Organic & Biomolecular Chemistry

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

A selective turn-on fluorescent probe for Cd^{2+} based on a boron difluoride β-dibenzoyl dye and its application in living cells†

Cite this: *Org. Biomol. Chem.*, 2013, **11**, 3014

Li Xin,[a,b] Yu-Zhe Chen,*[a] Li-Ya Niu,[a] Li-Zhu Wu,[a] Chen-Ho Tung,[a] Qing-Xiao Tong*[b] and Qing-Zheng Yang[a]

Received 21st February 2013,
Accepted 14th March 2013

DOI: 10.1039/c3ob40376a

www.rsc.org/obc

Herein we report the first example of a difluoroboron dibenzoyl based fluorescent probe for Cd^{2+} detection. The probe displays high selectivity and sensitivity toward Cd^{2+} over Zn^{2+} in aqueous solution under physiological conditions. Fluorescence imaging experiments demonstrate its potential application for detecting Cd^{2+} in living cells.

Introduction

Fluorophores serve as versatile tools for probing environments and living systems. The development of different fluorescent compounds as reporters for biological probes may be suitable for specific applications and benefits from the availability of structurally diverse fluorescent dyes with different spectroscopic properties. Therefore, searching for new fluorescent species as detection tools is still an important issue. Fluorescent boron difluoride dyes such as "Bodipy" and β-diketone–boron difluoride complexes are noted for their large molar absorption coefficient, intense fluorescence, insensitivity to solvent and pH, and good aqueous stability. Compared to the commercially available Bodipy, the applications of β-diketone–boron difluoride dyes are yet to be fully exploited. Studies have revealed their promising applications in biological systems,[1] organic light-emitting diodes (OLED),[2,3] *in vivo* detection of amyloid-β deposit,[4] as medium-sensitive two-photon fluorescent probes,[5] or as anion sensors with C–H···X interactions.[6] In addition to the common advantages of Bodipy, β-diketone–boron difluoride dyes have large Stokes shifts, large dipole moments, large two-photon absorption cross sections and versatile reactivities,[7–10] which made them potential candidates for constructing fluorescent chemosensors. Despite the boron dye system having been investigated infrequently since 1905, no fluorescent chemosensors for cation detection based on the boron difluoride β-dibenzoyl skeleton have been reported so far.

Environmental pollution caused by cadmium has long been a worldwide public health issue due to the fact that cadmium and its complexes are widely used and are toxic, even at low doses.[11] There is evidence of increasing cadmium content in food, which poses severe harm to human health and environment. Cadmium can accumulate in the human body and cause pulmonary cancer, renal dysfunction, calcium metabolism disorders and other relevant forms of diseases.[12–14] Therefore, there is a great need for selective detection and monitoring of the presence and concentration of cadmium ions in the environment and in biological systems. Fluorescent chemosensors[15] are of great potential for tracing cadmium distribution in cells and revealing Cd-carcinogenesis and other biological effects for living systems. However, only a few fluorescent sensors for cadmium have been reported in living cells so far.[16] Furthermore, cadmium ions usually exhibit very similar properties to zinc ions, which are the second most abundant transition metal ions in the human body. It is still a challenge to develop fluorescent chemosensors that can discriminate between cadmium and zinc ions.[17] Therefore, it is highly desirable to develop Cd^{2+}-selective sensors that can avoid the interference of Zn^{2+} and other biologically relevant metal cations.

In this report, we present a new fluorescent probe **1** based on a boron difluoride β-dibenzoyl dye for highly selective detection of cadmium over zinc ions (Fig. 1). Herein, 2,2′-dipicolylamine (DPA) is employed as a Cd^{2+} receptor. DPA has proven to be a versatile metal chelator with good affinity and high biocompatibility especially for Cd^{2+} and Zn^{2+}.[18] It is introduced into the boron difluoride β-dibenzoyl fluorescence skeleton *via* an acetyl amine group. A 2-methoxyethoxy group is introduced to the boron difluoride β-dibenzoyl compound to

[a]*Key Laboratory of Photochemical Conversion and Optoelectronic Materials, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, 29 Zhongguancun East Road, Beijing 100190, China. E-mail: chenyuzhe@mail.ipc.ac.cn*
[b]*Department of Chemistry, Shantou University, Shantou, Guangdong 515063, China. E-mail: qxtong@stu.edu.cn*

†Electronic supplementary information (ESI) available: Absorption spectra of **1** upon addition of Cd^{2+}, pH effect on the emission of **1** and Cd–**1**, calculation of the binding constant and the detection limit, solvent effect on the fluorescence, NMR and MS data of **1** and Cd–**1**. See DOI: 10.1039/c3ob40376a

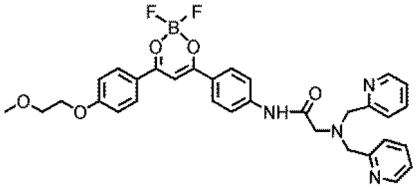


Fig. 1 Fluorescent probe **1**.

improve the water-solubility of the fluorescent sensor. We demonstrate in this contribution that boron difluoride β-dibenzoyl dyes can be successfully applied in selectively detecting cations by rational design. As expected, upon addition of Cd^{2+}, **1** shows a significant fluorescence enhancement in aqueous media. Confocal laser scanning microscopy (CLSM) experiments demonstrate that **1** could be used as a fluorescent probe for Cd^{2+} in living cells.

Results and discussion

Probe **1** was synthesized in six steps as shown in Scheme 1, with 4-amino ethylbenzoate as the starting material. It was protected by the di-*tert*-butyl dicarbonate to form **a**. Then **b**, an important intermediate, was obtained by the Claisen condensation between 1-(4-(2-methoxyethoxy)phenyl)ethanone and **a**. After reaction with bromo acetyl bromide and nucleophilic substitution of the deprotected **d** with DPA, **e** was achieved. The reaction of **e** with 1.5 equiv. of $BF_3{\cdot}OEt_2$ in CH_2Cl_2 afforded the desired product **1** in good yield. The intermediates **b–e**, which would be present in solution as their enol forms, were specified in the experimental parts.[19] The structure of **1** was confirmed by ^1H NMR, ^{13}C NMR, and HRMS data. The detailed experimental procedures and characterizations are shown in the ESI.†

The sensing performance of probe **1** towards Cd^{2+} ions was investigated in CH_3CN–HEPES buffer (1 : 1, v/v, 20 mM, pH 7.4). Fig. 2a shows the emission spectral change of **1** (5 μM) upon the addition of Cd^{2+}. The ion-free form of **1** has a weak fluorescence peak at 463 nm, while the addition of Cd^{2+} results in an obvious fluorescence enhancement, with no shifting in the excitation and emission maxima. The fluorescence quantum yield increases from 0.057 for **1** to 0.31 for Cd–**1**. The weak fluorescence of **1** was attributed to the efficient photo-

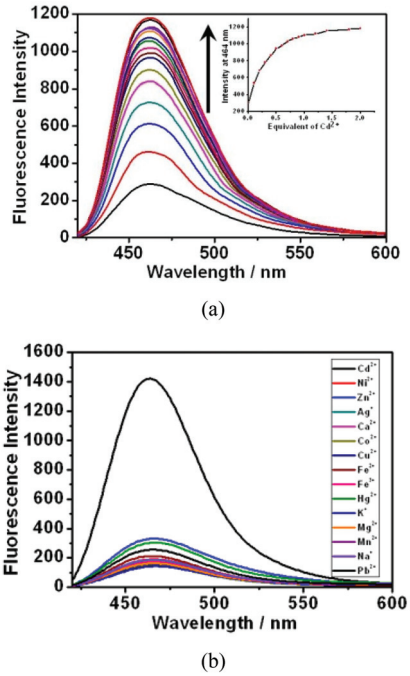
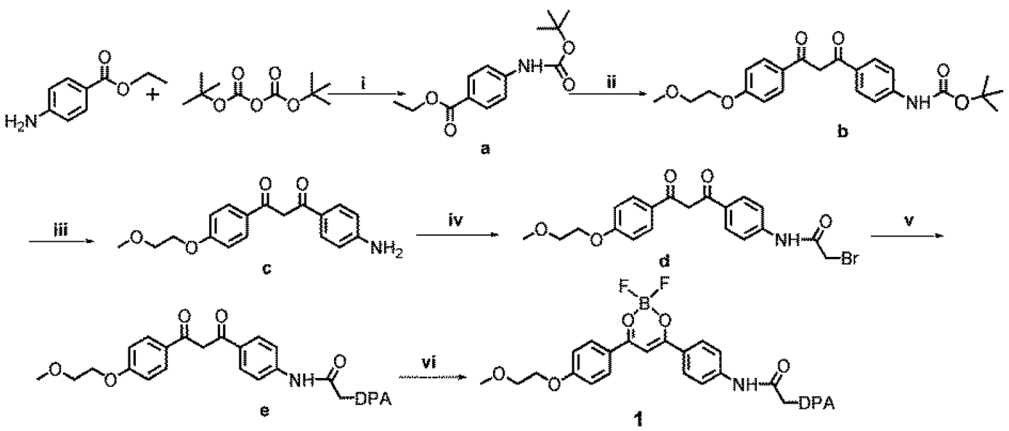


Fig. 2 (a) Changes in the fluorescence emission spectra of **1** (5 μM) in CH_3CN–HEPES buffer (1 : 1 v/v, 20 mM, pH 7.4) upon titration of Cd^{2+} (0–10 μM), λ_{ex} = 400 nm. The inset of (a) shows the relative emission intensity (I_{max} = 464 nm) *vs.* the equivalent of Cd^{2+}. (b) Fluorescence intensity change profiles of **1** (5 μM) in the presence of various cations. Metal ions (1 equiv. relative to **1**) were added in the form of $Cd(NO_3)_2$, $NiCl_2$, $CoCl_2$, $Zn(NO_3)_2$, $AgNO_3$, $CuCl_2$, $FeCl_2$, $Fe(NO_3)_3$, $HgCl_2$, $MnCl_2$, $Pb(NO_3)_2$. Others (30 equiv. relative to **1**): KCl, $CaCl_2$, NaCl, $MgSO_4$.



Scheme 1 Synthesis of probe **1**. (i) Dioxane, reflux, 24 h; (ii) 1-(4-(2-methoxyethoxy)phenyl)ethanone, NaH, THF, 0 °C → r.t., 24 h; (iii) trifluoroacetic acid (TFA), 0 °C → r.t., 3 h; (iv) bromoacetyl bromide, 4-dimethylaminopyridine (DMAP), CH_2Cl_2, 0 °C → r.t.; (v) 2,2′-dipicolylamine (DPA), K_2CO_3, KI, CH_3CN, reflux; (vi) boron trifluoride diethyl etherate ($BF_3{\cdot}OEt_2$), CH_2Cl_2, reflux.

induced electron transfer (PET) from the amino group to the fluorophore. Upon binding to Cd^{2+}, the electron-donating capability of the amino group was reduced effectively, suppressing the PET process and resulting in a considerable increase in fluorescence. To the best of our knowledge, this is the first difluoroboron dibenzoyl derivative used as a fluorophore to detect Cd^{2+} ions.

The titration experiment showed that **1** forms a 1 : 1 complex with Cd^{2+} (Fig. 2a). The ESI-MS of a mixture of **1** and $Cd(ClO_4)_2$ also reveals the formation of a 1 : 1 complex through a metal coordination interaction, where the peak at *m*/*z* 812.9 can be assigned to the species $[1 + Cd + ClO_4]^+$ (see ESI†). The binding constant ($K = 5.59 \times 10^5$ M^{-1}, $R^2 = 0.999$), which was calculated *via* a linear analysis of the fluorescence intensity *versus* the Cd^{2+} ion concentration,[20,21] suggests a strong binding ability of **1** to Cd^{2+} (Fig. 2S†). The detection limit[22,23] was estimated to be as low as 2.19×10^{-7} M ($R^2 = 0.996$) according to the titration experiment, indicating a high sensitivity of **1** to Cd^{2+} (Fig. 3S†).

The binding model of **1** towards Cd^{2+} was investigated by ^1H NMR in d_3-acetonitrile [Fig. 3, **1** and **1**–$Cd(ClO_4)_2$]. All protons were assigned referring to the literature.[24] Compared to **1**, the chemical shifts of Cd–**1** have an obvious down field shift except for H_a (from 11.48 to 9.37). It suggests that the oxygen atoms of the amide group and the DPA unit participate together in binding to a Cd^{2+} ion, which decreases proton exchange and reduces π-electron density.[25–27] The coordination of the oxygen atom further enhances the binding capacity of the probe to Cd^{2+}, and thus gives excellent selectivity. No resonance signal changes upon addition of a second equivalent of Cd^{2+}, which verifies the binding ratio of 1 : 1 as mentioned above.

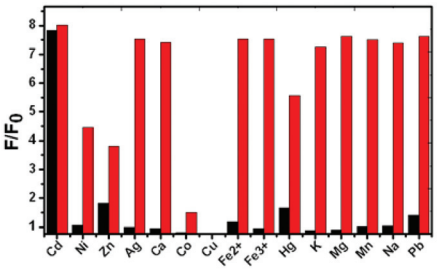


Fig. 4 The fluorescence response of **1** (5 μM) towards Cd^{2+} and other metal ions in CH_3CN–HEPES buffer (1 : 1 v/v, 20 mM, pH 7.4; λ_{ex} = 400 nm) at 464 nm at room temperature. Black bar: other competing metal ions (1 equiv.) were added to **1**. Red bar: Cd^{2+} (1 equiv.) was added to **1** containing the above metal ions (100 equiv.).

The titration of **1** with various physiologically and environmentally relevant metal ions showed excellent selectivity to Cd^{2+} (Fig. 2b and 4). Only Cd^{2+} induced a dramatic fluorescence enhancement in the emission spectrum. Cations such as Mg^{2+}, Ca^{2+}, Fe^{3+}, K^+, which are abundant in living cells, are not able to cause interference with Cd^{2+}. It is worth noting that **1** shows good selectivity for Cd^{2+} over Zn^{2+}, which is the main competitor for cadmium ion chemosensors. Common heavy and transition metal ions, such as Hg^{2+}, Pb^{2+} and Cu^{2+} induced slight enhancement or even quenching in fluorescence. The competition experiment also revealed that **1** showed turn-on fluorescence even in the presence of 100 equivalents of other competing ions with the exception of Cu^{2+} and Co^{2+}, which limited the turn-on Cd^{2+} response of **1** as reported elsewhere[28,29] (Fig. 4).

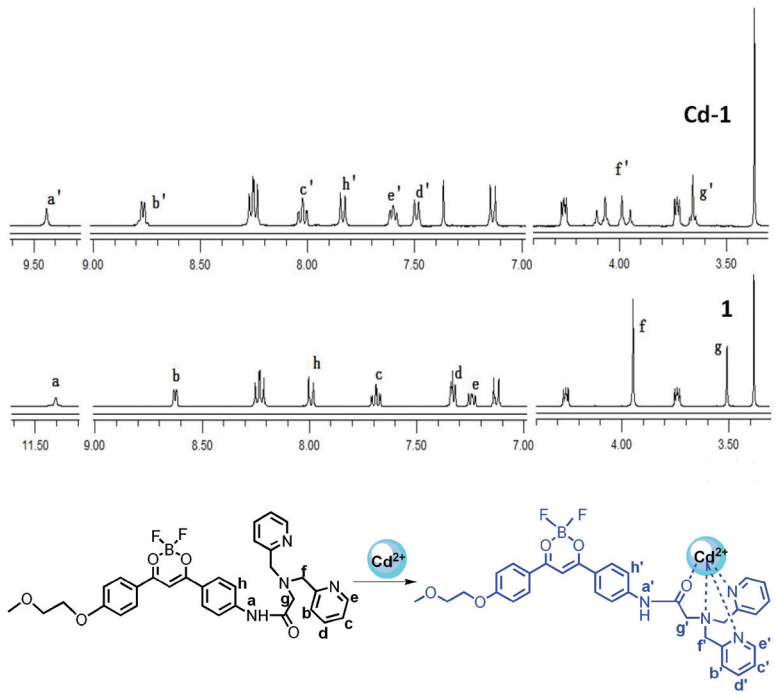


Fig. 3 (a) ^1H NMR spectra of **1** in the presence of 1 equiv. of Cd^{2+} in CD_3CN; (b) the proposed binding mode of Cd–**1**.

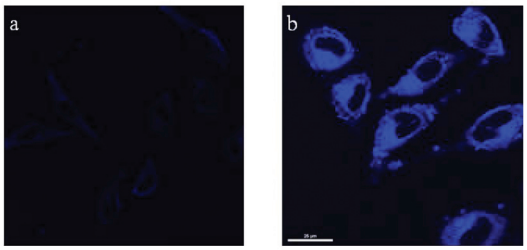


Fig. 5 Confocal fluorescence of living HeLa cells: (a) fluorescence image of HeLa cells incubated with **1** (5 μM); (b) fluorescence image of HeLa cells incubated with **1** after adding Cd^{2+} (25 μM).

The influence of pH on the emission intensity was studied from pH 5.82 to 7.85 (Fig. 4S†). Probe **1** exhibited intense fluorescence at pH 5.82 due to the protonation of the amino group under acidic conditions, and decreasing intensity above pH 6.17, which was attributed to the PET from the amino to the difluoroboron dibenzoyl fluorophore. A weaker pH effect was observed for the complex Cd–**1** in the pH range of 6.17–7.85. Therefore, the physiological pH would be suitable for Cd^{2+} detection.

To further demonstrate the practical application of the probe, we carried out fluorescence imaging experiments in living cells. HeLa cells incubated with **1** (5 μM) at 37 °C for 15 min showed almost no fluorescence (Fig. 5a). Treatment of HeLa cells with Cd^{2+} (25 μM) for 10 min and subsequent treatment of cells with **1** (5 μM) for another 15 min showed an obvious blue fluorescence (Fig. 5b). The results suggest that **1** can penetrate the cell membrane and can potentially be used for imaging of Cd^{2+} in living cells and *in vivo*.

Conclusions

In conclusion, we have designed and synthesized a difluoroboron dibenzoyl-based fluorescent probe **1** for Cd^{2+} based on a PET mechanism. Probe **1** displayed high selectivity and sensitivity towards Cd^{2+} with significantly enhanced fluorescence intensity due to the formation of a 1 : 1 metal–ligand complex. It can distinguish Cd^{2+} from other metal ions, especially from Zn^{2+} in aqueous solution. This is the first example of metal ion detection with difluoroboron dibenzoyl derivatives as fluorophores. The living cell image experiments further demonstrate its value in the practical applications of biological systems. Efforts to employ difluoroboron dibenzoyl derivatives as fluorophores to detect various metal ions and to further improve their water-solubility are underway.

Experimental section

General information

All the reagents and solvents are of commercial quality and used without further purification. ^1H and ^{13}C NMR spectra were recorded on an Advance Bruker 400 M spectrometer and referenced to solvent signals. Mass spectra (EI) were obtained in the positive ion mode on a Waters GCT premier. HRMS was measured with a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer with ESI positive mode. Fluorescence spectra were recorded at ambient temperature on a Perkin Elmer LS50B spectrofluorimeter and UV/Vis spectra were recorded on a Shimadzu UV-1601PC spectrophotometer.

Preparation of 1

Caution: Sodium hydride is potentially explosive and must be handled with care, shock and high temperatures must be avoided.

Synthesis of a. A solution of 4-amino ethylbenzoate (3 g, 18.2 mmol) and di-*tert*-butyl dicarbonate (6.3 g, 29 mmol) in 1,4-dioxane (67 mL) was stirred at 80 °C for 24 h. The reaction was cooled to room temperature and then evaporated to dryness. The residue was purified by column chromatography on silica gel with petroleum ether : ethyl acetate (1 : 3) as the eluent to give **a** (4.34 g, yield: 90%). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.98 (2H, d, J = 8.8 Hz), 7.43 (2H, d, J = 8.4 Hz), 6.73 (1H, br), 4.37 (2H, q, J = 7.2 Hz), 1.51 (9H, s), 1.37 (3H, t, J = 7.2 Hz).

Synthesis of 1-(4-(2-methoxyethoxy)phenyl)ethanone. 4-Hydroxyacetophenone (1.8 g, 13.2 mmol), 1-bromo-2-methoxyethane (2.4 g, 17.1 mmol) and K_2CO_3 (4.15 g, 30 mmol) in DMF (40 mL) were heated at 90 °C for 24 h. The mixture was then cooled, diluted with water and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$, and the solvent was evaporated. The residue was purified by column chromatography on silica gel with petroleum ether : ethyl acetate (1 : 10) as the eluent to give 1-(4-(2-methoxyethoxy)phenyl)ethanone as a white solid (2.32 g, yield: 90.7%). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.92 (2H, d, J = 9.2 Hz), 6.95 (2H, d, J = 8.8 Hz), 4.17 (2H, t, J = 4.4 Hz), 3.76 (2H, t, J = 4.4 Hz), 3.44 (3H, s), 2.54 (3H, s).

Synthesis of b. 1-(4-(2-Methoxyethoxy)phenyl)ethanone (1.8 g, 15 mmol) was dissolved in 100 mL dry THF, then the mixture was cooled to 0 °C under N_2. Sodium hydride was added to the mixture slowly. After 15 min, **a** (1.35 g, 9 mmol) was added. Then the mixture was stirred for 8 h at room temperature. The reaction was quenched with water, and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4, and the solvent was evaporated to dryness. The residue was purified by column chromatography on silica gel with petroleum ether : ethyl acetate (1 : 4) as the eluent to give **b** in its enol form as a pale yellow powder (1.56 g, yield: 42%). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 17.08 (1H, s), 7.94 (4H, m), 7.48 (2H, d, J = 8.8 Hz), 7.00 (2H, d, J = 8.8 Hz), 6.76 (1H, s), 6.73 (1H, s), 4.19 (2H, t, J = 4.8 Hz), 3.78 (2H, t, J = 4.8 Hz), 3.46 (3H, s), 1.53 (9H, s).

Synthesis of c. The solution of **b** (0.7 g, 1.7 mmol) in dichloromethane (40 mL) was added slowly to TFA (6 mL) at 0 °C. The mixture was stirred for 3 h at room temperature and evaporated to dryness. The residue was redissolved in 10 mL of 2 M NaOH, extracted with CH_2Cl_2, dried over anhydrous K_2CO_3 and evaporated to obtain a yellow solid. The residue was

purified by column chromatography on silica gel with petroleum ether : ethyl acetate (3 : 1) as the eluent to give **c** as its enol form as a yellow powder (0.32 g, yield: 60%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 17.23 (1H, s), 7.95 (2H, d, J = 8.8 Hz), 7.86 (2H, d, J = 8.8 Hz), 7.01 (2H, d, J = 8.8 Hz), 6.72 (1H, s), 6.70 (2H, m), 4.20 (2H, t, J = 4.4 Hz), 4.13 (2H, s), 3.80 (2H, t, J = 4.4 Hz), 3.48 (3H, s).

Synthesis of d. A solution of bromo acetyl bromide (0.2 g, 1 mmol) in dry CH_2Cl_2 (80 mL) was added dropwise to a solution of **c** (0.2 g, 0.64 mmol) and DMAP (0.014 g, 0.12 mmol) in dry CH_2Cl_2 (20 mL) stirred in an ice bath. After 6 h at room temperature, the mixture was diluted with water and extracted with CH_2Cl_2, dried over anhydrous $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel with petroleum ether : dichloromethane (1 : 1) as the eluent to afford **d** in its enol form as a pale yellow powder (0.17 g, yield: 60%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 17.01 (1H, s), 8.35 (1H, s), 7.97 (4H, m), 7.69 (2H, m), 7.01 (2H, d, J = 8.0 Hz), 6.76 (1H, s), 4.20 (2H, t, J = 4.8 Hz), 4.05 (2H, s), 3.74 (2H, t, J = 4.8 Hz), 3.47 (3H, s).

Synthesis of e. A suspension of **d** (0.1 g, 0.23 mmol), DPA (72 mg, 0.36 mmol), K_2CO_3 (0.1 g, 0.72 mmol) and KI (15 mg, 0.09 mmol) in 40 mL of acetonitrile was refluxed for 24 h under N_2. After evaporation of the acetonitrile, the residue was diluted with water and extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4 and evaporated to obtain a viscous yellow liquid. The residue was purified by column chromatography on silica gel with 1 : 1 methanol : dichloromethane as the eluent to give **e** in its enol form as a yellow oil (63 mg, yield: 50%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 17.10 (s, 1H), 11.27 (s, 1H), 8.63 (2H, d, J = 4.4 Hz), 7.95 (6H, m), 7.62 (2H, dt, J = 7.8 Hz, 1.6 Hz), 7.28 (2H, d, J = 7.6 Hz), 7.19 (2H, dd, J = 5.6 Hz, 1.6 Hz), 7.01 (2H, d, J = 8.8 Hz), 6.77 (1H, s), 4.20 (2H, t, J = 4.8 Hz), 3.95 (4H, s), 3.79 (2H, t, J = 4.8 Hz), 3.51 (2H, s), 3.46 (3H, s). MS (EI): calcd for $[M]^+$ 552.24, found 552.23.

Synthesis of 1. $BF_3{\cdot}OEt_2$ (86 mg, 0.6 mmol) was added to a solution of dry **e** (0.2 g, 0.4 mmol) in dry CH_2Cl_2 (80 mL). The mixture was refluxed for 3 h under N_2. After cooling to room temperature, the mixture was diluted with water and extracted with CH_2Cl_2, dried over anhydrous $MgSO_4$ and evaporated to obtain a pale yellow viscous liquid. The residue was purified by column chromatography on silica gel with 40 : 1 dichloromethane : methanol as the eluent to give **1** as a yellow powder (14 mg, yield: 60%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 11.48 (1H, s), 8.64 (2H, d, J = 4.8 Hz), 8.15 (4H, dd, J = 7.2 Hz, 2.0 Hz), 8.00 (2H, d, J = 8.8 Hz), 7.64 (2H, dt, J = 7.6 Hz, 1.6 Hz), 7.29 (2H, d, J = 6.0 Hz), 7.21 (2H, dd, J = 4.8 Hz, 2.4 Hz), 7.07 (2H, d, J = 3.2 Hz), 7.05 (1H, s), 4.24 (2H, t, J = 4.8 Hz), 3.97 (4H, s), 3.80 (2H, t, J = 4.8 Hz), 3.55 (2H, s), 3.47 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 181.21, 180.77, 170.94, 164.79, 157.99, 149.56, 145.15, 137.00, 131.47, 130.41, 126.95, 124.79, 123.54, 122.91, 119.46, 115.28, 92.04, 70.83, 67.98, 60.53, 59.44, 59.15. MS (EI): calculated for $[M]^+$ 600.24, found for 600.38. HRMS (ESI) m/z calcd for $C_{32}H_{32}BF_2N_4O_5$ (MH^+), 601.2436; found 601.2432.

Fluorescence quantum yield of 1

Quantum yields of **1** were determined using quinine sulfate in 0.1 N H_2SO_4 as standards according to a published method.[30] The quantum yields were calculated according to the following equation:

$$\Phi = \Phi_S \times I/I_S \times OD_S/OD \times \eta^2/\eta_S{}^2 \quad (1)$$

in which Φ is the quantum yield, I is the integrated intensity, η is the refractive index (η_{H_2O} = 1.333 was used here), OD is the optical density. The subscript S refers to the standard.

Cell culture and fluorescence imaging

HeLa cells were cultured in culture media (DMEM/F12 supplemented with 10% FBS, 50 unit mL^{-1} penicillin, and 50 μg mL^{-1} of streptomycin) at 37 °C under a humidified atmosphere containing 5% CO_2 for 24 h. The cells were treated and incubated with 25 μM of $Cd(NO_3)_2$ at 37 °C under 5% CO_2 for 10 min, and washed three times with phosphate buffered saline (PBS). Then 5 μM of **1** were added to the above cells, incubated for another 15 min at 37 °C under 5% CO_2, and washed three times with PBS. For the control experiment, the cells were incubated with 5 μM of **1** in culture media for 15 min. Confocal fluorescence imaging was performed with a Nikon A1R multiphoton microscope with a 60× oil-immersion objective lens. Fluorescence was excited at 405 nm with a Si laser and emission was collected by a 425–475 nm band pass filter.

Acknowledgements

We are grateful for financial support from the 973 Program (2013CB933800, 2013CB834803), the National Natural Science Foundation of China (21222210, 91027041, 51273108), and the Chinese Academy of Sciences (100 Talents Program). We thank Prof. P. Wang (TIPC, CAS) for providing HeLa cells and Dr M. Zheng (TIPC, CAS) for her help in confocal laser scanning microscopy.

Notes and references

1 J. Contreras, J. S. Xie, Y. J. Chen, H. Pei, G. Q. Zhang, C. L. Fraser and S. F. Hamm-Alvarez, *Acs Nano*, 2010, **4**, 2735–2747.
2 B. Domercq, C. Grasso, J. L. Maldonado, M. Halik, S. Barlow, S. R. Marder and B. Kippelen, *J. Phys. Chem. B*, 2004, **108**, 8647–8651.
3 Y. Qin, I. Kiburu, S. Shah and F. Jakle, *Org. Lett.*, 2006, **8**, 5227–5230.
4 C. Z. Ran, X. Y. Xu, S. B. Raymond, B. J. Ferrara, K. Neal, B. J. Bacskai, Z. Medarova and A. Moore, *J. Am. Chem. Soc.*, 2009, **131**, 15257–15261.
5 E. Cogne-Laage, J. F. Allemand, O. Ruel, J. B. Baudin, V. Croquette, M. Blanchard-Desce and L. Jullien, *Chem.–Eur. J.*, 2004, **10**, 1445–1455.

6 H. Maeda and Y. Kusunose, *Chem.–Eur. J.*, 2005, **11**, 5661–5666.
7 Y. L. Chow, C. I. Johansson, Y. H. Zhang, R. Gautron, L. Yang, A. Rassat and S. Z. Yang, *J. Phys. Org. Chem.*, 1996, **9**, 7–16.
8 C.-T. Poon, W. H. Lam, H.-L. Wong and V. W.-W. Yam, *J. Am. Chem. Soc.*, 2010, **132**, 13992–13993.
9 C. Risko, E. Zojer, P. Brocorens, S. R. Marder and J. L. Bredas, *Chem. Phys.*, 2005, **313**, 151–157.
10 (*a*) G. Q. Zhang, J. B. Chen, S. J. Payne, S. E. Kooi, J. N. Demas and C. L. Fraser, *J. Am. Chem. Soc.*, 2007, **129**, 8942–8943; (*b*) G. Q. Zhang, G. M. Palmer, M. W. Dewhirst and C. L. Fraser, *Nat. Mater.*, 2009, **8**, 747–751; (*c*) G. Q. Zhang, R. E. Evans, K. A. Campbell and C. L. Fraser, *Macromolecules*, 2009, **42**, 8627–8633.
11 A. M. S. Mendes, G. P. Duda, C. W. A. do Nascimento and M. O. Silva, *Sci. Agric.*, 2006, **63**, 328–332.
12 T. Y. Jin, J. Lu and M. Nordberg, *Neurotoxicology*, 1998, **19**, 529–535.
13 M. P. Waalkes, *Mutat. Res.*, 2003, **533**, 107–120.
14 M. Waisberg, P. Joseph, B. Hale and D. Beyersmann, *Toxicology*, 2003, **192**, 95–117.
15 (*a*) E. M. Nolan and S. J. Lippard, *Chem. Rev.*, 2008, **108**, 3443–3480; (*b*) D. G. Cho and J. L. Sessler, *Chem. Soc. Rev.*, 2009, **38**, 1647–1662; (*c*) M. E. Jun, B. Roy and K. H. Ahn, *Chem. Commun.*, 2011, **47**, 7583–7601; (*d*) L.-Y. Niu, H. Li, L. Feng, Y.-S. Guan, Y.-Z. Chen, C.-F. Duan, L.-Z. Wu, Y.-F. Guan, C.-H. Tung and Q.-Z. Yang, *Anal. Chim. Acta*, 2013, DOI: 10.1016/j.aca.2013.03.013; (*e*) J. Fan, M. Hu, P. Zhan and X. Peng, *Chem. Soc. Rev.*, 2013, **42**, 29–43.
16 H. N. Kim, W. X. Ren, J. S. Kim and J. Yoon, *Chem. Soc. Rev.*, 2012, **41**, 3210–3244 and references therein.
17 (*a*) X. Peng, J. Du, J. Fan, J. Wang, Y. Wu, J. Zhao, S. Sun and T. Xu, *J. Am. Chem. Soc.*, 2007, **129**, 1500–1501; (*b*) X. Zhou, P. Li, Z. Shi, X. Tang, C. Chen and W. Liu, *Inorg. Chem.*, 2012, **51**, 9226–9231.
18 Z. C. Xu, J. Yoon and D. R. Spring, *Chem. Soc. Rev.*, 2010, **39**, 1996–2006.
19 (*a*) G. Gilli, F. Bellucci, V. Ferretti and V. Bertolasi, *J. Am. Chem. Soc.*, 1989, **111**, 1023–1028; (*b*) V. Bertolasi, P. Gilli, V. Ferretti and G. Gilli, *Chem.–Eur. J.*, 1996, **2**, 925–934.
20 S. Feryforgues, M. T. Lebris, J. P. Guette and B. Valeur, *J. Phys. Chem.*, 1988, **92**, 6233–6237.
21 K. K. Sadhu, S. Sen and P. K. Bharadwaj, *Dalton Trans.*, 2011, **40**, 726–734.
22 O. Oter, K. Ertekin, C. Kirilmis and M. Koca, *Sens. Actuators, B*, 2007, **122**, 450–456.
23 M. Shortreed, R. Kopelman, M. Kuhn and B. Hoyland, *Anal. Chem.*, 1996, **68**, 1414–1418.
24 Z. C. Xu, K. H. Baek, H. N. Kim, J. N. Cui, X. H. Qian, D. R. Spring, I. Shin and J. Yoon, *J. Am. Chem. Soc.*, 2010, **132**, 601–610.
25 M. J. McDonough, A. J. Reynolds, W. Y. G. Lee and K. A. Jolliffe, *Chem. Commun.*, 2006, 2971–2973.
26 H.-H. Wang, Q. Gan, X.-J. Wang, L. Xue, S.-H. Liu and H. Jiang, *Org. Lett.*, 2007, **9**, 4995–4998.
27 L. Xue, H. H. Wang, X. J. Wang and H. Jiang, *Inorg. Chem.*, 2008, **47**, 4310–4318.
28 L. Fabbrizzi, M. Licchelli, P. Pallavicini, A. Perotti and D. Sacchi, *Angew. Chem., Int. Ed.*, 1994, **33**, 1975–1977.
29 S. C. Burdette, C. J. Frederickson, W. M. Bu and S. J. Lippard, *J. Am. Chem. Soc.*, 2003, **125**, 1778–1787.
30 S. Hamai and F. Hirayama, *J. Phys. Chem.*, 1983, **87**, 83–89.