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Self-assembled tetrazine cryptophane for ion pair recognition and guest release by cage disassembly†

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Hereby, we describe the synthesis of a self-assembled *syn*-cryptophane using dynamic nucleophilic aromatic substitution of tetrazines. ^{1}H NMR cage titrations reveal that the tetramethylammonium cation binds under slow exchange conditions while counter-anions show a fast exchange regime. Finally, the cryptophane can be disassembled by the addition of thiols allowing guest release.

Cryptophanes are a class of cage molecules that are built from two cyclotriveratrylene (CTV) moieties usually linked by alkylene-dioxy arms,[1] although other linkers such as disulfide bridges,[2] *p*-phenylene groups[3] or triazole rings[4] have been reported. These molecular hosts attract great interest as they display remarkable recognition properties toward small molecules like epoxides,[5] methane and halogenomethane,[2a,6] anions,[7] cations,[8] or even atoms like radon or xenon.[7b,9] However, cryptophanes have not yet been used for ion-pair recognition due to their homotopicity. Promising related applications are currently being investigated; for instance, the development of hyperpolarized ^{129}Xe biosensors for MRI,[10] the design of devices for methane sensing,[11] and the trapping of radioactive Cs^+ found in nuclear waste.[12] Both covalent and self-assembled cryptophane cages[13] have been reported and the photoswitching of metallo cryptophanes has also been described; however, without the release of a guest.[14] Nevertheless, controlled guest release triggered by the disassembly of a cryptophane host remains unexplored. To avoid the possible dissociation of cryptophane-like coordination cages at low concentrations, which could limit their applications, we turned our attention to the construction of covalent cryptophane cages by subcomponent self-assembly using reversible covalent bonds. We hypothesized that a cryptophane bearing tetrazine units as linkers should solve both issues, *i.e.* heterotopicity and controlled guest release, simultaneously. Indeed, 1,2,4,5-tetrazines are known to bind cations[15] by nitrogen lone pairs and anions[16] through anion–π interactions. Thus, their embedment in a receptor should raise ion pair recognition. Moreover, the dynamic nucleophilic aromatic substitution of tetrazines (S_NTz), recently reported by the group of Carrillo, facilitates their assembly into a functional receptor and the subsequent addition of thiols was shown to promote their disassembly.[17] Intrigued by the versatility of such a simple building block, we therefore set out to investigate its incorporation into a heteroditopic self-assembled cryptophane for ion pair recognition, capable of guest release using a simple chemical stimulus. The dynamic nature of dative bonds has already been employed for guest release in metallacages;[18] however, this is the first example of a covalent cage using S_NTz. In this communication, we describe the synthesis of a tris-tetrazine *syn*-cryptophane **3**, the binding of the tetramethylammonium cation in slow exchange, together with the recognition of a counter anion in fast exchange harnessed by anion–π and coulombic interactions. Finally, on-demand guest release was achieved by the disassembly of the receptor using alkanethiols (Scheme 1).

The synthesis of cryptophane **3** is straightforward and involves the reaction of two equivalents of *rac*-CTV-OH **1** and three equivalents of dichloro-*s*-tetrazine **2** in acetonitrile in the presence of triethylamine at room temperature for 3.5 h (15% yield) (Scheme 2). Cryptophane **3** was fully characterized by NMR and HRMS techniques (see ESI†). The ^{1}H NMR spectrum of **3** in acetonitrile-d_3 displays OMe protons (c) as a singlet at 3.58 ppm, two AB doublets for the H_e and H_a protons at 3.73 and 4.87 ppm, respectively, and finally aromatic protons (b) at 7.08 ppm and (a) at 7.42 ppm (Fig. 2(b), 0.0 equiv.). It is important to note that cryptophane **3** was obtained exclusively as the achiral *syn*-diastereomer after silica gel chromatography. Experiments starting from enantiopure CTV **1** did not gave any trace of *anti*-cryptophane, but led to the formation of oligomers. Single crystals

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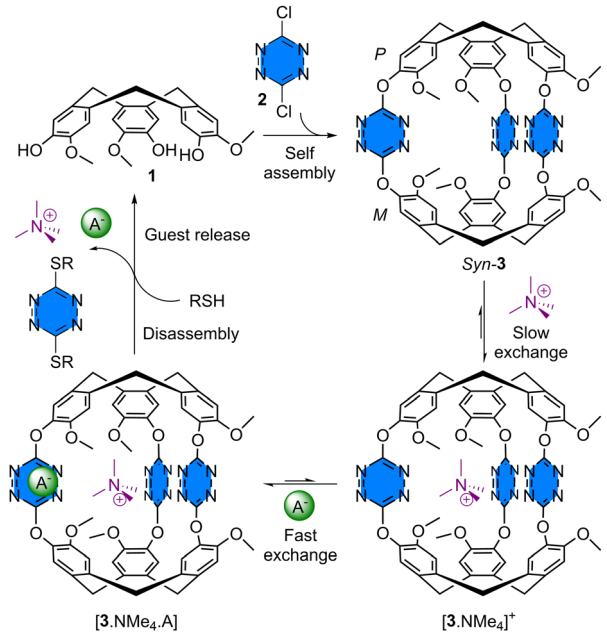
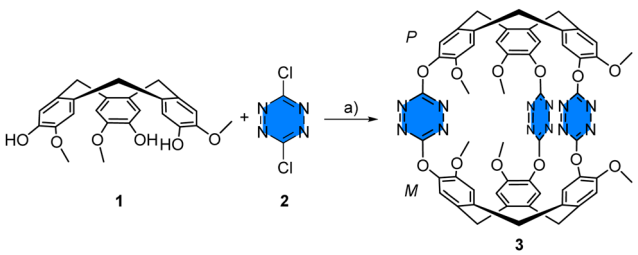


Scheme 1 Self-assembly of cryptophane **3** using CTV-OH **1** and dichloro-*s*-tetrazine **2**. Receptor **3** is then able to recognize ion pairs with different exchange dynamics and finally the disassembly is made possible using alkanethiols.



Scheme 2 Synthesis of cryptophane **3**. (a) Conditions: Et_3N, acetonitrile, r.t., 3.5 h, 15% yield.

of **3** were grown from slow evaporation of an acetonitrile solution at 4 °C. The resolution is only partial, as the crystals degrade due to fast desolvatation and the cif file could not pass the checkcif process. However, the resolution was sufficient enough to assess the stereochemistry of cryptophane **3** (Fig. 1). Optimization of the *syn*-cryptophane **3** was also performed by DFT calculations in the gas phase (Fig. S2, ESI†) and is in accordance with the partial structure determined by X-ray analysis.

Next, we evaluated the affinity of cryptophane **3** for the tetramethylammonium chloride (NMe_4Cl) ion pair. A solution of **3** (0.79 mM) was titrated by aliquots of an NMe_4Cl solution (19 mM) in acetonitrile-d_3 followed by ^{1}H NMR (Fig. 2(b)). From this titration, we first observed the appearance of a new set of signals corresponding to the formation of a complex in slow exchange compared to the NMR time scale. Such behavior is expected for the recognition of tetramethylammonium by cryptophanes.[1c] Cryptophane **3** can interact with cations, not only through the usual cation–π interactions with the CTV unit, but also engage in

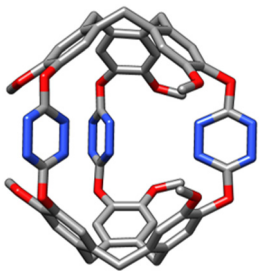


Fig. 1 X-ray diffraction structure of **3**. H atoms are omitted.

additional interactions with the lone pair of the tetrazine's nitrogens.[15] Protons corresponding to $[\mathbf{3}{\cdot}NMe_4]^+$ can be observed at 3.50 ppm for protons (c), at 3.76 and 4.89 ppm for protons H_e and H_a respectively and at 7.09 and 7.48 ppm for aromatic protons (b) and (a), after the addition of one equivalent of NMe_4Cl. A binding constant of K_a = 460 was calculated for the recognition of the tetramethylammonium cation considering a 1 : 1 stoichiometry. Following the addition of more aliquots (Fig. 2(b)), the signals (a) and (b) corresponding to the complex $[\mathbf{3}{\cdot}NMe_4]^+$ are shifted towards lower fields, corresponding to the formation of a second species, a ternary complex with chloride $[\mathbf{3}{\cdot}NMe_4Cl]$. These shifts are modest; however, they support the recognition of the anion. By plotting the variation of the $[\mathbf{3}{\cdot}NMe_4Cl]$ chemical shifts against the host guest ratio, we obtained a binding constant of K_a = 124 considering a 1 : 1 stoichiometry (Fig. S9, ESI†). This behavior is remarkable as it accounts for following the recognition of ion pairs using two different ^{1}H NMR probes and thus allows the determination of the two binding constants separately without additional experiments. In addition, the DFT optimized structure of $[\mathbf{3}{\cdot}NMe_4Cl]$ gives valuable information on the interactions stabilizing the complex. Multiple short contacts are observed between the tetramethylammonium guest and the aromatic rings of the CTVs, which can be described as cation π-interactions (d_{H-C} = 2.74 and 2.78 Å), as well as with the nitrogen of the tetrazines (d_{H-N} = 2.14, 2.44 and 2.38 Å) (Fig. S3, ESI†). An additional interaction between the chloride and the tetrazine (d_{C-N} = 3.27 Å) can be observed characteristic of an anion–π interaction (Fig. 3).

In order to get more insights into the two equilibria, we decided to titrate cryptophane **3** with tetramethylammonium acetate (NMe_4OAc), providing a counter anion with a trigonal geometry. Satisfyingly, the ^{1}H NMR titration also shows a slow exchange compared to the NMR time scale characterized by the appearance of a new set of signals upon the addition of the titrating solution (Fig. 2(c)). Integration of the chemical shifts corresponding to the complex and the free cryptophane **3** gives a binding constant of K_a = 2000 for NMe_4^+ (1 : 1 stoichiometry). The difference with the binding constant obtained with NMe_4Cl probably arises from a less tight ion pair in NMe_4OAc. After the addition of more aliquots, the signals of protons (a) and (b) shift downfield, corresponding to an ion pair ternary complex $[\mathbf{3}{\cdot}NMe_4OAc]$. At the early stage of the titration, the chemical shift of the methyl of the acetate moiety is deshielded (1.76 ppm) due to the formation of the ternary complex, but will eventually match the chemical shift of acetate in solution

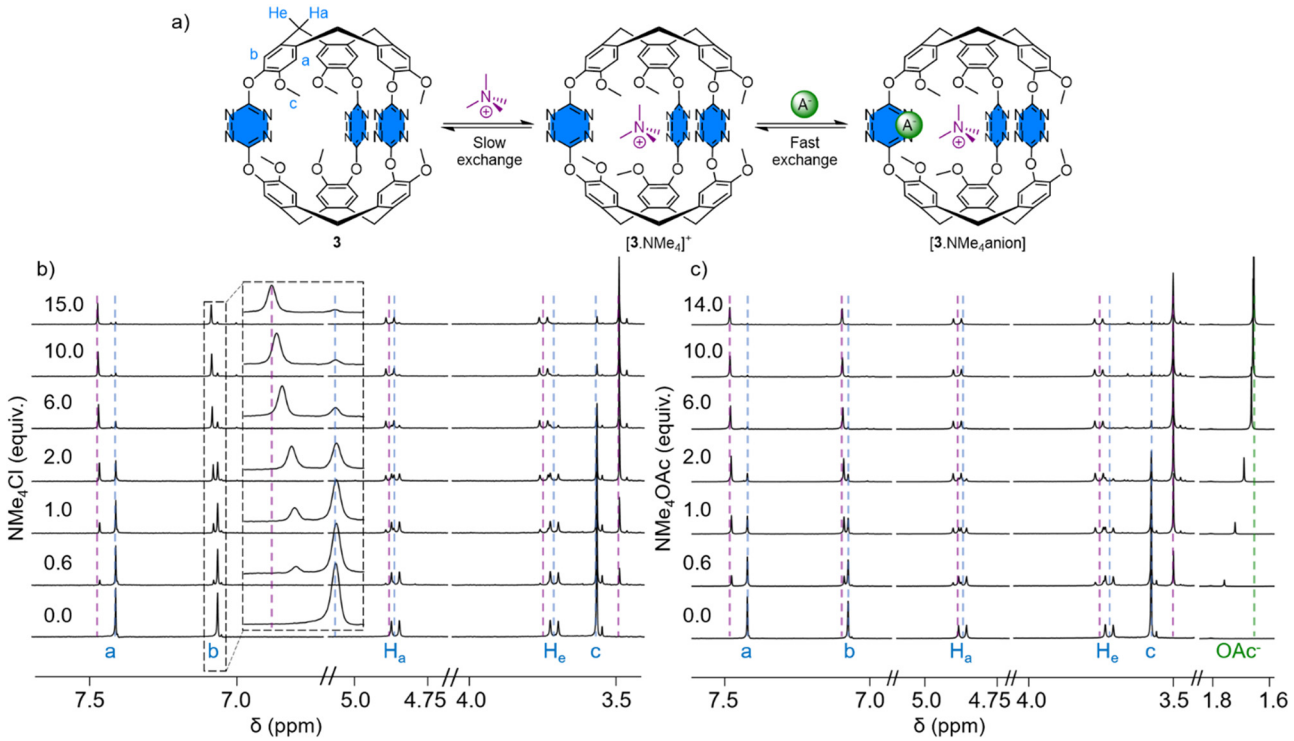


Fig. 2 (a) Equilibria for the recognition of ion pairs by cryptophane **3** and proton assignment. (b) Titration of **3** (0.79 mM) by NMe_4Cl (19 mM); inset shows the enlargement of the chemical shifts between 7.05 and 7.10 ppm and (c) titration of **3** (1 mM) by NMe_4OAc (24 mM) (500 MHz, acetonitrile-d_3, 298 K).

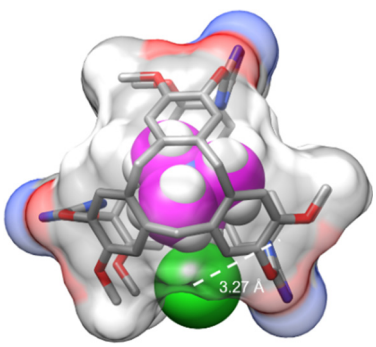


Fig. 3 DFT optimized structures of [**3**·Me_4NCl].

(1.65 ppm) due to the large number of equivalents added (14 equiv.). The binding constant K_a = 67 of $[\mathbf{3}\cdot NMe_4]^+$ for acetate was determined from the variation of the chemical shift of proton (a) assuming a 1 : 1 stoichiometry (Fig. S8, ESI†). Hence, tetramethylammonium ion pairs are recognized by cryptophane **3**, which exhibit different exchange rates depending on the nature of the ion.

Tetrazines are good acceptors for anion–π interactions, we thus decided to evaluate if the concomitant complexation of the tetramethylammonium cation was necessary for the complexation of the anion by the cryptophane cage or if the affinity of cryptophane **3** for anions remains without tetramethylammonium trapped inside. Titrations of **3** with tetrabutylammonium chloride or acetate did not show any binding event in acetonitrile-d_3 (Fig. S6 and S7, ESI†). Since anions are recognized by the cationic complex $[\mathbf{3}\cdot NMe_4]^+$ but not by **3** alone, we have clear evidence for a positive allosteric effect which could be attributed to synergistic coulombic and anion–π interactions.

Lastly, we investigated the disassembly of the cryptophane **3** taking advantage of the dynamic nucleophilic aromatic substitution of tetrazines. In the presence of alkanethiols and a base, the group of Carrillo demonstrated the disassembly of tetrazine cages.[17] However, this has never been achieved on an inclusion complex. To this end, 1-hexanethiol (10 equiv.) and triethylamine (10 equiv.) were added to an equimolar mixture of **3** and NMe_4OAC (Fig. 4). After stirring for one hour at room temperature, we observed the ^{1}H NMR signals of the CTV **1** confirming the disassembly of cryptophane **3**. In addition, the chemical shift of NMe_4^+ is at 3.00 ppm, close to the 3.15 ppm observed for NMe_4^+ in acetonitrile-d_3. This experiment demonstrates the possibility of disassembling cryptophane **3** in the presence of alkanethiol and a base leading to the release of the tetramethylammonium cation.

In conclusion, a self-assembled tetrazine cryptophane receptor **3** was obtained in one step. This achiral heteroditopic molecular cage forms an inclusion complex with the tetramethylammonium cation, which can simultaneously bind anionic species to provide [**3**·NMe_4Anion] complexes. No affinity for anionic guests without a cation inside the cavity was measured, suggesting a positive allosteric effect. This result can be explained by a synergy between anion–π, cation–π and coulombic interactions. Moreover, the slow and fast exchange kinetics observed, respectively, with cationic and anionic guests, allowed the determination of both binding constants in a single titration. Cryptophane disassembly was achieved by

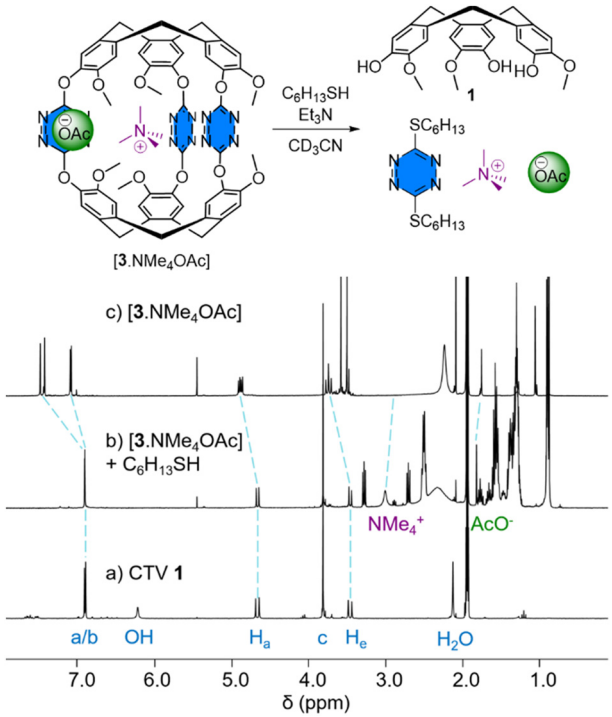


Fig. 4 Superposition of the ^{1}H NMR spectra (400 MHz, acetonitrile-d_3, 298 K) of (a) CTV **1** (see atom labelling on Fig. 1), (b) the mixture obtained after one hour of stirring of an equimolar solution of **3** (1.6 mM, acetonitrile-d_3) and NMe_4OAc (1.6 mM, acetonitrile-d_3) in the presence of 1-hexanethiol (10 equivalents) and triethylamine (10 equivalents) and (c) the [**3**·NMe_4OAc] complex.

exchange reaction in the presence of thiols and a base, triggering the release of the encapsulated guest molecule. Finally, post-functionalization of the tetrazine units *via* inverse electron demand Diels–Alder [4+2] cycloadditions would be of interest to modify the cryptophane structure. Studies are currently in progress in our group.

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Conflicts of interest

There are no conflicts to declare.

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