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One-step synthesis of a fluorescein derivative and mechanistic studies

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ABSTRACT

We report a convenient method for the one-step synthesis of a fluorescein derivative under acidic conditions. Mechanistic studies indicate that the acid-promoted condensation of *o*-tolualdehyde and 4-chlororesorcinol to form the fluorescein derivative proceeds through a cyclization-oxidation pathway while an alternative oxidation–cyclization pathway remains possible.

Fluorescein and its derivatives such as dichlorofluorescein (DCF) and Oregon Green[1] (Fig. 1) are widely used fluorescent dyes in chemistry, biology, and medicine.[2] Historically, fluoresceins with a carboxy group at the 2-position are the dominant species due to their synthetic accessibility. However, this functional group, negatively charged under physiological conditions, is not crucial for fluorescence emission because the benzoate group is orthogonal to the xanthene ring.[3] Moreover, the negative charge may be detrimental to cell permeability.[4] For these two reasons, the carboxy group has been replaced by other functional groups with more desirable properties. 2-Me Tokyo Green is the first of its class in which the carboxy group is replaced by an alkyl group with significant fluorescence intensity.[5] Later, Pennsylvania Green was introduced and shown to be superior to Tokyo Green for biological applications due to the lower pK_a value of the phenolic hydroxy group.[6] We and others recently reported the synthesis and applications of Pittsburgh Green and its derivatives in chemistry, biology, geology, and environmental and pharmaceutical sciences.[4,7] Pittsburgh Green was found to be more permeable than DCF in a biological system.[4] However, in our attempts to image the presence of ozone in live cells, an *O*-butenylated Pittsburgh Green was ineffective.[7c] This failure prompted us to prepare the new DCF analog **1a** and its *O*-butenylated derivative.[7c] Herein, we report both a convenient synthesis of the Pittsburgh Green derivative **1a** ('Pittsburgh Green II') in one step from commercially available compounds as well as mechanistic insights.

As Scheme 1 shows, the condensation of *o*-tolualdehyde and 4-chlororesorcinol proceeded in the presence of methanesulfonic acid to form tetraol **2a** in 93% yield according to the protocol from the Van Vranken laboratory.[8] The purification of this tetraol (45 g) only required recrystallization in organic solvents, which should be amenable to a large-scale synthesis. The treatment of tetraol **2a** with *p*-TsOH (8 equiv) in toluene at 110 °C produced mixture **X**. Subsequently, this mixture was subjected to DDQ[8] to form xanthenone **1a**. This sequence was inconvenient for a gram-scale synthesis because it was difficult to separate the desired product **1a** from DDQ and its reduced derivative DHQ. Perhaps for the same reason, similar compounds were purified by HPLC.[8] Nonetheless, similar protocols were used by others to prepare related compounds.[9] In order to circumvent the tedious purification process associated with the use of DDQ, the Nagano group performed an oxidation reaction without DDQ, resulting in a very low yield.[10] The Peterson

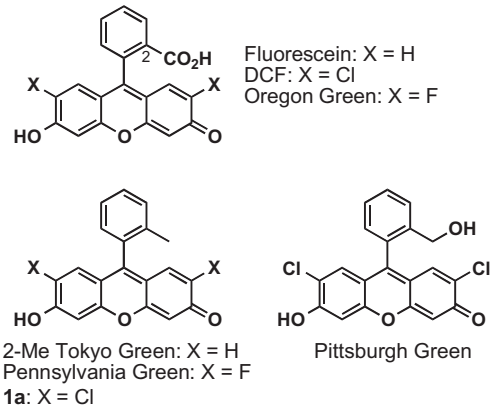

Figure 1. Structures of fluorescein and its derivatives.

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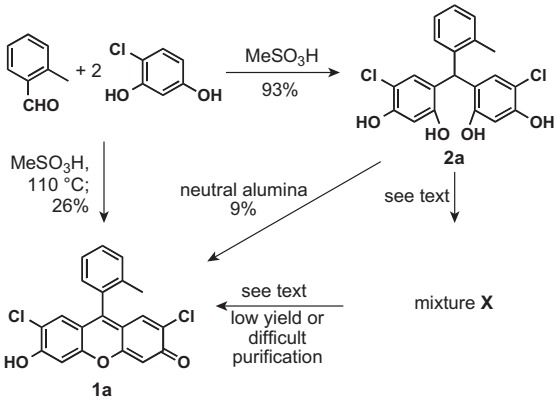


Scheme 1. Synthesis of xanthenone **1a**.

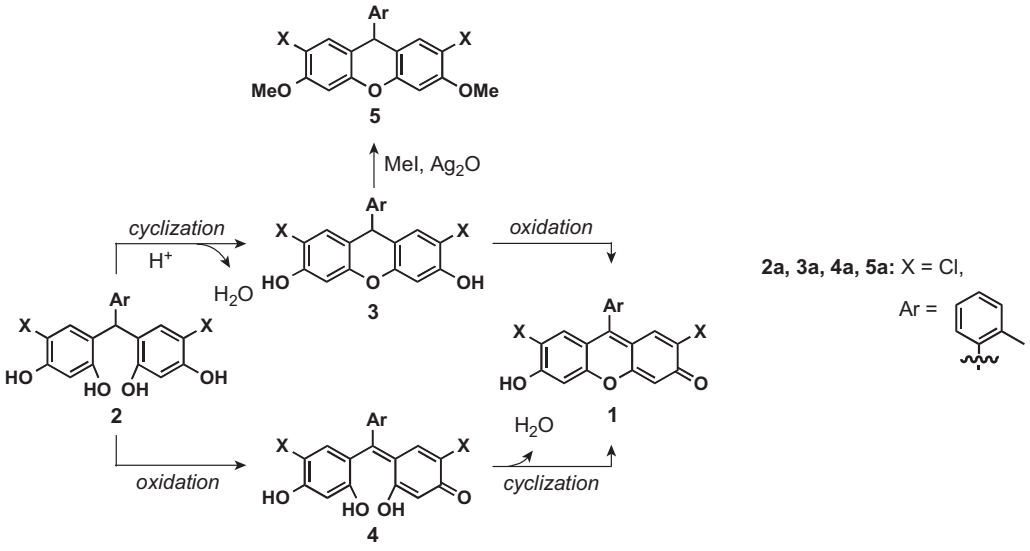
group converted an analogous intermediate to Pennsylvania Green upon heating in the presence of *p*-TsOH, albeit with moderate yields (37–38% yields).[6b] The low efficiency of these reactions may be attributed to a retro-Friedel–Crafts process.[11] Consistent with these reports, heating mixture **X** with and without an acid proved to be low yielding (<5%) and not reproducible in our laboratory. These frustrations prompted us to consider an alternative approach for the synthesis of **1a**.

When we were following the literature protocol to cyclize tetraol **2a** to xanthenone **1a** via mixture **X**, we noticed that a nonfluorescent compound in mixture **X** became fluorescent after heating the TLC plate. This could be due to the facile conversion of xanthene **3a** to xanthenone **1a** (Scheme 2) on a silica gel surface. To test this hypothesis, tetraol **2a** was dissolved in 1:1 MeOH/CH_2Cl_2 and added to silica gel and neutral alumina. After removing the solvents under reduced pressure, the silica gel and alumina containing tetraol **2a** were heated in an oven for 48 h at 140 °C (CAUTION: *The organic solvents must be thoroughly removed by a high vacuum before placing the silica gel in the oven*). The conversion of tetraol **2a** to xanthenone **1a** was visually obvious because while tetraol **2a** and mixture **X** were white, xanthenone **1a** was red. After rinsing the red silica gel and alumina with 1:4 MeOH/CH_2Cl_2, xanthenone **1a** was isolated in 9% yield from alumina and in a lower yield from silica gel. Although this protocol was convenient, the purification was not practical in a preparative scale because many byproducts and the starting material were coeluted with the desired xanthenone **1a** in column chromatography.

During these synthetic studies, we realized that both of the steps (*o*-tolualdehyde+4-chlororesorcinol→**2a** and **2a**→**1a**) were promoted by acid. Therefore, it was hypothesized that these steps could proceed in one step under acidic conditions. In effect, we treated a solution of *o*-tolualdehyde and 4-chlororesorcinol with $MeSO_3H$ at 110 °C, which produced xanthenone **1a** in 26% isolated yield. The purification did not require HPLC to obtain **1** in >95% purity. A protocol devoid of column chromatography is also described in the experimental section. These convenient protocols may provide an easy access to fluorescein derivatives for others engaged in the synthesis of this class of compounds.[1,5,6,8,9,11,12]

It should be noted that the mechanism for the conversion of tetraol **2a** to xanthenone **1a** is debatable. Specifically, as Scheme 2 indicates, the first step may be the acid-catalyzed cyclization to form xanthene **3a** or air-oxidation of the triarylmethane C–H to form triol **4a**. In a similar system, Gee et al. indicated that xanthene **3** was an intermediate en route to xanthenone **1**.[13] In contrast, Van Vranken and co-workers implied that the intermediate might have been triol **4** because tetraol **2** did not undergo cyclization to form xanthene **3**.[8] The formation of triol **4** might be reasonable after benzylic C–H oxidation followed by dehydration. To shed further insight into this mechanism, we decided to closely examine the conversion of tetraol **2a** to xanthenone **1a**.

During the conversion of tetraol **2a** to xanthenone **1a**, three additional compounds were observed by TLC analysis. Attempts to purify these compounds from mixture **X** were not fruitful, possibly due to air oxidation of xanthene **3a** to xanthenone **1a** and rapid cyclization of triol **4a** to xanthenone **1a** among others. LC–MS analysis of mixture **X** using reverse-phase chromatography did not provide conclusive data due to poor separation of multiple compounds. The ^{1}H NMR spectrum of mixture **X**[14] (Fig. S6, Supplementary data) showed the presence of xanthene **3a**, as indicated by a singlet peak at 5.88 ppm that was absent in both tetraol **2a** and xanthenone **1a**. The mass spectroscopic analysis of mixture **X** indicated the presence of xanthene **3a** and xanthenone **1a**, but triol **4a** could not be observed. The structure of the air-sensitive xanthene **3a** could be further corroborated after the treatment of mixture **X** with MeI and NaH, which produced the more air-stable methyl ether **5a**. This ether derivative was semipurified using a preparative TLC and analyzed by ^{1}H NMR spectroscopy and mass spectroscopy (Figs. S7 and S8, Supplementary data). This result



Scheme 2. Two plausible pathways for the conversion of tetraol **2** to xanthenone **1**.

indicates that the cyclization–oxidation pathway (**2a**→**3a**→**1a**) accounts for the formation of xanthenone **1a**. A cyclization similar to the conversion of **2a** to **3a** under the acidic conditions was previously observed,[15] although the mechanism is not yet clear.

As for the alternative pathway (**2a**→**4a**→**1a**), it is noteworthy that at least one fluorescent compound other than xanthenone **1a** was observed transiently by TLC analysis during the conversion of **2a** to **1a**, but this fluorescent compound could not be isolated. This fluorescent intermediate might be triol **4a**, which could cyclize to form xanthenone **1a**. Therefore, the cyclization–oxidation and oxidation–cyclization pathways might be concurrently operating.

In summary, we developed a one-step protocol for the preparation of xanthenone **1a**, and subsequent mechanistic studies supported the cyclization–oxidation pathway. It should be noted that this study by no means excludes the oxidation–cyclization pathway. Further mechanistic studies are warranted to improve the efficiency of this important synthetic process for chemistry, biology, and material sciences.

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Supplementary data

Supplementary data (experimental procedure and characterization data of compounds **1a**, **2a**, **3a**, and **5a**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.07.084.

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