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PAPER

Naked-eye visible and fluorometric dual-signaling chemodosimeter for hypochlorous acid based on water-soluble *p*-methoxyphenol derivative†

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The oxidation of a simple *p*-methoxyphenol derivative by HClO induces an intramolecular charge transfer from the end phenyl units to the middle benzoquinone, which leads to colorimetric and fluorescent changes. This detection can be run in aqueous solution with high selectivity over other reactive oxygen species.

Introduction

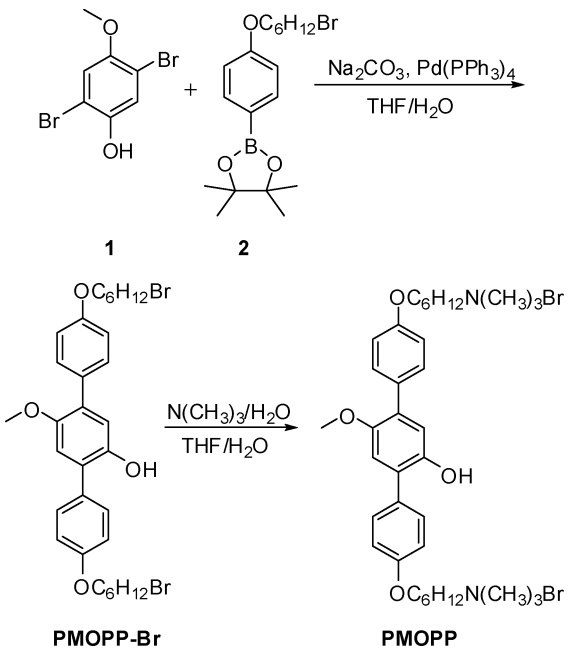
As a kind of reactive oxygen species (ROS), hypochlorous acid (HClO) plays a crucial role in our daily lives and in host defence against invading pathogens.[1–2] Under physiological conditions (with a pK_a of 7.46 at 35 °C), approximately half of the HClO dissociates to the hypochlorite anion (ClO^-), one of the most powerful natural oxidants.[3–5] In living organisms, hypochlorous acid is generated by the reaction of hydrogen peroxide with chloride ions under the catalysis of the heme enzyme myeloperoxidase (MPO), which is synthesized and secreted by activated phagocytes.[6–9] However, because of its high reactivity and non-specificity,[10] excessive hypochlorous acid can lead to damage of host tissue that is implicated in a wide range of human diseases, such as kidney disease,[11–12] atherosclerosis,[13–16] and arthritis.[17–19] Therefore, sensitive and selective detection of hypochlorous acid is of important significance.

Recently, a few probes for hypochlorous acid have been reported based on the strong oxidation property of HClO.[5,20–26] For example, Nagano's group[20] and Libby's group[5] reported rhodamine- and fluorescein-based fluorescent probes for HClO, respectively, but the fluorescent probes involved complicated synthesis; Ma's group also developed two fluorescent probes for HClO,[21,22] which work in an organic co-solvent system. Moreover, to the best of our knowledge, none of these probes can respond to HClO with colorimetric and fluorescent changes simultaneously. A dual-signaling chemodosimeter can combine the sensitivity of fluorescence with the convenience and esthetic appeal of a colorimetric assay.[27,28]

It is known that *p*-methoxyphenol can be selectively oxidized to benzoquinone by hypochlorous acid.[20] Based on this reaction, we designed a simple *p*-methoxyphenol derivative, namely PMOPP, by putting the *p*-methoxyphenol as the middle unit of a terphenyl conjugated system. We anticipate that the oxidation of the *p*-methoxyphenol unit by HClO would induce an intramolecular charge transfer (ICT) from the end phenyl units to the middle benzoquinone, which could in turn lead to colorimetric and fluorescent changes. In order to be applied in aqueous systems, the two end phenyl units are equipped with quaternary ammonium parts.

Results and discussion

PMOPP can be easily prepared in two steps. As shown in Scheme 1, the Suzuki coupling reaction of 2,5-dibromo-4-methoxyphenol (**1**) and 2-(4-(6-bromohexyloxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**2**) gave the precursor PMOPP-Br, which was then followed by quaternization with trimethylamine to afford PMOPP.



Scheme 1 Synthesis of PMOPP.

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† Electronic supplementary information (ESI) available: ESI-MS spectra of PMOPP and PQP, and NMR spectra of the key compounds. See DOI: 10.1039/c1ob05550j

The final product shows good water-solublity owing to the charged trimethylammonium groups.

In order to mimic the environment in living organisms, the optical responses of PMOPP toward HClO were examined in 10 mM phosphate buffered saline (PBS) solution (pH 7.4). The absorption spectra of PMOPP upon addition of hypochlorite are shown in Fig. 1. After addition of hypochlorite, the absorption at 314 nm decreases; synchronously, a new band at 393 nm appears. A clear isosbestic point at 341 nm indicates that the reaction of hypochlorite with PMOPP is a clean process. The broad absorption band centred at *ca.* 393 nm could be attributed to an ICT transition from the end phenyl units to the middle benzoquinone. It is noteworthy that the process is accompanied by a color change from colorless to yellow (the inset of Fig. 1), which makes PMOPP capable for the naked-eye detection of HClO. Compared to fluorometric sensors, naked-eye detect in a straightforward and inexpensive manner, offers qualitative and quantitative information without using expensive equipment.[29]

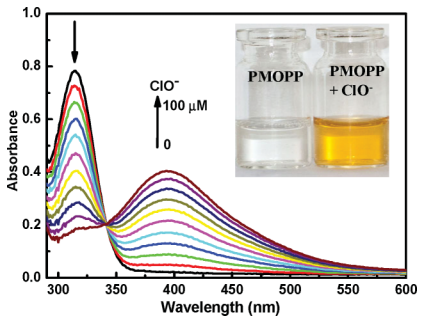


Fig. 1 UV/vis spectra of PMOPP (final concentration: 50 μM) in 0.01 M PBS solution (pH 7.4) after the addition of ClO^- (ranging from 0 to 100 μM). Inset: the color change of the solution.

The fluorescent response of PMOPP to hypochlorite was subsequently investigated. As indicated in Fig. 2, the fluorescence of PMOPP decreases dramatically upon increasing the hypochlorite concentration. After treatment with 24 μM hypochlorite, the fluorescence of the solution is quenched completely. Correspondingly, the fluorescent quantum yield decreased from 72.6% to 0.8%. In the range of 1–10 μM (the inset of Fig. 2), the fluorescence intensity is directly proportional to the ClO^- concentration. The detection limit for HClO is determined to be 0.8 μM (S/N = 3) from the fluorescence titration profile of PMOPP (5 μM) with HClO.

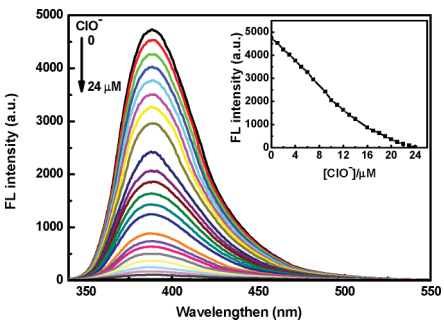
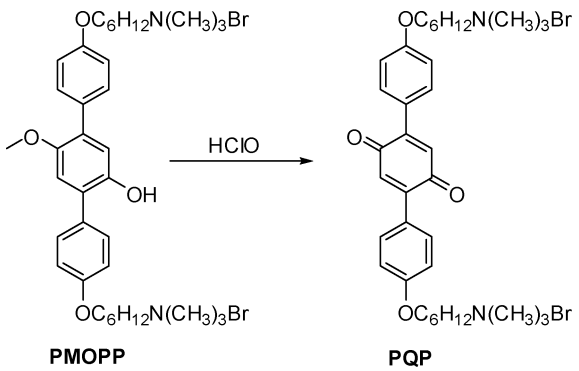


Fig. 2 Fluorescence spectra of PMOPP (final concentration: 5 μM) in 0.01 M PBS solution (pH 7.4) after the addition of ClO^- (ranging from 0 to 24 μM). The excitation wavelength is 320 nm.

The value is comparable to the reported probes for hypochlorous acid.[21,22]

We presumed that the color change and fluorescence quenching could be attributed to the oxidation of PMOPP to its benzoquinone derivative PQP (Scheme 2). To further confirm the process, we carried out ^{1}H NMR titrations. Superimposed ^{1}H NMR spectra of PMOPP after addition of 0–3 equiv. of hypochlorite are shown in Fig. 3. Upon the addition of hypochlorite, the signals assigned to OH at $\delta \approx 6.7$ ppm and OCH_3 at $\delta \approx 3.1$ ppm are gradually weakened, instead a new resonance at $\delta \approx 3.2$ ppm appears, which may correspond to $ClCH_3$. Accordingly, the signals assigned to the phenyl group also show changes in chemical shift. The ^{1}H NMR titrations confirm that the *p*-methoxyphenol is actually oxidized to benzoquinone by hypochlorite. More direct evidence comes from the ESI-MS result, in which the oxidation product PQP is observed (see the ESI†).



Scheme 2 Reaction of PMOPP with HClO.

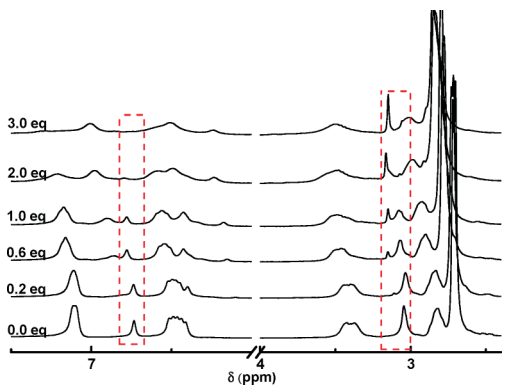


Fig. 3 ^{1}H NMR spectra of PMOPP (12 mM) in D_2O after addition of 0–3 equiv. of ClO^-.

Finally, we investigated the reactivity of PMOPP toward various ROS, including HClO, H_2O_2, $O_2^{\bullet-}$, NO, $^{\bullet}OH$, $ONOO^-$. The photograph in Fig. 4 shows the solution color after addition of various ROS in PBS solution. It is clear that the solution remains colorless upon addition of various ROS, except HClO. Similarly, PMOPP exhibits a selective fluorometric detection for hypochlorite (Fig. 5). It is obvious that fluorescence quenching occurs only upon addition of HClO.

Fig. 4 Color changes of PBS solution (pH = 7.4, 0.01 M) of PMOPP (50 μM) in the presence of the following ROS: from left to right: none, HClO, H_2O_2, $O_2^{\cdot-}$, NO, ·OH and $ONOO^-$. HClO: 50 μM $Ca(ClO)_2$. H_2O_2: 20 mM H_2O_2. $O_2^{\cdot-}$: 20 mM KO_2 was added and the mixture was stirred at 37 °C for 30 min. NO: 20 mM sodium nitroferricyanide(III) was added and the mixture was stirred at 25 °C for 30 min. ·OH: 10 mM ferrous perchlorate and 20 mM H_2O_2. $ONOO^-$: 20 mM sodium peroxynitrite.

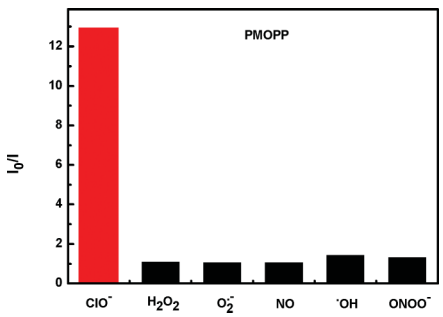


Fig. 5 Fluorescence intensity changes of PMOPP (final concentration: 5 μM) in PBS solution (pH = 7.4, 0.01 M) with various ROS generating systems. The fluorescence intensity (388 nm) was determined with excitation at 320 nm. HClO: 10 μM $Ca(ClO)_2$. H_2O_2: 100 μM H_2O_2. $O_2^{\cdot-}$: 100 μM KO_2 was added and the mixture was stirred at 37 °C for 30 min. NO: 100 μM sodium nitroferricyanide (III) was added and the mixture was stirred at 25 °C for 30 min. ·OH: 50 μM ferrous perchlorate and 100 μM H_2O_2. $ONOO^-$: 100 μM sodium peroxynitrite.

Conclusions

In conclusion, we have designed and synthesized a simple and water-soluble *p*-methoxyphenol derivative that shows colorimetric and fluorometric dual-signaling responses for hypochlorite acid in the aqueous system. The detection process gives rise to a color change that is clearly visible to the naked eye. This makes the colorimetric chemodosimeters helpful for "in-the-field" measurement. Furthermore, both the colorimetric and fluorometric detection exhibit high selectivity over the usual reactive oxygen species. The advantages of dual-signaling, color-change and running environment in physiological conditions make the new probe promising for the detection of hypochloric acid, especially in biological system, and this work is under way.

Experimental

General information

^{1}H NMR and ^{13}C NMR spectra were obtained in the indicated solvent with tetramethylsilane as an internal standard on MECUYRVX300 spectrometers. Elemental analysis of carbon, hydrogen, and nitrogen were performed on a Carlorerba-1106 microanalyzer. Mass spectra were measured on a ZAB 3F-HF mass spectrophotometer. LC-mass spectra were measured on a Waters ZQ-Mass ESI. UV-vis absorption measurements were performed on a Shimadzu 2550 spectrophotometer. Fluorescence measurements were performed on a Hitachi F-4500 fluorescence spectrophotometer.

All chemicals were purchased from commercial sources and were used without further purification unless otherwise noted. 2,5-Dibromobenzene-1,4-diol and 2,5-dibromo-4-methoxyphenol (**1**) were synthesized following literature procedures.[30]

Synthesis of 2-(4-(6-bromohexyloxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (2)

A flask charged with 1-bromo-4-(6-bromohexyloxy)benzene (1.01 g, 3.0 mmol), bis(pinacolato)diborane (1.26 g, 3.3 mmol), potassium acetate (0.88 g, 9.0 mmol), $Pd(dppf)Cl_2$ (0.73 g, 0.1 mmol), and 20 mL of anhydrous dioxane was degassed for 15 min. The mixture was stirred at 80 °C for 12 h. After being cooled to room temperature, the mixture was poured into ice water, and then extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4. After the solvent was removed by rotary evaporation, the residue was purified by silica gel column chromatography (15 : 1 petroleum ether/ethyl acetate) to afford a white solid. Yield: 0.90 g, 78%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.72 (d, 2H, J = 8.4 Hz), 6.87 (d, 2H, J = 8.4 Hz), 3.97 (t, 2H, J = 6.3 Hz), 3.41 (t, 2H, J = 6.6 Hz), 1.88 (m, 2H), 1.78 (m, 2H), 1.49 (m, 4H), 1.32 (s, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.8, 136.7, 114.0, 83.7, 67.7, 34.0, 32.9, 29.2, 28.1, 25.5, 25.1, 25.0. EI-MS: m/z 382 (M^+). Anal. Calcd for $C_{18}H_{28}BBrO_3$: C, 56.43; H, 7.37. Found: C, 56.36; H, 7.63.

4,4″-Bis(6-bromohexyl)oxy-5′-methoxy-1,1′:4′,1″-terphenyl-2′-ol (PMOPP-Br)

A flask charged with **1** (0.14 g, 0.5 mmol), **2** (0.45 g, 1.2 mmol), $Pd(PPh_3)_4$ (0.02 g, 0.02 mmol), and sodium carbonate (1.06 g, 10.0 mmol), in 20 mL THF/H_2O (3 : 1) was degassed for 15 min. The mixture was refluxed for 24 h. After being cooled down to room temperature, the mixture was neutralized by acetic acid, and then extracted with $CHCl_3$. The organic layer was washed with water and brine and dried with anhydrous Na_2SO_4. After the solvent was removed by rotary evaporation, the residue was purified by silica gel column chromatography (5 : 1 petroleum ether/ethyl acetate) to give a pale yellow solid. Yield: 0.25 g, 79%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51 (d, 2H, J = 8.1 Hz), 7.45 (d, 2H, J = 8.7 Hz), 7.03 (d, 2H, J = 8.1 Hz), 6.96 (s, 1H), 6.94 (d, 2H, J = 7.5 Hz), 6.83 (s, 1H), 4.98 (s, 1H), 4.05–3.99 (m, 4H), 3.77 (s, 3H), 3.47–3.42 (m, 4H), 1.91–1.83 (m, 8H), 1.53 (br, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.8, 158.3, 150.6, 146.4, 130.7, 130.5, 130.2, 129.2, 126.6, 117.8, 115.2, 114.1, 113.6, 67.9, 67.7, 56.4, 33.9, 32.7, 29.2, 29.1, 28.0, 25.3. EI-MS: m/z 632 (M^+) Anal. Calcd for $C_{31}H_{38}Br_2O_4$: C, 58.69; H, 6.04. Found: C, 58.75; H, 6.20.

6,6′-[(2′-Hydroxy-5′-methoxy-1,1′:4′,1″-terphenyl-4,4″-diyl)bis-(oxy)]bis(*N*,*N*,*N*-trimethylhexan-1-aminium) dibromide (PMOPP)

To a solution of PMOPP-Br (0.15 g, 0.2 mmol) in 10 mL THF was added $N(CH_3)_3$ aqueous solution (~2.4 mL). After stirring for

0.5 h, 1 mL H_2O was added to the mixture. Then excess $N(CH_3)_3$ aqueous (~2.4 mL) was added and the mixture was stirred for 48 h. After the solvent was removed by rotary evaporation, the crude product was recrystallized from ethanol. Yield: 0.10 g, 58%. ^1H NMR (300 MHz, D_2O): 7.01 (br, 4H), 6.73 (s, 1H), 6.49–6.41 (br, 6H), 3.42–3.38 (br, 4H), 3.05 (s, 3H), 2.82 (br, 4H), 2.72 (s, 9H), 2.70 (s, 9H), 1.26 (br, 8H), 0.90 (br, 8H). ^{13}C NMR (75 MHz, D_2O): δ 157.8, 150.1, 147.5, 130.7, 129.4, 127.0, 118.0, 114.5, 68.3, 66.6, 56.0, 53.0, 28.8, 25.7, 25.1, 22.6. ESI-MS: m/z 671 (M^+ – Br). Anal. Calcd for $C_{37}H_{56}Br_2N_2O_4$: C, 59.04; H, 7.50. Found: C, 59.26; H, 7.51.

General procedure for spectroscopic measurements

A 5 mM stock solution of PMOPP was prepared in PBS buffer. Testing solutions were prepared by adding 2 or 20 μL of the probe stock solution to 2 mL PBS buffer in a test tube and then adding an appropriate aliquot of each ROS stock solution. All of the absorption and fluorescence sensing of ROS was run immediately after the ROS were added.

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