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1,9-Dihydro-3-phenyl-4*H*-pyrazolo[3,4-*b*]quinolin-4-one, a novel fluorescent probe for extreme pH measurement

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The synthesis of 1,9-dihydro-3-phenyl-4*H*-pyrazolo[3,4-*b*]quinolin-4-one and its pH-dependent fluorescent properties for extreme pH measurement are presented.

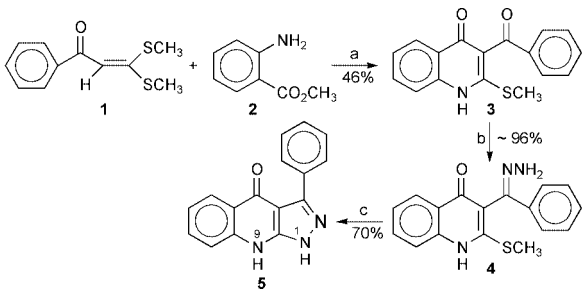
The accurate measurement of pH is very important because it usually plays a key role in a variety of systems. The most popular and direct device for pH measurement is the glass pH electrode. However, the known limitations of the glass pH electrodes (*e.g.* its electrical interference or mechanical damage to small cells, and the presence of acid error and especially alkaline error[1]) make them unsuitable for certain applications: intracellular pH and microscopy studies as well as the measurements of extreme pH values below 1 or above 9. In contrast to the electrochemical methods, optical measurements, based on fluorescent probes that are either protonated or deprotonated, have no such drawbacks.[2] Moreover, fluorescent measurements are convenient to microscopy studies, and can reflect the H^+ distribution and change within cells.[3] It is not surprising, therefore, that the researches on pH-dependent fluorescent probes have received a great attention,[4] particularly on the probes which are pH-sensitive to the near neutral pH value of normal body fluids for the point of biological application. To the best of our knowledge, however, relatively less attention was paid on the fluorescent probes which are pH-sensitive in the lower pH region (pH < 5) or the higher pH region (pH > 9), though in some cases pH changes (*e.g.* within the stomach) can enter the extreme pH ranges.[1,5] It is also a challenge to design a fluorescent probe with linear response over a broad range, because pH measurement can be accurately made only over a range of about two pH units, *i.e.* p*K*a ± 1. Although some attempts have been made to broaden the response range of pH measurement by using a mixture of multiple pH indicators,[6] this makes the system rather complex. Another feasible approach to the problem is to develop a probe with multiple steps of H^+ binding.[7] Unfortunately, the typically used pH-dependent groups such as –COOH, –OH, *etc*., have a high affinity for common metal ions, thus resulting in complexation and nonspecific response of the probe to H^+.

The objective of this research was to design a novel fluorescent probe which is both pH-selective and pH-sensitive for extreme pH ranges, by assembling several electronegative atoms in the different positions of a highly conjugated molecule. Scheme 1 shows the synthetic route to such a fluorescent probe, 1,9-dihydro-3-phenyl-4*H*-pyrazolo[3,4-*b*]quinolin-4-one **5**.[8] The reaction of 2-benzoylketene dithioacetal **1**[9] with **2** gave methylthio-substituted quinolone **3**. Upon treatment with hydrazine, **3** was converted quantitatively to hydrazone **4** which was then transformed into the designed product **5** by further heating in pyridine.

In order to increase the selectivity for H^+, the use of carboxyl which is easy to complex with metal ions was avoided. pH-dependent amino groups, though they are also strong ligands for metal ions, were mainly chosen as H^+ receptors because of their convenient arrangement in synthesis and the wealth of proton binding data available.[10] The arrangement of multiple H^+ receptors is conducive to obtaining a broad pH response range, and three nitrogen atoms and one oxygen atom were set in the probe **5**. To achieve the fluorescent response to extreme pH values, the environmental difference among the electronegative atoms in the conjugated molecule should be as large as possible. The potential binding sites for H^+ in **5** were therefore placed in quite different environments. Particularly following the known data,[11] one nitrogen atom [*e.g.* N(9)] was arranged in an electron-deficient position and another one N(1) in an electron-rich position, expecting the generation of considerably different p*K*a values. In addition, for improved selectivity, the arrangement of all the electronegative atoms in the structure should not provide a suitable cavity or a convenient formation of five- and six-membered ring complexes for metal ions. As shown in Scheme 1, the prepared probe **5** does not possess any favorable complexation sites for metal ions. The benzene ring in the position 3 of the probe would render a steric hindrance for any possible complexation of either the adjacent oxygen or nitrogen atom with metal ions.

The experimental results showed that the probe has a notable fluorescence quantum yield of φ_{base} = 0.14 in basic media (pH = 13.0) or φ_{acid} = 0.12 in acidic media (pH = 1.0) with an appreciable fluorescence lifetime of 11.12 ± 0.04 ns,[12] and its fluorescence spectra are highly dependent on pH (Fig. 1). Titration of fluorescence intensity with pH gave two p*K*a values of 2.61 and 12.44 (Fig. 1, insets),[13] which correspond to the protonations of the two nitrogen atoms N(9) and N(1) in the probe, respectively. It is understandable that only two of the electronegative atoms exhibit pH-sensitivity, because the basicity of the other two (one nitrogen and one oxygen) is largely weakened by their lone electron pairs participating in the system conjugation. As a result, the probe is capable of measuring the extreme pH values over the two pH ranges of 1.8–3.4 and 11.6–13.3, respectively. Further, the probe was stable and no obvious changes in fluorescence were observed within 5 months at rt. It should be pointed out that the probe is unsuitable for neutral pH measurements, since a nonlinear response of fluorescence intensity to pH was observed in that region.

To test the selectivity of the probe, the effects of various diverse ions upon the emission spectra were examined. The results showed that the selectivity of this probe for H^+ over other



Scheme 1 *Reagents and conditions*: (a) propanoic acid, reflux 48 h; (b) hydrazine hydrate (30%), ethanol reflux; (c) anhydrous pyridine, argon protection, reflux 60 h.

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Table 1 The tolerable concentration of foreign ions for the measurement of pH[a]

M^{n+}	Ca^{2+}	Cu^{2+}	Fe^{2+}	Fe^{3+}	K^+	Mg^{2+}	Mn^{2+}	Zn^{2+}	NO_3^-	PO_4^{3-}	CO_3^{2-}	SO_4^{2-}
$c/10^{-4}$ M[b]	10	10	0.70	0.40	20	0.80	40	2.6	0.50	2.0	0.20	4.0
$c/10^{-5}$ M[c]	20	4.5	3.6	3.4	20	0.32	0.20	2.2	1.0	1.0	8.3	20

[a] The tolerable concentration was estimated by the criterion at which a species gave a relative error of no more than 5% in the analytical signal (fluorescence intensity) of the probe (2.0×10^{-3} g dm^{-3}) in the presence of 0.1 M NaCl. [b] Measured in acid medium (pH = 2.0). [c] Measured in basic medium (pH = 12.0).

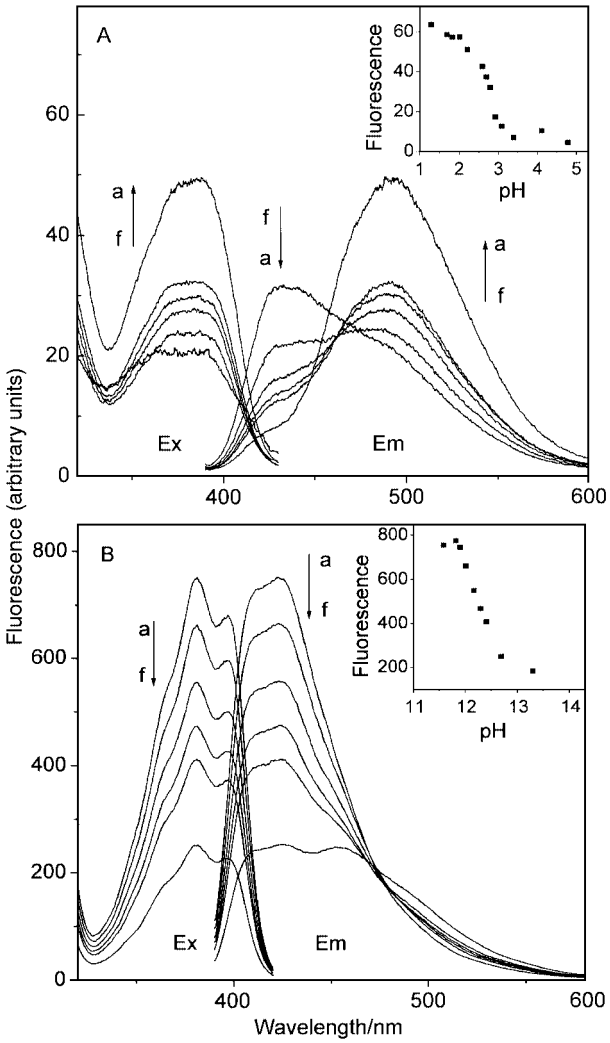

Fig. 1 pH-dependence of the fluorescence spectra of **5** (5×10^{-3} g dm^{-3}) in 0.1 M NaCl at various pH values (A): 1.29 (a), 2.59 (b), 2.72 (c), 2.81 (d), 3.09 (e), 3.41 (f); and (B): 11.82 (a), 12.01 (b), 12.17 (c), 12.29 (d), 12.41 (e), 12.69 (f). Different pH values were obtained by adding small amounts of 0.1 M HCl or NaOH to the solution. The excitation was at 385 nm for both of the emission spectra (Em) of (A) and (B); the emission was at 493 and 433 nm for the excitation spectra (Ex) of (A) and (B), respectively. Inset shows the variation of fluorescence emission intensity with pH at 493 (A) and 433 nm (B), respectively.

ions (Table 1) is of specificity, making it very useful for accurate measurement of extreme pH values.

The mechanism of the probe's fluorescence response to pH is complex due to the influence of protonation/deprotonation and the possible presence of enol–amide tautomerism, but it may be interpreted largely according to photoinduced electron transfer (PET) principle, which has been widely used to design a variety of fluorescent ion sensing molecules.[14] Nitrogen atoms often serve as both H^+ receptors and electron donors in the PET process. In acid media, the probe **5** has a longer fluorescence emission of 493 nm at the optimal excitation of 385 nm (Fig. 1A), and the H^+ binding equilibrium of the N(9) atom yields an isosbestic point at 462 nm. Further protonation of the probe may inhibit the related PET, resulting in fluorescence enhancement (Fig. 1, curve a). On the other hand, the deprotonation of the N(1) atom in basic media presumably revives the corresponding PET, causing fluorescence quenching at 433 nm (Fig. 1B).

In summary, a novel fluorescent pH probe **5** has been prepared by arranging several electronegative atoms in the different environments of a conjugated molecule. Although this probe is far from being an ideally broad pH sensor, it greatly complements glass pH electrodes. Further work in this area would be beneficial to designing more excellent fluorescent pH probes.

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