New Lamellarin Alkaloids from the Indian Ascidian *Didemnum obscurum* and Their Antioxidant Properties[⊥]

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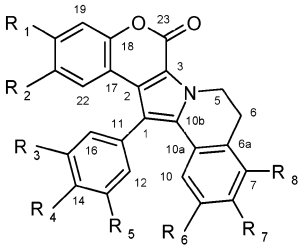
Received December 9, 2003

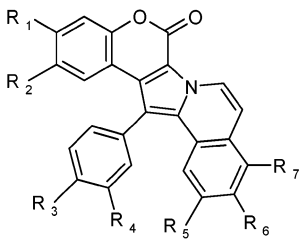
Three new lamellarin alkaloids, lamellarins γ (**1**), α (**2**), and ϵ (**3**), along with eight known lamellarin alkaloids, lamellarins M (**4**), K (**5**), K-diacetate (**6**), K-triacetate (**7**), U (**8**), I (**9**), C-diacetate (**10**), and X-triacetate (**11**), have been isolated from the Indian ascidian *Didemnum obscurum*. The structures of **1**−**11** were established using standard spectroscopic techniques. The structure of lamellarin K-triacetate (**7**) was further confirmed by X-ray crystallographic analysis. The antioxidant properties of lamellarin γ, lamellarin γ-monoacetate, lamellarins K, U, and I, and lamellarin C-diacetate were evaluated.

In recent years, marine ascidians (tunicates) have been widely recognized as a major source of bioactive natural products. The lamellarins are a group of DOPA-[2-amino,3-(3′,4′-dihydroxyphenyl)propionic acid]-derived pyrrole alkaloids, which were first isolated from the prosobranch mollusc *Lamellaria* sp.[1] and later obtained from the genus *Didemnum*.[2,3] Some lamellarin alkaloids have demonstrated significant cytotoxicity, immunomodulatory,[4] and anti-HIV[5] activities. The lamellarin group of alkaloids can be divided into two groups, viz., compounds possessing a Δ^5 olefin and those in which this olefin is absent. These two groups can readily be distinguished by their ^{1}H NMR spectrum.[1−6] In the ^{1}H NMR spectrum of the former group of compounds the H-5 and H-6 resonate at δ ~9.20 (1H, d, J = 7.5 Hz) and 7.40 (1H, d, J = 7.5 Hz), respectively, while in the latter case they resonate at δ ~4.63 (1H, m 5-Ha) and 4.68 (1H, m, 5-H_b); 3.08 (2H, bt, J = 6.0 Hz, 6-H_2). The position of methoxyl groups at C-7, C-8, C-9, C-13, C-14, C-15, and C-21 can be routinely determined by NOESY correlations with adjacent protons.

As a part of our ongoing search for biologically active compounds from Indian ascidians of the Gulf of Mannar,[5,6] we have examined the red colonial tunicate *Didemnum obscurum* collected off Tiruchandur, Tamilnadu, India, during February 2002. The DCM/MeOH extract was subjected to gel filtration chromatography and grouped into two fractions, fraction I and fraction II. Fraction I was purified by silica gel column chromatography followed by a reversed-phase (C_{18}) HPLC column to afford three new lamellarin alkaloids, lamellarins γ (**1**), α (**2**), and ϵ (**3**), and four known alkaloids, lamellarins M (**4**),[3] K (**5**),[3] U (**8**),[6] and I (**9**).[3] Fraction II was acetylated (Ac_2O/NaOAc) and purified over silica gel column chromatography, followed by a reversed-phase (C_{18}) HPLC column to afford the acetylated derivatives of four known lamellarin alkaloids, lamellarins K-diacetate (**6**), K-triacetate (**7**),[3] C-diacetate (**10**),[6] and X-triacetate (**11**).[6] The structures of the known alkaloids were characterized by comparison of their physical and spectral data with those reported in the literature. In addition the structure of lamellarin K-triacetate was confirmed by X-ray crystallographic analysis.



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
1	OH	OMe	OMe	H	OMe	OMe	OMe	OH
1a	OAc	OMe	OMe	H	OMe	OMe	OMe	OH
5	OH	OMe	H	OH	OMe	OMe	OMe	OH
6	OAc	OMe	H	OAc	OMe	OMe	OMe	OH
7	OAc	OMe	H	OAc	OMe	OMe	OMe	OAc
8	OH	OMe	H	OMe	OH	OMe	OMe	H
9	OH	OMe	H	OMe	OMe	OMe	OMe	OMe
10	OAc	OMe	H	OAc	OMe	OMe	OMe	OMe



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
2	OH	OMe	OMe	OH	OMe	OMe	H
3	OH	OMe	OMe	OMe	OMe	OMe	OH
4	OH	OMe	OH	OMe	OMe	OMe	OH
11	OAc	OMe	OMe	OAc	OMe	OMe	OAc

Lamellarin γ (**1**) was isolated as an optically inactive white solid, mp 163−165 °C. The molecular formula $C_{30}H_{27}NO_9$, which requires 18 degrees of unsaturation, was

[⊥] IICT: Communication # 040328.

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10.1021/np030503t CCC: $27.50
Published on Web 06/23/2004

Table 1. Spectral Data of Lamellarin γ (**1**)

position	^{1}H NMR[a] multiplicity (J in Hz)	^{13}C NMR[b]	HMBC[c] (J = 7.0 Hz)
C-1		115.41	
C-2		128.09	
C-3		113.12	
C-5	4.78, t, (7.0)	42.08	C-3, C-6, C-6a
C-6	3.13, t, (7.0)	29.66	C-5, C-6a, C-7, C-10a
C-6a		135.43	
C-7		145.54	
C-8		135.43	
C-9		150.36	
C-10	6.37, s	101.87	C-6a, C-8, C-9, C-10a
C-10a		123.18	
C-10b		123.64	
C-11		128.29	
C-12	7.09, d, (1.7)	112.02	C-11, C-13, C-14, C-15
C-13		148.89	
C-14	7.04, s	114.11	C-13, C-15, C-16
C-15		149.84	
C-16	7.09, d, (1.7)	123.64	C-11, C-13, C-14, C-15
C-17		110.18	
C-18		146.26	
C-19	6.96, s	104.08	C-17, C-18, C-20, C-21
C-20		146.44	
C-21		143.33	
C-22	6.58, s	103.45	C-2, C-17, C-18, C-20, C-21
C-23		155.57	
C-24	3.89, s	56.27	C-8
C-25	3.34, s	55.58	C-9
C-26	3.96, s	56.19	C-13
C-27	3.87, s	61.01	C-15
C-28	3.47, s	55.08	C-21

[a] Measured in $CDCl_3$, 400 MHz. [b]Measured in $CDCl_3$, 75 MHz. [c] Measured in $CDCl_3$, 500 MHz.

determined by high-resolution FAB mass measurement in conjunction with the ^{1}H and ^{13}C NMR data. The IR bands at 3417 and 1698 cm^{-1} indicated the presence of phenolic and aromatic ester functionalities, respectively. The UV absorptions at λ_{max} 365, 335, 331, 310, 278, and 206 nm are similar to those of lamellarin F isolated from *D. chartaceum*.[2] The ^{1}H NMR data of lamellarin γ (**1**) (Table 1) revealed the presence of five methoxyl signals at δ 3.34 (3H, s, H-25), 3.47 (3H, s, H-28), 3.96 (3H, s, H-26), 3.89 (3H, s, H-24), and 3.87 (3H, s, H-27); a 1,3,5-trisubstituted benzene ring at δ 7.04 (1H, s, H-14) and 7.09 (2H, d, J = 1.7 Hz, H-12 and H-16); and three aromatic singlets at δ 6.37 (1H, s, H-10), 6.58 (1H, s, H-22), and 6.96 (1H, s, H-19). Further, its ^{1}H NMR spectrum showed two mutually coupled methylene protons at δ 4.78 (2H, t, J = 7.0 Hz, H_2-5) and 3.13 (2H, t, J = 7.0 Hz, H_2-6) and also two D_2O exchangeable signals at δ 5.95 (1H, s) and 5.70 (1H, s). However, compound **1** formed a monoacetate (**1a**) upon acetylation with AC_2O/NaOAc. The foregoing spectral data indicated that compound **1** belongs to a 5,6-dihydro lamellarin group of alkaloids.[6] The positions of the methoxyl and phenolic hydroxyl groups were determined by the analysis of HMQC, HMBC (Table 1), and ^{1}H−^{1}H NOESY data.

In the ^{1}H−^{1}H NOESY spectrum of **1**, the methoxyl signals at δ 3.47 (3H, s, H-28), 3.87 (3H, s, H-27), 3.96 (3H, s, H-26), and 3.34 (3H, s, H-25) showed correlations with the protons δ 6.58 (1H, s, H-22), 7.04 (1H, s, H-14), 7.09 (1H, J = 1.7, H-12), and 6.37 (1H, s, H-10), respectively. In the HMBC spectrum of **1** the H-22 aromatic proton signal at δ 6.58 (s) showed correlations with carbons at C-2 (128.09), C-17 (110.18), C-18 (146.26), C-20 (146.44), and C-21 (143.33), and the aromatic H-19 proton signal at δ 6.96 (s) showed correlations with carbons at C-17 (110.18), C-18 (146.26), C-20 (146.44), and C-21 (143.33), respectively. Similarly in the trisubstituted aromatic ring the two proton signals at δ 7.09 (2H, d, J = 1.7 Hz, H-12 and H-16) showed correlations with carbons C-11 (128.29), C-13 (148.89), C-14 (114.11), and C-15 (149.84), and the proton signal H-14 at δ 7.04 (s) showed correlations with carbons C-13 (148.89), C-15 (149.84), and C-16 (123.64). The H-10 aromatic proton signal at δ 6.37 (s) showed correlations with carbons at C-6a (135.43), C-8 (135.43), C-9 (150.36), and C-10a (123.18). From the foregoing spectral data the structure of lamellarin γ was assigned as **1**. The difference between lamellarins γ and F is the pattern of the phenyl substituent at C-1. In lamellarin γ, a 1,3,5-trisubstituted phenyl is attached at this position, whereas in lamellarin F the 1,3,4-trisubstituted phenyl group is attached at C-1.

Lamellarin α (**2**) was isolated as an optically inactive white solid, mp > 260 °C. The molecular formula $C_{29}H_{23}NO_8$ was determined by HRFABMS. The UV absorptions at λ_{max} 206, 281, 302, 323, 339, 369, and 387 nm showed additional absorption bands compared to compound **1**, which indicated an additional degree of unsaturation attributed to a 5,6 double bond as observed in lamellarin N.[6] The IR spectrum showed bands at 3358 (phenol) and 1693 cm^{-1} (aromatic ester). The ^{1}H NMR spectra of lamellarin α (**2**) and lamellarin N were similar and differed only in the presence of an additional methoxy at C-8. The ^{1}H NMR spectrum of **2** displayed four methoxyl signals at δ 3.48 (3H, s, H-24), 3.99 (3H, s, H-25), 3.89 (3H, s, H-26), and 3.52 (3H, s, H-27), two *ortho*-coupled aromatic protons at δ 9.25 (1H, d, J = 7.4 Hz, H-5) and 7.06 (1H, d, J = 7.4 Hz, H-6) assigned to the isoquinoline system,[6] three aromatic signals at δ 7.18 (1H, dd, J = 8.5, 1.8 Hz, H-16), 7.21 (1H, d, J = 8.5 Hz, H-15), and 7.08 (1H, d, J = 1.8 Hz, H-12) assigned to a 1,3,4-trisubstituted benzene ring, four aromatic singlets at δ 7.09 (1H, s, H-10), 7.15 (1H, s, H-7), 7.02 (1H, s, H-19), and 6.72 (1H, s, H-22), and two overlapping D_2O exchangeable broad singlets at δ 5.82 (2H, brs). The foregoing spectral data indicated that compound **2** belonged to the lamellarin group of alkaloids[7] and also possessed a structure similar to lamellarin N.[6] The position of the methoxyl groups was determined by the analysis of the ^{1}H−^{1}H NOESY spectrum. In its NOESY spectrum the methoxyl groups at δ 3.48 (3H, s, H-24), 3.99 (3H, s, H-25), 3.89 (3H, s, H-26), and 3.52 (3H, s, H-27)) showed correlations with δ 7.15 (1H, s, H-7), 7.09 (1H, s, H-10), 7.21 (1H, d, J = 8.5 Hz, H-15), and 6.72 (1H, s, H-22), respectively. However, lamellarin α (**2**) is a synthetically known compound.[8]

Lamellarin ϵ (**3**) was also isolated as an optically inactive white solid, mp 271−275 °C. The molecular formula $C_{30}H_{25}NO_9$ was determined by HRFABMS. The UV [λ_{max} 209, 277, 304, 325, 329, 367, and 387 nm] and IR [3357 (phenol) and 1694 (aromatic ester) cm^{-1}] spectra of **3** are similar to those of compound **2** and are closely related to those reported for lamellarin X.[6] The ^{1}H NMR spectrum of **3** was found to be similar to that of lamellarin X, differing only in the replacement of a hydroxyl group with a methoxy group. The ^{1}H NMR spectrum of **3** contained five methoxyls at δ 4.00 (3H, s, H-24), 3.44 (3H, s, H-25), 3.90 (3H, s, H-26), 3.94 (3H, s, H-27), and 3.50 (3H, s, H-28), two *ortho*-coupled peripheral aromatic protons at δ 9.20 (1H, d, J = 7.5 Hz, H-5) and 7.42 (1H, d, J = 7.5 Hz, H-6) assigned to the isoquinoline system, three aromatic signals at δ 7.14 (1H, d, J = 1.8 Hz, H-12), 7.15 (1H, d, J = 8.1 Hz, H-15), and 7.20 (1H, dd, J = 8.1, 1.8 Hz, H-16) attributed to a 1,3,4-trisubstituted benzene ring, three aromatic singlets at δ 6.79 (1H, s, H-10), 7.01 (1H, s, H-19), and 6.67 (1H, s, H-22), and two broad D_2O exchangeable singlets at δ 5.80 (1H, brs) and 6.23 (1H, brs). These spectral data indicated that

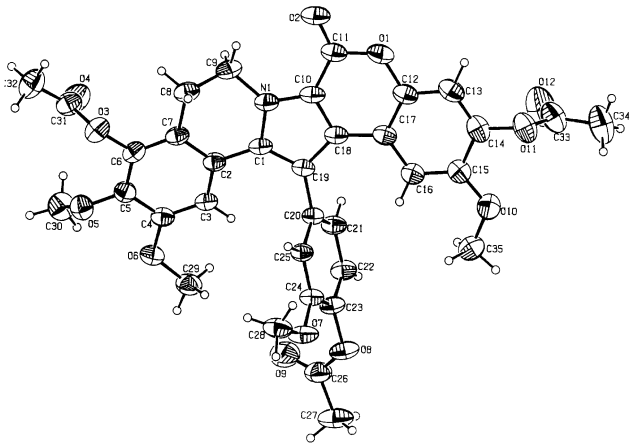

Figure 1. ORTEP projection of lamellarin K-triacetate (**7**).

Table 2. Free Radical Scavenging Activities of the Isolates Using the DPPH-HPLC Method

compd tested	% radical scavenging activity[a]				
	1[b]	2	3	4	5
1	4.98	84.28	87.37	90.82	92.42
1a	0	21.68	43.36	65.04	86.72
5	0	34.71	48.26	69.51	88.83
8	0	25.18	41.31	60.72	79.32
9	1.93	75.11	159.20	237.81	316.41
10	0	0	30.95	61.90	92.84

[a] % radical scavenging = $[(PA_{BLANK} - PA_{SAMPLE}) \times 100]/PA_{BLANK}$. [b] Concentrations in mg/mL.

compound **3** also belonged to the lamellarin class of alkaloids and possessed a structure similar to lamellarin X.[6] The positions of the methoxyl and phenolic hydroxyl groups were determined by the analysis of $^1H-^1H$ NOESY spectra. In the $^1H-^1H$ NOESY spectrum of **3** the methoxyl groups at δ 3.44 (3H, s, H-25), 3.90 (3H, s, H-26), 3.94 (3H, s, H-27), and 3.50 (3H, s, H-28) showed correlations with aromatic protons at δ 6.79 (1H, s, H-10), 7.14 (1H, d, J = 1.8 Hz, H-12), 7.15 (1H, d, J = 8.1 Hz, H-15), and 6.67 (1H, s, H-22), respectively.

Lamellarin K-triacetate (**7**) was crystallized as colorless monoclinic needles from methanol. The computer-generated drawing of the final X-ray model of lamellarin K-triacetate (**7**) is shown in Figure 1. The X-ray structure showed that the crystal was racemic, so the atropisomer shown is accompanied by an equal amount of its enantiomer. The racemic nature of lamellarin K-triacetate (**7**) was also indicated by its lack of optical activity. The structure of lamellarin K-triacetate is divided into two planar portions. The dihedral angle between the two mean planes [(C-1−C-19) and (C-20−C-25)] is 110.7° (±3).

The lamellarin group of alkaloids were reported to be cytotoxic,[3] and some showed good antitumor activity against both multidrug resistance and their corresponding parental cell lines.[9] Many antioxidant compounds possess anticancer or anticancerogenic properties.[10−12] Hence the lamellarin compounds **1**, **1a**, **5**, **8**, **9**, and **10** were tested for antioxidant activity. The antioxidant activity was evaluated using a standardized HPLC method, which involves scavenging of DPPH free radical.[13,14] The Trolox equivalent antioxidant capacity (TEAC) was expressed as the ratio of the IC_{50} of Trolox to that of the standard antioxidants (α-tocopherol and probucol) and test compounds. The percent radical scavenging activity of the test compounds at various concentrations is given in Table 2. The concentration of 50% DPPH free radical scavenging (IC_{50}) and the TEAC values are given in Table 3. As evident from the IC_{50} values

Table 3. 50% DPPH Free Radical Scavenging Activity (IC_{50}) Concentrations of Test Compounds and Their Trolox Equivalent Antioxidant Capacity (TEAC) Compared to Probucol and α-Tocopherol

compound	free radical scavenging activity, IC_{50}[a]	TEAC[b]
1	3.28 mM	0.02
1a	5.63 mM	0.01
5	5.80 mM	0.01
8	6.70 mM	0.01
9	2.96 mM	0.02
10	5.87 mM	0.01
probucol	52.66 μM	1.29
α-tocopherol	63.57 μM	1.07
Trolox	67.78 μM	1.00

[a] IC_{50} values were determined by linear regression analysis using at least five different concentrations in triplicate. [b] TEAC = IC_{50} Trolox/IC_{50} test compound.

(Table 3), all the tested compounds possessed antioxidant activity only at higher concentrations (in mM) against the standard antioxidants used, which are active in the μM range. α-Tocopherol, a lipophilic derivative of Trolox, has a similar TEAC (1.07) value, which is consistent with the results reported earlier.[13] The decrease in the potency of antioxidant activity of the test compounds may be attributed to the absence of a hydroxyl group at C-8 in compounds **1**, **1a**, **5**, **8**, **9**, and **10**, which is in agreement with the previous report that the hydroxyl groups at positions C-8 and C-20 were important structural requirements for cytotoxic activity.[15] Also the presence of different functional groups at C-14 (H in **1**, **1a**; OH in **5**; OMe in **8**, **9**; OAc in **10**) did not produce marked changes in the antioxidant activity. Similar results were reported by Ishibashi et al.[15] that a hydroxyl group at C-14 was not necessary for the activity. Among the tested compounds, **1** and **9** were more potent than their counterparts. The rank order from the most active to the least active compound was **9** > **1** > **1a** > **5** > **10** > **8** (based on IC_{50} and TEAC values from Table 3).

Experimental Section

General Experimental Procedures. Melting points were obtained on a Mel-Temp apparatus and are uncorrected. The optical rotations were measured on a JASCO DIP-370 polarimeter. UV and IR spectra were recorded on Shimadzu-240 and Perkin-Elmer 240-C instruments, respectively. The ^1H NMR (500 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian Gemini 500 MHz spectrometer using TMS as internal standard. Chemical shifts are reported in parts per million, and coupling constants (J) are expressed in hertz. The MS were recorded on a VG Auto Spec-M instrument. Preparative scale HPLC was performed using a Supelcosil C_{18} column (60 A^0, 12 μm, 25 cm × 21.2 mm).

Animal Material. The ascidian *Didemnum obscurum* F. Monniot, 1969 (Didemnidae) was collected from the Tiruchandur coast in the Gulf of Mannar, Tamilnadu, India, during February 2002. A voucher specimen (IIC-467) is on deposit at the National Institute of Oceanography, Goa, India.

Extraction and Isolation. The freshly collected ascidian specimens (1.5 kg wet weight) were soaked in MeOH at the site of collection until workup. The initial methanol extract was decanted and the ascidian material extracted with fresh 1:1 CH_2Cl_2/MeOH (3 × 3 L) at room temperature. The combined extract including initial methanol extract was filtered, and the solvent was removed under reduced pressure to a predominantly aqueous suspension, extracted into ethyl acetate (3 × 1 L), and concentrated under reduced pressure to give a dark brown gummy mass (10 g). This crude extract (10 g) was subjected to gel filtration chromatography (Sepha-

dex LH-20, 1:1 CH_2Cl_2/MeOH, 47 mm × 820 mm) by collecting a total of 30 continuous fractions (25 mL each). Following the TLC pattern the 30 continuous fractions were pooled into two fractions, fractions I and II. Fraction I was subjected to a reversed-phase (C_{18}) HPLC column [acetonitrile/water (60:40)], at a flow rate of 3.5 mL/min, to afford three new lamellarin alkaloids, lamellarins γ (**1**), α (**2**), and ε (**3**), and four known alkaloids, lamellarins M (**4**,[3] 3 mg), K (**5**,[3] 4 mg), U (**8**,[6] 2 mg), and I (**9**,[3] 2 mg). Fraction II was acetylated (Ac_2O/NaOAc) after the usual workup, and the crude acetylated product was subjected to silica gel column chromatography eluting with chloroform and chloroform/methanol mixtures. The fractions thus obtained were purified by using reversed-phase (C_{18}) HPLC column chromatography [acetonitrile/water (60:40)], at a flow rate of 3.5 mL/min, to afford four known lamellarin alkaloids as acetates, lamellarins K-diacetate (**6**, 10 mg), K-triacetate (**7**,[3] 5 mg), C-diacetate (**10**, 2 mg), and X-triacetate (**11**, 3 mg).

Lamellarin γ (1): white solid (6 mg); mp 163−165 °C; IR (KBr) ν_{max} 3417, 2915, 1698, 1428, 1244, and 1030 cm^{-1}; UV (MeOH) λ_{max} (log ε) 206 (1.8775), 278 (1.6549), 310 (1.6342), 331 (1.549), and 365 (0.8659); ^1H NMR (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (75 MHz, $CDCl_3$), see Table 1; HRFABMS *m/z* 546.1752 (calcd for $C_{30}H_{27}NO_9$, 546.1764); FABMS obsd *m/z* (%) 546 ($M^+ + 1$, 25), 545 (M^+, 25), 465 (18), 329 (6), 307 (25), 289 (14), 259 (8), 228 (26), 176 (12), 154 (100), 136 (93), 120 (18), 107 (30), 89 (22), 75 (60).

Lamellarin γ (1a): white solid (2 mg); ^1H NMR (400 MHz, $CDCl_3$) 4.80 (2H, m, Ha, Hb-5), 3.15 (2H, m, Ha, Hb-6), 6.38 (1H, s, H-10), 7.03 (1H, d, J = 2.0 Hz, H-12), 7.07 (1H, d, J = 2.0 Hz, H-12), 7.03 (1H, d, J = 2.0 Hz, H-14), 7.09 (1H, d, J = 2 Hz, H-16), 7.08 (1H, d, J = 2 Hz, H-19), 6.73 (1H, s, H-22), 3.89 (3H, s, H-24), 3.34 (3H, s, H-25), 3.96 (3H, s, H-26), 3.87 (3H, s, H-27), 3.42 (3H, s, H-28) 5.95 (1H, brs); [M^+, $C_{32}H_{30}NO_{11}$]; FABMS obsd *m/z* (%) 588.

Lamellarin α (2): white solid (5 mg); mp 228−30 °C; IR (KBr) ν_{max} 3358, 2925, 1693, 1658, 1631, 1552, 1428, 1269, 1223, 1042, 858, and 755 cm^{-1}; UV (MeOH) λ_{max} (log ε) 206 (1.994), 281 (1.910), 302 (1.7889), 323 (1.5374), 369 (1.398), and 387 (1.49712); ^1H NMR (400 MHz, $CDCl_3$) 9.25 (1H d, J = 7.4 Hz, H-5), 7.06 (1H, d, J = 7.4 Hz, H-6), 7.15 (1H, s, H-7), 7.09 (1H, s, H-10), 7.08 (1H, d, J = 1.8 Hz, H-12), 7.21 (1H, d, J = 8.5 Hz, H-15), 7.18 (1H, dd, J = 8.5, 1.8 Hz, H-16), 7.02 (1H, s, H-19), 6.72 (1H, s, H-22), 3.48 (3H, s, H-24), 3.99 (3H, s, H-25), 3.89 (3H, s, H-26), 3.52 (3H, s, H-27), 5.82 (2H, brs); HRFABMS *m/z* 514.1499 (calcd for $C_{29}H_{23}NO_8$, 514.1501); FABMS obsd *m/z* (%) 514 ($M^+ + 1$, 12), 368 (4), 340 (4), 314 (4), 137 (12), 119 (10), 109 (20), 95 (42), 83 (43), 67 (38), 55 (100).

Lamellarin ε (3): white solid (5 mg); mp 271−275 °C; IR (KBr) ν_{max} 3357, 2931, 1694, 1614, 1532, 1430, 1351, 1234, 1044, 860, 802, and 757 cm^{-1}; UV (MeOH) λ_{max} (log ε) 209 (2.6066), 277 (2.3723), 304 (2.333), 325 (2.1037), and 367 (1.6923); ^1H NMR (400 MHz, $CDCl_3$) 9.20 (1H d, J = 7.5 Hz, H-5), 7.42 (1H, d, J = 7.5 Hz, H-6), 6.79 (1H, s, H-10), 7.14 (1H, d, J = 1.8 Hz, H-12), 7.15 (1H, d, J = 8.1 Hz, H-15), 7.20 (1H, dd, J = 8.1, 1.8 Hz, H-16), 7.01 (1H, s, H-19), 6.67 (1H, s, H-22), 4.00 (3H, s, H-24), 3.44 (3H, s, H-25), 3.90 (3H, s, H-26), 3.94 (3H, s, H-27), 3.50 (3H, s, H-28), 6.23 (1H, brs), 5.80 (1H, brs); HRFABMS *m/z* 544.1603 (calcd for $C_{30}H_{25}NO_9$, 544.1607); FABMS obsd *m/z* (%) 544 ($M^+ + 1$, 6), 414 (8), 392 (6), 330 (16), 308 (12), 260 (6), 192 (18), 176 (62), 154 (74), 136 (86), 123 (22), 107 (44), 95 (52), 81 (40), 69 (74), 55 (100), 43 (85).

Crystal data for lamellarin K-triacetate (7): molecular formula $C_{35}H_{31}NO_{12}$, crystal size (mm) 0.19 × 0.25 × 0.28, colorless needles; crystal system monoclinic; space group $P2_1/c$; unit cell dimensions a = 10.5305(6) Å, b = 12.3030(7) Å, c = 24.7235(15) Å, β = 92.945(1)°; volume 3198.9(3) $Å^3$; Z = 4; formula weight = 657.61; density (calc) = 1.365 Mg/m^3; absorption coefficient 0.104 mm^{-1}; F(000) 1376. The reflection data were collected on a Bruker Smart Apex CCD diffractometer using graphite-monochromatic Mo Kα radiation (λ = 0.71073 Å), a total of 19 590 reflections were collected in the range 1.65° ≤ θ ≤ 27.99°, of which 3772 were unique reflections with $I \geq 2\sigma(I)$ and were used for refinement. The final R was 0.065. The structure was solved by direct methods using SHELXTL-Plus. Hydrogen atoms were included at calculated positions and were refined as a riding model along with the atoms to which they were attached.

Antioxidant Activity. Sample Preparation. DPPH, Trolox, probucol, and α-tocopherol were soluble in methanol. Compounds **1**, **5**, **8**, and **9** were soluble in methanol. Compounds **1a** and **10** were soluble in DMSO. Different concentrations (100 μL) of standard antioxidants or test compounds in methanol were prepared and added to 100 μL of DPPH in methanol (final concentration 250 μM), vortexed for a few seconds, and left to stand in the dark for 20 min at room temperature. Fresh DPPH stock solutions were used on each day of analysis. The sample was filtered through a 0.2 μm nylon membrane filter (Pall Gelman Laboratory), and an aliquot (20 μL) of the sample was injected for HPLC analysis.

Chromatographic Conditions. The reversed-phase HPLC system consisted of a Shimadzu HPLC system (LC-10 Ai, Japan) consisting of a pump (LC-10 Ai), a system controller (SCL-10AVP), an auto-injector (SIL-10 ADVP), and a diode array detector (SPD-M10 AVP). Data analysis and processing were done by class LC-10 software (version 1.6). Analyses were carried out using a LiChrospher 100 RP-18e column (250 × 4 mm, 5 μM) (Merck, Darmstadt, Germany). Isocratic elution was carried out with methanol/water (80:20, v/v) at a flow rate of 1 mL/min. The DPPH peaks were monitored at 517 nm.

Calculations. The difference in the reduction of DPPH peak area (PA) between the blank and the sample was used for determining the percent radical scavenging activity of the sample. % Radical scavenging = $(PA_{BLANK} - PA_{SAMPLE}) \times 100/PA_{BLANK}$.

Acknowledgment. We are thankful to Dr. V. K. Meenakshi, Department of Zoology, APC Mahalaxmi College for Woman, Tuticorin-628 002, Tamilnadu, India, for identifying the ascidian, the Department of Ocean Development, New Delhi, India, for financial assistance, Dr. J. S. Yadav, Director IICT for his encouragement, and CSIR, New Delhi, for providing fellowships to P.K. and V.L.N.R.

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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NP030503T