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Supporting information for article:

A natural, single-residue substitution yields a less active peptaibiotic: the structure of bergofungin A at atomic resolution

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Table S1 Backbone and side chain torsion angles (°) for bergofungin A.

	φ^a	ψ	ω	χ^1	χ^2	$\chi^{3/3,1}$	$\chi^{3,2}$	χ^4	ϑ^4
Val1	-119.8(9)	-71.5(9)	177.6(7)	179.2(8)/-59(1)					
Aib2	-53.4(1.0)	-36.9(9)	-178.4(7)						
Aib3	-48.4(1.0)	-42.1(1.0)	-173.6(8)						
Aib4	-57.5(1.0)	-39.4(1.0)	178.6(7)						
Val5	-59.5(1.0)	-44.3(1.0)	179.4(8)	171.3(7)/-64.9(9)					
Gly6	-62.7(1.0)	-40.4(1.1)	-176.8(7)						
Leu7	-71.9(1.0)	-39.2(1.0)	175.7(7)	-56(1)	-177(1)/-52(1)				
Aib8	-65.3(1.0)	-36.2(1.0)	-158.0(7)						
Aib9	-51.4(1.0)	-46.2(1.0)	-172.3(7)						
Hyp10	-62.5(1.0)	-20.7(1.0)	-179.6(7)	-20.8(8)	34.4(8)	-34.1(8)	-82.3(8)	22.0(9)	-0.6(9)
Gln11	-83.6(1.0)	-17.2(1.2)	-167.7(8)	-49(1)	-47(2)	140(1)/-40(1)			
Aib12	-53(1)	-42.8(1.1)	-172.8(7)						
Hyp13	-60.7(1.0)	-25.8(1.2)	180.0(8)	-25.0(9)	39.2(1.0)	-37.3	-79.7(9)	22.6(1.0)	1.4(1.0)
Aib14	-66.8(1.1)	-20.7(1.1)	-172.8(8)						
Fol15	-103(1)	65.2(9)[b]		-77.1(8)	75.4(9)/-101.5(8)				

[a] The symbols are named according to the IUPAC-commision

[b] Denotes the angle N_15 - CA_15 - C_15 - O_15

Table S2 Intra- and intermolecular hydrogen bonds of bergofungin A

Intramolecular	D...A (Å)	<CON (°)	H...A (Å)	<DHA (°)	Symmetry
O1←N4	2.93(1)	130.9(5)	2.18	145.9	
O2←N5	2.942(9)	127.3(5)	2.33	128.7	
O3←N6	2.977(9)	115.8(6)	2.51	115.2	
O3←N7	3.08(1)	162.9(6)	2.27	154.9	
O4←N8	3.07(1)	155.5(6)	2.22	168.9	
O5←N9	2.911(9)	155.1(6)	2.32	125.6	
O8←N11	2.97(1)	141.7(6)	2.17	153.2	
O9←N12	3.008(8)	122.9(5)	2.18	162.0	
O11←N14	3.097(9)	134.0(6)	2.34	146.5	
O12←N15	2.950(9)	128.6(6)	2.13	158.7	
N1→O14	2.80(1)	138.9(6)	1.98	158.1	x-1/2,y-1/2,z-1
N2→O15	2.95(1)	137.5(5)	2.14	156.8	x-1/2,y-1/2,z-1
N3→O14	3.22(1)	138.8(6)	2.38	164.4	x-1/2,y-1/2,z-1
OH15→O0	2.708(9)	116.5(6)	1.93	159.1	x+1/2,y-1/2,z+1
OD1(10)→O7	2.760(9)	165.1(6)	1.97	160.9	-x+1/2,y-1/2,-z
NE2(11)→OD1(10)	2.93(1)	95.2(5)	2.12	157.9	-x+1/2,y+1/2,-z
NE2(11)→O6	2.87(1)	136.0(1)	2.08	152.5	-x+1/2,y+1/2,-z
OD1(13)-OE1(11)	2.760(1)	138.4(6)	1.98	159.4	x, y-1,z
H_2O16→O10	2.96(1)	121.7(7)			
H_2O16→OE1(11)	2.99(6)	110.8(7)			

As in Figure 2 of the main body of the article, the atoms involved in type 1←5 hydrogen bonds are marked in green and the ones participating in 1←4 hydrogen bonds in blue. In cyan are marked the atoms involved in the ordered solvent hydrogen bond.

Table S3 Matthews coefficients (Matthews, 1968) and solvent content of peptaibol crystals whose structures are solved by X-ray crystallography.

Peptaibol	Reference	Matthews ratio ($Å^3$ Da^{-1})	Solvent content (% v/v)
Trichovirin I 4A	Gessmann *et al*., 2012b	1.61	23.8
Alamethicin F-30	Fox & Richards, 1982	1.55	21.0
Bergofungin A	present work	1.51	19.2
Samarosporin I	Gessmann *et al*., 2012a	1.47	17.0
Trichotoxin A50E	Chugh *et al.*, 2002	1.44	14.5
Antiamoebin	Snook *et al*., 1998	1.41	12.8

S1. Bacterial growth inhibition assay

Configuring the test plate: Volumes/punched hole: 50 µl; agar wells: 9 mm diameter; Test solutions/test plate: 1 x 50 µl reference substance. Test plates were cultivated for 18 h at 37 °C (Anonymous, 1986).

Reading the inhibition zones (IZ): As IZ, the zone in which no growth can be determined with the naked eye is measured; the tiniest colonies on the edge of the inhibition zone were therefore not taken into consideration.

The growth in the inhibition zones around the holes containing active substances is thereby compared to the growth in the growth control (no active substance).With each test approach, the reference substance was brought along as a control; if there were deviations from allowed IZ, the test was repeated.

For inhibition of bacterial growth ciprofloxacin was used as reference substance. For inhibition of fungal growth amphotericin B served as reference substance.

Analysis at 50 µg test substance/diffusion zone:

The inhibition of bacterial growth was determined using bacterial cultures grown in Müller-Hinton-bouillon (Mueller & Hinton, 1941), which was configured at barely visible opacity with the McFarland Standard Nr. 0.5, which indicates a bacterial density of 10^8/ml. This bacterial suspension was diluted with Müller-Hinton-bouillon using 2 dilution series in the agar diffusion test.

Yeast was propagated in YPD (yeast extract peptone dextrose): 1% yeast extract, 10 g; 2% peptone, 20 g; 2% agar, 20 g; 2% dextrose (glucose), 20 g; Sigma-Y1375.

All bacteria and yeast suspensions were freshly made. The cultures were incubated at 37 °C for 16 h. Afterwards, the bacterial concentration from each culture was determined and 34 ml of nutrient agar was loaded with 10^7 cells. These cultures were stored at 6-8 °C and could be used for seven days for the preparation of test plates: 34 ml of nutrient agar were liquefied and inoculated with the calculated amount of bacterial suspension at 48-50 °C, which is a temperature range suitable for vegetative forms of bacteria. The inoculated nutrient media (34 ml each) were immediately poured into the prepared test plates (3 mm layer). With a punching device, 12 holes per test plate were punched out. The results are shown in Table S4. For comparison, the minimum inhibitory concentrations of samarosporin (Inoue *et al.*, 1976) are cited in Table S5.

Table S4 Bacteria growth assay of bergofungin*

tested substance	concentration (µg/ml)	*E. coli* SG458	*Pseudomonas aeruginosa* SG137	*Staphylococcus aureus* 134/93	*Candida albicans* H8
Bergofungin	50	0	0	0	0
Bergofungin	1000	0	0	0	0
Ciprofloxacin	5	25/33	29	0	0
Amphotericin B	10	0	0	0	21

* The values in columns 3-6 are the diameters of the inhibition zones in mm.

Table S5 Minimum inhibitory concentration (MIC) of samarosporin (Inoue *et al.*, 1976)

	E. coli B	*Erwinia catotonova*	*Staphylococcus aureus* FDA 209P	*Candida albicans* YU-1200
MIC (µg/ml)	625	156	156	625

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