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pH-sensitive fluorescent deoxyuridines labeled with 2-aminofluorene derivatives

Ji Won Lee [a], Ye-seul Son [a], Jung-Yean Lee [a], Mi Hyun Kim [b], Sang-Keun Woo [b], Kyo Chul Lee [b], Yong Jin Lee [b], Gil Tae Hwang [a,*]

[a] Department of Chemistry, Kyungpook National University, Daegu 41566, Republic of Korea
[b] Molecular Imaging Research Center, Korea Institute of Radiological and Medical Sciences (KIRAMS), Seoul 01812, Republic of Korea

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ABSTRACT

Two fluorescent 2′-deoxyuridines, $\mathbf{U}^{\mathbf{AF}}$ and $\mathbf{U}^{\mathbf{DAF}}$, labeled with 2-aminofluorene and 2-dimethylaminofluorene units, respectively, and having values of pK_a of 4.27 and 4.66, respectively, display 'turn-on' emission responses in acidic solutions. They can also penetrate into HeLa cell membranes, where they exhibit their strong fluorescence under acidic conditions.

1. Introduction

The proton is one of the most important targets in physiological and pathological processes because intracellular pH plays a critical role in many cellular, enzymatic, and tissue activities, including cell growth and apoptosis, ion transport, endocytosis, multidrug resistance, and enzyme activity.[1] In particular, abnormal values of intracellular pH are found in such diseases as ischemic stroke,[2] cystic fibrosis,[3] subarachnoid hemorrhage,[4] epilepsy,[5] Parkinson's disease,[6] Alzheimer's disease[7] and cancer.[8] Therefore, monitoring the pH of living cells in real time remains an important challenge.

Fluorescent pH probes offer several attractive features, including high sensitivity, short response time, real-time monitoring and nondestructive identification; these features set such probes apart from other pH measurement methods using, for example, electrodes,[9] semiconductor sensors[10] or nuclear magnetic resonance (NMR) spectroscopy.[9a,11] In particular, fluorescent aromatic amines are useful as pH-responsive probes because they can display photo-induced intramolecular charge transfer (ICT), in which an electron migrates from the amino group to the aromatic ring upon light absorption in their free-base forms,[12] whereas protonation of their amino groups terminates their electron-donating ability. In other words, ICT is influenced in different ways by the pH. Thus, the pH-dependence of the absorption and emission behavior of aromatic amines is of great interest and has been studied to investigate the protonation/deprotonation reactions of aromatic amines in their ground and excited states.[1,12d]

Fluorescent nucleosides bearing aromatic amino moieties are especially attractive because, as nucleoside derivatives, they can be taken up into cells through the action of either concentrative nucleoside transporters or equilibrative nucleoside transporters;[13] in the absence of a nucleoside unit, microinjection[14] or carrier-mediated endocytosis[15] would have to be used to import fluorescent compounds into cells, and these approaches tend to perturb the physiology of the cell resting state. Although several pH-sensitive fluorescent nucleosides have been described,[16] there have been few reports of deoxyuridine-bearing aromatic amines as ICT molecules and fluorophores.[16b,c] Because deoxyuridine labeled with a fluorophore is a nucleoside that can act as a microenvironment-sensitive probe,[17] its labeling with aromatic amines would affect its absorption and emission maxima and lead to protonation/deprotonation-induced on/off fluorescent switching that could be used in aqueous solution to sense pH.

Herein, we report two efficient pH-responsive fluorescent probes comprising deoxyuridine derivatives bearing 2-aminofluorene (**AF**) and 2-dimethylaminofluorene (**DAF**) units, designated $\mathbf{U}^{\mathbf{AF}}$ and $\mathbf{U}^{\mathbf{DAF}}$, respectively.

* Corresponding author. Fax: +82 53 950 6330; e-mail address: giltae@knu.ac.kr (G.T. Hwang).

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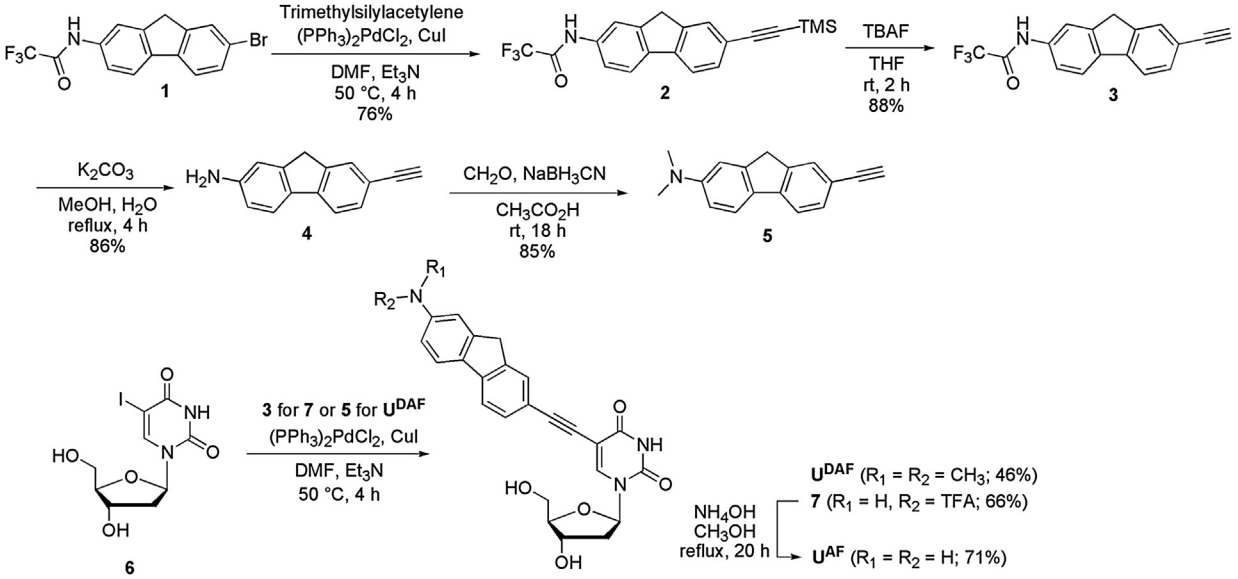
2. Results and discussion

Scheme 1 presents the synthetic strategy we used to prepare the fluorescent nucleosides $\mathbf{U}^{\mathbf{AF}}$ and $\mathbf{U}^{\mathbf{DAF}}$. 2-Ethynyl-7-trifluoroacetylaminofluorene (**3**) was prepared through palladium-catalyzed Sonogashira coupling of **1**[18] with TMS-acetylene followed by deprotection with TBAF. Deacetylation of **3** with K_2CO_3, followed by reductive amination with formaldehyde, yielded compound **5**. Subsequent Sonogashira couplings of 2′-deoxy-5-iodouridine (**6**) with the acetylene derivatives **3** and **5** afforded the fluorescent nucleosides **7** and $\mathbf{U}^{\mathbf{DAF}}$, respectively. We obtained $\mathbf{U}^{\mathbf{AF}}$ through deprotection of **7** with NH_4OH. We employed a previously reported procedure to synthesize $\mathbf{U}^{\mathbf{FL}}$, bearing the fluorene (**FL**) unit as a reference probe.[17d]

acidic conditions. The most remarkable feature of the absorption spectra of **DAF** and $\mathbf{U}^{\mathbf{DAF}}$ was that their primary bands grew dramatically upon acidification relative to those of **AF** and $\mathbf{U}^{\mathbf{AF}}$, suggesting that the protonated form containing a **DAF** moiety absorbed more light at a given wavelength than the protonated form containing an **AF** moiety.

We then examined the fluorescence of all fluorophores in aqueous solutions having various pH values upon excitation at wavelengths near their isosbestic points (Fig. 1). We observed no consistency in the fluorescence intensity of **FL** upon changing the pH (Fig. 1a), whereas the signals of $\mathbf{U}^{\mathbf{FL}}$ were relatively red-shifted and quenched upon acidification (Fig. 1b). This behavior may have originated from the mesomeric effect and weak acidic properties of the uracil base; N1 of uracil could act as a weak



Scheme 1. Synthesis of $\mathbf{U}^{\mathbf{AF}}$ and $\mathbf{U}^{\mathbf{DAF}}$.

First, we recorded the absorption spectra (see Supplementary data, Fig. S1 and Tables S1–S3) of **FL**, **AF**, and **DAF** (i.e., free fluorene derivatives, not coupled to deoxyuridine units) in 10 mM phosphate buffer at pH 3.10–9.31 at constant ionic strength (0.16 M NaCl). We observed no significant changes in the absorption spectrum of **FL** in response to the pH change. In contrast, lowering the pH of the solution increased the intensity of the primary absorption band of **AF** and **DAF** near 260 nm, originating from the protonated **FL** moiety, while decreasing the intensity of the secondary band (at approximately 280 nm), attributable to the ICT transition arising from mixing of the $\pi \rightarrow \pi^*$ transition of the aromatic ring with the lone pair transition from the amino moiety, with isosbestic points at 270 and 290 nm, respectively.

Next, we recorded the absorption spectra of the fluorescent nucleosides containing each **FL** derivative ($\mathbf{U}^{\mathbf{FL}}$, $\mathbf{U}^{\mathbf{AF}}$, and $\mathbf{U}^{\mathbf{DAF}}$) in the same buffer solution at pH 3.10–9.31 (see Supplementary data, Fig. S2 and Tables S4–S6). Again, we observed no significant changes in the absorption spectrum of $\mathbf{U}^{\mathbf{FL}}$, whereas the intensities of the primary bands of $\mathbf{U}^{\mathbf{AF}}$ and $\mathbf{U}^{\mathbf{DAF}}$ (approximately 325 and 328 nm, respectively) increased as the intensities of the secondary bands (approximately 340 and 350 nm, respectively) decreased, with isosbestic points at 330 and 350 nm, respectively. These findings are consistent with the protonated amino moieties lacking the electron lone pair necessary for the ICT transition; thus, the absorption spectra of $\mathbf{U}^{\mathbf{AF}}$ and $\mathbf{U}^{\mathbf{DAF}}$ were similar to that of $\mathbf{U}^{\mathbf{FL}}$ under electron-releasing moiety to the fluorophore through a double bond and triple bond, and this mesomeric effect of $\mathbf{U}^{\mathbf{FL}}$ was more pronounced under basic conditions because the uracil moiety became a better electron donor by partial deprotonation of its 3-NH unit (pK_a=9.5).[19] Under acidic conditions, however, the electron releasing properties of a uracil moiety is decreased, which might induce red shifts and decrease in emission intensity.

Fig. 1c and e shows the fluorescence spectra of **AF** and **DAF** in aqueous solutions having various pH values upon excitation at their isosbestic points (270 and 290 nm, respectively). Generally, lowering the pH of the solution of **AF** or **DAF** decreased the intensity of its emission band (approximately 375 and 390 nm, respectively), originating from an ICT-excited state, and increased the intensity of a new emission band (approximately 310 nm), assigned to fluorescence from the excited acidic form of the fluorophore. Notably, upon acidification, where was a difference in the rate of increase in emission intensity of the protonated form of the fluorophore between **AF** and **DAF**. For **DAF**, the emission intensity of the excited acidic form increased at a faster rate than the rate of decrease in emission intensity at the ICT-excited state. This phenomenon originated from the significant increase in absorbance properties of protonated **DAF** under acidic conditions. Therefore, the fluorescence intensity of **DAF** increased significantly upon acidification relative that of **AF**.