Supporting Information

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A Very Short Route to Enantiomerically Pure Coumarin-bearing Fluorescent Amino Acids**

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**General.** $^1$H- and $^{13}$C-NMR (respectively, 400 MHz and 100 MHz) were recorded in the solvent indicated on a Brüker Avance 400 spectrometer. Chemical shifts are reported in ppm downfield from TMS ($\delta$), coupling constants ($J$) are reported in Hz. Assignments of $^{13}$C-NMR spectra chemical shifts were achieved by means of DEPT, HMQC and HMBC experiments for compound (S)-6 and postulated for other compounds (5, (R)-6 to 15) assuming that they would exhibit the same shape. Mass spectra were recorded on a Q-Tof Micromass spectrometer equipped with Z-spray source. Optical rotations were measured on a Jasco model P-1030 polarimeter.

**Materials.** Asp and Glu derivatives were purchased from Bachem, 3-hydroxyphenol, 3,4-dimethoxyphenol, 4-chlororesorcinol, 3-ethoxyphenol from Acros and 3-methoxyphenol, 5-methoxyresorcinol, 3,5-dimethoxyphenol, 3,5-dihydroxyphenol from Aldrich.

**Method A. General procedure for the synthesis of amino acids derived $\beta$-ketoesters.** To a solution of the protected amino acid in THF (4 mL/mmol), carbonyldiimidazole (1.1 equiv) was added at room temperature. The resulting reaction mixture was stirred two hours at room temperature, then cooled at 0°C before addition of monoethyl malonic acid magnesium salt (0.54 equiv). The reaction mixture was stirred overnight at room temperature as a slow CO$_2$ release was observed. It was taken up in ether (15-20 mL/mmol of amino acid) and acidified with concentrated HCl (1N or 6N) at 0°C; the etheral extract was washed with 10% NaHCO$_3$, 4:1 H$_2$O:KHSO$_4$(1M), H$_2$O and brine, dried (Na$_2$SO$_4$) and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (1:1 EtOAc:cyclohexane).

(2S)-2-Benzylxocarbonylamino-4-oxo-hexanedioic acid 1-benzyl ester 6-ethyl ester (1). Following method A from Cbz-(L)-Asp-OBn (7.41 g, 20.74 mmol), compound (1) (6.83 g, 16.0 mmol, 77%) was obtained as a white solid. $^1$H-NMR (CDCl$_3$) : $\delta$ 1.26 (t, $^3$J(H,H) = 7.2, 3H), 3.15 (dd, $^2$J(H,H) = 18.6, $^3$J(H,H) = 4.2, 1H), 3.32 (dd, $^2$J(H,H) = 18.6, $^3$J(H,H) = 4.2, 1H), 3.42 (s, 2H), 4.17 (q, $^3$J(H,H) = 7.2, 2H), 4.52-4.71 (m, 1H), 5.12 (s, 2H), 5.17 (s, 2H), 5.78 (d, $^3$J(H,H) = 8.4,1H), 7.33 (m, 10H). $^{13}$C-NMR (CDCl$_3$) : $\delta$ 13.7 (n), 44.2 (k), 48.7 (i),
(2R)-2-tert-Butoxycarbonylamino-4-oxo-hexanedioic acid 1-benzyl ester 6-ethyl ester (2).

Following method A from Boc-(D)-Asp-OBn (2 g, 6.2 mmol), compound (2) (1.609 g, 4.1 mmol, 66%) was obtained as a pure white solid. $^1$H-NMR (CDCl$_3$) :  δ 1.27 (t, $^3$J(H,H) = 7.2, 3H), 1.45 (s, 9H), 3.12 (dd, $^2$J(H,H) = 18.4, $^3$J(H,H) = 4.4, 1H), 3.30 (dd, $^2$J(H,H) = 18.4, $^3$J(H,H) = 4.4, 1H), 3.43 (s, 2H), 4.14-4.22 (q, $^3$J(H,H) = 7.2, 2H), 4.58 (m, 1H), 5.16 (s, 2H), 5.48 (d, $^3$J(H,H) = 8.4, 1H), 7.3-.74 (m, 5H). MS (ESI, 30 V) : m/z (%) : 416 (100) [M$^+$ + Na], 360 (38).

(2S)-2-Benzoyloxycarbonylamino-5-oxo-heptanedioic acid 1-benzyl ester 7-ethyl ester (3).

Following method A from Cbz-(L)-Glu-OBn (10 g, 26.93 mmol), compound (3) (6.187 g, 14 mmol, 52%) was obtained as a pure white solid. $^1$H-NMR (CDCl$_3$) :  δ 1.26 (t, $^3$J(H,H) = 7.2, 3H), 1.9-2.01 (m, 1H), 2.1-2.28 (m, 1H), 2.5-2.7 (m, 2H), 3.37 (s, 2H), 4.18 (q, $^3$J(H,H) = 7.2, 2H), 4.41 (m, 1H), 5.11 (s, 2H), 5.18 (s, 2H), 5.44 (d, $^3$J(H,H) = 8, 1H), 7.25-7.41 (m, 10H). MS (ESI, 30 V) : m/z (%) : 464 (100) [M$^+$ + Na].

Method B. General procedure for the removal of Cbz and Bn groups on amino acids derived β-ketoesters . The β-ketoester obtained by method A was dissolved in 1:1 AcOEt:95% EtOH and HCl 1N (1 or 2 equiv) and subjected to hydrogenolysis under atmospheric pressure over 10% Pd on charcoal (0.05 equiv). The resulting reaction mixture was filtrated through celite, washed with 95% EtOH and concentrated in vacuo. The residue was taken up in water and lyophilised.

(1S)-(1-Carboxy-4-ethoxycarbonyl-3-oxo-butyl) ammonium chloride ((S)-4). Following method B from compound (1) (6.78 g, 15.8 mmol), compound ((S)-4) (3.72 g, 15.5 mmol, 97%) was obtained as a pure white solid. $^1$H-NMR (DMSO) :  δ 1.14 (t, $^3$J(H,H) = 7.2, 3H), 3.13 (dd, $^2$J(H,H) = 18.8, $^3$J(H,H) = 5.2, 2H), 3.21 (dd, $^2$J(H,H) = 18.8, $^3$J(H,H) = 5.2, 2H), 3.62 (d, $^3$J(H,H) = 16.4, 1H), 3.67 (d, $^2$J(H,H) = 16.4, 1H), 4.05 (q, $^3$J(H,H) = 7.2, 2H), 4.09-4.15 (m, 1H), 8.40 (s, 3H). $^{13}$C-NMR (DMSO) :  δ 14.0 (CH$_3$), 42.0 (CH$_2$), 47.3 and 48.7 (CH

49.5 (h), 61.2 (m), 66.7 and 67.2 (e and e’), 127.7, 127.8, 128.0, 128.1 and 128.2 (b, c, d, b’, c’ and d’), 134.8 and 135.7 (a and a’), 155.6 (g), 166.1(l), 170.2 (f) and 200.4 (j).
and CH\textsubscript{2} of the β-ketoester), 60.7 (CH\textsubscript{2}), 166.7 (C=O ester), 170.0 (C=O acid), 200.1 (C=O ketone).

(1R)-(1-Carboxy-4-ethoxycarbonyl-3-oxo-butyl) ammonium chloride ((R)-4). Following method B from compound (2) (1.43 g, 3.6 mmol), the Bn group was removed and the resulting residue was treated for removal of the Boc group in a 1:1 mixture of CHCl\textsubscript{3}:TFA (24mL) added at 0°C. After one hour at room temperature, the mixture was concentrated \textit{in vacuo}, the residue taken up in water and lyophilised giving compound ((R)-4) as a pure white solid (1g, 3.3 mmol, 91%). \textit{1}H-NMR (DMSO): \(\delta\) 1.15 (t, \(^3J(H,H) = 7.2\), 3H), 3.09 (dd, \(^2J(H,H) = 19\), \(^3J(H,H) = 6\), 1H), 3.16 (dd, \(^2J(H,H) = 19\), \(^3J(H,H) = 4.4\), 1H), 3.65 (s, 2H), 4.05 (q, \(^3J(H,H) = 7.2\), 2H), 4.11-4.21 (m, 1H), 8.16 (s, 3H).

Method C. General procedure for the condensation of phenols with amino acid-derived β-ketoesters. Amino acid-derived β-ketoesters and phenol derivative (1.5 equiv) were mixed before addition of 99% methanesulfonic acid (25 equiv) at 0°C and stirred at room temperature for one to two hours. The deep-red, homogenous reaction mixture was taken up in cold ether (-30°C) and centrifuged 20 minutes at 3600×g. After ether discarding, the residue was washed once more with cooled ether, centrifuged 20 minutes at 3600×g, taken up in water and lyophilised.

(1S)-1-Carboxy-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)ethyl ammonium trifluoroacetate (5). Following method C from β-ketoester ((S)-4) (197 mg, 0.82 mmol) and 3-hydroxyphenol (136 mg, 1.24 mmol), compound (5) (142 mg, 0.39 mmol, 47%) was obtained as a pure pale yellow solid. \textit{1}H-NMR (DMSO): \(\delta\) 3.08 (dd, \(^2J(H,H) = 15.2\), \(^3J(H,H) = 6.8\), 1H), 3.32 (dd, \(^2J(H,H) = 15.2\), \(^3J(H,H) = 5.2\), 1H), 4.12-4.22 (m, 1H), 6.16 (s, 1H), 6.71 (d, \(^4J(H,H) = 2.4\), 1H), 6.79 (dd, \(^3J(H,H) = 8.8\), \(^4J(H,H) = 2.4\), 1H), 7.56 (d, \(^3J(H,H) = 8.8\), 1H), 8.28 (s, 3H), 10.63 (s, 1H). \textit{13}C-NMR (DMSO): \(\delta\) 32.0 (l), 51.0 (k), 102.6, 110.7, 112.8, 113.1, 126.0, 149.6, 155.3, 160.0, 161.4, 170.0. \([\alpha]_D^{20} = 8.4\) (c=0.485 in 1N HCl).

(1S)-1-Carboxy-2-(7-methoxy-2-oxo-2H-chromen-4-yl)ethyl ammonium methanesulfonate ((S)-6). Following method C from β-ketoester ((S)-4) (1.786 g, 7.45 mmol) and 3-methoxyphenol (1.387 g, 11.18 mmol), compound ((S)-6) (1.9 g, 5.3 mmol, 71%) was obtained as a pure white powder. \textit{1}H-NMR (DMSO): \(\delta\) 2.31 (s, 3H), 3.12 (dd, \(^2J(H,H) = 14.4\), \(^3J(H,H) = 8.8\), 1H), 3.35 (dd, \(^2J(H,H) = 14.4\), \(^3J(H,H) = 5.8\), 1H), 3.82 (s, 3H), 4.11-4.23 (m, 1H), 6.25 (s, 1H), 6.96 (dd, \(^3J(H,H) = 8.8\), \(^4J(H,H) = 2.4\), 1H), 6.70 (d, \(^4J(H,H)=2.4\), 1H).
= 2.4, 1H), 7.66 (d, $^2J(H,H) = 8.8$, 1H), 8.31 (s, 3H). $^{13}$C-NMR (DMSO) : $\delta$ 31.9 (m), 51.0 (l), 56.1 (k), 101.2 (j), 111.9 (i), 112.4 (h), 113.8 (g), 126.0 (f), 149.7 (e), 155.3 (d), 159.9 (c), 162.6 (b), 167.0 (a). MS (ESI, 20 V) : $m/z$ (%) : 264 (100) [M$^+$], 191 (65). $[\alpha]_D^{20} = 12.9$ (c=0.7 in 1N HCl).

(1R)-1-Carboxy-2-(7-methoxy-2-oxo-2H-chromen-4-yl)ethyl ammonium trifluoroacetate ([(R)-6]). Following method C from $\beta$-ketoester ([(R)-4]) (694 mg, 2.9 mmol) and 3-methoxyphenol (540 mg, 4.34 mmol), compound ([(R)-6]) (728 mg, 2.03 mmol, 70%) was obtained as a pure pale yellow solid after purification by semi-preparative HPLC. $^1$H-NMR (DMSO) : $\delta$ 2.99 (dd, $^2J(H,H) = 14.6$, 3H, 1H), 3.39 (dd, $^2J(H,H) = 14.6$, 3H, 1H), 3.81 (s, 3H), 3.85-3.98 (m, 1H), 6.22 (s, 1H), 6.96 (dd, $^3J(H,H) = 8.8$, 4H, 1H), 7.00 (d, $^4J(H,H) = 2.4$, 1H), 7.66 (d, $^3J(H,H) = 8.8$, 1H), 8.12 (s, 3H). MS (ESI, 30 V) : $m/z$ (%) : 286 (100) [M$^+$ -H +Na]. $[\alpha]_D^{20} = -12.1$ (c=0.468 in 1N HCl).

(1S)-1-Carboxy-2-(6,7-dimethoxy-2-oxo-2H-chromen-4-yl)ethyl ammonium trifluoroacetate (7). Following method C from $\beta$-ketoester ([(S)-4]) (556 mg, 2.32 mmol) and 3,4-dimethoxyphenol (537 mg, 3.48 mmol), compound (7) (426 mg, 1.04 mmol, 45%) was obtained as a pure pale yellow solid. $^1$H-NMR (DMSO) : $\delta$ 3.15 (dd, $^2J(H,H) = 14.6$, 3H, 1H), 3.35 (dd, $^2J(H,H) = 14.6$, 3H, 1H), 3.80 (s, 3H), 3.81 (s, 3H), 4.22-4.35 (m, 1H), 6.24 (s, 1H), 7.01-7.75 (m, 1H), 7.10 (s, 1H), 8.31 (s, 3H). $^{13}$C-NMR (DMSO + TFA) : $\delta$ 32.4, 51.9, 103.7, 111.6, 113.5, 117.1, 125.6, 150.1, 153.5, 156.7, 159.7, 169.7. MS (ESI, 30 V) : $m/z$ (%) : 294 (41) [M$^+$ -H +Li], 211 (98), 185 (43).

(1S)-1-Carboxy-2-(6-chloro-7-hydroxy-2-oxo-2H-chromen-4-yl)ethyl ammonium trifluoroacetate (8). Following method C from $\beta$-ketoester ([(S)-4]) (516 mg, 3.57 mmol) and 4-chlororesorcinol (515.7 g, 3.57 mmol), compound (8) (50 mg, 0.13 mmol, 16%) was obtained as a pure white solid. $^1$H-NMR (DMSO) : $\delta$ 3.07 (dd, $^2J(H,H) = 14.4$, 3H, 1H), 3.3 (dd, $^2J(H,H) = 14.4$, 3H, 1H), 4.16-4.28 (m, 1H), 6.23 (s, 1H), 6.89 (s, 1H), 7.73 (s, 1H), 8.3 (s, 3H). $^{13}$C-NMR (DMSO) : $\delta$ 32.4, 51.9, 103.7, 111.6, 113.5, 117.1, 125.6, 150.1, 153.5, 156.7, 159.7, 169.7. MS (ESI, 50 V) : $m/z$ (%) : 288 (100) [M$^+$ -H +Li], 211 (98), 185 (43).
(1S)-1-Carboxy-2-(7-ethoxy-2-oxo-2H-chromen-4-yl)ethyl ammonium trifluoroacetate (9). Following method C from β-ketoester ((S)-4) (547 mg, 2.3 mmol) and 3-ethoxyphenol (477 mg, 3.45 mmol), compound (9) (341 mg, 0.9 mmol, 39%) was obtained as a pure white solid. $^1$H-NMR (DMSO) : δ 1.31 (t, $^3 J(H,H) = 6.8$, 3H), 3.07 (dd, $^2 J(H,H) = 14.4$, $^3 J(H,H) = 9.6$, 1H), 3.35 (dd, $^2 J(H,H) = 14.4$, $^3 J(H,H) = 5.2$, 1H), 4.09 (q, $^3 J(H,H) = 6.8$, 2H), 4.12-4.24 (m, 1H), 6.20 (s, 1H), 6.9-6.99 (m, 2H), 7.65 (d, $^3 J(H,H) = 8.8$, 1H), 8.28 (s, 3H). $^{13}$C-NMR (DMSO + TFA) : 14.3, 31.9, 50.9, 64.1, 101.5, 111.7, 112.6, 115.1 (q, $J = 1147$), 125.8, 149.5, 155.3, 158.5 (q, J = 152), 159.9, 161.8, 170.0. MS (ESI, 60 V) : $m/z$ (%) : 322.2 (100) [M$^-$-2H +2Na]. $[\alpha]_D^{20} = 15.7$ (c=0.476 in 1N HCl).

(1S)-1-Carboxy-2-(5-hydroxy-7-methoxy-2-oxo-2H-chromen-4-yl)ethyl ammonium trifluoroacetate (10) and (1S)-1-Carboxy-2-(7-hydroxy-5-methoxy-2-oxo-2H-chromen-4-yl)ethyl ammonium trifluoroacetate (11). Following method C from β-ketoester ((S)-4) (536 mg, 2.24 mmol) and 5-methoxyresorcinol (471 mg, 3.36 mmol), compound (10) (300 mg, 0.8 mmol, 36%) and compound (11) (120 mg, 0.4 mmol, 18%) were obtained in a 2:1 ratio as pure pale yellow solids after separation by semi-preparative HPLC on a Kromasil C$_8$ column (AIT, 10µ, 20×250 mm). Compound (10) : $^1$H-NMR (DMSO) : δ 2.58-2.71(m, 1H), 3.55-3.68 (m, 1H), 3.72-3.8 (m, 1H), 3.81 (s, 3H), 5.90 (s, 1H), 6.30 (d, $^2 J(H,H) = 2$, 1H), 6.34 (d, $^2 J(H,H) = 2$, 1H), 7.65 (broad singles, 3H), 10.64 (s, 1H). $^{13}$C-NMR (DMSO + TFA) : δ 36.9, 51.6, 56.0, 95.8, 96.0, 101.6, 113.1, 115.1 (q, J = 1147), 150.0, 156.8, 158.5 (q, J = 152), 159.6, 161.8, 170.5. MS (ESI, 50 V) : $m/z$ (%) : 304.3 (76) [M$^+$+H +Na], 107.9 (100). Compound (11) : $^1$H-NMR (DMSO) : δ 2.80 (dd, $^2 J(H,H) = 12.8$, $^3 J(H,H) = 10.8$, 1H), 3.73 (s, 3H), 3.81 (dd, $^2 J(H,H) = 12.8$, $^3 J(H,H) = 4.0$, 1H), 3.94-4.09 (m, 1H), 3.81 (s, 3H), 5.96 (s, 1H), 6.30 (d, $^4 J(H,H) = 2.4$, 1H), 6.44 (d, $^4 J(H,H) = 2.4$, 1H), 7.9 (broad singles, 3H). MS (ESI, 30 V) : $m/z$ (%) : 302.2 (74) [M$^+$-H +Na], 236.2 (100). $[\alpha]_D^{20} = 10.1$ (c=0.323 in EtOH 95%).

(1S)-1-Carboxy-2-(5,7-dimethoxy-2-oxo-2H-chromen-4-yl)ethyl ammonium trifluoroacetate (12). Following method C from β-ketoester ((S)-4) (547 mg, 2.28 mmol) and 3,5-dimethoxyphenol (528 mg, 3.42 mmol), compound (12) (316 mg, 0.78 mmol, 34%) was obtained as a pure white solid. $^1$H-NMR (DMSO) : δ 2.84 (dd, $^2 J(H,H) = 12.8$, $^3 J(H,H) = 10.8$, 1H), 3.73 (dd, $^2 J(H,H) = 12.8$, $^3 J(H,H) = 4.0$, 1H), 3.81 (s, 3H), 3.85 (s, 3H), 3.99-4.11 (m, 1H), 6.01 (s, 1H), 6.51 (d, $^4 J(H,H) = 2.0$, 1H), 6.61 (d, $^4 J(H,H) = 2.0$, 1H), 8.17 (s, 3H). $^{13}$C-NMR (DMSO) : δ 36.9, 51.6, 56.0, 56.3, 94.0, 95.6, 102.8, 114.1, 149.7, 156.8,
(1S)-1-Carboxy-2-(5,7-dihydroxy-2-oxo-2H-chromen-4-yl)ethyl ammonium trifluoroacetate (13). Following method C from β-ketoester ((S)-4) (553 mg, 2.31 mmol) and 3,5-dihydroxyphenol (561 mg, 3.46 mmol), compound (13) (420.2 mg, 1.1 mmol, 48%) was obtained as a pure pale yellow powder. $^1$H-NMR (DMSO) : $\delta$ 2.80 (dd, $^2$$\text{J(H,H)}$ = 13, $^3$$\text{J(H,H)}$ = 11.6, 1H), 3.78 (dd, $^2$$\text{J(H,H)}$ = 13, $^3$$\text{J(H,H)}$ = 4, 1H), 4.05-4.2 (m, 1H), 5.88 (s, 1H), 6.17 (d, $^4$$\text{J(H,H)}$ = 2.2, 1H), 6.25 (d, $^4$$\text{J(H,H)}$ = 2.2, 1H), 8.16 (s, 3H), 10.40 (s, 1H). MS (ESI, 30 V) : m/z (%) : 288 (43) [M$^+$ -H +Na], 266 (73) [M$^+$], 151 (100). $[\alpha]^D$$_{20}$$ = 13.2 (c=0.448 in 1N HCl).

(1S)-1-Carboxy-3-(7-methoxy-2-oxo-2H-chromen-4-yl)propyl ammonium trifluoroacetate (14) and (1S)-1-Benzyloxy carbonyl-3-(7-methoxy-2-oxo-2H-chromen-4-yl)propyl ammonium trifluoroacetate (15). Following method C from compound (3) (300 mg, 0.7 mmol) and 3-methoxyphenol (869 mg, 7 mmol), compound (14) (98 mg, 0.26 mmol, 37%) and compound (15) (76 mg, 0.16 mmol, 23%) were obtained in 62:38 proportion as a pure orange solid and a pure white solid respectively after separation by semi-preparative HPLC. Compound (14) : $^1$H-NMR (DMSO) : $\delta$ 1.92-2.16 (m, 2H), 2.72-2.97 (m, 2H), 3.82 (s, 3H), 3.82-4.0 (m, 1H), 6.16 (s, 1H), 6.95 (dd, $^3$$\text{J(H,H)}$ = 8.8, $^4$$\text{J(H,H)}$ = 2.4, 1H), 6.98 (d, $^4$$\text{J(H,H)}$ = 2.4, 1H), 7.68 (d, $^3$$\text{J(H,H)}$ = 8.8, 1H), 8.17 (s, 3H). $^{13}$C-NMR (D$_2$O) : $\delta$ 29.6, 31.2, 55.5, 58.7, 103.8, 112.5, 115.1, 115.8, 128.5, 157.4, 159.7, 165.4, 167.4, 174.8. MS (ESI, 30 V) : m/z (%) : 300.2 (100) [M$^+$ -H +Na]. $[\alpha]^D$$_{20}$$ = 19.4 (c=0.504 in 1N HCl). Compound (15) : $^1$H-NMR (DMSO) : $\delta$ 1.88-2.11 (m, 2H), 2.68-2.89 (m, 2H), 3.81 (s, 3H), 3.73-4.02 (m, 1H), 5.17 (s, 2H), 6.10 (s, 1H), 6.88 (dd, $^3$$\text{J(H,H)}$ = 8.8, $^4$$\text{J(H,H)}$ = 2.4, 1H), 6.96 (d, $^4$$\text{J(H,H)}$ = 2.4, 1H), 7.23-7.42 (m, 5H), 7.57 (d, $^3$$\text{J(H,H)}$ = 8.8, 1H). $^{13}$C-NMR (DMSO) : $\delta$ 26.5, 28.7, 51.5, 56.0, 67.4, 101.1, 110.6, 111.9, 112.3, 125.9, 128.5, 128.6, 135.1, 154.7, 155.1, 160.1, 162.5, 169.2. MS (ESI, 40 V) : m/z (%) : 390.2 (100) [M$^+$ -H +Na].

(2S)-2-Fmoc-amino-3-(7-methoxy-2-oxo-2H-chromen-4-yl)propionic acid, Fmoc-Mca ((S)-16). The coumaryl amino acid ((S)-6) (510 mg, 1.42 mmol) dissolved in 1:1 dioxane:H$_2$O (7 mL/mmol) was treated at 0°C with 10% NaHCO$_3$. One minute later, FmocCl (404 mg, 1.57 mmol, 1.1 equiv) was added at 0°C; the reaction mixture was stirred one hour at 0°C then two hours at room temperature. The reaction mixture was taken up in AcOEt, the organic extract was washed with H$_2$O, 1N HCl and brine, dried (Na$_2$SO$_4$) and concentrated in vacuo.
The residue was purified by flash chromatography on silica gel (4:1 EtOAc:cyclohexane to pure AcOEt) to give a pure white solid (512.9 mg, 1.14 mmol, 80%). $^1$H-NMR (DMSO) : $\delta$ 3.00 (dd, $^2$$J$(H,H) = 14.4, $^3$$J$(H,H) = 11.2, 1H), 3.28 (dd, $^2$$J$(H,H) = 14.4, $^3$$J$(H,H) = 3.4, 1H), 3.80 (s, 3H), 4.13 (t, $^3$$J$(H,H) = 6.4, 1H), 4.17 (d, $^3$$J$(H,H) = 6.4, 2H), 4.2-4.31 (m, 1H), 6.20 (s, 1H), 6.95 (dd, $^3$$J$(H,H) = 8.8, $^4$$J$(H,H) = 2.4, 1H), 6.98 (d, $^4$$J$(H,H) = 2.4, 1H), 7.26 (dd, $J$ = 14.4, 7.2, 2H), 7.35 (t, $J$ = 7.6, 2H), 7.57 (dd, $J$ = 11.8, 7.6, 2H), 7.7 (d, $^3$$J$(H,H) = 8.8, 1H), 7.83 (dd, $J$ = 8.0, 3.6, 2H).

$^{13}$C-NMR (DMSO) : $\delta$ 21.1, 32.7, 46.5, 52.8, 56.0, 66.8, 66.4, 101.1, 112.1, 112.4, 120.2, 125.15, 125.9, 127.1, 127.7, 140.7, 143.67, 143.74, 152.7, 155.1, 156.0, 160.0, 162.4, 172.7.

(2R)-2-Fmoc-amino-3-(7-methoxy-2-oxo-2H-chromen-4-yl)propionic acid ((R)-16). Following the same procedure as for compound ((S)-16), the coumaryl amino acid ((R)-6) (631 mg, 1.76 mmol) led to compound ((R)-16) (556 mg, 1.23 mmol, 70%) as a pure white solid. $^1$H-NMR (DMSO) : $\delta$ 3.00 (dd, $^2$$J$(H,H) = 14.4, $^3$$J$(H,H) = 11.2, 1H), 3.28 (dd, $^2$$J$(H,H) = 14.4, $^3$$J$(H,H) = 3.4, 1H), 3.80 (s, 3H), 4.13 (t, $^3$$J$(H,H) = 6.4, 1H), 4.17 (d, $^3$$J$(H,H) = 6.4, 2H), 4.2-4.31 (m, 1H), 6.20 (s, 1H), 6.95 (dd, $^3$$J$(H,H) = 8.8, $^4$$J$(H,H) = 2.4, 1H), 6.98 (d, $^4$$J$(H,H) = 2.4, 1H), 7.26 (dd, $J$ = 14.4, 7.2, 2H), 7.35 (t, $J$ = 7.6, 2H), 7.57 (dd, $J$ = 11.8, 7.6, 2H), 7.7 (d, $^3$$J$(H,H) = 8.8, 1H), 7.83 (dd, $J$ = 8.0, 3.6, 2H).

$^{13}$C-NMR (DMSO) : $\delta$ 21.1, 32.7, 46.5, 52.8, 56.0, 66.8, 66.4, 101.1, 112.1, 112.4, 120.2, 125.15, 125.9, 127.1, 127.7, 140.7, 143.67, 143.74, 152.7, 155.1, 156.0, 160.0, 162.4, 172.7. MS (ESI, 40 V) : m/z (%) : 508 (54) [M$^+$ +Na], 179 (89), 141 (100).

(2S)-2-Cbz-amino-3-(7-methoxy-2-oxo-2H-chromen-4-yl)propionic acid (17). To a cold solution of NaOH (66 mg, 1.66 mmol, 2 equiv) in 1:1 dioxane:H$_2$O (2.7 mL, 1.6 mL/mmol of amino acid) was added the coumaryl amino acid ((S)-6) (300 mg, 0.83 mmol) at 0°C. A solution of benzyl chlorofor miate (157 mg, 0.92 mmol, 1.1 equiv) in dioxane (0.4 mL) was carefully added at 0°C ($pH \approx 9-11$). After one hour at room temperature, the reaction mixture was acidified with 1N HCl and extracted with AcOEt (40 mL). The organic extract was washed with brine, dried (Na$_2$SO$_4$) and concentrated in vacuo giving the compound (17) (290 mg, 0.83 mmol, 100%) as a pure brown solid. $^1$H-NMR (DMSO) : $\delta$ 2.97 (dd, $^2$$J$(H,H) = 14.4, $^3$$J$(H,H) = 10.8, 1H), 3.26 (dd, $^2$$J$(H,H) = 14.4, $^3$$J$(H,H) = 3.6, 1H), 3.77 (s, 3H), 4.22-4.32 (m, 1H), 4.91 (s, 2H), 6.15 (d, 1H), 6.95 (dd, $^3$$J$(H,H) = 8.8, $^4$$J$(H,H) = 2.8, 1H), 6.97 (d, $^4$$J$(H,H) = 2.8, 1H), 7.09-7.4 (m, 5H), 7.69 (d, $^3$$J$(H,H) = 8.8, 1H), 7.75 (d, $^3$$J$(H,H) = 8.0, 1H). $^{13}$C-NMR (DMSO + TFA) : $\delta$ 32.8, 52.8, 56.0, 65.4, 101.1, 112.1, 112.4, 120.2, 125.15, 125.9, 127.4, 127.8, 128.0, 128.4, 136.9, 152.6, 155.1, 156.0, 159.9, 162.4, 172.5. MS (ESI, 40 V) : m/z (%) : 442 (26) [M$^-$-H$^-$+2Na], 420 (39) [M$^-$+Na], 318 (100).
(2S)-2-(Boc-amino)-3-(7-methoxy-2-oxo-2H-chromen-4-yl)propionic acid (18). The coumaryl amino acid ((S)-6) (300 mg, 0.83 mmol) in dioxane (3.5 mL) was treated by 5% NaHCO$_3$ (3.5 mL, 175 mg, 2.08 mmol). At 0°C was added Boc$_2$O (218 mg, 1 mmol). The resulting reaction mixture was stirred one hour at 0°C and three hours at room temperature, taken up in AcOEt (180 mL) and acidified with 10% citric acid (30 mL). The organic extract was washed with H$_2$O (3 × 20 mL) and brine (3 × 50 mL), dried (Na$_2$SO$_4$) and concentrated in vacuo giving compound (18) (254 mg, 0.70 mmol, 84%) as a pure pale brown oil. $^1$H-NMR (DMSO): $\delta$ 1.25 (s, 9H), 2.82-2.96 (m, 1H), 3.13-3.27 (m, 1H), 3.81 (s, 3H), 4.1-4.2 (m, 1H), 6.13 (s, 1H), 6.9-7.0 (m, 2H), 7.16 (d, $^3$J(H,H) = 8.0, 1H), 7.68 (d, $J$ = 8.4, 1H). $^{13}$C-NMR (DMSO): $\delta$ 26.9, 27.6, 28.1, 31.3, 33.1, 52.6, 56.0, 78.3, 85.7, 101.1, 112.1, 112.2, 112.4, 125.9, 146.3, 152.9, 155.1, 155.4, 160.0, 162.4, 172.8. MS (ESI, 35 V): m/z (%) : 386 (38) [M$^+$ +Na], 330 (54), 141 (100).

(2S)-2-(Boc-amino)-3-(7-hydroxy-2-oxo-2H-chromen-4-yl)propionic acid (19) and (2S)-2-(Boc-amino)-3-(7-Boc-hydroxy-2-oxo-2H-chromen-4-yl)propionic acid (20). To compound (5) (574 mg, 1.58 mmol) in THF (10 mL) was added triethylamine (1.1 mL, 7.91 mmol) at room temperature, then di-tert-butyl dicarbonate (827 mg, 3.79 mmol) and DMAP (173 mg, 1.42 mmol). The resulting reaction mixture was stirred overnight at room temperature, taken up in AcOEt and washed with 1:1 H$_2$O:1N HCl. The organic extract was washed with H$_2$O and brine, dried (Na$_2$SO$_4$) and concentrated in vacuo leading to a mixture of N-monoprotected amino acid, compound (19) (354 mg, 0.79 mmol, 50%) and diprotected amino acid, compound (20) (144 mg, 0.39 mmol, 25%) in a 66:33 ratio as a brown oil. Peptide synthesis was performed using the crude mixture. Compound (19) : $^1$H-NMR (250 MHz, DMSO) : $\delta$ 1.26 (s, 9H), 1.42 (s, 9H), 2.9-3.3 (m, 2H), 4.2-4.4 (m, 1H), 6.41 (s, 1H), 7.2-7.5 (m, 3H), 7.93 (d, $^3$J(H,H) = 8.8, 1H). Compound (20) : $^1$H-NMR (250 MHz, DMSO) : $\delta$ 1.26 (s, 9H), 2.9-3.3 (m, 2H), 4.2-4.4 (m, 1H), 6.15 (s, 1H), 6.75-7.0 (m, 3H), 7.7 (d, 1H).

(2S)-2-[2S)-2-(9H-Fluoren-9-ylmethoxy carbonylamino)-3-(7-methoxy-2-oxo-2H-chromen-4-yl)propionylamino]propionic acid tert-butyl ester (21). To a mixture of amino acid ((S)-16) (61.5 mg, 0.14 mmol), (1S)-1-tert-butoxycarbonylethylammonium chloride (25 mg, 0.14 mmol) and BOP (93 mg, 0.21 mmol) was first added DMF (1 mL) and immediately after DIEA (24 mg, 0.182 mmol). The reaction mixture was stirred 180 minutes at room temperature, taken up in ether (30 mL) and NaHCO$_3$ sat (5 mL). The organic extract was washed with NaHCO$_3$ sat, 10% citric acid, H$_2$O and brine, dried (Na$_2$SO$_4$) and concentrated in vacuo giving compound (21). $^1$H-NMR (DMSO + TFA) : $\delta$ 1.22 (d, $^3$J(H,H) = 7.2, 3H), 1.33...
(s, 9H), 2.92 (dd, $^2J(H,H) = 14.0$, $^3J(H,H) = 10.0$, 1H), 3.13 (dd, $^2J(H,H) = 14.0$, $^3J(H,H) = 4.4$, 1H), 3.77 (s, 3H), 4.04-4.25 (m, 4H), 4.33-4.48 (m, 1H), 6.24 (s, 1H), 6.84-7.0 (m, 2H), 7.14-7.27 (m, 2H), 7.27-7.41 (m, 2H), 7.50-7.61 (m, 2H), 7.69 (d, $^3J(H,H) = 8.8$, 1H), 7.73-7.87 (m, 3H), 8.46 (d, $^3J(H,H) = 7.2$, 1H).

(2S)-2-[(2R)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-(7-methoxy-2-oxo-2H-chromen-4-yl)propionylamino]propionic acid tert-butyl ester (22). Following the same procedure as for compound (19) from amino acid ((R)-16) (37.5 mg, 0.083 mmol), compound (22) was obtained. $^1H$-NMR (DMSO) : $\delta$ 1.17 (d, $^3J(H,H) = 7.2$, 3H), 1.33 (s, 9H), 2.92 (dd, $^2J(H,H) = 14.0$, $^3J(H,H) = 10.0$, 1H), 3.11 (dd, $^2J(H,H) = 14.0$, $^3J(H,H) = 4.4$, 1H), 3.79 (s, 3H), 3.97-4.3 (m, 4H), 4.34-4.48 (m, 1H), 6.23 (s, 1H), 6.92 (dd, $^3J(H,H) = 8.8$, $^4J(H,H) = 2.4$, 1H), 6.96 (d, $^4J(H,H) = 2.4$, 1H), 7.15-7.3 (m, 2H), 7.3-7.45 (m, 2H), 7.53-7.68 (m, 2H), 7.74 (d, $^3J(H,H) = 8.8$, 1H), 7.78-7.95 (m, 3H), 8.47 (d, $^3J(H,H) = 7.2$, 1H).

Peptide Synthesis. Peptide synthesis was carried out using the stepwise solid-phase method of Merrifield on an Applied Biosystems (ABI) 431A peptide synthesizer with ABI small-scale Fmoc chemistry on an HMP resin and DCC/HOBt coupling method. Fmoc groups were removed by piperidine (20% in NMP). Peptide 1 was synthesized by first loading compound ((S)-16) to the resin and by introducing the amino acid successively by classical DCC/HOBt coupling method. For peptide 2, the amino acid sequence until the fluorescent amino acid was synthesized on the synthesizer and the Fmoc group of Arg was removed by 20% piperidine. To a solution of the Boc-protected amino acid 19 and 20 (1.18 mmol) in DMF (4 mL) was added BOP (1.18 mmol) and HOBt (1.18 mmol). The resulting mixture was activated for less than 5 min with DIEA (3.54 mmol) and coupled overnight to the peptidyl resin in a syringe.

The final peptidyl resin of both peptides was dried and cleaved with a mixture of TFA/TIPS/H$_2$O (9.5/0.25/0.25 in volume) for 4 h at room temperature. After partial evaporation, the filtrate from the cleavage reaction was precipitated with cold ether, and the precipitate was collected by centrifugation. The crude peptide was purified by semipreparative HPLC on a Nucleosil C$_{18}$ column (Vydac, 5 µm, 10 × 250 mm), and the fractions were analyzed by analytical HPLC on a C$_{18}$ column (Vydac, 5 µm, 4.6 × 250 mm). The pure fractions were collected, evaporated and lyophilized. The structure of the peptides was confirmed by electrospray mass (peptide 1 : $v_{el} = 34.2$ mL, MS = 2490.8 ; peptide 2 : $v_{el} = 32.4$ mL, MS = 2477.9).
Cell-peptide incubations and peptide visualization. 5×10^4 HeLa cells were plated onto eight-chamber culture slides and grown overnight in Dulbecco’s minimum essential medium complemented with 10% fetal bovine serum and 100 units/ml penicillin/streptomycin in a 5% CO\textsubscript{2} atmosphere at 37°C. Cells were treated with 10\textmu M peptide 2 for 2 h, washed twice with phosphate-buffered saline, fixed in 3% paraformaldehyde 20 min at room temperature and washed twice with phosphate-buffered saline. Culture slides were mounted using DAKOCytomation fluorescent mounting medium

Confocal microscopy. Data were obtained with a Zeiss LSM510 confocal laser scanning microscope. Excitation was provided with an argon ion laser set at 488 nm, and the emitted light was filtered with an appropriate long pass filter.