Tetrahedron 66 (2010) 2705–2712

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Novel thiazole and oxazole containing cyclic hexapeptides from a waterbloom of the cyanobacterium *Microcystis* sp.

Avi Raveh [a], Smadar Moshe [b], Zoharia Evron [b], Eliezer Flescher [b], Shmuel Carmeli [a,*]

[a] Raymond and Beverly Sackler School of Chemistry and Faculty of Exact Sciences, Sackler Faculty of Medicine, Tel Aviv University, Ramat-Aviv, Tel-Aviv 69978, Israel
[b] The Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Ramat-Aviv, Tel-Aviv 69978, Israel

ARTICLE INFO

Article history:
Received 16 October 2009
Received in revised form 30 December 2009
Accepted 1 February 2010
Available online 4 February 2010

Keywords:
Cyanobacteria
Microcystis
Cyclichexpeptides
Natural products

ABSTRACT

Eight novel thiazole and oxazole containing cyclic peptides, microcyclamides GL616, GL582, GL614A, GL614B, GL614C, GL546A, GL546B, and GL628, as well as the known microcyclamide A, were isolated from the hydrophylic extract of a *Microcystis* sp. water-bloom collected in Gilboa reservoir, Valley of Armageddon, Israel. The planar structure of the compounds was determined by homonuclear and inverse-heteronuclear 2D NMR techniques as well as high-resolution mass spectrometry. The absolute configuration of the asymmetric centers of the amino acids was studied using Marfey's method for HPLC following ozonolysis, hydrolysis and derivatization with Marfey's reagent. This is the first example where acidic and modified amino acids are incorporated in this group of ribosomally biosynthesized metabolites.

1. Introduction

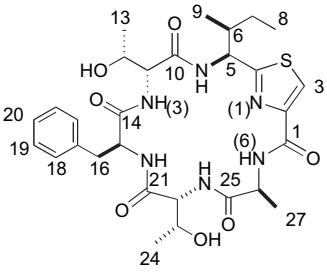
Certain genera of cyanobacteria, including *Microcystis*, *Planktothrix*, *Anabaena*, *Nostoc*, and *Nodularia*, form massive and frequently toxic blooms in fresh and brackish water-bodies. These genera of cyanobacteria habitually produce the hepatotoxic[1] and tumor promoting microcystins.[2] Aside from these toxins these genera of cyanobacteria produce a large number of bioactive metabolites with a diverse range of biological activities. These include families of protease inhibitors such as the micropeptins,[3] anabaenopeptins,[4] aeruginosins,[5] microginins,[6] microviridins,[7] and other metabolites with diverse activities such as the aeruginoguanidines,[8] kawaguchipeptins,[9] nostophycin,[10] nostopeptolides,[11] and nostodiones.[12] Many of the later metabolites are short modified peptides that are products of non-ribosomal biosynthesis. Cyclic peptides involving heterocyclic modifications (usually called cyclamides) were initially isolated from several marine organisms (through cyanobacterial diet, i.e., dolastatin E,[13] or cyanobacterial symbionts, i.e., trisoxazoline and bistratamides[14]). Such metabolites were characterized from marine (i.e., venturamides[15]) and cultured terrestrial cyanobacteria (i.e., raocyclamides and tenuecyclamides)[16] and recently also from water-bloom-forming strains of cyanobacteria such as *Microcystis* and *Nostoc* (i.e., microcyclamide,[17] aerucyclamides,[18] and banyascyclamides[19]). Contrary to the other peptide derived metabolites of water-bloom-forming cyanobacteria, this group of metabolites is ribosomally synthesized.[20] As part of our continuing interest in the chemical ecology of cyanobacterial water blooms and search for novel drugs for human diseases, we examined the extracts of a *Microcystis* sp. bloom collected during July 2007 from Gilboa reservoir, Valley of Armageddon, Israel. Mass spectral analysis of the bloom material extract[21] indicated that it contained considerable amounts of microcystins (microcystin LR, RR, LY, and [Dha]-microcystin RR, and three derivatives of microcystin LY), micropeptins, and a series of unidentified compounds with molecular weights around 600 mass units. Fractionation of the methanol/water extract of the bloom material (IL-363) afforded nine non-toxic secondary metabolites. Of these nine compounds, eight are new natural products: microcyclamide GL616 (**1**), microcyclamide GL582 (**2**), microcyclamide GL614A (**3**), microcyclamide GL614B (**4**), microcyclamide GL546A (**5**), microcyclamide GL546B (**6**), microcyclamide GL628 (**7**), microcyclamide GL614C (**8**), and one is known microcyclamide A (**9**).[17] The structure elucidation, inter-conversion, and biological activity of the compounds is discussed below.

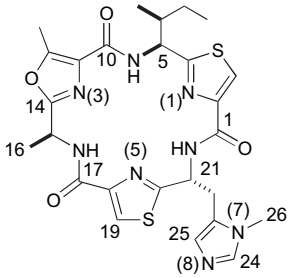
2. Results and discussion

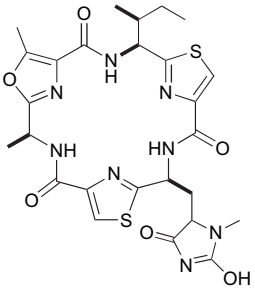
Microcyclamide GL616 (**1**) was isolated as a yellow glassy material. The molecular formula of **1**, $C_{29}H_{40}N_6O_7S$ (HREIMS, *m*/*z* 616.2679, 0.9 ppm error), indicated 13 unsaturations within the molecule. The hexapeptide nature of **1** was deduced from the six nitrogen atoms in the formula and six sp^2 carbon signals in the amide region of the ^{13}C NMR spectrum. The presence of five amide

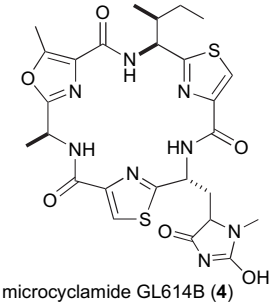
* Corresponding author. Tel.: +972 3 6408550; fax: +972 3 6409293.
E-mail address: carmeli@post.tau.ac.il (S. Carmeli).

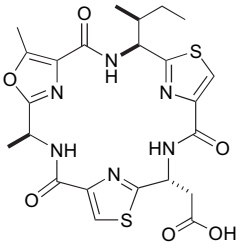
0040-4020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2010.02.008

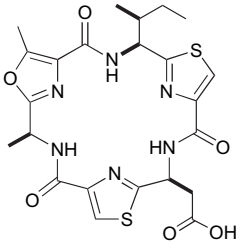

microcyclamide GL616 (**1**)

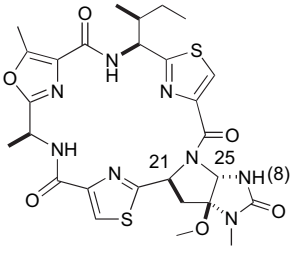

microcyclamide GL582 (**2**)

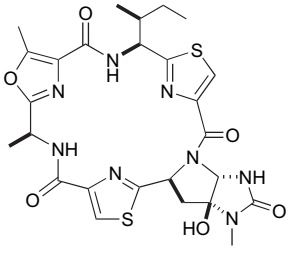

microcyclamide GL614A (**3**)

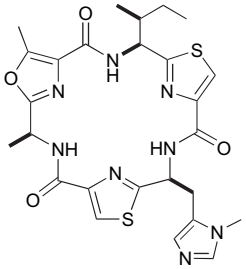

microcyclamide GL614B (**4**)


microcyclamide GL546A (**5**)


microcyclamide GL546B (**6**)


microcyclamide GL628 (**7**)


microcyclamide GL614C (**8**)


microcyclamide A (**9**)

protons, one singlet aromatic proton and two doublet methyl carbinol units in the ^{1}H NMR spectrum suggested the presence of one cyclically modified amino acid. Five standard amino acid residues—two serinyl-moieties, one alanyl-moiety, one isoleucyl-moiety and a phenylalanyl-moiety—and one modified amino acid–a thiazole—were established on the basis of analyses of ^{1}H, ^{13}C (Table 1), COSY, HMQC, and HMBC spectra. The thiazole-isoleucyl unit could be established on the basis of the long range H–C correlations between the aromatic protons (H-3) and the neighboring carbons C-1, C-2, and C-4, of H-5 with C-4 and on the basis of the COSY correlations of H-5 with N(2)H and H-6, H-6 with H_2-7 and H_3-9, and H_2-7 with H_3-8. The two threonine moieties were established from the COSY correlations of H-11 with H-12 and N(3)H, H-12 with 12-OH and Me-13 and the HMBC correlation of H-11 with C-10 and of H-22 with H-23 and N(5)H, H-23 with 23-OH and Me-24 and the HMBC correlation of H-22 with C-21. The phenylalanyl-moiety was deduced on the basis of COSY correlations between H-15 and H-16, H-16′ and N(4)H and between H-19 and H-19′ and H-18, H-18′ and H-20 and the HMBC correlation of C-16 with H-18,18′ and C-14 with H-15, H-16 and H-16′. The alanyl-moiety was established based on the COSY correlations of H-26 with N(6)H and H_3-27, and on the basis of the HMBC correlation of H-26 and H_3-27 with C-25. HMBC correlations were used to connect the above fragments to the planar structure of **1**. C-1 presented correlation with N(6)H, C-10 with N(2)H, C-14 with N(3)H, C-21 with N(4)H and C-25 with N(5)H. Ozonolysis, acid hydrolysis and derivatization of **1** with Marfey's reagent,[22] followed by HPLC analysis, demonstrated the L-configuration of alanine, isoleucine, and phenylalanine and the D-stereochemistry of threonine. These arguments established structure **1** for microcyclamide GL616.

Microcyclamide GL582 (**2**) and microcyclamide A (**9**) eluted from the HPLC column one after the other. They share the same molecular weight (HREIMS, *m*/*z* 582.1830, M^{+}, for **2** and 582.1831 for **9**, and molecular formula $C_{26}H_{30}N_8O_4S_2$). Their ^{1}H and ^{13}C NMR spectra (see Table 2) were similar and presented only small chemical shifts differences for the N(2)H to N(6)H segment of the molecules. Their NMR data suggested that the two compounds, **2** and **9**, differ at least in one of the four-stereogenic centers present in their structure. Analyses of their ^{1}H, ^{13}C, HMQC, HMBC (Table 2), and COSY spectra established three molecular fragments: thiazole-isoleucyl, methyl oxazole-alanyl and thiazole-*N*-methyl histidine. The latter fragments were connected to the planar structure of **2** based on the HMBC correlations of N(6)H and H-21 with C-1, of N(2)H with C-10 and of N(4)H with C-17. Similar arguments were used to establish the same planar structure for **9**. Literature search revealed that **9** is identical with the *Microcystis aeruginosa* metabolite microcyclamide A.[17] Ozonolysis, acid hydrolysis, and derivatization of **2** with Marfey's reagent,[22] followed by HPLC analysis, established the absolute configuration of the amino acids obtained in this hydrolytic procedure as: L-isoleucine, L-alanine, and D-aspartic acid (obtained from the ozonolysis of the methyl histidine side-chain). The absolute configuration of the asymmetric centers of **2** is thus, 5*S*, 6*S*, 15*S*, and 21*R*. Using the same procedure for **9**, established the absolute configuration of the asymmetric centers as 5*S*, 6*S*, 15*S*, and 21*S*, in accordance with the published data.[17]

Microcyclamide GL614A (**3**) and microcyclamide GL614B (**4**) also eluted from the HPLC column one after the other and were isolated

Table 1
NMR data of microcyclamide GL616 (**1**) in DMSO-d_6[a]

Position	δ_C, mult.[b]	δ_H, mult., J (Hz)	LR H–C correlations[c]	COSY correlations
1	160.6 s		N(6)H,3	
2	148.5 s		3	
3	124.0 d	8.18 s		
4	172.0 s		3, 5	
5	53.8 d	5.28 dd 9.1, 4.3	N(2)H, 6	6
6	41.1 d	1.83 ddq 6.1, 5.6, 6.7	5, 7, 8, 9	5, 7, 9
7	26.3 t	1.12 m; 1.38 m	6, 8, 9	6, 8
8	11.7 q	0.86 t, 7.3	6, 7	7
9	14.3 q	0.78 d, 6.7	6, 7	6
N(2)H		8.11 d, 9.1		5
10	170.4 s		N(2)H	
11	61.5 d	4.03 dd 7.2, 4.5	N(3)H,12, 12-OH	N(3)H, 12
12	65.5 d	3.87 ddq 7.2, 6.4, 6.1	N(3)H, 12, 12-OH	11, 12-OH, 13
12-OH		4.82 d 6.4		12
13	20.4 q	1.09 d 6.1	12,12-OH	12
N(3)H		8.16 brd 4.5		11
14	170.6 s		N(3)H, 15, 16, 16′	
15	52.1 d	4.58 ddd 8.7, 8.4, 4.0	N(4)H, 16	N(4)H, 16, 16′
16	37.6 t	2.73 dd 13.7, 8.4 2.95 dd 13.7, 4.0	15, 18	15
17	137.0 s		18, 19	
18,18′	129.3 d	7.10 d 7.5	19, 20	19, 19′
19,19′	127.0 d	7.01 dd 7.5, 7.0	18, 20	18, 18′, 20
20	126.1 d	6.96 t 7.0	18, 19	19
N(4)H		7.16 d 8.7		15
21	169.7 s		N(4)H, 22, 23	
22	61.3 d	3.82 dd 7.0, 5.3	N(5)H, 23	N(5)H, 23
23	65.7 d	4.00 ddq 6.6, 5.3, 6.1	22, 23-OH	22, 24
23-OH		4.83 d 6.6		23
24	20.2 q	0.93 d 6.1	23, 23-OH	23
N(5)H		8.07 d 7.0		22
25	171.4 s		N(5)H,26,27	
26	48.6 d	4.68 dq 7.1, 6.4	N(6)H, 27	N(6)H, 27
27	17.3 q	1.20 d 6.4	26	26
N(6)H		8.38 d 7.1		26

[a] ^{1}H (400 MHz), ^{13}C (100 MHz).
[b] Multiplicity and assignment from HSQC experiment.
[c] Determined from HMBC experiment, $^nJ_{CH}$=8 Hz, recycle time 1 s, presented as a correlation of a proton with carbon in the row.

as white glassy materials. Both presented a HRCIMS quasimolecular ion peak (MH^+) at m/z 615.1818 (**3**) and 615.1825 (**4**) matching the molecular formula, $C_{26}H_{30}N_8O_6S_2$. The ^{1}H and ^{13}C NMR spectra of **3** and **4** (see Table 3) were similar and presented only small chemical shifts differences for the C-20 to N(6)H segment of the molecule, suggesting opposite stereochemistry at C-21, as found for **2** and **9**. Comparison of the NMR spectra of **2** and **9** with those of **3** and **4** revealed that they differ only in the C-21 to C-26 region in the molecules (see Tables 2 and 3). The 32 mass unit difference between **2** and **9** and **3** and **4** is in accordance with the loss of 3 sp^2 carbons (resonating at ca. 130, 136, and 120 ppm) that appear in the spectra of **2** and **9** and the appearance of 1 sp^3 methine and 2 oxygenated sp^2 carbons (resonating at ca. 60, 157, and 174 ppm) in the ^{13}C NMR spectra of **3** and **4**. The C-21 to C-23 fragment of both **3** and **4** was established on the basis of COSY correlations. The N–Me protons (H_3-26) present HMBC correlations with both C-23 and the carbon resonating at δ_C 156.8 ppm (in both compounds), suggesting that the N–Me moiety is situated between them. H-22, -22′ and -23 present HMBC correlations with the carbon at δ_C 174 ppm. The chemical shift of the latter carbon is in accordance with an amide carbonyl and the correlation with H-23 suggests their vicinity. The chemical shift of the carbon resonating at δ_C 156.8 ppm is in agreement with an imide carbonyl or enolimide carbon. Although no direct correlation between the latter carbonyls was observed, their chemical shifts and the molecular formula of **3** and **4** suggest that they are connected through an *N*-imine nitrogen to produce a 2-hydroxy-1-methyl-1-*H*-imidazol-4(5*H*)-one-5-yl moiety, which seems to be derived from the *N*-methyl-imidazole moiety of **2** and **9**. Ozonolysis, acid hydrolysis, and derivatization of **3** and **4** with Marfey's reagent,[22] followed by HPLC analysis, established the absolute configuration of the amino acids obtained in this hydrolytic procedure as: L-isoleucine, L-alanine for both **3** and **4**, and L-aspartic acid for **3** and D-aspartic acid for **4** (obtained from the ozonolysis of the dioxoimidazole ring system). The absolute configuration of the asymmetric centers of **3** is thus, 5*S*, 6*S*, 15*S*, and 21*S* and 5*S*, 6*S*, 15*S*, and 21*R* for **4**. The absolute configuration of C-23 was not established due to the oxidation process that occurred under the reaction conditions. On the basis of these arguments the structures of microcyclamide GL614A and microcyclamide GL614B were established as **3** and **4**, respectively.

In common with **2** and **9**, and **3** and **4** microcyclamides GL546A (**5**) and GL546B (**6**) eluted from the column one after the other. They presented similar HRCIMS molecular ion clusters as well, m/z 547.1437 for **5** and m/z 547.1430 for **6**, in accordance with a molecular formula of $C_{23}H_{26}N_6O_6S_2$. The ^{1}H and ^{13}C NMR spectra of **5** and **6** (see Table 4) were similar. When compared with that of **2**, **3**, **4**, and **9** it was obvious that the methyl-imidazole moiety is missing in **5** and **6** since the aromatic singlet protons and the *N*-methyl resonances were missing from their spectra. The chemical shifts of the signals assigned to positions 1–19 were similar to those of **2**, **3**, **4**, and **9** suggesting that this part of the molecules is similar in all compounds. For positions 20 to N(6)H some small chemical shifts differences were observed, which might suggest a difference in the absolute configuration of C-21 (see Table 4). This molecular fragment in **5** and **6**, was established by COSY, HSQC, and HMBC correlations (Table 4) as an aspartyl moiety, which its C-termini condensed to a thiazole and it N-termini to a second thiazole moiety. Ozonolysis, acid hydrolysis, and derivatization of **5** and **6** with Marfey's reagent,[22] followed by HPLC analysis, established the absolute configuration of the amino acids obtained in this hydrolytic procedure as: L-isoleucine, L-alanine for both **5** and **6**, and D-aspartic acid for **5** and L-aspartic acid for **6**. The absolute configuration of the asymmetric centers of **5** is thus, 5*S*, 6*S*, 15*S*, and 21*R* and 5*S*, 6*S*, 15*S*, and 21*S* for **6**. On the basis of this evidence the structures of microcyclamide GL546A and microcyclamide GL546B were established as **5** and **6**, respectively.

Microcyclamide GL628 (**7**) and microcyclamide GL614C (**8**) are similar in their structure as evident from their NMR spectra. Comparison of their NMR data revealed that **7** is a methyl ether of the tertiary lactim, **8**. The HRCIMS quasimolecular $[MH]^+$ ion of **7** at m/z 629.1958 matched the molecular formula $C_{27}H_{32}N_8O_6S_2$ while that of **8**, m/z 637.1627 $[M+Na]^+$ matched the molecular formula $C_{26}H_{30}N_8O_6S_2$, thus confirming the difference between these to compounds. The oxidation state of **7** and **8** seems to be similar to that of **3** and **4**, while **7** differs from them by a carbon and two protons. The proton and carbon NMR spectra of **7** and **8** in DMSO-d_6 appeared as a mixture of two conformers while those taken in $CDCl_3$ displayed one conformer and were well resolved. Comparison of the NMR data of **7**, in $CDCl_3$ with that of **2** (data not shown) revealed the similarity of the C-4 to C-19 fragment in both compounds and confirmed the difference in the C-20 to C-3 fragment. These differences suggested that an oxidized form, in **7**, substitutes the methyl histidine moiety of **2**. In the ^{1}H NMR spectrum of **7**, the doublet N(6)H was missing, while a new singlet amide proton (δ_H 6.28 ppm) and a new singlet methine (δ_H 5.19 ppm) were observed and the chemical shift of the *N*Me was upfield shifted (δ_H 3.29 ppm), relative to that of **2**. In the ^{13}C NMR, of **7**, the three double bond carbons of the imidazole moiety (of **2**) were missing and instead an uryl amide carbon (δ_C 157.6 ppm), a tertiary oxygenated carbon (δ_C 100.4 ppm), and a methine carbon (δ_C 72.1 ppm,

Table 2
NMR data of microcyclamide GL582 (**2**) and microcyclamide A (**9**) in DMSO-d_6[a]

Position	Microcyclamide GL582 (**2**)			Microcyclamide A (**9**)	
	δ_C, mult.[b]	δ_H, mult., J (Hz)	LR H–C correlations[c]	δ_C, mult.[b]	δ_H, mult., J (Hz)
1	159.5 s		21, N(6)H, 3	159.3 s	
2	148.0 s		3	147.7s	
3	125.4 d	8.28 s		125.2 d	8.28 s
4	168.0 s		3, 5, N(2)H	167.8 s	
5	54.6 d	5.32 dd 7.4, 5.8	N(2)H, 6, 7, 7′, 9	54.4 d	5.31 dd 7.8, 6.4
6	40.2 d	2.01 m	5, 7, 7′, 8, 9	40.2 d	2.01 m
7	25.5 t	1.18 ddq 13.2, 8.1, 7.4	6, 8, 9	25.3 t	1.18 m
		1.58 ddq 13.2, 5.0, 7.4			1.59 m
8	11.7 q	0.94 t 7.4	6, 7, 7′	11.5 q	0.94 t 7.4
9	14.7 q	0.81 d 6.8	5, 6, 7, 7′	14.5 q	0.81 d 6.7
N(2)H		8.52 d 7.4			8.42 d 7.8
10	159.4 s		5, N(2)H, 13	159.6 s	
11	128.0 s		13	127.8 s	
12	153.6 s		13	153.5 s	
13	11.4 q	2.60 s		11.2 q	2.60 s
14	161.4 s		15, 16, N(4)H	161.4 s	
15	44.3 d	5.18 dq 5.9, 6.6	16, N(4)H	44.1 d	5.19 dq 7.0, 6.6
16	19.3 q	1.54 d 6.6	15	19.1 q	1.53 d 6.6
N(4)H		8.56 d 5.9			8.58 d 7.7
17	159.8 s		15, N(4)H, 19	159.3 s	
18	147.9 s		19	147.7 s	
19	126.0 d	8.46 s		125.9 d	8.48 s
20	169.2 s		19, 21, 22, 22′, N(6)H	169.0 s	
21	49.9 d	5.99 ddd 8.9, 8.4, 5.5	22, 22′	49.6 d	6.01 ddd 8.6, 8.5, 5.4
22	31.0 t	3.38 dd 15.3, 8.4	21	30.8 t	3.36 dd 15.4, 8.5
		3.48 dd 15.3, 5.5			3.50 dd 15.4, 5.4
23	129.9 s		21, 22, 22′, 24, 25, 26	130.2 s	
24	136.2 d	8.77 brs	25, 26	135.7 d	8.98 s
25	120.2 d	7.29 brs	22, 22′, 24	119.0 d	7.45 s
26	33.1 q	3.79 brs	24	33.2 q	3.84 s
N(6)H		8.90 d 8.9			8.94 d 8.6

[a] ^{1}H (400 MHz), ^{13}C (100 MHz).
[b] Multiplicity and assignment from HSQC experiment.
[c] Determined from HMBC experiment, $^nJ_{CH}$=8 Hz, recycle time 1 s, presented as a correlation of a proton with carbon in the row.

δ_H 5.19 ppm) were observed. Analyses of ^{1}H, ^{13}C, HMQC, HMBC (Table 5), and COSY spectra, of **7**, established the similarity of the C-4 to C-19 segment in **2** and **7**. The cross peaks in the COSY spectrum established the connectivity of H-21, H-22 and H-22′, as well as, those of H-25 and N(8)H. The pyrrolidine ring contains the nitrogen bearing C-21, C-22, the carbon at δ_C 100.4 (C-23), which is connected to C-22, and the carbon at δ_C 72.1 (C-25), which in turn is connected to the tri-substituted nitrogen. The HMBC correlations of H-21 with the carbons at δ_C 100.4 and 72.1 ppm, of H-22 and 22′ with the carbons at δ_C 100.4, 72.1, and 56.5 (C-21) ppm, of the proton at δ_H 5.19 ppm with the carbons at δ_C 100.4, 56.5, and 41.1 (C-22) ppm and of the methoxy protons (δ_H 3.29 ppm) with the carbon at δ_C 100.4 ppm, established the structure of a pyrrolidine ring. The HMBC correlations of H-25 with the carbonyl at δ_C 157.6, of the amide proton at δ_H 6.28 ppm with C-23 and of the *N*Me protons with the carbonyl at δ_C 157.6 ppm and C-23 close a second five member ring, which is fused to the pyrrolidine ring through carbons 23 and 25. HMBC correlations between C-20 and H-19, H-21, H-22 and H-22′ connected the pyrrolidine moiety to one of the thiazole moieties while the correlations of H-25, and H-3 with C-1 connected the pyrrolidine moiety to the carbonyl of the other thiazole thus, establishing the planar structure of **7**. The relative configuration of the pyrrolidine ring was established through the NOE correlations, from a ROESY experiment, between H-21 and H-22′, H-22 and H-25 and H-25 and 23-OMe to be *syn* between H-25 and 23-OMe and *anti* between H-21 and H-25. Based on these arguments the proposed relative configuration of the chiral centers of this moiety is 21*S**, 23*S**, and 25*S**. The absolute configuration of the chiral centers was established by the same procedure described for compounds **1**–**6** as L-isoleucine, L-alanine, and L-aspartate. Establishing the absolute configuration of C-21 as *S* by Marfey's method defines the absolute configuration of the chiral centers of the pyrrolidinone moiety as 23*S* and 25*S* and structure **7** for microcyclamide GL628.

Microcyclamide GL614C (**8**) was isolated as a white glassy material. The NMR spectra of **8** resembled those of **7**. The only difference observed was the absence of the methyl ether signals (δ_H 3.04 ppm, δ_C 50.9 ppm in **7**), in the spectra of **8**. Analyses of ^{1}H, ^{13}C, HMQC, HMBC, COSY, and ROESY spectra of microcyclamide GL614C and Merfay's analysis[22] established structure **8** as shown.

Histidine in proteins, peptides and as a free acid is known to be susceptible to oxidation. Hara[23] studied the oxidation of monomeric and protein histidine, by photosynthesizers or metal catalysis and found aspartic acid and urea to be the final products. Others have shown that histidine in proteins and peptides can specifically be oxidized by irradiation with visible light in the presence of a suitable photo sensitizer[24] or in the presence of a suitable metal such as zinc, copper or iron[25,26] to produce the 2-oxo-imidazole derivative without breaking the peptide bond between the amino acids of the peptide. Tomita et al.[27] studied the photoxidation of *N*-benzoylhistidine and isolated a handful of products, among them *N*-benzoyl-aspartate and 6a-hydroxy-2-oxo-4-(phenylcarbonyl) octahydropyrrolo-[3,2-*d*]imidazole-5-carboxylic acid similar to that of **8**. They proposed a mechanism for the reaction, which is based on a [2+4] singlet oxygen addition to the imidazole diene system. More recent studies with urocanic acid suggest that the imidazole moiety can react with singlet oxygen in [2+4] and [2+2] fashions.[28] We propose that the methyl histidine moiety of microcyclamides **2** and **9** underwent, either an enzyme mediated or spontaneous, oxidative process that resulted in the production of compounds **3**–**6** and **8** (see Scheme 1). Compound **7** is most

Table 3
NMR data of microcyclamide GL614A (**3**) and microcyclamide GL614B (**4**) in DMSO-d_6[a]

Position	Microcyclamide GL614A (**3**)		Microcyclamide GL614B (**4**)		
	δ_C, mult.[b]	δ_H, mult., J (Hz)	δ_C, mult.[b]	δ_H, mult., J (Hz)	LR H–C correlations[c]
1	159.4 s		159.4 s		N(6)H, 3
2	148.4 s		148.5 s		3
3	125.0d	8.23 s	125.1 d	8.30 s	
4	168.0 s		167.8 s		3, 5, N(2)H
5	54.8 d	5.26 dd 7.5, 6.0	54.8 d	5.26 dd 7.7, 6.0	N(2)H, 6, 7, 7′, 9
6	39.8 d	2.02 m	40.5 d	2.01 m	5, 7, 7′, 8, 9
7	25.6 t	1.20 ddq 13.1, 8.0, 7.3	25.6 t	1.16 ddq 13.2, 8.0, 7.4	6, 8, 9
		1.61 ddq 13.1, 4.8, 7.3		1.62 ddq 13.2, 4.7, 7.4	
8	11.4 q	0.93 t 7.3	11.4 q	0.94 t 7.4	6, 7, 7′
9	14.8 q	0.81 d 6.7	14.7 q	0.83 d 6.7	5, 6, 7, 7′
N(2)H		8.44 d 7.5		8.35 d 7.7	
10	160.0 s		159.9 s		5, N(2)H, 13
11	128.6 s		128.1 s		13
12	153.6 s		153.6 s		13
13	11.6 q	2.59 s	11.7 q	2.59 s	
14	161.4 s		161.4 s		15, 16, N(4)H
15	44.4 d	5.15 dq 5.7, 6.6	44.5 d	5.15 dq 5.4, 6.7	16, N(4)H
16	19.3 q	1.53 d 6.6	19.3 q	1.54 d 6.7	15
N(4)H		8.55 d 5.7		8.51 d 5.4	
17	159.6 s		159.6 s		N(4)H, 19
18	148.1 s		148.1 s		19
19	125.8 d	8.43 s	125.9 d	8.43 s	
20	170.5 s		170.6 s		19, 21, 22, 22′
21	47.9 d	5.78 ddd 9.4, 9.2, 3.4	47.6 d	5.92 ddd 9.7, 8.9, 4.2	22, 23
22	36.7 t	2.38 ddd 14.7, 4.3, 3.4	36.8 t	2.43 m	21, 23
		2.54 ddd 14.7, 9.2, 3.7		2.49 m	
23	59.7 d	4.09 dd 4.3, 3.7	59.3 d	4.00 dd 7.0, 3.3	22, 26
24	156.6 s		156.8 s		26
24-OH		10.11 s		—	
25	174.2 s		174.4 s		22, 22′, 23
26	27.6 q	2.68 s	27.5 q	2.73 s	
N(6)H		8.98 d 9.4		8.73 d 9.7	

[a] ^{1}H (400 MHz), ^{13}C (100 MHz).
[b] Multiplicity and assignment from HSQC experiment.
[c] Determined from HMBC experiment, $^nJ_{CH}$=8 Hz, recycle time 1 s, presented as a correlation of a proton with carbon in the row.

Table 4
NMR data of microcyclamide GL546A (**5**) and microcyclamide GL546B (**6**) in DMSO-d_6[a]

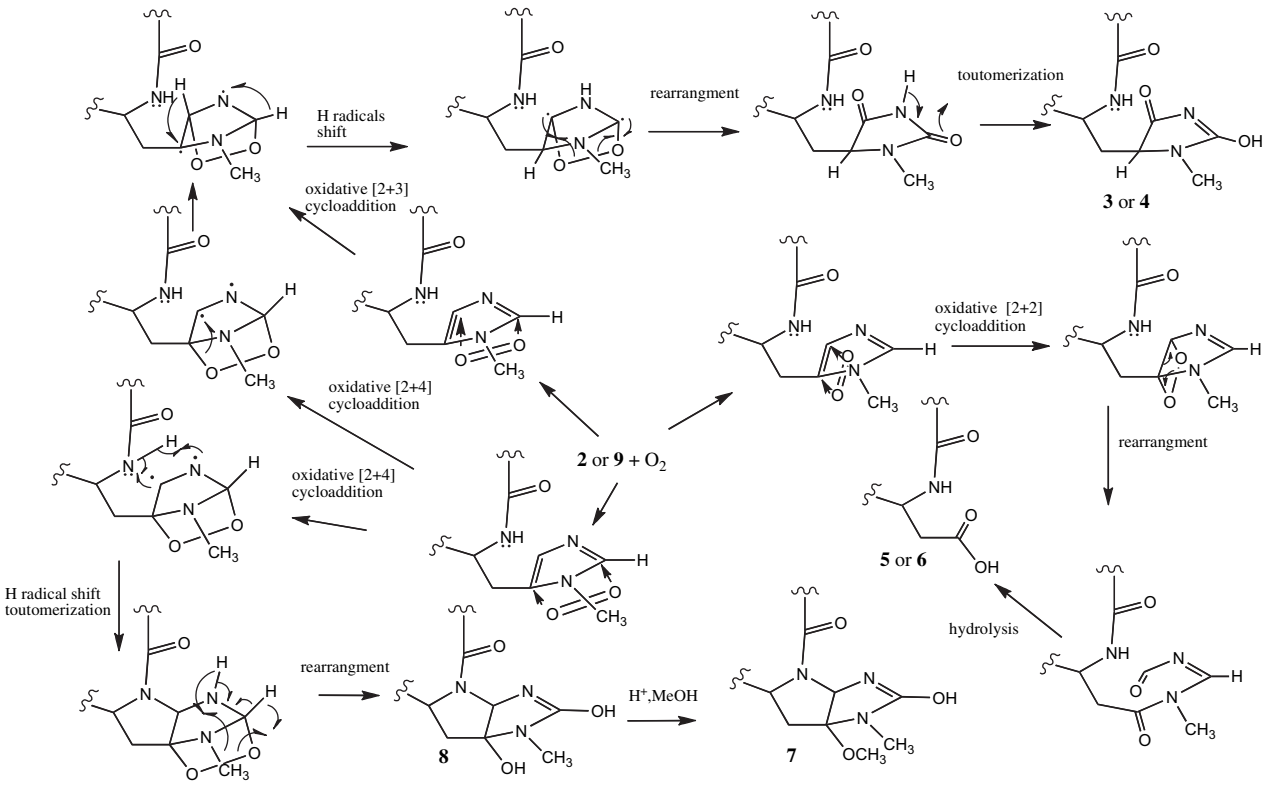
Position	Microcyclamide GL546A (**5**)			Microcyclamide GL546B (**6**)	
	δ_C, mult.[b]	δ_H, mult., J (Hz)	LR H–C correlations[c]	δ_C, mult.[b]	δ_H, mult., J (Hz)
1	159.2 s		N(6)H, 21, 3	159.0 s	
2	148.4 s		3	148.5 s	
3	125.4 d	8.37 s		125.2 d	8.35 s
4	168.2 s		3, 5	168.2 s	
5	54.5 d	5.31 dd 7.8, 6.0	6, 7, 9	54.5 d	5.30 dd 7.5, 6.1
6	40.5 d	1.97 m	7, 8, 9	40.5 d	1.96 m
7	25.5 t	1.15 ddq 13.6, 8.8, 7.3	6, 8	25.4 t	1.16 ddq 13.1, 7.7, 7.2
		1.35 ddq 13.6, 4.7, 7.3			1.57 ddq 13.1, 4.8, 7.2
8	11.7 q	0.91 t 7.3	6, 7	11.6 q	0.92 t 7.2
9	14.7 q	0.78 d 6.8	5, 6	14.8 q	0.79 d 6.7
N(2)H		8.54 d 6.0			8.53 d 6.1
10	159.8 s		N(2)H, 5, 13	159.8 s	
11	128.0 s		13	128.7 s	
12	153.6 s		13	153.6 s	
13	11.4 q	2.60 s		11.4 q	2.59 s
14	161.5 s		15, 16	161.5 s	
15	44.2 d	5.19 dq 7.8, 6.8	14, 16, N(4)H	44.1 d	5.19 dq 7.6, 6.7
16	19.3 q	1.53 d 6.8	15	19.3 q	1.52 d 6.7
N(4)H		8.38 d 7.8			8.38 d 7.6
17	159.5 s		N(4)H, 19	159.**5** s	
18	147.9 s		N(4)H, 19	147.7 s	
19	125.9 d	8.41 s		125.8 d	8.39 s
20	170.3 s		19, 21	170.9 s	
21	48.4 d	5.80 dt 8.2, 5.2	N(6)H, 22	48.6 d	5.77 dt 8.2, 5.6
22	40.8 t	3.07 d 5.2	N(6)H, 21	41.4 t	2.98 brs
23	171.7 s			170.9 s	
N(6)H		8.77 d 8.2			8.78 d 8.2

[a] ^{1}H (400 MHz), ^{13}C (100 MHz).
[b] Multiplicity and assignment from HSQC experiment.
[c] Determined from HMBC experiment, $^nJ_{CH}$=8 Hz, recycle time 1 s, presented as a correlation of a proton with carbon in the row.

Table 5
NMR data of microcyclamide GL628 (**7**) and microcyclamide GL614C (**8**) in $CDCl_3$[a]

Position	Microcyclamide GL628 (**7**)			Microcyclamide GL614C (**8**)	
	δ_C, mult.[b]	δ_H, mult., *J* (Hz)	LR H–C correlations[c]	δ_C, mult.[b]	δ_H, mult., *J* (Hz)
1	162.2 s		3, 25	162.3 s	
2	148.8 s		3	149.0 s	
3	126.1 d	8.12 s		126.0 d	8.10 s
4	167.9 s		3, 5, 6, N(2)H	168.1 s	
5	56.4 d	5.27 dd 6.1, 4.6	6, 7, 7′, 8, 9	56.4 d	5.21 dd 5.6, 6.2
6	39.7 d	2.33 m	5, 7, 7′, 8, 9	39.8 d	2.31 m
7	26.3 t	1.33 ddd 13.3, 4.7, 7.4	6, 8, 9	26.4 t	1.33 ddq 13.3, 8.8, 7.4
		1.66 m			1.67 ddq 13.3, 5.3, 7.4
8	11.8 q	1.04 t 7.4	7, 7′	11.8 q	1.05 t 7.4
9	14.0 q	0.90 d 6.8	5, 7, 7′	14.3 q	0.90 d 6.8
N(2)H		8.50 d 6.1			8.47 d 6.2
10	161.2 s		5, N(2)H, 13	161.2 s	
11	128.7 s		13	128.5 s	
12	153.6 s		13	153.5 s	
13	11.5 q	2.65 s		11.5 q	2.64 s
14	161.5 s		15, 16, N(4)H	161.3 s	
15	45.2 d	5.17 dq 5.3, 6.7	N(4)H, 16	45.1 d	5.15 dq 5.3, 6.7
16	20.1 q	1.63 d 6.7	N(4)H, 15	20.0 q	1.62 d 6.7
N(4)H		8.41 d 5.3			8.40 d 5.3
17	159.9 s		N(4)H, 19	160.0 s	
18	149.0 s		N(4)H, 19	149.0 s	
19	124.8 d	8.13 s		124.4 d	8.12 s
20	168.7 s		19, 21, 22, 22′	168.6 s	
21	56.5 d	6.38 dd 2.0, 6.5	22, 22′, 25	56.8 d	6.39 dd 6.4, 2.0
22	41.1 t	2.62 dd 13.2, 6.5; 2.64 brd 13.2	25	40.9 t	2.68 brs
23	100.4 s		21, N(8)H, 22, 22′, 25, 26	96.7 s	
24	157.6 s		N(8)H, 25, 26	156.9 s	
25	72.1 d	5.19 s	21, 22, 22′	76.2 d	5.16 s
26	24.1 q	2.39 brs		23.9 q	2.44 brs
OMe/OH	51.0 q	3.04 s			
N(8)H		6.28 s			6.28 s

[a] ^1H (500 MHz), ^{13}C (125 MHz).
[b] Multiplicity and assignment from HSQC experiment.
[c] Determined from HMBC experiment, $^nJ_{CH}$=8 Hz, recycle time 1 s, presented as a correlation of a proton with carbon in the row.



Scheme 1. Suggested route from methyl histidine to the different oxidation products.

probably an artifact of isolation from the methylation of **8**. We propose that compounds **3** and **4** are produced from **9** and **2**, respectively, through a [2+3] singlet oxygen addition to the imidazole diene or, a [2+4] addition and 1,2-oxygen migration, followed by 1,2-H radicals shift and rearrangement of the cyclic peroxide to the dioxo-imidazolidine derivatives, **3** and **4**. Such oxidation products were not characterized previously for histidine, proteins or peptides that contain histidine. A [2+2] singlet oxygen addition to the methyl-imidazole C-23–C-25 double bond affords the dioxetane, which collapse to the corresponding open carbonyl and upon hydrolysis led to the formation of the carboxylic acids, **5** and **6**. Compounds **5** and **6** might as well biosynthesized directly by incorporation of aspartic acid instead of histidine to the natural products. A [2+4] singlet oxygen addition to the imidazole diene system followed by H-radical shift, C–N bond formation and collapse of the dioxan ring to the hydroxy carbonyl derivative, led to compound **8**.

Microcyclamide A (**9**) was reported to have moderate cyctotoxicity against P388 murine leukemia cells.[17] To investigate the anticancer potential of compounds **1–8** they were assayed against one solid tumor (A549-lung) and one leukemia (Molt-4) cell lines at concentrations of 1 and 10 μg/mL.[29] Microcyclamide GL582 (**2**) displayed a weak cytotoxicity (20% growth inhibition) against Molt-4 cell line at a concentration of 10 μg/mL. The other compounds were non active at these concentrations toward both cell lines.

3. Experimental

3.1. Instrumentation

Low- and high-resolution EI and CI mass spectra were measured on a Fisons VG AutospecQ instrument. Low- and high-resolution ESI mass spectra were recorded on a Waters 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 ARX-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 1H, 100.62 MHz for ^{13}C. COSY-45, gTOCSY, gROESY, gHSQC, gHMQC, and gHMBC spectra were recorded using standard Bruker pulse sequences. HPLC separations were preformed on a Jasco HPLC system (model PU-2080-plus pump and model MD-2010-plus diode array detector), a Merck Hitachi (model L-6200 intelligent pump, model L-4200 UV–vis) a,nd an Agilent 1100 series HPLC system (model Agilent 1100 DAD and Agilent 1100 pump).

3.2. Biological material

Microcystis sp., TAU strain IL-363, was collected, in August 2008, from Gilboa reservoir Valley of Armageddon, Israel. A sample of the cyanobacterium is deposited at the culture collection of Tel Aviv University.

3.3. Isolation procedure

The freeze-dried cells (35 g) were extracted with 7:3 MeOH/H_2O. The crude extract (7.2 g) was evaporated to dryness and separated in two portions on an ODS (YMC-GEL, 120A, 4.4×6.4 cm) flash column with increasing amounts of MeOH in water. Fraction 7 (3:4 MeOH/H_2O, 617 mg) was subjected to a Sephadex LH-20 column in 1:1 chloroform/methanol to obtain 10 fractions that were combined to three pools, **7a–c**, based on NMR and TLC. Pool **7b** (fractions 4–7, 35 mg) was separated on a reversed phase HPLC column (YMC-Pack C8, 250×20 mm, 5 μm, DAD at 238 nm, flow rate 5 mL/min) eluted with 1:1 acetonitrile/0.1% TFA in H_2O, to obtain six fractions, 7b1–6. Fraction 7b5 (t_R 35.2 min, 17.1 mg, 0.049% yield based on the dry weight of the bacteria) was established as the new microcyclamide GL616 (**1**). Fraction 9 of the initial separation (1.4 gr, 8:2 MeOH/H_2O) was separated on a Sephadex LH-20 column (5×30 cm) eluted with 1:1 $CHCl_3$/MeOH into 15 fractions. The 15 fractions were combined based on their NMR spectra into five pools 9a–e. Pool 9c (fractions 6–10, 340 mg) was separated on a reversed phase HPLC column (YMC-Pack C8, 250×20 mm, 5 μm, DAD at 238 nm, flow rate 5 mL/min) eluted with 55:45 acetonitrile/0.1% TFA in H_2O, to obtain eight semi-pure compounds 9c1–8. Fraction 9c1 was further separated on the same HPLC column (YMC-Pack C8, 250×20 mm, 5 μm, DAD at 238 nm, flow rate 5 mL/min) eluted with 43:57 acetonitrile/0.1% TFA in H_2O to obtain a single pure compound (t_R 16.1 min, 6.1 mg, 0.017% yield based on the dry weight of the bacteria), established as the new microcyclamide GL582 (**2**). Fraction 9c2 was further separated on the same HPLC column (YMC-Pack C8, 250×20 mm, 5 μm, DAD at 238 nm, flow rate 5 mL/min) eluted with 40:60 acetonitrile/0.1% TFA in H_2O to obtain a single pure compound (t_R 16.6 min, 2.0 mg, 0.006% yield based on the dry weight of the bacteria), which was established as the known microcyclamide A (**9**).[17] Fraction 9c3 was further separated on the same HPLC column eluted with 35:65 acetonitrile/0.1% TFA in H_2O to obtain a single pure compound (t_R 54.2 min, 4.1 mg, 0.012% yield based on the dry weight of the bacteria), it's structure was established as the new microcyclamide GL614A (**3**). Fraction 9c4 was further separated on the same HPLC column eluted with 50:50 acetonitrile/0.1% TFA in H_2O to obtain a single pure compound (t_R 19.4 min, 2.7 mg, 0.008% yield based on the dry weight of the bacteria), it's structure was established as the new microcyclamide GL614B (**4**). Fraction 9c5 was further separated on the same HPLC column eluted with 43:57 acetonitrile/0.1% TFA in H_2O to obtain a single pure compound (t_R 26.7 min, 7.1 mg, 0.02% yield based on the dry weight of the bacteria), established as the new microcyclamide GL546A (**5**). Fraction 9c6 was further separated on the same HPLC column eluted with 43:57 acetonitrile/0.1% TFA in H_2O to obtain a single pure compound (t_R 28.2 min, 0.7 mg, 0.002% yield based on the dry weight of the bacteria), it's structure was established as the new microcyclamide GL546B (**6**). Fraction 9c7 was further separated on the same HPLC column eluted with 43:57 acetonitrile/0.1% TFA in H_2O to obtain a single pure compound (t_R 18.9 min, 2.0 mg, 0.006% yield based on the dry weight of the bacteria), established as the new microcyclamide GL628 (**7**). Fraction 9c8 was further separated on the same HPLC eluted with 32:68 acetonitrile/0.1% TFA in H_2O to obtain a single pure compound (t_R 19.4 min, 2.4 mg, 0.007% yield based on the dry weight of the bacteria), it's structure was established as the new microcyclamide GL614C (**8**).

*3.3.1. Microcyclamide GL616 (**1**).* Yellowish glassy material. $[\alpha]_D^{20}$ +6.0 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.10), 280 (3.40) nm; for NMR data see Table 1; HREIMS *m*/*z* 616.2678 (M^+, calcd for $C_{29}H_{40}N_6O_7S$, 616.2679).

*3.3.2. Microcyclamide GL582 (**2**).* White glassy material. $[\alpha]_D^{20}$ −104 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.40) nm; for NMR data see Table 2; HREIMS *m*/*z* 582.1830 (M^+, calcd for $C_{26}H_{30}N_8O_4S_2$, 582.1831).

*3.3.3. Microcyclamide GL614A (**3**).* White glassy material. $[\alpha]_D^{20}$ −168 (*c* 0.008, MeOH); UV (MeOH) λ_{max} (log ε) 222 (4.26) nm; for NMR data see Table 3; HRCIMS *m*/*z* 615.1818 (MH^+, calcd for $C_{26}H_{31}N_8O_6S_2$, 615.1802).

*3.3.4. Microcyclamide GL614B (**4**).* Colorless glassy material. $[\alpha]_D^{20}$ −184 (*c* 0.005, MeOH); UV (MeOH) λ_{max} (log ε) 223 (3.96) nm; for

NMR data see Table 3; HRCIMS m/z 615.1825 (MH^+, calcd for $C_{26}H_{31}N_8O_6S_2$, 615.1802).

*3.3.5. Microcyclamide GL546A (**5**).* Colorless glassy material. $[\alpha]_D^{20}$ −70 (c 0.057, MeOH); UV (MeOH) λ_{max} (log ε) 222 (4.26) nm; for NMR data see Table 4; HRCIMS m/z 547.1437 (MH^+, calcd for $C_{23}H_{27}N_6O_6S_2$, 547.1428).

*3.3.6. Microcyclamide GL546B (**6**).* Colorless glassy material. $[\alpha]_D^{20}$ −46 (c 0.013, MeOH); UV (MeOH) λ_{max} (log ε) 223 (3.96) nm; for NMR data see Table 4; HRCIMS m/z 547.1430 (MH^+, calcd for $C_{23}H_{27}N_6O_6S_2$, 547.1428); HRESIMS m/z 569.1254 (MNa^+, calcd for $C_{23}H_{26}N_6NaO_6S_2$, 569.1253).

3.3.7. Microcyclamide GL628 (7). Colorless glassy material. $[\alpha]_D^{20}$ −180 (c 0.006, MeOH); UV (MeOH) λ_{max} (log ε) 224 (4.10) nm; for NMR data see Table 5; HRCIMS m/z 629.1958 (MH^+, calcd for $C_{27}H_{33}N_8O_6S_2$, 629.1959).

*3.3.8. Microcyclamide GL614C (**8**).* Colorless glassy material. $[\alpha]_D^{20}$ −61 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.26) nm; for NMR data see Table 5; HRESIMS m/z 637.1599 (MNa^+, calcd for $C_{26}H_{30}N_8NaO_6S_2$, 637.1627).

*3.3.9. Microcyclamide A (**9**).* Colorless glassy material. $[\alpha]_D^{20}$ −184 (c 0.005, MeOH); UV (MeOH) λ_{max} (log ε) 223 (3.96) nm; for NMR data see Table 2; EIMS m/z 582.1 (MH^+).

3.4. Determination of the absolute configuration of the amino acids

Compounds **1**–**8** (0.3 mg portions) were dissolved in dichloromethane (10 mL) and cooled to −70 °C. Ozone was bubbled through the cold sample for 30 s and the reaction mixture was heat to rt. The solvent was removed with air stream and the residue was dissolved in 6 M HCl (1 mL). The reaction mixture was then placed in a sealed glass bomb at 110 °C for 20 h. After removal of HCl, by repeated evaporation in vacuo, the hydrolyzate was resuspended in water (40 μL). A solution of (1-fluoro-2,4-dinitrophenyl)-5-L-alanine amide (FDAA) (4.2 mMol) in acetone (150 mL) and 1 N $NaHCO_3$ (20 mL) were added to each reaction vessel and the reaction mixture was stirred at 40 °C for 2 h. A 2 N HCl solution (10 mL) was added to each reaction vessel and the solution was 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 on LichroCART 250×4.6 mm Purospher STAR, 5 μm column at a flow rate of 1 mL/min and UV detection at 340 nm. For **1**, a linear gradient elution from 9:1 50 mM triethylammonium phosphate (TEAP) buffer (pH 3)/acetonitrile to 1:1 TEAP/acetonitrile within 60 or 120 min was used. For compounds **2**–**8**, a 60 min gradient from 0.1% TFA in water to 6:4 acetonitrile/0.1% TFA in water was used. HPLC analysis of Marfey's derivatives of **1** established the following retention times of the derivatized amino acids: L-Ala, 34.1 min; D-Thr, 26.9 min using 60 min gradient, and L-Phe, 90.1 min; L-Ile, 89.4 min; using 120 min gradient. HPLC analysis of Marfey's derivatives of compounds **2**–**8** established the following retention times of the derivatized amino acids: L-Ala, 40.3 and L-Ile, 50.6 min (+/−0.2 min) for all the compounds, D-Asp, 36.6 min for microcyclamide GL582 (**2**), L-Asp, 39.3 min for microcyclamide GL614A (**3**), D-Asp, 36.1 min microcyclamide GL614B (**4**), D-Asp, 36.2 min for microcyclamide GL546A (**5**), L-Asp, 39.8 min for microcyclamide GL546B (**6**), L-Asp, 40.2 min for microcyclamide GL628 (**7**), and L-Asp, 40.4 min for microcyclamide GL614C (**8**).

Acknowledgements

We thank S. Hauftmann and N. Tal for acquisition of the mass spectral data. This study was supported by the Israeli Science Foundation grant 776/06 to S.C. and E.F.

Supplementary data

1D and 2D NMR spectra of **1**–**8** are available. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2010.02.008.

References and notes

1. Yoshizawa, S.; Matsushima, R.; Watenabe, M. F.; Hard, K.; Ichihara, A.; Carmichael, W. W.; Fujiki, H. *J. Cancer Res.Clin. Oncol.* **1990**, *116*, 609–614.
2. Ito, E.; Takai, A.; Kondo, F.; Masui, H.; Imanishi, S.; Harada, K. *Toxicon* **2002**, *40*, 1017–1025.
3. Sano, T.; Kaya, K. *J. Nat. Prod.* **1996**, *59*, 90–92.
4. Harada, K.; Fujiki, H.; Shimada, T.; Sozuki, M.; Sano, H.; Adachi, K. *Tetrahedron Lett.* **1995**, *36*, 1511–1514.
5. Ishida, K.; Okita, Y.; Matsuda, H.; Okino, T.; Murakami, M. *Tetrahedron* **1999**, *55*, 10971–10988.
6. Okino, T.; Matsuda, H.; Murakami, M.; Yamaguchi, K. *Tetrahedron Lett.* **1993**, *34*, 501–504.
7. Reshef, V.; Carmeli, S. *Tetrahedron* **2006**, *62*, 7361–7369.
8. Ishida, K.; Matsuda, H.; Okita, Y.; Murakami, M. *Tetrahedron* **2002**, *58*, 7645–7652; Gesner-Apter, S.; Carmeli, S. *Tetrahedron* **2008**, *64*, 6628–6634.
9. Ishida, K.; Matsuda, H.; Murakami, M.; Yamaguchi, K. *J. Nat. Prod.* **1997**, *60*, 724–726.
10. Fujii, K.; Sivonen, K.; Kashiwagi, T.; Hirayama, K.; Harada, K. *J. Org. Chem.* **1999**, *64*, 5777–5782.
11. Golakoti, T.; Yoshida, W. Y.; Chaganty, S.; Moore, R. E. *Tetrahedron* **2000**, *56*, 9093–9102.
12. Kobayashi, A.; Kajiyama, S.-i.; Inawaka, K.; Kanzaki, H.; Kawazu, K. *Z. Naturforsch., C: Biosci.* **1994**, *49*, 464–470; Ploutno, A.; Carmeli, S. *J. Nat. Prod.* **2001**, *64*, 544–545.
13. Ojika, M.; Nemoto, T.; Nakamura, M.; Yamada, K. *Tetrahedron Lett.* **1995**, *36*, 5057–5058.
14. Hambley, T. W.; Hawkins, C. J.; Lavin, M. F.; Van Der Brenk, A.; Watters, D. J. *Tetrahedron* **1992**, *48*, 341–348; Degan, B. M.; Hawkins, C. J.; Lavin, M. F.; McCaffrey, E. J.; Parry, D. L.; Watters, D. J. *J. Med. Chem.* **1989**, *32*, 1354–1359; Foster, M. P.; Concepcion, G. P.; Caraan, G. B.; Ireland, C. M. *J. Org. Chem.* **1992**, *57*, 6671–6675.
15. Linington, R. G.; Gonzalez, J.; Urena, L.-D.; Romero, L. I.; Ortega-Barria, E.; Gerwick, W. H. *J. Nat. Prod.* **2007**, *70*, 397–401.
16. Admi, V.; Afek, U.; Carmeli, S. *J. Nat. Prod.* **1996**, *59*, 396–399; Banker, R.; Carmeli, S. *J. Nat. Prod.* **1999**, *61*, 1248–1251.
17. Ishida, K.; Nakagawa, H.; Murakami, M. *J. Nat. Prod.* **2000**, *63*, 1315–1317.
18. Portmann, C.; Blom, J. F.; Gademann, K.; Juttner, F. *J. Nat. Prod.* **2008**, *71*, 1193–1196; Portmann, C.; Blom, J. F.; Kaiser, M.; Brun, R.; Juttner, F.; Gademann, K. *J. Nat. Prod.* **2008**, *71*, 1891–1896.
19. Ploutno, A.; Carmeli, S. *Tetrahedron* **2002**, *58*, 9949–9957.
20. Ziemert, N.; Ishida, K.; Quillardet, P.; Bouchier, C.; Hertweck, C.; Tandeau de Marsac, N.; Dittmann, E. *Appl. Environ. Microbiol.* **2008**, *74*, 1791–1797.
21. Welker, M.; Brunke, M.; Preussel, K.; Lippert, I.; von Dohren, H. *Microbiology* **2004**, *150*, 1785–1796.
22. Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596.
23. Hara, H. *Nippon Nogeikagaku Kaishi* **1960**, *34*, 493.
24. Makino, H.; Inada, Y. *Biochim. Biophys. Acta* **1973**, *295*, 543–548.
25. Chang, S. H.; Teshima, G. M.; Milby, T.; Gillece-Castro, B.; Canova-Davis, E. *Anal. Biochem.* **1997**, *244*, 221–227.
26. Zaho, F.; Ghezzo-Schoneich, E.; Aced, G. I.; Hong, J.; Millby, T.; Schoneich, C. *J. Biol. Chem.* **1997**, *272*, 9019–9029.
27. Tomita, M.; Irie, M.; Ukita, T. *Biochemistry* **1969**, *8*, 5149–5160.
28. Roa, R.; O'shea, K. E. *Tetrahedron* **2006**, *62*, 10700–10708.
29. Rotem, R.; Heyfets, A.; Fingrut, O.; Blickstein, D.; Shaklai, M.; Flescher, E. *Cancer Res.* **2005**, *65*, 1984–1993.