

Synthesis and Characterization of the Conjugated Peptide Lunatin-Folate

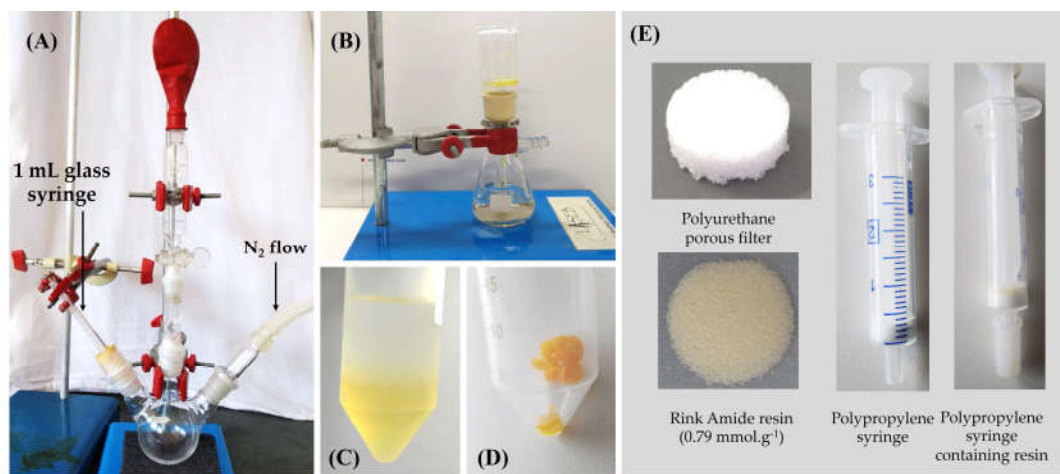


Figure S1 – Images of the reaction systems used in liquid and solid-phase synthesis. (A) Reflux apparatus for FA-Pra synthesis, (B) vacuum filtration of DCU, (C) FA-Pra suspended in cold diethyl ether, (D) filtered FA-Pra and (E) resin, porous filter and syringe used in solid-phase synthesis.

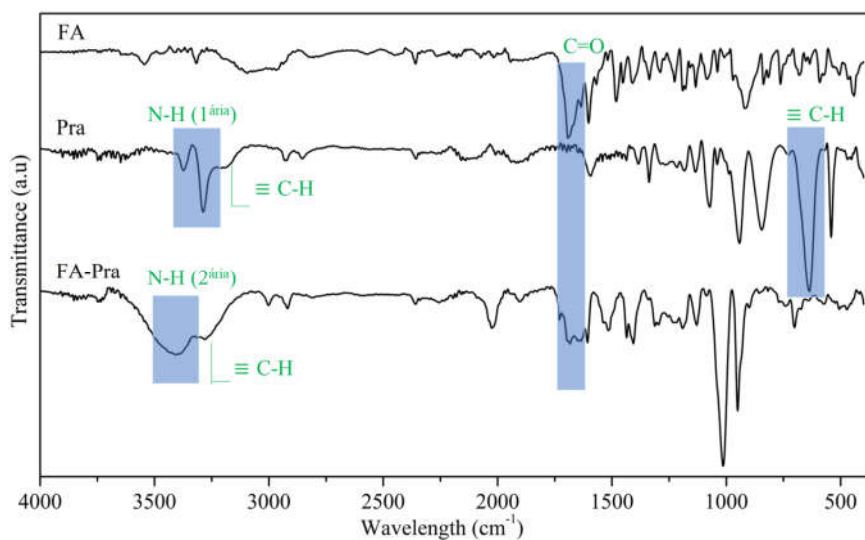


Figure S2- ATR-FTIR spectrum of the FA-Pra synthesis product.

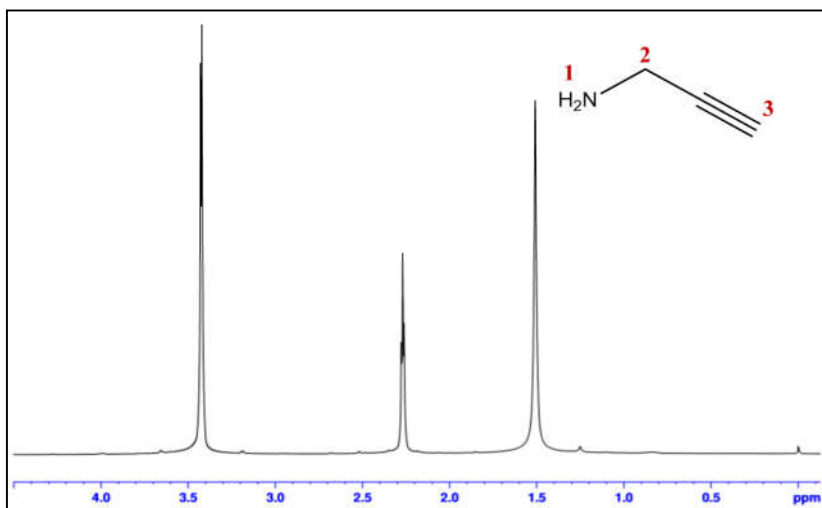


Figure S3 - ^1H NMR spectrum at 300 MHz of Pra ($0.03 \text{ mmol}\cdot\text{mL}^{-1}$) in CDCl_3 containing 0.5% (v/v) TMS.

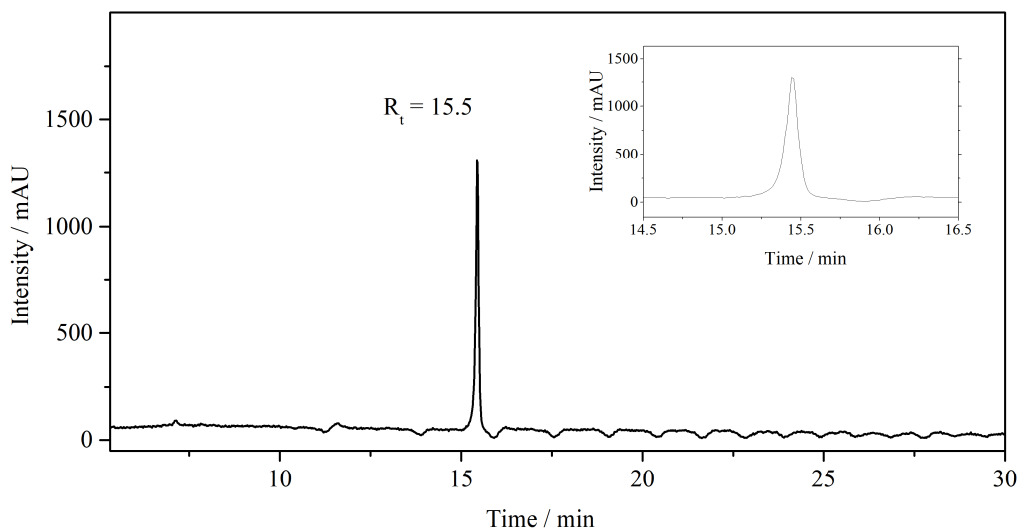


Figure S4 - Chromatographic profile of the crude sample of the tripeptide FIG- NH_2 . The analysis was performed using reversed-phase HPLC in a C18 Vydac 218TP column ($4.6 \text{ mm} \times 250 \text{ mm}$, GraceTM) equilibrated with 0.1% aqueous TFA followed by a linear gradient of acetonitrile in 0.1% TFA of 0–100% acetonitrile for 0–30 min. The flow was $0.80 \text{ mL}\cdot\text{min}^{-1}$ and detection was at 214 nm.

Chemical shift of FIG- NH_2 : 14 ppm (t, J 6.17 Hz, 1H); 3.02 ppm (d, J 6.76 Hz, 2H); 6.94 ppm (s, 1H); 7.32 ppm (s, 1H); 8.36 ppm (s, 2H); 4.00 ppm (s, 1H); 1.62 ppm (m, 1H); 1.29 ppm (m, 3H); 0.98 ppm (m, J 7.31 Hz, 2H); 0.70 ppm (m, J 6.50 Hz, 3H); 3.72 ppm (dd, J 5.9, 2H); and 8.18 ppm (t, J 5.28, 2H).

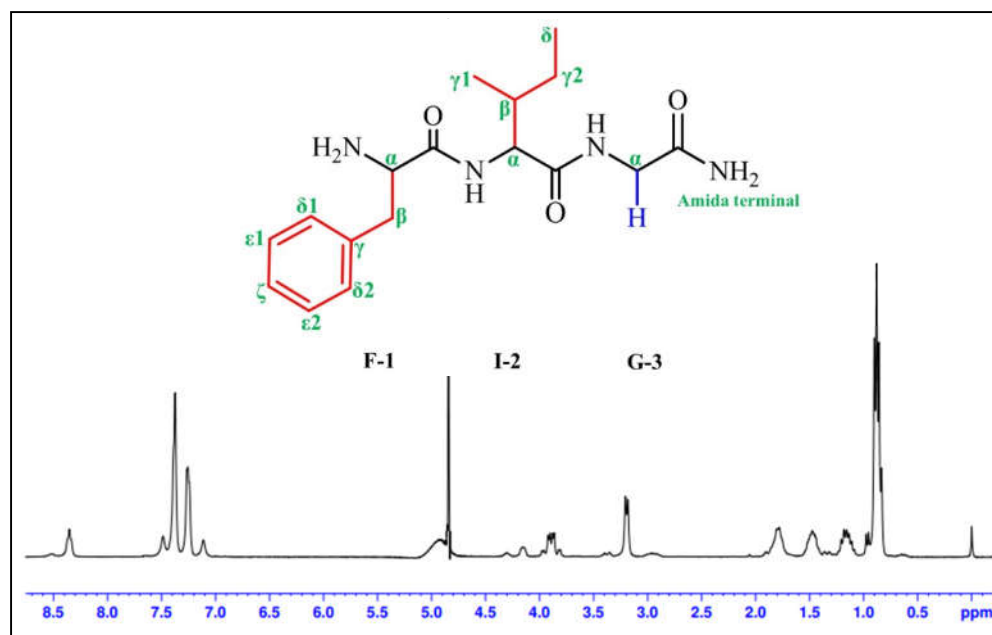


Figure S5 - ^1H NMR spectrum at 300 MHz of the tripeptide FIG- NH_2 (50 mmol.L $^{-1}$) in water containing 10% (v/v) D $_2\text{O}$ and 1% (v/v) DSS.

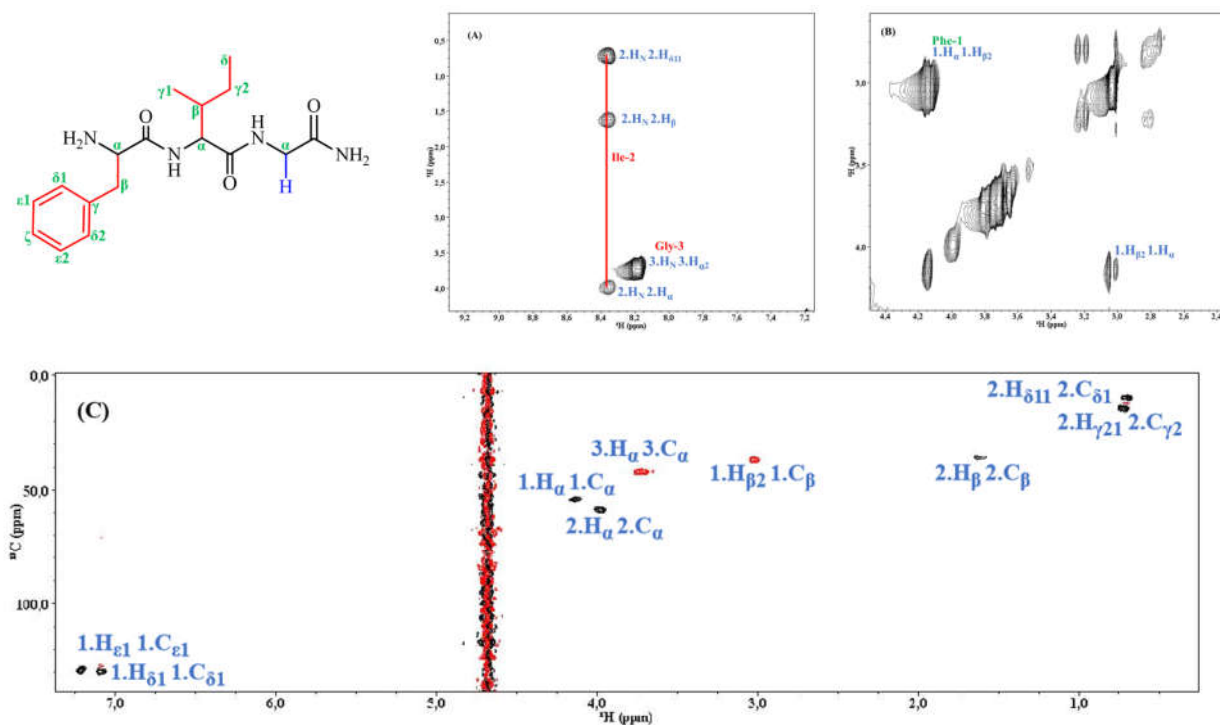


Figure S6 - $[\text{}^1\text{H}, \text{}^1\text{H}]$ TOCSY NMR spectrum at 300 MHz of Br-FIG- NH_2 (30 mmol.L $^{-1}$) in DMSO- d_6 containing 0.5% (v/v) TMS. (A) Enlargement of the amide hydrogen region; (B) Enlargement of the side-chain hydrogen region. (C) $[\text{}^1\text{H}, \text{}^{13}\text{C}]$ HSQC NMR spectrum at 300 MHz of the tripeptide FIG- NH_2 (50 mmol.L $^{-1}$) in water containing 10% (v/v) D $_2\text{O}$ and 1% (v/v) DSS. Edited HSQC contour plot reveals methylene ($-\text{CH}_2$) correlations in the inverted red phase, while methine ($-\text{CH}$) and methyl ($-\text{CH}_3$) groups appear in the positive black phase.

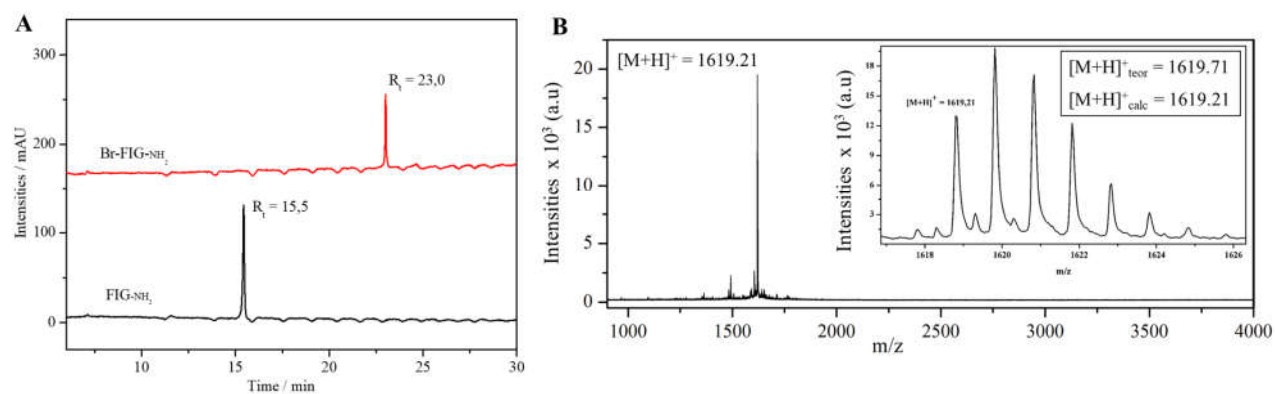


Figure S7 - (A) Chromatographic profile of the raw sample of the tripeptide Br-FIG-NH₂. The analysis was performed using reversed-phase HPLC in a C18 Vydac 218TP column (4.6 mm x 250 mm, GraceTM) equilibrated with 0.1% aqueous TFA followed by a linear gradient of acetonitrile in 0.1% TFA of 0–100% acetonitrile for 0–30 min. The flow was 0.80 ml. min⁻¹ and detection was at 214 nm. **(B)** Mass spectrum obtained by MALDI-ToF in MS mode of the crude sample of the tripeptide Br-FIG-NH₂. $[M+H]^+_{theor}$ represents the theoretical mass/charge, and $[M+H]^+_{calc}$ represents the calculated mass in g.mol⁻¹.

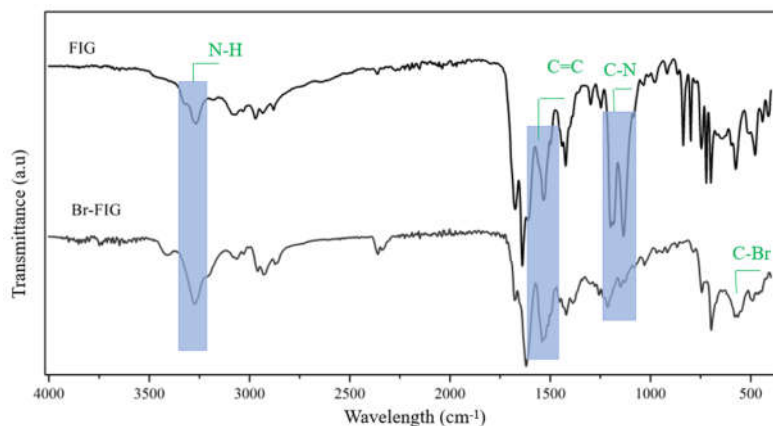


Figure S8 - ATR-FTIR spectrum of the product Br-FIG-NH₂ from step 1 and the tripeptide FIG-NH₂.

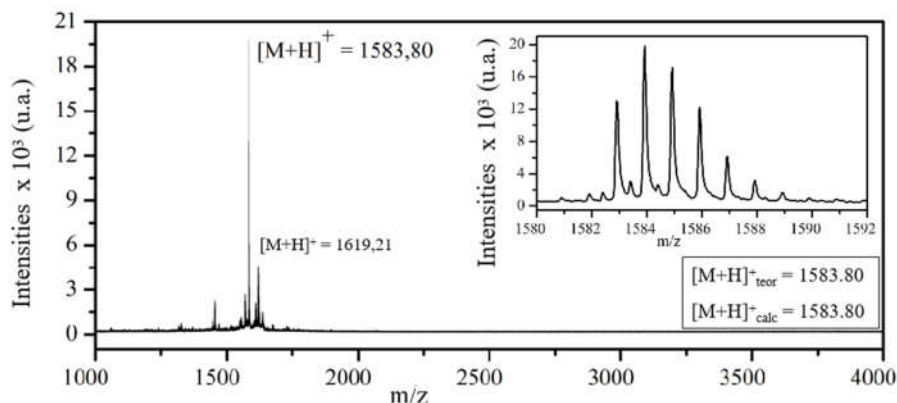


Figure S9 - Mass spectrum obtained by MALDI-ToF in MS mode of the crude sample of the tripeptide N₃-FIG^[2a]-NH₂. $[M+H]^+_{theor}$ represents the theoretical mass/charge, and $[M+H]^+_{calc}$ represents the calculated mass in g.mol⁻¹.

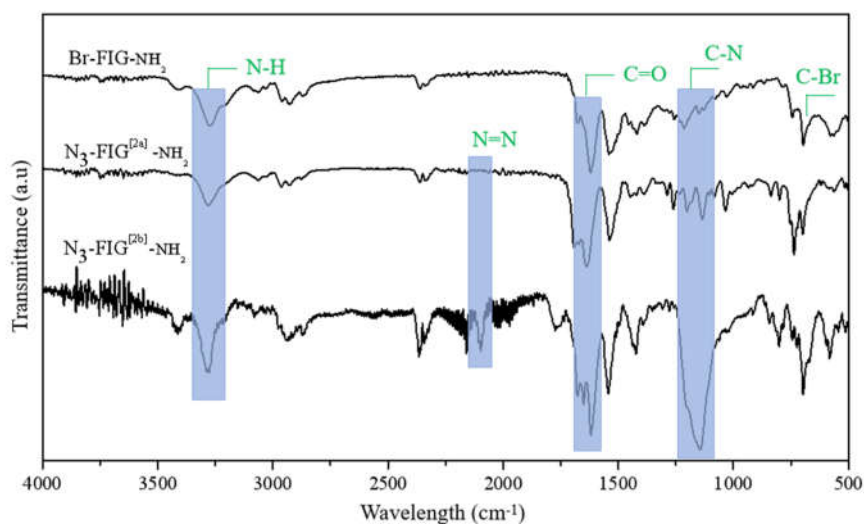


Figure S10 - ATR-FTIR spectra of the products from step 2 and the Br-FIG-NH₂ derivative.

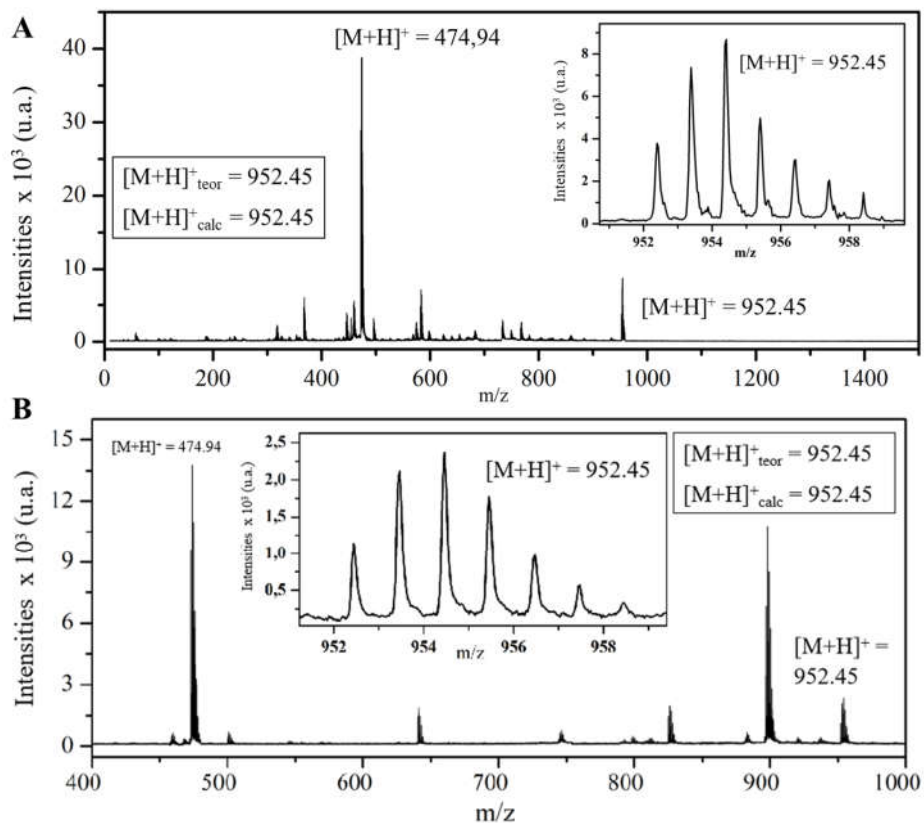


Figure S11- Mass spectrum obtained by MALDI-ToF in MS mode of the crude sample of the tripeptide (A) FA-Trz-FIG^[3a-24h] and (B) FA-Trz-FIG^[3b]

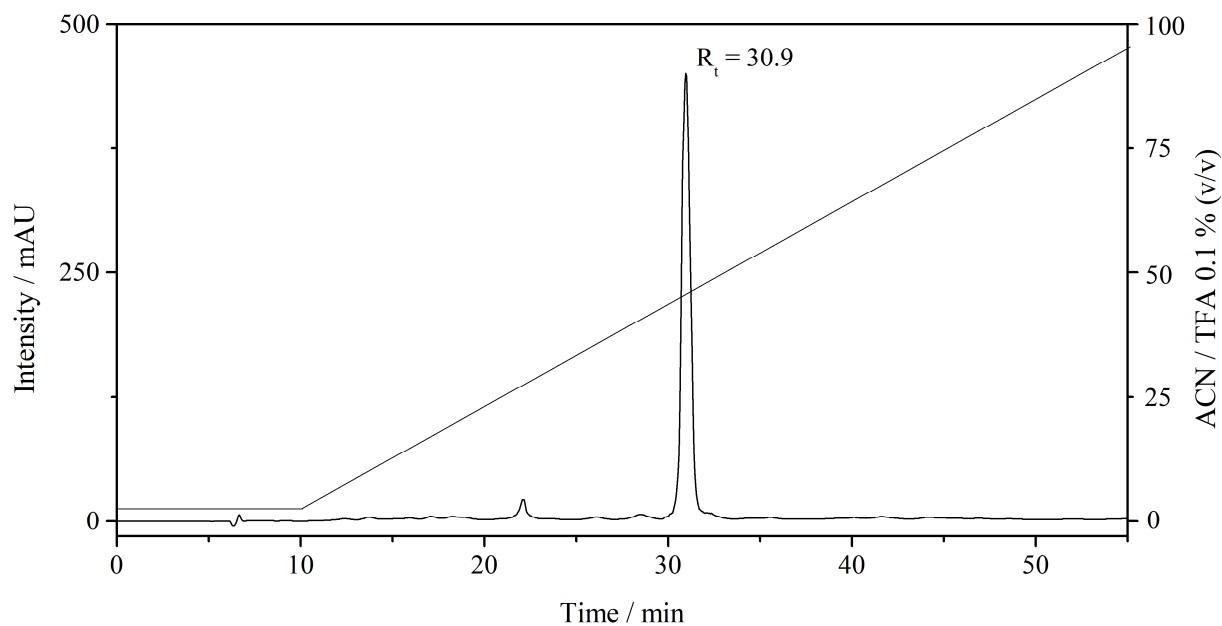


Figure S12 - Chromatogram of Lunatin-1. The analysis was performed using reversed-phase HPLC in a C18 Vydac 218TP column (4.6 mm $\times$ 250 mm, Grace™) equilibrated with 0.1% aqueous TFA starting with an isocratic elution of 5% acetonitrile containing 0.1% TFA followed by a linear gradient of 5–95% acetonitrile over 55 min. The flow was 0.80 ml.min⁻¹ and detection was at 214 nm.

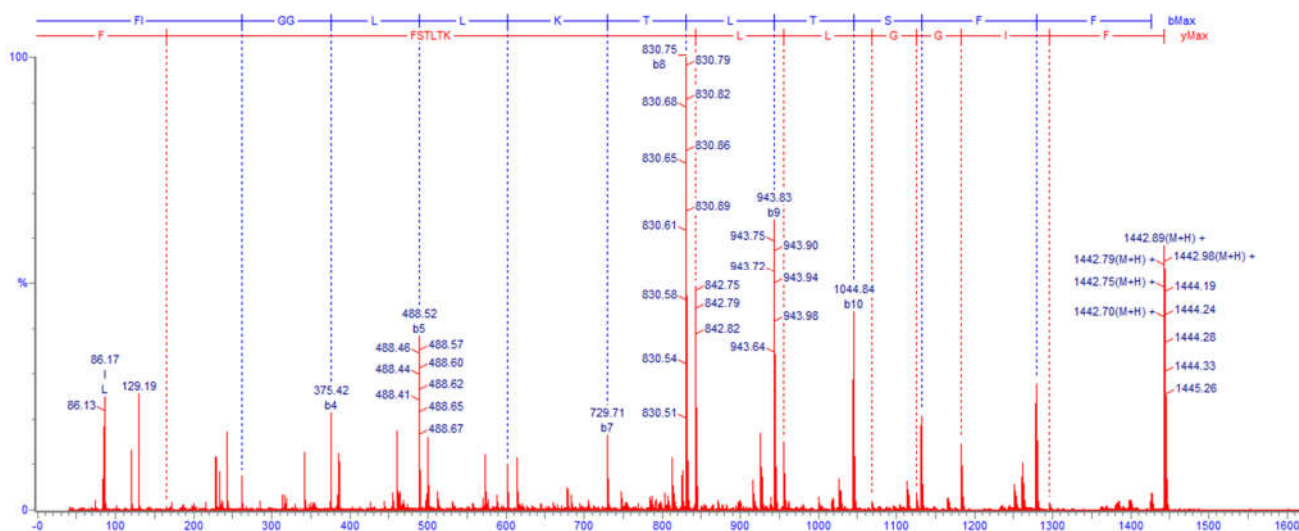


Figure S13 - MALDI-ToF mass spectrum in MS/MS mode of the $[M+H]^+$ ion m/z 1442.87 Da corresponding to the Lunatin-1 peptide.