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# New polychlorinated pyrrolidinones from the Red Sea marine sponge *Lamellodysidea herbacea*

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Chemical investigation of the dichloromethane extract of the Red Sea marine sponge *Lamellodysidea herbacea* led to the isolation of seven new polychlorinated derivatives **3–6** and **8–10** in addition to the known dysidamide **1**, dysidamide B **2** and dysidamide C **7**. Their structures were established by extensive NMR spectroscopic data. The absolute configuration of compound **9** was determined by X-ray crystallographic diffraction analysis. Dysidamide **1** exhibited neurotoxic effects towards both mesencephalic and cortical murine neurones at 0.8 µg/ml.

## 1. Introduction

Marine sponges of the genus *Dysidea* have yielded secondary metabolites of a great structural diversity such as sesqui- and sesterterpenes,<sup>1–3</sup> polybrominated derivatives of diphenyl ether<sup>1</sup> and dibenzo-*p*-dioxin types<sup>4,5</sup> and polychlorinated derivatives<sup>6</sup> including amino acids<sup>7</sup> as well as peptides.<sup>8</sup> Some of them exhibited interesting pharmacological activities such as inhibition of iodide transport in thyroid cells,<sup>9</sup> neurotoxicity<sup>10</sup> and inhibition of protein phosphatase.<sup>11</sup>

In our ongoing chemical studies on marine sponges, we have examined a sample of *Lamellodysidea herbacea* (formerly *Dysidea herbacea*) collected in the Red Sea. Chemical investigations of the dichloromethane extract led to the isolation of seven new polychlorinated derivatives **3–6** and **8–10** in addition to the known dysidamide **1**, dysidamide B **2** and dysidamide C **7**, previously reported.<sup>12,13</sup> This paper describes the isolation and structure elucidation of these new polychlorinated derivatives.

## 2. Results and discussion

The marine sponge *Lamellodysidea herbacea* was collected by hand using SCUBA (–20 m) in the Red Sea and was air-dried. Silica gel flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH) of the dichloromethane extract followed by Sephadex LH-20 and/or reversed-phase HPLC yielded compounds **1–10** in pure form. The known compounds dysidamide **1**, dysidamide B **2** and dysidamide C **7**, originally isolated from the marine sponge *Dysidea herbacea*<sup>12,13</sup> were rapidly identified by comparison with literature values. An X-ray diffraction analysis<sup>14</sup> of dysidamide **1** showed the presence of two conformers in the unit cell, in which all asymmetric carbon centres exhibited (*S*) configuration, as previously reported.<sup>15</sup>

Compounds **3–6** and **8–10** were identified through extensive 1D and 2D NMR spectroscopic data.

Compound **3** was isolated as a colourless glass,  $[\alpha]_D = -4.0$  (*c* 0.15, CH<sub>2</sub>Cl<sub>2</sub>). Electrospray mass spectrum of compound **3** exhibited a cluster ion at *m/z* 440, 442, 444, 446, 448 characteristic of the presence of five chlorine atoms in the molecule. The molecular formula C<sub>15</sub>H<sub>22</sub>NO<sub>3</sub>Cl<sub>5</sub> was deduced from HREIMS and <sup>13</sup>C NMR spectrum. In the IR spectrum, absorption bands at 3606, 1741 and 1697 cm<sup>-1</sup> indicated the presence of an hydroxyl (or NH) and two carbonyl groups, respectively. NMR spectral data were similar to those of dysidamide **1**, pointing out the presence

**Keywords:** *Lamellodysidea herbacea*; Sponge; Dysidamide; Polychlorinated pyrrolidinones.

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**Table 1.**  $^1\text{H}$  NMR data of compounds **1–4** and **10** recorded in  $\text{CDCl}_3$  ( $\delta_{\text{H}}$ , multiplicity,  $J$  in Hz)

No	1	2	3	4	10
4	4.13 (d, 7.5)	4.10 (br d, 7.3)	4.14 (d, 7.7)	4.12 (d, 7.3)	
5	4.38 (dt, 7.5, 5.3)	4.28 (m)	4.36 (dt, 7.6, 5.2)	4.31 (ddd, 7.3, 4.5, 7.0)	4.52 (t, 6.5)
6	2.17 (m)	2.04 (ddd, 14.1, 6.0, 7.0)	2.16 (m)	2.03 (brdd, 14.0, 6.9)	2.20 (dd, 6.0, 6.8)
		1.85 (m)		1.88 (ddd, 14.3, 5.9, 4.5)	
7	2.97 (m)	2.48 (m)	2.98 (m)	2.48 (m)	2.98 (m)
8		5.93 (d, 2.9)		5.94 (d, 2.8)	
9	1.21 (s)	1.20 (s)	1.19 (s)	1.22 (s)	1.31 (s)
10	1.22 (s)	1.20 (s)	1.21 (s)	1.21 (s)	1.40 (s)
11	1.37 (d, 6.4)	1.17 (d, 6.7)	1.36 (d, 6.5)	1.19 (d, 6.0)	1.47 (d, 6.4)
2'	3.55 (dd, 18.1, 1.6)	3.20 (dd, 18.1, 5.9)	3.19 (dd, 18.1, 5.8)	3.58 (dd, 18.1, 2.6)	3.68 (dd, 18.0, 1.8)
	3.14 (dd, 18.1, 9.4)	2.98 (dd, 18.1, 6.9)	3.03 (dd, 18.1, 7.1)	3.11 (dd, 18.1, 9.3)	3.25 (dd, 18.0, 9.2)
3'	3.29 (m)	2.77 (m)	2.78 (m)	3.30 (ddq, 2.7, 9.2, 6.5)	3.31 (m)
4'		5.95 (d, 3.3)	5.95 (d, 3.3)		
5'	1.34 (d, 6.4)	1.19 (d, 6.6)	1.18 (d, 6.7)	1.35 (d, 6.5)	1.39 (d, 6.6)

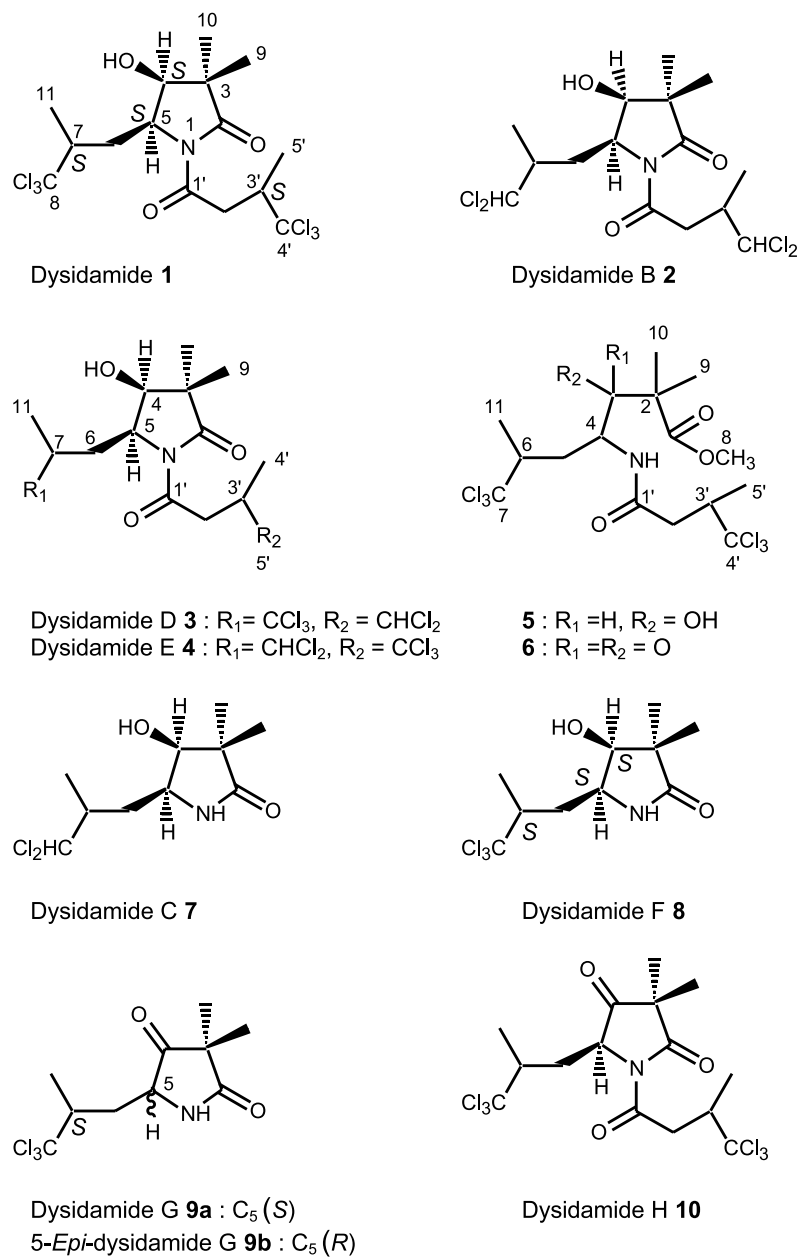
of a pyrrolidinone ring system (see Table 1). In comparison with dysidamide **1**, we observed in the  $^1\text{H}$  NMR spectrum the presence of one additional signal at  $\delta$  5.95 (1H, d,  $J$  = 3.3 Hz). The  $^{13}\text{C}$  NMR spectrum showed the loss of the quaternary carbon signal at  $\delta$  104.9 and the presence of one additional methine carbon signal at  $\delta$  77.8. Furthermore, we noticed that the methine proton at  $\delta$  50.7 was shifted at  $\delta$  40.1. This information suggested the replacement of one  $\text{CCl}_3$  group by a  $\text{CHCl}_2$  functionality in **3**. This hypothesis was strengthened by COSY spectrum. Starting from the additional proton at  $\delta$  5.95 ( $\text{H}4'$ ), correlations could be observed to the methine proton at  $\delta$  2.78 ( $\text{H}3'$ ), which in turn coupled to both methylene protons at  $\delta$  3.19–3.03 ( $\text{H}2'$ ) and methyl protons at  $\delta$  1.18 ( $\text{H}5'$ ), confirming the presence of a  $\text{Me}-\text{CH}(\text{CHCl}_2)-\text{CH}_2-$  side chain in **3**. A second spin system was observed in the COSY spectrum with correlations between the methyl doublet at  $\delta$  1.36 ( $\text{H}11$ ) to the methine proton at  $\delta$  2.98 ( $\text{H}7$ ), which in turn coupled to the methylene protons at  $\delta$  2.16 ( $\text{H}6$ ). These protons further coupled to the methine proton at  $\delta$  4.36, which coupled to the methine proton at  $\delta$  4.14 ( $\text{H}4$ ), as in dysidamide **1**. Key HMBC correlations between both methylene protons at  $\delta$  3.19–3.03 ( $\text{H}2'$ ) and methine proton at  $\delta$  4.36 ( $\text{H}5$ ) with the carbonyl  $\text{C}1'$  at  $\delta$  172.9, unambiguously located each chain on the pyrrolidinone ring as depicted in Figure 2. NOESY correlations between the methyl protons at  $\delta$  1.21 in position 10 and both methine protons at  $\delta$  4.36 ( $\text{H}5$ ) and 4.14 ( $\text{H}4$ ) and between the methyl protons at  $\delta$  1.19 in position 9 with the methylene protons at  $\delta$  2.16 indicated that the relative stereochemistry of the new compound **3** was identical to that of dysidamide **1**. We propose the name dysidamide D for this compound **3**.

Compound **4** was isolated as a colourless glass,  $[\alpha]_{\text{D}} = -3.5$  ( $c$  0.3,  $\text{CH}_2\text{Cl}_2$ ). The molecular formula,  $\text{C}_{15}\text{H}_{22}\text{NO}_3\text{Cl}_5$ , deduced from HREIMS indicated that compound **4** was isomer of compound **3**. The  $^1\text{H}$  NMR spectrum rather matched that of dysidamide **B** 2 by the presence of the non equivalent methylene protons at  $\delta$  2.03–1.88. However, we noticed in the  $^1\text{H}$  NMR spectrum the loss of one doublet signal at  $\delta$  5.95 (see Table 1). The COSY spectrum delineated the presence of two spin systems, whose position was secured by HMBC correlations, as presented in Figure 3. NOESY correlations between the methyl protons  $\text{Me}-10$  at  $\delta$  1.21 and the methine protons at  $\delta$  4.31 ( $\text{H}5$ ) and 4.12 ( $\text{H}4$ ) and between the methyl protons at  $\delta$  1.22 and the

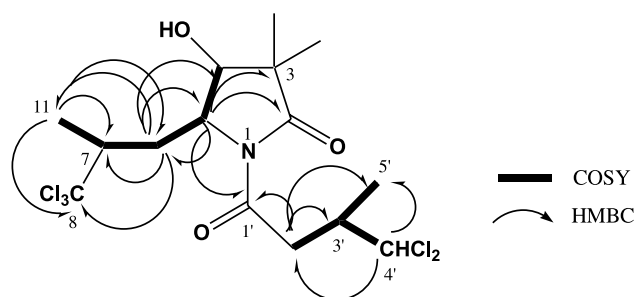
methylene protons at  $\delta$  2.03–1.88 were observed, assigning the stereochemistry of **4** identical to that of dysidamide **1**. Thus, combined 2D NMR data allowed to propose the structure depicted in Figure 1 for compound **4** (see also Tables 1 and 5), we named dysidamide E.

Compound **5** was obtained as white needles, mp 173–174 °C,  $[\alpha]_{\text{D}} = -41.8$  ( $c$  1.1,  $\text{CH}_2\text{Cl}_2$ ). The molecular formula  $\text{C}_{16}\text{H}_{25}\text{NO}_4\text{Cl}_6$  was obtained by combined HREIMS and  $^{13}\text{C}$  NMR spectral data. The spectral data of **5** were similar to those obtained for dysidamide **1** (see Table 2). The significant difference in  $^1\text{H}$  NMR spectrum was the presence of two additional signals at  $\delta_{\text{H}}$  5.84 and  $\delta$  3.70. This last signal gave a carbon resonance in  $^{13}\text{C}$  NMR spectrum at  $\delta$  52.4, indicating the presence of a methoxyl group in the molecule. HMBC correlations between these methoxyl protons at  $\delta$  3.70 to the carbonyl at  $\delta$  178.9 suggested the presence of a methyl ester. HMBC correlations between the two singlet methyl protons at  $\delta$  1.19 and 1.28 to the carbonyl at  $\delta$  178.9, placed the methyl ester function in position 2 on the molecule. We also observed the presence of two resonances at  $\delta$  106.1 and 105.0 due to two  $\text{CCl}_3$  groups. Also in this case, the COSY spectrum confirmed the presence of the two chains identical to those of dysidamide **1**. In addition, the deshielded proton at  $\delta$  5.84, which did not match any carbon resonance in the HSQC spectrum, showed an HMBC correlation to the carbonyl at  $\delta$  169.7, showing the presence of an amide function. At last, key HMBC correlations between the methine proton at  $\delta$  2.48–1.45 ( $\text{H}5$ ) to the carbonyl at  $\delta$  169.7, suggested the arrangement reported in Figure 1 for compound **5**.

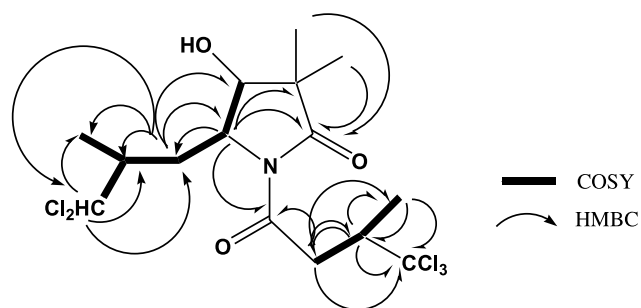
Compound **6** was obtained as white needles, mp 95–96 °C;  $[\alpha]_{\text{D}} = -29.6$  ( $c$  0.44,  $\text{CH}_2\text{Cl}_2$ ), that analysed for  $\text{C}_{16}\text{H}_{23}\text{NO}_4\text{Cl}_6$  by HREIMS. The IR spectrum showed absorptions due to the presence of three carbonyl groups ( $\lambda$  1749, 1723 and  $1683\text{ cm}^{-1}$ ). The spectral data of **6** were similar to those of compound **5** (see Table 2). The only noticeable difference in the  $^1\text{H}$  NMR spectrum was the loss of the methine proton at  $\delta$  3.59. We observed in the  $^{13}\text{C}$  NMR spectrum an additional signal at  $\delta$  206.9. These spectral differences were readily accommodated by the replacement of the secondary alcohol group in **5** by a ketone function in position 3 of the molecule. This proposal was



**Figure 1.** Structures of compounds **1–10** isolated from the marine sponge *Lamellodysidea herbacea*.



**Figure 2.** COSY and selected HMBC correlations used to determine the structure of **3**.



**Figure 3.** COSY and selected HMBC correlations used to determine the structure of **4**.

**Table 2.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of compounds **5–6** recorded in  $\text{CDCl}_3$ 

N <sup>o</sup>	<b>5</b>			<b>6</b>		
	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (m, <i>J</i> in Hz)	HMBC (H→C)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (m, <i>J</i> in Hz)	HMBC (H→C)
1	178.9			173.5		
2	45.8			40.5		
3	78.9	3.59 (d, 1.1)	1, 2, 5, 9, 10	206.9		
4	46.7	4.33 (ddd, 12.6, 11.7, 1.1)	5, 6, 11	51.6	5.09 (ddd, 11.8, 9.5, 2.3)	5, 6, 11
5	39.5	2.48 (ddd, 12.8, 12.8, 0.8) 1.45 (ddd, 13.9, 2.5, 10.1)	4, 6, 7, 11, 1'	36.9	2.12 (brdd, 14.2, 0.7) 1.68 (ddd, 14.2, 10.2, 2.3)	4, 6, 7, 11, 1'
6	52.0	2.39 (ddq, 10.1, 1.7, 6.3)	4, 5, 11, 1'	51.7	2.47 (m)	4, 5, 11, 1'
7	106.1			105.3		
8	52.4	3.70 (s)	1	52.9	3.73 (s)	1
9	20.1	1.19 (s)	1, 2, 3, 10	21.9	1.42 (s)	1, 2, 3, 10
10	24.0	1.28 (s)	1, 2, 3, 9	22.2	1.43 (s)	1, 2, 3, 9
11	16.8	1.41 (d, 6.3)	5, 6, 7	16.3	1.42 (d, 4.2)	5, 6, 7
1'	169.7			170.0		
2'	40.4	2.92 (dd, 14.8, 2.5) 2.13 (dd, 14.8, 10.2)	1', 3', 5'	40.3	2.97 (dd, 14.9, 3.1) 2.20 (dd, 14.9, 9.6)	1', 3', 5'
3'	51.6	3.17 (ddq, 10.2, 2.5, 6.4)	1', 2', 4', 5'	51.6	3.17 (ddq, 3.1, 9.6, 6.5)	1', 2', 4', 5'
4'	105.0			104.8		
5'	16.8	1.33 (d, 6.4)	2', 3', 4'	16.9	1.34 (d, 6.5)	2', 3', 4'
NH1'		5.84 (d, 9.8)	3, 4, 1'		6.02 (d, 9.5)	3, 4, 1'

confirmed by interpretation of combined 2D NMR spectra (see Table 2).

Compound **8** was isolated as white needles, mp 171–172 °C,  $[\alpha]_{\text{D}} = -36.0$  (*c* 0.84,  $\text{CH}_2\text{Cl}_2$ ). Its ESI mass spectrum exhibited a cluster of peaks at *m/z* 288, 290, 292, 294 with a pattern characteristic of a trichlorinated compound. The molecular formula  $\text{C}_{10}\text{H}_{16}\text{NO}_2\text{Cl}_3$  was determined on the basis of HREIMS and NMR data. Comparison of the NMR data of **8** with those of dysidamide **1** indicated that **8** also contained a pyrrolidinone ring system. The  $^1\text{H}$  NMR spectrum also showed the presence of a methylene group at  $\delta$  2.33–1.73, one methine at  $\delta$  2.79 and one methyl doublet at  $\delta$  1.40, indicating that **8** possessed the same  $\text{MeCH}(\text{CCl}_3)\text{CH}_2-$  side chain as dysidamide **1** on C5. NOESY correlations between the methyl protons in position 10 at  $\delta$  1.15 and protons H-4 and H-5 and between the methyl protons in position 9 at  $\delta$  1.12 with the methylene protons at  $\delta$  2.33–1.73 were also observed.

The structure of **8** was secured by hydrolysis of dysidamide **1**. Under mild basic conditions with 0.1%  $\text{K}_2\text{CO}_3$  in MeOH, dysidamide **1** gave rise to a compound, whose NMR data were in complete accordance with those of the natural product **8**, suggesting (*S*) configuration for all asymmetric centres. We propose the name dysidamide F for compound **8**.

This is the first report of this compound as a natural product.

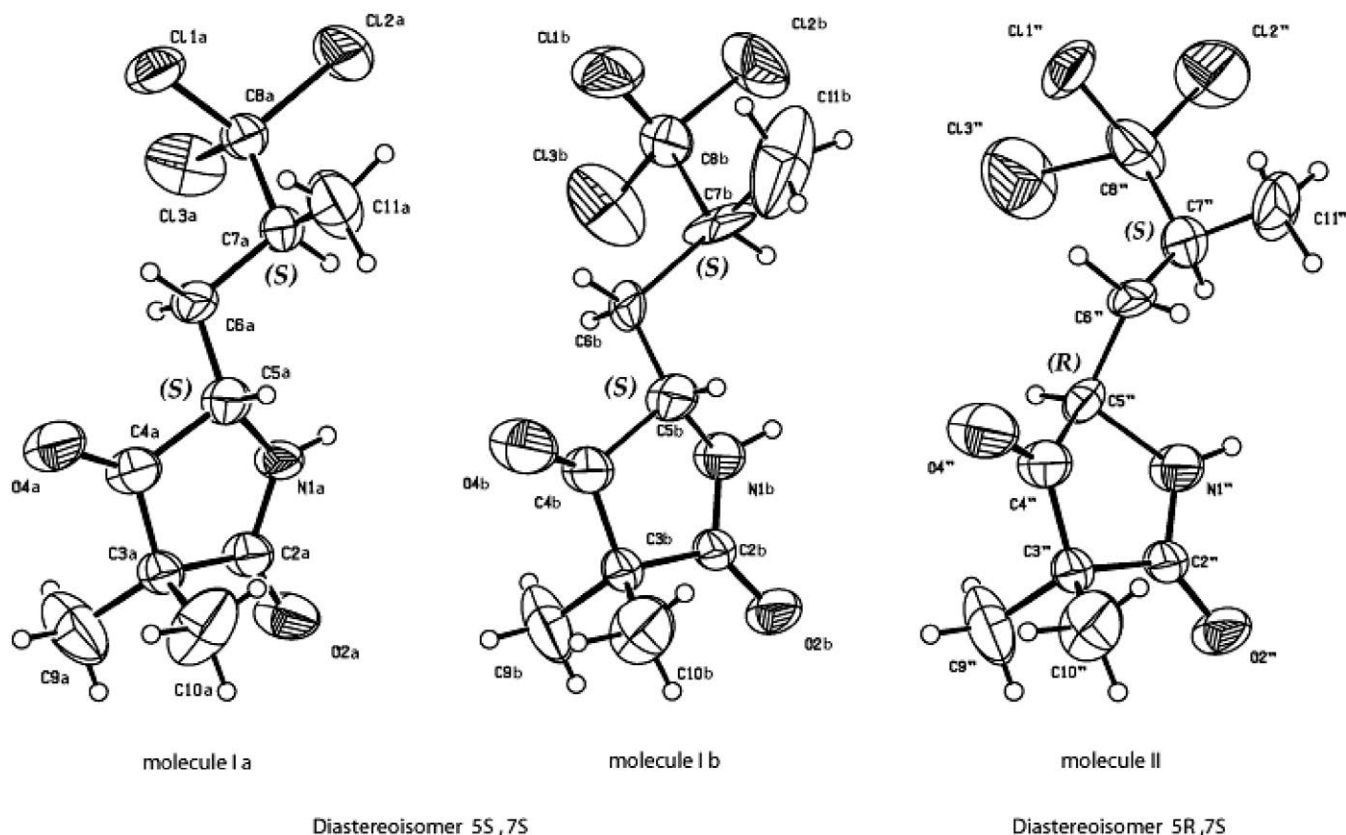
This compound was previously obtained after hydrolysis of dysidamide **1**.<sup>12</sup>

Compound **9** was isolated as colourless needles, mp 109 °C,  $[\alpha]_{\text{D}} = -38.5$  (*c* 0.75,  $\text{CH}_2\text{Cl}_2$ ). Its ESI mass spectrum exhibited a characteristic cluster of three chlorine atoms for the  $[\text{M}+\text{H}]^+$  peaks at *m/z* 286, 288, 290, 292. From the lower peak at *m/z* 286.0161 ( $\Delta -0.8$  mmu) by HREIMS, a molecular formula of  $\text{C}_{10}\text{H}_{14}\text{NO}_2\text{Cl}_3$  was deduced. Its IR spectrum showed the presence of hydroxyl or NH groups ( $\lambda$  3419  $\text{cm}^{-1}$ ), two carbonyl functions ( $\lambda$  1775, 1711  $\text{cm}^{-1}$ ), one gem dimethyl ( $\lambda$  1416, 1389  $\text{cm}^{-1}$ ). The  $^1\text{H}$  NMR spectrum recorded in  $\text{CD}_3\text{OD}$  was similar to that of compound **8** but showed that all  $^1\text{H}$  signals were split into two in a  $3/4:1/4$  proportion, suggesting the presence of two diastereoisomers (see Table 3). Compound **9** was crystallized by slow evaporation from a mixture of MeOH/ $\text{H}_2\text{O}$  (7/3) for X-ray studies. The crystals belong to the triclinic system, space group *P1*, chiral. Anomalous dispersion from the chlorine atoms of the molecule permitted us to deduce the relative stereochemistry of the asymmetric carbons, namely C5 and C7.

The crystal is made of two pairs of molecules with occupancy factor = 0.5 in the unit cell. In each pair, one of the two molecules, (Ia), exhibits the *S*-configuration at both quaternary carbons C5(*S*) and C7(*S*) (see Fig. 4). In the crystal packing, molecule (Ia) is connected to either its conformer (Ib) or to its diastereoisomer (II) by two

**Table 3.**  $^1\text{H}$  NMR data of compounds **8**, **9a** and **9b** recorded in  $\text{CD}_3\text{OD}$  ( $\delta_{\text{H}}$ , multiplicity, *J* in Hz)

N <sup>o</sup>	<b>8</b>	<b>9a</b>	<b>9b</b>
4	3.97 (d, 5.3)		
5	3.86 (ddd, 5.3, 4.5, 9.5)	4.24 (dd, 3.4, 10.9)	4.22 (dd, 5.3, 9.5)
6	2.33 (ddd, 14.1, 10.2, 1.5) 1.73 (ddd, 14.1, 10.2, 4.0)	1.91 (ddd, 13.9, 10.5, 3.4) 2.13 (ddd, 13.9, 10.9, 2.3)	1.80 (ddd, 13.9, 9.5, 5.3) 2.36 (m)
7	2.79 (m)	2.87 (ddq, 10.5, 2.3, 6.5)	3.03 (brdq, 9.5, 6.5)
8	—	—	—
9	1.12 (s)	1.20 (s)	1.21 (s)
10	1.15 (s)	1.23 (s)	1.22 (s)
11	1.40 (d, 6.5)	1.41 (d, 6.5)	1.42 (d, 6.5)



**Figure 4.** ORTEP-plots showing the molecule Ia: C5(*S*), C7(*S*), Ib: C5(*S*), C7(*S*) and II: C5(*R*), C7(*S*) of compound **9**. Atomic displacement ellipsoids drawn at the 30% probability level, hydrogen atoms drawn as spheres of arbitrary radius.

hydrogen bonds, involving N1a and O2'' or O2b ( $d = 2.02$  Å, angle N–H–O =  $151.8^\circ$ ) and O2a and N1'' or N1b ( $d = 2.11$  Å, angle N–H–O =  $171.0^\circ$ ), respectively (see Fig. 5).

In the unit cell, molecule (Ia) adopts a N4-envelope conformation as deduced from the torsion angles (see Table 4). The second molecule (Ib) adopts a half-chair conformation and also exhibits *S*-configuration at both quaternary carbons. The third molecule (II) appears to be C5(*R*), C7(*S*) and adopts a half-chair conformation (see Fig. 4).

Hence, the crystal is made of two diastereoisomers in the following proportion  $\frac{3}{4}:\frac{1}{4}$  which is somewhat consistent with the  $^1\text{H}$  NMR results aforementioned. We propose the name dysidamide G for compound **9a** and 5-*epi*-dysidamide G for **9b** (see Fig. 1).

Compound **10** was isolated as a colourless glass;  $[\alpha]_D = -62.8$  ( $c$  0.6,  $\text{CH}_2\text{Cl}_2$ ). Its ESIMS exhibited a pseudo-molecular ion cluster peak at  $m/z$  474, 476, 478, 480, 482 and 484 indicating the presence of six chlorine atoms in the molecule. The molecular formula was deduced from HREIMS as being  $\text{C}_{15}\text{H}_{19}\text{NO}_3\text{Cl}_6$ , implying the presence of four unsaturations in the molecule. The IR spectrum of **10** showed the presence of three carbonyl groups ( $1776$ ,  $1741$ ,  $1708$   $\text{cm}^{-1}$ ) and one gem dimethyl group ( $1385$ ,  $1373$   $\text{cm}^{-1}$ ). The  $^1\text{H}$  NMR spectrum was similar to that of dysidamide **1** but revealed the disappearance of the methine signal at  $\delta$  4.13. The  $^{13}\text{C}$  NMR spectrum showed an

additional carbonyl signal at  $\delta$  209.4. Extensive 1D and 2D spectra were consistent with structure presented in Figure 1. In particular, HMBC correlations between the gem methyl protons at  $\delta$  1.40, the methine proton at  $\delta$  4.52 and the methylene protons at  $\delta$  2.20 with the carbonyl at  $\delta$  209.4 supported this structure. NOESY correlation between the methyl at  $\delta$  1.31 in position 9 and the methylene protons at  $\delta$  2.20 was observed. We propose the name dysidamide H for compound **10**.

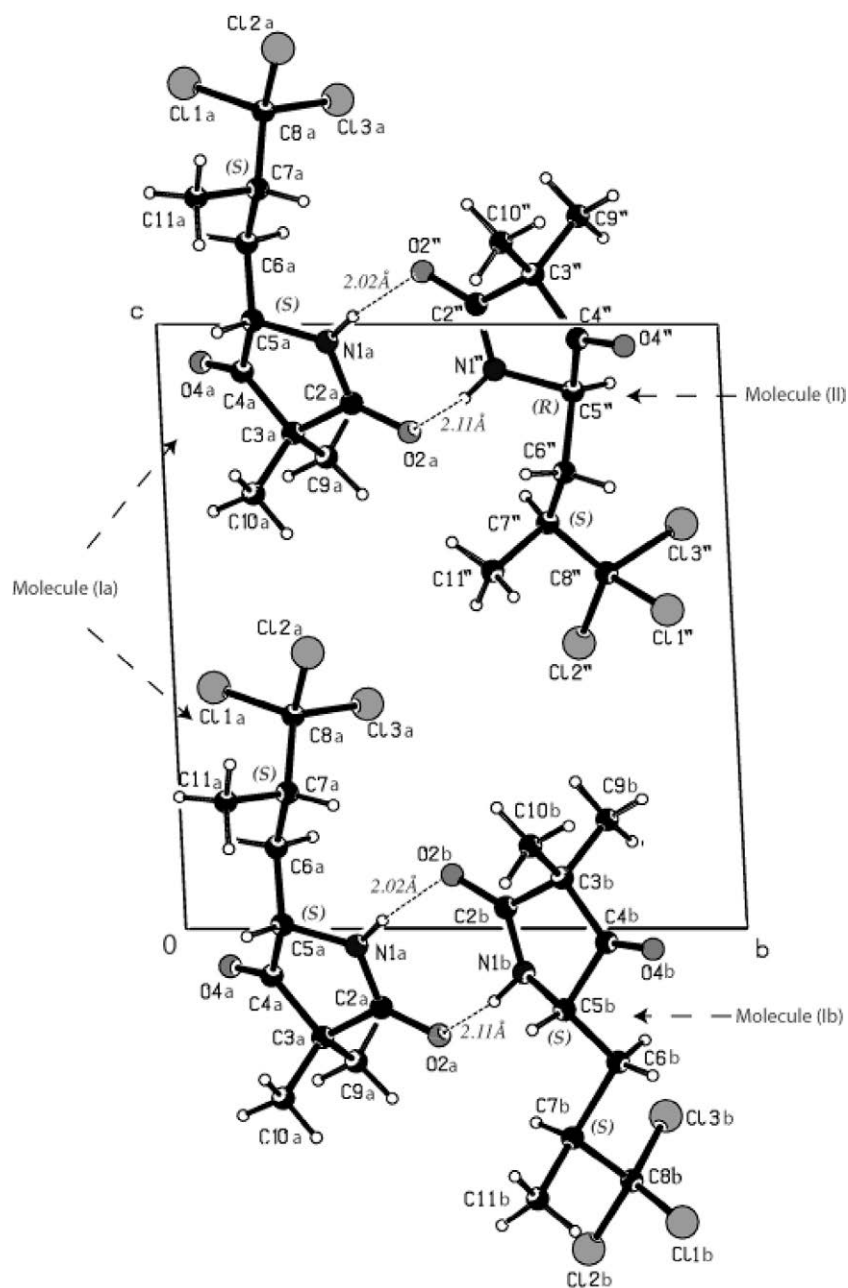
Previous intensive studies of the same species, referred to as *Dysidea herbacea* demonstrated that large quantities of the filamentous cyanobacterium *Oscillatoria spongeliae* are responsible for the occurrence of the isolated chlorinated compounds.<sup>16,17</sup> Our work also illustrates and re-enforces the chemical diversity within the genus *Lamellodysidea*.

Dysidamide **1** was tested towards both mesencephalic and cortical murine neurones. In these two neuronal cultures, dysidamide **1** provoked the entire and rapid death of neurones, even at doses of  $0.8$   $\mu\text{g}/\text{ml}$ . No significant effect was observed at  $100$   $\text{ng}/\text{ml}$ . Further investigations are in progress to evaluate the neurotoxic effect of the other compounds in the series.

### 3. Experimental

#### 3.1. General experimental procedures

Silica gel column chromatographies were carried out using



**Figure 5.** Crystal packing of compound **9**: interpretation of the unit cell content in the *bc* plane. Molecule (Ia) (left column) can be H-bonded either to its conformer (Ib) or its diastereoisomer (II). Note that in the crystallographic refinement, N1b is equivalent to N1'', C2b to C2'', C3b to C3'', C4b to C4'', C9b to C9'', C10 to C10'', O2b to O2'', O4b to O4'', and so are the corresponding attached hydrogens.

**Table 4.** List of selected torsion angles (°) for compound **9**

Diastereoisomer				C5(S) C7(S)		C5(R) C7(S)
				Molecule Ia	Molecule Ib	Molecule II
‘Five-member ring’						
N1	C2	C3	C4	12.9	−10.1	−10.1
C2	C3	C4	C5	−21.5	−15.8	27.7
C3	C4	C5	N1	21.8	30.9	−32.2
C4	C5	N1	C2	−14.2	−38.0	27.2
C5	N1	C2	C3	1.5	31.6	−12.9
‘Tail’						
C4	C5	C6	C7	−179.7	−172.7	169.1
N1	C5	C6	C7	−68.6	−68.2	70.2
C5	C6	C7	C8	170.2	171.8	95.9
C5	C6	C7	C11	−65.4	−73.0	−127.2



**Table 5.**  $^{13}\text{C}$  NMR data of compounds **1–4** and **10** recorded in  $\text{CDCl}_3$  and compounds **7–9** recorded in  $\text{CD}_3\text{OD}$ 

N°	1	2	3	4	7	8	9a	9b	10
2	178.9	178.8	178.9	178.9	183.9	183.9	180.2	180.2	176.1
3	46.4	46.5	46.3	46.5	46.7	46.7	47.4	47.4	49.1
4	72.9	73.0	73.1	72.9	77.9	77.9	215.1	215.3	209.4
5	57.0	56.7	56.9	56.8	54.8	55.3	60.2	59.9	61.4
6	33.1	31.9	33.2	31.7	33.6	34.6	37.7	37.7	35.1
7	53.5	46.5	53.5	42.8	42.1	53.3	52.7	52.2	50.4
8	105.8	78.4	105.9	78.3	80.5	107.6	106.6	106.6	105.1
9	19.3	19.3	19.3	19.2	18.6	18.8	21.2	21.4	20.8
10	23.9	24.1	24.1	24.0	23.5	23.7	21.3	21.4	22.9
11	16.9	15.1	16.9	15.5	14.9	16.7	16.1	17.0	15.8
1'	172.3	172.7	172.9	172.2					171.0
2'	41.9	40.9	40.8	41.9					42.4
3'	50.7	40.0	40.1	50.7					50.4
4'	104.9	77.8	77.8	105.0					104.7
5'	17.2	15.4	15.2	17.3					17.4

Kieselgel 60 (230–400 mesh, E. Merck). Fractions were monitored by TLC using aluminium-backed sheets (Si gel 60 F254, 0.25 mm thick). Analytical reversed-phase HPLC (Kromasil RP18 column K2185,  $4.6 \times 250$  mm, MeOH/H<sub>2</sub>O) were performed with a L-6200A pump (Merck-Hitachi) equipped with a UV-vis detector ( $\lambda = 210$  nm) L-4250C (Merck-Hitachi) and a chromatointegrator D-2500 (Merck-Hitachi).

IR spectra were recorded on a Nicolet IMPACT 400D FT-IR spectrophotometer.

Mass spectra were recorded on an API Q-STAR PULSAR I of Applied Biosystem and on a JEOL MS 700BE for low and high-resolution, respectively.

$^{13}\text{C}$  NMR spectra were obtained on a Bruker AC300 at 75.47 MHz.  $^1\text{H}$  NMR spectra 1D and 2D (COSY, HSQC, HMBC, NOESY) were obtained on a Bruker AVANCE 400.

### 3.2. Animal material

Specimens of *Lamellodysidea herbacea* (Keller, 1889) (Order Dictyoceratida, Family Dysideidae) were collected by J. Vacelet in the Red Sea at a depth of 20 m in January 1985 during the Ardoukoba expedition. A voucher specimen is available from the Muséum d'Histoire Naturelle in Marseille as collection number MHNM 13660. In the new classification of Hooper and Van Soest, the species *D. herbacea* and *D. chlorea* have been transferred from *Dysidea* to the new genus *Lamellodysidea*.<sup>18</sup> This new genus has been split from *Dysidea* because of the consistent presence in these sponges of an encrusting basal plate and the lack of orientation of the skeleton with respect to the surface. Many sponge specimens from the Indo-Pacific area have been previously identified to *Dysidea herbacea*, which has been originally described from the Red Sea. However, it is likely that this species has been confused with other species such as *D. chlorea*,<sup>18</sup> which could explain the reported chemical diversity.

### 3.3. Extraction and isolation

The air-dried sponge (288 g) was extracted with 3L methanol at room temperature for 3 days. The concentrated

extract (60 g) was partitioned into hexane,  $\text{CH}_2\text{Cl}_2$ , MeOH soluble fractions. The  $\text{CH}_2\text{Cl}_2$  soluble extract was then fractionated by rapid filtration on a silicagel (Merck silica gel 70–230 mesh) column using dichloromethane with increasing amounts of methanol as eluent. Fraction eluted with 2% MeOH (5.4 g) was subjected to successive reversed-phase HPLC using MeOH/H<sub>2</sub>O 7/3 as eluent (flow rate: 1 ml/min, det. wavelength of 210 nm) to yield in order of elution dysidamide B **2** (4.3 mg, at  $t = 12.66$  mn), dysidamide D **3** (1.5 mg at  $t = 15.44$  mn), dysidamide E **4** (3.0 mg, at  $t = 15.90$  mn), dysidamine **1** (450 mg at  $t = 17.99$  mn), compound **5** (11.4 mg at  $t = 19.55$  mn), compound **6** (4.4 mg at  $t = 19.56$  mn), dysidamide H **10** (6.4 mg at  $t = 20.86$  mn). Fraction eluted with 5% MeOH (5.5 g) was first separated on a Sephadex LH20 in 5 subfractions. Subfraction 3 (3.6 g) was successively subjected to reversed-phase column and RP-18 HPLC using MeOH/H<sub>2</sub>O 7/3 as eluent to yield dysidamide C **7** (1 mg), dysidamide F **8** (10 mg) and dysidamide G **9a** with 5-*epi*-dysidamide G **9b** (26 mg).

**3.3.1. Compound 1. Dysidamide, 4-hydroxy-3,3-dimethyl-1-(4,4,4-trichloro-3-methyl-1-oxobutyl)-5-(3,3,3-trichloro-2-methylpropyl)-2-pyrrolidinone.** 0.450 g, 0.15% dry weight white crystals; mp 133 °C;  $[\alpha]_{\text{D}} = -16.1$  ( $c$  2.76,  $\text{CH}_2\text{Cl}_2$ ), IR  $\nu \text{ cm}^{-1}$ : 3447, 1722, 1700, 1385, 1385, 1387,  $^1\text{H}$  and  $^{13}\text{C}$  NMR data recorded in  $\text{CDCl}_3$ : see Tables 1 and 5; HREIMS  $[\text{M} + \text{H}]^+$  found at  $m/z$  473.9758 ( $\Delta + 0.5$  mmu) for  $\text{C}_{15}\text{H}_{22}\text{NO}_3$   $^{35}\text{Cl}_6$ .

**3.3.2. Compound 2. Dysidamide B, 1-(4,4-dichloro-3-methyl-1-oxobutyl)-5-(3,3-dichloro-2-methylpropyl)-4-hydroxy-3,3-dimethyl-2-pyrrolidinone.** 0.0043 g, 0.0015% dry weight colourless glass;  $[\alpha]_{\text{D}} = +10.5$  ( $c$  0.43,  $\text{CH}_2\text{Cl}_2$ ), IR  $\nu \text{ cm}^{-1}$ : 36.7, 1743, 1701, 1388, 1374;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data recorded in  $\text{CDCl}_3$ : see Tables 1 and 5; HREIMS  $[\text{M} + \text{H}]^+$  found at  $m/z$  406.0500 ( $\Delta - 1.0$  mmu) for  $\text{C}_{15}\text{H}_{24}\text{NO}_3$   $^{35}\text{Cl}_4$ .

**3.3.3. Compound 3. Dysidamide D, 1-(4,4-dichloro-3-methyl-1-oxobutyl)-4-hydroxy-3,3-dimethyl-5-(3,3,3-trichloro-2-methylpropyl)-2-pyrrolidinone.** 0.0015 g,  $0.05 \times 10^{-2}$  % dry weight colourless glass;  $[\alpha]_{\text{D}} = -4.0$  ( $c$  0.15,  $\text{CH}_2\text{Cl}_2$ ); IR  $\nu \text{ cm}^{-1}$ : 3606, 1741, 1697, 1386, 1371,  $^1\text{H}$  and  $^{13}\text{C}$  NMR data recorded in  $\text{CDCl}_3$ : see Tables 1



and 5; HREIMS  $[M+H]^+$  found at  $m/z$  440.0118 ( $\Delta - 0.2$  mmu) for  $C_{15}H_{23}NO_3^{35}Cl_5$ .

**3.3.4. Compound 4. Dysidamide E, 5-(3,3-dichloro-2-methylpropyl)-4-hydroxy-3,3-dimethyl-1-(4,4,4-trichloro-3-methyl-1-oxobutyl)-2-pyrrolidinone.** 0.003 g,  $0.10 \times 10^{-2}$  % dry weight colourless glass;  $[\alpha]_D = -3.5$  ( $c$  0.3,  $CH_2Cl_2$ ); IR  $\nu$   $cm^{-1}$ : 3614, 1745, 1703, 1395, 1381;  $^1H$  and  $^{13}C$  NMR data recorded in  $CDCl_3$ ; see Tables 1 and 5; HREIMS  $[M+H]^+$  found at  $m/z$  440.0112 ( $\Delta - 0.7$  mmu) for  $C_{15}H_{23}NO_3^{35}Cl_5$ .

**3.3.5. Compound 5. 7,7,7-Trichloro-3-hydroxy-2,2,6-trimethyl-4-(4,4,4-trichloro-3-methyl-1-oxobutylamino)-heptanoic acid methyl ester.** 0.0114 g,  $0.4 \times 10^{-2}$  % dry weight white needles; mp 173–174 °C,  $[\alpha]_D = -41.8$  ( $c$  1.1,  $CH_2Cl_2$ ), IR  $\nu$   $cm^{-1}$ : 3423, 1698, 1676, 1510, 1393, 1374;  $^1H$  and  $^{13}C$  NMR data recorded in  $CDCl_3$ ; see Table 2; HREIMS  $[M+H]^+$  found at  $m/z$  505.9988 ( $\Delta - 0.5$  mmu), for  $C_{16}H_{26}NO_4^{35}Cl_6$ .

**3.3.6. Compound 6. 7,7,7-Trichloro-2,2,6-trimethyl-3-oxo-4-(4,4,4-trichloro-3-methyl-1-oxobutylamino)-heptanoic acid methyl ester.** 0.004 g,  $0.14 \times 10^{-2}$  % dry weight, white needles; mp 95–96 °C;  $[\alpha]_D = -29.6$  ( $c$  0.44,  $CH_2Cl_2$ ), IR  $\nu$   $cm^{-1}$ : 3414, 1749, 1723, 1683, 1512, 1427; 1389, 1376,  $^1H$  and  $^{13}C$  NMR data recorded in  $CDCl_3$ ; see Table 2; HREIMS  $[M+H]^+$  found at  $m/z$  503.9821 ( $\Delta - 1.5$  mmu), calcd 503.9836 for  $C_{16}H_{24}NO_4^{35}Cl_6$ .

**3.3.7. Compound 7. Dysidamide C, 5-(3,3-dichloro-2-methylpropyl)-4-hydroxy-3,3-dimethyl-2-pyrrolidinone.** 0.001 g,  $0.03 \times 10^{-2}$  % dry weight white needles;  $^{13}C$  NMR data recorded in  $CD_3OD$ ; see Table 5; ESIMS  $[M+H]^+$  found at  $m/z$  254.0578 for  $C_{10}H_{18}NO_2Cl_2$ .

**3.3.8. Compound 8. Dysidamide F, 4-hydroxy-3,3-dimethyl-5-(3,3,3-trichloro-2-methylpropyl)-2-pyrrolidinone.** 0.010 g,  $0.3 \times 10^{-2}$  % dry weight white needles; mp 171–172 °C;  $[\alpha]_D = -36.0$  ( $c$  0.84,  $CH_2Cl_2$ ); IR  $\nu$   $cm^{-1}$ : 3610, 3423, 1716, 1392, 1386;  $^1H$  and  $^{13}C$  NMR data recorded in  $CD_3OD$ ; see Tables 3 and 5; HREIMS  $[M+H]^+$  found at  $m/z$  288.0319 ( $\Delta - 0.6$  mmu) for  $C_{10}H_{17}NO_2^{35}Cl_3$ .

**3.3.9. Compound 9. Dysidamide G with 5-epi-dysidamide G, 3,3-dimethyl-4-oxo-5-(3,3,3-trichloro-2-methylpropyl)-2-pyrrolidinone.** 0.026 g,  $0.9 \times 10^{-2}$  % dry weight colourless needles; mp 109 °C,  $R_f$  ( $CH_2Cl_2$ /acetone 1:1) 0.11;  $[\alpha]_D = -38.5$  ( $c$  0.75,  $CH_2Cl_2$ ), IR  $\nu$   $cm^{-1}$ : 3419, 1775, 1711, 1416, 1389;  $^1H$  and  $^{13}C$  NMR data recorded in  $CD_3OD$ ; see Tables 3 and 5; HREIMS  $[M+H]^+$  found at  $m/z$  286.0161 ( $\Delta - 0.8$  mmu) for  $C_{10}H_{15}NO_2^{35}Cl_3$ .

**3.3.10. Compound 10. Dysidamide H, 3,3-dimethyl-4-oxo-1-(4,4,4-trichloro-3-methyl-1-oxobutyl)-5-(3,3,3-trichloro-2-methylpropyl)-2-pyrrolidinone.** 0.0064 g,  $0.22 \times 10^{-2}$  % dry weight colourless glass;  $[\alpha]_D = -62.8$  ( $c$  0.6,  $CH_2Cl_2$ ), IR  $\nu$   $cm^{-1}$ : 1776, 1741, 1708, 1385, 1373;  $^1H$  and  $^{13}C$  NMR data recorded in  $CDCl_3$ ; see Tables 1 and 5; HREIMS  $[M+H]^+$  found at  $m/z$  471.9579 ( $\Delta + 0.5$  mmu) for  $C_{15}H_{20}NO_3^{35}Cl_6$ .

### 3.4. X-ray crystallographic analysis

A total of 8577 (4051 unique) reflections were collected up to  $2\theta_{max} = 50.0^\circ$  in 234 frames of  $2^\circ$  oscillation range using an Enraf-Nonius Kappa-CCD diffractometer with graphite monochromated Mo-K radiation ( $\lambda = 0.71073$  Å) at room temperature. The structure was solved by Patterson method to locate four over six chlorides, the rest of atoms was derived from successive Fourier difference syntheses. Structural parameters were refined based on  $F^2$  using the programs from the SHELX97 package.<sup>19</sup> All non-hydrogen atoms were refined anisotropically, except for C11b and C11". Hydrogen atoms were assigned idealized locations and given isotropic displacement parameters  $1.15 \times$  that of the bonded carbon atoms (but  $1.2 \times$  for the hydrogens in methyl groups).

Crystallographic data for  $C_{10}H_{14}Cl_3NO_2$  (FW = 286.57), colourless thin needles [the crystal size,  $0.1 \times 0.3 \times 0.35$  mm<sup>3</sup>], triclinic,  $P1$  (#1),  $a = 6.010$  (4) Å,  $b = 10.341$  (3) Å,  $c = 11.103$  (4) Å,  $\alpha = 92.26^\circ$ ,  $\beta = 94.12$  (2)°,  $\gamma = 97.24^\circ$ ,  $V = 681.9$  (6) Å<sup>3</sup>,  $Z = 2$ ,  $D_{calcd} = 1.396$  g/cm<sup>3</sup>,  $\mu$  (Mo-K $\alpha$ ) =  $0.658$  mm<sup>-1</sup>, observed unique reflections = 4047, 358 parameters refined using 11 restraints. For 3210  $F > 4\sigma$  (F):  $R1 = 0.0590$  (based on F),  $wR2 = 0.1614$  (based on  $F^2$ ) and goodness of fit factor = 1.051 (based on  $F^2$ ).  $\Delta\rho_{max} = 0.29 \times 10^3$  Å<sup>-3</sup>,  $\Delta\rho_{min} = -0.21$  eÅ<sup>-3</sup>.

Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre (CIF file) as supplementary publication number CCDC 244215. Copies of the data can be obtained free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1, EZ, UK [fax: +44(0)1223-336033 or e-mail: [http://deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk)].

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