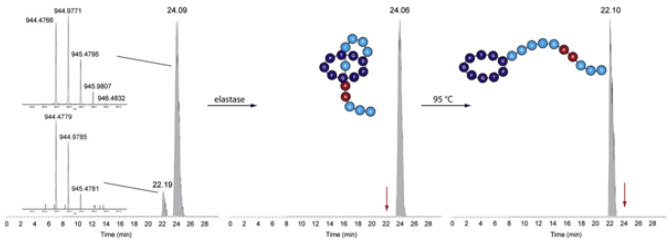
Application Number	127770
Method	HPLC
Results	

Title: see Note:

Substances	capistruin R15A/F16A

Product(s)

Phase	REF	Webshop
NUCLEODUR C18 ec	760001.20	Shop now

Matrix	cell culture
Sample(s)	For cloning and site directed mutagenesis as well as for heterologous production and extraction of capistruin and capistruin derivatives see the cited literature. Stability studies of capistruin R15A/F16A An extract of the supernatant of a 6 L culture was prepared as described in the cited literature and dissolved in 1.6 mL 20 mM Tris/HCl (pH 8.0). In a 30 mL reaction vial, 25 mL of the extract was incubated in the presence or absence of 0.025 units elastase for 1 h at 25 °C (20 mM Tris/HCl (pH 8.0) was used to fill up to 30 mL). Afterwards the samples were heated for 1 h at 95 °C, cooled down to 25 °C, and incubated again in the presence or absence of additional 0.025 units elastase for 1 h

	at 25 °C. After each single incubation step, 20 µL of the reaction mixtures was analyzed by LC-MS.
Conditions	Eluent A: water with 0.05 % formic acid Eluent B: acetonitrile with 0.045 % formic acid Gradient: 10 - 40 % B in 30 min, 40 - 95 % B in 5 min, 95 % B for 2 min Flow rate: 0.2 mL/min Temperature: 40 °C Injection volume: 20 µL
Detection	LC-MS (ion trap)
Note	Extracted ion chromatograms (EIC) of an extracted culture supernatant revealed two compounds (RT 22.19 min and RT 24.09 min) corresponding to the calculated mass of the doubly protonated ion of capistruin R15A/F16A ([M+2H]2+ calculated = 944.4716). Elastase treatment resulted only in a degradation of the 22 min compound. The remaining 24 min compound could be converted into the one at 22 min upon incubation at 95 °C. Further incubation of the heat-converted sample with elastase resulted in a complete digestion, indicating that the 24 min compound corresponds to the lasso-structured conformation and the 22 min compound resembles the branched-cyclic peptide.
Author	Knappe, T.A. et al.
Source	Chemistry&Biology 16, 1290 - 1298, December 24, 2009
Disclaimer	Concerning questions to literature applications please contact the cited author/s. Chemicals mentioned in applications are not manufactured or sold by MACHEREY-NAGEL. Applications mentioned in MACHEREY-NAGEL literature or databases are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of the applications.