Supporting Information
for
Position-dependent impact of hexafluoroleucine and trifluoroisoleucine on protease digestion

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Characterization and identification of synthesized peptides, characterization of the enzymatic digestion reactions, and identification of proteolytic cleavage products, HPLC methods, and synthesis protocol for Fmoc-HfLeu-OH
**Synthesis of Fmoc-HfLeu-OH**

**General information**

All reactions were run under an argon atmosphere unless otherwise indicated. Room temperature refers to 22 °C. Reagents and anhydrous solvents were transferred via oven-dried syringe or cannula. Flasks were flame-dried under vacuum and cooled under a constant stream of argon. Reactions were monitored by thin layer chromatography using Merck KGaA silica gel 60 F_{254} TLC aluminium sheets and visualized with ceric ammonium molybdate, vanillin staining solution or potassium permanganate staining solution. Chromatographic purification was performed as flash chromatography on Macherey-Nagel GmbH & Co. KG silica gel 60 M, 0.04–0.063 mm, using a forced flow of eluent (method of Still). Concentration under reduced pressure was performed by rotary evaporation at 40 °C at the appropriate pressure. Yields refer to chromatographically purified and spectroscopically pure compounds. NMR measurements were recorded on a JEOL-ECX400 (operating at 400 MHz for $^1$H NMR, 101 MHz for $^{13}$C NMR and 376 MHz for $^{19}$F NMR). Chemical shifts δ are reported in ppm with the solvent resonance as the internal standard. Coupling constants J are given in Hertz (Hz). Multiplicities are classified by the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, br = broad or m = multiplet and combinations thereof. High resolution mass spectra were obtained on an Agilent ESI-ToF 6220 (Agilent Technologies, Santa Clara, CA, USA).

**Scheme S1**: Synthesis of Fmoc-HfLeu-OH 9.
Compounds 3 to 7 were synthesized according to literature [1,2]. Obtained NMR data (\(^1\)H, \(^{13}\)C, and \(^{19}\)F) are consistent with literature [1,2].

**Synthesis of Fmoc-HfLeu-OEt (8)**

(S)-7 (1.04 g, 3.89 mmol) was dissolved in 10% Na\(_2\)CO\(_3\), aq (4 mL) and cooled to 0°C. Dioxane (1 mL) was added and the suspension was stirred for 15 min at 0 °C after which FmocOSu (1.44 g, 4.28 mmol) was added. The mixture was stirred for 3 h at 0 °C and at room temperature overnight. The reaction was diluted with H\(_2\)O (50 mL) and extracted with Et\(_2\)O (4 x 25 mL). The combined organic layers were concentrated in vacuo and the residue was subjected to column chromatography (n-hexane/Et\(_2\)O, 3:1) to give (S)-8 (1.12 g, 2.29 mmol, 59%) as a waxy solid.

TLC: \(R_f = 0.45\) (n-hexane/Et\(_2\)O, 5:1).

\(^1\)H-NMR (400 MHz, CDCl\(_3\)): \(\delta = 7.77\) (d, \(J = 7.5, 2H\)); 7.58 (d, \(J = 7.2, 2H\)); 7.40 (t, \(J = 7.5, 2H\)); 7.32 (t, \(J = 7.0, 2H\)); 5.40 (d, \(J = 7.50, 1H\)); 4.47 (dt, \(J = 20.0; 13.40, 3H\)); 4.32 – 4.18 (m, 3H); 3.18 (s, 1H); 2.40 (d, \(J = 14.5, 1H\)); 2.05 (d, \(J = 10.0, 1H\)); 1.30 (t, \(J = 7.1, 3H\)).

\(^{13}\)C-NMR (101 MHz, CDCl\(_3\)) \(\delta = 170.68, 143.75, 143.53, 141.45, 141.44, 140.84, 130.32, 127.92, 127.90, 127.19, 127.17, 125.07, 124.98, 120.32, 120.15, 120.11, 67.29, 62.53, 51.96, 47.21, 37.15, 27.27, 14.13.

\(^{19}\)F-NMR (376 MHz, CDCl\(_3\)) \(\delta = -67.27\) – -67.44 (m), -67.63 – -67.79 (m).

HRMS calculated for C\(_{23}\)H\(_{31}\)F\(_6\)NNaO\(_4\) [M+Na]^+: 512.1267; observed: 512.1294.

**Synthesis of Fmoc-HfLeu-OH (9)**

A solution of (S)-8 (55.0 mg, 11.2 mmol) in HCl\(_{conc}\) (2 mL) was stirred at room temperature for 24 h. The crude product was lyophilized and purified via a LaPrep\(^\circ\) low-pressure HPLC system (VWR, Darmstadt, Germany) using a Kinetex RP-C18 endcapped HPLC-column (5 \(\mu\)M, 100 Å, 250 \(\times\) 21.2 mm, Phenomenex\(^\circ\), USA). Deionized water and acetonitrile (ACN), both containing 0.1% (v/v) TFA served as eluents. A linear gradient of 30–100% ACN + 0.1% (v/v) TFA over 18 min with a flow rate of 20.0 mL/min was applied. UV-detection occurred at 280 nm. This gave (S)-9 (36.3 mg, 7.87 mmol, 70%) as a white powder.

\(^1\)H-NMR (400 MHz, DMSO-D\(_6\)): \(\delta = 7.82\) (d, \(J = 7.6, 2H\)); 7.77 (d, \(J = 8.7, 1H\)); 7.63 (d, \(J = 7.5, 2H\)); 7.36 (t, \(J = 7.4, 2H\)); 7.26 (t, \(J = 7.4, 2H\)); 4.35 – 4.23 (m, 2H); 4.17 (t, \(J = 6.7, 1H\)); 4.04 (br, 1H); 2.30 – 2.17 (m, 1H); 2.13 – 2.01 (m, 1H).
$^{13}$C-NMR (101 MHz, DMSO-D$_6$): $\delta = 175.95$ (s); 158.87 (s); 144.17 (s); 144.10 (s); 141.23 (s); 141.22 (s); 128.27 (s); 128.25 (s); 127.62 (s); 127.61 (s); 125.69 (s); 125.68 (s); 125.63 (s); 125.61 (s); 120.65 (s); 120.61 (s); 66.23 (s); 51.54 (s); 47.11 (s); 29.52 (s); 26.34 (s).

$^{19}$F-NMR (376 MHz, DMSO-D6): $\delta = -65.91$ – -66.13 (m); -66.38 – -66.62 (m).

HRMS calculated for C$_{21}$H$_{17}$F$_6$NO$_4$ [M+Na]$^+$: 484.0954; observed: 484.0942.

**Peptide synthesis, purification and characterization**

**Peptide synthesis**

Peptides containing HfLeu were synthesized on an Activo P11 Automated Peptide Synthesizer (Activotec, Cambridge, United Kingdom) working under nitrogen atmosphere. All other peptides, either non-fluorinated or TfIle containing, were synthesized manually under standard conditions.

**Peptide characterization**

High resolution mass spectra were recorded on an Agilent 6220 ESI–ToF LC–MS spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) to identify the pure peptide products. The samples were dissolved in a 1:1 mixture of water and acetonitrile containing 0.1% (v/v) TFA and injected directly into the spray chamber by a syringe pump using a flow rate of 10 µL min$^{-1}$. A spray voltage of 3.5 kV was used, the drying gas glow rate was set to 5 L min$^{-1}$ and the nebulizer to 30 psi. The gas temperature was 300 °C.

To verify purity of the synthesized peptides analytical HPLC was carried out on a Chromaster 600 bar DAD-System with CSM software (VWR/Hitachi, Darmstadt, Germany). The system works with a low-pressure gradient containing a HPLC-pump (5160) with a 6-channel solvent degasser, an organizer, an autosampler (5260) with a 100 µL sample loop, a column oven (5310) and a diode array flow detector (5430). A LUNA™ C8 (2) column (5 µm, 250 x 4.6 mm, Phenomenex®, Torrance, CA, USA) was used. As eluents water and ACN, both containing 0.1% (v/v) TFA were used, the flow rate was adjusted to 1 mL/min and the column was heated to 24 °C. The used gradient method is shown in Table S1. The UV-detection of the peptides occurred at 220 nm. The data were analyzed with EZChrom Elite software (version 3.3.2, Agilent Technologies, Santa Clara, CA, USA).
Table S1: Used linear gradient for the purity determination of the synthesized peptides.

| Time [min] | Water + 0.1% (v/v) TFA [%] | ACN + 0.1% (v/v) TFA [%] |
|------------|---------------------------|--------------------------|
| 0          | 95                        | 5                        |
| 18         | 30                        | 70                       |
| 19         | 0                         | 100                      |
| 21         | 0                         | 100                      |
| 21.5       | 95                        | 5                        |
| 24         | 95                        | 5                        |

Table S2: Identification of the synthesized peptides by ESI–ToF mass spectrometry and analytical RP-HPLC.

| Peptide     | Retention time [min] | Charge | m/z calculated | m/z observed |
|-------------|----------------------|--------|----------------|--------------|
| FA          | 10.597               | +1     | 967.5364       | 967.5396     |
|             |                      | +2     | 484.2721       | 484.2736     |
| P2-LeuFA    | 12.500               | +1     | 1009.5463      | 1009.5849    |
|             |                      | +2     | 505.2956       | 505.2970     |
| P2-HfLeuFA  | 12.393               | +1     | 1117.4622      | 1117.5306    |
|             |                      | +2     | 559.2573       | 559.2691     |
| P2-IleFA    | 12.137               | +1     | 1009.5463      | 1009.5849    |
|             |                      | +2     | 505.2956       | 505.2971     |
| P2-TfIleFA  | 12.493               | +1     | 1063.4622      | 1063.5576    |
|             |                      | +2     | 532.2814       | 532.2845     |
| P1'-LeuFA   | 11.773               | +1     | 1009.5463      | 1009.5863    |
|             |                      | +2     | 505.2956       | 505.7982     |
| P1'-HfLeuFA | 11.917               | +1     | 1117.4622      | 1117.5272    |
|             |                      | +2     | 559.2573       | 559.2684     |
| P1'-IleFA   | 11.370               | +1     | 1009.5463      | 1009.5858    |
|             |                      | +2     | 505.2956       | 505.2975     |
| P1'-TfIleFA | 11.870               | +1     | 1063.4622      | 1063.5556    |
|             |                      | +2     | 532.2814       | 532.2816     |
| P2'-LeuFA   | 11.847               | +1     | 1009.5463      | 1009.5866    |
|             |                      | +2     | 505.2956       | 505.2981     |
| P2'-HfLeuFA | 12.197               | +1     | 1117.4622      | 1117.5305    |
|             |                      | +2     | 559.2573       | 559.2693     |
| P2'-IleFA   | 11.557               | +1     | 1009.5463      | 1009.5864    |
|             |                      | +2     | 505.2956       | 505.2980     |
| P2'-TfIleFA | 12.283               | +1     | 1063.4622      | 1063.5576    |
|             |                      | +2     | 532.2814       | 532.2835     |
Enzymatic digestion studies

Characterization of the enzymatic digestion reactions was carried out via analytical HPLC on a LaChrom-ELITE-HPLC-System from VWR International Hitachi (Darmstadt, Germany). The system contains an organizer, two HPLC-pumps (L-
2130) with solvent degasser, an autosampler (L-2200) with a 100 µL sample loop, a
diode array flow detector (L-2455), a fluorescence detector (L-2485) and a high
pressure gradient mixer. As eluents water and ACN, both containing 0.1% (v/v) TFA
were used, and a flow rate of 3 mL/min was applied. The used linear gradients are
shown in Table S3. For the non-fluorinated peptides method A was used to follow the
digestion process, and for the fluorinated peptides method B was applied. For
chromatograms where an insufficient baseline separation was observed,
measurements were repeated using methods C [FA (pepsin), P2-LeuFA
(proteinase K), P2-IleFA (pepsin), P2-IleFA (proteinase K), P1'-LeuFA (elastase),
P1'-LeuFA (proteinase K), P1'-IleFA (proteinase K)] or D [P2-HfleuFA (proteinase K),
P2-TfIleFA (pepsin), P2-TfIleFA (proteinase K), P1'-TfIleFA (elastase), P2'-TfIleFA
(proteinase K)]. The obtained data were analyzed with EZChrom Elite software
(version 3.3.2, Agilent Technologies, Santa Clara, CA, USA).

Table S3: Used linear gradients to follow the digestion process by FL-RP-HPLC.

| Method | Time [min] | Water + 0.1% (v/v) TFA [%] | ACN + 0.1% (v/v) TFA [%] |
|--------|------------|-----------------------------|--------------------------|
| A      | 0          | 95                          | 5                        |
|        | 5          | 70                          | 30                       |
|        | 5.5        | 70                          | 30                       |
|        | 6          | 95                          | 5                        |
|        | 9          | 95                          | 5                        |
| B      | 0          | 95                          | 5                        |
|        | 5          | 60                          | 40                       |
|        | 5.5        | 60                          | 40                       |
|        | 6          | 95                          | 5                        |
|        | 9          | 95                          | 5                        |
| C      | 0          | 95                          | 5                        |
|        | 15         | 70                          | 30                       |
|        | 15.5       | 70                          | 30                       |
|        | 16         | 95                          | 5                        |
|        | 17         | 95                          | 5                        |
| D      | 0          | 95                          | 5                        |
|        | 15         | 55                          | 45                       |
|        | 15.5       | 55                          | 45                       |
|        | 16         | 95                          | 5                        |
|        | 17         | 95                          | 5                        |
Identification of the proteolytic cleavage products (Table S4–S7) occurred according to the mass-to-charge ratios determined with an Agilent 6220 ESI–ToF–MS instrument (Agilent Technologies, Santa Clara, CA, USA). For this, the quenched peptide-enzyme-solutions after 120 min and 24 h incubation were analyzed. The solutions were injected directly into the spray chamber using a syringe pump with a flow rate of 10 µL min⁻¹. Spray voltage was set to 3.5 kV, a drying gas flow rate of 5 L min⁻¹ was used, the nebulizer was set to 30 psi, and the gas temperature to 300 °C. The fragmentor voltage was 200 V. Not all corresponding fragments could be detected.

| Peptide | Fragment               | [M + H]⁺ calculated | [M + H]⁺ observed |
|---------|------------------------|--------------------|-------------------|
| FA      | Abz-KAFAAAAK           | 967.5364           | 967.5376          |
|         | Abz-KAAF                | 555.2559           | 555.2938          |
|         | AAAAK                  | 431.2617           | 431.2627          |
| P2-LeuFA| Abz-KALeuFAAAK         | 1009.5463          | 1009.5883         |
|         | Abz-KALeuF             | 597.3029           | 597.2609          |
| Peptide       | Fragment          | [M + H]<sup>+</sup> calculated | [M + H]<sup>+</sup> observed |
|--------------|-------------------|-------------------------------|-------------------------------|
| P2-HfLeuFA   | Abz-KAHfLeuFAAAAK | 1117.4622                     | 1117.5298                     |
| P2-IleFA     | Abz-KAAlleFAAAAK  | 1009.5463                     | 1009.5851                     |
|              | Abz-KAAlleF       | 597.3029                      | 597.3435                      |
|              | AAAAK             | 431.2617                      | 431.2647                      |
| P2-TfIleFA   | Abz-KATfIleFAAAAK | 1063.4622                     | 1063.5577                     |
| P1'-LeuFA    | Abz-KAAFLeuAAAK   | 1009.5463                     | 1009.5866                     |
|              | Abz-KAAFLeu       | 776.2559                      | 776.3214                      |
|              | HfLeuAAK          | 581.2246                      | 581.2246                      |
|              | Abz-KAAF          | 555.2559                      | 555.2934                      |
|              | AAAK              | 360.2246                      | 360.2239                      |
| P1'-HfLeuFA  | Abz-KAAFHiLeuAAK  | 1117.4622                     | 1117.5280                     |
|              | Abz-KAAFHiLeu     | 776.2559                      | 776.3214                      |
|              | HfLeuAAK          | 581.2246                      | 581.2246                      |
|              | Abz-KAAF          | 555.2559                      | 555.2934                      |
|              | AAAK              | 360.2246                      | 360.3630                      |
| P1'-IleFA    | Abz-KAAFlleAAK    | 1009.5463                     | 1009.5825                     |
|              | Abz-KAAF          | 555.2559                      | 555.2951                      |
|              | IleAAK            | 473.3087                      | 473.3104                      |
| P1'-TfIleFA  | Abz-KAAFTfIleAAK  | 1063.4622                     | 1063.5604                     |
|              | Abz-KAAF          | 555.2559                      | 555.2954                      |
|              | TfIleAAK          | 527.2246                      | 527.2827                      |
| P2'-LeuFA    | Abz-KAAFALeuAAK   | 1009.5463                     | 1009.5872                     |
|              | Abz-KAAF          | 555.2559                      | 555.2922                      |
|              | AlleAAK           | 473.3087                      | 473.3087                      |
| P2'-HfLeuFA  | Abz-KAAFAHfLeuAAK | 1117.4622                     | 1117.5331                     |
|              | AHfLeuAAK         | 581.2246                      | 581.2550                      |
|              | Abz-KAAF          | 555.2559                      | 555.2965                      |
| P2'-IleFA    | Abz-KAAFAlleAAK   | 1009.5463                     | 1009.5875                     |
|              | Abz-KAAF          | 555.2559                      | 555.2943                      |
|              | AlleAAK           | 473.3087                      | 473.3112                      |
| P2'-TfIleFA  | Abz-KAAFATfIleAAK | 1063.4622                     | 1063.5575                     |
|              | Abz-KAAF          | 555.2559                      | 555.2945                      |
|              | ATfIleAAK         | 527.2246                      | 527.2822                      |

Table S5: Identification of the cleavage products of the different peptides by ESI–ToF mass spectrometry after digestion with pepsin.
| Peptide      | Fragment                        | [M + H]⁺ calculated | [M + H]⁺ observed |
|--------------|---------------------------------|---------------------|-------------------|
| Abz-KAAF     | 668.3400                        | 668.3820            |
| Abz-KAAF     | 555.2559                        | 555.2971            |
| LeuAAAK      | 473.3087                        | 473.3126            |
| AAK          | 360.2246                        | 360.2271            |
| P1'-HfLeuFA  | Abz-KAAFHfLeuAAAK               | 1117.4622           | 1117.5325         |
| Abz-KAAF     | 776.2559                        | 776.3236            |
| HfLeuAAAK    | 581.2246                        | 581.2553            |
| Abz-KAAF     | 555.2559                        | 555.2956            |
| AAK          | 360.2246                        | 360.2273            |
| P1'-IleFA    | Abz-KAAFleAAAK                  | 1009.5463           | 1009.5908         |
| Abz-KAAF     | 555.2559                        | 555.2969            |
| IleAAAK      | 473.3087                        | 437.3087            |
| P1'-TfIleFA  | Abz-KAAFATfIleAAAK              | 1063.4622           | 1063.5634         |
| Abz-KAAF     | 555.2559                        | 555.2969            |
| TfIleAAAK    | 527.2246                        | 527.2843            |
| P2'-LeuFA    | Abz-KALKeuAAA                   | 1009.5463           | 1009.5889         |
| Abz-KAAF     | 555.2559                        | 555.2970            |
| LeuAAK       | 473.3087                        | 473.3121            |
| P2'-HfLeuFA  | Abz-KAFAHfLeuAAK                | 1117.4622           | 1117.5307         |
| Abz-KAAF     | 626.2930                        | 626.3344            |
| HfLeuAAK     | 510.1875                        | 510.2170            |
| P2'-IleFA    | Abz-KAFAlleAAK                  | 1009.5463           | 1009.5899         |
| Abz-KAAF     | 555.2559                        | 555.2970            |
| AlleAAK      | 473.3087                        | 473.3121            |
| P2'-TfIleFA  | Abz-KAFAFATfIleAAK              | 1063.4622           | 1063.5627         |
| FATfIleAAK   | 674.2930                        | 674.3530            |
| Abz-KAFA     | 626.2930                        | 626.3333            |
| Abz-KAAF     | 555.2559                        | 555.2969            |
| ATfIleAAK    | 527.2246                        | 527.2845            |
| TfIleAAK     | 456.1875                        | 456.2462            |

Table S6: Identification of the cleavage products of the different peptides by ESI–ToF mass spectrometry after digestion with elastase.
| Peptidic Sequence | M/z 1 | M/z 2 |
|-------------------|-------|-------|
| Abz-KAHfLeuFA    | 776.2559 | 776.2559 |
| FAAAAK           | 578.3301 | 578.2440 |
| **P2-IleFA**     | Abz-KAlleFAAAAK | 1009.5463 | 1009.5884 |
|                  | Abz-KAlleFAAAA | 881.4513 | 881.4960 |
|                  | Abz-KAlleFAAA  | 810.4142 | 810.4960 |
|                  | Abz-KAlleFAA   | 739.3771 | 739.4176 |
|                  | Abz-KAlleFA    | 668.3400 | 668.3787 |
|                  | FAAAAK         | 578.3301 | 578.3322 |
|                  | AAK             | 360.2246 | 360.2256 |
|                  | AAK             | 289.1875 | 289.1880 |
|                  | AK              | 218.1504 | 218.1505 |
| **P2-TfIleFA**   | Abz-KATfIleFAAAAK | 1063.4622 | 1063.5576 |
|                  | Abz-KATfIleFAAA | 864.3301 | 864.4210 |
|                  | Abz-KATfIleFAA  | 793.2930 | 793.3844 |
|                  | Abz-KATfIleFA   | 722.2559 | 722.3470 |
|                  | FAAAAK         | 578.3301 | 578.3271 |
|                  | AAK             | 360.2246 | 360.2224 |
| **P1'-LeuFA**    | Abz-KAAFLeuAAAK | 1009.5463 | 1009.5887 |
|                  | Abz-KAAFLeuAAA  | 881.4513 | 881.4815 |
|                  | Abz-KAAFLeuAA   | 810.4142 | 810.4472 |
|                  | AAFLeuAAAK      | 762.4513 | 762.4458 |
|                  | Abz-KAAFLeuA    | 739.3771 | 7394120 |
|                  | ALeuAAAK        | 691.4142 | 691.4142 |
|                  | FLLeuAAAK       | 620.3771 | 620.3783 |
|                  | Abz-KAA         | 408.1875 | 408.1875 |
|                  | Abz-KA          | 337.1504 | 337.1504 |
|                  | AK              | 289.1504 | 289.1858 |
| **P1'-HfLeuFA**  | Abz-KAFAHfLeuAAAK | 1117.4622 | 1117.5330 |
|                  | Abz-KAFAHfLeuAA | 918.3301 | 918.3982 |
|                  | AAFHfLeuAAAK    | 870.3673 | 870.3975 |
|                  | AFHfLeuAAAK     | 799.3301 | 799.3600 |
|                  | Abz-KAA         | 408.1875 | 408.1875 |
|                  | Abz-KA          | 337.1504 | 337.1864 |
| **P1'-IleFA**    | Abz-KAFAIleAAAK  | 1009.5463 | 1009.5853 |
|                  | Abz-KAFAIleAA   | 810.4142 | 810.4500 |
|                  | Abz-KAFAIleA    | 739.3771 | 739.4144 |
|                  | IleAAAK         | 620.3771 | 620.3747 |
| **P1'-TfIleFA**  | Abz-KAFAFtIleAAAK | 1063.4622 | 1063.5578 |
|                  | Abz-KAFAFtIleAA | 864.3301 | 864.4205 |
|                  | Abz-KAFAFtIleA  | 793.2930 | 793.3844 |
|                  | AFTIleAAAK      | 745.3301 | 745.3814 |
|                  | AAK             | 289.1875 | 289.1857 |
|                  | AK              | 218.1504 | 218.1483 |
| **P2'-LeuFA**    | Abz-KAFAFLeuAAK  | 1009.5463 | 1009.5894 |
|                  | Abz-KAFAFLeuAA  | 881.4513 | 881.4938 |
|                  | Abz-KAFAFLeuA   | 810.4142 | 810.4938 |
|                  | AFAFLeuAAK      | 691.4142 | 691.4168 |
|                  | Abz-KAAF        | 626.2930 | 626.3324 |
|                  | LeuAAK          | 402.2716 | 402.2730 |
|                  | Abz-KA          | 337.1504 | 337.1872 |
| **P2'-HfLeuFA**  | Abz-KAFAHfLeuAAK | 1117.6622 | 1117.5330 |
|                  | Abz-KAFAHfLeuA  | 918.3301 | 918.4349 |
|                  | AAFHfLeuAAK     | 870.3673 | 870.4001 |
|                  | AFAHfLeuAAK     | 799.3301 | 799.3612 |
Table S7: Identification of the cleavage products of the different peptides by ESI-ToF mass spectrometry after digestion with proteinase K.

| Peptide          | Fragment               | [M + H]\(^+\) calculated | [M + H]\(^+\) observed |
|------------------|------------------------|----------------------------|-------------------------|
| FA               | Abz-KAAFAAAAK          | 967.5364                   | 967.5376                |
|                 | AFAAAAK                | 649.3673                   | 649.2762                |
| P2-LeuFA         | Abz-KAKeuFAAAAK        | 1009.5463                  | 1009.5863               |
|                 | Abz-KAKeuFAAAA         | 810.4142                   | 810.4536                |
|                 | Abz-KAKeuFAAA         | 739.4116                   | 739.4179                |
|                 | Abz-KAKeuFA            | 668.3404                   | 668.3816                |
|                 | Abz-KAKeu              | 450.2345                   | 450.2719                |
|                 | AAK                    | 289.1504                   | 289.1880                |
| P2-HfLeuFA       | Abz-KAHfLeuFAAAAK      | 1117.4622                  | 1117.5298               |
|                 | Abz-KAHfLeuFAAAA       | 918.3301                   | 918.3970                |
|                 | Abz-KAHfLeuFAA         | 847.2930                   | 847.3608                |
|                 | Abz-KAHfLeuFA          | 776.2559                   | 776.3233                |
|                 | Abz-KAHfLeu            | 558.1504                   | 558.2168                |
|                 | AAK                    | 289.1875                   | 289.1884                |
| P2-IleFA         | Abz-KAllleFAAAAK       | 1009.5463                  | 1009.5872               |
|                 | Abz-KAllleFAA          | 810.4142                   | 810.4551                |
|                 | Abz-KAllleFA           | 739.3771                   | 739.4192                |
|                 | Abz-KAllle             | 668.3400                   | 889.3806                |
|                 | Abz-KAllleF            | 597.3029                   | 597.3422                |
|                 | AAK                    | 289.1875                   | 289.1891                |
| P2-TfIleFA       | Abz-KATfIleFAAAAK      | 1063.4622                  | 1063.5604               |
|                 | Abz-KATfIleFAAAA       | 864.3301                   | 864.4204                |
|                 | Abz-KATfIleFAA         | 793.2930                   | 793.3895                |
|                 | Abz-KATfIleFA          | 722.2559                   | 722.3512                |
|                 | Abz-KATfIleF           | 651.2188                   | 651.3140                |
|                 | AAAAK                  | 431.2617                   | 430.0513                |
|                 | AAK                    | 289.1875                   | 289.1888                |
| P1'-LeuFA        | Abz-KAAFLeuAAK         | 1117.4622                  | 1117.5271               |
|                 | Abz-KAAFLeuAA          | 810.4142                   | 810.4552                |
|                 | Abz-KAAFLeuA           | 739.3771                   | 739.4182                |
|                 | FLeuAAAK               | 620.3771                   | 620.3813                |
|                 | Abz-KAA                | 408.1875                   | 408.2276                |
|                 | AAK                    | 289.1504                   | 289.1885                |
| P1'-HfLeuFA      | Abz-KAAFHfLeuAAAK      | 1117.4622                  | 1117.5271               |
|                 | FHfLeuAAAK             | 728.2930                   | 728.3226                |
| Peptide  | Mass P1' | Mass P2' |
|----------|----------|----------|
| Abz-KAA  | 408.1875 | 408.2261 |
| Abz-KAAFlleAAK | 1009.5463 | 1009.5878 |
| Abz-KAAFlleAA | 810.4142 | 810.4545 |
| Abz-KAAFlleA | 739.3771 | 739.4169 |
| FlleAAK | 620.3771 | 620.3795 |
| Abz-KAA | 408.1875 | 408.2265 |
| AAK | 289.1504 | 289.1891 |
| Abz-KAAFlleAAK | 1063.4622 | 1063.5580 |
| Abz-KAAFlleAA | 864.3301 | 864.4253 |
| Abz-KAAFlleA | 793.2930 | 793.3853 |
| FlleAAAK | 647.2930 | 674.3504 |
| Abz-KAA | 408.1875 | 408.2260 |
| AAK | 289.1875 | 289.1880 |
| AK | 218.1504 | 218.1508 |
| Abz-KAAFlleAAK | 1009.5463 | 1009.5878 |
| Abz-KAAFlleAA | 739.3771 | 739.4169 |
| FlleAAK | 620.3771 | 620.3795 |
| Abz-KAA | 408.1875 | 408.2265 |
| AAK | 289.1875 | 289.1883 |
| Abz-KAAFlleAAK | 1117.4622 | 1117.5304 |
| Abz-KAAFlleAA | 847.2930 | 847.3603 |
| FlleAAK | 510.1875 | 510.2172 |
| AAK | 289.1875 | 268.1885 |
| Abz-KAAFlleAAK | 1009.5463 | 1009.5878 |
| Abz-KAAFlleAA | 626.2930 | 626.338 |
| FlleAAK | 620.3771 | 620.3791 |
| Abz-KAA | 408.1875 | 408.2260 |
| Abz-KAAFlleAAK | 1063.4622 | 1063.5580 |
| FATlleAAK | 674.2930 | 674.3496 |
| Abz-KAA | 408.1875 | 408.2239 |

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