Bhc-diol as a Photolabile Protecting Group for Aldehydes and Ketones

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All reagents and solvents were purchased from commercial sources and used without further purification with the following exceptions. Toluene was dried by passing it through activated alumina under nitrogen pressure (Solv-Tek, Berryville, VA). Pyridine and acetonitrile were refluxed with calcium hydride under nitrogen, and then distilled. ^1H NMR and ^{13}C NMR spectra were recorded on a Varian MercuryPlus 400 MHz spectrometer. FTIR spectra were recorded on a Bruker Vector 22 spectrophotometer. UV spectra were recorded on a Cary 300 Bio UV-Visible spectrophotometer (Varian). HPLC analysis (analytical and preparative) was performed on a Varian ProStar HPLC system with an autosampler and diode array detector using Microsorb C-18 reverse phase columns. Mass spectrometry was performed on a Sciex API-1 Plus quadrupole mass spectrometer with an electrospray ionization source. KMOPS buffer consisted of 100 mM KCl and 10 mM MOPS titrated to pH 7.2 with KOH. Thin layer and column chromatography were performed on precoated silica gel 60 F₂₅₄ plates (EM Science or Sorbent Technologies) and 230-400 mesh silica gel 60 (EM Science), respectively. Melting points were determined on a Mel-Temp (Laboratory Devices, Inc.), and are uncorrected.

6-Bromo-4-chloromethyl-7-acetyloxycoumarin (11). Under a nitrogen atmosphere, 6-bromo-4-chloromethyl-7-hydroxycoumarin (**10**, 780 mg, 2.69 mmol) and acetic anhydride (0.64 mL, 6.8 mmol) were stirred in anhydrous pyridine (3 mL) for 4 h. The pyridine was removed under vacuum, and the resulting crude product was purified by flash chromatography through silica gel (hexanes/EtOAc, 1:1) to afford **11** as a white solid (760 mg, 85%, mp 180 °C dec). ^1H NMR (CDCl_3) δ 7.91 (1H, s), 7.21 (1H, s), 6.58 (1H, s), 4.63 (2H, s), 2.41 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) 168.06, 159.45, 153.68, 150.99, 148.31, 128.61, 116.96, 116.59, 113.30, 112.40, 41.19, 21.03; FTIR (neat) 1732, 1599, 1396, 1217, 1181, 1137, 1022, 906, 862, 728, 658, 629 cm^{-1} .

Phosphonium salt 12. Under a nitrogen atmosphere, a mixture of 6-bromo-4-chloromethyl-7-acetyloxycoumarin (**11**, 650 mg, 1.96 mmol) and triphenyl phosphine (1.028 g, 3.92 mmol) in acetonitrile was heated to 85 °C. After 30 min, the suspended mixture dissolved. Heating was continued for 18 h, during which time the white phosphonium salt precipitated. The mixture was cooled, filtered, and the filtrand was washed several times with boiling benzene to yield phosphonium salt **12** as a white solid (948 mg, 82%), which was carried to the next step without further purification.

6-Bromo-4-(1,2-ethylene)-7-hydroxycoumarin (Bhc-ethylene, 13). Phosphonium salt **12** (310 mg, 0.522 mmol) was taken up in a 37% formaldehyde solution (4 mL, aqueous). The mixture was stirred for 15 min, and then an aqueous solution of 15% Na_2CO_3 (0.5 mL) was added intermittently by a syringe. Each subsequent addition was made after the orange-yellow color of the phosphorone formed had disappeared. When the addition of the base was complete, the mixture was stirred at room temperature for 2 h, and then extracted with CHCl_3 (3 $\times$). The

combined CHCl_3 extracts were dried over anhydrous sodium sulfate and evaporated. The crude product was purified by flash chromatography through silica gel (hexanes/EtOAc, 6:4) to afford **13** as a white solid (100 mg, 72%, mp 210-230 °C dec). ^1H NMR ($(\text{CD}_3)_2\text{CO}$) δ 8.00 (1H, s), 7.21 (1H, dd, J = 17.2, 11.6 Hz), 6.94 (1H, s), 6.35 (1H, s), 6.14 (1H, dd, J = 17.2, 1.2 Hz), 5.77 (1H, dd, J = 11.2, 0.8 Hz); ^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{CO}$) 160.08, 157.31, 154.88, 150.18, 130.47, 129.19, 123.31, 112.80, 108.38, 106.08, 103.87; FTIR (neat) 3392, 1700, 1607, 1412, 1365, 1310, 1268, 1223, 1155, 954 cm^{-1} ; MS m/z 267 (MH^+ , ^{81}Br), 265 (MH^+ , ^{79}Br).

6-Bromo-4-(1,2-dihydroxyethyl)-7-hydroxycoumarin (Bhc-diol, 14). Bhc-ethylene (**13**, 200 mg, 0.749 mmol) and one small piece of osmium tetroxide (which was not weighed because of its toxicity and the difficulty in accurately weighing it, due to rapid sublimation) were added to a solution of 4-methylmorpholine *N*-oxide monohydrate (101 mg, 0.747 mmol) in water (4 mL) and acetone (2 mL). The resulting solution was vigorously stirred at room temperature for 18 h, during which time the mixture turned a light brown color. The reaction was neutralized to pH 7 with 3 N sulfuric acid. The acetone was evaporated, and the resulting aqueous solution was extracted with EtOAc (5 $\times$). The combined organic extracts were evaporated, and the resulting crude product was purified by flash chromatography through silica gel (hexanes/EtOAc, 2:8) to afford **14** as a white solid (180 mg, 80%, mp 200-220 °C dec). ^1H NMR ($(\text{CD}_3)_2\text{CO}$) δ 8.05 (1H, s), 6.92 (1H, s), 6.41 (1H, s), 5.13 (1H, dd, J = 4.4, 4.4 Hz), 3.88 (1H, dd, J = 12.0, 4.4 Hz), 3.71 (1H, dd, J = 11.2, 5.6 Hz); ^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{CO}$) 160.78, 157.55, 156.15, 155.57, 129.94, 113.09, 111.09, 106.56, 104.44, 71.42, 67.03; FTIR (neat) 3332, 1726, 1604, 1437, 1158, 1120, 882, 773, 695, 540 cm^{-1} ; MS m/z 303 (MH^+ , ^{81}Br), 301 (MH^+ , ^{79}Br).

General Procedure for the Synthesis of 6-Bromo-7-Hydroxycoumarin Acetals and Ketals.

Under a nitrogen atmosphere, aldehyde or ketone **15** (2 equiv.) in 10 μL of 1-butanol was added to a mixture of Bhc-diol (**14**, 1 equiv.), pyridinium *p*-toluenesulfonate (PPTS, 1 equiv), and anhydrous MgSO_4 (100 mg) in anhydrous toluene (2 mL). The reaction mixture was stirred at 110 °C for 8 h, and then filtered, washing the solid filtrand with CHCl_3 . The filtrate was evaporated, and the resulting crude product was purified by flash chromatography through silica gel (EtOAc/hexanes, 1:1) to give the Bhc-protected aldehyde or ketone **16**. With the exception of **16d**, all Bhc-diol protected aldehydes and ketones were isolated as a mixture of diastereomers.

Bhc-diol-benzaldehyde (16a). Bhc-diol (15 mg, 0.05 mmol), PPTS (13 mg, 0.05 mmol), benzaldehyde (**15a**, 10 μL , 0.10 mmol); yield = 11 mg (57%, mp 106-180 °C dec). ^1H NMR (CDCl_3) δ 7.50 (6H, m), [7.07 (s), 7.06 (s) (1H)], [6.67 (s), 6.55 (s) (1H)], [6.14 (s), 6.02 (s) (1H)], 5.40 (1H, m), [4.73 (dd, J = 7.6, 7.6 Hz), 4.57 (dd, J = 8.4, 8.4 Hz) (1H)], [4.09 (dd, J = 8.4, 5.6 Hz), 3.86 (dd, J = 7.2, 7.2 Hz) (1H)]; ^{13}C NMR (100 MHz, CDCl_3) (160.44, 160.33), (155.37, 155.27), (154.91, 154.85), (152.14, 151.85), 136.57, 135.58, (129.96, 129.76), (128.68, 128.62), 126.67, (126.54, 126.44), (112.24, 112.15), 110.70, 109.60, 106.68, 105.18, (104.74, 104.67), (73.80, 73.30), (70.62, 70.23); FTIR (neat) 3407, 2922, 1698, 1602, 1396, 1308, 1271, 1222, 1153, 1095, 1021, 873, 756, 699 cm^{-1} ; MS m/z 391 (MH^+ , ^{81}Br), 389 (MH^+ , ^{79}Br).

Bhc-diol-piperonal (16b). Bhc-diol (15 mg, 0.05 mmol), PPTS (13 mg, 0.05 mmol), piperonal (**15b**, 15 mg, 0.10 mmol); yield = 6.0 mg (28%, mp 176-195 °C dec). ^1H NMR (CDCl_3) δ [7.58 (s), 7.50 (s) (1H)], 7.06 (3H, m), [6.87 (s), 6.85 (s) (1H)], [6.64 (s), 6.54 (s) (1H)], 6.02 (2H, m), [6.02 (s), 5.92 (s) (1H)], [4.40 (dd, J = 6.8, 6.8 Hz), 5.35 (dd, J = 6.8, 5.6 Hz) (1H)], [4.74 (dd, J

= 7.6, 7.6 Hz), 4.54 (dd, $J = 8.0, 8.0$ Hz) (1H)], [4.07 (dd, $J = 7.6, 5.2$ Hz), 3.82 (dd, $J = 7.6, 7.6$ Hz) (1H)]; ^{13}C NMR (100 MHz, CDCl_3) (160.38, 160.27), (155.34, 155.25), (154.96, 154.90), (152.10, 151.77), (148.97, 148.84), 148.01, (130.44, 129.46), (126.70, 126.55), (120.96, 120.76), (112.28, 112.20), (110.74, 109.61), (108.29, 108.22), 106.92, (106.72, 106.69), (105.05, 104.65), (104.76, 104.70), (101.40, 101.37), (73.76, 73.28), (70.62, 70.12); FTIR (neat) 3423, 2960, 2923, 1722, 1605, 1400, 1260, 1094, 1029, 869, 801 cm^{-1} ; MS m/z 435 (MH^+ , ^{81}Br), 433 (MH^+ , ^{79}Br).

Bhc-diol-acetophenone (16c). Bhc-diol (20 mg, 0.07 mmol), PPTS (33 mg, 0.14 mmol), acetophenone (**15c**, 40 μL , 0.35 mmol); yield = 6.0 mg (22%, mp 170-220 $^\circ\text{C}$ dec). ^1H NMR (CDCl_3) δ 7.51 (6H, m), [7.05 (s), 7.01 (s) (1H)], [6.64 (s), 6.40 (s) (1H)], [5.40 (dd, $J = 7.2, 7.2$ Hz), 5.07 (dd, $J = 7.6, 5.2$ Hz) (1H)], [4.65 (dd, $J = 8.8, 6.8$ Hz), 4.24 (dd, $J = 8.0, 8.0$ Hz) (1H)], [3.89 (dd, $J = 8.0, 5.2$ Hz), 3.71 (dd, $J = 8.0, 8.0$ Hz) (1H)], [1.83 (s), 1.78 (s) (3H)]; ^{13}C NMR (100 MHz, CDCl_3) 160.59, 155.40, (154.81, 154.63), 152.56, 141.98, (128.60, 128.53), (128.47, 128.34), 126.66, 125.27, 124.70, 112.15, 111.11, 110.20, 106.63, (104.61, 104.51), 74.41, 72.94, (69.86, 69.33), (28.16, 27.74); FTIR (neat) 3416, 2922, 1697, 1602, 1395, 1308, 1224, 1155, 1096, 874, 760, 699 cm^{-1} ; MS m/z 405 (MH^+ , ^{81}Br), 403 (MH^+ , ^{79}Br).

Bhc-diol-cyclohexanone (16d). Bhc-diol (15 mg, 0.05 mmol), PPTS (13 mg, 0.05 mmol), cyclohexanone (**15d**, 10 μL , 0.10 mmol); yield = 7.5 mg (39%, mp 180-230 $^\circ\text{C}$ dec). ^1H NMR (CDCl_3) δ 7.54 (1H, s), 7.04 (1H, s), 6.58 (1H, s), 5.26 (1H, dd, $J = 8.0, 8.0$ Hz), 4.53 (1H, dd, $J = 8.0, 8.0$ Hz), 3.77 (1H, dd, $J = 8.0, 8.0$ Hz), 1.70 (10H, m); ^{13}C NMR (100 MHz, CDCl_3) 160.66, 155.26, 154.72, 152.69, 126.63, 112.38, 111.56, 109.89, 106.62, 104.60, 72.94, 69.09, 35.67, 34.81, 25.01, 23.89, 23.79; FTIR (neat) 3386, 2935, 2862, 1707, 1604, 1395, 1272, 1231, 1155, 1107, 1036, 875, 700 cm^{-1} ; MS m/z 383 (MH^+ , ^{81}Br), 381 (MH^+ , ^{79}Br).

Determination of the Quantum Efficiency for Single Photon Excitation. KMOPS-buffered solutions (3 mL) of the substrates (100 μM) in quartz cuvettes (21-Q-10, Starna, Atascadero, CA) were irradiated with 365-nm UV light from a mercury lamp (Spectroline SB-100P; Spectronics Corporation, Westbury, NY). The spectral output of the lamp is a distribution across the UV-A wavelengths (310-400 nm) with an intense band at 365 nm. The duration of each irradiation period ranged from 5 to 360 s. After each period of irradiation, a 20 μL aliquot of the solution was removed for analysis by HPLC, using an external standard method to determine concentrations. The compound was eluted with an isocratic mixture of 50% acetonitrile and 50% water containing 0.1% trifluoroacetic acid (flow rate of 1 mL/min). Absorbance was detected at 325 nm (**16a**), 328 nm (**16c** and **16d**), and 329 nm (**16b**). The progress curves were plotted as simple decaying exponentials. Quantum efficiencies (Q_{ul}) were calculated according to a published method,¹ using $Q_{ul} = (I\sigma t_{90\%})^{-1}$, where I is the irradiation intensity in $\text{ein}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$, σ is the decadic extinction coefficient (10^3 times ϵ , the molar extinction coefficient) in $\text{cm}^2\cdot\text{mol}^{-1}$, and $t_{90\%}$ is the irradiation time in seconds for 90% conversion to product. The UV intensity of the lamp I was measured by using potassium ferrioxalate actinometry² in the same setup. Release of piperonal (**15b**) or acetophenone (**15c**) was monitored at 313 and 240 nm, respectively, and the progress curves plotted as an exponential rise to max. Concentrations were determined using an external standard.

Determination of the Dark Hydrolysis Rate. Substrates were dissolved in a KMOPS-buffered solution and stored in the dark at room temperature. HPLC analysis was carried out periodically as described for single photon photolysis.

Control Photolysis Experiments. Piperonal (**15b**, 97 μ M) and acetophenone (**15c**, 104 μ M) were each dissolved in KMOPS buffer. A small amount of MeOH was used as a co-solvent to effect dissolution. The solutions were exposed to the 365-nm mercury lamp for 1 min, and any change in concentration was monitored by HPLC as in the quantum efficiency determination. KMOPS-buffered solutions of Bhc-diol (**14**, 100 μ M) and carbonyl compound (**15b**, 104 μ M; **15c**, 97 μ M) were photolyzed as before, and any changes in concentration of Bhc-diol, piperonal, or acetophenone were monitored by HPLC.

Measurement of the Two-Photon Uncaging Cross-Section. Measurements were carried out in microcuvettes (10 $\times$ 1 $\times$ 1 mm illuminated dimensions) with an effective filling volume of 20 μ L (26.10F-Q-10, Starna, Atascadero, CA), using light from a fs-pulsed and mode-locked Ti:Sapphire laser (Mira 900 pumped by a Verdi, Coherent, Santa Clara, CA) focused on the center of the cuvette chamber with a 25 mm focal length lens optimized for IR lasers (06LXP003/076, Melles-Griot, Irvine, CA). The two-photon uncaging cross-section (δ_{u2}) was estimated by referencing to fluorescein, a compound with a known two-photon fluorescence quantum yield ($Q_{f2} = 0.9$ mol/ein) and absorbance cross-section ($\delta_{aF} = 30$ GM at 740 nm).³

$$\delta_{u2} = \frac{N_p \Phi Q_{f2} \delta_{aF} C_F}{\langle F(t) \rangle C_s}$$

where N_p is the number of product molecules formed per unit time (molecules/s, determined by HPLC analysis as in the 1-photon analysis); Φ is the collection efficiency of the detector (SED033 on an IL-1700, International Light, Newburyport, MA) used to measure the fluorescence of fluorescein emitted at a right angle to the beam and passed through a 535/45 nm bandpass filter (Chroma Technologies, Brattleboro, VT); C_F is the concentration of fluorescein (mol/L); $\langle F(t) \rangle$ is the time averaged fluorescent photon flux (photons/s) collected by the detector; and C_s is the initial concentration of caged substrate (mol/L).¹

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