# Dysideaprolines A−F and Barbaleucamides A−B, Novel Polychlorinated Compounds from a *Dysidea* Species

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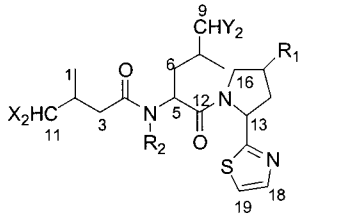
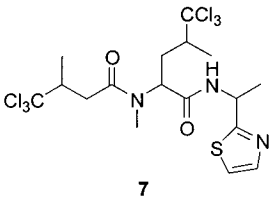
Chemical investigation of a marine sponge, *Dysidea* sp., collected at Bararin Island, Philippines, has afforded the novel metabolites **1**−**6**, proline-derived analogues of dysidenin (**7**). We have termed compounds **1**−**6** dysideaprolines A−F, respectively. Also isolated were compounds **8** and **9**, structural analogues of barbamide (**10**), a metabolite originally isolated from the cyanobacterium *Lyngbya majuscula*. We have termed these novel compounds barbaleucamides A (**8**) and B (**9**). It is most probable that the compounds presented here are actually derived from a symbiotic cyanobacterium found in close association with the *Dysidea* sp. Structure elucidation of the isolated metabolites involved high-field 2D NMR spectroscopy including $^1$H−$^1$H COSY, HSQC, and HMBC.

The marine sponge *Dysidea* sp. (Demospongia, Dictyoceratida, Dysideidae) has proven to be a prolific producer of structurally diverse secondary metabolites including bromophenols,[1] sesquiterpenes,[2] sesterterpenes,[3] sterols,[4] and polychlorinated compounds including dysidenin (**7**),[5] dysidamides,[6] dysideathiazoles,[7] and chlorinated diketopiperazines.[8] Many compounds originally isolated from *Dysidea* sp. have also been reported to have intriguing pharmacological activity. Thus, dysidenin (**7**) inhibits iodide transport in thyroid cells,[9] dysiherbaine is a neurotoxic amino acid,[10] and dysidiolide has been reported as a protein phosphatase inhibitor.[11] We now report on a chemical investigation where Si gel chromatography and semi-preparative reversed-phase HPLC of the $CH_2Cl_2$ extract of a *Dysidea* sp. collected from Bararin Island, Philippines, have afforded dysidenin (**7**) and the novel compounds **1**−**6**, **8**, and **9**.



**1** $R_1$ = H, $R_2$ = $CH_3$, X = Cl, Y = Cl
**2** $R_1$ = $CH_3$, $R_2$ = $CH_3$ , X = Cl, Y = Cl
**3** $R_1$ = H, $R_2$ = H, X = Cl, Y = Cl
**4** $R_1$ = H, $R_2$ = $CH_3$, X = H, Y = Cl
**5** $R_1$ = H, $R_2$ = $CH_3$, X =Cl, Y = H
**6** $R_1$ = H, $R_2$ = $CH_3$, X = Cl , $Y_2$ = ClH



**7**

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## Results and Discussion

High-resolution mass measurements for compound **1** of *m/z* 502.0665 $[M + H]^+$ and an isotope pattern typical of a tetrachlorinated compound indicated a molecular formula of $C_{19}H_{27}Cl_4N_3O_2S$. The presence of four chlorine atoms and one sulfur atom, together with $^1$H and $^{13}$C NMR data (Table 1) which supported the presence of a thiazole residue, suggested that **1** was structurally related to the well-known *Dysidea* metabolite dysidenin (**7**). The presence of a 5,5-dichloroisovaleroic acid residue and a *N*-methyl-5,5-dichloroleucine residue could be discerned from $^1$H, $^{13}$C NMR and supporting $^1$H−$^1$H COSY, HSQC, and HMBC spectral data (Table 1). This left only one nitrogen atom, three methylene groups, and one methine group to be accounted for. By supposing that the *N*-methyl-5,5-dichloroleucine residue could be linked to the thiazole residue by a proline-derived pyrrolidine ring, structure **1** could be proposed. Corroborating HMBC data included a key connectivity between H-13 and C-12 and C-17. Other supporting data are shown in both Table 1 and Figure 1.

Ozonolysis of **1** followed by acid hydrolysis liberated proline, which subsequent chiral HPLC analysis showed to be the L-form. The absolute stereochemistry at C-13 was thus determined to be *S*. The stereochemistry of the other asymmetric centers for **1** was not pursued. To date, all carbons bearing di- or trichloromethyl groups in polychlorinated compounds isolated from *Dysidea* sp. have been reported to have the *S* configuration. These assignments have been based on X-ray crystallography experiments[7] or total syntheses.[12] It is also considered that the chlorinated leucine residue is most probably derived from L-leucine,[13] and this appears to be the case for all *Dysidea* sp. metabolites reported to date. It would therefore seem reasonable to assume that, in **1**, the absolute configuration at C-5 is *S*. However, as will be discussed later, the configuration at C-5 for the dysideaprolines is most probably *R*.

The $^1$H and $^{13}$C NMR spectral characteristics of compound **2** (Table 2) were similar to those of **1** but clearly demonstrated the presence of an additional methyl group. This was consistent with a molecular formula of $C_{20}H_{29}Cl_4N_3O_2S$ for **2** based on an accurate mass measurement of *m/z* 516.0820 $[M + H]^+$ and an isotope pattern typical of a tetrachlorinated compound. The mass spectrum also

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**Table 1.** NMR Data for Dysideaproline A (**1**) in DMSO-$d_6$ at 25 °C

| H/C | δH (*J* in Hz) | $\delta^{13}C^a$ | $^1H-^1H$ COSY | HMBC[b] |
|---|---|---|---|---|
| 1 | 1.13 d (6.6) | 14.5 q | H-2 | H-11 |
| 2 | 2.71 m | 40.1 d | H-1,H-3a, H-11 | H-1 |
| 3a | 2.53 dd (6.9, 16.2) | 35.5 t | H-2, H-3b | H-1, H-11 |
| 3b | 2.66 dd (5.4, 16.2) | | H-3a | H-1, H-11 |
| 4 | | 170.6 s | | H-5, H-10 |
| 5 | 5.38 t (7.6) | 51.7 d | H-6a, H-6b | H-6a, H-6b, H-7, H-10 |
| 6a | 1.51 m | 31.51 t | H-5, H-6b, H-7 | H-5, H-7, H-8, H-9 |
| 6b | 1.97 m | | H-5, H-6a, H-7 | H-5, H-7, H-8, H-9 |
| 7 | 2.07 m | 39.4 d | H-6a, H-6b, H-8, H-9 | H-5, H-6a, H-6b, H-8 |
| 8 | 1.10 d (6.6) | 14.1 q | H-7 | H-6a, H-6b, H-7, H-9 |
| 9 | 6.43 d (3.3) | 79.2 d | H-7 | H-6a, H-6b, H-8 |
| 10 | 2.81 s | 30.3 q | | H-5 |
| 11 | 6.42 d (3.3) | 79.2 d | H-2 | H-1 |
| 12 | | 168.3 s | | H-5, H-6a, H-6b H-13, H-16a, H-16b |
| 13 | 5.35 dd (3.8, 8.8) | 58.6 d | H-14a, H-14b | H-14, H-16a, H-16b |
| 14a | 2.07 m | 31.53 t | H-13, H-14b, H-15 | H-13, H-15, H-16a, H-16b |
| 14b | 2.26 m | | H-13, H-14a, H-15 | H-13, H-15, H-16a, H-16b |
| 15 | 1.95 m | 24.0 t | H-14a, H-14b, H-16a, H-16b | H-13, H-14a, H-14b, H-16a, H-16b |
| 16a | 3.41 m | 46.3 t | H-15, H-16b | H-13, H-14a, H-14b, H-15 |
| 16b | 3.57 m | | H-15, H-16a | H-13, H-14a, H-14b, H-15 |
| 17 | | 172.2 s | | H-13, H-14a, H-14b, H-18, H-19 |
| 18 | 7.70 d (3.2) | 142.0 d | H-19 | H-19 |
| 19 | 7.59 d (3.2) | 119.4 d | H-18 | H-18 |

[a] Multiplicity deduced from APT experiment. [b] Proton showing long-range correlation to indicated carbon.

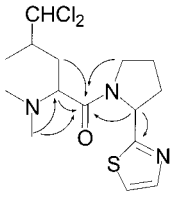



**Figure 1.** Selected HMBC correlations from **1**.

shared an identical fragment ion of *m/z* 348.0100 ($C_{12}H_{18}{}^{35}Cl_4NO_2$) present in the mass spectrum of **1**. This fragment is derived from cleavage of the C-12−N bond, clearly suggesting that the molecular composition of the left-hand of the molecule is identical for compounds **1** and **2**. The presence of the 5,5-dichloroisovaleroic acid residue and the *N*-methyl-5,5-dichloroleucine residue could be discerned from $^1H$ and $^{13}C$ NMR spectral data (Tables 2 and 3), as could the thiazole unit. This left only the pyrrolidine ring as a site for the additional methyl group. The placement of this methyl group at C-15 was supported, as were all proton and carbon assignments for **2**, by $^1H-^1H$ COSY, HSQC, and HMBC spectral data. NOE difference spectroscopy indicated that the methyl group and H-13 were anti to each other. Thus, in addition to an enhancement of the signal for 15-Me, irradiation at δ 2.25 (H-15) resulted in enhancements of H-16a and H-13, placing these two protons on the same face of the pyrrolidine ring. If we assume the *S* configuration at C-13, as in **1**, then the configuration at C-15 must also be *S*. The presence of a 4-methylproline-derived thiazole ring is unprecedented in natural product chemistry. The 4-methylproline residue has been encountered in the nostopeptins,[14] the spumigins,[15] the mycoplanecins,[16] and the leucinsostatins,[17] all microbial-derived peptides.

High-resolution mass measurements of *m/z* 488.0517 [M + H]$^+$ for compound **3** and an isotope pattern typical of a tetrachlorinated compound indicated a molecular formula of $C_{18}H_{25}Cl_4N_3O_2S$. The $^1H$ NMR spectrum of **3** in DMSO-$d_6$ at 25 °C demonstrated the presence of two rotamers in a 2:1 ratio. The most significant difference in this spectrum from that of **1**, however, was the absence of a signal corresponding to an *N*-methyl group and the presence of a signal at δ 8.5 (for the major rotamer) suggestive of a free NH group. This suggested that **3** was simply the *N*-demethylated analogue of **1**. This was, of course, supported by the molecular formula and by an upfield shift in H-5 in the $^1H$ NMR spectrum of **3** relative to that in **1**. All proton and carbon assignments listed in Tables 2 and 3 for the major rotamer of **3** were corroborated by HSQC and HMBC spectroscopy.

High-resolution mass measurements of *m/z* 434.1442 [M + H]$^+$ for compound **4** and its isotope pattern indicated a molecular formula of $C_{19}H_{29}Cl_2N_3O_2S$. A fragment peak, derived from cleavage of the C-12−N bond, was observed at *m/z* 280.0869 ($C_{12}H_{20}{}^{35}Cl_2NO_2$). The $^1H$ NMR spectrum indicated that **4** was related to compounds **1**−**3**, although it recorded only a single chloromethyl unit (δ 6.4, H-9). The presence of a 6H doublet at δ 0.91 was suggestive of an isopropyl unit. This clearly implied that only the leucine-derived residue *or* the isovaleroic acid-derived residue of **4** was chlorinated. Comparison of the $^1H$ and $^{13}C$ NMR spectra of **4** (Tables 2 and 3) with that of **1**−**3** suggested that the isovaleroic acid-derived unit was non-chlorinated. Thus, the $^1H$ NMR spectrum did not show signals at ∼δ 2.5 and 2.7 (H-3a/H-3b) as in the spectra of **1**−**3**. Rather, these signals were replaced in the spectrum of **4** by a 2H doublet at δ 2.26. Significantly, $^1H-^1H$ COSY indicated a spin system between signals at δ 0.91 (H-1/H-11), 2.03 (H-2), and 2.26 (H-3). Further support for the proposed structure of **4** was afforded by HMBC spectroscopy, which demonstrated long-range correlations between H-3 and C-1, C-2, C-4, and C-11.

High-resolution mass measurements of *m/z* 434.1426 [M + H]$^+$ for compound **5** indicated a molecular formula of $C_{19}H_{29}Cl_2N_3O_2S$. Furthermore, a fragment peak at *m/z* 280.0871 ($C_{12}H_{20}{}^{35}Cl_2NO_2$) arising from cleavage of the C-12−N bond indicated that the left-hand side of the molecule had the same molecular composition as that found in **4**. The most plausible difference between **4** and **5** was that, in **5**, the leucine-derived residue was non-chlorinated. This was supported by $^1H$ and $^{13}C$ NMR spectral data including HSQC and HMBC connectivities (Tables 2 and 3). On acid hydrolysis, *N*-methylleucine was liberated, which, on subsequent Marfey analysis,[18] was shown to have the D-configuration. This was somewhat surprising since, as mentioned earlier, the chlorinated leucine residue in

**Table 2.** $^1$H NMR Data for Compounds **2**−**6** in DMSO-$d_6$ at 25 °C

| | δH m (*J* in Hz) | | | | |
|---|---|---|---|---|---|
| position | **2** | **3** | **4** | **5** | **6** |
| 1 | 1.08 d (6.6) | 1.01 d (6.6) | 0.91 d (6.7) | 1.09 d (6.6) | 1.12 d (6.5) |
| 2 | 2.65 m | 2.58 m | 2.03 oct (6.8) | 2.67 m | 2.70 m |
| 3a | 2.47 m | 2.18 m | 2.26 d (6.8) | 2.46 dd (7.6, 16.3) | 2.50 dd (7.3, 16.1) |
| 3b | 2.61 dd (4.5, 16.2) | 2.42 dd (4.9, 14.5) | 2.26 d (6.8) | 2.59 dd (5.1, 16.3) | 2.64 dd (4.9, 16.1) |
| 5 | 5.31 dd (6.6, 9.0) | 4.76 br dt (5.5, 9.1) | 5.38 dd (6.2, 8.8) | 5.37 dd (6.6, 8.3) | 5.38 dd (5.8, 9.3) |
| 6a | 1.38 m | 1.53 m | 1.48 m | 1.51 m | 1.53 m |
| 6b | 1.90 p (6.6) | 1.90 m | 1.89 m | 1.51 m | 1.79 m |
| 7 | 2.02 m | 2.19 m | 2.02 m | 1.37 m | 1.69 m |
| 8 | 1.03 d (6.6) | 1.10 d (6.6) | 1.08 d (6.6) | 0.87 d (7.0) | 1.00 d (6.5) |
| 9a | 6.39 d (2.6) | 6.40 d (2.8) | 6.42 d (2.6) | 0.88 d (7.0) | 3.57 dd (6.3, 10.7) |
| 9b | | | | | 3.70 dd (3.8, 10.7) |
| 10 (N-C*H*$_3$) | 2.71 s | | 2.77 s | 2.80 s | 2.82 s |
| 10 (N-*H*) | | 8.49 d (8.5) | | | |
| 11 | 6.38 d (2.6) | 6.35 d (3.1) | 0.91 d (6.8) | 6.39 d (3.2) | 6.42 d (3.2) |
| 13 | 5.19 t (8.4) | 5.29 dd (2.0, 8.1) | 5.32 dd (3.6, 8.0) | 5.30 dd (3.2, 8.2) | 5.34 dd (3.5, 8.0) |
| 14a | 2.47 m | 2.22 m | 2.02 m | 2.05 m | 2.07 m |
| 14b | 1.57 m | 2.25 m | 2.22 m | 2.22 m | 2.26 m |
| 15a | 2.27 m | 1.93 m | 1.88 m | 1.88 m | 1.92 m |
| 15b | | 1.97 m | 1.93 m | 1.95 m | 1.97 m |
| 15-Me | 0.93 d (6.6) | | | | |
| 16a | 3.63 dd (7.2, 9.2) | 3.60 br dt (7.1, 9.5) | 3.35 m | 3.40 m | 3.47 m |
| 16b | 2.81 dd (9.2, 13.2) | 3.88 br dt (2.9, 8.1) | 3.53 m | 3.55 m | 3.58 m |
| 18 | 7.62 d (3.2) | 7.67 d (3.2) | 7.68 d (3.3) | 7.68 d (3.3) | 7.70 d (3.2) |
| 19 | 7.53 d (3.2) | 7.54 d (3.2) | 7.55 d (3.3) | 7.55 d (3.3) | 7.58 d (3.2) |

**Table 3.** $^{13}$C NMR Data for Compounds **2**−**6** in DMSO-$d_6$ at 25 °C

| | δC m[a] | | | | |
|---|---|---|---|---|---|
| position | **2** | **3** | **4** | **5** | **6** |
| 1 | 15.2 q | 14.2 q | 22.3 q | 14.5 q | 14.5 q |
| 2 | 40.9 d | 40.6 d | 24.7 d | 40.2 d | 40.2 d |
| 3 | 35.9 t | 37.7 t | 41.1 t | 35.6 t | 35.5 t |
| 4 | 171.1 s | 169.9 s | 172.0 s | 170.1 s | 170.8 s |
| 5 | 53.0 d | 48.1 d | 51.2 d | 51.7 d | 51.5 d |
| 6 | 32.4 t | 33.9 t | 31.5 t | 36.8 t | 32.0 t |
| 7 | 40.0 d | nd[b] | nd[b] | 24.0 d | 31.0 d |
| 8 | 14.7 q | 14.7 q | 14.9 q | 23.0 q | 18.0 q |
| 9 | 80.0 d | 79.1 d | 79.1 d | 22.1 q | 50.8 t |
| 10 | 31.0 q | | 30.3 q | 30.2 q | 30.2 q |
| 11 | 80.0 d | 79.0 d | 22.3 q | 79.1 d | 79.1 d |
| 12 | 169.4 s | 169.6 s | 168.6 s | 170.1 s | 168.7 s |
| 13 | 60.1 d | 58.4 d | 58.5 d | 58.4 d | 58.5 d |
| 14 | 41.5 t | 33.9 t | 31.5 t | 31.5 t | 31.5 t |
| 15 | 33.7 d | 23.5 t | 23.9 t | 23.9 t | 24.0 t |
| 15-Me | 16.9 q | | | | |
| 16 | 54.7 t | 46.4 t | 46.3 t | 46.4 t | 46.3 t |
| 17 | 173.3 s | 172.5 s | 172.5 s | 172.6 s | 172.4 s |
| 18 | 142.5 d | 143.0 d | 142.0 d | 142.0 d | 142.0 d |
| 19 | 120.1 d | 119.5 d | 119.5 d | 119.5 d | 119.5 d |

[a] Multiplicity deduced from APT experiment. [b] nd not detected, presumably obscured by DMSO-$d_6$ signal.

other *Dysidea* metabolites has been shown to be derived from L-leucine.[13]

Compounds **4** and **5** are notable in that they are the first dysidenin-type compounds in which one of the isovaleroic acid- or leucine-derived units is chlorinated while the other unit of the molecule is not. There are clearly interesting implications for the biosynthesis of these and related compounds. Isovaleroic acid is a presumed catabolite of leucine, and it would appear that chlorination of this unit can occur after incorporation into the dysideaproline scaffold. The presence of a non-chlorinated leucine residue as in **5** also indicates that chlorination need not be a prerequisite for incorporation of leucine. There is thus no reason to suppose that chlorine introduction in these and related 5,5-dichloroleucine-bearing metabolites necessarily occurs as early as at the free leucine or leucine thioester though it is entirely feasible that chlorine introduction may be an early step in the biosynthesis of 5,5,5-trichloroleucine-bearing metabolites.[13] Of course, the fact that we are dealing here with D-leucine-derived units, as opposed to L-leucine-derived units as in other *Dysidea* metabolites, should be taken into account when making biosynthetic comparisons. Also, noteworthy is the fact that the dichlorinated compounds, **4** and **5**, are *N*-methylated, whereas the tetrachlorinated compound **3** was the only non-*N*-methylated dysideaproline analogue encountered during our investigation.

Compound **6** added further insights to the above speculation on the biosynthesis of the dysideaprolines. The molecular formula of **6** was established as $C_{19}H_{28}Cl_3N_3O_2S$ on the basis of an accurate mass measurement of *m*/*z* 468.1066 $[M + H]^+$ and its isotope pattern. A fragment based on cleavage of the C-12−N bond was observed at *m*/*z* 314.0481 ($C_{12}H_{19}{}^{35}Cl_3NO_2$). There was no diagnostic signal in the $^{13}$C NMR spectrum (Table 3) indicative of a single trichloromethyl group. Rather, signals at δ 6.42 and 79.1 in the $^1$H (Tables 2 and 3) and $^{13}$C NMR spectra, respectively, clearly indicated the presence of a dichloromethyl group, while signals at δ 3.57, 3.70, and 50.8 supported the presence of a monochloromethyl group. The monochlorinated group was placed at C-9 on the basis of HMBC correlations between H-9a and H-9b with C-6, C-7, and C-8 and between H-7 and C-5, C-6, C-8, and C-9. The dichlorinated methyl group showed HMBC correlations between H-11 and C-1, C-2, and C-3, confirming its placement at C-11. Compound **6** is thus a chlorinated analogue of **5** and supports the hypothesis that chlorination of the dysideaprolines occurs at a late stage in the biosynthetic pathway.

Compound **7** had an optical rotation and UV, IR, MS, and $^1$H and $^{13}$C NMR spectral characteristics consistent with those reported for dysidenin.[5,19] Interestingly, given the isolation of **7**, no examples of hexachlorinated dysideaprolines were encountered in this investigation.

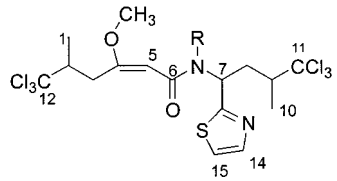
Compound **8** had a molecular formula of $C_{16}H_{20}Cl_6N_2O_2S$ on the basis of an accurate mass measurement of *m*/*z* 514.9475 $[M + H]^+$ and an isotope pattern typical of hexachlorinated compounds. It showed some similarity with compounds **1**−**7** in that signals from a thiazoline ring could be discerned in the $^1$H and $^{13}$C NMR spectra (Table

**Table 4.** NMR Data for Barbaleucamide A (**8**) in DMSO-$d_6$ at 25 °C

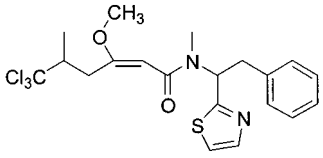
| H/C | $\delta$H m (*J* in Hz) | $\delta^{13}$C m[a] | $^1$H−$^1$H COSY | HMBC[b] |
|---|---|---|---|---|
| 1 | 1.20 d (6.5) | 15.3 q | H-2 | H-2, H-3a, H-3b |
| 2 | 2.97 m | 52.1 d | H-1, H-3a, H-3b | H-1, H-3a, H-3b |
| 3a | 2.94 m | 33.5 t | H-2, H-3b | H-1, H-5 |
| 3b | 3.56 dd (10.5, 13.0) | | H-2, H-3a | |
| 4 | | 167.4 s | | H-2, H-3a, H-3b, OC*H*$_3$, H-5 |
| 5 | 5.54 br s | 95.4 d | | |
| 6 | | 166.0 s | | N*H*, H-5, H-7 |
| 7 | 5.40 m | 47.7 d | NH, H-8a, H-8b | H-8a, H-8b, N*H* |
| 8a | 2.15 m | 36.9 t | H-7, H-8b, H-9 | NH, H-7, H-9, H-10 |
| 8b | 2.55 m | | H-7, H-8a | |
| 9 | 2.70 m | 51.1 d | H-10, H-8a, H-8b | H-8a, H-8b, H-10 |
| 10 | 1.35 d (6.5) | 15.4 q | H-9 | H-8a, H-8b |
| 11 | | 106.0 s | | H-8a, H-8b, H-10 |
| 12 | | 105.7 s | | H-1, H-2, H-3a, H-3b |
| 13 | | 172.9 s | | H-7, H-14, H-15 |
| 14 | 7.77 d (3.3) | 142.3 d | H-15 | H-15 |
| 15 | 7.65 d (3.3) | 120.0 d | H-14 | H-14 |
| OC*H*$_3$ | 3.95 s | 55.3 q | | |
| N*H* | 8.61 br d (9.0) | | H-7 | |

[a] Multiplicity deduced from APT experiment. [b] Proton showing long-range correlation to indicated carbon.

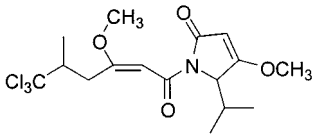
4). The presence of six chlorine atoms, two methyl doublets at $\delta$ 1.20 (H-1) and $\delta$ 1.35 (H-10), and signals at $\delta$ 105.7 (C-12) and 106.0 (C-11) which are suggestive of trichloro-bearing carbons indicated there were two moieties present in **8** that are derived from 5,5,5-trichloroleucine. The presence of a methoxy group and an isolated olefinic proton, as discerned from NMR data, also suggested that compound **8** contained a trichloromethyl/methyl enol ether of a $\beta$-keto-amide portion analogous to that found in barbamide (**10**)[20] and dysidin (**11**).[21] Given this unit and the presence of a thiazoline ring, it was now necessary to account for the other trichloromethyl group, one methyl group, one methylene group, and two methine groups. This could easily be accommodated in the structure now given for **8**. Supporting $^1$H−$^1$H COSY correlations included those from the spin system NH-H-7-H-8-H-9-H-10 (Table 4). The HMBC spectral data, including a key connectivity between H-7 and C-6 and C-13, presented in Table 4, added further support. A NOE difference spectroscopy experiment supported the regiochemistry of the double bond as the *E* configuration; irradiation of $\delta$ 5.54 (H-5) showed a strong enhancement of $\delta$ 3.95 (OC$H_3$). The absolute stereochemistry at C-2/C-9 and C-7 was not pursued. In barbamide (**10**), biosynthetic experiments have demonstrated that its trichloromethyl group is derived from the pro-4*S* methyl group of (2*S*)-leucine.[22] The stereochemistry at C-7 has been determined as *S*.[23] For the dysideathiazoles (e.g., **11**),[7] which, as in **8**, have a thizaole ring derived from 5,5,5-trichloroleucine, X-ray crystallography determined all chiral centers to be *S*.



**8** R = H
**9** R = $CH_3$



**10**



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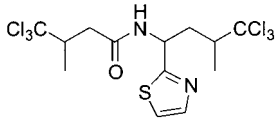
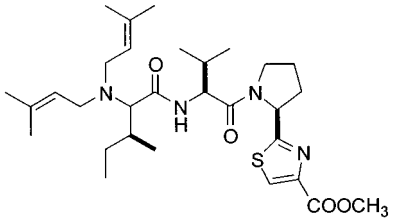


**12**

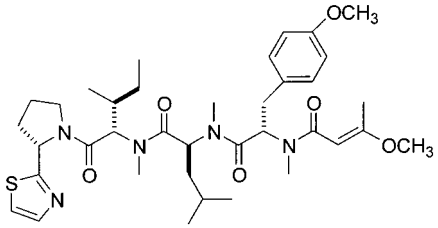
In our hands, compound **8** was unstable even on refrigeration, decomposing during spectral characterization and structure elucidation studies. An even more rapidly decomposing analogue (**9**) was encountered. HRESIMS indicated a molecular formula of $C_{17}H_{22}Cl_6N_2O_2S$ for **9** based on an accurate mass measurement of *m*/*z* 528.9667 and an isotope pattern typical of a hexachlorinated compound. The additional presence of a 3H signal at $\delta$ 2.82 relative to the $^1$H NMR spectrum of **8,** a downfield shift in H-7 ($\delta$ 6.20), and the absence of a signal for an amide N*H* indicated that **9** was simply the *N*-methylated analogue of **8**. We have termed these compounds barbaleucamides A (**8**) and B (**9**) respectively, in recognition of the discovery of barbamide (**10**).[20]

Considerable interest has been shown in the biosynthesis of *Dysidea* polychlorinated compounds due to the presence of the unique 5,5-dichloroleucine and/or 5,5,5-trichloroleucine residue in many of these compounds. The true biogenic source of polychlorinated metabolites isolated from *Dysidea* is considered to be symbiotic cyanobacteria closely associated with the sponge mass. Thus, *Oscillatoria spongeliae* has been shown to be the true source of chlorinated diketopiperazines and a dysidenin analogue isolated from a *D. herbacea* specimen collected from the Great Barrier Reef, Australia.[24] More recently, dysidenin (**7**) has been reported from a Panamanian collection of the free-living cyanobacterium *Lyngbya majuscula*.[19] In light of these observations, the thiazole-pyrrolidine moiety present in compounds **1**−**6** is intriguing, as the only other secondary metabolites it has been reported in are aeruginosamide (**13**),[25] isolated from the cyanobacterium *Microcystis aeruginosa* and lyngbyapeptin A (**14**)[26] from *Lyngbya* spp.

The barbaleucamides (**8**, **9**) also support the contention that many *Dysidea* compounds are of cyanobacterial origin



**13**



**14**

given their obvious similarity to barbamide (**10**).[20] They are interesting, from a biosynthetic perspective, in that they represent two of the few examples where a chlorinated leucine derivative, which plays such an important role, in the synthesis of many chlorinated sponge and cyanobacterial metabolites is actually a precursor of a thiazoline ring.

Finally, it is noteworthy that lyngbyapeptin A (**14**) should contain both the rarely encountered thiazole-pyrrolidine moiety present in compounds **1**−**6** and the, just as rare, methyl enol ether of a $\beta$-keto-amide group found in compounds **8** and **9**. Clearly, cyanobacteria are capable of formidable "combinatorial chemistry", which is why, after a chemical investigation of a sponge, we are left reflecting on the biosynthetic versatility of these prolific prokaryotes!

## Experimental Section

**General Experimental Procedures.** $^1$H and $^{13}$C NMR spectra were recorded in DMSO-$d_6$ at 25 °C on a Varian spectrometer operating at 500 and 125 MHz, respectively, using residual solvent signals as an internal reference. All 2D NMR experiments were performed on the same spectrometer and in the same solvent. HSQC experiments were optimized for $^1J_{CH} = 150$ Hz, and HMBC experiments were optimized for $^nJ_{CH} = 7$ Hz, respectively. HRESIMS were recorded in positive mode on a Mariner Electrospray-Time of Flight Biospectrometry Workstation (PerSeptive Biosystems).

**Animal Material.** The *Dysidea* sp. (NCI 1517) was supplied by Prof. D. J. Faulkner, Scripps Oceanographic Institute, La Jolla, CA. It was collected on a reef flat near Bararin Island, Philippines, at a depth of 20−40 feet in April 1993. This sponge was described as Bermuda grass-like with a greenish-blue exterior and interior on collection. The following description was given by Prof. J. A Hooper, Director, Queensland Centre for Biodiversity, Queensland Museum, Australia, after histological examination: "Growth form. Encrusting mats, grass- or algae-like. Colour. greenish-blue *in situ*, beige in ethanol. Oscules not visible. Texture. Very soft, compressible, mucusy. Surface ornamentation. Small conules interconnected by ridges. Opaque, membraneous. Ectosomal skeleton. Membraneous, with primary fibres protruding and forming distinct surface conules. Choanosomal skeleton. Primary and secondary fibres cored by detritus, where in the former they occupy most of fibre diameter, completely obscuring any pith elements that may be present. Both primary and secondary fibres occasionally branch and rejoin, forming an irregular, very open loose reticulation. Primary fibers mostly parallel to and near the surface, occasionally extending down to the base of the sponge, secondary fibres irregular sparse. Collagen medium and even throughout. Many different foreign sponge spicules incorporated into fibres, with fewer sand grains or calcitic debris. Choanocyte chambers not seen due to poor preservation."

According to Prof. J. A. Hooper (persnal communication), this species has been recorded from three separate collections from the Philippines (Northern and Southern Islands) and is therefore probably a common member of the regional fauna. It cannot be assigned to a known taxon and is probably new to science. It has been registered in the Queensland Museum as QM G318546.

**Extraction and Isolation.** The freeze-dried sponge (160 g) was soaked in $CH_2Cl_2$ (800 mL) overnight, and the solvent was decanted and concentrated in vacuo. This afforded 7.3 g of dark green oil. This extract was applied to a Si gel column and elution initiated with hexane−$CH_2Cl_2$ (2:3) followed by mixtures containing progressively increasing amounts of $CH_2Cl_2$ to 100% $CH_2Cl_2$. Elution then proceeded with $CH_2Cl_2$ mixtures containing progressively increasing amounts of *i*-PrOH. The $CH_2Cl_2$−*i*-PrOH (19:1) fraction was concentrated in vacuo and subjected to semipreparative reversed-phase HPLC chromatography ($C_{18}$ Alltech Alltima; 1.0 × 25.0 cm, 5 $\mu$M particle size; flow rate 3 mL/min) using a $CH_3CN$−0.01% TFA linear gradient (10−100% $CH_3CN$ over 45 min then 100% $CH_3CN$ for 15 min). Detection was at 210 nm. Compound **1** (675 mg) was collected between 43.0 and 45.0 min. Compound **2** (16.7 mg) was collected between 45.0 and 46.0 min and dysidenin (**7**) (43.5 mg) between 46.0 and 47.0 min.

The $CH_2Cl_2$−*i*-PrOH (9:1) fraction from the above Si gel chromatography step was also subjected to semipreparative reversed-phased HPLC as above. Compound **3** (4.9 mg) was collected between 36.0 and 36.8 min and compound **4** (19.3 mg) between 37.2 and 37.9 min. A fraction was also collected between 37.8 and 38.4 min. This fraction was dried and subjected to semipreparative HPLC on the same column but this time using a 50% isocratic gradient at 4 mL/min. Compound **5** (2.0 mg) was collected between 45 and 46.0 min and compound **6** (2.0 mg) between 47.0 and 48.0 min.

The $CH_2Cl_2$−*i*-PrOH (49:1) fraction from the Si gel chromatography step was also subjected to semipreparative reversed-phased HPLC as above. Compound **8** (29.4 mg) eluted between 46.0 and 47.5 min; compound **9** (27.3 mg) eluted between 51.5 and 53.0 min. These two compounds decomposed over time, even on refrigeration. Repeated repurifications during spectral characterization and structure elucidation studies ultimately resulted in only sub-milligram quantities of these compounds remaining.

The hexane−$CH_2Cl_2$ (1:4) fraction from the Si gel chromatography step was also subjected to semipreparative reversed-phased HPLC as above, affording herbadysidolide[27] (35.0 mg) collected between 44.0 and 45.0 min.

**Dysideaproline A (1):** pale yellow oil; $[\alpha]^{23}_D$ +16.5° (*c* 0.02, MeOH); UV (MeOH) $\lambda_{max}$ ($\epsilon$) 213 (8295), 239 (4400) nm; IR (film) $\nu_{max}$ 2980, 2929, 2885, 1640, 1453, 1402, 1269, 1095, 748 cm$^{-1}$; $^1$H NMR see Table 1; $^{13}$C NMR see Table 1; HRESIMS *m/z* 502.0665 [M + H]$^+$ (calcd for $C_{19}H_{28}{}^{35}Cl_4N_3O_2S$, 502.0656); 348.0100 (30) (calcd for $C_{12}H_{18}{}^{35}Cl_4NO_2$, 348.0092). In DMSO-$d_6$, dysideaproline A (**1**) exists as a 4:1 mixture of rotamers. NMR data are given for the major rotamer only.

**Dysideaproline B (2):** pale yellow oil; $[\alpha]^{23}_D$ +37.5° (*c* 0.02, MeOH); UV (MeOH) $\lambda_{max}$ ($\epsilon$) 214 (5885), 240 (3270) nm; IR (film) $\nu_{max}$ 2965, 2929, 2875, 1644, 1456, 1418, 1307, 1267, 1100, 745 cm$^{-1}$; $^1$H NMR see Table 2; $^{13}$C NMR see Table 3; HRESIMS *m/z* 516.0820 [M + H]$^+$ (calcd for $C_{20}H_{30}{}^{35}Cl_4N_3O_2S$, 516.0813); 348.0100 (22) (calcd for $C_{12}H_{18}{}^{35}Cl_4NO_2$, 348.0092). As with dysideaproline A (**1**), compound **2** exists as a 4:1 mixture of rotamers and NMR data are given for the major rotamer only.

**Dysideaproline C (3):** pale yellow oil; $[\alpha]^{23}_D$ +23.5° (*c* 0.02, MeOH); UV (MeOH) $\lambda_{max}$ ($\epsilon$) 212 (6210), 239 (3920) nm; IR (film) $\nu_{max}$ 2970, 2929, 2870, 1631, 1535, 1441, 1204, 750 cm$^{-1}$; $^1$H NMR see Table 2; $^{13}$C NMR see Table 3; HRESIMS *m/z* 488.05017 [M + H]$^+$ (calcd for $C_{18}H_{26}{}^{35}Cl_4N_3O_2S$, 488.0499). As with dysideaproline A (**1**), compound **3** exists as a mixture of rotamers (2:1 ratio) and NMR data are given for the major rotamer only.

**Dysideaproline D (4):** pale yellow oil; $[\alpha]^{23}_D$ +28.8° (*c* 0.02, MeOH); UV (MeOH) $\lambda_{max}$ ($\epsilon$) 215 (5800), 237 (4095) nm; IR (film) $\nu_{max}$ 2957, 2929, 2868, 1640, 1420, 1305, 1266, 1201, 1097, 743 $cm^{-1}$; $^1H$ NMR see Table 2; $^{13}C$ NMR see Table 3; HRESIMS *m/z* 434.1442 $[M + H]^+$ (calcd for $C_{19}H_{30}{}^{35}Cl_2N_3O_2S$, 434.1436); 280.0869 (100) (calcd for $C_{12}H_{20}Cl_2NO_2$, 280.0871). As with dysideaproline A (**1**), compound **4** exists as a 4:1 mixture of rotamers and NMR data are given for the major rotamer only.

**Dysideaproline E (5):** pale yellow oil; $[\alpha]^{23}_D$ +45.5° (*c* 0.02, MeOH); UV (MeOH) $\lambda_{max}$ ($\epsilon$) 214 (5500), 235 (4335) nm; IR (film) $\nu_{max}$ 2955, 2929, 2864, 1641, 1453, 1402, 1320, 1268, 1135, 750 $cm^{-1}$; $^1H$ NMR see Table 2; $^{13}C$ NMR see Table 3; HRESIMS *m/z* 434.1426 $[M + H]^+$ (calcd for $C_{19}H_{30}{}^{35}Cl_2N_3O_2S$, 434.1436); 280.0820 (100) (calcd for $C_{12}H_{20}Cl_2NO_2$, 280.0871). As with dysideaproline A (**1**), compound **5** exists as a mixture of rotamers and NMR data are given for the major rotamer only.

**Dysideaproline F (6):** pale yellow oil; $[\alpha]^{23}_D$ +18.8° (*c* 0.02, MeOH); UV (MeOH) $\lambda_{max}$ ($\epsilon$) 215 (6040), 236 (4135) nm; IR (film) $\nu_{max}$ 2953, 2927, 2868, 1635, 1456, 1419, 1307, 1266, 1106, 726 $cm^{-1}$; $^1H$ NMR see Table 2; $^{13}C$ NMR see Table 3; HRESIMS *m/z* 468.1066 $[M + H]^+$ (calcd for $C_{19}H_{29}{}^{35}Cl_3N_3O_2S$, 468.1046); 314.0480 (65) (calcd for $C_{12}H_{19}Cl_3NO_2$, 314.0481). As with dysideaproline A (**1**), compound **6** exists as a mixture of rotamers and NMR data are given for the major rotamer only.

**Dysidenin (7):** pale yellow oil; $[\alpha]_D$ −10.0° (*c* 0.02, MeOH); $^1H$ and $^{13}C$ NMR consistent with lit.;[5,23] HRESIMS *m/z* 543.9710 $[M + H]^+$ (calcd for $C_{17}H_{24}{}^{35}Cl_6N_3O_2S$, 543.9720).

**Barbaleucamide A (8):** pale yellow oil; $^1H$ NMR see Table 4; $^{13}C$ NMR see Table 4; HRESIMS *m/z* 514.9475 $[M + H]^+$ (calcd for $C_{16}H_{21}{}^{35}Cl_6N_2O_2S$, 514.9455).

**Barbaleucamide B (9):** pale yellow oil; $^1H$ NMR (DMSO-$d_6$) $\delta$ 7.78 (2H, m, H-14/H-15), 6.20 (1H, m, H-7), 5.80 (1H, br s, H-5), 3.84 (3H, s, OC$H_3$), 2.82 (3H, s, N−C$H_3$), 1.30 (3H, d, $J$ = 6.5 Hz, H-10), 1.10 (3H, d, $J$ = 6.5 Hz, H-1); HRESIMS *m/z* 528. 9667 $[M + H]^+$ (calcd for $C_{17}H_{23}{}^{35}Cl_6N_2O_2S$, 528.9611).

**Determination of Absolute Configuration at C-13 in Dysideaproline A (1).** Compound **1** (0.5 mg) was subjected to ozonolysis ($CH_2Cl_2$, −78 °C, 15 min) and then evaporated to dryness under $N_2$. A solution of the ozonolysis product in 6 N HCl (1 mL) was incubated at 110 °C for 18 h. The dried hydrolyzate was subjected to chiral HPLC analysis on a Chirex phase 3126 (D) column (Phenomenex, 0.46 × 25.0 cm) using 2 mM $CuSO_4$−$CH_3CN$ (95:5) as the mobile phase at a flow rate of 0.7 mL/min. Detection was at 254 nm. The $t_R$ for authentic standards of L- and D-proline (Sigma) were 14.0 and 28.5 min, respectively. The proline liberated from the acid hydrolysate of **1** showed a $t_R$ value of 14.0 min.

**Determination of Absolute Configuration at C-5 in Dysideaproline E (5).** After acid hydrolysis of compound **5** (0.2 mg, 6 N HCl, 110 °C, 18 h), the dried hydrolysate was subjected to Marfey derivatization and analysis.[18] HPLC analysis was conducted on a $C_{18}$ Alltech Alltima column (0.46 × 15.0 cm, 5 $\mu$M) using a MeCN in 0.01% TFA linear gradient (10−100% over 25 min, flow rate = 1 mL/min). Detection was at 340 nm. The $t_R$ for the Marfey products of authentic standards of *N*-methyl-L- and *N*-methyl-D-leucine (Sigma) were 14.5 and 14.9 min, respectively. Three peaks at 13.5, 14.5, and 14.9 min were observed in the Marfey profile of **5**. Skeptical that **5** contained both *N*-methyl-L- and *N*-methyl-D-leucine, and anticipating two peaks attributable to epimerization of the thiazole-proline residue during the acid hydrolysis step, we repeated the HPLC analysis using MS detection. Selective ion monitoring at *m/z* 398 showed a peak at 14.9 min consistent with the presence of the *N*-methyl-D-leucine Marfey product. This was confirmed by co-injection. Selective ion monitoring at *m/z* 407 showed two peaks at 13.5 and 14.5 min attributable to the epimerized thiazole-proline residues.

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