Supplementary Data

NisC, the cyclase of the lantibiotic nisin, can catalyze cyclization of designed non-lantibiotic peptides

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Table S1 : Monoisotopic masses of hydrolysis fragments of the NisC-cyclized peptide RADhbVADhbCKGCK. The peptide was hydrolyzed for 15 min in 6 N HCl at 120 °C. Vertical lines in sequence indicate hydrolysis of peptide bond and underlined residues are absent.

Peptides and peptide fragments	Observed (Da) $[M + H]^+$	Calculated (Da) $[M + H]^+$
H-RADhbVADhbCKGCK-OH	1101.61	1101.57
+ H_2O	1119.61	1119.58
+ H_2O + O	1135.64	1135.58
+ 2 H_2O	1137.60	1137.60
H-RADhbVADhbCK|G|CK-OH	1062.59	1062.56
+ H_2O	1080.61	1080.57
H-RADhbVADhbC|K|GCK-OH	991.52	991.48
+ H_2O	1009.53	1009.49
H-RADhbVADhbC|KG|CK-OH	934.49	934.46
+ H_2O + O	950.48	950.46
+ H_2O	952.47	952.47
H-RADhbVADhbC|K|GC|K-OH	863.41	836.39
+ H_2O	881.43	881.40
H-RADhbVADhbC|KG|C|K-OH	806.39	806.37
+ O	822.40	822.37
+ H_2O	824.38	824.38
H-RADhbVA|DhbCKG|CK-OH	748.40	748.41

H-RADhbVA**DhbS**C|KGCK-OH[a]	719.34	719.33
H-R|ADhb|V|ADhbC|KG|CK-OH	697.55	697.30
H-RADhbVADhbC|KGCK-OH	685.37	685.35
H-RADhbVA|Dhb|C|KGCK-OH	620.33	620.32
H-RA**DhbS**VA|DhbCKGCK-OH[a]	533.30	533.29
H-RADhb|VA**DhbS**C|KGCK-OH[a]	409.21	409.16
Fragments that were not observed. If they would have been observed this might have indicated a non-intertwined peptide (Figure 3H). The fact that they were not observed indicates intertwined peptide (Figure 3G)		
H-RADhb|VADhbCKG|CK-OH	-	560.29
H-RADhb|VADhbCK|GCK-OH	-	503.27
H-RADhb|VADhbC|KGCK-OH	-	375.17
H-RADhbV|ADhbCKG|CK-OH	-	461.22
H-RADhbV|ADhbCK|GCK-OH	-	404.20
H-RADhbVA|DhbCKG|CK-OH	-	390.18
H-RADhb|VADhbCKG|CK-OH	-	578.31

[a] **DhbS** represents β-methylcysteine which is the result of opening of the methyl-lanthionine at the sulphur atom, the sulphur originating from the cysteine shifting to the former dehydrobutyrine.

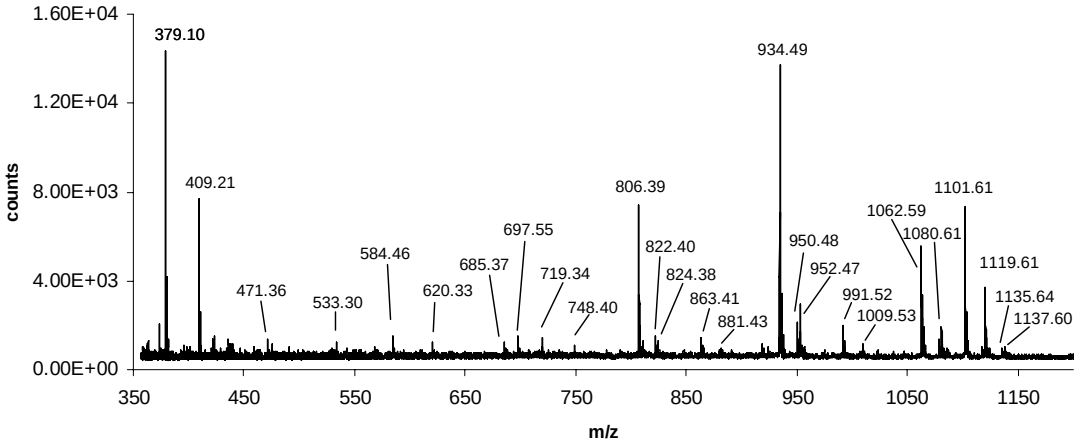

Figure S1: Maldi-tof spectrum of fragments of the NisC-cyclized peptide RADhbVADhbCKGCK. This peptide was hydrolyzed for 15 min in 6 N HCl at 120 °C. The spectrum was recorded in reflectron mode. The peak of 379.10 Da originates from the used matrix α-cyano-4-hydroxy-cinnamic acid. The observed masses are explained in Table S1.

Figure S1 shows a series of fragments that are generated upon partial hydrolysis of the thioether ring-containing RADhbVADhbCKGCK peptide with 6 N HCl at 120 °C. Fragments having higher mass than 800 Da are relatively abundant. They are explained by various hydrolysis events of peptide backbone resulting in the loss of lysine and glycine residues, whose hydrolysis-mediated loss apparently takes place more easily. The less abundant fragments of masses below 800 Da are mostly explained by opening of the thioether bond. The sulphur atom can shift either to the former dehydrobutyrine, generating β-methylcysteine, or react back yielding a free cysteine again. The lower part of Table S1 shows a series of peptides that were not found. If any of them would have been observed this might have indicated a non-

intertwined peptide as depicted in Fig. 3H. The fact that none of them was observed indicates the intertwined ring-containing peptide depicted in Figure 3G. All the observed fragments can be expected for both the intertwined (Fig. 3G) and the alternative structure (Fig. 3H); however, all additional hydrolysis events (+ H_2O) are easier explained by the intertwined peptide. They are possible in the non-intertwined peptide but then they should be exactly between residues 6 and 7, the small lanthione ring (Fig. 3H). Moreover, the complete absence of fragments that in the case of a non-intertwined peptide as depicted in Figure 3H could have been generated easily by only two hydrolysis events is compelling evidence towards the intertwined ring structure (Fig. 3G).