Fluoro- and Chromogenic Chemodosimeters for Heavy Metal Ion Detection in Solution and Biospecimens

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1. Introduction

Heavy metal ions are of great concern, not only among the scientific community, especially chemists, biologists, and environmentalists, but increasingly among the general population, who are aware of the some of the disadvantages associated with them. In spite of the fact that some heavy metal ions play important roles in living systems, they are very toxic and hence capable of causing serious environmental and health problems.[1−6] Some heavy metal ions, such as Fe(III), Zn(II), Cu(II), Co(II), Mn(II), and Mo(VI), are essential for the maintenance of human metabolism. However, high concentrations of these ions can lead to many adverse health effects.[1,2,7−20] It is also a fact that others such as Hg(II), Cd(II), Pb(II), and As(III) are among the most toxic ions known that lack any vital or beneficial effects. Accumulation of these over time in the bodies of humans and animals can lead to serious debilitating illnesses.[2,21−30] Therefore, the development of increasingly selective and sensitive methods for the determination of heavy metal ions is currently receiving considerable attention.[7,23,31−36]

Several methods, including atomic absorption spectroscopy, inductively coupled plasma atomic emission spectrometry, electrochemical sensoring, and the use of piezoelectric quartz crystals make it possible to detect low limits.[37−40] However, these methods require expensive equipment and involve time-consuming and laborious procedures that can be carried out only by trained professionals.

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Duong Tuan Quang was born in 1970 in Thanhhoa, Vietnam, and graduated from Hue University in 1992, where he obtained his M.S. degree two years later and began his career as a lecturer in Chemistry soon afterwards. He received his Ph.D. degree in 2003 from Institute of Chemistry, Vietnamese Academy of Science and Technology. In 2006, he worked as a postdoctoral fellow in Professor Jong Seung Kim's laboratory, Dankook University, Seoul, Korea. He was promoted as an associate professor in 2009 and went to Korea University as a research professor in 2010. His main task involved the development of chromogenic and fluorogenic molecular sensors to detect specific cations and anions.

Alternatively, analytical techniques based on fluorescence detection are very popular because fluorescence measurements are usually very sensitive (parts per billion/trillion), easy to perform, and inexpensive.[23,37,41−45] Furthermore, the photophysical properties of a fluorophore can be easily tuned using a range of routes: charge transfer, electron transfer, energy transfer, the influence of the heavy metal ions, and the destabilization of nonemissive $n-\pi^*$ excited states.[5] Consequently, a large number of papers involving fluorescent chemosensors (see definition in section 2) have been published.

In general to date, fluorescent chemosensors for anions and cations have proven popular, but those for many heavy metal ions such as Hg(II), Pb(II), Cu(II), Fe(III), and Ag(I) present challenges because these ions often act as fluorescence quenchers. Cu(II) is a typical ion that causes the chemosensor to decrease fluorescent emissions due to quenching of the fluorescence by mechanisms inherent to the paramagnetic species.[46−48] Such decreased emissions are impractical for analytical purposes because of their low signal outputs upon complexation. In addition, temporal separation of spectrally similar complexes by time-resolved fluorimetry is subsequently prevented.[49]

Compared to the relatively well-developed fluorescent chemosensors, fluorescent chemodosimeters (see definition in section 2) have recently emerged as a research area of

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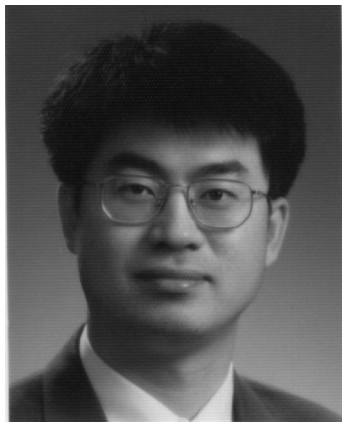


Jong Seung Kim was born in 1963. He received a Ph.D. degree from Department of Chemistry and Biochemistry at Texas Tech University in Lubbock, Texas, U.S.A., in 1993 under the supervision of Professor Richard A. Bartsch. In 1993, he joined Professor Jay K. Kochi's research lab at the University of Houston as a postdoctoral fellow. He started his teaching career at Department of Chemistry at Konyang University in Nonsan, Korea, in 1994. He then transferred to Department of Chemistry at Dankook University in Seoul, Korea, in 2003 and developed the calixarenes anchored chromogenic and fluorogenic molecules to detect specific cations, anions, and neutral molecules. In 2007, he moved to Department of Chemistry at Korea University in Seoul as a professor. His current research is in four major fields: calixarenes, chemosensors, DDS, and MRI contrasting agent. To date his research records 250 scientific publications and 30 domestic and international patents.

significant importance. Chemodosimeters are used to detect an analyte through a highly selective and usually irreversible chemical reaction between the dosimeter molecule and the target analyte. This leads to an observable signal that has an accumulative effect and hence is directly related to the concentration of the analyte. Interestingly, chemodosimeters provide an ideal way to design fluorescence "turn-on" probes for the quenching of the heavy metal ions listed above because, in most cases, the fluorescent product does not coordinate to the metal ions. As a result, an increasing number of papers on fluorescent chemodosimeters for heavy metal ions have appeared in recent years. Furthermore, the chemodosimeter often provides signaling changes in both the absorption wavelength and color. These signaling changes have been widely used as "detection events" because they require only the use of inexpensive equipment, or in some cases no equipment at all, as color changes can be detected by the naked eye. Thus, both the fluorogenic and chromogenic behaviors of the chemodosimeter are often studied together. The development of chemodosimeters has recently emerged as an active research area of significant importance. It has attracted a tremendous amount of attention as a result of its high sensitivity and rapid response. In spite of this, to date a comprehensive report addressing fluoro- and chromogenic chemodosimeters for cation sensing, particularly the most recent ones for the heavy metal ions, has not been published. In an effort to fill this gap, therefore, we will now review the literature of the last five years covering the chemodosimetric approach for detecting heavy metal ions.

2. Comparison of Chemodosimeters and Chemosensors

Although Anthony W. Czarnik reported the first relevant research in 1992, since then only a few papers concerning the chemodosimetric approach appeared until 2005. Nevertheless, the number of researchers has increased considerably

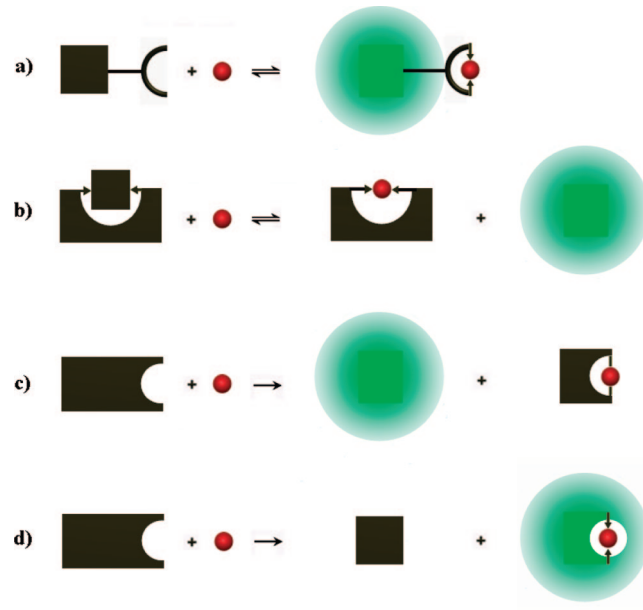


Figure 1. Schematic operating protocols of chemosensors a and b and chemodosimeters c and d.

over the past few years. Although various definitions of chemodosimeters have appeared in papers elsewhere, the differentiation between a chemodosimeter and a chemosensor is sometimes unclear. Therefore, before detailing the relevant papers, a definition of these terms is in order.

Chae and Czarnik first described the term chemodosimeter as an abiotic molecule used to achieve analyte recognition with concomitant irreversible transduction of a human-observable signal.[50] Such a chemodosimetric approach involves the use of reactions induced by a specific analyte such as an anion or cation or other molecule. These reactions result in a significant chemical transformation involving both the breaking and formation of several covalent bonds. They are generally irreversible and reflect a cumulative response related directly to the concentration of the analyte.[41,50−54] Chemosensors, on the other hand, are molecules of an abiotic origin that interact with the analyte to yield measurable signals with a real-time response (usually less than a few seconds). The contrast is that the general operating principle of chemosensors is based on coordination events. Hence, the reaction of a chemosensor with the analyte and the accompanying signal changes are reversible.[41,50,53] In fact, coordination is a typical reversible chemical reaction in which any changes in the concentration of the anion determine the relative amounts of both coordinated and free moiety. Figure 1 represents the operating protocols of both chemosensors and chemodosimeters. The most widely used chemosensor protocol, seen in Figure 1a, involves the binding site/signaling subunit approach. Here the two units are covalently linked to give an optical response following coordination to a selective analyte.[31,41,50,53,55−58] The other chemosensor protocol (Figure 1b) follows the displacement approach.[59−64] This involves the use of a binding site and a signal reporter. However, in this case, the two units are not covalently linked. On addition of the analyte, it coordinates with the binding site and with subsequent release of the signaling subunit. Chemodosimeters are based on such units. However, the irreversible reactions induced by specific analytes result in products chemically different from the starting chemodosimeters. In the first case (Figure 1c), the analyte covalently links to one or more atoms, which subsequently leave the

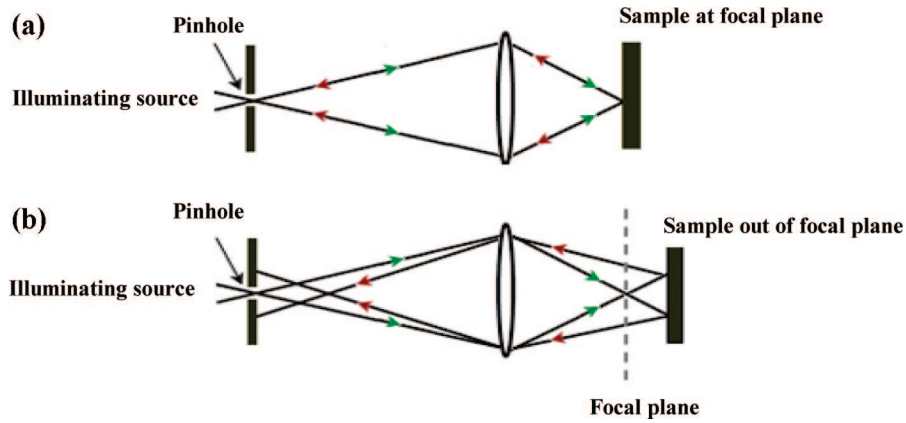


Figure 2. Comparison of light reflected from the sample placed in the confocal microscope: (a) sample on focal plane and (b) sample out of focal plane.

chemodosimeter together. In the case of Figure 1d, a small molecule is separated from the chemodosimeter while the analyte coordinatively binds to its remaining part. In the chemodosimeter approach, the objective is to find a specific reactivity; the goal for both the binding site signaling unit and the displacement protocols is selective coordination. For the purposes of this review, only papers on chemodosimeters that conform to these aforementioned requirements are considered.

3. Advances in Fluorescence Techniques for Imaging Biological Specimens

Fluorescence microscopy has proven to be an effective tool for many analytical applications involving both environmental and biological examples.[42,65] Whereas traditional fluorescence microscopy can be routinely used for the detection of analytes in solution, it is an unsuitable technique for living organisms. This is due to the fact that, whereas many fluorescence systems rely on the absorption of visible or ultraviolet light and emission at longer wavelengths, light from this region of the spectrum does not easily penetrate living tissue. Although in some cases of fixed tissue, it can be imaged several hundred micrometers deep into the specimen, living tissue is a hostile environment. There is major light scattering and, as a result, little fluorescence from the area of interest reaches the detector. At certain depths, the scattering is such that traditional fluorescence imaging techniques are no longer effective. In some cases, observation of the fluorescence image can be improved by increasing the intensity of the excitation and/or the collection time per pixel. However, the photobleaching phenomenon that occurs when a fluorophore permanently loses its ability to fluoresce, due to photon-induced chemical damage and covalent modification, always sets a limit on the amount of light to which the specimen can be safely exposed.[66] Some fluorophores bleach quickly after emitting only a few photons, while other more robust ones can undergo thousands or millions of cycles before bleaching. Another issue is that, with thick tissue samples, when the image is defocused, its features blur and the edges become less sharp.[67]

Careful sample preparation can sometimes solve several of these imaging issues. Physically cutting a specimen into thinner slices can allow the illuminating light to more easily reach the sample, the signal can be collected with minimal scattering, and there is less out-of-focus light to confuse the interpretation. However, this method is not often used with living tissue since cells do not remain alive for long when sectioned thinly. It is the most appropriate for fixed-tissue observation.

In an alternative approach, the development of the confocal microscope, which uses point illumination and a pinhole in an optically conjugate plane in front of the detector to eliminate out-of-focus signal, provided an important new imaging tool for scientists.[68−71] This technique allows an opportunity of using optically cross-sectioned samples without the necessity of physically slicing them into thin sections. Furthermore, as depicted in Figure 2, out-of-focus information from both above and below the focal plane is greatly reduced, which yields sharper images compared to conventional microscopy techniques. However, the use of confocal microscopes can also cause the entire specimen to be bleached due to the fact that fluorescence is generated throughout all depths of the sample (Figure 3). Moreover, a large fraction of the fluorescence photons is scattered while en route out of the sample; this is especially true in the case of deep tissue. The scattered photons cannot be collimated and thus cannot be fed through the beam path of a descanned confocal system.

Recently, two-photon microscopy has been developed and effectively used to yield better fluorescent images of biological samples.[72−76] In this technique, the combination of two low-energy photons, typically from the same laser, causes a higher-energy electronic transition in a fluorophore. The phenomenon of two-photon excitation is depicted in a Jablonski diagram as shown in Figure 3.[42] Compared to the one-photon techniques on which confocal microscopy is based, two-photon excitation provides more advantages for microscopic analysis of scattering specimens. The excitation wavelengths of the deep-red and infrared region, used in two-photon microscopy, can penetrate substantially deeper into a sample than either visible light or the UV laser of a confocal microscope.[77−79] Because of the nature of nonlinear excitation (i.e., generated fluorescence depends on the square of the number of photons per time and cm^2), scattered excitation photons are too dilute to cause appreciable fluorescence. Therefore, excitation is mainly limited to a small focal volume. This is true even in the case of deep tissue where almost all the incidence photons are scattered. Moreover, depth resolution is obtained via the nonlinearity of the excitation process. A pinhole for the rejection of out-of-focus fluorescence is therefore unnecessary.[80−82] As a result, unlike wide-field and confocal microscopy where scattered fluorescence photons are either lost, or even worse, contribute to background, in two-photon microscopy, all the

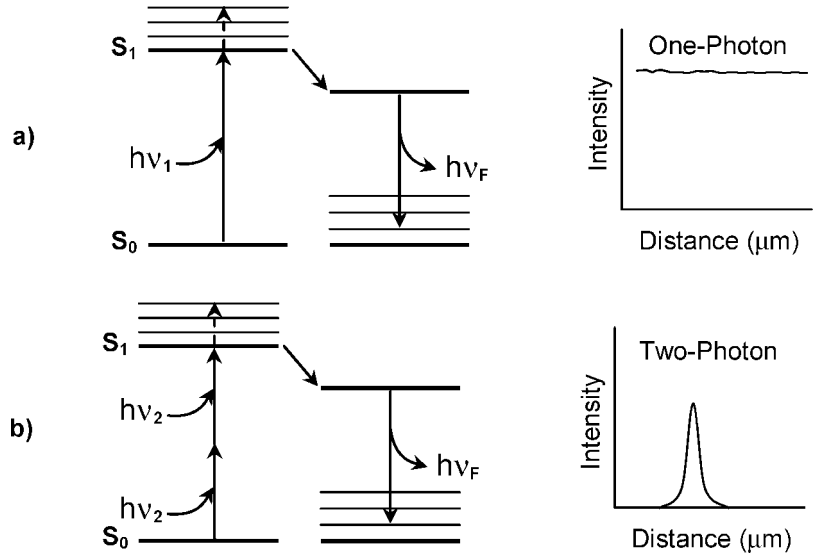


Figure 3. Jablonski diagram and fluorescence intensity versus distance from the sample surface for (a) one-photon excitation and (b) two-photon excitation.

fluorescence photons, both ballistic and scattered, if detected, constitute useful signal.[83,84]

4. Molecular Chemodosimeters

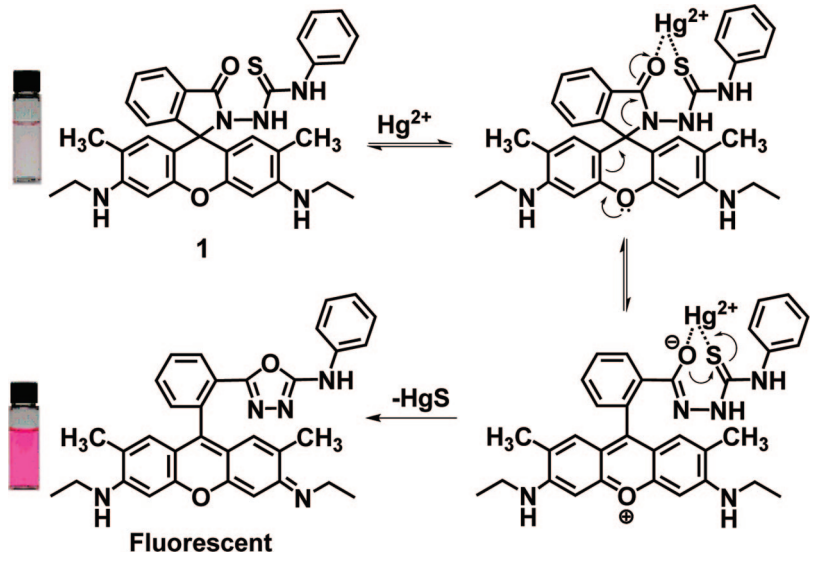
4.1. Ring-Opening of Spirocyclic Systems

Czarnik, who worked at Ohio State University, was the first to introduce a potentially new approach in the field of optical sensing termed chemodosimetry.[50] In 1997, he and his co-workers reported work utilizing a ring-opening reaction of a rhodamine-B derivative for the design of a Cu(II) chemodosimeter.[53] Following this pioneering work, after an initial slow start, many exciting papers on ring-opening reactions have been published.

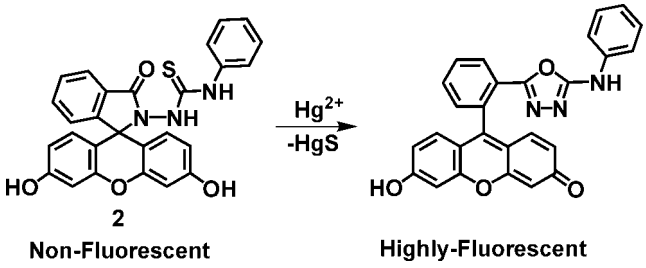
In an effort to develop a technique for Hg(II) determination, the chemodosimeter molecule **1** was prepared and studied by Tae and co-workers.[51] As predicted, a combination of the spirolactam ring-opening process[53,85] and the Hg(II)-triggered 1,3,4-oxadiazole cyclization[86] of the thiosemicarbazide provided a novel chemodosimeter for Hg(II). After the addition of Hg(II) (1.0 equiv) to a solution of **1** in water−methanol (4/1, v/v), a 26-fold fluorescence enhancement was produced with a concomitant 4-nm red-shift in the wavelength of the maximum emission, from 553 to 557 nm. Metal-ion selectivity experiments indicated that the emission from **1** was unaffected by any of the following species: Cu(II), Pb(II), Cd(II), Ni(II), Co(II), Fe(II), Mn(II), Mg(II), Ca(II), Ba(II), Li(I), K(I), Na(I), Rh(III), and Cr(II). Although the addition of Ag(I) or Zn(II) caused small fluorescence enhancements, the presence of these metal ions did not interfere with the Hg(II)-induced fluorescence response. Sensitivity tests showed that detection limits for **1** were <2.0 ppb, below the Environmental Protection Agency (EPA) drinking water limits for inorganic Hg(II).[87] Moreover, the interaction of Hg(II) with **1** caused the solution to change from colorless to pink, indicating the formation of rhodamine-6G in a ring-opened form.[50,85,88] The color change was visible by the naked eye at a probe concentration of 10.0 μM; no significant color changes promoted by other metal ions were observed (see Scheme 1).

Like many related xanthenone-based fluorophores, fluoresceins are also widely used in aqueous and biological

Scheme 1



Scheme 2



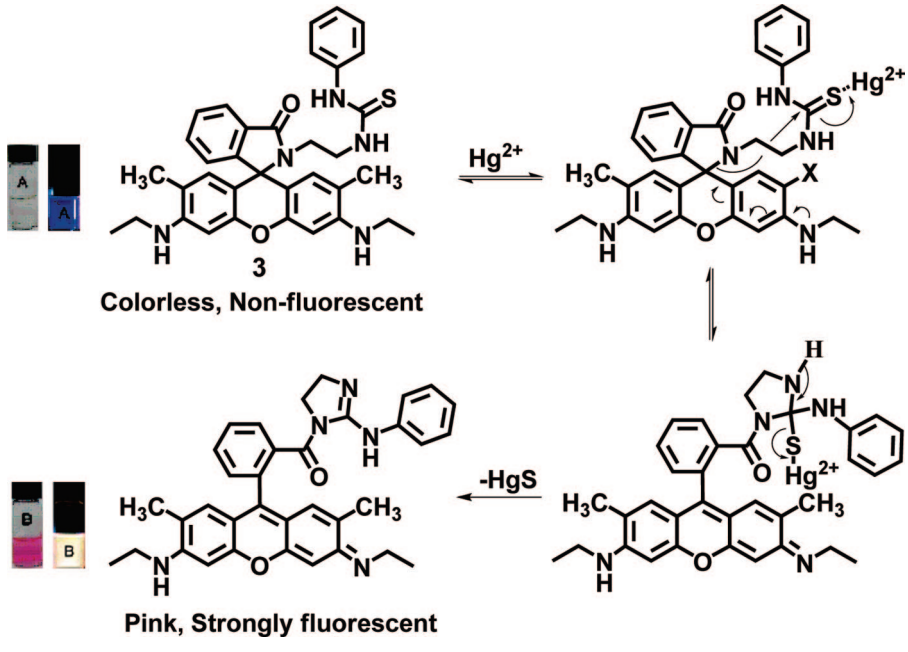
applications. Some of the most advantageous features of the fluorescein fluorophore are good water solubility, visible excitation and emission, maximum brightness at physiological pH, and both a high extinction coefficient and high quantum yield. On the basis of a structurally similar framework, the probe **2** molecule[89] exhibits virtually the same properties as **1**. Both use a Hg(II)-triggered irreversible reaction with a spirolactam ring-opening, followed by cyclization of a thiosemicarbazide to form 1,3,4-oxadiazole. Probe **2** was also remarkably selective and sensitive for Hg(II) over other metal ions. Titrations of **2** with Hg(II) revealed a stoichiometric 1:1 reaction and a linear fluorescence response up to 1.0 μM Hg(II). The detection limit of 1.0 μM of **2** for Hg(II) was 2.0 ppb. However, it requires a relatively longer response time (15−20 min) than **1** (<1 min) (see Scheme 2).

Recently, Kim and co-workers synthesized the molecular probe **3**,[90] *N*-(rhodamine-6G)lactam-*N*′-phenylthioureaethylenediamine. This is potentially a selective probe for the detection of Hg(II) in aqueous solution. Compound **3** differs from **1** in the spacer used between the (rhodamine-6G)lactam and the phenylthiourea group. As a consequence of using this spacer, instead of forming the 1,2,3-oxadiazole with **1**, the addition of Hg(II) to a solution of **3** induced the *N* atom of the spirolactam to attack the C atom of the thiourea. This leads to a ring-opening of the spirolactam of the rhodamine, followed by removal of HgS and the formation of intramolecular guanylation. A background mixture of Fe(II), Co(II), Ni(II), Cu(II), Zn(II), Pb(II), Cd(II), Ca(II), Mg(II), K(I), and Na(I) had no effect on the emission/absorption of **3** and did not interfere with the Hg(II)-induced fluorescence/absorption response. This indicated that the spirolactam ring-opening, followed by the cyclic guanylation, was very selective for Hg(II) over the other competitive metal ions tested. Introduction of Hg(II) also caused an emission color change from blue to yellow, visible by the eye when 10.0 μM solutions of **3** and **3** + Hg(II) were excited with 365 nm light from a handheld UV lamp. For the absorption, a new band centered at 532 nm appeared with increasing intensity, inducing a definite color change from colorless to pink upon introduction of Hg(II) to a 10.0 μM solution of **3** (see Scheme 3).

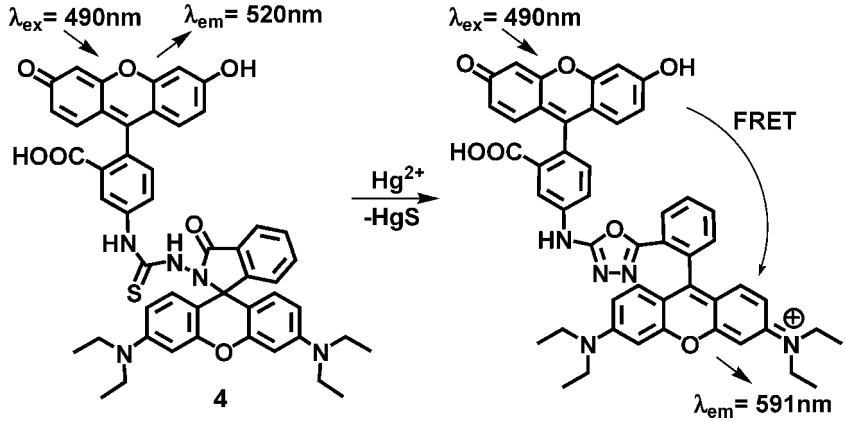
Most optical chemosensors/chemodosimeters reported so far have been based on the increase or decrease in fluorescence intensity at a single emission wavelength after addition of the analyte.[51,89,91−102] In spite of being flexible in design and synthesis, there are still some drawbacks to this kind of sensor. The most important is that the fluorescence intensity can vary for reasons other than analyte concentration. This unexpected variation can result from any of the following: a change in pH, photobleaching, compartmentalization within cells leading to changes in the microenvironment, and finally light scattering. In order to avoid such issues, an alternative is to acquire intensity measurements at two emission wavelengths at which the intensity responds differently.[103−108]

One such chemodosimeter following the ratiometric approach just described was reported by Shang et al.[109] The authors synthesized the molecule **4**, composed of a fluorescein fluorophore and a rhodamine-B hydrazide linked together by a thiourea spacer. Addition of Hg(II) to a solution of **4** caused a spirolactam ring-opening and led to the release of a fluorescent rhodamine-B moiety. Subsequently, an intramolecular fluorescence resonance energy transfer (FRET)[34,110−115] from fluorescein to the rhodamine group occurred. Because of the fluorescein emission, the free probe exhibited a single emission band centered at 520 nm when excited at 490 nm. The introduction of Hg(II) produced another band centered at 591 nm. This corresponds to the emission of the ring-opened rhodamine-B moiety. A decrease in fluorescence intensity at 520 nm and a concomitant

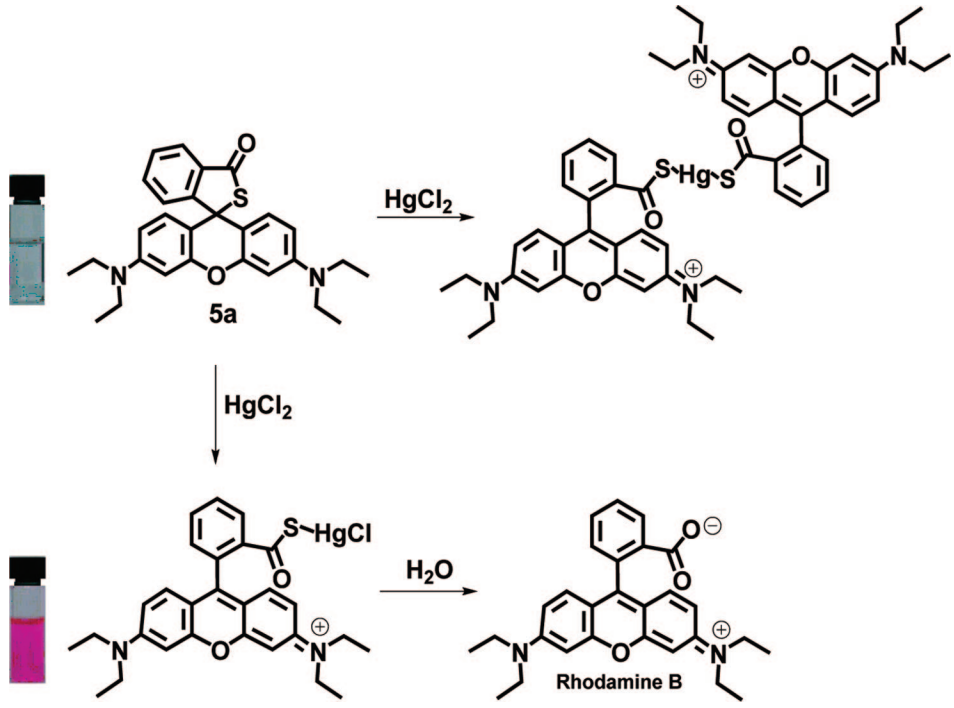
Scheme 3



Scheme 4



Scheme 5

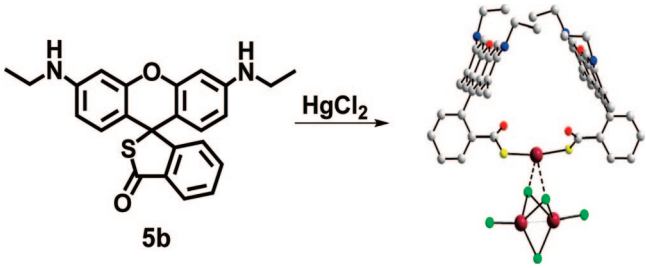


increase in fluorescence intensity at 591 nm took place on addition of increasing amounts of Hg(II). Therefore, the determination of Hg(II) could be performed by measuring the ratio of fluorescence intensities at 591 and 520 nm, respectively. Solution studies revealed a 1:1 reaction stoichiometry of **4** for Hg(II), a detection limit of 0.05 μM, and a linear response up to 10.0 μM Hg(II). Probe **4** also exhibited a color change from yellow to magenta upon addition of Hg(II), allowing for detection with the naked eye. Metal-ion selectivity experiments indicated that the emission and color from **4** was unaffected by Fe(III), Co(II), Ni(II), Cr(III), Zn(II), Pb(II), Cd(II), Ca(II), Mg(II), Ba(II), and Mn(II) (see Scheme 4).

Chemodosimeters derived from rhodamine-B derivatives commonly contain an additional benzene ring that can cause difficulties in water compatibility. To overcome this, Shi and Ma synthesized rhodamine-B thiolactone **5a**,[116a] a small molecule lacking unwanted aromatic moieties and, hence, with a concomitant increase in water solubility. Addition of Hg(II) caused a 10-nm red-shift in the wavelength of the maximum emission from 575 to 585 nm, most likely due to the formation of different fluorescent species. When 5.0 μM of **5a** was employed, a linear fluorescence response up to 5.0 μM Hg(II), and a low detection limit of 20.0 nM were observed. The other attractive feature of this probe is the fact it can be operated in a ca. 100% aqueous solution around a neutral pH span of 5−8. This greatly increases its potential for use in biological contexts. This probe is found to be highly selective for Hg(II) over other metal ions, including Ca(II), Cd(II), Co(II), Cu(II), Fe(III), K(I), Mg(II), Mn(II), Pb(II), and Zn(II). An additional advantage is the fact that the interaction of 5.0 μM of **5a** with 50.0 μM Hg(II) induced a colorless-to-pink change that remained unaffected by several other metal ions, thus providing a means of Hg(II) detection by the naked eye, even in the presence of the other species studied (see Scheme 5).

Yoon and co-workers used X-ray crystallography to explicitly prove the geometry of the bound Hg(II) ion ring-opened rhodamine-6G complex, formed from the Hg(II) ion-induced spirothiolactone ring-opened system.[116b] With respect to fluorescence enhancement after metal ion-binding, compound **5b** showed a very high selectivity toward the Hg(II) ion over other metal ions in the CH_3CN−HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid) buffer (0.01

Scheme 6



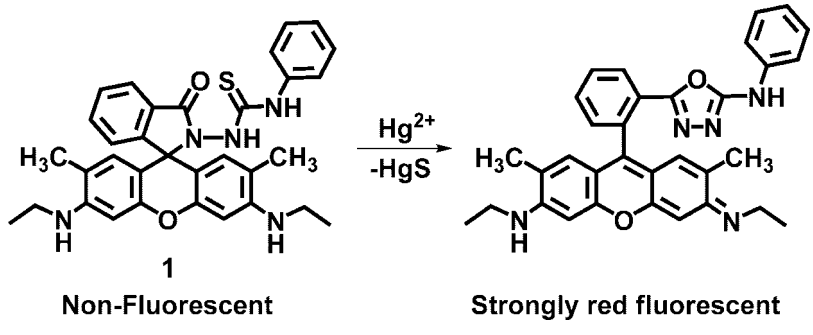
M, pH 7.4) (1:99, v/v). This behavior is similar to that of **5a**. An enhancement of up to a 200-fold "off−on" type fluorescence for **5b** was observed after the addition of the Hg(II). Evidence for a 2:1 binding mode (ligand/Hg(II)) included that from electrospray-ionization mass spectroscopy (ESIMS), the Job plot, and X-ray crystal structure data. The spirothiolactone ring-opened structure, as well as the coordination of the two sulfur atoms to Hg(II), was clearly confirmed. Since the nature of probe **5b** is based on a reversible coordination event, it really can be considered a chemosensor (see Scheme 6).

There is a big demand for good sensors for the detection of heavy metal ions because, as mentioned above, although some have vital and beneficial effects, the toxicity of others is of particular concern. For chemosensors/chemodosimeters that have applications in living cells, they are required to be water-soluble, have good permeability and long emission wavelengths, be nontoxic, and be capable of functioning at physiological pH. Rhodamine derivatives were found to possess all these important features and hence have been used for the development of a range of chemosensors. Recently, some chemodosimeters based on rhodamine derivatives have also been reported as displaying the ability to monitor heavy metal ions in living cells. Probe **1**, discussed above, can react with Hg(II) to form a strong fluorescent 1,3,4-oxadiazole derivative that has the potential to operate in aqueous solution at long wavelengths. This is where the background autofluorescence in cells is minimal. While incorporating all these advantageous features, Tae and co-workers developed **1** as a chemodosimeter for monitoring Hg(II) ions in living cells and vertebrate organisms.[117] After the living cells were treated with 50.0 μM of **1** for 20 min and incubated with 50.0 μM of Hg(II) for 10 min at 37 °C, subsequent examination of the fluorescence microscopic images of the cells showed that **1** entered the cell membranes and reacted with the mercury ions. The detection limits of **1** for Hg(II) in the in vivo system were also evaluated and shown to be capable of monitoring ca. 40.0 μM of Hg(II). The fact that only insignificant fluorescence enhancement was observed, on the addition of a range of other biologically relevant ions such as Mg(II), Zn(II), Ca(II), Fe(II), as well as toxic metal ions prevalent in the environment such as Cd(II) and Pb(II), indicates a good selectivity of **1** for Hg(II). Moreover, further experiments were designed to test whether or not **1** could be used to detect Hg(II) in living organisms. The results showed good fluorescent images from the living organisms of zebrafish treated with 5.0 nM of Hg(II) for 12 h and 10.0 μM of **1** for 30 min. However, results indicated that there was a nonselective accumulation of Hg(II) in different locations within the zebrafish (see Scheme 7).

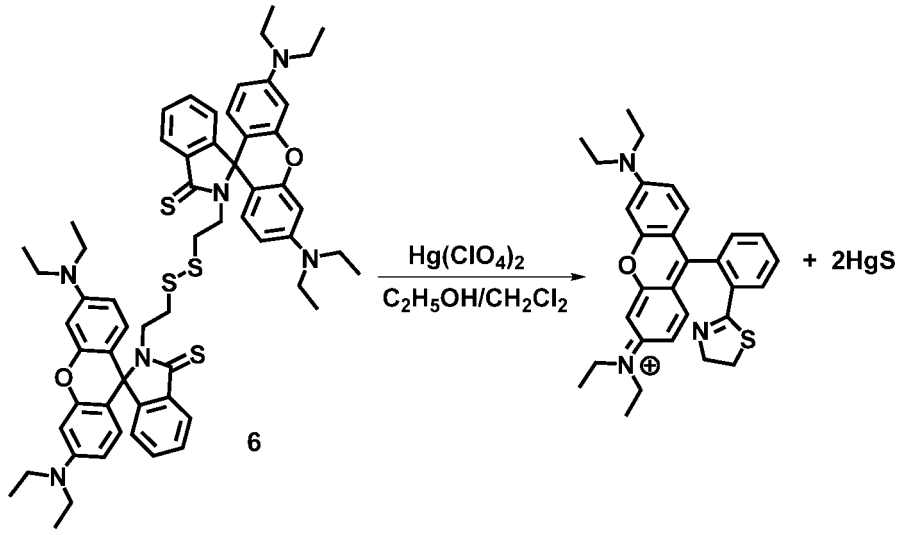
A thiorhodamine-based chemodosimeter with a disulfide linker (**6**) was synthesized and studied by Wang and coworkers.[118] This compound can induce a fluorescence enhancement almost immediately (within 10 s); this is particularly important for practical applications. Solution studies revealed a 1:2 reaction stoichiometry of **6** for Hg(II), and a linear response up to 10.0 μM of Hg(II). The probe also demonstrated an excellent selectivity for Hg(II) over other cations. When 5.0 μM was employed, the addition of 50.0 μM of any of Cu(II), Cd(II), Pb(II), Zn(II), Fe(II), Co(II), Ni(II), Ca(II), Mg(II), Li(I), K(I), Na(I), Cu(I), Ag(I), and Fe(III) caused only a slight fluorescence enhancement, whereas a ~50-fold fluorescence enhancement was observed following the introduction of 10.0 μM of Hg(II). In addition, these metal ions did not interfere with the Hg(II)-induced response of **6**. On the basis of these beneficial features, probe **6** was developed as a chemodosimeter for the detection of Hg(II) in living cells. HK-2 cells were first treated with 10.0 mM of **6** for 30 min at 37 °C followed by Hg(II) at various concentrations. The fluorescence images showed a Hg(II)-concentration dependence. The higher the Hg(II) concentration used, the stronger were the fluorescence images observed. Probe **6** was found to have a detection limit of 0.1 mM for the imaged intracellular Hg(II) (Scheme 8).

A chemodosimeter based on the dual rhodamine−urea derivative **7**[119] was prepared and studied for use in the detection of Hg(II) in living cells. A ca. 10-fold fluorescence enhancement of **7** occurred at 550 nm, with the solution changing from colorless to pink following the addition of Hg(II). The "trigger" emission for **7** was found to be selective for Hg(II) and occurred in the presence of mixtures of a range of other metal ions including Ag(I), Zn(II), Cu(II), Pb(II), Cd(II), Ni(II), Co(II), Mn(II), Mg(II), Ca(II), Ba(II), Li(I), K(I), Na(I), and Cr(II). The detection limit for Hg(II) in methanol was below 2.0 ppb when 10^{-7} M **7** was employed. Favorable spectroscopic properties prompted the authors to further investigate fluorescence imaging of Hg(II) in living cells. HeLa cells were first incubated with 10.0 μM of **7** for 30 min at 37 °C, then treated with 10.0 and 100 μM of Hg(II) for 30 min at 37 °C. Confocal laser scanning microscopy

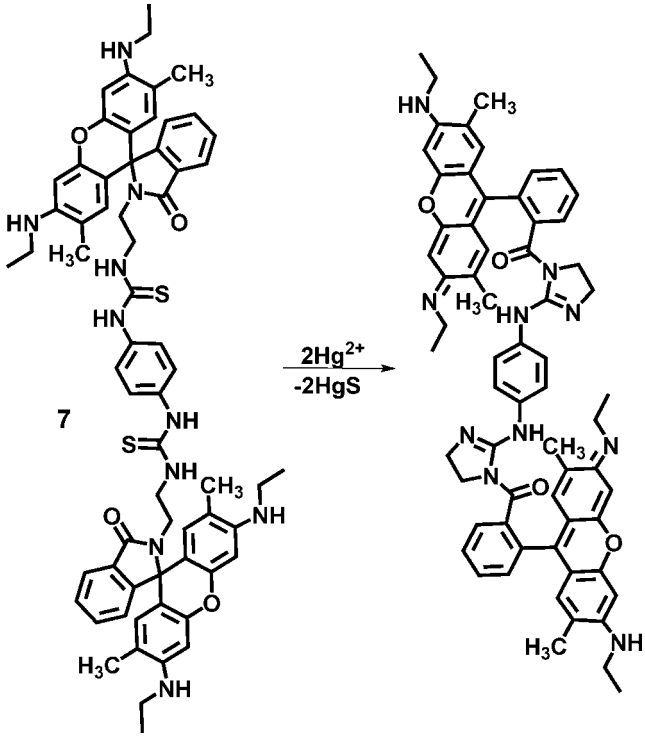
Scheme 7



Scheme 8



Scheme 9



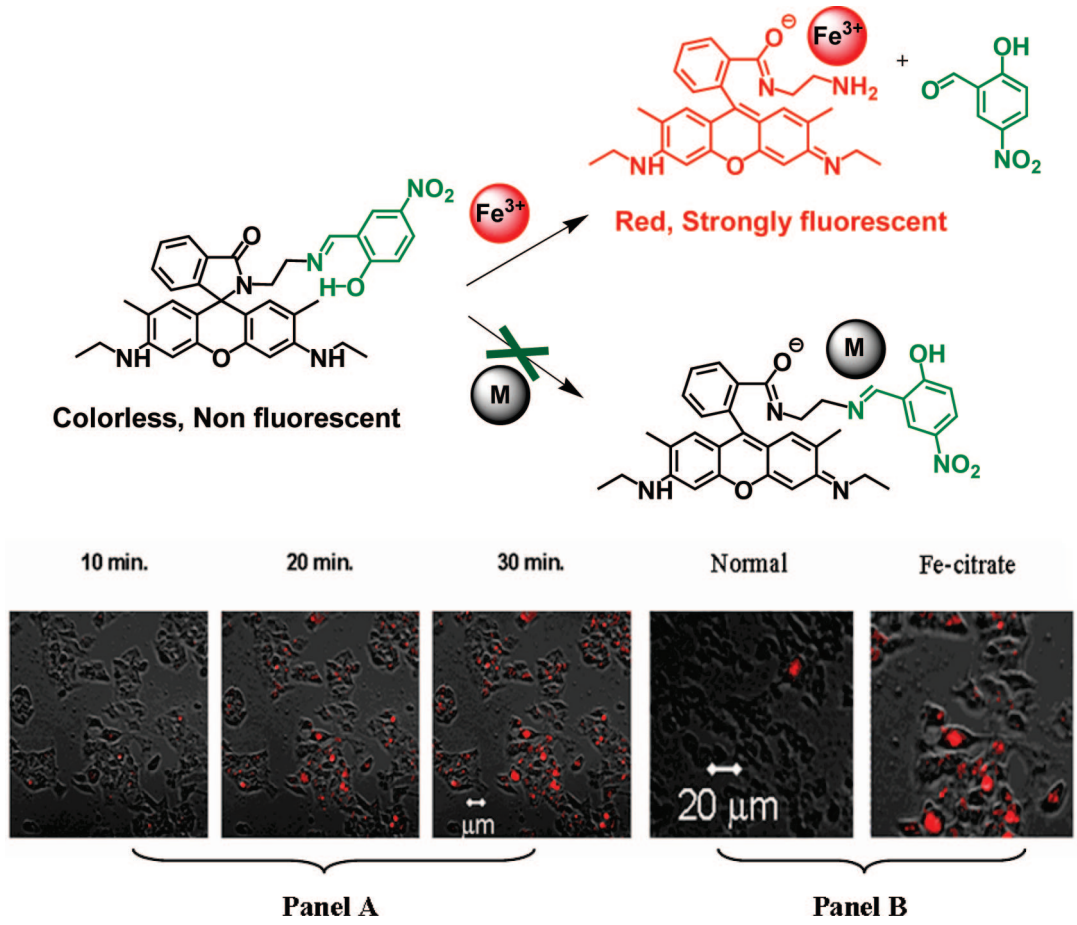
experiments showed that the higher the concentration of Hg(II), the stronger was the fluorescence of the treated cells.

The addition Hg(II) to a solution of **7** most likely caused a spirolactam ring-opening, followed by guanylation of thiourea,[13,90] because the formation of a 1,3,4-oxadiazole was impossible because of an ethylene spacer between the rhodamine and thiourea portions of the molecule (see Scheme 9).

More recently, a rhodamine-6G Schiff-based chemodosimeter for Fe(III) (**8**) was synthesized and studied by Kim and co-workers.[120] The foundation for this research stemmed not only from the high affinity of Fe(III) toward the ethylenediamine framework[121,122] but also its strong Lewis acid activity over other metal ions that facilitate hydrolysis of the Schiff base.[123−125] Addition of Fe(III) to an aqueous solution of **8** induced a color change from colorless to pink. This color change was concomitant with a strong green fluorescence emission band at 551 nm, indicative of rhodamine-B formation (open-ring form). Of the seven metal ions screened, only Fe(III) promoted a significant fluorescence enhancement, thus making **8** highly Fe(III)-selective. Furthermore, the Fe(III)-triggered fluorescence response was not affected by other metal ions such as K(I), Na(I), Fe(II), Ca(II), Mg(II), Mn(II), Ag(I), Hg(II), Ba(II), Cd(II), Co(II), Cu(II), Ni(II), Pb(II), and Zn(II). Titrations of **8** with Fe(III) revealed a 1:1 reaction stoichiometry and a detection limit of <1.0 μM. Unlike other commonly encountered chemodosimeters, the addition of Fe(III) to a solution of **8** led to the formation of a chelation product. However, this probe has an authentic chemodosimetric nature because the interaction of Fe(III) with **8** involved irreversible hydrolysis of the corresponding imine linkage as clearly shown by the ^{1}H NMR and fast atom bombardment mass spectroscopic (FAB-MS) data. As a result of its remarkable selectivity for Fe(III) as well as its water compatibility, **8** was then developed for monitoring Fe(III) in living cells. In this work, the Hep G2 cell line was chosen because Fe(III) can easily enter liver-origin cells and it is a well-established iron-overloading method.[126] The number of fluorescent cells as well as the intensity gradually increased over time, indicating that fluorescence is dependent on an intracellular reaction (panel A). Both the larger number of fluorescent cells and stronger intensities were much more evident for the iron-overloaded cells compared to the normal untreated cells (panel B). It appears, therefore, that this chemodosimetric reaction with free Fe(III) in liver cells, and the lack of any detrimental effect on the Fe(III)-based enzyme, means that this system can be used as a free Fe(III) detector in vivo (see Scheme 10).

Among the heavy metal ions, Cu(II) is of particular interest given its vital role in many cellular processes. These include those occurring in the human nervous system, gene expression, and the functioning and structural enhancement of proteins.[19,20,127] Conversely, if the concentration of copper exceeds that required in such cellular processes, it becomes toxic and can cause oxidative stress and disorders associated with neurodegenerative diseases such as Alzheimer's.[128] As mentioned in the introduction, fluorescence "turn-off" probes can result in false-positive results caused by other quenchers in real-world samples and, hence, are unpopular for practical analytical applications. However, for most of the reported

Scheme 10

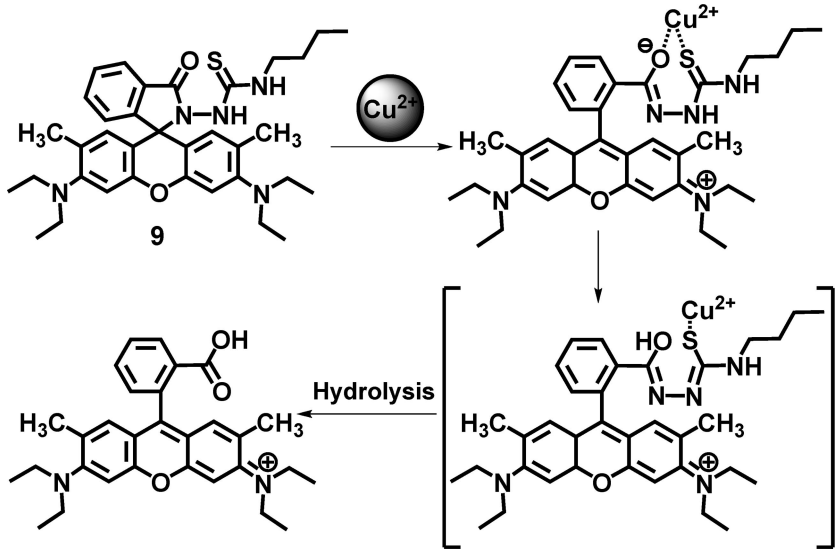


Cu(II) fluorescent chemosensors, because of the paramagnetic nature of Cu(II), the binding of the metal ion causes a quenching of the fluorescence emission and leads to a turn-off signal.[46−48,129−132] Fortunately, chemodosimeters function differently from general chemosensors and can provide an ideal way to design fluorescence "turn-on" probes for paramagnetic metal ions such as Cu(II). This is due to the fact that the fluorescent product does not coordinate to the metal ion. Recently, Huang and co-workers synthesized and studied the chemodosimeter **9**, a rhodamine-B derivative that can be used as a turn-on probe for monitoring Cu(II) in living cells.[133] A solution of **9** in CH_3CN is colorless and weakly fluorescent. This is indicative of the existence of the rhodamine-B hydrazide spiro-ring form. The addition of Cu(II) induces the appearance of a purple−red color and an intense orange−red fluorescence. It was proposed that the absorption and fluorescence behaviors result from consecutive processes. The addition of Cu(II) caused a Cu(II)-promoted ring-opening, followed by a redox reaction between **9** and Cu(II), leading to a reduction of Cu(II) to Cu(I). Next, irreversible hydrolysis of the corresponding imine linkage occurs, leading to the formation of the intensely fluorescent product. Such reactions can produce a fluorescent nonchelating compound that effectively avoids the fluorescence quenching caused by the paramagnetic nature of Cu(II). Probe **9** can operate successfully in aqueous solution at physiological pH. It gives a linear response in the 4.5−160 ppb range for Cu(II) and has a lower detection limit of 10 ppb. The time-dependent response, based on absorption, shows that when 20.0 μM of **9** and ≤100 μM of Cu(II) are used, the response is observed within 1 min. An almost instantaneous response was observed in the case of lower concentrations of Cu(II) (≤1.0 μM). As a result of these promising results, this probe was developed for real-time tracking of Cu(II) in living cells. There was a significant difference in confocal fluorescence images between HeLa cells stained with Cu(II) and HeLa cells only. Bright-field images showed that the fluorescence signals were localized in the perinuclear region of the cytosol. This is indicative of the subcellular Cu(II) distribution being internalized into the living cells from the growth medium. Moreover, a more intense intracellular fluorescence can be observed using two-photon fluorescence microscopy (see Scheme 11).

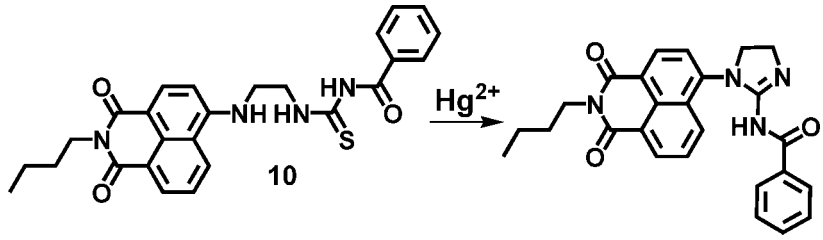
4.2. Intramolecular Cyclic Guanylation of Thiourea Derivatives

Guanidines are important compounds serving not only as building blocks for many biologically relevant compounds[134−136] but also as useful base catalysts in organic syntheses.[137−139] Accordingly, much attention has been paid to the synthesis of guanidines. This has resulted in the development of different methods for the guanylation of amines including the reaction modulation of amines with thiourea derivatives promoted by Mukaiyama's reagent[140] and the catalytic hydroamination of carbodiimides using Ti, V imido complexes and samarium diiodide (SmI_2) as catalysts.[141,142] In addition, the Hg(II) ion can be used to effectively trigger both inter- and intramolecular guanylation. Although the Hg(II)-promoted intramolecular transformation of thiourea into guanidine has been known for a long time, it had rarely attracted attention as a potential fluorogenic reaction because most thiourea deriva-

Scheme 11



Scheme 12

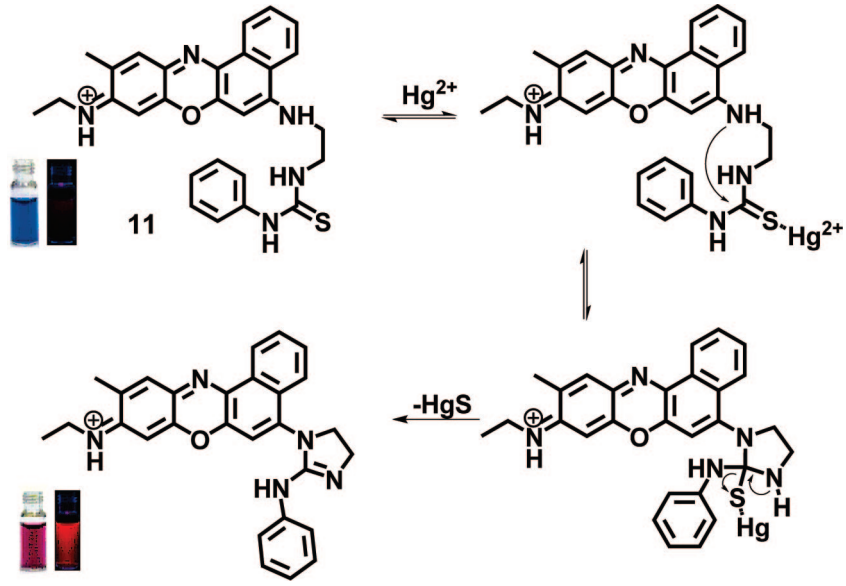


tives have the same color or fluorescence as that of the guanidine analogues. Liu and Tian were the first to report a fluorescent chemodosimeter based on this Hg(II)-promoted intramolecular cyclic guanylation.[143] In this work, a new napthalimide derivative (**10**) was synthesized and studied. When the derivative **10** itself was excited at 290 nm, it emitted dark-green light with a maximum emission at 530 nm. The addition of Hg(II) to a solution of **10** caused a cyclization of thiourea to form a deep, dark-turquoise fluorescent chemodosimeter with a maximum emission at 475 nm. A blue-shift in the wavelength of the maximum emission was explained as a result of the conversion of the thiourea group into the much weaker electron-donating imidazoline moiety. This resulted in a significant reduction in electron delocalization within the 1,8-naphthalimide fluorophore. Solution studies revealed a 1:1 stoichiometry for the Hg(II)-promoted cyclic guanylation of the chemodosimeter and a high selectivity of **10** for Hg(II) over other metal ions tested. The change in fluorescent color occurred over 1 h after addition of the Hg(II), but in the case of Ag(I), it required 20 h. Metal-ion selectivity experiments indicated that emission from **10** was unaffected by other metal ions such as Cu(II), Co(II), Ni(II), Zn(II), and Pb(II). Addition of Hg(II) to a solution of **10** induced an increase in emission at 475 nm with a concomitant decrease in emission at 530 nm. Therefore, **10** can provide a single-excitation dual-emission ratiometric detection of Hg(II) by a comparison of the intensity ratios at 475 and 530 nm before and after Hg(II) addition. However, the detection limit for Hg(II) remains unreported (see Scheme 12).

Although many efforts have been made to improve Hg(II) chemodosimeters with respect to metal ion selectivity, long-range excitation, emission wavelengths, and high quantum yield,[117,144,145] their biological applications are hampered by poor solubility in water and modest sensitivities. With such problems in view, Kim and co-workers recently focused on the synthesis of a new derivative of Nile Blue (**11**) and investigated its chemodosimetric properties for sensitive Hg(II) detection in 100% aqueous solution.[36] Probe **11** proved to be a highly efficient colorimetric and fluorimetric sensor for Hg(II) in the pH range 2−9 at room temperature. This chemodosimeter showed an excellent selectivity for Hg(II) over other cations. When 5.0 μM of probe **11** was employed, excess biologically active metal ions such as highly concentrated Na(I), K(I), Ca(II), and Mg(II) (200 equiv) did not interfere significantly with the Hg(II)-induced fluorescence response. The selectivity of **11** for Hg(II) was unaffected by heavy- and transition-metal ions such as Zn(II), Cd(II), Co(II), Fe(II), Ba(II), and Ni(II) (25.0 μM of each). In particular, the thiophilic metal ions Cu(II) and Ag(I) gave no detectable fluorescence changes in a solution of **11**. Fluorescence titrations of **11** with Hg(II) revealed a detection limit of 1.0 ppb. This is below the allowed level of Hg(II) ion in drinking water regulated by the U.S. EPA (2.0 ppb).[87] Furthermore, the selectivity and sensitivity of **11** toward Hg(II) were also confirmed in both blood plasma and albumin samples. Hence, the successful synthesis of **11** together with its selective and sensitive detection of Hg(II) in aqueous systems provides potential guidelines for future chemodosimeter research oriented toward biological or environmental applications (see Scheme 13).

On the basis of the colorimetric properties of azo-compounds and the guanylation of thiourea derivatives, Kim and co-workers synthesized and studied a select range of azo-component-containing chemodosimeters (**12**−**14**).[146] The addition of Hg(II) to an aqueous solution of **12**, **13**, or **14** gave an instantaneous color change from deep yellow to

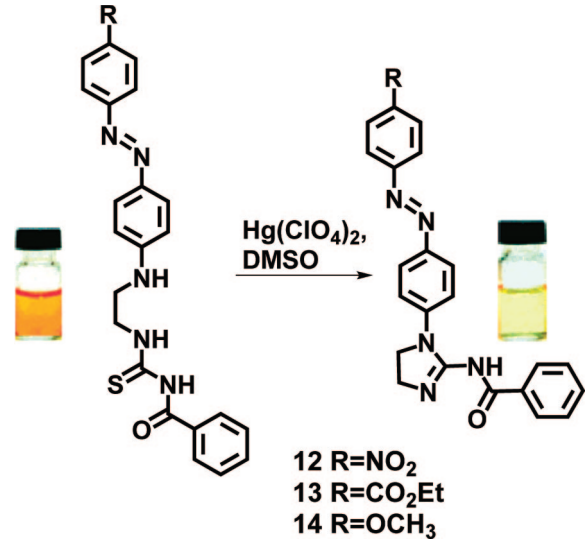
Scheme 13



colorless, along with a blue-shifted absorption band. The chemodosimetric mechanism responsible for the blue-shift in the UV/vis spectra is believed to result from a Hg(II)-triggered guanylation of thiourea, followed by desulfurization to form an imidazoline moiety. This is responsible for the decrease in the intramolecular charge-transfer properties of the azo-chromophore. Control experiments for a compound similar to **12**, but without the benzoylthiourea group, showed no spectral changes on addition of even 100 equiv of Hg(II). This indicates a crucial role of this group in the mercury-induced desulfurization. With respect to naked-eye detection of Hg(II), **12** appeared to be the best of the three receptors examined because it caused the biggest blue-shift (70 nm). **13** and **14** gave blue-shifts of 60 and 16 nm, respectively. The blue-shift response of **12** to Hg(II) was selective over other metal ions, which included Al(III), Pb(II), Zn(II), Cu(II), Cd(II), Co(II), Ca(II), Ba(II), Sr(II), Mg(II), Na(I), K(I), and Cs(I). A background mixture of these ions had no effect on the absorption of **12** and did not interfere with the Hg(II)-induced color response. Additionally, solution studies indicated a 1:1 stoichiometric reaction with a detection limit of 1.0 μM Hg(II) in a 1.0 μM solution of **12** (see Scheme 14).

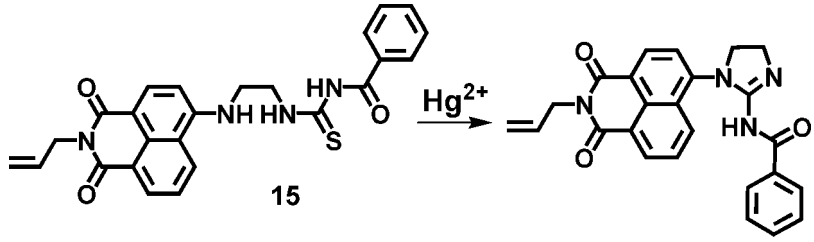
As seen in the use of many fluorescent probes, tracking metal ions in living cells is often carried out using confocal microscopy. This technique permits the acquisition of high-resolution, three-dimensional images of cultured cells. However, its use in deep tissue imaging is limited. Because of its deeper penetration, two-photon excitation can be a superior alternative to confocal microscopy. Additional advantages are the fact that, because two-photon excitation occurs only at the focal point of the microscope, photobleaching and photodamage, which are two major limiting factors in imaging living cells, are minimized. Recently, Tian and co-workers described a chemodosimeter **15**, bearing benzoylthiourea and naphthyllimide units, that was capable of tracking Hg(II) in solutions as well as living cells under single-photon and two-photon excitation.[147] Under single-photon excitation (SPE) at 405 nm or two-photon excitation (TPE) at 800 nm, the addition of Hg(II) to an acetonitrile/water solution of **15**, or the uptake of both **15** and Hg(II) into the living cells,

Scheme 14



caused a clear spectral blue-shift of approximately 50 and 32 nm, respectively. Change in the maximum wavelength emission for **15** was highly selective for Hg(II) over a wide range of other metal ions. There was no spectral shift, even in the case of a 500-fold amount of each ion, for Ca(II), K(I), Na(I), Mg(II), Ni(II), Fe(III), Cu(II), Pb(II), Zn(II), Mn(II), and Co(II). However, for Ag(I), the fluorescent response was similar to that of **15** with Hg(II), which limits the selectivity of this particular chemodosimeter. The sensitivity for Hg(II) in solutions was as low as concentrations of 5.0×10^{-10} M as detected by SPE and 2.5×10^{-8} M by TPE. In euglena gracilis (EG 277) living cells, the SPE spectra were disturbed by autofluorescence from the flavin fluorophore in the cells when the cellular Hg(II) content and concentration of **15** were low. The TPE spectra were still of high quality since this chemodosimeter has a larger two-photon absorption (TPA) cross section than the native fluorophores. The conclusion, therefore, is that two-photon excitation has a higher potential in the detection of Hg(II) in living cells (see Scheme 15).

Scheme 15



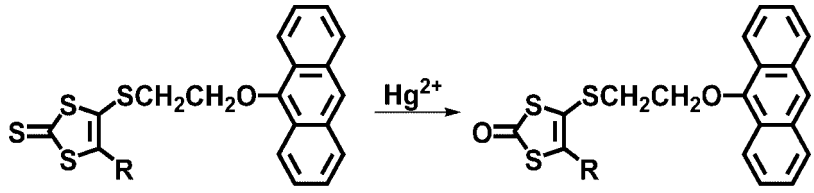
4.3. Conversion of Thiocarbonyl Compounds into Their Carbonyl Analogues

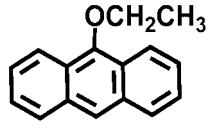
Conversion of thiocarbonyl compounds into their carbonyl analogues has attracted the interest of synthetic organic chemists.[148] These conversions include oxidative procedures that involve both inorganic and organic reagents, as well as hydrolytic reactions.[149−152] In the hydrolysis of thiocarbonyl compounds, processes catalyzed by metal ions result in clean reactions because the metal ion acts as an electrophile toward the thiocarbonyl group, and this results in a weakening of the C═S bond.[148] Of the most frequently employed metal ions, Hg(II), Ag(I), Au(III), Fe(III), and Cu(I), the Hg(II) ions are especially effective. For example, the desulfurization of thioesters proceeds rapidly using solutions of Hg(II) carboxylates in chloroform or pyridine.[153]

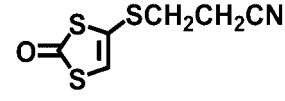
Zhu and co-workers reported the chemodosimeters **16** and **17**, which are based on 1,3-dithiole-2-thione derivatives containing an anthracene unit.[145] Both compounds showed weak fluorescence with rather low quantum yields, given that there is an overlap between the fluorescence spectrum of the anthracene portion and the absorption spectrum of the 1,3-dithiole-2-thione portion,[154−158] leading to FRET from the excited anthracene to the 1,3-dithiole-2-thione. In contrast, there was almost no spectral overlap between the anthracene portion and 1,3-dithiol-2-one. As a result, the corresponding energy transfer process between **18** and **19** was unfavorable. The result, therefore, was that **18** and **19** showed strong fluorescence with a relatively high quantum yield, ∼18-fold greater than **16** and **17**. According to previous studies,[159] because of the interaction of the Hg(II) ions (soft acid) with the sulfur atoms (soft base) of 1,3-dithiole-2-thione, the carbon atom of the C═S bond becomes more electrophilic and hence is more easily attacked by water. This leads to the conversion of **16** and **17** into **18** and **19**, respectively. Thus, in these cases, the presence of water was required for the transformation. When excited at 370 nm, **18** showed three emission maxima, with the strongest one centered at 418 nm. An ∼10-fold fluorescence enhancement was observed after the addition of Hg(II) (1.0 equiv) to a solution of **18** (10.0 mM) for 30 min at 40 °C. Metal-ion selectivity experiments indicated that the emission from **18** was insignificantly affected by the following: Ag(I), Ba(II), Co(II), Mn(II), Ni(II), Pb(II), Zn(II), and Fe(III). Competition experiments showed that Hg(II)-triggered fluorescence enhancement was not hampered by the presence of Ag(I), Ba(II), Co(II), Mn(II), Ni(II), Pb(II), and Zn(II). Interference from other high oxidation state metal ions such as La(III), Ce(III), Nd(III), Y(III), and Gd(III) can also be neglected. The detection limit of **18** for Hg(II) was found to be 5.0×10^{-8} M under the experimental conditions (see Scheme 16).

On the basis of the 8-hydroxyquinoline (8-HQ) scaffold, Chang and co-workers synthesized and studied **20** as a potential selective chemodosimeter for Hg(II).[160] Thioamides are in general readily oxidized, and in this system, the thioamide group could act as a strong intramolecular quenching entity for the 8-HQ-based fluorophore.[50] In 30% aqueous acetonitrile, the thioamide **20** showed a very weak fluorescence centered around 475 nm. After the addition of various metal ions, including Na(I), K(I), Mg(II), Ca(II), Ni(II), Cu(II), Zn(II), Cd(II), and Hg(II), it was found that only Hg(II) induced an enhancement in fluorescence intensity. This was so large that, with only 1.0 equiv of Hg(II), the fluorescence intensity at 479 nm increased 167-fold with a moderate shift in the emission maximum from 468 to 479 nm. Other representative metal ions examined revealed virtually insignificant responses with a relatively constant

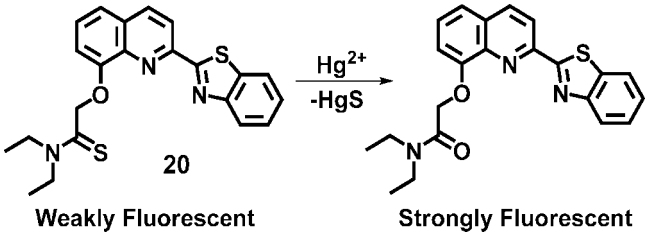
Scheme 16







Scheme 17

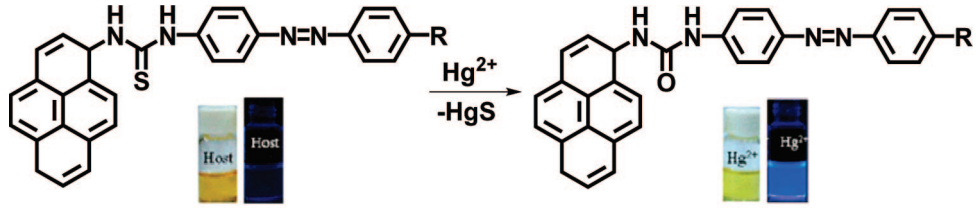


fluorescence enhancement factor less than 2.0-fold. An exception was Cd(II), which gave a 3.8-fold increase. The titration of **20** with Hg(II) in 30% aqueous acetonitrile revealed a detection limit of 5.4×10^{-7} M. This system was also tested for the analysis of Hg(II) using a practical sample. The possible interference from other metal ions was assessed by measuring Hg(II)-induced fluorescence changes of **20** in the presence of background metal ions. Among the tested metal ions, Cd(II) and Ag(I) were found to interfere by diminishing the fluorescence intensity of the **20**−Hg(II) system by up to 10 and 13%, respectively. This potentially limits the practical applicability of **20** toward the analysis of samples with high concentrations of these two ions. However, a favorable profile of a Hg(II) ion-dependent fluorescence change was observed in the presence of background physiologically important metal ions.[161] This indicates suitability for the analysis of real samples in a pseudophysiological environment (see Scheme 17).

Yen and co-workers reported the synthesis of the chemodosimeters **21**−**23** and the photochemical elucidation of their selective color and fluorescence changes toward Hg(II).[162] In each of these, the thiourea group acted as an ionophore. The azobenzene moiety underwent a color change due to the electronic effects of the substrate species in the event of the Hg(II) ion-induced chemodosimetric desulfurization. The pyrene group was ultimately responsible for the fluorogenic behavior. The chemodosimeter **21** exhibited three characteristic maximum absorptions at 279, 350, and 397 nm. The addition of Hg(II) to a solution of **21** in DMSO/H_2O (v/v = 9/1) resulted in red-shifts of the maximum absorptions from 279 and 350 nm to 285 and 368 nm, respectively. There were color changes from gold to pale yellow and an isosbestic point at 392 nm. An important feature of the chemodosimeter **21** was its high selectivity toward Hg(II) over other metal ions such as Na(I), K(I), Mg(II), Ca(II), Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Ag(I), Pb(II), Zn(II), and Cd(II). The miscellaneous competitive cations did not cause any significant spectral change in **21** and did not interfere with Hg(II)-induced absorption changes. Of all the various metal ions tested in the fluorescence experiments, including Na(I), K(I), Mg(II), Ca(II), Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Ag(I), Pb(II), Zn(II), Cd(II), and Hg(II), only Hg(II) induced a large fluorescence intensity enhancement (63-fold) with fluorescence changes from dark to bright blue. The fluorescence changes were unaffected by the presence of miscellaneous competitive cations. Solution studies revealed a 1:1 reaction stoichiometry of **21** for Hg(II). The colorimetric and fluorescent detection limits in dimethylsulfoxide (DMSO)/H_2O (v/v = 9/1) were 10.0 and 0.5 μM, respectively. The authors also modified the substituents on the phenyl ring of the azobenzene group with the purpose of creating an even better chemodosimeter. The 4-trifluoromethylazobenzene group was replaced by either a more electron-withdrawing group, 4-nitroazobenzene, or a less electron-withdrawing group, azobenzene. However, both **22** and **23** showed photochemical changes similar to **21** after addition of Hg(II). The greatest change in quantum yield was for **21** from 0.0043 to 0.253 whereas only smaller changes were observed for both **22** and **23**. It can be concluded, therefore, that **21** appears to be the best for Hg(II) detection by fluorescence. Furthermore, on the basis of the successful color changes, test strips were prepared with the purpose of providing rapid facile detection of the metal ions. The results showed that an immediate obvious color change was observed only with the Hg(II) solution. Colorimetric changes of the test strips were also used for sensing different Hg(II) concentrations, exhibiting that these were detected by the naked eye. This technique can be used to detect a concentration of Hg(II) as low as 5.0×10^{-4} M (see Scheme 18).

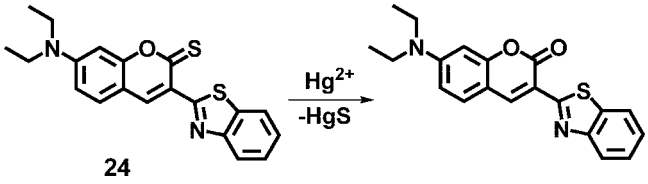
Coumarin and its derivatives are widely used fluorophores possessing favorable physical and optical properties and stabilities.[163] A simple substitution of the oxygen atom of the ligand with a sulfur frequently imparts a dramatic change in the resulting compound's ability to form various complexes.[164−167] With these in mind, Chang and co-workers synthesized a new thiocoumarin-based chemodosimeter (**24**) and studied the Hg(II)-promoted desulfurization of this thiocoumarin into a coumarin derivative for Hg(II) signaling.[168] The thiocoumarin **24** exhibited a strong absorption band at 523 nm. Addition of Hg(II) to **24** caused a solution color change from pink to yellowish green, with a blue-shift in the maximum absorption from 523 to 467 nm. Ratiometric behavior was observed in the optical absorption spectra: an absorption increase at 467 nm, a decrease at 523 nm, and an isosbestic point at 487 nm. A comparison of the I_{467}/I_{523} ratios before and after addition of 100 equiv of Hg(II) provided an 840-fold ratiometric change. The other metal ions, on the other hand, showed relatively constant I_{467}/I_{523} values between 0.30 and 0.39. The titration of **24** with Hg(II)

Scheme 18



21 R = CF_3
22 R = NO_2
23 R = H

Scheme 19



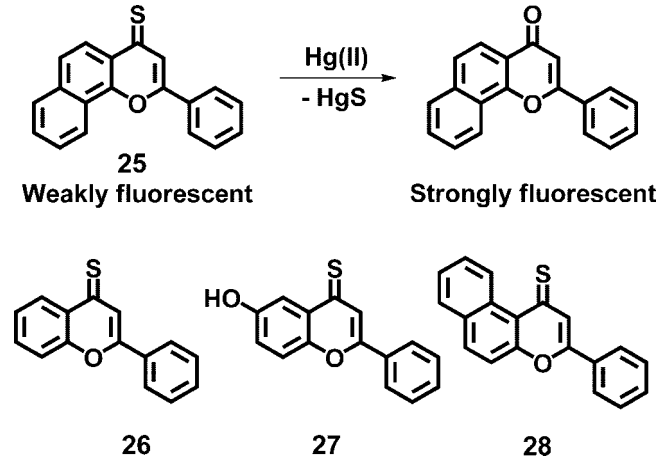
24

revealed a detection limit of 1.7×10^{-6} M. Thiocoumarin **24** exhibited relatively weak fluorescence centered at 511 nm in HEPES-buffered 50% aqueous acetonitrile. Addition of 1.0 equiv of Hg(II) to **24** afforded a 25.8-fold fluorescence enhancement and a change in the fluorescence color from dark to bright green. Among the thiophilic metal ions tested, only Ag(I) showed a minor enhancement at 557 nm. Other metal ions exhibited almost no change in emission behavior. From the concentration dependence of the fluorescence change, the detection limit of **24** for the determination of Hg(II) was estimated to be $\sim 8.9 \times 10^{-7}$ M. Metal-ion selectivity experiments indicated that both absorption and emission from **24** were unaffected by Na(I), K(I), Mg(II), Ca(II), Fe(II), Co(II), Ni(II), Cu(II), Zn(II), and Pb(II). Even the representative thiophilic ions Ag(I) and Cd(II) had no effect. The selective fluorescence signaling of **24** with Hg(II) in the presence of potentially interfering metal ions was also investigated. The fluorescence of the **24**−Hg(II) system containing **24** and 10 equiv of Hg(II) was affected by <10% in the presence of 100 equiv of other physiologically or environmentally important background metal ions (see Scheme 19).

Very recently, Chang and co-workers reported a simple chemodosimeter system (**25**) for selective Hg(II) signaling that is based on the Hg(II)-induced transformation of flavothione into flavone.[169] The flavothione **25** exhibits a weak fluorescence centered around 440 nm, as well as a pronounced off−on type signaling toward Hg(II). In a series of experiments with various metal ions, the fluorescence intensity at 438 nm was markedly enhanced (by 45-fold) only with Hg(II); other metal ions gave negligible responses (I/I_o varied between 0.57 for Na(I) and 1.79 for Cd(II)). Except for Cu(II), competition experiments confirmed that the fluorescence increase was not significantly affected by the presence of other metal ions at 100 equiv. In the presence of Cu(II), the fluorescence increase was somewhat less effective, reaching 83% of the **25**−Hg(II) system. The decreased fluorescence might be due to the quenching nature of the paramagnetic Cu(II). The detection limit of **25** for Hg(II) was estimated to be 1.6×10^{-6} M. The authors also assessed the potential of a series of other flavothiones **26**, **27**, and **28** for Hg(II) signaling. However, under the same experimental conditions used for **25**, none of these were sufficiently strong for an effective off−on-type Hg(II)-signaling system (see Scheme 20).

Because of their diverse molecular structures and optical properties that have great potential for use in optoelectronics and biologics, fluorescent organic nanoparticles (FONs) have become the subject of ever-increasing attention in recent years.[170−173] Li and Yan reported the effective ratiometric fluorescent chemodosimeter (**29**) for Hg(II). It was based on organic nanoparticles of the thioureathiadiazole−pyridine linked molecule (TTP).[174] TTP molecules readily self-assembled into colloidal nanoparticles as a result of reprecipitation when water was added as a nonsolvent to a THF solution. As evidenced by ^{1}H NMR data, two TTP monomers

Scheme 20



25 **26** **27** **28**

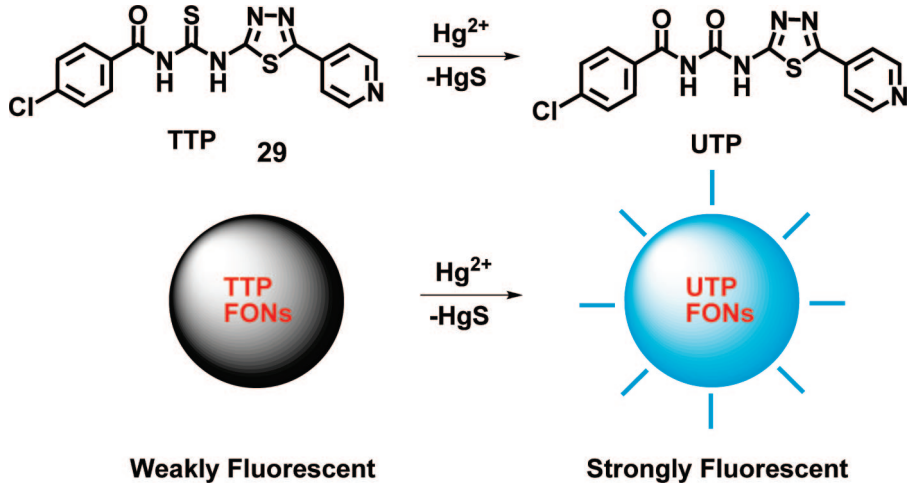
formed a dimer through hydrogen bonds and $\pi-\pi$ stacking interactions. TTP formed dimers as the preaggregate, and these dimers were the actual building blocks for the solid state and were brought together via van der Waals interactions. The weak and diffuse nature of the van der Waals driving forces for the association of the dimers led to the formation of TTP nanoparticles. When excited at 330 nm, the free TTP FONs showed a typical maximum emission at 403 nm. Upon addition of Hg(II), ratiometric behavior could be observed with an emission increase at 501 nm and a decrease at 403 nm. An obvious blue−green emission from the solution was easily observed by the naked eye under the illumination of UV light at 365 nm. The effects of potentially interfering metal ions, such as Na(I), Ca(II), Ni(II), Cd(II), Cu(II), Ag(I), Pb(II), Mn(II), and Mg(II), were found to be fairly insignificant in aqueous solution. In comparison with TTP FONs, the TTP monomer did not appear to be a selective sensor for Hg(II), the specific target metal ion. The ratios of the intensities (I_{501}/I_{403}) varied over a small range between 0.1 and 0.21 for the metal ions screened, including the Hg(II). It is reasonable to conclude that the TTP FONs play an important role in the selective luminescence response to Hg(II) (see Scheme 21).

4.4. Mercuration

The mercuration reaction has been known for a long time. The final organic mercury products, sometimes called organomercurials, contain covalent bonds between carbon and mercury. Numerous organomercurials have been synthesized with some finding uses in organic synthesis.[175−181] Some have also been used for the detection and determination of sulfide and hydrogensulfide ions,[182−185] whereas, in other cases, the mercuration reaction itself provides a means of Hg(II) sensing based on spectroscopic changes. The following section discusses some chemodosimeters based on such reactions that are used for Hg(II) detection.

Chang and co-workers reported a dichlorofluorescein-based chemodosimeter **30** for the selective determination of Hg(II).[186] The chemodosimeter **30** exhibited characteristic absorption bands at 475 and 505 nm in acetate-buffered aqueous 10% DMSO solution. The addition of Hg(II) caused these bands to gradually decrease with a concomitant red-shift to 483 and 533 nm, respectively. The color of the solution changed from yellowish green to orange. Metal-ion selectivity experiments accessed by the absorbance ratio of A_{533}/A_{483} indicated that **30** was selective for Hg(II) (A_{533}/

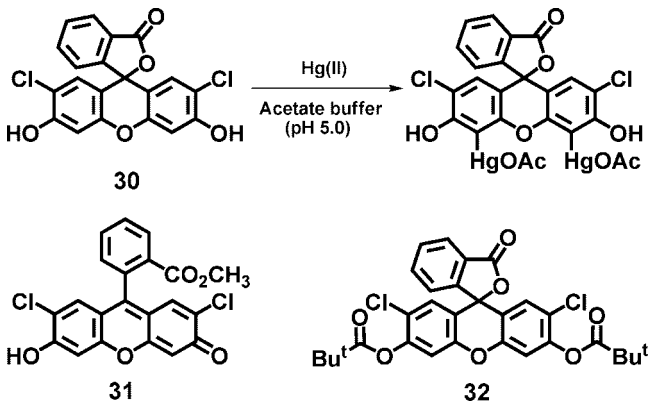
Scheme 21



A_{483} = 1.02). Other metal ions had ratios ranging from 0.058 (Ni(II)) to 0.085 (Ag(I)). Compound **30** exhibited a maximum emission at 528 nm with a band effectively quenched after addition of Hg(II). While Hg(II) gave a 900-fold fluorescence quenching, other metal ions failed to induce noticeable changes in the fluorescence emission. Unlike many other chemodosimeters, which often give rise to fluorescence enhancement on addition of Hg(II), the interaction of **30** with Hg(II) resulted in a decrease in the fluorescence intensity. This can be explained by the fact that, in most cases, the signaling molecules did not contain Hg(II) whereas, in this case, the Hg(II) was covalently bonded to the final signaling molecule. This leads to fluorescence quenching. Hg(II) is a heavy metal ion that can quench fluorescence by several mechanisms.[187−190] Moreover, the addition of Hg(II) caused a significant emission color change from bright green to almost colorless, which was visible by eye when a 5.0 × 10^{-6} M solution of **30** was excited with 47 nm light by a handheld UV lamp. A background mixture of Na(I), K(I), Mg(II), Ca(II), Ba(II), Fe(II), Co(II), Ni(II), Cu(II), Zn(II), Ag(I), Cd(II), Hg(II), and Pb(II) had no effect on the emission of **30** and did not interfere with the Hg(II)-induced fluorescence response. When 2.5 × 10^{-5} M **30** was used, the detection limit for Hg(II) was estimated to be 7.5 × 10^{-6} M (see Scheme 22).

A series of fluorescein derivatives were also studied for their chromogenic and fluorogenic behaviors. The spectroscopic response of **31** after the addition of metal ions was similar to that of **30**. However, **31** showed somewhat less sensitive Hg(II) signaling (detection limit = 1.5 × 10^{-5} M). In contrast, the spectroscopic properties of the derivative **32** remained almost unchanged after Hg(II) addition. These additional experiments clearly showed the importance of the phenolic moieties of the xanthene in the signaling process.

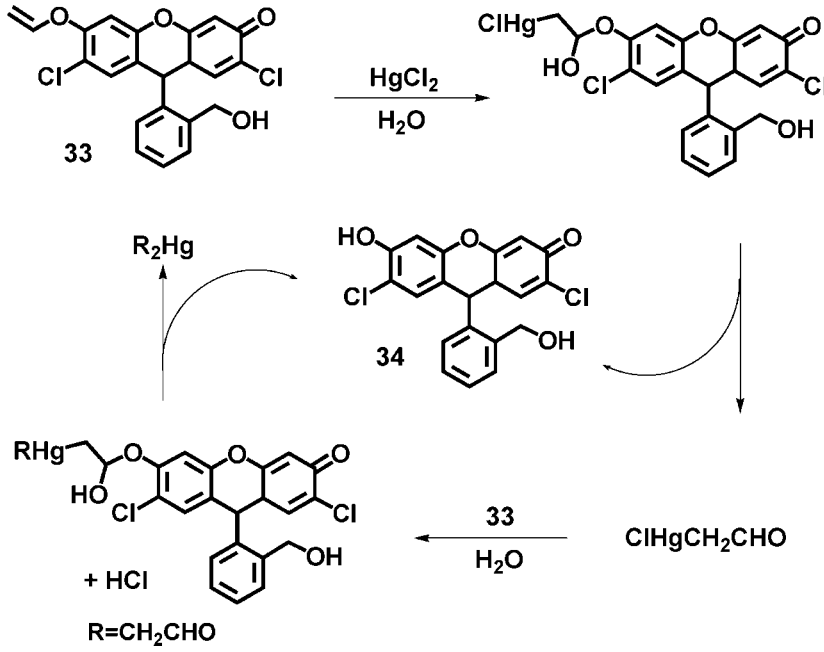
Scheme 22



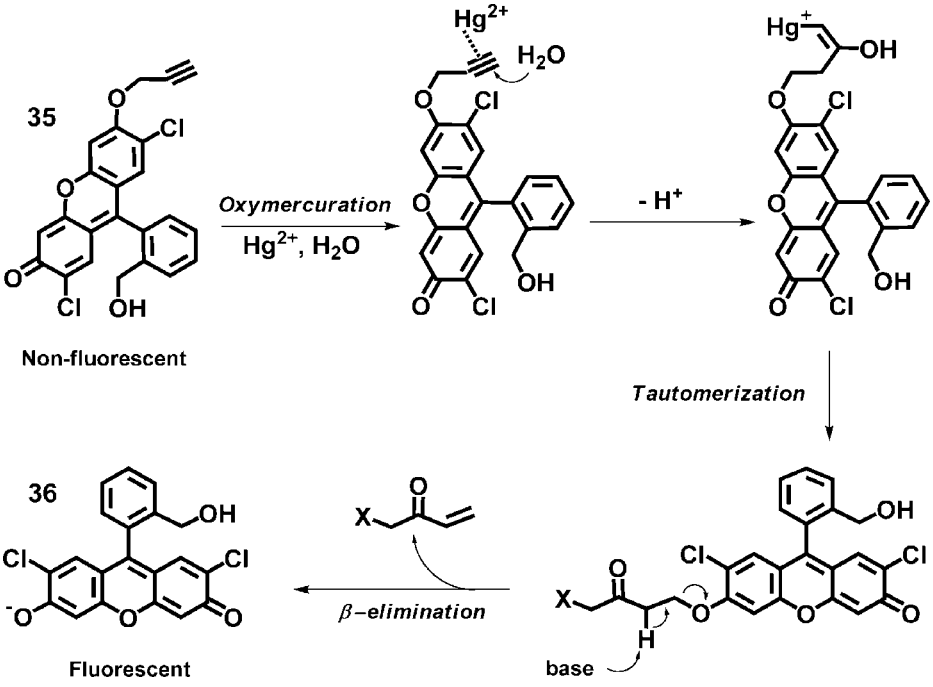
It is known that some prokaryotes that live in the sediments of aqueous environments can convert inorganic Hg(II) into methylmercury, a potent neurotoxin that concentrates throughout the food chain in the tissues of fish and marine mammals. The ingestion of methylmercury by humans from seafood is connected to serious sensory, motor, and cognitive disorders.[25,191−194] However, to date, most spectroscopic probes developed are specific to inorganic mercury ions; those for methylmercury remain rare. Recently, Ahn and co-workers designed a structurally simple but efficient fluorescent chemodosimeter (**33**) for methylmercury.[195] A solution of **33** in phosphate-buffered saline (PBS) buffer containing 5% DMSO, which is very weakly fluorescent, can be used as a turn-on probe for Hg(II) while it undergoes Hg(II)-promoted hydrolysis to give the strongly fluorescent fluorescein **34**. Metal-ion selectivity experiments showed an excellent selectivity of **33** for Hg(II) over other cations, including Mg(II), Ca(II), Ba(II), Cr(II), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Pb(II), and Ag(I). This has been attributed to the sparse hydrolysis of the vinyl ether with these particular cations, which means that they do not interfere significantly with the Hg(II) response. A detection limit < 1.0 ppb and a linear response up to 1 000 ppb were obtained. The chemodosimeter **33** was also studied for monitoring CH_3HgCl species in living organisms using three-day-old zebrafish. The results of the fluorescence microscopy analysis showed that the CH_3HgCl species in zebrafish was successfully detected using fluorescence. The CH_3HgCl species were found in the eye, heart, fin, gall bladder, and eggs of the zebrafish, but not in the brain and liver. These preliminary in vivo studies clearly demonstrate that the probe has potential for analyzing the accumulation of methylmercury species in other cells and organisms (see Scheme 23).

Koide and co-workers also reported a mercuration-based chemodosimeter (**35**) for sensing Hg(II) and CH_3HgCl species.[196] After the addition of Hg(II) to a solution of **35**, the metal ion catalyzed the hydration of alkynes to form the corresponding ketones. This was followed by a β-elimination process, resulting in a strongly fluorescent molecule **36** (ca.

Scheme 23



Scheme 24



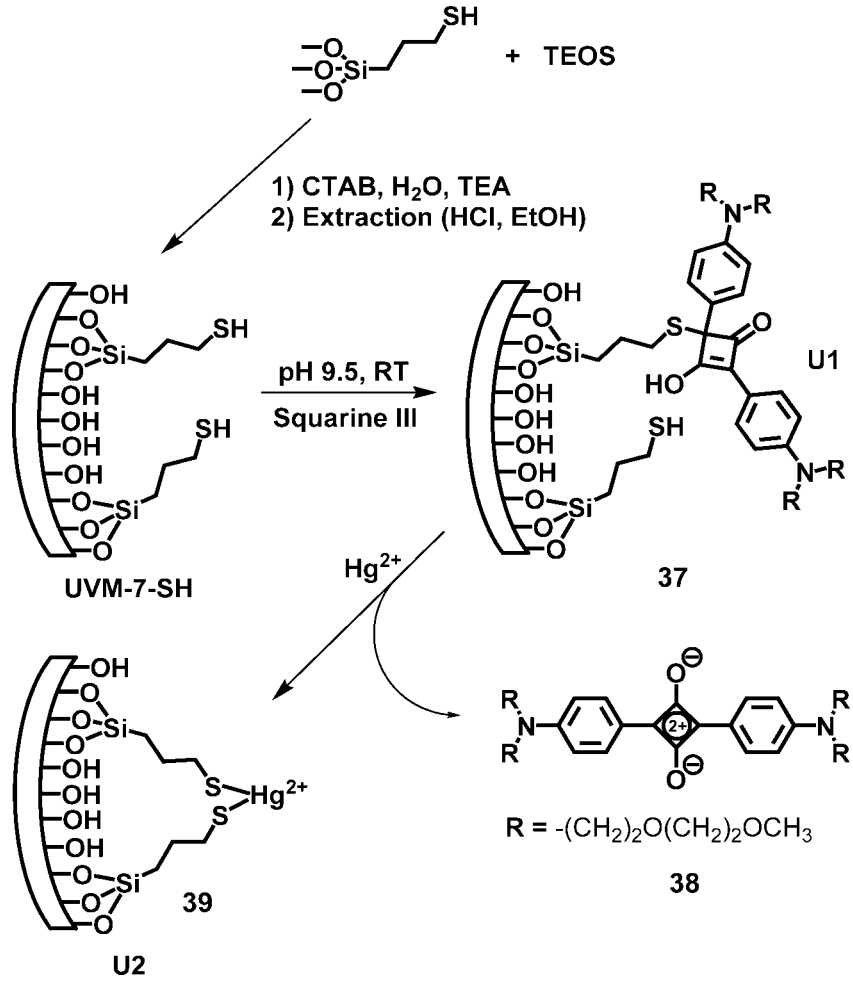
200-fold fluorescence enhancement compared to **35**). When 0.1 μM of **35** was employed, a detection limit in the ppb range was obtained. The metal ions Li(I), Na(I), Mg(II), Ca(II), Ba(II), Ni(II), Zn(II), Cu(II), Cr(III), Co(II), Mn(II), Pb(II), Cd(II), Fe(III), and Ag(I) all interfered only nominally (<8%) with the Hg(II) response. Furthermore, **35** proved to be compatible with strong oxidants such as *N*-chlorosuccinimide (NCS). This compatibility was crucial because most mercury samples typically contain oxidants. The authors also studied the ability of the chemodosimeter **35** to monitor mercury species in biological samples. Their results suggest that **35** can be used to monitor mercury concentrations in fish and potentially other species. Moreover, **35** was also found to be capable of detecting leached mercury from amalgam dental fillings (see Scheme 24).

5. Hybrid Chemodosimetric Materials

The combination of supramolecular and nanomaterial chemistry, related to chemosensors/chemodosimeters, brings new perspectives regarding the applicability of heterosupramolecular concepts to sensing protocols. Although a great deal of hybrid sensory materials based on chemosensors have been prepared,[197−206] those based on chemodosimeters are still very rare.

Recently, Martínez-Máñez, Rurack, and co-workers designed a 3D hybrid chemodosimetric material (**37**) capable of use as a sensor for Hg(II) detection.[207] In the first step, a thiol-containing derivative of mesoporous UVM-7 was prepared through co-condensation protocols designed to favor good sulfhydryl group (SH) dispersion. The UVM-7 was then

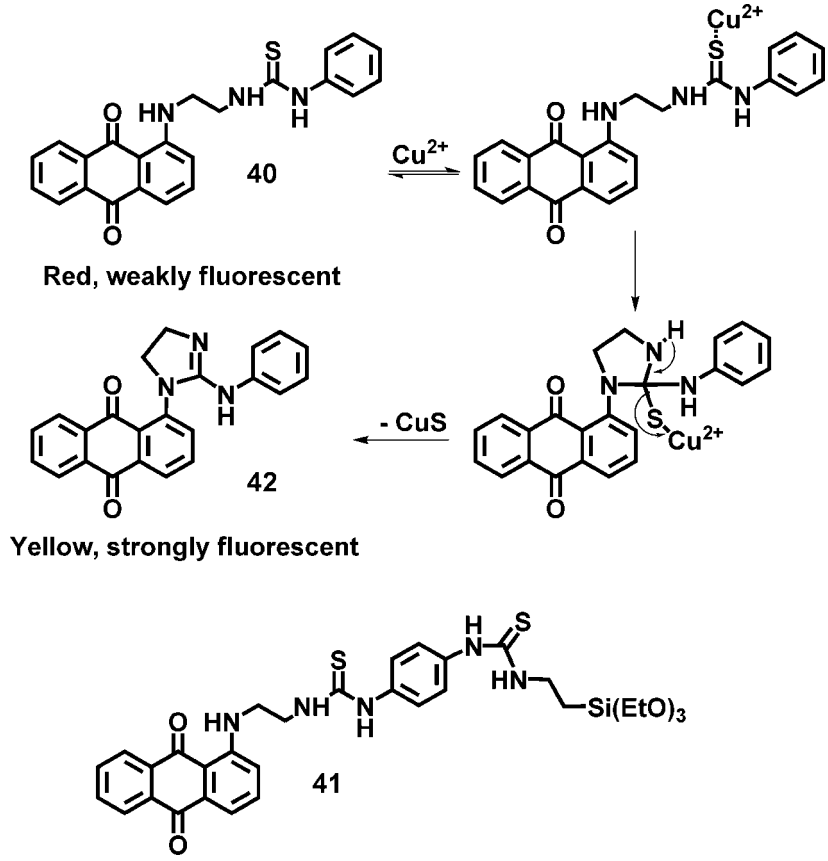
Scheme 25



treated with HCl to yield UVM-7−SH. Treatment of UVM-7−SH with a squaraine dye derivative in acetonitrile/water, under a slightly basic medium, produced a white solid (**37**), containing the 2,4-bis(4-dialkylaminophenyl)-3-hydroxy-4-alkylsulfanylcyclobut-2-enone (APC) derivative covalently anchored to the silica matrix. Reaction of Hg(II) with the APC fragment in **37** released the squaraine dye (**38**) into solution, which turned deep blue and fluoresced strongly. The response was noteworthy because both absorption and emission were found at the far end of the visible spectral window (642 and 670 nm, respectively).[207,208] Here, interference due to matrix absorption or autofluorescence of the sample is usually negligible. Additionally, this dramatic hyper- and bathochromic shift on mercury-induced release of **38** allowed for straightforward naked-eye detection of Hg(II) using a facile procedure. The absorption response of **37** to Hg(II) was selective over Pb(II), Ni(II), Cd(II), Zn(II), Cu(II), and Fe(III). The presence of other thiophilic cations such as Pb(II) and Cd(II) only led to very minor dye release at relatively high concentrations of 1.0 mM. **37** also showed no response to the presence of alkali and alkaline earth metal ions or anions ubiquitously present in the water, such as chloride, carbonate, sulfate, and phosphate. In this paper, the role of the inorganic skeleton in the sensing processes was also studied. The authors showed that the presence of nanoscopic channels in **37**, in which the proximal thiol groups were located at a concave surface, seemed to facilitate enhanced binding of the target rather than the flat surfaces of the 2D materials. The latter can vary locally and be very inhomogeneous with flat, convex, and concave microdomains. This is especially true for the reference solid analogous to **37** that is prepared from 2D silica materials. Once Hg(II) enters the pores, it reacts more readily with the APC moieties in the confined space, resulting in a more efficient release of the squaraine dye to the solution. The sensitive response of **37** to Hg(II) was also found to be related to the particle size. The experiment showed that, under the same conditions, in the presence of Hg(II), nanoscale **37** led to a squaraine absorbance up to 5 times higher than a reference solid with a typical microscale MCM-41 structure. Thus, when using mesoporous supports, **37** displayed a more sensitive response in the presence of Hg(II), stressing the importance of the textural (nano- versus microscale) shape of the particulate material for signaling performance. It is noteworthy that the chemodosimeter **37** can be partially regenerated by simple washing with concentrated HCl. This transforms the product (**39**) back into UVM-7−SH, which is then ready for chemodosimeter regeneration by reaction with the squaraine derivative **38** (see Scheme 25).

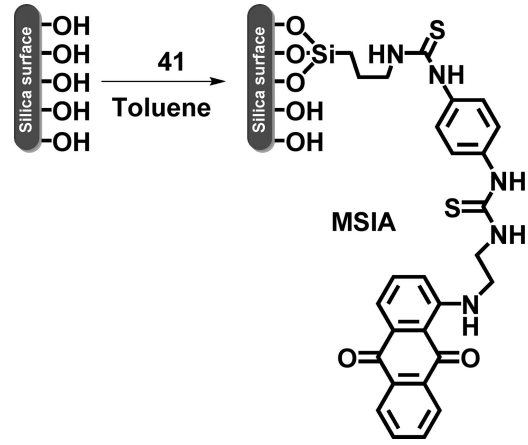
By combining the good features and advantages of both chemodosimeters and nanomaterials, Kim et al. designed an anthraquinone-based chemodosimeter (**40**). The mesoporous silica-immobilized anthraquinone (MSIA) component is responsible for the Cu^{2+}-induced desulfurization subsequently followed by cyclization. This provides highly selective UV and fluorescence changes.[13] A solution of **40** in CH_3CN was deep red and displayed an intensive intramolecular charge transfer (ICT) absorption band centered at 500 nm. The

Scheme 26



addition of Cu(II) induced a distinct blue-shift of 50 nm and produced a pale yellow solution. The highly desirable feature whereby no significant spectral changes were observed with the introduction of other metal ions such as Li(I), Na(I), K(I), Rb(I), Cs(I), Cd(II), Ag(I), Pb(II), Zn(II), Sr(II), Ba(II), or Ca(II) was confirmed. In order to develop **40** into a hybrid chemodosimetric material, it was attached to a (3-aminopropyl)triethoxysilane. This produces a precursor of MSIA (**41**), which is then coupled with mesoporous silica in toluene to produce the final MSIA material. In the absence of specific guest ions, a suspension of the MSIA produced a weakly fluorescent red color, consistent with the spectrum of **40**. In the presence of Cu(II), however, the MSIA suspension showed a remarkably enhanced fluorescence emission centered at 560 nm with a concomitant color change from red to yellow. Conversely, no significant changes in fluorescence emission or color were observed in control experiments with Li(I), Na(I), K(I), Mg(II), Ba(II), Ca(II), Sr(II), Co(II), Cd(II), Pb(II), Zn(II), and Ag(I). From the spectroscopic changes observed, Kim et al. concluded that the MSIA possessed a high selectivity for Cu(II) ions over other metal cations in that it showed a similar spectroscopic response to that of **40**. These results led to the conclusion that the MSIA-based chemodosimeter has considerable potential for environmental applications as a new organic−inorganic hybrid sensor for the detection of Cu(II). In the mechanism of the functioning chemodosimeter **40**, two different conformations can be adopted: a nonplanar structure in ethyl acetate or a planar ICT structure in CH_3CN.[209,210] Kim et al. made the assumption that the 1-NHR substituent is in the same plane as the 9,10-anthraquinone moiety, with the lone pair of electrons of the amine in full resonance with the anthraquinone π-orbitals. However, after the Cu(II)-assisted cyclization, the

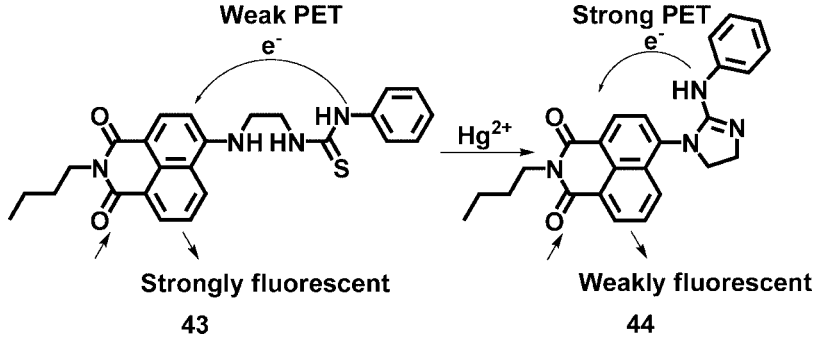
Scheme 27



1-NRR′ substituent of **42** is expected to be too sterically congested to adopt a planar ICT structure. This structural change significantly reduced the ICT character of the anthraquinone moiety, leading to a blue-shifted UV band and the fluorescence emissions of **40** and MSIA, also when these chemodosimeters interact with Cu(II) (see Schemes 26 and 27).

Leng, Tian, and co-workers described the chemodosimeter **43** as well as its hybrid material with gold nanoparticles (Au−NPs).[211] In a mixed DMSO−H_2O solution (1:1 v/v), **43** displayed a fluorescence emission with a maximum at 542 nm. The addition of Hg(II) to a solution of **43** induced a significant decrease in fluorescence emissions concomitant with a hypsochromic shift near 12 nm. The quenching was attributed to the enhanced photoinduced electron transfer (PET)[34,110,212−220] process from the aniline subunit to the naphthalimide chromophore as a result of their close proxim-

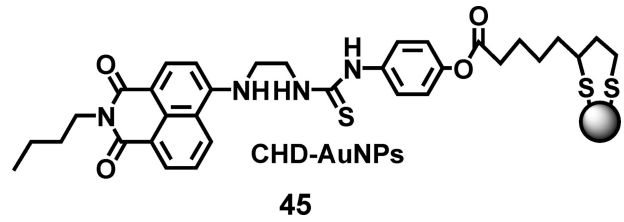
Scheme 28



ity within the new product (**44**). This probe was found to be highly selective for Hg(II) over many other ions such as Co(II), Cu(II), Hg(II), Ni(II), Pb(II), Zn(II), Cd(II), Mn(II), and Sn(II). Although Ag(I) and Hg(II) also gave an analogous reaction, their reactivity was lower and required a much greater time frame to reach comparable levels of emission intensities. Moreover, a background combined of all the above metal ions did not interfere with the Hg(II)-induced fluorescence response. This clearly indicates a higher reactivity of **43** toward Hg(II) over Ag(I). It appears, therefore, that **43** can be used as a potential fluorescent chemodosimeter for Hg(II) (see Scheme 28).

To improve practical applications in an aqueous environment, CHD−AuNPs (chemodosimeter-functionalized gold nanoparticles (**45**)) were prepared. The immobilization of the chemodosimeter onto the surface of the AuNPs, using the characteristic optical properties and the good dispersity of AuNPs in water, was exploited. Although the fluorescence response of the CHD−AuNPS, following introduction of Hg(II), was insignificant, the absorption spectra displayed interesting changes. In fact, there was almost no change in the wavelength range of <500 nm after addition of Hg(II) to a suspension of CHD−AuNPS, showing a low sensitivity of CHD−AuNPs for Hg(II) in water. The sensing ability was low because of the poor aqueous solubility of the chromophore monolayer on the AuNP surface.[221,222] The CHD−AuNPs were less compatible with water and they were not well dispersed, thus limiting their effective interaction toward the Hg(II) ions. Realizing that the sensing ability of the chemosensor could be modulated by a suitable surfactant,[221] Leng, Tian, and co-workers used the surfactant sodium dodecyl sulfate (SDS) to modulate the sensing capability of the CHD−AuNPs in water. Interestingly, addition of Hg(II) to an aqueous suspension of CHD−AuNPs, at a particular SDS concentration induced a significant increase in the absorbance at wavelengths of <500 nm, with only a slight variation at 520 nm. Enhancement of the sensing ability of the CHD−AuNPs in conjunction with the SDS environment can be explained by formation of micellar units. In the SDS environment, water compatibility is improved, leading to the organic chromophore monolayer on the surface of AuNPs more easily approaching the metal ions within the micellar environment. Moreover, the electrostatic interaction between the anionic SDS micellar surface and Hg(II) led to an increase in the local concentration of the metal ions in the vicinity of the detector. This ensures a more effective communication between the chromophores and the Hg(II) ions. Taking into consideration the changes in the absorption spectra of CHD−AuNPs, Leng, Tian, and co-workers proposed a route to sensing Hg(II) based on a ratiometric approach. The quantitative detection of the

Scheme 29



mercury ions in aqueous environments can be achieved by using the absorbance ratio at 300 and 520 nm, which is approximately proportional to the Hg(II) concentration (see Scheme 29).

6. Conclusions

Since 1992, when Czarnik described the first synthetic chemodosimeter for the selective determination of Hg(II) ions, the field of irreversible molecular sensors for heavy metal ions has been growing in interest. This review addresses the literature covering chromo- and fluorogenic chemodosimeters and chemodosimetric materials as sensory systems for sensing heavy metal ions in both solution and biological samples over the last five years. The chemodosimetric approach follows a specific chemical reaction between the dosimeter molecule and the target species. This allows for circumvention of the quenching phenomenon caused by the paramagnetic nature and the heavy atom effect, which means that the fluorescent product does not coordinate to metal ions as in the binding site/signaling subunit approach. Consequently, turn-on probes are favored over those exhibiting fluorescence quenching. This is due to the fact that the readout mechanism for the sensor response often proves undesirable for practical analytical applications.

The classification of chemodosimeters is based on structural changes of the reactants. It has been shown that the ring-opening of the spirocyclic systems has been widely studied, especially that of rhodamine derivatives. This is because, on the one hand, metal-induced ring-opening is very selective for a few heavy metal ions, whereas on the other hand, rhodamines possess important features suitable for designing sensors with excellent capabilities such as good water solubility, long emission wavelength, nontoxicity, and the ability to function at physiological pH.

In this review, we have also reported certain advances in the design of hybrid chemodosimetric materials that use a combination of supramolecular chemistry and nanomaterial chemistry. Although only a few relevant publications have appeared so far, their potential applications in heavy metal ion sensing should greatly encourage researchers to develop many more inorganic−organic composite materials in the

not-too-distant future. In general, a wide range of chemodosimeters have been developed and have proven to be good sensing systems in terms of wavelength, nontoxicity, selectivity, and sensitivity. However, only a few chemodosimeters function well in aqueous solutions, although many other systems display sensing properties in nonaqueous solvents. This is a very limiting factor in developing real applications. There is much research needed in these fields in order to develop better sensing systems for the detection of heavy metal ions in real-world applications.

7. Acknowledgments

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