Fluorescent Sensors for Transition Metals Based on Electron-Transfer and Energy-Transfer Mechanisms

Luigi Fabbrizzi,* Maurizio Licchelli, Piersandro Pallavicini, Angelo Perotti, Angelo Taglietti and Donata Sacchi

Abstract: Fluorescent sensors for 3d divalent metal ions have been designed by means of a supramolecular approach: an anthracene fragment (the signalling subunit) has been linked to either a cyclic or a noncyclic quadridentate ligand (the receptor). Occurrence of the metal–receptor interaction is signalled through the quenching of anthracene fluorescence. When the receptor (i.e., the dioxotetramine subunit of sensors **2** and **3**) is able to promote the one-electron oxidation of the metal, quenching takes place through a photoinduced metal-to-fluorophore *electron-transfer* mechanism. In the case of sensors containing a tetraamine binding subunit (**4** and **5**), quenching proceeds by an *energy-transfer* process. Selective metal binding and recognition can be achieved by varying the pH, and metal ions can be distinguished (e.g., Cu^{II} from Ni^{II}) by spectrofluorimetric titration experiments in buffered solutions. Whereas systems **2**, **3** and **5** show reversible metal binding behaviour, the *cyclam*-containing system **4** irreversibly incorporates transition metals (due to the *kinetic macrocyclic effect*) and cannot work properly as a sen-

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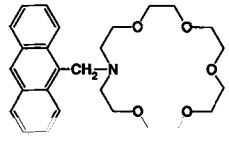
copper complexes · electron transfer · energy transfer · fluorescent sensors · nickel complexes

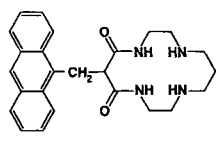
In the supramolecular world, a *sensor* is a two-component system in which the specific receptor for the intended substrate is connected to a subunit capable of signalling the occurrence of the receptor–substrate interaction. The signal is given by a drastic change of a property: thus sensor efficiency is related to the ease of detecting such a property and measuring its intensity over a substantial concentration range, possibly down to trace level, as well as to receptor specificity. In this context, *fluorescence* is a convenient property to investigate. Fluorescence is visible, can be determined in real time without excessively sophisticated and expensive instrumentation and, if the appropriate fluorophore is chosen, can be safely monitored at a concentration level as low as 10^{-7} M. Efficient sensing should involve variation of the investigated property by at least two orders of magnitude: in spectrofluorimetric measurements, such a situation would correspond to full *quenching* or to complete *revival* of the emission intensity.

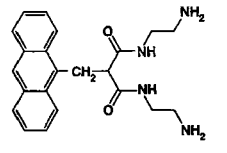
During the last decade, a number of fluorescent sensors has been designed for s-block metal ions.[1] Most of them operate by a photoinduced electron-transfer (PET) mechanism.[2] In a classic example from the de Silva group, the binding component of the sensor is an NO_5 crown, which is linked through the amine nitrogen atom to the powerful light-emitting fragment anthracene by a methylene group.[3] The uncomplexed sensor **1** is not fluorescent, as the photoexcited fluorophore is deactivated by a nonradiative mode through the transfer of an electron from the highly re-

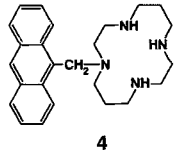


(e.g., of a K^+ ion), the metal–ligand interaction decreases the amine oxidation potential drastically and prevents the electron transfer. As a consequence, the intense and characteristic anthracene emission is largely restored.

We were interested in developing fluorescent sensors for transition metals using the same supramolecular approach. In particular, we noted that the fluorophore (anthracene, also in the present case) was connected to a multidentate system containing nitrogen donor atoms. In fact, amine and amide groups display a special affinity for d-block metal ions. Thus, the following two-component systems were considered, in which the anthracene fragment has been linked to quadridentate aza crowns (**2** and **4**, derivatives of the classical macrocycles dioxo-







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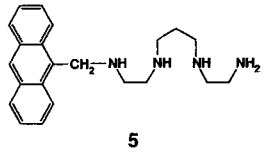

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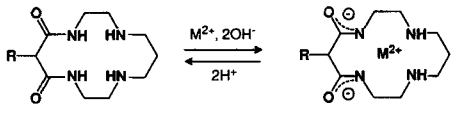
cyclam[4] and cyclam,[5] and of their open-chain counterparts, **3** and **5**).

It should be noted that PET sensors like **1** must contain a group to supply the electrons in addition to the fluorescent subunit and the receptor, for example a tertiary amine group near the light-emitting fragment. Such a device may be unnecessary in transition-metal sensing with a PET mechanism. In fact, as 3d metals typically exhibite rich redox activity, the electron transfer can take place directly from the fluorophore to the complexed ion (or vice versa). In this case, one should choose as a receptor a ligand able to promote the redox activity of the target metal ion, that is, favouring the conversion to an adjacent oxidation state. In addition, 3d ions possess empty levels of suitable energy which can be involved in *energy-transfer* processes from the photoexcited fluorophore (according to the Dexter mechanism).[6] This offers a second opportunity (not available to non-transition metals) for fluorescence quenching and sensing. The aim of this work is to demonstrate that supramolecules **2**–**5** act as efficient sensors for transition metals, taking advantage of either electron-transfer or energy-transfer mechanisms. A preliminary investigation of the sensing properties of **3** has been recently reported.[7]

Results and Discussion

Using dioxotetraamines as receptors: Dioxocyclam is a powerful ligand capable of binding divalent metal ions of the late 3d series, with simultaneous deprotonation of the two amido groups, as outlined in Scheme 1.[8] In order to define the pH dependence of metal complexation by the dioxocyclam derivative **2** and to study the corresponding effects on the light-emitting properties of the appended fluorophore, titration experiments were carried out inside a spectrofluorimetric cuvette. First, a solution of **2** and excess strong acid in MeCN and water (80:20) was titrated with a standard NaOH solution. No alteration of the intense characteristic emission of anthracene was observed over the investigated pH interval (2–12). However, if the titration was carried out in a similar solution that also contained 1 equiv of Cu^{II}, the intensity of the emission band, I_F, began to decrease at pH = 3.6, and fluorescence was completely quenched at pH = 5.3. Specifically, the plot of I_F against pH displays a sigmoidal profile, as illustrated in Figure 1. Fluorescence quenching has to be associated with the incorporation of the Cu^{II} ion by the dioxocyclam subunit of **2** according to an equilibrium of the type outlined in Scheme 1. This is confirmed by a similar titration experiment carried out in the spectrophotometric cuvette: at pH = 3.7 an absorption band centred at 510 nm developed, to reach a limiting value at pH = 6.0. Notably, the plot of the molar absorbance at 510 nm, A_{510}, against pH shows a sigmoidal profile mirroring that of the I_F/pH plot illustrated in Figure 1. The band at 510 nm corresponds to a species of type **6** (see Scheme 1), pink-violet in colour, in which the Cu^{II} centre is coordinated by two amine groups and two deprotonated amide nitrogen atoms of the dioxocyclam subunit. Similar behaviour has been reported for the open-chain analogue **3**.[7] The corresponding I_F/pH plot for the system Cu^{II}/**3** is reproduced in Figure 2 for the purpose of comparison. The I_F/pH profiles have a similar shape, but do not coincide: the displacement of the profile of **2** to pH values about 1.5 units lower reflects the higher solution stability of Cu^{II} complexes with cyclic ligands compared with those with open-chain counterparts (the *thermodynamic* macrocyclic effect).[9]



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Scheme 1. Complexation of a divalent 3d metal ion (M = Cu, Ni) by a dioxotetraamine subunit (R = H, dioxocyclam; R = anthracene-9-ylmethyl, **2**).

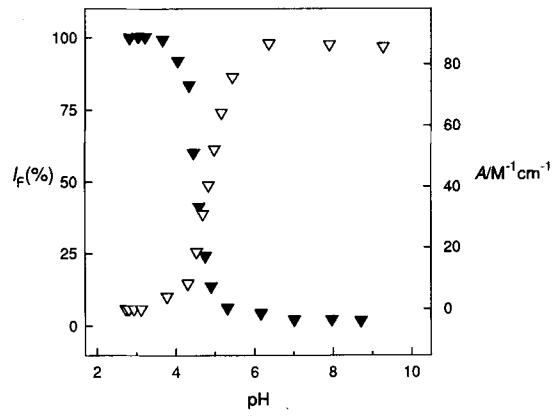

Fig. 1. pH dependence of the fluorescence intensity (I_F, ▼) and of the molar absorbance of the band at 510 nm (A, ▽) for a solution containing equimolar amounts of **2** and of Cu^{II} in MeCN/H_2O (4:1).

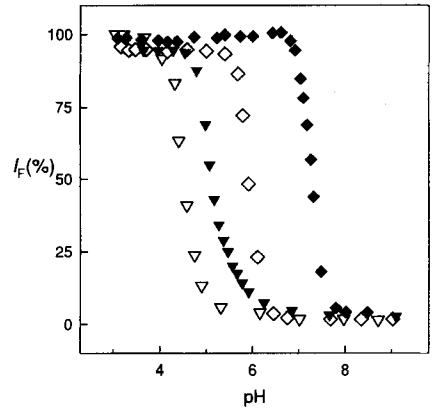

Fig. 2. pH dependence of the fluorescence intensity (I_F) for solutions containing equimolecular amounts of a dioxotetraamine sensor and of either Cu^{II} or Ni^{II} in MeCN/H_2O (4:1). a) **2** and Cu^{II} (▽); b) **2** and Ni^{II} (▼); c) **3** and Cu^{II} (◇); iv) **3** and Ni^{II} (◆).

The Ni^{II} ion behaves similarly, as indicated by titration experiments monitored through spectrofluorimetric and spectrophotometric techniques. In the presence of Ni^{II} the anthracene emission was quenched, and the I_F/pH plot shows a sigmoidal profile centred at pH = 5. Spectrophotometric titration showed that an absorption band centred at 450 nm formed and develops around the same pH. Such a band corresponds to a dioxocyclamato(2−) complex of type **6** (the yellow low-spin Ni^{II} chromophore). Figure 2 shows that the I_F/pH sigmoidal profile for the Ni^{II}/**2** system is centred at a pH about one unit higher than that observed for the Cu^{II}/**2** systems: this reflects the generally observed greater stability of Cu^{II} complexes with polyaza ligands compared with Ni^{II}, in agreement with the Irving–Williams series.[10]

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Notably, only Cu^{II} and Ni^{II} among 3d divalent cations had an effect on the light-emitting properties of **2**. On titration of solutions containing equivalent amounts of **2** and Mn^{II}, Fe^{II}, Co^{II} and Zn^{II}, no decrease of fluorescence was observed. Such behaviour cannot be ascribed to any photophysical effect, but simply to the fact that under the experimental conditions the above-mentioned ions were not chelated by the dioxocyclam ring of **2**. In this connection, one should consider that the very endothermic effect associated with the deprotonation of the two amido groups (see Scheme 1) can be compensated only by metal ions late in the 3d series (e.g., Cu^{II} and Ni^{II}), which can take advantage of a very favourable contribution from the ligand field stabilization energy. Divalent cations earlier in the series profit little from a much less favourable ligand-field effect and cannot induce deprotonation of the two amide groups of the dioxotetraamine ligand. Thus, the cyclic receptor of the supramolecular sensor **2** does not recognize the size of the cation, as typically observed in the interaction of sensors of type **1** with the spherical ions of the s block, but recognizes its position in the Periodic Table.

A deeper insight into Figure 2 suggests a way to discriminate between Cu^{II} and Ni^{II} ions using the fluorescent sensor **2**. A pH value can be chosen within the interval delimited by the two titration profiles at which the Cu^{II} ion is formed, at least in part, and the Ni^{II} complex is not. Thus, an MeCN/water solution (4:1, v/v) containing **2** was adjusted to pH = 4.7 with CH_3COOH/CH_3COO^- buffer. Figure 2 suggests that at this pH the Ni^{II} ion is not chelated by the dioxocyclam receptor, whereas Cu^{II} is complexed at about 50%. The above solution was first titrated with a solution of Ni^{II}. Addition of more than 1 equiv did not have any effect on the emission band of the anthracene (Fig. 3). Then the same solution was titrated with Cu^{II}: fluorescence decreased linearly, to reach 50% of the original intensity after the addition of 1 equiv, as predicted from the titration graph in Figure 2.

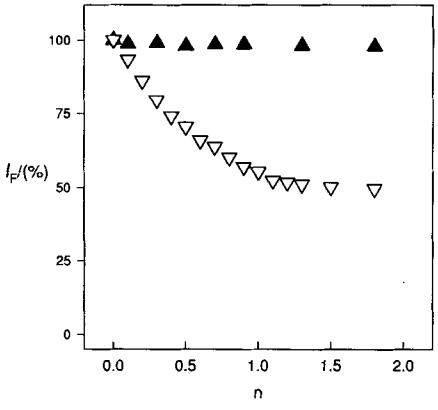


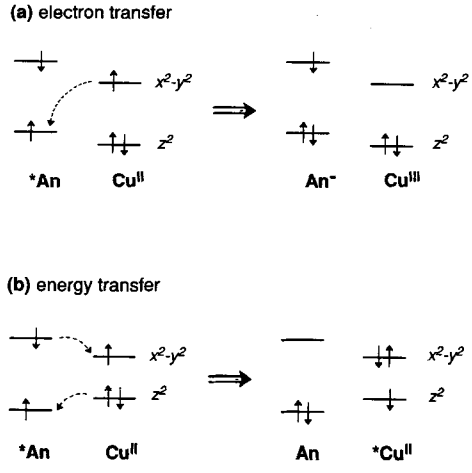
Fig. 3. Discrimination of Cu^{II} and Ni^{II} by the fluorescent sensor **2** in an $MeCN/H_2O$ solution (4:1), buffered at pH = 4.7 with acetate buffer. Titration with Ni^{II} does not alter fluorescence (▲). On addition of Cu^{II}, I_F decreases linearly (▽) to reach 50% of the original value with the addition of 1 equiv. n = number of added equivalents.

Similar recognition of Cu^{II} and Ni^{II} and discrimination under buffered conditions has been observed in the case of the open-chain analogue **3**.[7] However, for the noncyclic system the separation between the two profiles was about 2 pH units wide, which makes a larger pH interval available for selective titration under buffered conditions. In particular, in a solution buffered at pH = 7.1, addition of Ni^{II} did not induce any decrease in fluorescence, and addition of 1 equiv of Cu^{II} *fully* quenched fluorescence.

Thus, sensors **2** and **3** differ in the following aspects, which depend on the cyclic or noncyclic structure of the receptor: a) titration profiles for **3** for both Cu^{II} and Ni^{II} are shifted to higher pH values: this reflects the higher solution stability of the metal complexes of the macrocyclic receptor compared with those of its open-chain counterpart (the previously mentioned *thermodynamic* macrocyclic effect—the cyclic ligand is preoriented for coordination and does not lose any energy when encircling the metal);[11] b) the separation of Cu^{II} and Ni^{II} profiles is narrower for the cyclic system **2** than for **3**: this may reflect the fact that on complexation the high-spin Ni^{II} ion moves to the low-spin state (which reduces its radius by 10% or more).[12] This should allow Ni^{II} to fit better into the rather rigid dioxocyclamato(2−) ring and to profit from the macrocyclic effect to a larger extent that the greater Cu^{II} cation.

Nevertheless, fluorescent sensors **2** and **3** exhibit the same qualitative behaviour, independently of the cyclic or noncyclic nature of the receptor. Incorporation/release of the metal centre is a fast and reversible process, which can be driven back and forth by addition of strong base and strong acid, with concurrent quenching/revival of fluorescence, indefinitely (apart from dilution effects on emission band monitoring). It will be shown later that the reversible binding of a transition metal is rather unusual for cyclic polyaza receptors and can be observed for dioxotetraamine ligands but not for the oxo-free analogues like cyclam. In fact, for a polyamine receptor, the cyclic or noncyclic nature causes significant differences in the application (vide infra).

The nature of metal-induced fluorescence quenching in sensors containing the dioxotetraamine subunit: A transition-metal centre can induce the nonradiative deactivation of a proximate photoexcited fluorophore through two distinct mechanisms: *electron transfer* or *energy transfer*.[13] Both possibilities are open to Cu^{II} and Ni^{II} cations that interact with sensors **2** and **3**. As far as the electron-transfer mechanism is concerned, coordination by deprotonated amide groups favours access to the Cu^{III} and Ni^{III} state; the $[Cu^{III}(dioxocyclamato(2-)]^+$ species was the first reported macrocyclic complex of trivalent copper(III) stable in aqueous solution.[14] Thus, electron transfer from the complexed Cu^{II} centre to the photoexcited anthracene moiety An* of **2** (or **3**), as illustrated in Scheme 2a, should be feasible. On the other hand, transition metal ions like Cu^{II} and Ni^{II} possess empty or half-filled orbitals of convenient energy, which can be



Scheme 2. Quenching of the excited anthracene subunit by the proximate Cu^{II} centre in supramolecular systems of type **2**–**5**: a) electron-transfer mechanism; b) energy-transfer mechanism (Dexter type).

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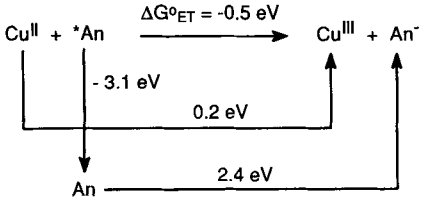
involved in an energy-transfer mechanism of the Dexter type. Scheme 2b qualitatively illustrates such a possibility in the case of the Cu^{II} ion.

The nature of the quenching mechanism can be assessed through spectrofluorimetric investigations carried out in a frozen glass solution at low temperature (e.g., liquid nitrogen). An electron-transfer process (Scheme 2a) generates charge separation, which would induce a drastic rearrangement of the solvation sphere. Immobilization of solvent molecules in the glass prevents such a reorganization, precluding the electron-transfer process and restoring fluorescence.[15] In contrast, an energy-transfer process like that illustrated by Scheme 2b does not involve any charge separation and consequent mobilization of solvent molecules: it is simply an internal double electron-exchange process and cannot be affected by the state of the medium, be it liquid or frozen. Thus, fluorescence quenching should be maintained even in a glass environment. In this context, the sensor **2** and Cu^{II} were dissolved in the glass-forming solvent ethanol and two equivalents of standard base were added: complexation took place and anthracene fluorescence was fully quenched. However, on freezing the same solution at 77 K, the emission band of anthracene was largely restored. This behaviour unequivocally assigns the mechanism of fluorescence quenching of the anthracene subunit following the interaction with the Cu^{II} ion with **2** to an *electron-transfer* process. Such a process should involve the release of an electron from the Cu^{II} centre to the photoexcited fluorophore An*, as indicated in the equation in Scheme 3.



Scheme 3. Thermodynamic cycle for the evaluation of the free energy change, ΔG_{ET}, associated with the electron transfer from Cu^{II} to the photoexcited anthracene fragment within system **2**.

The occurrence of such an electron transfer is fully justified on a thermodynamic basis. In fact, the free energy change associated with the electron transfer process, ΔG_{ET}, calculated from the cycle in Scheme 3 through the combination of photochemical and electrochemical quantities, has a distinctly negative (favourable) value ($\Delta G_{ET} = -E_{0-0(An)} + e(E^{\circ}_{Cu^{III}/Cu^{II}} - E^{\circ}_{An/An^-}) = -0.5$ eV, where $E_{0-0(An)}$ corresponds to the energy of the emission band; E°_{An/An^-} and $E^{\circ}_{Cu^{III}/Cu^{II}}$ have been obtained from voltammetric investigations in MeCN solution). Fluorescence revival was also observed in a glass ethanolic solution of **2** and Ni^{II}. In this case too, the electron-transfer process can be accounted for on a thermodynamic basis ($\Delta G_{ET} = -0.35$ eV). An analogous temperature-dependent pattern was observed for the Cu^{II} and Ni^{II} complexes of the open-chain analogue **3**. Thus, supramolecules **2** and **3** are genuine PET sensors for transition metals.

Using tetraamines as receptors: The light-emitting properties of the two-component system **5**, which contains a fully saturated open-chain receptor, were investigated by pH titration experiments similar to those described in the previous section. When a solution of **5** containing excess acid was titrated with standard base, the emission band of anthracene was observed in strongly acidic conditions, under which all the amine nitrogen atoms of the appended multidentate ligand are protonated. Fluorescence intensity began to decrease when the excess acid had been neutralized and further equivalents of base had been added, as shown in Figure 4. Quenching was most effectively achieved

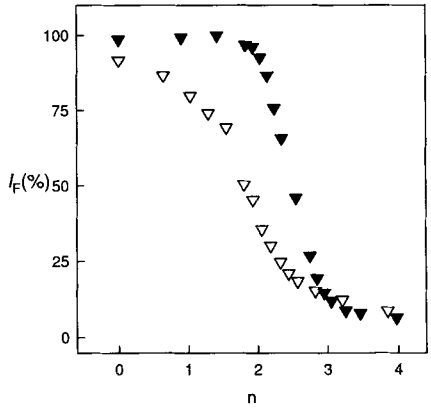


Fig. 4. Spectrofluorimetric titrations of tetraamine sensors **4** (▾) and **5** (▿) in an MeCN/H_2O (4:1) solution.

during the addition of the second and third equivalents. This indicates that the second and third protons are released from the two ammonium groups closest to the anthracene subunit. Deprotonation makes available an electron pair on the nitrogen atom: one electron of the pair is transferred to the proximate photoexcited fluorophore. The fastest electron transfer and the most efficient quenching effect should be provided by the secondary amine group immediately adjacent to the anthracene moiety. However, the simple spectrofluorimetric titration does not permit us to define the deprotonation sequence. pH-dependent fluorescence quenching of this type has been observed in the case of anthrylamines.[2]

Figure 5 is an I_F/pH plot for the titration described above. One equivalent of Cu^{II} was added to the same solution, which was then titrated. Figure 5 shows that I_F decreased sigmoidally, but at a pH 1.5 units lower than observed for the metal-free solution. In this case too, fluorescence quenching has to be attributed to the binding of the metal ion by the chelating subunit. In fact, in the same pH interval (2.0–3.5) a d–d band centred at 530 nm formed and developed. A band of the same energy and comparable intensity was observed for the Cu^{II} com-

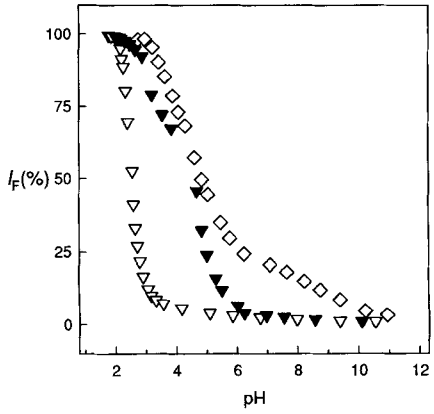


Fig. 5. pH dependence of the fluorescence intensity (I_F) for solutions containing: a) the tetraamine sensor **5** alone (◇); b) equimolar amounts of **5** and Cu^{II} (▿); c) equimolar amounts of **5** and Ni^{II} (▾).

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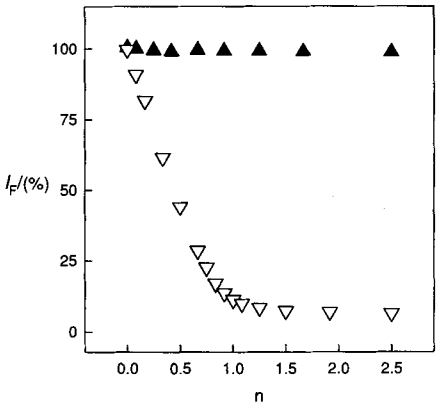

Fig. 6. Differentiation of Cu^{II} and Ni^{II} by the fluorescent sensor **5** in an $MeCN/H_2O$ solution (4:1), buffered at pH = 2.9 with chloroacetate buffer. Titration with Ni^{II} does not alter fluorescence (▲). On addition of Cu^{II}, I_F decreases linearly (▽); full quenching is observed with the addition of 1 equiv. n = number of added equivalents.

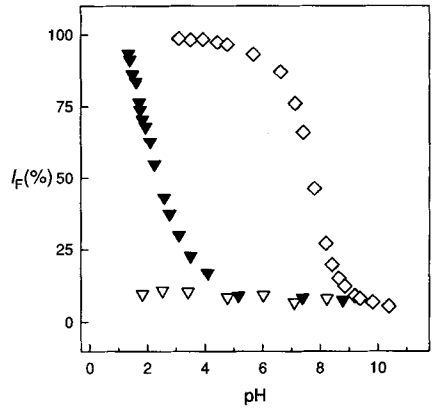

Fig. 7. pH dependence of the fluorescence intensity (I_F) for solutions containing: a) **4** alone (◇); b) equimolar amounts of **4** and Cu^{II}, data obtained by titrating the acidified solution with standard base (▼); c) the same solution as (b), data from back titration with standard acid (▽).

plex of *N,N'*-bis-(2-aminoethyl)propane-1,3-diamine (2.3.2-tet), that is, the metal-binding component of **5**. This behaviour definitely demonstrates that fluorescence quenching in the pH range 2.0–3.5 has to be associated with metal chelation by the tetraamine fragment.

Spectrofluorimetric titration in the presence of Ni^{II} gave roughly similar results. However, the quenching profile was not as symmetrical as that observed for Cu^{II} (Fig. 5). It is possible that such a profile results from the superimposition of the quenching effect caused by the deprotonation of the anthrylammonium group of **5** (which takes place first) and of the quenching effect exerted by the complexed Ni^{II} centre (which takes place 1 pH unit later). In any case, Ni^{II} binding took place at a pH value 2 units higher than for Cu^{II}, which reflects its lower thermodynamic stability in solution. The distinct separation of the two I_F/pH profiles would allow sensor **5** to discriminate between Cu^{II} and Ni^{II} under the appropriate pH conditions. In fact, addition of Ni^{II} to a solution of **5**, buffered at pH 2.9 ($ClCH_2COOH/ClCH_2COO^-$ buffer) did not alter anthracene emission (see Figure 6), whereas subsequent addition of Cu^{II} induced quantitative quenching. Notice that owing to the relatively large separation of the two titration profiles a pH exists at which Ni^{II} is not complexed at all, whereas the Cu^{II} complex of **5** is 100% formed, causing full quenching of fluorescence. In this respect, sensors containing either tetraamine or dioxotetraamine binding moieties displayed a similar behaviour. However, a substantial difference exists between dioxotetraamine and tetraamine systems. Sensors of the former type display the same qualitative behaviour independent of the cyclic or non-cyclic nature of the receptor. In contrast, the closed structure of **4** lends it properties that differ drastically from those of **5** when complexed with transition metals and thus precludes its use as a sensor (vide infra). The spectrofluorimetric titration curve (I_F/OH^-) for a metal-free solution of **4** is shown in Figure 4: in this case, fluorescence quenching took place during the addition of the third equivalent of base, which allows us to label the third deprotonation step ($LH_2^{2+} + OH^- = LH^+ + H_2O$) as that involving the anthrylammonium group. Figure 7 shows the I_F/pH profiles for the spectrofluorimetric titration of **4** in the absence and presence of 1 equiv of Cu^{II}. Metal-induced fluorescence quenching took place early in the pH scale due to the formation of an especially stable Cu^{II} complex of the tetraaza macrocyclic subunit (d–d band centred at 506 nm). However, back-titration with strong acid, even when the acid was added in large excess, did not induce any fluorescence revival, in sharp contrast to that observed with the open-chain analogue **5** and with both **2** and **3**, which displayed complete reversibility. Such behaviour reflects the extreme kinetic inertness of tetraaza macrocyclic complexes, in particular of cyclam and its derivatives, with respect to demetallation (the kinetic macrocyclic effect)[16] and has been ascribed to the fact that the four donor atoms of the ring are mechanically held in the coordination sites; they cannot rotate outwards so their lone pairs are not accessible to the incoming H^+ ions. Noticeably, the kinetic macrocyclic effect does not operate with dioxocyclam and its derivatives (e.g., **2**). In this case, the protons bind the partially negatively charged carbonyl oxygen atoms of the two deprotonated amido groups, increasing the double-bond character of the carbon–nitrogen bonds and drastically reducing the coordinating tendencies of the amide nitrogen atoms, which induces a rapid demetallation.[17] Reversibility is a mandatory requirement for receptors (and sensors). Due to its unique electronic features, dioxocyclam is the only tetraaza crown that can act as a receptor for transition metal ions and can be used to build a sensor.

Metal-induced fluorescence quenching was observed also in the case of the Ni^{II}/**2** system in the pH interval 5.7–7.1. In the present case, the sigmoidal quenching profile is displaced towards higher pH values compared with Cu^{II}, which reflects the lower thermodynamic stability of Ni^{II} polyamine complexes. Moreover, it was observed that equilibration after each addition during the spectrofluorimetric titration took some minutes. This may be because the Ni^{II} cation is intrinsically sluggish and its interaction with the partially protonated cyclam subunit of **4** is especially slow. As noted for Cu^{II}, back-titration with acid did not induce any fluorescence revival, further evidence of the kinetic macrocyclic effect. The two-component system **4**, in spite of the high affinity of its binding subunit towards transition metals, definitely cannot work properly as a sensor.

It should be noted that the kinetically inert metallocyclam subunit has been previously linked to the inorganic fluorophore $[Ru(bipy)_3]^{2+}$.[18, 19] The proximate transition metal centre quenched the photoexcited fluorophore. In contrast, anthracene derivatives of open-chain polyamines have been used as fluorescent sensors for multipoint recognition of anions.[20]

The nature of metal induced fluorescence quenching in sensors containing the tetraamine subunit: The mechanism responsible for the quenching of the photoexcited anthracene moiety in systems **4** and **5** could be assessed through spectrofluorimetric

investigations of frozen solutions at 77 K. Solutions of **4** or **5** in the glass-forming solvent ethanol did not show any emission at room temperature. However, freezing at the temperature of liquid nitrogen largely restored fluorescence, confirming that quenching in liquid solution is caused by the transfer of an electron from the closest amine group (tertiary for **4**, secondary for **5**) to the photoexcited fluorophore. On addition of Cu^{II} to the same solutions, the metal centre is bound by the tetraamine fragment, as shown by the appearance of the violet colour (and by the development of the appropriate d–d band). Such solutions do not fluoresce at room temperature *nor* at 77 K. This indicates that quenching takes place through an *energy-transfer* process (Dexter mechanism) of the type illustrated in Scheme 2b. An energy-transfer mechanism is also responsible for the intramolecular fluorescence quenching observed in the Ni^{II} derivatives of **4** and **5**, whose ethanolic solutions, both liquid and frozen, did not show any fluorescence emission.

It should be noted that a *metal-to-fluorophore* electron-transfer mechanism, as observed for systems containing the dioxotetraamine binding subunit, is not thermodynamically allowed for the present systems, essentially owing to the lesser tendency of tetramine ligands to favour access to the Cu^{III} and Ni^{III} states compared with the diamine-deprotonated diamide donor set. The metal-to-fluorophore electron-transfer process is characterized by positive or slightly negative values of ΔG_{ET}: $[Cu^{II}(\mathbf{4})]^{2+}$, + 0.3 eV; $[Cu^{II}(\mathbf{5})]^{2+}$, + 0.4 eV; $[Ni^{II}(\mathbf{4})]^{2+}$, −0.1 eV; $[Ni^{II}(\mathbf{5})]^{2+}$, 0.0 eV. The *fluorophore-to-metal* electron-transfer process, on the other hand, is quite favoured from a thermodynamic point of view. ΔG_{ET} values are distinctly negative for both Cu^{II} and Ni^{II} derivatives (e.g., $[Cu^{II}(\mathbf{4})]^{2+}$, −1.1 eV; $[Ni^{II}(\mathbf{4})]^{2+}$, −0.3 eV). However, in spite of the thermodynamic feasibility of the fluorophore-to-metal electron-transfer process, the energy-transfer mechanism dominates in tetramine systems **4** and **5**. This may be ascribed to the facts that: a) the emission band of the anthracene fragment (ending at 450 nm) overlaps with the absorption bands of the metal chromophore (d–d in nature), which provides the basis for the double electron transfer; b) the fluorophore and the metal centre are quite close and the flexible nature of the connecting bridge allows an easy Van der Waals contact of the two subunits.

In the dioxotetraamine systems **2** and **3**, condition (a) (overlap of emission and absorption bands) is still fulfilled. However, the anthracene moiety is linked to a chelate ring made rigid and planar by π delocalization, which hinders the fluorophore–metal contact. This prevents energy transfer and allows a genuine electron transfer to take place.

Zinc(II)-promoted fluorescence enhancement in sensors containing the tetraamine subunit: Zn^{II}, which has a d^{10} electron configuration, cannot be involved in any energy-transfer process. Moreover, Zn^{II} does not show any redox activity at all, in whatever coordinating environment, which excludes any possibility of a photoinduced electron-transfer process. Thus, Zn^{II} is an inoffensive metal centre from a photophysical point of view, and, in this respect, is similar to s-block cations. In any case, possible photophysical effects associated with Zn^{II} binding could not be explored with sensors **2** and **3**, as dioxotetraamine receptors do not bind to the non-transition metal ion Zn^{II}. The situation is different with polyamine receptors that form solution-stable complexes with Zn^{II} at not too high a pH value.[21]

Spectrofluorimetric titration of **5** in the presence of Zn^{II} yielded a singular profile, as shown in Figure 8. After an initial quenching, fluorescence began to revive from pH = 3.7 to achieve, at pH = 6 and more, almost the original intensity. Noticeably, the first part of the profile superimposes well on that obtained for a metal-free solution of **5**. In this pH interval, Zn^{II} does not interact with the tetraamine receptor and, following deprotonation, the free anthrylamine group can transfer an electron to the photoexcited fluorophore, quenching fluorescence. At pH = 3.7, the Zn^{II}–tetraamine complex begins to form. Interaction with the metal centre drastically reduces the oxidation potential of the anthrylamine group, stopping electron transfer and restoring fluorescence. Thus, the left edge of the well in the I_F/pH profile corresponds to the pH region in which the anthrylamine group has already lost the proton, but has not yet bound the Zn^{II} ion.

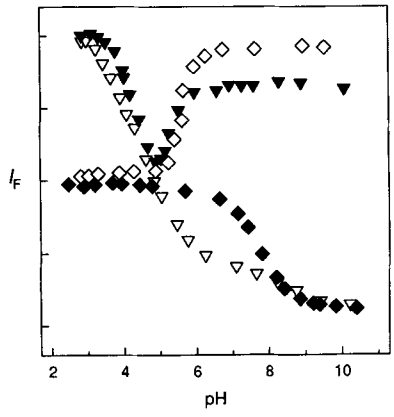

Fig. 8. pH dependence of the fluorescence intensity (I_F) for solutions containing: a) system **5** alone (▽); b) equimolar amounts of **5** and Zn^{II} (▼); c) system **4** alone (◆); iv) equimolar amounts of **4** and Zn^{II} (◇).

The system containing the cyclic tetraamine receptor, **4**, displays quite different behaviour. In the presence of Zn^{II}, no change in the intensity of fluorescence was observed from the strongly acidic region up to pH = 4.9 (Fig. 8). At this pH, anthracene emission intensity increases sigmoidally to a constant value. Notably, a sigmoidal profile centred at pH = 5.8 was also observed in the 386 nm absorbance against pH curve, determined through spectrophotometric titration of an equimolar solution of **4** and Zn^{II} plus excess acid with standard base. As such an absorption band is sensitive to the Zn^{II}–anthrylamine interaction, one should conclude that the sigmoidal fluorescence enhancement is associated with metal binding by the cyclic receptor. While Zn^{II}-induced fluorescence enhancement in anthrylamine systems is an expected phenomenon,[22] the question must be why the uncomplexed system, even in a distinctly acidic solution, does not display the full fluorescence emission of the anthracene fragment, which occurs only following metal coordination. In this context, potentiometric titration experiments have shown that the fully protonated cyclam subunit of **4** is a strong acid, as far as the first two dissociation steps are concerned (i.e., pK_{A1}, $pK_{A2} < 2$). This means that from pH = 2 to pH = 4.9, when the deprotonation of the anthrylammonium group takes place, the tetraamine ring contains two ammonium groups (the anthrylammonium and, due to electrostatic repulsive effects, probably the group in the *trans* position) and two free secondary amine groups (those adjacent to anthrylammonium). It is possible that an electron-transfer process takes place from one of these secondary amine groups to the photoexcited fluorophore. Fluorescence quenching is only partial (about 40%), probably owing to the relatively large distance between the donor and the acceptor. It is possible that the long-distance electron-transfer rate competes with the rate of the radiative decay of the photoexcited anthracene fragment. In any case,

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following Zn^{II} binding (from pH = 4.9 upwards), all the amine nitrogen atoms of the cyclam subunit are engaged in coordination and any electron release to the proximate photoexcited fluorophore is prevented.

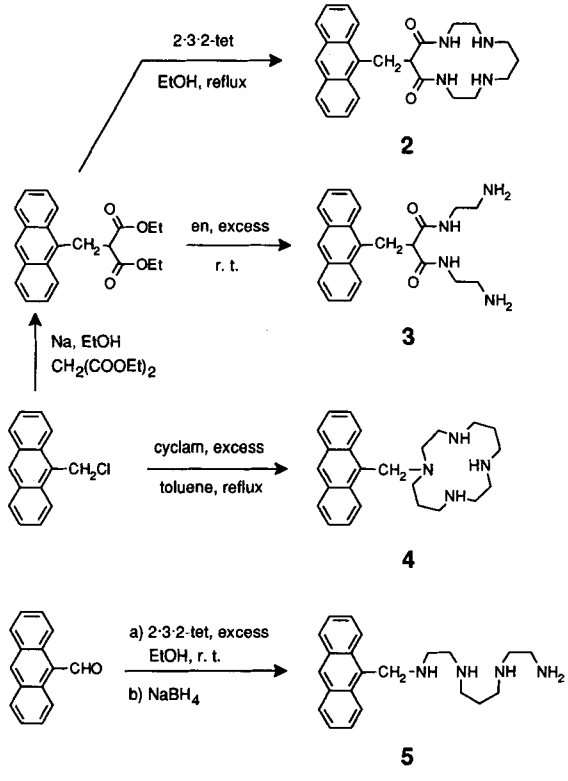
Conclusions

Requirements for the design of fluorescent sensors for s-block cations have been clearly defined:[123] a) *recognition:* the binding subunit (a crown ether or a cryptand) recognizes the size of the cation; b) *signalling:* the metal–ligand interaction suspends electron transfer to the photoexcited fluorophore and awakens fluorescence (OFF–ON switching). The present investigation has tried to outline the principles underlying the design of fluorescent sensors for transition metals. As far as signalling is concerned, 3d metals, in view of their electronic properties, can quench the fluorescence of a proximate fluorophore (ON–OFF switching), through either electron-transfer or energy-transfer mechanisms. Occurrence of *electron transfer* can be controlled through the design of a receptor capable of promoting the redox activity of the envisaged metal: this offers a further attractive element of selectivity. The *energy-transfer* mechanism is simply related to the electronic configuration of the metal ($3d^n$, $0<n<10$), that is, to the presence of an empty or half-filled d level of lower but not too low energy compared with that of the HOMO level of the photoexcited fluorophore. Predominance of either electron-transfer (when thermodynamically allowed) or energy-transfer mechanisms should be related to the structural features of the supramolecular sensor, in particular to the properties of the spacer connecting the signalling unit and the receptor, namely, its rigid or flexible nature and its ability to carry electrons. This important aspect has not been considered in detail in the present study and deserves further investigation. As far as recognition (point a) is concerned, binding selectivity in the receptor subunit can be achieved by several approaches. In this work, ligands containing donor atoms which are also Brønsted bases were considered and pH control was used to tune cation binding. Discrimination was especially efficient in the case of dioxotetraamine receptors **2** and **3**, for which the endothermic deprotonation of the amide groups emphasized the different binding tendencies of divalent transition metals (which are related to the electronic configuration). Other elements of selectivity could be taken into account for receptor design: the nature and number of donor atoms, whether the structure of the ligating backbone is linear or branched, and the stereochemical demands of the metal centre under investigation. In this context, it is helpful that a number of useful tricks for ligand design and engineering have been made available during the last one hundred years of coordination chemistry.

Experimental Procedure

UV–visible spectra were measured on a Hewlett–Packard 8452 diode array spectrophotometer. Spectrophotometric titrations were performed in acetonitrile/water (4:1 v/v) solution (50 mL, 5×10^{-4} mol L^{-1}); standard aqueous solutions of $HClO_4$ (0.1 and 1 mol L^{-1}) and NaOH (0.1 and 1 mol L^{-1}) were used. Emission spectra were recorded with a Perkin–Elmer LS-50 luminescence spectrometer (excitation wavelength 372 nm; maximum emission intensity at 415 nm) and were all uncorrected for instrumental response. Spectrofluorimetric titrations were performed in acetonitrile/water (4:1 v/v) solutions (50 mL, 10^{-6} mol L^{-1}); standard $HClO_4$, NaOH and $M^{II}(ClO_4)_2$ solutions were used. The pH scale was calibrated prior to each titration experiment in aqueous acetonitrile by the Gran method [24]. Buffered solutions were obtained with 2–6 *lutidine* (pH 7.14), *sodium chloroacetate* (pH 2.86) and sodium acetate (pH 4.68). Emission spectra at 77 K were measured in dry ethanol (10^{-6} mol L^{-1}) with quartz sample tubes and the same luminescence spectrometer equipped with a low-temperature luminescence accessory (Perkin–Elmer). Fluorescence spectra were taken in a frozen ethanolic solution of the sensor (**2**–**5**), then in an ethanolic solution containing equivalent amounts of the sensor and of the metal ion. For dioxotetramine systems **2** and **3**, the typical intense anthracene fluorescence was observed before and after the addition of the metal ion (+ 2 equiv OH^-). For tetramine systems **4** and **5**, metal-free solutions displayed anthracene fluorescence, which was quenched in the presence of the metal. Mass spectra were obtained with a Finnigan Mat 8222 mass spectrometer.

9-chloromethylanthracene and anthracene-9-carbaldehyde (Fluka) were used without further purification. *N,N'*-bis-(2-aminoethyl)propane-1,3-diamine (2.3.2-tet) was prepared as described for the analogous tetramine 3.2.3-tet [5], distilled at reduced pressure (125 °C; 5×10^{-2} Torr) and stored over NaOH under refrigeration. 1,4,8,11-tetraazacyclotetradecane (cyclam) was prepared following the literature method [5]. Experimental details for the synthesis of 2-(anthracen-9-ylmethyl)malonic acid diethyl ester, **7**, have already been reported [7]. Supramolecular systems **2**–**5** were prepared according to Scheme 4.



Scheme 4. Synthesis of fluorescent sensors **2**–**5**. en = ethylenediamine; 2.3.2-tet = *N,N'*-bis-(2-aminoethyl)propane-1,3-diamine; cyclam = 1,4,8,11-tetraazacyclotetradecane.

6-(Anthracen-9-ylmethyl)-1,4,8,11-tetraazacyclotetradecane-5,7-dione (**2**): A solution of **7** (0.65 g, 1.9 mmol) and 2.3.2-tet (0.3 g, 1.9 mmol) in dry ethanol (40 mL) was refluxed under a dinitrogen atmosphere for 7 d. On cooling, a waxy solid separated; it was filtered off and washed several times with diethyl ether. Yield 43%. M.p. 256–259 °C; MS (70 eV, EI, direct introduction, 280 °C): *m/z* (%): 418 (32%) [M^+], 191 (100%) [$C_{14}H_9-CH_2^+$]; $C_{25}H_{30}N_4O_2$ (418.2): calcd C 71.74, H 7.22, N 13.39; found C 71.79, H 7.05, N 13.54.

***N,N'*-Bis-(2-aminoethyl)-2-anthracen-9-ylmethylmalonamide** (**3**): Ethylenediamine (30 mL, freshly distilled over CaO) and **7** (0.5 g) were stirred at room temperature under a dinitrogen athmosphere for 7 d. Excess ethylenediamine was distilled off under reduced pressure; on treatment of the yellow residue with diethyl ether, a pale yellow precipitate formed, which was filtered off and recrystallized from ethanol. Yield 74%. M.p. 205–208 °C; MS (70 eV, EI, direct introduction, 200 °C): *m/z* (%): 378 (73%) [M^+]; 349 (52%) [$M-CHNH_2^+$], 191 (100%) [$C_{14}H_9-CH_2^+$]. $C_{22}H_{26}N_4O_2$ (378.5): calcd C 69.82, H 6.92, N 14.80; found C 70.21, H 7.15, N 14.53.

1-(Anthracen-9-ylmethyl)-1,4,8,11-tetraazacyclotetradecane (**4**): Cyclam (4.42 g, 22 mmol) was dissolved in hot toluene (230 mL) and 9-chloromethylanthracene (1 g, 4.4 mmol) was added portionwise to the boiling solution. The resulting mixture was refluxed for 6 h, then filtered and allowed to cool. Unreacted cyclam, which is insoluble in cold toluene, was filtered off and the solution washed with aqueous 5% NaOH and water and dried over $MgSO_4$. After removing solvent by rotary evapo-

ration, an oily residue was obtained which solidifies on cooling. Yield 54%. M.p. 137–140 °C; $C_{25}H_{34}N_4$ (390.6): calcd C 76.88, H 8.77, N 14.34; found C 77.23, H 8.65, N 14.21.

***N*-(2-Aminoethyl)-*N'*{2-[(anthracen-9-ylmethyl)amino]ethyl}propane-1,3-diamine (5)**: 2.3.2-Tet (3.2 g, 20 mmol) and anthracene-9-carbaldehyde (0.81 g, 4 mmol) were dissolved in ethanol (50 mL) and allowed to react for 36 h at room temperature. Then $NaBH_4$ (1.7 g, 45 mmol) was added portionwise and the resulting solution warmed at 50 °C for 4 h. Ethanol was distilled off under reduced pressure, the residue treated with water (40 mL) and extracted with dichloromethane (3 × 30 mL). After this was dried over $MgSO_4$ and solvent removed by rotary evaporation, a semi-solid residue was obtained that was characterized as the tetrahydrobromide salt and its copper(II) complex.

The tetrahydrobromide salt 5·4HBr: Compound **5** (0.5 g, 1.43 mmol) was dissolved in ethanol and treated with excess aqueous 48% HBr. A yellowish precipitate formed which was filtered and dried under vacuum. Yield 73%. $C_{22}H_{34}N_4Br_4$ (674.15): calcd C 39.20, H 5.08, N 8.31; found C 39.53, H 4.95, N 8.12.

[Cu(5)](ClO$_4$)$_2$: An ethanol solution (20 mL) of **5** (0.1 g, 0.28 mmol) was treated with an equimolar amount of $Cu(ClO_4)_2 \cdot 6H_2O$ (0.53 mL of an aqueous 0.54 mol L^{-1} solution). Violet crystals were obtained on slow evaporation. Yield: 58%. $C_{22}H_{30}N_4Cl_2CuO_8$ (612.8): calcd C 43.11, H 4.93, N 9.14; found C 42.86, H 4.84, N 9.03.

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