BODIPY-Derived Piperazidine Fluorescent Near-Neutral PH Indicator and Its Bioimaging

Min Deng, Chengduan Yang, Deyan Gong, Anam Iqbal, Xiaoliang Tang, Weisheng Liu, and Wenwu Qin ¹

Key Laboratory of Nonferrous Metal Chemistry and Resources Utilization of Gansu Province and State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, P. R. China.

Two new 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dyes (**1** and **2**) with piperazine subunits on position 3 or 5 were synthesized. Their absorption and steady-state fluorescence properties were investigated as a function of solvent. In water/MeCN (9:1, V/V) solution, the dyes show fluorescent enhancement upon increasing the acidity of the solution. They can be used in water/MeCN (9:1, V/V) solution as fluorescent pH probes excitable with visible light, with pK_a values near-neutral (around 6.5 and 6.8). Dyes **1** and **2** are readily taken up by biological cells in the cytosol where it has no obvious toxic effects.

Keywords: BODIPY; piperazine; near-neutral pH; biological cells;

¹ Corresponding author: Fax: +86-931-8912582 E-mail address: qinww@lzu.edu.cn

(W. Qin)

1. Introduction

Fluorescent pH probes are obligato tools for measuring changes of intracellular proton concentrations[1]. Measurement of pH by fluorescence-based technique is well established for both imaging and sensing applications[1, 2]. Compared to most other techniques, the fluorescence-based technique offers significant advantages owing to its generally nondestructive character, low background noise, high sensitivity and specificity, and the wide range of indicator dyes available[3-7]. To quantitatively determine pH, it is imperative to match the pK_a of the probe to the pH of the investigated system. Since the pH in the cytosol is close to neutral (generally between ~6.8 and 7.4), fluorescent probes with a pK_a around 7 are necessary for cytosolic pH determinations.

Due to their excellent characteristics, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene[8-10] (better known as difluoroboron *dipyrromethene* or BODIPY) derivatives may be important beneficial fluorophores in the design of fluorescent sensors. The excellent qualities of BODIPY comprised relatively high fluorescence quantum yields and molar absorption coefficients, narrow emission bandwidths with high peak intensities, high elevated stability towards chemicals and light, and the extra feature of excitation / emission wavelengths in the visible region. Furthermore, the spectroscopic properties of BODIPY derivatives can be fine-tuned by synthetically introducing suitable substituents at the right positions of the BODIPY[8] core.

Difluoroboradiaza-*s*-indacenes with hydroxyaryl subunits have been reported as

pH probes in aqueous-organic mixed media[11, 12]. These on / off pH probes showed a photo-induced electron transfer (PET) from the twisted and conjugatively uncoupled phenolate to the BODIPY subunit, causing fluorescence quenching at high pH. 8-(4'-Hydroxyphenyl) substituted BODIPY derivatives[11] sense the alkaline pH range, while the tetrahydroxylated calix[4]arene derivative[12] is sensitive in the near-neutral pH range. In our previous work, we have synthesized and spectroscopically characterized two water-soluble, on/off pH indicators with pK_a around 7.5 based on *o*-chlorophenol linked to the *meso* (8-) position[13, 14] or via a vinyl bridge to the 3-position[15] of BODIPY. A series of pH indicators based on BODIPY with *meso*-anilino substituents have been described recently for imaging acidic endosomes in cancer cells.[16] These derivatives are almost nonfluorescent in basic media due to PET.

We chose N-methylpiperazine[17] and N-ethylpiperazine with the favorable pK_a around 9 to serve as pH-sensitive ligand for the near-neutral pH range. Substitution of piperazidine will generally lead to altered the pK_a values. Due to the electron-withdrawing character of BODIPY, it is expected that conjugatively linking BODIPY fluorescent reporter subunit via an ethenyl bridge to piperazidine will decrease the pK_a of piperazidine in sensors **1** and **2** by approximately 1 unit compared to free piperazidine[18].

2. Experimental section

2.1 Instrument and reagent

All chemical solvents and reagent were obtained from Bailingwei. ^1H and ^{13}C

NMR spectra were recorded with a Bruker DRX-400 and DRX-400/4 spectrometer with tetramethylsilane (0.00 ppm) as an internal standard and CDCl₃ as solvent. Chemical shift multiplicities are reported as s=singlet, d=doublet, t=triplet, q=quartet and m=multiplet. ¹³C spectra were referenced to ppm with the CDCl₃ (77.67 ppm) signal. Mass spectra were recorded in E.I. mode. Melting points were obtained with an X-4 precise micro melting point cryoscope (Beijing Fukai Instrument Co.) and are uncorrected. All measurements were performed at room temperature.

2.2 The synthesis of sensor **1**

BODIPY derivatives **1** and **2** were prepared using the same procedure, starting from 3,5-dichloro-4,4-difluoro-8-(4-tolyl)-4-bora-3a,4a-diaza-*s*-indacene (**3**).

The synthetic route is depicted in Scheme 1. 150 mg (0.43 mmol) of compound **3** was dissolved in 25 ml acetonitrile and 0.5 ml of triethylamine was added to it. After 15 minutes, 48 µl (0.43 mmol) of compound N-methylpiperazine was added to it. The reaction mixture was stirred at room temperature for 72 h. After solvent was removed under reduced pressure, the crude solid was purified by column chromatography on silica purified through column chromatography over silica (dichloromethane/methanol, 50/1, v/v) to obtain a reddish brown powder 130 mg of **1** with 75 % yield. Reddish solid, mp 127-128 °C; ¹H NMR (CDCl₃, 400 MHz) δ 2.36 (s, 3H, H-a), 2.41 (s, 3H, H-b), 2.63 (t, 4H, J = 4.8 Hz, H-c), 3.99 (t, 4H, J = 4.8 Hz, H-d), 6.18 (d, 1H, J = 4.0 Hz, H-e), 6.246 (d, 1H, J = 5.2 Hz, H-g), 6.28 (d, 1H, J = 4.0 Hz, H-f), 6.848 (d, 1H, J = 5.2 Hz, H-h), 7.22 (d, 2H, J = 8.0 Hz, aromatic), 7.29 (d, 2H, J = 8.0 Hz, aromatic); ¹³C NMR (CDCl₃, 100 MHz): δ 21.45, 45.74, 50.29,

54.96, 113.03, 113.72, 119.02, 128.98, 129.50, 130.46, 131.35, 131.45, 131.55, 135.15, 135.64, 139.26, 162.01; MS (ESI) m/z calcd. For $C_{21}H_{22}BClF_2N_4$ 414.2; found 415.2 (M+1); 395.2 (M-F, 100%).

2.3 The synthesis of sensor **2**

The crude solid was purified by column chromatography on silica purified through column chromatography over silica (dichloromethane/methanol, 50/1, v/v) to obtain a reddish brown powder 135 mg of **2** with 73 % yield. Reddish solid, mp 150-152 °C; 1H NMR ($CDCl_3$, 400 MHz) δ 1.12 (t, 3H, $J = 7.2$ Hz, H-a'), 2.41 (s, 3H, H-b), 2.49 (q, 2H, $J = 7.2$ Hz, H-a), 2.67 (t, 4H, $J = 4.8$ Hz, H-c), 4.00 (t, 4H, $J = 4.8$ Hz, H-d), 6.177 (d, 1H, $J = 4.0$ Hz, H-e), 6.25 (d, 1H, $J = 5.2$ Hz, H-g), 6.27 (d, 1H, $J = 4.0$ Hz, H-f), 6.844 (d, 1H, $J = 5.2$ Hz, H-h), 7.22 (d, 2H, $J = 8.0$ Hz, aromatic), 7.29 (d, 2H, $J = 8.0$ Hz, aromatic); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 11.87, 21.44, 50.41, 52.13, 52.80, 112.94, 113.78, 118.80, 128.96, 129.29, 130.46, 131.27, 131.34, 131.49, 135.20, 135.60, 139.20, 161.91; MS (ESI) m/z calcd. For $C_{22}H_{24}BClF_2N_4$ 428.2; found 429.2 (M+1, 100%).

< Scheme 1 >

2.4. Determination of K_a from Direct Fluorimetric Titration

The K_a values of **1** and **2** were determined in water/MeCN (9:1, V/V) solution at 25 °C by direct fluorometric titration as a function of pH using the fluorescence emission spectra. Nonlinear fitting of Eq.1[19, 20] to the steady-state fluorescence data F recorded as a function of $[H^+]$ yields values of K_a , the fluorescence signals F_{min} and F_{max}

at minimal and maximal $[H^+]$, respectively, and n (the number of protons bound per fluorescent pH probe).

$$F = \frac{F_{\max} [H^+]^n + F_{\min} K_a}{K_a + [H^+]^n} \quad (1)$$

Because the fits of Eq. 1 to the fluorescence data F with n , K_a , $F_{\min}$, and $F_{\max}$ as freely adjustable parameters always gave values of n close to 1, n was kept fixed at 1 in the final curve fittings, from which the estimated values of K_a are reported here. Equation 1 assumes (i) photo-excitation with light of constant incident spectral radiant power, (ii) that the absorbance A of the sample is small (≤ 0.1) and (iii) the rate of proton binding in the excited state can be neglected.

2.5. General method for measurements of photophysical properties

UV-vis absorption spectra were recorded on a Varian UV-Cary 5000 spectrophotometer and for the corrected steady-state emission spectra, a Hitachi F-7000 spectrofluorimeter was employed. Freshly prepared samples were used to perform all UV-vis absorption and emission measurements. For the determination of the fluorescence quantum yields ϕ_f of **1** and **2**, only dilute solutions with an absorbance below 0.1 at the excitation wavelength ($\lambda_{\text{ex}} = 480 \text{ nm}$) were used. Rhodamine 6G in water ($\phi_f = 0.76$) was used as fluorescence standard [21]. The ϕ_f values reported in this work are the averages of multiple (generally three), fully independent measurements. In all cases, correction for the solvent refractive index was applied.

All measurements were done at 25 °C with an air conditioner.

2.6. Crystal structure determination

Crystals of **1** were obtained by slow evaporation of the mixed solvent (dichloromethane/cyclohexane) (2:1, v/v) in air over one weeks, yielding clear dark orange block crystals with approximate dimensions of $0.18 \times 0.14 \times 0.11 \text{ mm}^3$ suitable for X-ray diffraction.

Crystals of **2** were obtained by slow evaporation of the ethyl acetate in air over two weeks, yielding clear dark violet block crystals with approximate dimensions of $0.34 \times 0.32 \times 0.31 \text{ mm}^3$ suitable for X-ray diffraction. The basic crystallographic data of **1** and **2** are compiled in Table S1.

The data for **1** and **2** was measured on a Bruker Smart APEX II CCD diffractometer at $155 \pm 10 \text{ K}$ and $297 \pm 10 \text{ K}$ respectively equipped with graphite-monochromatized Mo $K\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$ and $\lambda = 1.54184 \text{ \AA}$). The structures were solved by direct methods. All non-hydrogen atoms were subjected to anisotropic refinement by full-matrix least-squares methods on F^2 by using the program package SHELXS-97[22]. Hydrogen atoms were placed at calculated positions.

Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 1443775 for **1** and CCDC 1443776 for **2**. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk or www.ccdc.cam.ac.uk/conts/retrieving.html).

2.7. Cell culture, fluorescence imaging and cytotoxicity assay

Cell Culture and fluorescence imaging. To determine the cell permeability of **1** or **2**, Baby Hamster Syrian Kinney (BHK) cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS (fetal bovine serum). The cell lines were maintained in a humidified atmosphere containing 5% CO₂ at 37 °C. After removal of the culture medium, cells were incubated with **1** or **2** (10 µM) in 1.0 mL of fresh culture medium for 2 h. Before imaging measurement, the cells were washed three times with PBS to remove the residual **1** or **2**. Cells were observed under an Olympus FV1000-IX81 laser confocal microscope.

Cytotoxicity Test. BHK Cells were seeded at a density of 10⁴ cells per well (100 µL total volume/well) in 96-well assay plates for 24 h. Then, the as-prepared **1** or **2**, at the indicated concentrations (0, 5, 10, 15, 20, 25 µg / ml), were added to the cell culture medium. Cells were incubated with **1** or **2** for 24 h. To determine toxicity, 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium, inner salts (MTS) was added to each well of the microtiter plate and the plate was incubated in the CO₂ incubator for additional 4 h. Absorbance values were determined with Bio-Rad model-680 microplate reader at 490 nm (corrected for background absorbance at 630 nm). The cell viability was estimated according to the following equation: cell viability (%) = mean of absorbance value of treatment group/mean absorbance value of control × 100%.

2.8. Theoretical calculations

The calculation was performed using the Gaussian 09 suite of programs[23]. The

ground state structures of two compounds were based on the corresponding crystal structures and were not further optimized. The density functional theory (DFT) with Becke's three parameter hybrid functional with the Lee–Yang–Parr correlation functional (B3LYP) and 6–31+G(d) basis set were used to treat all atoms. The excited state related calculations were carried out with the time dependent density functional theory (TD-DFT) with the ground state structure was obtained. There are no imaginary frequencies in frequency analysis, and the contours of the HOMOs and LUMOs were plotted to understand the nature of the excited state. The energy and oscillator strength for the twenty lowest singlet-singlet absorptions of each compound were obtained from the TD-DFT output files. The calculated absorption spectra were visualized directly with Gauss View 5.0.

3. Results and Discussion

3.1. Crystal structure of **1** and **2**.

As shown in Figure 1, the two planar pyrrole subunits and the boron atom constitute a plane for which the deviation of all non-hydrogen atoms is within the 0.005–0.070 Å range in the BODIPY ring system. The angle between the two pyrrole moieties is 10.43° (for **1**) and 7.45° (for **2**), which is near the average 5.79° (range 0°–18.1°) found in the Cambridge Structural Database (CSD, updated to October 2008). The two fluorine atoms are equidistant above and under the plane of the pyrrole moieties, and the F–B–F plane is 109.91° (for **1**) and 110.07° (for **2**) to the plane of the BODIPY core. The methyl phenyl ring at the meso-position makes an angle of 54.79° (for **1**) and 60.41° (for **2**) with the BODIPY plane, which is in the

range of most BODIPY derivatives (40.3°–90°), but is smaller than the average value of 76.4° found in the CSD. The dihedral angles between piperzine ring and pyrroles is 41.15° (for **1**) and 41.80° (for **2**).

< Fig. 1 >

3.2. Spectroscopic properties 1 and 2.

Sensors **1** and **2** were dissolved in several solvents respectively (cyclohexane, THF, MeCN) to investigate their solvatochromic properties. Figure 2 shows the normalized UV–vis absorption and fluorescence emission spectra of **1** dissolved in various solvents. The absorption spectrum is of comparable shape as those of the described BODIPY dyes[24-28], with an intense absorption band somewhat above 470 nm (is attributed to the 0–0 vibrational band of $S_0 \rightarrow S_1$ transition) in acetonitrile. Additionally, a weaker, broad band is found in the absorption spectra at around 340 nm. This broader and weaker absorption band is attributed to the 0–0 vibrational band of $S_0 \rightarrow S_2$ ($\pi - \pi^*$) transition. The main absorption band of **1** is at ~ 515 nm in cyclohexane, and a shoulder at the short wavelength side can be observed – centered at approximately 490 nm – which is assigned to the 0–1 vibrational band of the same transition. In more polar solvents no vibrational fine structure can be observed. The absorption spectra of **2** (Fig S1) is, in general, similar to those of **1** in the solvents used.

The probes **1** or **2** in various solvents shows an emission spectrum with a maximum at ~550 nm, shaped like the mirror image of the absorption spectrum, displaying the typical emission features of BODIPY[24-28]. The ϕ_f value for **1** and **2** are low in

solvents (about 0.05) can be attributed to an efficient quenching via an excited-state ICT process from the nitrogen atom of piperazine to the strongly electron-deficient BODIPY acceptor. The absorption spectra are affected by solvent polarity; the maximum being blue-shifted (by ~44 nm) when the solvent is changed from cyclohexane (518 nm) to acetonitrile (474 nm). The maximum wavelength of the fluorescence band is hardly affected by solvent polarity (Figure 2, Table 1): there is only a 7 nm difference between the wavelengths of the emission maxima $\lambda_{em(max)}$ in toluene (560 nm) and DMSO (553 nm). The blue shift of the absorption spectrum from cyclohexane to acetonitrile can hardly be attributed to a difference in solvent polarizability (Figure S2). If it is attributed to dipolar interactions, this blue shift suggests that the dipole moment of the ground state is larger than that of the excited state.[29]

< Fig. 2>

< Table 1>

3.3. Fluorimetric titrations of **1** and **2**.

Protonation of the piperazine function drastically alters the electron-donating properties of the tertiary amine group and consequently it is expected to switch off the ICT to the borondipyrromethene moiety. The changes of the absorption spectra of **1** as a function of pH are shown in Figure 3a. It is clear that changing the pH leads to several isosbestic points and is accompanied by a hypsochromic shift of the major absorption maximum of **1** (from 485 nm at pH 3.36 to 474 nm at pH 8.86) upon increasing the pH.

The fluorescence emission spectra of **1** in water/MeCN (9:1, V/V) solution are shown as a function of pH in Figures 3c. The fluorescence signals increase considerably at lower pH. The highest ϕ_f value (0.12, $\lambda_{ex} = 480$ nm) is found at low pH (pH 3.22). Binding of H^+ by the piperazine raises the energy of the intramolecular CT state and hence suppresses the formation of this state. Blocking this non-radiative decay channel of the locally excited state leads to a large fluorescence enhancement.

Analogous absorption effects are found for **2** (Figure 3b), where the major absorption maximum shifts hypsochromically from 485 nm at pH 3.72 to 474 nm at pH 8.68. In analogy to **1**, the fluorescence emission maximum at ~550 nm of **2** remains almost unaffected by pH (Figure 3d). The fluorescence intensity increases significantly with decreasing pH. The highest ϕ_f value (0.13, $\lambda_{ex} = 480$ nm) is found at low pH (pH 3.82). Figure S3 and S4 displays the absorption and emission spectra of sensors **1** and **2** as a function of pH (7–12) in water/MeCN (9:1, V/V) solution. In alkaline conditions, the absorption and emission spectra hardly be influenced by pH in the range of 8-12.

< Fig. 3 >

The acidity constant K_a and the stoichiometry (n) of the binding of H^+ by the piperazine (tertiary amine) group of new fluorescent pH indicators in water/MeCN (9:1, V/V) solution were determined by fitting eq 1 to the fluorescence emission spectral data. The results of the direct fluorimetric titration using the emission data of Figure 3c ($\lambda_{ex} = 480$ nm, $\lambda_{em} = 550$ nm) are displayed in Figure 4a and yield a pK_a value of 6.45 ± 0.05 and a well-defined 1:1 stoichiometry between **1** and H^+ , indicate

the formation of indicative of ammonium ion. A similar titration using the emission spectra of Figure 3d ($\lambda_{\text{ex}} = 480 \text{ nm}$, $\lambda_{\text{em}} = 550 \text{ nm}$) yield a pK_a value of 6.74 ± 0.11 and a well-defined 1:1 stoichiometry between **2** and H^+ (figure 4b). These results are in perfect agreement with the curve-fittings using different wavelength of sensor **1** (Fig S5, at 535 nm: $pK_a=6.43\pm0.06$, at 555 nm: $pK_a=6.52\pm0.06$) and sensor **2** (Fig S6 at 530nm: $pK_a=6.73\pm0.08$ at 560m: $pK_a=6.88\pm0.15$). The average pK_a obtained from Fig S7 are 6.50 ± 0.07 (**1**) and 6.80 ± 0.11 (**2**). These results show that the obtained pK_a values are independent of the selected wavelengths. All estimated n values are indicative of a 1 to 1 binding between dyes **1** or **2** and H^+ .

< Fig. 4>

3.4. Cell culture

For practical applications, it is necessary that the molecular probe described here is able to detect pH under biological conditions. To test whether **1** or **2** fulfills these additional requirements, measurements on live cells were performed. Based on the fluorescence intensity upon excitation at 488 nm of the BHK cells during dye loading (10 μM), it can be concluded that **1** or **2** efficiently accumulates inside the cytosol, but is excluded from the cell nucleus (Fig. 5).

Moreover, cell cytotoxicity experiment of **1** or **2** was evaluated using BHK cell lines through the MTS assay. As shown in Fig S9, the **1** and **2** exhibited low cytotoxicity, which may be due to the low toxicity of BODIPY. Taken together, it is clearly demonstrated that the sensor is membrane-permeable, and the fluorescence change in the BHK cells was due to the synchronous presence of **1** and **2**. The result

confirmed that the probe **1** or **2** can be used to image intracellular pH levels in living cells.

< Fig. 5>

4. Conclusion

We have synthesized two new 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene dyes (**1-2**) with piperazine subunits. Their absorption and steady-state fluorescence properties were investigated as a function of solvent. In water/MeCN (9:1, V/V) solution, the dyes **1** and **2** undergo a reversible protonation-deprotonation reaction in the near-neutral pH range. They can be used in water/MeCN (9:1, V/V) solution as fluorescent pH probes excitable with visible light, with pK_a values were 6.5 ± 0.07 and 6.8 ± 0.11 . For cytosolic pH measurements, a better match between the pK_a of the indicator and pH (6.8–7.4) is desirable. Dyes **1** and **2** are readily taken up by biological cells in the cytosol where it is no obvious toxic effects.

Acknowledgments

The authors would like to thank the Natural Science Foundation of China (no. 21271094, 21371083). This work was supported by the National Science Foundation for Fostering Talents in Basic Research of the National Natural Science Foundation of China (Grant no. J1103307).

Supporting Information Available:

Compound characterization data, this material can be found in the Supporting Information.

References

- [1] R.P. Haugland, The handbook: a guide to fluorescent probes and labeling technologies: Molecular probes. 2005.
- [2] J. Han, K. Burgess, Fluorescent indicators for intracellular pH, Chem. Rev. 110(2009) 2709-2728.
- [3] J.-P. Desvergne, A.W. Czarnik, Chemosensors of ion and molecule recognition: Springer. Berlin. 1997.
- [4] X. Li, X. Gao, W. Shi, H. Ma, Design strategies for water-soluble small molecular chromogenic and fluorogenic probes, Chem. rev. 114(2013) 590-659.
- [5] G.K. Vegesna, J. Janjanam, J. Bi, F.-T. Luo, J. Zhang, C. Olds, et al., pH-activatable near-infrared fluorescent probes for detection of lysosomal pH inside living cells, J. Mater. Chem. B. 2(2014) 4500-4508.
- [6] Q. Wan, S. Chen, W. Shi, L. Li, H. Ma, Lysosomal pH Rise during Heat Shock Monitored by a Lysosome - Targeting Near - Infrared Ratiometric Fluorescent Probe, Angew. Chem. 126(2014) 11096-11100.
- [7] L. Fan, Q. Liu, D. Lu, H. Shi, Y. Yang, Y. Li, et al., A novel far-visible and near-infrared pH probe for monitoring near-neutral physiological pH changes: imaging in live cells, J. Mater. Chem. B. 1(2013) 4281-4288.
- [8] A. Treibs, F.H. Kreuzer, Difluoroboryl - Komplexe von Di - und Tripyrrylmethenen, Liebigs Ann. Chem. 718(1968) 208-223.
- [9] A. Loudet, K. Burgess, BODIPY dyes and their derivatives: syntheses and spectroscopic properties, Chem. Rev. 107(2007) 4891-4932.

- [10] G. Ulrich, R. Ziessel, A. Harriman, The chemistry of fluorescent bodipy dyes: versatility unsurpassed, *Angew. Chem., Int. Ed.* 47(2008) 1184-1201.
- [11] T. Gareis, C. Huber, O.S. Wolfbeis, J. Daub, Phenol/phenolate-dependent on/off switching of the luminescence of 4, 4-difluoro-4-bora-3a, 4a-diaza-s-indacenes, *Chem. Commun.* (1997) 1717-1718.
- [12] C.N. Baki, E.U. Akkaya, Boradiazaindacene-appended calix [4] arene: fluorescence sensing of pH near neutrality, *J. org. chem.* 66(2001) 1512-1513.
- [13] M. Baruah, W. Qin, N. Basaric, W.M. De Borggraeve, N. Boens, BODIPY-based hydroxyaryl derivatives as fluorescent pH probes, *J org chem*, 70(2005) 4152-4157.
- [14] W. Qin, M. Baruah, A. Stefan, M. Van der Auweraer, N. Boens, Photophysical Properties of BODIPY - Derived Hydroxyaryl Fluorescent pH Probes in Solution, *ChemPhysChem.* 6(2005) 2343-2351.
- [15] W. Qin, M. Baruah, W.M. De Borggraeve, N. Boens, Photophysical properties of an on/off fluorescent pH indicator excitable with visible light based on a borondipyrromethene-linked phenol, *J Photochem. Photobiol., A.* 183(2006) 190-197.
- [16] Y. Urano, D. Asanuma, Y. Hama, Y. Koyama, T. Barrett, M. Kamiya, et al., Selective molecular imaging of viable cancer cells with pH-activatable fluorescence probes, *Nat. med.* 15(2009) 104-109.
- [17] A. Castro, J.R. Leis, M.E. Peña, Decomposition of N-methyl-N-nitrosotoluene-p-sulphonamide in basic media: hydrolysis and transnitrosation reactions, *J. Chem. Soc., Perkin Trans. 2.* (1989) 1861-1866.
- [18] N. Boens, W. Qin, M. Baruah, W.M. De Borggraeve, A. Filarowski, N. Smisdom, et al., Rational Design, Synthesis, and Spectroscopic and Photophysical Properties of a Visible - Light - Excitable, Ratiometric, Fluorescent Near - Neutral pH Indicator Based on BODIPY, *Chem.—Eur. J.* 17(2011)

10924-10934.

[19] E. Cielen, A. Tahri, K. Ver Heyen, G.J. Hoornaert, F.C. De Schryver, N. Boens, Synthesis and spectroscopic characterisation of fluorescent indicators for Na⁺ and K⁺, J. Chem. Soc., Perkin Trans. 2. (1998) 1573-1580.

[20] E. Cielen, A. Stobiecka, A. Tahri, G.J. Hoornaert, F.C. De Schryver, J. Gallay, et al., Synthesis and characterisation of Thio-H, a new excitation and emission ratioable fluorescent Ca²⁺/Mg²⁺ indicator with high brightness, J. Chem. Soc., Perkin Trans. 2. (2002) 1197-1206.

[21] J. Olmsted, Calorimetric determinations of absolute fluorescence quantum yields, J. Phys. Chem. 83(1979) 2581-2584.

[22] G. Sheldrick, SHELX-97, Program for crystal structure solution and refinement, University of Göttingen, Germany, (1997) 1456.

[23] M. Frisch, G. Trucks, H. Schlegel, G. Scuseria, M. Robb, J. Cheeseman, et al., Gaussian 09, revision A. 1; Gaussian, Inc: Wallingford, CT. 2009.

[24] T.L. Arbeloa, F.L. Arbeloa, I.L. Arbeloa, I. Garcia-Moreno, A. Costela, R. Sastre, et al., Correlations between photophysics and lasing properties of dipyrromethene-BF₂ dyes in solution, Chem. Phys. Lett. 299(1999) 315-321.

[25] A. Costela, I. García-Moreno, C. Gomez, R. Sastre, F. Amat-Guerri, M. Liras, et al., Photophysical and lasing properties of new analogs of the boron-dipyrromethene laser dye PM567 in liquid solution, J. Phys. Chem. A. 106(2002) 7736-7742.

[26] V.M. Martinez, F.L. Arbeloa, J.B. Prieto, T.A. López, I.L. Arbeloa, Characterization of rhodamine 6G aggregates intercalated in solid thin films of laponite clay. 1. Absorption spectroscopy, J. Phys. Chem. B. 108(2004) 20030-20037.

- [27] V. Leen, V.Z. Gonzalvo, W.M. Deborggraeve, N. Boens, W. Dehaen, Direct functionalization of BODIPY dyes by oxidative nucleophilic hydrogen substitution at the 3- or 3,5-positions, *Chem. Commun.* 46(2010) 4908-4910.
- [28] A. Filarowski, M. Lopatkova, P. Lipkowski, M. Van der Auweraer, V. Leen, W. Dehaen, Solvatochromism of BODIPY-Schiff Dye, *J. Phys. Chem. B.* 119(2014) 2576-2584.
- [29] W. Qin, V. Leen, T. Rohand, W. Dehaen, P. Dedecker, M. Van der Auweraer, et al., Synthesis, spectroscopy, crystal structure, electrochemistry, and quantum chemical and molecular dynamics calculations of a 3-anilino difluoroboron dipyrromethene dye, *J. Phys. Chem. A.* 113(2008) 439-447.

Figure captions

Fig. 1. ORTEP representation of compounds **1** (a) and **2** (b) with displacement ellipsoids at the 20% probability level.

Fig. 2: Normalized absorption (dash curves) and fluorescence spectra (solid curves) of sensor **1** in various solvents.

Fig. 3: Absorption spectra of sensors **1** (a) and **2** (b) in water/MeCN (9:1, V/V) solution as a function of pH. Corresponding fluorescence emission spectra c and d. ($\lambda_{\text{ex}} = 480 \text{ nm}$).

Fig. 4: The solid line represents the best fit of eq 1 with $n = 1$ to the fluorimetric titration data of sensors **1** (a) and **2** (b) obtained from the emission spectra of Figure 3c and 3d ($\lambda_{\text{ex}} = 480 \text{ nm}$).

Fig. 5: Confocal fluorescence images of BHK cells of the sensors **1** and **2**. The bright-field images of **1** (a) and **2** (c); the confocal fluorescence images of **1** (b) and **2** (d).

Table 1. Spectroscopic / Photophysical Data of sensors **1** and **2** in Different Solvents at 25 °C.

Scheme 1. Synthesis of sensors **1** and **2**.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Table 1

Solvent	$\lambda_{\text{abs}}(\text{max}) / \text{nm}$		$\lambda_{\text{em}}(\text{max}) / \text{nm}$		Φ_f	
	1	2	1	2	1	2
Cyclohexane	518	518	557	557	0.055±0.001	0.052±0.001
Toluene	511	511	560	560	0.053±0.002	0.057±0.002
Tetrahydrofuran	488	486	557	550	0.046±0.001	0.043±0.001
Dichloromethane	489	486	556	553	0.052±0.002	0.053±0.002
Dimethyl sulfoxide	484	485	553	554	0.052±0.001	0.049±0.002
Acetonitrile	474	474	556	556	0.047±0.003	0.055±0.001

Scheme 1

Biographies

Min Deng received her B.S. degree from Lanzhou University, PR China, in 2014. She is currently a master candidate at College of Chemistry and Chemical Engineering, Lanzhou University, PR China. Her research interests focus on developing fluorescent chemosensors.

Chengduan Yang received his B.S. degree from Weifang University, PR China, in 2013. He is currently a master candidate at College of Chemistry and Chemical Engineering, Lanzhou University, PR China. Her research interests focus on developing fluorescent chemosensors.

Deyan Gong received his B.S. degree from Xi'an University of Technology, PR China in 2014. He is currently a master candidate at College of Chemistry and Chemical Engineering, Lanzhou University, PR China. His research interests focus on developing fluorescent chemosensors.

Xiao-Liang Tang received his Ph.D. degree from Lanzhou University, PR China, in 2009. He is currently an associate professor at College of Chemistry and Chemical Engineering, Lanzhou University, PR China. His research interests focus on developing function materials and fluorescent chemosensors.

Wei-Sheng Liu received his Ph.D. degree from Lanzhou University, PR China, in 1996. He is currently a professor at College of Chemistry and Chemical Engineering, Lanzhou University, PR China. His research interests focus on developing function materials and fluorescent chemosensors.

Wen-Wu Qin received his Ph.D. degree from Lanzhou University, PR China, in 2003.

He is currently a professor at College of Chemistry and Chemical Engineering, Lanzhou University, PR China. His research interests focus on developing fluorescent chemosensors

Graphical Abstract

Sensors **1** and **2**