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Eight micropeptins from a *Microcystis* spp. bloom collected from a fishpond near Kibbutz Lehavot HaBashan, Israel

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ABSTRACT

Eight new micropeptin-type cyclic depsipeptides, micropeptins LH920, LH1021, LH1048, LH1062, LH911A, LH911B, LH911C, and LH925, along with five known micropeptins, three known anabaenopeptins and two known microcystins (LR and RR), were isolated from a water bloom biomass of *Microcystis* spp. assembly that was collected from a fishpond near Kibbutz Lehavot HaBashan, Israel. The structures of the pure compounds were elucidated using 1D and 2D NMR techniques, as well as high-resolution mass spectrometry. The stereochemistry of the new natural products was determined using Marfey's method for amino acids. The inhibitory activity of the compounds was determined for the serine proteases, trypsin, and chymotrypsin.

1. Introduction

The micropeptins are cyclic depsipeptides characterized by the unique 3-amino-6-hydroxy-piperidone (Ahp) moiety and a lactone ring. The Ahp moiety is formed by cyclization of glutamyl-γ-aldehyde to the amide nitrogen of the neighboring amino acid. The lactone ring contains six amino-acid residues and derives from a cyclization of the C-terminal carboxyl usually to the side-chain hydroxyl of threonine[1] or, in a single case, to the hydroxyl of 3-hydroxy-4-methylproline (Hmp).[2] A side-chain of one to four amino-, hydroxyl- or fatty acids is attached to the amide nitrogen of the lactone forming amino acid. So far, 131 micropeptins have been isolated[3] from fresh and brackish water toxic-bloom forming genera of cyanobacteria[4] and from filamentous bloom-forming marine cyanobacteria.[5] Most of the micropeptin-type metabolites display inhibitory activity against serine proteases. The selectivity of the inhibitory activity to the trypsin clade or chymotrypsin clade of enzymes is governed by the nature of the amino acid at the fifth position from the C-terminus of the peptide, where basic amino acids select for the trypsin clade and lipophilic or aromatic amino acids select for the chymotrypsin clade. The micropeptins usually appear, in blooms and cultures, as a mixture of several isoforms with comparable protease inhibition potency, which does not explain their co-existence in the same bloom or their ecological role. Evidence accumulated over the past 30 years suggest that these groups of protease inhibitors affect the ability of these cyanobacteria to survive in their ecological niche by preventing the detoxification of microcystins,[6] thus having a negative impact on population growth and on survival of zooplankton species.[7] The involvement of the micropeptins and anabaenopeptins in the lysis of cultured cyanobacteria cell lines was demonstrated and may imply their role in strains dynamic in water blooms.[8] As part of our ongoing research on the chemistry and chemical ecology of cyanobacteria blooms in water bodies,[9] a biomass of yellow-brown bloom material composed of an assembly of several *Microcystis* spp. (TAU IL-376) was collected in November 2007 from a fishpond near Kibbutz Lehavot HaBashan, Israel. The extract of this bloom material afforded eight new micropeptins; micropeptins LH920 (**1**), LH1021 (**2**), LH1048 (**3**), LH1062 (**4**), LH911A (**5**), LH911B (**6**), LH911C (**7**), and LH925 (**8**) along with the known, micropeptins MZ939A[10] and SF909,[11] cyanopeptolins S,[12] SS,[13] and 1020,[14] anabaenopeptins, A,[15] B,[15] and F,[16] and microcystins LR[17] and RR.[18] The isolation and structure elucidation of the new secondary metabolites and their biological activity are discussed below.

2. Results and discussion

The lyophilized *Microcystis* biomass was extracted and separated in two batches. The 7:3 MeOH/H_2O extract from each batch was separated on a reversed phase (ODS) open column followed by separation on Sephadex LH-20 and repeated preparative reversed-phase HPLC to afford **1**–**8** and the 10 known metabolites mentioned

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above. Micropeptin LH920 (**1**) was isolated as a transparent glassy material exhibiting an HRESIMS sodiated quasi-molecular ion at *m*/*z* 943.4576, which was consistent with a molecular formula of $C_{46}H_{64}N_8O_{12}$ and 19 degrees of unsaturation. Examination of the NMR spectra of **1** in DMSO-d_6 (Tables 1 and 2 and Table S1 in Supplementary data) revealed its peptide nature, i.e., nine carboxylic carbons in the ^{13}C NMR spectrum and four doublet and one triplet amide protons in the ^{1}H NMR spectrum. Some characteristic signals in the ^{1}H NMR spectrum of **1** suggested that it was a micropeptin-type compound, i.e., a broad singlet hydroxyl signal at δ_H 5.99, a downfield shifted quartet ester-oxymethine at δ_H 5.37, a singlet *N*Me group at δ_H 2.75, and a doublet methyl signal at δ_H 1.16.[19] Taking into account the *N*Me-aromatic amino acid and the *N*,*N*-disubstituted-amino acid of the micropeptins, the five amide doublet protons revealed that this micropeptin was composed of seven amino acids and a fatty acid. Analysis of the COSY, TOCSY, and HSQC 2D NMR experiments (Table S1 in Supplementary data) allowed the assignment of the side chains of a valine, a glutamine,

Table 1
^{1}H NMR data of compounds **1**–**8** in DMSO-d_6

Moiety	Position	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[b]	**6**[a]	**7**[b]	**8**[a]
1Val/Ile	2	4.70, dd	4.62, dd	4.69, dd	4.67, dd	4.58, dd	4.60, dd	4.60, m	4.62, m
	3	2.04, m	2.04, m	2.05, m	2.04, m	2.01, m	1.74, m	1.74, m	1.75, m
	4	0.72, d	0.72, d	0.71, d	0.70, d	0.75, d	1.05, m	1.03, m	1.04, m
							1.25, m	1.25, m	1.25, m
	5	0.86, d	0.84, d	0.85, d	0.84, d	0.85, d	0.80, t	0.80, t	0.80, t
	6	—	—	—	—	—	0.83, d	0.83, d	0.83, d
	NH	7.41, d	7.48, d	7.39, d	7.44, d	7.66, d	7.72, d	7.71, d	7.69, d
[2]*N*MeTyr/Phe	2	4.91, br d	4.87, m	4.88, br d	4.86, br d	5.12, dd	5.06, dd	5.12, dd	5.12, dd
	3	2.70, br t	2.69, br t	2.70, br t	2.68, br t	2.79, br t	2.79, dd	2.79, br t	2.80, dd
		3.09, br d	3.11, br d	3.10, br d	3.08, br d	3.30, m	3.29, m	3.29, m	3.29, m
	5,5′	7.00, d	6.99, d	6.98, d	6.98, d	7.22, d	7.23, d	7.22, d	7.22, d
	6,6′	6.77, d	6.76, d	6.76, d	6.76, d	7.25, t	7.26, t	7.25, t	7.25, t
	7-OH/7	9.36, s	9.32, s	9.32, s	9.34, br s	7.18, t	7.18, t	7.19, t	7.18, t
	NMe	2.75, s	2.74, s	2.74, s	2.74, s	2.74, s	2.72, s	2.72, s	2.72, s
[3]Phe/Ile/Val	2	4.77, d	4.74, dd	4.74, d	4.72, dd	4.37, d	4.31, m	4.37, d	4.36, d
	3	1.80, dd	1.79, dd	1.79, dd	1.78, m	1.74, m	1.92, m	1.75, m	1.75, m
		2.86, dd	2.85, br t	2.85, br t	2.84, br t				
	4	—	—	—	—	0.60, m	−0.19, d	0.60, m	0.60, m
						1.06, m		1.04, m	1.06, m
	5,5′/5	6.84, d	6.82, d	6.82, d	6.82, d	0.59, br d	0.46, d	0.59, br d	0.59, br d
	6,6′/6	7.19, t	7.17, t	7.16, t	7.16, t	−0.26, d	—	−0.26, d	−0.26, d
	7	7.13, t	7.13, t	7.13, t	7.12, t	—	—	—	—
[4]Ahp	3	3.60, m	3.60, m	3.60, m	3.59, m	4.43, m	4.45, m	4.43, m	4.44, m
	4	1.58, m	1.60, m	1.57, m	1.57, m	1.72, m	1.73, m	1.72, m	1.73, m
		2.39, br q	2.38, br q	2.39, br q	2.36, m	2.60, br q	2.61, br q	2.61, br q	2.61, br q
	5	1.53, m	1.54, m	1.53, m	1.54, m	1.73, m	1.72, m	1.73, m	1.74, m
		1.65, m	1.68, m	1.68, m	1.65, m		1.77, m		
	6	5.05, br s	5.04, br s	5.04, br s	5.04, br s	4.90, br s	4.91, br s	4.90, br s	4.90, br s
	6-OH	5.99, br s	6.05, br d	6.00, d	6.06, d	6.12, d	6.11, br s	6.14, br s	6.11, d
	NH	7.05, d	7.06, d	7.07, d	7.09, d	7.27, d	7.26, d	7.26, d	7.28, d
[5]Gln/Arg/Lys	2	4.15, ddd	4.13, ddd	4.21, m	4.19, m	4.31, m	4.30, m	4.28, m	4.31, m
	3	1.57, m	1.58, m	1.36, m	1.35, m	1.42, m	1.45, m	1.46, m	1.48, m
		2.05, m	2.05, m	1.88, m	1.86, m	2.04, m	2.03, m	2.02, m	2.05, m
	4	1.98, m	1.94, m	1.37, m	1.36, m	1.41, m	1.44, m	1.27, m	1.25, m
	5	—	—	3.02, m	3.02, m	3.06, m	3.06, m	1.46, m	1.49, m
								1.53, m	1.59, m
	6	—	—	—	—	—	—	2.82, m	2.95, m
	NH	8.43, d	8.43, d	8.47, d	8.46, d	8.57, d	8.59, d	8.53, br d	8.54, d
	NH_2/NH/	6.63, br s	6.66, br s	7.77, br t	7.76, br t	7.46, br t	7.63, br s	8.20, br s	—
	NMe	7.16, br s	7.12, br s					2.53, br t	2.71, s
[6]Thr	2	4.61, d	4.60, d	4.56, d	4.53, d	4.64, d	4.65, d	4.62, m	4.61, m
	3	5.37, br q	5.38, br q	5.38, q	5.39, q	5.49, q	5.49, br q	5.49, br q	5.49, br q
	4	1.16, d	1.17, d	1.14, d	1.15, d	1.20, d	1.20, d	1.21, d	1.21, d
	NH	7.90, d	7.89, d	7.83, d	7.87, d	7.64, d	7.62, d	7.67, d	7.67, d
[7]Thr/Glu/Ga	2	—	4.41, dd	4.38, dt	4.39, dt	4.25, m	4.25, m	4.26, m	4.26, m
	3	—	3.99, ddq	1.73, m	1.76, m	3.82, dd	3.82, dd	3.82, dd	3.82, dd
				1.88, m	1.89, m	3.97, dd	3.98, dd	3.98, dd	3.97, dd
	4/3-OH	—	1.03, d	2.18, m	2.32, m	6.09, d	6.06, d	6.09, d	6.07, d
	5	—	4.88, d	—	—	—	—	—	—
	NH	—	7.65, d	7.99, d	8.00, d	—	—	—	—
OMe		—	—	—	3.55, s	—	—	—	—
[7/8]Gly	2	3.78, dd	3.72, dd	—	—	—	—	—	—
		3.85, dd	3.78, dd						
	NH	8.06, t	8.06, t	—	—	—	—	—	—
[8/9]Hex/Oct	2	2.11, t	2.10, t	2.11, m	2.11, m	—	—	—	—
	3	1.51, m	1.48, tt	1.48, m	1.47, m	—	—	—	—
	4	1.25, m	1.22, m	1.23, m	1.22, m	—	—	—	—
	5	1.26, m	1.24, m	1.23, m	1.22, m	—	—	—	—
	6	0.85, t	0.83, t	1.22, m	1.21, m	—	—	—	—
	7	—	—	1.24, m	1.23, m	—	—	—	—
	8	—	—	0.83, t	0.83, t	—	—	—	—

[a] 400 MHz.
[b] 500 MHz.

Table 2
^{13}C NMR data of compounds **1**–**8** in DMSO-d_6

Moiety	Position	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[b]	**6**[a]	**7**[b]	**8**[a]
1Val/Ile	1	172.0, s	172.2, s	172.1, s	172.1, s	172.7, s	172.8, s	172.9, s	172.8, s
	2	56.0, d	56.2, d	55.9, d	56.0, d	56.4, d	55.5, d	55.5, d	55.5, d
	3	30.9, d	30.8, d	30.5, d	30.8, d	31.0, d	37.4, d	37.5, d	37.5, d
	4	17.4, q	17.5, q	17.3, q	17.4, q	17.9, q	24.8, t	24.6, t	24.7, t
	5	19.4, q	19.4, q	19.4, q	19.4, q	19.4, q	11.1, q	11.2, q	11.2, q
	6	—	—	—	—	—	16.0, q	16.0, q	16.0, q
^{2}*N*MeTyr/Phe	1	169.3, s	169.5, s	169.3, s	169.4, s	169.3, s	169.1, s	169.2, s	169.2, s
	2	61.0, d	61.0, d	61.1, d	61.1, d	60.7, d	60.6, d	60.6, d	60.6, d
	3	33.0, t	32.9, t	33.0, t	33.0, t	34.4, t	34.3, t	34.3, t	34.3, t
	4	127.6, s	127.7, s	127.6, s	127.6, s	137.9, s	137.9, s	137.9, s	137.8, s
	5,5′	130.5, d×2	130.6, d×2	130.5, d×2	130.5, d×2	129.8, d×2	129.7, d×2	129.7, d×2	129.7, d×2
	6,6′	115.5, d×2	115.5, d×2	115.5, d×2	115.5, d×2	128.7, d×2	128.6, d×2	128.7, d×2	128.7, d×2
	7	156.4, s	156.4, s	156.4, s	156.4, s	126.8, d	126.7, d	126.8, d	126.8, d
	NMe	30.4, q	30.5, q	30.5, q	30.5, q	30.5, q	30.2, q	30.3, q	30.3, q
^{3}Phe/Ile/Val	1	170.5, s	170.5, s	170.5, s	170.5, s	169.9, s	169.8, s	169.8, s	169.8, s
	2	50.4, d	50.4, d	50.4, d	50.5, d	54.3, d	55.9, d	54.2, d	54.3, d
	3	35.5, t	35.4, t	35.4, t	35.5, t	33.1, d	27.4, d	33.1, d	33.1, d
	4	136.9, s	136.9, s	136.9, s	136.9, s	23.8, t	18.1, q	23.6, t	23.8, t
	5,5′/5	129.6, d×2	129.6, d×2	129.5, d×2	129.6, d×2	10.5, q	18.1, q	10.4, q	10.4, q
	6,6′/6	127.9, d×2	127.9, d×2	127.8, d×2	127.9, d×2	14.0, q	—	14.0, q	14.0, q
	7	126.3, d	126.4, d	126.3, d	126.3, d	—	—	—	—
^{4}Ahp	2	169.1, s	168.9, s	169.0, s	168.2, s	169.5, s	169.4, s	169.5, s	169.5, s
	3	48.9, d	48.9, d	48.8, d	48.9, d	49.1, d	49.0, d	49.1, d	49.1, d
	4	21.7, t	21.7, t	21.7, t	21.7, t	21.7, t	21.6, t	21.7, t	21.7, t
	5	29.4, t	29.4, t	29.4, t	29.4, t	29.9, t	29.8, t	29.9, t	29.9, t
	6	73.9, d	73.9, d	73.9, d	73.9, d	74.2, d	74.1, d	74.1, d	74.1, d
^{5}Gln/Arg/Lys	1	169.8, s	169.8, s	169.8, s	169.7, s	170.3, s	170.2, s	170.4, s	170.4, s
	2	51.9, d	52.0, d	51.5, d	51.5, d	51.9, d	52.0, d	51.8, d	51.6, d
	3	26.5, t	26.5, t	27.5, t	27.4, t	27.3, t	27.3, t	29.4, t	29.4, t
	4	31.6, t	31.6, t	25.0, t	25.1, t	25.2, t	25.1, t	22.4, t	22.3, t
	5	173.8, s	173.8, s	40.3, t	40.3, t	40.5, t	40.3, t	24.7, t	23.2, t
	6	—	—	—	—	—	—	48.3, t	56.8, t
	7/NMe	—	—	157.0, s	156.8, s	156.7, s	156.9, s	32.6, q	42.4, q×2
^{6}Thr	1	169.3, s	169.3, s	169.2, s	169.2, s	169.2, s	169.1, s	169.1, s	169.2, s
	2	54.8, d	55.0, d	54.7, d	54.9, d	54.5, d	54.5, d	54.6, d	54.7, d
	3	72.1, d	72.0, d	72.1, d	72.0, d	72.3, d	72.2, d	72.1, d	72.1, d
	4	18.0, q	18.1, q	17.8, q	18.0, q	17.9, q	17.9, q	18.0, q	18.0, q
^{7}Thr/Glu/Ga	1	—	170.9, s	172.5, s	172.3, s	171.9, s	171.9, s	172.0, s	172.0, s
	2	—	57.7, d	52.0, d	51.8, d	70.6, d	70.7, d	70.7, d	70.7, d
	3	—	67.0, d	27.3, t	27.1, t	68.4, t	68.4, t	68.3, t	68.3, t
	4	—	19.7, q	—	—	—	—	—	—
	5	—	—	174.5, s	173.1 s	—	—	—	—
OMe		—	—	—	51.5, q	—	—	—	—
$^{7/8}$Gly	1	170.0, s	169.3, s	—	—	—	—	—	—
	2	42.1, t	42.1, t	—	—	—	—	—	—
$^{8/9}$Hex/Oct	1	172.9, s	172.7, s	172.6, s	172.7, s	—	—	—	—
	2	35.3, t	35.3, t	35.2, t	35.2, t	—	—	—	—
	3	25.0, t	25.1, t	25.4, t	25.4, t	—	—	—	—
	4	31.0, t	31.0, t	28.6, t	28.6, t	—	—	—	—
	5	22.0, t	22.1, t	28.7, t	28.7, t	—	—	—	—
	6	14.0, q	14.0, q	31.3, t	31.4, t	—	—	—	—
	7	—	—	22.2, t	22.2, t	—	—	—	—
	8	—	—	14.1, q	14.1, q	—	—	—	—

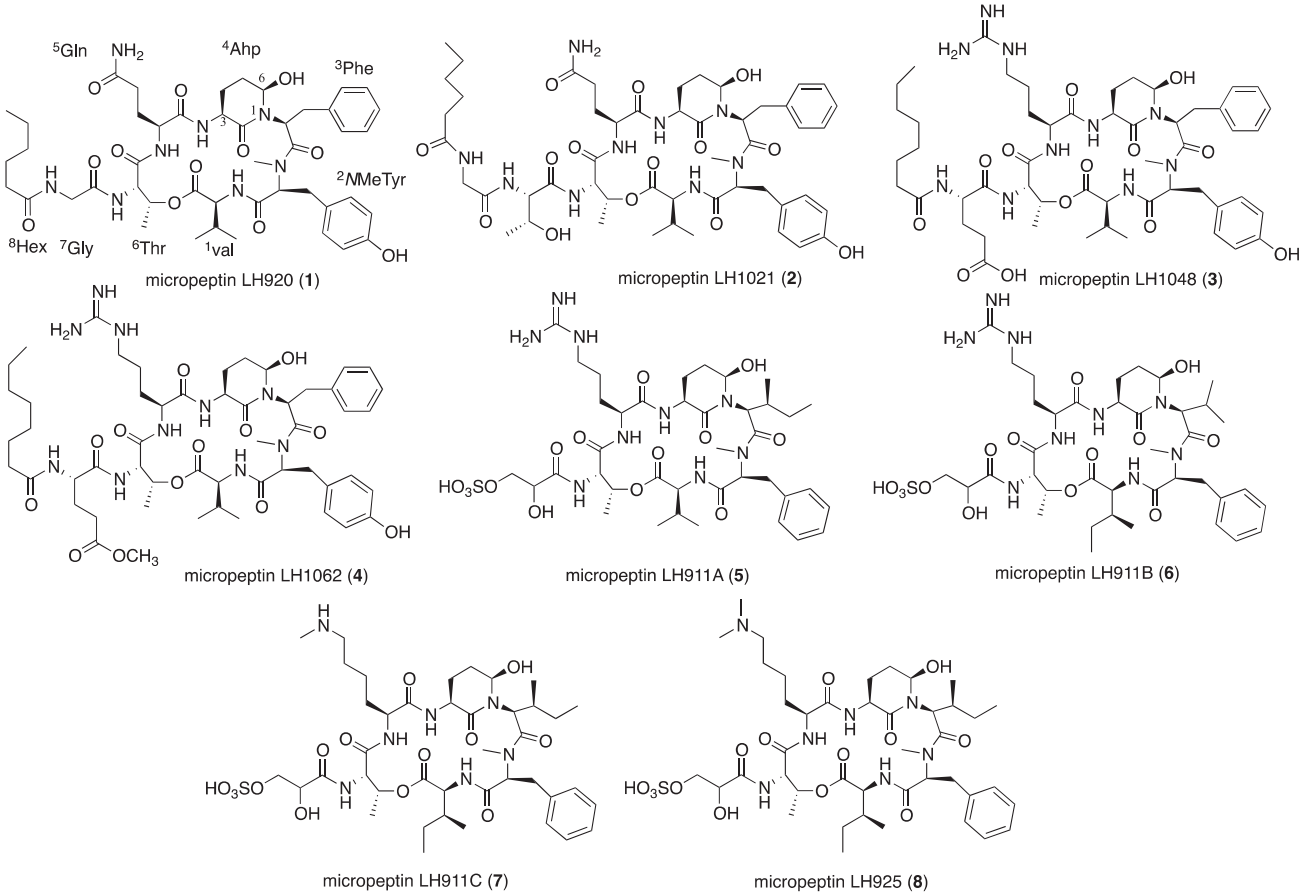
[a] 100 MHz.
[b] 125 MHz.

a threonine, a glycine, a hexanoic acid, two short fragments in agreement with *N*,*N*-disubstituted aromatic amino acids, a 1,4-disubstituted phenol ring, a monosubstituted phenyl ring, an amino hydroxy piperidone (Ahp) moiety, and *n*-pentyl.

The latter fragments were extended to the complete structures, and the carboxamide carbons were assigned to the side chains, by analysis of the correlation map of an ^{1}H–^{13}C HMBC experiment (Table S1 in Supplementary data). This procedure established the structures of Val, *N*MeTyr, *N*,*N*-disubstituted Phe, Ahp, Gln, 3-*O*-substituted-Thr, Gly, and hexanoic acid (HA) residues. The sequence of the acid residues of the peptide: Val, *N*MeTyr, *N*,*N*-disubstituted-Phe, Ahp, Gln, Thr, Gly, and HA was assigned on the basis of HMBC and ROESY correlations as follows: HMBC correlations between the carboxamide of *N*MeTyr and Val-NH, the carboxamide of *N*,*N*-disubstituted-Phe and the *N*Me of *N*MeTyr, the carboxamide of Gln and NH of Ahp, the carboxamide of Thr and NH of Gln, the carboxamide of Gly and the NH of Thr and the carboxamide of HA and NH of Gly, and the NOE correlations between H-2,3a,3b of *N*,*N*-disubstituted-Phe and Ahp H-6. The ester linkage between Thr and Val was established through the HMBC correlation of Thr H-3 and the carboxyl of Val. Acid hydrolysis of **1** and derivatization with Marfey's reagent,[20] followed by HPLC analysis, demonstrated the L-configuration of valine, *N*Me-tyrosine (by comparison with synthetic standard), phenylalanine, threonine, and glutamic acid residues. Jones oxidation[21] of **1**, followed by a similar hydrolysis, derivatization and HPLC analysis, determined the 3*S*-configuration for the Ahp residue (oxidation and subsequent hydrolysis liberated L-glutamic acid from Ahp). The configuration of C-6 of the Ahp was determined as *R* on the basis of the coupling constants of H-6 (<1 Hz), which points to an equatorial orientation of this proton, the chemical shift of the pseudoaxial H-4 (δ_H 2.39 br q J=12.8 Hz), which is shifted downfield by the axial 6-hydroxy-group, the trans

diaxial relationship with H-3 and the NOE correlation between H-4pax and the 6-OH.[11] Marfey's analysis using 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA)[20] fails to distinguish L-threonine from L-*allo*-threonine and L-isoleucine from L-*allo*-isoleucine and thus additional evidence was needed to support the assignment of the absolute configuration of C-3 of threonine. The observed *J*-value (0–1 Hz) between H-2 and H-3 of the *N*,*O*-disubstituted threonine in **1**, suggested that, as in the case of all known micropeptins, it should be L-threonine and not L-*allo*-threonine.[22] On the basis of the arguments described above the structure of micropeptin LH920 was established as **1**.

Micropeptins LH1048 (**3**) and LH1062 (**4**) were isolated as glassy solids that presented high-resolution ESIMS protonated quasi-



Micropeptin LH1021 (**2**) was isolated as a glassy solid presenting a high-resolution ESIMS sodiated quasi-molecular ion, *m*/*z* 1044.5032, which corresponds to the molecular formula, $C_{50}H_{71}N_9O_{14}$ and 20 degrees of unsaturation. The ^{1}H NMR spectrum of **2** resembled that of **1** except for the presence of seven extra protons; a doublet amide proton (δ_H 7.65); an exchangeable doublet proton (δ_H 4.88); two protons next to electronegative atoms (δ_H 4.41, dd and 3.99, ddq) and a doublet methyl (δ_H 1.03). Correlations of the later protons in the COSY spectrum revealed an additional threonine moiety relative to **1**. The chemical shifts of the ^{1}H (Table 1) and ^{13}C (Table 2) NMR signals of the remaining amino acids and the fatty acid that compose **2**, were almost identical to those of **1**. Analysis of the COSY, TOCSY, HSQC, and HMBC 2D NMR experiments (Table S2 in Supplementary data) established the structures of Val, *N*MeTyr, *N*,*N*-disubstituted-Phe, Ahp, Gln, 3-*O*-substituted-Thr, Thr, Gly, and HA residues. HMBC correlations between the carboxamide of *N*MeTyr and Val-NH, the carboxamide of *N*,*N*-disubstituted-Phe and the *N*Me of *N*MeTyr, the carboxamide and C-6 of Ahp and Phe-H-2, the carboxamide of Gln and NH of Ahp, the carboxamide of 3-*O*-substituted-Thr and NH of Gln, the carboxamide of Thr and H-2 and NH of 3-*O*-substituted-Thr, the carboxamide of Gly and H-2 and NH of Thr, and the carboxamide of HA and NH of Gly, and correlation of 3-*O*-substituted-Thr H-3 with the carboxyl of Val established the sequence and point of cyclization of **2**. A similar procedure to that applied for **1** assigned the absolute configuration of all of the amino-acid residues, in micropeptin LH1021, to be L. Based on the results discussed above the structure of micropeptin LH1021 was established as **2**.

molecular ions, *m*/*z* 1049.5670 and 1063.5840, which corresponded to the molecular formulas, $C_{52}H_{76}N_{10}O_{13}$ and $C_{53}H_{78}N_{10}O_{13}$, respectively, and 20 degrees of unsaturation, each. Their 1D NMR spectra (Tables 1 and 2) were almost identical and indicated an extra methoxy-group in **4**, in accordance with the mass spectral data. When the ^{1}H NMR spectra (Table 1) of **3** and **4** were compared with that of **1**, the absence of the primary amide protons of Gln from the spectra of **3** and **4** was noticed and instead a broad signal integrating for two protons and a very broad exchangeable signal appeared at 3.02 ppm and around 7.00 ppm, respectively, in the spectra of **3** and **4**. Analysis of the COSY, TOCSY, HSQC, and HMBC 2D NMR experiments of **3** and **4** (Tables S3 and S4 in Supplementary data) established the structures of Val, *N*MeTyr, *N*,*N*-disubstituted-Phe, Ahp, Arg, 3-*O*-substituted-Thr, Glu (Glu-*O*Me, in **4**), and octanoic acid (OA) for both compounds. For **3**, HMBC correlations between the carboxamide of *N*MeTyr and Val-NH, the carboxamide of *N*,*N*-disubstituted-Phe and H-2 and *N*Me of *N*MeTyr, the carboxamide of Arg and NH of Ahp, the carboxamide of 3-*O*-substitued-Thr and NH of Arg, the carboxamide of Glu and

H-2 and NH of 3-*O*-substituted-Thr, and the carboxamide of OA and NH of Glu and NOE correlations (from ROESY experiment) between Ahp H-6 and Phe H-3 and H-3′ established the sequence of the peptide. Correlation of 3-*O*-substituted-Thr H-3 with the carboxyl of Val established the point of cyclization of **3**. For **4**, a similar pattern of correlations was observed except for the HMBC correlation of the carboxamide of 3-*O*-substituted-Thr and the NH of Arg. The connectivity between these two was established by the observation of NOE correlation of 3-*O*-substituted-Thr H-2 with the amide proton of Arg, in the ROESY map. The absolute configuration of all of the amino acids that compose micropeptins LH1048 and LH1062 were determined as L, by the same procedure applied for **1**. Based on these arguments, the structures of micropeptins LH1048 and LH1062 were established as **3** and **4**, respectively.

Micropeptin LH911A (**5**) was isolated as a transparent glassy solid, which presented a high-resolution ESIMS sodiated molecular cluster ion, *m*/*z* 934.3964, corresponding to the molecular formula, $C_{39}H_{61}N_9O_{14}S$ and 14 degrees of unsaturation. Examination of the NMR spectra of **5** in DMSO-d_6 (Tables 1 and 2 and Table S5 in Supplementary data) revealed that this micropeptin markedly differed in the composition of its acid residues from those of **1**–**4**. Analysis of the COSY, TOCSY, HSQC, and HMBC 2D NMR experiments of **5** (Table S5 in Supplementary data) established the structure of Val, *N*MePhe, *N*,*N*-disubstituted-Ile, Ahp, Arg, 3-*O*-substituted-Thr, and 3-sulfo-glyceric acid (3-sulfo-GA).[12] The carboxamide of the arginine residue was assigned based on an NOE correlation between the Ahp amide proton and Arg H-2, and an HMBC correlation of the Ahp amide proton with the carboxamide that resonated at 170.3 ppm. HMBC correlations between the carboxamide of *N*MePhe and Val-NH, the carboxamide of *N*,*N*-disubstituted-Ile and the *N*Me of *N*MePhe, C-6 of Ahp, and *N*,*N*-disubstituted-Ile H-2, the carboxamide of Arg and NH of Ahp, the carboxamide of 3-*O*-substituted-Thr and NH of Arg, the carboxamide of 3-sulfo-GA and H-2 and NH of 3-*O*-substituted-Thr, and correlation of 3-*O*-substituted-Thr H-3 with the carboxyl of Val established the sequence and point of cyclization of **5**. The absolute configuration of the chiral centers of the amino acids of **5** was established by application of Marfey's procedure,[20] as described for **1**. This procedure established the L configuration of all of the α-carbons of the amino acids in **5** and allowed determining the 6*R* configuration of the aminal carbon of Ahp, as described for **1**. As mentioned above, Marfey's analysis fails to distinguish L-threonine from L-*allo*-threonine and L-isoleucine from L-*allo*-isoleucine. The observed *J*-value (0–1 Hz) between H-2 and H-3 of the *N*,*O*-disubstituted threonine in **5**, suggested that, as in the case of all known micropeptins, it should be L-threonine.[22] In the case of the *N*,*N*-disubstituted-Ile, the ^{13}C NMR chemical shifts measured for compound **5**, 10.5 (C-5) and 14.0 (C-6) ppm, were found to be similar to those reported for L-Ile (10.3 and 13.9 ppm, C-5 and C-6, respectively),[23] while different from those reported for *allo*-Ile (12.2 and 14.1 ppm, C-5 and C-6, respectively).[24] On the basis of the arguments described above the structure of micropeptin LH911A was established as **5**.

Micropeptin LH911B (**6**) presented 1D NMR spectra (Tables 1 and 2) almost identical with those of **5**. It displayed a quasi-molecular ion at *m*/*z* 910.3989 $[M-H]^-$ in the HRESIMS, which correspond to the molecular formula, $C_{39}H_{61}N_9O_{14}S$, and 14 degrees of unsaturation, identical to that of **5**. Analysis of the COSY, TOCSY, HSQC, and HMBC 2D NMR experiments of **6** (Table S6 in Supplementary data) established the structures of Ile, *N*MePhe, *N*,*N*-disubstituted-Val, Ahp, Arg, 3-*O*-substituted-Thr, and 3-sulfo-GA, as in **5**. The carboxamide of the arginine residue was assigned based on an NOE correlation between the Ahp amide proton and Arg H-2, and an HMBC correlation of the Ahp amide proton with the carboxamide that resonated at 170.2 ppm. HMBC correlations between the carboxamide of *N*MePhe and Ile NH, the carboxamide of *N*,*N*-disubstituted-Val and the *N*Me of *N*MePhe, C-6 of Ahp and *N*,*N*-disubstituted-Val H-2, the carboxamide of Arg and NH of Ahp, the carboxamide of 3-*O*-substituted-Thr and NH of Arg, the carboxamide of 3-sulfo-GA and H-2 and NH of 3-*O*-substituted-Thr, and correlation of 3-*O*-substituted-Thr H-3 with the carboxyl of Ile established the sequence and point of cyclization of **6**. This analysis defined that the Val and Ile switched positions in **5** and **6**. The absolute configuration of the chiral centers of the amino acids of **6** was established by application of Marfey's procedure,[20] as described for **5**, leading to the assignment of structure **6** to micropeptin LH911B.

Micropeptin LH911C (**7**), shared the same nominal mass with **5** and **6**, but its HRESIMS exhibited a sodiated quasi-molecular ion at *m*/*z* 934.4233, consistent with a molecular formula of $C_{41}H_{65}N_7O_{14}S$ and 13 degrees of unsaturation, thus differing from **5** and **6**. Correspondingly, **7** displayed 1D NMR spectra (Tables 1 and 2) significantly different from that of **5** and **6**, indicating the replacement of the Val unit by additional Ile (in **7**) and the Arg by ω-*N*MeLys. Interpretation of the COSY, TOCSY, HSQC, and HMBC 2D NMR experiments of **7** (Table S7 in Supplementary data) revealed the presence of Ile, *N*MePhe, *N*,*N*-disubstituted-Ile, Ahp, ω-*N*MeLys, 3-*O*-substituted-Thr, and 3-sulfo-GA. The carboxamide of the lysine residue was assigned based on an NOE correlation between the Ahp amide proton and ω-*N*MeLys H-2, and an HMBC correlation of the Ahp amide proton with the carboxamide that resonated at 170.4 ppm. HMBC correlations between the carboxamide of *N*MePhe and Ile-NH, the carboxamide of *N*,*N*-disubstituted-Ile and the *N*Me of *N*MePhe, C-6 of Ahp and *N*,*N*-disubstituted-Ile H-2, the carboxamide of ω-*N*MeLys and NH of Ahp, the carboxamide of 3-sulfo-GA and H-2 and NH of 3-*O*-substituted-Thr, and correlation of 3-*O*-substituted-Thr H-3 with the carboxyl of Ile, and NOE correlation of ω-*N*MeLys with the α-amide proton and H-2 of 3-*O*-substituted-Thr, established the sequence and point of cyclization of **7**. Application of Marfey's procedure,[20] as described for **5**, assigned structure **7** to micropeptin LH911C.

Micropeptin LH925 (**8**) was isolated as a transparent glassy solid, which presented a high-resolution ESIMS sodiated quasi-molecular ion, *m*/*z* 948.4383, corresponding to the molecular formula, $C_{42}H_{67}N_7O_{14}S$ and 13 degrees of unsaturation. Micropeptin LH925 presented 1D NMR spectra almost identical with those of **7**. The only differences observed in the ^1H NMR spectra were the downfield shift of the lysine H_2-6 and *N*Me by 0.13 and 0.18 ppm, in **8**, the absence of the ω-*N*H in **8**, and the doubled integration of the *N*Me signal in **8**. In the ^{13}C NMR spectra, **8** differed from **7** in the upfield shift of the lysine C-5 signal (by 1.5 ppm) and the downfield shift of C-6 and *N*Me signals (by 8.5 and 9.6 ppm, respectively). These differences were in accordance with the presence of a *N*,*N*-dimethyl group at the ω-amine of Lys in **8**. Interpretation of the COSY, TOCSY, HSQC, and HMBC 2D NMR experiments of **8** (Table S8 in Supplementary data) revealed the presence and the sequence of Ile, *N*MePhe, *N*,*N*-disubstituted-Ile, Ahp, ω,ω-NMe$_2$Lys, 3-*O*-substituted-Thr, and 3-sulfo-GA. Application of Marfey's procedure,[20] as described for **5**, assigned structure **8** to micropeptin LH925.

The crude cyanobacteria extract exhibited significant inhibition of the serine proteases trypsin and chymotrypsin at a concentration of 1 mg/mL. The activity-guided purification of the proteases inhibiting components of the extract revealed that micropeptins **1**–**4** were responsible for the inhibition of chymotrypsin, while micropeptins **3**–**8** were responsible for the inhibition of trypsin (see Table 3). The selectivity and potency of **1** and **2** against chymotrypsin is comparable with that of micropeptin 103[25] (IC_{50} 1.1 μM) and micropeptin MM978[26] (IC_{50} 4.6 μM). Micropeptin 103 contains the same sequence as **1** except of *N*MeTrp instead of *N*MeTyr, in **1** and micropeptin MM978 contains the same sequence as **1** except of Ile instead of Val and Thr instead of Gly. Micropeptins

Table 3
Protease inhibition properties of **1**–**8**[a]

Compound	Trypsin	Chymotrypsin
Micropeptin LH920 (**1**)	—	4.7
Micropeptin LH1021 (**2**)	—	1.1
Micropeptin LH1048 (**3**)	2.0	5.3
Micropeptin LH1062 (**4**)	3.0	3.0
Micropeptin LH911A (**5**)	1.9	—
Micropeptin LH911B (**6**)	3.1	—
Micropeptin LH911C (**7**)	11.7	—
Micropeptin LH925 (**8**)	>45.5	—

[a] IC_{50}, μM.

LH1048 (**3**) and LH1062 (**4**) inhibited both trypsin and chymotrypsin in a similar potency. They share this unique property with micropeptin HU909[27] (IC_{50} chymotrypsin 2.8 μM, trypsin 1.1 μM) and related micropeptins from the same bloom, all of which display remarkably different amino acid sequences. Interestingly, cyanopeptolin 1020 that was isolated along with **3** and **4** from the same extract was reported to potently inhibit trypsin (IC_{50} 0.67 nM), but not chymotrypsin.[14] Micropeptins LH911A (**5**) and B (**6**) were found to be somewhat less potent against trypsin than the known cyanopeptolin S[12] (IC_{50} 0.2 μM) that was also isolated in the current study. Cyanopeptolin S contains two Ile residues, instead of Val and Ile in **5** and **6**, and identical with them in the rest of the sequences. The *N*-methylated lysine containing, **7** and **8**, displayed lower potency to trypsin than **5** and **6** suggesting that the methylation resulted in higher steric hindrance and thus lowering their binding affinity to the active pocket of the enzyme.

3. Experimental section

3.1. Instrumentation

Mass spectra were recorded on a Waters MALDI Synapt instrument. UV spectra were recorded on an Agilent 8453 spectrophotometer. Optical rotation values were obtained on a Jasco P-1010 polarimeter at the sodium D line (589 nm). NMR spectra were recorded on a Bruker DRX-500 spectrometer at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C and a Bruker Avance 400 Spectrometer at 400.13 MHz for ^{1}H, 100.62 MHz for ^{13}C. DEPT, COSY-45, gTOCSY, gROESY, gHSQC, gHMQC, gHMBC, spectra were recorded using standard Bruker pulse sequences. HPLC separations were performed on a JASCO HPLC system (model PU-2080-plus pump, model LG-2080-04 Quaternary gradient unit and model MD-2010-plus Multiwavelength detector) and on a Merck Hitachi HPLC system (model L-7100 pump and model L-7450A Diode Array detector). Protease inhibition assays were measured on an EL_x808, BIO-TEK Instrument ELISA Reader.

3.2. Biological material

Microcystis sp., TAU strain IL-376, was collected from a fishpond near Kibbutz Lehavot HaBashan, Israel on November 4, 2007. Samples of the cyanobacteria are deposited at the culture collection of Tel Aviv University.

3.3. Isolation procedure

The freeze-dried cells (IL-376, 1680 g) were extracted with 7:3 MeOH/H_2O (3×3 L). The active crude extract (92.0 g, full inhibition of trypsin and chymotrypsin at 1 mg/mL) was evaporated to dryness and separated, in 10 portions, on an ODS (YMC-GEL, 120A, 4.4×6.4 cm) flash column with increasing amounts of MeOH in water. Six of the fractions (3:7 MeOH/H_2O to 9:1 MeOH/H_2O) that inhibited trypsin and chymotrypsin in at 0.45 mg/mL were each further separated on a Sephadex LH-20 column eluted with a 1:1 chloroform/methanol or 2:1:1 petroleum ether/chloroform/methanol solutions to obtain fractions that contain different mixture of the micropeptins and accompanying compounds. These fractions were separated repeatedly on different preparative HPLC columns [YMC ODS-A, 10 μm, 250 mm×20.0 mm; YMC C-8, 10 μm, 250 mm×20.0 mm; Phenomenex, Cosmosil C-8, 5 μm, 250 mm×25.0 mm; DAD at maximum absorbance or 210 nm, flow rate 5.0 mL/min] in a variety of MeOH/water or acetonitrile/water containing 0.1% trifluoroacetic acid mixtures to the pure compounds. The pure compounds isolated (weight and yield, % of crude extract weight) were: micropeptin LH920 (**1**, 9.9 mg, 0.00059%); micropeptin LH1021 (**2**, 10.8 mg, 0.00064%); micropeptin LH1048 (**3**, 16.0 mg, 0.00095%); micropeptin LH1062 (**4**, 2.0 mg, 0.00012%); micropeptin LH911A (**5**, 3.0 mg, 0.00018%); micropeptin LH911B (**6**, 6.0 mg, 0.00036%); micropeptin LH911C (**7**, 8.7 mg, 0.00052%); micropeptin LH925 (**8**, 4.9 mg, 0.00029%); micropeptin SF909 (1.2 mg, 0.00007%); micropeptin MZ939A (2.0 mg, 0.00012%); cyanopeptolin S (98.6 mg, 0.00587%); cyanopeptolin SS (6.5 mg, 0.00037%); cyanopeptolin 1020 (8.6 mg, 0.00051%); microcystin LR (2.8 mg, 0.00017%); microcystin RR (8.3 mg, 0.00049%); anabaenopeptin A (2.3 mg, 0.00013%), anabaenopeptin B (4.6 mg, 0.00027%); and anabaenopeptin F (6.7 mg, 0.00040%).

3.4. Micropeptin LH920 (1)

Transparent glassy material; $[\alpha]_D^{29}$ −26 (*c* 0.41, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.38), 227 (3.80), 279 (3.07) nm; For NMR data see Tables 1 and 2 and S1; HRESIMS *m*/*z* 943.4576 (MNa^+, calcd for $C_{46}H_{64}N_8NaO_{12}$ *m*/*z* 943.4541). Retention time of AA Marfey derivatives: L-threonine 29.6 min (L-Thr 29.6, D-Thr 31.9 min), L-glutamic acid 31.7 min (L-Glu 31.7, D-Glu 32.5 min), L-valine 39.5 min (L-Val 39.5, D-Val 42.7 min), L-phenylalanine 44.5 min (L-Phe 44.5, D-Phe 46.6 min), L-*N*Me-tyrosine 48.7 min (L-*N*MeTyr 48.7 min).

3.5. Micropeptin LH1021 (2)

Transparent glassy material; $[\alpha]_D^{29}$ −34 (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.51), 226 (3.89), 279 (3.11) nm; For NMR data see Tables 1 and 2 and S2; HRESIMS *m*/*z* 1044.5032 (MNa^+, calcd for $C_{50}H_{71}N_9NaO_{14}$ *m*/*z* 1044.5018). Retention time of AA Marfey derivatives: L-threonine 29.4 min (L-Thr 29.4, D-Thr 31.9 min), L-glutamic acid 31.4 min (L-Glu 31.4, D-Glu 32.2 min), L-valine 39.6 min (L-Val 39.6, D-Val 42.7 min), L-phenylalanine 44.0 min (L-Phe 44.0, D-Phe 46.2 min), L-*N*Me-tyrosine 48.5 min (L-*N*MeTyr 48.5 min).

3.6. Micropeptin LH1048 (3)

Glassy white solid; $[\alpha]_D^{30}$ −28 (*c* 0.34, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.40), 227 (3.80), 279 (3.04) nm; For NMR data see Tables 1 and 2 and S3; HRESIMS *m*/*z* 1049.5670 (MH^+, calcd for $C_{52}H_{77}N_{10}O_{13}$ *m*/*z* 1049.5672). Retention time of AA Marfey derivatives: L-threonine 29.6 min (L-Thr 29.6, D-Thr 32.1 min), L-arginine 29.9 min (L-Arg 29.9, D-Arg 29.1 min), L-glutamic acid 31.7 min (L-Glu 31.7, D-Glu 32.4 min), L-valine 39.8 min (L-Val 39.8, D-Val 42.9 min), L-phenylalanine 44.3 min (L-Phe 44.3, D-Phe 46.4 min), L-*N*Me-tyrosine 48.7 min (L-*N*MeTyr 48.7 min).

3.7. Micropeptin LH1062 (4)

Glassy white solid; $[\alpha]_D^{30}$ −36 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.36), 227 (3.80), 279 (3.20) nm; For NMR data see Tables 1 and 2 and S4; HRESIMS *m*/*z* 1063.5840 (MH^+, calcd for

$C_{53}H_{79}N_{10}O_{13}$ *m/z* 1063.5828). Retention time of AA Marfey derivatives: L-threonine 29.4 min (L-Thr 29.4, D-Thr 32.0 min), L-arginine 29.9 min (L-Arg 29.9, D-Arg 29.1 min), L-glutamic acid 31.6 min (L-Glu 31.6, D-Glu 32.4 min), L-valine 39.9 min (L-Val 39.9, D-Val 42.9 min), L-phenylalanine 44.5 min (L-Phe 44.5, D-Phe 46.5 min), L-*N*Me-tyrosine 48.6 min (L-*N*MeTyr 48.6 min).

3.8. Micropeptin LH911A (5)

Glassy white solid; $[\alpha]_D^{29}$ −63 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.46) nm; For NMR data see Tables 1 and 2 and S5; HRESIMS *m/z* 934.3964 (MNa^+, calcd for $C_{39}H_{61}N_9NaO_{14}S$ *m/z* 934.3956). Retention time of AA Marfey derivatives: L-threonine 29.6 min (L-Thr 29.6, D-Thr 32.3 min), L-arginine 30.1 min (L-Arg 30.1, D-Arg 29.3 min), L-glutamic acid 32.0 min (L-Glu 32.0, D-Glu 32.8 min), L-valine 39.9 min (L-Val 39.9, D-Val 42.9 min), L-isoleucine 43.2 min (L-Ile 43.2, D-Ile 46.2 min), L-*N*Me-phenylalanine 43.9 min (L-*N*MePhe 43.9 min).

3.9. Micropeptin LH911B (6)

Glassy white solid; $[\alpha]_D^{29}$ −61 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.22) nm; For NMR data see Tables 1 and 2 and S6; HRESIMS *m/z* 910.3989 ($M-H^-$, calcd for $C_{39}H_{60}N_9O_{14}S$ *m/z* 910.3980). Retention time of AA Marfey derivatives: L-threonine 29.7 min (L-Thr 29.7, D-Thr 32.3 min), L-arginine 30.1 min (L-Arg 30.1, D-Arg 29.3 min), L-glutamic acid 31.8 min (L-Glu 31.8, D-Glu 32.7 min), L-valine 39.8 min (L-Val 39.8, D-Val 42.9 min), L-isoleucine 43.1 min (L-Ile 43.1, D-Ile 46.3 min), L-*N*Me-phenylalanine 43.9 min (L-*N*MePhe 43.9 min).

3.10. Micropeptin LH911C (7)

Glassy white solid; $[\alpha]_D^{29}$ −79 (*c* 0.47, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.33), 258 (2.36) nm; For NMR data see Tables 1 and 2 and S7; HRESIMS *m/z* 934.4233 (MNa^+, calcd for $C_{41}H_{65}N_7NaO_{14}S$ *m/z* 934.4208). Retention time of AA Marfey derivatives: L-threonine 29.5 min (L-Thr 29.5, D-Thr 32.1 min), L-glutamic acid 31.7 min (L-Glu 31.7, D-Glu 32.5 min), L-valine 39.9 min (L-Val 39.9, D-Val 42.9 min), L-isoleucine 43.1 min (L-Ile 43.1, D-Ile 46.2 min), L-*N*Me-phenylalanine 43.8 min (L-*N*MePhe 43.8 min), ω-*N*Me-lysine 44.8 min (L-*N*MeLys, 44.8 min).

3.11. Micropeptin LH925 (8)

Glassy white solid; $[\alpha]_D^{29}$ −78 (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.38) nm; For NMR data see Tables 1 and 2 and S7; HRESIMS *m/z* 949.4421 (MNa^+, calcd for $C_{42}H_{68}N_7NaO_{14}S$ *m/z* 949.4443). Retention time of AA Marfey derivatives: L-threonine 30.1 min (L-Thr 30.1, D-Thr 32.7 min), L-glutamic acid 31.8 min (L-Glu 31.8, D-Glu 32.7 min), L-isoleucine 43.9 min (L-Ile 43.9, D-Ile 46.7 min), L-*N*Me-phenylalanine 44.3 min (L-*N*MePhe 44.3 min), ωω-NMe_2-lysine 30.8 min (L-NMe_2Lys, 30.8 min).

3.12. Determination of the absolute configuration of the amino acids

Small portions (0.5 mg) of compounds **1**–**8** were dissolved in 6 N aqueous HCl (1 mL). The reaction mixture was then placed in a sealed glass bomb at 110 °C for 16 h. In another experiment, 0.2 mg portions of compounds **1**–**8** were first oxidized with Jones' reagent (one drop from solution of 1.34 g $K_2Cr_2O_7$, 1 mL of H_2SO_4 in 8 mL of H_2O) in 1 mL of acetone at 0 °C for 10 min. The mixture was allowed to warm to room temperature and a few drops of MeOH were added. The bluish residue that developed was filtered and the solvent was evaporated in vacuo. Following the usual work-up, the resultant product was dissolved in 6 N aqueous HCl (1 mL) and placed in a sealed glass bomb at 110 °C for 16 h. After the removal of the hydrochloric acid, by repeated evaporation in vacuo, the hydrolyzate was resuspended in water (250 μL). A 0.03 M solution of (1-fluoro-2,4-dinitrophenyl)-5-L-alanine amide (FDAA) in acetone (1.2 mol per 1 mol AA) and 1 N aqueous $NaHCO_3$ (20 μL per AA) were added to each reaction vessel and the reaction mixtures were stirred at 70 °C for 2 h. A 2 N HCl solution (10 μL per AA) was added to each reaction vessel and the solutions were evaporated in vacuo. The *N*-[(-dinitrophenyl)-5-L-alanine amide]-amino acid derivatives, from hydrolyzates, were compared with similarly derivatized standard amino-acids by HPLC analysis: LiChrospher 60 RP-select B, 5 μm, 4.0×250 mm, flow rate: 1 mL/min, UV detection at 340 nm, linear gradient elution from 100%, 0.1% TFA in H_2O to 1:1, 0.1% TFA in H_2O/ACN, within 60 min. The determination of the absolute configuration of each amino acid was confirmed by spiking the derivatized hydrolyzates with the derivatized authentic amino acids.

3.13. Protease inhibition assays

The procedures used to determine the inhibitory activity of the new compounds against trypsin and chymotrypsin were described in a previous paper.[26]

Acknowledgements

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Supplementary data

The ^1H, ^{13}C, HMQC or HSQC, HMBC COSY, and ROESY NMR spectra, table of full NMR data, and HRESIMS data of compounds **1**–**8**. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2013.09.054. These data include MOL files and InChIKeys of the most important compounds described in this article.

References and notes

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