

## PAPER

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View Journal | View IssueCite this: *RSC Adv.*, 2019, 9, 28636

# A coumarin–dihydroperimidine dye as a fluorescent chemosensor for hypochlorite in 99% water†

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The hypochlorite anion ( $\text{OCl}^-$ ), a reactive oxygen species (ROS), is an important microbicidal agent in the immune system. Accurate and selective detection of  $\text{OCl}^-$  in environmental and biological samples by a fluorescent molecular sensor is an important subject. All previously reported sensors, however, have suffered from tedious multi-step synthesis for the sensors and the use of large amounts of organic solvents for the analysis. Herein, we report that a coumarin–dihydroperimidine dye prepared by facile condensation behaves as a fluorescent sensor for  $\text{OCl}^-$  in 99% water. The sensor exhibits weak fluorescence, but  $\text{OCl}^-$ -selective dehydrogenation of its dihydroperimidine unit creates a strong blue fluorescence. This turn-on fluorescence response facilitates selective and sensitive detection of  $\text{OCl}^-$  in the physiological pH range. *Ab initio* calculation revealed that the fluorescence enhancement by  $\text{OCl}^-$  is triggered by intramolecular proton transfer from the coumarin –OH to the imine nitrogen of the formed perimidine moiety.

Received 18th July 2019

Accepted 5th September 2019

DOI: 10.1039/c9ra05533a

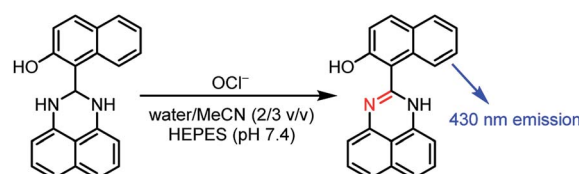
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## Introduction

Reactive oxygen species (ROS) play crucial roles in several life functions.<sup>1</sup> Among them, hypochlorous acid ( $\text{HClO}$ ) is one of the most biologically important ROS.<sup>2</sup>  $\text{HClO}$  undergoes deprotonation at physiological pH and produces the hypochlorite anion ( $\text{OCl}^-$ ),<sup>3</sup> which behaves as a microbicidal agent in the immune system.<sup>4</sup>  $\text{OCl}^-$  is produced *in vivo* by the reaction of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) with  $\text{Cl}^-$  via an enzymatic reaction on myeloperoxidase (MPO).<sup>5</sup> Controlled generation of  $\text{OCl}^-$  is necessary to inhibit invading microbes. Uncontrolled  $\text{OCl}^-$  generation, however, causes several diseases such as neuron degeneration, arthritis, and cancer,<sup>6</sup> because  $\text{OCl}^-$  reacts with several biomolecules such as amino acids, proteins, and nucleosides.<sup>7</sup> In addition,  $\text{HClO}$  is widely used in daily life for sterilization and disinfection of water supplies, and high residual concentrations of  $\text{OCl}^-$  in water is hazardous to human and animal health.<sup>8</sup> Analytical methods that quantitatively detect small amount of  $\text{OCl}^-$  in environmental and biological samples on inexpensive instrumentations with simple pre-treatment are necessary.

Fluorometric analysis with  $\text{OCl}^-$ -selective molecular sensors is one promising method for this purpose since this facilitates simple quantification or imaging of  $\text{OCl}^-$  with a common

fluorescence spectrometer or microscope apparatus.<sup>9</sup> A number of fluorescent  $\text{OCl}^-$  sensors have been reported;<sup>10–17</sup> however, many of them require tedious multi-step procedures for the synthesis of sensors or a solution containing a large amount of organic solvents for sensing due to the low solubility of the sensors in water. Among the previously reported  $\text{OCl}^-$  sensors, a “dihydroperimidine”-based sensor designed by Goswami *et al.*<sup>18</sup> has the simplest structure, which can be prepared by a facile condensation. As shown in Scheme 1, they synthesized a naphthol–dihydroperimidine dye by the condensation of 1,8-diaminonaphthalene with 1-formyl-2-naphthol as a fluorophore. The sensor shows a sensitive turn-on fluorescence response via an  $\text{OCl}^-$ -selective dehydrogenation of the dihydroperimidine unit. The sensor, however, requires a solution containing 60% MeCN owing to its low solubility in water. Based on this molecular design, Fan *et al.*<sup>19</sup> synthesized a sensor by the condensation of 1,8-diaminonaphthalene with 7-diethylamino-1,4-benzoxazin-2-one as a fluorophore. Although the sensor exhibits a selective and sensitive response towards  $\text{OCl}^-$ , it still requires a large amount of organic solvent (80%



**Scheme 1** A naphthol–dihydroperimidine dye exhibiting a turn-on fluorescence response toward  $\text{OCl}^-$ .<sup>18</sup>

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† Electronic supplementary information (ESI) available: Table S1, Fig. S1–S10, and Cartesian coordinates for the molecules. See DOI: 10.1039/c9ra05533a

DMF) for the sensing. Design of a sensor that can be synthesized by a simple procedure and has a high water solubility is therefore desirable.

We used coumarin as a fluorophore due to its relatively high water solubility,<sup>20</sup> high fluorescence quantum yield,<sup>21</sup> large Stokes shift,<sup>22</sup> high stability,<sup>23</sup> and good cell permeability.<sup>24</sup> As shown in Scheme 2, the sensor **1**, synthesized by a simple condensation of 1,8-diaminonaphthalene with 8-formyl-7-hydroxy-4-methylcoumarin, is soluble in water containing only 1% organic solvents. The sensor shows a weak fluorescence, but OCl<sup>−</sup>-selective dehydrogenation of its dihydroperimidine unit creates a strong fluorescence at 462 nm. This turn-on response facilitates sensitive detection of OCl<sup>−</sup>. Several spectroscopic analysis and *ab initio* calculations revealed that this turn-on response by OCl<sup>−</sup> is triggered by intramolecular proton transfer from the coumarin −OH to the imine nitrogen of the formed perimidine unit.

## Results and discussion

### Synthesis and fluorescence properties of the sensor

The sensor **1** was prepared by the reaction shown in Scheme 2. 8-Formyl-7-hydroxy-4-methylcoumarin prepared by formylation of 7-hydroxy-4-methylcoumarin (yield: 45%)<sup>25</sup> and 1,8-diaminonaphthalene were dissolved in EtOH, and the solution was stirred at 80 °C for 2.5 h in an aerated condition. The solid formed was recovered by filtration and washed thoroughly with EtOH, affording **1** as pale pink solids with 69% yield (overall yield: 31%). The purity of **1** was confirmed by <sup>1</sup>H NMR, <sup>13</sup>C NMR and FAB-MS analysis (Fig. S1–S3, ESI†). **1** is soluble in common organic solvents such as DMSO, CHCl<sub>3</sub>, DMF, and MeCN and in aqueous solutions with 1% organic solvents such as DMSO and MeCN. Fig. S4 (ESI†) shows the absorption spectra of 1% MeCN solutions containing different concentrations of **1**. The linear relationship between the absorbance at 325 nm and the concentration of **1** (0–20 μM) indicates that it follows the Beer's law, suggesting that **1** is fully soluble in the solutions. Note that

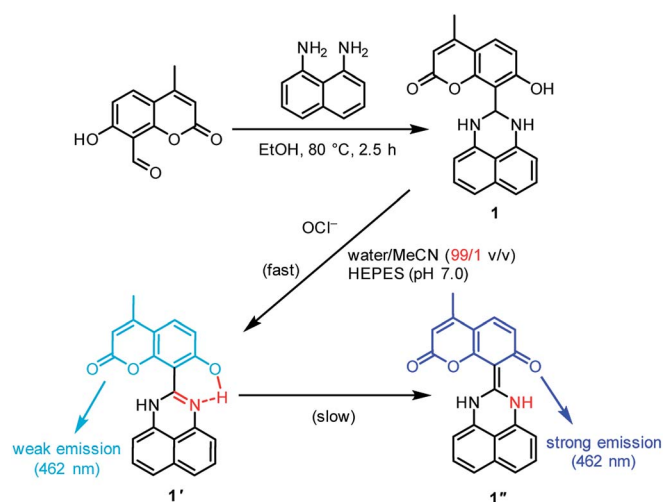
the molar extinction coefficient of **1** at 325 nm was determined to be 10 039 M<sup>−1</sup> cm<sup>−1</sup>.

Fluorescence spectra of **1** (10 μM) were measured in a buffered water/MeCN mixture (99/1 v/v) with pH 7.0 (HEPES 0.1 M) at 25 °C (λ<sub>ex</sub> = 344 nm). As shown in Fig. 1, **1** itself shows a very weak fluorescence (fluorescence quantum yield, Φ<sub>F</sub> = 0.002). In contrast, addition of 50 equiv. of OCl<sup>−</sup> to the solution followed by stirring for 20 min creates a strong blue fluorescence at 462 nm (Φ<sub>F</sub> = 0.082). Other anions (F<sup>−</sup>, Cl<sup>−</sup>, AcO<sup>−</sup>, NO<sub>2</sub><sup>−</sup>, NO<sub>3</sub><sup>−</sup>, ClO<sub>4</sub><sup>−</sup>, and HSO<sub>4</sub><sup>−</sup>), ROS [hydroxyl radical (·OH), singlet oxygen (<sup>1</sup>O<sub>2</sub>), H<sub>2</sub>O<sub>2</sub>, superoxide radical (·O<sub>2</sub><sup>−</sup>), and *tert*-butyl hydroperoxide (*t*-BuOOH)], or RNS [NO and peroxynitrite (ONOO<sup>−</sup>)], when added to the solution containing **1**, scarcely change the fluorescence spectra, indicating that OCl<sup>−</sup> selectively triggers fluorescence enhancement of **1**.

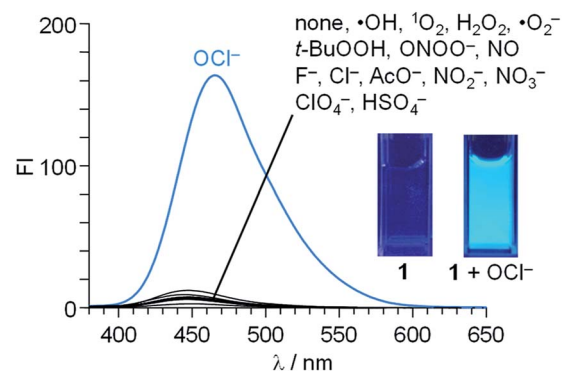
Fig. 2a shows the results of fluorescence titration of **1** with OCl<sup>−</sup>. Stepwise addition of OCl<sup>−</sup> increases the intensity of the 462 nm fluorescence. As shown in Fig. 2b, the change in the ratio of fluorescence intensity at 462 nm (F<sub>i</sub>/F<sub>0</sub>) with the OCl<sup>−</sup> concentrations clearly shows linear relationship, indicating that **1** facilitates accurate OCl<sup>−</sup> sensing at ~100 μM. The lower detection limit was determined to be 3.3 μM based on the signal-to-noise (S/N) ratio using the equation (DL = 3 × SD/S),<sup>26</sup> where SD is the standard deviation of blank analysis (SD = 0.19, *n* = 10) and *S* is the slope of the fluorescence intensity *versus* the OCl<sup>−</sup> concentrations (*S* = 0.18 μM<sup>−1</sup>). This detection limit (3.3 μM) is lower than the physiological OCl<sup>−</sup> concentrations (5–25 μM) in the human body,<sup>27</sup> suggesting that **1** facilitates sensitive OCl<sup>−</sup> detection even in high-water-content solution.

### Reaction of the sensor with OCl<sup>−</sup>

As shown in Scheme 2, the turn-on fluorescence response of **1** upon addition of OCl<sup>−</sup> is triggered by the transformation to **1'**, *via* dehydrogenation of the dihydroperimidine moiety of **1**. This transformation is confirmed by <sup>1</sup>H, <sup>13</sup>C NMR and FAB-MS analysis of a DMSO-*d*<sub>6</sub> solution containing **1** and OCl<sup>−</sup> (Fig. S5–S7, ESI†). Partial <sup>1</sup>H NMR charts of **1** and **1'** measured in DMSO-*d*<sub>6</sub> are shown in Fig. 3, where the 2D COSY spectra were used for the assignment of the respective chemical shifts



**Scheme 2** Synthesis of the sensor **1**, and proposed mechanism for selective turn-on fluorescence response by OCl<sup>−</sup>.



**Fig. 1** Fluorescence spectra (λ<sub>ex</sub> = 344 nm) of **1** (10 μM) in a buffered water/MeCN mixture (99/1 v/v; HEPES 0.1 M, pH 7.0) at 25 °C with 50 equiv. of each respective analytes. All spectra were obtained after stirring the solution for 20 min.



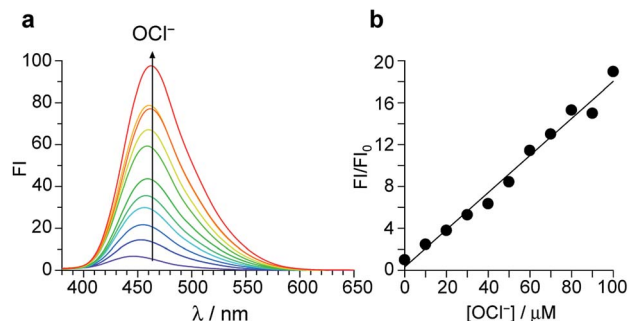


Fig. 2 (a) Change in fluorescence spectra of **1** (10  $\mu\text{M}$ ) upon titration with  $\text{OCl}^-$  in a buffered water/MeCN mixture (99/1 v/v; HEPES 0.1 M, pH 7.0) at 25  $^\circ\text{C}$ . (b) Change in the ratio of fluorescence intensity at 462 nm ( $\text{FI}/\text{FI}_0$ ) versus the  $\text{OCl}^-$  concentration. The respective data were obtained after stirring the solution for 20 min.

(Fig. S8 and S9, ESI $^\dagger$ ). As shown in Fig. 3a, **1** shows an  $\text{H}^a$  proton at the 2-position of the dihydroperimidine unit at 6.0 ppm. However, as shown in Fig. 3b, addition of  $\text{OCl}^-$  to the solution leads to almost complete disappearance of the  $\text{H}^a$  proton. In addition, **1** shows two N–H protons of the dihydroperimidine moiety at 7.0 ppm. After the addition of  $\text{OCl}^-$ , its chemical shift moves to 7.1 ppm, and its integral value becomes almost 1. These data indicate that  $\text{H}^a$  and one N–H proton of **1** are removed by the reaction with  $\text{OCl}^-$ . The dehydrogenation of **1** by  $\text{OCl}^-$  is confirmed by FAB-MS analysis. As shown in Fig. S3 (ESI $^\dagger$ ), **1** shows a peak at  $m/z$  344.1 assigned to  $[\mathbf{1}]^+$ . In contrast, as shown in Fig. S7 (ESI $^\dagger$ ), a solution containing **1** and  $\text{OCl}^-$

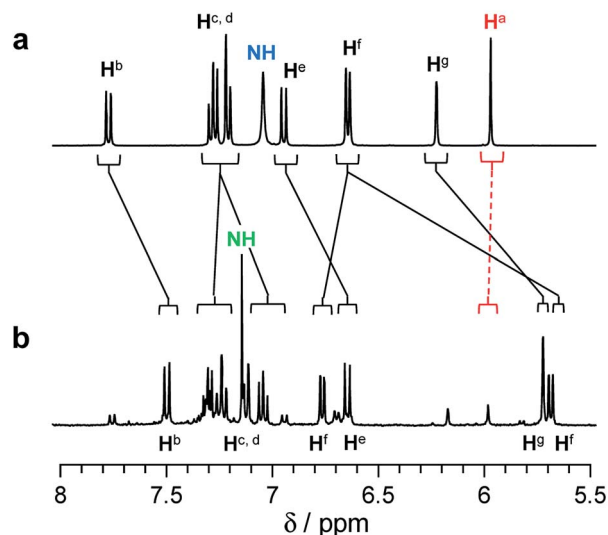
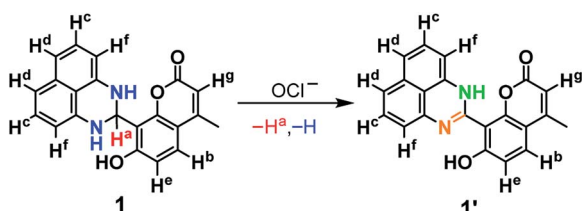


Fig. 3  $^1\text{H}$  NMR chart of **1** (24 mM) measured in  $\text{DMSO}-d_6$  (a) without and (b) with 8 equiv of  $\text{OCl}^-$  (400 MHz, 30  $^\circ\text{C}$ ).

shows a peak at  $m/z$  342.1 assigned to the dehydrogenated product  $[\mathbf{1}']^+$ . These NMR and FAB-MS data clearly suggest that dehydrogenation of the dihydroperimidine moiety of **1** via the oxidation by  $\text{OCl}^-$  gives **1'** containing the perimidine moiety.

As shown in Scheme 2, the enol-imine form (**1'**) is rapidly produced by dehydrogenation of **1** by  $\text{OCl}^-$  and shows a weak fluorescence ( $\Phi_F = 0.009$ ). Then, **1'** undergoes tautomerization to the keto-amine form (**1''**) via a proton transfer of the coumarin –OH to the imine nitrogen of the perimidine unit, as often observed for similar *o*-hydroxyl Schiff bases,<sup>28,29</sup> and exhibits a strong fluorescence ( $\Phi_F = 0.082$ ). This sequence is confirmed by time-dependent changes in the absorption and fluorescence spectra of **1** monitored after addition of  $\text{OCl}^-$ . As shown in Fig. 4a, addition of  $\text{OCl}^-$  immediately increases the fluorescence intensity at 462 nm within 1 min (blue to red line), although the intensity is weak. Then, the intensity gradually increases with time and plateaus after 15 min, creating a strong fluorescence, where the emission wavelengths scarcely change during the measurements. This indicates that the reaction of **1** with  $\text{OCl}^-$  creates two different emitting species. As shown in Fig. 4b, absorption spectrum of **1** also changes immediately after the  $\text{OCl}^-$  addition within 1 min (blue to red line). Then, the spectrum changes gradually with a decrease in *ca.* 320 nm absorbance and an increase in *ca.* 375 nm absorbance. The isosbestic point at 344 nm clearly indicates that, as shown in Scheme 2, the reaction of **1** with  $\text{OCl}^-$  rapidly produces

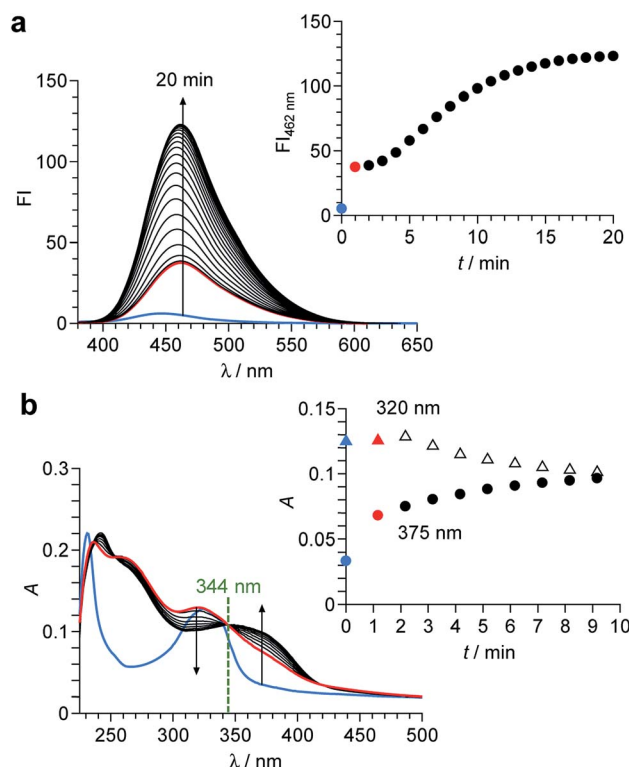


Fig. 4 (a) Time-dependent change in fluorescence spectra ( $\lambda_{\text{ex}} = 344$  nm) of **1** (10  $\mu\text{M}$ ) in a buffered water/MeCN mixture (99/1 v/v; HEPES 0.1 M, pH 7.0) at 25  $^\circ\text{C}$  after addition of 50 equiv. of  $\text{OCl}^-$ . The inset shows change in the intensity at 462 nm. (b) Time-dependent change in absorption spectra of **1** after addition of 50 equiv. of  $\text{OCl}^-$ . The inset shows change in the absorbance at 320 nm and 375 nm.



a weakly-fluorescent enol-imine form ( $1'$ ) and its slow tautomerization by the intramolecular proton transfer creates a strongly-fluorescent keto-amine form ( $1''$ ).

The tautomerization of  $1'$  to  $1''$  is promoted by polar water molecules. It is well known that, for the tautomerization of *o*-hydroxy Schiff bases,<sup>30,31</sup> the enol-imine form is stabilized in less polar solvents such as benzene, while the keto-amine form is stable in polar solvents such as EtOH. Fig. S10 (ESI†) shows the change in fluorescence intensity of  $1$  after addition of  $\text{OCl}^-$  in MeCN solutions with different water contents. In all solutions, the weakly-fluorescent enol-imine form ( $1'$ ) is rapidly produced by the addition of  $\text{OCl}^-$ . The strongly-fluorescent keto-amine form ( $1''$ ) is not produced in low-water-content solutions (30% and 60%), whereas increasing the water content produces  $1''$ . This indicates that increasing water content increases the polarity of solutions and promotes  $1'$ -to- $1''$  tautomerization. However, as shown in Fig. 3b and S5–S7 (ESI†),  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and FAB-MS analysis of the product obtained by the reaction of  $1$  with  $\text{OCl}^-$  in DMSO- $d_6$  detected  $1'$ . This is because the  $1'$ -to- $1''$  tautomerization is not promoted in less polar DMSO. These findings clearly support the  $1 \rightarrow 1' \rightarrow 1''$  transformation by the reaction of  $1$  with  $\text{OCl}^-$  in high-water-content solutions, as shown in Scheme 2.

### Ab initio calculations

The mechanism for the turn-on fluorescence response of  $1$  was clarified by *ab initio* calculations. The structures and optical properties of  $1$ ,  $1'$ , and  $1''$  species were calculated by the density functional theory (DFT) and the time-dependent DFT (TD-DFT), respectively, within the Gaussian 03 program with water as a solvent. As summarized in Table S1 (ESI†), singlet electronic transition of  $1$  mainly consists of HOMO  $\rightarrow$  LUMO+2 ( $S_0 \rightarrow S_4$ ) transition. Its calculated transition energy (3.76 eV, 330 nm) is

close to the absorption maximum ( $\lambda_{\text{max}}$ ) of  $1$  at 323 nm (Fig. 4b, blue line). As shown in Fig. 5 (left),  $\pi$ -electrons of both HOMO and LUMO+2 of  $1$  are localized on the dihydroperimidine moiety, indicating that photoexcitation of the coumarin fluorophore is not populated. This therefore results in almost no fluorescence of  $1$ .

As shown in Fig. 5 (center), optimized structure of  $1'$  has a planar structure, where the coumarin and perimidine units lie on the same plane. It is noted that the structural optimization spontaneously creates an H-bonding interaction between the imine nitrogen and coumarin  $-\text{OH}$  units, in which the N–O distance of  $\sim 2.5$  Å indicates strong electrostatic interaction between these units.<sup>32</sup> The structural regulation by the H-bonding may create the planar structure. As shown in Table S1 (ESI†), the electronic transition of  $1'$  mainly consists of HOMO-1  $\rightarrow$  LUMO+1 ( $S_0 \rightarrow S_6$ ) transition. Its energy (3.97 eV, 312 nm) is also close to that for the absorption maximum (320 nm) of  $1'$  (Fig. 4b, red line). As shown in Fig. 5 (center), relatively large distribution of  $\pi$ -electrons are observed on both HOMO-1 and LUMO+1 for  $1'$ . This is because the H-bonding interaction of the coumarin  $-\text{OH}$  increases the electron density of coumarin unit.<sup>33</sup> The enhanced photoexcitation of the coumarin units may therefore result in weak fluorescence of  $1'$ .

As shown in Fig. 5 (right), optimized structure of  $1''$  also has a planar structure owing to the C=C bond formation between the coumarin and dihydroperimidine units. Singlet electronic transition of  $1''$  is mainly contributed by HOMO-1  $\rightarrow$  LUMO+1 ( $S_0 \rightarrow S_4$ ) transition (Table S1, ESI†). Its transition energy (3.64 eV, 340 nm) is also close to that for the absorption band (375 nm) of  $1''$  (Fig. 4b). As shown in Fig. 5 (right), almost all of the  $\pi$ -electrons of both HOMO-1 and LUMO+1 for  $1''$  are localized on the coumarin units because complete deprotonation of the coumarin  $-\text{OH}$  significantly increases the electron density of

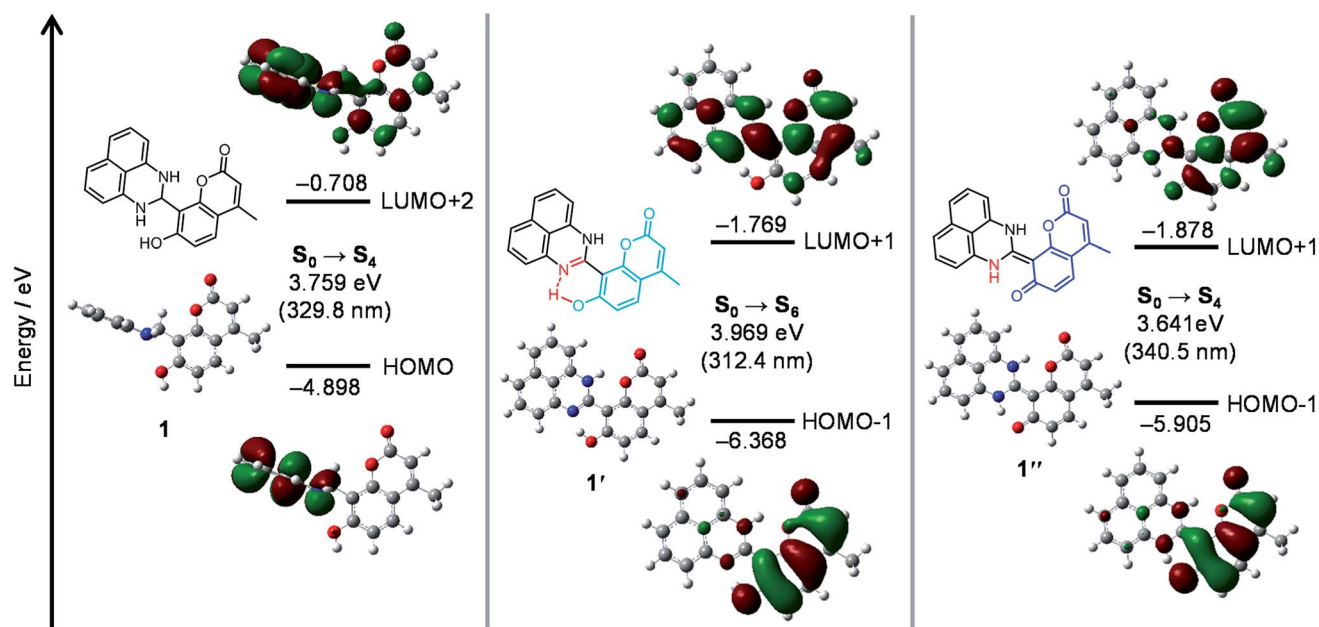


Fig. 5 Energy diagrams and interfacial plots of main molecular orbitals of (left)  $1$ , (center)  $1'$  and (right)  $1''$ , calculated at the DFT level (B3LYP/6-31+G\*).



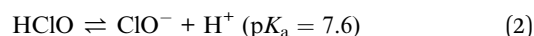
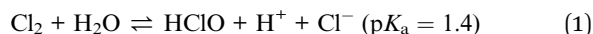


the coumarin unit.<sup>33</sup> This therefore results in strong coumarin fluorescence from **1''**.

The total energies of **1'** and **1''** in water were determined to be  $-717846.94$  and  $-717850.01$  kcal mol<sup>-1</sup>, respectively. The lower energy of **1''** ( $\Delta E = 3.07$  kcal mol<sup>-1</sup>) indicates that the keto-amine form (**1''**) is indeed more stable in water than the enol-imine form (**1'**). This further supports the **1**  $\rightarrow$  **1'**  $\rightarrow$  **1''** transformation by the reaction of **1** with OCl<sup>-</sup> in high-water-content solutions. These DFT results clearly indicate that the dihydroperimidine unit acts as a proton acceptor for the coumarin -OH (Scheme 2). The OCl<sup>-</sup>-triggered formation of the perimidine unit leads to H-bonding interaction between the imine nitrogen and coumarin -OH and creates weak emission (**1'**). Water-assisted tautomerization of **1'** to **1''** leads to complete proton transfer from the coumarin -OH and creates strong emission (**1''**).

### Effect of pH

It is noted that pH of the solution is critical for the OCl<sup>-</sup> sensing. Fig. 6 shows the fluorescence intensity of **1** at 462 nm measured at different pH with and without 50 equiv. of OCl<sup>-</sup>, where the mole fraction distributions of Cl<sub>2</sub>, HClO, and OCl<sup>-</sup> are also shown based on their equilibria in water,<sup>34,35</sup> using the following equations:



The fluorescence enhancement of **1** by OCl<sup>-</sup> occurs at neutral physiological pH (6–8), and does not occur at acidic or basic pH. In acidic media (pH < 6), protonation of OCl<sup>-</sup> (HClO formation; eqn (2)) cancels the basicity of OCl<sup>-</sup> and, hence, suppresses dehydrogenation of the dihydroperimidine unit of **1**. In contrast, basic media (pH > 8) stabilize OCl<sup>-</sup>, but the fluorescence enhancement does not occur. This is probably because, as observed for several OCl<sup>-</sup> sensors,<sup>10,14,15</sup> the oxidation ability of OCl<sup>-</sup> decreases in basic media and inhibits

dehydrogenation of the dihydroperimidine unit. These data suggest that **1** facilitates fluorometric sensing of OCl<sup>-</sup> in physiological pH media (pH 6–8).

## Conclusions

We synthesized a coumarin-dihydroperimidine dye (**1**), acting as a fluorescent sensor for OCl<sup>-</sup> in 99% water. **1** shows a weak fluorescence, but OCl<sup>-</sup>-selective dehydrogenation of its dihydroperimidine unit creates a strong blue fluorescence. **1** facilitates selective and sensitive OCl<sup>-</sup> detection at physiological pH. The turn-on response of **1** occurs *via* two-step reactions. The dehydrogenation by OCl<sup>-</sup> rapidly produces the enol-imine form (**1'**) involving the H-bonding interaction between the imine nitrogen and coumarin -OH. This increases the electron density of the coumarin unit, resulting in weak fluorescence. **1'** undergoes tautomerization to the keto-amine form (**1''**) due to the stabilization in polar water media. The complete proton transfer from the coumarin -OH to the imine nitrogen significantly increases the electron density of the coumarin unit, exhibiting a strong fluorescence. The molecular design based on the dihydroperimidine unit as an OCl<sup>-</sup>-driven proton sensor, may contribute to the design of efficient fluorescent sensors for OCl<sup>-</sup> in environmental and biological samples.

## Experimental

### General

All chemicals were used as received. ·OH was generated by the Fenton reaction.<sup>36</sup> <sup>1</sup>O<sub>2</sub> was generated from the H<sub>2</sub>O<sub>2</sub>/MoO<sub>4</sub><sup>2-</sup> system in alkaline media.<sup>37</sup> NO was generated using sodium nitroferrocyanide(III) dehydrate.<sup>38</sup> ONOO<sup>-</sup> was generated from the SIN-1 reagent (Dojindo Molecular Technologies, Japan). ·O<sub>2</sub><sup>-</sup> was generated using potassium superoxide (KO<sub>2</sub>).<sup>36</sup> Fluorescence spectra were measured on a JASCO FP-6500 fluorescence spectrophotometer with a 10 nm path length cell (both excitation and emission slit widths, 5.0 nm) at 298 ± 1 K using a temperature controller.<sup>39</sup> Absorption spectra were measured on an UV-visible photodiode-array spectrometer (Shimadzu; Multispec-1500) equipped with a temperature controller (S-1700).<sup>40</sup> All measurements were performed under aerated conditions. <sup>1</sup>H and <sup>13</sup>C NMR charts were obtained using a JEOL JNM-ECS400 spectrometer. FAB-MS analysis was performed on a JEOL JMS 700 Mass Spectrometer. Fluorescence quantum yields ( $\Phi_{\text{F}}$ ) were determined with quinine sulfate dihydrate (in 0.1 M HClO<sub>4</sub> solution) as a standard.<sup>41,42</sup>

### Synthesis of the sensor (**1**) [8-(2,3-dihydro-1H-perimidin-2-yl)-7-hydroxy-4-methyl-2H-chromen-2-one]

8-Formyl-7-hydroxy-4-methylcoumarin (200 mg, 0.98 mmol)<sup>25</sup> and 1,8-diaminonaphthalene (188 mg, 1.20 mmol) were dissolved in EtOH (20 ml), and the solution was stirred at 80 °C for 2.5 h. The solid formed was recovered by filtration and washed thoroughly with EtOH, affording **1** as pale pink solids. Yield: 234.4 mg (69.4%). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>, TMS),  $\delta$  (ppm): 10.38 (1H, s), 7.74 (1H, d,  $J = 8.8$  Hz), 7.24–7.28 (2H, m), 7.18–

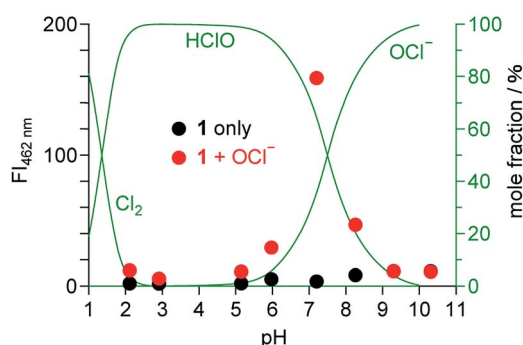


Fig. 6 Fluorescence intensity of **1** (10  $\mu$ M) monitored at 462 nm in water/MeCN mixtures (99/1 v/v) at 25 °C with different pH, (red) with and (black) without OCl<sup>-</sup> (50 equiv.). The mole fraction distributions of Cl<sub>2</sub>, HClO, and OCl<sup>-</sup> calculated based on the equilibria (eqn (1) and (2)) are shown by green lines.



7.20 (2H, m), 7.03 (2H, s), 6.94 (1H, d,  $J = 8.8$  Hz), 6.64 (2H, d,  $J = 7.2$  Hz), 6.23 (1H, s), 5.99 (1H, s), 2.44 (3H, s).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ , TMS),  $\delta$  (ppm): 161.0, 159.6, 153.8, 152.4, 142.9, 134.2, 126.9, 126.7, 117.2, 113.5, 113.2, 112.0, 110.9, 110.3, 106.3, 59.9, 55.9, 18.3. FAB-MS:  $m/z$ : calcd for  $\text{C}_{21}\text{H}_{16}\text{O}_3\text{N}_2^+$  ( $\text{M}^+$ ) 344.1161; found (ESI $^+$ ): 344.1158.

### Calculation details

*Ab initio* calculations were performed with tight convergence criteria at the DFT level within the Gaussian 03 package, using the B3LYP/6-31+G(D) basis set for all atoms. The excitation energies and oscillator strengths of the compounds were calculated by TDTFT<sup>43</sup> at the same level of optimization using the PCM with water as a solvent.<sup>44</sup> Cartesian coordinates are summarized at the end of ESI $^+$ .

### Conflicts of interest

There are no conflicts to declare.

### Acknowledgements

This work was supported by the Grant-in Aid for Scientific Research (No.15K06556) from the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT).

### Notes and references

- 1 A. Manke, S. Luanpitpong, C. Dong, L. Wang, X. He, L. Battelli, R. Derk, T. A. Stueckle, D. W. Porter, T. Sager, H. Gou, C. Z. Dinu, N. Wu, R. R. Mercer and Y. Rojanasakul, *Int. J. Mol. Sci.*, 2014, **15**, 7444.
- 2 A. Gomes, E. Fernandes and J. L. F. C. Lima, *J. Biochem. Biophys. Methods*, 2005, **65**, 45.
- 3 J. Shepherd, S. A. Hilderbrand, P. Waternan, J. W. Heinecke, R. Weissleder and P. Libby, *Chem. Biol.*, 2007, **14**, 1221.
- 4 A. J. Kettle and C. C. Winterbourn, *Redox Rep.*, 1997, **3**, 3.
- 5 T. Aoki and M. Munemori, *Anal. Chem.*, 1983, **55**, 209.
- 6 T. Sugiyama, M. Fujita, N. Koide, I. Mori, T. Yoshida, H. Mori and T. Yokochi, *Microbiol. Immunol.*, 2004, **48**, 957.
- 7 S. L. Hazen, F. F. Hsu, K. Duffin and J. W. Heinecke, *J. Biol. Chem.*, 1996, **271**, 23080.
- 8 L. C. Adam and G. Gordon, *Anal. Chem.*, 1995, **67**, 535.
- 9 Q. Xu, K.-A. Lee, S. Lee, K. M. Lee, W.-J. Lee and J. Yoon, *J. Am. Chem. Soc.*, 2013, **135**, 9944.
- 10 J. Xu, H. Yuan, C. Qin, L. Zeng and G.-M. Bao, *RSC Adv.*, 2016, **6**, 107525.
- 11 L. Long, Y. Wu, L. Wang, A. Gong, F. Hu and C. Zhang, *Chem. Commun.*, 2015, **51**, 10435.
- 12 L. Long, D. Zhang, X. Li, J. Zhang, C. Zhang and L. Zhou, *Anal. Chim. Acta*, 2013, **775**, 100.
- 13 Y.-R. Zhang, X.-P. Chen, J. Shao, J.-Y. Zhang, Q. Yuan, J.-Y. Miao and B.-X. Zhao, *Chem. Commun.*, 2014, **50**, 14241.
- 14 Y. Huang, Y. Zhang, F. Huo, J. Chao and C. Yin, *Sens. Actuators, B*, 2019, **287**, 453.
- 15 J. Zha, B. Fu, C. Qin, L. Zeng and X. Hu, *RSC Adv.*, 2014, **4**, 43110.
- 16 K. Wang, P. Sun, X. Chao, D. Cao, Z. Mao and Z. Liu, *RSC Adv.*, 2018, **8**, 6904.
- 17 J.-T. Hou, H. S. Kim, C. Duan, M. S. Ji, S. Wang, L. Zeng, W. X. Ren and J. S. Kim, *Chem. Commun.*, 2019, **55**, 2533.
- 18 S. Goswami, A. Manna, S. Paul, C. K. Quah and H.-K. Fun, *Chem. Commun.*, 2013, **49**, 11656.
- 19 J. Fan, H. Mu, H. Zhu, J. Wang and X. Peng, *Analyst*, 2015, **140**, 4594.
- 20 J. Li, C.-F. Zhang, Z.-Z. Ming, G.-F. Hao, W.-C. Yang and G.-F. Yang, *Tetrahedron*, 2013, **69**, 4743.
- 21 C. M. Krauter, J. Mohring, T. Buckup, M. Pernpointner and M. Motzkus, *Phys. Chem. Chem. Phys.*, 2013, **15**, 17846.
- 22 L. Yuan, W. Lin, Y. Yang, J. Song and J. Wang, *Org. Lett.*, 2011, **13**, 3730.
- 23 Y. Feng, Y. Yang, Y. Wang, F. Qiu, X. Song, X. Tang, G. Zhang and W. Liu, *Sens. Actuators, B*, 2019, **288**, 27.
- 24 J. Li, C.-F. Zhang, S.-H. Yang, W.-C. Yang and G.-F. Yang, *Anal. Chem.*, 2014, **86**, 3037.
- 25 X. Huang, Y. Dong, Q. Huang and Y. Cheng, *Tetrahedron Lett.*, 2013, **54**, 3822.
- 26 N. Sharma, S. I. Reja, V. Bhalla and M. Kumar, *Dalton Trans.*, 2014, **43**, 15929.
- 27 M. C. Mancini, B. A. Kairdolf, A. M. Smith and S. Nie, *J. Am. Chem. Soc.*, 2008, **130**, 10836.
- 28 C. Albayrak, G. Kastan, M. Odabasoglu and R. Frank, *Spectrochim. Acta, Part A*, 2013, **114**, 205.
- 29 H. Dal, Y. Suzen and E. Sahin, *Spectrochim. Acta, Part A*, 2007, **67**, 808.
- 30 H. Dal, *Asian J. Chem.*, 2014, **26**, 2759.
- 31 G. Kastan, *J. Mol. Struct.*, 2012, **1017**, 38.
- 32 G. A. Jeffrey, *An introduction to hydrogen bonding*, Oxford University Press, New York, 1997.
- 33 H. Zhi, J. Wang, S. Wang and Y. Wei, *J. Spectrosc.*, 2013, **2013**, 147128.
- 34 M. Whiteman and J. P. E. Spencer, *Biochem. Biophys. Res. Commun.*, 2008, **371**, 50.
- 35 S. Nakagawara, T. Goto, M. Nara, Y. Ozawa, K. Hotta and Y. Arata, *Anal. Sci.*, 1998, **14**, 691.
- 36 K. Setsukinai, Y. Urano, K. Kakinuma, H. J. Majima and T. Nagano, *J. Biol. Chem.*, 2003, **278**, 3170.
- 37 N. Umezawa, K. Tanaka, Y. Urano, K. Kikuchi, T. Higuchi and T. Nagano, *Angew. Chem., Int. Ed.*, 1999, **38**, 2899.
- 38 Z.-N. Sun, F.-Q. Liu, Y. Chen, P. K. H. Tam and D. Yang, *Org. Lett.*, 2008, **10**, 2171.
- 39 Y. Shiraishi, M. Nakamura, K. Yamamoto and T. Hirai, *Chem. Commun.*, 2014, **50**, 11583.
- 40 Y. Shiraishi, K. Tanaka, E. Shirakawa, Y. Sugano, S. Ichikawa, S. Tanaka and T. Hirai, *Angew. Chem., Int. Ed.*, 2013, **52**, 8304.
- 41 C. Wurth, M. Grabolle, J. Pauli, M. Spieles and U. R. -Genger, *Anal. Chem.*, 2011, **83**, 3431.
- 42 B. Bag and P. K. Bharadwaj, *J. Phys. Chem. B*, 2005, **109**, 4377.
- 43 R. E. Stratmann, G. E. Scuseria and M. J. Frisch, *J. Chem. Phys.*, 1998, **109**, 8218.
- 44 M. Cossi, V. Barone, R. Cammi and J. Tomasi, *Chem. Phys. Lett.*, 1996, **255**, 327.

