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Ultra-Sensitive and Selective Hg²⁺ Chemosensors Derived from Substituted 8-Hydroxyquinoline Analogues

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Abstract

Novel analogues of 8-hydroxyquinoline with phosphinates or thiophosphinates functions and styryls fluorophores in *para* position to the nitrogen atom were prepared via multi-step syntheses, using phosphorylation and Wittig coupling reactions. A strong affinity between the quinoline analogues and heavy metals ions such as Pb²⁺, Cd²⁺ and Hg²⁺ was highlighted. The interaction of the metals ions with the nitrogen of the styrylquinoline leads to a large redshift of the absorption and emission spectra in agreement with an increase of the photoinduced charge transfer character of the styryl fluorophore. In the presence of metal ions there is also appearance of a green fluorescence emission by excitation at 420 nm or 840 nm thanks to a significant increase of the two-photon. Under optimal conditions, a mercury concentration of 15 ppt in a partially aqueous medium can be detected using the thiophosphinate derivative without interference from other metal ions.

Introduction

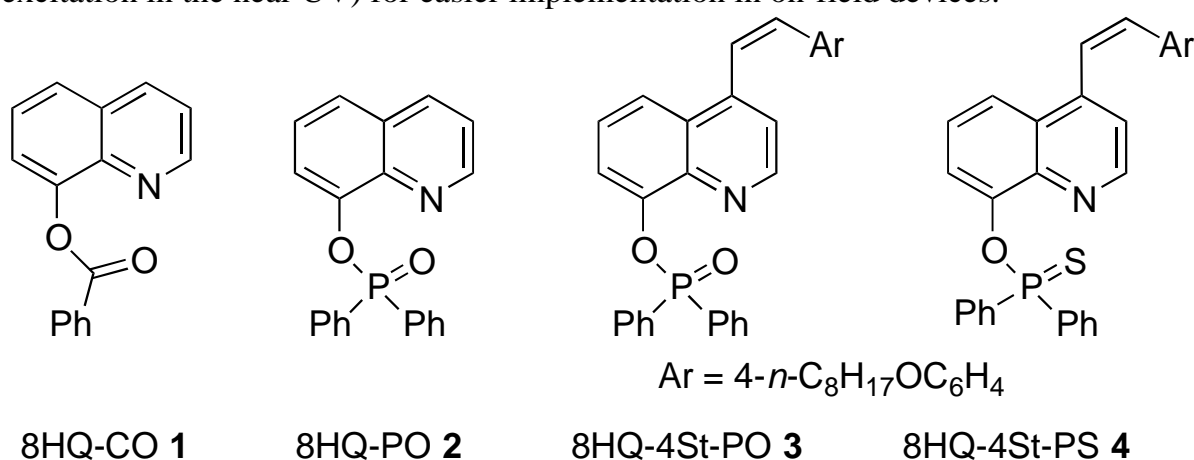
The design of sensors for the detection of heavy-metal ions has been a field of continuing research since the control of water resources and natural environments has become a worldwide major challenge.¹ Among heavy metal ions, lead, nickel, cadmium and mercury salts specifically need to be detected,² the last two being classified among “priority hazardous substances”, toxic, persistent and bioaccumulative chemicals. Hg²⁺ ion is considered as highly toxic causing environmental and health problems.^{1,2} The level of this ion is therefore subjected to strict regulations and should presently not exceed 1 µg L⁻¹.² Apart from analytical techniques (atomic absorption or emission, inductively coupled plasma spectroscopy), the methodology based on fluorescent molecular sensors has attracted considerable interest due to the superior sensing sensitivity and selectivity they offer.³ Some examples described the use of chemodosimeter which have some drawbacks

because of their irreversibility.⁴ However, among fluorescent molecular sensors currently available, only a few are competitive in terms of sensitivity, selectivity, while direct measurements in aqueous medium remain a crucial issue.⁵ The development of sensitive and selective fluorescent sensors is thus a subject of constant interest. In the course of our recent ongoing program directed toward the synthesis of novel fluorophores⁶ and on the basis of recent work on 8-hydroxyquinoline (8-HQ) analogues,⁷ we designed novel sensors for the detection of Hg^{2+} and wish to describe therein the synthesis, photophysical and complexing properties.

Results and discussion

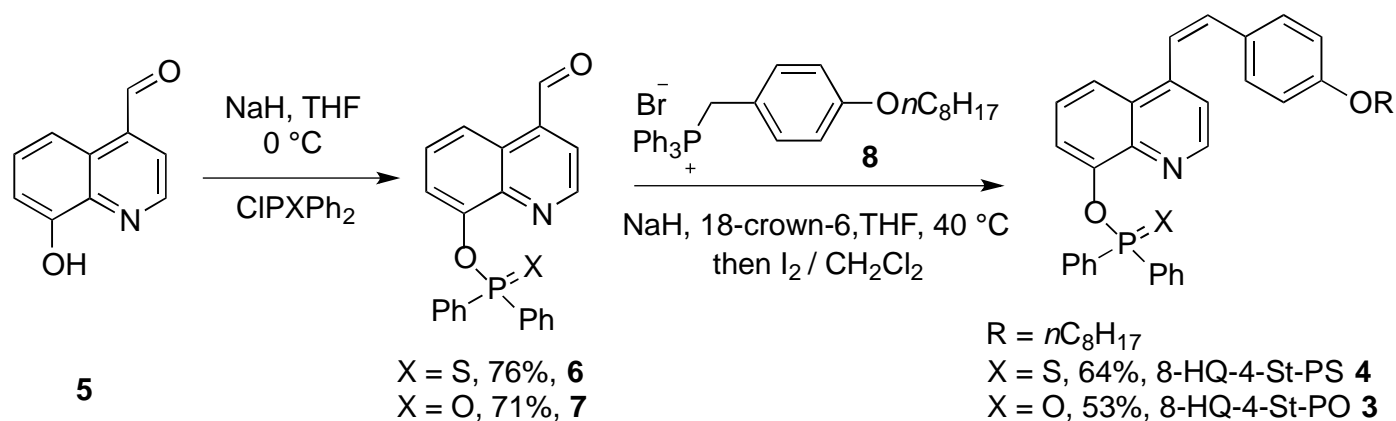
Synthesis

We chose the 8-hydroxyquinoline moiety as one of the most important chelators for metal ions⁸ and anticipated that the introduction of a phosphane oxide or thiophosphinate moiety would enhance the selectivity towards mercury ions. We therefore engaged in the synthesis of thiophosphinate 8-HQ-4-St-PS **4** and 8-HQ-4-St-PO **3** (Scheme 1), the arylvinylidene side chain being selected in order to shift the fluorescence emission in the visible region (as well as excitation in the near UV) for easier implementation in on-field devices.



Scheme 1 Hydroxyquinoline fluorescent sensors

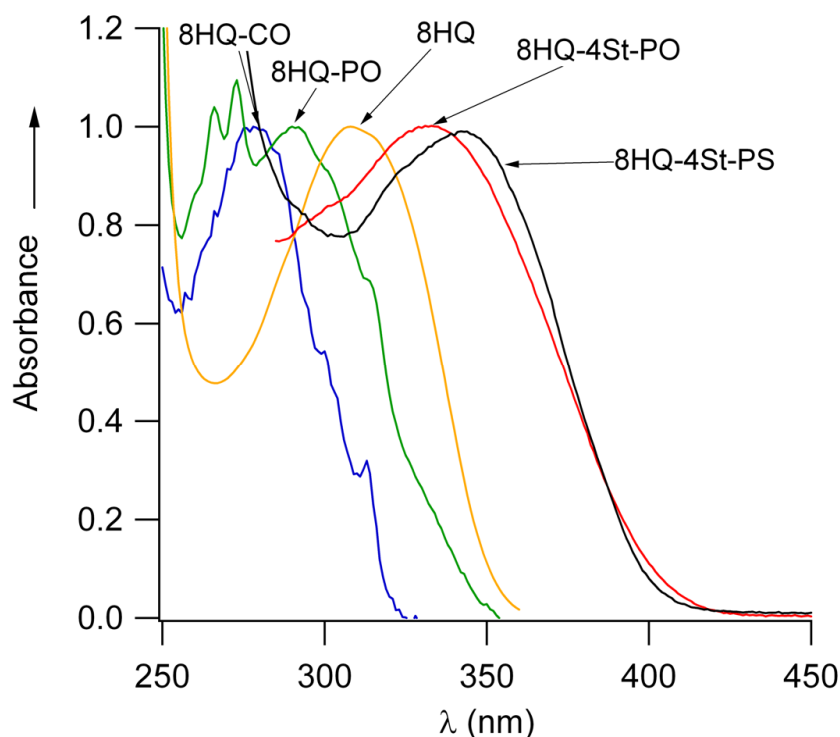
The synthesis of model 8HQ-PO **2** was straightforward starting from 8HQ in the presence of sodium hydride and diphenylphosphanoyl chloride at 0°C in THF.⁹ The introduction of the donating vinylidene groups required a multistep synthesis starting from the known aldehyde-substituted 8-hydroxyquinoline **5** (Scheme 2).¹⁰ The aldehyde **5** was phosphorylated with diphenylthiophosphanyl chloride¹¹ in good yield. The corresponding phosphorous derivative **6** was then engaged in a Wittig reaction in the presence of the phosphonium salt **8**.¹² The *Z* isomer¹³ 8-HQ-4-St-PS **3** was isolated in 64% yield after an isomerization reaction in the presence of iodine. A similar synthesis was accomplished for the preparation of 8-HQ-4-St-PO **4** and led to the desired sensor in 38% yield over two steps.



Scheme 2 Synthesis of chemosensors 8-HQ-4-St-PS **3** and 8-HQ-4-St-PO **4**.

Photophysical properties of the ligands

The absorption and fluorescence properties of the quinoline derivatives are collected in Table 1, and their absorption and emission spectra are shown in Figure 1a) and b), respectively. The fluorophores show an absorption band in the UV region. The phosphane oxide derivative and the carbonyl compound have comparable extinction coefficient. As expected the introduction of a styryl derivative on the *para* position of the quinoline derivative induces a noticeable red-shift of the absorption and the emission spectra due to the intramolecular charge transfer character of the transition. As previously described for the carbonyl derivatives,^{7a} the proximity of the phosphoryl oxygen (sulfur) lone pair to the acceptor nitrogen of the fluorophores induces a radiationless deactivation process through a $^3(n-\pi)^*$ state.



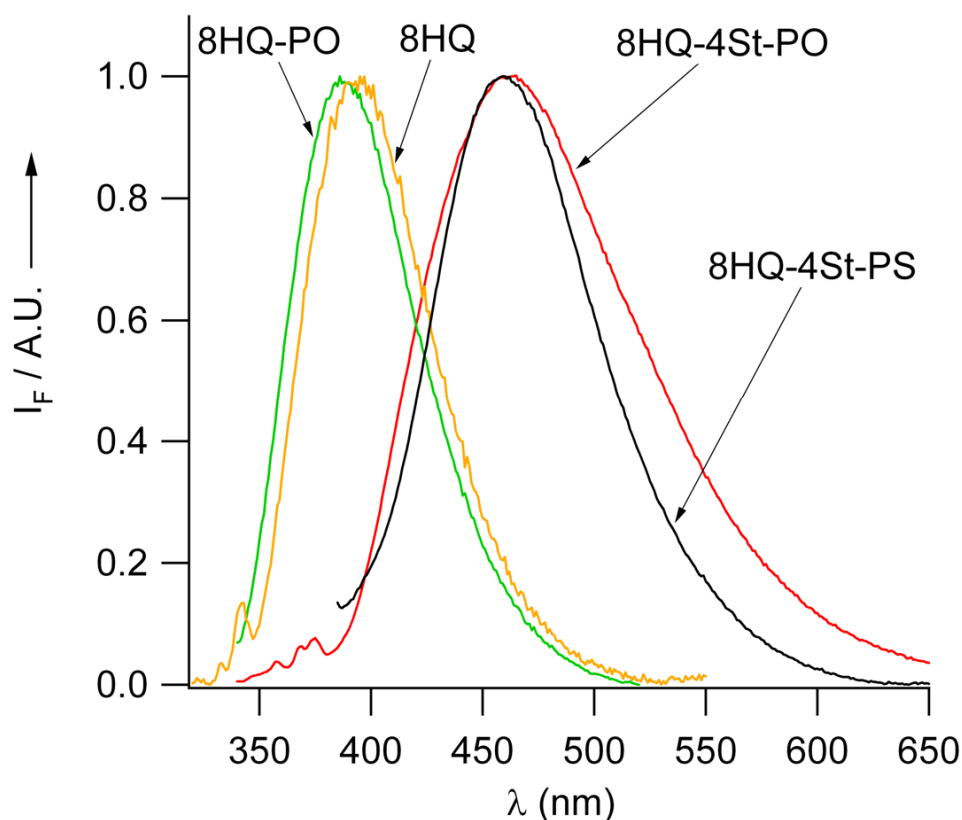


Figure 1: a) Absorption of quinoline derivatives in CH₃CN. b) Corrected normalised emission spectra of the quinoline derivatives in CH₃CN.

Table 1. Photophysical properties of quinoline derivatives in CH₃CN. Maxima of the one-photon absorption λ_{abs} [nm] and of steady-state emission λ_{em} (nm), molar absorption coefficient ϵ (M⁻¹ cm⁻¹), fluorescence quantum yield Φ_{F} .

Product	$\lambda_{\text{abs}}^{\text{max}}$ (nm)	ϵ^{max} (M ⁻¹ cm ⁻¹)	$\lambda_{\text{em}}^{\text{max}}$ (nm)	Φ_{F}
8HQ	308	2400	393	$3.8 \cdot 10^{-3}$
8HQ-CO [a]	275	4700	385	$7.6 \cdot 10^{-4}$
8HQ-PO	290	5000	396	$2.7 \cdot 10^{-3}$
8HQ-4St-PO	332	7600	461	$2.6 \cdot 10^{-3}$
8HQ-4St-PS	342	7600	459	$2.6 \cdot 10^{-3}$

[a] ref 7a

Cation-induced photophysical changes:

The complexation of the phosphane oxide and the phosphane sulfide derivatives was investigated in the presence of heavy metal ions in acetonitrile. The complexation of mercury salts by the phosphane oxide derivative 8HQ-PO **2** (figure 2) was studied in acetonitrile with a ligand concentration of 22 μM upon addition of increasing aliquots of mercury perchlorates. As expected, a red shift of the absorption band from 332 nm to 380 nm occurred, confirming the role of the quinoline nitrogen atom in the coordination of the metal ion.

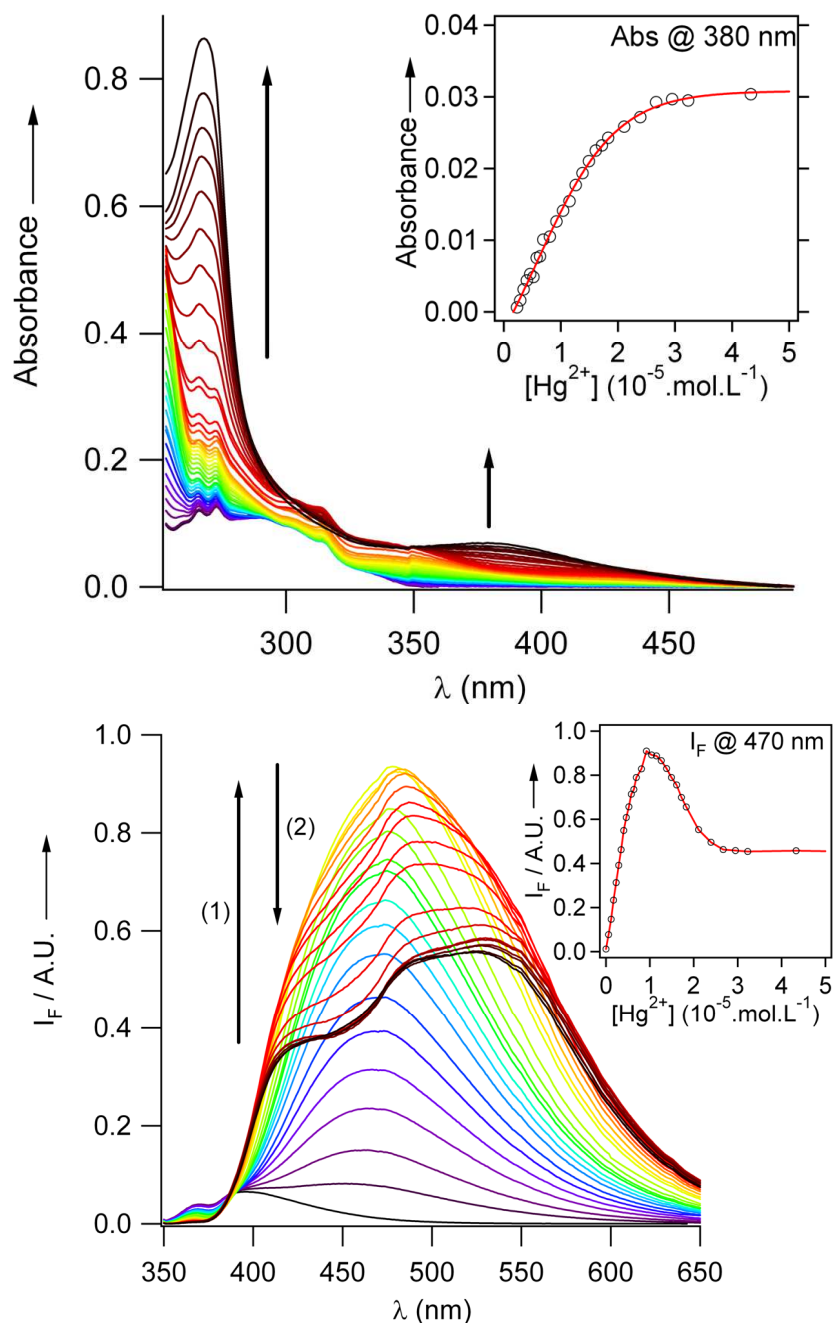


Figure 2: (a) Absorption and (b) corrected emission spectra of 8HQ-PO **2** in the presence of increasing concentration of Hg^{2+} in CH_3CN ($C_L = 22 \mu\text{M}$, $\lambda_{\text{exc}} = 335 \text{ nm}$)

Thereby, when using an excitation wavelength of 337 nm, an emission band appears at 490 nm and a decrease of the fluorescence is observed for the higher concentrations. The analysis of the evolution of the spectra using SPECFIT software revealed that different complexes are formed with the following different stoichiometries 1:1 and 1:2 (ML and ML₂). The decrease of the fluorescence intensity for larger concentration of mercury can be explained by stronger interaction between the fluorophore and the mercury and by a possible electron transfer process.

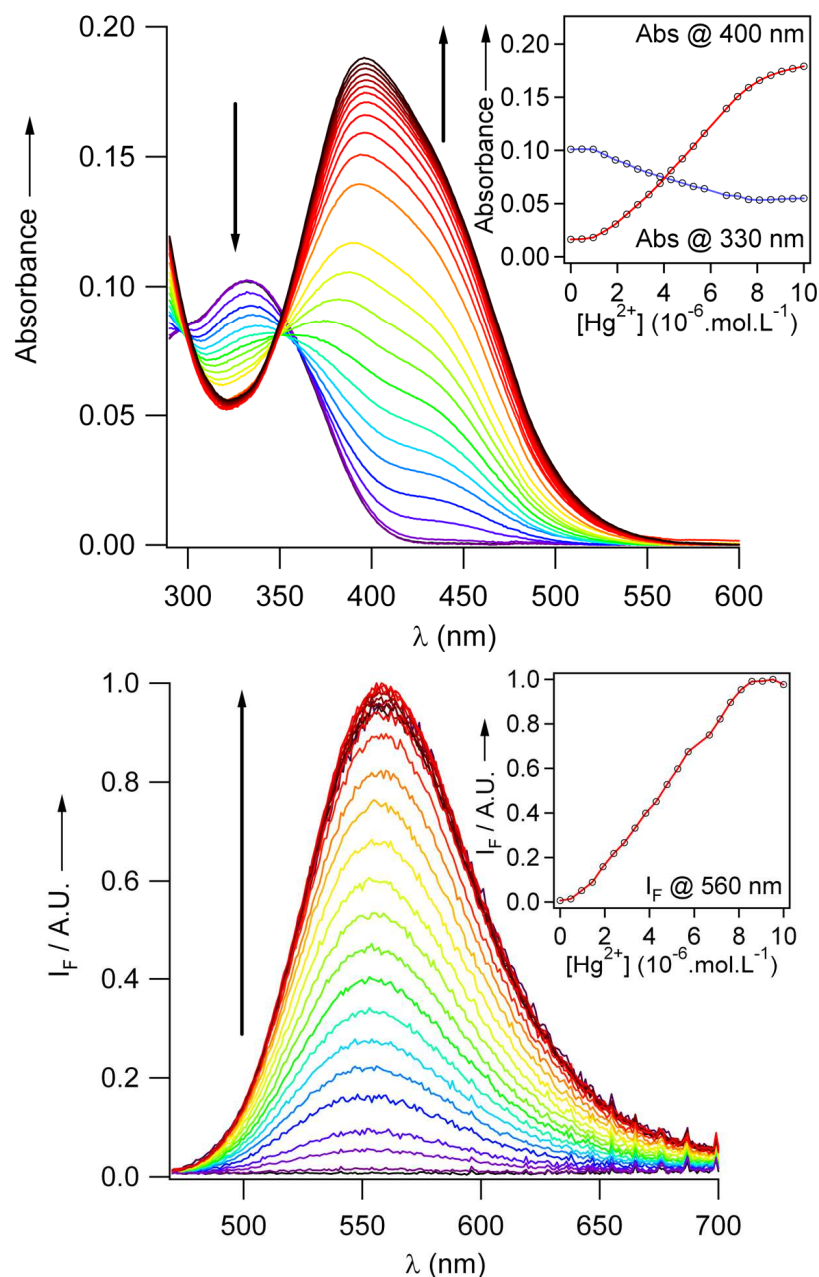


Figure 3: (a) Absorption and (b) corrected emission spectra of 8HQ-4St-PO **3** in the presence of increasing concentration of Hg^{2+} in CH_3CN ($C_L = 22 \mu\text{M}$, $\lambda_{\text{exc}} = 420 \text{ nm}$)

Table 2. Stability constants of quinoline derivatives with Pb^{2+} , Cd^{2+} and Hg^{2+} in CH_3CN

	8HQ-CO ^[a]	8HQ-PO		8HQ-4St-PO	8HQ-4St-PS	
	$\log \beta_{\text{ML}}$	$\log \beta_{\text{ML}}$	$\log \beta_{\text{ML}2}$	$\log \beta_{\text{ML}}$	$\log \beta_{\text{ML}}$	$\log \beta_{\text{ML}2}$
Hg^{2+}	4.5 ± 0.7	7.0 ± 0.2	12.6 ± 0.2	5.8 ± 0.5	7.5 ± 0.5	13.5 ± 0.7
Pb^{2+}	1.2 ± 0.2	6.7 ± 0.2	12.0 ± 0.3	6.3 ± 0.2	4.7 ± 0.1	11.2 ± 0.3
Cd^{2+}	-	-	-	6.6 ± 0.2	5.2 ± 0.2	-

[a] ref 7a

The stability constants derived for the phosphane oxide derivatives and the phosphane sulfide derivatives are given on table 2. For the 8HQ-4St-PO **3**, the addition of mercury induces a larger photophysical effect in relation with an efficient photoinduced intramolecular charge transfer phenomenon. The absorption band shifts from 345 nm to 410 nm in the presence of mercury. In emission direct emission of the complex at 420 nm induces the formation of a non structured band at 560 nm. For the phosphane sulfide derivative 8HQ-4St-PS **4**, similar photophysical effects have been observed upon complexation of heavy metal ions. In comparison to the results obtained by Jiang's group,^{7a} the higher stability constants obtained here confirms that the introduction of a phosphane oxide or a phosphane sulfide function increases the ability of the probe to coordinate heavy metal ions. Because of the high affinity of the phosphane sulfide derivative for mercury, the highest complexation constant was observed for the 8HQ-4St-PS **4**. It should be noticed that for the compound **3** and **4** only the 1-1 stoichiometry complex is observed because of the steric hindrance due to the incorporation of the styryl conjugated group.

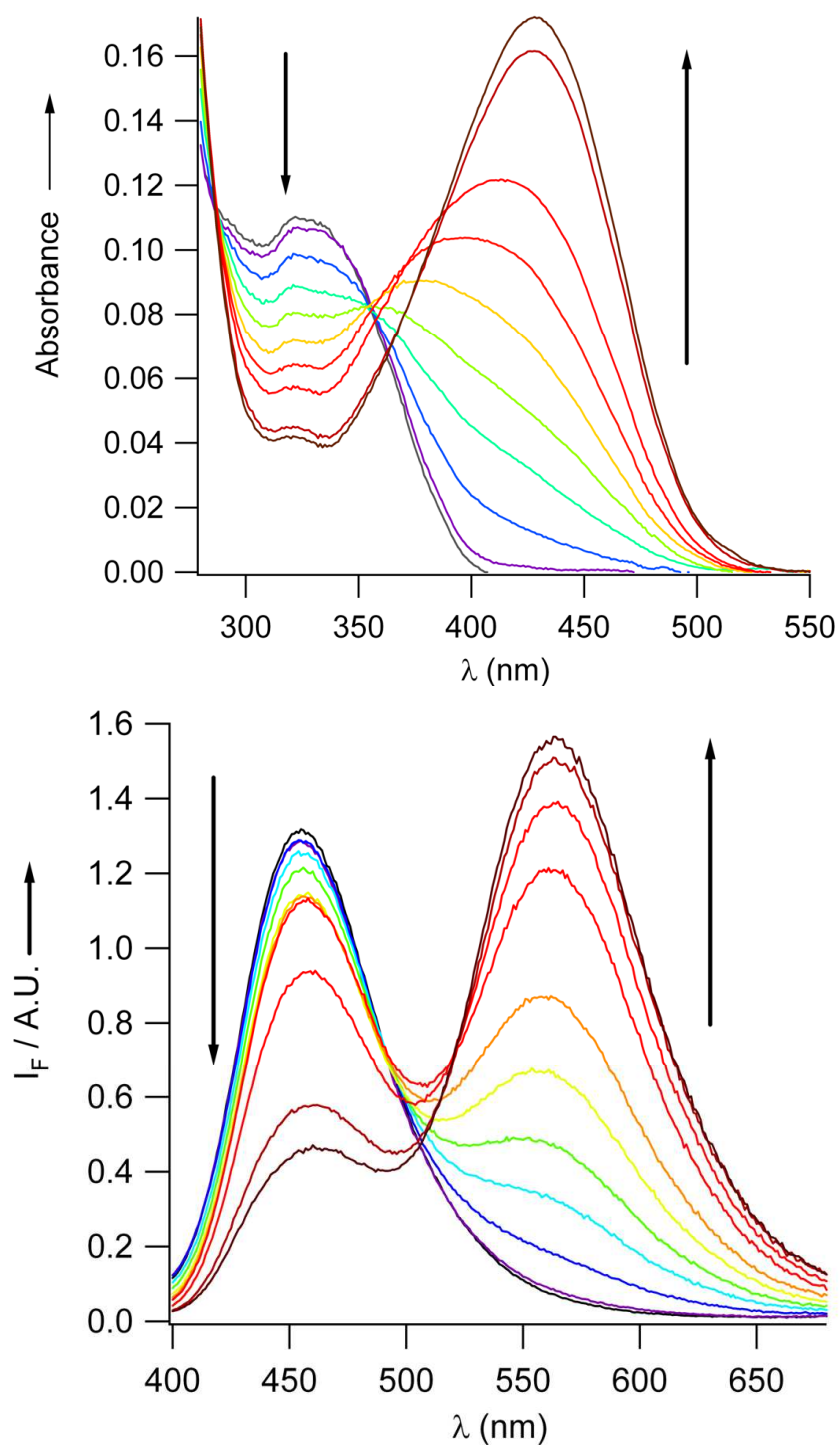


Figure 4: (a) Absorption and (b) corrected emission spectra of 8HQ-4St-PS 4 in the presence of Hg^{2+} in CH_3CN ($C_L = 15 \mu\text{M}$, $\lambda_{\text{exc}} = 350 \text{ nm}$)

Two-photon absorption

In addition to standard one-photon excitation, we also investigated the potential use of two-photon excitation (TPE) for selective excitation of the probe. There has been a lot of efforts in designing bright biphotonic fluorescent probes^{14,15} in the last decade mainly driven by the advantages TPE provides for biological imaging.¹⁶ In the field of fluorescent probes, TPE could offer an additional benefit by allowing selective excitation in the presence of fluorescent contaminants. We have investigated the TPE response of the thiophosphinate 8-HQ-4-St-PS **4** for mercury detection, expecting that only the complex would have significant TPE response in the particular spectral range of interest. This indeed proved to be the case (Figure 2a), as pure 8-HQ-4-St-PS **4** did not show detectable fluorescence when two-photon excited in the same range. Taking advantage of the large contrast between the TPE response of 8-HQ-4-St-PS **4** and of its mercury complex and of the high sensitivity and affinity for mercury of the 8-HQ-4-St-PS **4**, we further demonstrated that monitoring fluorescence by two-photon excitation at optimum wavelength (typically in the 800-900 nm range) indeed allowed monitoring Hg^{2+} concentration (Figure 2b).

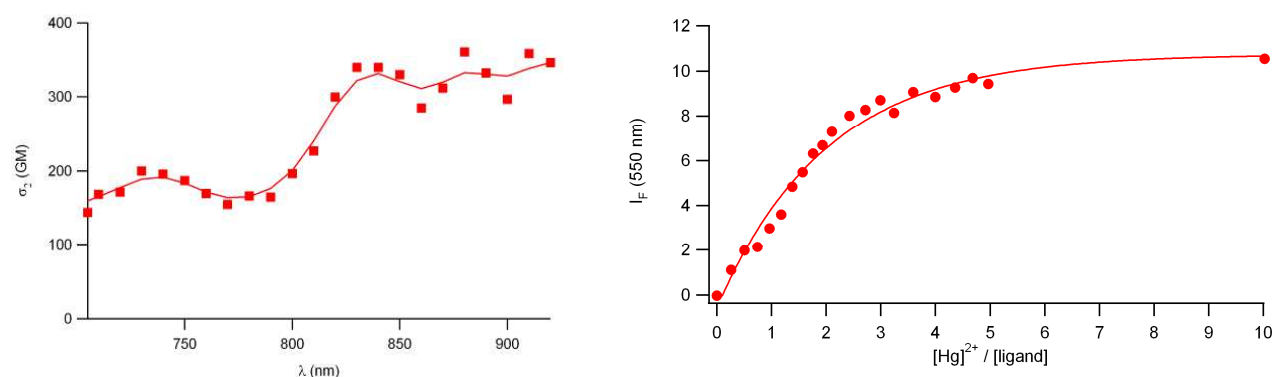
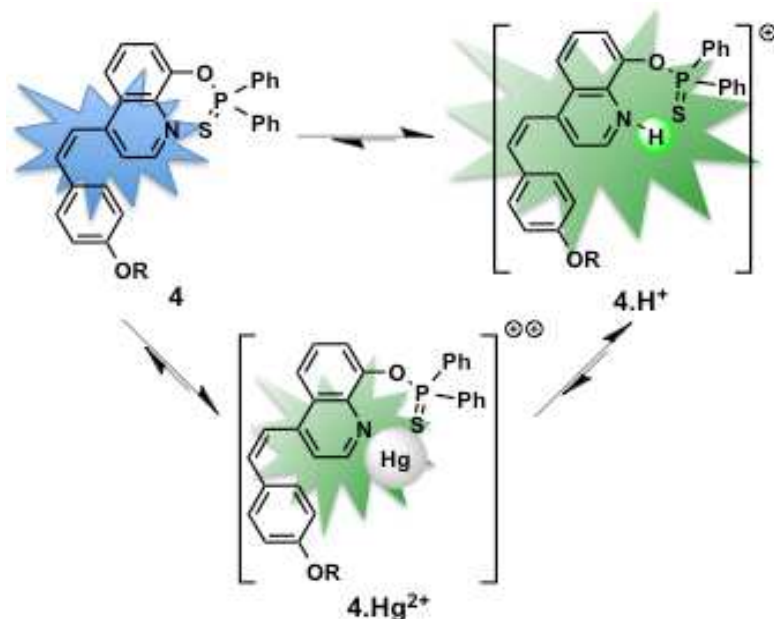


Figure 2. (a) Two-photon absorption of 8-HQ-4-St-PS **4** in the presence of an excess of Hg^{2+} in CH_3CN ($C_L = 10^{-4}$ M, $C_{\text{Hg}(\text{ClO}_4)_2} = 10^{-3}$ M). No TPEF signal is observed in the absence of Hg^{2+} . (b) Fluorescence signal of 8-HQ-4St-PS **4** in the presence of increasing concentration of Hg^{2+} in CH_3CN (two-photon excitation at 840 nm) $C_L = 10^{-4}$ M.

Practical determination of mercury in water

In view of practical applications, complexation studies were then carried out in a partially aqueous medium $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ 40/60. Titration experiments show that 8-HQ-4St-PS **4** was able to coordinate mercury in aqueous medium (see supporting information). Analysis of the spectra revealed that under these conditions a 1:1 complex is formed with mercury with a $\log K_{11} = 3.8 \pm 0.1$. In $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (40 : 60 : v:v),¹⁷ the pK_a of 8-HQ-4St-PS **4** was measured to be 3.23 ± 0.02 , which is lower than the complexation constant of 8-HQ-4St-PS **4** with mercury. In addition, the quantum yield of the protonated form **4.H⁺** being 10 fold higher than the mercury complex **4.Hg²⁺**, we optimized the sensing experimental conditions ($\text{pH} = 3$) to exploit the replacement of a proton by mercury to ensure larger fluorescence variations, and consequently improved sensitivity (Scheme 3).¹⁸



Scheme 3 Involved equilibrium upon complexation with Hg^{2+}

As previously observed, the addition of mercury induced a bathochromic shift of the absorption band, and a quenching of the fluorescence was observed in relation with the replacement of the proton by mercury (Figure 3a). Scheme 2 outlines the involved equilibrium upon complexation with mercury in these operating conditions. Taking advantage of this highly efficient quenching process, a calibration curve can be established. The measured detection limit was found to be 0.5 nanomol per liter, and the theoretical detection limit, calculated as three times the standard deviation of the background/noise ratio, was found to be 0.075 nanomol per liter (15 ppt).

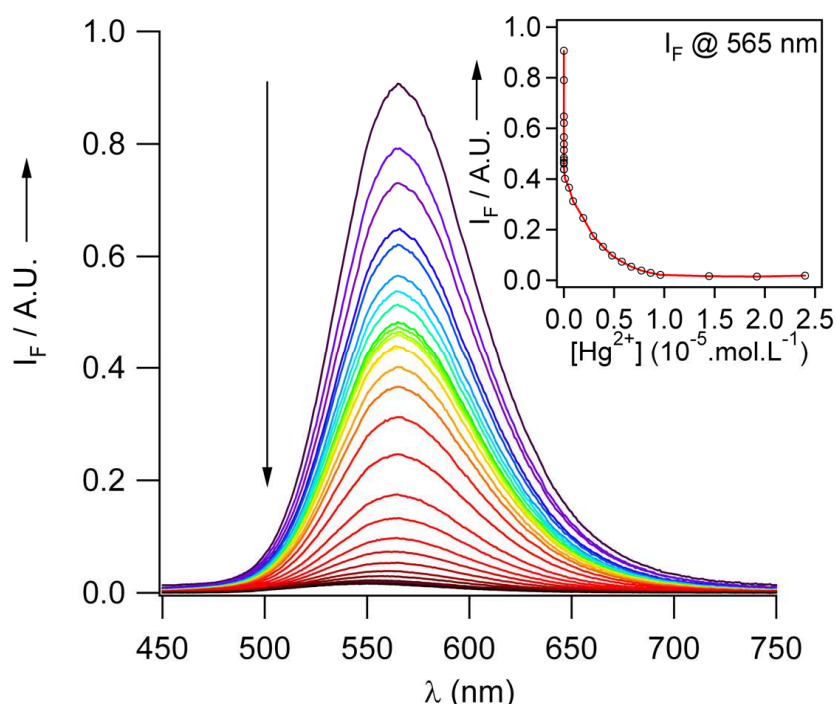


Figure 3. Corrected emission spectra of 8-HQ-4-St-PS **4** in the presence of increasing concentration of Hg^{2+} in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ 40:60 v/v, ($C_L = 13 \mu\text{M}$, $\lambda_{\text{exc}} = 420 \text{ nm}$).

The selectivity of the probe was also tackled. As shown in Figure 4, no significant change of the fluorescence were observed upon addition of high concentrations of interfering cations such as Li^+ , Na^+ , Mg^{2+} , K^+ , Ca^{2+} , Mn^{2+} , Fe^{2+} , Co^{2+} , Ni^+ , Cu^{2+} , Zn^{2+} , Ag^+ , Cd^{2+} and Pb^{2+} at 1 or 0.1 mM. Moreover, after addition of 1 equiv. of Hg^{2+} , the fluorescence was still quenched immediately, indicating that the presence of other cations does not affect the sensing process and its sensitivity.

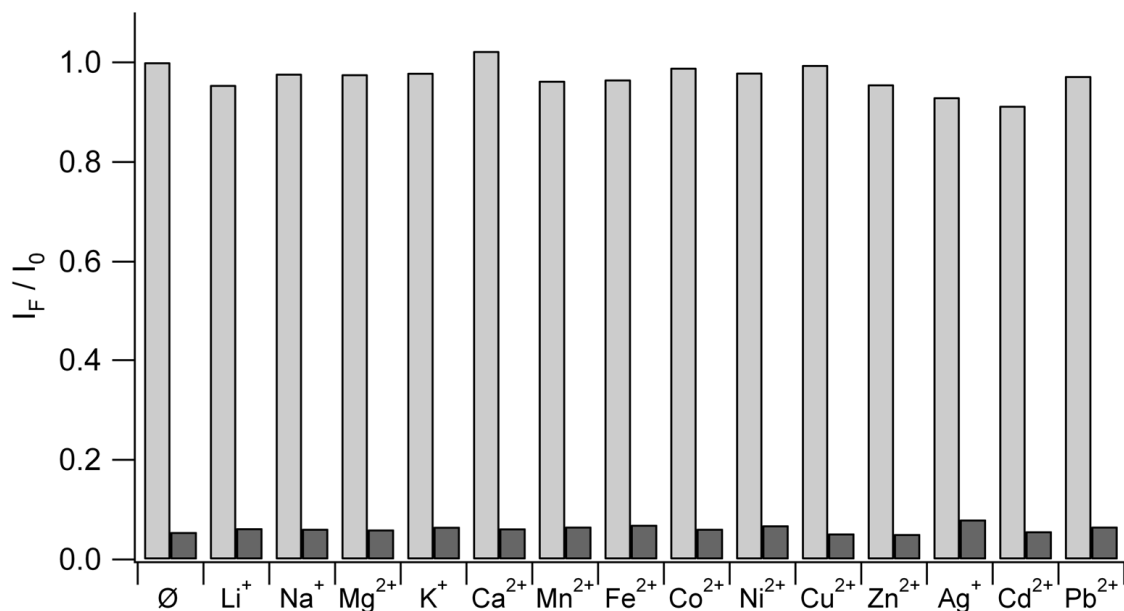


Figure 4 Light gray) Response I_F/I_0 of 8-HQ-4-St-PS **4** (13 μM) in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (40:60 v/v) at $\text{pH} = 3$ plus interfering ions Li^+ , Mg^{2+} , Ca^{2+} , Co^{2+} , Zn^{2+} and Cd^{2+} at 1 mM, and Na^+ , K^+ , Mn^{2+} , Fe^{2+} , Ni^+ , Cu^{2+} , Ag^+ and Pb^{2+} at 0.1 mM ($\lambda_{\text{exc}} = 420$ nm, $\lambda_{\text{em}} = 565$ nm). (Dark gray) idem in the presence of Hg^{2+} (13 μM).

Conclusion

In conclusion, we have synthesized a series of fluorescent chemosensors based on hydroxyquinoline bearing chalcogenic phosphanes such as phosphane oxide and sulfide derivatives. This original derivative showed an intense absorption band in the near-UV region and a fluorescence emission in the visible region. The large Stokes shift value and the unresolved vibronic structure of the fluorescence spectra strongly support the intramolecular charge transfer (ICT) character of the low-energy transition, which is thus expected to be highly sensitive to complexation by a cationic species. Indeed, addition of a heavy metal cation such as Hg^{2+} induced a bathochromic shift of the absorption and emission spectra thanks to the increase of electron-withdrawing character of the complexed quinoline moiety. In view of practical application, the cation complexation was studied in a mixture $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ under optimized pH conditions ($\text{pH}=3$) and the presence of mercury cations induced a major fluorescence quenching which allowed reaching a detection limit in the picomolar range (75 pM-15 ppt). This outstandingly low detection limit does therefore meets the requirements defined by the World Health Organization for drinking water. Further efforts will be dedicated to insert the fluorescent sensor in microfluidic chip.

Experimental

General informations: all reactions were performed under a dry argon atmosphere by using standard Schlenk techniques. Column chromatography was performed with E. Merck 0.040-0.063 mm Geduran silica gel. ^1H NMR, ^{13}C NMR and ^{31}P NMR were recorded on Bruker AV 300 and 400 instruments. All signals were expressed as ppm (δ) and internally referenced to residual protio solvent signals. Coupling constants (J) are reported in Hz and refer to apparent peak multiplicities. Mass spectrometry analyses were performed at IMAGIF/ICSN and at the University P. et M. Curie. Compounds **5**¹⁰ and **8**¹¹ were prepared according to published procedures. The preparation of diphenylthiophosphanyl chloride was realized according to literature.¹¹

Syntheses of 8-hydroxyquinoline analogues

Thiophosphinate 6. To a solution of 4-formyl-8-hydroxyquinoline **5** (300 mg, 1.7 mmol) in tetrahydrofuran (10 mL) at 0°C was added 60% w/w NaH in mineral oil (69 mg, 1.7 mmol). The resulting mixture was stirred for 15 minutes at 0°C, then diphenylthiophosphinic chloride (429 mg, 1.7 mmol) was added dropwise. The mixture was stirred 3 h at room temperature. The mixture was quenched with water and extracted with dichloromethane (2×20 mL). The organic phase was dried over MgSO_4 and the volatiles removed under vacuum. The residue was purified by flash silica gel chromatography (cyclohexane/ethyl acetate 80/20) to give 450 mg (71%) of the product. ^1H NMR (300 MHz, CDCl_3): δ = 10.47 (s, 1H), 9.18 (d, 1H, J = 4.2Hz), 8.76 (d, 1H, J = 7.3Hz), 8.22-8.14 (m, 4H), 7.79 (d, 1H, J = 4.2Hz), 7.65-7.56 (m, 1H), 7.51-7.46 (m, 7H) ^{13}C NMR (75.5 MHz, CDCl_3): δ = 192.6, 150.0, 147.3, 136.5, 135.2, 133.7, 132.1, 131.4 (d, J = 11.7 Hz), 129.0, 128.4 (d, J = 13.8 Hz), 126.2, 125.2, 121.1 (d, J = 5.7 Hz), 120.8. ^{31}P (121.5 MHz, CDCl_3): δ = 84.2. HRMS (ESI+): m/z : calcd for $\text{C}_{90}\text{H}_{94}\text{O}_4\text{P}$: 1269.6884; found: 1269.6937 $[\text{M}+\text{H}]^+$.

Thiophosphinate 4. To a solution of thiophosphinate **6** (100 mg, 0.25 mmol) and phosphonium salt **8** (154 mg, 0.275 mmol) in tetrahydrofuran (1 mL) at 0°C was added 60% w/w NaH in mineral oil (20 mg, 0.50 mmol) and 18-crown-6 (13.2 mg, 0.05 mmol). The resulting mixture was stirred at room temperature for 2 hours, then quenched with water and extracted with dichloromethane (2×10 mL). The organic phase was dried over MgSO_4 and the volatiles removed under vacuum. The crude product was redissolved in dichloromethane (5 mL), a catalytic amount of iodine was added. The solution was stirred at room temperature for 3 h under light exposure then washed with saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$ and dried over MgSO_4 . The residue was purified by flash silica gel chromatography (cyclohexane/ethyl acetate 80/20 then 70/30) to give 94 mg (64%) of the product. ^1H NMR (300 MHz, CDCl_3): δ = 8.79 (d, 1H, J = 3Hz), 8.20-8.27 (m, 4H), 7.80 (d, 1H, J = 6.2Hz), 7.64 (d, 1H, J = 6.2Hz), 7.40-7.55 (m, 7H), 7.35-7.27 (m, 3H), 6.91 (d, 1H, J = 9Hz), 6.85 (d, 2H, J = 12.4Hz), 6.76 (d, 2H, J = 12.4Hz), 6.60 (d, 1H, J = 9Hz), 3.85 (t, 2H, J = 6.6Hz) 1.65-1.72 (m, 2H), 1.25-1.43 (m, 10H), 0.87 (t, 3H, J = 6.7Hz). ^{13}C NMR (75 MHz, CDCl_3): δ = 159.8, 149.9, 147.4, 144.4, 135.5, 133.8, 131.8 (d, J = 11.4Hz), 130.4, 128.3 (d, J = 13.9Hz), 128.0, 125.6, 123.2, 121.4, 120.1, 114.1, 67.9, 31.8, 29.3, 29.2, 26.0, 22.6, 14.1. ^{31}P (121.5 MHz, CDCl_3): δ = 83.4. HRMS (ESI+): m/z : calcd for $\text{C}_{39}\text{H}_{37}\text{NO}_2\text{PS}$: 592.2439; found: 592.2449 $[\text{M}+\text{H}]^+$.

Phosphinate 7. To a solution of 4-formyl-8-hydroxyquinoline **5** (300 mg, 1.7 mmol) in tetrahydrofuran (10 mL) at 0°C was added 60% w/w NaH in mineral oil (69 mg, 1.7 mmol). The resulting mixture was stirred for 15 minutes at 0°C, then diphenylphosphinic chloride (402 g, 1.7 mmol) was added dropwise. The mixture was stirred 3 h at room temperature. The mixture was quenched with water and extracted with dichloromethane (2×20 mL). The organic phase was dried over MgSO₄ and the volatiles removed under vacuum. The residue was purified by flash silica gel chromatography (cyclohexane/ethyl acetate 80/20) to give 450 mg (71%) of the product. ¹H NMR (300 MHz, CDCl₃): δ = 10.49 (s, 1H), 9.27 (d, 1H, *J* = 4.1Hz), 8.72 (d, 1H, *J* = 8.7Hz), 8.06-8.14 (m, 4H), 7.92 (dt, 1H, *J* = 7.7Hz, *J* = 1.3Hz), 7.82 (d, 1H, *J* = 4.1Hz), 7.57 (t, 1H, *J* = 8.5Hz), 7.53-7.38 (m, 6H). ¹³C NMR (75 MHz, CDCl₃): δ = 192.6, 150.3, 132.5, 132.1 (d, *J* = 10.6Hz), 129.4, 128.5 (d, *J* = 13.5Hz), 126.1, 120.6. ³¹P (121.5 MHz, CDCl₃): δ = 32.1. HRMS (ESI+): *m/z*: calcd for C₉₀H₉₄O₄P: 1269.6884; found: 1269.6937 [M+H]⁺.

Phosphinate 3. To a solution of phosphinate **7** (100 mg, 0.26 mmol) and phosphonium salt **8** (160 mg, 0.286 mmol) in tetrahydrofuran (1 mL) at 0°C was added 60% w/w NaH in mineral oil (21 mg, 0.52 mmol) and 18-crown-6 (13.7 mg, 0.052 mmol). The resulting mixture was stirred at room temperature for 2 hours, then quenched with water and extracted with dichloromethane (2×10 mL). The organic phase was dried over MgSO₄ and the volatiles removed under vacuum. The crude product was redissolved in dichloromethane (5 mL), a catalytic amount of iodine was added. The solution was stirred at room temperature for 3 h under light exposure then washed with saturated aqueous Na₂S₂O₃ and dried over MgSO₄. The residue was purified by flash silica gel chromatography (cyclohexane/ethyl acetate 80/20 then 70/30) to give 80 mg (53%) of the product. ¹H NMR (300 MHz, CDCl₃): δ = 8.88 (d, 1H, *J* = 3Hz), 8.11-8.19 (m, 4H), 7.83 (d, 1H, *J* = 7.7Hz), 7.77 (d, 1H, *J* = 8.3Hz), 7.40-7.65 (m, 8H), 6.89 (d, 2H, *J* = 8.6Hz), 6.84 (d, 1H, *J* = 12.0Hz), 6.74 (d, 1H, *J* = 12.0Hz), 6.59 (d, 2H, *J* = 8.7Hz), 3.85 (t, 2H, *J* = 6.6Hz), 1.69-1.76 (m, 2H), 1.25-1.44 (m, 10H), 0.87 (t, 3H, *J* = 6.8Hz). ¹³C NMR (75 MHz, CDCl₃): δ = 158.8, 149.9, 144.7, 133.9, 132.2 (d, *J* = 10.6Hz), 130.4, 128.4 (d, *J* = 13.5Hz), 127.9, 126.2, 123.1, 121.4 (d, *J* = 14.9Hz), 119.9, 114.1, 67.9, 31.8, 29.3, 29.2, 26.0, 22.6, 14.1. ³¹P (121.5 MHz, CDCl₃): δ = 31.8. HRMS (ESI+): *m/z*: calcd for C₂₅H₃₀NO₂: 376.2277; found: 376.2272 [M-C₁₂H₈OP]⁺. (ESI-): *m/z*: calcd for C₁₂H₁₀O₂P: 217.0418; found: 217.0423 [M-C₂₅H₂₈NO]⁻.

Spectroscopic measurements: UV/Vis absorption spectra were recorded on a Varian Cary5000 spectrophotometer and corrected emission spectra were obtained on a Jobin–Yvon Spex Fluorolog-3 spectrofluorimeter. The fluorescence quantum yields were determined by using quinine sulfate dihydrate in sulfuric acid (0.5 N; $\phi_F = 0.546^{19}$) as standards. For the emission measurements, the absorbance at the excitation wavelengths were below 0.1 and so the concentrations were below 10⁵ molL⁻¹. The complexation constants were determined by global analysis of the evolution of all absorption and/or emission spectra by using the Specfit Global Analysis System V3.0 for 32-bit Windows system. This software uses singular value decomposition and non-linear regression modelling by the Levenberg–Marquardt method.²⁰

Two-photon excited fluorescence spectroscopy: Two-photon excited fluorescence spectroscopy was performed using a modelocked Ti:sapphire laser generating 150 fs wide pulses at a 76 MHz rate, with a time-averaged power of several hundreds of mW (Coherent Mira 900 pumped by a 5 W Verdi). The laser light is attenuated using a combination of half-wave plates and a Glan-laser polariser and the excitation power is further controlled using neutral density filters of varying optical density mounted in a computer-controlled filter wheel. After five-fold expansion through two achromatic doublets, the laser beam is focused by a microscope objective (10x, NA 0.25, Olympus, Japan) into a standard 1 cm absorption cuvette containing the sample. The applied average laser power arriving at the sample was between 0.5

and 15 mW, leading to a time-averaged light flux in the focal volume on the order of 0.1 - 1 mW/ μm^2 . The generated fluorescence is collected in epi-fluorescence mode, through the microscope objective, and reflected by a dichroic mirror (675dcxru, Chroma Technology Corporation, USA). Residual excitation light is removed using a barrier filter (e650-2p, Chroma) and the fluorescence is coupled into a 600 μm multimode fiber by an achromatic doublet. The fiber is connected to a compact CCD-based spectrometer (BTC112-E, B&W Tek, USA), which measures the two-photon excited emission spectrum. The emission spectra are corrected for the wavelength-dependence of the detection efficiency using correction factors established through the measurement of reference compounds having known fluorescence emission spectra. Briefly, the set-up allows for the recording of corrected fluorescence emission spectra under multiphoton excitation at variable excitation power and wavelength.

Absolute values for the two-photon excitation action cross sections $\sigma_2\Phi$ were obtained according to the method described by Xu & Webb, using 10^{-4} M fluorescein in 0.01M NaOH(aq) as a reference.²¹ **4** was dissolved in CH_3CN at a concentration of $3.8 \cdot 10^{-5}$ M. The complexation studies were performed by using different concentrations of mercury.

Solvents and salts: Acetonitrile from Aldrich (spectrometric grade) and millipore filtered water (conductivity $<6 \times 10^8 \text{ } \square^{-1}\text{cm}^{-1}$ at 20°C) were employed as solvent for absorption and fluorescence measurements. Sodium thiocyanate, potassium thiocyanate, lithium perchlorate, magnesium perchlorate, calcium perchlorate, manganese perchlorate, iron perchlorate, cobalt perchlorate, nickel perchlorate, copper perchlorate, zinc perchlorate, silver perchlorate, cadmium perchlorate and lead(II) thiocyanate, from Aldrich or Alfa Aesar, were of the highest quality available and vacuum dried over P_2O_5 prior to use.

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Notes and references

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† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

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