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A New Selective Chromogenic and Turn-On Fluorogenic Probe for Copper(II) in Water−Acetonitrile 1:1 Solution

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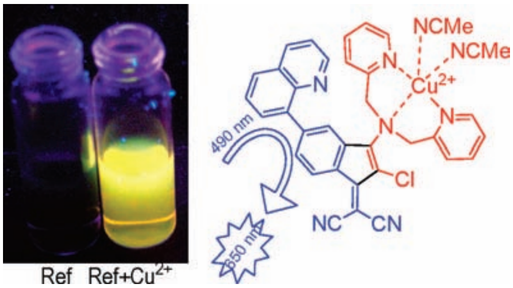
ABSTRACT



A quinoline-indene derivative is described as a new highly selective and sensitive chromogenic and turn-on fluorogenic probe for the naked-eye detection of copper(II) cation in water−acetonitrile 1:1 mixture.

Copper is a vital trace element, the third most abundant in humans, and is present at low levels in a variety of cells and tissues with the highest concentrations in the liver.[1] Its concentration in the neuronal cytoplasm may contribute to the etiology of Alzheimer's[2] or Parkinson's disease.[3] Cu^{2+} is implicated in several physiological responses. Proteins containing copper ions at their active site are usually involved as redox catalysts in biological processes such as electron transfer or oxidation of various organic substrates.[4] Research advances concerning the active-site chemistry of dioxygen activating copper enzymes have shown that single copper center O_2-derived reactive Cu-oxygen species are implicated in the action of biological oxygenases.[5] However, oxidative damage has been linked to chronic Cu overload or exposure to excess Cu caused by accidents, occupational hazards, and environmental contamination.[6] Therefore fast detection of Cu^{2+} in water or physiological samples is of toxicological and environmental concern.[7] Despite the fact that selective colorimetric probes for naked-eye Cu^{2+} detection are im-

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(1) Mathie, A.; Sutton, G. L.; Clarke, C. E.; Veale, E. L. *Pharm. Ther.* **2006**, *111*, 567.

(2) (a) Mare, S.; Penugonda, S.; Robinson, S. M.; Dohgu, S.; Banks, W. A.; Ercal, N. *Peptides* **2007**, *28*, 1424. (b) Deraeve, C.; Boldron, C.; Maraval, A.; Mazarguil, H.; Gornitzka, H.; Vendier, L.; Pitié, M.; Meunier, B. *Chem. Eur. J.* **2008**, *14*, 682.

(3) (a) Kim, B.-E.; Nevitt, T.; Thiele, D. J. *Nat. Chem. Biol.* **2008**, *4*, 176. (b) Lee, J. C.; Gray, H. B.; Winkler, J. R. *J. Am. Chem. Soc.* **2008**, *130*, 6898.

(4) Koval, I. A.; Gamez, P.; Belle, C.; Selmeczi, K.; Reedijk, J. *Chem. Soc. Rev.* **2006**, *35*, 814.

(5) Maiti, D.; Lucas, H. R.; Sarjeant, A. A. N.; Karlin, K. D. *J. Am. Chem. Soc.* **2007**, *129*, 6998.

(6) Gaetke, L. M.; Chow, C. K. *Toxicology* **2003**, *189*, 147.

(7) (a) Pamukoglu, M. Y.; Kargi, F. *J. Hazard. Mater.* **2007**, *148*, 2744. (b) Welsha, P. G.; Lipton, J.; Mebane, C. A.; Marr, J. C. A. *Ecotoxicol. Environ. Saf.* **2008**, *69*, 199. (c) Buck, K. N.; Ross, J. R. M.; Flegal, A. R.; Bruland, K. W. *Environ. Res.* **2007**, *105*, 5. (d) Van Genderen, E.; Gensemer, R.; Smith, C.; Santore, R.; Ryan, A. *Aquat. Toxicol.* **2007**, *84*, 279. (e) Kumar, P.; Tewari, R. K.; Sharma, P. N. *Plant Cell Rep.* **2008**, *27*, 399.

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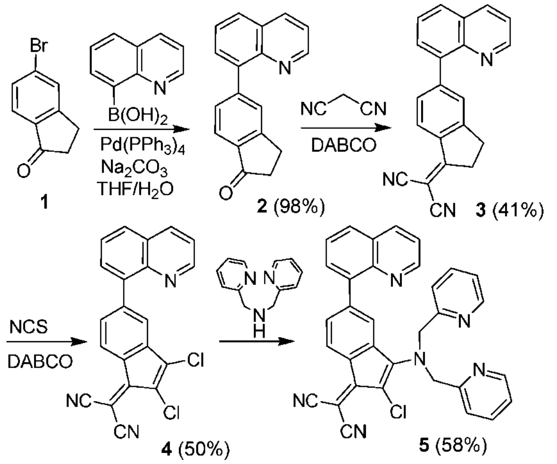
portant,[8] research efforts have been oriented to the generation of selective fluorescent probes[9] for Cu^{2+}, which is known as a fluorescence quencher because of its paramagnetic nature.[10] Therefore several ON−OFF fluorescent probes for Cu^{2+} have been reported.[11] Although the displacement approach may serve to reverse the quenching effect,[12] an increasing number of true OFF−ON fluorescent probes for Cu^{2+} have been reported.[13] Interference with related metal ions and limited efficacy in the presence of water are common drawbacks of several chemical probes, and therefore the preparation of new selective fluorescent probes for Cu^{2+} is necessary for achieving selectivity. In this paper we report a new selective chromogenic and OFF−ON fluorogenic probe for Cu^{2+} in water−acetonitrile 1:1 solution. We designed and synthesized a dicyanomethylene indene equipped with quinoline and 2,2′-dipicolylamine moieties in which coordination of the metal ion would impact the conjugation of the potentially fluorescent push−pull chromophore (Scheme 1). Suzuki reaction[14] of 5-bromoindanone **1** and quinolin-8-ylboronic acid gave ketone **2** in near quantitative yield. Knoevenagel condensation[14] of **2** with malononitrile and diazabicyclo[2.2.2]octane (DABCO) in benzene gave indene **3** (66%), which was subjected to reaction with *N*-chlorosuccinimide (NCS) and DABCO in dichloromethane (DCM) to give dichloroindene **4** (70%), which reacted with 2,2′-dipicolylamine in DCM to give the molecular probe **5** in 81% yield. Compounds **2**−**5** were characterized by the usual spectroscopic and analytical techniques.

Scheme 1. Synthesis of Chemical Probe **5**



A 10^{-4} M solution of compound **5** in MeCN displays a strong purple color ($\lambda_{max} = 545$ nm, $\varepsilon = 5893$ M^{-1} cm^{-1} and $\lambda_{max} = 393$ nm, $\varepsilon = 12700$ M^{-1} cm^{-1}). Addition of 2 equiv of Cu^{2+} to 10^{-4} M solutions of **5** in water−acetonitrile 1:1 resulted in a dramatic change of color from purple to orange. This response was selective for Cu^{2+} under these conditions and addition of 2 equiv of the other cations Ag^{+}, Ni^{2+}, Sn^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}, Fe^{3+}, Sc^{3+}, Al^{3+}, and Hg^{2+} as their perchlorate or triflate salts resulted in no appreciable changes (Figure 1).

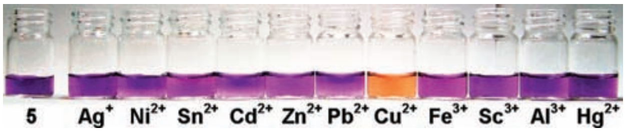


Figure 1. Color changes of a 10^{-4} M solution of **5** in MeCN/H_2O 1:1 in the presence of 2 equiv of every cation.

A slight visual discoloration was observed after addition of 5 or more equiv of Fe^{3+} or, to a lesser extent, of Hg^{2+}, added as perchlorate salts, to 10^{-4} M solutions of **5** in MeCN/H_2O 1:1 without emission of fluorescence under UV light. Therefore large amounts of these cations may interfere with Cu^{2+} sensing under visible light, but they cannot interfere by fluorescence. Quantitative UV−vis titration of a 10^{-4} M solution of **5** in MeCN/H_2O 1:1 and Cu^{2+}, added as perchlorate salt, showed that the original absorption maximum centered at 550 nm diminished as Cu^{2+} was added (Figure 2), generating one isosbestic point at 490 nm. The

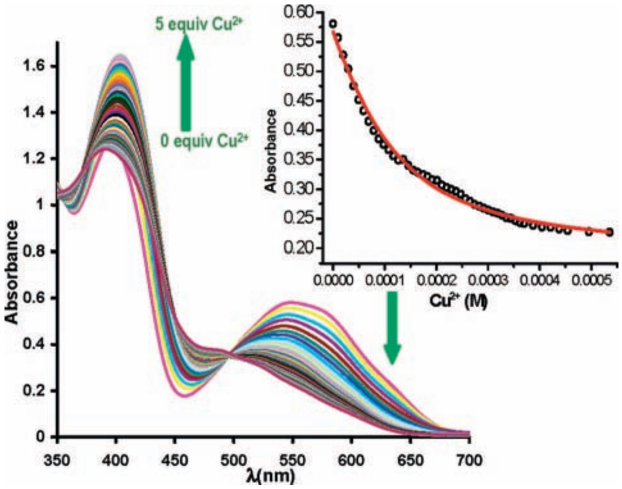


Figure 2. UV−vis titration curves and titration profile of a 10^{-4} M solution of **5** in MeCN/H_2O 1:1 with Cu^{2+}.

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(8) (a) Sheng, R.; Wang, P.; Gao, Y.; Wu, Y.; Liu, W.; Ma, J.; Li, H.; Wu, S. *Org. Lett.* **2008**, *10*, 5015. (b) Basurto, S.; Riant, O.; Moreno, D.; Rojo, J.; Torroba, T. *J. Org. Chem.* **2007**, *72*, 4673. (c) Liu, J.; Lu, Y. *Chem. Commun.* **2007**, 4872. (d) Schmittel, M.; Lin, H.-W. *Angew. Chem., Int. Ed.* **2007**, *46*, 893. (e) Lee, S. J.; Lee, S. S.; Lee, J. Y.; Jung, J. H. *Chem. Mater.* **2006**, *18*, 4713.

(9) (a) Prodi, L.; Bolletta, F.; Montalti, M.; Zaccheroni, N. *Coord. Chem. Rev.* **2000**, *205*, 5959. (b) Valeur, B.; Leray, I. *Coord. Chem. Rev.* **2000**, *205*, 3.

(10) De Silva, A. P.; Gunarante, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515.

(11) (a) Zeng, Q.; Cai, P.; Li, Z.; Qin, J.; Tang, B. Z. *Chem. Commun.* **2008**, 1094. (b) Xie, J.; Ménand, M.; Maisonneuve, S.; Métivier, R. *J. Org. Chem.* **2007**, *72*, 5980. (c) Park, S. M.; Kim, M. H.; Choe, J.-I.; No, K. T.; Chang, S.-K. *J. Org. Chem.* **2007**, *72*, 3550. (d) Guo, Z.; Zhu, W.; Shen, L.; Tian, H. *Angew. Chem., Int. Ed.* **2007**, *46*, 5549. (e) Lee, S. J.; Lee, S. S.; Lah, M. S.; Hong, J.-M.; Jung, J. H. *Chem. Commun.* **2006**, 4539. (f) Kim, S. H.; Kim, J. S.; Park, S. M.; Chang, S.-K. *Org. Lett.* **2006**, *8*, 371.

(12) Royzen, M.; Dai, Z.; Canary, J. W. *J. Am. Chem. Soc.* **2005**, *127*, 1612.

titration profile fitted nicely a 1:1 binding model, and the association constant was calculated: log $K = 4.23 \pm 0.04$.

Job plot analysis of the UV−vis titrations carried out in MeCN/H_2O 1:1 revealed a maximum at a 50% mole fraction, in accord with the proposed 1:1 binding stoichiometry (Figure 3). The detection limit of a 10^{-4} M solution of **5** in MeCN/H_2O 1:1, calculated by the blank variability method,[15] was 4.17×10^{-7} M. Analogously, titration of a 10^{-4} M solution of **5** in MeCN/H_2O 7:3 and Cu^{2+} fitted a 1:1 binding model; the association constant was calculated: log $K = 4.73 \pm 0.04$. The sensing action of **5** and Cu^{2+} was equally effective in MeCN/Tris-HCl (pH 7.2) 9:1 and in a mixture of a 10^{-4} M solution of **5** and 1 equiv of every cation Ag^+, Ni^{2+}, Sn^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}, Fe^{3+}, Sc^{3+}, and Al^{3+} in MeCN/H_2O 1:1, although the presence of an additional 1 equiv of Hg^{2+} caused slight decoloration without losing sensitivity to Cu^{2+} (see Supporting Information). UV titrations permitted calculation of the binding constants for the 1:1 models of complexation of **5** and Fe^{3+} or Hg^{2+} in MeCN/H_2O 1:1, which were log $K_{Fe^{+3}} = 3.44 \pm 0.04$ and log$K_{Hg^{+2}} = 3.17 \pm 0.01$. Additional experiments (see Supporting Information) showed that the UV titration of **5** and Cu^{2+} in the presence of several or all of these cations was performed with no or very little interaction.

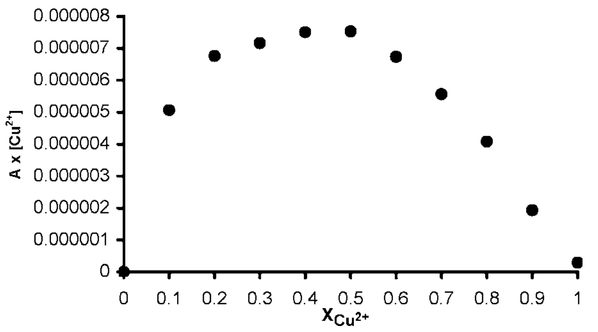

Figure 3. Job plot analysis of a 10^{-4} M solution of **5** in MeCN/H_2O 1:1 with Cu^{2+}.

Under the common TLC-UV light, $\lambda = 366$ nm, a distinct orange fluorescent emission was observed after addition of 2 equiv of Cu^{2+} to a 10^{-4} M solution of **5** in H_2O/MeCN 1:1 (Figure 4, right). The fluorescence was much more intense when the same experiment was conducted in acetonitrile (Figure 4, left). Fluorescence of solutions of **5** under the 366 nm light was negligible.

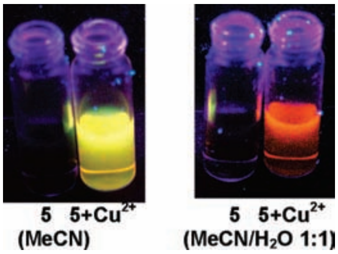

Figure 4. Fluorescence changes of 10^{-4} M solutions of **5** in MeCN and MeCN/H_2O 1:1 in the presence of 2 equiv of Cu^{2+} under a 366 nm UV light.

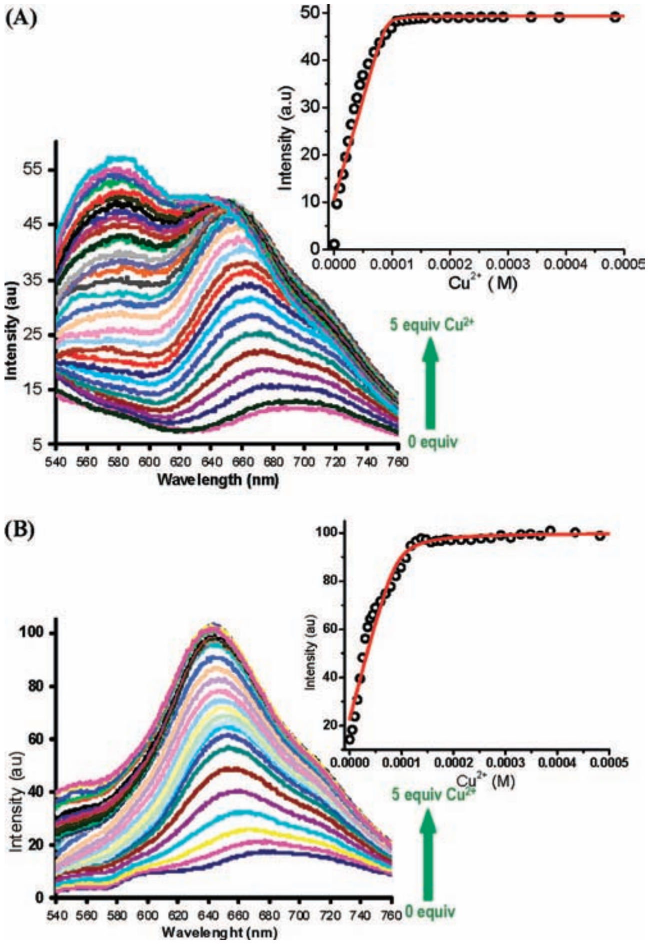

Figure 5. (A) Fluorescence titration curves and titration profile of a 10^{-4} M solution of **5** in MeCN/H_2O 1:1 with Cu^{2+}. (B) Fluorescence titration curves and titration profile of a 10^{-4} M solution of **5** in MeCN with Cu^{2+}.

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(13) (a) Yu, M.; Shi, M.; Chen, Z.; Li, F.; Li, X.; Gao, Y.; Xu, J.; Yang, H.; Zhou, Z.; Yi, T.; Huang, C. *Chem. Eur. J.* **2008**, *14*, 6892. (b) Li, G.-K.; Xu, Z.-X.; Chen, C.-F.; Huang, Z.-T. *Chem. Commun.* **2008**, 1774. (c) Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2007**, *129*, 9838. (d) Kovács, J.; Rödler, T.; Mokhir, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 7815. (e) Wen, Z.-C.; Yang, R.; He, H.; Jiang, Y.-B. *Chem. Commun.* **2006**, 106. (f) Martínez, R.; Zapata, F.; Caballero, A.; Espinosa, A.; Tárraga, A.; Molina, P. *Org. Lett.* **2006**, *8*, 3235. (g) Martínez, R.; Ratera, I.; Tárraga, A.; Molina, P.; Veciana, J. *Chem. Commun.* **2006**, 3809. (h) Xiang, Y.; Tong, A.; Jin, P.; Ju, Y. *Org. Lett.* **2006**, *8*, 2863. (i) Qi, X.; Jun, E. J.; Xu, L.; Kim, S.-J.; Hong, J.S. J.; Yoon, Y. J.; Yoon, J. *J. Org. Chem.* **2006**, *71*, 2881. (j) Singhal, N. K.; Ramanujam, B.; Mariappanadar, V.; Rao, C. P. *Org. Lett.* **2006**, *8*, 3525. (k) Zhang, H.; Han, L.-F.; Zachariasse, K. A.; Jiang, Y.-B. *Org. Lett.* **2005**, *7*, 4217. (l) Xu, Z.; Xiao, Y.; Qian, X.; Cui, J.; Cui, D. *Org. Lett.* **2005**, *7*, 889.

(14) Basurto, S.; García, S.; Neo, A. G.; Torroba, T.; Marcos, C. F.; Miguel, D.; Barberá, J.; Ros, M. B.; De la Fuente, M. R. *Chem. Eur. J.* **2005**, *11*, 5362.

(15) Massart, D. L.; Vandeginste, B. G. M.; Buydens, L. M. C.; De Jong, S.; Lewi, P. J.; Smeyers-Verbeke, J. *Handbook of Chemometrics and Qualimetrics: Part A*; Eselvier: Amsterdan, The Netherlands, 1997; Chapter 13, p 379.

Quantitative fluorescence titration of a 10^{-4} M solution of **5** in MeCN/H_2O 1:1 and Cu^{2+} ($\lambda_{exc} = 495$ nm) showed a 5-fold increase in the intensity of emission centered at 650 nm as Cu^{2+} was added (Figure 5A). The titration profile fitted nicely a 1:1 binding model, and the association constant was calculated: $\log K = 6.48 \pm 0.48$. The detection limit of a 10^{-4} M solution of **5** in MeCN/H_2O 1:1, calculated in fluorescence emission by the blank variability method,[15] was 4.08×10^{-8} M. A large emission at 580 nm, seen in Figure 5A, did not appear by fluorescence titration of **5** (10^{-4} M, MeCN) and Cu^{2+} ($\lambda_{exc} = 490$ nm) in the absence of water, which showed a high increase in the intensity of emission at 650 nm (Figure 5B). Quantum yield of the complex in MeCN, measured at $\lambda_{exc} = 392$ nm, using DPA as standard, was $\Phi_{\mathbf{5}\text{-Cu(II)}} = 0.034$. The titration profile fitted a 1:1 binding model, and the calculated association constant was $\log K = 5.48 \pm 0.15$.

We carried out ^1H NMR titration experiments on a 10^{-2} M solution of **5** in CD_3CN/D_2O 9:1 with Cu^{2+} (Figure 6).

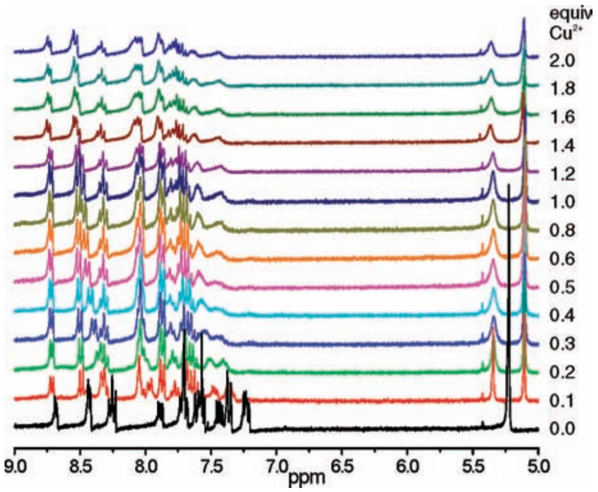

Figure 6. ^1H NMR titration of a 10^{-2} M solution of **5** in CD_3CN/D_2O 9:1 with Cu^{2+}.

By addition of increasing amounts of Cu^{2+} in CD_3CN/D_2O 9:1, the methylene signal at δ 5.2 splits into two signals at δ 5.1 and 5.3, corresponding to two different proton environments, one of them much more affected by the presence of Cu^{2+} probably by closer proximity. The effect is more pronounced by performing the titration in CD_3CN (see Supporting Information).

NMR titration indicated that the complexation is effected by the *N*,*N*-bis(pyridin-2-ylmethyl)amine group and that it is affected by the presence of water, being compatible with the results from UV and fluorescence titrations. Probably, a 1:1 complex (Figure 7) is formed in which Cu^{2+} disrupts conjugation of amine and cyano groups, causing a ipsochromic shift of the main absorption band and, at the same time, inhibiting quenching of the fluorescence by the amino group, giving rise to the appearance of fluorescence from the uncomplexed moiety of the molecule. In fact, compound **3** is a yellow fluorescent solid under the 366 nm UV light. Several 1:1 Cu^{2+}-bis(pyridinylmethyl)amine complexes are known.[16]

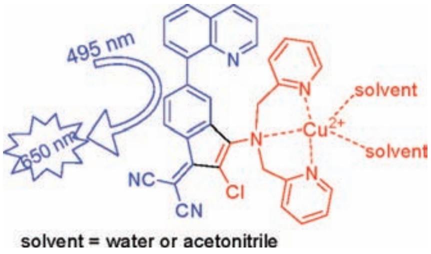

Figure 7. Proposed model for the sensing action of **5** and Cu^{2+}.

In summary, we have described a new chromogenic and OFF−ON fluorogenic probe for the naked-eye detection of copper(II) cation in mixtures of water−acetonitrile, in conditions of complete selectivity and nanomolar sensitivity. It should be useful for fast copper(II) cation detection from environmental samples in the range of trace amounts (μg L^{-1}), required by organisms, by UV and fluorescence spectroscopy.

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Supporting Information Available: Experimental procedures, physical and spectral data of all new products, additional colorimetric and fluorimetric studies, calculations of Job plot, detection limits, and quantum yields. This material is available free of charge via the Internet at http://pubs.acs.org.

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[16] (a) Nielsen, A.; Veltzé, S.; Bond, A. D.; McKenzie, C. J. *Polyhedron* **2007**, *26*, 1649. (b) Hazell, A.; Hazell, R.; McKenzie, C. J.; Nielsen, L. P. *Dalton Trans.* **2003**, 2203. (c) Foxon, S.; Xu, J.-Y.; Turba, S.; Leibold, M.; Hampel, F.; Heinemann, F. W.; Walter, O.; Würtele, C.; Holthausen, M.; Schindler, S. *Eur. J. Inorg. Chem.* **2007**, 429.