Supporting Information

Control of an Unusual Photo-Claisen Rearrangement in Coumarin Caged Tamoxifen by an Extended Spacer

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I. Materials and Methods

Materials

Unless otherwise noted, reagents and solvents were purchased and used as received from commercial suppliers including 7-(diethylamino)-4-methyl-2H-chromen-2-one (98%, TCI America), 4-Bromorescorcinol (98%, TCI America), methanesulfonyl chloride (≥99.7%, Sigma-Aldrich), 4-nitrophenyl chloroformate (>98%, TCI America), *N*,*N'*-dimethyl ethylenediamine (99%, Sigma-Aldrich), dichloromethane (CH_2Cl_2) and ethyl acetate (EtOAc) both from Sigma-Aldrich. NMR spectral data are reported in deuterium-labeled solvents including $CDCl_3$, DMSO-d_6 and CD_3OD, each purchased from Sigma-Aldrich. Flash column chromatography employed in the purification of reaction products was performed using silica gel (200–400 mesh), and product purity was characterized by thin layer chromatography (TLC) performed using Merck® TLC plates (250 μm thick).

Analytical Methods

Structural identity of caged compounds and their intermediates was characterized by standard analytical methods including NMR (^{1}H, ^{13}C) spectroscopy, mass spectrometry, and UV–vis spectrometry. NMR spectra were acquired at 500 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR in a Varian nuclear magnetic resonance spectrometer. Chemical shift values for ^{1}H NMR spectra are reported in ppm with a reference to tetramethylsilane (TMS) as an internal standard (δ = 0.00 ppm), or to residual signals from the specific NMR solvent used. All NMR spectra

were acquired at 297.3 K by standard default pulse sequences. Mass spectrometric analysis was performed in an electrospray ionization (ESI) mode with a Micromass AutoSpec Ultima Magnetic sector mass spectrometer. Measurement of an exact mass was performed by a high resolution VG 70-250-S mass spectrometer using the EI mode. UV–vis absorption spectrometry was performed with a Perkin Elmer Lamda 20 spectrophotometer.

Compound homogeneity was determined by ultrahigh performance liquid chromatography (UPLC) with a Waters Acquity System combined with a photodiode array (PDA) detector. The UPLC analysis was performed with a C4 BEH column (100 × 2.1 mm, 300 Å) at a flow rate of 0.2 mL min^{-1}. Its elution method is based on a linear gradient made of two mobile solvents, water and acetonitrile each with TFA (0.1% v/v) (eluent A and B respectively). This initial mobile phase 1% B (0–1.4 min) was linearly increased to 80% B (1.4–13.4 min), a decrease to 50% B (13.4–13.8 min), a decrease to 1% B (13.8–14.4 min) and finally an isocratic elution at 1% B (14.4–18 min). This elution method was applied for the kinetic analysis of drug release.

4-Hydroxytamoxifen (4-OHT)

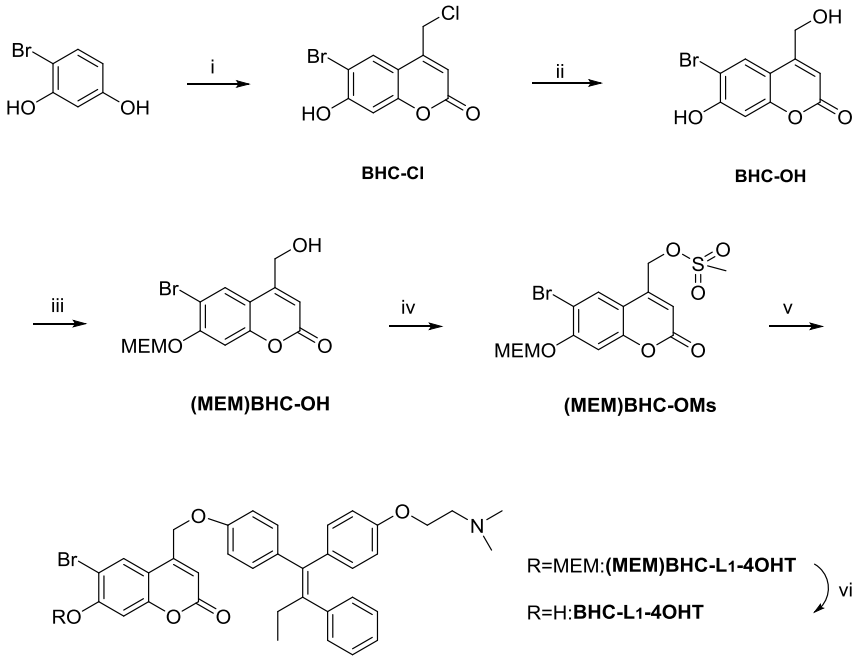
Synthesis of 4OHT (Z- and E-isomer) was performed according to a literature method as described earlier.[1] It was obtained as a white solid. R_f (10% v/v CH_3OH/CH_2Cl_2) = 0.18. MS (ESI) *m/z* (relative intensity, %) = 388 (100) $[M+H]^+$. ^1H NMR (500 MHz, CD_3OD): δ 7.16–7.06 (m; 6H, ArH), 7.02–7.00 (dd, J_1 =2, J_2 = 6 Hz, 1H, ArH), 6.94–6.92 (dd, J_1 =2, J_2 =6 Hz, 1H, ArH), 6.76–6.74 (dd, J_1 = 2, J_2 = 6 Hz, 1H, ArH), 6.64–6.62 (dd, J_1 =2, J_2 = 6 Hz, 1H, ArH), 6.57–6.55 (dd, J_1 = 2, J_2 = 6 Hz, 1H, ArH), 6.39–6.37 (dd, J_1 =2, J_2 =6 Hz, 1H, ArH), 4.14–4.12 (t, J = 5 Hz, 1H, 1/2CH_2O), 3.98–3.96 (t, J = 5 Hz, 1H, 1/2CH_2O), 2.86–2.84 (t, J = 5 Hz, 1H, 1/2CH_2N), 2.77–2.75 (t, J = 5 Hz, 1H, 1/2CH_2N), 2.50–2.44 (m, 2H, CH_2), 2.40 (s; 3H, CH_3N), 2.34 (s; 3H, CH_3N), 0.91–0.88 (t, J = 5 Hz; 3H, CH_3).

ONB-L_1-4OHT (1) and ONB-L_3-4OHT (6)

Each of these caged compounds was synthesized by coupling of 4OHT to an *ortho*-nitrobenzyl (ONB) cage molecule through an ether or extended carbamate linker as we reported in our previous study.[2]

BHC-L_1-4OHT (2)

BHC-Cl (6-bromo-4-(chloromethyl)-7-hydroxy-2H-chromen-2-one). To methane sulfonic acid (10 ml) was added 4-bromoresorcinol (1 g, 5.28 mmol) and ethyl 4-chloroacetoacetate (1.07 mL, 7.94 mmol). The mixture was stirred at room temp for 2 h before adding ice (30 g). The mixture was stirred for 10 min and the precipitate was collected by filtration through a filter paper. The solid was rinsed with water (4 × 20 mL) and drying under high vaccum afforded the desired product BHC-Cl as an off white solid (1.46 g, 95%). R_f (1:2 EtOAc/hexane) = 0.35. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.56 (s, 1H, HO-Ar), 7.99 (s, 1H, ArH), 6.91 (s, 1H, ArH), 6.47 (s, 1H, CHC(=O)), 4.99 (s, 2H, CH_2Cl) ppm.



reagents and conditions: i) Ethyl 4-chloroacetoacetate, methanesulfonic acid; ii) H_2O, reflux, 24 h; iii) 2-methoxyethoxymethyl chloride (MEMCl), DIPEA, CH_2Cl_2; iv) methanesulfonyl chloride, DIPEA, CH_2Cl_2; v) 4OHT, K_2CO_3/Cs_2CO_3, THF; vi) CF_3CO_2H, CH_2Cl_2.

BHC-OH (6-bromo-7-hydroxy-4-(hydroxymethyl)-2H-chromen-2-one).[3] BHC-Cl (600 mg, 2.07 mmol) was suspended in water (120 mL) and refluxed at 120°C for 2 days in the dark. The mixture was concentrated to approximately 20 mL. The precipitated solid was collected, washed with water (10 mL) and dried *in vacuo*. It was triturated with 1:1 EtOAc/hexane (10 mL), and the desired product BHC-OH was obtained as an off-white solid (502 mg, 89%). R_f (2:1 EtOAc/hexane) = 0.39. MS (ESI) *m/z* (relative intensity, %) = 270.9 (100) and 272.9 (89) $[M + H]^+$; 292.9 (21) and 294.9 (19) $[M + Na]^+$. ^{1}H NMR (500 MHz, CD_3OD): δ 7.81 (s, 1H, ArH),

6.84 (s, 1H, ArH), 6.37 (s, 1H, CHC(=O)), 4.76 (s, 2H, CH_2O) ppm. UV–vis spectroscopy: λ_{max} = 331 nm (ε = 1812.5 $M^{-1}cm^{-1}$).

(MEM)BHC-OH. To a cold solution of BHC-OH (300 mg, 1.11 mmol) and *N*,*N*-diisopropyl-*N*-ethylamine (DIPEA; 214 μL, 1.20 mmol) dissolved in dichloromethane (12 ml) was added 2-methoxyethoxymethyl chloride (MEM-Cl; 136 μl, 1.20 mmol) dropwise. The reaction mixture was stirred in the ice water bath for 2 h and at room temp for 30 min. The mixture was poured into 0.5 M citric acid (10 ml), stirred for 30 min, and its organic layer was extracted with dichloromethane (2 × 10 mL). A combined extract was washed with brine solution (20 mL), dried over Na_2SO_4 and concentrated *in vacuo* to yield the product (MEM)BHC-OH as a grey-white solid (371.0 mg, 93%). R_f (1:1 EtOAc/hexane) = 0.22. MS (ESI) *m/z* (relative intensity, %) = 381.0 (100) and 383.0 (90) $[M + Na]^+$. ^1H NMR (500 MHz, $CDCl_3$): δ 7.69 (s, 1H, ArH), 7.20 (s, 1H, ArH), 6.51 (s, 1H, CHC(=O)), 5.40 (s, 2H, OCH_2O), 4.85 (s, 2H, CH_2OH), 3.87–3.85 (m, 2H, OCH_2), 3.57–3.56 (m, 2H, OCH_2), 3.38 (s, 3H, OCH_3) ppm.

(MEM)BHC-OMs. To a mixture of (MEM)BHC-OH (82 mg, 0.23 mmol) and DIPEA (120 μL, 0.69 mmol) in dichloromethane (4 mL) cooled in an ice water bath was added methanesulfonyl chloride (20 μL, 0.25 mmol). The reaction mixture was stirred in the same bath for 2 h. and mixed with dichloromethane (4 mL). An organic layer was separated and washed with saturated Na_2CO_3 (4 mL), 0.5 M citric acid (4 mL), water (4 mL), and brine (4 mL). After drying over Na_2SO_4; the solution was concentrated to give the product (MEM)BHC-OMs as a white solid. This product was used immediately in the next step without further purification (88 mg, 88%). R_f (1:1 EtOAc/hexane) = 0.28. MS (ESI) *m/z* (relative intensity, %) = 458.9 (100) and 460.9 (96) $[M + Na]^+$.

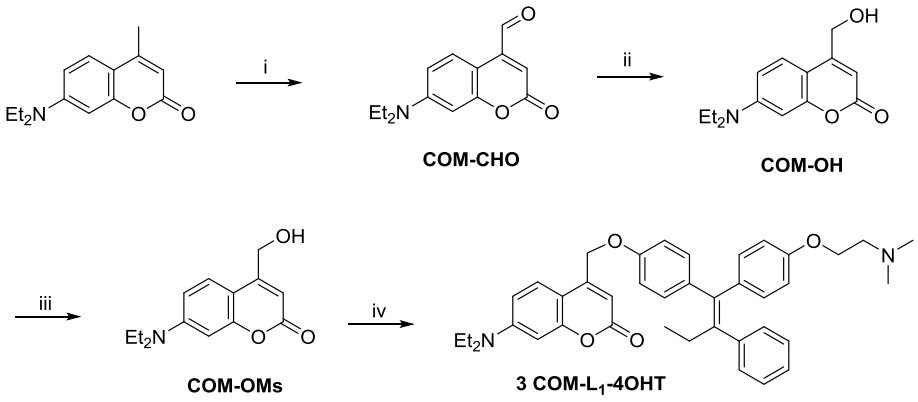
(MEM)BHC-L_1-4OHT. To a solution of (MEM)BHC-OMs (88 mg, 0.25 mmol) and 4OHT (38 mg, 0.098 mmol) dissolved in THF (5 mL) was added K_2CO_3 (81 mg, 0.59 mmol) and Cs_2CO_3 (192 mg, 0.59 mmol). The mixture was stirred at room temp for 48 h, and concentrated *in vacuo*. It was suspended in dichloromethane (16 mL), and washed with 0.5 M citric acid (2×10 mL) and brine (10 mL). After drying over Na_2SO_4, the solution was concentrated, and the crude product was purified by flash silica column chromatography by eluting with 3% v/v

CH_3OH/CH_2Cl_2. The product (MEM)BHC-L_1-4OHT was obtained as a white solid (27 mg, 38%). R_f (5% v/v CH_3OH /CH_2Cl_2) = 0.47. MS (ESI) *m/z* (relative intensity, %) = 728.0 (100) $[M + H]^+$. HRMS (ESI) calcd for $C_{40}H_{42}BrNO_7$ $[M + H]^+$ 728.2217, found 728.2236. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.78 and 7.67 (s, 1H, ArH (BHC)), 7.24 and 7.20 (s, 1H, ArH (BHC)), 7.20–7.09 (m, 6H, ArH (4OHT)), 6.98 and 6.96 (s, 1H, ArH (4OHT)), 6.90 and 6.89 (s, 1H, ArH (4OHT)), 6.82 and 6.80 (s, 1H, ArH (4OHT)), 6.78 and 6.76 (s, 1H, ArH (4OHT)), 6.63 and 6.62 (s, 1H, ArH (4OHT)), 6.56 and 6.54 (s, 1H, ArH (4OHT)), 6.60 and 6.48 (s, 1H, CHC(=O) (BHC)), 5.42 and 5.40 (s, 2H, OCH_2O), 5.18 and 5.02 (s, 2H, OCH_2C=CH), 4.26 (br, 1H, CH_2O), 4.12 (br, 1H, CH_2O), 3.88–3.85 (m, 2H, CH_2O (MEM)), 3.58–3.56 (m, 2H, CH_2O(MEM)), 3.39 and 3.37 (s, 3H, OCH_3 (MEM), 3.03 and 2.96 (br, 2H, CH_2N), 2.58 (br, 3H, CH_3N), 2.53 (br, 3H, CH_3N), 2.50–2.46 (m, 2H, $CH_2(CH_3)$), 0.95–0.92 (m, 3H, CH_3 (CH_2)) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ 149.42, 132.19, 132.01, 130.88 130.70, 129.65, 127.91, 127.57, 127.49, 126.11, 114.46, 114.15, 113.68, 113.38, 112.04, 111.90, 104.25, 94.19, 71.37, 68.58, 65.58, 59.12, 57.64, 29.06, 13.60 ppm.

BHC-L_1-4OHT (2). (MEM)BHC-L_1-4OHT (22 mg, 0.030 mmol) was dissolved in a mixture of dichloromethane (1 mL) and trifluoroacetic acid (TFA; 1 mL), and stirred at room temp for 3 h. The volatile solvents were evaporated and the residue was dissolved in EtOAc (2 mL). It was treated with N_2 flow until a TFA residue was fully removed. Finally, the residue was triturated in ether (2 × 1 mL), and the deprotected product **2** BHC-L_1-4OHT was obtained as a white solid (18 mg, 78%). Purity (UPLC): 98%. MS (ESI) *m/z* (relative intensity, %) = 640.0 (100) $[M+H]^+$. HRMS (ESI) calcd for $C_{36}H_{34}BrNO_5$ $[M + H]^+$ 640.1693, found 640.1711. ^{1}H NMR (500 MHz, CD_3OD): δ 7.95 and 7.86 (s, 1H, ArH (BHC)), 7.20–7.07 (m, 7H, 1ArH (BHC) and 6 ArH (4OHT), 7.03 and 7.01 (s, 1H, ArH (4OHT)), 6.87–6.80 (m, 3H, ArH (4OHT)), 6.72 and 6.70 (s, 1H, ArH (4OHT)), 6.67 and 6.65 (s, 1H, ArH (4OHT)), 6.45 and 6.2 (s, 1H, CHC(=O) (BHC)), 5.33 and 5.16 (s, 2H, OCH_2C=CH), 4.36–4.34 (t, *J* = 5 Hz, 1H, CH_2O), 4.19–4.17 (t, *J* = 5 Hz, 1H, CH_2O), 3.56–3.54 (m, 1H, CH_2N), 3.46–3.43 (m, 1H, CH_2N), 2.95 (s, 3H, CH_3N), 2.88 (s, 3H, CH_3N), 2.50–2.44 (m, 2H, $CH_2(CH_3)$), 0.92–0.90 (m, 3H, $CH_3(CH_2)$) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ 162.83, 162.77, 159.28, 159.20, 158.22, 157.93, 157.33, 157.08, 155.72, 152.66, 152.57, 143.64, 143.16, 143.10, 139.05, 138.70, 138.43, 138.14, 133.17, 133.15, 131.82, 130.86, 130.84, 129.64, 129.60, 128.96, 128.93, 127.40, 127.26, 127.17, 123.11, 115.70, 115.46,

114.91, 114.61, 112.35, 110.50, 108.13, 108.06, 104.36, 104.30, 66.72, 66.50, 63.16, 62.91, 57.81, 57.77, 43.90, 43.85, 29.95, 29.85, 13.83 ppm.

COM-L_1-4OHT (3)



reagents and conditions: i) SeO_2, Xylene, 150°C; ii) $NaBH_4$, MeOH, 0°C to rt; iii) methanesulfonyl chloride, Et_3N, CH_2Cl_2, 0°C to room temp; iv) 4OHT, K_2CO_3, Cs_2CO_3, DMF, rt to 60°C

COM-CHO (7-(diethylamino)-2-oxo-2H-chromene-4-carbaldehyde). Synthesis of COM-CHO was performed by oxidation of 7-(diethylamino)-4-methyl-2H-chromen-2-one using selenium dioxide (SeO_2) as reported elsewhere.[4] To a stirred suspension of selenium dioxide (1.97 g, 17.75 mmol) in xylene (40 mL) was added 7-diethylamine-4-methylcoumarin (3 g, 13.0 mmol). The mixture was heated at 150°C for 3 h while being stirred vigorously. After cooling to room temp, a second portion of selenium dioxide (1.31 g, 11.8 mmol) was added, and the mixture was heated at 150°C for an additional 3 h. The mixture was concentrated *in vacuo*, and the residue was dissolved in 1:1 EtOAc/hexane (50 mL) and filter through filter paper. The filtrate was concentrated, and the solid residue was purified by flash column chromatography by elution with 1:5 to 1:2 EtOAc/hexane. The desired product COM-CHO was obtained as a syrup (0.53 g, 24 %). R_f (1:2 EtOAc/hexane) = 0.36. MS (ESI) *m/z* (relative intensity, %) = 246 (33%) $[M+H]^+$, 268 (100) $[M + Na]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.03 (s, 1H, CHO), 8.32–8.30 (d, J = 10 Hz, 1H, ArH), 6.64–6.62 (dd, J_1 = 2, J_2 = 10 H, 1H, ArH), 6.53 (s, 1H, ArH), 6.46 (s, 1H, CHC=O), 4.84 (s, 2H, CH_2O), 3.45–3.41 (q, J = 7, 4H, $2CH_2N$), 1.24–1.21 (t, J = 7, 6H, $2CH_3$).

COM-OH (7-(diethylamino)-4-(hydroxymethyl)-2H-chromen-2-one). COM-CHO (115 mg, 0.47 mmol) was dissolved in methanol (3 mL) and cooled in an ice water bath. It was reduced by

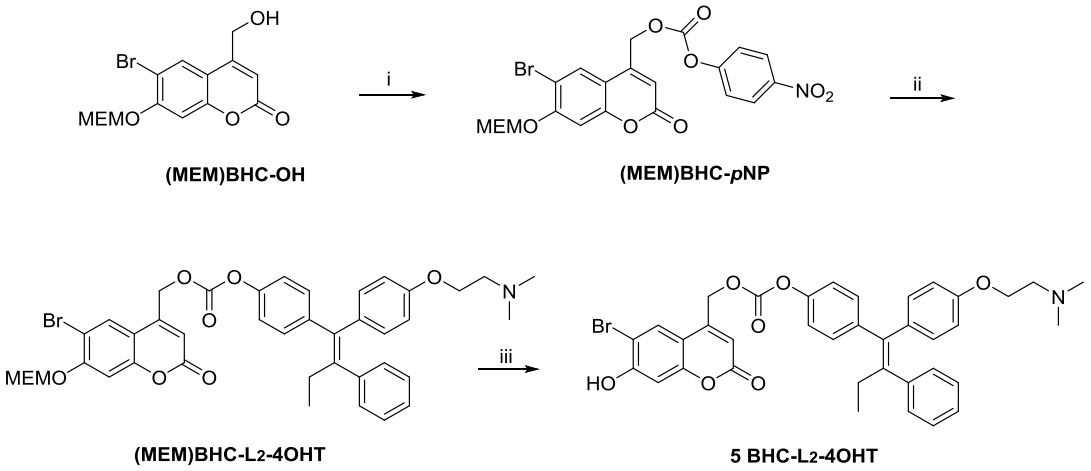
sodium borohydride (13 mg, 0.47 mmol) for 40 min at the same temperature.[5] To quench the reaction, water (3 mL) was added, and the mixture was stirred for 10 min. The mixture was extracted with dichloromethane (3 × 5 mL), and the combined extract was washed with water (5 ml) and brine (5 mL). After drying over sodium sulfate, the solution was concentrated to afford the desired product as a pale yellow solid (113.7 mg, 98%). R_f (1:1 EtOAc/hexane) = 0.26. MS (ESI) *m/z* (relative intensity, %) = 248 (100 %) $[M+H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.34–7.32 (d, *J* = 10 Hz, 1H, ArH), 6.60 (br, 1H, ArH), 6.55 (s, 1H, ArH), 6.27 (s, 1H, CHC=O), 4.84 (s, 2H, CH_2OH), 3.43–3.39 (q, *J* = 7 Hz, 4H, $2CH_2N$), 1.22–1.19 (t, *J* =7 Hz, 6H, $2CH_3$).

COM-OMs: To a cold solution of COM-OH (50 mg, 0.20 mmol) dissolved in dichloromethane (5 mL) was added Et_3N (31 μL, 0.22 mmol) and followed by methanesulfonyl chloride (MsCl; 17 μL, 0.22 mmol). The mixture was stirred at 5°C for 5 min and at room temp for 1.5 h. When the reaction was completed as monitored by TLC, the mixture was washed with 1M H_3PO_4 (2 × 3 mL), saturated $NaHCO_3$ (3 mL) and water (2 × 3 mL). Evaporation of the organic phase afforded COM-OMs as a pale yellow solid (68 mg, 100 %). It was used immediately in the next step without further purification. R_f (2 % v/v CH_3OH/CH_2Cl_2) = 0.44. MS (ESI) *m/z* (relative intensity, %) = 326 (100 %) $[M+H]^+$.

COM-L_1-4OHT (3): To a stirred solution of 4OHT (30 mg, 0.078 mmol) in DMF (2 mL) was added K_2CO_3 (32 mg, 0.23 mmol) and Cs_2CO_3 (76 mg, 0.23 mmol). The mixture was stirred at 60°C for 5 min under an nitrogen atmosphere, and COM-OMs (28 mg, 0.085 mmol) dissolved in DMF (0.5 mL) was added. The final reaction mixture was stirred at 60°C for 2 h. After cooling, the reaction mixture was diluted with dichloromethane (5 mL), washed with saturated NH_4Cl (2 × 5 mL) and water (5 mL). The organic solution was dried, concentrated, and purified by flash column chromatography by eluting with 8% v/v CH_3OH/CH_2Cl_2. The desired product **3** COM-L_1-4OHT was obtained as a brown solid (26 mg, 54%). R_f (8% v/v CH_3OH/CH_2Cl_2) = 0.50. Purity (UPLC): 98%. MS (ESI) *m/z* (relative intensity, %) = 617 (100 %) $[M + H]^+$. HRMS (ESI) calcd for $C_{40}H_{44}N_2O_4$ $[M + H]^+$ 617.3374, found 617.3373. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.41–7.39 (d, *J* = 10 Hz, 0.5H, 1/2ArH(COM)), 7.30–7.28 (d, *J* = 10 Hz, 0.5H, 1/2ArH(COM)), 7.19–7.09 (m, 6H, ArH(4OHT)), 6.95–6.93 (m, 1H, ArH (4OHT)), 6.91–6.89 (m, 1H, ArH (4OHT)), 6.80–6.78 (m, 2H, 2ArH (4OHT)), 6.77–6.75 (m, 1H, 1ArH (4OHT)), 6.62–6.59 (m, 1H, 1ArH (4OHT)), 6.56–6.53 (m, 2H, 1ArH (4OHT) and 1ArH (COM)), 6.51 and 6.50 (s, 1H,

ArH (COM), 6.32 and 6.22 (s, 1H, CHC=O (COM)), 5.16 and 5.00 (s, 2H, CH_2O (COM)), 4.13–4.11 (t, *J* = 5 Hz, 1H, 1/2CH_2O (4OHT)), 3.97–3.95 (t, *J* = 5 Hz, 1H, 1/2CH_2O (4OHT), 3.44–3.38 (m, 4H, 2CH_2N (COM)), 2.81–2.79 (t, *J* = 5 Hz, 1H, 1/2CH_2N (4OHT)), 2.72–2.70 (t, *J* = 5 Hz, 1H, 1/2CH_2N (4OHT)), 2.50–2.46 (q, *J* = 5 Hz, 2H, CH_2 (4OHT)), 2.40 (s, 3H, CH_3N (4OHT), 2.34 (s, 3H, CH_3N (4OHT)), 1.23–1.18 (m, 6H, 2CH_3 (COM)), 0.94–0.91 (m, 3H, CH_3 (4OTH)). ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.00, 150.38,129.68, 129.66, 114.96, 114.08, 113.56, 67.17, 67.16, 65.62, 44.72, 30.08, 12.43, 12.42 ppm.

BHC-L_2-4OHT (5)



reagents and conditions: i) *p*-Nitrophenyl chloroformate, DIPEA, $CHCl_3$ ii) 4OHT, DMAP, CH_3CN, CH_2Cl_2. iii) TFA,CH_2Cl_2

(MEM)BHC-*p*NP. To a cold solution of *p*-nitrophenyl chloroformate (62 mg, 0.31 mmol) dissolved in anhydrous THF (3 mL) was added a solution of $CHCl_3$ (3 mL) containing (MEM)BHC-OH (100 mg, 0.28 mmol) and *N*,*N*-diisopropylethylamine (DIPEA; 146 μL, 0.84 mmol) in an ice bath. This mixture was stirred at 5^oC for 30 min and at room temp overnight. A second portion of *p*-nitrophenyl chloroformate (62 mg, 0.31 mmol) was added to the reaction mixture followed by the addition of 4-dimethylaminopyridine (DMAP, 36 mg, 0.31 mmol). The final mixture was stirred at room temp for additional 4 h, and concentrated *in vacuo*. A flash silica column chromatography, eluting with 1:5, then 1:1 EtOAc/hexane, gave the product as a white solid (66 mg, 45%). R_f (1:2 EtOAc/hexane) = 0.60. MS (ESI) *m/z* (relative intensity, %) = 546.0 (100) and 548.0 (94) $[M + Na]^+$. ^1H NMR (500 MHz, $CDCl_3$): δ 8.33–8.31 (d, *J* = 10 Hz,

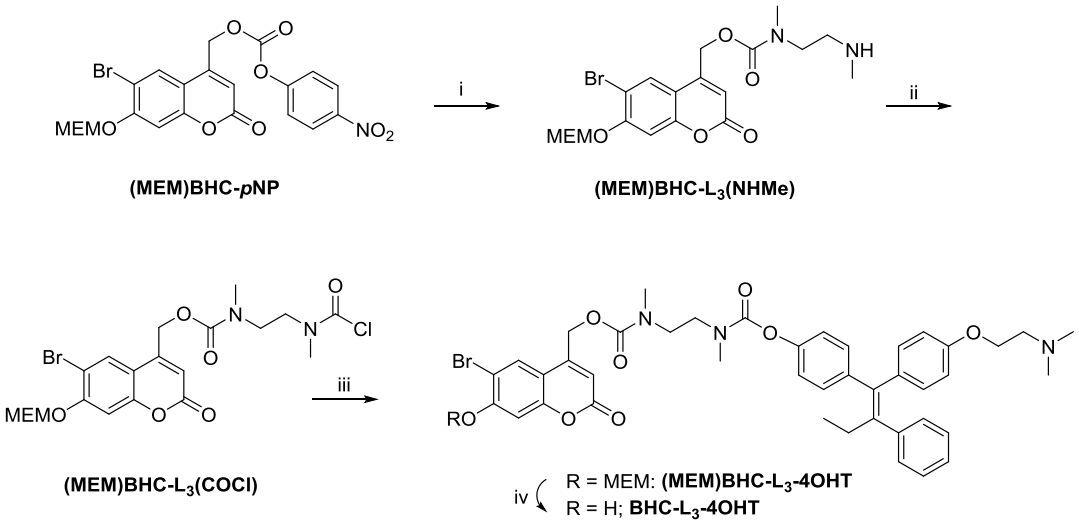
2H, ArH (ortho to NO_2; $PhNO_2$)), 7.70 (s, 1H, ArH (BNC)), 7.44–7.42 (d, *J* = 10 Hz, 2H, ArH (meta to NO_2; $PhNO_2$)), 7.26–7.25 (d, *J* = 5 Hz , 1H, ArH (BNC), 6.50 (s, 1H, CHC=O (BHC), 5.42 (s, 2H, 1/2 OCH_2O(MEM)), 5.41 (s, 2H,1/2OCH_2C(BHC)), 3.88–3.86 (t, *J* = 5 Hz , 2H, OCH_2(MEM)), 3.58–3.56 (t, *J* = 5 Hz , 2H, CH_2O (MEM)), 3.38 (s, 3H, OCH_3(MEM)) ppm.

(MEM)BHC-L_2-4OHT. To a solution of (MEM)BHC-*p*NP (38 mg, 0.072 mmol) dissolved in a mixture of acetonitrile (1.5 mL) and dichloromethane (1.5 mL) was added 4OHT (25 mg, 0.065 mmol) and DMAP (9 mg, 0.072 mmol). The mixture was stirred in the dark overnight and diluted with dichloromethane (5 mL). It was washed with 0.5 M citric acid (5 mL) and brine (5 mL), and then dried over Na_2SO_4. After concentration, the crude product was purified by flash silica column chromatography by eluting with 3 to 10% v/v CH_3OH/CH_2Cl_2. The desired product was isolated as a white solid (34.5 mg, 62%). R_f (5% v/v CH_3OH/CH_2Cl_2) = 0.42. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.72 and 7.67 (s, 1H, ArH (BHC)), 7.28–7.08 (m, 9H, ArH (1BHC and 8 4OHT)), 6.91–6.84 (m, 3H, ArH (4OHT)), 6.78 and 6.76 (s, 1H, ArH (4OHT)), 6.56 and 6.55 (s, 1H, ArH (4OHT)), 6.50 and 6.42 (s, 1H, CHC(=O) (BHC)), 5.42 and 5.41 (s, 2H, OCH_2O), 5.39–5.38 (d, *J* = 5 Hz, 1H, OCH_2C=), 5.31–5.30 (d, *J* = 5 Hz, 1H, OCH_2C=), 4.32 (br, 1H, CH_2O), 4.16 (br, 1H, CH_2O), 3.88–3.85 (m, 2H, CH_2O(MEM)), 3.58–3.55 (m, 2H, CH_2O(MEM)), 3.38 and 3.37 (s, 3H, OCH_3 (MEM), 3.08 (br, 2H, CH_2N), 2.63 (br, 3H, CH_3N), 2.59 (br, 3H, CH_3N), 2.50–2.45 (m, 2H, $CH_2(CH_3)$), 0.95–0.91 (m, 3H, $CH_3(CH_2)$) ppm.

BHC-L_2-4OHT (5). To a solution of (MEM)BHC-L_2-4OHT (26 mg, 0.034 mmol) dissolved in dichloromethane (1 mL) was added TFA (1 mL). The mixture was stirred for 3 h in the dark. Volatile solvents were removed by evaporation and the residue was dissolved in EtOAc (0.5 mL) before titrated into ether (5 mL). White precipitates were collected and washed with ether (2 × 1 mL). Drying under higher vacuum afforded **5** BHC-L_2-4OHT as a white solid (24 mg, quantitative). Purity (UPLC): 98%. MS (ESI) *m/z* = 683.9 $[M + H]^+$. HRMS (ESI) calcd for $C_{37}H_{34}BrNO_7$ $[M + H]^+$ 684.1591, found 684.1587. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.62 and 7.56 (s, 1H, ArH (BHC)), 7.24–7.07 (m, 9H, ArH (1BHC and 8 4OHT)), 6.88–6.84 (m, 3H, ArH (4OHT)), 6.76 and 6.74 (s, 1H, ArH (4OHT)), 6.52 and 6.51 (s, 1H, ArH (4OHT)), 6.42 and 6.36 (s, 1H, CHC(=O) (BHC)), 5.33–5.33 (d, *J* = 1 Hz, 1H, 1/2OCH_2C=), 5.26–5.26 (d, *J* = 1 Hz, 1H, 1/2OCH_2C=), 4.47–4.45 (t, *J* = 5 Hz, 1H, 1/2CH_2O), 4.31–4.30 (t, *J* = 5 Hz, 1H, 1/2CH_2O), 3.53–3.51 (t, *J* = 5 Hz, 1H, 1/2CH_2N), 3.43–3.41 (t, *J* = 5 Hz, 1H, 1/2CH_2N), 2.98 (s, 3H,

CH_3N), 2.91 (s, 3H, CH_3N), 2.49–2.43 (m, 2H, $CH_2(CH3)$), 0.94–0.90 (m, 3H, $CH_3(CH_2)$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 139.47, 131.89, 130.67, 129.56, 127.97, 126.97, 123.97, 120.49, 119.59, 114.21, 110.66, 104.44, 86.93, 81.98, 73.66, 63.14, 48.59, 43.82, 40.06, 13.48 ppm.

BHC-L_3-4OHT (7)



reagents and conditions: i) N^1,N^2-dimethylethane-1,2-diamine, DIPEA, THF; ii) triphosgene, DIPEA, CH_2Cl_2; iii) 4OHT, DIPEA, DMAP, CH_2Cl_2; iv) TFA, CH_2Cl_2, rt

(MEM)BHC-L_3(NHMe). To a cold solution of *N*,*N'*-dimethylethylenediamine (12 mg, 0.14 mmol) and DIPEA (18 mg, 24 μL, 0.14 mmol) dissolved in THF (1 mL) in an ice water bath was added (MEM)BHC-*p*NP (66 mg, 0.13 mmol; prepared above for BHC-L_5-4OHT). The mixture was stirred in the ice -water bath for 1 h, and concentrated *in vacuo*. This crude product was purified by short flash silica column chromatography by eluting with 5 to 20% v/v CH_3OH/CH_2Cl_2, affording (MEM)BHC-L_3(NHMe) as a pale yellow syrup (42 mg, 71%). R_f (50% v/v CH_3OH/CH_2Cl_2) = 0.27. MS (ESI) *m/z* (relative intensity, %) = 473 (100) and 475 (90) $[M + H]^+$; 494.9 (38) and 496.9 (31) $[M + Na]^+$; ^1H NMR (500 MHz, $CDCl_3$): δ 7.71 (s, 1H, ArH (BNC)), 7.21 (s, 1H, ArH (BNC), 6.37 (s, 1H, CHC=O (BHC), 5.41(s, 2H, OCH_2O(MEM)), 5.26 and 5.25 (s, 2H,OCH_2C (BHC)), 3.87–3.85 (m , 2H, OCH_2 (MEM)), 3.57–3.55 (m, 2H, CH_2O (MEM)), 3.49–3.46 (m, 2H, CH_2N), 3.38 (s, 3H, OCH_3 (MEM)), 3.04 and 3.01 (s, 3H, CH_3N), 2.83–2.77 (m, 2H, CH_2N), 2.48 and 2.45 (s, 3H, CH_3N) ppm.

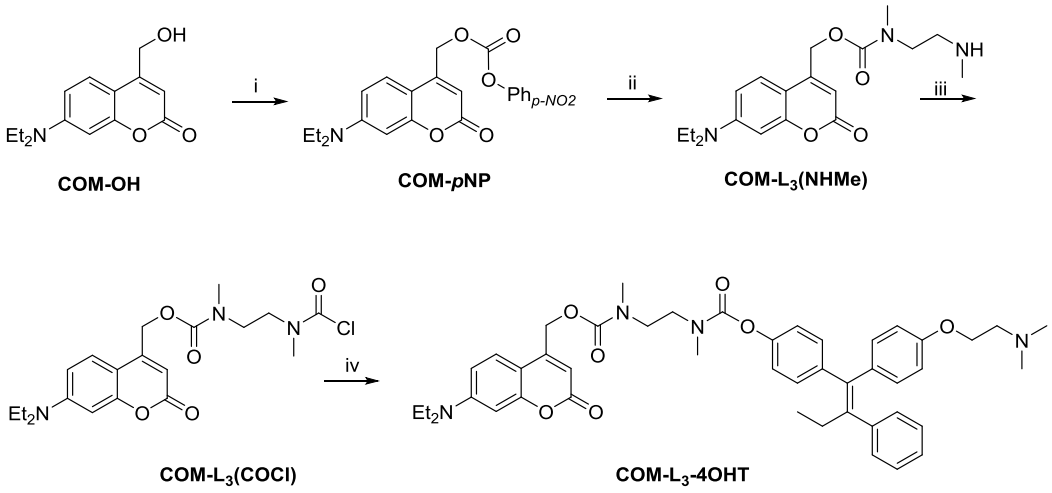
(MEM)BHC-L_3(COCl). To a cold solution of (MEM)BHC-L_3(NHMe) (42 mg, 0.089 mmol) dissolved in dichloromethane (1.5 mL) cooled in an ice bath was added DIPEA (35 mg, 47 μL, 0.27 mmol) and triphosgene (27 mg, 0.089 mmol). The mixture was stirred at 5°C for 30 min, concentrated *in vacuo*, and purified by flash silica column chromatography by eluting with 2% v/v CH_3OH/CH_2Cl_2. The desired product was isolated as a white syrup (38.0 mg). R_f (5% CH_3OH/CH_2Cl_2) = 0.56.

(MEM)BHC-L_3-4OHT. To a cold solution of (MEM)BHC-L_3(COCl) (38.0 mg, 0.071 mmol) dissolved in dichloromethane (1.5 mL) in an ice bath was added 4OHT (27 mg, 0.070 mmol), DIPEA (18 mg, 25 μL, 0.14 mmol) and DMAP (9 mg, 0.070 mmol). The reaction mixture was then stirred at room temp overnight. It was concentrated *in vacuo*, and the residue was purified by flash silica column chromatography by eluting with 5–8% v/v CH_3OH/CH_2Cl_2. The desired product was isolated as a white solid (40 mg, 63%). R_f (10% v/v CH_3OH/CH_2Cl_2) = 0.52. MS (ESI) *m/z* (relative intensity, %) = 886.3 (100) and 888.3 (92) $[M + H]^+$, 501.3 (31). HRMS (ESI) calcd for $C_{46}H_{52}BrN_3O_{10}$ $[M+H]^+$ 886.2909, found 886.2906. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.71–7.68 (m, 1H, ArH (BNC)), 7.20–7.05 (m, 9H, 8ArH (4OHT) and 1ArH (BNC)), 6.88–6.70 (m, 4H, ArH (4OHT), 6.53 (m, 1H, ArH (4OHT), 6.36–6.32 (m, 1H, CHC=O (BHC), 5.40 (s, 2H, OCH_2O(MEM)), 5.30–5.18 (m, 2H,OCH_2C (BHC)), 4.34 and 4.18 (b, 2H, CH_2O (4OHT)), 3.86–3.84 (m , 2H, OCH_2 (MEM)), 3.64–3.51 (m, 6H, CH_2O (MEM) and $2CH_2N$), 3.38 (s, 3H, OCH_3 (MEM)), 3.16–2.92 (m, 8H, $2CH_3N$ and CH_2N(4OHT)), 2.64 (b, 6H, $2CH_3N$(4OHT)), 2.47–2.44 (m, 2H, $CH_2(CH_3)$(4OHT)), 0.92–0.88 (m, 3H, $CH_3(CH_2)$(4OHT)) ppm.

BHC-L_3-4OHT (7). To a solution of (MEM)BHC-L_3-4OHT (39 mg, 0.044 mmol) dissolved in dichloromethane (1 mL) was added TFA (1 mL). The reaction mixture was stirred at room temp for 2h in the dark. Volatile solvents were removed, and the residue was dissolved in EtOAc (1 ml). Titration of this solution to ether (10 ml) led to formation of white precipitates. The solid was collected, washed with ether (1 ml) and dried under higher vacuum. The product **7** BHC-L_3-4OHT was obtained as a white solid (34 mg, 86%). Purity (UPLC): 98%. MS (ESI) *m/z* (relative intensity, %) = 798.2 (90) and 800.2 (100) $[M + H]^+$. HRMS (ESI) calcd for $C_{42}H_{44}BrN_3O_8$ $[M + H]^+$ 798.2385, found 798.2378. ^{1}H NMR (500 MHz, CD_3OD): δ 7.88–7.81 (m, 1H, ArH (BNC)), 7.21–7.05 (m, 10H, 9ArH (4OHT) and 1ArH (BNC)), 6.86–6.62 (m, 4H, ArH (4OHT), 6.29–

6.17 (m, 1H, CHC=O (BHC), 5.33–5.17 (m, 2H,OCH_2C (BHC)), 4.37–4.35 (m, 1H, 1/2CH_2O (4OHT)), 4.21–4.19 (m, 1H, 1/2CH_2O (4OHT)), 3.70–3.43 (m, 4H, 2CH_2N), 3.16–2.92 (m, 8H, 2CH_3N and CH_2N(4OHT)), 2.64 (br, 6H, 2CH_3N(4OHT)), 2.46–2.40 (m, 2H, $CH_2(CH_3)$(4OHT)), 0.91–0.85 (m, 3H, $CH_3(CH_2)$(4OHT)) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ 133.12, 132.64, 131.81, 131.31, 130.83,129.61, 129.02, 128.93, 127.26, 122.64, 121.71, 115.52, 114.67, 104.44, 63.67, 63.10, 62.85, 57.79, 57.75, 49.28, 48.35, 47.83, 43.88, 43.83, 35.40, 29.90, 15.43, 13.76 ppm.

COM-L_3-4OHT (8)



reagents and conditions: i) *p*-Nitrophenyl chloroformate, DIPEA, THF, $CHCl_3$, 0°C; ii) N^1,N^2-dimethylethane-1,2-diamine, THF; iii) triphosgene, DIPEA, CH_2Cl_2, iv) 4OHT, Et_3N, DMAP, DMF

COM-*p*NP. To a solution of *p*-nitrophenyl chloroformate (171 mg, 0.85 mmol) dissolved in anhydrous THF (5 mL) was added a solution of COM-OH (200 mg, 0.81 mmol) and *N*,*N*-diisopropylethylamine (DIPEA; 0.3 mL) dissolved in $CHCl_3$ (5 mL). This mixture was stirred at room temp overnight in the dark. A second portion of *p*-nitrophenyl chloroformate (0.171 mg, 0.85 mmol) was added to the reaction mixture followed by the addition of 4-dimethylaminopyridine (DMAP; 104 mg, 0.85 mmol). The final mixture was stirred at room temp for additional 4 h, and concentrated *in vacuo*. The residue was dissolved in EtOAc (100 mL), and washed with 1M H_3PO_4 (2 × 50 mL), water (50 mL), saturated $NaHCO_3$ solution (2 × 50 mL), water (2 × 50 mL) and finally a brine solution (50 mL). The organic phase was collected, dried over Na_2SO_4, and evaporated to dryness, yielding COM-*p*NP as a pale yellow

solid (265 mg, 79%). R_f (1:1 EtOAc/hexane) = 0.65. MS (ESI) *m/z* (relative intensity, %) = 413 (100 %) $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.32.34–8.30 (d, *J* = 10 Hz, 2H, ArH-pNP), 7.43–7.41 (d, *J* = 10 Hz, 2H, ArH-pNP), 7.33–7.31 (d, *J* = 10 Hz, 1H, ArH), 6.66–6.64 (d, *J* = 10 Hz, 1H, ArH), 6.57 (s, 1H, ArH), 6.24 (s, 1H, CHC=O), 5.40 (s, 2H, CH_2O), 3.45–3.41 (q, *J* = 7 Hz, 4H, $2CH_2N$), 1.23–1.10 (t, *J* = 7, 6H, $2CH_3$) ppm.

COM-L$_3$(NHMe). To a solution of *N,N'*-dimethylethylenediamine (10.7 mg, 0.12 mmol) dissolved in THF (1 mL) cooled in an ice-water bath was added COM-*p*NP (33 mg, 0.081 mmol) dissolved in dichloromethane (1 mL) dropwise. The mixture was stirred in the ice-water bath for 2 h, was quenched with water (1 mL) and extracted with EtOAc (3 × 1 mL). The combined organic solution was concentrated *in vacuo*, and the crude product was purified by flash silica column chromatography by eluting with 10–30% v/v CH_3OH/EtOAc, affording COM-L_2(NHMe) as a pale yellow syrup (20.8 mg, 71% yield). R_f (50% v/v CH_3OH/EtOAc) = 0.1. MS (ESI) *m/z* (relative intensity, %) = 362.2 (100 %) $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.31–7.29 (d, *J*=10 Hz, 1H, ArH), 6.58– 6.56 (d, *J* =10 Hz , 1H, ArH), 6.50 (s, 1H, ArH), 6.12 (s, 1H, CHC=O), 5.25 (s, 2H, CH_2O), 3.49–3.47 (m, 2H, CH_2NC=O), 3.43–3.39 (q, *J*=7, 4H, $2CH_2N$), 3.03 and 3.00 (s, 3H, CH_3N), 2.85–2.75 (m, 2H, CH_2N(H)), 2.49 and 2.44 (s, 3H, CH_3N), 1.22–1.19 (t, *J* = 7, 6H, $2CH_3$) ppm.

COM-L$_3$(COCl). To a cold solution of COM-L_3(NHMe) (20.8 mg, 0.058 mmol) dissolved in dichloromethane (1mL) in an ice-water bath was added triethylamine (48 μL, 0.35 mmol) and triphosgene (17.1 mg, 0.058 mmol). The mixture was stirred at 5°C for 40 min, concentrated *in vacuo,* and purified by flash silica column chromatography by quick elution with 2% CH_3OH/CH_2Cl_2. The desired product COM-L_3(COCl) was isolated as a pale yellow solid (26.5 mg), and used in the next step reaction immediately. R_f (2% v/v CH_3OH/CH_2Cl_2) = 0.64.

COM-L$_3$-4OHT (8). To 4OHT (24.5 mg, 63 μmol) dissolved in DMF (0.6 mL) cooled in an ice-water bath were added triethylamine (24 μL, 173 μmol), 4-dimethylaminopyridine (3.5 mg, 28.8 μmol) and COM-L_2(COCl) (26.5 mg, 67 μmol) dissolved in dichloromethane (0.6 mL). This mixture was stirred in the ice bath (5°C) for 1 h and then at room temp overnight in the dark. It was concentrated *in vacuo*, and the residue was purified by flash silica column chromatography by eluting with 2–10% CH_3OH/CH_2Cl_2. The desired product **8** COM-L_3-4OHT

was isolated as a pale yellow solid (16.4 mg, 32%). R_f (5% v/v CH_3OH/CH_2Cl_2) = 0.22. Purity (UPLC): 95%. MS (ESI) *m/z* (relative intensity, %) = 775.5 (100 %) $[M + H]^+$. HRMS (ESI) calcd for $C_{46}H_{54}N_4O_7$ $[M + H]^+$ 775.4065, found 775.4062. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33–7.27 (m, 1H, ArH(COM)), 7.24–7.07 (m, 8H, ArH(4OHT)), 6.90–6.86 (m, 1H, ArH (4OHT)), 6.84–6.82 (m, 1H, ArH (4OHT)), 6.75–6.73 (m, 2H, 2ArH (4OHT)), 6.58–6.50 (m, 3H, 1ArH (4OHT) and 2ArH (COM)), 6.12–6.04 (m, 1H, CHC=O (COM)), 5.30–5.19 (m, 2H, CH_2O (COM)), 4.11–4.09 (t, *J* = 5 Hz, 1H, 1/2CH_2O (4OHT)), 3.95–3.93 (t, *J* = 5 Hz, 1H, 1/2CH_2O (4OHT), 3.64–3.45 (m, 4H, 2CH_2NCO), 3.42–3.38 (m, 2CH_2N (COM)), 3.15–2.99 (m, 6H, 2CH_3NCO), 2.79 (m, 1H, 1/2 CH_2N(4OHT)), 2.69 (m, 1H, 1/2CH_2N (4OHT)), 2.50–2.42 (m, 2H, CH_2 (4OHT)), 2.38 (s, 3H, CH_3N (4OHT), 2.32 (s, 3H, CH_3N (4OHT)), 1.21–1.19 (t, *J* = 5 Hz, 6H, 2CH_3 (COM)), 0.93–0.87 (m, 3H, CH_3 (4OHT)). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.66, 150.70, 150.32, 142.33, 135.99, 135.52, 131.94, 131.72, 130.67, 130.40, 129.67, 127.90, 127.87, 126.14, 126.03, 124.53, 124.42, 121.22, 120.35, 114.13, 113.40, 108.67, 106.05, 97.82, 65.67, 65.41, 62.66, 62.51, 47.55, 46.93, 46.84, 45.70, 45.65, 44.75, 35.32, 35.02, 29.12, 28.98, 13.55, 12.45 ppm.

In vitro Cell Studies

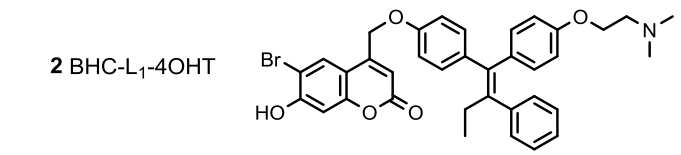
Single-photon Uncaging *In Vitro*.[2, 6] Kinetic studies for 4OHT release induced by long wavelength UVA light exposure were performed using a UV lamp (Spectroline® XX-15A; with a maximal intensity of emission at 365 nm). Typically, an aqueous solution of **2** BHC-L_1-4OHT (78 μM in 20% (v/v) aqueous methanol) was prepared and exposed to light at a distance of 5 cm. A series of aliquots were taken as a function of exposure time (0–15 min), and analyzed by UPLC and UV–vis absorption spectrometry to monitor the amount of 4OHT release and the byproduct formed by photon-catalyzed Claisen rearrangement. Some of the fractions were further characterized by mass spectrometry (ESI, HPLC–MS) to identify the molecular mass of the products. Quantum efficiency (Φ) for the uncaging reaction was calculated using the photon flux ($q_{n,p}$) of the UV lamp determined by the standard protocol for ferrioxalate actinometry.[7]

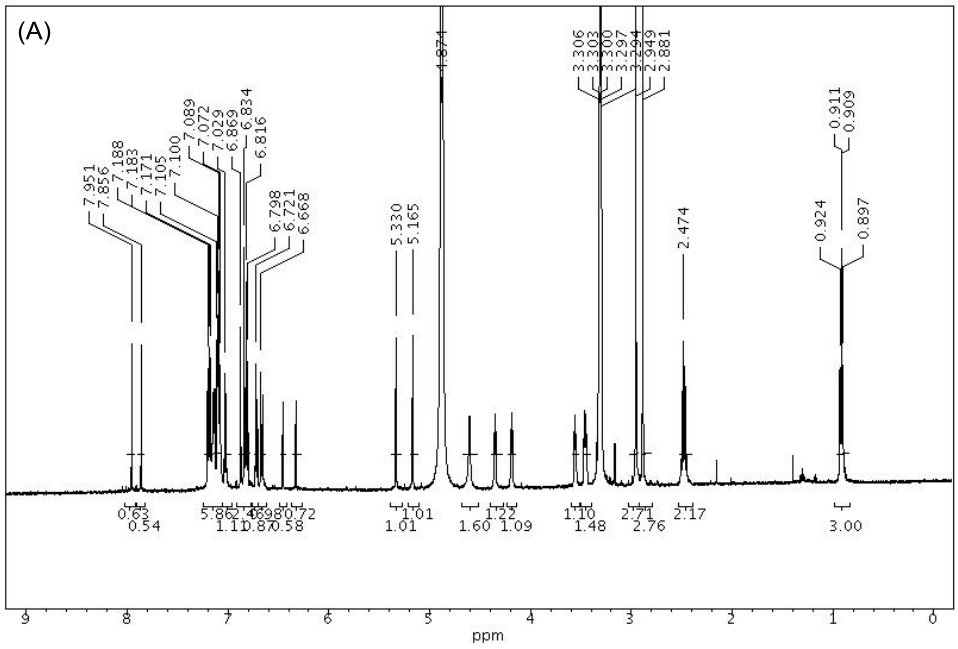
Photocontrolled Induction of GFP Expression *In Vitro*.[2] MEF UbcCre-ERT2 mTmG cells were seeded at 2.5×10^5 cells/well in growth media on two 8-chamber coverglass slides (Thermo-scientific) overnight. The growth media was removed from the cells, and replaced with 250 μL of 4OHT or the caged compounds **2** BHC-L_1-4OHT, or **7** BHC-L_3-4OHT, each at 250 nM. After incubation at 37°C for 5 min, one slide was irradiated under a UVA lamp (365 nm) (Spectroline® XX-15A) for 3 min, while the other was kept in the dark at room temperature for 3 min. Additional fresh media (250 μL) was added to each well, and the cells were incubated for 24 h at 37°C. The cells were washed 2 times with PBS, fixed in 4% (w/v) paraformaldehyde in PBS for 10 min, dried, and mounted in ProLong gold with DAPI. Images were taken on a Leica inverted SP5X confocal fluorescence microscope (Leica Microsystems) with 40 × magnification in sequential scanning mode (mTomato: ex 555 nm, em 570–650 nm; GFP: ex 488 nm, em 500–540 nm; DAPI: ex 350 nm, em 450–490 nm).

Two-photon Uncaging *In Vitro*.[8] Studies for 4OHT release induced by two-photon exposure were performed using a Mai Tai BB (Spectra Physics) laser with 80 fs pulse width and a repetition rate of 80 MHz. Typically, an aqueous solution of **1**, **2**, **3**, **6** and **7** was made and a droplet hung from a coverslip. The laser focal point was scanned through the droplet at varying powers for varying time points. The uncaged compound solution was then diluted into media and added to cultured UbcCreERT2 mTmG MEFs for 24 h. The cells were subsequently analyzed for GFP expression by flow cytometry. Briefly, approximately 200,000 treated MEFs were lifted, filtered and resuspended in PBS with 2% (w/v) fetal calf serum (FCS). These were then analyzed using an LSR II Fortessa (BD) for expression of GFP.

II. Copies of NMR (^{1}H, ^{13}C) Spectra

2 BHC-L_1-4OHT





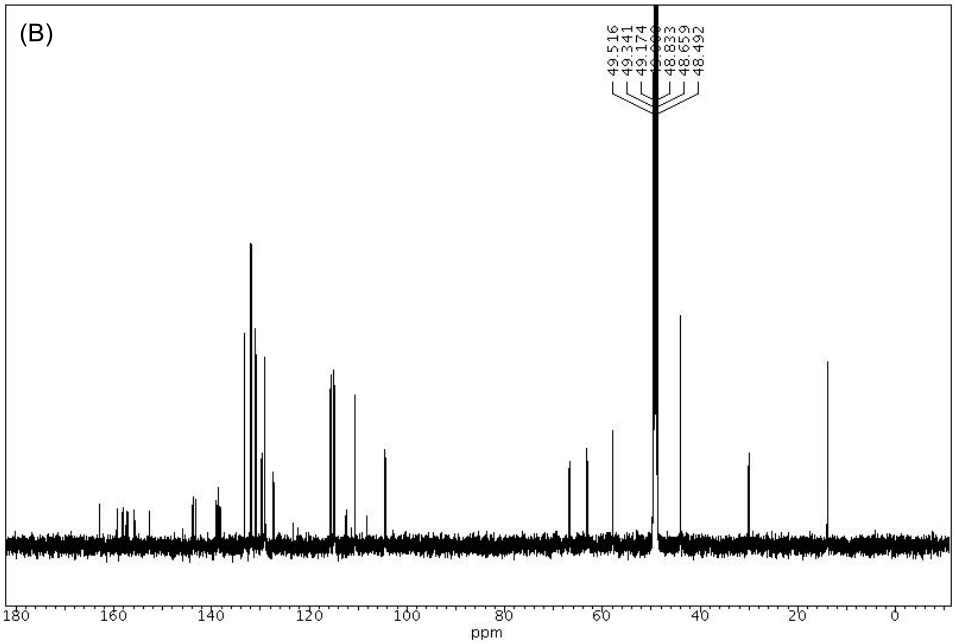


Figure S1. (A, B) ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) spectra of **2** BHC-L_1-4OHT.

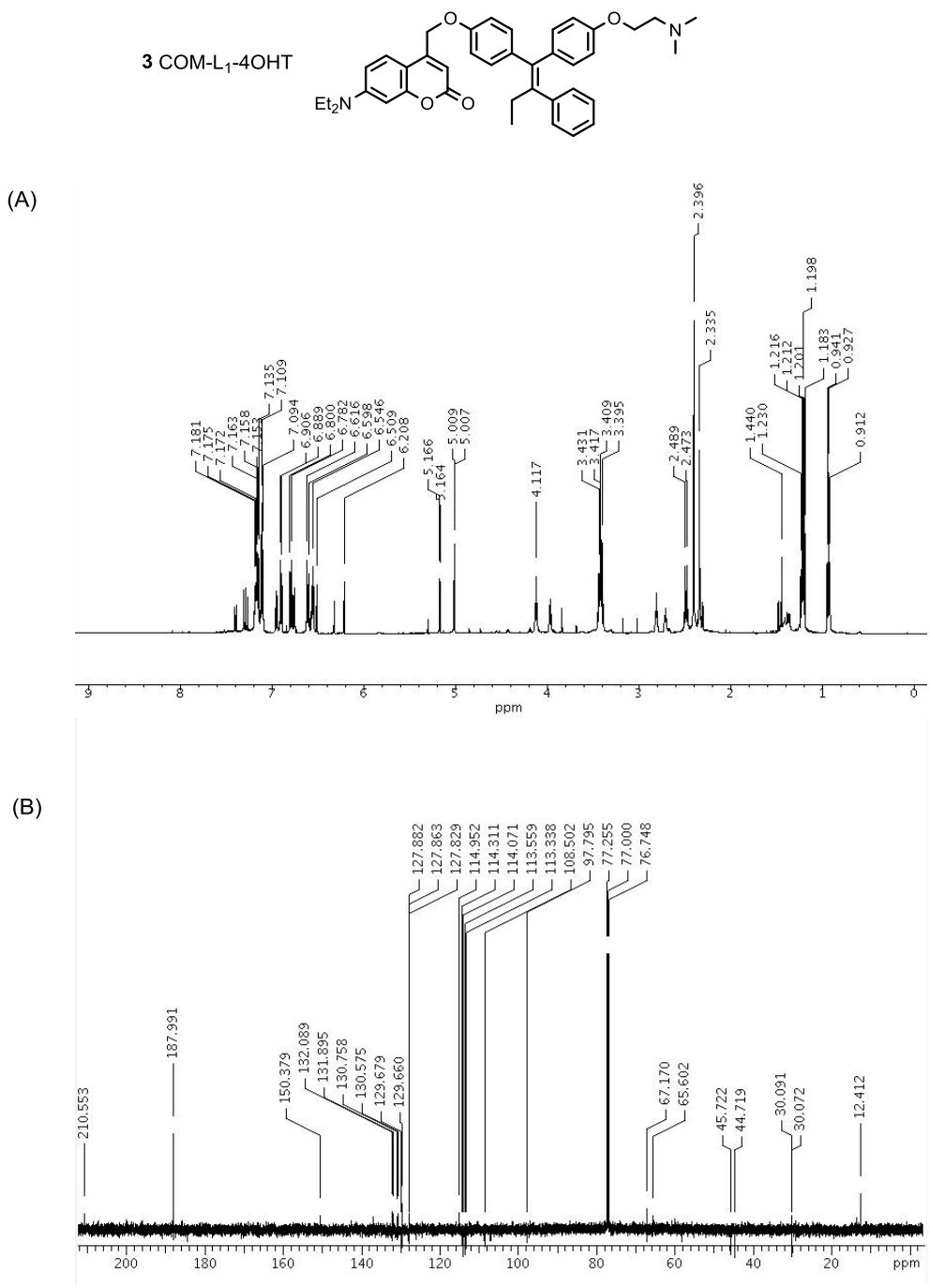


Figure S2. (A, B) ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3** COM-L_1-4OHT.

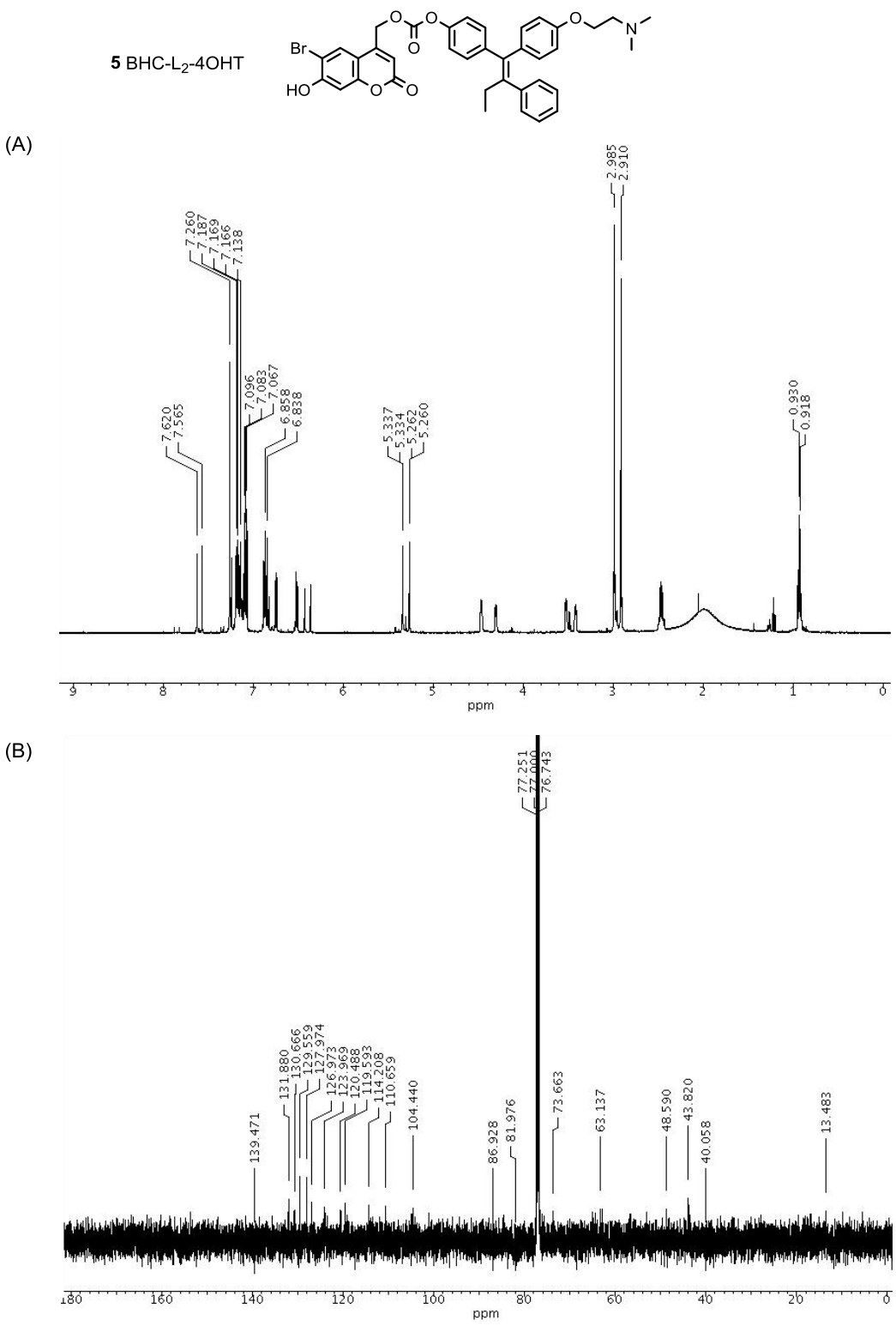


Figure S3. (A, B) ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **5** BHC-L_2-4OHT.

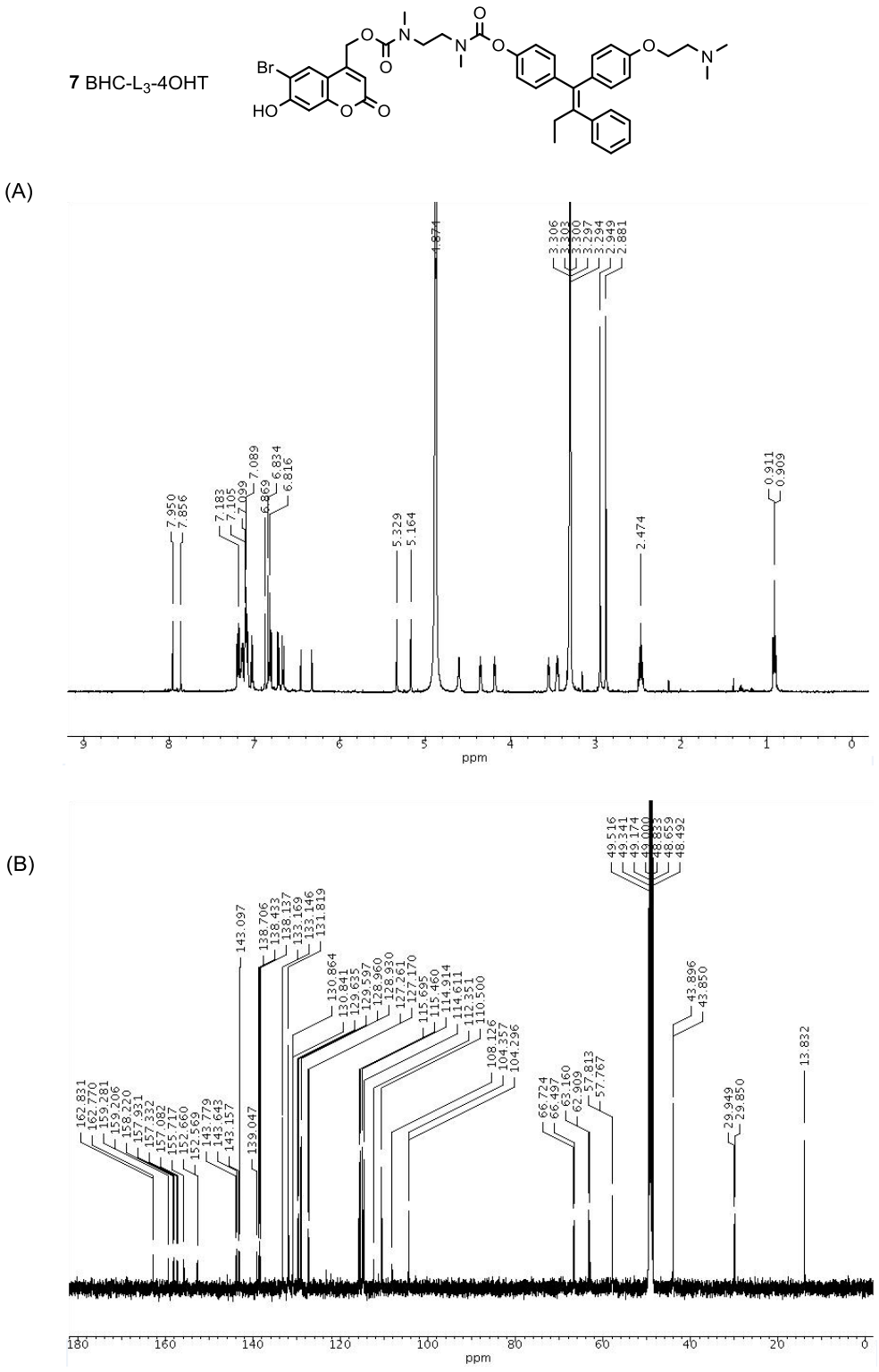


Figure S4. (A, B) ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) spectra of **7** BHC-L_3-4OHT.

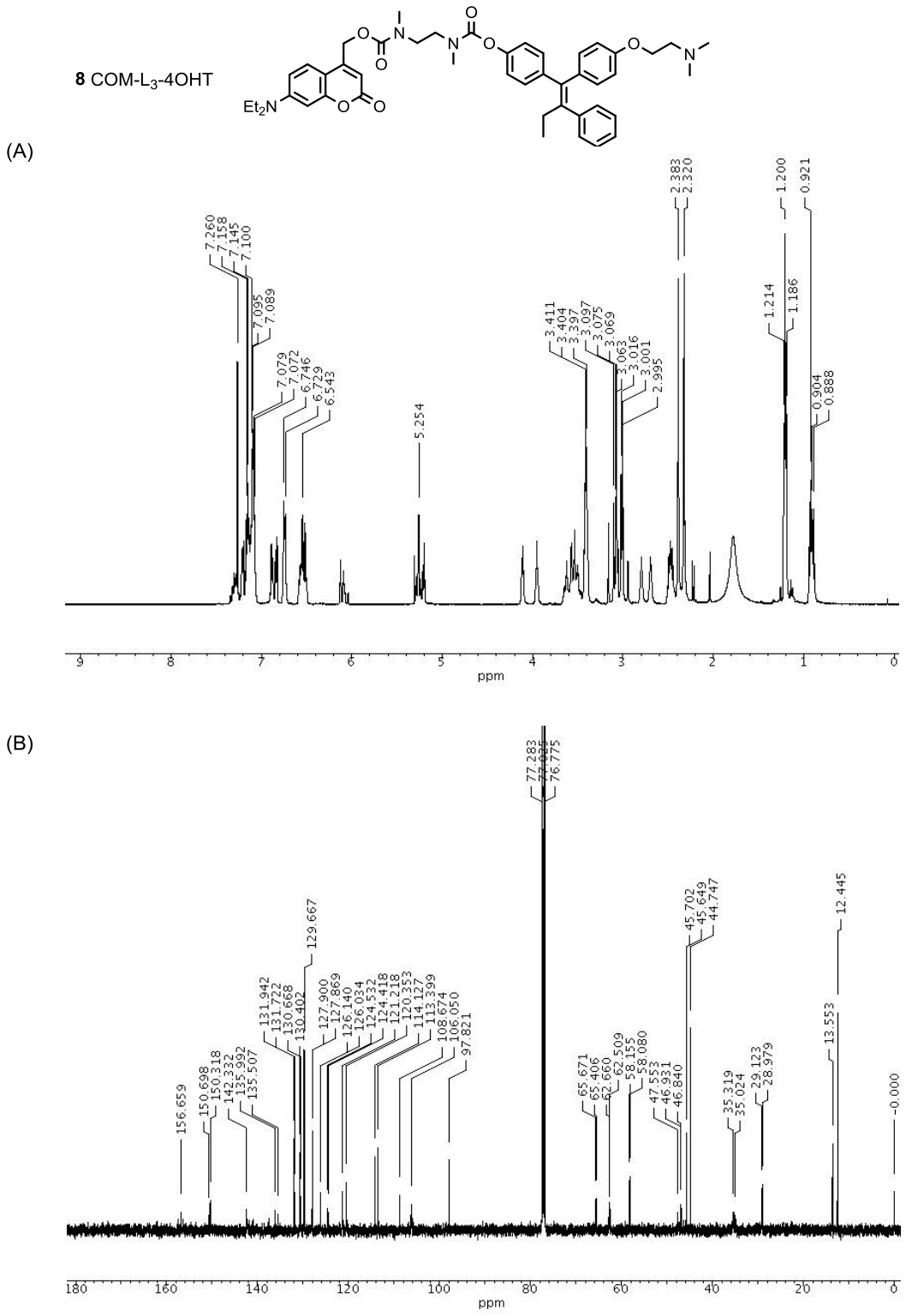


Figure S5. (A, B) ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **8** COM-L_3-4OHT.

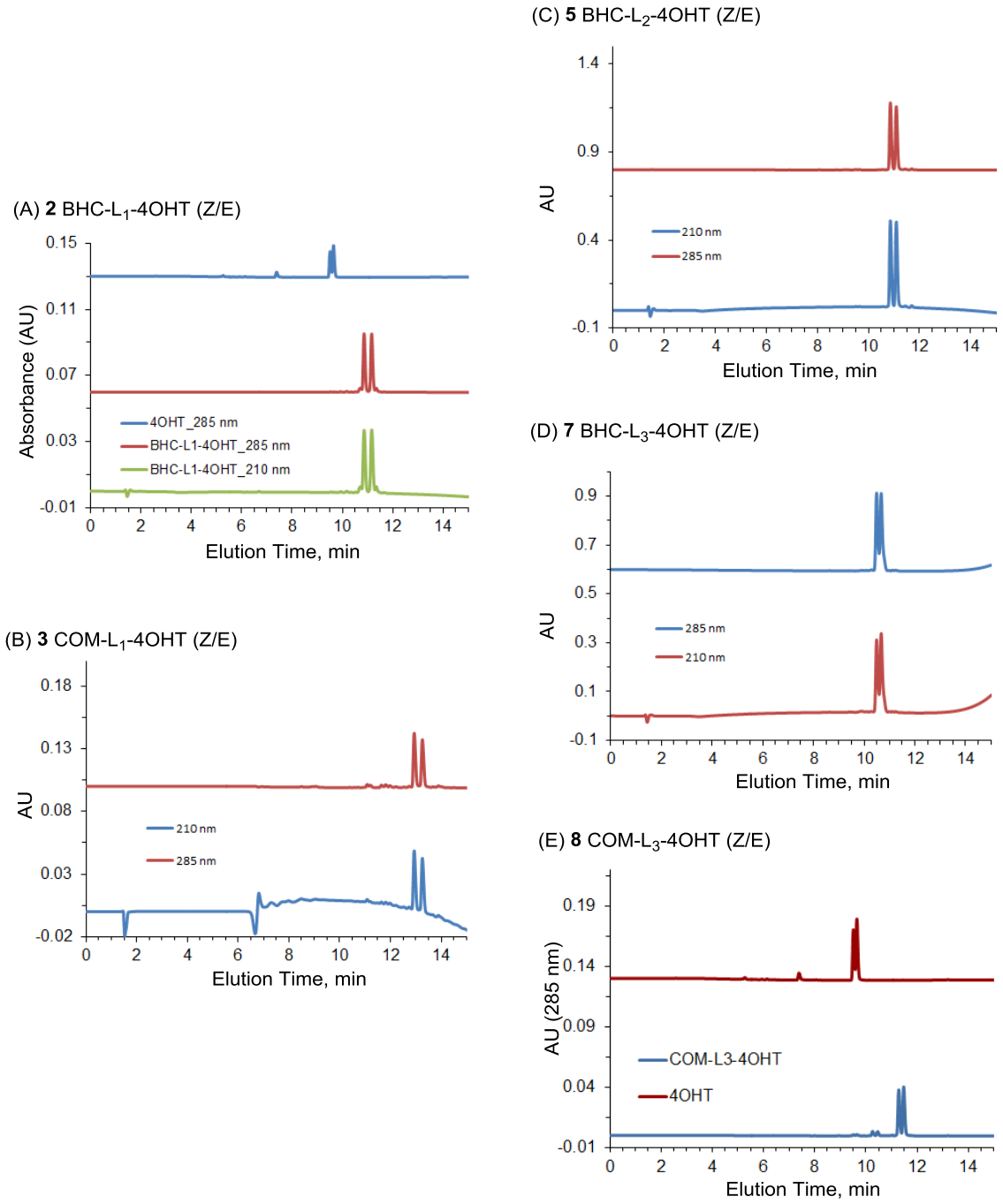


Figure S6. UPLC traces of caged compounds **2** BHC-L_1-4OHT, **3** COM-L_1-4OHT, **5** BHC-L_2-4OHT, **7** BHC-L_3-4OHT, and **8** COM-L_3-4OHT. See ref 2 for ONB caged compounds **1** and **6**.

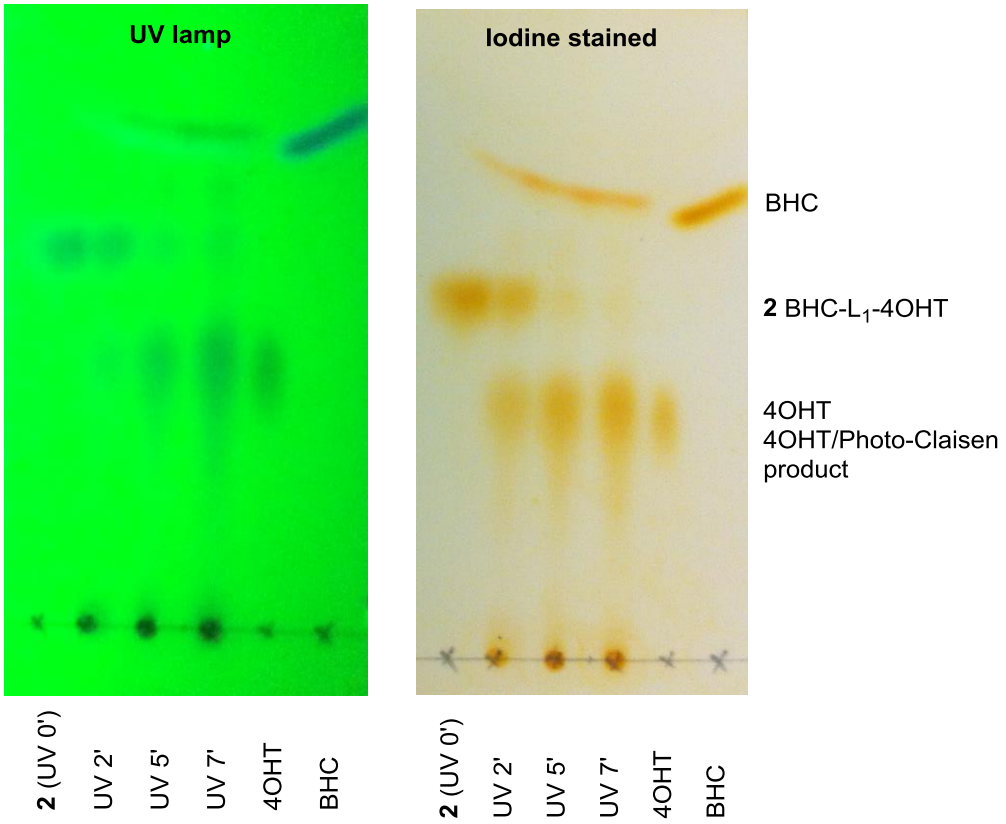


Figure S7. TLC analysis of reaction products after photolysis (2, 5 and 7 min; 365 nm) of **2** BHC-L_1-4OHT, showing the lack of resolution between free 4OHT and the photo-Claisen byproduct. Visualization method: UV lamp (left), iodine staining (right).

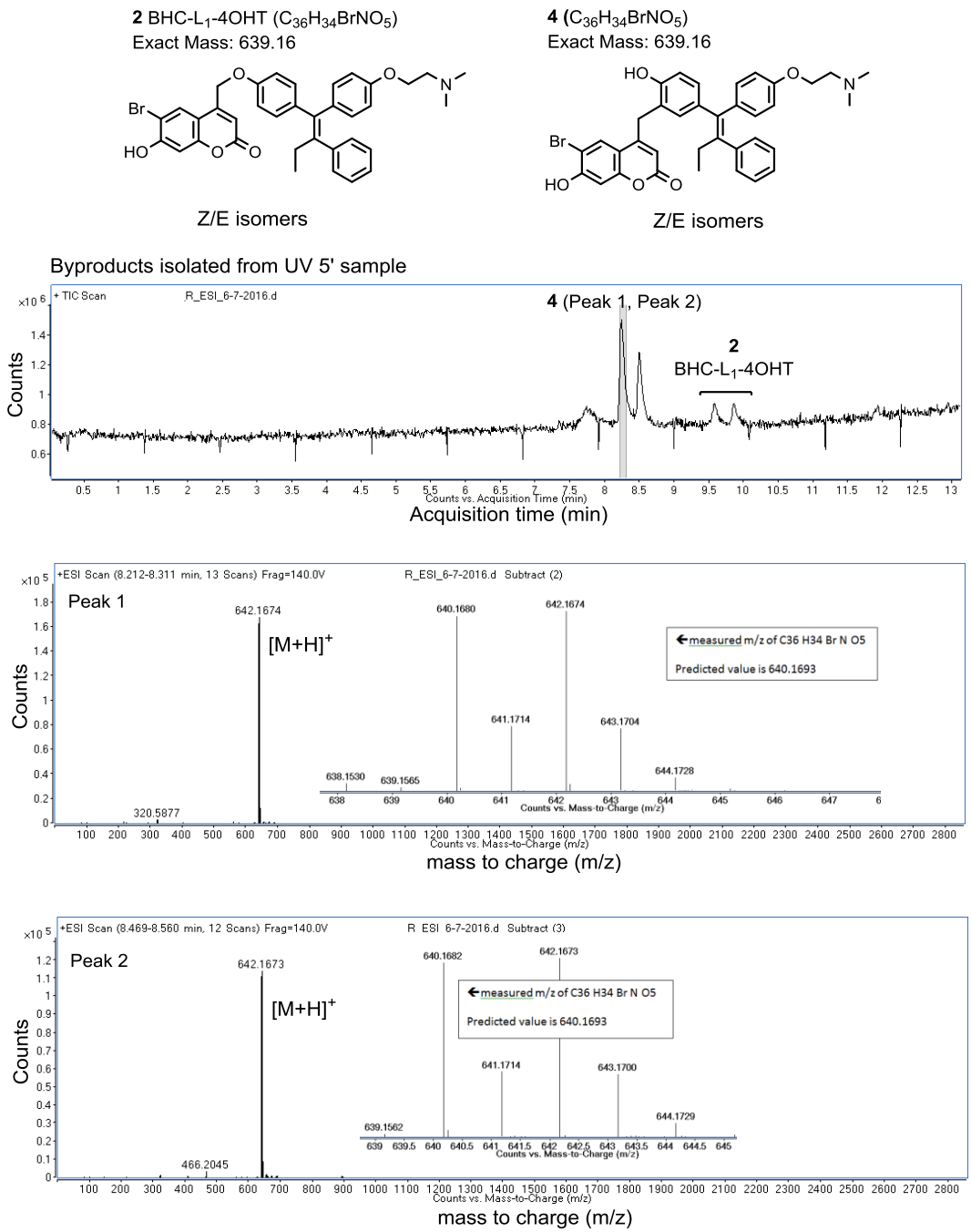


Figure S8. (Top) Structures of **2** BHC-L_1-4OHT and **4** the proposed photo-Claisen byproduct, and (Bottom) LC-MS analysis of the isolated byproduct (the same sample shown in Figure 2C; bottom), confirming that both peak 1 and peak 2 (Z/E isomers) are identical in their mass as anticipated from **4**.

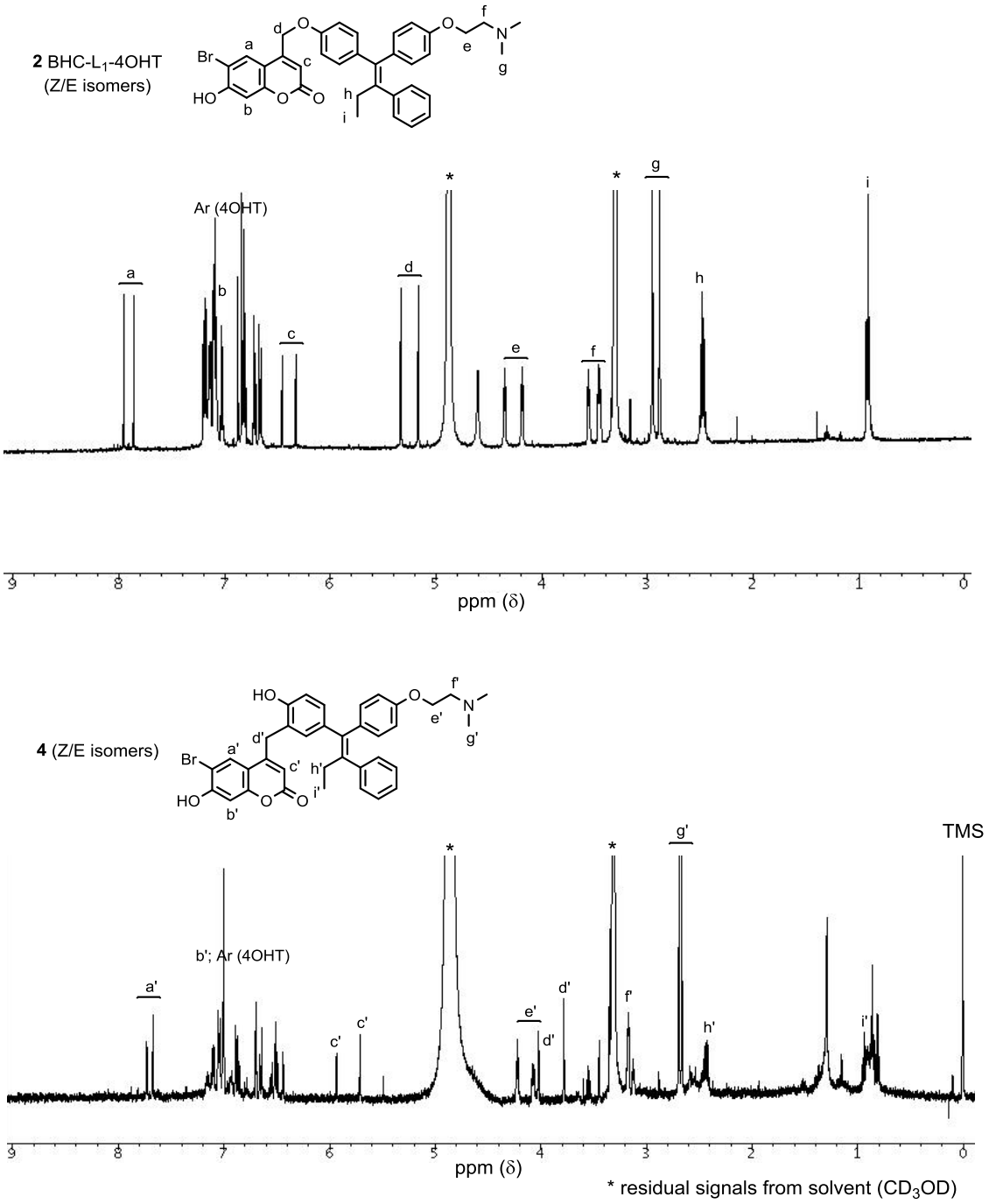


Figure S9. ^{1}H NMR (500 MHz, CD_3OD) spectra of **2** BHC-L_1-4OHT (Z/E isomers) and its photoarrangement byproduct **4** (Z/E isomers).

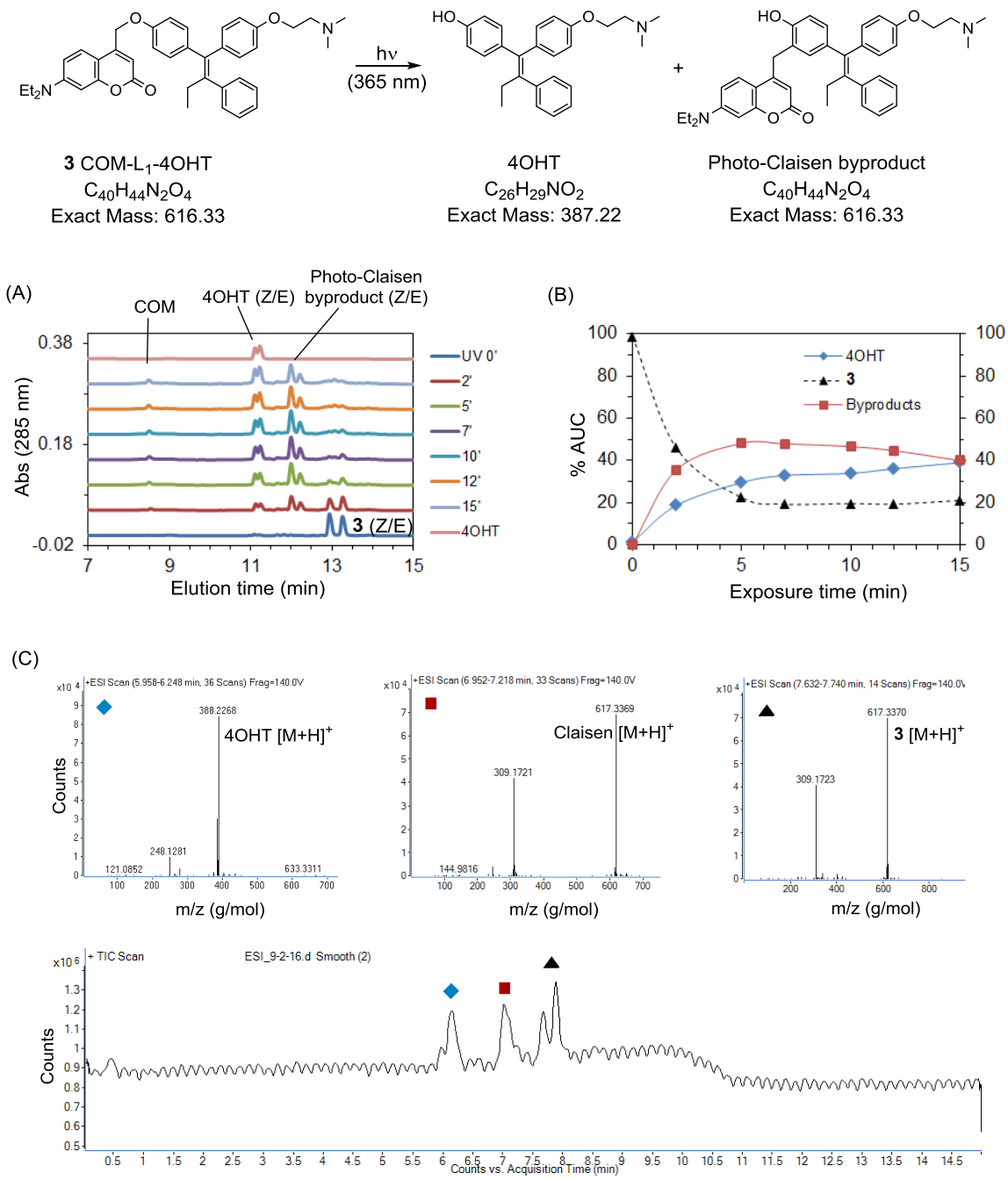


Figure S10. Mechanism of photolysis of **3** COM-L_1-4OHT that accounts for the release of 4OHT in addition to generation of the photo-Claisen byproduct. (A) Overlaid UPLC traces acquired after photolysis of **3** (78 μM in 20% v/v aq. methanol); (B) A plot of area under curves (%AUC) for 4OHT, **3** and the byproduct as a function of exposure time; (C) LC-MS analysis after 10 min photolysis of **3**, confirming the masses anticipated for 4OHT and the photo-Claisen byproduct.

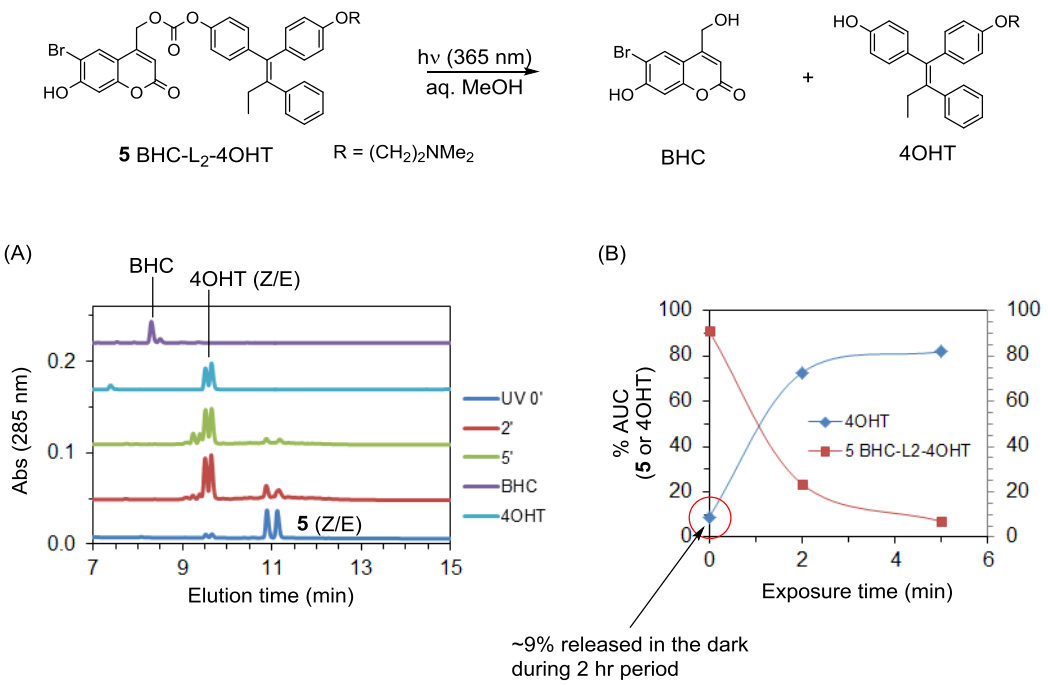


Figure S11. Mechanism of photolysis of **5** BHC-L_2-4OHT that accounts for release of 4OHT. (A) Overlaid UPLC traces acquired after photolysis of **5** (125 μM in 20% v/v aq. methanol). (B) A plot of %AUC of 4OHT or remaining **5** against UV exposure time.

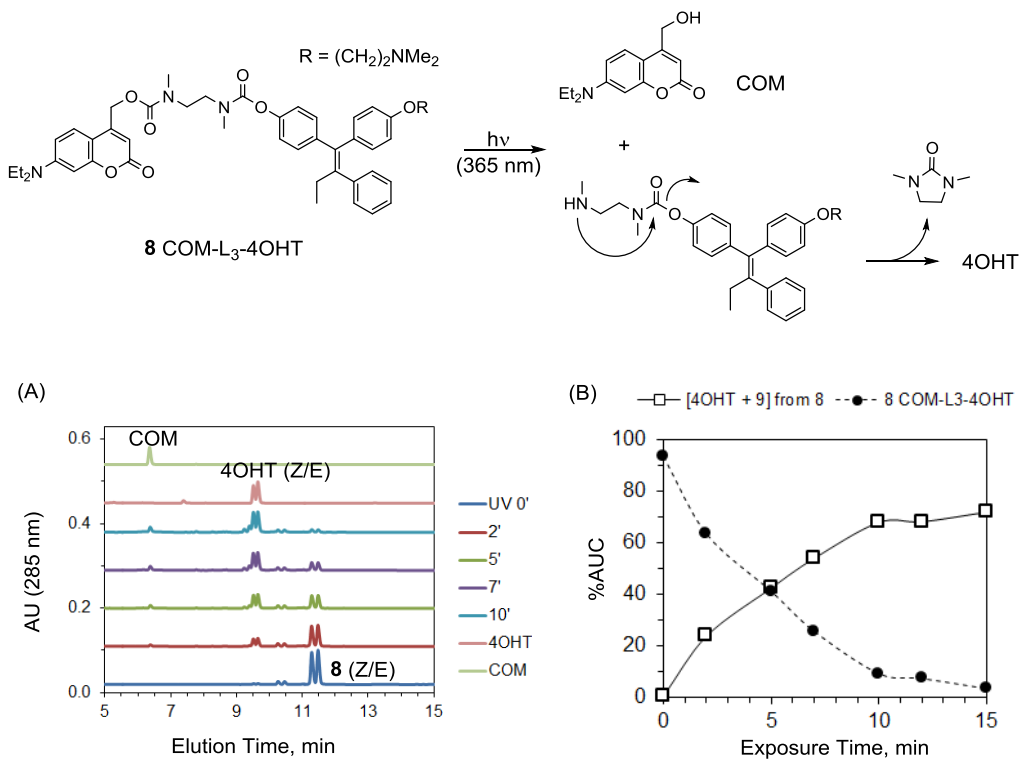


Figure S12. (Top) Mechanism of photolysis of **8** COM-L_3-4OHT that accounts for the release of 4OHT via self-immolation of the spacer. (Bottom) Overlaid UPLC traces acquired after photolysis of **8** (129 μM in 20% v/v aq. methanol) as a function of UV exposure time.

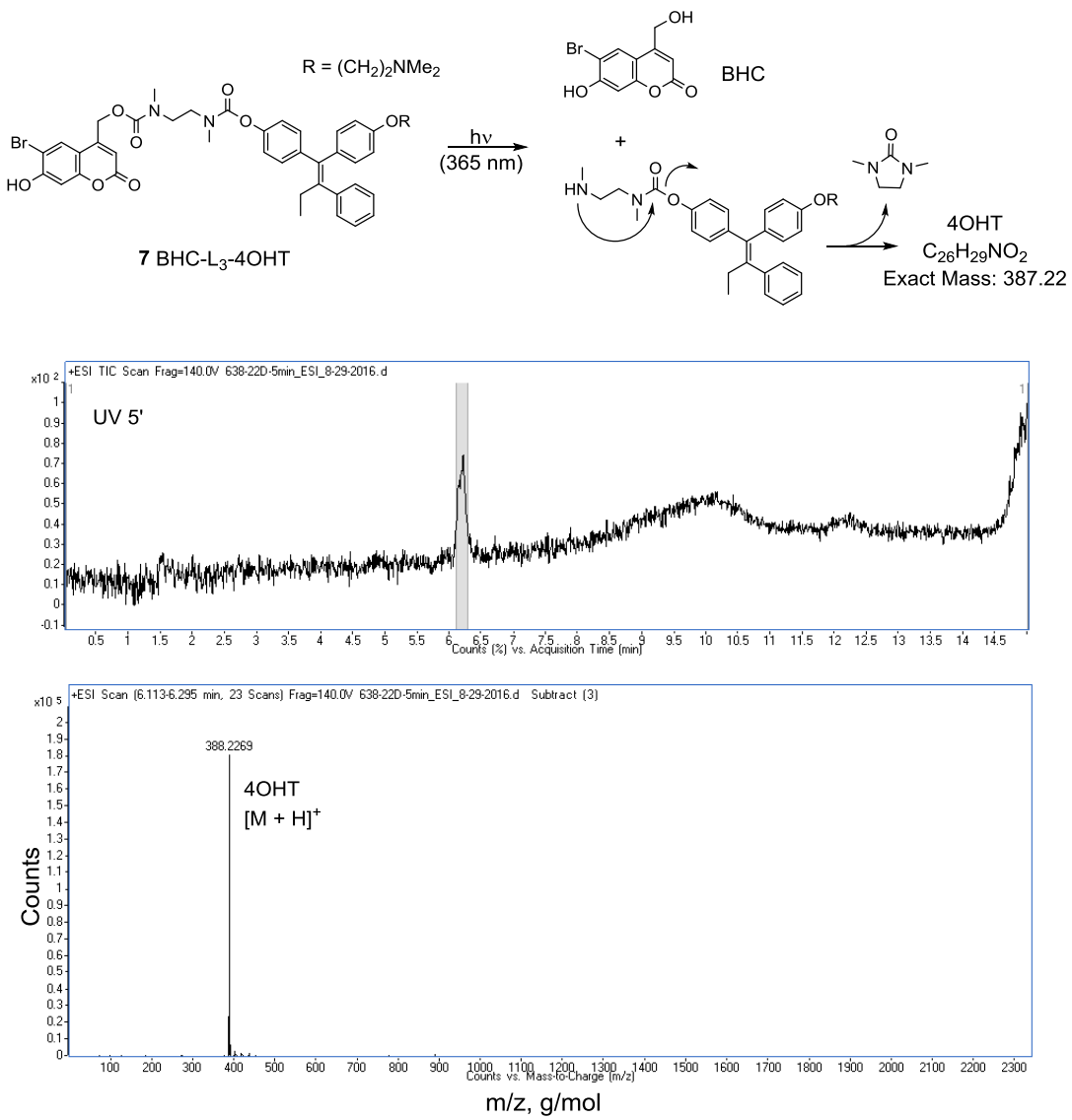


Figure S13. (Top) Mechanism of photolysis of **7** BHC-L_3-4OHT that accounts for the release of 4OHT via self-immolation of the spacer. (Bottom) An LC-MS trace acquired after photolysis (5 min) of **7** (110 μM in 20% v/v aq. methanol).

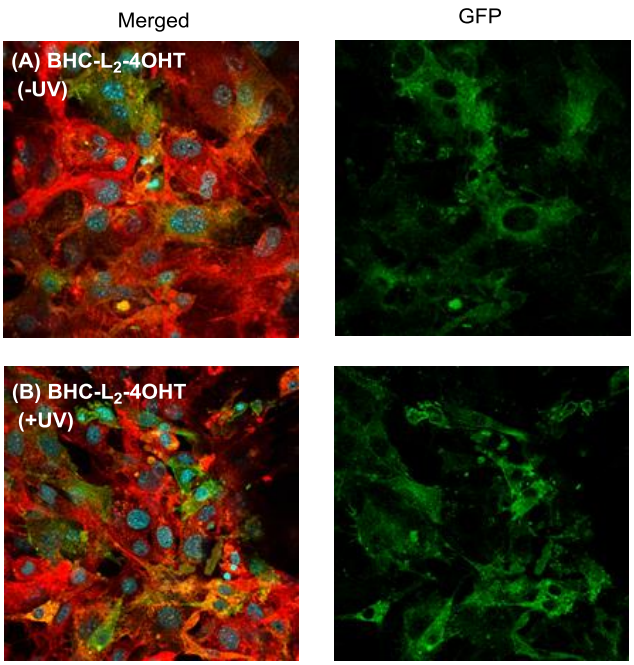


Figure S14. Confocal fluorescence microscopy of Cre-ERT2 mediated GFP expression in UbcCreERT2 mTmG MEFs demonstrating the lack of UV mediated control of **5** BHC-L_2-4OHT activation. MEFs treated with 250 nM of **5** BHC-L_2-4OHT (A) without or (B) with UVA exposure for 3 min. TdTomato fluorescence (red), and GFP fluorescence (green) are shown. Nuclei were labeled with DAPI (blue).

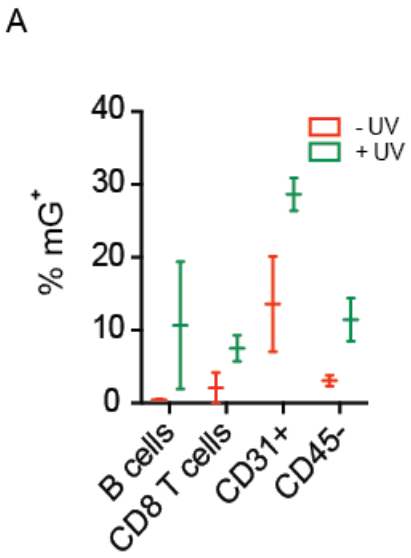
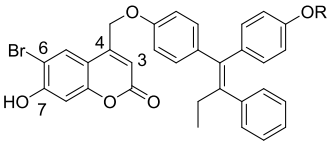
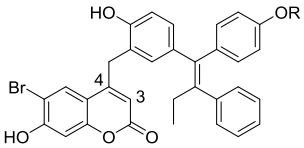
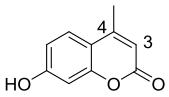
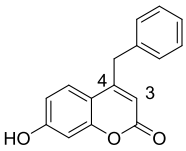
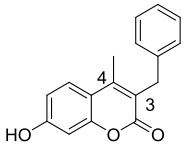
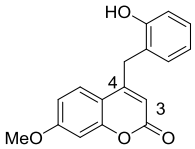
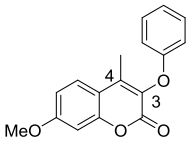


Figure S15. In a preliminary experiment, 125 μL of 1 mM **7** BHC-L_3-4OHT was administered to UbcCreERT2 mTmG mice. Three hours post injection, the right ear was exposed to 365 nm irradiation for 1 hour. At 24 hours, mice were sacrificed and the ears (A) were enzymatically dissociated and analyzed by flow cytometry for expression of GFP. n = 2. Experiment performed once. All experimental procedures conformed to ethical principles and guidelines approved by the University of California, San Francisco (UCSF) Institutional Animal Care and Use Committee.

Table S1. Comparison of ^{1}H NMR spectral data (δ, ppm) between coumarin compounds substituted with a benzyl group at the C3 and C4 position.

Coumarin Molecules	C3	C4	Solvent (reference)
2 (Z/E isomers) 	6.2, 6.45 (CH)	5.33, 5.16 (CH_2)	CD_3OD (present study)
4 (Z/E isomers) 	5.85, 5.95 (CH)	3.8, 4.0 (CH_2)	CD_3OD (present study)
	6.1 (CH)	2.36 (CH_3)	DMSO-d_6 (ref [9])
	NR*	4.13 (CH_2)	DMSO-d_6 (ref [9])
	-	2.38 (CH_3)	DMSO-d_6 (ref [9])
	5.85 (CH)	4.04 (CH_2)	DMSO-d_6 (ref [10])
	-	2.07 (CH_3)	DMSO-d_6 (ref [10])

*NR = not recorded in the literature

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