Theonellapeptolide IIIe, a New Cyclic Peptolide from the New Zealand Deep Water Sponge, *Lamellomorpha strongylata*

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Received September 5, 1997

The structure, stereochemistry, and conformation of theonellapeptolide IIIe (**1**), a new 36-membered ring cyclic peptolide from the New Zealand deep-water sponge *Lamellomorpha strongylata*, is described. The sequence of the cytotoxic peptolide was determined through a combination of NMR and MS–MS techniques and confirmed by X-ray crystal structure analysis, which, with chiral HPLC, established the absolute stereochemistry.

Since 1982, more than 5000 specimens of marine organisms have been collected from the oceans around New Zealand in a continuous endeavor to find bioactive natural products. Over that period, compounds with a wide degree of structural diversity and bioactivity have been discovered.[1–3] To explore further the potential pharmaceutical resources of deep-water marine organisms, an expedition to the Chatham Rise, 200 km off the east coast of the South Island, was carried out in early 1995. Preliminary assays on the crude extracts indicated that deep-water organisms are a rich source of bioactive compounds.[4]

A bioassay-guided separation of the extract from *Lamellomorpha strongylata* from the collection resulted in the isolation of three distinct classes of cytotoxic compounds. We have previously reported the characterization of calyculins A, B, E, and F, calyculinamides A and B, and swinholide H,[4] which comprise two of the classes of cytotoxins from this sponge. We now present the structure of theonellapeptolide IIIe (**1**), the major component in the third cytotoxic fraction from the extract.

Results and Discussion

A frozen sample of *L. strongylata* (1 kg) was homogenized, extracted with MeOH–CH_2Cl_2 (1:1) and subjected to rounds of C18, DIOL, and LH-20 chromatography, as described previously.[4] LH-20 chromatography (MeOH–CH_2Cl_2, 1:1) of the more polar DIOL fractions resulted in a fraction with weak P-388 activity (IC_{50} 25 μg/mL), which eluted just prior to the calyculinamides A and B. The peptidic nature of the fraction was indicated by both the ^{1}H NMR (doublets between δ 7.39 and 9.00) and the ^{13}C NMR (resonances between δ 169 and 176) spectra. Purification, by repeated reversed-phase HPLC, yielded five new active compounds, theonellapeptolides IIIa, IIIb, IIIc, IIId, and IIIe, in order of elution.

Theonellapeptolide IIIe (**1**), the most abundant peptide, exhibited an MH^+ ion at *m/z* 1419 in the LR-FABMS. The molecular formula $C_{71}H_{127}N_{13}O_{16}$ was determined by HRFABMS (*m/z* 1550.8577, $[M + Cs]^+$, Δ −0.1). A negative result against ninhydrin suggested that the *N*-terminus was blocked, or part of a cyclic peptide, while the observation of a lactone carbonyl stretch (1734 cm^{-1}) in the IR spectrum, coupled with the unsaturation requirements of the molecular formula, indicated that **1** was most likely a peptolide rather than a peptide. The ^{1}H NMR spectrum showed seven amide proton signals (δ 9.00, 8.76, 8.43, 8.19, 7.65, 7.45, 7.39) and six *N*-methyl singlets (δ 3.33, 3.30, 3.24, 3.19, 2.75, 2.69). Searching the MarinLit[5] database revealed structural similarity of this new compound to the known theonellapeptolides.[6–8]

Amino acid analysis by GC–MS of the *N*-trifluoroacetamide-*n*-butyl ester derivatives[9,10] formed after complete acid hydrolysis of the peptolide confirmed 13 amino acids: Ala, *N*-MeAla, β-Ala, *N*-Meβ-Ala, Ile, *N*-MeIle ($\times$ 2), Leu, *N*-MeLeu ($\times$ 2), Thr, and Val ($\times$ 2). An additional carbonyl resonance in the ^{13}C NMR spectrum, as well as signals corresponding to isolated methylene (δ 3.86, s, 2H) and methoxy (δ 3.16, s, 3H) functionalities in the ^{1}H NMR spectrum, indicated the probable existence of a 2-methoxyacetyl group, blocking the *N*-terminus of the peptolide.

To determine the amino acid sequence, the ring-opened methyl ester (**2**) was prepared by methanolysis. Detailed examination of MS–MS data from the FABMS of **2** established the sequence shown in Figure 1. Of particular note was the observation of daughter ions at *m/z* 299, 172, and 100 in the link scan from the ion at *m/z* 400. These ions corresponded to the loss of Thr, *N*-MeLeu or *N*-MeIle, and 2-methoxyacetyl (from *m/z* 172), respectively, thus confirming the *N*-terminus region.

In analyzing FABMS data it is not possible to distinguish between isobaric amino acids, such as Ala and β-Ala, *N*-MeAla and *N*-Meβ-Ala, Leu and the Ile's, and *N*-MeLeu and the *N*-MeIle's. Therefore, the suggested sequence had to be confirmed by other methods. This confirmation of the amino acid sequence in **1** was accomplished by ^{1}H–^{1}H decoupling experiments and extensive analyses of the TOCSY, COSY, ROESY, HSMQC,[11] and HMBC NMR spectra. The spin systems

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S0163-3864(97)00417-5 CCC: $15.00 © 1998 American Chemical Society and American Society of Pharmacognosy
Published on Web 05/12/1998

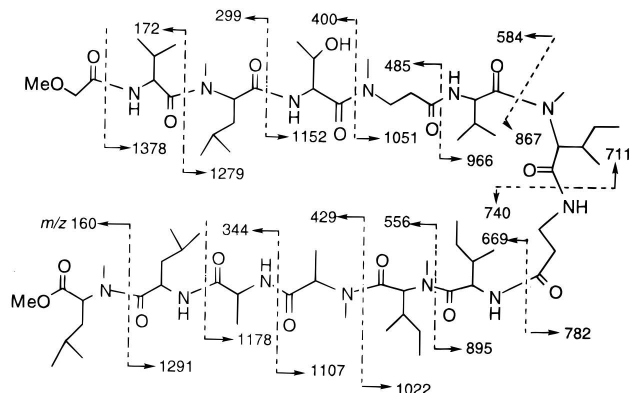

Figure 1. FABMS−MS fragmentation of **2** (protonated).

(see Table 1) for each of the individual amino acid partial structures, except for *N*-MeAla (residue 10) and *N*-MeLeu (residue 13), were defined by COSY and 2D TOCSY experiments. The *N*-MeAla subunit was subsequently defined by specific $^{1}H-^{1}H$ decoupling experiments, which showed that the unassigned methyl doublet at δ 1.44 was coupled into an α-methine multiplet at δ 5.20. For the *N*-MeLeu (residue 13) examination of the 2D TOCSY spectrum indicated that an unassigned methine signal at δ 3.74 gave correlations to ^{1}H resonances at δ 2.05, 1.06, and 0.98, as well as a weak correlation to δ 1.66, but the δ 3.74 methine resonance was not correlated to any carbons in the HMBC spectrum; however, a correlation between the protons at δ 3.74 and 2.05 in the COSY spectrum and correlations to δ 2.05 from both δ 3.74 and 1.66 in 1D TOCSY experiments were observed, which suggested that the signal at δ 3.74 was indeed the α-proton for an *N*-MeLeu. An ^{1}H *N*-methyl signal (δ 3.30) and the ^{1}H resonance at δ 2.05, which were correlated with a carbon (δ 63.0) resonance in the HMBC spectrum, supported the given assignments.

Correlations between NH, *N*-Me, or α-protons to carbonyl carbons in the HMBC spectrum afforded much of the connectivity data. For example, the correlations from H3 of Thr (residue 3) to the carbonyl resonance of *N*-MeLeu (residue 13) suggested that the Thr and *N*-MeLeu were linked by an ester bond, and so established the ring system of the peptolide. Complete assignments for NMR resonances, based on 1D and 2D NMR experiments, are summarized in Table 1. Correlations observed in the ROESY experiment confirmed the sequence of peptide bonds deduced in the HMBC experiment. Interestingly, the ROESY spectrum also showed cross peaks between the NH of β-Ala (residue 7) and the *N*-Me of *N*-MeIle (residue 6), between the NH's of Ala and Leu (residues 11 and 12), and between the NH of Ala and the *N*-Me of *N*-MeAla (residues 11 and 10), indicating *syn* orientations about *N*-MeIle/β-Ala, Ala/Leu, and Ala/*N*-MeAla peptide bonds. The same *syn* orientations were also observed in the X-ray analysis (see below), suggesting at least comparable geometries for the solution and solid phases for **1**. The peptide sequence deduced from analysis of the NMR data was consistent with the MS−MS analysis.

At this point, the stereochemistry of the component amino acids had not been addressed. This was resolved by a combination of X-ray crystallography and chiral HPLC. The structure was solved by direct methods and refined using the SHELXTL system of programs and all the unique intensity data.[12,13] The best-converged conventional *R*-factor was 12.9% for 12 029 reflections with $F_o > 4\sigma(F_o)$. All numerical data came from programs in the SHELXTL system.[12,13]

In addition to the theonellapeptolide molecule in the unit cell, there were at least 21 sites for the oxygen atoms of water molecules, 14 of which were at distances appropriate for H-bonding to carbonyl O and N atoms of the main chain. These water molecules are labeled in the Supplementary Tables. The other seven water molecules participated in water-to-water H-bonding. The atomic displacement parameters were consistent with some disorder among these water molecules. Six sites, involved in interatomic distance interactions between 1.6 and 2.0 Å, must be only partially occupied. These features have so far hindered attainment of better conventional agreement factors. This can be seen in deviations of some bond lengths from ideal values. Nevertheless, the structure and relative stereochemistry are fully characterized by this study.

Because the X-ray analysis gave only the relative stereochemistry of the cyclic peptolide, the absolute configuration of at least one of the individual amino acid residues needed to be determined. This was achieved by chiral HPLC analysis of the constituent amino acids.[14] A sample of the acid hydrolysis product was derivatized with dansyl chloride (Dan−Cl).[15] The dansylated derivatives of amino acids were co-injected with standard Dan-L-Thr (2*S*,3*R* stereogenic centers) and Dan-D,L-Thr, respectively. The Dan−Thr from the hydrolysate was found to coelute with standard Dan-L-Thr, establishing 2*S*,3*R* stereochemistry at the Thr stereogenic centers. From the relative stereochemistries of theonellapeptolide IIIe (**1**), established by X-ray crystallography, it was then possible to assign the stereochemistry at all stereogenic centers. Thus, the chiral amino acids of **1** could be assigned as: L-Val, L-Thr, D-Val, D-*allo*-Ile, L-*N*-MeIle (× 2), L-*N*-MeAla, L-Ala, D-Leu, and D-*N*-MeLeu (x 2) (see Figure 2).

For reasons of clarity each amino acid residue has been numbered from the *N*-terminus, and this system has been used in place of crystallographic numbering for descriptive purposes. The crystallographic numbering scheme and data are available in the Supporting Information.

Based on the general A−B bond length (2.79 ± 0.12 Å) for N−H···O bonds, three intramolecular H-bonds can be assumed: NH of Val (residue 5) to O of Val (residue 1) 2.89 Å, NH of Thr (residue 3) to O of Val (residue 5) 2.85 Å, and NH of Leu (residue 12) to O of *allo*-Ile (residue 8) 2.91 Å, with a further, weaker H-bond that should also be taken into consideration, NH of *allo*-Ile (residue 8) to O of Leu (residue 12), 3.08 Å. In terms of crystallographic numbering (Supporting Information) these H-bonds correspond to N9−O13, N11−O9, N2−O6, and N6−O2. The latter three of these H-bonds are across the peptolide ring and stabilize the complex conformation found in **1** (see Figures 2 and 3). The presence of a β-Ala and an *N*-Meβ-Ala allows for considerable flexibility in the conformation of the cyclic peptolide ring, which despite this flexibility, can be considered as an antiparallel array of two strands in which the turns are secured at one end by a *N*-Meβ-

Table 1. Correlated ^{1}H and ^{13}C Spectral Data for Theonellapeptolide IIIe (**1**)

amino acid (residue no.)		δ^{a}			
		^{13}C(# H)[b]	^{1}H (mult, *J*(Hz))[c]	HMBC[d]	ROESY[e]
L-Val (1)	1	174.4 (0)			
	2	54.5 (1)	5.03 (m)	174.4, 170.4, 32.1, 19.6, 17.6	3.19, 2.01, 0.98
	3	32.1 (1)	2.01 (m)	54.5, 19.6, 17.6	5.03, 3.19, 0.98, 0.91
	4	17.6 (3)	0.91	19.6	5.03, 2.01
	5	19.6 (3)	0.98	17.6	5.03, 2.01,
	NH		7.45 (d, 9.3)	170.4	5.03, 3.86, 2.72, 0.98
D-*N*-Me-Leu (2)	1	172.8 (0)			
	2	56.4 (1)	5.28 (m)	174.4, 172.8, 38.0, 31.9, 25.9	9.0, 8.19, 2.05, 1.48
	3	38.0 (2)	2.05 (m)	56.4, 25.9	1.48, 0.94
			1.48 (m)	25.9, 56.4	5.28
	4	25.9 (1)	1.48 (m)	24.1	3.19, 2.05, 0.94
	5	10.7 (3)	0.94	38.0, 25.9, 24.1	5.28, 3.19, 2.05, 1.48
	6	21.4 (3)	1.06	38.0, 25.9	2.05
	NCH_3	31.9 (3)	3.19 (s)	174.4, 56.4	5.03, 2.01, 1.48, 0.98, 0.94
L-Thr (3)	1	169.2 (0)			
	2	53.7 (1)	5.00 (m)	172.8, 169.2, 18.2	7.65, 2.75, 1.31
	3	71.2 (1)	5.47 (dd, 8.7, 6.3)	172.3, 53.7	7.65, 3.53, 2.75, 1.31
	4	18.2 (3)	1.31 (d, 6.3)	71.2, 53.7	5.47, 5.00
	NH		9.00 (d, 6)		5.47, 5.28, 5.00
N-Me-β-Ala (4)	1	172.9 (0)			
	2	34.7 (2)	2.30 (m)	172.9, 45.4	
			2.51 (m)	172.9, 45.4	8.19, 4.58, 2.30
	3	45.4 (2)	2.72 (m)		
			4.58 (m)	34.7	2.72, 2.51, 2.30
	NCH_3	35.0 (3)	2.75 (s)	169.2, 45.4	7.65, 5.47, 5.00, 2.51
D-Val (5)	1	174.0 (0)			
	2	55.4 (1)	5.06 (m)	172.9, 174.0, 31.0, 18.3	8.19, 3.24, 2.06, 0.99, 0.98
	3	31.0 (1)	2.06 (m)	55.4, 18.3	8.19, 5.06, 3.24, 0.98
	4	18.3 (3)	0.98	31.0	8.19, 5.06, 2.06
	5	19.7 (3)	0.99	31.0	
	NH		8.19 (d, 8.7)	172.9, 55.4	5.28, 5.06, 2.51, 2.06, 0.98
L-*N*-Me-Ile (6)	1	170.8 (0)			
	2	61.4 (1)	5.22 (m)	174.0, 170.8, 33.1, 31.6, 16.1	7.65, 3.24, 2.29, 1.13
	3	33.1 (1)	2.29 (m)		3.24, 1.43, 1.13
	4	16.1 (3)	1.13 (d, 6.3)	61.4, 33.1, 25.3	5.22, 2.29, 1.43
	5	25.3 (2)	1.43 (m)		2.29, 0.96
	6	21.8 (3)	0.96	25.3	
	NCH_3	31.6 (3)	3.24 (s)	174.0, 61.4	5.06, 2.29, 2.06
β-Ala (7)	1	172.5 (0)			
	2	35.9 (2)	2.51 (m)	172.5	8.76, 5.47, 3.53, 1.31
			2.69 (m)		8.76, 3.53
	3	35.8 (2)	3.53 (m)	172.5, 170.8	7.65, 5.47, 4.54, 2.51
			4.54 (m)	172.5, 170.8	3.53, 2.69
	NH		7.65 (d, 5.4)	170.8	5.47, 5.22, 5.00, 4.54, 3.53, 3.24
D-*allo*-Ile (8)	1	175.9 (0)			
	2	53.6 (1)	5.38 (m)	175.9, 172.5, 38.8, 15.5	8.76, 3.33, 1.75, 1.55, 0.98
	3	38.8 (1)	1.75 (m)	53.6, 26.9, 15.5, 12.4	5.38, 3.33, 1.04, 0.98
	4	15.5 (3)	0.98		
	5	26.9 (2)	1.22 (m)	53.6, 38.8, 15.5, 12.4	1.55
			1.55 (m)	53.6, 38.8, 12.4	5.58, 1.22, 1.04
	6	12.4 (3)	1.04		
	NH		8.76 (d, 9.3)	172.5	5.38, 2.69, 2.51, 0.98
L-*N*-Me-Ile (9)	1	172.7 (0)			
	2	55.5 (1)	5.18 (m)	175.9, 172.7, 33.8, 31.7, 29.7	3.33, 2.69
	3	33.8 (1)	2.28 (m)		3.33, 0.78
	4	15.8 (3)	0.78 (d, 12)	55.5, 33.8, 25.6	2.69, 2.28, 1.42,
	5	25.6 (2)	1.42 (m)	15.8	0.82
	6	9.8 (3)	0.82 (dd, 9.6, 6.3)	33.8, 25.6	2.28, 1.42
	NCH_3	31.7 (3)	3.33 (s)	175.9, 55.5	5.38, 5.18, 2.28, 1.75, 1.42
L-*N*-Me-Ala (10)	1	171.1 (0)			
	2	57.5 (1)	5.20 (m)	172.7, 171.7, 29.7, 15.0	2.69, 1.44
	3	15.0 (3)	1.44 (d, 6.9)	171.1, 57.5	5.20, 2.69
	NCH_3	29.7 (3)	2.69 (s)	172.7, 57.5	5.20, 5.18, 1.44, 0.78
L-Ala (11)	1	174.7 (0)			
	2	52.3 (1)	4.51 (m)	174.7, 17.9	1.29
	3	17.9 (3)	1.29 (d, 7.5)	174.7, 52.3	7.39, 4.51
	NH		7.39 (d, 6.3)	171.1, 52.3, 17.9	8.43, 4.51, 2.69, 1.29
D-Leu (12)	1	174.9 (0)			
	2	48.8 (1)	5.15 (m)	174.9, 40.8	8.43, 3.30, 1.87
	3	40.8 (2)	1.34 (m)		1.87, 0.98
			1.87 (m)	174.9, 48.8, 25.4	8.43, 5.15, 1.34
	4	25.4 (1)	1.87 (m)	21.3	1.04, 0.98
	5	21.3 (3)	1.04	40.8, 25.4, 23.9	1.87
	6	23.9 (3)	0.98	40.8, 25.4, 21.3	
	NH		8.43 (d, 8.4)	174.7, 48.8	7.39, 5.15, 1.87, 1.34

Table 1 (Continued)

amino acid (residue no.)		δ^a			
		^{13}C(# H)[b]	^{1}H (mult,*J*(Hz))[c]	HMBC[d]	ROESY[e]
D-*N*-Me-Leu (13)	1	172.3 (0)			
	2	63.0(1)	3.74 (m)		3.30, 2.05, 1.06
	3	38.3 (2)	2.05 (m)	172.3, 63.0, 25.6, 23.8, 22.5	3.74, 1.06, 0.98
	4	25.6 (1)	1.66 (m)	38.3, 23.8	3.30
	5	23.8 (3)	0.98	38.3	
	6	22.5 (3)	1.06	38.3, 25.6	3.74, 2.05, 1.66
	NCH_3	38.8 (3)	3.30 (s)	174.9, 63.0	5.15, 3.74, 1.66
MeO−CH_2CO	1	170.4 (0)			
	2	72.1 (2)	3.86 (s)	170.4, 59.0	7.45, 3.16
	OCH_3	59.0 (4)	3.16 (s)	72.1	3.86

[a] Referenced to residual solvent C_6D_6 $\delta_H = 7.27$, $\delta_C = 128.4$. [b] ^{13}C spectra recorded on a Varian XL300 at 75 MHz. Number of attached H determined by DEPT. [c] ^{1}H spectra recorded on a Varian UNITY 300 at 300 MHz. [d] $J_{nxh} = 8.3$, 5.0 arrayed mixing times. [e] A mixing time of 0.15 s was used.

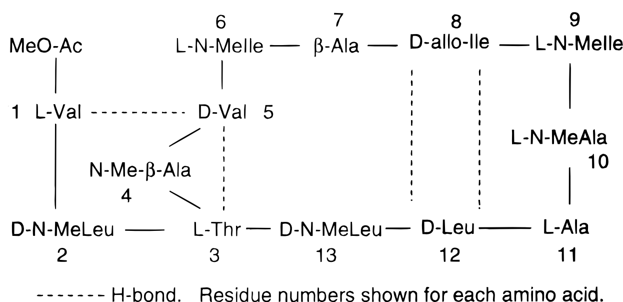

Figure 2. Theonellapeptolide IIIe (**1**).

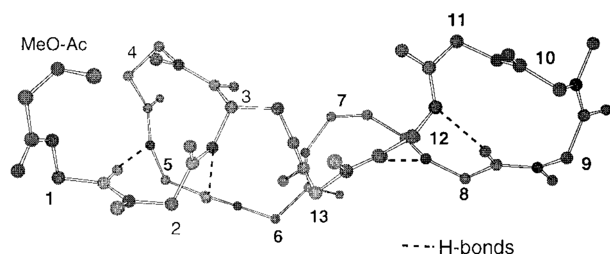

Figure 3. Backbone projection of theonellapeptolide IIIe (**1**).

Ala (residue 4) and at the other by a *cis*-peptide bond [*N*-MeIle (residue 9)/*N*-MeAla (residue 10)]. The resulting conformation is a triple loop,[12] which is more clearly depicted in the backbone projection diagram (Figure 3). The two major loops are defined by the two H-bonds between the *allo*-Ile and the Leu (residues 8 and 12), which forms an antiparallel β-pleated sheet structure. The third loop is defined by weak NH···O associations from the NH of β-Ala (residue 7) to Thr (residue 3) and MeLeu (residue 13) 3.28 Å and 3.31 Å (in crystallographic numbering N7−O1 and N7−O11). That portion of the peptide outside the macrocycle is static, being held in place by two H-bonds from Val (residue 5) to Thr (residue 3) and Val (residue 1). The overall conformation of **1** can best be described as resembling a "taco shell" with all the larger aliphatic groups aligned along the lip of the taco shell, while the base is composed of the smaller amino acids (Ala, β-Ala, *N*-Meβ-Ala). The very defined structure of theonellapeptolide **1** can only be visualized in stereoprojection (see Supplementary Figures).

The reported theonellapeptolides I and II differ mainly in variation among the aliphatic side chains. For example, theonellapeptolide Ie,[7] which has the same molecular formula as **1**, has a similar peptolide backbone, but varies in the fifth, ninth, eleventh, and thirteenth amino acid residues from *N*-terminus, corresponding to substitutions of D-Val for D-Leu, L-*N*-MeIle for L-*N*-MeVal, L-Ala for β-Ala, and D-*N*-MeLeu for D-*N*-Me*allo*-Ile, respectively. These modifications result in a 36-, rather than a 37-membered peptolide ring for **1**. An X-ray structure has been reported in a conference abstract[16] for theonellapeptolide Id. Theonellapeptolide Id differs from Ie only in substitution of a β-Ala for a *N*-Meβ-Ala. Despite the five amino acid substitutions and the difference in ring size, there is a quite remarkable similarity in the overall conformation and H-bonding patterns between theonellapeptolides IIIe (**1**) and Id, which suggests that the theonellapeptolide family quite probably have similar tertiary structures.

Theonellapeptolide IIIe exhibited modest cytotoxicity (7.4 μg/mL) against the P-388 cell line. This sample was also tested for antiviral and anti-HIV activities; however, only cytotoxicity and no protection was observed. Other reported biological activities for the theonellapeptolide family are ion-transport properties for theonellapeptolide Id (Na^+, K^+, and Ca^{2+}) and Ie (Na^+, K^+ across human erythrocyte membranes).[6−8] It is pertinent to note that the theonellapeptolide III compounds were isolated from a *Lamellomorpha* sp. sponge, which is in a different order from *Theonella swinhoei*, from which both the theonellapeptolides I and II were isolated.[6−8]

As it is rare to find so many interesting structures and associated bioactivities in one species, further chemical and biological studies are currently underway on *L. strongylata*. In particular, the question of what symbiotic microorganisms are present and their role in the production of the bioactive metabolites will be addressed.

Experimental Section

General Experimental Procedures. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO J-20 recording spectropolarimeter. IR spectra were recorded on a Shimadzu FTIR-8201PC infrared spectrophotometer. GC−MS spectra were measured on a Kratos MS80RFA mass spectrometer equipped with a Carlo Erba MFC500 GC. FABMS and HRFABMS were recorded on a JEOL SX102A spectrometer. ^{1}H and all 2D NMR spectra were recorded on a Varian UNITY 300 NMR spectrometer. ^{13}C NMR spectra were recorded on a Varian XL 300 NMR spectrometer.

Extraction and Isolation. The formal description

of *L. strongylata* and the extraction and separation of the theonellapeptolide fraction from the calyculin and swinholide fractions have been reported previously.[4] Final purification of the peptolides was carried out by reversed-phase HPLC on a Philips PU4100 using an ODS column (4.6 mm × 25 cm) eluting with H_2O (0.1% TFA)−MeOH (0.1% TFA) (11:89). A limited sample (2 mg) of the crude peptide was injected in each run. Cutting the appropriate peak in multiple runs gave pure theonellapeptolide IIIe (**1**) (94 mg).

Theonellapeptolide IIIe (1): colorless plates (Me_2CO-H_2O 1:1); mp 184−186 °C; $[\alpha]^{22}_D$ −48.6° (*c* 1.0, MeOH); IR ($CHCl_3$) ν_{max} 3352, 3006, 2966, 2935, 1734, 1672, 1628, 1533, 1468, 1173 cm^{-1}; ^1H NMR ($CD_3OD-C_6D_6$, 2:8) see Table 1; ^{13}C NMR (CD_3OD/C_6D_6, 2:8) see Table 1; HRFABMS, *m/z* 1550.8577 $[M + Cs]^+$, (calcd for $C_{71}H_{127}N_{13}O_{16}Cs$, 1550.8578).

Acid Hydrolysis of 1. Theonellapeptolide IIIe (**1**) (1 mg) was placed in a hydrolysis tube, and HCl (6 M) (1 mL) was added. After sealing under N_2, the tube was heated at 110 °C (17 h). The acid solution was evaporated to dryness under vacuum to give the product (1 mg).

Methanolysis of 1. Theonellapeptolide IIIe (**1**) (9 mg) was dissolved in dry MeOH (2 mL) with NaOMe−MeOH (4%) (0.6 mL). The MeOH solution was stirred at room temperature (3 h), before pouring into iced H_2O and extracting with EtOAc (3 × 1 mL). Evaporation of the solvent gave a product that was purified by reversed-phase HPLC [H_2O (0.1% TFA)−MeOH 18:82] to give the methanolysis product **2** (6 mg): HRFABMS *m/z* 1582.8860 $[M + Cs]^+$, (calcd for $C_{72}H_{131}N_{13}O_{17}Cs$ 1582.8840).

Amino Acid Analysis by GC−MS. The *N*-trifluoroacetamide-*n*-butyl esters of the amino acids were prepared under standard conditions.[10] The acid hydrolysate of **1** (1 mg) was transferred to a Reacti-vial (1 mL) and dried under vacuum before HCl−*n*-BuOH (2 M) (400 μL) was added. The vial was sealed with a Teflon-lined cap and, after sonication (15 s), was heated at 110 °C in an oil bath (10 min), followed by further sonication (10 s) and heating (20 min). After cooling, the solvent was evaporated under vacuum. A trifluoroacetic anhydride−CH_2Cl_2 mixture (750 μL) (1:2) was added to the sample, which was then heated (150 °C for 5 min). The remaining reagent and solvent were removed under N_2 at 50 °C and the product then dissolved in dry CH_2Cl_2 (600 μL) for GC analysis. The GC−MS analysis was performed on a DB Wax column (30 m × 0.25 mm) with an initial column temperature of 50 °C, which was held for 15 min postinjection and then increased to 130 °C at 1°/min, to 250 °C at 23°/min, and finally held at 250 °C for 20 min. The individual amino acids were identified on the basis of retention time against standards and by analysis of the MS data.

X-ray Structure Determination of Theonellapeptolide IIIe (1). A colorless crystal of theonellapeptolide IIIe (**1**), obtained as a polyhydrate from Me_2CO-H_2O (1:1), was used in the data collection. Data reported here were derived from an irregular chunk of dimensions (0.6 × 0.4 × 0.15) mm^3. Crystal data: formula $C_{71}H_{127}N_{13}O_{16}/(H_2O)_{18}$, FW = 1418/(324); orthorhombic, $P2_12_12_1$, a = 12.507(3), b = 19.573(4), c = 41.416(8) Å, V = 10,139(8) $Å^3$, Z = 4, ρ (calculated) = 1.142 Mg/m^3, T = −70 °C.

The data collection nominally covered a hemisphere. Intensities of 18 590 unique reflections were collected on a Siemens SMART area detector system, fitted with a nitrogen low-temperature gas flow device, using Mo Kα (λ = 0.710 73 Å) X-radiation. These data were processed using program SAINT, which corrects for Lp effects and crystal decay for the duration of the experiment. All crystallographic data are reported in the Supplementary Tables.

Determination of Absolute Configuration. A sample of the acid hydrolysate of theonellapeptolide IIIe (**1**, 1 mg) was dissolved in aqueous Li_2CO_3 (0.04 M) (3 mL) and reacted with dansyl chloride (Dan−Cl 2.2 mg−AcCN 1 mL) (2.3 mL)[15] at room temperature (2 h). The solvent was removed under vacuum. The Dan−amino acids were further purified on a DIOL cartridge with CH_2Cl_2−ether−HOAc (5:1:1) as eluent. The first fraction (0.8 mL) was collected and dried under N_2 (0.8 mg). The Dan-derivatives were analyzed on a reversed-phase HPLC column (ODS, 4.6 × 220 mm) run isocratically using L-Val (4 mM)−copper acetate (2 mM)−sodium acetate (0.3 M) (pH 7) as chiral eluent (1 mL/min). Standard Dan-L-Thr and Dan-DL-Thr were co-injected with the dansyl derivatives.

Acknowledgment. We thank the University of Canterbury, PharmaMar SA, Canterbury Medical Research Foundation, and the New Zealand Lotteries Board (Scientific) for financial support; Dr. M. R. Boyd and Dr. M. J. Currens (NCI/DTP/LDDRD) for HIV assays, Ms. G. Ellis (University of Canterbury) for other biological assays, and Mr. B. M. Clark (University of Canterbury) for mass spectral analyses.

Supporting Information Available: Copies of tables of final fractional atomic coordinates and the full list of bond lengths and angles from the X-ray crystallographic study of **1**, stereoprojection figure (15 pages). Ordering information is given on any current masthead page.

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NP970417R