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We report the synthesis of a tetrapyridinium phenyl extended calix[4]pyrrole showing high selectivity and binding affinity for the *cis*-conformers of *N*-phenyl-amides in water. The conformational selectivity is remarkable owing to the energetic preference of the amides to adopt the *trans*-conformation. In turn, the high binding affinities derived from the combination of hydrogen bonding interactions and the hydrophobic effect. We used two binding models for the mathematical analyses of the titration data and rationalized the different magnitudes calculated for the corresponding binding constants (apparent and intrinsic).

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Conformational selectivity and high-affinity binding in the complexation of *N*-phenyl amides in water by a phenyl extended calix[4]pyrrole†

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We describe the synthesis of a tetrapyridinium phenyl extended calix[4]pyrrole that is soluble in neutral water solution at mM concentrations. We show that, in pure water, the synthesized calix[4]pyrrole receptor selectively binds the *cis*-(*E*) conformers of secondary *N*-phenyl-amides and tertiary *N*-methyl-*N*-phenyl-formamide with binding affinities larger than 10^3 M^{-1}. The conformational selectivity is remarkable owing to the energetic preference of amides to adopt the *trans*-(*Z*) conformation in solution. In this respect, we used two binding models for the mathematical analyses of the titration data and calculated apparent and intrinsic binding constants. The combined action of hydrogen bonding and the hydrophobic effect that operates in the binding of the amides in water is responsible for the large affinities displayed by the receptor.

Introduction

Carboxamides (*a.k.a* organic amides) are important functional groups in both natural (*e.g.* peptides) and synthetic (*e.g.* nylons) polymers. The existence of many methods for their synthesis and their high thermodynamic stability under a wide range of chemical conditions render amides very useful covalent linkers in many chemical constructs. The pseudo-double-bond character of the carbon(C=O)–nitrogen σ-bond restricts its rotation, and therefore, secondary and tertiary amides exist as two isomeric *cis–trans* rotamers (Fig. 1). In solution, secondary amides show a marked preference for the *trans*-rotamer (*Z*-isomer).[1,2] On the one hand, the energetic difference between the two rotamers lies in the range of 0.5 to 2.5 kcal mol^{-1} for both secondary and tertiary amides.[3] On the other hand, because the free energy barrier for the *cis–trans* interconversion is moderate ($\Delta G = 16$–22 kcal mol^{-1})¶ the process is typically slow on the NMR time-scale but fast on the human time-scale.[3] Steric contributions on either side of the amide group seem to be the most significant factors for easing the barrier crossing, as well as controlling the relative population of *cis–trans* isomers.

The *cis–trans* isomerism of the amide bond plays an important role in determining folding,[4] functional[5] and ligand

interactions in proteins and peptides.[6–10] Likewise, the photo-physical behaviour of simple phenanthrene carboxamides was shown to be dependent on the amide conformation.[11] The equilibrium between secondary amide rotamers is known to be solvent dependent.[12] In addition, *cis*-amide rotamers have been stabilized by intramolecular CH–π interactions,[13] lone pair–π interactions,[14,15] and the hydrophobic effect.[16] In organic solvents, the formation of host–guest hydrogen bonded complexes has also been used to stabilize *cis*-amides.[15,17–20] In water, the combination of hydrogen bonding and the hydrophobic effect has provided thermodynamically stable complexes of synthetic receptors with small linear and cyclic peptides.[21,22] However, to the best of our knowledge, examples of synthetic receptors displaying selective binding of the *cis*-rotamers of mono-amides in water and producing thermodynamically highly stable complexes are not known.

Sessler *et al.* described the binding of small tertiary amides (*i.e.* DMF and DEF) using octa-methyl calix[4]pyrrole in benzene solution. The resulting complexes displayed binding affinities of the order of 10 M^{-1} and were mainly stabilized by the establishment of hydrogen-bonding interactions between the

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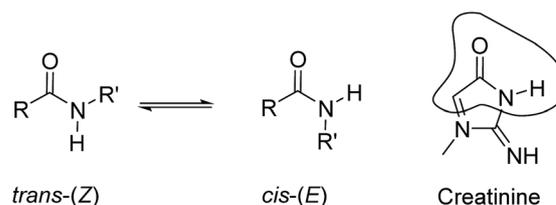
Fig. 1 Equilibrium between the *cis*-(*E*) and *trans*-(*Z*) conformers of a secondary amide. The structural analogy of the *cis*-(*E*) isomer with the lactam tautomeric form of creatinine is highlighted.

pyrrole NHs and the oxygen atom of the amide's carbonyl group.[23] More recently, we reported the use of phosphonate calix[4]pyrrole cavitands, featuring a polar aromatic cavity, for the efficient binding of creatinine in organic solvents.[24] In solution and in the solid-state, the inclusion of creatinine in the cavity of the receptor was mainly driven by the establishment of multiple hydrogen bonds. Nevertheless, additional intermolecular CH–π interactions were also present in the above creatinine complex. To us, the lactam tautomer of the bound creatinine resembled a *cis*-rotamer of a secondary amide and suggested the potential use of aryl-extended calix[4]pyrroles for its selective binding in water solution (Fig. 1).

Herein, we report the synthesis of a water soluble tetra-cationic pyridinium aryl-extended calix[4]pyrrole receptor **1**. We also describe the results of the binding studies of receptor **1** with a series of acyclic mono-amides in water. The conformational selectivity displayed by receptor **1** in the exclusive binding of the *cis*-rotamers of *N*-phenyl-amides with affinity constants larger than 10^3 M^{-1} is highlighted.‡

Results and discussion

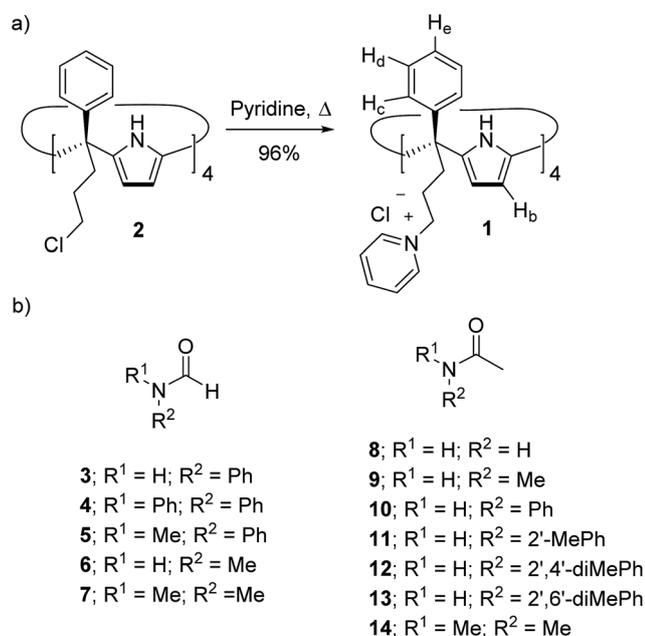### Synthesis of tetra-pyridinium calix[4]pyrrole receptor 1

Water soluble aryl-extended calix[4]pyrroles bearing ionizable carboxylic acid functions have been previously reported in the literature.[25–27] These receptors required basic media for their water solubilisation. In order to avoid these conditions, not compatible with biological standards, we designed α,α,α,α-tetra-phenyl calix[4]pyrrole **1** (Scheme 1a) featuring four *meso*-(3-pyridinium-propyl) substituents at its lower rim. The water solubilization of calix[4]pyrrole **1** was inspired by previous

studies on water soluble deep cavitands bearing benzimidazolones at their upper rims.[28] In these examples, the deep cavitands were rendered water soluble by converting the lower-rim tetra-chloride derivatives into quaternary ammonium salts by treatment with an excess of pyridine. Receptor **1** was synthesized in two steps from commercially available starting materials (Scheme 1a). To start with, the α,α,α,α-tetra-chloro calix[4]pyrrole derivative **2** was prepared using a synthetic methodology recently developed by us.[29] Treatment of tetra-chloride **2** at 110 °C in an excess of pyridine provided the tetra-pyridinium calix[4]pyrrole salt **1** as a yellow precipitate in excellent yield and purity. Calix[4]pyrrole **1** was soluble in neutral water at concentrations up to 15 mM.§ A dilution experiment (1 mM to 0.4 mM) showed negligible changes in the proton signals of the acquired ^{1}H NMR spectra. At diluted concentrations, the proton signals of **1** are sharp and well-defined and their number is in agreement with C_{4v} symmetry (ESI†). Most likely, both alternate and cone conformations of **1** are present in solution. The two conformers are in equilibrium and display a fast chemical exchange on the chemical shift time-scale.[30,31]

Binding studies of 1 with formamides in water

Molecular modelling. Earlier studies with aryl-extended calix [4]pyrroles related to **1** and their cavitand derivatives demonstrated that, in the solid-state and organic media, *N,N*-dimethylformamide[32] and creatinine[24] were suitable guests for inclusion in their polar aromatic cavities defined by the four *meso*-phenyl substituents. Based on these precedents, we envisaged that α,α,α,α-aryl-extended calix[4]pyrrole **1** might show conformational selectivity in the recognition of size and shape complementary secondary and tertiary small amides in water. Simple molecular modelling studies (Fig. 2) revealed that the inclusion of *N*-phenyl-formamide **3** in the aromatic cavity of the cone conformation of **1** was energetically more favorable ($\Delta E = 7.3$ kcal mol^{-1}) for the *cis*-rotamer (Fig. 2a).

The *cis*-**3**⊂**1** complex is stabilized by four hydrogen-bonding interactions established between the amide carbonyl oxygen

Scheme 1 (a) Synthesis of tetra-phenyl tetra-pyridinium calix[4] pyrrole receptor **1** and (b) line-drawing structures of the formamides (**3**–**7**) and acetamides (**8**–**14**) discussed in this work. When applicable only the *cis*-rotamer is depicted. See Fig. 1 for the *cis*/*trans* equilibrium.
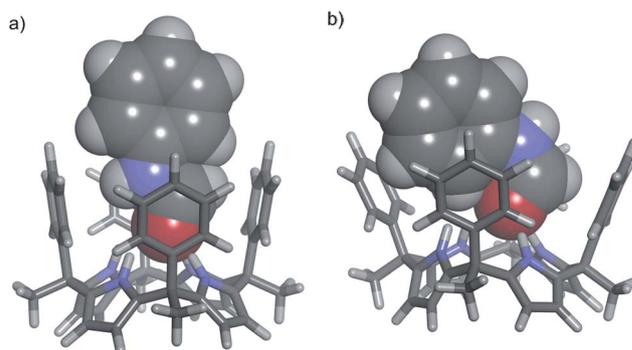

Fig. 2 Energy minimized structures (PM3, water COSMO model as implemented in SCIGRESS Version FJ 2.6 (EU 3.1.9)) of the inclusion complexes: (a) *cis*-**3**⊂**1** and (b) *trans*-**3**⊂**1**. A difference of energy of 7.3 kcal mol^{-1} was calculated in favor of the *cis*-**3**⊂**1** complex. Calix[4] pyrrole **1** is shown in stick representation and amides (*cis*-**3** and *trans*-**3**) are shown as CPK models. *Meso*-(3-pyridinium-propyl) groups were pruned to methyl groups to ease the calculations.

atom and the NHs of the four pyrrole units. Additional CH–π and NH–π[17,33,34] interactions are also established between the included *cis*-**3** amide and the *meso*-phenyl walls of **1**.[35] On the other hand, the inclusion of *trans*-**3** in the cone conformation of **1**, *trans*-**3**⊂**1** complex, evidenced significant steric clashes between the *N*-phenyl substituent and two of the four *meso*-phenyl groups of the receptor (Fig. 2b).

¹H NMR spectroscopy titrations. We decided to experimentally probe the interaction of **1** with *N*-phenyl-formamide **3** in water using ¹H NMR titration experiments. We mentioned above that the steric factors are usually the most important contributors to the relative populations of the *cis/trans* rotamers in small secondary and tertiary amides, and that the isomer-ratio is also affected by the solvent.[36] In this respect, the ¹H NMR spectrum of a 10 mM solution of *N*-phenyl-formamide **3** in water showed a 32 : 68 *cis/trans* ratio, based on the integral values of the two singlets resonating at $\delta = 8.65$ and 8.28 ppm, respectively, which are assigned to the hydrogen atoms of the formyl group in each rotamer (ESI†).

The incremental addition of formamide **3** to a 1 mM water solution of the tetra-pyridinium receptor **1** induced significant chemical changes to several proton signals of the receptor. In particular, the triplet of the *para*-aromatic proton, H_e, of the *meso*-phenyl groups of **1** moved upfield ($\Delta\delta_{max} = -0.21$ ppm). In contrast, the β-pyrrole protons (H_b) moved downfield ($\Delta\delta_{max} = +0.09$ ppm).¶ These observations indicated the existence of a fast chemical exchange on the ¹H NMR time-scale between free and bound receptor **1**. Remarkably, after the addition of 0.6 equiv. of **3** only the sharp singlet corresponding to the hydrogen atom of the formyl group for the free *trans*-isomer became visible. At this point, we also observed a complex signal of aromatic protons resonating at $\delta = 7.5$ ppm that was assigned to the phenyl group of the formamide. The signals assigned to free *trans*-**3** grew in intensity as the concentration of **3** was increased (Fig. 3). In contrast, the signal of the formyl proton in the free *cis*-rotamer was not observed even when 2 equiv. of **3** were added.

The titration data were mathematically analyzed using the HypNMR 2008 software Version 4.0.66.[37] The fit of the chemical shift changes experienced by the selected proton signals of receptor **1** (H_b, H_c, H_d, and H_e, see Scheme 1 for proton assignment) to a binding isotherm of a theoretical 1 : 1 binding model was good. We estimated an apparent binding constant value $K_a > 10^4$ M^{-1} for the formed complex and determined the chemical shift values of the protons of the receptor in the complex (ESI†). These latter values allowed the calculation of the corresponding complexation induced shifts (CISs).

At first sight, the determined magnitude of the apparent binding constant might be considered as the weighted-average of two putative 1 : 1 inclusion complexes: *cis*-**3**⊂**1** and *trans*-**3**⊂**1**. In order to assess the conformational selectivity exhibited by receptor **1** in the binding of **3**, we decided to titrate it against tertiary *N,N*-diphenyl-formamide **4**. The hydrogen-bonding inclusion of formamide **4** in the cavity of **1** should produce the **4**⊂**1** complex exhibiting a binding geometry closely resembling that of the *trans*-**3**⊂**1** complex (Fig. 2b). Our idea was to use the binding constant value of **4**⊂**1** as the reference for the *trans*-**3**⊂**1** counterpart.

The extensive sonication of a suspension of *N,N*-diphenyl-formamide **4** with a 1.7 mM water solution of **1** led to the dissolution of 2 equiv. of **4** but did not induce appreciable changes in the chemical shift values of the receptor's protons. Thus, we estimated a binding constant value $K_a(\mathbf{4}⊂\mathbf{1}) < 10$ M^{-1}. On the one hand, this result strongly supports a conformational selectivity towards the *cis*-rotamer in the binding of *N*-phenyl-formamide **3** with receptor **1**. On the other hand, it also requested a change in the mathematical analysis of the titration data in order to assess a more accurate value of the binding constant for the *cis*-**3**⊂**1** complex.

Therefore, we reanalyzed the titration data of **1** with **3** using a theoretical binding model that considers the existence of the equilibrium between the two rotamers of **3** and the exclusive formation of a 1 : 1 complex with the *cis*-rotamer and receptor **1**.

In doing so a theoretical binding isotherm was calculated from the simulated speciation profile produced with the Specfit Software Version 3.0.40 (differential kinetics module). We considered the equilibrium constant between the two rotamers of **3** and the determined chemical shift values for the protons in the free and bound receptor **1** as fixed values. The value of $K(\textit{cis}\text{-}\mathbf{3}/\textit{trans}\text{-}\mathbf{3}) = 32/68 = 0.47$ was determined from the ¹H NMR spectrum of **3**. The complexation induced shift values (CISs) for the protons of **1** were those calculated from the previous fit (*vide supra* and ESI† for details). The fit of the experimental titration data to the more elaborate theoretical binding model only served to estimate that the binding constant value $K_a(\textit{cis}\text{-}\mathbf{3}⊂\mathbf{1})$ was larger than 10^4 M^{-1}. The magnitude of this estimate represents a remarkable binding affinity for the complexation of a small polar molecule in water using a synthetic receptor.[38,39]

The fact that the *cis*-rotamer is present in solution to a significant extent (32%) is the main reason for the observed coincidence between the binding constant values estimated using the two bindings models, that is $K_a(\textit{cis}\text{-}\mathbf{3}⊂\mathbf{1}) \sim K_{app}$. We will show below that when the *cis*-rotamer is present in solution at low levels, the mathematical analyses of the titration data using the two binding models will produce very different values for $K_a(\textit{cis}⊂\mathbf{1})$ and K_{app}.

Next, we performed a reverse titration of *N*-phenyl-formamide **3** with receptor **1** (Fig. 4). The ¹H NMR spectrum
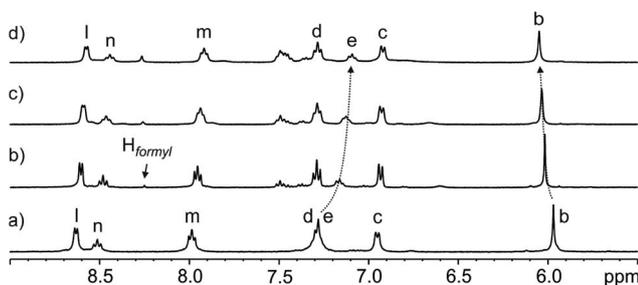

Fig. 3 Selected region of the ¹H NMR (400 MHz, D₂O, 298 K) spectra of the titration of calix[4]pyrrole **1** with *N*-phenyl-formamide **3**: (a) **1**; (b) **3** + **1** (0.6 : 1 molar ratio); (c) **3** + **1** (1 : 1 molar ratio) and (d) **3** + **1** (2 : 1 molar ratio). The H$_{formyl}$ signal corresponds to the hydrogen atom of the formyl group for *trans*-**3**. See Scheme 1a for proton assignments of receptor **1**.
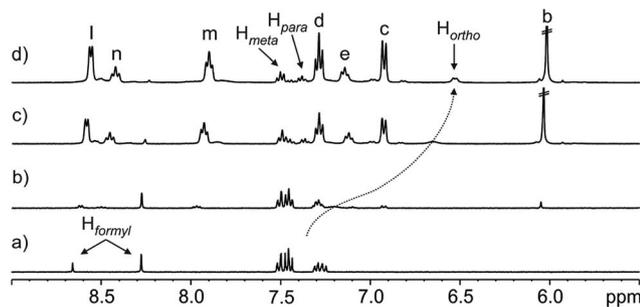
Fig. 4 Selected region of the ^{1}H NMR (400 MHz, D$_2$O, 298 K) spectra of the titration of *N*-phenyl-formamide **3** with calix[4]pyrrole **1**: (a) **3**; (b) **1** + **3** (0.05 : 1 molar ratio); (c) **1** + **3** (1 : 1 molar ratio) and (d) **1** + **3** (2 : 1 molar ratio). In (a) the H$_{formyl}$ signals correspond to *cis*-**3** (left) and *trans*-**3** (right) providing a 32 : 68 molar ratio. See Scheme 1a for proton assignments of receptor **1**. In (d) the *ortho*, *meta* and *para* phenyl protons of bound **3** are also indicated.

of a 1.2 mM water solution of **3** containing just 0.05 equiv. of **1** (Fig. 4b) showed the formyl proton of the *trans*-**3** rotamer as a sharp singlet resonating at the same chemical shift as in the absence of **1**. On the contrary, the formyl singlet assigned to the *cis*-**3** rotamer was not detectable at this stage of the titration. This observation indicated that while the equilibrium involving the two free rotamers of **3** displayed slow chemical exchange on the chemical shift time-scale, the *cis*-**3** rotamer was also involved in a binding equilibrium, probably with bound *cis*-**3**, featuring intermediate chemical exchange on the chemical shift time-scale. The intermediate kinetics of the latter chemical exchange process produced broadening beyond detection for the formyl proton in the *cis*-**3** rotamer. This result also provides irrefutable support to the selective binding of the *cis*-**3** rotamer by receptor **1**.

In complete agreement with the previous statement, an increase in the concentration of **1** did not affect the appearance and chemical shift value of the formyl proton signal for the *trans*-**3** rotamer, however, its intensity decreased. We also observed an increase in the intensity of three aromatic signals, two triplets moving downfield and one doublet that shifted upfield. We assigned these three signals to the protons of the *N*-phenyl group of the *cis*-**3** rotamer, which are involved in a fast chemical exchange on the chemical shift time-scale between its free and bound states. The different kinetics of the chemical exchanges experienced by the protons of the *cis*-**3** rotamer, that is, the formyl proton is involved in an intermediate exchange and the aromatic protons are involved in a fast exchange, result from notably different CISs. The formyl proton of bound *cis*-**3** is included in the aromatic cavity of **1** experiencing the strong shielding exerted by the *meso*-phenyl substituents.‖

On the other hand, the *N*-phenyl unit of bound *cis*-**3** resides almost completely outside of the aromatic cavity of **1**, with its *ortho*-protons being the only ones affected by the shielding effect (Fig. 2a and 4).

In order to evaluate the scope of the conformational selectivity featured by tetra-phenyl calix[4]pyrrole receptor **1** in the binding of other formamides, we investigated the complexation

of **1** with *N*-methyl-*N*-phenyl-formamide **5**. Based on the integral values of the two separate formyl protons, formamide **5** is present in water solution as an 82 : 18 *cis/trans* mixture of rotamers (K(*cis*-**5**/*trans*-**5**) = 4.56).

The direct ^{1}H NMR titration of **1** with incremental amounts of **5** showed an analogous behavior with respect to the chemical shift changes of the proton signals of receptor **1** to the one previously described for **3**. The mathematical analysis of the titration data using the binding model that considers the equilibrium between rotamers and the exclusive formation of a 1 : 1 complex with the *cis*-**5** isomer allowed us to estimate that the affinity constant value K_a(*cis*-**5**⊂**1**) must be > 10^4 M^{-1}. We also performed an ITC titration experiment for formamide **5** and calix[4]pyrrole **1** (ESI†). The calculated apparent binding constant was $8.6 \pm 0.1 \times 10^3$ M^{-1}, a value that is in line with the estimated one from the ^{1}H NMR titration analysis. The ITC results showed that the binding process was enthalpically driven (-7.1 kcal mol^{-1}) and entropically opposed (1.75 kcal mol^{-1}). Clearly, this thermodynamic signature is not the expected one for purely hydrophobic binding.[40]

We also performed a reverse titration by adding increasing amounts of **1** to a 2.9 mM water solution of **5**. The proton signals assigned to the *trans*-**5** rotamer did not experience noticeable chemical shift changes. In contrast, those of the *cis*-**5** counterpart, especially the formyl proton and the methyl protons, moved significantly upfield. From these titration data, we could estimate the CISs experienced by these protons because, although their signals broadened beyond detection in the initial and middle phases of the titration, they became observable again in the presence of 2 equiv. of **1**. In the *cis*-**5**⊂**1** complex, the formyl group resonates at $\delta = 5.01$ ppm ($\Delta\delta = -3.39$ ppm) and the methyl resonates at $\delta = 1.25$ ppm ($\Delta\delta = -2.07$ ppm). The large calculated CISs confirm the deep inclusion of *cis*-**5** in the aromatic cavity of **1**.

A 2D NOESY experiment showed intermolecular cross-peaks between the singlet of the methyl protons of bound *cis*-**5** and the aromatic protons (H$_c$ and H$_d$) of the *meso*-phenyl substituents of the calix[4]pyrrole **1**.**

We also assessed the affinity constant of calix[4]pyrrole **1** for *N*-methyl-formamide **6**. Formamide **6** is present in water as an 8 : 92 mixture of *cis/trans* rotamers. Nevertheless, molecular modelling studies and the previous results obtained in the binding of the secondary and tertiary formamides **3** and **5** strongly supported the inclusion of both isomers of **6** in receptor **1**. The energy minimized 1 : 1 inclusion complexes, *cis*-**6**⊂**1** and *trans*-**6**⊂**1**, featured close to isoenergetic values (ESI†).

Therefore, the ^{1}H NMR titration data for the interaction of **1** with **6** were analyzed using a simple 1 : 1 binding model obtaining a good fit and returning an affinity constant value of K_a(**6**⊂**1**) = 4.4×10^3 M^{-1}. The removal of the phenyl group in **6** compared to **5** slightly diminished the binding affinity for receptor **1** (Table 1). Probably, this difference is caused by a combination of reduction in hydrophobicity and the hydrogen-bonding accepting character of the oxygen atom in formamide **6** (secondary *vs.* tertiary).

In agreement with this hypothesis, the addition of an extra methyl group into *N,N*-dimethyl-formamide **7** increased its

binding affinity for **1**, compared to **6**, placing it at the same level observed for the analogous but more lipophilic *N*-phenyl derivatives **3** and **5**.

It is worth noting that in the reverse ^{1}H NMR titration of *N*-methyl-formamide **6** with receptor **1**, after the first addition of the receptor, the separate methyl proton signals of the two rotamers of **6** coalesce into a broad singlet that shifted upfield upon increasing the concentration of **1** (ESI†). This result clearly supports the binding of two rotamers of **6**, which are involved in a chemical exchange equilibrium with the free counterparts featuring fast/intermediate dynamics on the chemical shift time-scale.

Also note that, in the examples of *N*-phenyl-formamides **3** and **5**, only the proton signals assigned to the *cis*-rotamer experienced broadening and chemical shift changes. We used this observation as evidence of the conformational selectivity in the binding process. The results described above indicate that receptor **1** might be considered as a minimal synthetic chaperone selecting the *cis*-conformation of the bound *N*-phenyl-formamides and increasing their relative concentration in solution in the bound form.

Binding studies of 1 with acetamides in water

Molecular modelling. Molecular modelling studies suggested that acetamide **8** and the *cis*-rotamer of *N*-methyl-acetamide **9**, as well as those of other secondary *N*-aryl amide derivatives like **10–13**, were suitable candidates for inclusion in the aromatic cavity of **1** (Fig. 5a). The modelled acetamides closely resembled the *cis*-rotamer of *N*-methyl-formamides (ESI†) in terms of size complementarity with the dimensions of the aromatic cavity of **1** in the cone conformation.

In contrast, tertiary *N,N*-dimethyl acetamide **14** presents an additional methyl group to be included in the cavity of **1**. The
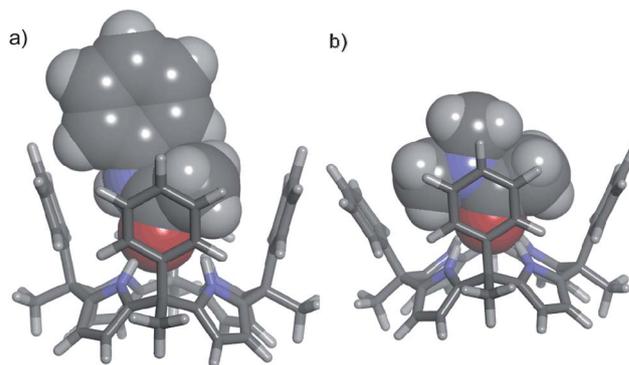

Fig. 5 Energy minimized structures (PM3, water COSMO model as implemented in SCIGRESS Version FJ 2.6 (EU 3.1.9)) of the inclusion complexes: (a) *cis*-**10**⊂**1** and (b) **14**⊂**1**. Calix[4]pyrrole **1** is shown in stick representation and amides (*cis*-**10** and **14**) are shown as CPK models. *Meso*-(3-pyridinium-propyl) groups were pruned to methyl groups to ease the calculations.

energy minimized structure of the **14**⊂**1** complex displayed a severe distortion of the cone conformation of the receptor owing to steric clashes between the *meso*-phenyl substituents and the two included methyl groups of the amide (Fig. 5b).

^{1}H NMR spectroscopy titrations. The titration of receptor **1** with increasing amounts of acetamide **8** afforded exclusively the **8**⊂**1** complex and, accordingly, it was analyzed using a simple 1 : 1 binding model returning $K_a(\mathbf{8}⊂\mathbf{1}) = 6.8 \times 10^3$ M^{-1}. The magnitude of the binding constant is in agreement with the value determined for the isosteric *N*-methyl-formamide, $K_a(\mathbf{6}⊂\mathbf{1}) = 4.4 \times 10^3$ M^{-1}.

In water, *N*-methyl-acetamide **9** and *N*-phenyl-acetamide **10** displayed *cis/trans* isomeric ratios of 2 : 98 and 1 : 99, respectively, based on the integral values of their methyl acetamide proton signals. In particular, the assignment of the methyl proton signal for the *cis*-**10** rotamer to a small singlet resonating at $\delta = 1.90$ ppm was confirmed by magnetization transfer experiments (ESI†). The titration data of **1** with both acetamides, **9** and **10**, were analyzed in an analogous manner. For the sake of brevity, we will only describe in detail the titration experiments performed with **10** and the corresponding data analyses.

The incremental addition of **10** (up to 15 equiv.) to a 1.2 mM solution of **1** in water produced very small changes in the chemical shift values of the diagnostic protons of the receptor (H$_b$, H$_c$, H$_d$ and H$_e$) used for signaling of complex formation in the formamide series.

We also performed a reverse ^{1}H NMR titration experiment adding incremental amounts of calix[4]pyrrole **1** to a 1.5 mM solution of **10** (ESI†). As expected the proton signals of *trans*-**10** did not experience chemical shift changes and only a small reduction of their intensities was observed. Unfortunately, it was not possible to accurately quantify the amount of *trans*-**10** rotamer that has been isomerized into the *cis*-**10** counterpart using the integral values of selected signals in the acquired ^{1}H NMR spectra due to its selective complexation.

We rationalized these results assuming that, as expected, receptor **1** does not bind the *trans*-**10** rotamer and that, owing to

Table 1 *Cis/trans* rotamer ratio in water at 298 K for the amides used as guests in this study and binding constant values (M^{-1}) of the 1 : 1 inclusion complexes with receptor **1**. See Scheme 1 for the line-drawing of the molecular structures of the amides

Formamides	R^1	R^2	*cis/trans*	$K_{a(cis⊂\mathbf{1})}{}^a$	$K_{app}{}^b$	$K_{a(1:1)}{}^b$
3	H	Ph	32 : 68	>10^4	>10^4	
4	Ph	Ph	n.a.			<10
5	Me	Ph	82 : 18	>10^4	>10^4	
6	H	Me	8 : 92		4.4 × 10^{3,c}	
7	Me	Me	n.a.			>10^4

Acetamides	R^1	R^2	*cis/trans*	$K_{a(cis⊂\mathbf{1})}{}^a$	$K_{app}{}^b$	$K_{a(1:1)}{}^b$
8	H	H	n.a.			6.8 × 10^3
9	H	Me	2 : 98	1.2 × 10^4	210	
10	H	Ph	1 : 99	5.0 × 10^3	51	
14	Me	Me	n.a.			27

^{a} A theoretical binding model considering the *cis/trans* equilibrium between amide rotamers and the exclusive formation of the *cis*⊂**1** complex was used. ^{b} Using a 1 : 1 theoretical binding model. ^{c} Weighted-average of $K_{a(cis⊂\mathbf{1})}$ and $K_{a(trans⊂\mathbf{1})}$. Errors (standard deviations) are estimated to be lower than 20%. n.a. = not applicable.

the low concentration of the *cis*-**10** rotamer in solution, the formation of the *cis*-**10**⊂**1** complex (in the direct and reverse titrations) takes place to a reduced extent. To verify our hypothesis, we simulated the speciation profile of a direct titration considering $K(cis\text{-}\mathbf{10}/trans\text{-}\mathbf{10}) = 0.01$ and $K_a(cis\text{-}\mathbf{10}\subset\mathbf{1}) = 1 \times 10^4$ M^{-1}. Surprisingly to us, the simulated profile indicated a significant formation of the *cis*-**10**⊂**1** complex in the concentration range used for the experimental titration (ESI†). Owing to the low saturation levels of complex formation attained during the direct and reverse titration of **1** with **10** we could not determine an accurate binding constant value for the *cis*-**10**⊂**1** complex. Nevertheless, we obtained a reasonable fit of the experimental data using the theoretical binding model that considers the equilibrium between rotamers and the exclusive formation of a 1 : 1 complex with the *cis*-isomer. The fit was performed by fixing the values of $K(cis\text{-}\mathbf{10}/trans\text{-}\mathbf{10})$, δ_{free} and δ_{bound}†† (0.01, 5.97 ppm, 6.08 ppm). The only variable to refine was $K_a(cis\text{-}\mathbf{10}\subset\mathbf{1})$. The best fit was obtained assuming $K_a(cis\text{-}\mathbf{10}\subset\mathbf{1}) = 5.0 \times 10^3$ M^{-1}. This result suggests a reduction in the binding constant of the inclusion complex of the *cis*-rotamer of acetamide **10** compared to the isosteric *N*-methyl-*N*-phenyl-formamide **5**. Remarkably, the mathematical analysis of the same titration data using the simple 1 : 1 binding model provided an apparent binding constant of 51 M^{-1}.

This example showcases the significant difference in the calculated values of $K_a(cis\text{-}\mathbf{10}\subset\mathbf{1})$ and K_{app} when the *cis*-rotamer is present in solution at very low concentration. It also serves to justify the use of the elaborate binding model in the estimation or accurate determination of $K_a(cis\subset\mathbf{1})$ values.

We also determined the *cis*/*trans* ratios for the *N*-aryl acetamide derivatives **11**–**13** in water. We expected to observe an increase in the amount of the *cis*-rotamer compared to **10** as was previously described in organic solvents.[18] Unfortunately, in water, the percentage of the *cis*-isomers for the series of acetamides **11**–**13** did not increase over 3%. This limitation precluded the undertaking of the experimental quantification of the corresponding $K_a(cis\subset\mathbf{1})$ values for these *N*-aryl acetamides.

Finally, the affinity constant value of receptor **1** for *N*,*N*-dimethyl-acetamide **14** was determined to be $K_a(\mathbf{14}\subset\mathbf{1}) = 27$ M^{-1} using a simple 1 : 1 binding model. This value supports the conformational selectivity of the receptor in the binding of *cis*-**9**. It also serves to quantify in three orders of magnitude the binding selectivity expressed by receptor **1** in the binding of *N*,*N*-dimethyl-formamide **7** compared to the homologated and more hydrophobic *N*,*N*-dimethyl-acetamide **14**.

Conclusions

In summary, we report the synthesis of a tetra-α aryl-extended calix[4]pyrrole receptor **1** bearing four *meso*-(3-pyridinium-propyl) groups providing water solubility. We studied the binding of a series of primary, secondary and tertiary formamides and acetamides with receptor **1** in water. The mathematical analyses of the ^{1}H NMR titrations of *N*-phenyl-formamides **3** and **5** using a simple 1 : 1 binding model, K_{app}, or a more elaborate theoretical model including the *cis*/*trans*

isomerization and the exclusive formation of the *cis*⊂**1** complex, $K_{cis\subset\mathbf{1}}$, returned similar values, which were typically larger than 10^3 M^{-1}. We demonstrated that calix[4]pyrrole **1** selectively binds the *cis*-rotamers of these two formamides. In contrast, both rotamers of formamides **6** and **7**, the *N*-methyl analogues of **3** and **5**, are bound by receptor **1** without substantial changes in affinity values. Remarkably, primary (**8**) and secondary (**9** and **10**) acetamides are also bound in water by receptor **1** with high affinities. For the secondary acetamides (**9** and **10**), receptor **1** also features conformational selectivity for their *cis*-rotamers. In these examples, the mathematical analyses of the ^{1}H NMR titration data for the secondary acetamides provided very different binding constant values depending on the binding model used. We showed that this result is a direct consequence of the low level of *cis*-rotamers present in solution. *N*,*N*-Diphenyl-formamide **4** and *N*,*N*-dimethyl-acetamide **14** show a reduced affinity for **1** owing to size complementary issues with the receptor's polar cavity. Taken together, the reported findings demonstrate that calix[4]pyrrole **1** functions as a minimal chaperone analogue increasing the amount of amide *cis*-rotamers in solution through selective binding. The reported association constant values for most of the amide⊂**1** complexes are among the highest reported to date for the binding of small polar molecules[38] and even small peptides[41–46] in water using synthetic receptors. The amides are bound in the functionalized aromatic cavity of calix[4]pyrrole **1** by a combination of hydrogen-bonding, NH–π and CH–π interactions and the hydrophobic effect. We foresee that further elaboration of the aromatic cavity of water soluble *meso*-aryl extended calix[4] pyrroles could have an impact on improving their recognition properties, such as achieving higher binding constants or selectivity. Studies to further develop and understand these receptors are currently ongoing in our laboratory.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Notes and references

‡ The results of the binding studies of related water soluble aryl-extended calix[4] pyrroles with creatinine and a homologous series of lactams will be reported elsewhere.

§ Some foam appeared in the solutions after vigorous shaking at high concentrations indicating the amphiphilic nature of tetra-pyridinium calix[4]pyrrole **1**. In order to avoid misleading results, we performed our experiments at concentrations below 5 mM.

¶ The pyridinium proton signals also displayed chemical shift changes upon addition of incremental amounts of the guest. However, we decided not to use these nuclei to determine binding constants from the ^{1}H NMR titration data because they are not close to the receptor's binding site.

‖ A broad signal resonating at $\delta = 5.32$ ppm was assigned to the formyl proton of bound cis-**3**. The computationally determined chemical shift value (DFT) of this proton in the inclusion complex cis-**3**⊂**1** is in complete agreement with the assignment.

** Using DFT calculations we computed the chemical shift values of the protons for bound cis-**5**. We were gratefully surprised to find a nice agreement with the experimental ones (ESI†).

†† The chemical shift value of the beta-pyrrole protons in the cis-**5**⊂**1** complex was considered to be a good estimate for the δ_{bound} value in cis-**10**⊂**1**.

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