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Selective Mercuration of 2-Hydroxy Nile Red and Its Application towards Chemodosimetric Hg^{2+}-selective Signaling

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Selective mercuration at the 1,6-positions of 2-hydroxy derivative of Nile Red and its application towards Hg^{2+}-selective signaling was investigated. The 2-hydroxy Nile Red exhibited a selective UV-vis absorption and fluorescent signaling behavior towards Hg^{2+} ions over common coexisting physiologically important metal ions in aqueous environment. ^{1}H NMR studies revealed that the mercuration was selectively effected at the 1,6-positions of 2-hydroxy Nile Red, which is quite different from that at the 6,8-positions for the parent Nile Red.

Key Words: Hg^{2+} signaling, Mercuration, Nile Red, Chemodosimeter, Fluorescence signaling

Introduction

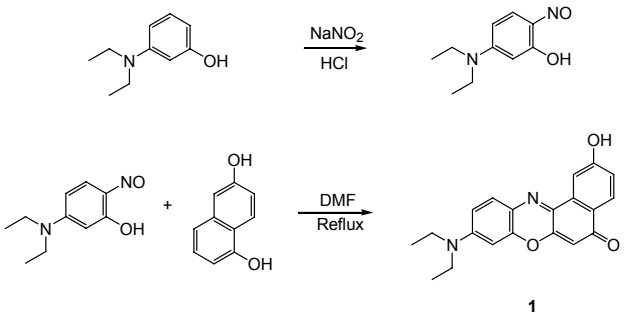
Selective detection of transition and heavy metal ions is very important in various fields of chemical and biological sciences, as well as in the protection of our environment.[1] Among many important transition metal ions, Hg^{2+} ions attract much research interest due to its toxic environmental impact.[2] There are many sophisticated systems for the efficient and selective detection[3] and visualization of Hg^{2+} ions.[4] However, a great deal of effort has been continuously devoted toward the construction of devices that are able to signal and visualize the presence of Hg^{2+} in varying origins.[5]

Recently, mercuration of important dyes has been used for the purpose of specific imaging and labeling of proteins. Mercuration of fluorescein and resorufin after subsequent transmetalation with $AsCl_3$ afforded biarsenical ligands, FlAsH and ReAsH, respectively, which are employed for affinity chromatography, fluorescence measurements, and localization of tetracysteine-tagged proteins.[6,7] Similarly, mercuration of Nile Red has provided dimercurated derivative for the preparation of biarsenical compound for the imaging of conformational changes of proteins containing tetracysteine motif.[8]

Nile Red and related benzophenoxazine derivatives have been utilized as fluorescent dyes for the labeling of biomolecules.[9] A series of water soluble Nile Red derivatives for the development of fluorescent probes for biotechnology have been prepared accordingly.[10] Chemiluminescent energy-transfer cassettes based on fluorescein and Nile Red[11] and near-IR fluorescence probes derived from Nile Red[12] were also reported. Other Nile Red derivatives are currently used as probes for lipid- and drug-binding proteins,[13] tools for hydrophobic characterization of intracellular lipids,[14] and solvatochromic nucleoside for indicating micropolarity around DNA.[15] During the course of search for a new probe system based on Nile Red derivatives, we found that Nile Red and its 2-hydroxy analogue exhibited quite different mercuration profile. In this paper, we report a selective mercuration of 2-hydroxy Nile Red and its potential for application toward chromogenic and fluorogenic Hg^{2+}-selective signaling. The process of the mercuration reaction mode of 2-hydroxy Nile Red could be confirmed by ^{1}H NMR spectroscopy.

Results and Discussion

The 2-hydroxy derivative of Nile Red **1** was prepared by the reaction of 5-diethylamino-2-nitrosophenol, which was obtained by the reaction of 3-diethylaminophenol with $NaNO_2$, with 1,6-dihydroxynaphthalene following the reported procedure (Scheme 1).[16] First, the UV-vis signaling behavior of **1**



Scheme 1. Preparation of the 2-hydroxy derivative of Nile Red **1**

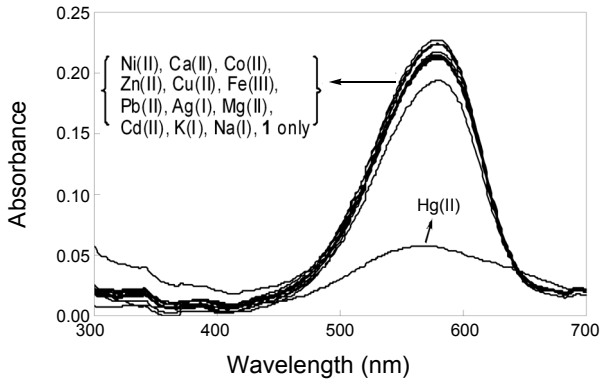


Figure 1. UV-vis spectra of **1** in the presence of various metal ions. [**1**] = 5.0×10^{-6} M, [M^{n+}] = 5.0×10^{-4} M. In acetate buffered (pH 4.7, 10 mM) H_2O-MeOH (50:50, v/v).

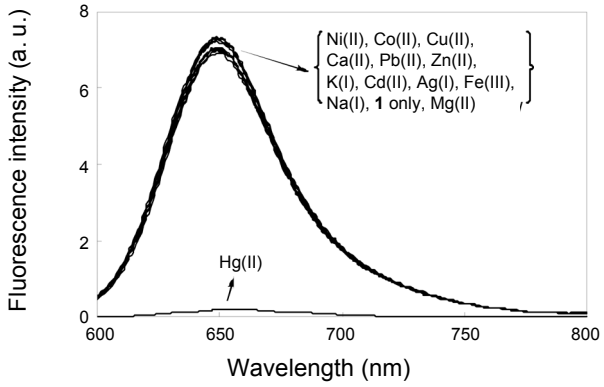


Figure 2. Fluorescence spectra of **1** in the presence of various metal ions. [**1**] = 5.0×10^{-6} M, [M^{n+}] = 5.0×10^{-4} M. λ_{ex} = 580 nm. In acetate buffered (pH 4.7, 10 mM) H_2O-MeOH (50:50, v/v).

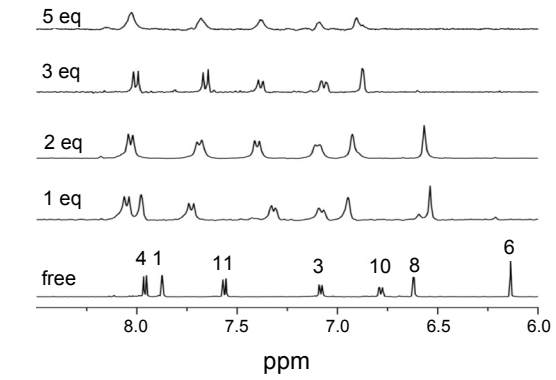
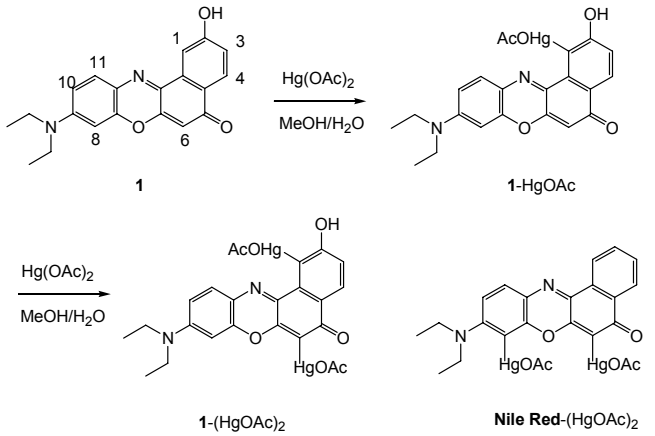


Figure 3. Partial ^{1}H NMR spectra of **1** in the presence of increasing amounts of Hg^{2+} ions. For numbering, see Scheme 2. [**1**] = 1.0×10^{-2} M in DMSO-d_6.



Scheme 2. Selective 1,6-dimercuration of 2-hydroxy Nile Red

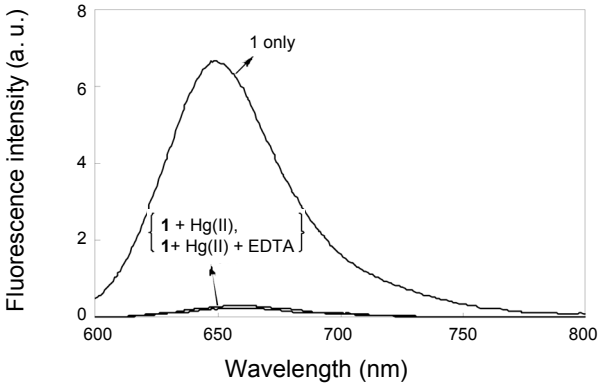


Figure 4. Fluorescence spectra of **1** in the presence of Hg^{2+} and EDTA. [**1**] = 5.0×10^{-6} M, [Hg^{2+}] = 1.5×10^{-4} M. [EDTA] = 2.5×10^{-4} M. λ_{ex} = 580 nm. In acetate buffered (pH 4.7, 10 mM) H_2O-MeOH (50:50, v/v).

toward representative alkali, alkaline earth, and transition metal ions was investigated. Compound **1** revealed a strong absorption at 580 nm in acetate buffered aqueous 50% methanol solution at pH 4.7 (Figure 1). Upon treatment with various metal ions, the absorption spectrum was affected particularly with Hg^{2+} ions; the absorption intensity was significantly diminished with a slight blue-shift to 568 nm ($\Delta\lambda_{max}$ = −12 nm). The solution color changed from deep blue to light blue. Other surveyed metal ions revealed no significant changes.

Compound **1** exhibited an intense fluorescence emission centered at 649 nm in aqueous 50% methanol at pH 4.7. The Hg^{2+} ions quenched the fluorescence of **1** effectively and the fluorescence maximum was slightly red-shifted to 658 nm (Figure 2). The quenching was quite effective and the intensity ratio I/I_o at 649 nm was 0.027 with 100 equiv of Hg^{2+} ions. The significant quenching of the fluorescence resulted in almost no fluorescence of the solution under illumination with a UV lamp. Other metal ions exhibited relatively minor effects on the fluorescence intensity and profile, and the I/I_o values at 649 nm varied between 0.99 for Mg^{2+} and 1.04 for Ca^{2+}.

The observed UV-vis and fluorescence signaling behaviors of **1** are due to the mercuration of 2-hydroxy Nile Red (Scheme 2). The dimercuration of parent Nile Red was known to be selectively effected at the 6,8-positions of the benzophenoxazine moiety.[8] However, for 2-hydroxy Nile Red, mercuration was found to be effected at the 1,6-positions. The mercuration process was followed by measurements of the ^{1}H-NMR spectrum of **1** in the presence of varying amounts of $Hg(OAc)_2$ in DMSO-d_6 solution.

With 1 equiv of Hg^{2+} ions, all the ^{1}H NMR resonances of compound **1** were somewhat broadened and downfield shifted without any significant changes in individual resonances (Figure 3). That might be due to the interaction of the Hg^{2+} ions with, most probably, the phenolic oxygen atom of **1** as in the sensing of Hg^{2+} ions by benzothiazolium hemicyanine dye.[17] However, with 2 equiv of Hg^{2+} ions, the resonance of the 1-position proton at 8.0 ppm completely disappeared, while other resonances were not so significantly affected, consistent with the formation of monomercurated compound **1**-HgOAc. Upon addition of 3 equiv of Hg^{2+} ions, subsequent disappearance of the resonance for the 6-position proton at 6.6 ppm was observed as expected for the formation of dimercurated product **1**-$(HgOAc)_2$. With subsequent addition of Hg^{2+} ions (5 equiv), all the remaining resonances were significantly broadened.

The abovementioned chemodosimetric signaling behavior of **1** was further evidenced by treatment with EDTA (Figure 4). Diminished fluorescence of the **1**-Hg^{2+} system, which was obtained by the treatment of **1** with 30 equiv of Hg^{2+} ions, was not affected by subsequent treatment with 50 equiv of the EDTA solution. This observation manifests the irreversible mercura-

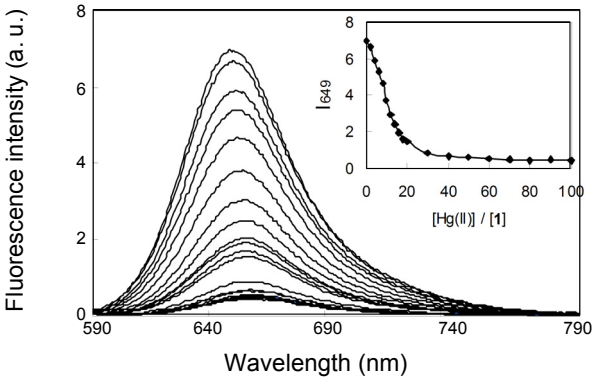


Figure 5. Fluorescence titration of **1** with Hg^{2+} ions in the presence of physiologically relevant metal ions as background. [**1**] = 5.0×10^{-6} M, λ_{ex} = 580 nm. [Na^+] = 138 mM, [K^+] = 4.0 mM, [Mg^{2+}] = 1.0 mM, [Ca^{2+}] = 3.0 mM, [Zn^{2+}] = 0.02 mM, and [Cu^{2+}] = 0.015 mM. In acetate buffered (pH 4.7, 10 mM) H_2O-MeOH (50:50, v/v).

tion process of the **1**-Hg^{2+} system as depicted in Scheme 2.

Quantitative signaling behavior of **1** toward Hg^{2+} ions was assessed by fluorescence titration. As the amount of Hg^{2+} ions increased, the fluorescence of **1** decreased steadily until approximately 20 equiv of Hg^{2+} ions. The emission maximum slightly shifted toward a longer wavelength. The signaling was not significantly interfered by the presence of other physiologically relevant metal ions. The fluorescence titration of **1** with Hg^{2+} ions in the presence of representative physiologically important metal ions ([Na^+] = 138 mM, [K^+] = 4.0 mM, [Mg^{2+}] = 1.0 mM, [Ca^{2+}] = 3.0 mM, [Zn^{2+}] = 0.02 mM, and [Cu^{2+}] = 0.015 mM) as background resulted in similar titration curve (Figure 5). From this titration the detection limit of **1** for the signaling of Hg^{2+} ions was estimated to be 2.8×10^{-5} M.[18]

To gain further insight into the signaling behavior of **1**, a time course trace of the signaling of Hg^{2+} ions of **1** was measured. Changes in absorbance at 580 nm indicated that the signaling was somewhat slow and completed within 2 h after sample preparation, which is definitely undesirable behavior for an ideal system of fast chemodosimetric signaling. This sluggishness is due to the fact that signaling is based on the chemical transformation of the probe with analytes, as reported in other chemodosimeters.[19] In fact, the mercuration of parent Nile Red required somewhat harsh conditions of overnight reaction using acetic acid at 50 °C.[8] Attempts to signal Hg^{2+} by Nile Red was also carried out, but the mercuration was much more sluggish and required several days to obtain a constant signal. The presence of the hydroxyl group of **1** was found to considerably enhance the mercuration process.

In summary, we have investigated selective mercuration of 2-hydroxy Nile Red and its chemodosimetric signaling behavior toward Hg^{2+} ions. The 2-hydroxy Nile Red exhibited significant chromogenic and fluorescence responses toward Hg^{2+} ions by selective mercuration in aqueous environments. The ^{1}H NMR measurements evidenced the 1,6-dimercuration of 2-hydroxy Nile Red, which was quite different from that of 6,8-dimercuration of the parent Nile Red. The results obtained also suggest that one should pay extra attention to the design of Nile Red-based signaling and visualizing systems for the possibility of mercuration of the Nile Red moiety.

Experimental

General. Nile Red, *N*,*N*-diethyl-3-aminophenol, and 1,6-dihydroxy naphthalene were purchased from TCI Chemical Co. and used without further purification. The 2-hydroxy derivative of Nile Red was prepared following a reported procedure.[16] All solvents were purchased from Aldrich Chemical Co. as 'spectroscopic grade'. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were obtained on a Varian VNS NMR spectrometer and referenced to the residual solvent signal. UV-vis spectra were recorded with a Jasco V-550 spectrophotometer. Fluorescence spectra were measured on an Aminco-Bowman Series 2 Spectrophotometer.

Preparation of compound 1.[16]

5-(Diethylamino)-2-nitrosophenol: Sodium nitrite (0.12 g, 1.8 mM) was dissolved in water (1 mL) and a solution of *N*,*N*-diethyl-3-aminophenol (0.20 g, 1.2 mM) in aqueous HCl (1.3 mL, 6 N) was added in several small portions at 0 °C. After stirring for 3 h at 0 °C , the resulting precipitate was filtered and dried to yield the product (85%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.30 (d, J = 10.2 Hz, 1H), 6.88 (d, J = 10.2 Hz, 1H), 6.05 (s, 1H), 3.57 (m, 4H), 1.2-1.0 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.7, 138.8, 131.2, 116.9, 112.9, 110.2, 52.4, 10.0.

9-(Diethylamino)-2-hydroxy-5*H*-benzo[*a*]phenoxazin-5-one (1): To a solution of 5-(diethylamino)-2-nitrosophenol (0.25 g, 1.3 mM) in DMF (40 mL) was added 1,6-dihydroxy naphthalene (0.20 g, 1.3 mM) and the mixture was refluxed for 4 h under nitrogen atmosphere. After evaporating the solvent, the residue was purified by column chromatography (silica gel, ethyl acetate:isopropyl alcohol = 2:1, v/v) to yield a dark green colored product (70%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.44 (s, 1H), 7.97 (d, J = 8.5 Hz, 1H), 7.88 (d, J = 2.4 Hz, 1H), 7.59 (d, J = 8.8 Hz, 1H), 7.09 (dd, J = 8.6 and 2.4 Hz, 1H), 6.81 (dd, J = 8.9 and 2.5 Hz, 1H), 6.65 (d, J = 2.5 Hz, 1H), 6.16 (s, 1H), 3.49 (m, 4H), 1.16 (m, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 182.0, 161.1, 152.0, 151.1, 148.8, 139.2, 134.2, 127.9, 124.3, 118.8, 110.4, 108.6, 104.5, 96.5, 44.9, 12.9.

Mercuration of 1. A mixture of compound **1** (10 mM) and $Hg(OAc)_2$ (10 - 50 mM) was dissolved in DMSO-d_6 solution and the ^{1}H-NMR spectrum was measured. Monomercurated product **1**-HgOAc; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.94 (d, J = 8.4 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.18 (d, J = 8.1 Hz, 1H), 6.82 (d, J = 7.8 Hz, 1H), 6.61 (s, 1H), 6.18 (s, 1H), 3.47 (br m, 4H), 1.15 (br m, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 181.9, 173.6, 163.6, 152.7, 151.3, 147.4, 138.4, 137.0, 130.4, 128.4, 125.3, 122.7, 110.5, 105.0, 96.5, 44.9, 22.2, 12.9. Dimercurated product **1**-$(HgOAc)_2$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.98 (d, J = 8.7 Hz, 1H), 7.56 (d, J = 9.0 Hz, 1H), 7.20 (d, J = 8.4 Hz, 1H), 6.87 (d, J = 8.4 Hz, 1H), 6.64 (s, 1H), 3.49 (br m, 4H), 1.17 (br m, 6H). ^{13}C NMR spectral data could not be obtained due to the limited solubility of **1**-$(HgOAc)_2$ in common NMR solvents.

UV-vis and fluorescence measurements. Stock solutions of compound **1** and Nile Red were prepared as 5.0×10^{-4} M in methanol. Stock solutions of metal perchlorate were prepared as 1.0×10^{-2} M in water. The working solutions were prepared by adding different volumes of stock solutions of probe, metal perchlorates, and buffer, followed by dilution to 3.0 mL by

adding calculated amounts of methanol and water. The pH of the solution was fixed at 4.7 with an acetate buffer solution. The final concentrations of the measuring solution were [**1**] = 5.0×10^{-6} M, [M^{n+}] = 5.0×10^{-4} M, and [acetate buffer] = 1.0×10^{-2} M in a methanol-water solution (1:1, v/v) for UV-vis and fluorescence measurements (excitation wavelength = 580 nm). The measuring solution was thoroughly mixed and the absorption and fluorescence spectra were measured after 2 h of sample preparation. Fluorescence titration was carried out in a methanol-water (1:1, v/v) solution. Working solutions were prepared by adding 0 to 100 equiv of Hg^{2+} ions to the solution of **1** (5.0×10^{-6} M) and the fluorescence spectrum was measured with an excitation wavelength of 580 nm. Fluorescent titration of **1** with Hg^{2+} ions was also carried out similarly in the presence of physiologically relevant ions ([Na^+] = 138 mM, [K^+] = 4.0 mM, [Mg^{2+}] = 1.0 mM, [Ca^{2+}] = 3.0 mM, [Zn^{2+}] = 0.02 mM, and [Cu^{2+}] = 0.015 mM) as background.

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References

1. de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev*. **1997**, *97*, 1515.
2. Krämer, R. *Angew. Chem. Int. Ed.* **1998**, *37*, 772.
3. (a) Dujols, V.; Ford, F.; Czarnik, A. W. *J. Am. Chem. Soc*. **1997**, *119*, 7386. (b) Torrado, A.; Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc*. **1998**, *120*, 609. (c) Zheng, Y.; Huo, Q.; Kele, P.; Andreopoulos, F. M.; Pham, S. M.; Leblanc, R. M. *Org. Lett*. **2001**, *3*, 3277. (d) Martinez, R.; Espinosa, A.; Tarraga, A.; Molina, P. *Org. Lett*. **2005**, *7*, 5869. (e) Royzen, M.; Dai, Z.; Canary, J. W. *J. Am. Chem. Soc*. **2005**, *127*, 1612. (f) Yang, Y.-K.; Yook, K.-J.; Tae, J. *J. Am. Chem. Soc.* **2005**, *127*, 16760. (g) Xiang, Y.; Tong, A.; Jin, P.; Ju, Y. *Org. Lett.* **2006**, *8*, 2863. (h) Qi, X.; Jun, E. J.; Xu, L.; Kim, S.-J.; Hong, J. S.; Yoon, Y. J.; Yoon, J. *J. Org. Chem*. **2006**, *71*, 2881. (i) Martinez, R.; Zapata, F.; Caballero, A.; Espinosa, A.; Tarraga, A.; Molina, P. *Org. Lett*. **2006**, *8*, 3235. (j) Liu, J.; Lu, Y. *J. Am. Chem. Soc*. **2007**, *129*, 9838. (k) Zhan, X.-Q.; Qian, Z.-H.; Zheng, H.; Su, B.-Y.; Lan, Z.; Xu, J.-G. *Chem. Commun*. **2008**, 1859. (l) Lee, M. H.; Lee, S. W.; Kim, S. H.; Kang, C.; Kim, J. S. *Org. Lett*. **2009**, *11*, 2101. (m) Choi, M. G.; Kim, Y. H.; Namgoong, J. E.; Chang, S.-K. *Chem. Commun*. **2009**, 3560. (n) Hu, D.; Sheng, Z.; Gong, P.; Zhang, P.; Cai, L. *Analyst* **2010**, *135*, 1411. (o) Kim, H. J.; Kim, Y.; Kim, S. J.; Park, S. Y.; Lee, S. Y.; Kim, J. H.; No, K.; Kim, J. S. *Bull. Korean Chem. Soc*. **2010**, *31*, 230.
4. (a) Nolan, E. M.; Lippard, S. J. *J. Am. Chem. Soc*. **2003**, *125*, 14270. (b) Zeng, L.; Miller, E. W.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc*. **2006**, *128*, 10. (c) Que, E. L.; Chang, C. J. *J. Am. Chem. Soc*. **2006**, *128*, 15942. (d) Zheng, H.; Qian, Z. H.; Xu, L.; Yuan, F. F.; Lan, L. D.; Xu, J. G. *Org. Lett*. **2006**, *8*, 859. (e) Santra, M.; Ryu, D.; Chatterjee, A.; Ko, S.-K.; Shin, I.; Ahn, K. H. *Chem. Commun.* **2009**, 2115. (f) Yang, Y.-K.; Ko, S.-K.; Shin, I.; Tae, J. *Org. Biomol. Chem*. **2009**, *7*, 4590. (g) Jana, A.; Kim, J. S.; Jung, H. S.; Bharadwaj, P. K. *Chem. Commun.* **2009**, 4417. (h) Lin, W.; Cao, X.; Ding, Y.; Yuan, L.; Long, L. *Chem. Commun.* **2010**, 3529.
5. Nolan, E. M.; Lippard, S. J. *Chem. Rev.* **2008**, *108*, 3443.
6. Adams, S. R.; Campbell, R. E.; Gross, L. A.; Martin, B. R.; Walkup, G. K.; Yao, Y.; Llopis, J.; Tsien, R. Y. *J. Am. Chem. Soc*. **2002**, *124*, 6063.
7. Adams, S. R.; Tsien, R. Y. *Nat. Protoc.* **2008**, *3*, 1527.
8. Nakanishi, J.; Nakajima, T.; Sato, M.; Ozawa, T.; Tohda, K.; Umezawa, Y. *Anal. Chem*. **2001**, *73*, 2920.
9. Jose, J.; Burgess, K. *Tetrahedron* **2006**, *62*, 11021.
10. Jose, J.; Burgess, K. *J. Org. Chem.* **2006**, *71*, 7835.
11. Han, J.; Jose, J.; Mei, E.; Burgess, K. *Angew. Chem. Int. Ed*. **2007**, *46*, 1684.
12. Sun, C.; Yang, J.; Li, L.; Wu, X.; Liu, Y.; Liu, S. *J. Chromatogr. B* **2004**, *803*, 173.
13. Black, S. L.; Stanley, W. A.; Filipp, F. V.; Bhairo, M.; Verma, A.; Wichmann, O.; Sattler, M.; Wilmanns, M.; Schultz, C. *Bioorg. Med. Chem*. **2008**, *16*, 1162.
14. Diaz, G.; Melis, M.; Batetta, B.; Angius, F.; Falchi, A. M. *Micron* **2008**, *39*, 819.
15. Okamoto, A.; Tainaka, K.; Fujiwara, Y. *J. Org. Chem.* **2006**, *71*, 3592.
16. Briggs, M. S. J.; Bruce, I.; Miller, J. N.; Moody, C. J.; Simmonds, A. C.; Swann, E. *J. Chem. Soc. Perkin Trans. 1* **1997**, 1051.
17. Tatay, S.; Gaviña, P.; Coronado, E.; Palomares, E. *Org. Lett*. **2006**, *8*, 3857.
18. Shortreed, M.; Kopelman, R.; Kuhn, M.; Hoyland, B. *Anal. Chem.* **1996**, *68*, 1414.
19. (a) Hennrich, G.; Walther, W.; Resch-Genger, U.; Sonnenschein, H. *Inorg. Chem.* **2001**, *40*, 641. (b) Cho, Y.-S.; Ahn, K. H. *Tetrahedron Lett*. **2010**, *51*, 3852. (c) Do, J. H.; Kim, H. N.; Yoon, J.; Kim, J. S.; Kim, H.-J. *Org. Lett*. **2010**, *12*, 932.