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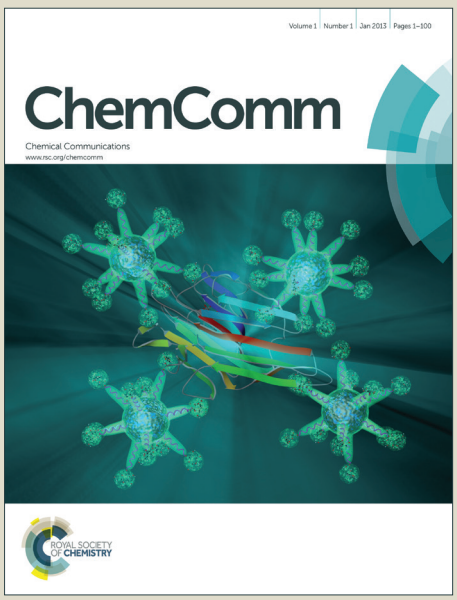

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ARTICLE TYPE

A Fluorescent Heteroditopic Hemicryptophane Cage for the Selective Recognition of Choline Phosphate

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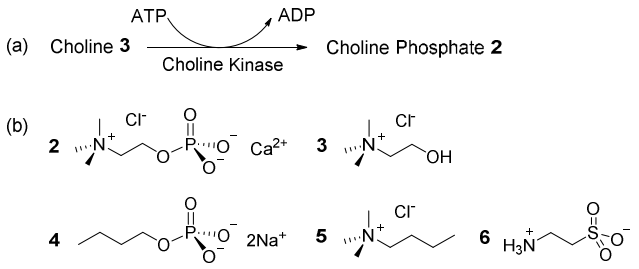
The first fluorescent hemicryptophane cage was synthesized and developed as an efficient and selective sensor for choline phosphate. The heteroditopic character of the host in the recognition process was evidenced. NMR experiments highlight a full encapsulation of the guest, inducing the chiralisation-like behavior of the achiral choline phosphate.

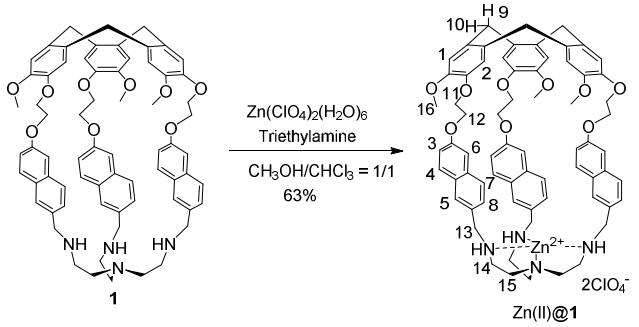
The design of artificial molecular receptors is very attractive as they can mimic biological systems such as enzymes.[1-5] A large number of bio-inspired compounds have been designed and their recognition properties towards guests of biological interest have been studied. Among the classes of molecular containers, such as calixarenes, resorcinarenes, curcubiturils, or cyclodextrines,[6] cryptophanes and hemicryptophanes both based on cyclotriveratrylene (CTV) unit have recently received a growing interest.[7,8] Cryptophanes are homotopic host compounds and have been shown to efficiently complex small molecules like methane or epoxides, or atoms like xenon. The related hemicryptophanes combining a CTV unit with another C_3 symmetrical moiety are ditopic hosts presenting recognition properties towards biological molecules like carbohydrates, ammonium and zwitterionic neurotransmitters.[9] For example, we have recently demonstrated that using ^{1}H NMR and ITC techniques, a water-soluble hemicryptophane host is able to selectively recognize choline.[9h] Despite their high efficiency and good selectivity of hemicryptophanes as receptors, the development of fluorescent hemicryptophanes for molecular recognition studies has never been described to date. However, it should be noted that fluorescence spectroscopy appears as an efficient method for the detection of biological molecules due to its high sensitivity, simple manipulation and facile visualization of bio-processes.

It is known that phosphorylated molecules play a crucial role in many biological processes.[10,11] In particular choline phosphate, generated by the conversion of choline and ATP under the choline kinase along with the production of ADP (Scheme 1a), is an important biological phosphorylated compound. It is an essential intermediate in the biosynthetic pathway of phosphatidylcholine, a major component of the membrane of all eukaryotic cells.[12,13] Thus, both effective complexation and optical detection of choline phosphate may have application as inhibitor or indicator in biological environment, allowing therefore a better understanding of this biosynthetic pathway. Moreover, the selective sensing of choline phosphate among choline may be developed as a method to monitor choline kinase activity in real time. Nevertheless, to the best of our knowledge, there is no fluorescent sensor capable of selective recognition of choline phosphate.



Scheme 1 The biological process involved in the generation of choline phosphate (a) and the guests studied in this work (b).

As literatures have revealed that metal-based sensors are particularly relevant for the sensing of phosphorylated molecules,[14] we decided to combine the recognition properties of hemicryptophanes with those of Zn(II) complexes to design a heteroditopic host for the recognition of zwitterionic choline phosphate (Scheme 2). It is anticipated that (i) the CTV unit of Zn(II)@**1** will bind the ammonium part of choline phosphate, (ii) the Zn(II) center will stabilize the phosphate part and (iii) the naphthalene fluorophores which are used as hydrophobic "walls" connecting the Zn(II) binding moiety to the CTV unit, will confer fluorescent properties of the host structure.



Scheme 2 Synthesis of the fluorescent Zn(II)@**1** complex.

Ligand **1** was first synthesized in four steps, from vanillic alcohol, with 4% overall yield, according to a previous procedure (Scheme S1).[15] Then the Zn(II) cation was coordinated by the

tris(2-aminoethyl)amine (tren) unit via a reaction between ligand **1** and $Zn(ClO_4)_2$ in a $CHCl_3/CH_3OH$ mixture (1/1, v/v) (Scheme 2). The ^1H NMR spectrum of the Zn(II)@**1** exhibits broad signals at room temperature (298 K), probably because of the conformational rigidification of the whole structure induced by the metal complexation (Fig. 1). Variable temperature ^1H NMR experiments in the range of 298-373 K were then performed, and they show that the signals became increasingly narrow and well defined as the temperature raised. In particular, at 373 K, a set of expected sharp peaks with a C_{3v} symmetrical conformation was well displayed.

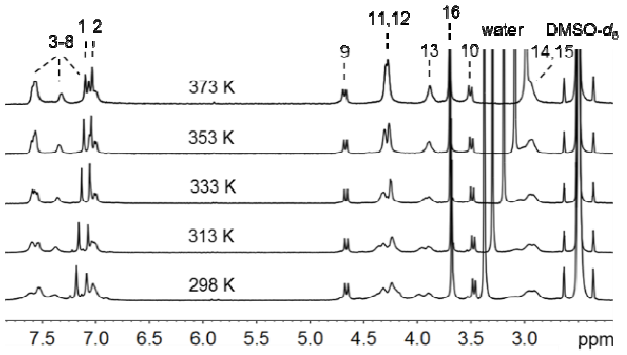


Fig. 1 ^1H NMR spectra of Zn(II)@**1** at different temperature in DMSO-d_6. For the proton assignment, see Scheme 2.

The complexation of choline phosphate **2** by Zn(II)@**1** was studied by evaluating the fluorescent changes of a 5 µM solution of Zn(II)@**1** upon progressive addition of the guest in DMSO containing 2% water. As shown in Fig. 2a, addition of choline phosphate **2** resulted in significant fluorescence quenching of the host at 350 nm with 5 equivalents reaching the end of titration. A binding constant of 4.2×10^5 M^{-1} was obtained according to a 1:1 model confirmed by Job plot (Fig. S1a). Choline **3** could also give rise to obvious fluorescence quenching of Zn(II)@**1** while 90 equiv. of **3** were needed to reach the end of titration (Fig. S1b and S2). In addition, a much smaller binding constant compared with choline phosphate **2** was achieved (7.0×10^3 M^{-1}). The fluorescence quenching of the host induced by guest **2** or **3** could be attributed to the promotion of the photo-induced electron transfer (PET) process from the tren nitrogens to the naphthalene fluorophores because of the weakening of the tren-Zn(II) bonds after guest coordination.[14f] It should be noted that in spite of a moderate binding between Zn(II)@**1** and choline **3**, the host could fully distinguish choline phosphate from choline since as shown in Fig. 2b, 2 equiv. of choline phosphate **2** lead to remarkable fluorescence quenching of the host (75%), while only 9% quenching is generated by 2 equiv. of choline **3**. This high selectivity of Zn(II)@**1** towards **2** over **3** is very meaningful since the two biologically important compounds simultaneously participate in one crucial process of metabolism (Scheme 1a). Moreover, this selectivity was also maintained in a more competitive solvent. In DMSO/H_2O (80/20, v/v), the marked fluorescence quenching of the host induced by choline phosphate **2** also occurred and a binding constant of 4.1×10^3 M^{-1}, larger than that with choline (1.2×10^2 M^{-1}), was presented (Fig. S3). This result also underlines that the Zn(II)@**1** cage is very efficient for the sensing of choline phosphate even in competitive media.

We then decided to investigate whether the heteroditopicity of the host played a crucial role in the recognition process or if the complexation was only driven by either phosphate-zinc or cation-π interaction. Thus, the binding experiments of Zn(II)@**1** with butylphosphate **4** and butyl-trimethylammonium **5**, which only contain one kind of the recognized units, were carried out. As shown in Fig. 2b, when in the presence of 2 equiv. of whatever **4** or **5**, a much smaller fluorescence quenching of Zn(II)@**1** was observed (75%, 48% and 3% quenching induced by guests **2**, **4** and **5**, respectively), indicating that only the guest bearing both the phosphate and ammonium parts allows an efficient fluorescence quenching. The corresponding titration experiments of host **1** with **4** and **5** were also performed and binding constants of 6.3×10^4 M^{-1} and 2.2×10^3 M^{-1} were respectively achieved, which are distinctly smaller than that obtained with the zwitterionic choline phosphate **2** (4.2×10^5 M^{-1}), again emphasizing the synergistic effects of both the CTV and Zn(II) moieties in the complexation (Fig. S4).

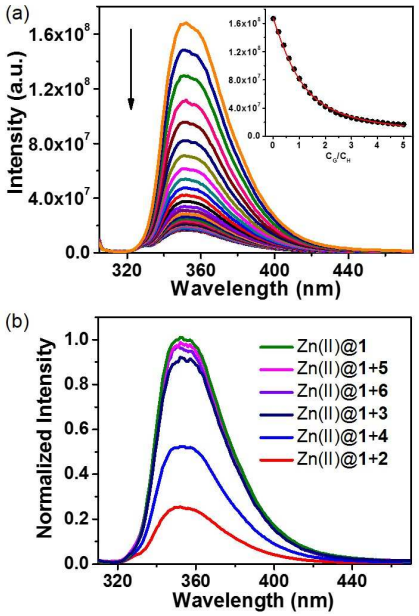


Fig. 2 (a) Fluorescence titrations of 5 µM Zn(II)@**1** excited at 300 nm with choline phosphate **2** in DMSO containing 2% water. Inset: the fluorescence intensity at 350 nm as a function of the equivalents of added choline phosphate **2**. (b) Normalized spectra of 5 µM Zn(II)@**1** upon addition of 2 equiv. of different guests in DMSO containing 2% water.

Previously, our group also reported two other heteroditopic hemicryptophane hosts for the recognition of zwitterions.[9d,9e] Both of the two previous receptors showed the largest binding constant for taurine **6**, up to 5.0×10^5 M^{-1} (scheme 1b).[9e] Hence, we decided to study the fluorescent response of the present cage Zn(II)@**1** for this competitive zwitterion. It was found that taurine **6** only gave rise to negligible fluorescence quenching of Zn(II)@**1** in DMSO containing 2% water (Fig 2b and Fig S5), highlighting (i) the high sensing selectivity of this new host for choline phosphate **2** and (ii) the possibility to tune the structure of hemicryptophane hosts in order to complex selectively a zwitterionic guest of biological interest. In this case, the specificity probably arise from the high binding affinity of Zn(II) moiety towards phosphorylated molecules.[14b,14d] Indeed, the fluorescent sensing of choline phosphate using the ligand **1** was also investigated, and only very small fluorescent changes were

observed (Fig S6), emphasizing the crucial role of Zn(II) moiety in this recognition process.

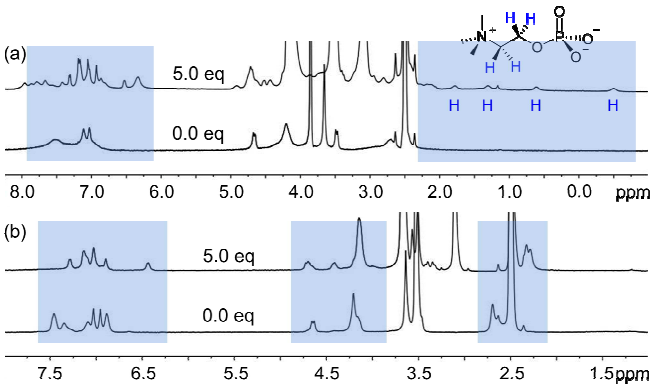


Fig. 3 ^{1}H NMR spectra of 1 mM Zn(II)@**1** in the absence and presence of 5.0 equiv. of choline phosphate **2** in DMSO-d_6/D_2O (80/20, v/v) at 298 K (a) and at 353 K (b). The four new peaks respectively at -0.50, 0.61, 1.32 and 1.79 ppm in Fig. 3a are attributed to the four diastereotopic protons of the methylene groups of the encaged choline phosphate **2**.

More insights into the interaction mechanism were obtained from a series of NMR experiments. Progressive additions of choline phosphate **2** to a solution of Zn(II)@**1** in DMSO-d_6/D_2O (80/20, v/v) at room temperature lead to more complicated signals for aromatic protons (down-field region) and to new peaks (up-field region) that cannot be assigned to the free choline phosphate **2**, indicating the existence of multiple conformers in solution for the host-guest complex (Fig. 3a and Fig. S7). Heating the host-guest mixture up to 353 K resulted in the disappearance of these additional signals and the appearance of sharp and well defined peaks for the whole spectrum (Fig. 3b and Fig. S8). A subsequent return to room temperature for the mixture restored the initial spectrum (Fig. S8). These results imply that the exchange between the free and complexed choline phosphate appears slow on the NMR time scale at room temperature while it is fast at high temperature (353 K). It is interesting to note that the four new peaks respectively at -0.50, 0.61, 1.32 and 1.79 ppm display almost the same integration ratio (1:1:1:1). Indeed, these four new peaks in the up-field region of the spectrum can be attributed to the four diastereotopic protons of methylene group of the encaged choline phosphate which suffer from a shielding effect by the naphthalene linkers. The complexed choline phosphate is encapsulated in a chiral environment, hence any points of the cavity are chirotopic.[16] As a consequence, methylene groups of choline phosphate also become diastereotopic after inclusion giving rise to the four new peaks. Even though the latter are quite weak and broad, some clear cross-peaks between the four diastereotopic hydrogens were also observed in the COSY NMR spectrum (Fig. S9). Thus, both the low chemical shifts of these shielded protons and the chiralisation-like behavior[17] of the guest strongly suggest that the choline phosphate is fully encaged inside the molecular cavity of Zn(II)@**1**. Interestingly, a similar but less extent NMR titration behavior also occurred with choline **3**. At room temperature, progressive addition of choline to Zn(II)@**1** in DMSO-d_6/D_2O (80/20, v/v) gave rise to new peaks in the up-field region of the ^{1}H NMR spectra (0.5-2.0 ppm), indicating a slow complexation exchange on the NMR time scale (Fig. S10), whereas at higher temperature (353 K), the gradual shifts of the initial signals and the absence of new peaks during titrations suggest a fast exchange on the NMR time scale (Fig. S11).

^{31}P NMR titration experiments were also carried out to investigate the interaction mechanism. After addition of Zn(II)@**1** to choline phosphate **2** in DMSO-d_6/D_2O (80/20, v/v), the signal composed initially of only one sharp peak splits into two peaks with opposite direction of chemical shifts: one narrow down-field shifted peak and a broad up-field shifted signal (Fig. S12). The former strongly favored after addition of 0.5 equiv. of host was attributed to the encapsulated choline phosphate, whereas the latter whose intensity decreased was assigned to the free guest. These results support the encapsulation event between the host and guest and the slow complexation process on the NMR time scale at room temperature, which are in good agreement with those obtained from ^{1}H NMR titrations.

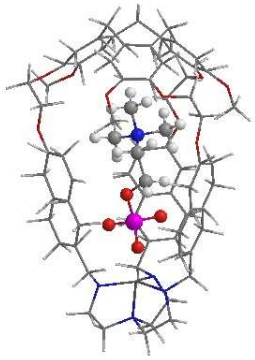


Fig. 4 DFT optimized structure of the complex between Zn(II)@**1** and choline phosphate **2**.

Finally, we examined the interactions involved in the recognition process by Density Functional Theory (DFT) calculations. In the fully optimized geometry of the complex, choline phosphate is encapsulated inside the Zn(II)@**1** cavity (Fig. 4).[18] The phosphate unit of the guest is linked to the zinc atom through a coordination bond (Zn···O distance: 1.9 Å). The distances between the aromatic rings of both the CTV unit and the naphthalene linkers of the host, and the ammonium moiety of the guest suggest that they interact through several CH···π interactions (several distances between 2.8 and 3.3 Å). Thus, both the CTV and Zn(II) units contribute to the efficient and selective binding of choline phosphate, emphasizing the heteroditopic character of the host. Interestingly, C-H···π interactions also occur between the methylene groups of the guest and the naphthalene linkers (several distances between 2.8 and 3.3 Å), accounting for the high-field shifts of these protons observed in ^{1}H NMR titrations, and also highlighting their diastereotopic behavior.

Conclusions

In conclusion, we have described a hemicryptophane cage combining a CTV unit and a Zn(II) metal center holding fluorescent properties. This fluorescent hemicryptophane has been developed as the first fluorescent sensor for zwitterionic choline phosphate in competitive media. The heteroditopic character of the host towards the guest was evidenced since the guest bearing both the ammonium and phosphate parts can give

rise to the most significant fluorescence quenching and largest binding constant. Moreover, it was found that the exchange between the free and complexed guest is slow on the NMR time scale at room temperature while it is fast at higher temperature (353 K). NMR experiments also indicate the formation of an inclusion complex between the cage and the guest, and a chiralisation-like behavior of the achiral choline phosphate occurs inside the cavity. The exact binding mode has been examined by DFT calculations. Studies of stereoselective recognition properties of chiral zwitterions using enantiopure fluorescent metal-based hemicryptophanes are in progress.

Notes and references

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† Electronic Supplementary Information (ESI) available: [Synthesis of complex **1**, fluorescent Job plot and titrations, NMR titrations and computational method]. See DOI: 10.1039/b000000x/

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