# Lagunamides A and B: Cytotoxic and Antimalarial Cyclodepsipeptides from the Marine Cyanobacterium *Lyngbya majuscula*

Ashootosh Tripathi,[†] Jonathan Puddick,[‡] Michèle R. Prinsep,[‡] Matthias Rottmann,[§] and Lik Tong Tan*[,†]

*Natural Sciences and Science Education, National Institute of Education, Nanyang Technological University, 1 Nanyang Walk, Singapore 637616, Singapore, Department of Chemistry, University of Waikato, Private Bag 3105, Hamilton 3205, New Zealand, Swiss Tropical and Public Health Institute, Parasite Chemotherapy, Socinstrasse 57, PO Box, CH-4002 Basel, Switzerland, and University of Basel, CH-4003 Basel, Switzerland*

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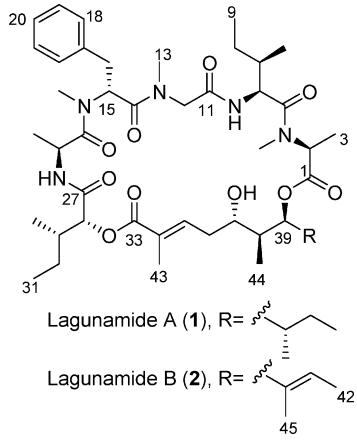
Lagunamides A (**1**) and B (**2**) are new cyclic depsipeptides isolated from the marine cyanobacterium *Lyngbya majuscula* obtained from Pulau Hantu Besar, Singapore. The planar structural characterization of these molecules was achieved by extensive spectroscopic analysis, including 2D NMR experiments. In addition to Marfey's method and $^3J_{H-H}$ coupling constant values, a modified method based on Mosher's reagents and analysis using LC-MS was deployed for the determination of the absolute configuration. Lagunamides A and B displayed significant antimalarial properties, with $IC_{50}$ values of 0.19 and 0.91 $\mu$M, respectively, when tested against *Plasmodium falciparum*. Lagunamides A and B also possessed potent cytotoxic activity against P388 murine leukemia cell lines, with $IC_{50}$ values of 6.4 and 20.5 nM, respectively. Furthermore, these cyanobacterial compounds exhibited moderate antiswarming activities when tested against *Pseudomonas aeruginosa* PA01.

Marine cyanobacterial strains belonging to the *Lyngbya* genus are bountiful producers of structurally intriguing and biologically active secondary metabolites.[1] Some of the important biological activities associated with these secondary metabolites include antimicrobial, antimalarial, cytotoxic, and neurotoxic properties.[2,3] Among the diverse classes of compounds being discovered from this genus, a substantial number belong to either the polypeptide or hybrid polyketide−polypeptide structural classes.[1−3]

As part of the drug discovery program in our laboratory, we chanced upon a persistent strain of the marine cyanobacterium *Lyngbya majuscula* from the intertidal region in the western lagoon of Pulau Hantu Besar, Singapore. Subsequent chemical workup of its organic extracts yielded a number of new, as well as known, cytotoxic compounds.[4,5] Further purification of the bioactive polar fraction obtained from the vacuum flash chromatography (VFC) of the organic extract led to the isolation of two new cyclic depsipeptides, lagunamides A (**1**) and B (**2**). These compounds are structurally related to a series of potent cytotoxic marine cyanobacterial compounds including the aurilides, kulokekahilide-2, and pulau'amide.[6−8]




## Results and Discussion

Collections of a shallow water strain of *L. majuscula* were made during low tides from the western lagoon of Pulau Hantu Besar, Singapore, in June 2007. The cyanobacterial biomass was extracted repeatedly with $CHCl_3$/MeOH (1:1) and fractionated by VFC with increasing polarity of organic solvents. Preliminary brine shrimp lethality bioassay (BSLA) of the 100% EtOAc-eluted fraction showed a high incidence of toxicity (100% at 10 ppm). The bioactive fraction was then subjected to SEP PAK $C_{18}$ solid-phase fractionation followed by reversed-phase HPLC, yielding lagunamides A (**1**) and B (**2**) as colorless, amorphous solids.

Lagunamide A (**1**) possesses a molecular formula of $C_{45}H_{71}N_5O_{10}$ as determined from HRESIMS based on the $[M + Na]^+$ ion peak at *m*/*z* 864.5093. The $^1$H and $^{13}$C NMR data, recorded in $CDCl_3$, of the apparently chromatographically homogeneous material revealed complex overlapping of signals, which may be due to the existence of two or more conformers of **1**. Nevertheless, the $^1$H NMR spectrum indicated the peptidic nature of **1** and suggested the presence of at least two secondary amide proton signals. Changing the solvent to $CD_3OD$ (Table 1) provided reasonably well dispersed $^1$H and $^{13}$C NMR signals, which could be attributed to the preponderance of a single conformer for compound **1** in deuterated methanol. The $^1$H NMR spectrum further showed the presence of at least three *N*-methyl amide groups at $\delta$ 2.90, 3.06, and 3.32. Furthermore, the $^{13}$C NMR spectrum (in $CD_3OD$) of **1** indicated the presence of seven carbonyl carbons attributable to ester/amide functionalities and a monosubstituted phenyl ring ($\delta$ 137.4, 129.6, 128.0, and 126.4) system (Table 1).

Detailed analysis of the 1D and 2D NMR data of **1** revealed a structural framework consisting of peptide and polyketide sections (depicted as substructures **A** and **B** in Figure 1). Extensive NMR analysis of **1** allowed the construction of five standard amino acid and one hydroxy acid moieties in substructure **A**. Five standard amino acid residues, *N*-methylalanine (*N*-Me-Ala), isoleucine (Ile), *N*-methylglycine (*N*-Me-Gly), *N*-methylphenylalanine (*N*-Me-Phe), and alanine (Ala), were deduced by following the extension of the spin system of each residue by $^1$H−$^1$H COSY (Figure 1) and TOCSY experiments. A sixth residue in substructure **A** (Figure 1) exhibited $^1$H NMR resonances at $\delta$ 4.92 (H-28), 1.68 (H-29), and 0.97−1.51 (H-30 to H-32), suggesting an isoleucine residue. However, the HSQC spectrum indicated the attachment of H-28 to

* To whom correspondence should be addressed. E-mail: liktong.tan@nie.edu.sg. Tel: +65 6790 3820. Fax: +65 6896 9432.
[†] Nanyang Technological University.
[‡] University of Waikato.
[§] Swiss Tropical and Public Health Institute and University of Basel.

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**Table 1.** NMR Spectroscopic Data for Lagunamides A (**1**) and B (**2**) in $CD_3OD$ (400 MHz)

| | | lagunamide A (**1**) | | | lagunamide B (**2**) | |
|---|---|---|---|---|---|---|
| unit | C/H no. | $\delta_C$[a] | $\delta_H$ ($J$ in Hz)[b] | HMBC[c] | $\delta_C$[a] | $\delta_H$ ($J$ in Hz)[b] |
| *N*-Me-Ala | 1 | 171.7 | | | 171.6 | |
| | 2 | 59.2 | 3.96, q (6.9) | 1, 3, 4 | 60.0 | 3.79, m |
| | 3 | 12.8 | 1.43, d (6.9) | 1, 2 | 12.5 | 1.38, d (6.8) |
| | 4 | 36.5 | 3.32, s | 2, 5 | 36.5 | 3.36, s |
| Ile | 5 | 172.0 | | | 170.6 | |
| | 6 | 53.7 | 5.06, d (6.2) | 5, 7, 8, 11 | 53.0 | 5.08, d (7.1) |
| | 7 | 38.4 | 1.83, m | | 38.5 | 1.86, m |
| | 8a | 23.7 | 1.64, m | | 23.9 | 1.74, m |
| | 8b | | 1.32, m | | | 1.43, m |
| | 9 | 10.3 | 0.93, m | 7, 8 | 10.9 | 0.97, m |
| | 10 | 15.0 | 1.06, d (6.8) | 6, 7, 8 | 14.8 | 1.04, d (6.8) |
| *N*-Me-Gly | 11 | 170.2 | | | 170.2 | |
| | 12 | 51.6 | 4.22, d (18.3) | 11, 13 | 51.5 | 4.19, d (18.4) |
| | | | 3.59, d (18.3) | 11, 13 | | 3.58, d (18.4) |
| | 13 | 35.5 | 2.90, s | 12, 14 | 35.8 | 2.93, s |
| *N*-Me-Phe | 14 | 171.5 | | | 171.9 | |
| | 15 | 54.0 | 5.47, dd (10.3, 5.2) | 14, 16, 17, 23 | 54.0 | 5.44, dd (10.3, 5.0) |
| | 16a | 34.8 | 3.04, m | 15, 17, 18, 19 | 34.9 | 3.02, m |
| | 16b | | 2.94, dd (14.3, 5.2) | 15, 17, 18, 19 | | 2.95, dd (14.1, 5.0) |
| | 17 | 137.4 | | | 137.4 | |
| | 18/22 | 128.0 | 7.20, m | 16, 19, 21 | 128.0 | 7.21, m |
| | 19/21 | 129.6 | 7.18, m | 20, 18, 22 | 129.6 | 7.15, m |
| | 20 | 126.4 | 7.28, m | 19, 21 | 126.4 | 7.31, m |
| | 23 | 29.5 | 3.06, s | 15, 24 | 29.4 | 3.05, s |
| Ala | 24 | 173.8 | | | 173.8 | |
| | 25 | 45.3 | 4.52, q (6.9) | 24, 26, 27 | 45.6 | 4.47, q (6.9) |
| | 26 | 14.7 | 0.87, d (6.9) | 24, 25 | 14.4 | 0.87, d (6.9) |
| Hila | 27 | 171.7 | | | 172.0 | |
| | 28 | 76.7 | 4.92, m | 27, 29, 30, 32, 33 | 76.6 | 4.92, d (11.4) |
| | 29 | 37.3 | 1.68, m | 30, 31 | 37.4 | 1.86, m |
| | 30a | 26.3 | 1.51, m | 29, 31 | 26.4 | 1.54, m |
| | 30b | | 1.30, m | | | 1.34, m |
| | 31 | 10.6 | 0.97, m | 28, 29, 30 | 10.3 | 0.96, m |
| | 32 | 13.5 | 1.14, m | 28, 29 | 13.5 | 0.96, m |
| Dtea | 33 | 169.3 | | | 169.5 | |
| | 34 | 127.6 | | | 127.8 | |
| | 35 | 145.6 | 7.32, brd | 34,36, 43 | 145.8 | 7.31, brd |
| | 36a | 29.6 | 2.27, m | 34, 35, 37 | 29.4 | 2.25, m |
| | 36b | | 2.08, m | | | 2.04, m |
| | 37 | 70.4 | 3.70, brd (3.0) | 35, 44 | 70.6 | 3.79, brd |
| | 38 | 40.4 | 2.18, m (3.0, 3.8) | | 40.4 | 2.19, m |
| | 39 | 78.0 | 4.84, m (3.8, 10.0) | 1, 38, 40, 44 | 82.8 | 4.90, m |
| | 40 | 37.5 | 1.82, m (10.0) | 39, 41, 45 | 132.4 | |
| | 41a | 27.2 | 1.32, m | 42 | 126.4 | 5.62, m |
| | 41b | | 1.16, m | 45 | | |
| | 42 | 11.3 | 0.91, m | 41a | 9.7 | 1.63, m |
| | 43 | 11.4 | 1.93, s | 33, 34, 35 | 11.4 | 1.93, s |
| | 44 | 9.0 | 0.90, m | 37, 38, 39 | 8.5 | 0.76, d (7.0) |
| | 45 | 11.8 | 0.91, m | 40 | 12.1 | 1.64, s |

[a] Recorded at 100 MHz. [b] Recorded at 400 MHz. Coupling constants (Hz) are in parentheses. [c] Protons correlated to carbon resonances in $\delta_C$ column.

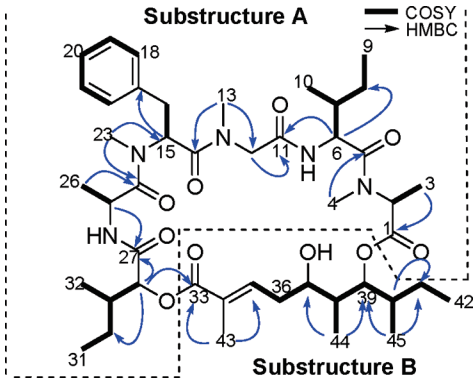


**Figure 1.** Planar structure of lagunamide A (**1**) with key COSY and HMBC correlations indicated.

an oxymethine carbon ($\delta$ 76.7) and thus was more consistent with a 2-hydroxyisoleucic acid (Hila) moiety (Figure 1). The sequence *N*-Me-Ala−Ile−*N*-Me-Gly−*N*-Me-Phe−Ala−Hila of these amino/hydroxy acid residues in **1** was deduced from HMBC correlations between H-2/C-1, H-4 and H6/C-5, H-6/C11, H-12/C-11, $H_3$-13/C-12 and C-14, H-15/C-14, $H_3$-23/C-24, H-25 and $H_3$-26/C-24, H-25/C-27, and H-28/C-27 (Table 1) to generate substructure **A** (Figure 1).

The structure elucidation of substructure **B** was accomplished by COSY analysis of proton signals from the olefinic proton, H-35, via the allylic methylene protons, H-36a/H-36b, the oxymethine proton at H-37, and the methine proton at H-38. The continued COSY correlations can be traced forward between methyl protons at $H_3$-44 to the methine proton at H-38, extending to the methine protons at H-39 and H-40, and further to the methyl proton signal at $H_3$-45. Additional COSY correlations between H-40/$H_2$-41ab and $H_2$-41ab/$H_3$-42 completed substructure **B**. This substructure was supported by HMBC long-range correlations observed from the resonances of $H_3$-43 to C-33 and C-35, $H_3$-44 to C-37 and C-39, and $H_3$-45 to C-39 and C-41 (Figure 1).

In a similar manner, the HMBC correlations observed from $H_3$-43 to C-33, C-34, and C-35, as well as correlations from $H_3$-44 to

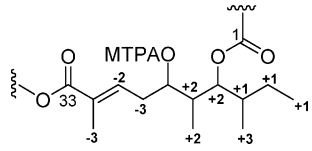


**Figure 2.** $\Delta\delta_{(S-R)}$ values ($\times 10^{-2}$ ppm) of the MTPA esters of lagunamide A (**1**).

C-37 and C-39, acted as the missing link to complete substructure **B** (Figure 1) of **1**. Furthermore, the presence of a hydroxy group at C-37 was suggested on the basis of the molecular formula and the characteristic chemical shifts of H-37 ($\delta$ 3.70) and C-37 ($\delta$ 70.4). The *E*-geometry of the double bond $\Delta^{34,35}$ was assigned on the basis of the $^{13}$C NMR chemical shifts of the methyl group $CH_3$-43 at $\delta$ 11.4.[9]

Substructures **A** and **B** were connected on the basis of HMBC data. The α-proton (H-28) of Hila showed a cross-peak to the C-33 carbonyl carbon in substructure **B**. Furthermore, the correlation of H-39 in substructure **B** with the C-1 carbonyl carbon in substructure **A** through an ester bond satisfies the final degree of unsaturation to complete a 26-membered ring in lagunamide A (**1**).

Several chemical and NMR techniques were employed to determine the configuration of **1**. Lagunamide A was hydrolyzed with 6 N HCl and was subjected to the advanced Marfey's method, revealing the absolute configurations of Ala, *N*-Me-Phe, *N*-Me-Ala, and Ile to be L, D, L, and L-*allo*, respectively.[10−12]

Due to the unavailability of pure Hila standards, we decided to synthesize the isomers by diazotization of the corresponding amino acids in dilute perchloric acid.[13] The replacement of the amino group by a hydroxy group is known to occur with retention of configuration at the α-carbon owing to anchiomeric participation of the neighboring carboxy function.[14] The synthesized Hila isomers were subjected to derivatization of the secondary alcohol with Mosher's acids (*S*- and *R*-MTPA) and subsequent analysis by LC-MS (refer to scheme in Supporting Information). The modified technique successfully enabled the assignment of the absolute configuration of Hila as D-*allo*.

The absolute configuration of C-37 was defined by preparation of the *S*- and *R*-MTPA esters of the hydroxy group at C-37 of **1**.[15] The $\Delta\delta_{(S-R)}$ values (Figure 2) showed unambiguously that C-37 possesses the *S*-configuration. The relative configurations of the other three carbons (C-38, C-39, and C-40) were determined using $^3J_{H,\,H}$ values as well as NOESY and ROESY correlations (Figure 3).

The protons at H-37 and H-38 displayed a small coupling constant ($^3J_{H\text{-}37,\ H\text{-}38} = 3.0$ Hz, obtained through HOM2DJ experiment in $CD_3OD$ at 400 MHz), indicating them to be in a *syn*-conformation. Having the absolute configuration at C-37 determined as *S* allowed the construction of four (out of six) possible conformations for C-37 and C-38. A NOESY correlation observed between $H_3$-44 and H-37 would be satisfied by two possible relative conformations; however only one, 37*S* and 38*S**, can satisfy the NOESY correlation between $H_2$-36 and H-38 (Figure 3a). Likewise, the protons H-38 and H-39 displayed a small coupling constant ($^3J_{H\text{-}38,\ H\text{-}39} = 3.8$ Hz, obtained through HOM2DJ experiment), which is consistent with a *gauche*-conformation and produced four possible conformations. Of these, however, only 38*S** and 39*S** can satisfy the NOESY correlation between $H_3$-44 and H-40 (Figure 3b).

The continued stereochemical analysis of the spin system through C-39 to C-40 revealed a large coupling constant between H-39 and H-40 ($^3J_{H\text{-}39,\ H\text{-}40} = 10.0$ Hz), indicating the protons to be in an *anti*-orientation. Moreover, the NOESY correlation between $H_3$-45 and H-39 would satisfy two possible relative conformations of either 39*S**,40*S** or 39*S**,40*R**. However a NOESY correlation observed between $H_3$-45 and H-38 suggested the relative configuration to be 39*S**,40*S** (Figure 3c). Having

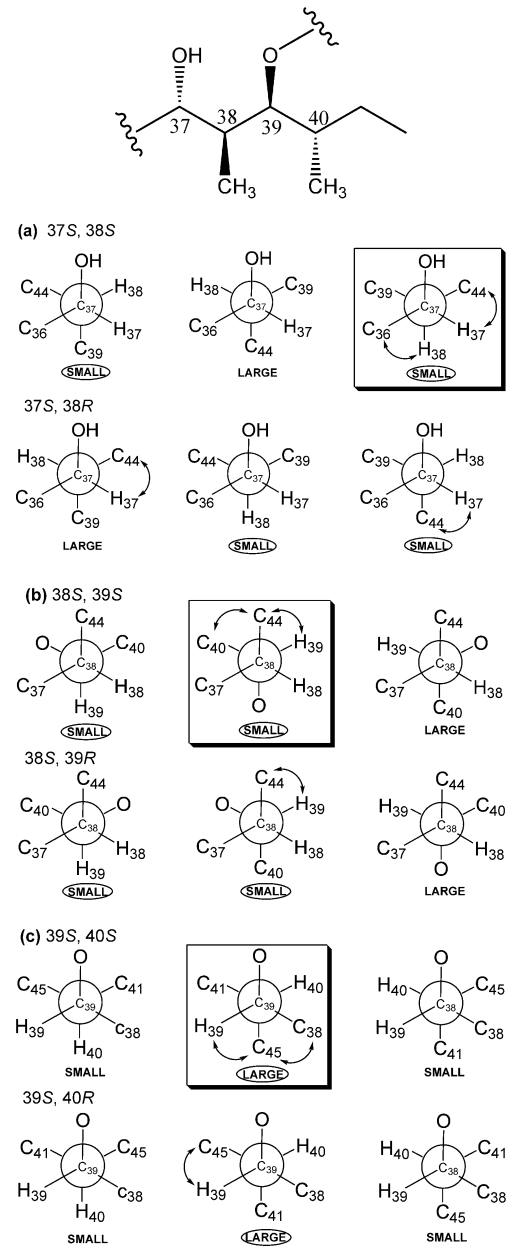


**Figure 3.** Newman projections for (a) C-37/C-38; (b) C-38/C-39; and (c) C-39/C-40. Labels below projections denote predicted size of the $^3J_{H-H}$ coupling constant between protons displayed. Predicted values highlighted by a circle are consistent with observed $^3J_{H-H}$ coupling constant values. Observed NOESY correlations are presented as double-headed arrows.

confirmed the absolute configuration of C-37 as *S*, the absolute configurations of 37*S*, 38*S*, 39*S*, and 40*S* were established for the polyketide moiety in lagunamide A (**1**).

Lagunamide B (**2**) was isolated by RP-HPLC from the same fraction containing compound **1**. The HRESIMS of the molecule provided a molecular formula of $C_{45}H_{69}N_5O_{10}$ showing a $[M + Na]^+$ ion peak at 862.4937. Similarly to **1**, compound **2** adopted at least two or more conformers in the $^1$H NMR spectrum when measured in $CDCl_3$. Lagunamide B (**2**) had high structural similarity to **1**, as evidenced by nearly identical $^1$H and $^{13}$C NMR chemical shifts when measured in $CD_3OD$ (Table 1). However, it displayed subtle differences within the polyketide portion, which could be traced in the spin system from $CH_3$-42 to the olefinic protons at H-41 in comparison to methylene $H_2$-41 in **1**.

Hydrolysis and stereoanalysis of the peptide portion of **2** were undertaken as described above for lagunamide A (**1**). The absolute

configurations of the five amino/hydroxy acid residues Ala, *N*-Me-Phe, *N*-Me-Ala, Ile, and Hila were determined to be L, D, L, L-*allo*, and D-*allo*, respectively. The absolute configurations from C-37 to C-39 were proposed to be identical to those of **1** on the basis of highly comparable NMR spectroscopic data. An *E*-geometry of the double bond $\Delta^{40,41}$ in **2** was assigned on the basis of the $^{13}C$ NMR chemical shift of C-45 at $\delta$ 11.8.[9]

Lagunamides A (**1**) and B (**2**) were tested for their antimalarial activity against the NF54 strain of the malarial parasite and showed effective *in vitro* activity against *Plasmodium falciparum* with $IC_{50}$ values of 0.19 and 0.91 $\mu$M, respectively. This is the first report of antimalarial activity for this class of aurilide-related molecules.[6−8] Interestingly, the only structural difference between **1** and **2** is the additional olefinic group between C40−C41 in **2**, and this minor difference is reflected in the enhanced (about 4.7-fold increase) antimalarial activity observed in **1**. Furthermore, the antimalarial activity of **1** is found to be similar to that of dolastatin 15 but less potent than that of dolastatin 10, which is the most potent antimalarial marine cyanobacterial compound known.[16] The lagunamides also exhibited potent cytotoxic properties against the P388 cancer cell line with $IC_{50}$ values of 6.4 nM for **1** and 20.5 nM for **2**.

Lagunamides A (**1**) and B (**2**) displayed antiswarming activity when tested against the Gram-negative bacterial strain *Pseudomonas aeruginosa* PA01. Both compounds, when tested at 100 ppm, exerted moderate antiswarming activities (62% for **1** and 56% for **2** compared to control). *P. aeruginosa* is an opportunistic nosocomial pathogen, and the discovery of the lagunamides having antiswarming activities on *P. aeruginosa* PA01 is therefore significant. It is however unknown at this point if the antiswarming activities of these cyanobacterial compounds operate by interference of the bacterial quorum sensing system. The discovery of the diverse biological activities of the lagunamides in this present study once again demonstrates the importance of marine cyanobacteria as a source of potential therapeutic agents.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a Bellingham Stanley ADP 440 polarimeter. UV and IR spectra were measured on a Varian Cary 50 UV visible spectrophotometer and a PerkinElmer spectrum 100 FT-IR spectrophotometer, respectively. $^1H$, $^{13}C$, and 2D NMR spectra were recorded in $CD_3OD$ on a 400 MHz Bruker NMR spectrometer using the residual solvent signal ($\delta_H$ at 3.31 ppm and $\delta_C$ at 49.1 ppm) as internal standards. HRESIMS data were obtained using a Bruker Daltonics MicrOTOF mass spectrometer. HPLC isolation of lagunamides A (**1**) and B (**2**) was conducted on a Shimadzu LC-8A Preparative LC equipped with a Shimadzu SPD-M10A VP diode array detector, while an Agilent 1100 series coupled with an Agilent LC/MSD trap XCT mass spectrometer equipped with an ESI interface system was used for the detection of the Marfey-derivatized alanine, *N*-methyl-phenylalanine, *N*-methyl-alanine, and isoleucine as well as for Mosher's derivatized α-hydroxy acids in compound **1**. Cell viability in 96-well plates was measured using a Bio Rad Benchmark Plus microplate reader.

**Biological Material.** The filamentous benthic marine cyanobacterium *Lyngbya majuscula* (~1.5 L) was collected from the western lagoon of Pulau Hantu Besar, Singapore, during low tides on June 25, 2007, and stored before workup (70% EtOH(aq), −20 °C). A voucher specimen of this cyanobacterial bloom material is maintained at NIE under the code TLT/PHB/002.

**Extraction and Isolation.** The cyanobacterium (~169 g dry wt) was exhaustively extracted using $CHCl_3$/MeOH (1:1) to produce an organic extract (~1 g). The extract was then fractionated on normal-phase silica VFC using a stepwise gradient solvent system from hexane, to EtOAc, and MeOH. The fraction eluted with 100% EtOAc was found to possess 100% activity in the brine shrimp toxicity assay at 10 ppm and was passed through a Sep-Pak RP-18 cartridge eluting with 100% MeOH. The eluent was concentrated *in vacuo*, and the resulting dark green gum was subjected to C18 RP-HPLC (Phenomenex Sphereclone 5 $\mu$m ODS, 250 × 10.00 mm, MeOH/$H_2O$ (78:22) at 3.0 mL/min, UV detection at 230 nm) to obtain lagunamides A (**1**, 20.1 mg, 2% of extract, $t_R$ = 30.1 min) and B (**2**, 11.2 mg, 1.1% of extract, $t_R$ = 21.2 min).

**Lagunamide A (1):** white, amorphous powder; $[\alpha]^{25}_D$ −36 (*c* 0.5, MeOH); UV(MeOH) $\lambda_{max}$ 229 nm (log $\varepsilon$ 2.89); IR (neat) 3436, 2929, 2599, 2341, 2054, 1739, 1634, 1519, 1246 $cm^{-1}$; $^1H$ NMR (400.13 MHz, $CD_3OD$) and $^{13}C$ NMR (100.62 MHz, $CD_3OD$) data, see Table 1; HRESIMS *m/z* $[M + Na]^+$ 864.5106 (calcd for $C_{45}H_{71}N_5O_{10}Na$, 864.5093).

**Lagunamide B (2):** white, amorphous powder; $[\alpha]^{25}_D$ −39 (*c* 0.5, MeOH); UV(MeOH) $\lambda_{max}$ 221 nm (log $\varepsilon$ 2.91); IR (neat) 3487 (br), 3436 (br), 2962, 2566, 2374, 2170, 1736, 1641, 1462, 1199, 1044 $cm^{-1}$; $^1H$ NMR (400.13 MHz, $CD_3OD$) and $^{13}C$ NMR (100.62 MHz, $CD_3OD$) data, see Table 1; HRESIMS *m/z* $[M + Na]^+$ 862.4940 (calcd for $C_{45}H_{69}N_5O_{10}Na$, 862.4937).

**Advanced Marfey's Analysis of Amino Acids.** Lagunamide A (**1**, 1.0 mg) was hydrolyzed in 6 N HCl (1 mL) in a sealed reaction vial at 110 °C for 18 h. Trace HCl was removed under a stream of $N_2$ gas, and the resulting hydrolysate was redissolved in $H_2O$ (0.6 mL) and divided into two equal portions. Each portion was combined with either a 1% solution of 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (L-FDAA, Marfey's reagent) (50 $\mu$L) in acetone or a racemic mixture of a 1% solution of 1-fluoro-2,4-dinitrophenyl-5-DL-alaninamide (DL-FDAA, 50 $\mu$L) in acetone and 1 M $NaHCO_3$ (25 $\mu$L), and the two mixtures were heated at 40 °C for 45 min. Both reaction mixtures were cooled to room temperature (rt), quenched by addition of 2 N HCl (25 $\mu$L), dried, and redissolved in MeCN (500 $\mu$L). The aliquots were subjected to reversed-phase LCMS (Agilent 1100 series) according to the advanced Marfey's method (Phenomenex, Luna, 150 × 2.0 mm, 5 $\mu$m, 100 Å; MeCN in 0.1% (v/v) aqueous HCOOH; at 0.20 mL/min) using a linear gradient (10−50% MeCN over 60 min).[10−12] An Agilent 1100 series MSD spectrometer was used for detection in API-ES (negative mode). The retention times and ESIMS product ions ($t_R$ in min, *m/z* $[M - H]^-$) of the L-FDAA monoderivatized amino acids in the hydrolysate of the first portion were observed to be Ile (49.2, 382.0), *N*-Me-Phe (51.9, 430.1), Ala (31.0, 340.0), and *N*-Me-Ala (37.4, 354.0), while the reaction with racemic DL-FDAA in the second portion gave rise to two peaks for each corresponding amino acid moiety. The retention times and ESIMS product ions ($t_{R1}/t_{R2}$, min, *m/z* $[M - H]^-$) were observed to be Ile (49.2/56.1, 382.0), *N*-Me-Phe (51.1/51.9, 430.1), Ala (31.0/51.1, 340.0), and *N*-Me-Ala (37.4/38.1, 354.0). Peaks eluted with longer $t_R$ could be attributed to the D-FDAA derivative of the amino acids. Consequently, the absolute configurations of the moieties in the hydrolysate of **1** were confirmed as L-Ile, D-*N*-Me-Phe, L-Ala, and L-*N*-Me-Ala.

**Marfey's Analysis for Isoleucine.** Lagunamide A (**1**, 0.5 mg) was hydrolyzed in 6 N HCl at 110 °C for 20 h and derivatized with Marfey's reagent (L-FDAA) as described above. Two portions each of 0.5 mg standard L-Ile and L-*allo*-Ile were dissolved in 100 $\mu$L of $H_2O$. A 1.0% solution of L-FDAA (100 $\mu$L) and 1 N $NaHCO_3$ (20 $\mu$L) were added to one portion each of L-Ile and L-*allo*-Ile, and to the other portions were added a 1.0% solution of D-FDAA (100 $\mu$L) and 1 N $NaHCO_3$ (20 $\mu$L). All four mixtures were then heated at 40 °C for 45 min. The solutions were cooled to rt, neutralized with 2 N HCl (10 $\mu$L), and evaporated to dryness. The residues were then resuspended in MeCN (500 $\mu$L). The aliquots were subjected to reversed-phase LCMS (Agilent 1100 series) according to the Marfey's method (Phenomenex, Luna, 150 × 2.0 mm, 5 $\mu$m, 100 Å; MeCN in 0.1% (v/v) aqueous HCOOH; at 0.20 mL/min) using a linear gradient (30−70% MeCN over 40 min).[10−12] An Agilent 1100 series MSD spectrometer was used for detection in API-ES (negative mode). The derivatized Ile residue in the hydrolysate of **1** eluted at the same retention time as the derivatized standard L-*allo*-Ile (15.8 min) but not that of L-Ile (15.0 min), D-Ile (= D-FDAA-derivatized L-Ile, 18.4 min), and D-*allo*-Ile (= D-FDAA-derivatized L-*allo*-Ile, 18.8 min).

**Preparation of 2-Hydroxyisoleucic Acid (Hila).** L-Ile (100 mg, 0.75 mmol) was dissolved in 0.2 N perchloric acid (50 mL) at 0 °C. To this was added a cold (0 °C) solution of $Na_2SO_3$ (1.4 g, 20 mmol) in $H_2O$ (20 mL) with rapid stirring. With continued stirring the reaction mixture was allowed to reach rt until evolution of $N_2$ subsided (about 30 min). The solution was then boiled for 3 min, cooled to rt, and saturated with NaCl before extraction with $Et_2O$ and drying under vacuum to give 2*S*,3*S*-Hila (L-Hila). The three other stereoisomers 2*R*,3*R*-Hila (D-Hila), 2*R*,3*S*-Hila (D-*allo*-Hila), and 2*S*,3*R*-Hila (L-*allo*-Hila) were

synthesized in a similar manner from D-Ile, D-*allo*-Ile, and L-*allo*-Ile, respectively.[13,14]

**Absolute Configuration of Hila Moiety in 1.** Determination of the absolute configuration for the Hila residue in **1** was accomplished by a modified method based on Mosher's reagents and analysis using LCMS. Lagunamide A (**1**, 1.0 mg) was hydrolyzed in 6 N HCl (1 mL) in a sealed reaction vial at 110 °C for 18 h. Trace HCl was removed under a stream of $N_2$ gas, the resulting hydrolysate was divided into two equal portions (0.5 mg each), and pyridine (0.5 mL) was added to each. α-Methoxy-α-trifluoromethylphenylacetic acid (*R*-MTPACl) (2.5 mg) was added to one portion and *S*-MTPACl (2.5 mg) to the other. The reaction was carried out for 10 h at rt, and the solvent was evaporated under $N_2$. In a similar manner, each isomer of Hila (0.5 mg each) was derivatized with either *R*- or *S*-MTPACl (1.0 mg each in 0.5 mL of pyridine). All derivatized samples were subjected to reversed-phase LCMS (Agilent 1100 series) (Phenomenex, Luna, 150 × 2.0 mm, 5 μm, 100 Å; MeCN in 0.1% (v/v) aqueous HCOOH; at 0.20 mL/min) using a linear gradient (30−70% MeCN over 90 min). An Agilent 1100 series MSD spectrometer was used for detection in API-ES (negative mode). The retention times and ESIMS product ions ($t_R$ in min) of the *S*-MTPACl-monoderivatized standard hydroxy amino acids were observed to be L-Hila (44.1 min), L-*allo*-Hila (44.5 min), D-Hila (= *R*-MTPACl-derivatized L-Hila, 42.9 min), and D-*allo*-Hila (= *R*-MTPACl-derivatized L-*allo*-Hila, 43.4 min). Consequently, the absolute configuration of the Hila moiety in the hydrolysate of **1** derivatized with *S*-MTPACl was confirmed as D-*allo*-Hila since it eluted at 43.4 min.

**MTPA (α-Methoxy-α-Trifluoromethylphenylacetic Acid) Esters of 1.** Two portions of lagunamide A (**1**, 0.5 mg each) were reacted with *R*- or *S*-MTPACl (5.0 mg) in pyridine (0.5 mL) for 10 h at rt, and the solvent was then evaporated under $N_2$. The corresponding esters were subjected to NMR analysis.

***S*-MTPA Ester:** $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 7.201 (H-35), 2.206 (H-36a), 2.015 (H-36b), 3.542 (H-37), 1.301 (H-38), 4.839 (H-39), 2.248 (H-40), 1.207 (H-41a), 1.413 (H-41b), 0.918 (H-42), 1.907 (H-43), 0.901 (H-44), 0.923 (H-45).

***R*-MTPA Ester:** $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 7.223 (H-35), 2.232 (H-36a), 2.047 (H-36b), 3.556 (H-37), 1.283 (H-38), 4.810 (H-39), 2.236 (H-40), 1.191 (H-41a), 1.401 (H-41b), 0.902 (H-42), 1.939 (H-43), 0.881 (H-44), 0.891 (H-45).

**Antiswarming Assay Based on *Pseudomonas aeruginosa* PA01.** An antiswarming assay based on the bacteria *P. aeruginosa* PA01 was carried out using either lagunamide A or B (0.5 mg) added into 5 mL of molten STA (soft top agar) [constituted with 200 mL of deionized $H_2O$, 1.3 g of agar (Technical agar # 3 Oxoid), 2 g of tryptone (Difco), 1 g of NaCl (Sigma)] and poured immediately over the surface of a solidified Luria Bertani agar (40 g/L from MoBio) plate as an overlay. Once the overlaid agar had solidified, the plate was point inoculated with the bacterial strain (5 μL) and incubated (37 °C for 72 h). The experiment was performed in triplicate along with a MeOH-treated plate as a control, and the extent of swarming was determined by measuring the area of the colony using a leaf surface meter (area meter AM200, ADC Bioscientific Ltd.).

**Cytotoxicity Assay.** Test solutions were added to P388 murine leukemia cells (ATCC CCL46) in a series of eight 2-fold dilutions, and cells were then incubated (35 °C, 72 h). Media, solvents, cells, and positive controls were included in each assay run. 3-(4,5-Dimethylthiazoyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to wells; then cells were incubated for a further 4 h. Cell viability was determined by measurement of formazan production via a spectrophotometer at 540 nm. The percentage inhibition of cell growth was determined by comparison of test well absorbance to that of a control, and a plot of logarithm of sample concentration versus absorbance was used to determine the $IC_{50}$ (ng/mL) value (concentration of the test sample required to reduce the P388 cell line growth by 50%).

***In Vitro* Antimalarial Assay.** *Plasmodium falciparum* drug-sensitive NF54 and chloroquine-resistant K1 strains were cultivated in a variation of the medium previously described,[17,18] consisting of RPMI 1640 supplemented with 0.5% ALBUMAX II, Hepes (25 mM), $NaHCO_3$ (25 mM, pH 7.3), hypoxanthine (0.36 mM), and neomycin (100 μg/mL). Human erythrocytes served as host cells. Cultures were maintained in an atmosphere of $O_2$:$CO_2$:$N_2$ (3:4:93) in humidified modular chambers at 37 °C. Compounds were dissolved in DMSO (10 mM), diluted in hypoxanthine-free culture medium, and titrated in duplicates over a 64-fold range in 96-well plates. Infected erythrocytes (1.25% final hematocrit and 0.3% final parasitemia) were added into the wells. After 48 h incubation, [$^3$H]hypoxanthine (0.5 μCi) was added per well and plates were incubated for an additional 24 h. Parasites were harvested onto glass-fiber filters, and radioactivity was counted using a Betaplate liquid scintillation counter (Wallac, Zurich). The results were recorded and expressed as a percentage of the untreated controls. $IC_{50}$ values were estimated by linear interpolation.[19]

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**Supporting Information Available:** $^1$H, $^{13}$C, and 2D NMR spectra in $CD_3OD$ for lagunamides A (**1**) and B (**2**) and biological data. This material is available free of charge via the Internet at http://pubs.acs.org.

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