JOC The Journal of Organic Chemistry

Article

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Exhaustive Syntheses of Naphthofluoresceins and Their Functions

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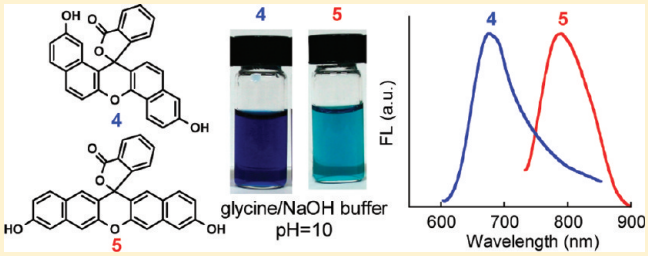
Supporting Information

ABSTRACT: Naphthofluorescein and/or seminaphthofluorescein derivatives possessing the additional benzene units to one or both sides of fluorescein were exhaustively constructed through Friedel–Crafts type reactions between corresponding aroylbenzoic acids and dihydroxynaphthalenes. Compound **4** works as a one-dye pH indicator, which shows red in strong acid condition and blue in basic solution. Compound **23** (diacetate of compound **4**) shows good transitivity to the HEK 293 cells and acts as a fluorescent pigment for the living cell imaging. Compounds **5**, **6**, and **9** show fluorescent emission in the NIR region (>700 nm) and imply the potentialities of NIR fluorescent probes.



INTRODUCTION

Because of their significant contributions, fluorescent probes have become indispensable tools in the fields of biology and chemical biology.[1] Advantages of fluorescent probes for bioimaging include (1) less damage to living cells, (2) high sensitivity, and (3) real-time tracing of target biological materials. Among fluorescent probes, fluorescein[2] and cyanine[3] derivatives are the most common. Fluorescein derivatives have a good transitivity into the cell and can increase lipophilicity by introducing acyl groups on their two hydroxy groups. Additionally, once the acyl groups are hydrolyzed by enzymes in the cell, the active forms are well retained by the cell.[4] Furthermore, a methodology to control the on/off fluorescent properties based on Pet[2c] or d-Pet[5] mechanism has also been developed. However, their excitation and emission wavelengths are around 500 nm and overlap with autofluorescence in biological materials such as tryptophan and porphyrin. For biological applications, avoidance of overlap with autofluorescence is desirable. Hence, probes with excitation and emission wavelengths in the near-infrared region (NIR) (>700 nm) are preferable. Fluorescent probes with excitation and emission wavelengths in the NIR are also suitable to visualize deeper tissues with less damage to the organism using low energy light.[6] Some cyanine derivatives have excitation and emission wavelength around the NIR, and consequently, the aforementioned autofluorescence problems do not occur.[7] Although cyanine derivatives are designed for longer wavelengths, their chemical structures are complicated and often cause changes that reduce cell transitivity.

Under such a background, we hypothesized that if the benzene units could be expanded to one or both sides of the dibenzopyran system in the fluorescein skeleton, then the excitation and emission wavelengths could be elongated to the NIR. Furthermore, these compounds should have practical benefits due to their structural similarities with fluorescein, as fluorescein has a long history of modifications and applications. In this paper, we introduce the exhaustive synthesis of naphthofluoresceins **2**–**10** (Figure 1) and evaluate their properties, including UV–vis and fluorescent spectra. Among them, compounds **7**[8] and **10**[9] are known as fluorescent materials. Several biochemical applications of **7** such as hydrogen peroxide and superoxide radical anion imaging probes have been reported.[8b,8d]

RESULTS AND DISCUSSION

Synthesis of Compounds 2–10. Based on the synthetic route to fluorescein (**1**),[10] we initially examined a double Friedel–Crafts reaction with 2 equiv of 2,7-dihydroxynaphthalene (**11**) and phthalic anhydride (**12**) in methanesulfonic acid. The reactivity on the naphthalene ring for electrophilic substitution is higher at the 1-position of **11** than at the 3-position. Consequently, compound **2**, which is due to a double Friedel–Crafts reaction at 1-position, was generated in 6% yield instead of **3** (4%) and **5** (1%). Additionally, a large amount of thick paste was formed (Scheme 1).

An alternative and selective synthetic route for linear naphthofluorescein **5** was also developed (Scheme 2). Methoxymethyl (MOM)-protected bromonaphthalene **14**, which was produced from corresponding diol **13**[11] in 86% yield, was treated with *n*-BuLi and allowed to react with 0.5 equiv of phthalic anhydride (**12**) to give **15** in 81% yield. The phenolic MOM groups of **15** were removed by 4 N HCl in dioxane to afford **16** in 73% yield. Intramolecular dehydration

Received: February 8, 2012
Published: March 19, 2012

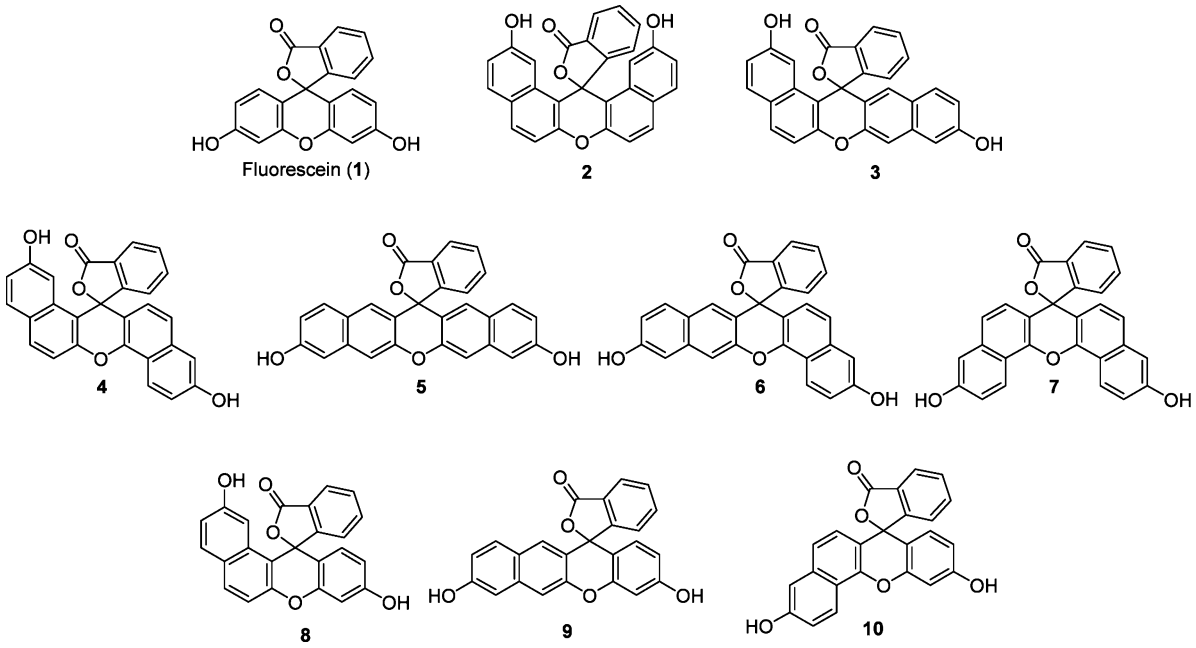
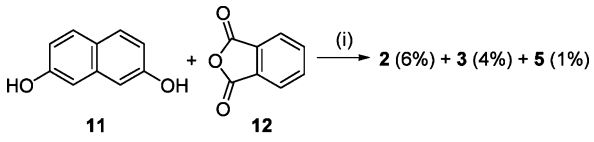


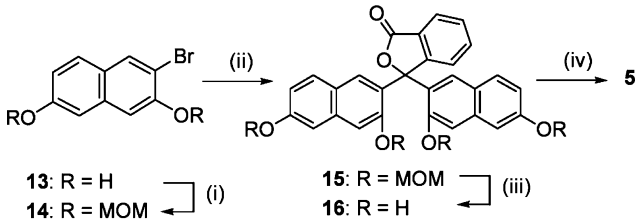
Figure 1. Structures of fluorescein (**1**), naphthofluoresceins (**2**–**7**), and seminaphthofluoresceins (**8**–**10**).

Scheme 1. Synthetic Route for Naphthofluoresceins 2, 3, and 5



(i) $MeSO_3H$, 100 °C

Scheme 2. Synthetic Route for Naphthofluorescein 5



(i) MOMCl, NaH, 86%, (ii) *n*-BuLi, **12**, 81%, (iii) 4N HCl, 73%, (iv) $MeSO_3H$, 77%

occurred under acidic conditions to give desired **5** in 77% yield. Colorless crystals of **5** precipitated from toluene–ethyl acetate. According to X-ray analysis, the crystals of **5** are monoclinic and belong to the $P2_1/c$ space group. One unit cell is composed of two compound **5** molecules (molecule A and molecule B) and two toluene molecules (Figure 2). Of particular interest is that compared to a planar hexagonal shape (720°), the two binaphthopyranes in molecules A and B show high planarities based on the sum of the internal angles of the central pyrane ring (719.33° and 719.27° for A and B, respectively).

Scheme 3 outlines the synthesis of compound **4**. According to the literature, methoxytetralone **17** was converted to **18** in two steps.[12] A methyl group of **18** was removed by BBr_3 (**19**; 88% yield) and then two hydroxy groups of **19** were protected by MOM to give **20** in 81% yield. Lithiated **20** was reacted with

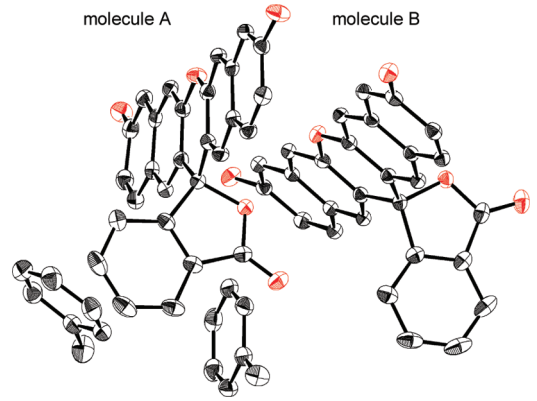
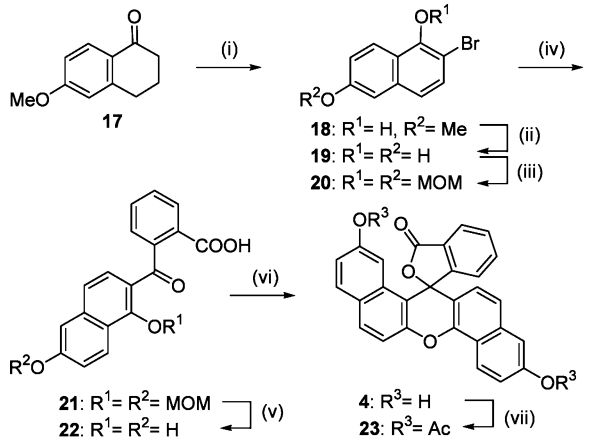


Figure 2. Crystal structure of the naphthofluorescein **5**.

Scheme 3. Synthetic Route for Naphthofluorescein 4 and 23



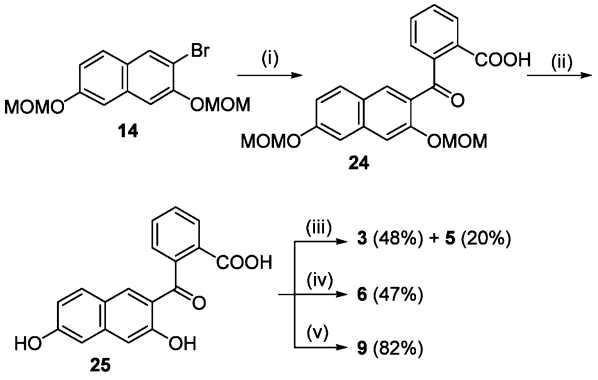
(i) ref.12, (ii) BBr_3, 88%, (iii) MOMCl, NaH, 81%, (iv) *n*-BuLi, **12**, 61%, (v) 4N HCl, 96%, (vi) $MeSO_3H$, **11**, 76%, (vii) Ac_2O, 92%

dx.doi.org/10.1021/jo300177b | J. Org. Chem. 2012, 77, 3492–3500

1 equiv of phthalic anhydride (**12**) to afford key intermediate 2-aroyl-1-benzoic acid **21** in 61% yield. After deprotection of the MOM group in HCl and dioxane (**22**; 96%), **22** was treated with 2,7-dihydroxynaphthalene (**11**) to afford **4** in 76% yield. For the living cell imaging, compound **4** was treated with acetic anhydride to afford the diacetate **23** in 92% yield.

Aroylbenzoic acid **25** was constructed from **14** and phthalic anhydride (**12**) in 68% yield, and subsequent deprotection of the MOM group occurred in 67% yield. Using aroylbenzoic acid **25**, a variety of naphthofluoresceins were synthesized. Thus, **25** was reacted with 2,7-dihydroxynaphthalene (**11**) to give **3** (48%) and **5** (20%), with 1,6-dihydroxynaphthalene (**26**) to afford **6** (47%), and with resorcinol to give **9** in 82% yield (Scheme 4).

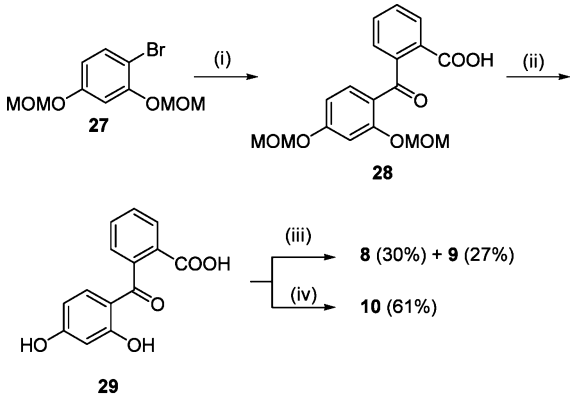
Scheme 4. Synthetic Route for 3, 5, 6 and 9



(i) *n*-BuLi, **12**, 68%, (ii) 4N HCl, 67%,
(iii) $MeSO_3H$, 2,7-dihydroxynaphthalene (**11**),
(iv) $MeSO_3H$, 1,6-dihydroxynaphthalene (**26**), 47%
(v) $MeSO_3H$, resorcinol, 82%

The aroylbenzoic acid route might be applicable to synthesize one benzene-unit expanded seminaphthofluoresceins **8**–**10** (Scheme 5). Thus, aroylbenzoic acid **29**,[13] which was synthesized from **27**,[14] was coupled with 2,7-dihydroxynaphthalene (**11**) to afford **8** and regioisomer **9** in yields of 30 and 27%, respectively. Additionally, coupling **29** with 1,6-dihydroxynaphthalene (**26**) produced **10** in 61% yield.

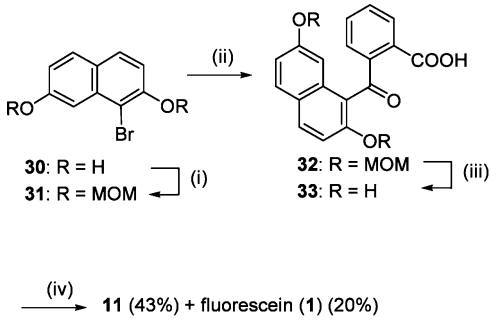
Scheme 5. Synthetic Route for 8–10



(i) *n*-BuLi, **12**, 60%, (ii) 4N HCl, 90%,
(iii) $MeSO_3H$, **11**, (iv) $MeSO_3H$, **26**, 61%

The aroylbenzoic acid route from **33**, a 2,7-dihydroxynaphthalene possessing a keto acid in its 1 position, is a dead end. For example, in the case of reaction between **33** and resorcinol in methanesulfonic acid, unexpected retro Friedel–Crafts reaction should have precedence over desired Friedel–Crafts reaction to give 2,7-dihydroxynaphthalene (**11**) in 43% yield and fluorescein (**1**) in 20% yield, which should be generated from fragmented phthalic anhydride (**12**) and resorcinol (Scheme 6).

Scheme 6. Unexpected Retro-Friedel–Crafts Reaction



(i) MOMCl, NaH, 99%, (ii) *n*-BuLi, **12**, 96%, (iii) 4N HCl, 94%,
(iv) $MeSO_3H$, resorcinol

Coloration of 2–10 under Various pH Conditions. With the series of benzene-unit expanded fluoresceins **2**–**10** in hand, we then scrutinized the coloration of **2**–**10** under various pH conditions (−1 to 14). Figure 3 shows pictures and UV–vis spectra of naphthofluoresceins **2**–**7** (see Supporting Information Figure S-1 for seminaphthofluoresceins **8**–**10**). The compounds have low solubilities in aqueous solutions and precipitate in the acidic region. Homogeneous solutions occur in basic solutions or in extremely acidic solutions (i.e., methanesulfonic acid).

Compounds **2**–**10** display peaks near 490–580 nm in the acidic region (red lines in each spectra) and near 595–708 nm in the basic region (blue lines (pH = 11) in the corresponding spectra). The data indicates that (1) the HOMO–LUMO gap of dianionic form in a basic solution is narrower than that of in cationic form in an acidic region (Figure 4, Figure S-2 and S-3 for the DFT calculations, Supporting Information), and (2) linear naphthofluorescein **5** shows longer λ_{max} wavelengths in both acidic and basic solutions compared to other bent naphthofluoresceins.

Intriguingly, compounds **2** and **4** show a clear color contrast; they are vibrant red in strongly acidic conditions and bright blue in basic conditions. In particular, the absorbance for compound **4** in strongly acidic conditions is almost same as that in pH 10. Hence, we attempted a trial of compound **4** as a one-dye pH-test paper. White filter paper was impregnated with compound **4** and dried. Then the color responses against pH were examined. As expected, the filter paper is suitable as a pH-test paper. It turns red in acidic conditions and blue in basic conditions (Figure 5).

Fluorescence Properties of 2–10 under Basic Conditions (pH 11). Despite the UV–vis spectrum possessing large absorptions at pH 10 and 11 near 700 nm, linear naphthofluorescein **5** is a sickly green or blue-green color to the naked eye because λ_{max} is beyond the human visible range (>700 nm). If the synthesized compounds provide a fluorescent

dx.doi.org/10.1021/jo300177b | *J. Org. Chem.* 2012, 77, 3492–3500

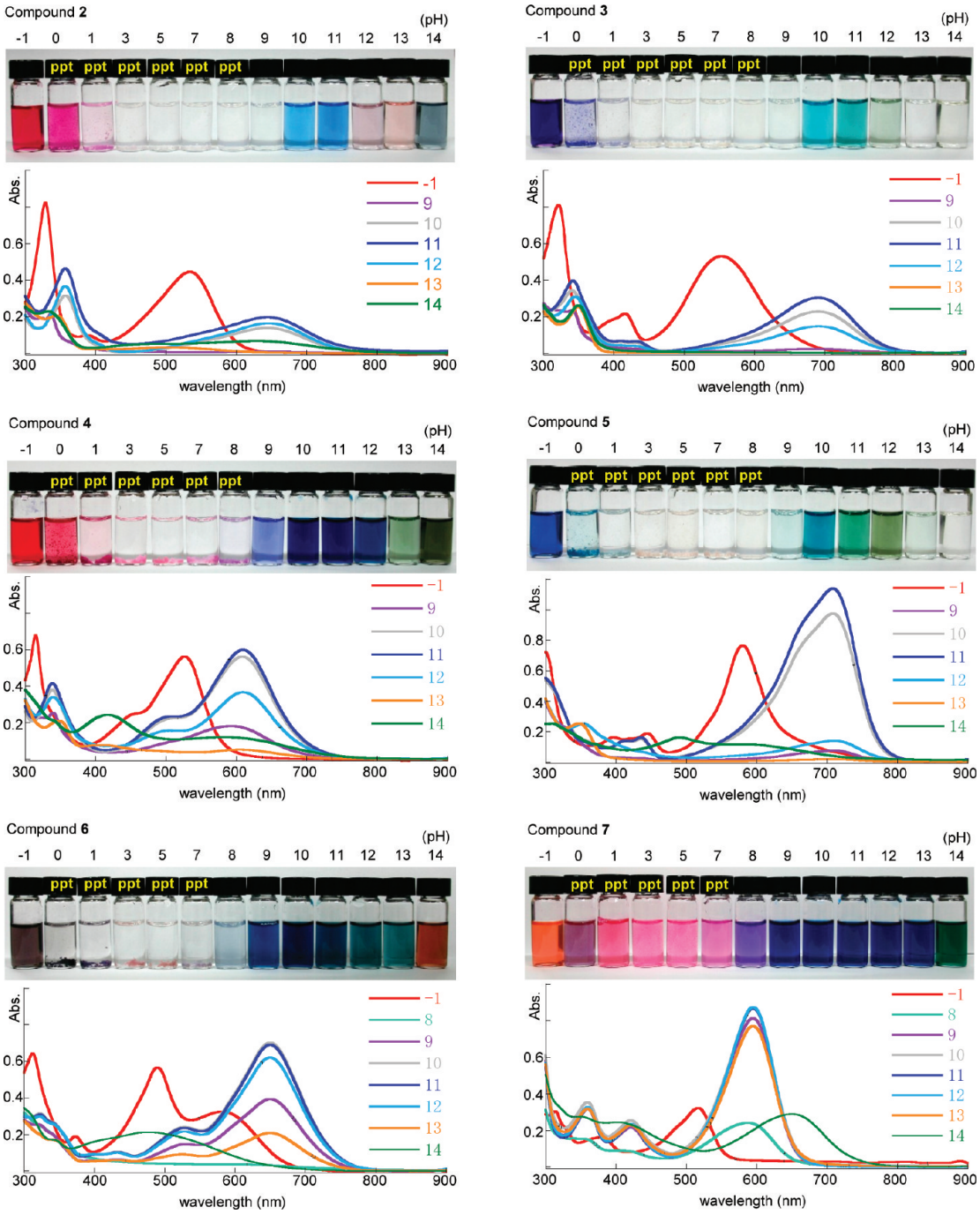


Figure 3. Coloration of the naphthofluoresceins **2**–**7** under various pH conditions. Conditions: photo; [compound] = 1.0×10^{-4} M, UV–vis spectra; [compound] = 2.0×10^{-5} M, pH = −1 ($MeSO_3H$ aq.), pH = 1 (HCl-KCl buffer), pH = 3, 5, 7 (citrate-phosphate buffer), pH = 8, 9 (Tris-HCl buffer), pH = 10–14 (glycine-NaOH buffer). ppt = precipitates.

response, their excitation and emission wavelengths should be in the NIR. Therefore, to investigate the potential of **2**–**10** as probes in the chemical biology field, we examined their fluorescent properties in aqueous glycine-NaOH buffer conditions (pH 11).

Figure 6 shows the normalized fluorescent spectra of typical compounds, while Table 1 summarizes the characteristic parameters such as λ_{max} of absorbance, excitation and emission wavelengths, quantum yield (Φ), and Stokes shift of the benzene unit expanded fluoresceins.

Compounds **2**, **3**, and **8** do not have meaningful emissions under basic aqueous conditions. Comparing to the emission wavelengths of other compounds, all new compounds **4**–**6**, and **9** (solid lines in Figure 6) have longer wavelengths than known compounds **7**, **10**, and fluorescein (**1**) (dotted lines in Figure 6). Especially, the emission peaks of compounds **5**, **6**, and **9** exceed 700 nm and are in the NIR region. In addition, compounds **6** (133 nm) and **9** (196 nm) exhibit quite large Stokes shifts, which are advantageous in avoiding the overlap between emission and scattering light.

For a preliminary living cell imaging experiment, we chose compound **23** (diacetate of compound **4**) as an appropriate fluorophore under the condition of common fluorescence microscopy and filters. HEK 293 or NIH 3T3 cells were

dx.doi.org/10.1021/jo300177b | J. Org. Chem. 2012, 77, 3492–3500

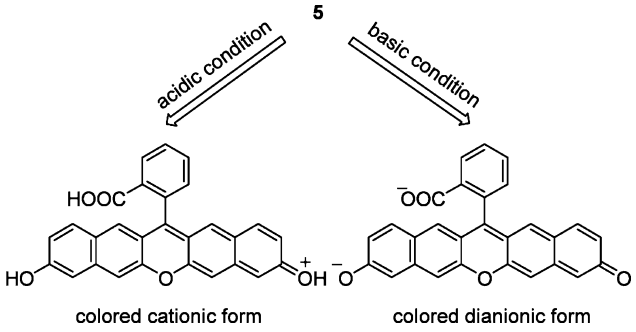


Figure 4. Proposed colored forms in acidic or basic conditions.

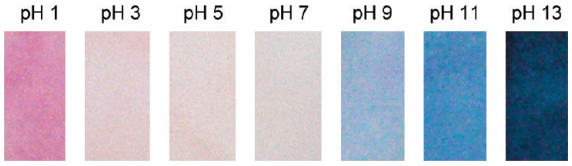


Figure 5. Naphthofluorescein **4** as one-dye pH indicator.

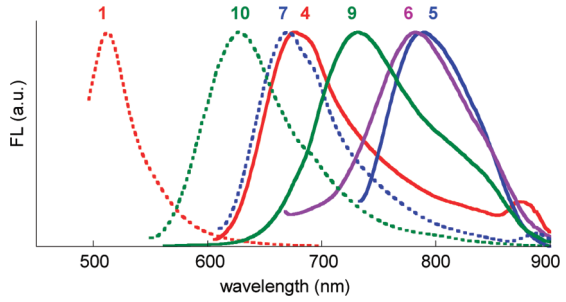


Figure 6. Fluorescence spectra of fluorescein (**1**), naphthofluoresceins (**4**–**7**), and seminaphthofluoresceins (**9**–**10**). Conditions; glycine-NaOH buffer conditions (pH 11). exciting wavelength; **1** (491 nm), **4** (583 nm), **5** (715 nm), **6** (647 nm), **7** (591 nm), **9** (536 nm), **10** (536 nm).

incubated in PBS buffer containing 0.01–0.10 mM of compound **23** for 15, 30, 60 min at rt. After replacing the medium with PBS buffer, the cells were observed by a standard fluorescence microscopy. Under all conditions examined, the fluorescence images for both types of cells were obtained without obvious cytotoxicity as shown in Figure 7. These data indicated that compound **23** was smoothly transferred into the cells and released active compound **4**, suggesting the clear fluorescence response from the cells and the low cytotoxicity.

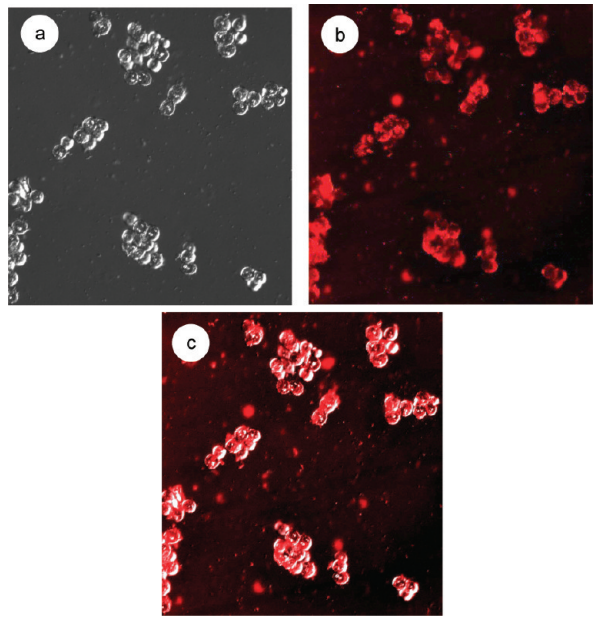


Figure 7. Raw and fluorescence images of HEK 293 cells. HEK 293 cell were incubated in 0.05 mM of compound **23** for 60 min. (a) Raw image, (b) fluorescence image, and (c) overlapping image of (a) and (b).

CONCLUSIONS

We have exhaustively constructed benzene unit expanded fluoresceins, mainly through an aroylbenzoic acid route, and elucidated their properties. Compound **4** works as a one-dye pH indicator, which is red in strongly acid conditions and blue in basic conditions. Although the quantum yields of **5**, **6**, and **9** are moderate, they have important functions, such as a simple framework, and may aid in designing functional fluorescein derivatives. In particular, compound **9** emits in the NIR with a large Stokes shift and is water-soluble. We are currently investigating these compounds for application for living cell under NIR fluorescence microscopy conditions as well as improvement of quantum yield based on increasing rigidity of the framework.

Table 1. UV–Vis–NIR and FL Spectral Properties of Compounds 1–10

compound	$\lambda_{Abs.\ max}$ (nm)	ε ($\lambda_{Abs.\ max}$)	λ_{ex} (nm)	ε (λ_{ex})	λ_{em} (nm)	Φ (%)[a]	Stokes shift (nm)
1	491	78,000	491	78,000	513	97[b]	22
2	645	9,800					
3	691	15,000					
4	610	30,000	583	25,000	674	0.23	91
5	708	57,000	715	56,000	790	0.17	75
6	650	35,000	647	34,000	780	0.4	133
7	595	44,000	591	43,000	670	14[b]	79
8	540	20,000					
9	536	29,000	536	29,000	732	0.24	196
10	538	44,000	536	44,000	629	35	93

[a]The fluorescence quantum yields of **4**–**6** and **9**–**10** (Φ) were determined using a solution of compound **7** in glycine-NaOH buffer (pH 11) as a reference standard (Φ = 0.14). [b]Taken from ref 8a.

dx.doi.org/10.1021/jo300177b | J. Org. Chem. 2012, 77, 3492–3500

EXPERIMENTAL SECTION

General Procedure for the Protection of Phenolic Hydroxy Group with MOMCl. The preparation of the MOM-protected bromonaphthalene **14** is typical. To a stirred solution of 3-bromonaphthalene-2,7-diol (**13**, 9.0 g, 37.6 mmol) in dry DMF (50 mL), NaH (60% dispersion in mineral oil, 4.5 g, 112.5 mmol, 3 equiv) was added portionwise under ice-bath cooling. After 1 h, MOMCl (6.8 mL, 89.5 mmol, 2.4 equiv) was added dropwise to the suspension and stirred for 4.5 h at room temperature. The reaction mixture was poured into the mixed solvent of ethyl acetate and water. The organic layer was separated and washed successively with water (twice) and brine. After being dried over sodium sulfate, the solvent was evaporated in vacuo to give a residue. The residue was purified by column chromatography (SiO_2; *n*-hexane to *n*-hexane/ethyl acetate = 10/1) to afford **14** (10.6 g, 32.4 mmol, 86%) as a white powder. Compound **14**: 86% yield; white powder; mp 50−51 °C; IR (neat) 2956, 1628, 1595, 1504, 1371, 1149 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.60 (d, J = 8.9 Hz, 1H), 7.39 (s, 1H), 7.29 (d, J = 2.4 Hz, 1H), 7.10 (dd, J = 8.9, 2.4 Hz, 1H), 5.35 (s, 2H), 5.27 (s, 2H), 3.55 (s, 3H), 3.51 (s, 3H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 155.5, 151.4, 134.4, 131.7, 128.0, 125.8, 117.8, 111.2, 110.2, 109.0, 95.0, 94.3, 56.3, 56.1; MS (EI$^+$) m/z (rel. int.) 328 (M^+, 75), 326 (M^+, 75), 296 (13), 266 (17), 247 (12), 182 (23), 180 (23), 113 (100). HRMS (EI$^+$) Calcd for $C_{14}H_{15}{}^{79}BrO_4$ (M^+): 326.0154. Found: 326.0159.

Compound **20**: 81% yield from **19**; colorless oil; IR (neat) 1624, 1585, 1500, 1363, 1236 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.11 (d, J = 8.9 Hz, 1H), 7.53 (d, J = 8.9 Hz, 1H), 7.40 (d, J = 8.9 Hz, 1H), 7.36 (d, J = 2.4 Hz, 1H), 7.26 (dd, J = 8.9, 2.4 Hz, 1H), 5.29 (s, 2H), 5.25 (s, 2H), 3.71 (s, 3H), 3.52 (s, 3H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 155.4, 150.6, 134.9, 130.4, 125.4, 124.5, 124.0, 119.5, 110.4, 109.8, 100.1, 94.3, 58.2, 56.1; MS (EI$^+$) m/z (rel. int.) 328 (M^+, 100), 326 (M^+, 100), 298 (55), 296 (55). HRMS (EI$^+$) Calcd for $C_{14}H_{15}{}^{79}BrO_4$ (M^+): 326.0154. Found: 326.0150. Calcd for $C_{14}H_{15}{}^{81}BrO_4$ (M^+): 328.0133. Found: 328.0130. Anal. Calcd for $C_{14}H_{15}BrO_4$: C, 51.40; H, 4.62. Found: C, 51.18; H, 4.64.

Compound **31**: 99% yield; colorless oil; IR (neat) 2902, 1628, 1510, 1360, 1151 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.77 (d, J = 2.5 Hz, 1H), 7.71 (d, J = 8.9 Hz, 1H), 7.70 (d, J = 8.9 Hz, 1H), 7.29 (d, J = 8.9 Hz, 1H), 7.15 (dd, J = 8.9, 2.5 Hz, 1H), 5.35 (s, 2H), 5.34 (s, 2H), 3.57 (s, 3H), 3.54 (s, 3H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 156.5, 152.1, 134.3, 129.7, 128.3, 126.2, 117.5, 114.6, 109.3, 109.0, 95.4, 94.5, 56.5, 56.3; MS (EI$^+$) m/z (rel. int.) 328 (M^+, 81), 326 (M^+, 81), 298 (28), 296 (26), 268 (27), 266 (27), 153 (61), 136 (53), 106 (46), 89 (66), 77 (100). HRMS (EI$^+$) Calcd for $C_{14}H_{15}O_4{}^{79}Br$ (M^+): 326.0153. Found: 326.0148.

General Procedure for the Aroylbenzoic Acid Derivatives. The preparation of compound **21** is typical. A solution of *n*-BuLi (1.63 M *n*-hexane solution; 1.35 mL, 2.21 mmol) was added dropwise to a solution of **20** (600 mg, 1.83 mmol) in dry THF (7 mL) under N_2 atmosphere at −78 °C. After 1 h of stirring, the solution of lithiated **20** in THF was added dropwise to a solution of phthalic anhydride (**12**, 251.5 mg, 1.70 mmol) in dry THF (10 mL). The resultant solution was stirred at −78 °C for 1 h and then allowed to warm to room temperature with stirring for 2.5 h. The reaction mixture was poured into the mixed solvent of ethyl acetate and 0.1 M aq. HCl. The organic layers were successively washed with water and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to dryness. The residue was purified by column chromatography (SiO_2; *n*-hexane-EtOAc, 3:1 → 1:1) to afford **21** (437 mg, 61%) as a pale-yellow oil.

Compound **21**. 61% yield; pale-yellow oil; IR (neat) 3444, 1718, 1664, 1620, 1469, 1240 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.29 (d, J = 8.9 Hz, 1H), 8.01−7.98 (m, 1H), 7.61−7.55 (m, 2H), 7.52 (d, J = 8.9 Hz, 1H), 7.46 (d, J = 8.9 Hz, 1H), 7.47−7.42 (m, 1H), 7.36 (d, J = 2.4 Hz, 1H), 7.27 (dd, J = 8.9, 2.4 Hz, 1H), 5.31 (s, 2H), 5.05 (s, 2H), 3.52 (s, 3H), 3.40 (s, 3H); ^{13}C NMR (68 MHz, CD_3OD) δ 192.4, 169.9, 158.7, 156.7, 144.2, 139.9, 132.6, 132.5, 130.8, 130.6, 129.1, 128.9, 127.2, 125.4, 123.6, 120.4, 110.6, 102.7, 95.3, 58.1, 56.5 (one peak overlapped); MS (EI$^+$) m/z (rel. int.) 396 (M^+, 27), 334 (100), 304 (11), 284 (19). HRMS (EI$^+$) Calcd for $C_{22}H_{20}O_7$ (M^+): 396.1209. Found: 396.1210.

Compound **24**: 68% yield from **12** and **14**; white powder; mp 132−134 °C; IR (KBr) 2893, 1697, 1655, 1622, 1464, 1288 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$ 40 °C) δ 8.16 (brs, 1H), 7.95 (d, J = 7.0 Hz, 1H), 7.76 (d, J = 8.9 Hz, 1H), 7.64−7.50 (m, 2H), 7.35 (d, J = 7.3 Hz, 1H), 7.35−7.25 (m, 2H), 7.10 (dd, J = 8.9, 2.2 Hz, 1H), 5.28 (s, 2H), 4.98 (s, 2H), 3.47 (s, 3H), 3.22 (s, 3H); ^{13}C NMR (68 MHz, CD_3OD) δ 194.6, 167.5, 156.6, 153.0, 143.7, 137.3, 132.0, 131.5, 130.9, 130.1, 129.3, 129.1, 127.1, 126.5, 123.3, 117.6, 109.0, 108.3, 93.8, 93.7, 55.8, 55.7; MS (EI$^+$) m/z (rel. int.) 396 (M^+, 13), 334 (50), 284 (77), 256 (55), 129 (64), 73 (100). HRMS (EI$^+$) Calcd for $C_{22}H_{20}O_7$ (M^+): 396.1209. Found: 396.1202.

Compound **28**: 60% yield from **12** and **27**; pale-yellow oil; IR (neat) 3458, 2966, 1714, 1651, 1601, 1261 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 7.96 (dd, J = 7.7, 1.4 Hz, 1H), 7.77 (d, J = 9.5 Hz, 1H), 7.59 (ddd, J = 7.7, 7.7, 1.4 Hz, 1H), 7.51 (ddd, J = 7.7, 7.7, 1.4 Hz, 1H), 7.25 (dd, J = 7.7, 1.4 Hz, 1H), 6.77−6.72 (m, 2H), 5.21 (s, 2H), 4.83 (s, 2H), 3.44 (s, 3H), 3.14 (s, 3H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 195.5, 170.5, 161.9, 158.2, 145.6, 132.9, 132.2, 129.7, 128.6, 128.3, 126.2, 121.1, 108.7, 103.1, 94.2, 94.1, 56.3, 56.1; MS (EI$^+$) m/z (rel. int.) 346 (M^+, 34), 313 (16), 284 (100), 241 (13), 149 (12). HRMS (EI$^+$) Calcd for $C_{18}H_{18}O_7$ (M^+): 346.1053. Found: 346.1052.

Compound **32**: 96% yield from **12** and **31**; yellow powder; mp 102−103 °C; IR (KBr) 2952, 1705, 1651, 1514, 1242, 1147 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.87 (d, J = 7.3 Hz, 1H), 7.85 (d, J = 8.9 Hz, 1H), 7.72 (d, J = 8.9 Hz, 1H), 7.59−7.47 (m, 4H), 7.25 (d, J = 8.9 Hz, 1H), 7.14 (dd, J = 8.9, 2.4 Hz, 1H), 5.19 (s, 2H), 4.97 (s, 2H), 3.43 (s, 3H), 3.21 (s, 3H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 196.7, 172.6, 156.5, 154.3, 142.0, 133.3, 132.6, 131.1, 130.8, 130.7, 129.5, 129.5, 129.1, 125.2, 122.0, 117.3, 113.3, 107.4, 94.6, 94.3, 56.2, 56.1; MS (EI$^+$) m/z (rel. int.) 396 (M^+, 8), 334 (12), 284 (67), 256 (55), 129 (64), 73 (100). HRMS (EI$^+$) Calcd for $C_{22}H_{20}O_7$ (M^+): 396.1209. Found: 396.1198.

Compound **15**: Compound **15** was synthesized in a similar manner to that for **21** with exception of 2 equiv of **14**; 73% yield; white foam; IR (neat) 2956, 1765, 1633, 1504, 1466, 1431, 1377, 1223, 1146 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.97 (dd, J = 7.5, 1.1 Hz, 1H), 7.82 (dd, J = 7.5, 1.1 Hz, 1H), 7.68 (s, 2H), 7.64 (ddd, J = 7.5, 7.5, 1.1 Hz, 1H), 7.57 (d, J = 8.9 Hz, 2H), 7.54 (ddd, J = 7.5, 7.5, 1.1 Hz, 1H), 7.33 (s, 2H), 7.27 (d, J = 2.4 Hz, 2H), 7.05 (dd, J = 8.9, 2.4 Hz, 2H), 5.26 (s, 4H), 4.95 (ABq, νAB = 3.5 Hz, JAB = 7.0 Hz, 4H), 3.50 (s, 6H), 3.02 (s, 6H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 170.4, 155.8, 153.4, 152.4, 135.4, 133.5, 129.5, 128.9, 127.7, 127.4, 126.6, 125.5, 124.6, 124.3, 117.2, 109.5, 108.6, 94.4, 94.1, 90.6, 56.1, 55.9; MS (EI$^+$) m/z (rel. int.) 626 (M^+, 9), 595 (3), 581 (85), 565 (100), 537 (11), 521 (12). HRMS (EI$^+$) Calcd for $C_{36}H_{34}O_{10}$ (M^+): 626.2152. Found: 626.2181.

General Procedure for the Deprotection of MOM group. The synthesis of **16** is typical. A solution of 4 M hydrogen chloride in 1,4-dioxane (3.7 mL) was added dropwise to a solution of **15** (467.5 mg, 0.76 mmol) in 1,4-dioxane (5 mL) and stirred for 3 h at room temperature. The reaction mixture was poured into a mixture of ethyl acetate and 0.1 M aq. HCl. The organic layer was separated, washed successively with water and brine, dried over Na_2SO_4 and evaporated in vacuo to give a residue. The residue was purified by column chromatography (SiO_2; *n*-hexane/EtOAc = 1/2) to furnish **16** (246.3 mg, 73%) as a green foam.

Compound **16**: 73% yield; green foam; IR (KBr) 3375, 1739, 1635, 1448, 1381, 1348, 1292, 1219 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 7.93 (d, J = 7.6 Hz, 1H), 7.87 (d, J = 7.6 Hz, 1H), 7.69 (dd, J = 7.6, 7.6 Hz, 1H), 7.57−7.53 (m, 1H), 7.55 (s, 2H), 7.45 (d, J = 8.9 Hz, 2H), 6.91 (s, 2H), 6.87 (d, J = 2.4 Hz, 2H), 6.80 (dd, J = 8.9, 2.4 Hz, 2H); ^{13}C NMR (68 MHz, CD_3OD) δ 173.0, 157.1, 155.2, 154.3, 137.9, 134.6, 130.8, 129.8, 129.1, 127.7, 126.1, 125.7, 123.9, 116.5, 110.0, 107.6, 93.3 (one peak overlapped); MS (EI$^+$) m/z (rel. int.) 450 (M^+, 5), 432 (14), 387 (73), 371 (25), 358 (25), 329 (24), 300 (81), 104 (100). HRMS (EI$^+$) Calcd for $C_{28}H_{18}O_6$ (M^+): 450.1103. Found: 450.1121.

dx.doi.org/10.1021/jo300177b | J. Org. Chem. 2012, 77, 3492−3500

Compound **22**: 96% yield from **21**; brown oil; IR (neat) 3240, 1705, 1628, 1591, 1238 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 8.26 (d, J = 8.9 Hz, 1H), 8.07 (dd, J = 7.6, 1.4 Hz, 1H), 7.68 (ddd, J = 7.6, 7.6, 1.4 Hz, 1H), 7.57 (ddd, J = 7.6, 7.6, 1.4 Hz, 1H), 7.36 (dd, J = 7.6, 1.4 Hz, 1H), 7.04 (dd, J = 8.9, 2.4 Hz, 1H), 6.95 (d, J = 2.4 Hz, 1H), 6.90 (d, J = 8.9 Hz, 1H), 6.84 (d, J = 8.9 Hz, 1H); ^{13}C NMR (68 MHz, CD_3OD) δ 203.8, 168.5, 163.7, 160.7, 141.8, 141.1, 133.4, 131.3, 130.5, 130.3, 128.3, 128.0, 127.1, 119.8, 118.6, 117.9, 113.1, 110.4; MS (EI^+) m/z (rel. int.) 308 (M^+, 59), 290 (91), 284 (100), 262 (82), 256 (51), 129 (40). HRMS (EI^+) Calcd for $C_{18}H_{12}O_5$ (M^+): 308.0685. Found: 308.0694.

Compound **25**: 67% yield from **24**; yellow powder; mp 221−222 °C; IR (KBr) 3354, 1697, 1641, 1597, 1448, 1329, 1211 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 8.14 (dd, J = 7.3, 1.4 Hz, 1H), 7.74 (ddd, J = 7.3, 7.3, 1.4 Hz, 1H), 7.67 (ddd, J = 7.3, 7.3, 1.4 Hz, 1H), 7.57 (s, 1H), 7.47 (d, J = 8.9 Hz, 1H), 7.46 (dd, J = 7.3, 1.4 Hz, 1H), 7.03 (s, 1H), 6.91 (d, J = 2.4 Hz, 1H) 6.83 (dd, J = 8.9, 2.4 Hz, 1H); ^{13}C NMR (68 MHz, CD_3OD) δ 204.3, 168.4, 160.2, 158.6, 141.8, 141.5, 136.6, 133.4, 132.5, 131.4, 130.8, 130.7, 128.6, 123.2, 120.7, 118.0, 110.5, 107.8; MS (EI^+) m/z (rel. int.) 308 (M^+, 33), 290 (55), 284 (100), 262 (58), 256 (51), 241 (30), 129 (65), 73 (100). HRMS (EI^+) Calcd for $C_{18}H_{12}O_5$ (M^+): 308.0685. Found: 308.0679.

Compound **29**: 90% yield from **28**; pale-yellow powder; mp 203−205 °C; IR (KBr) 3398, 1714, 1624, 1356, 1282, 1227, 1122 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 8.08 (dd, J = 7.8, 1.4 Hz, 1H), 7.69 (ddd, J = 7.8, 7.8, 1.4 Hz, 1H), 7.61 (ddd, J = 7.8, 7.8, 1.4 Hz, 1H), 7.36 (dd, J = 7.8, 1.4 Hz, 1H), 6.92 (d, J = 8.9 Hz, 1H), 6.31 (d, J = 2.4 Hz, 1H), 6.21 (dd, J = 8.9, 2.4 Hz, 1H); ^{13}C NMR (68 MHz, CD_3OD) δ 202.5, 168.5, 166.4, 166.4, 141.8, 135.9, 133.3, 131.3, 130.5, 130.4, 128.5, 114.8, 108.9, 103.5; MS (EI^+) m/z (rel. int.) 258 (M^+, 18), 256 (60), 213 (18), 129 (33), 73 (43). HRMS (EI^+) Calcd for $C_{14}H_{10}O_5$ (M^+): 258.0528. Found: 258.0527.

Compound **33**: 94% yield from **32**; brown powder; mp 168−169 °C; IR (KBr) 3375, 1705, 1633, 1518, 1292, 1213 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 7.97 (dd, J = 7.4, 1.4 Hz, 1H), 7.85 (d, J = 8.6 Hz, 1H), 7.61 (d, J = 8.6 Hz, 1H), 7.58 (ddd, J = 7.4, 7.4, 1.4 Hz, 1H), 7.51 (ddd, J = 7.4, 7.4, 1.4 Hz, 1H), 7.17 (dd, J = 7.4, 1.4 Hz, 1H), 6.91 (d, J = 8.6 Hz, 1H), 6.87 (d, J = 2.4 Hz, 1H), 6.82 (dd, J = 8.6, 2.4 Hz, 1H); ^{13}C NMR (68 MHz, CD_3OD) δ 202.1, 170.3, 163.7, 158.1, 144.6, 137.7, 135.5, 132.8, 132.1, 131.4, 131.3, 131.0, 128.5, 124.5, 116.3, 116.1, 114.8, 109.3; MS (EI^+) m/z (rel. int.) 308 (M^+, 5), 284 (11), 160 (100), 104 (53). HRMS (EI^+) Calcd for $C_{18}H_{12}O_5$ (M^+): 308.0685. Found: 308.0680.

General Procedure for the Benzene-ring Expanded Fluorescein Derivatives. The synthesis of **5** is typical. A solution of **16** (127.5 mg, 0.28 mmol) in toluene (1.5 mL) and methanesulfonic acid (0.8 mL) was stirred for 30 min at 65 °C. The reaction mixture was poured into a mixture of ethyl acetate and water. The organic layer was separated, washed successively with water (twice) and brine, dried over Na_2SO_4 and evaporated in vacuo to give a residue. The residue was purified by column chromatography (SiO_2; toluene-EtOAc = 2:1) to afford **5** (94.7 mg, 77%) as a pale-blue powder.

Compound **5**: 77% yield from **16**; pale-blue powder; mp 291 °C (decomp.); IR (KBr) 3423, 1736, 1637, 1506, 1441, 1346, 1290 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 8.10 (dd, J = 6.5, 1.4 Hz, 1H), 7.85−7.74 (m, 2H), 7.57 (s, 2H), 7.55 (d, J = 8.9 Hz, 2H), 7.37 (dd, J = 6.6, 1.4 Hz, 1H), 7.30 (s, 2H), 7.10 (d, J = 2.4 Hz, 2H), 6.95 (dd, J = 8.9, 2.4 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 172.3, 159.2, 155.0, 152.1, 138.8, 137.6, 132.3, 131.9, 129.4, 128.5, 127.3, 127.2, 126.1, 120.6, 120.0, 112.6, 109.3, 86.1; MS (EI^+) m/z (rel. int.) 432 (M^+, 10), 388 (52), 371 (15), 284 (23), 241 (23), 185 (27), 129 (55), 73 (100). HRMS (EI^+) Calcd for $C_{28}H_{16}O_5$ (M^+): 432.0998. Found: 432.0996.

Compound **4**: 76% yield from **22** and **11**; pale-pink powder; mp >300 °C; IR (KBr) 3311, 1724, 1610, 1398, 1242 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 10.12 (s, 1H), 9.83 (s, 1H), 8.42 (d, J = 8.9 Hz, 1H), 8.16 (d, J = 7.3 Hz, 1H), 8.03 (d, J = 8.9 Hz, 1H), 7.79 (d, J = 8.9 Hz, 1H), 7.72 (dd, J = 7.3, 7.3 Hz, 1H), 7.66 (dd, J = 7.3, 7.3 Hz, 1H), 7.53 (d, J = 8.9 Hz, 1H), 7.37 (d, J = 8.9 Hz, 1H), 7.26 (dd, J = 8.9, 2.2 Hz, 1H), 7.19 (d, J = 7.3 Hz, 1H), 7.11 (d, J = 2.2 Hz, 1H), 6.93 (dd, J = 8.9, 2.2 Hz, 1H), 6.51 (d, J = 8.9 Hz, 1H), 6.30 (d, J = 2.2 Hz, 1H); ^{13}C NMR (68 MHz, DMSO-d_6) δ 169.3, 157.4, 156.6, 154.5, 150.3, 144.4, 135.9, 135.4, 132.8, 132.6, 131.1, 130.1, 126.3, 125.6, 125.4, 123.6, 123.5, 123.1, 122.5, 119.2, 116.8, 116.5, 114.7, 110.5, 109.4, 106.7, 106.4, 83.5; MS (EI^+) m/z (rel. int.) 432 (M^+, 13), 387 (63), 371 (100), 284 (90), 256 (53), 129 (40); HRMS (EI^+) Calcd for $C_{28}H_{16}O_5$ (M^+): 432.0998. Found: 432.1014.

Compound **3**: 48% yield from **25** and 2,7-dihydroxynaphthalene (**11**); pale-purple powder; mp 196−199 °C; IR (KBr) 3410, 1736, 1624, 1520, 1444, 1354, 1286 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 8.21 (d, J = 7.6 Hz, 1H), 7.89 (d, J = 8.6 Hz, 1H), 7.73−7.61 (m, 2H), 7.69 (d, J = 8.8 Hz, 1H), 7.52 (s, 1H), 7.51 (d, J = 8.9 Hz, 1H), 7.29 (d, J = 8.9 Hz, 1H), 7.16 (s, 1H), 7.08−7.05 (m, 2H), 6.93 (dd, J = 8.8, 2.4 Hz, 1H), 6.88 (dd, J = 8.6, 2.2 Hz, 1H), 6.44 (d, J = 2.2 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.2, 159.1, 158.7, 157.9, 153.7, 149.6, 138.2, 137.8, 135.3, 134.9, 132.9, 131.8, 129.4, 128.5, 128.1, 127.7, 127.6, 125.1, 120.7, 120.3, 117.9, 116.8, 112.1, 108.9, 108.6, 86.1 (some peaks overlapped); MS (EI^+) m/z (rel. int.) 432 (M^+, 2), 387 (5), 371 (10), 284 (23), 129 (56), 73 (100). HRMS (EI^+) Calcd for $C_{28}H_{16}O_5$ (M^+): 432.0998. Found: 432.1001.

Compound **6**: 47% yield from **25** and 1,6-dihydroxynaphthalene (**26**); purple powder; mp 182−183 °C; IR (KBr) 3336, 1736, 1637, 1288, 1252 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 8.50 (d, J = 8.9 Hz, 1H), 8.11−8.08 (m, 1H), 7.77−7.73 (m, 2H), 7.76 (s, 1H), 7.57 (d, J = 8.9 Hz, 1H), 7.32 (d, J = 8.9 Hz, 1H), 7.32 (s, 1H), 7.25 (dd, J = 8.9, 2.4 Hz, 1H), 7.23−7.21 (m, 1H), 7.15 (d, J = 2.4 Hz, 1H), 7.12 (d, J = 2.4 Hz, 1H), 6.97 (dd, J = 8.9, 2.4 Hz, 1H), 6.67 (d, J = 8.9 Hz, 1H); ^{13}C NMR (68 MHz, CD_3OD) δ 171.5, 158.4, 158.0, 154.9, 150.0, 150.0, 148.7, 137.8, 137.5, 136.6, 131.0, 130.7, 129.0, 127.4, 126.6, 125.8, 125.0, 124.8, 124.7, 122.8, 119.6, 119.2, 118.4, 111.8, 110.6, 110.3, 108.3, 85.4; MS (EI^+) m/z (rel. int.) 432 (M^+, 17), 387 (70), 284 (100), 256 (60), 149 (28), 97 (35). HRMS (EI^+) Calcd for $C_{28}H_{16}O_5$ (M^+): 432.0998. Found: 432.1009.

Compound **9**: 82% yield from **25** and resorcinol; orange powder; mp 185−187 °C; IR (KBr) 3354, 1732, 1624, 1444, 1240, 1165 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 10.09 (s, 1H), 9.92 (s, 1H), 7.98 (d, J = 7.3 Hz, 1H), 7.74 (dd, J = 7.3, 7.3 Hz, 1H), 7.68 (dd, J = 7.3, 7.3 Hz, 1H), 7.59 (d, J = 8.9 Hz, 1H), 7.57 (s, 1H), 7.26 (d, J = 7.3 Hz, 1H), 7.25 (s, 1H), 7.05 (s, 1H), 6.87 (d, J = 8.9 Hz, 1H), 6.66 (s, 1H), 6.53 (d, J = 8.9 Hz, 1H), 6.48 (d, J = 8.9 Hz, 1H); ^{13}C NMR (68 MHz, DMSO-d_6) δ 168.6, 159.6, 156.7, 152.4, 152.0, 148.6, 135.7, 135.6, 130.1, 129.9, 128.8, 127.8, 125.8, 124.8, 124.5, 123.9, 118.3, 117.3, 112.3, 110.2, 109.7, 107.1, 102.4, 82.8; MS (EI^+) m/z (rel. int.) 382 (M^+, 12), 337 (68), 284 (90), 256 (50), 207 (44), 129 (64), 73 (100); HRMS (EI^+) Calcd for $C_{24}H_{14}O_5$ (M^+): 382.0841. Found: 382.0837.

Compound **8**: 30% yield from **29** and 2,7-dihydroxynaphthalene (**11**); red powder; mp 178−179 °C; IR (KBr) 3255, 1732, 1628, 1522, 1446, 1240 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 8.09 (dd, J = 5.7, 1.6 Hz, 1H), 7.81 (d, J = 8.9 Hz, 1H), 7.66−7.57 (m, 3H), 7.18 (d, J = 8.9 Hz, 1H), 7.00 (dd, J = 5.9, 1.6 Hz, 1H), 6.82 (dd, J = 8.6, 2.4 Hz, 1H), 6.63 (d, J = 1.4 Hz, 1H), 6.49 (dd, J = 9.2, 1.4 Hz, 1H), 6.45 (d, J = 9.2 Hz, 1H), 6.32 (d, J = 2.4 Hz, 1H); ^{13}C NMR (68 MHz, CD_3OD) δ 172.1, 160.4, 157.4, 156.3, 152.5, 151.6, 136.6, 134.2, 133.6, 131.8, 130.6, 129.3, 127.9, 127.1, 126.3, 124.3, 116.9, 115.7, 113.8, 112.1, 108.1, 107.7, 102.9, 86.4; MS (EI^+) m/z (rel. int.) 382 (M^+, 13), 337 (52), 321 (100), 284 (30), 256 (15). HRMS (EI^+) Calcd for $C_{24}H_{14}O_5$ (M^+): 382.0841. Found: 382.0845.

Preparation of the Benzene-ring Expanded Fluorescein Derivatives 2, 3 and 5 through the Conventional Double Friedel−Crafts Reaction. A solution of phthalic anhydride (150 mg, 1.01 mmol) and 2,7-dihydroxynaphthalene (322.4 mg, 2.03 mmol) in methanesulfonic acid (2 mL) was stirred at 100 °C for 10 h. The reaction mixture was poured into iced-water and a precipitate was collected by filtration and dried under reduced pressure. A soluble fraction from the thick paste was extracted with a mixed solvent of chloroform and methanol (1:10) and evaporated in vacuo to give a residue. The residue was purified by reverse phase column chromatography (water/methanol = 1/3) to give a mixture of **3** and **5** and pure **2** (25.0 mg, 6% yield). The mixture of **3** and **5** was purified by preparative TLC on silica gel (*n*-hexane/ethyl acetate = 2/1

dx.doi.org/10.1021/jo300177b | J. Org. Chem. 2012, 77, 3492−3500

developing about 10 times) to afford **3** (16.3 mg, 4% yield) and **5** (6.3 mg, 1% yield)

Compound **2**. 6% yield; pale-pink powder; mp >300 °C; IR (KBr) 3421, 1720, 1637, 1616, 1442, 1406, 1238 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 8.22 (d, J = 7.3 Hz, 1H), 7.83 (d, J = 8.6 Hz, 2H), 7.66 (d, J = 8.6 Hz, 2H), 7.63−7.55 (m, 2H), 7.26 (d, J = 8.6 Hz, 2H), 7.14 (d, J = 7.6 Hz, 1H), 6.89 (dd, J = 8.6, 2.0 Hz, 2H), 6.76 (d, J = 2.0 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.4, 158.6, 156.7, 151.5, 137.6, 134.7, 133.2, 132.0, 131.4, 128.7, 127.0, 125.8, 117.5, 116.4, 110.4, 109.2, 89.0 (one peak overlapped); MS (EI^+) m/z (rel. int.) 432 (M^+, 1), 416 (11), 387 (13), 371 (18), 284 (23), 241 (23), 213 (21), 185 (27), 129 (55), 97 (26), 73 (100). HRMS (EI^+) Calcd for $C_{28}H_{16}O_5$ (M^+): 432.0998. Found: 432.1019. Anal. Calcd for $C_{28}H_{16}O_5$: C, 77.77; H, 3.73. Found: C, 77.57; H, 3.45.

Compound **19**: A solution of 1 M BBr_3 in dichloromethane (4.6 mL) was added dropwise to a solution of **18** (1.05 g, 4.15 mmol) in dichloromethane (7 mL) under ice-bath cooling, and stirred for 16 h at room temperature. The reaction mixture was poured into a mixture of chloroform and water. The organic layer was separated, washed with brine, dried over Na_2SO_4 and evaporated in vacuo to give a residue. The residue was purified by column chromatography (SiO_2; *n*-hexane/EtOAc = 5/1) to give **19** (0.87 g, 88%) as a white powder; mp 89 °C (decomp); IR (KBr) 3452, 1624, 1587, 1385, 1171 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 8.03 (d, J = 8.9 Hz, 1H), 7.30 (d, J = 8.9 Hz, 1H), 7.03 (d, J = 8.9 Hz, 1H), 6.99 (d, J = 8.9 Hz, 1H), 6.98 (s, 1H); ^{13}C NMR (68 MHz, CD_3OD) δ 156.7, 150.4, 136.6, 130.6, 124.8, 121.7, 120.3, 118.6, 109.8, 101.8; MS (EI^+) m/z (rel. int.) 240 (M^+, 21), 238 (M^+, 21), 153 (69), 136 (52), 107 (52), 89 (68), 77 (100), 58 (25). HRMS (EI^+) Calcd for $C_{10}H_7O_2{}^{79}Br$ (M^+): 237.9629. Found: 237.9622.

Compound **23**: A solution of **4** (11.0 mg, 0.025 mmol) and acetic anhydride (48.1 μL, 0.51 mmol) in acetic acid (29 μL) was stirred for 18 h at 100 °C. The reaction mixture was poured into a mixture of chloroform and aqueous $NaHCO_3$. The organic layer was separated, washed with water (twice) and brine, dried over Na_2SO_4 and evaporated in vacuo to give a powder. The powder was washed with a mixed solvent of *n*-hexane/EtOAc = 10/1 to give **23** (12.0 mg, 92%) as a white powder; mp 292−293 °C; IR (KBr) 1766, 1412, 1369, 1200 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.59 (d, J = 8.9 Hz, 1H), 8.19 (dd, J = 7.3, 1.1 Hz, 1H), 7.96 (d, J = 8.9 Hz, 1H), 7.81 (d, J = 8.9 Hz, 1H), 7.65 (ddd, J = 7.3, 7.3, 1.1 Hz, 1H), 7.60 (d, J = 8.9 Hz, 1H), 7.59 (ddd, J = 7.3, 7.3, 1.1 Hz, 1H), 7.53 (d, J = 2.2 Hz, 1H), 7.41 (d, J = 8.9 Hz, 1H), 7.41 (dd, J = 8.9, 2.2 Hz, 1H), 7.10 (dd, J = 8.9, 2.2 Hz, 1H), 7.08 (dd, J = 7.3, 1.1 Hz, 1H), 6.89 (d, J = 2.2 Hz, 1H), 6.77 (d, J = 8.9 Hz, 1H), 2.37 (s, 3H), 2.20 (s, 3H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 169.7, 169.1, 168.6, 154.7, 150.6, 150.0, 149.3, 144.7, 135.5, 134.5, 132.3, 131.8, 130.3, 129.8, 129.2, 126.5, 125.2, 123.8, 123.7, 123.6, 123.4, 121.8, 121.3, 119.5, 118.5, 117.9, 115.5, 113.3, 108.3, 83.4, 21.3, 21.2; MS (EI^+) m/z (rel. int.) 516 (M^+, 13), 429 (25), 413 (100), 387 (50), 371 (50), 300 (15), 97 (11), 57 (16). HRMS (EI^+) Calcd for $C_{32}H_{20}O_7$ (M^+): 516.1209. Found: 516.1205.

Treatment of Living Cells with Compound 23. NIH3T3 and HEK293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with penicillin (100 units/mL), streptomycin (100 μg/mL), and 5 and 7.5% (v/v) fetal bovine serum, respectively, in a humidified incubator containing 5% CO_2 gas. The clear image of cells was not obtained in a culture dish under our fluorescence microscope. Therefore, the confluent cells were trypsinized, and then the isolated 5 × 10^6 cells were treated with 1.0 mL of 1× PBS containing 0.1% (v/v) glucose, 0.058% (v/v) L-glutamine and compound **23** (final 10, 50, 100 μM) dissolved in DMSO (final less than 1%) for 15, 30, and 60 min at room temperature. After the treatment, the medium containing fluorescein derivative was removed and the cells were suspended in 20 μL of 1× PBS containing 0.1% (v/v) glucose and 0.058% (v/v) L-glutamine. Fluorescence images of the treated cells were captured using an Axio Imager M1 (Carl Zeiss) with a CCD camera, a Mercury Arc lamp with a G-365 nm excitation filter, and a BP-445/50 nm emission filter, or a BP-545/25 nm excitation filter, and a BP-625/53 nm emission filter. The cell viability after dye treatment was assessed by trypan blue staining. More than 90% cells were still alive even after treatment with 100 μM dye for 60 min.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Coloration of seminaphthofluoresceines **8**−**10** under various pH conditions, calculated UV−vis spectrum, HOMO and LUMO of the dianion and cation of compound **5**, ^1H and ^{13}C NMR spectra for all new compounds, and CIF of compound **5** (CCDC 862009). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was partly supported by KAKENHI (No. 22390003), Kyoto Prefectural University Research Fund, A-STEP from JST (No. 231Z03077), and a Grant-in-Aid for JSPS Fellows to E.A.

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