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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- α aryl-extended calix[4]pyrrole scaffolds in chlorinated solvents and in a 9 : 1 CDCl₃ : CD₃CN 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 ¹H 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**₂⊂**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 CDCl₃ : CD₃CN 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**₂⊂**1** and (CD₃CN)₂⊂**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.^{1,2} 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,^{3–5} stabilization of reactive species⁶ and mediation of chemical reactivity,^{7,8} among others.^{9–12}

The first examples of molecular cages date back to the middle 80s'. In pioneering works, Cram¹³ and Collet¹⁴ 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.^{15,16} 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.^{17,18} 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.¹⁹ 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.²⁰ The derived octa-imine cage was able to include a series of guests forming kinetically stable hemi-carceplexes owing to constrictive binding.² The ground state of the cage complexes had twisted portals that were too small to

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allow the free passage of the guests. The guest exchange was associated with the untwisting of the structure of the host, the loss of many close contacts and the maximization of the cross section of at least one portal. Thus, the calculated free energy barriers for the guest exchange were significantly larger ($>20 \text{ kcal mol}^{-1}$) than the absolute value of the free binding energy ($>-3 \text{ kcal mol}^{-1}$).

The binding affinity and selectivity displayed by synthetic molecular cages tends to be outperformed by those of biological receptors.^{21,22} For example, the lack of converging polar groups in the inner cavities of most synthetic molecular containers restricts their binding selectivity to non-polar guests being size and shape complementary. On the other hand, the extensive use of aromatic panels to shape the inner cavity of the synthetic molecular containers makes the incorporation of polar converging groups synthetically challenging. This serves as an explanation for the reduced number of endohedrally functionalized synthetic molecular cages described in literature. Nevertheless, in recent times, several approaches have been disclosed in trying to overcome this limitation.²³ For example, pyrrole units were incorporated in imine-based dynamic covalent cages to endow their cavities with polar hydrogen bond donor groups (Fig. 1).^{24,25}

Beer and co-workers described the synthesis of bis-(tripyrrolyl) cryptand-like hexa-imine cages *via* a [2 + 3] Schiff base condensation of triformyl-alkyl-tripyrrolyls and α,ω -alkyl diamines (Fig. 1a).²⁶ The X-ray structures of the hexa-imine cryptands revealed the inclusion of one molecule of the reactant diamine establishing multiple hydrogen bonding interactions with the converging polar functions inwardly directed with respect to the container's cavity. The authors suggested that the formation of the cages was templated by the diamines.

In the same vein, Roelens and co-workers, reported the quantitative self-assembly of a tris-pyrrolic hexa-imine macrobicyclic cage *via* [2 + 3] condensation of a 1,3,5-tris(aminomethyl)benzene derivative and pyrrole-2,5-dicarboxyaldehyde.²⁷ A one pot reduction of the hexa-imine yielded the corresponding hexa-amine cage that selectively bound β -D-monosaccharides of the gluco series.

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

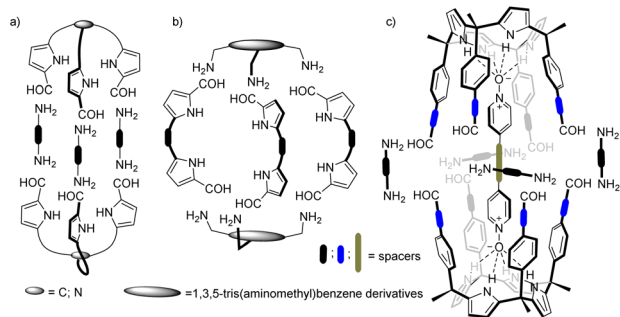


Fig. 1 Schematic representation of pyrrole-based cages formed by Schiff base condensation of polyamines and poly-formyl building blocks.

1,3,5-(tris-aminomethyl)benzene derivatives and diformyl-dipyrrolyl linkers having pyridine and naphthyridine spacers (Fig. 1b). The cage systems acted as shape-persistent POCs for the selective adsorption of CO_2 .^{28–30}

Our group introduced the use of $\alpha,\alpha,\alpha,\alpha$ -aryl-extended calix[4]pyrrole scaffolds for the construction of hydrogen-bonded³¹ and mechanically-bonded³² molecular capsules with polar interiors. We also described the template-directed self-assembly of octa-imine cages having polar interiors *via* the [2 + 4] Schiff base condensation reaction of a tetra-formyl aryl-extended calix[4]pyrrole and 1,2-diamines (Fig. 1c).³³ We used 4-(4-pyridinylethynyl)pyridine-*N,N'*-dioxide as ditopic template. The terminal pyridyl-*N*-oxides knobs of the template bound two $\alpha,\alpha,\alpha,\alpha$ -aryl-extended calix[4]pyrrole tetra-formyl units in cone conformation placing them in close proximity. The orientation of the formyl groups was also suitable for the stitching of the dimeric cage through imine bonds using 1,2-diamine linkers. Unfortunately, the template became tightly bound (locked) in the cage interior rendering the characterization of its binding properties inaccessible.

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_3 : CD_3CN solvent mixture and pure CDCl_3 or CD_2Cl_2 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** \subset **1** inclusion complex was thermodynamically more stable than the **5** \subset **1** counterpart. We investigated the kinetics for the formation of the inclusion complexes and the guests' exchange using ^1H NMR spectroscopy. In the 9 : 1 CDCl_3 : CD_3CN 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_3 solution were fast (min). To our surprise, the energy barrier of the guests' exchange in pure

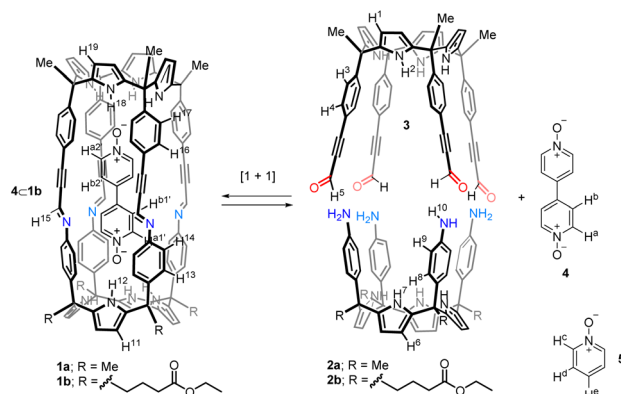


Fig. 2 Equilibrium of the [1 + 1] Schiff base condensation reaction between the “four wall” tetra-amine calix[4]pyrroles **2** and tetra-formyl counterpart **3** assisted by 4,4'-bispyridyl *N,N'*-dioxide **4** as template. Line drawing structure of pyridine *N*-oxide **5** is also shown.



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 CDCl_3 : CD_3CN 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.^{34,35} Tetra-formyl calix[4]pyrrole **3** (Fig. 2) was also prepared unevenly using literature procedures.³³

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 CDCl_3 solution. Based on our previous results on the assembly of octa-imine dynamic covalent cages,³³ 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 ^1H 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 CDCl_3 , the equimolar mixture of the components used in the self-assembly of the $4 \subset 1\text{a}$ 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 CDCl_3 produced a solution. The ^1H 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 (NH^{12} , and NH^{18}) 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 ($\text{H}^{\text{a}1}$, $\text{H}^{\text{a}2}$, $\text{H}^{\text{b}1}$, and $\text{H}^{\text{b}2}$). On the one hand, this result indicated that the bound bis-*N*-oxide **4** was no longer D_2 symmetric. On the other

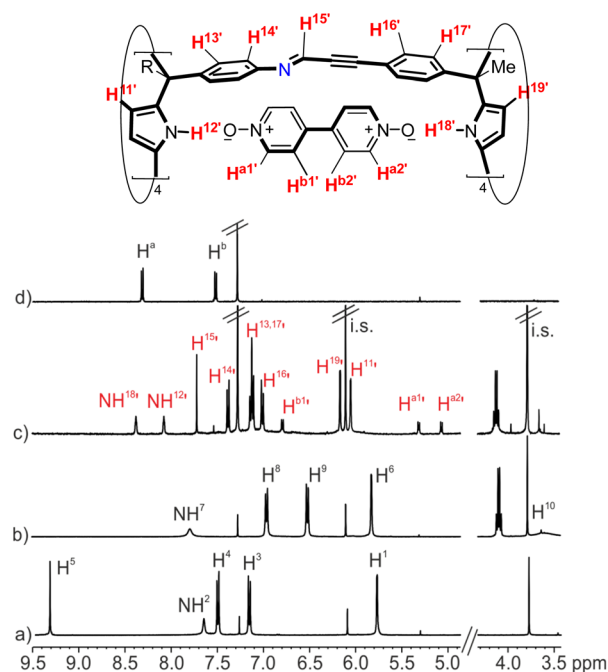


Fig. 3 (Top) Line drawing structure of the $4 \subset 1\text{b}$ complex with the corresponding proton assignment. (Bottom) Selected regions of the ^1H NMR spectra (400 MHz, at 298 K) of CDCl_3 solutions of (a) tetra-formyl calix[4]pyrrole **3**, (b) tetra-amine tetra-ester calix[4]pyrrole **2b**, (c) tetra-imine cage complex $4 \subset 1\text{b}$ 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 $4 \subset 1\text{b}$ 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 β -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 (H^{15}). Taken together, these results supported the predominant formation of the tetra-imine cage complex $4 \subset 1\text{b}$ in solution displaying an averaged C_{4v} symmetry. Using integral values of selected proton signals of the $4 \subset 1\text{b}$ 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 $4 \subset 1\text{b}$ complex were smaller than the ones observed in inclusion complexes of pyridine-*N*-oxide **5** with the modular calix[4]pyrroles **2b** and **3**.³³ This observation suggested that the hydrogen bonds present in the $4 \subset 1\text{b}$ 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 $4 \subset 1\text{b}$ 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 ^1H DOSY NMR experiment³⁶ 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†).³⁷ 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 CDCl₃ : CD₃CN 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.³⁸ 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 CDCl₃ : CD₃CN solvent mixture. To our delight, after 3 h, the ^1H 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 (CD₃CN)₂⊂**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 CDCl₃ : CD₃CN 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 CDCl₃ : CD₃CN 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 CDCl₃ : CD₃CN solvent mixture, we decided to study the self-assembly of cage **1b** in pure chlorinated solvents, such as CDCl₃ and CD₂Cl₂.

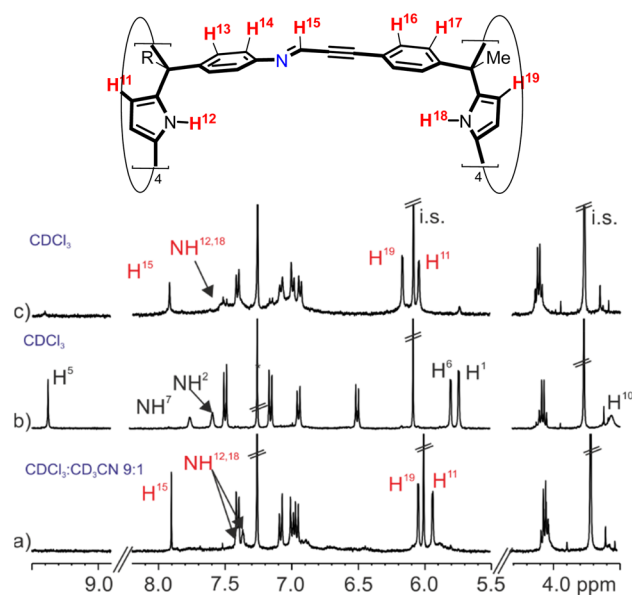


Fig. 4 (Top) Line drawing structure of tetra-imine cage **1b** with the corresponding proton assignment. (Bottom) Selected regions of the ^1H NMR spectra (400 MHz, at 298 K) of an equimolar solution of tetra-formyl calix[4]pyrrole **3** and tetra-amine tetra-ester calix[4]pyrrole **2b** in (a) 9 : 1 CDCl₃ : CD₃CN solvent mixture after 3 h; (b) CDCl₃ following its preparation and (c) CDCl₃ 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 ^1H NMR spectrum of a 2 mM equimolar mixture of tetra-formyl **3** and tetra-amine **2b** in chlorinated solvents (either CDCl₃ or CD₂Cl₂), 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 CDCl₃ and 72 h, 303 K CD₂Cl₂) 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 CDCl₃ (Fig. S23†) was almost coincident with the one measured above for the **4**⊂**1b** cage complex in the same solvent.

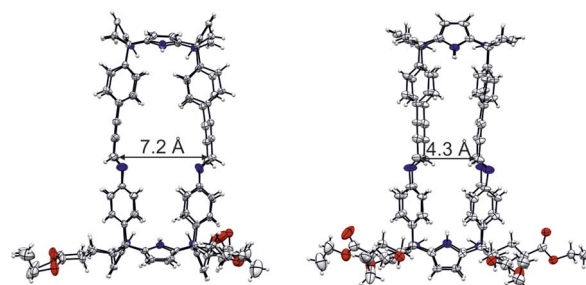


Fig. 5 Side views of the X-ray crystal structure of tetra-imine cage **1b**. The sizes of the two different portals are displayed. The structure is shown in ORTEP view with thermal ellipsoids set at 50% probability. Hydrogen atoms are shown as fixed-size spheres of 0.3 Å radius. Included guest and solvent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



It is worthy to note that the ^1H NMR spectrum of cage **1b** in CDCl_3 solution showed broader proton signals than in a 9 : 1 CDCl_3 : CD_3CN 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 (CHCl_3 or CH_2Cl_2) in its cavity providing sensible packing coefficient (PC)³⁹ 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 CDCl_3 : CD_3CN 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 (CDCl_3 or CD_3CN) 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 ^1H 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 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 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 ^1H 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 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 s^{-1} ($k_{\text{exch}} = k' + k_{\text{off}} \sim 10^{-2} \text{ s}^{-1}$).⁴⁰ 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 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 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 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 **5₂C1b** (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 ($\text{H}^{\text{e1'}}$, $\text{H}^{\text{e2'}}$, $\text{H}^{\text{d1'}}$, $\text{H}^{\text{d2'}}$, $\text{H}^{\text{e1''}}$ and $\text{H}^{\text{e2''}}$).

The downfield shift experienced by the pyrrole NHs in the **5₂C1b** 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 ⁴¹D3-def2-TZVP⁴² level of theory using Turbomole v7.0,^{43,44} of the two cage complexes also supported this conclusion: **4C1b** (average $d_{(\text{H}^{\text{N}} \cdots \text{O}^{\text{N}})} = 3.29 \pm 0.07 \text{ \AA}$) and **5₂C1b** (average $d_{(\text{H}^{\text{N}} \cdots \text{O}^{\text{N}})} = 2.88 \pm 0.02 \text{ \AA}$). 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 ($d_{(\text{H}^{\text{N}} \cdots \text{O}^{\text{N}})}$) on the range of 3.0 to 2.8 Å.⁴⁵ 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 CDCl_3 solution. Surprisingly to us, and based on the integral values of selected proton signals in the ^1H NMR spectra, the addition of the *N*-oxides (**4** and **5**) to the pre-assembled cage **1b** in 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.



and free **4** were still the major species in solution. The **4**⊂**1b** complex was present to a 20% extent. This result was in contrast to the quantitative formation in just few minutes of the **4**⊂**1b** complex from **1b** in CDCl₃ solution. During the course of several weeks, we observed that the intensity of the proton signals of the **4**⊂**1b** complex increased at the expenses of those of (CH₃CN)₂⊂**1b** and **4** (Fig. 7c). After one month the **4**⊂**1b** complex was quantitatively formed in solution.

We also employed the bimolecular irreversible kinetic model ($A + B \rightarrow C$) to mathematically analyze the time dependent concentration changes experienced by (CD₃CN)₂⊂**1b** and **4**⊂**1b** throughout the irreversible inclusion/exchange process.⁴⁸ The fit of the data returned the rate constant value for the formation of the **4**⊂**1b** complex (inclusion of **4** in **1b**) as $k_{on} = 1.0 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$, which corresponded to a free energy barrier of $\Delta G^\ddagger \sim 21.6 \text{ kcal mol}^{-1}$ (Fig. 7 top). This result indicated that the energy barrier for the formation of the **4**⊂**1b** cage complex was almost identical starting from **5**₂⊂**1b** in CDCl₃ solution or (CD₃CN)₂⊂**1b** in 9 : 1 CDCl₃ : CD₃CN solvent mixture. In contrast, the energy barrier for the inclusion of **4** dropped to $\sim 15 \text{ kcal mol}^{-1}$ when the (CDCl₃)₂⊂**1b** cage complex was used

as starting material in CDCl₃ solution (*vide supra*). In short, the displacement/exchange of two polar guests (**5** and CD₃CN) from the cavity of **1b** caused by the inclusion of **4** was associated to an increase in the free energy barrier of $> 6 \text{ kcal mol}^{-1}$ compared to the replacement of the less polar CDCl₃ molecules. We assumed that this increase was mainly associated with the breaking of polar intermolecular interactions (hydrogen bonds, CH- π and π - π). Nevertheless, owing to the superior binding properties of **5** for the aryl-extended calix[4]pyrrole receptors, we were surprised to find out that the energy barriers of the exchange of the pyridine-*N*-oxide **5** and CD₃CN were almost identical.

For this reason, we were keen in determining the relative thermodynamic stability of the **5**₂⊂**1b** and (CD₃CN)₂⊂**1b** cage complexes in the 9 : 1 CDCl₃ : CD₃CN solvent mixture.

Studies of the inclusion of pyridine-*N*-oxide **5 in the (CD₃CN)₂⊂**1b** cage complex.** We added 2 equiv. of pyridine-*N*-oxide **5** to a $\sim 1 \text{ mM}$ solution of the (CD₃CN)₂⊂**1b** cage solvate assembled in the 9 : 1 CDCl₃ : CD₃CN solvent mixture. The ¹H NMR spectrum of the mixture acquired immediately after the addition ($< 10 \text{ min}$) displayed three set of signals for the protons of **1b**. The less intense set of signals corresponded to the intact (CD₃CN)₂⊂**1b** cage solvate. The other two sets of proton signals were assigned to the (CD₃CN·**5**)⊂**1b** heterocomplex and the **5**₂⊂**1b** homocomplex. After 1.5 h, the two inclusion complexes were exclusively present in solution in a 60 : 40 molar ratio that did not change over time (24 h) (Fig. 8c). These results assigned lower energy barriers to the stepwise inclusion of pyridine-*N*-oxide **5** in the cavity of the (CD₃CN)₂⊂**1b** cage complex than the $21.6 \text{ kcal mol}^{-1}$ calculated for the bis-*N*-oxide **4**. Moreover, they also showed that working under stoichiometric control for the formation of the **5**₂⊂**1b** homocomplex, the 2 : 1 species was not the most favored complex in solution. Instead, the 1 : 1 heterocomplex (CD₃CN·**5**)⊂**1b** was present to a larger extent. The (CD₃CN·**5**)⊂**1b** complex was quantitatively formed in the presence of equimolar amounts of **1b** and **5** ($K_a[(\text{CD}_3\text{CN}\cdot\mathbf{5})\subset\mathbf{1b}] > 10^4 \text{ M}^{-1}$, see ESI for details, Fig. S37[†]). However, the quantitative formation of the **5**₂⊂**1b** analogue required a large excess of **5** ($> 4 \text{ equiv.}$). Consequently, we conclude that the formation of the (**5**)₂⊂**1b** complex occurred through a stepwise binding process featuring negative cooperativity ($K_a[(\text{CD}_3\text{CN}\cdot\mathbf{5})\subset\mathbf{1b}] > 4 \times K_a[\mathbf{5}_2\subset\mathbf{1b}]$). That is, the **5**₂⊂**1b** complex is relatively higher in energy than its immediate precursor (CD₃CN·**5**)⊂**1b**. Probably, the existence of steric clashes between the two pyridine-*N*-oxides occupying the rather limited volume of the cavity of the **5**₂⊂**1b** complex was responsible for this energy increase. All together, these findings might explain the unexpected similar values calculated for the energy barriers of the guests exchange reactions leading to the **4**⊂**1b** complex, either starting from **4** and **5**₂⊂**1b** in pure CDCl₃ solution or **4** and (CD₃CN)₂⊂**1b** in 9 : 1 CDCl₃ : CD₃CN solvent mixture.

It is worthy to note, that in CDCl₃ solution and using ¹H NMR spectroscopy, we did not detect the formation of the heterocomplex (CDCl₃·**5**)⊂**1b**. The exclusive formation of the **5**₂⊂**1b** complex in the presence of 3 equiv. of **5** implied a cooperative binding process in CDCl₃ solution. This result is in striking contrast with the negative cooperativity observed in the 9 : 1 CDCl₃ : CD₃CN solvent mixture. We hypothesized that

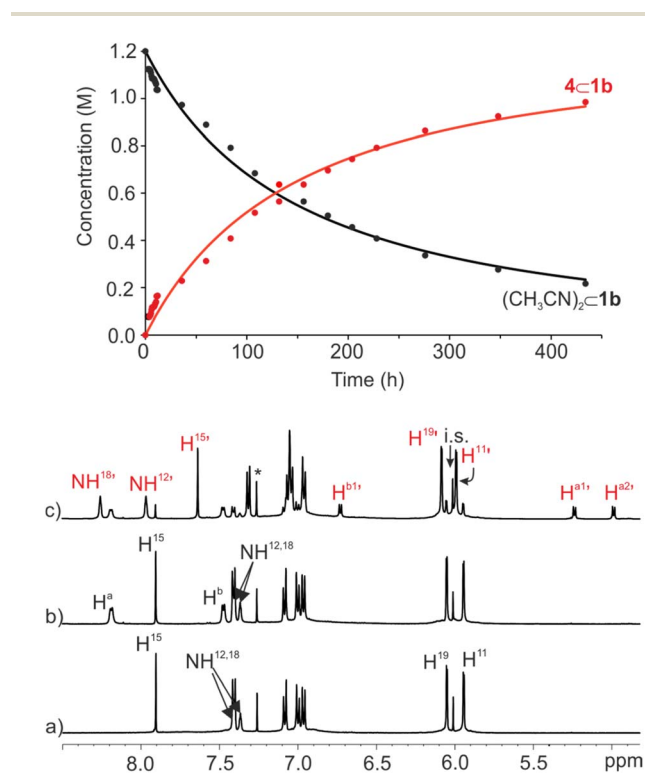


Fig. 7 (Top) Changes in the concentration of (CD₃CN)₂⊂**1b** (black) and **4**⊂**1b** (red) versus time (h) following the addition of 2 equiv. of bis-pyridyl *N*-oxide **4** (initial concentrations: [**1b**] = 1 mM and [**4**] = 2 mM). Solid lines represent the fit of the kinetic data to the rate law for a second order irreversible reaction using the parameter estimation module of COPASI Software Version 4.25. (Bottom) Selected regions of the ¹H NMR spectra (500 MHz, at 298 K) of 1 mM solution of imine cage **1b** in CDCl₃ : CD₃CN 9 : 1 mixture: (a) before, (b) following the addition of bis-pyridyl *N*-oxide **4**, and (c) after standing at r.t. for 18 days. i.s.: internal standard. See Fig. 2 for proton assignment. Red primed letters indicate the proton signals of the **4**⊂**1b** cage complex.



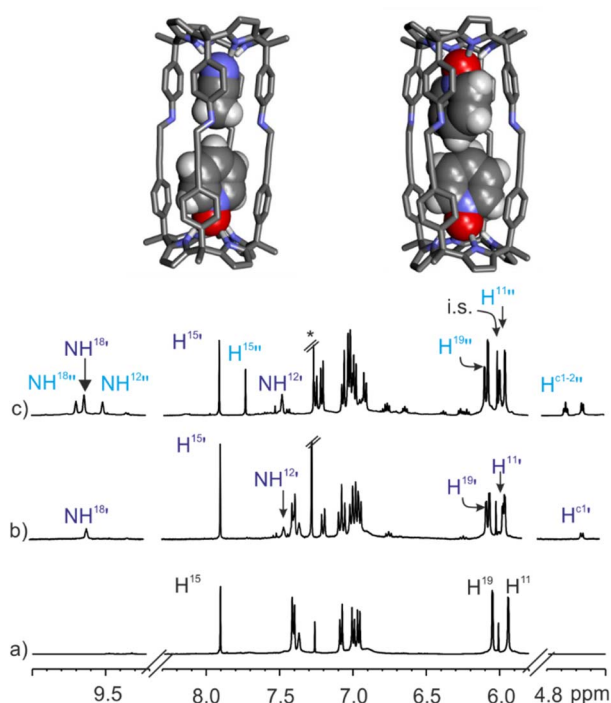


Fig. 8 (Top) Energy minimized structures (BP86-D3-def2-TZVP DFT) of $(\text{CD}_3\text{CN}\cdot 5)\subset 1\mathbf{b}$ (main isomer observed in solution) and $5_2\subset 1\mathbf{b}$ complexes. (Bottom) Selected regions of the ^1H NMR spectra (500 MHz, at 298 K) of 1 mM solution of imine cage $1\mathbf{b}$ 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 $(\text{CD}_3\text{CN}\cdot 5)\subset 1\mathbf{b}$ 1 : 1 complex and light blue doubled primed letters those of the 2 : 1 complex $5_2\subset 1\mathbf{b}$.

in CDCl_3 the heterocomplex $(\text{CDCl}_3\cdot 5)\subset 1\mathbf{b}$ was energetically less favored than the homo counterpart $5_2\subset 1\mathbf{b}$ despite the steric clashes between the included *N*-oxides.

The careful analysis of the ^1H NMR spectrum of the $(\text{CD}_3\text{CN}\cdot 5)\subset 1\mathbf{b}$ 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 $1\mathbf{b}$.^{**} 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 $(\text{CD}_3\text{CN}\cdot 5)\subset 1\mathbf{b}$ in comparison to the 57% calculated for the $5_2\subset 1\mathbf{b}$ (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- α 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 ($\sim 65\%$).

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

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 $4\subset 1\mathbf{b}$ 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 (CDCl_3 or CD_3CN) that must be displaced from the solvated cages' interior, as well as, to the dissimilar sizes of the incoming guests.

Notably, in 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 (ΔG^\ddagger) for the above displacement reaction and that of the formation of the inclusion complex $4\subset 1\mathbf{b}$ from the acetonitrile solvate $(\text{CD}_3\text{CN})_2\subset 1\mathbf{b}$, 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 CD_3CN), 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 $1\mathbf{b}$, 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.†



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 ¹H 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,⁴⁹ performed at different temperatures (323–238 K) using a 95 : 5 CHCl₃ : CDCl₃ solution of cage **1b** and by selectively exciting the CHCl₃ signal did not help in revealing the proton signals of the putative CHCl₃ included in the cage. Most likely, the chemical exchange between the free and bound CHCl₃ was fast on the chemical shift timescale.⁵⁰

** At the BP86-D3-def2-TZVP DFT level of theory using Turbomole v7.0, we computed an energy difference of 1.5 kcal mol⁻¹ for the two isomers of the (CDCl₃ · 5) **1b** in favor to the one experimentally observed.

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