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A highly selective naked-eye probe for hypochlorite with the *p*-methoxyphenol-substituted aniline compound

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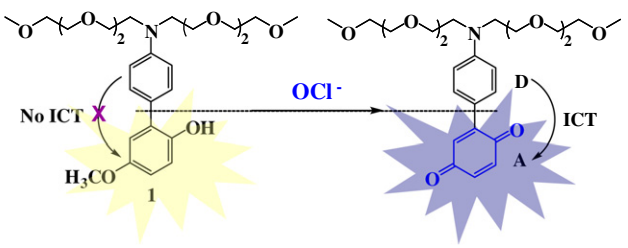
ABSTRACT

A highly selective naked-eye detection of ClO^- is successfully established with probe **1** by taking advantage of the oxidation transformation of *p*-methoxyphenol into benzoquinone with ClO^- and the ICT absorption within the electron donor–acceptor compound. The color of the solution of probe **1** was changed, obviously upon addition of ClO^- and ClO^- with concentration as low as 1.74 μM can be analyzed in aqueous solution with probe **1**. Moreover, the interferences of other anions can be neglected.

Hypochlorite anion (OCl^-) and hypochlorous acid (HOCl) are the biologically important reactive oxygen species (ROS).[1] In the physiological pH solution HOCl is partially dissociated into OCl^-. In organisms hypochlorite is generated from the reaction of H_2O_2 and chloride ion catalyzed by myeloperoxidase (MPO) enzyme.[2] Hypochlorite possesses important antibacterial properties and plays an essential role in the prevention of microorganism invasion. But, abnormal level of hypochlorite can lead to tissue damage and diseases such as atherosclerosis, arthritis, and cancers.[3] This may relate to the fact that hypochlorite in the physiological condition can react with DNA, RNA, fatty acids, cholesterol, and proteins. However, the exact action mechanism of hypochlorite in these diseases is not fully understood. Therefore, development of convenient and specific probes for OCl^- is highly desired for investigations of the functions of OCl^- in biological systems. Additionally, hypochlorite and hypochlorous acid are widely used in our daily lives as a disinfectant and as a bleaching agent. Simple detection methods for OCl^- and HOCl in the environment are also needed.

Recently, several sensitive and selective probes have been reported for the detection of OCl^- (and HOCl) by taking advantage of the strong oxidation capacity of OCl^- (and HOCl). For instance, the groups of Nagano,[4] Ma,[5] Shin[6] and Libby[7] have independently reported Rhodamine (or Fluorescein)-based fluorescent probes for OCl^- (and HOCl). Yang et al.[8] have recently described a highly selective BODIPY-based fluorescent sensor for HOCl by making use of the transformation of *p*-methoxyphenol into benzoquinone in the presence of HOCl. Very recently, Ma et al.[9] have designed a selective fluorescent probe for HOCl with the ferrocene–anthracene dyad. The fluorescent probes are sensitive but the signal readout still needs the aid of the bulk instruments. In comparison, colorimetric probes, in particular the naked-eye detection methods have obvious advantages. In this regard, selective naked-eye probe for OCl^- (and HOCl) is not available, to the best of our knowledge. Herein we want to describe a new highly selective naked-eye probe for the detection of OCl^- with the *p*-methoxyphenol-substituted aniline compound (**1**, Scheme 1).

The molecular design rationale for this naked-eye probe **1** is illustrated in Scheme 1 and explained as follows: (1) it is known that benzoquinone-substituted aniline is colorful (from purple to blue dependent on the chemical structure and the surrounding medium) due to the intramolecular charge-transfer (ICT) between the quinone and aniline units.[10] But, the ICT absorption disappears and it becomes almost colorless when the quinone unit is reduced to hydroquinone or substituted hydroquinone; (2) *p*-methoxyphenol can be



Scheme 1. Design rationale for probe **1**.

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oxidized by OCl^- into benzoquinone (in solutions which are not highly basic) according to the previous report;[8] (3) in order to improve the solubility of probe **1** in aqueous solution glycol chains are introduced in probe **1**. The results indicate that probe **1** is colorless and it becomes blue after the addition of ClO^-, and thus probe **1** can be used for the naked-eye detection of ClO^-. The synthetic approach for probe **1** is outlined in Scheme 2. The synthesis started from the reaction of compound **2** with *p*-bromoaniline leading to compound **3**. Compound **4** was transformed into compound **5** after sequential reaction with *n*-BuLi and $B(OMe)_3$. After Suzuki reaction of **3** with **5**, and removal of MOM group, probe **1** was obtained as a pale yellow oil in 37.4% yield. The chemical structure of probe **1** was established with ^1H NMR, ^{13}C NMR, and HRMS spectral data.[11]

Probe **1** can be dissolved in water and aqueous solutions of probe **1** with concentrations as high as 0.5 mM can be prepared. Figure 1a shows the absorption spectrum of probe **1** and those after addition of different amounts of ClO^- in PBS buffer (0.1 M, pH 7.4). Probe **1** absorbs strongly around 310 nm and exhibits almost no absorption above 400 nm. However, a new absorption around 572 nm emerged gradually and the absorption around 310 nm decreased simultaneously. Under the present condition the reaction of probe **1** with ClO^- was rather fast and it reached equilibrium almost immediately after the addition of ClO^-. Based on the fact that the corresponding benzoquinone-substituted aniline exhibited typical ICT absorption around 572 nm,[10] this new absorption band was likely due to the oxidation of *p*-methoxyphenol unit in probe **1** into the benzoquinone unit leading to the formation of the corresponding benzoquinone-substituted aniline. In fact, mass spectral signals at 494.5 ($M+2H+H^+$) and 516.5 ($M+2H+Na^+$), where M corresponds to the molecular weight of the corresponding benzoquinone-substituted aniline, were detected for the solution of probe **1** after the addition of ClO^- (see Fig. S2). In agreement with the absorption spectral change for probe **1** after introducing ClO^-, the colorless aqueous solution of probe **1** became blue (see the inset of Fig. 1a). It should be noted that the color change for probe **1** after addition of ClO^- is dependent on the concentration of probe **1**; the higher the concentration of probe **1** the larger the absorbance variation at 572 nm which leads to more obvious color change. When the concentration of probe **1** is over 50 μM the color change can be distinguished for probe **1** in 0.1 M PBS at pH 7.4 after the addition of 1.0 equiv of

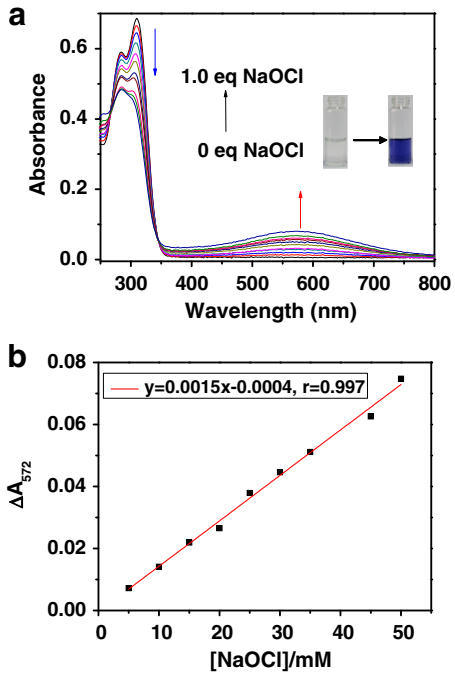
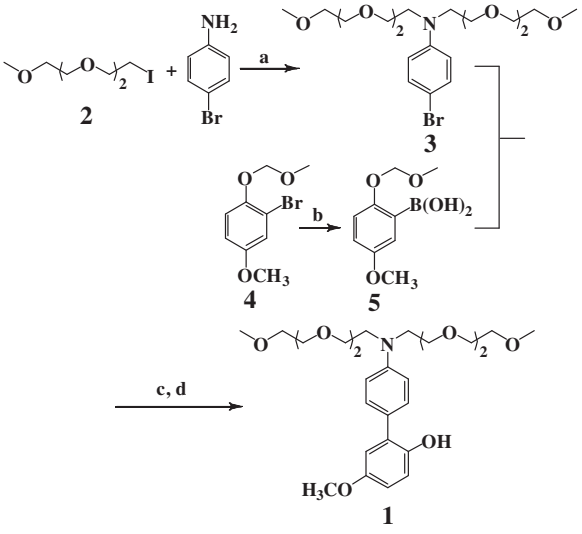


Figure 1. (a) Absorption spectra of probe **1** (50 μM) in the presence of different concentrations of OCl^- in 0.1 M PBS at pH 7.4. Inset: the color change for probe **1** (400 μM) after the addition of 1.0 equiv NaOCl. (b) The plot of the absorption spectra at 572 nm versus concentration of hypochlorite tested.

ClO^- (see Supplementary data). Figure 1b depicts the linear variation of the absorbance at 572 nm, ΔA ($A - A_0$), where A_0 and A were the absorbance of the solution at 572 nm in the absence and presence of ClO^- versus the concentration of ClO^- in the solution ($y = 0.0015\ C_{NaOCl^-}\ 0.0004$ ($n = 9$, $r = 0.997$)). The corresponding detection limit for ClO^- was estimated to be 1.74 μM (S/N = 3) with probe **1**. Compared to the reported detection methods[5,6,9] for ClO^- this naked-eye detection assay is not sensitive. Further optimizing the assay conditions and even modifying the structural design are necessary.

The detection of ClO^- with probe **1** was also examined in aqueous solutions of different pH values. Figure 2 depicts the plot of the variation of the absorbance at 572 nm for the solution of probe **1** (50 μM) and ClO^- (50 μM) versus the pH value of the solution. Obvious absorbance change was detected for solutions with pH values from 5 to 9 and large absorbance variation was observed in solutions with pH 6–7. These results are understandable by considering the following points: (1) at low pH the solution is acidic and as a result the aniline unit may be protonated which



Scheme 2. The synthetic procedure for probe **1**. Reagents and conditions: (a) K_2HPO_4, CH_3CN, reflux; (b) *n*-BuLi, $B(OMe)_3$, THF, −78 °C; (c) $Pd(PPh_3)_4$, $NaHCO_3$, toluene/H_2O, reflux; (d) CF_3COOH, CH_2Cl_2.

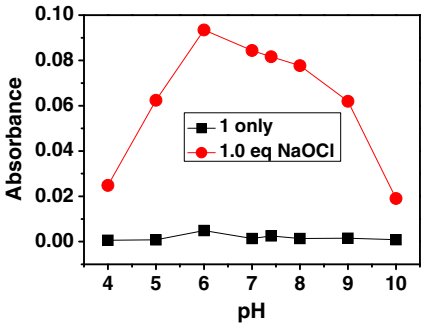


Figure 2. The variation of the absorbance at 572 nm for probe **1** (50 μM) in PBS (0.1 M) at different pH values in the absence (■) and presence (●) of 1.0 equiv of ClO^-.

may weaken the corresponding ICT absorption; (2) at high pH value HOCl is easily dissociated into ClO^- and accordingly the oxidation capacity of the solution is weak. This study indicates that probe **1** can be employed for the detection of ClO^- in aqueous solutions with pH 5–9.

The absorption spectra of probe **1** in the presence of other reactive oxygen species (ROS) and reactive nitrogen specied (RNS) including H_2O_2, $^1O_2$, $O_2^{\cdot-}$, ROO·, NO·, and ·OH were measured in PBS buffer (0.1 M) at pH 7.4. The absorption spectrum of probe **1** was almost unaltered in the presence of these ROS&RNS. Figure 3a depicts the absorbance variation at 572 nm for probe **1** in the presence of ClO^- (1.0 equiv vs probe **1**) and other ROS&RNS (10.0 equiv for each species vs probe **1**). It is obvious that large absorbance change was only observed for probe **1** after the addition of ClO^-. This is consistent with the observation that the solution of probe **1** became blue only after the addition of ClO^- as shown in Figure 3b, where the photos of the solutions of probe **1** in the presence of ClO^- and other ROS&RNS species were displayed. These results demonstrate that probe **1** is highly selective toward ClO^-. Alternatively, the absorption spectra of probe **1** were also measured after sequential addition of 10.0 equiv of each ROS&RNS species and 1.0 equiv of ClO^-. Absorbance at 572 nm increased significantly after the addition of 10.0 equiv of NO· from sodium nitroferricyanide (III) and 1.0 equiv of ClO^-, but the absorbance at 572 nm was almost not enhanced for probe **1** in the presence of 1.0 equiv of ClO^- and 10.0 equiv of H_2O_2, $O_2^{\cdot-}$, RCOO·, and HO· (see Supplementary data). This is because of the fact that ClO^- can react with H_2O_2, $O_2^{\cdot-}$, the precursors for RCOO·, and HO·,[12,13] and as a result ClO^- was consumed in the solution and oxidation of *p*-methoxyphenol cannot occur accordingly.

The interferences of other anions for the detection of ClO^- (HOCl) were also examined. For this purpose, the absorption spectrum of probe **1** was measured separately in the presence of F^-, Cl^-, Br^-, I^-, ClO_3^-, ClO_4^-, CO_3^{2-}, SO_4^{2-}, PO_4^{3-}, $H_2PO_4^-$ and OH^- (10.0 equiv for each anion vs probe **1**), respectively. For comparison the absorption spectrum of probe **1** was also recorded after the addition of 1.0 equiv of ClO^-. As shown in Figure 4, new absorption around 572 nm was detected for probe **1** only upon addition of ClO^- and the absorption spectrum of probe **1** kept almost unchanged in the presence of other anions. Therefore, it can be concluded that the interferences of other anions for this naked-eye detection of ClO^- (HOCl) can be neglected. Additionally, cations such as Fe^{2+}, Fe^{3+}, Cu^{2+}, and Zn^{2+} also show no interferences for the detection of ClO^- with probe **1** since the absorption spectrum of probe **1** was almost not affected by these metal ions (Fig. S1).

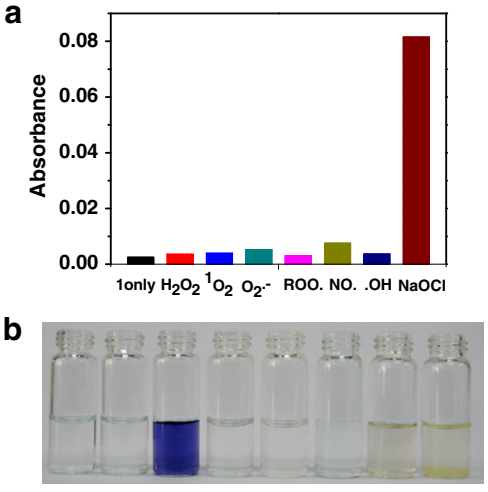


Figure 3. (a) The variation of the absorbance at 572 nm for probe **1** (50 μM) in PBS (0.1 M) buffer at pH 7.4 after the addition of OCl^- (1.0 equiv) and other ROS&RNS species including H_2O_2, $^1O_2$, $O_2^{\cdot-}$, ROO·, NO· and ·OH (10 equiv for each species). (b) The photos of the solutions of probe **1** (400 μM) in PBS (0.1 M) buffer at pH 7.4 from left to right: solution of **1** and those after the addition H_2O_2 (10 equiv), OCl^- (1.0 equiv), $^1O_2$ (10 equiv), $O_2^{\cdot-}$ (10 equiv), ROO· (10 equiv), NO· (10 equiv) and ·OH (10 equiv), respectively.

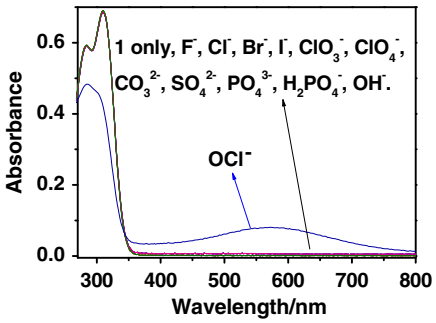


Figure 4. Absorption spectra of probe **1** (50 μM) upon addition of NaOCl (1.0 equiv) and other anions (10.0 equiv) in 0.1 M PBS buffer at pH 7.4.

In summary, probe **1** was designed and studied for the selective detection of ClO^- by taking advantage of the oxidation transformation of *p*-methoxyphenol into benzoquinone with ClO^- and the ICT absorption within the electron donor–acceptor compound. Absorption spectral investigations clearly indicate that probe **1** is highly selective toward ClO^- and the interferences of other anions can be neglected. Of interest is the color change for probe **1** upon addition of ClO^-. Thus, a highly selective naked-eye detection of ClO^- (HOCl) is successfully established with probe **1** and ClO^- with concentration as low as 1.74 μM that can be analyzed in aqueous solution. It is anticipated that probe **1** can be used for practical detection of ClO^- by modifying the structure of probe **1** which may further improve the sensitivity. Furthermore, instead of aniline, *p*-methoxyphenol can be connected with electron donor units which emit intrinsically and accordingly new naked-eye and fluorescent probes for ClO^- may be constructed.

Acknowledgments

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Supplementary data

Supplementary data (synthesis and characterization data of compounds **3**, **5,** and **1**; methods for preparation of ROS&RNS; absorption spectra, and relevant data for the ensemble) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.09.041.

References and notes

1. (a) Gomes, A.; Fernandes, E.; Lima, J. L. F. C. *J. Biochem. Biophys. Methods* **2005**, *65*, 45–80; (b) Oushiki, D.; Kojima, H.; Terai, T.; Arita, M.; Hanaoka, M.; Hanaoka, K.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2010**, *132*, 2795–2801; (c) Li, X. H.; Zhang, G. X.; Ma, H. M.; Zhang, D. Q.; Li, J.; Zhu, D. B. *J. Am. Chem. Soc.* **2004**, *126*, 11543–11548; (d) Bizyukin, A. V.; Korkina, L. G.; Velichkovskii, B. T. *Bull. Exp. Biol. Med.* **1995**, *119*, 347–351.
2. (a) Harrison, J. E.; Schultz, J. *J. Biol. Chem.* **1976**, *251*, 1371–1374; (b) Panizzi, P.; Nahrendorf, M.; Wildgruber, M.; Waterman, P.; Figueiredo, J. L.; Aikawa, E.; McCarthy, J.; Weissleder, R.; Hilderbrand, S. A. *J. Am. Chem. Soc.* **2009**, *131*, 15739–15744.
3. (a) Pattison, D. I.; Davies, M. J. *Chem. Res. Toxicol.* **2001**, *14*, 1453–1456; (b) Podrez, E. A.; Abu-soud, H. M.; Hazen, S. L. *Free Radical. Biol. Med.* **2000**, *28*, 1717–1725; (c) Pattison, D. I.; Davies, M. J. *Biochemistry* **2006**, *45*, 8152–8162; (d) O'Brien, P. J. *Chem. Biol. Interact.* **2000**, *129*, 113–139; (e) Kettle, A. J.; Winterbourn, C. C. *Redox. Rep.* **1997**, *3*, 3–15.

4. Setsukinai, K.; Urano, Y.; Kakinuma, K.; Majima, H. J.; Nagano, T. *J. Biol. Chem.* **2003**, *278*, 3170–3175.
5. Chen, X. Q.; Wang, X. C.; Wang, S. J.; Shi, W.; Wang, K.; Ma, H. M. *Chem. Eur. J.* **2008**, *14*, 4719–4724.
6. Yang, Y. K.; Cho, H. J.; Lee, J.; Shin, I.; Tae, J. *Org. Lett.* **2009**, *11*, 859–861.
7. Shepherd, J.; Hilderbrand, S. A.; Waterman, P.; Heinecke, J. W.; Weissleder, R.; Libby, P. *Chem. Biol.* **2007**, *14*, 1221–1231.
8. Sun, Z. N.; Liu, F. Q.; Chen, Y.; Tam, P. K. H.; Yang, D. *Org. Lett.* **2008**, *10*, 2171–2174.
9. Chen, S. M.; Lu, J. X.; Sun, C. D.; Ma, H. M. *Analyst* **2010**, *135*, 577–582.
10. (a) Zhang, H. B.; Liu, L.; Chen, Y. J.; Wang, D.; Li, C. J. *Adv. Synth. Catal.* **2006**, *348*, 229–235; (b) Zeng, Y.; Zhang, G. X.; Zhang, D. Q. *Anal. Chim. Acta* **2008**, *627*, 254–257; (c) Zeng, Y.; Zhang, G. X.; Zhang, D. Q.; Zhu, D. B. *Tetrahedron Lett.* **2008**, *49*, 7391–7394; (d) Zeng, Y.; Zhang, G. X.; Zhang, D. Q.; Zhu, D. B. *J. Org. Chem.* **2009**, *74*, 4375–4378.
11. The spectral data of probe **1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.30 (d, 2H, J = 8.1 Hz), 6.88 (d, 1H, J = 8.5 Hz), 6.81 (d, 2H, J = 7.9 Hz), 6.77–6.75 (m, 2H), 5.10 (s, 1H), 3.77 (s, 3H), 3.65 (m, 20H), 3.54 (m, 4H), 3.37 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 153.7, 147.7, 147.0, 130.0, 129.2, 124.5, 116.4, 115.4, 113.8, 112.4, 72.2, 70.9, 70.8, 68.7, 59.3, 56.0, 51.2; HRMS calcd for $C_{27}H_{41}NO_8$: 507.2832; found: 507.2836.
12. Long, C. A.; Blelski, B. H. J. *J. Phys. Chem.* **1980**, *84*, 555–557.
13. Kanazawa, H.; Harata, Y.; Onami, T. *Sci. Rep. Fukushima Univ.* **1991**, 37–47.