Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Fluorogenic Diazaborine Formation of Semicarbazide with Designed Coumarin Derivatives

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I. General Considerations

Chemicals and cells

Trifluoroacetic acid, DCM, dimethylformamide, tetrahydrofuran, N-hydroxysuccinimide, semicarbazide, fetal bovine serum (FBS) and LB Broth were purchased from Fisher Scientific. Boc-D-Dap-OH and dicyclohexylcarboiimide were purchased from Chem-Impex International. *Staphylococcus aureus* (ATCC 6538) was purchased from ATCC as a lyophilized cell pellet. N-Boc-Bromoethylamine, fluorescein isothiocyanate, potassium carbonate, 2-bromo hydroxybenzaldehyde, potassium acetate, triethylamine and dry dioxane and [1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) were purchased from Sigma Aldrich. Diethanolamine was purchased from Alfa Aesar. B_2Pin_2 was purchased from Frontier Scientific. All of the conjugation experiments were performed in PBS (1x, pH 7.4) buffer.

Instruments

UV spectra were collected on a Nanodrop UV-vis spectrometer (Thermo Scientific, Waltham, MA, USA). Fluorescence data were collected on a Cary Eclipse fluorescence spectrophotometer with slit width set to 5 nm. Voltages varied, as noted in the designated fluorescence sections. NMR data were collected on a Varian 600 MHz NMR spectrometer. Mass-spec data were generated using an Agilent 6230 LC-TOF mass spectrometer. HPLC purification and analysis was performed using a Waters Alliance 2695 system. Fluorescence images were taken on a Zeiss Axio Observer A1 inverted microscope. Flow cytometry data were collected using a BD FACSAria cell sorter housed in the Biology department at Boston College.

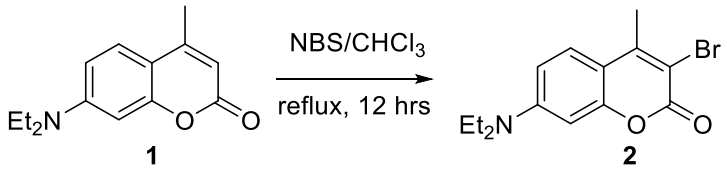
General methods for LC-MS and HPLC analysis

Method for LC-MS: (Agilent 6230 LC TOF): Agilent Extend C18 (1.8 µm, 2.1× 50 mm) analytical column using mobile phase water-acetonitrile-(0.1% formic acid) with a flow rate 0.2 mL/min. Gradient used: isocratic 5% CH_3CN for 3 min, then gradient from 5% to 85% CH_3CN over 15 min (3-18 min), then gradient from 85% to 95% CH_3CN over 2 min (18-20 min), then isocratic 95% CH_3CN for 2 min (20-22 min).

Method for semi-preparative HPLC (Waters Alliance 2695 HPLC): Phenomenex C18 (10 µm, 10 × 250 mm) semi-preparative column using water-acetonitrile-(0.1% trifluoroacetic acid) mobile phase with a flow rate of 5 mL/min. Gradient used: isocratic 0% CH_3CN for 5 min (0-10 min), then gradient from 0 to 50% CH_3CN over 30 minutes (5-40 min).

Method for analytical HPLC: (Waters Alliance 2695 HPLC): Phenomenex C18 (5 µm, 2.0 × 150 mm) analytical column using mobile phase water-acetonitrile-(0.1% formic acid) with a flow rate of 0.2 mL/min. Gradient used: isocratic 5% CH_3CN for 5 min, then gradient from 5 to 95% CH_3CN over 14 min over 14 m in (5-19 min), then isocratic 95% CH_3CN for 2 min (19-21 min).

II. Synthesis and Characterization of the Coumarin Analogues

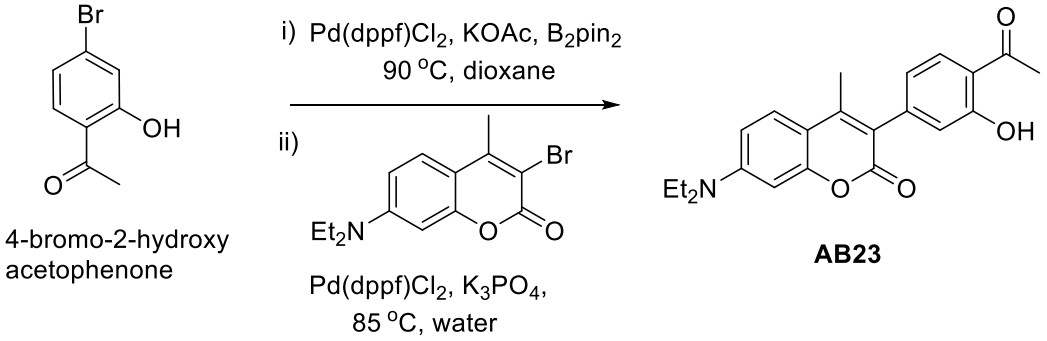


A mixture of 7-(diethylamino)-4-methyl-2H-chromen-2-one (**1**) (2.5 g, 13.5 mmol) and N-bromosuccinimide (3.75 g, 19.5 mmol, 1.5 eq) was added in chloroform (150 mL). Then, a catalytic amount of benzoyl peroxide (175 mg, 0.67 mmol, 0.05 eq) was added to the reaction mixture and refluxed for 12 h. The solvent was removed under a vacuum and the residue was digested in water and ethyl acetate (1:1; total volume 400 mL). Product was extracted in ethyl acetate (200 mL × 2). The combined organic layer was washed with brine (200 mL) and dried over sodium sulfate. After removing the solvent, the crude product was purified on silica gel column using ethyl acetate:hexane (1:9) as eluent to yield a yellow solid product (3.09 g, 74%). The product formation was confirmed by ^{1}H-NMR and mass, which matches with the previous reported data (*Chem. Eur. J.* **2012**, *18*, 4953 –4964).

^{1}H NMR (Chloroform-*d*, 500 MHz): *δ* 7.57 (d, 1H, *J* = 8.8 Hz), 6.92 (dd, 1 H, *J* = 2.5, *J* = 8.9 Hz), 6.85(d, 1 H, *J* = 2.5 Hz), 3.91 (s, 3 H), 2.63 (s, 3 H).

^{13}C NMR (Chloroform-*d* 126 MHz): *δ* 158.08, 154.41, 151.45, 150.61, 128.20, 126.03, 108.99, 105.58, 97.22, 44.77, 19.08, 12.42.

MS-ESI^{+}: *m/z* calculated for $C_{14}H_{16}BrNO_2$ $[M+H]^{+}$ 310.0443, found 310.0425.



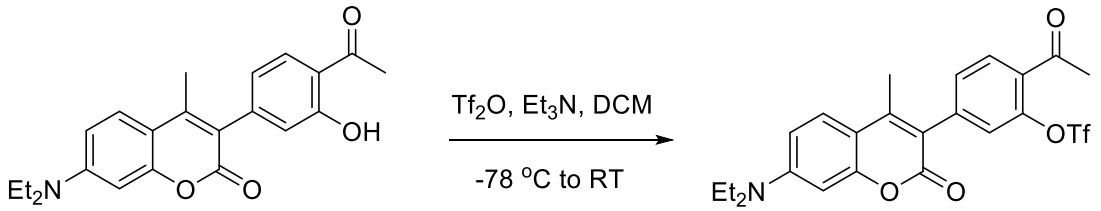
4-bromo-2-hydroxy acetophenone (215 mg, 1 mmol), B_2Pin_2 (280 mg, 1.1 mmol), $Pd(dppf)Cl_2$ (22 mg, 0.03 mmol, 3 mol %) and potassium acetate (294 mg, 3 mmol) were dissolved in anhydrous dioxane (5 mL). The reaction mixture was bubbled with argon for 10 minutes and it was allowed to stir at 90 °C for 90 minutes. Completion of the reaction was monitored by LC-MS. The reaction mixture was cooled to room temperature, and then **2** (216 mg, 0.7 mmol), $Pd(dppf)Cl_2$ (22 mg, 0.03 mmol, 3 mol %), K_3PO_4 (445 mg, 2.1 mmol) and water (1mL) were added subsequently in the same pot. Further, reaction mixture was heated to 85 °C for overnight. The reaction mixture was filtered through sintered funnel and digested in water (100 mL). The product was extracted with ethyl

acetate (3 × 100 mL). The combined organic layer was washed with brine (100 mL) and dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified on silica gel using hexane:ethyl acetate (4:1) as eluent. The desired product was obtained as a yellow solid (170 mg, 62%).

^{1}H NMR (Chloroform-*d*, 500 MHz): *δ* 12.30 (s, 1H), 7.80-7.78 (d, *J* = 7, 1H), 7.47-7.45 (d, *J* = 7.4, 1H), 6.91-6.89 (m, 2H), 6.64-6.62 (dd, *J* = 7.2, *J* = 4, 1H), 6.54 (d, *J* = 3.8, 1H), 3.46-3.41 (q, *J* = 6.8, 4H), 2.65 (s, 3H), 2.26 (s, 3H), 1.24-1.21 (t, *J* = 6.1, 6H).

^{13}C NMR (Chloroform-*d* 126 MHz): *δ* 204.13, 162.13, 161.24, 155.22, 150.57, 148.87, 144.17, 130.53, 126.25, 121.51, 120.44, 118.79, 108.77, 97.37, 44.77, 26.65, 24.83, 16.25, 12.44.

MS-ESI$^+$: *m/z* calculated for $C_{22}H_{23}NO_4$ $[M+H]^+$ 366.1707, found 366.1679.



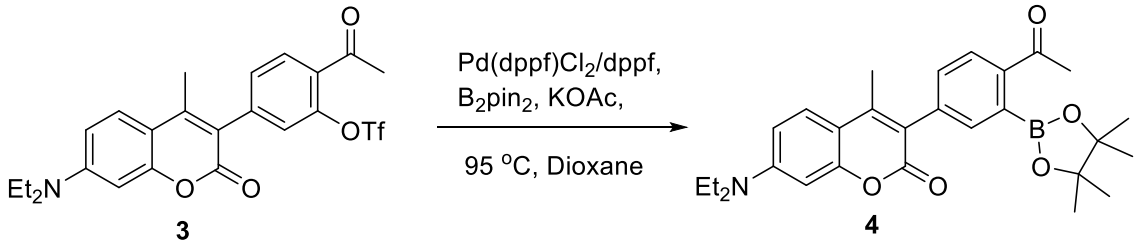
AB23 **3**

AB23 (100 mg, 0.274 mmol) was dissolved in anhydrous dichloromethane (1 mL) and triethylamine (160 µL, 1.15 mmol) was added while stirring. The reaction mixture was cooled to -78 °C and trifluoromethane sulfonic anhydride (102 µL, 0.6 mmol) was added slowly during 5 min. The reaction mixture was allowed to stir at room temperature for 0.5 hr under an argon environment. After that, the reaction was quenched with saturated sodium bicarbonate (1 mL) and the mixture was allowed to stir for 5 min. The product was extracted with dichloromethane (3 × 20 mL). The combined organic layer was washed with brine (30 mL) and dried over Na_2SO_4. After removing the solvent, the crude product was purified on silica gel column using ethyl acetate:hexane (1:4) as eluent to yield a dark red gummy product (126 mg, 93%).

^{1}H NMR (Chloroform-*d*, 600 MHz): *δ*, 7.87-7.86, (d, *J* = 7.1, 1H), 7.49-7.47 (m, 2H), 7.29 (d, *J* = 4.2, 1H), 6.63 (d, *J* = 7.2, 1H), 6.53 (d, *J* = 3.8, 1H) 3.46-3.42 (q, *J* = 6.8, 4H), 2.67 (s, 3H), 2.27 (s, 3H), 1.23-1.21 (t, *J* = 6.5, 6H).

^{13}C NMR (Chloroform-*d* 126 MHz): δ 196.28, 161.14, 155.36, 150.93, 149.76, 146.42, 141.71, 131.07, 130.52, 126.43, 125.20, 117.66, 108.98, 97.33, 60.36, 44.81, 29.47, 16.30, 12.43.

MS-ESI$^+$: *m/z* calculated for $C_{23}H_{22}F_3NO_6S$ $[M+H]^+$ 498.1196, found 498.1178.



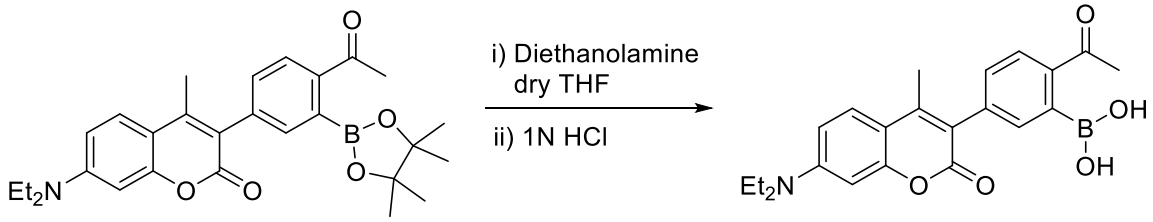
3 **4**

3 (100 mg, 0.2 mmol), B_2Pin_2 (126 mg, 0.5 mmol), $Pd(dppf)Cl_2$ (18 mg, 0. 020 mmol, 10 mol %), and potassium acetate (60 mg, 0.6 mmol) were was dissolved in anhydrous dioxane (1.2 mL). The reaction mixture was bubbled with argon for 10 minutes and it was allowed to stir at 95 ^{o}C for 50 minutes. Completion of the reaction was monitored by LC-MS. Then water (30 mL) was added to the reaction and the product was extracted with ethyl acetate (3 × 30 mL). The combined organic layer was washed with brine (100 mL) and dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified on silica gel using hexane:diethylether (11:9) as eluent. The desired product was obtained as a yellow gummy (68 mg, 72%).

^{1}H NMR (Chloroform-*d*, 500 MHz): *δ* 7.88-7.86 (d, *J* = 7.2, 1H), 7.43-7.41 (d, *J* = 6.5, 1H), 7.41-7.40 (m, 2H), 6.62-6.60 (dd, *J* = 7.1, *J* = 3.9, 1H), 6.53 (d, *J* = 3.8, 1H), 3.45-3.42 (q, *J* = 6.7, 4H), 2.61 (s, 3H), 2.20 (s, 3H), 1.46 (s, 12H), 1.22-1.20 (t, *J* = 6.4, 6H).

^{13}C NMR (Chloroform-*d* 126 MHz): δ 199.52, 161.36, 155.23, 150.42, 148.73, 139.83, 134.59, 131.26, 128.24, 126.13, 120.11, 109.29, 108.61, 97.44, 83.67, 77.00, 44.74, 30.30, 25.70, 24.86, 16.38, 12.45.

MS-ESI^{+}: *m/z* calculated for $C_{28}H_{33}BNO_4$ $[M\text{-}H_2O]^{+}$ 457.2424, found 457.2443.



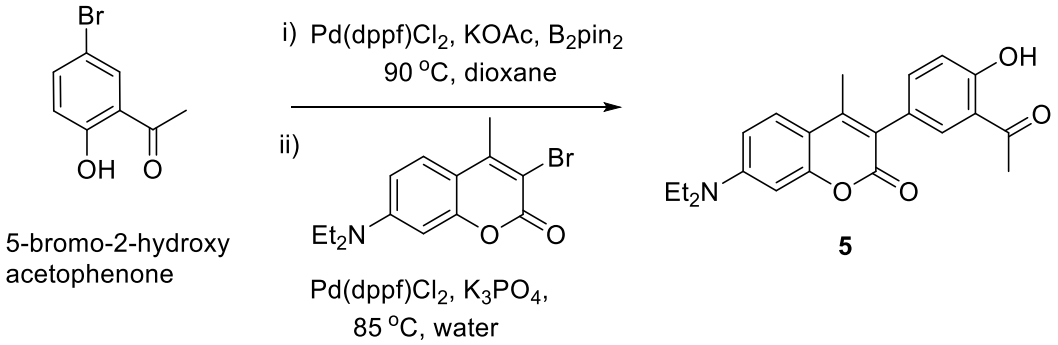
4 **AB21**

4 (60 mg, 0.126 mmol) was dissolved in anhydrous THF (500 µL) and diethanolamine (150 mg, 1.25 mmol) was added into the solution at room temperature. The reaction was allowed to stir for 3 hours and then quenched with 1N HCl (30 mL). Further, the solution was neutralized using $NaHCO_3$ (5%) solution in water. Then the product solution was directly purified through HPLC. The HPLC purification with acetonitrile/water (0.1% TFA) (Gradient: 5% ACN to 95% ACN during 50 min) mixture yielded the pure product as a yellow powder after lyophilization (72%, 35 mg).

^{1}H NMR (Methanol-d_4, 600 MHz): *δ* 8.11-8.10 (d, *J* = 6.2, 1H), 7.63-7.61 (d, *J* = 7.2, 1H), 7.46-7.45 (d, *J* = 6.4, 1H), 7.33 (s, 1H), 6.80 (dd, *J* = 5.7, *J* = 3.4, 1H), 6.58-6.57 (d, *J* = 4.9, 1H), 3.51-3.48 (q, *J* = 7.1, 4H), 2.68 (s, 3H), 2.25 (s, 3H), 1.23-1.21 (t, *J* = 6.3, 6H).

^{13}C NMR (Methanol-d_4,151 MHz): δ 200.98, 162.27, 155.07, 150.72, 150.20, 140.66, 138.60, 132.75, 130.77, 128.66, 119.16, 109.21, 96.70, 84.64, 78.76, 44.36, 23.68, 15.13, 11.30.

MS-ESI$^+$: *m/z* calculated for $C_{22}H_{25}BNO_5$ $[M+H]^+$ 394.1826, found 394.1843.

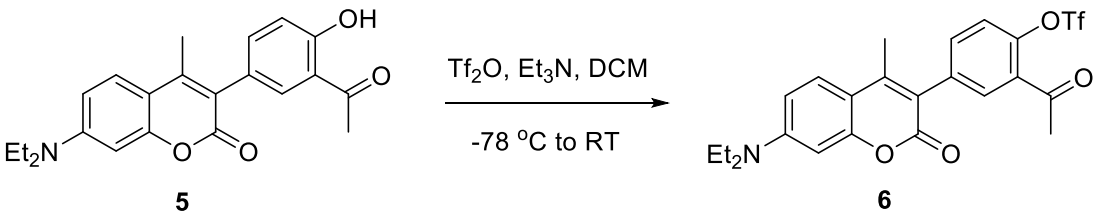


5-bromo-2-hydroxy acetophenone (215 mg, 1 mmol), B_2Pin_2 (280 mg, 1.1 mmol), Pd(dppf)Cl_2 (22 mg, 0.03 mmol, 3 mol %) and potassium acetate (294 mg, 3 mmol) were was dissolved in anhydrous dioxane (5 mL). The reaction mixture was bubbled with argon for 10 minutes and it was allowed to stir at 90 °C for 90 minutes. Completion of the reaction was monitored by LC-MS. The reaction mixture was cooled to room temperature, and then **2** (216 mg, 0.7 mmol), Pd(dppf)Cl_2 (22 mg, 0.03 mmol, 3 mol %), K_3PO_4 (445 mg, 2.1 mmol) and water (1mL) were added subsequently in the same pot. Further, reaction mixture was heated to 85 °C for overnight. The reaction mixture was filtered through sintered funnel and digested in water (100 mL). The product was extracted with ethyl acetate (3 × 100 mL). The combined organic layer was washed with brine (100 mL) and dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified on silica gel using hexane:ethyl acetate (4:1) as eluent. The desired product was obtained as a yellow solid (182 mg, 67%).

^{1}H NMR (Chloroform-*d*, 600 MHz): *δ* 12.31 (s, 1H), 7.71 (b, 1H), 7.46-7.45 (d, *J* = 6.9, 1H), 7.40-7.39 (d, *J* = 7, 1H), 7.04-7.03 (d, *J* = 6.9, 1H), 6.63-6.62 (dd, *J* = 7.0, *J* = 4, 1H), 6.55 (d, *J* = 4, 1H), 3.45-3.42 (q, *J* = 6.8, 4H), 2.63 (s, 3H), 2.26 (s, 3H), 1.24-1.21 (t, *J* = 7.5, 6H).

^{13}C NMR (Chloroform-*d* 151 MHz): *δ* 204.51, 162.19, 161.77, 155.07, 150.38, 148.68, 138.80, 132.88, 126.12, 125.91, 119.53, 118.30, 109.32, 108.70, 97.44, 77.21, 77.00, 76.79, 44.76, 26.69, 16.39, 15.26, 12.45.

MS-ESI$^+$: *m/z* calculated for $C_{22}H_{23}NO_4$ $[M+H]^+$ 366.1707, found 366.1723.

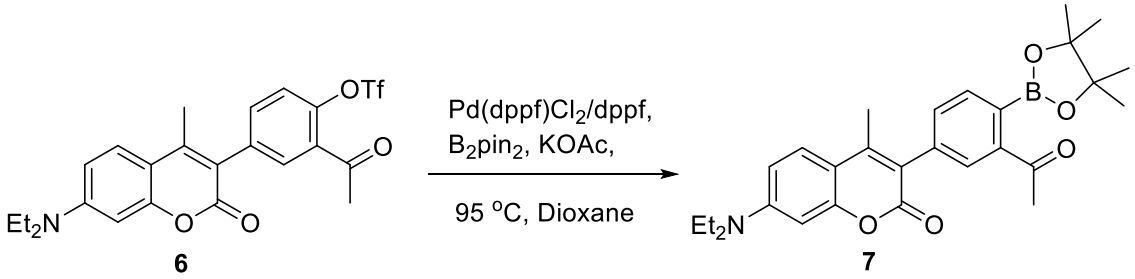


5 (100 mg, 0.274 mmol) was dissolved in anhydrous dichloromethane (1 mL) and triethylamine (160 µL, 1.15 mmol) was added while stirring. The reaction mixture was cooled to -78 ^{o}C and trifluoromethane sulfonic anhydride (102 µL, 0.6 mmol) was added slowly during 5 min. The reaction mixture was allowed to stir at room temperature for 0.5 hr under an argon environment. After that, the reaction was quenched with saturated sodium bicarbonate (1 mL) and the mixture was allowed to stir for 5 min. The product was extracted with dichloromethane (3 × 20 mL). The combined organic layer was washed with brine (30 mL) and dried over Na_2SO_4. After removing the solvent, the crude product was purified on silica gel column using ethyl acetate:hexane (1:4) as eluent to yield a dark red gummy product (120 mg, 91%).

^{1}H NMR (Chloroform-*d*, 600 MHz): *δ*, 7.76, (d, *J* = 5.9, 1H), 7.55-7.53 (dd, *J* = 6.4, *J* = 3.7, 1H), 7.48-7.47 (d, *J* = 6.5, 1H), 6.65-6.63 (dd, *J* = 6.7, *J* = 3.8, 1H), 6.55-6.54 (d, *J* = 6.3, 1H), 3.46-3.42 (q, *J* = 6.8, 4H), 2.65 (s, 3H), 2.25 (s, 3H), 1.24-1.22 (t, *J* = 6.3, 6H).

^{13}C NMR (Chloroform-*d* 151 MHz): *δ* 196.50, 155.28, 150.80, 149.52, 145.89, 133.25, 126.35, 122.48, 119.67, 117.95, 117.54, 108.91, 97.36, 44.80, 29.55, 16.47, 12.43.

MS-ESI^{+}: *m/z* calculated for $C_{23}H_{22}F_3NO_6S$ $[M+H]^+$ 498.1196, found 497.1182.



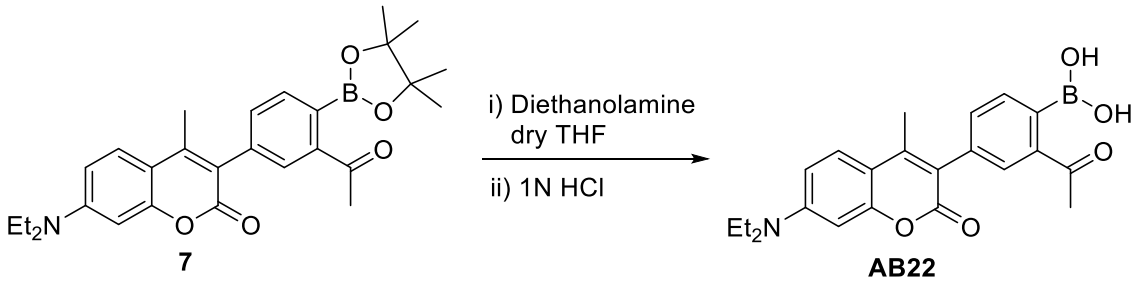
6 **7**

6 (100 mg, 0.2 mmol), B_2Pin_2 (126 mg, 0.5 mmol), $Pd(dppf)Cl_2$ (18 mg, 0. 020 mmol, 10 mol %), and potassium acetate (60 mg, 0.6 mmol) were was dissolved in anhydrous dioxane (1.2 mL). The reaction mixture was bubbled with argon for 10 minutes and it was allowed to stir at 95 ^{o}C for 50 minutes. Completion of the reaction was monitored by LC-MS. Then water (30 mL) was added to the reaction and the product was extracted with ethyl acetate (3 × 30 mL). The combined organic layer was washed with brine (100 mL) and dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified on silica gel using hexane:diethylether (11:9) as eluent. The desired product was obtained as a yellow gummy (62 mg, 70%).

^{1}H NMR (Chloroform-*d*, 600 MHz): *δ* 7.78 (b, 1H), 7.58-7.57 (d, *J* = 6.8, 1H), 7.47-7.43 (m, 2H), 6.63-6.61 (dd, *J* = 7.3, *J* = 3.7, 1H), 6.54 (d, *J* = 3.5, 1H), 3.45-3.41 (q, *J* = 6.6, 4H), 2.60 (s, 3H), 2.18 (s, 3H), 1.44 (s, 12H), 1.22-1.21 (t, *J* = 5.9, 6H).

^{13}C NMR (Chloroform-*d* 151 MHz): δ 199.72, 161.64, 155.20, 150.42, 148.76, 140.82, 136.24, 134.56, 132.18, 130.73, 126.13, 125.48, 119.94, 109.27, 108.65, 97.43, 44.74, 30.30, 24.87, 16.21, 12.46.

MS-ESI^{+}: *m/z* calculated for $C_{26}H_{34}BNO_5$ $[M-H_2O]^+$ 457.2424, found 457.2415.

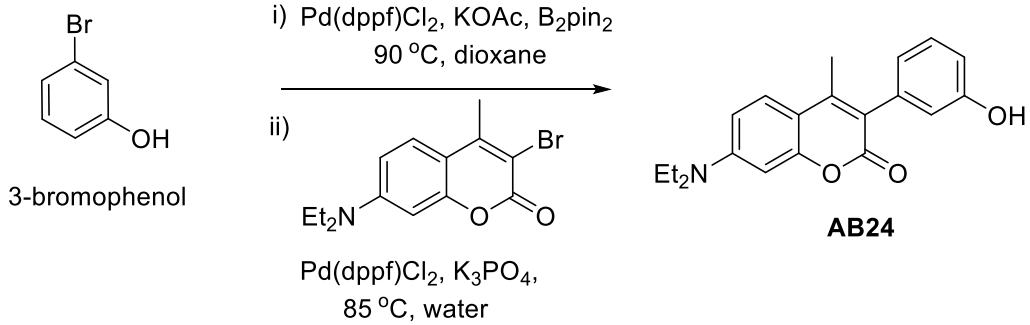


7 (50 mg, 0.105 mmol) was dissolved in anhydrous THF (500 µL) and diethanolamine (150 mg, 1.25 mmol) was added into the solution at room temperature. The reaction was allowed to stir for 3 hours and then quenched with 1N HCl (30 mL). Further, the solution was neutralized using $NaHCO_3$ (5%) solution in water. Then the product solution was directly purified through HPLC. The HPLC purification with acetonitrile/water (0.1% TFA) (Gradient: 5% ACN to 95% ACN during 50 min) mixture yielded the pure product as a yellow powder after lyophilization (74%, 28 mg).

^{1}H NMR (Methanol-d_4, 600 MHz): δ 7.98 (b, 1H), 7.66-7.64 (d, J = 7.8, 1H), 7.56-7.55 (d, J = 6.7, 1H), 7.50-7.49 (d, J = 6.9, 1H), 6.84-6.82 (d, J = 7.8, 1H), 6.63 (b, 1H), 3.53-3.50 (q, J =7, 4H), 2.65 (s, 3H), 2.28 (s, 3H), 1.24-1.21 (t, J = 6.9, 6H).

^{13}C NMR (Methanol-d_4,151 MHz): δ 203.88, 165.25, 161.81, 161.55, 157.64, 152.97, 142.37, 138.50, 137.87, 133.78, 132.94, 129.16, 121.94, 112.26, 99.89, 47.35, 26.39, 17.84, 13.91.

MS-ESI$^+$: *m/z* calculated for $C_{22}H_{25}BNO_5$ $[M+H]^+$ 394.1826, found 394.1838.



3-bromophenol (173 mg, 1 mmol), B_2Pin_2 (280 mg, 1.1 mmol), $Pd(dppf)Cl_2$ (22 mg, 0.03 mmol, 3 mol %) and potassium acetate (294 mg, 3 mmol) were was dissolved in anhydrous dioxane (5 mL). The reaction mixture was bubbled with argon for 10 minutes and it was allowed to stir at 90 °C for 90 minutes. Completion of the reaction was monitored by LC-MS. The reaction mixture was cooled to room temperature, and then **2** (216 mg, 0.7 mmol), $Pd(dppf)Cl_2$ (22 mg, 0.03 mmol, 3 mol %), K_3PO_4 (445 mg, 2.1 mmol) and water (1mL) were added subsequently in the same pot. Further, reaction mixture was heated to 85 °C for overnight. The reaction mixture was filtered through sintered funnel and digested in water (100 mL). The product was extracted with ethyl acetate (3 × 100 mL). The combined organic layer was washed with brine (100 mL) and dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified on

silica gel using hexane:ethyl acetate (4:1) as eluent. The desired product was obtained as a yellow solid (163 mg, 67%).
^{1}H NMR (Chloroform-*d*, 600 MHz): δ 7.45-7.44, (d, *J* = 7.9, 1H), 7.26-7.22 (m, 1H), 6.77-6.74 (m, 2H), 6.72 (m, 1H), 6.63-6.61(dd, *J* = 8.1, *J* = 4,1H) , 6.53 (d, *J* = 7.4, 1H), 6.34 (b, 1H), 3.44-3.40 (d, *J* = 7.2, 1H), 2.20 (s, 3H), 1.22-1.20 (t, *J* = 6.8, 6H).
^{13}C NMR (Chloroform-*d*,151 MHz): δ 162.79, 156.33, 154.95, 150.29, 149.30, 136.11, 129.40, 126.14, 122.02, 120.83, 117.58, 115.12, 109.51, 108.81, 97.41, 44.74, 16.28, 12.45.
MS-ESI^{+}: *m/z* calculated for $C_{20}H_{21}NO_3$ $[M+H]^{+}$ 324.1597, found 324.1587.

UV-Vis Analysis of the Coumarin Analogues
The UV-Vis profiles of the coumarin analogues were obtained by diluting a DMSO stock of each analog (AB21= 1 mM, AB22=2 mM, AB23=3 mM, AB24= 2.6 mM) to 50 µM in PBS (1x, pH 7.4). Spectra are shown in **Figure 2b**.

Fluorescence Analysis of the Coumarin Analogues
Fluorescence profiles of the coumarin analogues were obtained by diluting a DMSO stock of each analog (AB21= 1 mM, AB22=2 mM, AB23=3 mM, AB24= 2.6 mM) to 10 µM in PBS (1X, pH 7.4). Voltages applied to obtain a spectrum for each analog varied (AB21/22=800, AB23=700, AB24/25=600). Spectra are shown in **Figure 2c**.

LC/MS Analysis of the Conjugation of Coumarin Analogues to Semicarbazide
DMSO stocks of the coumarin analogues (AB21= 1 mM, AB22=2 mM) were diluted to 200 µM in PBS (1x, pH 7.4) and incubated with 200 µM semicarbazide (18 mM stock in PBS) for 30 minutes, then analyzed by LC/MS. AB21 and AB22 showed essentially complete conjugation. The LC traces are shown in **Figure 3a**.

Conjugation of Coumarin Analogues to Semicarbazide in the Presence of Serum
DMSO stocks of the coumarin analogues (AB21= 1 mM, AB22=2 mM, AB23=3 mM, AB24= 2.6 mM) were diluted to 100 µM in 1x PBS buffer with or without 20% fetal bovine serum (FBS), respectively. Then semicarbazide was added to these solutions from a stock solution to a final concentration of 100 µM. The reactions were incubated for 30 minutes before analysis via analytical HPLC. In the absence of FBS, AB21 and AB22 showed ~ 80% and 70% conjugation, respectively. The percentage of conjugation decreased minimally with the addition of FBS to ~75% and 60%, respectively. The LC traces are shown in **Figure S1**.

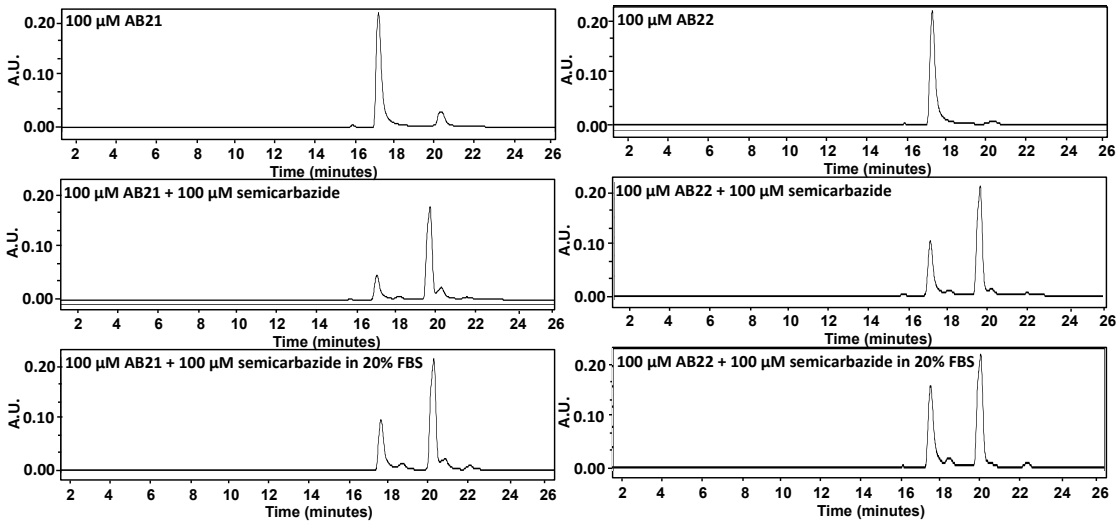


Figure S1. Analytical HPLC analysis of AB21 and AB22 conjugation to semicarbazide in the presence of 20% fetal bovine serum.

Fluorescence Analysis of the Conjugation of Coumarin Analogues to Semicarbazide

Fluorescence profiles of the coumarin analogues conjugated to semicarbazide were obtained by diluting a DMSO stock of each analogue (AB21= 1 mM, AB22=2 mM, AB23=3 mM, AB24= 2.6 mM, AB25=5.3 mM) to 200 µM in PBS (1X, pH 7.4), adding 200 µM semicarbazide (18 mM stock in PBS), and incubating for 30 minutes. Fluorescence spectra were then obtained immediately after diluting the conjugation mixture 20X in PBS (1X, pH 7.4). Voltages applied to obtain a spectrum for each analog varied (see Figure S2 legend). Fluorescence spectra with coumarin analog fluorescence overlays are shown in **Figure 2c** and **Figure S2**.

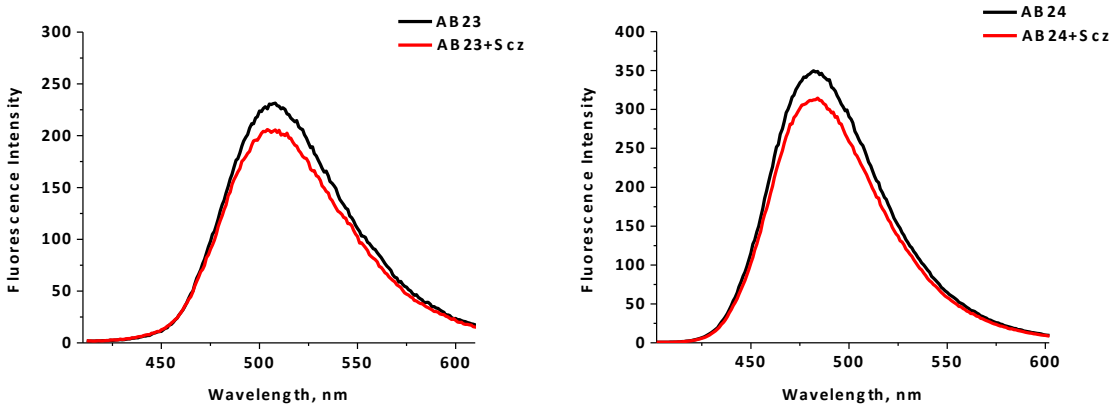


Figure S2. Addition of semicarbazide to AB23 and 24 generates no increase in fluorescence due to lack of conjugation (Voltage = 800 (AB21), 700 (AB22/23), 600 (AB24/25)).

Quantum Yield of Coumarin Analogs and Diazaborine Conjugates

The quantum yield of the coumarin derivatives were measured according to a literature protocol (*Biochemistry*, **2017**, *56*, 1585). The UV-Vis spectra of 10 µM AB21-24 were obtained which exhibit absorbance around 0.1 at the absorption maximum. The concentration of fluorescein necessary to obtain the same absorbance was estimated to be 2 µM (ε=50,358 $M^{-1}cm^{-1}$ in 1M NaOH). The UV-Vis spectrum of this concentration of fluorescein was obtained in 0.1M NaOH. Fluorescence readings were then obtained for AB21-24 as in **Figure 2c**, as well as for diazaborine conjugates as outlined in **Figure 2c** and **Figure S2**. The integrations of the fluorescence peak areas were then obtained. For fluorescein, a 10x dilution of the estimated concentration to produce the same absorbance as AB21-24 was necessary to obtain a readable fluorescence peak area within the range of fluorescence detection. The fluorescence readings for fluorescein were obtained in 0.1M NaOH at 0.2 µM. Integration of the fluorescence peak area was also obtained. To account for this dilution factor, the integrated area of fluorescein was multiplied by 10 for the quantum yield calculation. The data and extracted quantum yield are given in **Table S1**.

Table S1. Summary of AB21-24 quantum yield data obtained from comparison to the quantum yield of the standard, fluorescein.[1]

	Voltage	Integrated Area	Absorbance	Quantum Yield
AB21	700 V	4,032	0.066	0.014
AB21+Scz	700 V	16,862	0.066	0.060
AB22	700 V	8,311	0.096	0.020
AB22+Scz	700 V	41,877	0.096	0.102
AB23	700 V	20,518	0.033	0.145
AB23+Scz	700 V	14,524	0.033	0.103
AB24	600 V	28,737	0.063	0.455
AB24+Scz	600 V	19,932	0.063	0.315
Fluorescein	600 V	6,768	0.071	0.950
Fluorescein	700 V	28,905	0.071	0.950

Kinetics of AB21 and AB22 Conjugation to Semicarbazide

The kinetics of conjugation of AB21 and AB22 conjugating to semicarbazide were measured at 50 µM AB21/22 and 50 µM semicarbazide, as shown in **Figure S3**. Measurements were taken at 30 second intervals at a voltage of 600V until no further increase in fluorescence was observed. The reaction kinetics were fit according to a hyperbolic equation, as described below:

$$\frac{dP}{dt} = k_2(C_0 - P)(C_0 - P) \quad \textbf{(1)}$$

in which **P** is the product concentration, $\mathbf{C_0}$ is the starting material concentration, which is 50 µM for both reactants, and $\mathbf{k_2}$ is the rate constant.

$$\frac{d(C_0-P)}{dt} = -k_2(C_0 - P)^2 \quad \textbf{(2)}$$

$$\frac{d(C_0-P)}{(C_0-P)^2} = -k_2 dt \quad \textbf{(3)}$$

Integrate both sides of the equation to obtain:

$$\frac{1}{C_0-P} - \frac{1}{C_0} = k_2 t \quad \textbf{(4)}$$

Solving the equation for P gives:

$$P = \frac{C_0 t}{t+\frac{1}{C_0 k_2}} \quad \textbf{(5)}$$

Fitting the kinetic data of Figure S3 to a hyperbolic equation:

$$y = \frac{P_1 x}{x+P_2} \quad \textbf{(6)}$$

k_2 can be determined by:

$$P_2 = \frac{1}{C_0 k_2} \rightarrow k_2 = \frac{1}{C_0 P_2}$$

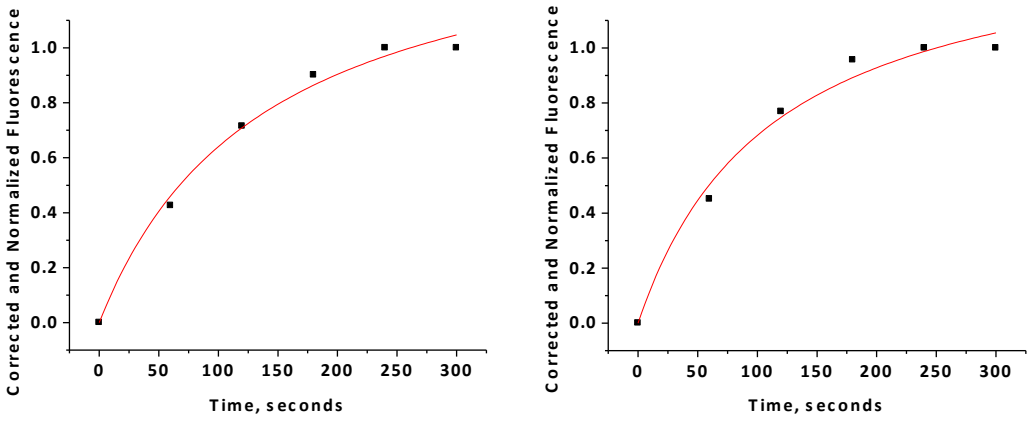


Figure S3. Kinetics of AB21 (left, k_2=144 ± 28 $M^{-1}s^{-1}$) and AB22 (right, k_2=177 ± 44 $M^{-1}s^{-1}$) conjugation to semicarbazide.

III. Synthesis of D-Dap-Scz

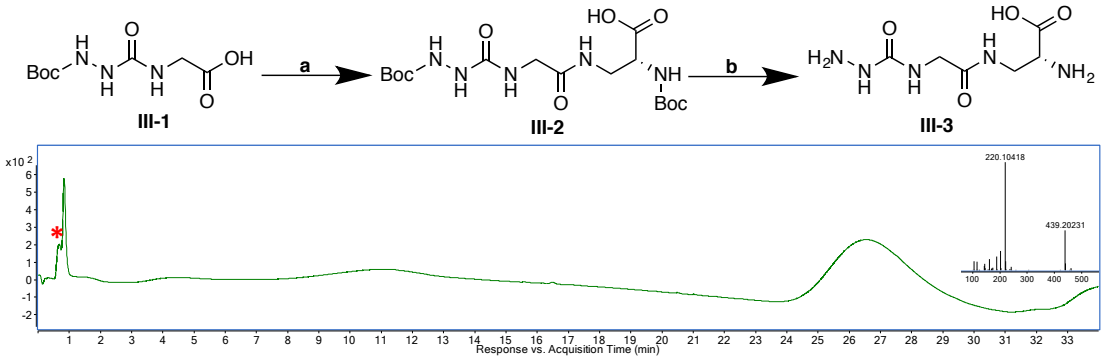


Figure S4. Synthetic scheme and LC/MS characterization of D-Dap-Scz (* indicates injection peak).

Synthesis of III-2

The synthesis of **III-1** has been previously reported in *J. Am. Chem. Soc.*, 2016, **139**, 871-878. **III-1** (0.11 mmol) was dissolved in dimethylformamide (200 µL) on ice for 5 minutes to dissolve. N-Hydroxysuccinimide (NHS, 0.16 mmol) and dicyclohexylcarboiimide (0.10 mmol) were added, and the mixture was allowed to stir on ice for an additional 5 minutes. The reaction mixture was then stirred at room temperature for 2 hours after which all of the starting material was consumed (note: the reaction mixture turns opaque due to the formation of dicyclohexyl urea). Boc-D-Dap-OH (0.16 mmol) was then dissolved in 1:1 10% sodium carbonate: tetrahydrofuran (400 µL) and added to the stirring reaction mixture, which was then stirred for 14 hours at room temperature. The reaction mixture was then acidified with 6N HCl and extracted with ethyl acetate (3x30 mL). The combined organic layers were washed with brine and dried over sodium sulfate. The solvent was then evaporated from the reaction mixture, and the product was purified via reverse phase HPLC using a semiprep column to yield a white powder after lyophilization (24 mg, 52%).

^{1}H NMR (DMSO-d_6) δ: 1.37 (18H), 3.55-3.62 (3H), 4.00 (2H), 6.36 (1H), 6.95 (1H), 7.85 (1H), 8.54 (1H), 12.61 (1H).

MS-ESI (m/z): Calculated for $[M+H]^+$ 420.40, Observed 420.21.

Synthesis of III-3

III-2 (24 mg, 0.057 mmol) was treated with 50% TFA in DCM (2 mL) for 1 hour. The solvent was evaporated, and the product was washed with DCM (3x10 mL). The product was then dissolved in 5 mL water and lyophilized to yield a white powder (12 mg, 100%). The LC trace and mass are shown in **Figure S4a** (blank trace in **Figure S4b** shows injection peak observed as shoulder in **Figure S4a** is extremely absorbant at the 220 nm, the wavelength being monitored).

^{1}H NMR (DMSO-d_6) δ: 3.45 (1H), 3.60 (1H), 2.69 (2H), 3.90-3.97 (1H), 6.55 (2H), 6.91 (2H), 7.41 (1H), 8.18 (1H), 9.13 (1H).

MS-ESI (m/z): Calculated for $[M+H]^+$ 220.10, Observed 220.10.

LC/MS Analysis of D-Dap-Scz for Conjugation with AB21/22

DMSO stocks of the coumarin analogs (AB21= 1 mM, AB22=2 mM, AB23=3 mM, AB24= 2.6 mM) were diluted to 50 µM in PBS (1X, pH 7.4) and incubated with 100 µM D-Dap-Scz (9 mM stock in PBS) for 30 minutes, then analyzed by LC/MS. AB21 and AB22 showed ~ 70% and 80% conjugation, respectively, whereas AB23, AB24 and AB25 displayed no conjugation. The LC traces and masses are shown in **Figure S5**.

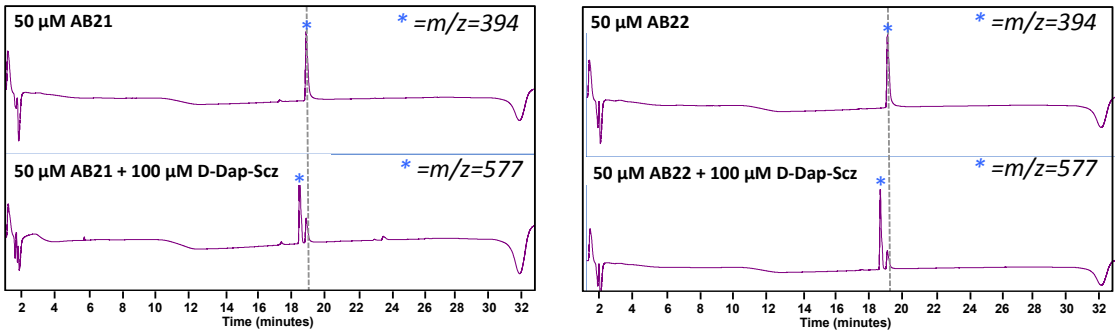


Figure S5. LC/MS analysis of the conjugation of AB21 and AB22 (indicated by gray dashed lines) to D-Dap-Scz. The reactions completed within 30 minutes of incubation with ~70% and 80% yield for AB21 and AB22 respectively. The lack of quantitative conversion is likely due to product inhibition as we previously reported (Figure S7 and related discussions in *J. Am. Chem. Soc.*, **2017**, *139*, 871)

Fluorescence of Coumarin Analogs Conjugating to D-Dap-Scz

Fluorescence profiles of the coumarin analogs conjugated to D-Dap-semicarbazide were obtained by diluting a DMSO stock of each analog (AB21= 1 mM, AB22=2 mM, AB23=3 mM, AB24= 2.6 mM, AB25=5.3 mM) to 50 µM in PBS (1X, pH 7.4), adding 100 µM D-Dap-semicarbazide (9 mM stock in PBS), and incubating for 30 minutes. Fluorescence spectra were then obtained immediately after diluting the conjugation mixture 5x in PBS (1x, pH 7.4). Voltages applied to obtain a spectrum for each analog varied (see Figure S6 legend for details). Fluorescence spectra are shown with ABX overlaid in **Figure S6**.

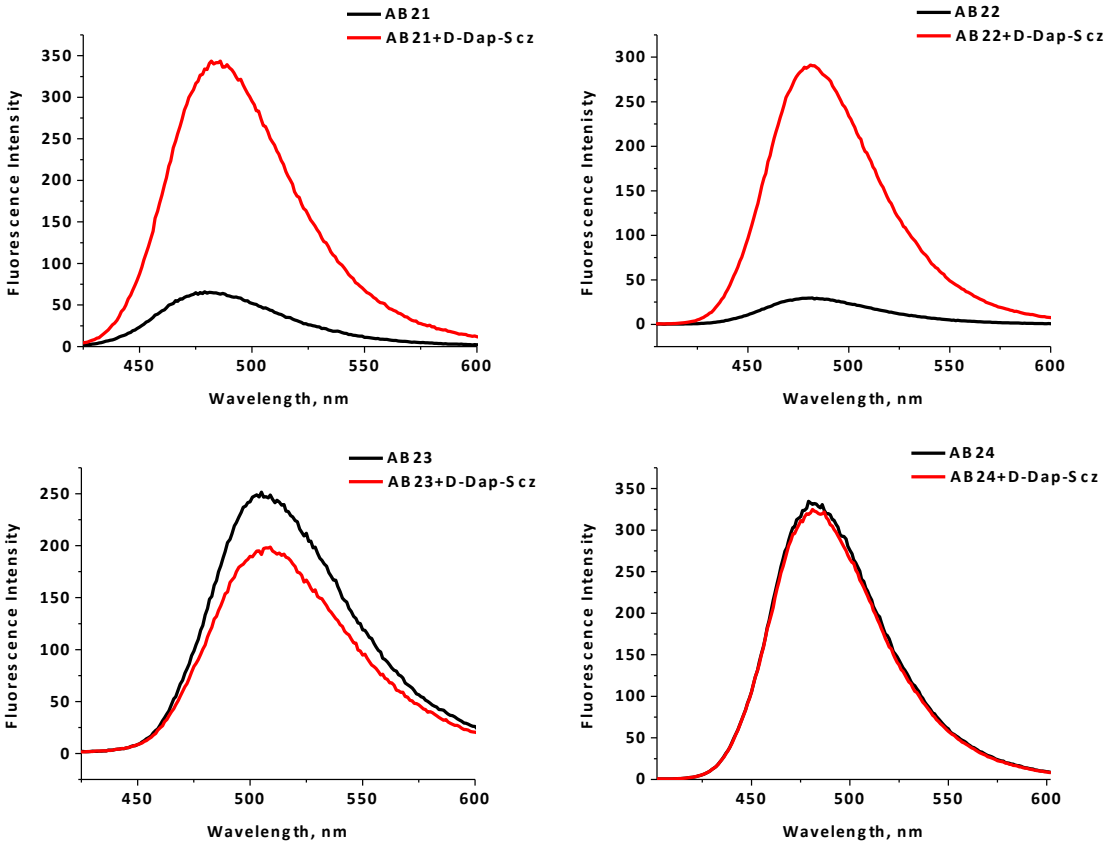


Figure S6. Fluorescence change of AB21-24 when conjugated to D-Dap-Scz (Voltage= 700 (AB21/22), 600 (AB22/24)).

IV. Synthesis of FPBA-FITC

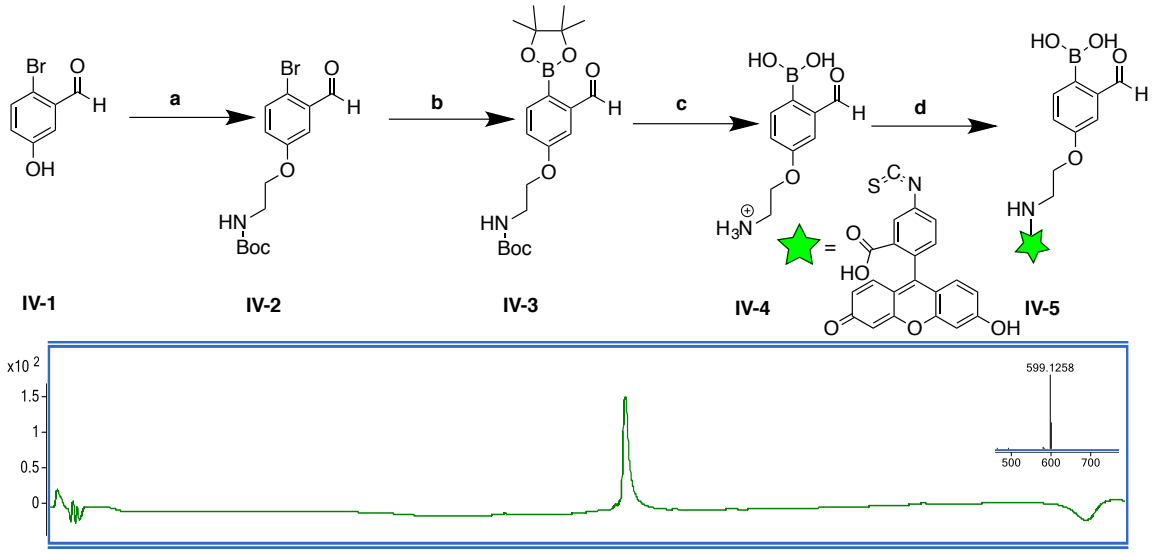


Figure S7. Synthetic Scheme and LC/MS characterization of FPBA-FITC.

Synthesis of IV-2

IV-1 (1.00 g, 0.0050 mol) was refluxed with N-Boc-bromoethylamine (1.30 g, 0.0055 mol) and potassium carbonate (2.10g, 0.015 mol) in DMF (5 mL) at 80°C for 15 hours. The crude product was dissolved in 50 mL water, and extracted with ethyl acetate (3x50 mL). The combined organic layers were washed with brine, then dried over sodium sulfate and concentrated under reduced pressure. The crude material was then purified by silica gel chromatorgraphy with 1:10 ethyl acetate:hexane (550 mg, 32% yield).

^{1}H NMR (Chloroform-d_6) δ : 1.44 (s, 9H), 3.54 (q, J=5.5 Hz, 2H) 4.03-4.05 (q, J=5.3 Hz, 2H), 4.97 (s, 1H), 7.01-7.03 (dd, J=8.8, 3.2 Hz, 1H), 7.38 (d, J=3.2 Hz, 1H), 7.52 (d, J=8.8 Hz, 1H), 10.28 (s, 1H).

^{13}C NMR (Chloroform-d_6) δ: 31.02, 42.53, 63.04, 82.37, 116.38, 120.82, 125.65, 136.65, 137.30, 158.47, 160.86, 194.24.

MS-ESI (m/z): Calculated for $[M+H]^+$ 345.21, Observed 244.99 (-Boc).

Synthesis of IV-3

IV-2 (550 mg, 0.0016 mol), B_2pin_2 (1.02g, 0.0040 mol), $Pd(dppf)Cl_2$•DCM (65 mg, 0.000080 mol) and potassium acetate (490 mg, 0.0048 mol) were combined in dry dioxane (10 mL), and purged with argon for 15 minutes. The reaction was then allowed to stir under argon at 85°C for 1 hour. The crude product was dissolved in 50 mL water, then extracted with ethyl acetate (3x50 mL). The combined organic layers were washed with brine and dried over sodium sulfate before being concentrated under reduced pressure. The crude mixture was purified via silica gel chromatography with 3:20 ethyl acetate:hexane (439 mg, 70% yield).

^{1}H NMR (Chloroform-d_6) δ: 1.35 (s, 12H), 1.43 (s, 9H), 3.54 (q, J=5.4 Hz, 2H), 4.07 (q, J=5.7 Hz, 2H), 5.00 (s, 1H), 7.09-7.11 (d, J=8.3 Hz, 1H), 7.36 (s, 1H), 7.84-7.86 (d, J=8.3 Hz,

1H), 10.64 (s, 1H).

^{13}C NMR (Chloroform-d_6) δ: 24.83, 28.35, 39.93, 60.35, 67.30, 84.16, 111.25, 119.86, 130.11, 138.00, 143.54, 155.80, 160.86, 194.60.

MS-ESI (m/z): Calculated for $[M+H]^+$ 391.27, Observed 236.07 (-Boc, -4xCH_3).

Synthesis of FPBC-FITC (IV-5)

IV-4 (100 mg, 0.26 mmol) was stirred with diethanolamine (270 mg, 2.6 mmol) in tetrahydrofuran (1 mL) overnight at room temperature. The mixture was then acidified with 1 N HCl, and stirred in 5 mL ethyl acetate for 30 minutes. The crude product was then extracted with ethyl acetate (3x10 mL), and the combined organic layers were washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. The crude product was then directly dissolved in 2 mL dry dichloromethane and 2 mL TFA (50% TFA in DCM), and stirred for 30 minutes. The mixture was then evaporated under reduced pressure, and crude **IV-4** (10 mg, 0.062 mmol) was directly stirred with fluorescein isothiocyanate (24 mg, 0.062 mmol) for 1 hour in triethylamine (18 µL, 0.124 mmol) and 2 mL DMF. The reaction mixture was then diluted in 10 mL 2:1 Water:Acetonitrile and purified via reverse phase HPLC using a semiprep column to yield a orange powder after lyophilization (9 mg, 25%).The LC trace and mass are shown in **Figure S7**.

MS-ESI (m/z): Calculated for $[M+H]^+$ 599.39, Observed 599.13.

V. *S. aureus* Labeling with D-Dap-Scz and FPBA-FITC

S. aureus from a single colony was grown overnight in LB broth at 37 °C with agitation. An aliquot was taken and diluted (1:100) in fresh broth and cultured for another 2 hours until the cells reached the beginning of logarithmic phase (OD_{600} ~ 0.2). Then, D-Dap-Scz (9 mM stock in 1× PBS, 7.4) was added to the bacterial cell culture (final concentration 0 µM, 5 µM, 25 µM, 50 µM, 100 µM, or 500 µM) and the cultures were allowed to continue to grow before 400 µL/200 µL aliquots were taken at 0.5, 1, 2/4 hours. The aliquots were spun down at 5000 rcf, washed once with 1 mL PBS (1×, pH 7.4), spun down again, and the cell pellet was then incubated with 50 µL of 50 µM FPBA-FITC at 37 °C for 30 minutes. The cells were spun down and washed two additional times, then diluted in 1 mL PBS (1×, pH 7.4). The cells were further diluted in PBS (1×, pH 7.4) to reach a cell density of ~$3x10^6$ cells/mL before analysis on a BD FACSAria cell sorter (BS Biosciences). Data analysis was performed with FlowJo (Tree Star, Inc.), from which the median fluorescence intensities of the stained cells were extracted. Results are shown in **Figure S8**.

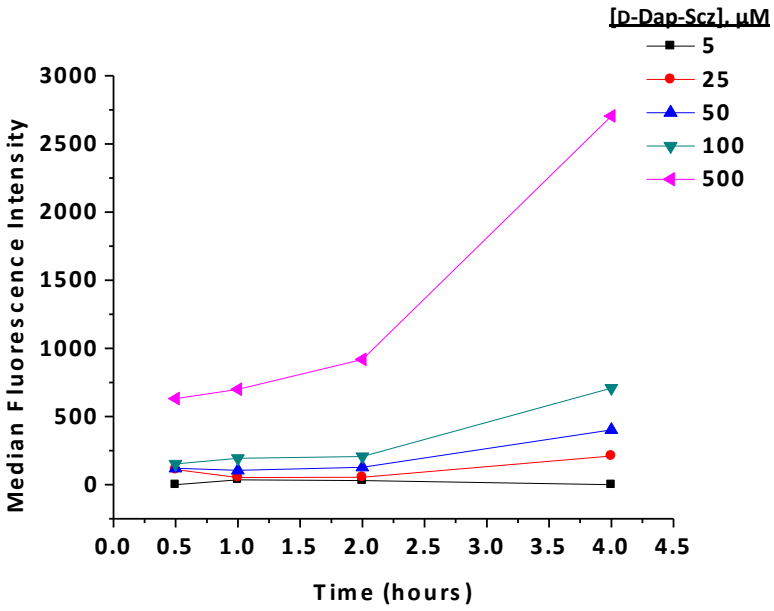
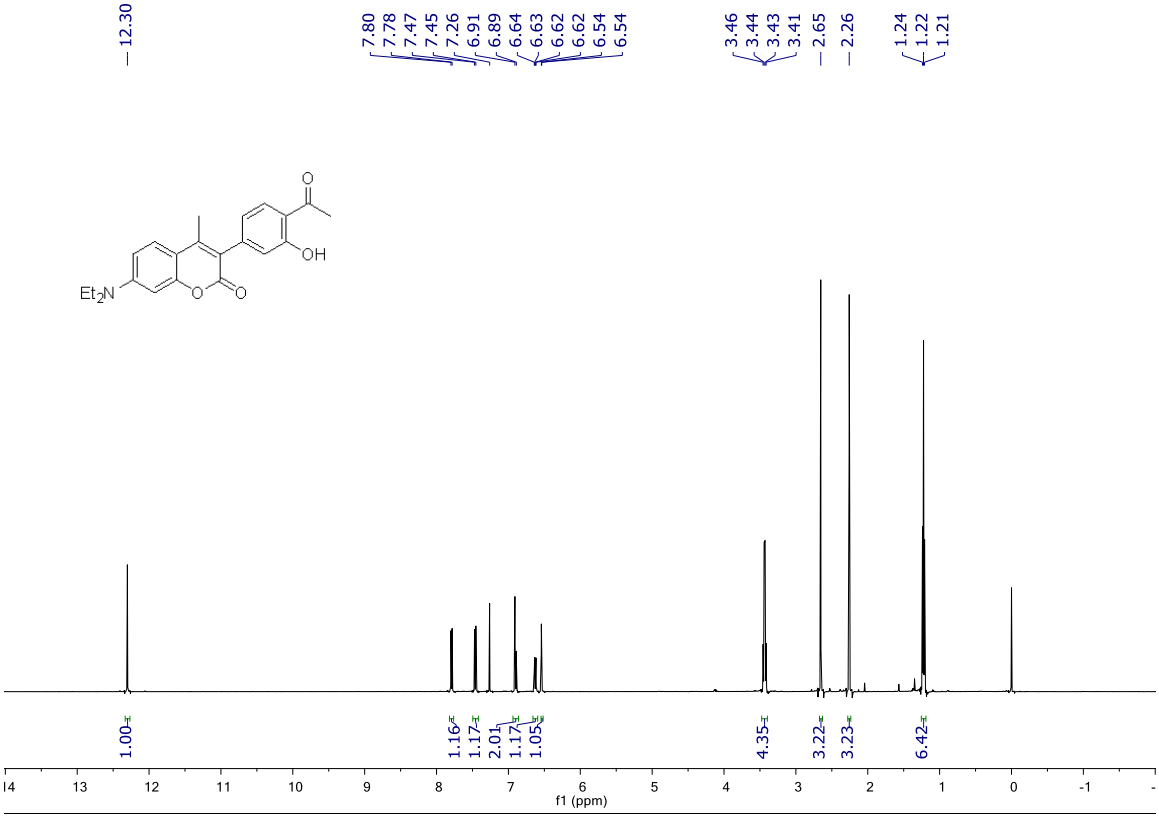


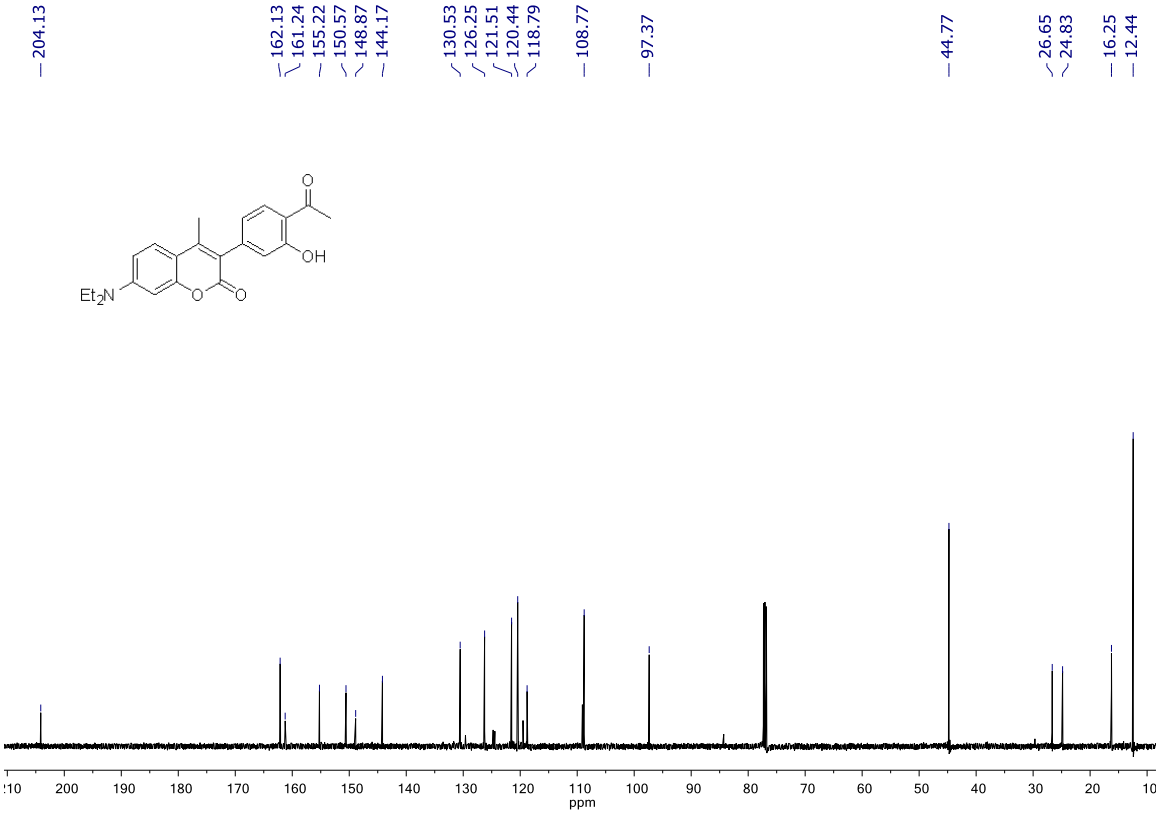
Figure S8. Flow cytometry of *S. aureus* metabolically labeled with D-Dap-Scz and stained with FPBA-FITC to determine the optimal concentration and incubation times for D-Dap-Scz incorporation.

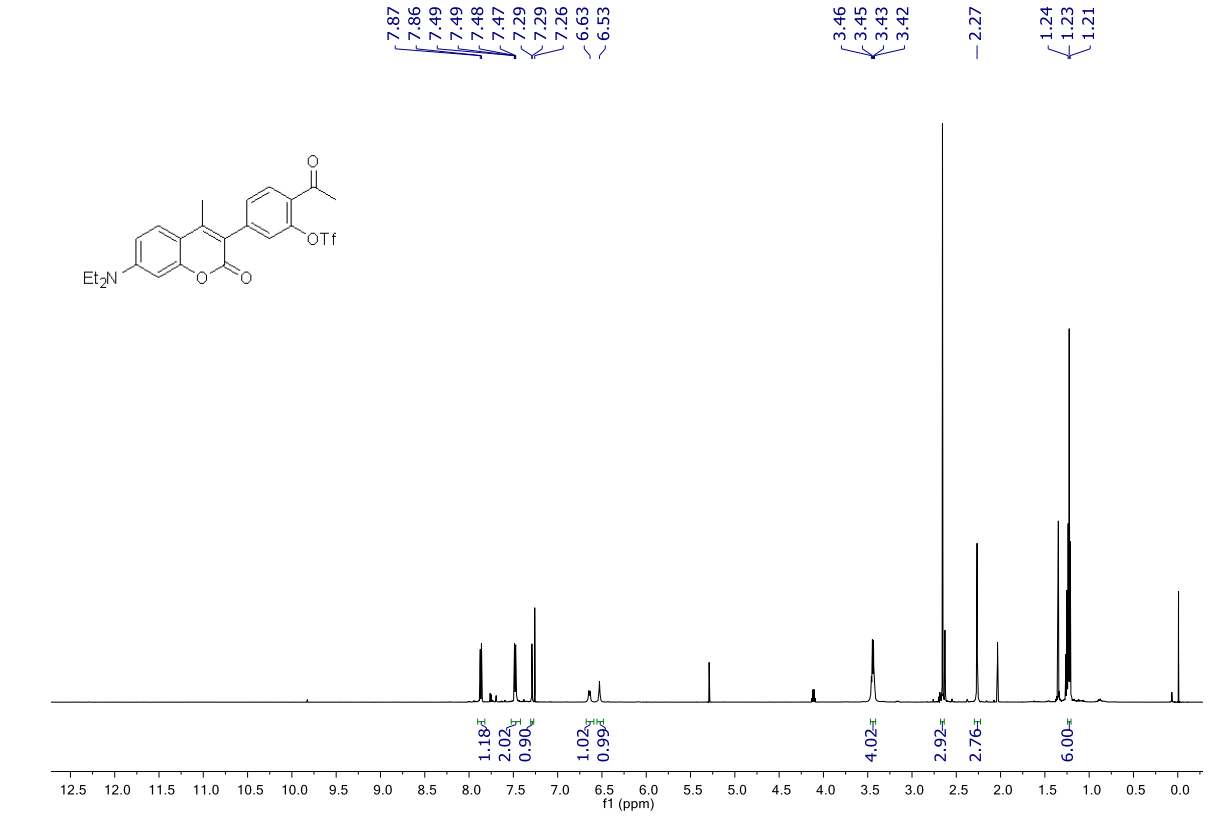
VI. *S. aureus* Cell Labeling via Fluorogenic Conjugation

S. aureus from a single colony was grown overnight in LB broth at 37 ^{o}C with agitation. An aliquot was taken and diluted (1:100) in fresh broth and cultured for another 2 hours until the cells reached the beginning of logarithmic phase (OD_{600} ~ 0.2). Then, D-Dap-Scz (9 mM stock in 1× PBS, 7.4) was added to the bacterial cell culture (final concentration 0 µM, 100 µM, or 500 µM) and the cultures were allowed to continue growth before 500 µL aliquots were taken at 4 hours. The aliquots were spun down at 5000 rcf, washed once with 1 mL PBS (1×, pH 7.4), spun down again, and the cell pellet was then incubated with 100 µL of 100 µM AB21/22 at 37 ^{o}C for 30 minutes. Parallel experiments were performed with AB24 and FPBA-FITC as controls. The cells were then fluorescently imaged using a DAPI filter. Images are shown in **Figure 4b**.

Appendix: ^{1}H and ^{13}C-NMR of AB21-4 and Intermediates



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3.42
2.27
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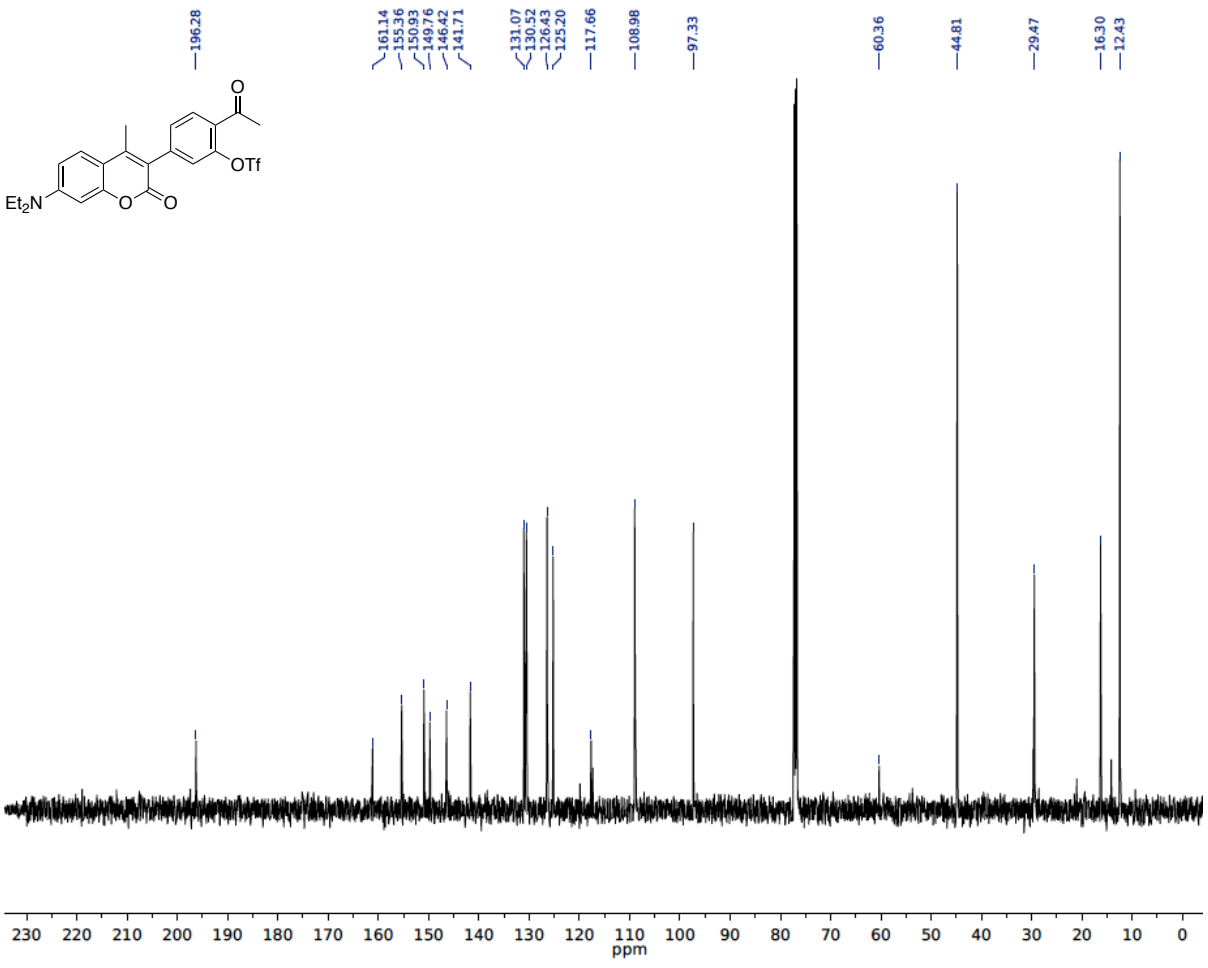
1.23
1.21
O
OTf
Et2N
O
O
1.18
2.02
0.90
1.02
0.99
4.02
2.92
2.76
6.00
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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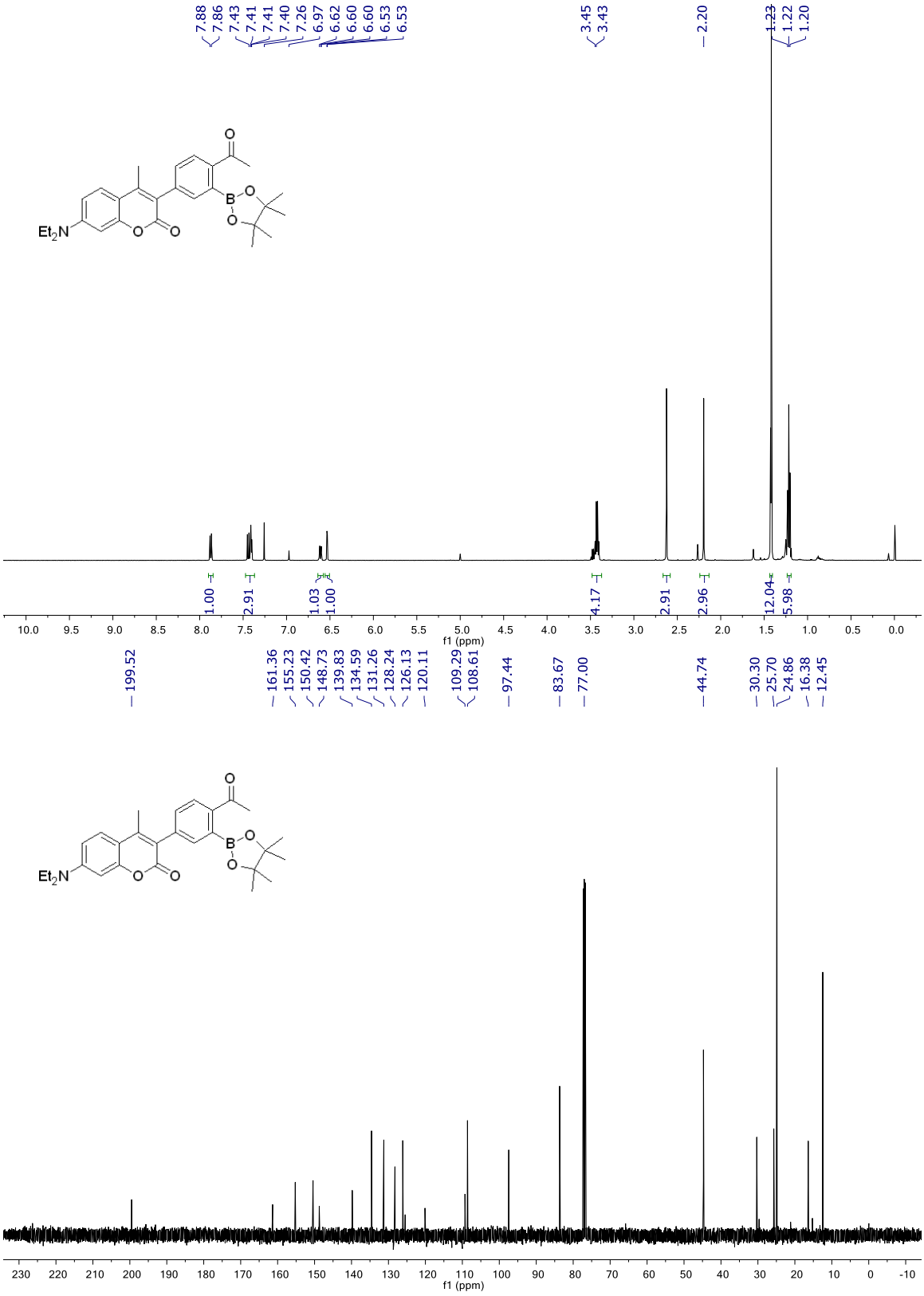
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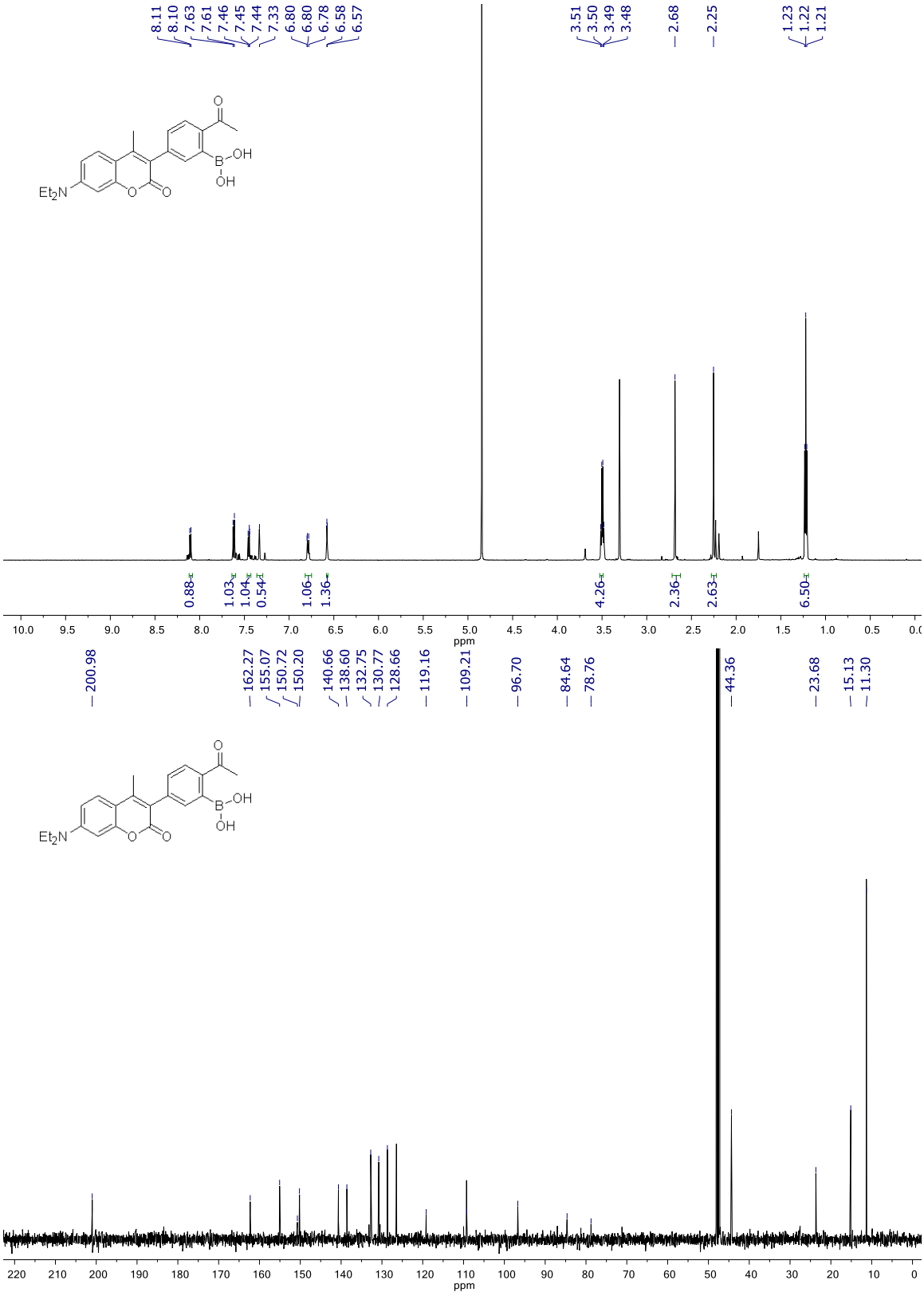
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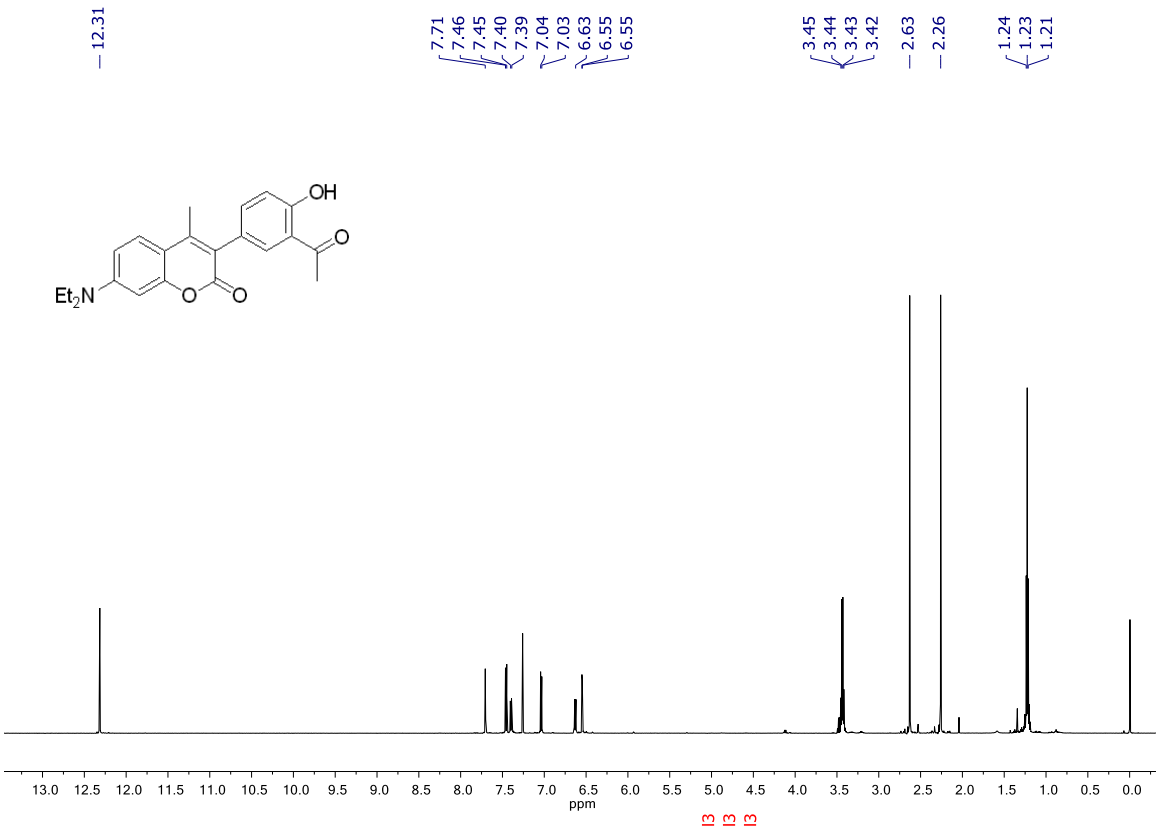
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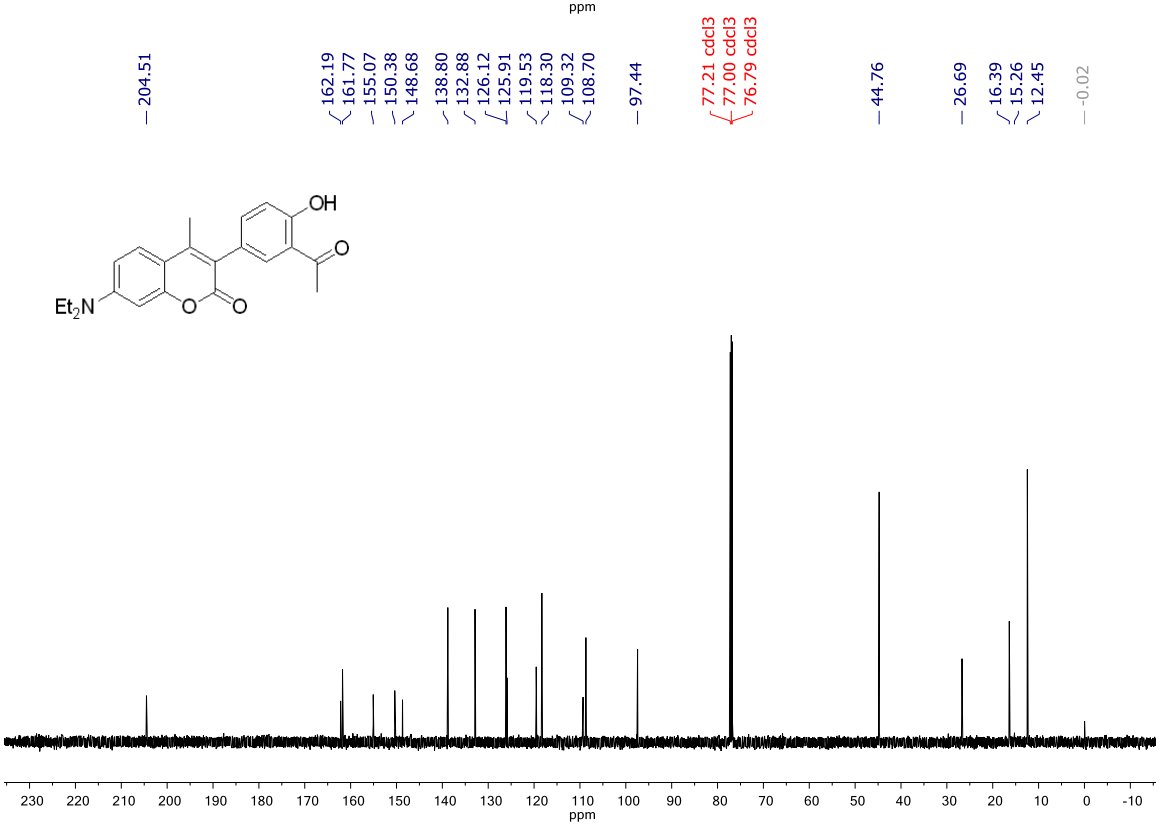
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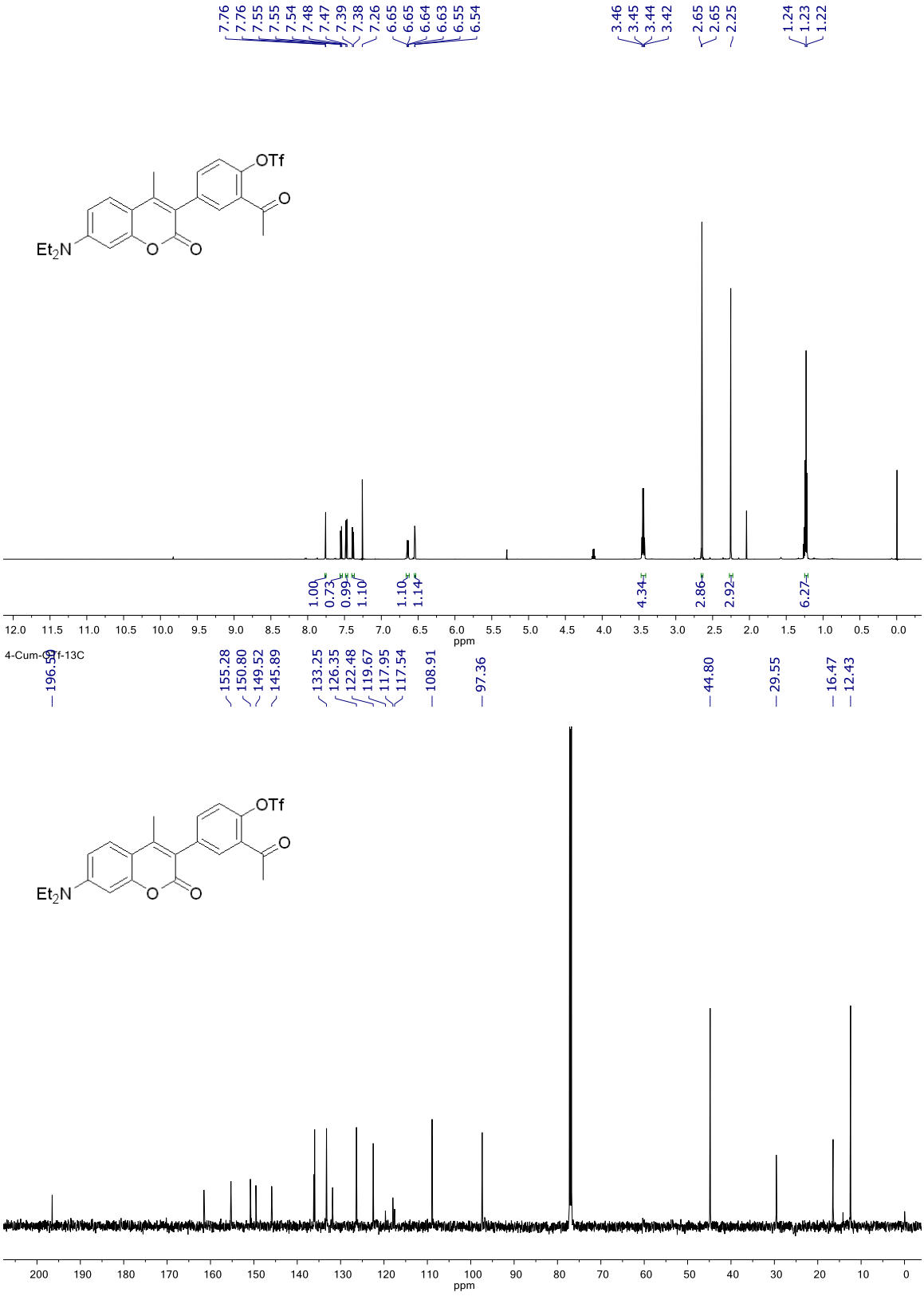
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6.63
6.55
6.54
3.46
3.45
3.44
3.42
2.65
2.65
2.25
1.24
1.23
1.22
OTf
O
Et2N
O
O
1.00
0.73
0.99
1.10
1.10
1.14
4.34
2.86
2.92
6.27
ppm
4-Cum-OTf-13C
196.59
155.28
150.80
149.52
145.89
133.25
126.35
122.48
119.67
117.95
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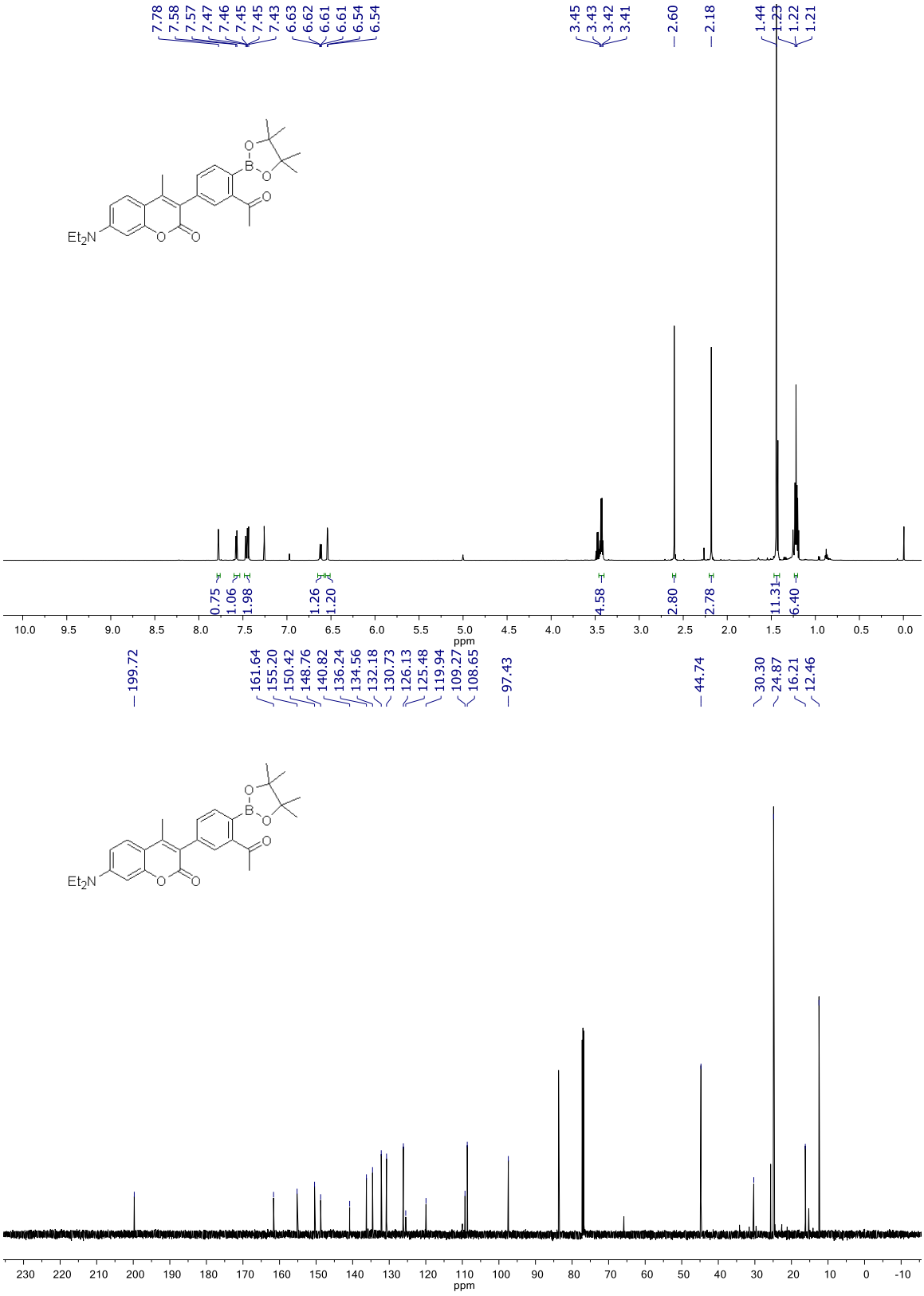
97.36
44.80
29.55
16.47
12.43
OTf
O
Et2N
O
O
ppm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7.57
7.47
7.46
7.45
7.45
7.43
6.63
6.62
6.61
6.61
6.54
6.54
3.45
3.43
3.42
3.41
2.60
2.18
1.44
1.23
1.22
1.21
Et2N
0.75
1.06
1.98
1.26
1.20
4.58
2.80
2.78
11.31
6.40
ppm
199.72
161.64
155.20
150.42
148.76
140.82
136.24
134.56
132.18
130.73
126.13
125.48
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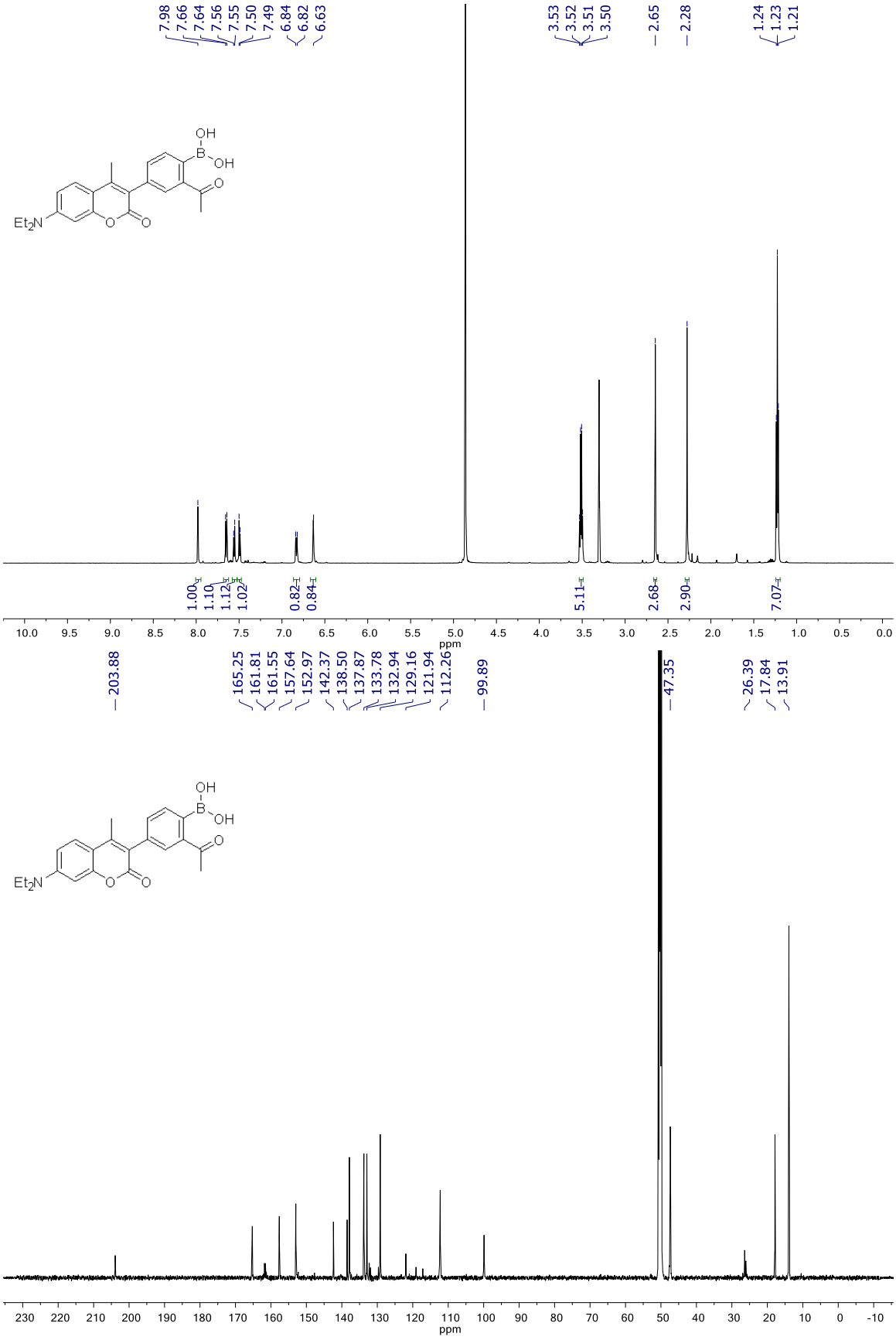
109.27
108.65
97.43
44.74
30.30
24.87
16.21
12.46
Et2N
ppm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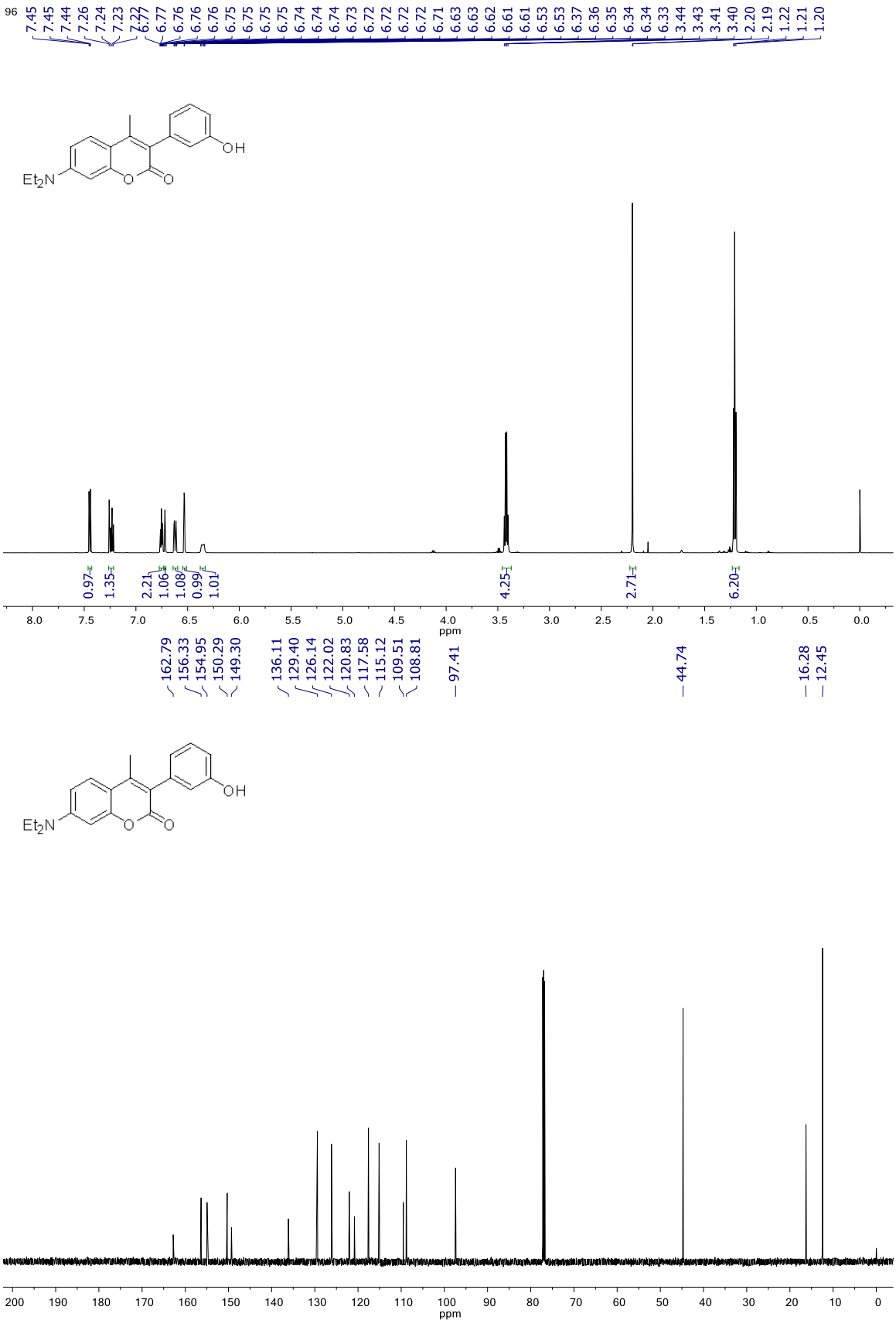
7.66
7.64
7.56
7.55
7.50
7.49
6.84
6.82
6.63
3.53
3.52
3.51
3.50
2.65
2.28
1.24
1.23
1.21
OH
B
OH
O
Et2N
O
O
1.00
1.10
1.12
1.02
0.82
0.84
5.11
2.68
2.90
7.07
ppm
203.88
165.25
161.81
161.55
157.64
152.97
142.37
138.50
137.87
133.78
132.94
129.16
121.94
112.26
99.89
47.35
26.39
17.84
13.91
ppm


96
7.45 7.45 7.44 7.26 7.24 7.23 7.22 6.77 6.77 6.76 6.76 6.76 6.75 6.75 6.75 6.75 6.74 6.74 6.74 6.73 6.72 6.72 6.72 6.72 6.71 6.63 6.63 6.62 6.61 6.61 6.53 6.53 6.37 6.36 6.35 6.34 6.34 6.33 3.44 3.43 3.41 3.40 2.20 2.19 1.22 1.21 1.20
OH
Et2N
O
O
0.97 1.35 2.21 1.06 1.08 0.99 1.01 4.25 2.71 6.20
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
ppm
162.79 156.33 154.95 150.29 149.30 136.11 129.40 126.14 122.02 120.83 117.58 115.12 109.51 108.81 97.41 44.74 16.28 12.45
OH
Et2N
O
O
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

References

1. C. Wurth, M. Grabolle, J. Pauli, M. Spieles, and U. Resch-Genger, *Nat. Protoc.*, 2013, *8*, 1535-1550.