Depsipeptide Companeramides from a Panamanian Marine Cyanobacterium Associated with the Coibamide Producer

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S Supporting Information

ABSTRACT: Two new cyclic depsipeptides, companeramides A (1) and B (2), have been isolated from the phylogenetically characterized cyanobacterial collection that yielded the previously reported cancer cell toxin coibamide A (collected from Coiba Island, Panama). The planar structures of the companeramides, which contain 3-amino-2-methyl-7-octynoic acid (Amoya), hydroxy isovaleric acid (Hiva), and eight α-amino acid units, were established by NMR spectroscopy and mass spectrometry. The absolute configuration of each companeramide was assigned using a combination of Marfey’s methodology and chiral-phase HPLC analysis of complete and partial hydrolysis products compared to commercial and synthesized standards. Companeramides A (1) and B (2) showed high nanomolar in vitro antiplasmodial activity but were not overtly cytotoxic to four human cancer cell lines at the doses tested.

Cyanobacteria are well known as sources of cytotoxins and have yielded medicinally relevant metabolites with antiproliferative and neurologically active properties. They are also known as sources of cytotoxins and have yielded medicinally relevant metabolites with antiproliferative and neurologically active properties. One example of such a metabolite is coibamide A, isolated from a Panamanian marine cyanobacterium. This metabolite was shown to have antiproliferative and neurologically active properties. The study also highlighted the importance of understanding the structural complexity of these metabolites.

The chemical investigation of previously unexplored marine cyanobacteria in Coiba National Park, Panama, is ongoing and to date has yielded the selective HDAC inhibitory santacrucamides from a cf. Sympleca species, the polyketide δ-lactone coibacins from a cf. Oscillatoria species, which exhibit selective antileishmanial activity, and the potent cancer cell toxin coibamide A. The latter antiproliferative depsipeptide was reported previously from a field collection of filamentous cyanobacteria identified morphologically as comprising primarily a Leptolyngbya species, although the major organism identified by phylogenetic analysis (16S rDNA) in the field collection was more similar to the Sympleca type strains (PAC-10-3, GenBank KC207936) and has been proposed to belong to a new genus. Collections of the same cyanobacterial assemblage have consistently provided relatively high yields of the two new cyclic depsipeptides, companeramides A (1) and B (2), so named to convey their repeated isolation as companion (Spanish “compañera”) compounds to coibamide, while avoiding their association with a cyanobacterial genus name that may be subject to change. Companeramides A (1) and B (2) show differential activity against chloroquine-sensitive and
A marine cyanobacterial assemblage comprising a small filament *Leptolyngbya* species, based on morphological analysis, was collected in 2004 by hand using scuba from a reef pinnacle in Coiba National Park, Panama. The alcohol-preserved tissue was extracted with CH$_2$Cl$_2$−MeOH, and the extract fractionated by normal-phase silica gel vacuum liquid chromatography (NP-VLC). In preliminary biological activity profiling, the 100% EtOAc fraction was cytotoxic to NCI-H460 human lung tumor cells with an IC$_{50}$ of 300 ng/mL and also showed preliminary activity in the ICBG panel of antiparasitic assays: malaria (IC$_{50}$ 6 μg/mL), American trypanosomiasis (IC$_{50}$ > 50 μg/mL). RP$_{18}$ solid-phase extraction (SPE), HPLC, and further cytotoxicity testing of this fraction resulted in the discovery of the potent antiproliferative metabolite coibamide A (Table 1). The 1H NMR spectrum for 1 displayed nine distinguishable signals for ester/amide-type carbonyls (δ$_C$ 169.4−174.4), one signal for an oxygenated sp$^3$-hybridized carbon (δ$_C$ 75.4), and two signals at δ$_C$ 83.8 and 68.6, diagnostic of a terminal acetylenic moiety (Table 1). The HMBC spectrum displayed prominent three-bond HMBC correlations from the four N-methyl singlets, permitting N-methyl values (N-Me-Val), one N-methyl leucine (N-Me-Leu), and one N-methyl alanine (N-Me-Ala) residue. Further analysis of the COSY, HSQC, HSQC-TOCSY, and HMBC experiments identified the regular amino acids alanine (Ala), proline (Pro), and two isoleucines (Ile), as well as hydroxyisovaleric acid (Hiva) (Table S1, Supporting Information). The molecular formula for 1 dictated a tenth residue comprising C$_{57}$H$_{97}$O$_{11}$N$_9$. COSY and TOCSY NMR experiments were used to identify a spin system in which a methine doublet of quartets (δ$_H$ 2.51, H-2) was correlated to both a methyl doublet (δ$_H$ 1.31, H$_2$-9) and a second downfield methine multiplet (δ$_H$ 3.97, H-3). This H-3 multiplet was in turn relay-coupled to the signals for three contiguous methylenes (H$_2$-4 to H$_2$-6) and also to an NH doublet at δ$_H$ 6.75 (NH-3; Figure 1). HMBC correlations from the distal methylene 1H signal (H$_2$-6, δ$_H$ 2.17) to nonprotonated sp$^3$-hybridized C-7 (δ$_C$ 83.8) and methine C-8 (δ$_C$ 68.6) signals completed the hydrocarbon chain with a terminal acetylene. Finally, three-bond HMBC correlations from the H$_2$-9 doublet to methine C-3 (δ$_C$ 51.2) and the C-1 carbonyl signal (δ$_C$ 174.3) aided in the establishment of this α-methyl-β-amino acid as 3-amino-2-methyl-7-octynoic acid (Amoya).

Despite several closely overlapping α-proton signals (H-17, H-32, H-38, H-44) correlated to overlapped carbonyl signals (C-10, C-31, and C-49) in the HMBC spectrum for companionamide A (1), all nine amino and one hydroxy acid subunits were sequenced from HMBC and ROESY data collected at 700 MHz. Two obvious fragments consisting of N-Me-Val−Ala−N-Me-Leu−Pro (fragment 1) and Ile−N-Me-Val−2−Ile−N-Me-Ala−Hiva (fragment 2) were assembled by HMBC correlations between N-methyl, amide, and/or α-H and the carbonyl 13C signals for neighboring residues (Figure 1 and Table S1, Supporting Information). At the N-terminus of fragment 2, the ester linkage between Hiva and the Amoya residue was defined by HMBC correlations from the Hiva α-H...
### Table 1. $^1$H and $^{13}$C NMR Data for Companeramides A (1) and B (2) in CDCl$_3$ (700 MHz)

| unit            | position | $\delta_H$ (J, Hz) | $\delta_C$ | unit            | position | $\delta_H$ (J, Hz) | $\delta_C$ |
|-----------------|----------|--------------------|-----------|-----------------|----------|--------------------|-----------|
| AMOYA           | 1        | 174.3, C           |           | AMOYA           | 1        | 175.4, C           |           |
|                 | 2        | 2.51, dq (7.2, 7.2) | 46.4, CH  |                 | 2        | 2.63, m            | 46.0, CH  |
|                 | 3        | 3.97, m            | 51.2, CH  |                 | 3        | 3.81, m            | 52.4, CH  |
| 3-NH            | 4        | 6.75, br d (6.5)   | 3-NH      | 4                | 7.44, d (6.0) |           |           |
|                 | 5        | 1.71, obs          | 31.3, CH$_3$ | 5                | 1.93, m | 30.2, CH$_3$       |           |
|                 | 6        | 1.63, obs          |           |                 | 1.73, m |           |           |
|                 | 7        | 1.54, m            | 25.2, CH$_2$ | 7                | 1.53, m | 25.5, CH$_2$       |           |
|                 | 8        | 1.48, obs          |           |                 | 1.45, m |           |           |
| N-Me-Val-1      | 10       | 170.1, C           |           | N-Me-Val-1      | 10       | 170.2, C           |           |
|                 | 11       | 4.58, d (11.2)     | 62.8, CH  | 11               | 4.72, d (11.2) | 62.4, CH  |           |
|                 | 12       | 2.29, obs          | 25.6, CH  | 12               | 2.27, m | 26.5, CH           |           |
|                 | 13       | 0.95, d (6.4)      | 19.4, CH$_3$ | 13               | 0.96, obs | 19.9, CH$_3$       |           |
|                 | 14       | 0.88, d (6.7)      | 18.4, CH$_3$ | 14               | 0.81, obs | 18.5, CH$_3$       |           |
|                 | 15       | 3.14, s            | 30.2, CH$_3$ | 15               | 3.23, s | 30.9, CH$_3$       |           |
| Ala             | 16       | 174.4, C           |           | Val-1           | 16       | 173.4, C           |           |
|                 | 17       | 4.72, obs          | 46.2, CH  | 17               | 4.58, dd (8.3, 7.8) | 55.5, CH  |           |
|                 | 18       | 8.86, d (6.3)      | 164.4, CH | 18               | 8.84, d (7.8) |           |           |
| N-Me-Leu        | 19       | 4.67, dd (9.7, 4.9)| 58.6, CH  | 19               | 0.91, obs | 18.8, CH$_3$       |           |
|                 | 20       | 1.83, ddd (14.5, 8.5, 4.9) | 36.7, CH$_3$ | 20               | 0.93, obs | 18.7, CH$_3$       |           |
|                 | 21       | 1.59, m            |           | N-Me-Ala-1      | 21       | 169.8, C           |           |
|                 | 22       | 1.41, obs          | 24.2, CH  | 22               | 4.96, q (6.8) | 56.1, CH  |           |
|                 | 23       | 0.92, d (6.6)      | 22.2, CH$_3$ | 23               | 1.32, d (6.8) | 15.3, CH$_3$       |           |
|                 | 24       | 0.91, d (6.8)      | 24.3, CH$_3$ | 24               | 2.74, s | 29.1, CH$_3$       |           |
|                 | 25       | 2.69, s            | 28.8, CH$_3$ |                |         |                   |           |
| Pro             | 26       | 172.4, C           |           | Pro             | 26       | 4.83, obs          | 55.4, CH  |
|                 | 27       | 4.92, ddd (7.4, 6.4) | 55.4, CH  | 27               | 2.10, m | 29.8, CH$_3$       |           |
|                 | 28       | 2.11, m            | 29.6, CH$_3$ | 28               | 2.29, m | 25.6, CH$_3$       |           |
|                 | 29       | 2.00, m            | 1.97, m   | 29               | 3.83, m | 48.0, CH$_3$       |           |
|                 | 30       | 1.94, m            | 3.72, m   | 30               | 3.20, m | 170.2, C           |           |
| Ile-1           | 31       | 3.76, dt (9.9, 7.5)| 170.0, C  | 31               | 4.76, m | 54.4, CH           |           |
|                 | 32       | 4.74, ddd (8.9, 6.9)| 54.0, CH  | 32               | 7.48, d (8.5) |           |           |
|                 | 33       | 6.91, d (8.9)      | 1.70, obs | 33               | 0.93, obs | 15.5, CH$_3$       |           |
|                 | 34       | 1.69, obs          | 38.6, CH  | 34               | 1.39, m | 24.7, CH$_2$       |           |
|                 | 35       | 0.92, d (6.5)      | 15.4, CH$_3$ | 35               | 1.06, m | 11.7, CH$_3$       |           |
|                 | 36       | 3.19, m            | 24.3, CH$_2$ | 36               | 0.87, obs | 11.7, CH$_3$       |           |
|                 | 37       | 1.06, obs          |           | N-Me-Val-2      | 37       | 169.3, C           |           |
|                 | 38       | 0.83, ddd (7.5, 4.8) | 11.0, CH$_3$ | 38               | 4.90, d (11.1) | 62.0, CH  |           |
|                 | 39       | 4.71, obs          | 61.7, CH  | 39               | 2.33, m | 25.8, CH           |           |
|                 | 40       | 2.27, obs          | 25.7, CH  | 40               | 0.98, obs | 19.9, CH$_3$       |           |
|                 | 41       | 0.94, d (6.3)      | 19.9, CH$_3$ | 41               | 0.78, d (6.5) | 18.7, CH$_3$       |           |
|                 | 42       | 0.77, d (6.8)      | 17.9, CH$_3$ | 42               | 3.06, s | 30.8, CH$_3$       |           |
|                 | 43       | 3.06, s            | 30.5, CH$_3$ |                |         |                   |           |
|                 | 44       | 172.3, C           |           | Val-2           | 44       | 172.4, C           |           |
|                 | 45       | 4.72, obs          | 53.0, C   | 45               | 6.80, d (9.3) | 54.7, CH  |           |
|                 | 46       | 6.92, d (8.8)      | 172.3, C  | 46               | 2.00, m | 31.1, CH           |           |
|                 | 47       | 1.74, obs          | 37.3, CH  | 47               | 0.93, obs | 19.2, CH$_3$       |           |
|                 | 48       | 0.84, d (6.7)      | 15.0, CH$_3$ | 48               | 0.91, obs | 18.4, CH$_3$       |           |
Table 1. continued

| unit | position | \( \delta_{\text{H}} \) mult. (J, Hz) | \( \delta_{\text{C}} \) mult. | unit | position | \( \delta_{\text{H}} \) mult. (J, Hz) | \( \delta_{\text{C}} \) mult. |
|------|----------|-------------------------------|-----------------|      |----------|-------------------------------|-----------------|
| 47   | 1.48, obs| 23.4, CH\(_3\)                  |                 | N-Me-Ala-2 | 47 | 5.40, q (7.0) | 52.2, CH |
|      | 1.07, obs|                                |                 |            | 48 | 1.35, obs   | 13.8, CH\(_3\) |
| 48   | 0.82, dd (7.4, 5.0) | 11.0, CH\(_3\)                  |                 |            | 50 | 2.89, s     | 30.7, CH\(_3\) |
| N-Me-Ala | 49 | 170.1, C                  |                 |            | 50 | 3.23, 3.06, 2.89, 2.74 |                  |
| 50   | 5.26, q (7.3) | 51.7, CH                  |                 |            | 51 | 1.31, d (7.3) | 13.5, CH\(_3\) |
| 51   | 1.31, d (7.3) | 13.5, CH\(_3\)                  |                 |            | 52 | 2.92, s     | 30.5, CH\(_3\) |
| 52   | 2.92, s     | 30.5, CH\(_3\)                  |                 |            | 52 | 4.80, d (7.7) | 75.5, CH |
| Hiva | 53 | 171.1, C                  |                 |            | 53 | 2.19, m     | 30.1, CH |
| 54   | 4.80, d (8.3) | 75.4, CH                  |                 |            | 54 | 1.08, d (6.9) | 18.2, CH\(_3\) |
| 55   | 2.19, obs   | 30.1, CH                  |                 |            | 55 | 1.00, d (6.5) | 18.2, CH\(_3\) |
| 56   | 1.04, d (6.6) | 18.6, CH\(_3\)                  |                 |            |      |                |                 |
| 57   | 0.93, d (5.7) | 17.8, CH\(_3\)                  |                 |            |      |                |                 |

Figure 1. Key 2D NMR correlations for (A) companeramide A (1) and (B) companeramide B (2).
cally logical. HMBC and ROESY correlations supported an extended fragment 2 for 2, in which the Ile present in 1 was substituted for a Val, providing a sequence of Ile-1-N-Me-Val-2-Val-2-N-Me-Ala-Hiva. Again, the two fragments could be connected by a ROESY correlation between the Pro methylene (H_2-29) signal and Ile α-H-31 signal in combination with a HMBC correlation from the Pro α-H-26 signal (δ_H 4.83) to the overlapped Ile carbonyl C-30 signal (δ_C 170.2). Finally, the macrocycle could be closed by a ROESY correlation between the signals at δ_H 7.44 (AmoYA NH-3) and 4.72 (α-H-11) together with an HMBC correlation from the NH-3 doublet to the C-10 carbonyl signal (δ_C 170.2) of N-Me-Val-1. The resulting planar structure of companeramide B (2, Figure 1B) was again supported by MS/MS data (Figure S16, Supporting Information).

The absolute configurations of the amino and hydroxy acid units in companeramides A (1) and B (2) were determined by both Marfey’s methodology and direct chiral-phase HPLC analyses of acid hydrolysates (0.5 mg, 6 N HCl, 110 °C, overnight). For each natural product, a portion (ca. 0.25 mg) of the acid hydrolysate was derivatized with N-α-(2,4-dinitro-5-fluorophenyl)-l-α-aminolactide (l-FDLA, Marfey’s reagent) and analyzed by comparative RP-HPLC with FDAA-derivatized D- and L-amino acid standards. The additional 0.25 mg of each natural product acid hydrolysate was reconstituted with H_2O, analyzed by chiral-phase HPLC, and compared with the retention times of authentic R- and S-Hiva standards. For companeramide A (1), an l-configuration for Ala, N-Me-Ala, Pro, and both Ile, N-Me-Leu, and N-Me-Val residues, as well as S-Hiva, was established. The hydrolysate from companeramide B (2) contained l-Pro, two l-N-Me-Val, two l-Val, l-Ile, and both d- and l-N-Me-Ala, as well as S-Hiva. In order to correctly assign the relative position of the d- and l-N-Me-Ala residues in companeramide B (2), a partial hydrolysis and purification of resulting fragments containing N-Me-Ala was attempted, for subsequent complete hydrolysis and Marfey’s analyses. To this end, 2 (4 mg) was partially hydrolyzed with 3 N HCl, and the product mixture analyzed by LC-MS to identify fragments containing a single N-Me-Ala residue. A tetrapeptide fragment (m/z 413.9) identified as N-Me-Val-Val-N-Me-Ala-Pro from MS/MS data (Figure S17, Supporting Information) was purified from the product mixture and subjected to complete hydrolysis and derivatization with N-α-(2,4-dinitro-5-fluorophenyl)-l-leucinolactide (l-FDLA, advanced Marfey’s reagent) for analysis. The retention times for the derivatized amino acids from the latter (tetrapeptide fragment 1 of 2) corresponded to FDAA-derivatized standards for N-Me-l-Val, l-Val, N-Me-d-Ala, and l-Pro. No other partial hydrolysis fragments could be isolated in reasonable amount for Marfey’s analysis; however, assignment of the N-Me-d-Ala in the northern hemisphere (fragment 1) of 2 led N-Me-l-Ala to be assigned in the southern hemisphere (fragment 2) of 2.

The β-amino acid AmoYA present in companeramides A (1) and B (2) was first identified in the molluscan metabolite ochinidin, but has since been reported as a component of several marine cyanobacterial metabolites including ulongapeptin, guineamide C, and malevamide C. While the absolute configurations of the Amoya residues in guineamide C and malevamide C have not been determined, those in ulongapeptin were assigned as 2S, 3S by Marfey’s analysis using comparison with a synthetic mixture of C-2 diastereomers (2S, 3R and 2R, 3R) derivatized with both d- and l-FDLA enantiomers. The (2S,3S)-configuration was also assigned to the Amoya unit in ochinidin based on NOE studies and 1H NMR coupling constant analysis; however, the total synthesis of ochinidin suggested that a revision of the structure was necessary. Therefore, it was planned to use Marfey’s analysis for assignment of the Amoya unit in companeramides A and B, facilitated by the availability of the synthetic 3(R)-amino-2(RS)-methyloctanoate (AmoYA) standards.

Two portions of the synthetic material were derivatized separately with d- or l-FDLA to provide HPLC retention times for all four possible Amo diastereomers, with retention times of l-FDLA-(2R,3S)-Amo and l-FDLA-(2S,3S)-Amo being inferred from the retention times of the enantiomeric d-FDLA-(2S,3R)-Amo and d-FDLA-(2R,3R)-Amo standards, respectively. Hydrogenation of each companeramide to reduce the terminal alkyne was followed by acid hydrolysis to release Amo for separate derivatization with d- and l-FDLA. Reported retention times for the four Amo diastereomers using similar HPLC conditions were used to assign the order of elution of the pairs of standards generated in our protocol. For each companeramide, the d-FDLA-Amo product coeluted with the d-FDLA-derivatized (2S,3R)-Amo standard, and similarly the l-FDLA-Amo product coeluted with the l-FDLA-derivatized (2S,3S)-Amo, supporting a 2S,3R-AmoYA unit in companeramides A (1) and B (2).

Companeramides A (1) and B (2) showed no significant cytotoxicity at 1 μM against four human cancer cell lines (NCI-H460 non-small-cell lung carcinoma, MDA-MB-231 breast adenocarcinoma, SF-295 glioblastoma, and SK-OV3 ovarian carcinoma cells). Instead preliminary antiparasitic activity of the parent fractions led to testing of the two pure compounds against three strains of the malaria parasite *Plasmodium falciparum* in a fluorescence-based assay. Neither compound was as active as the chloroquine control against the chloroquine-sensitive D6 or chloroquine-insensitive Dd2 and 7G8 strains (Table 2, Figure S18, Supporting Information).

**Table 2. Antiplasmodial Activities for 1 and 2 Compared to Chloroquine against Chloroquine-Sensitive (D6) and Chloroquine-Resistant (Dd2 and 7G8) Strains of Plasmodium falciparum**

| test compound | IC_{50} (nM) against plasmodial strain |
|---------------|--------------------------------------|
|                | D6        | Dd2       | 7G8       |
| companeramide A (1) | 570       | 1000      | 1100      |
| companeramide B (2) | 220       | 230       | 700       |
| chloroquine   | 5         | 80        | 71        |

However, the differential activity between the two companeramides across all strains is noteworthy considering their structural similarity. The chloroquine-sensitive D6 strain was approximately twice as sensitive to companeramide A (1) as the chloroquine-resistant Dd2 and 7G8 strains. In contrast, companeramide B (2) showed comparable activity against the chloroquine-sensitive D6 and chloroquine-resistant strain Dd2, but was about three times less active against the chloroquine-resistant 7G8 strain.

Companeramides add to the growing repertoire of cyanobacterial depsipeptides that show some degree of antiplasmodial activity, including dolastatin 10, the venturamides, carbens and dragonamides, and symplastin A. While some of these compounds (e.g., dolastatin 10) are potently toxic to mammalian cells as well as to the malarial parasite, others such as the venturamides and carbenin A show relatively little
toxicity to mammalian cells. At the concentrations tested, it is not possible to distinguish any selective antiplasmodial activity for companionamide A (1), although the results for companionamide B (2) suggest potential antiplasmodial selectivity. The requisite testing of 2 for mammalian cell toxicity at higher micromolar concentrations was not pursued in the interest of saving material for other assays in which the target activity may be more pronounced than the moderate antiplasmodial activity presented here. It is interesting to speculate that related large cyclic alkyne depsipeptides that are reported to be nontoxic, such as the malevamides, 18 may possess antiparasitic activity. Noteworthy also is that despite the structural relationship between the companionamides and ulongapeptin, only the heptameric ulongapeptin displays nanomolar cytotoxicity. 16

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1010 polarimeter. UV and IR data were obtained on a Hitachi U-2000 spectrophotometer and a Nicolet IR100 FT-IR instrument, respectively. NMR spectra were acquired on Bruker Avance 700 MHz and Bruker Avance DRX 600 MHz spectrometers with the residual CHCl3 solvent used as an internal standard (δH: 77.23, δδ: 7.26). LR FABMS and HRFOMS (ES+) mass spectra were recorded on ABSciex 3200 QTRAP and Waters Micromass mass spectrometers. The isolation of compounds 1 and 2 was performed using a Waters HPLC system consisting of two Waters S15 pumps, a Rheodyne 7725i injector, and a Waters 996 photodiode array detector. Marley’s and chiral-phase HPLC analyses were conducted on a Shimadzu HPLC system equipped with two LC-20AD pumps and an SPD-M20A photodiode array detector. General reagents were from Sigma-Aldrich Corp and VWR International.

Collection and Identification. The marine cyanobacterial assemblage (McPhail laboratory voucher number PAC-6/25/04-2) was first collected in June 2004 by hand using scuba from a reef pinnacle off the west coast of Coiba National Park. The material collected for chemical extraction (1 L) was stored in 50% EtOH for 28 days, evaporated to dryness, and reconstituted in H2O (50 μL). FDDA solutions in acetone (0.1%, 100 μL) and 1 N NaHCO3 (50 μL) were added to each hydrolysate and heated to 37 °C for 1 h. The solutions were allowed to cool to rt, neutralized with 2 N HCl (2 μL) and evaporated to dryness. The residues were resuspended in DMSO–H2O (1:1, 100 μL) and analyzed by RP18 HPLC (Waters Symmetry Shield C18 column, 3.9 × 150 mm, 1 μL/min, UV detection at 340 nm) using a linear gradient of 9:1 40 mM ammonium acetate buffer (pH 5.2)–CH3CN to 1:1 40 mM ammonium acetate buffer–CH3CN over 60 min. The absolute configurations of the amino acids in the hydrolysates of 1 and 2 were determined by direct comparison with the retention times (tR, min) for Marley’s derivatives of authentic standards.

The retention times (min) of the FDDA-derivatized α-amino acids in the hydrolysate of 1 matched those of L-Pro (12.8; D-Pro, 13.5), N-Me-L-Val (23.2; D-N-Me-Val, 27.5), L-allo-Ile (21.4; L-Ile, 21.2, D-Ile, 28.3; D-L-allo-Ile, 28.8), L-Ala (12.3; D-Ala, 16.9), N-Me-L-Ala (14.3; N-Me-D-Ala, 16.6), and N-Me-L-Leu (26.6; N-Me-D-Leu, 30.3). The retention times (min) of the derivatized amino acids in the hydrolysate of 2 matched L-Pro (12.8; D-Pro, 13.5), N-Me-L-Val (23.2; D-N-Me-Val, 27.5), L-Val (17.3; D-Val, 24.0), L-allo-Ile (21.4; L-Ile, 21.2, D-Ile, 28.3 and D-allo-Ile, 28.8), and both N-Me-L-Ala (14.3) and N-Me-D-Ala (16.6).

Chiral-phase HPLC analysis was used to determine the absolute configurations of the Hiva residues in the two depsipeptides. A portion of the acid hydrolysate of 1 (0.25 mg) and 2 (0.25 mg) was reconstituted in H2O prior to chiral-phase HPLC analysis (88:15 2 mM CuSO4–CH3CN; column Phenomenex Chirex 3126 (D), 4.6 × 250 mm, flow 1.0 mL/min, UV detection at 254 nm). The Hiva residue in the hydrolysates of 1 and 2 eluted with the same retention time (tR, min) as the S-Hiva standard (40.0) but not that of (S)-Hiva standard (40.0) before (S)-Hiva. The absolute configurations of the Hiva residues in the two depsipeptides were determined by direct comparison with the retention times (tR, min) for Marley’s derivatives of authentic standards.

To assign the position of N-Me-L-Ala versus N-Me-D-Ala in companionamide B (2), 4 mg of 2 was partially hydrolyzed in 3 N HCl (2 mL, constant stirring, 100 °C); the reaction was monitored by LC-MS at 30 min intervals and was halted after 3 h by cooling to 20 °C, after which the acid was removed under high vacuum. The residual hydrolysate was suspended in 75% CH3CN–H2O (100 μL) for LC-MS/MS analysis (ES+) to identify fragments containing single N-Me-Ala residues. Tetrapeptide N-Me-Val-Val-N-Me-Ala-Pro (m/z 513.9, 0.1 mg) was purified from the total hydrolysate by RP18 HPLC using a linear gradient of 5–100% CH3CN–H2O (0.1% TFA over 60 min (Phenomenex Synergi Hydro column, 10 × 250 mm, 2.5 mL/min, 340 nm). Subsequent complete hydrolysis of the tetrapeptide (6 N HCl, microwave irradiation, 1000 W, 50 s) was followed by Marley’s derivatization of the resulting hydrolysate (with L-FDLa and commercial amino acid standards (with L-FDLa and D-FDLa). In each case, 1 M NaHCO3 (20 μL) was added to a 50 mM amino acid solution in H2O, followed by FDLa (1% w/v in acetone, 44 μL), and the reaction mixture heated to 40 °C (1 h). The reactions were quenched by cooling to rt and acidification (20 μL of 1 N HCl), before drying under high vacuum and resuspension in 100 μL of DMSO for LC-MS analysis (Thermo Aquasil C18 column, 3 × 150 mm, 25–55% linear gradient of CH3CN–H2O/0.1% TFA over 45 min, 1 mL/min,
three strains of *Plasmodium falciparum*. Experimental details for DNA extraction and amplification of cyanobacterial 16S rRNA, phylogenetic analysis, and phylogenetic alignment tree. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**
The authors declare no competing financial interest.

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**DEDICATION**

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