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# Ratiometric Signaling of Hypochlorite by the Oxidative Cleavage of Sulfonhydrazide-Based Rhodamine–Dansyl Dyad

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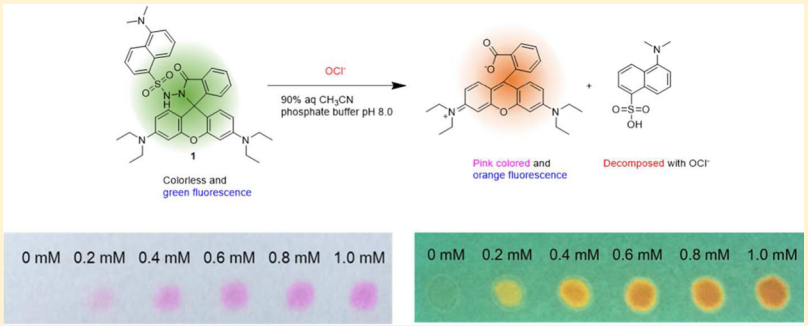
Supporting Information




**ABSTRACT:** A reaction-based probe **1** for hypochlorite signaling was designed by the conjugation of two fluorophores, rhodamine and dansyl moieties, by the reaction of rhodamine B base with dansylhydrazine. Probe **1** exhibited pronounced hypochlorite-selective chromogenic and fluorescent signaling behavior over other oxidants used in practical applications, such as hydrogen peroxide, peracetic acid, and ammonium persulfate, as well as commonly encountered metal ions and anions. Signaling was attributed to the hypochlorite-induced oxidative cleavage of the sulfonhydrazide linkage of the probe. In particular, favorable ratiometric fluorescence signaling was possible by utilizing the emissions of the two fluorophores. A detection limit of $1.13 \times 10^{-6}$ M (0.058 ppm) was estimated for the determination of hypochlorite. A paper-based test strip was prepared and was used as a semiquantitative indicator for the presence of hypochlorite in aqueous solutions. The probe was also successfully applied for the determination of hypochlorite in practical tap water samples.

## INTRODUCTION

Hypochlorous acid (HOCl) is an important reactive oxygen species in biology; it plays several fundamental roles in living systems, such as antimicrobial activity in the natural defense system as well as protein unfolding and aggregation.[1] In addition, it has been widely employed in various industrial applications, such as in the paper and textile industries for bleaching and to control slime and algae in piping and tubes.[2] Furthermore, HOCl has also been applied as a safe sanitizer for the treatment of drinking water and sanitization of swimming pools.[3] In the guideline of the World Health Organization, the minimum residual concentration of free chlorine in drinking-water at the point of delivery should be 0.2 mg/L to ensure safe water supply by sanitizing certain bacteria and other microbes in tap water.[4] Because of its biological and environmental importance, it is imperative to develop highly sensitive and selective optical signaling probes for HOCl or hypochlorite, its ionized form. In recent years, various fluorescent probes have been devised for the signaling of HOCl.[5] However, most of the sensors and probes are targeting for the imaging of HOCl in biological media, such as in live-cell imaging.[6] On the contrary, only a few studies on the optical sensing of HOCl using functional dyes for real applications, such as for the analysis of tap and swimming pool water, have recently attracted research interest.[7]

For the selective signaling of HOCl, a number of chromogenic and luminescent chemodosimeters have been developed. These designed probes are principally based on its strong oxidative property.[5] Representative examples of the sensors developed include the well-controlled HOCl-induced oxidation of oximes of hydroxycoumarin and fluorescein to aldehydes,[8] oxidation of boron dipyrromethene (BODIPY)-based diphenyl selenide and diphenyl telluride into selenoxide and telluroxide, respectively,[9,10] and the oxidative conversion of BODIPY-appended *p*-methoxyphenol into its quinone derivative.[11] On the other hand, the ring-opening and oxidation of rhodamine-based thioether[12] as well as the cleavage of the ether moiety having photoelectron transfer quenching of the 4-amino or 4-hydroxylphenyl ether moiety of rhodamine derivatives have been utilized for the imaging of HOCl in various cells.[13] In addition, rhodamine-based thiospirolactone[14] and hydroxamic acid[15] have also been successfully used for the detection of HOCl in various chemical and biological systems, such as in living human neutrophil cells and zebrafish. In particular, a number of rhodamine hydrazide-based HOCl probes,[5] designed from rhodamine hydrazide itself[16] to simple derivatives functionalized with the phenylhydrazo[17] and

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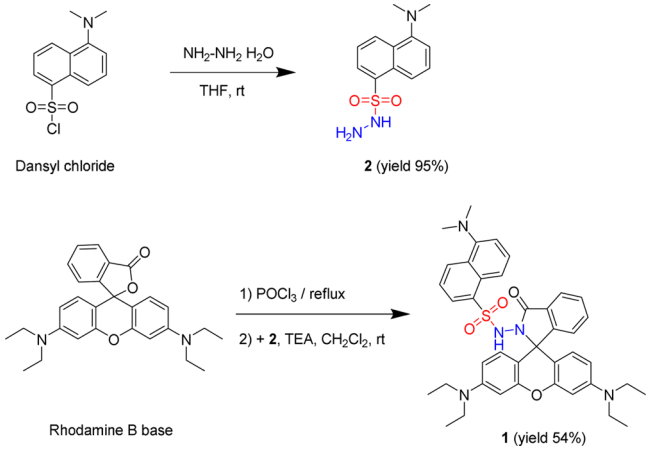
naphthoylhydrazo groups,[18] have been reported. The transformations of rhodamine−thiosemicarbazide into rhodamine-1,3,4-oxadiazole as well as benzoylhydrazide of rhodamine−coumarin conjugates were also successfully used for the ratiometric signaling of HOCl.[19,20]

The sulfonhydrazide functionality, which is utilized as a useful protecting group for carbonyl-containing compounds in synthetic organic chemistry, is known to be readily cleaved by HOCl.[21] However, to the best of our knowledge, this valuable reaction or processes related to the hydrolysis of sulfonhydrazide have not been used for the design of any probe for HOCl. In this study, we designed a hypochlorite-selective reaction-based probe based on a rhodamine−dansyl dyad by utilizing the selective oxidative cleavage of the sulfonhydrazide linkage group.[22] We used the rhodamine−dansyl dyad as the signal transducer and the sulfonhydrazide linkage as the triggering switch, which responds to the presence of hypochlorite. The designed probe exhibited prominent chromogenic and ratiometric fluorogenic signaling behavior toward hypochlorite.

## RESULTS AND DISCUSSION

**Synthesis of Rhodamine−Dansyl Dyad 1.** Sulfonhydrazide probe **1** based on a rhodamine−dansyl dyad was prepared by the reaction of rhodamine B base with dansylhydrazine **2** (Scheme 1). Dansylhydrazine was obtained by treating dansyl chloride with hydrazine following a literature procedure for the preparation of 4-nitrobenzenesulfonhydrazide.[23] Attempts to synthesize probe **1** by treating rhodamine B hydrazide with dansyl chloride were unsuccessful. The rhodamine and dansyl moieties were used for designing a reaction-based probe with the aim of constructing a more versatile ratiometric system for the selective signaling of hypochlorite using two fluorophores. Similar signaling could be realized by the use of other simple sulfonhydrazides such as *p*-toluenesulfonyl hydrazide-based dyad; however, the resulting signal obtained is a simple off−on-type fluorescence change. With the probe currently designed, the changes in the characteristic emissions of both rhodamine and dansyl fluorophores could be utilized to obtain more desirable ratiometric signaling behavior.

**Scheme 1. Preparation of Rhodamine−Dansyl Dyad-Based Sulfonhydrazide Probe 1**




**Colorimetric and Fluorescence Signaling Behavior of 1.** The signaling behavior of probe **1** toward oxidants typically used for practical applications was investigated by UV−vis and fluorescence measurements. As shown in Figure 1, in a 90% aqueous acetonitrile solution buffered at pH 8.0 with a phosphate buffer (final concentration = 10 mM), probe **1** exhibited no significant absorption above 450 nm. However, after treatment with 10 equiv of various oxidants, only $OCl^-$ exhibited a prominent absorption band at 555 nm (Figures 1 and S1, Supporting Information). The color of the solution concomitantly changed from colorless to pink, which was readily detected without any device. Other oxidants used for practical applications, such as $H_2O_2$, peracetic acid (PAA), *tert*-butyl hydroperoxide (TBHP), $O_2^-$, Oxone, persulfate, percarbonate, and perborate, did not induce any noticeable response. The $OCl^-$-selective signaling of **1** could be illustrated by the enhancement in absorbance, expressed by $A/A_0$ at 555 nm, where $A$ and $A_0$ denote the absorbance in the presence and absence of analytes, respectively. Hypochlorite induced a 31-fold enhancement in absorbance at 555 nm, while other oxidants exhibited negligible changes (Figure S1, Supporting Information).

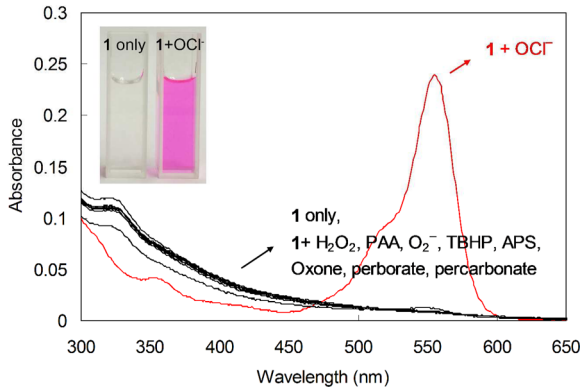



**Figure 1.** UV−vis spectra of **1** in the presence of oxidants typically used for practical applications. (inset) Photograph of **1** in the absence and presence of $OCl^-$. [**1**] = $5.0 \times 10^{-6}$ M, [oxidant] = $5.0 \times 10^{-5}$ M in a 90% aqueous acetonitrile solution buffered at pH 8.0 (phosphate, 10 mM). PAA = peracetic acid, TBHP = *tert*-butyl hydroperoxide, and APS = ammonium persulfate.

However, probe **1** revealed a moderate-intensity emission centered at 494 nm, mainly attributed to the dansyl fluorophore. As expected, the rhodamine moiety did not exhibit any emission, attributed to the ring-closed lactam structure of **1**. However, in the presence of 10 equiv of oxidants typically used for practical applications, the emission profile dramatically changed exclusively with $OCl^-$ (Figures 2 and S2, Supporting Information). In fact, the isosbestic point of probe **1** was 460 nm. However, when probe **1** was excited at 460 nm, dansyl fluorophore did not exhibit any emission signaling (Figure S3, Supporting Information). To realize ratiometric signaling using both dansyl and rhodamine fluorophores of **1**, the probe was excited at 400 nm instead of its isosbestic point. Under illumination with a UV lamp, the solution color changed from green to orange. The emission band of probe **1** at 494 nm completely disappeared, and a new strong band was observed at 578 nm, attributed to the ring-opened form of the rhodamine fluorophore. With this change, the emission intensity ratio at two characteristic wavelengths 578 and 494 nm $I_{578}/I_{494}$ dramatically increased (130-fold) from 0.32 to 42.1. To obtain more efficient signaling conditions, we surveyed the signaling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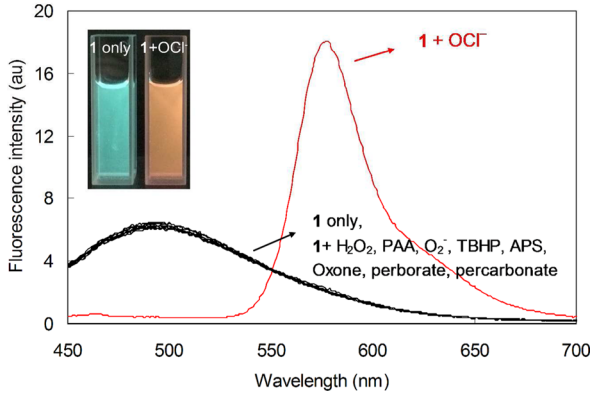



**Figure 2.** Changes in the fluorescence spectra of **1** in the presence of oxidants typically used for practical applications. (inset) UV-illuminated photograph of **1** in the absence and presence of $OCl^-$. [**1**] = $5.0 \times 10^{-6}$ M, [oxidant] = $5.0 \times 10^{-5}$ M in a 90% aqueous acetonitrile solution buffered at pH 8.0 (phosphate, 10 mM). $\lambda_{ex}$ = 400 nm.

behavior of **1** toward $OCl^-$ in various solvents with different water percentages (Figures S4–S6, Supporting Information). Among the tested solvents, signaling in dimethyl sulfoxide (DMSO) was not possible due to the scavenging effect of DMSO for hypochlorite.[24] After systematic survey, we found that probe **1** exhibited relatively optimized $OCl^-$-selective signaling behavior in terms of selectivity and ratiometrically analyzable behavior in 90% aqueous acetonitrile buffered at pH 8.0 with a phosphate buffer.

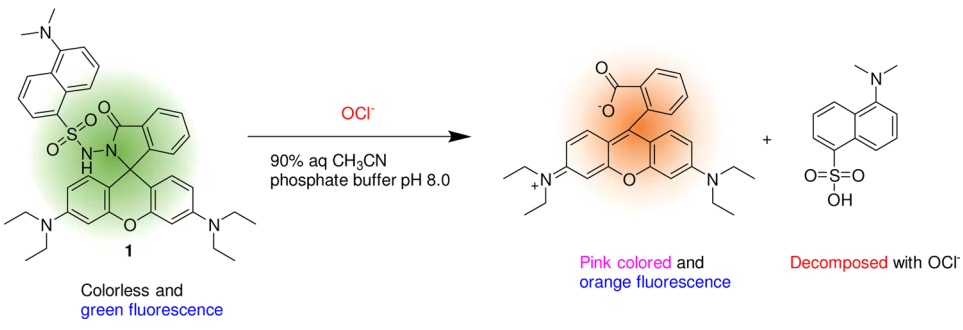
Selective signaling for $OCl^-$ is attributed to the oxidative hydrolysis of sulfonhydrazide **1** (Scheme 2). In fact, the $OCl^-$-induced hydrolysis of sulfonyl hydrazone as a protecting group has already been reported in synthetic organic chemistry.[21] However, we could not find any study on a similar $OCl^-$-induced reaction used for sulfonhydrazides. Upon reaction with $OCl^-$, probe **1** first decomposed to rhodamine B base and dansyl acid. The rhodamine B base thus generated was found to be stable under the experimental conditions. However, dansyl acid was found to decompose further, caused by the strong oxidation property of $OCl^-$ (Figure S7, Supporting Information). The proposed signaling process of **1** toward $OCl^-$ was confirmed by $^1$H NMR and mass measurements. Figure 3 shows the $^1$H NMR spectrum of the purified signaling product of **1** after treatment with $OCl^-$; the characteristic resonances of rhodamine B base were clearly observed. As previously described, after signaling with $OCl^-$, the relatively stable rhodamine B base could be recovered, while dansyl acid was decomposed further and could not be identified. In the mass spectrum, a diagnostic peak was observed at $m/z$ = 442.2 for rhodamine B base ($C_{28}H_{30}N_2O_3$).

Probe **1** showed satisfactory selectivity toward $OCl^-$ over other common metal ions and anions (Figures 4, S8 (for metal ions), and S9 (for anions), Supporting Information). The surveyed metal ions of alkali, alkaline earth, and transition metal ions did not exhibit any response. Notably, probe **1** did not exhibit any response toward $Cu^{2+}$ and $Hg^{2+}$ under the signaling conditions. Typically, rhodamine hydrazide-based sensors and probes exhibit significant responses to these two ions.[25] In fact, the *p*-toluenesulfonyl hydrazide analogue of **1** is known to exhibit $Cu^{2+}$-selective signaling behavior in aqueous acetonitrile.[26] Anions also did not exhibit significant responses to **1** (inset of Figure 4). This prominent selectivity of **1** for $OCl^-$ was also confirmed by the plot of the fluorescence intensity ratio at 578 and 494 nm ($I_{578}/I_{494}$): it varied between 0.28 (for $Ca^{2+}$) and 0.33 (for $Hg^{2+}$) for the tested metal ions and between 0.30 (for $ClO_4^-$) and 0.34 (for $Br^-$) for the surveyed anions.

For the selective determination of $OCl^-$ in practical samples, it is imperative to investigate the $OCl^-$ signaling of **1** in the presence of common metal ions or anions as background. With the tested metal ions, the changes in the fluorescence intensity ratio $I_{(1+\text{metal ion}+OCl-)}/I_{(1+OCl-)}$ at 578 nm varied in a narrow range between 0.96 (for $Pb^{2+}$) and 1.01 (for $Cd^{2+}$) (Figure 5). With the surveyed anions, the fluorescence signaling of $OCl^-$ was also not affected by the presence of anions: the ratio $I_{(1+\text{anion}+OCl-)}/I_{(1+OCl-)}$ at 578 nm marginally fluctuated between 0.99 (for $Br^-$) and 1.03 (for $NO_3^-$) (Figure S10, Supporting Information). These observations clearly imply that probe **1** could be applied for the determination of $OCl^-$ in chemical and environmental analytes, where various metal ions and anions are frequently present as coexisting ions.

The signaling of $OCl^-$ was completed within 1 min after sample preparation, as evidenced by the time-dependent absorption changes at 555 nm (Figure S11, Supporting Information). In addition, to test the applicability of the probe for the determination of $OCl^-$ in tap water under various circumstances that are expected for the water supply systems of different regions and seasonal changes, temperature-dependent signaling behavior of **1** toward $OCl^-$ was investigated. As shown in Figure S12 (Supporting Information), there was no discernible variation in the signaling rate of probe **1** for $OCl^-$ in the measured temperature range (10–50 °C). Furthermore, the stability of probe **1** was satisfactory: probe **1** exhibited no noticeable responses after 5 h of sample preparation. Effects of pH on the $OCl^-$ signaling of **1** were also verified by monitoring the changes in absorbance of **1** at 555 nm as a function of the pH of the measuring solution. As shown in Figure S13

**Scheme 2. Signaling Process for $OCl^-$ of Rhodamine–Dansyl Dyad 1**



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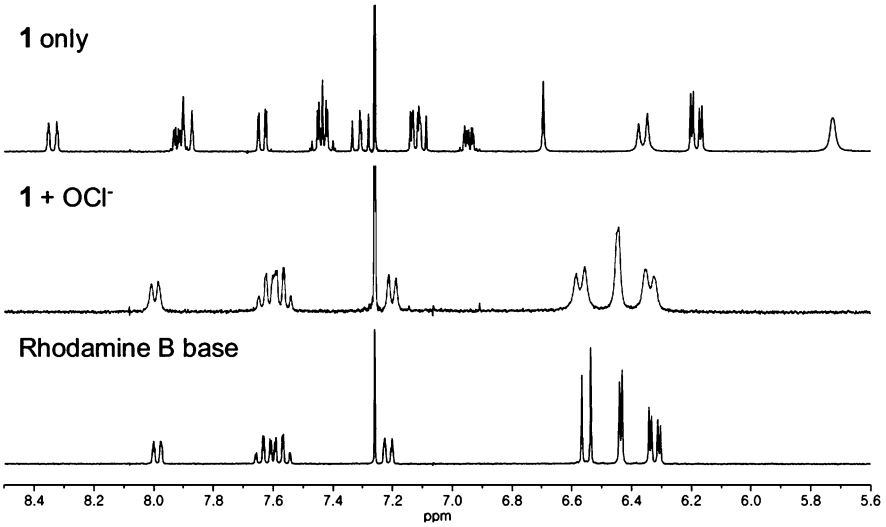


**Figure 3.** Partial $^1$H NMR spectrum of **1** alone, **1** in the presence of $OCl^-$, and rhodamine B base. [**1**] = [rhodamine B base] = 5.0 mM in $CDCl_3$. The spectrum of **1** + $OCl^-$ was obtained after the purification of the signaling product by passing it through a short silica plug.

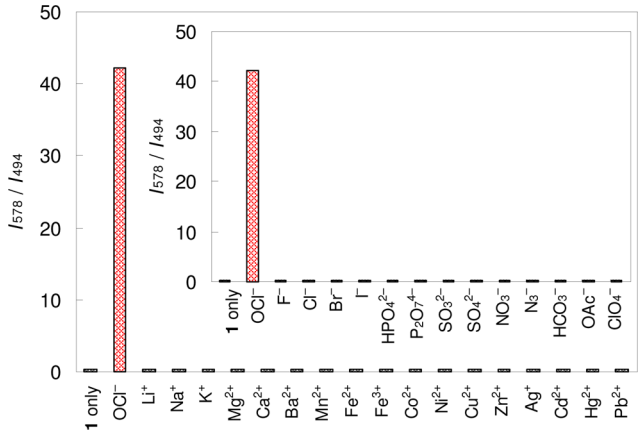


**Figure 4.** Changes in the fluorescence intensity ratio ($I_{578}/I_{494}$) of **1** in the presence of common metal ions or anions. [**1**] = $5.0 \times 10^{-6}$ M, [$OCl^-$] = [$M^{n+}$] = [$A^{n-}$] = $5.0 \times 10^{-5}$ M in a 90% aqueous acetonitrile solution buffered at pH 8.0 (phosphate, 10 mM). $\lambda_{ex}$ = 400 nm.

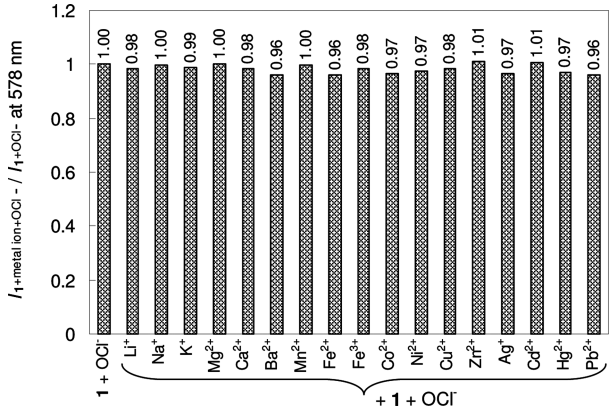


**Figure 5.** Competitive fluorescence signaling of $OCl^-$ by **1** in the presence of various metal ions as background. [**1**] = $5.0 \times 10^{-6}$ M, [$OCl^-$] = [$M^{n+}$] = $5.0 \times 10^{-5}$ M in a 90% aqueous acetonitrile solution buffered at pH 8.0 (phosphate). $\lambda_{ex}$ = 400 nm.

(Supporting Information), signaling became more efficient as the pH of the measuring solution increased from pH 4.5 to 8.0, followed by slow decrease as the pH increased to 10. From this plot, we confirmed that the $OCl^-$ signaling of probe **1** is optimized in a 90% aqueous acetonitrile solution phosphate buffered at pH 8.0.

The quantitative signaling behavior of $OCl^-$ by **1** was investigated by fluorescence titration. The fluorescence intensity measured at 494 nm decreased, while the emission at 578 nm steadily increased with increase in [$OCl^-$] (Figure 6). Ratiometry using the fluorescence intensity ratio at 578 and

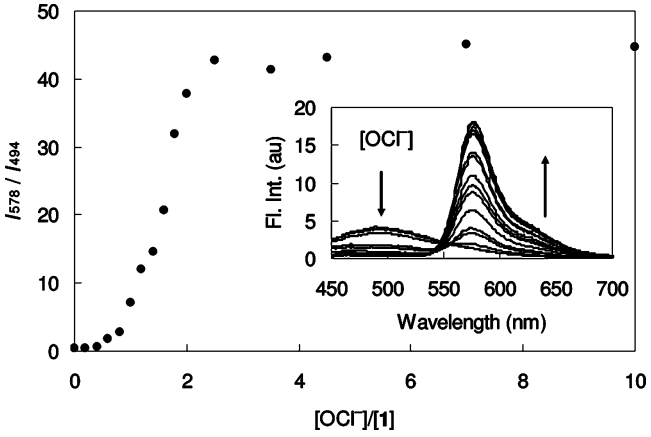


**Figure 6.** Concentration-dependent ratiometric signaling of $OCl^-$ by **1**. [**1**] = $5.0 \times 10^{-6}$ M, [$OCl^-$] = 0–$5.0 \times 10^{-5}$ M in a 90% aqueous acetonitrile solution buffered at pH 8.0 (phosphate). $\lambda_{ex}$ = 400 nm.

494 nm ($I_{578}/I_{494}$) was useful for the construction of a calibration plot. From concentration-dependent signaling measurements, a detection limit of $1.13 \times 10^{-6}$ M (0.058 ppm) was estimated for the determination of $OCl^-$ in a 90% aqueous acetonitrile.[27]

**Signaling of $OCl^-$ in Practical Samples.** Probe **1** was applied for the signaling of $OCl^-$ in real tap water samples. First, the possibility of visualizing $OCl^-$ in distilled water was tested using a test strip (Figure 7). For this purpose, a filter paper was impregnated with an acetonitrile solution of probe **1**, affording a filter-paper-based test strip for the sensing of $OCl^-$. When this test strip was treated with distilled water containing


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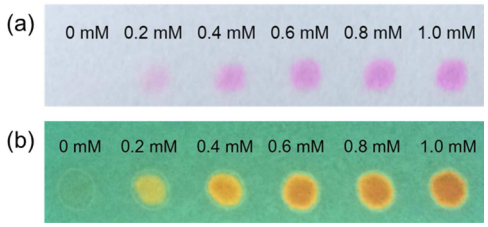


**Figure 7.** Photographs of the test strip image under (a) daylight and (b) UV illumination of **1** in the presence of varying amounts of $OCl^-$. $[OCl^-]$ varied from 0 to $1.0 \times 10^{-3}$ M in distilled water.

varied amounts of $OCl^-$, spots of pronounced colors were observed, which can be easily discernible by the naked eye as well as under UV illumination. This observation implies that probe **1** could be useful as a preliminary, convenient sensing kit for measuring the concentration of hypochlorite in practical samples. Next, the signaling of $OCl^-$ in tap water using the probe **1** was examined. As shown in Figure 8, only tap water

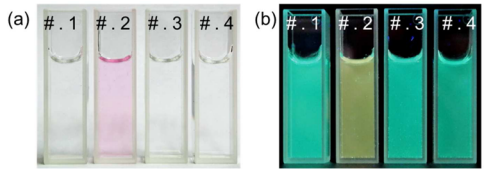


**Figure 8.** Photographs of the solution under (a) daylight and (b) UV illumination of **1** in the varying practical samples. $[\mathbf{1}] = 5.0 \times 10^{-6}$ M, in a 90% aqueous acetonitrile solution buffered at pH 8.0 (phosphate). (#.1) distilled water, (#.2) tap water, (#.3) commercial mineral water (Sam Da Soo, Jeju, Korea), (#.4) purified water (from a water purifier, CHP-570L, WoongJin, Korea).

sample exhibited prominent chromogenic and fluorogenic signaling behavior. Other practical samples, such as commercial mineral water (Sam Da Soo, Jeju, Korea) and purified water (from a water purifier, CHP-570L, WoongJin, Korea), did not induce any noticeable response. By the simple addition of a stock solution of the probe **1** to tap water analytes, changes discernible by the naked eye were observed in the analytes containing residual hypochlorites under measurement conditions. In addition, we conducted the standard addition method to determine the concentration of hypochlorite in tap water (Figure S14, Supporting Information). The concentration of hypochlorite in tap water was found to be $2.15 \times 10^{-6}$ M (0.11 ppm), which is in good agreement with the result obtained by using the standard *N*,*N*-diethyl-*p*-phenylenediamine (DPD) method ($0.12 \pm 0.01$ ppm).[28] On the basis of this observation, we believe that using the designed probe **1**, the presence and semiquantitative determination of hypochlorite in tap water as well as possibly in other drinking water supply systems could be rapidly achieved.

## CONCLUSION

A new hypochlorite-selective reaction-based dual-signaling probe by the conjugation of rhodamine B base with dansylhydrazine was developed. Rhodamine–dansyl dyad was used as the ratiometric signal transducer, and the sulfonhydrazide linkage was used as the reaction-based switch, which responds to the presence of hypochlorite. The probe exhibited highly selective hypochlorite-induced chromogenic and fluorescent responses in the presence of coexisting common metal ions and anions. In addition, the probe exhibited pronounced selectivity toward hypochlorite over other oxidants typically used for practical applications, such as hydrogen peroxide, peracetic acid, and ammonium persulfate. The designed probe could be used as a selective signaling tool for the detection of hypochlorite at micromolar concentrations. In addition, the practical application for the monitoring of hypochlorite in tap water using a test strip and solution color change was investigated.

## EXPERIMENTAL SECTION

**General.** Rhodamine B base, dansyl chloride, phosphorus oxychloride, hydrazine monohydrate, and sodium hypochlorite were purchased from Aldrich Chemical Co. All other chemicals and solvents were obtained from commercial sources and used as received. $^1$H NMR (300 and 600 MHz) and $^{13}$C NMR (75 and 150 MHz) spectra were recorded on Varian Gemini 2000 and Varian VNS NMR spectrometers using residual solvent signals as reference. UV–vis spectra were recorded on a Scinco S-3100 spectrophotometer equipped with a Peltier temperature controller. Fluorescence spectra were obtained on a PTI QuantaMaster steady-state spectrofluorometer. Mass spectra were recorded on a Micromass Autospec mass spectrometer. Elemental analysis data were obtained using a Thermo Electron Corporation Flash EA 1112 analyzer. Column chromatography was performed with silica gel (Merck, 240 mesh).

*Preparation of Dansylhydrazine* **2**. Dansylhydrazine **2** was obtained by treating dansyl chloride with hydrazine following a literature procedure for the preparation of 4-nitrobenzenesulfonhydrazide.[23] A solution of dansyl chloride (0.27 g, 1.0 mmol) in tetrahydrofuran (10 mL) was added to an aqueous hydrazine monohydrate solution (0.23 mL, 5.0 mmol). The progress of the reaction was monitored by thin-layer chromatography, and after the completion of the reaction, 20 mL of ethyl acetate was added to the reaction mixture. The resulting solution was washed three times using 10 mL of distilled water. The organic part was separated and evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel, $CH_2Cl_2$) to yield dansylhydrazine **2** (0.25 g, 95%) as a yellowish white powder. $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 8.60 (d, $J$ = 8.5 Hz, 1H), 8.30 (dd, $J$ = 7.3, 1.1 Hz, 1H), 8.28 (d, $J$ = 8.5 Hz, 1H), 7.56 (m, 2H), 7.20 (d, $J$ = 7.4 Hz, 1H), 6.01 (s, 1H), 2.89 (s, 6H). $^{13}$C NMR (150 MHz, $CDCl_3$) $\delta$ 152.1, 131.9. 131.5, 130.7, 130.0, 129.9, 128.8, 123.1, 118.7, 115.5, 45.4. HRMS: (FAB$^+$); $m/z$ calcd for $C_{12}H_{15}N_3O_2S^+$ $[M]^+$: 265.0885, found 265.0880.

*Preparation of Rhodamine–Dansyl Dyad* **1**. First, rhodamine B base (0.44 g, 1.0 mmol) was added to a solution of $POCl_3$ (15 mL), and the solution was refluxed for 6 h. After the completion of the reaction, the mixture was evaporated under reduced pressure, and the residue was dissolved in dichloromethane. Next, dansylhydrazine **2** (0.27 g, 1.0 mmol) and triethylamine (1 mL) were added to the above dichloromethane solution, and the resulting mixture was stirred for 12 h. Then, the reaction mixture was washed three times using 10 mL of distilled water. Finally, the organic part was separated and evaporated under reduced pressure, and the crude product was purified by column chromatography (silica gel, $CH_2Cl_2/CH_3OH$ = 29:1, v/v) to yield rhodamine–dansyl dyad (**1**) (0.37 g, 54%) as a pale white powder. $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 8.34 (d, $J$ = 8.4 Hz, 1H), 7.94–7.85 (m, 2H), 7.64 (dd, $J$ = 7.3, 1.3 Hz, 1H), 7.47–7.40 (m, 2H), 7.31 (dd, $J$ = 8.6, 7.5 Hz, 1H), 7.15–7.09 (m, 2H), 6.97–6.92 (m, 1H), 6.70 (s, 1H), 6.36 (d, $J$ = 8.6 Hz, 2H), 6.19 (dd, $J$ = 8.8, 2.6 Hz, 2H), 5.73 (s, 2H), 3.30 (q, $J$ = 7.2 Hz, 8H), 2.90 (s, 6H), 1.18 (t, $J$ = 7.1 Hz, 12H). $^{13}$C NMR (150 MHz, $CDCl_3$) $\delta$ 168.3, 153.0, 152.2, 151.4, 148.6, 134.1, 133.7, 130.0, 129.4, 129.4, 129.3, 128.6, 128.3, 128.0, 127.3, 124.4, 123.4, 122.9, 119.6, 114.6, 107.6, 104.3, 97.6, 66.6, 45.6, 44.2, 12.7. HRMS: (FAB$^+$); $m/z$ calcd for $C_{40}H_{44}N_5O_4S^+$ $[M + H]^+$: 690.3114, found 690.3119. Anal. Calcd for $C_{40}H_{43}N_5O_4S$: C, 69.64; H, 6.28; N, 10.15. Found: C, 69.32; H, 6.36; N, 10.22%.


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**Competitive Signaling Experiments for Metal Ions and Anions.** The competitive signaling behavior of probe **1** was measured in a mixture of phosphate buffer (pH 8.0) and acetonitrile (1:9, v/v). Test solutions were prepared by placing the stock solution of metal ion or anion (0.01 mM, 15 $\mu$L), the stock solution of **1** in acetonitrile (0.5 mM, 30 $\mu$L), and $OCl^-$ solution (0.01 mM, 15 $\mu$L) consecutively in a vial. The final concentrations of **1**, buffer, metal ion or anion ($M^{n+}$ or $A^{n-}$), and $OCl^-$ were $5.0 \times 10^{-6}$ M, $1.0 \times 10^{-2}$ M, $5.0 \times 10^{-5}$ M, and $5.0 \times 10^{-5}$ M, respectively. For fluorescence measurements, an excitation wavelength of 400 nm was used.

**$^1$H NMR Measurement of the Signaling Product.** To obtain evidence for the transformation of probe **1** to rhodamine B base by hypochlorite, the $^1$H NMR spectrum of the purified signaling product for a mixture of **1** and hypochlorite was recorded. The $^1$H NMR spectra of **1** ($5.0 \times 10^{-3}$ M) and rhodamine B base ($5.0 \times 10^{-3}$ M) were obtained in chloroform-*d* (0.6 mL). The $^1$H NMR spectrum of the signaling product was obtained after the purification by a short silica plug of the signaling product of probe **1** (22 mg, 0.05 mmol) treated with 0.1 N sodium hypochlorite (1.0 mL, 0.1 mmol) in a 50% aqueous acetonitrile solution.

**Application of Test Strip for Hypochlorite Determination.** A laboratory filter-paper-based test strip was prepared for the determination of hypochlorite. First, filter paper (Whatman #.3) was dipped into a solution of probe **1** (1.0 mM, acetonitrile) and then dried in atmosphere. Then, solutions with varied concentrations of hypochlorite (0–5.0 mM) were dropped on the prepared test paper. After these dots of solution dried for few minutes in air, pink colored and strongly fluorescent orange colored spots were developed. Images of the test paper under daylight and UV-lamp light were taken using a digital camera. To indicate the presence and semiquantitatively determine hypochlorite in tap water, a stock solution of probe **1** (10 $\mu$L, 1.0 mM, acetonitrile) in phosphate buffer (0.1 mL, pH 8.0, 10 mM) was added to tap water analytes, and tap water was further added to adjust the final volume to 10 mL. The absorption and fluorescence data of the resulting solutions were measured.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.inorgchem.5b01284.

UV–vis absorbance ratio ($A/A_0$), fluorescence spectra, time course plot, and pH dependency of probe **1** as well as NMR spectra of **1** and **2**. (PDF)

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### Notes

The authors declare no competing financial interest.

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