Supporting information

Toward Solid Phase Peptide Fragments Ligation by a Traceless-Ugi Multicomponent Reaction Approach

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Table of contents

Materials and equipment

All the chemical reagents and solvents from commercial sources were used without further purification. Coupling reagents and amino acid derivatives were purchased from Matrix Innovation Inc. (Quebec, QC, Canada). Rink Amide ChemMatrix® resin (0,41 mmol/g) was purchased from PCAS Biomatrix, Rink Amide AM polystyrene resin (0.56 mmol/g) from ChemImpex (Wood Dale, IL, USA) and TentaGel S NH2 (130 µm, 0.29 mmol/g) from Rapp Polymere (Tübingen, Germany). All other reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Reactions on solid support were performed in filter columns (2 and 10 mL) from Roland Vetter Laborbedarf OHG (Ammerbuch, Germany). RP-HPLC analyses were achieved on a Shimadzu Prominence instrument (Columbian, MD, USA) using a Phenomenex Kinetex column (4.6 mm x 100 mm, 2.6 µm XB-C18, 100 Å, 1.5 mL/min) with a 10.5 min gradient from water (0.1% TFA) and CH_3CN (0.1% TFA) (CH_3CN 10-100%) and detection at 220 nm and 254 nm. LC/MS analyses were performed on a Shimadzu Prominence LCMS-2020 equipped with an ESI and APCI ion source. Microwave experiments were conducted on a Biotage Initiator microwave instrument (Charlotte, NC, USA) with 0.2-0.5 and 0.5-2 mL microwave vials. Peptides were synthetized on a Prelude peptide synthetizer from Protein Technologies (Tucson, AZ, USA). High-resolution mass spectrometry was performed on a Waters Synapt G2-Si (Quadrupole/TOF) with a Waters UPLC binary pump and FTN injector. The mass spectrometer was operated in High resolution mode and calibration done with a sodium formate (Sigma) solution and lock-mass correction using a Leucine-enkephaline solution (Waters).

Peptide synthesis

Peptides were synthesized by standard Fmoc solid-phase synthesis. Briefly, amino acid couplings were performed with a solution of Fmoc-Xaa-OH (3 equiv.), HCTU (3 equiv.) and NMM (6 equiv.) in DMF for 20 min. The coupling step was repeated once and the resin washed with DMF (5×). The Fmoc protecting group was removed by treating the resin twice with a solution of 20% piperidine in DMF (v/v) for 8 min followed by washing with DMF (5×).

Solid-phase fragment coupling with standard coupling reagents

Coupling with HATU. The resin bearing the C-terminal fragment **1** was swelled in DMF for 10 minutes. The N-terminal fragment (1.2 equiv.), HATU (1.2 equiv.) and NMM (2.4 equiv.) were dissolved in DMF and added to the resin. After stirring the mixture for 3 h, the resin was filtered and washed with DMF (5×). The Fmoc group was removed with a solution of 20% piperidine in DMF (v/v) and the peptide was simultaneously deprotected and cleaved from the resin with a solution of TFA/H_2O/TIPS (95:2.5:2.5) for 1 h at room temperature. After filtration and washing with TFA, the filtrate was evaporated under reduced pressure and the resulting mixture analyzed and purified by RP-HPLC and characterized by ESI-MS.

H-Gly-Phe-Gly-Tyr-Leu-Gly-Gly-Leu-Gly-Lys-Phe-Gly-NH_2 (**6**) (White powder, 4.7 mg, 45% isolated yield): RP-HPLC t_R= 7.05 min; ESI-MS *m/z*: 1171.65 $[M+H]^+$; calcd for $C_{57}H_{83}N_{14}O_{13}$ 1171.63.

Coupling with PyAOP. The resin bearing the C-terminal fragment **1** was swelled in DMF for 10 minutes. The N-terminal fragment **2** (1.2 equiv.), PyAOP (1.2 equiv.) and DIPEA (2.4 equiv.) were dissolved in DMF and added to the resin. After stirring the mixture for 3 h, the resin was filtered and washed with DMF (5×). The Fmoc group was removed with a solution of 20% piperidine in DMF (v/v) and the peptide was simultaneously deprotected and cleaved from the resin with a solution of TFA/H_2O/TIPS (95:2.5:2.5) for 1 h at room temperature. After filtration and washing with TFA, the filtrate was evaporated under reduced pressure and the resulting mixture analyzed and purified by RP-HPLC and characterized by ESI-MS.

H-Gly-Phe-Gly-Tyr-Leu-Gly-Gly-Leu-Gly-Lys-Phe-Gly-NH_2 (**6**) (White powder, 4.6 mg, 33% isolated yield): RP-HPLC t_R= 7.05 min; ESI-MS *m/z*: 1171.65 $[M+H]^+$; calcd for $C_{57}H_{83}N_{14}O_{13}$ 1171.63.

Coupling with DIC/6-Cl-HOBt. The resin bearing the C-terminal fragment **1** was swelled in NMP for 10 minutes.[1] The N-terminal fragment **2** (1.2 equiv.), DIC (1.2 equiv.) and 6-Cl-HOBt (1.2 equiv.) were dissolved in NMP and added to the resin. After stirring the mixture for 3 h, the resin was filtered and washed with NMP (5×) and DMF (5×). The Fmoc group was removed with a solution of 20% piperidine in DMF (v/v) and the peptide was

simultaneously deprotected and cleaved from the resin with a solution of TFA/H_2O/TIPS (95:2.5:2.5) for 1 h at room temperature. After filtration and washing with TFA, the filtrate was evaporated under reduced pressure and the resulting mixture analyzed and purified by RP-HPLC and characterized by ESI-MS.

H-Gly-Phe-Gly-Tyr-Leu-Gly-Gly-Leu-Gly-Lys-Phe-Gly-NH_2 (**6**) (White powder, 4.5 mg, 35% isolated yield): RP-HPLC t_R= 7.05 min; ESI-MS *m/z*: 1171.65 $[M+H]^+$; calcd for $C_{57}H_{83}N_{14}O_{13}$ 1171.63.

H-Gly-Phe-Gly-Tyr-Leu-Phe-Gly-Leu-Gly-Lys-Phe-Gly-NH_2 (**16a**): 54% crude purity; RP-HPLC t_R= 7.75 min; MS (ESI) *m/z*: 1261.70 $[M+H]^+$; calcd for $C_{64}H_{89}N_{14}O_{13}$ 1261.65.

Références

1. Bacsa, B.; Horvati, K.; Bosze, S.; Andreae, F.; Kappe, C. O. Solid-phase synthesis of difficult peptide sequences at elevated temperatures: A critical comparison of microwave and conventional heating technologies. *J. Org. Chem.* **2008,** *73*, (19), 7532-7542.

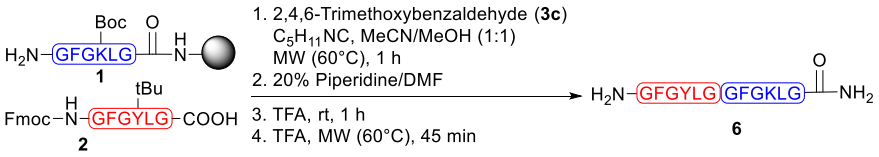
Tables

Table S1. Selection of the optimal aldehyde and deprotection conditions for the synthesis of peptide **6**

Aldehyde	**Treatment with TFA**		**Unprotected : Protected**	**Crude purity** (%)[a]
	rt (min)	**MW (60°C)** (min)	**Ratio** (%)[a]	
p-methoxybenzaldehyde (**3a**)	60	0	0	---
	60	60	32	21
2,4-dimethoxybenzaldehyde (**3b**)	60	0	0	---
	60	60	69	57
2,4,6-trimethoxybenzaldehyde (**3c**)	60	0	9	4
	60	15	84	64
	60	30	93	72
	60	45	>99	77

[a]Crude purities and conversion ratios from peptide **5a**-**c** to peptide **6** were determined by % area of UV signal at 220 nm in HPLC analysis of crude product.

Table S2. Evaluation of different Rink Amide resins for the synthesis of peptide **6**[a]



Resin	Conversion rate (%)[a]	Crude purity (%)[b]
ChemMatrix ®	99	88
Polystyrene	94	75
TentaGel™	91	68

[a]Conversion rate of C-terminal fragment **1** into peptide **6**. [b]Crude purities were determined by % area of UV signal at 220 nm in HPLC analysis of crude product.

Table S3. Calculated and observed mass for peptides **6-14**

Peptide	Formula	$[M+H]^+$		$[M+Na]^+$		$[M+2H]^{2+}$		$[M+H+Na]^{2+}$	
		Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed
6	$C_{57}H_{83}N_{14}O_{13}$	1171.6259	1171.6355	1193.6083	1193.6168	586.3166	586.3215	597.3078	597.3235
7	$C_{87}H_{121}N_{20}O_{20}$	1765.9061	1765.9084	1787.8885	1787.8922	883.4567	883.4606	894.4479	894.4610
8	$C_{77}H_{111}N_{18}O_{19}$	1591.8267	1591.8297	1613.8092	1613.8109	796.4170	796.4182	807.4083	807.4063
9	$C_{79}H_{115}N_{18}O_{18}$	1603.8631	1603.8645	1625.8456	1625.8402	802.4352	802.4349	813.4265	813.4269
10	$C_{97}H_{140}N_{23}O_{23}S$	2027.0208	----	2049.0033	----	1014.0140	1014.0192	1025.0053	1025.0135
11	$C_{99}H_{144}N_{23}O_{22}S$	2039.0572	----	2061.0396	----	1020.0322	1020.0392	1031.0235	1031.0310
12	$C_{102}H_{146}N_{23}O_{23}$	2061.0956	----	2083.0781	----	1031.0515	1031.0591	1042.0427	1042.0536
13	$C_{122}H_{175}N_{28}O_{27}S$	2496.2897	----	2518.2722	----	1248.6485	1248.6575	1259.6397	1259.6523
14	$C_{137}H_{206}N_{33}O_{32}S$	2857.5222	----	2879.5047	----	1429.2648	1429.2590	1439.7524	----

Table S4. Crude purity and isolated yield for peptides **6-14**

Peptide	Crude purity (%)	Starting resin (mg)	Purified mass (mg)	Isolated yield (%)[a]
6	88	57.0	9.3	57
7	55	54.0	4.0	24
8	81	35.5	4.9	45
9	83	45.0	6.6	46
10	89	42.9	4.3	26
11	85	20.8	4.5	54
12	75	36.0	7.9	69
13	43	43.8	3.3	20
14	33	44.1	1.2	6

[a]Yields are calculated with the experimental loading of 0.31 mmol/g for Rink Amide CM.

Figures

Figure S1. HPLC profiles (λ = 220 nm) and ESI-MS spectra of C-terminal fragments.

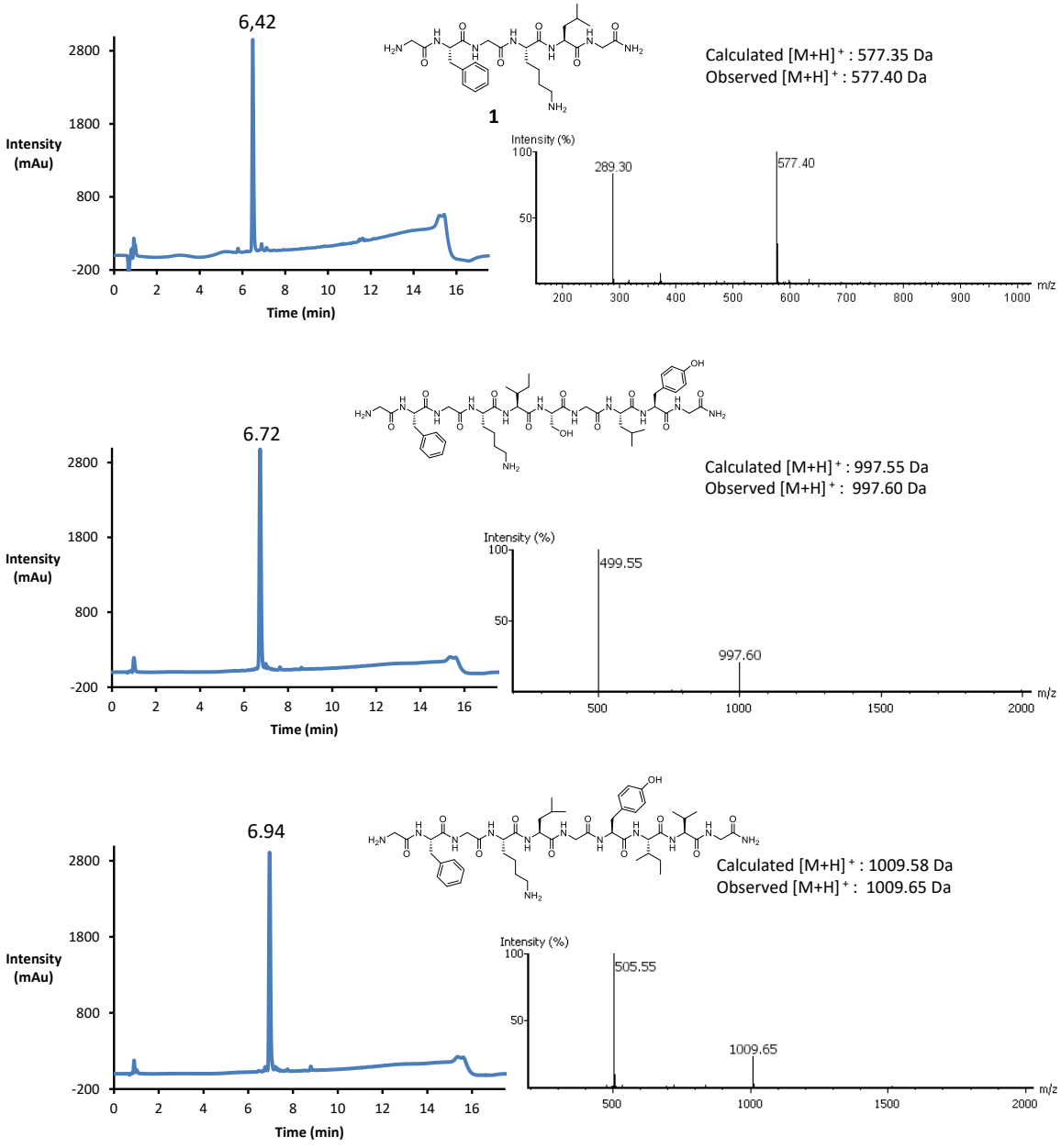


Figure S1. (Continued)

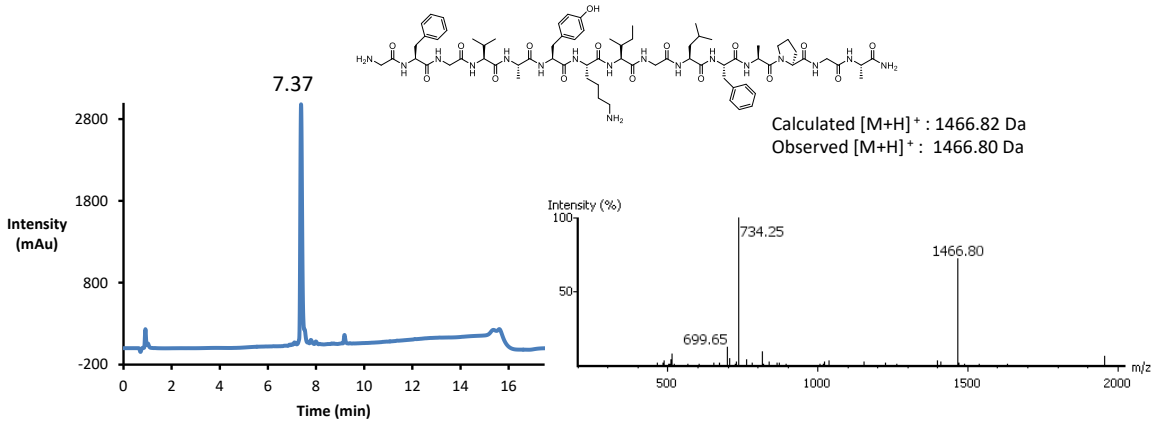
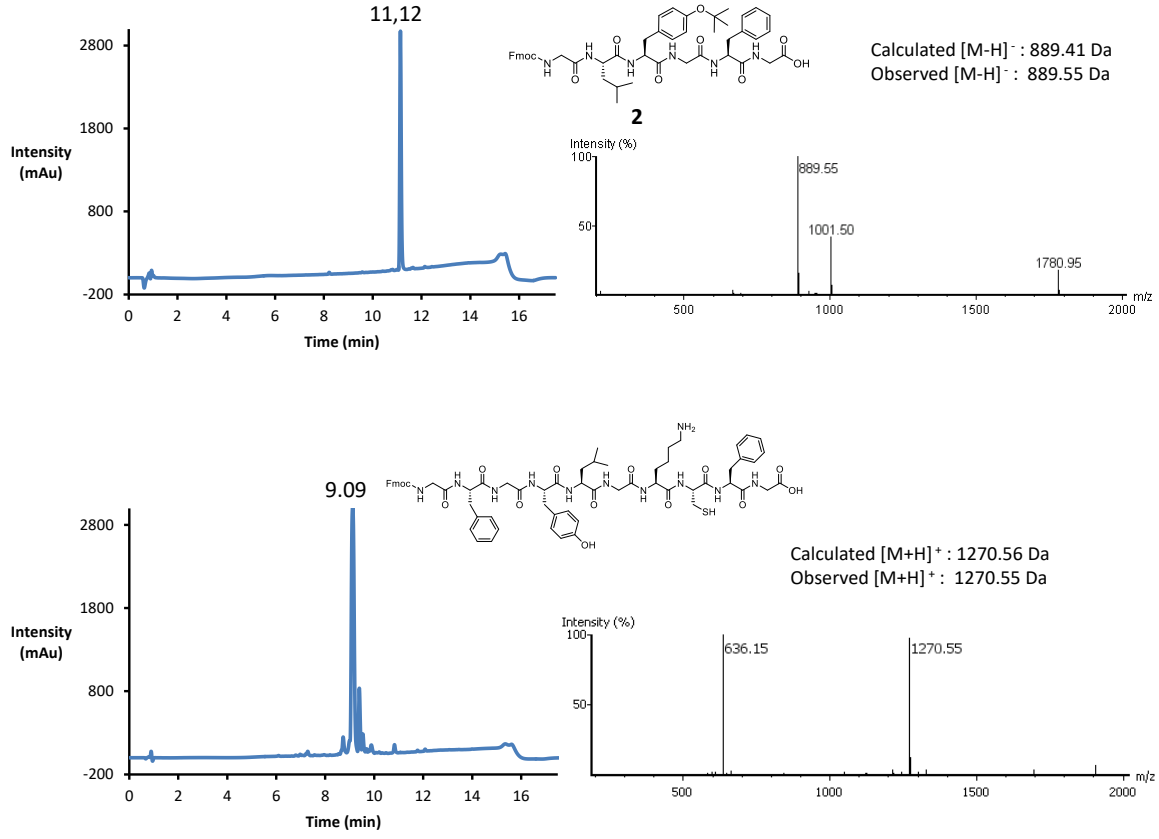


Figure S2. HPLC profiles (λ = 220 nm) and ESI-MS spectra of N-terminal fragments.



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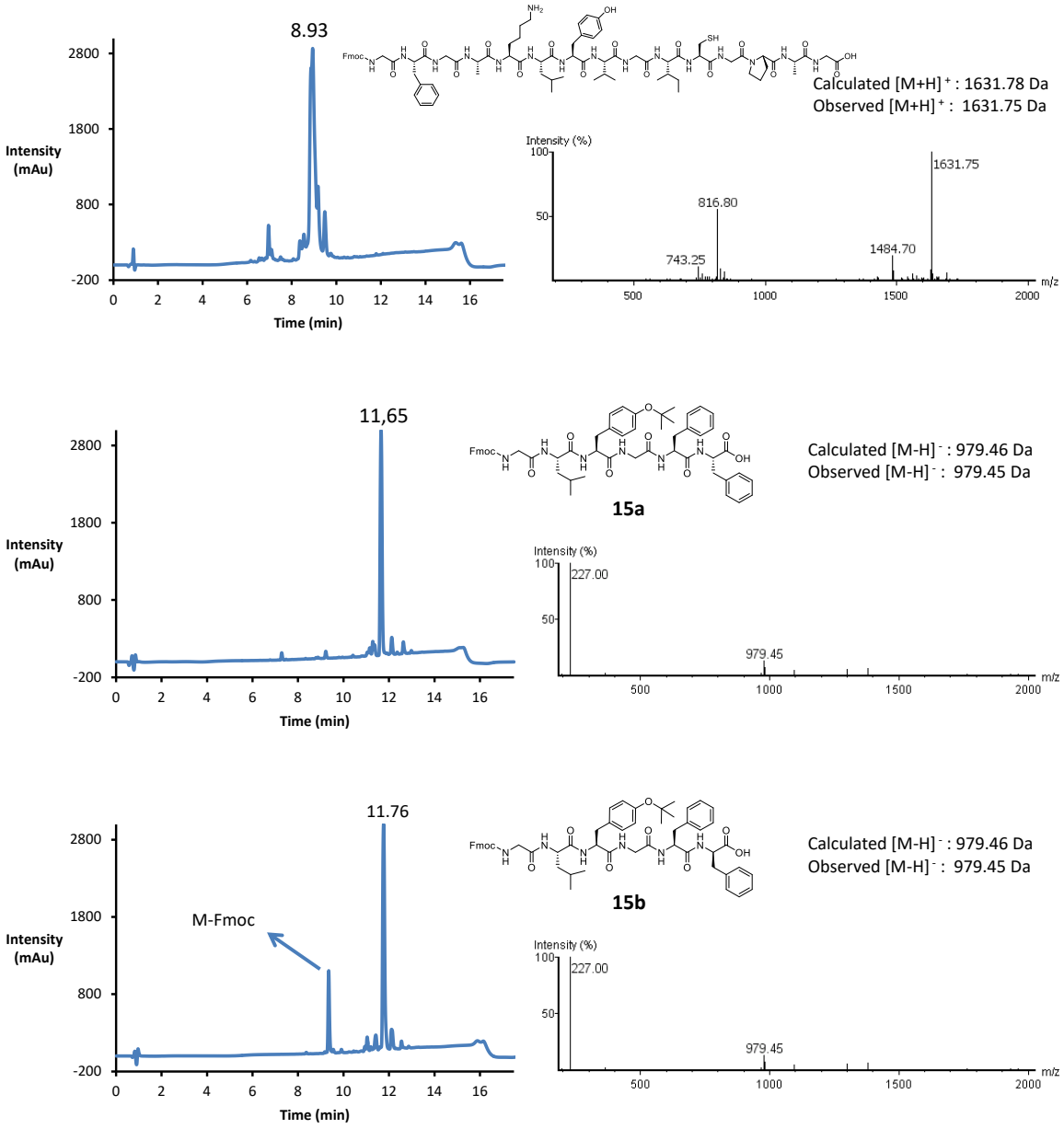
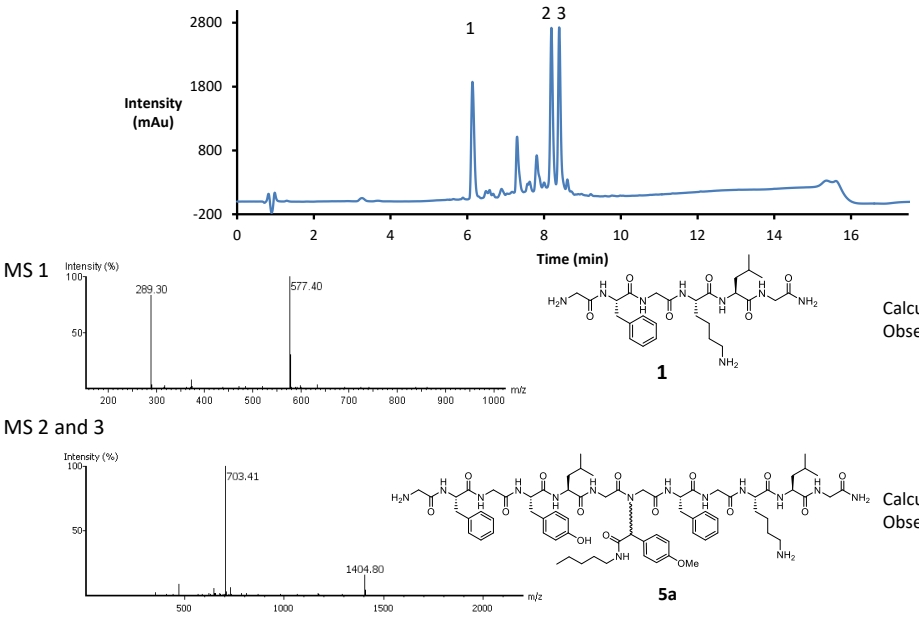


Figure S3. HPLC profile (λ=220 nm) and ESI-MS spectra of backbone amide protected peptide **5a**.

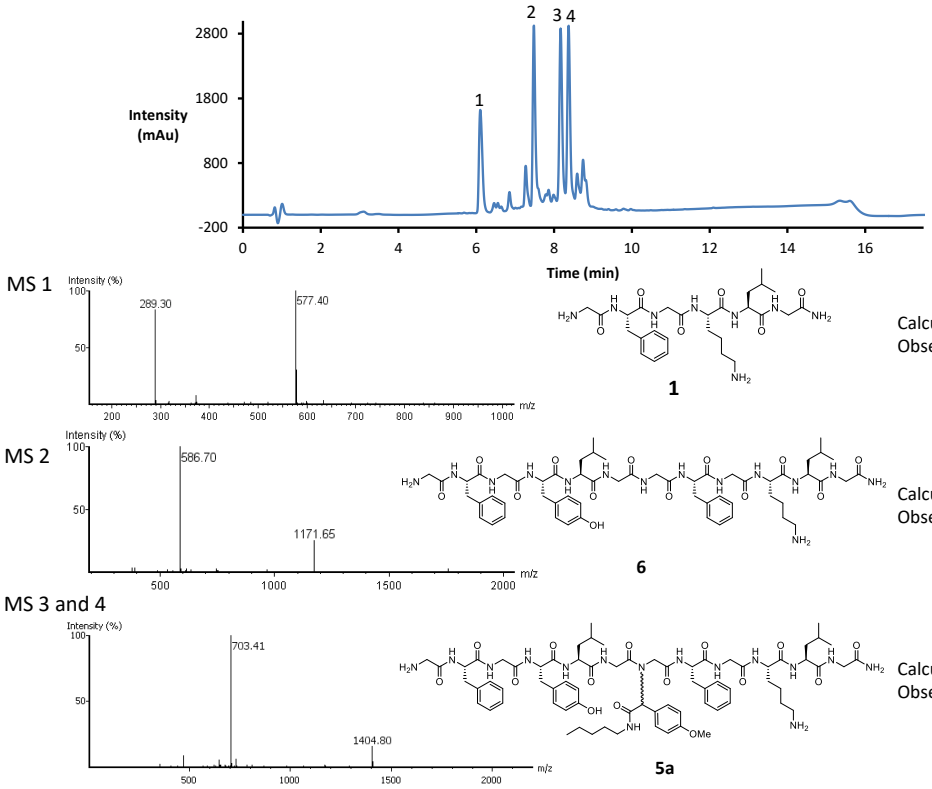
After treatment with TFA for 1 h at rt



Calculated $[M+H]^+$: 577.35 Da
Observed $[M+H]^+$: 577.40 Da

Calculated $[M+H]^+$: 1404.77 Da
Observed $[M+H]^+$: 1404.80 Da

After treatment with TFA for 1 h at rt and 1 h under MW at 60°C



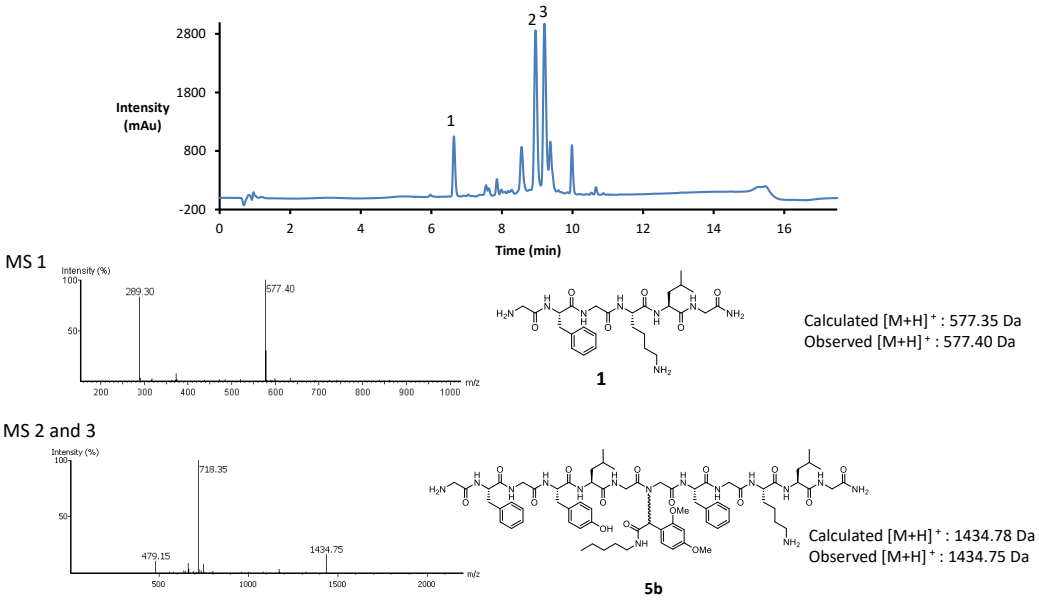
Calculated $[M+H]^+$: 577.35 Da
Observed $[M+H]^+$: 577.40 Da

Calculated $[M+H]^+$: 1171.63Da
Observed $[M+H]^+$: 1171.65 Da

Calculated $[M+H]^+$: 1404.77 Da
Observed $[M+H]^+$: 1404.80 Da

Figure S4. HPLC profile (λ = 220 nm) and ESI-MS spectra of backbone amide protected peptide **5b**.

After treatment with TFA for 1 h at rt



After treatment with TFA for 1 h at rt and 1 h under MW at 60°C

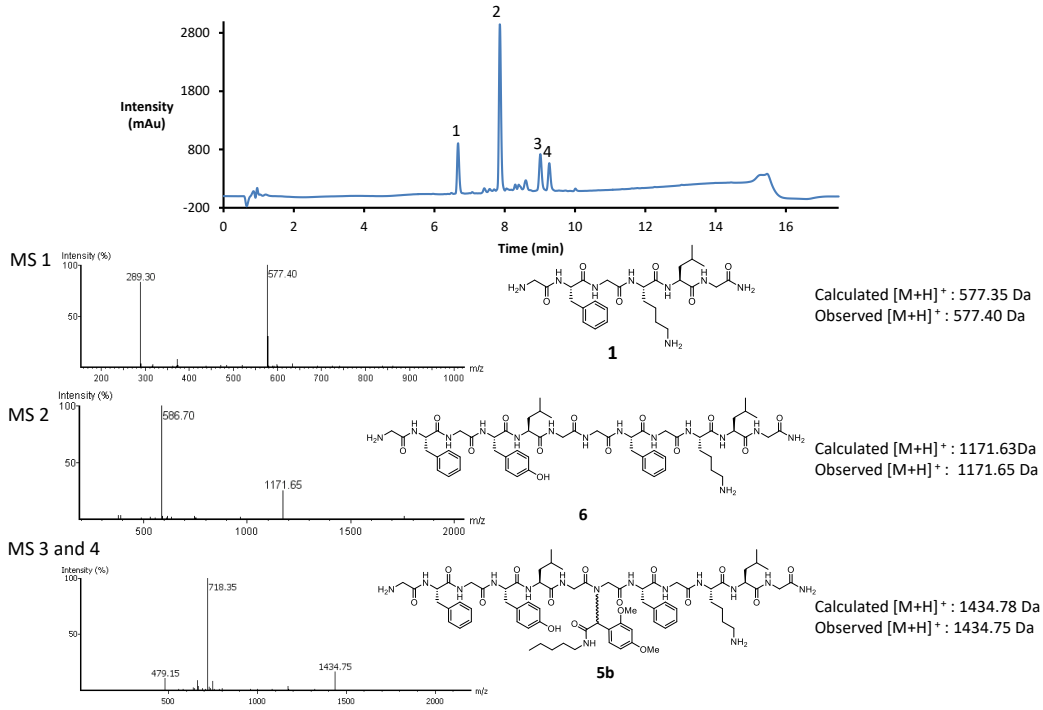


Figure S5. HPLC profile (λ = 220 nm) and ESI-MS spectra of backbone amide protected peptide **5c**.

After treatment with TFA for 1 h at rt

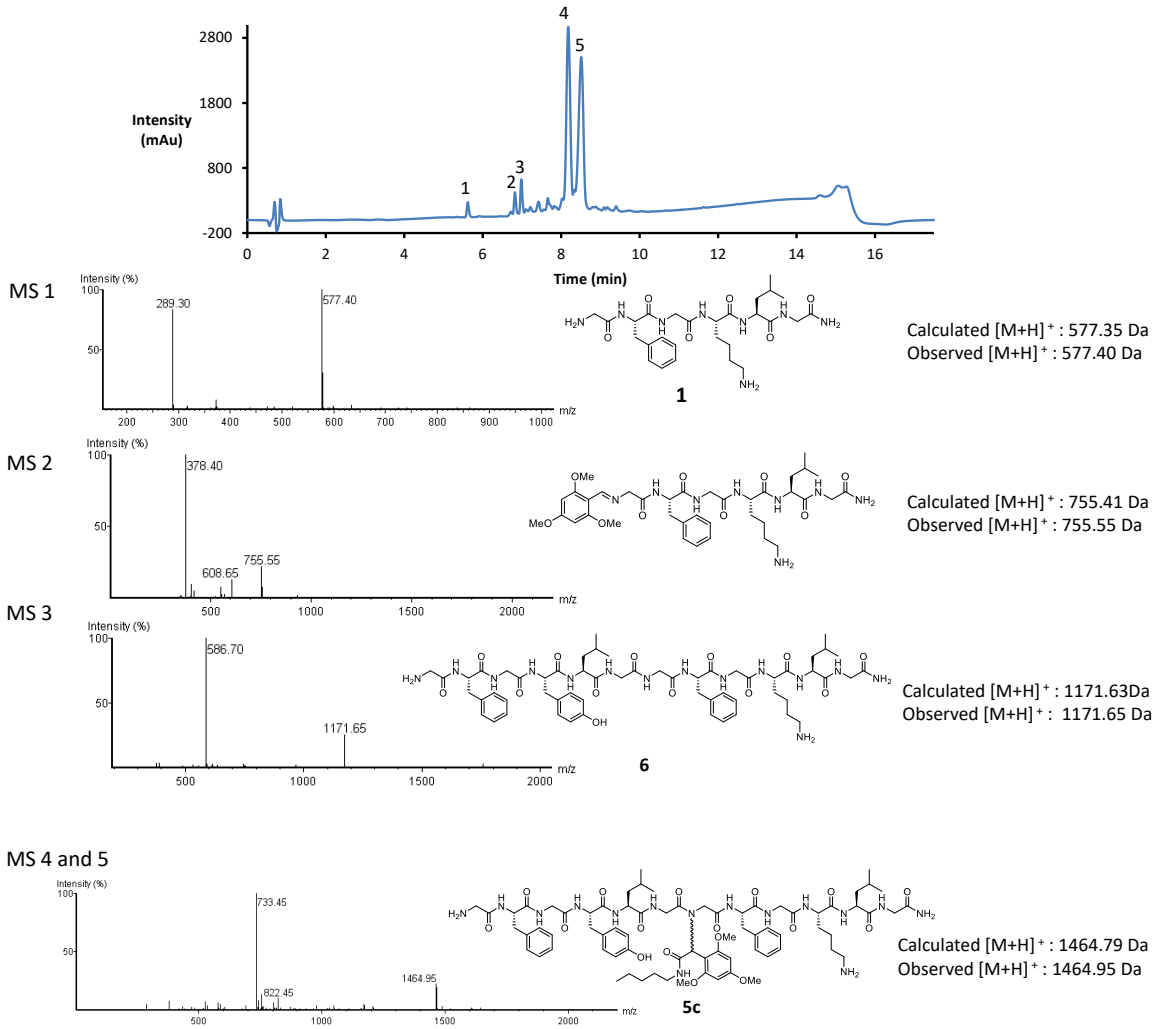
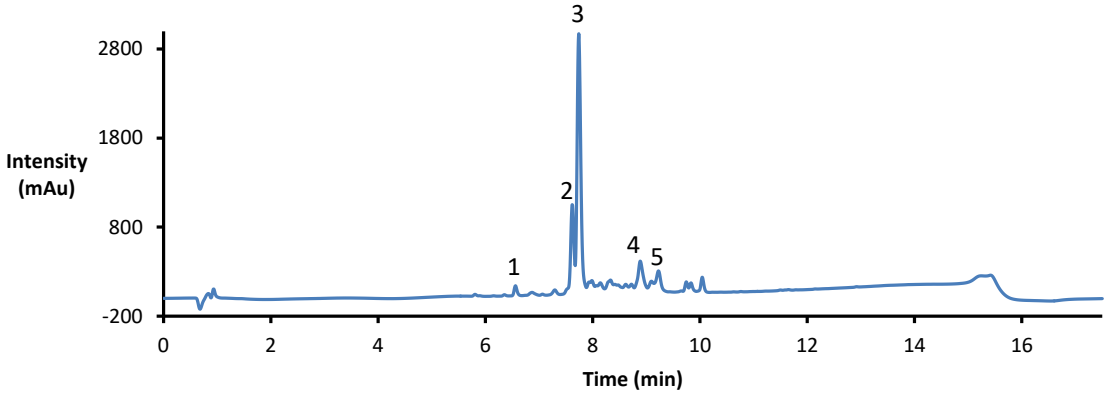
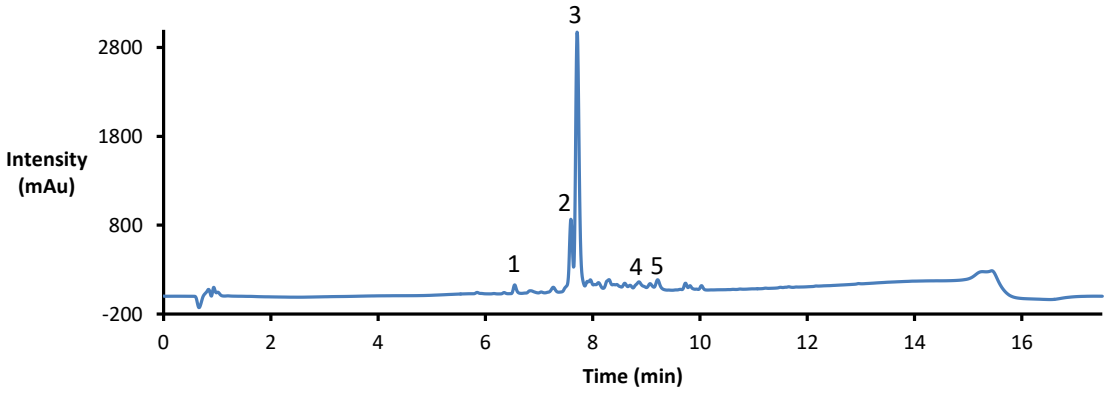


Figure S5. (Continued)

After treatment with TFA for 1 h at rt and 15 min under MW at 60°C



After treatment with TFA for 1 h at rt and 30 min under MW at 60°C



After treatment with TFA for 1 h at rt and 45 min under MW at 60°C

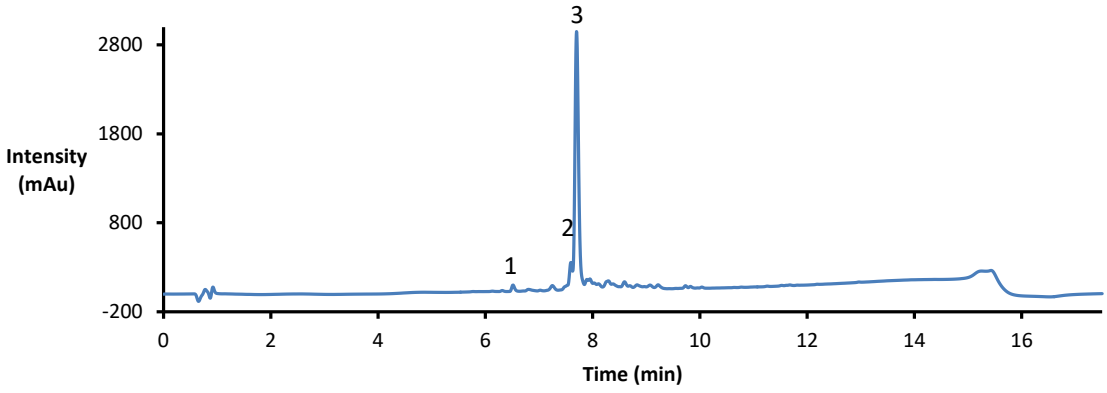


Figure S6. HPLC-MS profiles (λ = 220 nm) and HRMS spectra for peptides **6-14**

H-Gly-Phe-Gly-Tyr-Leu-Gly-Gly-Leu-Gly-Lys-Phe-Gly-NH_2 (**6**)

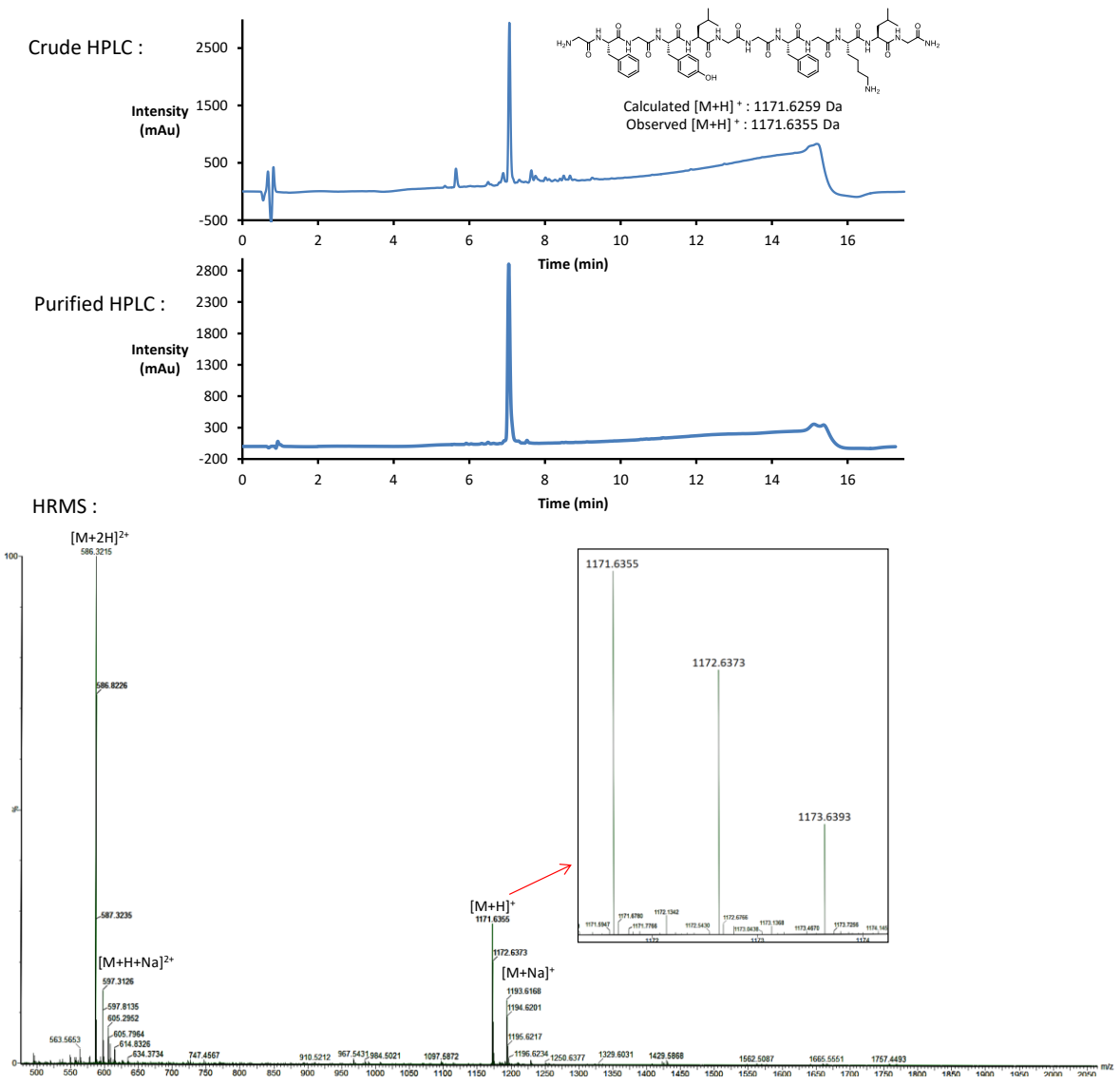


Figure S6. (Continued)

H-Gly-Phe-Gly-Tyr-Leu-Gly-Gly-Phe-Gly-Tyr-Leu-Gly-Gly-Leu-Gly-Lys-Phe-Gly-NH_2 (**7**)

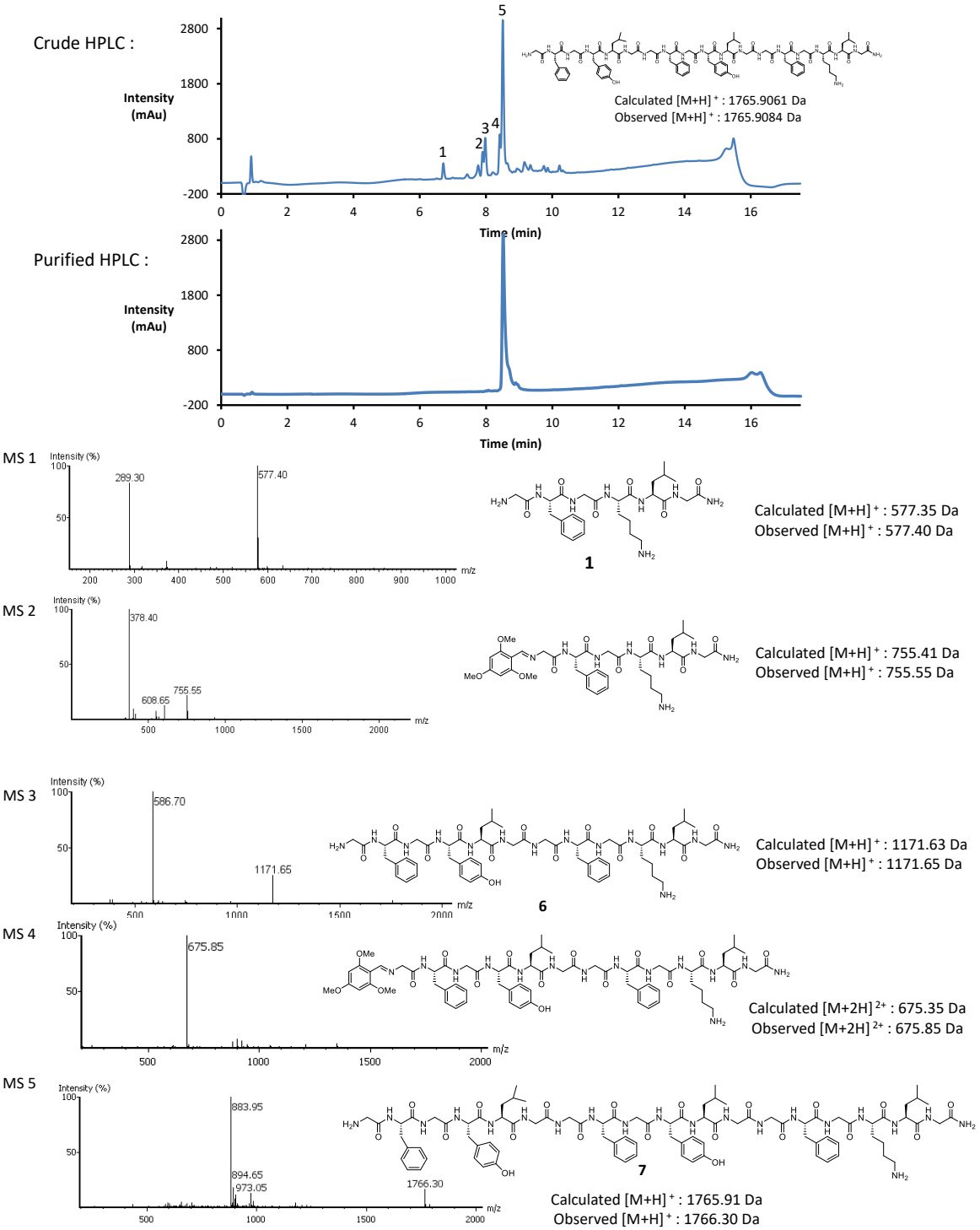


Figure S6. (Continued)

HRMS :

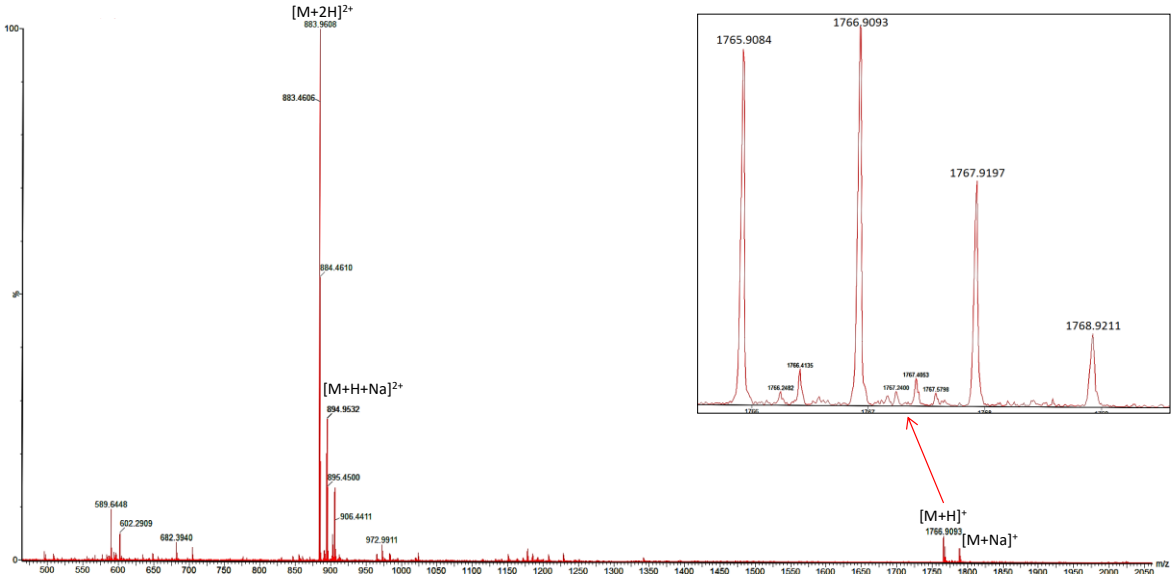


Figure S6. (Continued)

H-Gly-Phe-Gly-Tyr-Leu-Gly-Gly-Phe-Gly-Lys-Ile-Ser-Gly-Leu-Tyr-Gly-NH_2 (**8**)

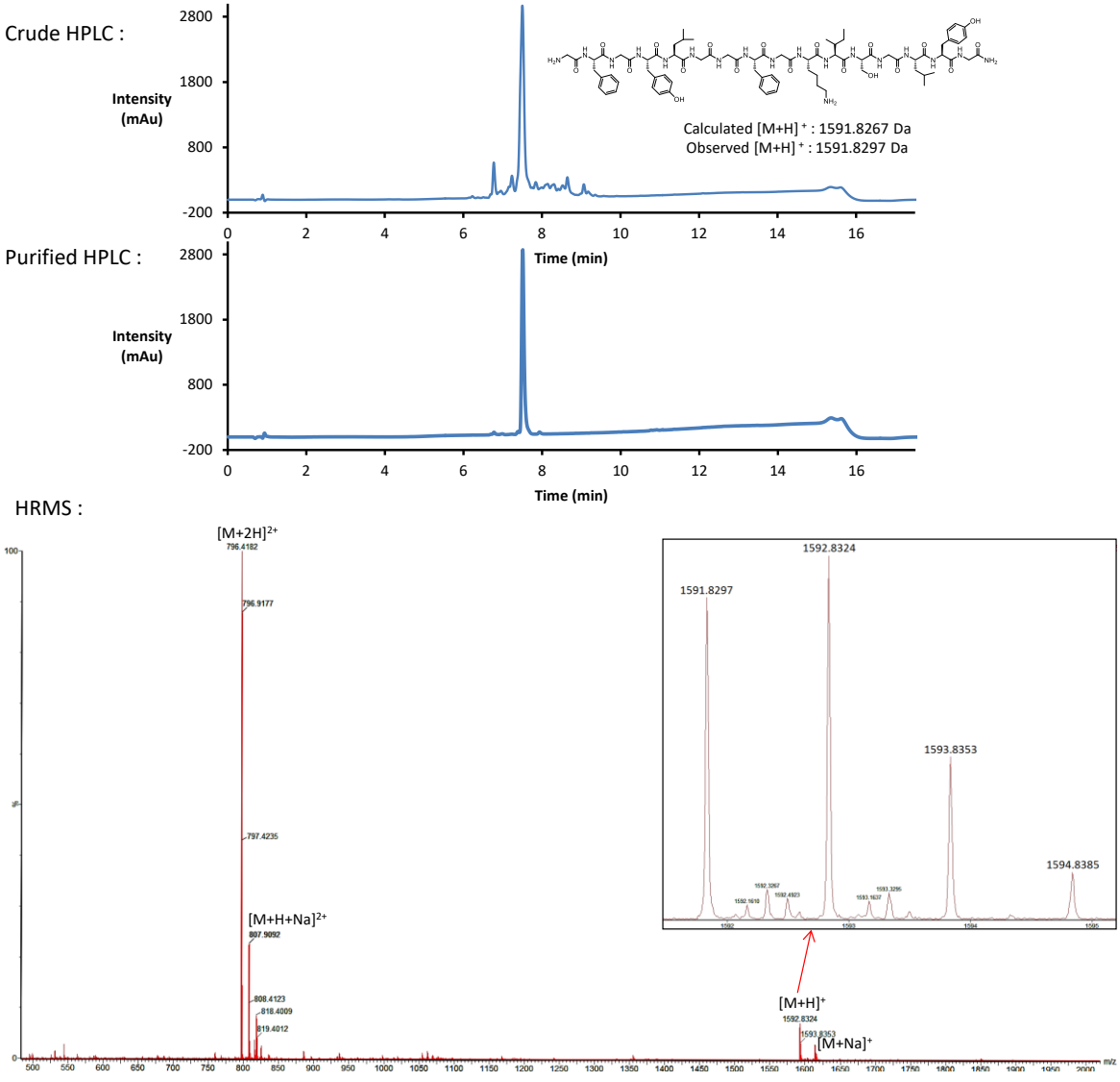


Figure S6. (Continued)

H-Gly-Phe-Gly-Tyr-Leu-Gly-Gly-Phe-Gly-Lys-Leu-Gly-Tyr-Ile-Val-Gly-NH_2 (**9**)

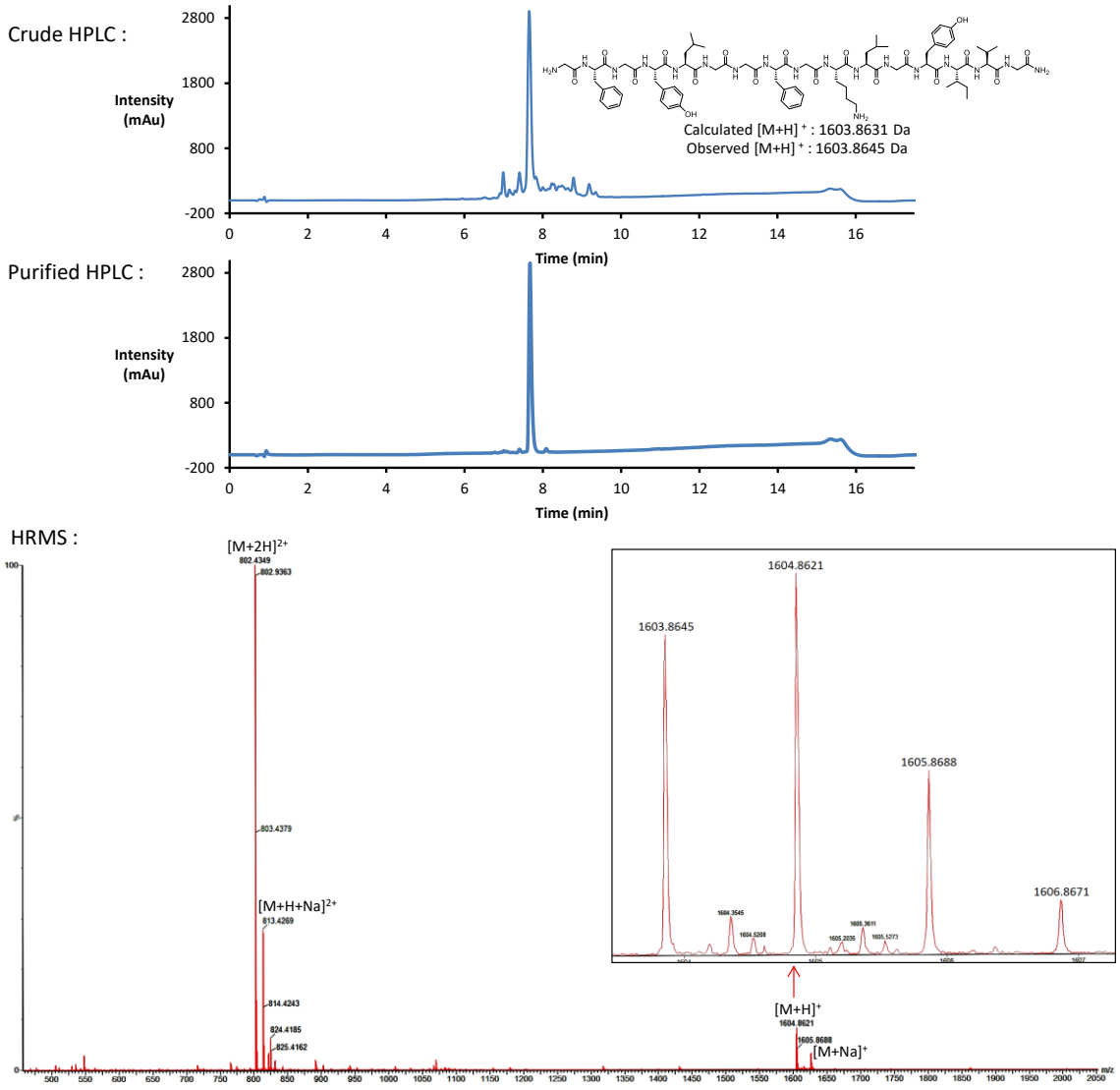


Figure S6. (Continued)

H-Gly-Phe-Gly-Tyr-Leu-Gly-Lys-Cys-Phe-Gly-Gly-Phe-Gly-Lys-Ile-Ser-Gly-Leu-Tyr-Gly-NH_2 (**10**)

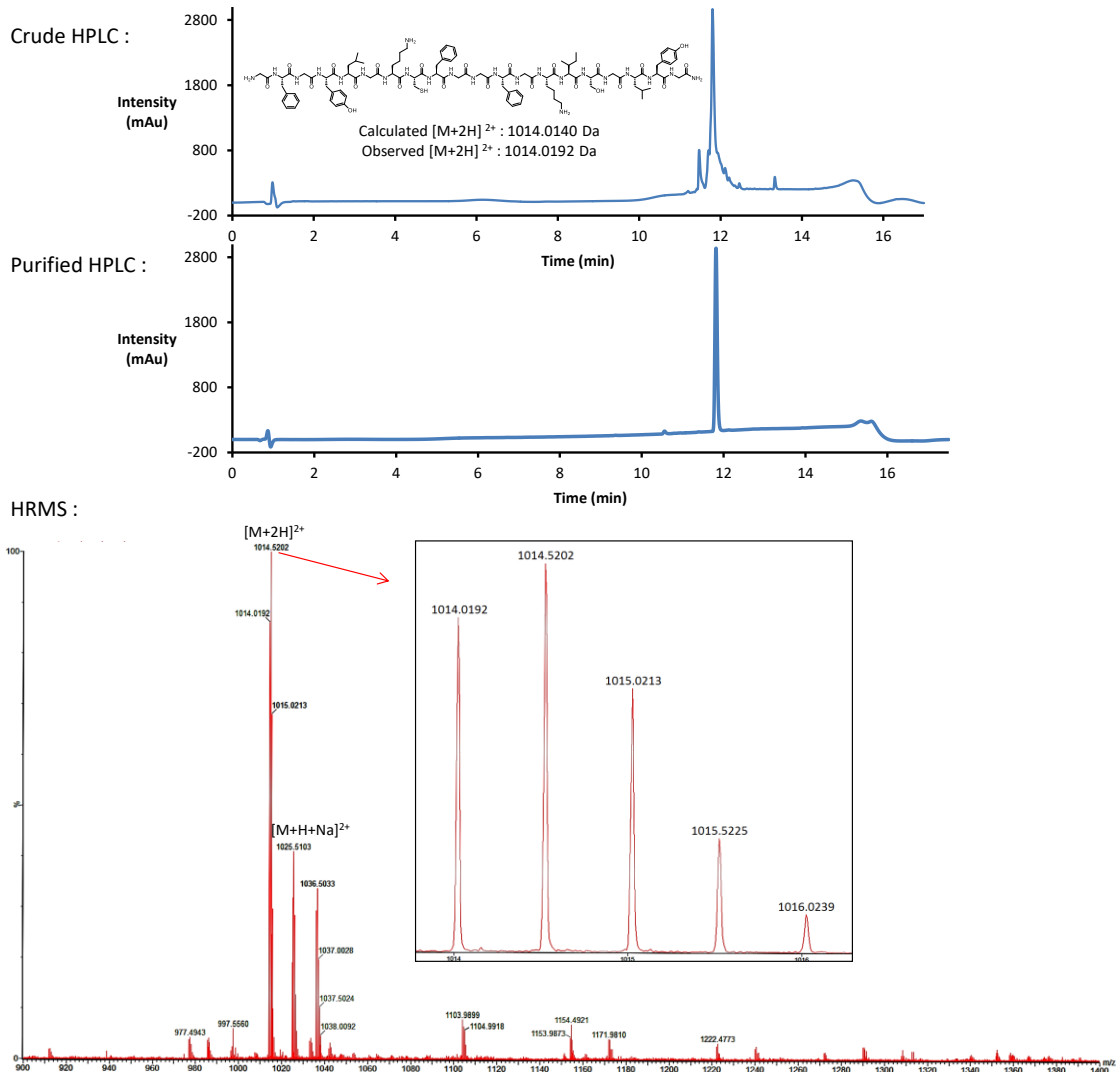


Figure S6. (Continued)

H-Gly-Phe-Gly-Tyr-Leu-Gly-Lys-Cys-Phe-Gly-Gly-Phe-Gly-Lys-Leu-Gly-Tyr-Ile-Val-Gly-NH_2 (**11**)

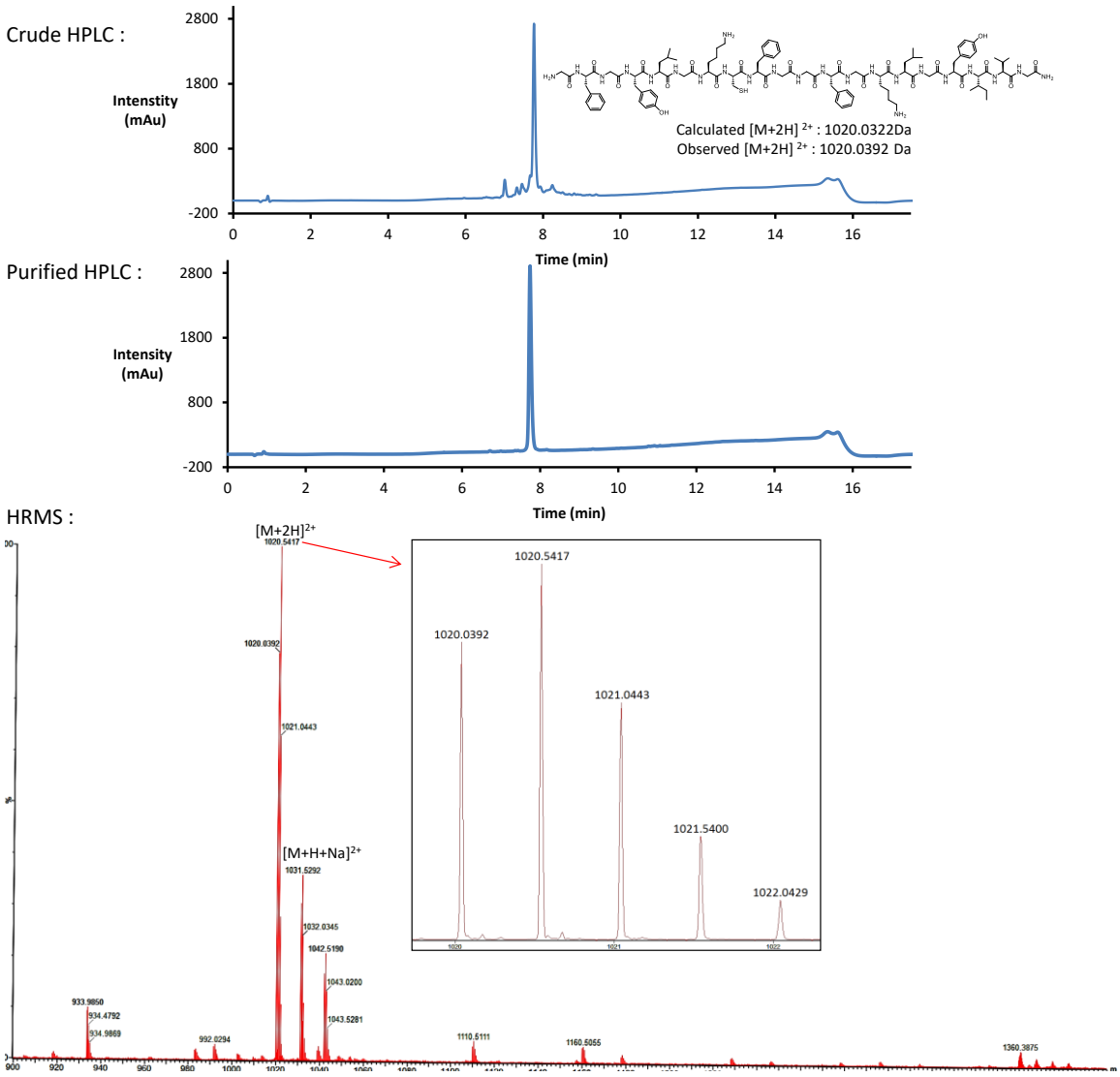


Figure S6. (Continued)

H-Gly-Phe-Gly-Tyr-Leu-Gly-Gly-Phe-Gly-Val-Ala-Tyr-Lys-Ile-Gly-Leu-Phe-Ala-Pro-Gly-Ala-NH_2 (**12**)

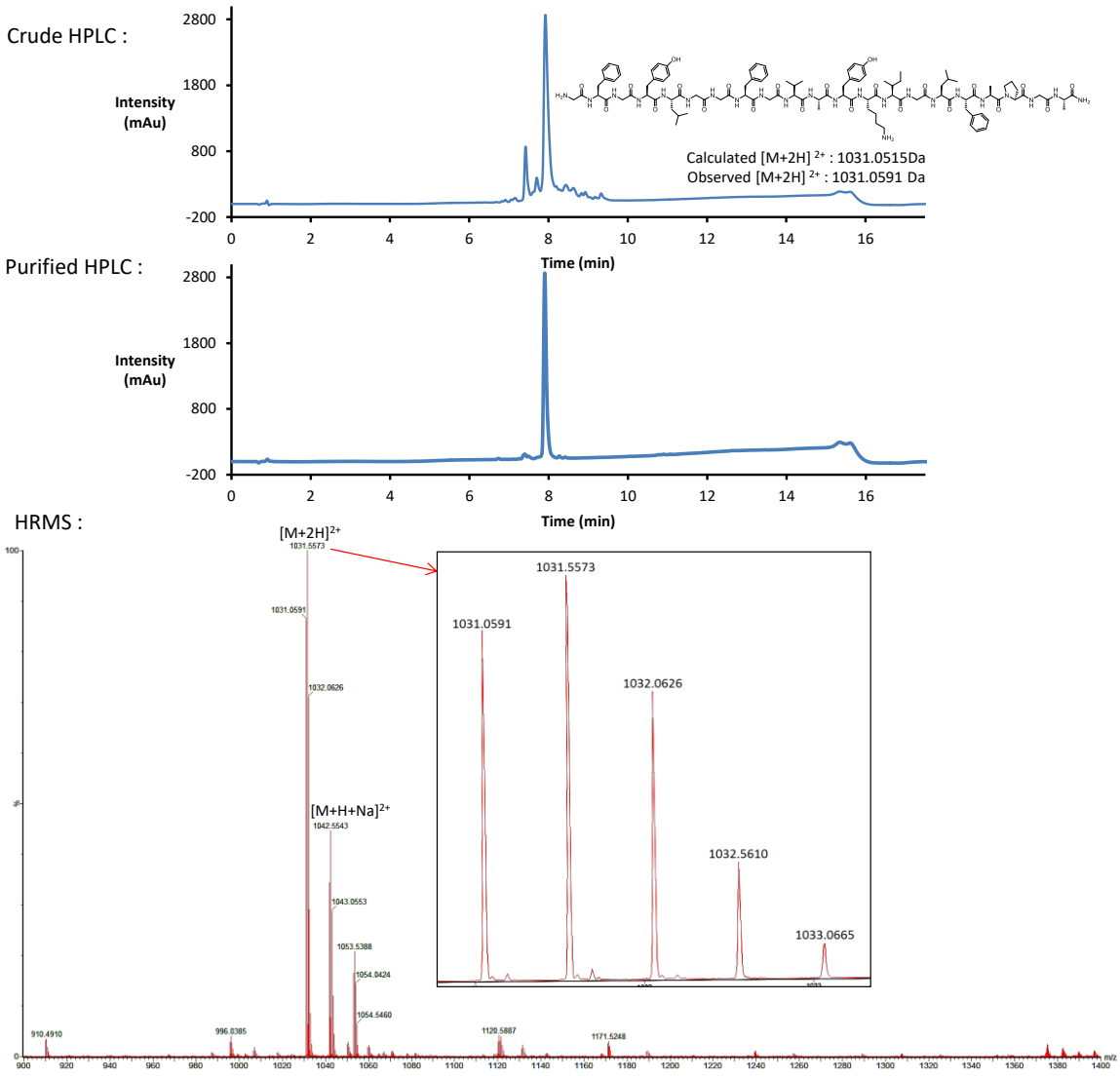


Figure S6. (Continued)

H-Gly-Phe-Gly-Tyr-Leu-Gly-Lys-Cys-Phe-Gly-Gly-Phe-Gly-Val-Ala-Tyr-Lys-Ile-Gly-Leu-Phe-Ala-Pro-Gly-Ala-NH_2 (**13**)

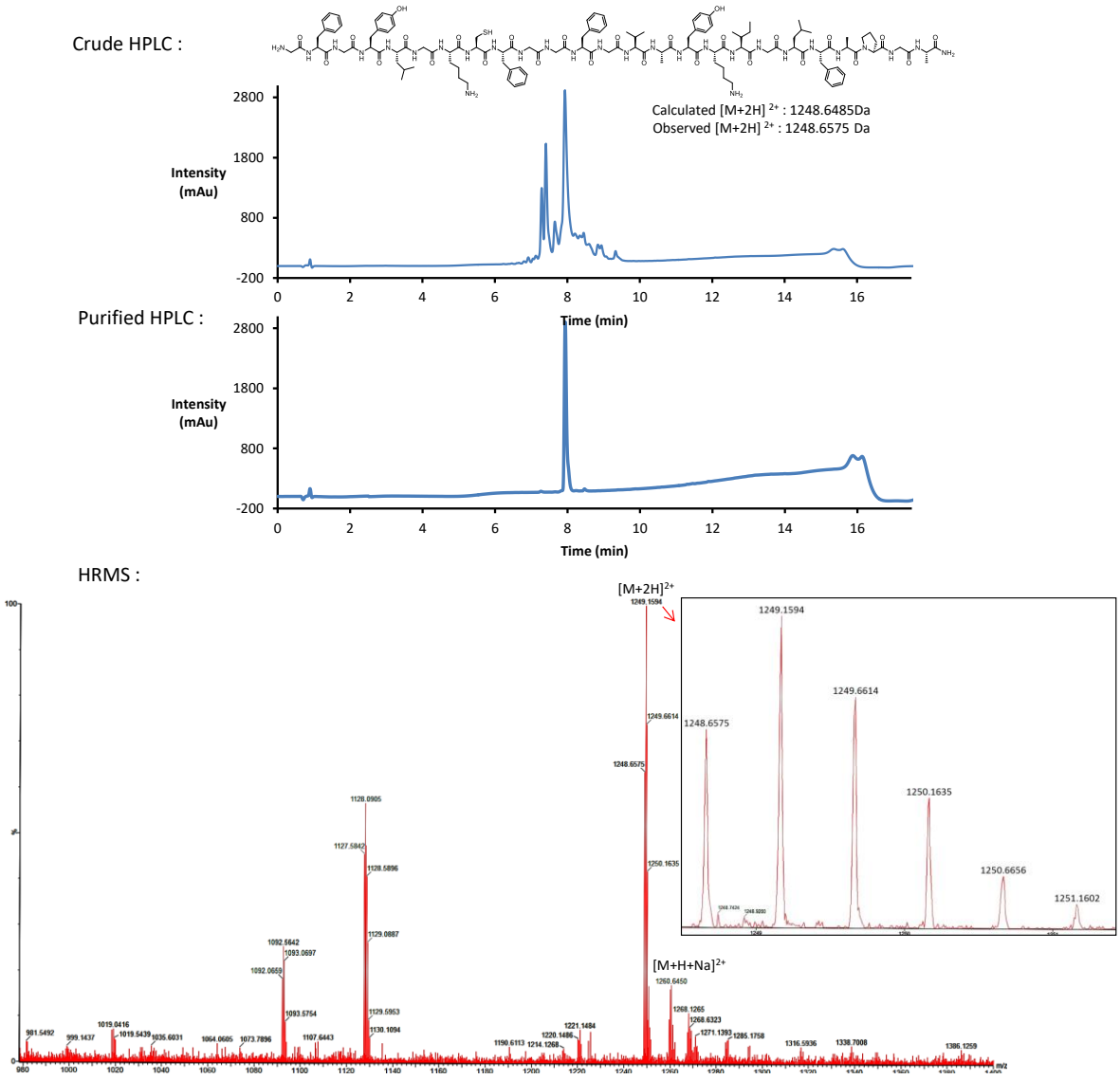


Figure S6. (Continued)

H-Gly-Phe-Gly-Ala-Lys-Leu-Tyr-Val-Gly-Ile-Cys-Gly-Pro-Ala-Gly-Gly-Phe-Gly-Val-Ala-Tyr-Lys-Ile-Gly-Leu-Phe-Ala-Pro-Gly-Ala-NH_2 (**14**)

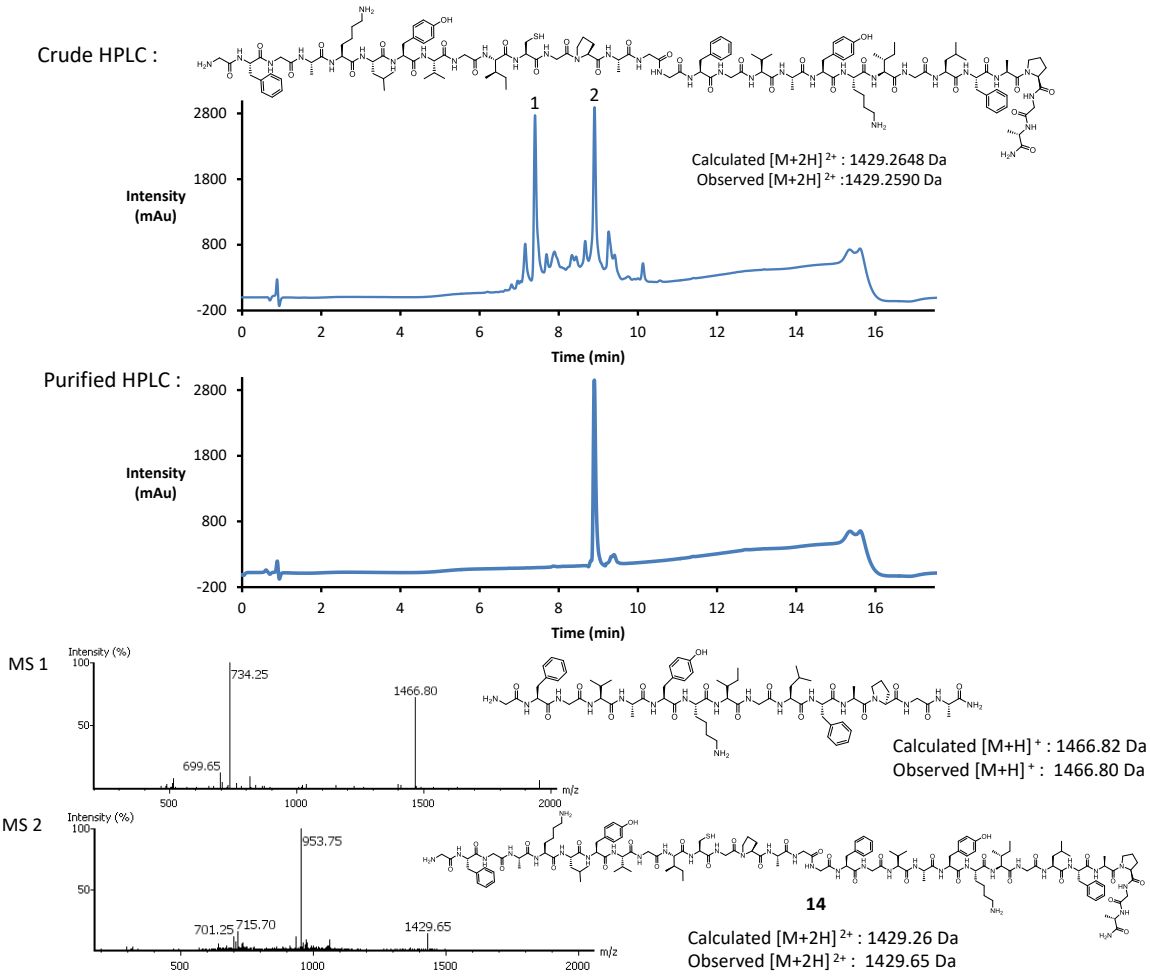


Figure S6. (Continued)

HRMS :

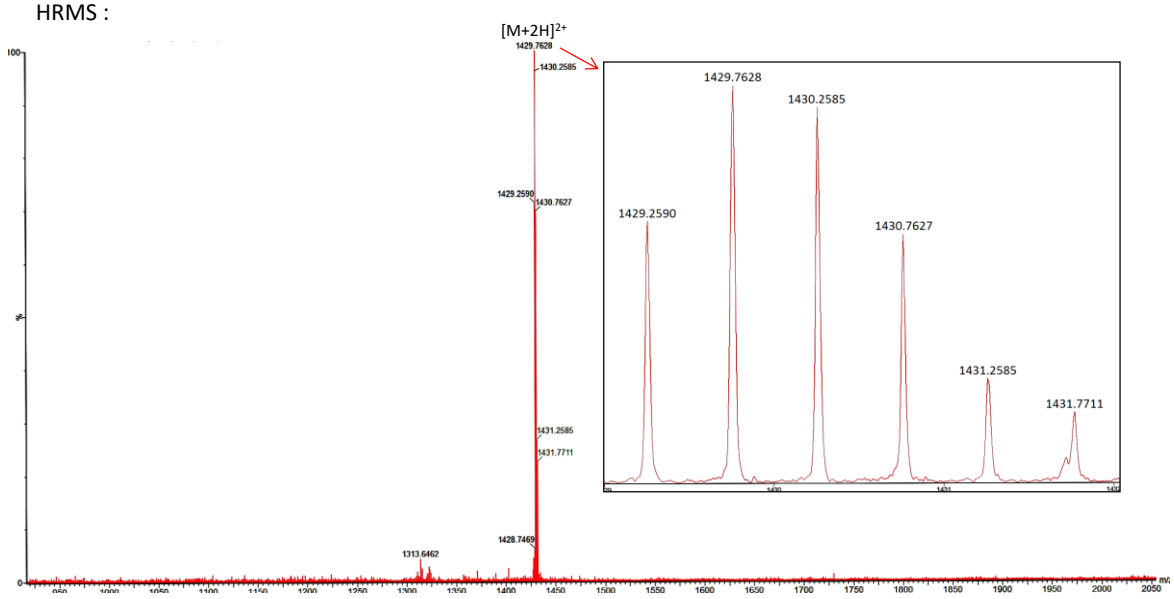
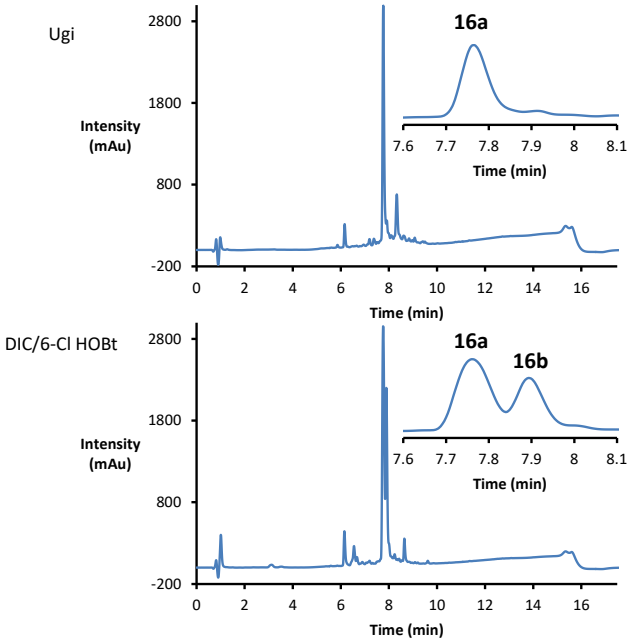
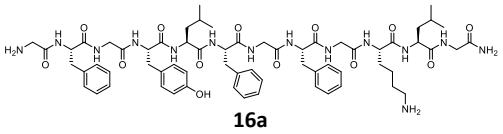


Figure S7. HPLC profiles (λ = 220 nm) and ESI-MS spectra of peptides **16a** and **16b**.

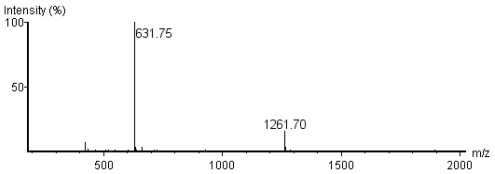
Coupling of Fmoc-GFGYLF-OH **15a** to C-terminal fragment **1**



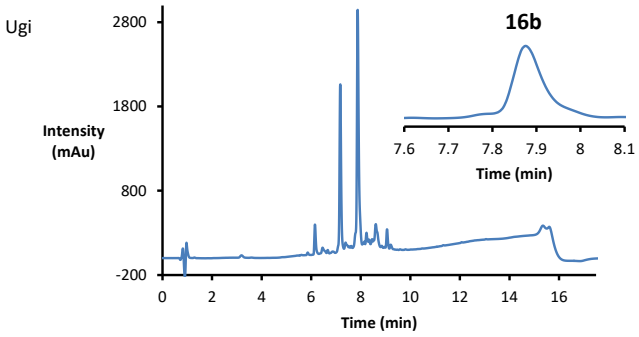


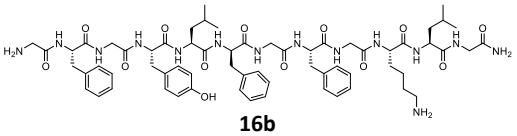
16a

Calculated $[M+H]^+$: 1261.65 Da
Observed $[M+H]^+$: 1261.70 Da



Coupling of Fmoc-GFGYLf-OH **15b** to C-terminal fragment **1**





16b

Calculated $[M+H]^+$: 1261.65 Da
Observed $[M+H]^+$: 1261.70 Da