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List of abbreviations

C-terminus	carboxy terminus
FTICR	Fourier transform ion cyclotron resonance
HCN	proton, carbon, nitrogen
MALDI	matrix-assisted laser desorption/ionization
MS	mass spectrometry
MS/MS	tandem mass spectrometry
N-terminus	amino terminus
NanoESI	nano-electrospray ionization
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
Q-TOF	quadrupole time of flight
rmsd	root mean square deviation
TOF	time of flight

Supplementary figures

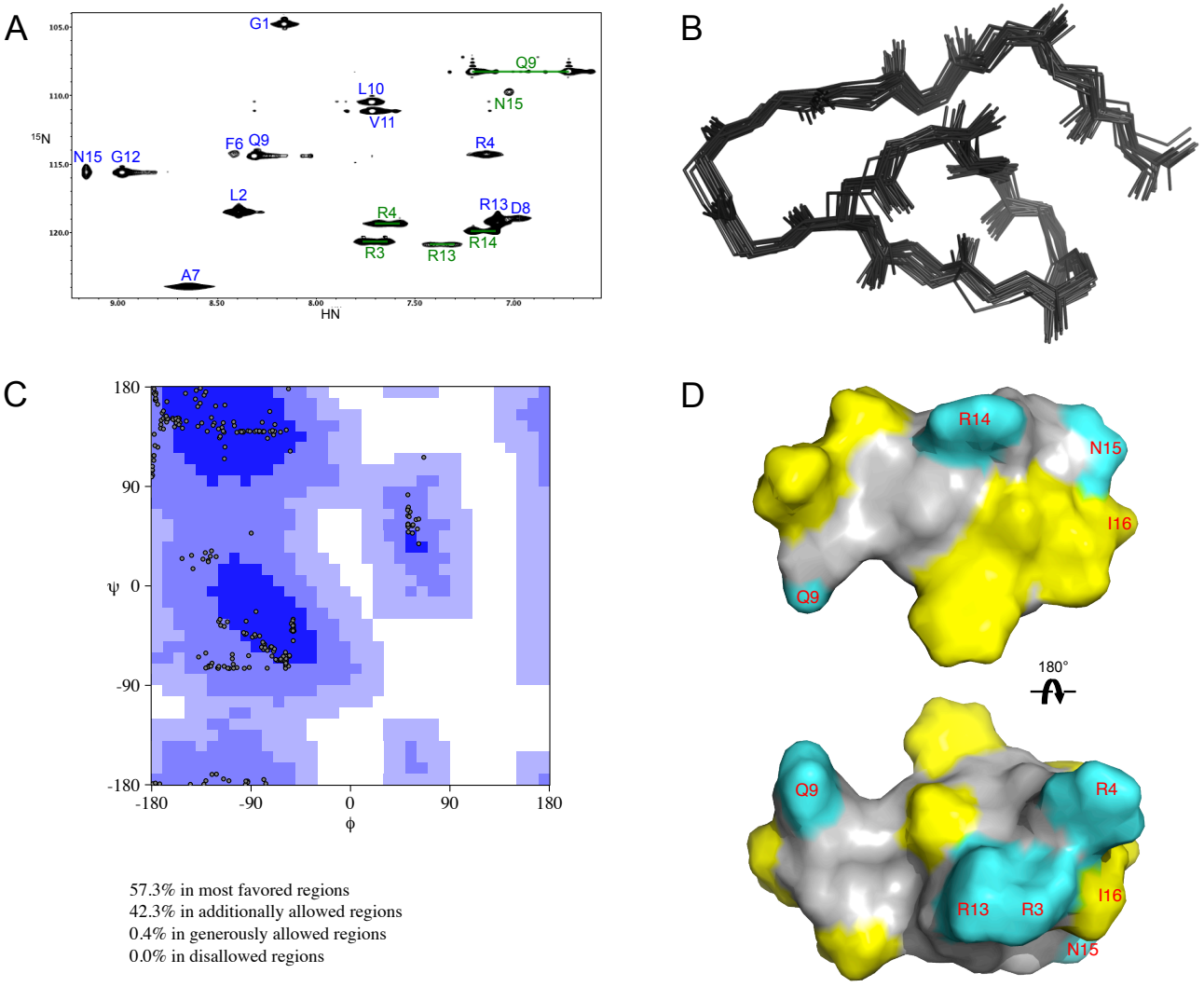

Figure S1, related to Figure 1. **A)** 2D-[^{1}H–^{15}N]-HSQC spectrum of lassomycin.12 backbone resonances are labeled in blue. Sidechain amide resonances are paired together with green lines and labeled in green. The second sidechain resonance for Asn15 (7.45 ppm) is not shown because of its extremely weak intensity. **B)** Backbone overlay of the 20 lowest energy conformers of lassomycin. **C)** Ramachandran plot of the dihedral angles (ϕ versus ψ) of the 20 best solution structures for lassomycin. **D)** Surface representation of lassomycin. Hydrophobic side chains are highlighted in yellow and hydrophilic sidechains are shown in cyan. Hydrophilic residues and the C-terminal residue (Ile16) are labeled.

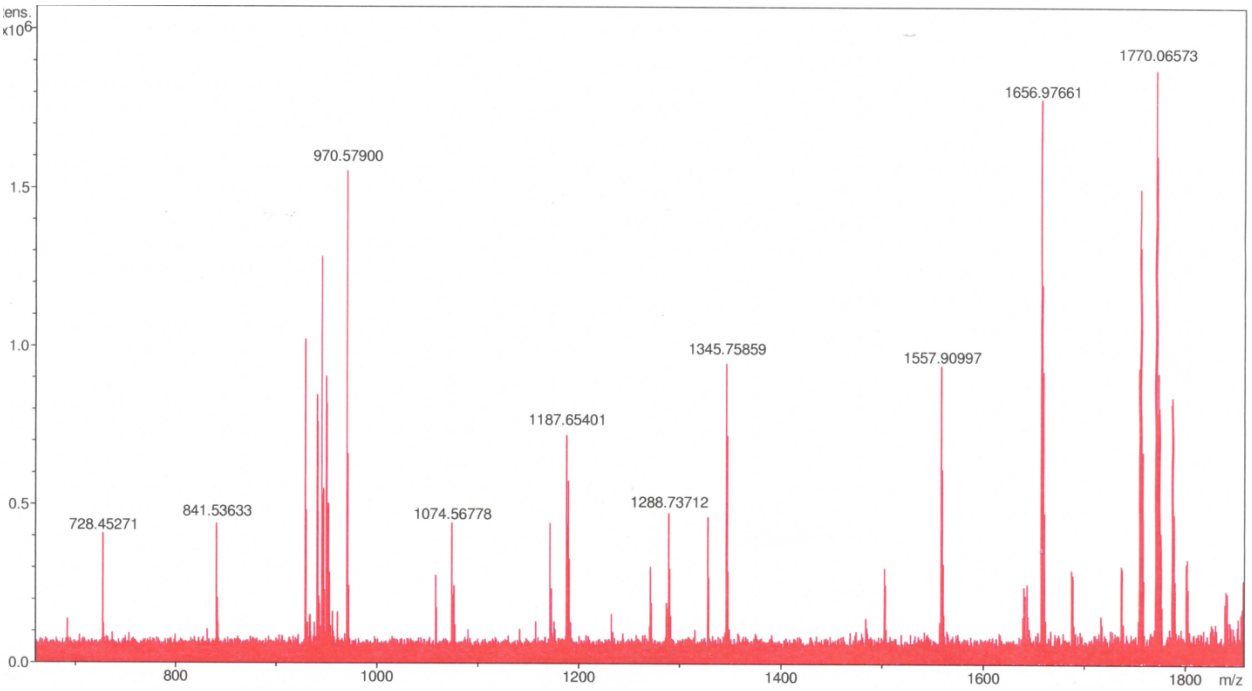

Figure S2 related to exact mass determination. MALDI FTICR spectrum of lassomycin.

Supplementary tables

Table S1 related to MS/MS sequencing. MALDI FTICR and MALDI TOF/TOF analysis of partially hydrolyzed lassomycin.

Amino acids (X)	Proposed formula $[X+H]^+$	Calculated mass $[X+H]^+$	Observed mass $[X+H]^+$	Error (ppm)
Series 1 (ion series A & B)				
ELVGRRNI-$COOCH_3$[a]	$C_{41}H_{76}N_{15}O_{12}$	970.5792	970.5790[b]	0.3
LVGRRNI-$COOCH_3$	$C_{36}H_{69}N_{14}O_9$	841.5367	841.5363	0.4
VGRRNI-$COOCH_3$	$C_{30}H_{58}N_{13}O_8$	728.4526	728.4527	-0.2
GRRNI-$COOCH_3$	$C_{25}H_{49}N_{12}O_7$	629.3842	629.3841	0.1
RRNI-$COOCH_3$	$C_{23}H_{46}N_{11}O_6$	572.3627	572[c]	–
RNI-$COOCH_3$	$C_{17}H_{34}N_7O_5$	416.2616	416[c]	–
Series 2 (ion series C)				
GLRRLFAD	$C_{42}H_{69}N_{14}O_{10}$	929.5316	929.5316	0.0
GLRRLFAD+H_2O	$C_{42}H_{71}N_{14}O_{11}$	947.5421	947.5424	-0.3
GLRRLFADE[a]	$C_{47}H_{78}N_{15}O_{14}$	1076.5847	1076.5840	0.7
GLRRLFADEL	$C_{53}H_{89}N_{16}O_{15}$	1189.6688	1189.6691	-0.2
GLRRLFADELV	$C_{58}H_{98}N_{17}O_{16}$	1288.7372	1288.7371	0.1
GLRRLFADELVG	$C_{60}H_{101}N_{18}O_{17}$	1345.7587	1345.7586	0.1
GLRRLFADELVGR	$C_{66}H_{113}N_{22}O_{18}$	1501.8598	1501.8578	1.3
Series 3 (ion series D)				
M+H_2O with loss E-L-V[a]	$C_{67}H_{117}N_{26}O_{17}$	1557.9085	1557.9100	-1.0
M+H_2O with loss E-L	$C_{72}H_{126}N_{27}O_{18}$	1656.9769	1656.9766	0.2
M+H_2O with loss E	$C_{78}H_{137}N_{28}O_{19}$	1770.0609	1770.0657	-2.7
1-16 (molecular ion)				
GLRRLFADQLVGRRNI-$COOCH_3$	$C_{83}H_{143}N_{30}O_{20}$	1880.1089	1880.1079	0.5
GLRRLFADELVGRRNI-$COOCH_3$[a]	$C_{83}H_{142}N_{29}O_{21}$	1881.0930	1881.0914	0.9
GLRRLFADELVGRRNI-$COOCH_3$+H_2O	$C_{83}H_{144}N_{29}O_{22}$	1899.1035	1899.1044	-0.5

[a]De-amidation during acid hydrolysis converted Q to E
[b]Exact masses measured on MALDI FTICR MS
[c]Observed in MALDI TOF/TOF MS/MS but not in MS mode on FTICR MS

Table S2 related to NMR spectroscopy. Experimental parameters used to acquire NMR spectra on [^{13}C, ^{15}N]lassomycin to obtain chemical shift assignments, coupling constants, and NOE restraints.

Exp. Name[a]	Nuclei[b]	x-sw[c]	y-sw	z-sw	x-pts	y-pts	z-pts	References
^{13}C-HSQC (full)	^{1}H, ^{13}C	11990	28155		1024	128		
^{15}N-HSQC	^{1}H, ^{15}N	11990	2800		1024	128		(Kay et al., 1992)
HNHA	^{1}H, $^{1}H_{\alpha}$, ^{15}N	11990	8000	1945	1024	96	32	(Kuboniwa et al., 1994; Vuister and Bax, 1993)
CBCA(CO)NH	^{1}H, ^{13}C, ^{15}N	11990	12000	1945	1024	64	32	(Muhandiram and Kay, 1994)
HCCH-TOCSY	^{1}H, ^{1}H, ^{13}C	11990	9000	12001	1024	144	28	(Sattler et al., 1995)
HNCO	^{1}H, $^{13}C(O)$, ^{15}N	11990	3770	1945	1024	64	32	(Grzesiek and Bax, 1992; Ikura et al., 1990; Kay et al., 1994; Muhandiram and Kay, 1994)
HNCACB	^{1}H, ^{13}C, ^{15}N	11990	16089	1945	1024	64	32	(Kay et al., 1994; Muhandiram and Kay, 1994; Wittekind and Mueller, 1993)
^{13}C-NOESYHSQC	^{1}H, ^{1}H, ^{13}C	11990	8000	8000	1024	128	32	(Farrow et al., 1994)
^{15}N-NOESYHSQC	^{1}H, ^{1}H, ^{15}N	11990	7998	1945	1024	128	32	(Zhang et al., 1994)
^{15}N-TOCSYHSQC	^{1}H, ^{1}H, ^{15}N	11990	8000	1945	1024	128	32	(Zhang et al., 1994)

[a]Experiments were acquired at 800 MHz.
[b]The nucleus acquired in each dimension (e.g. 1H,15N indicates hydrogen x, nitrogen y).
[c]x,y,z-pts and sw are the number of complex points and sweep width in each respective dimension (x is the directly detected dimension).

Table S3 related to NMR spectroscopy. ^{1}H Chemical shift assignments of lassomycin.

	HN	**Hα**	**Hβ**	**others**
Gly 1	8.16	4.79, 3.36		
Leu 2	8.39	4.53	1.88, 1.36	γCH 1.59, δCH_3 0.87, 0.85
Arg 3	NA[a]	4.48	1.52, 1.19	γCH_2 1.21, 1.17, δCH_2 3.17, 2.88, $\eta_2 NH_2$ 7.76, 7.66
Arg 4	7.14	4.40	1.66, 1.54	γCH_2 1.44, 1.34, δCH_2 3.02, $\eta_2 NH_2$ 7.69, 7.58
Leu 5	NA	4.41	1.10, 0.81	γCH 1.03, δCH_3 0.67, 0.61
Phe 6	8.41	3.98	3.64, 3.03	
Ala 7	8.65	4.25	1.38	
Asp 8	6.98	4.65	2.95, 1.88	
Gln 9	8.32	3.59	1.80, 1.65	γCH_2 2.15, 2.10, εNH_2 7.21, 6.73
Leu 10	7.72	4.40	1.61, 1.38	γCH 1.40, δCH_3 0.86, 0.83
Val 11	7.72	4.39	1.87	γCH_3 0.83, 0.83
Gly 12	8.98	5.39, 3.56		
Arg 13	7.08	5.17	1.20, 1.06	γCH_2 1.25, 1.13, δCH_2 2.85, 2.68, $\eta_2 NH_2$ 7.42, 7.32
Arg 14	NA	NA	1.80, 1.54	γCH_2 1.51, δCH_2 3.09, 2.99, $\eta_2 NH_2$ 7.20, 7.10
Asn 15	9.16	5.32	2.69	δNH_2 7.47, 7.03
Ile 16[b]	NA	4.07	1.77	γCH_2 1.44, 1.23 γCH_3 0.89, δCH_3 0.86, εCH_3 3.55

[a]NA = not assigned. Due to spectral overlap, a chemical shift could not be definitively assigned to the HA of Arg 14 and the HN proton of Arg3, Leu5, Arg14 or Ile16. [b]Carboxy group of Ile is methylated (εCH_3).

Table S4 related to NMR spectroscopy. Nitrogen and carbon chemical shift assignments of lassomycin

	N	Cα	Cβ	others
Gly 1	104.75	45.02		
Leu 2	118.46	53.33	43.05	Cγ 27.28, Cδ 26.05, 24.92
Arg 3	NA[a]	54.50	28.52	Cδ 43.90, $N\eta_2$ 110.11
Arg 4	114.27	57.97	34.42	Cγ 28.09, Cδ 43.31, $N\eta_2$ 108.79
Leu 5	NA	53.76	46.89	Cγ 31.83, Cδ 25.70, 24.71
Phe 6	114.24	60.05	36.78	
Ala 7	123.92	53.92	20.54	
Asp 8	118.95	52.05	39.51	
Gln 9	114.39	59.38	29.57	Cγ 34.63, Nε 108.24
Leu 10	110.45	56.22	45.10	Cδ 25.31, 24.85
Val 11	111.10	60.14	35.01	Cγ 18.56, 16.22
Gly 12	115.58	44.17		
Arg 13	119.20	54.35	33.20	Cγ 27.81, Cδ 43.81, $N\eta_2$ 110.32
Arg 14	NA	NA	33.75	Cγ 27.25, Cδ 44.29, $N\eta_2$ 109.33
Asn 15	115.56	52.02	41.47	Nδ 109.72
Ile 16[b]	NA	60.86	39.11	Cγ 28.17, Cγ' 18.20, Cδ 14.31, Cε 54.82

[a]NA = not assigned. Due to spectral overlap, a chemical shift could not be definitively assigned to the Cα of Arg14 and the amide N of Arg3, Leu5, Arg14 or Ile16. [b]Carboxy group of Ile is methylated (Cε).

Table S5 related to structure calculations. Structural statistics of the solution structure of lassomycin

Structural statistics	
Distance and angle restraints	
total cross peak assignments	449
short ($\|i–j\| \leq 1$)	369
medium ($1 < \|i–j\| < 5$)	19
long ($\|i–j\| \geq 5$)	61
number of ϕ angles	1
Average target function value	0.01
rmsd (Å) for residues 1-16	
backbone	0.35 ± 0.10
heavy atoms	1.00 ± 0.23

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