Two Novel Cyclic Peptides with Antifungal Activity from the Cyanobacterium *Tolypothrix byssoidea* (EAWAG 195)

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Two novel cyclic tridecapeptides, tolybyssidins A (**1**) and B (**2**), were isolated from the culture medium of mass cultured cyanobacterium *Tolypothrix byssoidea* (EAWAG 195) by means of bioguided isolation. The gross structures of these peptides were determined by 1D and 2D NMR experiments and tandem mass spectrometry. Both peptides contain the nonnatural amino acid dehydrohomoalanine (Dhha) as well as proteinogenic amino acids albeit with D- or L-configuration. The compounds exhibit moderate antifungal activity against the yeast *Candida albicans*.

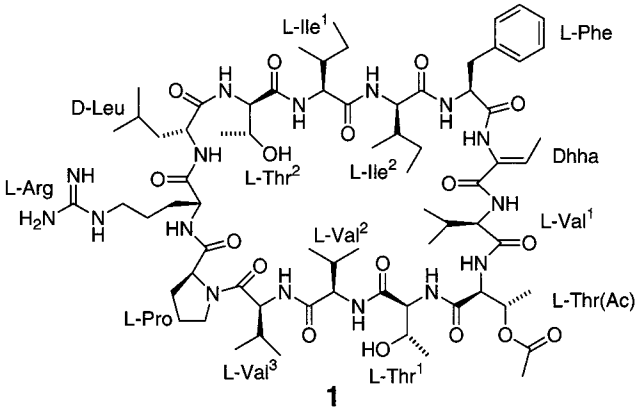
Cyanobacteria have received considerable attention in the past as a source of natural products with unique structures and biological activities.[1] Malyngolide,[2] majusculamide C,[3] and kawaguchipeptin A and B[4] are well-described examples of bioactive agents from this group of ubiquitous prokaryotic organisms. Herein, we report on the isolation and structure determination of two novel cyclic tridecapeptides with moderate antifungal activity, tolybyssidins A and B, both isolated from the cyanobacterium *Tolypothrix byssoidea* (Hass.) Kirch. (EAWAG 195).

Results and Discussion

T. byssoidea (EAWAG 195) was isolated from a sample collected in 1967 in Pokhara (Nepal) from a granite block and was subsequently mass cultured. The culture medium was filtered to remove cell mass. Thereafter, the cell-free supernatant was subjected to an Amberlite XAD-2 column. The Amberlite resin was subsequently eluted first with MeOH and then DCM. The biologically active MeOH extract was separated by open column chromatography using $CHCl_3$ and MeOH as a step gradient mixture. The final separation and purification of the two peptides was carried out by reversed-phase HPLC with a MeCN/H_2O gradient.

Tolybyssidin A was isolated as a white amorphous powder. The MALDI mass spectrum showed a positive ion peak $[M + Na]^+$ at 1488 *m/z*, indicating a molecular weight of 1465 for **1**. The ^{1}H NMR spectrum in methanol-d_3 displayed 12 amide proton signals between 9.50 and 7.23 ppm that completely vanished upon exchange into fully deuterated methanol. In addition, signals occurred in the aromatic region coding for one phenyl group; also, one triplet, seven doublets, and eight additional signals (each 3H), whose multiplicities could not be determined due to overlapping, were found between 0.74 and 1.25, corresponding to 17 aliphatic methyl groups. The distribution of signals with doublets in the amide-proton region and signals covering the complete range between 5 and 0.7 ppm was a clear indication that the investigated compound was a peptide. Surprisingly for the case of a peptide a singlet at 2.03 ppm appeared indicative of an OCO*CH*$_3$ group.

Further spectroscopic evidence supporting the classification of the compound as a peptide was derived from the ^{13}C NMR and DEPT 135 spectra. These spectra revealed



1

the carbonyl signal of an acetate moiety at δ_c 173.6, one signal of a carbon corresponding to a guanidine (δ_c 162.6, C-6 of arginine), and 12 (amide-) carbonyl signals in the range between 166.1 and 174.6 ppm. By using DEPT 135 and DEPT 90 experiments, 27 methine, 10 methylene, and 17 methyl signals were identified. These signals, along with three additional quaternary carbon signals at 130.3, 137.9, and 162.2 ppm, indicated that a total of 71 carbons were present in **1**. From these data, **1** was undoubtedly identified as a peptide. Since the compound gave a negative response in the ninhydrin test, **1** had to be either a cyclic peptide or an acyclic peptide with a protected N-terminus.

Sequential resonance assignment was performed largely following the protocol developed by Wüthrich and coworkers.[5] A first step interpretation of the 80 ms TOCSY and DQF-COSY spectra led to the classification of a total number of 13 different spin systems corresponding to the following amino acids: Phe, Val, Thr, Arg, Leu, Ile, Ac-Thr, Pro, and Dhha. However, since the sequential resonance assignment procedure usually requires the primary sequence to be known in advance, the amino acid identifications were mainly based upon complete ^{13}C assignments of the amino acids and comparison of these values with the random coil carbon shifts. In almost all cases very close correspondence was observed with deviations being smaller than 1 ppm. The presence of arginine instead of ornithine was established by confirming the guanidine residue via long-range correlations between H_2-5 (δ_H 3.09, m, 2H) and C-6 (δ_c 162.6) as well as by COSY and HMBC correlations linking H-2, H_2-3, H_2-4, and H_2-5 of the arginine moiety. The detailed ^{1}H and ^{13}C assignments are given in Table 1.

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **1** in CD_3OH

amino acid		^{1}H *J* (Hz)	^{13}C	amino acid		^{1}H *J* (Hz)	^{13}C
Phe	1		174.6 (s)	Pro	1		173.7 (s)
	2	4.55 (m)	57.6 (d)		2	4.39 (dd, 6.2 each)	62.2 (d)
	3	2.99 (dd, 7.6, 13.3)	37.3 (t)		3	1.94 (m)	30.6 (t)
		3.19 (dd, 7.6, 13.2)				2.21 (m)	26.1 (t)
	4		137.9 (s)		4	2.07 (m)	49.2 (t)
	5,9	7.29[a]	130.5 (d)		5	3.64 (m)	
	6,8	7.30[a]	129.7 (d)			3.92 (m)	
	7	7.23[a]	128.0 (d)				
	NH	8.75 (d, 9.7)					
Dhha	1		166.1 (s)	Arg	1		174.6 (s)
	2		130.3 (s)		2	4.31 (m)	54.2 (d)
	3	6.61 (q, 7.0)	134.1 (d)		3	1.65 (m)	30.3 (t)
	4	1.31 (d, 7.0)	13.3 (q)		4	1.51 (m)	27.7 (t)
	NH	9.50 (s)			5	3.09 (m)	40.5 (t)
					6		162.2 (s)
					NH	8.23 (d, 8.0)	
Val[1]	1		171.0 (s)	Leu	1		174.5 (s)
	2	4.37[a]	61.5 (d)		2	4.43 (m)	53.4 (d)
	3	1.85 (m)	31.6 (d)		3	1.67 (m)	41.8 (t)
	4	0.97[a]	19.9 (q)		4	1.59 (m)	25.9 (d)
	5	0.95 (d, 7.1)	19.4 (q)		5	0.92[a]	23.1 (q)
	NH	8.14 (d, 7.7)			6	0.91[a]	22.8 (q)
					NH	8.0 (d 7.4)	
Thr(Ac)	1		172.9 (s)	Thr[2]	1		172.4 (s)
	2	4.39 (dd, 7.2 each)	58.0 (d)		2	4.65 (dd, 8.3 each)	59.9 (d)
	3	5.15 (m)	71.6 (d)		3	4.23 (m)	68.1 (d)
	4	1.15 (d, 6.3)	20.0 (q)		4	1.21 (d, 6.2)	17.7 (q)
	5		173.6 (s)		NH	8.44 (d, 8.3)	
	6	2.03 (s)	22.8 (q)				
	NH	7.82 (d, 7.2)					
Thr[1]	1		171.0 (s)	Ile[1]	1		173.3 (s)
	2	4.05 (dd, 7.2 each)	60.9 (d)		2	4.43 (dd, 9.7 each)	59.0 (d)
	3	4.32[a]	68.7 (d)		3	1.83 (m)	39.6 (d)
	4	1.25 (d, 6.3)	20.4 (q)		4	1.10 (m)	27.5 (t)
	NH	8.29 (d, 7.2)				1.36 (m)	
					5	0.88 (t, 7.1)	15.1 (q)
					6	0.82 (d, 5.6)	12.1 (q)
					NH	8.55 (d, 9.7)	
Val[2]	1		173.3 (s)	Ile[2]	1		173.2 (s)
	2	4.46[a]	60.1 (d)		2	4.10 (dd, 6.2 each)	58.1 (d)
	3	2.12[(m)]	32.2 (d)		3	1.85 (m)	40.4 (d)
	4	0.97[a]	20.2 (q)		4	1.01 (m)	
	5	0.77 (d, 6.6)	18.5 (q)			1.34 (m)	27.6 (t)
	NH	7.82 (d, 7.2)			5	0.82[a]	14.7 (q)
					6	0.74 (d, 6.9)	12.2 (q)
					NH	7.23 (6.2)	
Val[3]	1		172.3 (s)				
	2	4.28 (dd, 6.6, 7.7)	58.7 (d)				
	3	2.13 (m)	31.4 (d)				
	4	0.92[a]	19.9 (q)				
	5	0.91[a]	19.4 (q)				
	NH	7.90 (d, 6.6)					

[a] Multiplicity of the signals is unclear due to overlapping

These spin systems were subsequently linked sequentially via (*i*,*i*+1) H^N,H^α NOEs. NOEs were observed for Phe NH/Ile^2 α-H, Dhha NH/Phe α-H, Thr^1 NH/Thr(Ac) α-H, Val^2 NH/Thr^1 α-H, Val^3 NH/Val^2 α-H, Arg NH/Pro α-H, Leu NH/Arg α-H, Thr^2 NH/Leu α-H, Ile^1 NH/Thr^2 α-H, and Ile^2 NH/Ile^1 α-H. Furthermore, amide protons typically displayed intraresidual and interesidual (*i*,*i*−1) (H^N,C′) HMBC correlations supporting the sequential assignment. Such HMBC correlations were observed for Phe α-H/Phe C═O, Dhha *β*-H/Dhha C═O, Val^1 α-H/Dhha C═O, Val^1 α-H/Val^1 C═O, Thr(Ac) α-H/Thr(Ac) C═O, Thr^1 α-H/Thr(Ac) C═O, Thr^1 α-H/Thr^1 C═O, Val^2 α-H/Thr^1 C═O, Val^2 α-H/Val^2 C═O, Val^3 α-H/Val^2 C═O, Val^3 α-H/Val^3 C═O, Pro α-H/Val^3 C═O, Pro α-H/Pro C═O, Arg α-H/Pro C═O, Arg α-H/Arg C═O, Leu α-H/Arg C═O, Leu α-H/Leu C═O, Thr^2 α-H/Leu C═O, Thr^2 α-H/Thr^2 C═O, Ile^1 α-H/Thr^2 C═O, Ile^1 α-H/Ile^1 C═O, Ile^2 α-H/Ile^1 C═O, Ile^2 α-H/Ile^2 C═O, and Phe α-H/Ile^2 C═O. These data, together with a molecular weight of 1465 as determined by MALDI mass spectrometry, are consistent with the molecular formula $C_{71}H_{116}N_{16}O_{17}$. No HMBC correlations between Thr(Ac) α-H and Val^1 C═O as well as no NOEs between Thr(Ac) NH and Val^1 α-H could be found. However, the ring connection between Val^1 and Thr(Ac) could be confirmed by an NOE observed between Val^1 H_3-5 and Ac-Thr H_3-6 and with the analysis of the tandem mass spectrum of **1** showing the fragment ion [M + H − Dhha-Val-Thr(Ac)-Thr-Val-Val]$^+$ at *m*/*z* 842.

The following MS fragments that helped to establish the amino acid sequence of the molecule **1** were detected in the tandem mass spectrum: *m*/*z* (rel int) 1466.5 [M + H]$^+$ (72), 1423 [M + H − $(CH_3)_2CH$]$^+$ (25), 1323 [M + H − Thr-(Ac)]$^+$ (73), 1284 [M + H − Dhha-Val]$^+$ (7), 1222 [M + H − Thr(Ac)-Thr]$^+$ (6), 1123 [1423 − H − Val-Val-Thr]$^+$ (39), 1119 [1423 − $(CH_3)_2$CH-Phe-Ile]$^+$ (1), 1024 [M + H − Ile-Phe-Dhha-Val]$^+$ (100), 907 [1423 + H − $(CH_3)_2$CH-Phe-Ile-Ile-Thr]$^+$ (11), 842 [M + H − Dhha-Val-Thr(Ac)-Thr-

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **2** in CD_3OH

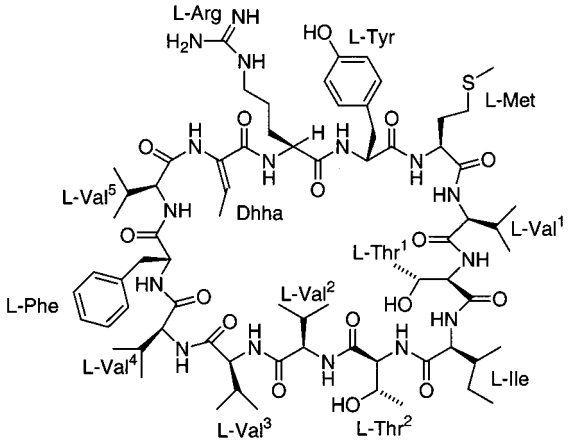
amino acid		^{1}H *J* (Hz)	^{13}C	amino acid		^{1}H *J* (Hz)	^{13}C
Val[1]	1		173.9 (s)	Phe	1		173.3 (s)
	2	4.43[a]	59.2 (d)		2	4.54 (m)	56.9 (d)
	3	1.81 (m)	32.6 (d)		3	2.58 (dd, 8.7, 16.2)	40.6 (t)
	4	0.84 (d, 5.7)	19.9 (q)			3.17 (dd, 8.9, 13.8)	
	5	0.84 (d, 5.7)	15.2 (q)		4		138.8 (s)
	NH	8.58 (d, 9.6)			5,9	7.20[a]	130.5 (d)
					6,8	7.30	131.3 (d)
					7	7.29 (m)	129.8 (d)
					NH	8.78 (d, 8.7)	
Thr[1]	1		175.0 (s)	Val[5]	1		173.8 (s)
	2	4.40[a]	61.2 (d)		2	3.97 (dd, 5.9, 8.5)	61.3 (d)
	3	4.03 (m)	68.8 (d)		3	2.1 (m)	32.2 (d)
	4	1.22 (d, 5.8)	14.3 (q)		4	0.94[a]	19.4 (q)
	NH	8.30 (d, 7.4)			5	0.94[a]	14.6 (q)
					NH	7.76 (d, 8.5)	
Ile	1		175.9 (s)	Dhha	1		166.2 (s)
	2	4.41[a]	59.2 (d)		2		130.9 (s)
	3	1.64 (m)	32.8 (d)		3	6.64 (q, 8.3)	134.0 (d)
	4	1.16 (m)	27.8 (t)		4	1.29 (d, 7.3)	13.3 (q)
		1.49 (m)			NH	9.51 (s)	
	5	0.92 (t, 7.6)	19.9 (q)				
	6	0.87 (d, 7.1)	12.2 (q)				
	NH	8.04 (d, 9.1)					
Thr[2]	1		173.9 (s)	Arg	1		175.1 (s)
	2	4.62 (dd, 8.8 each)	61.2 (d)		2	4.06 (dd, 8.7 each)	56.6 (d)
	3	4.00 (m)	66.5 (d)		3	1.66 (m)	32.7 (t)
	4	1.20 (d, 5.8)	15.9 (q)			1.85 (m)	
	NH	8.48 (d, 8.8)			4	1.53 (m)	27.7 (t)
					5	2.80 (dd, 6.0, 13.0)	40.4 (t)
					6		159.9 (s)
					NH	7.39 (d, 8.7)	
Val[2]	1		175.9 (s)	Tyr	1		174.3 (s)
	2	4.40[a]	59.7 (d)		2	4.05 (m)	58.2 (d)
	3	1.91 (m)	30.4 (d)		3	2.76 (dd, 7.6, 13.2)	38.1 (t)
	4	0.88 (d, 7.1)	18.5 (q)			3.28[a]	
	5	0.94[a]	15.1 (q)		4		129.8 (s)
	NH	8.12 (d, 8.0)			5	7.08 (d, 8.3)	130.6 (d)
					6	6.80 (d, 8.5)	116.3 (d)
					7		157.1 (s)
					8	6.72 (d, 8.3)	115.3 (d)
					9	7.02 (d, 8.4)	130.6 (d)
					NH	8.93 (d, 8.5)	
Val[3]	1		173.8 (s)	Met	1		172.4 (s)
	2	4.23[a]	62.3 (d)		2	4.71[a]	58.2 (d)
	3	2.12 (m)	31.6 (d)		3	1.81 (m)	34.8 (t)
	4	0.95[a]	19.5 (q)			2.04 (m)	
	5	0.95[a]	14.6 (q)		4	2.55[a]	32.3 (t)
	NH	7.92 (d, 7.6)				2.60[a]	
					5	2.10 (s)	15.8 (q)
					NH	8.99 (d, 8.4)	
Val[4]	1		176.2 (s)				
	2	4.10 (dd, 7.5 each)	61.6 (d)				
	3	1.64 (m)	30.3 (d)				
	4	0.80 (d, 6.1)	19.4 (q)				
	5	0.76 (d, 6.4)	14.7 (q)				
	NH	7.25 (d, 7.5)					

[a] Multiplicity of the signals is unclear due to overlapping.

Val-Val]$^+$ (6), 722 [M + H − Phe-Ile-Ile-Thr-Leu-Arg]$^+$ (4), 699 [M − Thr-Leu-Arg-Pro-Val-Val-Thr]$^+$ (5).

Tolybyssidin B (**2**) was obtained as a yellow-white solid. MALDI MS and ^{13}C NMR data were consistent with the molecular formula $C_{72}H_{115}N_{16}O_{16}S$. The ^{1}H NMR data indicated **2** to be a peptide as well. Due to the limited quantity of the isolated material, carbon signals of **2** had to be exclusively detected indirectly through HSQC and HMBC correlations. The detailed ^{1}H and ^{13}C assignments are given in Table 2. The NMR spectroscopic identification of **2** was performed analogously to **1**. In this process the spin systems of the amino acid residues were again identified by interpretation of the TOCSY and COSY spectra and sequentially linked through NOEs and HMBC correlations. NOEs were observed for Met NH/Tyr α-H, Val[1] NH/Met α-H, Thr[1] NH/Val[1] α-H, Ile NH/Thr[1] α-H, Thr[2] NH/Ile α-H, Val[2] NH/Thr[2] α-H, Val[3] NH/Val[2] α-H, Val[4] NH/Val[3] α-H, Phe NH/Val[4] α-H, Val[5] NH/Phe α-H, Dhha-NH/Val[5] α-H, and Tyr NH/Arg α-H.

The ring connection between Dhha and Arg could be unambiguously confirmed by analysis of the tandem mass spectrum of **2** with the fragment ion [M − H − Phe-Val]$^+$ at *m*/*z* 1244 and the fragment ion of this fragment [1244 − Dhha-Arg]$^+$ at *m*/*z* 1004. The following fragments, supporting the sequence of the molecule **2**, were detected in the tandem mass spectrum: *m*/*z* (rel int) 1473 [M − H_2O]$^+$ (3), 1447 [M − H − $CH(CH_3)_2$]$^+$ (24), 1445 [M − H − $CH(OH)CH_3$]$^+$ (100), 1383 [M − H − $OH(C_6H_4)CH_2$]$^+$ (10), 1372 [1473 − Thr]$^+$ (30), 1347 [1447 − Val]$^+$ (17), 1310 [M + H − Val-Dhha]$^+$ (10), 1284 [1383 − Val]$^+$ (15), 1245



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[1445 − Val − Thr]$^+$ (5), 1244 [M − H − Phe-Val]$^+$ (10), 1217 [1447 − Met-Val]$^+$ (4), 1183 [1284 − Thr]$^+$ (<1), 1052 [1183 − Met]$^+$ (1), 1004 [1217 + H − Thr-Ile]$^+$ (<1).

Analysis of the acid hydrolysate as Marfey derivatives[6] indicated two amino acids (Pro and Leu) of **1** to be D and 10 amino acids (Val^1, Val^2, Val^3, Thr(Ac), Thr^1, Thr^2, Arg, Ile^1, Ile^2, and Phe) to be L. All amino acid residues of **2** were determined to be L.

Tolybyssidins A (**1**) and B (**2**) inhibit the growth of the yeast *Candida albicans* at a concentration of 32 μg/mL for **1** and 64 μg/mL for **2**, respectively**.** A MIC value of 8 μg/mL was detected for the reference compound miconazole.

Experimental Section

General Experimental Procedures. Optical rotations were measured in MeOH on a Perkin-Elmer model 241 polarimeter. IR spectra were recorded in KBr pellets on a Perkin-Elmer 2000 FT infrared spectrophotometer. UV spectra were obtained in methanol on a UVIKON 930 spectrophotometer. Melting points were measured on a Mettler FP 5. MALDI MS spectra were measured on a Perseptive Biosystems Voyager Elite spectrometer and tandem MS spectra on a Finnigan LCQ Ion Prep spectrometer. NMR spectra were recorded on a Bruker DRX-500 spectrometer operating at a basic ^{1}H frequency of 500 MHz at 298 K, using the solvent line (CD_3OH, ^{1}H δ 3.31, ^{13}C δ 49.0) for referencing. For homonuclear proton 2D experiments (DQF-COSY,[7] TOCSY,[8] and NOESY[9]) standard experiments were performed with suppression of the methanol OH line by low-power presaturation. [^{1}H,^{13}C] HSQC[10] and HMBC[11] experiments utilized pulsed-field gradients for coherence selection.[12] HPLC separations were performed with a Merck-Hitachi pump connected to a Rheodyne HPLC injector, a Merck variable wavelength monitor, and Knauer HPLC columns (Hypersil ODS, 5 μm, 250 × 16). Si gel (Si gel 60, 40−60 μm, Merck) was applied for open column chromatography. For TLC controls, RP-18 F_{254} precoated sheets (0.25 mm, Merck) were used. All solvents were of HPLC grade.

Organisms and Culture Conditions. *T. byssoidea* (Hass.) Kirch., designated strain EAWAG 195, was isolated from a sample collected in Nepal, 1967. The culture is deposited at the Culture Collection of Algae at the Swiss Federal Institute for Water Resources and Water Pollution Control (EAWAG), Dübendorf, Switzerland. The cyanophyte was cultivated in 10 L bottles containing a modified inorganic culture medium. The cultures were illuminated continuously with fluorescent lamps (Philips TLM/33 Rs 40 W) at 29 μmol/(s·m^2), aerated with a mixture of 2% CO_2 in air, and incubated at a temperature of 24 ± 1 °C. The cyanobacterial cultures were harvested after 25−30 days. The supernatant was separated from the cells by filtration and adsorbed on a column filled with 250 g of Amberlite XAD-2 resin (nonpolar, surface area 330 m^2/g). Subsequently, the column was eluted with MeOH.

Isolation of 1 and 2. The MeOH extract (0.8 g) obtained from 90 L of microscopically cell-free culture medium was applied to an open column (4 × 100 cm, Si Gel). Elution was carried out with $CHCl_3$/MeOH as step gradient to obtain 10 fractions (20−100 mg). Bioactive fraction 6 (80 mg, eluted with $CHCl_3$/MeOH, 25:75) was subjected to reversed-phase HPLC (UV detection, 220 nm) using 1:1 MeCN/H_2O as an eluent to yield four fractions (5−30 mg). Bioactive fraction 3 (30 mg) was rechromatographed with reversed-phase HPLC with a 80:20 MeCN/H_2O eluent to yield **1** (8 mg) and **2** (3 mg) as pure compounds.

HPLC Analysis of the Marfey Derivatives of 1 and 2. To the acid hydrolysate (6 N HCl, 16 h, 110 °C) of a 200 μg portion of **1** and **2**, respectively, were added 100 μL of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (L-FDAA) in Me_2CO (10 mg/mL) and 200 μL of 1 M $NaHCO_3$, and the reaction mixture was kept at 80 °C for 3 min. Then 100 μL of 2 N HCl and 400 μL of 50% MeCN were added and analyzed by reversed-phase HPLC: Lichrosorb RP-18 (250 × 4 mm); gradient elution from 95:5:0.1 MeCN/H_2O/TFA to 40:60:0.1 MeCN/H_2O/TFA in 60 min; UV detection 340 nm, flow-rate 1.5 mL/min. Retention times of the standard amino acids (min): L-Leu (41.42), D-Leu (45.31), L-Phe (41.76), D-Phe (44.51), L-Ile (40.70), D-Ile (44.94), L-Val (37.07), D-Val (40.56), L-Pro (31.44), D-Pro (32.78), L-Arg (27.82), D-Arg (28.68), L-Thr (26.84), D-Thr (28.42), L-Thr(Ac) (25.27), D-Thr(Ac) (26.05), L-Met (37.07), D-Met (38.25), L-Tyr (48.56), D-Tyr (50.96).

Retention times of the amino acids of **1**: D-Leu (45.31), L-Phe (41.76), L-Ile (40.70), L-Val (37.07), D-Pro (32.78), L-Arg (27.82), L-Thr (26.84), L-Thr(Ac) (25.27).

Retention times of the amino acids of **2**: L-Phe (41.98), L-Ile (40.96), L-Val (36.86), L-Arg (27.98), L-Thr (26.84), L-Met (37.07), L-Tyr (48.56).

Preparation of the Acetyl Derivative of Thr (Acetyl-Threonine, Thr(Ac)). Thr (5.6 mg), anhydrous pyridine (0.5 mL), and acetic anhydride (0.5 mL) were kept dark and at room temperature for 18 h. The reaction mixture was diluted with 2 mL of water and stored at 4 °C for 1 h. Then it was applied to a reversed-phase cartridge, which had been washed with 10 mL of water. Pyridine, acetic anhydride, and non-acetylated Thr were eluted with water, followed by the elution of acetylated compound with $CHCl_3$. Evaporation of $CHCl_3$ under reduced pressure below 30 °C yielded 2.5 mg of the acetyl derivative, which was completely dried in a vacuum-drying oven at 25−28 °C. The identity of Thr(Ac) was proven by ^{1}H NMR spectroscopy measured in CD_3OD. In comparison with the ^{1}H NMR spectrum of non-acetylated Thr, the derivatized compound showed the expected additional CH_3 signal at 1.80 ppm, and a double acetylation was excluded.

Antifungal Assay. The MIC determinations for **1** and **2** were performed as described previously.[13] The yeast *C. albicans* (ATCC 26790) was applied as a test organism.

Tolybyssidin A (1): white, amorphous solid; $[\alpha]^{20}_D$ −13° (*c* 0.2, MeOH); mp 177−187 (dec); UV (MeOH) λ_{max} 207 nm; IR (KBr) ~3400, 1735, 1640 (br), 1510, 1370; ^{1}H and ^{13}C NMR data, see Table 1; MS data (tandem MS) see Results and Discussion, MALDI MS *m/z* 1488 [M + Na]$^+$.

Tolybyssidin B (2): yellow-white, waxy solid; $[\alpha]^{20}_D$ −18° (*c* 0.3, MeOH); mp 127−137 (dec); UV (MeOH) λ_{max} 222, 278 nm; IR (KBr) ~3400, 1640 (br), 1510; ^{1}H and ^{13}C NMR data, see Table 2; MS data (tandem MS) see Results and Discussion, MALDI MS *m/z* 1514 [M + Na]$^+$.

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