Supporting Information
for
A new synthetic protocol for coumarin amino acid

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Experimental and analytical data
Experimental

**General remarks:** All reagents were purchased from commercial suppliers and used without further purification. Flash chromatography was carried out with silica gel (200–300 mesh). Analytical TLC was performed with silica gel GF\textsubscript{254} plates, and the products were tested by UV detection. \textsuperscript{1}H NMR spectra were recorded at 500 MHz (Varian DD2) and \textsuperscript{13}C NMR spectra were recorded at 125 MHz (Varian DD2). Chemical shifts (δ) are reported in ppm with TMS as internal standard, and spin–spin coupling constants (J) are given in Hz. \textsuperscript{19}F NMR spectra were recorded at 470 MHz (Varian DD2) and were reported in ppm with TFA as internal standard. HRMS (ESI) were measured on an Agilent Technologies 6110 mass spectrometer.

**4-(Chloromethyl)-7-hydroxy-2H-chromen-2-one (3a):**

Resorcinol (11.0 g, 100 mmol) was carefully dissolved in H\textsubscript{2}SO\textsubscript{4} solution (95%, 90 mL) at 0 °C with stirring. Ethyl 4-chloroacetoacetate (18.1 g, 110 mmol) was slowly added to the solution and the reaction mixture was stirred at 0 °C to room temperature for 5 h. TLC indicated that resorcinol was completely consumed and a fluorescent product was formed. The solution was poured slowly into an ice/water mixture (700 mL) and a large amount of solid was precipitated. The precipitate was filtered and
washed with water several times. It was then dried in an oven to afford a white solid, which is product 3a (16.3 g, 77.4 mmol, 77.4% yield); ESI-MS m/z: 211 [M + H]$^+$; $^1$H NMR (500 MHz, DMSO-d$_6$) $\delta$ 4.94 (s, 2H), 6.41 (s, 1H), 6.75 (d, $J = 2.5$ Hz, 1H), 6.84 (dd, $J_1 = 2.5$ Hz, $J_2 = 8.8$ Hz, 1H), 7.67 (d, $J = 8.8$ Hz, 1H), 10.64 (s, 1H); $^{13}$C NMR (125 MHz, DMSO-d$_6$) $\delta$ 41.36, 102.50, 109.32, 111.02, 113.06, 126.50, 150.95, 155.27, 160.13, 161.46.

Diethyl ((7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)phosphonate (4a): Compound 3a (860 mg, 4.10 mmol) was suspended in triethyl phosphite (2.72 g, 16.38 mmol), a catalytic amount of potassium iodide was added and the mixture was heated under reflux at 155 °C under N$_2$ for 4 h. TLC indicated that compound 3a was completed consumed. The reaction mixture was concentrated using an oil pump to afford compound 4a as orange-colored oil, which was used in the following reaction without purification. ESI-MS m/z: 313 [M + H]$^+$.

7-Hydroxy-4-vinyl-2H-chromen-2-one (5a): Compound 4a (4.10 mmol) from the last step was dissolved in anhydrous THF (40 mL) in an ice bath. NaH (60%, 0.82 g, 20.5 mmol)
was added to the solution batchwise and it was then stirred at 0 °C for 2 h. Formaldehyde solution (40%, 3 g, 40 mmol) was added dropwise and the reaction mixture was then stirred at rt for 5 h. TLC indicated the formation of three new spots with fluorescence. The solution was evaporated to dryness and then partitioned between ether and brine (v:v, 1:1, 100 mL total). The aqueous layer was further extracted with ether (2 × 50 mL). TLC indicated that the fluorescent products were in the organic layers. The organic layers were combined and condensed and then purified by flash chromatography (petroleum ether:ethyl acetate, 3:1) to afford compound 5a as a yellow solid (210 mg, 1.12 mmol, 27.3% yield over the last two steps). ESI-MS m/z: 189 [M + H]+, 211 [M + Na]+; 1H NMR (500 MHz, DMSO-d₆) δ 5.74 (dd, J₁ = 1.0 Hz, J₂ = 11.3 Hz, 1H), 6.21 (dd, J₁ = 1.0 Hz, J₂ = 17.1 Hz, 1H), 6.35 (s, 1H), 6.74 (d, J = 2.5 Hz, 1H), 6.81 (dd, J₁ = 2.5 Hz, J₂ = 8.8 Hz, 1H), 7.15 (dd, J₁ = 11.3 Hz, J₂ = 17.1 Hz, 1H), 7.74 (d, J = 8.8 Hz, 1H); 13C NMR (125 MHz, DMSO-d₆) δ 102.57, 106.20, 110.36, 111.02, 113.02, 123.96, 126.45, 130.19, 150.65, 155.32, 161.20.

Diethyl 2-acetamido-2-(2-(7-hydroxy-2-oxo-2H-chromen-4-yl)ethyl)malonate (6a): Potassium tert-butoxide (89.6 mg, 0.800 mmol), diethyl
acetamidomalonate (231 mg, 1.10 mmol) and catalytic amount of
tetrabutylammonium bromide (TBABr) were dissolved in anhydrous THF
(30 mL) and stirred at 0 °C for 1 h. Compound 5a (50 mg, 0.27 mmol) was
added to the solution and the reaction mixture was heated under reflux at
55 °C overnight. The reaction mixture was concentrated to dryness and
partitioned between ethyl acetate and water. TLC indicated that the fluorescent
product was in the organic layer. The organic layer was condensed and
purified by flash chromatography (CHCl₃:CH₃OH, 20:1) to afford a light yellow
solid product 6a (80 mg, 0.20 mmol, 73.1% yield). ESI-MS m/z: 406 [M + H]⁺,
428 [M + Na]⁺; ¹H NMR (500 MHz, DMSO-d₆) δ 1.13 (t, J = 7.1 Hz, 6H), 1.97 (s,
3H), 2.43 (m, 2H), 2.61 (m, 2H), 4.13 (q, J = 7.1 Hz, 4H), 6.09 (s, 1H), 6.72 (d,
J = 2.4 Hz, 1H), 6.81 (dd, J₁ = 2.4 Hz, J₂ = 8.8 Hz, 1H), 7.46 (d, J = 8.8 Hz,
1H), 8.46 (s, 1H), 10.56 (s, 1H dis. with D₂O); ¹³C NMR (125 MHz, DMSO-d₆) δ
13.74, 22.15, 25.79, 31.64, 61.83, 65.65, 102.54, 110.04, 110.77, 112.97,
125.80, 155.17, 155.45, 160.15, 161.14, 167.20, 169.40.

**rac-(2-(7-Hydroxycoumarin-4-yl)ethyl)glycine (1a):**

Compound 6a (80 mg, 0.20 mmol) was dissolved in HCl
(12 M, 30 mL) and heated under reflux at 110 °C in a
sealed tube for 4 h. The reaction mixture was evaporated
to dryness and partitioned between ethyl acetate and water (v:v, 1:1, 100 mL total). The aqueous layer was extracted by ethyl acetate (2 × 50 mL). The acid aqueous phase was then lyophilized to give colorless, crystalline product 1a as a salt of HCl (57 mg, 0.19 mmol, 95% yield). ESI-MS m/z: 264 [M + H]^+, 286 [M + Na]^+; ^1H NMR (500 MHz, DMSO-d$_6$) δ 2.21-2.07 (m, 2H), 3.0-2.85 (m, 2H), 4.02 (s, 1H), 6.13 (s, 1H), 6.77 (d, J = 2.5 Hz, 1H), 6.84 (dd, J$_1$ = 2.5 Hz, J$_2$ = 8.8 Hz, 1H), 7.67 (d, J = 8.8 Hz, 1H), 8.64 (s, 3H), 10.75 (s, 1H dis. with D$_2$O); ^13C NMR (125 MHz, DMSO-d$_6$) δ: 26.87, 28.65, 51.58, 102.54, 109.58, 110.83, 113.08, 126.25, 155.13, 155.26, 160.34, 161.41, 170.63.

4-(Chloromethyl)-6-fluoro-7-hydroxy-2H-chromen-2-one (3b): To a solution 4-fluoro-1,3-dihydroxybenzene (2.89 g, 22.6 mmol) in ice-cold concentrated sulfuric acid (98%, 10 mL) was added ethyl 4-chloroacetoacetate (3.72 g, 22.6 mmol) dropwise. The reaction mixture was then allowed to react at 0 °C to room temperature for 5 h. TLC indicated the disappearance of 4-fluoro-1,3-dihydroxybenzene and the formation of a new fluorescent compound. The reaction mixture was poured into ice-water (100 mL), and large amount of maroon solid was formed immediately. The mixture was filtered, washed with water several times and dried to afford solid product (4.00 g, 78.0% yield). Compound 3b (C$_{10}$H$_6$ClFO$_3$)
ESI-MS $m/z$: 229 [M + H]$^+$, 251 [M + Na]$^+$; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$

4.95 (s, 2H), 6.49 (s, 1H), 6.95 (d, $J = 7.69$ Hz, 1H), 7.66 (d, $J = 12.13$ Hz, 1H).

**Diethyl (6-fluoro-7-hydroxy-2-oxo-2$H$-chromen-4-yl) methylphosphonate (4b):** Compound 3b (3.00 g, 13.2 mmol) and a catalytic amount of potassium iodide were dissolved in triethyl phosphate (12.79 g, 77.0 mmol) to form a brown solution. It was then heated under reflux at 155 °C for 4 h under N$_2$. The reaction mixture was concentrated using an oil pump to afford a brown solid. It was resuspended in ethyl acetate and the mixture was put in a freezer set at $-20$ °C. A solid product was precipitated overnight, which was then filtered, washed with ethyl acetate several times and dried to afford brown solid product 4b (2.80 g, 64.2% yield). The product was used in the following reaction without purification.

**6-Fluoro-7-hydroxy-4-vinyl-2$H$-chromen-2-one (5b):** NaH (60%, 256 mg, 6.4 mmol) was added slowly to a solution of compound 4b (422 mg, 1.28 mmol) in anhydrous THF (15 mL) in an ice bath. The reaction mixture was then stirred at 0 °C for 0.5 h. After an aqueous solution of formaldehyde (40%, 0.96 g, 12.8 mmol) was added
dropwise, the reaction mixture was stirred at room temperature for 5 h. After concentration at reduced pressure, the mixture was partitioned between ether and brine (v/v, 1/1). The ethereal layer was evaporated to dryness to afford a light brown solid (5b) (220 mg, 83.0% yield). Compound 5b (C_{11}H_{7}FO_{3}) ESI-MS m/z: 207 [M + H]^+, 229 [M+Na]^+; $^1$H NMR (500 MHz, DMSO-d$_6$) δ 5.75 (dd, $J_1 = 0.8$ Hz, $J_2 = 11.3$ Hz, 1H), 6.19 (dd, $J_1 = 0.8$ Hz, $J_2 = 17.2$ Hz, 1H), 6.47 (s, 1H), 6.93 (d, $J = 7.2$ Hz, 1H), 7.15 (dd, $J_1 = 11.3$ Hz, $J_2 = 17.2$ Hz, 1H), 7.77 (d, $J = 11.8$ Hz, 1H).

Diethyl 2-acetamido-2-(2-(6-fluoro-7-hydroxy-2-oxo-2H-chromen-4-yl)ethyl)malonate (6b): Potassium tert-butoxide (673 mg, 6.00 mmol), diethyl acetamidomalonate (652 mg, 3.00 mmol) and a catalytic amount of tetrabutylammonium bromide (TBABr) were dissolved in anhydrous THF (30 mL) and stirred at 0 °C for 1 h. Compound 5b (206 mg, 1.00 mmol) was added to the solution and the reaction mixture was heated under reflux at 55 °C for 4 h. The reaction mixture was concentrated to dryness and partitioned between ethyl acetate and water. TLC indicated that the fluorescent product was in the organic layer. The organic layer was condensed and the residue was purified by flash chromatography (CHCl$_3$:CH$_3$OH, 20:1) to afford a
light yellow solid product 6b (364 mg, 86.0% yield). Compound 4b (C_{20}H_{22}FNO_{8}) ESI-MS m/z: 424 [M + H]^+, 446 [M + Na]^+

2-Amino-4-(6-fluoro-7-hydroxy-2-oxo-2H-chromen-4-yl) butanoic acid (1b): Compound 6b (80 mg, 0.19 mmol) was dissolved in HCl (30 mL, 12 M) and heated under reflux at 110 °C for 4 h in a sealed tube. The reaction mixture was concentrated under reduced pressure and precipitated with ethyl acetate. The precipitate was filtered and washed with ethyl acetate several times to afford product 1b as a HCl salt (50 mg, 83% yield). Compound 1b (C_{13}H_{12}FNO_{5}) ESI-MS m/z: 282 [M + H]^+, 304 [M + Na]^+; \(^{1}\)H NMR (500 MHz, DMSO-\(d_6\)) \(\delta\) 2.15-2.06 (m, 2H), 2.91-2.82 (m, 2H), 4.06 (m, 1H), 6.21 (s, 1H), 6.95 (d, \(J = 7.3\) Hz, 1H), 7.63 (d, \(J = 11.7\) Hz, 1H), 8.44 (s, 3H); \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)) \(\delta\) 26.87, 28.65, 51.58, 102.54, 109.58, 110.83, 113.08, 126.25, 155.13, 155.26, 160.34, 161.41, 170.63; \(^{19}\)F NMR (470 MHz, DMSO-\(d_6\)) \(\delta\) −143.44.

6-Chloro-4-(chloromethyl)-7-hydroxy-2H-chromen-2-one (3c): To a solution of 4-chloro-1,3-dihydroxybenzene (14.4 g, 100 mmol) in ice-cold concentrated sulfuric acid (98%, 30 mL)
was added ethyl 4-chloroacetoacetate (16.4 g, 100 mmol) dropwise. The reaction mixture was then allowed to react at 0 °C to room temperature for 5 h. TLC indicated the disappearance of 4-fluoro-1,3-dihydroxybenzene and the formation of a new fluorescent compound. The reaction mixture was poured into ice-water (100 mL), and a large amount of yellow solid was formed immediately. The mixture was filtered, washed with water several times and dried to afford a solid product (17.1 g, 70.0% yield). Compound 3c (C10H6Cl2O3) ESI-MS m/z: 246 [M + H]+, 268 [M + Na]+; 1H NMR (500 MHz, DMSO-d6) δ 4.99 (s, 2H), 6.48 (s, 1H), 6.94 (s, 1H), 7.86 (s, 1H).

Diethyl (6-chloro-7-hydroxy-2-oxo-2H-chromen-4-yl) methylphosphonate (4c): Compound 3c (2.83 g, 11.6 mmol) and a catalytic amount of potassium iodide were dissolved in triethyl phosphate (7.64 g, 46.0 mmol) to form a brown solution. It was then heated under reflux at 155 °C for 4 h under N2. The reaction mixture was concentrated using an oil pump to afford a brown solid. It was resuspended in ethyl acetate and the mixture was put in a freezer set at −20 °C. The solid product was precipitated overnight, which was filtered, washed with ethyl acetate several times and dried to afford brown solid product 4c (2.48 g, 62.0% yield). The product was used in the following reaction without further purification.
6-Chloro-7-hydroxy-4-vinyl-2H-chromen-2-one (5c): NaH (60%, 0.20 g, 5 mmol) was added slowly to a solution of compound 4c (240 mg, 0.69 mmol) in anhydrous THF (10 mL) in an ice bath. The reaction mixture was then stirred at 0 °C for 0.5 h. After an aqueous solution of formaldehyde (40%, 0.52 g, 6.9 mmol) was added dropwise, the reaction mixture was stirred at room temperature for 5 h. After concentration at reduced pressure, the mixture was partitioned between ether and brine (v/v, 1:1). The ethereal layer was evaporated to dryness to afford a light brown solid, which was purified by flash chromatography (petroleum ether:ethyl acetate, 3:1) to afford product 5c (137 mg, 89.2% yield). Compound 5c (C_{11}H_{7}ClO_{3}) ESI-MS m/z: 223 [M + H]^+; ¹H NMR (500 MHz, DMSO-d_6) δ 5.74 (d, J = 11.1 Hz, 1H), 6.17 (d, J = 17.1 Hz, 1H), 6.43 (s, 1H), 6.91 (s, 1H), 7.20 (dd, J_1 = 11.1 Hz, J_2 = 17.1 Hz, 1H), 7.92 (s, 1H).

Diethyl 2-acetamido-2-(2-(6-chloro-7-hydroxy-2-oxo-2H-chromen-4-yl)ethyl)malonate (6c): Potassium tert-butoxide (189 mg, 1.68 mmol), diethyl acetamidomalonate (373 mg, 1.70 mmol) and a catalytic amount of tetrabutylammonium bromide (TBABr) were dissolved in anhydrous THF (30 mL) and stirred at 0 °C for 1 h. Compound 5c (125 mg, 0.56 mmol)
was added to the solution and the reaction mixture was heated under reflux at 55 °C for 4 h. The reaction mixture was concentrated to dryness and partitioned between ethyl acetate and water. TLC indicated that the fluorescent product was in the organic layer. The organic layer was condensed and the residue was purified by flash chromatography (CHCl₃:CH₃OH, 20:1) to afford a light yellow solid product 6c (212 mg, 86.0% yield). Compound 6c (C₂₀H₂₂ClNO₈) ESI-MS m/z: 440 [M + H]⁺, 462 [M + Na]⁺; ¹H NMR (500 MHz, DMSO-d₆) δ 1.14 (t, 6H), 1.98 (s, 3H), 2.38-2.41 (m, 2H), 2.59-2.63 (m, 2H), 4.12-4.17 (m, 4H), 6.17 (s, 1H), 6.91 (s, 1H), 7.57(s, 1H), 8.49 (s, 1H).

2-Amino-4-(6-chloro-7-hydroxy-2-oxo-2H-chromen-4-yl)butanoic acid (1c): Compound 6c (212 mg, 0.48 mmol) was dissolved in HCl (50 mL, 12 M) and heated under reflux at 110 °C for 6 h in a sealed tube. The reaction mixture was concentrated under reduced pressure and precipitated with ethyl acetate. The precipitate was filtered and washed with ethyl acetate several times to afford product 1c as a HCl salt (135 mg, 84.3% yield). Compound 1c (C₁₃H₁₂ClNO₅) ESI-MS m/z: 298 [M + H]⁺, 320 [M + Na]⁺; ¹H NMR (500 MHz, DMSO-d₆) δ 2.20-2.03 (m, 2H), 2.97-2.78 (m, 2H), 4.02 (t, J = 6.0 Hz, 1H), 6.18 (s, 1H), 6.95 (s, 1H), 7.79 (s, 1H); ¹³C NMR (125 MHz, DMSO-d₆) δ 26.62,
Protein expression

To determine whether compound 1a is incorporated into the protein with high efficiency and fidelity, an amber stop codon was substituted for Ile38 in E.coli Thioredoxin-1 (TRX). The plasmid pEVOL-CouRS-D8 was cotransformed with a plasmid carrying the Thioredoxin38TAG gene (pET-TRX38TAG) into E. coli BL21-DE3 cells. Bacteria were grown at 37 °C in LB medium for 2.5 h until OD$_{600}$ = 1.0, at which point 2 mM racemic 1a was added to the culture. The bacteria were grown at 37 °C for 30 min and then protein expression was induced by the addition of 0.02% arabinose and 1 mM isopropyl-$\beta$-D-thiogalactopyranoside (IPTG). After another 5 h, cells were harvested by centrifugation. The TAG38 mutant thioredoxin was purified by Ni-NTA affinity chromatography under native conditions. Analysis of the purified protein by SDS-PAGE showed that full-length thioredoxin was expressed only in the presence of 1a (Figure 2).
Figure S1: Absorption spectra of 1b and 1c at different pH. (A) Absorption spectrum of 50 μM 1b in 200 mM sodium phosphate buffer (pH 5.8–8.0), 200 mM sodium acetate buffer (pH 3.7–5.6) or 50 mM Tris-HCl Buffer (pH 8.2–8.9). (B) Absorption spectrum of 25 μM 1c in 200 mM sodium phosphate buffer (pH 5.8–8.0) or sodium acetate buffer (pH 3.7–5.6).