A Versatile Fluorescent System for Sensing of H^+, Transition Metals, and Aromatic Carboxylates

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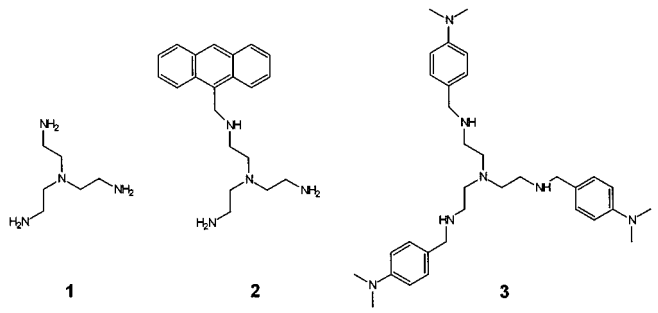
The fluorescent emission of the *N*,*N*-dimethylaniline fragment of the tetramine system **3** varies with pH, displaying a maximum of the fluorescent intensity, I_F, at pH = 5. Binding of transition metals (Cu^{II}, Ni^{II}) induces fluorescence quenching and modifies the I_F vs. pH profile, giving rise to an OFF-ON-OFF type of sensor. The $[Zn^{II}(\mathbf{3})]^{2+}$ complex recognises carboxylate anions, both aliphatic and aromatic. Recognition is signalled via fluorescence quenching only for aromatic carboxylates.

In principle, a molecular fluorescent sensor for any kind of analyte can be built by following the fluorophore-spacer-receptor approach, i.e. by covalently linking a light-emitting fragment to a ligating subunit capable of recognising the desired substrate. Most importantly, an intra-molecular mechanism must exist that associates substrate binding and emitting activity of the fluorophore.[1−4] Thus, the recognition process can be signalled through either fluorescence enhancement or quenching. In this sense, a molecular fluorescent sensor can be well illustrated with the switch-light bulb metaphor, as an external input (a variation of analyte concentration, corresponding to a finger) operates the switch (the receptor subunit), thus turning on/off the light emission by the fluorescent fragment (the light bulb).[5]

We describe here a versatile fluorosensor for ionic analytes of varying kind, based on the tripodal tetramine tren, **1**.



1 **2** **3**

Tren is a quite good ligand for transition metal ions, imposing in most cases a trigonal bipyramidal coordination geometry.[6] Whereas four coordination sites are occupied by the amine groups of tren, the axial position, opposite to the tertiary nitrogen atom, is vacant and available for an anion. As a consequence, a metallo-tren subunit may behave as a receptor for ionic analytes. In this regard, we have recently reported on a Zn^{II}-tren system with a covalently appended anthracene fragment, $[Zn^{II}(\mathbf{2})]^{2+}$, which is able to recognise the $-COO^-$ residue.[7] However, recognition is signalled only for those anions displaying electron donor/acceptor tendencies, which quench the nearby fluorophore via an electron transfer mechanism.

We have now appended at each terminal amine nitrogen atom of tren an *N*,*N*-dimethylaniline subunit (DMA): *N*2-(4-dimethylaminobenzyl)-*N*1,*N*1-bis[2-(4-dimethylaminobenzylamino)ethyl]ethane-1,2-diamine, trenDMA$_3$, **3**. The DMA fragment, when irradiated at 300 nm, gives rise to a charge transfer (CT) excited state, which emits light at 360 nm. The interaction of the DMA-photoexcited fragment with analytes hosted by the tetramine receptor may induce drastic variations of the emission intensity, opening the way to fluorosensing of protons, transition metals and anions. Moreover, the protonation of the dimethylamine group of DMA, in strongly acidic solutions, destroys the CT-photoexcited state and quenches the fluorescence. This unique feature opens a further opportunity for selective signalling of ionic analyte recognition in aqueous solution.

Interaction with Protons

The emitting behaviour of **3** over the pH range 2−10 was investigated through a spectrofluorimetric titration experiment. In particular, a solution of **3** (10^{-4} mol L^{-1}) containing excess acid was titrated with a standard NaOH solution. The profile of the fluorescence intensity, I_F (%), vs. pH is displayed in Figure 1.

At very low pH (<2), the solution is very poorly emitting: in these acidic conditions, the dimethylamine group of each DMA fragment of **3** is protonated and the CT excited state cannot be achieved. On increasing pH, the intensity (I_F) of the emission band centered at 370 nm steeply increases, due to the stepwise deprotonation of the dimethylammonium groups and concurrent formation of the corresponding photoexcited state. At pH = 5.3, I_F reaches its maximum

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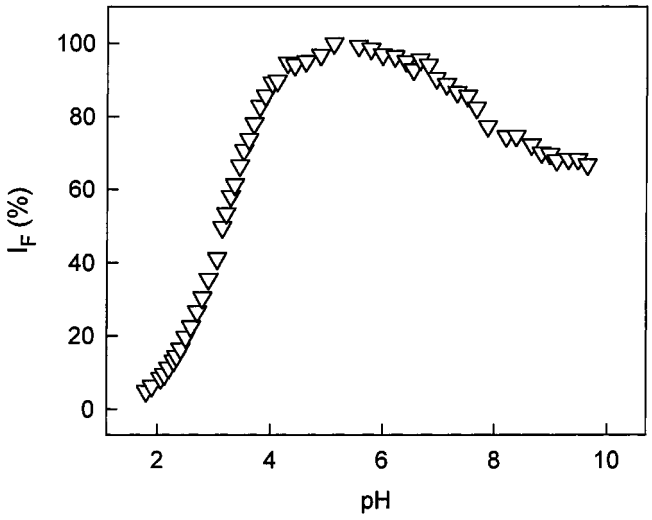

Figure 1. pH dependence of the fluorescence (λ_{exc} = 300 nm; λ_{em} = 370 nm) intensity for a solution of **3** in water

value then decreases smoothly. An emission spectrum of **3** measured at pH 8.5 is reported in Figure 2.

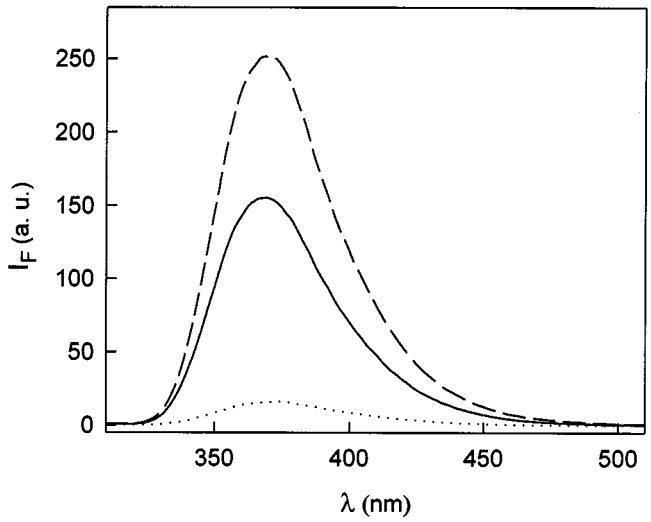

Figure 2. Emission spectra (λ_{exc} = 300 nm) in aqueous solution at pH 8.5 of: (___) free ligand **3**, (····) copper complex, (– – –) zinc complex

Progressive quenching of fluorescence after pH = 5.3 is to be ascribed to the stepwise deprotonation of the ammonium groups of the tren subunit adjacent to the DMA fragments. In fact, the amine group possesses reducing tendencies and can transfer an electron to a nearby photoexcited fluorophore, inducing fluorescence quenching.[8][9] It is possible that not only the adjacent secondary amine groups, but also the tertiary one, probably the first to deprotonate, is involved in the electron transfer process to the DMA fragment. The rather low solubility of **3** prevented a whole investigation of the stepwise protonation equilibria through potentiometric titration and determination of the pertinent pK_A values.

It should be noted that **3** belongs to the class of OFF-ON-OFF fluorosensors.[10] Sensors of this type are especially valuable, as, in contrast to usual indicators, they signal, via the fluorescent emission, the pH of the solution, within a given window. The narrower the window, the most precise and useful the sensor. In this sense, **3** is a rather poor sensor, as its peaks is poorly defined, and the window is spread over a too large pH interval.

Sensing of 3d Metal Ions

A solution of **3** containing 1 equiv. of Cu^{II} and excess acid was titrated with standard NaOH and the emission of the solution was spectrofluorimetrically monitored. In the acidic region, the I_F vs. pH profile superimposes well on the profile observed for the metal free solution. However, after reaching its maximum value at pH = 3.8, I_F decreases abruptly and full quenching is observed at pH = 5.5. The addition of Cu^{II} did not induce any changes in the shape and energy of the emission band (see Figure 3).

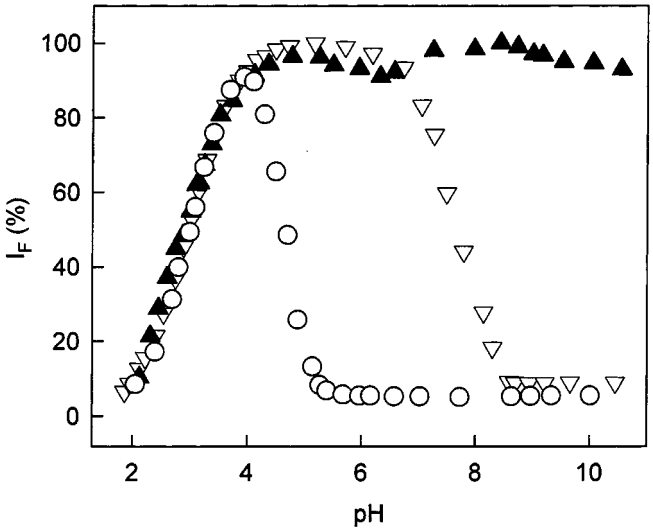

Figure 3. pH dependence of the fluorescence intensity (λ_{exc} = 300 nm; λ_{em} = 370 nm) for solutions containing equimolecular amounts of **3** in water and Cu^{II} (○); Ni^{II} (▽); Zn^{II} (▲)

Quenching is to be associated to the metal binding by the tren subunit of **3**, a process occurring in the pH = 4–5.5 interval. Transition metals typically quench proximate fluorophores via either electron transfer (eT) or energy transfer (ET) processes.[11,12] The two mechanisms can be discriminated by carrying out spectrofluorimetric studies on solutions frozen at the liquid nitrogen temperature. In fact, the occurrence of an eT process induces charge separation within the two-component system, to which a substantial rearrangement of solvent molecules is associated. Freezing prevents this rearrangement and interrupts the eT process: as a consequence, fluorescence is restored. On the other hand, an ET process induces a circular exchange of electrons, with no charge separation and no solvational rearrangement: as a consequence, the fluorophore remains quenched even when the solution is frozen at liquid nitrogen temperature and the solvent molecules are immobilised.[13] In particular, we observed that freezing at 77 K of

an equimolar solution of **3** and Cu^{II} in EtOH, did not induce any fluorescence revival, thus indicating that an ET mechanism operates. In this connection, it should be considered that Cu^{II} possesses a half-filled level of low energy (d_{z^2}, in a trigonal bipyramidal geometry), suitable for the occurrence of a double electron exchange process with a neighboring excited singlet. However other deactivation pathways (e.g. internal conversion) may be competitive in the presence of the metal ion. An intersystem crossing process could be accelerated by the proximate transition metal ion to generate a triplet state which is often non-emissive even at low temperatures.

Quite interestingly, the **3**-Cu^{II} equimolar system behaves as an extremely efficient OFF-ON-OFF pH sensor, giving rise to a well defined peak and to a narrow pH-window (2/5.5). The observed behaviour is very similar to that displayed by previously reported systems,[10] consisting of covalently linked amine, pyridine, and anthracene fragments. In those pH sensors, the quenching mechanism before and after the I_F peak (both located at pH = 6) were both of eT nature: (i) from the photoexcited anthracene subunit, An*, to the pyridinium moiety, and (ii) from the amine group to An*. In the presently investigated **3**-Cu^{II} system, the protonation of the amine group of DMA, and an ET mechanism (involving Cu^{II} and DMA*) operate.

Also when titrating **3** in presence of 1 equiv. of Ni^{II}, a peak is observed in the I_F vs. pH profile, fluorescence decrease and quenching being observed at a higher pH than in the case of Cu^{II} (see Figure 3). This is due to the intrinsically lower affinity of the tetramine subunit towards Ni^{II}, compared to Cu^{II} (constants for the M^{II} + tren = $[M^{II}(tren)]^{2+}$ equilibrium, in aqueous solution are $10^{14.95}$ and $10^{19.58}$, respectively).[14] Thus, also the **3**-Ni^{II} system gives rise to an OFF-ON-OFF behaviour even if with a rather large peak width.

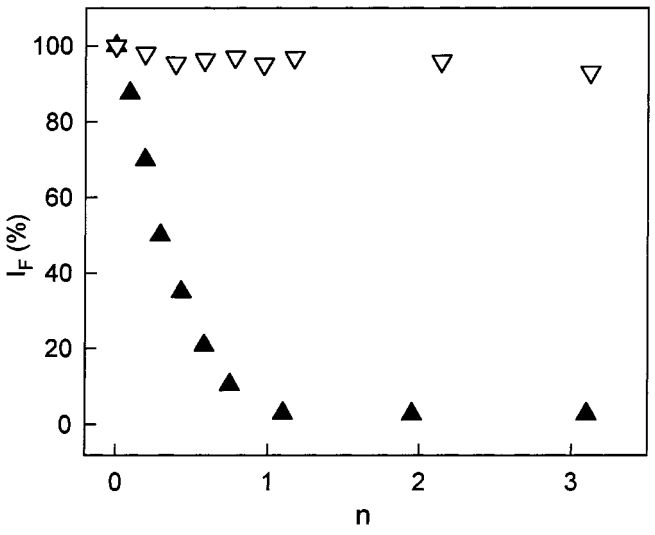

Figure 4. Discrimination of Cu^{II} and Ni^{II} by fluorescent sensor **3** in water solution buffered at pH 6 with MES buffer. Titration with Ni^{II} does not alter fluorescence (▽). On addition of Cu, I_F (λ_{exc} = 300 nm; λ_{em} = 370 nm) decreases linearly (▲); full quenching is observed with the addition of 1 equiv. *n* = number of added equivalents

On the other hand, the remarkably different affinity of the tren subunit towards Cu^{II} and Ni^{II} allows an efficient discrimination of the two metal ions to be carried out. In this connection, an aqueous solution 10^{-4} M in **3** was buffered to pH = 6 and titrated with a Ni^{II} standard solution: no alteration of the emission spectrum of the DMA fragment was observed, even after the addition of several equiv. of Ni^{II} (see Figure 4). Failure of I_F modification was due to the fact that at pH = 6 the Ni^{II} ion is not complexed by the tetramine receptor subunit. Then, the same solution was titrated with Cu^{II}. A linear decrease of I_F was observed and complete fluorescence quenching was achieved after the addition of 1 equiv. of Cu^{II}.

The Zn^{II} ion behaves quite differently with respect to Cu^{II} and Ni^{II}. In fact, on titration with standard base of an acidified solution of **3**, containing 1 equiv. of Zn^{II}, the typical increase of I_F with increasing pH was observed, which reached its maximum value at pH = 5 (see Figure 3). At this pH, the dimethylammonium group of each DMA fragment is deprotonated, which restores DMA emission. However, at pH ≥ 6, I_F does not begin to decrease, as observed in a metal-free solution (due to the occurrence of an eT process from the proximate amine groups) or in presence of either Cu^{II} or Ni^{II} (due to an ET mechanism involving the tetramine complexed transition metal), but maintains its maximum value until definitely basic conditions. This behaviour must be ascribed to the complexation of Zn^{II} by the tren subunit, which should occur at pH ≥ 6.5. In fact, metal coordination prevents the occurrence of the amine-to-DMA* eT process. On the other hand, the Zn^{II} centre, which has a d^{10} electronic configuration and does not exhibit any redox activity, cannot be involved in any ET or eT process with the nearby photo-excited fragments. The photophysical innocence of Zn^{II} as well as the coordinative unsaturation of its tren complex open the way to the design of fluorosensors for anions, whose recognition processes are based on the metal-ligand interaction (vide infra).

Sensing of Aromatic Carboxylates

The tren subunit typically imposes a trigonal bipyramidal stereochemistry to the complexed metal centre. The axial position opposite to the tertiary amine group is vacant and available for the coordination by a solvent molecule or by an anion. Thus, a metal−tren moiety can behave as a receptor for anionic substrates displaying coordinating tendencies. In this connection, the Zn^{II} derivative of a tren subunit equipped with an anthracenyl substituent (trenan, **2**) was observed to recognise aromatic carboxylates (in view of the special affinity of the $-COO^-$ group for the Zn^{II} centre), but to sense, via the anthracene fluorescence quenching, only those benzoates bearing either a donor, e.g. $-N(CH_3)_2$, or an acceptor substituent, e.g. $-NO_2$, on the 4-position of the ring.[7] Quenching was ascribed to an intra-complex eT process involving the benzoate substituent and the facing photo-excited anthracene fragment. Plain benzoate binds the Zn^{II} centre in **2**, but its recognition is not signalled by any modification of the anthracene emission.

We have tested the recognising and sensing tendencies of the Zn^{II}-**3** system towards carboxylates by carrying out spectrofluorimetric titration experiments in MeOH solution. In particular, a solution of the $[Zn^{II}(\mathbf{3})]^{2+}$ complex was titrated with a solution containing the sodium salt of the envisaged carboxylate anion. It was observed that addition of aromatic carboxylates induces quenching of the emission of the DMA fluorophore, I_F being reduced to less than 20% of the original value. I_F vs. equiv. of anion profiles for benzoate, 9-antracenoate, 4-nitrobenzoate, are reported in Figure 5.

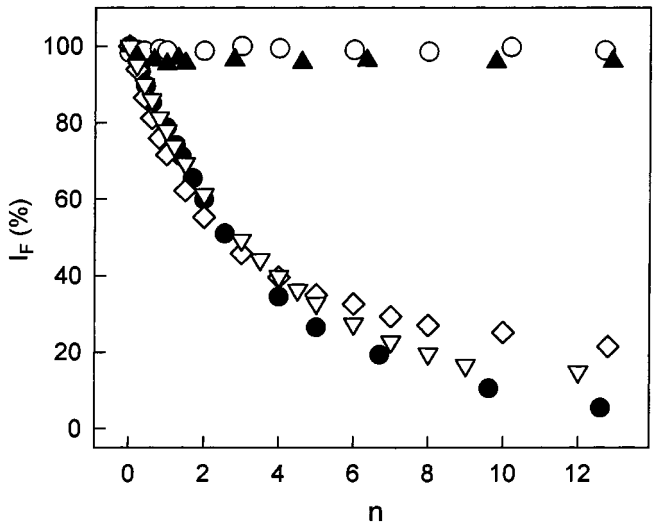

Figure 5. Spectrofluorimetric titrations (λ_{exc} = 300 nm; λ_{em} = 370 nm) of the receptor $[Zn^{II}(\mathbf{3})]^{2+}$ in methanolic solution (10^{-4} mol L^{-1}) with standard methanolic solutions of benzoate (◇); 9-anthracenoate (▽); 4-nitrobenzoate (●); acetate (▲); cyclohexylcarboxylate (○); n = number of added equivalents

Non-linear least-squares analysis of titration profiles reported in Figure 5 indicated the formation of 1:1 adducts.[15] The log$K$ values associated to the equilibrium:

$$[Zn^{II}(\mathbf{3})]^{2+} + X^- \rightleftharpoons [Zn^{II}(\mathbf{3})(X)]^+ \quad (1)$$

are reported in Table 1.

Table 1. log$K$ values for the $[Zn^{II}(\mathbf{3})(anion)]^+$ adduct formation

Anion	logK	quenching[a]
benzoate	4.69±0.04	yes
4-nitrobenzoate	4.43±0.06	yes
9-anthracenoate	4.47±0.04	yes
acetate	4.35±0.08	no
cyclohexylcarboxylate	4.08±0.08	no
chloride	3.69±0.08	no
nitrate	not bound[b]	=
perchlorate	not bound[b]	=

[a] Anion binding induces partial quenching of the emission of the DMA fragment of **3**. – [b] No alteration of titration profiles of benzoate were detected during competition experiments (see text).

Titration with aliphatic carboxylates (acetate and cyclohexylcarboxylate) did not induce any modification of the DMA emission intensity, as seen in Figure 5. However, the binding of the aliphatic carboxylates is demonstrated by competitive titration experiments involving aromatic carboxylates. As an example, titration of a solution of $[Zn^{II}(\mathbf{3})]^{2+}$ containing 10 equiv. of acetate with benzoate produces a less steep profile than that obtained in absence of acetate, indicating competition of the two substrates for the Zn^{II} site. The log$K$ values associated to the adducts of aliphatic carboxylates and other inorganic anions were calculated from the I_F vs equiv. of benzoate profiles, obtained for spectrofluorimetric titrations of solutions containing varying amounts of the investigated anion: their values are reported in Table 1. It can be seen that the values for aliphatic carboxylates are comparable with those observed for aromatic carboxylates.

It derives that the $[Zn^{II}(\mathbf{3})]^{2+}$ system is a discriminating sensor, which recognises the $-COO^-$ group, but communicates recognition only if the $-COO^-$ group is appended to an aromatic backbone. The explanation of this behaviour is not straightforward.[16] It is suggested that aromatic carboxylates interact with the DMA substituents via π stacking. This modifies the energy of the π orbitals of the DMA subunits. It should happen that the the energy of the DMA HOMO level is reduced to such an extent that its combination with the donating DMA orbital is minimized and, as a consequence, the generation of the charge transfer excited state prevented.

Experimental Section

General Considerations: All the reagents (Aldrich) were used without further purifications. – NMR data were obtained on a Bruker AMX 400 MHz. – All fluorescence measurements were carried out on a Perkin Elmer LS-50 luminescence spectrometer equipped with a 1.0 cm quartz cells. – Emission spectra at 77 K were measured in dry ethanol by using quartz sample tubes and the same luminescence accessory (Perkin-Elmer, Norwalk, CT, USA). – All pH measurements were made with an Orion 420A (Cambridge, MA, USA) digital pH meter using a combined glass-calomel electrode.

Synthesis of *N*2-(4-Dimethylaminobenzyl)-*N*1,*N*1-bis[2-(4-dimethylaminobenzylamino)ethyl]ethane-1,2-diamine (TrenDMA_3, **3**): 1.53 g (10.27 mmol) of 4-dimethylaminobenzaldehyde were dissolved in toluene (30 mL) and slowly added to a solution of tren (3.42 mmol) in toluene (100 mL). The reaction mixture was heated at 40°C for 24 hours. Solvent was removed under reduced pressure, and the yellow-orange oil (the imine intermediate) was dissolved in MeOH. $NaBH_4$ was carefully added in small portions, and then the reaction mixture was heated at 50°C overnight. The solvent was removed under reduced pressure and the sticky solid obtained was dissolved in 50 mL of water. The aqueous phase was extracted with CH_2Cl_2 (3× 50 mL). The organic phase was dried with $MgSO_4$ and then the solvent was distilled off, obtaining **3** as a yellow-brown oil. Yield: 70%. – MS (70 eV); *m/z* (%): 545 (6) $[M^+]$ – ^1H NMR (400 MHz, $CDCl_3$): δ = 7.1 (d, J = 9 Hz, 6 H aromatic), 6.6 (d, J = 9 Hz, 6 H aromatic), 3.55 (s, 6 H) 2.8 (s, 18 H), 2.55 (t, J = 6 Hz, 6 H), 2.45 (t, J = 6 Hz, 6 H).

Spectrofluorimetric Titrations: pH titrations were performed on 10^{-4} mol L^{-1} water solutions of **3** adjusted at pH < 2 by adding small amounts of a standard solution of $HClO_4$ (1 or 0.1 mol L^{-1}). Then additions of standard solutions of NaOH (1 or 0.1 mol L^{-1}) were made until a basic pH (> 10) was obtained. Emission spectra

(excitation wavelength 300 nm) were taken after each addition of base. Titrations at buffered pH were carried out in water solutions (40 mL, 10^{-4} mol L^{-1}) . Buffered solutions were obtained with MES (pH 6). $M^{II}(ClO_4)_2$ standard (M = Zn, Cu, Ni) solutions were used and spectra recorded after every addition. Anion titrations were carried out in methanol solutions (40 mL, 10^{-4} mol L^{-1}) of an equimolecular mixture of **3** and $Zn(ClO_4)_2$. Standard solutions in methanol of sodium carboxylate salts were added, and spectra recorded after each addition.

Acknowledgments

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[16] One of the Referees suggested an alternative explanation: in particular, a non-emissive exciplex between the aromatic carboxylate and the excited aminobenzene fragment could form. Such an exciplex would be expected to undergo a fast intersystem crossing due to the carbonyl oxygen lone pairs. This would not be the case for the non-aromatic carboxylates.

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