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A highly selective turn-on fluorescent chemodosimeter for Cr(VI) and its application in living cell imaging†

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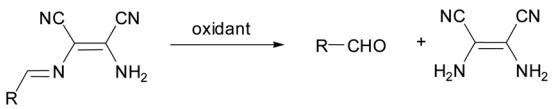
A BODIPY-based fluorescent probe, functionalized with an aminomaleonitrile was synthesized as a "turn-on" fluorescent chemodosimeter for Cr(VI) in aqueous solution based on the Cr(VI) promoted oxidation reaction. Moreover, it can fluorescently respond to Cr(VI) in living cells.

Chromium(VI) is an environmental pollutant and is universally known to promote some diseases such as damage exposed skin, irritate mucous membranes, produce pulmonary sensitivity, create dental erosion, cause loss of weight, induce renal damage, and target the respiratory tract and skin due to its highly carcinogenic and mutagenic properties.[1] In addition, experimental evidence links chromium(VI) with various types of cancer.[2] The increased hazardous status is attributed to the high oxidation potential and the correlation between oxygen consumption and single strand breakage (SSB) in DNA by Cr(VI) suggests the participation of an active oxygen-coordinated chromium species in this process.[3] World Health Organisatin (WHO) recommends chromium(VI) to be limited to 0.05 μg L^{-1} (0.17 μM) within groundwater and Cr(VI) is one of six materials whose uses are regulated by the RoHS directive of European Union.[4,5] Therefore, the accurate determination of Cr(VI) at trace level is important in the field of environmental science and industry. Up to date, there have been many detection methods developed for Cr(VI) analysis, such as FAAS[6] or ET-AAS,[7] ICP-AES,[8] ICP-MS,[9] colorimetric method,[10] fluorescence,[1,11,12] chemiluminesence,[13] electrochemical method,[14–25] SERS,[26] X-ray fluorescence (XRF),[27] *etc.*

Among these methods, fluorescent sensing appeared to be the most attractive method for detecting Cr(VI) with the distinct advantages of high sensitivity, selectivity and easy operation. However, there have been only few fluorescent probes for the facile detection of Cr(VI). Furthermore, most of the reported fluorescent Cr(VI) probes are based on a fluorescence quenching mechanism and suffer from at least one undesirable limitation, such as limited selectivity, low sensitivity, poor water solubility, use of harmful organic solvents, poor detection limit or narrow useable pH range.[1,11,12] In general, the fluorescence turn-on response for detecting metal ions is highly preferable in practical applications because the fluorescent turn-off response can experience interference by other external factors. Therefore, the exploration of new fluorescent turn-on probes for analyzing Cr(VI) with appropriate sensitivity, high selectivity remains a challenge. One alternative strategy to achieve fluorescence turn on involves the use of reaction-based indicator systems, chemodosimeters, and has attracted a great deal of attention.[28] Recently, more and more borondipyrromethene (BODIPY) derivatives have been widely employed as chemoprobes for detecting ions owing to their remarkable photophysical properties, such as high molar extinction coefficients, high fluorescence quantum yields, excellent photostability, easy structural modification and appropriate redox potential.[29–32]

In this communication, we demonstrated a novel BODIPY based turn-on fluorescent chemodosimeter integrated with diaminomaleonitrile unit for the detection of Cr(VI) in aqueous solution. It is known that de-diaminomaleonitrile reaction can be proceeded by an oxidant (Scheme 1).[33,34] Thus we hypothesized that incorporation of formyl-BODIPY with diaminomaleonitrile can generate a new potential probe for an oxidant.



Scheme 1 De-diaminomaleonitrile leading to aldehyde by an oxidant.

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Chemoprobe **1** was readily obtained in two steps (Scheme 2). The intermediate, 2-formyl-BODIPY **3**, was prepared on the basis of a known procedure using the classical Vilsmeier reaction.[35] Reaction of intermediate **3** with diaminomaleonitrile afforded compound **1** in 56% yield. The structure of the final product **1** was confirmed by NMR, and HRMS analysis (See ESI†).

The sensing behaviour of **1** was investigated by fluorescence measurements with different anions *i.e.*, F^-, Cl^-, Br^-, I^-, OAc^-, $C_2O_4^{2-}$, NO_3^-, CO_3^-, HCO_3^-, PO_4^{3-}, SO_4^{2-}, HSO_4^-, ClO^-, ClO_4^-, S^{2-}, OH^-, $Cr_2O_7^{2-}$, SO_3^{2-} and different cations *i.e.*, Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Al^{3+}, Pb^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cr^{3+}, Ag^+, Cd^{2+}, Hg^{2+}, and the oxidants such as Mn^{7+}, Ce^{4+} and H_2O_2 (Fig. 1). Considering that probe **1** is not completely water soluble we screened various organic solvents and the combinations of DMF–H_2O. A combination of DMF–H_2O (3 : 7, v/v) proved to be highly efficient for the fluorescent sensing process. As shown in Fig 1, probe **1** displayed a rather week fluorescent emission at 507 nm. However, it became strongly fluorescent upon addition of Cr(VI). The compound **1** behaves as a "turn-on" type of fluorescence probe towards Cr(VI). Whereas other ions did not induce any discernible spectral changes. Next, the fluorescence titration of probe **1** with Cr(VI) was conducted in DMF–H_2O (3 : 7, v/v, Fig. 2). Upon incremental addition of Cr(VI) to **1** solution, the fluorescence intensity increased remarkably and a fluorescence enhancement factor of more than 4-fold at 507 nm after reaching the titration equilibrium (5 equiv.) was estimated within 5 min, displaying a quick response to Cr(VI). For practical applicability, the proper fluorescence titrations at different pH conditions (pH 4.0–10.0) were also carried out (Fig. S8, ESI†). Experimental results show that both free probe **1** and fluorescence enhancement responses to Cr(VI) are stable in the range of pH from 6.0 to 10.0. This property of probe **1**

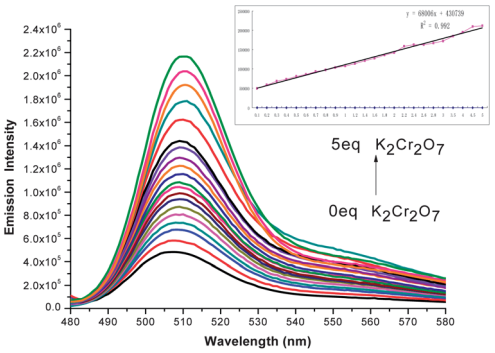
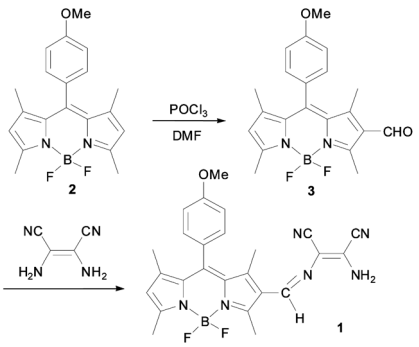


Fig. 2 The fluorescence spectra of **1** (10 μM) upon addition of $Cr_2O_7^{2-}$ (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 equiv., $K_2Cr_2O_7$) in H_2O : DMF = 3 : 7. Inset: the linear relationship of fluorescence titration.



Scheme 2 Design and synthesis of chemodosimeter **1**.

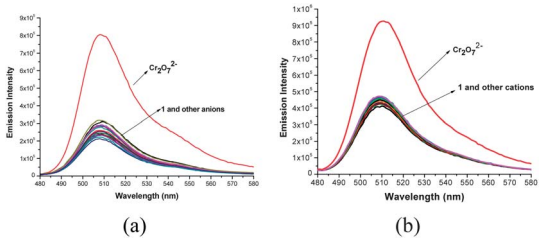


Fig. 1 (a) Changes in the fluorescence spectra of probe **1** (1 μM) upon addition of various anions (1 equiv.) ($K_2Cr_2O_7$, $Na_2C_2O_4$, $Mg(ClO_4)_2$, Na_2SO_3, NaClO, NaF, NaBr, $NaHSO_4$, $AgNO_3$, NaCl, $NaHCO_3$, Na_2CO_3, Na_2S, $NaNO_2$, NaI, Na_2SO_4, K_3PO_4, NaOAc, 1 μM) in PBS/DMF (3 : 7, v/v, pH = 6.8). (b) Changes in the fluorescence spectra of probe **1** (1 μM) upon addition of various cations and H_2O_2 ($K_2Cr_2O_7$, LiCl, NaCl, KCl, $MgSO_4$, $CaCl_2$, $Ba(NO_3)_2$, $Al(NO_3)_3$, $Pb(NO_3)_2$, $KMnO_4$, $FeCl_3$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $CuCl_2$, $Cr_2(SO_4)_3$, $AgNO_3$, $Zn(OAc)_2$, $CdCl_2$, $HgCl_2$, $(NH_4)_2Ce(NO_3)_6$, and H_2O_2, 1 μM) in PBS/DMF (3 : 7, v/v, pH = 6.8). Every drop of ion interval is 30 s. λ_{ex} = 470 nm.

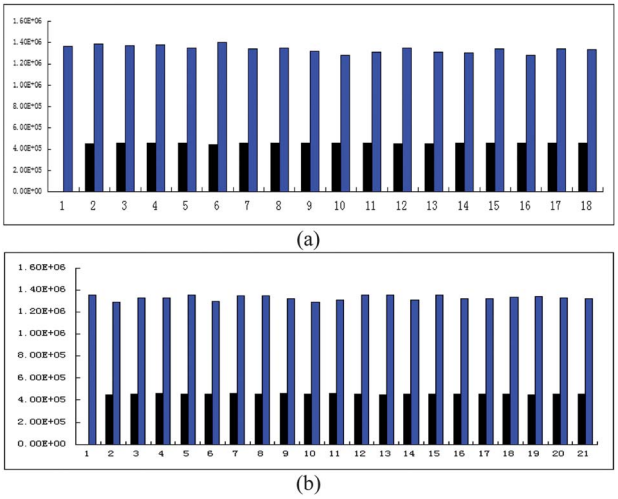


Fig. 3 (a) Fluorescence responses of probe **1** (10 μM) to various anions: $Cr_2O_7^{2-}$ only, $C_2O_4^{2-}$, ClO_4^-, SO_3^{2-}, ClO^-, F^-, Br^-, HSO_4^-, NO_3^-, Cl^-, HCO_3^-, CO_3^{2-}, S^{2-}, NO_2^-, I^-, SO_4^{2-}, PO_4^{3-}, OAc^- in H_2O : DMF (3 : 7, v/v), black bars represent addition of different anions (5 equiv.) to the solution. Blue bars represent the change of the emission that occurs upon the subsequent addition of Cr(VI) (5 equiv.) to the above solutions. Wait for 30 s each test, λ_{ex} = 470 nm. (b) Fluorescence responses of probe **1** (10 μM) to various cations: $K_2Cr_2O_7$ only, Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Al^{3+}, Pb^{2+}, $KMnO_4$, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cr^{3+}, Ag^+, Zn^{2+}, Cd^{2+}, Hg^{2+}, $(NH_4)_2Ce(NO_3)_6$, and H_2O_2, in H_2O : DMF (3 : 7, v/v), black bars represent addition of different cations (5 equiv.) to the solution. Blue bars represent the change of the emission that occurs upon the subsequent addition of Cr(VI) (5 equiv.) to the above solutions. Wait for 30 s each test, λ_{ex} = 470 nm.

suggests that no buffer solutions are required for the detection of Cr(VI), which is convenient for the practical application.

To further examine the effective applications of the chemodosimeter, the fluorescence responses of **1** to Cr(VI) in the presence of typical competing anions, cations and several oxidants such as $KMnO_4$, CAN, and H_2O_2 were studied. As shown in Fig. 3, most of competing ions only exhibited minimum interference in the detection of Cr(VI). The observation that upon treatment of Cr(VI) in the presence of different competing ions probe **1** displayed green fluorescence further supports that probe **1** is useful for selectively sensing Cr(VI) even under competition from other related ions. Moreover, as shown in Fig. 4, no observable both of color and fluorescence changes were caused for the anions and cations except for Cr(VI). This results further demonstrated the high selectivity of probe **1** for visual detection of Cr(VI) (Fig. 4, Fig. S12, ESI†).

The plausible sensing mechanism of probe **1** towards Cr(VI) can be ascribed to the de-diaminomaleonitrile reaction of the aldehyde group by Cr(VI) under mild conditions which is similar to the specific deprotection reaction promoted hypochlorite.[33,34] Furthermore, ^{1}H NMR spectroscopy was employed to provide direct evidence to confirm the assumed sensing mechanism. As shown in Fig. 5, upon treatment with Cr(VI) ion, the ^{1}H NMR spectrum of **1** solution changed. The characteristic signal corresponding to the aldehyde group proton (–CHO) emerged at 9.9 ppm and the characteristic signal corresponding to the –N=CH (H_a in Fig. 5) proton disappeared gradually. At the same time, the singlet signal corresponding to the pyrrole proton on formyl-BODIPY also appeared. at 6.4 ppm which was shifted downfield from the signal corresponding to the pyrrole proton on **1** at 6.3 ppm slightly.[35] All these results clearly indicated that the de-diaminomaleonitrile reaction occurred with the addition of Cr(VI) into the aqueous solution of **1**.

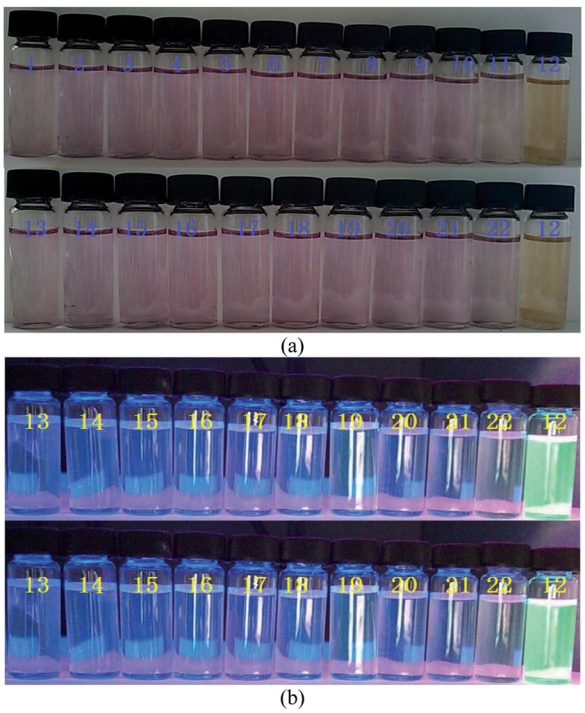


Fig. 4 (a) Color and (b) fluorescence (365 nm lamp) change of **1** (10 μM) to various cations and H_2O_2 (only **1**, LiCl, NaCl, KCl, $MgSO_4$, $CaCl_2$, $Ba(NO_3)_2$, $Al(NO_3)_3$, $Pb(NO_3)_2$, $Cr_2(SO_4)_3$, $KMnO_4$, $K_2Cr_2O_7$, $FeCl_3$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $CuCl_2$, $AgNO_3$, $Zn(OAc)_2$, $CdCl_2$, $HgCl_2$, $(NH_4)_2Ce(NO_3)_6$, and H_2O_2, 10 μM) in H_2O : DMF (3 : 7, v/v) solution.

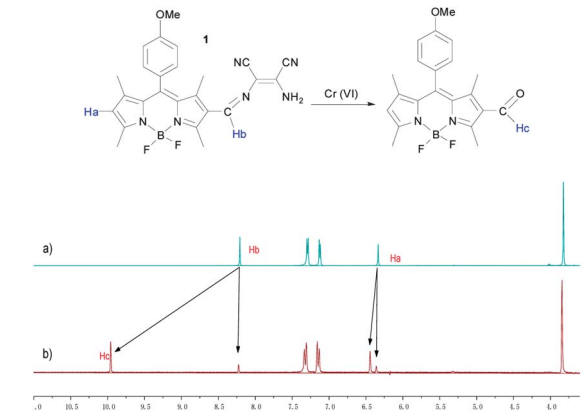


Fig. 5 Proposed sensing mechanism and partial ^{1}H NMR spectra of **1** (5 μM) in DMSO-d_6 upon addition of (a) 0, (b) 1 equiv. of $Cr_2O_7^{2-}$.

We next evaluated the potential utility of probe **1** for the fluorescence imaging of Cr(VI) in living cells. Hela cells treated with 10 μM probe **1** alone exhibited very weak background fluorescence, Fig. 6(1a–c). Whereas Hela cells treated with probe **1** and then further incubated with Cr(VI) displayed enhanced green fluorescence, Fig. 6(2a–c). These data indicate that probe **1** is cell membrane permeable and capable of fluorescent imaging of Cr(VI) in living cells.

In summary, on the basis of a specific de-diaminomaleonitrile reaction, we have demonstrated a novel strategy in designing fluorescent probes for the highly selective detection of Cr(VI). The probe **1**, which features high selectivity and pH-independency, was successfully utilized in detecting Cr(VI) in aqueous media and living cells. We hope the results present

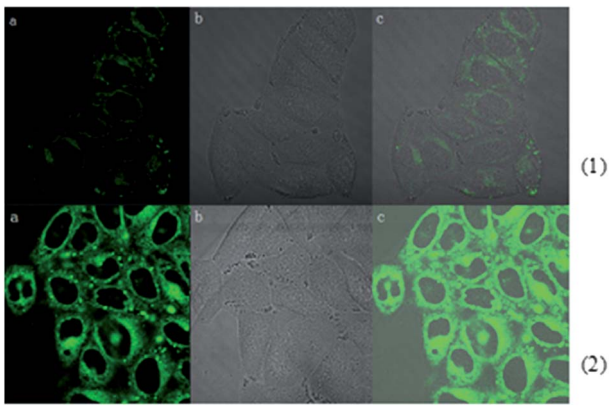


Fig. 6 Confocal fluorescence images of living Hela cells: (1a) cells loaded with 10 μM probe at 25 °C for 1 h (λ_{ex} = 488 nm; band path, 490–650 nm); (1b) bright field images; (1c) overlaid images of panels 1a and 1b; (2a) probe **1**-loaded cells with 10 μM Cr(VI) at 25 °C for 4 h (λ_{ex} = 488 nm; band path, 490–650 nm); (2b) bright field images; (2c) overlaid images of panels 2a and 2b.

here may contribute to the development of novel chemodosimeter for the fluorescence detection of Cr(VI).

Acknowledgements

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