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Fluorescent Hg^{2+} Sensors: Synthesis and Evaluation of a Tren-Based Starburst Molecule Containing Fluorinated 1,2,4-Oxadiazoles

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A new tren-based starburst molecule containing fluorinated 1,2,4-oxadiazoles as fluorophores has been synthesized and its sensing behavior toward several metal cations has been investigated by UV/Vis, fluorescence, ^1H NMR and ^{19}F NMR spectroscopy. Selective sensing for Hg^{2+} ions through a PET-based mechanism was evidenced, suggesting application as fluorescent sensor for Hg^{2+} of the off-on type.

Introduction

Fluorescent chemosensors for the detection of heavy metal ions by a simple fluorescence turn-on (off-on) or quenching (on-off) are a fundamental tool for a rapid and nondestructive analysis of biochemical and environmental matrices.[1] In this field, a fundamental topic is represented by the design of selective sensors for Hg^{2+}, which is one of the most hazardous pollutants for the environment.[2] Many examples of fluorescent sensors for Hg^{2+} have been reported.[3] The majority of them is based on fluorescence quenching (on-off response),[4] whereas others involve fluorescence enhancement (off-on response).[5] Moreover, due to its coordination chemistry, which takes advantage of a tripodal structure, several sensors containing the tris(2-aminoethyl)amine (tren) moiety have been reported.[6] Among these, only a few tren-based sensors were selective for Hg^{2+}.[7] A structural similarity with tren-based sensors could be envisaged in some fluorinated 1,2,4-oxadiazole-containing starburst molecules **1**, with a triethanolamine core, which were proposed as blue emitters in OLEDs (Figure 1).[8] For some of these derivatives, emission is very weak or not observed at all, and this was attributed to intramolecular self-quenching by photoinduced electron transfer (PET) between the tertiary amino and the oxadiazole moieties. However, upon protonation of the tertiary amino nitrogen atom, emission of the oxadiazole fluorophore was detected (Figure 1).[8] 1,2,4-Oxadiazoles are widely studied for their applications either in medicinal chemistry or in materials science,[9] as well as for their rearrangement reactions[10] and photochemical reactivity.[11] Nevertheless, at the best of our knowledge, no 1,2,4-oxadiazole-containing fluorophore sensor has been previously reported. Starting from these considerations, we were encouraged to synthesize 1,2,4-oxadiazole-containing starburst molecules as scaffold for the design of a sensor whose fluorescence could be turned on upon complexation of a cation. In this work we report the synthesis and the evaluation of a tren-based molecule where the tren moiety is responsible for metal complexation.

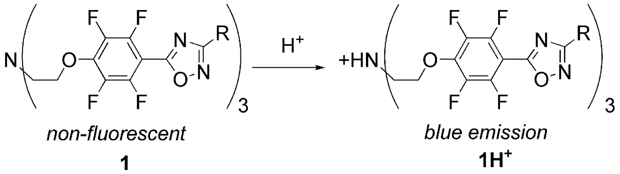

Figure 1. pH-sensitive starburst oxadiazoles.

Results and Discussion

We previously reported that 5-(polyfluorophenyl)-1,2,4-oxadiazoles easily undergo a classic nucleophilic aromatic substitution of the 4′-fluoro moiety under mild experimental conditions.[12,13] To take advantage of such reactivity, we synthesized 5-(pentafluorophenyl)-1,2,4-oxadiazole **3** to be used in the functionalization of the terminal amino groups of tren. The synthesis of oxadiazole **3** was accomplished in a two-step procedure. Initially, amidoxime **2** was treated with pentafluorobenzoyl chloride to yield the corresponding *O*-(pentafluorobenzoyl)amidoxime. This product was then heated under solvent-free conditions to give oxadiazole **3** (Scheme 1). Reaction of **3** with tren, in a 3:1 molar ratio, was performed in a DMF suspension of K_2CO_3. Re-

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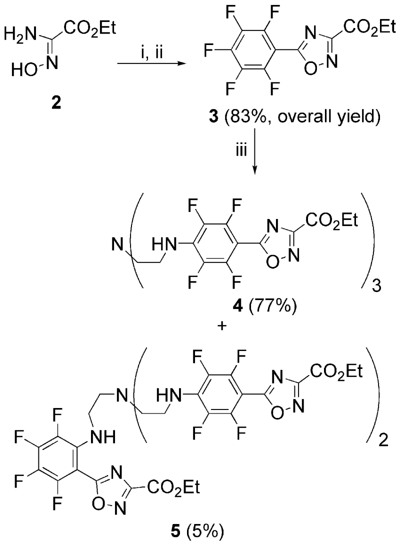
moval of the solvent and chromatography of the residue produced the target compound **4** (77%) together with some amounts of regioisomer **5** (5%), arising from S_NAr on the *ortho* position (Scheme 1).



Scheme 1. Synthesis of compound **4**. (i) C_6F_5COCl, K_2CO_3, acetone, room temp., 1 h; (ii) 180 °C, solvent-free, 1 h; (iii) tren, K_2CO_3, DMF, room temp., 2 h.

The structure of compound **4** was confirmed by means of spectroscopic data (^1H NMR, ^{13}C NMR, ^{19}F NMR, FT-IR and MS), and its photophysical behavior was first evaluated through UV/Vis and fluorescence spectroscopy. UV/Vis spectra recorded in acetonitrile show a strong absorption band centered at 313 nm (ε = 79100 $\text{M}^{-1}\,\text{cm}^{-1}$). Moreover, compound **4** is non-fluorescent under neutral conditions, whereas its emission spectra showed a distinctly enhanced fluorescence, centered at 430 nm, under acidic conditions, by adding 2 equiv. of HCl. This suggests that fluorescence of compound **4** is quenched through a PET from the tertiary nitrogen atom of tren, as expected (see Supporting Information). From the UV/Vis spectra of **4** (10 µM) in acetonitrile upon addition of 10 equiv. of various perchlorate salts of metal ions, such as Hg^{2+}, Pb^{2+}, Cu^{2+}, Zn^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Mg^{2+}, Li^+, Na^+, K^+, and Ag^+, no spectral change could be observed. On the other hand, by measuring fluorescence emission spectra of **4** (1 µM in CH_3CN) in the presence of 10 equiv. of metal salts, a strong emission band, centered at 420 nm, appears in the presence of Hg^{2+} ions (I/I_0 = 23) (Figure 2). In contrast, other ions lead to much smaller spectral changes (Cu^{2+} and Pb^{2+}: $I/I_0 < 9$) or almost no spectral changes (Zn^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Mg^{2+}, Li^+, Na^+, K^+, and Ag^+) (Figure 2), which indicates that **4** shows a good sensitivity toward Hg^{2+} and selectivity over other competitive cations. Figure 3 shows the results of fluorescence titration of **4** with Hg^{2+}. The Hg^{2+} addition causes an increase in fluorescence, which reaches its maximum upon addition of 1.5 equiv. of Hg^{2+}; with 1 equiv. of Hg^{2+} an enhancement factor I/I_0 = 22.5 was observed.

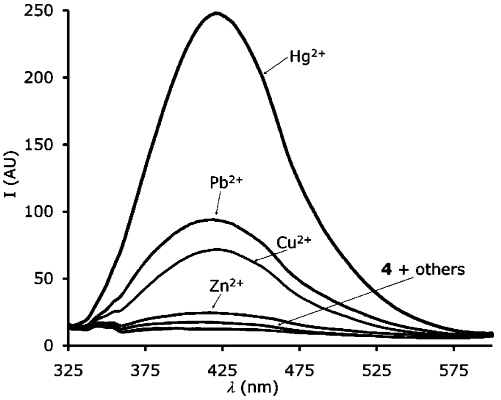


Figure 2. Fluorescence spectra of **4** (1 µM) with addition of ClO_4^- salts of Hg^{2+}, Pb^{2+}, Cu^{2+}, Zn^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Mg^{2+}, Li^+, Na^+, K^+, and Ag^+ (10 equiv., each) in CH_3CN with an excitation at 313 nm.

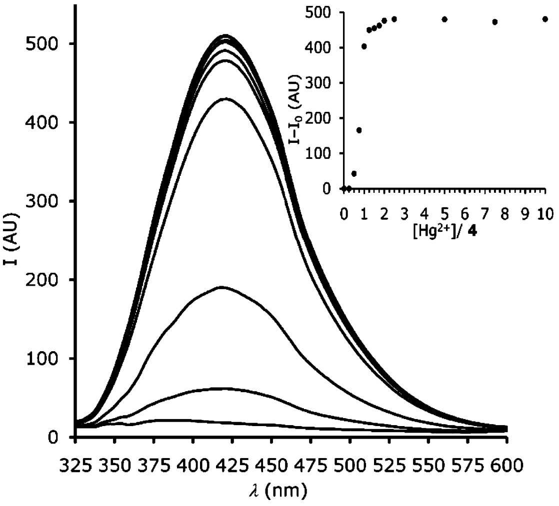
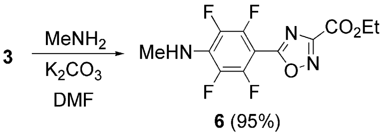


Figure 3. Change in fluorescence spectra (λ_{ex} = 313 nm) of **4** (2 µM) measured in CH_3CN upon addition of Hg^{2+}. Inset: change in fluorescence intensity at 420 nm.

In order to assess the possibility of an Hg–fluorophore interaction involved in the observed fluorescence enanchement, compound **6** was synthesized as a model for a fluorophoric group in compound **4** (Scheme 2). Indeed, fluorescence spectra of compound **6**, were not affected by Hg^{2+} addition (see Supporting Information), thus excluding any involvement of Hg–fluorophore interactions in the sensing activity of compound **4**.



Scheme 2. Synthesis of reference compound **6**.

To obtain further information about the binding mode of sensor **4**, ^{1}H NMR titration experiments were performed in CD_3CN. After addition of increasing amounts of Hg^{2+}, the only protons affected were those of the tren moiety, whose signals were broadened and shifted downfield, whereas the proton signals of the ethoxycarbonyl group remained unchanged (Figure 4). Similarly, ^{19}F NMR experiments showed a major downfield shift of signals relative to fluorine atoms in *ortho* positions to the amino moiety (see Supporting Information).

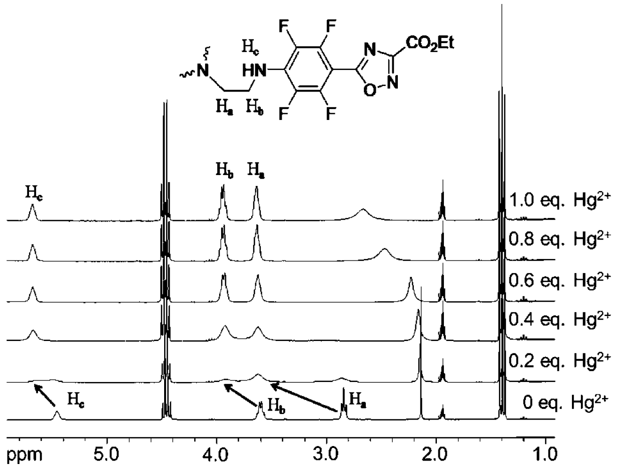


Figure 4. ^{1}H NMR spectroscopic data of **4** (10^{-2} M) in CD_3CN in the presence of various amounts of $Hg(ClO_4)_2$.

These pieces of evidence confirm that only the tren portion is involved in the Hg^{2+} complexation, suggesting the formation of a 1:1 complex. As a consequence of metal binding, which inhibits the PET from the tertiary nitrogen atom of tren to the oxadiazole ring, the fluorescence of the oxadiazole luminophore is restored (Figure 5).

In order to evaluate a practical applicability of the Hg sensor in aqueous media, the sensing ability of compound **4** was tested in H_2O/CH_3CN mixtures in the presence of Hg^{2+}, by adding increasing amounts of water. As the water content increases, a decrease of the fluorescence was observed. In this context a mixture containing 5% H_2O (v/v) was selected as a good compromise for further investigations. Under these conditions, compound **4** continues to show a higher selectivity toward Hg^{2+}, and competitive binding experiments reveal that the Hg sensing seems not to be affected by the presence of various metal cations (Figure 6). Under these conditions, with 1 equiv. of Hg^{2+} an enhancement factor $I/I_0 = 1.2$ was observed.

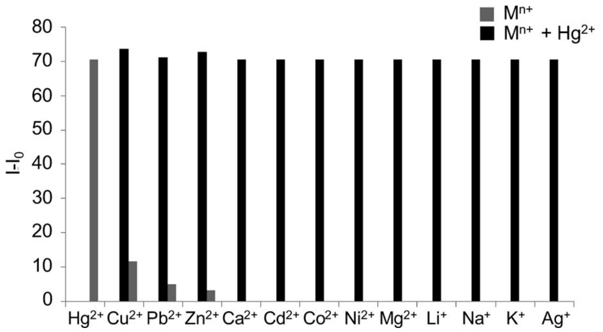


Figure 6. Fluorescence changes (430 nm) for **4** [2 μM in H_2O/CH_3CN (5% v/v)] upon addition of metal cations M^{n+} (40 μM) or $M^{n+} + Hg^{2+}$ (40 μM + 40 μM).

From the titration profile at 430 nm of **4** with Hg^{2+} (under mixed solvents conditions), the nonlinear fitting of the titration curve (on the basis of a 1:1 stoichiometry) allowed the determination of the association constant of **4** with the Hg^{2+} ion, calculated to be $4.06 \pm 0.04 \times 10^4$ M^{-1} (Figure 7).[14]

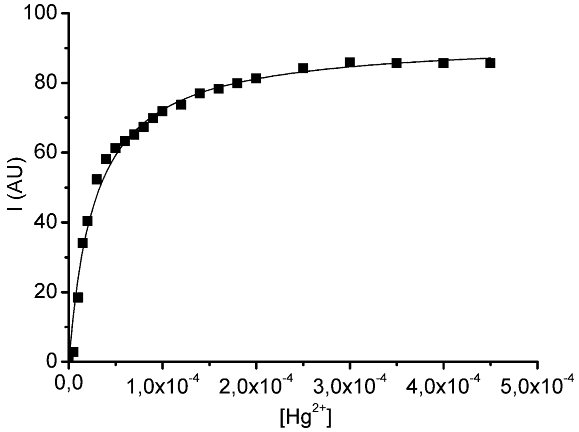


Figure 7. Change in fluorescence intensity at 430 nm (λ_{ex} = 313 nm) of **4** [2 μM in H_2O/CH_3CN (5% v/v)] upon addition of Hg^{2+}.

The 1:1 stoichiometry was also confirmed by means of the Benesi–Hildebrand method;[15] a plot of $1/(I - I_0)$ vs. $1/[Hg^{2+}]_0$ shows a linear relationship, and also the associa-

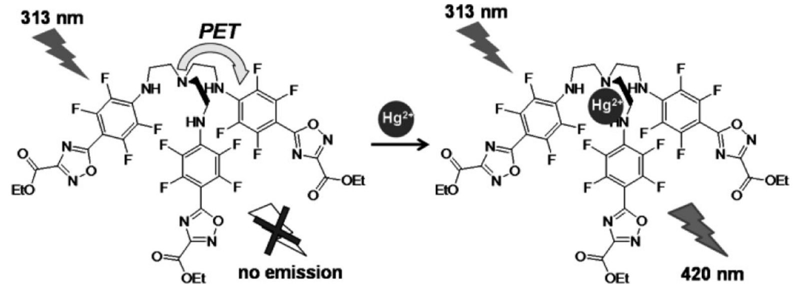


Figure 5. Rationale for the sensing mechanism of compound **4**.

tion constant of **4** with the Hg^{2+} ion (calculated to be $4.07 \pm 0.05 \times 10^4$ M^{-1}) is in agreement with the value found from non-linear fitting (see Supporting Information).

Conclusions

A new tren-based sensor with good selectivity toward Hg^{2+} has been designed and synthesized in few steps and high yields. The sensing mechanism of this new fluorogenic chemosensor, containing for the first time an 1,2,4-oxadiazole as luminophore, is based on the inhibition of a photoinduced electron transfer from tren to oxadiazole, after coordination of the metal ion with a 1:1 stoichiometry. The activity of this sensor has been investigated also in mixed aqueous/organic solvents. Moreover, the coordinating ability of the tripodal nitrogen atoms in the tren seems not to be affected by the link with the electron-withdrawing fluoroaryl moiety. This opens the way to the development of new sensors based on the easily obtainable fluoroarylated tren core.

Experimental Section

Synthesis of Compound 4: To a stirred solution of tris(2-aminoethyl)amine (44.8 µL, 0.3 mmol) in dry DMF (3 mL), K_2CO_3 (0.138 g, 1 mmol) and compound **3** (0.277 g, 0.9 mmol) were added, and the suspension was maintained at room temperature under efficient stirring for 2 h. The reaction mixture was then diluted with water (50 mL) and extracted with EtOAc (200 mL). The organic phase was dried with Na_2SO_4, filtered, and concentrated in vacuo. The obtained residue was chromatographed to give unreacted **3** (28 mg, 10 %), compound **4** (233 mg, 77 %) and compound **5** (15 mg, 5 %).

Compound 4: M.p. 161–163 °C (EtOH). ^{1}H NMR (300 MHz, [D$_3$]acetonitrile): δ = 1.39 (t, J = 7.2 Hz, 3 H, CH_2CH_3), 2.84 (t, J = 6.0 Hz, 2 H, NCH_2), 3.57–3.63 (m, 2 H, $NHCH_2$), 4.46 (q, J = 7.2 Hz, 2 H, OCH_2) 5.46 (br. s, 1 H, exchangable with D_2O) ppm. ^{13}C NMR (^{1}H-decoupled, 75 MHz, [D$_3$]acetonitrile,): δ = 14.2, 43.1, 54.1, 63.8, 133.9, 134.9 (d, $J_{C,F}$ = 128.5 Hz), 146.8 (d, $J_{C,F}$ = 261.8 Hz), 158.3, 163.0, 170.7 ppm. ^{19}F NMR (283 MHz, [D$_3$]acetonitrile): δ = –140.4, –162.7 ppm. UV (CH_3CN) λ_{max} = 313 nm (ε = 79100 M^{-1}cm^{-1}). FT-IR (Nujol): $\tilde{\nu}$ = 3379, 1751, 1734, 1655 cm^{-1}. GC–MS: m/z (%) = 1010 (100) [M$^+$]. $C_{39}H_{30}F_{12}N_{10}O_9$ (1010.70): calcd. C 46.35, H 2.99, N 13.86; found C 46.40, H 2.90, N 13.90.

Spectroscopic Material and Methods: Stock solutions (0.01 M) of the metal perchlorate salts were prepared in CH_3CN or H_2O/CH_3CN. Stock solutions of **4** and **6** were prepared in CH_3CN or H_2O/CH_3CN. For all fluorescent tests, the excitation wavelength was 313 nm with excitation and emission slit widths of 3 nm.

Supporting Information (see footnote on the first page of this article): Experimental procedures, NMR spectra of compounds **3**–**6**, and additional spectra and titration data.

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