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A Reaction-Based Sensing Scheme for Gold Species: Introduction of a (2-Ethynyl)benzoate Reactive Moiety

Hyewon Seo,[†] Mi Eun Jun,[†] Olga A. Egorova,[‡] Kyung-Ha Lee,[§] Kyong-Tai Kim,[§] and Kyo Han Ahn*,[†]

Department of Chemistry and Center for Electro-Photo Behaviors in Advanced Molecular Systems, Pohang University of Science and Technology (POSTECH), San 31, Hyoja-dong, Pohang, 790-784, Republic of Korea, Department of Analytic Technology Research, Samsung Display, Tangjeong-meon, Asan-si, Chungnam, 336-841, Republic of Korea, and Department of Life Science, Pohang University of Science and Technology (POSTECH), San 31, Hyoja-dong, Pohang, 790-784, Republic of Korea

ahn@postech.ac.kr

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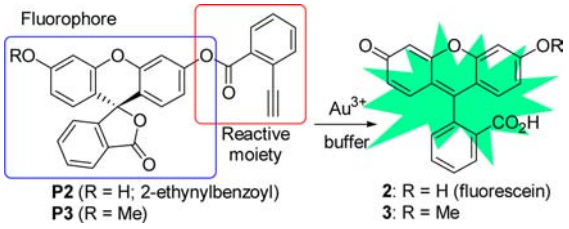
ABSTRACT



To alleviate side reactions identified in an *N*-propargyl-rhodamine lactam sensing system, we devised the novel reaction-based sensing scheme for gold species based on the alkynophilicity. A fluorescein (2-ethynyl)benzoate underwent Au(III)-promoted ester hydrolysis selectively over other metal ions with high sensitivity, which accompanies a turn-on fluorescence change in pH 7.4 HEPES buffer. The work offers a versatile reactive moiety for the development of gold probes with improved sensing properties.

Gold chemistry has been spotlighted in recent years because of the unique optical properties of gold nanoparticles[1] and catalytic[2] and biological activities[3] of gold complexes. Widespread use of gold species in academia and industry thus needs efficient and convenient detection methods to evaluate and monitor the residual gold species in various circumstances. For analysis of gold species, various spectroscopic (atomic absorption/emission spectroscopy with various atomizers), mass, and electrochemical (anodic stripping voltammetry) methods are available.[4] In contrast, fluorogenic sensing systems for gold ions have appeared very recently. Given that the fluorescent method is sensitive and easy-to-use, it would complement conventional analytical methods. Furthermore, the fluorescent probes are indispensible in the bioimaging area.

Recently we developed a rhodamine-derived *N*-propargyl-spirolactam **P1** as a fluorescent probe for gold species. **P1** undergoes tandem spirolactam ring opening and

[†] Department of Chemistry and Center for Electro-Photo Behaviors in Advanced Molecular Systems, POSTECH.
[‡] Samsung Display.
[§] Department of Life Science, POSTECH.

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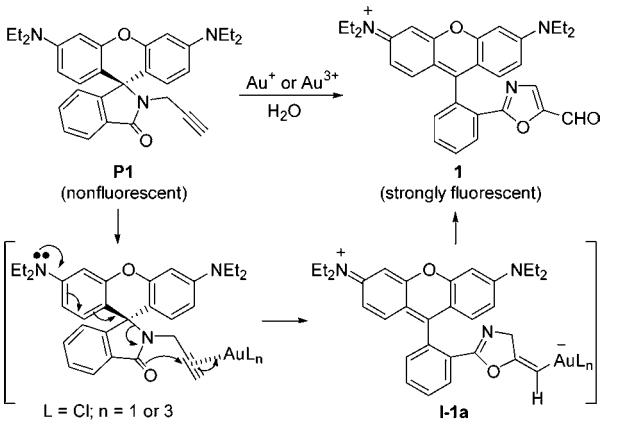
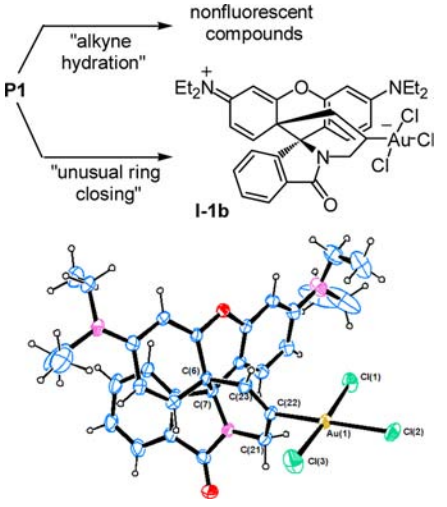
benzoxazole ring formation promoted by gold species, which accompanies a turn-on fluorescence change (Scheme 1).[5] This strategy was based on our previous reaction-based approach to sense silver ions, that is, the silver ion-promoted tandem spirolactam ring opening and oxazoline ring formation of an *N*-iodoethyl-rhodamine B lactam.[6]

Scheme 1. Reaction-Based Sensing of Gold Ions with **P1**



Concurrently, others have reported reaction-based fluorescent probes for gold species based on the unique alkynophilicity of gold ions.[7,8] **P1** provided fluorescent formyloxazole **1**, rather than the corresponding proto-deauration products of the vinylgold intermediate (**I-1a**); this unexpected result prompted us to characterize the reaction intermediates in the gold-mediated cyclization of *N*-propargylbenzamides as model compounds.[9] We found that the gold sensing process could also produce two types of nonfluorescent compounds (Scheme 2), along with formyloxazole **1** that constitutes 60−70% of the total mass. Simple hydration of the acetylenic moiety promoted by Lewis acidic gold species could compete with the spirolactam ring-opening process, producing the corresponding fluorescent compounds. Furthermore, an unusual ring-closing process is found to compete with the sensing process: A tricyclic vinylgold(III) species **I-1b**, which is nonfluorescent, has been identified by its single crystal X-ray structure.[10] This resolved structure also adds a valuable piece of evidence for the participation of aryl rings in the related gold-mediated cyclization reactions.[11] If a reaction-based sensing protocol produces nonfluorescent side products, as in the case of **P1**, then the product distribution would vary depending on the sensing condi-

Scheme 2. Competing Reactions in the Gold Sensing Process with **P1** and Crystal Structure of **I-1b** (ORTEP Drawing with 20% Probability)



tions, which would undermine the reliability of the quantification data. Therefore, we set out to investigate a related but different approach that may alleviate the side reactions observed in the rhodamine-based probe. We envisioned that we could avoid fluorescence interference from the side reactions by separating the reaction site from the fluorophore. Herein, as a proof-of-concept we report such a reaction-based sensing scheme for gold species that circumvents the aforementioned problems.

Our newly designed probes for gold species are (2-ethynyl)benzoates of fluorescein, **P2** (**P2a**, **P2b**) and **P3**, where the reactive moiety, (2-ethynyl)benzoate, is separated from the fluorophore. For example, activation of the ethynyl group in **P2** by Au^{3+} would generate the corresponding oxonium intermediate **I-2**, which would subsequently undergo hydrolysis to regenerate the strongly fluorescent fluorescein **2** (Scheme 3), along with isochromen-1-one **4**. A related gold-promoted hydrolysis process was ingeniously applied to a glycosylation reaction by Yao and co-workers.[12] According to the present sensing

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(10) Crystal data for **I-1b** ($C_{31}H_{33}AuCl_3N_3O_2$): M = 782.92, monoclinic, space group P_{21}/n; a = 10.9486(11) Å, b = 11.7414(12) Å, c = 24.580(3) Å, β = 100.062(2)°, V = 3111.1(5) Å^3; Z = 4, T = 293(2) K, μ (λ = 0.71073 Å) = 5.019 mm^{-1}, ρ_{calcd} = 1.671 g/cm^3, 17 756 reflections measured, 6348 unique (R_{int} = 0.0686), R_1 = 0.0412, wR_2 = 0.1012 ($I > 2\sigma(I)$), R_1 = 0.0597, wR_2 = 0.1104 (all data), GOF = 0.999, CCDC No. 747441.

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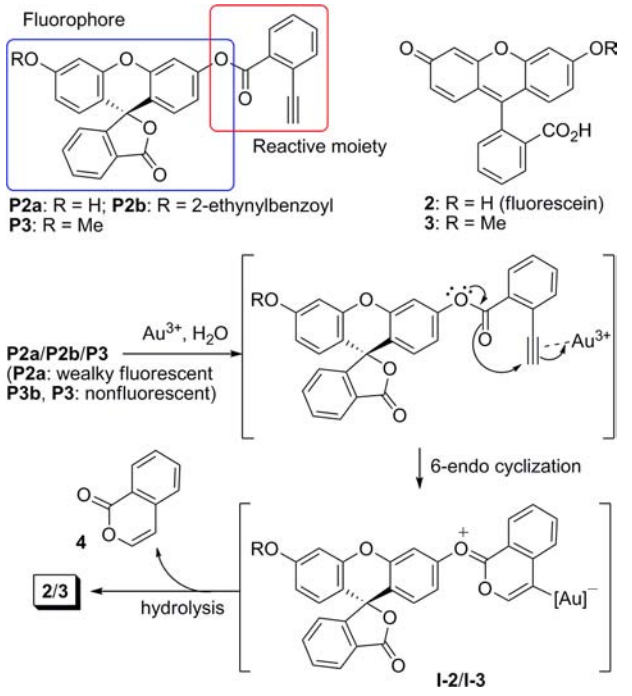
scheme, the hydration at the ethynyl group, if any, does not lead to the fluorescent compounds. Also, the unusual ring-opening process observed in the case of the rhodamine lactam is not conceivable.

Scheme 3. New Fluorescein-Derived Probes for Gold Species (**P2**) Equipped with Outer Reactive Moiety of (2-ethynyl)-benzoate, and Its Sensing Mechanism



The mono- and bis-(2-ethynylbenzoate) of fluorescein (**P2a**, **P2b**) and a methyl ether derivative **P3** were readily prepared by EDC-DMAP coupling of fluorescein or its methyl ether derivative **3** with (2-ethynyl)benzoic acid, respectively (see the Supporting Information). We have found that (2-ethynyl)benzoic acid[13] undergoes polymerization on standing, and thus its precursor, methyl (2-ethynyl)benzoate, should be hydrolyzed prior to use. Otherwise, 2-iodobenzoic acid can be first coupled with fluorescein, and then the ethynyl group is introduced by Sonogashira coupling.[14] In both cases, the ester coupling reactions yielded a mixture of mono- and bis-benzoates, which were separable by silica gel column chromatography. Similarly, **P3** was synthesized from fluorescein methyl ether **3**. Fluorescein bis(benzoate) **P2b** thus could be prepared in about 30% isolated yield under stoichiometric reaction conditions.

P2b was found to be the probe of choice based on preliminary evaluation studies for the **4** fluorescein benzoates. **P2b** barely emitted, whereas **P2a** emitted fluorescence, albeit small, to give an apparent background signal (Figure S1). In addition, the fluorescence response of **P2a** to $AuCl_3$ was slower than that of **P2b**. In the case of **P3**, it also showed a slower response to $AuCl_3$ (Figure S2). Furthermore, its sensing product **3** emitted weakly, giving about one-fifth of the emission intensity of fluorescein (Figure S3). Of particular note is that **3** barely emitted in cells, giving poor fluorescent images (in the following). Therefore, use of fluorescein methyl ether **3** for cell imaging purposes is not recommended in spite of some reported probes based on it.[15]

A solution of **P2b** (5 μM) dissolved in pH 7.4 buffer (5 mM HEPES containing 0.25% DMSO) emitted barely when excited at 470 nm.[16] Upon addition of $AuCl_3$, however, the solution of **P2b** emitted strong green fluorescence with λ_{max} at 508 nm. A time course of the fluorescence titration shows that the response is fast and complete within 1 h at ambient temperature (Figure 1). These results suggested that the $AuCl_3$-promoted ester hydrolysis indeed took place; the fluorescence was found to result from fluorescein by comparison with an authentic sample (Figure S5). Although it causes significant fluorescence changes, we have not examined AuCl in detail, as Au(I) readily undergoes a disproportionation into Au(III) and Au(s) species in aqueous media [$3Au^{+}(aq.) \rightarrow Au^{3+}(aq.) + 2Au^{0}$].[17]

Next, we evaluated the fluorescence response of **P2b** toward various metal salts such as Mg(II), Ba(II), Cr(II), Mn(II), Fe(III), Co(II), Ni(II), Pd(II), Cu(II), Ag(I), Zn-(II), Cd(II), Hg(II), Pt(II), and Au(III) (as their chloride salts except for $AgNO_3$).

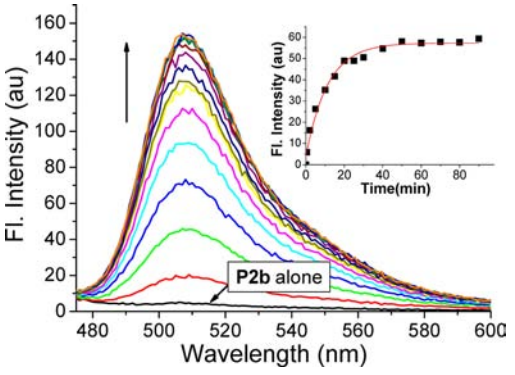


Figure 1. Time-dependent fluorescence changes of **P2b** (5 μM) in the presence of $AuCl_3$ (2 equiv) in pH 7.4 buffer (5 mM HEPES containing 0.25% DMSO) at ambient temperature, taken under excitation at 470 nm. Inset: a plot of the fluorescence intensity vs time.

As shown in Figure 2, **P2b** responds only to the gold species among the metal species examined. A competition

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(16) Excited at lower wavelength than the emission maximum of the solution of **P2b** and $AuCl_3$ (λ_{max} = 490 nm, Figure S4) to avoid self-absorption.

assay, however, showed that coexistence of some metal species caused emission enhancement but Ag(I) and Pt(II) species caused a small decrease in the fluorescence (Figure S6). The reason for this interesting behavior is unclear at the moment.

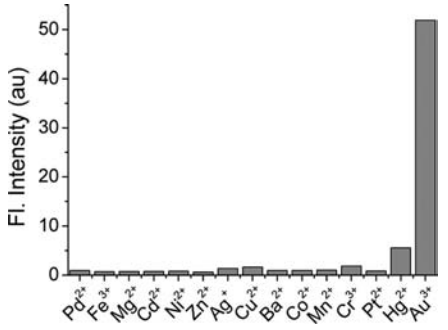

Figure 2. Fluorescence response of **P2b** (5 μM) toward various metal species (2 equiv) in pH 7.4 buffer (5 mM HEPES containing 0.25% DMSO) at ambient temperature, obtained after 20 min under excitation at 470 nm.

The fluorescence response of **P2b** was linear in the range of 1−30 μM $AuCl_3$ (Figure S7). On the basis of the signal-to-noise ratio of 3, the detection limit of **P2b** for $AuCl_3$ was estimated to be below 0.4 μM (Figure S8).

Progress of the sensing reaction was followed by TLC, and the product was identified by NMR analysis for a reaction mixture containing **P2b** and $AuCl_3$ at a higher concentration (~2 mM of substrates in the aqueous media). Under the conditions, only the Au(III)-promoted ester hydrolysis occurred predominantly, and other possible side reactions such as simple hydration of the ethynyl moiety were negligible (Figure S9). The results show that the new approach alleviates the side reactions observed in the rhodamine-based system.

Interestingly, the sensing behavior of **P2b** toward $AuCl_3$ was sensitive to pH. A solution of **P2b** and $AuCl_3$ (2 equiv) at different pH values (pH 1−10) showed strong emission only near pH 7 but little at other pH values except at pH 10 where a substantial increase was observed (Figure S10). At high pH conditions, nonspecific ester hydrolysis seems to occur because **P2b** is an ester-type probe.

Finally, we briefly evaluated **P2b** for the fluorescent imaging of $AuCl_3$ in cells. Bright-field and fluorescent microscopic images of HeLa cells incubated with both **P2b** (10 μM) and $AuCl_3$ (100 μM) showed green fluorescence. In contrast, little fluorescence was observed from the cells treated only with the probe (Figure S11). However, we found that hydrolysis of the ester probe by esterase in the cells interferes with the gold-promoted sensing process, which limits its application for the bioimaging purpose. We also examined **P3** in the fluorescent cell imaging. As noted above, the corresponding reaction product **3** gave poor fluorescence (Figure S12); hence, fluorescein methyl ether **3** is not suitable for cell imaging. As an alternative application of **P2b**, we used the probe to quantify residual gold species in a $AuCl_3$-catalyzed reaction product that is purified by silica gel column chromatography (Figure S13).

In summary, we have devised a new reaction-based sensing scheme for gold species by utilizing their alkynophilicity. One of the (2-ethynyl)benzoates derived from fluorescein underwent Au(III)-promoted hydrolysis, which accompanies a turn-on fluorescence change in aqueous media. The sensing reaction is selectively promoted by Au(III) species among various metal species examined. The new reactive moiety is promising for the development of improved fluorescent probes for gold species, as it alleviates the side reactions indentified in the rhodamine-based probe. A further study to prevent the ester-type probes' sensitivity toward esterase is underway and will be reported in due course.

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Supporting Information Available. Synthesis and characterization of **P2** and **P3**, pH-dependent fluorescence behavior, and other fluorescence titration data. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.