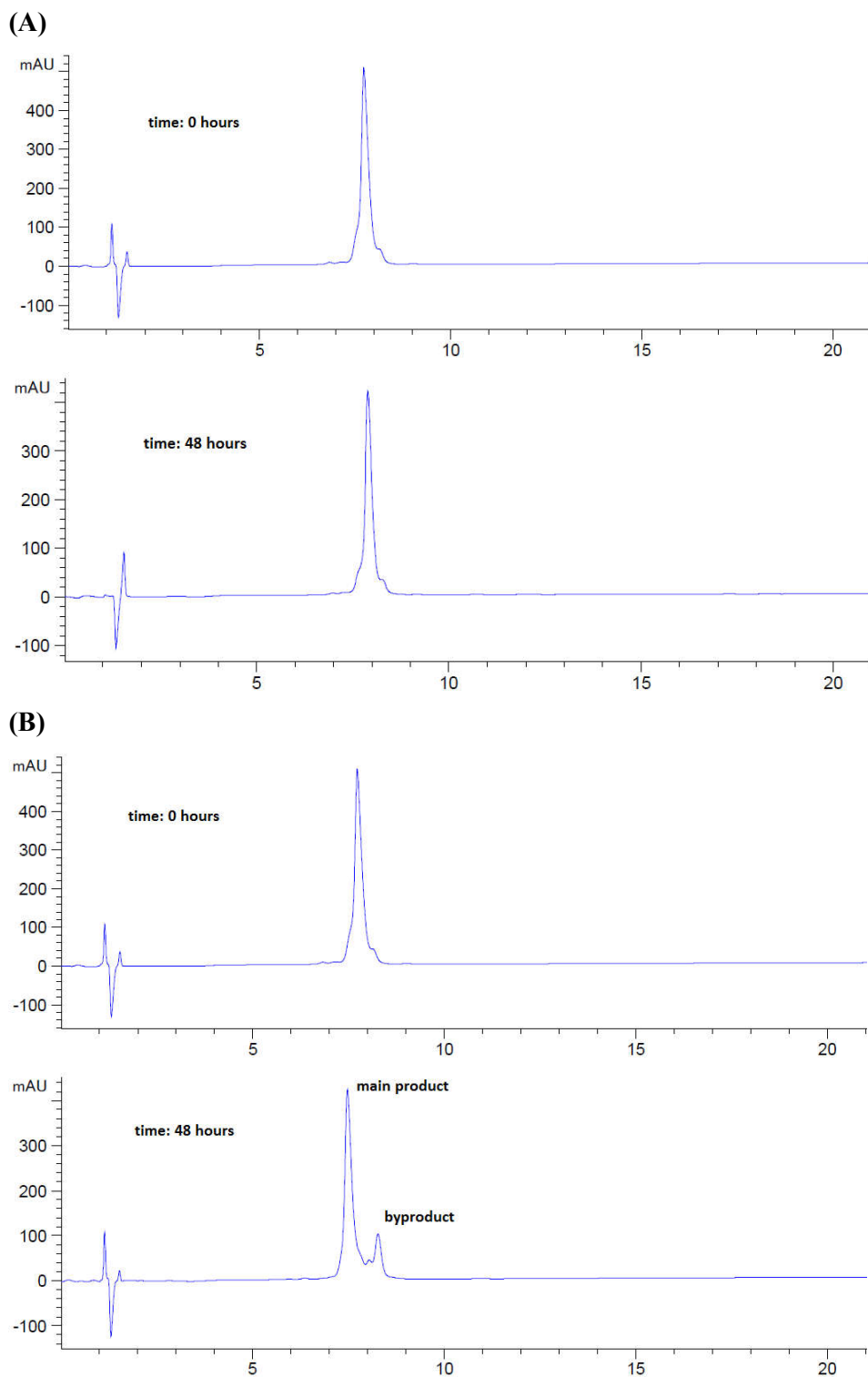


## **Supplementary Materials**

### **Characterization of Asparagine Deamidation in Immunodominant Myelin Oligodendrocyte Glycoprotein Peptide Potential Immunotherapy for the Treatment of Multiple Sclerosis**

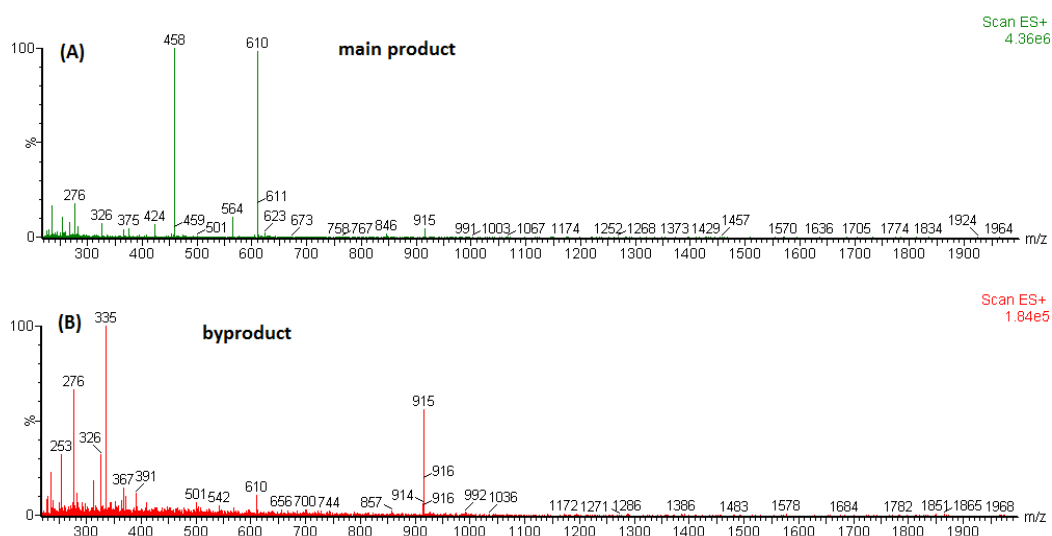
**RP-HPLC Conditions:**

- i) Temperature: 25 °C
- ii) Column: Purospher RP-18 (5µm, Hibar 100-4, 6 mm),
- iii) Solvents: H<sub>2</sub>O (0.08% TFA), AcN (0.08% TFA),
- iv) Gradient elution: from 18% AcN to 40% AcN over 30 min



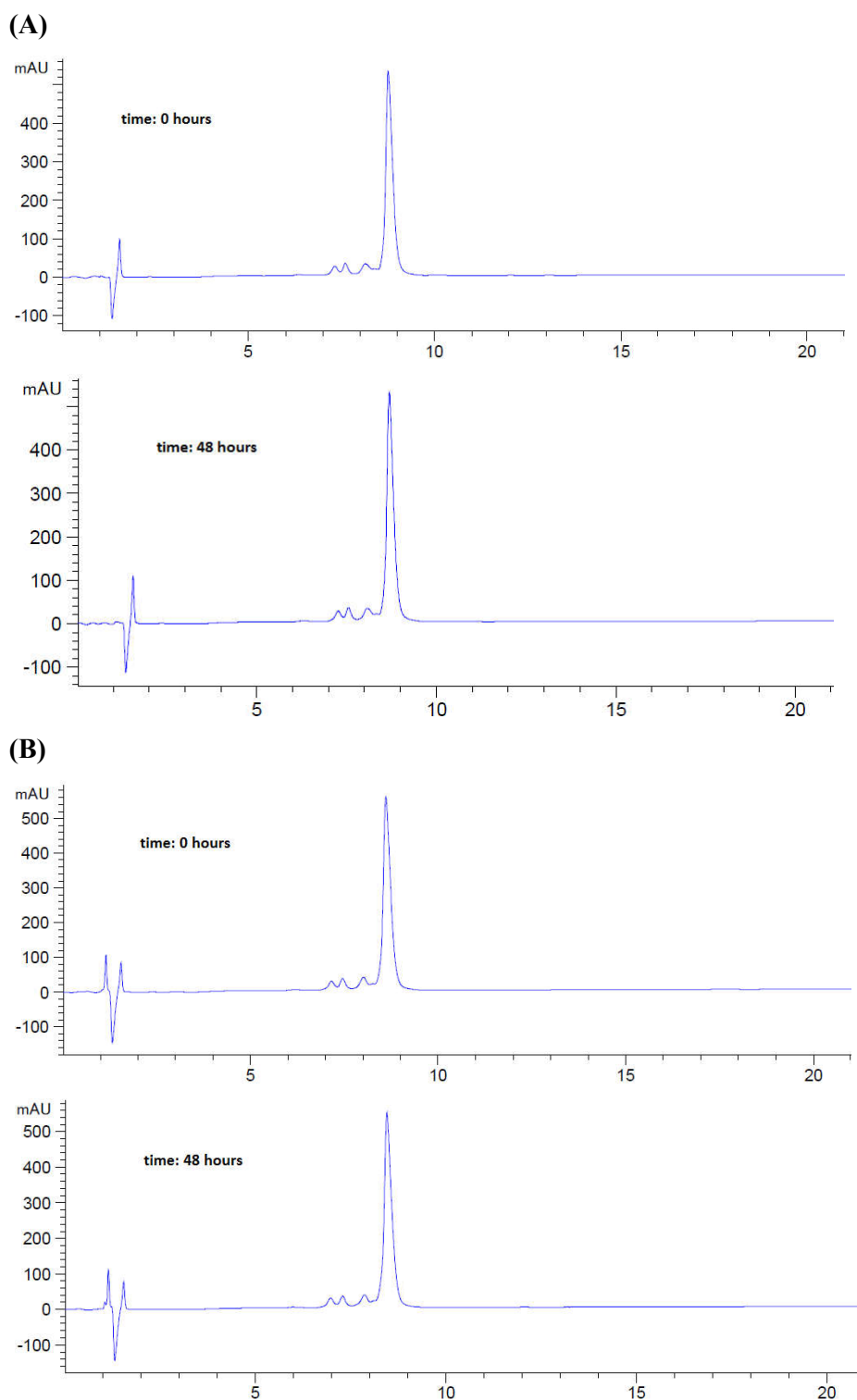
**Figure S1:** RP-HPLC chromatogram of Pep 1 (MOG<sub>4155</sub>) at 214.4 nm, at time zero and 48 h after dilution; **(A)** dissolved in water, **(B)** dissolved in bicarbonate buffer pH 9.0

$t_R$  (main product): 7.47 min (83.1%) and  $t_R$  (byproduct): 8.27 min (13.9%)



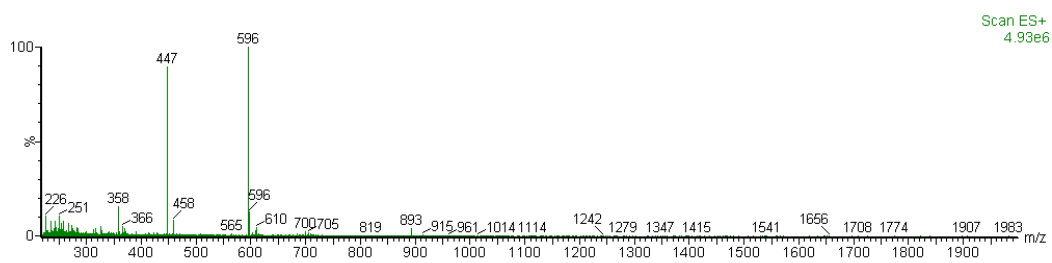
**Figure S2:** ESI-MS spectra of **Pep 1** (MOG<sub>4155</sub>) dissolved in bicarbonate buffer, pH 9.0; of main product **(A)** and byproduct **(B)**

MW theoretical (main product): 1826.12 Da



**Figure S3:** RP-HPLC chromatogram of **Pep 2** [MOG<sub>4155</sub> (Ala<sup>53</sup>)] at 214.4 nm, at time zero and 48 h after dilution; **(A)** dissolved in water, **(B)** dissolved in bicarbonate buffer pH 9.0

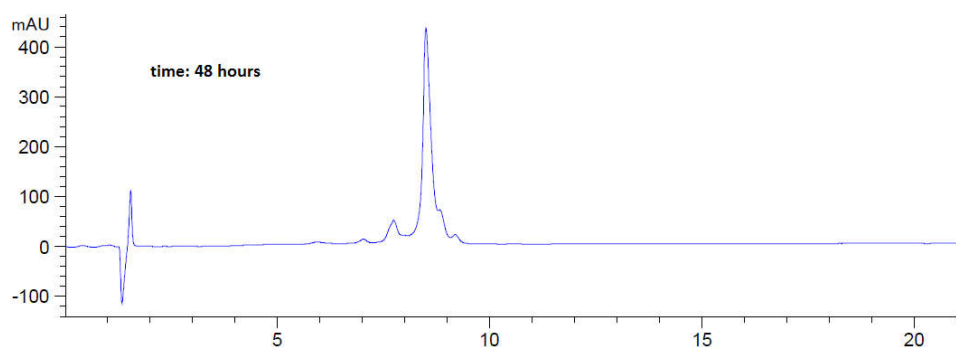
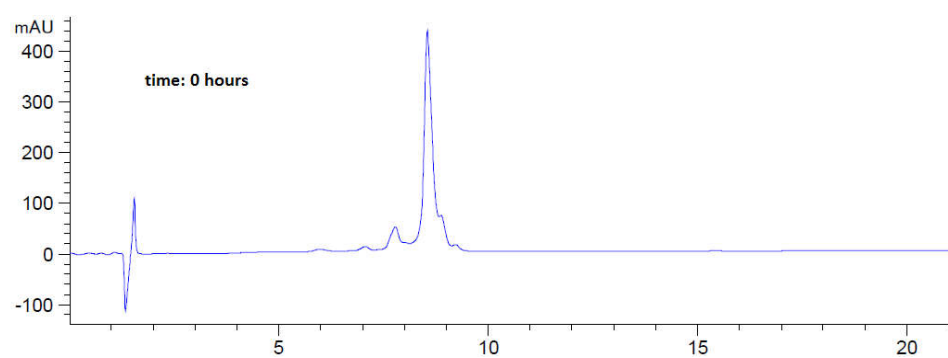
$t_R$  (main product): 8.45 min (100%) and  $t_R$  (byproduct): do not observed



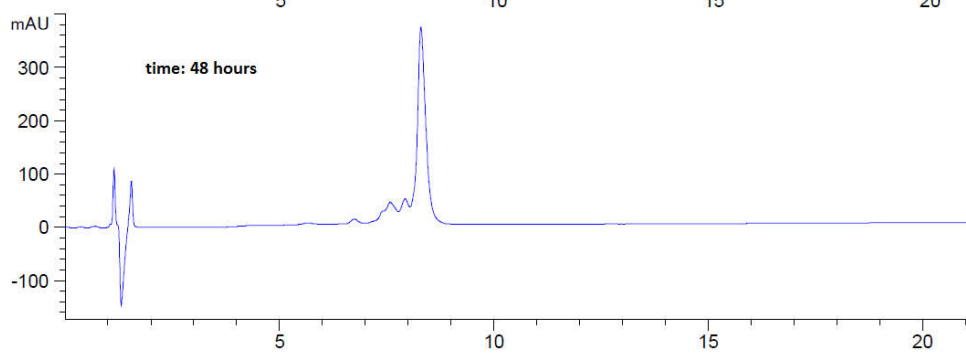
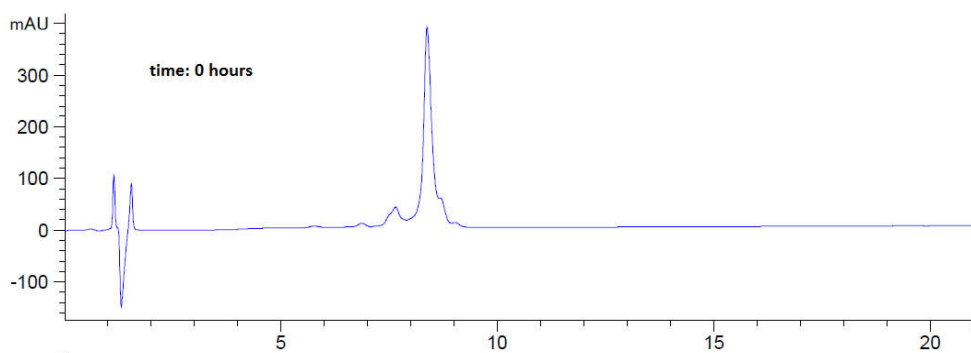
**Figure S4:** ESI-MS spectra of **Pep 2** [MOG<sub>4155</sub> (Ala<sup>53</sup>)]

MW<sub>theoretical</sub> (main product): 1783.11 Da

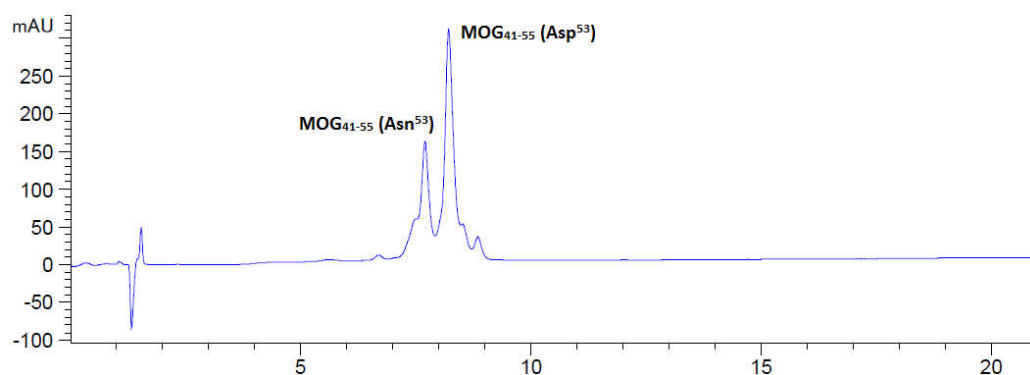
**(A)**



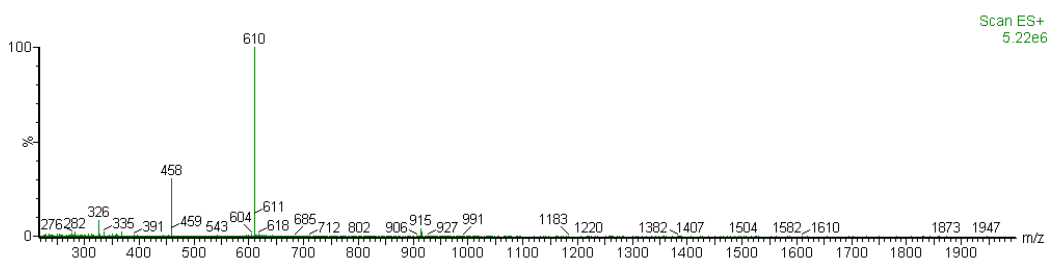
**(B)**



(C)

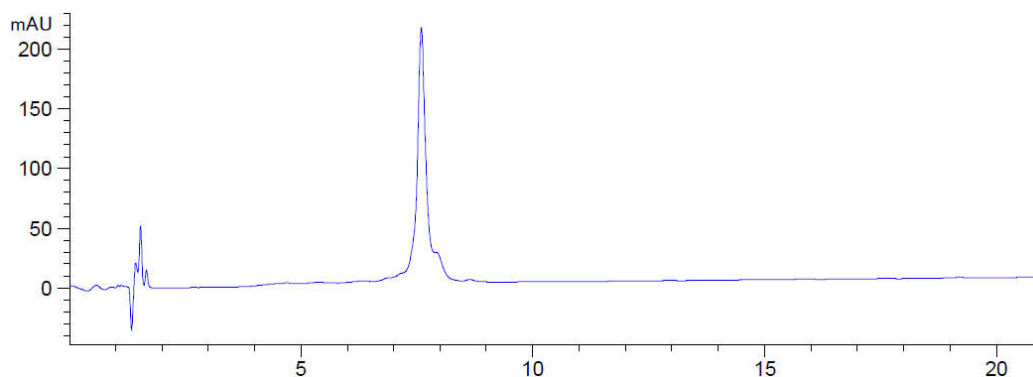


**Figure S5:** RP-HPLC chromatogram of **Pep 3** [MOG<sub>4155</sub> (Asp<sup>53</sup>)] at 214.4 nm, at time zero and 48 h after dilution; (A) dissolved in water, (B) dissolved in bicarbonate buffer pH 9.0;  $t_R$  (main product): 8.3 min (100%) and  $t_R$  (byproduct): do not observed (C) RP-HPLC chromatogram after co-injection of MOG<sub>4155</sub> (**Pep 1**) (dissolved in bicarbonate buffer) and MOG<sub>4155</sub> (Asp<sup>53</sup>) (**Pep 3**) (dissolved in water) at 214.4 nm



**Figure S6:** ESI-MS spectra of **Pep 3** [MOG<sub>4155</sub> (Asp<sup>53</sup>)]

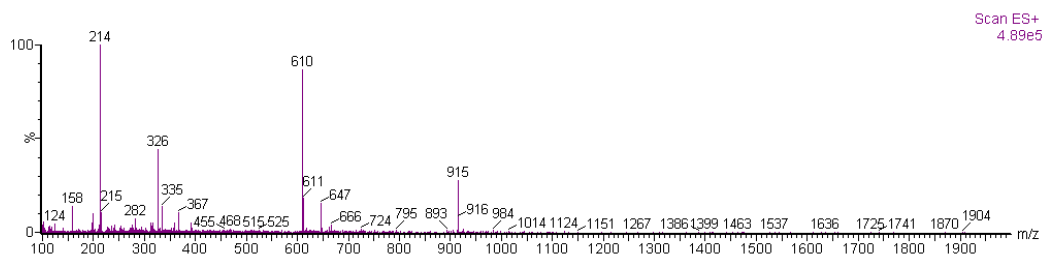
MW theoretical (main product): 1827.12 Da



**Figure S7:** RP-HPLC chromatogram of **Pep 4** [MOG<sub>4155</sub> (isoAsp<sup>53</sup>)] at 214.4 nm, dissolved in water

$t_R$  (main product): 7.6 min (100%)

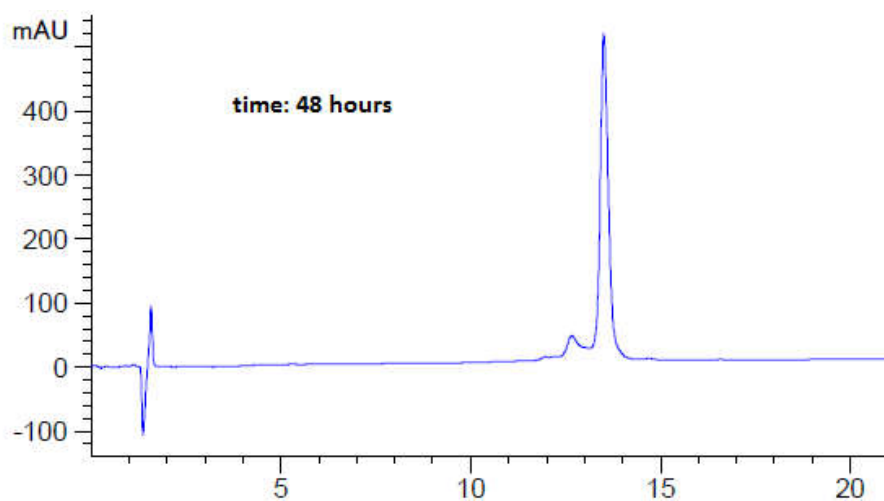




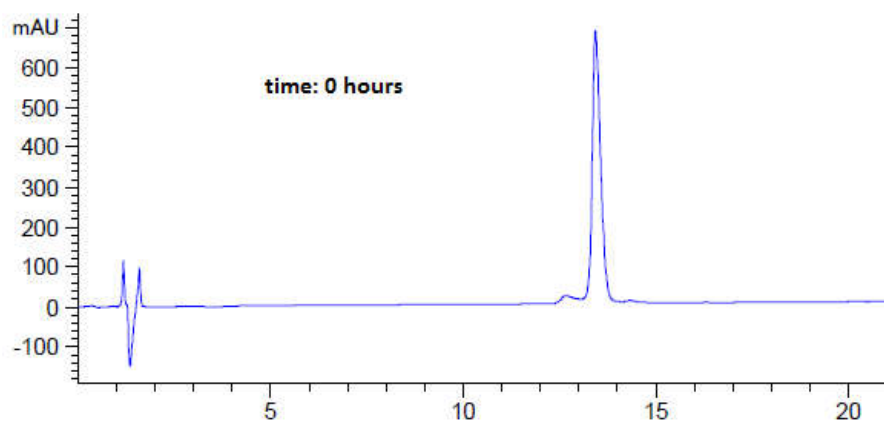
**Figure S8:** ESI-MS spectra of **Pep 4** [MOG<sub>4155</sub> (isoAsp<sup>53</sup>)]

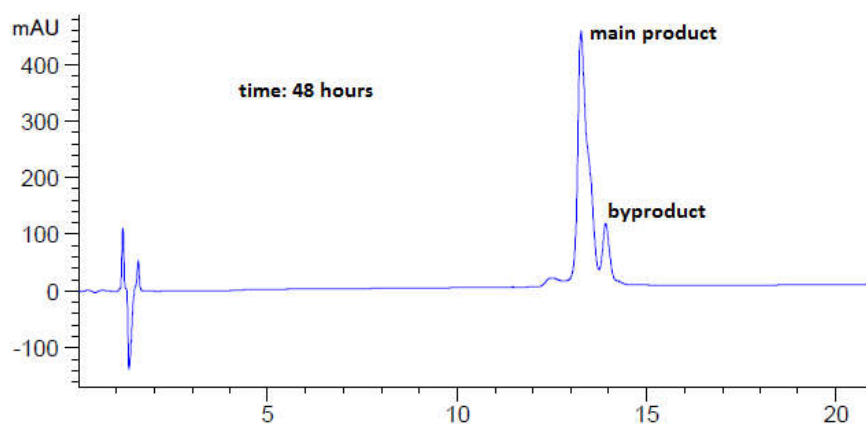
MW<sub>theoretical</sub> (main product): 1827.12 Da

**(A)**



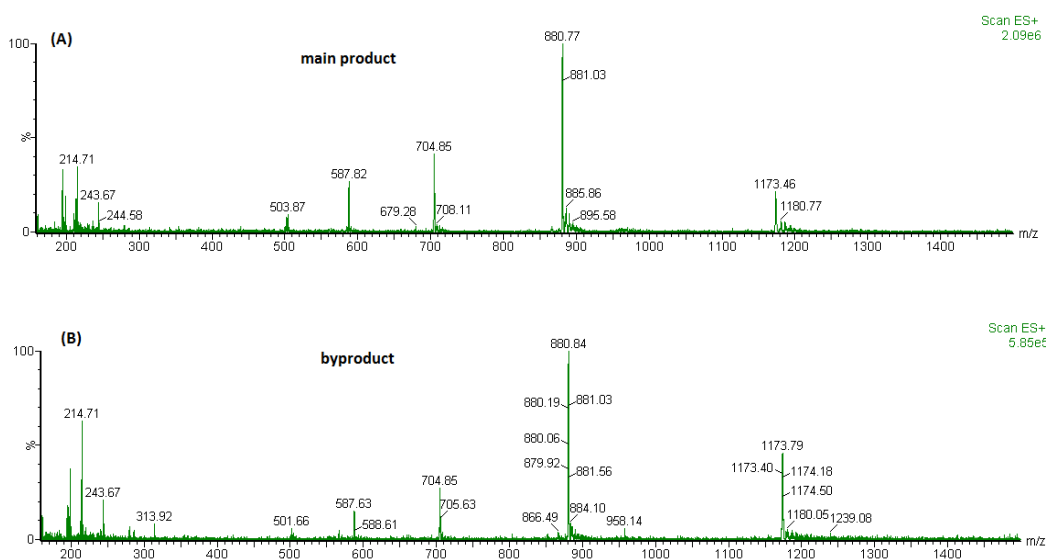
**(B)**





**Figure S9:** RP-HPLC chromatogram of **Pep 5** [(KG)<sub>5</sub>MOG<sub>3555</sub>] at 214.4 nm, at time zero and 48 h after dilution; **(A)** dissolved in water, **(B)** dissolved in bicarbonate buffer pH 9.0

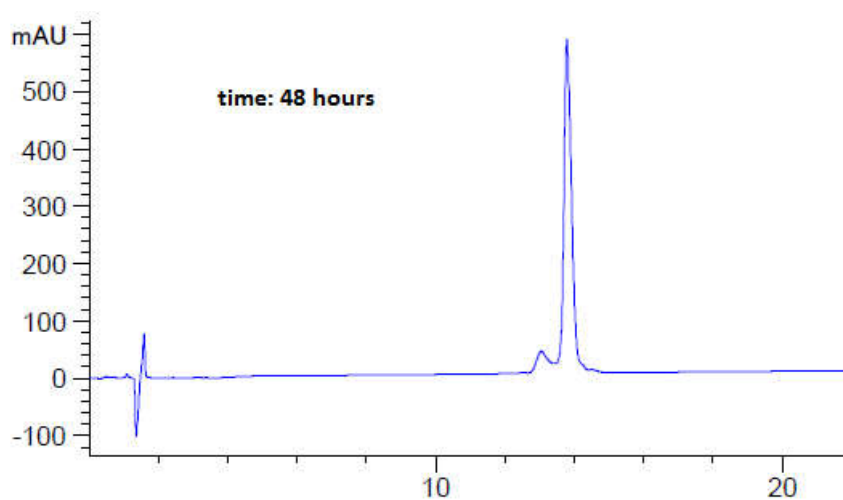
$t_R$  (main product): 13.3 min (88.6%) and  $t_R$  (byproduct): 13.9 min (11.4%)



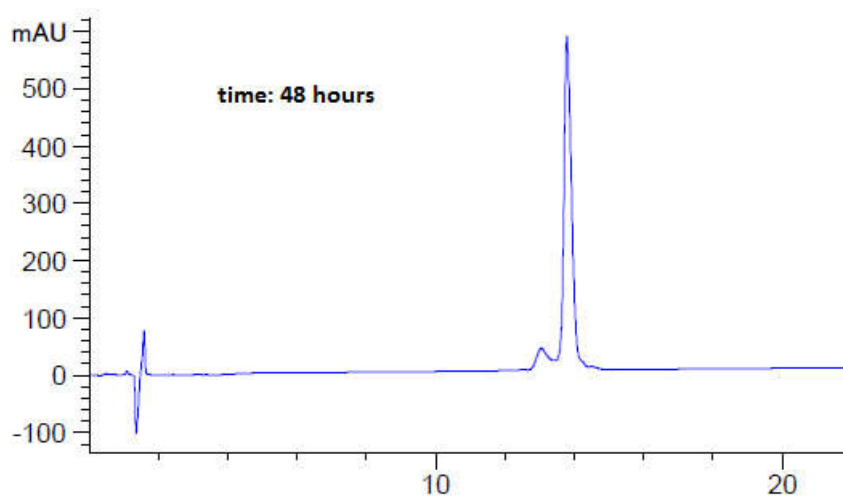
**Figure S10:** ESI-MS spectra of **Pep 5** [(KG)<sub>5</sub>MOG<sub>3555</sub>] dissolved in bicarbonate buffer, pH 9.0; of main product **(A)** and byproduct **(B)**

MW<sub>theoretical</sub> (main product): 3518.1 Da

(A)



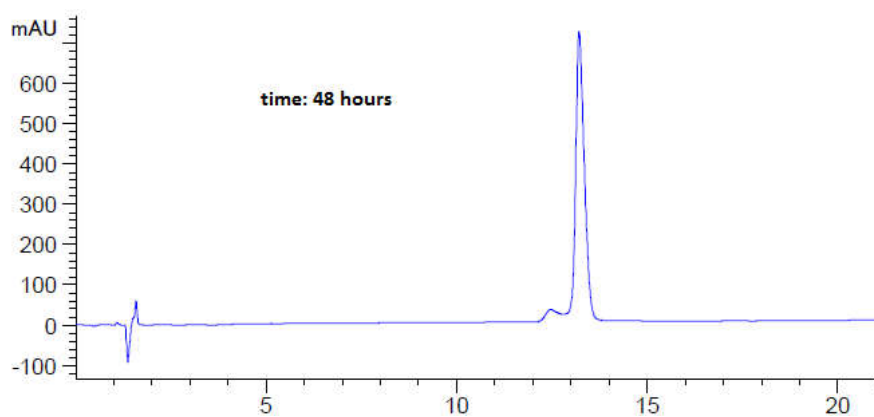
(B)



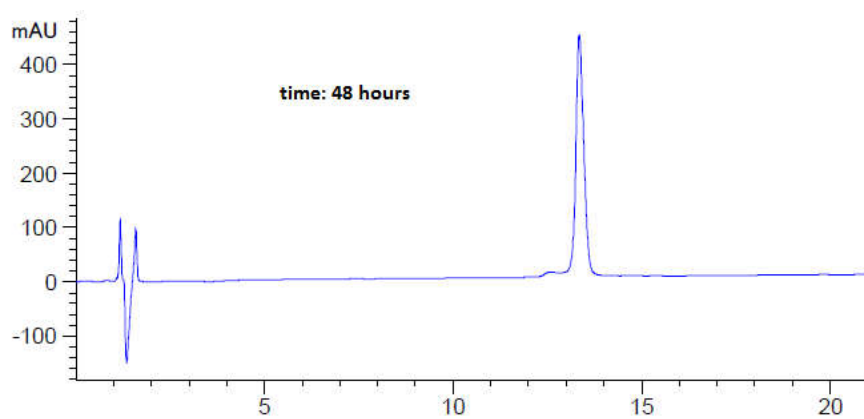
**Figure S11:** RP-HPLC chromatogram of **Pep 6** [(KG)<sub>5</sub>MOG<sub>3555</sub>(Asp<sup>53</sup>)] at 214.4 nm, at 48 h after dilution; **(A)** dissolved in water, **(B)** dissolved in bicarbonate buffer pH 9.0

$t_R$  (main product): 13.8 min

(A)

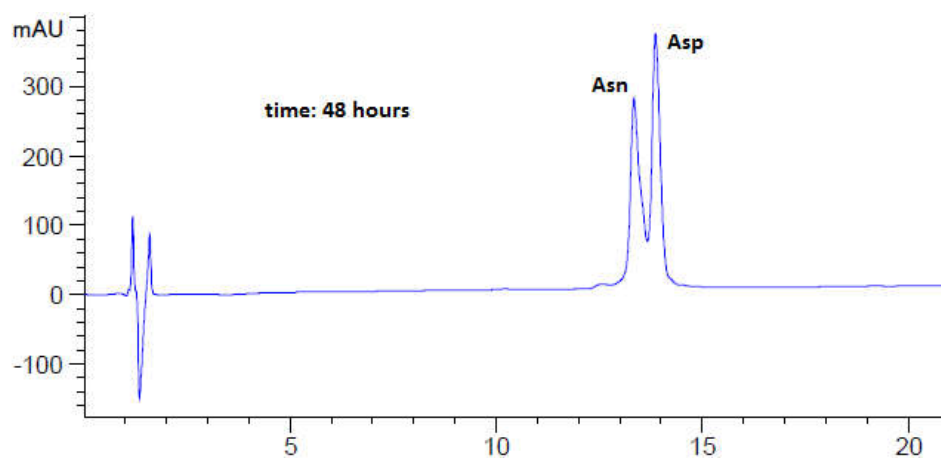


(B)



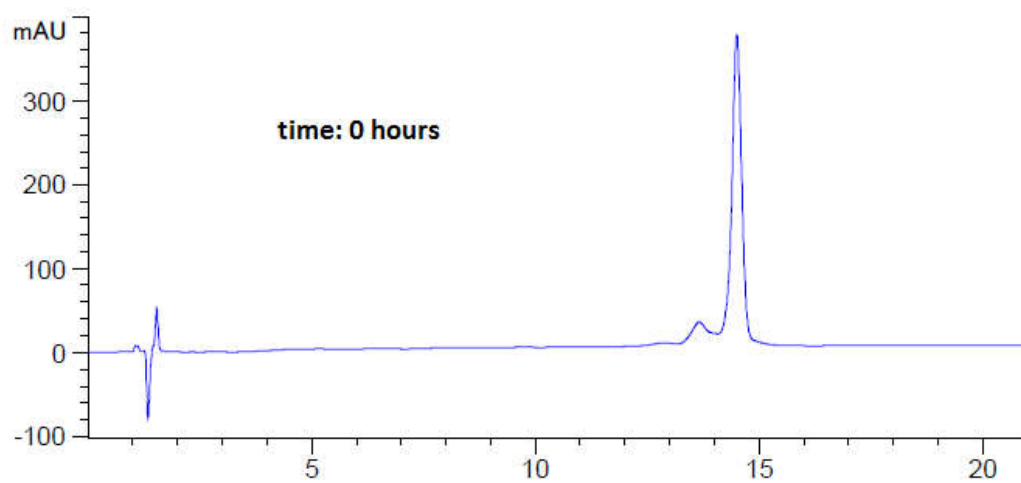
**Figure S12:** RP-HPLC chromatogram of **Pep 7** [(KG)<sub>5</sub>MOG<sub>3555</sub>(isoAsp<sup>53</sup>)] at 214.4nm, at 48 h after dilution; **(A)** dissolved in water, **(B)** dissolved in bicarbonate buffer pH 9.0

$t_R$  (main product): 13.3 min

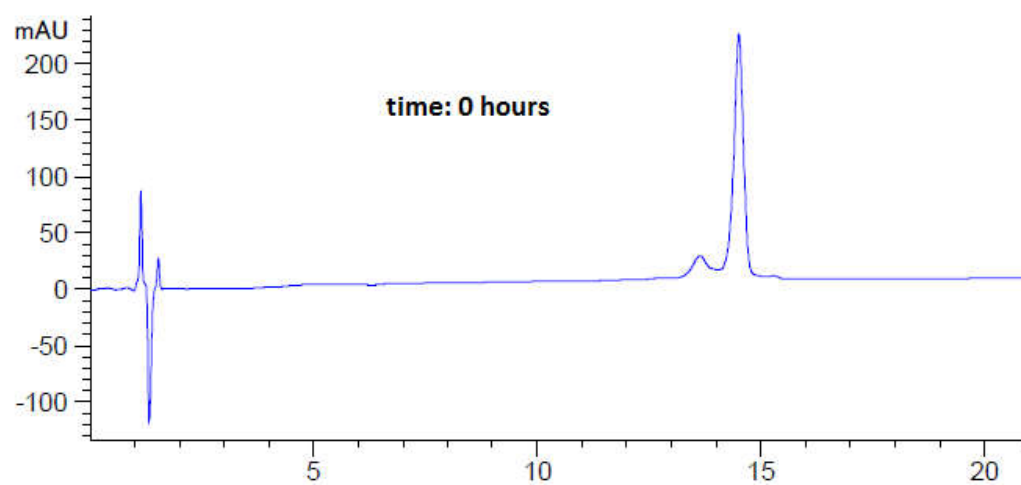


**Figure S13:** RP-HPLC chromatogram of co-injection [(KG)<sub>5</sub>MOG<sub>3555</sub>(Asn<sup>53</sup>)-  
(KG)<sub>5</sub>MOG<sub>3555</sub>(Asp<sup>53</sup>)] at 214.4 nm, at 48 h after dilution in bicarbonate buffer pH 9.0  
 $t_{R(\text{pep } 5)}$ : 13.3 min,  $t_{R(\text{pep } 6)}$ : 13.9 min

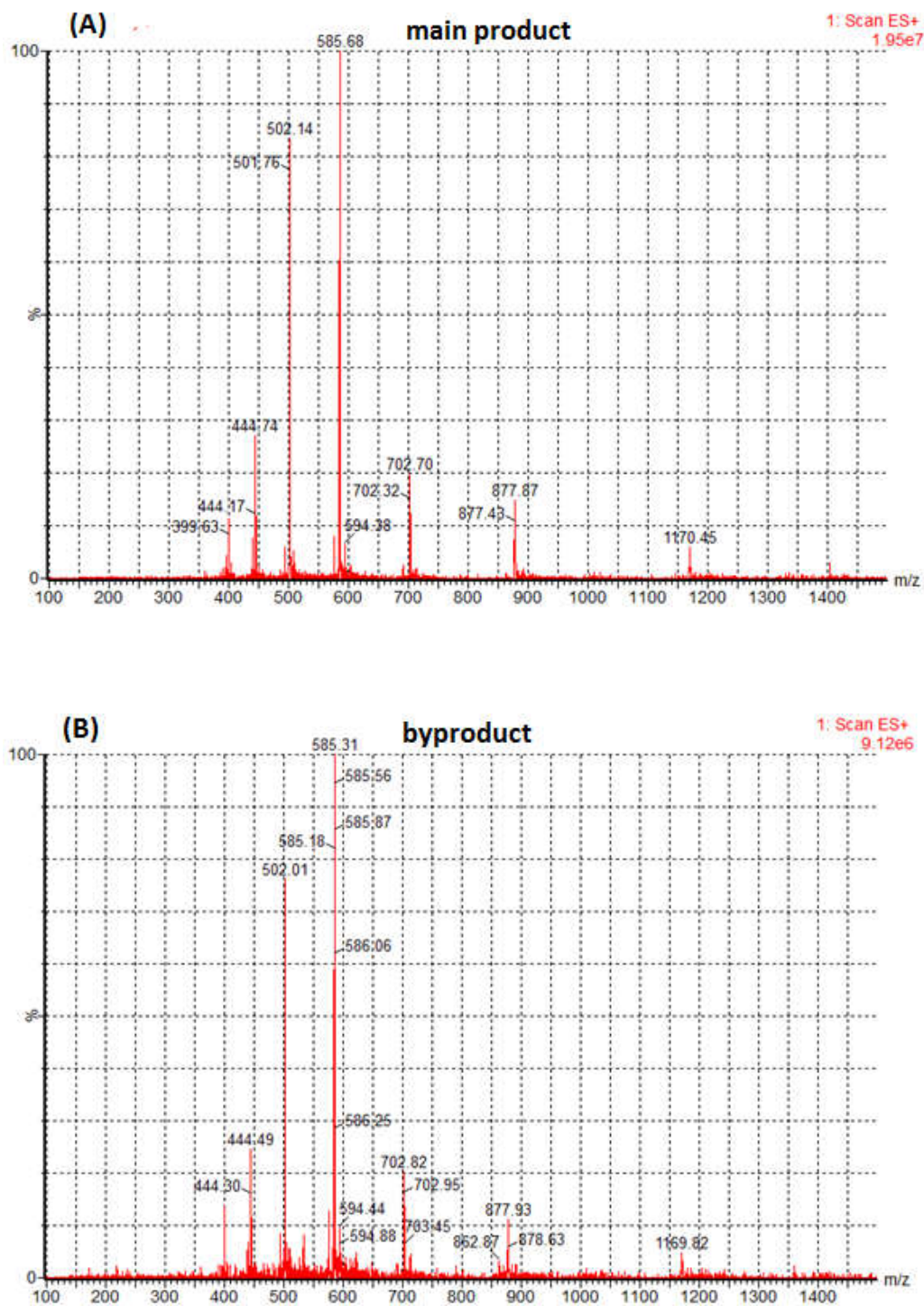
**(A)**



**(B)**



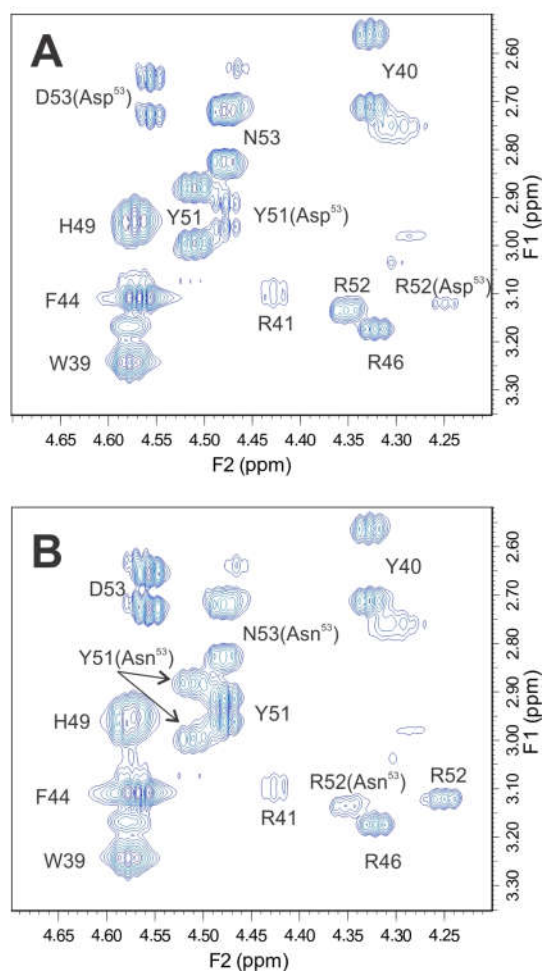
**Figure S14:** RP-HPLC chromatogram of Pep 8 [(KG)<sub>5</sub>MOG<sub>3555</sub>(Ser<sup>42</sup>)] at 214.4 nm, at time zero after dilution; **(A)** dissolved in water, **(B)** dissolved in bicarbonate buffer pH 9.0



**Figure S15:** ESI-MS spectra of **Pep 8** [(KG)<sub>5</sub>MOG<sub>355</sub>(Ser<sup>42</sup>)] dissolved in bicarbonate buffer, pH 9.0; of main product **(A)** and byproduct **(B)**

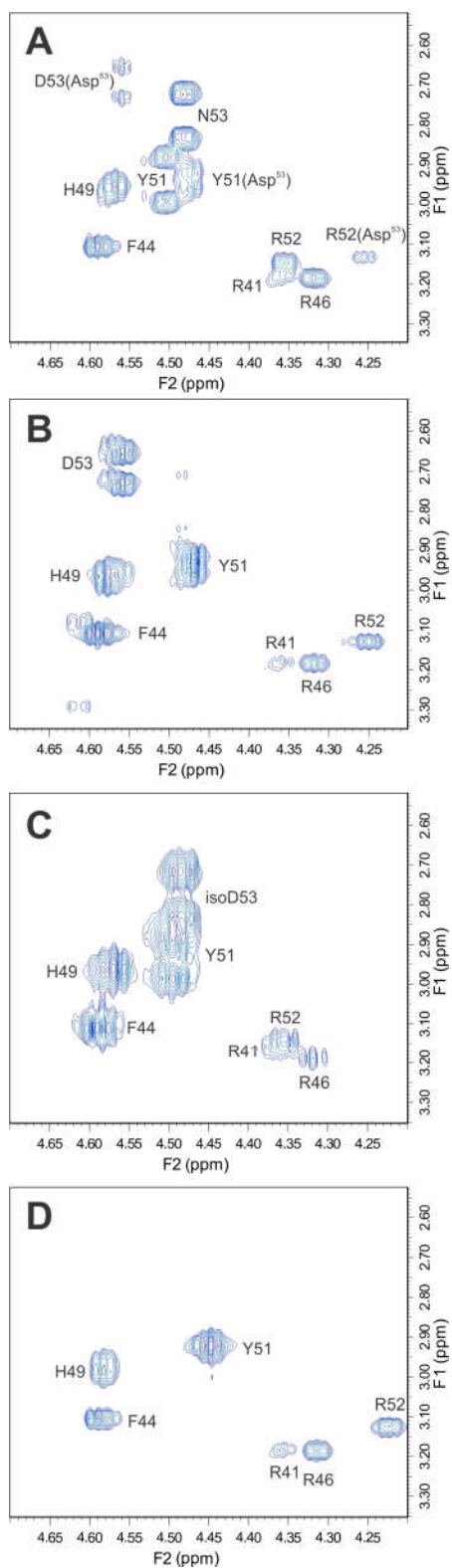
MW theoretical (main product): 3508.07 Da

MW theoretical (by product): 3509.06 Da



**Figure S16:** Expanded regions of TOCSY spectra of: **(A)** (KG)<sub>5</sub>MOG<sub>3555</sub> (**Pep 5**) and **(B)** isolated byproduct, after semipreparative-HPLC purification, (KG)<sub>5</sub>MOG<sub>3555</sub>(Asp<sup>53</sup>) (**Pep 6**) of (KG)<sub>5</sub>MOG<sub>3555</sub> (**Pep 5**) recorded in bicarbonate buffer, pH 9.0





**Figure S17:** Expanded regions of TOCSY spectra of: **(A)** MOG<sub>4155</sub> (**Pep 1**), **(B)** MOG<sub>4155</sub>(Asp<sup>53</sup>) (**Pep 3**), **(C)** MOG<sub>4155</sub>(isoAsp<sup>53</sup>) (**Pep 4**) and **(D)** MOG<sub>4155</sub>(Ala<sup>53</sup>) (**Pep 2**) recorded in bicarbonate buffer, pH 9.0

**Table S1:** Chemical shifts in ppm of  $\alpha$ ,  $\beta$  protons of residues 51 and 53 and  $\alpha$ ,  $\delta$  protons of residue 52 for peptides 1 to 7 in bicarbonate buffer (pH 9.0) referenced according to DSS- $d_6$ . These chemical shifts define the position of representative cross-peaks in expanded TOCSY spectra (Figures 2, S16 and S17) relevant for the explanation of degradation of peptides containing Asn at position 53

	<b>Pep 1</b> MOG <sub>41-55</sub>		<b>Pep 2</b> MOG <sub>41-55</sub> (Ala <sup>53</sup> )	<b>Pep 3</b> MOG <sub>41-55</sub> (Asp <sup>53</sup> )	<b>Pep 4</b> MOG <sub>41-55</sub> (isoAsp <sup>53</sup> )
	major	minor			
Tyr51 H <sup><math>\alpha</math></sup>	4.50	4.47	4.45	4.47	4.50
Tyr51 H <sup><math>\beta</math></sup>	2.88 2.30	2.92 2.95	2.92	2.91 2.95	2.86 2.98
Arg52 H <sup><math>\alpha</math></sup>	4.36	4.25	4.22	4.25	4.38
Arg52 H <sup><math>\delta</math></sup>	3.15	3.13	3.12	3.12	3.16
Asn53 H <sup><math>\alpha</math></sup>	4.48				
Asn53 H <sup><math>\beta</math></sup>	2.72 2.83				
Asp53 H <sup><math>\alpha</math></sup>		4.56		4.56	
Asp53 H <sup><math>\beta</math></sup>		2.66 2.73		2.65 2.73	
isoAsp53 H <sup><math>\alpha</math></sup>					4.48
isoAsp53 H <sup><math>\beta</math></sup>					2.72 2.84

	<b>Pep 5</b> (KG) <sub>5</sub> MOG <sub>35-55</sub>		<b>isolated byproduct of Pep 5</b>		<b>Pep 6</b> (KG) <sub>5</sub> MOG <sub>35-55</sub> (Asp <sup>53</sup> )	<b>Pep 7</b> (KG) <sub>5</sub> MOG <sub>35-55</sub> (isoAsp <sup>53</sup> )
	major	minor	major	minor		
Tyr51 H <sup><math>\alpha</math></sup>	4.51	4.48	4.48	4.51	4.48	4.52
Tyr51 H <sup><math>\beta</math></sup>	2.30, 2.88	2.91 2.96	2.91 2.96	2.30 2.88	2.91 2.96	2.88 3.00
Arg52 H <sup><math>\alpha</math></sup>	4.35	4.25	4.25	4.35	4.25	4.35
Arg52 H <sup><math>\delta</math></sup>	3.13	3.12	3.12	3.13	3.12	3.13
Asn53 H <sup><math>\alpha</math></sup>	4.48			4.48		
Asn53 H <sup><math>\beta</math></sup>	2.72 2.82			2.72 2.83		
Asp53 H <sup><math>\alpha</math></sup>		4.56	4.56		4.56	
Asp53 H <sup><math>\beta</math></sup>		2.65 2.73	2.65 2.73		2.66 2.73	
isoAsp53 H <sup><math>\alpha</math></sup>						4.48
isoAsp53 H <sup><math>\beta</math></sup>						2.72 2.82