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Efficient and Scalable Synthesis of 4-Carboxy-Pennsylvania Green Methyl Ester: A Hydrophobic Building Block for Fluorescent Molecular Probes

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Abstract

Fluorinated fluorophores are valuable tools for studies of biological systems. However, amine-reactive single-isomer derivatives of these compounds are often very expensive. To provide an inexpensive alternative, we report a practical synthesis of 4-carboxy-Pennsylvania Green methyl ester. Derivatives of this hydrophobic fluorinated fluorophore, a hybrid of the dyes Oregon Green and Tokyo Green, are often cell permeable, enabling labeling of intracellular targets and components. Moreover, the low pKa of Pennsylvania Green (4.8) confers bright fluorescence in acidic cellular compartments such as endosomes, enhancing its utility for chemical biology investigations. To improve access to the key intermediate 2,7-difluoro-3,6-dihydroxyxanthen-9-one, we subjected bis-(2,4,5-trifluorophenyl)methanone to iterative nucleophilic aromatic substitution by hydroxide on scales of > 40 g. This intermediate was used to prepare over 15 grams of pure 4-carboxy-Pennsylvania Green methyl ester in 28% overall yield without requiring chromatography. This compound can be converted into the amine reactive *N*-hydroxysuccinimidyl ester in essentially quantitative yield for the synthesis of a wide variety of fluorescent molecular probes.

Keywords

fluorophore; bioorganic chemistry; chemical biology; molecular probes; fluorine; conjugation

Introduction

Fluorescent molecular probes are powerful tools for visualizing and quantifying biological processes.^{1–3} Fluorophores derived from fluorescein (**9**, Figure 1) have been extensively used in this way because these compounds can exhibit excellent photophysical properties under physiological and more basic conditions (fluorescein Abs. $\lambda_{\text{max}} = 490 \text{ nm}$, Em. $\lambda_{\text{max}} = 517 \text{ nm}$, $\epsilon = 76,900\text{--}87,600 \text{ M}^{-1} \text{ cm}^{-1}$, Φ (aqueous, pH > 8) = 0.92–0.95).^{3–8} However,

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because the phenol of fluorescein exhibits the relatively high pKa of 6.3–6.8,⁹ this dye is much less fluorescent in weakly acidic environments commonly found in biological systems, such as the lumen of endosomes (pH ~6.5). Fluorescein is also relatively susceptible to photobleaching.¹⁰ These limitations are reduced in certain halogenated analogues^{11–13} such as Oregon Green (2',7'-difluorofluorescein, **10**, Figure 1).⁶ The fluorine substituents of **10** decrease the pKa of the phenol to 4.8 and substantially enhance photostability. However, single isomer 5- and 6-carboxy derivatives of Oregon Green are costly because condensation of 4-carboxyphthalic anhydride with relatively expensive 4-fluororesorcinol is used to generate a mixture of regioisomers that need to be further protected as the diacetate for separation.⁶ The preparation of the analogous carboxyfluorescein regioisomers is less expensive as they can be isolated in pure form on a multigram scale by recrystallization of methanesulfonate ester precursors.¹⁴ Moreover, because the acidic phenol and 3-carboxylic acid functionalities of Oregon Green are both ionized under physiological conditions, this highly polar dianion does not easily penetrate biological membranes for studies of targets in the interior of living cells.

To attempt to overcome some of the limitations of Oregon Green, we designed and synthesized a hybrid fluorophore termed Pennsylvania Green (**12**, Figure 1).^{15,16} Pennsylvania Green incorporates the 2', 7'-difluoro substituents of Oregon Green (**10**, Figure 1), but replaces the 3-carboxylic acid with the methyl group found in more hydrophobic Tokyo Green (**11**, Figure 1).⁷ This hybrid fluorophore has been synthesized as the 4-carboxy methyl ester derivative (**8**) to install a convenient handle for bioconjugation, structurally analogous to 5-carboxy-Oregon Green. This ester can be hydrolyzed to the corresponding carboxylic acid (**13**), and converted to the highly amine-reactive *N*-hydroxysuccinimidyl ester (**14**), in nearly quantitative yield.^{15,16} 4-Carboxy-Pennsylvania Green (**13**) exhibits excellent photophysical properties (Abs. λ_{max} = 494 nm, Em. λ_{max} = 515 nm, ϵ = 62,000 M⁻¹ cm⁻¹, Φ (aqueous, pH 7.4) = 0.89) that are similar to 5-carboxy Oregon Green. However, amide derivatives of this hydrophobic analogue can be much more cell permeable, making this compound a potentially useful probe of intracellular components, targets, and processes in living cells.¹⁵

To synthesize Pennsylvania Green and derivatives, 2,7-difluoro-3,6-dihydroxy-xanthen-9-one (**5**, Scheme 1) has been used as a key building block.¹⁶ However, two previously reported routes^{16,17} to this compound are challenging to execute on multigram scales. To improve access to fluorophores derived from this intermediate, we report here a scalable synthesis of xanthone **5** from bromoarene **1** and conversion to multigram quantities of fluorophore **8** (Scheme 1).

Xanthone **5** was first prepared in 2002 by Lawrence and co-workers¹⁷ using the nine-step route shown in Scheme 2 (Panel A). In this report, synthesis of 0.58 grams of **5** in 28% overall yield is described. Based on this precedent, in 2006 we developed¹⁶ a shorter six-step route (Scheme 1, Panel B) to **5** involving cobalt-mediated carbonylation¹⁸ of iodoarene **24** under microwave irradiation to generate benzophenone **25**, followed by demethylation to yield the common intermediate **22** previously described by Lawrence.¹⁷ Although microwave-promoted carbonylation of **24** was effective on small scales of less than one gram, this reaction was not successful for larger scale preparations, and multiple

chromatographic steps were required for purification of intermediates generated using this route. In 2007, we described¹⁵ a second more concise route for the synthesis of 4-carboxy-Pennsylvania Green involving condensation of a functionalized benzaldehyde with 4-fluororesorcinol, but challenging purification steps that hinder production of gram quantities of this fluorophore were also observed. More recently, in 2012, we reported¹⁹ an improved synthesis of xanthone **5** based on repeated nucleophilic aromatic substitution of hexafluorobenzophenone **4** by hydroxide (Scheme 1, Panel C). We describe here the optimization and extension of this methodology to allow synthesis of multigram quantities of 4-carboxy-Pennsylvania Green methyl ester (**8**).

Results and Discussion

A multigram synthesis of 2,7-difluoroxanthone (**5**), based on our iterative nucleophilic aromatic substitution approach,¹⁹ is shown in Scheme 1. The synthesis of key intermediate **5** started with commercially available 1-bromo-2,4,5-trifluorobenzene (**1**). This compound was treated with isopropylmagnesium chloride in dry THF at $-78\text{ }^{\circ}\text{C}$ to promote metal-halogen exchange,²⁰ followed by the addition of 2,4,5-trifluorobenzaldehyde **2** to afford diphenylmethanol **3** in 95% yield. Oxidation with TEMPO and NaOCl provided bis-(2,4,5-trifluorophenyl)methanone (**4**) in excellent yield and high purity after recrystallization from hexanes. During optimization of conversion of **1** to **3**, we observed that at least 1.05 equiv of both *i*-PrMgCl and **2** are required for addition to 1.0 equiv of **1** to achieve the highest conversion and minimize impurities and unreacted **2**. This slight excess of 0.05 equiv of **2** was oxidized to 2,4,5-trifluorobenzoic acid in the subsequent step, allowing facile removal by extraction into basic aqueous solution. This optimized process provided benzophenone **4** as a crystalline solid in 90% overall yield from **1**, and did not require purification of intermediate **3**.

As summarized in Table 1, we examined the sensitivity of fluorinated benzophenone **4** on a small scale to base, temperature, and time. The weak base NaOAc was unreactive even after reflux for 12 h (entry 1). However, reflux of **4** in 1.5 M aqueous KOH afforded bis(4-hydroxyphenyl)methanone (**26**) in > 90% yield (entry 2). When the concentration of aqueous KOH was increased to 10 M, bis(2,4-dihydroxyphenyl)methanone (**22**) was obtained in 60% yield, with xanthone **5** isolated in 5% yield (entry 3). Importantly, conversion to **5** was dramatically accelerated and driven to completion by heating to $200\text{ }^{\circ}\text{C}$ under these conditions in a sealed tube. Although this approach provided an excellent yield of **5** (96%) after 4 hours, the ability to safely scale up this method was limited due to the high pressures involved (entry 4).

Based on the observed product distribution, treatment of **4** with KOH initially substitutes the reactive fluorines at the 4 and 4'-positions with hydroxyl groups. Subsequent slower addition of hydroxide at the 2-position, with further substitution at the 2'-position, is followed by cyclization to yield xanthone **5**. To drive this reaction to completion on multigram scales at ambient pressure, we found that reaction of **4** with 10 M KOH required refluxing conditions for extended periods of time. However, in glass vessels, silicon-derived impurities were generated under these conditions that were difficult to separate from **5**. To avoid these impurities, we often used an all-Teflon flask for this reaction. Although this

method could produce **5** in a one-pot procedure, higher yields of **5** (60%) were obtained on large scale (> 10 g) by a two pot process initially involving heating of **4** to 80 °C for 3 h in a glass round bottom flask containing DMSO and 10 M aqueous KOH to produce a mixture of **26** and **22**. These more water-soluble intermediates were extracted, dried, and subsequently refluxed for 48 h in an all-Teflon flask charged with only fresh 10 M aqueous KOH. We used this approach to prepare several ~ 25 g batches of pure **5**. Alternatively, simple one-pot reflux of **4** in a glass round-bottom flask with aqueous 10 M KOH and DMSO for 48 h could also be used to prepare **5**, often in higher yield, presumably due to improved thermal transfer properties of glass compared with Teflon, but under these conditions silica impurities contributed to ~10% of the isolated mass of **5**. However, crude **5** prepared in this way could be readily converted without further purification to bis MEM-ether **6**, and when this method was used, any silica impurities were easily removed from this much less polar derivative.

The phenol of xanthone **5** was protected to afford bis MEM ether **6** (Scheme 1). Initial studies of reaction of **5** in DMF containing MEM-Cl and NaH (5 equiv) for 16 h at 4–22 °C provided reasonably high yields of **6** (65%), but a large volume of DMF was necessary to solubilize **5** under these conditions. To overcome this limitation on multigram scales, **5** was alkylated at 50 °C with MEM-Cl and the weaker base DIEA (3 equiv) in THF. Under these conditions, **6** could be isolated in 75% yield after only 3 h. This reaction was quenched with water, diluted with CH₂Cl₂, and the organic layer was isolated. Extraction of the organic phase with aqueous KOH (1 M) was used to remove impurities and any unreacted starting material (**5**). Crystallization of **6** from hexanes/CH₂Cl₂ provided pure **6** as a white powder (75% yield). This approach allowed preparation of ~ 40 g batches of **6** with standard laboratory equipment.

To further optimize the synthesis of 4-carboxy-Pennsylvania Green methyl ester (**8**), commercially available methyl 4-iodo-3-methylbenzoate (**7**) in dry THF was subjected to metal-halogen exchange at –78 °C with *i*-PrMgCl.LiCl complex in THF (Turbo Grignard).^{21,22} A solution of MEM-protected xanthone **6** in THF at –78 °C was slowly added, the solution was warmed to room temperature for 12 h, and **6** was completely consumed. To stop the reaction, and cleave the MEM protecting groups, the reaction was cooled to 4 °C and 6 M aqueous HCl was slowly added. As previously reported in other systems,²² if *i*-PrMgCl in THF (without LiCl) was used for the metal-halogen exchange, this reagent generated a much less reactive aryl Grignard reactant. Under these conditions, at least 5 equiv of the aryl Grignard, much higher concentrations, and a reaction time of 24 h was required to drive the reaction to completion. By contrast, the *i*-PrMgCl.LiCl complex in THF (Turbo Grignard) generated a more reactive aryl Grignard reagent, shortened the reaction time, and required only 1.5 equiv for the conversion of **6** to **8**.

Conclusion

To provide access to multigram quantities of 4-carboxy-Pennsylvania Green methyl ester (**8**), we developed a new scalable route to 2,7-difluoroxanthone (**5**). This route involves the use of iterative nucleophilic aromatic substitution of bis-(2,4,5-trifluorophenyl)methanone (**4**) by hydroxide to install four carbon-oxygen bonds in a one-pot process. Optimization of

addition of the Grignard reagent derived from methyl 4-iodo-3-methylbenzoate (**7**) to bis-MEM ether **6** allowed preparation of 15 g of pure fluorophore **8** in a single five linear step process and 28% overall yield without requiring chromatography. Considering only the cost of materials, the optimized synthetic procedure described here allows access to 4-carboxy-Pennsylvania Green (**13**) for ~\$5/gram, which is orders of magnitude less expensive than comparable single-isomer amine-reactive fluorophores available through commercial sources. The ability to economically produce multi-gram quantities of 4-carboxy-Pennsylvania Green, especially considering its potential for high compatibility with solid phase peptide synthesis, should facilitate the preparation of large quantities or libraries of fluorescent molecular probes that would be inaccessible, much more costly, or difficult to prepare using other approaches.

Experimental

General Methods. All reagents and solvents were commercially available and were used without further purification unless otherwise noted. NMR spectra of the intermediates were recorded on Bruker NMR spectrometers using TMS as an internal standard. EI-MS spectra were obtained on a Finnigan MAT 95 mass spectrometer and ESI-MS spectra were obtained on a Kratos MS 80 mass spectrometer. Elemental analysis was obtained using a vario EL spectrometer. HPLC analysis of **8** shown in the Supporting Information was performed on an Agilent 1100 liquid chromatograph equipped with a Hamilton reverse-phase PRP-1 column (7 μm , 100 \AA , 4.1 \times 250 mm); flow rate 0.8 mL/min, detection wavelength: 254 nm, temperature: 25 $^{\circ}\text{C}$. Mobile phase gradient: 30:70 to 0:100 water/acetonitrile over 30 minutes (purity > 97%). THF (Sigma-Aldrich, 99.0%) was rendered anhydrous by use of a Glass Contour solvent purification system from SG Water USA.

Bis(2,4,5-trifluorophenyl)methanone (4**).** A one-necked round-bottom flask (1 L) equipped with a Teflon-coated magnetic stirring bar was removed from a drying oven (150 $^{\circ}\text{C}$), sealed with a rubber septum while hot, and purged with dry nitrogen. After cooling to room temperature (22 $^{\circ}\text{C}$), 1-bromo-2,4,5-trifluorobenzene (50.0 mL, 90.0 g, 0.427 mol, Oakwood Products) and 400 mL of anhydrous THF was syringed into the flask. The flask was submerged in a bath of acetone cooled to -78°C with dry ice. To this solution was added a solution of isopropylmagnesium chloride in THF (2.0 M, 224 mL, 0.448 mol, Sigma-Aldrich) with stirring over 5 min. The resulting mixture was stirred for an additional 10 min, the dry ice/acetone bath (-78°C) was removed, and a bath containing ice water was substituted. This reaction mixture was stirred for an additional 30 min, the ice water bath was removed, and the flask resubmerged in the dry ice/acetone bath (-78°C). This reaction mixture was stirred for 10 min, followed by addition of 2,4,5-trifluorobenzaldehyde (50.4 mL, 71.7 g, 0.448 mol, Oakwood Products) by syringe. The resulting mixture was stirred for an additional 10 min, the -78°C bath was removed, the flask was warmed to room temperature, and the reaction mixture was stirred at room temperature for 3 h. The reaction was quenched by addition of saturated aqueous NH_4Cl solution (50 mL). The resulting biphasic mixture was diluted with water (300 mL) and Et_2O (200 mL), the two phases were separated, and the aqueous fraction was extracted with Et_2O (2×300 mL). The combined organic fractions were dried over anhydrous Na_2SO_4 and concentrated by rotary evaporation to provide a viscous oil. This crude **3** was dissolved in CH_2Cl_2 (1.0 L) and transferred to an

Erlenmeyer flask (5 L). To this solution of **3** was added (2, 2, 6, 6-tetramethylpiperidine-1-yl)oxyl free radical (TEMPO, 3.33 g, 21.0 mmol, 5 mol%, Oakwood Products), KBr (10.1 g, 85.4 mmol, Alfa Aesar), NaHCO₃ (72.0 g, 0.93 mol) and saturated aqueous sodium chloride (500 mL). The resulting mixture was vigorously stirred (1000 rpm), and aqueous NaOCl (0.72 M, 1.5 L, 1.10 mol, Clorox household-grade bleach) was added. The reaction mixture was stirred for 3 h, transferred to a separatory funnel, and the phases were separated. The aqueous layer was extracted with CH₂Cl₂ (2 × 350 mL), the layers were combined, dried over anhydrous Na₂SO₄, and concentrated by rotary evaporation to give a crude oil. This oil was quantitatively pipetted onto a plug of packed CH₂Cl₂-saturated silica gel (50 g, 65 mm diameter) and **4** was eluted with CH₂Cl₂ (400 mL). The filtrate was transferred to a one-necked round-bottom flask (500 mL) and the solvent was removed by rotary evaporation to provide a viscous oil that crystallized upon addition of hexanes (250 mL). The colorless crystals were collected by vacuum filtration and air-dried for 3 h to give pure benzophenone **4** (111.5 g, 90%). Pure **4** has the following properties: mp 80–81 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.57 (qd, *J* = 8.8, 6.4 Hz, 2H), 6.95 (qd, *J* = 9.2, 6.4 Hz, 2H); ¹³C NMR (126 MHz, CDCl₃) δ 184.7 (s), 156.8 (dd, *J* = 254.7, 9.8 Hz), 153.3 (ddd, *J* = 260.0, 14.5, 12.2 Hz), 147.1 (ddd, *J* = 248.1, 12.7, 3.1 Hz), 123.2 (d, *J* = 14.9 Hz), 118.8 (d, *J* = 20.1 Hz), 111.6 – 98.1 (m); IR (thin film) ν_{\max} : 3073, 1667, 1623, 1509, 1437, 1420, 1333, 1293, 1208, 1138, 889 cm⁻¹; HRMS (ESI) *m/z* 291.0263 (M+H⁺, C₁₃H₅F₆O requires 291.0245); Anal. calcd. for C₁₃H₄F₆O: C, 53.81; H, 1.39; N, 0, found: C, 53.58; H, 1.36; N, <0.05.

2,7-difluoro-3,6-dihydroxy-9H-xanthen-9-one (**5**). To a glass one-necked round-bottom flask (500 mL) equipped with a Teflon-coated magnetic stirring bar was added **4** (43.5 g, 150 mmol, 1.0 equiv), aqueous KOH (10 M, 145 mL, 1.45 mol, 9.67 equiv), and DMSO (145 mL). This mixture was stirred at 80 °C for 3 h. The solution was poured into a beaker (2 L) containing ice (500 g) and concentrated aqueous HCl (145 mL, 1.74 mol). The resulting slurry was extracted with Et₂O (3 × 150 mL). The organic fractions were combined and dried over anhydrous sodium sulfate (45 g). This solution was concentrated by rotary evaporation (30 °C, 10 mmHg) in a one-necked all-Teflon round-bottom flask (500 mL, ChemGlass). This Teflon flask was equipped with a Teflon-coated magnetic stirring bar and an aqueous solution of KOH (10 M, 300 mL, 3.00 mol, 20 equiv) was added. The flask was vigorously refluxed (bath temp = 150 °C) for 48 h. The resulting slurry was poured, while hot, into a 2 L beaker containing ice (1 Kg) and concentrated aqueous HCl (300 mL, 3.60 mol). This mixture was briefly stirred with a rod and placed in a refrigerator at 4 °C for 3 h. A colorless precipitate was collected by vacuum filtration, washed with water (3 × 250 mL), and dried by vacuum filtration. The colorless solid was transferred to a one-necked round-bottom flask (500 mL), and exposed to high vacuum (12 h) to remove residual water. The solid was ground with a mortar and pestle to provide xanthone **5** as a white powder (23.9 g, 60%). Pure **5** has the following properties: mp > 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.56 (apps, 2H), 7.76 (d, *J* = 10.8, 2H), 7.07 (d, *J* = 6.8, 2H); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 173.2 (s), 153.1 (s), 152.0 (d, *J* = 15.0 Hz), 148.8 (d, *J* = 242.5 Hz), 112.6 (s), 111.0 (d, *J* = 20.0 Hz), 104.7(s); IR (thin film) ν_{\max} : 3356, 1627, 1475, 1309, 1188, 1160 cm⁻¹; HRMS (ESI) *m/z* 263.0154 (M-H, C₁₃H₅F₂O₄ requires 263.0156); Anal. calcd. for C₁₃H₆F₂O₄: C, 59.10; H, 2.29; N, 0, found: C, 58.91; H, 2.28; N, <0.05.

2,7-difluoro-3,6-bis((2-methoxyethoxy)methoxy)-9H-xanthen-9-one (**6**). To an oven-dried one-necked round-bottom flask (500 mL) equipped with a Teflon-coated magnetic stirring bar was added xanthone **5** (30.0 g, 113.4 mmol, 1.0 equiv). The flask was sealed with a rubber septum, purged with dry nitrogen, and anhydrous THF (250 mL) and *N,N*-diisopropylethylamine (60.2 mL, 44.6 g, 344 mmol, 3.0 equiv) and 2-methoxyethoxymethyl chloride (MEM-Cl, 39.2 mL, 42.6 g, 344 mmol, 3.0 equiv, AK Scientific) were added sequentially by syringe. The resulting mixture was heated to 50 °C and stirred for 3 h. The reaction was allowed to return to ambient temperature and was diluted with CH₂Cl₂ (250 mL). The resulting mixture was washed with aqueous KOH (1 M, 3 × 100 mL). The combined aqueous phases were extracted with CH₂Cl₂ (200 mL) and the combined organic fractions were dried over anhydrous Na₂SO₄. Concentration by rotary evaporation yielded a pale yellow solid. The solid was further purified by recrystallization by the following protocol: The solid was dissolved in CH₂Cl₂ (200 mL) and transferred to an Erlenmeyer flask (500 mL). To this flask was also added a boiling stone and hexanes (200 mL). The resulting solution was heated to a vigorous boil on a hot plate and the total volume of the solution was reduced to 200 mL. The Erlenmeyer flask containing the solution was removed from the hot plate, allowed to stand for 12 h, and crystallization was observed. Collection of the colorless needles by vacuum filtration, washing with hexanes (100 mL), and drying by exposure to air for 3 h provided pure MEM-protected xanthone **6** (38.0 g, 75%). Pure **6** has the following properties: mp > 300 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.97 (d, *J* = 10.4, 2H), 7.28 (d, *J* = 6.8, 2H), 5.47 (s, 4H), 3.93 (t, *J* = 4.4, 4H), 3.61 (t, *J* = 4.3, 4H), 3.41 (s, 6H); ¹³C NMR (125 MHz, CDCl₃) δ 174.6 (s), 153.4 (s), 151.0 (d, *J* = 25.0 Hz), 149.9 (d, *J* = 246 Hz), 117.2 (s), 115.4 (d, *J* = 6.25 Hz), 111.9 (d, *J* = 21.0 Hz), 104.8 (s), 94.3 (s), 71.4 (s), 68.6 (s), 59.1 (s); IR (thin film) ν_{max}: 2892, 1630, 1503, 1468, 1369, 1272 cm⁻¹; HRMS (ESI) *m/z* 439.1224 (M-H, C₂₁H₂₁F₂O₈ requires 439.1205); Anal. calcd. for C₂₁H₂₂F₂O₈: C, 52.27; H, 5.04; N, 0, found: C, 57.19; H, 4.92; N, <0.05.

4-Carboxy-Pennsylvania Green methyl ester (**8**)

An oven-dried one-necked round-bottom flask (500 mL) equipped with a Teflon-coated magnetic stirring bar was removed from a drying oven, sealed while hot with a rubber septum, and purged with dry nitrogen. After cooling to room temperature, methyl 4-iodo-3-methylbenzoate (**7**, 22.6 g, 81.6 mmol, 1.5 equiv) was added and the flask resealed. Anhydrous THF (100 mL) was added by syringe, the flask was submerged in a dry ice/acetone bath (−78 °C), and the solution was stirred for 10 min. Isopropylmagnesium chloride lithium chloride complex in THF (1.3 M, 62.8 mL, 81.6 mmol, 1.5 equiv, Acros) was added dropwise over 10 min. The dry ice/acetone bath was removed, replaced with an ice bath, and the reaction mixture stirred for 1 h. During this 1 h period, **6** (24.0 g, 54.8 mmol, 1.0 equiv) was added to a separate oven-dried one-necked round-bottom flask (250 mL). This flask was sealed with a rubber septum, purged with dry nitrogen, and anhydrous THF (100 mL) was added to completely dissolve **6**. After this 1 h period, the flask containing the aryl Grignard was resubmerged in the dry ice/acetone bath (−78 °C) and the reaction mixture stirred for 10 min. The solution of **6** was cannulated into the flask at −78 °C containing the aryl Grignard over the course of 5 min. The dry ice/acetone bath was removed, the reaction mixture was warmed to room temperature, and the reaction was stirred for an additional 12 h. The flask was cooled in an ice-water bath for 5 min. Aqueous

HCl (6 M, 150 mL, 0.9 mol) was added dropwise over 10 min. The resulting slurry was stirred for 1 h at 4 °C, diluted with 300 mL of H₂O, and stirred for an additional 30 min. The red-orange precipitate was collected by vacuum filtration, washed with water (3 × 150 mL), and air-dried thoroughly (1.5 h). The yellow-orange pellet was slurried in CH₂Cl₂/hexane (3:1, 150 mL). The suspension was stirred at ambient temperature overnight, followed by 4 °C for 1 h, and then was filtered. The filter cake was washed with water (2 × 300 mL) and air dried to give **8** as an orange-red solid (> 95% pure, 15.0 g, 70%). Pure **8** has the following properties: mp 220–224 °C; ¹H NMR (500 MHz, CD₃OD/0.15 M NaOCD₃) δ 8.08 (dt, *J* = 1.6, 0.7 Hz, 1H), 8.03 (ddd, *J* = 7.8, 1.6, 0.8 Hz, 1H), 7.26 (d, *J* = 7.8 Hz, 1H), 6.71 (d, *J* = 7.4 Hz, 2H), 6.62 (d, *J* = 11.4 Hz, 2H), 3.39 (s, 3H), 2.12 (s, 3H); ¹³C NMR (126 MHz, CD₃OD/0.15 M NaOCD₃) δ 174.9 (s), 172.5 (d, *J* = 17.4 Hz), 157.5 – 157.3 (m), 156.2 (s), 155.5 (s), 140.4 (s), 136.6 (s), 136.0 (s), 132.5 (s), 128.9 (d, *J* = 177 Hz), 111.7 (s), 111.5 (s), 110.9 (d, *J* = 8.5 Hz), 106.5 (d, *J* = 5.5 Hz), 49.9 (s), 19.7 (s); IR (thin film) ν_{\max} : 3465, 3000, 1796, 1712, 1213 cm⁻¹; HRMS (ESI) *m/z* 397.0888 (MH⁺, C₂₂H₁₄O₅F₂ requires 397.0884).

Supplementary Material

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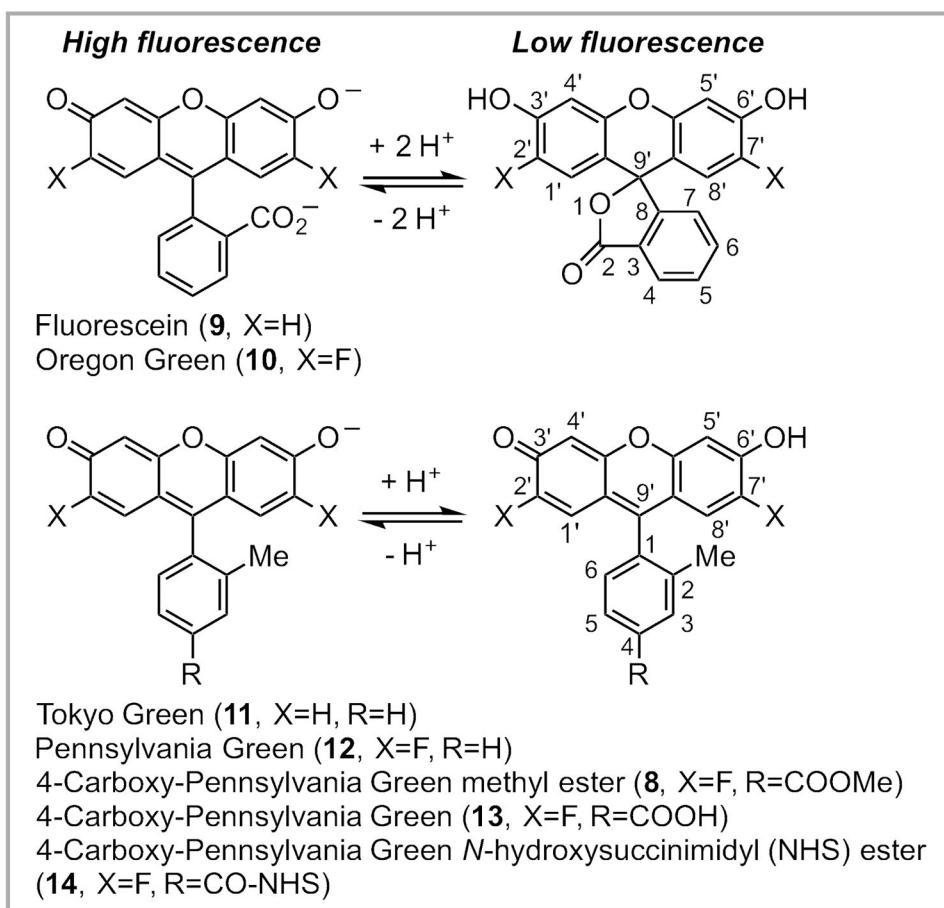
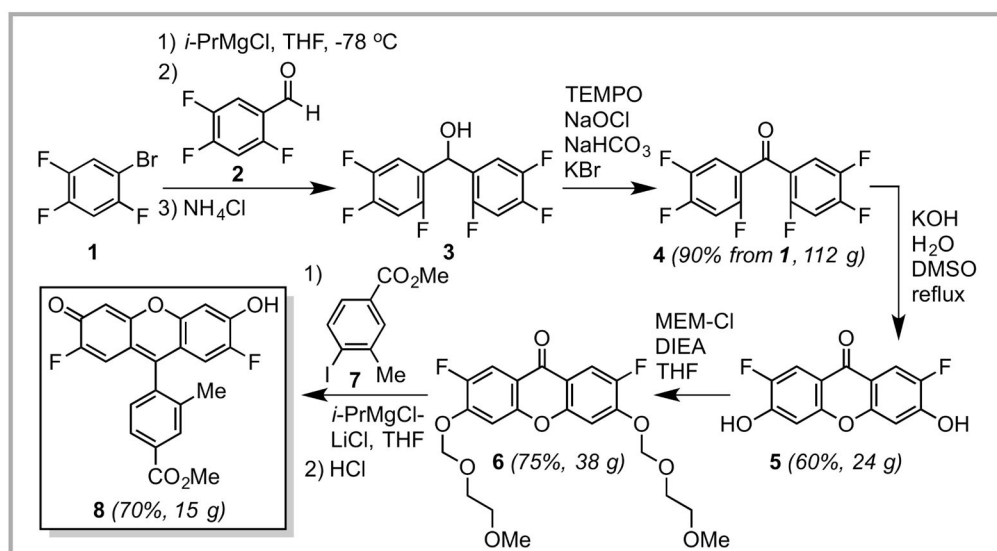
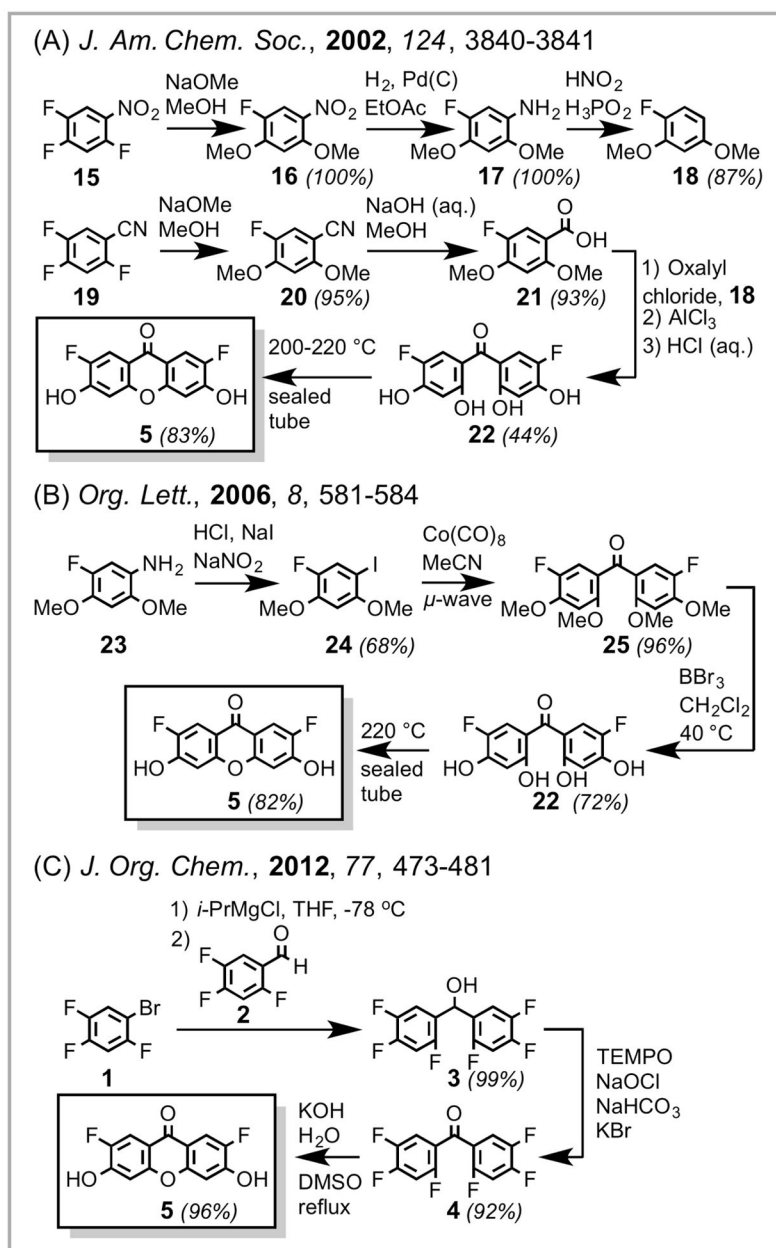


Figure 1.
Structures of fluorophores.

**Scheme 1.**

A practical multigram synthesis of 4-carboxy-Pennsylvania Green methyl ester (**8**).



Scheme 2.
Previously reported syntheses of xanthone **5**.

Table 1

Conversion of benzophenone **4** to xanthone **5**.

Entry	Reaction conditions (0.5 g scale)	Recovered 4 (%)	26 (%)	22 (%)	5 (%)
1	NaOAc/AcOH, H ₂ O, 12 h, reflux	> 90	trace	trace	0.0
2	1.5 M KOH, H ₂ O, 12 h, reflux	0	95	< 5	trace
3	10 M KOH, H ₂ O, 18-crown-6, 12 h, 100 °C	0	10	60	5
4	10 M KOH, H ₂ O, 4 h, 200 °C, sealed glass tube	0	trace	trace	96

