Tetrahedron Vol. 47, No. 12/13, pp. 2087–2096, 1991
Printed in Great Britain

0040-4020/91 $3.00+.00

Mirabimides A-D, New N-Acylpyrrolinones from the Blue-Green Alga *Scytonema mirabile*

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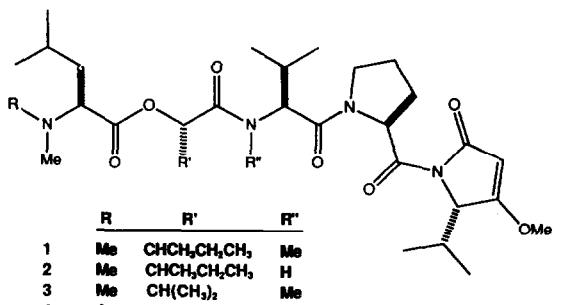
(*Received in USA* 17 *December* 1990)

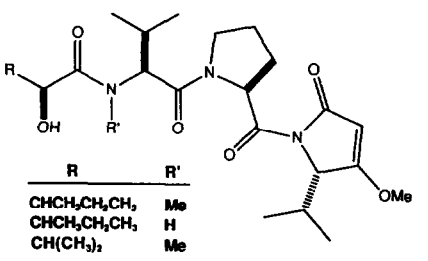
Abstract. Mirabimide A, the major imide from the terrestrial blue-green alga *Scytonema mirabile* (Dillwyn) Bornet (strain BY-8-1), is N-[2-(N,N-dimethylleucyl)oxy-3-methylpentanoyl-N-methylvalyl-prolyl]-4-methoxy-5-isopropyl-3-pyrrolin-2-one. Mirabimides B-D are the valyl, 2-oxy-3-methylbutanoyl, and N-acetyl-N-methylleucyl analogs, respectively. The absolute configurations of the five asymmetric carbons in the mirabimides are proposed to be all *S*, but the assignments for the dimethylleucine units in A-C and the hydroxyacid units in B-D are tentative.

Naturally-occurring N-acylpyrrolinones are associated with sponges of the genus *Dysidea*,[2] the marine blue-green alga *Lyngbya majuscula*,[3] and gliding bacteria.[4] We report here the isolation and structure determination of four new cytotoxic[5] N-acylpyrrolinones, mirabimides A-D (**1** - **4**), from the terrestrial blue-green alga *Scytonema mirabile* (Dillwyn) Bornet (strain BY-8-1).

Isolation and structure elucidation. Isolation of the mirabimides was monitored by NMR analysis and biological activity.[5] The freeze-dried alga was extracted with 70% ethanol and the crude extract was subjected to reversed-phase flash chromatography. Fractions were collected by elution with 2:8, 1:1, 7:3 and 1:9 water/methanol mixtures followed by methanol, acetonitrile and ethyl acetate. The 1:9 water/methanol fraction, which contained the mirabimides along with tantazoles,[6] mirabilene isonitriles[7] and polymethoxy-1-alkenes,[8] was further purified by reversed-phase HPLC on low carbon C-18 silica. Using 85:15 methanol/0.1N aqueous ammonium acetate buffer as the eluant, three mirabimide-containing fractions could be found in the HPLC chromatogram. The most polar one (fraction A) contained a mixture of hydrolyzed mirabimides (**5** - **7**). The remaining two fractions, which were the least polar ones in the chromatogram, contained



	R	R'	R''
1	Me	$CHCH_3CH_2CH_3$	Me
2	Me	$CHCH_3CH_2CH_3$	H
3	Me	$CH(CH_3)_2$	Me
4	Ac	$CHCH_3CH_2CH_3$	Me



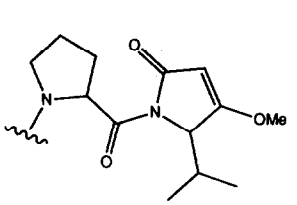
	R	R'
5	$CHCH_3CH_2CH_3$	Me
6	$CHCH_3CH_2CH_3$	H
7	$CH(CH_3)_2$	Me

mirabimides A (**1**), B (**2**) and C (**3**) in fraction P and mirabimide D (**4**) in fraction Q. When the elution was carried out in the absence of buffer, mirabimides could not be found in any of the fractions; however, on the following day, a mixture of **5** - **7** could be eluted from the silica column. Final purification of mirabimides A-D and the hydrolysis products **5** - **7** was achieved by semipreparative reversed-phase HPLC.

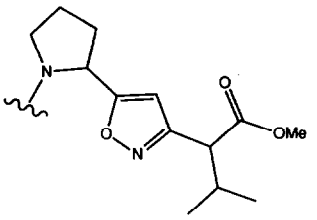
The molecular weights and compositions of all of the compounds were determined by a combination of nmr analysis and fast-atom-bombardment mass spectrometry. The ultraviolet spectra [λ_{max} 216 nm (ε 17,000) and 240 nm (ε 14,500)] and CD curves ([θ]$_{260}$ = 0; [θ]$_{225}$ = -4500; [θ]$_{212}$ = 4800; [θ]$_{195}$ = 3000) for the four mirabimides were similar. Two-dimensional NMR techniques, viz. inverse detected heteronuclear correlation spectroscopy (HMQC[9] and HMBC[10]) coupled with homonuclear COSY and hypercomplex phase sensitive NOESY[11] experiments, were used to determine the structures of the various amino and hydroxy acid units and to sequence these units into a gross structure for the acyclic depsipeptide portion of the molecule; however, it was not possible to use these techniques to unambiguously deduce the structure of the pyrrolinone ring system and attach it to the rest of the molecule. An INADEQUATE experiment with uniformly ^{13}C,^{15}N enriched **5** allowed us to determine the pyrrolinone structure and mass spectrometry showed that it was connected to the pyrrole unit. Finally the stereochemistry was determined by optical analysis of each component of a mixture of amino and hydroxy acids obtained by acid hydrolysis. The absolute configurations of the amino acids, for example, were obtained by appropriate derivatization of the amino acids in the acid hydrolyzate and analysis by chiral GC-MS.

Mirabimide A (1). The ^1H and ^{13}C NMR spectral data (Table 1) coupled with a high resolution FAB mass measurement indicated that **1** had the molecular formula $C_{33}H_{56}O_7N_4$. Chemical shifts and a H-H COSY experiment allowed us to write six partial structures which accounted for all of the protons in the spectrum, i.e. a N,N-dimethylleucyl unit, an O-substituted 2-oxy-3-methylpentanoyl unit, a N-substituted N-methylvalyl unit, a 1,2-disubstituted pyrrole unit, an sp^2 methine connected allylically to an sp^3 methine bearing an isopropyl group, and a methoxy group. Inverse detected H-C correlation experiments (HMQC and HMBC) confirmed these assignments and allowed us to connect the N,N-dimethylleucyl unit through the 2-oxy-3-methylpentanoyl unit to the N-methylvalyl unit (see Table 1 for HMBC correlations). The pyrrolyl unit, however, did not show meaningful correlations with its adjacent groups and the structure of the pyrrolinone unit could not be deduced unambiguously from the correlations between its protons and carbons. Since H-2 of the pyrrolyl unit showed an appreciable NOE to H-3 of the N-methylvalyl unit and H-2 of the N-methylvalyl unit showed NOE correlations to H-5 and H-5' of the pyrrole, the N-methylvalyl carbonyl had to be connected to the pyrrolyl nitrogen. The remaining COSY and HMBC correlations were found to fit two alternative structures, i.e., (1) a prolyl unit connnected to the nitrogen of a 5-isopropyl-4-methoxy-3-pyrrolin-2-one (**a**) or C-2 of a pyrrolyl unit connected to C-5 (or C-3) of an isoxazole ring with a methine bearing an isopropyl group and a carbomethoxy group connected to C-3 (or C-5) of the same isoxazole ring (**b**). The detection of proline by amino acid analysis and the isolation of 5-isopropyl-4-methoxy-3-pyrrolin-2-one (**8**) on mild hydrolysis indicated that structure **a** was present.

Further proof of structure was obtained as follows: Extract of alga cultured on ^{13}C sodium bicarbonate and ^{15}N sodium nitrate was fractionated to give labeled **5** (23% ^{13}C and 90% ^{15}N), using conditions where the mirabimide A hydrolyzed during the silica gel chromatography step. An INADEQUATE spectrum confirmed the



a



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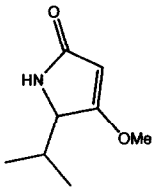

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structures of the four acid units and also unambiguously established the structure of the pyrrolinone unit. The following carbons showed coupling to nitrogen: pyrrolinone C-2 and C-5; proline CO, C-2 and C-5; valine C-1, C-2 and N-methyl; 2-oxypentanoyl C-1; leucine C-2 and N-dimethyl carbons. The arguments described above sequence the prolyl, N-methylvalyl, 2-oxy-3-methylpentanoyl and N,N-dimethylleucyl units, but failed to connect the above unit to the pyrrolinone unit. FAB mass spectrum of mirabimide A showed the parent ion cluster at m/z 621 and strong fragment ions at m/z 369 and 253. These two ions represent fragmentation of the bond between the proline nitrogen and valine carbonyl and thus confirm the connection between the pyrrolinone and proline units.

Mirabimide B (2). The FAB mass spectrum (MH^+ at m/z 607) indicated that **2** differed from **1** by 14 mass units. The ^1H and ^{13}C NMR spectra of **2** and **1** showed only slight differences. An N-methyl signal was missing from both the ^1H and ^{13}C NMR spectra of **2**. A substantial NH proton signal was present at 7.04 ppm for **2** and small chemical shift differences were observed for the valyl and 2-oxy-3-methylpentanoyl protons. Compound **6** was shown to be the hydrolysis product of **2** by NMR and MS analysis.

Mirabimide C (3). Mirabimide C (**3**) showed one less methylene signal in both its ^1H and ^{13}C NMR spectra. In the C-methyl region of the ^1H NMR spectrum there was one more doublet and one less triplet. COSY, HMQC and HMBC experiments indicated that these differences were due to the substitution of the isoleucic acid residue by an α-hydroxyisovaleric acid unit.[12] The FAB mass spectrum confirmed this structure with the MH^+ ion at m/z 607 and fragment ions at m/z 355 and 253 resulting from cleavage of the Mva-Pro bond. NMR and MS analysis indicated that compound **7** was the hydrolysis product of mirabimide C.

Mirabimide D (4). The ^1H NMR spectrum of mirabimide D (**4**) displayed appreciable changes in the chemical shifts and intensities of several signals relative to those of **1**. The leucine N-methyl signal had shifted upfield and integration indicated three protons rather than six. Another methyl signal had appeared at 2.05 ppm and the leucine H-2 had shifted 2 ppm downfield to 5.28 ppm. These differences could be explained by substitution of one of the two leucine N-methyl groups in mirabimide A by an acetyl group. The ^{13}C NMR spectrum of **4** revealed two new signals at 171.17 (s) and 29.80 (q) ppm and some changes in the chemical shifts of the other leucine signals, all in agreement with the structural modification proposed above. The proposed structure was confirmed by HMQC and HMBC correlations, and by the FAB mass spectrum which showed the MH^+ ion at m/z 649 (MK^+ shifted to m/z 687) and fragment ions at m/z 397 and 253 (cleavage of Mva-Pro bond).

Table 1. NMR Data for mirabimide A.[a]

Unit[b]	C No.	δ_C mult.	δ_H mult.	J(Hz)	HMBC Corr.[b]
Pyrrolinone	2	170.59 s			Pyr-3,5
	3	94.89 d	5.23 s		Pyr-5
	4	180.21 s			Pyr-OMe,5,6
	4-OMe	59.00 q	3.93 s		
	5	64.20 d	4.53 d	2.0	Pyr-3,6,7,7'
	6	28.79 d	2.52 dhep	2.0,7.2	Pyr-5,7,7'
	7	18.36 q	1.05 d	7.2	Pyr-5,6,7'
	7'	15.80 q	0.82 d	7.2	Pyr-5,6,7
Pro	CO	172.05 s			Pro-2,3,3'
	2	60.94 d	5.42 dd	8.5,5.5	Pro-3',4,4',5'
	3	29.20 t	2.26 m		Pro-2,4,4',5,5'
			1.77 m		
	4	24.92 t	1.97 m		Pro-2,3,3',5,5'
			1.83 m		
	5	48.13 t	3.74 ddd	-10.5,7.5,6.5	Pro-3,3',4'
			3.63 dt	-10.5,7.0	
Mva	1	168.35 s			Mva-2
	2	59.52 d	5.05 d	11.0	Mva-3,4,4',NMe
	2-NMe	29.89 q	3.05 s		Mva-2
	3	27.40 d	2.22 dhep	11.0,7.1	Mva-2,4,4'
	4	19.32 q	0.91 d	7.1	Mva-2,4'
	4'	18.40 q	0.80 d	7.1	Mva-2,4
Ila	1	170.39 s			Mva-NMe,Ila-2
	2	73.39 d	5.19 d	4.5	Ila-3',4,4'
	3	36.38 d	1.91 dddq	4.5,7.2,7.6,	
				6.9	Ila-2,3',4,4',5
	3'	14.48 q	0.99 d	6.9	Ila-2,4,4'
	4	26.49 t	1.46 ddq	-13.6,7.4,7.2	Ila-2,3',5
			1.27 ddq	-13.6,7.6,7.4	
	5	11.60 q	0.94 t	7.4	Ila-4,4'
Dml	1	172.29 s			Ila-2,Dml-2,3,3'
	2	65.50 d	3.28 t	7.5	Dml-3,3',NMe_2,4
	NMe_2	41.11 q	2.35 s		Dml-2,NMe
	3	39.27 t	1.55 dt	-13.4,7.5	
			1.50 dt	-13.4,7.2	Dml-2,4,5,5'
	4	24.92 d	1.68 ddqq	7.5,7.2,6.8,	
				6.4	Dml-2,3,3',5,5'
	5	22.68 q	0.92 d	6.8	Dml-3,3',4,5'
	5'	22.42 q	0.88 d	6.4	Dml-3,3',4,5

[a]9.2 mg of mirabimide A in 0.5 mL acetone-d_6. [b]Mva = N-methylvaline; Ila = isoleucic acid; Dml = N,N-dimethylleucine.

The ester bond in mirabimides A, B, and C could be readily cleaved under very mild acidic conditions; e.g., these esters were hydrolyzed to **5**, **6** and **7**, respectively, by 1% AcOH in 2:8 water/methanol solution or during chromatography on RP-18 silica that had not been encapped by silylation. In contrast, mirabimide D was completely resistant to hydrolysis under these conditions.

Absolute stereochemistry. Mirabimides A-D exhibited similar CD curves, indicating that the absolute stereochemistry of the pyrrolinone moiety was identical in all four compounds. Attempts to establish its absolute stereochemistry by optical analysis of the 5-isopropyl-4-methoxy-3-pyrrolin-2-one (**8**) obtained by mild basic hydrolysis of **5** failed; lack of an α_D and CD curve indicated that **8** had completely racemized during the hydrolysis. The absolute stereochemistry of the pyrrolinone, proline and N-methylvaline units in mirabimide A were determined as follows: Permanganate oxidation of **5** followed by acid hydrolysis, Fisher esterification of the resulting acid hydrolyzate with methanol, and acylation with trifluoroacetic anhydride led to L-valine, L-proline, and L-N-methylvaline, as determined by gas chromatography on a chiral column. This meant that Pyr C-5, Pro C-2 and Mva C-2 in **5** as well as **1** are all ***S***. In another experiment **5** was hydrolyzed to L-isoleucic acid (L-N-methylvaline and L-proline also obtained) which indicated Ila C-2 to be ***S*** in both **5** and **1**. The absolute stereochemistry of the N,N-dimethylleucine unit was not determined, but is probably L (see below).

Compounds **6** and **7** and mirabimide D (**4**) were hydrolyzed, esterified, and N-acylated with trifluoroacetic anhydride to give amino acids derivatives which were analyzed by gas chromatography on a chiral column. Compound **6** gave L-valine and L-proline, compound **7** gave L-N-methylvaline and L-proline, and **4** gave L-N-methylvaline, L-N-methylleucine and L-proline.

These results suggested that C-2 in all of the amino acid residues and C-5 in the pyrrolinone units of mirabimides A-D were all ***S***. The isolation of L-N-methylleucine from **4** implied that the N,N-dimethylleucyl unit in mirabimides A, B and C was L. The isolation of L-isoleucic acid from **5** suggested that the isoleucic acid units in **2** and **4**, as well as the α-hydroxyisovaleric acid unit in **3**, were also L.

Experimental Section

Spectral Analysis. NMR spectra were determined in acetone-d_6, unless otherwise noted, on a spectrometer operating at 500 MHz for proton and 125 MHz for carbon-13. Proton chemical shifts are referenced to the residual acetone signal (2.04 ppm) and carbon-13 chemical shifts are referenced to the solvent signal (206.0 ppm). Homonuclear ^{1}H connectivities were determined by using the COSY experiment. Homonuclear ^{1}H NOEs were obtained by Hypercomplex Phase Sensitive NOESY experiments using a 3s recycling delay and 500 ms mixing period. Heteronuclear ^{1}H-^{13}C connectivities were determined by HMQC and HMBC experiments. UV and CD spectra were recorded in MeOH at 25°C. Mass spectra, including high resolution mass measurements, were determined in FAB mode.

Culture Conditions. *Scytonema mirabile* (Dillwyn) Bornet, designated strain number BY-8-1, was cultured as previously described.[8]

Isolation. Freeze-dried alga (82 g) was extracted with 3 x 3L portions of 7:3 EtOH/water solution (24 h for each extraction). The total extract (38.4 g in five portions) was flash chromatographed on a RP-18 column (30 mL,

YMC-GEL, ODS 120A). The chromatogram was developed with 100 mL of each of the following solvents: 2:8, 1:1, 1:3 and 1:9 H_2O/MeOH mixtures, MeOH, MeCN and ethyl acetate. Seven fractions (100 mL) were collected.

Fraction 4 from the RP-18 column was applied to a preparative HPLC column (Alltech Econosphere RP-18 10μ 22 X 250 mm) using 85:15 MeOH/0.1N aq. ammonium acetate buffer as the eluant (5 mL/min). The separation was monitored by UV at 254 nm and fourteen fractions were collected. Three fractions contained mirabimides, viz. fraction A (t_R 26.5 min, 30 mg), fraction P (t_R 62.5 min, 35 mg) and fraction Q (t_R 64.0 min, 9.0 mg). Further purification of these fractions on a semipreparative HPLC column (YMC AM-324, ODS, 120A, 10 X 300 mm) using 85:15 MeOH/water as the eluant (2 mL/min) afforded mirabimide D (**4**, 3.2 mg, t_R 17.5 min) from fraction Q and mirabimides A (**1**, 16.6 mg, t_R 15.0 min), B (**2**, 1.5 mg, t_R 14.0 min) and C (**3**, 2.1 mg, t_R 13.0 min) from fraction P. HPLC of fraction A on the same semipreparative column using 3:1 MeOH/water (2 mL/min) as the eluant gave the hydrolysis products **5** (22.5 mg, 16 min), **6** (0.4 mg, 20.5 min) and **7** (0.5 mg, 13 min).

Mirabimide A (1): FAB MS (glycerol) *m/z* 659 $[MK]^+$, 643 $[MNa]^+$, 621$[MH]^+$, 369 [Dml-Ila-Mva]$^+$, 253 [Pro-Pyr + 2H]$^+$ and 210 [Mva-Pro]$^+$; high resolution FAB MS 621.4219 $(MH)^+$, calcd for $C_{33}H_{57}O_7N_4$, mmu error 0.8. UV (EtOH) 216 nm (ε 17,000), 240 (14,500); CD (EtOH) $[\theta]_{260}$ 0, $[\theta]_{225}$ - 4500, $[\theta]_{212}$ 4800; for ^1H and ^{13}C NMR data see Table I.

Mirabimide B (2). FAB MS (glycerol) m/z 645 $[MK]^+$, 629 $[MNa]^+$, 607 $[MH]^+$; UV (EtOH) 213 nm (ε 16,900), 237 (14,600); CD (EtOH) $[\theta]_{260}$ 0, $[\theta]_{225}$ - 4500, $[\theta]_{212}$ 4800. ^1H NMR data δ (multiplicity, J in Hz, assignment): **pyrrolinone** - 5.23 (s, H-3), 3.94 (s, OMe on C-4), 4.52 (d, J = 3.4 Hz, H-5), 2.50 (heptet of doublets, J = 7.3 and 3.4 Hz, H-6), 1.05 (d, J = 7.3 Hz, H_3-7), 0.81 (d, J = 7.3 Hz, H_3-7'); **proline** - 5.46 (dd, J = 6.0 and 8.7 Hz, H-2), 2.32 (m, H-3), 1.78 (dddd, J = - 12.9, 6.9, 6.0, and 5.1 Hz, H-3'), 2.00 & 1.91 (m, H-4 and H-4'), 3.81 (dt, J = - 9.4 and 6.9 Hz, H-5), 3.68 (dt, J = - 9.4 and 6.9 Hz, H-5'); **valine** - 4.59 (dd, J = 8.6 and 6.0 Hz, H-2), 7.04 (brd, J = 8.6 Hz, NH on C-2), 2.12 (dqq, J = 6.0, 6.3 and 6.9 Hz, H-3), 0.99 (d, J = 6.3 Hz, H_3-4), 0.89 (d, J = 6.9 Hz, H_3-4'); **isoleucic acid** - 5.13 (d, J = 3.3 Hz, H-2), 2.00 (dddq, J = 3.3, 7.2, 7.4 and 6.9 Hz, H-3), 0.95 (d, J = 6.9 Hz, 3-Me), 1.42 (ddq, J = -13.6, 7.2 and 6.9 Hz, H-4), 1.27 (ddq, J = -13.6, 7.4 and 6.9 Hz, H-4'), 0.91 (t, J = 6.9 Hz, H_3-5); **N,N-dimethylleucine** - 3.36 (t, J = 7.7 Hz, H-2), 2.35 (s, NMe_2), 1.57 (dd, J = 7.7 and 6.9 Hz, H_2-3), 1.70 (tqq, J = 6.9, 6.8 and 6.0 Hz, H-4), 0.92 (d, J = 6.0 Hz, H_3-5), 0.88 (d, J = 6.8 Hz, H_3-5'). ^{13}C NMR δ (multiplicity, carbon position, proton HMBC correlations): **pyrrolinone** - 170.69 (s, C-2, Pyr 3), 95.20 (d, C-3, none), 180.41 (s, C-4, Pyr 4-OMe,5,6), 59.30 (q, 4-OMe, none), 64.44 (d, C-5, Pyr 3,7,7'), 29.05 (d, C-6, Pyr 5,7,7'), 18.68 (q, C-7, Pyr 5,7'), 16.11 (q, C-7', Pyr 5,7); **proline** - 172.35 (s, CO, Pro 2,3,3'), 60.94 (d, C-2, Pro-3,3'), 29.50 (t, C-3, Pro 2,4,4',5,5'), 25.36 (t, C-4, Pro 2,3,3',5,5'), 48.34 (t, C-5, Pro 3,3'); **valine** - 169.85 (s, C-1, Val 2), 55.67 (d, C-2, Val 3,4,4'), 31.81 (d, C-3, Val 2,4,4'), 20.00 (q, C-4, Val 2,3,4'), 17.73 (q, C-4', Val 2,3,4); **isoleucic acid** - 169.49 (s, C-1, Ila 2), 76.57 (d, C-2, Ila 3-Me,4, 4'), 37.82 (d, C-3, Ila 2,3-Me,4,4',5), 14.79 (q, 3-Me, Ila 2,4,4'), 26.84 (t, C-4, Ila 2,3-Me,5), 11.94 (q, C-5, Ila 4,4'); **N,N-dimethylleucine** - 172.39 (s, C-1, Ila 2 and Dml 2,3,3'), 65.81 (d, C-2, Dml 3,3',NMe_2,4), 41.48 (q, NMe_2, Dml 2,NMe), 39.29 (t, C-3, Dml 2,5,5'), 25.36 (d, C-4, Dml 2,3,3',5,5'), 23.15 (q, C-5, Dml 3,3',4,5'), 22.39 (q, C-5', Dml 3,3',4,5).

Mirabimide C (3). FAB MS (glycerol) m/z 645 $[MK]^+$, 629 $[MNa]^+$, 607 $[MH]^+$, 355 [Dml-Hib-Mva]$^+$, 253; UV (EtOH) 215 nm (ε 16,900), 237.2 (14,700); CD (EtOH) $[\theta]_{260}$ 0, $[\theta]_{225}$ - 4500, $[\theta]_{212}$ 4800. ^1H NMR data δ (multiplicity, J in Hz, assignment): **pyrrolinone** - 5.23 (s, H-3), 3.93 (s, OMe on C-4), 4.53 (d, J = 2.6 Hz, H-5),

2.52 (heptet of doublets, J = 7.3 and 2.6 Hz, H-6), 1.05 (d, J = 7.3 Hz, H_3-7), 0.83 (d, J = 7.3 Hz, H_3-7'); **proline** - 5.42 (dd, J = 5.1 and 8.6 Hz, H-2), 2.28 (m, H-3), 1.77 (m, H-3'), 1.98 & 1.84 (m, H-4 and H-4'), 3.79 (ddd, J = - 10.1, 6.9 and 4.3 Hz, H-5), 3.63 (ddd, J = - 10.1, 10.4 and 6.9 Hz, H-5'); **N-methylvaline** - 5.08 (d, J = 9.5 Hz, H-2), 3.08 (s, NMe), 2.24 (dqq, J = 9.5 and 6.9 Hz, H-3), 0.91 (d, J = 6.9 Hz, H_3-4), 0.81 (d, J = 6.9 Hz, H_3-4'); **2-hydroxyisovaleric acid (Hiv)** - 5.075 (d, J = 6.8 Hz, H-2), 2.16 (octet, J = 6.8 Hz, H-3), 0.99 (d, J = 6.8 Hz, H_3-4), 1.01 (d, J = 6.8 Hz, H_3-4'); **N,N-dimethylleucine** - 3.28 (t, J = 7.7 Hz, H-2), 2.38 (s, NMe_2), 1.55 (ddd, J = 6.8, 7.7, and -13.7, H-3), 1.50 (ddd, J = 6.9, 7.7, and -13.7 Hz, H-3'), 1.67 (nonet, J = 6.8 Hz, H_2-4), 0.92 (d, J = 6.8 Hz, H_3-5), 0.88 (d, J = 6.8 Hz, H_3-5'). ^{13}C NMR δ (multiplicity, carbon position, proton HMBC correlations): **pyrrolinone** - 170.73 (s, C-2, Pyr 3,5), 95.07 (d, C-3, none), 180.40 (s, C-4, Pyr 4-OMe,5,6), 59.20 (q, 4-OMe, none), 64.41 (d, C-5, Pyr 3,6,7,7'), 28.63 (d, C-6, Pyr 5,7,7'), 18.59 (q, C-7, Pyr 5,6,7'), 15.96 (q, C-7', Pyr 5,6,7); **proline** - 172.25 (s, CO, Pro 2,3,3'), 60.76 (d, C-2, Pro-3',4,4',5), 28.99 (t, C-3, Pro 2,4,4',5,5'), 25.11 (t, C-4, Pro 2,3,3',5,5'), 48.40 (t, C-5, Pro 3,3',4,4'); **N-methylvaline** - 168.63 (s, C-1, Mva 2), 29.40 (q, NMe, Mva 2), 59.72 (d, C-2, Mva 3,4,4'), 27.67 (d, C-3, Mva 2,4,4'), 19.48 (q, C-4, Mva 2,3,4'), 18.60 (q, C-4', Mva 2,3); **2-hydroxyisovaleric acid (Hiv)** - 170.65 (s, C-1, Mva NMe and Hib 2), 75.51 (d, C-2, Hib 3',4,4'), 30.53 (d, C-3, Hib 2,4,4'), 18.81 (q, C-4, Hib 2,3,4'), 17.96 (q, C-4', Hib 2,3,4); **N,N-dimethylleucine** - 172.51 (s, C-1, Hib 2 and Dml 2,3,3'), 65.67 (d, C-2, Dml 3,3',NMe_2), 41.32 (q, NMe_2, Dml 2,NMe), 39.47 (t, C-3, Dml 2,5,5'), 25.21 (d, C-4, none), 22.64 (q, C-5, Dml 3,3',4,5'), 22.85 (q, C-5', Dml 3,3',4,5).

Mirabimide D (4). FAB MS (glycerol) m/z 687 $[MK]^+$, 671 $[MNa]^+$, 649 $[MH]^+$, 397 [Aml-Ila-Mva]$^+$, 253, 210; UV (EtOH) 213 nm (ε 17,100), 239.5 (14,400); CD (EtOH) $[\theta]_{260}$ 0, $[\theta]_{225}$ - 4500, $[\theta]_{212}$ 4800; ^{1}H NMR data δ (multiplicity, J in Hz, assignment): **pyrrolinone** - 5.23 (s, H-3), 3.93 (s, OMe on C-4), 4.53 (d, J = 2.8 Hz, H-5), 2.52 (heptet of doublets, J = 7.2 and 2.8 Hz, H-6), 1.05 (d, J = 7.2 Hz, H_3-7), 0.82 (d, J = 7.2 Hz, H_3-7'); **proline** - 5.42 (dd, J = 5.4 and 8.4 Hz, H-2), 2.27 (m, H-3), 1.77 (m, H-3'), 1.98 & 1.83 (m, H-4 and H-4'), 3.75 (ddd, J = - 10.5, 7.5 and 6.5 Hz, H-5), 3.62 (dt, J = - 10.5 and 7.0 Hz, H-5'); **N-methylvaline** - 5.04 (dd, J = 10.9 Hz, H-2), 3.02 (s, NMe on C-2), 2.22 (d septets, J = 10.9 and 6.7 Hz, H-3), 0.91 (d, J = 6.7 Hz, H_3-4), 0.80 (d, J = 6.7 Hz, H_3-4'); **isoleucic acid** - 5.17 (d, J = 4.1 Hz, H-2), 1.91 (dddq, J = 4.1, 7.1, 7.6 and 6.6 Hz, H-3), 0.96 (d, J = 6.6 Hz, 3-Me), 1.45 (ddq, J = - 13.5, 7.3, and 7.1 Hz, H-4), 1.27 (ddq, J = - 13.5, 7.6 and 7.3 Hz, H-4'), 0.94 (t, J = 7.3 Hz, H_3-5); **N-acetyl-N-methylleucine (Aml)** - 5.28 (t, J = 7.5 Hz, H-2), 2.94 (s, NMe), 2.05 (s, NCOMe), 1.74 (dt, J = 7.5 and 7.2 Hz, H_2-3), 1.56 (tqq, J = 7.2,7.1 and 6.6 Hz, H-4), 0.94 (d, J = 7.1 Hz, H_3-5), 0.92 (d, J = 6.6 Hz, H_3-5'). ^{13}C NMR δ (multiplicity, carbon position, proton HMBC correlations): **pyrrolinone** - 170.81 (s, C-2, Pyr 3,5), 95.16 (d, C-3, Pyr 5), 180.47 (s, C-4, Pyr 4-OMe,5,6), 59.24 (q, 4-OMe, none), 64.50 (d, C-5, Pyr 3,6,7,7'), 29.08 (d, C-6, Pyr 5,7,7'), 18.36 (q, C-7, Pyr 5,6,7'), 15.80 (q, C-7', Pyr 5,6,7); **proline** - 172.29 (s, CO, Pro 2,3,3'), 60.68 (d, C-2, Pro-3,3',4,4',5), 29.50 (t, C-3, Pro 2,4,4',5,5'), 25.18 (t, C-4, Pro 2,3,3',5,5'), 48.41 (t, C-5, Pro 2,3,3',4,4'); **N-methylvaline** - 169.57 (s, C-1, Mva 2), 59.90 (d, C-2, Mva 3,4,4',NMe), 30.00 (q, NMe, Mva 2), 27.64 (d, C-3, Mva 2,4,4'), 19.58 (q, C-4, Mva 2,3,4'), 18.61 (q, C-4', Mva 2,3,4); **isoleucic acid** - 170.28 (s, C-1, Mva NMe and Ila 2), 74.51 (d, C-2, Ila 3-Me,4, 4'), 36.54 (d, C-3, Ila 2,3-Me,4,4',5), 14.41 (q, 3-Me, Ila 2,4,4'), 26.85 (t, C-4, Ila 2,3,3-Me,5), 11.86 (q, C-5, Ila 3,4,4'); **N-acetyl-N-methylleucine (Aml)** - 172.21 (s, C-1, Ila 2 and Aml 2,3), 54.73 (d, C-2, Aml 3,3',NMe), 32.50 (q, NMe, Aml 2), 171.17 (s, N-acetyl CO, Aml 2,NMe,N-Ac), 29.80 (q, N-acetyl Me), 38.09 (t, C-3, Aml 2,4,5,5'), 24.92 (d, C-4, Aml 2,3,3',5,5'), 23.43 (q, C-5, Aml 3,3',4,5'), 22.42 (q, C-5', Aml 3,3',4,5).

Hydrolysis Product of Mirabimide A (5). FD MS m/z 479 $[M]^+$; FAB MS (glycerol) m/z (rel intensity) 502 (0.6) $[MNa]^+$, 480 (1.5) $[MH]^+$, 253 (20), 228 (100); high resolution FABMS m/z 480.3065 (calcd for $C_{25}H_{42}N_3O_6$, -0.9 mmu error), 253.1540 ($C_{13}H_{21}N_2O_3$, - 1.2 mmu error), 228.1608 ($C_{12}H_{22}NO_3$, 0.8 mmu error); UV (EtOH) 213 nm (ε 17,100), 239.5 (14,400); CD (EtOH) $[\theta]_{260}$ 0, $[\theta]_{225}$ - 4500, $[\theta]_{212}$ 4800. ^1H NMR data δ (multiplicity, J in Hz, assignment): **pyrrolinone** - 5.23 (s, H-3), 3.93 (s, OMe on C-4), 4.53 (d, J = 2.9 Hz, H-5), 2.52 (heptet of doublets, J = 7.3 and 2.9 Hz, H-6), 1.05 (d, J = 7.3 Hz, H_3-7), 0.82 (d, J = 7.3 Hz, H_3-7'); **proline** - 5.43 (dd, J = 4.9 and 8.3 Hz, H-2), 2.24 (m, H-3), 1.78 (m, H-3'), 1.97 & 1.85 (m, H-4 and H-4'), 3.82 (ddd, J = - 10.6, 7.5 and 6.5 Hz, H-5), 3.65 (dt, J = - 10.6 and 7.0 Hz, H-5'); **N-methylvaline** - 5.10 (d, J = 11.0 Hz, H-2), 2.96 (s, NMe), 2.28 (dqq, J = 11.0 and 6.8 Hz, H-3), 0.95 (d, J = 6.8 Hz, H_3-4), 0.81 (d, J = 6.8 Hz, H_3-4'); **isoleucic acid** - 4.42 (d, J = 2.4 Hz, H-2), 1.69 (hextet of doublets, J = 6.9 and 2.4 Hz, H-3), 0.72 (d, J = 6.9 Hz, 3-Me), 1.42 (dp, J = - 13.5 and 6.9 Hz, H-4), 1.27 (dp, J = - 13.6 and 6.9 Hz, H-4'), 0.94 (t, J = 6.9 Hz, H_3-5). ^{13}C NMR δ (multiplicity, carbon position, proton HMBC correlations): **pyrrolinone** - 170.81 (s, C-2, Pyr 3,5), 95.20 (d, C-3, Pyr 5), 180.51 (s, C-4, Pyr 4-OMe,5,6), 59.32 (q, 4-OMe, none), 64.52 (d, C-5, Pyr 3,6,7,7'), 28.87 (d, C-6, Pyr 5,7,7'), 18.36 (q, C-7, Pyr 5,6,7'), 16.07 (q, C-7', Pyr 5,6,7); **proline** - 172.26 (s, CO, Pyr 5 and Pro 2,3), 60.91 (d, C-2, Pro-3,3',4,4',5), 29.20 (t, C-3, Pro 2,4,4',5,5'), 25.19 (t, C-4, Pro 2,3,3',5,5'), 48.36 (t, C-5, Pro 2,3,3',4,4'); **N-methylvaline** - 168.38 (s, C-1, Mva 2,3), 30.20 (q, NMe, Mva 2), 60.63 (d, C-2, Mva 3,4,4',NMe), 27.45 (d, C-3, Mva 2,4,4'), 19.52 (q, C-4, Mva 2,3,4'), 18.67 (q, C-4', Mva 2,3,4); **isoleucic acid** - 175.70 (s, C-1, Mva NMe,2 and Ila 2), 70.62 (d, C-2, Ila 3-Me,4, 4'), 37.70 (d, C-3, Ila 2,3-Me,4,4',5), 12.93 (q, 3-Me, Ila 2,3,4,4'), 27.52 (t, C-4, Ila 2,3,3-Me,5), 11.95 (q, C-5, Ila 3,4,4').

Hydrolysis Product of Mirabimide B (6). FAB MS (glycerol) m/z 488 $[MNa]^+$, 466 $[MH]^+$. ^1H NMR data δ (multiplicity, J in Hz, assignment): **pyrrolinone** - 5.24 (s, H-3), 3.93 (s, OMe on C-4), 4.53 (d, J = 2 Hz, H-5), 2.51 (heptet of doublets, J = 7 and 2 Hz, H-6), 1.05 (d, J = 7 Hz, H_3-7), 0.82 (d, J = 7 Hz, H_3-7'); **proline** - 5.48 (dd, J = 5.5 and 9 Hz, H-2), 2.25 (m, H-3), 1.8 (m, H-3'), 2.0 & 1.85 (m, H-4 and H-4'), 3.85 (dt, J = -10 and 7 Hz, H-5), 3.65 (dt, J = -10 and 7 Hz, H-5'); **valine** - 4.59 (dd, J = 9 and 6.0 Hz, H-2), 7.40 (brd, J = 9 Hz, NH on C-2), 2.12 (m, H-3), 0.99 (d, J = 7 Hz, H_3-4), 0.89 (d, J = 7 Hz, H_3-4'); **isoleucic acid** - 4.02 (br s, H-2), 1.7 (m, H-3), 0.80 (d, J = 7 Hz, 3-Me), 1.5 (m, H-4), 1.3 (m, H-4'), 0.91 (t, J = 6.9 Hz, H_3-5).

Hydrolysis Product of Mirabimide D (7). FAB MS (glycerol) m/z 488 $[MNa]^+$, 466 $[MH]^+$. ^1H NMR data δ (multiplicity, J in Hz, assignment): **pyrrolinone** - 5.23 (s, H-3), 3.93 (s, OMe on C-4), 4.53 (d, H-5), 2.52 (heptet of doublets, H-6), 1.05 (d, H_3-7), 0.82 (d, H_3-7'); **proline** -5.43 (dd, H-2), 2.25 (m, H-3), 1.80 (m, H-3'), 1.98 & 1.84 (m, H-4 and H-4'), 3.79 (ddd, H-5), 3.63 (ddd, H-5'); **N-methylvaline** - 5.09 (d, H-2), 2.97 (s, NMe), 2.28 (dqq, H-3), 0.95 (d, H_3-4), 0.80 (d, H_3-4'); **2-hydroxyisovaleric acid** - 4.26 (d, J = 3.0 Hz, H-2), 1.95 (heptet of doublets, J = 6.6 Hz, H-3), 0.99 (d, J = 6.6 Hz, H_3-4), 0.75 (d, J = 6.6 Hz, H_3-4').

Conversion of Mirabimide D (4) and Compounds 5, 6 and 7 to Methyltrifluoroacetamidates. Compound **4** (similarly **5**, **6** and **7**) was dissolved in 5.5 M HCl (0.3 mL) and heated to 108 °C for 18 h. The solvent was removed by a nitrogen stream, 2N methanolic HCl (0.3 mL) was added, and the mixture was heated for 1h and evaporated to dryness. The resulting amino acid methyl esters were treated with 0.5 mL of 1:1 trifluoroacetic anhydride/methylene chloride solution at 100 °C for 5 min, the excess reagent was removed with a stream of nitrogen, and the residue was dissolved in dichloromethene (0.5 mL) for GCMS analysis on a 25 m X 0.25 mm Chirasil-Val column (Alltech). Using the following conditions, viz. 12 psi head pressure (flow rate estimated to be about 0.6

mL/sec) and column temperature increased from 60 to 110 °C at 2°/min after injection, t_R for the D(*R*) and L(*S*) N-methyl-N-trifluoroacetyl valine methyl ester are 14:36 and 15:21 min, t_R for the D(*R*) and L(*S*) N-trifluoroacetyl valine methyl ester are 14:58 and 16:42 min, t_R for the D(*R*) and L(*S*) N-methyl-N-trifluoroacetyl leucine methyl ester are 18:40 and 19:17 min, and t_R for the D(*R*) and L(*S*) N-trifluoroacetyl proline methyl ester are 25:02 and 25:55 min, respectively. The retention times for N-methyl-N-trifluoroacetyl valine methyl ester, N-methyl-N-trifluoroacetyl leucine methyl ester and N-trifluoroacetyl proline methyl ester from degradation of **4** were found to be 15:22, 19:20 and 25:54 min, respectively. The retention times for N-methyl-N-trifluoroacetyl valine methyl ester and N-trifluoroacetyl proline methyl ester from degradation of **5** were found to be 15:20 and 25:57 min, respectively. The retention times for N-trifluoroacetyl valine methyl ester and N-trifluoroacetyl proline methyl ester from degradation of **6** were found to be 16:42 and 25:55 min respectively. Finally, the retention times for N-methyl-N-trifluoroacetyl valine methyl ester and N-trifluoroacetyl proline methyl ester from degradation of **7** were found to be 15:21 and 25:50 min, respectively.

In another experiment compound **5** (0.5 mg) in acetone (0.1 mL) was first reacted with excess aqueous $NaIO_4$ solution containing a small amount of $KMnO_4$ for a few hours at room temperature. Methanol was added and the mixture was allowed to stand until the permanganate color had discharged. The solvent was removed with a nitrogen stream and the residual oxidized **5** was converted to methyltrifluoroacetamidates and analyzed by GCMS as described above. The retention times for N-methyl-N-trifluoroacetyl valine methyl ester, N-trifluoroacetyl valine methyl ester and N-trifluoroacetyl proline methyl ester from degradation of oxidized **5** were found to be 15:20, 16:45 and 25:51 min, respectively.

Hydrolysis of Compound 5 to L-Isoleucic Acid. Compound **5** (5 mg) in 5.5 N HCl (1 mL) was refluxed under N_2 for 16 h. The solution was diluted with water and passed through a 2 x 0.9 cm column of C-18. After washing the column thoroughly with water, the 2(*S*)-hydroxy-3(*S*)-methylpentanoic acid (L-isoleucic acid) was eluted from the column with methanol. The methanol was evaporated carefully and the product was dried and purified by shortl-path distillation. The ^{1}H NMR spectrum and positive CD curve were identical with those of authentic 2(*S*)-hydroxy-3(*S*)-methylpentanoic acid (L-isoleucic acid).[13]

Hydrolysis of Compound 5 to 5-Isopropyl-4-methoxy-3-pyrrolin-2-one (8). A solution of **5** (3 mg) in 4:1 MeOH/4N aqueous KOH (5 mL) was allowed to stand at room temperature for 24 h and evaporated. The residue in water was applied to a 2 x 0.9 cm C-18 column and the material (1.5 mg) eluted with 7:3 MeOH/water (5 mL) was subjected to HPLC (YMC AM-324, ODS, 120A, 10 X 300 mm) using 75:25 MeOH/water as the eluant at a flow rate of 2 mL/min. The resulting **8** (0.5 mg, t_R 10.1 min) exhibited the same optical properties (no $[\alpha]_D$ or CD spectrum) and the same ^{1}H NMR spectrum as that reported for **8** from dysidin.[2a] ^{1}H NMR ($CDCl_3$) δ (multiplicity, J in Hz, assignment): 7.15 (br s, NH), 5.03 (d, J = 1 Hz, H-3), 3.80 (s, OMe on C-4), 4.01 (dd, J = 3 and 1 Hz, H-5), 2.10 (heptet of doublets, J = 6.5 and 3 Hz, H-6), 1.03 (d, J = 6.5 Hz, H_3-7), 0.80 (d, J = 6.5 Hz, H_3-7').

Acknowledgments. This research was supported by Grant No. CA12623 from the National Cancer Institute, Department of Health and Human Services. We thank Faith Caplan and Linda K. Larsen for determining the cytotoxicities and Drs. Thomas Corbett and Frederick Valeriote (Wayne State University School of Medicine) for evaluating the compounds for selective cytotoxicity. Initial studies on the isolation and characterization of compound **5** were carried out by Dr. Shanta Banarjee.

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