness in water, because of an abnormal acidity of the amide protons within its structure. Consequently, and because of the concurrence of rotational isomerism on acidic conditions (fixed at higher pH values), the compound was found to display a dual behavior as a conformationally locked/flexible molecular host, being able to recognize appropriate aromatic substrates, on a lock-and-key or induced fit fashion, by a conjunction of π-π, C-H···π and, crucially, hydrophobic interactions.

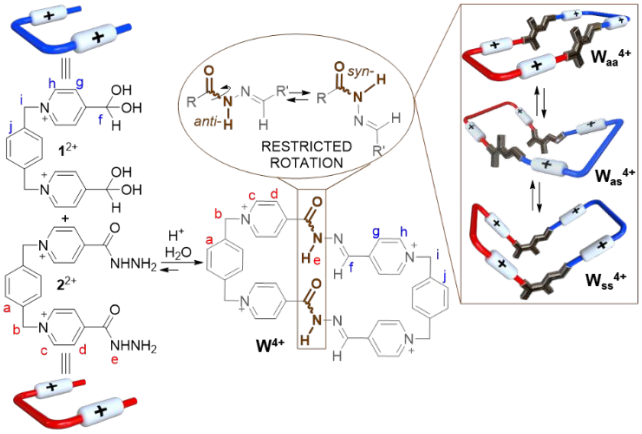
Introduction

Currently commercially available macrocycles such as cyclodextrins,[1] crown ethers,[2] calixarenes[3] or cucurbiturils,[4] are prominent examples of the myriad of molecular receptors developed over the years since the seminal works of Pedersen,[5-6] Lehn[7-8] and Cram.[9]

Nevertheless, such fully static hosts have many weaknesses that constrain the adjustment of their properties,[10] as well as the exploration of new biomimetic concepts that could potentially evolve the field of host-guest chemistry in particular, and supramolecular chemistry in general.[11-13] Firstly, the synthesis of those macrocycles, and therefore of fine-tuned analogues, is hampered by cyclization steps being carried out typically under kinetic control and, therefore, resulting on low yields on the desired target.[14,15] Likewise, rigid receptors are usually preferred over conformationally flexible analogues,[16] prevailing therefore in their host-guest chemistry Fischer's lock and key[17] over Koshland's induced fit.[18]

The above-discussed limitations are even more pronounced in two scenarios. Firstly, in aqueous media, where most of the weak intermolecular forces, commonly used as driving force of the molecular recognition, are mainly disrupted due to the dominant effect of water on the association processes.[19-20] Additionally, the most consistent approach for the self-assembly of molecular receptors is still based on metal-directed methodologies,[21] where the precise geometry of the different coordination modes of the metallic ions, as well as their various degrees of kinetic/thermodynamic stability, offer a reliable strategy for the prospective design of self-assembled species with well-defined shapes and geometries. Conversely, reports on the thermodynamically-controlled synthesis of wholly organic and functional macrocyclic hosts are still quite scarce.[14]

To improve in some extent some of the above-mentioned drawbacks, and following our continuous interest on the development of self-assembled receptors in aqueous media,[22-27] we have turned our attention to the use of hydrazone-based dynamic covalent chemistry for the task.[28] This methodology has very recently erupted in the literature, allowing the thermodynamically-controlled preparation in aqueous media of entirely organic molecular receptors and mechanically interlocked molecules.[29-34] Even though the strategy has been proven quite successful, in most of the cases the amphiphilic character of the building blocks results rather into hydrophobically-driven self-catenation or knotting,[29-33] than into fully functional and empty kinetically stable hosts.[34]



Scheme 1. Depiction of the planned self-assembly of the *white box* $\mathbf{W}^{4+}$, and its potential conformational isomerism.

In this scenario, we propose herein the development of the *white box* (**W**$^{4+}$), a new self-assembled cyclophane analogue to the well-known *blue box* (cyclobis(paraquat-*p*-phenylene)cyclophane) developed by Stoddart and coworkers.[15, 16] In essence, to avoid the kinetically-controlled synthesis of the receptor, we projected its self-assembly of **W**$^{4+}$ in water by acyl hydrazone bonding (**Scheme 1**), using complementary hydrophilic *bis*(pyridinium)-xylylene tweezers **1**$^{2+}$ and **2**$^{2+}$, building blocks prepared in excellent yield as the corresponding dibromides starting from 1,4-bis(bromomethyl)benzene.[35] The π-deficient and hydrophilic character of the components, was designed not only to minimize the propensity to self-thread observed in similar systems,[29-33] but to promote host-guest interactions in a *blue box*-like fashion (i.e. by maximization of host-guest π-π, C-H$\cdots\pi$ and hydrophobic interactions with four parallel pyridinium rings).[15, 16, 36]

Results and discussion

In a first effort to produce cyclophane **W**$^{4+}$, we attempted typical reaction conditions for thermodynamic control on the hydrazone bonding,[30] monitoring by ^{1}H-NMR the TFA-catalyzed reaction at 80 °C of an equimolar 1.5 mM mixture of **1**·2Br and **2**·2Br in D_2O. After 24 hours, the results were quite misleading, with the starting materials being transformed into a complex mixture of presumably, oligomeric and isomeric imine-containing species.[36] In order to simplify the analysis of the outcome of the self-assembly, we decided to use the very same reaction conditions, but with an excess of 2,7-dihydroxynaphthalene (2,7-DHN, 1.5 eq.), acting as a template. The aromatic substrate would, *a priori*, not only decrease the energetic penalties of the process, but, we argued that in order to maximize host-guest interactions, the macrocycle would prefer to adopt a *blue box*-like conformation, that is **W**$_{aa}^{4+}$, the only conformer able to potentially align the π-deficient pyridinium moieties of each side of the rectangle.[36] In that manner, the observation of conformers on the NMR would be conveniently precluded.

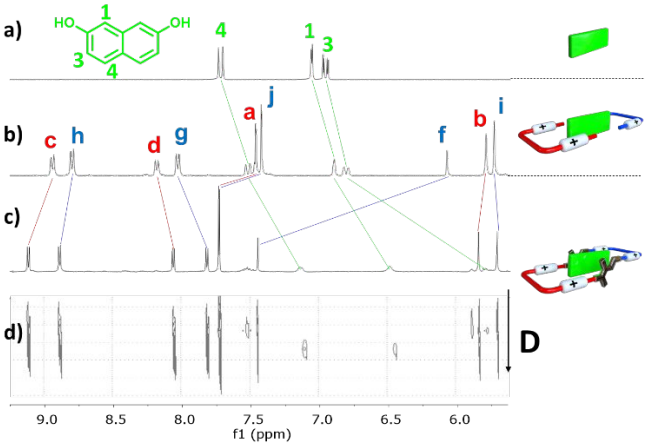


FIGURE 1. ^{1}H NMR (D_2O, t.a., 500 MHz) of: a) 1.5 mM DHN; b) equimolar 1.5 mM mixture of **1**·2Br, **2**·2Br and 2,7-DHN at r.t. and t = 0; c) the same mixture after 24 hour heating at 80 °C; d) DOSY NMR for mixture c). Proton numbering on **Scheme 1**

In this case, after 24 hours, the results obtained for the acid-catalyzed reaction were in good agreement with the expected inclusion complex **W**$^{4+}$⊂2,7-DHN (**Figure 1**). Essentially, compared with the free guest, signals of the substrate appear consistently shielded, being H_3 particularly affected due to the typical occurrence of [C-H$\cdots\pi$] interactions with the phenylene moieties of **W**$^{4+}$($\Delta\delta H_3$ = -1.2 ppm, $\Delta\delta H_a$ = 0.27 ppm and $\Delta\delta H_j$ = 0.3 ppm).[42] Furthermore, the resonances observed for H_f (δH_f = 7.35 ppm) and C_f (δC_f = 143.9 ppm), are completely congruent with the formation of the expected imine bonds. Finally, DOSY NMR also allowed us to support the formation of the inclusion complex, with all its resonances on the aggregate diffusing as a whole on the corresponding spectrum (**Figure 1d**)

The synthesis of **W**·4Br⊂2,7-DHN could be achieved on a preparative scale (0.4 mmol), allowing for the expulsion of the template from the cavity of the receptor and the isolation of virtually pure **W**·4PF_6 on a 83% yield, simply by addition of excess KPF_6 to the aqueous reaction media, filtration and washing of the obtained solid with AcOEt. 1D/2D NMR experiments in CD_3CN showed a sole main species, in good agreement with the expected cyclophane **W**$^{4+}$. The identity of the macrocycle was corroborated as well by ESI-MS, showing both the typical loss of PF_6^- counterions on the macrocyclic structure, as well as HPF_6 fragments as a result of acid-base reactions occurring on the gas phase (**Figure 2**),[43] a fact that could be later correlated with an increased acidity of the amide protons of the structure (*vide infra*).

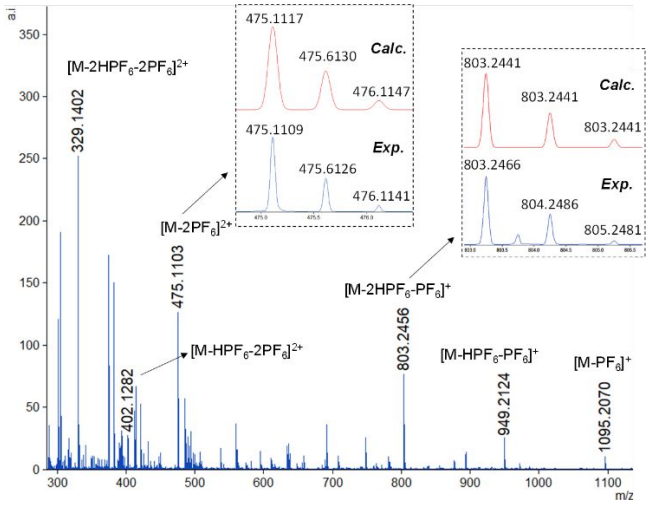


FIGURE 2. LR ESI-MS spectrogram for **W**·4PF_6 showing the loss of PF_6^- and HPF_6 fragments. Insets, HR ESI-MS for some of the most relevant peaks.

The convenient ease on the synthesis of the cyclophane by anion exchange is not surprising. Due to the suppression of the hydrophobic effect, **W**$^{4+}$ can be anticipated as a quite deficient molecular host in organic solvents.[15-16, 22-23] To corroborate that hypothesis, we unfruitfully attempted in CD_3CN the synthesis of the corresponding inclusion complexes of **W**·4PF_6 and variety of electron rich aromatics, with the corresponding ^{1}H NMR showing the absence of appreciable host-guest interactions.[35]

Turning our attention to the study of **W**$^{4+}$ in aqueous media, **W**·4Cl could be easily obtained in an excellent yield of 89% by ion metathesis of the hexafluorophosphate salt dissolved in CH_3CN.[35] In this case, the ^{1}H NMR of the empty receptor in D_2O at r.t., matched with the existence of various

imine-containing isomeric species. As it can be seen on the spectrum shown in **Figure 3**, two sets of sharp resonances appear on the spectrum accompanied by another, quite complex, set of signals in a minor proportion. VT ^{1}H NMR experiments, competition reaction with excess of 4-(dihydroxymethyl)-1-methylpyridin-1-ium iodide, and the non-alteration of the observed signals over time, account for the kinetic stability of the products detected on these conditions.[35] Based on these experimental observations, the extended bibliography on the occurrence of rotational isomerism of acylhydrazones,[29-34,37-41] as well as the different 1D/2D NMR experiments recorded for **W**·4Cl in D_2O, we established the hypothesis of the macrocycle appearing as a mixture of the three potentially stable rotamers that were tentatively assigned as $\mathbf{W_{aa}}^{4+}$, $\mathbf{W_{ss}}^{4+}$ and $\mathbf{W_{as}}^{4+}$ (**Scheme 1**),[36] being the three in slow exchange on the NMR timescale on a 0.40:0.35:0.25 proportion, respectively ($K_{aa\rightarrow as}$ = 0.82, $K_{as\rightarrow ss}$ = 1.40, $K_{aa\rightarrow ss}$ = 1.15).[44] An EXSY NMR experiment recorded for the product, was specially in good agreement with the hypothesis of the conformers, with signals for the three potential species showing appropriate cross peaks on the diagonal of the corresponding 2D spectrum.[35] Furthermore, addition of an equimolar amount of 2,7-DHN to a D_2O solution of the conformationally flexible receptor, spontaneously recovered the very same NMR data obtained for $\mathbf{W}^{4+}\subset$2,7-DHN. Going back to the analysis of the VT ^{1}HNMR experiment, we clearly detected an increase on the exchange velocity for the signals assigned to different isomers, allowing for the rough assessment of coalescence temperatures for some of those, and the estimation of a $\Delta G^{\#}$ ~ 17.3 Kcal/mol for the interconversion processes,[35] a value clearly matching that frequently observed for the rotational barrier of amide bonds within acylhydrazones.[40-41]

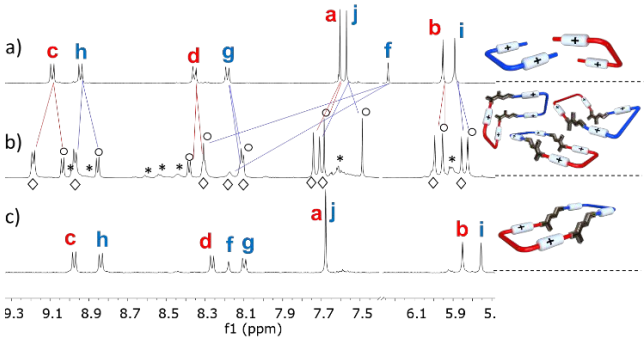


FIGURE 3. ^{1}H NMR (D_2o, t.a., 500 MHz) of: a) equimolar 1.5 mM mixture of **1**·2Br, **2**·2Br at r.t.; b) product obtained after ion metathesis of **W**·4PF_6 with TBACl (pH = 4); c) same as b) in a buffered solution at pH = 7.2. Legend: $\mathbf{W_{aa}}^{4+}$, diamonds; $\mathbf{W_{ss}}^{4+}$ circles; $\mathbf{W_{as}}^{4+}$, stars. Proton numbering on **Scheme 1**.

In order to further validate our hypothesis of the obtained macrocycle $\mathbf{W}^{4+}$ being observed as a mixture of isomers on NMR on aqueous media, we argued that deprotonation of the amide protons at a sufficiently basic pH would eliminate the proposed rotational isomerism, as a result of the delocalization of the negative charges over two of the pyridinium rings (**Figure 4**). As a corollary of this prediction, a decreased acidity should be observed for the macrocycle, a fact that was conveniently verified by an UV-Vis titration yielding a p*Ka* = 6.5 for an acid-base process where two protons are released to the aqueous media.[35]

As shown on **Figure 4**, the appearance of the conjugated base $\mathbf{W}^{2+}$ on increasing the pH, clearly results on a substantial decrease of the originally observed main absorption for $\mathbf{W}^{4+}$ (λ_{max} = 325 nm, associated to π-π^* transitions), and the concomitant appearance of a new band clearly indicating an intramolecular charge-transfer (λ_{max} = 400 nm), associated with the delocalization of negative charge over the pyridinium rings upon deprotonation. In addition, the ^{1}H-NMR for a buffered aqueous solution of the macrocycle at pH = 7.2 (**Figure 3c**), clearly simplifies the initially observed complex spectra for $\mathbf{W}^{4+}$ to a sole set of resonances for the cyclophane, a compelling evidence for the compound appearing as the conformationally rigid species $\mathbf{W}^{2+}$ at a sufficiently basic pH.

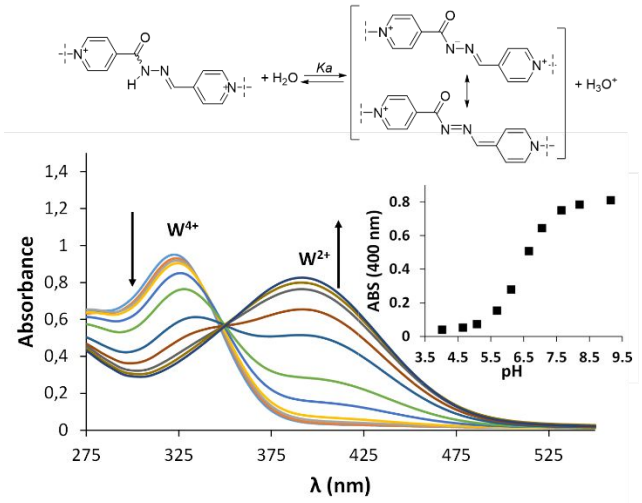


Figure 4. A) Schematic depiction of the acid-base equilibrium for $\mathbf{W}^{4+}$ in water. B) UV-Vis spectra for the titration of 30 μM **W**·Cl with solutions of citric acid/Na_2HPO_4 buffer of increasing pH. Insets on B): Absorption for the compound at λ = 400 nm plotted against the pH.

Attracted by the pH-dependent nature of our macrocycle, we proceeded to study its ability as molecular receptor in water. Consequently, 2,7-DHN was used as archetypical aromatic guest, and its association with the host studied by ^{1}H NMR titrations in buffered solutions. Firstly, we conducted the experiments at pH = 7.8, assuring that our receptor would be mainly deprotonated. The obtained data showed the host-guest complexation to be fast on the NMR timescale, allowing for its convenient fitting to a 1:1 association model using the software Dynafit.[45] The obtained value of K_a = 4960 ± 330 M^{-1} for $\mathbf{W}^{2+}\subset$2,7-DHN, is in good agreement to the reported values for similar systems.[15, 21-27]

The same experiments at pH = 4, guaranteeing the protonation of the compound, yielded really interesting results. As shown on **Figure 5**, it can be clearly observed that upon addition of substrate, only one out of the three sets of signals assigned to the rotamers on slow exchange, that tentatively assigned to $\mathbf{W_{aa}}^{4+}$ (**Figure 3**), shows a shifting on its resonances accounting for a host-guest complex on rapid equilibrium with this rotamer on the NMR timescale. Conversely, the signals attributed to the other two isomers, $\mathbf{W_{ss}}^{4+}$ and $\mathbf{W_{as}}^{4+}$, are practically unaltered, on their chemical shifts, during the titration. Fitting of the obtained data to the global process [$\mathbf{W_{aa}}^{4+} \leftrightarrows \mathbf{W_{as}}^{4+} \leftrightarrows \mathbf{W_{ss}}^{4+}$] $\leftrightarrows \mathbf{W_{aa}}^{4+}\subset$2,7-DHN (**Figure 5**), using the software Dynafit,[45] allowed for the

estimation of a *Ka* = 2630 ± 200 M^{-1} for the induced fit process,[47] a value that accounts as well for the hydrophobic effect, and not the total charge of the macrocycle, being mainly responsible for the observed aggregation proccesses.[14,22-27]

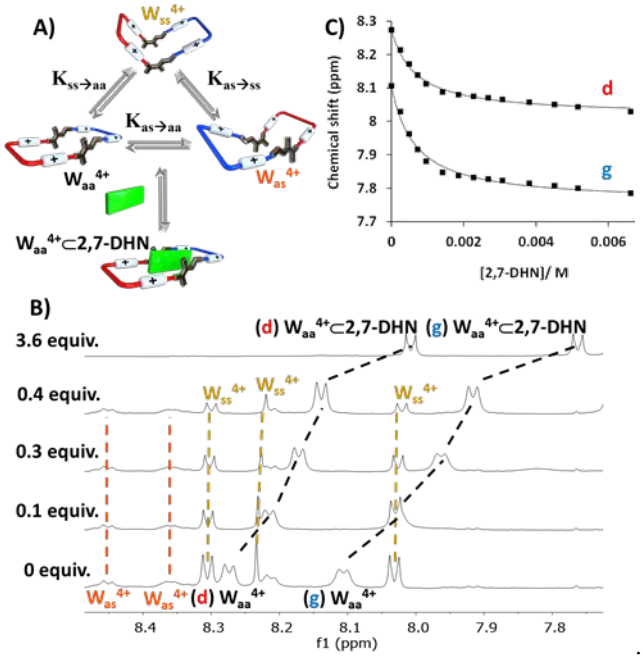


FIGURE 5. A) Proposed equilibria involved on the induced fit process. B) Selected spectra form the titration of **W**·4Cl (1.8 mM in D_2O, pH= 4), with increasing amounts of 2,7-DHN, showing a selected region of the ^1H NMR spectra (D_2O, t.a., 500 MHz). C) Fitting of the observed variation on the chemical shifts of protons H_d and H_g plotted against the total concentration of added guest.

The assignment of the rotamer $\mathbf{W_{aa}}^{4+}$ as the one involved on the induced fit process, is argued on the basis of different evidences. First, the slow interconversion rate between rotamers, and the fast exchange regime for the complexation of the guest, producing only one set of 12 sharp resonances, directly confirms both the induced fit mechanism and exonerates isomer $\mathbf{W_{as}}^{4+}$ as being the one selected on the process (**Figure 5B**).[44] In order to clarify whether $\mathbf{W_{aa}}^{4+}$ or $\mathbf{W_{ss}}^{4+}$ is the symmetric conformation chosen on the recognition process, we performed a series of NMR experiments to evaluate the association at pH = 4 of $\mathbf{W}^{4+}$ with appropriate guests of increasing sizes (1,5-DHN < carbazole < phenanthrene < pyrene). The obtained results are very similar to those discussed for $\mathbf{W}^{4+}\subset$2,7-DHN, with the aggregates showing not only the expected deshielding for the nuclei on the guest because of π-π interactions, but as well diagnostic protons on the longitudinal axis of the guests clearly involved in C-H···π interactions, a fact that fits extremely well with fixed longitudinal insertion modes for all the substrates within the $\mathbf{W_{aa}}^{4+}$ conformer, as is also typically shown in similar systems.[15, 24-26, 35]

Finally, to shed some light on the structures of both $\mathbf{W_{aa}}^{4+}$ and its inclusion complex $\mathbf{W_{aa}}^{4+}\subset$2,7-DHN, those were minimized using DFT methods at the M06/6-31G(d,p) level in water. The obtained results (**Figure 6**), show minor differences between the conformation of free and complexed cyclophane, supporting the longitudinal insertion mode of the guest and the coplanar conformation of $\mathbf{W_{aa}}^{4+}$, being the distance between the mean plane of 2,7-DHN and each of the long sides of the guest around 3.3-3.4 Å, optimal for the establishment of π–π-interactions as in similar systems.[15, 24-26, 35]

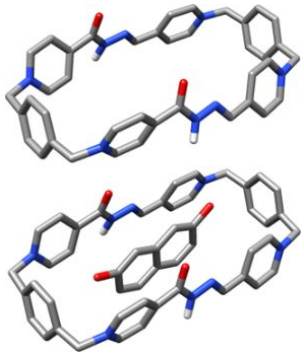


FIGURE 6. DFT optimized structure (M06/6-31G(d,p)) of $\mathbf{W_{aa}}^{4+}$ (top) and $\mathbf{W_{aa}}^{4+}\subset$2,7-DHN (bottom).

Conclusions

In summary, we have reported herein the successful template-assisted self-assembly of a new pH-dependent and conformationally modulable molecular receptor, the *white box*, an acyl hydrazone-extended analogue of Stoddart's *blue box*, able to recognize a variety of aromatic substrates in aqueous media by a combination of π-π, C-H···π and, decisively, hydrophobic interactions. The host-guest chemistry of the receptor could be studied in detail, being a nice example of key-and-lock recognition in neutral/basic conditions, and induced fit on more acidic media. The reported results open the door not only for the easy design and synthesis of new fine-tuned analogues of the *white box* (for instance by replacing the aldehyde groups on $\mathbf{1}^{2+}$ by structurally-diverse ketones), but also for the exploration of refreshing concepts in dynamic supramolecular chemistry, as exemplified by the induced-fit mechanism reported herein.

ASSOCIATED CONTENT

Supporting Information

Complete experimental procedures, 1D/2D NMR experiments and ESI MS data for new compounds, extra figures and computational details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interests.

† By analogy to Stoddart's *blue box*, we have named the host developed herein as the *white box*, obviously based not in its shade, but in honor to the common surname of three of the authors of the present work.

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(36) The presence of two acyl hydrazone moieties potentially confer our designed receptor both configurational (*E*-/*Z*-) and conformational (*syn*-/*anti*-) isomerism. Considering the well-known prevalence of the less sterically-hindered *E*- form on the imine bond on most aryl acyl hydrazones,[33-34, 37-40] the isomerism would be, in principle, restricted to the appearance of the *syn*-/*anti*-conformers of the amide bonds. In consequence, the two acyl hydrazone moieties on our envisaged receptor would result in three potential conformers for $\mathbf{W}^{4+}$ (labeled as $\mathbf{W_{ss}}^{4+}$, $\mathbf{W_{sa}}^{4+}$ and $\mathbf{W_{aa}}^{4+}$, **Scheme 1**).

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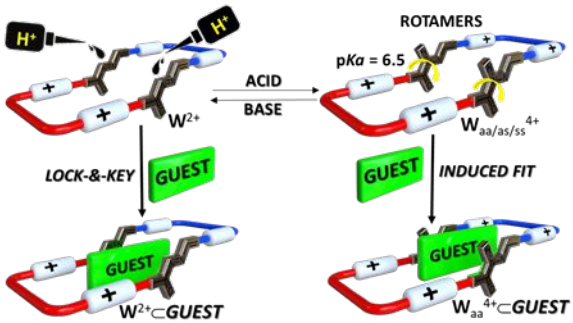
(46) For instance, on the ^{1}H NMR for $\mathbf{W}^{4+}\subset$2,7-DHN, strong C-H$\cdots\pi$ interactions are solely observed for equivalent protons $H_{2,7}$ on the longitudinal axis of the PAH ($\Delta\delta$ = -3.1 ppm), with the other equivalent protons $H_{1,3,6,8}$ and $H_{4,5,9,10}$ being far less deshielded and pointing out a fixed longitudinal insertion mode for the PAH. Any other conformation of the host within the aggregate different than that of $\mathbf{W_{aa}}^{4+}$, would clearly result on a quite different pattern of deshielding. For an extensive discussion on the assessment of insertion modes on bipyridinium-based metallacycles of similar dimension, see reference 26.

(47) The quite large residuals observed on the fitting shown on **Figure 5C**, most likely account for the '*non-correct*' conformers, W_{as} and W_{ss}, being able to weakly complex 2,7-DHN in a much lesser extent than W_{aa}. Due to the limitations of the NMR technique, no signs of those other potential inclusion complexes were observed on the recorded spectra, so the consequent equilibria could not be included on a more complex binding model.

TOC


H+
H+
W2+
ACID
BASE
ROTAMERS
pKa = 6.5
Waa/as/ss4+
LOCK-&-KEY
GUEST
GUEST
INDUCED FIT
GUEST
GUEST
W2+⊂GUEST
Waa4+⊂GUEST