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# Influence of the solvent in the self-assembly and binding properties of [1 + 1] tetra-imine bis-calix[4]pyrrole cages†

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We report the self-assembly of shape-persistent [1 + 1] tetra-imine cages **1** based on two different tetra- $\alpha$  aryl-extended calix[4]pyrrole scaffolds in chlorinated solvents and in a 9 : 1 CDCl<sub>3</sub> : CD<sub>3</sub>CN solvent mixture. We show that the use of a bis-*N*-oxide **4** (4,4'-dipyridyl-*N,N'*-dioxide) as template is not mandatory to induce the emergence of the cages but has a positive effect on the reaction yield. We use <sup>1</sup>H NMR spectroscopy to investigate and characterize the binding properties (kinetic and thermodynamic) of the self-assembled tetra-imine cages **1** with pyridine *N*-oxide derivatives. The cages form kinetically and thermodynamically stable inclusion complexes with the *N*-oxides. For the bis-*N*-oxide **4**, we observe the exclusive formation of 1 : 1 complexes independently of the solvent used. In contrast, the pyridine-*N*-oxide **5** (mono-topic guest) produces inclusion complexes displaying solvent dependent stoichiometry. The bis-*N*-oxide **4** is too short to bridge the gap between the two endohedral polar binding sites of **1** by establishing eight ideal hydrogen bonding interactions. Nevertheless, the bimolecular **4**⊂**1** complex results as energetically favored compared to the **5**<sub>2</sub>⊂**1** ternary counterpart. The inclusion of the *N*-oxides, **4** and **5**, in the tetra-imine cages **1** is significantly faster in chlorinated solvents (minutes) than in the 9 : 1 CDCl<sub>3</sub> : CD<sub>3</sub>CN solvent mixture (hours). We provide an explanation for the similar energy barriers calculated for the formation of the **4**⊂**1** complex using the two different ternary counterparts **5**<sub>2</sub>⊂**1** and (CD<sub>3</sub>CN)<sub>2</sub>⊂**1** as precursors. We propose a mechanism for the in-out guest exchange processes experienced by the tetra-imine cages **1**.

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## Introduction

Molecular cages possess three-dimensional cavities with multiple portals that are capable of including sizable guests. Included guests reside in the “inner phase”, whose properties are different from those of the outer phase *i.e.* the bulk solution.<sup>1,2</sup> The portals connecting the two phases and their sizes control the in and out exchange of the guests. These singular features make cage containers attractive molecular designs for different applications such as molecular recognition,<sup>3–5</sup> stabilization of reactive species<sup>6</sup> and mediation of chemical reactivity,<sup>7,8</sup> among others.<sup>9–12</sup>

The first examples of molecular cages date back to the middle 80s'. In pioneering works, Cram<sup>13</sup> and Collet<sup>14</sup> reported

the preparation of molecular containers using exclusively irreversible covalent bonds. The large kinetic stability of the purely covalent cages contrasted with its high synthetic cost and the low yield in which they were produced. Dynamic covalent chemistry (DCC) constitutes an efficient synthetic strategy for the modular self-assembly of three-dimensional molecular containers in high yields.<sup>15,16</sup> DCC combines the strength of covalent bonds with the error-checking nature of reversible interactions. The formation of imine bonds is a well-established reversible chemical transformation suitable for DCC. In fact, many shape-persistent organic cages, *a.k.a.* porous organic cages (POCs), were self-assembled from reversible condensation reactions between simple aldehyde and amine building blocks.<sup>17,18</sup> The resulting poly-imine POCs displayed a well-defined interior cavity that did not collapse into more dense or twisted structures owing to the conformational restrictions imposed by the imine bonds.<sup>19</sup> The first Schiff base molecular container was described in 1991 by Quan and Cram, *via* the condensation reaction between two molecules of a tetra-aldehyde resorcin[4]arene cavitand and four molecules of 1,3-diaminobenzene.<sup>20</sup> The derived octa-imine cage was able to include a series of guests forming kinetically stable hemi-carceplexes owing to constrictive binding.<sup>2</sup> The ground state of the cage complexes had twisted portals that were too small to

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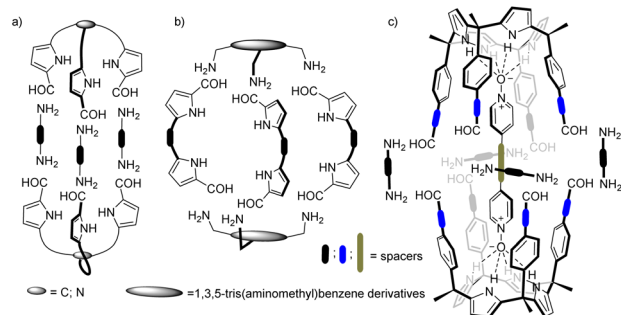
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More recently, Sessler and co-workers also prepared a series of hexa-imine cages *via* [2 + 3] condensation reaction between

Herein, we describe the self-assembly of related tetra-imine cages **1** using bis-pyridyl-*N,N'*-dioxide **4** as template (Fig. 2). We also report the self-assembly of the tetra-imine cages **1** in the absence of template **4** using a 9:1 CDCl<sub>3</sub>:CD<sub>3</sub>CN solvent mixture and pure CDCl<sub>3</sub> or CD<sub>2</sub>Cl<sub>2</sub> solvents. These results allowed the study and characterization of the binding properties of the tetra-imine cages **1** towards bis-*N*-oxide **4** and pyridine-*N*-oxide **5**. In the case of pyridine-*N*-oxide **5**, we detected the formation of 1:1 and 1:2 complexes. Despite **4** was too short to establish an ideal ditopic interaction with the two polar hemispheres of **1**, the **4**⊂**1** inclusion complex was thermodynamically more stable than the **5**<sub>2</sub>⊂**1** counterpart. We investigated the kinetics for the formation of the inclusion complexes and the guests' exchange using <sup>1</sup>H NMR spectroscopy. In the 9:1 CDCl<sub>3</sub>:CD<sub>3</sub>CN solvent mixture the inclusion of **4** in cage **1** was quite slow in reaching equilibrium (days). On the contrary, the uptakes of the guests in pure CDCl<sub>3</sub> solution were fast (min). To our surprise, the energy barrier of the guests' exchange in pure



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$\text{CDCl}_3$  ( $5_2 \subset 1 + 4 \rightleftharpoons 4 \subset 1 + 2 \times 5$ ) coincided with that of the uptake of **4** by the capsular solvate  $(\text{CD}_3\text{CN})_2 \subset 1$  assembled in the 9 : 1  $\text{CDCl}_3$  :  $\text{CD}_3\text{CN}$  solvent mixture.

## Results and discussion

### Synthesis of precursors

Tetra-amine “four wall” calix[4]pyrrole **2a** and its lipophilic tetra-ester analogue **2b** were synthesized using reported methodologies.<sup>34,35</sup> Tetra-formyl calix[4]pyrrole **3** (Fig. 2) was also prepared uneventfully using literature procedures.<sup>33</sup>

### Template-assisted self-assembly of tetra-imine cages **1**

Firstly, we investigated the self-assembly of cage **1a** using equimolar amounts of *meso*-tetramethyl tetra(4-amino)phenyl calix[4]pyrrole **2a**, tetra-formyl calix[4]pyrrole **3** and bis-pyridyl-*N*-oxide **4** in  $\text{CDCl}_3$  solution. Based on our previous results on the assembly of octa-imine dynamic covalent cages,<sup>33</sup> we assumed that the inclusion of the ditopic template was mandatory for the emergence of the tetra-imine cage **1a**. Molecular modelling (MM3) studies showed the existence of a good fit (size, shape and function complementarity) for the bis-pyridyl *N*-oxide **4** included in the polar cavity of **1a** (Fig. S11b†).

We monitored the self-assembly of **1a** by  $^1\text{H}$  NMR spectroscopy and used 1,3,5-trimethoxybenzene as internal standard (i.s.). Owing to the reduced solubility of the *meso*-tetramethyl tetra(4-amino)phenyl calix[4]pyrrole **2a** in  $\text{CDCl}_3$ , the equimolar mixture of the components used in the self-assembly of the **4**  $\subset$  **1a** cage complex produced a suspension. This had a negative impact on the yield and reaction time needed to reach the equilibrium for the self-assembly of the tetra-imine cage **1a** compared to its lipophilic version **1b**. Cage **1b** contained *meso*-tetra(4-ethylbutanoate) substituents in the hemisphere deriving from the tetra-amine calix[4]pyrrole (see ESI† for details). For the sake of brevity and similarity of results, we will concentrate in describing those obtained with the tetra-imine cage **1b**. The 2 mM equimolar mixture of tetra-formyl calix[4]pyrrole **3**, *meso*-alkyl substituted tetra-amine **2b** and bis-*N*-oxide **4** in  $\text{CDCl}_3$  produced a solution. The  $^1\text{H}$  NMR spectrum acquired approximately 3 h following the preparation of the solution mixture showed sharp and well-defined proton signals (Fig. 3c).† Notably, none of the proton signals of the calix[4]pyrrole precursors **3** and **2b** were detected. These findings suggested the presence of a predominant and structurally well-defined species in solution.

We detected two signals for the pyrrole NHs ( $\text{NH}^{12\prime}$ , and  $\text{NH}^{18\prime}$ ) resonating as broad singlets at  $\delta = 8.1$  and 8.4 ppm, respectively. The downfield shifts experienced by these signals compared to those in the free calix[4]pyrroles **3** and **2b** were indicative of their involvement in hydrogen bonding interactions with the oxygen atoms of the pyridyl-*N*-oxide ends of template **4**. The aromatic proton signals of template **4** appeared as four upfield shifted ( $\Delta\delta = 3.2$ – $0.4$  ppm) doublets ( $\text{H}^{\text{a}1\prime}$ ,  $\text{H}^{\text{a}2\prime}$ ,  $\text{H}^{\text{b}1\prime}$ , and  $\text{H}^{\text{b}2\prime}$ ). On the one hand, this result indicated that the bound bis-*N*-oxide **4** was no longer  $D_2$  symmetric. On the other

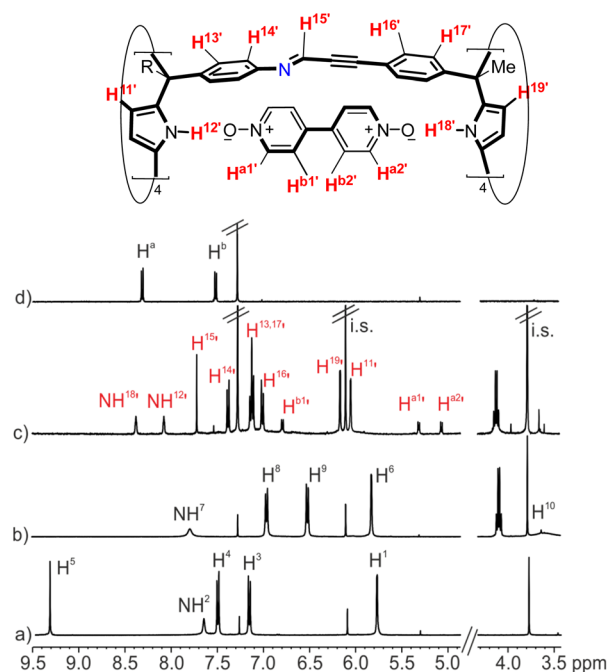


Fig. 3 (Top) Line drawing structure of the **4**  $\subset$  **1b** complex with the corresponding proton assignment. (Bottom) Selected regions of the  $^1\text{H}$  NMR spectra (400 MHz, at 298 K) of  $\text{CDCl}_3$  solutions of (a) tetra-formyl calix[4]pyrrole **3**, (b) tetra-amine tetra-ester calix[4]pyrrole **2b**, (c) tetra-imine cage complex **4**  $\subset$  **1b** and (d) bis-pyridyl *N*-oxide **4**. i.s.: internal standard. See Fig. 2 for proton assignment of **2b** and **3**. Red and primed letters correspond to protons of the **4**  $\subset$  **1b** complex.

hand, it suggested that the pyridyl-*N*-oxide ends of **4** were included in chemically different calix[4]pyrrole aromatic cavities and experienced the strong shielding effect provoked by the four *meso*-phenyl groups. In addition, the aromatic protons of these *meso*-phenyl substituents appeared as two sets of doublets. The  $\beta$ -pyrrole protons moved downfield and appeared as two separate singlets. Finally, we detected a sharp singlet centered at  $\delta = 7.7$  ppm, which combined with the disappearance of the formyl proton of calix[4]pyrrole **3**, hinted for its assignment to an imine proton ( $\text{H}^{15\prime}$ ). Taken together, these results supported the predominant formation of the tetra-imine cage complex **4**  $\subset$  **1b** in solution displaying an averaged  $C_{4v}$  symmetry. Using integral values of selected proton signals of the **4**  $\subset$  **1b** complex and those of the i.s., we determined that the yield for the assembly of the complex was  $\sim 65\%$ . We hypothesized that the remaining calix[4]pyrroles units might be involved in the formation of large polymeric aggregates whose proton signals broadened beyond detection. It is worthy to note that the downfield shifts experienced by the pyrrole NHs in the **4**  $\subset$  **1b** complex were smaller than the ones observed in inclusion complexes of pyridine-*N*-oxide **5** with the modular calix[4]pyrroles **2b** and **3**.<sup>33</sup> This observation suggested that the hydrogen bonds present in the **4**  $\subset$  **1b** cage complex were longer and that **4** was too short to adequately cover the gap between the two calix[4]pyrrole binding sites of **1b**. In short, the ditopic hydrogen-bonding interaction in **4**  $\subset$  **1b** was not optimal.



Cage complex **4**⊂**1b** was fully characterized by a complete set of high-resolution spectra (NMR and Mass Spectrometry, see ESI† for details, Fig. S5–S9 and S39†). A  $^1\text{H}$  DOSY NMR experiment<sup>36</sup> assigned identical diffusion coefficient to the two components of the cage complex,  $D = 3.58 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ , evidencing their involvement in the same species (Fig. S10†).<sup>37</sup> Using as reference the larger diffusion constant determined for the tetra-formyl calix[4]pyrrole **3** ( $D = 5.68 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ ), we assigned a 1.6 fold increase in the hydrodynamic radius of the **4**⊂**1b** complex diffusing as an hypothetically spherical object. Notably, the calculated spherical hydrodynamic radii for **3** and **4**⊂**1b** were in good agreement with those of the spheres including their energy minimized structures (Fig. S11†).

### Solvent-assisted self-assembly of tetra-imine cage **1b**

**9 : 1  $\text{CDCl}_3$  :  $\text{CD}_3\text{CN}$  solvent mixture.** Aiming at investigating the binding properties of the tetra-imine cage **1b**, we decided to explore its self-assembly in the absence of template **4**. In previous works, we described that one molecule of acetonitrile was nicely accommodated in the polar cavity of the cone conformer of  $\alpha,\alpha,\alpha,\alpha$ -aryl-extended calix[4]pyrroles. The nitrogen atom of the bound acetonitrile molecule established four convergent hydrogen-bonds with the pyrrole NHs locking the receptor in the cone conformation.<sup>38</sup> We hypothesized that the preorganization of the modular components, **2b** and **3**, of tetra-imine cage **1b** in cone conformation might facilitate its self-assembly.

Thus, we prepared a 2 mM equimolar solution of the tetra-amine calix[4]pyrrole **2b** and the tetra-formyl counterpart **3** in a 9 : 1  $\text{CDCl}_3$  :  $\text{CD}_3\text{CN}$  solvent mixture. To our delight, after 3 h, the  $^1\text{H}$  NMR spectrum of the solution mainly showed the proton signals diagnostic of the self-assembly of the tetra-imine cage **1b** (Fig. 4a).§ Most likely, two molecules of acetonitrile were included and hydrogen-bonded to the two polar binding sites of the cavity of cage **1b**, resulting in the solvate inclusion complex  $(\text{CD}_3\text{CN})_2\subset\text{1b}$ . Using the integral values of selected proton signals of **1b** and those of the i.s., we determined that the self-assembly took place to an extent close to 65%. Notably, the yield of the self-assembly process of **1b** in a 9 : 1  $\text{CDCl}_3$  :  $\text{CD}_3\text{CN}$  solvent mixture was very similar to the one obtained for the template-assisted cage complex **4**⊂**1b**.

Luckily, single crystals suitable for X-ray diffraction grew from the sequential slow diffusion of dihalogenated benzene derivatives into a 9 : 1  $\text{CDCl}_3$  :  $\text{CD}_3\text{CN}$  solvent mixture containing the tetra-imine cage **1b**.¶ The analysis of the diffraction data revealed the solid-state structure of **1b**. The four imine bonds displayed *E*-configuration. The imine CHs were outwardly directed with respect to internal cavity. They converged in pairs generating two differently sized portals of tetra-imine cage **1b** (Portal1: average  $d_{\text{Cim1}\cdots\text{Cim2}} = 7.2 \text{ \AA}$ ; Portal2: average  $d_{\text{Cim1}\cdots\text{Cim4}} = 4.3 \text{ \AA}$ ) (Fig. 5).

**Chlorinated solvents.** Encouraged by the results obtained in the 9 : 1  $\text{CDCl}_3$  :  $\text{CD}_3\text{CN}$  solvent mixture, we decided to study the self-assembly of cage **1b** in pure chlorinated solvents, such as  $\text{CDCl}_3$  and  $\text{CD}_2\text{Cl}_2$ .

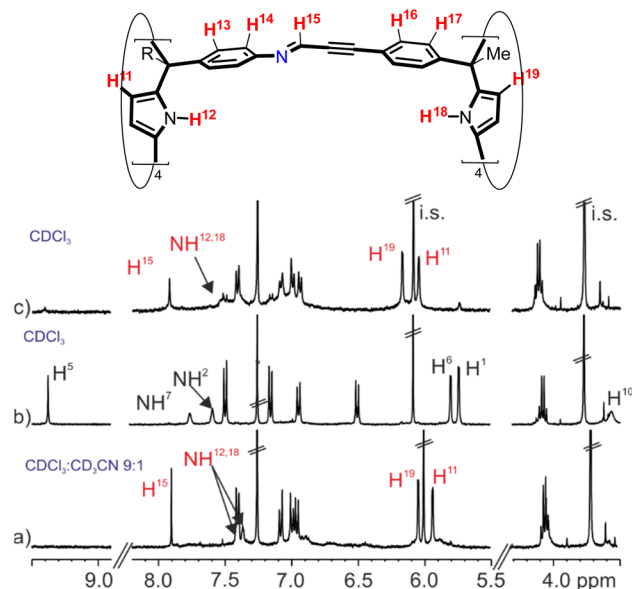


Fig. 4 (Top) Line drawing structure of tetra-imine cage **1b** with the corresponding proton assignment. (Bottom) Selected regions of the  $^1\text{H}$  NMR spectra (400 MHz, at 298 K) of an equimolar solution of tetra-formyl calix[4]pyrrole **3** and tetra-amine tetra-ester calix[4]pyrrole **2b** in (a) 9 : 1  $\text{CDCl}_3$  :  $\text{CD}_3\text{CN}$  solvent mixture after 3 h; (b)  $\text{CDCl}_3$  following its preparation and (c)  $\text{CDCl}_3$  after heating the solution at 308 K for 24 h. i.s.: internal standard. See Fig. 2 for proton assignments of **2b** and **3**. Red letters correspond to protons of the tetra-imine cage **1b**.

The  $^1\text{H}$  NMR spectrum of a 2 mM equimolar mixture of tetra-formyl **3** and tetra-amine **2b** in chlorinated solvents (either  $\text{CDCl}_3$  or  $\text{CD}_2\text{Cl}_2$ ), acquired following its preparation (<15 min), mainly showed the proton signals of the two modular components. The proton signals of tetra-imine cage **1b** showed a reduced intensity (Fig. 4b). Heating the solutions of the chlorinated solvents (48 h, 308 K for  $\text{CDCl}_3$  and 72 h, 303 K  $\text{CD}_2\text{Cl}_2$ ) produced the tetra-imine cage **1b** as the main species in solution. We calculated a yield of ~50% for the self-assembly of **1b** in the chlorinated solvents (Fig. 4c). The diffusion coefficient of **1b** determined in  $\text{CDCl}_3$  (Fig. S23†) was almost coincident with the one measured above for the **4**⊂**1b** cage complex in the same solvent.

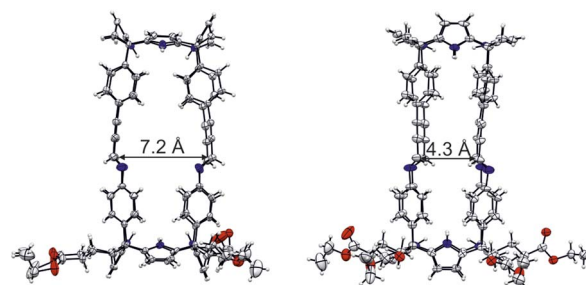


Fig. 5 Side views of the X-ray crystal structure of tetra-imine cage **1b**. The sizes of the two different portals are displayed. The structure is shown in ORTEP view with thermal ellipsoids set at 50% probability. Hydrogen atoms are shown as fixed-size spheres of 0.3 Å radius. Included guest and solvent molecules are omitted for clarity.





It is worthy to note that the  $^1\text{H}$  NMR spectrum of cage **1b** in  $\text{CDCl}_3$  solution showed broader proton signals than in a 9 : 1  $\text{CDCl}_3$  :  $\text{CD}_3\text{CN}$  solvent mixture (Fig. 4c and a, respectively). This observation, together with the reduction in yield of the self-assembly process suggested that in pure chlorinated solvents the tetra-imine cage **1b** was thermodynamically less stable and conformationally more flexible than the  $(\text{CD}_3\text{CN})_2\text{C1b}$  analogue. The large equilibration time, the use of heat, and the observation of intermediate species supported the thermodynamic nature of the tetra-imine cage **1b**.

Molecular modelling studies (MM3) of the tetra-imine cage **1b** showed a nice fit of two chlorinated solvent molecules ( $\text{CHCl}_3$  or  $\text{CH}_2\text{Cl}_2$ ) in its cavity providing sensible packing coefficient (PC)<sup>39</sup> values 43–57% (see Fig. S29 and Table S1 in the ESI†).||

To sum up, in pure chlorinated solvents, the Schiff base condensation reaction producing **1b** proceeds with lower yields (50%). It required longer time and temperature (24–48 h, >300 K) to finish (reach equilibrium) than the template assisted methodology (65%, 3 h r.t.) or the use of the 9 : 1  $\text{CDCl}_3$  :  $\text{CD}_3\text{CN}$  solvent mixture (65%, 3 h r.t.). These results evidenced that the preorganization of the modular components of **1b** in cone conformation was clearly beneficial for its self-assembly.

### Binding studies in chloroform

The self-assembly of tetra-imine cage **1b**, putatively including two solvent molecules ( $\text{CDCl}_3$  or  $\text{CD}_3\text{CN}$ ) in its cavity, opened the possibility to study its binding properties using pyridyl-*N*-oxides **4** and **5** as guests. All thermodynamic and kinetic studies of the inclusion complexes of **1b** were performed using an excess of guest with respect to the cage concentration. We considered that the oligomeric aggregates of **2b** and **3** that might be present in solution, but not visible in the  $^1\text{H}$  NMR spectrum, would also bind the pyridyl-*N*-oxide guests. For this reason, in the following binding studies, we used 2 equiv. of the bis-*N*-oxide **4** and 4 equiv. of mono-*N*-oxide **5** with respect to the initial concentration of the modular components (**3** and **2b**) used for the self-assembly of **1b**.

**Studies of the inclusion of the ditopic bis-pyridyl-*N*-oxide **4** in cage **1b** self-assembled in  $\text{CDCl}_3$  solution: kinetic and thermodynamic characterization of the **4C1b** complex.** We added bis-*N*-oxide **4**, 2 equiv. with respect to the cage's modular components, to a  $\text{CDCl}_3$  solution of the self-assembled **1b** cage ( $[\mathbf{3}] = [\mathbf{2b}] = 2 \text{ mM}$  afforded  $[\mathbf{1b}] \sim 1 \text{ mM}$ , ~50% yield, after heating the solution at 308 K for 24 h). We analyzed the resulting solution using  $^1\text{H}$  NMR spectroscopy immediately following the addition of the *N*-oxide (~5 min). We observed the exclusive presence of the diagnostic proton signals of the **4C1b** cage complex (Fig. S30†). We also detected the aromatic proton signals of the free *N*-oxide **4** with a reduced intensity. Collectively, these results indicated that the kinetics of complex formation was fast (<5 min) and that a binding constant larger than  $10^4 \text{ M}^{-1}$  could be estimated for the complex ( $\Delta G(\mathbf{4C1b}) \sim -5 \text{ kcal mol}^{-1}$ ). Moreover, the chemical exchange process between the free and the bound *N*-oxide **4** was slow not only on the proton chemical shift timescale (separate signals) but also

on the EXSY timescale (lack of cross peaks, mixing time = 0.3 s or 1 s, Fig. S31†). Therefore, the dissociation rate constant of the complex  $k_{\text{off}}(\mathbf{4C1b})$  must be smaller than  $0.01 \text{ s}^{-1}$  ( $k_{\text{exch}} = k' + k_{\text{off}} \sim 10^{-2} \text{ s}^{-1}$ ).<sup>40</sup> This value allowed us to assign a lower limit for the free energy barrier of the complex dissociation as  $\Delta G_{\text{off}}^{\ddagger}(\mathbf{4C1b}) > 20 \text{ kcal mol}^{-1}$ . Considering the estimates of  $K_{\text{a}}(\mathbf{4C1b}) > 10^4 \text{ M}^{-1}$  and  $k_{\text{off}}(\mathbf{4C1b}) < 0.01 \text{ s}^{-1}$ , we deduced a sensible value for  $k_{\text{on}}(\mathbf{4C1b})$  from the product  $K_{\text{a}}(\mathbf{4C1b}) \times k_{\text{off}}(\mathbf{4C1b}) = 10^2 \text{ M}^{-1} \text{ s}^{-1}$ . The calculation of  $k_{\text{on}}(\mathbf{4C1b})$  indicated that the formation of the **4C1b** complex was not diffusion controlled. It also served to assign a lower estimate to the energy barrier of its formation:  $\Delta G_{\text{on}}^{\ddagger}(\mathbf{4C1b}) \sim 15 \text{ kcal mol}^{-1}$ . Because the dissociation of the **4C1b** complex required the rupture of stronger intermolecular interactions than its formation, it was not surprising to involve a higher energy barrier ( $\Delta G_{\text{off}}^{\ddagger}(\mathbf{4C1b}) > 20 \text{ kcal mol}^{-1}$ ).

We concluded that the weakly bound  $\text{CDCl}_3$  molecules occupying the interior of cage **1b** were readily replaced (low energy barrier) by the incoming *N*-oxide forming the thermodynamically and kinetically highly stable **4C1b** cage complex.

**Studies of the inclusion of pyridine-*N*-oxide **5** in cage **1b** self-assembled in  $\text{CDCl}_3$ .** We performed analogous binding experiments using pyridine *N*-oxide **5**. The addition of excess of **5** (3 mM, > 3 equiv.) to an approximately 1 mM  $\text{CDCl}_3$  solution of **1b** produced the immediate appearance of a new and exclusive set of proton signals for **1b**. We assigned the new set of signals to the protons of **1b** in the 2 : 1 cage complex **5<sub>2</sub>C1b** (Fig. S32†). One pyridine-*N*-oxide **5** was included in each one of the two hemispheres of cage **1b**. Because the two hemispheres are chemically non-equivalent, the aromatic protons of the pyridine-*N*-oxides resonated as six diastereotopic and separated upfield shifted signals ( $\text{H}^{\text{c1'}}$ ,  $\text{H}^{\text{c2'}}$ ,  $\text{H}^{\text{d1'}}$ ,  $\text{H}^{\text{d2'}}$ ,  $\text{H}^{\text{e1'}}$  and  $\text{H}^{\text{e2'}}$ ).

The downfield shift experienced by the pyrrole NHs in the **5<sub>2</sub>C1b** complex (*i.e.*  $\Delta\delta = 0.86$ ) were larger than those observed for the **4C1b** counterpart. This result evidenced that the hydrogen bonds in the former cage complex were shorter than in the latter. DFT theoretical calculations, at the BP86 <sup>41</sup>D3-def2-TZVP<sup>42</sup> level of theory using Turbomole v7.0,<sup>43,44</sup> of the two cage complexes also supported this conclusion: **4C1b** (average  $d_{(\text{H}^-\cdots\text{O}^-\text{N})} = 3.29 \pm 0.07 \text{ \AA}$ ) and **5<sub>2</sub>C1b** (average  $d_{(\text{H}^-\cdots\text{O}^-\text{N})} = 2.88 \pm 0.02 \text{ \AA}$ ). For comparison, the X-ray crystal structures of aryl-extended calix[4]pyrrole complexes with pyridyl-*N*-oxide guests previously reported in the literature revealed an average hydrogen bonding distances ( $d_{(\text{H}^-\cdots\text{O}^-\text{N})}$ ) on the range of 3.0 to 2.8 Å.<sup>45</sup> Taken together, these results reinforced our hypothesis that the length of the bis-*N*-oxide **4** was too short to establish eight simultaneous optimal hydrogen bonding interactions with the two hemispheres of cage **1b** (*vide supra*).

**General observations of the binding experiments performed in  $\text{CDCl}_3$  solution.** Surprisingly to us, and based on the integral values of selected proton signals in the  $^1\text{H}$  NMR spectra, the addition of the *N*-oxides (**4** and **5**) to the pre-assembled cage **1b** in  $\text{CDCl}_3$  solution did not increase significantly its concentration. In short, the amplification effect exerted by the *N*-oxides in the dynamic combinatorial library resulting by mixing **2b** and **3** was almost negligible.



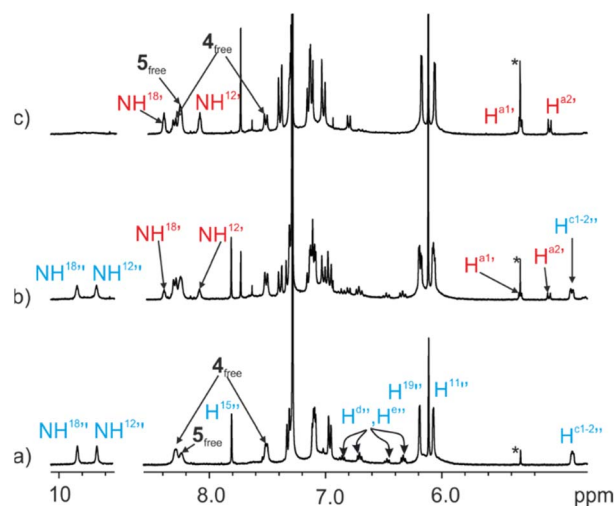
Considering the fast kinetics observed for the inclusion of **4** and **5** in the cavity of cage **1b** in this solvent, we envisaged that it did not require the partial cleavage of imine bonds. We hypothesized that the guest uptake mechanisms involved a gating process through the calix[4]pyrrole *meso*-phenyl substituents. Because we did not observe significant differences in the uptake kinetics of the two guests, we propose a similar uptake mechanism for both of them. That is, the incoming guests are squeezed on passing through the enlarged portals of cage **1b** resulting from a “French doors” mechanism of the *meso*-phenyl substituents.<sup>46,47</sup> The inclusion of **4** in **1b** can be considered as an elementary process. In contrast, the formation of the **5**<sub>2</sub>⊂**1b** complex is stepwise and requires separate “french doors” mechanisms for the two hemispheres.

We estimated in the previous section that the energy barrier for the uptake of bis-*N*-oxide **4** by cage **1b** should be of the order of 15 kcal mol<sup>−1</sup>. Most likely, **5** is included in the cavity of **1b** with a lower or similar energy barrier. The energy barrier corresponded to: (a) the concerted rotation of at least four *meso*-substituent in one of the capsule's hemisphere; (b) the breaking of the interactions with the released CDCl<sub>3</sub> molecule per s; (c) the squeezing of the incoming planar *N*-oxides and the expelled CDCl<sub>3</sub> molecule through opposed and wider portals of **1b** available in the transition state of the guest uptake.

**Guest exchange experiments in CDCl<sub>3</sub> solution.** We investigated the kinetics of the exchange of the two pyridine-*N*-oxide molecules bound in the **5**<sub>2</sub>⊂**1b** cage complex by one molecule of the ditopic bis-*N*-oxide **4**. To this end, we added approximately 2 equiv. of the 4',4'-bipyridyl-*N,N'*-dioxide **4** to a 1 mM CDCl<sub>3</sub> solution of the self-assembled **5**<sub>2</sub>⊂**1b** cage complex containing excess of **5**. The resulting mixture produced a solution that was monitored at different time intervals using <sup>1</sup>H NMR spectroscopy (Fig. S35†).

The <sup>1</sup>H NMR spectrum acquired immediately after the addition of the ditopic guest **4** showed exclusively the proton signals corresponding to the **5**<sub>2</sub>⊂**1b** cage complex and those of the free bis-pyridyl *N*-oxides **4** and excess of **5** (Fig. 6a). After 12 hours, the signals of the protons of the **4**⊂**1b** complex became visible. With time, they grew at the expenses of those of the **5**<sub>2</sub>⊂**1b** counterpart (Fig. 6b). It took more than 1 month for the ditopic *N*-oxide **4** to completely replace the two copies of pyridine-*N*-oxide **5** initially included in the cavity of **1b** (Fig. 6c).

We fit the experimental kinetic data of the guests' exchange experiment (Fig. S36†) to the theoretical kinetic model of an irreversible bimolecular reaction (A + B → C). Using the parameter estimation module of the COPASI Software Version 4.25<sup>48</sup> we obtained a good fit returning a rate constant value of  $k_{\text{exch}} = 8.7 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$ . This value was translated into a free energy barrier of *ca.*  $\Delta G^\ddagger = 21.6 \text{ kcal mol}^{-1}$  for the guests' exchange reaction (298 K). Notably, the free energy barrier calculated for the exchange reaction  $\mathbf{4} + \mathbf{5}_2 \subset \mathbf{1b} \rightleftharpoons 2 \mathbf{5} + \mathbf{4} \subset \mathbf{1b}$  using kinetic <sup>1</sup>H NMR experiments was in line with our lower estimate for the exchange barrier of **4** (>20 kcal mol<sup>−1</sup>), determined for the reaction  $\mathbf{4} + \mathbf{4} \subset \mathbf{1b} \rightleftharpoons \mathbf{4} + \mathbf{4} \subset \mathbf{1b}$ , owing to the lack of exchange cross-peaks in the corresponding EXSY experiment (see section describing the results of the inclusion of **4** in **1b**).



**Fig. 6** Selected regions of the <sup>1</sup>H NMR spectra (400 MHz, at 298 K) of a CDCl<sub>3</sub> solution of imine cage complex **5**<sub>2</sub>⊂**1b** (1 mM) in the presence of excess of bis-*N*-oxide **4**: (a) immediately after the addition; (b) 4 days after the addition; (c) 1 month after the addition. i.s.: internal standard. See Fig. 2 for proton assignment. Primed letters indicate the proton signals of the **4**⊂**1b** (red) and **5**<sub>2</sub>⊂**1b** (blue) complexes. Note that the polymeric aggregates of **2b** and **3** present in solution also bind the *N*-oxides based on the integrals of the proton signals detected for the free species. However, the proton signals of the aggregates are not detected in the <sup>1</sup>H NMR spectra due to extensive broadening.

We reasoned that both exchange processes should occur *via* structurally related transition states involving the breaking of a similar number and type of intermolecular interactions. As could be expected, the guests' exchange experiment starting from the **4**⊂**1b** complex and adding guest **5** in excess did not produce, after more than a month, noticeable changes in the <sup>1</sup>H NMR spectrum of the mixture. The results above assigned a larger thermodynamic stability to the **4**⊂**1b** complex ( $\Delta\Delta G > 3 \text{ kcal mol}^{-1}$ ) compared to the three particles **5**<sub>2</sub>⊂**1b** counterpart. It also supported the use of the theoretical kinetic model for an irreversible bimolecular reaction in the fit of the guests' exchange kinetic data.

### Binding studies in 9 : 1 CDCl<sub>3</sub> : CD<sub>3</sub>CN solvent mixture

Having kinetically and thermodynamically characterized the inclusion complexes of cage **1b** with the pyridine-*N*-oxides derivatives **4** and **5** in CDCl<sub>3</sub> solution, we became interested in their characterization in 9 : 1 CDCl<sub>3</sub> : CD<sub>3</sub>CN solvent mixture.

**Studies of the inclusion of the ditopic pyridyl-*N*-oxide **4** starting from the (CD<sub>3</sub>CN)<sub>2</sub>⊂**1b** cage complex.** The addition of an excess of the ditopic 4',4'-bipyridyl *N,N'*-dioxide **4** to a 1 mM solution of the self-assembled cage **1b** in 9 : 1 CDCl<sub>3</sub> : CD<sub>3</sub>CN solvent mixture produced, after 10 min, a <sup>1</sup>H NMR spectrum displaying exclusively the proton signals of the starting (CH<sub>3</sub>CN)<sub>2</sub>⊂**1b** cage complex and the free bis-pyridyl *N*-oxide **4** (Fig. 7b). The solution mixture was left standing at r.t. for 24 h and reanalyzed using <sup>1</sup>H NMR spectroscopy. After the elapsed time, we observed the emergence of a new set of proton signals corresponding to the **4**⊂**1b** complex. Notably, (CH<sub>3</sub>CN)<sub>2</sub>⊂**1b**







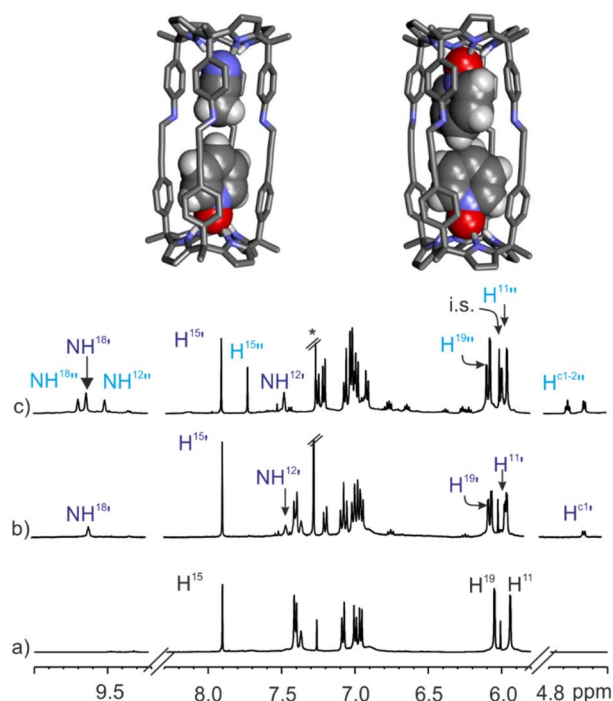


Fig. 8 (Top) Energy minimized structures (BP86-D3-def2-TZVP DFT) of  $(\text{CD}_3\text{CN}\cdot 5)\subset 1\text{b}$  (main isomer observed in solution) and  $5_2\subset 1\text{b}$  complexes. (Bottom) Selected regions of the  $^1\text{H}$  NMR spectra (500 MHz, at 298 K) of 1 mM solution of imine cage **1b** in  $\text{CDCl}_3 : \text{CD}_3\text{CN}$  9 : 1 mixture before (a), after the addition of pyridyl *N*-oxide **5** (0.3 mM) (b) and an excess of guest (2 mM) (c). i.s.: internal standard. See Fig. 2 for proton assignment. Dark blue primed letters indicate the signals of the major isomer of  $(\text{CD}_3\text{CN}\cdot 5)\subset 1\text{b}$  1 : 1 complex and light blue doubled primed letters those of the 2 : 1 complex  $5_2\subset 1\text{b}$ .

in  $\text{CDCl}_3$  the heterocomplex  $(\text{CDCl}_3\cdot 5)\subset 1\text{b}$  was energetically less favored than the homo counterpart  $5_2\subset 1\text{b}$  despite the steric clashes between the included *N*-oxides.

The careful analysis of the  $^1\text{H}$  NMR spectrum of the  $(\text{CD}_3\text{CN}\cdot 5)\subset 1\text{b}$  cage complex formed in the 9 : 1  $\text{CDCl}_3 : \text{CD}_3\text{CN}$  solvent mixture allowed us to identify two separate signals for the pyrrole NHs resonating at  $\delta = 9.6$  and 7.4 ppm (Fig. 8b). This observation was indicative of the formation of a single isomer, in which the pyridine-*N*-oxide **5** must be preferentially located in one of the two hemispheres of **1b**.<sup>†</sup> A 2D ROESY experiment located the included *N*-oxide **5** in the hemisphere having the *meso-p*-phenyl alkynyl substituents. The co-inclusion of one molecule of acetonitrile in the opposite hemisphere produced a packing coefficient of 53% for the heterocomplex  $(\text{CD}_3\text{CN}\cdot 5)\subset 1\text{b}$  in comparison to the 57% calculated for the  $5_2\subset 1\text{b}$  (Fig. 8 top).

## Conclusions

In summary, we described the self-assembly of tetra-imine shape-persistent cages **1** based on two different “four wall” tetra- $\alpha$  aryl-extended calix[4]pyrrole scaffolds. We showed that the presence of a bis-*N*-oxide template is not mandatory for the emergence of the cages. The tetra-imine cages **1** self-assembled

in chlorinated solvents and in a 9 : 1  $\text{CDCl}_3 : \text{CD}_3\text{CN}$  mixture of solvents.

We demonstrated that the use of a templating guest produced a moderate increase in the yield of the self-assembly process (50% to 65%). Interestingly, the use of bound acetonitrile molecules pre-organizing the calix[4]pyrrole modular components of **1** in cone conformation had a similar effect (~65%).

The X-ray crystal structure of the tetra-imine cage **1b** revealed that the imine bonds were exclusively present as *E*-isomers. The imine hydrogen atoms converged in pairs defining two different sized-portals of **1b**.

The tetra-imine cages **1** formed kinetically and thermodynamically stable complexes with the bis-*N*-oxide **4** and pyridine-*N*-oxide **5**. On the one hand, bis-*N*-oxide **4** produced exclusively 1 : 1 complexes. On the other hand, the stoichiometry of the complexes of **1** with the mono-*N*-oxide **5** was shown to be solvent dependent.

The kinetics of the inclusion processes of the guests in **1** were also solvent dependent. In chlorinated solvents, the inclusion of both guests (**4** and **5**) was quantitative and required minutes. In contrast, in the 9 : 1  $\text{CDCl}_3 : \text{CD}_3\text{CN}$  solvent mixture and working under identical stoichiometric control, the **4**⊂**1b** complex was exclusively formed after several weeks, while **5** rendered an equilibrium mixture of 1 : 1 and 2 : 1 complexes after several hours.

We ascribed the different kinetics (*i.e.* transition state energies) observed for the formation of the inclusion complexes to the nature of the solvent molecules ( $\text{CDCl}_3$  or  $\text{CD}_3\text{CN}$ ) that must be displaced from the solvated cages' interior, as well as, to the dissimilar sizes of the incoming guests.

Notably, in  $\text{CDCl}_3$  solution, the displacement reaction of the pyridine-*N*-oxide molecules in the  $5_2\subset 1$  complex by the bis-*N*-oxide **4** was also quantitative but required more than a month. The calculated energy barriers ( $\Delta G^\ddagger$ ) for the above displacement reaction and that of the formation of the inclusion complex **4**⊂**1b** from the acetonitrile solvate  $(\text{CD}_3\text{CN})_2\subset 1\text{b}$ , assembled in a 9 : 1  $\text{CDCl}_3 : \text{CD}_3\text{CN}$  solvent mixture, almost coincided.

Owing to the different polarities and sizes of the displaced guests (**5** or  $\text{CD}_3\text{CN}$ ), we surmised that the  $5_2\subset 1$  complex must be energetically disfavored due to steric clashes between the included guest molecules. Indeed, the study of the inclusion process of **5** in the tetra-imine **1b**, performed in the 9 : 1  $\text{CDCl}_3 : \text{CD}_3\text{CN}$  solvent mixture, supported our hypothesis. That is, the quantitative formation of the  $5_2\subset 1$  complex required the use of **5** in a large stoichiometric excess (>4 equiv.).

## Conflicts of interest

There are no conflicts to declare.

## Data availability

All of the related experimental data are provided in the ESI.<sup>†</sup>





## Author contributions

Conceptualization, P. B.; methodology, C. M.; formal analysis, P. B.; G. A.; writing—original draft preparation, P. B., C. M. and G. A.; writing—review and editing, P. B., G. A. and C. M.; supervision, P. B.; All authors have read and agreed to the published version of the manuscript.

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## Notes and references

† Analogous reaction conditions using tetra-methyl tetra-amino calix[4]pyrrole **2a** initially produced broad and ill-defined signals. Remarkably, a white solid precipitated out from this mixture. The suspension was left overnight at room temperature. After 12 h the solid was completely dissolved to yield a yellowish clear solution. The <sup>1</sup>H NMR spectrum of this solution showed sharp and well-defined signals that were assigned to the formation of the tetra-imine cage complex **4** **1a**.

§ The reaction performed at more diluted conditions (1 mM) produced similar yields. However, larger reaction times were needed to observe the complete consumption of the starting material.

¶ Deposition number CCDC 2208504 contains the supplementary crystallographic data for this paper.

|| 1D GOESY experiments,<sup>49</sup> performed at different temperatures (323–238 K) using a 95 : 5 CHCl<sub>3</sub> : CDCl<sub>3</sub> solution of cage **1b** and by selectively exciting the CHCl<sub>3</sub> signal did not help in revealing the proton signals of the putative CHCl<sub>3</sub> included in the cage. Most likely, the chemical exchange between the free and bound CHCl<sub>3</sub> was fast on the chemical shift timescale.<sup>50</sup>

\*\* At the BP86-D3-def2-TZVP DFT level of theory using Turbomole v7.0, we computed an energy difference of 1.5 kcal mol<sup>-1</sup> for the two isomers of the (CDCl<sub>3</sub> · 5) **1b** in favor to the one experimentally observed.

- 1 F. Hof, S. L. Craig, C. Nuckolls and J. Rebek, *Angew. Chem., Int. Ed.*, 2002, **41**, 1488–1508.
- 2 *Container Molecules and Their Guests*, ed. D. J. Cram and J. M. Cram, The Royal Society of Chemistry, Cambridge, England, 1997.
- 3 L. Tapia, I. Alfonso and J. Solà, *Org. Biomol. Chem.*, 2021, **19**, 9527–9540.
- 4 G. Montà-González, F. Sancenón, R. Martínez-Mañez and V. Martí-Centelles, *Chem. Rev.*, 2022, **122**, 13636–13708.
- 5 A. Platzek, S. Juber, C. Yurtseven, S. Hasegawa, L. Schneider, C. Drechsler, K. E. Ebbert, R. Rudolf, Q.-Q. Yan, J. J. Holstein, L. V. Schäfer and G. H. Clever, *Angew. Chem., Int. Ed.*, 2022, e202209305.
- 6 A. Galan and P. Ballester, *Chem. Soc. Rev.*, 2016, **45**, 1720–1737.
- 7 R. Saha, B. Mondal and P. S. Mukherjee, *Chem. Rev.*, 2022, **122**, 12244–12307.
- 8 G. Olivo, G. Capocasa, D. Del Giudice, O. Lanzalunga and S. Di Stefano, *Chem. Soc. Rev.*, 2021, **50**, 7681–7724.
- 9 D. Ajami and J. Rebek, *Acc. Chem. Res.*, 2013, **46**, 990–999.
- 10 S. La Cognata, R. Mobili, C. Milanese, M. Boiocchi, M. Gaboardi, D. Armentano, J. C. Jansen, M. Monteleone, A. R. Antonangelo, M. Carta and V. Amendola, *Chem.–Eur. J.*, 2022, **28**, e202201631.
- 11 W. Liu and J. F. Stoddart, *Chem.*, 2021, **7**, 919–947.
- 12 S. Pullen, J. Tessarolo and G. H. Clever, *Chem. Sci.*, 2021, **12**, 7269–7293.
- 13 D. J. Cram, S. Karbach, Y. H. Kim, L. Baczynskyj and G. W. Kallemeyn, *J. Am. Chem. Soc.*, 1985, **107**, 2575–2576.
- 14 J. Gabard and A. Collet, *J. Chem. Soc., Chem. Commun.*, 1981, 1137–1139.
- 15 S. J. Rowan, S. J. Cantrill, G. R. L. Cousins, J. K. M. Sanders and J. F. Stoddart, *Angew. Chem., Int. Ed.*, 2002, **41**, 898–952.
- 16 J. Lindsey, *New J. Chem.*, 1991, **15**, 153–180.
- 17 J.-M. Lehn, *Chem. Soc. Rev.*, 2007, **36**, 151–160.
- 18 M. E. Belowich and J. F. Stoddart, *Chem. Soc. Rev.*, 2012, **41**, 2003–2024.
- 19 G. Zhang and M. Mastalerz, *Chem. Soc. Rev.*, 2014, **43**, 1934–1947.
- 20 M. L. C. Quan and D. J. Cram, *J. Am. Chem. Soc.*, 1991, **113**, 2754–2755.
- 21 L.-P. Yang, X. Wang, H. Yao and W. Jiang, *Acc. Chem. Res.*, 2020, **53**, 198–208.
- 22 R. A. Tromans, T. S. Carter, L. Chabanne, M. P. Crump, H. Li, J. V. Matlock, M. G. Orchard and A. P. Davis, *Nat. Chem.*, 2019, **11**, 52–56.
- 23 For recent examples of endohedrally functionalized molecular containers see ref. 5, 12 and 21 and references therein.
- 24 F. Wang, C. Bucher, Q. He, A. Jana and J. L. Sessler, *Acc. Chem. Res.*, 2022, **55**, 1646–1658.
- 25 T. Guchhait, P. Pradhan, L. Panda and M. S. K. Rao, *ChemistrySelect*, 2022, **7**, e202202671.
- 26 O. D. Fox, T. D. Rolls, M. G. B. Drew and P. D. Beer, *Chem. Commun.*, 2001, 1632–1633.
- 27 O. Francesconi, A. Ienco, G. Moneti, C. Nativi and S. Roelens, *Angew. Chem., Int. Ed.*, 2006, **45**, 6693–6696.
- 28 H. J. Han, J. H. Oh, J. L. Sessler and S. K. Kim, *Chem. Commun.*, 2019, **55**, 10876–10879.
- 29 F. Wang, E. Sikma, Z. Duan, C. Lei, Z. Zhang, S. M. Humphrey and J. L. Sessler, *J. Porphyrins Phthalocyanines*, 2020, **24**, 424–431.
- 30 F. Wang, E. Sikma, Z. Duan, T. Sarma, C. Lei, Z. Zhang, S. M. Humphrey and J. L. Sessler, *Chem. Commun.*, 2019, **55**, 6185–6188.
- 31 P. Ferreira, G. Moncelis, G. Aragay and P. Ballester, *Chem.–Eur. J.*, 2021, **27**, 12675–12685.
- 32 J. R. Romero, G. Aragay and P. Ballester, *Chem. Sci.*, 2017, **8**, 491–498.
- 33 A. Galán, E. C. Escudero-Adán and P. Ballester, *Chem. Sci.*, 2017, **8**, 7746–7750.
- 34 P. Ballester and G. Gil-Ramírez, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 10455–10459.



- 35 L. Escobar, F. A. Arroyave and P. Ballester, *Eur. J. Org. Chem.*, 2018, **2018**, 1097–1106.
- 36 L. Avram and Y. Cohen, *Chem. Soc. Rev.*, 2015, **44**, 586–602.
- 37 D. Sinnaeve, *Concepts Magn. Reson., Part A*, 2012, **40A**, 39–65.
- 38 D. Hernández-Alonso, S. Zankowski, L. Adriaenssens and P. Ballester, *Org. Biomol. Chem.*, 2015, **13**, 1022–1029.
- 39 S. Mecozzi, J. Rebek and Julius, *Chem.–Eur. J.*, 1998, **4**, 1016–1022.
- 40 A. Pastor and E. Martínez-Viviente, *Coord. Chem. Rev.*, 2008, **252**, 2314–2345.
- 41 J. P. Perdew, *Phys. Rev. B: Condens. Matter Mater. Phys.*, 1986, **33**, 8822–8824.
- 42 F. Weigend and R. Ahlrichs, *Phys. Chem. Chem. Phys.*, 2005, **7**, 3297–3305.
- 43 TURBOMOLE V7.0 2015, a development of University of Karlsruhe and Forschungszentrum Karlsruhe GmbH, 1989–2007, TURBOMOLE GmbH, since 2007; available from <http://www.turbomole.com>.
- 44 R. Ahlrichs, M. Bär, M. Häser, H. Horn and C. Kölmel, *Chem. Phys. Lett.*, 1989, **162**, 165–169.
- 45 C. R. Groom, I. J. Bruno, M. P. Lightfoot and S. C. Ward, *Acta Crystallogr., Sect. B*, 2016, **72**, 171–179.
- 46 L. Escobar, E. C. Escudero-Adán and P. Ballester, *Angew. Chem., Int. Ed.*, 2019, **58**, 16105–16109.
- 47 L. Escobar, D. Villarón, E. C. Escudero-Adán and P. Ballester, *Chem. Commun.*, 2019, **55**, 604–607.
- 48 S. Hoops, S. Sahle, R. Gauges, C. Lee, J. Pahle, N. Simus, M. Singhal, L. Xu, P. Mendes and U. Kummer, *Bioinformatics*, 2006, **22**, 3067–3074.
- 49 J. Stonehouse, P. Adell, J. Keeler and A. J. Shaka, *J. Am. Chem. Soc.*, 1994, **116**, 6037–6038.
- 50 M. Chas and P. Ballester, *Chem. Sci.*, 2012, **3**, 186–191.

