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Cucurbit[8]uril Controls the Folding of Cationic Diaryl Ureas in Water

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The ability of cucurbit[8]uril (CB[8]) to control the folding of diaryl ureas 1 and 2 in water was investigated. Compounds 1 and 2 contain two ureidyl C–N bonds which can each populate two conformational states resulting in a conformational ensemble comprising at least three states. We find that the presence of CB[8] results in the selective population of the (*E*,*E*)-2 conformer by the formation of CB[8]·(*E*,*E*)-2 complex at CB[8]:2 stoichiometries of 1: < 1; at higher stoichiometries, an unfolding process takes place during the formation of CB[8]·(*Z*,*Z*)-2_2. In contrast, compound 1 forms the 2:2 complex $CB[8]_2$·(*Z*,*Z*)-1_2 over a broad range of CB[8]:2 stoichiometries. The absolute stoichiometries of these complexes were established by diffusion ordered spectroscopic methods (DOSY). The folding of 2 into the (*E*,*E*)-2 conformer under the formation of CB[8]·(*E*,*E*)-2 is responsive to the presence of guests in its environment. For example, the addition of 8 results in the expulsion of (*E*,*E*)-2 from the cavity of CB[8] followed by its unfolding to the thermally preferred mixture of (*Z*,*Z*)- and (*Z*,*E*)-2 conformers. These results suggest that complexation within synthetic molecular containers—just like their natural counterparts the chaperones—may be an efficient route to control the folding behaviour of non-natural oligomers in aqueous solution.

Keywords: Cucurbit[*n*]urils; Foldamers; Molecular containers; Biomimetic systems

INTRODUCTION

Nature derives its functional macromolecules—proteins and nucleic acids—by folding oligopeptides and oligonucleic acids into specific three-dimensional conformations aided by non-covalent interactions between non-adjacent residues in the sequence of the oligomer. In some cases, this folding occurs autonomously and in others it is assisted by the presence of chaperone proteins that act as molecular containers that prevent protein aggregation and misfolding [1]. Inspired by the remarkable abilities of these Natural systems, supramolecular chemists have been studying the folding properties of a variety of non-natural oligomers—foldamers—into a variety of secondary, tertiary and even quaternary structures. Particularly well-known classes of foldamers include aromatic donor–acceptor stacks, phenyleneethynylenes and β-peptide systems [2]. As the ability to predict the folding properties of these foldamers have improved, research in this area has refocused on the development of functional systems [3] and those whose conformation can be controlled by environmental stimuli (e.g. photochemical, chemical, concentration, solvent) [4–7]. Recently, we reported that the folding of oligo(triazene-arylenes) that populate a complex conformational ensembles of nearly isoenergetic states can be controlled by the presence of members of the cucurbit[*n*]uril (CB[*n*]) family of molecular containers and that these systems respond to the presence of guests within their environment [8]. In this paper, we extend our previous work towards the controlled folding of diaryl ureas that populate a less complicated three-member conformational ensemble. The environment provided by the concave recognition surfaces of CB[8] molecular containers selectively stabilises the (*E*,*E*)-conformer of diaryl urea **2** which is not significantly populated thermally at room temperature.

Several groups have previously reported on the folding properties of urea-based foldamers [6,9]. Of highest relevance to our work are the reports by Gong and Meijer on the enforced folding of *N*,*N*′-diaryl ureas by intramolecular H-bonding

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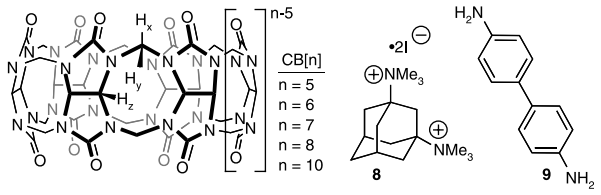


CHART 1 Chemical structures of CB[*n*], **8** and **9**.

interactions [10]. A number of research groups have contributed to elucidating the conformational preferences of related functional groups including oligo(amides), oligo(guanidines) and oligo(imides) [4,11]. Similarly, a number of conceptual elements of the present work build upon valuable precedent from the literature. For example, Lehn previously discussed the possibility of selectively stabilising specific conformations of oligo(diamidopyridines) in non-polar solvents by the application of small hydrogen-bonding modules (e.g. imides or barbiturates) [5,12]. Recently, Yashima's group showed that oligo(resorcinols) form double helices in water that can be unwound to linear conformations in response to chemical stimuli in the form of β-cyclodextrin (β-CD) molecular containers [13]. In this case, addition of adamantane carboxylic acid—which is an excellent guest for β-CD—sequesters β-CD and repopulates the double helical conformation. Fujita has previously shown that self-assembled molecular containers promote folding of certain peptides in water [14]. In a previous work, we showed that CB[*n*] molecular containers can be used to select a specific member of a 10-component nearly isoenergetic conformational ensemble [8]. This paper shows that the extremely strong binding that occurs within CB[*n*] molecular containers [15–17] and the associated free energy (ΔG) can even be used to selectively stabilise high energy conformations from within a conformational ensemble.

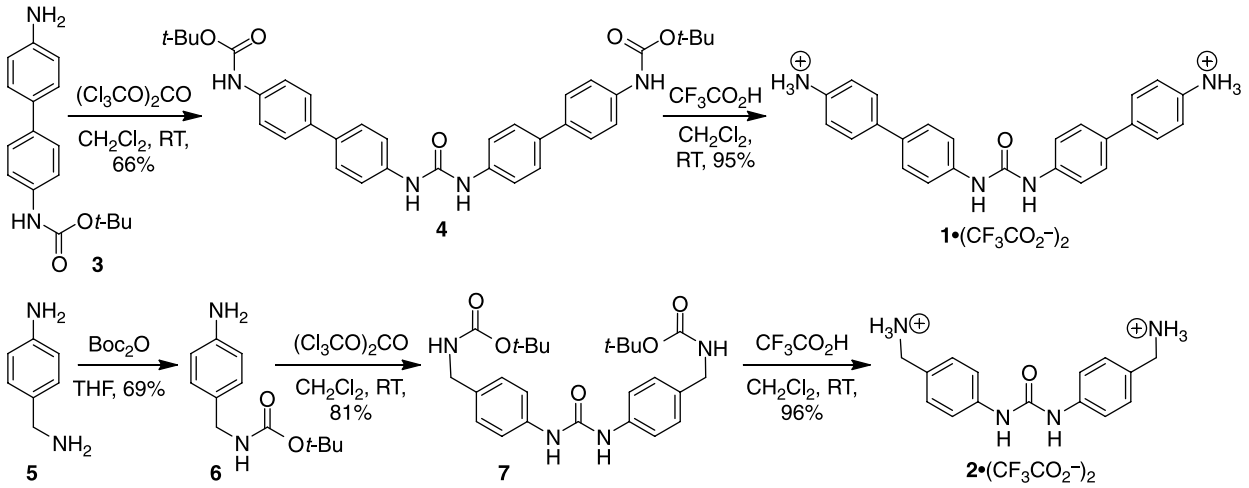
RESULTS AND DISCUSSION

In this section, we first discuss our design and synthesis of diarylureas **1** and **2** followed by an enumeration of the various conformations open to **1** and **2**. Second, we describe our rationale for selecting CB[8]—a prominent member of the cucurbit[*n*]uril family of macrocycles (Chart 1)—as the molecular container to drive the folding of diarylureas **1** and **2** in water. Third, we describe the supramolecular structures formed by **1** and **2** with CB[8] as a function of host:guest stoichiometry. Lastly, we show how chemical stimuli in the form of tight binding guests for CB[8] can be used to eject **2** from the CB[8]·**2** complex which subsequently undergoes an unfolding process.

Design and Synthesis of Diaryl Ureas 1 and 2

We decided to focus on controlling the folding properties of cationic water soluble diarylureas **1** and **2** by complexation within CB[8]. Our rationale in selecting **1** and **2** for the folding experiments was manifold. First, **1** and **2** each contain a single *N*,*N*′-diarylurea unit which can adopt three different conformations (*vide infra*) of drastically different shapes (U, S or W). Of these, the U-shaped conformer is highest in energy, but perhaps most useful as a turn element in non-natural folding processes. Second, **1** and **2** contain two ammonium groups that are known to impart a high binding affinity towards members of the cucurbit[*n*]uril family of macrocycles. We anticipated that this high affinity would also result in a relatively high selectivity toward the U, S or W-shaped conformers allowing an efficient control over the folding process.

For the preparation of **1**, we protected benzidine according to the literature procedure to deliver **3** (Scheme 1) [18]. Compound **3** was transformed into



SCHEME 1 Synthesis of compounds **1** and **2**.

4 by reaction with triphosgene in CH_2Cl_2 at room temperature. Compound **4** was deprotected with trifluoroacetic acid (TFA) to yield water soluble **1** as its trifluoroacetate salt in 95% yield. For the preparation of **2**, we mono-protected diamine **5**, according to a literature procedure, to yield **6**. The reaction of **6** with triphosgene (0.5 equiv.) in CH_2Cl_2 delivered **7** in 81% yield. Compound **7** was deprotected by treatment with TFA to yield **2** as its trifluoroacetate salt in 96% yield.

Selection of CB[*n*] Molecular Containers

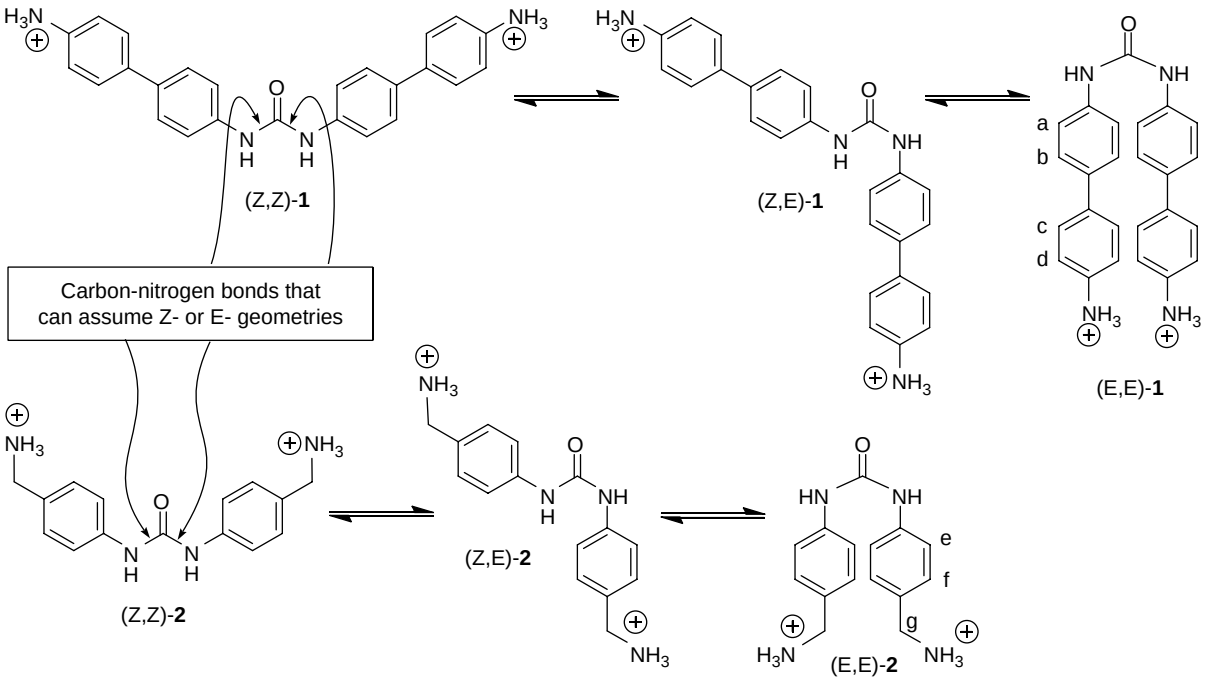
Cucurbit[*n*]uril ($n = 5–10$) molecular containers (Chart 1) are formed by the macrocyclisation of *n* glycoluril rings connected by *2n* methylene bridges [19]. CB[*n*] molecular containers are well known for their high affinity towards cationic guests (K_a up to $10^{12}\,M^{-1}$) in aqueous solution and for their exquisite selectivity (up to 10^6) based on only subtle structural changes [15,16]. For these reasons, the CB[*n*] family has been used in numerous application areas including enzyme assays, molecular machines, drug delivery, solution and gas remediation, and chemical sensors [20]. Our group recently reported that a single cationic arylene-triazene oligomer can be folded into four distinct conformations based on the presence of CB[7], CB[8] and CB[10] [8]. Of high relevance to the results reported in this paper on the controlled folding of diarylureas inside CB[8] are the reports of Kim who has shown that CB[8] enhances charge-transfer interactions between electron donor–electron acceptor pairs covalently connected by flexible spacers though an enforced folding process [21].

Enumeration of the Conformations Available to 1 and 2

Compounds **1** and **2** each contain a single *N,N′*-diaryl urea substructure. These *N,N′*-diaryl urea functional groups each contain two C–N bonds that can theoretically exist as either *E*- or *Z*-rotamers. Accordingly, there are four (2^2) possible conformations of **1** and **2** (*E,E*-; *E,Z*-; *Z,E*- and *Z,Z*-) of which three are unique. Scheme 2 enumerates the three conformations available to both **1** and **2**. Given the preference of *N,N′*-diphenyl ureas to assume the (*Z,Z*)- and (*Z,E*)-conformations [22, 23] we hoped to be able to use the binding energy of complexation within CB[8] to drive the formation of the CB[8]·(*E,E*)-**1** and CB[8]·(*E,E*)-**2** folded structures.

Compounds 1 and 2 Exhibit a Preferred Conformation

Before proceeding to study the ability of CB[8] to control the folding of **1** and **2** we sought to determine their uncomplexed conformational preferences in solution. The ^{1}H NMR spectra recorded for **1** and **2** show the presence of a single set of sharp resonances for **1** and **2**. In accord with theoretical calculations [22] and the known fast conformational interconversion of *E*- and *Z*-urea rotamers [23] the ^{1}H NMR



SCHEME 2 Three distinct conformations are available to **1** and **2**.

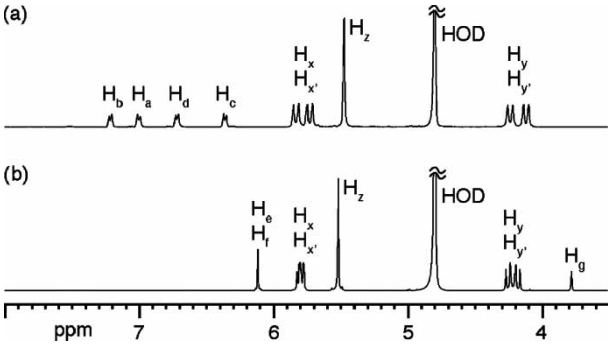


FIGURE 1 ^{1}H NMR spectra (D_2O, RT) recorded for equimolar mixtures of: (a) an equimolar mixture of CB[8] and **1** and (b) an equimolar mixture of CB[8] and **2**.

results suggest that **1** and **2** predominately exist as mixtures of the (*Z*,*Z*)- and (*Z*,*E*)-rotamers in solution.

Complexation of CB[8] With 1 and 2

We next set out to determine the conformations of **1** and **2** in the presence of CB[8]. Fig. 1 shows the ^{1}H NMR spectra recorded for equimolar mixtures of CB[8] and **1** or **2**. Quite gratifyingly, we observed a single set of sharp resonances that lead us to conclude that a single complex with a well-defined conformation was formed in each case. Relative to uncomplexed host and guest, the ^{1}H NMR spectra of equimolar mixture of CB[8] and **1** and CB[8] and **2** show two diagnostic features: (1) the resonances for the protons on the aromatic rings of **1** and **2** undergo substantial upfield shifts and (2) two pairs of doublets are observed for the diastereotopic CH_2 groups of CB[8]. These observations establish that the aromatic rings of **1** and **2** are symmetrically included in the cavity of CB[8] such that the top and bottom ureidyl C═O portals of CB[8] become non-equivalent. In the sections below, we examine the influence of host:guest stoichiometry on the complexes formed and determine the absolute stoichiometry by diffusion ordered spectroscopy (DOSY) [24].

Influence of Host:guest Stoichiometry

Given the well-defined folding properties exhibited by **1** and **2** in the presence of equimolar amounts of CB[8], we wondered whether host:guest stoichiometry might influence the folding behaviour by enabling aggregates of different absolute stoichiometry. Somewhat surprisingly, we found that the complex formed at a 1:1 ratio of CB[8]:**1** was surprisingly robust and did not change as the stoichiometry changed from 2:1 to 1:2 suggesting a high thermodynamic stability (Supporting Information). In line with our expectations, we observed significant changes in the ^{1}H NMR spectra recorded for mixtures of CB[8] and **2** as the relative

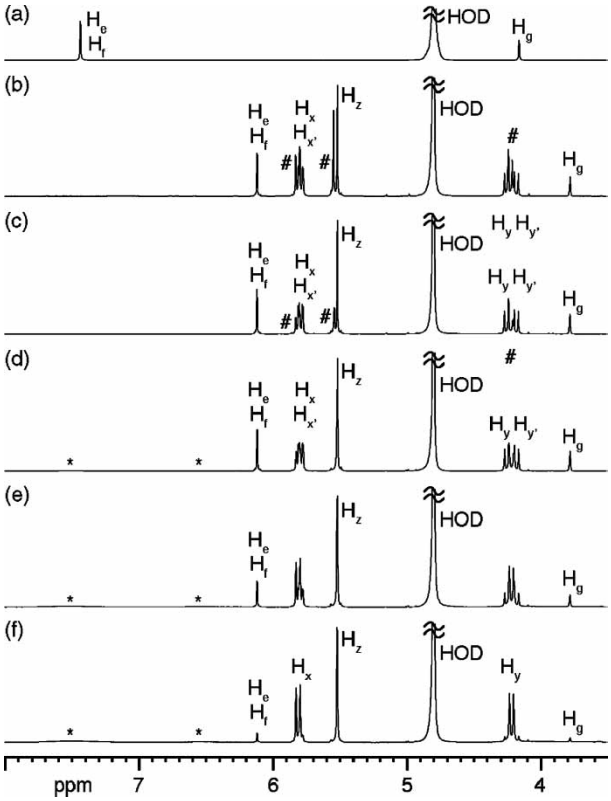
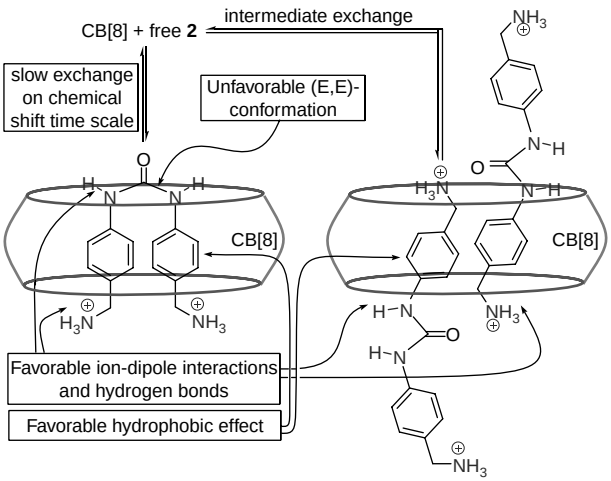


FIGURE 2 ^{1}H NMR spectra (500 MHz, D_2O, RT) recorded for mixtures of CB[8] and **2** of different relative stoichiometry: (a) 0:1, (b) 2:1, (c) 1.5:1, (d) 1:1, (e) 1:1.5 and (f) 1:2. The asterisk indicates the location of the resonances for CB[8]·$\mathbf{2}_2$ undergoing dynamic exchange. Resonances marked with a # symbol arise from uncomplexed CB[8].

stoichiometry is changed from 2:1 to 1:2 (Fig. 2). Interestingly, the two pairs of doublets previously observed for the two different pairs of diastereotopic CH_2-groups on the top and bottom of the CB[8] macrocycle change to a single pair of doublets which suggests that a symmetric complex of CB[8]·$\mathbf{2}_2$ stoichiometry builds up as the CB[8]:**2** stoichiometry



SCHEME 3 Representations of the geometries for CB[8]·(*E*,*E*)-**2** and CB[8]·(*Z*,*Z*)-$\mathbf{2}_2$ and their interconversion via free CB[8] and **2**.

increases. Also of interest are the guest resonances (H_e, H_f and H_g) which broaden dramatically and shift downfield as the CB[8]:**2** stoichiometry increases to 1:2. To rationalise these observations, we postulate an equilibrium between CB[8]·(*E,E*)-**2**, CB[8]·(*Z,Z*)-$\mathbf{2}_2$ and free **2** wherein the rate of exchange between CB[8]·(*E,E*)-**2**, CB[8] and free **2** is slow on the chemical shift timescale but the rate of exchange between CB[8]·(*Z,Z*)-$\mathbf{2}_2$, CB[8] and free **2** is in the intermediate exchange regime. This set of equilibria are summarised graphically in Scheme 3. When there is excess CB[8] relative to **2**, each molecule of **2** forms a 1:1 complex (CB[8]·**2**) with excess CB[8] remaining in its uncomplexed form. Within this 1:1 complex, urea **2** pays the energetic cost of adopting the (*E,E*)-**2** conformation by maximising the number of H-bonds and ion–dipole interactions to CB[8] and by increasing the hydrophobic driving force. In this manner, the conformation of **2** is sensitive to environmental conditions in the form of the presence and relative concentration of CB[8].

Determination of Host:guest Stoichiometry by Diffusion Ordered Spectroscopy (DOSY)

Although one can accurately determine the *relative stoichiometry* of a non-covalent aggregate by integration of the ^{1}H NMR resonances of each of its components (e.g. 1:1, 2:2, 3:3, … *n*:*n*), the determination of absolute stoichiometry requires advanced techniques. For example, cold-spray ionisation and electrospray ionisation mass spectrometry can be used to determine the molecular weight of a non-covalent structure although interpretation may be ambiguous given that the measurements take place on the desolvated structures in the gas phase [25]. Equilibrium solution phase molecular weight determination techniques include vapour pressure osmometry [26] and analytical ultracentrifugation [27] but these techniques impose their own measurement concentration regimes which may not correspond to those conditions used in the NMR measurements. Accordingly, we turned to diffusion ordered spectroscopy (DOSY) [24]—which allows one to determine the diffusion coefficients (D_s) and thereby estimate molecular size—to determine the absolute stoichiometry of the complexes between CB[8] and **1** or **2**. To provide a reliable internal standard for these measurements, we used the CB[8]·**8** complex which was previously characterised by X-ray crystallography [16]. Fig. 3a shows a plot of intensity versus gradient field strength for a equimolar mixture of CB[8]·**8** and CB[8]$_n$·$\mathbf{2}_n$. Fitting these curves into the theoretical equation [24] (equation (1)) allowed us to extract diffusion coefficients for CB[8]·**8** ($D_s = 2.74 \times 10^{-10}\,m^2\,s^{-1}$) and CB[8]$_n$·$\mathbf{2}_n$ ($D_s = 2.72 \times 10^{-10} m^2\,s^{-1}$). In equation 1, I and I_0 are signal intensities, D is the diffusion coefficient, γ is the gyromagnetic ratio, g is the gradient strength, δ is the length of the gradient and Δ is the diffusion time. The ratio of these values of D_s is 0.99 which indicates they have comparable molecular size which allows us to formulate the aggregate formed between CB[8] and **2** under conditions of excess CB[8] as CB[8]·**2**. When the CB[8]:**8**:**2** ratio is 2:1:2 (Fig. 3b) the ratio of diffusion constants for CB[8]·**8** ($D_s = 2.79 \times 10^{-10}\,m^2\,s^{-1}$) and CB[8]·$\mathbf{2}_2$ ($D_s = 2.44 \times 10^{-10}\,m^2\,s^{-1}$) is consistent with the formulation of a 1:2 stoichiometry for CB[8]·$\mathbf{2}_2$. A strikingly different result was obtained when an equimolar mixture of CB[8] and **1** was analysed by DOSY (Fig. 3c). The diffusion constants for CB[8]·**8** ($D_s = 2.64 \times 10^{-10}\,m^2\,s^{-1}$) and CB[8]$_n$·$\mathbf{1}_n$ ($D_s = 1.80 \times 10^{-10}\,m^2\,s^{-1}$) in this experiment are quite different (ratio = 0.68) which indicates that $n > 1$ for this species. For roughly spherical molecules, the theoretical ratio of diffusion constants of monomer to dimer and monomer to trimer are 0.79 and 0.69, respectively, which suggests that $n = 3$ for CB[8]$_n$·$\mathbf{1}_n$. Such an analysis, however, neglects the influence of the rod-like geometry anticipated for CB[8]$_2$·$\mathbf{1}_2$ on its diffusion coefficient [28]. For rod-like dimers, the theoretical ratio of diffusion constants for monomer to rod-like dimer span the range 0.67–0.72 depending on its length to width ratio (Supporting Information).

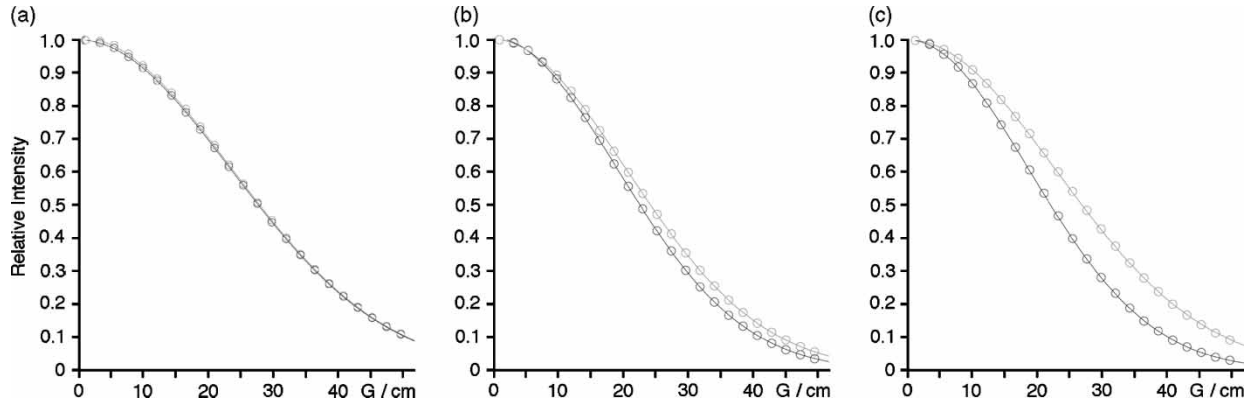


FIGURE 3 Plots of signal intensity versus field gradient and fitting lines which allowed extraction of the diffusion coefficients for mixtures of: (a) CB[8]·**8** (o) and CB[8]·**2** (o), (b) CB[8]·**8** (o) and CB[8]·$\mathbf{2}_2$ (o), and (c) CB[8]·**8** (o) and CB[8]$_2$·$\mathbf{1}_2$ (o).

Accordingly, we formulate the aggregate obtained with CB[8] and **1** as CB[8]$_2$·**1**$_2$. The observation of a single resonance for the NH protons of guest **1** in the ^{1}H NMR spectrum of CB[8]$_2$·**1**$_2$ recorded in H_2O:D_2O (9:1) supports the further formulation of this complex as CB[8]$_2$·(*Z*,*Z*)-**1**$_2$.

$$I = I_0 e^{-D\gamma^2 g^2 \delta^2 (\Delta - \delta/3)} \quad (1)$$

Molecular Modelling

Unfortunately, we were unable to obtain X-ray crystal structures for either the CB[8]$_2$·(*Z*,*Z*)-**1**$_2$ or CB[8]·(*E*,*E*)-**2**, so we turned to molecular modelling to elucidate the structural features of these complexes (Supporting Information). The geometries depicted schematically in Scheme 3 for CB[8]·(*E*,*E*)-**2** and CB[8]·(*Z*,*Z*)-**2**$_2$ are based on those obtained from MMFF calculations. Fig. 4 shows a depiction of the geometry of CB[8]$_2$·(*Z*,*Z*)-**1**$_2$ and CB[8]·(*E*,*E*)-**1** based on the results of MMFF calculations.

Compound 2 Changes Shape in Response to Chemical Stimuli

Given the ability of CB[8] to stabilise the (*E*,*E*)-conformer of **2**—the thermally least favourable conformer—we wondered whether it would be possible to forcibly unfold (*E*,*E*)-**2** by the addition of competitive guests. We first studied the influence of **8** as a competitive guest since **8** is known to form a very tight CB[8]·**8** complex ($K_a = 1.1 \times 10^{11}\,M^{-1}$) and undergoes slow exchange on the chemical shift timescale which allows us to use ^{1}H NMR as the analytical tool to monitor the process [16]. Fig. 5 shows the ^{1}H NMR spectra recorded as a solution of CB[8]·(*E*,*E*)-**2** is treated with **8** (0, 0.67, 1.33 equiv.). The ^{1}H NMR spectra clearly indicate that the addition of **8** results in the ejection of (*E*,*E*)-**2** from the cavity of CB[8] by the formation of a very strong CB[8]·**8** complex. Once **2** is free in solution it returns to its equilibrium mixture of the (*Z*,*Z*)-**2** and (*Z*,*E*)-**2** conformers. In this process, the binding free energy associated with the formation of CB[8]·**8** acts as a stimulus to trigger the unfolding of **2**.

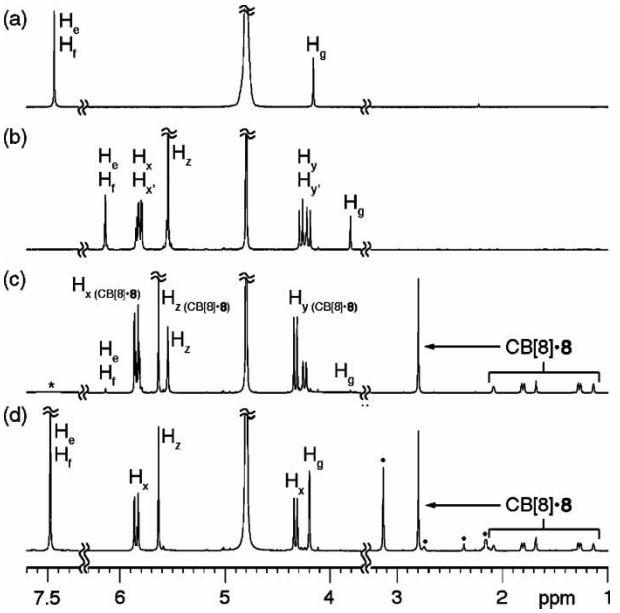


FIGURE 5 ^{1}H NMR spectra (500 MHz, D_2O, RT) recorded for: (a) **2**, (b) CB[8]·(*E*,*E*)-**2**, (c) after addition of 0.67 equiv. **8** and (d) after addition of 1.33 equiv. of **8**. The resonances marked with an asterisk result from intermediate exchange within the CB[8]·**2**$_2$ complex. The resonances marked with a bullet (·) arise from uncomplexed **8**.

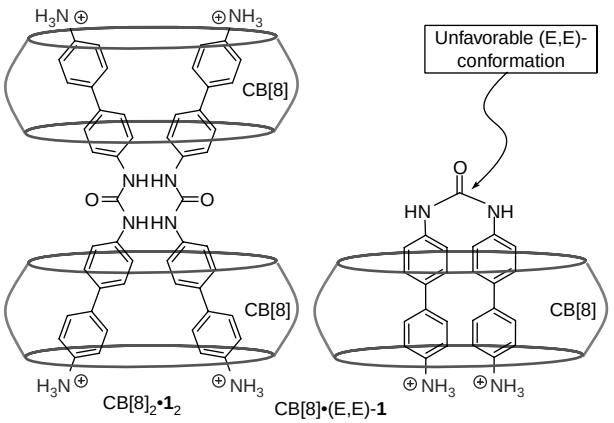


FIGURE 4 Depiction of the geometry of CB[8]$_2$·(*Z*,*Z*)-**1**$_2$ and CB[8]·(*E*,*E*)-**1** based on MMFF calculations.

The Dissociation of CB[8]$_2$·**1**$_2$ Is Triggered by Benzidine

Given the stability of CB[8]$_2$·**1**$_2$ over a broad range of CB[8]:**1** stoichiometries we wondered whether it would be possible to dissociate this aggregate by the addition of cationic guests as competitors. Initially, we studied the addition of **8** as competitor. Given the excellent size, shape and electrostatic match between CB[8] and **8**, and the accordingly high thermodynamic stability of the CB[8]·**8** complex ($K_a = 1.1 \times 10^{11}\,M^{-1}$), it was not surprising that addition of 1.33 equiv of **8** results in the complete dissociation of the CB[8]$_2$·**1**$_2$ complex under formation of CB[8]·**8** (Supporting Information). Somewhat more surprisingly, we found that benzidine **9** is also effective at triggering the dissociation of CB[8]$_2$·**1**$_2$. Fig. 6(a) shows the ^{1}H NMR spectrum recorded for a 1:4 mixture of CB[8] and **9** which establishes that CB[8] readily forms the 1:2 complex CB[8]·**9**$_2$ with a slow exchange with free **9** on the chemical shift timescale. Fig. 6(b)–(d) shows the ^{1}H NMR spectra that result upon the addition of **9** (0, 1, 2 equiv). Remarkably, the addition of a mere 2 equiv of **9** results in the quantitative formation of CB[8]·**9**$_2$

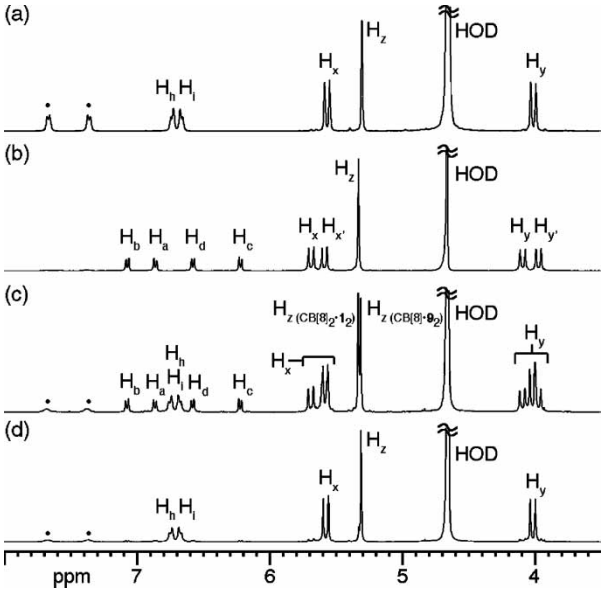


FIGURE 6 ^{1}H NMR spectra (400 MHz, D_2O, RT) recorded for: (a) CB[8]·$\mathbf{9}_2$ and excess **9**, (b) CB[8]$_2$·(*Z*,*Z*)-$\mathbf{1}_2$, (c) after addition of 1 equiv. of **9** and (d) after addition of 2 equiv. **9**. Uncomplexed **1** is insoluble in D_2O and forms a precipitate. Resonances for uncomplexed **9** are marked with a bullet (·).

and concomitant with the release of free **1**. To gain insight into the efficiency of **9** as a competitor we performed MMFF calculations of the geometry of CB[8]·$\mathbf{9}_2$ (Supporting Information). Fig. 7 shows a representation of the geometry of CB[8]·$\mathbf{9}_2$ based on the calculations. Interestingly, the two equivalents of **9** are skewed with respect to the C8-axis of CB[8] presumably to maximise cation–dipole interactions between the four ^+H_3N-groups on the two equivalents of **9** and the ureidyl carbonyl rims of CB[8]. The two equivalents of **9** assume a crossed geometry probably to minimise electrostatic repulsion between the ^+H_3N-groups. The fact that we do not observe the CB[8]·**9** complex in these experiments suggests cooperativity in the formation of CB[8]·$\mathbf{9}_2$. We attribute the ability of two equivalents of **9** to act as an efficient competitor in the dissociation of CB[8]$_2$·(*Z*,*Z*)-$\mathbf{1}_2$ to its ability to form four good $^+H_3N\cdots O{=}C$ cation–dipole interactions per molecule of CB[8]

CONCLUSIONS

We have designed and synthesised diarylureas **1** and **2** whose conformational space consists of (*E*,*E*)-, (*Z*,*E*)- and (*Z*,*Z*)-conformers. The (*Z*,*Z*)- and (*Z*,*E*)-conformers are populated at room temperature; the (*E*,*E*)-conformer is ≈ 5 kcal mol^{-1} higher in energy. We find that the presence of CB[8] selectively stabilises the (*Z*,*Z*)-**1** conformation during the formation of CB[8]$_2$·(*Z*,*Z*)-$\mathbf{1}_2$ whose absolute stoichiometry was established by DOSY measurements.

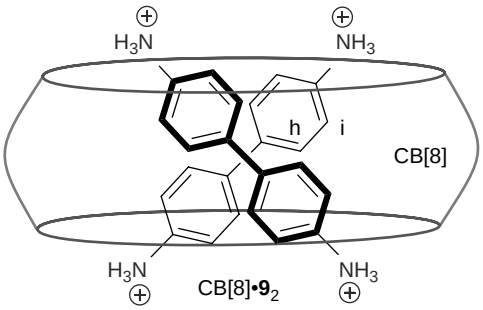


FIGURE 7 Representation of the geometry of CB[8]·$\mathbf{9}_2$ based on MMFF calculations.

CB[8]$_2$·(*Z*,*Z*)-$\mathbf{1}_2$ is stable over a broad range of CB[8]:**1** stoichiometries. In contrast, compound **2** selectively populates the (*E*,*E*)-**2** conformer when the CB[8]:**2** stoichiometry is 1: <1 due to the formation of CB[8]·(*E*,*E*)-**2**. At higher CB[8]:**2** stoichiometries, (*E*,*E*)-**2** undergoes an unfolding process during the formation of CB[8]·$\mathbf{2}_2$. Both CB[8]$_2$·(*Z*,*Z*)-$\mathbf{1}_2$ and CB[8]·(*E*,*E*)-**2** respond to chemical stimuli in the form of competitive guests **8** and **9** by releasing their guests which subsequently undergo unfolding processes to form the equilibrium population of conformers. We attribute the different behaviour of **1** and **2** (e.g. 2:2 versus 1:1 complexation) to the different lengths of the spacing group between the ^+H_3N-groups of **1** and **2**. The linking unit in **2** is too short to allow the formation of CB[8]$_2$·(*Z*,*Z*)-$\mathbf{2}_2$.

Previous work has shown that it is possible to selectively populate a single member of a nearly isoenergetic conformational ensemble by complexation within molecular containers or with other chemical stimuli [5,8]. In this work, we demonstrate that it is possible to select for otherwise unobserved conformations (e.g. (*E*,*E*)-**2**) by application of CB[*n*] molecular containers. In this instance, the very high binding affinities (K_a up to 10^{12} M^{-1}) and very high selectivities (K_{rel} up to 10^6) readily obtained with CB[*n*] molecular containers provide the driving free energy needed to stabilise these otherwise unfavourable conformers [15,16]. Although we have only selectively stabilised the U-shaped conformer of **2** (e.g. (*E*,*E*)-**2**) it should be possible to functionalise the ^+H_3N-groups of **2** with biological oligomers (e.g. peptides or oligonucleotides) whose intramolecular folding would be responsive to the presence of CB[8]. In such a situation, the ability to trigger unfolding upon application of chemical stimuli (e.g. **8** or **9**) could enable a wide range of applications.

EXPERIMENTAL SECTION

General experimental details have been published previously [16]. Compound **3** was prepared by the literature procedure [18]. Starting materials were

obtained from commercial suppliers and used without further purification. Supporting Information is available upon request from the authors.

Compound 4

Triphosgene (15.0 mg, 0.051 mmol) was added cautiously in small portions (exothermic reaction) to a stirred solution of compound **3** (80.0 mg, 0.28 mmol) and Et_3N (0.10 ml, 0.62 mmol) in CH_2Cl_2 (1 ml) at RT under N_2. After 4 h the solid was filtered, washed with CH_2Cl_2 (2 × 10 ml) and concentrated. Recrystallisation from $CHCl_3$/hexanes gave **4** as an off-white solid (60.0 mg, 0.10 mmol, 66%). Mp 270°C dec. TLC ($CHCl_3$/MeOH, 25:1) R_f 0.28. IR (KBr, cm^{-1}): 3330s, 2963m, 2929s, 2853m, 1698s, 1664m, 1584s, 1512s, 1327m, 1276s, 1236s, 1166s, 1058m. ^1H NMR (400 MHz, DMSO-d_6): δ 9.40 (s, 2H), 8.75 (s, 2H), 7.60–7.50 (m, 16H), 1.48 (s, 18H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 152.8, 152.4, 138.7, 138.5, 133.5, 133.3, 126.4, 126.2, 118.5, 118.4, 79.1, 28.1. MS (FAB, Magic Bullet): *m/z* 595 (100, $[M + H]^+$). HRMS (FAB, Magic Bullet/PEG): *m/z* 595.2921 ($[M + H]^+$, $C_{35}H_{39}N_4O_5$, calcd 595.2920).

Compound 1

Compound **4** (33.0 mg, 0.055 mmol) was dissolved in TFA:CH_2Cl_2 (1:1) (1 ml) and stirred at RT for 4 h. Concentration and drying at high vacuum afforded **1** (33.0 mg, 0.053 mmol, 96%) as a pale yellow solid. Mp >300°C. IR (KBr, cm^{-1}): 3332w, 2924m, 2617w, 1670s, 1600s, 1548s, 1501s, 1432w, 1318w, 1204s, 1137s. ^1H NMR (400 MHz, DMSO-d_6): δ 9.17 (s, 2H), 7.65 (d, $J = 8.4$ Hz, 4H), 7.58 (m, 8H), 7.21 (d, $J = 8.4$ Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.6 (q, $^2J_{CF} = 34.4$ Hz), 139.4, 136.8, 134.7, 132.6, 127.2, 126.8, 121.4, 118.6, 116.3 (q, $^2J_{CF} = 292.9$ Hz). MS (FAB, Magic Bullet): *m/z* 394 (100, $[M - 2CF_3COOH]^+$). HRMS (FAB, Magic Bullet/PEG): *m/z* 394.1801 ($[M - 2CF_3COOH]^+$, $C_{25}H_{22}N_4O$, calcd 394.1794).

Compound 6

To a stirred solution of 4-aminobenzylamine (2.00 g, 16.4 mmol) in anhydrous THF (15 ml), *N*,*N*-diisopropylethylamine (3.25 ml, 19.7 mmol) was added and the reaction mixture was cooled to 0°C [29]. Di-*tert*-butyldicarbonate (3.57 g, 16.4 mmol) was added in one portion and the stirring was continued for 4 h at 0°C. Then the reaction mixture was allowed to warm to RT and stirring was continued for another 12 h. The solid was filtered, the filtrate was evaporated under reduced pressure, the residue was dissolved in toluene (100 ml), washed with brine (1 × 20 ml), 0.1 N KOH (1 × 20 ml), brine (1 × 20 ml) and dried over $MgSO_4$. The solvent was evaporated to yield the crude product, which was recrystallised from $CHCl_3$/hexanes to yield **6** as a white solid (2.50 g, 11.3 mmol, 69%). The ^1H NMR data match those reported in the literature [29].

Compound 7

Triphosgene (190 mg, 0.64 mmol) was added cautiously in small portions (exothermic reaction) to a stirred solution of compound **6** (0.90 g, 4.05 mmol) and Et_3N (1.25 ml, 8.91 mmol) in CH_2Cl_2 (8 ml) at RT under N_2 [30]. After 4 h, the solvent was evaporated to dryness under reduced pressure and the residue was resuspended in EtOAc (15 ml). The white solid ($Et_3N{\cdot}HCl$) was filtered and washed well with EtOAc (3 × 10 ml). The combined filtrate was washed with water (2 × 15 ml), brine (1 × 15 ml) and dried over anh. Na_2SO_4. The solvent was evaporated to afford the crude product (750 mg). Column chromatography (SiO_2, $CHCl_3$/MeOH 50:1) gave compound **7** (740 mg, 1.57 mmol, 81%) as a yellow solid. Mp 204–205°C. TLC ($CHCl_3$/MeOH, 25:1) R_f 0.32. IR (KBr, cm^{-1}): 3346m, 2978m, 2931w, 1695s, 1601s, 1546s, 1514s, 1366m, 1238m, 1169s. ^1H NMR (400 MHz, DMSO-d_6): 8.58 (s, 2H), 7.36 (d, $J = 8.4$ Hz, 4H), 7.31 (t, $J = 6.0$ Hz, 2H), 7.12 (d, $J = 8.4$ Hz, 4H), 4.04 (d, $J = 6.0$ Hz, 4H), 1.38 (s, 18H). ^{13}C NMR (100 MHz, DMSO-d_6): 155.8, 152.5, 138.3, 133.5, 127.5, 118.1q, 77.7, 43.0, 28.3. MS (FAB, Magic Bullet/Li): *m/z* 477 (100, $[M + Li]^+$). HRMS (FAB, Magic Bullet/Li/PEG): *m/z* 477.2710 ($[M + Li]^+$, $C_{25}H_{34}N_4O_5Li$, calcd 477.2689).

Compound 2

Compound **7** (710 mg, 1.51 mmol) was dissolved in TFA:CH_2Cl_2 (1:1) (16 ml) and stirred at RT for 4 h. Concentration and drying at high vacuum afforded **2** (710 mg, 1.43 mmol, 95%) as a pale yellow solid. Mp 124–125°C. IR (KBr, cm^{-1}): 3274w, 2925m, 1674s, 1605w, 1542m, 1521m, 1317m, 1240m, 1197m, 1138m. ^1H NMR (400 MHz, D_2O, TMSP as external reference): δ 7.45 (s, 8H), 4.17 (s, 4H). ^{13}C NMR (100 MHz, D_2O, 1,4-dioxane as ext. reference): δ 163.1 (q, $^2J_{CF} = 35.1$ Hz), 155.7, 138.7, 129.9, 128.1, 121.4, 116.4 (q, $^2J_{CF} = 290.3$ Hz), 42.7. MS (FAB, Magic Bullet): *m/z* 271 (52, $[M - 2CF_3COOH + H]^+$), 254 (100, $[M - 2CF_3COOH - NH_2]^+$). HRMS (FAB, Magic Bullet/PEG): *m/z* 271.1566 ($[M - 2CF_3COO + H]^+$, $C_{15}H_{19}N_4O$, calcd 271.1559).

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