ORGANIC LETTERS

2010
Vol. 12, No. 21
4756−4759

Chromene "Lock", Thiol "Key", and Mercury(II) Ion "Hand": A Single Molecular Machine Recognition System

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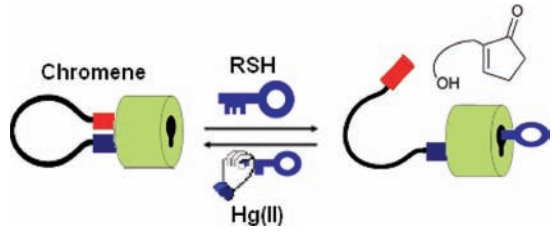
Received July 29, 2010

ABSTRACT



A regenerative, molecular machine-like "ON−OFF−ON" chemosensor based on a chromene molecule with the pyran ring "OFF−ON−OFF" cycle is reported for the first time. It behaves as a molecular lock that requires a thiol "key" to open the lock and a mercury(II) ion "hand" that unlatches the key for unsheathing the key to close the lock.

There is considerable interest and intense activity in constructing molecular-level devices with molecular recognition functionality and signal transduction ability in chemistry,[1] biology,[2] and sensors.[3] Especially important and significant in this regard are sensors that detect sulfhydryl-containing amino acids and peptides, cysteine (Cys), homocysteine (Hcy), and glutathione (GSH), as they play many crucial roles in many physiological processes.[4] In recent years, great effort has been put into the development of thiol detection by means such as high-performance liquid chromatography,[5] capillary electrophoresis,[6] Ellman's reagent,[7] cleavage reactions by thiol,[8] cyclization reactions with aldehyde,[9] and others.[10]

One of the most attractive approaches involves the construction of receptors of a thiol-based reaction through the powerful

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10.1021/ol101771j
Published on Web 10/04/2010

"click" chemisty.[11,12] A few excellent receptors have been exploited such as OND,[12a] squaraine,[12b,c] coumarin dyes,[12d,e] maleimide,[12f,g] propiolate,[12h] and quinone.[12i,j]

Very recently, we demonstrated a colorimetric probe for thiol based on the ring-opening mechanism of the chromene molecule, 7-nitro-2,3-dihydro-1*H*-cyclopenta[*b*]chromen-1-one, in aqueous solution.[13] Herein, we report a new, regenerative, fluorescence "ON−OFF−ON" probe for thiol and the mercury(II) ion, 7-chloro-2,3-dihydro-1*H*-cyclopenta[*b*]chromene-1-one (**1**) (Figure 1, prepared by Baylis−Hillman

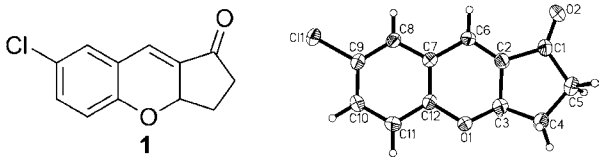
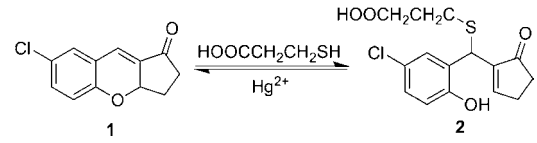


Figure 1. Structure and thermal ellipsoids of probe **1** are drawn at the 50% probability level.

and intramolecular Michael addition reactions (Figure S1, Supporting Information)).[14] We also report a single molecular lock based on the following phenomenon: a thiol-chromene "click" nucleophilic pyran ring-opening reaction with a thiol key to open the lock and Hg^{2+}-promoted desulfurization and subsequent intramolecular Michael addition to the pyran ring with the mercury(II) ion as a "hand" unsheathing the key to close the lock.

Scheme 1



Scheme 1 depicts the reversible character of the thiol-chromene "click" nucleophilic pyran ring-opening reaction of **1** and Hg^{2+}-induced cyclization of **2** to chromene as a sensing mechanism. The nucleophilic addition of mercaptopropionic acid (MPA) to the probe **1** leads rapidly to the formation of the ring-opened, low fluorescent product **2** and demonstrates a fluorescence quenching effect. 1D ^{1}H NMR, ^{13}C NMR, 2D ^{1}H−^{1}H COSY, and ESI mass spectrometry confirmed a thiol-quantitative reaction (Figure S2, Supporting Information). A kinetic study of the response of MPA to probe **1** under pseudofirst-order conditions (30 μM probe **1** and 300 μM MPA) is shown in Figure S3 (Supporting Information). The nucleophilic ring-opening reaction was completed in less than 3 min at room temperature in aqueous media.

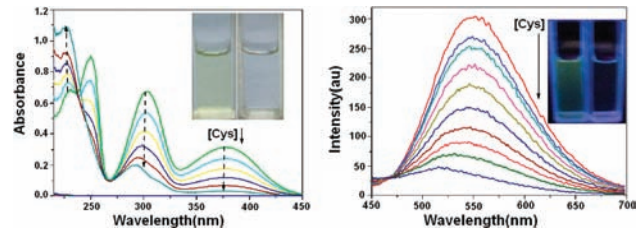


Figure 2. (Left) Absorption spectral changes of **1** (30 μM) in Tris-HCl 10 mM containing 0.15% EtOH, pH 8.0 aqueous buffer upon addition of Cys. Cys was added gradually at [Cys] = 0−90 μM. Each spectrum was recorded 3 min after Cys addition. (Right) Fluorescence spectral changes of **1** (3 μM) upon addition of Cys (0−9 μM) (λ_{ex} = 380 nm, λ_{em} = 550 nm; slit, 10 nm/10 nm) in 10 mM Tris-HCl containing 0.15% EtOH, pH 8.0 aqueous buffer upon addition of Cys. Each spectrum was recorded 3 min after Cys addition. Inset: color (left) and visual fluorescence (right) change photographs of **1** (100 μM) upon addition of Cys in Tris-HCl/ethanol (200:1, v/v, 10 mM, pH 8.0) buffer solution under illumination with a UV 365 nm lamp.

Figure 2 (left) shows the change in the UV/visible spectrum when the Cys solution was added to Tris-HCl buffer (10 mM, pH 8.0) containing probe **1** (30 μM). With increasing Cys concentration, the probe **1** absorption peaks at 250, 302, and 377 nm gradually decreased, and a new peak appeared at 227 nm (blue-shifted 23, 75, and 150 nm, respectively) with an isosbestic point at 236 nm, indicating the formation of the product **2**. Figure 2 (right) displays the

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(14) See Supporting Information. The probe **1** molecule: ^{1}H NMR (600 MHz, 25°C, DMSO-d_6): δ 7.54 (s, 1H), 7.34 (d, 1H), 7.25 (s, 1H), 6.96 (d, 1H), 5.32 (t, 1H), 2.58−2.63 (m, 1H), 2.45−2.49 (m, 1H), 2.36−2.42 (m, 1H), 2.00−2.08 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 27.99, 37.05, 75.91, 117.87, 123.21, 126.30, 127.07, 129.55, 131.91, 132.73, 157.46, 201.08. Elemental analysis (calcd %) for $C_{12}H_9ClO_2$: C, 65.32; H, 4.11. Found: C, 65.64; H, 4.21. ESI-MS *m/z* 220.9 (30%, $[M]^+$): calculated 220.7 $[M]^+$. Crystal data for **1**: $C_{12}H_9ClO_2$, FW = 220.64, crystal size: 0.1 × 0.1 × 0.1 mm, orthorhombic, space group *Pbca* (No.61), a = 15.103(3) Å, b = 6.0078(12) Å, c = 21.747(4) Å, β = 90.000°, V = 1973.2(7) Å^3, Z = 8, T = 183 K, θ_{max} = 25.35°, 7615 reflections measured, 1766 unique (R_{int} = 0.041). Final residual for 137 parameters and 1575 reflections with $I > 2\sigma(I)$: R_1 = 0.0442, wR_2 = 0.1037, and GOF = 1.09.

emission peak of **1** at 550 nm (λ_{ex} =380 nm) that decreased rapidly upon addition of Cys and eventually stopped with a saturation point around 3 equiv of Cys. When 3 equiv of Cys was added to the solution of **1**, a more than 10-fold decrease in fluorescent intensity at 550 nm was observed.

Simultaneously, an elaborate assay was carried out by fluorescence titration (Figure S4, Supporting Information) and showed a linear response of probe **1** to increasing amounts of even low Cys concentration from 3×10^{-7} to 3.9×10^{-6} M. GSH and HCy can also play the same roles. These indicate that probe **1** can respond to thiol at low micromolar levels. Other amino acids did not affect the detection of Cys (Figure S5, Supporting Information).

It is very exciting and noteworthy that probe **1** could be regenerated only by adding Hg^{2+} ions to the solution containing compound **2** (a regenerative process of **1**, Figure S6, Supporting Information). Common metal ions such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Al^{3+}, Ga^{3+}, Sn^{2+}, Zr^{4+}, Ce^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, Er^{3+}, Tb^{3+}, Ho^{3+}, Yb^{3+}, Ag^+, and Pb^{2+} did not generate the same result, which indicates these ions cannot cause the regeneration of **1** (Figure 3 (above)). These novel facts show

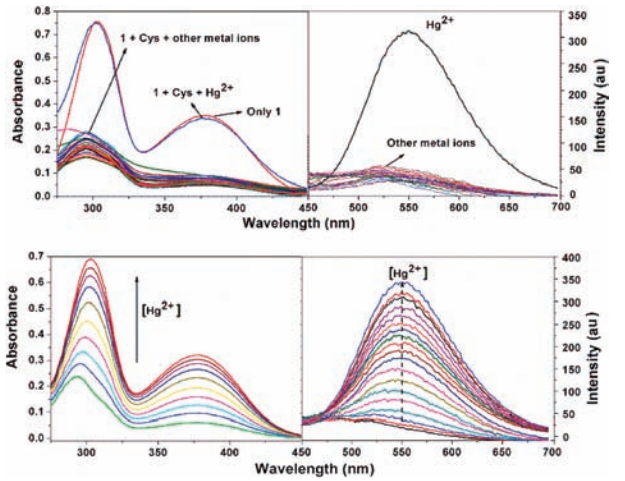
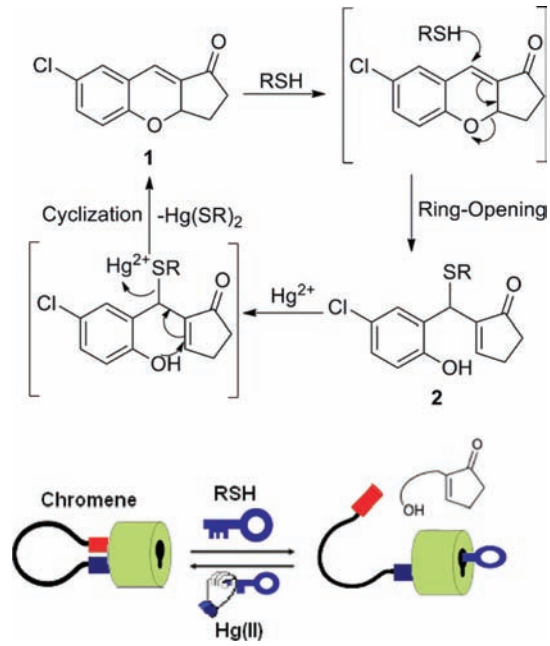


Figure 3. (Above) Absorption spectral changes (30 μM) and fluorescence spectral (3 μM) changes of **2** (λ_{ex} = 380 nm, λ_{em} = 550 nm; slit, 10 nm/10 nm) in 10 mM Tris-HCl, 0.5 mM EDTA, containing 0.15% EtOH, pH 8.0 aqueous buffer upon addition of 10 equiv of various metal ions including: Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Al^{3+}, Ga^{2+}, Sn^{2+}, Zr^{4+}, Ce^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, Er^{3+}, Tb^{3+}, Ho^{3+}, Yb^{3+}, Ag^+, Pb^{2+}, and Hg^{2+}. (Below) Hg^{2+} ion was added gradually with [Hg^{2+}] = 0−90 μM for UV/visible spectra with [**2**] = 30 μM and [Hg^{2+}] = 0−9 μM for fluorescence spectra with [**2**] = 3 μM. Each spectrum was recorded 10 min after Hg^{2+} addition.

that compound **2** can act as a fluorogenic chemosensor for the Hg^{2+} ions with high selectivity and sensitivity (Figure 3 (below)). The detection limit for Hg^{2+} in aqueous buffer is low micromolar level, e.g., 0.15−9.0 μM. The regeneration of probe **1** should involve a Hg^{2+}-promoted desulfurization such as the one reported by Ros-Lis:[12c,15] intramolecular Michael addition of **2** to the pyran ring. In other words, Hg^{2+} ions trigger C−S bond cleavage of compound **2**, and subsequent intramolecular nucleophilic attack of the phenolic oxygen on the cyclopentenone group results in the recovery of the cyclized probe **1** (Scheme 1). Furthermore, the regeneration of probe **1** after exposure to thiols and Hg^{2+} was examined by spectra. The change of spectra is regenerative over several cycles of "ON−OFF−ON" (Figure S7, Supporting Information). The results strongly proved that probe **1** can be not only used to detect thiols and Hg^{2+} but also readily regenerated.

On the basis of the sensor "ON−OFF−ON" cycle, we propose a cyclic mechanism (Scheme 2 (above), Figure S7,

Scheme 2



Supporting Information). This reversible system of the thiol-chromene "click" ring-opening process and Hg^{2+}-promoted pyran ring cyclization behaves as a molecular lock that can be unlocked with a thiol key and can be locked with an Hg^{2+} ion "hand", unsheathing the key (Scheme 2 (below)): probe **1** and product **2** represent the locked states and unlocked states, respectively.

On the basis of structure information for compound **2**, we propose a fluorescence mechanism of **1**, quenched upon the addition of thiols. The probe **1** molecule is planar with a maximum deviation of 0.4914 (2) Å for only the C3 atom and has a strong fluorescence emission at 552 nm. When MPA is added to **1**, the rigid plane of the chromene **1** is destroyed because of the pyran ring opening. To clarify this change, an energy-minimized structure of **2** was produced, with a molecular mechanical calculation which suggests that

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the dihedral angle between the cyclopentene plane and the benzene ring plane is 66.58° (Figure S8, Supporting Information).

In summary, the current study has demonstrated for the first time a regenerative, single molecular machine-like "ON−OFF−ON" chemosensor, based on a chromene molecule with a pyran ring "OFF−ON−OFF" cycle process. The sensor can function as a new, simple fluorescence-off probe for thiol with very high sensitivity and selectivity through the thiol-chromene "click" ring-opening reaction in aqueous media, and it can act as a novel fluorescence-on probe of the Hg^{2+} ion with high selectivity based on a Hg^{2+}-promoted desulfurization, intramolecular Michael addition to the pyran ring. Thus, these results are significant and interesting for a new generation of molecular recognition systems. The above phenomenon behaves as a molecular lock, which requires an inserted key to open and a hand to unlatch the key to close. Further studies on novel molecular machine-like, regenerative probes and their applications are now underway in our laboratory.

Acknowledgment. This work was supported by the National Natural Science Foundation of China (No. 20801032, 21072119), Shanxi Provincial Natural Science Foundation (No. 2009021006-2), the Shanxi Province Foundation for Returnee (200815), the Program for the Top Young Academic Leaders of Higher Learning Institutions of Shanxi (TYAL), and the Shanxi Province Foundation for Selected Returnee (No. 2010).

Supporting Information Available: Experimental procedures, spectroscopic data, kinetic study, ^{1}H NMR, ^{13}C NMR, ESI-MS data, and crystal data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

OL101771J