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A fluorescein-derived anthocyanidin-inspired pH sensor

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ABSTRACT

A new multicolor pH-dependent fluorophore was synthesized via Pd-mediated cross-coupling chemistry of the mono-triflate of fluorescein with *p*-hydroxyphenylboronic acid. The novel indicator, named anthofluorescein, is highly sensitive to pH changes between 7 and 10 and displays a green to red fluorescence shift, making it a valuable candidate for biological studies.

The direct visualization of general biochemical events, specific molecular targets, or defined biochemical processes are indispensable for both in vitro[1–4] and, increasingly, in vivo applications.[3–6] These applications, because of the remarkable advances in imaging techniques, have begun to use a multitude of different fluorescent labels, probes, and sensors in multi-channel multiplex studies[7–9] which include the real-time analysis of cells and whole organisms.[10–13]

Due to the particular needs of each and every study, fluorescent dyes and probes with a myriad of defined optical, chemical, and biological properties are in high demand. Much effort has been focused on the development of optical pH chemosensors,[14–18] with specific attention paid to highly sensitive indicators within the physiological pH range.[19,20] In this area fluorescein and its derivatives are perhaps the most widely used fluorescent pH probes,[21,22] due in part to their excellent spectral and physical properties.

Anthocyanidins are the chromophores of a well-known family of natural dyes and are characterized by their broad spectral window, which is controlled by the various substituents on the benzopyrylium core and the pH.[23] Inspired by the general structures of the anthocyanidins **1**, the work described herein focuses on the modification of fluorescein **2** via substitution of one of its phenolic groups with *p*-hydroxyphenyl (see Fig. 1), a feature found in many anthocyanidins.[23] Due to its extended conjugation, compound **3** (which has been named anthofluorescein) was expected to display optical properties similar to both fluorescein and the anthocyanidins, but without the chemical instability of the latter.[23,24]

To assess the usefulness of the sensor prior to synthesis, the potential bathochromic shift in the excitation/emission wavelength of the different prototropic forms of derivative **3** was calculated via a series of configuration interaction singles, CIS/6-31g(d,p) calculations. These predicted that the 4′′-oxo dianion form (D-I in the Supplementary data) would display a maximum absorbance at 530 nm and emission at 570 nm, the emission being red shifted by over 50 nm relative to fluorescein (see Supplementary data). In contrast, the 3′-oxo dianion form (D-II in the Supplementary data) should keep the original spectroscopic properties of fluorescein, with absorption maximum at 480 nm and emission maximum at 513 nm. These dianionic forms were predicted to have significant oscillator strengths. Due to the different pK_a values of the two phenolic OH groups, we envisaged that we would observe a dual-color performance tuned by the pH of the solution.

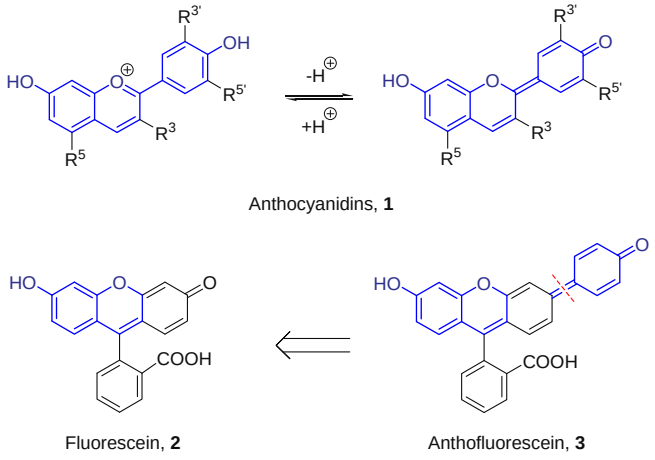

Figure 1. Structures of anthocyanidins, fluorescein (quinoid form), and anthofluorescein (quinoid form). Similarities in the structures are highlighted in blue.

Compound **3** was synthesized via sequential microwave-assisted mono-triflation of fluorescein **2** using one equivalent of *N*-phenylbis(trifluoromethanesulfonimide)[25] (a mild triflation reagent) followed by Suzuki aryl palladium-catalyzed cross-coupling of **4** with *p*-hydroxyphenylboronic acid (see Scheme 1) to give rise to anthofluorescein **3**. Heterogeneous catalysis using polystyrene resin captured palladium was successful but lower yields were obtained in this case (41%).[26]

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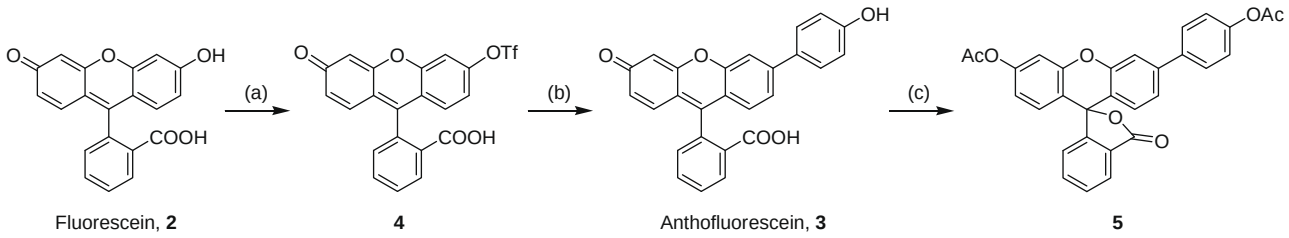

Scheme 1. Reagents and conditions: (a) $PhN(Tf)_2$, K_2CO_3, DMF, μω 80 °C, 20 min, 61%; (b) *p*-hydroxyphenylboronic acid, $Pd(OAc)_2$, K_2CO_3, PPh_3, H_2O:dioxane (1:9), μω 120 °C, 30 min, 78%; (c) Ac_2O:Pyr (1:1), 16 h, 87%.

A study of the absorptivity and the fluorescent properties of the novel dye confirmed that the optical properties of anthofluorescein were quite different from those of fluorescein, with both the absorption and emission spectra highly influenced by pH (as is also observed for the anthocyanidins). Spectrophotometric analysis of anthofluorescein solutions (50 μM) at distinct pH values (Fig. 2) showed that absorbance increased with pH, with the largest impact being observed between pH 7 and 10 (see Fig. 2 for the apparent colors of the solution), with a maximum absorptivity at pH 11 (ε = 25,000 M^{-1} cm^{-1}). Absorptivity greatly decayed at pH 13, indicating a large change in the prototropic composition of the solution.

We attribute the changes seen in the fluorescence emission spectra between pH 7 and pH 13 (see Fig. 3) as being predominantly due to the prototropic equilibrium existing between the two dianionic forms of the molecule (for a more detailed explanation see Supplementary data). The experimental quantum yield[27] of the dye at pH 8, where maximal emission was observed, was very low (0.02), which in practice would limit applicability for cell assays. However, further analysis indicated that the quantum yield was highly influenced by the viscosity of the solution, with the quantum yield rising to 0.3 at pH 8 in 20% glycerol, presumably due to a decrease in the rate of internal conversion mediated by the *p*-hydroxyphenyl group torsional motion, similar to that ascribed to the diethylamino torsional motion in Rhodamine B.[28] It would be expected that the changes in emission intensity and quantum yield would be mirrored by changes in fluorescence lifetime and, as such, the molecule could find application as a, potentially, very sensitive probe of local temperature and viscosity in fluorescence lifetime imaging microscopy.

A non-fluorescent derivative of anthofluorescein **3**, diacetylated derivative **5**, was synthesized (see Scheme 1) in order to enhance the cellular penetrability of anthofluorescein and to reduce extracellular background. Compound **5** was incubated with HeLa cells for 2 h and then imaged using a 488/20 excitation filter (in the absence of an emission filter). As expected, intracellular deacetylation led to the formation of anthofluorescein **3**, which was identified by fluorescently yellow cells (Fig. 4), highlighting the viability of the cells. Due to the higher viscosity of the cell cytoplasm (expected to be more akin to glycerol than water)[29] the anthofluorescein dye was strongly emissive within the cells, confirming the previous viscosity observations.

In conclusion a new pH-sensitive dye synthesized from fluorescein using a straightforward microwave-assisted two-step proce-

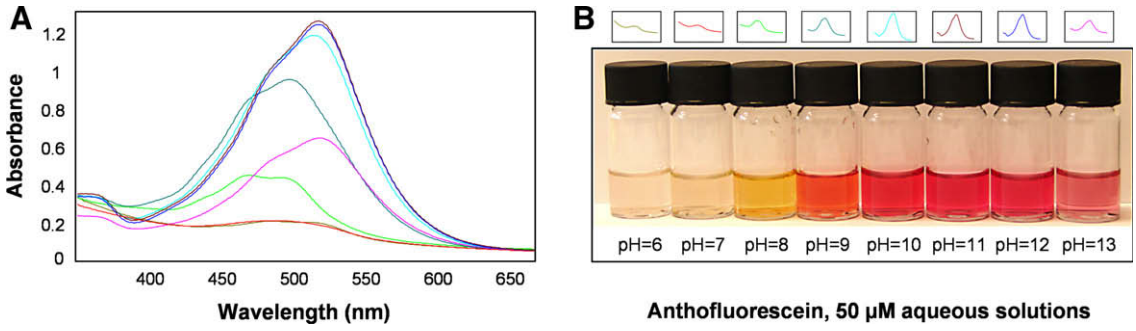

Figure 2. (A) Absorption spectra of 50 μM solutions of anthofluorescein **3** at pH 6–13. (B) Images of the dye solutions at various pH values.

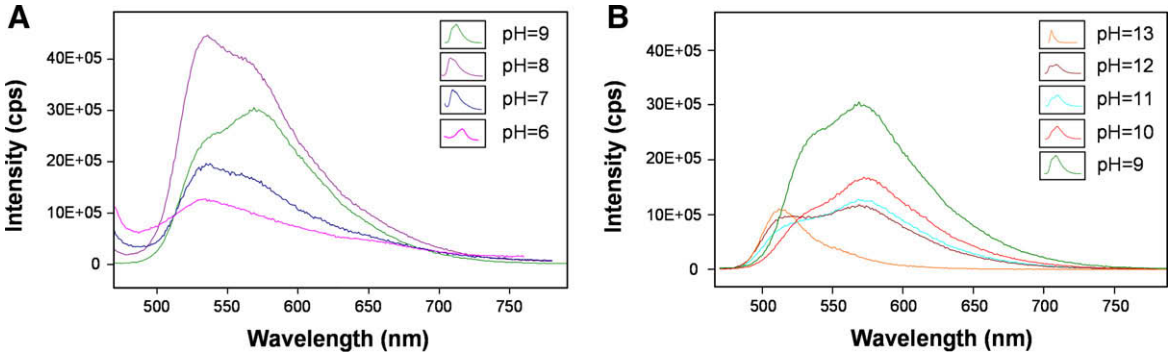

Figure 3. Emission spectra of anthofluorescein **3**, with excitation at 450 nm: (A) pH 6–9 and (B) pH 9–13.

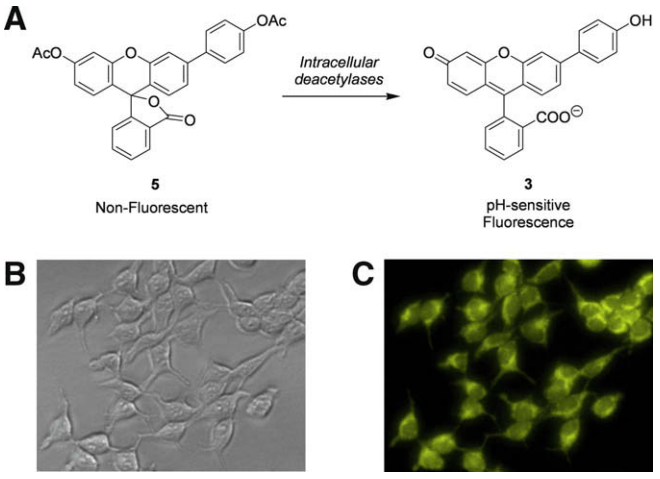

Figure 4. (A) Intracellular deacetylation of compound **5** to give anthofluorescein 3. HeLa cells were incubated for 2 h with a 25 μM solution of diacetylated anthofluorescein **5**. (B) Brightfield. (C) Fluorescent image with 488 nm excitation and no emission filter.

dure consisting of mono-triflation and subsequent Suzuki aryl cross-coupling with *p*-hydroxyphenylboronic acid has been developed. The new dye, named anthofluorescein, was characterized by a highly sensitive absorption and fluorescence emission particularly in the pH range from 7 to 10. A diacetylated derivative of anthofluorescein successfully labeled living HeLa cells, which indicates that it might become a valuable tool for cell labeling and viability studies and, with the assistance of advanced microscopy techniques, for uses such as a ratiometric reporter of pH, viscosity, and/or temperature. Finally, the synthetic method provides a facile route to the development of new multicolor fluorescence dyes with red-shifted absorption and emission, which are in high demand as fluorescence probes.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.03.223.

References and notes

1. Johnsson, N.; Johnsson, K. *ACS Chem. Biol.* **2007**, *2*, 31–38.
2. Unciti-Broceta, A.; Díaz-Mochón, J.; Mizomoto, H.; Bradley, M. *J. Comb. Chem.* **2008**, *10*, 179–184.
3. Domaille, D. W.; Que, E. L.; Chang, C. J. *Nat. Chem. Biol.* **2008**, *4*, 168–175.
4. Giepmans, B. N.; Adams, S. R.; Ellisman, M. H.; Tsien, R. Y. *Science* **2006**, *312*, 217–224.
5. Weissleder, R.; Ntziachristos, V. *Nat. Med.* **2003**, *9*, 123–128.
6. Zhang, J.; Campbell, R. E.; Ting, A. Y.; Tsien, R. Y. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 906–918.
7. Díaz-Mochón, J. J.; Tourniaire, G.; Bradley, M. *Chem. Soc. Rev.* **2007**, *36*, 449–457.
8. Pernagallo, S.; Unciti-Broceta, A.; Díaz-Mochón, J. J.; Bradley, M. *Biomed. Mater.* **2008**, *3*, Art. No. 034112 (6pp).
9. Unciti-Broceta, A.; Diezmann, F.; Ou-Yang, C. Y.; Fara, M. A.; Bradley, M. *Bioorg. Med. Chem.* **2009**, *17*, 959–966.
10. Lavis, L. D.; Raines, R. T. *ACS Chem. Biol.* **2008**, *3*, 142–155.
11. Hell, S. W. *Nat. Biotechnol.* **2003**, *11*, 1347–1355.
12. Weissleder, R. *Science* **2006**, *312*, 1168–1171.
13. Alexander, L.; Dhaliwal, K.; Simpson, J.; Bradley, M. *Chem. Commun.* **2008**, 3507–3509.
14. Charier, S.; Ruel, O.; Baudin, J.-B.; Alcor, D.; Allemand, J.-F.; Meglio, A.; Jullien, L. *Angew. Chem., Int. Ed.* **2004**, *43*, 4785–4788.
15. Wong, L. S.; Birembaut, F.; Bocklesby, W. S.; Frey, J. G.; Bradley, M. *Anal. Chem.* **2005**, *77*, 2247–2251.
16. Cho, J. K.; Wong, L. S.; Dean, T. W.; Ichihara, O.; Muller, C.; Bradley, M. *Chem. Commun.* **2004**, 1470–1471.
17. Cho, J. K.; White, P. D.; Klute, W.; Dean, T. W.; Bradley, M. *J. Comb. Chem.* **2003**, *5*, 632–636.
18. Wong, L. S.; Brocklesby, W. S.; Bradley, M. *Sens. Actuators B-Chem.* **2005**, *107*, 957–962.
19. Bradley, M.; Alexander, L.; Duncan, K.; Chennaoui, M.; Jones, A. C.; Sanchez-Martin, R. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 313–317.
20. Vasylevska, A. S.; Karasyov, A. A.; Borisov, S. M.; Krause, C. *Anal. Bioanal. Chem.* **2007**, *387*, 2131–2141.
21. Lavis, L. D.; Rutkoski, T. J.; Raines, R. T. *Anal. Chem.* **2007**, *79*, 6775–6782.
22. Smith, J. P.; Drewes, L. R. *J. Biol. Chem.* **2006**, *281*, 2053–2060.
23. Castañeda-Ovando, A.; Pacheco-Hernández, M. L.; Páez-Hernández, M. E.; Rodríguez, J. A.; Galán-Vidal, C. A. *Food Chem.* **2009**, *113*, 859–871.
24. Jurd, L.; Geissman, T. A. *J. Org. Chem.* **1963**, *28*, 2394–2397.
25. Miller, E. W.; Albers, A. E.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 16652–16659.
26. Cho, J. K.; Najman, R.; Dean, T. W.; Ichihara, O.; Muller, C.; Bradley, M. *J. Am. Chem. Soc.* **2006**, *128*, 6276–6277.
27. Williams, A. T. R.; Winfield, S. A.; Miller, J. N. *Analyst* **1983**, *108*, 1067.
28. Lopez Arbeloa, I.; Rohatgi-Mukherjee, K. K. *Chem. Phys. Lett.* **1986**, *129*, 607–614.
29. Daniels, B. R.; Masi, B. C.; Wirtz, D. *Biophys. J.* **2006**, *90*, 4712–4719.