LyeTx I) or to its C-terminal portion (LyeTx I-K-HYNIC) were synthesized and purified, as previously reported [14].

Both synthesis were performed by stepwise solid-phase using the N-9-fluorenylmethyloxycarbonyl (Fmoc) strategy on a rink amide resin (0.63 $mmol \cdot g^{-1}$). Side chain protecting groups were as follows: *t*-butyl for threonine, *t*-butyloxycarbonyl for lysine and tryptophan, (triphenyl) methyl for histidine, asparagine and glutamine. Couplings were performed with 1,3-diisopropylcarbodiimide/1-hydroxybenzotriazole in DMF for 60–180 min. Deprotections (15 min, twice) were conducted by piperidine: DMF (1:4; v:v). Cleavage from the resin and final deprotection were performed with TFA/water/triisopropylsilane (95.0/2.5/2.5, v:v) at room temperature during 90 min. Post-precipitation of the products with cold diisopropyl ether, the crude peptide complexes were extracted with water:acetonitrile (1:1; v:v), followed by freeze-drying.

The crude synthetic products were purified by RP-HPLC on a C8 column (Discovery® BIO Wide Pore C8 column, 5 μm, 250.0 mm × 4.6 mm), previously equilibrated with 0.1 % (v:v) TFA in water (eluent A) and eluted by a linear gradient of 0.1 % (v:v) TFA in acetonitrile (eluent B), as specified in Table 1A.

The collected fractions were assessed by matrix-assisted laser desorption ionization time of flight mass spectrometer (MALDI-ToF-MS) analysis on AutoFlex III (Bruker Daltonics®, Germany). Briefly, samples were spotted onto a sample plate (MTP 384 Anchorchip, Bruker Daltonics®, Germany) mixed with a saturated solution of α-cyano-4-hydroxycinnamic acid and allowed to dry at room temperature (dried-droplet method). The mass spectrometer (MS) spectra were acquired in the positive reflector mode with external calibration (Peptide Calibration Standard II, Bruker Daltonics®, Germany).

Purity assessment of the peptide LyeTx I derivatives modified with the chelating agent HYNIC

The purified synthetic products were analyzed by RP-HPLC on a C18 analytical column (PepMap C18™ column, 5 μm, 150.0 mm × 4.6 mm), previously equilibrated with 0.1 % (v:v) TFA in water (eluent A) and eluted by a linear gradient of 0.1 % (v:v) TFA in acetonitrile (eluent B), as specified in Table 1B. The peaks of the peptides were collected and analyzed by MALDI-ToF-MS on AutoFlex III (Bruker Daltonics®, Germany), as described in the previous section.

In vitro evaluation of the maintenance of the antimicrobial activity of the peptide LyeTx I derivatives modified with the chelating agent HYNIC

The maintenance of the antimicrobial activity after peptide LyeTx I modifications with HYNIC was evaluated by microdilution test, according to the Clinical and Laboratory Standards Institute [15]. Bacterial strains of reference, *S. aureus* (ATCC® 6538) and *E. coli* (ATCC® 10536), were grown on tryptic soy agar at 37 °C for 18 h. Then, 0.5 McFarland scale bacterial suspensions (10^8 $CFU \cdot mL^{-1}$) were prepared on tryptic soy broth (TSB). The readouts were carried by determination of minimum inhibitory concentration (MIC), defined as a reduction of 100 % in bacterial growth post-incubation with the peptide LyeTx I derivatives at 37 °C for 24 h. LyeTx I obtained by chemical synthesis and without the coupled chelating agent was used as treatment control. Only TSB (no bacterial suspension and no peptide) was used as negative control. TSB plus bacterial suspension (no peptide) were used as positive control. MIC was expressed as median ($n = 3$). Each replicate was performed with a different bacterial colony, in duplicate.

Radiolabeling and radiochemical purity of LyeTx I-K-HYNIC with ^{99m}Tc

The radiolabeling procedure of LyeTx I-K-HYNIC with ^{99m}Tc and radiochemical purity analysis were performed as previously reported elsewhere [16], with some modifications. Briefly, in a sealed vial, tricine (20 mg) and EDDA (5 mg) were solubilized in 0.9 % NaCl (w:v) solution (200 μL). Next, LyeTx I-K-HYNIC (5, 10 or 20 μg) and 1 $mg \cdot mL^{-1}$ $SnCl_2 2H_2O$ solution (100, 200, 250 or 300 μL) in 0.25 $mol \cdot L^{-1}$ HCl were added. Then, the pH was adjusted (5, 6, 7, 8 or 9). Finally, $Na^{99m}TcO_4$ (37 MBq; q.s. ad = 1000 μL) was added to the vial and the final solution was heated (100 °C) in water bath (5,

Table 1 Solvent conditions for RP-HPLC

(A) Crude synthetic product purification		(B) Purified synthetic product analysis		(C) LyeTx I-K-HYNIC-^{99m}Tc evaluation	
Time (min)	Gradient of eluent B (%)	Time (min)	Gradient of eluent B (%)	Time (min)	Gradient of eluent B (%)
0–8.2	0	0–3.7	0	0–5.0	0
8.2–12.4	0–30	3.7–33.5	0–100	5.0–30.0	0–55
12.4–50.0	30–55	33.5–39	100	30.0–35.0	55–100
50.0–54.0	55–100			35.0–45.0	100
54.0–62.5	100				

Flow = 1.0 $mL \cdot min^{-1}$. Detection = 214 nm