Photodynamic Fluorescent Metal Ion Sensors with Parts per Billion Sensitivity

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Abstract: We report herein the synthesis and spectroscopic study of the first spiropyrandinolines that function as sensors for metal ions in the parts per billion range. These systems operate by either photochemically or chemically induced reversible formation of merocyanine metal ion complexes. The application of this novel photodynamic sensing material to sensor technology is discussed.

Introduction

The design and synthesis of functional molecules that could serve as molecular devices for sensors, switching, and signal transduction is an area of intense activity and of tremendous potential significance.[1] A key feature for applications involving sensing is reversibility, i.e. off-rate, in any given recognition process, a property that also figures prominently in the development of efficient switching mechanisms.[2]

The development of optical methods for the detection and estimation of clinically and environmentally important species, such as metal ions, is an important area of contemporary sensor research.[1e−g] Cation receptors that lead to changes in fluorescence on metal ion binding, which are referred to as fluorescent chemosensors or fluoroionophores, offer distinct advantages over those that employ other signaling mechanisms in terms of sensitivity and selectivity. Several research groups have developed systems that couple metal cation binding to changes in fluorescence emission.[3,4]

The spiropyran nucleus is an attractive starting point in such constructions as the photoreversibility of such systems is well-documented.[5] While the nonphotolytic thermal equilibrium between **1** and **2** favors the neutral spiropyran by ca. 2.5 kcal/mol,[6] we have found that incorporation of the spiropyran into the 19-membered ring lactone **3** leads to a 3 kcal/mol change in the relative energies of **3** and **4**, effectively replacing $h\nu_1$ in Scheme 1 with the strain energy of the macrocyclic ring (Scheme 2).[7] Since the development of devices that are dependent on a single wavelength of light would be inherently easier to manipulate, we have examined other alternatives to $h\nu_1$ for the opening the spiropyran **1** to merocyanine **2**.

Several years ago, Taylor and Phillips independently reported that suitably substituted spiropyrans **5** (R = Me) could be used to bind metal ions in the open merocyanine form as shown in **6** (Scheme 3).[8] We subsequently established that this binding is photoreversible and that this system can be used for the photodynamic transport of metal ions across an organic membrane.[9] Photoreversibility of metal ion binding has also been demonstrated in spiropyran−crown ether conjugates by Inouye and co-workers.[5a] It was clear from our preliminary studies with the **5 → 6** system that a significant increase in sensitivity (**5 → 6** operating in the millimolar metal ion concentration range) would be required for the application of this strategy to the synthesis of metal ion sensors. We describe herein the extension of this spiropyran-based technology to the design and synthesis of the first highly fluorescent *photoreversible* spiropyranindoline "real-time" metal ion sensors with considerably greater sensitivity (parts per billion range) and lower fatigue rates than previously described systems.[5a]

Results and Discussion

The first compound that we examined is the 8-hydroxyquinolol-derived spiropyran **11**, which was prepared by a modifica-

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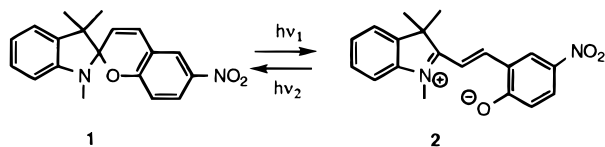
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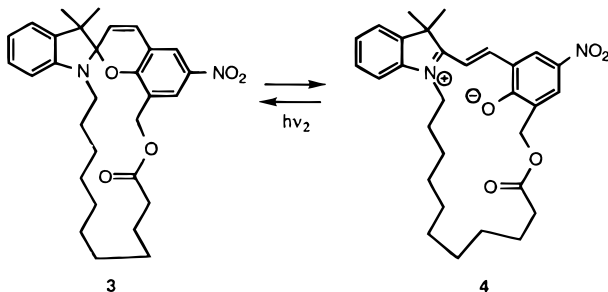
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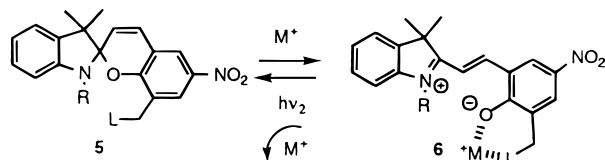(9) Winkler, J.; Deshayes, K.; Shao, B. *J. Am. Chem. Soc.* **1989**, *111*, 769−770.
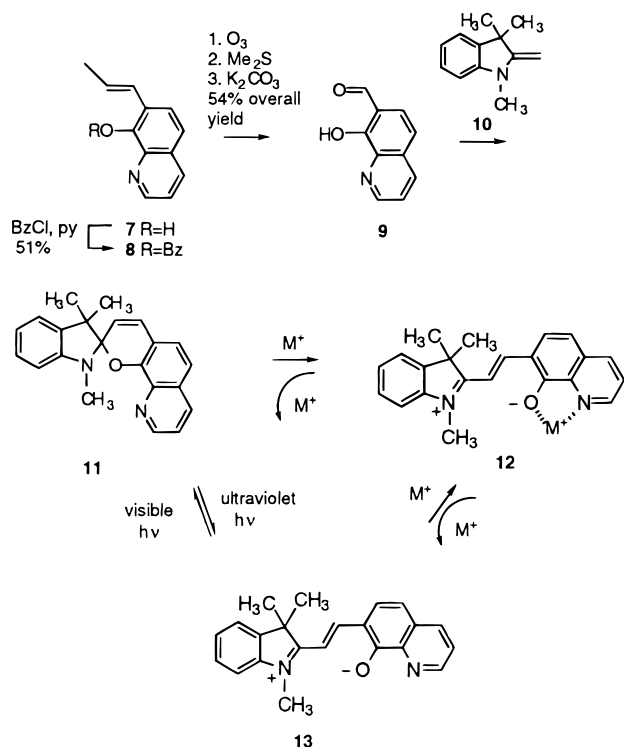
Scheme 1

Scheme 2

Scheme 3

Scheme 4

tion of the procedure reported by Phillips as outlined in Scheme 4.[10] We found it advantageous to perform the ozonolytic cleavage of **7**[11] on the corresponding benzoate **8** (benzoyl chloride, pyridine, 51%). Ozonolysis of **8** and reaction of the crude product with dimethyl sulfide and then ethanolic potassium carbonate gave the 7-formylquinolol (**9**), in 53% yield over the two steps. Condensation of **9** with Fisher's base **10**[12] gave the spiropyran **11** (butanone, reflux, 16 h, 57%) as a purple solid (mp 193 °C).

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Minimal fluorescence at 610 nm is observed with a 10^{-5} M ethanolic solution of quinolinespiropyranindoline (SP) **11** upon excitation at 550 nm ($\Phi_F = 0.0015$),[13] the absorbance maximum of the metal-free merocyanine (MC) **13**, a result that is consistent with the predominance of **11** in the absence of exogenous metal ion or UV light (Scheme 4). Addition of 1 equiv of $ZnCl_2$ to the solution of **11** and excitation at 572 nm (the absorbance λ_{max} of chelate **12** in ethanol where $M^+ = Zn^{2+}$) led to a 14-fold increase in emission intensity at 610 nm (Figure 1). Similar effects have been observed with other divalent and trivalent cations, and our results are summarized in Figure 2.

This dramatic increase in fluorescence intensity is due not only to an increase in the concentration of **12** but also to the coordination of metal ion by the fluorophore. While the difference in the changes in the long-wavelength absorbance maxima of an ethanolic solution of **11** upon photochemical generation of **13** ($\Delta A_{550\ nm} = 0.79$)[14] and upon addition of 1 equiv of $ZnCl_2$ to **11** to form **12** ($\Delta A_{572\ nm} = 0.52$) are small, the changes in the fluorescence emission intensities are dramatic. These results are consistent with data reported for metal-bound and metal-free 8-hydroxyquinoline (8-HQ)[15] and have been recently attributed to the role of the metal cation in inhibiting photoinduced tautomerization of the 8-hydroxyquinoline moiety which leads to nonradiative deexcitation.[16] Emission in **12** (metal-bound) is enhanced relative to **13** (metal-free) when the pathways for radiationless relaxation of the excited state available to **13** are no longer available in the Zn^{2+}-chelated species **12**.

These results have been extended to mixed aqueous systems with similar results, i.e. the sensitivity of **11** for Zn^{2+} in 1:1 ethanol/water is 3.3 ppb. We have established that this modest attenuation in sensitivity is due to the decrease of the fluorescence of **12** in aqueous media and not due to a decrease in the denticity of the zwitterion **12** for the metal ion in the presence of water.

The magnitude of the fluorescence enhancement of **11** in the presence of Cd^{2+}, another group IIb metal ion, is smaller than that observed with Zn^{2+}, which could be attributed to the larger size of Cd^{2+} relative to Zn^{2+}. Enhanced emission of **11** was also observed in the presence of certain alkaline earth metals. Exposure of **11** to $MgCl_2$ results in an increase of fluorescence intensity of the same magnitude as that observed with Zn^{2+}. Since the binding affinities of 8-hydroxyquinoline, the chromophore responsible for metal ion binding in **11**, for Mg^{2+} and Zn^{2+} are different,[17] the similarity of the resulting emission enhancements of **11** may be explained by differences in the stoichiometry or geometry of the respective metal complexes.

The formation of chelates of **12** with other transition metals (Cu^{2+}, Ni^{2+}, Co^{2+}, and Fe^{3+}) can be monitored by an increase in the long-wavelength absorbance maxima. For example, addition of 1 equiv of $CuCl_2$ to an ethanolic solution of **11** results

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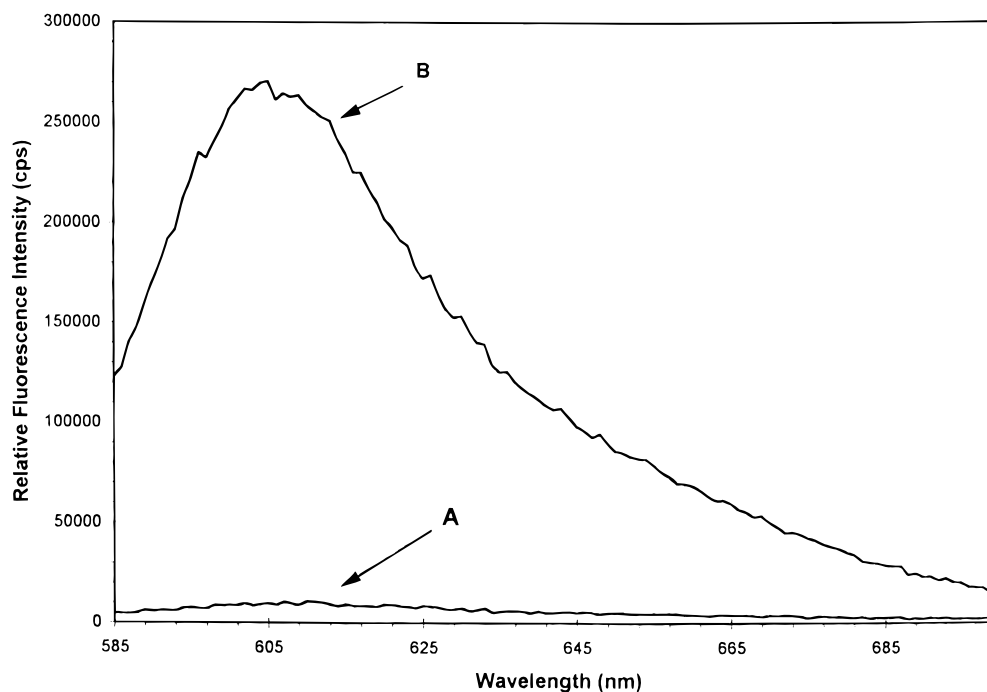
Figure 1. Fluorescence emission spectra of spiropyran **11** (10^{-5} M ethanol, room temperature): (A) emission of "metal-free" solution; (B) emission after addition of 1 equiv of $ZnCl_2$.
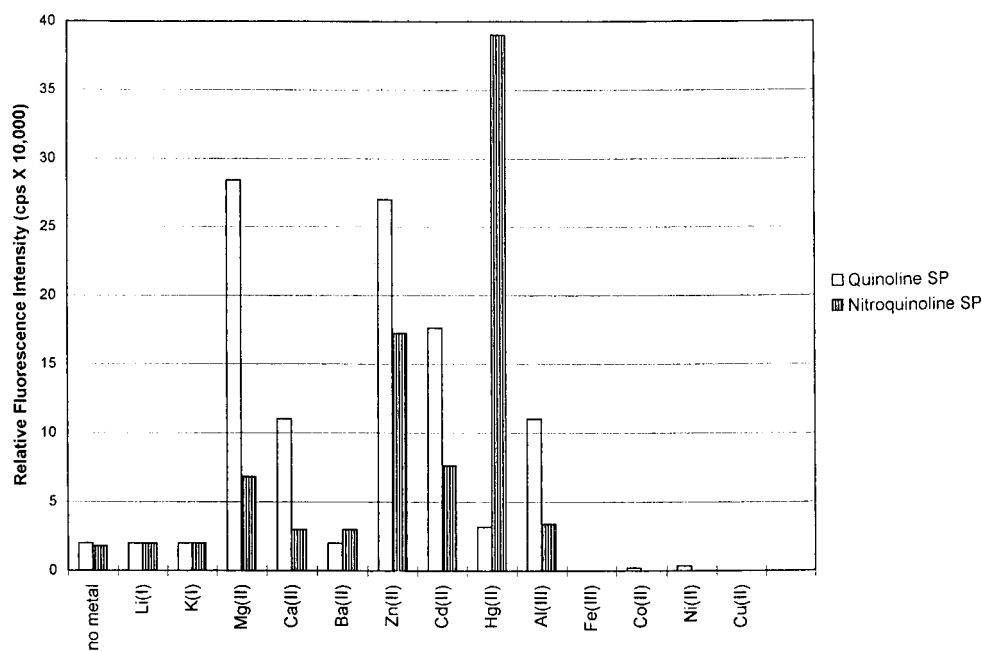

Figure 2. Relative fluorescence emission intensities of **11** (10^{-5} M ethanol) and **15** (10^{-5} M in benzene) at room temberature for "metal-free" solutions and these spiropyrans in the presence of several metal chlorides.

in a blue shift of the absorbance maximum and an increase in the absorbance value ($\Delta A_{548\ nm} = 0.22$). In the Cu^{2+} chelate of **12**, the proximity of the paramagnetic metal ion to the unpaired electrons of the ligand leads to spin−orbit coupling and intersystem crossing, resulting in complete quenching of fluorescence emission from the **12** chelate (Figure 2). Similar effects were observed with Ni^{2+}, Co^{2+}, and Fe^{3+}.

While irradiation of metal chelate **12** ($M^+ = Zn^{2+}$) did not liberate metal ion with regeneration of **11**, the liberation of the metal ion could be achieved chemically, i.e. by addition of 1 equiv of nitrilotriacetic acid, $N(CH_2CO_2H)_3$, which sequesters the metal ion, liberating the closed spiropyran **11**. The chemically regenerated **11** can then be recycled for another metal ion binding event. While this result demonstrates that

the metal binding is chemically reversible, it does not establish the facile photochemical reversibility that was characteristic of our earlier study with the piperidinomethyl spiropyrans **5** ⇌ **6** (Scheme 3).[9]

We reasoned that the phenolate in **12** (Scheme 4) was a better ligand for the metal ion than *p*-nitrophenolate **6** (Scheme 3). The *p*-nitro group withdraws electron density from the phenolate oxygen, decreasing the denticity of **6** and thereby facilitating reversible binding. We therefore prepared the nitroquinolinespiropyran **15** (Scheme 5) by condensation of Fisher's base **10** with 7-formyl-8-hydroxy-5-nitroquinoline (**14**).[11] In contrast to the modest absorbance bands (554 and 592 nm) that were observed with ethanolic solution of **11**, strong absorbance at 560 nm was observed with an ethanolic solution of the nitro-

Scheme 5
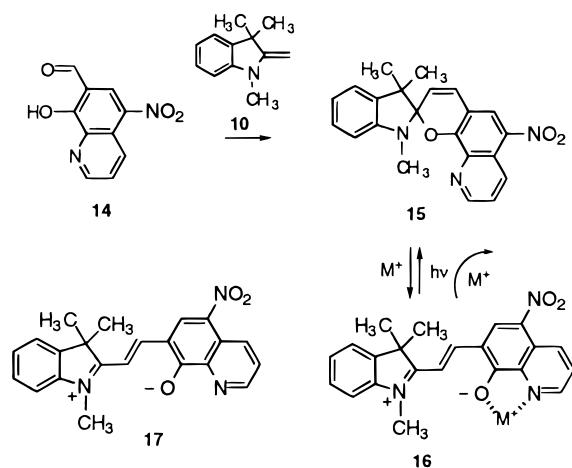
quinolinespiropyran **15**. The introduction of the nitro substituent shifts the spiropyran $\rightleftharpoons$ merocyanine equilibrium in polar solvents such as ethanol to favor the open form **17**.[18] In less polar solvents, i.e. benzene or ethyl acetate, the predominant form is **15**. Like spiropyranquinoline **11**, the fluorescence emission of **15** is also sensitive to group IIb and several other transition metals but inert to alkali metal chlorides (Figure 2). However, unlike **11**, **15** is also relatively inert to alkaline earth cations.

Addition of 1 equiv of $ZnCl_2$ to a benzene solution of **15** resulted in a 9-fold enhancement of fluorescence emission (Figure 3). The magnitude of this increase in relative intensity is less than that observed with **11**. Likewise, only a 3−4-fold increase in emission intensity of **16** was observed upon addition of 1 equiv of $MgCl_2$ or $CdCl_2$. These differences may be due simply to the reduced chelating ability of the nitrated hydroxy-quinoline chromophore. The metal ion binding constants of 5-nitro-8-hydroxyquinoline are typically lower than those of 8-hydroxyquinoline. It is interesting to note that $HgCl_2$ does not follow the trend exhibited by other group IIb metals. A nearly 20-fold increase of emission intensity of **16** was observed upon addition of 1 equiv of $HgCl_2$. Studies to determine the basis for these observed differences are currently underway.[19]

We were delighted to find that the ejection of metal ion from **16** occurs upon brief irradiation of the Zn^{2+} chelate with visible light (150-W tungsten flood lamp, 30 s to 5 min). Rapid dissociation of approximately 60% of the fluorescent Zn^{2+} chelate **16** accompanied by regeneration of **15** occurs with expulsion of the metal ion (Figure 3). The regenerated spiropyran is fully functional and has been run through 10 cycles of metal binding−release without detectable fatigue. Incomplete photochemical reversal of metal ion binding with **16**, in contrast to the complete reversal observed on exposure of **12** to nitrilotriacetic acid, may be due the position of the equilibrium between **15** and **16** in the presence of Zn^{2+}, which was irreversibly sequestered on exposure of **12** to nitrilotriacetic acid.

Conclusions

We have therefore established that chemically and photo-chemically reversible metal ion binding can be detected using fluorescence spectroscopy with sensitivity for Zn^{2+} in the parts per billion range (6.5 ppb for **11** in ethanol and 30 ppb for **15**

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(19) Similar differences in metal ion-binding with Hg^{2+} were observed in our earlier studies (ref 9).

in benzene). The covalent attachment of these metal-ion sensors to fiber optic surfaces, which could lead to a practically useful metal-ion sensing device, is currently underway in our laboratory, and our results will be reported in due course.

Experimental Section

General Methods. All reagents and solvents were obtained from Aldrich, Eastman, Fisher, or Sigma. Tetrahydrofuran was distilled from sodium/benzophenone. Methylene chloride, benzene, and toluene were distilled from calcium hydride. All reactions were performed under a dry argon atmosphere unless specified otherwise. Reaction progress was monitored by analytical thin-layer chromatography (TLC), performed with 0.25-mm Whatman silica gel glass plates containing F-254 indicator. Visualization on TLC was achieved by either UV light (254 nm), phosphomolybdic acid indicator, potassium permanganate, or ceric sulfate indicator. Flash chromatography was performed with 32−63-mm silica gel packing. Proton magnetic resonance spectra were recorded at 500 MHz and carbon spectra were recorded at 125 MHz on a Bruker AMX-500, with chemical shifts reported as parts per million downfield using $CHCl_3$ (^1H, δ 7.24; ^{13}C, δ 77.0) or C_6H_6 (^1H, δ 7.28; ^{13}C, δ 128.0) as an internal standard. Melting points were determined using a Thomas-Hoover apparatus and are uncorrected. Infrared spectra were acquired on a Perkin-Elmer 1600 FT-IR. Neat IR spectra were acquired using a NaCl plate. High-resolution mass spectra were obtained at the University of Pennsylvania Mass Spectrometry Service Center on either a VG micromass 70/70H high-resolution double-focusing electron impact/chemical ionization spectrometer or a VG ZAB-E spectrometer. UV/visible absorbance spectra were acquired on a Hewlett-Packard 8452A diode array spectrophotometer, and fluorescence emission spectra were acquired on a Photon Technologies International A1010 luminescence spectrometer. Emission measurements were performed in a four-sided quartz cell at room temperature and are uncorrected for detector response. Standard solutions (10^{-3} M) for spectroscopic measurements were prepared using spectrophotometric quality solvents (absolute ethanol was obtained from Pharmco) and volumetric flasks. Appropriate dilutions were carried out using volumetric pipets.

8-(Benzoyloxy)-7-propenylquinoline (8). An oven-dried 250-mL round-bottomed flask was placed under Ar and charged with 4 g (21.6 mol) of 7-propenyl-8-quinolinol **7** and 150 mL of anhydrous pyridine. The solution was cooled at 0 °C, and to it was added 7.5 mL of benzoyl chloride. The reaction mixture was allowed to stir at 0 °C for 5 min and heated at 50 °C for 16 h. The reaction mixture was concentrated under reduced pressure, and the residue was taken up in 100 mL of ethyl ether. The organic phase was washed with 1 M HCl and then 2 M Na_2CO_3, dried over $MgSO_4$, filtered, and concentrated to afford 6 g of **9** as a clear oil. Purification over silica (gradient of 10 to 40% EtOAc/hexanes) afforded a 3.15 g of a white solid (51%): ^1H NMR (500 MHz, $CDCl_3$) δ 8.97 (d, J = 4.3 Hz, 1H), 8.09 (d, J = 1.2 Hz, 2H), 8.05 (d, J = 8.2 Hz, 1H), 7.72−7.30 (m, 6H), 6.77 (d, J = 15.9 Hz, 1H), 6.45 (dq, J = 15.9, 6.6 Hz, 1H), 1.89 (d, J = 6.6 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.1, 150.3, 142.7, 141.3, 136.0, 133.3, 132.3, 130.6, 130.4, 130.3, 129.6, 129.4, 128.4, 128.2, 125.3, 123.9, 120.9, 26.7, 18.8; IR (KBr, cm^{-1}) 3033, 1738, 1449, 1260, 1176, 1093; HRMS (CI, NH_3) calcd for $C_{19}H_{15}NO_2$ (M + 1) 290.1181, found (M + 1) 290.1176, error 2 ppm; TLC R_f = 0.30 (20% EtOAc/hexanes).

7-Formyl-8-quinolinol (9). An oven-dried 100 mL round-bottomed flask was charged with 1.9 g (6.57 mmol) of 8-(benzoyloxy)-7-propenylquinoline (**8**), 120 mL of methanol, and 12 mL of dichloromethane and cooled to −78 °C in a dry ice−2-propanol bath. A stream of ozone was bubbled through the reaction for 30 min. TLC was used to monitor the consumption of starting material. At −78 °C, Ar was bubbled through the bluish reaction mixture for 20 min to remove excess ozone. Dimethyl sulfide (1.45 mL, 19.7 mmol) was added, and the reaction mixture was allowed to warm to 25 °C and stirred for 16 h. The reaction mixture was then concentrated under reduced pressure. To the crude residue dissolved in 100 mL of ethanol was added 0.99 g (7.23 mmol) of K_2CO_3 and 0.52 mL (32.8 mmol) of
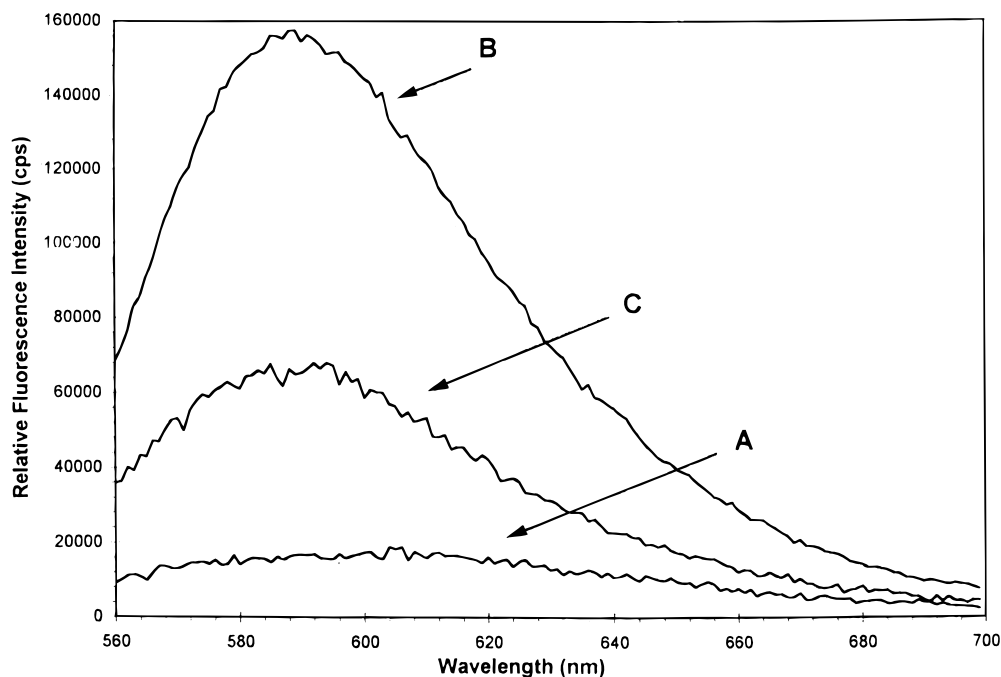
Figure 3. Fluorescence emission spectra of spiropyran **15** (10^{-5} M benzene, room temperature): (A) emission of "metal-free" solution; (B) emission after addition of 1 equiv of $ZnCl_2$; (C) emission after 30 s of irradiaion with visible light.

water. The mixture was heated at 70 °C for 2 h, cooled to room temperaure, and then evaporated under reduced pressure. The residue was taken up in 100 mL of chloroform. The organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated to afford 1 g of **9** as a yellow oil. Crystallization from a methanol−dichloromethane mixture afforded 608 mg (53%, two steps) of 7-formyl-8-quinolinol as an off-white solid: mp > 220 °C; ^{1}H NMR (500 MHz, CDCl$_3$) δ 10.36 (s, 1H), 8.94 (d, J = 3.0 Hz, 1H), 8.15 (d, J = 8.1 Hz, 1H), 7.74 (d, J = 8.5 Hz, 1H), 7.57 (dd, J = 7.8, 3.9 Hz, 1H), 7.35 (d, J = 7.8 Hz, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 192.9, 159.1, 149.4, 139.1, 136.1, 132.3, 125.8, 124.5, 118.4, 117.6; IR (KBr, cm^{-1}) 3082, 2367, 2344, 1676, 1508, 1391, 1228; HRMS calcd for $C_{10}H_7NO_2$ (M + 1) 173.0555, found (M + 1) 173.0548, error 4 ppm.

Spiropyran 11. An oven-dried 25-mL round-bottomed flask, equipped with magnetic stirrer, was placed under Ar and charged with 200 mg (1.16 mmol) of 7-formyl-8-quinolinol (**9**) and 15 mL of 2-butanone. To this was added 0.185 mL (1.05 mmol) of 1,3,3-trimethyl-2-methyleneindoline (**10**). The resulting reaction mixture was heated at reflux for 16 h. After cooling to 25 °C, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash chromatography over silica (1% to 10% MeOH/ CH_2Cl_2 with 1% Et$_3$N) to afford 197 mg (57%) of **11** as a light purple solid: mp 193 °C (lit. mp 193 °C); ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.48 (dd, J = 4.2, 1.7 Hz, 1H), 7.40 (dd, J = 8.2, 1.5 Hz, 1H), 7.13 (dd, J = 7.7, 1.0 Hz, 1H), 6.99 (dd, J = 6.9, 0.9 Hz, 1H), 6.94 (s, 2H), 6.86(m, 1H), 6.60 (dd, J = 8.2, 4.0 Hz, 1H), 6.56 (d, J = 10.1 Hz, 1H), 6.41 (d, J = 7.7 Hz, 1H), 5.51 (d, J = 10.1 Hz, 1H), 2.62 (s, 3H), 1.47 (s, 3H), 1.11 (s, 3H); ^{13}C NMR (125 MHz, C$_6$D$_6$) δ 149.2, 136.5, 135.9, 129.7, 129.2, 128.6, 127.8, 125.7, 125.3, 122.1, 121.7, 121.3, 119.9, 119.4, 118.7, 116.6, 107.0, 76.7, 64.1, 52.0, 28.9, 25.8, 24.4, 20.4; IR (neat, cm^{-1}) 2976, 2943, 2604, 2497, 1477, 1397, 1172, 1037; HRMS (CI, NH$_3$) calcd for $C_{22}H_{20}N_2O$ (M + 1) 329.1654, found (M + 1) 329.1650, error 1.5 ppm.

Metal Ion Binding Experiments Using 11. Photochromism at Low Temperature. A 10^{-4} M solution of quinolinespiropyran **11** in ethanol was prepared. A 0.5-mL aliquot of this solution was placed in a quartz EPR tube (3 mm diameter). The Dewar−cuvette apparatus was filled with a methanol/liquid N$_2$ bath, and the EPR tube containing the solution of spiropyran was cooled to −60 °C. The temperature of the sample solution inside the EPR tube was monitored using a temperature electrode. A blank was also prepared for use as a background measurement. The sample was irradiated with a 450-W

Hanovia medium-pressure mercury lamp in a borosilicate glass immersion well for 2 min and then the absorbance or emission spectrum was immediately acquired (within 2 min). The sample was then irradiated with a 150-W flood lamp, and the absorbance or emission spectrum was again acquired.

Metal Ion Binding and Chemical Reversibility Experiments. A 10^{-5} M solution of the quinolinespiropyran **11** in ethanol was prepared. A 3-mL aliquot of the solution was placed in a quartz absorbance or fluorescence cuvette equipped with magnetic stirrer. The absorbance spectrum was acquired or the sample was excited at 550 nm (the λ_{max} corresponding to the merocyanine form), and the emission spectrum was acquired (average of three runs). Using a 10-μL syringe, the appropriate volume of a 3×10^{-3} M solution of the metal chloride in ethanol was added to the sample and the resulting solution was allowed to stir in the dark. The absorbance or emission spectrum was acquired (excitation at 572 nm) every 2 min until a maximum absorbance or emission intensity was obtained (a maximum was reached immediately in the case of absorbance and in ca. 1 min in the case of emission). A 0.3 M solution of nitrilotriacetic acid disodium salt in 10% EtOH/H$_2$O was prepared, and a 100-fold dilution was made to afford a 3×10^{-3} M solution of NTA in 10% EtOH/H$_2$O. An appropriate volume of this solution was added to the spiropyran−metal chelate solution using a 10-μL syringe. The absorbance or emission spectrum (excitation at 550 nm) was then acquired. This binding−release cycle was repeated as necessary to determine the extent of fatigue.

Preparation of 15. Spiropyran 15. An oven-dried 50-mL round-bottomed flask was placed under Ar charged with a magnetic stirring bar, 58 mg (0.267 mmol) of 8-hydroxy-7-formyl-5-nitroquinoline (**14**), 20 mL of freshly distilled tetrahydrofuran, and 2.1 mL of DMSO. The heterogeneous mixture was heated to 60 °C until dissolution was achieved. To this yellow solution was added 57 μL (0.32 mmol) of 1,3,3-trimethyl-2-methyleneindoline (**10**), and the resulting mixture was stirred under Ar at 25 °C for 16.5 h. The solvent was removed under reduced pressure, and the residue was purified using flash chromatography (pure CH_2Cl_2 and 1% Et$_3$N, then gradient of MeOH in CH_2Cl_2 and 1% Et$_3$N) affording 76 mg (77%) of the desired spiropyran **15** as a gold−green solid: ^{1}H NMR (500 MHz, C$_6$D$_6$) δ 8.91 (dd, J = 8.8, 1.6 Hz, 1H), 8.37 (dd, J = 4, 1.6 Hz, 1H), 8.01 (s, 1H), 7.25 (t, J = 7.5 Hz, 1H), 7.08 (d, J = 7.5 Hz, 1H), 6.99 (t, J = 7.5 Hz, 1H), 6.52 (d, J = 7.5 Hz, 1H), 6.28 (d, J = 10.2 Hz, 1H), 5.50 (d, J = 10.2 Hz, 1H), 2.63 (s, 3H), 1.46 (s, 3H), 1.15 (s, 3H); ^{13}C NMR (125 MHz, C$_6$D$_6$) δ 155.8, 150.1, 147.9, 138.3, 137.1, 135.2, 131.8, 125.2, 124.3,

124.1, 121.9, 120.6, 120.4, 114.2, 107.6, 107.5, 52.5, 28.7, 25.8, 20.2; IR (neat, cm^{-1}) 2917, 2849, 1609, 1574, 1514, 1470, 1390, 1274, 1223, 1110; TLC R_f = 0.20 (10% MeOH/CH$_2$Cl$_2$); HRMS (CI/CH$_4$) calcd for C$_{22}$H$_{19}$N$_3$O$_3$ (M + H) 374.1504, found 374.1513, error 3 ppm.

Metal Ion Binding and Photohromism Experiments Using 15. Photochromism at Low Temperature. A 10^{-4} M solution of nitroquinolinespiropyran **15** in benzene was prepared and allowed to equilibrate overnight in the absence of light at 25 °C. A 0.5-mL portion of this solution was placed in a quartz EPR tube (3 mm diameter). The Dewar−cuvette apparatus was filled with an ice−water bath, and the EPR tube containing the solution of spiropyran was cooled to ca. 8 °C. A blank was also prepared for use as a background measurement. The sample was irradiated with a 450-W Hanovia medium-pressure mercury lamp in a borosilicate glass immersion well for 2 min, and then the absorbance spectrum was immediately acquired (within 2 min). The sample was then irradiated with a 150-W flood lamp, and the absorbance spectrum was acquired.

A 10^{-5} M solution of the nitroquinolinespiropyran **15** in benzene was prepared and allowed to equilibrate overnight at 25 °C and in the dark. A 3-mL aliquot of the solution was placed in a quartz absorbance or fluorescence cuvette equipped with magnetic stirrer. The absorbance spectrum was acquired or the sample was excited at 550 nm (the λ_{max} corresponding to the merocyanine form) and the emission spectrum was acquired (average of three runs). Using a 10-μL syringe, the appropriate volume of a ca. 10^{-3} M solution of the metal chloride in ethanol was added to the sample and the resulting solution was allowed to stir in the dark. A control experiment was also conducted in which

10 μL of metal-free ethanol was added to the benzene solution of nitroquinolinespiropyran. No significant change in the fluorescence emission was observed. The absorbance or emission spectrum was acquired (excitation at 550 nm) every 2 min until a maximum absorbance or emission intensity was obtained (a maximum was reached immediately in the case of absorbance and in ca. 4 min in the case of emission). The sample was then irradiated with a 150-W flood lamp for 30 s. Longer irradiation (up to 5 min) with visible light did not have any effect on the extent of photoreversible metal ion binding in any case. The absorbance or emission spectrum (excitation at 550 nm) was immediately acquired. The sample was allowed to stir at 25 °C in the dark, and the absorbance or emission spectrum was acquired (excitation at 550 nm) every 2 min until a maximum absorbance or emission intensity was obtained (again, this occurred immediately in the case of absorbance and in ca. 4 min in the case of emission). This cycle was repeated as necessary to determine the extent of photochromic fatigue.