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Fluorescence regulation of 4-aminobenzofluoran and its applications for Cu^{2+}-selective fluorescent probe and bioimaging

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ABSTRACT

Fluoran dyes, a large family of colourants, have been widely used as thermosensitive dyes in print industry by taking advantage of the thermo-controlled interconversions between the colorless spirolactone and the colored zwitterion forms. But few attentions have been paid to their fluorescence properties. In this paper, the fluorescence regulation of 4-aminobenzofluoran dye **1** by introducing of electron-deficient acetyl group to tune the energy level of the highest occupied molecular orbital (HOMO) of the amino group has been carried out. Based on this strategy, the *N*-acetylated product **BF** derived from its precursor **1** shows stable UV–Vis and fluorescence spectra in water from pH 1.0 to 12.0 due to the blocking of the photoinduced electron transfer (PET) process from the amino group to the excited state of fluoran chromophore. Moreover, **BF** is cell permeable and can be used as signal reporter for the design of fluorescence probes by taking advantage of the fluorescence controlled mechanism of the ring opening and closing chemical processes of the spirolactone platform. By using **BF** as precursor, a highly sensitive and selective fluorescent probe **BF-Cu** for Cu^{2+} was synthesized by introducing a Cu^{2+}-responsive water soluble 2-hydroxylethyl hydrazine functional group. We expect that the fluorescence recovery of fluoran dyes via the regulation of the HOMO energy level of the amino group enable an additional synthetic effort on the development of fluorescence fluoran dyes and expansion of their applications.

1. Introduction

Because of their excellent photophysical properties, such as high molar extinction coefficients, excellent fluorescence quantum yields and great photostability, rhodamine dyes have been frequently employed for the design of fluorescent probes for various analytes [1–3]. Meanwhile, the unique spirolactone structure of these dyes provides a universal platform to realize the analyte-triggered fluorescence off-on switch [4–6]. It is well known that fluoran dyes, another type of dyes containing xanthene chromophore, were widely used as thermosensitive dyes in print industry by taking advantage of the thermo-controlled interconversions between the colorless spirolactone and the colored zwitterion forms (Scheme 1) [7,8]. However, few attentions have been paid to the development of fluoran-based probes. To the best of our knowledge, only a small amount of fluoran-based chromogenic chemosensors were developed for sensing of ions [9,10], and no fluoran-based fluorescent probe has been developed up to date.

Previously, our group had developed a few fluorescent probes by using xanthene-based dyes as signal reporter [11]. As a continuous project for fluorescent probe development, we reported herein, the fluorescence regulation of 4-aminobenzofluoran **1** (Chart 1) and its applications for the design of fluorescent probes and biological applications. During the investigation of the UV–Vis and fluorescence spectra of the dye, we found that **1** showed a stable ring-opened zwitterionic structure in water from pH 1.0 to 12.0. It exhibited two absorption bands at ca. 515 nm and 560 nm, respectively, which can be assigned to the π-π* transitions of the ring-opened xanthene ring. With the decrease in pH from 4.0 to 1.0, the absorption band at 560 nm diminished gradually, along with the increase of the absorption band at 515 nm due to the

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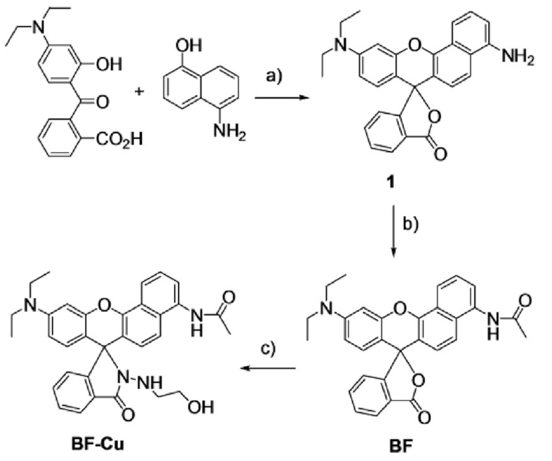


Scheme 1. The synthetic route of **1**, **BF** and **BF-Cu**: a) CH_3SO_3H, 80 °C for 24 h; b) acetyl chloride, Et_3N, dry CH_2Cl_2, 0 °C to rt, 2 h; c) 2-hydroxyethyl hydrazine, dry ethanol, N_2, refluxed for 12 h.

protonation of the amino group. However, **1** only showed fluorescence at ca. 595 nm in a low pH environment (pH < 4.0). Accordingly, even though **1** exists in a ring-opened zwitterionic structure from pH 1.0 to 12.0, its fluorescence signal from pH 4.0 to 12.0 is quenched by the free amino group via a PET mechanism from the electron-donating amino group to the fluoran chromophore (Scheme 2a; Fig. 1b).

2. Experimental

2.1. Materials and equipments

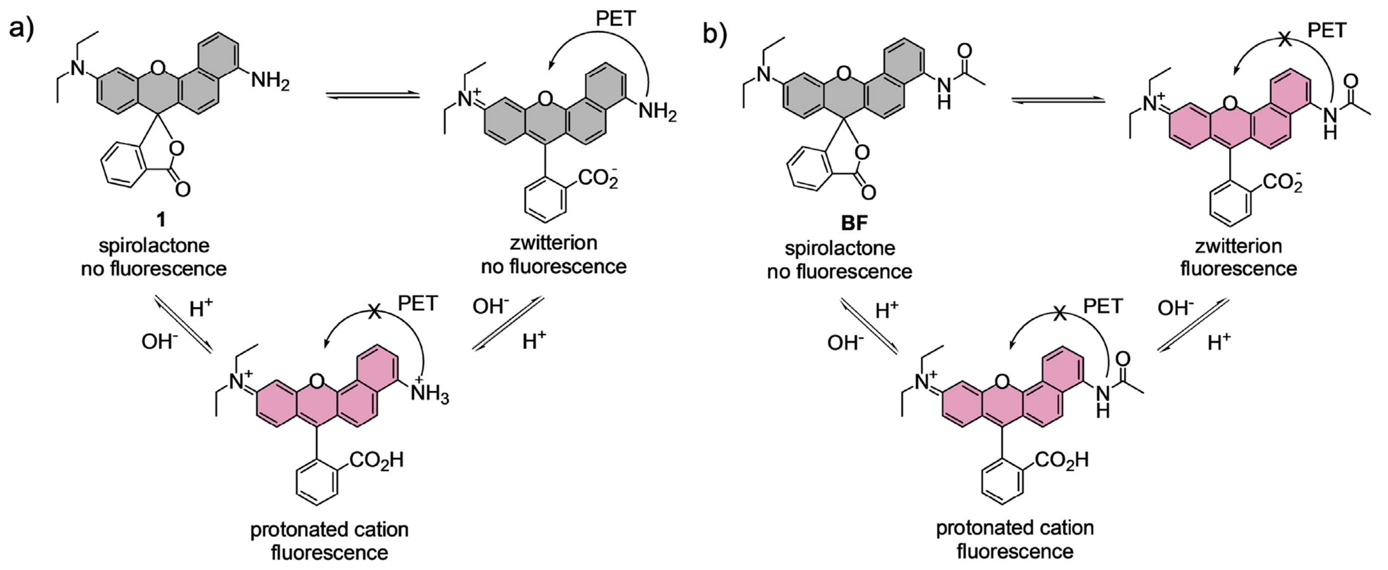
All reagents were obtained from commercial suppliers and used without further purification unless otherwise mentioned. Absorption spectra were measured on UV-2550 UV/Vis spectrophotometer (Hitachi Japan) with 1 cm quartz cell. Fluorescence measurements were carried out on F-4600 fluorescence spectrofluorometer (Hitachi Japan) with a Xenon lamp and 1.0 cm quartz cells. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker spectrometer with chemical shifts reported in ppm (in $CDCl_3$, TMS as internal standard). High resolution mass spectrometry (HRMS) were obtained using an Agilent 6510 Q-TOF LC/MS instrument (Agilent Technologies, Palo Alto, CA) equipped with electrospray ionization (ESI) source. Melting points were recorded on a Boethius Block apparatus. pH measurements were carried out on a Mettler Toledo MP 220 pH meter. Cells were imaged on a confocal microscope (Olympus FV 1000-IX81).

2.2. General methods

Stock solutions (10 mM) of the nitrate salts of Ag^+, Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^+, Mg^{2+}, Na^+, Ni^{2+}, Pb^{2+} and Zn^{2+} ions were prepared in distilled water. Stock solutions of **BF** (5 mM), **BF-Cu** (5 mM) were prepared in DMF. UV–Vis absorption and fluorescence spectra were recorded after incubation of the probe with the appropriate ions. In all cases, the proportion of DMF was maintained to be no more than 0.2%. Unless otherwise stated, all data were collected at 30 min after the addition of guest ions.

2.3. Synthesis of benzofluoran *1*

2-(4-Diethylamino-2-hydroxybenzoyl)benzoic acid (3.13 g, 10 mmol) and 1-amino-5-naphthol (1.59 g, 10 mmol) were mixed in CH_3SO_3H (10 mL) and heated at 90 °C for 24 h. The reaction mixture was poured into stirred ice water and its pH was adjusted to 7–8 with 1.0 M sodium hydroxide solution. The resulted reaction mixture was stirred for 20 min and then extracted with CH_2Cl_2 (50 mL × 3). The separated organic layers were dried over Na_2SO_4 and evaporated to dryness in vacuo, and the residue was purified by recrystallization from acetonitrile to afford a dark gray solid of **1** in 75.0% yield (3.27 g), m.p. 128–130 °C. HRMS (ESI) *m*/*z* cacld. 437.1865 $[M + H]^+$, found *m*/*z* 437.1866 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.08–8.04 (m, 2H), 7.66–7.58 (m, 2H), 7.45–7.37 (m, 2H), 7.16 (dd, J = 6.4 Hz, 1.4 Hz, 1H), 6.89 (dd, J = 7.2 Hz, 0.7 Hz, 1H), 6.72 (d, J = 8.9 Hz, 1H), 6.66 (d, J = 8.9 Hz, 2H), 6.43 (dd, J = 8.8 Hz, 2.5 Hz, 1H), 4.6–3.0 (broad, 2H, $-NH_2$), 3.40 (q, J = 7.1 Hz, 4H), 1.21 (t, J = 7.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.00, 153.93, 152.72, 149.69, 147.89, 142.35, 134.95, 129.58, 128.96, 127.11, 125.17, 124.97, 124.22, 124.18, 122.87, 116.10, 113.04, 112.92, 111.80, 109.03, 105.24, 98.02, 84.78, 44.65, 12.68.



Scheme 2. Proposed conversions of **1** a) and **BF** b) in water under different pH conditions.