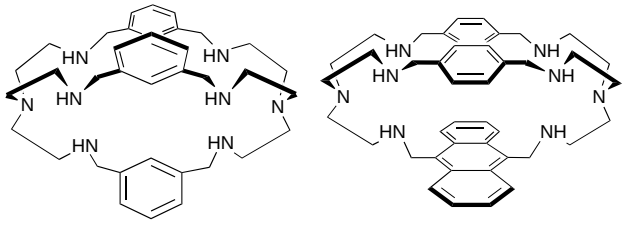
A fluorescent cage for anion sensing in aqueous solution

Luigi Fabbrizzi,*† Ilaria Faravelli, Giancarlo Francese, Maurizio Licchelli, Angelo Perotti and Angelo Taglietti

Dipartimento di Chimica Generale, Università di Pavia, I-27100 Pavia, Italy

The dizinc(II) complex of the bistren cage 2 selectively includes the isostructural N_3^- and NCO^- ions in aqueous solution, but only N_3^- inclusion is signalled through the quenching of the fluorescent emission of the anthracene spacer.

Molecular recognition of anions is in most cases based on electrostatic interactions (which include hydrogen bonding). Typically, the receptor offers a concave array of sites suitable for anion binding: ammonium or guanidium groups, amide hydrogen atoms.[1] In general, the intrinsically low energy of the electrostatic interaction does not compete successfully with the anion hydration energy and recognition studies have to be carried out in non-aqueous and poorly polar solvents. Efficient binding of anions in water would require the use of stronger host–guest interactions. This may be the case of the metal–ligand interactions. In fact, metal containing receptors perform efficient anion recognition in aqueous solution. As an example, the dicopper(II) complex of the bistren cage **1** selectively includes polyatomic anions, *e.g.* N_3^-, NCO^-, HCO_3^-. While anion inclusion is demonstrated by X-ray diffraction studies on the solid complexes,[2] recognition in aqueous solution can be monitored spectrophotometrically and is characterised by values of the association constant, K_{assn}, as high as 10^5 dm^3 mol^{-1}.[3] Each of the two donor atoms of the included ambidentate anion occupies a vacant coordination site of each Cu^{II} centre (the axial position of a trigonal bipyramid) and selectivity derives from the matching between the anion 'bite' (*i.e.* the distance between the two donor atoms) and the distance between the two vacant axial positions.



1 **2**

The function of recognition can be implemented to sensing if the receptor system contains a reporter subunit capable of signalling substrate binding through the variation of a chosen well detectable property. Fluorescence seems a particularly convenient property since it can be monitored in real time, even at very low concentration levels, by using a rather simple instrumentation. Fluorosensors for a large number of biotic and abiotic analytes have been designed during the past decade by appending a fluorescent fragment to the envisaged receptor framework:[4] in all cases, an efficient mechanism has to be provided for either quenching or reviving fluorescence, following substrate recognition. In contrast to the many efficient fluorosensors reported for cations to date, fluorescent sensors for anions are still rare.[4]

We have now synthesised the cage **2**, in which one of the spacers connecting the two tren compartments is the strongly fluorescent 9,10-anthracenyl fragment. The synthetic procedure, first involved the appending of two tren subunits to a 9,10-anthracenyl fragment; then, the terminal amine groups of each subunit were connected by 1,4-xylyl spacers.‡ Cage **2** incorporates pairs of metal ions, *e.g.* two Cu^{II}, and, following a cascade mechanism, can include ambidentate anions like N_3^- and NCO^-. However, we could not use the dicopper(II) complex as a fluorosensor, since Cu^{II} itself quenches the fluorescence of any proximate fluorophore through either an electron transfer (eT) or an energy transfer (ET) mechanism.[5] Therefore, we switched to Zn^{II}, which still forms stable complexes with amine ligands and, being redox inactive and having a filled 3d level, cannot be involved in either an eT or an ET process. Polyamine systems containing two Zn^{II} ions have been used to sense the imidazolate anion.[6]

The emitting behaviour of **2** (= L) in the pH range 2–12 was investigated by titrating with standard base a solution containing its octahydrochloride, $[H_8L]Cl_8$, plus excess acid, in the spectrofluorimetric cuvette. The corresponding fluorescence intensity, I_F, *vs.* pH profile, is shown in Fig. 1 (circles). At low pH values full anthracene fluorescence is observed. Then, I_F decreases in the pH range 4–6 following a sigmoidal pattern. Noticeably, the plot of I_F *vs.* the equiv. of added OH^- shows that fluorescence quenching takes place during the addition of the third equivalent of base (after that the excess acid had been neutralised). Amine groups display reducing properties and can transfer an electron to the proximate excited anthracenyl fragment, *An, quenching its fluorescence. It is possible that, after the neutralisation of the two protons released from the apical ammonium groups, the third proton leaves one of the ammonium groups adjacent to the An fragment allowing an eT process to take place. Then, a similar titration experiment was carried out on a solution containing also 2 equiv. of Zn^{II}. The corresponding I_F *vs.* pH profile (triangles) superimposes well on that observed for the titration of the cage alone until pH = 7. Here, I_F stops decreasing and increases again to reach a maximum at pH = 8.5. We ascribe this behaviour to the fact that from pH = 7 Zn^{II} ions enter the two tren compartments engaging all the amine nitrogen atoms in the coordinative bonds and preventing the electron transfer to *An. The maximum

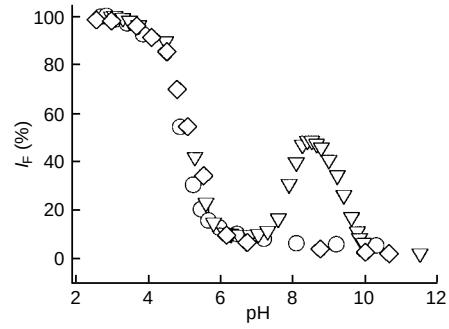


Fig. 1 pH dependence of the fluorescence intensity (I_F) for aqueous solutions containing: (○) **2** (10^{-4} M) plus excess acid; (▽) **2**, 2 equiv. of Zn^{II}, plus excess acid; (◇) **2**, 2 equiv. of Zn^{II}, 1 equiv. of N_3^-, plus excess acid.

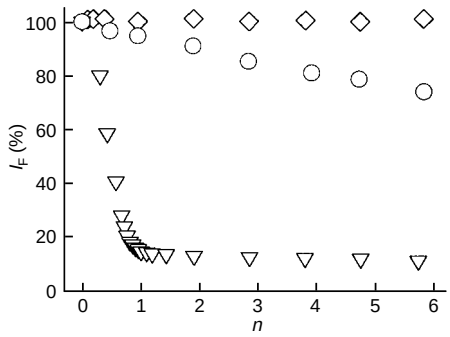


Fig. 2 Spectrofluorimetric titrations of aqueous solutions containing $[Zn^{II}(\mathbf{2})]^{2+}$ (10^{-4} M) and buffered to pH = 8.5: (▽) addition of N_3^-; (◇) addition of NO_3^- (no modification of I_F was observed also on addition of HCO_3^-, SO_4^{2-}, Cl^-, Br^-, NCO^-); (○) addition of N_3^- to a solution containing also 5 equiv. of NCO^-. *n* = Number of added equivalents of anion.

fluorescence should correspond to the maximum concentration of the $[Zn^{II}_2L_2]^{4+}$ complex. A further pH increase makes I_F decrease again. This may be due to the formation of a species in which an OH^- ion is coordinated to each metal centre. In particular, in the $[Zn^{II}_2L_2]^{4+}$ complex a water molecule is expected to occupy the axial position of each metal centre, completing five-coordination. Therefore, hydroxide containing species result from the deprotonation of the Zn^{II} bound water molecules.§ The OH^- anion can undergo an eT process and quench the proximate *An fragment. Intramolecular fluorescence quenching by coordinated OH^- ions is typically observed in Zn^{II} anthrylpolyamine complexes. Finally, in order to test the sensing behaviour of the dizinc(II) cage towards polyatomic anions, a titration with standard NaOH was carried out on a solution containing **2**, 2 equiv. of Zn^{II}, 1 equiv. of N_3^-, plus excess acid. In the corresponding profile (diamonds in Fig. 1) no fluorescence revival is observed above pH = 7, but I_F keeps decreasing until complete quenching. We ascribe this behaviour to the formation of a $[Zn^{II}_2L(N_3)]^{3+}$ inclusion complex.¶ Quenching may be due to an eT process from the electron rich N_3^- ion to the close *An fragment.

From molecular modelling, distances as short as 3 Å can be calculated between the closest nitrogen atoms of N_3^- and carbon atoms of the anthracene fragment, which may allow a fast through-space eT process to occur. Intermolecular quenching of anthracene by the N_3^- ion has been previously observed in aqueous ethanol and has been ascribed to an eT mechanism.[7] Comparison of the I_F *vs*. pH profiles of Figs. 1 and 2 indicates that at pH ≈ 8 the dizinc(II) cage could behave as a fluorescent sensor for the N_3^- ion: in particular, azide inclusion should be signalled through a substantial quenching of the anthracene fluorescence. Indeed, by titrating with a standard N_3^- solution an aqueous solution 10^{-4} M in **2**, containing 2 equiv. of Zn^{II} and buffered to pH = 8.0, a linear decrease of fluorescence was observed until the addition of 1 equiv. of azide (Fig. 2).

Least-squares analysis of the titration profile gave a value of $\log K_{assn}$ of 5.8 ± 0.1. Then, the receptor solution (1 equiv. of **2**, 2 equiv. of Zn^{II}, adjusted to pH = 8) was titrated with a series of anions: NO_3^-, HCO_3^-, SO_4^{2-}, Cl^-, Br^-. In all cases, no I_F decrease was observed even after the addition of 10 equiv. Moreover, the N_3^- titration profile as shown in Fig. 2 was not altered when the receptor solution contained also 10 equiv. of each one of the above anions. This indicates that these anions do not compete with N_3^- for inclusion: in particular, the corresponding K_{assn} values should be lower than $10^{3.6}$. The case of NCO^- is unique. On addition of NCO^- to the receptor solution, no I_F decrease was observed. However, the titration profile of N_3^- was remarkably affected by the presence of NCO^-: the greater the NCO^- concentration, the less steep the I_F decrease, indicating severe competition for inclusion within the cage. The profile reported in Fig. 2 refers to a solution containing 5 equiv. of NCO^-. From this a $\log K_{assn}$ of 6.5 can be calculated for NCO^-. Thus, NCO^- has a slightly greater affinity for **2** than N_3^-, but, due to its less pronounced reducing tendencies, when included in the cage, it is unable transfer an electron to the nearby *An fragment. N_3^- and NCO^- anions have a similar bite length (2.34 and 2.42 Å, respectively) and the rather high values of K_{assn} should reflect the favourable matching with the distance between the two vacant axial positions of the two Zn^{II} centres. The other linear triatomic anion, NCS^-, quenches fluorescence, but according to a much less steep profile, to which a much lower value of $\log K_{assn}$ corresponds: 2.45 ± 0.05. NCS^- is a one-electron reducing agent of strength comparable to that of N_3^- ($NCS^{\cdot}/NCS^-$ potential: 1.62 V *vs*. NHE; $N_3^{\cdot}/N_3^-$: 1.33),[8] which accounts for the occurrence of an intracomplex photoinduced eT process and fluorescence quenching. However, the much greater bite length (2.75 Å) should induce an endothermic rearrangement of the cage framework, making inclusion 2200 times much less favourable than for N_3^-.

This study demonstrates that the zinc(II) containing cage **2** is an efficient and selective receptor for ambidentate anions. First, it discriminates polyatomic anions from monoatomic anions, which have a less pronounced tendency to bridge two metal centres and whose inclusion is then disfavoured. Then, it recognises polyatomic anions on the basis of their bite length. Finally, the presence of the 9,10-anthracenyl spacer permits the inclusion of anions prone to a photo-induced eT process being signalled through fluorescence quenching.

Notes and References

† E-mail: fabbrizz@ipv36.unipv.it

‡ A solution of terephthalaldehyde (4.0 mmol in 250 ml of MeOH) was added dropwise over 2 h under magnetic stirring to a solution of the bistren derivative of the 9,10-anthracenyl fragment[6] (2.0 mmol in 750 ml of MeOH). The stirred solution was then heated to 50 °C and 2.5 g of $NaBH_4$ were added in small portions over 3 h. The solution was then stirred overnight at room temperature. MeOH was distilled off under reduced pressure, and the residue was dissolved in 100 ml of water. The aqueous solution was extracted with CH_2Cl_2 (3 × 50 ml). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure to give a gold–yellow solid (yield: 55%). FAB MS *m/z* (%): 699 (100) M − H^+. **2** was used in solution studies as its hydrochloride, **2**·8HCl, which was satisfactorily analysed.

§ The following species were found to be present at equilibrium, in the 2–12 pH range, in a dioxane–water mixture (4 : 1, v/v), as ascertained through non-linear least-squares analysis of potentiometric titration data: $[Zn^{II}(H_3L)]^{5+}$, $[Zn^{II}_2L]^{4+}$, $[Zn^{II}_2L(OH)]^{3+}$, $[Zn^{II}_2L(OH)_2]^{2+}$. The dioxane–water medium was used to ensure a concentration of **2** ⩾ 10^{-3} M, as required, for potentiometric studies. A potentiometric titration study in pure water could not be carried out owing to the poor solubility of **2** (≈ 10^{-4} M).

¶ Potentiometric titration experiments in dioxane–water (4 : 1 v/v) indicated that the $[Zn^{II}_2L(N_3)]^{3+}$ complex is present as a major species in the pH range 6.5–9.0.

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