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Supplementary Information

Novel peptide dendrimer LTP efficiently facilitates transfection of mammalian cells

Ksenia V. Kozhikhova ^{*a}, Sergey M. Andreev ^a, Igor P. Shilovskiy ^a, Anastasiia V. Timofeeva ^{a,b}, Alina R. Gaisina ^a, Artem A. Shatilov ^{a,b}, Evgeny A. Turetskiy ^{a,b}, Igor M. Andreev ^c, Valeriy V. Smirnov ^{a,b}, Anton S. Dvornikov ^d, Musa R. Khaitov ^a

- a. NRC Institute of Immunology FMBA, Moscow, Russian Federation
- b. I.M. Sechenov First Moscow State Medical University, Moscow, Russian Federation
- c. Timiryazev Institute of Plant Physiology, RAS, Moscow, Russian Federation
- d. Pirogov Russian National Research Medical University, Moscow, Russian Federation

*E-mail: kv.kozhikhova@nrcii.ru.

Additional experimental information

Content:

Linear peptides: NC-769, NC-772, NC-777, NC-783, NC-784, NC-788, NC-789, NC-790

Lipopeptides: AM-2, NC-784/Cl, NC-773

Peptide dendrimers: NC-780, LTP, NC-798

HPLC: preparative RP-HPLC was conducted on a Shimadzu Prominence system (Shimadzu corporation) with C18 Grace Vydac 218 TP1022 column (20 × 250 mm). A linear gradient of 0–70% eluent B ran for 25 min at 10 mL/min with detection at 226 nm. Eluent A contained 0.1% TFA/H₂O; eluent B contained pure acetonitrile (Sigma-Aldrich).

MS: The peptide structures were confirmed by matrix assisted laser desorption/ionization and time-of-light analysis (MALDI-TOF, Microflex™ LT MALDI-TOF, Bruker Daltonics) mass spectrometry.

1) **NC-769:** RRLSYSRRRFC (Arg-Arg-Leu-Ser-Tyr-Ser-Arg-Arg-Arg-Phe-Cys)
M_w = 1500 g/mol (360 mg, 49 %), HPLC (t = 11,357) (Fig. S1), MS (MALDI-TOF) m/z 1499,148 (M⁺) (Fig. S2).

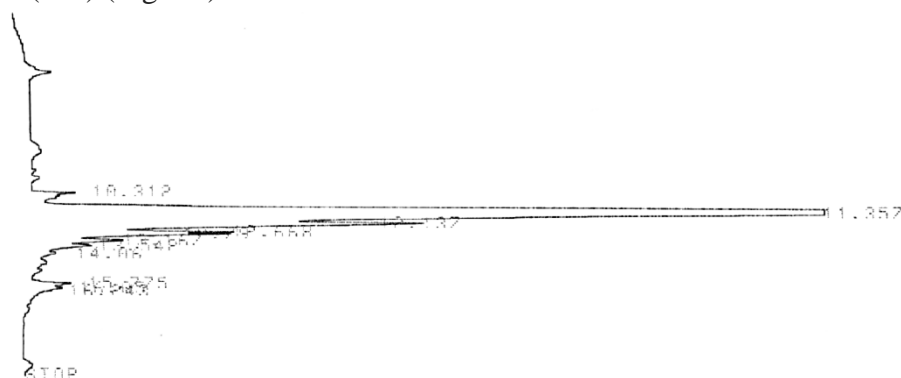


Fig. S1. HPLC chromatogram of NC-769

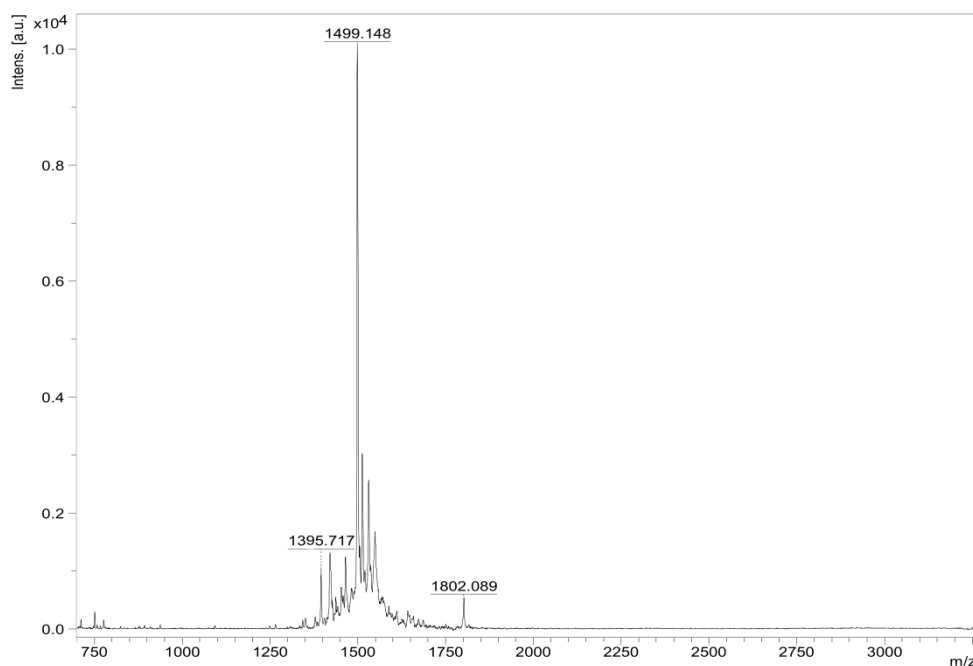


Fig. S2. Mass spectrum (MALDI-TOF) of NC-769

2) **NC-772**: CKRRRRRRRRRRR (Cys-Lys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg)
Mw = 1967 g/mol (430 mg, 45 %), HPLC (t = 13,017) (Fig. S3), MS (MALDI-TOF) m/z
1967,421 (M+) (Fig. S4).

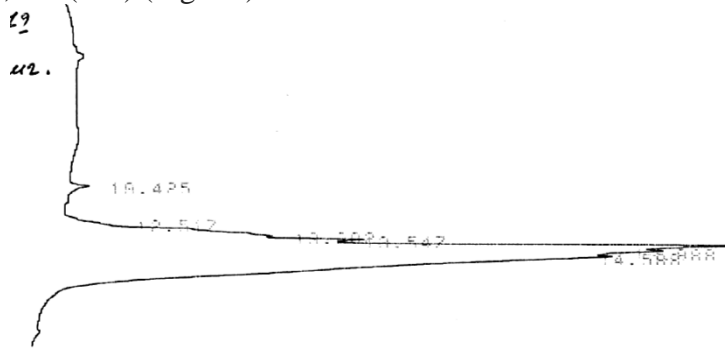


Fig. S3. HPLC chromatogram of NC-772

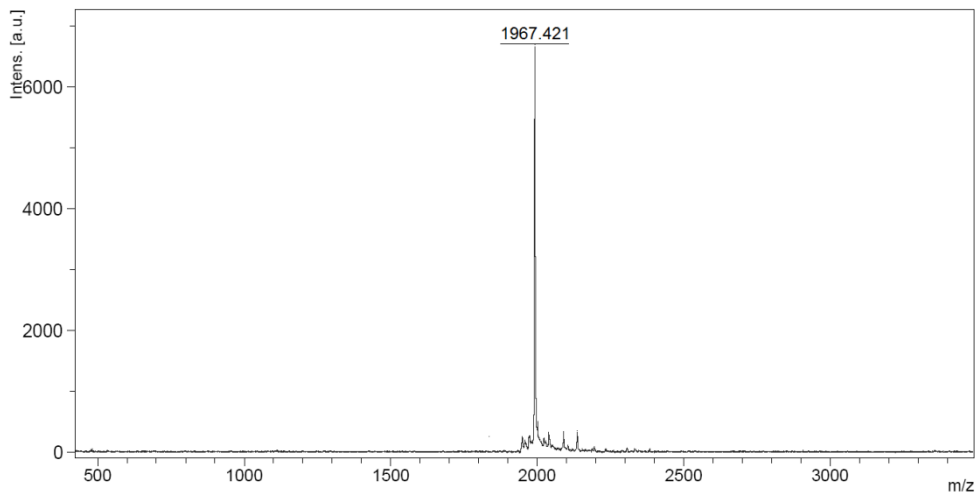


Fig. S4. Mass spectrum (MALDI-TOF) of NC-772

3) **NC-777**: LPSRDRQHLPL (Leu-Pro-Ser-Arg-Asp-Arg-Gln-His-Leu-Pro-Leu)
Mw = 1331 g/mol (300 mg, 46 %), HPLC (t = 12,883) (Fig. S5), MS (MALDI-TOF) m/z
1331,447 (M+) (Fig. S6).

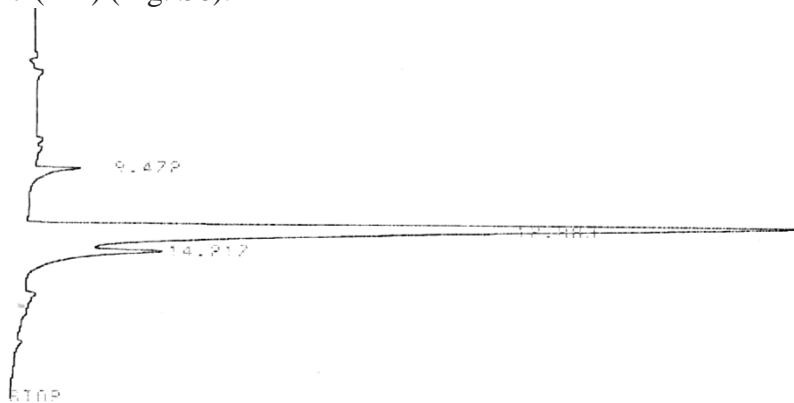


Fig. S5. HPLC chromatogram of NC-777

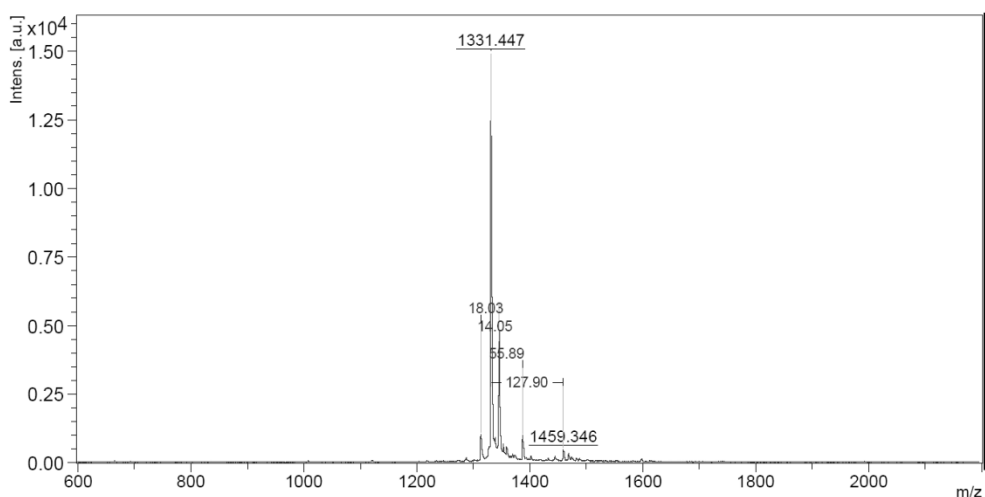


Fig. S6. Mass spectrum (MALDI-TOF) of NC-777

4) **NC-783:** KRRGGGKLLKLLKLLKLLKLC (Lys-Arg-Arg-Gly-Gly-Gly-Lys-Leu-Leu-Lys-Leu-Leu-Leu-Lys-Leu-Leu-Lys-Cys)
 Mw = 2505 g/mol (380 mg, 31 %), HPLC (t = 22,73) (Fig. S7), MS (MALDI-TOF) m/z 2505,312 (M⁺) (Fig. S8).

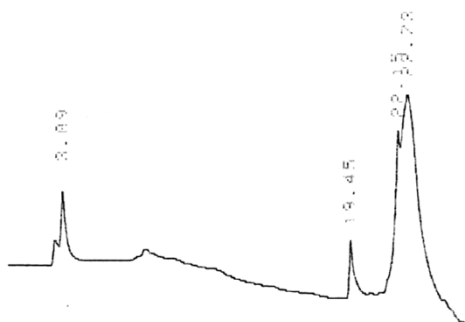


Fig. S7. HPLC chromatogram of NC-783

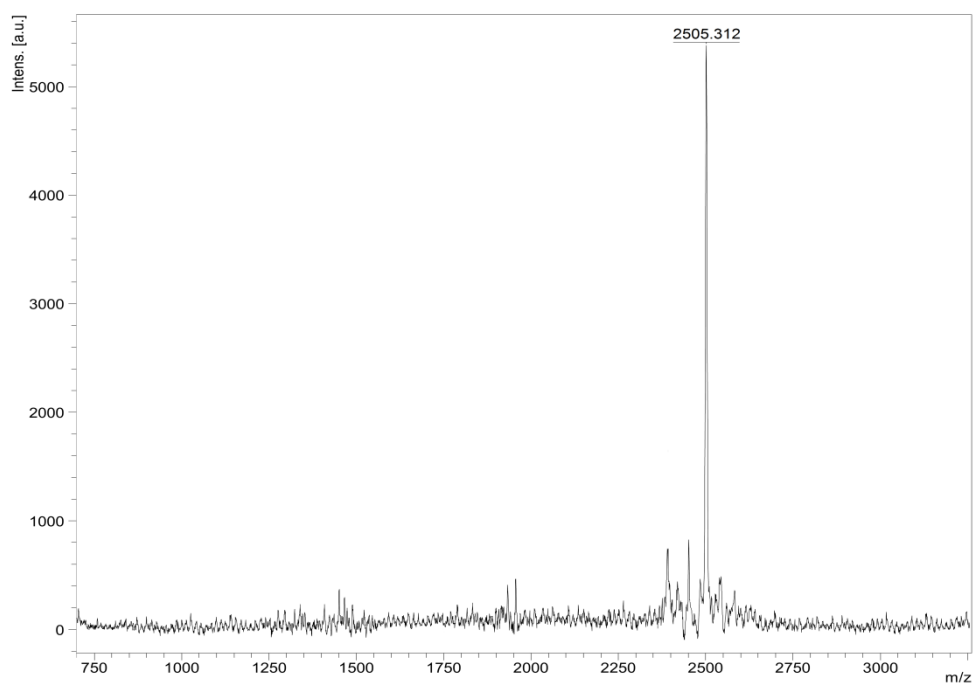


Fig. S8. Mass spectrum (MALDI-TOF) of NC-783

5) **NC-784:** GRKKRRQRRRG-NH₂ (Gly-Arg-Lys- Lys-Arg-Arg-Gln-Arg-Arg-Arg-Gly-NH₂)
Mw = 1454 g/mol (340 mg, 48 %), HPLC (t = 8,85) (Fig. S9), MS (MALDI-TOF) m/z 1452,998 (M⁺) (Fig. S10).

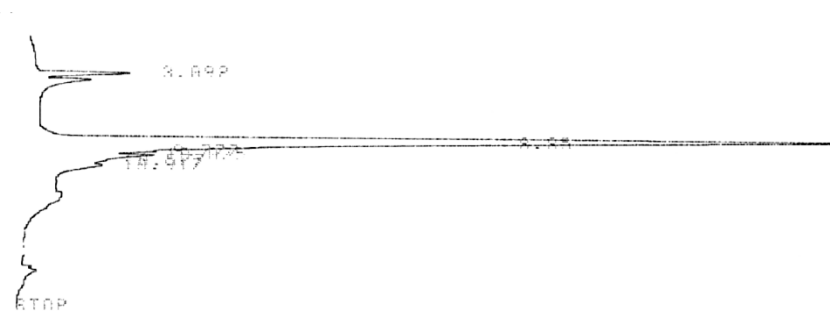


Fig. S9. HPLC chromatogram of NC-784

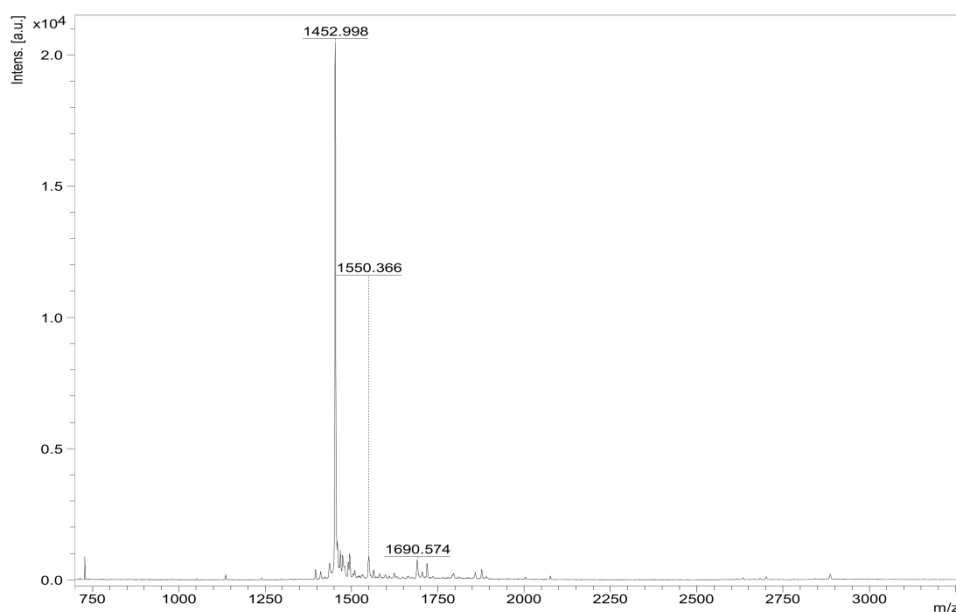


Fig. S10. Mass spectrum (MALDI-TOF) of NC-784

6) **NC-788:** CPWKRMEKKRSHL (Cys-Pro-Trp-Lys-Arg-Met-Glu-Lys-Lys-Arg-Ser-His-Leu)
Mw = 1698 g/mol (350 mg, 42 %), HPLC (t = 12,33) (Fig. S11), MS (MALDI-TOF) m/z 1699,377 (M⁺) (Fig. S12).

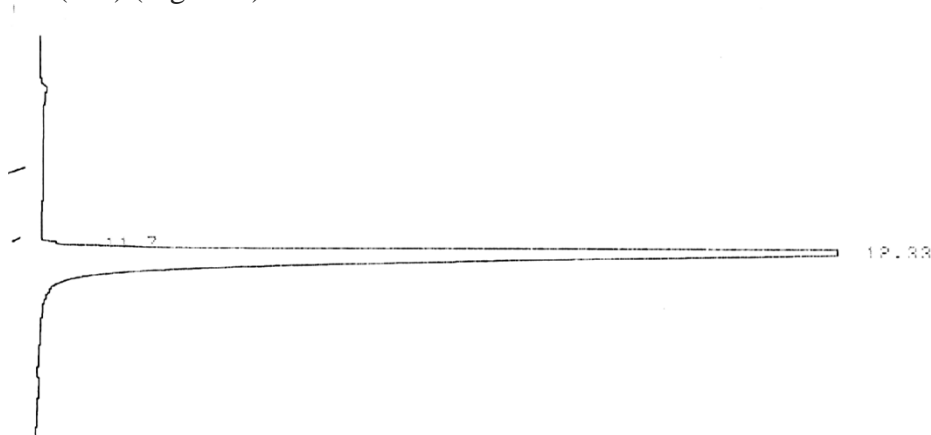


Fig. S11. HPLC chromatogram of NC-788

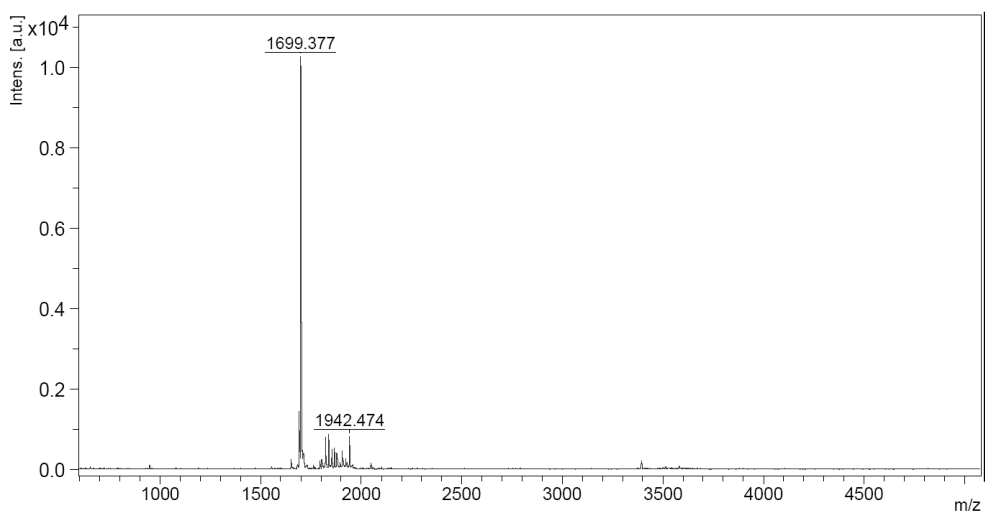


Fig. S12. Mass spectrum (MALDI-TOF) of NC-788

7) **NC-789**: GRKKRRQRRRCG (Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-Cys-Gly)
 Mw = 1557 g/mol (330 mg, 44 %), HPLC (t = 9,833) (Fig. S13), MS (MALDI-TOF) m/z
 1558,052 (M+) (Fig. S14).

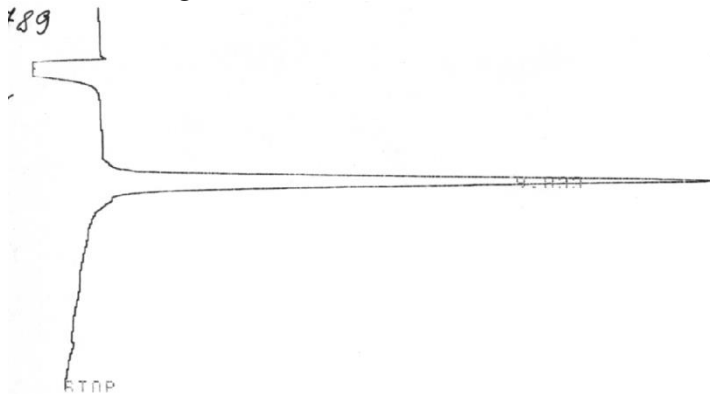


Fig. S13. HPLC chromatogram of NC-789

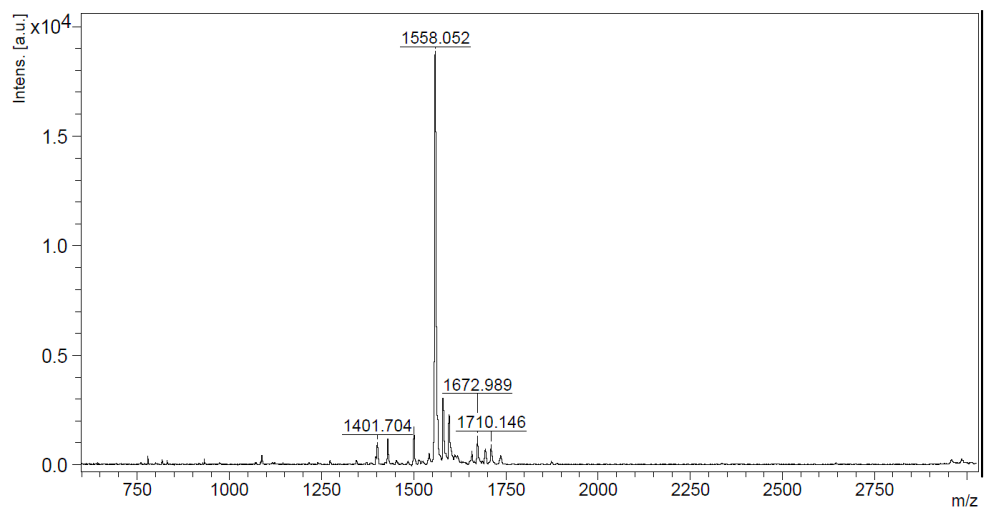


Fig. S14. Mass spectrum (MALDI-TOF) of NC-789

8) **NC-790:** CGRKKRRQRRRCG (Cys-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-Cys-Gly)
Mw = 1660 g/mol (350 mg, 43 %), HPLC (t = 16,203) (Fig. S15), MS (MALDI-TOF) m/z
1658,128 (M+) (Fig. S16).

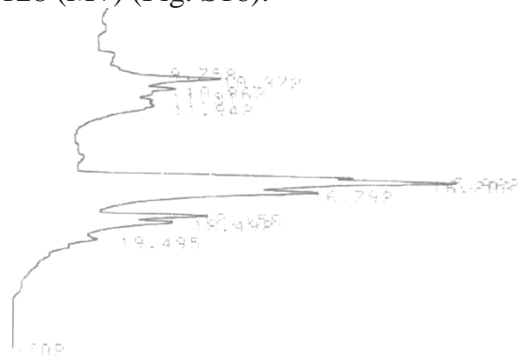


Fig. S15. HPLC chromatogram of NC-790

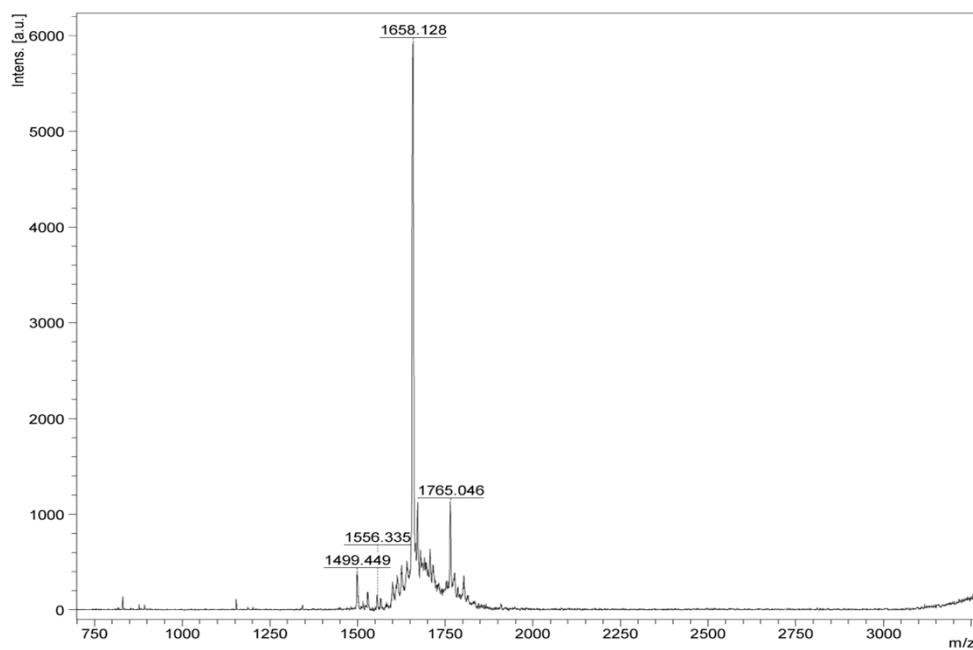


Fig. S16. Mass spectrum (MALDI-TOF) of NC-790

9) **AM-2:** (Mir)₂KRPAPAR-NH₂ ((Myristoyl)₂Lys-Arg-Pro-Ala-Arg-Pro-Ala-Arg-NH₂) 1371
Mw = 1371 g/mol (260 mg, 39 %), HPLC (t = 12,562) (Fig. S17), MS (MALDI-TOF) m/z
1372,071 (M+) (Fig. S18).

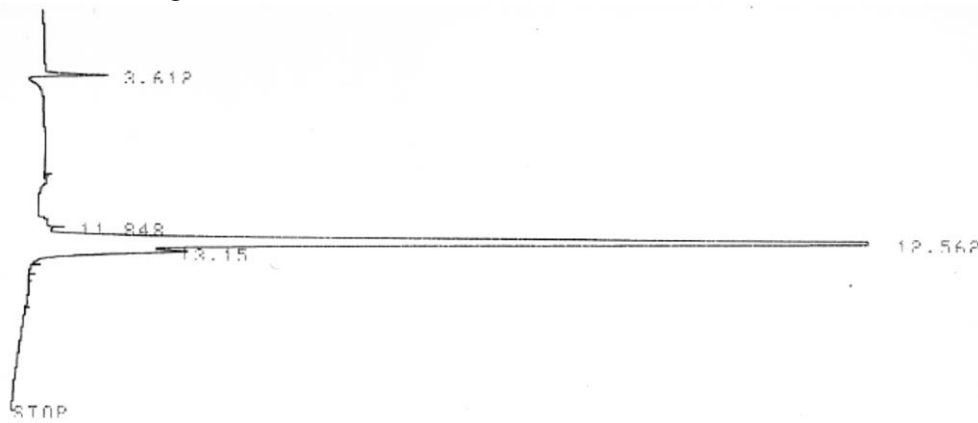


Fig. S17. HPLC chromatogram of AM-2

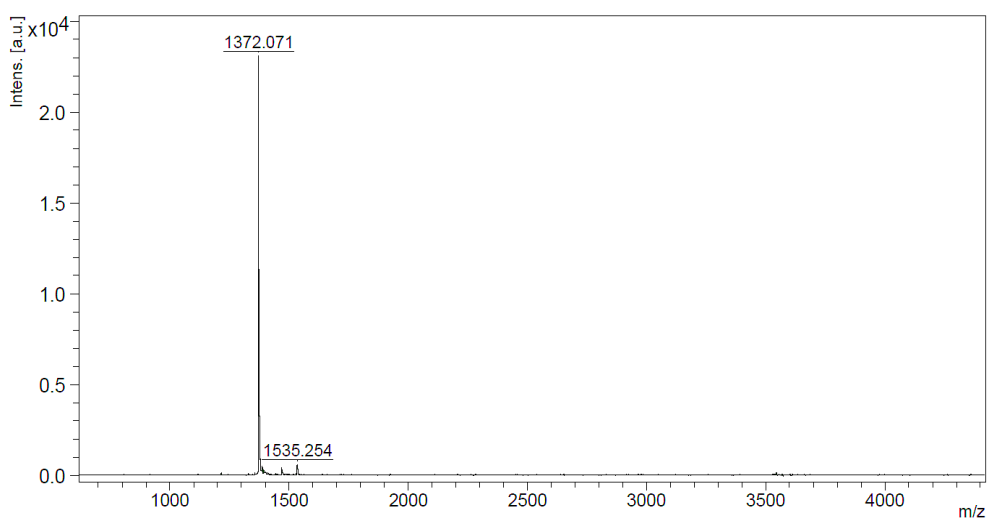


Fig. S18. Mass spectrum (MALDI-TOF) of AM-2

10) **NC-784/Cl**: Palm-GRKKRRQRRRG-NH₂ (Palmitoyl-Gly-Arg-Lys- Lys-Arg-Arg-Gln-Arg-Arg-Arg-Gly-NH₂)

M_w = 1692 g/mol (240 mg, 29 %), HPLC (t = 9,208) (Fig. S19), MS (MALDI-TOF) m/z 1692,161 (M⁺) (Fig. S20).

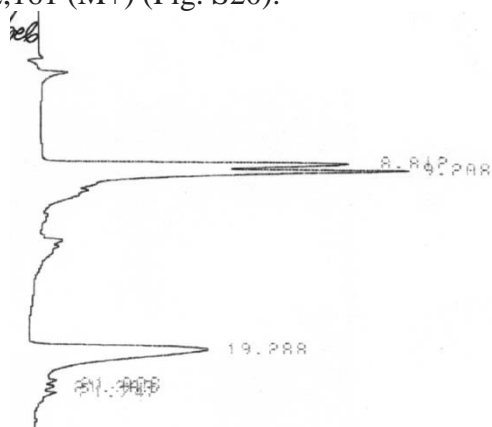


Fig. S19. HPLC chromatogram of NC-784/Cl

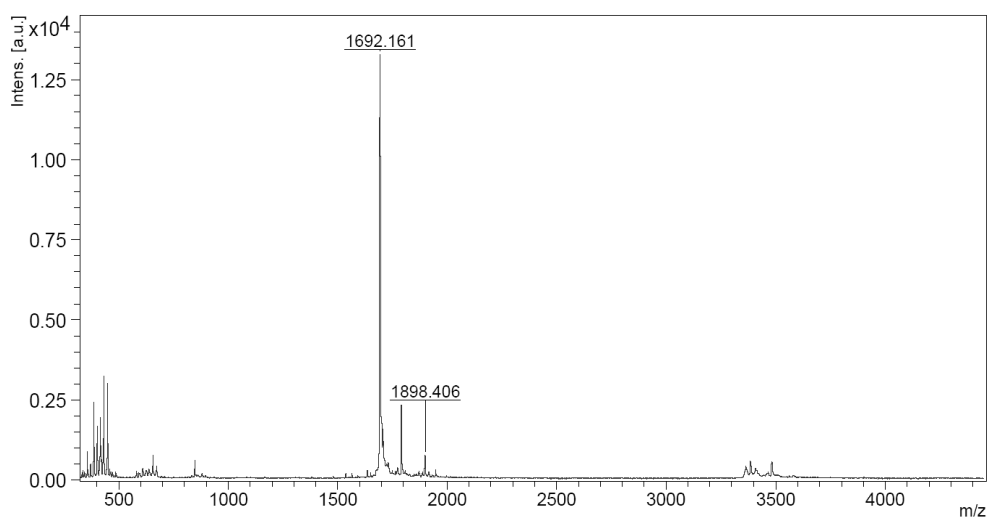


Fig. S20. Mass spectrum (MALDI-TOF) of NC-784/Cl

11) **NC-773:** Palm-CKRRRRRRRRRRR (Palmitoyl-Cys-Lys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg)
Mw = 2207 g/mol (340 mg, 31 %), HPLC (t = 19,318) (Fig. S21), MS (MALDI-TOF) m/z 2206,828 (M+) (Fig. S22).

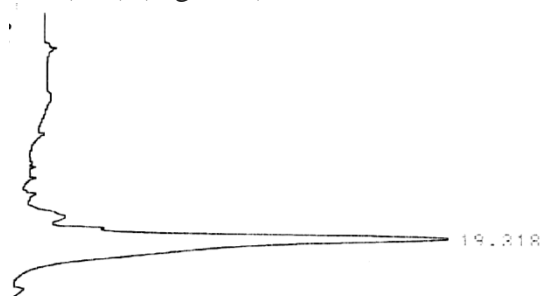


Fig. S21. HPLC chromatogram of NC-773

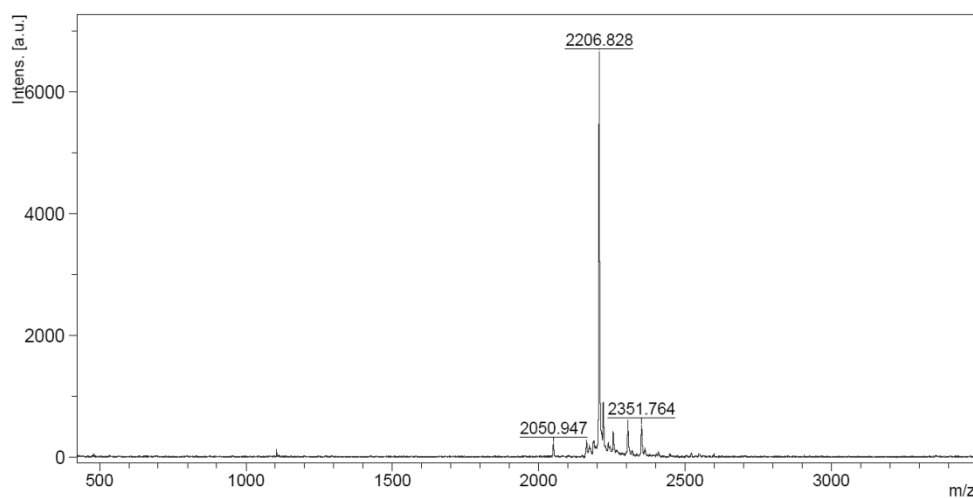


Fig. S22. Mass spectrum (MALDI-TOF) of NC-773

12) **NC-780:** ((K)₂K)₂KAC-NH₂ 1090 (((Lys)₂Lys)₂Lys)₂Lys-Ala-Cys-NH₂)
Mw = 1090 g/mol (220 mg, 42 %), HPLC (t = 14,092) (Fig. S23), MS (MALDI-TOF) m/z 1090,628 (M+) (Fig. S24).

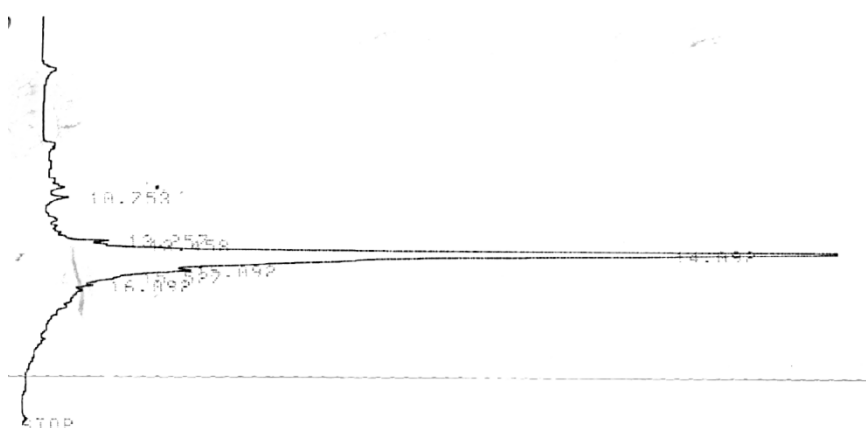


Fig. S23. HPLC chromatogram of NC-780

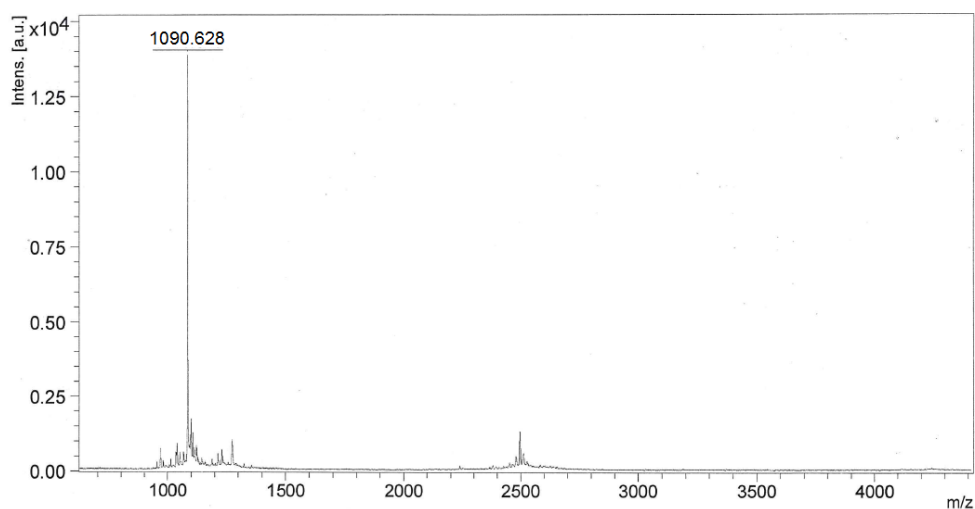


Fig. S24. Mass spectrum (MALDI-TOF) of NC-780

13) **LTP:** (((R)₂K)₂K)₂KAC-NH₂ (((Arg)₂Lys)₂Lys)₂Lys-Ala-Cys-NH₂)

M_w = 2338 g/mol (460 mg, 40 %), HPLC (t = 9,908) (Fig. S25), MS (MALDI-TOF) m/z 2337,291 (M⁺) (Fig. S26), MS (ESI) m/z 390.6 (M+6), 468.5 (M+5), 585.3 (M+4), and 780.3 (M+3) (Fig. S27)

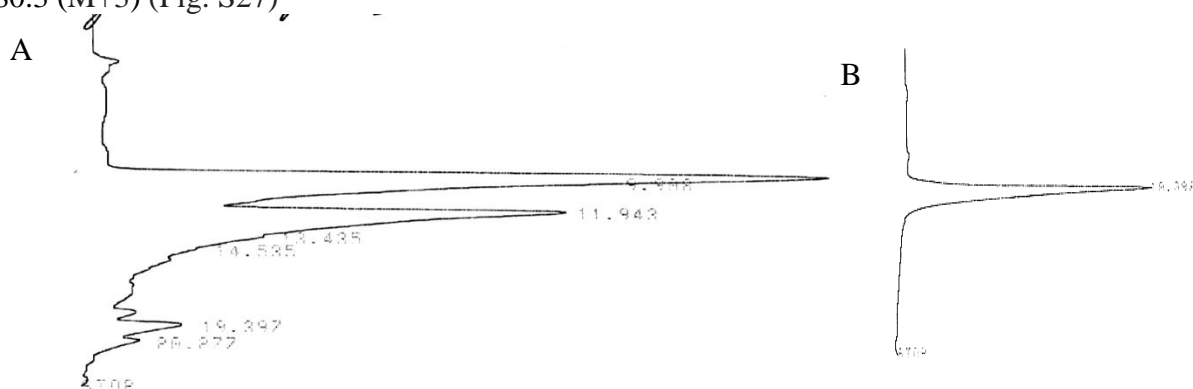


Fig. S25. HPLC chromatogram of LTP: preparative (A), analytical (B)

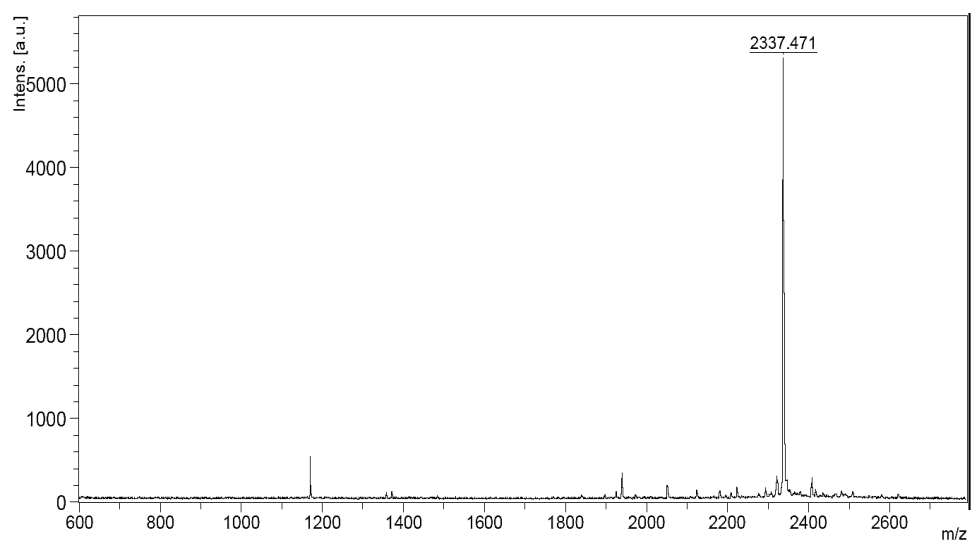


Fig. S26. Mass spectrum (MALDI-TOF) of LTP

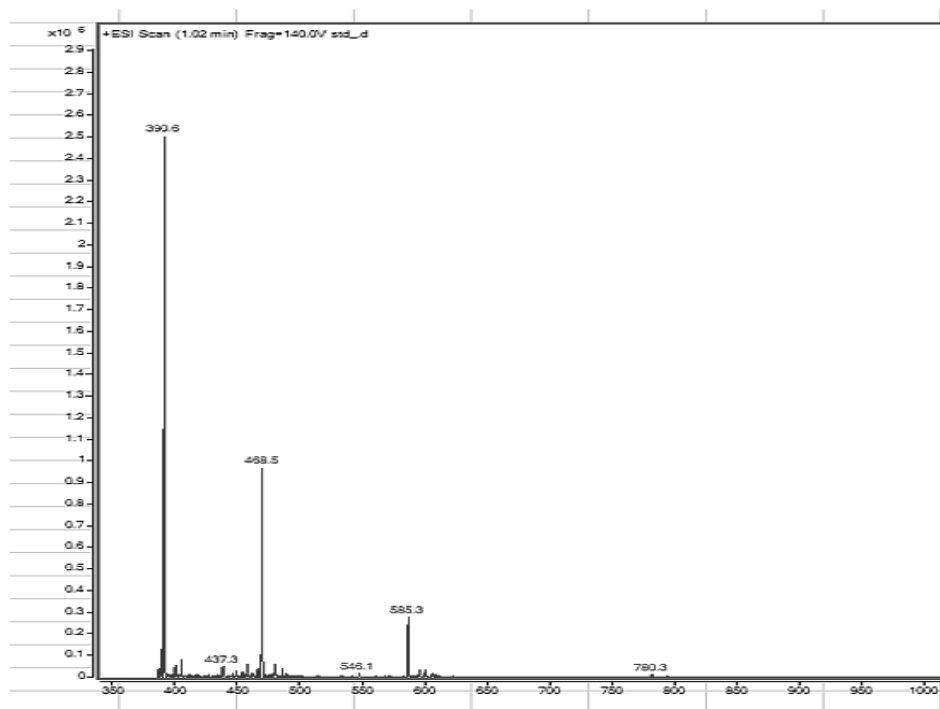


Fig. S27. Mass spectrum (ESI) of LTP. The ESI spectrum contains ion peaks with m/z : 390.6 (six-charged ion), 468.5 (five-charged ion), 585.3 (four-charged ion), and 780.3 (three-charged ion). Thus, the molecular weight of the peptide is 2338 Da, that corresponds to the calculated data.

14) **NC-798:** (((K)₂K)₂K)₂KAC-NH₂ (((Lys)₂Lys)₂Lys)₂Lys-Ala-Cys-NH₂
 Mw = 2114 g/mol (390 mg, 38 %), HPLC (t = 12,39) (Fig. S28), MS (MALDI-TOF) m/z 2114,048 (M⁺) (Fig. S29).

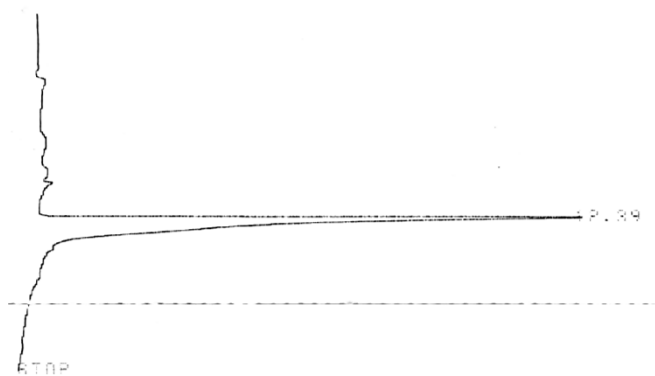


Fig. S28. HPLC chromatogram of NC-798

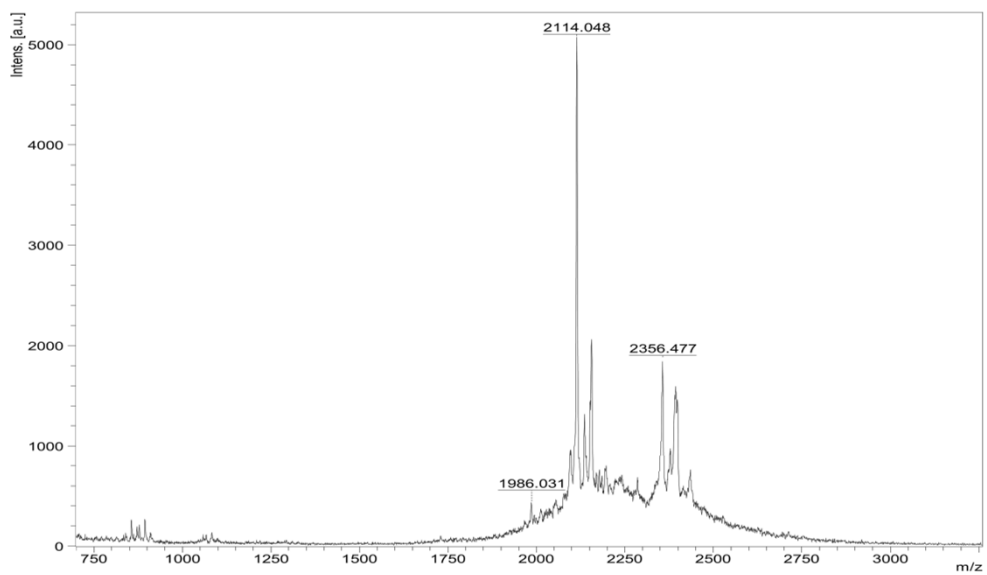


Fig. S29. Mass spectrum (MALDI-TOF) of NC-798