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New Lamellarin Alkaloids from an Unidentified Ascidian from the Arabian Sea

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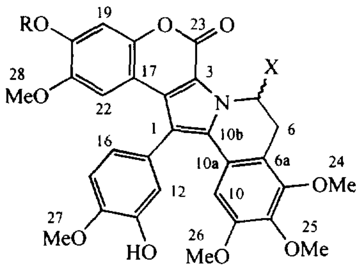
Abstract: An unidentified ascidian from the Arabian Sea contained nine new alkaloids of the lamellarin class together with lamellarin N. The structures of the 20-sulfate derivatives of lamellarins T, U, V, and Y (**1-4**) and lamellarins T - X (**5-9**) were identified by interpretation of spectroscopic data. This is the first report of lamellarin sulfates. © 1997 Elsevier Science Ltd. All rights reserved.

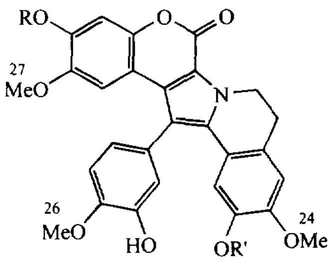
The lamellarins are a group of DOPA-derived pyrrole alkaloids that were first isolated from the prosobranch mollusc *Lamellaria* sp.[1] They were later obtained from ascidians of the genus *Didemnum*.[2-4] Some lamellarin alkaloids have demonstrated significant cytotoxicity and immunomodulatory activities.[1,3] In this paper,[5] we report a study of an unidentified ascidian (collection # IIC-197),[6] obtained from the Trivandrum coast of India, that contained lamellarin N and nine new lamellarin alkaloids.

Specimens of the ascidian IIC-197 were stored in methanol then extracted with 1:1 methanol-dichloromethane. The methanol-soluble material from the combined extracts was chromatographed on Sephadex LH-20 to obtain two UV-active fractions that were further purified by reversed phase HPLC to obtain lamellarin T 20-sulfate (**1**, 0.033% dry weight), lamellarin U 20-sulfate (**2**, 0.028% dry weight), lamellarin V 20-sulfate (**3**, 0.035% dry weight), lamellarin Y 20-sulfate (**4**, 0.003% dry weight) and lamellarins T (**5**, 0.014% dry weight), U (**6**, 0.008% dry weight), V (**7**, 0.014% dry weight), W (**8**, 0.028% dry weight), X (**9**, 0.003% dry weight) and N (**10**, 0.019% dry weight). Lamellarin N (**10**) had previously been isolated only as the corresponding triacetate (**11**).

The major metabolite, lamellarin T 20-sulfate (**1**), was isolated as an optically inactive white solid. The molecular formula, $C_{30}H_{26}NO_{12}SNa$, which requires 18 degrees of unsaturation (not counting the sulfate),



1 R = SO_3Na, X = H (T 20-sulfate)
3 R = SO_3Na, X = OH (V 20-sulfate)
5 R = X = H (T)
7 R = H, X = OH (V)



2 R = SO_3Na, R' = Me (U 20-sulfate)
4 R = SO_3Na, R' = H (Y 20-sulfate)
6 R = H, R' = Me (U)

was determined by high resolution mass measurement in conjunction with the ^{1}H and ^{13}C NMR data. The IR spectrum contained bands at 3500 (phenolic OH), 1695 (aromatic ester), and 1050 cm^{-1} (sulfate). The UV absorptions at 330 (sh), 309, 274, 265 (sh), and 204 nm underwent a bathochromic shift on addition of base, indicating the presence of a phenol. The ^{1}H NMR spectrum (DMSO-d_6) contained 5 methoxy signals at δ 3.30, 3.36, 3.75, 3.80 and 3.84, two mutually-coupled methylene signals at 3.09 (br t, 2 H, J = 6 Hz), 4.63 (m, 1 H) and 4.68 (m, 1 H), and signals at 6.90 (d, 1 H, J = 2 Hz), 6.91 (dd, 1 H, J = 8, 2 Hz) and 7.17 (d, 1 H, J = 8 Hz), assigned to a 1,3,4-trisubstituted benzene ring, 6.62 (s, 1 H), 6.69 (s, 1 H) and 7.49 (s, 1 H), due to aromatic protons and an exchangeable signal at 9.32 (s, 1 H). The ^{13}C NMR spectrum contained a carbonyl signal at δ 154.2, two methylene signals at 41.7 and 21.4, 5 methoxy signals and 22 signals in the aromatic region, which required that the compound be hexacyclic. These data are indicative of a lamellarin alkaloid with no substituent at C-5, exemplified by lamellarin C (**12**).[1]

The positions of the methoxyl groups, the phenol and the sulfate were determined by analysis of HMQC and HMBC experiments. The aromatic proton singlets at δ 6.69 and 7.49 showed one-bond correlations to the carbon signals at 105.1 and 108.5, respectively. The signal at δ 7.49, which is shifted downfield from a "normal" position (cf. 6.80 in **5**) by the adjacent sulfate group,[7] showed long-range correlations to C-17, C-18, C-20 and C-21, while the proton signal at 6.69 was correlated to C-2, C-18, C-20 and C-21 and the methoxyl signal at δ 3.36 showed a 3-bond correlation to C-21. In the trisubstituted aromatic ring, the methoxyl signal at δ 3.84 showed a 3-bond correlation to C-14 and the phenolic proton at 9.32 showed long-range correlations to C-12, C-13 and C-14. The singlet at δ 6.62, which is shifted upfield by the ring current of the trisubstituted aromatic ring, showed a long-range correlation to C-6a, which is also correlated to the H-5 signal: these two observations, in conjunction with the C-10 chemical shift and the HMBC correlations from the 3 remaining methoxyl groups, established the substution pattern about the final aromatic ring. The NMR assignments in Table 1 agree well with literature values for similar compounds.[2-4]

Table 1. NMR data for lamellarin T 20-sulfate (**1**) and lamellarin T (**5**) in DMSO-d_6.

		lamellarin T 20-sulfate (**1**)			lamellarin T (**5**)	
C#	δ_C	δ_H	mult, J (Hz)	HMBC	δ_H	mult, J (Hz)
1	115.7					
2	126.7					
3	113.4					
5	41.7	4.63	m	C-6a	4.63	m
		4.68	m	C-6a	4.68	m
6	21.4	3.09	t, 2 H, 6	C-5, C-6a. C-7, C-10a	3.07	br t, 2 H, 6
6a	120.1					
7	150.3					
8	142.0					
9	151.4					
10	105.2	6.62	s, 1 H	C-6a, C-8, C-9, C-10b	6.61	s, 1 H
10a	122.4					
10b	134.7					
11	126.9					
12	117.7	6.90	d, 1 H, 2	C-1, C-14, C-16	6.88	d, 1 H, 2
13	147.6					
14	147.8					
15	113.6	7.18	d, 1 H, 8	C-11, C-13	7.16	d, 1 H, 8
16	121.5	6.91	dd, 1 H, 8, 2	C-1, C-12, C-14	6.90	dd, 1 H, 8, 2
17	112.0					
18	144.5					
19	108.9	7.49	s, 1 H	C-17, C-18, C-20, C-21	6.80	s, 1 H
20	142.4					
21	146.8					
22	105.1	6.69	s, 1 H	C-2, C-18, C-20, C-21	6.65	s, 1 H
23	154.2					
24	60.2	3.80	s, 3 H	C-7	3.80	s, 3 H
25	60.5	3.75	s, 3 H	C-8	3.74	s, 3 H
26	56.1	3.30	s, 3 H	C-9	3.30	s, 3 H
27	55.1	3.84	s, 3 H	C-14	3.84	s, 3 H
28	54.8	3.36	s, 3 H	C-21	3.37	s, 3 H
OH-13		9.32	s, 1 H	C-12, C-13, C-14	9.30	s, 1 H
OH-20					9.67	s, 1 H

Lamellarin T (**5**) was isolated as an optically inactive white powder. The molecular formula, $C_{30}H_{27}NO_9$, which was determined by high resolution mass spectrometry in conjunction with the ^1H and ^{13}C NMR data, suggested the replacement of the sulfate group in **1** by a phenol. Comparison of the ^1H NMR data with those of sulfate **1** (see Table 1) revealed the presence of a second phenolic proton signal at δ 9.67. Furthermore, the H-19 signal had moved from δ 7.49 in the sulfate **1** to 6.80 in the phenol **5**, with almost no change in the chemical shifts of all other signals. To confirm the proposed relationship, lamellarin T 20-sulfate (**1**) was converted into lamellarin T (**5**) by solvolysis in 1:1 pyridine/dioxane at 80 °C for 1 hr.

Table 2. NMR data for lamellarin U 20-sulfate (**2**), lamellarin U (**6**) and lamellarin Y 20-sulfate (**4**) in DMSO-d_6.

		lamellarin U 20-sulfate (**2**)			lamellarin U (**6**)		lamellarin Y 20-sulfate (**4**)	
C#	δ_C	δ_H	mult, J (Hz)	HMBC	δ_H	mult, J (Hz)	δ_H	mult, J (Hz)
1	114.8							
2	126.7							
3	113.1							
5	42.0	4.63	m	C-6, C-6a	4.63	m	4.62	br t, 2 H, 6
		4.68	m	C-6, C-6a	4.68	m		
6	27.6	3.10	t, 2 H, 6	C-5, C-6a, C-7, C-10a	3.08	br t, 2 H, 6	3.06	br t, 2 H, 6
6a	127.0							
7	111.8	6.98	s, 1 H	C-6, C-8, C-9, C-10a	6.97	s, 1 H	6.93	s, 1 H
8	148.9							
9	147.0							
10	108.6	6.70	s, 1 H	C-6a, C-8, C-9, C-10b	6.68	s, 1 H	6.51	s, 1 H
10a	119.2							
10b	135.4							
11	127.0							
12	117.8	6.89	d, 1 H, 2	C-1, C-14, C-16	6.87	d, 1 H, 2	6.82	d, 1 H, 2
13	147.6							
14	147.7							
15	113.5	7.16	d, 1 H, 8	C-11, C-13	7.15	d, 1 H, 8	7.12	d, 1 H, 8
16	121.5	6.90	dd, 1 H, 8, 2	C-1, C-12, C-14	6.89	dd, 1 H, 8, 2	6.83	dd, 1 H, 8, 2
17	112.0							
18	144.6							
19	108.8	7.47	s, 1 H	C-17, C-18, C-20, C-21	6.79	s, 1 H	7.44	s, 1 H
20	142.3							
21	146.7							
22	105.1	6.71	s, 1 H	C-2, C-20, C-21	6.67	s, 1 H	6.66	s, 1 H
23	154.2							
24	55.6	3.76	s, 3 H	C-8	3.76	s, 3 H	3.78	s, 3 H
25	54.5	3.25	s, 3 H	C-9	3.25	s, 3 H		
26	56.0	3.82	s, 3 H	C-14	3.82	s, 3 H	3.84	s, 3 H
27	55.1	3.35	s, 3 H	C-21	3.37	s, 3 H	3.32	s, 3 H
OH-9							8.82	s, 1 H
OH-13		9.30	s, 1 H	C-12, C-13, C-14	9.27	s, 1 H	9.28	s, 1 H
OH-20					9.64	s, 1 H		

Lamellarin U 20-sulfate (**2**) was obtained, after lyophilization of an HPLC fraction, as an optically inactive white powder. The molecular formula, $C_{29}H_{24}NO_{11}SNa$, which again requires 18 degrees of unsaturation, suggested that **2** differed from **1** by replacement of a methoxyl group by hydrogen. As expected, the ^{1}H NMR spectrum contained only four methoxyl signals at δ 3.25, 3.35, 3.76 and 3.82. The additional aromatic proton signal took the form of a singlet at δ 6.98, which, since it is not coupled to any other signal, can only be placed at C-7. The HMQC and HMBC experiments revealed a one-bond correlation to δ 111.8 (C-7) and long-range correlations to C-6, C-8, C-9 and C-10a. The proposed structure for **2** was fully supported by analysis of the NMR data (Table 2).

Lamellarin U (**6**) was isolated as a white solid. The molecular formula, $C_{29}H_{25}NO_8$, suggested that lamellarin U was the desulfated analog of sulfate **2**. The absence of the 20-sufate group was supported by the observation of an upfield shift of the H-19 signal from δ 7.47 in **2** to 6.79 in **6** and the presence of an additional exchangable phenolic proton signal at 9.64. All other ^{1}H NMR signals were essentially the same for both compounds **2** and **6** (Table 2).

Lamellarin V 20-sulfate (**3**) was obtained, after lyophilization of an HPLC fraction, as an optically inactive white powder. The molecular formula, $C_{30}H_{26}NO_{13}SNa$, which again requires 18 degrees of unsaturation, suggested that **3** differed from **1** by addition of a hydroxyl group. Examination of the ^{1}H NMR spectrum (Table 3) revealed that many of the signals were doubled, a situation that we had observed previously for lamellarin A (**13**), which occurs as an inseparable mixture of possible atropisomers due to restricted rotation about the C1-C11 bond.[1] The H-5 signal was observed at δ 6.83 (m, 1 H), replacing the

Table 3. ^{1}H NMR data (400 MHz, DMSO-d_6) for lamellarin V 20-sulfate (**3**) and lamellarin V (**7**).

C#	δ_H	δ_H	mult, J (Hz)	δ_H	δ_H	mult, J (Hz)
5	6.83		m, 1 H	6.79		m, 1 H
6	3.06	3.10	br t, each 0.5 H, 4	3.02	3.06	m, each 0.5 H
	3.29	3.30	br d, each 0.5 H, 2	3.28	3.30	m, each 0.5 H
10	6.67	6.68	s, each 0.5 H	6.62	6.63	s, each 0.5H
12	6.75	7.07	d, each 0.5 H, 2	6.73	7.04	d, each 0.5 H, 2
15	7.13	7.26	d, each 0.5 H, 8	7.11	7.23	d, each 0.5 H, 8
16	6.75	7.07	dd, each 0.5 H, 2, 8	6.73	7.04	dd, each 0.5 H, 2, 8
19	7.51		s, 1 H	6.81		s, 1 H
22	6.66	6.67	s, each 0.5 H	6.62	6.66	s, each 0.5 H
24	3.78		s, 3 H	3.77		s, 3 H
25	3.75		s, 3 H	3.74		s, 3 H
26	3.30		s, 3 H	3.29	3.30	s, each 1.5 H
27	3.84	3.85	s, each 1.5 H	3.82	3.83	s, each 1.5 H
28	3.35	3.36	s, each 1.5 H	3.36		s, 3 H
OH-5	6.54		m, 1 H	6.45		br d, 1 H
OH-13	9.29	9.44	s, each 1.5 H	9.26	9.40	s, each 1.5 H
OH-20				9.67	9.71	s, each 1.5 H

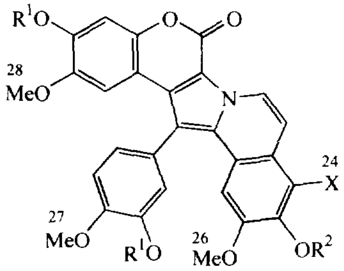
Table 4. NMR data for lamellarin W (**8**) and lamellarin X (**9**) in DMSO-d_6.

	lamellarin W (**8**)				lamellarin X (**9**)	
C#	δ_C	δ_H	mult, *J* (Hz)	HMBC	δ_H	mult, *J* (Hz)
1	111.9					
2	128.7					
3	107.1					
5	122.1	9.03	d, 1 H, 7.5	C-3, C-6, C-6a, C-10b	9.00	d, 1 H, 7.5
6	106.8	7.38	d, 1 H, 7.5	C-5, C-6a, C-7, C-10a	7.45	d, 1 H, 7.5
6a	118.5					
7	148.0					
8	141.8					
9	153.0					
10	101.4	7.04	s, 1 H	C-6a, C-8, C-9, C-10b	6.86	s, 1 H
10a	120.6					
10b	132.8					
11	127.0					
12	118.1	6.98	d, 1 H, 2	C-1, C-14, C-15, C-16	7.69	d, 1 H, 2
13	147.7					
14	148.0					
15	113.7	7.22	d, 1 H, 8	C-11, C-13	7.23	d, 1 H, 8
16	121.9	6.99	dd, 1 H, 8, 2	C-1, C-12, C-14	7.00	dd, 1 H, 8, 2
17	108.0					
18	147.9					
19	103.7	6.86	s, 1 H	C-17, C-18, C-20, C-21	6.80	s, 1 H
20	146.2					
21	144.6					
22	105.6	6.72	s, 1 H	C-2, C-18, C-20, C-21	6.73	s, 1 H
23	154.3					
24	61.6	3.93	s, 3 H	C-7		
25	60.7	3.81	s, 3 H	C-8	3.72	s, 3 H
26	55.0	3.37	s, 3 H	C-9	3.37	s, 3 H
27	56.1	3.85	s, 3 H	C-14	3.85	s, 3 H
28	54.8	3.36	s, 3 H	C-21	3.38	s, 3 H
OH-13		9.37	s, 1 H	C-12, C-13, C-14		
OH-20		9.84	s, 1 H	C-19, C-21		

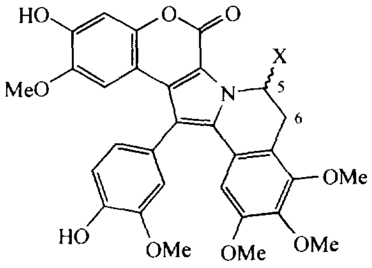
placement of the methoxy and phenol groups was entirely dependent on comparison of the ^{1}H NMR data (Table 4) with those of lamellarins B,[1] M ,[3] N (**10**) and W (**8**), which suggested that the four methoxyl groups should be placed at C-8, C-9, C-14 and C-21 with the phenolic hydroxyl groups at C-7, C-13 and C-20.

Lamellarin N (**10**) was isolated as a pale yellow solid. The molecular formula, $C_{28}H_{21}NO_8$, and UV spectrum suggested that **10** was related to **8** and **9** and that one of the phenolic hydroxyl or methoxyl groups was replaced by hydrogen. The ^{1}H NMR spectrum contained 3 methoxyl and 3 phenol groups. When compared with the ^{1}H NMR data for **8** and **9**, there was an additional aromatic proton signal that appeared as a singlet and must therefore be located at C-7. Interpretation of the NMR data led to the conclusion that **10** was methylene signals at 4.63 and 4.68 in **1**, at a similar chemical shift to the corresponding signal in **13**. The H-19 signal was observed at δ 7.51, which allowed the sulfate group to be placed at C-20. Hydrolysis of lamellarin V 20-sulfate (**3**) gave lamellarin V (**7**), which had been isolated as a pale yellow solid of molecular formula $C_{30}H_{27}NO_{10}$. As expected, the H-19 signal was shifted upfield to δ 6.81.

Lamellarin Y 20-sulfate (**4**) was obtained as a minor metabolite, in the form of a white powder. The molecular formula, $C_{28}H_{22}NO_{11}SNa$, which again requires 18 degrees of unsaturation, suggested that **4** differed from **2** by loss of 14 amu, which corresponds to the replacement of a methoxy group by a phenol. The ^{1}H NMR spectrum (Table 2) confirmed this proposal since it contained only three methoxyl signals at δ 3.32, 3.78 and 3.84 and an additional phenolic signal at 8.82. Comparison of the NMR data for **2** and **4**, particularly the H-10 chemical shifts, suggests that the second phenol is at C-9, although this could not be confirmed because insufficient material was available to record the ^{13}C NMR, HMQC and HMBC spectra.



8 $R^1 = H, R^2 = Me, X = OMe$ (W)
9 $R^1 = H, R^2 = Me, X = OH$ (X)
10 $R^1 = R^2 = X = H$ (N)
11 $R^1 = R^2 = Ac, X = H$



12 $X = H$ (C)
13 $X = OH$ (A)
14 $X = H, {}^{5,6}\Delta$ (B)

Lamellarin W (**8**) was obtained as a pale yellow amorphous solid. The molecular formula, $C_{30}H_{25}NO_9$, which requires 19 degrees of unsaturation, was determined by high resolution mass measurement in conjunction with the ^{1}H and ^{13}C NMR data. The UV spectrum contained absorptions at 385, 364, 336 (sh), 302, 279 and 205 nm, similar to those of lamellarin B (14),[1] which indicated that the additional degree of unsaturation consisted of a 5,6 double bond. The ^{1}H NMR spectrum (Table 4) contained signals at 9.03 (d, 1 H, $J = 7.5$ Hz) and 7.38 (d, 1 H, $J = 7.5$ Hz) that are typical of an isoquinoline system. In addition, there were 5 methoxy signals and two phenolic proton signals, which suggested that **8** might be the product of dehydration of lamellarin V (7). Careful analysis of the ^{13}C NMR, HMQC and HMBC data (Table 4) confirmed the substitution pattern and the proposed structure for lamellarin W (**8**).

Lamellarin X (**9**) was isolated as a very minor metabolite in the form of a white powder. The molecular formula, $C_{29}H_{23}NO_9$, and UV spectrum suggested that lamellarin X (**9**) was related to **8** by replacement of a methoxy group by a phenol. Without ^{13}C NMR data, due to the small sample size, the lamellarin N, which had previously been reported only as a triacetate derivative.[3] This was confirmed by acetylation of lamellarin N (**10**) to form the triacetate **11**, which had identical ^{1}H NMR data to that reported in the literature.[3]

In the NCI 60 cell-line panel, lamellarin N (**10**) showed some selectivity toward the melanoma cell lines SK-MEL-5 (LC_{50} 1.87 x 10^{-7} M) and UACC-62 (LC_{50} 9.88 x 10^{-6} M). The lamellarin sulfates, which represent a new series of compounds, decomposed before they could be tested.

EXPERIMENTAL SECTION

General Methods: Melting points were obtained on a Mel-Temp apparatus and are uncorrected. Optical rotations were measured on an Autopol III polarimeter using a 1 dm cell. IR and UV spectra were recorded on Perkin-Elmer 1600 and Lambda 3B instruments, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 400 and Varian Inova 300 spectrometers and HMQC and HMBC experiments were recorded on a Varian Unity 500 spectrometer. Chemical shifts are reported in ppm based on $\delta_{TMS} = 0$ and coupling constants (*J*) are reported in Hz. High resolution FAB mass spectra were run on a ZAB-E4F mass spectrometer ar the Regional Mass Spectrometry Facility at UC Riverside.

Collection, Extraction and Isolation: A specimen of the unidentified ascidian (IIC- 197) was collected in January 1995 at a depth of 30 feet by skin diving, near Trivandrum in the Arabian Sea, India. A voucher specimen (IIC-197) has been lodged at the museum of the National Institute of Oceanography, Goa, India. The specimens were soaked in MeOH at the site of collection until workup. The MeOH was decanted and concentrated *in vacuo* to obtain an aqueous suspension that was lyophilized to give a solid residue. The specimens (43g dry wt.) were then lyophilized, extracted with MeOH:CH_2Cl_2 (1:1, 2 x 700 mL) and concentrated to obtain a solid residue. The combined residues were extracted with MeOH (2 x 300 mL), and the concentrated extract (226 mg) was chromatographed on Sephadex LH-20 using 1:1 MeOH:CH_2Cl_2 as eluant to afford two major UV-active fractions. Both fractions were subjected to flash chromatography on a reversed phase C-18 column using a gradient of MeOH and water. The first fraction afforded a fraction containing a mixture of three compounds and a pure sample of lamellarin T 20-sulfate (**1**, 14 mg, 0.033% dry wt.). The mixture was separated by reversed phase HPLC on a preparative Dynamax 60A C-18 column, using CH_3CN:H_2O (1:4) as eluant, to afford lamellarin U 20-sulfate (**2**, 12 mg, 0.028% dry wt.), lamellarin V 20-sulfate (**3**, 15 mg, 0.035% dry wt.) and lamellarin Y 20-sulfate (**4**, 1.2 mg, 0.003% dry wt.). The second fraction afforded lamellarin W (**8**, 12 mg, 0.028% dry wt.), lamellarin N (**10**, 8 mg, 0.019% dry wt.), a fraction which was purified by reversed phase HPLC using 3:1 MeOH-water as eluant to obtain lamellarin X (**9**, 1.2 mg, 0.003% dry wt.) and a fraction containing a mixture of three compounds, which was separated by reversed phase HPLC using 3:1 MeOH-water to afford lamellarin T (**5**, 6 mg, 0.014% dry wt.), lamellarin U (**6**, 3.5 mg, 0.008% dry wt.) and lamellarin V (**7**, 6 mg, 0.014% dry wt.).

Lamellarin T 20-sulfate (1): white powder; IR (AgCl) 3595, 1695, 1415, 1270, 1240, 1050, 840 cm^{-1}; UV (MeOH) λ_{max} 330 (sh, ε 16 600), 309 (ε 21 000), 274 (ε 24 000), 265 (sh, ε 19 350), 204 nm (ε 47 000); UV (MeOH + NaOH) λ_{max} 336 (sh), 308 (ε 20 000), 274 (ε 22 000), 265 (sh), 205 nm (ε 58 000); ^{1}H NMR (400

MHz, DMSO-d_6) see Table 1; ^{13}C NMR (75 MHz, DMSO-d_6) see Table 1; FABMS *m/z* 670 [M+Na]$^+$, 648 [M+H]$^+$; HRFABMS *m/z* 670.0980, calcd. for $C_{30}H_{26}NO_{12}SNa_2$ [M+Na]$^+$ 670.0971.

Lamellarin U 20-sulfate (2): white solid, mp 222-226 °C; IR (AgCl) 3480, 1695, 1410, 1275, 1240, 1045, 840 cm^{-1}; UV (MeOH) λ_{max} 327 (sh), 309 (ε 20 000), 273 (ε 23 500), 265 (sh), 202 nm (ε 40 000); UV (MeOH + NaOH) λ_{max} 332 (sh), 306 (ε 20 000), 273 (ε 23 000), 264 (sh), 209 nm (ε 40 000); ^{1}H NMR (400 MHz, DMSO-d_6) see Table 2; ^{13}C NMR (75 MHz, DMSO-d_6) see Table 2; FABMS *m/z* 594 [M-Na]$^-$; HRFABMS *m/z* 594.1087, calcd. for $C_{29}H_{24}NO_{11}S$ [M-Na]$^-$ 594.1070.

Lamellarin V 20-sulfate (3): white solid, mp 174-178 °C; IR (AgCl) 3470 (br), 1695, 1410, 1270, 1240, 1045 cm^{-1}; UV (MeOH) λ_{max} 328 (sh), 304 (ε 14 000), 273 (ε 17 000), 264 (sh), 202 nm (ε 29 000); UV (MeOH + NaOH) λ_{max} 333 (sh), 303 (ε 15 000), 274 (ε 16 600), 264 (sh), 204 nm (ε 52 000); ^{1}H NMR (400 MHz, DMSO-d_6) see Table 3; FABMS *m/z* 640 [M-Na]$^-$; HRFABMS *m/z* 640.1104, calcd. for $C_{30}H_{26}NO_{13}S$ [M-Na]$^-$ 640.1125.

Lamellarin Y 20-sulfate (4): white powder; IR (AgCl) 3475, 1695, 1410, 1270, 1240, 1045 cm^{-1}; UV (MeOH) λ_{max} 328 (sh), 309 (ε 21 000), 273 (ε 23 000), 265 (sh), 204 nm (ε 35 000); UV (MeOH + NaOH) λ_{max} 333 (sh), 306 (ε 18 000), 273 (ε 20 000), 264 (sh), 204 nm (ε 56 000); ^{1}H NMR (400 MHz, DMSO-d_6) see Table 2; FABMS *m/z* 580 [M- Na]$^-$; HRFABMS *m/z* 580.0931, calcd. for $C_{28}H_{22}NO_{11}S$ [M-Na]$^-$ 580.0913.

Lamellarin T (5): white powder, mp 214-218 °C; IR (AgCl) 3400, 1690, 1455, 1415, 1270 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) see Table 1; ^{1}H NMR (200 MHz, CDCl$_3$) δ 3.11 (m, 2 H, H-6), 3.36 (s, 3 H, Me-26), 3.51 (s, 3 H, Me-28), 3.86 (s, 3 H, Me-25), 3.89 (s, 3 H, Me-24), 3.98 (s, 3 H, Me-27), 4.65-4.82 (m, 2 H, H-5), 6.56 (s, 1 H, H-10), 6.65 (s, 1 H, H-22), 6.93 (s, 1 H, H-19), 6.99 (dd, 1 H, *J* = 2, 8 Hz, H-16), 7.04 (d, 1H, *J* = 8 Hz, H-15), 7.12 (d, 1 H, *J* = 2 Hz, H-12); ^{13}C NMR (50 MHz, CDCl$_3$) δ 29.9 (C-6), 42.1 (C-5), 55.5 (C-28), 55.1 (C-26), 56.3 (C-27), 60.9 (C-25), 61.0 (C-24), 103.4 (C-19), 104.1 (C-22), 105.3 (C-10), 110.2 (C-17), 111.2 (C- 15), 113.9 (C-3), 115.2 (C-1), 117.4 (C-12), 120.1 (C-6a), 123.0 (C-16), 123.1 (C-10a), 128.0 (C-11), 128.1 (C-2), 135.3 (C-10b), 142.2 (C-8), 143.2 (C-20), 145.5 s (C-18), 146.4 (C-21), 146.4 (C-13, C-14), 150.6 (C-7), 151.8 (C-9), 155.6 (C-23); FABMS *m/z* 546 [M+H]$^+$; HRFABMS *m/z* 546.1772, calcd. for $C_{30}H_{28}NO_9$ [M+H]$^+$ 546.1772.

Lamellarin U (6): white solid, mp 200-204 °C; ^{1}H NMR (400 MHz, DMSO-d_6) see Table 2; FABMS *m/z* 516 [M+H]$^+$; HRFABMS *m/z* 516.1661, calcd. for $C_{29}H_{26}NO_8$ [M+H]$^+$ 516.1658.

Lamellarin V (7): white powder, mp 162-166 °C; ^{1}H NMR (400 MHz, DMSO-d_6) see Table 3; ^{1}H NMR (400 MHz, acetone-d_6) δ 3.13 (br d, 0.5 H, *J* = 4 Hz, H-6), 3.18 (br d, 0.5 H, *J* = 4 Hz, H-6), 3.40 (s, 1.5 H, Me-26), 3.41 (s, 1.5 H, Me-26), 3.50 (s, 3 H, Me-28), 3.53 (br d, 0.5 H, *J* = 2 Hz, H-6), 3.59 (br d, 0.5 H, *J* = 2 Hz, H-6), 3.84 (s, 3 H, Me-25), 3.91 (s, 3 H, Me-24), 3.98 (s, 1.5 H, Me-27), 3.99 (s, 1.5 H, Me-27), 6.77 (s, 0.5 H, H-10), 6.79 (s, 0.5 H, H-10), 6.79 (s, 1 H, H-22), 6.87 (s, 1 H, H-19), 6.89 (dd, 0.5 H, *J* = 8, 2 Hz, H-16), 6.92 (d, 0.5 H, *J* = 2 Hz, H-12), 6.99 (m, 1 H, H-5), 7.18 (dd, 0.5 H, *J* = 8, 2 Hz, H-16), 7.21 (d, 0.5 H, *J* = 2 Hz, H-12), 7.24 (d, 0.5 H, *J* = 8 Hz, H-15), 7.36 (d, 0.5 H, *J* = 8 Hz, H-15); FABMS *m/z* 561 M$^+$; HRFABMS *m/z* 561.1636, calcd. for $C_{30}H_{27}NO_{10}$ 561.1635.

Lamellarin W (8): pale yellow solid, mp 224-228 °C; IR (AgCl) 3490 (br), 1675, 1480, 1425, 1275 cm^{-1}; UV (MeOH) λ_{max} 385 (ε 6 000), 364 (ε 5 000), 336 (sh), 302 (ε 13 000), 279 (ε 15 000), 205 nm (ε 30 000);

UV (MeOH + NaOH) λ_{max} 396 (ε 6 000), 316 (ε 16 000), 296 (ε 15 000), 256 (sh), 209 nm (ε 52 000); ^{1}H NMR (400 MHz, DMSO-d_6) see Table 4; ^{13}C NMR (100 MHz, DMSO-d_6) see Table 4; FABMS m/z 544 [M+H]+; HRFABMS *m/z* 544.1662, calcd. for $C_{30}H_{26}NO_9$ $[M+H]^+$ 544.1607.

Lamellarin X (9): white powder; IR (AgCl) 3400 (br), 1685, 1425, 1275 cm^{-1}; UV (MeOH) λ_{max} 386 (ε 7 000), 365 (sh), 338 (sh), 324 (sh), 303 (ε 14 000), 276 (ε 15 000), 204 nm (ε 30 000); UV (MeOH + NaOH) λ_{max} 402 (ε 4 000), 317 (ε 11 000), 298 (sh), 207 nm (ε 56 000); ^{1}H NMR (400 MHz, DMSO-d_6) see Table 4; FABMS *m/z* 530 $[M+H]^+$; HRFABMS *m/z* 530.1445, calcd. for $C_{29}H_{24}NO_9$ $[M+H]^+$ 530.1451.

Lamellarin N (10): white powder, mp 271-275 °C (dec.); IR (AgCl) 3400 (br), 1680, 1425, 1270 cm^{-1}; UV (MeOH) λ_{max} 386 (ε 9 000), 365 (ε 6 000), 336 (sh), 322 (sh), 279 (ε 22 500), 204 nm (ε 29 500); UV (MeOH + NaOH) λ_{max} 402 (ε 11 300), 383 (sh), 315 (sh), 295 (ε 16 800), 242 (sh), 204 nm (ε 50 000); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.23 (s, 6 H), 3.86 (s, 3 H), 6.75 (s, 1 H), 6.86 (s, 1 H), 7.00 (dd, 1 H, J = 8, 2 Hz), 7.01 (d, 1 H, J = 2 Hz), 7.16 (s, 1 H), 7.18 (s, 1 H), 7.19 (d, 1 H, J = 7.5 Hz), 7.22 (d, 1 H, J = 8 Hz), 8.99 (d, 1 H, J = 7.5 Hz), 9.37 (s, OH), 9.82 (s, OH), 9.92 (s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 54.6 (q), 55.1 (q), 56.2 (q), 103.8 (d), 105.4 (d), 105.7 (d), 106.5 (s), 108.3 (s), 110.5 (s), 111.6 (d), 112.4 (d), 113.7 (d), 117.5 (s), 118.3 (d), 122.0 (d), 122.2 (d), 124.7 (s), 127.4 (s), 128.8 (s), 133.9 (s), 144.6 (s), 146.3 (s), 147.7 (s), 147.8 (s), 148.0 (s), 148.3 (s), 148.6 (s), 154.4 (s); FABMS *m/z* 499 M^+; HRFABMS *m/z* 499.1273, calcd. for $C_{28}H_{21}NO_8$ 499.1273.

Acetylation of lamellarin N (10): A solution of lamellarin N (**10**, 1 mg) in acetic anhydride (2 drops) and pyridine (2 drops) was allowed to stand overnight at 25 °C. Water (1 mL) was added to the reaction mixture, which was then extracted with CH_2Cl_2 (2 x 2 mL). The organic phase was dried over Na_2SO_4 and evaporated, using high vacuum to remove a trace of pyridine, to obtain lamellarin N triacetate (**11**, 1 mg), which posessed an NMR spectrum identical to that reported by Carroll *et al.*[3]

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