

Mitsoamide: A cytotoxic linear lipopeptide from the Madagascar marine cyanobacterium *Geitlerinema* sp.*

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Abstract: A new cytotoxic and linear peptide (IC₅₀ 460 nM to NCI-H460 human lung tumor cells) was isolated from the marine cyanobacterium *Geitlerinema* sp. The planar structure of mitsoamide was deduced by 1D and 2D NMR experiments in combination with MS analyses. The structure of mitsoamide contains an unusual polyketide unit (3,7-dimethoxy-5-methyl-nonanedioic acid, DMNA), incorporates a homolysine (HomoLys) residue and possesses a highly unusual piperidine aminal moiety. The configurations of the relatively common amino acids present in mitsoamide (Ala, Ile, *N*-Me-Ile, Phe, Val) were determined by chiral HPLC analysis of the acid hydrolysate.

Keywords: cyanobacteria; cytotoxins; lipopeptides; polyketides; marine.

INTRODUCTION

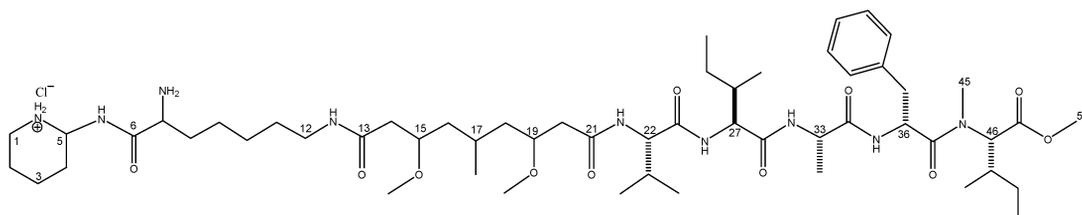
A number of the most therapeutically interesting natural products are mixed polyketide/nonribosomal peptides, such as bleomycin, the epothilones, FK506, and rapamycin [1–3]. The biosynthesis of these clinically important natural products is achieved through the action of two related enzyme families: the nonribosomal peptide synthetases (NRPSs) [1] and polyketide synthases (PKSs) [1,2]. These large, multimodular proteins are subdivided into discrete domains, each possessing a distinct catalytic function that contributes to the biosynthetic assembly line process.

Cyanobacteria are prolific producers of peptides that are commonly modified by the incorporation of polyketide portions to produce mixed peptide-polyketide hybrids [4]. These arise from the interdigitated functioning of type I NRPSs [5] and type I PKSs [6] as revealed by the growing number of sequenced biosynthetic gene clusters from this biological source which includes curacin A [7], barbamide [8], jamaicamide [9], lyngbyatoxin [10], microcystin [11], and cryptophycin [12]. In general, the metabolites of cyanobacteria constitute a highly diverse set of structures in which many novel biosynthetic transformations are revealed, including unusual halogenations, unsaturations, cyclizations, and addition of pendant carbon atoms to polyketide chains. As a result, many cyanobacterial natural products have unique and potent biological properties, including cancer cell toxicity through interaction with tubulin or actin [13,14], neurotoxicity via interference with normal ion channel function [15], and antifungal or other antimicrobial properties [16,17].

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As part of our effort to discover and characterize structurally novel and biologically interesting substances from marine cyanobacteria [18–20], we initiated a detailed examination of a Malagasy marine cyanobacterium, *Geitlerinema* sp., which showed potent brine shrimp toxicity in its crude organic extract (LC_{50} 0.01 $\mu\text{g}/\text{mL}$). Using this assay to guide the isolation of bioactive constituents, we obtained an amazing array of new and potently active molecules, including a small quantity of curacin A [21], a curacin A-related substance under continuing investigation, several new swinholide-related metabolites including ankarharolide A [14], and from quite a polar fraction, a new linear lipopeptide which we have named mitsoamide (**1**). This paper describes our isolation and structure elucidation of this latter metabolite which possesses several novel structural features including a homolysine (HomoLys) residue, a 3,7-dimethoxy-5-methyl-nonadioic acid residue, and a piperidine aminal, and was strongly cytotoxic to the H-460 human lung cancer cell line (IC_{50} 460 nM).



Structure of mitsoamide (**1**).

RESULTS AND DISCUSSION

The marine cyanobacterium *Geitlerinema* sp. was collected from Mitso-Ankara Island near NosyBe, Madagascar, extracted with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (2:1) and fractionated by Si VLC. The resulting fractions were tested for brine shrimp toxicity and revealed that the 50:50 EtOAc/MeOH eluting material was strongly active (63 % toxicity 1 ppm). This material was further purified on analytical reversed-phase high-performance liquid chromatography (RP-HPLC) to give a small quantity of mitsoamide (**1**, 3 mg, 0.3 % of extract; LC_{50} 450 nM to the brine shrimp).

Mitsoamide (**1**) was isolated as yellow oil with a molecular mass of 1093.6835 by HR +ESI TOF MS that indicated a formula of $\text{C}_{55}\text{H}_{96}\text{ClN}_9\text{O}_{11}$. This was consistent with the ion cluster at m/z 1093.7/1095.7 observed in both fast atom bombardment mass spectrometry (FABMS) and matrix-assisted laser desorption ionization with time-of-flight mass spectrometry (MALDI–TOFMS) experiments, which confirmed the presence of one chlorine atom. 1D NMR spectra exhibited resonances typical for a peptide natural product, including several exchangeable NH signals between 6–8 ppm, a number of resonances in the α -proton region, and a packet of carbonyl carbons around δ 170 (Table 1). Expanding outward from these NH and α -protons, standard analysis by ^1H - ^1H correlation spectroscopy (COSY), multiplicity edited heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) NMR, in combination with literature values, quickly revealed the side chains corresponding to alanine (Ala), valine (Val), two isoleucine (Ile) residues, and phenylalanine (Phe). However, one of the Ile residues showed no α -proton to NH correlation, and by HMBC, was shown to be *N*-methyl substituted (δ_{H} 2.99, δ_{C} 31.2). Interestingly, this latter residue was also shown by HMBC to possess a methyl ester (δ_{H} 3.64, δ_{C} 52.07), and thus *N,O*-dimethyl Ile formed the carboxy terminus of mitsoamide (**1**).

Table 1 NMR data for mitsosamide (1) in CD₃CN (400 MHz).

| Unit | Position | δ_{C} | δ_{H} (<i>J</i> in Hz) | HMBC ^a |
|---------|-------------------|---------------------|---------------------------------------|-------------------|
| Aminal | 1 | 47.31 | 3.67, m 3.15, m | 2,3,5 |
| | 2 | 24.96 | 1.90, m 1.76, m | 1 |
| | 3 | 18.58 | 1.21, m 1.20, m | 1 |
| | 4 | 29.41 | 2.07, m 1.86, m | 2,3,5 |
| | 5 5-NH | 59.38 | 4.27, dd (4.1,3.7) 7.19, d (4.1) | 1,2,3,4,6 6 |
| HomoLys | 6 | 172.02 | | |
| | 7 | 53.31 | 4.47, ddd (4.3,4.5,4.3) | 6,8,9 |
| | 7-NH ₂ | | 7.25, d (7.4) | 6 |
| | 8 | 32.10 | 1.87, m 1.62, m | 6 |
| | 9 | 23.04 | 1.36, m | 10 |
| | 10 | 18.60 | 1.40, m | 11 |
| | 11 | 29.17 | 1.46, m 1.31, m | 12 |
| DMNA | 12 | 38.96 | 3.13, m 3.01, m | 11 |
| | 12-NH | | 6.70, dd (6.1,4.4) | 13 |
| | 13 | 171.70 | | |
| DMNA | 14 | 41.16 | 2.35, m 2.23, m | 13,15,16 |
| | 15 | 78.40 | 3.59, m | 17 |
| | 15-OMe | 56.26 | 3.30, s | 15 |
| | 16 | 35.89 | 1.49, m 1.39, m | 14,15,17 |
| | 17 | 19.0 | 1.39, m | 16,18 |
| | 17-Me | 18.54 | 0.91, d (3.4) | 16,17,18 |
| | 18 | 36.18 | 1.49, m 1.39, m | 17,19,20 |
| | 19 | 78.41 | 3.59, m | 17 |
| | 19-OMe | 56.38 | 3.31, s | 19 |
| | 20 | 41.44 | 2.43, m 2.33, m | 18,19,21 |
| Val | 21 | 171.42 | | |
| | 21-NH | | 7.12, d (7.7) | 21 |
| | 22 | 54.80 | 4.61, dd (8,7.7) | 23,24,25,26 |
| | 23 | 32.04 | 2.07, m | 22,24,25 |
| | 24 | 13.92 | 0.82, d (6.3) | 22,23 |
| | 25 | 17.84 | 0.92, d (7.9) | 22,23 |
| | 26 | 169.95 | | |

(continues on next page)

Table 1 (Continued).

| Unit | Position | δ_{C} | δ_{H} (<i>J</i> in Hz) | HMBC ^a |
|---------------------|----------|---------------------|---------------------------------------|-------------------|
| Ile | 26-NH | | 6.86, d (7.4) | 26 |
| | 27 | 58.03 | 4.14, dd (7.4,7.1) | 28,29,30,32 |
| | 28 | 36.87 | 1.86, m | 27,29,30 |
| | 29 | 15.58 | 0.89, d (7.1) | 27,28,30 |
| | 30 | 24.74 | 1.36 | 28,31 |
| | | | 1.06 | |
| | 31 | 10.8 | 0.94, d (3.4) | 28,30 |
| | 32 | 171.38 | | |
| Ala | 32-NH | | 7.14, d (6.8) | 32 |
| | 33 | 49.42 | 4.29, d (6.8,6.2) | 34,35 |
| | 34 | 18.26 | 1.16, d (6.2) | 33,35 |
| | 35 | 172.16 | | |
| Phe | 35-NH | | 7.44, d (7.7) | 35 |
| | 36 | 52.5 | 4.90, dd (7.7,7.4) | 37,38,44 |
| | 37 | 38.44 | 3.01 | 36,38,39/40 |
| | | | 2.92 | |
| | 38 | 137 | | |
| | 39/40 | 130 | 7.25 | 37,38,41/42 |
| | 41/42 | 128.6 | 7.29 | 38,39/40,43 |
| | 43 | 127.1 | 7.22 | 41/42 |
| | 44 | 173.89 | | |
| <i>N</i> -Me-Ile-Me | 45 (NMe) | 31.20 | 2.99, s | 46 |
| | 46 | 61.74 | 4.63, d (10.7) | 47,51 |
| | 47 | 31.2 | 1.60, m | 46,48,49 |
| | 48 | 14.72 | 0.93, d (8) | 47 |
| | 49 | 26.46 | 1.50 | 47,50 |
| | | | 1.10 | |
| | 50 | 10.8 | 0.96, d (10) | 47,49 |
| | 51 | 170.49 | | |
| | 52 (OMe) | 52.07 | 3.64, s | 51 |

^aProton showing HMBC correlation to indicated carbon.

Mitsoamide (**1**) incorporated several unique structural features not previously or rarely observed in marine cyanobacterial natural products. A carbon at δ 47.31 with protons at δ 3.17 and δ 3.65 was characteristic of a methylene group adjacent to nitrogen in a cyclic or asymmetric system. Long-range ^1H - ^{13}C HMBC (various mixing times) and ^1H - ^1H COSY experiments showed a sequential series of four methylene groups (see Table 1), which then connected to a methine at δ 4.27 (δ 59.38). This latter signal was also coupled to a NH proton at δ 7.19. To unambiguously confirm this unusual spin system, a series of selective 1D total correlation spectroscopy (TOCSY) experiments were performed. Irradiation of the proton at H-1 α (δ 3.65) showed couplings to H-1 β (δ 3.17), H-2 α (δ 1.90), H-2 β (δ 1.76), H-3 α (δ 1.20), H-3 β (δ 1.21), H-4 α (δ 1.86), H-4 β (δ 2.07), and H-5 (δ 4.27). However, no coupling from the distal exchangeable NH proton was observed. Irradiation of the proton at H-5 (δ 4.27) displayed similar couplings as seen above but with additional coupling to the NH proton at δ 7.19. Hence, this methine was attached to both the nitrogen between it and the CH₂-1 methylene as well as the latter amide-type NH, defining an aminal functionality (Fig. 1). Additional confirmation of this unusual structural feature was gained by comparison with various reference compounds as well with calculated ^1H and ^{13}C shifts for mitsoamide (see Supporting Information) [22].

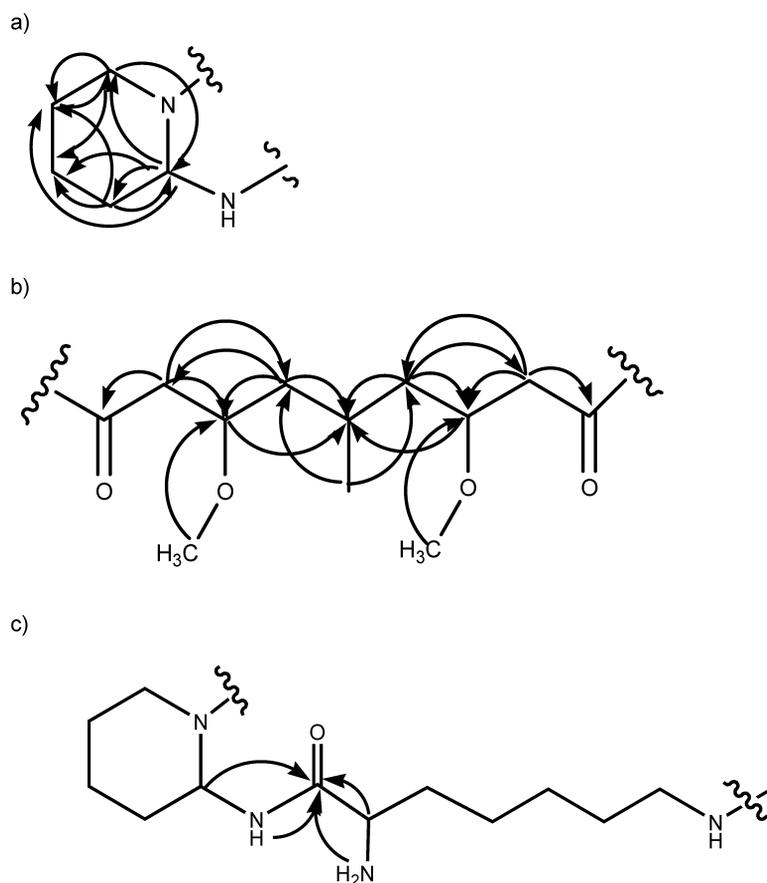


Fig. 1 Selected HMBC correlations of mitsosamide (**1**); (a) amino acid (piperidin-2-amine, PPA); (b) polyketide DMNA; (c) connection of PPA and HomoLys residues in **1**.

A second uncommon structural feature in mitsosamide (**1**) was the relatively unusual amino acid HomoLys. Initially, routine ^1H - ^{13}C HMBC and ^1H - ^1H COSY experiments were used to define a spin system that began with a deshielded methine at δ 4.47 and extended through five sequential methylene groups. Again, application of selective 1D TOCSY was very powerful in confirming this portion of the molecule. Irradiation of α -proton H-7 (δ 4.47) revealed couplings to exchangeable NH signals (δ 7.25), H-8 α (δ 1.87), H-8 β (δ 1.62), H₂-9 (δ 1.36), H₂-10 (δ 1.40), H-11 α (δ 1.46), H-11 β (δ 1.31), and H-12 α (δ 3.13). Irradiation of H-12 α (δ 3.13) displayed similar couplings as seen above but with the exclusion of the distal NH₂ (δ 7.25) and addition of the proximate NH (δ 6.70). Irradiation of H-10 (δ 1.40), showed similar couplings as seen above with the inclusion of both exchangeable NH signals (δ 7.25, 6.70). In complementary fashion, irradiation of H-11 α (δ 1.46) displayed all of the same couplings observed from irradiation of H-10 with the exception of the δ 7.25 NH protons. This composite of results established a 2,7-diamino substitution pattern and thus identified this residue as HomoLys (Fig. 2). HomoLys, while rare in natural products, has been previously characterized in several cyanobacterial peptides, including microcystin LR and nodularin-Har [23,24].

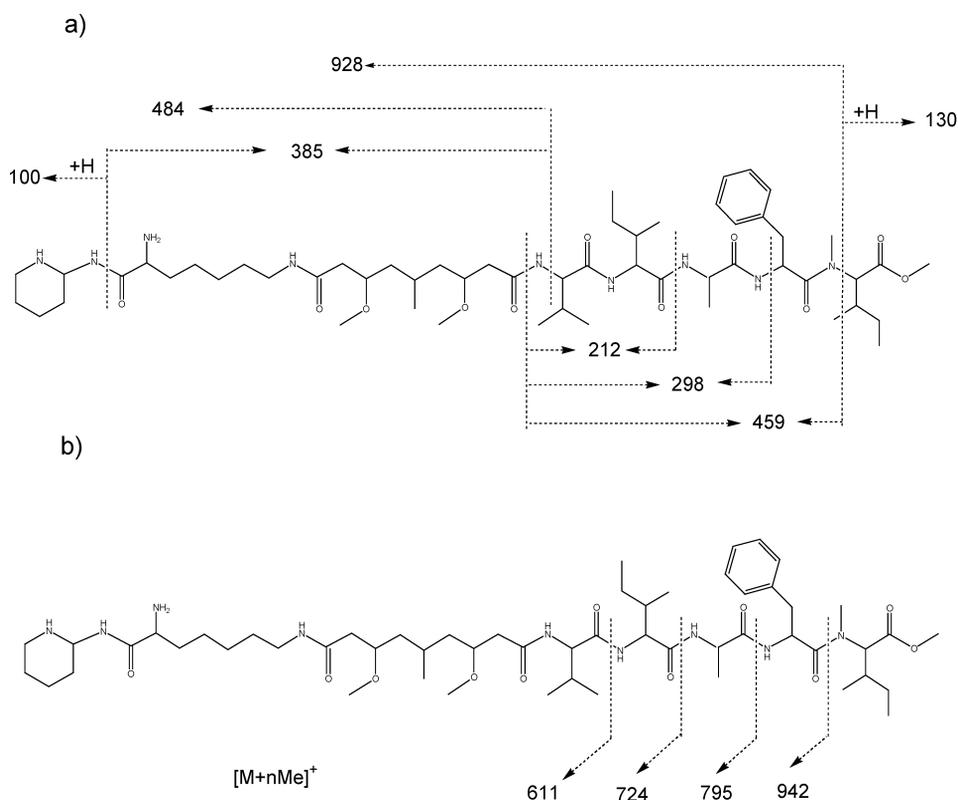


Fig. 2 Key fragments from (a) ESI+ TOF MS/MS and (b) FAB MS.

A final unusual section of mitsoamide (**1**) was established by ^1H - ^1H COSY, long-range ^{13}C - ^1H correlation experiments (HMBC) with different mixing times and selective 1D TOCSY. By ^{13}C NMR, two nearly isochronous methoxy groups (δ 56.26, 56.38), two oxygen-bearing methines (δ 78.40, 78.41), four methylenes (δ 35.89, 36.18, 41.16, 41.44), one methyl (δ 18.54), and one high-field methine (δ 19.0) were unaccounted for in **1**, and hence, likely formed a polyketide fragment. HMBC correlations revealed that the methyl group (δ 18.54) was attached to the high-field methine at δ 19.0, which in turn was sequentially located between two methylenes (δ 35.89, 36.18) and the two methoxy methines. These mid-field methines were in turn adjacent to two similarly shifted methylenes, δ 2.23/2.35 and δ 2.33/2.43 with attached carbons at δ 41.16 and δ 41.44. From chemical shift reasoning, these latter methylenes were likely adjacent to carbonyl functionalities; HMBC correlations from δ 2.23/2.35 to δ 171.70 and δ 2.33/2.43 to δ 171.42 confirmed this deduction. By 1D TOCSY, irradiation of the H-20 α proton (δ 2.33) resulted in couplings to protons at H-20 β , H-19, H₂-18, H-17, and H₃-17-Me. Thus, the final remaining residue in mitsoamide A (**1**) was the essentially symmetric polyketide 3,7-dimethoxy-5-methyl-nonanedioic acid) (DMNA, Fig. 1b).

The above spectroscopic reasoning defined eight residues in mitsoamide: Ala, Val, Ile, Phe, *N,O*-dimethylisoleucine (diMeIle), piperidin-2-amine (PPA), HomoLys, and the polyketide DMNA. By comparing the carbon, proton, nitrogen, and oxygen count from these eight residues, $\text{C}_{55}\text{H}_{94}\text{N}_9\text{O}_{11}$, to the molecular formula established by HRMS, $\text{C}_{55}\text{H}_{96}\text{ClN}_9\text{O}_{11}$, the remaining unassigned atoms were two protons and one chlorine. Hence, on the basis of the observed chemical shifts for **1**, mitsoamide was a hydrochloride salt, likely present at the basic aminal nitrogen. Indeed, a similar hydrochloride salt was observed for the aminal portion of siastatin B [22].

Long-range ^1H - ^{13}C correlation experiments (HMBC) with different mixing times were used in combination with ESI-MS/MS fragmentation patterns to define the sequence of residues in mitsamide (**1**). Connection between the PPA and HomoLys residues was made by observing long-range ^1H - ^{13}C correlations between H-5, NH-5, and H-7 with the C-6 carbonyl at δ 172.02. Similarly, H-14 α , H-12 α , and NH-12 all showed correlations to the C-13 carbonyl (δ 171.70), thus connecting the HomoLys and DMNA units. These connectivities were reinforced by ESI-MS/MS fragment ions at m/z 385 for HomoLys-DMNA-NH and m/z 484 for PPA-HomoLys-DMNA-NH. Additional fragment ion masses at m/z 942, 975, 724, and 611, resulting from consecutive Phe, Ala, and Ile cleavages, indicated an *N,O*-diMeIle-Phe-Ala-Ile sequence at the carboxy terminus. In turn, ions at m/z 212 (Val-Ile) $^+$, 298 (Val-Ile-Ala-NH) $^+$, and 459 (Val-Ile-Ala-Phe-NMe) $^+$ identified that Val was attached to the Ile residue to extend this sequence to *N,O*-diMeIle-Phe-Ala-Ile-Val. The Phe-Ala-Ile-Val sequence was confirmed by sequential HMBC correlations from each NH to the preceding carbonyl (Table 1). Combination of these two sequences, PPA-HomoLys-DMNA plus Val-Ile-Ala-Phe-*N,O*-diMeIle, along with the hydrochloride salt feature of the PPA residue, completed the planar structure of mitsamide **1**.

The absolute configurations of the common amino acids in **1** were established by chiral HPLC in comparison with authentic standards following hydrolysis with 6 N HCl. All of the amino acids were shown to possess the L-configuration except Phe which was found to have the D-configuration (Supporting Information). Assignment of the remaining five stereocenters in **1** (C-5, C-7, C-15, C-17, C-19) was precluded by decomposition of some of these constituents during acid hydrolysis of **1** (e.g., the PPA residue), lack of the appropriate reference standards (e.g., the DMNA unit), and the relatively small quantity of mitsamide isolated.

Mitsamide (**1**) was evaluated for cytotoxicity to the NCI-H460 human lung tumor cell line, and showed an LC₅₀ of 460 nM, very similar to the potency observed in the brine shrimp toxicity assay. While mitsamide's structure is generally consistent with other cyanobacterial natural products in that it is composed of polyketide and amino acid subunits, there are several unique structural units and arrangements never or rarely seen previously. The central polyketide section possesses amide linkages to amino acids at both termini, and is thus inconsistent with established pathways for mixed NRPS/PKS pathways. It is conceivable that mitsamide biosynthesis begins with this polyketide section, perhaps a pentaketide that is truncated by one carbon atom or a tetraketide wherein the initiating unit is malonate rather than acetate, which then transitions to a series of NRPS modules to produce the pentapeptide (Val, Ile, Ala, Phe, and Ile) section. The pattern of oxidation in the polyketide section (methoxy groups at C-15 and C-19) suggests that C-17 also derives from C-1 of acetate, and hence, the methyl group attached at this position likely derives from C-2 of acetate as described for curacin A^{7,25} and jamaicamide A [9]. An epimerase domain should be present in the penultimate NRPS module, converting L-Phe to D-Phe during the biosynthetic assembly process, and an N-methyl transferase must be associated with the terminating module to create N-methyl Ile. Cleavage of the mixed PKS/NRPS product from the assembly enzyme might occur similarly to that of melithiazol to directly produce the terminal carbomethoxy ester. This hypothesis would implicate the presence of another NRPS module to incorporate Ala (e.g., a hexapeptide is initially formed) which is then truncated to form the methyl ester of the preceding residue [26]. A subsequent condensation between the zeta amino function of the unusual dipeptide, HomoLys plus piperidine aminal, would complete the biosynthesis of mitsamide (**1**).

GENERAL EXPERIMENTAL PROCEDURES

Optical rotations were measured on a Perkin Elmer 243 polarimeter, and UV and FT-IR spectra were obtained employing Hewlett Packard 8452A and Nicolet 510 instruments, respectively. All NMR spectra were recorded on Bruker Avance DRX300, DPX400, and DRX600 spectrometers with spectra referenced to residual solvent signal of CH₃CN ($\delta_{\text{H/C}}$ 1.94/118.69). LR FAB mass spectra were acquired on a Kratos MS50TC instrument. MALDI-TOF and TOF-MS-ES data were recorded on Applied

Biosystems 4700 TOF-TOF and Waters Micromass LCT Classic mass spectrometers, respectively. HPLC was carried out using a Waters system consisting of a Rheodyne 7725i injector, two 515 pumps, a pump control module, and a 996 photodiode array detector. All solvents were purchased as HPLC grade. Analytical chiral HPLC analyses were performed on CHIRIOBIOTIC T (Teicoplanin) – 10 μm (size 4.6×100 mm) column.

COLLECTION, EXTRACTION, AND ISOLATION PROCEDURES

The marine cyanobacterium *Geitlerinema* cf. sp. (voucher number MMA-18Apr00-02) was collected in April 2000 from Nosy Mitso-Ankaraha Island, NosyBe, Madagascar from a depth of 16 m by SCUBA. After collection, the microalga was stored in 2-propanol at -20 °C until workup. A total of 164.0 g (dry wt) of the cyanobacterium was extracted repeatedly with CH_2Cl_2 -MeOH (2:1, v/v) to produce 1.0 g of crude organic extract. The crude extract was found to be active at 10 ppm (90 % mortality) in the brine shrimp toxicity assay. A portion of the crude extract (950 mg) was fractionated using VLC on Si gel with a stepwise gradient of hexanes-EtOAc and EtOAc-MeOH to give 13 fractions. Fraction 9 (eluting at 50:50 EtOAc/MeOH) showed a cytotoxic effect (63 % toxicity at 1 ppm to brine shrimp) and was further purified on analytical RP-HPLC [Phenomenex Spherclone ODS 5 μm , 250×10.0 mm, MeOH/ H_2O (90:10)] to yield 3 mg of **1**.

Amino acid analysis of 1. Mitsoamide (**1**, 850 μg) was hydrolyzed in 6 N HCl at 105 °C for 19 h, and dried under a stream of N_2 . The residue was reconstituted with 510 μL of H_2O and 200 μL EtOH prior to chiral HPLC analysis [Chiral Chirobiotic T (Teicoplanin) column, 4.6×250 mm; UV detection at 215 and 254 nm], using mobile phase I, 70/30 H_2O /EtOH, flow rate 0.5 mL/min; mobile phase II, 50/50 H_2O /EtOH, flow rate 1 mL/min; mobile phase III, 90/10 EtOH/ H_2O , flow rate 1 mL/min; mobile phase IV, 85/15 EtOH/ H_2O , flow rate 1 mL/min; mobile phase V, 80/20 H_2O /EtOH, flow rate 0.7 mL/min. Elution times of authentic standards in mobile phase I (t_{R} , min): L-Phe (3.84), D-Phe (3.15), mobile phase II (t_{R} , min): L-Ala (3.25), D-Ala (2.83), mobile phase III (t_{R} , min): L-Val (5.90), D-Val (8.50), mobile phase IV (t_{R} , min): L-Ile (5.58), D-Ile (10), mobile phase V (t_{R} , min): L-*N,O*-diMeIle (7.8), D-*N,O*-diMeIle (6.8). The hydrolysate of **1** was chromatographed alone and co-injected with standards to confirm assignments of L-Val, L-Ile, L-Ala, and D-Phe (Supporting Information). The presence of L-*N,O*-diMeIle was confirmed by methylation of standard D- and L-*N*-MeIle with diazomethane and comparison with the hydrolysate. Because hypermethylation of the standards could occur, the hydrolysate of mitsoamide was also treated with diazomethane before this latter analysis.

CYTOTOXICITY ASSAY

Cytotoxicity was measured in NCI-H460 lung tumor cells using the method of Alley et al. [27] with cell viability being determined by MTT reduction [28]. Cells were seeded in 96-well plates at 6000 cells/well in 180 μl . Twenty-four hours later, the test chemical dissolved in DMSO and diluted into medium without fetal bovine serum was added at 20 μl /well DMSO was less than 0.5 % final concentration. After 48 hr, the medium was removed and cell viability determined.

Mitsoamide (1): $[\alpha]_{\text{D}}^{24} + 17^\circ$ (*c* 1, CHCl_3); UV (CHCl_3) λ_{max} 206 nm ($\log \epsilon$ 3); IR ν_{max} (neat) 2925, 2861, 1716, 1694, 1540, 1463, 1370, 1186, 1077, 995 cm^{-1} ; ^1H NMR (CD_3CN , 400 MHz) and ^{13}C NMR (CD_3CN , 100 MHz), see Table 1; LR FABMS (nba matrix) m/z $[\text{M} + \text{HCl}]^+$ 1093.7, m/z $[\text{M}]^+$ 1057.7 [29]; MALDI-TOF m/z $[\text{M} + \text{HCl}]^+$ 1093.7 [29]; HR ESI+ TOF MS m/z $[\text{M} + \text{HCl}]^+$ 1093.6835 (calc. for $\text{C}_{55}\text{H}_{96}\text{ClN}_9\text{O}_{11}$, 1093.6918).

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SUPPORTING INFORMATION

^1H NMR, ^{13}C NMR, ^1H - ^1H COSY, HSQC, HMBC, 1D TOCSY, and MS of compound **1**, and chiral HPLC of the hydrolysis products of compound **1**. This material is available free of charge via the Internet at <<http://pubs.acs.org>>.

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