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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3000–3004

Preparation and biochemical evaluation of fluorenone-containing lipid analogs

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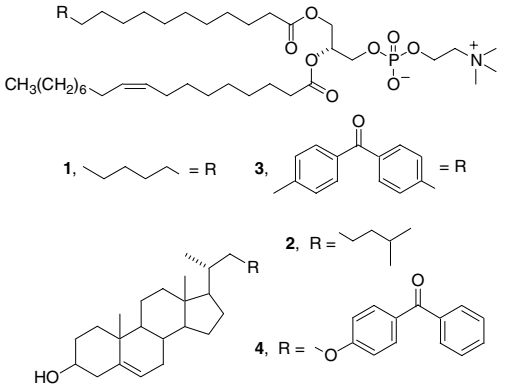
Received 31 January 2006; revised 21 February 2006; accepted 21 February 2006
Available online 10 March 2006

Abstract—Syntheses are described of fatty acid analogs **5** and **6**, and cholesterol (**2**) analogs **7** and **8** containing fluorenone groups, which are both photoactivable and fluorescent. The potential of the analogs of **2** as biochemical research tools has been demonstrated by the findings that **7** and **8** can replace **2** in apolipoprotein A-I-induced cellular efflux of **2** and that fluorescence is easily visible at the surface of smooth muscle cells equilibrated with **8**.

For use as photoaffinity labels in a collaborative study of cellular lipid efflux and high density lipoprotein (HDL) formation, we have recently prepared a number of benzophenone-containing analogs of phosphatidylcholine (**1**)[1] and cholesterol (**2**).[2] The analogs of **1** are exemplified by **3**, with a benzophenone group incorporated at the end of the C1 fatty acid chain. The cholesterol analogs include **4**, known as FCBP, which can replace at least 47% of cellular **2** without perturbing normal cellular function,[3] and which has proved to be a valuable research tool for elucidating the mechanisms of cholesterol efflux.[3,4]

The widely used benzophenone group offers several distinct advantages as a photophore,[5–7] including its hydrophobicity, which makes it especially attractive for covalent attachment to lipids. On the other hand, the potential of the structurally closely similar fluorenone moiety has barely been explored. Two examples of the use of substituted fluorenones as protein photoaffinity labeling agents have appeared, citing the conformational rigidity of the fluorenone group as important.[7–9] However, the fact that fluorenones are fluorescent as well as photoactivable appears not to have been investigated



for its possible value in biochemical research. We decided to incorporate fluorenone groups into analogs of **1** and **2** to determine if such compounds could be used as fluorescent molecular probes. In this letter, the syntheses of fluorenone-containing fatty acid analogs **5** and **6**, and cholesterol analogs **7** and **8** are described, along with preliminary assessment of the potential biochemical utility of **7** and **8**.

Syntheses of **5** and **6** were readily accomplished, as in the case of their benzophenone-containing counterparts,[1] by use of Suzuki coupling reactions (Scheme 1). In the case of **5**, commercially available 2-bromofluorenone (**9**) was coupled[10,11] with methyl 10-undecenoate (**10**), which

Keywords: Fluorenones; Lipids; Cholesterol; Fatty acids.
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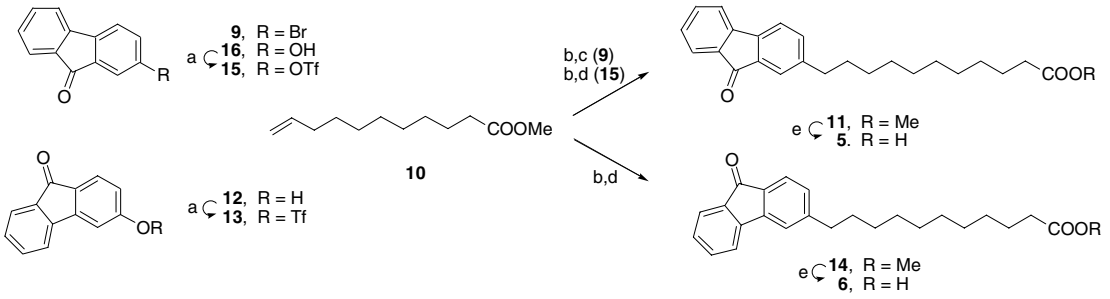
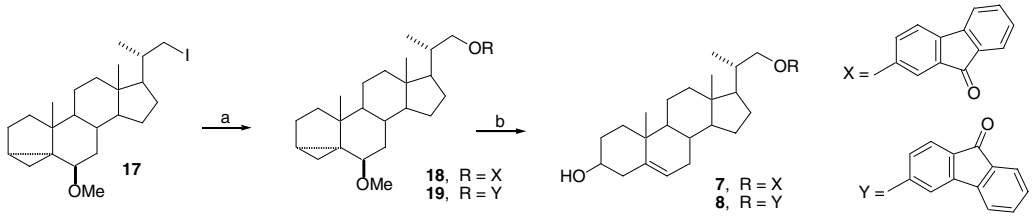
doi:10.1016/j.bmcl.2006.02.061



Scheme 1. Reagents and conditions: (a) Tf_2O, 2,6,di-*tert*-butyl-4-methylpyridine, CH_2Cl_2, −78 °C, 1 h, 0 °C, 1.5 h; (b) 9-BBN, THF, 0 °C, 2 h, rt, 3 h; (c) **9**, Cs_2CO_3, $AsPh_3$, Pd(dppf)Cl_2, THF, DMF, H_2O, 85 °C, overnight; (d) **13**, K_3PO_4, Pd(dppf)Cl_2, THF, overnight at reflux; (e) KOH, 95% EtOH, rt, 4 h, 10% HCl.



Scheme 2. Reagents and conditions: (a) NaH, THF, **16** (for **18**) or **12** (for **19**), DMSO, THF, reflux, 17 h; (b) TsOH, dioxane, H_2O, 80 °C, 5 h.

had been subjected to hydroboration with 9-BBN, to afford ester **11** in 54% yield.[12] Saponification of **11** then gave 80% of pure **5**.[13] For reasons that remain unclear, we had difficulty reproducing a literature synthesis[14] of 3-bromofluorenone, so it was decided instead to prepare 3-hydroxyfluorenone (**12**) and use its triflate derivative **13** for the Suzuki coupling.[15] 3-Methoxyfluorenone was prepared by the procedure of Kym et al.[16] in an improved 70% overall yield. Ether cleavage of **12** was then effected with HBr/CH_3COOH[17] in 95% yield, as compared to the 37% yield obtained with $BF_3{\cdot}SMe_2$.[16] After conversion of **12** to triflate **13** in 90% yield,[18] Suzuki coupling with the 9-BBN[15] adduct from **10** gave 42% of ester **14**,[19] which was quantitatively saponified to **6**.[13] Synthesis of isomeric ester **11** was also performed in 47% yield by this route from triflate **15**, formed in 98% yield from commercially available 2-hydroxyfluorenone (**16**).[18]

The fluorenone-containing cholesterol analogs **7** and **8** were readily prepared from the *i*-steroid C22 iodide **17**[20] which we had used previously for the synthesis of several cholesterol derivatives with modified side chains (Scheme 2).[21,22] Alkylation of the anions of hydroxyfluorenones **16** and **12** with **17** by the procedure of Heathcock et al.[23] gave fluorenylated *i*-steroids **18** and **19** in 69% and 53% yield, respectively.[24] Acidic hydrolysis then converted **18** to **7** (74% yield) and **19** to **8** (77% yield).[25]

The yellow fluorenone-containing analogs **7** and **8** were evaluated as cholesterol (**2**) surrogates first by examining their ability to replace **2** in the efflux of **2** induced by apolipoprotein A-I, the major protein of HDL. An assay to measure such efflux was recently developed in human skin fibroblasts and vascular smooth muscle cells (SMC)[2,3] for use with several benzophenone-containing analogs of **2** that were found successfully to replace **2** in this complex pathway of multiple cell-surface and intracellular steps.[2] When this assay, described briefly in the legend for Figure 1, was applied to **7** and **8**, these compounds also decreased efflux of [^{3}H]-**2** to an extent comparable to that of **2** itself, establishing that **7** and **8** may be useful research tools in biochemical experiments.

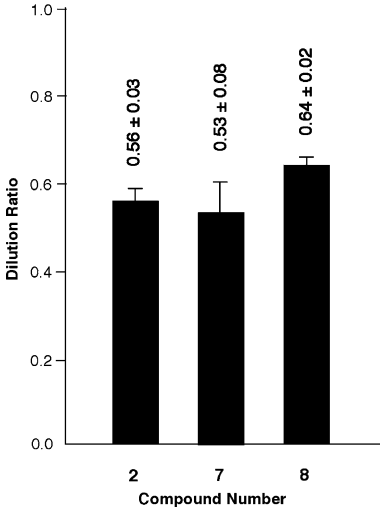


Figure 1. The dilution of [^{3}H]cholesterol ([^{3}H]-**2**) radioactivity in fibroblast monolayers by **2** or FC analogs **7** and **8**. The dilution ratio is the reduction in [^{3}H]-**2** efflux to the cellular medium when fibroblast monolayers were equilibrated (48 h, 37 °C) with 10 μCi [^{3}H]-**2** plus unlabeled **2** or analog equal to the total sterol content of cells and medium (10% plasma v/v) compared with cells labeled with the same level of tracer [^{3}H]-**2** only. Complete equilibration between sterol pools is indicated by a dilution ratio comparable to that produced by the added unlabeled **2**, designed to be approximately 50%. Values shown represent means ± 1 SD of three independent experiments, each including triplicate dishes of fibroblasts incubated as described in Ref. 2.

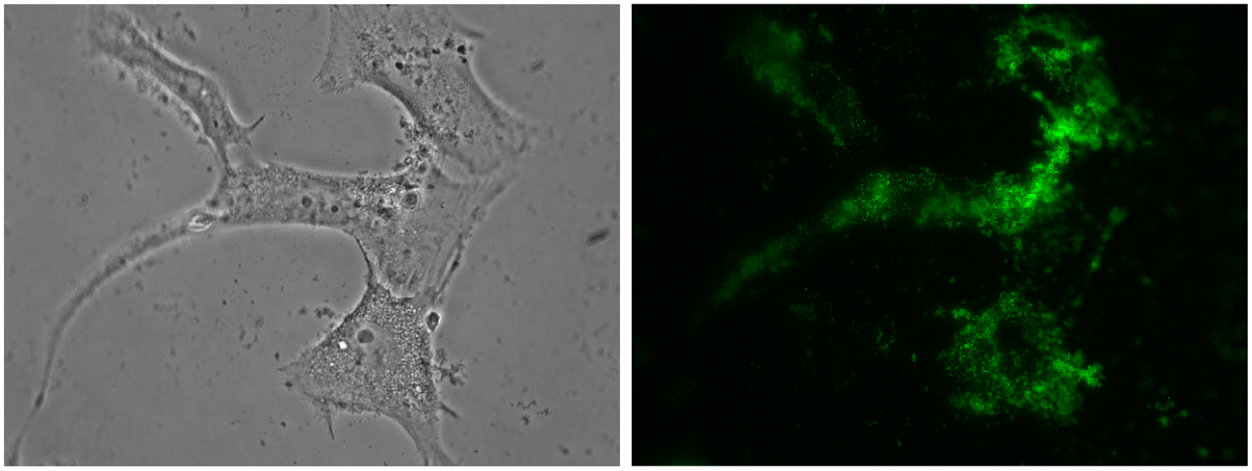


Figure 2. Human aortic smooth muscle cells (SMC) were cultured in 10% bovine fetal serum (BFS) as previously described in Ref. 3. A solution of **8** in DMSO (1 mg/mL) was incorporated into BFS to a concentration (30 μg/mL) equivalent to 50% of BFS-cholesterol. After incubation with the cells for 24 h at 37 °C, the medium was removed. The cells were washed and visualized with a Nikon Eclipse TS/100-F microscope, with Fifc filter. The images shown (magnification 60×) are: top, SMC by phase contrast microscopy; and, bottom, fluorescence emission microscopy of the same cells.

The potential utility of the fluorescence of these analogs was also demonstrated. Compounds **5**–**8** all exhibit intense green fluorescence in the 500–550 nm range.[26] Preliminary assessment of these compounds as biochemical fluorophores was conducted by incubation of **8** with SMC.[3] As shown in Figure 2, after **8** was equilibrated into SMC, highly visible fluorescence was concentrated at the cell surface, particularly at the leading edge of pseudopodia, in a pattern similar to that observed by immunofluorescence for caveolin,[3] a key protein involved in cholesterol efflux.

Acknowledgment

This research was supported by Grant HL67294 from the National Institutes of Health.

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12. Preparation of **11**. From **9**: according to the method of Johnson and Braun,[10] to a solution of 336 mg (1.70 mmol) of methyl 10-undecenoate (**10**) in 5 mL of dry THF was added 4.1 mL of 0.5 M 9-BBN in THF at 0 °C. The mixture was stirred at 0 °C for 2 h, and at rt for 3 h, and then was introduced into a mixture of 550 mg (2.12 mmol) of 2-bromofluorenone (**9**), 2.07 mg (6.37 mmol) Cs_2CO_3, 130 mg (0.424 mmol) $AsPh_3$, and 346 mg (0.424 mmol) $Pd(dppf)Cl_2$ in a mixture of 8 mL of THF, 8 mL DMF, and 2 mL H_2O. The resulting mixture was heated at 85 °C overnight, cooled, and passed through a short pad of silica gel with 4:1 hexane/EtOAc. The solvent was evaporated and the residue was dissolved in 150 mL EtOAc, washed with brine, dried, filtered, and evaporated to give 1.30 g of residue, which was chromatographed on silica gel (25:1–20:1 hexane/EtOAc) to give 348 mg (54%) of yellow **11**, mp 59.7–61.3 °C, which was recrystallized from hexane/EtOAc to give **11**: mp 60.9–62.3 °C; ^{1}H NMR (500 MHz) δ 7.64 (m, 1H), 7.48 (m, 3H), 7.42 (m, 1H), 7.26 (m, 2H), 3.68 (s, 3H), 2.64 (t, J = 7.5 Hz, 2H), 2.31 (t, J = 7.5 Hz, 2H), 1.63 (m, 4H), 1.29 (m, 12H); ^{13}C NMR (125 MHz) δ 194.6, 174.6, 144.9, 144.7, 142.3, 134.9, 134.9, 134.7, 134.6, 128.8, 124.6, 124.5, 120.4, 120.2, 51.7, 36.0, 34.3, 31.4, 29.7, 29.7, 29.7, 29.5, 29.4, 29.4, 25.2. Anal. Calcd for $C_{25}H_{30}O_3$: C, 79.33; H, 7.99. Found: C, 79.23; H. 7.98. From **15**: exactly as in the preparation of **14** described below, 400 mg (2.02 mmol) of **10** was treated with 9-BBN and then coupled with **15** to afford, after chromatography, 299 mg (47%) of yellow solid **11**, contaminated with a trace of **15**.
13. Preparation of **5** and **6**. For **5**, to a solution of 235 mg (0.62 mmol) of **11** in 8 mL of 95% ethanol was added 208 mg (1.86 mmol) of KOH. The mixture was stirred at rt for 4 h and 5 mL of 10% HCl was added to adjust the pH to 2. The solvent was evaporated and the residue was extracted with EtOAc, washed with brine, dried, filtered, and evaporated to give 340 mg of residue, which was chromatographed on silica gel (9:1–2:1 hexane/EtOAc) to give 180 mg (80%) of yellow **5**, mp 91.2–93.0 °C, which was recrystallized from hexane/EtOAc to give **5**: mp 93.6–94.3 °C; λ_{max} (CH_2Cl_2) 263 nm (ε 6.0×10^4), 255 nm (ε 5.4×10^4); ^{1}H NMR δ 7.64 (m, 1 H), 7.48 (m, 3H),

7.42 (m, 1H), 7.28 (m, 2H), 2.64 (t, J = 7.5 Hz, 2H), 2.38 (t, J = 7.5 Hz, 2H), 1.64 (m, 4H), 1.31 (m, 12H); ^{13}C NMR δ 194.6, 180.4, 144.9, 144.7, 142.3, 134.94, 134.92, 134.6, 134.5, 128.8, 124.6, 124.5, 120.4, 120.3, 36.0, 34.4, 31.4, 29.7, 29.65, 29.62, 29.5, 29.4, 29.3, 24.9. Anal. Calcd for $C_{24}H_{28}O_3$: C, 79.09; H, 7.74. Found: C, 79.08; H, 7.79. For **6**, as in the preparation of **5**, 143 mg (0.38 mmol) of **14** was saponified to give, after silica gel chromatography, 137 mg (100%) of yellow **6**, mp 112–114 °C, which was recrystallized from hexane/EtOAc to give **6**: mp 114.1–115.5 °C; λ_{max} (CH_2Cl_2) 265 nm (ε 5.6×10^4), 260 nm (ε 5.7×10^4); ^{1}H NMR (500 MHz) δ 7.65 (m, 1H), 7.58 (m, 1H), 7.50 (m, 2H), 7.28 (m, 2H), 7.10 (m, 1H), 2.70 (t, J = 7.5 Hz, 2H), 2.36 (t, J = 7.5 Hz, 2H), 1.66 (br, 4H), 1.32 (br, 12H); ^{13}C NMR (125 MHz) δ 194.0, 180.1, 151.2, 145.1, 144.7, 135.0, 134.7, 132.3, 129.3, 129.2, 124.6, 124.4, 120.8, 120.4, 36.8, 34.2, 31.4, 29.7, 29.7, 29.6, 29.5, 29.5, 29.3, 24.9. Anal. Calcd for $C_{24}H_{28}O_3$: C, 79.09; H, 7.74. Found: C, 78.79; H. 7.79.

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18. Preparation of **13** and **15**. For **13**, to a solution of 630 mg (3.21 mmol) of 3-hydroxyfluorenone (**12**), mp 232–234 °C (lit.[16] mp 215–220 °C), and 1.65 g (8.04 mmol) of 2,6-di-*tert*-butyl-4-methylpyridine in 20 mL of dry CH_2Cl_2 was added 0.65 mL (3.85 mmol) Tf_2O dropwise at −78 °C. The mixture was stirred at −78 °C for 1 h and at 0 °C for 1.5 h, and then was evaporated to give 2.79 g of residue, which was chromatographed on silica gel (20:1 hexane/EtOAc) to give 952 mg (90%) of yellow **13**: mp 114.0–115.8 °C; ^{1}H NMR δ 7.70 (m, 2H), 7.51 (m, 2H), 7.37 (m, 2H), 7.18 (m, 1H); ^{13}C NMR δ 191.6, 154.0, 147.3, 142.6, 135.4, 134.4, 133.8, 130.6, 126.2, 124.9, 121.8, 121.2, 121.1, 116.8, 114.2. For **15**, exactly as in the preparation of **13**, 350 mg (1.78 mmol) of 2-hydroxyfluorenone (**16**) was converted to 577 mg (98%) of yellow **15**: mp 99.3–100.4 °C; ^{1}H NMR (500 MHz) δ 7.73 (m, 1H), 7.62 (m, 1H), 7.58 (m, 3H), 7.40 (m, 2H); ^{13}C NMR δ 191.0, 150.2, 144.3, 143.2, 136.4, 135.6, 134.5, 130.1, 127.4, 125.1, 122.0, 121.1, 117.9, 116.8.
19. Preparation of **14**. According to the procedure of Ohe, Miyaura, and Suzuki,[15] to a solution of 198 mg (1.00 mmol) of **10** in 5 mL of dry THF was added 2.4 mL of 0.5 M 9-BBN in THF at 0 °C. The mixture was stirred at 0 °C for 2 h, and at rt for 2 h, and then was introduced into a mixture of 300 mg (0.91 mmol) of **13**, 291 mg (1.37 mmol) of K_3PO_4, and 56 mg (0.069 mmol) of Pd (dppf) Cl_2 in 4 mL THF. The resulting mixture was heated at reflux overnight, cooled, and passed through a short pad of silica gel with 4:1 hexane/EtOAc. The solvent was evaporated and the residue was chromatographed on silica gel (20:1 hexane/EtOAc) to afford 144 mg (42%) of yellow **14**, mp 58.1–59.8 °C, which was recrystallized from hexane/EtOAc to give **14**: mp 60.0–61.3 °C; ^{1}H NMR δ 7.65–7.44 (m, 4H), 7.33–7.25 (m, 2H), 7.10 (m, 1H), 3.68 (s, 3H), 2.67 (t, J = 7.5 Hz, 2H), 2.32 (t, J = 7.5 Hz, 2H), 1.64 (br, 4H), 1.30 (br, 12H); ^{13}C NMR δ 193.9, 174.5, 151.1, 145.0, 144.6, 135.0, 134.6, 132.3, 129.3, 129.2, 124.6, 124.4, 120.8, 120.4, 51.7, 36.8, 34.4, 31.4, 29.7, 29.7, 29.7, 29.5, 29.5, 29.4, 25.2. Anal. Calcd for $C_{25}H_{30}O_3$: C, 79.33; H, 7.99. Found: C, 79.41; H, 8.09.
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24. Preparation of **18** and **19**. For **18**, according to a procedure by Heathcock et al.[23] to a suspension of 69 mg (2.66 mmol) of NaH in 5 mL of THF was added a solution 196 mg (1.0 mmol) of **16** in 5 mL of dry THF, followed by 1 mL DMSO and 460 mg (1.0 mmol) of **17** dissolved in 5 mL THF. The resulting mixture was heated at reflux for 17 h, cooled, and poured into 15 mL water. The water layer was saturated with NaCl and extracted with ether. The combined ether layers were washed with water and brine, dried, filtered, and evaporated to give 0.8 g of residue, which was chromatographed on silica gel (9:1–4:1 hexane/EtOAc) to give 330 mg (69%) of yellow **18**: mp 108–110 °C; ^{1}H NMR δ 7.60 (m, 1H), 7.40 (m, 3H), 7.18 (m, 2H), 6.96 (m, 1H), 3.95 (dd, J = 9.0, 3.0 Hz, 1H), 3.72 (dd, J = 9.0, 7.0 Hz, 1H), 3.33 (s, 3H), 2.78 (m, 1H), 2.06–0.80 (m, 20H), 1.14 (d, J = 6.6 Hz, 3H), 1.04 (s, 3H), 0.78 (s, 3H), 0.68 (m, 1H), 0.44 (m, 1H); ^{13}C NMR δ 194.2, 161.0, 145.2, 136.9, 136.0, 135.0, 134.5, 128.0, 124.5, 121.5, 121.0, 119.7, 110.2, 82.6, 73.9, 56.8, 56.4, 53.0, 48.2, 43.6, 43.2, 40.3, 36.7, 35.5, 35.2, 33.6, 30.7, 28.1, 25.2, 24.5, 22.9, 21.7, 19.5, 17.6, 13.3, 12.5. For **19**, as in the preparation of **18**, 98 mg (0.5 mmol) of **12** was treated with 30 mg (1.25 mmol) of NaH and then 228 mg (0.5 mmol) of **17** to afford, after silica gel chromatography, 139 mg (53%) of yellow **19**: mp 96.4–98.4 °C; ^{1}H NMR δ 7.62 (m, 2H), 7.48 (m, 2H), 7.30 (m, 1H), 7.02 (m, 1H), 6.72 (m, 1H), 4.01 (dd, J = 9.0, 3.0 Hz, 1H), 3.80 (dd, J = 9.0, 7.0 Hz, 1H), 3.33 (s, 3H), 2.78 (m, 1H), 2.02–0.82 (m, 20H), 1.16 (d, J = 6.6 Hz, 3H), 1.04 (s, 3H), 0.79 (s, 3H), 0.66 (m, 1H), 0.45 (m, 1H); ^{13}C NMR δ 192.8, 165.6, 147.2, 143.7, 135.7, 134.3, 129.5, 127.1, 126.6, 124.1, 120.3, 113.6, 107.8, 82.6, 73.9, 56.8, 56.5, 53.0, 48.2, 43.6, 43.2, 40.3, 36.7, 35.5, 35.3, 33.6, 30.8, 28.1, 25.2, 24.5, 23.0, 21.7, 19.5, 17.6, 13.3, 12.6.
25. Preparation of **7** and **8**. For **7**, a solution of 120 mg (0.229 mmol) of **18** and 11 mg (0.058 mmol) of *p*-TsOH in a mixture of 3 mL dioxane and 1 mL water was heated at 80 °C for 5 h. The mixture was cooled to rt, quenched with 10 mL of saturated $NaHCO_3$ solution, and extracted with CH_2Cl_2. The CH_2Cl_2 layers were dried, filtered, and evaporated to afford 103 mg of residue, which was chromatographed on silica gel (4:1 hexane/EtOAc) to give 87 mg (74%) of yellow **7**: mp 222–225 °C; after recrystallization from EtOAc: mp 230–231 °C; λ_{max} (CH_2Cl_2) 269 nm (ε 7.0×10^4); ^{1}H NMR (500 MHz) δ 7.61 (m, 1H), 7.42 (m, 3H), 7.21 (m, 2H), 6.98 (m, 1H), 5.37 (br, 1H), 3.98 (dd, J = 9.0, 3.0 Hz, 1H), 3.74 (dd, J = 9.0, 7.0 Hz, 1H), 3.56 (m, 1H), 2.32 (m, 2H), 2.05 (m, 2H), 1.88 (m, 4H), 1.68–0.98 (m, 14H), 1.16 (d, J = 6.6 Hz, 3H), 1.04 (s, 3H), 0.76 (s, 3H); ^{13}C NMR (125 MHz) δ 194.3, 161.0, 145.2, 141.0, 137.0, 136.1, 135.1, 134.6, 128.0, 124.6, 121.9, 121.6, 121.1, 119.8, 110.2, 73.8, 72.0, 56.7, 52.9, 50.3, 42.8, 42.5, 39.8, 37.5, 36.7, 36.7, 32.2, 32.1, 31.9, 28.1, 24.6, 21.3, 19.7, 17.6, 12.2. Anal. Calcd for $C_{35}H_{42}O_3$: C, 82.31; H, 8.29. Found: C, 82.07; H, 8.30. For **8**, as in the preparation of **7**, acidic hydrolysis of 70 mg (0.13 mmol) of **19** afforded, after silica gel chromatography, 52 mg (77%) of yellow **8**: mp, after recrystallization from CH_2Cl_2, 221–222 °C; λ_{max} (CH_2Cl_2) 278 nm (ε 4.0×10^4), 254 nm (ε 4.0×10^4); ^{1}H NMR (500 MHz) δ 7.64 (m, 2H), 7.48 (m, 2H), 7.30 (m, 1H), 7.04 (m, 1H), 6.74 (m, 1H), 5.37 (br, 1H), 4.04 (dd, J = 9.0, 3.0 Hz, 1H), 3.82 (dd, J = 9.0, 7.0 Hz, 1H),

3.56 (m, 1H), 2.30 (m, 2H), 2.08–1.87 (m, 6H), 1.67–0.96 (m, 14 H), 1.18 (d, $J = 6.6$ Hz, 3H), 1.04 (s, 3H), 0.77 (s, 3H); ^{13}C NMR (125 MHz) δ 192.0, 165.6, 147.2, 143.7, 141.0, 135.7, 134.4, 129.5, 127.1, 126.6, 124.1, 121.8, 120.3, 113.6, 107.9, 73.9, 72.0, 56.7, 52.8, 50.3, 42.8, 42.5, 39.8, 37.5, 36.75, 36.7, 32.2, 32.1, 31.9, 28.1, 24.6, 21.3, 19.7, 17.7, 12.2. Anal. Calcd for $C_{35}H_{42}O_3$: C, 82.31; H, 8.29. Found: C, 82.17; H, 8.36.

26. Emission maxima λ (nm) in CH_2Cl_2: **5**, 538; **6**, 540; **7**, 555; **8**, 515. This long wavelength fluorescence of fluorenones has been ascribed to emission from an excimer: Heidt, J. R.; Heidt, J.; Josefowicz, M.; Kaminski, J. *J. Fluoresc.* **2001**, *11*, 65; Murphy, R. S.; Moorlag, C. P.; Green, W. H.; Bohne, C. *J. Photochem. Photobiol., A* **1997**, *110*, 123.