Hectochlorin and Morpholine Derivatives from the Thai Sea Hare, *Bursatella leachii*

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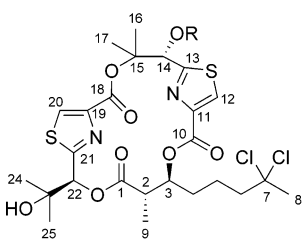
Investigation of the EtOAc extract from the Thai sea hare, *Bursatella leachii,* resulted in the isolation of a potent stimulator of actin assembly, hectochlorin (**1**), and its new derivative, deacetylhectochlorin (**2**). Compound **2** exhibited more potent cytotoxicity than **1** against different human carcinoma cell lines. In addition, a new morpholine-2,5-dione analogue, *syn*-3-isopropyl-6-(4-methoxybenzyl)-4-methylmorpholine-2,5-dione (**3**), was co-isolated.

Sea hares in the genera *Aplysia*, *Dolabella*, and *Stylocheilus* (order Anaspidea, subclass Opisthobranch, phylum Mollusca) have afforded numerous bioactive secondary metabolites over the past 30 years,[1a,b] for example, the antitumor macrolide aplyronine A,[1c] the antitumor peptides dolastains 10 and 15,[1d,e] and the cytotoxic lipopeptides malyngamides O and P,[1f] respectively. However, only two secondary metabolites from the sea hare *Bursatella* have been reported in the literature including an unusual metabolite, bursatellin,[2] and malyngamide S.[3] The ability of sea hares to concentrate dietary derived defensive metabolites from different algal species[4] makes these mollusks attractive for discovering new bioactive compounds. Therefore, we have investigated the Thai sea hare, *Bursatella leachii*, collected from the Gulf of Thailand, in which chemical study has been not yet reported. Cytotoxic investigation from the crude EtOAc extract of the Thai sea hare, *B. leachii*, yielded hectochlorin (**1**) and its new derivative, deacetylhectochlorin (**2**), together with a new structurally unrelated molecule, *syn*-3-isopropyl-6-(4-methoxybenzyl)-4-methylmorpholine-2,5-dione (**3**). Hectochlorin[5] and structurally related compounds including lyngbyabellins A,[6] B,[7] C,[8] and D[9] were initially isolated from the strains of the cyanobacteria *Lyngbya majuscula*. Moreover, the related dolabellin was reported from the sea hare *Dolabella auricularia*.[10] Hectochlorin was recently reported as a potent fungicide and a strong promotor of actin polymerization.[5] Compound **3** is a new morpholine-2,5-dione-possessing depsipeptide analogue of a modified amino acid, *N*-methylvaline, and 2-hydroxy-3-(4-methoxyphenyl)-propionate. To our knowledge, **3** is the second example of a morpholine-2,5-dione-containing natural product since the discovery of ergosecalinine (**4**) isolated from the ergot *Claviceps purpurea* in 1959.[11] Herein, we wish to report the isolation, structure elucidation, stereochemistry assignment, and biological activity of the compounds.

Extraction of internal organs of the mollusks with MeOH afforded a green gum, which was solvent-partitioned to yield the crude EtOAc extract. Repeat separation of the extract by Si gel and Sephadex LH-20 chromatography afforded the recently reported cyanobacterial metabolite hectochlorin (**1**, 0.25% yield of the crude extract). Further purification with C-18 reversed-phase HPLC yielded two new compounds, deacetylhectochlorin (**2**) and *syn*-3-isopropyl-6-(4-methoxybenzyl)-4-methylmorpholine-2,5-dione (**3**), in 0.09 and 0.007% yield of the crude extract, respectively.



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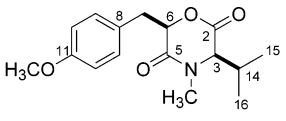
2 R = H



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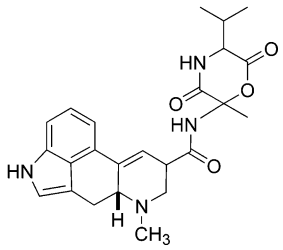

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Compound **1** was obtained as white crystals. HREIMS of **1** established a molecular ion M^+ peak at *m/z* 664.1082 for the molecular formula $C_{27}H_{34}Cl_2N_2O_9S_2$ (calcd 664.1083). **1** was identified as hectochlorin by extensive analyses of 1D and 2D NMR data and comparison with the previously published data.[5]

Compound **2** was obtained as a white amorphous solid from reversed-phase HPLC. The close relationship of **2** to **1** became evident by inspecting the ^1H, ^{13}C, H,H COSY, HMQC, and HMBC spectra of the compounds (Table 1). HREIMS of **2** showed a molecular ion M^+ peak at *m/z* 622.0984, corresponding to the molecular formula $C_{25}H_{32}Cl_2N_2O_8S_2$ (calcd 622.0977). The molecular formula of **2** corresponded to 10 degrees of unsaturation and differed from **1** by 42 amu. In contrast to **1**, **2** lacked the signals of an acetyl group, which established **2** as deacetylhectochlorin. The ^1H and ^{13}C NMR spectra of **2** were nearly identical to those of **1**, which contained two units of 2-alkylthiazole-4-carboxylic acid (C-10 to C-13 and C-18 to C-21) and a 7,7-dichloro-3-acyloxy-2-methyloctanoate (DCAO) fragment (C-1 to C-9). The 2-alkylthiazole-4-carboxylic acid units were indicated by two singlets of the methine proton signals at δ 8.25 (H-12) and 8.15 (H-20) in the ^1H NMR

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Table 1. ^1H and ^{13}C NMR Spectral Data of Hectochlorin (**1**) and Deacetylhectochlorin (**2**) Recorded in $CDCl_3$

	1		**2**			
position	$^1H^a$ δ_H (mult., J in Hz)	$^{13}C^b$ δ_C	$^1H^a$ δ_H (mult., J in Hz)	$^{13}C^b$ δ_C	H,H COSY[a]	HMBC[a,c] ($^nJ_{CH}$, n = 2, 3)
1		172.8		174.0		
2	3.15 (1H, m)	42.7	3.77 (1H, m)	42.3	H-3, H_3-9	
3	5.33 (1H, m)	75.1	5.22 (1H, m)	76.9	H-2, H_2-4ab	
4a	1.69 (1H, m)	31.0	1.76 (1H, m)	30.5	H-3, H_2-5	
4b	1.78 (1H, m)		2.02 (1H, m)			
5	1.69 (2H, m)	21.0	1.76 (2H, m)	20.6	H_2-4ab, H_2-6	
6a	2.11 (1H, m)	49.4	2.19 (1H, m)	49.5	H_2−5	
6b	2.25 (1H, m)					
7		90.4		90.2		
8	2.07 (3H, s)	37.3	2.10 (3H, s)	37.3		C-6,[d] C-7
9	1.28 (3H, d, 7.4)	15.2	1.28 (3H, d, 6.8)	15.2	H-2	C-1, C-2,[d] C-3
10		160.9		159.2		
11		146.8		142.9		
12	8.14 (1H, s)	128.3	8.25 (1H, s)	129.0		C-10,[d] C-11, C-13
13		166.1		177.5		
14	6.78 (1H, s)	74.7	5.55 (1H, brs)	73.8		
15		82.0		85.7		
16	1.82 (3H, s)	24.6	1.85 (3H, s)	24.3		C-14, C-15, C-17
17	1.59 (3H, s)	22.0	1.57 (3H, s)	20.1		C-14, C-15, C-16
18		160.2		158.6		
19		147.3		146.4		
20	7.91 (1H, s)	127.6	8.15 (1H, s)	128.6		C-18,[d] C-19, C-21
21		165.0		167.6		
22	5.61 (1H, s)	77.9	5.38 (1H, s)	78.9		C-1,[d] C-21, C-23,[d] C-24,[d] C-25[d]
23		71.6		71.7		
24	1.29 (3H, s)	26.8	1.23 (3H, s)	26.7		C-22,[d] C-23, C-25[d]
25	1.35 (3H, s)	26.1	1.44 (3H, s)	26.4		C-22,[d] C-23, C-24
26		168.5				
27	2.16 (3H, s)	21.0				

[a] Recorded at 300 MHz. [b] Recorded at 75 MHz. [c] If not indicated otherwise, correlations were observed after optimization for $^nJ_{CH}$ = 8 Hz. [d] Correlations after optimization for $^nJ_{CH}$ = 4 Hz.

spectrum as well as eight sp^2 carbon signals at δ 159.2 (C-10), 142.9 (C-11), 129.0 (C-12), 177.5 (C-13), 158.6 (C-18), 146.4 (C-19), 128.6 (C-20), and 167.6 (C-21) in the ^{13}C NMR spectrum. The NMR data further assigned the presence of the DCAO fragment as the following. Analysis of the H,H COSY spectrum and additional HMBC correlations of the methyl protons at δ 2.10 (H_3-8) to the *gem*-dichloromethyl carbon at δ 90.2 (C-7) and the methylene carbon at δ 49.5 (C-6) clearly established the aliphatic chain (C-2 to C-9). The carbonyl signal at δ 174.0 was assigned to C-1 of the DCAO fragment by HMBC correlation observed from the methyl protons at δ 1.28 (H_3-9).

The α,β-dihydroxyisovalerate (DHIV, C-21 to C-25) unit in **2** was observed due to the HMBC correlations from the *gem*-dimethyl protons at δ 1.23 (H_3-24) and 1.44 (H_3-25) to the pseudo-α-carbon at δ 78.9 (C-22) and the quaternary carbon at δ 71.7 (C-23). The downfield signals of C-22 (δ_H 5.38 and δ_C 78.9) and C-23 (δ_C 71.7) were satisfied with an ester bond linkage and a tertiary alcohol functionality as observed in a DHIV unit of **1**, respectively. The connections of the DHIV unit to the DCAO and the first thiazole (C-18 to C-21) residues were assembled since H-22 showed HMBC correlations to C-1 and C-21. Chemical shifts at δ_H 5.22 and δ_C 76.9 of C-3 in the DCAO unit further indicated an acyloxy substituted on this position. This was satisfied with the ester bond linkage between C-3 and C-10 of the second thiazole unit (C-10 to C-13). Similarly, the second DHIV unit (C-13 to C-17) was presented in **2** on the basis of HMBC correlations from the *gem*-dimethyl protons at δ 1.85 (H_3-16) and 1.57 (H_3-17) to the pseudo-α-carbon at δ 73.8 (C-14) and the quaternary carbon at δ 85.7 (C-15). The methine proton signal of H-14 in **2** (δ 5.55) is shifted upfield by ~1 ppm compared with **1** (δ 6.78), consistent with a hydroxyl group at C-14 in **2** rather than an acetoxy group in **1**. The downfield signal of C-15 was consequently satisfied with the ester bond linkage to the carbonyl carbon C-18 of the first thiazole unit. The IR spectrum of **2** showed strong absorptions at 1739 and 1717 cm^{-1}, confirming the presence of the ester moieties, and a strong broad band at 3391 cm^{-1}, further supporting the hydroxyl groups in the molecule. Finally, the cyclic nature of **2** was unambiguously furnished by ring closure of C-13 and C-14, although the HMBC correlation from H-14 to C-13 was virtually absent. This cyclic structure completed the 10 degrees of unsaturation required by the molecular formula of **2**. Therefore, **2** was established as a new deacetylhectochlorin.

To determine the absolute stereochemistry in **2**, the acetyl group of **1** was removed by hydrazine hydrolysis of **1** in MeOH at 0 °C for 2 h. The ^1H and ^{13}C NMR spectra and the specific rotation of the hydrolysis product were identical to those of **2**. The absolute stereochemistry of **2** was then suggested by comparison of the CD spectra of the natural and the transformed **2**, which displayed the similar positive Cotton effect at 228 nm and two negative effects at 209 (210) and 257 (258) nm, respectively. On the basis of the above information, the absolute configuration of **2** was the same as that of **1**. The absolute stereochemistry of **1** was recently determined as 2*S*, 3*S*, 14*S*, 22*S* by X-ray crystallography.[5]

Both **1** and **2** were directly detected by Si gel TLC from a fraction prior to purification with reversed-phase HPLC with R_f values of 0.50 and 0.38 (solvent system: EtOAc−hexane, 3:1), respectively. This evidence ruled out the possibility that **2** was an artifact from acid hydrolysis of **1** during the HPLC purification process using a mixture of CH_3CN−MeOH−1% TFA in water (2:1:1) as the eluting solvent. **1** was recently isolated from the cyanobacteria *Lyngbya majuscula* (Oscillariaceae) and exhibited potent stimulating activity of actin assembly.[5] Therefore, the real producer of **1** might be the cyanobacteria fed on by the

Table 2. ^1H and ^{13}C NMR Spectral Data of **3** Recorded in $CDCl_3$

position	^1H[a] δ_H(mult., J in Hz)	^{13}C[b] δ_C	H,H COSY	HMBC[c] ($^nJ_{CH}$ n= 2, 3)	NOESY
2		164.2			
3	3.82 (1H, d, 5.4)	66.3	H-14	C-2, C-14, C-15, C-16	$-NCH_3$, H_3-15, H_3-16
5		164.7			
6	5.00 (1H, dd, 4.0, 8.0)	79.9	H_2-7ab		H-9/H-13
7a	3.17 (1H, dd, 8.0, 14.4)	38.9	H-6	C-6, C-8, C-9/C-13	H-9/H-13, H-14, H_3-15
7b	3.33 (1H, dd, 4.0, 14.4)				H-9/ H-13
8		127.6			
9/13	7.18 (1H, d, 8.4)	130.8	H-10/H-12	C-7, C-9/C-13, C-11	H-6, H_2-7ab
10/12	6.84 (1H, d, 8.4)	114.1	H-9/H-13	C-8, C-11, C-10/C-12	$-OCH_3$
11		158.9			
14	1.99 (1H, m)	29.7	H-3, H_3-15, H_3-16	C-2, C-3, C-15, C-16	H-7a, $-NCH_3$
15	0.82 (3H, d, 6.6)	17.6	H-14	C-3, C-14, C-16	H-7a, $-NCH_3$
16	1.09 (3H, d, 6.6)	19.8	H-14	C-3, C-14, C-15	$-NCH_3$
$-NCH_3$	3.00 (3H, s)	34.2		C-3, C-5	H-3, H-14, H_3-15, H_3-16
$-OCH_3$	3.78 (3H, s)	55.3		C-11	H-10/H-12

[a] Recorded at 600 MHz. [b] Recorded at 150 MHz with a 5 mm inverse probe. [c] Correlations after optimization for $^nJ_{CH}$ = 8 Hz.

mollusk. The co-occurrence of **1** and **2** in the extract from *B. leachii* suggested that **2** could be a metabolism product formed from **1** as a result of the ester bond cleavage occurring in the acidic digestive gland of the mollusk. This eventual metabolism was earlier implied in the biogenesis of dolabellin found in the sea hare *Dolabella auricularia*.[9]

syn-3-Isopropyl-6-(4-methoxybenzyl)-4-methylmorpholine-2,5-dione (**3**) was obtained as a white solid in trace amount (0.5 mg, 0.007% yield of the crude extract). Structure elucidation of the compound was achieved by NMR (Table 2) and mass data. Compound **3** has the molecular formula $C_{16}H_{21}NO_4$ (MW 291.3422), accounting for 7 degrees of unsaturation, as determined by ESITOFMS (*m/z* 314.1679 $[M + Na]^+$; 292.1653 $[M + H]^+$, calcd for $C_{16}H_{22}NO_4$, 292.1549). The ^1H NMR spectrum of **3** exhibited a 1,4-disubstituted aromatic ring, in which proton signals were observed at δ 7.18 (H-9/H-13, 2H, d 8.4) and 6.84 (H-10/H-12, 2H, d, 8.4). The $^3J_{H,H}$ couplings of H-6 (δ 5.00, dd, J = 8.0, 4.0 Hz) to both methylene protons H-7ab (H-7a, δ 3.17, dd, J = 8.0, 14.4 Hz and H-7b δ 3.33, dd, J = 4.0, 14.4 Hz) suggested a series of ABX coupling signals as a $-CH(X)-CH_2-$ residue. The H,H COSY experiment revealed the presence of an additional partial structure, $-CH(X)-CH(CH_3)_2$, of the *N*-methylvaline residue (C-3 and C-14 to C-16, see Table 2). The presence of *N*-methylvaline in the molecule was established due to the HMBC correlation of the *N*-methyl protons at δ 3.00 to the methine carbon at δ 66.3 (C-3) and that of the methine proton at δ 1.99 (H-14) to the ester carbonyl carbon at δ 164.2 (C-2). The HMBC correlation observed for the *N*-methyl protons and the carbonyl carbon at δ 164.7 (C-5) thus suggested the amide functionality in the molecule. The IR spectrum showing two strong absorption bands at 1744 and 1651 cm^{-1} further confirmed the presence of ester and amide functionalities in the molecule. The methoxyl substituted at C-11 was indicated from HMBC correlations of the methoxyl proton signal at δ 3.78 (11-OCH_3) and the aromatic proton signals at δ 7.18 (H-9/H-13) and 6.84 (H-10/H-12) to the oxygenated quaternary carbon at δ 158.9 (C-11). The substitution at C-8 was the remaining $-CH(X)-CH_2-$ residue since the methylene protons at δ 3.17 (H-7a) and 3.33 (H-7b) showed HMBC correlations to the aromatic carbons at δ 127.6 (C-8) and 130.8 (C-9/C-13). The downfield chemical shifts of H-6 at δ 5.00 and C-6 at δ 79.9 were satisfied by an ester bond linkage between C-6 and C-2 of the *N*-methylvaline fragment. Although the HMBC correlations of H-6 and H-7ab to C-5 were virtually absent, the morpholine nucleus of **3** was assembled with the ring closure between the amide carbonyl (C-5) and the methine carbon (C-6). With this ring closure, the 7 degrees of unsaturation required by the molecular formula of **3** were finally satisfied.

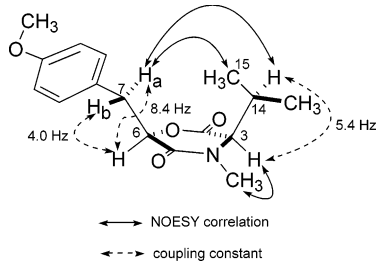

Figure 1. Selected NOESY correlations and the *J* values observed in **3**.

The relative stereochemistry of **3** was revealed by analyses of *J* values from the ^1H NMR spectrum and the 2D NOESY experiment (Figure 1). H-3 was coupled to H-14 with $^3J_{3-14}$ = 5.4 Hz, and H-6 was coupled to H-7ab with $^3J_{6-7a}$ = 8.4 and $^3J_{6-7b}$ = 4.0 Hz. Accordingly, the dihedral angles of $H^3-C-C-H^{14}$, $H^6-C-C-H^{7a}$, and $H^6-C-C-H^{7b}$ were approximately 60°, 180°, and 60°, respectively. Interestingly, only H-7a displayed NOESY correlations to H-14 and H_3-15. The data implied that the methoxyl benzyl and the isopropyl substituents were in the pseudoaxial orientation and H-6 and H-3 were in the pseudoequatorial orientation. Therefore, **3** was established as *syn*-3-isopropyl-6-(4-methoxybenzyl)-4-methylmorpholine-2,5-dione.

Compound **3** is the second example of morpholine-2,5-dione-containing natural products after the discovery of ergosecalinine (**4**) in the fungus *Claviceps purpurea*.[11] From a biogenetic point of view, **3** could be derived by condensation of 2-hydroxy-3-(4-methoxyphenyl)propionate, a product of the Shikimate pathway, and a modified amino acid, *N*-methylvaline, which is often found as an amino acid component in cyanobacterial metabolites.[12] Unfortunately, the absolute configuration assignment and biological testing of **3** were not done due to limited amount of the compound.

Compounds **1** and **2** were subjected to cytotoxic testing against oral human epidermoid carcinoma of nasopharynx (KB), human small cell lung cancer (NCI-H187), and breast cancer (BC) cell lines. Compound **2** showed potent cytotoxicity against KB and NCI-H187 with ED_{50}'s of 0.31 and 0.32 μM, while **1** displayed ED_{50}'s of 0.86 and 1.20 μM, respectively. In addition, **2** showed moderate cytotoxic activity against the BC cancer cell line (ED_{50} 1.03 μM). Both compounds were inactive to in vitro antimalarial and antituberculous assays.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter using a sodium lamp operating at 589 nm. Circular dichroism (CD) spectra were recorded on a JASCO J-715 spectropolarimeter. IR and UV spectra were obtained on a Perkin-Elmer 2000 FT-IR spectrometer and a Spectronic 3000 UV spectrometer, respectively. ^{1}H and ^{13}C, DEPT, H,H COSY, HMQC, HMBC, and NOESY experiments were obtained from a Bruker AVANCE DPX-300 FT-NMR or a Bruker AMX-600 spectrometer in $CDCl_3$. The HREIMS spectra were obtained with a JEOL JMS-700 mass spectrometer, operating at 10 kV ionization voltages. The ESITOFMS spectrum was obtained with a Q-TOF mass spectrometer (Micromass, Manchester, UK) equipped with a Z-spray type ESI ion source. All experiments were performed in the positive ion mode.

Animal Material. Eleven specimens of the sea hare *Bursatella leachii* (1.5 kg, wet weight) were collected by hand from Sichang Island, Chonburi Province, Thailand, in July 2002, and frozen on site before extraction at our laboratory. The voucher specimens and the live photo of this animal are available from the Supporting Information and our laboratory. The genus *Bursatella* belonging to the class Anaspediae, order Opisthobranchia, and family Mullusca is categorized into a single species worldwide, *B. leachii*, which for convenience is divided into several geographical subspecies. The animal is covered all over with simple and compound villi (papillae) of unequal size, giving it a ragged appearance. The color was greenish brown, with reticulate markings, black spots, and clear, brighter green areas, each with a peacock-blue ocellus, inner edges of the parapodia, mantle cavity, and pedal sole paler.[13]

Extraction and Isolation. The internal organs (440 g, wet weight) of the specimens (11 animals) were blended into small pieces and macerated three times with MeOH (1.5 L, each). The combined extracts were concentrated under reduced pressure, and the residue was partitioned between EtOAc and H_2O to obtain the crude EtOAc extract (6.4 g) as a dark green oil. The crude EtOAc extract was chromatographed on a Si gel column by eluting stepwise with CH_2Cl_2 and MeOH to give four fractions. The second fraction (2.3 g) was chromatographed on a Si gel column using gradient mixtures of hexane−EtOAc−MeOH, yielding fractions containing hectochlorin (**1**) and deacetylhectochlorin (**2**), Si gel TLC (solvent system: hexane−EtOAc, 1:3) showing **1** and **2** at R_f values of 0.50 and 0.38, respectively. **1** (16.2 mg, 0.25% of the crude extract) was obtained by repeat purification using Sephadex LH-20 (CH_2Cl_2−EtOAc, 1:1). The fraction containing **2** was subjected to C-18 reversed-phase HPLC [LiChroCART column (250 × 10 mm), 1 mL/min, detection at 254 nm] using CH_3-CN−MeOH−1% TFA in water (2:1:1) as the eluting solvent, yielding **2** (6.0 mg, t_R 22 min, 0.09% of the crude extract) and the fraction containing **3**. Upon further purification with preparative TLC (hexane−EtOAc, 1:3), 0.5 mg of **3** was obtained (R_f 0.47, 0.007% yield of crude extract).

Hectochlorin (1): white crystals; $[\alpha]^{20}_D$ −7.47° (*c* 0.28, MeOH);[14] CD (MeOH) $\Delta\epsilon_{207}$ −13.7, $\Delta\epsilon_{230}$ +8.9, $\Delta\epsilon_{276}$ −0.2; UV (MeOH) λ_{max} (log ϵ) 239 (3.66); IR (film) 3456, 3118, 2983, 2939, 1747, 1692, 1481, 1371, 1314, 1270, 1215, 1149 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1; HREIMS (direct inlet) M^+ *m/z* 664.1082 (calcd for $C_{27}H_{34}Cl_2N_2O_9S_2$, 664.1083).

Deacetylhectochlorin (2): white amorphous solid; $[\alpha]^{20}_D$ −22.78° (*c* 0.10, MeOH); CD (MeOH) $\Delta\epsilon_{209}$ −32.6, $\Delta\epsilon_{228}$ +28.1, $\Delta\epsilon_{257}$ −18.8; UV (MeOH) λ_{max} (log ϵ) 239 (3.83); IR (film) 3391, 3155, 2982, 2939, 1739, 1717, 1479, 1382, 1327, 1241, 1140 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1; HREIMS (direct inlet) M^+ *m/z* 622.0984 (calcd for $C_{25}H_{32}Cl_2N_2O_8S_2$, 622.0977).

Preparation of 2 from 1. A 6 mg (9 μM) sample of **1** was hydrolyzed with 30 μL of hydrazine monohydrate and 1 mL of MeOH at 0 °C for 2 h. The mixture was twice partitioned between CH_2Cl_2 and water, the organic layers were combined and dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The extract was purified by C18 reversed-phase HPLC [LiChroCART column, (250 × 10 mm), 2 mL/min, detection at 254 nm] using MeOH and water (3:1) as the eluting solvent to provide **2** (3 mg, t_R 14.1 min): white solid; $[\alpha]^{20}_D$ −26.02° (*c* 0.10, MeOH); CD (MeOH) $\Delta\epsilon_{210}$ −25.5, $\Delta\epsilon_{228}$ +24.6, $\Delta\epsilon_{258}$ −17.1; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.28 (3H, d, J = 6.3 Hz, H-9), 1.44 (3H, s, H-25), 1.23 (3H, s, H-24), 1.60 (3H, s, H-17), 1.76 (2H, m, H-5), 1.76 (1H, m, H-4a), 2.07 (1H, m, H-4b), 1.86 (3H, s, H-16), 2.10 (3H, s, H-8), 2.16 (2H, m, H-6), 3.71 (1H, m, H-2), 5.25 (1H, m, H-3), 5.40 (1H, s, H-22), 5.52 (1H, brd, H-14), 8.13 (1H, s, H-20), 8.22 (1H, s, H-12); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 15.3 (C-9), 20.6 (C-5), 20.0 (C-17), 24.1 (C-16), 26.5 (C-25), 26.7 (C-24), 30.5 (C-4), 37.3 (C-8), 42.3 (C-2), 49.5 (C-6), 71.7 (C-23), 73.7 (C-14), 77.0 (C-3), 79.0 (C-22), 85.8 (C-15), 90.2 (C-7), 128.6 (C-20), 129.0 (C-12), 142.7 (C-11), 146.3 (C-19), 158.5 (C-18), 159.0 (C-10), 167.7 (C-21), 177.9 (C-13), 174.1 (C-1).

***syn*-3-Isopropyl-6-(4-methoxybenzyl)-4-methylmorpholine-2,5-dione (3):** white solid; $[\alpha]^{20}_D$ −58.21° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log e) 223 (2.93), 250 (2.20), 273 (2.08), 281 (1.99); IR (film) 2920, 1744, 1651, 1514, 1249 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C ($CDCl_3$, 150 MHz), see Table 2; ESITOFMS *m/z* 314.1679 $[M + Na]^+$; 292.1653 $[M + H]^+$ (calcd for $C_{16}H_{22}NO_4$, 292.1549).

Cytotoxicity Assay. Cytotoxic activity against breast cancer (BC), oral human epidermoid carcinoma of nasopharynx (KB), and human small cell lung cancer (NCI-H187) cell lines was measured by the sulforhodamine B (SRB) method. [15]

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Supporting Information Available: Photo of the Thai sea hare, *Bursatella leachii*; ^{1}H NMR spectrum of **1**; ^{1}H, ^{13}C, DEPT-135, HMQC, COSY, HMBC, MS, and CD spectra of **2**; ^{1}H NMR and CD spectra of the transformed deacetylhectochlorin; ^{1}H, ^{13}C, HMQC, COSY, HMBC, NOESY, and MS spectra of **3**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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