JOC The Journal of Organic Chemistry

Article

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Fluorescein Analogues as Photoremovable Protecting Groups Absorbing at ~520 nm

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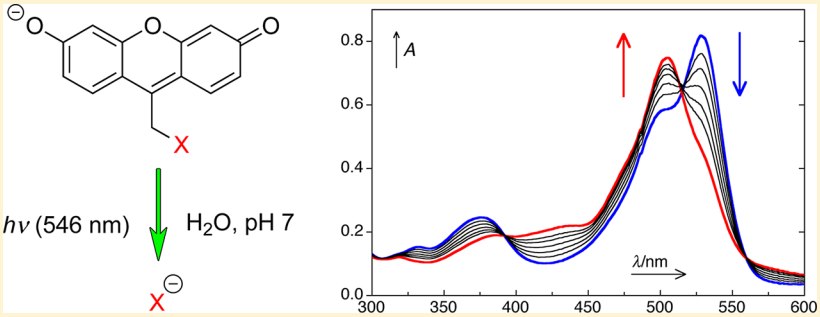
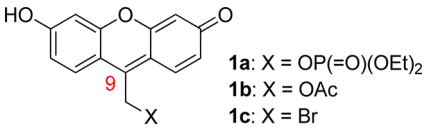
Supporting Information



ABSTRACT: A new photoremovable protecting group, (6-hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl (**1**), with a molar absorption coefficient ε of ~4 × 10^4 m^{-1} cm^{-1} at ~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 ≤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.[1,2] 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 s^{-1} following excitation with a short light pulse.[3]

In an effort to extend the wavelength range of coumarin PPGs we undertook to synthesize and study the (6-hydroxy-3-oxo-3*H*-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.[4,5] Most xanthene 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,[6−9] trifluoromethyl,[8] alkyl,[10,11] or alkenyl[12] groups, have been synthesized and their structure properly elucidated.



RESULTS AND DISCUSSION

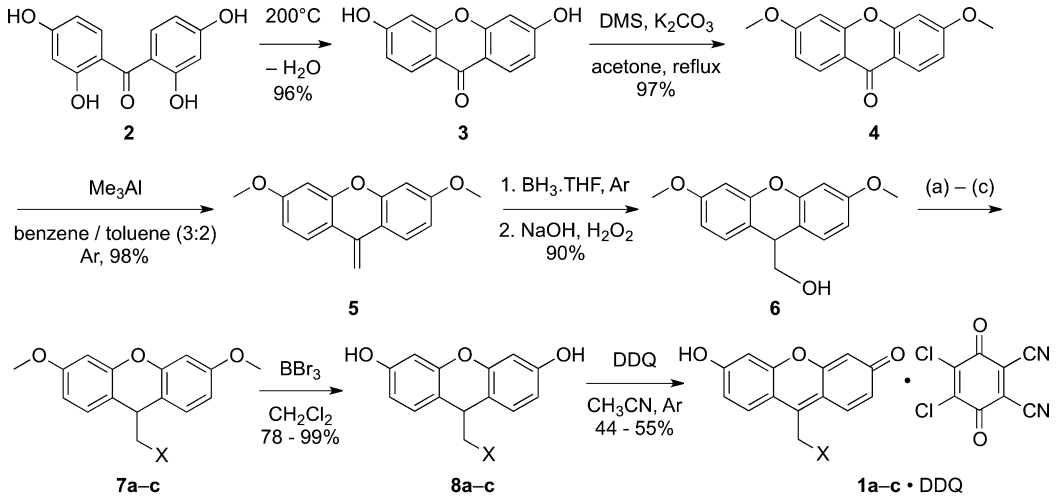
Synthesis. 3,6-Dihydroxy-9*H*-xanthen-9-one (**3**) was prepared from 2,2′,4,4′-tetrahydroxybenzophenone **2** by cyclizing condensation in water in an autoclave at 200 °C according to a known procedure (Scheme 1).[13] The two hydroxy groups of **3** were protected using dimethyl sulfate to give **4**, which was subsequently treated with trimethylaluminium in a Wittig-like fashion[14] to give 3,6-dimethoxy-9-methylene-9*H*-xanthene (**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**,[15] and bromide **7c**.[16] Deprotection of the methoxy groups[17] in **7a**–**c** using 13 equiv of boron tribromide gave the 9*H*-xanthene-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.[18] However, instead of the anticipated (6-hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl derivatives **1a**–**c**, 1:1 complexes with DDQ (**1a**–**c**·DDQ) precipitated as fine red powders from

Special Issue: Howard Zimmerman Memorial Issue

Received: July 17, 2012
Published: July 24, 2012

   dx.doi.org/10.1021/jo301455n | J. Org. Chem. 2013, 78, 1833–1843

Scheme 1. Synthesis of Complexes 1a−c·DDQ[a]



[a]Reagents and conditions: (a) X = OP(=O)(OEt)$_2$: ClP(=O)(OEt)$_2$, CH_2Cl_2, Ar, 78%; (b) X = OAc: Ac_2O, TEA, DMAP, Ar, 98%; (c) X = Br: CBr_4, PPh_3, CH_2Cl_2, Ar, 97%. DMS = dimethyl sulfate.

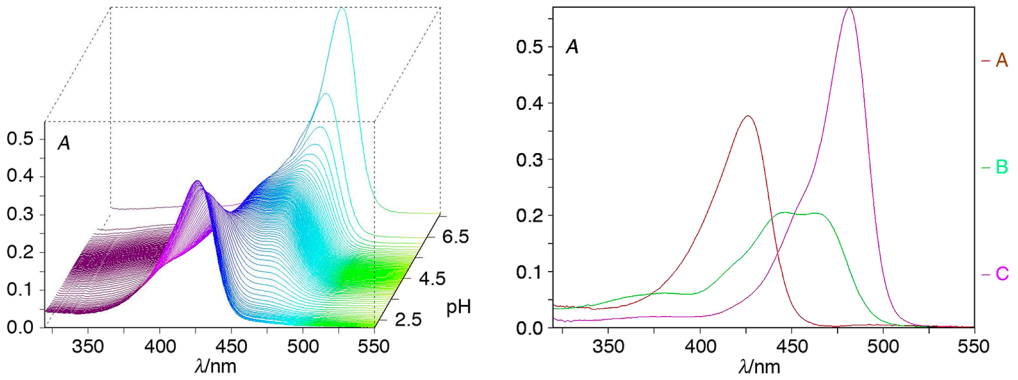


Figure 1. Spectrophotometric titration of 6-hydroxy-9-methyl-3*H*-xanthen-3-one **11**. Left: Plot of the absorption spectra at pH values ranging from 2 to 7. Right: Species spectra (A, cation **11**$^+$; B, neutral **11**; C, anion **11**$^-$) resulting from global analysis.

the solution in 44−55% yield (calculated with respect to the molecular weight of the complex; a 1.25-molar excess of DDQ was used for the reaction). Their chemical composition was determined by a combination of complementary analytical methods described below. The overall optimized chemical yield of **1a−c**·DDQ was 20−25% over 7 steps.

Charge-transfer (CT) complexes are known to be formed between DDQ and many electron donors[19] such as durene,[20] perylene, pyrene,[21] phenantroline, and aromatic amines.[22] The reduction potential of DDQ ($E[A/A^{\bullet-}]$ = +0.51 V in CH_3CN vs SCE)[19] is higher than that of tetracyanoethylene (+0.24 V in CH_3CN vs SCE).[23] On the other hand, xanthene dyes, such as fluorescein ($E[D^{\bullet+}/D]$ = −1.22 V for fluorescein in acetonitrile vs SCE)[24] are good electron donors. Thus the formation of the CT complexes **1a−c**·DDQ is in line with previous experience.

Several other reagents to oxidize compounds **8** were tested. For example, 2,3,5,6-tetrafluoro-1,4-benzoquinone (fluoranil)[25] was used in a stoichiometric amount under various preparative conditions; however, **8a** was found to be inert to the oxidation. In a different unsuccessful synthetic approach, we attempted to carry out epoxidation[26] of the C=C bond in **5**, the product of which could have subsequently been hydrolyzed and converted to the target molecules **1**.

Identification of the Complexes 1·DDQ. Elemental analyses showed that the isolated products are equimolar complexes of compounds **1** and DDQ. For all three products both direct inlet and HPLC−HRMS analyses provided clearly the molecular ions of **1a−c**·DDQ with isotope patterns typical for the presence of two chlorine atoms (Supplementary Figures S14, S22, and S30−31). Moreover, characteristic vibrations of the cyano group were observed in the IR spectra (Supplementary Figure S21).

The solubility of **1a**·DDQ is very low in all common organic solvents, except for DMSO and DMF. All attempts to isolate the free compounds **1a−c** were unsuccessful. The components of the complexes **1a−c**·DDQ could not be separated chromatographically. We were also unable to crystallize the compounds from DMSO or DMF to produce crystals suitable for X-ray analysis.

To determine whether the formation of CT complexes of **1** with DDQ is a general behavior of 6-hydroxy-3*H*-xanthen-3-one derivatives, we studied the interactions of DDQ with succinylfluorescein **9**, synthesized according to a known procedure (Experimental Section, Scheme 4),[10] with its methyl ester **10** and with the parent compound 6-hydroxy-9-methyl-3*H*-xanthen-3-one **11** (Experimental Section, Scheme 5). DDQ was not used in the synthesis of these compounds.

DDQ is only moderately stable in methanol;[27] its half-life was estimated to be ∼4 h by UV spectroscopy. The formation of the complexes **9−10**·DDQ in methanol solutions of **9−10**

dx.doi.org/10.1021/jo301455n | J. Org. Chem. 2013, 78, 1833−1843

containing equimolar amounts of DDQ was observed by direct inlet and LC–HRMS, which clearly showed the molecular ions of the complexes and the corresponding isotope patterns typical for the presence of 2 chlorine atoms (Supplementary Figures S43 and S44), as in the case of **1a–c**·DDQ. However, in dilute (30 μM) solutions of **9–11** with up to 100-fold excess of DDQ, absorption and fluorescence spectroscopy did not provide any evidence for complex formation.

The complexes **1a–c**·DDQ dissolved relatively well (up to ~10 mM) in 0.1 M aqueous buffer solutions (pH 7–8) or water. DDQ is known to decompose rapidly in water.[19] It reacts within seconds forming 2,3-dichloro-5-cyano-6-hydroxy-1,4-benzoquinone (**12**) and HCN. We conclude that compounds **1a–c** are no longer associated with DDQ when the solid complexes **1a–c**·DDQ are dissolved in aqueous solvents. Slow decomposition of **1a–c** occurred in phosphate buffer (pH 7, I = 0.1 M) in the dark at room temperature with a half-life of 7 days in 30 mM solution.

Strong bands at λ_{max} ~528 (**1a**), 522 (**1b**), and 519 nm (**1c**), typical for xanthene dyes,[28] were found in their absorption spectra in aqueous phosphate buffer, pH = 7 (ε_{max} ~4 × 10^4 m^{-1} cm^{-1}; Supplementary Figures S13, S20, and S29). The bathochromic shifts of the first absorption band associated with the electronegative substituents on 9-methyl indicated that excitation to the first singlet state is accompanied by charge transfer to carbon 9, as expected.

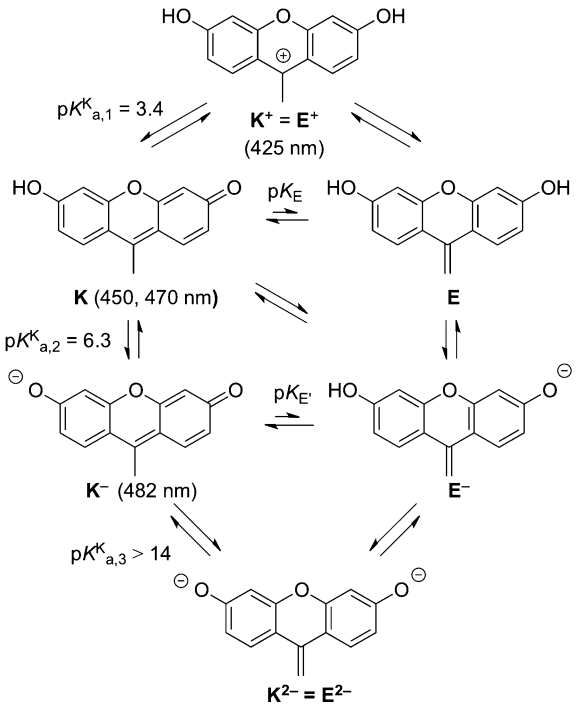
Titrations. To determine the acidity constants of the 6-hydroxy-3*H*-xanthen-3-one chromophore we first used the parent compound 6-hydroxy-9-methyl-3*H*-xanthen-3-one **11**. A series of spectra was measured by addition of 0.1 M HCl to a solution of **11** in 0.1 M aqueous sodium acetate (Figure 1). The acidity constants resulting from a global analysis of the spectra and fitting with a titration model allowing for two acidity constants are $pK_{a,c,1}$ = 3.44 ± 0.11 and $pK_{a,c,2}$ = 6.31 ± 0.03 (standard deviations obtained by three independent titration runs, I = 0.1 M, 25 ± 0.3 °C). Similar measurements with the bromide **1c** gave $pK_{a,1}$ = 2.9 ± 0.1 and $pK_{a,2}$ = 6.1 ± 0.1.

Succinylfluorescein **9** exists in the form of several tautomers that undergo slow equilibration at 20 °C.[10,29] Similarly, two tautomeric forms of neutral **11** and its anion may participate in the protomeric equilibria, the keto form **K** and the enol form **E** (Scheme 2). However, the characteristic absorption spectra of **11** in the visible range (Figure 1) indicate that the keto forms of the neutral species and the anion, **K** and $\mathbf{K}^-$, predominate in aqueous solutions up to pH 13. In the hydrogen-bond acceptor solvent DMSO, **11** is converted largely to the enol form (Figures S40 and S41; Supporting Information). Following injection of a concentrated DMSO solution into water, the reketonization reaction could be monitored by the rising absorption in the visible range. The ketonization rate constants were slowest in neutral solutions pH 6–9, $k_{rise} \approx 1 \times 10^{-2}$ s^{-1}, and increased linearly with pH as it was decreased below 5 (acid catalysis) or increased above 10 (base catalysis).

A ~20% increase in the absorbance of **11** at 482 nm was observed upon addition of 1 M HCl to a solution of **11** in 1 M aqueous KOH. Therefore, the third acidity constant of **11** is probably not far greater than 14. At pH 7, the compound **11** is mostly in the anionic form $\mathbf{K}^-$ (83%); therefore the compounds **1a–c** carrying an electronegative substituent at the exocyclic carbon will be almost exclusively anionic at pH 7.

Calculations. Density functional theory (DFT) calculations were done with the Gaussian package of programs[30] to calculate the energy difference between the keto and enol forms

Scheme 2. Protonation and Tautomerization Equilibria of 11[a]



[a]**K** and **E** are the keto and enol forms, respectively.

K and **E** of **11** (see Scheme 2). Geometries were fully optimized at the B3LYP level of theory with the 6-31+g(d) basis set and the PCM solvation model for water; vibrational frequencies were calculated. The free energy differences were found to be $\Delta_{K\rightarrow E}G(298\ K)$ = 26.0 kJ mol^{-1} and $\Delta_{K^-\rightarrow E^-}G(298\ K)$ = 54.8 kJ mol^{-1}, corresponding to enolization constants pK_E = 4.6 and $pK_{E'}$ = 9.4 for the neutral and anionic forms, respectively.

The same calculations were done for the acetate derivative **1b**. The acetate substituent favored the enol forms to give pK_E = −0.06 and $pK_{E'}$ = 6.3 for the neutral and anionic forms, respectively.

Photochemistry. Solutions of **1a** (10 μM) in aqueous phosphate buffers (0.1 M, pH = 7.0–7.4) were irradiated with green light isolated from a high-pressure mercury arc (λ = 546 nm). The course of the reaction was followed by UV–vis spectroscopy (Figure 2 and Supplementary Figure S45). The absorption maximum of **1a** at λ ~528 nm was replaced by a new maximum at λ ~500 nm during irradiation indicating the formation of a new product. Similar changes in the absorption spectra were observed when either **1b** or **1c** were irradiated in phosphate buffer solutions (Supplementary Figures S48 and S49, respectively). The ^{1}H NMR and ^{31}P NMR spectral changes upon irradiation of **1a** are presented in Supplementary Figures S46 and S47. The primary photoproduct(s) are not stable and the absorption spectra change upon standing in the dark for hours.

The leaving group (*O*,*O*-diethyl phosphate) was released in high chemical yield upon irradiation of **1a** (>90%, as detected by both ^{1}H and ^{13}C NMR; Supplementary Figures S46, S51, and S52). In addition, two photoproducts, 3,6-dihydroxy-9*H*-xanthen-9-one (**3**) and 6-hydroxy-3-oxo-3*H*-xanthene-9-carboxylic acid (**13**), were identified (Scheme 3). Their chemical yields varied with the solvent used. Following irradiation of

dx.doi.org/10.1021/jo301455n | J. Org. Chem. 2013, 78, 1833–1843

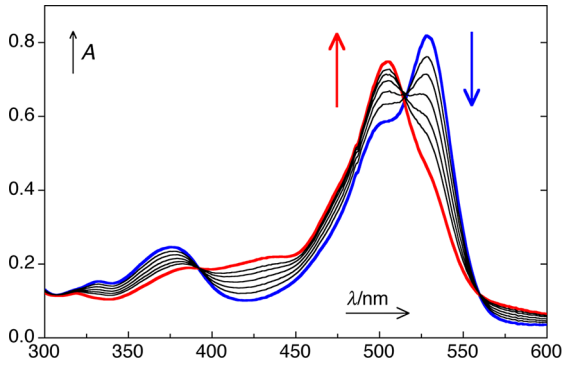
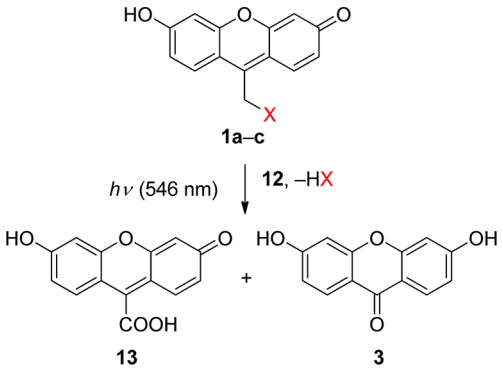


Figure 2. Irradiation of **1a** (first spectrum, λ_{max} = 528 nm, blue) at λ = 546 nm in phosphate buffer (0.1 M, pH = 7.0) as monitored by absorption spectroscopy. The last spectrum (λ_{max} = 505 nm, red) was taken after 15 min of irradiation.

Scheme 3. Photochemistry of 1a–c



water/methanol (1:1, v/v) solutions, the xanthenone **3** was isolated in 70% yield, whereas **13** was not detected. In aqueous phosphate buffer solutions of **1a** (10 μM), **13** formed as the major photoproduct. In a more concentrated solution (10 mM), 40% of the starting material precipitated during irradiation. The product **13** was then obtained in 50% yield (calculated on the basis of the starting material consumed) by precipitation upon acidification of the remaining filtered solution using aq CF_3COOH, whereas only a small amount of **3** (<5%) was detected in the reaction mixture.

The same photoproducts were formed by irradiation of degassed solutions of **1a**. The hydrolysis product of DDQ, compound **12**, that is formed by dissolving the solid complexes **1a–c**·DDQ in water presumably serves to oxidize the expected primary product 6-hydroxy-9-hydroxymethyl-3*H*-xanthen-3-one to **13** either photochemically or in the dark. Careful removal of air oxygen did not affect the course of the reaction and no other oxidation agents were present.

The absorption maxima of the pure (isolated) acid **13** in a phosphate buffer and in methanol are λ_{max} = 489 and 492 nm, respectively; its structural characterization is provided in the Supporting Information. Formation of **13** by condensation of resorcinol and chloral hydrate in sulfuric acid has been claimed in the literature;[31] we have reproduced that procedure, but the red product so obtained was found to be a complex mixture. The absorption spectra of the irradiated buffer solutions of **1b** and **1c** clearly show that the acid **13** was also formed as the major photoproduct (Supplementary Figures S48 and S49).

The quantum yields of photorelease from the compounds **1a–c** are listed in Table 1. Degassing did not affect the observed UV changes nor the quantum yields.

Table 1. Quantum Yields of Disappearance (Φ_{disapp}) for 1a–c in Aqueous Solutions

compound	Φ_{disapp}[a] (%)
1a	1.7 ± 0.3
1b	0.27 ± 0.08
1c	2.4 ± 0.2

[a]Quantum yields of disappearance were obtained spectrophotometrically (Figure 2) by irradiation of the compounds in aq phosphate buffer solutions (pH = 7.0, I = 0.1 M) with light pulses from a NOPA at λ = 538 ± 7 nm. *meso*-Diphenylhelianthrene was used as an actinometer.[32] Each value represents an average of at least five measurements; standard deviations of the means are given.

Fluorescence. The fluorescence spectra of **11** in aqueous solution are similar to those of fluorescein.[33] A series of fluorescence spectra was measured by adding 0.1 M aqueous HCl to a solution of **11** in 0.1 M phosphate buffer, pH 7. Global analysis of the spectra recorded in the range pH 2–7 indicated two spectral components, which are attributed to the emission spectra of the anion **11**$^-$ and of the neutral compound (Figure 3). Remarkably, the emission band of the neutral (λ_{max} = 510 nm, strong shoulder at 540 nm) extends to longer wavelength than that of the anion (λ_{max} = 507 nm, weak shoulder at 540 nm).

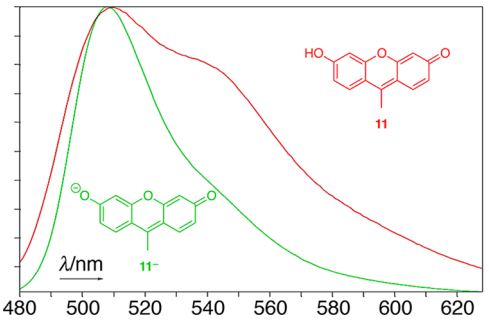


Figure 3. Fluorescence emission spectra (uncorrected, normalized) of **11** and its anion **11**$^-$ in aqueous solution.

Fitting of a titration function to these data gave an apparent acidity constant p$K_{a,2}$* = 5.4 ± 0.1 for the ionization of S_1(**11**). However, the equilibrium **11*** → **11**$^-$* + H^+ is presumably not fully established during the excited state lifetime. The fluorescence decay of **11***/**11**$^-$* obeyed a single exponential rate law with a lifetime of τ = 3.5 ± 0.2 ns at all wavelengths and pH values from 3–11.

The fluorescence decay of the bromide **1c** was measured with freshly prepared solutions in 0.1 M aqueous phosphate buffer, pH 7. With an ordinary (nonflow) cuvette the observed fluorescence emission (λ_{max} = 530 nm and a shoulder at 570 nm) obeyed a single exponential rate law (τ = 3.6 ± 0.1 ns). The signal increased in intensity with continued irradiation, but its lifetime and spectrum remained the same. We therefore suspected that the observed signal must largely be due to the photoproduct. We then used a flow cell to reduce dual irradiation of the sample. Now the decay obeyed a biexponential rate law with lifetimes τ_1 = 0.38 ± 0.03 ns (amplitude 23%) and τ_2 = 3.6 ± 0.1 ns (77%) (Figure 4).

dx.doi.org/10.1021/jo301455n | J. Org. Chem. 2013, 78, 1833–1843

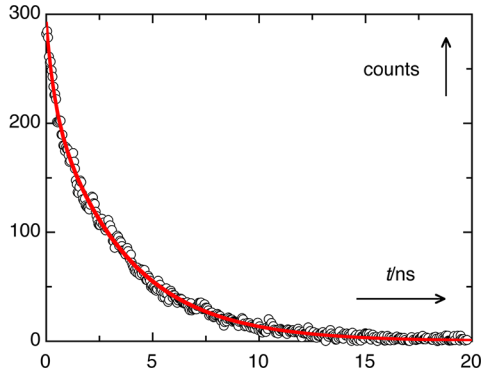


Figure 4. Fluorescence decay of **1c** in aq phosphate, pH 7.

Assuming that the weak component decaying faster can be attributed to the bromide **1c** and the slower decay to its photoproduct, this indicates that the release of bromide from **1c** occurs with a lifetime of 0.4 ns.

CONCLUSION

The (6-hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl complexes **1a**–**c**·DDQ have been synthesized in 7 steps with an overall chemical yield of 20–25%. In aqueous solutions, nonassociated **1a**–**c** were shown to release diethyl phosphate, acetate, and bromide, respectively, upon irradiation at over 500 nm. Compounds **1a**–**c** are among the rare examples of visible-light-triggered caged systems. The 9-methylxanthenone chromophore thus holds promise as a photoremovable protecting group for use with the second harmonic of Nd:YAG lasers.

EXPERIMENTAL SECTION

Materials and Methods. The reagents and solvents of the highest purity available were used as purchased or were purified/dried when necessary. Acetone, acetonitrile, dichloromethane, and tetrahydrofuran were dried by standard procedures and kept over high-temperature-dried 3 Å molecular sieve (8–12 mesh) under dry N_2; they were freshly distilled for each experiment. Synthetic steps were performed under ambient atmosphere unless stated otherwise. All glassware was flame-dried prior to use when water- and/or air-sensitive reagents were used. Oxygen was removed from solutions by three freeze–pump–thaw cycles or bubbling with inert gas (N_2 or Ar) for at least 15 min. All column chromatography purification procedures were performed with silica.

NMR spectra were recorded on 300, 400, 500, or 600 MHz spectrometers in acetonitrile-d_3, chloroform-*d*, dichloromethane-d_2, dimethylsulfoxide-d_6, methanol-d_4, trifluoroacetic acid-*d*, water-d_2, or their mixtures. The signals in ^{1}H and ^{13}C NMR were referenced to the residual peak of a (major) solvent except for H_2O, and those of the ^{31}P NMR were unreferenced. The deuterated solvents (except for CF_3COOD and D_2O) were kept over high-temperature-dried 3 Å molecular sieve (8–12 mesh) under dry N_2. Mass spectra were recorded on a GC-coupled (30 m DB-XLB column) spectrometer in a positive mode with EI or FAB. MALDI-MS analyses were performed using an automatic spectrometer with *p*-nitroaniline as a matrix. UV spectra were obtained with matched 1.0 cm quartz cells. IR spectra were obtained on an FT spectrometer. Fluorescence emission spectra (uncorrected) were recorded on a Spex Fluorolog spectrophotometer 111C equipped with a 150-W xenon arc for excitation and an R928 photomultiplier. Excitation spectra were corrected with a built-in Rhodamine-6G quantum counter. Fluorescence lifetime measurements were done by excitation with subpicosecond pulses at 515 nm from a Clark-MXR Ti:Sa laser CPA 2001 coupled to a non-colinear optical parametric amplifier (NOPA). The fluorescence spectra and decays were measured with a Hamamatsu C5680 streak camera operated with 20-ps time windows. Exact masses were performed using a triple quadrupole electrospray ionization mass spectrometer in positive or negative modes. Melting points were obtained in open-end-capillary tubes using a noncalibrated digital melting point apparatus or a non=calibrated Kofler's hot stage melting point apparatus. Elemental analyses were performed on an automatic analyzer. Lyophillization was performed at 5 Pa and −110 °C. The solution pH values were determined using a glass electrode calibrated with certified buffer solutions at pH = 4, 7, or 10. A 40-W medium pressure mercury arc equipped with the corresponding band-pass or cutoff filters was used for irradiation.

Quantum Yield Measurements. The quantum yield measurements were performed using a Ti:Sa laser coupled to a non-colinear optical parametric amplifier (NOPA) producing light at λ = 538 ± 7 nm (bandwidth at half height). The number of photons entering the sample cell was ∼4.8 × 10^{-9} einstein s^{-1} (the quantum flux was determined precisely before and after each measurement, and the average of the two measurements was used for the quantum yield calculation). The absorbance $A(\lambda_{max})$ of all sample solutions was kept below 2.0. The course of the photoreactions was followed spectrophotometrically (UV–vis absorption). *meso*-Diphenylhelianthrene (*m*DPH) was used as an actinometer. *m*DPH is known to undergo a uniform and well-described[32] self-sensitized photooxidation reaction in a solution of air-saturated toluene to form the corresponding endoperoxide. All measurements were done at ambient temperature. Each sample was measured at least five times and quantum yields were obtained with less than 15% standard deviation.

3,6-Dihydroxy-9*H*-xanthen-9-one (3). A stirred suspension of 2,2′,4,4′-tetrahydroxybenzophenone (**2**, 4.00 g, 16.3 mmol) in distilled water (24 mL) was heated in an autoclave at 200 °C for 6 h. The mixture was cooled to 20 °C, and 3,6-dihydroxy-9*H*-xanthen-9-one was obtained as a cluster of needles. It was filtered off, washed with hot distilled water (3 × 10 mL), and dried under reduced pressure to give a pure title product. Yield: 3.55 g (96%). Light orange needles. Mp: 330 °C (decomp) (lit. 320 °C;[34] 347 °C[35]). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 6.82 (d, 2H, J = 2.0 Hz), 6.86 (dd, 2H, J_1 = 8.7 Hz, J_2 = 2.1 Hz), 7.98 (d, 2H, J = 8.7 Hz), 10.82 (s, 2H, −OH). ^{13}C NMR (100.5 MHz, DMSO-d_6): δ (ppm) 102.0, 113.5, 113.9, 127.6, 157.3, 163.2, 173.8. MS (ESI$^-$; CH_3OH + 2% NH_3, γ ∼0.1 mg cm^{-3}): m/z = 227.2 (M − H^+, 100), 228.25 (M^-, 12.8). FTIR (cm^{-1}): 3382, 3095, 1629, 1611, 1575, 1454, 1393, 1352, 1324, 1291, 1273, 1254, 1245, 1243, 1169, 1115, 1104, 986, 847, 831, 790, 693, 665, 635. UV–vis (C_2H_5OH, c 1.46 × 10^{-6} mol dm^{-3}): λ_{max}/nm (ε/M^{-1} cm^{-1}) = 209 (29000), 239 (42500), 267 (11400), 280 (8050), 312 (24400), 321 (23600). Anal. Calcd for $C_{13}H_8O_4$: C, 68.42; H, 3.53; O, 28.04. Found: C, 67.81; H, 3.70; O, 28.49. This compound has also been characterized elsewhere.[13,36,37]

3,6-Dimethoxy-9*H*-xanthen-9-one (4). A mixture of anhydrous potassium carbonate (3.30 g, 23.9 mmol) in acetone (75 mL) was added to a stirred suspension of 3,6-dihydroxy-9*H*-xanthen-9-one (**3**, 840 mg, 3.68 mmol). Dimethyl sulfate (6.3 mL, 66.4 mmol) was then added dropwise in 20 min. The resulting solution was stirred for 30 min at 20 °C and then refluxed for 20 h. After cooling to 0 °C, aqueous ammonia (c 0.5 mol L^{-1}, 15 mL) was added dropwise, and the reaction mixture was stirred for an additional 1 h at 20 °C. A white precipitate, obtained by addition of water (100 mL) to the mixture, was filtered off, washed with distilled water (3 × 10 mL), and dried under reduced pressure to give pure **4**. Yield: 914 mg (97%). White solid. Mp: 184.5–186.0 °C (lit. 187–188 °C).[38,39] ^{1}H NMR (500 MHz, CD_2Cl_2): δ (ppm) 3.90 (s, 6H,) 6.84 (dd, 2H, J_1 = 2.4 Hz, J_2 =1.0 Hz), 6.90 (ddd, 2H, J_1 = 8.8 Hz, J_2 = 2.4 Hz, J_3 = 0.7 Hz), 8.13 (d, 2H, J = 8.8 Hz). ^{13}C NMR (126 MHz, CD_2Cl_2): δ (ppm) 56.2, 100.5, 113.1, 116.1,

dx.doi.org/10.1021/jo301455n | J. Org. Chem. 2013, 78, 1833–1843

128.1, 158.3, 165.0, 175.3. MS (ESI$^+$; CH_3OH/H_2O, 1:1 (v/v) + NH_4OAc (5 mM), γ ~0.1 mg cm^{-3}): m/z = 257.1 (M + H$^+$, 100), 258.1 (M + 2 H$^+$, 16.2). MS (EI; 70 eV, 150 °C): m/z = 256.1 (M). FTIR (cm^{-1}): 1612, 1501, 1428, 1357, 1302, 1257, 1211, 1157, 1099, 1018, 979, 925, 825, 763, 663. UV−vis (C_2H_5OH, c 1.27 × 10^{-5} mol dm^{-3}): λ_{max}/nm (ε/M^{-1} cm^{-1}) = 209 (26700), 240 (43000), 266 (11000), 307 (22700). Anal. Calcd for $C_{15}H_{12}O_4$: C, 70.31; H, 4.72; O, 24.97. Found: C, 70.18; H, 4.75; O, 25.07. This compound has also been characterized elsewhere.[38,39]

3,6-Dimethoxy-9-methylene-9*H*-xanthene (5). Trimethylaluminium (1.1 mL, 2.2 mmol, 2 M solution in toluene) was added dropwise to a stirred suspension of 3,6-dimethoxy-9*H*-xanthen-9-one (**4**, 500 mg, 1.95 mmol) in a mixture of dry benzene (15 mL) and dry toluene (10 mL) under argon atmosphere at 25 °C to give a yellow mixture. It became homogeneous after warming to 50 °C. After cooling to 25 °C, trimethylaluminium (2.2 mL, 4.4 mmol, 2 M solution in toluene) was slowly added (caution: methane as a side product is released). The reaction mixture was then heated to 65 °C for 90 min, cooled to 0 °C, and ice (20 g) and aq HCl (0.1 M, 3 mL) were cautiously added (methane is released). The mixture was extracted with CH_2Cl_2 (3 × 15 mL), the combined organic layers were dried with Na_2SO_4, and the solvent was removed under reduced pressure to give the title product. Yield: 485 mg (98%). Yellow solid. Mp: 144.5−145.8 °C (lit. 146−147 °C[40]). ^{1}H NMR (400 MHz, CD_2Cl_2): δ (ppm) 3.83 (s, 6H), 5.29 (s, 2H), 6.62 (d, 2H, J = 2.6 Hz), 6.72 (dd, 2H, J_1 = 8.8 Hz, J_2 = 2.6 Hz), 7.66 (d, 2H, J = 8.8 Hz). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 3.80 (s, 6H), 5.39 (s, 2H), 6.72 (d, 2H, J = 2.6 Hz), 6.78 (dd, 2H, J_1 = 8.8 Hz, J_2 = 2.6 Hz), 7.78 (d, 2H, J = 8.9 Hz). ^{13}C NMR (100.5 MHz, DMSO-d_6): δ (ppm) 55.4, 97.2, 100.8, 111.3, 113.3, 125.2, 130.5, 150.7, 160.4. MS (EI; 70 eV, 150 °C): m/z = 254.1 (M). FTIR (cm^{-1}): 1625, 1619, 1599, 1567, 1467, 1462, 1440, 1425, 1386, 1330, 1265, 1247, 1207, 1171, 1160, 1109, 1098, 1078, 1028, 983, 946, 925, 838, 818, 783, 636. UV−vis (C_2H_5OH, c 8.71 × 10^{-6} mol dm^{-3}): λ_{max}/nm (ε/M^{-1} cm^{-1}) = 220 (27700), 231 (32600), 273 (3200). Anal. Calcd for $C_{16}H_{14}O_3$: C, 75.57; H, 5.55; O, 18.88. Found: C, 75.55; H, 5.59; O, 18.86. This compound has also been characterized elsewhere.[40,41] Note: **5**, both in the solid state or dissolved in polar solvents, is oxidized rapidly to a mixture of green products; therefore it should be stored in dark under N_2 atmosphere. If necessary, it can be easily purified by recrystallization from *n*-hexane.

(3,6-Dimethoxy-9*H*-xanthen-9-yl)methanol (6). BH_3·THF (1.0 M in THF, 3.5 mL, 3.5 mmol) was added to a stirred solution of **5** (200 mg, 0.79 mmol) in dry THF (25 mL) over a period of 20 min at 0 °C. The reaction mixture was stirred for 4 h at 20 °C and cooled to 0 °C, and then water (2 mL, 10% solution in THF), aq NaOH (3.0 M, 2.5 mL, 7.50 mmol), and aq hydrogen peroxide (30%, 2.7 mL, 26.4 mmol) were cautiously added. The resulting mixture was stirred for 1.5 h at 20 °C, then poured into water (50 mL), and neutralized with aq HCl (1 M, ~8 mL) to pH = 7, and the organic material was extracted with diethyl ether (3 × 20 mL). The combined organic layers were washed with brine (50 mL), dried with Na_2SO_4, and filtered, and the solvent was removed under reduced pressure to give the title product. No further purification was necessary. Yield: 193 mg (90%). Pale yellow solid. Mp: 83.9−85.0 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) 3.43 (t, 2H, J = 5.7 Hz), 3.75 (s, 6H), 3.86 (t, 1H, J = 6.1 Hz), 4.81 (t, 1H, J = 5.3 Hz, −OH), 6.63 (d, 2H, J = 2.5 Hz), 6.68 (dd, 2H, J_1 = 8.4 Hz, J_2 = 2.6 Hz), 7.23 (d, 2H, J = 8.5 Hz) (Supplementary Figure S1). ^{13}C NMR (126 MHz, DMSO-d_6): δ (ppm) 39.7, 55.2, 68.2, 100.8, 109.4, 114.9, 130.2, 152.0, 158.8 (Supplementary Figure S2). MS: (ESI$^+$; CH_3OH/H_2O, 1:1 (v/v) + 5 mM NH_4OAc, γ ~0.1 mg cm^{-3}): m/z = 255.1 (M − OH$^-$, 32.4), 272.1 (M, 3.1), 273.0 (M + H$^+$, 100), 289.7 (M + NH_4^+, 3.9), 561.5 (M_2 + NH_4^+, 33.4). FTIR (cm^{-1}): 3299, 3053, 2941, 2921, 2875, 2831, 1633, 1614, 1574, 1500, 1462, 1435, 1425, 1325, 1276, 1259, 1205, 1184, 1161, 1149, 1097, 1053, 1034, 1022, 983, 976, 925, 824, 815, 638, 617. UV−vis (C_2H_5OH, c 1.19 × 10^{-5} mol dm^{-3}): λ_{max}/nm (ε/M^{-1} cm^{-1}) = 212 (46400), 278 (300). Anal. Calcd for $C_{16}H_{16}O_4$: C, 70.58; H, 5.92; O, 23.50. Found: C, 70.63; H, 6.06; O, 23.31.

Diethyl-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl) Methyl Phosphate·DDQ Complex (1a·DDQ). *(3,6-Dimethoxy-9H-xanthen-9-yl)methyl Diethyl Phosphate (**7a**).* Diethyl chlorophosphate (0.11 mL, 0.75 mmol) in dry dichloromethane (20 mL) was added to a solution containing **6** (170 mg, 0.625 mmol) and 4-dimethylaminopyridine (91 mg, 0.75 mmol). The resulting solution was stirred at 20 °C under argon atmosphere for 24 h, and water (20 mL) was added. The reaction mixture was extracted with ethyl acetate (3 × 20 mL); the combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4, and filtered; and the solvent was removed under reduced pressure. The remaining yellow oil was purified by column chromatography (*n*-hexane/ethyl acetate, 60:40, v/v) to give the pure title product. Yield: 199 mg (78%). Slightly yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.07 (dt, 6H, J_1 = 7.1 Hz, J_2 = 0.8 Hz), 3.72 (m, 4H), 3.76 (s, 6H), 4.02 (t, 2H, J = 4.9 Hz), 4.24 (dt, 1H, J_1 = 4.5 Hz, J_2 = 1.5 Hz), 6.67 (d, 2H, J = 2.5 Hz), 6.73 (dd, 2H, J_1 = 8.5 Hz, J_2 = 2.6 Hz), 7.30 (d, 2H, J = 8.6 Hz). ^{1}H NMR (600 MHz, CD_2Cl_2): δ (ppm) 1.19 (dt, 6H, J_1 = 7.1 Hz, J_2 = 0.9 Hz), 3.80 (s, 6H), 3.87 (m, 4H), 4.01 (t, 2H, J = 5.9 Hz), 4.18 (t, 1H, J = 5.9 Hz), 6.64 (d, 2H, J = 2.5 Hz), 6.68 (dd, 2H, J_1 = 8.4 Hz, J_2 = 2.6 Hz), 7.21 (d, 2H, J = 8.4 Hz) (Supplementary Figure S3). ^{13}C NMR (126 MHz, CD_2Cl_2): δ (ppm) 16.2, 38.7, 55.8, 64.0, 72.6, 101.7, 110.3, 113.6, 130.5, 153.5, 160.5 (Supplementary Figure S4). ^{31}P NMR (162 MHz): δ (ppm) −1.47. MS (FAB): m/z = 406.1 (M^{2-}, 0.74), 407.1 (M$^-$, 4.66), 408.1(M, 1.34), 409.1 (M + H$^+$, 3.85), 410.1 (M + 2H$^+$, 1.0). MALDI-MS (positive mode): m/z = 406.978, 407.977, 408.979. FTIR (cm^{-1}): 2980, 2940, 2906, 2836, 2360, 2332, 1733, 1634, 1614, 1599, 1574, 1500, 1464, 1437, 1427, 1394, 1369, 1326, 1258 (P═O), 1202, 1196, 1162 (P−O−C), 1121, 1101, 1014 (P−O−C), 971 (P−O−C), 891, 830, 800, 733. UV−vis ($CHCl_3$): λ_{max}/nm (relative intensities) = 241 (100), 278 (62). Anal. Calcd for $C_{20}H_{25}O_7P$: C, 58.82; H, 6.17. Found: C, 58.90; H, 6.25.

*(3,6-Dihydroxy-9H-xanthen-9-yl)methyl Diethyl Phosphate (**8a**).* Boron tribromide (1 M in dichloromethane, 9.56 mL, 9.56 mmol) was added dropwise to a solution of (3,6-dimethoxy-9*H*-xanthen-9-yl)methyl diethyl phosphate (**7a**, 300 mg, 0.735 mmol) in dry CH_2Cl_2 (30 mL) under nitrogen atmosphere at −78 °C. The reaction mixture was stirred and left to warm to −10 °C in 24 h. Water (30 mL) was then added, and the mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (50 mL) and dried over $MgSO_4$, and the solvent was removed under reduced pressure. The resulting solid was dried under reduced pressure to give the pure title product. Yield: 276 mg (99%). Beige powder. Mp: 135 °C (decomp). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.09 (t, 6H, J = 7.1 Hz), 3.74 (m, 4H), 3.94 (t, 2H, J = 5.2 Hz), 4.12 (t, 1H, J = 4.3 Hz) 6.45 (d,

dx.doi.org/10.1021/jo301455n | J. Org. Chem. 2013, 78, 1833−1843

2H, J = 2.2 Hz), 6.53 (dd, 2H, J_1 = 8.3 Hz, J_2 = 2.2 Hz), 7.15 (d, 2H, J = 8.4 Hz), 9.56 (s, 2H, −OH) (Supplementary Figure S5). ^{13}C NMR (126 MHz, DMSO-d_6): δ (ppm) 15.70, 15.76, 37.1, 62.87, 62.93, 72.2, 102.3, 110.8, 111.3, 129.8, 152.3, 157.3 (Supplementary Figure S6). ^{31}P NMR (162 MHz): δ (ppm) −4.77. MS (ESI$^+$; CH_3OH/H_2O, 1:1 (v/v) + 5 mM NH_4OAc, γ ∼0.1 mg cm^{-3}): m/z = 380.7 (M^+), 381.7 (M + H^+). FTIR (cm^{-1}): 3296, 2960, 2925, 2873, 2853, 2357, 2332, 1736, 1612, 1589, 1502, 1487, 1458, 1365, 1309, 1285, 1262, 1227 (P═O), 1208, 1198, 1174, 1147, 1098, 1069, 1032 (P−O−C), 984 (P−O−C), 859, 851, 820, 801, 778, 668, 651. UV−vis (CH_3OH, c 1.20 × 10^{-5} mol dm^{-3}): λ_{max}/nm (ε/M^{-1} cm^{-1}) = 211 (34700), 279 (4200). Anal. Calcd for $C_{18}H_{21}O_7P$: C, 56.84; H, 5.57. Found: C, 56.90; H, 5.77.

*Diethyl (6-Hydroxy-3-oxo-3H-xanthen-9-yl)methyl Phosphate·DDQ Complex (**1a**·DDQ).* 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 23 mg, 0.1 mmol) was added to a solution of (3,6-dihydroxy-9*H*-xanthen-9-yl)methyl diethyl phosphate (**8a**, 31 mg, 0.08 mmol) in dry acetonitrile (2 mL) at 20 °C, and the mixture was stirred at this temperature for 20 min. The resulting red precipitate was filtered off, washed with acetonitrile (20 mL), and dried under reduced pressure to give the pure title complex. Yield: 25 mg (50%). Orange powder. Mp 170 °C (decomp). ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) 1.04 (t, 3H, J = 7.0 Hz), 1.17 (t, 3H, J = 7.0 Hz), 3.74 (m, 2H), 3.94 (m, 2H), 5.89 (d, 1H, J = 7.1 Hz), 6.47 (dd, 2H, J_1 = 3.8 Hz, J_2 = 2.4 Hz), 6.63 (dt, 2H, J_1 = 8.7 Hz, J_2 = 2.2 Hz), 7.53 (d, 1H, J = 8.6 Hz), 7.57 (d, 1H, J = 8.6 Hz), 9.66 (s, 2H, −OH) (Supplementary Figure S7). The peak at 5.89 (d, 1H, J = 7.1 Hz) is split to a doublet by the ^{31}P atom, which was proved by a phosphorus decoupled ^{1}H[^{31}P] NMR. ^{1}H NMR (300 MHz, phosphate buffer in D_2O, pH = 7.4, I = 0.1 M): δ (ppm) 0.99 (t, 3H, J = 6.9 Hz), 1.07 (t, 3H, J = 6.9 Hz), 3.79−3.94 (m, 4H), 6.55 (s, 2H), 6.90 (d, 2H, J = 9.2 Hz), 7.14 (d, 1H, J = 5.4 Hz), 7.84−8.85 (2 × bs, 2H) (Supplementary Figure S10). ^{13}C NMR (126 MHz, DMSO-d_6): δ (ppm) 15.4, 15.5, 15.6, 15.7, 63.6, 68.1, 68.2, 101.3, 101.5, 103.1, 103.2, 110.3, 110.6, 111.9, 112.7, 114.2, 128.7, 129.4, 134.0, 151.6, 151.8, 158.1 (Supplementary Figure S8) (the weak C_q signals from DDQ were not observed; the compound probably decomposes in DMSO). ^{13}C NMR (500 MHz, phosphate buffer in D_2O, pH = 7.4, I = 0.1 M): δ (ppm) 15.1, 15.2, 66.4, 96.9, 98.6, 114.9, 117.2, 132.3, 133.6, 139.5, 143.1, 158.7, 166.2 (Supplementary Figure S11). ^{31}P NMR (162 MHz, DMSO-d_6): δ (ppm) −2.92 (q, J = 6.4 Hz) (Supplementary Figure S9). FTIR (cm^{-1}): 3151, 2712, 2586, 2367, 2336, 1722 (C═O), 1599, 1561, 1552, 1480, 1463, 1424, 1413, 1384, 1361, 1323, 1266, 1240 (P═O), 1152, 1125, 1090, 1039 (P−O−C), 992, 954 (P−O−C), 925, 863, 793, 641. UV−vis (aq phosphate buffer, pH = 7.0, I = 0.1 M): λ_{max}/nm (ε/M^{-1} cm^{-1}) = 214 (37300), 245 (46600), 332 (6500), 528 (39100) (Supplementary Figure S13). HRMS (TOF ES$^+$): calcd for $C_{26}H_{20}Cl_2N_2O_9P$ (M + H^+): 605.0283 ($C_{26}H_{19}{}^{35}Cl^{35}ClN_2O_9P$ + H^+), 607.0254 ($C_{26}H_{19}{}^{35}Cl^{37}ClN_2O_9P$ + H^+). Found: 605.0283, 607.0272 (Supplementary Figure S14). Anal. Calcd for $C_{26}H_{19}Cl_2N_2O_9P$: C, 51.59; H, 3.16; N, 4.63. Found: C, 51.16; H, 3.48; N, 4.52.

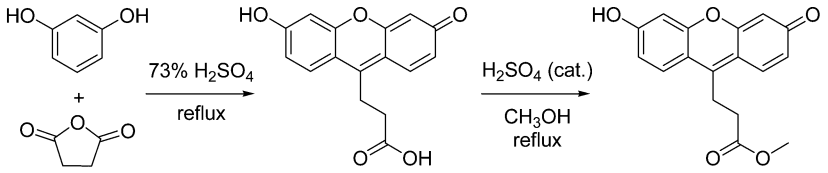
(6-Hydroxy-3-oxo-*3H*-xanthen-9-yl)methyl Acetate·DDQ Complex (1b·DDQ). *(3,6-Dimethoxy-9H-xanthen-9-yl)methyl Acetate (**7b**).* A mixture of (3,6-dimethoxy-9*H*-xanthen-9-yl)methanol (**6**, 0.336 g, 1.9 mmol), triethylamine (0.8 mL, 5.7 mmol), 4-dimethylaminopyridine (0.0179 g, 0.15 mmol), and acetic anhydride (0.7 mL, 7.4 mmol) was stirred at 20 °C for 14 h under an Ar atmosphere. The reaction mixture was shaken with diethyl ether (10 mL) and hydrochloric acid (2 M, 10 mL). The organic layer was washed twice with saturated aq $NaHCO_3$ (2 × 20 mL), dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure to give a slightly brownish oil which was purified by thin-layer chromatography ($CHCl_3$/ethyl acetate, 30:1, v/v) to obtain a pure title product. Yield: 0.38 g (98%). Slightly yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.90 (s, 3H), 3.76 (s, 6H), 4.06 (d, 2H, J = 5.9 Hz), 4.20 (t, 1H, J = 5.8 Hz), 6.67 (d, 2H, J = 2.5 Hz), 6.71 (dd, 2H, J_1 = 8.4 Hz, J_2 = 2.6 Hz), 7.26 (d, 2H, J = 8.5 Hz) (Supplementary Figure S15). ^{13}C NMR (126 MHz, DMSO-d_6): δ (ppm) 20.5, 36.1, 55.2, 68.9, 101.0, 109.9, 113.2, 129.9, 152.2, 159.2, 169.9 (Supplementary Figure S16). MALDI-MS: (negative mode): m/z = 313.358, 314.341. MS (FAB): m/z = 314.0 (M, 3.08), 315.1 (M + H^+, 9.56), 316.1 (M + 2H^+, 2.24), 317.0 (M + 3H^+, 0.67). FTIR (cm^{-1}): 3000, 2943, 2908, 2836, 2365, 1735 (C═O), 1693, 1650, 1631, 1612, 1599, 1573, 1499, 1462, 1436, 1425, 1377, 1358, 1327, 1286, 1250 (O−CH_2), 1224, 1195, 1160, 1120, 1100, 1028, 974, 926, 830, 806, 736, 716, 659, 645. UV−vis ($CHCl_3$): λ_{max}/nm (relative intensity) = 241 (100), 278 (67). Anal. Calcd for $C_{18}H_{18}O_5$: C 68.78; H 5.77; O 25.45. Found: C 68.66; H 5.96; O 25.38.

*(3,6-Dihydroxy-9H-xanthen-9-yl)methyl Acetate (**8b**).* This compound was synthesized from (3,6-dimethoxy-9*H*-xanthen-9-yl)methyl acetate (**7b**) by the same procedure as that used for (3,6-dihydroxy-9*H*-xanthen-9-yl)methyl diethyl phosphate (**8a**). Yield: 97%. Beige powder. Mp 150 °C (decomp). ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) 1.92 (s, 3H), 4.00 (d, 2H, J = 6.0 Hz), 4.07 (t, 1H, J = 5.9 Hz), 6.46 (d, 2H, J = 2.0 Hz), 6.52 (dd, 2H, J_1 = 8.2 Hz, J_2 = 2.1 Hz), 7.11 (d, 2H, J = 8.3 Hz), 9.56 (s, 2H, −OH) (Supplementary Figure S17). ^{13}C NMR (126 MHz, DMSO-d_6): δ (ppm) 20.4, 36.0, 68.9, 102.3, 110.7, 111.6, 129.7, 152.0, 157.1, 169.7 (Supplementary Figure S18). MS (ESI$^-$; CH_3OH/H_2O, 1:1 (v/v) + 5 mM NH_4OAc, γ ∼0.1 mg cm^{-3}): m/z = 285.0 (M − H^+, 100), 286.0 (M^-, 16). FTIR (cm^{-1}): 3353, 2359, 2341, 1739 (C═O), 1725, 1700, 1608, 1558, 1506, 1456, 1446, 1374, 1358, 1280, 1272, 1256, 1239, 1226, 1217, 1209, 1166, 1148, 1109, 1036, 989, 955, 840, 668, 660. UV−vis (C_2H_5OH, c 1.16 × 10^{-5} mol dm^{-3}): λ_{max}/nm (ε/M^{-1} cm^{-1}) = 211 (48400), 278 (4700). Anal. Calcd for $C_{16}H_{14}O_5$: C 67.13; H 4.93. Found: C 67.01; H 5.13.

*(6-Hydroxy-3-oxo-3H-xanthen-9-yl)methyl Acetate·DDQ Complex (**1b**·DDQ).* This compound was synthesized from (3,6-dihydroxy-9*H*-xanthen-9-yl)methyl acetate (**8b**) by the same procedure as that used for diethyl (6-hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl phosphate·DDQ complex (**1a**·DDQ). Yield: 44%. Dark brown powder. Mp 190 °C (decomp). ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) 1.94 (s, 3H), 6.24 (s, 1H), 6.47 (dd, 2H, J_1 = 4.8 Hz, J_2 = 2.3 Hz,), 6.63 (m, 2H), 7.57 (m, 2H), 9.67 (s, 2H, −OH) (Supplementary Figure S19). ^{13}C NMR (126 MHz, DMSO-d_6): δ (ppm) 20.2, 67.6, 98.7, 101.2, 109.8, 110.5, 112.9, 114.1, 128.6, 129.3, 151.4, 151.7, 157.9, 158.1, 169.4. FTIR (cm^{-1}): 2513, 2360, 2331, 2229, 2221 (C≡N), 1755 (C═O), 1643, 1593, 1556, 1446, 1402, 1272, 1253, 1199, 1176, 1114, 1068, 1041, 991, 946, 927, 839, 818, 650, 613, 590 (Supplementary Figure S21). UV−vis (aq phosphate buffer, pH = 7.0, I = 0.1 M): λ_{max}/nm (ε/M^{-1} cm^{-1}) = 244 (53200), 328 (7300), 375 (11200), 495 (35700), 524 (40900) (Supplementary Figure S20). HRMS (TOF MS ESI$^-$): calcd for $C_{24}H_{11}Cl_2N_2O_7$ (M −H^+) 508.9943

dx.doi.org/10.1021/jo301455n | J. Org. Chem. 2013, 78, 1833−1843

Scheme 4. Synthesis of Succinylfluorescein 9 and Its Methyl Ester 10



9 10

($C_{24}H_{12}{}^{35}Cl^{35}ClN_2O_7$ − H^+), 510.9914 ($C_{24}H_{12}{}^{35}Cl^{37}ClN_2O_7$ − H^+), found 508.9943, 510.9951 (Supplementary Figure S22).

9-(Bromomethyl)-6-hydroxy-3*H*-xanthen-3-one·DDQ Complex (1c·DDQ). *9-(Bromomethyl)-3,6-dimethoxy-9H-xanthene (**7c**).* Triphenylphosphine (0.59 g, 2.25 mmol) and carbon tetrabromide (1.63 g, 4.92 mmol) were added to a solution of **6** (0.51 g, 1.86 mmol) in dichloromethane (12 mL). The reaction mixture was stirred under Ar atmosphere at 20 °C for 10 h, and subsequently washed with aq $NaHCO_3$ (2 × 20 mL, satd) and brine (2 × 20 mL). The organic layer was dried over Na_2SO_4 and filtered, and the solvent was removed under reduced pressure. The residue was purified by column chromatography ($CHCl_3$/MeOH, 20:1, v/v). Yield: 0.61 g (97%). Yellow greenish solid. Mp 134.3−135.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 3.75 (s, 6H), 3.78 (d, 2H, J = 4.1 Hz), 4.49 (t, 1H, J = 4.0 Hz), 6.63 (d, 1H, J = 2.6 Hz), 6.70 (dd, 2H, J_1 = 8.5 Hz, J_2 = 2.6 Hz), 7.31 (d, 2H, J = 8.6 Hz) (Supplementary Figure S23). ^{13}C NMR (126 MHz, DMSO-d_6): δ (ppm) 37.4, 44.3, 55.2, 100.7, 109.9, 113.7, 129.7, 152.1, 159.2 (Supplementary Figure S24). MS (FAB): m/z = 334.0 ($C_{16}H_{15}{}^{79}BrO_3$, 22), 334.9 ($C_{16}H_{15}{}^{79}BrO_3$ + H^+, 100), 335.9 ($C_{16}H_{15}{}^{81}BrO_3$, 31), 336.9 ($C_{16}H_{15}{}^{81}BrO_3$ + H^+, 68). FTIR (cm^{-1}): 2359, 2336, 1656, 1631, 1604, 1566, 1551, 1502, 1469, 1462, 1437, 1422, 1327, 1291, 1257, 1201, 1185, 1168, 1149, 1097, 1029, 980, 845, 830, 821, 806, 800, 788, 668 (C−Br), 626, 618. UV−vis ($CHCl_3$): λ_{max}/nm (relative intensity) = 241 (100), 278 (59). Anal. Calcd for $C_{16}H_{15}BrO_3$: C, 57.33; H, 4.51. Found: C, 57.37; H, 4.75.

*9-(Bromomethyl)-9H-xanthene-3,6-diol (**8c**).* This compound was synthesized from **7c** according to the same procedure as that used for (3,6-dihydroxy-9*H*-xanthen-9-yl)methyl diethyl phosphate (**8a**). Yield: 99%. Ochre powder. Mp 120 °C (decomp). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 3.70 (d, 2H, J = 4.3 Hz), 4.34 (t, 1H, J = 4.2 Hz), 6.42 (d, 1H, J = 2.4 Hz), 6.51 (dd, 2H, J_1 = 8.3 Hz, J_2 = 2.4 Hz,) 7.16 (d, 2H, J = 8.4 Hz), 9.57 (s, 2H, −OH) (Supplementary Figure S25). ^{13}C NMR (126 MHz, DMSO-d_6): δ (ppm) 37.6, 44.3, 102.2, 110.9, 112.3, 129.6, 152.1, 157.3 (Supplementary Figure S26). MALDI-MS (positive mode): m/z = 305.073, 307.084. MS (ESI^-; CH_3OH/H_2O, 1:1 (v/v) + 5 mM NH_4OAc, γ ∼0.1 mg cm^{-3}): m/z = 305.0 ($C_{14}H_{11}{}^{79}BrO_3$ − H^+, 30), 306.0 ($C_{14}H_{11}{}^{79}BrO_3{}^-$, 6.0), 307.0 ($C_{14}H_{11}{}^{81}BrO_3$ − H^+, 32.6), 308.0 ($C_{14}H_{11}{}^{81}BrO_3{}^-$, 5.5). FTIR ($cm^{-1}$): 3348, 3217, 2360, 2341, 1612, 1589, 1504, 1450, 1296, 1265 (CH_2), 1172, 1095, 987, 933, 840, 756, 648 (C−Br). UV−vis (C_2H_5OH, c 1.22 × 10^{-5} mol dm^{-3}): λ_{max}/nm (ε/M^{-1} cm^{-1}) = 203 (54800), 211 (54500), 279 (14000). Anal. Calcd for $C_{14}H_{11}BrO_3$: C, 54.75; H, 3.62. Found: C, 54.84; H, 3.99.

*9-(Bromomethyl)-6-hydroxy-3H-xanthen-3-one·DDQ Complex (**1c**·DDQ).* This compound was synthesized from 9-(bromomethyl)-9*H*-xanthene-3,6-diol (**8c**) by the procedure used for the preparation of diethyl (6-hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl phosphate·DDQ complex (**1a**·DDQ). Yield: 55%. Crimson powder. Mp >105 °C (decomp). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 6.53 (d, 1H, J = 2.4 Hz), 6.60 (m, 2H, J_1 = 2.4 Hz, J_2 = 2.4 Hz, J_3 = 2.3 Hz), 6.67 (s, 1H), 6.68 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.5 Hz), 7.51 (d, 1H, J = 8.7 Hz), 8.32 (d, 1H, J = 8.8 Hz), 9.97 (bs, 1H), 10.12 (bs, 1H) (Supplementary Figure S27). ^{13}C NMR (126 MHz, DMSO-d_6): δ (ppm) 94.2, 102.3, 102.0, 110.4, 112.0, 113.8, 113.9, 124.8, 127.8 128.1, 150.5, 152.6, 158.6, 158.9 (Supplementary Figure S28). FTIR (cm^{-1}): 2970, 2359, 2334, 1736 (C═O), 1364, 1601, 1575, 1568, 1456, 1418, 1404, 1318, 1267, 1209, 1188, 1129, 1118, 1083, 1048, 931, 848, 826, 815, 771, 764, 668, 653 (C−Br), 633. UV−vis (aq phosphate buffer, pH = 7.0, I = 0.1 M): λ_{max}/nm (ε/M^{-1} cm^{-1}) = 243 (44300), 329 (5200), 517 (44900) (Supplementary Figure S29). HRMS (TOF MS ESI^-): calcd for $C_{22}H_8BrCl_2N_2O_5$ (M − H^+) 528.8999 ($C_{22}H_9{}^{79}Br^{35}Cl^{35}ClN_2O_5$ − H^+), 530.8979 ($C_{22}H_9{}^{81}Br^{35}Cl^{35}ClN_2O_5$ − H^+), found 528.8995, 530.8986 (Supplementary Figures S30 and S31).

6-Hydroxy-3-oxo-3*H*-xanthene-9-propanoic Acid (Succinylfluorescein, 9; Scheme 4). A stirred mixture of succinic acid anhydride (2.50 g, 25 mmol) and resorcinol (2.75 g, 25 mmol) in aq sulfuric acid (30 mL, 73% (v/v)) was heated to 140 °C for 6 h. The reaction mixture was cooled to 20 °C and poured into water (500 mL). The stirred solution was alkalinized with aq NaOH (50%) to pH = 13, while the temperature was kept at ∼20 °C. Acetic acid was added to the solution until pH = 4 was obtained, and the brown precipitate was filtered. The filtrate was washed with water (3 × 25 mL), dried under reduced pressure, washed with hot 1,4-dioxane (15 mL) and hot methanol (15 mL), and dried under reduced pressure to give pure **9**. Yield: 5.7 g (80%). Dark brown solid. Mp 300 °C (decomp) (lit. 155−160 °C (decomp)).[10] ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 3.40 (d, 2H, J = 7.2 Hz), 5.81 (t, 1H, J = 7.3 Hz), 6.50 (d, 1H, J = 2.3 Hz), 6.56 (d, 1H, J = 2.3 Hz), 6.62 (dt, 2H, J_1 = 8.6 Hz, J_2 = 2.3 Hz), 7.43 (dd, 2H, J_1 = 8.6 Hz, J_2 = 2.0 Hz), 9.74 (s, 1H, −OH), 9.89 (s, 1H, −OH), 12.36 (bs, 1H, −COOH) (Supplementary Supplementary Figure S32). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm) 34.6, 66.2, 102.2, 102.6, 110.8, 111.8, 111.9, 112.7, 115.8, 124.3, 126.5, 128.3, 151.2, 152.9, 157.9, 158.1, 172.9 (Supplementary Figure S33). MS (EI^+, 70 eV): m/z = 284 (35), 255 (1), 239 (100), 229 (50), 223 (7), 213 (10), 200 (3), 181 (5), 165 (6), 152 (11), 137 (7), 115 (9). FTIR (KBr, cm^{-1}) = 3450 (br), 3053, 2968, 1745, 1633, 1599, 1463, 1391, 1329, 1250, 1202, 1159, 1116, 1037, 846. UV−vis (aq phosphate buffer, pH = 7.0, I = 0.1 M), (c 1.00 × 10^{-5} mol dm^{-3}): λ_{max}/nm (ε/M^{-1} cm^{-1}) = 238 (53800), 486 (95000) (Supplementary Figure S34). HRMS (ESI^+): calcd for $C_{16}H_{13}O_5$ (M + H^+) 285.0757. Found: 285.0758. This compound has also been characterized elsewhere.[10]

Methyl 3-(6-Hydroxy-3-oxo-3*H*-xanthene-9-yl)-propanoate (10). A stirred mixture of succinylfluorescein (**9**, 1.00 g, 4.0 mmol) and H_2SO_4 (0.15 mL, 95% (v/v)) in

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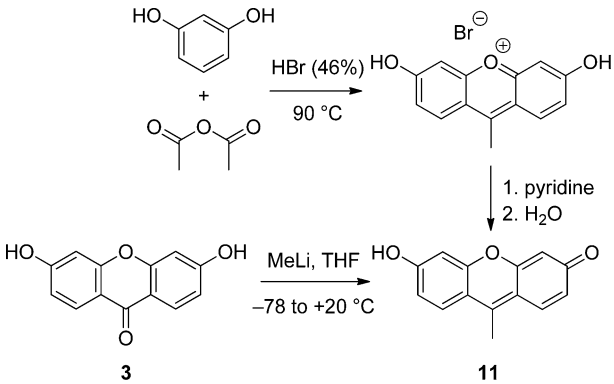
MeOH (300 mL) was heated to 65 °C for 7 days. The reaction mixture was then cooled to 20 °C. The stirred solution was neutralized to pH = 6 with aq Na_2CO_3 (satd). A reddish precipitate was formed, filtered, and purified by column chromatography ($CHCl_3$/MeOH, 9:1, v/v). The purified product was redissolved in cold aq NaOH (0.2 M) keeping pH = 9 and then was reprecipitated by adjusting pH to 4 with aq HCl (0.2 M). The precipitate was filtered, washed with water (3 × 15 mL), and dried under reduced pressure to give a red solid. Yield: 150 mg (13%). Mp 187−189 °C (decomp) (lit. 187−190 °C (decomp)[10]). Note: A product of methanolysis was also identified simultaneously in a freshly prepared solution in methanol (Supplementary Figures S35 and S36); a tautomeric form containing an exocyclic double bond was then formed overnight, and it was the only form present in DMSO-d_6 (Supplementary Figures S37 and S38)). ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) 1.81 (t, 2H, J = 7.9 Hz), 2.35 (t, 2H, J = 7.7 Hz), 2.76 (t, 0.75H, J = 7.8 Hz), 3.43 (s, 3H), 3.64 (t, 0.75H, J = 7.9 Hz), 3.66 (s, 1.13H), 6.50 (d, 2H, J = 1.8 Hz), 6.64 (d, 0.75H, J = 1.7 Hz) 6.66 (d, 2H, J = 2.0 Hz), 6.87 (d, 1H, J = 8.6 Hz), 7.30 (d, 2H, J = 8.5 Hz), 7.99 (d, 1H, J = 9.3 Hz) (Supplementary Figure S35). ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 3.51 (d, 2H, J = 7.2 Hz), 3.65 (s, 3H), 5.79 (t, 1H, J = 7.2 Hz), 6.50 (d, 1H, J = 2.2 Hz), 6.56 (d, 1H, J = 2.2 Hz), 6.58−6.65 (m, 2H), 7.41 (d, 1H, J = 8.5 Hz), 7.44 (d, 1H, J = 8.6 Hz), 9.76 (s, 1H, −OH), 9.90 (s, 1H, −OH) (Supplementary Figure S37). ^{13}C NMR (126 MHz, CD_3OD): δ (ppm) 23.8, 30.8, 35.5, 41.7, 52.1, 52.5, 76.0, 103.1, 104.4, 113.0, 113.2, 129.1, 130.3, 154.7, 156.1, 159.7, 173.7, 175.2 (Supplementary Figure S36). ^{13}C NMR (126 MHz, DMSO-d_6): δ (ppm) 34.3, 51.6, 102.2, 102.6, 110.8, 110.9, 111.9, 112.6, 115.6, 124.4, 127.0, 128.3, 151.2, 152.9, 157.9, 158.2, 171.9 (Supplementary Figure S38). MS (EI$^+$, 70 eV): m/z = 298 (38), 299 (8), 240 (17), 239 (100), 238 (6), 237 (8), 213 (14). FTIR (KBr, cm^{-1}) = 3045 (br), 2949, 1737, 1641, 1596, 1459, 1395, 1330, 1274, 1207, 1116, 847. UV−vis (aq phosphate buffer, pH = 7.0, I = 0.1 M/CH_3OH, 1:1, (v/v)), (c 1.00 × 10^{-5} mol dm^{-3}): λ_{max}/nm (ε/M^{-1} cm^{-1}) = 241 (43500), 498 (77000) (Supplementary Figure S39). HRMS (ESI$^+$): calcd for $C_{17}H_{14}O_5$ (M + H$^+$) 299.0914. Found: 299.0915. This compound has also been characterized elsewhere.[10]

6-Hydroxy-9-methyl-3*H*-xanthen-3-one (11; Scheme 5). Method A. Hydrobromic acid (6.7 mL, aq, 46%, 57 mmol) was added dropwise to stirred acetic anhydride (28 mL, 294 mmol) at 0 °C over a period of 20 min. Resorcinol (11.0 g, 100 mmol) was then added to the reaction mixture in one portion.

Scheme 5. Synthesis of 6-Hydroxy-9-methyl-3*H*-xanthen-3-one 11



The solution was heated to 90 °C (the reaction mixture became red and a red precipitate appeared). The reaction mixture was then cooled to 0 °C and filtered. The solid precipitate was washed with glacial acetic acid (3 × 5 mL) and ice-cold propan-2-ol (5 mL), and the remaining solid was dried under vacuum over phosphorus pentoxide to an afford orange solid of 3,6-dihydroxy-9-methylxanthenium bromide. Yield 4.86 g (32%). Mp >170 °C (decomp). ^{1}H NMR (600 MHz, CF_3COOD): δ (ppm) 3.25 (s, 3H) 7.42 (d, 2H, J = 2.2 Hz), 7.45 (dd, 2H, J_1 = 9.3 Hz, J_2 = 2.2 Hz), 8.40 (d, 2H, J = 9.3 Hz). ^{13}C NMR (126 MHz, CF_3COOD): δ (ppm) 16.7, 105.0, 119.8, 122.4, 132.8, 161.4, 170.6, 171.9. MS (EI): 226.2 (100), 227.2 (14) 228.2 (2), 229.2 (0.1). FTIR (KBr, cm^{-1}) = 2916, 2721, 2582, 2359, 1721, 1632, 1599, 1551, 1530, 1474, 1469, 1461, 1410, 1356, 1310, 1269, 1228, 1206, 1168, 1124, 866, 838, 821, 702, 648. UV−vis (1 M aq HCl): λ_{max}/nm (relative intensity) = 205 (46), 226 (69), 249 (51), 291 (8), 426 (100). Anal. Calcd for $C_{14}H_{11}BrO_3$: C, 54.75; H, 3.61. Found: C, 54.49; H, 3.62. All obtained 3,6-dihydroxy-9-methylxanthenium bromide (4.52 g, 1.47 mmol) was suspended in dry pyridine (80 mL) by sonication and left at 20 °C for an additional 90 min. Water (400 mL) was then added to the slowly stirred solution, and a precipitate was formed. The precipitate was filtered, washed with water (5 × 50 mL), and dried under vacuum over phosphorus pentoxide to a crimson solid. Yield: 3.08 g (93%).

Method B. MeLi (4.80 mL, 1.6 M solution in hexane, 7.7 mmol) was added dropwise to a stirred solution of 3,6-dihydroxy-9*H*-xanthen-9-one (**3**, 0.50 g, 2.2 mmol) in dry THF (100 mL) at −78 °C under inert atmosphere. After 30 min of stirring at −78 °C another portion of MeLi (1.37 mL, 1.6 M solution in hexane, 2.20 mmol) was added dropwise. The reaction mixture was stirred for an additional 45 min at −78 °C, allowed to slowly warm up to 20 °C (in 30 min) and then was stirred at 20 °C for 1 h. The solvents were removed under reduced pressure. The solid residue was dissolved in dichloromethane (20 mL), washed with acidic water (10 mL, pH = 3) and brine (10 mL), dried over $MgSO_4$, and filtered, and the solvent was removed under reduced pressure to give a deep red solid. Yield: 451 mg (91%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 5.25 (s, 2H), 6.49 (d, 2H, J = 2.4 Hz), 6.60 (dd, 2H, J_1 = 8.7 Hz, J_2 = 2.4 Hz), 7.65 (d, 2H, J = 8.7 Hz), 9.92 (s, 2H, −OH) (Supplementary Figure S40). ^{13}C NMR (126 MHz, DMSO-d_6): δ (ppm) 95.3, 102.3, 112.0, 112.1, 125.2, 131.0, 150.8, 158.1 (Supplementary Figure S41). MS (ESI$^-$; CH_3OH + 5 mM NH_3, γ ~0.1 mg cm^{-3}): m/z = 223.5 (M − H$^+$, 100), 226.3 (M$^-$, 15.8), 450.8 (M$_2$ − H$^+$, 35.2), 451.9 (M$_2^-$, 9.9). MS (EI$^+$, 70 eV, m/z, %): 226 (100), 211 (25). FTIR (KBr, cm^{-1}) = 3257, 2359, 2336, 1558, 1447, 1387, 1315, 1268, 1251, 1198, 1178, 1109, 1040, 927, 839, 814, 806, 778, 654. UV−vis (0.1 M aq NaOH): λ_{max}/nm (relative intensity) = 238 (60), 279 (14), 312 (9), 481 (100). UV−vis (C_2H_5OH), (c 1.55 × 10^{-5} mol dm^{-3}): λ_{max}/nm (ε/M^{-1} cm^{-1}) = 211 (25456), 232 (30733), 274 (9570), 497 (26312). HRMS (ESI$^+$): calcd for $C_{14}H_{11}O_3$ (M + H$^+$) 227.0703. Found: 227.0694. HRMS (ESI$^-$): calcd for $C_{14}H_9O_3$ (M − H$^+$) 225.0557. Found: 225.0556.

2,3-Dichloro-5-cyano-6-hydroxy-1,4-benzoquinone (12). Water (5 mL) was added to a solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 15 mg) in methanol (5 mL) in one portion at 20 °C; the solution changed color from yellow to red while released HCN was detected by the corresponding analytic techniques or sensorically. Methanol was removed under reduced pressure, and remaining water was lyophilized to give a yellow powder. No further purification was

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performed. Yield: 13 mg (90%). Mp 196−199 °C. ^{13}C NMR (75.5 MHz, 0.1 M D_2O phosphate buffer with 5% CD_3CN (v/v)): δ (ppm) 89.1, 117.5, 137.7, 144.9, 173.3, 175.7, 175.8 (Supplementary Figure S42). FTIR (KBr, cm^{-1}) = 3009, 2970, 2950, 2227, 1739, 1698, 1589, 1577, 1369, 1219, 1109, 889, 822, 746, 600, 528. HRMS (ESI$^-$): calcd for $C_7Cl_2NO_3$ ($M - H^+$) 215.9261 ($C_7{}^{35}Cl^{35}ClNO_3$), 217.9231 ($C_7{}^{37}Cl^{35}ClNO_3$). Found: 215.9258, 217.9229.

Photochemical Experiments. *Irradiation in UV Cuvettes (General Procedure).* Solutions of **1a−c**·DDQ ($c \sim 1 \times 10^{-5}$ mol dm^{-3}) in 0.1 M phosphate buffer (4 mL, pH = 7.0) were irradiated with a medium pressure 40-W mercury lamp through a band-pass optical filter (λ_{irr} = 546 nm). The progress of the reaction was monitored using by UV−vis spectrometry.

Irradiation in NMR Tubes (General Procedure). Small amounts of **1a−c**·DDQ (2−3 mg) were dissolved in 0.1 M phosphate buffer (D_2O based, pH = 7.4) or methanol-d_4 or methanol-d_4/water-d_2 1:1 (v/v) mixture (500 μL) in an NMR tube. The solutions were irradiated with the same irradiation source as described above. The reaction progress was monitored by ^{1}H NMR and ^{31}P NMR.

Characterization of the Photoproducts. Diethyl phosphoric acid, acetic acid, and 3,6-dihydroxy-9*H*-xanthen-9-one (**3**) were characterized by their ^{1}H, ^{13}C, and ^{31}P (when applicable) NMR, MS and/or HRMS (for photoproducts from **1a** only), and comparison of the data to those of the authentic samples.

6-Hydroxy-3-oxo-3*H*-xanthene-9-carboxylic Acid (13). Compound **13** was obtained upon irradiation of **1a** in aqueous phosphate buffer. A ~10 mmol dm^{-3} solution of **1a**·DDQ in 0.1 M phosphate buffer (D_2O, pH = 7.4) was irradiated in an NMR tube. Part (40%) of the starting material precipitated and was filtered off. The filtrate was acidified with aq CF_3COOH to form a red precipitate, which was filtered, washed with aq CF_3COOH (3 × 1 mL), and dried. Yield: 50% (calculated on the basis of the starting material consumed). Dark red powder. ^{1}H NMR (300 MHz, 0.1 M phosphate buffer in D_2O, pH = 7.4): δ (ppm) 6.67 (d, 1H, J = 2.0 Hz), 6.84 (dd, 2H, J_1 = 9.0 Hz, J_2 = 2.0 Hz), 7.63 (d, 2H, J = 9.0 Hz) (Supplementary Figure S51). ^{1}H NMR (300 MHz, CD_3OD/CD_3CN, 1:1, v/v): 6.56 (d, 2H, J = 2.6 Hz), 6.64 (dd, 2H, J_1 = 8.6 Hz, J_2 = 2.6 Hz), 7.23 (d, 2H, J = 8.6 Hz). ^{13}C NMR (126 MHz, 0.1 M phosphate buffer in D_2O, pH = 7.4): δ (ppm) 104.2, 108.5, 123.6, 131.1, 154.0, 159.7, 171.9 (−COOH), 180.5 (C_{ar}=O) (Supplementary Figure S52). HRMS (TOF MS ES$^+$): calcd for $C_{14}H_9O_5$ ($M + H^+$) 257.0444, found 257.0452 (Figure S53). UV−vis (CH_3OH): λ_{max}/nm (relative intensity) = 491 (100), 321 (16), 242 (101); UV−vis (0.1 M aq phosphate buffer, pH = 7.4): λ_{max}/nm (relative intensity) = 489 (100), 318 (11), 241 (81) (Supplementary Figure S50). According to claims in the literature, this compound has also been prepared before.[31,42] These procedures were reproduced to give solid samples with the described characteristics (color and appearance), but they were found to be complex mixtures.

Characterization of the 9·DDQ and 10·DDQ Complexes. Succinylfluorescein (**9**) or methyl 3-(6-hydroxy-3-oxo-3*H*-xanthene-9-yl)propanoate (**10**) was mixed with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in methanol in the 1:1 molar ratio while keeping the concentration of **9**/**10** equal to 1.0×10^{-5} mol dm^{-3}. The solutions were left for 2 h at 20 °C, and the solvent was evaporated to yield a dark brown solid that was analyzed by HRMS.

9·DDQ. HRMS (ESI$^-$): calcd for $C_{24}H_{11}Cl_2N_2O_7$ 508.9943 ($C_{24}H_{12}{}^{35}Cl^{35}ClN_2O_7 - H^+$), 510.9914 ($C_{24}H_{12}{}^{35}Cl^{37}ClN_2O_7 - H^+$). Found 508.9945, 510.9913 (Supplementary Figure S43).

10·DDQ$_2$. HRMS (ESI$^-$): calcd for $C_{25}H_{13}Cl_2N_2O_7$ 523.0100 ($C_{25}H_{14}{}^{35}Cl^{35}ClN_2O_7 - H^+$), 525.0070 ($C_{25}H_{14}{}^{35}Cl^{37}ClN_2O_7 - H^+$). Found 523.0106, 525.0087 (Supplementary Figure S44).

■ ASSOCIATED CONTENT

Supporting Information

UV, NMR, IR and HRMS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Support for this work was provided by the Swiss National Science Foundation, the Grant Agency of the Czech Republic (GA203/09/0748), and the project CETOCOEN (CZ.1.05/2.1.00/01.0001) granted by the European Regional Development Fund (P.S., P.K.). The authors express their thanks to Jaromír František, Blanka Vrbková, Lubomír Prokeš, Michal Čajan, Otakar Humpa, Daniel Häussinger, Heinz Nadig, and Robert Vícha for their help with the mass spectrometry, NMR measurements, and elemental analyses. We thank Prof. Reinhard Schmidt for providing us the mDPH actinometer and Miloš Černík, Radek Marek, Lukáš Maier, Jan Taimr, and Kamil Paruch for fruitful discussions.

■ DEDICATION

This paper is dedicated to the memory of Professor Howard E. Zimmerman, University of Wisconsin, Madison (July 5, 1926 − February 11, 2012).

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