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PAPER

A cell compatible fluorescent chemosensor for Hg²⁺ based on a novel rhodamine derivative that works as a molecular keypad lock

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A novel fluorescent chemosensor based on rhodamine derivative (**Rh1**) has been designed and synthesized for detection of Hg²⁺ ions, which exhibits high sensitivity and selectivity over other metal ions in aqueous solution and living cells. Moreover, this “Off–On”-type fluorescent sensor could successfully mimic a molecular level keypad lock in the presence of Cu²⁺ ions. Stimulated by the two chemical inputs (Hg²⁺ and Cu²⁺), **Rh1** undergoes transformation of the structure between spirocyclic and ring-opened spirocyclic, which results in the change of fluorescence. Significantly, the outputs of the system depend on not only the proper combination but also on the correct order of the input signals, which is the most important feature of the keypad lock system. Only a specific sequence of inputs, *i.e.* the correct password, results in strong fluorescence emission at 555 nm which can be used to “open” this molecular keypad lock. Therefore, this molecular keypad lock has the potential for application in security devices, which could be used to authorize a user, to verify authentication of a product, or to initiate a higher process.

Introduction

In recent years, various molecular switches, logic gates and logic circuits based on chemical systems capable of elaborating binary (Boolean) information have been proposed because of the wide variety of organic molecular designs, synthesis, and light-emitting properties that are available.¹ The combination of these molecular logic gates and logic circuits has been used for mimicking various electronic devices at the molecular level. The primary motivation for this is to develop novel paradigms for information processing that, by moving beyond silicon-based technology, could lead to electronic devices of extremely small size, low power consumption and unprecedented performance. Implementation of various mimicking electronic devices, such as memory units,² comparators,³ and demultiplexers,⁴ performing digital operations with numerous chemical systems is now possible. Recently, a keypad lock, which is an important electronic logic device, has been mimicked at the molecular level.⁵ This device can be used for numerous applications in which access to an object or data is to be restricted to a limited number of persons having the exact password to open the keypad lock. The most important advantage of a keypad lock system over simple logic gate is that its output signals are dependent not only on the proper combination of inputs but also

on the correct order by which these inputs are introduced. Therefore, the development of such molecular devices, capable of authorizing password entries, is a particularly attractive research goal as it represents a new approach for protecting information on molecular scale.

To mimic various electronic devices, numerous chemical systems responding to a large variety of activating input signals (*e.g.*, light, electrical, magnetic, and chemical) have been developed.^{1,6} Among these chemical systems, the most important one is fluorescent chemosensors with high selectivity and sensitivity for a specific analyte, such as heavy metal ions, anions, and pH. In particular, a fluorescent chemosensor for heavy metal ions not only can be used to detect the presence of the heavy metal ions (such as Hg²⁺ ions) in aqueous solution and living cells, but also to integrate these heavy metal ions as chemical-driven molecular machines in future molecular computing. Therefore, there is an urgent need to develop innovative and convenient fluorescent chemosensors for heavy metal ions (such as Hg²⁺ ions) because of the dual important application of a single molecule.

For fluorescent chemosensors, suitable fluorescence indicators that are sensitive to heavy metal ions (such as Hg²⁺ ions) concentrations and exhibit changes in fluorescence intensity have been used as molecular recognition materials. Among the numerous indicators, rhodamine derivatives are an excellent candidate due to their high fluorescence sensitivity to heavy metal ions, in addition to the excellent spectroscopic properties of large molar extinction coefficients, high fluorescence quantum yields and visible wavelength excitation. They can change their geometric structures among two states as a result of stimuli. Typically, in the absence of metal ions, the molecules prefer their

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spirolactam ring-closed geometry,⁷ which shows little absorption and fluorescence, whereas upon the addition of specific metal ions, the chelation or reaction of metal ions with sensor molecules will simultaneously open the spirolactam ring, which gives rise to a strong fluorescence emission.⁸ Simultaneously, they display a strong color development against the colorless blank during the sensing event, which is an important feature that would facilitate “naked-eye” detection. Therefore, many molecular switches and logic gates using rhodamine derivatives as indicator have been proposed.⁹ However, up to now, there has been no report on molecular keypad locks based on rhodamine derivatives as far as we know.

Herein, we intend to report the synthesis and characterization of a novel fluorescent chemosensor to detect Hg^{2+} both in aqueous solution and in living cells with rhodamine derivative **1** (**Rh1**) as fluorescent indicator. Moreover, **Rh1** has been identified to respond as On and Off state of fluorescence depending upon the sequence of addition of Hg^{2+} and Cu^{2+} ions into the solution. Based on this property, a molecular keypad lock based on rhodamine derivative **Rh1** was constructed whose fluorescence is in the On state in response to a specific sequence of chemical inputs (Hg^{2+} and Cu^{2+} ions).

Experimental section

Reagents and materials

Rhodamine 6G and hydrazine hydrate were supplied by Shanghai Chemical Reagents (Shanghai) and used as received. Lawesson's Reagent (2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide) and 2-hydroxy-1-naphthaldehyde were purchased from Alfa Aesar. Toluene was treated by sodium chips and then distilled. Ethanol was treated by magnesium ribbon and then distilled. Other chemicals are of analytical reagent grade and used without further purification except when specified. All inorganic metal salts were analytical grade and used without further purification. The water used in our present work was deionized. NaAc-HAc buffer solution was prepared using proper amount of NaAc and HAc (analytical grade) under adjustment by a pH meter.

Characterization

Absorption spectra were recorded with a Shimadzu UV-3000 spectrophotometer. Fluorescence spectra were measured with a Hitachi F-4500 fluorescence spectrophotometer with a 10 mm quartz cuvette. The excitation and emission wavelength band-passes were both set at 5 nm. ^1H and ^{13}C NMR spectra were recorded using a mercury-300BB spectrometer (Varian, USA) operated at 300 MHz with tetramethylsilane (TMS) as internal standard. Mass spectra were performed on Agilent 1100 MS series and AXIMA CFR MALDI/TOF (Matrix assisted laser desorption ionization/Time-of-flight) MS (COMPACT). All of the measurements were done at room temperature, about 298 K.

Synthesis of the materials

Synthesis of rhodamine-6G thiohydrazide. Rhodamine-6G hydrazide was prepared according to a literature method.¹⁰ The method used for the synthesis of rhodamine-6G thiohydrazide

was according to the procedure described in the literature.¹¹ Rhodamine-6G hydrazide (0.6 g, 1.3 mmol) and Lawesson's Reagent (0.52 g, 1.3 mmol) were dissolved in dry toluene, and the resulting mixture was refluxed and stirred for 4 h under N_2 atmosphere. The solvent was removed under reduced pressure to give a pink solid. The crude product was purified by flash column chromatograph using CH_2Cl_2 as eluent to afford rhodamine-6G thiohydrazide (yield: 16%). ^1H NMR (CDCl_3), δ (ppm): 1.30–1.35 (t, 6H, NCH_2CH_3), 1.91 (s, 6H, xanthene- CH_3), 3.22–3.24 (q, 4H, NCH_2CH_3), 4.83 (s, N- NH_2), 5.30 (s, NHCH_2CH_3), 6.14 (s, 2H, xanthene-H), 6.44 (s, 2H, xanthene-H), 7.08 (dd, 1H, Ar-H), 7.49 (dd, 2H, Ar-H), 8.12 (dd, 1H, Ar-H). FAB-MS m/z : [$1 + \text{H}$] $^+$ calc. for $\text{C}_{26}\text{H}_{28}\text{N}_4\text{OS}$, 444.2; found, 445.2.

Synthesis of rhodamine derivative Rh1. To a solution of rhodamine-6G thiohydrazide (0.047 g, 0.1 mmol) in absolute alcohol (30 mL) was added 2-hydroxy-1-naphthaldehyde (0.017 g, 0.1 mmol). After the addition, the mixture was refluxed with stir for 12 h. The solvent was evaporated under reduced pressure, and the crude product was purified by flash column chromatograph using CH_2Cl_2 as eluent to afford **Rh1** (yield: 80%) as a yellow solid. ^1H NMR (CDCl_3), δ (ppm): 1.29–1.34 (t, 6H), 1.92 (s, 6H), 3.18–3.25 (q, 4H), 5.30 (s, 2H), 6.30 (s, 2H), 6.60 (s, 2H), 7.12 (dd, 1H), 7.20 (dd, 1H), 7.40 (dd, 1H), 7.49 (m, 2H), 7.58 (m, 1H), 7.81 (t, 2H), 8.22 (m, 2H), 9.69 (s, 1H), 12.79 (s, 1H). ^{13}C NMR (CDCl_3), δ (ppm): 14.74, 16.78, 38.38, 64.68, 96.33, 105.89, 108.53, 119.44, 119.88, 120.13, 120.29, 122.26, 123.46, 127.21, 127.33, 127.64, 128.00, 128.10, 129.08, 130.00, 132.45, 134.00, 135.71, 136.41, 158.10, 158.33, 161.09, 166.786. FAB-MS m/z : [$1 + \text{H}$] $^+$ calc. for $\text{C}_{26}\text{H}_{28}\text{N}_4\text{OS}$, 598.24; found, 599.4.

Cell culture and imaging

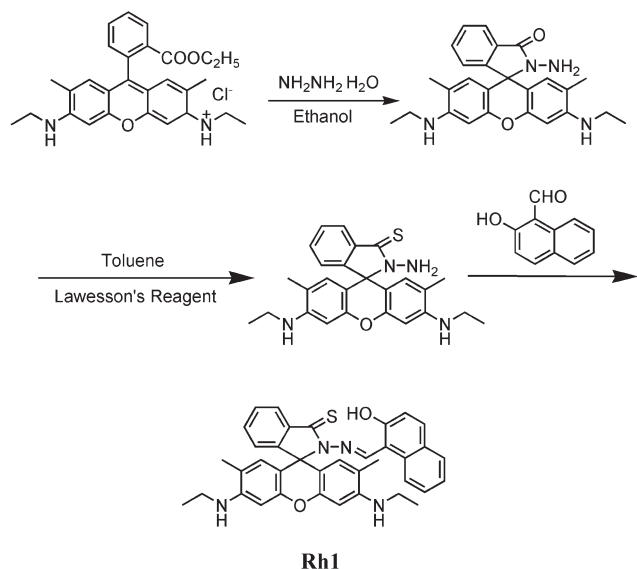
The Rat Schwann cells (RSC 96) were provided by Norman Bethune College of Medicine Jilin University (China). Cells were grown in H-DMEM (Dulbecco's Modified Eagle's Medium, High Glucose) supplemented with 10% FBS (Fetal Bovine Serum) in an atmosphere of 5% CO_2 , 95% air at 37 °C. Cells were plated on 6-well plate at 5×10^6 cells per well and allowed to adhere for 12 h. Fluorescence imaging was performed with an Olympus fluorescence microscope (BX51, Olympus, Japan). Immediately before the experiments, the cells were washed with phosphate-buffered saline (PBS) and then incubated with 10 μM of **Rh1** (in the culture medium) for 10 min at 37 °C. Experiments to assess Hg^{2+} uptake were performed in the same media supplemented with 50 μM $\text{Hg}(\text{ClO}_4)_2$ for 0.5 h.

Results and discussion

Rhodamine derivatives are generally non-fluorescent and colorless, whereas when they coordinate with some specific metal ions, ring-opening of the corresponding spirolactam gives rise to a strong fluorescence emission and a pink color. Bearing this in mind, we envisaged that, if introducing proper binding sites, it would be possible to achieve a rhodamine spirolactam based chemosensor highly selective for heavy metal ions (such as Hg^{2+} ion) *via* color/fluorescence changes. Based on the theory of hard and soft acids and bases (HSAB theory), Hg^{2+} is a representative example of soft acid, and S^{2-} is a soft base. Therefore, a sulfur-based functional group must be a good candidate as the S is a

strong binding site for Hg^{2+} ions. Based on the above reasons, we designed and synthesized a fluorescent chemosensor based on rhodamine derivative **Rh1** for detecting Hg^{2+} ions that works as a molecular keypad lock. **Rh1** contains an S atom, an N atom attached to the N-bearing spiro ring and an O atom from phenolic hydroxyl of 2-hydroxy-1-naphthaldehyde, which might be a highly selective chemosensor for detecting Hg^{2+} ions since the S, N, O binding sites are a good choice to be parts of a selective receptor. The synthetic procedure of this material is outlined in Scheme 1. Rhodamine-6G hydrazide was prepared according to a literature method,¹⁰ and treatment of it with Lawesson's reagent in refluxing toluene gave, after flash column chromatography on silica, the corresponding rhodamine-6G thiohydrazide in a yield of *ca.* 16%. **Rh1** was successfully synthesized with 2-hydroxy-1-naphthaldehyde and rhodamine-6G thiohydrazide as an intermediate. Both **Rh1** and rhodamine-6G thiohydrazide were characterized using various analytical spectroscopic techniques (see the Experimental Section), which agreed well with the proposed structures.

As mentioned above, we speculated the S, N, O binding sites might be sensitive towards the Hg^{2+} ion, thus we tested the effect of Hg^{2+} ions on the fluorescence behavior of **Rh1**. The fluorescence spectra were recorded upon excitation at 500 nm at room temperature with the gradual addition of small amounts of Hg^{2+} ions (0–10 equiv, 0–100 μM) into **Rh1** solution, and shown in Fig. 1a. In the absence of Hg^{2+} , as expected, the **Rh1** solution exhibited very weak fluorescence in the range from 510 nm to 650 nm. Upon the addition of increasing concentrations of Hg^{2+} , a new emission band peaking at 555 nm appeared and developed, which can be ascribed to the delocalized xantheno moiety of rhodamine group.¹² The fluorescence response of **Rh1** to other metal ions under the same condition was also investigated. As illustrated in Fig. 1b, no significant spectral changes of **Rh1** were observed in the presence of K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Zn^{2+} , Cd^{2+} , Cu^{2+} and Pb^{2+} (1 mM), indicating that **Rh1** could recognize Hg^{2+} from other metal ions even those that exist in high concentrations.



Scheme 1 Synthesis procedure of **Rh1**.

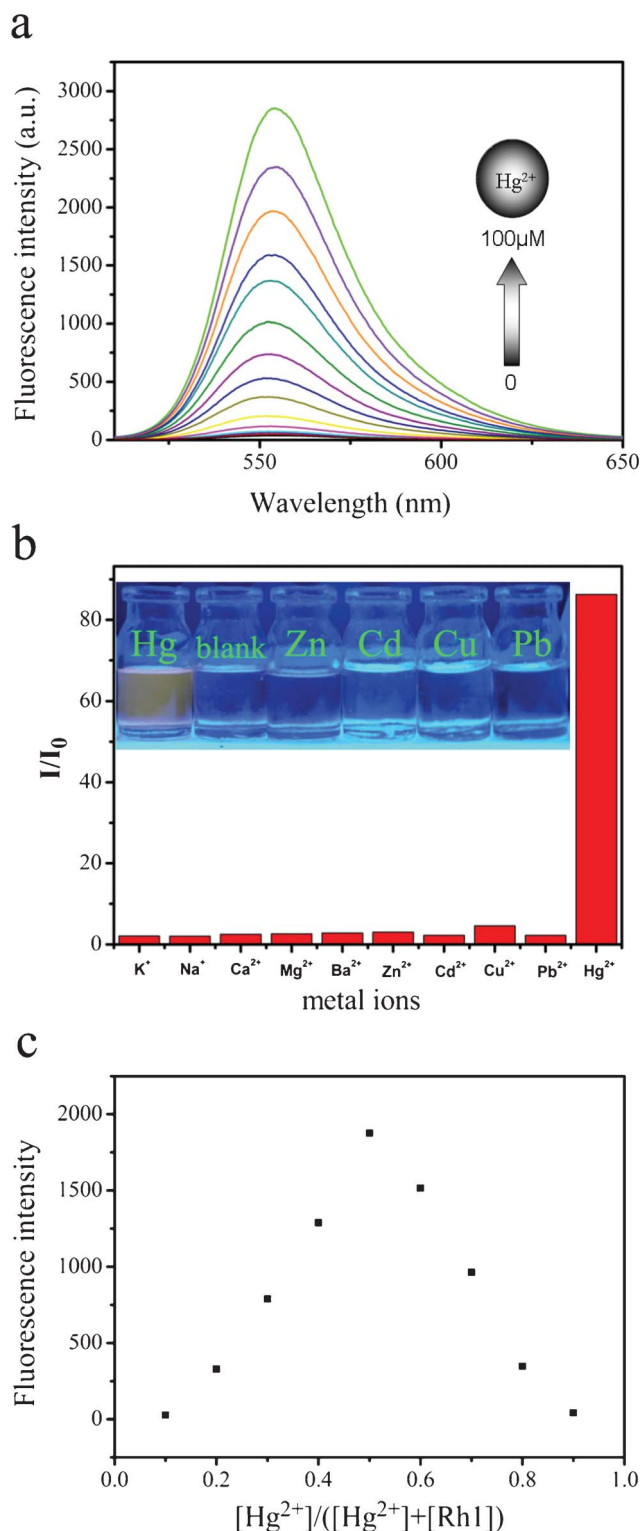
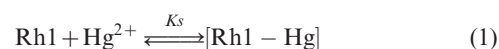


Fig. 1 (a) Emission spectra of **Rh1** (10 μM) in the presence of increasing concentration of Hg^{2+} in buffered (NaAc-HAc, pH = 7) water/ CH_3CN (1 : 1, v/v). λ_{ex} = 500 nm. (b) Normalized fluorescence response I/I_0 of **Rh1** (10 μM) in the presence of various metal ions (1×10^{-3} M). λ_{ex} = 500 nm and λ_{em} = 555 nm. Inset shows fluorescence change upon addition of Hg^{2+} , Zn^{2+} , Cd^{2+} , Cu^{2+} , Pb^{2+} and blank (5 equiv). (c) Job's plot for determining the stoichiometry of **Rh1** and Hg^{2+} ions (the total concentration of **Rh1** and Hg^{2+} ions was 10 μM).

To understand the recognition abilities of **Rh1** towards Hg^{2+} ions, a Job's plot analysis was conducted to determine the binding stoichiometry of the **Rh1**- Hg^{2+} complex, by maintaining the total **Rh1** and Hg^{2+} ions constant ($10\ \mu\text{M}$) and changing the mole fraction of Hg^{2+} from 0 to 1. From the Job's plot shown in Fig. 1c, we can observe that the significant increase in fluorescence intensity of **Rh1** resulted from the complexation with Hg^{2+} . The fluorescence intensity went through a maximum at a molar fraction of about 0.5 of Hg^{2+} , indicating that a 1 : 1 stoichiometry was the most possible one for the binding mode of Hg^{2+} and **Rh1**.

The association constant for **Rh1** binding to Hg^{2+} was determined from the absorption titration data. Upon gradual addition of Hg^{2+} into the solution, a new absorption peaking around 527 nm emerged with increasing intensity which corresponds to the appearance of a pink color, suggesting the formation of the ring-opened tautomer of **Rh1** upon Hg^{2+} binding (Fig. 2a and b). Since **Rh1** binds with Hg^{2+} to form a

complex with a complexing ratio of 1 : 1, the equilibrium can thus be described as follows:



Here, K_s denotes the association constant. The relative absorbance α is defined as the ratio of free **Rh1** to the total amount of **Rh1** in buffered water/ CH_3CN . It can be experimentally determined by measuring the absorbance values in the presence of different concentrations of Hg^{2+} :

$$\alpha = \frac{A_t - A_0}{A_t - A_0} \quad (2)$$

where A_0 and A_t are the limiting absorbance values for $\alpha = 1$ (in the absence of Hg^{2+}) and $\alpha = 0$ (**Rh1** is completely complexed with Hg^{2+}). According to the derivation following the mass law,¹³ the relationship between α and Hg^{2+} concentration can be represented as follows:

$$\frac{\alpha}{1 - \alpha} = \frac{1}{K_s[\text{Hg}]} \quad (3)$$

From the above discussion, it is apparent that the absorbance of **Rh1** has a distinct functional relationship with the Hg^{2+} concentration, and the association constant K_s , which provides the basis for the detection of the K_s value. The fitted curve to incorporate experimental data for Hg^{2+} is presented in Fig. 2c, which gave an association constant K_s value of $8.42 \times 10^4\ \text{M}^{-1}$ for **Rh1** binding to Hg^{2+} . The strong binding ability of **Rh1** towards Hg^{2+} could be ascribed to the introduction of the S atom.

In view of the good sensing performances of **Rh1** to Hg^{2+} in aqueous solution, fluorescence imaging experiments were carried out in living cells on a Olympus fluorescence microscope to further demonstrate the practical applicability of **Rh1** in living cells. As shown in Fig. 3b, after staining Rat Schwann cells with

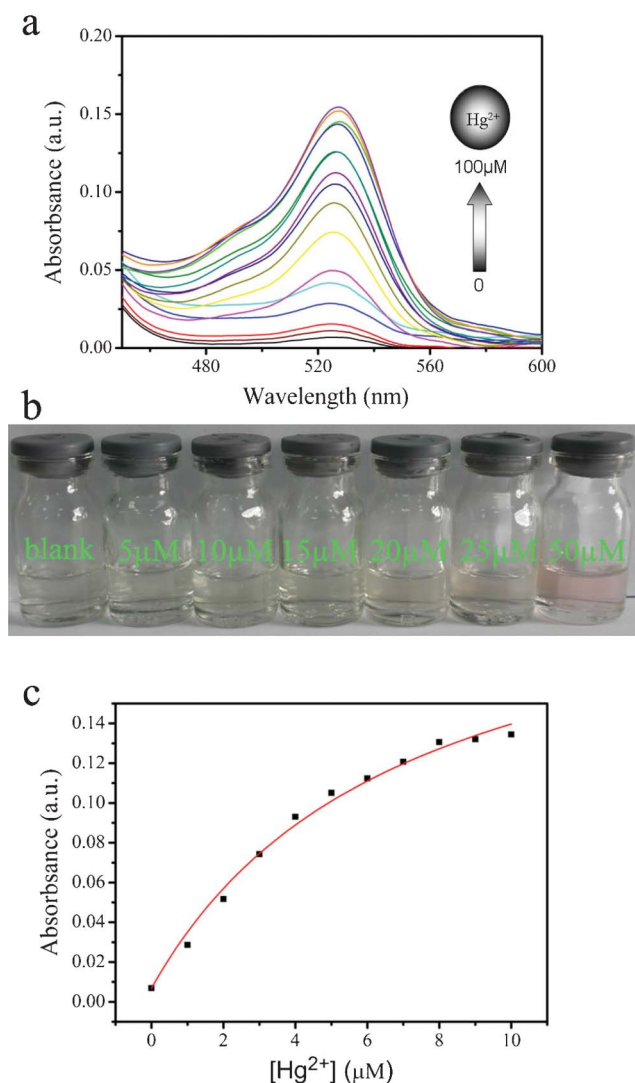


Fig. 2 (a) Absorption spectra of **Rh1** ($10\ \mu\text{M}$) in the presence of increasing concentration of Hg^{2+} in buffered (NaAc-HAc, pH = 7) water/ CH_3CN (1 : 1, v/v). (b) Color changes of **Rh1** ($10\ \mu\text{M}$) upon addition of different amounts of Hg^{2+} . (c) Absorbance as a function of $[\text{Hg}^{2+}]$ calculated from eqn (3): ■, data points experimentally obtained.

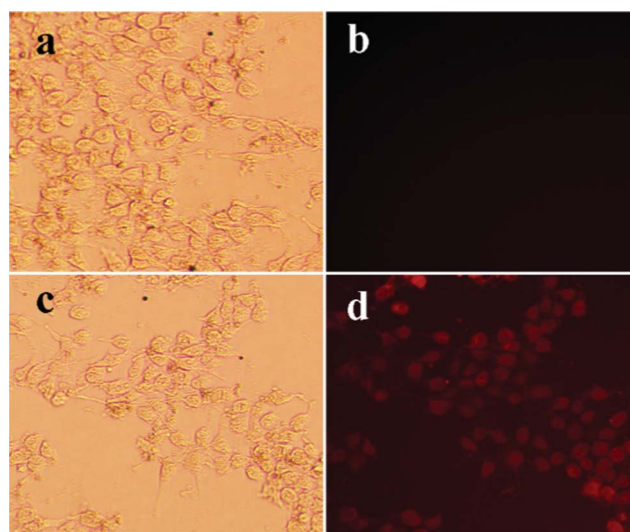


Fig. 3 Fluorescence images of Hg^{2+} ions in Rat Schwann cells with **Rh1** ($10\ \mu\text{M}$). Bright-field transmission image (a, c) and fluorescence image (b, d) of Rat Schwann cells incubated with $0\ \mu\text{M}$ and $50\ \mu\text{M}$ of Hg^{2+} ions for 30 min, respectively (excited with green light).



Fig. 4 Change in color of **Rh1** (10 μM) in buffered (NaAc-HAc, pH = 7) water/ CH_3CN (1 : 1, v/v) with metal ions: blank, K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Zn^{2+} , Cd^{2+} , Cu^{2+} and Pb^{2+} (1×10^{-3} M, from left to right).

Rh1 (10 μM) for 10 min, they displayed no detectable fluorescence signal in living cells. Upon addition of 50 μM Hg^{2+} ions, the fluorescence intensity increases dramatically to show a clear red intracellular fluorescence (Fig. 3d). The results indicate that **Rh1** can provide a fluorescence enhancement with

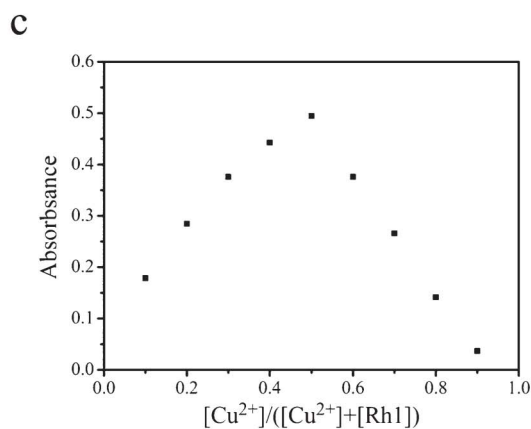
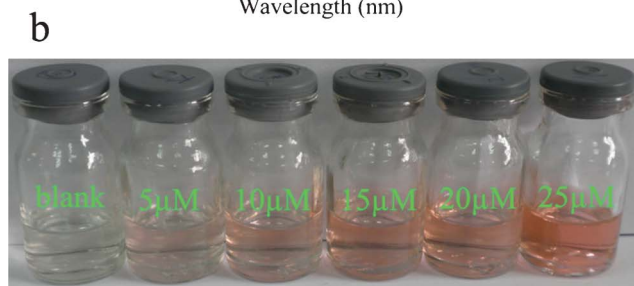
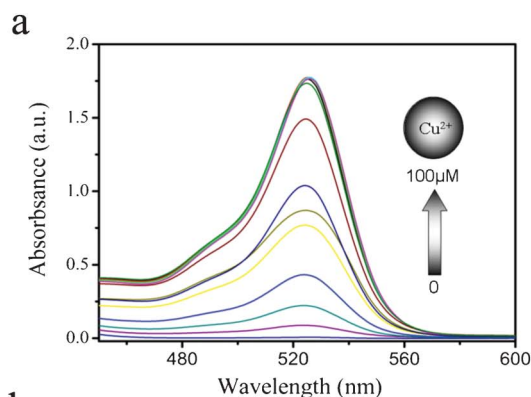


Fig. 5 (a) Absorption spectra of **Rh1** (10 μM) in the presence of increasing concentration of Cu^{2+} in buffered (NaAc-HAc, pH = 7) water/ CH_3CN (1 : 1, v/v). (b) Color changes of **Rh1** (10 μM) upon addition of different amounts of Cu^{2+} . (c) Job's plot for determining the stoichiometry of **Rh1** and Cu^{2+} ions (the total concentration of **Rh1** and Cu^{2+} ions was 10 μM).

excellent cell-permeability and biocompatibility for tracing the Hg^{2+} ion in cells, specifically and rapidly. Therefore, **Rh1** could be considered for application for *in vitro* imaging of Hg^{2+} ion in living cells and potentially *in vivo*.

In the experiment of testing the selectivity of **Rh1**, an interesting phenomenon attracted our attentions. There was no obvious color change in the presence of interfering effect metal ions, such as K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Zn^{2+} , Cd^{2+} , and Pb^{2+} as shown in Fig. 4. However, the solution of **Rh1** exhibits an obvious pink color with the addition of Cu^{2+} , thus almost no fluorescence change is observed (insert in Fig 1b). Therefore, we investigated the detailed response of **Rh1** to Cu^{2+} ion.

Unlike the varying tendency of fluorescence emission, upon addition of small amounts of Cu^{2+} , the absorbance was significantly enhanced with a new peak appearing at 527 nm (Fig. 5a), whereas the absorption spectra of free **Rh1** exhibited only a very weak band above 500 nm, which was ascribed to the spirolactam form of **Rh1**. Upon the addition of 5 equiv of Cu^{2+} , the new peak was enhanced up to $\log \epsilon = 5.25$, accompanied with an obvious color change from colorless to pink simultaneously (Fig. 5b). The Job's plot analysis in Fig. 5c was conducted to determine the binding stoichiometry of the **Rh1**- Cu^{2+} complex. It was observed that a maximum absorption was reached when the molar fraction of Cu^{2+} ions was about 0.5, confirming that a 1 : 1 stoichiometry is most possible for the binding mode of Cu^{2+} and **Rh1**. The association constant for **Rh1** binding to Cu^{2+} was also determined from the absorption titration data. Since **Rh1** bound with Cu^{2+} to form a complex with a complexing ratio of 1 : 1, the data still can be fitted by eqn (3). Fig. 6 shows the fitted curve to incorporate the experimental data for Cu^{2+} , which gives an association constant K_s value of $1.98 \times 10^4 \text{ M}^{-1}$ for **Rh1** binding to Cu^{2+} . Upon addition of small amounts Cu^{2+} , the enhancement in absorbance clearly suggested the formation of the delocalized xanthane moiety of the rhodamine group, along with a distinct color change from colorless to pink. However, the fluorescence of the open-ring form of **Rh1** was quenched by Cu^{2+} , which could be explained based on the well-known paramagnetic effect of the $\text{Cu}(\text{II}) d^9$ system.¹⁴

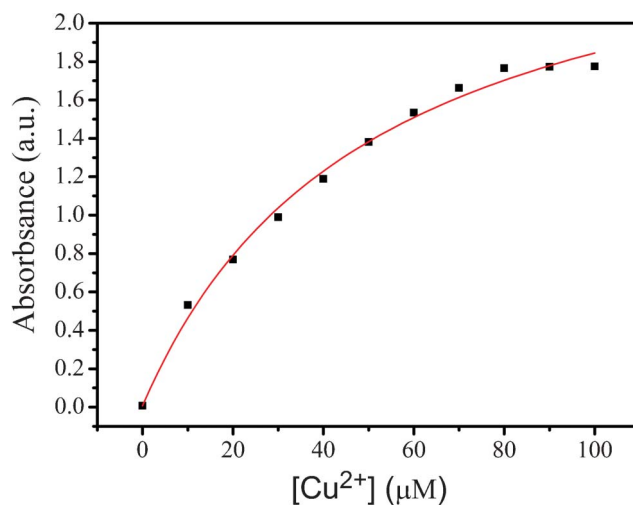


Fig. 6 Absorbance as a function of $[\text{Cu}^{2+}]$ calculated from eqn (3): ■, data points experimentally obtained.

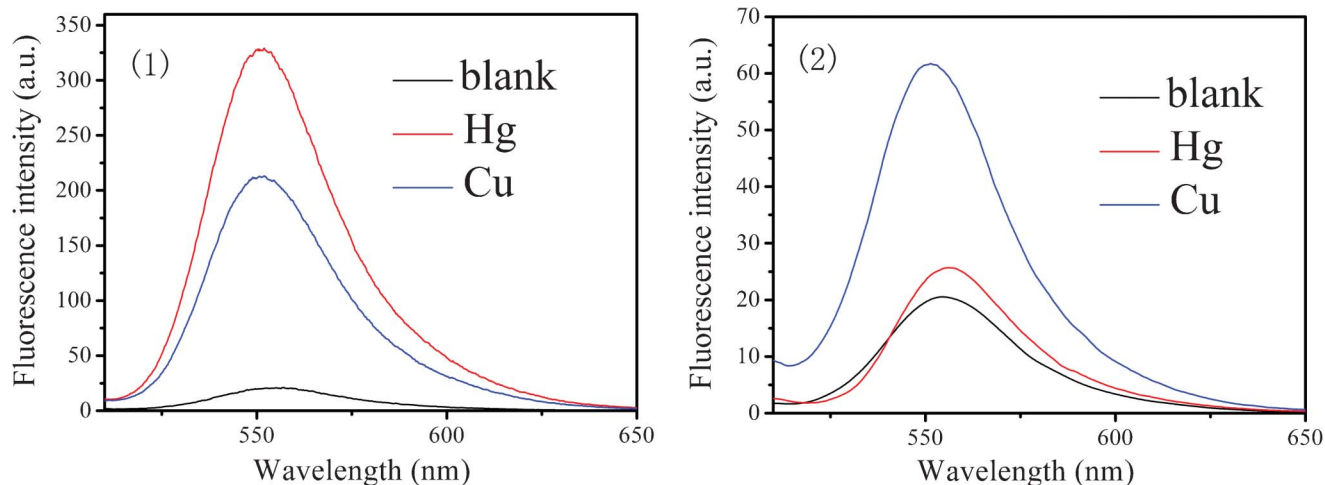


Fig. 7 Fluorescence emission output of **Rh1** with different input sequences: (1) Hg^{2+} as the first input followed by Cu^{2+} as the second one; (2) Cu^{2+} as the first input followed by Hg^{2+} as the second one.

Since the K_s values of **Rh1**- Hg^{2+} and **Rh1**- Cu^{2+} are comparable, Hg^{2+} and Cu^{2+} would compete against each other to coordinate with **Rh1** when they were coexist in a system, resulting in a transformation between **Rh1**- Hg^{2+} and **Rh1**- Cu^{2+} with fluorescence. Based on this, we investigated the different fluorescence state “ON and OFF” of **Rh1** with changing the addition sequence, using Hg^{2+} (2 equiv.) and Cu^{2+} (10 equiv.) as inputs (Fig. 7). When the first input is Hg^{2+} , the fluorescence emission at 555 nm significantly enhanced. Although the sequential addition of Cu^{2+} led to a relative decline of the fluorescence intensity, it was still in the “On” state. On reversal of the input sequence, *i.e.* for Cu^{2+} as the first input and Hg^{2+} as the second one, it caused the fluorescence intensity far below its initial maximum, *i.e.* “Off” state. The two input signals of Hg^{2+} and Cu^{2+} were defined as *In 1* and *In 2*, respectively. These inputs can be encoded with binary digits applying positive logic conventions (off = 0, on = 1). If we regard fluorescence intensity of 75 as the threshold values, *Output* = 0 when its corresponding spectral value was lower than 75; *Output* = 1 when its corresponding spectral value was higher than 75.

As discussed above, Fig. 8 presents the outputs in the fluorescence channel, obtained for different addition sequences. Only in input sequence of Hg^{2+} and Cu^{2+} , the fluorescent intensity was higher than the threshold value (*Output* = 1), resulting in strong fluorescence. However, changing the sequence of the inputs, it could not initiate a strong fluorescence at 555 nm (*Output* = 0). Therefore, according to this sequence-dependent phenomenon, a molecular keypad lock was constructed.

To simplify the input sequence as password of the molecular keypad lock, inputs Hg^{2+} and Cu^{2+} were designated as “K” and “E”, respectively. When the input signal “K” was added first and followed by “E”, the emission at 555 nm was in the “On” state, and it created a secret password “KEY” (Y defines On state). Inverting the addition sequence of inputs, *i.e.* the first input is “E” and the second input is “K”, gave an obvious fluorescence quenching (Off, designated as the character “N”). Thus, the wrong password “EKN” failed to open the lock. Therefore, only the authorized user who knows the exact password “KEY” can open the lock, which is a new approach

for protecting information at the molecular scale (Fig. 9). Due to the fact that the use of numerical digits (0–9) and letters (A–Z) as PIN numbers in a two-digit password allows a total of more than 700 different combinations,^{5j} it added to the complexity of

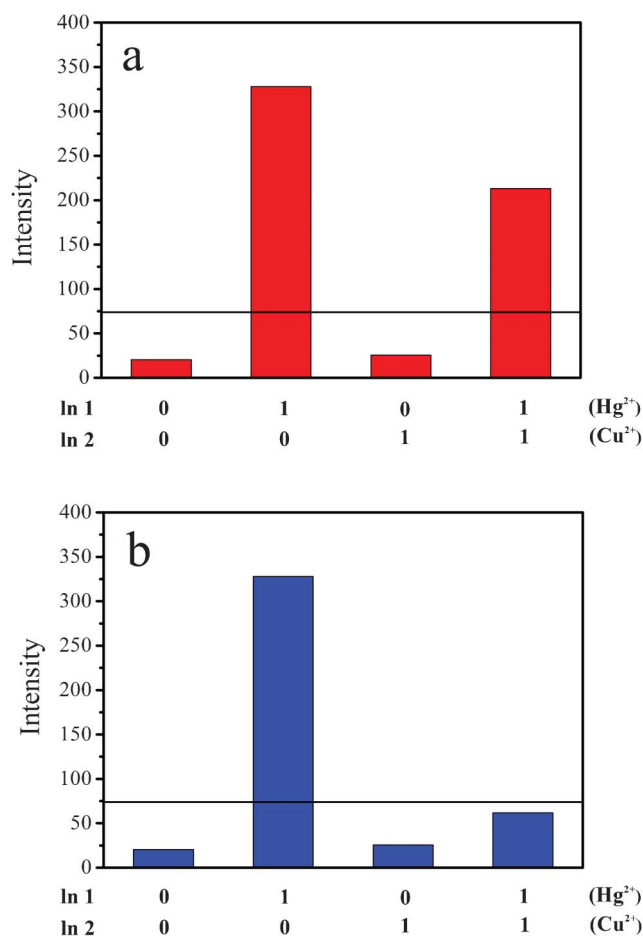


Fig. 8 Fluorescence output resulting from different input sequences: (a) *In 1* (Hg^{2+} first) and *In 2* (Cu^{2+} second); b) *In 2* (Cu^{2+} first) and *In 1* (Hg^{2+} second).

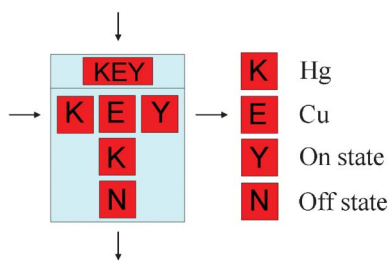


Fig. 9 Fluorescent keypad to access a secret password at 555 nm with different input sequences.

cracking the keypad lock, and improved remarkably the security of the molecular device.

Conclusions

In summary, a novel fluorescent chemosensor based on rhodamine derivative **Rh1** was designed and synthesized for the detection of Hg^{2+} in aqueous solution and living cells. The fluorescent indicator of **Rh1** can efficiently recognize Hg^{2+} over other metal ions by the fluorescence intensity increase, which could be ascribed to the delocalized xanthene moiety of the rhodamine group. Moreover, this “Off-On”-type fluorescent sensor could successfully mimic a molecular level keypad lock in the presence of Cu^{2+} ions. Only the correct password resulted in strong fluorescence emission at 555 nm which can be used to “open” this molecular keypad lock. Wrong passwords failed to open the lock, which can result in the “alarm” signal indicating the wrong password. Therefore, this molecular keypad lock has the potential for protecting information at the molecular scale, which would be used to authorize a user, to verify authentication of a product, or to initiate a higher process.

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References

- (a) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and E. T. Rice, *Chem. Rev.*, 1997, **97**, 1515; (b) B. L. Feringa, *Molecular witches*, Wiley-VCH, Weinheim 2001; (c) F. M. Raymo, *Adv. Mater.*, 2002, **14**, 401; (d) V. Balzini, A. Credi and M. Venturi, *ChemPhysChem*, 2003, **4**, 49; (e) X. Guo, D. Zhang, T. Wang and D. Zhu, *Chem. Commun.*, 2003, 914; (f) A. P. de Silva and N. D. McClenaghan, *Chem.–Eur. J.*, 2004, **10**, 574; (g) D. H. Qu, Q. C. Wang and H. Tian, *Angew. Chem.*, 2005, **117**, 5430; (h) D. H. Qu, F. Y. Ji, Q. C. Wang and H. Tian, *Adv. Mater.*, 2006, **18**, 2035; (i) J. Andreasson, S. D. Straight, G. Kodis, C. D. Park, M. Hambourger, M. Grevaldo, B. Albinson, T. A. Moore, A. L. Moore and D. Gust, *J. Am. Chem. Soc.*, 2006, **128**, 16259; (j) A. Credi, *Angew. Chem., Int. Ed.*, 2007, **46**, 5472; (k) U. Pischel, *Angew. Chem., Int. Ed.*, 2007, **46**, 4026; (l) K. Szacilowski, *Chem. Rev.*, 2008, **108**, 3481; (m) S. D. Straight, P. A. Liddell, Y. Terazono, T. A. Moore, A. L. Moore and D. Gust, *Adv. Funct. Mater.*, 2007, **17**, 777; (n) R. Ballardini, P. Ceroni, A. Credi, M. T. Gandolfi, M. Maestri, M. Semararo, M. Venturi and V. Balzani, *Adv. Funct. Mater.*, 2007, **17**, 740.
- R. Baron, A. Onopriyenko, E. Katz, O. Lioubashevski, I. Willner, S. Wang and H. Tian, *Chem. Commun.*, 2006, 2147.
- Y. Liu, W. Jiang, H. Y. Zhang and C. J. Li, *J. Phys. Chem. B*, 2006, **110**, 14231.
- J. Andreasson, S. D. Straight, S. Bandyopadhyay, R. H. Mitchell, T. A. Moore, A. L. Moore and D. Gust, *J. Phys. Chem. C*, 2007, **111**, 14274.
- (a) D. Margulies, C. E. Felder, G. Melman and A. Shanzer, *J. Am. Chem. Soc.*, 2007, **129**, 347; (b) G. Strack, M. Ornatska, M. Pita and E. Katz, *J. Am. Chem. Soc.*, 2008, **130**, 4234; (c) M. Kumar, R. Kumar and V. Bhalla, *Chem. Commun.*, 2009, 7384; (d) M. Zhou, X. Zheng, J. Wang and S. Dong, *Chem.–Eur. J.*, 2010, **16**, 7719; (e) J. Andreasson, S. D. Straight, T. A. Moore, A. L. Moore and D. Gust, *Chem.–Eur. J.*, 2009, **15**, 3936; (f) J. Halamek, T. K. Tam, S. Chinnapareddy, V. Bocharova and E. Katz, *J. Phys. Chem. Lett.*, 2010, **1**, 973; (g) J. Wang and C. S. Ha, *Analyst*, 2010, **135**, 1214; (h) J. Halamek, T. K. Tam, G. Strack, V. Bocharova, M. Pita and E. Katz, *Chem. Commun.*, 2010, 2405; (i) S. Kumar, V. Luxami, R. Saini and D. Kaur, *Chem. Commun.*, 2009, 3044; (j) M. Suresh, A. Ghosh and A. Das, *Chem. Commun.*, 2008, 3906; (k) Z. Guo, W. Zhu, L. Shen and H. Tian, *Angew. Chem., Int. Ed.*, 2007, **46**, 5549; (l) M. Kumar, A. Dhir and V. Bhalla, *Org. Lett.*, 2009, **11**, 2567; (m) P. Singha, J. Kaura and W. Holzer, *Sens. Actuators, B*, 2010, **150**, 50.
- (a) A. P. de Silva and S. Uchiyama, *Nat. Nanotechnol.*, 2007, **2**, 399; (b) D. Margulies, G. Melman and A. Shanzer, *J. Am. Chem. Soc.*, 2006, **128**, 4865.
- T. Nguyen and M. B. Francis, *Org. Lett.*, 2003, **5**, 3245.
- B. Valeur, *Molecular Fluorescence: Principles and Applications*, Wiley-VCH, Verlag GmbH: New York, 2001.
- (a) Y. Xiang and A. Tong, *Org. Lett.*, 2006, **8**, 1549; (b) Y. Xiang, A. Tong, P. Jin and Y. Ju, *Org. Lett.*, 2006, **8**, 2863; (c) Y. K. Yang, K. J. Yook and J. Tae, *J. Am. Chem. Soc.*, 2005, **127**, 16760; (d) J. Y. Kwon, Y. J. Jang, Y. J. Lee, K. M. Kim, M. S. Seo, W. Nam and J. Yoon, *J. Am. Chem. Soc.*, 2005, **127**, 10107; (e) H. Zheng, Z. H. Qian, L. Xu, F. F. Yuan, L. D. Lan and J. G. Xu, *Org. Lett.*, 2006, **8**, 859; (f) J. S. Wu, I. C. Hwang, K. S. Kim and J. S. Kim, *Org. Lett.*, 2007, **9**, 907; (g) D. Wu, W. Huang, C. Duan, Z. Lin and Q. Meng, *Inorg. Chem.*, 2007, **46**, 1538; (h) J. Chen, A. F. Zheng, A. H. Chen, Y. Gao, C. He, X. Kai, G. Wu and Y. Chen, *Anal. Chim. Acta*, 2007, **599**, 134; (i) K. P. Prathish, D. James, J. Jaisy and T. Prasada Rao, *Anal. Chim. Acta*, 2009, **647**, 84; (j) Y. Xiang, Z. Li, X. Chen and A. Tong, *Talanta*, 2008, **74**, 1148; (k) M. Suresh, A. Shrivastav, S. Mishra, E. Suresh and A. Das, *Org. Lett.*, 2008, **10**, 3013; (l) Y. Zhao, X. B. Zhang, Z. X. Han, L. Qiao, C. Y. Li, L. X. Jian, G. L. Shen and R. Q. Yu, *Anal. Chem.*, 2009, **81**, 7022; (m) V. Bhalla, R. Tejpal and M. Kumar, *Sens. Actuators, B*, 2010, **151**, 180.
- (a) Y. K. Yang, K. J. Yook and J. Tae, *J. Am. Chem. Soc.*, 2005, **127**, 16760; (b) X. F. Yang, X. Q. Guo and Y. B. Zhao, *Talanta*, 2002, **57**, 883.
- (a) Z. X. Han, X. B. Zhang, Z. Li, Y. J. Gong, X. Y. Wu, Z. Jin, C. M. He, L. X. Jian, J. Zhang, G. L. Shen and R. Q. Yu, *Anal. Chem.*, 2010, **82**, 3108; (b) H. Zheng, Z. H. Qian, L. Xu, F. F. Yuan, L. D. Lan and J. G. Xu, *Org. Lett.*, 2006, **8**, 859; (c) W. Liu, L. Xu, R. Sheng, P. Wang, H. Li and S. Wu, *Org. Lett.*, 2007, **9**, 3829.
- D. Wu, W. Huang, C. Duan, Z. Lin and Q. Meng, *Inorg. Chem.*, 2007, **46**, 1538.
- (a) X. B. Zhang, C. C. Guo, Z. Z. Li, G. L. Shen and R. Q. Yu, *Anal. Chem.*, 2002, **74**, 821; (b) Y. Zhao, X. B. Zhang, Z. X. Han, L. Qiao, C. Y. Li, L. X. Jian, G. L. Shen and R. Q. Yu, *Anal. Chem.*, 2009, **81**, 7022.
- (a) J. S. Kim and D. T. Quang, *Chem. Rev.*, 2007, **107**, 3780; (b) M. Suresh, A. Shrivastav, S. Mishra, E. Suresh and A. Das, *Org. Lett.*, 2008, **10**, 3013.