Neopeltolide, a Macrolide from a Lithistid Sponge of the Family Neopeltidae[⊥]

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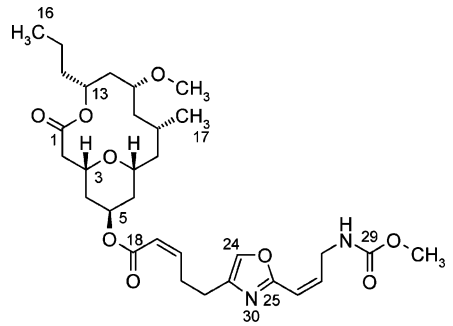
A new marine-derived macrolide designated as neopeltolide (**1**) has been isolated from a deep-water sponge of the family Neopeltidae. Its structure was elucidated on the basis of spectroscopic data interpretation. Neopeltolide (**1**) is a potent inhibitor of the in vitro proliferation of the A-549 human lung adenocarcinoma, the NCI-ADR-RES human ovarian sarcoma, and the P388 murine leukemia cell lines, with IC_{50}'s of 1.2, 5.1, and 0.56 nM, respectively. Neopeltolide (**1**) also inhibited the growth of the fungal pathogen *Candida albicans* with a minimum inhibitory concentration of 0.62 μg/mL.

Marine sponges of the polyphyletic order "Lithistida" have been the source of a wealth of natural products exhibiting a variety of biological activities.[1] Sponges of the family Neopeltidae include the genera *Callipelta*, *Daedalopelta, Homophymia,* and *Neopelta*, and only very limited work has been performed on their natural products chemistry. Shallow-water collections of the sponge *Homophymia conferta* yielded aurantoside C as a mildly toxic compound to brine shrimp.[2] Shallow-water collections of a sponge of the genus *Callipelta* from New Caledonia yielded the callipeltins, a series of peptides with anti-HIV activity,[3,4] and the callipeltosides,[5,6] a series of glycosidic macrolides with moderate cytotoxic properties. As part of our ongoing effort to discover compounds with useful therapeutic properties, we report herein the isolation of a novel compound designated as neopeltolide (**1**) from a deep-water specimen of a sponge of the family Neopeltidae. The sponge most closely matches the taxonomic description for the genus *Daedalopelta.*[7] This article describes the isolation, structure elucidation, and cytotoxicity of neopeltolide (**1**).



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Results and Discussion

Two samples of the sponge *Daedalopelta* were collected using the Johnson-Sea Link human-occupied submersible on a single dive at depths of 442 and 433 m off the north Jamaican coast. Samples for this study were stored at −20 °C immediately after collection. The frozen sponge was extracted exhaustively with ethanol. After concentration by distillation under reduced pressure, the extract was partitioned between *n*-butanol and water. The *n*-butanol partition was further separated by vacuum-column chromatography on a silica gel stationary phase using a step gradient of ethyl acetate in heptane to yield an enriched fraction, which, after reversed-phase HPLC, led to the purification of neopeltolide (**1**) as a colorless oil, $[\alpha]_D$ +24.

Inspection of the ^{13}C NMR spectrum coupled with HRMS data suggested a molecular formula of $C_{31}H_{46}O_9N_2$ for **1** [$(M + H^+)$ *m*/*z* observed 591.32692, calcd 591.32816, $\Delta = -1.2$ mmu)], indicating the presence of 10 degrees of unsaturation. ^{13}C NMR resonances attributable to two ester carbonyls (δ_C 173.0, 166.9), two heterosubstituted sp^2-hybridized carbons [δ_C 161.9 (C), 159.6-(C)], and six additional olefinic carbons (δ_C 150.0 (CH), 142.3 (C), 139.2 (CH), 135.9 (CH), 121.7 (CH), 115.7 (CH)) accounted for seven of the degrees of unsaturation, suggesting that **1** is tricyclic.

The planar structure of neopeltolide (**1**) was established from the analysis of its one- and two-dimensional NMR data. Especially useful was a series of 1D-DPFGSE-TOCSY[8] experiments, which allowed for the assignment of the upfield portion of the ^1H NMR spectrum, in which a significant level of overlap was present. Combination of the spin systems defined by the 1D-DPFGSE-TOCSY experiments coupled with $^1H-^{13}C$ one-bond connectivities determined from the edited-HSQC experiment[9] and $^1H-^1H$ scalar coupling determined from the 2D-DQF-COSY[10] spectrum led to the assignment of proton spin systems shown as bold bonds in Figure 1.

For example, selective irradiation of a proton observed at δ_H 4.04 led to observation of resonances at δ_H 5.17, 2.66, 2.26, 1.78, and 1.46, indicating that they are part of the same scalar spin system. The HSQC spectrum defined the protons observed at δ_H 2.66 and 2.26 as geminal protons on a carbon observed at 43.2 ppm. Similarly, the protons observed at δ_H 1.78 and 1.46 ppm were geminal protons on a carbon appearing at δ_C 36.2 ppm. Evaluation of the 2D DQF-COSY spectrum allowed for sequencing of the resonances as follows: the methylene group observed at δ_H 2.66/2.26 was coupled to the methine proton observed at δ_H 4.04, which was in turn coupled to the methylene group observed at δ_H 1.78/1.46, which was in turn coupled to the methine observed at 5.17 ppm. A second 1D-DPFGSE-TOCSY experiment, in which the proton observed at δ_H 3.55 was irradiated, led to the observation of a spin system containing two methylene groups (δ_H 1.64, 1.49; δ_C 37.4 and δ_H 1.36, 1.25; δ_C 45.2), as well as the methine proton observed at 5.17 ppm. Interpretation of the DQF-COSY spectrum led to the following spin system: the methine proton observed at δ_H 5.17 was coupled to the methylene group observed at δ_H 1.64/1.49, which was in turn coupled to the methine proton observed at δ_H 3.55, and which in turn was coupled to the methylene protons observed at δ_H 1.36/1.25. The DQF-COSY spectrum clearly indicated that the proton observed at δ_H 5.17 was coupled to protons

[⊥] Dedicated to the late Dr. Kenneth L. Rinehart of the University of Illinois at Urbana−Champaign for his pioneering work on bioactive natural products.

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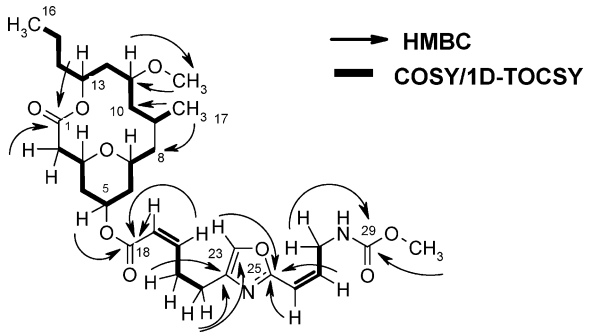

Figure 1. Planar structure of neopeltolide (**1**) showing partial structures derived from the 2D-DQF-COSY and 1D-DPFGSE TOCSY spectra and linkage via key HMBC correlations.

in the two flanking methylene groups and allowed for the full assignment of atoms 2−8 shown in Figure 1. Similar experiments were run to define all spin systems in **1**.

The spin systems shown in Figure 1 were tied together through interpretation of the HMBC spectrum as described below and were fully confirmed by a proton-detected H2BC experiment.[11] Although the carbon skeleton between atoms 2−16 should constitute a single spin system, spin systems C-2−C-8 and C-9−C-16 could not be tied together unequivocally from the DQF-COSY or 1D-DPFGSE-TOCSY experiments due to overlap of the resonances for H-8a and H-9. HMBC correlations observed between H_3-17 and C-8 and C-10 clearly linked these two partial structures through the C-8−C-9 bond. Correlations from the methylene resonances observed at δ_H 2.66 and 2.26 (H-2ab) to the ester carbon observed at δ_C 173.0 (C-1) allowed for incorporation of the first ester moiety into the molecule. The ^{1}H NMR chemical shift of the proton observed at δ_H 5.14 (H-13) was consistent with that predicted for an acylated methine, and correlations observed in the HMBC spectrum between this proton and C-1 allowed for closure of the macrolide ring. The methoxy protons observed at δ_H 3.23 (s, 3H) showed correlations in the HMBC spectrum to the carbon observed at δ_C 77.1 (C-11), allowing for assignment of this methoxyl functionality at C-11. Correlations from H-3 to C-7 in the HMBC spectrum allowed for closure of a tetrahydropyran ring between atoms C-3 and C-7.

The olefinic protons observed at δ_H 5.86 (H-19) and 6.33 (H-20) showed correlations in the HMBC spectrum to the ester carbon observed at δ_C 166.7, defining the presence of an α,β-unsaturated ester in **1**. Correlations in the HMBC spectrum between H-5 observed at δ_H 5.17 and this same ester carbon allowed the C-19−C-22 fragment to be attached to the macrolide via an ester linkage. The presence of an oxazole in **1** was suggested by the ^{1}H NMR resonance observed at δ_H 7.64 and the carbon resonances observed at δ_C 142.2 (C), 135.8 (CH), and 161.8 (C), which were similar to those observed in leucascandrolide A.[12] Correlations observed in the HMBC spectrum from H-21ab, H-22ab, and H-24 to the carbon observed at δ_C 142.2 assigned this as C-23. Correlations observed in the HMBC spectrum from H-24, H-26, and H-27 to the carbon observed at δ_C 161.9 assigned this as C-25 and connected the C-25−C-28 fragment to the molecule. A carbamate functionality was attached at C-28 on the basis of correlations in the HMBC spectrum from H-28ab observed at δ_H 4.28 to the remaining carbonyl observed at δ_C 159.5. The chemical shift of the carbon at C-28 was δ_C 41.0, consistent with nitrogen substitution on this carbon. A methoxy group was attached to C-29 on the basis of correlations in the HMBC spectrum from the methyl resonance observed at δ_H 3.62 to the carbamate carbonyl C-29. The C-19−C-20 double bond was assigned *Z* geometry due to the $J_{H19,20}$ value of 11.6 Hz and the observation of a strong correlation in the NOESY spectrum between H-19 and H-20 and the lack of correlation between H-19 and H-21ab that would be predicted for *E* geometry. The C-26−C-27 double bond was also assigned *Z* geometry due to the $J_{H26,27}$ of 11.6 Hz, the observation of a strong correlation in the NOESY spectrum between H-25 and H-26, and the lack of correlation between H-26 and H-28ab that would be predicted for *E* geometry.

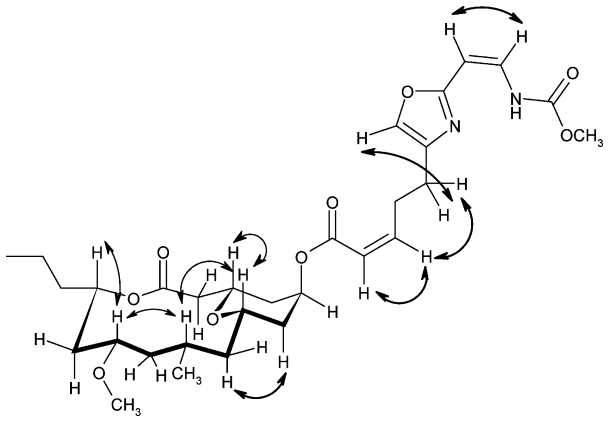

Figure 2. Key NOE correlations to define the relative stereochemistry of neopeltolide (**1**) and geometry of the olefins.

The relative placement of the nitrogen and oxygen in the oxazole ring was assigned on the basis of comparison of the chemical shifts to those reported for the same moiety in leucasandrolide A, as well as through interpretation of the proton-detected ^{1}H−^{15}N HMBC spectrum.[13] Correlations observed from both H-22ab and H-24 to a nitrogen observed at δ_N 254.3 placed the oxazole nitrogen on C-23. In addition to chemical shift arguments, the observation of correlations from H-27 and H-28ab to a nitrogen observed at δ_N 77.2 in the proton-detected ^{1}H−^{15}N HMBC spectrum clearly placed the nitrogen on C-28. This completed the planar structure of **1**.

The relative stereochemistry of **1** was assigned on the basis of analysis of coupling constants, the 2D-NOESY spectrum, and a series of 1D-DPFGSE NOE experiments (Figure 2).[8] H-3 was assigned an axial conformation on the basis of the observation of an axial coupling constant of 11.6 Hz to H-4b. In turn, H-7 was assigned as axial on the basis of observation of an axial coupling constant of 11.6 Hz to H-6b. Strong correlations observed in the NOESY spectrum between H-3 and H-7 further support the 1,3 diaxial assignment for these protons. The H-5 signal was observed as a multiplet lacking any large vicinal couplings, no correlations or enhancements were observed with the axial proton H-3 or H-7, and it was therefore assigned as equatorial. All of the protons H-3, H-7, H-9, H-11, and H-13 were assigned on the same side of the macrolide ring on the basis of the following NOESY correlations that were confirmed via 1D-NOE experiments. The H-3 signal showed a correlation with H-7, which had a correlation with H-9, and in turn was correlated with H-11, which in turn was correlated with H-13. All of the ring substituents, the C-17 CH_3, the C-11 OCH_3, and the C-13 propyl functionality were found to adopt pseudoequatorial conformations around the macrolide ring. The absolute stereochemistry of neopeltolide (**1**) has not been determined due to lack of available material.

The macrolide portion of neopeltolide (**1**) shows similarities to the macrolide ring seen in the callipeltosides[5,6] isolated from the sponge *Callipelta*, lyngbyaloside[14] from *Lyngbya* sp., lyngbouilloside[15] from *Lyngbya bouilloni*, and the aurisides[16] isolated from the sea hare *Dolabella auricularia*, with a primary difference being the reduction of the C-7 hemiketal to an ether functionality (tetrahydropyran ring system) and the much simpler propyl side chain functionality. Polycavernoside A,[17] isolated from the red alga *Polycavernosa tsudai*, is a potent toxin that has a 14-membered macrolide, but also has a tetrahydropyran ring as found in neopeltolide (**1**). Neopeltolide also shows strong similarities to leucascandrolide A isolated from the calcareous sponge *Leucascandra caveolata*.[12] Leucascandrolide A has a 16-membered macrolide ring with a tetrahydropyran ring, rather than the hemiketal, and the same oxazole-containing side chain as found in neopeltolide (**1**). Tan et al. have hypothesized that the true

Table 1. ^{1}H and ^{13}C NMR Data for Neopeltolide (**1**) (CD_3OD, 600 MHz)

position		δ_C, mult.	δ_H (*J* in Hz)	COSY	HMBC[b]	H2BC[c]	NOESY
1		173.0, qC					
2	a	43.2, CH_2	2.66, dd (15.1, 4.1)	2b, 3	1, 3	3	2b, 3
	b		2.26, dd (15.1, 11.0)	2a, 3	1, 3, 4	3	2a, 4b
3		71.3, CH	4.04, ddt (4.1, 2.1, 11.7)	2ab, 4b	2, 5, 7	2, 4	2a, 4a, 7
4	a	36.2, CH_2	1.78, m	4b, 5	5, 6	3, 5	2a, 4b, 3, 5
	b		1.46, m	4a,3, 5	3	3, 5	2b, 4a
5		69.2 CH	5.17, m	4ab, 6ab	3, 7, 18	4	4ab, 6
6	a	37.4, CH_2	1.64, m	5, 6b, 7	4, 5	5	5, 6a, 7, 8b
	b		1.49, m	5, 6a, 7		5	
7		77.0, CH[a]	3.55, bt (10.3)	6ab, 8ab	5, 8, 9	6, 8	3, 6a, 8b, 9
8	a	45.2, CH_2	1.36, m	7, 8b	9		
	b		1.25, m	8a	6, 9, 10, 17		17
9		32.6, CH	1.38, m	17abc, 10a	11	10, 17	7, 11, 17
10	a	43.5, CH_2	1.54, m	9, 10b, 11	8, 9, 11, 12	9	10b, 11, OCH_3-11, 17
	b		1.08, m	10a, 11	8, 9, 11, 12, 17		9, 10a
11		77.1, CH[a]	3.64, m	10ab, 12ab	9, 10, 12, OCH_3-11, 13	10	9, 10a, OCH_3-11
OCH_3-11		56.4, CH_3	3.23, s		11		10a, 11
12	a	41.0, CH_2	1.83, m	11, 12b, 13	13	13	11, 12b
	b		1.28, m	11, 12a		13	12a, 13
13		73.9, CH	5.14, dt (4,8, 9.6)	12a, 14ab	1, 15	14	11, 12b, 14b
14	a	37.9, CH_2	1.68, m	13, 14b, 15ab	13, 15, 16	13, 15	14b, 16
	b		1.48, m	13, 14a, 15ab	15, 16	13, 15	
15	ab	20.0, CH_2	1.33, m	14ab, 16	13, 16	14, 16	
16	abc	14.1, CH_3	0.92, t (7.6)	15ab	14, 15	15	14ab, 15ab
17	abc	26.0, CH_3	0.94, d (6.9)	9	8, 9, 10	9	8ab, 10a
18		166.9,qC					
19		121.7, CH	5.86, dt (11.7, 1.4)	20	18, 21	20	20
20		150.0, CH	6.33, dt (11.7, 7.6)	19, 21ab	18, 22	19, 21	19, 22
21	ab	29.0, CH_2	2.98, m	20, 22ab	18w, 19, 20, 22, 23	20, 22	22, 24
22	ab	26.4, CH_2	2.68, dd (7.6 7.6)	21ab, 24	20, 21, 23, 24, N-30	21	20, 21, 24
23		142.3, qC					
24		135.9, CH	7.64, s	22ab	23, 25, N-30		22ab, 23ab
25		161.9, qC					
26		115.7, CH	6.24, dt (11.7, 2.1)	27, 28ab	25, 27, 28	27	27
27		139.2, CH	6.02, dt (11.7, 6.2)	26, 28ab	25, N-31	26	26, 28ab
28	ab	41.0, CH_2	4.28, bd (4.8)	26, 27	27, 29, N-31	27	27
29		159.6, qC					
OCH_3-29	abc	52.6, CH_3	3.62, s		29		
N-30		δ_N 254.3					
N-31		δ_N 77.2					

[a] Assignments may be interchanged. [b] HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon. [c] H2BC correlations, optimized for 6 Hz, are from proton(s) stated to the indicated carbon.

biosynthetic origin of the callipeltosides, aurisides, and polycavernoside may be cyanobacteria that either live in association with the macroorganisms or are derived from dietary sources.[16] D'Ambrosio et al. have proposed a microbial origin for the leucascandrolides, which are structurally unprecedented for calcareous sponges.[12] It is interesting to note the presence of neopeltolide (**1**), a structurally related compound in a lithistid sponge that occurs in deep-water habitats where limited if any light is present, and one might speculate that heterotrophic cyanobacteria may be present and responsible for the biosynthesis of at least some of the metabolites found in deep-water lithistid sponges. Alternatively, in all cases the compounds could be produced by heterotrophic bacteria living in association with the organism from which they have been isolated.

Neopeltolide (**1**) is a potent inhibitor of the growth of the fungal pathogen *Candida albicans*. It showed a growth inhibitory zone of 17 mm when tested at a concentration of 25 μg/disk in the *C. albicans* disk diffusion assay and a minimum inhibitory concentration (MIC) in liquid culture of 0.625 μg/mL. Neopeltolide (**1**) is also a potent inhibitor of tumor cell proliferation in vitro. In the MTT assay to determine effects of **1** on cell proliferation, IC_{50} values of 1.2 nM against the A549 human lung adenocarcinoma, 5.1 nM against the NCI/ADR-RES ovarian sarcoma, and 0.56 nM against the P388 murine leukemia were apparent. In the PANC-1 pancreatic cancer cell line and the DLD-1 colorectal adenocarcinoma cell line, both of which have p53 mutations, neopeltolide (**1**) showed strong inhibition of cell proliferation at nanomolar concentrations but did not give the typical sigmoidal curve, and instead showed 50% cell kill over an extended dose range. Accordingly, it may be cytostatic to these cell lines rather than cytotoxic. Similar to callipeltoside A, cell cycle analysis by flow cytometric methods indicated that neopeltolide (**1**) caused a block of the cell cycle at G1 at doses of 100 nM in the A549 lung adenocarcinoma cell line (Table 2). Preliminary investigation into the mechanism of action of neopeltolide (**1**) suggests that it does not act via interaction with tubulin or actin, and additional studies are underway.

Table 2. Effects of Neopeltolide on the Cell Cycle of A549 Cells

treatment	dose (nM)	cells in G1 phase (%)	cells in S phase (%)	cells in G2/M phase (%)
nontreated control		47.13	39.05	13.82
vehicle control		46.55	36.46	13.99
paclitaxel	100	1.56	0.00	98.44
neopeltolide	100	63.03	22.02	14.95
	10	59.66	30.27	10.07
	1	46.11	39.95	13.94
	0.1	46.4	40.01	13.59

Experimental Section

General Experimental Procedures. The IR spectra were collected on a Midac M-1200 with Galactic GRAMS/386 software. The UV spectra were collected on a Hitachi U-3010 spectrophotometer. The optical rotation was measured on a JASCO DIP-370 digital polarimeter. NMR data were collected on a JEOL ECA-600 spectrometer operating at 600.17 MHz for ^{1}H, 150.9 MHz for ^{13}C, and 60.8 MHz for ^{15}N

(instrument reference set to liquid NH_3). The edited-gHSQC spectrum was optimized for 140 Hz, the gHMBC spectrum was optimized for 8 Hz, and the H2BC spectrum was optimized for 6 Hz. The 1D-DPFGSE-TOCSY experiments were conducted with a 5.78 [kHz] spin lock field using a DIPSI2 modulation. Mix times varied depending upon the extent of the correlations but were typically 50 ms. Chemical shifts were referenced to solvent, e.g., CD_3OD, δ_H observed at 3.31 ppm and δ_C observed at 49.0 ppm. Chemical shifts for ^{15}N were referenced to liquid NH_3 with long-range $J_{H,N}$ optimized for 6 Hz. The HRFABMS were measured using a Kratos MS50TC mass spectrometer. Flow cytometry was conducted on a BD FACSCalibur apparatus.

Biological Material. The two specimens used in this study are most closely related to the genus *Daedalopelta* Sollas, 1888 (phylum Porifera; class Demospongiae; order "Lithistida"; family Neopeltidae) as described in *Systema Porifera: A Guide to the Classification of Sponges*.[7] Sample 23-VIII-93-5-008 [Harbor Branch Oceanographic Museum (HBOM) catalog number 003:000959] was collected by manned submersible from a rock outcrop at a depth of 442 m off the northwest coast of Jamaica. It has a cream-colored, stalked, folded plate, with a smooth, porous upper surface and very warty undersurface, approximately 20 cm diameter and 2 cm thick. Sample 23-VIII-93-5-010 [HBOM catalog number 003:01004] was collected nearby at a depth of 433 m. It has a cream-colored stalked plate, slightly folded, with a smooth, porous upper surface, and slightly warty undersurface, approximately 15 cm diameter and 1 cm thick. Both specimens have similar spicules. The ectosomal spicules are pseudophyllotriaenes with irregular narrow cladi, dentate margins, and strongly tuberculated upper surfaces; cladomes are approximately 300 μm in diameter, rhabdomes are smooth, approximately 130 μm long. The choanosomal spicules are monocrepid desmas that resemble complex branched tetraclones and are smooth except for strongly tuberculated, articulated tips. The microscleres are a single type of amphiaster in two size classes: the larger are up to 23 μm long with long spines, the smaller ones are approximately 13 μm long with shorter, denser spines. The distinction between the genera *Callipelta* and *Daedalopelta* is poorly defined in the literature. In *Callipelta*, the pseudophyllotriaenes are tuberculate, and there are two types of amphiasters. In *Daedalopelta*, the pseudophyllotriaenes may be tuberculate, and there is one type of amphiaster, in two size classes. *Callipelta* has been reported only from the Indo-Pacific, whereas *Daedalopelta* has been reported only from Florida and the Bahamas. The original type material has been lost, and the redescription of *Daedalopelta* is based on material from the Harbor Branch collection (HBOM 003:00949, collected from the Bahamas). The two specimens from this study differ in morphology from HBOM 003:00949, but are similar to each other. It is likely that they are a different species from HBOM 003:00949. Pisera and Levi[7] suggest that *Callipelta* and *Daedalopelta* may be merged in the future. For the present, the minor differences in spicule complement coupled with the geographic distribution support our assignment of the specimens to the genus *Daedalopelta*.

Extraction and Isolation. A 105 g sample of the frozen Neopeltidae sponge, 23-VIII-93-5-010, was extracted exhaustively by macerating with ethanol using a Waring blender (5 × 250 mL). The combined filtered extracts were concentrated by distillation under reduced pressure to yield 3.2 g of crude residue. The residue was partitioned between *n*-butanol and water (3 × 50 mL portions). After concentration, the *n*-butanol phase (0.7 g) was separated under vacuum-column chromatographic conditions on a Kieselgel 60 H (EM Science) stationary phase. A 150 mL Büchner funnel fitted with a medium-porosity fritted glass disk was used as the column. The stationary phase was packed to a total height of 4 cm. The butanol partition was applied as a slurry to the column in a mixture of heptane−ethyl acetate (4:1 v/v). Fractions were eluted using a 20% step gradient of ethyl acetate in heptane followed by a series of fractions containing increasing amounts of methanol in ethyl acetate [fraction 1: heptane−ethyl acetate, 4:1 (150 mL); fraction 2: heptane−ethyl acetate, 3:2 (150 mL); fraction 3: heptane−ethyl acetate, 2:3 (150 mL); fraction 4: heptane−ethyl acetate, 1:3 (150 mL); fraction 5: heptane−ethyl acetate, 1:4 (150 mL); fraction 6: ethyl acetate (150 mL); fraction 7: ethyl acetate−methanol, 3:1 v/v (150 mL); fraction 8: ethyl acetate−methanol, 1:1 v/v (150 mL); fraction 9: methanol (150 mL)]. Neopeltolide (**1**) eluted cleanly into fraction 3. Fraction 3 was further separated by HPLC using a Vydac C18 protein and peptide column (10 mm × 250 mm, 10 μm particle size), flow rate 3 mL/min; eluent: water−acetonitrile, 2:3; monitored by UV at 230 nm. Neopeltolide (**1**), yield 4.0×10^{-3}% of frozen weight, eluted after approximately 6.2 column volumes under these conditions.

Neopeltolide (1): colorless oil; $[\alpha]^{24}_D$ +24.0 (*c* 0.24, MeOH); UV (EtOH) λ_{max} (log ϵ) 218 (4.54), 262.4 (4.29) nm; IR (KBr) ν_{max} 3430, 3269, 2914, 1718, 1538, 1345, 1283, 1157 cm; ^1H and ^{13}C NMR data, see Table 1; HRMS *m*/*z* $[M + H]^+$ 591.32692 (calcd for $C_{31}H_{47}O_9N_2$, 591.32816).

Cytotoxicity Assays. Neopeltolide (**1**) was evaluated for its effects on proliferation of the A549 human lung adenocarcinoma (ATCC No. CCL-185), PANC-1 human pancreatic cancer (ATCC No. CRL-1469), DLD-1 human colorectal adenocarcinoma (ATCC No. CCL-221), NCI-ADR-RES (formerly MCF-7/ADR) human ovarian carcinoma, and P388 murine leukemia cell lines. The A549, PANC-1, and DLD-1 cell lines were obtained from the American Type Culture Collection (Rockville, MD). The P388 and NCI/ADR-RES cell lines were obtained from the NCI-Frederick Cancer DCTD Tumor/Cell Line Repository (Bethesda, MD). Assays were run using protocols described previously.[18] All samples were assayed a minimum of three times to derive the final IC_{50} value.

Antifungal Assays. The antifungal activity of neopeltolide (**1**) against *Candida albicans* (American Type Culture Collection strain 44506) was determined through the use of both agar diffusion and broth dilution assays. Agar diffusion assays were performed using Sabouraud dextrose agar plates seeded with the test microbe at 10^5/mL. Disks (6.35 m, Schliecher and Schuell) were impregnated with 25 μg of neopeltolide (**1**), allowed to dry, and then placed on the agar surface. After incubation at 37 °C for 24 h the zones of growth inhibition were determined. A control disk containing 100 IU nystatin was run on the same plate as compound **1**, with the zone of inhibition for nystatin being 28 mm. Broth dilution assays were performed as standard 96-well microtiter assays in a total volume of 50 μL with a seeding density of 10^3 cells/mL. The concentration range for neopeltolide (**1**) ranged from 5 to 0.4 μg/mL as 2-fold dilutions. Plates were incubated at 37 °C for 24 h, at which time the minimum inhibitory concentration (MIC) was determined as the lowest concentration in the test range that completely inhibited growth of *C. albicans*. 5-Fluorocytosine was used as an antifungal control for the assay: the MIC for 5-fluorocytosine was 0.62 μg/mL.

Cell-Cycle Analysis. A549 human lung adenocarcinoma cells were used to observe the effects of neopeltolide (**1**) on the cell cycle. Cell-cycle analysis was performed as follows: A549 cells were incubated in tissue culture media [TCM = Roswell Park Memorial Institute (RPMI) medium 1640 supplemented with 100 U/mL penicillin, 100 mg/mL streptomycin, 60 mg/mL L-glutamine, 18 mM HEPES, 0.05 mg/mL gentamicin, and 10% fetal bovine serum] at 37 °C in 5% CO_2 in air in the presence or absence of varying concentrations of neopeltolide (**1**), methanol (vehicle control), or paclitaxel (positive control) for 24 h. At the end of this incubation, cells were harvested, fixed in ethanol, and stained with 0.02 mg/mL of propidium iodide together with 0.1 mg/mL of RNAse A. Stained preparations were sent to the University of Florida Flow Cytometry Core Laboratory for analysis on a BD FACSCalibur instrument. Measurement of 10 000 events was collected per sample. Raw histogram data were further analyzed using the DNA analysis software ModFit (Verity, Topsham, ME).

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Supporting Information Available: ^1H NMR, ^{13}C NMR, 1D-DPFGSE-TOCSY, and 2D NMR spectra in CD_3OD for neopeltolide (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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