Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

Importance of Ile71 in β-actin on histidine methyltransferase SETD3 catalysis

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1. Characterisation of β-actin peptides

Table S1 Overview of synthetized βA peptide sequences and their respective m/z values.

Names	Sequences	m/z Calculated	m/z Found
βA (66-88)	TLKYP**I**EHGIVTNWDDMEKIWHH	2861.42	2861.97
βA-Gly71	TLKYP**G**EHGIVTNWDDMEKIWHH	2805.35	2805.65
βA-Ala71	TLKYP**A**EHGIVTNWDDMEKIWHH	2819.37	2820.27
βA-Abu71	TLKYP**Abu**EHGIVTNWDDMEKIWHH	2833.38	2833.79
βA-Nva71	TLKYP**Nva**EHGIVTNWDDMEKIWHH	2847.40	2848.39
βA-Nle71	TLKYP**Nle**EHGIVTNWDDMEKIWHH	2861.42	2861.88
βA-Ahp71	TLKYP**Ahp**EHGIVTNWDDMEKIWHH	2875.43	2875.76
βA-Aoc71	TLKYP**Aoc**EHGIVTNWDDMEKIWHH	2889.45	2889.83
βA-Cha71	TLKYP**Cha**EHGIVTNWDDMEKIWHH	2901.45	2900.63
βA-Phe71	TLKYP**F**EHGIVTNWDDMEKIWHH	2895.40	2895.99
βA-Val71	TLKYP**V**EHGIVTNWDDMEKIWHH	2847.40	2847.59
βA-Leu71	TLKYP**L**EHGIVTNWDDMEKIWHH	2861.42	2861.73
βA-Dap71	TLKYP**Dap**EHGIVTNWDDMEKIWHH	2834.38	2834.66
βA-Dab71	TLKYP**Dab**EHGIVTNWDDMEKIWHH	2848.39	2848.66
βA-DIle71	TLKYP**D-Ile**EHGIVTNWDDMEKIWHH	2861.42	2861.71
βA-hSer71	TLKYP**hSer**EHGIVTNWDDMEKIWHH	2849.38	2849.50

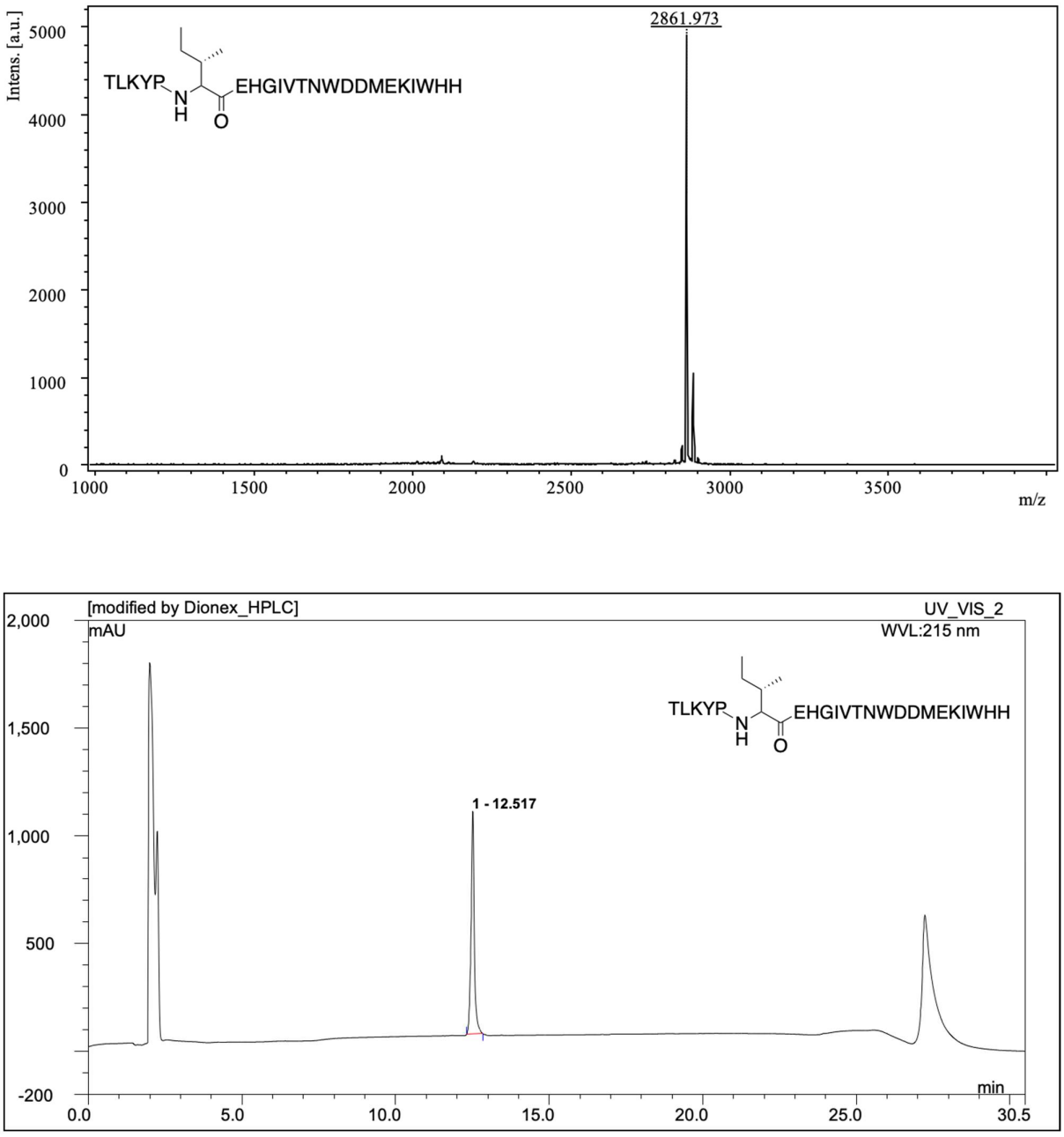

Fig. S1 MALDI-TOF MS data (top) calc. *m/z* 2861.4 and found *m/*z 2862.0. Analytical HPLC of the βA-Ile71 peptide after RP-HPLC purification (bottom). Peptide elutes at 12.52 min.

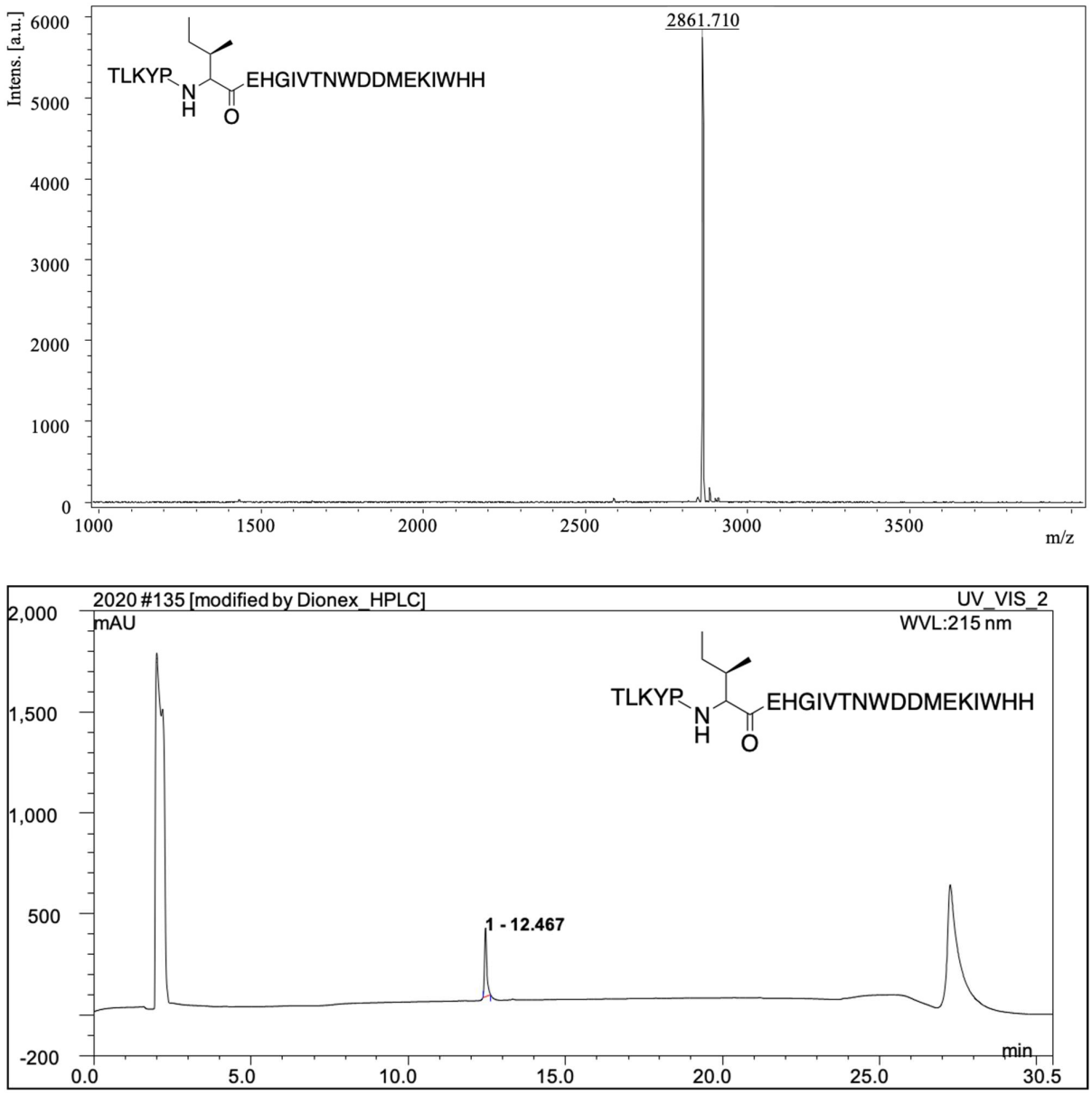

Fig. S2 MALDI-TOF MS data (top) calc. *m/z* 2861.4 and found *m/*z 2861.7. Analytical HPLC of the βA-DIle71 peptide after RP-HPLC purification (bottom). Peptide elutes at 12.47 min.

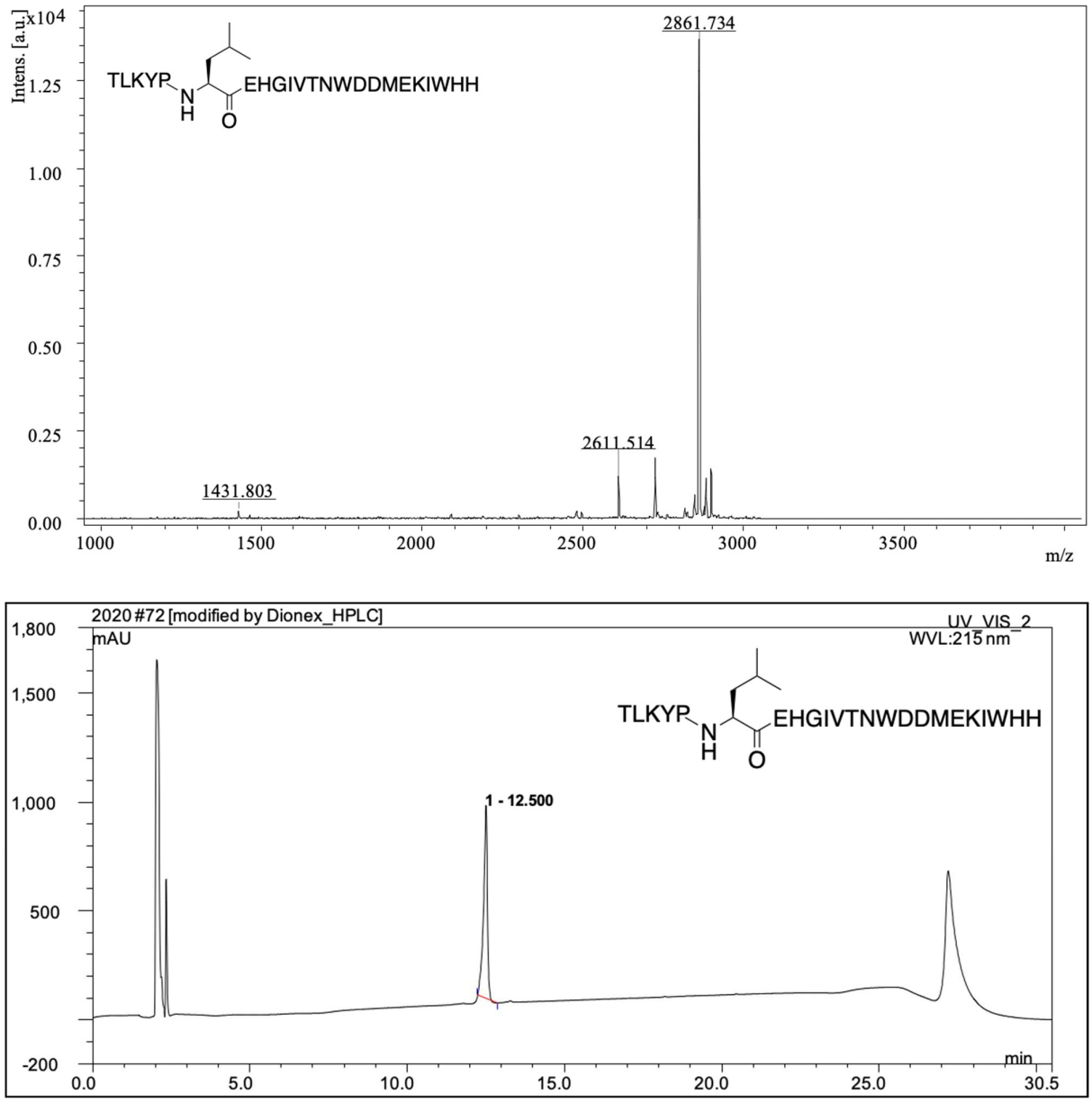

Fig. S3 MALDI-TOF MS data (top) calc. *m/z* 2861.4 and found *m/z* 2861.7. Analytical HPLC of the βA-Leu71 peptide after RP-HPLC purification (bottom). Peptide elutes at 12.5 min.

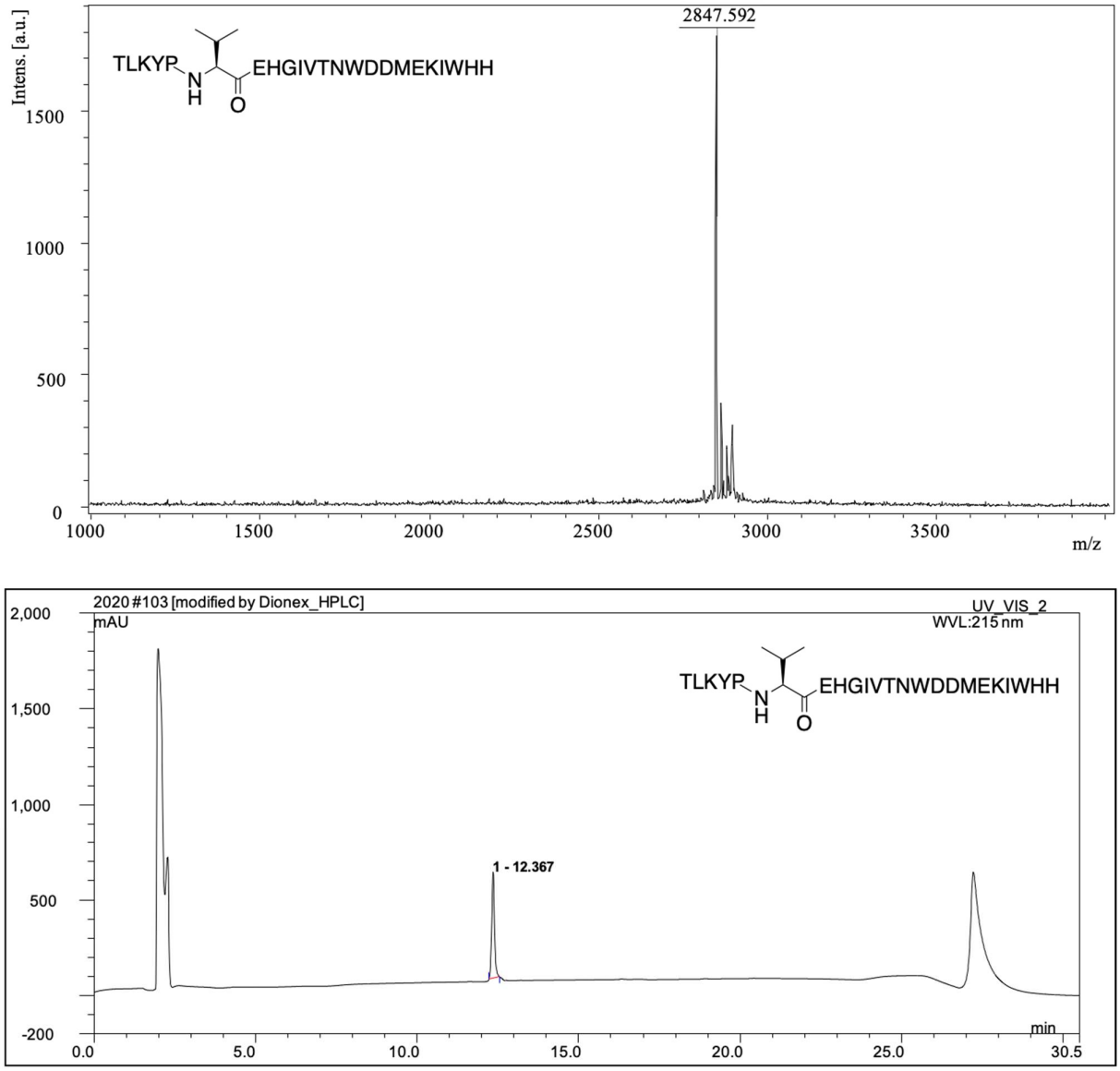

Fig. S4 MALDI-TOF MS data (top) calc. *m/z* 2847.4 and found *m/z* 2847.6. Analytical HPLC of the βA-Val71 peptide after RP-HPLC purification (bottom). Peptide elutes at 12.37 min.

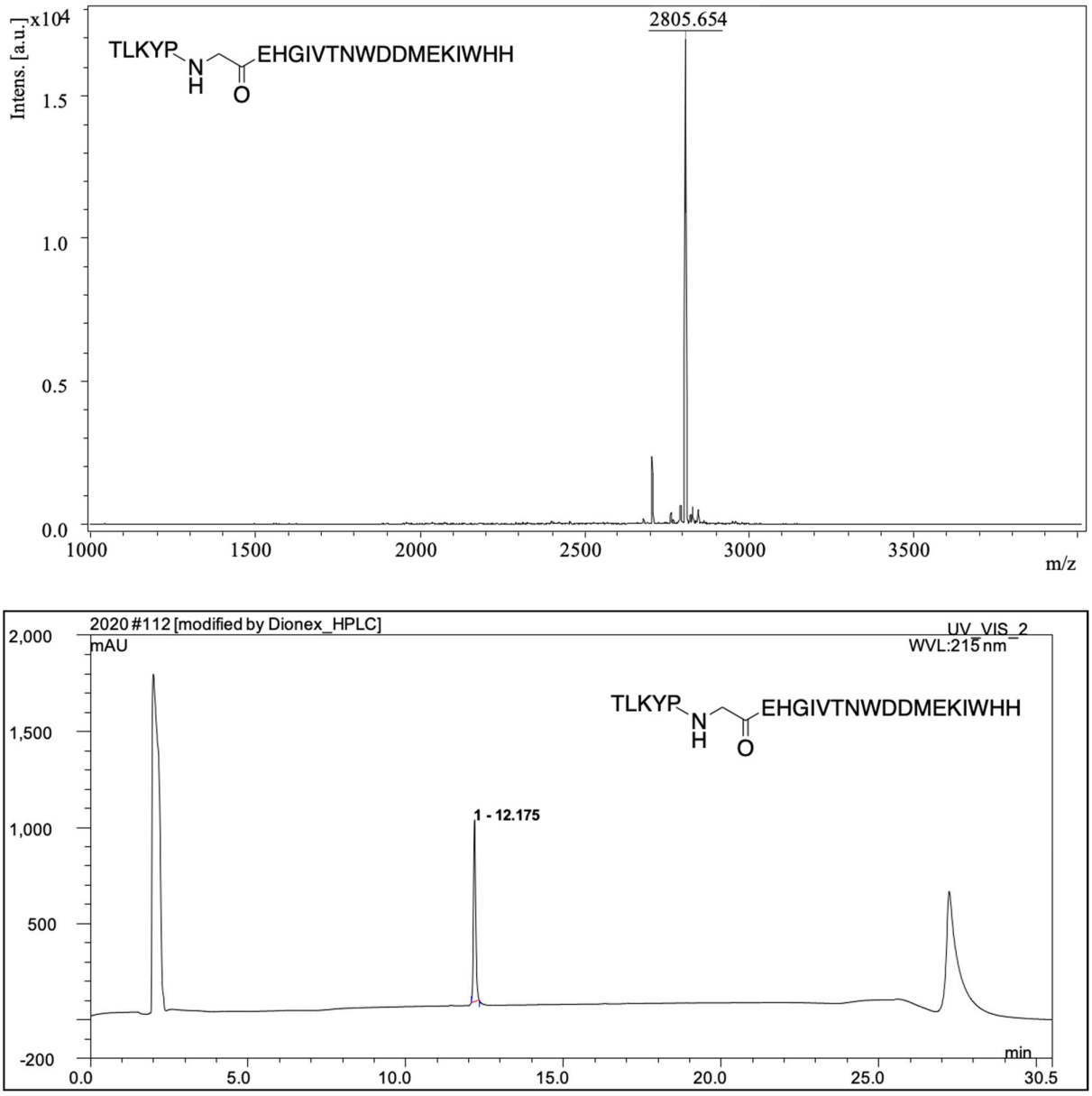

Fig. S5 MALDI-TOF MS data (top) calc. *m/z* 2805.4 and found *m/z* 2805.7. Analytical HPLC of the βA-Gly71 peptide after RP-HPLC purification (bottom). Peptide elutes at 12.18 min.

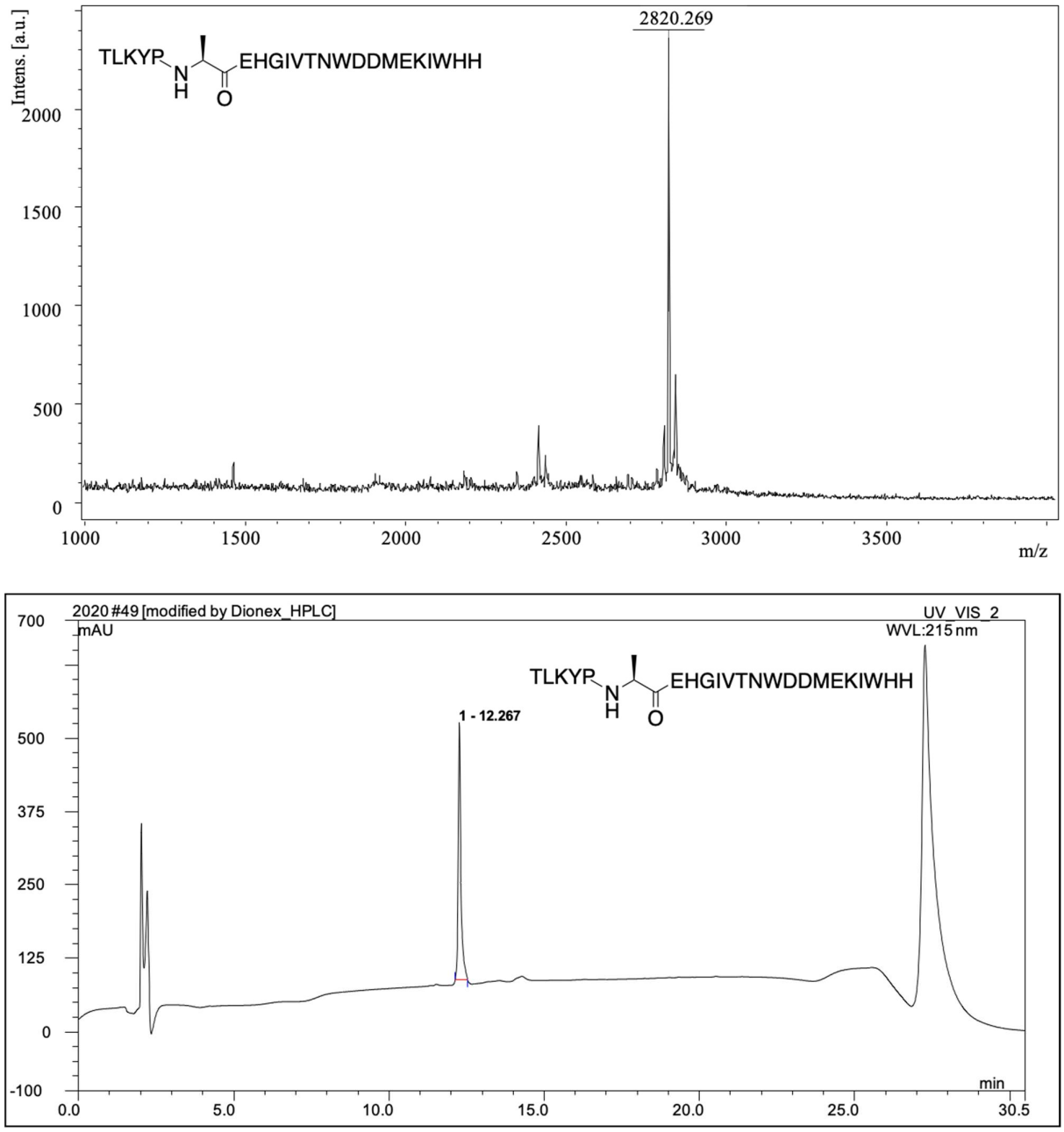

Fig. S6 MALDI-TOF MS data (top) calc. *m/z* 2820.4 and found *m/z* 2820.3. Analytical HPLC of the βA-Ala71 peptide after RP-HPLC purification (bottom). Peptide elutes at 12.27 min.

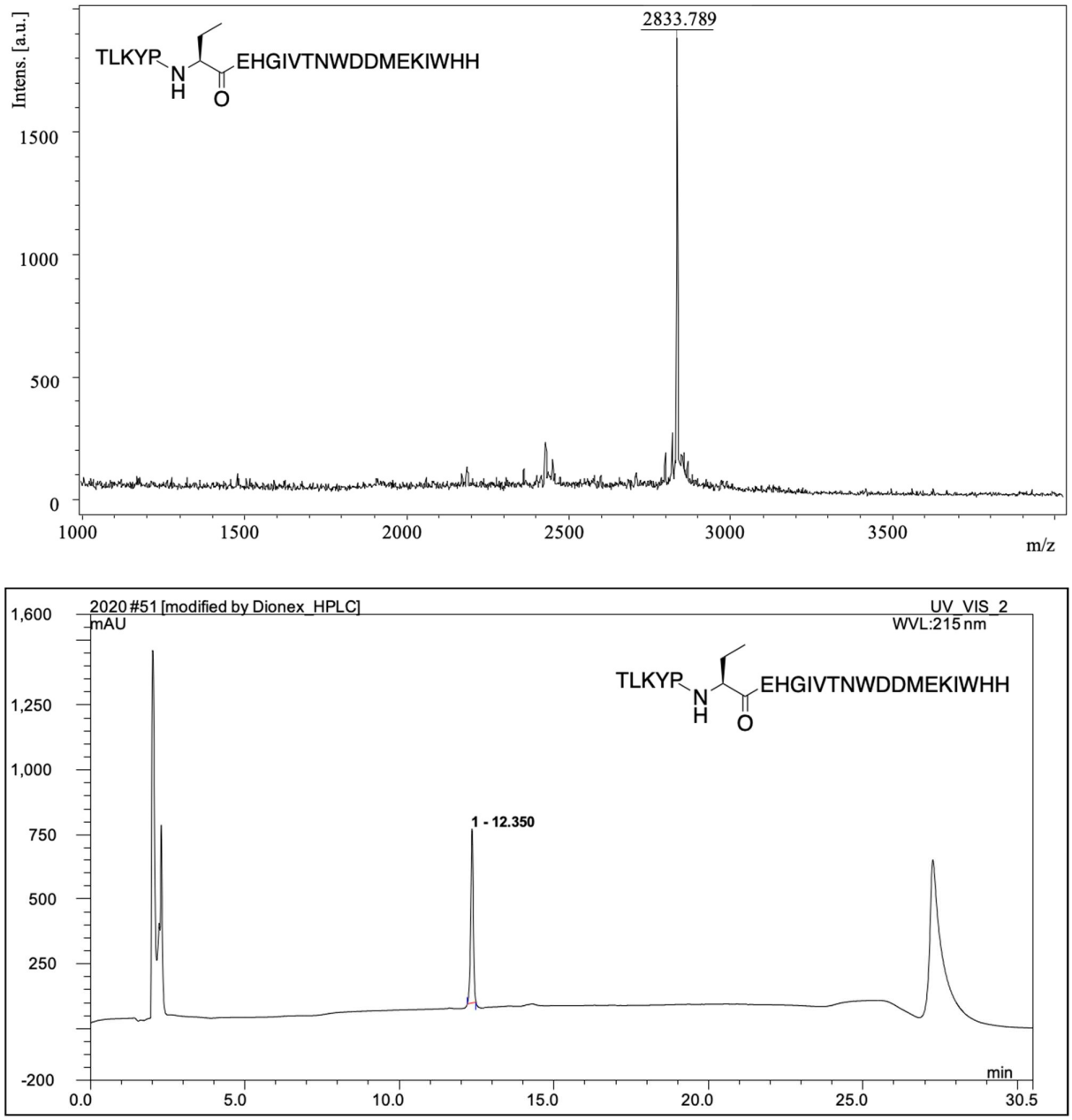

Fig. S7 MALDI-TOF MS data (top) calc. *m/z* 2833.4 and found *m/*z 2833.8. Analytical HPLC of the βA-Abu71 peptide after RP-HPLC purification (bottom). Peptide elutes at 12.35 min.

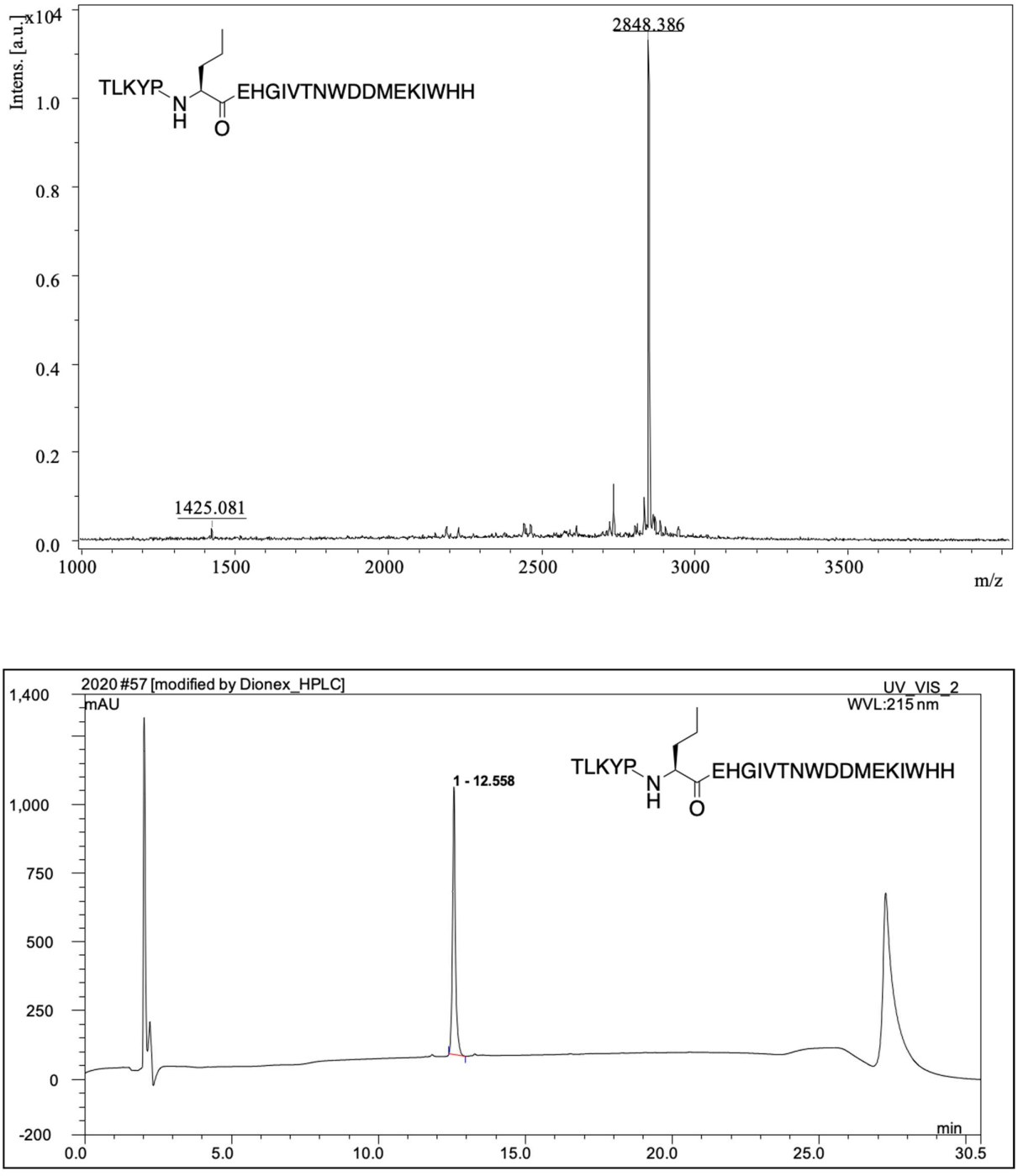

Fig. S8 MALDI-TOF MS data (top) calc. *m/z* 2847.4 found *m/z* 2848.4. Analytical HPLC of the βA-Nva71 peptide after RP-HPLC purification (bottom). Peptide elutes at 12.56 min.

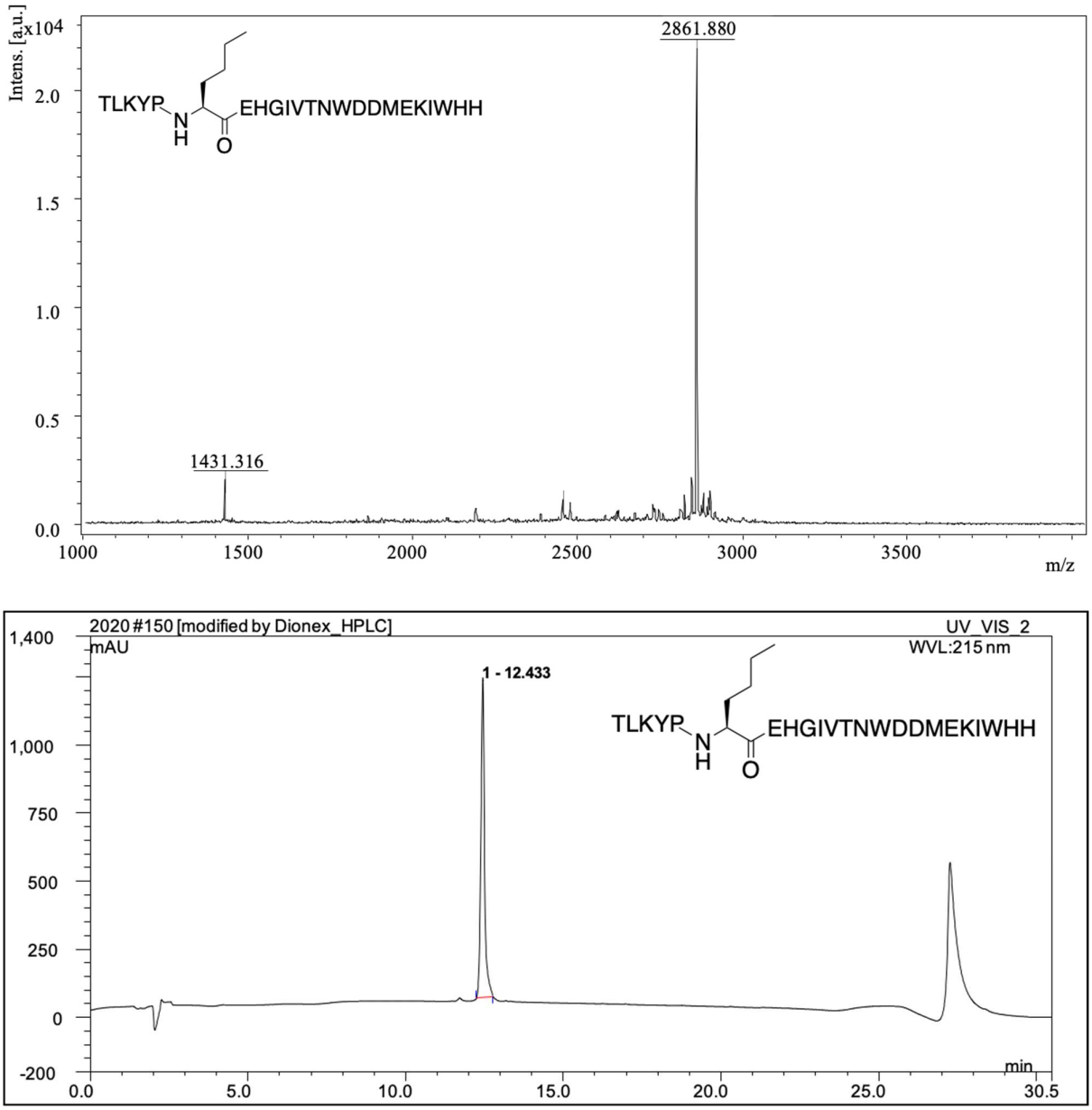

Fig. S9 MALDI-TOF MS data (top) calc. *m/z* 2861.4 and found *m/*z 2861.9. Analytical HPLC of the βA-Nle71 peptide after RP-HPLC purification (bottom). Peptide elutes at 12.43 min.

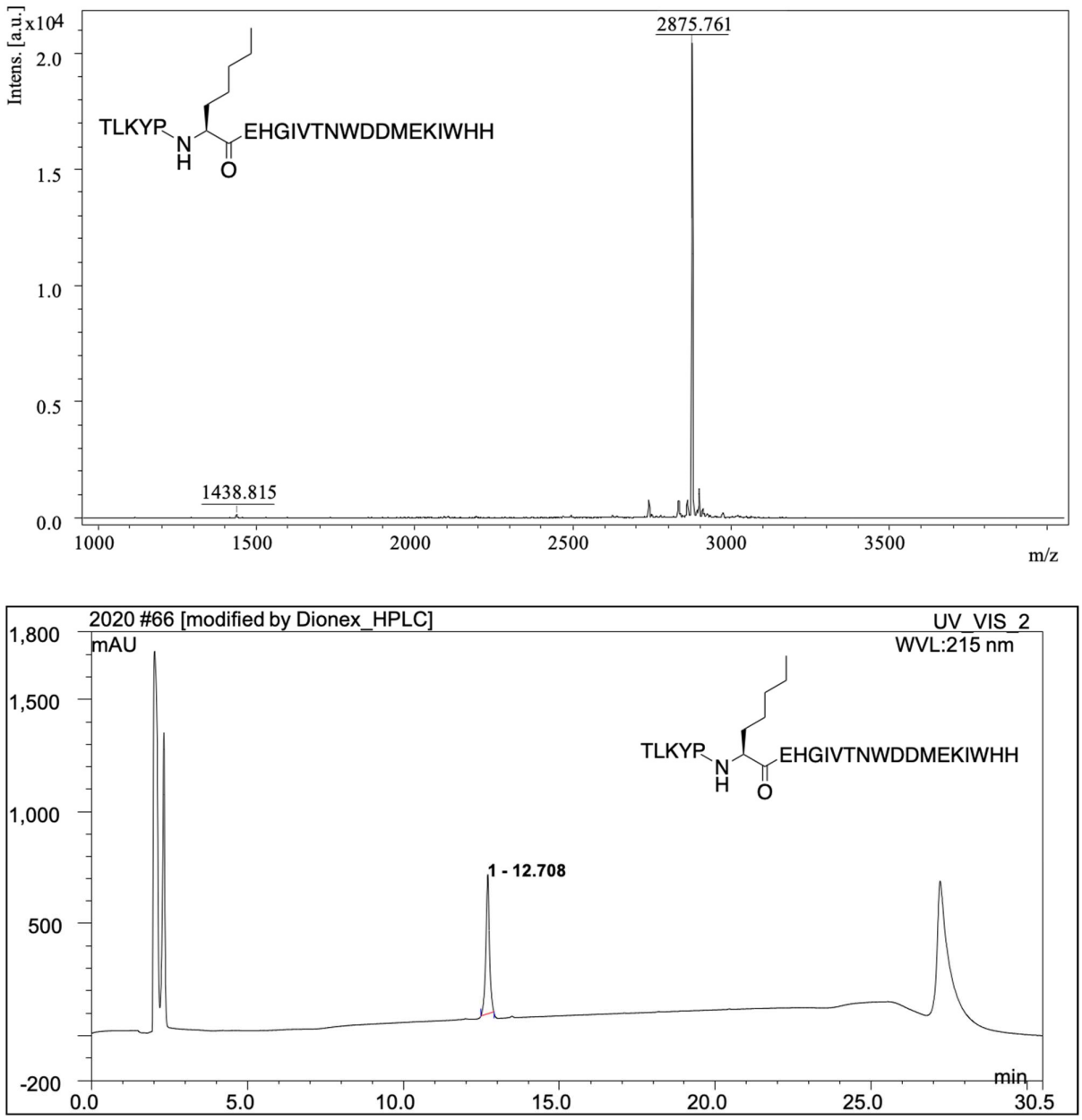

Fig. S10 MALDI-TOF MS data (top) calc. *m/z* 2875.4 and found *m/*z 2875.8. Analytical HPLC of the βA-Ahp71 peptide after RP-HPLC purification (bottom). Peptide elutes at 12.71 min.

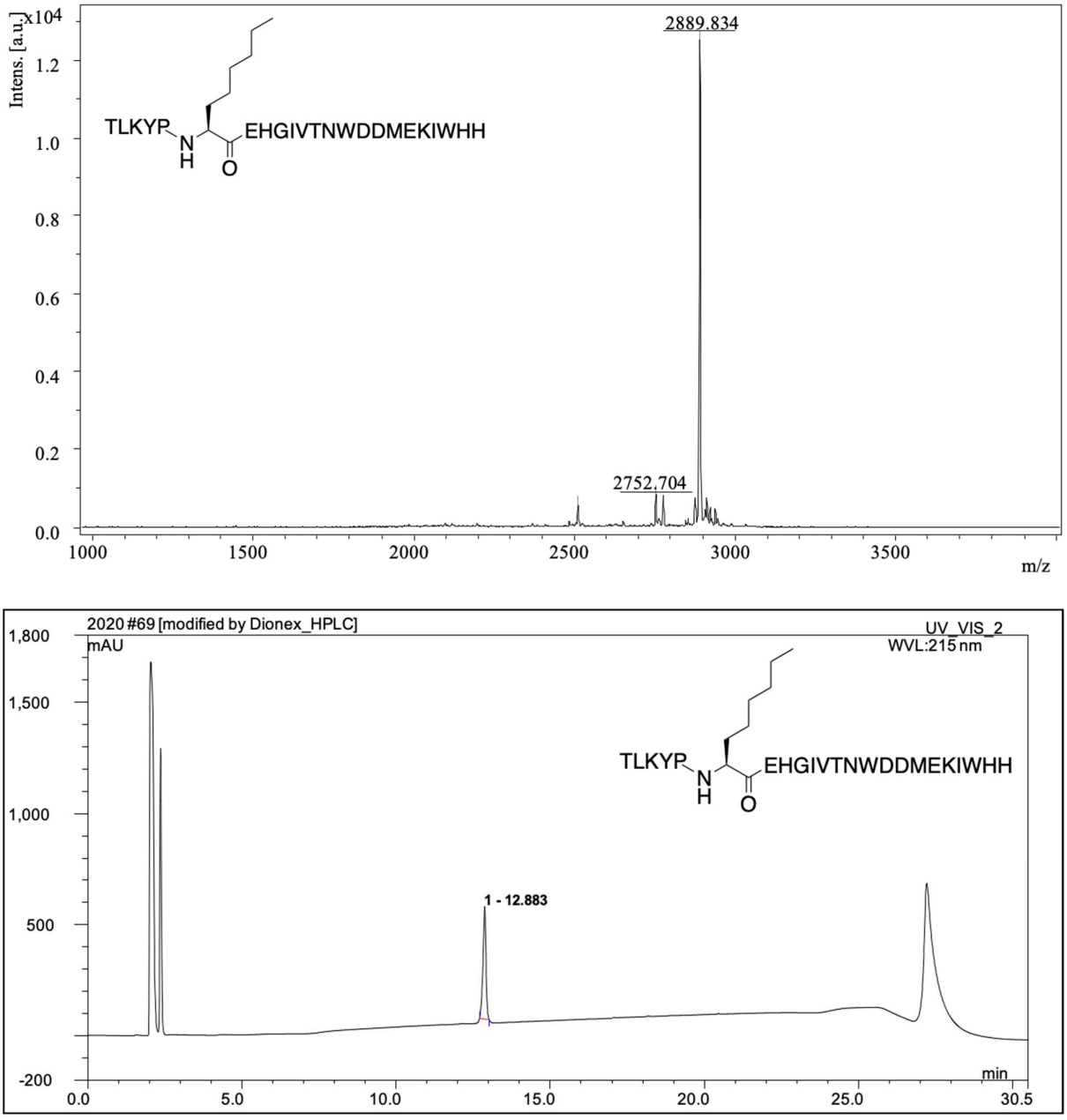

Fig. S11 MALDI-TOF MS data (top) calc. *m/z* 2889.5 and found *m/*z 2889.8. Analytical HPLC of the βA-Aoc71 peptide after RP-HPLC purification (bottom). Peptide elutes at 12.88 min.

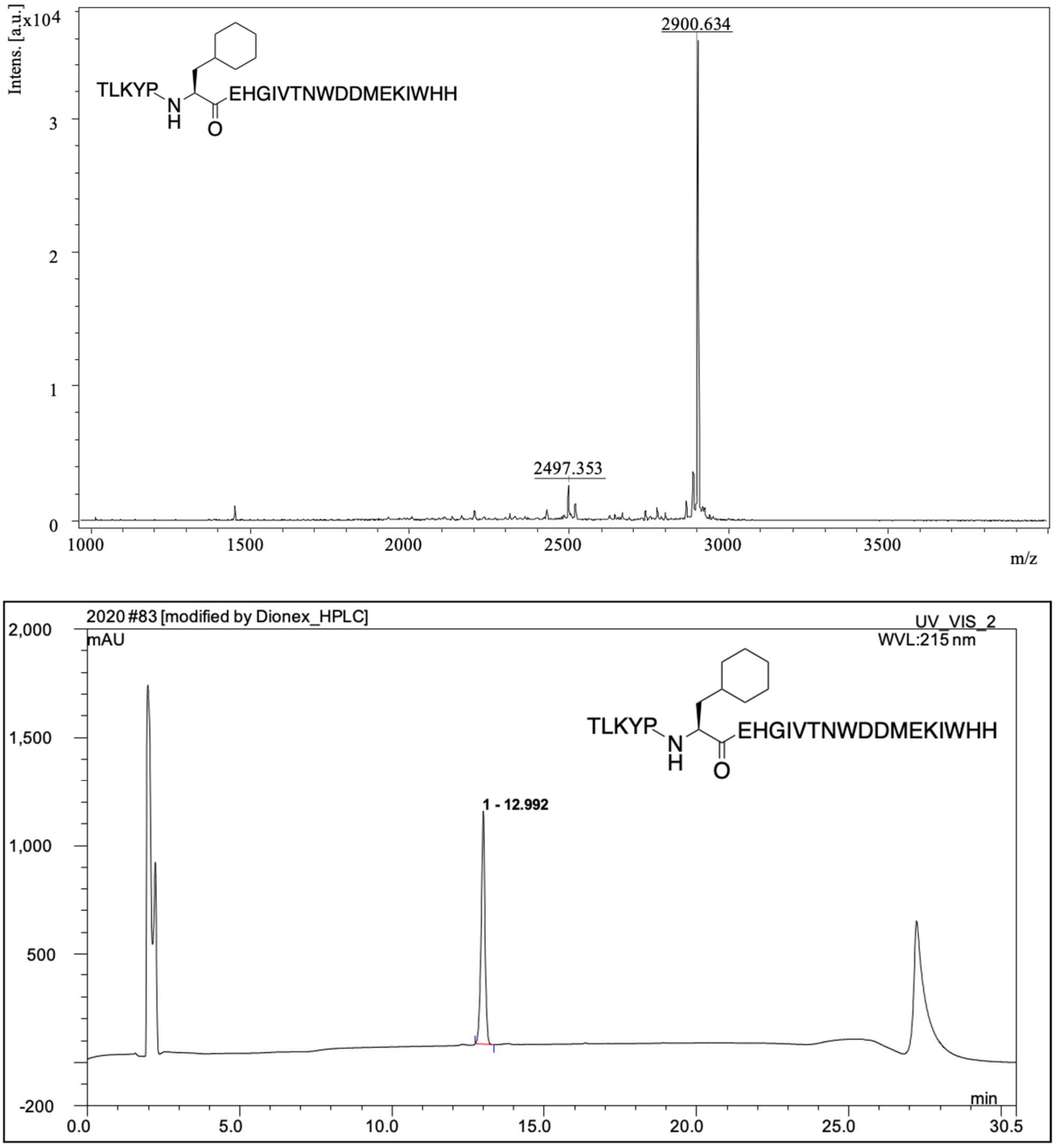

Fig. S12 MALDI-TOF MS data (top) calc. *m/z* 2901.5 and found *m/*z 2901.6. Analytical HPLC of the βA-Cha71 peptide after RP-HPLC purification (bottom). Peptide elutes at 12.99 min.

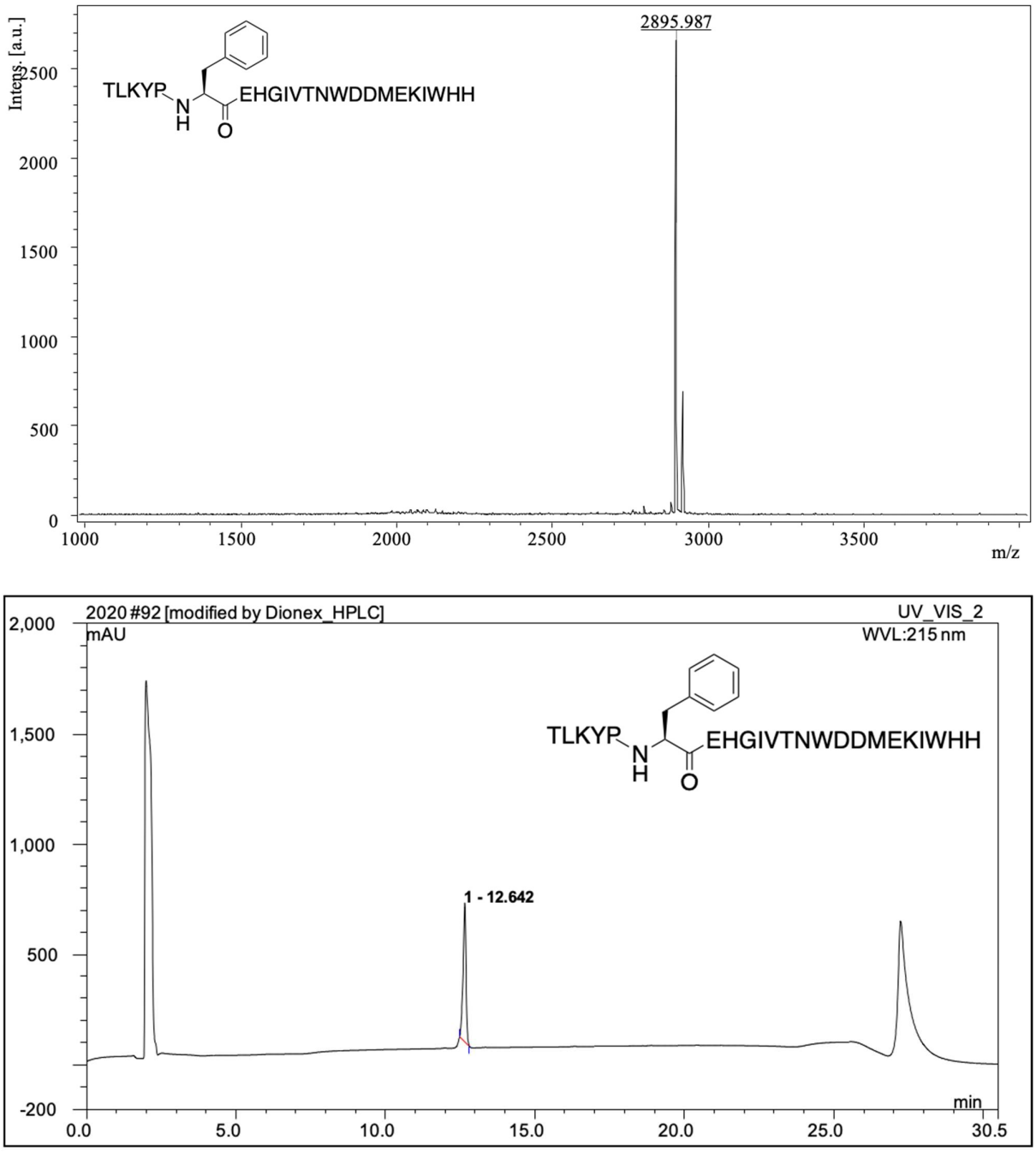

Fig. S13 MALDI-TOF MS data (top) calc. *m/z* 2895.4 and found *m/*z 2896.0. Analytical HPLC of the βA-Phe71 peptide after RP-HPLC purification (bottom). Peptide elutes at 12.64 min.

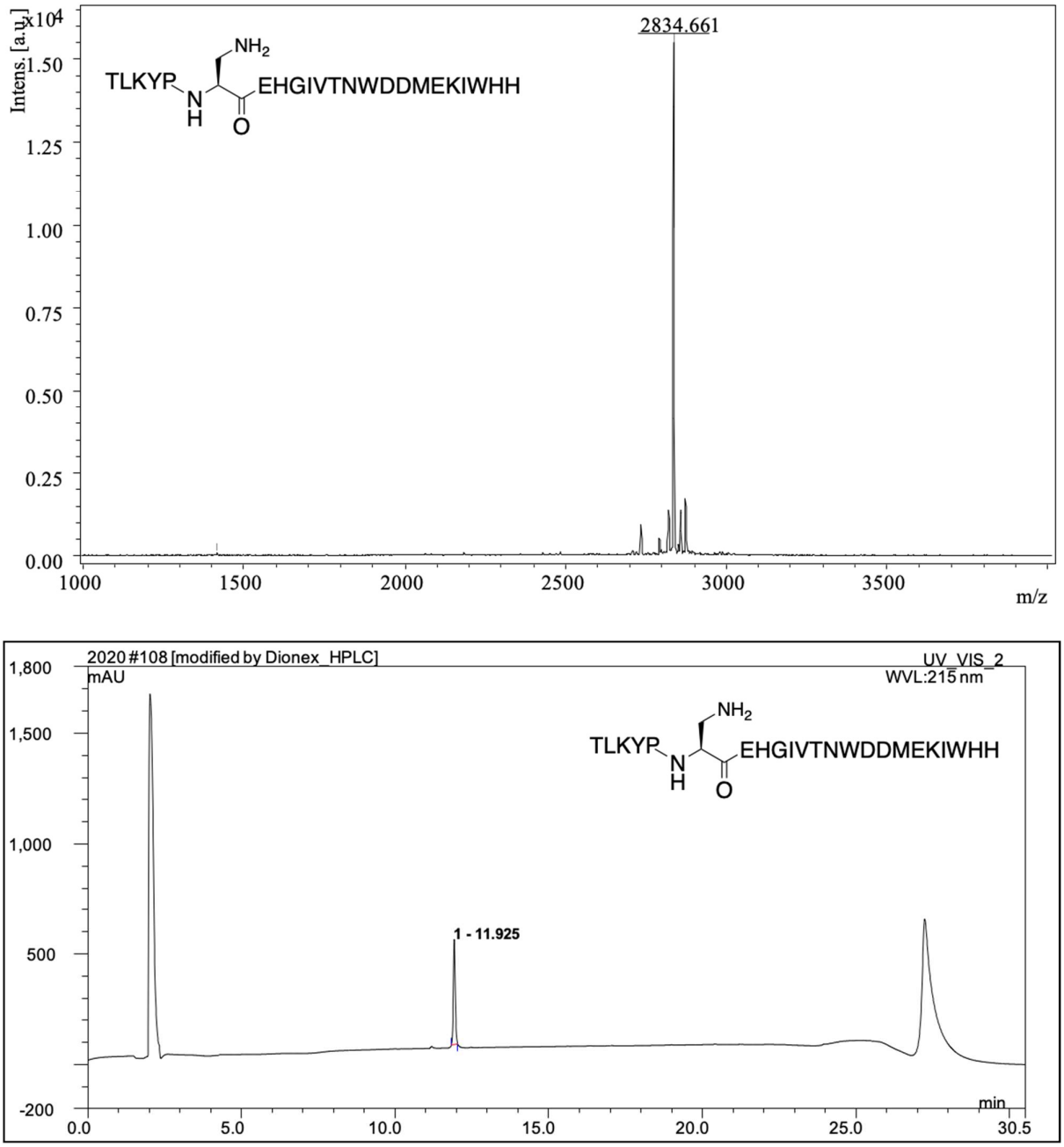

Fig. S14 MALDI-TOF MS data (top) calc. *m/z* 2834.4 and found *m/*z 2834.7. Analytical HPLC of the βA-Dap71 peptide after RP-HPLC purification (bottom). Peptide elutes at 11.93 min.

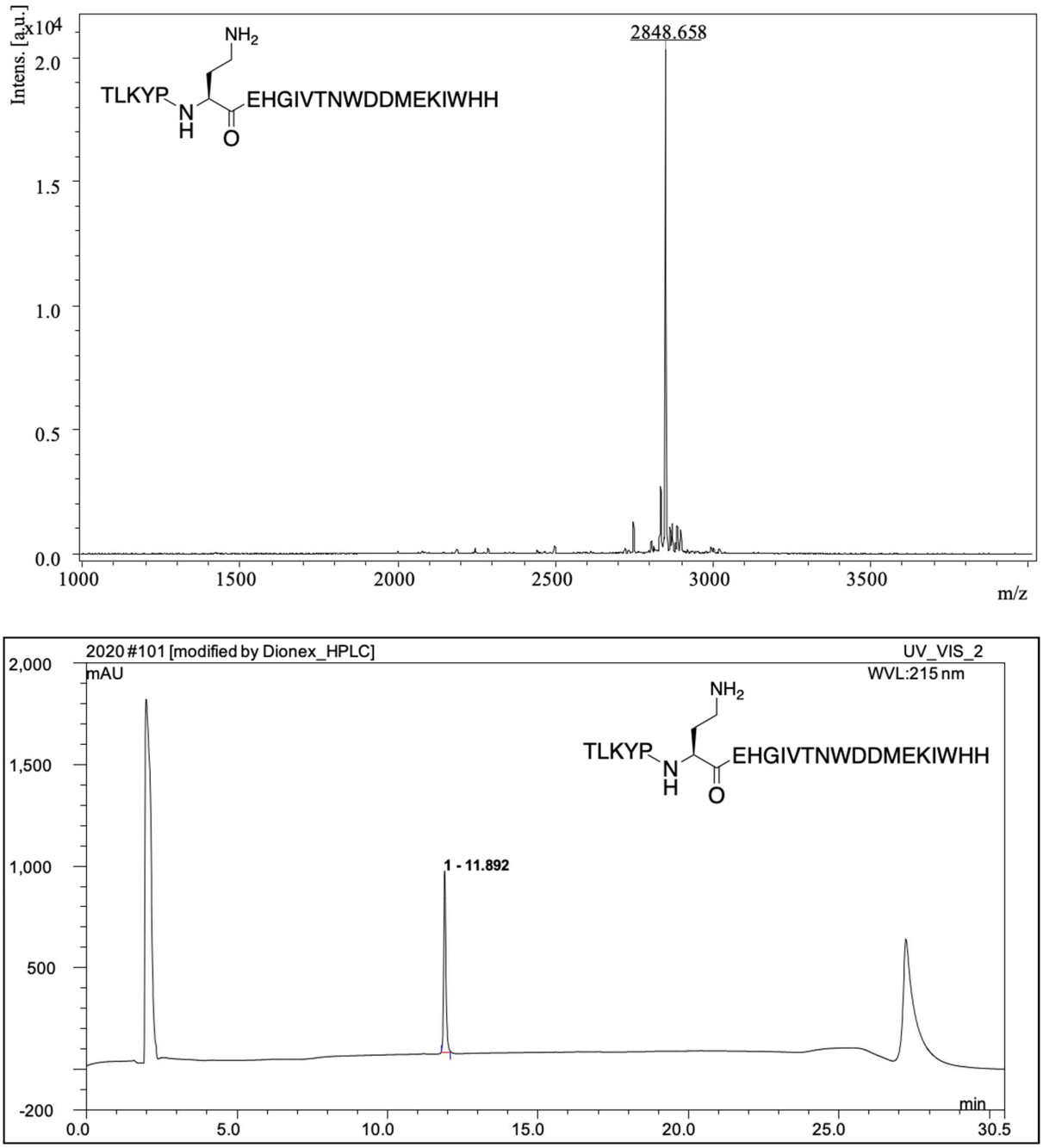

Fig. S15 MALDI-TOF MS data (top) calc. *m/z* 2848.4 and found *m/*z 2848.7. Analytical HPLC of the βA-Dab71 peptide after RP-HPLC purification (bottom). Peptide elutes at 11.89 min.

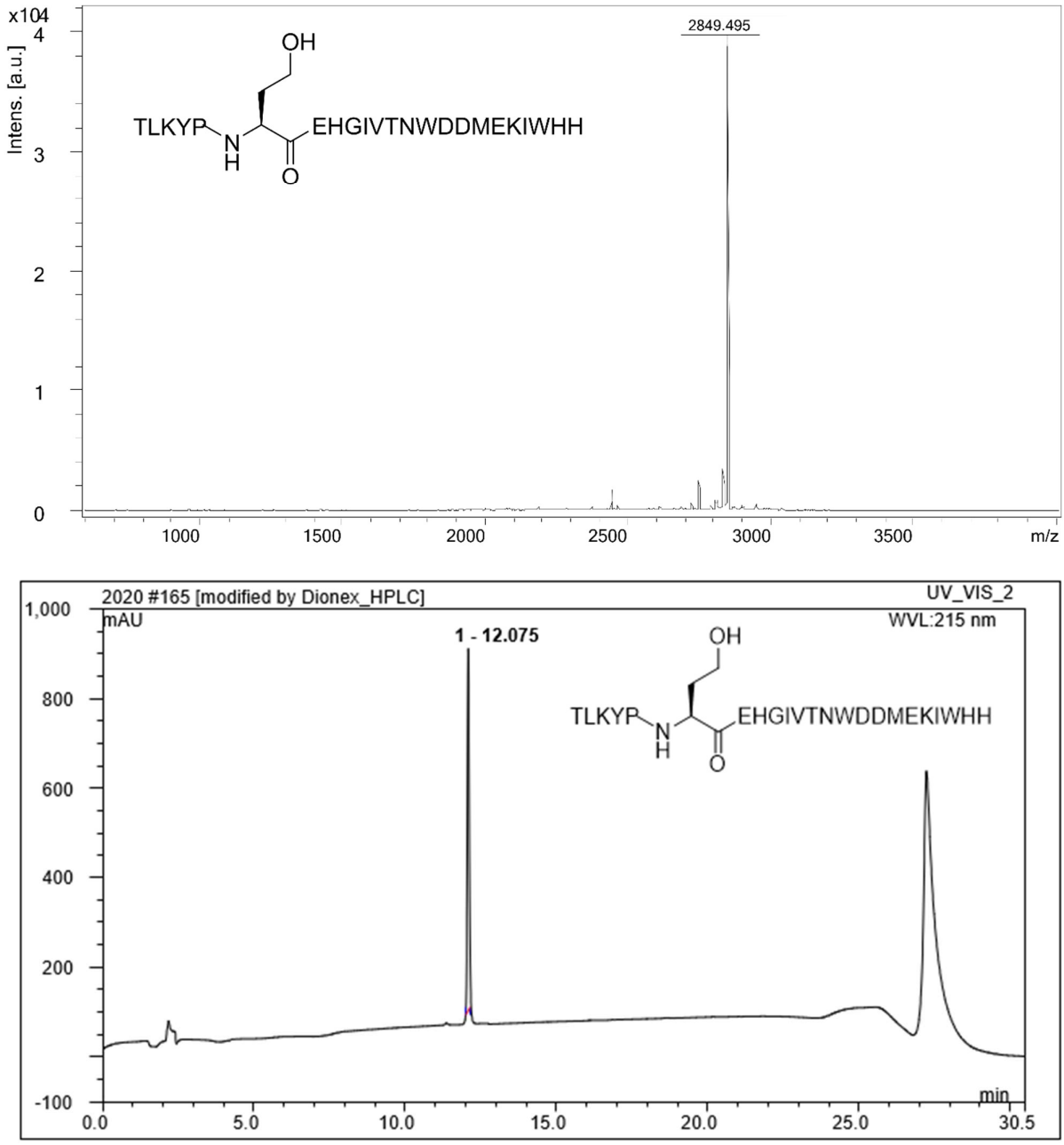

Fig. S16 MALDI-TOF MS data (top) calc. *m/z* 2849.4 and found *m/*z 2849.5. Analytical HPLC of the βA-hSer71 peptide after RP-HPLC purification (bottom). Peptide elutes at 12.08 min.

2. MALDI-TOF MS supporting figures

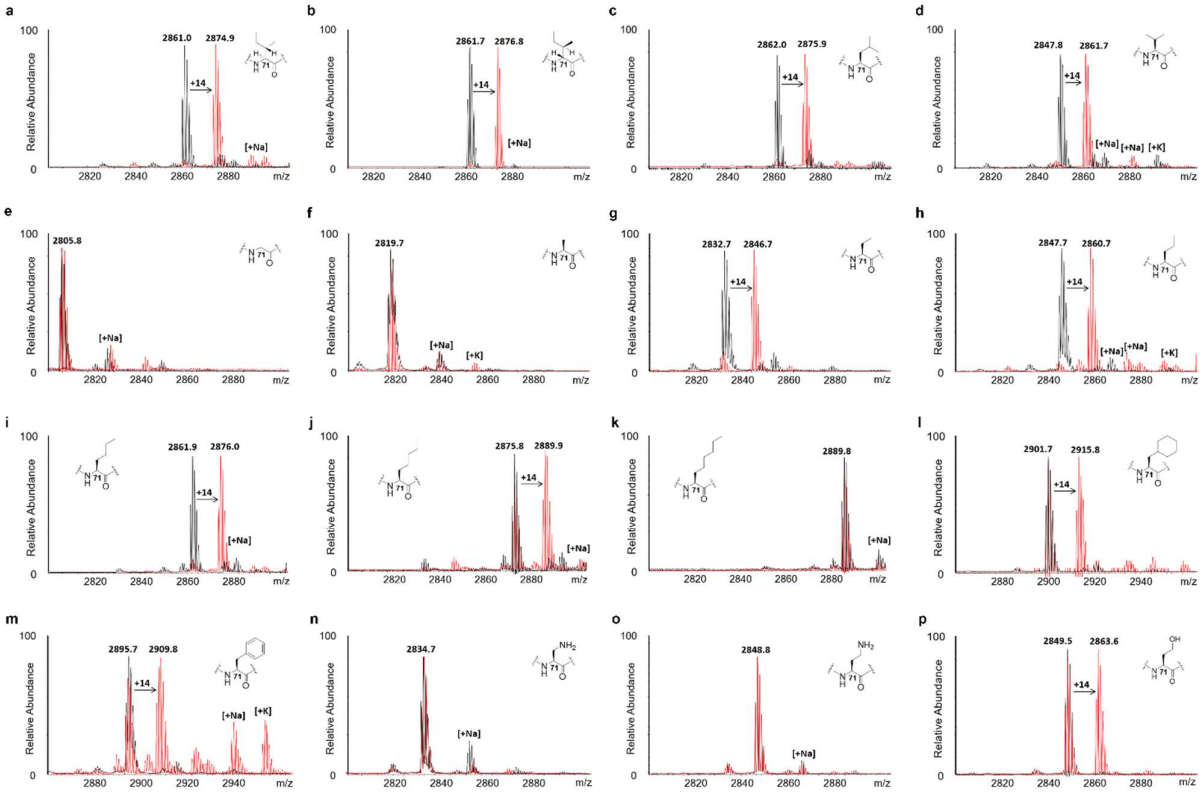

Fig. S17 MALDI-TOF MS data showing SETD3-catalysed (1 µM) methylation of βA peptides (10 µM) in the presence of SAM (100 µM) after 1 h at 37 ℃, pH 9. Control reactions in the absence of SETD3 are shown in black, whereas SETD3-catalysed reactions are shown in red. **a**) βA-Ile71, **b**) βA-DIle71, **c**) βA-Leu71, **d**) βA-Val71, **e**) βA-Gly71, **f**) βA-Ala71 **g**) βA-Abu71, **h**) βA-Nva71, **i**) βA-Nle71, **j**) βA-Ahp71, **k**) βA-Aoc71, **l**) βA-Cha71, **m**) βA-Phe71, **n**) βA-Dap71, **o**) βA-Dab71, **p**) βA-hSer71.

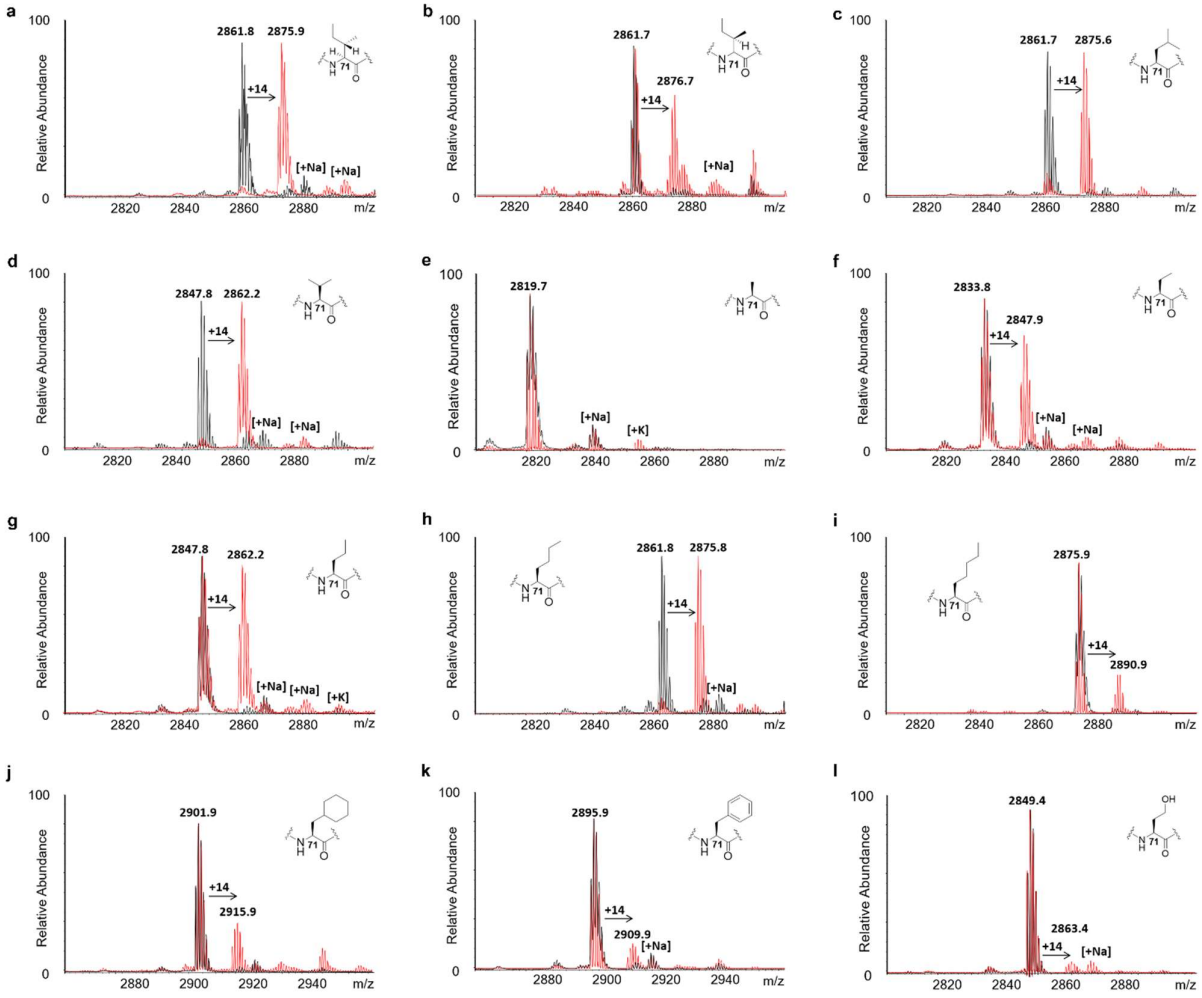

Fig. S18 MALDI-TOF MS data showing SETD3-catalysed (200 nM) methylation of βA peptides (10 μM) in the presence of SAM (100 μM) after 1 h at 37 ℃, pH 9. Control reactions in the absence of SETD3 are shown in black, whereas SETD3-catalysed reactions are shown in red. **a**) βA-Ile71, **b**) βA-DIle71, **c**) βA-Leu71, **d**) βA-Val71, **e**) βA-Ala71, **f**) βA-Abu71 **g**) βA-Nva71, **h**) βA-Nle71, **i**) βA-Ahp71, **j**) βA-Cha71, **k**) βA-Phe71, **l**) βA-hSer71.

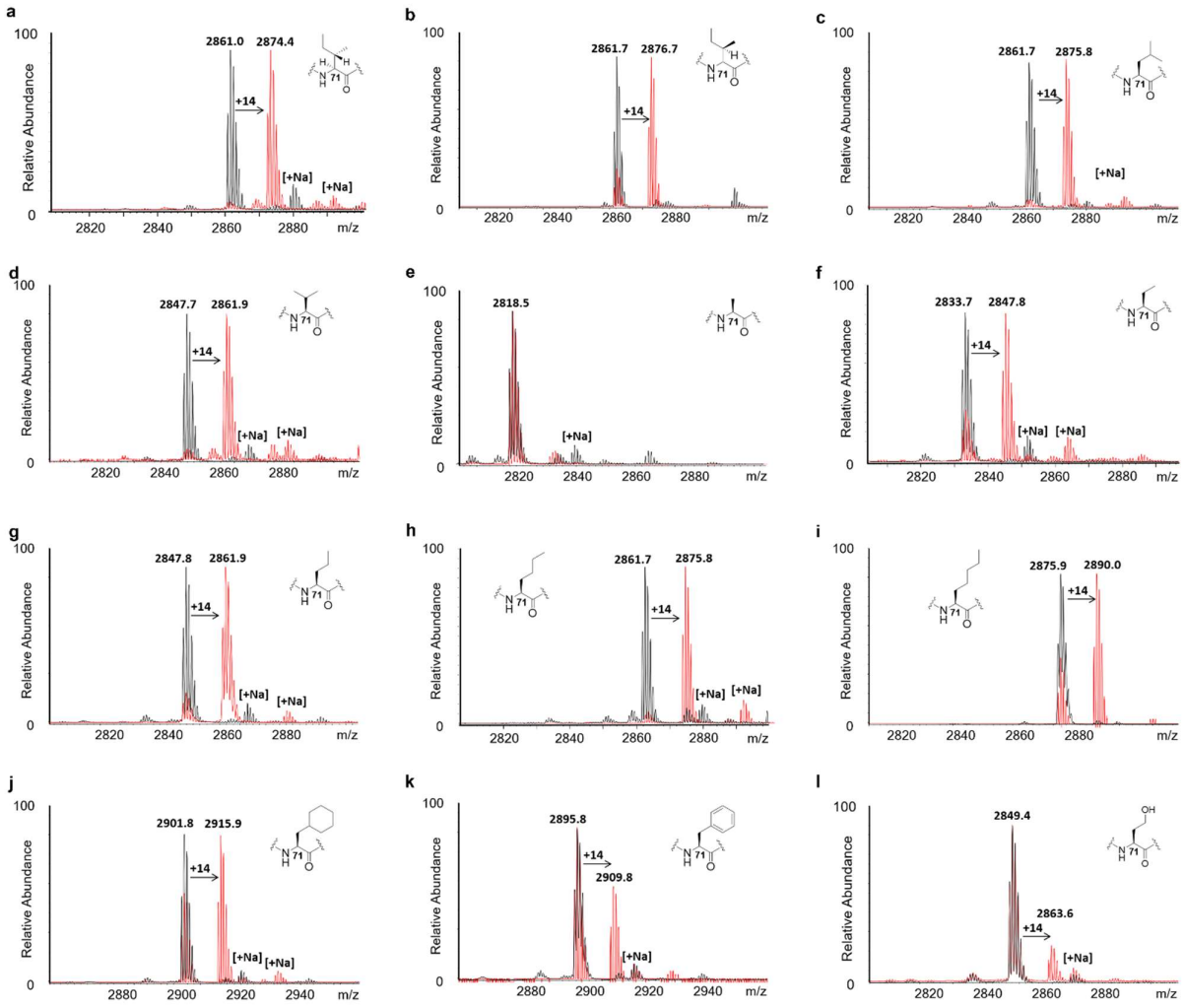

Fig. S19 MALDI-TOF MS data showing SETD3-catalysed (200 nM) methylation of βA peptides (10 μM) in the presence of SAM (100 μM) after 3 h at 37 ℃, pH 9 (**a-l**). Control reactions in the absence of SETD3 are shown in black, whereas SETD3-catalysed reactions are shown in red. **a**) βA-Ile71, **b**) βA-DIle71, **c**) βA-Leu71, **d**) βA-Val71, **e**) βA-Ala71, **f**) βA-Abu71 **g**) βA-Nva71, **h**) βA-Nle71, **i**) βA-Ahp71, **j**) βA-Cha71, **k**) βA-Phe71, **l**) βA-hSer71.

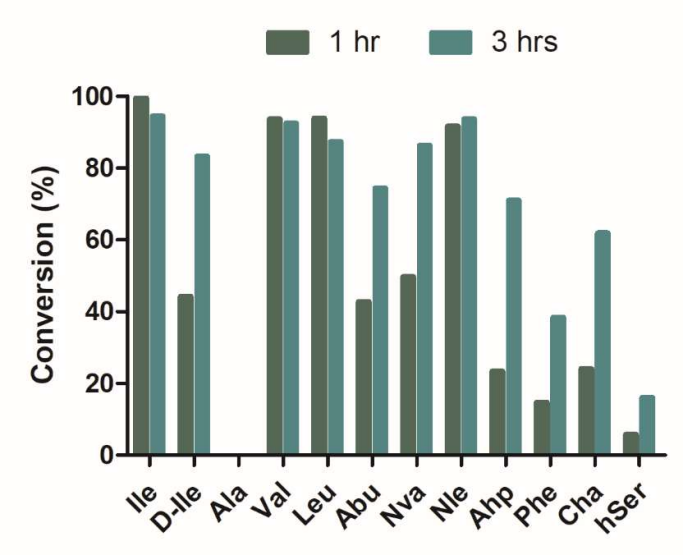

Fig. S20 Data showing percentage methylation of all βA peptides (10 μM) in the presence of 200 nM SETD3 and 100 μM SAM after 1 and 3 h.

3. Kinetics analysis supporting figures

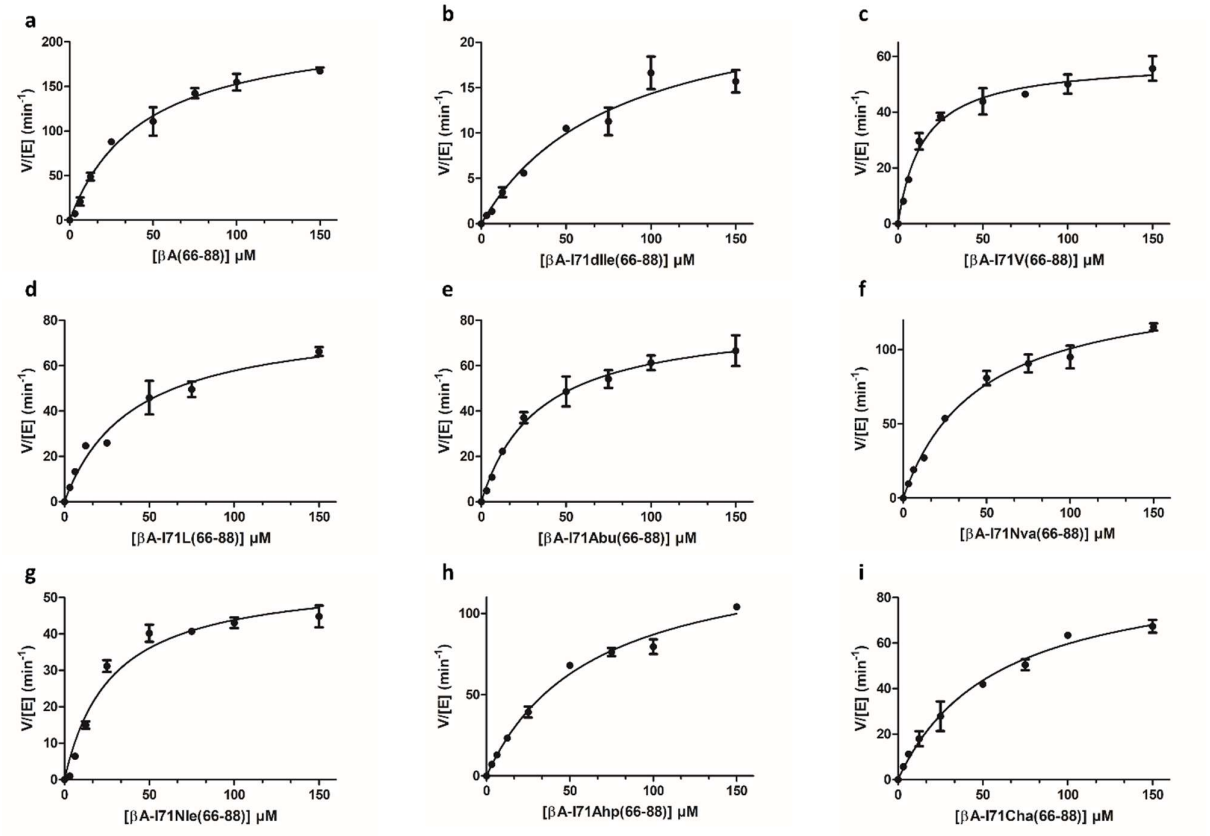

Fig. S21 MALDI-TOF MS kinetics analysis of SETD3-catalysed methylation of β-actin peptides containing isoleucine and isoleucine analogues at position 71. Michaelis-Menten plots of **a)** βA-Ile71, **b)** βA-D-Ile71, **c)** βA-Val71, **d)** βA-Leu71, **e)** βA-Abu71, **f)** βA-Nva71, **g)** βA-Nle71, **h)** βA-Ahp71, **i)** βA-Cha71.

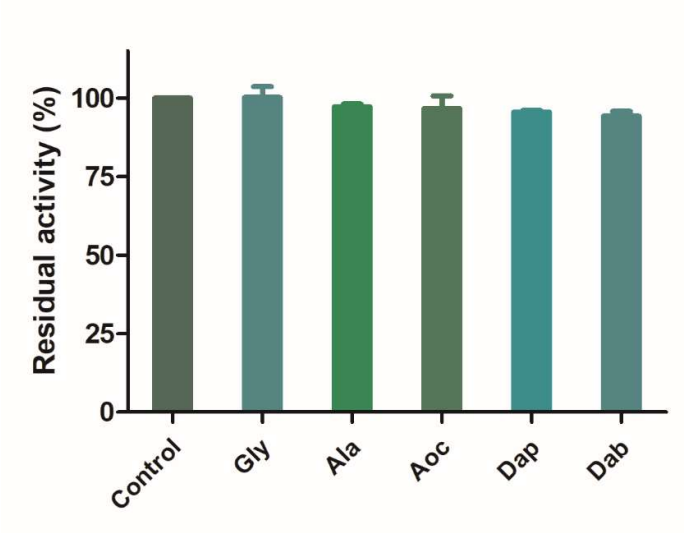

Fig. S22 A single point inhibition of human SETD3 (360 nM) by βA peptides (10 μM). Experiments were carried out in replicates (n = 2), error bars reported as standard error (SE).

4. Docking supporting figures

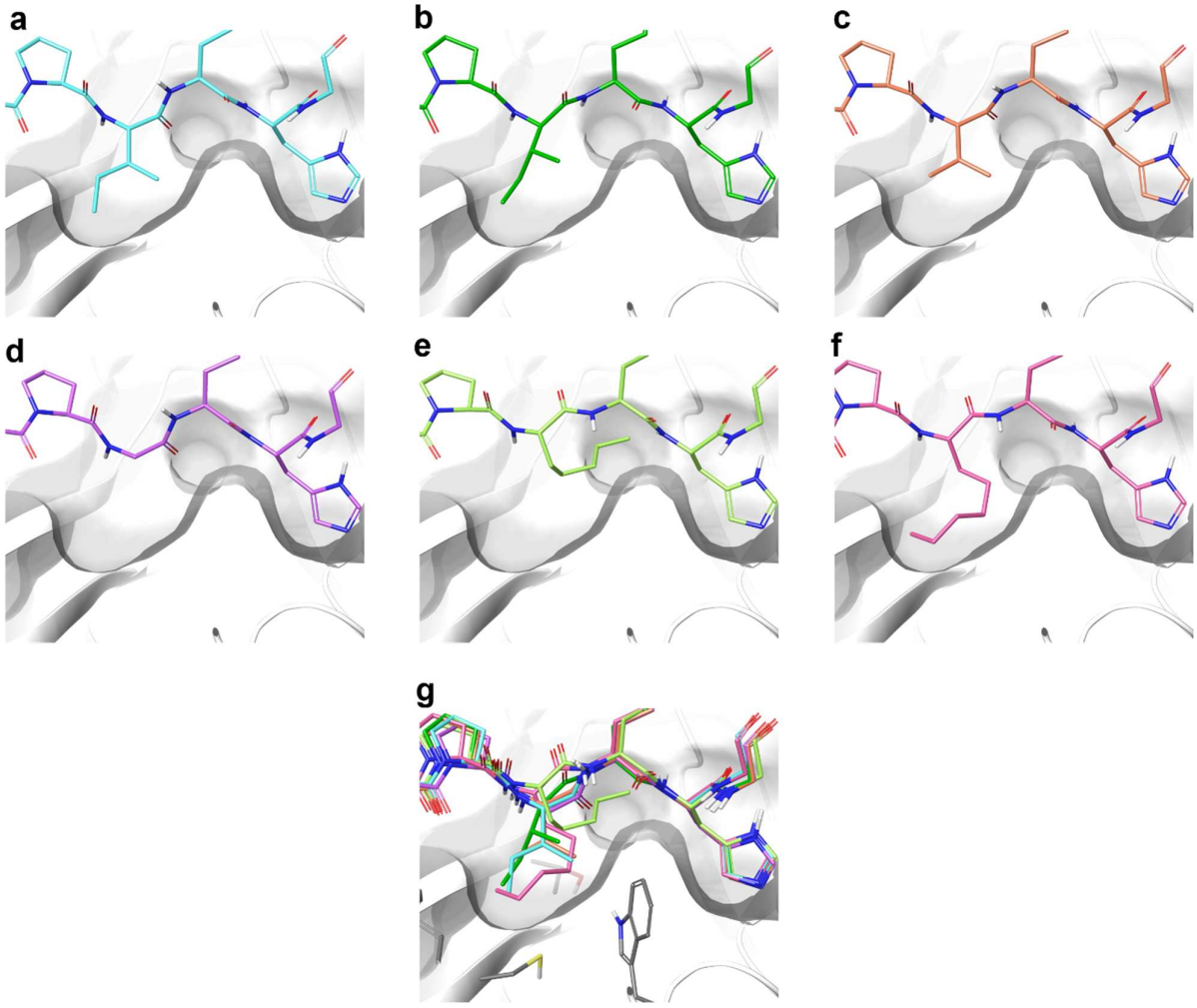

Fig. S23 Docking poses of a selection of βA peptides. a) βA-Ile71, b) βA-D-Ile71, c) βA-Val71, d) βA-Gly71, e) βA-Ahp71, f) βA-Aoc71, g) a-f superimposed.

5. MD supporting figures

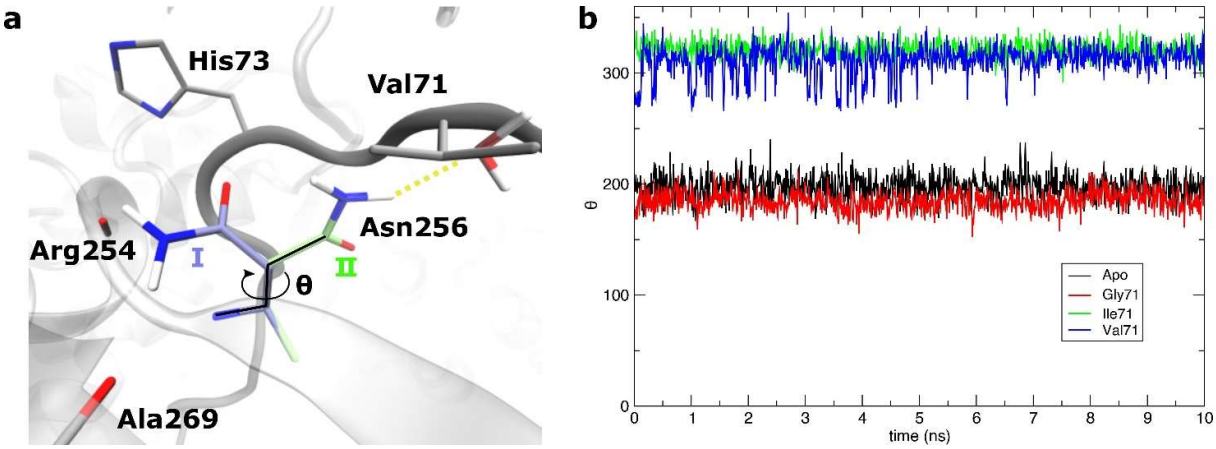

Fig. S24 Conformation of Asn256 during the MD simulations. a) Asn256 changes from (I) to (II) during MD simulation of SETD3 bound to βA-Gly71 and in the unbound (apo) simulation. The yellow dotted line marks a hydrogen bond between Asn256 and water in the simulation of βA-Gly71. b) Plot of the dihedral angle (θ) during the MD simulations.