

Supporting information

Design, Synthesis and Functional Analysis of Cyclic Opioid Peptides with Dmt-Tic Pharmacophore

Arijit Sarkar ^{1,2,†}, Anna Adamska-Bartłomiejczyk ^{3,†}, Justyna Piekielna-Ciesielska ³, Karol Wtorek ³, Alicja Kluczyk ⁴, Attila Borics ^{1,2} and Anna Janecka ^{3,*}

¹ Laboratory of Chemical Biology, Institute of Biochemistry, Biological Research Centre, Szeged, 62. Temesvári krt., H-6726 Szeged, Hungary; sarkar.arajit@brc.hu (A.S.); borics.attila@brc.hu (A.B.)

² Theoretical Medicine Doctoral School, Faculty of Medicine, University of Szeged, 97. Tisza L. krt., H-6722 Szeged, Hungary

³ Department of Biomolecular Chemistry, Faculty of Medicine, Medical University of Lodz, Mazowiecka 6/8, 92-215 Lodz, Poland; anna.adamska-bartlomiejczyk@umed.lodz.pl (A.A.-B.); justyna.piekielna-ciesielska@umed.lodz.pl (J.P.-C.); karol.wtorek@umed.lodz.pl (K.W.)

⁴ Faculty of Chemistry, University of Wroclaw, F. Joliot-Curie 14, 50-383 Wroclaw, Poland; alicja.kluczyk@chem.uni.wroc.pl

* Correspondence: anna.janecka@umed.lodz.pl; Tel.: +4842 272 57 06

† These authors contributed equally to this work.

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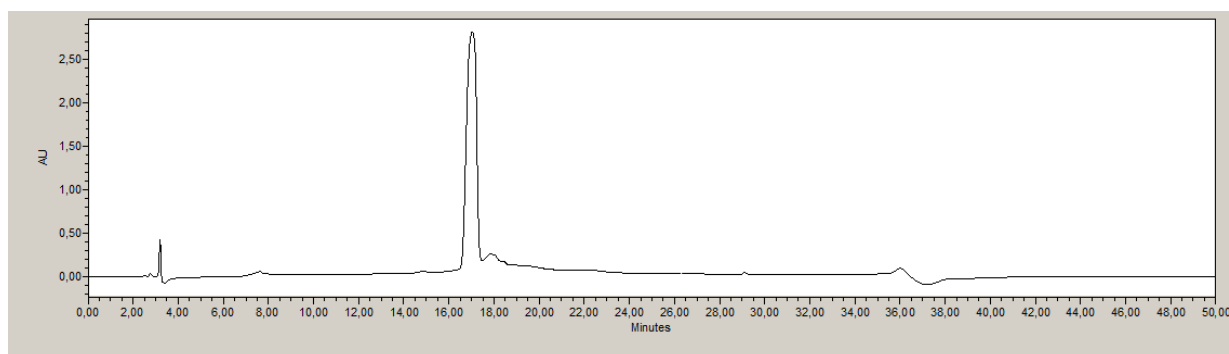


Figure S1. Analytical HPLC chromatogram of peptide Dmt-Tic-c[D-Lys-Phe-Phe-Asp]NH₂ (**1**).

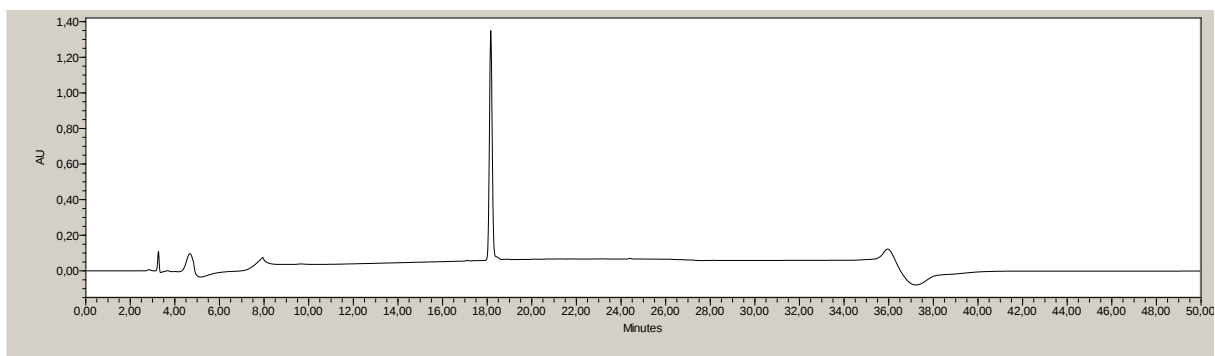


Figure S2. Analytical HPLC chromatogram of peptide Dmt-Tic-c[D-Lys-Phe-D-2Nal-Asp]NH₂ (**2**).

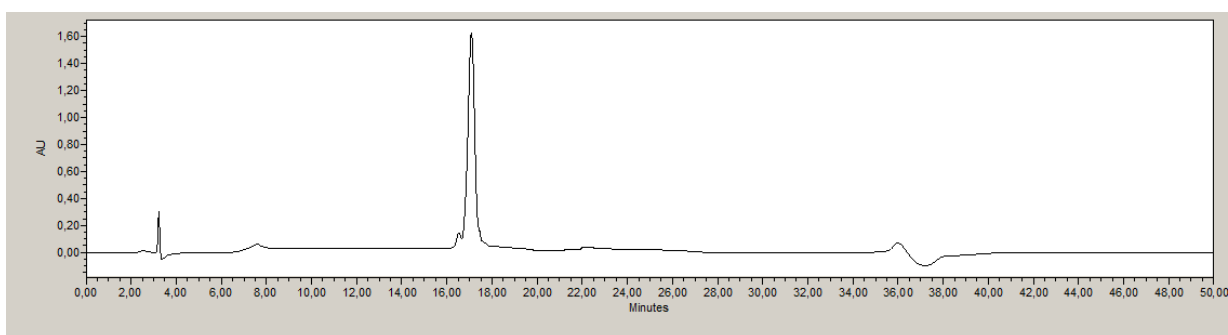


Figure S3. Analytical HPLC chromatogram of peptide Dmt-Tic-c[D-Lys-Phe-2,4F₂-Phe-Asp]NH₂ (**3**).

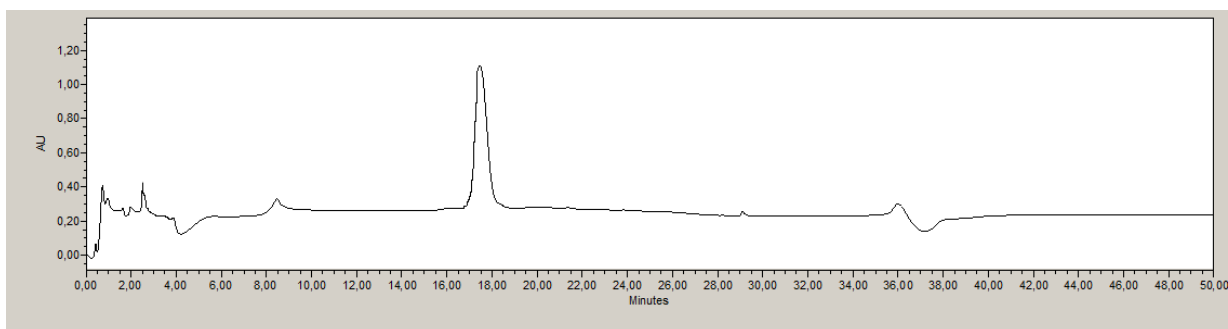


Figure S4. Analytical HPLC chromatogram of peptide Dmt-Tic-c[D-Dap-Phe-Phe-Asp]NH₂ (**4**).

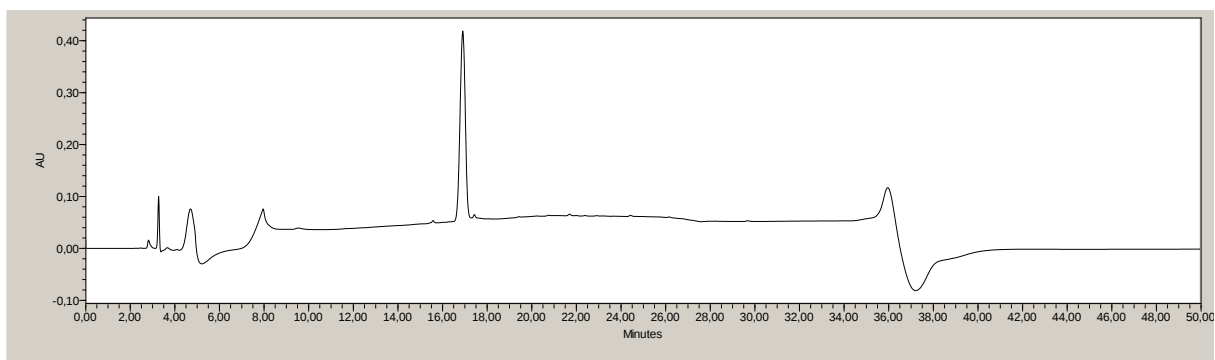


Figure S5 . Analytical HPLC chromatogram of peptide Dmt-Tic-c[D-Lys-Phe-Asp]NH₂ (**5**).

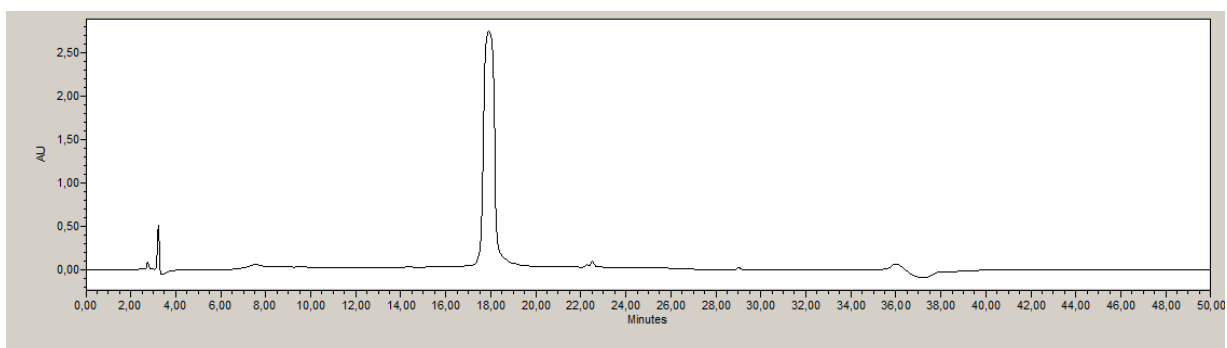


Figure S6. Analytical HPLC chromatogram of peptide Dmt-Tic-c[D-Lys-Phe-Asp]-Tic-Dmt-NH₂ (**6**).

Table S1. Physicochemical data of analogs **1-6**.

No.	Sequence	Formula	m/z [<i>M</i> + <i>H</i>] ^{+a}		HPLC <i>t</i> _R ^b [min]
			Calcd	Obsd	
1	Dmt-Tic-c[D-Lys-Phe-Phe-Asp]NH ₂	C ₄₉ H ₅₉ N ₈ O ₈	887.4450	887.4418	17.04
2	Dmt-Tic-c[D-Lys-Phe-D-2Nal-Asp]NH ₂	C ₅₃ H ₆₁ N ₈ O ₈	937.4606	937.4567	18.16
3	Dmt-Tic-c[D-Lys-Phe-2,4F ₂ -Phe-Asp]NH ₂	C ₄₉ H ₅₆ F ₂ N ₈ O ₈	923.4262	923.4244	17.21
4	Dmt-Tic-c[D-Dap-Phe-Phe-Asp]NH ₂	C ₄₆ H ₅₂ N ₈ O ₈	845.3981	845.3997	17.55
5	Dmt-Tic-c[D-Lys-Phe-Asp]NH ₂	C ₄₀ H ₄₉ N ₇ O ₇	740.3766	740.3760	16.87

6	Dmt-Tic-c[D-Lys-Phe-Asp]-Tic-Dmt-NH ₂	C ₆₁ H ₇₁ N ₉ O ₁₀	1090.5396	1090.5345	17.86
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^a Observed by ESI MS⁺ ionization.

^b Retention time on a Vydac C₁₈ column (4.6 x 250 mm, 5 μm,) using the solvent system of 0.1% TFA in water (A) and 80% acetonitrile in water containing 0.1% TFA (B) and a linear gradient of 0–100% solvent B over 50 min, with a flow rate of 1 mL/min.

High resolution mass spectra were recorded using Shimadzu IT-TOF (ion trap – time-of-flight) mass spectrometer equipped with standard ESI source (Shimadzu, Japan). For CID (collision-induced dissociation) experiments, the singly protonated precursor ions [M+H]⁺ were selected. The collision energy was adjusted to obtain the optimal fragmentation pattern. Argon was used as a collision gas. The obtained fragments were registered as an MS/MS (tandem mass spectrometry) spectrum.

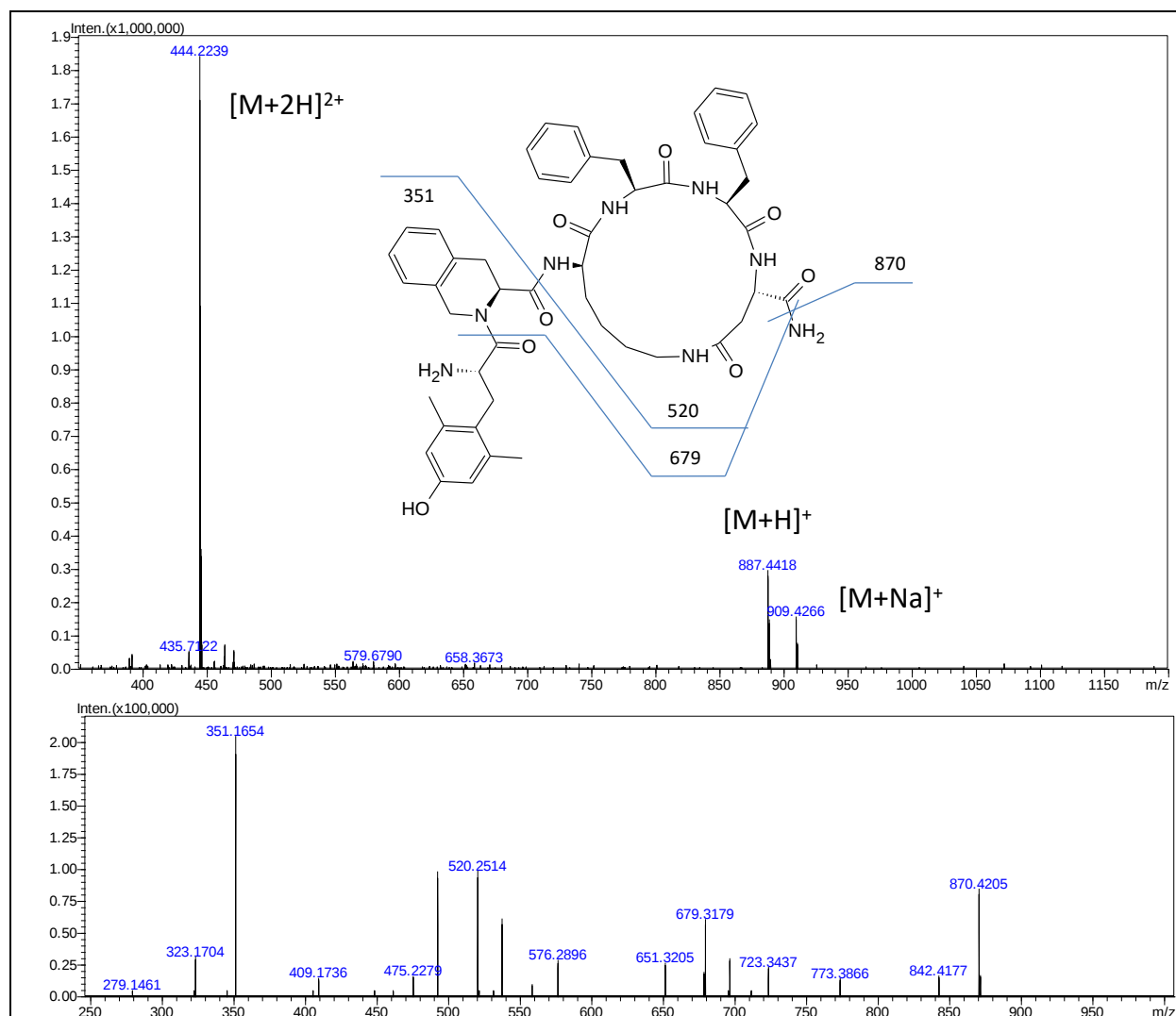


Figure S7. Top panel: High resolution MS spectrum of peptide $\text{Dmt-Tic-c[D-Lys-Phe-Phe-Asp]NH}_2$ (**1**). Bottom panel: High resolution MS/MS spectrum for the $[\text{M}+\text{H}]^+$ ion. In inset, the fragmentation scheme corresponding to MS/MS spectrum is proposed.

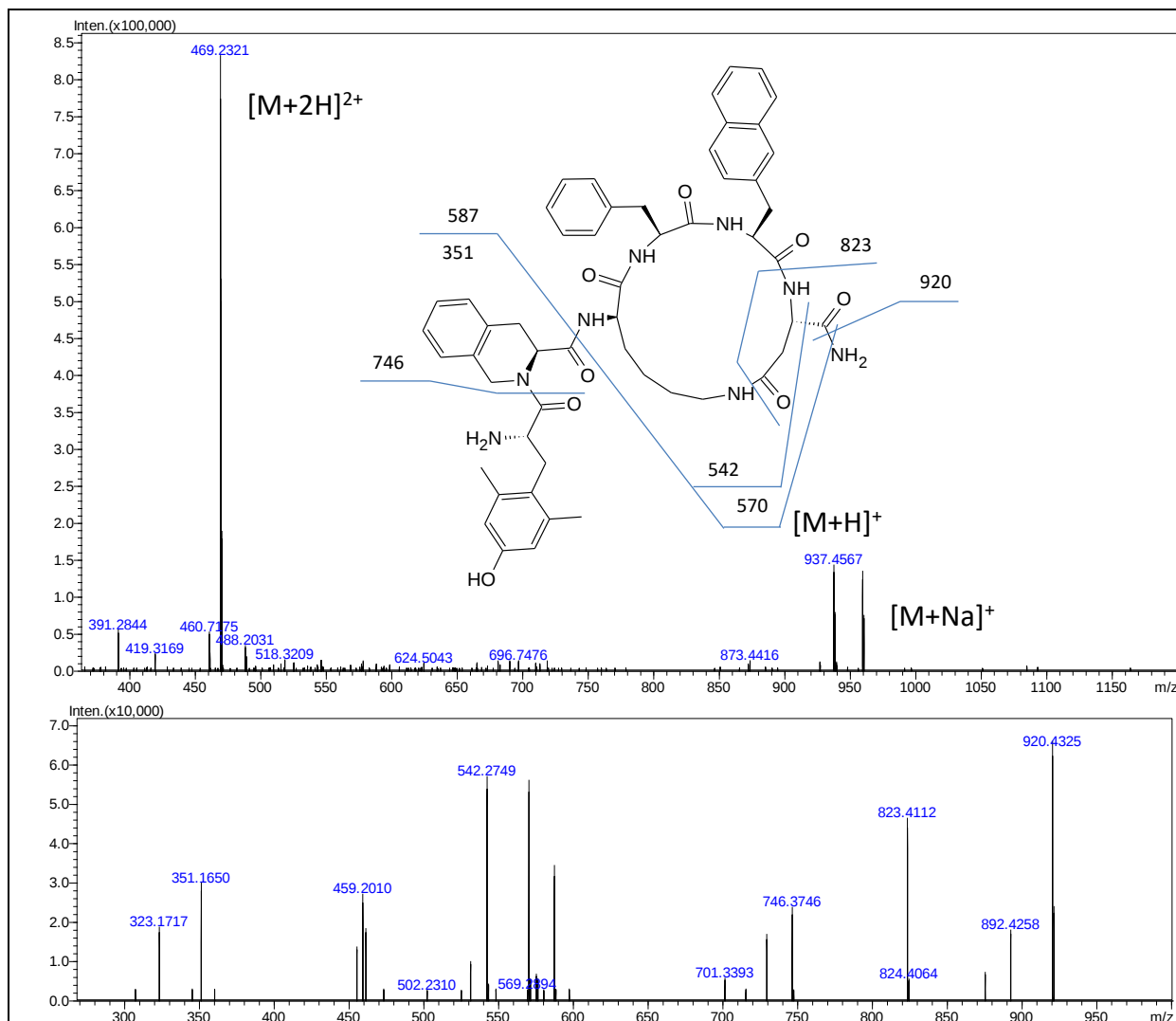


Figure S8. Top panel: High resolution MS spectrum of peptide Dmt-Tic-c[D-Lys-Phe-D-2Nal-Asp]NH₂ (2). Bottom panel: High resolution MS/MS spectrum for the [M+H]⁺ ion. In inset, the fragmentation scheme corresponding to MS/MS spectrum is proposed.

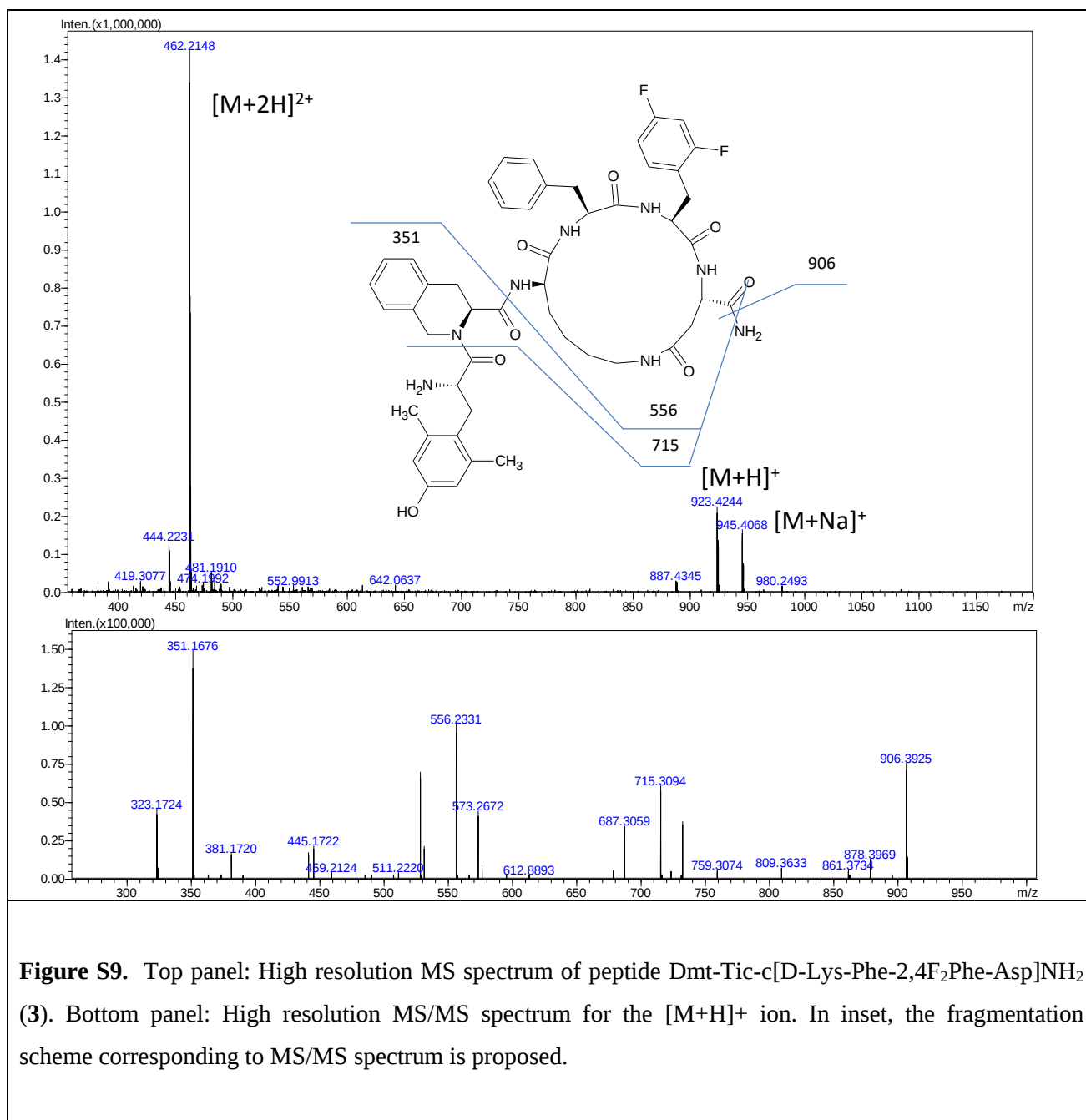
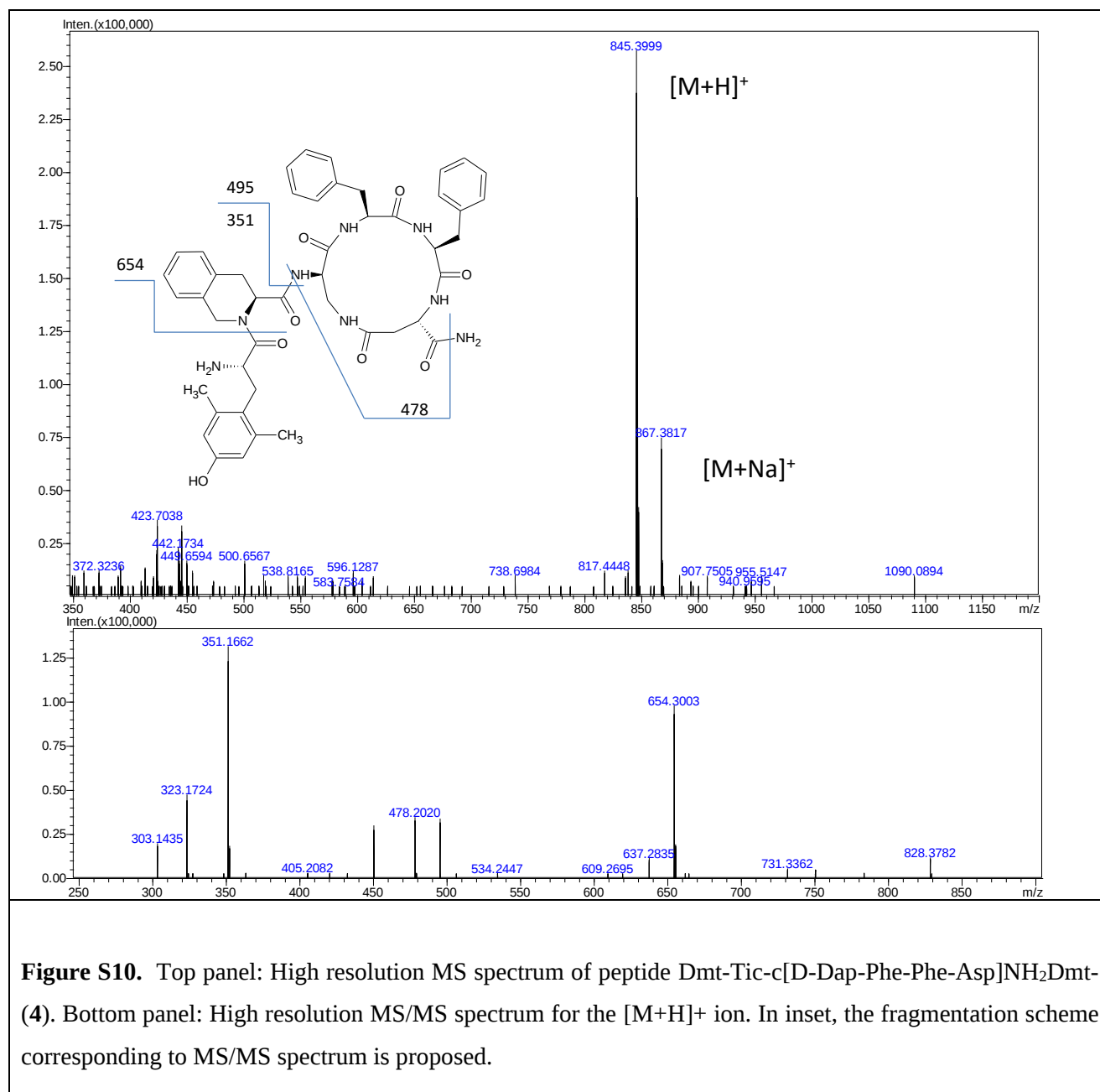
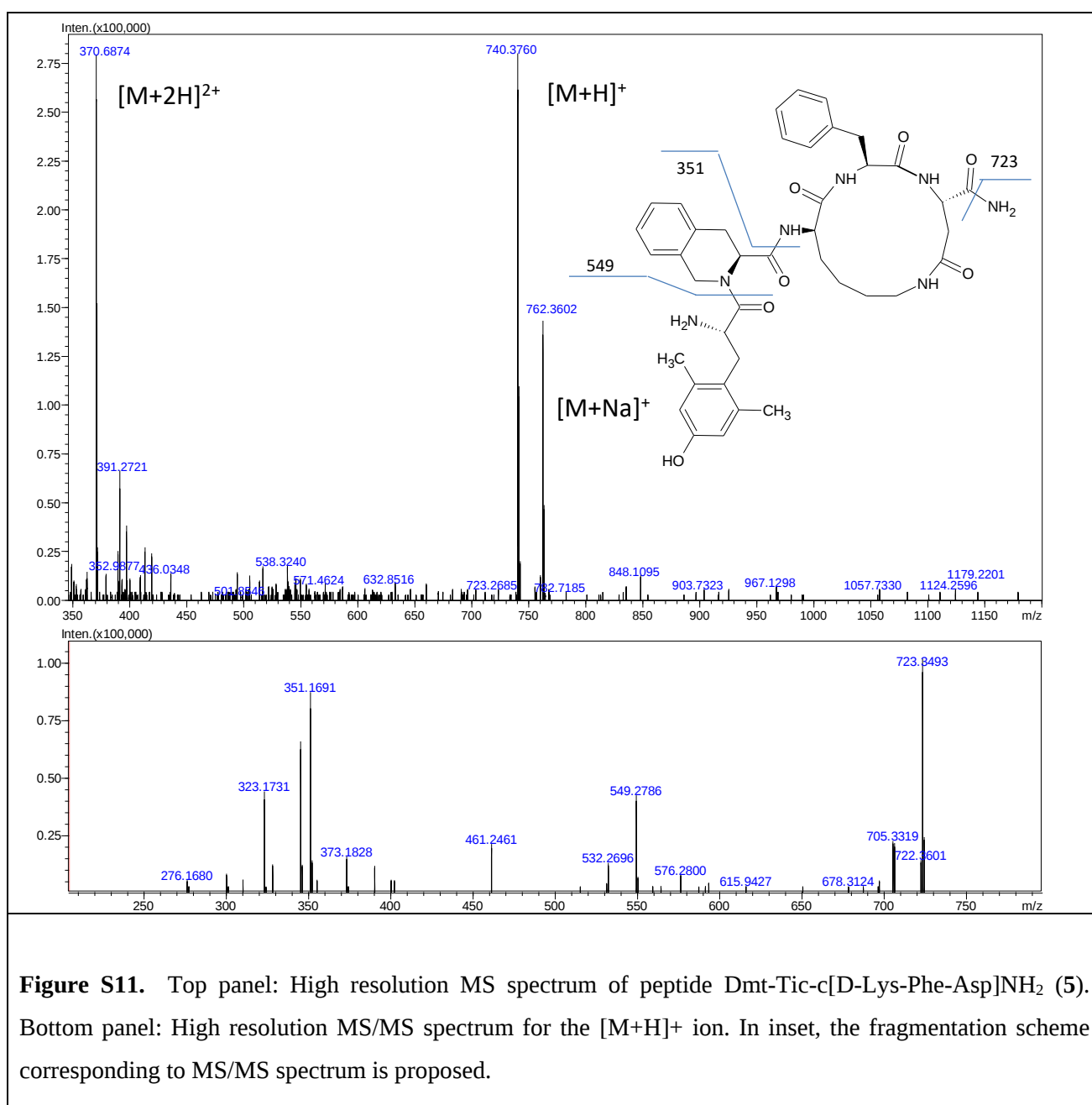


Figure S9. Top panel: High resolution MS spectrum of peptide Dmt-Tic-c[D-Lys-Phe-2,4F₂Phe-Asp]NH₂ (3). Bottom panel: High resolution MS/MS spectrum for the [M+H]⁺ ion. In inset, the fragmentation scheme corresponding to MS/MS spectrum is proposed.





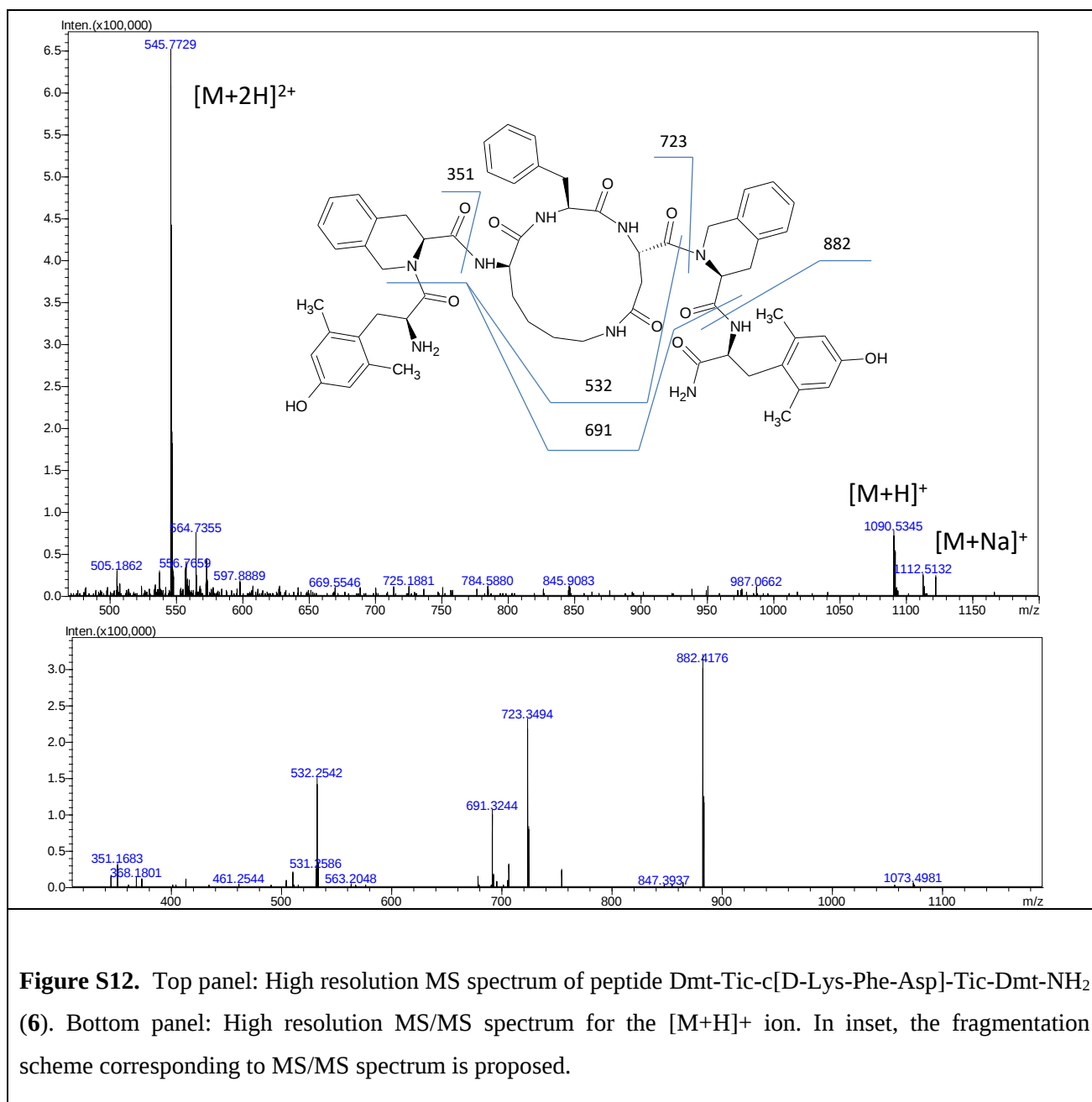


Figure S12. Top panel: High resolution MS spectrum of peptide Dmt-Tic-c[D-Lys-Phe-Asp]-Tic-Dmt-NH₂ (6). Bottom panel: High resolution MS/MS spectrum for the $[M+H]^+$ ion. In inset, the fragmentation scheme corresponding to MS/MS spectrum is proposed.