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Photophysical properties of an on/off fluorescent pH indicator excitable with visible light based on a borondipyrromethene-linked phenol

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Abstract

A borondipyrromethene-derived pH indicator (available as methyl ester (**1**) and sodium salt (**2**)) for the near-neutral pH range with ultra bright fluorescence in the red spectral region has been synthesized by linking *o*-chlorophenol to the 3-position of a difluoroboradiazaindacene derivative. Absorption and steady-state and time-resolved fluorescence measurements have been used to study the photophysical properties of the BODIPY-based pH probe. The fluorescence lifetime (3.8 ± 0.2 ns) and the very high (nearly 1.0) fluorescence quantum yield of dye **1** are not dependent on the solvent. In aqueous solution, the water-soluble compound **2** undergoes a reversible protonation–deprotonation reaction in the near-neutral pH range with a pK_a of 7.60, which is practically insensitive to low ionic strength. Fluorimetric titrations as a function of pH produce fluorescence emission enhancements at lower pH.
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Keywords: Fluorescent probe; PH sensor; Acidity constant; Borondipyrromethene; Spectroscopy

1. Introduction

Measurement of pH by fluorescence-based techniques is well established for both imaging and sensing applications [1]. Fluorescence offers significant advantages over other methods for physiological pH measurements due to its generally non-destructive character, high sensitivity and specificity, and the wide range of indicator dyes available [2]. Fluorescent pH indicators that can sense pH changes within the physiological range are an attractive target in molecular design and synthesis. Due to their excellent photophysical and optoelectronic properties, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene [3,4] (borondipyrromethene, BODIPY [5]) derivatives have become preferred fluorophores in new fluorescent probes that have applications in many different areas [6]. Some recent articles on BODIPY-based chemosensors describe probes for H^+ [7], Na^+ [8], K^+ [9], Ag^+ [10], and Zn^{2+} [11]. The valuable qualities of difluoroboradiaza-*s*-indacene fluorophores comprise relatively high absorption coefficients and fluorescence quantum yields (often approaching 1.0), narrow emission bandwidths with high peak intensities, elevated photostability and chemical stability, and excitation/emission wavelengths above 500 nm. Moreover, importantly, their spectroscopic properties can be fine-tuned by choosing appropriate substituents at the right positions.

Difluoroboradiaza-*s*-indacenes bearing hydroxyaryl subunits have been reported as pH indicators in aqueous-organic mixed media [12,13] and in aqueous solution [7]. The phenol derivatives [12] sense the alkaline pH range, while the calix[4]arene [13] and *o*-chlorophenol derivatives [7] are sensitive in the near-neutral pH range. The lack of fluorescence emission of the phenolate forms was attributed to an intramolecular charge transfer (ICT) between the phenolate anion and BODIPY subunits [7,12]. At lower pH a large fluorescence enhancement without spectral shift was observed.

Here, we report the synthesis and the pH dependent spectroscopic properties of *UBphen* – the methyl ester **1** and the water-soluble sodium salt **2** – substituted with a 2-(3-chloro-4-hydroxyphenyl)ethenyl group at the position 3 of the difluoroboradiaza-*s*-indacene core (Fig. 1). The basic structure of *o*-chlorophenol was preserved to obtain the most favorable pK_a value [7]. It is expected that the introduction of a styryl group at the 3-position will lead to a shift of both excitation and emission bands by ~100 nm to the red [14], elevated

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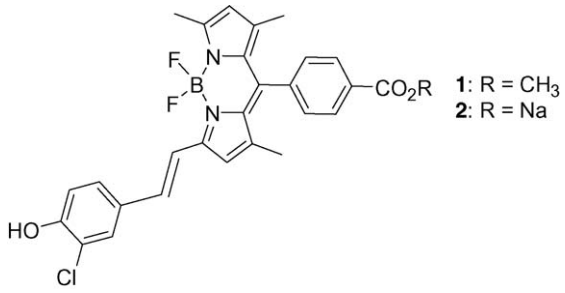

Fig. 1. Chemical structure of *UBphen*: methyl ester **1** and sodium salt **2**.

photostability, high fluorescence quantum yield and absorption coefficients, and a significant absorption wavelength shift upon (de)protonation.

2. Experimental

2.1. Materials

The chemicals for the synthesis were of reagent grade quality, procured from commercial sources, and used as received. All solvents for the spectroscopic measurements were of spectroscopic grade and were used without further purification. Potassium chloride (KCl, 99.999%) and MOPS (3-[*N*-morpholino]propanesulfonic acid, >99.5%) were obtained from Sigma–Aldrich and were used as such.

2.2. Instrumentation

^{1}H and ^{13}C NMR spectra were recorded on instruments operating at a frequency of 300 MHz for ^{1}H and 75 MHz for ^{13}C. ^{1}H NMR spectra were referenced to tetramethylsilane (0.00 ppm) as an internal standard. Chemical shift multiplicities are reported as s = singlet, d = doublet, and m = multiplet. ^{13}C spectra were referenced to the $CDCl_3$ (77.67 ppm) signal. Mass spectra were recorded in E.I. mode. Melting points are uncorrected.

2.3. Steady-state spectroscopy

The absorption measurements were done on a Perkin-Elmer Lambda 40 UV/Vis spectrophotometer. Corrected steady-state excitation and emission spectra were recorded on a SPEX Fluorolog. For the determination of the fluorescence quantum yields (ϕ_f), dilute solutions with an absorbance below 0.1 (1 cm optical path length) at the excitation wavelength λ_{ex} were used. Cresyl violet in methanol ($\lambda_{ex} = 546$ nm, $\phi_f = 0.55$) was used as a fluorescence standard [15]. The ϕ_f values reported in this work are the averages of multiple (generally 4) fully independent measurements. In all cases, correction for the refractive index was applied. All spectra were recorded at room temperature on nondegassed samples.

2.4. Time-resolved spectroscopy

Fluorescence decay traces of compound **1** were recorded by the single-photon timing method [16]. Details of the instrumentation used [17] and experimental procedures [18] have been described elsewhere. The samples were excited at 543 nm with a repetition rate of 4.09 MHz, yielding fluorescence decay histograms in 4096 channels. Using 10 mm × 10 mm cuvettes, fluorescence decays at several emission wavelengths were recorded. The absorbance at the excitation wavelength was always below 0.1. Histograms of the instrument response functions (using LUDOX scatterer), and sample decays were recorded until they typically reached 10^4 counts in the peak channel. The total width at half maximum of the instrument response function was ~60 ps. All fluorescence decays were recorded at 20 °C using nondegassed samples.

The fitting parameters were determined by nonlinear least-squares by minimizing the global reduced chi-square χ^2_g:

$$\chi^2_g = \sum_l^q \sum_i \frac{w_{li}(y^o_{li} - y^c_{li})^2}{\nu} \quad (1)$$

where the index l sums over q experiments, and the index i sums over the appropriate channel limits for each individual experiment. y^o_{li} and y^c_{li} denote respectively the observed and calculated (fitted) values corresponding to the ith channel of the lth experiment, and w_{li} is the corresponding statistical weight. ν represents the number of degrees of freedom for the entire multidimensional fluorescence decay surface.

The additional statistical criteria to judge the quality of the fit have been described elsewhere [19]. The decays were analyzed first individually in terms of decay times τ_i and their associated preexponential factors α_i. The final curve-fitting was done by global analysis in which decays recorded at four different emission wavelengths λ_{em} (from 570 to 630 nm in steps of 20 nm) were described by a monoexponential decay function with a linked lifetime τ and local preexponentials α. The goodness-of-fit was judged for each fluorescence decay trace separately as well as for the global fluorescence decay surface. All curve fittings presented here had χ^2 values below 1.1.

2.5. Determination of K_a from direct fluorimetric titration

The expression of the steady-state fluorescence signal F as a function of the ion concentration has been derived by Kowalczyk et al. for the case of a 1:1 complex between a fluorescent indicator and an analyte (here H^+) [20]. The expression has been extended to the case of a n:1 complex between cation and indicator (Eq. (2)) [21]. The K_a values of the fluorescent pH indicator **2** were determined by fluorimetric titration as a function of pH using the fluorescence excitation or emission spectra (at least four independent measurements were used to compute the average K_a value). Nonlinear fitting of Eq. (2) 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 (corresponding to the basic and acid forms of the pH probe, respectively), and n (the number of protons bound per fluorescent pH probe). Eq. (2) assumes that the absorbance of the sample is small (<0.1) and that the binding of

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