ELSEVIER

Coordination Chemistry Reviews
205 (2000) 85–108

COORDINATION CHEMISTRY REVIEWS

www.elsevier.com/locate/ccr

The design of luminescent sensors for anions and ionisable analytes

Luigi Fabbrizzi *, Maurizio Licchelli, Giuliano Rabaioli, Angelo Taglietti

Dipartimento di Chimica Generale, Università di Pavia, Via Taramelli, 12, I-27100 Pavia, Italy

Received 17 June 1999; accepted 31 December 1999

Contents

Abstract

A molecular luminescent sensor for anions can be built through a modular approach, i.e. by covalently linking an appropriate photoactive fragment to the receptor displaying a satisfactory affinity towards the desired substrate. Following the receptor-anion interaction, an intercomponent process must take place, e.g. an electron transfer (eT) or an energy transfer (ET) process, that distinctly modifies the emission of the luminophore, thus signalling the occurrence of the recognition event. In this article, specific molecular sensors are classified according to the type of receptor-anion interaction, whether hydrogen bonding or metal–ligand interactions. Receptors of the latter class are based on a Zn^{II} polyamine platform, which leaves at least a vacant coordination site for the incoming anion. Substrates include natural amino acids, NH_3^+–CH(**R**)–COO^-, for which the highest selectivity is

* Corresponding author. Tel.: + 39-382-507325; fax: + 39-382-528544.
E-mail address: fabbrizz@unipv.it (L. Fabbrizzi).

PII: S0010-8545(00)00239-3

observed when the receptor subunit specifically interacts with the **R** portion. An eT process involving **R** and the nearby excited luminophore may provide the signal transduction mechanism.

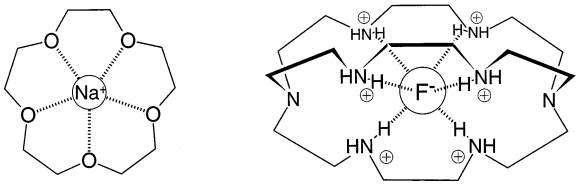
Keywords: Anion recognition; Amino acids; Fluorescent sensors; Electron transfer; Zinc(II) complexes

1. Introduction

The so-called multicomponent or modular approach represents the more straight and rational way to the design of a molecular sensor for any kind of analyte [1]. Following this approach, a receptor suitable for the desired substrate is chosen and it is linked, for instance through covalents interactions, to a signalling subunit. This subunit can be any molecular fragment, provided that it displays a property whose magnitude varies substantially following the interaction of the substrate with the receptor. When the signalling subunit is a luminescent fragment, the occurrence of the substrate recognition can be communicated to the outside by the enhancement or decrease of the light emission, a behaviour which in most cases can be visually perceived and is instrumentally monitored at especially low concentration levels [2]. An important requirement is that, following the receptor-substrate interaction, an intercomponent process is either established or interrupted between the receptor subunit and the luminescent fragment, so that the light emission is quenched or revived. If such an intercomponent process does not take place, the recognition event is not signalled and the receptor-luminophore system fails to behave as a sensor [3]. Thus, to design properly a molecular fluorescent sensor one must (i) choose the receptor for the envisaged substrate; (ii) select a convenient luminophore; and (iii) put an efficient intercomponent process in action, in order to associate recognition to signalling. Points (i) and (ii) are relatively easy and for their fulfilment one can take profit from a well developed literature in the fields of recognition chemistry and photophysics, respectively. Achievement of point (iii) is much less simple, as the occurrence of the intercomponent process can be hardly controlled, even if its mechanism, whether of the electron transfer (eT) or energy transfer (ET) type, can be well defined and accounted for, according to an a posteriori analysis [4,5].

The number of available luminescent sensors for classes of analytes is in some way proportional to the number of the corresponding receptors. Thus, many receptor-luminophore systems suitable for sensing of metal ions have been developed, which reflects the large body of knowledge on the coordination chemistry of metals, in particular of the d block and of the s block, accumulated during the last decades. Designing a receptor for anions is a much more difficult task, for reasons which will be summarized below, and this explains the rather scarce availability of the corresponding molecular luminescent sensors.

Receptors for anions, as those for cations, have to be concave and must be able to wrap the substrate in order to establish several binding interactions [6]. These

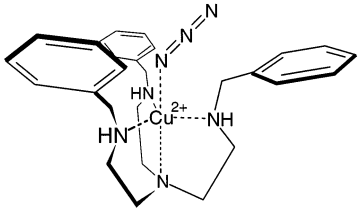


1 2

Fig. 1. Receptors for cations and anions: the sodium complex of 15-crown-5 (**1**); the inclusion complex of the fluoride ion within a hexaprotonated bistren cage (**2**).

interactions can be electrostatic (including the hydrogen bond). Since anions are larger than cations, the electrical charge is distributed over a greater surface area, thus making the electrostatic interaction with the receptor, which typically bear charges of opposite sign, less intense than observed for cations. For instance, the spherical ions F^- and Na^+ are isoelectronic. However, the smaller cation (r^+: 0.97 Å), due to its higher charge density, is able to establish relatively strong electrostatic interactions even with neutral multidentate ligands, either cyclic (the crown ether **1** [7], for instance) or non-cyclic. Complexation of the larger anion (r^-: 1.33 Å) requires much more elaborated receptors, containing prepositioned positively charged groups, like the hexammonium cage **2** [8] (see Fig. 1).

Anions themselves can behave as ligands towards metal ions, giving rise to complexes of varying stability, held together by coordinative interactions, whose nature changes from electrostatic to covalent, depending on the metal characteristics. Therefore metal complexes can be used as versatile anion receptors. The metal centre has to be coordinatively unsaturated, thus leaving one or more coordination sites vacant and available for the incoming anion, and has to be disposed to establish kinetically labile interactions, in order to provide the fast and reversible binding of the analyte, a mandatory prerequisite in recognition processes. An example of a coordinatively unsaturated metal complex capable of binding anions is shown in Fig. 2. A Cu^{II} centre is coordinated by the tripodal tetramine



3

Fig. 2. Anion binding to a copper(II) tetramine platform. The Cu^{II} complex of the tripodal tetramine tris-(*N*-benzyl-2-ethylamino)-amine possesses a vacant axial coordination site, available for anion binding. In adduct **3**, the azide anion exhibits a bent coordination mode due to the sp^2 nature of the terminal nitrogen donor atom.

tris-(2-aminoethyl)amine (tren). The branched polyamine ligand imposes a trigonal bipyramidal geometry, occupying four coordination sites. One of the axial position is vacant and can be occupied by a solvent molecule or by an anion (in this case, the N_3^- ion) [9].

Metal–ligand interactions are usually stronger than electrostatic interactions, and, when the metal belongs to the d block, have a defined directional character (whereas electrostatic interactions are typically a-directional). These features point toward a greater selectivity of the receptors based on the metal–ligand interaction with respect to receptors operating through simple electrostatic interactions, in the recognition of anionic substrates in solution.

The design of receptors for anions has been thoroughly and recently reviewed [10,11]. Aspects of optical sensing of anions through the metal–ligand interactions have been recently considered [12,13].

In this chapter, the building up of receptor–luminophore systems suitable for luminescent sensing of anions in solution will be considered.

2. Anion recognition and sensing based on electrostatic interactions

2.1. Non-cyclic polyammonium receptors

The first example of a fluorescent molecular sensor for anions is due to Czarnik [14]: in the two-component system **4**, an anthracene fragment is linked through a methylene group to a branched tetramine of the family of tren (in this case, each aliphatic chain has a $–CH_2–$ group more) (see Fig. 3).

In an aqueous solution at pH 6, the emission intensity by the anthracene subunit is low. At this pH, all the amine groups are protonated, but that one linked to the anthracenyl fragment, which has a benzylic nature and is less basic than the other ones. This benzylamine group plays an essential role, as it behaves as a donor and transfers an electron to the nearby photoexcited anthracene fragment, whose emission is substantially quenched. On addition of HPO_4^{2-}, the fluorescence is enhanced (of about 150%). It is suggested that the polyammonnium ion, **5**, acts as a receptor for hydrogenophosphate: in particular, hydrogen bonding interactions are established between the three ammonium groups and the three oxygen atoms of HPO_4^{2-}. Then a further hydrogen bond interaction is established between the benzylic amine group and the –OH fragment of the HPO_4^{2-} ion, as illustrated in formula **6** in Fig. 3; more probably, an intramolecular proton transfer takes place from –OH to the benzilic amine group and a hydrogen bonding interaction is then established between the benzylammonium group and the fourth oxygen atom of the PO_4^{3-} ion (which possesses 3/4 of negative charge), as shown in formula **7**. In any case, following either hydrogen bonding interaction with –OH or covalent bond formation with H^+, the benzylic nitrogen atom cannot make its lone electron pair available for the photoinduced electron transfer process. Thus, the fluorophore can release the photonic energy in the radiative mode: fluorescence is revived. The mechanism of fluorescence enhancement is also illustrated in the molecular orbital scheme in Fig. 4.

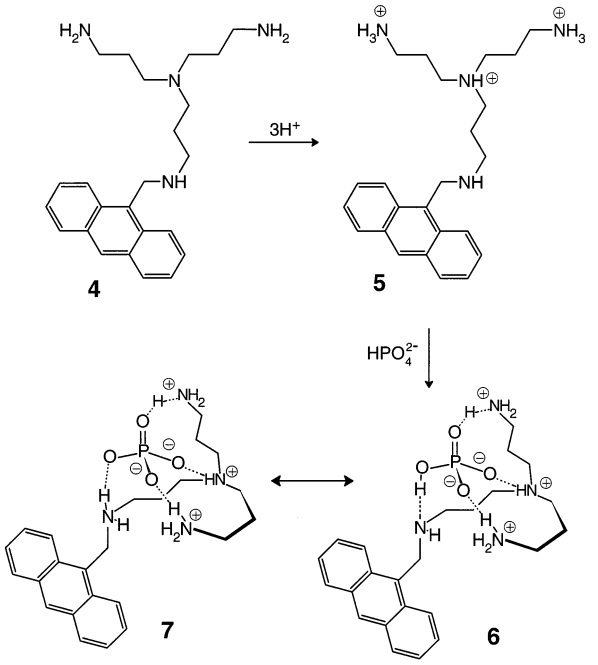


Fig. 3. Recognition and sensing of the HPO_4^{2-} anion. At pH 6, the anthracenyl functionalised tetramine **4** is triply protonated (**5**). **5** acts as a receptor for $H_2PO_4^-$, through the formation of hydrogen bonding interactions (**6**). It is possible that an intra-complex proton transfer takes place and the receptor-substrate adducts are better represented by formula **7**. Before the anion recognition, **5** is poorly fluorescent, as an electron transfer (eT) process from the anthrylamine nitrogen atom quenches light emission. Following anion binding, the anthrylamine group is engaged in either hydrogen binding (**6**) or ammonium formation (**7**). In any case the eT process is suspended and recognition signalled through fluorescence revival.

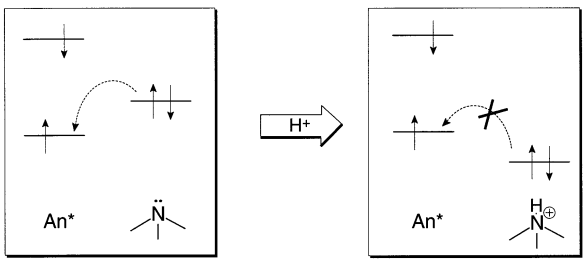
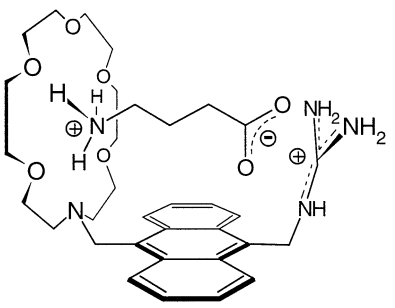


Fig. 4. An orbital diagram illustrating the occurrence of an eT process from an amine nitrogen atom to a nearby photexcited anthracene fragment (left side of the Figure). On protonation of the amine group, the eT process is thermodynamically prevented and anthracene fluorescence revived. This mechanism operates in the process described in Fig. 3.

The energy level associated to the lone pair on the benzylic nitrogen atom >N: is distinctly higher than that of the HOMO of the photoexcited fragment An*. Thus, the >N:-to-An* eT process is thermodynamically favoured. On interaction with HPO_4^{2-}, the lone pair of the benzylic nitrogen atom is strongly stabilised and, in particular, its energy level becomes lower than that of the HOMO level of An*, thus preventing the occurrence of the eT process and restoring the light emission. The benzylic amine nitrogen atom behaves as a switch: it keeps fluorescence off before the interaction with the substrate and turns on fluorescence to communicate the occurrence of the recognition process.

Due to its flexible nature, the polyammonium receptor of system **5** wraps the anion and provides a concave array of positive charges. The conditional constant for HPO_4^- at pH 6 is 0.82 log units. Flexibility disfavours selectivity: system **5**, at pH 6, senses other anions, in any case through fluorescence enhancement. In particular, the log K value for citrate is 2.3 (95% fluorescence enhancement), and for sulphate is 1.6 (114%). It may seem surprising that binding of the SO_4^{2-} ion, which does not bear any acidic hydrogen, quenches fluorescence. This may be ascribed to the fact that hydrogen bonding interactions with the sulphate ion promotes water dissociation and protonation of the benzylic amine group.

If the envisaged substrate is multifunctional, i.e. it contains distinct groups of different nature and charge, the receptor must provide distinct binding sites of appropriate characteristics. A representative case is that of amino acids, in their zwitterionic form: NH_3^+–CH(**R**)–COO^-. The first example of a molecular fluorescent sensor for amino acids has been presented by de Silva [15].



8

In the ditopic system **8**, an anthracene fragment displays a double function, of fluorophore and of spacer, as it links the two binding sites for the bifunctional substrate: a guanidinium subunit, suitable for electrostatic interaction with the carboxylate group of the amino acid, and a 18-crown-NO_5 moiety, which can establish hydrogen bonding interactions with the ammonium residue. The presence of a tertiary amine nitrogen atom in the crown, close to the anthracene subunit, is essential for the sensing behaviour: prior to substrate binding, the amine group undergoes the thermodynamically feasible transfer of an electron to the nearby excited fluorophore, thus quenching its emission. Therefore, one would expect that,

following the amino acid recognition, and, in particular, after that the non-bonding electron pair of the nitrogen in the crown has become involved in a hydrogen bonding interaction with the ammonium group of the amino acid, the eT process is suspended and the fluorescence is switched on. Indeed, interaction of **8** with a series of amino acids of general formula NH_3^+–$(CH_2)_n$–COO^- in an $MeOH/H_2O$ mixture is signalled through a significant enhancement of the anthracene emission. Interestingly, system **8** displays linear recognition features, the highest binding constant ($\log K = 1.92$) and the more significant increase of the emission intensity (3.5 times) being observed with $n = 4$. It appears that in the case of the latter amino acid (γ-amino butyric acid, GABA) the distance between the carboxylate and the ammonium residues fits well the separation of the binding sites provided by the heteroditopic receptor **8**. Quite interestingly, the natural amino acid glycine, as well as glutamic acid (NH_3^+–$CH(COO^-)$–$(CH_2)_2$–COO^-, the physiological precursor of GABA), induces a negligible fluorescence response, even if added in large excess with respect to the sensor.

2.2. Cyclic polyammonium receptors

If the receptor is cyclic, its binding tendencies toward a given substrate are enhanced. In particular, the thermodynamic stability of the cyclic receptor/substrate complex which forms in solution is substantially higher than that of the complex of the corresponding non-cyclic receptor. This effect (the so-called: thermodynamic macrocyclic effect [16]) reflects the circumstance that the receptor is already preoriented (preorganised, using Cram's language [17]) for binding and does not have to spend energy (of both enthalpic and entropic origin), when wrapping around the substrate. On the contrary, such an energy loss is observed in the case of the non-cyclic (and more flexible) receptors. Therefore, this cyclic effect on stability simply reflects the rigidity/flexibility contrast of the ligating framework. In particular, the effect is especially manifest when the binding sites are correctly positioned within the receptor's array and fit well the geometrical requirements of the substrate. These simple concepts, which underpin supramolecular and recognition chemistry, apply well to the design of molecular luminescent sensors for anions of the receptor–spacer–luminophore paradigm.

Representative examples of the approach outlined before are due to Lehn, in the design of sensitive fluorescent probes for adenosine triphosphate (ATP^{4-}) [18].

In particular, an aminoacridine fluorophore was linked through a trimethylenic chain to a nitrogen atom of a 24-crown-N_6O_2 subunit (formula 9 in Fig. 5). At pH 4, four of the six amine groups of the macrocycle are protonated: it is suggested that, in order to minimize electrostatic repulsions, the four protons binds the amine groups close to the ethereal oxygen atoms (**9**·4H+). Thus, the cyclic receptor offers two distinct doubly positively charged compartments, which are separated by $–CH_2CH_2OCH_2CH_2–$ bridges. This array is geometrically suitable for establishing electrostatic interactions with the triphosphate fragment of ATP^{4-}. Moreover, a

stacking interaction is established between the acridine subunit and the adenine fragment. The occurrence of the latter π interaction is responsible for a substantial increase of the of the acridine blue emission (at 452 nm), which signals to the outside the ATP^{4-} recognition event. Spectrofluorimetric titration experiments in a solution buffered at pH 4 indicated the formation of a stable 1:1 complex, whose hypothesized structural arrangement is sketched in Fig. 5 (**10**).

A further efficient fluorescent sensor of ATP^{4-} has been realised by Lehn [19], following a different approach (**11**, in Fig. 6). Also in the present case, the cyclic receptor contains two equivalent triamine compartments. Each compartment, at pH 6 is doubly protonated and is expected to act as a binding site for one of the negatively charged terminal parts of the triphospate fragment. However, the two triamine compartments are linked by napthyl fragments, which play the double role

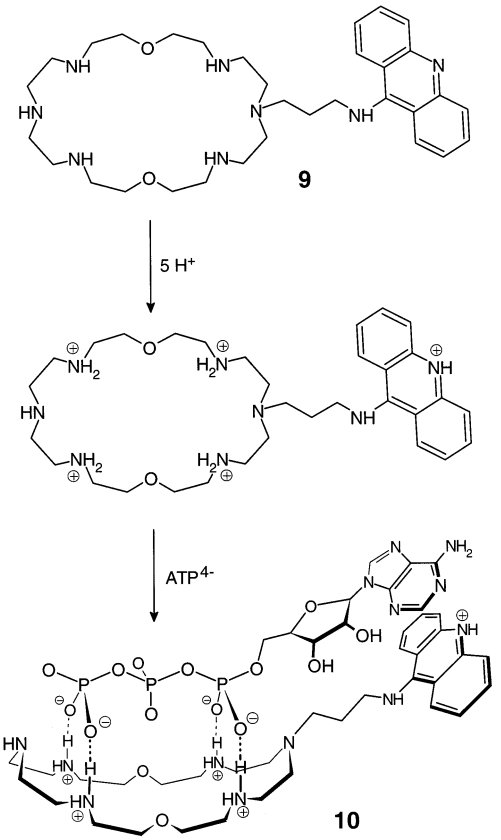


Fig. 5. Recognition and sensing of ATP^{4-}. Recognition results from: (i) the hydrogen bonding interactions between the cyclic polyammonium receptor and the triphosphate fragment; and (ii) a stacking interaction between the acridine subunit and the adenine fragment. The latter interaction makes acridine fluorescence substantially increase.

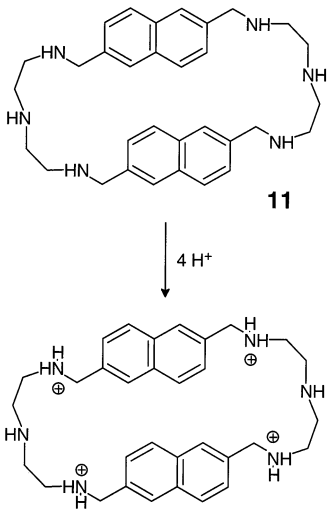


Fig. 6. Recognition and sensing of ATP^{4-}. Recognition results from the hydrogen bonding interactions between the cyclic tetraprotonated receptor and the triphosphate fragment and is signalled through quenching of the naphthalene emission. Quenching is ascribed to an electron transfer from the electron rich adenine fragment of ATP^{4-} to the excited fluorophore incorporated in the ring.

of fluorophore and spacer. Titration with ATP^{4-} of a solution of **12** buffered to pH 6 induces a progressive quenching of the naphthalene emission. It is suggested that in the tetraprotonated species the ammonium groups are symmetrically placed close to the fluorogenic spacers, providing a positively charged array suitable for interaction with the triphosphate subunit of ATP^{4-}. While the mechanism has not been investigated in detail, it is possible that fluorescence quenching is due to an electron transfer from the electron rich adenine fragment to the excited fluorophore incorporated in the ring. The formation constant for the sensor–analyte complex is quite high: $\log K = 5.1$, distinctly higher than that observed for the binding equilibrium of AMP^{2-}, which possesses a too short 'bite' to encompass the distance between the two compartments of the tetrammonium receptor ($\log K = 4.1$). On the other hand, ADP^{3-} displays a binding ability towards $\mathbf{11}{\cdot}4H^{+}$ ($\log K = 5.0$) comparable to that of ATP^{4-}. Lack of linear discrimination can be ascribed to the rather flexible nature of the two polyammonium compartments, which can fold and adjust themselves to encompass the length of the anionic substrate.

The octamine cage **12** (see Fig. 7) provides tighter recognition features [20]. At pH 6, all but the apical tertiary nitrogen atoms of each tripodal compartment are protonated. Also in the present case, the spacer linking the two polyammonium subunits are fluorogenic, thus acting as the signalling subunit of the sensor. The $\mathbf{12}{\cdot}6H^{+}$ cage, when void, is poorly fluorescent. In particular, the acridine emission is almost completely quenched, due to the formation of an excimer characterised by an especially low quantum yield (about 70 times lower than that of the monomeric

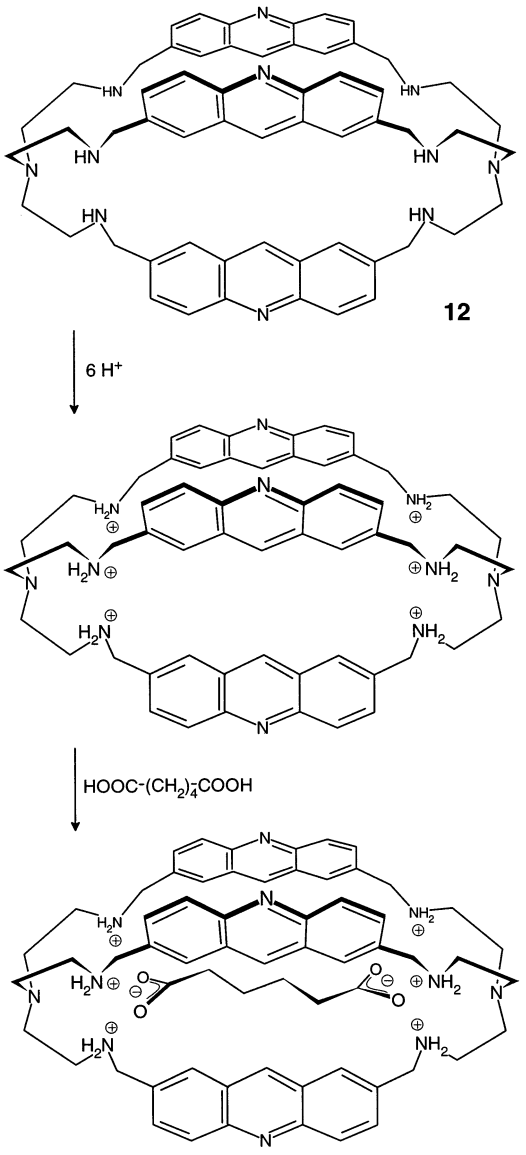


Fig. 7. Linear recognition and sensing of dicarboxylates. The **12**·6H$^+$ cage includes dicarboxylate anions of varying length. The best fit is observed with the COO^-–$(CH_2)_4$–COO^- anion. The void **12**·6H$^+$ cage is poorly fluorescent due to the formation of an intramolecular excimer. Following substrate inclusion, excimer formation is prevented and the monomer emission is fully revived up to three times.

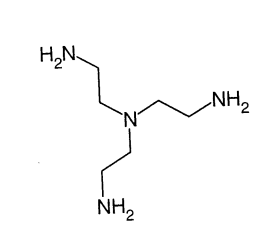
fluorophore). Inclusion of a substrate within the cage is expected to mechanically hinder the intramolecular interaction of the facing acridine subunits, thus preventing excimer formation and leaving the monomer emission intact. Indeed, **12**·6H$^+$

shows binding tendencies toward bicarboxylate anions of formula COO^- $-(CH_2)_n-COO^-$. When $n = 1$, 2, no emission enhancement is observed on anion addition. However, when $n \geq 3$, fluorescence is revived up to three times and more and respectable binding constants for the 1:1 complex formation are determined through spectrofluorimetric titration experiments ($n = 3$, $\log K = 2.7$; $n = 4$, $\log K = 3.6$; $n = 5$, $\log K = 3.2$), indicating pronounced chain length discrimination. In particular, it appears that dicarboxylate anions with $n < 3$ are too 'short' to encompass the distance between the two polyammonium binding compartments of $\mathbf{12}\cdot 6H^+$. Marked linear recognition effects are clearly observed also in the case of aromatic carboxylates. On addition of simple benzoate anion to $\mathbf{12}\cdot 6H^+$, no revival of the acridine emission is observed. However, 1,2-benzene-dicarboxylate makes the fluorescence intensity double, with a binding constant $\log K = 3.5$. Moreover, 1,3- and 1,4-benzene-dicarboxylates, which probably fit better the distance between the binding sites within $\mathbf{12}\cdot 6H^+$, give more stable complexes ($\log K = 4.1$ and 4.2, respectively) and induce conspicuous enhancement of the emission intensity (18.3 and 27.4 times, respectively).

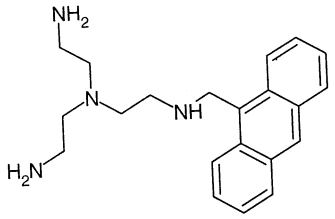
3. Anion recognition and sensing based on the metal–ligand interaction

Anions can behave as ligands and tend to bind metal ions (in particular those of the d block), displaying their own selectivity. Thus, metal ions can be used as receptors for anions. Metal containing receptors may present some favourable features with respect to the receptors relying only on the electrostatic and hydrogen bonding interactions. The metal–ligand interaction can be highly energetic: this depends on the electrical charge of the metal, on its electronic configuration and on the Ligand Field Stabilisation Energy anion coordination may induce. In any case, on average, the metal–ligand interaction is substantially higher than the metal-free electrostatic interaction. Moreover, the metal ion can present some geometrical preferences which can be conveniently utilised to impart selective binding tendencies toward the desired anion.

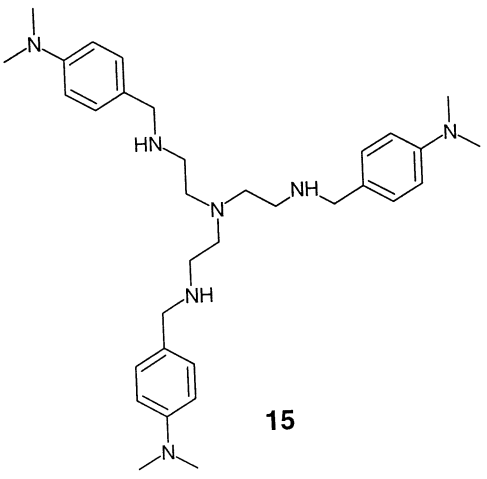
A crude metal ion, in its solvated form, cannot be used as an anion receptor, because it possesses too many binding sites (typically four or six) and it will be probably inclined to bind undiscriminately anions of varying nature and according to an uncontrolled stoichiometry. Thus, it is convenient to modulate the binding tendencies of the metal, for instance by blocking most of its coordinative position with an ancillary multidentate ligand and leaving a few binding sites vacant and available for the incoming anion. The ancillary ligand has in general an organic nature and can be equipped with some functionalities to introduce further elements of selectivity for anion recognition and/or to impart signalling capability.



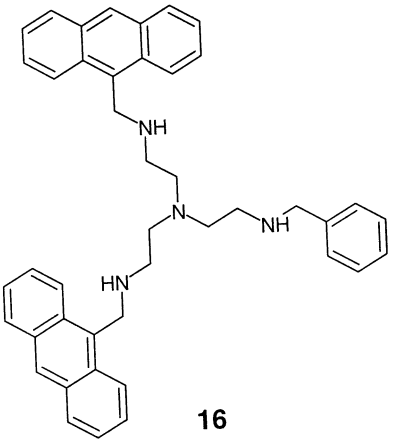
13



14



15



16

An example is provided by the the tripodal tetramine tren, **13**. It tends to form five-coordinate metal complexes of trigonal bipyramid stereochemistry, e.g. $[M^{II}(tren)]^{2+}$, in which one of the two axial positions is left available for the coordination by a further exotic monodentate ligand, either a solvent molecule S or an X^- anion [21]. The anion association constant refers to the equilibrium: $[M^{II}(tren)(S)]^{2+} + X^- = [M^{II}(tren)(X)]^+ + S$, in which S is replaced by X^-. The $[M^{II}(tren)]^{2+}$ receptor can evolve to a luminescent sensor if an appropriate light-emitting fragment is appended to the the tetramine framework. Sensing requires that an intercomponent process takes place between the metal-bound anion and the excited luminophore, whose emission, following anion binding, should vary to a substantial extent. However, the use of luminescence as a signal imposes serious restrictions on the choice of the metal centre which should act as the binding site of the anion receptor. In particular, genuine transition metal ions (electronic configuration d^n, $n = 1–9$), which display the greatest versatility in ligand coordination, must be ruled out, as they tend to irremediably quench any nearby luminophore, through either an electron transfer process (in view of their well established one-electron redox activity) or energy transfer process (double exchange mechanism, due to the presence of low energy partially filled d levels). However, the

non-transition metal ion Zn^{II} can be a good surrogate, as (i) it still forms fairly stable complexes with ligands bearing nitrogen and oxygen atoms (it follows Cu^{II} and Ni^{II} in the Irving–Williams series, but precedes all the other divalent 3d cations [22]) and (ii) cannot deactivate any nearby excited luminophore as it does not show any one-electron redox activity and possesses a completely filled d level.

The zinc(II) complex of the anthracene functionalised tren ligand, $[Zn^{II}(\mathbf{14})]^{2+}$, was tested for recognition and sensing of aromatic carboxylates (Zn^{II} polyamine complexes show good affinity toward the $-COO^-$ donor group) [23]. The plain benzoate ion binds the $[Zn^{II}(\mathbf{14})]^{2+}$ receptor, forming a stable 1:1 adduct, as indicated by a change in the absorption spectrum, monitored in the course of a spectrophotometric titration in ethanol. However, benzoate binding does not alter the typical emission of the nearby fluorophore. In fact, the anion does not possess any feature to interfere with the light-emission properties of the anthracene fragment, which therefore acts as a silent witness of the recognition process.

However, if an ethanolic solution of the $[Zn^{II}(\mathbf{14})]^{2+}$ complex is titrated with 4-*N*,*N*-dimethylaminebenzoate, the anthracene fluorescent emission is progressively quenched and complete quenching is observed at the 1:1 sensor/analyte ratio. The log *K* value for the association equilibrium is 5.45, as calculated from the spectrofluorimetric titration plot (I_F vs. anion eqvs). Quenching is ascribed to the thermodynamically favoured ($\Delta G^{\circ}_{eT} = -0.4$ eV) photoinduced electron transfer process, from the dimethylamine (DMA) donor group to the excited anthracene moiety, An* (see Fig. 8). In fact, the zinc(II)-benzoate interaction brings the DMA and An* fragments at a useful distance for the occurrence of a through-space eT process. In particular, occasional rotation around the $-CH_2-$ pivot may bring the anthracene subunit at the Van der Waals contact with the anion, thus allowing orbital overlapping and fast and efficient electron transfer. Similar behaviour is

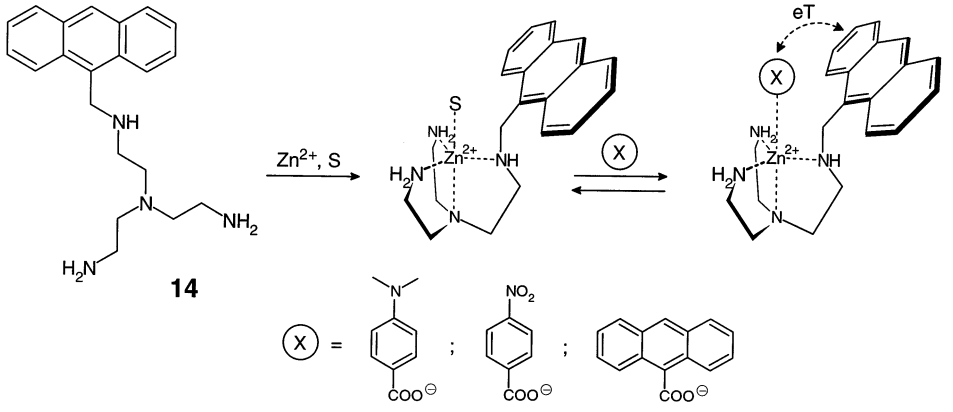


Fig. 8. Sensing of carboxylates based on the metal–ligand interaction. The tripodal tetramine **14** is first organised around a Zn^{II} centre. In absence of coordinating anions, the vacant axial position of the trigonal bipyramidal coordination polyhedron is occupied by a solvent molecule S. In an ethanolic solution, S can be replaced by an X^- anion, e.g. benzoate. When benzoate bears an electron donor or acceptor substituent, an eT process takes place and the anion binding is signalled by a fluorescence quenching.

observed when the benzoate ion bears an electron acceptor substituent, e.g. the $-NO_2$ group. On titration of $[Zn^{II}(\mathbf{14})]^{2+}$ with 4-nitrobenzoate, the anthracene emission is quenched, due to thermodynamically allowed An*-to-NO_2 eT process ($\Delta G^{\circ}_{eT} = -1.0$ eV). The log K for the 1:1 complex which forms in an EtOH solution is 4.73. It is worth noting that quenching is observed also when $[Zn^{II}(\mathbf{14})]^{2+}$ is titrated with the fluorescent anion 9-anthracenoate. In particular, all the fluorescence deriving from both fragments, *N*-substituted anthracenyl and 9-anthracenoate, is quenched. We know that the An*-to-An eT process is thermodynamically feasible ($\Delta G^{\circ}_{eT} = -0.2$ eV), but we cannot determine, through a simple spectrofluorimetric titration experiment, from which fragment to which fragment the electron moves.

Equipping the Zn^{II}-tren platform with fluorescent fragments of different nature may alter the selectivity of the sensing response. As an example, in system **15**, an *N*,*N*-dimethylaniline subunit has been appended to each terminal amine nitrogen atom of tren [24]. The DMA fragment, when irradiated at 300 nm, gives rises to a charge transfer (CT) excited state, which originates from the interaction of the $-N(CH_3)_2$ donor group and the $\pi-\pi^*$ excited state of the benzene fragment, and which undergoes emissive decay (non-structured band at 360 nm). The $[Zn^{II}(\mathbf{15})]^{2+}$ complex displays the typical emission features of DMA. In particular, the emission intensity of the $[Zn^{II}(\mathbf{15})]^{2+}$ complex in an MeOH solution is higher (about 1.7 times) than that of the metal free tetramine **15**, whose emission is partially quenched due to the competition of a photoinduced electron transfer from one of the amine nitrogen atoms of the tetramine to the proximated excited DMA subunit. Such a mechanism is arrested, and the fluorescence fully revived, when the lone pair on each nitrogen atom is engaged in the coordinative interaction with the Zn^{II} centre.

Carboxylates give stable 1:1 adducts with the $[Zn^{II}(\mathbf{15})]^{2+}$ receptor, in an MeOH solution, with log K values ranging from 4 to 5. However, only carboxylates bearing an aromatic residue (benzoate, 4-nitrobenzoate, 9-anthracenoate) are able to quench the emission of the DMA fluorophore (to 10–20% of its original value). On the other hand, binding of aliphatic carboxylates (acetate, cyclohexylcarboxylate) does not alter at all the DMA emission. Profiles of selected titration experiments of $[Zn^{II}(\mathbf{15})]^{2+}$ with anions are shown in Fig. 9.

Thus, $[Zn^{II}(\mathbf{15})]^{2+}$ is a discriminating molecular sensor, which recognises the $-COO^-$ residue, but signals the recognition only if the carboxylate group is linked to an aromatic backbone. This selective photophysical effect must be related to the establishing of an interaction between the aromatic moiety of the carboxylate anion and the aromatic substituents of the tetramine. The existence of such an interaction (probably of the π-stacking type) is also suggested by the fact that the binding constant for benzoate ($\log K = 4.69$) is distinctly higher than that observed for cyclohexylcarboxylate ($\log K = 4.07$), for which the additional intra-complex interaction (besides the Zn^{II}–COO^- binding) is precluded. It is possible that the π-interaction alters the energy of the levels of the DMA fragment in such a way that the interaction of the $\pi-\pi^*$ excited state with the level of the $-N(CH_3)_2$ fragment is minimised, which prevents the formation of the CT excited state. It is also possible that the photoexcited aminobenzene fragment and the facing aromatic carboxylate give rise to an exciplex. This exciplex could then undergo a fast intersystem crossing,

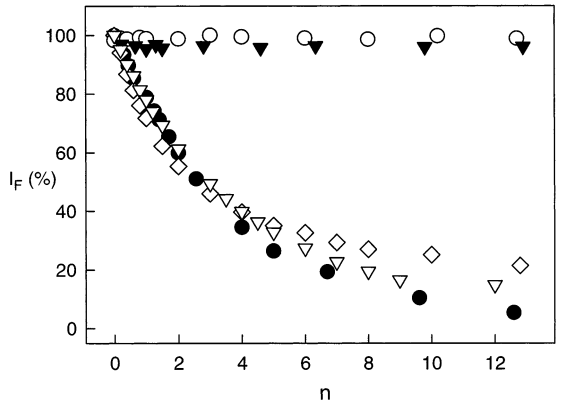


Fig. 9. Spectrofluorimetric titration profiles of the $[Zn^{II}(\mathbf{15})]^{2+}$ receptor in an MeOH solution (10^{-4} M) with standard solutions of benzoate (diamonds), 9-anthracenoate (open triangles), 4-nitrobenzoate (full circles), acetate (full triangles), cyclohexylcarboxylate (open circles). n is the number of added equivalents of carboxylate.

via the carbonyl oxygen lone pair, leading to a non-radiative decay. The $[Zn^{II}(\mathbf{15})]^{2+}$ receptor recognises selectively carboxylates over inorganic anions. For instance, the log K for Cl^- binding is 3.69 (and the DMA emission not altered). The NO_3^- and ClO_4^- anions are not bound by $[Zn^{II}(\mathbf{15})]^{2+}$ in an MeOH solution.

Some polyatomic anions are ambidentate, i.e. they contain at least two distinct donor atoms and can then coordinate two different metal centres: this is the case of the linear N_3^-, the triangular NO_3^-, the tetrahedral SO_4^{2-}. The proper receptor for an ambidentate anion should therefore possess two prepositioned metal ions, each one leaving a coordinative position available for one of the anion donor atoms. Selectivity should depend on how well the distance between the two donor atoms (the so-called anion bite) fits the distance between the two metal centres (or, more precisely, the distance between the vacant coordination sites). A very effective receptor for ambidentate anions is represented by the dicopper(II) complex of the octamine cage **17**, which consists of two tren subunits linked by 1,3-xylyl spacers. The $[Cu^{II}_2(\mathbf{17})]^{4+}$ complex is capable of including an ambidentate anion, e.g. N_3^-, NCO^- (see Fig. 10). X-ray diffraction studies have shown that the linear triatomic anion is co-linearly bound to the two Cu^{II} centres [25]. This coordinative situation is not obvious, as, in absence of the steric constraints imposed by the cage, the linear anion tends to bind the Cu^{II} centre in a bent mode. For instance, in the $[Cu^{II}(tren)(NCO)]^+$ complex, the Cu^{II}–N–C angle is 160° [26]. In the Cu^{II} complex of the tribenzyl-substituted tren ligand **3**, in which an N_3^- is axially bound, as illustrated in Fig. 2, the Cu^{II}–N–N angle reaches 121° [9]: this value is consistent with the sp^2 hybridisation of the terminal nitrogen atoms of N_3^-. The steric constraints imposed by the cage impart interesting selectivity effects in the recognition of ambidentate anions. In this sense, spectrophotometric studies have shown that the $[Cu^{II}_2(\mathbf{17})]^{4+}$ receptor gives 1:1 complexes of varying stability with ambidentate anions in aqueous solution [27].

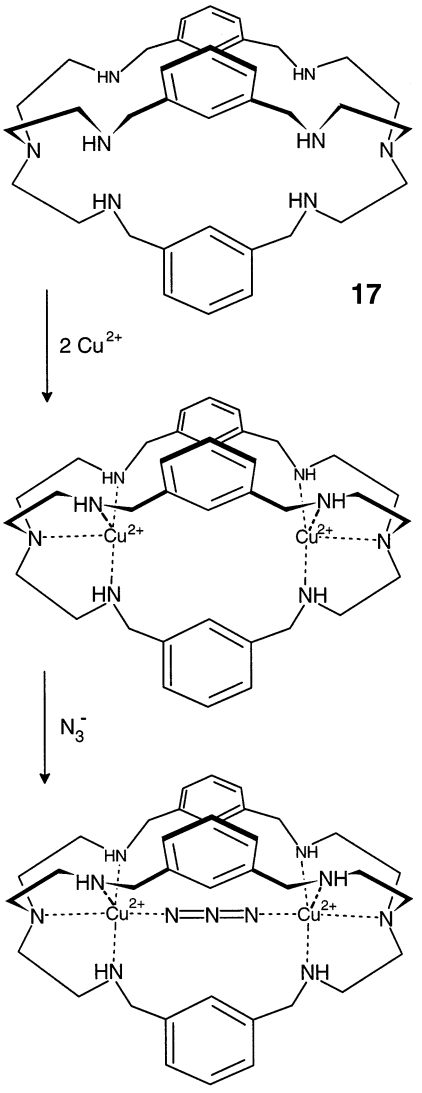


Fig. 10. Anion encapsulation within a dicopper(II) bistren cryptate. Each Cu^{II} centre occupies one of the two tetramine compartments of the cage, leaving a vacant coordination site. The two vacant sites can be occupied by the donor atoms of an ambidentate anion: N_3^- in the Figure. It should be noted that the homodimetallic cage forces the N_3^- anion to an innatural co-linear coordination mode, compared to the bent mode observed under unconstrained circumstances (see Fig. 2).

In particular, peak selectivity is observed when plotting the log K of the 1:1 adduct versus the anion bite length (see the plot in Fig. 11) The highest stability is observed with the N_3^- anion, whose bite (2.34 Å) fits well the distance of the two vacant positions on the Cu^{II} centres. The NCO^- and HCO_3^- anions exhibit a favourable bite length (2.42 and 2.28 Å, respectively) and show a good selectivity

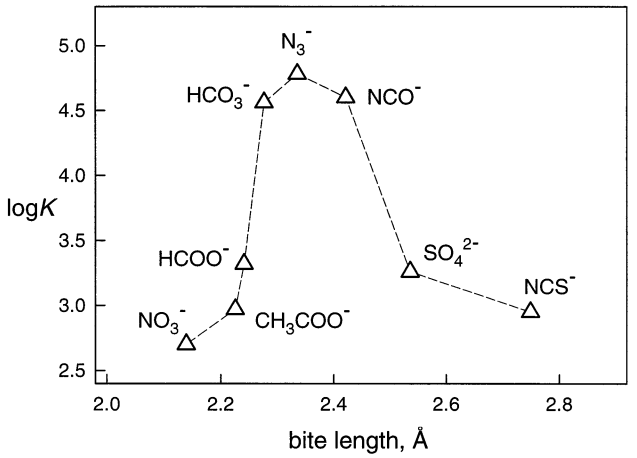


Fig. 11. Geometrical selectivity in the recognition of polyatomic anions by a dicopper(II) bistren cryptate. Log K values of inclusion constants determined in an aqueous solution buffered at pH 8. Bite length refers to the distance of the two coordinating donor atoms of the ambidentate anion. The dicopper(II) receptor recognises the bite of the anion and not its shape. The N_3^- ion provides the most favourable bite length (2.34 Å) to encompass the distance between the two vacant positions of the two Cu^{II} centres.

for $[Cu_2^{II}(\mathbf{17})]^{4+}$ receptor. Anions displaying a longer (NCS^-, 2.75 Å) or a shorter bite (NO_3^-, 2.14 Å) give much less stable adducts: the lower stability reflects the endoergonic rearrangement the cage has to experience when it expands or contracts its cavity in order to include properly the anion.

The selective recognition features of a dicopper(II) bistren receptor can be implemented to sensing (i) by replacing one of the xylyl spacers by the fluorescent fragment 9,10-anthracenyl (**18**); and (ii) by substituting Cu^{II} with the photophysically inactive Zn^{II} centre [28]. Thus, the dizinc(II) complex of **18** was formed, which is stable in aqueous solution over a substantial range of pH. At pH 8, the $[Zn_2^{II}(\mathbf{18})]^{4+}$ complex interacts with ambidentate anions, to give stable 1:1 inclusion complexes (see Fig. 12). In particular, by titrating with a standard N_3^- solution an aqueous solution 10^{-4} M in $[Zn_2^{II}(\mathbf{18})]^{4+}$ complex, a linear decrease of fluorescence was observed until the addition of 1 equiv. of azide. Least-squares analysis of the titration profile gave a value of $\log K$ of 5.8. Quenching may be due to the occurrence of an eT process from the electron rich N_3^- ion to the nearby 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 allow a fast through-space eT process to occur. When a solution of the $[Zn_2^{II}(\mathbf{18})]^{4+}$ complex was titrated with a series of anions: NO_3^-, HCO_3^-, SO_4^{2-}, Cl^-, Br^- no decrease of fluorescence intensity, I_F, was observed even after the addition of 10 eqvs. Moreover, competitive fluorimetric titration experiments showed that these anions, even when present in a 10-fold excess, do not compete with N_3^- for inclusion: this indicates that the corresponding $\log K$ values for the inclusion equilibria must be lower than 3.6. The case of NCO^- is different. On

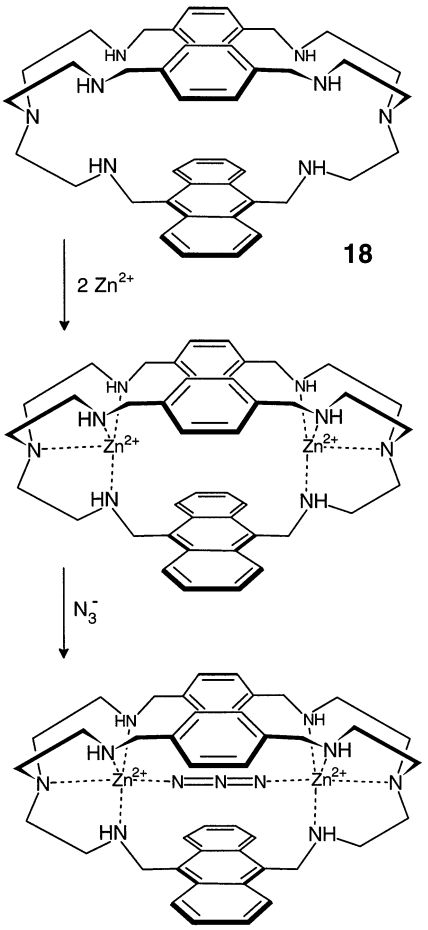


Fig. 12. Anion encapsulation within a dizinc(II) fluorescent cage. The inclusion of the N_3^- ion is signalled through the quenching of the emission by the 9,10-anthracenyl spacer. Quenching is associated with an eT process from the electron rich N_3^- ion to the proximate anthracene fragment. The inclusion of NCO^- does not alter the light emission, due to the anion resistance to undergo photoinduced electron abstaction.

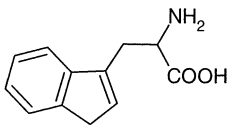
addition of NCO^- to the receptor solution, no I_F decrease was observed. However, the titration profile of N_3^- was remarkably affected by the presence of NCO^-: the greater NCO^- concentration, the less steep the I_F decrease, which indicates severe competition for anion inclusion within the cage. In particular, a log K of 6.5 can be calculated for the NCO^- inclusion equilibrium. Thus, NCO^- has a slightly greater affinity for $[Zn_2^{II}(\mathbf{18})]^{4+}$ than N_3^-, but, due to its less pronounced reducing tendencies, when included in the cage, it is unable to 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 the inclusion constant should reflect the favourable matching with the distance between the two vacant axial

positions of the two Zn^{II} centres. However, the affinity is inverted with respect to what observed for the inclusion in the $[Cu_2^{II}(\mathbf{17})]^{4+}$ cryptate, for which log K was slightly higher for N_3^- than for NCO^-. This may be due to the fact that cage **18** has 1,4-xylyl spacers, which are longer than the 1,3-xylyl spacers present in cage **17**, thus providing a more favourable cavity for the anion of higher bite length. The other linear triatomic anion, NCS^-, quenches fluorescence, but following a much less steep profile, to which a much lower value of log K corresponds: 2.45. NCS^- is a one-electron reducing agent even stronger than N_3^- (the $E°$ value for the NCS/NCS^- couple is: -1.62 V versus NHE; N_3^-/N_3: 1.33 V [29]), which accounts for the occurrence of an intra-complex photo-induced eT process and fluorescence quenching. However, the NCS^- bite length (2.75 Å) is too large for the cage relaxed to its more favourable arrangement. Thus, anion inclusion induces an endoergonic rearrangement, making the inclusion constant 2200 times lower than for NCO^-.

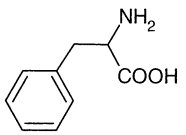
4. Sensing of aminoacids

Due to its special affinity toward the $-COO^-$ group, the Zn^{II}-tren platform can be proposed for the recognition of natural amino acids, of general formula: NH_3^+–CH(R)–COO^-. The availability of luminescent molecular sensors for amino acids is a longtime awaited opportunity by cell biologists [30]. In fact, one of the most efficient techniques for monitoring the concentration of any kind of analytes inside the cell, in real time and in real space, is represented by fluorescence confocal microscopy, which requires the use of fluorescent molecular sensors. One of the first and most fascinating examples in this field refers to the monitoring of the Ca^{2+} concentration inside the cell during muscle contraction, by means of a specific chelating agent equipped with a fluorophore [31].

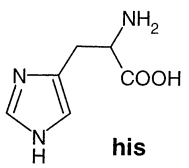
However, the prototype of Zn^{II}-tren base fluorescent sensors, $[Zn^{II}(\mathbf{14})]^{2+}$, shows a poor and scarcely selective affinity towards natural amino acids. For instance, in a 4:1 ethanol/water solution, $[Zn^{II}(\mathbf{14})]^{2+}$ forms poorly stable 1:1 adducts with amino acids: the log K values for the corresponding equilibria are in all cases around 2 (determined spectrophotometrically), independently upon the nature of the amino acid. Moreover, substrate binding does not modify the fluorescent emission of the anthracene fragment covalently linked to the tetramine framework. The poor affinity can be ascribed to the electrostatic repulsions between the Zn^{2+} ion and the ammonium residue of the α-amino acid. Also, the interaction cannot be selective, since it is addressed to a group, $-COO^-$, which is common to all the amino acids. It derives that, in order to increase selectivity, the receptor must be equipped with substituent providing further binding sites capable to establish a specific interaction with the desired substrate.



trp



phe



his

An example in this sense is provided system **16**, in which the tren framework has been armed with two antracenyl and one benzyl substituents [32]. The corresponding Zn^{II} complex, $[Zn^{II}(\mathbf{16})]^{2+}$, displays the typical anthracene emission and no spectral evidence exists for the formation of an excimer. $[Zn^{II}(\mathbf{16})]^{2+}$ shows a high affinity towards those natural amino acids that bear aromatic substituents: phenylalanine (phe) and tryptophane (trp). Log K values associated to the formation of the $[Zn^{II}(\mathbf{16})(phe)]^{2+}$ and $[Zn^{II}(\mathbf{16})(trp)]^{2+}$ adducts, in 4:1 ethanol/water mixture, buffered to pH 6.8, are 4.48 and 4.21, respectively (obtained through spectrophotometric titration experiments). It is worth noting that these values are remarkably higher than those observed for all the other amino acids (e.g. glycine, log $K = 3.06$).

The high stability of $[Zn^{II}(\mathbf{16})(phe)]^{2+}$ and $[Zn^{II}(\mathbf{16})(trp)]^{2+}$ adducts can be ascribed to the establishing of π-stacking interactions between the aromatic part of the Zn^{II}-bound amino acid and one of the facing poly-aromatic substituents of the tren framework. The binding situation in $[Zn^{II}(\mathbf{16})(trp)]^{2+}$ is tentatively illustrated in Fig. 13.

Noticeably, the $[Zn^{II}(\mathbf{16})]^{2+}$ system displays a selective behaviour also from the point of view of fluorescent sensing. In fact, titration of an aqueous ethanolic solution of $[Zn^{II}(\mathbf{16})]^{2+}$ with trp induces fluorescence quenching. The titration profile indicates the formation of a 1:1 adduct, whose log K value is the same as that obtained from the spectrophotometric titration. On the contrary, titration with the other amino acid bearing an aromatic substituent, phe, does not alter at all the anthracene emission $[Zn^{II}(\mathbf{16})]^{2+}$. The signalling mechanism operating in the $[Zn^{II}(\mathbf{16})(trp)]^{2+}$ adduct is brought by the analyte itself. The indole substituent of trp possesses electron donor tendencies and fluorescence quenching is due to a 'through-space' eT process from the secondary amine nitrogen atom of the trp subunit to the facing An* fragment. Such a mechanism cannot be provided by the other recognised amino acid, phe, whose substituent (a phenyl group) does not exhibit electron donor properties.

The selective behaviour of the $[Zn^{II}(\mathbf{16})]^{2+}$ receptor results from its capability to establish two different interactions with the NH_3^+–CH(R)–COO^- substrate: (i) the

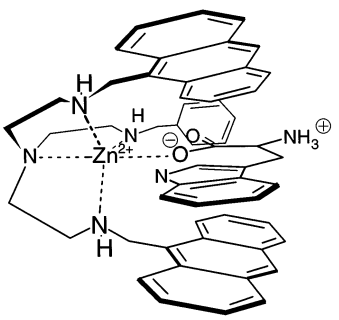


Fig. 13. Recognition of tryptophane by the Zn^{II} complex of tetramine **16**. The metal-carboxylate bond is implemented by a π-stacking interaction between the indole fragment of the amino acid and one of the aromatic substituents on the tetramine framework. An eT process from the indole fragment to the photoexcited anthracene fragment induces fluorescence quenching and signals the occurrence of the recognition process.

Zn^{II}–COO– metal–ligand interaction; and (ii) the π-stacking interaction between **R** and the aromatic substituents on the receptor framework. The example above agrees well with the general rule of molecular recognition: the higher the number of the interaction points between receptor and substrate, the higher the selectivity.

However, in order to obtain the most selective recognition of a desired amino acid one should try to ignore the common $–COO^-$ fragment and to put the attention on the sole **R** residue. In particular, a receptor subunit should be designed exclusively for the **R** moiety of the substrate. This case is nicely illustrated by the design of a molecular fluorescent sensor for histidine, whose **R** group is an imidazolyl fragment. A very effective receptor for imidazole can be designed by taking profit of its tendency to deprotonate and to act as an ambidentate ligand. Imidazole, imH, $pK_a = 14.5$, is a weaker acid than H_2O and does not deprotonate in aqueous solution. However, in presence of two prepositioned metal ions M^{II}, the ImH molecule simultaneously deprotonate and the Im^- anion which forms bridges the two metal centres [33], according to the equilibrium:

$$[M^{II}_2]^{4+} + ImH = [M^{II}(Im^-)M^{II}]^{3+} + H^+ \quad (1)$$

A basic requirement for reaction (1) to proceed is that the two M^{II} centres occupy fixed positions, in a rigid coordinative framework, at the correct distance. This situation is present in the superoxide dismutase enzyme (SOD), in which the imidazolate group of a histidine residue bridges a Cu^{II} ion and a Zn^{II} ion. The metalloenzyme is in charge of the elimination of the harmful radical O_2^-, through a disproportionation process which involves the Cu^{II}/Cu^{I} redox couple [34]. Artificial dicopper(II) analogues of the heterodimetallic core of SOD have been prepared [35]. In most cases, the two Cu^{II} ions are prepositioned in a two-compartment polyamine receptor and the two nitrogen atoms of the imidazolate bridging ligand completed the five-coordinate arrangement of each metal centre. On these bases, it was considered that a bistren system containing two Zn^{II} ions (instead of the irremediably photoactive Cu^{II}) could be a good candidate for imidazole recognition. In particular, in the homoditopic ligand **19**, the two tetramine subunits are covalently linked by a 9,10-anthracenyl spacer [36] (see Fig. 14).

Potentiometric titration experiments showed that the dizinc(II) complex containing an imidazolate bridge, $[Zn^{II}(\mathbf{19})(im^-)]^{3+}$ forms in a slightly basic solution and, in particular, it is the dominating species over the pH interval 9–10. Thus, the dizinc(II) bistren complex can behave as a receptor of imidazole. Moreover, titration with imidazole of an aqueous solution of the $[Zn^{II}(\mathbf{19})]^{4+}$ complex, buffered at pH 9.6, induced quenching of the anthracene fluorescence. Non-linear fitting of the I_F versus eqs. profile indicated the formation of the 1:1 receptor-substrate adduct, i.e. $[Zn^{II}(\mathbf{19})(im^-)]^{3+}$, with a conditional constant of 3.65 log units. Noticeably, titration with 1-methyl-imidazole, which cannot undergo deprotonation, did not induce any modification of the anthracene fluorescent emission, confirming that signalling is promoted by Zn^{II}–Zn^{II} bridging of the imidazolate fragment. Quenching in the $[Zn^{II}(\mathbf{19})(im^-)]^{3+}$ adduct has to be ascribed to the occurrence of an intra-complex electron transfer process from a π orbital of the electron rich im^- moiety to a π* orbital of the photo-excited anthracene fragment.

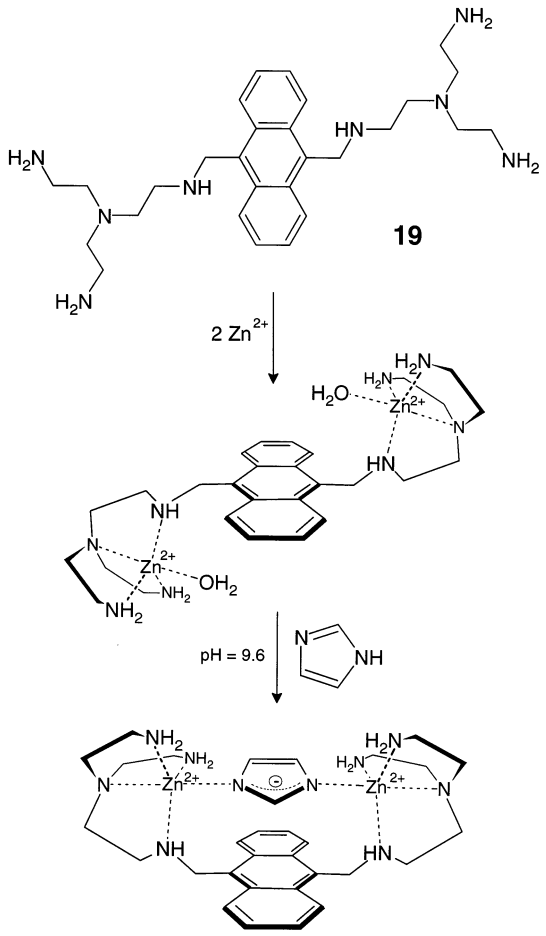


Fig. 14. Recognition and sensing of imidazole by a dizinc(II) bistren complex. At pH 9.6 imidazole deprotonates and bridges the two Zn^{II} centres. The electron rich imidazolate fragment transfers an electron to the nearby anthracene fragment, quenching its emission. The amino acid histidine is recognised and sensed by the same mechanism. All the other natural amino acids cannot compete.

Spectrofluorimetric titration of the $[Zn^{II}(\mathbf{19})]^{4+}$ complex with L-histidine induced a fluorescence quenching similar to that produced by plain imidazole, but characterised by a lower value for the binding constant ($\log K = 2.92$): this may reflect the existence of some steric repulsions between by the imidazole appended amino acid fragment and the receptor framework. Very interestingly, the titration profile is not modified when the solution contains even a large excess of any other amino acid. As an example, titration L-histidine to a solution buffered at pH 9.6 and containing the receptor $[Zn^{II}(\mathbf{19})]^{4+}$] plus 10 eqvs. of L-glycine gave the same titration profile obtained in absence of the competing amino acid. The lack of interference may be due to the fact that the only anionic group any amino acid other than histidine can

offer, i.e. the carboxylate fragment, does not display bridging tendencies toward the dimetallic core of $[Zn^{II}(\mathbf{19})]^4$ receptor. Thus, $[Zn^{II}(\mathbf{19})]^{4+}$ recognizes and senses L-histidine in presence of any other natural amino acid.

Acknowledgements

This work has been supported by C.N.R. (Progetto Finalizzato Biotecnologie).

References

[1] R.A. Bissel, A.P. de Silva, H.Q.N. Gunaratne, P.L.M. Lynch, G.E.M. Maguire, K.R.A.S. Sandanayake, Chem. Soc. Rev. 21 (1992) 187.
[2] A.P. de Silva, H.Q.N. Gunaratne, T. Gunnlaugsson, A.J.M. Huxley, C.P. McCoy, J.T. Rademacher, T.E. Rice, Chem. Rev. 97 (1997) 1515.
[3] L. Fabbrizzi, A. Poggi, Chem. Soc. Rev. 24 (1995) 197.
[4] V. Balzani, F. Scandola, Supramolecular Photochemistry, Ellis Horwood, Chichester, 1991, pp. 71–73.
[5] P. Suppan, Chemistry and Light, The Royal Society of Chemistry, Cambridge, 1994, pp. 66–68.
[6] J.-M. Lehn, Supramolecular Chemistry, Concepts and Perspectives, VCH, Weinheim, 1995, pp. 31–35.
[7] C.J. Pedersen, J. Am. Chem. Soc. 89 (1967) 7017.
[8] B. Dietrich, J.-M. Lehn, J. Guilhem, C. Pascard, Tetrahedron Lett. 30 (1989) 4125.
[9] P. Pallavicini, N. Sardone, unpublished results.
[10] F.P. Schmidtchen, M. Berger, Chem. Rev. 97 (1997) 1609.
[11] A. Bianchi, K. Bowman-James, E. Garcia-España, Supramolecular Chemistry of Anions, Wiley-VCH, New York, 1997.
[12] P.D. Beer, Chem. Commun. (1996) 689
[13] R.S. Dickins, T. Gunnlaugsson, D. Parker, R.D. Peacock, Chem. Commun. (1998) 1643
[14] M.E. Huston, E.U. Akkaya, A.W. Czarnik, J. Am. Chem. Soc. 111 (1989) 8735.
[15] A.P. de Silva, H.Q.N. Gunaratne, C. McVeigh, G.E.M. Maguire, P.R.S. Maxwell, E. O'Hanlon, Chem. Commun. (1996) 2191.
[16] D.K. Cabbiness, D.W. Margerum, J. Am. Chem. Soc. 92 (1970) 2151.
[17] D.J. Cram, Angew Chem. Int. Ed. Engl. 25 (1986) 1039.
[18] H. Fenniri, M.W. Hosseini, J.-M. Lehn, Helv. Chim. Acta 80 (1997) 786.
[19] M. Dhaenens, J.-M. Lehn, J.-P. Vigneron, J. Chem. Soc. Perkin Trans. 2 (1993) 1379.
[20] M.-P. Teulade-Fichou, J.-P. Vigneron, J.-M. Lehn, J. Chem. Soc. Perkin Trans. 2 (1996) 2169.
[21] L. Sacconi, Pure Appl. Chem. 17 (1968) 95.
[22] H. Irving, R.J.P. Williams, J. Chem. Soc. (1953) 3192.
[23] G. De Santis, L. Fabbrizzi, M. Licchelli, A. Poggi, A. Taglietti, Angew Chem. Int. Ed. Engl. 35 (1996) 202.
[24] L. Fabbrizzi, M. Licchelli, L. Parodi, A. Poggi, A. Taglietti, Eur. J. Inorg. Chem. (1999) 35.
[25] C.J. Harding, F.E. Mabbs, E.J.L. MacInnes, V. McKee, J Nelson, J. Chem. Soc. Dalton Trans. (1996) 3227.
[26] E.J. Laskowski, D.M. Duggan, D.N. Hendrickson, Inorg. Chem. 14 (1975) 2449.
[27] L. Fabbrizzi, P. Pallavicini, A. Perotti, L. Parodi, A. Taglietti, Inorg. Chim. Acta 238 (1995) 5.
[28] L. Fabbrizzi, I. Faravelli, G. Francese, M. Licchelli, A. Perotti, A. Taglietti, Chem. Commun. (1998) 971.
[29] P. Wardman, J. Phys. Chem. Ref. Data 18 (1989) 1710.
[30] A.W. Czarnik, Chem. Biol. 2 (1995) 423.

[31] R.Y. Tsien, Biochem 19 (1980) 2396.
[32] L. Fabbrizzi, M. Licchelli, L. Parodi, A. Poggi, A. Taglietti, J. Fluoresc. 8 (1998) 263.
[33] L. Fabbrizzi, P. Pallavicini, L. Parodi, A. Perotti, A. Taglietti, J. Chem. Soc. Chem. Comm. (1995) 2439.
[34] J.A. Cowan, Inorganic Biochemistry, VCH, New York, 1993, pp. 254–260.
[35] P.K. Coughlin, A.E. Martin, J.C. Dewan, E.-I. Watanabe, J.E. Bulkowski, J.-M. Lehn, S.J. Lippard, Inorg. Chem. 23 (1984) 1004.
[36] L. Fabbrizzi, G. Francese, M. Licchelli, A. Perotti, A. Taglietti, Chem. Commun. (1997) 581.