

Digital pH Fluorescent Sensing Shown by Small Organic Molecules

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Abstract A series of 2-(*N*-oxide pyridyl)-5-methoxythiazoles were synthesized and corresponding optical properties were also investigated. It is first found that a digital-type fluorescent responses in a sharp pH variation with dual-emission could be fulfilled based on a small fluorophore.

Keywords Fluorescence · pH sensor · Dual-emission · *N*-oxide

Introduction

Fluorescent sensors have been extensively applied in environmental science, medicine, pharmacy, and cellular biology [1–3]. Due to their on-off responses to the external species (stimuli), they were also regarded as the simplest tools that can transmit information on events occurring at a molecular scale to the macroscopic world [4]. A high sensitive ion indicator could provide a digital-type output which is more appreciated in both information processing systems and the involved detection applications. As exemplified by ratiometric fluorescent pH sensors, a ratio between two emission intensities could only provide a measurement in the general response range, i.e., about 2 pH units around pK_a [5–8]. A sharp response to ion concentration variation is thus difficult to obtain in the general organic systems. Considering that a small pH changes (<0.01 pH unit) might be

crucial in certain application, an inner filter effect has been utilized to improve the sensitivity of fluorescent pH sensors [9]. Other digital-typed fluorescent switches involved in a dynamic multichromophore array and copolymers [10, 11]. Up to now, there are no examples for a small fluorophore-based pH sensor with digital-type output.

Previously, we reported 5-methoxy-2-pyridylthiazoles (MPTs) as ratiometric fluorescent pH sensors with high quantum yields [12]. In the presented work, their pyridine *N*-oxides (MPTOs) are reported, which are not only a series of pH fluorescent sensors with dual-emission but also exhibit a digital-type response in an unusually narrow pH ranges (about 0.5 pH units).

Experimental

Materials and Methods

All chemicals were purchased from Acros and used as received without further purification. All reactions were performed under argon atmosphere using purified solvents by standard methods. For all spectrometric measurements, HPLC grade solvents were degassed before use. Aqueous solutions for spectrometric measurements were prepared by dissolving in doubly distilled and deionized water. Flash chromatography was performed with 100–200 mesh silica gel (Qingdao, China) and thin-layer chromatography (TLC) was carried out on silica coated glass sheets (Qingdao silica gel 60F-254).

UV–vis absorption spectra were measured with a Hitachi UV-3010 spectrophotometer and the fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and uncorrected. ^1H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra

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were recorded with a Burkert AV 300 (300 MHz) instrument using tetramethylsilane as the internal standard. IR spectra were recorded on a Perkin-Elmer 1200 FT-IR spectrometer. High-resolution mass spectra were taken on a Shimadzu GC-MS-QP2010. Elemental analyses were performed at Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, China.

All spectra measurements were recorded at 20 °C. The pH values were adjusted by addition of 0.1 M trifluoroacetic acid aqueous solution and then determined by a pH-meter at 20 °C.

Synthesis

5-Methoxy-2-(1-Oxide Pyridin-2-yl)thiazole (2-MPTO)

To a solution of picolinic acid *N*-oxide (0.5 g, 3.6 mmol) in 30 mL CH₂Cl₂ was added EDC (0.69 g, 3.6 mmol), HOBt (0.49 g, 3.6 mmol) and triethylamine (1.25 mL, 9 mmol). After stirring for 30 min under ice-bath, glycine methyl ester hydrochloride (0.5 g, 3.96 mmol) was added. The reaction mixture were allowed standing for further 16 h at room temperature, which were then washed by 10 % citric acid solution (20 mL), saturated NaHCO₃ solution (20 mL) and brine (20 mL). Organic layer was then dried over anhydrous Na₂SO₄ followed by condensed under reduce pressure. Separation through column chromatography using *n*-hexane and ethyl acetate as eluent provided the needed amide as white solid (0.43 g, yield: 56 %).

The obtained amide (100 mg, 0.546 mmol) and Lawesson's reagent (256 mg, 0.633 mmol) were refluxed in dried toluene for 16 h under Ar atmosphere. The resulting slurry was poured into a 10M NaOH aqueous solution in an ice-bath and extracted with ethyl acetate. The organic layer was followed by concentration under reduced pressure after being dried over anhydrous Na₂SO₄. Separation through column chromatography using *n*-hexane and ethyl acetate as eluent provided the product as an oil, which could be slowly recrystallized in ether under -5 °C (18 mg, yield: 18 %).

Mp: 14–16 °C. IR (film, cm⁻¹): 1531, 1490, 1453, 1419, 1262. ¹H NMR (CDCl₃, 300 MHz) δ=8.55 (d, *J*=4.5 Hz, 1H), 8.33 (d, *J*=8.0 Hz, 1H), 7.78–7.76 (m, 1H), 7.28–7.24 (m, 1H), 7.20 (s, 1H), 3.39 (s, 3H). ¹³C NMR (CDCl₃, 75 MHz) δ=165.4, 156.2, 151.8, 149.2, 136.8, 123.7, 122.6, 118.5, 61.0. Calcd for C₉H₈N₂O₂S: C 51.91, H 3.87, N 13.45. Found: C

51.66, H 4.01, N 12.98. Calcd for C₉H₈N₂O₂S: 208.2370, HRMS (*m/z*): 208.2369.

5-Methoxy-2-(1-Oxide Pyridin-3-yl)thiazole (3-MPTO)

Yield: 40 %. Mp: 47–49 °C. IR (KBr, cm⁻¹): 1529, 1491, 1431, 1276, 1217. ¹H NMR (CDCl₃, 300 MHz) δ=9.02 (s, 1H), 8.61–8.59 (m, 1H), 8.10 (d, *J*=7.9 Hz, 1H), 7.36–7.32 (m, 1H), 7.18 (s, 1H), 3.98 (s, 3H). ¹³C NMR (CDCl₃, 75 MHz) δ=163.7, 151.6, 149.9, 146.8, 132.5, 130.1, 123.5, 122.3, 61.6. Calcd for C₉H₈N₂O₂S: C 51.91, H 3.87, N 13.45. Found: C 51.70, H 3.75, N 13.05. Calcd for C₉H₈N₂O₂S: 208.2370, HRMS (*m/z*): 208.2369.

5-Methoxy-2-(1-Oxide Pyridin-4-yl)thiazole (4-MPTO)

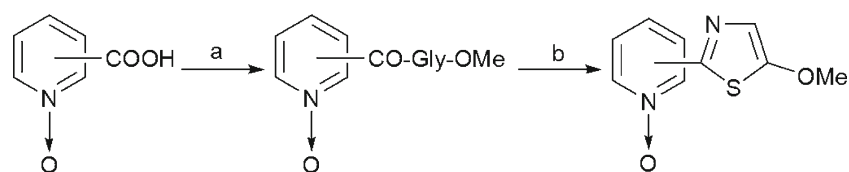
Yield: 24 %. Mp: 15–17 °C. IR (KBr, cm⁻¹): 1595, 1526, 1497, 1456, 1279. ¹H NMR (CDCl₃, 300 MHz) δ=8.65 (d, *J*=5.4 Hz, 2H), 7.66 (d, *J*=5.4 Hz, 2H), 7.24 (s, 1H), 4.00 (s, 3H). ¹³C NMR (CDCl₃, 75 MHz) δ=174.8, 152.1, 150.6, 141.1, 123.1, 119.5, 61.8. Calcd for C₉H₈N₂O₂S: C 51.91, H 3.87, N 13.45. Found: C 51.52, H 4.03, N 12.88. Calcd for C₉H₈N₂O₂S: 208.2370, HRMS (*m/z*): 208.2369.

Results and Discussion

Synthesis of all three MPTOs is shown in Scheme 1. Their optical data are collected in Table 1. Both 2- and 4-MPTO are highly fluorescent and comparable to their non-oxidized form.

Both the maxima of the absorption (see Fig. S1–3 in ESI) and fluorescence (see Fig. S6–9 in ESI) of MPTOs were red shifted with the decrease in pH values (Figs. 1 and 2). Such bathochromic absorption and emission are assigned to the protonated form, MPTO-H⁺. An isobestic point could be observed in the absorption evolution of every MPTO (see Fig. S4–6), which indicated the presence of equilibrium of protonation between the acidic and basic forms of MPTO. The p*K*_a(MPTO-H⁺/MPTO) values of the ground states of MPTOs were evaluated from the UV–vis absorption titration experiments, which are in good agreement with the p*K*_a of tertiary amine *N*-oxide [13, 14]. It is thus reasonable to ascribe that the protonation occurred at the oxygen site of pyridine *N*-oxide. The p*K*_a^{*}(MPTO-H⁺/MPTO) values were

Scheme 1 Synthesis of 5-methoxy-2-pyridylthiazole *N*-oxides



a. EDC, HOBt, Gly-OMe, TEA;
b. Lawesson's reagent, PhCH₃.

R = 2-(*N*-oxide pyridyl): **2-MPTO**
3-(*N*-oxide pyridyl): **3-MPTO**
4-(*N*-oxide pyridyl): **4-MPTO**

Table 1 Photophysical properties of MPTOs. Maxima of absorption^a λ_{abs} and of steady fluorescence emission^b λ_{em} , molar absorption coefficients $\epsilon_{\text{max}} \pm 5\%$, and fluorescence quantum yields^c $\Phi \pm 5\%$

	λ_{abs} (nm)	$\log(\epsilon_{\text{max}}/\text{M}^{-1} \text{cm}^{-1})$	λ_{em} (nm)	Φ	$\text{p}K_{\text{a}}$	$\text{p}K_{\text{a}}^*$
2-MPTO	322	3.56	395	0.81		
2-MPTO- H^+	363	3.81	450	0.54	2.2	13.8
3-MPTO	310	4.33	385	0.24		
3-MPTO- H^+	328	4.17	425	0.26	4.2	8.4
4-MPTO	318	3.98	410	0.73		
4-MPTO- H^+	366	4.66	450	0.76	4.2	13.2

^a All spectra were recorded at 291 K. General concentrations of analysts were 100 and 25 μM , respectively, for measurement of absorption and steady fluorescence emission

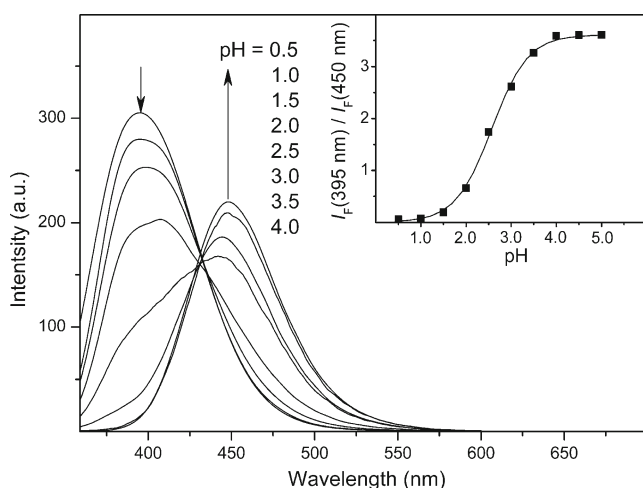
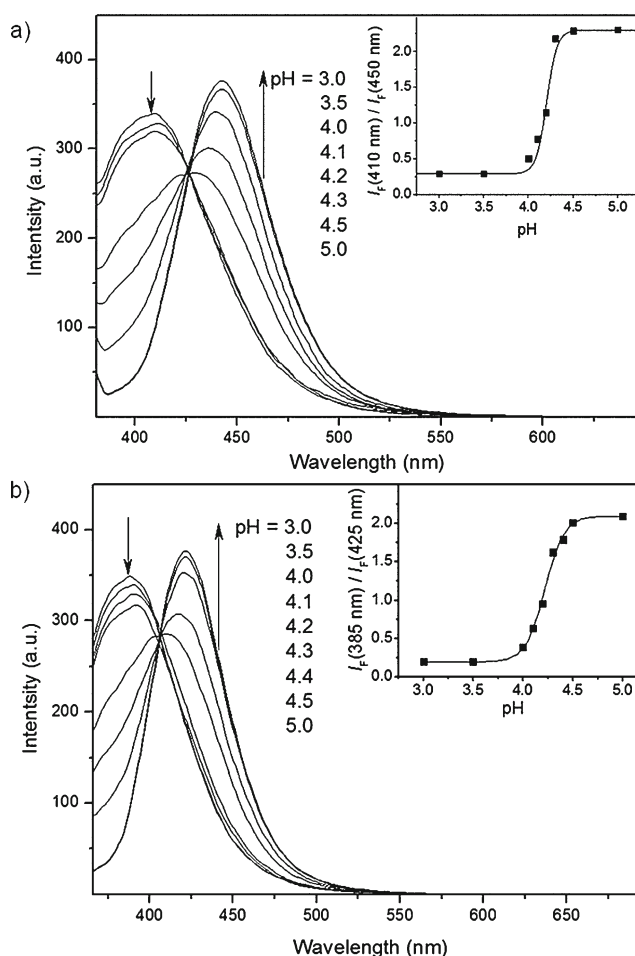
^b Excited at 332, 332 and 335 nm for 2-MPTO, 4-MPTO and 3-MPTO, respectively

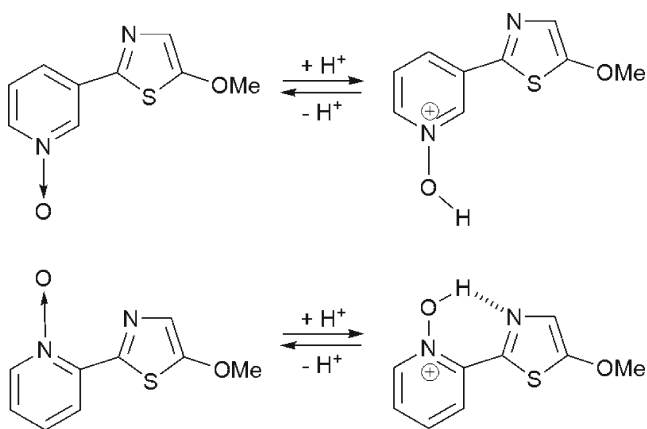
^c Relative quantum yields were evaluated using quinine sulfate ($\Phi=0.48 \pm 0.2$ in 0.5M H_2SO_4 , excited at 313 nm) as the standard compound

estimated according to the previously reported equation (Table 1) [12]. Compared with the ground states, an increased basicity of the excited states of MPTO suggested that an internal charge transfer to the pyridyl ring should be expected. In viewpoint of molecular structure, the more basic 2-MPTO- H^+ could be expected due to the hydrogen bond formed with the nitrogen atom of thiazole ring (Scheme 2). In the other hand, more strong internal charge transfer should result in higher basicity for the excited states of 2- and 4-MPTO, respectively, which should be ascribed to the strong electron-withdrawing abilities of 2- or 4-substituted pyridyl ring compared to 3-MPTO.

From the fluorescence evolution of 2-MPTO with pH values, a typical dual-emission response could be found in the range from 1.5 to 3.5, i.e. 2 pH units. However, in the evolution of the fluorescence titration experiments, an unusual narrow responding pH range, i.e. about 0.5 pH unit, was observed for both 3-MPTO and 4-MPTO, which is the first example of the exhibition that a digital-type fluorescent

output could be fulfilled in a small organic fluorophore (Fig. 2). We ascribed such step response to the protonation of the pyridine *N*-oxide group. For 2-MPTO, a little wide pH range observed may be introduced by the possible hydrogen bond (Scheme 2).

**Fig. 1** Emission evolution of 2-MPTO with pH values in the aqueous solution**Fig. 2** Emission evolution of 3-MPTO (up) and 4-MPTO (down) with pH values in the aqueous solution



Scheme 2 Protonation of MPTOs

Conclusions

In conclusion, we first discovered the digital-type output exhibited by small organic fluorophores. The unusual narrow responding range also provided the opportunity to more exact determination of pH value in aqueous system. Further investigation is on the way to reproduce such digital-type output in the physiological pH of blood, i.e. 7.34–7.35.

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