Supplementary Information

General Information

a. Materials

All reagents and solvents were obtained from Peptide Institute, Inc. (Osaka, Japan), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), Nacalai Tesque, Inc. (Kyoto, Japan), Watanabe Chemical Industries, Ltd. (Hiroshima, Japan), Merck KGaA (Darmstadt, Germany), and Sigma-Aldrich Co. LLC. (St. Louis, MO).

b. HPLC and Mass spectrometry

Analytical HPLC was performed on a Shimadzu liquid chromatograph Model LC-2010C (Kyoto, Japan) with a YMC-ODS AA12S05-1546WT (4.6 x 150 mm) and the following solvent systems: (A) 0.1% TFA in H_2O and (B) 0.1% TFA in CH_3CN (linear gradients: 1–60% (B) over 25 min in general, 10–80% (B) over 25 min for the experiment of Figure 5, 1–25% (B) over 25 min for the experiment of Figure S1 A-ii/iii) at a flow rate of 1 mL min^{-1} (40 °C) with detection at 220 nm unless otherwise noted. Preparative HPLC was carried out on a Shimadzu liquid chromatograph Model LC-8A or LC-20AP (Kyoto, Japan) with a YMC-ODS AA12S05-2530WT (30 x 250 mm) and the following solvent systems: (A) 0.1% TFA in H_2O and (B) 0.1% TFA in CH_3CN (linear gradients: 1-60% (B) over 100 min for BNP(1-32)) at a flow rate of 20 mL min^{-1} (room temperature) with detection at 220 nm. Mass spectra were observed with an Agilent G1956B LC/MSD detector using an Agilent 1100 series HPLC system; observed masses (most abundant masses) were derived from the observed m/z values for each protonation state of a target peptide.

c. SPPS

Automated Fmoc-SPPS was performed on an ABI 433A (Applied Biosystems, USA) peptide synthesizer using the standard method. The following side-chain-protected amino acids were employed: Arg(Pbf), Asn(Trt), Asp(O*t*Bu), Cys(Trt), Gln(Trt), Glu(O*t*Bu), His(Trt), Lys(Boc), Ser(*t*Bu), Thr(*t*Bu), Trp(Boc), Tyr(*t*Bu). In addition, the following protected amino acids were employed as N-terminal amino acids: Boc-Leu-OH (for peptide **1**), Boc- Ser(*t*Bu)-OH (for BNP(1-25)-MeNbz-Leu-NH_2 (**4**)), Boc-His(Trt)-OH (for peptide **9**).

d. Sequences of the target proteins

BNP(1-32): H-**SPKMVQGSGC**[10] **FGRKMDRISS**[20] **SSGLGCKVLR**[30] **RH**-OH,

Model peptide **11**: H-**HPGSRIVLSL**[10] **DVPIGLLQIL**[20] **LEQARARCAR**[30] **EQATTNARIL**[40] **ARV**-NH_2

Syntheses of fragments for model peptides

*H-**LYRANA**-MeNbz-**L**-NH_2* (**1a**)

Peptide was assembled on an Fmoc-Ala-MeNbz-Leu-Rink Amide resin (0.10 mmol) using automated Fmoc-SPPS procedure as described in general information. After Fmoc SPPS, the protected peptide on the resin is activated by 4-nitrophenyl chloroformate under reported conditions.[1] Then, deprotection with TFA/TIS/H_2O

(v/v, 95/2.5/2.5) for 2 h gave a crude product, which was purified by preparative HPLC to yield **1a** [70 mg, 0.057 mmol, 57% (calculated as **1a**·2TFA, M_{calcd}: 1221)]. Analytical HPLC: purity was 97% (t_R = 15.5 min); LRMS (ESI+) calcd $[M+H]^+$ 993.5, found 993.5.

*H-**LYRANF**-MeNbz-NH₂* (**1b**)

Peptide **1b** was prepared by the method similar to that described for **1a** (33 mg). Analytical HPLC: purity was 92% (t_R = 16.1 min); LRMS (ESI+) calcd $[M+H]^+$ 956.5, found 956.4.

*H-**LYRANN**-MeNbz-**L**-NH₂* (**1c**)

Peptide **1c** was prepared by the method similar to that described for **1a** (26 mg). Analytical HPLC: purity was 91% (t_R = 14.4 min); LRMS (ESI+) calcd $[M+H]^+$ 1037, found 1037.

*H-**LYRANL**-MeNbz-NH₂* (**1d**)

Peptide **1d** was prepared by the method similar to that described for **1a** (30 mg). Analytical HPLC: purity was 96% (t_R = 15.6 min); LRMS (ESI+) calcd $[M+H]^+$ 922.5, found 922.5.

*H-**LYRANV**-MeNbz-**L**-NH₂* (**1e**)

Peptide **1e** was prepared by the method similar to that described for **1a** (69 mg). Analytical HPLC: purity was 98% (t_R = 17.1 min); LRMS (ESI+) calcd $[M+H]^+$ 1022, found 1022.

*H-**CSPGYS**-NH₂* (**2**)

Peptide **2** was prepared by the method similar to that described in ref. 2 (110 mg). Analytical HPLC: purity was 97% (t_R = 9.0 min); LRMS (ESI+) calcd $[M+H]^+$ 612.2, found 612.2.

Syntheses of fragments of BNP(1-32)

BNP(1-25)-MeNbz-Leu-NH₂ (**4**)

Peptide **4** was prepared by the method similar to that described for **1a** (28 mg). Analytical HPLC: purity was 96% (t_R = 17.1 min); LRMS (ESI+) calcd 2858, found 2858.

BNP(26-32) (**5**)

Peptide **5** was prepared by the method similar to that described in ref. 3 (71 mg). Analytical HPLC: purity was 97% (t_R = 11.0 min); LRMS (ESI+) calcd $[M+H]^+$ 911.5, found 911.5.

Syntheses of fragments of Model peptide 11

*H-**HPGSRIVLSL**^{10} **DVPIGLLQIL**^{20} **LEQARAR**-MeNbz-**L**-NH₂* (**9**)

Peptide **9** was prepared by the method similar to that described in ref. 4 (47 mg). Analytical HPLC: purity was 95% (t_R = 18.8 min); LRMS (ESI+) calcd 3251, found 3251.

*H-**CAR**^{30} **EQATTNARIL**^{40} **ARV**-NH_2* (**10**)

Peptide **10** was prepared by the method similar to that described in ref. 4 (168 mg). Analytical HPLC: purity was 92% (t_R = 10.0 min); LRMS (ESI+) calcd 1771, found 1771.

General procedure of kinetics investigation of triazole-aided NCL

Peptide-MeNbz **1** (3.0 mM) and Cys-peptide **2** (2.0 mM) were dissolved in a ligation buffer (6.0 M Gn·HCl, 30 mM TCEP, 0.10 M Na_2HPO_4, pH 7.1) in the presence of various additives. In case of NCL using imidazole, a ligation buffer (6.0 M Gn·HCl, 30 mM TCEP, pH 7.1) was used. The reaction mixture was incubated at 37 °C. For each reaction, 5.0 μL aliquots were withdrawn from the reaction mixture at each time point and quenched with 45 μL of 5% TFA aq. and 5.0 μL of neutral TCEP solution (0.50 M, Sigma-Aldrich), and analyzed by analytical HPLC. The extent of ligation was quantified by the unreacted Cys-peptide **2** and the desired ligated product **3** with detection at 280 nm.

Figures

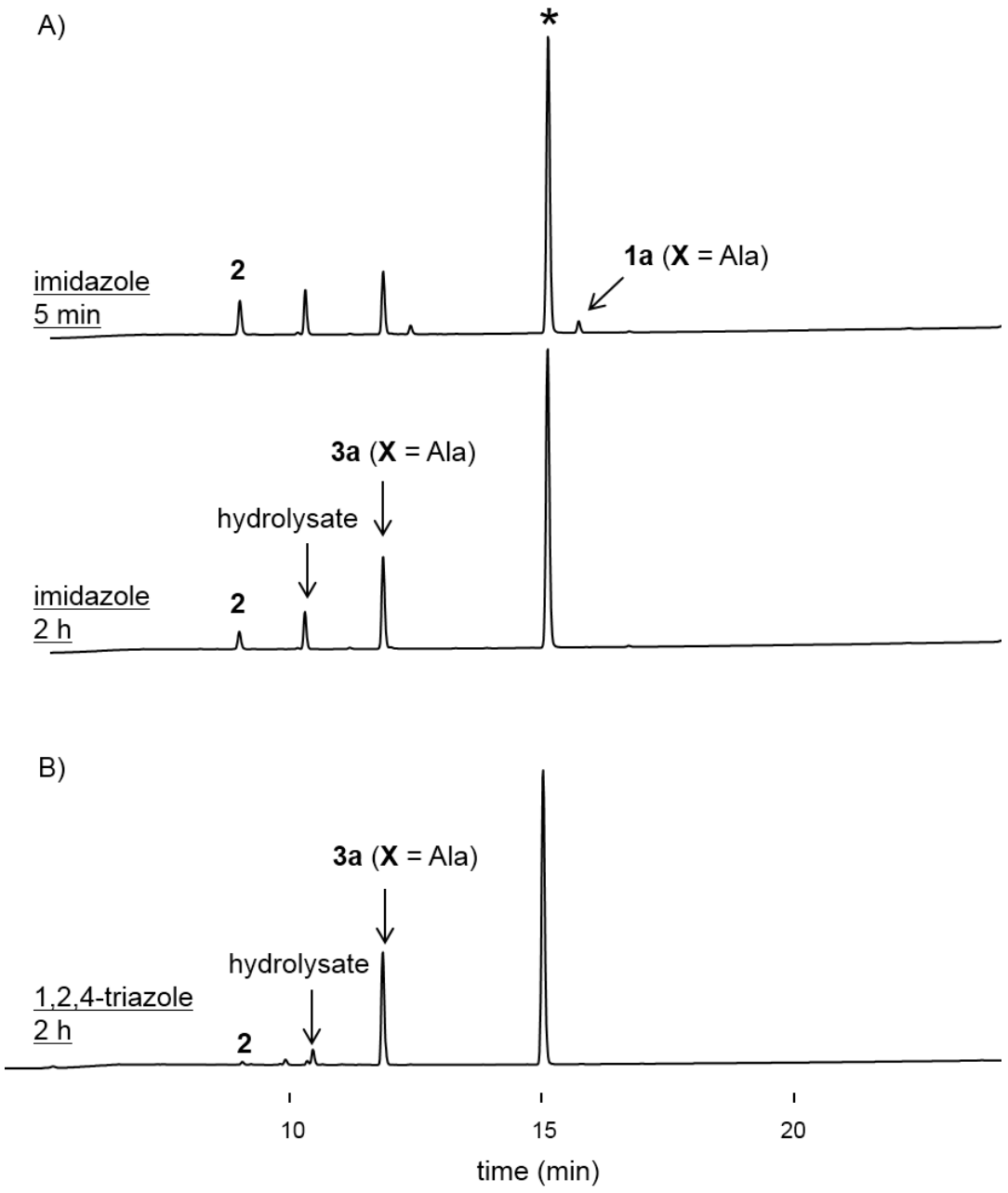


Figure S1. Comparison of efficiency of imidazole and 1,2,4-triazole for NCL using peptide-MeNbz. The NCL was performed between peptide-MeNbz (H-**LYRANA**-MeNbz-**L**-NH_2 (**1a**), 3.0 mM) and Cys-peptide (H-**CSPGYS**-NH_2 (**2**), 2.0 mM). In the case of imidazole-aided NCL (A), much amount of hydrolysate of peptide-MeNbz **1a** was observed compared to the case of 1,2,4-triazole-aided NCL (B) when the reaction is stopped. In other words, in the case of imidazole-aided NCL, peptide-MeNbz was exhausted before Cys-peptide is ligated to afford the desired product. *MeNbz-Leu-NH_2.

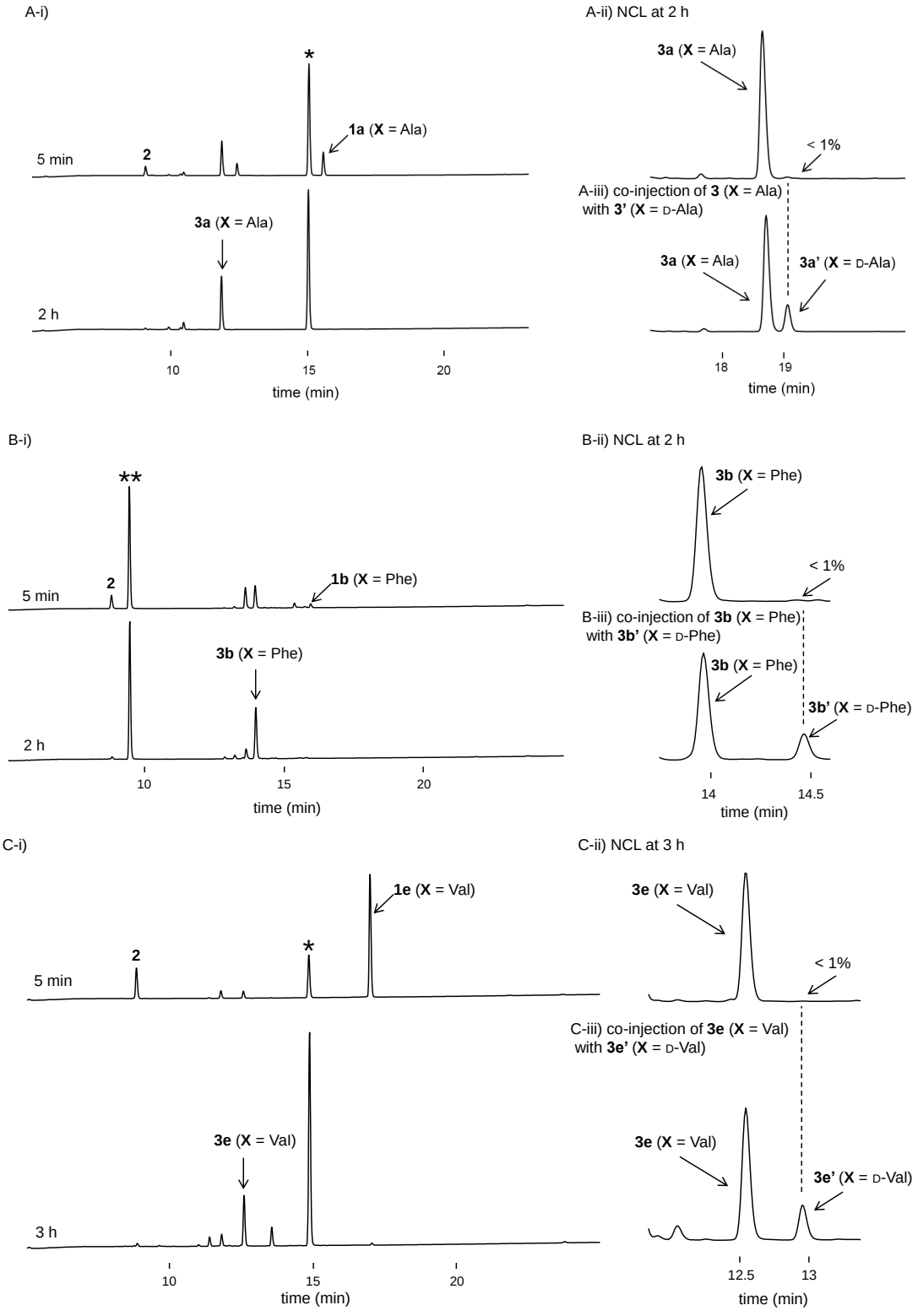


Figure S2. A,B,C-i) Representative HPLC traces of 1,2,4-triazole-aided NCL between peptide-MeNbz [H-**LYRANX**-MeNbz, A) **1a** (**X** = Ala); B) **1b** (**X** = Phe); C) **1e** (**X** = Val)] and Cys-peptide (H-**CSPGYS**-NH_2 (**2**)). A,B,C-ii, iii) Rate of epimerization was evaluated using the authentic corresponding epimer [ii) closeup view of HPLC chart, iii) co-injection with the authentic epimers]. * MeNbz-Leu-NH_2. **MeNbz-NH_2.

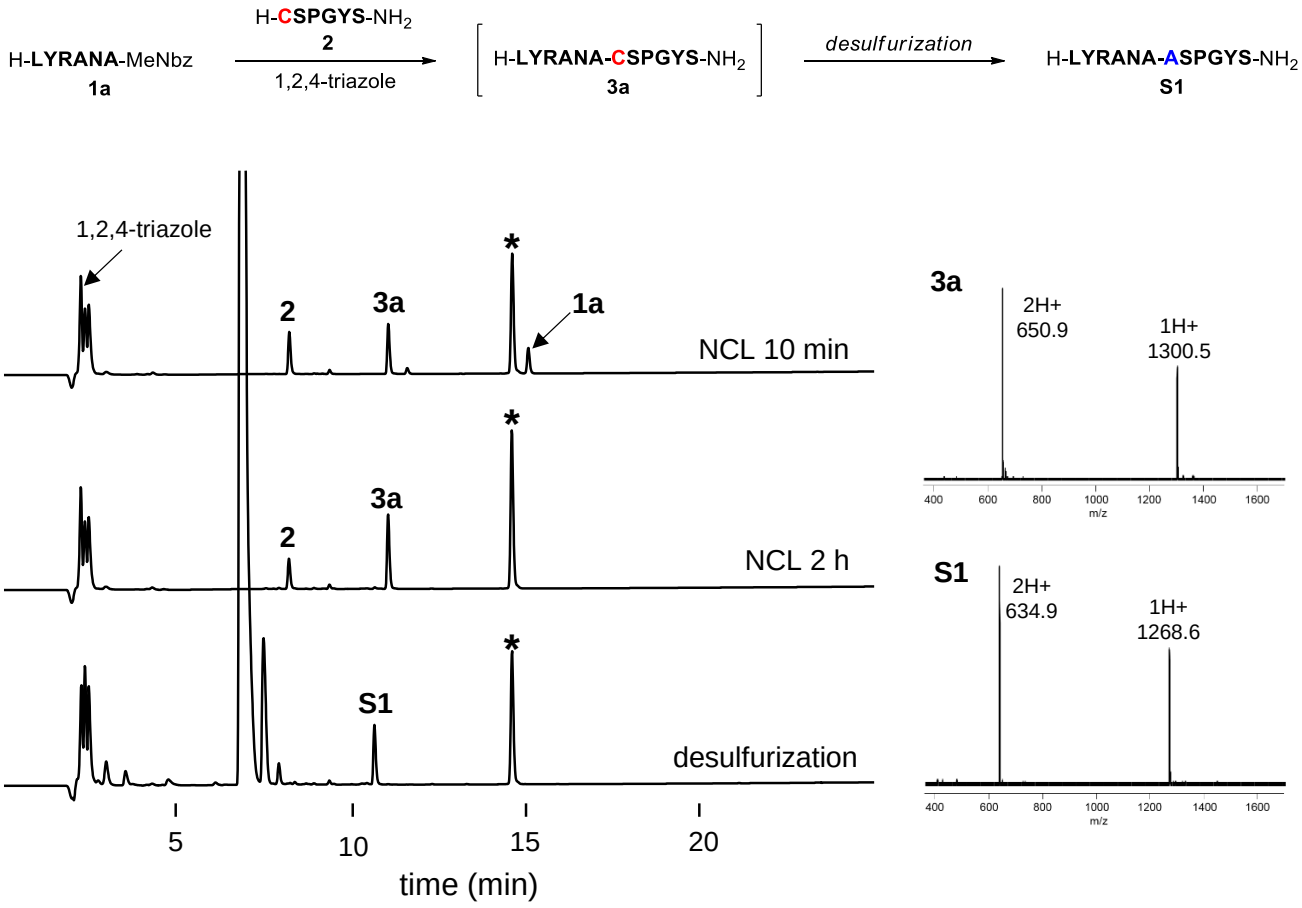
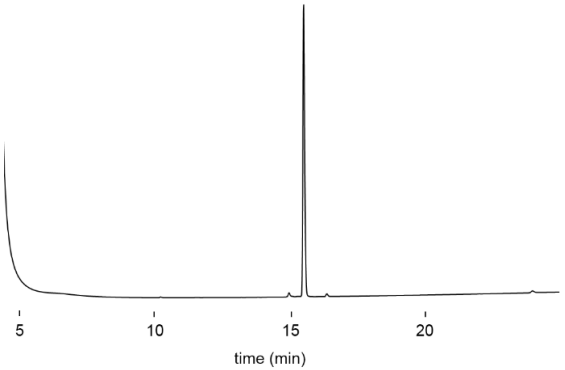


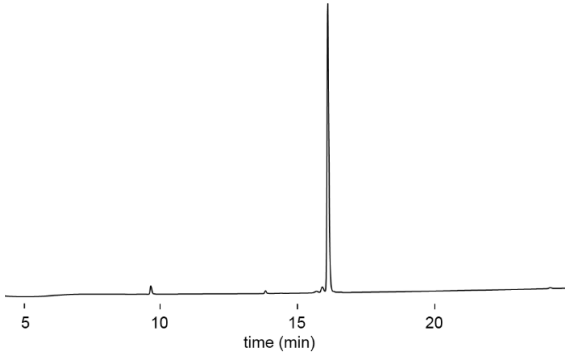
Figure S3. HPLC profiles and ESI MS spectra of one-pot desulfurization after thiol-additive-free NCL using 1,2,4-triazole: peptide-MeNbz (H-**LYRANA**-MeNbz-**L**-NH_2 (**1a**), 2.0 mM) and Cys-peptide (H-**CSPGYS**-NH_2 (**2**), 2.4 mM) were dissolved in a ligation buffer (3.0 M Gn·HCl, 30 mM TCEP, 5.0 M 1,2,4-triazole, pH 7.1), and the solution was incubated for 3 h. Then, to the ligation reaction mixture were added an equal volume of desulfurization buffer (6.0 M Gn·HCl, 0.10 M Na_2HPO_4, 450 mM TCEP, 80 mM glutathione, pH 4.4) containing VA-044 (final 50 mM). Then, the solution was incubated at 37 °C for 3h. *MeNbz-Leu-NH_2.

HPLC charts of purified peptides

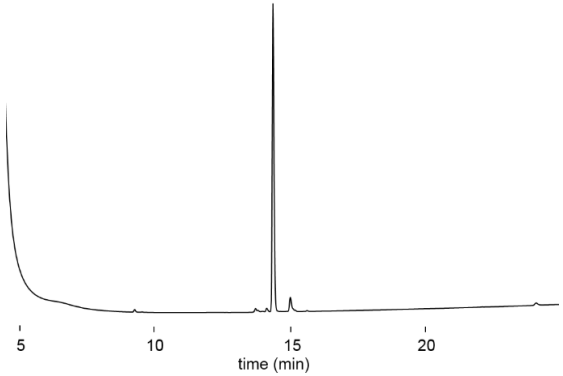
*H-**LYRANA**-MeNbz-**L**-NH$_2$* (**1a**)



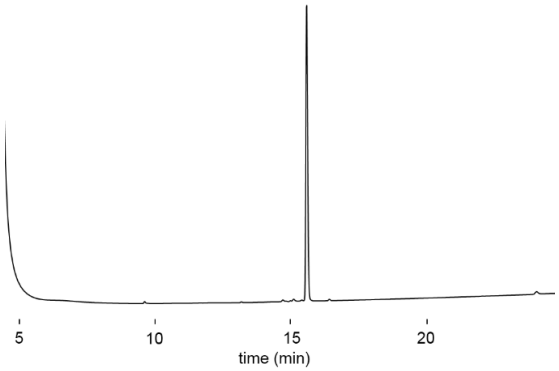
*H-**LYRANF**-MeNbz-NH$_2$* (**1b**)



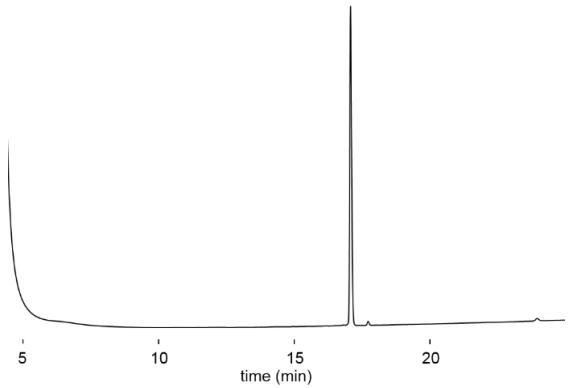
*H-**LYRANN**-MeNbz-**L**-NH$_2$* (**1c**)



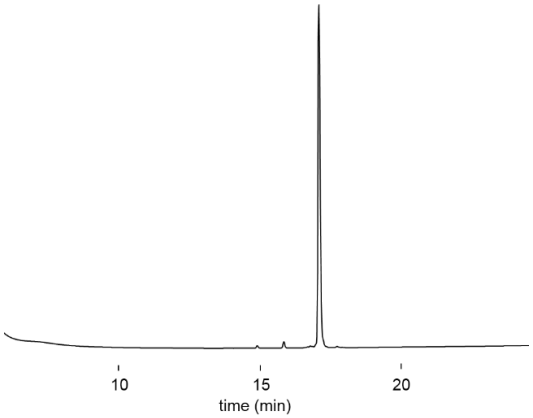
*H-**LYRANL**-MeNbz-NH$_2$* (**1d**)



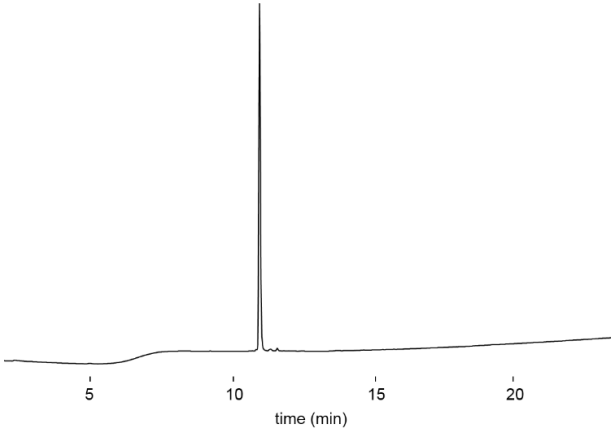
*H-**LYRANV**-MeNbz-**L**-NH$_2$* (**1e**)



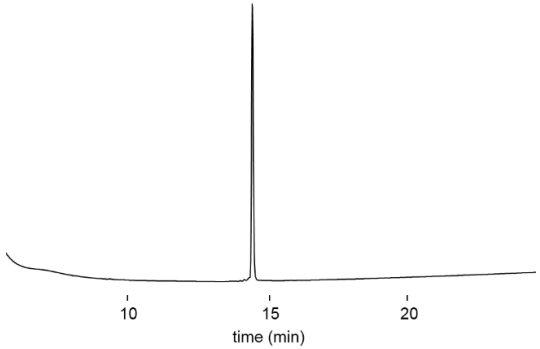
BNP(1-25)-MeNbz-Leu-NH_2 (**4**)



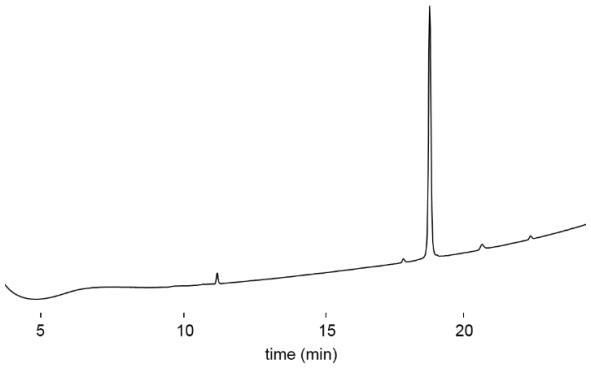
BNP(26-32) (**5**)



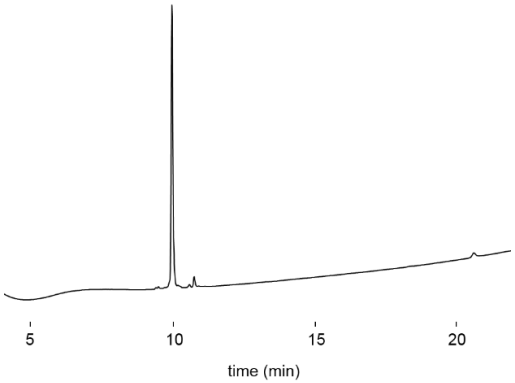
BNP(1-32) (**7**)



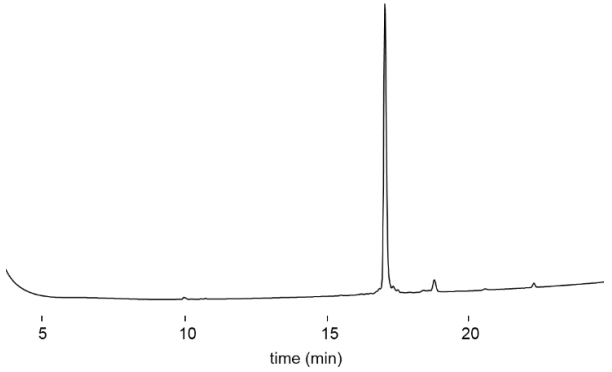
*H-**HPGSRIVLSL**^{10} **DVPIGLLQIL**^{20} **LEQARAR**-MeNbz-**L**-NH_2* (**9**)



*H-**CAR**^{30} **EQATTNARIL**^{40} **ARV**-NH_2* (**10**)

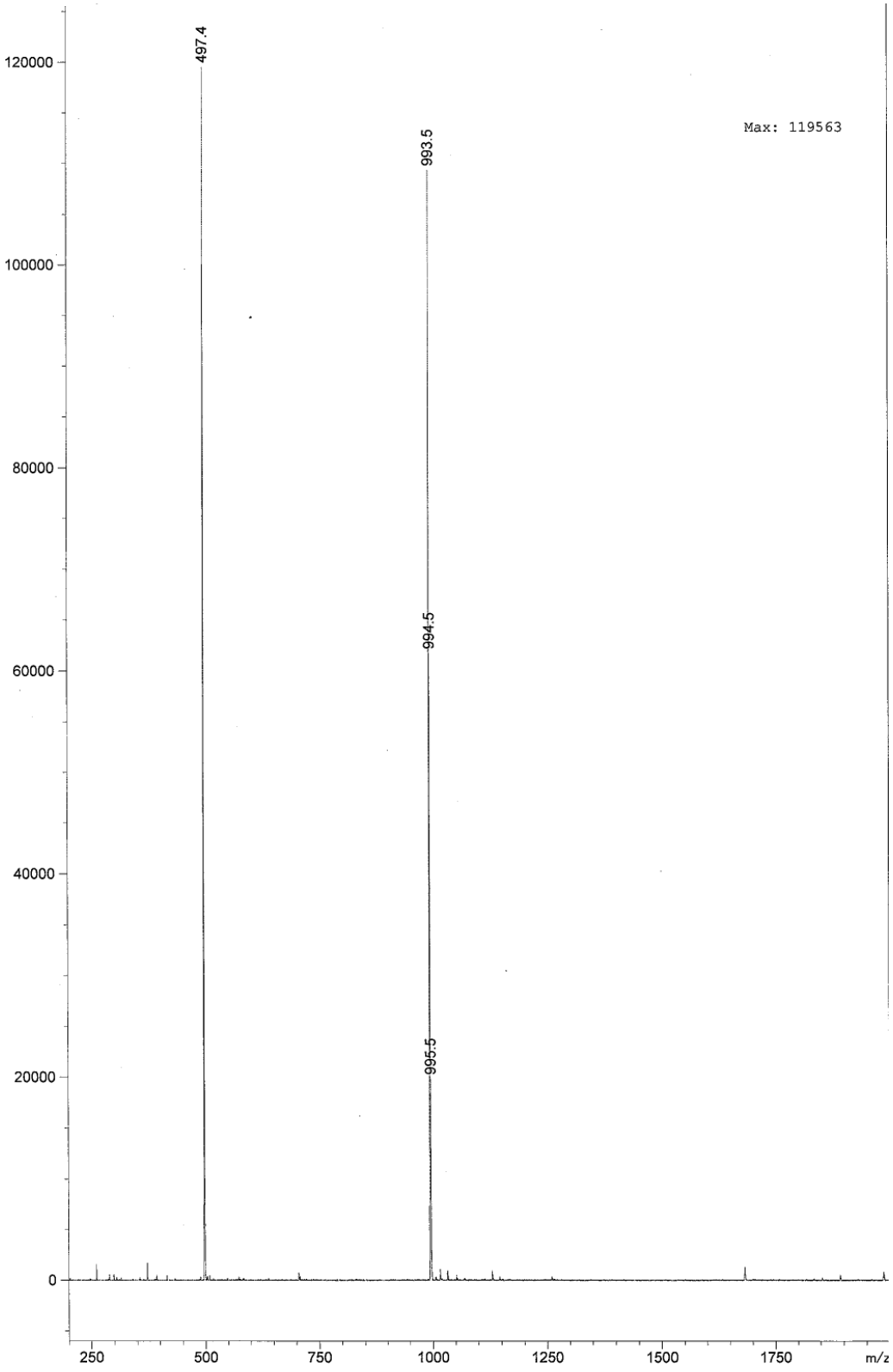


*Model peptide **11***

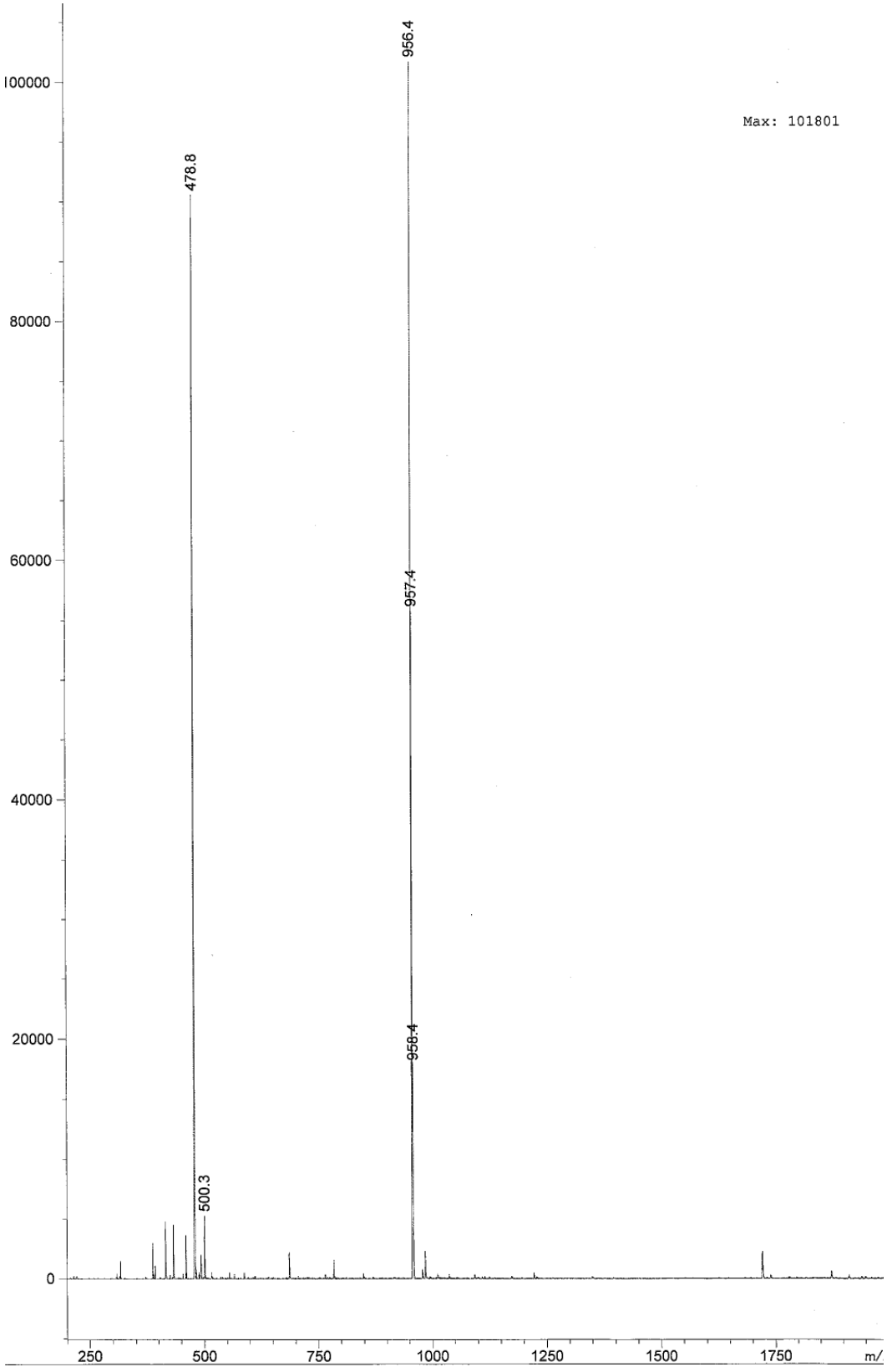


ESI-MS spectra of purified peptides

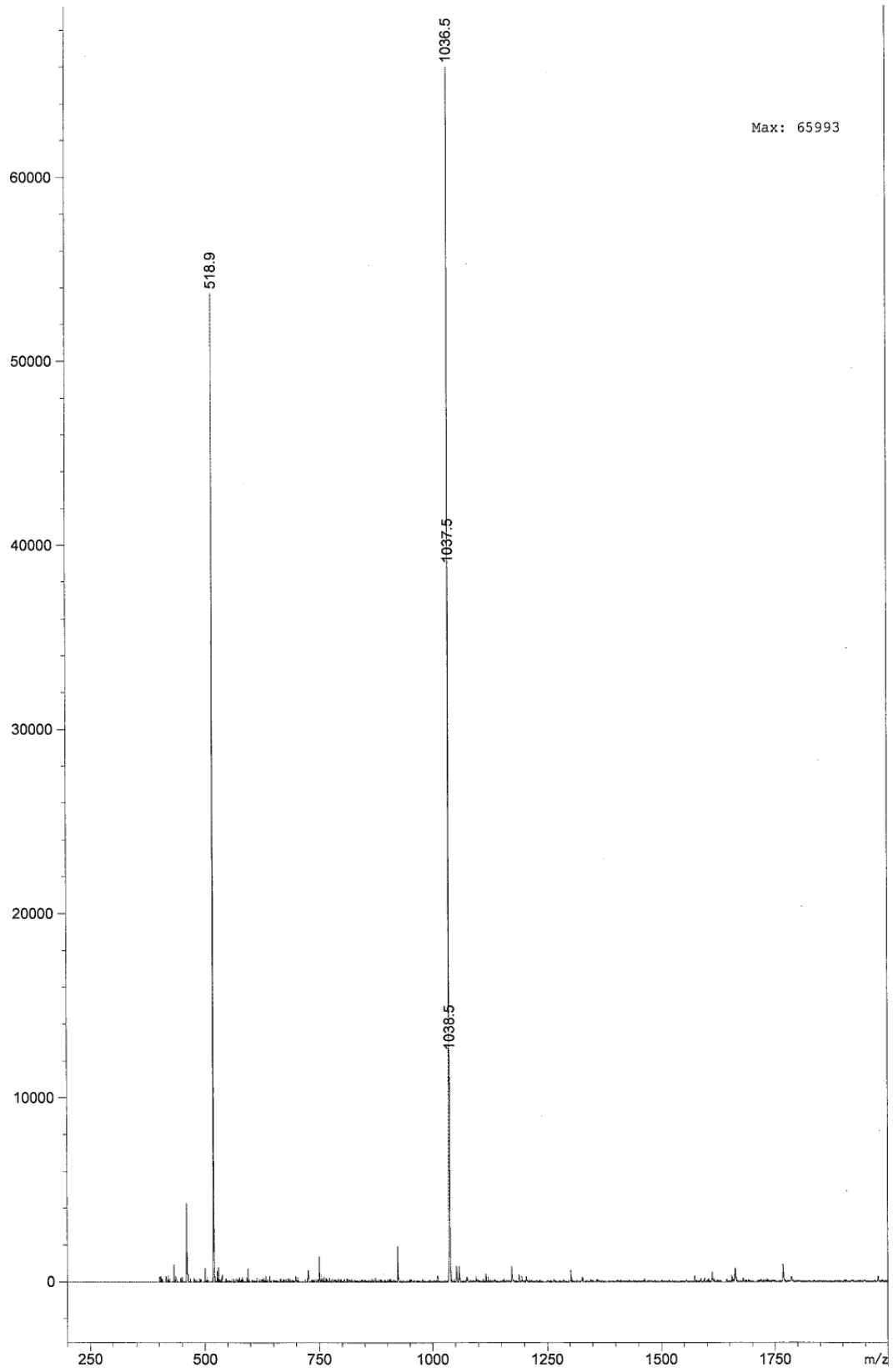
*H-**LYRANA**-MeNbz-**L**-NH₂* (**1a**)



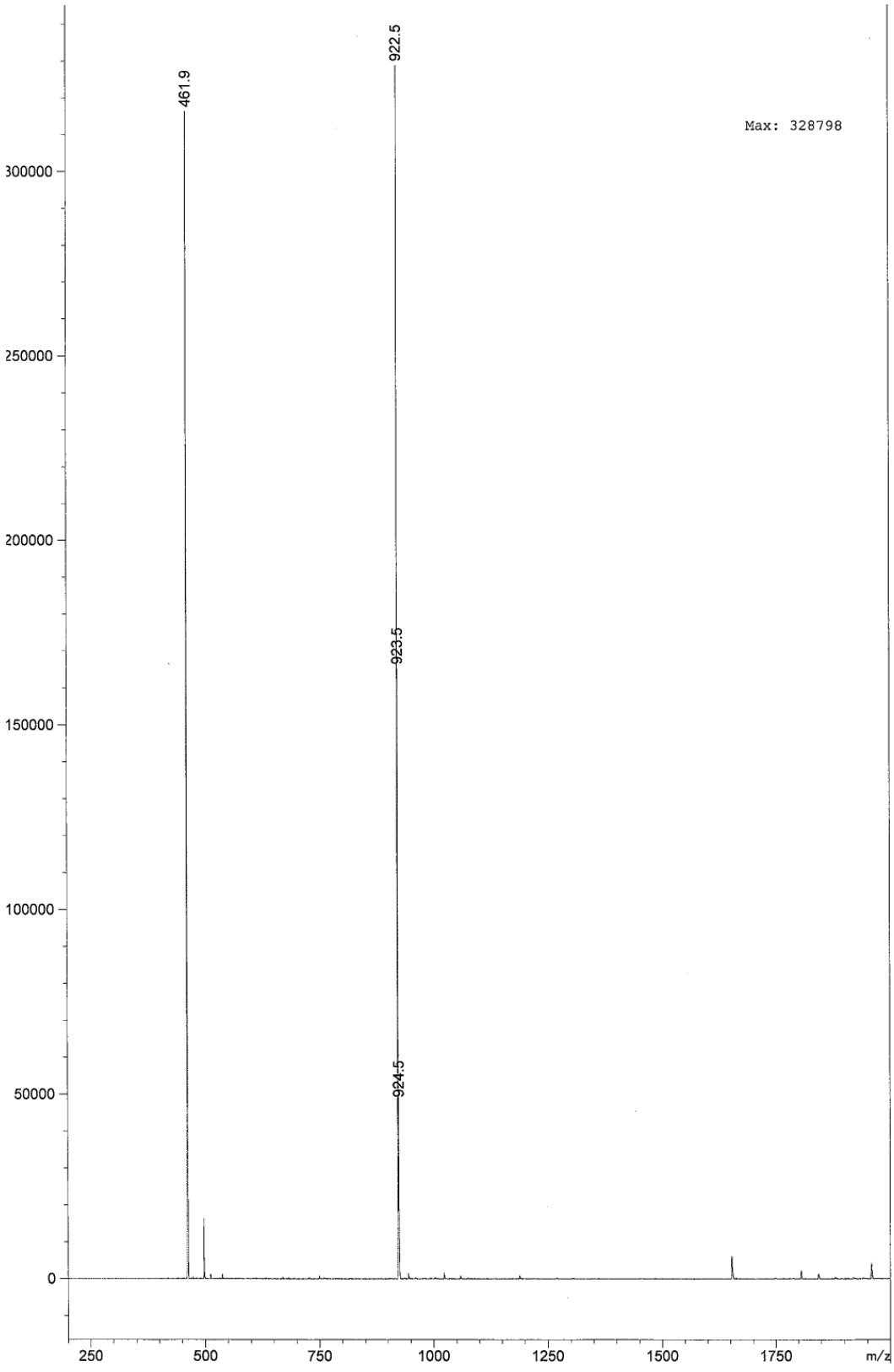
*H-**LYRANF**-MeNbz-NH₂* (**1b**)



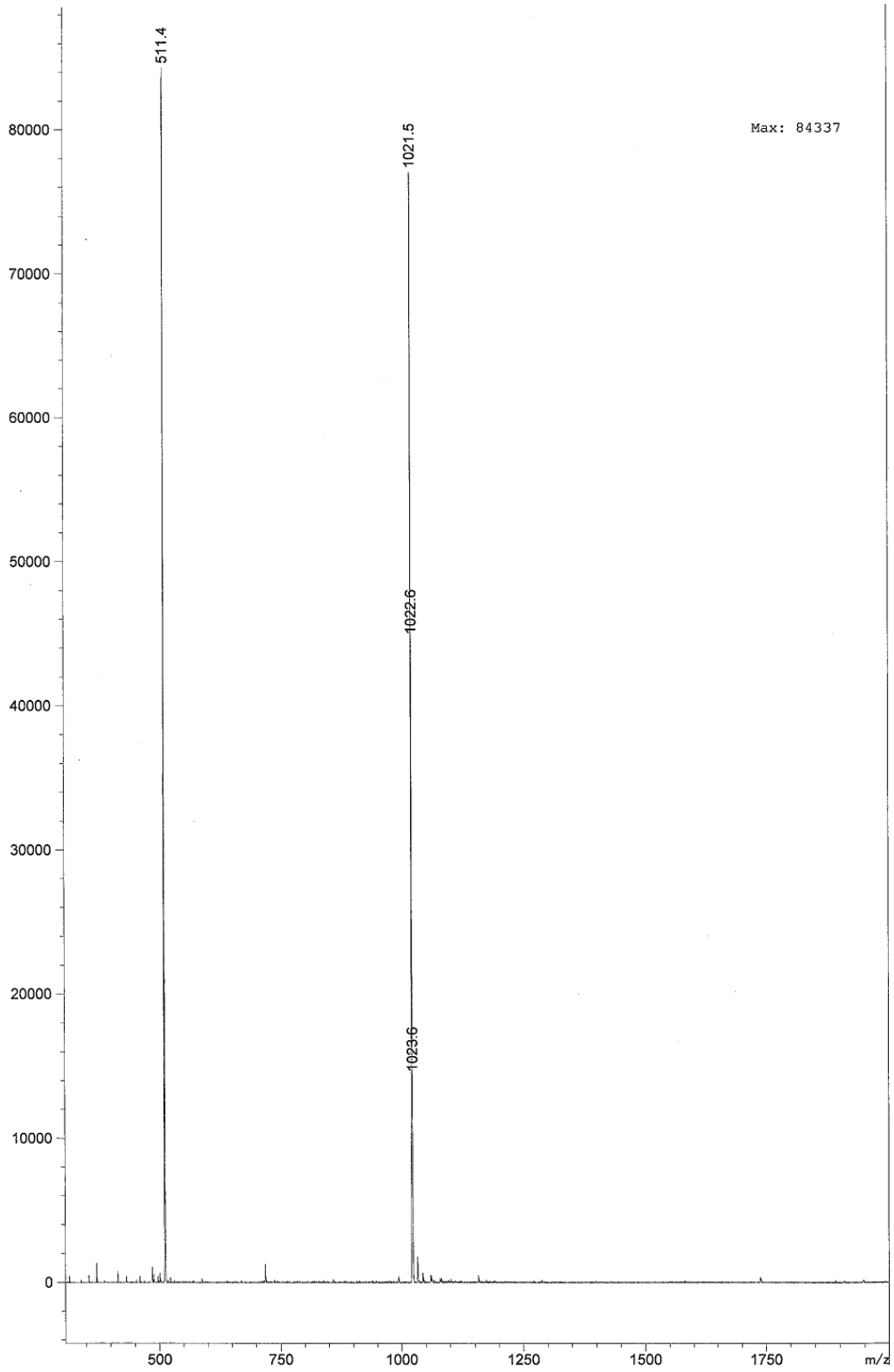
*H-**LYRANN**-MeNbz-**L**-NH₂* (**1c**)



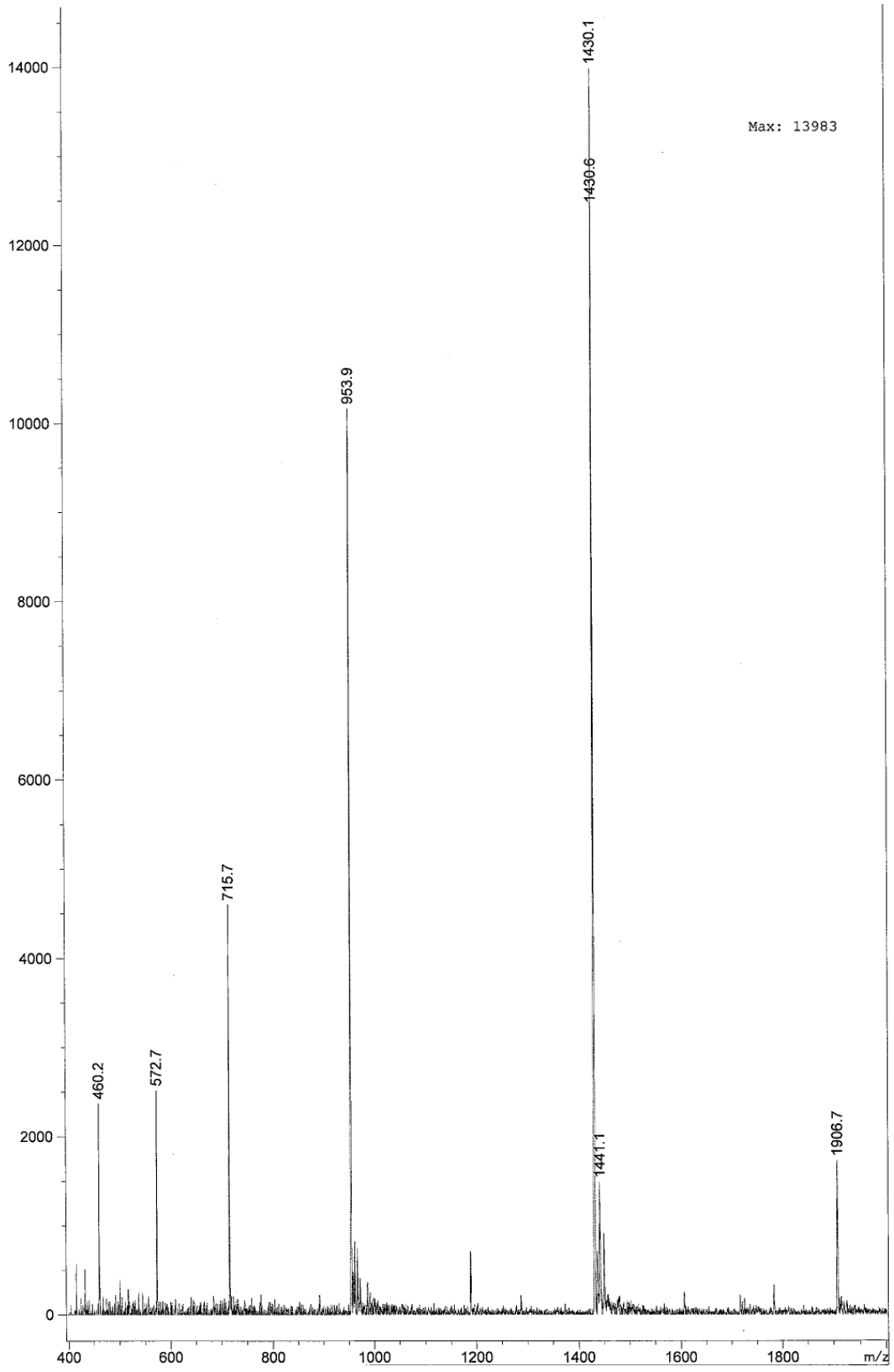
*H-**LYRANL**-MeNbz-NH₂* (**1d**)



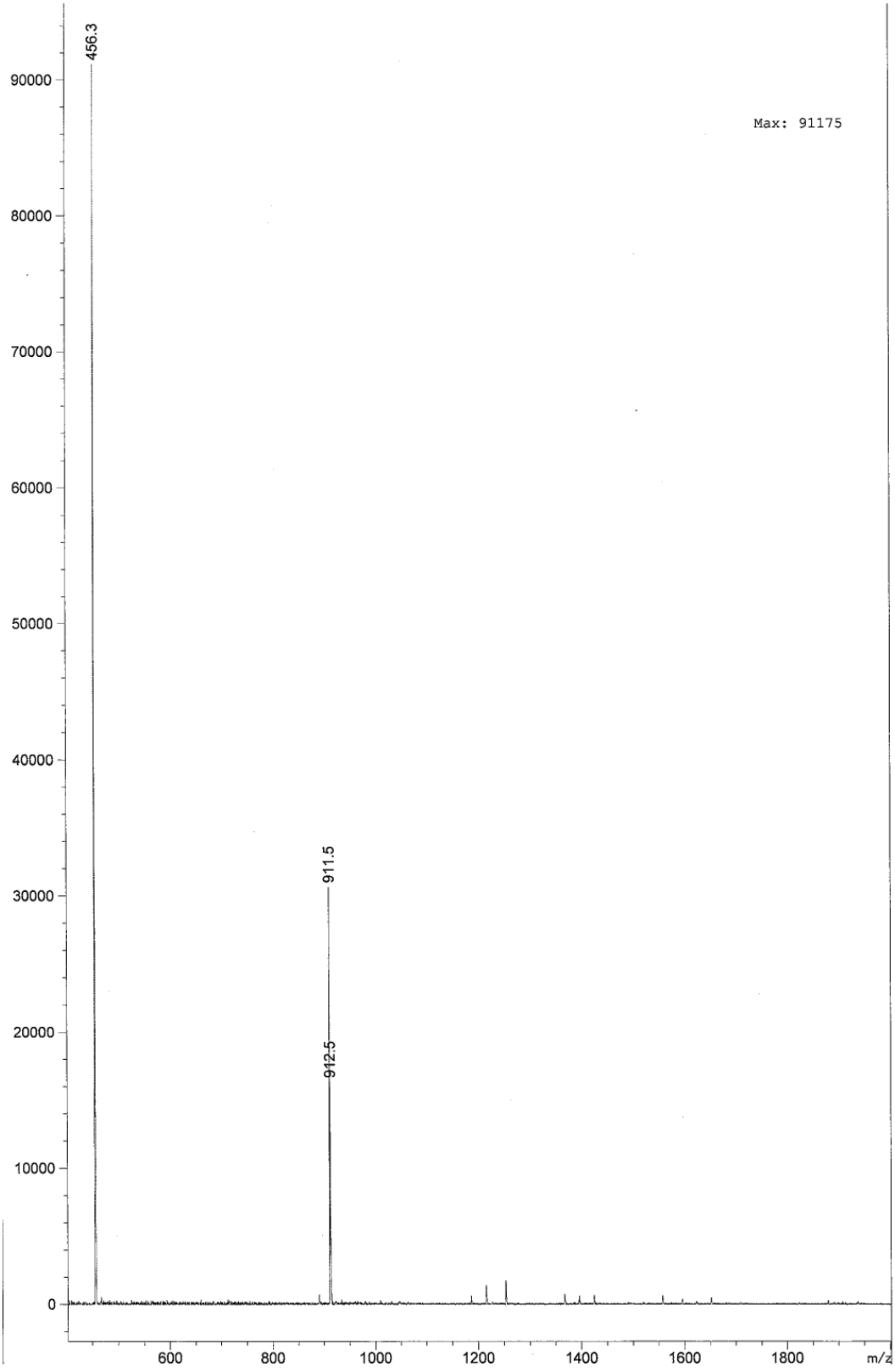
*H-**LYRANV**-MeNbz-**L**-NH₂* (**1e**)



BNP(1-25)-MeNbz-Leu-NH_2 (**4**)



BNP(26-32) (**5**)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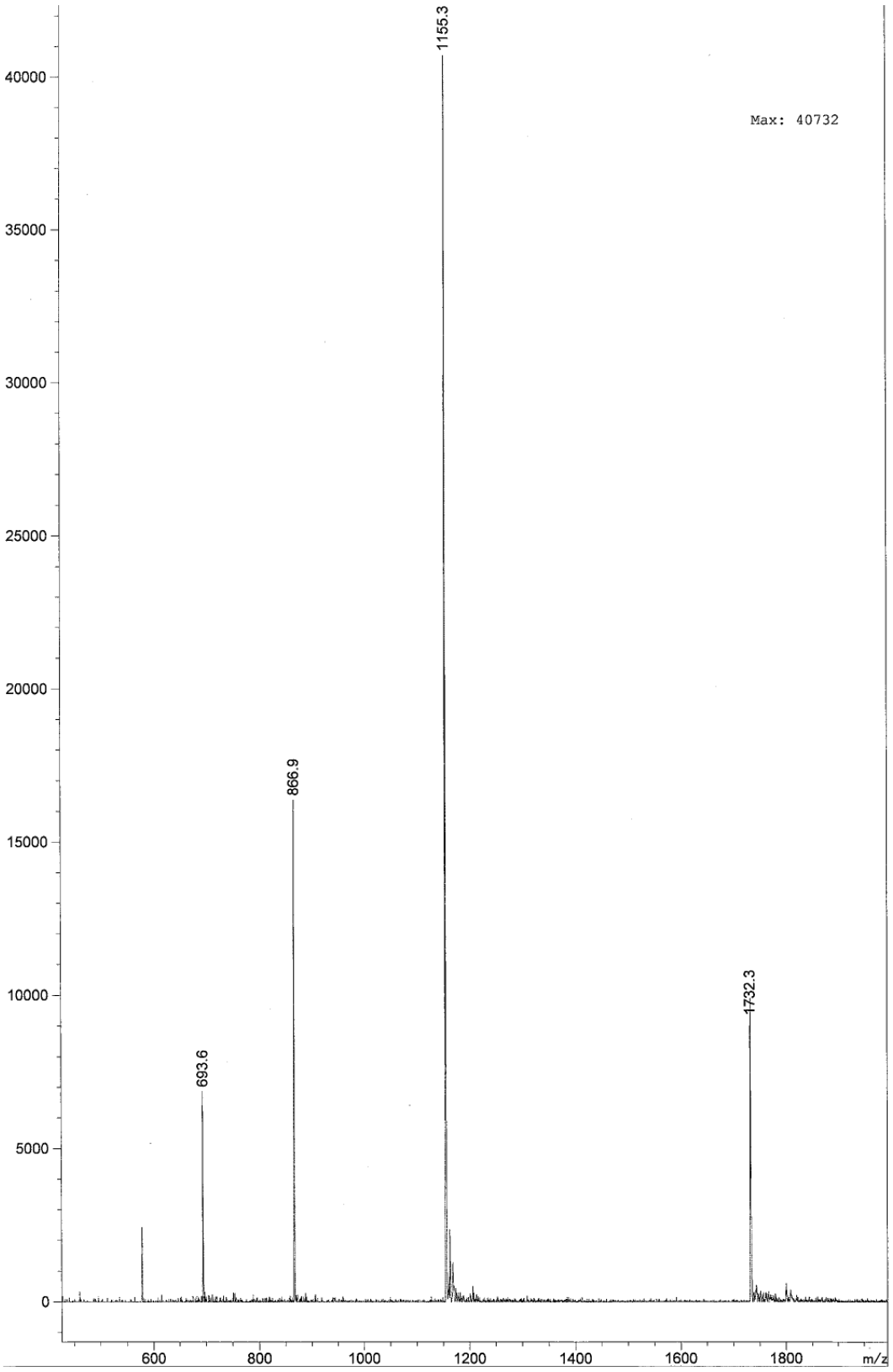
BNP(1-32) (**7**)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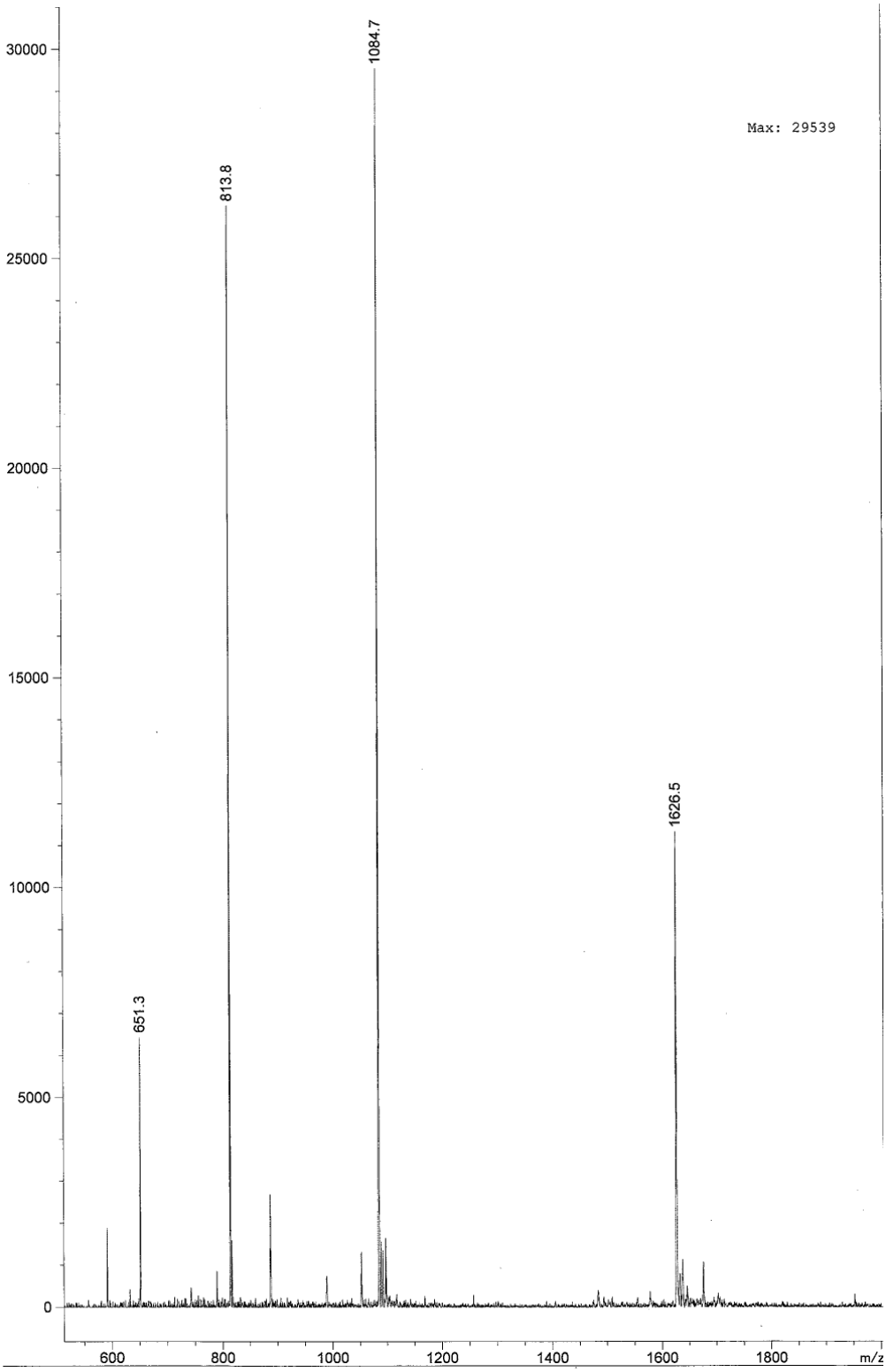
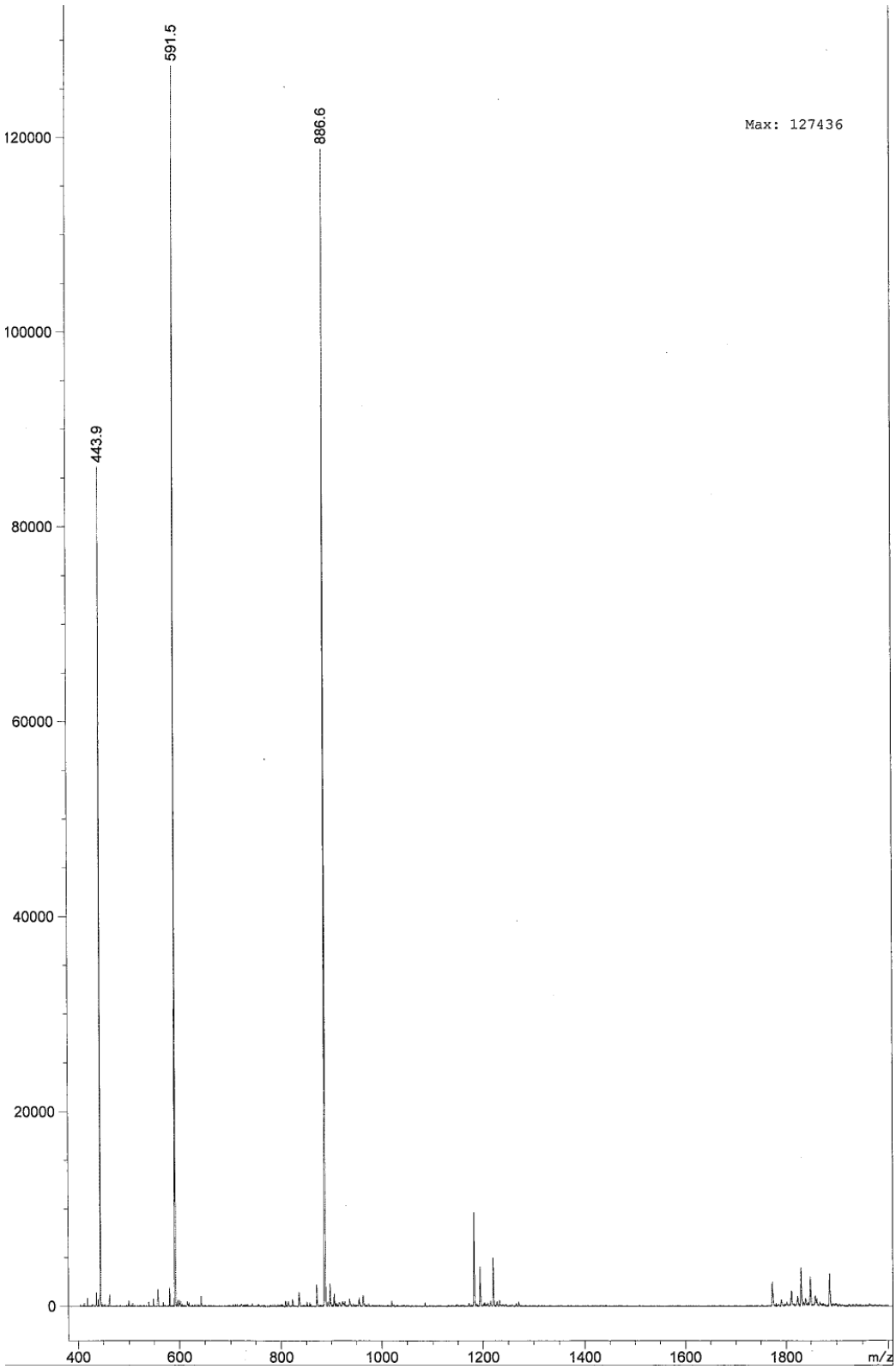
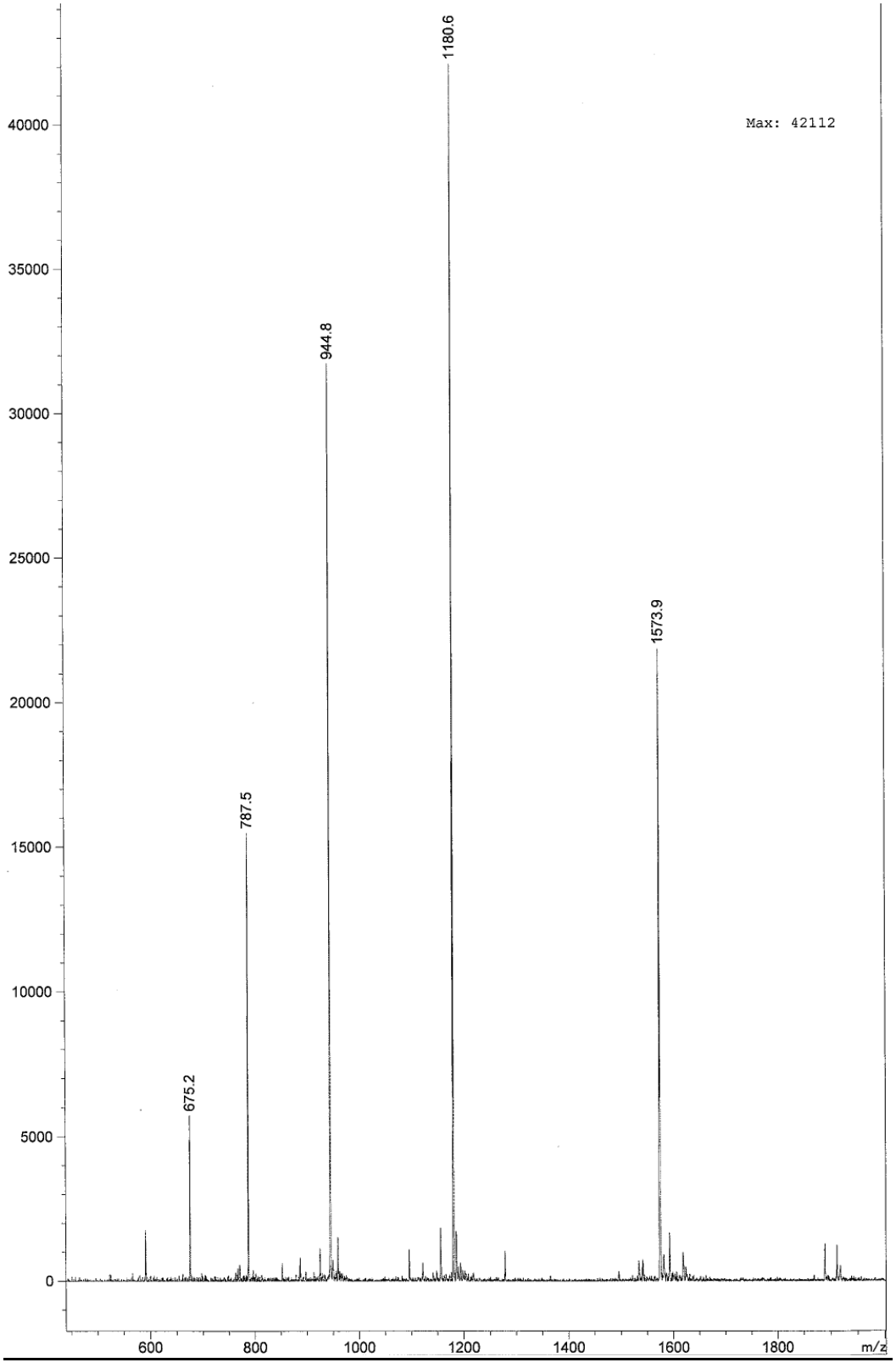
*H-**HPGSRIVLSL**[10] **DVPIGLLQIL**[20] **LEQARAR**-MeNbz-**L**-NH_2* (**9**)



*H-**CAR**^{30} **EQATTNARIL**^{40} **ARV**-NH_2* (**10**)



*Model peptide **11***



References

[1] J. B. Blanco-Canosa, B. Nardone, F. Albericio and P. E. Dawson, *J. Am. Chem. Soc.*, 2015, **137**, 7197.

[2] K. Sakamoto, S. Tsuda, M. Mochizuki, Y. Nohara, H. Nishio and T. Yoshiya, *Chem. Eur. J.*, 2016, **22**, 17940.

[3] S. Tsuda, M. Mochizuki, K. Sakamoto, M. Denda, H. Nishio, A. Otaka and T. Yoshiya, *Org. Lett.*, 2016, **18**, 5940.

[4] S. Tsuda, M. Mochizuki, H. Nishio and T. Yoshiya, *ChemBioChem*. 2016, **17**, 2133.