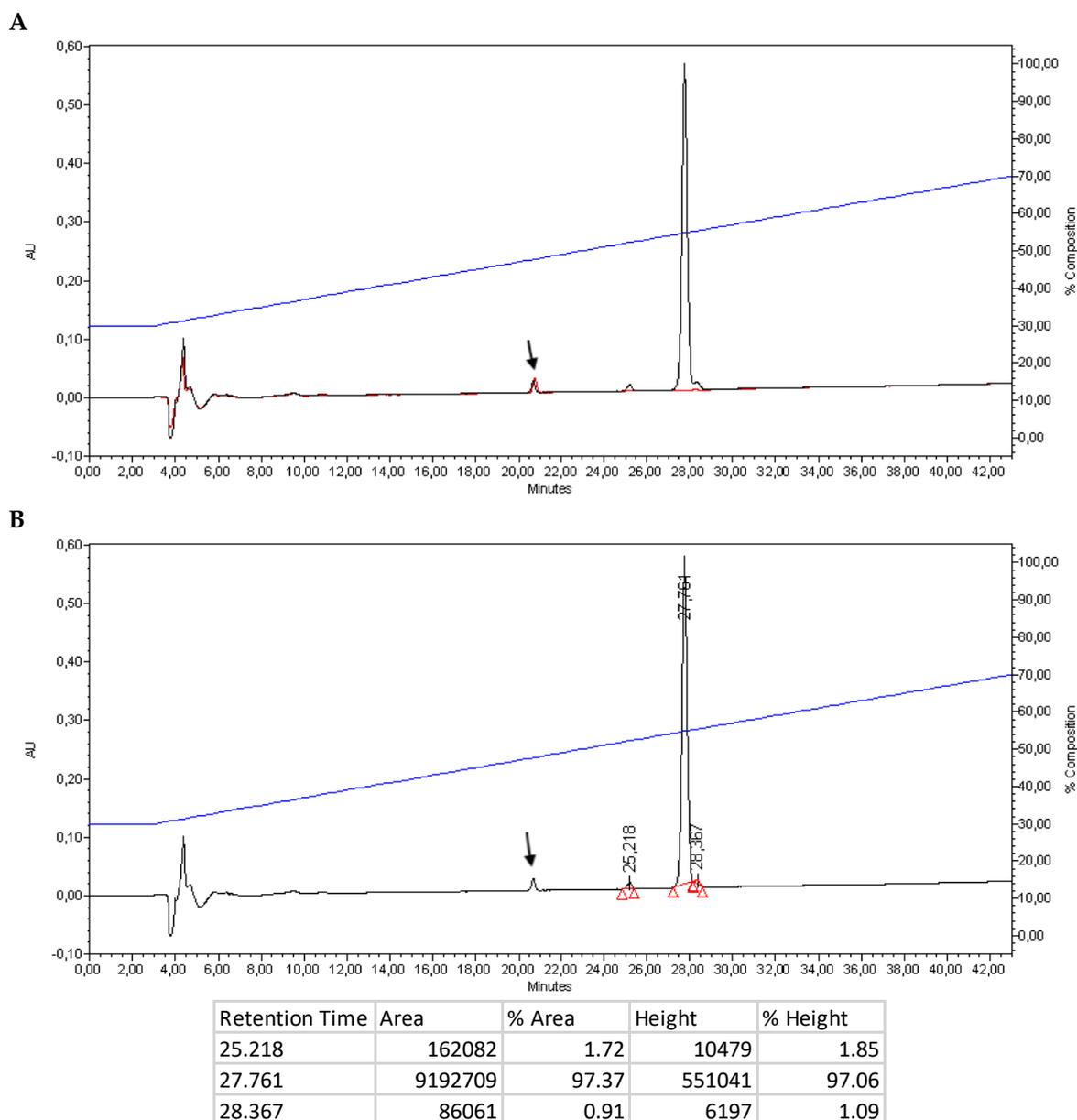
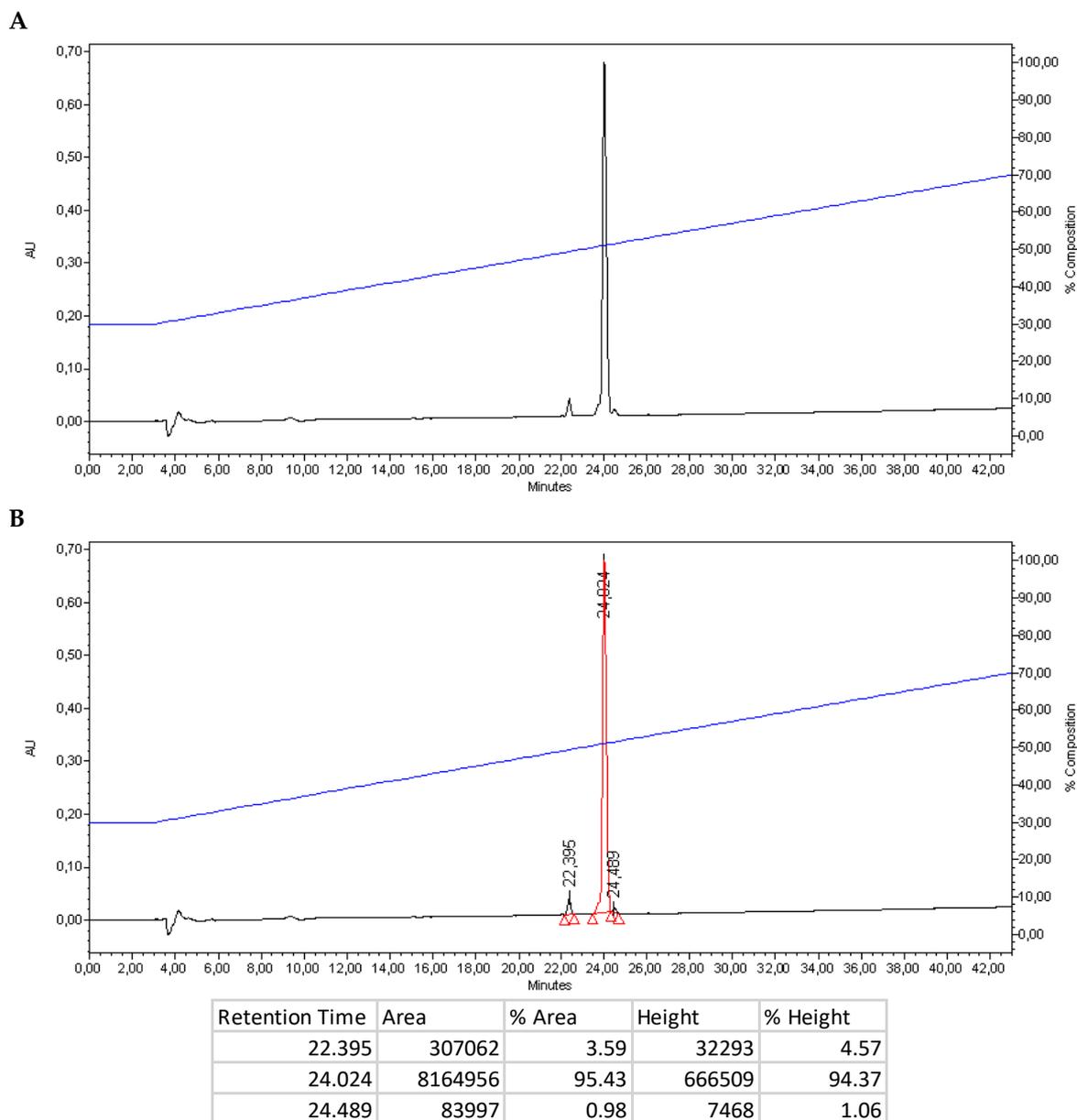


**Functional Characterization of Temporin-SHe, a New Broad-Spectrum Antibacterial and Leishmanicidal Temporin-SH Paralog from the Sahara Frog (*Pelophylax saharicus*)**

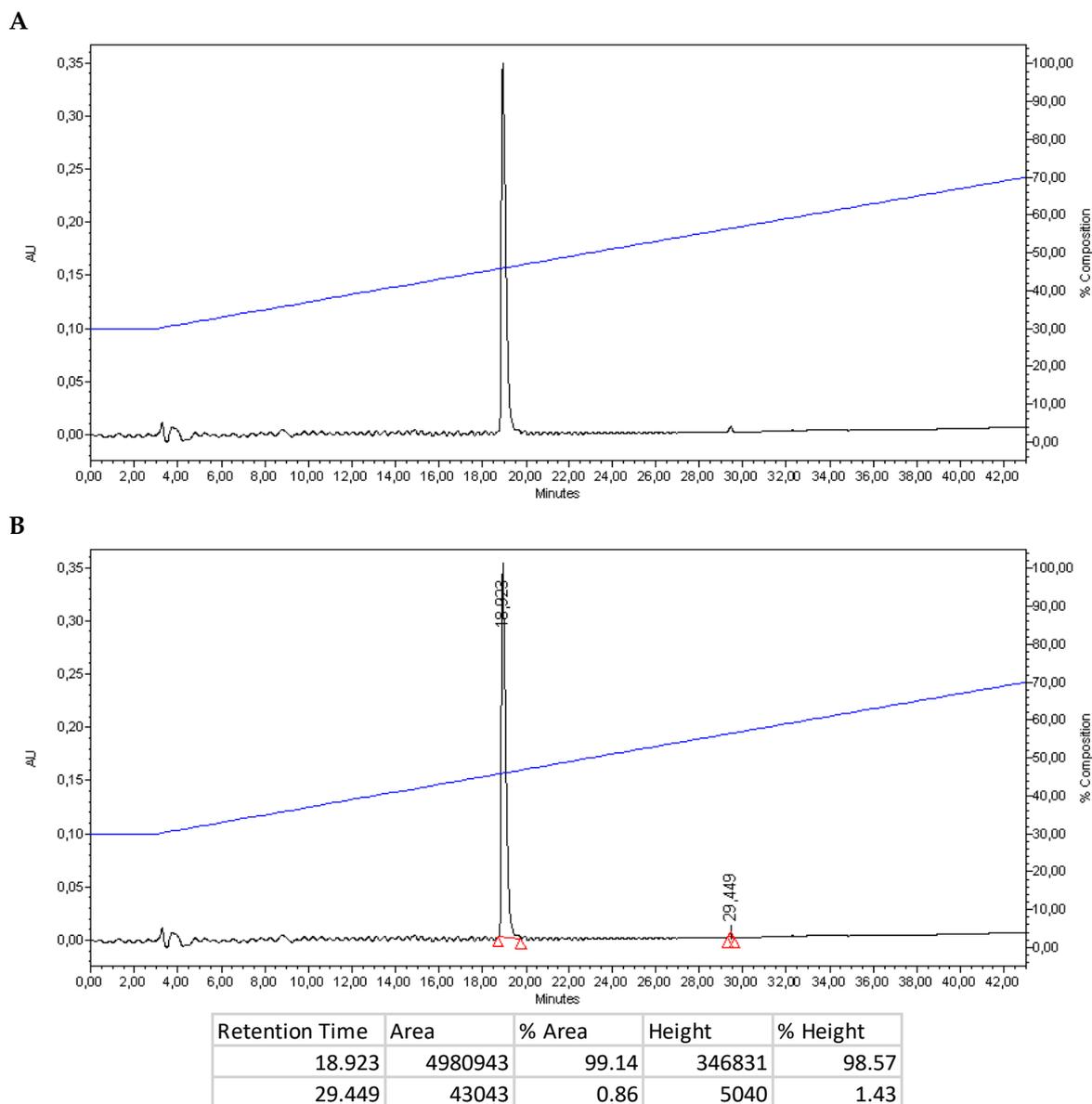
By André et al.



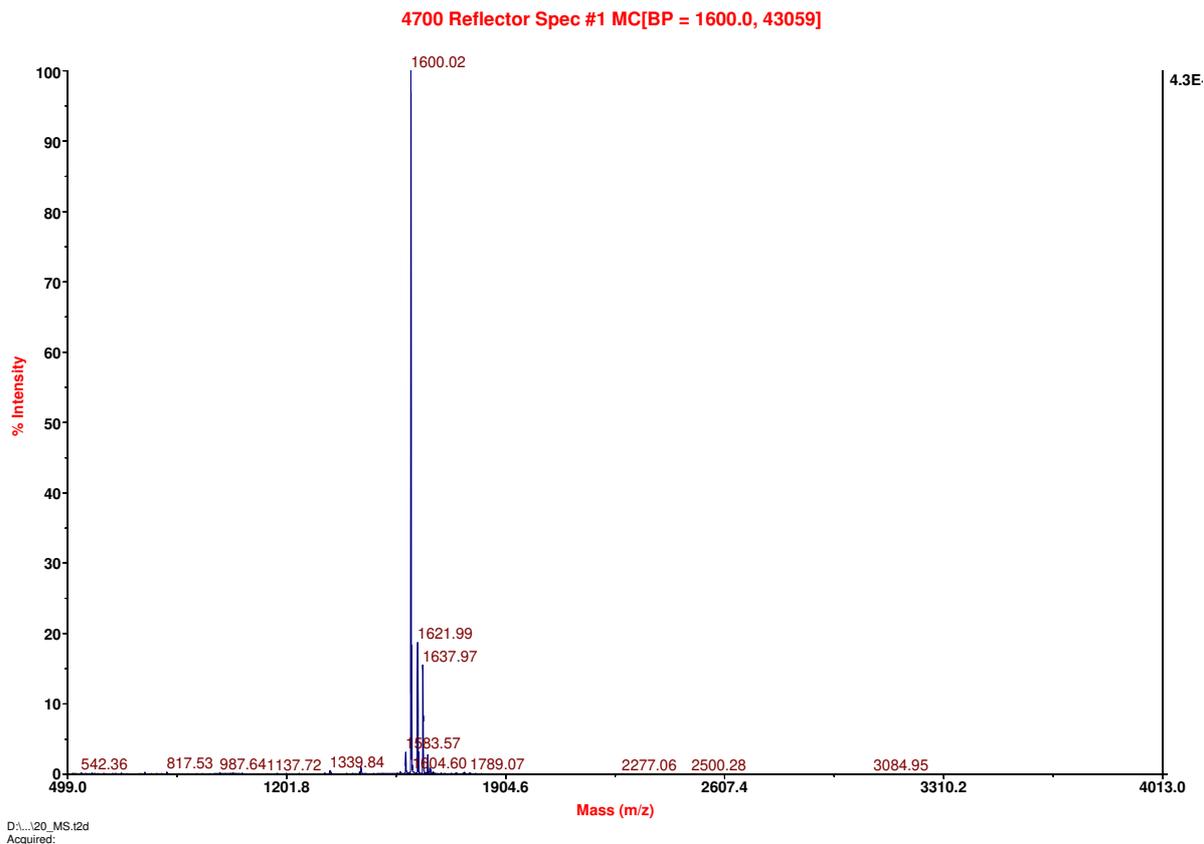
**Figure S1.** Analytical RP-HPLC of the synthesized temporin-SHe performed on a C18 column with a 30-70% linear gradient of acetonitrile (1%/min, see % composition axis) (blue line). Absorbance was monitored at 220 nm (AU: absorbance unit). (A) HPLC chromatogram showing elution of temporin-SHe at a retention time corresponding to 27.76 min (black line). Blank chromatogram (run without peptide) is also shown (red line) and revealed a small peak eluting at  $t = 20.7$  min (arrow). (B) Peak integration indicating 97.4% peptide purity (% area). The peak denoted by an arrow was not integrated as it was also present in the blank.



**Figure S2.** Analytical RP-HPLC of the synthesized temporin-SHd performed on a C18 column with a 30-70% linear gradient of acetonitrile (1%/min, see % composition axis) (blue line). Absorbance was monitored at 220 nm (AU: absorbance unit). **(A)** HPLC chromatogram showing elution of temporin-SHd at 24.02 min. **(B)** Peak integration indicating 95.4% peptide purity (% area).

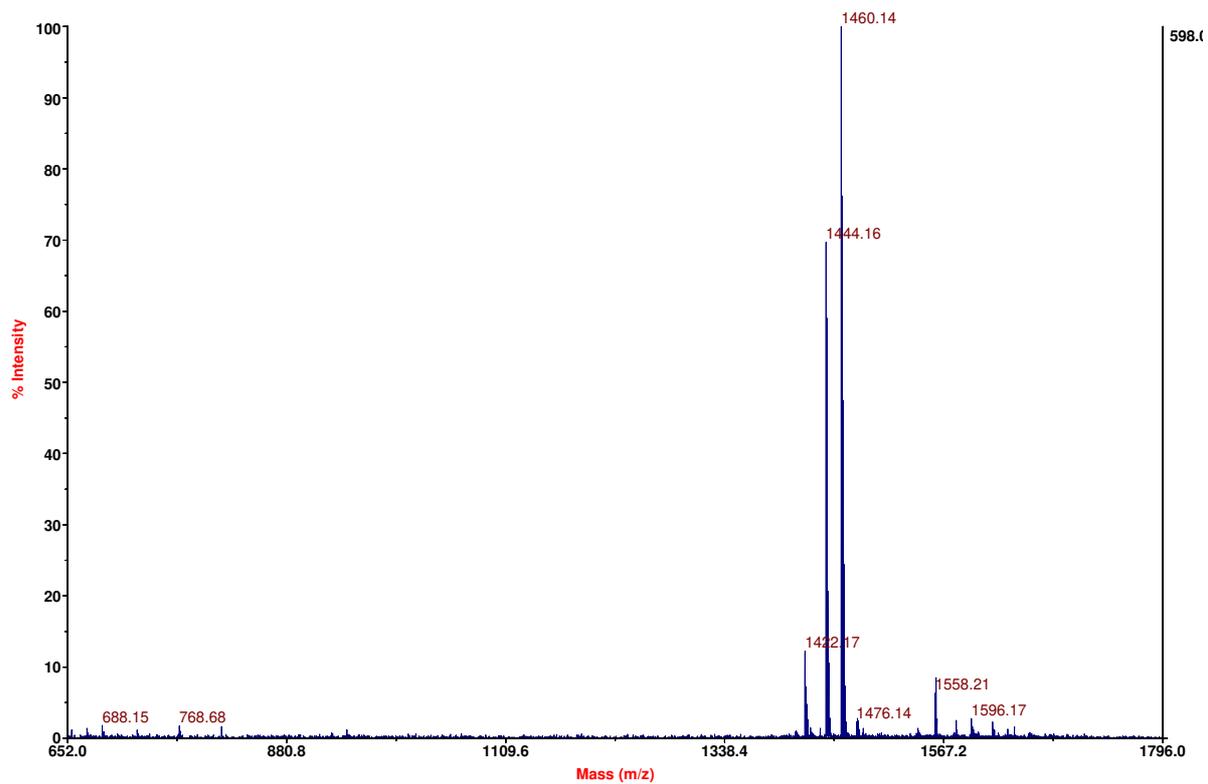


**Figure S3.** Analytical RP-HPLC of the synthesized [K<sup>3</sup>]temporin-SHa performed on a C18 column with a 30-70% linear gradient of acetonitrile (1%/min, see % composition axis) (blue line). Absorbance was monitored at 220 nm (AU: absorbance unit). **(A)** HPLC chromatogram showing elution of [K<sup>3</sup>]temporin-SHa at a retention time corresponding to 18.92 min. **(B)** Peak integration indicating 99% peptide purity (% area).



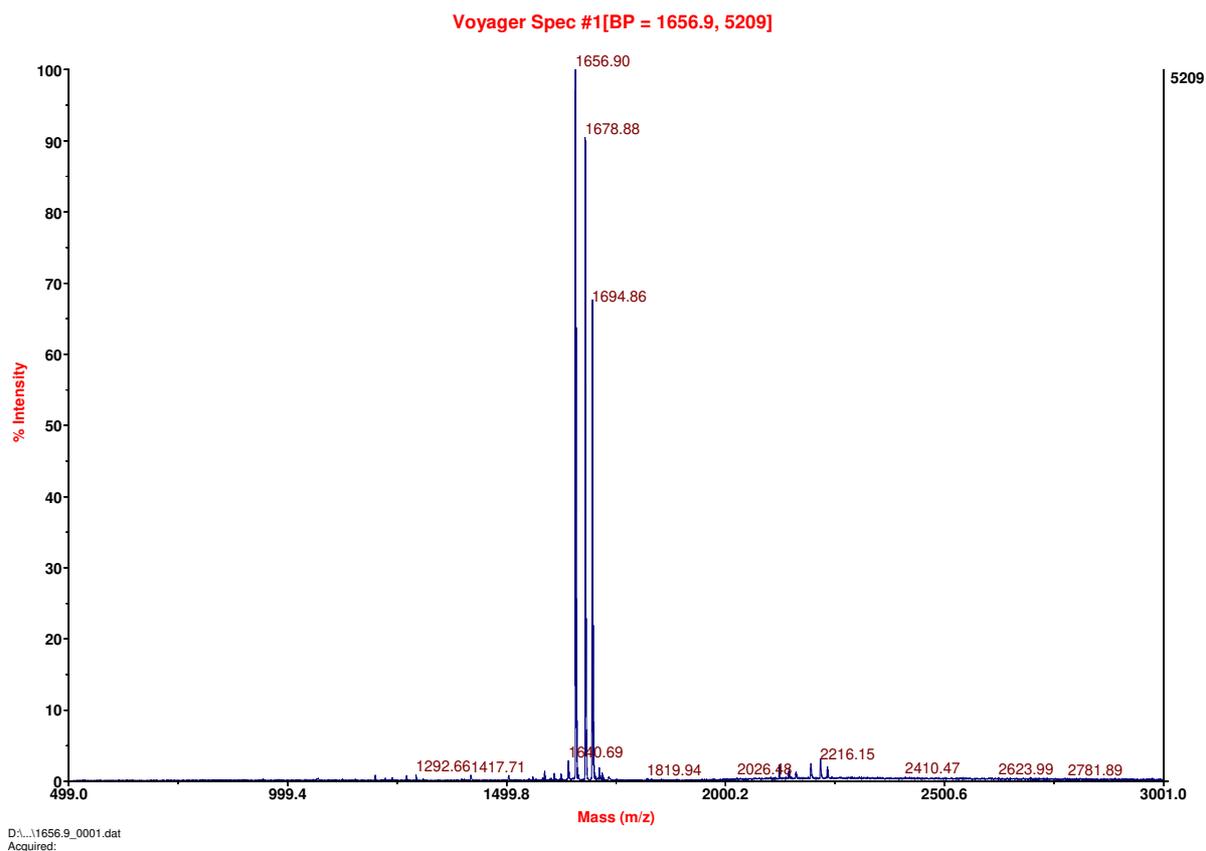
**Figure S4.** MALDI-TOF MS spectrum of the synthetic temporin-SHe. A mass product ( $[M+H]^+$ , 1600.02  $m/z$ ) corresponding to the theoretical monoisotopic mass of temporin-SHe is observed, with also the sodium and potassium adducts ( $[M+Na]^+$ , 1621.99  $m/z$ ;  $[M+K]^+$ , 1637.97  $m/z$ ).

4700 Reflector Spec #1[BP = 1460.1, 598]



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**Figure S5.** MALDI-TOF MS spectrum of the synthetic  $[K^3]$ temporin-SHa. A mass product ( $[M+H]^+$ , 1422.17  $m/z$ ) corresponding to the theoretical monoisotopic mass of  $[K^3]$ temporin-SHa is observed. Sodium and potassium adducts ( $[M+Na]^+$ , 1444.16  $m/z$ ;  $[M+K]^+$ , 1460.14  $m/z$ ) are also visible.



**Figure S6.** MALDI-TOF MS spectrum of the synthetic temporin-SHd. A mass product ( $[M+H]^+$ , 1656.90  $m/z$ ) corresponding to the theoretical monoisotopic mass of temporin-SHd is observed. Sodium and potassium adducts ( $[M+Na]^+$ , 1678.88  $m/z$ ;  $[M+K]^+$ , 1694.86  $m/z$ ) are also visible.