New Diketopiperazines from the Sponge *Dysidea chlorea*

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Received March 27, 1998

Twelve new polychlorinated diketopiperazines (**7**−**18**), along with six known ones (**1**−**6**) and a known sterol (**19**), were isolated from the sponge *Dysidea chlorea*, collected from Yap, Federated States of Micronesia. The structures of dysamides I−T (**7**−**18**) were elucidated by spectroscopic methods, and one (**8**) was confirmed by chemical conversion. The stereochemistry of the dysamides is discussed.

Sponges of the genus *Dysidea* (order Dendroceratida, family Dysideidae) have yielded a variety of organic compounds,[1] such as terpenoids,[2] steroids,[3] polybrominated diphenyl ethers,[4] and polychlorinated peptides.[5−9] It has been suggested[6,10] for some time that polyhalogenated metabolites may be produced by cyanobacterial symbionts, and this has recently been confirmed.[11−13]

Although sponges of the genus *Dysidea* have been the subject of intense chemical study,[1] only one chemical investigation of the sponge *D. chlorea* has been reported,[14] and in that study a polybrominated diphenyl ether was isolated from specimens collected in Palau. We have studied *D. chlorea* collected from Yap and have isolated 12 new chlorinated diketopiperazine derivatives, together with six known ones, but did not observe any polybrominated diphenyl ethers. Several polychlorinated diketopiperazines containing *N*-methyl di(or tri)chloroleucine have been previously reported from *D. herbacea*[6,7] and *D. fragilis*.[8,9] These included dysamide A (**2**)[8] and *N*-demethyl-2,3-dihydrodysamide C,[7] whose absolute stereochemistry has been determined by X-ray. In this paper, we describe the structure elucidation of the 12 new members of this polychlorinated diketopiperazine family.

Results and Discussion

D. chlorea de Laubenfels 1954 was extracted with MeOH and MeOH−CH_2Cl_2 (1:1), and the combined extracts, after removal of solvents, were subjected to solvent partitioning[15] to give hexane-, CH_2Cl_2-, and *n*-BuOH-soluble fractions. The CH_2Cl_2 solubles were chromatographed over a Si gel open column. Selected fractions from this chromatography were either recrystallized or rechromatographed on reversed-phase HPLC repeatedly to yield dysamides **1**−**18** and the known sterol **19** (Chart 1). The known compounds **1**−**5**[6−9] and **19**[16] were identified by comparison of their MS and ^{1}H NMR data with literature values. For **1**, **2**, and **19**, ^{13}C NMR data were also compared. The structure of dysamide E (**6**) has been presented in the proceedings of a meeting[17] without detailed experimental data; we provide here its NMR data.

All of the new dysamides are polychlorinated, a feature readily recognized by diagnostic clusters of isotope peaks in their mass spectra.[18] The presence of one or more trichloromethyls or dichloromethyls or both was evident from characteristic[6−9] ^{13}C NMR signals at δ 102−105 and 77, respectively. All the new compounds, except for **14**, had one double bond, whose stereochemistry could be distinguished by the chemical shift values of H-3 and H-4. In the (*Z*) isomers, H-3 (δ ca. 6.1) was observed farther downfield than in the (*E*) isomers (δ ca. 5.6), due to deshielding by the carbonyl group. Conversely, in the (*Z*) isomer H-4 resonated farther upfield (ca. δ 3.65) than in the (*E*) isomer (ca. δ 5.20).

Dysamide I (**7**)[19] had the molecular formula $C_{14}H_{18}Cl_6N_2O_2$ based on HRFABMS and NMR data (Tables 1 and 2), the latter being unambiguously assigned by COSY, HMQC, and HMBC experiments. This formula was the same as that of the known compound **1**, indicating they were isomers. The (*Z*)-configuration was assigned on the basis of the chemical shifts of H-3 and H-4 and was confirmed by a NOESY correlation between H-4 (δ 3.68) and N(7)-Me (δ 3.31). No NOE interaction between H-3 and N(7)-Me was observed.

Dysamide J (**8**) was the most unusual compound in this series owing to its conjugated diene moiety. Its molecular formula $C_{14}H_{17}Cl_5N_2O_2$ was deduced from HRFABMS and corroborated by NMR data (Tables 1 and 2). The IR spectrum showed bands at 1689 and 1631 cm^{-1}, consistent with the presence of saturated and α,β-unsaturated amide groups. The extended conjugated system in **8** was supported by UV absorption at 270 nm. The NMR data of **8** indicated that it had an *N*-methyl trichloroleucine residue, as does dysamide I (**7**). The remaining part of the structure was deduced from a long-range H/H coupling and an NOESY correlation between H-3 and the vinyl methyl and from HMBC correlations between the vinyl methyl (δ 2.03) and C-3, C-4, and C-5; and N(7)-Me (δ 3.05) and C-2 (δ 133.0). This structure was finally confirmed by chemical correlation with dysamide I (**7**). Thus, treatment of **7** with CH_3ONa−CH_3OH yielded dysamide **8** and its 2′-epimer **20**. An attempt to convert **7** to **8** using 4-(dimethylamino)-pyridine as base was unsuccessful.

Dysamide K (**9**) and dysamide L (**10**) had identical molecular formulas, $C_{14}H_{18}Cl_5N_2O_2$, based on their HR-FABMS and NMR data (Tables 1 and 2). This formula differed from that of compounds **1** and **7** by one chlorine. The presence of a dichloromethyl group in both **9** and **10** was indicated by a proton signal at δ 6.03 (d, J = 3.2 Hz) and a ^{13}C signal at δ ca. 77 (d). By comparison of their NMR data with those of compounds **1**, **3**, **5**, and **7**, dysamide K and dysamide L were assigned structures **9** and **10**, respectively.

Dysamide M (**11**), $C_{14}H_{20}Cl_4N_2O_2$ based on HRFABMS, showed two ^{13}C signals at δ 76.8 and 77.1 typical for the dichloromethyl group. These two signals were associated with ^{1}H signals at δ 5.67 (d, J = 4.8 Hz, H-6) and 6.06 (d,

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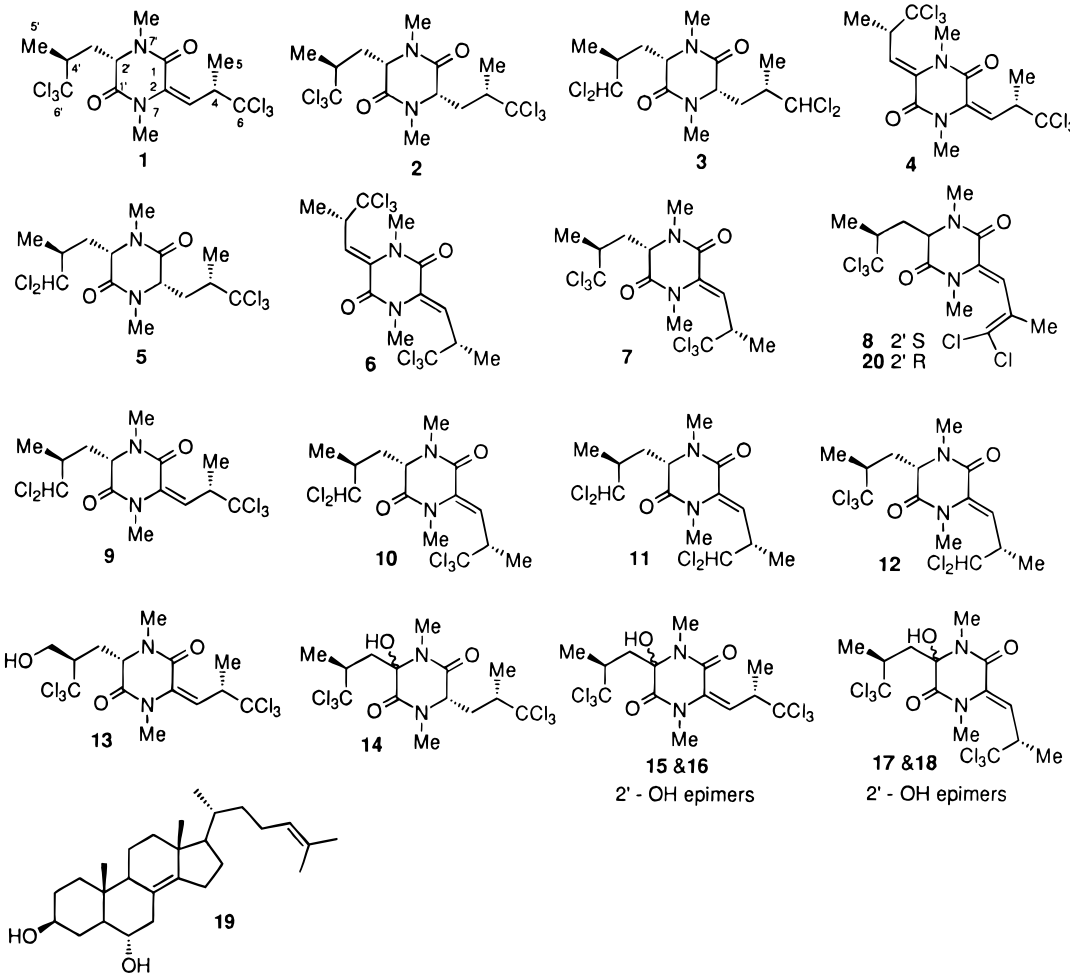
10.1021/np980123l CCC: $15.00
Published on Web 08/13/1998

Chart 1



J = 3.2 Hz, H-6′) based on correlations in the HMQC spectrum of dysamide M. Two spin systems (H-3 to H-6 and H-2′ to H-6′) could be delineated from the COSY spectrum of **11**. Therefore, structure **11** was assigned to dysamide M.

Dysamide N (**12**), a trace component, was assigned the formula $C_{14}H_{18}Cl_5N_2O_2$ based on HRFABMS data. The ^{1}H NMR data (Table 1) for H-3 to H-6 were nearly identical to those of dysamide M (**11**), and the rest of the data were the same as those for H-2′ to H-5′ in dysamide I (**7**). Thus, dysamide N was defined as **12**.

The IR spectra of dysamides **13**−**18** all had bands for an alcohol group: a primary alcohol in **13**, and tertiary alcohols in **14**−**18** according to NMR data.

The ^{1}H NMR and ^{13}C NMR data (Tables 3 and 4) of dysamide O (**13**), $C_{14}H_{18}Cl_6N_2O_3$, were similar to those of compound **1**[7,8] except that the three-proton doublet at δ 1.38 and the ^{13}C signal at δ 17.5 in the NMR spectra of **1** were absent in the data for **13**. Instead, signals for a primary alcohol group were observed in the proton spectrum at δ 4.22 (dt, J = 12.0, 5.0 Hz, H-5′), 3.67 (dt, J = 12.0, 7.5 Hz, H-5′), 2.23 (dd, J = 7.5, 5.0 Hz, OH), and in the carbon spectrum at δ 63.8 (t). The ^{13}C NMR peak at δ 63.8 was correlated to the ^{1}H resonances at δ 4.22 and 3.67 in the HMQC spectrum of **13**. Therefore, structure **13** was assigned to dysamide O, and this was supported by the COSY correlations that revealed two spin systems, H-3 to H-5 and H-2′ to H-5′.

The molecular formula of dysamide P (**14**), was established by HRFABMS, and this was corroborated by its NMR data (Tables 3 and 4). A trichloroleucine residue identical to that found in **1**, **2**, **5**, **7**, **8**, and **12** was established by NMR data. The other half of the structure was determined by interpretation of COSY, HMQC, and HMBC experiments. The hydroxyl group was located at C-2′ on the basis of HMBC correlations between C-2′ (δ 82.3) and H-3′ (δ 2.72, d, J = 14.3 Hz; 1.62, dd, J = 14.3, 8.0 Hz) and one N-Me (δ 3.06, s).

Dysamide Q (**15**) and R (**16**), both $C_{14}H_{18}Cl_6N_2O_3$ by HRFABMS, were isolated as a 7:1 mixture as judged by the integration of the ^{1}H NMR spectrum of the mixture. Analysis of COSY, HMQC, and HMBC data of both the major epimer **15** and the minor one, **16**, revealed that both possessed the same 2-hydroxy-5-trichloroleucine moiety found in **14**. The NMR spectral data for the remaining portion of **15** and **16** matched that of the unsaturated trichloroleucine residue present in **1**, **9**, and **13**. Hence, the epimeric structures **15** and **16** were proposed for dysamides Q and R, respectively.

Dysamides S (**17**) and T (**18**) were also isolated as a mixture, the ratio being 2:1 by NMR analysis. They had the same molecular formula, $C_{14}H_{18}Cl_6N_2O_3$, as that of dysamides Q and R. Comparison of the NMR data of the mixture of **17** and **18** with those of the mixture of **15** and **16** revealed that the former pair of epimers are the (2Z) isomers of the latter pair. The connectivities in each isomer were confirmed by COSY, HMQC, and HMBC data.

Initially, we suspected that the two sets of NMR signals for **15**/**16** and **17**/**18** mixture might be due to interconverting conformers. This was ruled out by variable tempera-

Table 1. ^{1}H NMR Data for Dysamides E (**6**), and I−N (**7**−**12**)[a]

position	dysamide E (**6**)	dysamide I (**7**)	dysamide J (**8**)	dysamide K (**9**)	dysamide L (**10**)	dysamide M (**11**)	dysamide N (**12**)
3	6.10 (d, 11.1)	6.19 (d, 11.1)	6.57 (s)	5.66 (d, 9.0)	6.17 (d, 11.2)	6.09 (d, 11.1)	6.11 (d, 11.1)
4	3.67 (dq, 11.1, 6.4)	3.68 (dq, 11.1, 6.4)		5.21 (dq, 9.0, 7.0)	3.66 (dq, 11.2, 6.9)	3.31 (m)	3.32 (m)
5	1.52 (d, 6.4)	1.56 (d, 6.4)	2.03 (s)	1.39 (d, 7.0)	1.57 (d, 6.9)	1.37 (d, 6.5)	1.38 (d, 6.9)
6						5.67 (d, 4.8)	5.66 (d, 4.8)
2′		4.00 (t, 7.4)	4.06 (dd, 6.4, 8.0)	3.97 (dd, 5.8, 8.5)	3.90 (dd, 5.8, 9.5)	3.90 (dd, 6.4, 10.1)	3.99 (dd, 6.9, 8.0)
3′	6.10 (d, 11.1)	2.48 (ddd, 14.3, 6.9, 2.1)	2.51 (ddd, 14.3, 6.4, 2.1)	2.16 (dt, 14.3, 6.4)	2.10 (dt, 13.8, 6.3)	2.09 (ddd, 14.3, 7.4, 6.4)	2.51 (ddd, 14.3, 6.9, 2.6)
		1.69 (dt, 14.3, 7.4)	1.70 (dt, 14.3, 7.4)	1.64 (ddd, 14.3, 6.9, 9.0)	1.64 (ddd, 13.8, 6.4, 9.5)	1.72 (ddd, 14.3, 6.5, 10.1)	1.76 (dt, 14.3, 7.9)
4′	3.67 (dq, 11.1, 6.4)	2.74 (ddq, 7.4, 2.1, 6.4)	2.80 (ddq, 7.4, 2.1, 6.5)	2.33 (dddq, 3.2, 6.4, 6.9, 6.5)	2.30 (dddq, 3.2, 6.3, 6.4, 6.9)	2.31 (dddq, 3.2, 6.4, 7.4, 6.5)	2.75 (m)
5′	1.52 (d, 6.4)	1.29 (d, 6.4)	1.32 (d, 6.5)	1.17 (d, 6.5)	1.09 (d, 6.9)	1.12 (d, 6.5)	1.31 (d, 6.3)
6′				6.03 (d, 3.2)	6.04 (d, 3.2)	6.06 (d, 3.2)	
N(7′)-Me	3.34 (s)	3.08 (s)	3.09 (s)	3.05 (s)	3.04 (s)	3.03 (s)	3.07 (s)
N(7)-Me	3.34 (s)	3.31 (s)	3.05 (s)	3.23 (s)	3.29 (s)	3.26 (s)	3.28 (s)

[a] Spectra were recorded in $CDCl_3$ at 500 MHz, referenced to residual solvent $CDCl_3$ (δ 7.26); δ (mult., J in Hz).

ture ^{1}H NMR studies. Heating of the mixture of dysamides Q (**15**) and R (**16**) in $CDCl_3$ at 50 °C or cooling of the mixture to −40 °C did not change the ratio of the sets of signals in the ^{1}H NMR spectrum. This was also the case for the mixture of dysamides S (**17**) and T (**18**). The epimers in each mixture were clearly separated by reversed-phase HPLC; however, each epimer rapidly equilibrated to a mixture with the same epimer ratio as present before HPLC separation. If our hypothesis regarding mixtures of epimers is correct for **15**/**16** and **17**/**18**, one would expect to see the same behavior for **14**. In fact, very weak signals (<5% of intensity observed for **14** signals) corresponding to a second epimer were observed in the ^{1}H and ^{13}C NMR spectra of **14**. These were not strong enough to confirm the structure of the minor component by HMQC or HMBC as in the case of **15**/**16** and **17**/**18**.

The difficulty in assigning the absolute stereochemistry of the polychlorinated compounds from sponges of the genus *Dysidea* has been noted.[5] However, based on recent X-ray studies,[5,7,8] on an asymmetric synthesis,[20] and on chemical correlations,[21,22] it has been established that the carbons bearing the tri (or di) chloromethyl groups in the polychlorinated compounds obtained from *Dysidea* sponges all have the (*S*) configuration. The (*S*) configuration of C-4 and C-4′ in dysamide A (**2**),[8] dysamide C (**4**),[17] and *N*-demethyl-2,3-dihydrodysamide C[7] is supported by X-ray data, as is the (*S*) configuration for C-2 and C-2′ in dysamide A (**2**)[8] and C-2′ in *N*-demethyl-2,3-dihydrodysamide C.[7] During chemical conversion of **7** to **8** by CH_3ONa−MeOH, compound **20**, a 2′-epimer of **8**, was also obtained. The change of the stereochemistry at C-2′ significantly altered the coupling pattern between H-2′ and H's-3′ ($J_{2',3'}$ = 12.2, 3.7 Hz), and between H-4′ and H's-3′ ($J_{3',4'}$ = 11.1, 2.1 Hz), and this would seem to provide a way to detect the presence of epimers at C-2 in the series of cyclic diketopiperazines with a hydrogen at C-2.

The optical rotations of the new dysamides **7**−**14** had the same sign as that of dysamides A (**2**),[8] C (**4**),[17] and *N*-demethyl-2,3-dihydrodysamide C,[7] whose absolute stereochemistries have been confirmed. In addition, the coupling constants between H-2′ (or H-2) and H's-3′ (or H's-3), and between H-3′ (or H-3) and H-4′ (or H-4) in these compounds were similar throughout the series and also to those of **1**−**6**. Hence, it seems likely that the stereochemistry at each of the chiral centers is (*S*) in compounds **1**−**12**. We speculate on biogenetic grounds that the configurations, where designated, in **13**−**18** are as shown to parallel those of the remainder of the series. The stereochemistry of C-2′ in **14**−**18** could not be assigned.

Overall, 18 diketopiperazine derivatives, **1**−**18**, and one known sterol, **19**, have been isolated and characterized. We assume, based on the work of Faulkner's group,[12,13] that the dysamides reported here also arise from cyanobacteria associated with the sponge. It has been noted earlier that collections of *Dysidea* species from a given location contain either chlorinated amino acid derivatives or polybrominated diphenyl ethers, but not mixtures of the two types of compounds.[13] We found no evidence for polybrominated diphenyl ethers in our collection of *D. chlorea* from Yap, which is consistent with these earlier observations.

Experimental Section

General Experimental Procedures. All solvents were redistilled. Merck Si gel 60 (230−240 mesh) was used for vacuum flash chromatography. HPLC was conducted using a UV detector and a Spherex 5 C-18 column. IR spectra were obtained on a Bio-Rad 3240-SPC FT instrument; UV spectra, on a Hewlett−Packard spectrophotometer. NMR experiments

Table 2. ^{13}C NMR Data for Compounds **6**−**11**, and **20**[a]

position	dysamide E (**6**)	dysamide I (**7**)[b]	dysamide J (**8**)[b]	dysamide K (**9**)	dysamide L (**10**)[c]	dysamide M (**11**)[c]	**20**
1	161.4	161.5	160.8	159.6	161.6	161.8	160.8
2	134.6	134.9	133.0	131.5	135.1	134.9	132.7
3	122.4	121.9	118.3	123.6	121.9	122.7	118.4
4	53.2	53.5	129.7[d]	51.3	53.4	43.5	129.5[e]
5	17.6	17.7	20.5	18.4	17.6	17.9	20.5
6	102.7	103.2	119.9[d]	104.1	103.3	76.8	120.2[e]
1′	161.4	166.7	166.1	165.3	166.7	166.7	165.4
2′	134.6	62.6	62.5	60.7	61.4	61.4	61.0
3′	122.4	37.1	37.2	36.3	36.0	35.7	35.7
4′	53.2	51.3	51.4	39.2	39.9	39.9	50.8
5′	17.6	17.7	17.9	15.4	15.2	15.1	16.0
6′	102.7	105.1	105.2	77.2	76.7	77.1	104.9
N(7′)-Me	35.2	33.8	33.7	33.2	33.3	33.2	33.2
N(7)-Me	35.2	35.2	33.0	31.1	35.2	35.1	32.6

[a] Spectra were recorded in $CDCl_3$ at 125 MHz, referenced to $CDCl_3$ (δ 77). [b] Assignments were aided by HMQC and HMBC experiments. [c] Assignments were aided by HMQC experiments. [d,e] Signals with the same letters may be interchanged.

Table 3. ^1H NMR Data for Compounds **13**−**18**[a]

position	dysamide O (**13**)	dysamide P (**14**)	dysamide Q (**15**)	dysamide R (**16**)	dysamide S (**17**)	dysamide T (**18**)
2		4.02 (t, 7.0)				
3	5.66 (d, 9.0)	2.52 (ddd, 14.3, 6.4, 2.6) 1.90 (dt, 14.3, 7.4)	5.62 (d, 9.5)	5.78 (d, 9.0)	6.35 (d, 11.5)	6.49 (d, 11.5)
4	5.20 (dq, 9.0, 7.0)	3.03 (m)	4.72 (dq, 9.5, 6.4)	5.19 (dq, 9.0, 6.4)	3.66 (dq, 11.5, 7.0)	3.83 (dq, 11.5, 6.0)
5	1.38 (d, 7.0)	1.43 (d, 7.0)	1.53 (d, 6.4)	1.37 (d, 6.4)	1.60 (d, 7.0)	1.43 (d, 6.0)
2′	4.18 (dd, 10, 5.0)					
3′	2.38 (dt, 14.5, 5.0)	2.72 (d, 14.3)	2.31 (d, 14.3)	2.58 (d, 14.3)	2.61 (d, 14.5)	2.14 (d, 14.4)
	2.10 (ddd, 14.5, 8.5, 5.0)	1.62 (dd, 14.3, 8.0)	1.94 (dd, 14.3, 7.4)	1.63 (dd, 14.3, 8.0)	1.72 (dd, 14.5, 8.5)	1.93 (dd, 14.4, 8.0)
4′	3.04 (m)	2.84 (dq, 8.0, 6.5)	2.54 (p, 6.4)	2.70 (m)	2.78 (m)	2.60 (m)
5′	4.22 (dt, 12.0, 5.0)	1.30 (d, 6.5)	1.41 (d, 6.9)	1.32 (d, 6.3)	1.23 (d, 7.0)	1.47 (d, 6.0)
	3.67 (dt, 12.0, 7.5)					
N(7′)-Me	3.09 (s)	3.06 (s)	3.05 (s)	3.10 (s)	3.08 (s)	3.05 (s)
N(7)-Me	3.21 (s)	3.12 (s)	3.26 (s)	3.34 (s)	3.40 (s)	3.47 (s)
OH	2.23 (dd, 7.5, 5.0)	4.81 (br s)	4.51 (br s)	4.64 (br s)	4.66 (br s)	4.55 (br s)

[a] Spectra were recorded in $CDCl_3$ at 500 MHz, referenced to residual solvent $CDCl_3$ (δ 7.26); δ (mult., J in Hz).

Table 4. ^{13}C NMR Data for Dysamides O−T (**13**−**18**)[a]

position	dysamide O (**13**)[b]	dysamide P (**14**)[c]	dysamide Q (**15**)[b]	dysamide R (**16**)[b]	dysamide S (**17**)[b]	dysamide T (**18**)[b]
1	159.4	165.0	159.2	159.1	160.9	Not observed
2	131.2	62.0	133.0	132.0	135.3	132.7
3	123.9	38.3	124.3	125.3	123.5	123.2
4	51.4	52.5	52.5	51.4	53.8	53.0
5	18.4	17.8	17.7	17.8	17.6	18.4
6	104.1	105.3	103.8	105.7	102.7	102.1
1′	166.3	167.5	166.8	167.2	169.0	167.9
2′	62.1	82.3	83.5	82.5	82.5	83.2
3′	34.2	44.3	42.0	43.0	41.7	41.2
4′	57.4	49.9	50.4	49.8	49.9	50.4
5′	63.8	18.1	19.1	18.1	17.4	19.4
6′	101.8	105.5	105.7	105.3	105.4	106.3
N(7′)-Me	33.0	27.8	26.3	27.2	27.2	26.8
N(7)-Me	31.1	34.1	31.9	32.0	35.9	35.0

[a] Spectra were recorded in $CDCl_3$ at 125 MHz, referenced to $CDCl_3$ (δ 77). [b] Assignments were aided by HMQC and HMBC experiments. [c] Assignments were aided by HMQC experiments.

were conducted with a Varian VXR-500 instrument equipped with a 3-mm $^1H/^{13}C$ switchable gradient microprobe (MDG-500−3) and a pulsed field gradient driver; signals are reported in parts per million (δ), referenced to the solvent used. FABMS were measured on a VG ZAB-E mass spectrometer; and optical rotations, on a Rudolph Autopol III automatic polarimeter.

Animal Material. The sponge was collected in August 1995, at Yap, Federated States of Micronesia, and frozen shortly after collection. The sponge forms a thin tissue-like encrusting mat with ragged fingerlike projections up to 4 cm high, the texture is very soft and easily torn and is commonly dark grayish green, often tinged with purple. The skeleton is irregular and sparse and the spongin fibers very lightly cored with spicule and sand debris. The choanosome is packed with cyanobacteria. Some specimens have a distinctive herbal smell. The sponge is closely comparable to *D. chlorea* de Laubenfels 1954 (order Dendroceratida, family Dysideidae) and is distinguished from close relatives *D. herbacea* and *D. fragilis* by tissue-paper thinness, the sparse coring of the fibers, and the fingerlike projections on the sponge surface. A voucher specimen has been deposited in The Natural History Museum, London (BMNH 1998.3.5.2), and at the University of Oklahoma (26YA95).

Extraction and Isolation. Freshly thawed specimens of the sponge (502 g wet wt; 42 g dry wt after extraction) were minced and soaked in MeOH (2 × 800 mL) followed by MeOH−CH_2Cl_2 (1:1) (2 × 800 mL), and finally CH_2Cl_2 (800 mL). All extracts were combined after removal of solvents *in vacuo* and subjected to solvent partitioning as described previously.[15] This gave, after evaporation of solvents *in vacuo*, hexane (8.55 g), CH_2Cl_2 (7.86 g), and *n*-BuOH (3.51 g) solubles. The CH_2Cl_2

solubles were fractionated by flash chromatography over Si gel using increasing amounts of EtOAc in hexane as eluent (10% EtOAc−hexane to EtOAc). Fourteen fractions were collected. The major dysamides **1** and **2**, and the sterol **19** were obtained from the fifth, tenth, and thirteenth fractions by recrystallization from various mixtures of $CH_3OH-CH_2Cl_2$. The fourth fraction was rechromatographed over reversed- phase HPLC using 20% H_2O-CH_3CN as eluent to yield compounds **1**, **4**, epimers **15** and **16**, and semipure compound **9**, which was further purified by reversed-phase HPLC using 25% H_2O-CH_3CN. Reversed-phase HPLC of the eighth fraction using 20% H_2O−MeOH as eluent afforded compounds **1**, **3**, **5**, **7**, **8**, **11**, **13**, **14**, epimers **17** and **18**, and a mixture of **6** and **7**, which was resolved by reversed-phase HPLC using 35% H_2O-CH_3CN as eluent. A mixture containing 70% of **10** was also obtained and purified by reversed-phase HPLC with 25% H_2O−MeOH as solvent. Similarly, fraction 9 was fractionated over reversed-phase HPLC (20% H_2O−MeOH) to furnish compounds **1**, **2**, **3**, **5**, **7**, **11**, and **12**. The following amounts and yields of each compound were obtained from the CH_2Cl_2 solubles: **1** (4 g, 9.52% of dry specimen wt), **2** (98 mg, 2.33×10^{-1}%), **3** (16 mg, 3.81×10^{-2}%), **4** (10.6 mg, 2.52×10^{-2}%), **5** (8.5 mg, 2.02×10^{-2}%), **6** (6.5 mg, 1.55×10^{-2}%), **7** (80 mg, 1.90×10^{-1}%), **8** (2.5 mg, 5.95×10^{-3}%), **9** (4.0 mg, 9.52×10^{-3}%), **10** (1.4 mg, 3.33×10^{-3}%), **11** (2.3 mg, 5.48×10^{-3}%), **12** (0.4 mg, 9.52×10^{-4}%), **13** (1.4 mg, 3.33×10^{-3}%), **14** (49.7 mg, 1.18×10^{-1}%), **15** and **16** (11.6 mg, 2.76×10^{-2}%), **17** and **18** (4.0 mg, 9.52×10^{-3}%), and **19** (69.0 mg, 1.64×10^{-1}%).

2,3-Dihydrodysamide C (1): $[\alpha]_D$ −153.2° (*c* 2.00, EtOH); FABMS *m/z* (rel int %) $[M + H]^+$ 457 (26.7), 459 (46.4), 461 (29.8), 463 (18.8), 465 (3.6); ^1H NMR, MS data identical with literature data.[8]

Dysamide A (2): $[\alpha]_D$ −34.5° (*c* 1.00, EtOH); FABMS *m/z* (rel int %) $[M + H]^+$ 459 (21.9), 461 (38.9), 463 (32.1), 465 (12.6), 467 (1.8); ^1H NMR, MS data identical with literature data.[8]

Dysamide B (3): $[\alpha]_D$ −31.0° (*c* 0.80, EtOH); EIMS *m/z* (rel int %) $[M]^+$ 390 (4.5), 392 (5.6), 394 (1.8); ^1H NMR, MS data identical with literature data.[8]

Dysamide C (4): $[\alpha]_D$ −10.4° (*c* 0.50, EtOH); FABMS *m/z* (rel int %) $[M + H]^+$ 455 (6.7), 457 (11.8), 459 (9.5), 461 (3.9); ^1H NMR, MS data identical with literature data.[8]

Dysamide D (5): $[\alpha]_D$ −6.0° (*c* 0.40, EtOH); EIMS *m/z* (rel int %) $[M]^+$ 424 (1.7), 426 (2.0), 428 (1.4); ^1H NMR, MS data identical with literature data.[9]

Dysamide E (6): $[\alpha]_D$ −308.0° (*c* 0.54, EtOH); HRFABMS *m/z* $[M + H]^+$ 456.9380 (calcd for $C_{14}H_{17}{}^{35}Cl_5{}^{37}ClN_2O_2$ 465.9392); ^1H and ^{13}C NMR, see Table 1 and Table 2, respectively.

Dysamide I (7): $[\alpha]_D$ −135.9° (*c* 0.85, EtOH); IR (neat) ν_{max} 1693, 1642 cm^{-1}; ^1H and ^{13}C NMR, see Table 1 and Table 2, respectively; HRFABMS *m/z* $[M + H]^+$ 458.9522 (calcd for $C_{14}H_{18}{}^{35}Cl_5{}^{37}ClN_2O_2$, 458.9548).

Dysamide J (8): $[\alpha]_D$ −186.8° (*c* 0.19, EtOH); IR (neat) ν_{max} 1689, 1631 cm^{-1}; UV (EtOH) λ_{max} 270 (ϵ 6960) nm; ^1H and ^{13}C NMR, see Table 1 and Table 2, respectively; HRFABMS *m/z* $[M + H]^+$ 422.9769 (calcd for $C_{14}H_{17}{}^{35}Cl_4{}^{37}ClN_2O_2$, 422.9781).

Dysamide K (9): $[\alpha]_D$ −72.7° (*c* 0.33, EtOH); IR (neat) ν_{max} 1683, 1639 cm^{-1}; ^1H and ^{13}C NMR, see Table 1 and Table 2, respectively; HRFABMS *m/z* $[M + H]^+$ 424.9944 (calcd for $C_{14}H_{19}{}^{35}Cl_4{}^{37}ClN_2O_2$, 424.9950).

Dysamide L (10): $[\alpha]_D$ −63.3° (*c* 0.12, EtOH); IR (neat) ν_{max} 1691, 1644 cm^{-1}; ^1H and ^{13}C NMR, see Table 1 and Table 2, respectively; HRFABMS *m/z* $[M + H]^+$ 424.9915 (calcd for $C_{14}H_{19}{}^{35}Cl_4{}^{37}ClN_2O_2$, 424.9938).

Dysamide M (11): $[\alpha]_D$ −35.0° (*c* 0.20, EtOH); IR (neat) ν_{max} 1689, 1640 cm^{-1}; ^1H and ^{13}C NMR, see Table 1 and Table 2, respectively; HRFABMS *m/z* $[M + H]^+$ 391.0340 (calcd for $C_{14}H_{20}{}^{35}Cl_3{}^{37}ClN_2O_2$, 391.0328).

Dysamide N (12): $[\alpha]_D$ −50.0° (*c* 0.04, EtOH); IR (neat) ν_{max} 1679, 1639 cm^{-1}; ^1H NMR, see Table 1; HRFABMS *m/z* $[M + H]^+$ 424.9917 (calcd for $C_{14}H_{19}{}^{35}Cl_4{}^{37}ClN_2O_2$, 424.9938).

Dysamide O (13): $[\alpha]_D$ −80.8° (*c* 0.13, EtOH); IR (neat) ν_{max} 3423, 1685, 1635 cm^{-1}; ^1H and ^{13}C NMR, see Table 3 and Table 4, respectively; HRFABMS *m/z* $[M + H]^+$ 474.9461 (calcd for $C_{14}H_{18}{}^{35}Cl_5{}^{37}ClN_2O_3$, 474.9497).

Dysamide P (14): $[\alpha]_D$ −19.3° [*c* 1.15, EtOH−CH_2Cl_2 (3:1)]; IR (neat) ν_{max} 3368, 1670 (br) cm^{-1}; ^1H and ^{13}C NMR, see Table 3 and Table 4, respectively; HRFABMS *m/z* $[M + H]^+$ 476.9637 (calcd for $C_{14}H_{20}{}^{35}Cl_5{}^{37}ClN_2O_3$, 476.9654).

Dysamide Q (15) and R (16): IR (neat) ν_{max} 3385, 1690, 1634 cm^{-1}; ^1H and ^{13}C NMR, see Table 3 and Table 4, respectively; HRFABMS *m/z* $[M + H]^+$ 476.9469 (calcd for $C_{14}H_{18}{}^{35}Cl_4{}^{37}Cl_2N_2O_3$, 476.9470).

Dysamide S (17) and T (18): IR (neat) ν_{max} 3395, 1695, 1636 cm^{-1}; ^1H and ^{13}C NMR, see Table 3 and Table 4, respectively; HRFABMS *m/z* $[M + H]^+$ 474.9462 (calcd for $C_{14}H_{18}{}^{35}Cl_5{}^{37}ClN_2O_3$, 474.9497).

Sterol (19): $[\alpha]_D$ +20.3° (*c* 1.25, EtOH); FABMS *m/z* $[M + H]^+$ 401; ^1H NMR and MS data identical with literature data.[16]

Conversion of Dysamide I to Dysamide J. To 5 mL of MeOH containing 6 mg of dysamide I (**7**), excess CH_3ONa (ca. 2 mg) was added. After being stirred 15 min at room temperature, the reaction mixture was diluted with 5 mL of H_2O and then extracted with CH_2Cl_2. The CH_2Cl_2 layer was separated and dried under a stream of N_2 to give a residue that was subjected to reversed-phase HPLC (30% H_2O-CH_3CN) to yield dysamide J (**8**) (1.7 mg) and its 2′-epimer (**20**) (3.0 mg).

Compound 20: $[\alpha]_D$ +188.0° [*c* 0.21, EtOH−CH_2Cl_2 (6:1)]; IR (neat) ν_{max} 1689, 1630 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.61 (s, H-3), 3.96 (dd, J = 3.7, 12.2 Hz, H-2′), 3.06 (s, N-Me), 3.04 (s, N-Me), 2.75 (ddq, J = 11.1, 2.1, 6.4 Hz, H-4′), 2.29 (dt, J = 2.1, 12.7 Hz, H-3′), 2.01 (s, H-5), 1.86 (ddd, J = 3.7, 11.1, 13.2 Hz, H-3′), 1.46 (d, J = 6.4 Hz, H-5′); ^{13}C NMR, see Table 2; HRFABMS *m/z* $[M + H]^+$ 422.9778 (calcd for $C_{14}H_{17}{}^{35}Cl_4{}^{37}ClN_2O_2$, 422.9781).

Acknowledgment. This work was supported by National Cancer Institute Grant CA 52955. We thank the government of Yap, Federated States of Micronesia, for permission to collect specimens.

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NP980123L