New Polychlorinated Amino Acid Derivatives from the Marine Sponge *Dysidea herbacea*

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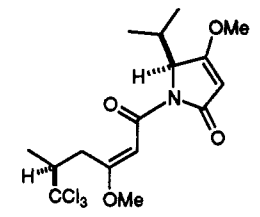
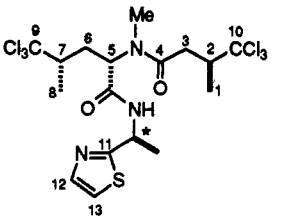
A new series of polychlorinated amino acid derivatives were isolated from the tropical marine sponge *Dysidea herbacea* (Dictyoceratida). Two major hexachlorinated metabolites, dysideathiazole (**4**) and *N*-methyldysideathiazole (**5**), and a minor pentachlorinated compound, 10-dechloro-*N*-methyldysideathiazole (**6**), were isolated from a specimen from Pohnpei. 10-Dechlorodysideathiazole (**7**) was isolated as a minor constituent of a Palauan specimen, together with the major metabolites 9,10-didechloro-*N*-methyldysideathiazole (**8**) and 10-dechloro-*N*-methyldysideathiazole (**6**). A second specimen from Palau contained dysideapyrrolidone (**9**). The structures of **4**–**9** were elucidated by spectroscopic methods. The absolute stereochemistry of compounds **4**–**6** was determined by X-ray crystallography of the natural products and of the carbamate derivative **17** to be *S* at all chiral centers.

Marine sponges of the genus *Dysidea* (Dictyoceratida) from temperate waters invariably contain terpenoids.[1] Certain specimens of *Dysidea herbacea* (Keller 1889), a tropical shallow-water species occurring throughout the Indo-Pacific[2,3] contain both sesquiterpenes and a group of unique amino acid derivatives notable for their trichloromethyl functionality, which has not been found elsewhere in nature. The polychlorinated amino acid derivatives are exemplified by dysidin (**1**),[4] dysidenin (**2**),[5] and isodysidenin (**3**),[6] from Australian and New Guinean specimens of *D. herbacea*. Recent research[7] has demonstrated that the polychlorinated compounds are associated with the sponge's major prokaryotic symbiont, the filamentous cyanobacterium *Oscillatoria spongeliae*,[8] providing additional evidence that the symbiont is responsible for their biosynthesis, as had been frequently suggested.[1,9–12] In this paper, we report the isolation and structural elucidation of a new series of polychlorinated amino acid derivatives from *D. herbacea*.

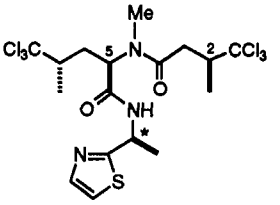
Specimens of *D. herbacea*, a thinly-encrusting blue to blue-gray sponge, were collected at Pohnpei and Palau and stored in methanol prior to extraction. Two major hexachlorinated metabolites, dysideathiazole (**4**) and *N*-methyldysideathiazole (**5**), and a minor pentachlorinated compound, 10-dechloro-*N*-methyldysideathiazole (**6**), were isolated from the specimen (sample 83–039) from Pohnpei. The major metabolites of a Palauan specimen (88–115) were 9,10-didechloro-*N*-methyldysideathiazole (**8**) and 10-dechloro-*N*-methyldysideathiazole (**6**) that were accompanied by a minor constituent, 10-dechlorodysideathiazole (**7**). Finally, dysideapyrrolidone (**9**), a related compound containing a pyrrolidone moiety in place of the thiazole ring, was isolated from another specimen (88-093) of *D. herbacea* from Palau.



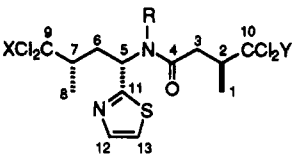
1 $[\alpha]_D = +141°$



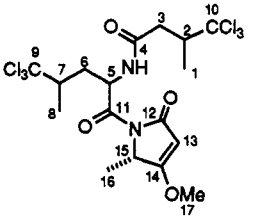
2 $[\alpha]_D = -98°$



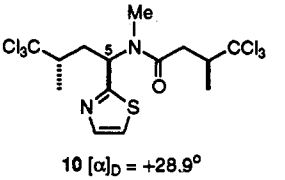
3 $[\alpha]_D = +47°$



	R	X	Y	$[\alpha]_D$
4	H	Cl	Cl	−78.8°
5	Me	Cl	Cl	−108.3°
6	Me	Cl	H	−98.9°
7	H	Cl	H	−57.5°
8	Me	H	H	−79.9°



9 $[\alpha]_D = +16.6°$



10 $[\alpha]_D = +28.9°$

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Dysideathiazole (4) was isolated as white needles, mp 176–177 °C. The molecular formula, $C_{13}H_{16}Cl_6N_2OS$, was obtained from high-resolution mass measurement of the molecular ion at m/z = 457.9117. The infrared bands at 3430 and 1680 cm^{-1} indicate the presence of a secondary amide group. The ^{13}C NMR spectrum contains an amide carbonyl signal at δ 170.1 (s) together with signals at 169.9 (s), 142.6 (d), and 119.3 (d) that are characteristic of a 2-substituted thiazole ring. In the ^1H NMR spectrum, the thiazole protons appear at δ 7.74 (d, 1 H, J = 3 Hz) and 7.32 (d, 1 H, J = 3 Hz). The ^1H and ^{13}C NMR data (Tables I and II), assigned by COSY, XHCORR, and COLOC experiments, were very similar to the comparable data for dysidenin (2),[5] comparison with which suggested the structure of dysideathiazole (4). The ^1H NMR data unambiguously defined the C-1 to C-3 and C-5 to C-8 spin systems which are also clearly recognizable in thiazoles 5–8, but determination of the stereochemistry required an X-ray experiment.

N-Methyldysideathiazole (5) was obtained as white needles, mp 96 °C. The molecular formula, $C_{14}H_{18}Cl_6N_2OS$, obtained from high-resolution mass spectrometry (m/z = 471.9271), indicated that *N*-methyldysideathiazole (5) was a homologue of dysideathiazole (4). The infrared spectrum of thiazole 5 contained a tertiary amide band at 1650 cm^{-1}, and the ^1H NMR (Table I) and ^{13}C NMR (Table II) spectra were almost identical to those of dysideathiazole (4) except for the addition of the *N*-methyl signals at δ 2.92 (s, 3 H) and 30.0 (q). The assignment of signals in the ^1H and ^{13}C NMR spectra was based on spin–spin decoupling and COLOC experiments. The stereochemistry of *N*-methyldysideathiazole (5) was assumed to be the same as that of dysideathiazole (4) because the coupling constants in the ^1H NMR spectra were almost identical. This was not the case for the isomeric compound *N*-methyl-5-*epi*-dysideathiazole (10), which was produced by treatment of thiazole 5 with hydrochloric acid in acetic acid at 95 °C. In the ^1H NMR spectrum of 10, the signals for the H-6 protons were shifted further apart than they were in 4 and 5 and the coupling constants associated with the H-5, H-6a, H-6b, H-7 spin system were significantly different.

10-Dechloro-*N*-methyldysideathiazole (6) was obtained as white prisms, mp 118–119 °C. The molecular formula, $C_{14}H_{19}Cl_5N_2OS$ (m/z = 437.9652), indicated that this metabolite might be related to *N*-methyldysideathiazole (5) by replacement of one chlorine atom by hydrogen, and interpretation of the ^1H and ^{13}C NMR spectra supported this hypothesis. In the ^{13}C NMR spectrum of 6, there appeared a signal at δ 78.0 (d) that was assigned to a $-CHCl_2$ group. The corresponding ^1H NMR signal occurred at δ 6.07 (d, 1 H, J = 3 Hz). These data are similar to those reported for the corresponding dechloro derivatives[13] of isodysidenin (3). Decoupling experiments revealed that the $-CHCl_2$ group was at C-10 rather than at C-9. Comparison of their spectral data, particularly the ^1H NMR coupling constants, suggested that the relative stereochemistry of 10-dechloro-*N*-methyldysideathiazole (6) was the same as that of dysideathiazole (4).

10-Dechlorodysideathiazole (7) was isolated as a colorless oil. The molecular formula, $C_{13}H_{17}Cl_5N_2OS$, m/z = 424.9578 [MH^+], the appearance in the ^{13}C and ^1H NMR spectra of signals attibutable to a $-CHCl_2$ group at δ 77.7 (d) and at δ 5.94 (d, 1 H, J = 3 Hz), respectively, and

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Table I. ^1H NMR (200, 360, or 500 MHz, $CDCl_3$, J in Hz) Data for Dysidenin (2),[5] Thiazoles 4–8 and 10, Pyrrolidone 9, and Derivatives 15–16

H no.	2[a]	4	5	6	7	8	9	10	15	16
1	1.36, d (7)	1.39, d (6.5)	1.41, d (6.5)	1.23, d (6.5)	1.23, d (6.3)	1.22, d (6.5)	1.36, d (6.4)	1.40, d (6.5)	1.41, d (6.5)	1.38, d (6.5)
2	3.3, m	3.25, dqd (9.5, 6.5, 3)	3.35, dqd (9, 6.5, 3)	~2.9, obscured m	~2.8, obscured m	~2.9, obscured m	3.12, ddd (9.8, 6.4, 2.6)	3.36, dqd (9, 6.5, 2.5)	3.34, dqd (9.5, 6.5, 3)	3.28, dqd (9.5, 6.5, 3)
3a	2.5, m	3.05, dd (15, 3)	3.09, dd (16, 3)	2.73, dd (16, 6)	2.59, m	2.70, dd (16, 6)	3.06, dd (14.8, 2.6)	3.05, dd (16, 2.5)	3.17, dd (15, 3)	3.17, dd (15, 3)
3b	2.5, m	2.29, dd (15, 9.5)	2.49, dd (16, 9)	2.44, dd (16, 7)	2.34, m	2.42, dd (16, 7)	2.27, dd (14.8, 9.8)	2.46, dd (16, 9)	2.52, dd (15, 9.5)	2.48, dd (15, 9.5)
5	5.27, dd (11, 4)	5.58, ddd (12, 9, 3)	6.33, dd (12, 3)	6.32, dd (12, 3)	5.56, m	6.26, dd (12, 4)	5.84, m	6.30, dd (10, 5)	5.18, dd (12, 3.5)	4.94, m
6a	3.10, m	2.60, dd (14, 12)	2.85, dd (13, 12)	~2.9, dd (13, 12)	~2,7, obscured m	2.54, ddd (14, 12, 2.5)	2.10, m	3.16, ddd (14, 10, 3)	~2.4, d (14, 12)	~2.4, m
6b	1.94, m	2.08, ddd (14, 10, 3)	2.24, ddd (13, 10.5, 3)	2.22, ddd (13, 11, 3)	2.14, ddd (13.5, 10, 3.5)	2.20, ddd (14, 10, 4)	1.86, m	1.80, ddd (14, 10, 5)	2.06, m	2.21, m
7	2.20, m	2.64, dq (10, 6.5)	2.31, dq (10.5, 6.5)	2.35, dq (11, 6.5)	~2.7, obscured m	2.05, dqdd (10, 6.5, 3, 2.5)	2.56, m	2.69, dqd (10, 6.5, 3)	2.34, m	2.28, m
8	1.33, d (7)	1.49, d (6.5)	1.44, d (6.5)	1.43, d (6)	1.48, d (6.4)	1.23, d (6.5)	1.55, d (6.4)	1.40, d (6.5)	1.37, d (6.5)	1.35, d (6.5)
9						5.82, d (3)				
10				6.07, d (3)	5.94, d (3)	6.07, d (3)				
11									9.54, s	3.65, m
12	7.60, d (3.5)	7.74, d (3)	7.72, d (3)	7.75, d (3)	7.75, d (3)	7.74, d (3)		7.78, d (3)		
13	7.26, d (3.5)	7.32, d (3)	7.35, d (3)	7.35, d (3)	7.33, d (3)	7.34, d (3)	5.06, s	7.36, d (3)		
15							4.51, q (6.6)			
16							1.48, d (6.6)			
17							3.88, s			
N-Me	3.04, s		2.92, s	2.90, s		2.88, s		2.94, s	3.03, s	2.95, s
NH	6.86, br d (8)	6.39, br d (9)			6.40, d (9)		6.55, d (9.1)			

[a] Selected data from ref 5, protons renumbered only for this table.

Table II. ^{13}C NMR (50 MHz, $CDCl_3$) Data for Dysidenin 2,[5] Thiazoles 4–8 and 10, Pyrrolidone 9, and Derivatives 15–16

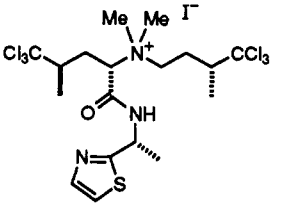
C no.	2[a]	4	5	6	7	8	9	10	15	16
1	17.3* (q)	16.9 (q)	17.2 (q)	15.2 (q)	16.6 (q)	15.2 (q)	16.8 (q)	17.3 (q)	17.2 (q)	17.2 (q)
2	51.4** (d)	51.6 (d)	51.4 (d)	40.5 (d)	48.7 (d)	40.5 (d)	50.8 (d)	51.7 (d)	51.5 (d)	51.5 (d)
3	37.4†† (t)	40.2 (t)	37.4 (t)	36.3 (t)	39.8 (t)	36.3 (t)	40.2 (t)	37.4 (t)	37.1 (t)	37.7 (t)
4	171.2§ (s)	170.1 (s)	171.0 (s)	171.0 (s)	170.6 (s)	171.4 (s)	170.1 (s)	170.3 (s)	171.7 (s)	172.4 (s)
5	54.0** (d)	48.9 (d)	51.6 (d)	51.6 (d)	52.0 (d)	51.4 (d)	51.6 (d)	51.7 (d)	61.7 (d)	63.2 (d)
6	31.0†† (t)	39.9 (t)	33.6 (t)	33.6 (t)	39.2 (t)	31.9 (t)	36.5 (t)	34.3 (t)	32.6 (t)	31.2 (t)
7	51.9** (d)	52.1 (d)	52.0 (d)	52.0 (d)	40.8 (d)	41.3 (d)	52.1 (d)	52.2 (d)	51.8 (d)	52.0 (d)
8	16.2* (q)	16.6 (q)	16.3 (q)	16.4 (q)	15.1 (q)	15.9 (q)	15.8 (q)	16.5 (q)	16.2 (q)	16.3 (q)
9	105.5† (s)	105.5 (s)	105.6 (s)	105.6 (s)	105.4 (s)	78.6 (d)	105.8 (s)	105.6 (s)	105.1 (s)	105.8 (s)
10	105.1† (s)	104.8 (s)	105.1 (s)	78.0 (d)	77.7 (d)	78.0 (d)	104.9 (s)	105.3 (s)	105.4 (s)	105.3 (s)
11	168.2§ (s)	169.9 (s)	168.3 (s)	168.7 (s)	170.1 (s)	168.9 (s)	169.0 (s)	167.1 (s)	197.4 (s)	52.5 (s)
12	142.3 (d)	142.6 (d)	142.3 (d)	142.4 (d)	142.6 (d)	142.3 (d)	171.1 (s)	142.6 (d)		
13	118.9 (d)	119.3 (d)	119.9 (d)	120.1 (d)	119.4 (d)	119.9 (d)	92.7 (d)	102.2 (d)		
14							181.2 (s)			
15							56.0 (d)			
16							17.0 (q)			
17							58.9 (q)			
N-Me	30.8 (q)		30.0 (q)	30.0 (q)		30.0 (q)		30.6 (q)	29.4 (q)	29.3 (q)

[a] Selected data from ref 5, carbons renumbered only for this table; *, **, †, § assignments of these signals may be interchanged.

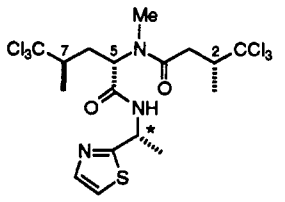
comparison of spectral data with those of thiazoles 4 and 6 indicated that the compound was a dechloro derivative of dysideathiazole (4). Decoupling experiments again showed that the $-CHCl_2$ group was at C-10 rather than at C-9.

9,10-Didechloro-*N*-methyldysideathiazole (8) was obtained as a colorless oil. The molecular formula, $C_{14}H_{20}Cl_4N_2OS$, was obtained by high resolution mass measurement (m/z = 405.0136 [MH^+]). The ^{13}C NMR spectrum contained two signals at δ 78.6 (d) and 78.0 (d) that were assigned to two $-CHCl_2$ groups. The ^1H NMR spectrum contained $-CHCl_2$ signals at δ 6.05 (d, 1 H, J = 3 Hz, H-10) and 5.81 (d, 1 H, J = 3 Hz, H-9), and analysis of the coupling constants strongly suggested that the didechloro derivative 8 had the same relative stereochemistry as the other compounds in the series. The optical rotations of the five new thiazoles 4–8 have the same sign, suggesting that their absolute configurations are identical, although one must recognize the usual caveat that absolute stereochemistry cannot be securely assigned on the basis of optical rotation data alone.

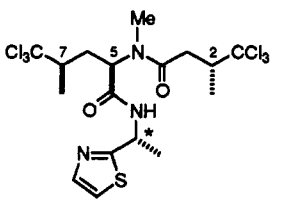
The absolute configuration of isodysidenin, based on a single-crystal X-ray analysis of the methylated derivative 11, was reported to be as depicted by structure 12.[6]



11



12



13

Comparison of optical rotation and ^1H NMR data of acid hydrolysis products established that dysidenin and isodysidenin differ only by being epimeric at C-5. The absolute stereochemistry of the remaining three chiral centers were assigned identical configurations in both compounds: *R* at both trichloromethyl-bearing carbons (carbons 2 and 7) and *R* at the carbon α to the thiazole ring (C-13 in the original paper, marked here as C*).[14] Dysidenin, which was originally reported without assignment of stereochemistry for the four chiral carbons, was thus assigned structure 13.[14] However, Ireland's group showed that thiazole amino acids undergo extensive racemization during acid hydrolysis, with racemization apparently initiated by protonation of the thiazole nitrogen followed by exchange of a proton at the carbon α to the thiazole ring.[15] Their reinvestigation of the absolute configuration of the *D. herbacea* compounds showed that the 2-(1'-aminoethyl)thiazoles present in dysidenin and isodysidenin have identical stereochemistry but opposite to that previously reported (i.e., *S* at carbon 13 in both compounds), and, by inference, that the natural products' correct absolute configuration is opposite to that reported from the X-ray study, i.e., they are correctly depicted by structures 2 and 3.[16] In 1985, the total synthesis of (+)-13-demethyldysidenin and (−)-13-demethylisodysidenin conclusively proved that the naturally-occurring compounds have the opposite absolute configuration from that shown in structures 12 and 13.[17]

Initial X-ray analyses of dysideathiazole (4) and *N*-methyldysideathiazole (5) provided information about the relative stereochemistry of thiazoles 4–8, indicating that these were either all *R* or all *S*. The difficulties described above in ascertaining the correct absolute configuration of dysidenin and isodysidenin led us to supplement our original X-ray data using chemical methods. Since the relative stereochemistry of the dysideathiazoles 4–8 had been defined by X-ray experiments and comparison of spectral data, we needed to unambiguously determine the absolute configuration at only one of the chiral carbons. We first attempted to hydrolyze *N*-methyldysideathiazole (5) to 4,4,4-trichloro-3-butanoic acid, for which the optical rotation and absolute configuration of both the *R* and *S* acids was known.[18,19] However, thiazole 5 proved resistant to hydrolysis by treatment with 6 N HCl and under more

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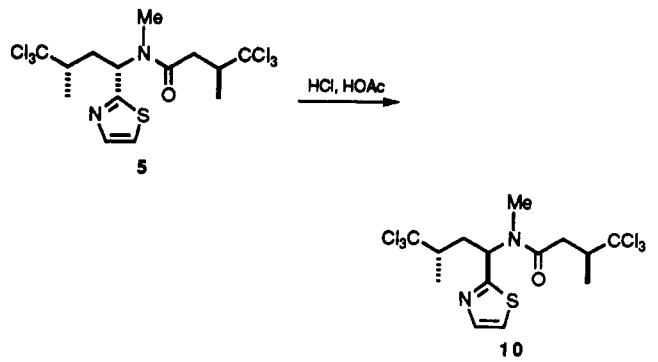
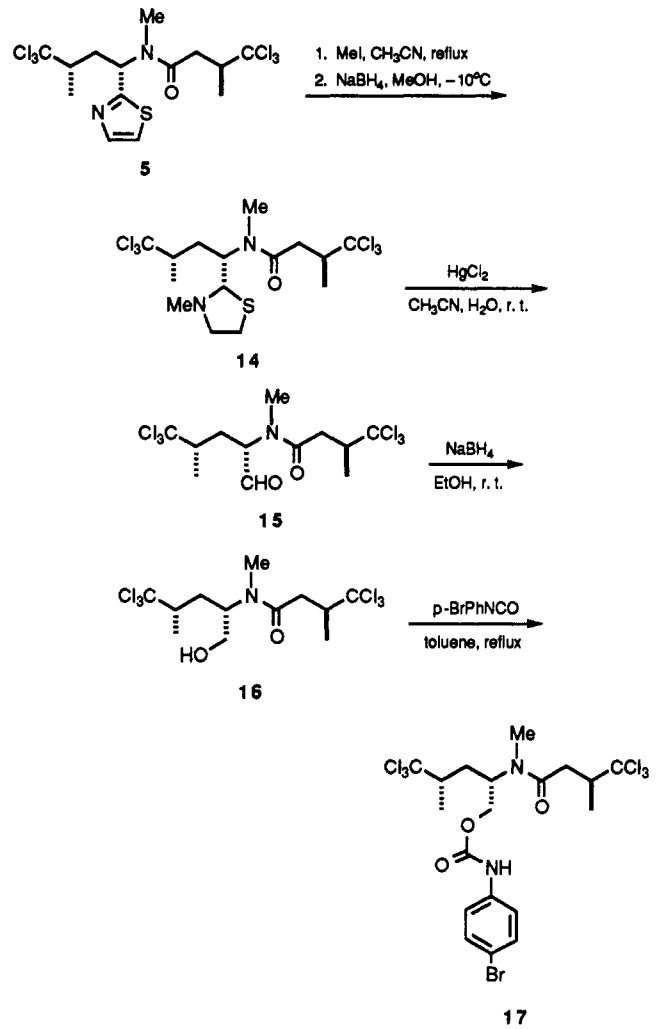
Scheme I



Scheme II



stringent conditions (see Experimental Section) gave low yields of the 5-*epi* derivative (10). An alternative strategy involved performing an X-ray analysis of a derivative of the natural product that contained either a residue of known absolute configuration or a different heavy atom. We chose the latter strategy. Using a procedure of Dondoni *et al.*,[20] the thiazole ring of *N*-methyldysideathiazole (5) was converted to the corresponding *N*-methylthiazolidine 14, which was then oxidized to the aldehyde 15. The aldehyde was reduced to the corresponding primary alcohol 16, which was treated with *p*-bromophenyl isocyanate to obtain the *p*-bromophenyl carbamate 17. This was

(20) Dondoni, A.; Fantin, G.; Fogagnolo, M.; Pedrini, P. *J. Org. Chem.* 1990, *55*, 1439.

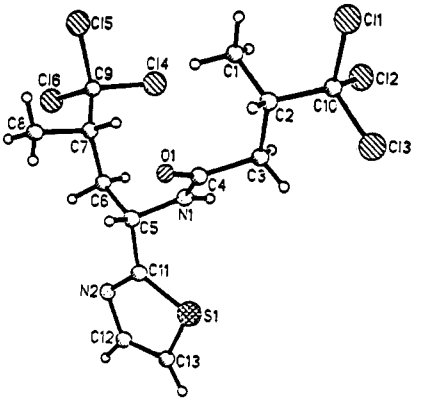


Figure 1. X-ray crystal structure of dysideathiazole (4).

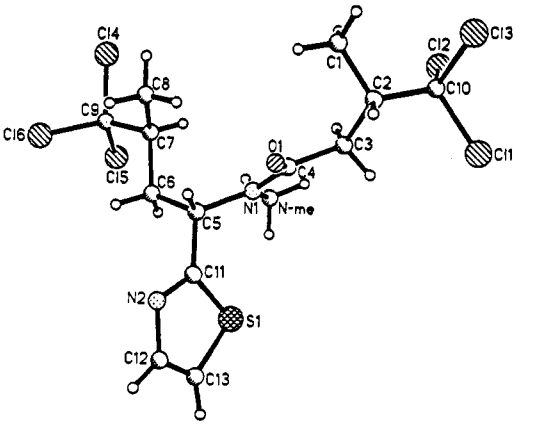


Figure 2. X-ray crystal structure of *N*-methyldysideathiazole (5).

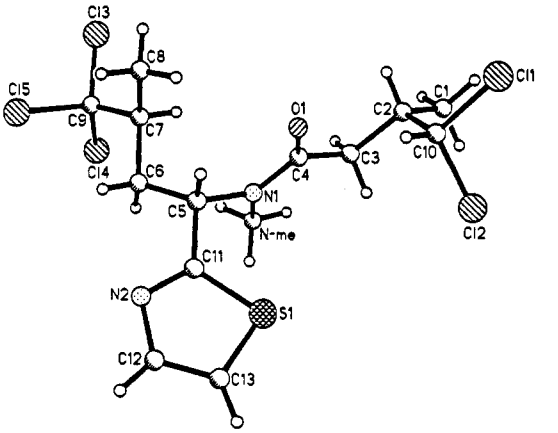


Figure 3. X-ray crystal structure of 10-dechloro-*N*-methyldysideathiazole (6).

crystallized and used in further X-ray experiments. The key point to note in these experiments is that since the anomalous scattering contribution of bromine is much greater than that of chlorine, the determination of the absolute stereochemistry would rest on a stronger experimental signal. The results of the X-ray crystallographic determinations are shown in Figures 1–4, depicting the absolute stereostructures of compounds 4, 5, 6, and 17, respectively. The absolute configuration at each of the chiral centers is *S* in all molecules.

It is interesting to note that thiazoles 4–6 and naturally-occurring dysidenin are of identical absolute configuration at C-2, C-5, and C-7 (all *S*) and have negative optical rotations of similar magnitude (presumably thiazoles 7

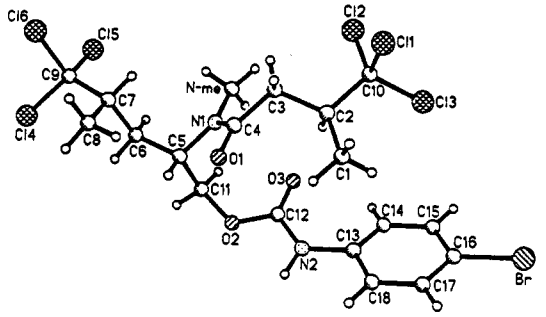


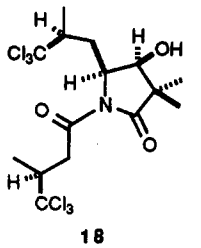
Figure 4. X-ray crystal structure of carbamate 17.

and 8 are also all *S*), whereas *N*-methyl-5-*epi*-dysideathiazole (**10**) and naturally occurring isodysidenin have the *R* configuration at C-5 and possess positive optical rotations. These observations suggest that the absolute configuration at C-5 strongly influences the optical rotation of the compounds in this series.

Dysideapyrrolidone (**9**) was isolated as white prisms, mp 165–166 °C. The molecular formula, $C_{17}H_{22}Cl_6N_2O_4$, was obtained from high-resolution mass measurement (*m/z* = 528.9800 [MH$^+$]). The ^{1}H NMR spectrum contained signals at δ 1.55 (d, 3 H, J = 6.4 Hz) and 1.36 (d, 3 H, J = 6.4 Hz) typical of methyl groups on carbons bearing the trichloromethyl moiety, as found in thiazoles **4** and **5**. The ^{13}C NMR spectrum also contained signals at δ 105.8 (s) and 104.9 (s), characteristic of the trichloromethyl group. No thiazole signals were present in the NMR spectra. In the ^{13}C NMR spectrum, signals at δ 92.7 (d) and 181.2 (s) suggested the presence of a substituted pyrrolidone moiety similar to that found in dysidin (**1**), which was previously reported from *D. herbacea*,[4] in malyngamide A (**19**), isolated from the marine filamentous cyanobacterium *Lyngbya majuscula*,[21] and in the mirabimides (**20–23**), from the terrestrial filamentous cyanobacterium, *Scytonema mirabile*.[22] The presence of the substituted pyrrolidone moiety was confirmed by heteronuclear correlation experiments (HMBC and XHCORR) which showed that a ^{1}H signal at δ 5.06 (s, 1 H) was coupled to an amide carbonyl signal at δ 171.1 (s) and to the signals at δ 92.7 (d) and 181.2 (s). To establish the configuration at C-15, a sample of dysideapyrrolidone **9** was oxidized to alanine by treatment with sodium periodate and potassium permanganate.[22] This was followed by derivatization with 2-propanol/acetyl chloride and pentafluoropropionic acid and analysis by chiral GC–MS. The dysideapyrrolidone derivative had a retention time identical within experimental error to that of the pentafluoropropionamide isopropyl esters of authentic L-(*S*)-alanine, and it was concluded that the configuration about C-15 in the pyrrolidone ring must thus be *S*.

The consistent association between *D. herbacea* and *O. spongeliae* is one of the best-studied sponge-prokaryote symbioses.[23] The resemblance of dysidin **1** to malyngamide A **19** first led to the proposal[21] that the cyanobacterial symbiont of *D. herbacea* is the actual producer of the polychlorinated compounds. Dysideapyrrolidone **9** shares

Chart I



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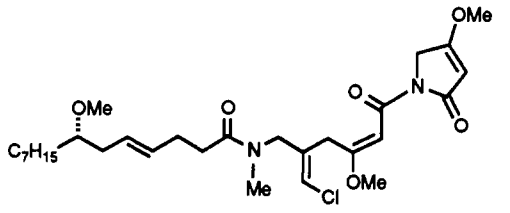
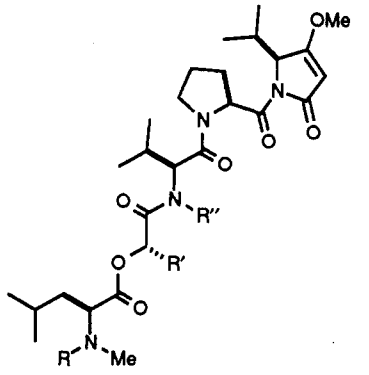
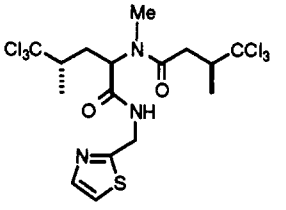


19



		R	R′	R″
20	mirabimide A	Me	$CH(CH_3)CH_2CH_3$	Me
21	mirabimide B	Me	$CH(CH_3)CH_2CH_3$	H
22	mirabimide C	Me	$CH(CH_3)_2$	Me
23	mirabimide D	Ac	$CH(CH_3)CH_2CH_3$	Me



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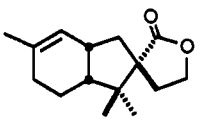

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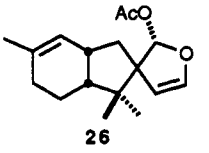

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structural features with the thiazoles **2–8** as well as the cyanobacterial metabolites **19** and **20–23** and thus provides additional circumstantial support for this hypothesis. The related compound, dysidamide (**18**), was recently reported from a Red Sea sample of *D. herbacea*.[24] We have recently shown[7] that the major chlorinated metabolite demethylisodysidenin (**24**)[13] from an Australian sample of *D. herbacea* was limited entirely to the associated cyanobacterial filaments, whereas the accompanying sesquiterpenes herbadysidolide (**25**)[25] and spirodysin (**26**)[26] were found only in the sponge cells.

(21) Cardellina, J. H., II; Marner, F.-J.; Moore, R. E. *J. Am. Chem. Soc.* **1979**, *101*, 240.
(22) Carmeli, S.; Moore, R. E.; Patterson, G. M. L. *Tetrahedron* **1991**, *47*, 2087.
(23) Wilkinson, C. R. Symbiotic interactions between marine sponges and algae. In *Algae and Symbioses: Plants, Animals, Fungi, Viruses, Interactions Explored*, Reisser, W., Ed.; Biopress Ltd.; Bristol, 1992; pp 111–151.
(24) Carmely, S.; Gebreyesus, T.; Kashman, Y.; Skelton, B. W.; White, A. H.; Yosief, T. *Aust. J. Chem.* **1990**, *43*, 1881.
(25) Charles, C.; Braekman, J. C.; Daloze, D.; Tursch, B.; Declercq, J. P.; Germain, G.; Van Meerssche, M. *Bull. Soc. Chim. Belg.* **1978**, *87*, 481.

It is interesting to speculate on the function of these compounds in the symbiotic association. The polychlorinated metabolites are not antimicrobial (inactive at 100 μg per disk in a standard disk assay using *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans*), but are strongly deterrent in fish-feeding experiments at concentrations well below the levels at which they occur *in vivo* (our unpublished data). It is possible that in this way these metabolites increase the fitness of the sponge–cyanobacterial association and perhaps serve other roles in maintaining the symbiosis.

Experimental Section

General Experimental Procedures. All solvents used were mass-spectral grade (Optima, Fisher Scientific) or freshly distilled from reagent grade. Acetyl chloride, 2-propanol, and pentafluoropropionic acid were obtained from Alltech, Inc., and other reagents were obtained from Aldrich Chemical Co.; all were used as received unless otherwise specified. Glassware used in reactions requiring anhydrous conditions was flame-dried, allowed to cool in a desiccator over calcium chloride, and flushed with dry nitrogen gas before use. Column chromatography was performed using Merck silica gel 60 (70–230 mesh ASTM). HPLC was carried out on a silica gel column (Whatman Partisil 10, 1 cm × 45 cm). ^{1}H- and ^{13}C NMR spectra were obtained in deuterated chloroform (Isotec, Inc.). High-resolution mass spectra were obtained from the Mass Spectrometry Facility, University of California, Riverside.

Extraction and Isolation. Specimens of *D. herbacea* (Keller 1889), a dictyoceratid sponge, were collected by hand using SCUBA. Specimen 83–039, a thin blue-gray encrusting sponge, was collected from −10 m at the eastern side of Mwang Passage, Pohnpei. The sponge was stored in methanol at 4 °C for 3 years. The methanol was decanted and fresh methanol added to the sponge which was sonicated to extract further material. The methanolic extracts were filtered, the methanol removed under reduced pressure, and the resulting aqueous residue sequentially extracted with hexanes, dichloromethane, and ethyl acetate. The remaining aqueous material was lyophilized. The hexane-soluble material, 1.5 g of a green oil, was chromatographed on silica gel, eluting with a solvent gradient from hexanes to ethyl acetate, to give a mixture of polychlorinated thiazoles. Repeated separation by HPLC on silica gel with hexanes–Et_2O (1:1) afforded pure dysideathiazole (**4**, 160 mg, 0.25% dry weight), *N*-methyldysideathiazole (**5**, 122 mg, 0.19% dry weight), and 10-dechloro-*N*-methyldysideathiazole (**6**, 3.2 mg, 0.005% dry weight). The known sesquiterpene herbadysidolide (**18**, 134 mg, 0.21% dry weight), previously described from *D. herbacea*,[15] was also isolated; physical data were consistent with published values. About 12% of the hexane-soluble material was a mixture of sterols, which proved to be characteristic of *D. herbacea* specimens containing chorinated metabolites; these sterols were not characterized further. Additional quantities of the thiazoles and herbadysidolide were present in the dichloromethane extract. Dysideathiazole (**4**) and *N*-methyldysideathiazole (**5**) were crystallized from hexanes–Et_2O (1:1).

Specimen 88–115, a thin purple encrusting sponge growing on dead coral, was collected from −3 m at Kaibaku marine lake, Palau. The sponge was stored in methanol at 4 °C for 2 years. The dichloromethane-soluble material from a methanolic extraction of the sponge was chromatographed as described above to obtain 10-dechlorodysideathiazole (**7**, 6.5 mg, 0.11% dry weight) and 9,10-didechloro-*N*-methyldysideathiazole (**8**, 51 mg, 0.82% dry weight). 10-Dechloro-*N*-methyldysideathiazole (**6**, 291 mg, 4.7% dry weight) and dysideathiazole (**4**, 2.1 mg, 0.034% dry weight) were also isolated from this specimen. Thiazole **8** was also isolated (32 mg, 0.038% dry weight) from specimen 85–006, a thin dark sponge collected at Iwayama Bay, Palau.

Specimen 88–093, a thin green encrusting sponge overgrowing a red coralline alga (*Jania* sp.), was collected from −3 m at Ngemelis Island drop-off, Palau. The sponge was stored in methanol at 4 °C for 2 years. The hexane-soluble material from a methanolic extraction of the sponge was chromatographed as described above to obtain dysideapyrrolidone (**9**, 22 mg, 0.013% dry weight) as well as 9,10-didechloro-*N*-methyldysideathiazole (**8**, 4.1 mg, 0.0024% dry weight).

(2*S*,5*S*,7*S*)-Dysideathiazole (4): white needles, mp 176–177 °C; $[\alpha]_D$ −71.8° (c = 2.07, $CHCl_3$); UV ($CHCl_3$) 202 nm (ε 6865), 241 (ε 5900); IR ($CHCl_3$) 3430, 3020, 3000, 1680, 1505, 1465, 1445, 1385 cm^{-1}; ^{1}H NMR: see Table I; ^{13}C NMR see Table II; EI-HRMS observed m/z = 457.9117, $C_{13}H_{16}Cl_6N_2OS$ requires 457.9115. Crystallographic information: A single crystal in the form of a clear colorless block, with dimensions 0.20 × 0.20 × 0.20 mm, was used to collect a room temperature (22 °C) data set using Cu Kα radiation. The data were collected on a Siemens R3m/V diffractometer to $2\theta < 115.0°$ using $2\theta - \theta$ scan with a variable scan speed of 1.5 to 30.0°/min in ω. Photographs displayed orthorhombic symmetry with accurate cell constants determined by a least-squares fit of 25 well-centered reflections in the range of $30° < 2\theta < 40°$, of a = 9.625(3) Å, b = 10.581(3) Å, and c = 20.386(6) Å. Systematic extinctions uniquely indicated the space group $P2_12_12_1$, where one molecule of composition $C_{13}H_{16}Cl_6N_2OS$ formed the asymmetric unit, with a calculated density D_x = 1.475 g cm^{-3}. A total of 3627 unique reflections were collected, of which 2327 (64%) were judged observed with $|F_o| > 4\sigma(F)$. The data were corrected for Lorentz and polarization effects, but not for absorption. The structure was solved by direct methods using the SHELXTL library of programmes. Several rounds of refinement using full-matrix least-squares techniques were applied to all non-hydrogen atoms with anisotropic thermal parameters and fixed riding hydrogens to converge to a final agreement factor R = 5.21% and wR = 5.31%, where $w^{-1} = \sigma^2(F) + 0.0005F^2$. The absolute configuration test (η = 1.02(9)) indicates the stereochemistry shown in Figure 1. The refinement of the enantiomeric structure led to R = 5.73% and wR = 5.99%, a statistically significant difference.

(2*S*,5*S*,7*S*)-*N*-Methyldysideathiazole (5): white needles, mp 96 °C; $[\alpha]_D$ −108.3° (c = 2.02, $CHCl_3$); UV ($CHCl_3$) 205 nm (ε 6530) 243 (ε 4325); IR ($CHCl_3$) 3020, 3000, 1650, 1505, 1470, 1405, 1385, 1305 cm^{-1}; ^{1}H NMR see Table I; ^{13}C NMR see Table II; EI-HRMS observed m/z = 471.9271, $C_{14}H_{18}Cl_6N_2OS$ requires 471.9271. Crystallographic information: A colorless block-shaped crystal of dimensions 0.25 × 0.25 × 0.35 mm was selected for crystallographic analysis and placed on a Siemens R3m/V diffractometer. Data were collected at room temperature (22 °C) using Cu Kα radiation. Systematic conditions identified the space group as $P2_12_12$, and a least-squares refinement of 25 selected reflections ($30° < 2\theta < 40°$) gave a = 10.183(2) Å, b = 32.762(7) Å, and c = 6.095(1) Å, which corresponds to one molecule of $C_{14}H_{18}Cl_6N_2OS$ comprising the asymmetric unit. A total of 3357 unique reflections were collected, of which 2168 were judged observed ($|F_o| > 4\sigma(F)$) and used in subsequent refinements. Lorentz and polarization corrections, but no absorption correction, were applied to the data. Full-matrix least-squares refinement converged to a final unweighted R = 5.66% and a wR = 5.40%, where $w^{-1} = \sigma^2(F) + 0.0005F^2$. Hydrogens were placed at calculated positions and refined with isotropic thermal factors using rigid body techniques. The absolute configuration, as indicated in Figure 2, was determined using the η method (η = 0.95(9)). Refinement of the enantiomer led to R = 6.27% and wR = 6.18%, a statistically significant difference.

(2*S*,5*S*,7*S*)-10-Dechloro-*N*-methyldysideathiazole (6): white prisms, mp 118–119 °C; $[\alpha]_D$ −98.9° (c = 0.47, $CHCl_3$); UV ($CHCl_3$) 244.5 (ε 4040); IR ($CHCl_3$) 3010, 1645, 1455, 1400 cm^{-1}; ^{1}H NMR see Table I; ^{13}C NMR see Table II; EI-HRMS, obsd m/z = 437.9652, $C_{14}H_{19}Cl_5N_2OS$ requires 437.9661. Crystallographic information: A clear colorless rectangular crystal of dimensions 0.25 × 0.25 × 0.35 mm was chosen to collect a room-temperature (22 °C) X-ray crystallographic data set using Cu Kα (1.541 78 Å) radiation. Preliminary photographs indicated orthorhombic symmetry, and a least-squares fit of 25 well-centered reflections ($25° < 2\theta < 40°$) gave a = 11.086(6) Å, b = 13.009(4) Å, and c = 13.363(4) Å. Systematic extinctions indicated the space group

(26) Kazlauskas, R.; Murphy, P. T.; Wells, R. J. *Tetrahedron Lett.* **1978**, 4949.

as $P2_12_12_1$, with one molecule of $C_{14}H_{19}Cl_5N_2OS$ forming the asymmetric unit, for a calculated density D_x = 1.519 g cm^{-3}. A total of 5798 independent reflections were collected using 2θ – θ scans, with a variable scan speed of 1.5 to 30.0°/min in ω, using a Siemens R3m/V diffractometer. Of these, 5140 (89%) were judged observed ($|F_o| > 4\sigma(F)$) and used in subsequent least-squares refinements. Lorentz and polarization corrections, but no absorption correction, were applied to the data. A full-matrix least-squares refinement gave a final unweighted R = 8.18% and a wR = 9.42%, where $w^{-1} = \sigma^2(F) + 0.0003F^2$. Hydrogens were placed at fixed positions with fixed isotropic thermal parameters and were allowed to refine using rigid body techniques. The absolute configuration was assessed using the η method (η = 1.02(7)) to indicate the stereochemistry shown in Figure 3.

(2*S*,5*S*,7*S*)-10-Dechlorodysideathiazole (7): colorless oil; $[\alpha]_D$ –57.5°(c = 0.65, $CHCl_3$); UV ($CHCl_3$) 244.5 (ε 4040); IR ($CHCl_3$) 3010, 1645, 1455, 1400 cm^{-1}; ^{1}H NMR see Table I; ^{13}C NMR see Table II; DCI-HRMS (NH_3) obsd m/z = 424.9578 [MH^+] $C_{13}H_{18}Cl_5N_2OS$ requires 424.9582.

(2*S*,5*S*,7*S*)-9,10-Didechloro-*N*-methyldysideathiazole (8): colorless oil; $[\alpha]_D$ -79.9°(c = 3.24, $CHCl_3$); UV ($CHCl_3$) 214 (ε 4270), 241 (ε 4880); IR ($CHCl_3$) 2965, 2925, 1640, 1400 cm^{-1}; ^{1}H NMR see Table I; ^{13}C NMR see Table II; DCI-HRMS (NH_3) obsd m/z = 405.0136 [MH^+] $C_{14}H_{21}Cl_4N_2OS$ requires 405.0129.

(2*S*,5*S*,7*S*,15*S*)-Dysideapyrrolidone (9): white prisms, mp 165–166 °C; $[\alpha]_D$ +16.6°(c = 0.42, $CHCl_3$); UV ($CHCl_3$) 215 (ε 5100), 238 (ε 14 000); IR ($CHCl_3$) 3010, 2970, 1720, 1650, 1320 cm^{-1}; ^{1}H NMR see Table I; ^{13}C NMR see Table II; FAB-HRMS obsd m/z = 528.9800 [MH^+] $C_{17}H_{23}Cl_6N_2O_4$ requires 528.9789.

Epimerization of *N*-Methyldysideathiazole. A solution of *N*-methyldysideathiazole (5, 65 mg) in glacial acetic acid (4 mL) containing concentrated hydrochloric acid (2 mL) was heated under nitrogen at 95 °C for 30 h. The reaction mixture was cooled, poured into ice (5 mL), and extracted with dichloromethane (3 × 5 mL). The combined extracts were washed with water and dried over sodium sulfate, and the solvent evaporated to obtain a residue that was chromatographed by HPLC on silica with hexanes–Et_2O (1:1) to afford the starting material (40 mg, 62% recovery) and *N*-methyl-5-*epi*-dysideathiazole (10, 6 mg, 10% yield), which was obtained as a colorless oil: $[\alpha]_D$ +28.9°(c = 0.75, $CHCl_3$); UV ($CHCl_3$) 244 (ε 4730); IR ($CHCl_3$) 3030, 3000, 2950, 1655, 1410 cm^{-1}; ^{1}H NMR see Table I; ^{13}C NMR see Table II.

Synthesis of Aldehyde 15. *N*-Methyldysideathiazole (5, 60 mg) was dissolved in CH_3CN (12 mL), and methyl iodide (4 mL) that had been freshly filtered through a column of activated alumina was added. The mixture was refluxed at 80–82 °C for 72 h under a positive atmosphere of dry nitrogen gas, after which the solvent was removed under reduced pressure. The resulting pale yellow solid residue was dissolved in dry MeOH (8 mL) and chilled to –10 °C using an ice–brine bath. $NaBH_4$ (12.6 mg) was added. The reaction mixture was stirred at –10 °C for 30 min and then quenched by the addition of acetone (1 mL). The solvent was removed under reduced pressure and the white residue partitioned between saturated aqueous $NaHCO_3$ (6 mL) and i-Pr_2O (3 x 5 mL). The ethereal extracts were combined and dried over Na_2SO_4 and the ether evaporated. The resulting crude thiazolidine mixture was dissolved in CH_3CN (3 mL), to which was added a solution of $HgCl_2$ (40 mg) in CH_3CN (4.8 mL) and H_2O (1.2 mL). This mixture was stirred at room temperature for 30 min and then concentrated under reduced pressure. i-Pr_2O (15 mL) was added, the mixture filtered through Celite, and the ether evaporated. The residue was washed with saturated aqueous NaCl (3 mL) and extracted with i-Pr_2O (3 x 5 mL). The ethereal extracts were combined and dried over Na_2SO_4 and the ether evaporated to give a residue that was chromatographed by HPLC on silica with hexanes–Et_2O (8:2) to afford the starting material (39 mg, 65% recovery) and 15 (9 mg, 17% yield) which was obtained as a colorless oil: $[\alpha]_D$ –45.1°(c = 1.90, $CHCl_3$); ^{1}H NMR see Table I; ^{13}C NMR see Table II.

Synthesis of Alcohol 16. The purified aldehyde 15 was dissolved in absolute EtOH (8 mL) to which was added 550 μL of a solution of $NaBH_4$ (22 mg) in absolute EtOH (10.0 mL). The mixture was stirred at room temperature for 45 min and then quenched with acetone (1.5 mL). The solvent was removed under reduced pressure and the residue washed with saturated aqueous $NaHCO_3$ (6 mL) and extracted with i-Pr_2O (3 x 5 mL). The ethereal extracts were combined and dried over Na_2SO_4, and the ether was evaporated to give 16 (9 mg, quantitative yield) which was obtained as a white amorphous solid: $[\alpha]_D$ –40.8°(c = 1.78, $CHCl_3$) ^{1}H NMR see Table I; ^{13}C NMR see Table II.

Synthesis of Carbamate 17. The alcohol 16 was dissolved in dry toluene (8 mL), and 400 μL of a solution of *p*-bromophenyl isocyanate (15.6 mg) in toluene (1.0 mL) was added. The mixture was refluxed at 104 °C for 73 h under a positive atmosphere of dry nitrogen gas, after which the solvent was removed under reduced pressure. The resulting residue was chromatographed by HPLC on silica with hexanes–Et_2O (7:3) to afford 17 (4 mg, 30% yield) which was crystallized as fine white needles (mp 133–134 °C) from Et_2O–CH_2Cl_2 (95:5) and submitted for X-ray analysis: ^{1}H NMR ($CDCl_3$) δ 1.35 (1 H, d, J = 6.5 Hz), 1.41 (1 H, d, J = 6.5 Hz), ~2.27 (2 H, obscured m), 2.45 (1 H, dd, J = 9.5, 15 Hz), ~2.92 (1 H, obscured m), 2.95 (3 H, s), 3.02 (1 H, dd, J = 1.5, 15 Hz), 3.27 (1 H, dqd, J = 3, 6.5, 9.5 Hz), 4.06 (1 H, dd, J = 3.5, 11 Hz), 4.36 (1 H, dd, J = 11, 12 Hz), 5.22 (1 H, m), 6.77 (1 H, m), 7.41 (4 H, d, J = 8.5 Hz). Crystallographic information: A colorless crystal of dimensions 0.10 x 0.30 x 0.63 mm was subjected to single-crystal X-ray analysis. The axial photographs and systematic conditions exhibit hexagonal symmetry ($P6_5$), where a = 17.658(2) Å, c = 15.504(2) Å, $\alpha = \beta = 90°$ and $\beta = 120°$, Z = 6, and a calculated density D_x = 1.475 g cm^{-3} for one molecule per asymmetric unit. The data were collected on a Siemens R3m/V diffractometer using Cu Kα (λ = 1.541 78Å) radiation. The 2θ – θ scan mode was used at a variable rate from 2.0 to 29.3°/min in ω. A total of 1990 independent reflections were measured, of which 1841 (93%) were considered observed ($|F_o| > 4\sigma(F)$) and used in subsequent least-squares refinement. Lorentz, polarization, and absorption correction factors were applied to the data. The structure was solved by direct methods with the Siemens SHELXTL PLUS (VMS). The positional and thermal parameters for the nonhydrogen atoms were refined anisotropically. The hydrogen atoms were placed at calculated positions with fixed isotropic thermal parameters and refined. The full-matrix least-squares refinement included a total of 279 variables. The model converged to a final unweighted and weighted R agreement factor of 5.96% and 7.95%, respectively, where $w^{-1} = \sigma^2(F) + 0.0015F^2$. The η test, a crystallographic absolute configuration test, was performed to give η = 1.03(7), indicating the stereochemistry displayed in Figure 4.

Derivatization of Dysideapyrrolidone (9). Dysideapyrrolidone (9, 0.42 mg) was dissolved in acetone (100 μL) and 60 μL of a solution of $NaIO_4$ (10.0 mg) and $KMnO_4$ (0.2 mg) in water (3.00 mL) added. The solution was stirred at room temperature for 7 h. MeOH (1.0 mL) was added and the solution stirred until the permanganate color was discharged (15 min). The solvent was evaporated under a nitrogen stream, and then 0.8 mL of a freshly-made solution of acetyl chloride (1 mL) in 2-propanol (5 mL) was added. The reaction vial was capped, heated for 45 min at 98–100 °C, and then uncapped and the excess reagent evaporated by a stream of nitrogen gas. The vial was then cooled on ice, and 0.8 mL of a freshly-made mixture of dichloromethane (3 mL) and pentafluoropropionic acid (1 mL) added. The vial was capped, heated for 15 min at 98–100 °C, and then cooled to room temperature and the excess reagent removed by a nitrogen stream. Pentafluoropropionamide isopropyl esters of D-alanine and L-alanine were made by the above method and used as standards for gas chromatograph–mass spectrometer analysis using a Hewlett-Packard 5890 Series II gas chromatograph fitted with an Alltech Chirasil-Val column (25-m length, 0.25-mm i.d., 0.16-μm film thickness) with helium (44 cm/s linear velocity at 10 psi). GC conditions were as follows: 4 min at 50 °C, heating to 180 °C at 4 °C/min, and then heating to 230 °C at 5 °C/min. Detection was by a Hewlett-Packard 5988A mass spectrometer with the mass spectral scan range set to 50–550 m/z. Retention times (peak maxima) were 10.81 min for D-alanine, 11.72 min for L-alanine, and 11.45 min for the dysideapyrrolidone derivative. Coinjection of the dysideapyrrolidone derivative with the pentafluoropropionamide isopropyl ester of D-alanine gave two peaks eluting at 10.86 and 11.52 min (peak maxima); coinjection of the dysideapyrrolidone derivative with the pentafluoropropionamide

isopropyl ester of L-alanine gave a single peak with a retention time (peak maximum) of 11.71 min.

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Supplementary Material Available: ^{1}H NMR spectra for compounds **4–10** and **15–17** and ^{13}C NMR spectra for compounds **4–10, 15,** and **16** (19 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.