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Efficient Two-Step Synthesis of 9-Aryl-6-hydroxy-3*H*-xanthen-3-one Fluorophores

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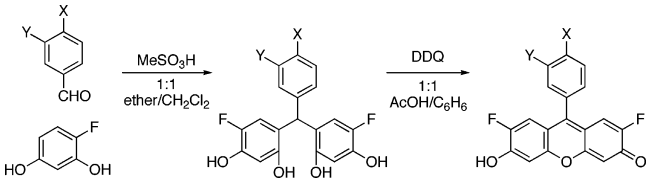
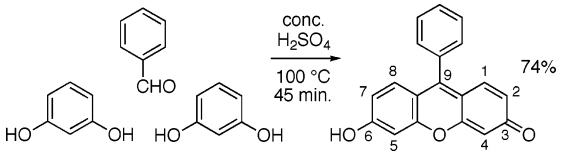


A two-step method for the synthesis of 9-aryl-6-hydroxy-3*H*-xanthen-3-one fluorophores involving condensation of aryl aldehydes and fluororesorcinol is shown to proceed through a triarylmethane intermediate. The condensation is complicated by retro-Friedel−Crafts reactions which can be minimized by controlling the amount of acid. The xanthenone ring system is prepared by a final oxidative cyclization with DDQ.

Metal-sensitive analogues of fluorescein referred to as fluo indicators have proven invaluable as intracellular fluorescent sensors of divalent ions.[1] Unlike fluorescein, the 9-aryl-6-hydroxy-3*H*-xanthen-3-one fluorophore of fluo dyes cannot form a spirolactone. The two traditional methods for preparing 9-aryl-6-hydroxy-3*H*-xanthen-3-ones involve harsh reaction conditions. The simplest methods involve a one-step condensation of acids,[2,3] esters,[4] or anhydrides[5] using anhydrous zinc chloride at temperatures at or above 140 °C. Using this method, 9-(4-aminophenyl)-6-hydroxy-3*H*-xanthen-3-one has been prepared in one step using anhydrous $ZnCl_2$ under a stream of HCl gas at 180 °C.[4] Alternatively, benzaldehydes can be condensed with resorcinols in concentrated sulfuric acid at 100 °C (Scheme 1).[6,7] A secondary oxidation step

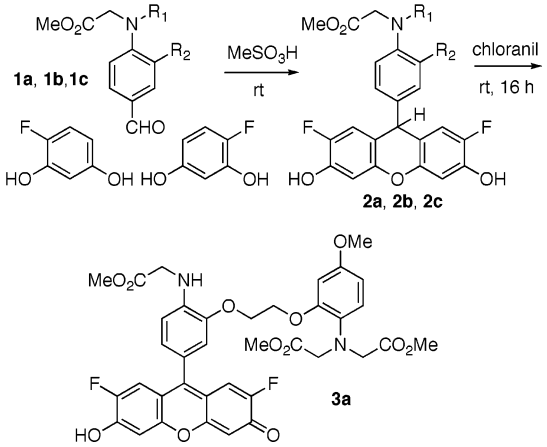
SCHEME 1



is not required because sulfuric acid acts as an oxidizing agent at these temperatures.

The unforgiving conditions required for these one-step procedures are incompatible with complex arene substituents such as those found on fluo indicators. In fact, fluo dyes have generally been prepared through multistep syntheses involving addition of arene nucleophiles to protected xanthones.[1,8] However, Gee and co-workers recently reported a relatively mild two-step protocol for construction of fluo analogues by condensing 4-aminobenzaldehydes **1a** (R_1 = H, R_2 = *O*-alkyl) with 4-fluororesorcinol at room temperature to form a dihydroxanthene **2a**. Subsequent oxidation with chloranil generated the xanthene fluorophore **3a** (Scheme 2).[9,10] Fluorinated xanthenes have superior fluorescence properties relative to nonfluorinated analogues.[10,11] For example, 2′,7′-difluoro-5(6)-carboxyfluorescein has a lower intrinsic pK_a and exhibits less photobleaching than 5-carboxyfluorescein.[11]

SCHEME 2



Unfortunately, when these conditions were applied to related aldehydes **1b** (R_1 = CH_2CO_2Me, R_2 = H) and **1c** (R_1 = CH_2CO_2Me, R_2 = OMe) with 4-fluororesorcinol,[12] none of the desired tricyclic dihydroxanthenes **2b** or **2c**, respectively, were formed. Instead, condensation of aldehyde **1b** with 4-fluororesorcinol generated only the acyclic

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(12) 4-Fluororesorcinol was prepared in two steps by fluorination of 1,3-dimethoxybenzene with SelectFluor, followed by demethylation with boron tribromide according to ref 11.

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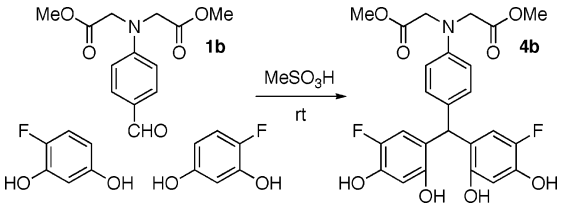
triarylmethane **4b** which was isolated in 51% yield (Scheme 3). To better understand the underlying chemistry behind xanthene formation and improve the mild two step conditions for synthesis of fluo dyes, we studied the condensation of benzaldehydes with 4-fluororesorcinol.

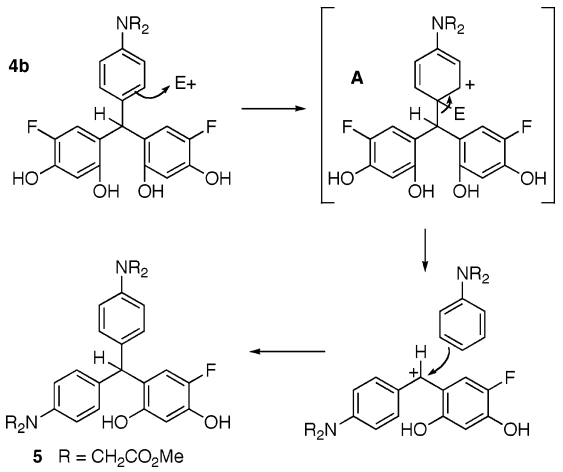
SCHEME 3



Triarylmethane **4b** could not be induced to cyclize to dihydroxanthene **2b** in methanesulfonic acid or under any other acidic conditions, implying that it is not an intermediate in the cyclization conditions (Scheme 3). Extended exposure of **4b** to methanesulfonic acid did not induce cyclization, but instead led to higher molecular weight products with a half-life of about 1 h. These observations suggest that dihydroxanthene **2a**, previously reported as a hydrate, is actually a triarylmethane corresponding to products **4b**−**e**. If so, the role of the oxidant in Scheme 1, and in all previous syntheses of 9-arylxanthenes from aldehydes, is to oxidize a triarylmethane intermediate analogous to **4b**, thereby facilitating cyclization/condensation to a xanthene.

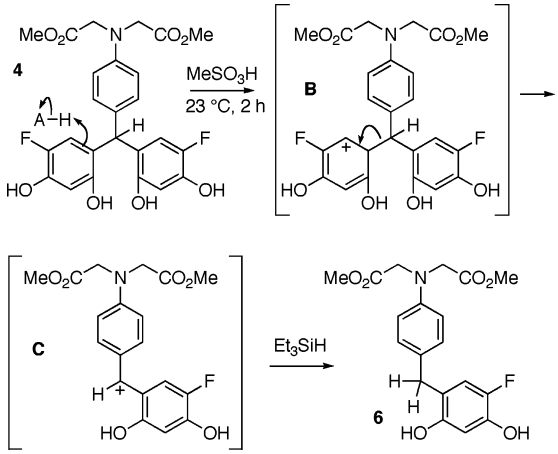
The condensation reactions were not clean due to rapid oligomerization. Surprisingly, when the reaction was slowed by diluting the methanesulfonic acid with ether/CH_2Cl_2, triarylmethane **5**, incorporating two aniline rings, was isolated from the product mixture in 16% yield. One explanation for the formation of triarylmethane **5** is that the electron-rich product **4b** is unstable toward retro-Friedel−Crafts fragmentations, initiated either by protons or benzhydryl cations (Scheme 4). Thus, each pernicious fragmentation of the triarylmethane product **4b** would directly deplete one molecule of product and generate a benzhydryl cation that could alkylate a second product molecule.[13] Indeed, the presence of high molecular weight products in the final reaction mixture supports this hypothesis.

SCHEME 4



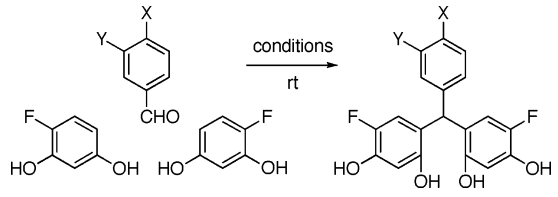
To test the fragmentation of triarylmethane **4b** under the conditions of the reaction it was treated with triethylsilane in methanesulfonic acid at room temperature over 2 h. Surprisingly, diarylmethane **6** was isolated in 81% yield. If fragmentation is the rate- determining step, then the result implies that acid-catalyzed fragmentation of the resorcinol substituent (Scheme 5) is at least four times faster than acid-catalyzed fragmentation of the aniline substituent. Taken together, the formation of triarylmethane **5** and the hydride trapping experiment show that all three arene rings of **4b** are susceptible to retro-Friedel−Crafts cleavage under the conditions of the reaction.

SCHEME 5



Since triarylmethane **4b** was unstable in neat methanesulfonic acid, shorter reaction times and lower concentrations of acids were considered. The rate of formation of triarylmethane intermediate **4b** is determined by the slow rate of dissolution of the resorcinol, but **4b** fragments as it forms. By predissolving the starting materials in 1:1 ether/CH_2Cl_2 and optimizing the amount of methanesulfonic acid, the yield of triarylmethane **4b** was increased to 86%. These conditions also seemed to give better yields for other benzaldehydes (Table 1).

TABLE 1. Improved Condensation of Benzaldehydes with 4-Fluororesorcinol



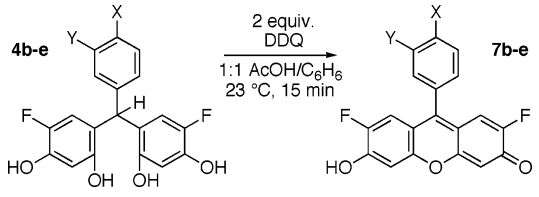
			yield (%)	
product	Y	X	neat $MeSO_3H$	8% $MeSO_3H$[a]
4b	H	$N(CH_2CO_2Me)_2$	51	86
4c	OMe	$N(CH_2CO_2Me)_2$	40	81
4d	H	H	31	85
4e	H	NMe_2	35	72

[a] 8:46:46 $MeSO_3H$/ether/CH_2Cl_2.

(13) The arenium ion derived from protonation of the aniline ring is expected to be more stable than the arenium ion derived from protonation of the resorcinol ring(s) by 11.8 kcal/mol (PM3).

The oxidative cyclization of triarylmethane **4b** with chloranil was slow and generated a significant number of byproducts. Unfortunately, the extent of the side reactions increases when the reaction was heated. The oxidative cyclization was much faster with DDQ and proceeded to completion in less than 20 min. The extent of the side reactions, most leading to oligomers, was decreased when protic acids were added to the reaction mixture. The cleanest transformations were affected in 1:1 benzene/acetic acid. Under these conditions, only two minor byproducts were visible by LCMS, one of which was the product of addition of DDQ (Table 2). The dimethylamino-substituted triarylmethane **4e** was very reactive and generated a larger quantity of the undesired byproducts, ultimately preventing purification.

TABLE 2. Oxidative Cyclization of Triarylmethanes 4b−e



product	Y	X	yield (%)
7b	H	$N(CH_2CO_2Me)_2$	71
7c	OMe	$N(CH_2CO_2Me)_2$	70
7d	H	H	39

Characterization of the arylxanthenones **7b−d** was complicated by their poor solubility and by slow tautomerization on the NMR time scale. Even at 100 °C, the protons at the 4 and 5 positions of the xanthenenone ring system (which were also coupled to fluorine) were still highly broadened. As observed by other investigators, many of the signals corresponding to the 6-hydroxy-3*H*-xanthen-3-one ring system were missing from the ^{13}C NMR spectra.[9,14]

In summary, we have shown that the condensation of benzaldehydes with 4-fluororesorcinols in methanesulfonic acid generates a triarylmethane. Since the arenes are electron rich, retro-Friedel−Crafts fragmentations compete with formation of the triarylmethane product. Ultimately, it was shown that the yield of triarylmethane could be improved by optimizing the concentration of acid. The triarylmethane does not cyclize unless subjected to an oxidant and for this purpose DDQ was shown to be superior to chloranil. The milder, improved conditions presented here for the synthesis of 9-arylxanthenes could provide access to a broader range of fluorophores.

Experimental Section

({4-[Bis-(5-fluoro-2,4-dihydroxy-phenyl)methyl]phenyl}-methoxycarbonylmethylamino)acetic Acid Methyl Ester (4b). To a flame-dried flask equipped with a magnetic stir bar were added 4-fluororesorcinol (50 mg, 0.390 mmol) and [(4-formylphenyl)methoxycarbonylmethylamino]acetic acid methyl ester (52 mg, 0.195 mmol). The solids were dissolved in 1.5 mL of anhydrous 1:1 Et_2O/CH_2Cl_2. The flask was flushed with argon, and $MeSO_3H$ (0.13 mL, 8% v/v) was added. The resulting pink/purple reaction mixture was stirred for 2 h at room temperature, diluted with Et_2O (10 mL), and poured into H_2O containing 170 mg of $NaHCO_3$. The pH of the aqueous layer was adjusted to 5−6 by dropwise addition of concentrated HCl, and organics were extracted with EtOAc (3 × 40 mL). The combined extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. The dark brown viscous oil was purified by silica gel flash chromatography (20−30% acetone/toluene + 1% AcOH) to give **4b** as a fluffy pink/orange powder (84 mg, 86%): mp 78−80 °C (toluene); ^1H NMR (500 MHz, DMSO-d_6, 298 K) δ 9.41 (s, 2 H), 8.98(s, 2 H), 6.75 (d, J = 8.8 Hz, 2 H), 6.44−6.39 m, 4 H), 6.25 (d, J = 12.5 Hz, 2 H), 5.60 (s, 1 H), 4.17 (s, 4 H), 3.64 (s, 6 H); ^{13}C NMR (125 MHz, DMSO-d_6, 298 K) δ 171.8, 151.2, 146.3, 144.7 (d, J = 230 Hz), 143.4 (d, J = 13 Hz), 133.1, 129.9, 122.1 (d, J = 62 Hz), 116.6 (d, J = 20 Hz), 112.1, 105.1, 53.1, 52.3, 41.3; IR (thin film) 3309.9, 2954.2, 1715.1, 1612.9, 1505.4, 1435.5, 1172.0 cm^{-1}; TLC R_f = 0.25 (30% acetone/toluene + 1% AcOH); LRMS (ESI) *m/z* (relative intensity) 504 (8), 526 (100); HRMS (CI) *m/z* calcd for $C_{25}H_{22}F_2NO_8Na$ $[M + Na]^+$ 526.1290, found 526.1302.

({4-[[4-Bis-methoxycarbonylmethylamino)phenyl]-(5-fluoro-2,4-dihydroxyphenyl)methyl]phenyl}methoxycarbonylmethylamino)acetic Acid (5). Isolated from the reaction mixture for **4b** after silica gel flash chromatography as a light brown solid (9.4 mg, 16%): mp 71−74 °C (DMSO); ^1H NMR (500 MHz, DMSO-d_6, 298 K) δ 9.48 (s, 1 H), 9.09 (s, 1 H), 6.80 (d, J = 8.8 Hz, 4 H), 6.43 (d, J = 8.8 Hz, 4 H), 6.42 (d, J = 7.8 Hz, 1 H), 6.33 (d, J = 12.5 Hz, 1 H), 5.40 (s, 1 H), 4.16 (s, 8 H), 3.64 (s, 12 H); ^{13}C NMR (125 MHz, DMSO-d_6, 298 K) δ 171.1, 150.5, 145.6, 144.2 (d, J = 230 Hz), 142.8 (d, J = 13 Hz), 132.9, 129.3, 121.8 (d, J = 5 Hz), 116.1 (d, J = 20 Hz), 111.5, 104.4, 52.4, 51.7, 46.5; IR (thin film) 3390.3, 3207.5, 2954.0, 1745.4, 1613.3, 1519.3, 1439.9, 1207.8, 1024.7 cm^{-1}; TLC R_f = 0.42 (30% acetone/toluene + 1% AcOH); LRMS (ESI) *m/z* (relative intensity) 635.0 (95), 613.2 (15), 581.2 (17), 537.2 (17), 275.1 (100); HRMS (CI) *m/z* calcd for $C_{31}H_{33}F_2NO_{10}Na$ $[M + Na]^+$ 635.2017, found 635.2012.

{[4-(2,7-Difluoro-6-hydroxy-3-oxo-3*H*-xanthen-9-yl)phenyl]methoxycarbonylmethylamino}acetic Acid Methyl Ester (7b). Triarylmethane **4b** (12.4 mg, 0.025 mmol) was dissolved in 1 mL of AcOH and diluted with 1 mL of benzene. DDQ (11.1 mg, 0.050 mmol) was added dropwise to **4b** as a solution in 1 mL of 1:1 AcOH/benzene. Upon addition of the first few drops of DDQ solution, the initial light yellow solution turned dark purple. After being stirred at room temperature for 15 min, the reaction mixture was concentrated in vacuo. The resultant dark brown solid (∼15 mg) was subjected to silica gel flash chromatography on a 70 mm × 50 mm pad of silica with 10% MeOH/$CHCl_3$ as eluant. The crude product was dissolved in a minimum volume of 15% MeOH/$CHCl_3$ and further purified by preparative RP-HPLC with gradient elution from 25 to 60% MeCN/0.1% TFA−H_2O. Pure fractions were collected and lyophilized to give **7b** as a dark brown powder (8.5 mg, 71%): mp 254−256 °C (H_2O); ^1H NMR (500 MHz, DMSO-d_6, 353 K) δ 7.30 (d, J = 8.8 Hz, 2 H), 6.94 (d, J = 11.8 Hz, 2 H), 6.87 (d, J = 8.8 Hz, 2 H), 6.75 (br s, 2 H), 4.32 (s, 4 H), 3.71 (s, 6 H); ^{13}C NMR (125 MHz, DMSO-d_6, 313 K) δ 170.5, 150.7, 130.7, 119.9, 111.9, 104.9, 52.2, 51.8; ^{19}F NMR (377 MHz, DMSO-d_6, 298 K) δ −74.0; IR (KBr) 3423.1, 2916.5, 2091.3, 1642.1, 1370.6, 1239.3 cm^{-1}; TLC R_f = 0.35 (10% MeOH/$CHCl_3$); LRMS (ESI) *m/z* (relative intensity) 484 (100); HRMS (CI) *m/z* calcd for $C_{25}H_{19}F_2NO_7Na$ $[M + Na]^+$ 506.1027, found 506.1031.

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Supporting Information Available: Experimental procedures for **1c**, **4c−e**, **6**, and **7c−d** and characterization data for all new compounds (**1c**, **4b−e**, **5**, **6**, and **7b−d**). This material is available free of charge via the Internet at http://pubs.acs.org.

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