Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Tuning of Protease Resistance in Oligopeptides through N-Alkylation

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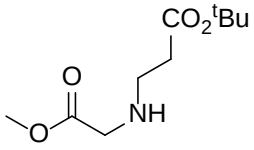
Monomer Synthesis

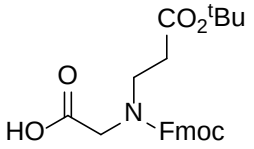
General Comments. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as the visualizing agent and an acidic mixture of anisaldehyde, ceric ammonium molybdate, or basic aqueous potassium permangante (KMnO4), and heat as developing agents. E. Merck silica gel (60, particle size 0.043–0.063 mm) was used for flash column chromatography. NMR spectra were recorded on Bruker Avance 500 MHz and Varian VNMRS 600 MHz instruments and calibrated using residual undeuterated solvent as an internal

reference ($CHCl_3$ @ 7.26 ppm ^{1}H NMR, 77.16 ppm ^{13}C NMR; DMSO @ 2.50 ppm ^{1}H NMR, 39.52 ppm ^{13}C NMR). The following abbreviations (or combinations thereof) were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Mass spectra (MS) were recorded on a quadrupole/time-of-flight tandem mass spectrometer (ESI), Waters GCT Premier high resolution time-of-flight mass spectrometer (EI, FD) or matrix assisted laser desorption/ionization (MALDI) using a 2,5 dihydroxy benzoic acid (DHB) matrix.



H*N*(EtCO$_2$^{t}Bu)-Gly-OMe. Glycine methyl ester hydrochloride (10.0 g, 79.6 mmol) was added to methanol (80 mL), followed by triethylamine (11.1 mL, 79.6 mmol, 1 eq) and *tert*-butyl acrylate (9.25 mL, 63.7 mmol, 0.8 eq). The reaction mixture was stirred at room temperature for 20 hours and concentrated *in vacuo*. The residue was partitioned between brine (100 mL) and ethyl acetate (100 mL), separated and the organic layer dried over Na_2SO_4. After concentration, the residue was purified by column chromatography (25% to 100% EtOAc in hexanes) to give the aza-Michael intermediate (**HN(EtCO$_2$^{t}Bu)-Gly-OMe**) (10.9 g, 79% yield) as a colorless oil.

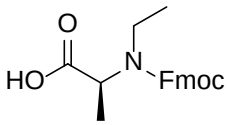
MS (*m/z*): calcd for $C_{10}H_{19}NO_4$, $[M]^+$, 217.1314; found, 217.13; **^{1}H NMR (500 MHz, $CDCl_3$)**: (500 MHz, Chloroform-*d*) δ 3.67 (s, 3H), 3.37 (s, 2H), 2.79 (t, *J* = 6.6 Hz, 2H), 2.37 (t, *J* = 6.6 Hz, 2H), 1.85 (s, 1H), 1.40 (s, 9H). **^{13}C NMR ($CDCl_3$, 126 MHz)**: δ 172.73, 171.79, 80.61, 51.77, 50.79, 45.04, 36.05, 28.15.



Fmoc-*N*(EtCO$_2$^{t}Bu)-Gly-OH. Intermediate **H*N*(EtCO$_2$^{t}Bu)-Gly-OMe** (10.9 g, 50.3 mmol) was dissolved in methanol (65 mL) and 1.43 N aq. NaOH (35 mL, 50 mmol, 1 eq) was added. The reaction mixture was warmed to 40 °C and stirred for 14 hours. The reaction mixture was cooled to room temperature and the methanol was removed *in vacuo*. To this crude sodium carboxylate solution was added sodium bicarbonate (6.34 g, 75.5 mmol, 1.5 eq) followed by Fmoc-Cl (13.0 g, 50.3 mmol, 1 eq) dissolved in dioxane (125 mL). The reaction mixture was stirred vigorously for 3 hours at room temperature and then

acidified with 1 N HCl (150 mL). The solution was extracted with EtOAc (100x 3), washed with brine and dried over Na_2SO_4. After concentration *in vacuo*, the residue was purified by column chromatography (DCM to 7% MeOH in DCM) to give **Fmoc-*N*(EtCO$_2$^{t}Bu)-Gly-OH** (10.1 g, 47% yield).

MS (*m/z*): calcd for $C_{24}H_{27}NO_6$, $[M]^+$, 425.1838; found, 425.18; **^{1}H NMR (500 MHz, CDCl$_3$)**: ^{1}H NMR (500 MHz, Chloroform-*d*) 1:1 mixture of rotamers. Pairs of rotamer chemical shifts listed in brackets. δ 9.92 (br s, 1H), [7.75 (d, *J* = 7.5 Hz), 7.71 (d, *J* = 7.6 Hz,) 2H], [7.58 (d, *J* = 7.4 Hz), 7.52 (d, *J* = 7.5 Hz) 2H], 7.42 – 7.24 (m, 4H), [4.52 (d, *J* = 6.2 Hz), 4.42 (d, *J* = 6.5 Hz) 2H], [4.26 (t, *J* = 6.2 Hz), 4.18 (t, *J* = 6.5 Hz) 1H], [4.11 (s), 4.04 (s) 2H], [3.57 (t, *J* = 6.5 Hz), 3.44 (t, *J* = 6.8 Hz) 2H], [2.57 (t, *J* = 6.5 Hz), 2.31 (t, *J* = 6.7 Hz) 2H], [1.44 (s), 1.43 (s) 9H]. **^{13}C NMR (CDCl$_3$, 151 MHz)**: (both observed rotamers) δ 175.12, 174.96, 171.83, 171.32, 156.51, 155.85, 143.90, 143.89, 141.49, 141.42, 127.87, 127.80, 127.27, 127.17, 124.96, 124.92, 120.11, 120.07, 81.15, 81.13, 67.84, 50.25, 49.95, 47.36, 47.28, 45.38, 44.67, 34.92, 34.72, 28.20, 28.16.

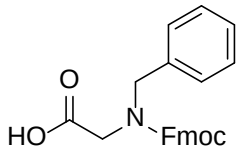


Fmoc-*N*(Et)-L-Ala-OH. In a 200 ml round bottom flask, finely ground (L)-alanine (1.78 g, 20 mmol) was dissolved in 50 ml of methanol under argon, followed by the addition of finely ground sodium hydroxide (0.88 g, 22 mmol). The mixture was sonicated at room temperature to a homogenous solution. The flask was transferred into an ice bath and acetaldehyde (1.76 g, 40 mmol) was added into the solution dropwise. The resulted solution was stirred at room temperature for 30 min. Then the solution was transferred into the ice bath again and NaBH4 (1.85 g, 50 mmol) was added portion wise. The mixture was warmed to room temperature and stirred for 1 hour. The mixture was acidified to isoelectric point with concentrated HCl (12 M) and then concentrated in vacuum. The white powder is triturated with acetone, filtered, dried and used directly in next step.

In a 500 ml round bottom flask, the white powder and sodium carbonate (10.6 g, 100 mmol) were dissolved in a mixture of 150 ml of H_2O and 75 ml of dioxane. A solution of Fmoc-Cl (6.4 g, 25 mmol) in 75 ml of dioxane was added dropwise into the flask at room temperature. The reaction was stirred for another 2 hours before it was acidified by HCl aqueous solution (3 M). The mixture

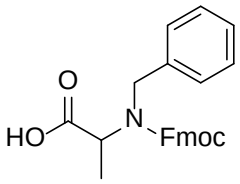
was extracted with 200 ml of dichloromethane for three times. The organic phases were combined, dried by $MgSO_4$ and concentrated by retovap. A flash chromatography (MeOH/dichloromethane = 1/20, R_f = 0.3) was performed to obtain 5.1 g product in 76% yield as white powder.

MS (*m/z*): calcd for $C_{20}H_{21}NO_4Na$, $[M+Na]^+$ 362.137,; found 362.136,; **^{1}H NMR (600 MHz, DMSO)**: 2:1 mixture of rotamers: δ 12.64 (b, 1H), 7.92 (d, *J* = 7.5 Hz, 2H), 7.71 – 7.65 (m, 2H), 7.45 (t, *J* = 7.4 Hz, 2H), 7.40 – 7.32 (m, 2H), 4.49 – 4.22 (m, 4H), 3.39 – 3.29 (m, 0.33H), 3.22 – 3.11 (m, 1H), 3.08 – 2.97 (m, 0.67H), 1.40 – 1.27 (m, 3H), 1.14 – 1.03 (m, 1H), 0.88 (t, *J* = 6.8 Hz, 2H). **^{13}C NMR (DMSO, 151 MHz)**: δ. 173.48, 155.42, 144.42, 144.34, 141.29, 141.15, 128.00, 127.49, 125.26, 120.49, 67.23, 66.83, 56.34, 55.14, 47.18, 41.19, 16.32, 15.64, 14.99, 14.65.



Fmoc-*N*(Bn)-Gly-OH. A mixture of N-Benzylglycine hydrochloride (10 g, 50.0 mmol, 1.00 equiv), dry dichloromethane (150 mL), and TMS-Cl (25.4 mL, 200 mmol, 4.00 equiv) were placed in 500 mL round-bottom flask. The mixture was refluxed for 2 h and then cooled in an ice bath. Diisopropylethylamine (15.1 mL, 86.5 mmol, 1.73 equiv) and Fmoc-Cl (8.67 g, 33.5 mmol, 0.67 equiv) were added to reaction mixture and stirred for 20 min at 0 ˚C. The mixture was warmed to room temperature and then stirred for 3 h. The reaction mixture was evaporated to dryness, and the residue was dissolved in ethylacetate, followed by washing with 1 M HCl solution (two times). The organic layer was washed with brine and dried over Na_2SO_4 for several hours, and the filtered solution was evaporated. The product was purified by column chromatography with dichloromethane and methanol (9:1) and the obtained product was recrystallized with dichloromethane and hexane (9.70 g, 74.7 % yield).

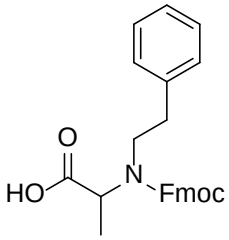
MS (*m/z*): calcd for $C_{24}H_{21}NO_4Na$, $[M+Na]^+$, 410.14; found, 410.16; **^{1}H NMR (600 MHz, $CDCl_3$; rotamer ratio = 1 : 1.23)**: δ 11.03 (s, 1H), 7.76 (d, *J* = 7.5 Hz, 2H), 7.65 – 7.47 (m, 2H), 7.47 – 7.16 (m, 8H), 7.10 (d, *J* = 6.8 Hz, 1H), 4.59 (d, *J* = 5.0 Hz, 3H), 4.52 (s, 1H), 4.28 (dd, *J* = 17.1, 10.5 Hz, 1H), 4.03 (s, 1H), 3.79 (s, 1H). **^{13}C NMR ($CDCl_3$, 101 MHz)**: δ 175.24, 175.15, 156.78, 156.43, 143.86, 143.82, 141.45, 136.47, 136.43, 128.92, 128.84, 128.28, 127.91, 127.88, 127.82, 127.81, 127.69, 127.22, 127.19, 125.02, 124.98, 124.92, 120.11, 120.08, 68.14, 67.88, 51.51, 51.30, 47.87, 47.42, 47.29, 46.96.



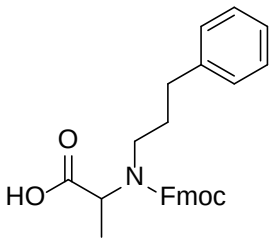
Fmoc-N(Bn)-L-Ala-OH. In a 200 ml round bottom flask, finely ground (L)-alanine (1.78 g, 20 mmol) was dissolved in 50 ml of methanol under argon, followed by the addition of finely ground sodium hydroxide (0.88 g, 22 mmol). The mixture was sonicated at room temperature to a homogenous solution. The flask was transferred into an ice bath and benzaldehyde (3.18 g, 30 mmol) was added into the solution dropwise. The resulted solution was stirred at room temperature for 30 min. Then the solution was transferred into the ice bath again and NaBH4 (1.85 g, 50 mmol) was added portion wise. The mixture was warmed to room temperature and stirred for 1 hour. The mixture was acidified to isoelectric point with concentrated HCl (12 M) and then concentrated in vacuum. The white powder is triturated with acetone, filtered, dried and used directly in next step.

In a 500 ml round bottom flask, the white powder and sodium carbonate (10.6 g, 100 mmol) were dissolved in a mixture of 150 ml of H_2O and 75 ml of dioxane. A solution of Fmoc-Cl (6.4 g, 25 mmol) in 75 ml of dioxane was added dropwise into the flask at room temperature. The reaction was stirred for another 2 hours before it was acidified by HCl aqueous solution (3 M). The mixture was extracted with 200 ml of dichloromethane for three times. The organic phases were combined, dried by $MgSO_4$ and concentrated by rotovap. A flash chromatography (MeOH/dichloromethane = 1/20) was performed to obtain 4.99 g product in 70% yield as white powder.

^{1}H NMR (600 MHz, $CDCl_3$, rotamer ratio 1:2): δ 7.72 (d, J = 7.9 Hz, 2H), 7.59 (d, J = 7.5 Hz, 0.66H), 7.42 (d, J = 7.5 Hz, 0.66H), 7.40 – 7.34 (m, 2.7H), 7.34 – 7.26 (m, 3.3H), 7.25 – 7.14 (m, 3.68H), 4.69 – 4.58 (m, 1.67H), 4.56 – 4.34 (m, 3H), 4.20 (d, J = 6.9 Hz, 1.33H), 1.40 (d, J = 7.3 Hz, 2H), 1.13 (d, J = 7.3 Hz, 1H). **^{13}C NMR ($CDCl_3$, 151 MHz)**: δ 177.11, 156.67, 143.86, 141.42, 137.90, 128.73, 127.79, 127.75, 127.44, 127.19, 127.10, 125.15, 125.04, 124.89, 120.07, 77.37, 77.16, 76.95, 68.04, 55.78, 54.88, 50.87, 50.27, 47.33, 15.77, 15.17.

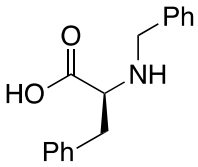


^{1}H NMR (600 MHz, $CDCl_3$, rotamer ratio 3:7): δ 7.74 (dd, J = 7.5, 3.7 Hz, 2H), 7.59 (t, J = 13.3 Hz, 2H), 7.43 – 7.16 (m, 8H), 6.92 (d, J = 7.1 Hz, 1H), 4.70 – 4.18 (m, 4H), 3.62 – 3.52 (m, 0.3H), 3.38 – 3.24 (m, 1H), 3.15 – 3.04 (m, 0.7H), 3.00 – 2.75 (m, 0.6H), 2.66 – 2.45 (m, 1.4H), 1.44 – 1.31 (m, 1H), 1.31 – 1.15 (m, 1H) **^{13}C NMR (151 MHz, $CDCl_3$):** δ 177.24, 156.52, 143.96, 143.90, 141.53, 138.76, 128.83, 128.55, 127.79, 127.24, 126.47, 124.84, 120.13, 120.11, 77.37, 77.16, 76.95, 67.53, 55.86, 55.15, 48.29, 47.60, 35.90, 35.46, 15.70, 15.16. Yield: 66%



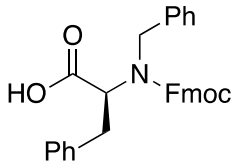
^{1}H NMR (600 MHz, $CDCl_3$, rotamer ratio 3:7): δ 7.78 – 7.70 (m, 2H), 7.61 – 7.52 (m, 2H), 7.44 – 7.23 (m, 6H), 7.22 – 7.05 (m, 3H), 4.61 – 4.46 (m, 2H), 4.38 – 4.28 (m, 0.6H), 4.27 – 4.14 (m, 1.4H), 3.42 (br, 0.3H), 3.24 – 3.10 (m, 1H), 3.01 – 2.91 (m, 0.7H), 2.68 – 2.53 (m, 0.6H), 2.46 – 2.34 (m, 1.4H), 1.99 – 1.57 (m, 2H), 1.46 – 1.34 (m, 2H), 1.34 – 1.18 (m, 1H). **^{13}C NMR (151 MHz, $CDCl_3$):** δ 177.11, 156.47, 144.02, 143.96, 141.51, 141.49, 141.36, 128.49, 128.36, 127.77, 127.20, 126.08, 124.87, 120.05, 77.37, 77.16, 76.95, 55.75, 47.46, 46.48, 33.13, 30.96, 15.70, 15.16.

yield: 61%



H-*N*(CH_2Ph)-L-Phe-OH. Phenylalanine (30.0 g, 0.182 mol) was dissolved in 480 mL of MeOH and sodium hydroxide was added (7.64 g, 0.191 mol, 1.05 eq). Benzaldehyde (26.0 mL, 0.255 mol, 1.40 eq) was added to the solution and the reaction mixture was stirred for 10 minutes at room temperature. The reaction mixture was then cooled to 0 °C and sodium borohydride (8.97 g, 0.237 mol, 1.30 eq) was slowly added. The mixture was stirred for 2 hours at 0 °C and HCl was added up to isoelectric point. The mixture was evaporated and obtained solid was triturated in acetone. The resulting residue was filtrated and purified by washing with water to give **H-*N*(CH_2Ph)-L-Phe-OH** (32.35 g, 69.7% yield).

^{1}H NMR (600 MHz, D_2O with TFA): 1:1 mixture of rotamers: δ 5.86 (dt, *J* = 15.1, 7.1 Hz, 3H), 5.80 (d, *J* = 7.5 Hz, 3H), 5.74 (d, *J* = 7.2 Hz, 2H), 5.64 (d, *J* = 6.7 Hz, 2H), 2.73 (d, *J* = 13.3 Hz, 1H), 2.63 (dd, *J* = 19.7, 10.6 Hz, 2H), 1.84 (dd, *J* = 15.3, 5.7 Hz, 1H), 1.72 (dd, *J* = 15.1, 7.9 Hz, 1H).



Fmoc-*N*(CH_2Ph)-L-Phe-OH. A mixture of N-Benzylglycine hydrochloride (30 g, 0.118 mol, 1.00 equiv), dry dichloromethane (600 mL), and TMS-Cl (44.9 mL, 0.354 mol, 3.00 equiv) were placed in round-bottom flask. The mixture was refluxed for 1 h and then cooled in an ice bath. Diisopropylethylamine (61.7 mL, 0.354 mol, 3.00 equiv) and Fmoc-Cl (36.7 g, 0.142 mol, 1.20 equiv) were added to reaction mixture and stirred for 20 min at 0 ˚C. The mixture was warmed to room temperature and then stirred for 2.5 h. The reaction mixture was evaporated to dryness, and the residue was dissolved in ethylacetate, followed by washing with 1 M HCl solution (x2). The organic layer was washed with brine and dried over Na_2SO_4 for several hours, and the filtrated solution was evaporated. The product was purified by column chromatography with dichloromethane and methanol (DCM/MeOH = 50:1 to 10:1) (33.3 g, 59.1 % yield).

MS (*m/z*): calcd for $C_{31}H_{27}NO_4$, $[M+]^+$, 500.18; found, 500.21; **^{1}H NMR (600 MHz, $CDCl_3$)**: 1:1 mixture of rotamers: δ 7.79 – 7.74 (m, 1H), 7.77 – 7.72 (m, 1H), 7.70 (dd, *J* = 18.4, 7.2 Hz, 1H), 7.55 – 7.46 (m, 2H), 7.44 – 7.33 (m, 3H), 7.33 – 7.23 (m, 4H), 7.26 – 7.12 (m, 5H), 7.07 (dd, *J* = 6.9, 2.5 Hz, 2H), 6.99 (ddd, *J* = 27.8, 7.4, 3.0 Hz, 3H), 6.63 – 6.59 (m, 1H), 5.02 (dd, *J* = 10.9, 4.4 Hz, 1H), 4.66 (dq, *J* = 10.8, 6.6, 5.2 Hz, 1H), 4.54 – 4.46 (m, 1H), 4.42 (d, *J* = 15.7 Hz, 1H), 4.24 (dt, *J* = 17.9, 5.4 Hz, 1H), 4.16 (dd, *J* = 9.7, 5.4 Hz, 1H), 3.89 – 3.81 (m, 1H), 3.74 (d, *J* = 15.8 Hz, 1H), 3.61 (d, *J* = 15.3 Hz, 1H), 3.37 (dd, *J* = 14.1, 5.4 Hz, 1H), 3.28 (dd, *J* = 14.1, 9.8 Hz, 1H), 2.82 (dd, *J* = 14.0, 5.5 Hz, 1H), 2.34 (dd, *J* = 14.0, 9.7 Hz, 1H).

^{13}C NMR ($CDCl_3$, 151 MHz): δ 175.47, 156.21, 143.76 , 143.74 , 141.46 , 141.38 , 141.34 , 137.75 , 137.57 , 136.45 , 129.25 , 129.06 , 128.62 , 128.49 , 128.45 , 128.41 , 128.34 , 127.98 , 127.82 , 127.72 , 127.60 , 127.43 , 127.41 , 127.20 , 127.14 , 127.10 , 126.71 , 126.51 , 125.01 , 124.94 , 124.57 , 124.35 , 120.15 , 119.99 , 119.98 , 67.70 , 66.68 , 62.24 , 60.88 , 52.47 , 47.42 , 47.27 , 35.69 , 35.25 .

Pentapeptide Synthesis

General Comments. TentaGel® S NH2 resin (90 µm, 0.26 mmol/g) was purchased from Rapp Polymere Gmbh. Rink amide linker was purchased from AnaSpec. The following chemicals were purchased from Sigma Aldrich: COMU®,97% (1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylamino-morpholinomethylene)]methanaminium hexafluorophosphate, N,N-Diisopropylethylamine (DIPEA) [99.5%], biotech grade, N,N-Dimethylformamide (DMF) [99.9%], Trifluoroacetic acid (TFA), CHROMASOLV® for HPLC [99.0%], Fmoc-L-Phe-OH [98%], and Fmoc-L-Glu(O^{t}Bu)-OH [98%]. Fmoc-L-Ala-OH, Fmoc-L-Gly-OH, Fmoc-Sar-OH, Fmoc-*N*-Me-Ala-OH, Fmoc-*N*-Me-Phe-OH, were purchased from Chem-Impex in their highest purity. The solvent dichloromethane (DCM) was purchased from Fisher Scientific. Disposable polypropylene columns (1.2 mL, Bio-Rad laboratories; 5 mL and 10 mL, Thermo Scientific) were used as reaction vessels for solid phase synthesis. Vessels containing reaction mixtures were rotated gently while fixed into Thermo Scientific Labquake Tube Shaker/Rotators. Wash volumes were approximately 3 mL of solvent per 100 mg of resin. Analytical High Performance Liquid Chromatography (HPLC) was carried out on reversed-phase ZORBAX Eclipse Plus C18 column (5 µm, 95 Å, 4.6x250 mm) and preparative HPLC was performed using a ZORBAX Eclipse XDB-C18 column (5 µm, 80 Å, 9.4x250 mm) on an Agilent 1200 series instrument (Agilent Technologies). A linear gradient of 10–100% ACN in water (0.1% TFA) over 30 min was used at a flow rate of 1 mL/min and 4 mL/min, respectively. Mass spectra (MS) were recorded on a time-of-flight (TOF) mass spectrometer using matrix assisted laser desorption ionization (MALDI) with α-cyano-4-hydroxycinnamic acid (CHCA) and on a TOF mass spectrometer using electro spray ionization (ESI) method.

Solid Phase Peptide Synthesis. In a polypropylene column equipped with a porous polyethylene disc, a stopper, and an end cap, TentaGel resin (600 mg, 0.26 mmol/gr) was loaded and pre-swollen in DMF for 1.5 h. Rink amide cleavable linker (252 mg, 0.47 mmol, 3 equiv) and COMU (200 mg, 0.47 mmol, 3 equiv) were dissolved in 6 mL of DMF. DIPEA (164 uL, 0.94 mmol, 6 equiv) was added and after 3 min of pre-activation the solution was poured to the DMF-drained resin. The reaction mixture was allowed to slowly rotate for 2 h. The resin was washed with DMF (x7) and deproteced by 20 % 4-methylpiperidine in DMF for 20 min. The resin was washed again with DMF (x2), DCM (x2), and DMF (x2) and then coupled with Fmoc-AA-OH. Cycles of coupling and

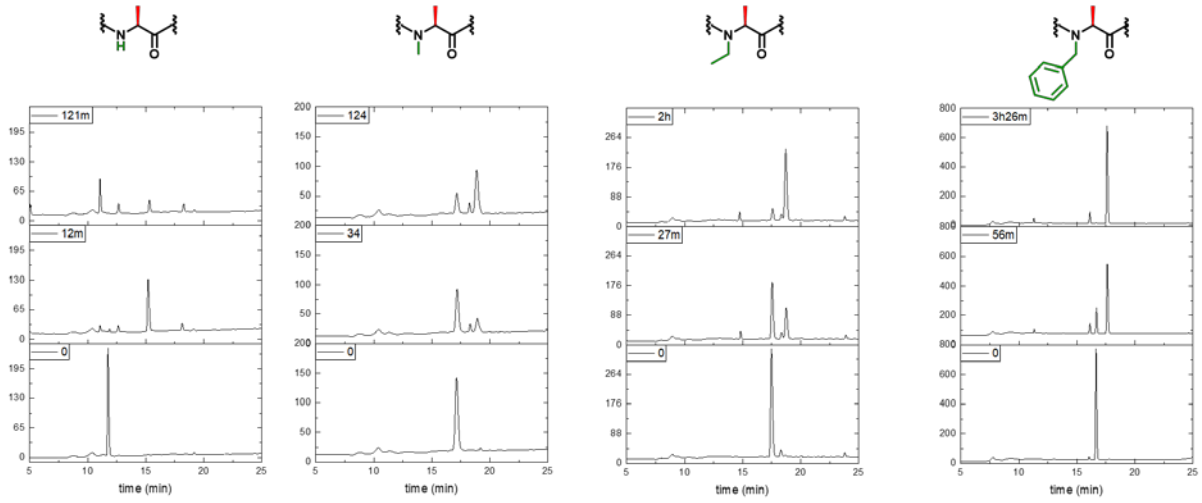
Fmoc deprotection were repeated in this fashion until the desired pentapeptides sequences were achieved. The pentapeptides were cleaved in a pre-prepared mixture of TFA/H_2O/triisopropylsilane; 95/2.5/2.5 for 1.5 h. The solutions were collected by filtration into polypropylene vials and after evaporated the oily crude products were washed with cold ether resulting in a white precipitation. The compounds were resolvated in water purified by high performance liquid chromatography (HPLC) using semi-prep C18 column (5 µm, 9.4x250 mm). Compounds were eluted with a linear gradient of 0–20/100% ACN in water (0.1% TFA) over 30 min at a flow rate of 5 ml/min. The compounds were collected and reinjected to an analytical HPLC C18 column (5 µm, 4.6x250 mm) and their molecular weights were confirmed by TOF- MS MALDI.

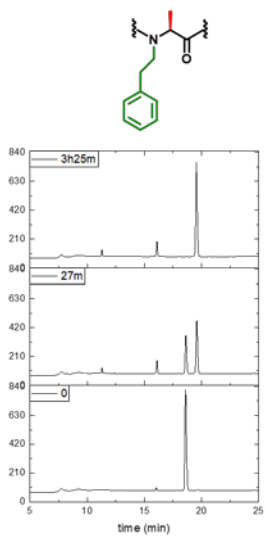
Middle unit	Pentapeptide	Observed Mass	Ions (+Na^+/H^+)	Calculated Mass	Main Observed Fragment Mass
Alanine (Ala)	$C_{17}H_{30}N_6O_7$	431.48	H^+	430.22	multiple
N-Me-Ala	$C_{18}H_{32}N_6O_7$	467.30	Na^+	444.23	375.19
N-Et-Ala	$C_{19}H_{34}N_6O_7$	481.48	Na^+	458.25	389.21
N-Bn-Ala	$C_{24}H_{36}N_6O_7$	543.54	Na^+	520.26	451.22
N-EtPhe-Ala	$C_{25}H_{38}N_6O_7$	557.43	Na^+	534.28	465.23
N-PrPhe-Ala	$C_{26}H_{40}N_6O_7$	571.61	Na^+	548.30	-
Phenylalanine (Phe)	$C_{23}H_{34}N_6O_7$	529.37	Na^+	506.25	multiple
N-Me-Phe	$C_{24}H_{36}N_6O_7$	543.30	Na^+	520.26	451.23
N-Bn-Phe	$C_{30}H_{40}N_6O_7$	619.46	Na^+	596.30	527.25
Glycine (Gly)	$C_{16}H_{28}N_6O_7$	417.42	H^+	416.20	multiple
Sarcosine (*N*-Me-Gly)	$C_{17}H_{30}N_6O_7$	431.32	H^+	430.22	ND
N-Bn-Gly	$C_{23}H_{34}N_6O_7$	529.38	Na^+	506.25	437.21

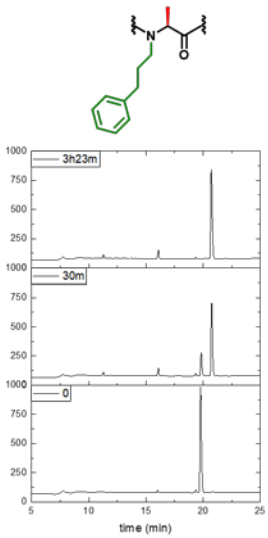
Table S1. Calculated and observed masses of synthesized peptides according to MALDI-MS, and corresponding main observed fragments seen by ESI-MS upon proteolysis by elastase.

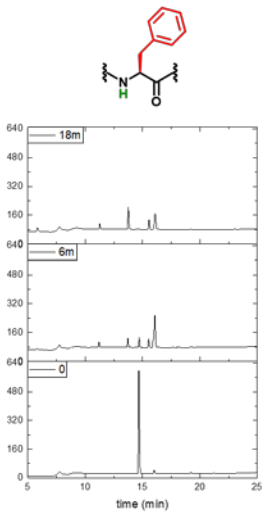
Proteolysis Experiments

Experiments to examine resistance against elastase were performed by incubation of 660 μM peptide in the presence 30 μM elastase (25:1) in a Tris buffer solution, (7.8pH, 30 mM, $CaCl_2$ 10 mM) at 37 ˚C. The solutions were sampled and quenched by the addition of 0.5% TFA. The degradation of the peptides was quantified by using reversed phase high performance liquid chromatography (RP-HPLC). The peptides peak intensities were followed for each sampling time and the trends were fitted to exponential decays. Data points were fitted normally to one-phase exponential decay, except from the case of Glycine (R_1=H, R_2=H), in which two phases were used. The first peak that evolved in the Glycine and Alanine chromatograms were detected by ESI-MS as the protonated peptides and not as a fragment, therefore in those two cases both the original and the second evolved peaks were considered as the unfragmented peptide.




3h25m
27m
0
time (min)


3h23m
30m
0
time (min)


18m
6m
0
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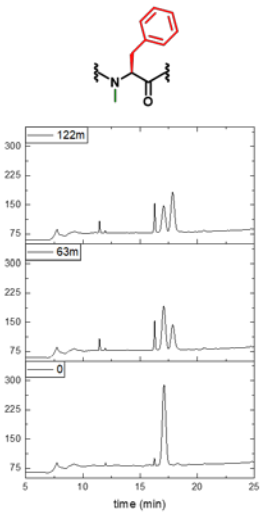
122m
63m
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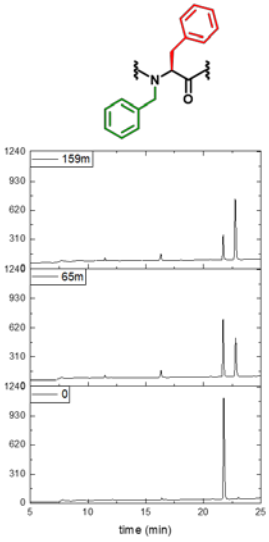
time (min)


159m
65m
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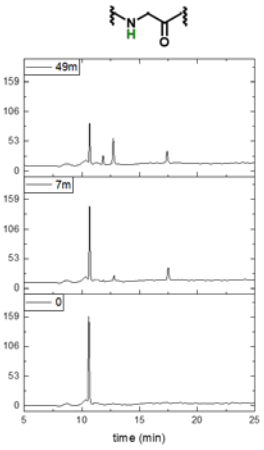
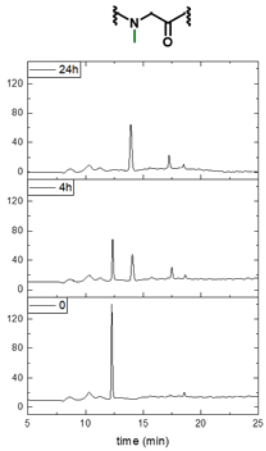
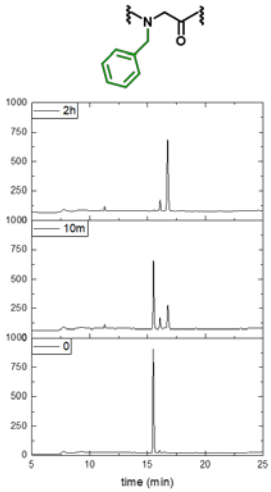
time (min)


49m
7m
0
time (min)


24h
4h
0
time (min)


2h
10m
0
time (min)

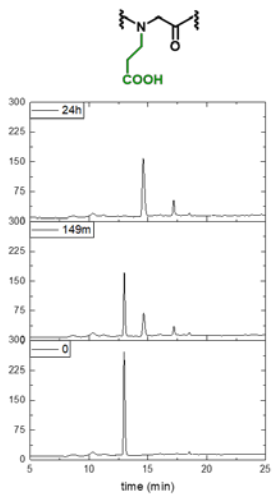


Figure S1. Representative chromatograms for each peptide incubated in the presence of elastase protease over time. Three representative sampling aliquots are shown for t=0 and two other time points sampled during the proteolysis.

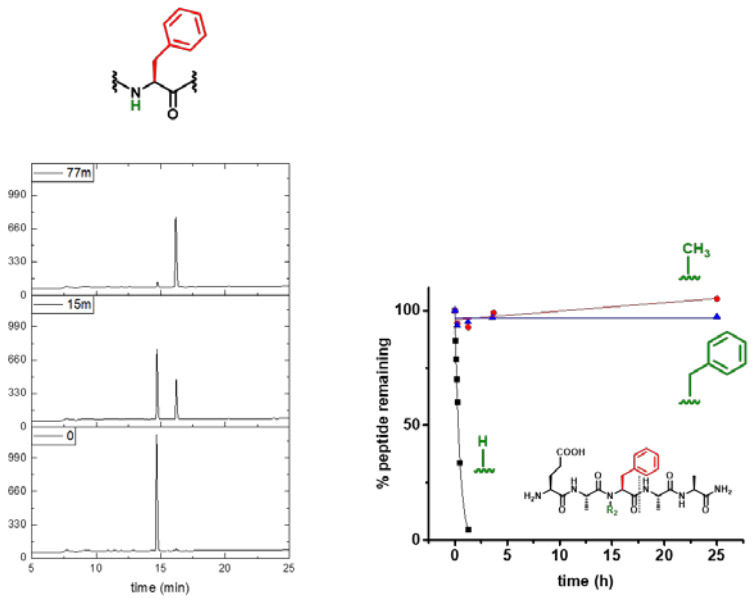
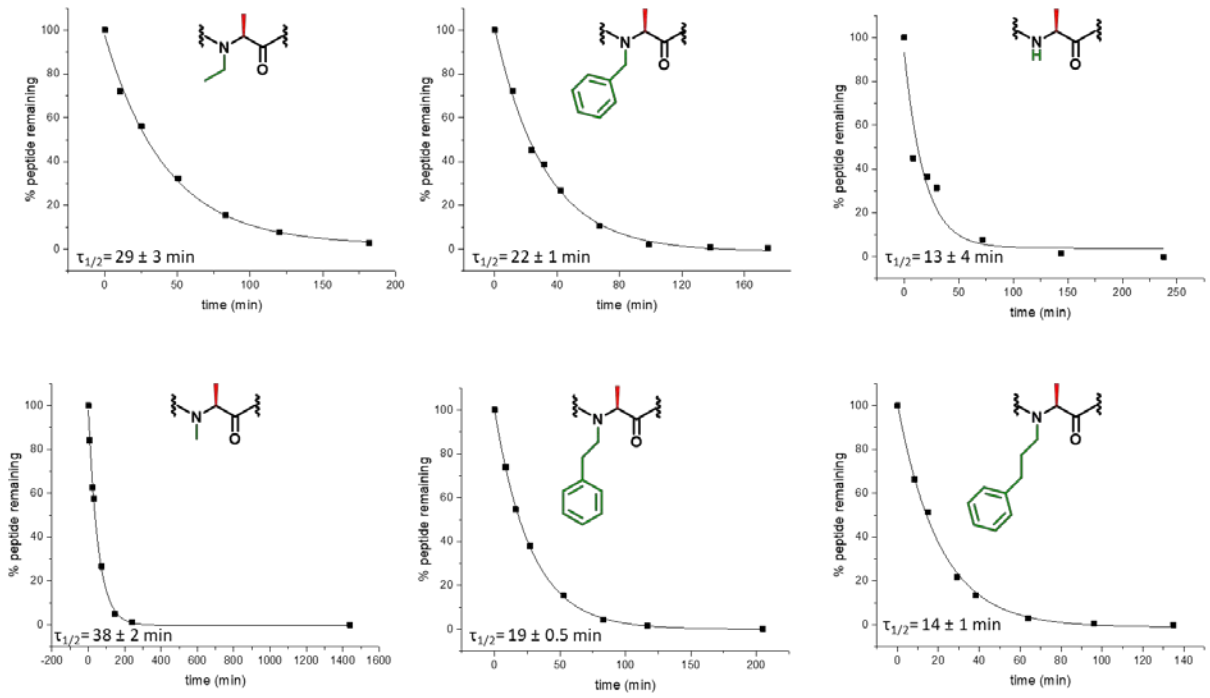


Figure S2. A) Chromatograms for peptide having a single phenylalanine unit incubated with Chymotrypsin protease. Three representative sampling aliquots are shown for t=0 and two other time points sampled during the proteolysis. B) Peptide degradation upon exposure to chymotrypsin when the middle unit is phenylalanine (black squares, $\tau_{1/2}$=19.5min), *N*-Me-Phe (red circles), or *N*-Bn-Phe (Blue triangles).



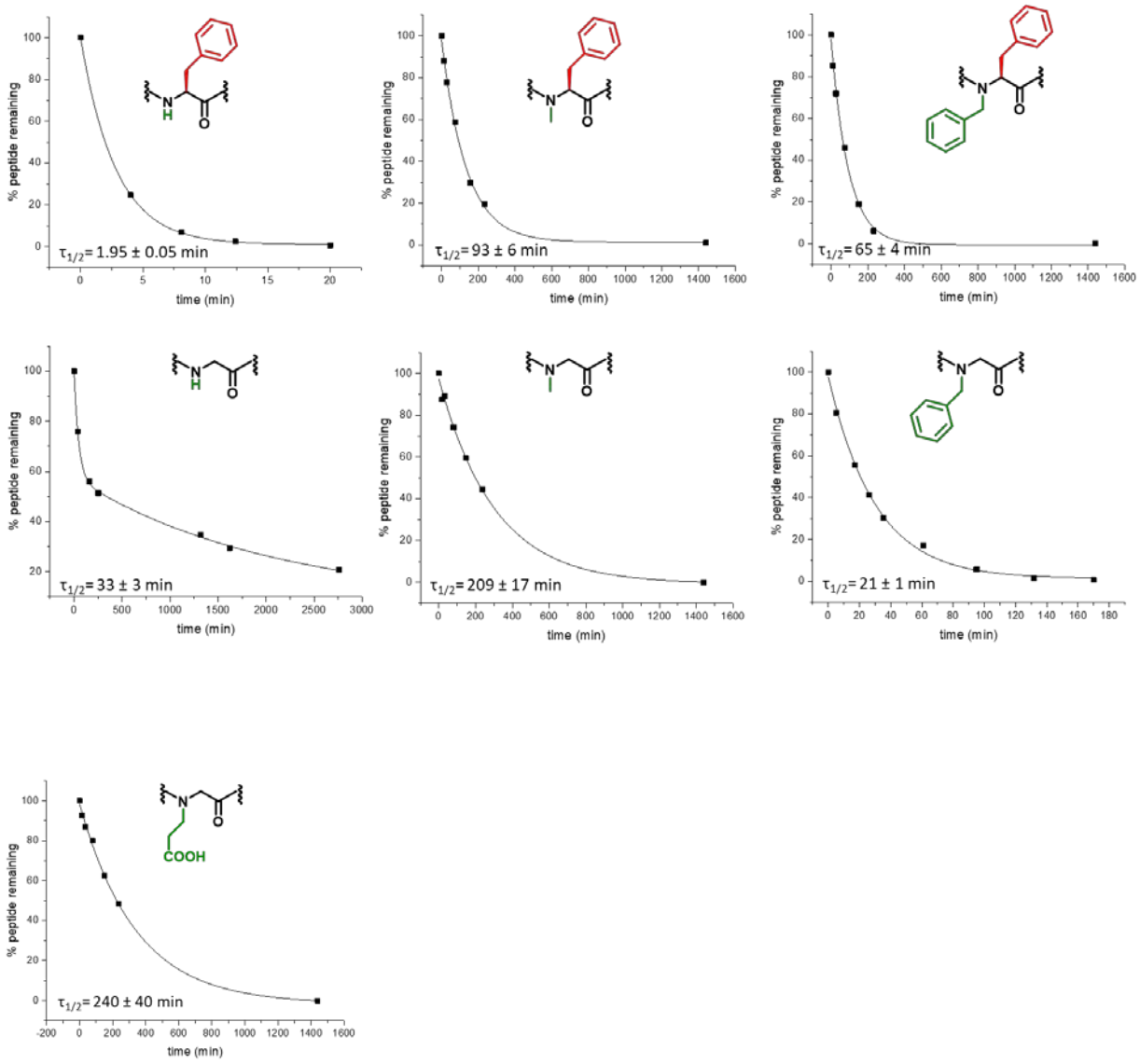


Figure S3. Peptide percentages plotted against time of proteolysis and the calculated half-lives of the examined peptides from the exponential decay fits.