CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.201001769

Near-Infrared Cell-Permeable Hg^{2+}-Selective Ratiometric Fluorescent Chemodosimeters and Fast Indicator Paper for $MeHg^{+}$ Based on Tricarbocyanines

Zhiqian Guo,[a] Weihong Zhu,*[a] Mingming Zhu,[b] Xumeng Wu,[a] and He Tian*[a]

Dedicated to Professor Jiacong Shen on the occasion of his 80th birthday

Abstract: Three tricarbocyanine dyes (IR-897, IR-877, and IR-925) with different thiourea substituents that function as dosimeter units through specific Hg^{2+}-induced desulfurization have been demonstrated in a fast indicator paper for Hg^{2+} and $MeHg^{+}$ ions. In comparison with available Hg^{2+}-selective chemodosimeters, IR-897 and IR-877 show several advantages, such as convenient synthesis, very long wavelengths falling in the near-infrared (NIR) region (650–900 nm) with high molar extinction coefficients, a ratiometric response, and quite low disturbance with Ag^{+} and Cu^{2+} ions. They exhibit large redshifts, which result in a clear color change from deep blue to pea green that can be easily monitored by the naked eye for a convenient indicator paper. In emission spectra, they display a characteristic turn-off mode at 780 nm and turn-on mode at 830 nm with titration of Hg^{2+} ions. Remarkably, the signal/noise (S/N) ratio with other thiophilic metal ions (Ag^{+} and Cu^{2+}) is greatly enhanced with ratiometric measurement of two channels: excitation spectra mode ($I_{810\,nm}/I_{670\,nm}$, monitored at 830 nm) and emission spectra mode ($I_{830\,nm}/I_{780\,nm}$, isosbestic absorption point at 730 nm as excitation). The distinct response is dependent upon the electron-donating effect of the thiourea substituents; that is, the stronger the electron-donating capability of the thiourea substituents, the faster the Hg^{2+}-promoted cyclization. Additionally, experiments with living SW1116 cells show that these three tricarbocyanine dyes with low toxicity can exhibit special characteristics that are favorable for visualizing intracellular Hg^{2+} and $MeHg^{+}$ ions in biological systems, including excellent membrane permeability, minimal interfering absorption and fluorescence from biological samples, low scattering, and deep penetration into tissues.

Keywords: chemodosimeters · fluorescence · mercury · sensors · tricarbocyanines

Introduction

Mercury (Hg^{2+}) ions, as one of the most toxic metal pollutants, are widespread in air, water, and soil.[1] Even more serious is the fact that mercury ions can be converted into organomercury and accumulated, mainly as methylmercury ($MeHg^{+}$) ions, in the human body. As a strong neurotoxin, methylmercury ions, which are mainly derivatives from the daily diet of fish and sea mammals, affect the central nervous system to cause prenatal brain damage, various cognitive and motion disorders, and Minamata disease.[2] Therefore, considerable efforts have been made to develop selective and efficient methods[3] to monitor and perform trace imaging of Hg^{2+} ions,[4] particularly fluorescence imaging of $MeHg^{+}$ ions in living cells and organisms.[5,6] Recently, cyanine dyes have become the focus of numerous analytical, biological, and especially in vivo fluorescence imaging,[7] because the near-infrared (NIR) region at around 650–900 nm exhibits special advantages including minimal interfering absorption and fluorescence from biological samples, low scattering, and deep penetration into tissues.[8,9] Among cyanine dyes, tricarbocyanines with a rigid chlorocyclohexenyl or

[a] Z. Q. Guo, Prof. Dr. W. H. Zhu, X. M. Wu, Prof. Dr. H. Tian
Key Laboratory for Advanced Materials and
Institute of Fine Chemicals
East China University of Science & Technology
Shanghai 200237 (P.R. China)
Fax: (+86) 21-6425-2758
E-mail: whzhu@ecust.edu.cn
tianhe@ecust.edu.cn

[b] M. M. Zhu
Department of Gastroenterology, Renji Hospital
Shanghai Jiao Tong University, School of Medicine
Shanghai Institute of Digestive Disease, Shanghai 200001 (P.R. China)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201001769.

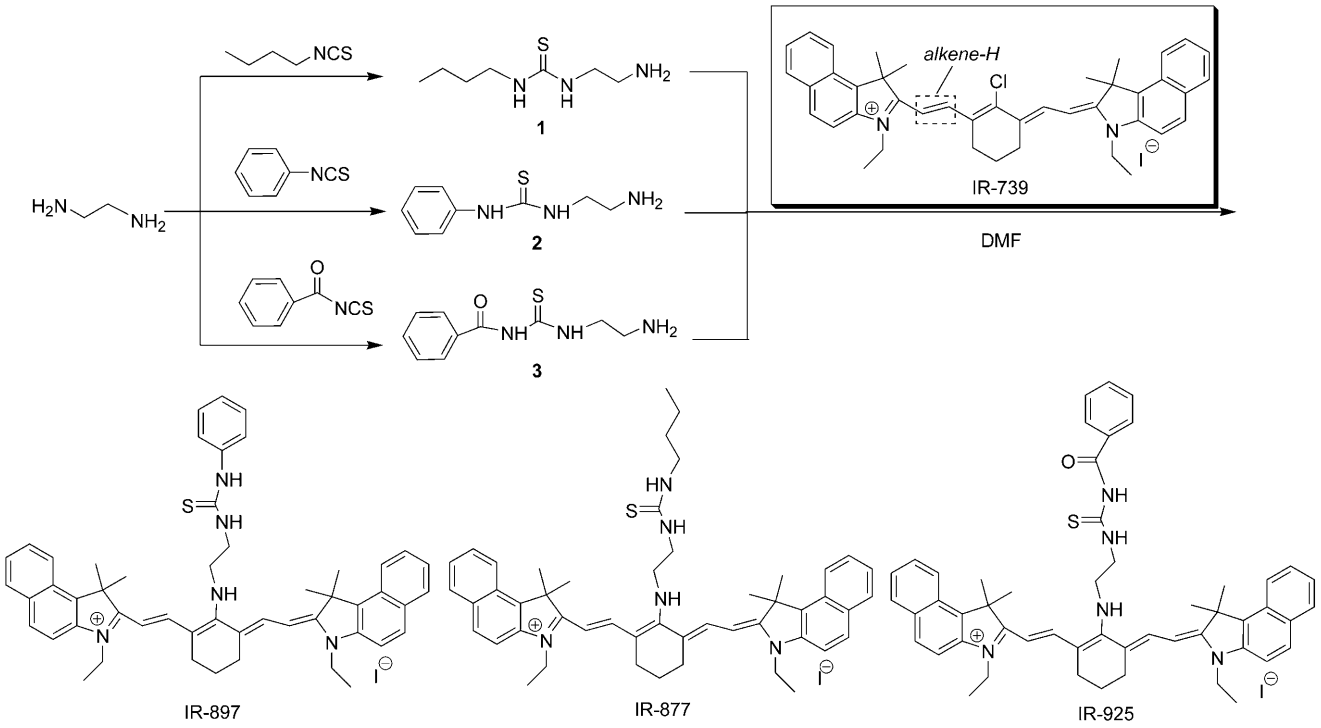
WILEY ONLINE LIBRARY
© 2010 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

chlorocyclopentenyl ring in the methine chain can increase the photostability, enhance the fluorescence quantum yield, and provide an ideal site for further modification with amino substitution.[10]

Chemodosimeters have great advantages in selectivity and sensitivity over other sensing strategies by utilization of a specific chemical reaction (usually irreversible) between guest molecules and target species.[11] They can perform a chemical reaction with a specific target species (usually a metal ion) that leads to the formation of a fluorescent or colored product displaying a unique spectroscopic change.[12] Indeed, several selective chemodosimeters for Hg^{2+} ions in water or organic solvents have been exploited on the basis of irreversible mercury-promoted desulfurization reactions, including hydrolysis, cyclization, and elimination reactions that stem from the strong thiophilic affinity of Hg^{2+} ions.[12] To the best of our knowledge, however, no study has taken these advantages for the development of a cyanine-based NIR colorimetric and ratiometric chemodosimeter for monitoring Hg^{2+} ions or organomercury compounds. Inspired by the chemodosimeters for Hg^{2+} ions and the principle of amino-substituted tricarbocyanine dyes as NIR fluorescence labels,[12,13b,14] we report herein three tricarbocyanine-derivative dyes (IR-897, IR-877, and IR-925; Scheme 1) with different thiourea substituents as Hg^{2+}-dosimeter units through a specific Hg^{2+}-induced desulfurization. The electron-donating effect of the chemodosimetric groups in the three tricarbocyanine dyes is determined, and it is found that the stronger the electron-donating capability of the thiourea substituents, the faster the Hg^{2+}-promoted cyclization. Remarkably, with Hg^{2+} ions, IR-877 and IR-897 exhibit large redshifts in absorption (176 and 162 nm, respectively) to fully meet "naked-eye" colorimetric changes, and they have been successfully developed as Hg^{2+}- and $MeHg^{+}$-ion indicator papers. Moreover, by monitoring either a single emission with two excitation sources (excitation spectra mode) or dual emission with an isosbestic absorption point as single excitation source, these tricarbocyanines can be successfully constructed as NIR ratiometric Hg^{2+} and $MeHg^{+}$ chemodosimeters, which allow for fast and accurate measurements with elimination of the influence of dye concentration and microenvironmental fluctuations in pH value, refractive index, and photobleaching.[13b,15] The above strategy of grafting the chemodosimeter unit into a tricarbocyanine provides a fascinating approach to the design of NIR chemodosimeters.

Results and Discussion

Synthetic strategy and characterization: The synthesis of the three tricarbocyanine dyes IR-877, IR-897, and IR-925 is depicted in Scheme 1. In order to gain insight into the role of the dosimetric groups for desulfurization, three thiourea derivative units were obtained by the reaction of ethylenediamine with isothiocyanatobenzene, *N*-butyl isothiocyanate, and benzoyl isothiocyanate, respectively. Finally, the target tricarbocyanine dyes were prepared from treatment of the precursor chlorocyclohexenyl-substituted cyanine dye (IR-739) with the thiourea derivatives in DMF, with a yield of



Scheme 1. Synthetic routes to IR-897, IR-877, and IR-925.

 © 2010 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

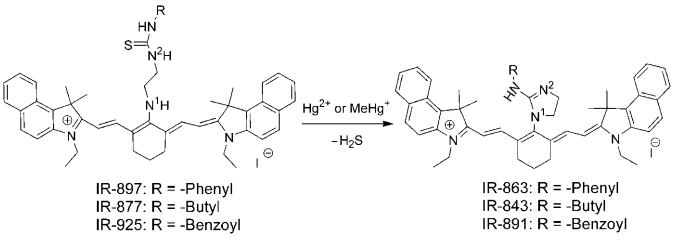
about 30%. Their chemical structures were fully characterized by ^{1}H NMR and ^{13}C NMR spectroscopy and by HRMS (see the Experimental Section). In an analysis of the ^{1}H NMR spectra of the chlorocyclohexenyl-substituted tricarbocyanine dye IR-739 and the aminocyclohexenyl-substituted tricarbocyanine dye IR-897, the chemical shifts of the alkene H atoms in the methine chain show significant upfield-shift from $\delta = 6.24$ and 8.48 ppm with IR-739 to $\delta =$ 5.62 and 7.77 ppm with IR-897. The same tendency is also observed with IR-877 and IR-925 (Table 1). The significant changes in the chemical shifts of the alkene H atoms are indicative of a reassignment in the electron distribution of the tricarbocyanine dye with an intramolecular charge-transfer (ICT) process.

Table 1. Photophysical characteristics of tricarbocyanine dyes in a mixture of methanol/water (80:20).

Dye	λ_{ab} [nm]	λ_{ab}[a] [nm]	$\Delta\lambda_{ab}$ [nm]	λ_{em}[b] [nm]	ε[c] ($\times 10^5$)	Φ[d]	δ (alkene *H*) [ppm]
IR-739	818	–	–	830	3.50	0.51	6.24, 8.48
IR-877	664	840	176	780	0.566	1.00	5.60, 7.73
IR-897	668	830	162	780	0.876	0.78	5.62, 7.77
IR-925	670	830	160	780	0.900	0.63	5.62, 7.85

[a] Wavelength of the absorption peak after addition of Hg^{2+} ions. [b] Wavelength of the emission peak. [c] Molar extinction coefficients [$M^{-1}cm^{-1}$] corresponding to the absorption band with no Hg^{2+} ion. [d] Relative quantum yields, determined by using IR-877 ($\Phi = 1.00$) as a reference.

Colorimetric detection of Hg^{2+} and $MeHg^+$ ions: In comparison with the absorption peak of precursor dye IR-739 located at $\lambda = 818$ nm (Figure S1 in the Supporting Information), IR-897 shows a very intense new band at $\lambda = 668$ nm ($\varepsilon = 8.76 \times 10^4\,M^{-1}cm^{-1}$) with a large blueshift of about 150 nm (Table 1), which could arise from an efficient excited-state ICT process from the donor (N^1, the secondary nitrogen atom linking with cyclohexenyl group; Scheme 2) to



Scheme 2. Mercury-promoted desulfurization reactions.

the acceptor (tricarbocyanine group).[14] As expected, IR-897 and IR-877 can be easily transformed into guanidine derivatives upon titration of Hg^{2+} ions,[16] which results in distinct color changes in a mixture of methanol/water (80:20) at room temperature (Figure 1 A and B). With an increasing concentration of Hg^{2+} ions into the IR-897 solution (10 μM), the absorption peak at $\lambda = 668$ nm decreased sharply and a

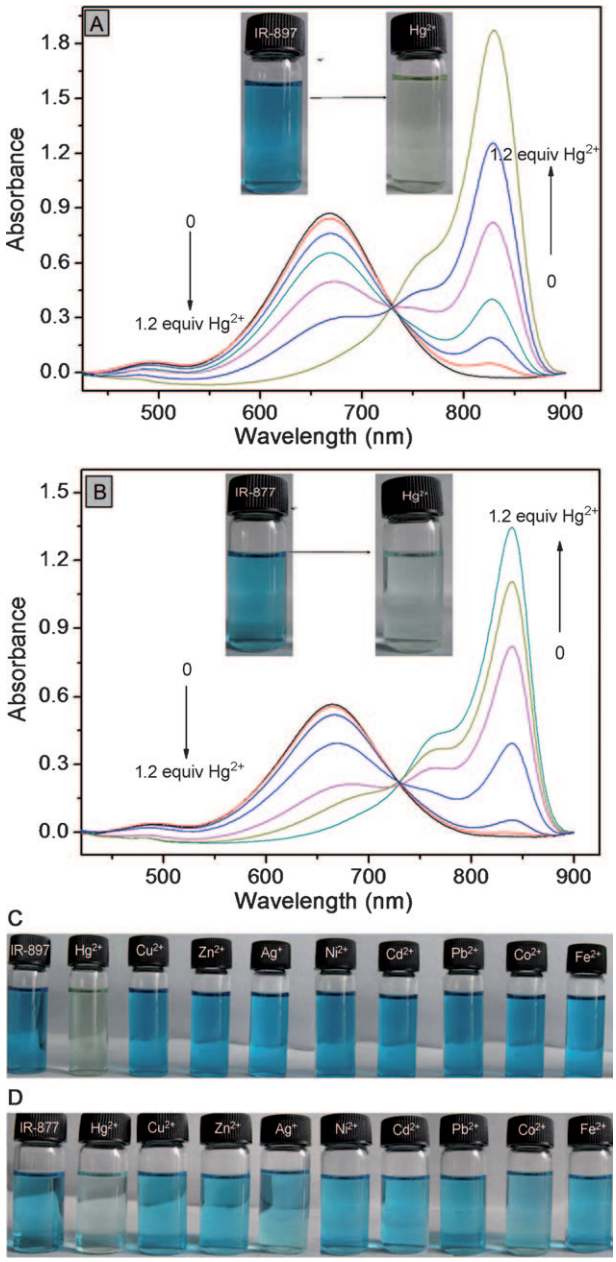


Figure 1. Changes in the absorption spectra of A) IR-897 (10 μM) and B) IR-877 (10 μM) upon titration of $HgCl_2$ (0, 2, 4, 6, 8, 9, 10, and 12 μM) in a mixture of methanol/water (80:20). Color changes of C) IR-897 (10 μM) and D) IR-877 (10 μM) in the presence of Hg^{2+} ions (10 μM) and other metal ions (30 μM). From left to right: IR-897 or IR-877 only, with Hg^{2+}, Cu^{2+}, Zn^{2+}, Ag^+, Ni^{2+}, Cd^{2+}, Pb^{2+}, Co^{2+}, and Fe^{2+} ions. All spectra were acquired 15 min after addition of the metal ions.

new band centered at $\lambda = 830$ nm ($\varepsilon = 1.87 \times 10^5\,M^{-1}cm^{-1}$) increased prominently with an isosbestic point at $\lambda = 730$ nm (Figure 1 A). The isosbestic point at $\lambda = 730$ nm clearly revealed that only two species coexist; these correspond to free IR-897 and desulfurization-product IR-863 (Scheme 2). In the cyclization process, the central bridging secondary amine (N^1H) of the tricarbocyanine is transformed into an imidazoline tertiary amine. This results in a decrease of electron-donating capability, so the efficiency of the excited-

© 2010 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

state ICT process from the N^1 atom to the acceptor tricarbocyanine was sharply diminished, and a large redshift in the absorption spectrum with visible color changes was thus observed (Figure 1).

As a consequence, IR-897, upon addition of Hg^{2+} ions, exhibits an extremely large redshift of about 162 nm in absorption and fully meets "naked-eye" colorimetric changes. Moreover, IR-897 also displays the same redshift from $\lambda = 668$ to 830 nm upon titration with methylmercury ions (Figure 2). Generally, colorimetric NIR sensors with distinct color changes have the advantage of a straightforward detection manner. As shown in Figure S2 in the Supporting Information, visible color changes were observed when test paper sheets adsorbed with IR-897 were dipped in $MeHg^+$ or Hg^{2+} solutions of various concentrations. Consequently, the first fast qualitative indicator paper for $MeHg^+$ and Hg^{2+} ions has been demonstrated.

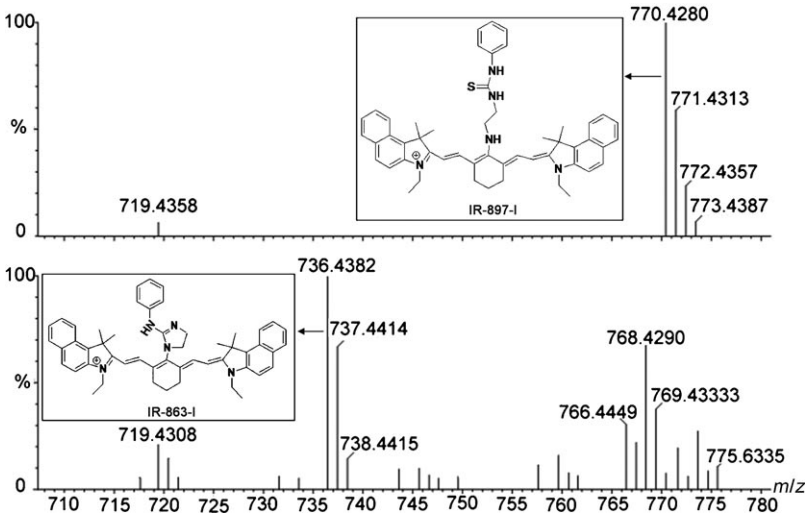


Figure 3. HR EI mass spectra of IR-897 and IR-897 with the addition of Hg^{2+} ions.

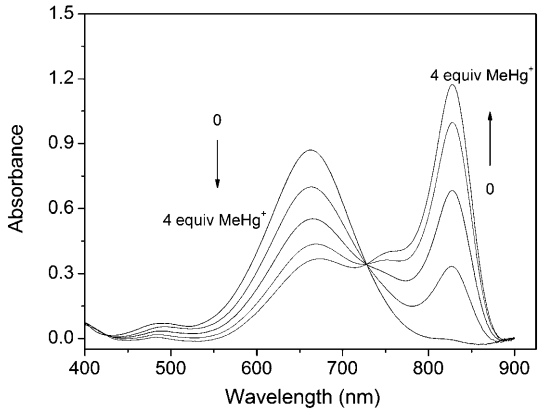


Figure 2. Changes in the absorption spectra of IR-897 (10 μM) upon titration of $MeHg^+$ (0, 1, 2, 3, and 4 equiv) in a mixture of methanol/water (80:20). All spectra were acquired 15 min after addition of the metal ions.

According to the linear Benesi–Hildebrand expression,[17] the measured absorbance $1/(A-A_0)$ at $\lambda = 668$ nm varies as a function of $1/[Hg^{2+}]$ in a linear relationship, which indicates 1:1 stoichiometry in the mercury-promoted cyclic guanylation of IR-897. The formation of 1:1 stoichiometry between an Hg^{2+} ion and IR-897 was also confirmed by high-resolution ESI-MS analysis (Figure 3). The peak at m/z 770.4280 (calcd as 770.4256) corresponded to [IR-897−I], and the peak at m/z 736.4382 (calcd as 736.4379) was assigned to the desulfurized product [IR-863−I]. Moreover, ^1H NMR studies provided further evidence consistent with the Hg^{2+}-promoted intramolecular cyclic guanylation of the thiourea moiety. Significant downfield shifts of the alkene protons in the methine chain were observed from $\delta = 5.62$ and 7.77 ppm to $\delta = 6.11$ and 8.40 ppm (Figure 4). The original three broad peaks at $\delta = 9.65$, 9.07, and 8.34 ppm that were assigned to the NH groups of IR-897 disappear upon addition of the Hg^{2+} ion, due to the intramolecular cyclic guanylation of the thiourea moiety. Similarly, the two groups of CH_2 peaks were downfield shifted from $\delta = 3.99$ and 4.15 ppm to $\delta = 4.36$ and 4.58 ppm.

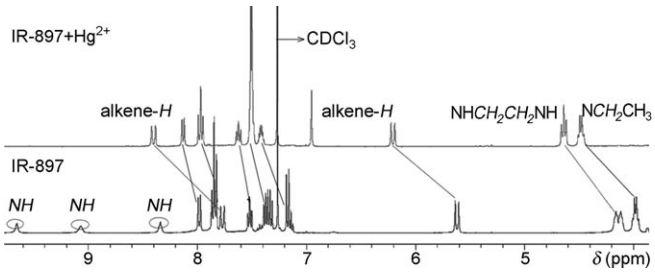


Figure 4. Partial ^1H NMR spectra for IR-897 showing the changes before and after the addition of Hg^{2+} ions.

NIR fluorescence ratiometric detection: The fluorometric detection of Hg^{2+} ions with different excitation wavelengths was also examined. As shown in Figure 5A, two different wavelengths ($\lambda = 670$ and 810 nm), corresponding to excitation of IR-897 and the cyclic guanylation product IR-863, were selected as the excitation wavelengths. Upon titration of IR-897 with Hg^{2+} ions, a distinct decrease was observed in the fluorescence intensity at $\lambda = 780$ nm ($I_{780\,nm}$) for IR-897 upon excitation at $\lambda = 670$ nm, until it was almost completely quenched to the baseline (Figure S4 in the Supporting Information). In contrast, upon excitation at $\lambda = 810$ nm,

 © 2010 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

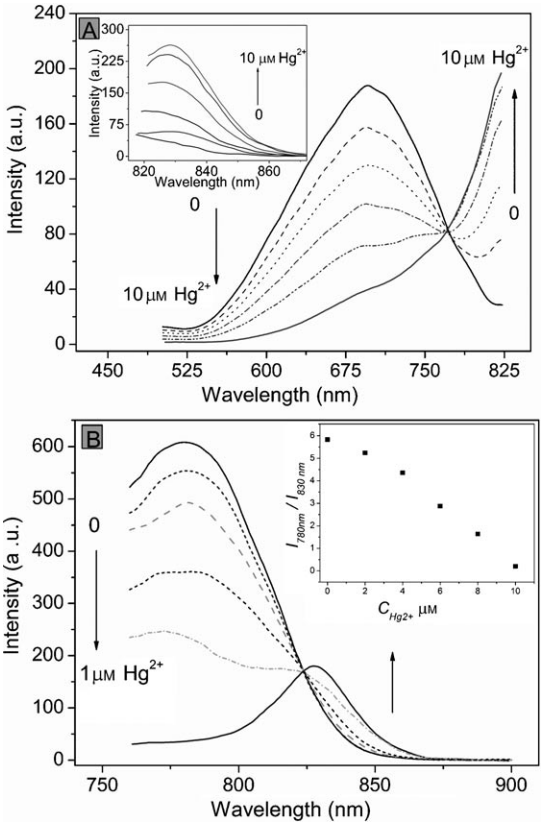


Figure 5. A) Excitation spectra of IR-897 (10 μM) in the presence of Hg^{2+} ions at concentrations of 0, 2, 4, 6, 8, and 10 μM, monitored at $\lambda = 830$ nm in a mixture of methanol/water (80:20). Inset: Emission spectra of IR-897 (10 μM) upon titration of Hg^{2+} ions. $\lambda_{ex} = 810$ nm. B) Emission spectra of IR-897 (1 μM) in the presence of Hg^{2+} ions at concentrations of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 μM, excited at $\lambda = 730$ nm, the isosbestic point of the absorption spectra, in a mixture of methanol/water (80:20). Inset: Fluorescence intensity ratio ($I_{780\,nm}/I_{830\,nm}$) of IR-897 as a function of Hg^{2+} concentration.

a "turn-on" fluorescence was observed at $\lambda = 830$ nm ($I_{830\,nm}$); this result corresponds to the mercury-promoted cyclic guanylation product IR-863 (Figure 5A, inset). The detection limit of IR-897 for Hg^{2+} ions was evaluated at the nanomolar level by monitoring the fluorescence titration curves of IR-897 (10^{-7} M) with Hg^{2+} ions. Moreover, a similar fluorescence phenomenon was also observed with the titration of Hg^{2+} ions with IR-877.

Ratiometric measurements that involve the ratio of absorbance or emission intensity at two different wavelengths can increase the selectivity and sensitivity to avoid troublesome self-calibrations and fluctuations in sensor concentration.[12c, m, 13b, 15] In this case, the ratiometric function can be built with two channels. As illustrated with IR-897, with two excitation sources at $\lambda = 670$ and 810 nm, corresponding to the absorption peaks of IR-897 and the cyclic guanylation product IR-863, we use the excitation spectra mode with monitoring at 830 nm to obtain the ratiometric function from the fluorescence ratio ($I_{810\,nm}/I_{670\,nm}$; Figure 5A). Another channel is to utilize a single excitation source with the wavelength of the isosbestic absorption point at $\lambda = 730$ nm. This results in a fluorescence ratio based on dual emission at $\lambda = 780$ and 830 nm ($I_{830\,nm}/I_{780\,nm}$), corresponding to the fluorescence peaks of IR-897 and IR-863 (Figure 5B). Clearly, the excitation wavelength of the isosbestic point ($\lambda = 730$ nm) and the emission wavelengths ($\lambda = 780$ and 830 nm) of IR-897 and IR-863 fall within the desirable NIR region; this offers distinct advantages for both in vitro and in vivo biological applications, in that absorption and emission maxima in the NIR region can avoid strong interference from UV-induced phototoxicity/autofluorescence and sensor-induced interference encountered in the shorter wavelength region.[7,9] Similarly, IR-877 can also be built as a ratiometric NIR Hg^{2+} chemodosimeter.

The selectivity for a specific analyte over other metal ions is an important feature of chemodosimeters. The specific selectivities of IR-897 and IR-877 toward Hg^{2+} ions were investigated, and these analytes displayed a remarkably high response for Hg^{2+} ions over other metal cations. As shown in Figure 1, no significant changes in the color of the IR-897 solutions were observed after adding a variety of metal ions: Cu^{2+}, Zn^{2+}, Ag^{+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Co^{2+}, and Fe^{2+}. Also, the dependence of the fluorescence ratios at $\lambda = 670$ and 810 nm ($I_{810\,nm}/I_{670\,nm}$) upon monitoring at 830 nm with various metal ions was measured (Figure 6A). Clearly, except for the Hg^{2+} ions ($I_{810\,nm}/I_{670\,nm} = 7.4$), the fluorescence ratios of the various metal cations ($I_{810\,nm}/I_{670\,nm} < 0.25$) can be neglected; this includes biologically active metal ions (Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+}), trace metals in organisms (Cu^{2+}, Zn^{2+}, Ag^{+}, Ni^{2+}, Co^{2+} Mn^{2+}, Fe^{3+}, and Fe^{2+}), and the prevalent toxic transition-metal cations (Cd^{2+} and Pb^{2+}). In particular, the selectivity for Hg^{2+} ions over Ag^{+} and Cu^{2+} ions becomes more distinct with the fluorescence-ratio strategy. As a matter of fact, the signal/noise (S/N) value in the fluorescence response with Hg^{2+} ions over Ag^{+} and Cu^{2+} ions increases drastically from 12 and 15 times (by using the ratio of fluorescence intensity and only monitoring at 780 nm; Figure 6B) to 30 and 40 times (by using the ratiometric method; Figure 6B), respectively. Similarly, in the absorption and emission spectra of IR-877, the high selectivity for Hg^{2+} ions over other metal ions, including the thiophilic Ag^{+} and Cu^{2+} metal ions, was also realized. Therefore, both IR-877 and IR-897 exhibit high sensitivity and selectivity for Hg^{2+} ions as NIR ratiometric fluorescence chemodosimeters.

Substituent effect on response: The effect of the thiourea substituents (Scheme 1) upon the recognition of Hg^{2+} ions was further studied. IR-877 exhibits a similar photochemical change to that of IR-897 upon addition of Hg^{2+} ions. As illustrated in Figure 1B, the band centered at $\lambda = 664$ nm shifts to $\lambda = 840$ nm with one isosbestic point at $\lambda = 730$ nm. A large redshift (176 nm) with visible color changes from dark blue to pea green is also observed (Figure 1B, inset). Not only does the measuring absorbance $1/(A - A_0)$ at $\lambda = 664$ nm vary as a function of $1/[Hg^{2+}]$ in a linear relationship, but also ESI MS analysis of IR-877 with Hg^{2+} ions confirms a 1:1 reaction stoichiometry (Figure S3 and S8 in

 © 2010 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

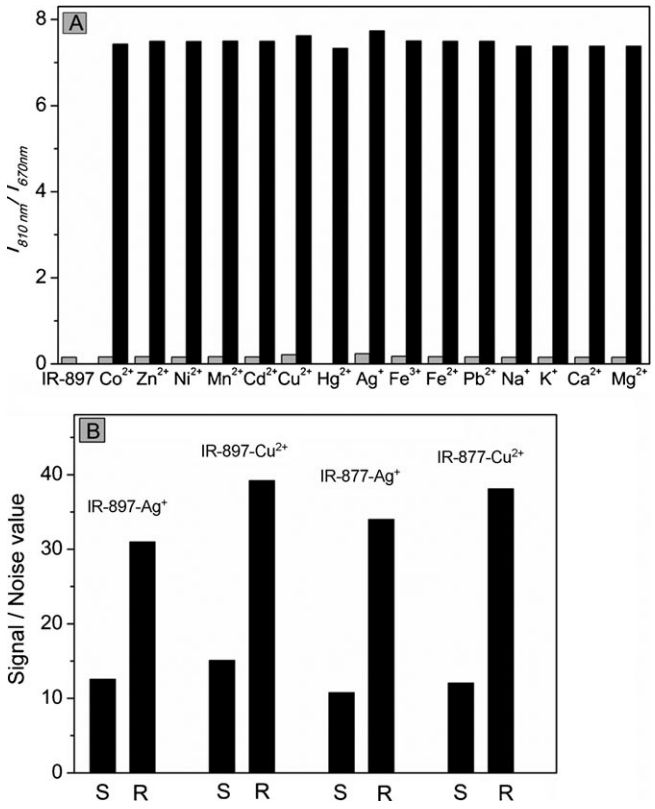


Figure 6. A) Fluorescence response of IR-897 (10 μM) in the presence of Hg^{2+} ions (10 μM) with various other metal ions (30 μM) in a mixture of methanol/water (80:20). The bars represent the fluorescence intensity ratio at $\lambda = 670$ and 810 nm ($I_{810\,nm}/I_{670\,nm}$) when monitored at 830 nm (excitation spectra mode). Gray bars: each cation was added. Black bars: each cation and Hg^{2+} ions were added. B) The values of signal/noise represent the changes in the absence and presence of Hg^{2+} ions (10 μM) with IR-897 and IR-877 (10 μM) compared with those of Ag^{+} (30 μM) and Cu^{2+} ions (30 μM); S: results of the method based on the fluorescence intensity change at 780 nm (excited at 670 nm); R: results of the ratiometric method monitored at 830 nm ($I_{670\,nm}/I_{810\,nm}$) based on the excitation mode.

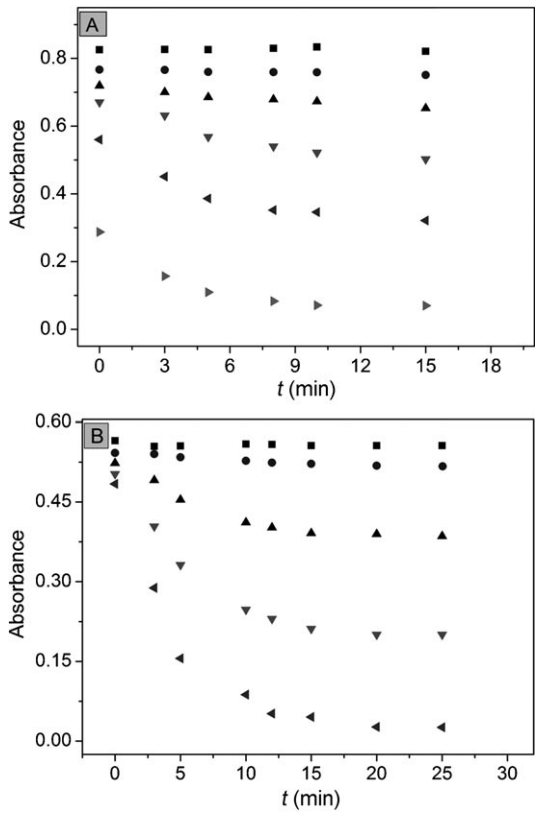


Figure 7. Absorbance response of A) IR-897 (10 μM) monitored at 668 nm and B) IR-877 (10 μM) monitored at 664 nm over time with the addition of Hg^{2+} ions (■: 2, ●: 4, ▲: 6, ▼: 8, ◀: 10, ▶: 12 μM)in a mixture of methanol/water (80:20).

the Supporting Information). In the ESI MS spectra, the peak at *m*/*z* 750.4553 (calcd as 750.4569) corresponds to [IR-877−I] and the peak at *m*/*z* 716.4659 (calcd as 716.4692) is assigned to a desulfurized product ($-H_2S$) [IR-843−I].

Unexpectedly, IR-925, which contains the electron-withdrawing group of a benzoyl substituent, displayed almost no response as the dosimeter unit within a reasonable time scale upon addition of Hg^{2+} ions (20 μM; Figure S9 in the Supporting Information). Even if the time period was extended to 24 h, the spectral changes of IR-925 with Hg^{2+} ions could be ignored with respect to those of IR-897 and IR-877. The kinetics of the Hg^{2+}-prompted chemodosimetric cyclization was studied by analysis of the response time towards Hg^{2+} ions with different thiourea substituents. The results reveal that the cyclization reaction of IR-897 (10 μM) with Hg^{2+} ion (12 μM) almost reaches completion within 5 min, as recorded by absorbance changes at $\lambda = 668$ and 830 nm and emission changes at $\lambda = 780$ nm (Figure 7A and Figure S10 in the Supporting Information). Similarly, the response of IR-877 is complete within about 12 min, as determined from the absorbance changes at $\lambda = 664$ and 840 nm, and emission changes at $\lambda = 780$ nm (Figure 7B and Figure S11 in the Supporting Information). However, establishment of the equilibrium of the reaction of IR-925 (10 μM) with Hg^{2+} ions (20 μM) takes over 24 h. The large difference in the response times is related to the corresponding substituents. Compared with the butyl substituent group in IR-877, the phenyl group in IR-897 shows a larger conjugated system with the thiourea unit and results in more electron richness for the sulfur atom of the thiourea unit. This prompts the cyclization reaction of IR-897 to occur quicker than that of IR-877. The electron-withdrawing properties of the benzoyl group in IR-925 distinctly decrease the electron density of the sulfur atom in the thiourea unit and make the desulfurized cyclization of IR-925 induced with Hg^{2+} ions much slower than those of IR-877 and IR-897. Consequently, the distinct responses of three tricarbocyanine dyes with Hg^{2+} ions are dependent upon the electron-donating effects of the thiourea substituents: the stronger the electron-donating ability of the thiourea substituents, the faster the Hg^{2+}-promoted cyclization.

Detection of intracellular Hg^{2+} ions with three tricarbocyanines: To further investigate the practical application of the

 © 2010 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

three tricarbocyanine dyes in living cells, human colon cancer cells (SW1116) were selected for our experiments. As mentioned above, the excellent chemical and spectroscopic properties of the tricarbocyanine dyes with Hg^{2+} ions in a mixture of methanol/water (80:20) indicate that IR-897 and IR-877 should be suitable to probe for Hg^{2+} ions in living cells. Brightfield-image measurement confirms that the cells are viable throughout the imaging studies (Figure S12a, S12d, and S12g in the Supporting Information). After treatment of SW1116 cells with IR-897, IR-877, and IR-925 (5 μM) alone for 20 min at 37 °C, the cells display strong fluorescence (Figure S12b, S12e, and S12h in the Supporting Information). Upon addition of Hg^{2+} ions (10 μM) and incubation for another 20 min at 37 °C, the SW1116 cells show a very weak fluorescence signal, which indicates that the strong red fluorescence is almost quenched by the cyclization reaction of IR-897 and IR-877 with intracellular Hg^{2+} ions (Figure S12c and S12f in the Supporting Information). In contrast, the cells treated with IR-925 and incubated with Hg^{2+} ions exhibit an almost negligible decrease in fluorescence intensity (Figure S12i in the Supporting Information). The results in living cells are in accordance with the investigation of the three tricarbocyanine dyes in solution, which showed a distinct response dependence upon the thiourea substituents. The above cell experiments also reveal that the three tricarbocyanine dyes have excellent membrane permeability. Thus, IR-877 and IR-897 can be expected to function as novel cell-permeable NIR Hg^{2+}-selective chemodosimeters in living cells by fluorescence microscopy.

Conclusion

Novel Hg^{2+}-selective NIR fluorescent chemodosimeters have been successfully designed by modifying an amine-substituted tricarbocyanine chromophore with dosimeter units. The distinct response is dependent upon the electron-donating effect of the thiourea substituents and reveals that the stronger the electron-donating ability of the thiourea substituents, the faster the Hg^{2+}-promoted cyclization. Two ratiometric strategies were performed to enhance the signal/noise (*S*/*N*) value; these were dependent upon the two modes of excitation spectra ($I_{810\,nm}/I_{670\,nm}$, monitored at 830 nm) and emission spectra ($I_{830\,nm}/I_{780\,nm}$, isosbestic absorption point at 730 nm as excitation). The long-wavelength NIR-region (650–900 nm) results with the ratiometric mode can greatly increase the selectivity and sensitivity and can avoid the troublesome self-calibration and fluctuations in sensor concentration. The excellent membrane permeability and low toxicity with cells are favorable for visualizing intracellular for Hg^{2+} and $MeHg^{+}$ ions in biological systems. Our approach can offer more convenient colorimetric and ratiometric NIR chemodosimeters for Hg^{2+} and organomercury ions for application in biological systems.

Experimental Section

Materials and methods: All solvents were of analytical grade. The intermediate of IR-739 was prepared by the established literature procedure. ^{1}H NMR and ^{13}C NMR spectra in $CDCl_3$ were measured on a Brücker AV-400 spectrometer with tetramethylsilane (TMS) as an internal standard. Electrospray-ionization high-resolution mass spectra were measured on a Micromass LCT instrument under standard conditions. UV/Vis spectra were obtained by using a Varian Cary 500 spectrophotometer (1 cm quartz cell) at 25 °C. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer (1 cm quartz cell) at 25 °C, and all fluorescence spectra were uncorrected by the photo-multiplier tube response. The width of the slit was 5 nm. Deionized water was used to prepare all aqueous solutions. Stock solutions of Hg^{2+}, Cu^{2+}, Fe^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+} ions were prepared from their chloride salts; solutions of Cd^{2+}, Pb^{2+}, and Ag^{+} ions were prepared from their nitrate salts. All spectroscopic measurements were performed in a mixture of methanol/water (80:20).

Synthesis of IR-877: IR-739 was synthesized by a general method.[18] IR-739 (1.0 g, 1.35 mmol) and **1** (0.46 g, 2.66 mmol) were dissolved in anhydrous DMF (50 mL). The mixture was stirred at 80–90 °C for 10 h under an argon atmosphere. The solvent was removed under reduced pressure and then the crude product was purified by silica gel chromatography with dichloromethane/methanol (20:1) to afford the desired product as a deep blue solid (350 mg): Yield 30%; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): $\delta = 0.93$ (t, $J = 7.2$ Hz, 3H, $NH(CH_2)_3CH_3$), 1.40 (t, $J = 7.0$ Hz, 8H, NCH_2CH_3, $NHCH_2CH_2CH_2CH_3$), 1.65 (t, $J = 8.0$ Hz, 2H, $NHCH_2CH_2CH_2CH_3$), 1.88 (t, $J = 6.2$ Hz, 2H, cyclohexane H), 1.99 (s, 12H, CH_3), 2.55 (t, $J = 6.2$ Hz, 4H, cyclohexane H), 3.60 (m, 2H, $NHCH_2CH_2CH_2CH_3$), 3.98–4.08 (m, 8H, $NHCH_2CH_2NH$, $NHCH_2CH_3$), 5.60 (d, $J = 13.0$ Hz, 2H, alkene H), 7.18 (d, $J = 8.8$ Hz, 2H, Ph H), 7.37 (t, $J = 7.2$ Hz, 2H, Ph H), 7.55 (t, $J = 7.2$ Hz, 2H, Ph H), 7.73 (d, $J = 13.0$ Hz, 2H, alkene H), 7.85 (t, $J = 8.8$ Hz, 4H, Ph H), 8.10 (d, $J = 8.6$ Hz, 2H, Ph H), 8.63 (br, 1H, NH), 8.81 ppm (br, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): $\delta = 11.81$, 13.90, 14.13, 20.26, 21.02, 22.64, 26.36, 28.46, 31.24, 31.57, 38.21, 44.85, 49.59, 52.91, 93.15, 109.43, 119.69, 122.02, 123.78, 127.39, 128.62, 129.52, 130.02, 130.82, 131.51, 136.94, 139.89, 168.37, 169.34 ppm; HRMS (TOF-ESI$^+$): *m*/*z*: calcd for $C_{49}H_{60}N_5S^+$: 750.4569 [$M-I^-$]; found: 750.4553.

Synthesis of IR-897: The reaction was performed with IR-739 (1.0 g, 1.35 mmol) and **2** (0.33 g, 1.7 mmol) by using the same method as that for IR-877. A blue solid was obtained (0.5 g): Yield 42%; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): $\delta = 1.40$ (t, $J = 7.2$ Hz, 6H, NCH_2CH_3), 1.88 (t, $J = 6.2$ Hz, 2H, cyclohexane H), 1.94 (s, 12H, CH_3), 2.54 (t, $J = 6.2$ Hz, 4H, cyclohexane H), 3.99 (q, $J = 7.2$ Hz, 4H, $NHCH_2CH_3$), 4.12 (t, $J = 4.8$ Hz, 2H, $NHCH_2CH_2NH$), 4.15 (t, $J = 4.8$ Hz, 2H, $NHCH_2CH_2NH$), 5.62 (d, $J = 13.0$ Hz, 2H, alkene H), 7.12–7.18 (m, 3H, Ph H), 7.33 (t, $J = 8.0$ Hz, 2H, Ph H), 7.38 (t, $J = 8.0$ Hz, 2H, Ph H), 7.52 (t, $J = 7.2$ Hz, 2H, Ph H), 7.77 (d, $J = 13.0$ Hz, 2H, alkene H), 7.82–7.86 (m, 6H, Ph H), 7.98 (d, $J = 8.5$ Hz, 2H, Ph H), 8.34 (br, 1H, NH), 9.07 (br, 1H, NH), 9.65 ppm (br, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): $\delta = 11.84$, 14.10, 21.18, 22.63, 26.05, 28.48, 38.32, 44.30, 49.75, 51.74, 93.50, 109.40, 119.81, 122.12, 123.59, 123.87, 124.67, 127.42, 128.39, 128.58, 129.75, 130.00, 130.89, 131.70, 137.47, 139.30, 139.75, 168.90, 182.30 ppm; HRMS (TOF-ESI$^+$): *m*/*z*: calcd for $C_{51}H_{56}N_5S^+$: 770.4256 [$M-I^-$]; found: 770.4280.

Synthesis of IR-925: The reaction was performed with IR-739 (1.0 g, 1.35 mmol) and **3** (0.4 g, 1.8 mmol) by using the same method as that for IR-877. A blue solid was obtained (0.40 g): Yield 32%; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): $\delta = 1.40$ (t, $J = 7.0$ Hz, 6H, NCH_2CH_3), 1.86 (t, $J = 6.2$ Hz, 2H, cyclohexane H), 1.98 (s, 12H, CH_3), 2.55 (t, $J = 6.2$ Hz, 4H, cyclohexane H), 3.99 (m, 4H, $NHCH_2CH_3$), 4.01 (t, 2H, $NHCH_2CH_2NH$), 4.19 (t, 2H, $NHCH_2CH_2NH$), 5.62 (d, $J = 13.0$ Hz, 2H, alkene H), 7.18 (t, $J = 8.5$ Hz, 2H, Ph H), 7.37 (t, $J = 7.2$ Hz, 2H, Ph H), 7.51–7.58 (m, 5H, Ph H), 7.82–7.88 (m, 6H, Ph H), 8.04 (d, $J = 8.0$ Hz, 2H, Ph H), 8.38 (d, $J = 7.0$ Hz, 2H, Ph H), 9.18 (br, 1H, NH), 9.27 ppm (br, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): $\delta = 11.81$, 14.13, 21.23, 22.65, 25.99, 28.30, 28.45, 31.58, 36.51, 38.02, 41.32, 49.70, 53.19, 93.16,

© 2010 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

109.39, 119.21, 122.08, 123.78, 127.36, 128.42, 128.61, 129.79, 129.96, 130.82, 131.78, 133.02, 137.18, 139.81, 168.67, 169.98 ppm.

Method for Hg^{2+} titration with IR-897 and IR-877: Stock solutions of Hg^{2+} ions (5 mM) were prepared in deionized water. Titration experiments were performed by adding a solution of IR-897 (10 μM, 3 mL) to a quartz cell of 1 cm optical pathlength and then adding Hg^{2+} ions (5 mM) incrementally by means of a micropipette. All spectra were recorded 15 min after the above operation. Test samples for selectivity experiments were prepared by adding three equivalents of the appropriate other metal ion to a solution of IR-897 (10 μM, 3 mL). All test solutions were shaken for 1 min and then kept at room temperature for 15 min. The experiment of Hg^{2+}-ion titration with IR-877 was carried out by a similar procedure to the described above.

Cell-culture methods: The cell line SW1116 was provided by Shanghai Institute of Digestive Disease, China. The cell line SW1116 in this study was cultured in Rosewell Park Memorial Institute 1640 (RPMI-1640) medium (Gibco, USA) supplemented with 10% fetal bovine serum and grown at 37°C in a humidified 5% CO_2 atmosphere. Cells were seeded onto 12-well plates at an initial density of 1×10^5 cells per well and allowed to adhere for 24 h.

Fluorescence imaging: After the SW1116 cells had been washed with phosphate-buffered saline (PBS), 5 μM tricarbocyanine dye in PBS was added to the cells. The cells were then incubated for 20 min at 25°C. Cell imaging was carried out after washing of the cells with PBS. The cells were further treated with $HgCl_2$ solution (10 μM) in PBS for 20 min. The treated cells were imaged by fluorescence microscopy (OLYMPUS BX51, Japan, with a 20× objective lens).

Acknowledgements

This work was financially supported by the NSFC/China, the National Basic Research 973 Program (2006CB806200), the Program for Professor of Special Appointment at Shanghai Institutions of Higher Learning, the Shanghai Shuguang Project (07SG34), the Specialized Research Fund for the Doctoral Program of Higher Education (SRFDP 200802510011), and the State Key Laboratory of Precision Spectroscopy (ECNU).

[1] a) G. Guzzi, C. A. M. La Porta, *Toxicology* **2008**, *244*, 1–12; b) N. Basu, A. Scheuhammer, N. Grochowina, K. Klenavic, D. Evans, M. O Brien, M. Chan, *Environ. Sci. Technol.* **2005**, *39*, 3585–3591.

[2] a) T. W. Clarkson, L. Magos, G. J. Myers, *N. Engl. J. Med.* **2003**, *349*, 1731–1739; b) C. M. L. Carvalho, E.-H. Chew, S. I. Hashemy, J. Lu, A. Holmgren, *J. Biol. Chem.* **2008**, *283*, 11913–11923.

[3] a) J. P. Desvergne, A. W. Czarnik, *Chemosensors of Ion and Molecule Recognition*, Springer, New York, **1997**; b) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, T. E. Rice, *Chem. Rev.* **1997**, *97*, 1515–1566; c) B. Valeur, I. Leray, *Coord. Chem. Rev.* **2000**, *205*, 3–40; d) R. Martínez-Mañez, F. Sancenón, *Chem. Rev.* **2003**, *103*, 4419–4476; e) J. S. Kim, D. T. Quang, *Chem. Rev.* **2007**, *107*, 3780–3799.

[4] a) E. M. Nolan, S. J. Lippard, *Chem. Rev.* **2008**, *108*, 3343–3480, and references therein; b) S. Yoon, A. E. Albers, A. P. Wong, C. J. Chang, *J. Am. Chem. Soc.* **2005**, *127*, 16030–16031; c) A. Coskun, E. U. Akkaya, *J. Am. Chem. Soc.* **2006**, *128*, 14474–14475; d) S. V. Wegner, A. Okesli, P. Chen, C. He, *J. Am. Chem. Soc.* **2007**, *129*, 3474–3475; e) Z. Q. Guo, W. H. Zhu, L. J. Shen, H. Tian, *Angew. Chem.* **2007**, *119*, 5645–5649; *Angew. Chem. Int. Ed.* **2007**, *46*, 5549–5553; f) D. Wu, A. B. Descalzo, F. Weik, F. Emmerling, Z. Shen, X. Z. You, K. Rurack, *Angew. Chem.* **2008**, *120*, 199–203; *Angew. Chem. Int. Ed.* **2008**, *47*, 193–197; g) L. Liu, D. Q. Zhang, X. P. Zheng, Z. Wang, D. B. Zhu, *J. Nanosci. Nanotechnol.* **2009**, *9*, 3975–3981; h) G. Mor-Piperberg, R. Tel-Vered, J. Elbaz, I. Willner, *J. Am. Chem. Soc.* **2010**, *132*, 6878–6879.

[5] a) F. Qian, C. Zhang, Y. Zhang, W. He, X. Gao, P. Hu, Z. Guo, *J. Am. Chem. Soc.* **2009**, *131*, 1460–1468; b) Z. Xu, K.-H. Baek, H. N. Kim, J. Cui, X. Qian, D. R. Spring, I. Shin, J. Yoon, *J. Am. Chem. Soc.* **2010**, *132*, 601–610.

[6] a) M. Santra, D. Ryu, A. Chatterjee, S. K. Ko, I. Shin, K. H. Ahn, *Chem. Commun.* **2009**, 2115–2117; b) Y. K. Yang, S. K. Ko, I. Shin, J. Tae, *Org. Biomol. Chem.* **2009**, *7*, 4590–4593; c) H. Strasdeit, *Angew. Chem.* **2008**, *120*, 840–842; *Angew. Chem. Int. Ed.* **2008**, *47*, 828–830; d) X. Chen, K. H. Baek, Y. Kim, S. J. Kim, I. Shin, J. Yoon, *Tetrahedron* **2010**, *66*, 4016–4021.

[7] a) C. Bouteiller, G. Clave, A. Bernardin, B. Chipon, M. Massonneau, P. Y. Renard, A. Romieu, *Bioconjugate Chem.* **2007**, *18*, 1303–1317; b) M. J. Yuan, Y. L. Li, J. B. Li, C. H. Li, X. F. Liu, J. Lv, J. L. Xu, H. B. Liu, S. Wang, D. B. Zhu, *Org. Lett.* **2007**, *9*, 2313–2316; c) R. Guliyev, A. Coskun, E. U. Akkaya, *J. Am. Chem. Soc.* **2009**, *131*, 9007–9013; d) G. T. Dempsey, M. Bates, W. E. Kowtoniuk, D. R. Liu, R. Y. Tsien, X. Zhuang, *J. Am. Chem. Soc.* **2009**, *131*, 18192–18193; e) U. Mayerhöffer, K. Deing, K. Gruß, H. Braunschweig, K. Meerholz, F. Würthner, *Angew. Chem.* **2009**, *121*, 8934–8937; *Angew. Chem. Int. Ed.* **2009**, *48*, 8776–8779; f) D. Oushiki, H. Kojima, T. Terai, M. Arita, K. Hanaoka, Y. Urano, T. Nagano, *J. Am. Chem. Soc.* **2010**, *132*, 2795–2801; g) X. Li, W. Shi, S. Chen, J. Jia, H. Ma, O. S. Wolfbeis, *Chem. Commun.* **2010**, 2560–2562; h) N. Ramsay, A. Jemth, A. Brown, N. Crampton, P. Dear, P. Holliger, *J. Am. Chem. Soc.* **2010**, *132*, 5096–5014.

[8] a) W. Leevy, S. T. Gammon, H. Jiang, J. R. Johnson, D. J. Maxwell, E. N. Jackson, M. Marquez, D. Worms, B. D. Smith, *J. Am. Chem. Soc.* **2006**, *128*, 16476–16477; b) Z. R. Zhang, S. Achilefu, *Org. Lett.* **2004**, *6*, 2067–2070; c) C. Li, T. R. Greenwood, Z. M. Bhujwalla, K. Glunde, *Org. Lett.* **2006**, *8*, 3623–3626.

[9] J. V. Frangioni, *Curr. Opin. Chem. Biol.* **2003**, *7*, 626–634.

[10] a) L. Strekowski, M. Lipowska, G. Patonay, *J. Org. Chem.* **1992**, *57*, 4578–4580; b) H. Lee, J. C. Mason, S. Achilefu, *J. Org. Chem.* **2006**, *71*, 7862–7865.

[11] a) V. Dujols, F. Ford, A. W. Czarnik, *J. Am. Chem. Soc.* **1997**, *119*, 7386–7387; b) J. Kovács, T. Rödler, A. Mokhir, *Angew. Chem.* **2006**, *118*, 7979–7981; *Angew. Chem. Int. Ed.* **2006**, *45*, 7815–7817; c) R. Martínez-Máñez, F. Sancenón, *Coord. Chem. Rev.* **2006**, *250*, 3081–3093; d) F. Song, S. Watanabe, P. E. Floreancig, K. Koide, *J. Am. Chem. Soc.* **2008**, *130*, 16460–16461.

[12] a) M.-Y. Chae, A. W. Czarnik, *J. Am. Chem. Soc.* **1992**, *114*, 9704–9705; b) G. Hennrich, H. Sonnenschein, U. Resch-Genger, *J. Am. Chem. Soc.* **1999**, *121*, 5073–5074; c) B. Liu, H. Tian, *Chem. Commun.* **2005**, 3156–3158; d) Y. K. Yang, K.-J. Yook, J. Tae, *J. Am. Chem. Soc.* **2005**, *127*, 16760–16761; e) J. Ros-Lis, M. D. Marcos, R. Martinez-Manez, K. Rurack, J. Soto, *Angew. Chem.* **2005**, *117*, 4479–4482; *Angew. Chem. Int. Ed.* **2005**, *44*, 4405–4407; f) S. K. Ko, Y. K. Yang, J. Tae, I. Shin, *J. Am. Chem. Soc.* **2006**, *128*, 14150–14155; g) K. C. Song, J. S. Kim, S. M. Park, K. C. Chung, S. Ahn, S.-K. Chang, *Org. Lett.* **2006**, *8*, 3413–3416; h) J. S. Wu, I. C. Hwang, K. S. Kim, J. S. Kim, *Org. Lett.* **2007**, *9*, 907–910; i) M. H. Lee, B. K. Cho, J. Yoon, J. S. Kim, *Org. Lett.* **2007**, *9*, 4515–4518; j) X. Chen, S. W. Nam, M. J. Jou, Y. Kim, S. J. Kim, S. Park, J. Yoon, *Org. Lett.* **2008**, *10*, 5235–5238; k) M. Yu, M. Shi, Z. Chen, F. Li, X. Li, Y. Cao, J. Xu, H. Yang, Z. Zhou, T. Yi, C. Huang, *Chem. Eur. J.* **2008**, *14*, 6892–6900; l) H. Zheng, G. Q. Shang, S. Y. Yang, X. Gao, J. G. Xu, *Org. Lett.* **2008**, *10*, 2357–2360; m) X. Zhang, Y. Xiao, X. Qian, *Angew. Chem.* **2008**, *120*, 8145–8149; *Angew. Chem. Int. Ed.* **2008**, *47*, 8025–8029; n) M. H. Lee, S. W. Lee, S. H. Kim, C. Kang, J. S. Kim, *Org. Lett.* **2009**, *11*, 2101–2104.

[13] a) E. Sasaki, H. Kojima, H. Nishimatsu, Y. Urano, K. Kikuchi, Y. Hirata, T. Nagano, *J. Am. Chem. Soc.* **2005**, *127*, 3684–3685; b) K. Kiyose, H. Kojima, Y. Urano, T. Nagano, *J. Am. Chem. Soc.* **2006**, *128*, 6548–6549; c) B. Tang, H. Huang, K. Xu, L. Tong, G. Yang, X. Liu, L. An, *Chem. Commun.* **2006**, 3609–3611; d) S. Tatay, P. Gaviñ, E. Coronado, E. Palomares, *Org. Lett.* **2006**, *8*, 3857–3860; e) B. Tang, L. J. Cui, K. H. Xu, L. L. Tong, G. W. Yang, L. G. An, *ChemBioChem* **2008**, *9*, 1159–1164; f) M. Zhu, M. Yuan, X. Liu, J. Xu, J. Lv, C. Huang, H. Liu, Y. Li, S. Wang, D. Zhu, *Org. Lett.* **2008**, *10*, 1481–1484; g) R. Shunmugam, G. J. Gabriel, C. E. Smith, K. A. Aamer, G. N. Tew, *Chem. Eur. J.* **2008**, *14*, 3904–3907; h) B. Tang, F.

 © 2010 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Yu, P. Li, L. Tong, X. Duan, T. Xie, X. Wang, *J. Am. Chem. Soc.* **2009**, *131*, 3016–3023.
[14] X. Peng, F. Song, E. Lu, Y. Wang, W. Zhou, J. Fan, Y. Gao, *J. Am. Chem. Soc.* **2005**, *127*, 4170–4171.
[15] a) M. A. Haidekker, T. P. Brady, D. Lichlyter, E. A. Theodorakis, *J. Am. Chem. Soc.* **2006**, *128*, 398–399; b) D. Srikun, E. Miller, D. W. Domaille, C. J. Chang, *J. Am. Chem. Soc.* **2008**, *130*, 4596–4597.
[16] a) J. Manimala, E. Anslyn, *Eur. J. Org. Chem.* **2002**, 3909–3922; b) R. A. Batey, D. A. Powell, *Org. Lett.* **2000**, *2*, 3237–3240; c) D. Boeglin, S. Cantel, A. Heitz, J. Martinez, J. A. Fehrentz, *Org. Lett.* **2003**, *5*, 4465–4468; d) S. Dahmenand, S. Bräse, *Org. Lett.* **2000**, *2*, 3563–3565.
[17] a) H. A. Benesi, J. H. Hildebrand, *J. Am. Chem. Soc.* **1949**, *71*, 2703–2707; b) M. Barra, C. Bohne, J. C. Scaiano, *J. Am. Chem. Soc.* **1990**, *112*, 8075–8579.
[18] N. Narayanan, G. Patonay, *J. Org. Chem.* **1995**, *60*, 2391–2395.

Received: June 23, 2010
Published online: October 29, 2010

 © 2010 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim