

## Fluorescein Analogues as Photoremovable Protecting Groups Absorbing at $\sim 520$ nm

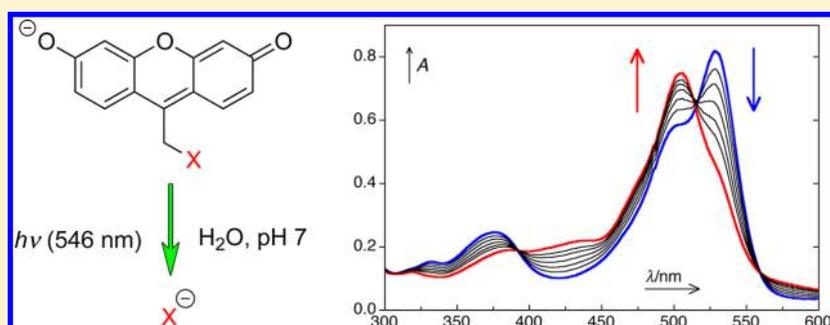
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### Supporting Information



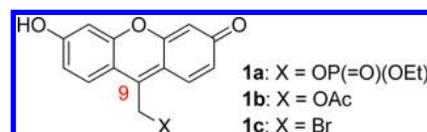
**ABSTRACT:** A new photoremovable protecting group, (6-hydroxy-3-oxo-3H-xanthen-9-yl)methyl (**1**), with a molar absorption coefficient  $\epsilon$  of  $\sim 4 \times 10^4 \text{ m}^{-1} \text{ cm}^{-1}$  at  $\sim 520$  nm for the release of carboxylates or phosphates is reported. Three derivatives of **1** (diethyl phosphate, acetate, and bromide) were isolated as complexes with DDQ and shown to release the ligands with quantum yields  $\leq 2.4\%$  in aqueous solution.

Photoremovable protecting groups (PPGs) are increasingly used as versatile tools allowing for the temporally and spatially controlled release of various bioagents in order to study the kinetics of chemical processes in living cells.<sup>1,2</sup> Attractive features of coumarin-derived PPGs are their strong absorption extending to the visible range and appearance rate constants of the free substrates that are on the order of  $10^9 \text{ s}^{-1}$  following excitation with a short light pulse.<sup>3</sup>

In an effort to extend the wavelength range of coumarin PPGs we undertook to synthesize and study the (6-hydroxy-3-oxo-3H-xanthen-9-yl)methyl derivatives **1a–c**. Encouraging MO calculations had indicated that strong charge transfer to the C9 position is associated with electronic excitation of the xanthenyl chromophore to the first excited singlet state, which should favor the heterolytic release of an attached leaving group.<sup>4,5</sup> Most xanthenes derivatives such as fluorescein are substituted with an aromatic ring at position C9. So far, only a limited number of compounds having a different substituent, such as cyano,<sup>6–9</sup> trifluoromethyl,<sup>8</sup> alkyl,<sup>10,11</sup> or alkenyl<sup>12</sup> groups, have been synthesized and their structure properly elucidated.

## RESULTS AND DISCUSSION

**Synthesis.** 3,6-Dihydroxy-9H-xanthen-9-one (**3**) was prepared from 2,2',4,4'-tetrahydroxybenzophenone **2** by cyclizing



condensation in water in an autoclave at  $200 \text{ }^\circ\text{C}$  according to a known procedure (Scheme 1).<sup>13</sup> The two hydroxy groups of **3** were protected using dimethyl sulfate to give **4**, which was subsequently treated with trimethylaluminum in a Wittig-like fashion<sup>14</sup> to give 3,6-dimethoxy-9-methylene-9H-xanthenone (**5**). Hydroboration resulted in formation of the corresponding primary alcohol **6** in 90% yield, which served as a common precursor for the preparation of the synthetic intermediates phosphate **7a**, acetate **7b**,<sup>15</sup> and bromide **7c**.<sup>16</sup> Deprotection of the methoxy groups<sup>17</sup> in **7a–c** using 13 equiv of boron tribromide gave the 9H-xanthen-3,6-diol derivatives **8a–c** in 78–99% yield. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) was then used to oxidize these compounds in dry acetonitrile.<sup>18</sup> However, instead of the anticipated (6-hydroxy-3-oxo-3H-xanthen-9-yl)methyl derivatives **1a–c**, 1:1 complexes with DDQ (**1a–c**-DDQ) precipitated as fine red powders from

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