Supporting Information for

Solid Phase Fluorescent Labeling of Peptides

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Table of contents

General procedure for the preparation of 2, 4, 7, 9: Thionyl chloride (0.14 g, 1.2 mmol) was added to a solution of 1*H*-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 ^{o}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. Na_2CO_3 soln. (3 × 50 mL) (for **7**), brine (50 mL), and dried over $MgSO_4$. 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 ^{o}C. ^{1}H NMR (300Hz, $CDCl_3$) 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); ^{13}C NMR ($CDCl_3$) 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 $[C_{18}H_{13}N_3O_4+Na]^+$, 358.0798; found, 358.0784.

(*S*)-(9*H*-Fluoren-9-yl)methyl-1-(1*H*-benzo[d][1,2,3]triazol-1-yl)-6-(2-(7-methoxy-2-oxo-2*H*-chromen-4-yl)acetamido)-1-oxohexan-2-ylcarbamate (N^{α}-Fmoc-L-Lys(Mca)-Bt) 4: Microcrystals (0.45g, 65 %). mp 144.0-146.0 ^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6) 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); ^{13}C NMR (75 MHz, DMSO-d_6) 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, 114.0, 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 $[C_{39}H_{35}N_5O_7+Na]^+$, 708.2428; found, 708.2455.

{(*S*)-1-(Benzotriazole-1-carbonyl)-5-[(2-oxo-2*H*-chromene-3-carbonyl)-amino]-pentyl}-carbamic acid 9*H*-fluoren-9-ylmethyl ester (*N*$^{\alpha}$-Fmoc-L-Lys(Cc)-Bt) 7: White microcrystals (0.53 g, 82 %); mp 113.0–115.0°C (lit. [36] mp 113.0–115.0°C), ^{1}H NMR (300 MHz, DMSO-d_6): δ 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), 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, *J* = 7.4Hz, 1H), 7.64-7.82 (m, 4H), 7.87 (d, *J* = 6.7 Hz, 2H), 7.95 (d, *J* = 7.7 Hz, 1H), 8.23 (d, *J* = 9.6 Hz, 1H), 8.28-8.32 (m, 2H), 8.70 (t, *J* = 5.5 Hz, 1H), 8.80 (s, 1H). ^{13}C NMR (DMSO-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 $C_{37}H_{31}N_5O_6$: C, 69.26; H, 4.87; N, 10.91%.

(*S*)-6-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-2-(2-oxo-2*H*-chromene-3-carboxamido)hexanoic acid (*N*$^{\alpha}$-(Cc)-L-Lys(Fmoc)-OH) 8: Solid of **5** (0.16 g, 0.5 mmol) was added in one portion to a solution of *N*$^{\varepsilon}$-Fmoc-L-lysine (0.20 g, 0.5 mmol) in MeCN-H_2O (5 mL : 3 mL), in the presence of Et_3N (0.70 mL, 0.5 mmol). The reaction mixture was then stirred at 20 ^{o}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 $MgSO_4$ .Evaporation of the solvent gave microclystals **8** (0.21 g, 79 %) which was recrystallized from DCM-hexanes. mp 87.9-89.9 ^{o}C. ^{1}H NMR (300

MHz, DMOS-d_6): δ 1.26-1.49 (m, 4H), 1.71-1.95 (m, 2H), 2.92-3.02 (m, 2H), 4.15-4.20 (m, 1H), 4.24-4.38 (m, 2H), 4.50 (q, J = 5.5Hz, 1H), 7.23-7.46 (m, 7H), 7.65 (d, J = 7.1 Hz, 2H), 7.60 (t, J = 7.4 Hz, 1H), 7.85 (d. J = 7.1 Hz, 2H), 7.98 (d, J = 7.7 Hz, 1H), 8.89 (s, 1H), 9.07 (d, J = 7.4 Hz, 1H), 13.00 (br s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 29.0, 31.2, 46.7, 52.3, 65.2, 116.2, 118.2, 118.4, 120.1, 125.1, 125.2, 127.0, 127.6, 130.4, 134.3, 140.7, 143.9, 148.0, 154.0, 156.1, 160.6, 160.7, 172.9. Found: C, 68.59; H, 5.57; N, 4.97. Calcd. for $C_{31}H_{28}N_2O_7$: C, 68.88; H, 5.22; N, 5.18

(*S*)-(9*H*-Fluoren-9-yl)methyl-6-(1*H*-benzo[d][1,2,3]triazol-1-yl)-6-oxo-5-(2-oxo-2*H*-chromene-3-carboxamido)hexylcarbamate (N^{α}-(Cc)-L-Lys(Fmoc)-Bt) 9: Microcrystals (0.46 g, 71 %). mp 106.9–108.9 ^{o}C. ^{1}H NMR (DMOS-d_6) δ 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 (DMSO-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 $[C_{37}H_{31}N_5O_6+Na]^+$, 664.2167; found, 664.2125.

HPLC profiles

Peptide 10: H-L-Ala-L-Lys(N^{ϵ}-Mca)-NH_2

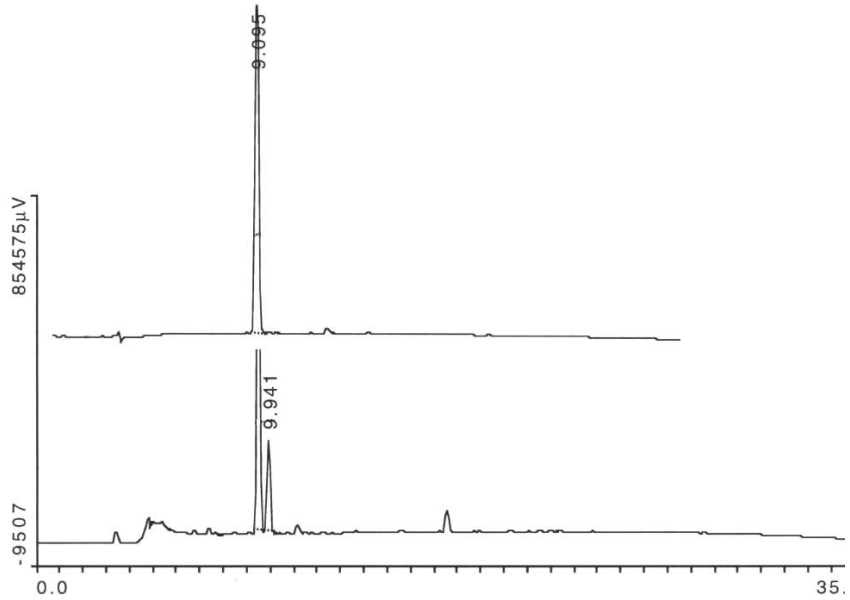

Figure S1: Bottom; the profile of crude peptide **10** (H-L-Ala-L-Lys(N^{ϵ}-Mca)-NH_2).

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

HRMS calcd. for $[C_{21}H_{28}N_4O_6+H]^+$, 433.2082; found, 433.2103.

Peptide 11: H-L-Ala-L-Lys(N^{ε}-Cc)-NH_2

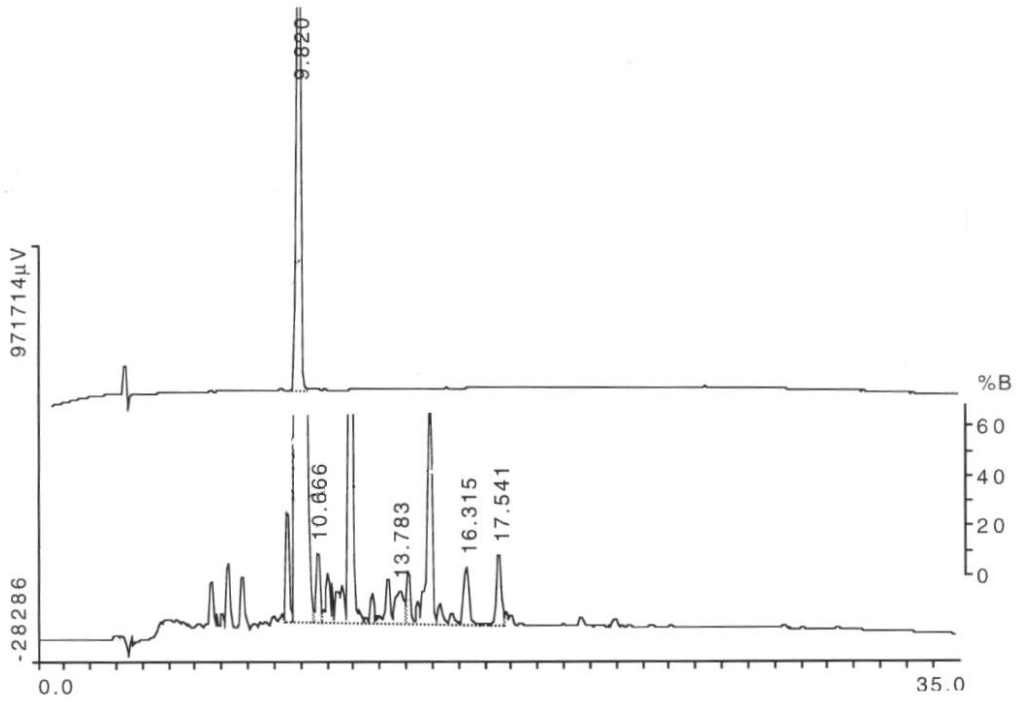

Figure S2: Bottom; the profile of crude peptide **11** (H-L-Ala-L-Lys(N^{ε}-Cc)-NH_2).

Top; the profile of peptide **11** after purification.

HRMS calcd. for $[C_{21}H_{28}N_4O_6+H]^+$, 389.1819; found, 389.1825.

Peptide 12: H-L-Pro-L-Phe-L-Lys(N^{ε}-Cc)-NH_2

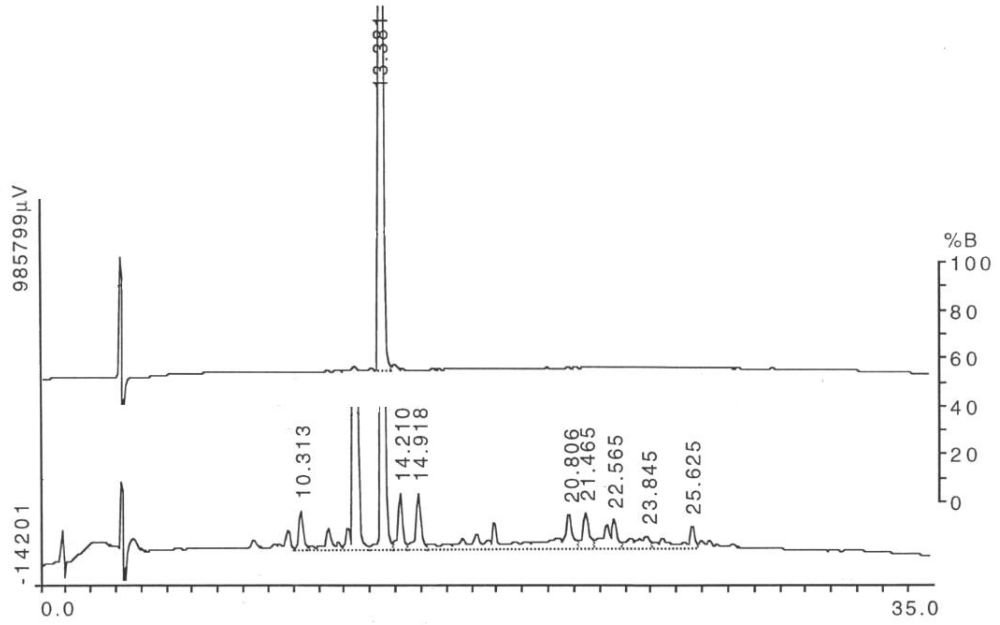

Figure S3: Bottom; the profile of crude peptide **12** (H-L-Pro-L-Phe-L-Lys(N^{ε}-Cc)-NH_2)

Top; the profile of peptide **12** after purification.

HRMS calcd. for $[C_{30}H_{35}N_5O_6+H]^+$, 562.2660; found, 562.2680.

Peptide 13: H-L-Trp-L-Lys(N^{ε}-Cc)-L-Met-L-Phe-NH_2

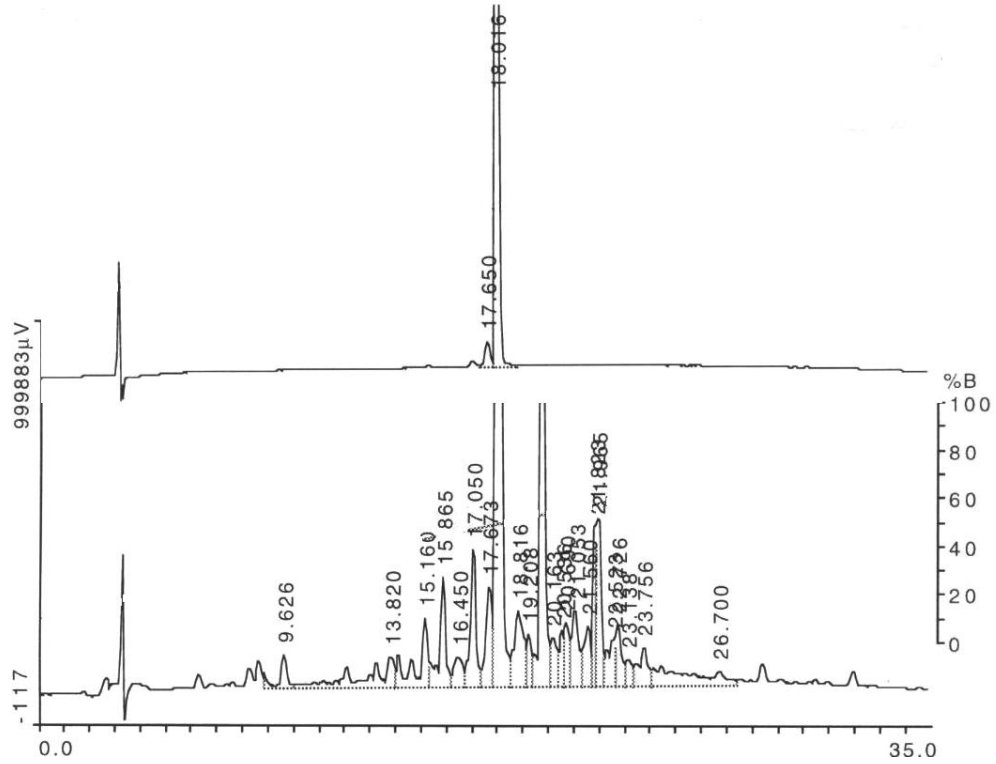

Figure S4: Bottom; the profile of crude peptide **13** (H-L-Trp-L-Lys(N^{ε}-Cc)-L-Met-L-Phe-NH_2)

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

HRMS calcd. for $[C_{41}H_{47}N_7O_7S+H]^+$, 782.3300; found, 782.3328.

Peptide14: H-L-Lys(N^{ε}-Cc)-L-Pro-Gly-L-Leu-L-Met-L-Trp-NH_2

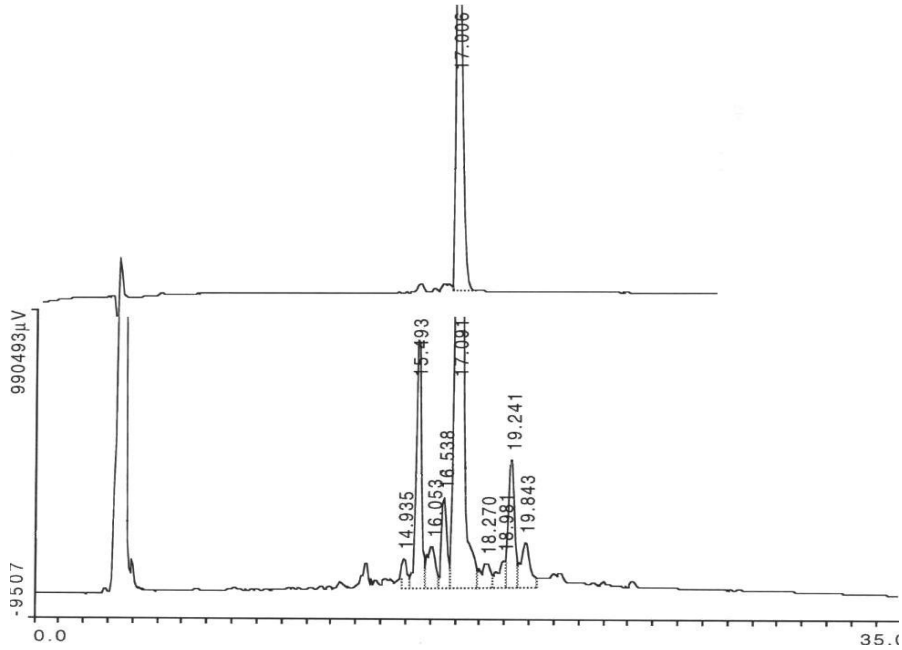

Figure S5: Bottom; the profile of crude peptide **14** (H-L-Lys(N^{ε}-Cc)-L-Pro-Gly-L-Leu-L-Met-L-Trp-NH_2)

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

HRMS calcd. for $[C_{45}H_{59}N_9O_9S+H]^+$, 902.4229; found, 902.4212.

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

		MW =	**901.4**	**[M+H]+ =**		**902.4**		
b-ions-H_2O						681.3	867.4	
a-ions (loss of CO)		273.1	370.2	427.2	540.3	671.3	857.4	
b-ions	N-term	301.1	398.2	455.2	568.3	699.3	885.4	C-term
Residue	**H**	**K(der)**	**P**	**G**	**L**	**M**	**W**	**-NH_2**
Residue mass	1.0	300.1	97.05	57.02	113.08	131.04	186.08	16.0
y-ions		902.4	602.3	505.3	448.3	335.2	204.1	
Loss of NH_3		885.4	585.3	488.3	431.3	318.2	187.1	

Peptide 15: H-L-Phe-L-Leu-L-Lys(N^{α}-Cc)-NH_2

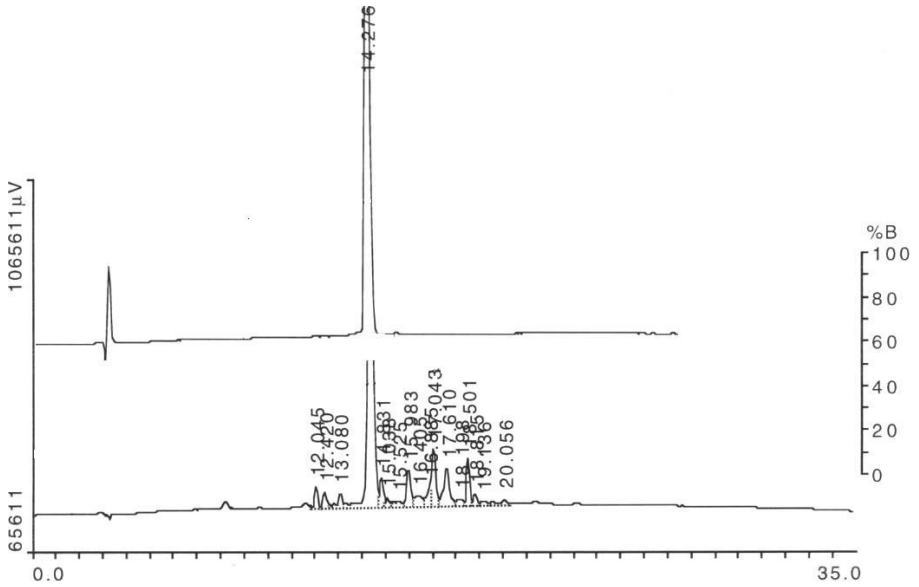

Figure S6: **Bottom**; the profile of crude peptide **15** (H-L-Phe-L-Leu-L-Lys(N^{α}-Cc)-NH_2)

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

HRMS calcd. for $[C_{31}H_{39}N_5O_6+H]^+$, 578.2973; found, 578.2987.

Peptide 16: (Cc)-L-Leu-L-Leu-NH_2

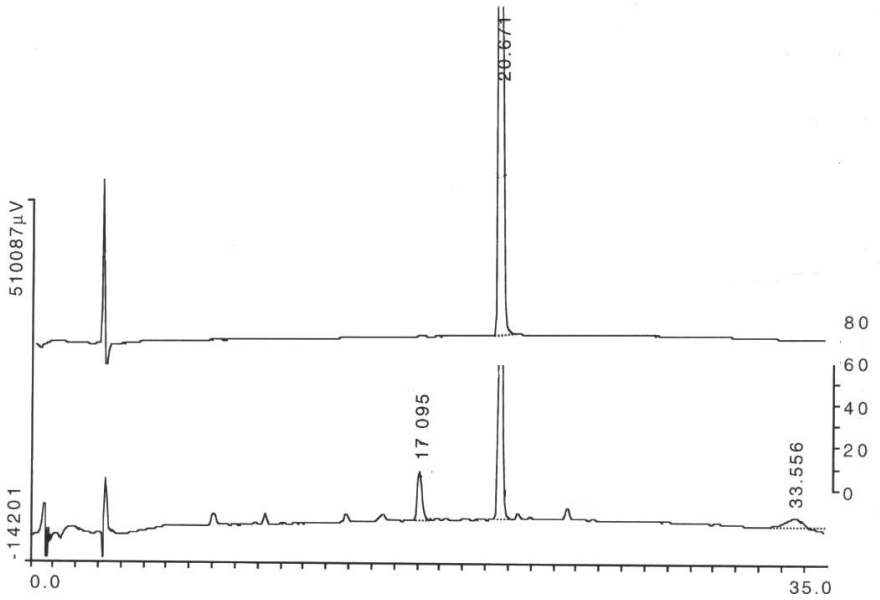

Figure S7: **Bottom**; the profile of crude peptide **16** ((Cc)-L-Leu-L-Leu-NH_2).

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-NH_2

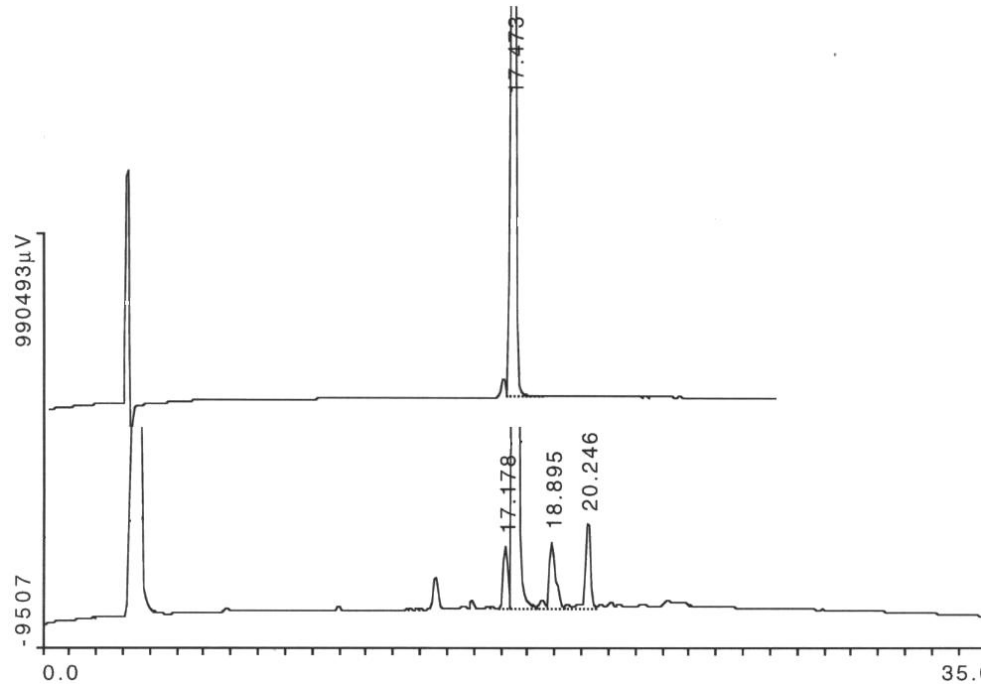

Figure S8: Bottom; the profile of crude peptide **17** ((Mca)-*L*-Leu-*L*-Leu-NH_2).

Top; The profile of peptide 17 after purification.

HRMS calcd. for $[C_{24}H_{33}N_3O_6+Na]^+$, 460.2442; found, 460.2455.

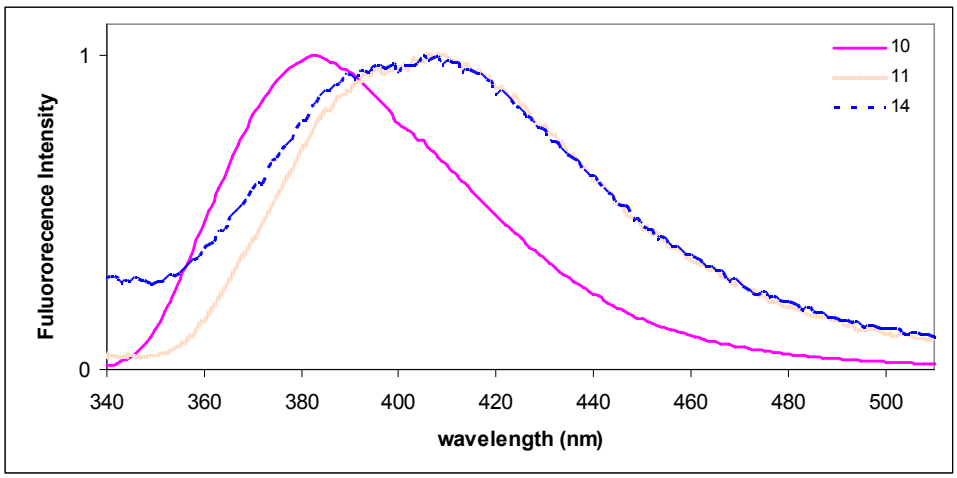

Figure S9: Fluorescence emission spectra of **10**, λ_{ex} = 323 nm, **11** λ_{ex} = 294 nm, **14** λ_{ex} = 290 nm in MeOH.

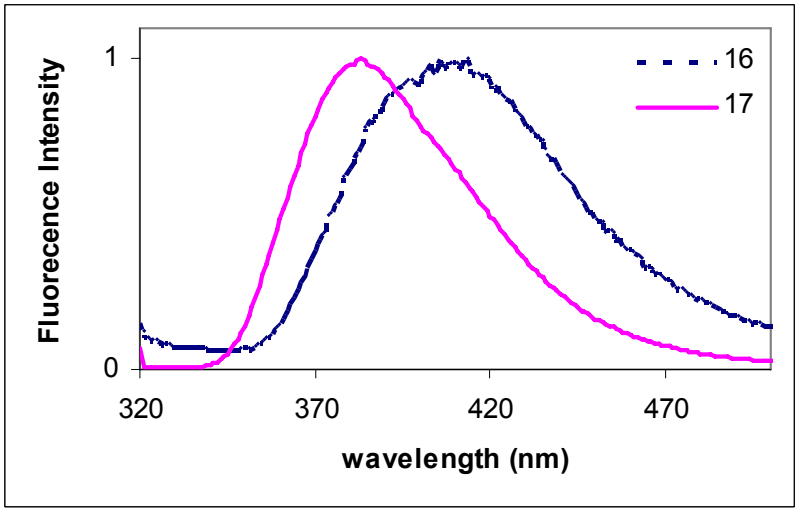

Figure S10: Fluorescence emission spectra of **16**, λ_{ex} = 413 nm and **17**, λ_{ex} = 383 nm in MeOH

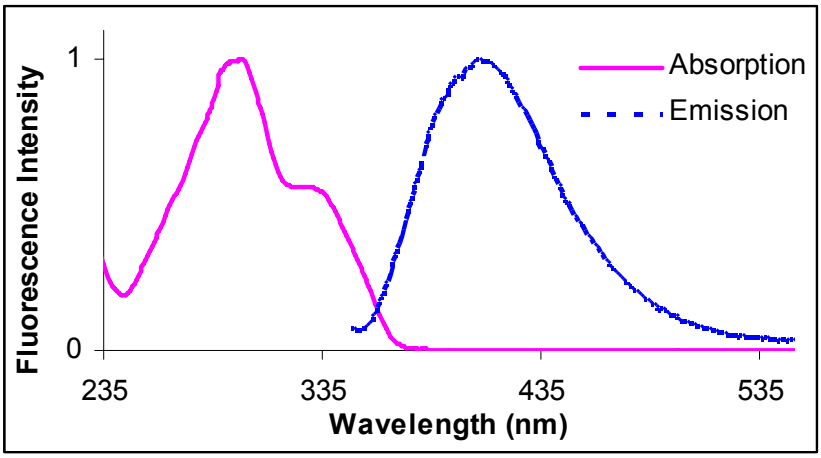

Figure S11: Absorption and fluorescence emission spectra of **15**, $\lambda_{Abs.}$ = 299 nm and λ_{Em} = 407 nm in MeOH