Isolation and Structural Elucidation of Crellastatins B–H: Cytotoxic Bis(steroid) Derivatives from the Vanuatu Marine Sponge *Crella* sp.

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Dedicated to the memory of Luigi Minale

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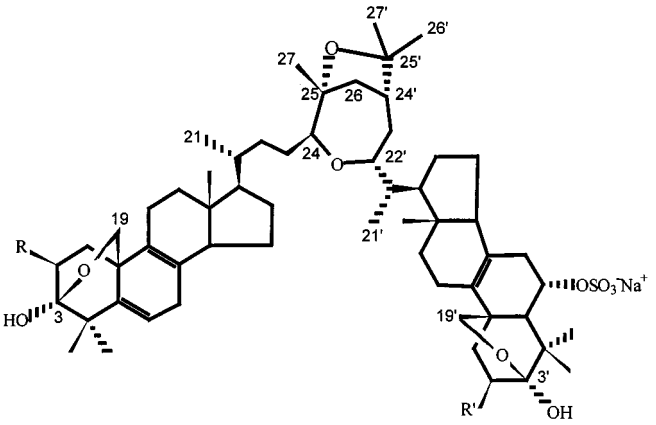
Seven new cytotoxic dimeric steroids, namely crellastatins B–H (**2**–**8**), have been isolated from the Vanuatu sponge *Crella* sp. They have been structurally characterized on the basis of 2D-NMR (500 MHz) and FAB-MS data. All the new compounds show the same unprecedented junction between the monomeric units, formed via their side-chains, whereas they differ from A (**1**) in the hydroxylation pattern of the two tetracyclic cores. Like crellastatin A (**1**), crellastatins B–H (**2**–**8**) exhibit in vitro antitumor activity against human bronchopulmonary non-small-cell lung carcinoma cell lines (NSCLC) with IC_{50} values in the range of 2–10 μg/mL.

Since the discovery of japindine,[1] the first example of a dimeric steroid to be isolated from a natural source, several examples of bis(steroid) derivatives have appeared in the literature.[2] Among these, the most pertinent with regard to their extraordinary biological activities are the cephalostatins[3] and the ritterazines,[4] which are isolated from marine organisms. They share a common structural feature, in which two highly oxygenated hexacyclic steroidal units are linked by a pyrazine ring. Recently, we reported the isolation of crellastatin A (**1**) from the Vanuatu marine sponge *Crella* sp.,[5] which constituted the first example of a dimeric steroid connected through its side chains. Further investigation of *Crella* sp. led to the isolation of four more related minor metabolites, crellastatins B−E (**2**−**5**), together with three new disulfated analogues, crellastatins F−H (**6**−**8**), which are the subject of this paper.

The lyophilized sponge *Crella* sp. (80 g) was extracted with methanol and the methanolic extract was subjected to a modified Kupchan partitioning procedure.[6] Preliminary pharmacological tests performed on the four extracts obtained were indicative of cytotoxic activity in the case of the $CHCl_3$ (1.6 g, IC_{50} < 3.3 μg/mL) and *n*BuOH (1.7 g, IC_{50} = 6.2 μg/mL) extracts.

The active $CHCl_3$ extract was subsequently fractionated by DCCC ($CHCl_3$/MeOH/H_2O, 7:13:8, ascending mode) followed by reversed-phase HPLC, to yield crellastatin A (**1**, 231 mg), crellastatin B (**2**, 19 mg), crellastatin C (**3**, 15 mg), crellastatin D (**4**, 4.5 mg), and crellastatin E (**5**, 3.0 mg).



	R	R′
1	OH	OH
2	H	OH
3	OH	H
4	H	H

Figure 1. Crellastatins A−D (**1**−**4**)

From the cytotoxic BuOH extract, which was likewise chromatographed by DCCC ($CHCl_3$/MeOH/H_2O, 7:13:8, ascending mode) and HPLC, we obtained further more polar disulfated metabolites, namely crellastatin F (**6**, 20.0 mg), crellastatin G (**7**, 10.5 mg), and crellastatin H (**8**, 4.5 mg).

Structure Determination of Crellastatins B−E (2−5)

By means of negative-ion fast-atom bombardment mass spectrometry (FAB-MS), which showed an intense ion peak at m/z = 991 $[M - H]^-$, together with ^{13}C- (Table 1) and

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Table 1. ^{13}C-NMR data of crellastatins B−H (**2**−**8**) (125 MHz, [D$_4$]methanol)

atom no.	**2**	**3**	**4**	**5** δ_C	**6**	**7**	**8**
1	31.7	43.0	34.1	42.8	44.5	44.7	48.0
2	30.7	68.4	30.5	68.4	67.0	67.2	69.0
3	100.0	99.8	99.8	99.7	100.0	100.0	221.4
4	44.1	42.7	43.1	42.6	41.0	41.0	47.4
5	148.9	148.1	148.8	147.8	51.5	51.8	53.0
6	115.8	116.0	115.7	116.0	75.8	75.9	75.9
7	30.3	30.2	30.2	30.0	35.5	35.6	36.3
8	130.3	130.5	130.2	130.5	127.8	128.8	128.0
9	127.4	126.8	127.2	126.8	131.0	131.5	134.1
10	38.6	38.6	38.4	38.4	41.0	41.0	40.7
11	23.6	23.4	23.5	23.6	23.4	23.2	22.5
12	38.4	38.5	38.3	38.2	38.6	38.6	38.2
13	42.9	42.9	42.9	42.5	42.4	42.6	42.4
14	53.5	53.5	53.4	53.3	53.1	53.6	52.4
15	24.2	24.1	24.1	24.2	25.2	25.2	24.5
16	30.7	30.7	30.5	30.6	30.9	30.8	30.5
17	55.1	54.7	54.9	54.5	54.1	54.4	53.7
18	11.6	11.8	11.8	11.8	11.3	11.9	10.9
19	73.7	73.0	73.6	73.0	70.1	70.1	22.2
20	36.3	36.4	36.4	37.2	36.2	36.4	36.5
21	19.3	19.4	19.3	19.4	19.5	19.4	19.1
22	36.5	36.4	36.2	36.4	36.9	36.7	36.7
23	25.1	25.4	25.1	25.4	24.7	24.7	25.2
24	86.1	86.0	85.1	85.8	86.3	86.3	85.8
25	88.1	88.2	88.0	87.8	88.3	88.2	87.8
26	36.5	36.5	36.5	36.2	36.5	36.6	36.2
27	27.4	27.3	27.3	27.3	27.1	27.2	26.7
28	25.7	25.4	25.6	25.1	18.0	18.0	19.0
29	26.4	25.9	26.4	25.6	28.3	28.3	33.1
1′	44.8	34.2	33.9	48.2	44.9	34.0	44.2
2′	67.5	29.5	29.7	70.0	67.2	29.6	66.9
3′	99.8	99.8	99.9	221.5	100.1	100.2	99.5
4′	41.0	42.2	42.1	47.8	41.0	42.8	40.5
5′	52.7	53.0	53.0	53.8	52.0	52.8	51.0
6′	75.6	75.9	75.9	76.0	76.0	76.3	75.8
7′	35.5	35.5	35.4	35.5	35.8	35.8	35.4
8′	127.9	128.5	128.4	128.3	126.2	127.8	126.4
9′	131.8	131.3	131.3	134.8	131.1	130.8	130.9
10′	40.7	40.9	40.8	41.7	40.8	41.0	41.2
11′	23.4	23.3	23.3	23.3	23.4	23.5	22.5
12′	38.4	38.4	38.3	38.2	38.7	38.6	38.2
13′	43.0	42.8	42.8	43.2	42.7	42.4	42.3
14′	54.0	54.0	53.9	53.0	53.4	54.1	54.0
15′	25.0	25.2	25.3	25.2	25.5	25.5	24.3
16′	29.8	29.7	29.4	29.7	29.6	29.7	29.6
17′	53.1	53.1	53.0	52.8	53.1	53.1	52.8
18′	11.6	11.5	11.5	11.5	11.7	11.4	11.4
19′	70.0	70.5	70.5	22.5	70.1	70.7	69.8
20′	44.1	44.0	43.9	44.2	44.0	44.0	43.7
21′	13.5	13.4	13.4	13.4	13.0	13.1	12.8
22′	71.6	71.6	71.5	71.8	71.7	71.7	71.5
23′	34.0	34.0	34.1	33.0	34.0	34.1	33.9
24′	46.9	45.8	45.7	46.0	45.8	45.9	45.5
25′	85.6	85.6	85.5	85.6	85.7	85.6	85.3
26′	25.4	25.7	25.7	25.5	25.7	25.7	25.3
27′	32.6	32.6	32.5	32.5	32.7	32.7	32.3
28′	17.9	17.6	17.6	19.5	18.0	17.8	17.7
29′	28.5	28.8	28.6	33.7	28.9	29.0	27.7

^{1}H-NMR data (Table 2), the molecular formula of crellastatin B (**2**) was shown to be $C_{55}H_{88}O_{11}S$, which is 16 mass units less than that of crellastatin A (**1**). The ^{1}H- and ^{13}C-NMR spectra closely resemble those of crellastatin A (**1**); in particular, the signals ascribable to the eastern hemisphere are superimposable on those of **1**, indicating that this hemisphere has the same gross structure. The signal at δ = 4.08 assigned to the hydroxymethine group at the C-2 position of crellastatin A (**1**) is absent in **2**, hence the latter is presumed to be 2-dehydroxycrellastatin A. In fact, the C-2 signal at δ = 68.2 in **1** is replaced by a methylene carbon signal (DEPT) at δ = 30.7 in **2**, while the C-1 signal is upfield shifted to δ = 31.7 (δ = 42.8 in **1**) and that of C-4 is downfield shifted to δ = 44.1 (δ = 42.6 in **1**). All these differences are consistent with the loss of the hydroxy group at C-2.

Crellastatin C (**3**) is an isomer of crellastatin B. In this case, the NMR data (Tables 1 and 2) for the western hemisphere are identical to those assigned to crellastatin A (**1**), whereas a perturbation is observed in ring A of the eastern hemisphere. In particular, the ^{13}C-NMR chemical shifts of C-1′ (δ = 34.2 vs. 44.7 in **1**), C-2′ (δ = 29.5 vs. 67.2 in **1**), and C-4′ (δ = 42.2 vs. 40.5 in **1**), as well as the absence of the proton signal at δ = 4.04, are consistent with **3** being 2′-dehydroxycrellastatin A.

Crellastatin D (**4**) shows a quasi molecular ion $[M - H]^-$ at *m*/*z* = 975, 32 mass units lower than that for crellastatin A (**1**). In the ^{1}H-NMR spectrum (Table 2), the signals at both δ = 4.08 and δ = 4.04, assigned to 2-H and 2′-H in **1**, are absent, thus suggesting that crellastatin D is a hybrid of crellastatins B and C. Comparison of NMR data (Tables 1 and 2) reveals that one half of crellastatin D is identical to the left hemisphere of crellastatin B while the other half is identical to the right hemisphere of crellastatin C. Therefore, **4** must be 2,2′-didehydroxycrellastatin A.

Analysis of FAB-MS data showed crellastatin E (**5**) to be isomeric with crellastatins B and C {FAB-MS (negative-ion mode): *m*/*z* = 991 $[M - H]^-$}. Inspection of the ^{1}H-NMR spectrum (Table 2) revealed the absence of the AB-system signals at δ = 3.94−3.83 assigned to the 19′-oxymethylene geminal protons of crellastatin A (**1**). One additional methyl singlet signal was observed at δ = 0.94 (s, 3 H). The structure of ring A of the eastern hemisphere of crellastatin E (**5**) could be deduced on the basis of diagnostic HMBC data (Figure 2). Both geminal methyl protons at C-4′ show HMBC correlations with a carbonyl carbon signal at δ = 221.5, thus suggesting the presence of a 3′-oxo functionality. This is further supported by a diagnostic downfield shift of 0.62 ppm (δ = 4.66 in **5** vs. 4.04 in **1**) of the signal of the adjacent 2′-hydroxymethine proton and smaller downfield shifts for the geminal methyl protons at C-4′ (δ = 1.38 and 1.45 in **5** vs. δ = 1.19 and 1.38 in **1**). The presence of the 3′-oxo functionality also has a marked effect on the chemical shifts of the C-4′, C-2′, and C-1′ signals in the ^{13}C-NMR spectrum (see Table 1). An additional methyl signal observed in both the ^{1}H- and ^{13}C-NMR spectra (δ_C = 22.5, δ_H = 0.94), showing numerous HMBC correlations with the centres of rings A and B (Figure 2), could be assigned to an angular methyl group at C-19′. Crellastatin E (**5**) may represent the biogenetic precursor of crellastatin A: the 3α-hydroxy 3,19-ether functionality present in crellastatin A might arise from oxidation of the angular 19-methyl group in crellastatin E to a hydroxymethylene group, with subsequent intramolecular cyclization to form the hemiketal system found in crellastatin A.

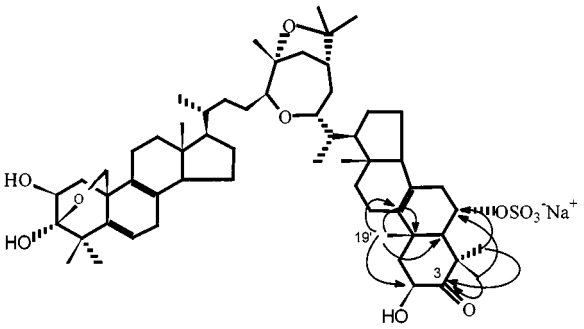
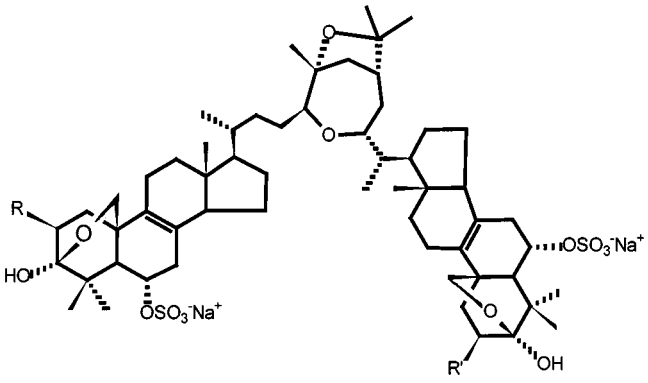


Figure 2. Crellastatin E (**5**) showing key HMBC correlations

Structure Determination of Crellastatins F−H (6−8)

Crellastatin F (**6**) represents the major component of the butanolic extract of the sponge *Crella* sp. The disulfated nature of the compound was evident from its higher polarity than that of crellastatins A−E, and from FAB-MS data, which showed a quasi-molecular ion peak at m/z = 1105 $[M - H]^-$. Analysis of ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2) suggested that the two hemispheres in **6** have a common 6-sulfated tetracyclic core. Although the differences between the two hemispheres appear only in the remote side chains, the nuclei of the two tetracyclic cores show small but significant differences in their ^{1}H- and ^{13}C-NMR resonances (Tables 1 and 2), which were assigned through a careful analysis of 2D-COSY, HMQC, and HMBC data. Analysis of the ROESY spectrum obtained for **6** shows the same sets of cross-peaks that are observed for crellastatin A, from which it can be concluded that both molecules have identical stereochemistry.



	R	R′
6	OH	OH
7	OH	H

Figure 3. Crellastatins F−G (**6**−**7**)

In the case of crellastatin G (**7**), FAB-MS (m/z = 1089) and NMR data (Tables 1 and 2) indicate that one of the two hydroxy groups at C-2 or C-2′ is absent, in a structural situation similar to that observed in crellastatins B (**2**) or C (**3**). Here, owing to the symmetrical nature of the disulfated crellastatins, determining which subunit contains the dehydroxylated ring A was somewhat problematic and merits special comment. In the HMBC spectrum, the angular methyl singlet signal at δ = 0.69, assigned to an 18′-Me group on the basis of HMBC correlations between 18′-H/C-17′, C-17′/21′-H, and 21′-H/C-22′, shows a correlation with the C-14′ signal at δ = 54.1, which, in turn, shows a long-range correlation with one of the 7′-H methylene protons at δ = 2.07. By following the coupling network from the 7′-CH_2 unit by means of ^{1}H-^{1}H COSY and HMBC correlations, all resonances of rings A and B could be determined. In this way, the presence of a methylene group at the C-2′ position was established (δ_H = 2.42, 1.75, δ_C = 29.6), and hence crellastatin G could be assigned the structure **7**.

Crellastatin H (**8**) is an isomer of crellastatin G, as was evident from FAB-MS analysis; m/z = 1089 $[M - H]^-$. In the ^{1}H-NMR spectrum, an additional methyl singlet signal is observed at δ = 0.93. Furthermore, one of the two hydroxymethine signals, attributable to the 2-H or 2′-H proton, is found to be significantly downfield shifted at δ = 5.04 (dd, J = 5.2, 11.7 Hz). These features, together with the observed disappearance of one of the two AB systems attributable to the C-19 (or C-19′) oxymethylene group, suggest the presence of a 19(19′)-methyl-3(3′)-oxo system. Once again, the complete structural characterization of the molecule requires a distinction to be made concerning which hemisphere contains the aforementioned 3-oxo functionality. As discussed above for other crellastatin derivatives, the starting point in this elucidation procedure is the angular C-18 (or C-18′) methyl group. The methyl singlet signal at δ = 0.72 shows diagnostic HMBC correlations (Figure 4), on the basis of which it can be established as belonging to the western hemisphere of the molecule. Furthermore, the C-18 methyl signal shows a correlation with the C-14 carbon signal at δ = 52.4, and its corresponding proton may be assigned to a signal at δ = 2.24 on the basis of HMQC analysis. The 14-H proton shows homoallylic coupling with one of the two 7-CH_2 protons at δ = 2.96. From analysis of the COSY spectrum, we can follow the ring B proton spin system as far as the 5-H proton, whose signal is observed as a well-resolved doublet, downfield shifted to δ = 2.45 (d, J = 11.0 Hz) due to the presence of the adjacent 3-oxo functionality. HMBC analysis of ring A further corroborates this conclusion (Figure 4). Therefore, **8** is established as being the 19-methyl-3-oxocrellastatin F.

Cytotoxic activities of crellastatins A−H (**1**−**8**) towards various clones of NSCLC tumor cells are shown in Table 3. The IC_{50} values are all comparable, indicating that neither the sulfate groups, the hydroxylation pattern at ring A, nor the 3α-hydroxy 3,19-ether functionality have any marked effect on the cytotoxicity of these metabolites. It would seem that the observed cytotoxicities are not attributable to any specific functionalities present in the crellastatins, but are merely due to the steroidal and dimeric nature of these metabolites. In view of their rigid and bulky architecture, the crellastatins could conceivably span or perturb the lipid bilayer of eukariotic cell membranes.

Table 2. Selected ^{1}H-NMR data of crellastatins B−H (**2**−**8**, 500 MHz, [D$_4$]methanol)

atom no.	**2**	**3**	**4**	**5** δ_H[a] (*J* in Hz)	**6**	**7**	**8**
1	2.19, 2.15	2.20, 2.15	2.20, 2.17	2.20, 2.15	1.95, 2.40 t (8.8)	1.95, 2.38	1.55, 2.61 t (11.7)
2	1.50, 2.20	4.08 dd (4.0, 9.2)	1.41, 2.15	4.05 dd (3.0, 9.2)	4.24 dd (5.1, 8.8)	4.22 dd (5.1, 9.6)	5.04 dd (5.2, 11.7)
5					1.83 d (11.7)	1.81 d (9.6)	2.45 d (11.0)
6	5.62 t (3.1)	5.60 t (3.0)	5.59 t (2.7)	5.60 t (2.9)	4.69	4.69	4.64
7	2.70	2.63 br. s, $W_{1/2}$ 7.3	2.64 br. s, *W*1/2 7.5	2.63 br. s, $W_{1/2}$ 6.9	2.94, 2.04	2.94, 2.17	2.96, 2.16
18	0.73 s	0.72 s	0.71 s	0.72 s	0.69 s	0.65 s	0.72 s
19	4.31 d (8.2), 3.67 dd (2.0, 8.2)	4.30 d, (8.1), 3.62 dd (1.5, 8.1)	4.28 d (8.0), 3.67 dd (1.7, 8.0)	4.30 d (8.3), 3.66 dd (1.9, 8.3)	3.90 br. dd (8.8), 3.83 d (8.8)	3.92 dd (2.2, 8.8) 3.83 br. dd (8.8)	0.93 s
28	1.22 s	1.22 s	1.22 s	1.22 s	1.37 s	1.37 s	1.45
29	1.13 s	1.17 s	1.17 s	1.13 s	1.23 s	1.23 s	1.51
1′	2.05, 2.02	2.27, 1.39	2.25, 1.46	2.50, 1.60	1.97, 2.21	1.67, 2.22	1.95, 2.39
2′	4.05 dd (4.9, 9.7)	2.16, 1.77	2.18, 1.76	4.66 dd (5.0, 9.5)	4.30 dd (5.1, 9.6)	2.42, 1.75	4.25 dd (5.0, 9.5)
5′	1.58 br. d (11.5)	1.58 br. d (10.5)	1.58 br. d (10.1)	2.26	1.81 d (11.0)	1.84 d (11.1)	1.89 d (11.7)
6′	4.64 dt (5.1, 11.5)	4.67 dt (5.1, 10.5)	4.65 dt (5.5, 10.1)	4.65 dd (4.9, 11.0)	4.69	4.71	4.70
7′	2.92 dd (5.1, 16.2) 2.05	2.91 dd (5.1, 17.0) 2.04	2.92 dd (5.5, 16.8) 2.04	2.92 dd (4.9, 17.2) 2.05	2.93, 2.07	2.92, 2.07	2.98, 2.27
18′	0.71 s	0.68 s	0.68 s	0.71 s	0.65 s	0.69 s	0.66 s
19′	3.94 dd (1.2, 8.8), 3.83 dd (1.5, 8.8)	3.93 dd (1.0, 9.0), 3.84 dd (1.4, 9.0)	3.94 dd (1.0, 8.8), 3.86 dd (1.6, 8.8)	0.94 s	3.90 d (8.7), 3.83 dd (2.5, 8.7)	3.93 d (8.9), 3.84 dd (2.1, 8.9)	3.93 d (8.8), 3.84 dd (2.7, 8.8)
28′	1.36 s	1.32 s	1.32 s	1.45 s	1.38 s	1.32 s	1.35
29′	1.17 s	1.20 s	1.30 s	1.38 s	1.30 s	1.29 s	1.18

[a] ^{1}H assignments aided by COSY and HMQC experiments.

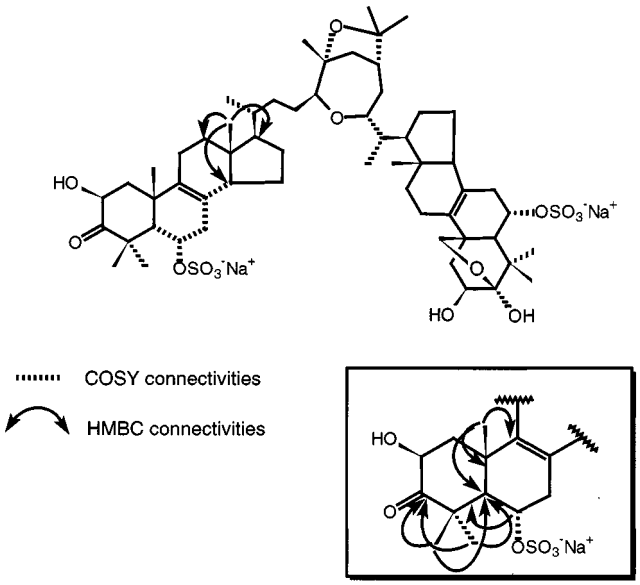


Figure 4. Crellastatin H (**8**) showing key HMBC and COSY correlations

Table 3. In vitro cytotoxic activity (IC_{50} in μg/mL) of crellastatins A−H (**1**−**8**)[a]

	NSCLC	C15	C65	C92	C98
crellastatin A (**1**)	1.52	< 1.1	4.3	6.3	4.2
crellastatin B (**2**)	1.2	–	–	–	–
crellastatin C (**3**)	3.4	2.3	4.6	6.1	4.8
crellastatin D (**4**)	5.94	–	–	–	–
crellastatin E (**5**)	9.87	–	–	–	–
crellastatin F (**6**)	2.7	1.3	–	–	–
crellastatin G (**7**)	2.5	–	–	–	–
crellastatin H (**8**)	2.3	–	–	–	–

[a] NSCLC: human bronchopulmonary non-small-cell lung carcinoma; C15, C65, C92, C98 are clones of the NSCLC cell line that differ in their doubling times.

Experimental Section

General Procedures: For general experimental procedures, see ref.[5]

Isolation: The sponge was collected off Santo (Lunganville) on the Vanuatu Islands in June 1996 and identified as *Crella* sp. (family Crellidae, order Poecilosclerida) by Dr. John Hooper (Museum of Queensland, Brisbane, Australia). A voucher specimen R1697 has been deposited at the ORSTOM Centre in Nouméa. The lyophilized sponges (80 g) were extracted with MeOH (3 × 2 L). The extracts were filtered, combined, and concentrated to give 20 g of a brown amorphous solid, which was successively extracted using a modified Kupchan[6] partitioning procedure as follows. The methanol extract (20 g) was first dissolved in a MeOH/H_2O (9:1) mixture and partitioned against *n*-hexane. The water content of the MeOH extract was then adjusted to 20% and 40% (v/v) and partitioning was carried out with CCl_4 and $CHCl_3$, respectively. The aqueous methanolic phase was finally concentrated to remove MeOH and extracted with *n*BuOH. The $CHCl_3$ extract (1.6 g) was fractionated by DCCC ($CHCl_3$/MeOH/H_2O, 7:13:8, ascending mode) followed by reversed-phase HPLC (μ-Bondapak C-18 column, eluting with MeOH/H_2O, 68:32) to afford **1** (231 mg, 0.29% dry weight of animal) as a colourless glassy solid, **2** (19 mg), **3** (15 mg), **4** (4.5 mg), and **5** (3.0 mg). The BuOH extract (1.3 g) was likewise fractionated by DCCC ($CHCl_3$/MeOH/H_2O, 7:13:8, ascending mode) followed by reversed-phase HPLC (μ-Bondapak C-18 column, eluting with MeOH/H_2O, 68:32) to afford **6** (20.0 mg), **7** (10.5 mg), and **8** (4.5 mg)

Crellastatin B (2): Amorphous powder. − $[\alpha]_D$ = +32 (*c* = 0.001, MeOH). − ^{1}H NMR ([D$_4$]methanol): Table 2. − ^{13}C NMR ([D$_4$]methanol): Table 1. − FAB-MS (negative ion mode); *m/z*: 991 $[M - H]^-$.

Crellastatin C (3): Amorphous powder. − $[\alpha]_D$ = +50.9 (*c* = 0.001, MeOH). − ^{1}H NMR ([D$_4$]methanol): Table 2. − ^{13}C NMR ([D$_4$]methanol):Table 1. − FAB-MS; *m*/*z*: 991 [M − H]$^-$.

Crellastatin D (4): Amorphous powder. − $[\alpha]_D$ = +51.6 (*c* = 0.001, MeOH). − ^{1}H NMR ([D$_4$]methanol): Table 2. − ^{13}C NMR ([D$_4$]methanol): Table 1. − FAB-MS; *m*/*z*: 975 [M − H]$^-$.

Crellastatin E (5): Amorphous powder. − $[\alpha]_D$ = +11.4 (*c* = 0.001, MeOH). − ^{1}H NMR ([D$_4$]methanol): Table 2. − ^{13}C NMR ([D$_4$]methanol): Table 1. − FAB-MS; *m*/*z*: 991 [M − H]$^-$.

Crellastatin F (6): Amorphous powder. − $[\alpha]_D$ = +60.3 (*c* = 0.001, MeOH). − ^{1}H NMR ([D$_4$]methanol): Table 2. − ^{13}C NMR ([D$_4$]methanol): Table 1. − FAB-MS; *m*/*z*: 1105 [M − H]$^-$.

Crellastatin G (7): Amorphous powder. − $[\alpha]_D$ = +68.7 (*c* = 0.001, MeOH). − ^{1}H NMR ([D$_4$]methanol): Table 2. − ^{13}C NMR ([D$_4$]methanol): Table 1. − FAB-MS; *m*/*z*: 1089 [M − H]$^-$.

Crellastatin H (8): Amorphous powder. − $[\alpha]_D$ = +27.8 (*c* = 0.001, MeOH). − IR (KBr): $\tilde{\nu}$ = 3420 cm^{-1} (OH), 2924 (CH), 1704 (C=O), 1458, 1200, 1060. − ^{1}H NMR ([D$_4$]methanol): Table 2. − ^{13}C NMR ([D$_4$]methanol): Table 1. − FAB-MS; *m*/*z*: 1089 [M − H]$^-$.

Cytotoxic Assays: Experiments were performed in 96-well microtiter plates (2×10^5 cells/mL). Cell growth was estimated by means of a colorimetric assay based on the conversion of a tetrazolium dye (MTT) to a blue formazan product using live mitichondria.[7] Eight determinations were performed at each concentration. Control growth was estimated from 16 determinations. The optical density at 570 nm, corresponding to solubilized formazan, was read for each well by means of a Titertek Multiskan MKII.

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