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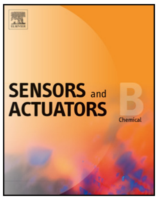

# New aliphatic and aromatic dialdehyde bridged turn-on probes for hypochlorite detection in biological samples based on bis(fluorescein)

Zheng Yang[a,1], Mengjia Wang[a,1], Mengyao She[a], Bing Yin[a,c], Yangyang Huang[a],
Ping Liu[a], Jianli Li[a,*], Shengyong Zhang[a,b]

[a] Ministry of Education Key Laboratory of Synthetic and Natural Functional Molecule Chemistry, College of Chemistry & Materials Science, Northwest University, Xi'an 710069, Shaanxi, PR China
[b] School of Pharmacy, Fourth Military Medical University, No.169, Changle West Road, Xi'an 710032, Shaanxi, PR China
[c] College of Chemistry, Beijing Normal University, No. 19, XinJieKouWai Street, HaiDian District, Beijing 100875, PR China

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## ABSTRACT

The design, synthesis and photo-physical properties of two novel dialdehyde bridged turn-on fluorescent probes with dual C=N moiety based on bis(fluorescein) for $OCl^-$ detection with high selectivity and sensitivity were reported. The well-defined linear plots between 5.0 μM and 500.0 μM of $OCl^-$ make the probes especially suitable for the quantification of $OCl^-$. The ring-opening progress was investigated both experimentally and computationally. Fluorescent imaging for $OCl^-$ in living MG-63 cells have also been successfully performed.

## 1. Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have gained significant scientific interest because of their crucial roles in a wide range of physiological processes [1,2]. Among the known ROS associated with biological processes, hypochlorous acid (HOCl), usually existing as hypochlorite ion ($OCl^-$) at physiological pH, has attracted much attention due to its critical role in water treatment and in the immune system [3]. The importance of hypochlorite is reflected by the fact that abnormal levels of hypochlorite have been implicated in a series of human diseases, such as cardiovascular diseases, damage of human red blood cells, neuron degeneration, lung injury, kidney disease, and cancer [4–7]. Hence, the development of smart, technologically innovative, and clinically applicable diagnostic highly selective and sensitive chemical probes for the detection of $OCl^-$ in environmental and biological systems is of particular importance [8–14].

Fluorescence has been widely used in imaging because it is a highly sensitive and non-destructive technique, with high spatial resolution when combined with microscopy [15–18]. By virtue of the unique photophysical properties such as long wavelength excitation and emission profiles, large extinction coefficient and high fluorescence quantum yields, probes derived from xanthene-based fluorophores have received a great deal of attention [19]. Recently, on the basis of ring-opening mechanism of the spirolactam or spirolacton, a number of xanthene derivatives have been utilized for the detection of biological relevant ions and bio-molecules [20–23]. Furthermore, Schiff functional group with C=N moiety has been proven to be an ideal receptor with significant attributes for metal ions and ROS including $Cu^{2+}$, $Hg^{2+}$, $Fe^{3+}$, NO and $OCl^-$ [24–29]. In combination with a fluorescence probe, it is believed to be highly suitable for fluorescent recognition and have the potential to improve the selectivity and the fluorescent properties significantly.

Herein in this paper, we report the design, synthesis and photophysical characterization of two novel dialdehyde bridged turn-on fluorescent probes with Schiff functional group for $OCl^-$ detection based on bis(fluorescein) (Scheme 1). High selectivity and sensitivity are observed over other ions or ROS for both probes and the fluorescent changes are shown to be mainly due to the spirolacton ring-opening power of $OCl^-$, which was reasonably proved by combined experimental and computational investigation.

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[*] Corresponding authors. Tel.: +86 029 88302604; fax: +86 29 88302601.
*E-mail addresses:* liuping@nwu.edu.cn (P. Liu), lijianli@nwu.edu.cn, chem311@nwu.edu.cn (J. Li).
[1] Contributed equally to this work.

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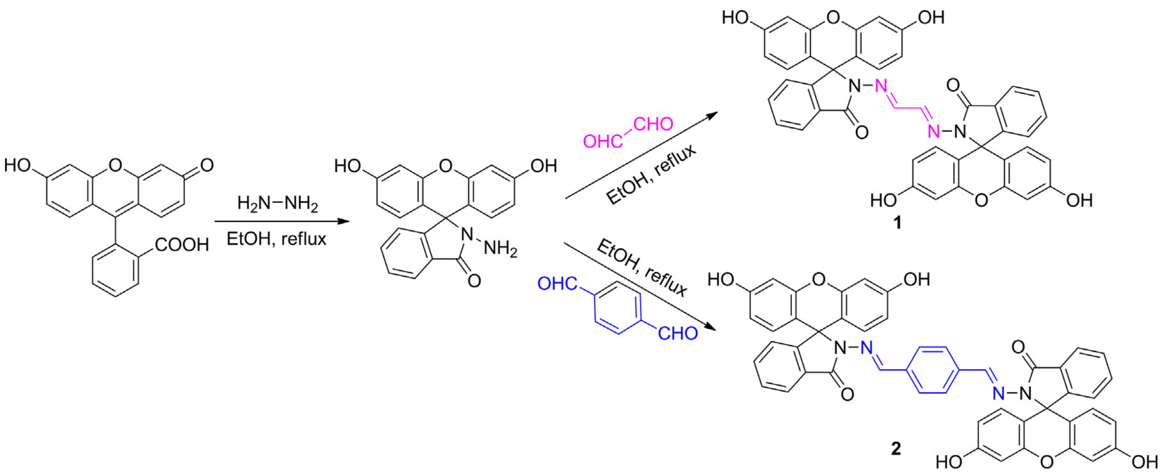

**Scheme 1.** Synthesis of the probes.

Furthermore, the wonderful properties make the probes well suitable for fluorescence imaging of $OCl^-$ in living cells.

## 2. Experimental

### 2.1. General methods and reagents

Elemental analyses were measured on a Vario EL III analyzer. The IR spectra were performed on the Bruker Tensor 27 spectrometer. NMR spectra were recorded on a Varian Inova-400 MHz spectrometer. High resolution mass spectra were performed on a Bruker micrOTOF-Q II ESI-Q-TOF LC/MS/MS Spectroscopy. X-ray crystal data were collected on a Bruker Smart APEX II CCD diffractometer. Fluorescent detections were performed on a Hitachi F-4500 fluorescence spectrophotometer. The cell imaging experiments were performed on an Olympus FV1000 confocal microscope.

### 2.2. Synthesis

#### 2.2.1. Synthesis of fluorescein hydrazide

Fluorescein (16.60 g, 0.05 mol) was dissolved in 250 mL ethanol solution and then 30.0 mL of hydrazine hydrate was added dropwise within 30 min. The reaction mixture was refluxed for 24 h until the fluorescence of the solution disappeared. The solvent was then evaporated under reduced pressure and recrystallized from ethanol to get fluorescein hydrazide as a white powder (15.58 g, yield: 90%), mp: 283–284 °C. Anal. Calcd. for $C_{20}H_{14}N_2O_4$: H: 4.07, C: 69.36, N: 8.09. Found: H: 4.05, C: 69.39, N: 8.08. $^1$H NMR (400 MHz, DMSO-d$_6$, TMS): δ: 9.82 (s, 2*H*), 7.78 (d, *J* = 6.2 Hz, 1*H*), 7.48 (s, 2*H*), 6.99 (d, *J* = 7.0 Hz, 1*H*), 6.59 (s, 2*H*), 6.43 (dd, *J* = 20.9, 8.6 Hz, 4*H*). $^{13}$C NMR (100 MHz, DMSO-d$_6$, TMS): δ: 165.8, 158.4, 152.6, 151.7, 132.8, 129.5, 128.3, 123.6, 122.6, 112.2, 110.1, 102.6 and 64.9. IR (KBr, $\upsilon\,cm^{-1}$): 3310, 1691, 1612, 1506, 1468, 1368, 1320, 1238, 1182, 1111, 1044, 995, 843, 758 and 690. MS (ESI) *m*/*z* = 345.0966 [M–H]$^-$, Calc. for $C_{20}H_{14}N_2O_4$ = 346.0954. Crystal data and structure refinement for fluorescein hydrazide: [$C_{20}H_{14}N_2O_4$]; *Mr* = 346.33; size: 0.30 × 0.29 × 0.27 mm$^3$; Monoclinic; *T* = 296(2) K; space group *P*2(1)/*n*; *a* = 11.300(3), *b* = 9.988(3), *c* = 19.645(5) Å; $\alpha$ = 90.00°, $\beta$ = 94.865(4)°, $\gamma$ = 90.00°; *V* = 2209.2(10) Å$^3$; *Z* = 4; *Dc* = 1.041 Mg m$^{-3}$, $\mu$ = 0.074 mm$^{-1}$; *F*(0 0 0) = 720; reflections collected = 12977; independent reflections = 5307 ($R_{int}$ = 0.0273); full-matrix least-squares refinement on $F^2$; goodness-of-fit on $F^2$ = 1.055; final $R_1$ = 0.0723 ($I > 2\sigma(I)$), $wR_2$ = 0.2250 ($I > 2\sigma(I)$); *R* indices (all data) $R_1$ = 0.0832 (all data), $wR_2$ = 0.2344 (all data); Largest diff. peak and hole = 0.644 and −0.615 eÅ$^{-3}$.

#### 2.2.2. Synthesis of 1

Fluorescein hydrazine (3.46 g, 0.01 mol) and the oxalaldehyde (0.29 g, 0.005 mol) were mixed in 50 mL ethanol. The reaction mixture was refluxed for 12 h. After cooling to the room temperature, the solvent was evaporated and then recrystallized from ethanol to get the white powder (2.95 g, yield: 72%). mp: more than 300 °C. Anal. Calcd. for $C_{42}H_{26}N_4O_8$: H: 3.67, C: 70.58, N: 7.84. Found: H: 3.70, C: 70.59, N: 7.85. $^1$H NMR (400 MHz, DMSO-d$_6$, TMS): δ: 9.99 (s, 4*H*), 8.08 (s, 2*H*), 7.87 (d, *J* = 7.4 Hz, 2*H*), 7.56 (dt, *J* = 23.8, 7.4 Hz, 4*H*), 7.02 (d, *J* = 7.5 Hz, 2*H*), 6.65 (s, 4*H*), 6.43 (q, *J* = 8.7 Hz, 8*H*).$^{13}$C NMR (100 MHz, DMSO-d$_6$, TMS): δ: 164.2, 158.8, 151.5, 145.5, 134.7, 129.2, 127.9, 127.1, 123.6, 112.6, 109.1, 102.8 and 64.9. IR (KBr, $\upsilon\,cm^{-1}$): 3356, 2608, 1727, 1607, 1468, 1325, 1292, 1258, 1206, 1181, 1108, 1044, 995, 972, 914, 872, 849, 798, 754, 719, 688 and 634. MS (ESI) *m*/*z* = 713.1829 [M–H]$^-$, Calc. for $C_{42}H_{26}N_4O_8$ = 714.1757. Crystal data and structure refinement for **1**: [$C_{50}H_{48}N_6O_{12}$]; *Mr* = 924.94; size: 0.37 × 0.26 × 0.21 mm$^3$; monoclinic; *T* = 296(2) K; space group *P*2(1)/*n*; *a* = 10.2598(11), *b* = 20.869(2), *c* = 11.7979(12) Å; $\alpha$ = 90.00°, $\beta$ = 114.757°, $\gamma$ = 90.00°; *V* = 2293.9(4) Å$^3$; *Z* = 2; *Dc* = 1.139 Mg m$^{-3}$, $\mu$ = 0.097 mm$^{-1}$; *F*(0 0 0) = 972; reflections collected = 11438; independent reflections = 4083 ($R_{int}$ = 0.0275); full-matrix least-squares refinement on $F^2$; goodness-of-fit on $F^2$ = 0.971; final $R_1$ = 0.0457 ($I > 2\sigma(I)$), $wR_2$ = 0.1190 ($I > 2\sigma(I)$); *R* indices (all data) $R_1$ = 0.0648 (all data), $wR_2$ = 0.1448 (all data); Largest diff. peak and hole = 0.277 and −0.275 eÅ$^{-3}$.

#### 2.2.3. Synthesis of 2

Probe **2** was synthesized in 75% yield using a similar method to the synthesis of 1. mp: more than 300 °C. Anal. Calcd. for $C_{48}H_{30}N_4O_8$: H: 3.82, C: 72.90, N: 7.09. Found: H: 3.81, C: 72.92, N: 7.09. $^1$H NMR (400 MHz, DMSO-d$_6$, TMS) $\delta$: 9.92 (s, 4H), 8.95 (s, 2H), 7.92 (d, *J* = 7.3 Hz, 2H), 7.62 (dt, *J* = 19.3, 7.3 Hz, 4H), 7.39 (s, 4H), 7.13 (d, *J* = 7.4 Hz, 2H), 6.66 (s, 4H), 6.46 (dd, *J* = 20.4, 8.6 Hz, 8H).$^{13}$C NMR (100 MHz, DMSO-d$_6$, TMS): δ: 163.8, 158.6, 152.2, 150.5, 147.9, 136.1, 134.2, 129.0, 128.0, 127.2, 123.6, 112.4, 110.1, 102.6 and 65.4. IR (KBr, $\upsilon\,cm^{-1}$): 3254, 1702, 1611, 1503, 1465, 1303, 1268, 1233, 1172, 1109, 994, 917, 839, 795, 758, 714, 688 and 620. MS (ESI) *m*/*z* = 789.1987 [M–H]$^-$, Calc. for $C_{48}H_{30}N_4O_8$ = 790.2064.

### 2.3. General procedure

Sensor **1** stock solution was prepared in 500 μM concentration. The solutions of ions tested are LiCl, NaCl, KCl, $MgCl_2$, $CaCl_2$, $AlCl_3$, $CrCl_3$, $MnCl_2$, $FeCl_3$, $CoCl_2$, $NiCl_2$, $CuCl_2$, $ZnCl_2$, $CdCl_2$, $SnCl_4$, $BaCl_2$, $HgCl_2$, $PbCl_2$, $Na_2SO_4$, $NaNO_3$, $Na_3PO_4$, $Na_2HPO_4$, $NaH_2PO_4$,