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A Highly Selective Fluorescence "Turn-On" Probe for Cu(II) Based on Reaction and Its Imaging in Living Cells

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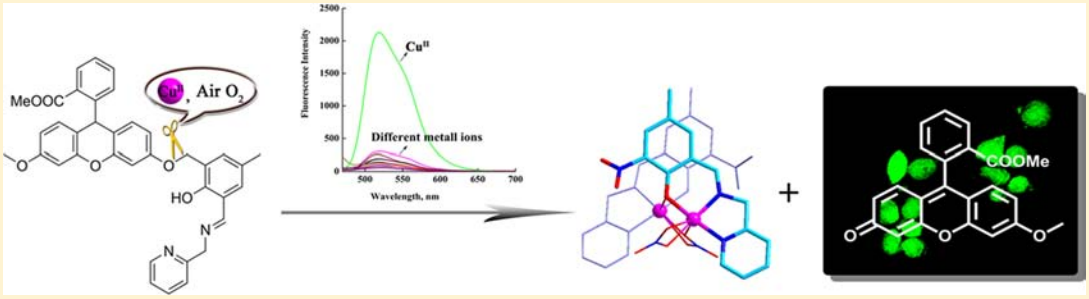
Supporting Information



ABSTRACT: A new oxidative C−O bond cleavage reaction-based probe FluHMPP was designed and prepared. FluHMPP displays excellent selective turn-on fluorescence response for Cu^{II} in aqueous solution under visible light excitation. The cleavage products are fully characterized. Fluorescein fragment is further oxidized to highly fluorescent MFME (3′-*O*-methylfluorescein methyl ester), and benzyl ether of imine fragment has been transformed to carboxyl. Confocal microscopy experiments have demonstrated that FluHMPP could also be used in live cells for the detection of Cu^{II}.

INTRODUCTION

Copper, as the third most abundant transition metal in the human body (after iron and zinc), plays a pivotal role involving cellular energy generation, oxygen transport and activation, and signal transduction.[1] Cells have developed sophisticated regulatory mechanisms to maintain a critical balance for ingestion of copper.[2] However, copper is highly toxic to organisms when its level exceeds cellular needs. For example, it is a powerful catalyst of the Fenton reaction, which can generate highly reactive hydroxyl radicals to interfere with cellular metabolism,[3] and it can displace other metal ions and change enzyme-catalyzed reactions.[4] Therefore, disruption of copper homeostasis is related to some neurodegenerative diseases such as Menkes syndrome, Wilson's disease, and Alzheimer's disease.[5] Despite the critical contributions of copper homeostasis to physiology and pathology, the detection of copper ions in living systems is still a challenge.

As one of the important means and techniques for detecting copper, fluorescent probes display evident advantages in selectivity and biological imaging.[6] Because of the fast electron/energy transfer involving intrinsic paramagnetic Cu^{II} centers, most probes showed a "turn-off" response.[7] Although some "turn-on" or ratiometric sensors have been reported and a few of them have been used with success in biological applications recently,[8] chemists still need to design novel ones, which can overcome these limitations, including ultraviolet excitation or operation at nonphysiological pH for fluorescence imaging.

Turn-on fluorescence detection based on reaction is an appealing strategy for paramagnetic metal ions. Furthermore, X-ray diffraction analysis of reaction product can effectively explain fluorescence enhancement mechanism. So far, most of the reaction-based probes for Cu^{II} exploit metal-promoted hydrolysis or desulfurization reaction, which may suffer from competing ions.[6b,9] However, there are only very few systems using the redox activity of Cu^{II}.[8a,10]

Imine derivatives have strong coordination ability and can modulate metal center electronic properties; they have been widely used in catalytic oxidation, analytical chemistry, and chemical simulation for biological systems.[11] Recently, C−O bond cleavage reaction-based systems were developed for turn-on sensing of Cu^{I} and Co^{II} by Taki[12] and Chang,[13] respectively. These results encouraged us to develop new systems for other substances in biological studies. Herein, we present the synthesis of FluHMPP (Scheme 1) in which a tridentate

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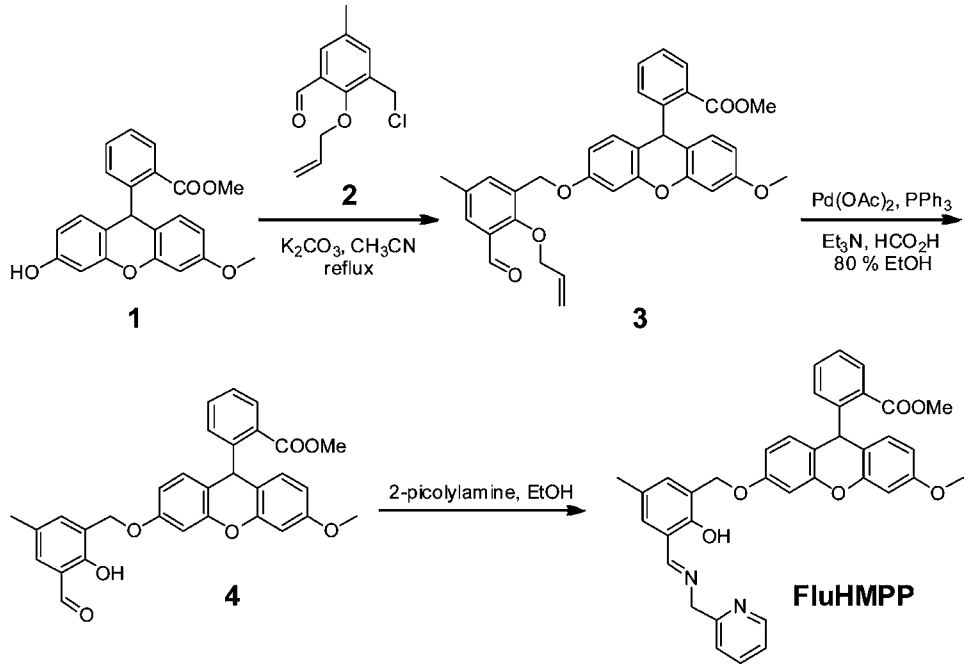
Scheme 1. Synthesis of FluHMPP



NNO imine ligand, 6-(hydroxymethyl)-4-methyl-2-((2-picolylimino)methyl)phenol (HMPP), is linked to the non-conjugated form of a fluorescein platform through a benzyl ether linkage. FluHMPP could selectively react with Cu^{II} through imine complexation and exhibits turn-on fluorescence response in aqueous media arising from oxidative cleavage. In addition, confocal microscopy experiments have demonstrated that FluHMPP is membrane permeable and can react with intracellular Cu^{II}.

EXPERIMENTAL SECTION

General Information and Materials. All solvents were of reagent grade. CH_3CN was purified by distillation over CaH_2 and transferred under argon. Oxygen-sensitive reaction was carried out under an argon atmosphere in a heat-dried flask. All reactions were monitored by thin-layer chromatography (TLC) using UV light. 2-(3-Hydroxy-6-methoxy-9*H*-xanthen-9-yl)benzoic acid methyl ester[12] (**1**) and 2-allyloxy-3-chloromethyl-5-methylbenzaldehyde[14] (**2**) were prepared according to literature procedures. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on a Bruker DRX400 spectrometer. Chemical shifts (δ) were denoted in ppm, and calibrated by using residual undeuterated solvent ($CHCl_3$ (7.27 ppm), or tetramethylsilane (0.00 ppm)) as internal reference for ^1H NMR and the deuterated solvent ($CDCl_3$ (77.00 ppm) or tetramethylsilane (0.00 ppm)) as internal standard for ^{13}C NMR. Mass spectra and the high-resolution mass spectrum (HRMS) were measured on a Bruker Esquire 6000 mass spectrometer and a Bruker maXis 4G mass spectrometer by means of the electronic spray ionization (ESI) technique, respectively. The melting points were measured on an X-6 melting point apparatus without calibration (Beijing Fuka Keyi Science and Technology Co., Ltd.). Elemental analyses were conducted using an Elementar VarioEL instrument. Tris−HCl solution (10 mM, pH 7.20) was prepared in H_2O-CH_3CN (7:3, v/v). A starting solution (H_2O-CH_3CN) of 100 mM NaOH (pH ≈ 13) was used for pH titrations. The pH values were lowered to 4.0 by the addition of aqueous HCl (H_2O-CH_3CN). All pH measurements were made with a pH-10C digital pH meter.

Caution: Although no problems were encountered during the preparation of perchlorate salts, suitable care should be taken when handling such potentially hazardous compounds.

Synthesis of FluHMPP. *Preparation of 2-[3-(2-Allyloxy-3-formyl-5-methyl-benzyloxy)-6-methoxy-9H-xanthen-9-yl]benzoic Acid Methyl Ester (**3**).* To a solution of **1** (1.81 g, 5.00 mmol) in degassed CH_3CN (100 mL) were added **2** (1.03 g, 5.00 mmol) and K_2CO_3 (1.38 g, 10 mmol). The reaction mixture was refluxed under Ar overnight. After removal of the insoluble materials by filtration, the filtrate was evaporated. The residue was dissolved in AcOEt (100 mL), and the organic layer was washed with water (100 mL × 2) and brine (100 mL), dried over $MgSO_4$, and concentrated. The crude residue was purified by flash chromatography (SiO_2, AcOEt/petroleum ether 1:2, v/v) to give **3** as a white solid (2.47 g, 90%). Mp 64.5−65.3 °C. Anal. Calcd for $C_{34}H_{30}O_7$: C, 74.17; H, 5.49. Found: C, 74.13; H, 5.43. ^1H NMR (400 MHz, $CDCl_3$): δ = 2.36 (s, 3H), 3.78 (s, 3H), 3.95 (s, 3H), 4.51 (d, J = 5.6 Hz, 2H), 5.06 (s, 2H), 5.26 (dd, J = 0.8, 10.4 Hz, 1H), 5.36 (dd, J = 1.2, 17.2 Hz, 1H), 5.99−6.09 (m, 1H), 6.22 (s, 1H), 6.53 (dd, J = 2.8, 8.8 Hz, 1H), 6.60 (dd, J = 2.4, 8.8 Hz, 1H), 6.65 (d, J = 2.4 Hz, 1H), 6.74 (d, J = 2.8 Hz, 1H), 6.93 (d, J = 8.8 Hz, 1H), 6.97 (d, J = 8.8 Hz, 1H), 7.12 (d, J = 7.6 Hz, 1H), 7.19 (t, J = 7.6 Hz, 1H), 7.32 (td, J = 1.2, 7.6 Hz, 1H), 7.55 (d, J = 2.4 Hz, 1H), 7.64 (d, 2.4 Hz, 1H), 7.80 (dd, J = 1.2, 7.6 Hz, 1H), 10.34 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.7, 37.7, 52.3, 55.3, 64.7, 78.4, 101.0, 102.0, 110.2, 110.6, 116.8, 117.5, 119.0, 126.1, 129.0, 129.2, 129.3, 129.5, 130.5, 130.7, 131.0, 131.7, 132.4, 132.5, 134.5, 136.9, 148.2, 151.3, 151.4, 157.9, 158.1, 159.2, 168.7, 189.9. ESI−MS m/z [$(M + 1)^+$]: 551.2.

*Preparation of 2-[3-(2-Hydroxy-3-formyl-5-methyl-benzyloxy)-6-methoxy-9H-xanthen-9-yl]benzoic Acid Methyl Ester (**4**).* A stirred mixture of **3** (1.71 g, 3.1 mmol), $Pd(OAc)_2$ (22 mg, 0.1 mmol), PPh_3 (157 mg, 0.6 mmol), Et_3N (3.93 g, 38.8 mmol), and HCO_2H (1.79 g, 38.8 mmol) in 80% EtOH was refluxed for 30 min. After removal of the solvent, the residue was partitioned between CH_2Cl_2 and 0.1 M HCl. The organic layer was dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography (SiO_2, petroleum ether/AcOEt 5:1, v/v) to afford **4** as a white solid (1.11 g, 70%). Mp 114.3−114.8 °C. Anal. Calcd for $C_{31}H_{26}O_7$: C, 72.93; H, 5.13. Found: C, 72.73; H, 5.02. ^1H NMR (400 MHz, $CDCl_3$): δ = 2.35 (s, 3H), 3.80 (s, 3H), 3.97 (s, 3H), 5.13 (s, 2H), 6.22 (s, 1H), 6.54 (dd, J = 2.4, 8.8 Hz, 1H), 6.63−6.67 (m, 2H), 6.78 (d, J = 2.4 Hz, 1H), 6.96 (t, J = 8.8 Hz, 2H), 7.13 (d, J = 8.0 Hz, 1H), 7.20 (td, J = 1.2, 8.0 Hz, 1H), 7.30−7.35 (m, 2H), 7.55 (s, 1H), 7.81 (d, J = 8.0 Hz, 1H), 9.86 (s, 1H), 11.19 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.3, 37.7, 52.3, 55.3, 63.8, 101.0, 102.1, 110.2, 110.8, 116.8, 117.3, 120.1, 125.2, 126.0,

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129.1, 129.3, 129.5, 130.5, 130.6, 131.7, 132.4, 133.0, 136.9, 148.3, 151.3, 151.4, 156.8, 158.2, 159.2, 168.7, 196.6. ESI−MS m/z [(M − 1)$^-$]: 509.4.

Preparation of FluHMPP. 2-Picolylamine (216 mg, 2 mmol) dissolved in 2 mL of abs EtOH was added to 50 mL of abs EtOH solution of **4** (1.02 g, 2 mmol), and the reaction mixture was stirred for 5 h at room temperature. After removal of the solvent, the crude product was subjected to flash chromatography (SiO_2, petroleum ether/AcOEt 10:1, v/v) to afford FluHMPP as a yellow solid (1.08 g, 90%). Mp 80.0−81.1 °C. Anal. Calcd for $C_{37}H_{32}N_2O_6$: C, 73.98; H, 5.37; N, 4.66. Found: C, 73.83; H, 5.18; N, 4.59. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.31 (s, 3H), 3.79 (s, 3H), 3.96 (s, 3H), 4.95 (s, 2H), 5.15 (s, 2H), 6.21 (s, 1H), 6.54 (dd, J = 2.4, 8.8 Hz, 1H), 6.65−6.68 (m, 2H), 6.81 (d, J = 2.4 Hz, 1H), 6.95 (d, J = 8.8 Hz, 2H), 7.08 (s, 1H), 7.13 (d, J = 8.0 Hz, 1H), 7.17−7.22 (m, 2H), 7.30−7.38 (m, 3H), 7.69 (td, J = 1.6, 7.6 Hz, 1H), 7.80 (dd, J = 0.8, 7.6 Hz, 1H), 8.51 (s, 1H), 8.59 (d, J = 4.8 Hz, 1H), 13.55 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.4, 37.7, 52.2, 55.3, 64.6, 65.0, 101.0, 102.1, 110.1, 110.9, 116.9, 117.0, 118.2, 121.9, 122.3, 124.2, 126.0, 127.6, 129.3, 129.4, 130.5, 130.5, 131.3, 131.8, 132.4, 132.6, 136.8, 148.3, 149.4, 151.3, 151.4, 156.5, 157.9, 158.5, 159.1, 166.7, 168.7. ESI−MS m/z [(M + 1)$^+$]: 601.4.

Synthesis of [Cu^{II}(MPP−NO$_2$)NO$_3$]$_2$. A 10 mL methanol solution of $Cu(NO_3)_2{\cdot}3H_2O$ (0.0121 g, 0.05 mmol) was added to a magnetically stirred 10 mL acetonitrile solution of FluHMPP (0.0300 g, 0.05 mmol). The mixture was stirred in air for 1 day whereby a yellow-green solution was formed. It was filtered and kept in air. Dark green rhombic single crystals of $[Cu^{II}(MPP{-}NO_2)NO_3]_2$ suitable for X-ray crystallography were obtained on slow evaporation of the filtrate within 5 days. Anal. Calcd for $C_{28}H_{24}Cu_2N_8O_{12}$: C, 42.48; H, 3.06; N, 14.15. Found: C, 42.65; H, 3.18; N, 13.89. HRESI−MS m/z [Cu^{II}(MPP−NO$_2$)$^-$]$^+$: 333.0177.

Determination of the Yield of the C−O Bond Cleavage Reaction. A 6 mL methanol solution of $Cu(NO_3)_2{\cdot}3H_2O$ (0.0121 g, 0.05 mmol) was added to a magnetically stirred 5 mL acetonitrile solution of FluHMPP (0.0300 g, 0.05 mmol). The mixture was stirred in dark for 2 h in air. After removal of the solvent, the crude product was subjected to flash chromatography (SiO_2, CH_2Cl_2/CH_3OH 50:1, v/v) to afford 3′-*O*-methylfluorescein methyl ester (MFME) as a yellow solid (14.5 mg, 80.5%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.64 (s, 3H), 3.92 (s, 3H), 6.46 (d, J = 1.6 Hz, 1H), 6.54 (dd, J = 1.6, 9.6 Hz, 1H), 6.74 (dd, J = 2.4, 9.2 Hz, 1H), 6.85 (d, J = 9.6 Hz, 1H), 6.89 (d, J = 9.2 Hz, 1H), 6.96 (d, J = 2.4 Hz, 1H), 7.31 (dd, J = 1.2, 7.6 Hz, 1H), 7.67 (td, J = 1.2, 8.0 Hz, 1H), 7.74 (td, J = 1.2, 7.6 Hz, 1H), 8.25 (dd, J = 1.2, 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 52.5, 56.1, 100.5, 105.9, 113.5, 115.0, 117.7, 129.0, 129.8, 130.0, 130.3, 130.5, 130.7, 131.3, 132.8, 134.8, 150.3, 154.4, 159.1, 164.2, 165.7, 185.8. ESI−MS m/z [(M + 1)$^+$]: 361.1.

Fluorescence Spectroscopy. Fluorescence spectra were recorded on a Hitachi F-4500 spectrofluorometer equipped with a xenon lamp as the excitation source. To reduce the fluctuation in the excitation intensity during measurement, the lamp was kept on for 1 h prior to the experiment. The path length was 1 cm with a cell volume of 3.0 mL. Fluorescence responses of FluHMPP to various metal ions were measured as follows. FluHMPP (final, 10 μM) was added to 1 mL of Tris−HCl (10 mM, pH 7.20), H_2O-CH_3CN (7:3, v/v), then aqueous solution of transition metal ions (NaCl, KCl, $MgCl_2$, $CaCl_2$, $Cr(ClO_4)_3$, $Co(ClO_4)_2$, $Ni(NO_3)_2$, $FeCl_3$, $Cu(NO_3)_2$, $ZnCl_2$, $Cd(NO_3)_2$, $Al(NO_3)_3$ and $Hg(ClO_4)_2$) or $[Cu(CH_3CN)_4]BF_4$ in CH_3CN were used to give a final concentration of 1 mM s- and 0.2 mM d-block metal ions. In the same way, aqueous solutions of various copper salts ($Cu(NO_3)_2$, $Cu(ClO_4)_2$, $CuSO_4$, $Cu(AcO)_2$, $Cu(CF_3SO_3)_2$, and $CuCl_2$) were used to detect the influence of the anions. The mixtures were kept in the dark for 2 h. All fluorescence spectra were measured with an excitation wavelength at 450 nm, and emission spectra were collected from 470 to 700 nm.

Product Analysis. For ESI−MS analysis, a 0.2 mM solution of $Cu(NO_3)$ in CH_3OH was added to a 10 μM FluHMPP solution in 1 mL of Tris−HCl (10 mM, pH 7.20), H_2O-CH_3CN (7:3, v/v) under aerobic conditions. The mixture was stirred for 2 h at room temperature. The samples were directly measured using ESI-TOF. For HPLC analysis, the above samples were analyzed with a reverse phase HPLC (Varian-ProStar system, 4.6 × 250 mm, Diamonsil C^{18} 5 μ) eluted with CH_3CN/H_2O. The retention time was compared to that of an authentic sample of 3′-*O*-methylfluorescein methyl ester (MFME).

Cell Culture and Fluorescence Microscopic Imaging. The Hela cell lines were provided by the Institute of Biochemistry and Cell Biology (China). Cells were grown in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum in an atmosphere of 5% CO_2 and 95% air at 37 °C humidified air for 24 h. One day before imaging, the cells were passaged and plated in phenol red-free medium on 14 mm glass coverslips in 12-well plates. Copper uptake was performed in the same medium with supplementation with $CuCl_2$ at 100 μM for 1 h. It was washed twice with PBS buffer, and then a solution of FluHMPP in CH_3CN (1 mM) was diluted into DMEM at 20 μM, added to the cells, and incubated for another 4 h. Before analysis, the coverslips were removed from the 12-well plate and plated on a glass slide. Next, fluorescence microscopic images were acquired. Excitation of loaded cells at 480 nm was carried out with a He−Ne laser. The confocal microscopic optical setup was in multichannel mode. All confocal images were collected with a Zeiss Leica inverted epifluorescence/reflectance laser scanning confocal microscope.

X-ray Diffraction Studies. Single-crystal X-ray diffraction measurements were carried out on a Bruker APEX-II CCD diffractometer operating at 50 KV and 30 mA using Mo Kα radiation (λ = 0.71073 Å). Data collection and reduction were performed using SMART and SAINT software.[15] An empirical absorption correction was applied using the SADABS program.[16] The structure was solved by direct methods and refined by full-matrix least-squares on F^2 using the SHELXL-97 program package.[17]

Crystal data and details of the structure determination for $[Cu^{II}(MPP{-}NO_2)NO_3]_2$ are summarized in Supporting Information Table S1. CCDC 910759 contains the supplementary crystallographic data for this Article. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi.

RESULTS AND DISCUSSION

Synthesis of Complexes. The synthesis of FluHMPP is shown in Scheme 1. **3** was synthesized via reaction of **2** with **1** using anhydrous potassium carbonate in refluxing CH_3CN. Removal of allyl of **3** using palladium catalyst followed by condensation with 2-picolylamine afforded FluHMPP. The structure of FluHMPP was confirmed by elemental analysis, ^{1}H NMR, ^{13}C NMR spectroscopies, and ESI mass spectrometry. In addition, complex $[Cu^{II}(MPP{-}NO_2)NO_3]_2$ was also obtained. Oxidation products were confirmed by ESI−MS, HPLC, and X-ray diffraction measurement.

Fluorescence Response and Product Analysis. Our research on a new Cu^{II} turn-on system is constructed of a nonfluorescent nonconjugated fluorescein platform and a receptor moiety (HMPP). Cu-mediated oxidative reaction renews the conjugated form of fluorescein via removal of the blocking receptor and thereby turns on the fluorescent signal of the probe. All fluorescence properties of FluHMPP were evaluated in Tris−HCl (10 mM, pH 7.20), H_2O-CH_3CN, (7:3, v/v). Predictably, FluHMPP has negligible fluorescence because the xanthene part of FluHMPP adopts a non-conjugated form. We studied the fluorescent changes of 10 μM FluHMPP by titration with various concentrations of Cu^{II} in air (Supporting Information Figure S1). An obvious enhancement of more than 30-fold in fluorescence intensity was observed with addition of 0.2 mM Cu^{II} within 2 h (Figure 1). HPLC (Supporting Information Figure S3) and ESI mass

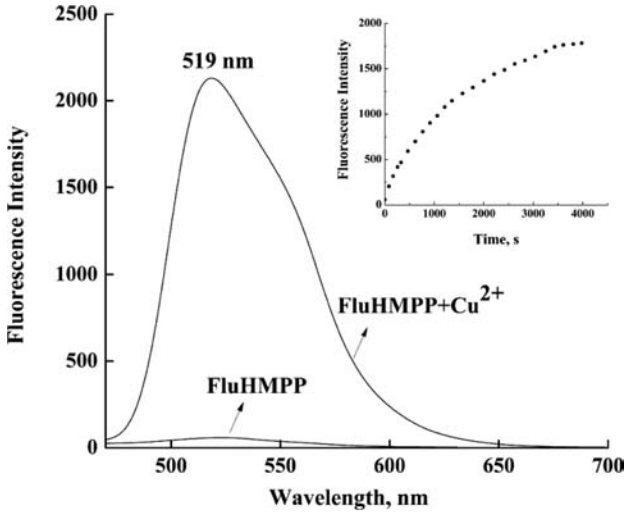

Figure 1. Fluorescence response of 10 μM FluHMPP before and after 2 h of reaction with 0.2 mM $Cu(NO_3)_2$ in Tris−HCl (10 mM, pH 7.20), H_2O-CH_3CN, (7:3, v/v). (Inset) Time course of the fluorescence intensity change at 519 nm in the reaction of FluHMPP (10 μM) and $Cu(NO_3)_2$ (0.2 mM) under aerobic conditions. The excitation wavelength was 450 nm.

spectrometry (Supporting Information Figure S4) analysis of the reaction mixture revealed that there is a new product, 3′-*O*-methylfluorescein methyl ester (MFME). This indicates that the benzyl ether C−O bond of FluHMPP was cleaved, and the resulting 2-(3-hydroxy-6-methoxy-9*H*-xanthen-9-yl)benzoic acid methyl ester was easily air-oxidized to afford MFME. Meanwhile, the ESI mass spectrum of the reaction mixture revealed a peak at m/z 436.2 (Supporting Information Figure S4) with isotope distribution pattern calculated for the Cu^{II} complex having an oxidized ligand of HMPP, $[Cu^{II}(MPP-COO)(CH_3CN)_2] + Na^+$. This suggests that the NNO imine part has been oxidized to the carboxylate-containing derivative, HMPP−COOH.

Crystal Structure of $[Cu^{II}(MPP-NO_2)NO_3]_2$ and Mechanism Research. The structure of the Cu^{II} complex with MPP−NO_2 is shown in Figure 2. Selected bond lengths and bond angles are given in Supporting Information Table S2. It

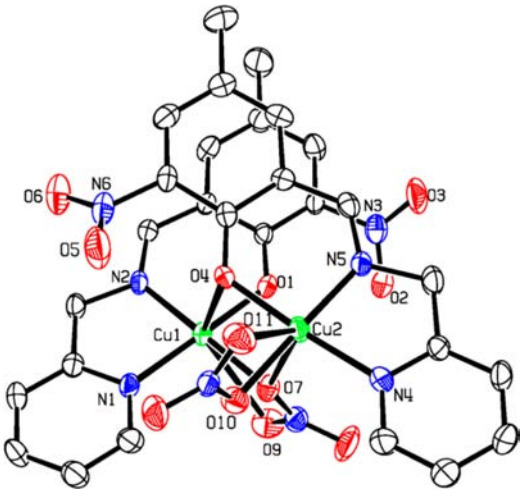

Figure 2. Thermal ellipsoid (30% probability level) plot of $[Cu^{II}(MPP-NO_2)NO_3]_2$. All hydrogen atoms were omitted for clarity.

crystallizes in the monoclinic system, with space group $P2_1/c$ from acetonitrile and methanol. The asymmetric unit contains two $(MPP-NO_2)^-$, two Cu^{II} ions, and two NO_3^-. A phenolic oxygen atom bridges two Cu^{II} ions, and a NO_3^- also bridges two Cu^{II} ions in μ_2-η^1:η^2 mode. However, when $Cu(ClO_4)_2$ was added to the reaction system instead of $Cu(NO_3)_2$, we acquired colorless crystals of $[Cu(CH_3CN)_4](ClO_4)$ previously reported.[18] These results indicate the formation of Cu^I during the reaction process. The possible mechanism we are considering is that Cu^{II} coordinates and reacts with the NNO receptor of FluHMPP, resulting in a transient Cu^I-complex (Supporting Information Figure S5),[19] and then Cu^I catalyzes benzyl ether C−O bond cleavage to form $[Cu^{II}(MPP-COOH)]^+$ and MFME with assistance of O_2 through an intramolecular mechanism similar to that reported in Taki's and Suzuki's systems.[12,20] Elimination of the carboxyl group from $[Cu^{II}(MPP-COOH)]^+$ followed by nitration affords $[Cu^{II}(MPP-NO_2)]^+$. Supporting Information Figure S6 shows a possible mechanism for the formation of $[Cu^{II}(MPP-NO_2)]^+$. Cu(II) ions play a significant catalytic role in the decarboxylation and nitration procedures.[21] The yield of C−O bond cleavage was 80.5%. In the Cu system reported by Taki, the fluorescence response had to be performed in the reducing environment provided by GSH. However, our system showed no fluorescence response for both Cu^{II} and Cu^I in the presence of GSH within 2 h (Supporting Information Figures S7 and S8).

Metal-Ion Selectivity and Effect of pH. The fluorescence selectivity of 10 μM FluHMPP was studied in the presence of 1 mM s- and 0.2 mM d-block metal ions after 2 h of mixing in air. Physiologically important metal ions such as Na^+, K^+, Fe^{3+}, and Zn^{2+} had no or negligible interference in the fluorescence of FluHMPP (Figure 3). Because of the formation of $[Cu(CH_3CN)_4]^+$, Cu^I cannot be quickly oxidized to Cu^{II}, so it showed a slightly enhanced fluorescence of FluHMPP in test time. However, Cu^I could also induce observable fluorescence enhancement if the reaction time was extended (Supporting Information Figure S9). The anion responses to the detection systems were further investigated. These results show that various kinds of anions Cu^{II} salts could cause fluorescence enhancement of FluHMPP (Scheme 2 and Supporting

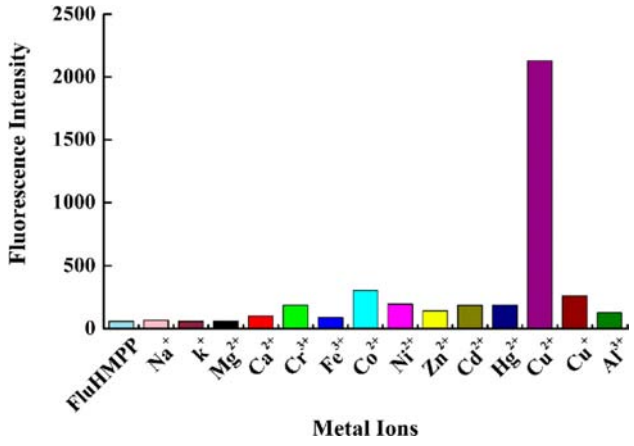

Figure 3. Metal ion selectivity of FluHMPP. The bars represent the fluorescence intensity at 519 nm after 2 h of reaction of 10 μM FluHMPP with each type of metal ion (1 mM s- and 0.2 mM d-block metal ions) in Tris−HCl (10 mM, pH 7.20), H_2O-CH_3CN, (7:3, v/v). The excitation wavelength was 450 nm.

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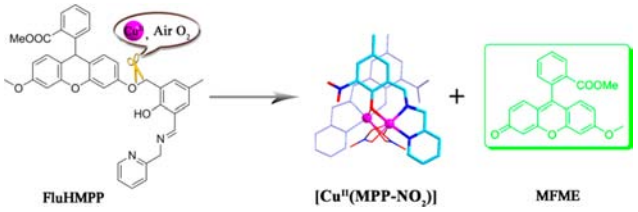
Scheme 2. Mechanism of Fluorescence Enhancement of FluHMPP Induced by Cu^{II}



Information Figure S10). In addition to excellent selectivity, it is essential that the probe can be operated in the physiological pH range for biological applications. Therefore, we investigated the pH effect on the fluorescence response of FluHMPP in the presence of Cu^{II}. As was seen from Figure 4, FluHMPP was

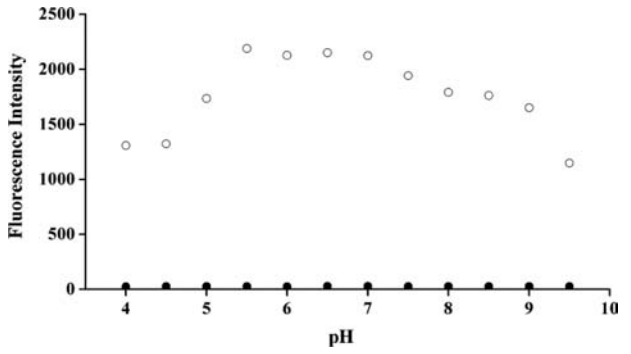


Figure 4. Effect of pH on the fluorescence intensity at 519 nm of FluHMPP (●) and of FluHMPP (10 μM) reacted with $Cu(NO_3)_2$ (0.2 mM) after 2 h (○) in Tris–HCl (10 mM, pH 7.20), H_2O–CH_3CN, (7:3, v/v) respectively. The excitation wavelength was 450 nm.

stable within the pH range of 4.0–9.5. On the other hand, it readily reacted with Cu^{II} within the biologically relevant pH range (5.5–7.5), indicating that FluHMPP possesses high sensing ability in living cells without interference from pH effects.

Fluorescence Images. Because of the favorable properties of FluHMPP in vitro, we investigated a practical application of FluHMPP, and bioimaging for Cu^{II} was examined in living cells using confocal fluorescence microscopy (Supporting Information Figure S11). HeLa cells treated with 20 μM FluHMPP alone in the growth medium for 4 h at 37 °C exhibited no fluorescence (Supporting Information Figure S11a and S11b). However, supplementing the cells with 100 μM of $CuCl_2$ in the growth medium for 1 h, followed by washing twice with PBS buffer prior to FluHMPP staining, results in a significant increase in the observed intracellular fluorescence (Supporting Information Figure S11d and S11e). Obvious changes indicated that FluHMPP was cell membrane permeable and capable of detecting Cu^{II} ions in the living cells.

CONCLUSIONS

We have synthesized and evaluated FluHMPP, a novel reaction-based probe for selective turn-on fluorescence detection of Cu^{II} in both aqueous media and living cells under visible light excitation. The results obviously suggested that only Cu^{II} could cause oxidative benzyl ether C–O bond cleavage via metal binding assisted by O_2. Moreover, single-crystal X-ray crystallographic analysis further provided strong support for our speculation about debenzylation and that in related literature.[12] Ongoing efforts are underway in our group to exploit the design strategy based on reaction to detect a variety of other substances in biological studies.

ASSOCIATED CONTENT

Supporting Information

X-ray crystallographic data of [Cu^{II}(MPP–NO_2)NO_3]$_2$ in CIF format, characterization, and additional spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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