Supporting Information for
Solid Phase Fluorescent Labeling of Peptides

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Table of contents

1. General Procedure for 2, 4, 7, 9……………………………………………….S2
2. Characterization data of 2, 4, 7, 8, 9……………………………………………S2
3. HPLC profiles of 10-17……………………………………………………………...S5
4. Fluorescence emission spectra of 10, 11, 14, 16 and 17 .........................S13
5. Absorption and fluorescence emission spectra of 15..............................S14
General procedure for the preparation of 2, 4, 7, 9: Thionyl chloride (0.14 g, 1.2 mmol) was added to a solution of 1H-benzotriazole (0.48 g, 4.0 mmol) in dry DCM (15 mL) at 20 °C and the reaction mixture was stirred for 20 min. To the reaction mixture was added 1, 3, 6, 8 (1.0 mmol), respectively, and the mixtures were stirred for 2 h at 20 °C. The white precipitate formed during the reaction was filtered off, the filtrate was diluted with additional DCM (80 mL) and the solution was washed with 6M HCl (3 × 50 mL) (for 2, 4, 9), with sat. Na2CO3 soln. (3 × 50 mL) (for 7), brine (50 mL), and dried over MgSO4. Removal of the solvent under reduced pressure gave 2, 4, 7, 9 which were recrystallized from DCM-hexanes.

4-(2-Benzotriazol-1-yl-2-oxoethyl)-7-methoxy-chromen-2-one, Mca-Bt 2:
Microcrystals (0.26 g, 78 %). mp 125.0-126.0 °C. 1H NMR (300Hz, CDCl3) δ 3.88 (s, 3H), 4.87 (s, 2H), 6.41 (s, 1H), 6.84-6.92 (m, 2H), 7.52-7.63 (m, 2H), 7.65-7.74 (m, 1H), 8.17 (d, J = 8.2 Hz, 1H), 8.24 (d, J = 8.2 Hz, 1H); 13C NMR (CDCl3) δ 38.4, 55.8, 101.2, 112.3, 112.8, 114.3, 114.7, 120.5, 125.5, 126.8, 130.9, 131.0, 146.4, 147.0, 155.6, 160.5, 163.0, 167.3. HRMS calcd. for [C18H13N3O4+Na]+, 358.0798; found, 358.0784.

(S)-(9H-Fluoren-9-yl)methyl-1-(1H-benzo[d][1,2,3]triazol-1-yl)-6-(2-(7-methoxy-2-oxo-2H-chromen-4-yl)acetamido)-1-oxohexan-2-ylcarbamate (Nα-Fmoc-L-Lys(Mca)-Bt) 4:
Microcrystals (0.45g, 65 %). mp 144.0-146.0 °C. 1H NMR (300 MHz, DMSO-d6) δ 1.37-1.53 (m, 4H), 1.78-2.00 (m, 2H), 3.00-3.12 (m, 2H), 3.65 (s, 2H), 3.83 (s, 3H), 3.83-3.90 (m, 1H), 4.18-4.28 (m, 1H), 4.29-4.38 (m, 2H), 6.23 (s, 1H), 6.90-7.00 (m, 2H), 7.32 (t, J = 7.1 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.66 (d, J = 8.7 Hz, 2H), 7.72 (d, J = 7.4 Hz, 2H), 7.81 (t, J = 7.7 Hz, 1H), 7.89 (d, J = 7.4 Hz, 2H), 8.19-8.32 (m, 3H); 13C NMR (75 MHz, DMSO-d6) δ 23.1, 28.4, 30.2, 38.6, 46.6, 54.3, 55.9, 55.9, 65.1,
100.9, 112.1, 112.6, 112.8, 120.2, 120.3, 125.3, 126.5, 126.9, 127.1, 127.7, 130.5, 131.3, 140.8, 143.8, 145.4, 151.2, 155.0, 156.5, 160.2, 162.4, 167.5, 172.2. HRMS calcd. for [C_{39}H_{35}N_{5}O_{7}+Na]^+; 708.2428; found, 708.2455.

\{(S)-1-(Benzotriazole-1-carbonyl)-5-[(2-oxo-2H-chromene-3-carbonyl)-amino]-pentyl]-carbamic acid \ 9H-fluoren-9-ylmethyl ester (Nα-Fmoc-L-Lys(Cc)-Bt) 7: White microcrystals (0.53 g, 82 %); mp 113.0–115.0°C (lit. \text{36} mp 113.0–115.0°C ), \text{1H} NMR (300 MHz, DMSO-\text{d}_6): \delta 1.49-1.68 (m, 4H), 1.82-2.08 (m, 2H), 3.22-3.40 (m, 2H), 4.18-4.38 (m, 1H), 4.38-4.42 (m, 2H), 4.38-4.42 (m, 2H), 5.42-5.53 (m, 1H), 7.28-7.35 (m, 2H), 7.25-7.46 (m, 3H), 7.46-7.52 (m, 2H), 7.61 (t, \text{J} = 7.4Hz, 1H), 7.64-7.82 (m, 4H), 7.87 (d, \text{J} = 6.7 Hz, 2H), 7.95 (d, \text{J} = 7.7 Hz, 1H), 8.23 (d, \text{J} = 9.6 Hz, 1H), 8.28-8.32 (m, 2H), 8.70 (t, \text{J} = 5.5 Hz, 1H), 8.80 (s, 1H). \text{13C} NMR (DMSO-\text{d}_6): 23.1, 28.4, 30.3, 46.6, 54.3, 65.9, 114.0, 116.1, 118.5, 119.0, 120.2, 125.1, 125.3, 126.8, 127.1, 127.6, 130.2, 130.6, 131.2, 134.0, 140.7, 143.7, 143.7, 145.3, 147.3, 153.8, 156.4, 160.3, 161.1, 172.1. Found: C, 69.01; H, 4.76; N, 11.03. Calcd. for C_{37}H_{31}N_{5}O_{6}: C, 69.26; H, 4.87; N, 10.91%.

(S)-6-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-2-(2-oxo-2H-chromene-3-carboxamido)hexanoic acid (Nα-(Cc)-L-Lys(Fmoc)-OH) 8: Solid of 5 (0.16 g, 0.5 mmol) was added in one portion to a solution of Nα-Fmoc-l-lysine (0.20 g, 0.5 mmol) in MeCN-H\text{2}O (5 mL : 3 mL), in the presence of Et\text{3}N (0.70 mL, 0.5 mmol). The reaction mixture was then stirred at 20 °C for 30 min. 6M HCl aq. (2 mL) was then added and the MeCN was removed under reduced pressure. The obtained residue was dissolved in DCM (50 mL), and the organic extract was washed with 6M HCl aq. (50 mL), brine (50 mL), and dried over with MgSO\text{4}. Evaporation of the solvent gave microcystals 8 (0.21 g, 79 %) which was recrystallized from DCM-hexanes. mp 87.9-89.9 °C. \text{1H} NMR (300...
(S)-(9H-Fluoren-9-yl)methyl-6-(1H-benzo[d][1,2,3]triazol-1-yl)-6-oxo-5-(2-oxo-2H-chromene-3-carboxamido)hexylcarbamate (N^α-(Cc)-L-Lys(Fmoc)-Bt) 9:

Microcrystals (0.46 g, 71 %). mp 106.9–108.9 °C. \(^1\)H NMR (DMOS-\(d_6\)) \(\delta\) 1.41-1.62 (m, 4H), 1.97-2.25 (m, 2H), 2.94-3.09 (m, 2H), 4.11-4.29 (m, 3H), 5.89-6.01 (m, 1H), 7.22-7.56 (m, 7H), 7.56-7.71 (m, 3H), 7.71-7.89 (m, 4H), 7.96 (d, \(J = 7.7\) Hz, 1H), 8.19-8.36 (m, 2H), 8.84-8.92 (m, 1H), 9.40 (d, \(J = 6.9\) Hz, 1H); \(^13\)C NMR (DMOS-\(d_6\)) 22.4, 28.9, 31.0, 31.1, 46.7, 53.0, 65.2, 114.0, 116.3, 118.1, 118.4, 120.1, 120.3, 125.1, 125.3, 126.9, 127.0, 127.6, 130.5, 130.7, 131.2, 134.5, 140.7, 145.4, 148.2, 154.0, 156.1, 160.5, 161.6, 170.9. HRMS calcd. for \([\text{C}_{37}\text{H}_{31}\text{N}_5\text{O}_6+\text{Na}]^+\), 664.2167; found, 664.2125.
HPLC profiles

Peptide 10: H-L-Ala-L-Lys(N’-Mca)-NH$_2$

**Figure S1: Bottom:** the profile of crude peptide 10 (H-L-Ala-L-Lys(N’-Mca)-NH$_2$).

**Top:** The profile of peptide 10 after purification.

HRMS calcd. for [C$_{21}$H$_{28}$N$_4$O$_6$+H]$^+$, 433.2082; found, 433.2103.
Peptide 11: H-L-Ala-L-Lys(\(N^\prime\)-Cc)-NH\(_2\)

Figure S2: Bottom; the profile of crude peptide 11 (H-L-Ala-L-Lys(\(N^\prime\)-Cc)-NH\(_2\)).

Top; the profile of peptide 11 after purification.

HRMS calcd. for [C\(_{21}\)H\(_{28}\)N\(_4\)O\(_6\)+H]\(^+\), 389.1819; found, 389.1825.
Peptide 12: H-L-Pro-L-Phe-L-Lys(N′-Cc)-NH₂

Figure S3: Bottom; the profile of crude peptide 12 (H-L-Pro-L-Phe-L-Lys(N′-Cc)-NH₂)

Top; the profile of peptide 12 after purification.

HRMS calcd. for [C₃₀H₃₅N₅O₆+H]⁺, 562.2660; found, 562.2680.
**Peptide 13: H-L-Trp-L-Lys(\(N'\)-Cc)-L-Met-L-Phe-NH\(_2\))**

*Figure S4: Bottom*; the profile of crude peptide 13 (H-L-Trp-L-Lys(\(N'\)-Cc)-L-Met-L-Phe-NH\(_2\))

*Top*; The profile of peptide 13 after purification.

HRMS calcd. for \([C_{41}H_{47}N_{7}O_{7}S+H]^+\), 782.3300; found, 782.3328.
**Peptide 14: H-L-Lys(N'-Cc)-L-Pro-Gly-L-Leu-L-Met-L-Trp-NH$_2$**

**Figure S5: Bottom;** the profile of crude peptide 14 (H-L-Lys(N’-Cc)-L-Pro-Gly-L-Leu-L-Met-L-Trp-NH$_2$)

**Top;** the profile of peptide 14 after purification.

HRMS calcd. for [C$_{45}$H$_{59}$N$_9$O$_9$S+H]$^+$, 902.4229; found, 902.4212.

**Table S1: MS/MS sequence of peptide 14**

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<tr>
<td>Loss of NH$_3$</td>
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Peptide 15: H-L-Phe-L-Leu-L-Lys(\(N^\alpha\)-Cc)-NH\(_2\).

**Figure S6: Bottom:** the profile of crude peptide 15 (H-L-Phe-L-Leu-L-Lys(\(N^\alpha\)-Cc)-NH\(_2\))

**Top:** the profile of peptide 15 after purification.

HRMS calcd. for [C\(_{31}\)H\(_{39}\)N\(_5\)O\(_6\)+H\(^+\)]\(^+\), 578.2973; found, 578.2987.
**Peptide 16: (Cc)-L-Leu-L-Leu-NH₂**

*Figure S7: Bottom*; the profile of crude peptide 16 ((Cc)-L-Leu-L-Leu-NH₂).

*Top*; The profile of peptide 16 after purification.

HRMS calcd. for \([C_{22}H_{29}N_3O_5+H]^+\), 416.2180; found, 416.2223.
Peptide 17: (Mca)-L-Leu-L-Leu-NH₂

Figure S8: **Bottom**: the profile of crude peptide 17 ((Mca)-L-Leu-L-Leu-NH₂).

**Top**: The profile of peptide 17 after purification.

HRMS calcd. for [C₂₄H₃₃N₃O₆+Na]⁺, 460.2442; found, 460.2455.
**Figure S9:** Fluorescence emission spectra of 10, $\lambda_{ex} = 323$ nm, 11 $\lambda_{ex} = 294$ nm, 14 $\lambda_{ex} = 290$ nm in MeOH.

**Figure S10:** Fluorescence emission spectra of 16, $\lambda_{ex} = 413$ nm and 17, $\lambda_{ex} = 383$ nm in MeOH
Figure S11: Absorption and fluorescence emission spectra of 15, $\lambda_{\text{Abs.}} = 299$ nm and $\lambda_{\text{Em}} = 407$ nm in MeOH.