

Rhodamine Triazole-Based Fluorescent  
Probe for the Detection of  $\text{Pt}^{2+}$ 

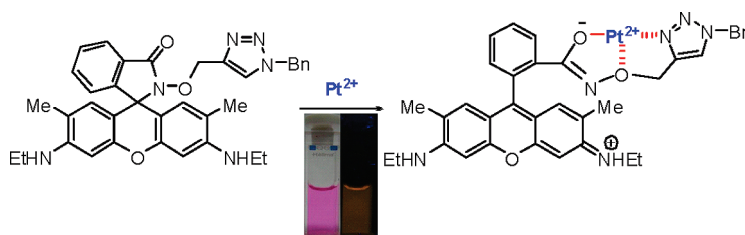
Hyemi Kim, Sunho Lee, Jihyun Lee, and Jinsung Tae\*

Department of Chemistry, Yonsei University, Seoul 120-749, Korea

jstae@yonsei.ac.kr

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## ABSTRACT



A rhodamine triazole-based fluorescent chemosensor has been developed for the selective detection of platinum ions in aqueous solutions. The rhodamine 6G hydroxamate linked with a propargyl group is converted to the corresponding triazole by a “click” reaction. The dual binding unit composed of a hydroxamate and a triazole shows high selectivity and sensitivity toward  $\text{Pt}^{2+}$  over a range of other metal ions in water. The fluorescent probe is applied to monitor cisplatin in aqueous solutions.

Platinum has many uses such as dental crowns, catalytic converters, fuel cells, jewelry, and anticancer drugs. Particularly, platinum complexes, such as cisplatin, carboplatin, and oxaliplatin, are widely used as anticancer drugs.<sup>1</sup> Although platinum-based chemotherapy is crucial for the treatment of many types of cancer, it is considered as potentially hazardous to human health.<sup>2</sup> Platinum salts can cause DNA alterations, cancers, autoimmune disorders, respiratory and hearing problems, and damages to organs, such as the intestine, kidney, and bone marrow.<sup>3</sup>

Analytical methods,<sup>4</sup> such as atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS), are often used to detect platinum in biological and environmental samples. However, these methods often need serious sample-preparation steps or require expensive

equipment. A fluorescent chemosensor method would be more desirable because it is less labor-intensive and highly sensitive. Recently, the Koide group reported fluorescent methods for the detection of platinum species using the Tsuji–Trost allylic oxidative insertion mechanism.<sup>5</sup> In this detection system, the ionic platinum species ( $\text{Pt}^{2+}$  and  $\text{Pt}^{4+}$ ) are reduced by phosphine to  $\text{Pt}^0$ . Herein, we report a fluorescent detection method of  $\text{Pt}^{2+}$  directly from aqueous solutions without reduction to  $\text{Pt}^0$  by using a rhodamine hydroxamate-based probe.

Whereas the “click triazole” has been frequently utilized as an interconnector between two functional entities in the areas of functional materials, biology, polymer science, drug discovery, etc.,<sup>6</sup> its potential as a metal coordination platform has been underexplored. Recently, 1,4-disubstituted 1,2,3-triazoles have been employed as a binding unit for metal ions, such as  $\text{K}^+$ ,<sup>7</sup>  $\text{Ca}^{2+}$ ,<sup>8</sup>  $\text{Zn}^{2+}$ ,<sup>9</sup>  $\text{Cu}^{2+}$ ,<sup>10</sup>  $\text{Hg}^{2+}$ ,<sup>11</sup>  $\text{Cd}^{2+}$ ,<sup>12</sup>

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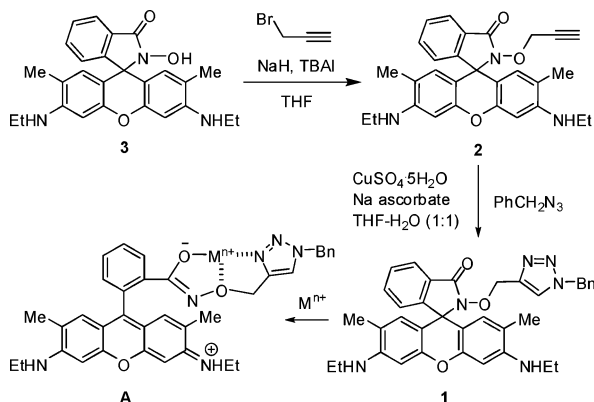
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$\text{Pd}^{2+}$ ,<sup>13</sup> and  $\text{Al}^{3+}$ ,<sup>14</sup> in the design of fluorescent chemosensors. However, rhodamine–triazole conjugates have not been reported as binding units to trigger the ring opening of the rhodamine spirolactam.<sup>15,16</sup>

Thus, we envisioned that introduction of a triazole moiety into the rhodamine hydroxamate could provide a well-organized coordination platform for metal ions (see a proposed complex structure **A** in Scheme 1). The triazole

**Scheme 1.** Synthesis of the Rhodamine–Triazole Conjugate **1**



could be readily synthesized by “click reaction”<sup>17</sup> using an azide and a terminal alkyne-functionalized rhodamine. Therefore, the rhodamine triazole **1** has been designed as a new fluorescent probe as shown in Scheme 1.

Compound **1** was prepared from the known rhodamine alkyne derivative **2**<sup>18</sup> which is prepared from the known rhodamine hydroxamic acid **3**<sup>19</sup> (1, propargyl bromide, NaH, THF; **2**, benzyl azide,  $\text{CuSO}_4$ , Na ascorbate,  $\text{THF-H}_2\text{O}$ ) as shown in Scheme 1.

Probe **1** shows neither color nor fluorescence in  $\text{H}_2\text{O}$  (DMSO 1% v/v) indicating that it exists in the spirocyclic form predominantly as expected. On treatment with 5.0 equiv of  $\text{Pt}^{2+}$  ions, probe **1** (5  $\mu\text{M}$ ) exerts strong fluorescence at 562 nm in  $\text{H}_2\text{O}$  (DMSO 1% v/v). In addition, the solution changes from colorless to pink-red color. Probe **1** could monitor  $\text{Pt}^{2+}$  ions in the pH 5–9 range (see Supporting Information).

Fluorescence titration of **1** (5  $\mu\text{M}$ ) with  $\text{Pt}^{2+}$  was conducted by using an  $\text{H}_2\text{O}$  (DMSO 1% v/v) solution at 25 °C. Upon each addition of  $\text{Pt}^{2+}$ , the solution is incubated for 30 min and then fluorescence intensity is measured. About 2.0 equiv of  $\text{Pt}^{2+}$  is required for the saturation of the fluorescence intensity under the titration conditions (Figure 1a). The Job’s plot<sup>20</sup> shows that **1** forms a 1:1 complex with  $\text{Pt}^{2+}$  (see Supporting Information). The binding constant ( $\log K = 5.2 \pm 0.7$ ) calculated in an  $\text{H}_2\text{O}$  (DMSO 1% v/v) solution from the fluorescence titration experiments based on the 1:1 binding model shows strong binding ability of **1** with  $\text{Pt}^{2+}$ . Addition of ethylenediamine to the mixture of **1** and  $\text{Pt}^{2+}$  decreases the fluorescence intensity of the solution, which implies the reversible binding between **1** and  $\text{Pt}^{2+}$  (see Supporting Information), and the fluorescence titration of  $\text{Pt}^{2+}$  at 0.5  $\mu\text{M}$  concentration of **1** demonstrates that the detection of  $\text{Pt}^{2+}$  is possible at the 125 nM level. Under these conditions, the fluorescence intensity of **1** is linearly proportional to the amount of  $\text{Pt}^{2+}$  (Figure 1b).

Next, the fluorescence responses of **1** (5  $\mu\text{M}$ ) to other biologically relevant metal ions in  $\text{H}_2\text{O}$  (DMSO 1% v/v) were examined. Upon additions of 5.0 equiv of metal ions ( $\text{Pt}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Cr}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Na}^+$ ,  $\text{Li}^+$ ,  $\text{Pd}^{2+}$ ,  $\text{Ru}^{3+}$ ,  $\text{Rh}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{K}^+$ ,  $\text{Ba}^{2+}$ ), only  $\text{Pt}^{2+}$  leads to a dramatic enhancement in fluorescence intensity in aqueous solution. Other metal ions develop no significant fluorescence intensity changes (Figure 2a). The competitive  $\text{Pd}^{2+}$  ions show very little fluorescence intensity changes, and the fluorescent intensity changes caused by the addition of  $\text{Pt}^{2+}$  are not influenced by the

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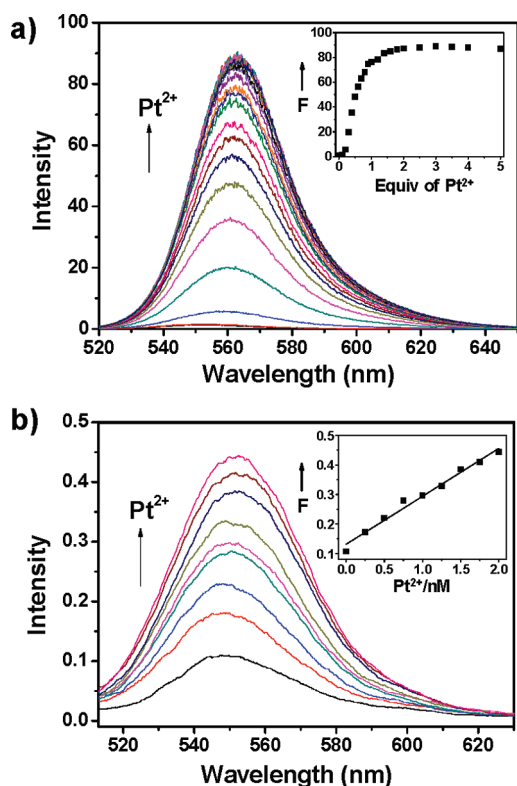
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**Figure 1.** Fluorescence intensity changes of **1** upon additions of  $\text{Pt}^{2+}$  in  $\text{H}_2\text{O}$  (DMSO 1% v/v). Each spectrum is observed after 30 min of each  $\text{Pt}^{2+}$  addition at  $25^\circ\text{C}$  with excitation at 500 nm. (a) **1** ( $5\ \mu\text{M}$ ). Inset: plot of fluorescence intensities at 562 nm depending on the equivalents of  $\text{Pt}^{2+}$ . (b) **1** ( $0.5\ \mu\text{M}$ ) upon additions of  $\text{Pt}^{2+}$  (by 125 nM). Inset: plot of fluorescence intensities at 553 nm depending on the concentration of  $\text{Pt}^{2+}$ .

presence of other metal ions as shown in Figure 2b. The fluorescent selectivity between  $\text{Pt}^{2+}$  and  $\text{Pd}^{2+}$  is especially promising because it is typically difficult to discriminate these similar metal ions.<sup>5</sup>

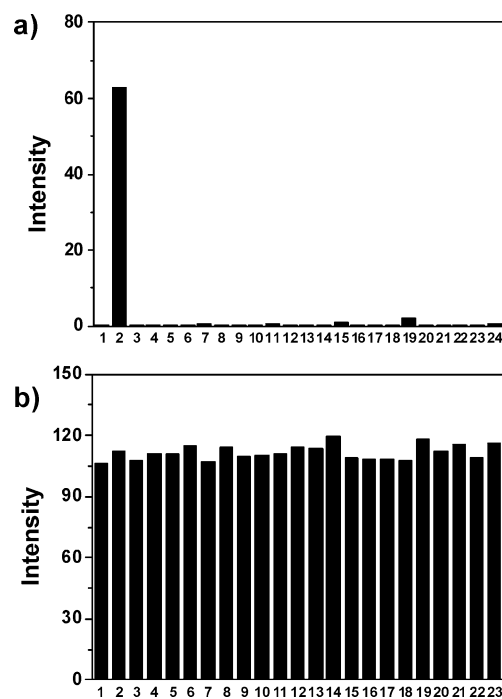
The fluorescence selectivity of **1** for  $\text{Pt}^{2+}$  is well matched when **1** is employed as a colorimetric detector. While the binding of **1** ( $5\ \mu\text{M}$ ) with  $\text{Pt}^{2+}$  results in clear colorless to pink-red color changes in aqueous solutions, no significant color changes are promoted by other metal ions except  $\text{Pd}^{2+}$  (Figure 3). Unlike the  $\text{Pt}^{2+}$ -selective fluorescence response of **1**, both  $\text{Pt}^{2+}$  and  $\text{Pd}^{2+}$  induce color changes of **1**.<sup>21</sup> This implies that, although both  $\text{Pt}^{2+}$  and  $\text{Pd}^{2+}$  can open the spirocyclic form of **1** to lead to color changes,  $\text{Pd}^{2+}$  quenches the expressed fluorescence efficiently.<sup>22</sup>

To demonstrate the potential application of this platinum ion probe, we conducted experiments to detect cisplatin<sup>23</sup>

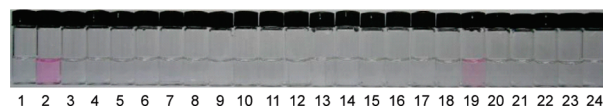
(21) The UV-vis absorption experiments also indicate that both  $\text{Pt}^{2+}$  and  $\text{Pd}^{2+}$  induce strong absorptions at  $\sim 541\ \text{nm}$  (see Supporting Information).

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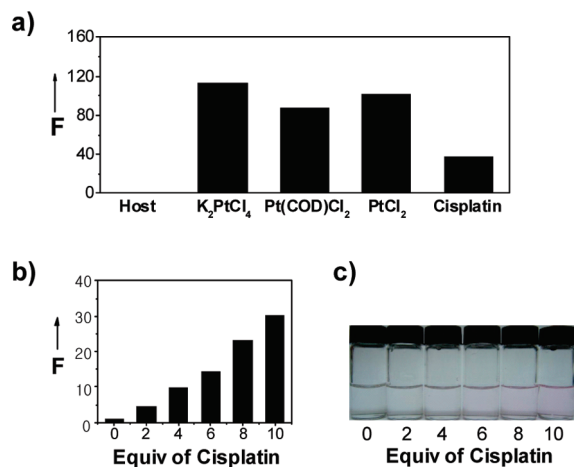
**Figure 2.** Fluorescence intensity changes of **1** ( $5\ \mu\text{M}$ ) in  $\text{H}_2\text{O}$  (DMSO 1% v/v) at 562 nm: (a) In the presence of metal ions (5.0 equiv): 1, none; 2,  $\text{Pt}^{2+}$ ; 3,  $\text{Fe}^{3+}$ ; 4,  $\text{Fe}^{2+}$ ; 5,  $\text{Hg}^{2+}$ ; 6,  $\text{Zn}^{2+}$ ; 7,  $\text{Pb}^{2+}$ ; 8,  $\text{Ca}^{2+}$ ; 9,  $\text{Co}^{2+}$ ; 10,  $\text{Mn}^{2+}$ ; 11,  $\text{Mg}^{2+}$ ; 12,  $\text{Cu}^{2+}$ ; 13,  $\text{Cd}^{2+}$ ; 14,  $\text{Al}^{3+}$ ; 15,  $\text{Cr}^{2+}$ ; 16,  $\text{Ag}^+$ ; 17,  $\text{Na}^+$ ; 18,  $\text{Li}^+$ ; 19,  $\text{Pd}^{2+}$ ; 20,  $\text{Ru}^{3+}$ ; 21,  $\text{Rh}^{2+}$ ; 22,  $\text{Ni}^{2+}$ ; 23,  $\text{K}^+$ ; 24,  $\text{Ba}^{2+}$ . (b) In the presence of  $\text{Pt}^{2+}$  (5.0 equiv) and other metal ions (5.0 equiv): 1, none; 2,  $\text{Fe}^{3+}$ ; 3,  $\text{Fe}^{2+}$ ; 4,  $\text{Hg}^{2+}$ ; 5,  $\text{Zn}^{2+}$ ; 6,  $\text{Pb}^{2+}$ ; 7,  $\text{Ca}^{2+}$ ; 8,  $\text{Co}^{2+}$ ; 9,  $\text{Mn}^{2+}$ ; 10,  $\text{Mg}^{2+}$ ; 11,  $\text{Cu}^{2+}$ ; 12,  $\text{Cd}^{2+}$ ; 13,  $\text{Al}^{3+}$ ; 14,  $\text{Cr}^{3+}$ ; 15,  $\text{Ag}^+$ ; 16,  $\text{Na}^+$ ; 17,  $\text{Li}^+$ ; 18,  $\text{Pd}^{2+}$ ; 19,  $\text{Ru}^{3+}$ ; 20,  $\text{Rh}^{2+}$ ; 21,  $\text{Ni}^{2+}$ ; 22,  $\text{K}^+$ ; 23,  $\text{Ba}^{2+}$ .



**Figure 3.** Color changes of **1** ( $5\ \mu\text{M}$ ) in the presence of metal ions (5.0 equiv) in  $\text{H}_2\text{O}$  (DMSO 1% v/v): 1, none; 2,  $\text{Pt}^{2+}$ ; 3,  $\text{Fe}^{3+}$ ; 4,  $\text{Fe}^{2+}$ ; 5,  $\text{Hg}^{2+}$ ; 6,  $\text{Zn}^{2+}$ ; 7,  $\text{Pb}^{2+}$ ; 8,  $\text{Ca}^{2+}$ ; 9,  $\text{Co}^{2+}$ ; 10,  $\text{Mn}^{2+}$ ; 11,  $\text{Mg}^{2+}$ ; 12,  $\text{Cu}^{2+}$ ; 13,  $\text{Cd}^{2+}$ ; 14,  $\text{Al}^{3+}$ ; 15,  $\text{Cr}^{3+}$ ; 16,  $\text{Ag}^+$ ; 17,  $\text{Na}^+$ ; 18,  $\text{Li}^+$ ; 19,  $\text{Pd}^{2+}$ ; 20,  $\text{Ru}^{3+}$ ; 21,  $\text{Rh}^{2+}$ ; 22,  $\text{Ni}^{2+}$ ; 23,  $\text{K}^+$ ; 24,  $\text{Ba}^{2+}$ .

in aqueous solutions. We first compared fluorescence intensity changes of **1** exerted by different platinum complexes ( $\text{K}_2\text{PtCl}_4$ ,  $\text{Pt}(\text{COD})\text{Cl}_2$ ,  $\text{PtCl}_2$ , and cisplatin). The platinum complexes were dissolved in  $\text{H}_2\text{O}$  (5 mM) for 1 h at  $25^\circ\text{C}$  and then added into the solution of probe **1** ( $5\ \mu\text{M}$ ) in  $\text{H}_2\text{O}$  (DMSO 1% v/v). The resulted solutions were incubated for 24 h at  $25^\circ\text{C}$  prior to the fluorescence measurement.<sup>24</sup> Although cisplatin shows relatively smaller fluorescence intensity changes compared with other platinum complexes, it is possible to detect cisplatin in aqueous solutions (Figure 4a). Fluorescence moni-

(24) It seems that the ligand exchange reactions of the platinum complexes take place slowly. See: Reedijk, J. *Platinum Met. Rev.* **2008**, 52, 2–11.



**Figure 4.** (a) Fluorescence intensities of **1** (5  $\mu$ M) in the presence of 10.0 equiv of different platinum complexes at 25 °C (at 562 nm). (b) Fluorescence intensity changes of **1** (5  $\mu$ M) upon addition of different amounts of cisplatin in H<sub>2</sub>O (DMSO 1% v/v) (at 562 nm). (c) Color change of **1** (5  $\mu$ M) upon addition of different amounts of cisplatin in H<sub>2</sub>O (DMSO 1% v/v).

toring of cisplatin using probe **1** (5  $\mu$ M) is possible at micromolar concentration in aqueous solutions according to the fluorescence experiment (Figure 4b), and colorimetric detection

of aqueous cisplatin is also possible at micromolar concentration as shown in Figure 4c.

In conclusion, we have described a highly selective and sensitive fluorescent chemosensor for the detection of Pt<sup>2+</sup> in aqueous solutions. The binding platform of a triazole–hydroxamate conjugate displays selective complexation with Pt<sup>2+</sup>,<sup>25</sup> and the fluorescent and colorimetric detections of cisplatin in aqueous solutions using probe **1** are also demonstrated.

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**Supporting Information Available:** Experimental procedures for the synthesis and copies of <sup>1</sup>H NMR and <sup>13</sup>C NMR of **1**, data for UV–vis, fluorescence of **1**, and other data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(25) The related hydroxamate binding platforms containing a simple binding unit, such as a pyridine and an amino group instead of a triazole, show nonselective binding properties.