Facile Derivatization of Pyridyloxazole-type Fluorophore via Click Chemistry

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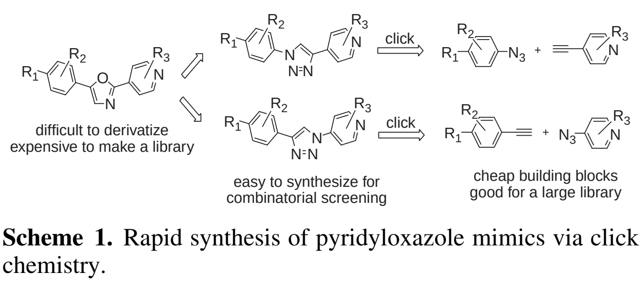
New derivatives of the pyridyloxazole-type fluorophore were synthesized in high efficiency through click chemistry. This method is potentially useful for the combinatory screening of fluorescent labels to target specific biological processes. Using this method, we successfully developed three new ratiometric dual-fluorescent pH sensors.

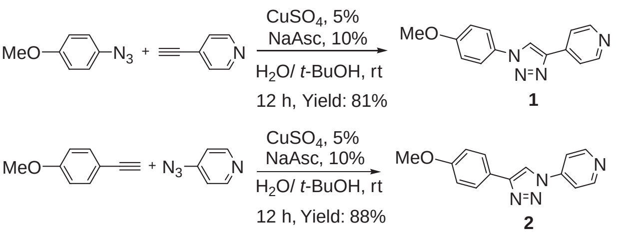
The development of powerful microscopy techniques and the availability of diverse fluorescent probes have made fluorescence microscopy an indispensable tool in chemical biology.[1] A current focus in the field is the search for fluorescent labels that can specifically target each biomacromolecule or organelle in the living cells. This task demands chemists to prepare libraries of functionalized fluorescent molecules from which some active ones may be selected through high-throughput screenings. Unfortunately, the backbone structure of most existing fluorescent molecules (fluorescein and pyranin, for instance) does not allow facile synthesis of such libraries of diversity-oriented derivatives.[2]

Here, we report a novel approach to overcome the difficulty in the combinatorial synthesis of fluorescent molecules. In this approach, we used the "click chemistry"[3] method to construct a functionalized fluorescent compound from simpler building blocks (Scheme 1). Undeniably this fluorophore would possess a triazole moiety that is not present in any existing fluorescent probes. However, we hypothesized that replacement of an aromatic ring by triazole would not dramatically alter the fluorescence property. Using this approach one might rapidly synthesize a library of different fluorescent molecules. From this library one then might be able to find the most active compound for a specific biological target. After the best compound is found, the triazole moiety could be further changed back to the original aromatic ring, or any other aromatic ring to give the most desirable fluorescence.

To demonstrate the utility of the approach, we chose a popular fluorescent molecule, 5-aryl-2-(4-pyridyl)oxazole.[4] This compound exhibits favourable photophysical features after one-photon excitation and strong fluorescence emission. One of its derivatives, PYMPO (Figure 1), has recently been successfully developed as a fluorescent pH probe for the study of biological samples in vivo.[5,6] Our questions were two-fold: (1) Whether could its triazole derivatives be easily synthesized via click chemistry? (2) Whether did the triazole derivatives exhibit similar fluorescent properties as PYMPO? To answer these questions, we synthesized compounds **1**[7] and **2**[8] from aryl or pyridyl alkynes and azides using the standard click chemistry method (Scheme 2).[9] The yields of the two reactions were high, and the reaction conditions were very mild (i.e. at room temperature, in aqueous media, and without inert gas protection) and compatible with different delicate functional groups.



Scheme 1. Rapid synthesis of pyridyloxazole mimics via click chemistry.



Scheme 2. Synthesis of 1,4-disubstituted 1,2,3-triazoles.

As shown in Figure 1, the fluorescence spectrum of compound **1** was similar to that of PYMPO where there were interestingly two pH-dependent peaks. When excited at 280 nm (the isosbesic point), the fluorescence emission spectrum of the protonated form of **1** was considerably red-shifted and broadened relative to that of the free form. As the pH increased a new band with an isoemission point at 455 nm appeared in the longer wavelength region. Due to these properties compound **1** could serve as a fluorescent probe to measure pH values in the acid to neutral range 1.5–7.0 through a ratiometric method, in the same manner as PYMPO (Figure 1). Compared to compound **1**, the variation of the two peak ratio from pH 1.5 to 7.0 was about 25 fold, whereas the variation of PYMPO was about 10 fold (See Inset in Figure 1). Therefore, compound **1** represented a more sensitive ratiometric sensor than PYMPO. On the other hand, compared to compound **1**, the synthesis and functionalization of the oxazole-containing PYMPO was much more sophisticated and low-yielding.[4] Thus, our experiment demonstrated not only the possibility, but also the desirable value of using triazole to replace the aromatic ring in the existing fluorophore.

Having established the validity of using click chemistry to rapidly synthesize a fluorescent compound, we next tried to examine whether the same approach could be used to prepare a library of fluorophores. Therefore, we selected several aryl or pyridyl alkynes and azides. Using the same click chemistry method, we easily synthesized six molecules in high yields, which were all found to be good fluorescent indicators (Table 1). The fluorescence properties of these newly synthesized compounds were then recorded in both acidic and basic buffers. To our surprise, as shown in Table 1 compound **1** exhibited two pH-dependent

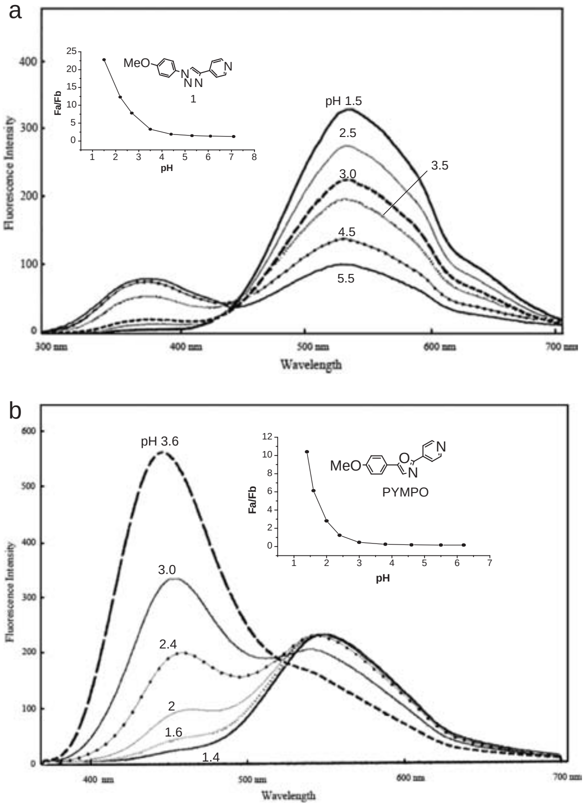
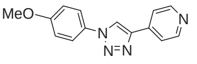


Figure 1. Fluorescence emission spectra of **1** and PYMPO at various pH values. Inset: evolution of the ratio of the fluorescence emission at acidic and base states of the indicator.

Table 1. Fluorescence spectral properties of a library of pyridyltriazole compounds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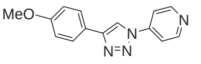
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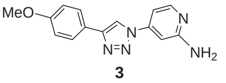
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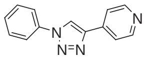
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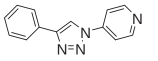
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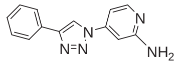
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Compound	λ_{ex}/nm	$\lambda_{em}{}^{a}$/nm	Φ^{a}	$\lambda_{em}{}^{b}$/nm	Φ^{b}
1	280	545	0.34	385	0.09
2	320	—	—	431	0.35
3	320	531	0.06	411	0.41
4	280	433	0.21	—	—
5	300	484	0.14	372	0.07
6	300	—	—	414	0.23

[a]Measurements were carried out in acidic buffers. [b]Measurements were carried out in basic buffers. Φ = fluorescence quantum yield, calculated using PYMPO as standard ($\Phi = 0.72^{4}$ in buffer).

peaks whereas its isomer, **2**, only displayed one. Similarly, comparing compounds **4** and **5** also demonstrated that the positioning of the triazole nitrogens played an important role in determining the pH-dependence of the fluorescence. Furthermore, compound **3** exhibited two pH-dependent peaks but compound **6** only displayed one pH-dependent peak. This indicated that the substituent could dramatically alter the pH-dependence property of the pyridyltriazole dye. This behaviour should be desirable because we wished to prepare a library of fluorophores that displayed dramatic property variations. In the present case, it was evident that compounds **1**, **3**, and **5** constituted novel fluorescent sensors for the pH changes that could function in a ratiometric fashion (i.e. there were two pH-dependent fluorescent peaks). Such a ratiometric measurement is important in studying biological samples because it minimizes any false signal due to the uneven concentration distribution.[10]

Using this method,we have successfully identified three new ratiometric dual-fluorescent pH indicators including compounds **1**, **3**, and **5**. Meanwhile, we have also observed some interesting substituent effects on the pH-dependence and ratiometric fluorescence property in the present sensor system. Currently, we are using this method to prepare libraries of PYMPO derivatives to develop sensors for other biologically significant analytes such as Ca^{2+} and Zn^{2+}. Furthermore, we are trying to apply the same click chemistry method to other classes of fluorophores, where we hope to develop new classes of fluorescence molecules that can be easily derivatized and tuned.

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References and Notes

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7 Characterization of **1**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.89 (3H, s), 7.06 (2H, d, $J = 8.6$ Hz), 7.69 (2H, d, $J = 8.6$ Hz), 7.79 (2H, d, $J = 5.9$ Hz), 8.24 (1H, s), 8.69 (2H, d, $J = 5.7$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.80, 115.05, 119.51, 120.14, 122.43, 130.27, 137.92, 145.82, 150.60, 160.30; HRMS (EI) m/z: calcd for $C_{14}H_{12}N_4O$ [M^+] 252.1011, found 252.1012.

8 Characterization of **2**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.87 (3H, s), 7.01 (2H, d, $J = 8.4$ Hz), 7.78 (2H, d, $J = 5.4$ Hz), 7.85 (2H, d, $J = 8.4$ Hz), 8.21 (1H, s), 8.80 (2H, d, $J = 5.4$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.26, 114.54, 118.42, 122.29, 126.89, 133.26, 142.52, 147.80, 151.69, 159.56; HRMS (EI) m/z: calcd for $C_{14}H_{12}N_4O$ [M^+] 252.1011, found 252.1009.

9 Synthesis of the triazole compound **1**: To a mixture of 1-azido-4-methoxybenzene (149 mg, 1 mmol) and ethynylbenzene (102 mg, 1 mmol) in water and *tert*-butyl alcohol (v/v = 1:1, 4 mL), sodium ascorbate (NaAsc, 0.1 mmol) was added, followed by the addition of $CuSO_4$ (12.5 mg, 0.05 mmol). The mixture was stirred overnight in the dark at room temperature. TLC analysis indicated complete consumption of the reactants in 12 h.

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