Isolation and Structure Elucidation of Enniatins L, M_1, M_2, and N: Novel Hydroxy Analogs

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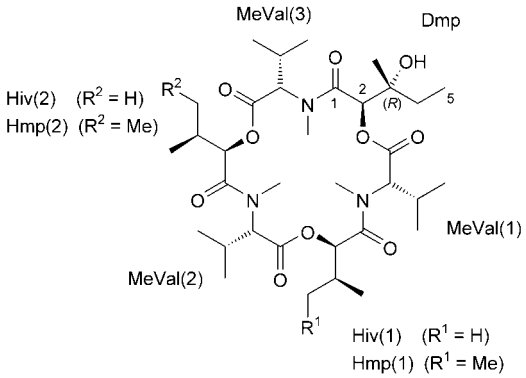
Four new cyclohexadepsipeptides, enniatins L (**1**), M_1 (**2**), M_2 (**3**), and N (**4**), have been isolated from an unidentified fungus (BCC 2629), together with the known enniatins B (**5**), H (**6**), and I (**7**), MK1688 (**8**), and enniatin B_4 (**9**). Compounds **1**–**4** are the first enniatin analogs with an OH group at the side chain of one of the 2-hydroxycarboxylic acid residues. The structures of **1**–**4** were elucidated by spectroscopic means and by X-ray crystallography.

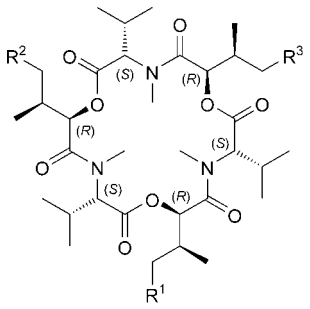
Introduction. – Enniatins are well-known cyclohexadepsipeptide antibiotics produced by various *Fusarium* species [1]. These compounds have been known to exhibit antibiotic [2][3], insecticidal [4][5], and phytotoxic activities [6][7]. They also inhibit acyl-CoA cholesterol acyltransferase (ACAT) [8]. Generally, enniatins consist of three D-configured 2-hydroxyisovaleric acid (Hiv) and three L-configured *N*-methyl amino acid residues linked alternately to furnish an 18-membered macrocycle.

Recently, we reported the isolation of the two new enniatins H and I, which bear one and two 2-hydroxy-3-methylpentanoic acid (Hmp) residues instead of Hiv, respectively, together with the known enniatins B and B_4, and their antimalarial and antituberculous activities were also evaluated [9]. In our further search for enniatin-producing fungi from the Thailand BIOTEC Culture Collection (BCC), we came across the strain BCC 2629, an unidentified fungus, from which four new compounds, enniatins L (**1**), M_1 (**2**), M_2 (**3**), and N (**4**), were isolated, along with the known enniatins B (**5**), H (**6**), I (**7**), MK1688 (**8**), and enniatin B_4 (**9**). The new compounds **1**–**4** are the first representatives of naturally-occurring enniatins possessing an OH group at one of the 2-hydroxy carboxylic acid residue side chains. Herein, we report the isolation, structure elucidation, and biological activities of these new analogs.

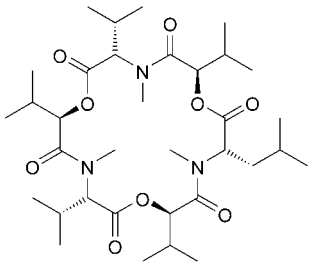
Results and Discussion. – Enniatins **1**–**9** were isolated by chromatographic fractionation of the MeOH extract of mycelia of the BCC-2629 fermentation broth. The four new compounds **1**–**4** and enniatin B_4 (**9**) were obtained as minor components, major metabolites being enniatins B (**5**), H (**6**), I (**7**), and MK 1688 (**8**). Unfortunately, attempted purification of **2** and **3** had met with failure, therefore, they were isolated as an inseparable *ca.* 1:1 mixture. The spectral data of the known enniatins **5**–**9** were identical to those reported in the literature [9–11].



1 $R^1 = R^2 = H$
2 $R^1 = Me$, $R^2 = H$
3 $R^1 = H$, $R^2 = Me$
4 $R^1 = R^2 = Me$



5 $R^1 = R^2 = R^3 = H$
6 $R^1 = Me$, $R^2 = R^3 = H$
7 $R^1 = R^2 = Me$, $R^3 = H$
8 $R^1 = R^2 = R^3 = Me$



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The ^{1}H- and ^{13}C-NMR spectra of enniatin L (**1**) suggested that this compound is an enniatin analog, possessing three *N*-methyl amino acid and three 2-hydroxycarboxylic acid units. The IR spectrum of **1** was very similar to those of the known enniatins **5**–**9**, showing ester (1741 cm^{-1}) and amide (1660 cm^{-1}) resonances, except for an additional OH absorption at 3442 cm^{-1}. High-resolution mass spectral (HR-MS) analysis gave rise to the molecular formula $C_{34}H_{59}N_3O_{10}$, in accord with an additional O-atom. NMR analysis (^{1}H, ^{13}C, DEPT, COSY, HMQC, and HMBC; in $CDCl_3$) revealed that all three *N*-methyl amino acid units were *N*-methylvaline (MeVal), whereas two of the three 2-hydroxycarboxylic acid units were 2-hydroxyisovaleric acid (Hiv). The remaining acid residue was elucidated as 2,3-dihydroxy-3-methylpentanoic acid (Dmp), which, so far, has not been found in previously reported enniatins. The ^{1}H-NMR signal for H–C(2), the α-H-atom, a sharp *singlet* at δ(H) 5.08, indicated that C(3) of Dmp was quaternary. HMBC Correlations of **1** clearly indicated that C(3) at δ(C) 73.6 was connected with a Me, an Et, and an OH group (*Fig. 1*).

The alternating connectivity pattern of the six acid residues was revealed by the analysis of HMBC and NOESY spectral data (*Fig. 1*). Most importantly, intense

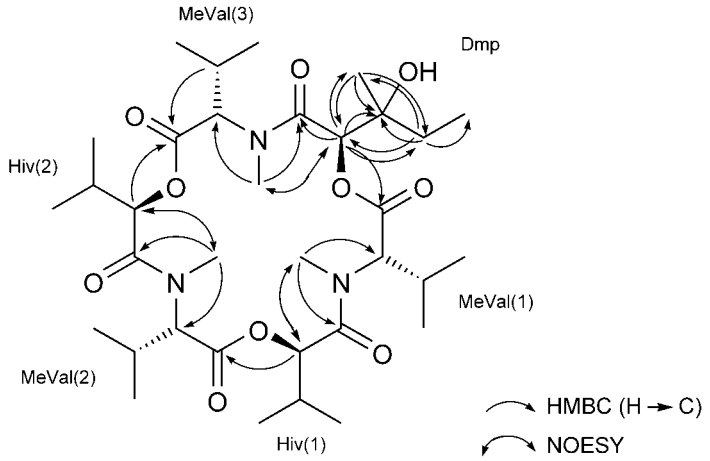

Fig. 1. *Selected HMBC and NOESY correlations for enniatin L* (**1**), *showing the connectivity of the six residues of the macrocycle as well as the partial structure of the Dmp residue*

NOESY correlations were observed for the three N-Me H-atoms at δ(H) 3.26 (MeVal(3)), 2.90 (MeVal(1)), and 3.37 (MeVal(2)) with respect to the three α-H-atoms of the 2-hydroxy acid units at δ(H) 5.08 (Dmp), 5.55 (Hiv(1)), and 5.20 (Hiv(2)). In contrast, the NOESY cross-signals between the N-Me and the α-H resonances of the corresponding MeVal units were absent or very weak relative to the above-mentioned correlations. These data are consistent with the established relative configurations and conformations of known enniatins, such as enniatin B [12], wherein the three N-Me groups are co-planar to the α-H-atoms of the neighboring (2*R*)-Hiv moiety in the macrocyclic ring, while the N-Me groups are antiperiplanar to the corresponding α-H-atoms of (2*S*)-MeVal. We also observed similar NOESY-correlation data for enniatins **5**–**9** in the same solvent ($CDCl_3$). The α-H-atoms and the C-atoms of the six units were clearly distinguished in the ^{1}H- and ^{13}C-NMR spectra (*Table 1*).

Enniatin N (**4**), which had the molecular formula $C_{36}H_{63}N_3O_{10}$ according to HR-MS, showed UV and IR spectra similar to those of **1**. Analysis of 2D-NMR (COSY, NOESY, HMQC, HMBC) spectra revealed that **4** consists of three MeVal, one Dmp, and two 2-hydroxy-3-methylpentanoic acid (Hmp) residues. The structural information derived from HMBC and NOESY data were very similar to those of **1**. Again, NOESY correlations of the three N-Me resonances at δ(H) 3.28 (MeVal(3)), 2.92 (MeVal(1)), and 3.38 (MeVal(2)), respectively, to the three α-H-atoms of the 2-hydroxy acid units at δ(H) 5.11 (Dmp), 5.65 (Hmp(1)), and 5.30 (Hmp(2)) strongly supported the proposed structure.

Attempts to separate compounds **2** and **3** by means of various chromatographic techniques had met with failure. Therefore, spectroscopic analyses were conducted for a *ca.* 1:1 mixture. The IR and UV Spectra of the mixture **2**/**3** were very similar to those of **1** and **4**. The ESI-TOF mass spectrum of the mixture showed two intense signals at *m*/*z* 684 (100%) and 706 (65%), correponding to $[M+H]^+$ and $[M+Na]^+$, respectiverly, which indicated that the two compounds had identical molecular

Table 1. *NMR Data of Enniatins L* (**1**) *and N* (**4**). At 500/125 MHz in $CDCl_3$; δ in ppm, J in Hz. For abbreviations, see text and chemical formulae.

Residue	Group	**1**		**4**	
		^{13}C	^{1}H	^{13}C	^{1}H
DMP	C(1)	168.3	–	168.4	–
	H–C(2)	75.1	5.08 (*s*)	75.0	5.11 (*s*)
	C(3)	73.6	–	73.7	–
	CH_2(4)	34.9	1.59 (*q*, *J* = 7.3)	34.8	1.61 (*q*, *J* = 7.5)
	Me(5)	7.7	0.97 (*t*, *J* = 7.5)	7.9	0.99 (*t*, *J* = 7.4)
	Me(4′)	22.6	1.32 (*s*)	22.6	1.34 (*s*)
	OH	–	4.72 (br. *s*)	–	n.d.[c])
MeVal(1)	C(1)	169.7	–	169.7	–
	H–C(2)	61.2	4.87 (*d*, *J* = 10.5)	61.4	4.88 (*d*, *J* = 10.8)
	H–C(3)	25.7	2.18 (*m*)	25.8	2.20 (*m*)
	Me(4)	19.8	0.99 (*d*, *J* = 6.8)	19.9[a])	1.02 (*d*, *J* = 6.6)
	Me(4′)	18.4	0.83 (*d*, *J* = 6.7)	18.4	0.85 (*d*, *J* = 6.7)
	N–Me	31.8	2.90 (*s*)	31.9	2.92 (*s*)
Hiv(1) or Hmp(1)	C–(1)	168.8	–	168.8	–
	H–C(2)	74.0	5.55 (*d*, *J* = 8.9)	73.1	5.65 (*d*, *J* = 9.1)
	H–C(3)	30.5	2.33 (*m*)	36.8	2.10 (*m*)
	Me(4), CH_2(4)	18.8	0.98 (*d*, *J* = 6.9)	24.4	1.52, 1.17 (2*m*)
	Me(5)	–	–	11.3[b])	0.92 (*t*, *J* = 7.4)
	Me(4′)	18.0	0.93 (*d*, *J* = 6.9)	14.8	0.95 (*d*, *J* = 6.6)
MeVal(2)	C(1)	170.7	–	170.7	–
	H–C(2)	61.0	5.00 (*d*, *J* = 9.8)	61.1	5.03 (*d*, *J* = 9.8)
	H–C(3)	28.6	2.24 (*m*)	28.7	2.26 (*m*)
	Me(4)	20.0	1.12 (*d*, *J* = 6.6)	20.0[a])	1.13 (*d*, *J* = 6.6)
	Me(4′)	19.8	0.92 (*d*, *J* = 6.9)	19.8[a])	0.97 (*d*, *J* = 6.7)
	N–Me	32.4	3.37 (*s*)	32.4	3.38 (*s*)
Hiv(2) or Hmp(2)	C(1)	170.7	–	170.7	–
	H–C(2)	74.0	5.20 (*d*, *J* = 9.9)	73.1	5.30 (*d*, *J* = 9.6)
	H–C(3)	29.7	2.24 (*m*)	36.0	2.07 (*m*)
	Me(4), CH_2(4)	18.6	0.95 (*d*, *J* = 6.9)	24.6	1.38, 1.05 (2*m*)
	Me(5)	–	–	11.2[b])	0.92 (*t*, *J* = 7.4)
	Me(4′)	17.7	0.87 (*d*, *J* = 6.8)	14.6	0.94 (*d*, *J* = 6.8)
MeVal(3)	C(1)	169.4	–	169.5	–
	H–C(2)	67.9	3.37 (br.)	67.7	3.43 (br.)
	H–C(3)	28.3	2.34 (*m*)	28.4	2.37 (*m*)
	Me(4)	21.3	1.05 (*d*, *J* = 6.5)	21.3	1.08 (*d*, *J* = 6.5)
	Me(4′)	19.1	0.95 (*d*, *J* = 7.0)	19.1	0.98 (*d*, *J* = 6.2)
	N–Me	38.7	3.26 (*s*)	38.8	3.28 (*s*)

[a]) [b]) Assignments may be interchanged. [c]) Not detected.

formulae, *i.e.*, $C_{35}H_{61}N_3O_{10}$. 2D-NMR Spectral analysis revealed that **2**/**3** consists of six MeVal, two Dmp, two Hmp, and two Hiv residues. These informations, as well as the observation that **1**–**4** were only minor components (in contrast to the major metabolites **5**–**8**) suggested that both **2** and **3** consist of three MeVal, one Dmp, one Hmp, and one Hiv residues each, and that only the connectivity pattern differs. ^{1}H- and ^{13}C-NMR assignments of each residue for **2** and **3** could not be distinguished due to strong signal overlapping. Therefore, these data are presented separately as sets of signals (*Table 2*).

Table 2. *NMR Data of a 1 : 1 Mixture of* **2**/**3**. At 500/125 MHz in $CDCl_3$; δ in ppm, J in Hz.

Residue	C-Atom	^{13}C	^{1}H	Residue	C-Atom	^{13}C	^{1}H
Dmp	C(1)	168.3	–	MeVal(2)	C(1)	170.6	–
(two units)	H–C(2)	74.9	5.09, 5.08 (2*s*)	(two units)	H–C(2)	61.0	5.01 (*d*, *J* = 9.8)
	C(3)	73.6	–		H–C(3)	28.6	2.24 (*m*)
	CH_2(4)	34.8, 34.7	1.60 (*q*, *J* = 7.4)		Me(4)	19.9, 19.7[a])	1.12, 1.11 (2*d*, *J* = 6.5 each)
	Me(5)	7.7	0.97 (*t*, *J* = 7.3)		Me(4′)	19.8[a])	0.98–0.92[e])
	Me(4′)	22.5	1.32 (*s*)		N–Me	32.4, 32.3	3.36, 3.35 (2*s*)
MeVal(1)	C(1)	169.7	–	Hiv(2)	C(1)	170.6	–
(two units)	H–C(2)	61.3, 61.2	4.87 (*d*, *J* = 10.2), 4.85 (*d*, *J* = 9.8)	(for **2**)	H–C(2)	74.0	5.20 (*d*, *J* = 9.9)
	H–C(3)	25.7	2.18 (*m*)		H–C(3)	29.7	2.24 (*m*)
	Me(4)	19.9[a])	0.99 (*d*, *J* = 7)		Me(4)	18.8[b])	0.98–0.92[e])
	Me(4′)	18.4, 18.3	0.83 (*d*, *J* = 6.7)		Me(4')	17.7	0.88 (*d*, *J* = 6.7)
	N–Me	31.8	2.90 (*s*)				
Hiv(1)	C(1)	168.8[b])	–	Hmp(2)	C(1)	170.6	–
(for **3**)	H–C(2)	74.1	5.55 (*d*, *J* = 8.9)	(for **3**)	H–C(2)	73.1	5.27 (*d*, *J* = 9.7)
	H–C(3)	30.4	2.33 (*m*)		H–C(3)	35.9	2.05 (*m*)
	Me(4)	18.6[c])	0.98–0.92[e])		CH_2(4)	24.3	1.37, 1.03 (2*m*)
	Me(4′)	18.0[c])	0.98–0.92[e])		Me(5)	11.2[d])	0.89 (*t*, *J* = 7.3)
					Me(4′)	14.5	0.98–0.92[e])
Hmp(1)	C(1)	168.7[b])	–	MeVal(3)	C(1)	169.5	–
(for **2**)	H–C(2)	73.0	5.62 (*d*, *J* = 8.2)	(two units)	H–C(2)	67.8, 67.6	3.41 (br.)
	H–C(3)	36.7	2.07 (*m*)		H–C(3)	28.4, 28.3	2.34 (*m*)
	CH_2(4)	24.5	1.51, 1.15 (2*m*)		Me(4)	21.2	1.06, 1.05 (2*d*, *J* = *ca.* 6.5 each)
	Me(5)	11.1[d])	0.89 (*t*, *J* = 7.3)		Me(4′)	19.1, 19.0[c])	0.98–0.92[e])
	Me(4′)	14.7	0.98–0.92[e])		N–Me	38.9, 38.8	3.26, 3.25 (2*s*)

[a])–[d]) Assignments may be interchanged. [e]) Overlapping signals.

The structure of enniatin N (**4**) was confirmed by X-ray single-crystal structure analysis (*Fig. 2*). The relative configuration in α-position (C(2)) of the amino- and hydroxy acid residues was found to be identical to that of other known enniatins. By means of correlation between **4** and the co-metabolites **5**–**9**, the (2*S*)-configuration for the three MeVal residues of **4**, and the (2*R*)-configuration for Dmp and the two Hmp residues were assigned. Hence, the configuration at the quaternary C(3)-atom in the Dmp unit of **4**, which could not be determined by NMR methods, was (*R*). The very similar ^{1}H- and ^{13}C-NMR data for the single Dmp moieties of compounds **1**–**4** (*Tables 1* and *2*) strongly suggested that **1**–**3** should also possess (3*R*)-configuration. It should also be noted that the (3*S*)-configuration of the two Hmp units of **4** is the same as in enniatins H (**6**), I (**7**), and MK1688 (**8**), as previously and independently established by means of precursor-directed biosynthesis [9].

The new enniatins L (**1**), M_1/M_2 (**2**/**3**), and N (**4**) were tested for their antimalarial, antituberculosis, and cytotoxic activities realtive to the known enniatin B (**5**). The new hydroxy congeners displayed similar growth-inhibitory activities as the reference compound, suggesting that the OH group does not play an important role in these assays (*Table 3*).

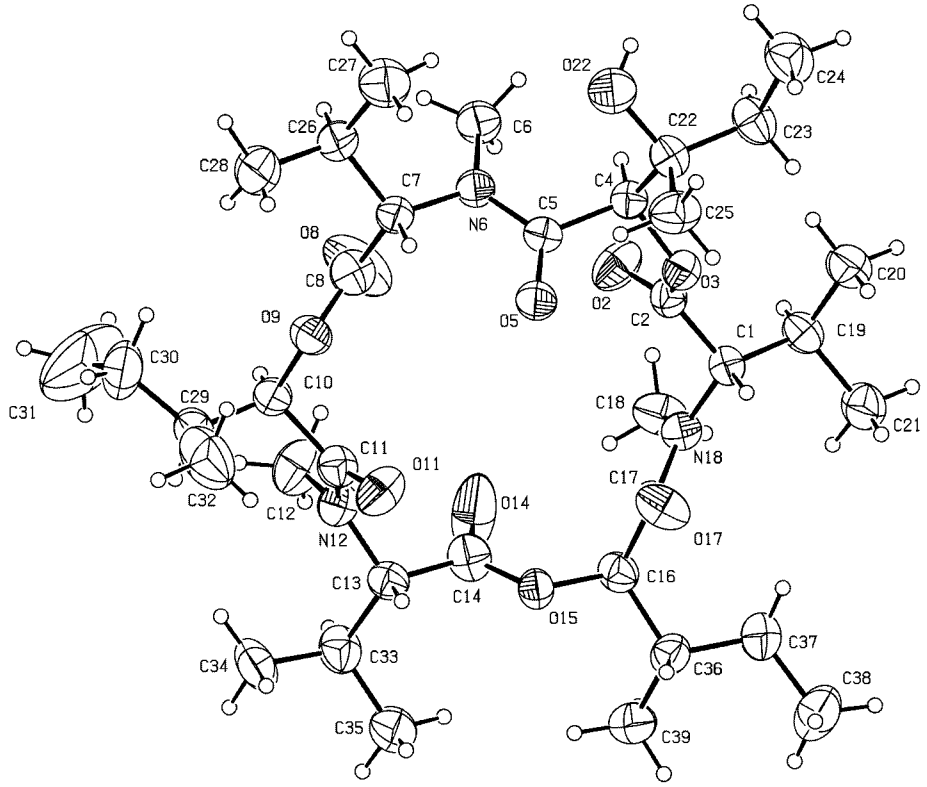

Fig. 2. *X-Ray crystal structure* (ORTEP plot) *of Enniatin N* (**4**)

Table 3. *Biological Activities of* **1**–**5** *against Malaria- and Tuberculosis-Inducing Microorganisms, as well as Cytotoxic Activities toward Different Cancer and Fibroblast Cells.* KB, oral human epidermal carcinoma cells; BC, human breast-cancer cells; NCI-H187, human small-cell lung-cancer cells; Vero, African-green-monkey kidney-fibroblast cells. For details, see the *Exper. Part*.

Compound	Malaria[a])	Tuberculosis[b])	Cytotoxicity[c])			
	IC_{50} [μg/ml]	*MIC* [μg/ml]	KB	BC	NCI-H187	Vero
1	3.3	12.5	3.6	3.8	2.1	6.4
2/**3**[d])	3.4	6.25	3.1	2.0	2.2	3.7
4	3.4	6.25	4.0	0.78	1.2	4.7
5	3.4	3.13	5.8	2.7	0.96	5.9

[a]) Against *P. falciparum* K1; the standard antimalarial drug dihydroartemisinin had an IC_{50} value of 0.0012 μg/ml. [b]) Against *M. tuberculosis* H37Ra; the standard antituberculosis drugs *isoniazid* and *kanamycin* had *MIC* values of 0.06 and 2.5 μg/ml, resp. [c]) The standard drug *ellipticine* exhibited IC_{50} values of 1.3, 1.5, 0.39, and 0.40 μg/ml for KB, BC, NCI-H187, and Vero cells, resp. [d]) Approximately a 1 : 1 mixture.

Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* (*Pharmacia*) or *Silica Gel 60 H* (*Merck*). HPLC: *Waters 600* controller; *Waters 996* photodiode-array detector; *Nova-Pak HR-C18* (40 × 100 mm; 6 μm) column.

M.p.: Electrothermal *IA9100* digital melting-point apparatus; uncorrected. Optical rotations: *JASCO DIP-370* digital polarimeter. UV Spectra: *Varian CARY-1E* spectrophotometer; λ_{max} in nm (log ε). IR Spectra: *Bruker VECTOR-22* spectrophotometer; in cm^{-1}. ^1H-, ^{13}C-, and 2D-NMR: *Bruker AV500* spectrometer; in $CDCl_3$; δ in ppm rel. to $SiMe_4$ as internal standard, *J* in Hz. MS: *Micromass LCT* spectrometer.

Fungal Material. The fungus used in this study had been isolated on May 27, 2000, by Dr. *Nigel L. Hywel-Jones* (Mycology Research Unit, BIOTEC) from spore attached to the synnema of *Hirsutella fomicarum* on ant collected at Khao Sok National Park, Surat Thani, southern Thailand. This fungus was deposited at the Thailand BIOTEC Culture Collection (BCC), registered as BCC 2629. The colony-growth rate was moderate on potato dextrose agar, reaching a diameter of 50 mm in 20 d, pure white, diffuse, thin and immersed with aerial mycelium, hyaline, septate and branching, up to 3 μm with sporulation of the fungus at 25° under normal day-light illumination. Anamorphic stage formed-genus *Acremonium*, however, it is not possible to identify into species level as this fungal genera is revising at the moment.

Fermentation, Extraction, and Isolation. The fungus BCC 2629 was maintained on potato dextrose agar at 25° for 21 d, after which the mycelium was cut into pieces and inoculated in four 250-ml *Erlenmeyer* flasks, each containing 25 ml of potato-dextrose broth (PDB). After incubation at 25° for 8 d on a rotary shaker (200 r.p.m.), these primary seed cultures were transferred into four 1000-ml *Erlenmeyer* flasks, each containing 250 ml of PDB, and incubated at 25° for another 8 d on a rotary shaker (200 r.p.m.). Each 25-ml portion of the secondary seed cultures was transferred into forty 1000-ml *Erlenmeyer* flasks containing the same liquid medium, and the fermentation was carried out at 25° for 40 d under stationary condition. Then, the cultures were filtered, and the residual mycelial cakes were extracted at r.t. with MeOH (1500 ml) for 2 d. To the filtrate was added H_2O (100 ml), and the mixture was washed with hexane (800 ml). The aq. MeOH layer was concentrated under reduced pressure. The residue was dissolved in AcOEt (300 ml), washed with H_2O (100 ml), and the org. layer was concentrated under reduced pressure, leaving a deep-yellow amorphous solid (2.2 g). This extract was purified by CC (1. *Sephadex LH-20*; CH_2Cl_2/MeOH; 2. SiO_2; MeOH/CH_2Cl_2) to obtain a mixture of enniatins (1.99 g). This mixture was subjected to repeated prep. HPLC (*ODS* column; MeCN/H_2O 70:30). The compounds were eluted in the following order: **1** > **2**, **3**, **5** > **9** > **4**, **6** > **7** > **8**. Repeated HPLC purification of each fraction with MeOH/H_2O 80:20 then afforded **1** (21 mg), **5** (126 mg), an inseparable 1:1 mixture of **2**/**3** (63 mg), **9** (27 mg), **4** (25 mg), **6** (246 mg), **7** (224 mg), and **8** (173 mg).

Enniatin L (**1**)[1]). Colorless powder. M.p. 132–133°. $[\alpha]_D^{25} = -102$ ($c = 0.22$, $CHCl_3$). UV (MeOH): 206 (4.11). IR (KBr): 3542, 2970, 1741, 1660, 1471, 1191, 1016. ^1H- and ^{13}C-NMR: see *Table 1*. HR-ESI-TOF-MS: 692.4908 ($[M + Na]^+$, $C_{34}H_{59}N_3NaO_{10}^+$; calc. 692.4908).

1:1 Mixture of Enniatins M_1 and M_2 (**2**/**3**). Colorless powder. M.p. 114–115°. $[\alpha]_D^{25} = -102$ ($c = 0.22$, $CHCl_3$). UV (MeOH): 207 (4.20). IR (KBr): 3442, 2968, 1744, 1659, 1469, 1189, 1012. ^1H- and ^{13}C-NMR: see *Table 2*. HR-ESI-TOF-MS: 684.4445 ($[M + H]^+$, $C_{35}H_{62}N_3O_{10}^+$; calc. 684.4436).

Enniatin N (**4**). Colorless crystals. M.p. 98–99°. $[\alpha]_D^{25} = -102$ ($c = 0.22$, $CHCl_3$). UV (MeOH): 206 (4.17). IR (KBr): 3416, 2968, 1743, 1659, 1467, 1193, 1010. ^1H- and ^{13}C-NMR: see *Table 1*. HR-ESI-TOF-MS: 676.4121 ($[M + H]^+$, $C_{36}H_{64}N_3O_{10}^+$; calc. 698.4591).

X-Ray Crystal Structure of Enniatin N (**4**)[2]). Crystals of compound **4** were grown from EtOH at r.t. Diffraction data were acquired from a colorless prism of size $0.25 \times 0.30 \times 0.50$ mm on a *Bruker-Nonius kappaCCD* diffractometer (graphite-monochromated MoK_α radiation; $\lambda = 0.71073$ Å) over a θ-range of 1.02–21.97°. No significant decay was observed during the data collection. From a total of 4859 unique reflection ($R_{int} = 0.045$), 4065 reflections were observed with $I \leq 2\sigma(I)$. The structure of **4** was solved by direct methods using SIR97 [13], and refined by full-matrix least-square optimization on F^2 using SHELXL97 [14]. All non-H atoms were refined anisotropically, and the H-atoms were fixed at calculated positions, and refined by means of a riding model. The final indices were $R = 0.0707$ and $wR = 0.1818$, with a goodness-of-fit on F^2 of 1.12 and a data-to-parameter ratio of 10.54. The final difference-electron-density map showed a maximum of + 0.24 and a minimum of − 0.15 $Å^{-3}$, resp. The following data were generated: molecular formula $C_{36}H_{67}N_3O_{12}$ (**4**·2 H_2O);

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[1]) Systematic name: (3*S*,6*R*,9*S*,12*R*,15*S*,18*R*)-6-[(1*R*)-1-hydroxy-1-methylpropyl]-3,9,12,15,18-pentaisopropyl-4,10,16-trimethyl-1,7,13-trioxa-4,10,16-triazacyclooctadecane-2,5,8,11,14,17-hexone.

[2]) Crystallographic data have been deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC-233901. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk) or *via* internet (http://www.cam.ac.uk/conts/retrieving.html).

M_r 733.94; trigonal system, space group $P\,3_1$; unit-cell parameters: $a = b = 14.8277(3)$, $c = 16.6565(3)$ Å; $V = 3171.5(1)$ Å^3; $D_x = 1.149$ g cm^{-3}; $Z = 3$, $\mu = 0.85$ cm^{-1}.

Biological Assays. The antimalarial assay for activity against *P. falciparum* K1 was performed according to a standard protocol [15], which follows the microculture radioisotope technique described by *Desjardins et al.* [16]. Growth inhibition against *M. tuberculosis* H37Ra was performed by means of the Microplate Alamar-Blue Assay (MABA) [17]. Cytotoxic activities of the pure compounds (or the 1 : 1 mixture in case of **2**/**3**) against oral human epidermal carcinoma (KB) cells, human breast-cancer (BC) cells, human small-cell lung-cancer (NCI-H187) cells, and African-green-monkey kidney fibroblasts (Vero cells) were evaluated colorimetrically [18].

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