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# A Highly Selective and Sensitive Fluorescence Probe for the Hypochlorite Anion

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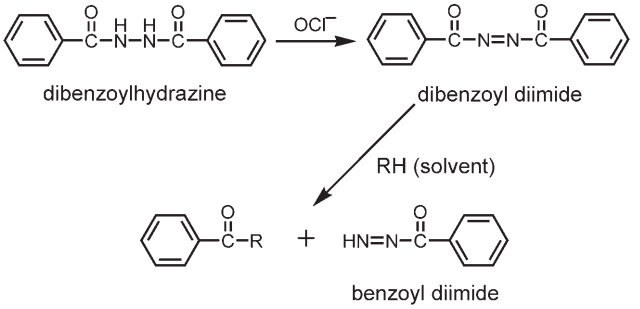
**Abstract:** A new rhodamine B-based fluorescent probe for the hypochlorite anion ($OCl^-$) has been designed, synthesized, and characterized. The probe comprises a spectroscopic unit of rhodamine B and an $OCl^-$-specific reactive moiety of dibenzoylhydrazine. The probe itself is nearly nonfluorescent because of its spirolactam structure. Upon reaction with $OCl^-$, however, a largely enhanced fluorescence is produced due to the opening of the spirolactam ring by the oxidation of the exocyclic hydrazide and subsequently the formation of the hydrolytic product rhodamine B. Most notably, the fluorescence-on reaction shows high sensitivity and extremely high selectivity for $OCl^-$ over other common ions and oxidants, which makes it possible for $OCl^-$ to be detected directly in their presence. In addition, the reaction mechanism has been investigated and proposed. The $OCl^-$ anion selectively oxidizes the hydrazo group in the probe, and forms the analogue of dibenzoyl diimide, which in turn hydrolyzes and releases the fluorophore. The reaction mechanism that is described here might be useful in developing excellent spectroscopic probes with cleavable active bonds for other species.

**Keywords:** cleavage reactions · fluorescence spectroscopy · fluorescent probes · hypochlorite anion · rhodamine B

## Introduction

Hypochlorite anion ($OCl^-$), one of the biologically important reactive oxygen species (ROS),[1] is produced in organisms by the reaction of $H_2O_2$ with $Cl^-$ ions under the catalysis of a heme enzyme, myeloperoxidase.[2] Endogenous $OCl^-$ is essential to life and has important antibacterial properties. However, excessive or misplaced production of $OCl^-$ can lead to tissue damage and diseases, such as atherosclerosis, arthritis, and cancers.[2,3] Therefore, much effort has recently been focused on the studies of the biological functions and/or deleterious effects of $OCl^-$,[3b,4] and a number of sensitive and selective analytical methods have been proposed for conducting such research, among which fluorescent probes play an important role in this respect due to their high-time and spatial resolution capability.[5,6] Unfortunately, only a limited number of fluorescent probes have been made available for $OCl^-$ detection so far, and in particular for $OCl^-$-selective detection.[6–8] For instance, 2,7-dichlorodihydrofluorescein, dihydrorhodamine 123 and 2-[6-(4′-amino)phenoxy-3*H*-xanthen-3-on-9-yl]benzoic acid have been suggested as probes for $OCl^-$ detection, but their fluorescence responses lack selectivity because of the cross-reactions with other ROS and oxidizing agents.[7–9] Nagano and co-workers[6] reported a fluorescence probe with good selectivity for hypochlorous acid, but its synthetic route was rather complicated.

It is noted that $OCl^-$ can selectively oxidize dibenzoylhydrazine into dibenzoyl diimide, which can further undergo a decomposition in some nucleophilic solvents (Scheme 1).[10]




Scheme 1. The $OCl^-$ anion oxidizes dibenzoylhydrazine into dibenzoyl diimide.

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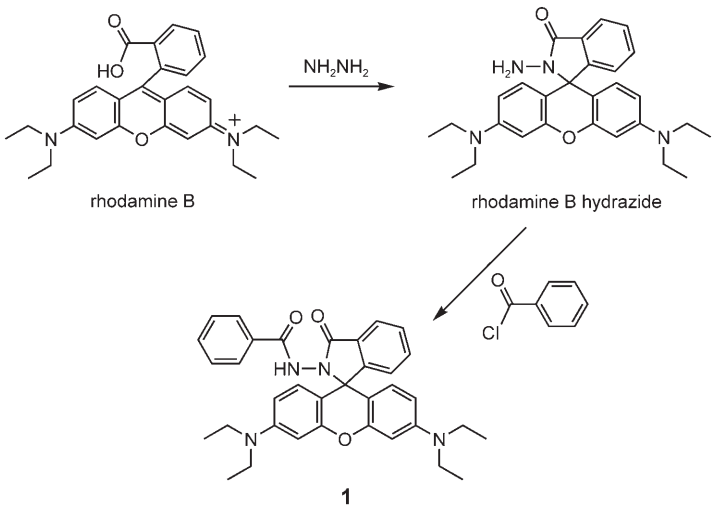
However, such a reaction has never been used to develop a selective fluorescent probe for $OCl^-$. In the present work, we attempted to accomplish this. Specifically, herein we describe the design of *N*-benzoyl rhodamine B–hydrazide **1** (Scheme 2) as a novel $OCl^-$ fluorescent probe by engineering a dibenzoylhydrazine moiety into rhodamine B, which is an excellent fluorophore.[11] The probe, **1**, can be synthesized by a simple synthetic procedure, and as expected, the probe exhibits a highly selective fluorescence response to $OCl^-$ only, instead of other common ions and oxidants.




Scheme 2. Synthesis of **1**.

## Results and Discussion

**Design and synthesis of 1**: Spectroscopic probes usually consist of two units:[12] 1) a spectroscopic or signaling unit, the properties of which should be changed upon reaction with the analyte of interest, and 2) a labeling or recognition unit that is responsible for the selective reaction with the analyte. To design a selective $OCl^-$ fluorescent probe, the fluorophore rhodamine B was chosen as the signaling unit. However, there still exists a challenge in choosing a suitable recognition unit because other oxidizing agents not only coexist, but also often have similar reactivity. In this respect, we take advantage of the selective oxidation of dibenzoylhydrazine by $OCl^-$. As a result, the probe **1** was readily prepared from rhodamine B by a two-step reaction (Scheme 2). The reaction of rhodamine B with hydrazine gave rhodamine B–hydrazide (yield: 79%), which was then converted into the designed product **1** (yield: 65%) by further reaction with benzoyl chloride. The structure of **1** was confirmed by $^1H$ NMR spectroscopy, mass spectrometry, and elemental analysis. A single crystal of **1** was grown at room temperature from the mixed solvents of petroleum ether/ethyl acetate (3:1, v/v) and characterized by X-ray crystallography. As shown in Figure 1, the probe has a five-membered spirolactam structure, which causes it to be colorless and non-

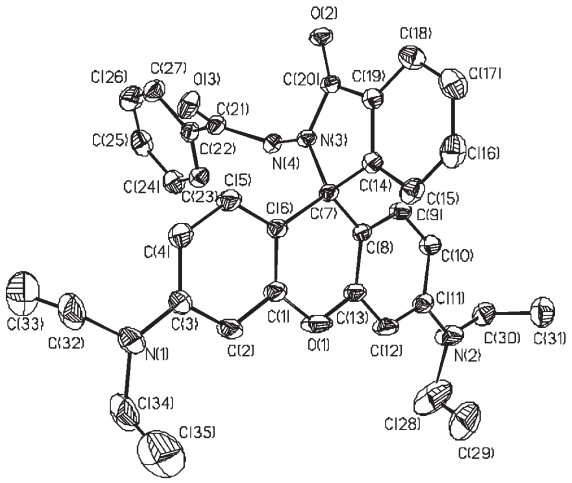



Figure 1. X-ray crystal structure of **1**.

fluorescent.[13] This is very desirable because it affords a low background signal in detection.

**Absorption spectral properties of 1**: The absorption spectra of **1** and its reaction solution with various concentrations of $OCl^-$ are shown in Figure 2. As expected, **1** has nearly no absorption in the visible region (curve 1) because of its closed spirolactam form.[13] Upon reaction with $OCl^-$, however, the pink color that is indicative of rhodamine B is largely restored with the increase in $OCl^-$ concentration (curves 2–20). This can be ascribed to the selective oxidation of dibenzoylhydrazine by $OCl^-$, which promotes the opening of the closed spirolactam ring. In addition, the probe is stable because no obvious change in the absorption spectrum was observed after the probe's solution was stored at room temperature for a week.

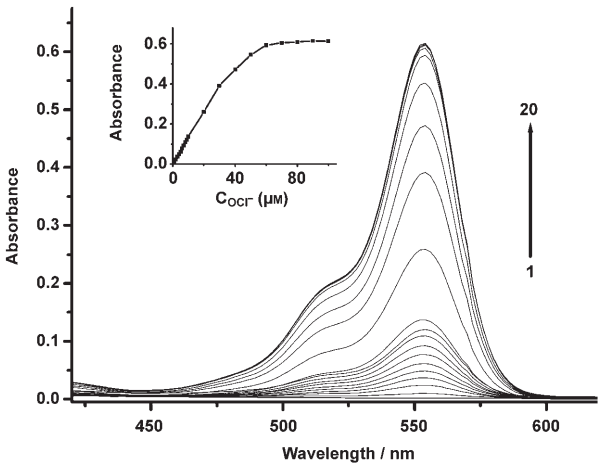



Figure 2. Absorption spectra of probe **1** (10 μM, curve 1) and its reaction solution with $OCl^-$ at various concentrations (1-100 μM, curves 2–20) against the corresponding reagent blank. The reaction was carried out in 0.03 M $Na_2B_4O_7$/NaOH buffer (pH 12) that contained 30% (v/v) THF. The inset depicts the absorbance at the absorption maximum of 554 nm in the curves 2–20 as a function of $OCl^-$ concentration.

**Fluorescence properties and selectivity studies**: Figure 3 shows the fluorescence spectra of **1** and its reaction solution with various species under the same conditions. Similar to its absorption properties, **1** itself is essentially nonfluorescent due to its five-membered spirolactam structure.[13] Upon the introduction of $OCl^-$ into the solution of **1**, however, fluorescence ($\lambda_{em}=578$ nm) that is characteristic of rhodamine B appeared.

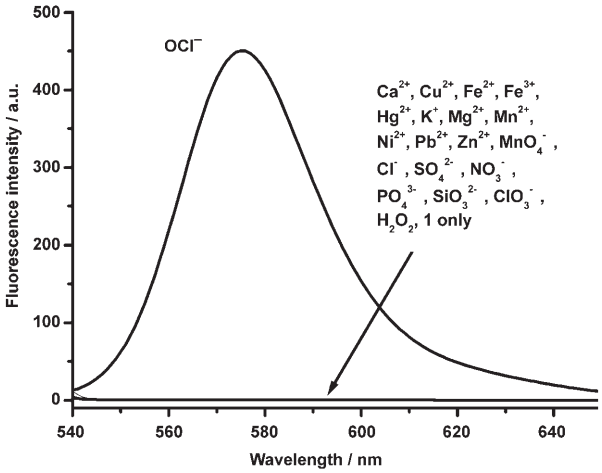



Figure 3. Fluorescence spectra ($\lambda_{ex}=520$ nm) of **1** (10 μM) at pH 12 in the presence of 0.5–500 equivalents of various species (500 equiv of $Cl^-$ or $K^+$; 100 equiv of $H_2O_2$, $NO_3^-$, $PO_4^{3-}$, $SiO_3^{2-}$, or $SO_4^{2-}$; 30 equiv of $Mg^{2+}$; 15 equiv of $Ca^{2+}$; 10 equiv of $ClO_3^-$, $Ni^{2+}$, or $Zn^{2+}$; 2 equiv of $Mn^{2+}$; 1 equiv of $Cu^{2+}$, $Fe^{2+}$, $MnO_4^-$, or $OCl^-$; 0.5 equiv of $Fe^{3+}$, $Hg^{2+}$, or $Pb^{2+}$ were used).

To test the selectivity of the reaction, a solution of probe **1** (10 μM) was prepared in 0.03 M $Na_2B_4O_7$/NaOH buffer (pH 12) that contained 30% (v/v) THF, and 0.5–500 equivalents of various species were then added. The reactions were conducted at room temperature (about 25 °C) for 30 min, and then the fluorescence spectrum was monitored. The species that were tested were $Ca^{2+}$, $Cu^{2+}$, $Fe^{3+}$, $Fe^{2+}$, $Hg^{2+}$, $K^+$, $Mg^{2+}$, $Mn^{2+}$, $Ni^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $MnO_4^-$, $H_2O_2$, $Cl^-$, $OCl^-$, $ClO_3^-$, $SO_4^{2-}$, $NO_3^-$, $PO_4^{3-}$, and $SiO_3^{2-}$. As shown in Figure 3, probe **1** displays a highly selective fluorescence-on response to $OCl^-$ only. This remarkable property makes **1** of great potential for detecting $OCl^-$ directly in the presence of other species.

**Optimization of experimental conditions**: Various experimental conditions, such as the concentration of probe **1**, reaction media, pH, temperature and time, were examined to optimize the reaction conditions. The results show that both the background and response signals increase with increasing concentration of **1**. In this study a concentration of 10 μM of **1** was used because sufficient fluorescence signal could be obtained at this concentration. The water solubility of **1** is limited, and the use of 30% (v/v) THF as a co-solvent is required in this system. Figure 4 depicts the effect of pH on the reaction. As can be seen, pH 12 can be used for the present system; milder pH conditions, such as about pH 10, can also be used, but the sensitivity is relatively low. Time-course studies revealed that the fluorescence intensity increased with the increase of reaction time at room temperature (about 25 °C). However, 30 min later a plateau of fluorescence enhancement was achieved. As a result, a reaction medium of 0.03 M $Na_2B_4O_7$/NaOH buffer (pH 12) that contained 30% (v/v) THF, and a reaction time of 30 min at room temperature were chosen for the present reaction, because under these conditions a plateau of fluorescence enhancement could be produced (see Figure 4 and Figures S1 and S2 in the Supporting Information). Obviously, under the above-determined conditions, the detection of $OCl^-$ should be performed in vitro instead of in vivo for biological studies.

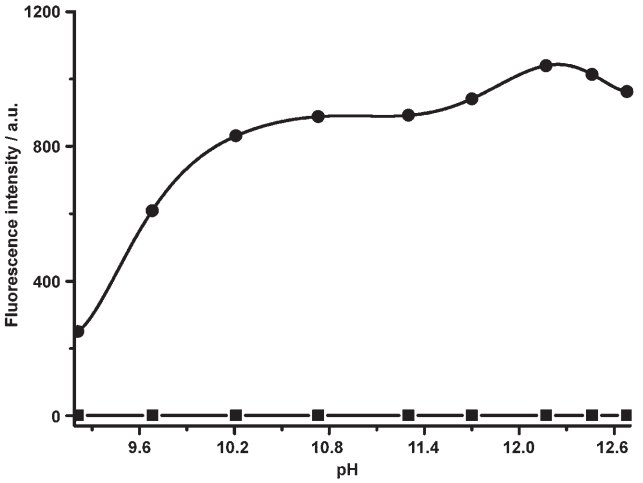



Figure 4. Effect of pH on the reaction of **1** (10 μM) with $OCl^-$ (40 μM); **1**+$OCl^-$ (●), **1** (■). Fluorescence intensity was measured at $\lambda_{ex/em}=520/578$ nm.

**Linearity**: Under the optimized conditions that were determined above, the enhancement value ($\Delta F$) of fluorescence intensity in contrast to the blank solution without $OCl^-$ could remain stable for at least 5 h, and it was directly proportional to the $OCl^-$ concentration ($C$) in a range of 1–10 μM (Figure 5). The linear regression equation was determined to be $\Delta F=52.69\times C-29.83$ ($n=10$, $\gamma=0.9992$) with a detection limit of 27 nM ($S/N=3$), which also shows a highly sensitive feature. Reproducibility tests ($n=6$) showed that the relative standard deviation of fluorescence was 4.0% for 10 μM $OCl^-$.

**Interference studies**: The effects of various ions on the determination of $OCl^-$ were investigated by analyzing synthetic sample solutions that contained 10 μM of $OCl^-$. The tolerable concentration was estimated by the criterion at which a species gave a relative error of no more than 10% as determined by the recovery of $OCl^-$. The results are listed in Table 1, from which it can be seen that the probe **1** exhibits a high selectivity for $OCl^-$ over a wide range of common

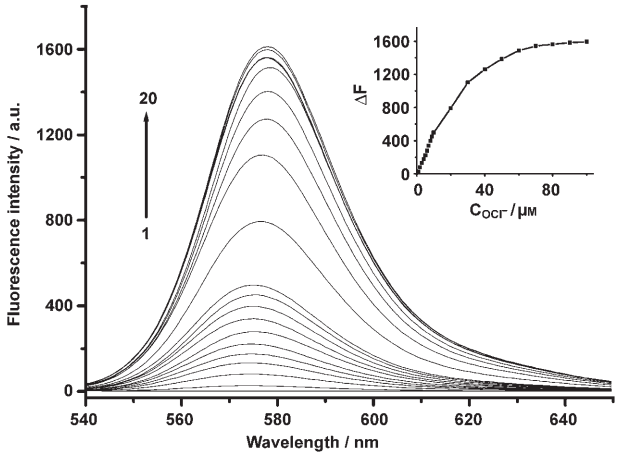



Figure 5. Fluorescent spectra of probe **1** (10 μM, curve 1) and its reaction solutions with various concentrations of $OCl^-$ (1–100 μM, curves 2–20) according to the general procedure. The inset shows the plot of fluorescence enhancement value $\Delta F$ against $OCl^-$ concentration.

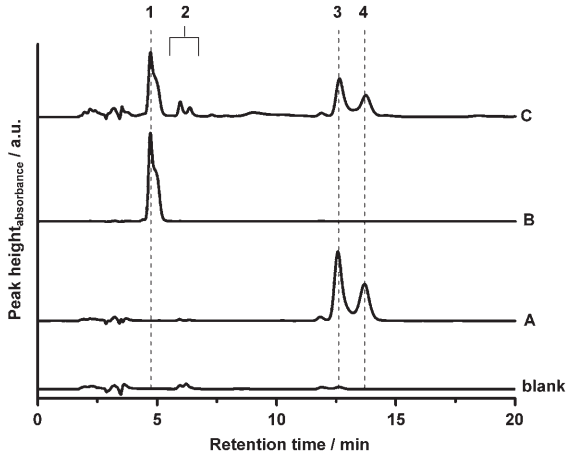



Figure 6. HPLC chromatograms of different reaction products in 0.03 M $Na_2B_4O_7$/NaOH buffer (pH 12) that contained 30% THF. A) 100 μM probe **1**, B) 100 μM rhodamine B, C) 100 μM **1** plus 100 μM $OCl^-$, and (blank) 100 μM $OCl^-$. HPLC analyses were performed on a HiQ sil C18W (4.6×200 mm) column by using a Jasco HPLC system that consisted of a PU-2086-plus pump and a UV-2075-plus detector at 300 nm with methanol/water (6:1, v/v) as eluent (flow rate: 0.8 mL min$^{-1}$). The assignment of the peaks: 1) 4.71 min, rhodamine B, 2) 5.75–6.81 min, unidentified products, 3) 12.57 min, and 4) 13.69 min, two tautomers of probe **1**.

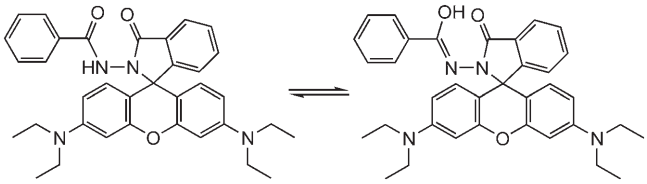
Table 1. Recovery of 10 μM $OCl^-$ in the presence of various coexisting species.

| Species | Concn [μM] | Molar ratio of the added species to $OCl^-$ | Recovery [%] |
|---|---|---|---|
| $K^+$ | 5000 | 500 | 96.2 |
| $Ca^{2+}$[a] | 150 | 15 | 98.7 |
| $Mg^{2+}$[a] | 300 | 30 | 100.4 |
| $Cu^{2+}$[a] | 10 | 1 | 93.9 |
| $Fe^{2+}$[a] | 10 | 1 | 103.1 |
| $Fe^{3+}$[a] | 5 | 0.5 | 101.3 |
| $Hg^{2+}$[a] | 5 | 0.5 | 107.0 |
| $Mn^{2+}$[a] | 20 | 2 | 101.0 |
| $Ni^{2+}$ | 100 | 10 | 101.8 |
| $Pb^{2+}$[a] | 5 | 0.5 | 106.4 |
| $Zn^{2+}$ | 100 | 10 | 98.5 |
| $Cl^-$ | 5000 | 500 | 96.2 |
| $ClO_3^-$ | 100 | 10 | 104.5 |
| $MnO_4^-$ | 10 | 1 | 106.0 |
| $NO_3^-$ | 1000 | 100 | 103.4 |
| $PO_4^{3-}$ | 1000 | 100 | 107.6 |
| $SiO_3^{2-}$ | 1000 | 100 | 102.9 |
| $SO_4^{2-}$ | 1000 | 100 | 106.9 |

[a] A higher concentration of these ions led to the precipitation of metal hydroxides at pH 12.

cations, anions, and even some oxidants, such as $ClO_3^-$ and $MnO_4^-$.

**Reaction mechanism**: The reaction mechanism in the present system was studied. The generation of rhodamine B as a product might be responsible for the recovery of both absorption and fluorescence. To prove this, the reaction products of probe **1** with $OCl^-$ were subjected to HPLC analysis. As shown in Figure 6, the chromatographic peak of rhodamine B appears at 4.71 min (peak 1 in curve B), whereas the probe **1** has two chromatographic peaks (curve A) that appear at 12.57 and 13.69 min, respectively, which may be ascribed to the two tautomers of the probe (see Scheme 3; this enolization phenomenon has been reported for similar

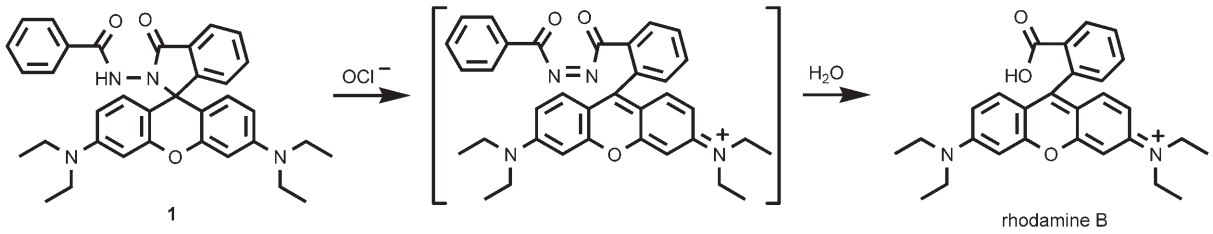


Scheme 3. Two possible tautomers of probe **1**.

compounds such as luminol[14]). After reaction with $OCl^-$, the two chromatographic peaks of **1** decrease markedly, and several new peaks emerge (curve C), among which the major peak at 4.71 min indicates the formation of rhodamine B.

To further confirm the generation of rhodamine B, the reaction solution also underwent MALDI-TOF MS analysis. The experimental results (see Figure S3 in the Supporting Information) showed that, besides the peak of probe **1** (*m*/*z*: 561 $[M+H]^+$), there are two more peaks that appear at *m*/*z*: 443 and 427, respectively. Among them, the peak at *m*/*z*: 443 represents the formation of rhodamine B (*m*/*z*: 443 $[M+H]^+$). Based on our data and previous observations that $OCl^-$ can selectively oxidize dibenzoylhydrazine into dibenzoyl diimide, which can further undergo a decomposition in some nucleophilic solvents (Scheme 1),[10] we propose that the fluorescence-on reaction in this system proceeds mainly through the route that is depicted in Scheme 4 to release the fluorophore of rhodamine B.




Scheme 4. The possible ring-opening reaction of **1** that is induced by the oxidative action of $OCl^-$.

## Conclusion

*N*-benzoyl rhodamine B–hydrazide has been characterized as a new fluorescent probe for $OCl^-$. The probe displays high sensitivity and extremely high selectivity for $OCl^-$, which makes it of great potential for the selective detection of $OCl^-$ in the presence of other common ions and oxidants. In addition, the reaction mechanism that is revealed here might be useful in developing excellent spectroscopic probes with cleavable active bonds for other species.

## Experimental Section

***N*-Benzoyl rhodamine B–hydrazide 1**: Probe **1** can be readily prepared from rhodamine B by a two-step reaction (Scheme 2). First, the rhodamine B–hydrazide was prepared by a similar procedure to that reported previously.[15] In brief, 85% hydrazine hydrate (4 mL) was added to a solution of rhodamine B (1 g, 2.09 mmol) in methanol (40 mL). The solution was refluxed for 6 h with stirring. Then, the reaction mixture was evaporated under reduced pressure to give an orange oil, which was then recrystallized from methanol/water to afford rhodamine B–hydrazide as a light-orange crystal (750 mg, 79%). ESI-MS: $m/z$: 457 $[M+H]^+$.

Benzoyl chloride (0.3 mL, 2.6 mmol) in THF (5 mL) was added dropwise to a mixed solution of rhodamine B–hydrazide (200 mg, 0.44 mmol) and triethylamine (0.4 mL, 2.9 mmol) in THF (5 mL)/water (1 mL) at 0–5 °C with stirring. Then, the mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure to give an orange oil. The oil was dissolved in $CH_2Cl_2$ (20 mL), and the solution was successively washed with dilute NaOH solution and brine. The organic phase was dried over anhydrous $Na_2SO_4$, concentrated and purified by silica-gel column chromatography with petroleum ether (60–90 °C)/EtOAc (3:1, v/v) as the eluent to afford **1** as a colorless crystal (160 mg, 65%). $^1$H NMR (300 MHz, [D$_6$]DMSO, 373 K): $\delta$ = 9.54 (s, 1H), 7.86 (d, $J$ = 7.2 Hz, 1H), 7.60–7.54 (m, 4H), 7.45 (t, $J$ = 6.8 Hz, 1H), 7.34 (t, $J$ = 7.0 Hz, 2H), 7.09 (d, $J$ = 7.0 Hz, 1H), 6.57 (d, $J$ = 8.5 Hz, 2H), 6.37–6.33 (m, 4H), 3.33 (q, $J$ = 6.9 Hz, 8H), 1.12 ppm (t, $J$ = 6.9 Hz, 12H); ESI-MS: $m/z$: 561 $[M+H]^+$; elemental analysis calcd (%) for $C_{35}H_{36}N_4O_3$: C 74.98, H 6.47, N 9.99; found: C 74.96, H 6.59, N 10.11; crystal data for **1**: triclinic; $a$ = 10.066(2), $b$ = 12.494(3), $c$ = 14.084(3) Å; $\alpha$ = 67.71(3), $\beta$ = 84.16(3), $\gamma$ = 67.70(3)°; $V$ = 1514.6(5) Å$^3$; $T$ = 293(2) K; space group = $P\bar{1}$; $Z$ = 30; $\rho_{cald}$ = 1.415 g cm$^{-3}$; $\mu(Mo_{K\alpha})$ = 0.71073 mm$^{-1}$; 6738 reflections measured, 6738 unique ($R_{int}$ = 0.1624); final $R_1$ = 0.1118, $wR_2$ = 0.2701; $S$ = 1.113.

**General procedure for $OCl^-$ determination**: Unless otherwise noted, all measurements were made in 0.03 M $Na_2B_4O_7$/NaOH buffer (pH 12) that contained 30% (v/v) THF according to the following procedure. In a 10 mL tube, the 1 mM stock solution of **1** (0.1 mL) in THF was diluted with THF (2.9 mL), followed by the addition of distilled, deionized water (2 mL) and 0.1 M $Na_2B_4O_7$-NaOH buffer (3 mL, pH 12). Then an appropriate volume of the $OCl^-$ sample solution (final concentration of $OCl^-$ was not more than 10 μM) was added, and the final volume was adjusted to 10 mL with distilled, deionized water. After 30 min at room temperature, a reaction aliquot (3 mL) was transferred to a 1 cm quartz cell to measure an absorbance or fluorescence intensity/spectrum with $\lambda_{ex/em}$ = 520/578 nm and both excitation and emission slit widths of 10 nm. In the meantime, a blank solution that contained no $OCl^-$ was prepared and measured under the same conditions for comparison.

## Acknowledgement

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[1] a) A. Gomes, E. Fernandes, J. L. F. C. Lima, *J. Biochem. Biophys. Methods* **2005**, *65*, 45–80; b) M. P. Fink, *Curr. Opin. Crit. Care* **2002**, *8*, 6–11; c) X. H. Li, G. X. Zhang, H. M. Ma, D. Q. Zhang, J. Li, D. B. Zhu, *J. Am. Chem. Soc.* **2004**, *126*, 11543–11548.

[2] a) A. J. Kettle, C. C. Winterbourn, *Redox Rep.* **1997**, *3*, 3–15; b) D. I. Pattison, M. J. Davies, *Chem. Res. Toxicol.* **2001**, *14*, 1453–1464; c) P. J. O'Brien, *Chem.–Biol. Interact.* **2000**, *129*, 113–139.

[3] a) E. A. Podrez, H. M. Abu-Soud, S. L. Hazen, *Free Radical Biol. Med.* **2000**, *28*, 1717–1725; b) D. I. Pattison, M. J. Davies, *Biochemistry* **2006**, *45*, 8152–8162.

[4] a) Y. Kawai, H. Kiyokawa, Y. Kimura, Y. Kato, K. Tsuchiya, J. Terao, *Biochemistry* **2006**, *45*, 14201–14211; b) D. I. Pattison, M. J. Davies, *Biochemistry* **2005**, *44*, 7378–7387.

[5] Z. Takats, K. J. Koch, R. G. Cooks, *Anal. Chem.* **2001**, *73*, 4522–4529.

[6] S. Kenmoku, Y. Urano, H. Kojima, T. Nagano, *J. Am. Chem. Soc.* **2007**, *129*, 7313–7318.

[7] A. V. Bizyukin, L. G. Korkina, B. T. Velichkovskii, *Bull. Exp. Biol. Med.* **1995**, *119*, 347–351.

[8] K. Setsukinai, Y. Urano, K. Kakinuma, H. J. Majima, T. Nagano, *J. Biol. Chem.* **2003**, *278*, 3170–3175.

[9] S. L. Hempel, G. R. Buettner, Y. Q. O'Malley, D. A. Wessels, D. M. Flaherty, *Free Radical Biol. Med.* **1999**, *27*, 146–159.

[10] J. E. Leffler, W. B. Bond, *J. Am. Chem. Soc.* **1956**, *78*, 335–341.

[11] a) J. Y. Kwon, Y. J. Jang, Y. J. Lee, K. M. Kim, M. S. Seo, W. Nam, J. Yoon, *J. Am. Chem. Soc.* **2005**, *127*, 10107–10111; b) T. Lescrinier, C. Hendrix, L. Kerremans, J. Rozenski, A. Link, B. Samyn, A. V. Aerschot, E. Lescrinier, R. Eritja, J. V. Beeumen, P. Herdewijn, *Chem. Eur. J.* **1998**, *4*, 425–433; c) V. Dujols, F. Ford, A. W. Czarnik, *J. Am. Chem. Soc.* **1997**, *119*, 7386–7387; d) G.-S. Jiao, L. H. Thoresen, T. G. Kim, W. C. Haaland, F. Gao, M. R. Topp, R. M. Hochstrasser, M. L. Metzker, K. Burgess, *Chem. Eur. J.* **2006**, *12*, 7816–7826.

[12] a) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, T. E. Rice, *Chem. Rev.* **1997**, *97*, 1515–1566; b) *Fluorescent Chemosensors for Ion and Molecule Recognition* (Ed.: A. W. Czarnik), ACS, Washington, **1992**; c) H. M. Ma, U. Jarzak, W. Thiemann, *New J. Chem.* **2001**, *25*, 872–874; d) L.

  

Prodi, F. Bolletta, M. Montalti, N. Zaccheroni, *Coord. Chem. Rev.* **2000**, *205*, 59–83.
[13] a) Y. K. Yang, K. J. Yook, J. Tae, *J. Am. Chem. Soc.* **2005**, *127*, 16760–16761; b) H. Zheng, Z. H. Qian, L. Xu, F. F. Yuan, L. D. Lan, J. G. Xu, *Org. Lett.* **2006**, *8*, 859–861; c) X. Q. Chen, H. M. Ma, *Anal. Chim. Acta* **2006**, *575*, 217–222.
[14] D. I. Roshchupkin, N. S. Belakina, M. A. Murina, *Biophysics (Biophys. Soc. Jpn.)* **2006**, *51*, 79–86.
[15] X.-F. Yang, X.-Q. Guo, Y.-B. Zhao, *Talanta* **2002**, *57*, 883–890.

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