Isolation and Biological Evaluation of 8-*epi*-Malyngamide C from the Floridian Marine Cyanobacterium *Lyngbya majuscula*[†]

Jason C. Kwan,[‡] Max Teplitski,[§] Sarath P. Gunasekera,[⊥] Valerie J. Paul,[⊥] and Hendrik Luesch*[,‡]

Department of Medicinal Chemistry, University of Florida, 1600 SW Archer Road, Gainesville, Florida 32610, Soil and Water Science Department, Genetics Institute, University of Florida Institute of Food and Agricultural Sciences, Gainesville, Florida 32610, and Smithsonian Marine Station, 701 Seaway Drive, Fort Pierce, Florida 34949

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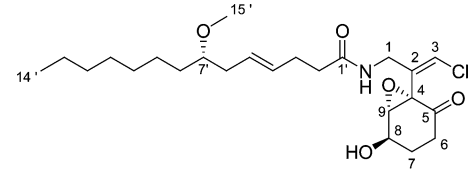
A new stereoisomer of malyngamide C, 8-*epi*-malyngamide C (**1**), and the known compound lyngbic acid [(4*E*,7*S*)-7-methoxytetradec-4-enoic acid] were isolated from a sample of *Lyngbya majuscula* collected near Bush Key, Dry Tortugas, Florida. The structure of **1** was determined by NMR and MS experiments. The absolute configuration of **1** was determined by selective Mitsunobu inversion of C-8 to give malyngamide C, as determined by NMR, MS, and comparison of specific rotation. Both **1** and malyngamide C were found to be cytotoxic to HT29 colon cancer cells (IC_{50} 15.4 and 5.2 μM, respectively) and to inhibit bacterial quorum sensing in a reporter gene assay.

In the late 1970s and early 1980s, malyngamides A−E, malyngamide C acetate, deoxymalyngamide C, and dideoxymalyngamide C[1−5] were isolated from *Lyngbya majuscula* and described by Richard E. Moore's laboratory, while Paul J. Scheuer's group isolated the related stylocheilamide[6,7] from the sea hare *Stylocheilus longicauda.* Subsequently, many more members of the series[8−19] were reported, largely by William Gerwick's lab and mostly isolated from *L. majuscula*. Some malyngamides have been isolated from sea hares[6,8,16,20] and a red alga,[11] but in these cases the biosynthetic origin is thought to be dietary and epiphytic cyanobacteria, respectively.

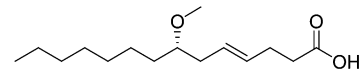
Malyngamides are small amides produced by marine cyanobacteria, of which there are 29 known examples (malyngamides A−X,[1−5,8−21] C acetate, deoxy C, dideoxy C,[1] and F acetate[10] and stylocheilamide[6]). The acid side chain is most commonly (4*E*,7*S*)-7-methoxytetradec-4-enoic acid (lyngbic acid), but it can be (4*E*,7*S*)-7-methoxy-9-methylhexadec-4-enoic acid (as in malyngamides D and E[5]) or (4*E*,7*S*)-7-methoxydodec-4-enoic acid (as in malyngamides G,[14] S,[8] and U−W[12,19]). In one notable example (malyngamide X[15,16]) the side chain is 7*R*-lyngbic acid. The other half of the molecule sometimes contains a vinylic chlorine and either a five-membered lactam or a six-membered cyclic ketone, lactone, or aromatic ring.

Various malyngamides have been reported to have cytotoxic activity, usually in the micromolar range. Ecologically, malyngamides A and B are known feeding deterrents,[22−24] but it is worth noting that the biological activity of some members of the series are largely unexplored. Here we present a previously unknown epimer of malyngamide C, 8-*epi*-malyngamide C (**1**), and evaluation of its cytotoxicity and quorum sensing inhibitory activity.

Samples of *L. majuscula* were collected off Bush Key, Florida, within the Dry Tortugas National Park in April 2007. The material was freeze-dried and extracted with EtOAc−MeOH to afford a nonpolar extract, which was subsequently partitioned between hexanes and MeOH−H_2O (80:20). The MeOH−H_2O fraction was further partitioned between *n*-BuOH and H_2O. The *n*-BuOH-soluble portion was subjected to silica gel chromatography and HPLC to give **1** and lyngbic acid (Figure 1).



1 8-*epi*-Malyngamide C



Lyngbic acid

Figure 1. Structures of 8-*epi*-malyngamide C (**1**) and lyngbic acid.

HRESI/APCIMS data for **1** suggested a molecular formula of $C_{24}H_{38}ClNO_5$ (*m/z* 456.2518 and 458.2492 for $[M + H]^+$). This molecular formula is identical to that of malyngamide C,[1] but although the ^1H and ^{13}C NMR spectra for **1** are similar to those of a previously isolated sample of malyngamide C, there are differences (see Supporting Information). Examination of the ^1H NMR, ^{13}C NMR, COSY, edited HSQC, and HMBC spectra of **1** (Table 1) allowed the construction of the same 2D structure as malyngamide C. Importantly, while the signal for H-8 in the ^1H NMR spectrum for malyngamide C shows one large coupling (9.7 Hz), the corresponding signal in **1** shows only small couplings (Figure 2). It was therefore suspected that **1** is an epimer of malyngamide C, with an equatorial H-8 instead of the axial H-8 observed in malyngamide C.

The carboxylic acid side chain of **1** is lyngbic acid (Figure 1), which was also isolated from the same silica chromatography fraction. HRESI/APCIMS data suggested a molecular formula of $C_{15}H_{28}O_3$ (*m/z* 257.2121 for $[M + H]^+$). ^1H and ^{13}C NMR data were identical to literature values[15,25−27] for lyngbic acid. In addition, the specific rotation was close to values previously reported for the *S* isomer (−12.6 versus −13.3,[15] −12.8,[25] and −13[27]), and therefore the configuration had to be 7*S*. Isolation of 7*S*-lyngbic acid supports a 7′*S* configuration for **1**. NOESY correlations between H-3 of **1** and H-1a and H-1b suggest a *Z* configuration for the C-2−C-3 double bond, as in malyngamide C.

In order to test our hypothesis that **1** is the 8-epimer of malyngamide C, selective inversion of this center was carried out using the Misunobu reaction, with *p*-nitrobenzoic acid (*p*-NBA) as

[†] Dedicated to the late Richard E. Moore of the University of Hawaii at Manoa for his pioneering work on bioactive natural products.

* Corresponding author. Tel: (352) 273-7738. Fax: (352) 273-7741. E-mail: luesch@cop.ufl.edu.

[‡] Department of Medicinal Chemistry, University of Florida.

[§] Soil and Water Science Department, University of Florida.

[⊥] Smithsonian Marine Station.

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Table 1. NMR Data for 8-*epi*-Malyngamide C (**1**) in $CDCl_3$ at 400 MHz (^1H) or 600 MHz (2D NMR, ^1H) and 100 MHz (^{13}C)

C/H no.	δ_H (*J* in Hz)	δ_C, mult[a]	$^1H-^1H$ COSY	HMBC[b]	NOESY
1a	3.96, ddd (−14.7, 5.7, 0.6)	41.4, t	H-1b, H-3, NH	2, 3, 4, 1′	H-3, NH
1b	3.84, ddd (−14.7, 5.5, 0.7)		H-1a, H-3, NH	2, 3, 4, 1′	H-3, NH
2		132.9, s			
3	6.39, dd (0.6, 0.7)	123.3, d	H-1a, H-1b	1, 2, 4	H-1a, H-1b, H_3-15′
4		60.9, s			
5		202.8, s			
6a	2.52, ddd (−17.3, 10.6, 6.1)	31.9, t	H-6b, H-7a, H-7b	5, 7, 8	H-6b, H-7a, H-7b
6b	2.45, ddd (−17.3, 5.3, 5.0)		H-6a, H-7a, H-7b	4, 5, 7, 8	H-6a, H-7a, H-7b
7a	2.10, dddd (−14.5, 6.1, 5.3, 3.8)	26.0, t	H-6a, H-6b, H-7b, H-8	5, 6, 9	H-6a, H-6b, H-7b, H-8
7b	1.98, dddd (−14.5, 10.6, 5.0, 0.7)		H-6a, H-6b, H-7a, H-8	5, 6, 9	H-6a, H-6b, H-7a, H-8
8	4.47, ddd (3.8, 2.5, 0.7)[c]	64.2, d	H-7a, H-7b, H-9	4	H-7a, H-7b, H-9
9	3.58, d (2.5)	64.3, d	H-8	2, 4, 7, 8	H-8
NH	6.16, dd (5.7, 5.5)		H-1a, H-1b	1, 1′	H-1a, H-1b,H_3-15′
1′		173.0, s			
2′	2.22, m (2H)	36.20, t	H-3a′, H-3b′	1′, 3′, 4′	
3a′	2.29, m	28.3, t	H_2-2′, H-3b′, H-4′	1′, 2′, 4′, 5′	
3b′	2.20, m		H_2-2′, H-3a′	1′, 4′, 5′	
4′	5.42, ddd	130.6, d	H-3a′, H-5′	3′, 5′, 6′	
5′	5.50, ddd	127.7, d	H-4′, H_2-6′	3′, 4′, 6′, 7′	H-7′, H_3-15′
6′	2.16, m (2H)	36.24, t	H-5′, H-7′	4′, 5′, 7′, 8′	
7′	3.15, quin	80.7, d	H_2-6′, H-8a′, H-8b′	5′, 6′, 8′, 9′	H-5′, H_3-15′
8a′	1.40, m	33.3, t	H-7′, H-8b′, H-9a′, H-9b′	7′, 9′, 10′	
8b′	1.36, m		H-7′, H-8a′, H-9a′, H-9b′	6′, 7′, 9′	
9a′	1.31, m	25.3, t	H-8a′, H-8b′, H-9b′	11′	
9b′	1.24, m		H-8a′, H-8b′, H-9a′		
10′	1.24, m	29.7, t			
11′	1.24, m	29.3, t			
12′	1.23, m	31.8, t			H_3-14′
13a′	1.26, m	22.6, t	H_3-14′	10′, 12′, 14′	H_3-14′
13b′	1.24, m		H_3-14′		H_3-14′
14′	0.87, t	14.1, q	H-13a′, H-13b′	12′, 13′	H_2-12′, H-13a′, H-13b′
15′	3.30, s	56.4, q		7′	H-3, H-5′, H-7′, NH

[a] Multiplicity derived from edited HSQC spectra. [b] Protons showing long-range correlation to indicated carbon. [c] Coupling constants derived from signals of coupling partners.

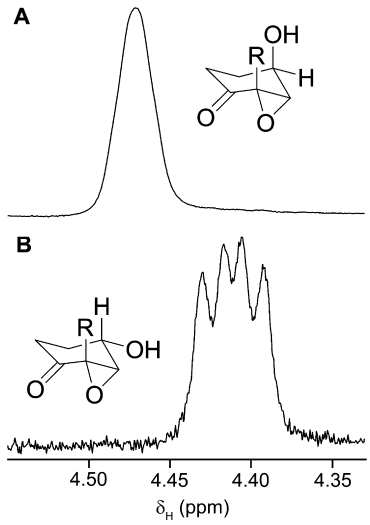


Figure 2. Comparison of the ^1H NMR signal for H-8 in (A) 8-*epi*-malyngamide C (**1**) and (B) malyngamide C (400 MHz, $CDCl_3$).

the nucleophile.[28] Deprotection using K_2CO_3[29] gave the inverted product, which had ^1H and ^{13}C NMR spectra identical to malyngamide C, which we isolated from sea hares collected in the Cook Islands in the Pacific Ocean (see Supporting Information). The spectroscopic data also matched those previously reported for malyngamide C.[1] Additionally, the product had a very similar specific rotation to that reported for malyngamide C (−29.3 versus −27.4[1]), indicating that both compounds have the same absolute configuration. This result therefore established the absolute configuration of **1** to be 4*S*,8*R*,9*S*,7′*S*.

Bioactivities for malyngamide C have not been previously reported, and consequently malyngamide C was tested alongside **1** in all assays. Both **1** and malyngamide C were found to be cytotoxic to HT29 colorectal adenocarcinoma cells, with IC_{50}s of 15.4 and 5.2 μM, respectively. We were also interested in the potential for **1** and malyngamide C to act on bacterial quorum sensing (QS) pathways. These pathways allow bacteria to regulate their behavior according to their own population density.[30] QS regulated behaviors include the expression of virulence factors and the onset of hardy biofilm phenotypes.[30] One group of compounds that are used for QS signaling are the acyl homoserine lactones, which all contain a five-membered lactone ring joined by an amide linkage to a fatty acid chain.[31] We found compounds **1** and malyngamide C were able to reduce 3-oxo-C_{12}-HSL signaling in a LasR-based quorum sensing reporter (Figure 3) at concentrations where these compounds did not inhibit bacterial cell growth. The potencies of **1** and malyngamide C are similar to that of tumonoic acid F,[32] another cyanobacterial compound that previously has been reported to inhibit bioluminescence in *Vibrio harveyi* (IC_{50} 62 μM).

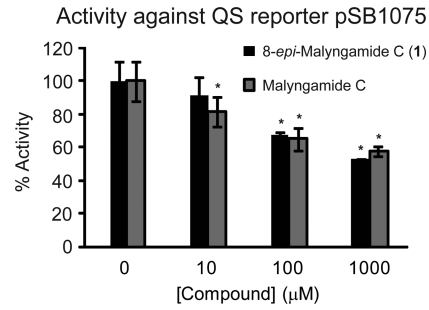
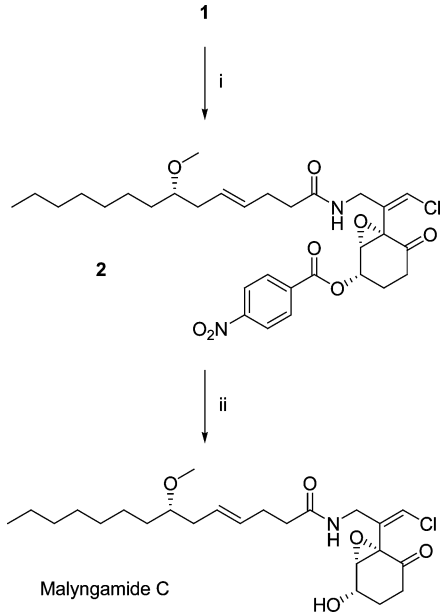


Figure 3. Activity against the quorum sensing reporter pSB1075, which expresses LasR and responds to 3-oxo-C_{12}-HSL. Asterisks (*) indicate reduction of luminescence with statistical significance of $P < 0.05$ (*t*-test) compared to untreated controls.

Scheme 1. Conversion of **1** to Malyngamide C by Mitsunobu Inversion[a]



[a] (i) PPh_3, *p*-NBA, DEAD, THF, rt for 14 h, then 40 °C for 3 h; (ii) K_2CO_3, rt for 1 h.

Experimental Section

General Experimental Procedures. Optical rotation was measured on a Perkin-Elmer 341 polarimeter. UV was measured on a SpectraMax M5 (Molecular Devices), and IR data were obtained on a Bruker Vector 22 instrument. ^{1}H and ^{13}C NMR spectra in $CDCl_3$ for **1**, as well as ^{1}H NMR spectra for **2** and malyngamide C, were recorded on a Varian Mercury 400 MHz spectrometer. ^{13}C NMR spectra for **2** and malyngamide C, as well as 2D NMR spectra for **1**, were recorded on a Bruker 600 MHz Avance II spectrometer using a 1 mm triple-resonance high-temperature superconducting cryogenic probe.[33] Spectra were referenced to residual solvent signals [$\delta_{H/C}$ 7.26/77.0 ($CDCl_3$)]. HSQC experiments were optimized for 145 Hz, and HMBC experiments were optimized for 7 Hz couplings. HRESI/APCIMS data were recorded on an Agilent LC-TOF mass spectrometer equipped with an APCI/ESI multimode ion source detector in positive ion mode. MTT assays were detected on a SpectraMax M5, and luminescence readings for quorum sensing assays were measured using a Perkin-Elmer Victor3.

Biological Material. Samples of *L. majuscula* were collected at a depth of 1 m on the west side of Bush Key, Florida (24°37.582′ N 82°52.099′ W) on April 23, 2007. The dimensions—sheath width 41.2 ± 0.61 μm (mean ± SE), cell width 34.3 ± 0.75 μm, cell length 1.9 ± 0.28 μm—fit the description of *L. majuscula* with short cells 2−4 μm in length.[34] Voucher specimens (DRTO0000019) are maintained at the Smithsonian Marine Station at Fort Pierce, Florida, and at South Florida Collections Management Center, Everglades National Park.

Specimens of the sea hare *Stylocheilus striatus* were collected in November 2005 from the shores of Rarotonga, Cook Islands, and preserved in 3% formalin in seawater. After two weeks the animals were transferred to EtOH for storage.

Extraction and Isolation of 8-*epi*-Malyngamide C (1) and Lyngbic Acid. After freeze-drying, *L. majuscula* from Bush Key, FL, was extracted with EtOAc−MeOH (1:1) to give 1.391 g of nonpolar material. This was partitioned between hexanes and MeOH−H_2O (80:20). The MeOH−H_2O-soluble portion (1.126 g) was further partitioned between *n*-BuOH and H_2O. The *n*-BuOH fraction (351.8 mg) was subjected to silica gel chromatography using a gradient system of increasing *i*-PrOH in CH_2Cl_2. The fraction eluting with 6% *i*-PrOH (55.0 mg) was further purified by reversed-phase HPLC [Synergi Hydro-RP (Phenomenex), 250 × 10 mm, 2.0 mL/min; UV detection at 220 and 240 nm] using a CH_3CN−0.1% HCOOH linear gradient (40−100% CH_3CN over 20 min, then 100% CH_3CN for 30 min), to furnish compound **1** (17.7 mg) at t_R 22.3 min and lyngbic acid (8.0 mg) at t_R 23.7 min.

Extraction and Isolation of Malyngamide C. Six *S. striatus* (5.5 g wet wt), stored in 15 mL of EtOH, were cut into small pieces and soaked in 100 mL of MeOH−EtOAc (1:1), sonicated for 2 h, and left overnight. The filtered extract was combined with the storage solution to furnish 93 mg (1.6% yield, wet wt) of extract. This was applied to a column of C_{18} (10 g) and eluted using a MeOH−H_2O step gradient system to give four subfractions. Subfraction 3 (12 mg), which eluted with MeOH−H_2O (3:1), was further separated by RP HPLC [Econosil RP-18 10 μm (Alltech), 250 × 10 mm, 3 mL/min; UV detection at 230 and 254 nm] using MeOH−H_2O (4:1) to give pure malyngamide C (8 mg, 0.14% wet wt) at t_R 20.1 min. Small amounts of *L. majuscula* that the sea hares were feeding on were also collected and showed the presence of malyngamide C by LC/MS.

8-*epi*-Malyngamide C (1): light brown oil; $[\alpha]^{20}_D$ −8.0 (*c* 0.36, MeOH); UV (MeOH) λ_{max} (log ε) 213 (3.30), 231 (2.82), 270 (2.31); IR (film) ν_{max} 3360 (br), 3070 (w), 2927, 2854, 2361 (w), 1716, 1654, 1542, 1520, 1495, 1457, 1267, 1090, 1071, 971, 914 cm^{-1}; ^{1}H NMR, ^{13}C NMR, COSY, edited HSQC, HMBC, NOESY, see Table 1 and Supporting Information; HRESI/APCIMS *m/z* $[M + H]^+$ 456.2518 and 458.2492 (ratio 3:1, calcd for $C_{24}H_{39}{}^{35}ClNO_5$, 456.2517, and $C_{24}H_{39}{}^{37}ClNO_5$, 458.2487).

Lyngbic acid: pale yellow oil; $[\alpha]^{20}_D$ −12.6 (*c* 0.8, MeOH) [lit. −13.3,[15] −12.8,[25] and −13[27]]; UV (MeOH) λ_{max} (log ε) 203 (3.08), 230 (sh, 2.54), 261 (2.10); IR (film) ν_{max} 2925, 2854, 2360, 2342, 1708, 1541 (w), 1456, 1361, 1270, 1193, 1096, 970, 722 (w), 669 (w) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.54−5.43 (m, 2H), 3.32 (s, 3H), 3.15 (quin, J = 5.8 Hz, 1H), 2.45−2.38 (m, 2H), 2.37−2.30 (m, 2H), 2.21−2.16 (m, 2H), 1.48−1.38 (m, 2H), 1.33−1.19 (m, 11H), 0.88 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.7, 130.1, 127.8, 80.7, 56.5, 36.3, 33.9, 33.3, 31.8, 29.7, 29.3, 27.7, 25.3, 22.7, 14.1; HRESI/APCIMS *m/z* $[M + H]^+$ 257.2121 (calcd for $C_{15}H_{29}O_3$ 257.2117).

8*S*-(*p*-Nitrobenzoyl)malyngamide C (2). A mixture of 5 mg of **1**, 7.7 mg of *p*-NBA (4.2 equiv), and 11.4 mg of PPh_3 (4.0 equiv) was dissolved in dry THF. While the solution was stirred on an ice bath, 7.75 mg of diethyl azodicarboxylate (DEAD) was added slowly (4.0 equiv, as a 40% w/v solution in toluene). After addition of DEAD, the mixture was stirred at room temperature for 14 h, then heated to 40 °C for 3 h. The reaction mixture was concentrated *in vacuo*, and the residue was applied to a 1 g prepacked C_{18} cartridge (Alltech) and eluted with MeOH, then further purified by HPLC [Alltima HP C18, 250 × 4.6 mm (Alltech), 1.0 mL/min; PDA detection] using a MeOH−H_2O linear gradient (40−100% MeOH over 20 min, then 100% MeOH for 10 min) to give pure **2** (4.9 mg, 74% yield) at t_R 22.8 min. Colorless, amorphous solid: $[\alpha]^{20}_D$ −12.5 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.94), 257 (3.74); IR (film) ν_{max} 3308 (br), 3079 (w), 2926, 2855, 1724, 1653, 1607, 1530, 1456, 1348, 1320, 1269, 1172, 1101, 1032 (w), 1015 (w), 971 (w), 929 (w), 872, 858, 784 (w), 755 (w), 721 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, J = 8.9 Hz, 2H), 8.27 (d, J = 8.8 Hz, 2H), 6.42 (d, J = 0.5 Hz, 1H), 6.05 (t, J = 5.1 Hz, 1H), 5.72 (dd, J = 10.1, 5.8 Hz, 1H), 5.54−5.42 (m, 2H), 4.03 (ddd, J = 14.8, 6.0, 0.8 Hz, 1H), 3.88 (dd, J = 14.8, 4.7 Hz, 1H), 3.80 (br, 1H), 3.31 (s, 3H), 3.15 (quin, J = 5.7 Hz, 1H), 2.70 (dt, J = 17.9, 4.0 Hz, 1H), 2.43 (ddd, J = 18.4, 12.0, 6.4 Hz, 1H), 2.38−2.30 (m, 2H), 2.30−2.10 (m, 6H), 1.48−1.37 (m, 2H), 1.37−1.17 (m, 10H), 0.87 (t, J = 6.8 Hz, 3H); HRESI/APCIMS *m/z* $[M + H]^+$ 605.2625 and 607.2609 (ratio 3:1, calcd for $C_{31}H_{42}{}^{35}ClN_2O_8$, 605.2630, and $C_{31}H_{42}{}^{37}ClN_2O_8$, 607.2601).

Malyngamide C. K_2CO_3 (15.9 mg) was suspended in 755 μL of MeOH and stirred. To this was added 2.45 mg of **2** in 500 μL of MeOH. The mixture was stirred at room temperature for 1 h, dried under air, and extracted between EtOAc and H_2O. The EtOAc fraction was washed with water, concentrated to dryness, and subjected to purification by HPLC [Alltima HP C18, 250 × 4.6 mm (Alltech), 1.0 mL/min; PDA detection] using a MeOH−H_2O linear gradient (40−100% MeOH over 20 min, then 100% MeOH for 10 min) to give pure malyngamide C (1.4 mg, 76% yield) at t_R 20.9 min. Colorless oil: $[\alpha]^{20}_D$ −29.3 (*c* 0.14, MeOH) [lit. −27.4[1]]; UV (MeOH) λ_{max} (log ε) 202 (3.90), 232 (sh, 3.00), 264 (2.98); IR (film) ν_{max} 3308 (br), 3054 (w), 2923, 2851, 2360 (w), 1717, 1654, 1526, 1456, 1372, 1264, 1085, 1028 (w), 969, 925 (w), 893 (w), 854 (w), 792 (w), 737, 703 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.40 (s, 1H), 6.08 (br t, J = 4.5 Hz, 1H), 5.54−5.40 (m, 2H), 4.41 (dd, J = 9.5, 5.4 Hz, 1H), 4.06 (ddd, J = 14.6, 6.3, 0.6 Hz, 1H), 3.82 (dd, J = 14.6, 4.6 Hz, 1H), 3.62 (s, 1H), 3.33 (s, 3H), 3.16 (quin, J = 5.8 Hz, 1H), 2.59 (dt, J = 17.7, 4.1 Hz,

1H), 2.46−2.11 (m, 7H), 2.07−1.88 (m, 3H), 1.50−1.39 (m, 2H), 1.37−1.16 (m, 11H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 202.0, 172.6, 133.4, 130.6, 127.6, 122.3, 80.8, 66.2, 65.4, 61.8, 56.4, 40.5, 36.34, 36.28, 35.4, 33.3, 31.8, 29.7, 29.2, 28.4, 25.2, 24.8, 22.6, 14.0; HRESI/APCIMS m/z $[M + H]^+$ 456.2520 and 458.2496 (ratio 3:1, calcd for $C_{24}H_{39}{}^{35}ClNO_5$, 456.2517, and $C_{24}H_{39}{}^{37}ClNO_5$, 458.2487).

Cell Viability Assay. HT29 cells were cultured in Dulbecco's modified Eagle medium (DMEM; Invitrogen) containing 10% fetal bovine serum (Hyclone), in a humidified atmosphere containing 5% CO_2 at 37 °C. Cells were seeded into 96-well plates at a density of 10 000 cells/well (100 μL medium/well). After 24 h, compound **1** and malyngamide C were added to wells at varying concentrations (as 1 μL stock solutions in EtOH). Etoposide was used as a positive control for cytotoxicity, and EtOH alone was used as a negative control. After another 48 h, the plate was developed with MTT dye according to the manufacturer's protocol (G4000, Promega).

Quorum Sensing Assay. The assay used the quorum sensing reporter plasmid pSB1075[35] transformed into *Escherichia coli* JM109. This reporter expresses the LasR receptor, which in the presence of the cognate signaling molecule 3-oxo-C_{12}-HSL will bind to the *lasI* promoter cloned upstream of a promoterless *luxCDABE* cassette, resulting in luminescence. *E. coli* JM109 pSB1075 was grown overnight (37 °C, with shaking) in LB, then diluted 100-fold with fresh LB and grown for another 2 h under the same conditions. After this time, the culture was diluted 100-fold with fresh LB again and incubated for another 1 h. The culture was diluted 10-fold with fresh LB before 100 μL was added to each well of a black 96-well plate, with 3-oxo-C_{12}-HSL and various amounts of test compounds already added. The final concentration of 3-oxo-C_{12}-HSL used was 1 nM, and bacteria were always grown in the presence of ampicillin to maintain the plasmid. Plates were incubated at 37 °C in a humid environment for 6.5 h before luminescence was quantified. As a control for effects on cell viability, nontransformed *E. coli* JM109 was treated with **1** or malyngamide C, and ampicillin was used as a positive control. In all cases, cell density (as measured by OD_{590}) was unaffected.

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Supporting Information Available: NMR spectra for compound **1**, ^{1}H NMR spectrum for compound **2**, and comparison of ^{1}H and ^{13}C NMR spectra of **1**, the Mitsunobu product, and malyngamide C. This material is available free of charge via the Internet at http://pubs.acs.org.

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