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**Influence of shifting positions of Ser, Thr, and Cys residues in prenisin on the efficiency of modification reactions and on the antimicrobial activities of the modified prepeptides**

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1 **Supporting material for:**  
2  
3 **The influence of shifting positions of Ser, Thr and Cys residues in**  
4 **prenisin on the efficiency of modification reactions and on the**  
5 **antimicrobial activity of the modified propeptides**

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7 **and Oscar P. Kuipers<sup>1\*</sup>**  
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15 3632093

16  
17 **Fig. S1-S9** Show mass spectra of prenisin and prenisin mutants analyzed in the study.

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20 **Fig. S10** Direct presence of dehydrated amino acids and/or thioether bridge protects  
21 against trypsin cleavage. Nisin and its eight mutants were subjected to tryptic  
22 digestion as described in the Materials and Methods section. The reaction mixture was  
23 subjected to Tricine-Page electrophoresis. (1) undigested prenisin, (2) prenisin, (3)  
24 prenisin 1Ala, (4) prenisin 2Ala, (5) prenisin 3Ala, (6) prenisin 4Ala, (7) prenisin  
25 1Ahinge, (8) prenisin 2Ahinge, (9) prenisin 3Ahinge, (10) prenisin 4Ahinge. Arrows  
26 indicate prenisin (upper arrow 2) and nisin (lower arrow 3).

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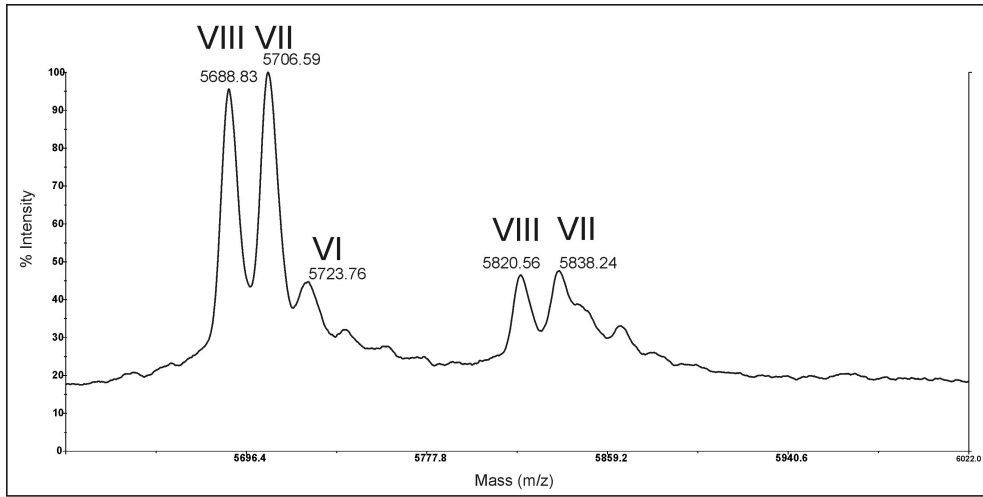


Fig. S1 Prenisin wild type

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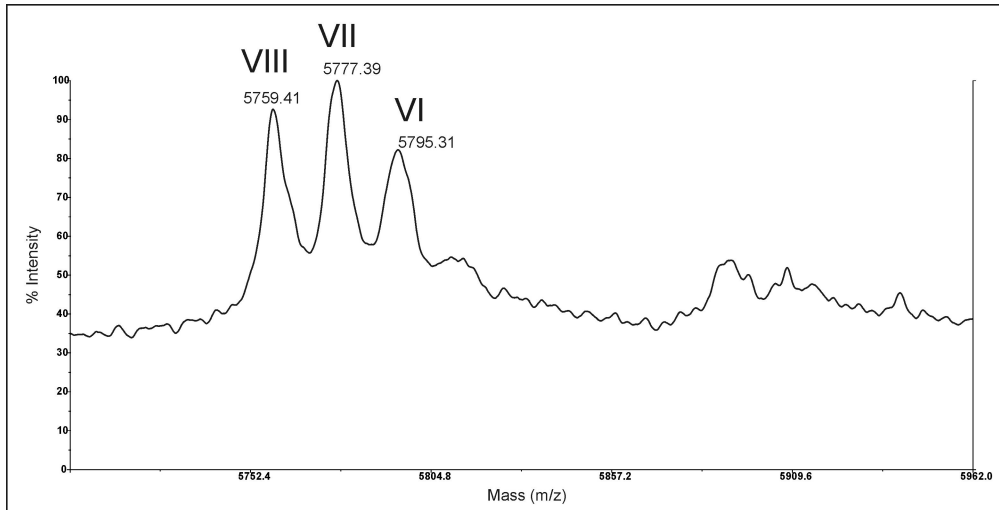


Fig. S2 Prenisin 1Aa

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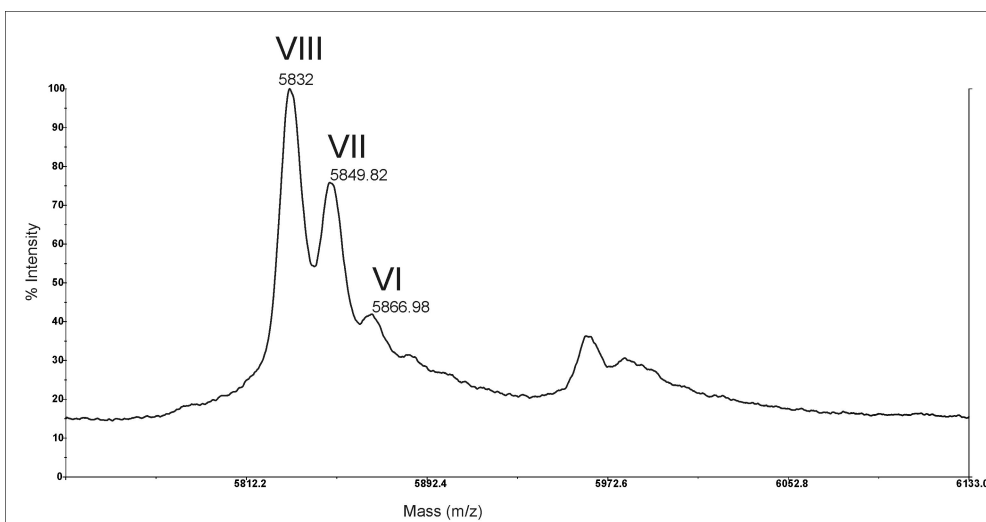


Fig. S3 Prenisin 2Ala

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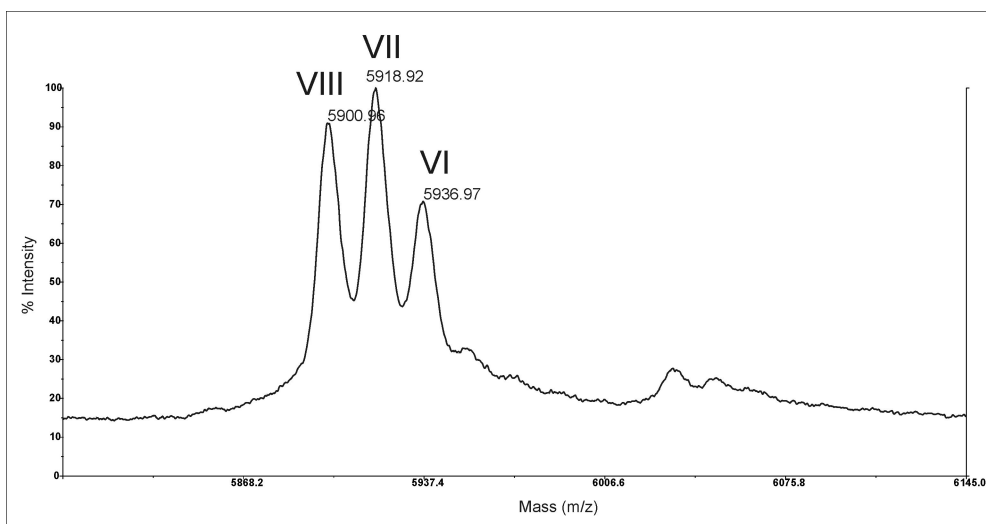
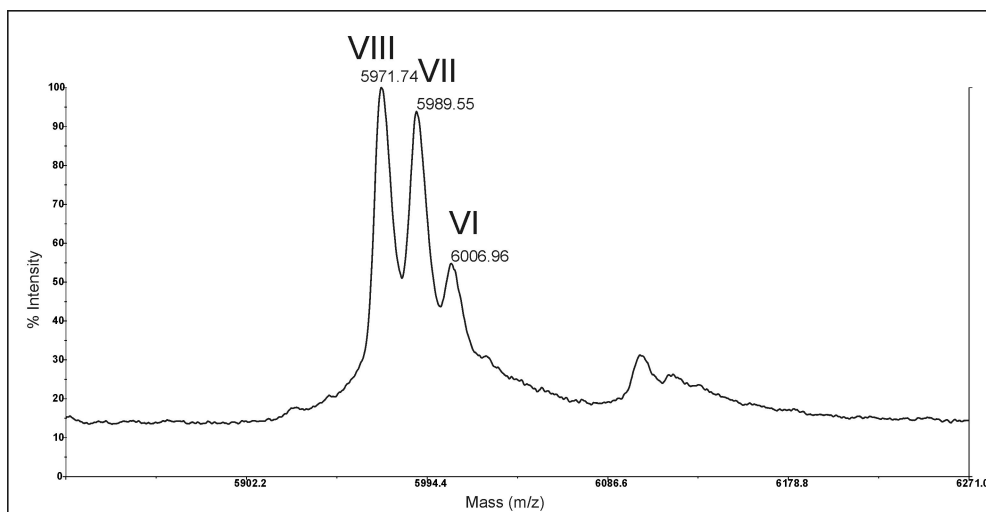


Fig. S4 Prenisin 3Ala

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Fig. S5 Prenisin 4Ala

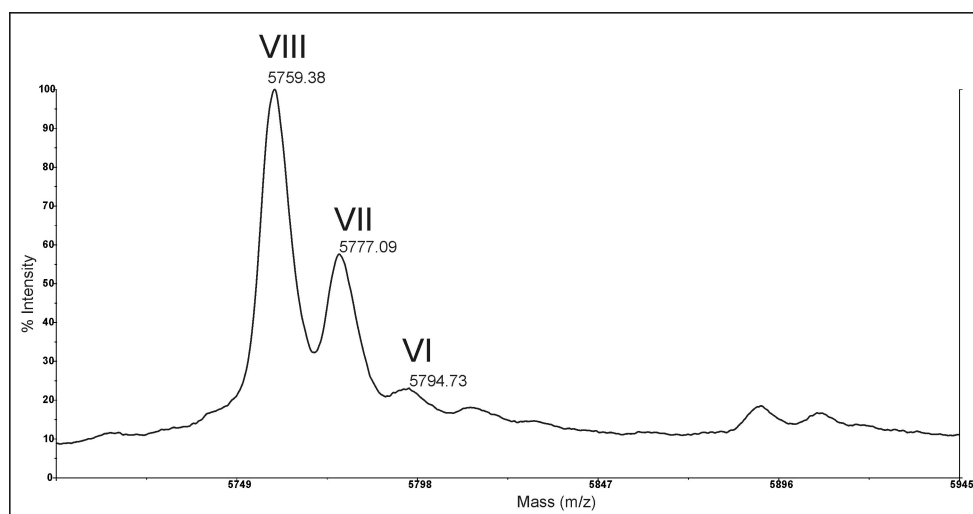
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Fig. S6 Prenisin 1AHinge

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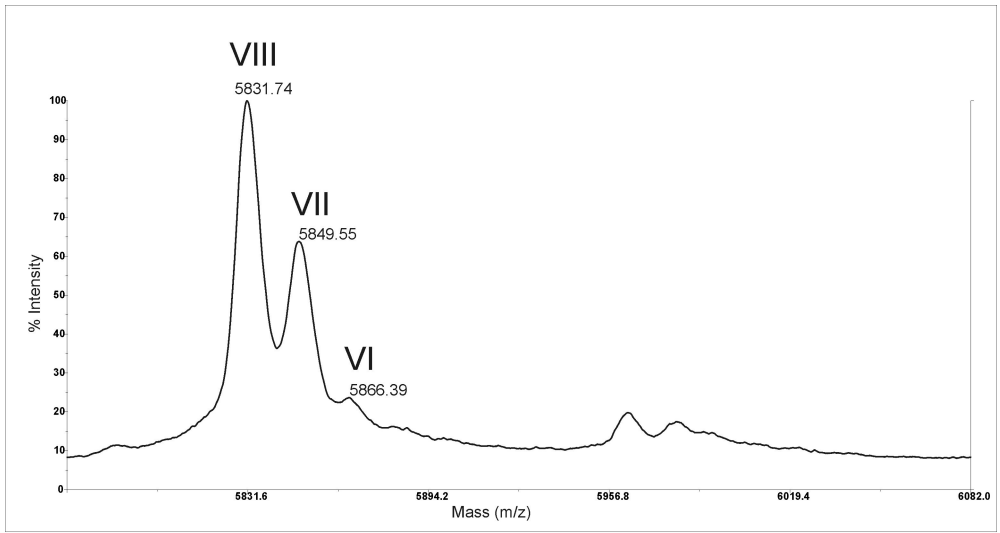


Fig. S7 Prenisin 2AHinge

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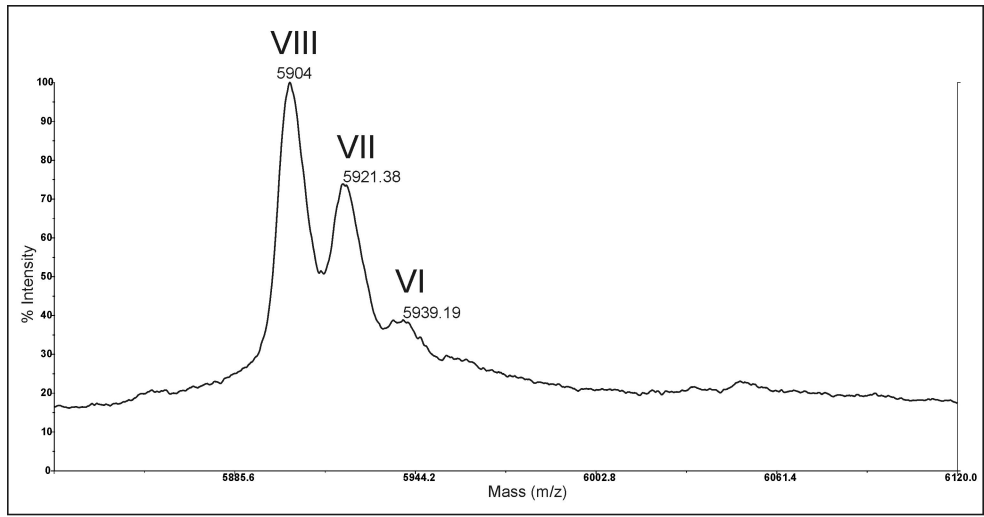
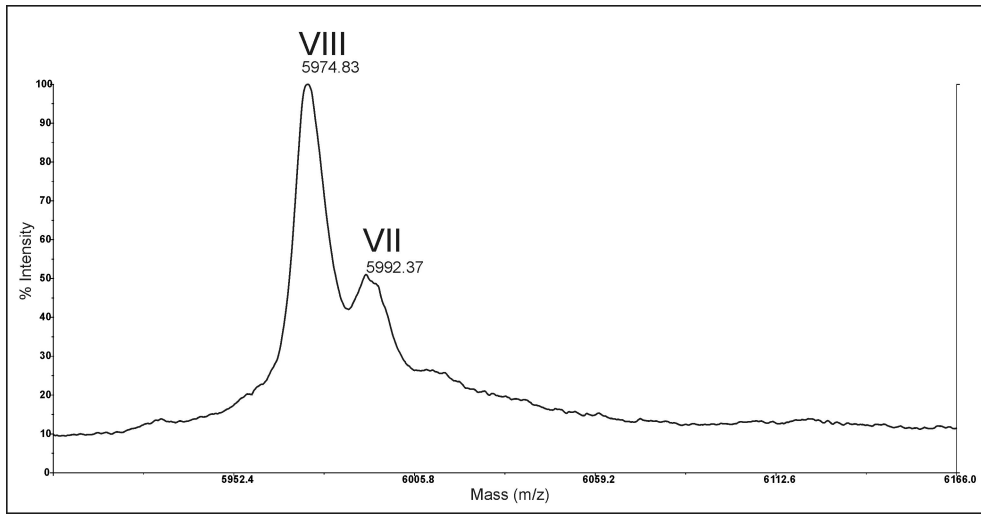


Fig. S8 Prenisin 3AHinge

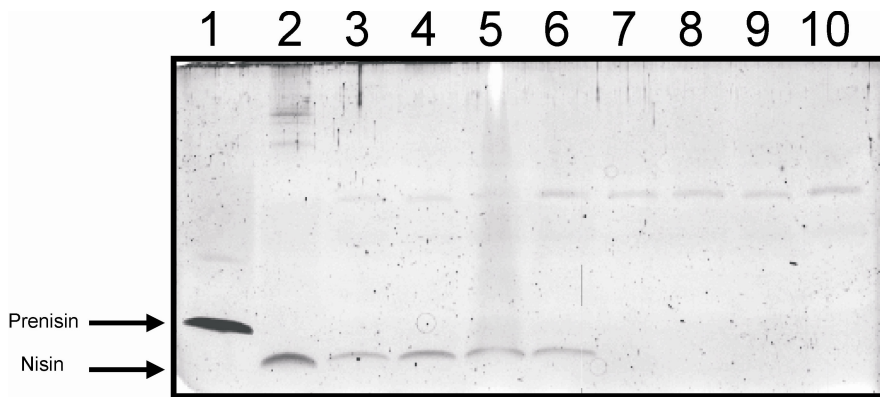
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Fig. S9 Prenisin 4AHinge



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Fig. S10