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# Malyngamide X: The First (7*R*)-Lyngbic Acid that Connects to a New Tripeptide Backbone from the Thai Sea Hare *Bursatella leachii*

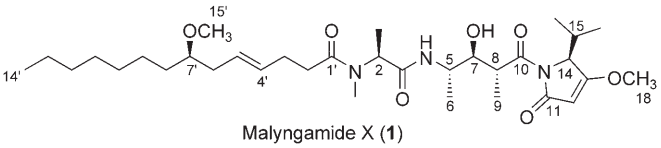
**Suchada Suntornchashwej,[a, b] Khanit Suwanborirux,*[a] Kazushi Koga,[b] and Minoru Isobe*[b, c]**

**Abstract:** Malyngamide X (**1**), the first (7*R*)-lyngbic acid connected to a new tripeptide backbone, was isolated from the Thai sea hare *Bursatella leachii*. The gross structure of **1** was established on the basis of 1D and 2D NMR and mass spectroscopic data. Combination of the NMR spectroscopic experiments with α-methoxy-α-(trifluoromethyl)-phenylacetic acid esters, 2,2,2-trifluoro-1-(9-anthryl)ethanol chiral solvating agent, and molecular mechanics of **1** and the synthetic molecular fragments allowed us to determine the absolute stereochemistry of all six stereogenic centers without hydrolytic degradation of the compound. Compound **1** displayed moderate cytotoxic, antitubercular, and antimalarial properties.

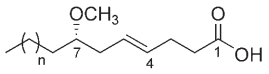
**Keywords:** chiral resolution · chiral solvating agent · malyngamide X · natural products · sea hare

## Introduction

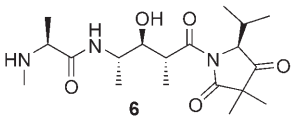
Malyngamides are structurally characterized as N-substituted amides of long-chain methoxylated fatty acids and exhibit a broad spectrum of bioactivities.[1] The fatty acyl portion in these compounds have structural similarities, with a 7*S* configuration and a *trans* double bond. They are (4*E*,7*S*)-7-methoxydodec-4-enoic acid (**2**),[2,3] (4*E*,7*S*)-7-methoxytetradec-4-enoic acid or lyngbic acid (**3**),[4] (4*E*,7*S*)-7-methoxy-9-methylhexadec-4-enoic acid (**4**),[5] and (4*E*,7*S*)-7-methoxyeicos-4-enoic acid (**5**).[6] Some of the malyngamides have been isolated from the extracts of sea hares (family Aplysiidae, phylum Mollusca), such as *Stylocheilus longicauda* (malyngamides O and P)[7] and *Bursatella leachii* (malyngamide S),[3] which are known to accumulate a wide variety of toxic metabolites from their diet. We have investigated, as part of our research for new biologically active compounds from marine organisms collected from the Gulf of Thailand, the chemical content of the sea hare *B. leachii* and reported two new hectochlorin and morpholine derivatives.[8] We report herein a new variety of the malyngamide family, malyngamide X (**1**), isolated from this animal from a different collection.




Malyngamide X (**1**)




**2**: *n* = 3
**3**: *n* = 5 (lyngbic acid)
**5**: *n* = 11

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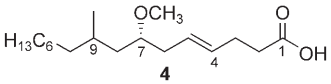


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Compound **1** is biosynthetically and pharmacologically interesting as it possesses a novel malyngamide skeleton and displays antimalarial and antitubercular activities. Unfortu-

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nately, we have a very limited amount of **1** in hand, and we failed to re-isolate it from other collections of the animals. We therefore employed several NMR spectroscopic analyses and molecular mechanics calculations as nonhydrolytic degradation methods for the stereochemical assignment of its six stereogenic centers. In particular, the stereochemistry of two isolated chiral carbons C14*S* and C7′*R* in **1** were successively determined through the application of NMR chiral solvation experiments with 2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE or Pirkle alcohol). The discovery of the (7*R*)-lyngbic acid moiety in **1** further highlights its unique structural feature. We now report the isolation, structure determination, and biological activity of malyngamide X.

The sea hare *B. leachii* was collected by hand from the Gulf of Thailand. Extraction of the internal organs of the animals with a mixture of EtOH and *n*PrOH afforded a green gum, which was solvent-partitioned with EtOAc. Biological evaluation of the crude EtOAc extract showed antimalarial activity with an $ED_{50}$ of 2.4 $\mu g\,mL^{-1}$ and cytotoxicity against the Vero cell line and KB and BC cancer cell lines with $ED_{50}$ of 16.2, 7.2, and 6.6 $\mu g\,mL^{-1}$, respectively. Fractionations of the extract on silica gel and Sephadex LH-20 columns were guided by colored TLC spots from Dragendorff reagent and by the results of biological activity determinations. Finally, 10 mg of **1** was isolated from the antimalaria-active fraction.

## Results and Discussion

### Gross Structure

Malyngamide X (**1**) was obtained as a pale-yellow oil. The molecular formula of **1** ($C_{33}H_{57}N_3O_7$) was determined from an intense pseudomolecular ion at *m/z* 608.4272 $[M+H]^+$ on accounting for seven degrees of unsaturation. The IR spectrum showed absorptions due to free OH or amide NH (3438 $cm^{-1}$), H-bonded OH or amide NH (br, 3200–3600 $cm^{-1}$), and amide carbonyl groups (1624, 1684, 1722 $cm^{-1}$) in the molecule. According to extensive analyses of 1D and 2D NMR spectra, **1** is a lipopeptide derived from (*E*)-7-methoxytetradec-4-enoic acid, *N*-methylalanine, 4-amino-3-hydroxy-2-methylpentanoic acid, and 5-isopropyl-4-methoxy-$\Delta^3$-pyrrolin-2-one (Figure 1, components I–IV, respectively).[9] The characteristic signals for component I include a carbonyl carbon at C1′ ($\delta_C$ 173.5 ppm), nine methylene signals for C2′ ($\delta_H$ 2.43, $\delta_C$ 33.8 ppm), C3′ ($\delta_H$ 2.37, $\delta_C$ 28.1 ppm), C6′ ($\delta_H$ 2.19, $\delta_C$ 36.4 ppm), C8′ ($\delta_H$ 1.44, $\delta_C$ 33.4 ppm), and C9′ to C13′ ($\delta_H$ 1.23–1.33, $\delta_C$ 25.3, 29.3, 29.8, 31.8, 22.7 ppm, respectively); a terminal methyl signal for C14′ ($\delta_H$ 0.88, $\delta_C$ 14.1 ppm), two olefinic signals for C4′ ($\delta_H$ 5.53, $\delta_C$ 127.3 ppm) and C5′ ($\delta_H$ 5.48, $\delta_C$ 131.1 ppm), a methoxy signal for OC15′$H_3$ ($\delta_H$ 3.32, $\delta_C$ 56.5 ppm), and an oxygenated methine signal for C7′ ($\delta_H$ 3.15, $\delta_C$ 80.8 ppm).[4,10] Although the aliphatic methylene protons of 9′-$H_2$ to 13′-$H_2$ appeared as overlapping signals in the $\delta_H$ 1.23–1.33 ppm region, its corresponding five carbon signals were all well-resolved to establish a chain length of 14 carbon atoms. The *E* geometry of the $\Delta^{4'}$ olefin was determined from the 4′-H/5′-H coupling constant of 15.6 Hz.

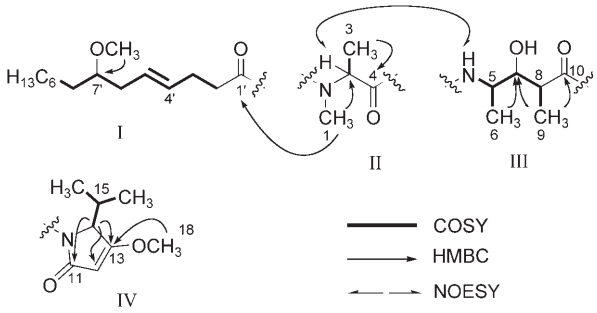


Figure 1. Components of **1**, their connectivities, and 2D NMR correlations.

The $CH_3CH(X)$ residue in component II (Figure 1) was delineated by the $^1H,^1H$-COSY correlations between the protons at C3 ($\delta_H$ 1.35 ppm) and C2 ($\delta_H$ 5.23 ppm). The HMBCs of the $NCH_3$ protons ($\delta_H$ 2.95 ppm) to C2 ($\delta_C$ 51.9 ppm) and of the C3 methyl protons to the carbonyl carbon atom (C4; $\delta_C$ 171.4 ppm) completed this component. In component III (Figure 1), the amide proton ($\delta_H$ 6.45 ppm) exhibited a $^1H,^1H$-COSY correlation to the methine proton at C5 ($\delta_H$ 4.06 ppm), which in turn correlated to the other methine proton at C7 ($\delta_H$ 3.62 ppm, $^3J_{5\text{-H},7\text{-H}}$ = 2.4 Hz) and the methyl protons at C6 ($\delta_H$ 1.21 ppm). Furthermore, 7-H coupled to the methine proton at C8 ($\delta_H$ 3.88 ppm, $^3J_{7\text{-H},8\text{-H}}$ = 9.0 Hz), which in turn showed correlation to the methyl protons at C9 ($\delta_H$ 1.14 ppm). The HMBCs of 6-$H_3$ to C7 ($\delta_C$ 77.4 ppm) and of 9-$H_3$ to C7 and the imide carbonyl C10 ($\delta_C$ 175.7 ppm) concluded the assignment. To date, only two other natural compounds have this component in the molecules: janolusimide (**6**) from the nudibranch *Janolus cristatus*[9a] and bleomycin $A_2$ from *Streptomyces verticillus*.[11] The last component IV (Figure 1) was established by HMBCs of the methine proton at C14 ($\delta_H$ 4.53 ppm) to the imide carbonyl C11 ($\delta_C$ 171.0 ppm) and

**Abstract in Thai:**

จากการศึกษาองค์ประกอบทางเคมีของกระต่ายป่าทะเล *Bursatella leachii* จากทะเลไทย ซึ่งเป็นสัตว์จำพวกหอยที่ไม่มีเปลือก โดยวิธีทางโครมาโตกราฟี สามารถแยกสาร malyngamide X (**1**) ซึ่งเป็นสารกลุ่ม malyngamides ชนิดแรก ที่มีส่วนกรดไขมันเป็น 7*R*-lyngbic acid ที่เชื่อมต่อกับส่วน tripeptide การพิสูจน์โครงสร้างทางเคมีทำได้โดยการวิเคราะห์ข้อมูล 1D, 2D NMR และ MS ส่วนการกำหนดลักษณะสเตริโอเคมีสัมบูรณ์ทั้ง 6 แห่ง ทำโดยวิธีการที่ไม่ทำลายสารโดยวิเคราะห์ข้อมูล NMR ที่ได้จากอนุพันธ์ MTPA esters และ TFAE chiral solvating agent ของสาร **1** และส่วนย่อยของโมเลกุลที่สังเคราะห์ขึ้นร่วมกับข้อมูลทาง molecular mechanics สาร 1 แสดงฤทธิ์ความเป็นพิษต่อเซลล์มะเร็ง ฤทธิ์ต้านวัณโรค และฤทธิ์ต้านมาลาเรียอย่างปานกลาง

  

the olefinic carbon atoms C12 ($\delta_C$ 94.6 ppm) and C13 ($\delta_C$ 179.9 ppm). The methoxy substitution on C13 was located through the HMBC cross-peak of the methoxy proton signal of 18-$H_3$ ($\delta_H$ 3.85 ppm) to the olefinic C13 and is responsible for a pair of unusual carbon chemical shifts at C12 and C13.[12] The isopropyl side chain was placed at C14 ($\delta_C$ 64.5 ppm) on the basis of $^1$H-$^1$H COSY correlations of both 16-$H_3$ ($\delta_H$ 0.77 ppm) and 17-$H_3$ ($\delta_H$ 1.10 ppm) to 15-H ($\delta_H$ 2.63 ppm), which was, in turn, coupled to 14-H. The connection of components I and II was deduced from the HMBC observed from the $NCH_3$ proton signal of 1-$H_3$ to C1′ of the fatty acid component. Further connection to component III was suggested by the interpretation of the NOE observed between 2-H and NH. Finally, the last component IV was placed at C10, and this completed the determination of the gross structure of malyngamide X.

## Stereochemistry of Malyngamide X

*Component III*

The relative stereochemistry of the three adjacent stereogenic centers along C5–C9 was established by NMR coupling (*J*) and NOE analysis as well as by molecular mechanics calculations. For a pair of vicinal carbons C7/C8, the typical *anti*-coupling value of $^3J_{7\text{-H},8\text{-H}} = 9.0$ Hz pointed to an *anti* H/H orientation, which was consistent with the $^3J_{H,H}$ values observed for other *anti*-configured α-methyl-β-hydroxy carbonyl compounds.[13] Cross-peaks in the NOESY spectrum from 9-$H_3$ to both 5-H and 7-H and between 5-H and 8-H revealed that 9-$H_3$ are *gauche* to C5 and C5 is *gauche* to 8-H; taken together, this is in agreement with the *anti* H/H configuration of 7*S**,8*R**.[14] For the determination of the configuration at C5, two diastereomers were analyzed to find the preferential conformation; thus, 5*S**,7*S**,8*R** and 5*R**,7*S**,8*R** were compared by calculations performed on MacroModel with an MM2 force field. In the case of the C5*S* isomer, the *anti* type A was found to be the most-stable conformer (Figure 2), with a dihedral angle C8–C7/C5–C6$H_3$ of 173° and the corresponding calculated $^3J_{5\text{-H},7\text{-H}}$ of 2.0 Hz. On the other hand, the C5*R* isomer assumes two low-energy conformers A′ and B′ (Figure 2), with the latter having a higher energy of 0.56 kcal mol$^{-1}$. The coupling constants and

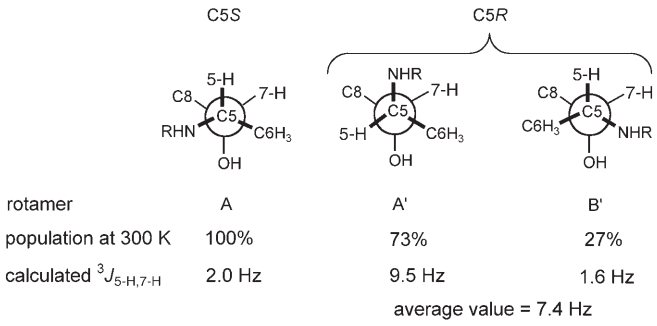


Figure 2. Conformers analyzed for the assignment of the relative configuration at C5 of **1**.

populations of A′ and B′ are 9.5 Hz (73%) and 1.6 Hz (27%), respectively; this gives a calculated average coupling constant $^3J_{5\text{-H},7\text{-H}}$ of 7.4 Hz. As the observed $^3J_{5\text{-H},7\text{-H}}$ was 2.6 Hz, the configuration of C5 was assigned to be *S*. Therefore, the relative stereochemistry of **1** was concluded to be 5*S**,7*S**,8*R**.

To establish the absolute configuration of **1**, we started at the C7 position, whose stereochemistry could be solved by modified Mosher analysis (Figure 3).[15] For this purpose,

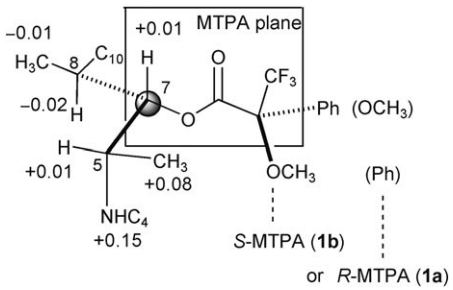


Figure 3. The $\Delta\delta_{SR}$ ($\delta_{1b}-\delta_{1a}$) values for the MTPA esters of **1** in $CDCl_3$.

(+)-MTPA ester **1a** and (−)-MTPA ester **1b** (MTPA = α-methoxy-α-(trifluoromethyl)phenylacetic acid) were prepared from **1**. The chemical shifts of the proton signals were assigned to each ester derivative from the $^1$H,$^1$H-COSY data. The $\Delta\delta_{SR}$ values (−0.02 for 8-H, −0.01 for 9-$H_3$, +0.01 for 5-H, +0.08 for 6-$H_3$, +0.15 ppm for NH) revealed a 7*S* configuration for **1** (Figure 3). With the relative stereochemistry for C5–C9 established, the absolute stereochemistry of the pentanoic fragment was consequently deduced to be 5*S*,7*S*,8*R*.

*Component IV*

NMR chiral solvating agents (CSAs) are optically pure compounds that bind in situ to the substrate through noncovalent, intermolecular forces.[16] They are widely applied for the analysis of enantiomeric excess through recording of the $^{19}$F or $^1$H NMR spectra of diastereomers formed from a pair of enantiomers with a chiral solvent for NMR spectroscopy. The solvated complexes show NMR nonequivalence due to differences in the shielding influences of the anthryl substituent at the chiral center of the CSA on protons of the enantiomers. The use of CSAs for absolute stereochemical assignments is very limited and can only be applied to a chiral compound whose diastereomeric solvated complexes with both enantiomers of the CSA are well-understood.[17]

We found that this method could be extended to the determination of the absolute configuration at isolated chiral carbon atoms of **1** when NMR spectroscopic experiments are carried out with molecular synthetic fragments of **1** with known stereochemistry at the observed chiral carbon. The senses of the chemical shift nonequivalence of protons at the chiral carbon atoms of each diastereomeric solvated complex were then analyzed to match their configurational relationships. By extension, the absolute stereochemistry at

  

the chiral carbon atom in the parent molecule can then be established with some confidence. The chemical shift nonequivalence was defined as $\Delta\delta_{RS}=\delta_R-\delta_S$, where $\delta_R$ and $\delta_S$ are the $^1$H NMR chemical shifts of the solvated substrate in (*R*)- and (*S*)-TFAE, respectively. The NMR spectroscopic experiments were carried out at low temperature and with a high CSA/substrate stoichiometry. This pushed the equilibrium towards formation of the diastereomeric solvated complexes and provided larger $\Delta\delta_{RS}$ values.[18] Although the choice of cosolvent has been reported to lead to variations in $\Delta\delta_{RS}$ values, the most commonly used, $CDCl_3$, appeared to be the first choice of cosolvent.[16]

Using TFAE (Pirkle alcohol) as a chiral solvating agent, we analyzed several synthetic pyrrolinone fragments with both *S* (**7a**–**9a** and **10**) and *R* (**8b** and **9b**) configurations (Figure 4) to develop a sense of the relation between the

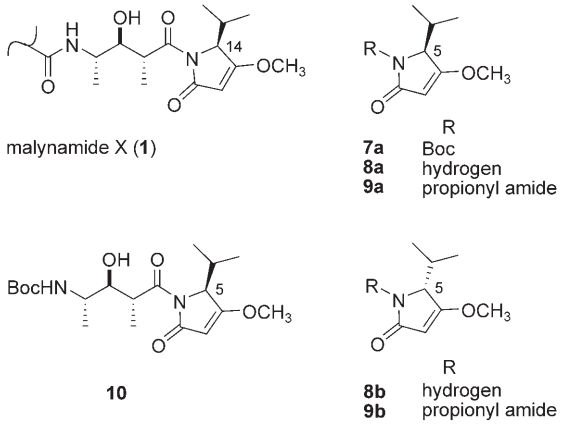


Figure 4. Compounds used for the determination of experimental $\Delta\delta_{RS}=\delta_R-\delta_S$ values for 5-H or 14-H ($CDCl_3$, 273 K, 600 MHz).

$\Delta\delta_{RS}$ value of 5-H and its configuration. Authentic samples were synthesized in three steps from *R* and *S* enantiomers of *N*-Boc-valine (Boc = *tert*-butoxycarbonyl), which was coupled with Meldrum acid then decarboxylated and O-methylated to afford the (5*R*)- and (5*S*)-isopropyl-4-methoxy-$\Delta^3$-pyrrolin-2-one product under conditions described by Akaji et al.[19]

As shown in Table 1, the chemical shifts of the 5*S*-H proton with (*R*)-TFAE-solvated complexes generally appeared at higher field than with (*S*)-TFAE-solvated complexes, and vice versa for those of 5*R*-H at lower field. This implies that if $\Delta\delta_{RS}$ of a proton at a chiral center of the *N*-acylpyrrolinone ring of **1** is negative, the absolute configuration of a chiral carbon is *S*. If $\Delta\delta_{RS}$ is positive, then the absolute configuration of the *N*-acylpyrrolinone ring in **1** should be *R*.

We now repeated the experiments with compound **1**. The 14-H proton signal of the solvated complex between **1** and (*R*)-TFAE appeared at $\delta_H$ 4.44 ppm, whereas that of the solvated complex with (*S*)-TFAE appeared at $\delta_H$ 4.46 ppm; this corresponds to a 14-H chemical shift nonequivalence $\Delta\delta_{RS}$ of −0.02 ppm (in the presence of 20 equiv TFAE, 14-H $\Delta\delta_{RS}=-0.07$). The observation that the 14-H $\Delta\delta_{RS}<0$ therefore agrees with the diastereomeric complexes of TFAE and *N*-acylpyrrolinones having the *S* configuration. We can thus conclude that C14 of **1** has the *S* configuration.

Table 1. Determination of the stereochemistry of component IV by NMR nonequivalence determined by the NMR chiral solvation method.

| Substrate[a] | $\delta$ (5-H or 14-H) [ppm][b] (*R*)-TFAE | (*S*)-TFAE | $\Delta\delta_{RS}$ (5-H or 14-H) [ppm][c] | Config. at C5 or C14 |
|---|---|---|---|---|
| **1** (5) | 4.44 | 4.46 | −0.02 | *S* |
| **1** (20) | 4.25 | 4.32 | −0.07 | *S* |
| **7a** (10) | 4.23 | 4.24 | −0.01 | *S* |
| **8a** (10) | 3.63 | 3.72 | −0.09 | *S* |
| **9a** (10) | 4.31 | 4.35 | −0.04 | *S* |
| **8b** (10) | 3.72 | 3.64 | +0.08 | *R* |
| **9b** (10) | 4.36 | 4.33 | +0.03 | *R* |
| **10** (10) | 4.33 | 4.38 | −0.05 | *S* |

[a] See Figure 4 for substrate structures. The values given in parentheses are the number of equivalents of TFAE present. [b] $^1$H NMR conditions: $CDCl_3$, 273 K, 600 MHz. [c] $\Delta\delta_{RS}=\delta_R-\delta_S$.

*Component II*

After four of the five stereogenic centers in the tripeptide portion of **1** were established, we solved the configuration at C2 by use of NOE data combined with a molecular mechanics study.[20] The lowest-energy conformations of models of the 2*S* and 2*R* isomers (3000 structures searched for each) were obtained from the MacroModel molecular modeling program with Monte Carlo conformational searching. After energy minimization of each isomer running on the MMFF94S force field in $CDCl_3$ solution, the lowest-energy structures of each isomer (Figure 5a and b, respectively) were then analyzed to determine a single structure that did not contradict any of the NOE interaction and coupling constant data of **1**. The distances obtained by NOE experiments were generally classified semiquantitatively into strong (<2.5 Å), medium (2.5–3.5 Å), and weak (3.5–5.0 Å) NOE interactions.[20b] The presence of the observed medium interresidual NOE cross-peak between 1-$H_3$ and 8-H was satisfied only by the conformation obtained for the 2*S* isomer (Figure 5b), in which this calculated NOE distance was 2.94 Å. Therefore, the stereostructure in the peptide portion of **1** was finally solved as having the 2*S*,5*S*,7*S*,8*R*,14*S* configuration. This stereochemistry was proved by the total synthesis of malyngamide X.[23]

*Component I*

The configuration at C7′ in **1** was first presumed to be *S*, as it is a homologue of other malyngamides.[22] However, the synthetic sample of (7′*S*)-**1** showed a different specific-rotation value from that of the natural compound, and this suggests that these are diastereomers of one another.[23] Therefore, we reconsidered the assignment at C7′ of **1**, and carried out NMR chiral solvation experiments on natural **1**, synthetic (7′*S*)-**1**, molecular synthetic fragments **11**–**12**, and known isomalyngamide A (**13**) (Figure 6). For the synthesis of the model fatty acyl fragments, the C7 stereochemistry and C14

  

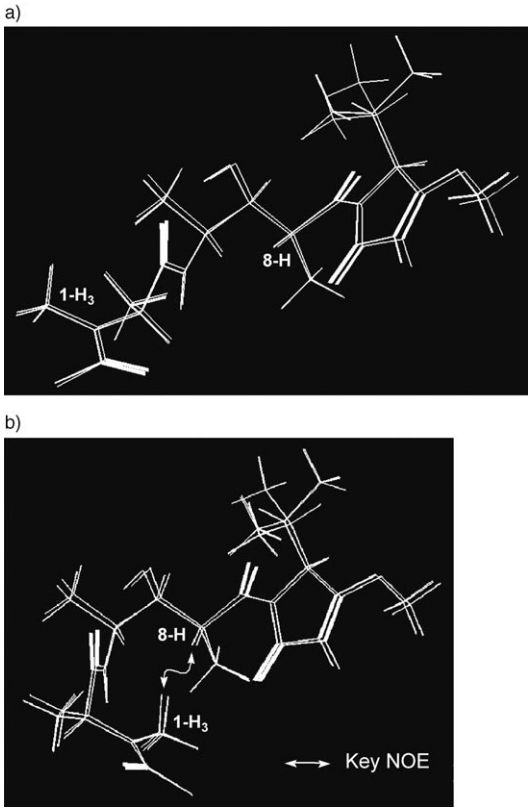


Figure 5. Overlay of conformers of (2*R*)-**1** (A) and (2*S*)-**1** (B) within 1.00 kcal mol$^{-1}$ of the global minimum, as obtained by the MacroModel Monte Carlo conformational search. Fatty acyl groups were deleted before the structures were superimposed.[21]

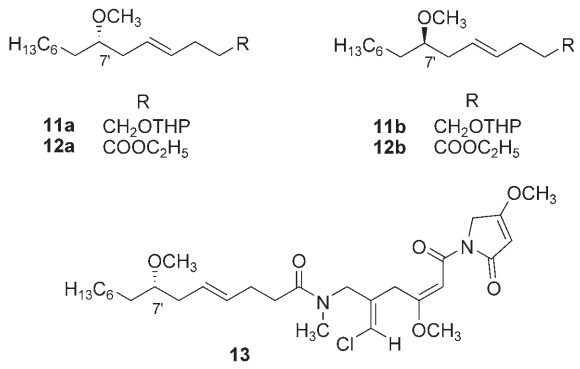


Figure 6. Compounds used for the determination of experimental $\Delta\delta_{RS}=\delta_R-\delta_S$ values observed for 7′-OCH$_3$ (CDCl$_3$, 273 K, 600 MHz).

skeleton were constructed from the two enantiomers of glycidyl tosylate ((*S*)-glycidyl tosylate for (7′*S*)-**1, 11a**, and **12a**, and (*R*)-glycidyl tosylate for **11b** and **12b**) coupled with O-protected pent-4-yn-1-ol, followed by *trans*-reduction and O-methylation. As shown in Table 2, the chemical shifts of the 7′*S*-OCH$_3$ protons with (*R*)-TFAE-solvated complexes appeared downfield relative to the (*S*)-TFAE-solvated complexes (7′*S*-OCH$_3$ of (7′*S*)-**1**, **11a**, **12a**: $\Delta\delta_{RS}>0$) and vice versa for those of C7′*R* (7′*S*-OCH$_3$ of **11b**–**12b**: $\Delta\delta_{RS}<0$).

Table 2. Determination of the stereochemistry of component I by NMR nonequivalence determined by the NMR chiral solvation method.

| Substrate[a] | $\delta$ (7′-OCH$_3$) [ppm][b] (*R*)-TFAE | (*S*)-TFAE | $\Delta\delta_{RS}$ (7′-OCH$_3$) [ppm][c] | Config. at C7′ |
|---|---|---|---|---|
| Natural **1** (5) | 3.29 | 3.30 | −0.01 | *R* |
| Natural **1** (20) | 3.19 | 3.23 | −0.04 | *R* |
| Synthetic (7′*S*)-**1** (5) | 3.30 | 3.29 | +0.01 | *S* |
| Synthetic (7′*S*)-**1** (20) | 3.24 | 3.22 | +0.02 | *S* |
| **11a** (5) | 3.24 | 3.23 | +0.01 | *S* |
| **12a** (5) | 3.26 | 3.26 | +0.00 | *S* |
| **11b** (5) | 3.24 | 3.25 | −0.01 | *R* |
| **12b** (5) | 3.25 | 3.27 | −0.02 | *R* |
| **13** (5) | 3.29 | 3.28 | +0.01 | *S* |
| **13** (20) | 3.20 | 3.17 | +0.03 | *S* |

[a] See Figure 6 for substrate structures. The values given in parentheses are the number of equivalents of TFAE present. [b] $^1$H NMR conditions: CDCl$_3$, 273 K, 600 MHz. [c] $\Delta\delta_{RS}=\delta_R-\delta_S$.

We also checked our conclusion with isomalyngamide A (**13**), a known (7′*S*)-malyngamide (Figure 7).[4d] The 7′*S*-OCH$_3$ $\Delta\delta_{RS}$ observed for **13** and TFAE was positive (in the presence of 5 equiv TFAE, 7′*S*-OCH$_3$ $\Delta\delta_{RS}=+0.01$ ppm, whereas with 20 equiv reagent, 7′*S*-OCH$_3$ $\Delta\delta_{RS}=+0.03$ ppm). This implies that, as the 7′-OCH$_3$ $\Delta\delta_{RS}$ value of natural **1** is positive, the absolute configuration at C7′ is *S*. The solvated complex between **1** and TFAE gave 7′-OCH$_3$ $\Delta\delta_{RS}<0$, or the opposite value for both **13** and synthetic (7′*S*)-**1**. In the presence of (*R*)-TFAE, the 7′-OCH$_3$ proton signals of **1** appeared at $\delta_H$ 3.288 ppm, whereas with (*S*)-TFAE, they appeared at $\delta_H$ 3.296 ppm; this corresponds to 7′-OCH$_3$ $\Delta\delta_{RS}=-0.01$ ppm (with 20 equiv TFAE, 7′-OCH$_3$ $\Delta\delta_{RS}=-0.04$ ppm). The results (7′*S*-OCH$_3$ $\Delta\delta_{RS}>0$ for (7′*S*)-**1**, **11a**, **12a**, **13**; 7′*R*-OCH$_3$ $\Delta\delta_{RS}<0$ for **11b**, **2b**) show that **1** is the first natural malyngamide derived from (7*R*)-lyngbic acid.

## Conclusions

To date, more than 30 structures of malyngamides have been isolated from cyanobacteria and sea hares. Some of them display potential activity as immunosuppressive (malyngamide G),[24] anticancer (malyngamide J),[25] and anti-HIV (serinol-derived malyngamides) agents.[6] Although the new malyngamide X (**1**) has been isolated from the sea hare *B. leachii*, the biosynthetic origins of **1** could be the cyanobacteria fed on by these molluscs, and this is the reason why structurally unrelated molecules are isolated from different collections of these sea hares.[8] Compound **1** contains six stereogenic centers, one of which is located at a remote position in the fatty acyl part. Generally, the C7′ configuration of malyngamides is assigned by analysis of the $[\alpha]_D$ values of the hydrolysate[26] or co-isolated free fatty acids and comparison with the literature values of free acid **3**.[5b] However, it is still very difficult when a limited amount of isolated natural compounds is available and the co-occurring free acid is not present in the extract.[22] Our studies showed that the ap-

  

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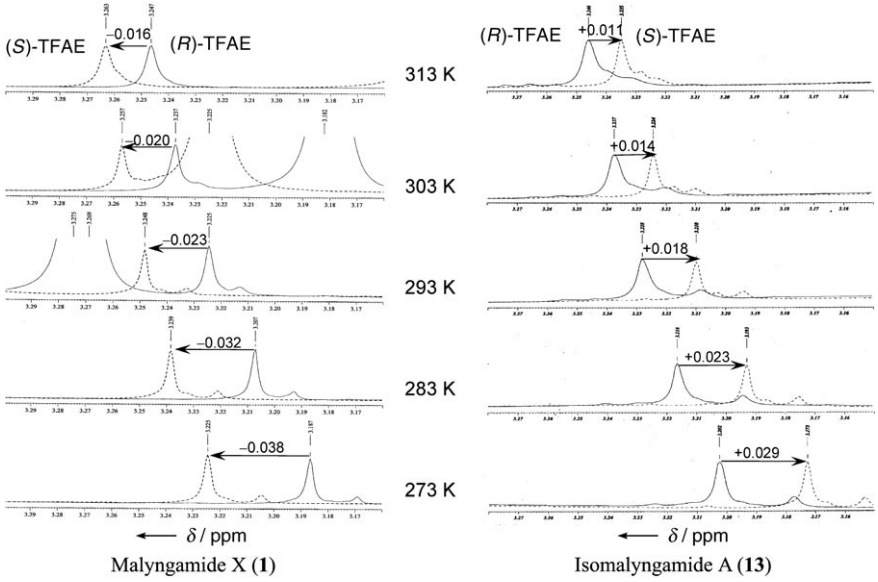


Figure 7. Variation of $\Delta\delta_{RS}$ for 7′-$OCH_3$ of **1** and **13** with temperature in the presence of 20 equiv (*R*)-TFAE (——) and (*S*)-TFAE (-----).

plication of the NMR chiral solvating agent TFAE is an efficient method for the absolute configurational assignment of isolated stereogenic centers such as C7′ and C14 of **1**. Compound **1** was completely recovered from the CSA–substrate mixtures after each NMR measurement. Note that this chiral solvation method is only valuable when the data are compared with those of similar authentic samples, which may be provided by synthesis in most cases. Therefore, several synthetic model fragments with both enantiomers could be used for confirming the assignment by using this nondestructive method. Finally, we solved the stereochemical assignment of **1** as 2*S*,5*S*,7*S*,8*R*,14*S*,7′*R*. The origin of the 7′*R* fattyl acyl group in **1** is unknown. It may be a metabolite of other marine organisms, as several (*R*)-methoxylated fatty acids have been isolated from sponge cells or bacteria living in symbiosis with sponges.[27]

Compound **1** showed moderate cytotoxicity against oral human epidermoid carcinoma of the nasopharynx (KB), human small cell lung cancer (NCI-H187), and breast cancer (BC) cell lines with $ED_{50}=8.20$, 4.12, and 7.03 μM, respectively. Compound **1** also exhibited antitubercular activity against the *Mycobacterium tuberculosis* H37Ra strain, with MIC = 80 μM, and antimalarial activity against *Plasmodium falciparum* (K1, multidrug-resistant strain) with $ED_{50}=5.44$ μM. Further studies on the total synthesis of malyngamide X and more-extensive biological evaluations are in progress.[23]

## Experimental Section

*General*

All moisture-sensitive reactions were carried out under a nitrogen or argon atmosphere. Optical rotations were measured on a Perkin–Elmer 341 polarimeter with a sodium lamp operating at 589 nm. IR and UV/Vis spectra were obtained on a Perkin–Elmer 2000 FTIR spectrometer and a Spectronic 3000 UV/Vis spectrometer, respectively. $^1$H and $^{13}$C NMR, distortionless enhancement by polarization transfer (DEPT), $^1$H,$^1$H-COSY, heteronuclear multiple-quantum coherence (HMQC), HMBC, and NOE experiments were carried out on a Bruker AVANCE DPX-300 FTNMR, a Bruker AV-400, or a Bruker AMX-600 spectrometer. ESI/Q/TOF MS spectra were recorded on a Micromass LCT mass spectrometer. FAB MS and EI MS spectra were obtained on a JEOL JMS-700 mass spectrometer. All experiments were performed in the positive-ion mode. Conformational analyses were calculated by the Monte Carlo method with an MMFF94S force field, and the calculation of NOE interaction distances in Å by MacroModel was performed on a Power IRIS GTX200BII instrument (Silicongraphics Limited).

*Animal Material*

Four specimens of the sea hare *B. leachii* (230 g wet weight) were collected by hand from Sichang Island, the Gulf of Thailand in October 2000. They were frozen on site before extraction. The voucher specimens and a photo of the live animal are available from our laboratory.

*Extraction and Isolation*

The internal organs (65 g wet weight) of the specimens (four animals) were blended into small pieces and macerated three times with a 1:1 mixture of EtOH/*n*PrOH (150 mL each). The combined extracts were concentrated under reduced pressure, and the residue was partitioned between EtOAc and $H_2O$ to give the crude EtOAc extract (1.4 g) as a dark-green oil. It was subjected to chromatography (silica gel, MeOH/$CHCl_3$ gradient); this gave four fractions. The fraction eluting with 5% MeOH in $CHCl_3$ gave a positive spot to Dragendorff spraying reagent of **1** (TLC: $R_f=0.34$, silica gel, $CHCl_3$/EtOAc/$Et_2NH$ = 1:3:0.05). It was further purified by a combination of flash column chromatography (silica gel, 1.5% $Et_2NH$ in $CHCl_3$/EtOAC = 1:3) and Sephadex LH-20 chromatography (hexane/$CHCl_3$/MeOH = 20:75:5); this afforded **1** as a pale-yellow oil (10 mg, 0.015% yield wet weight).

**1**: Pale-yellow oil; $[\alpha]_D^{27}=-6.8$ ($c=0.18$, $CHCl_3$); UV/Vis (MeOH): $\lambda_{max}$ ($\log\varepsilon$) = 219 (5.11), 241 nm (5.03 $dm^3mol^{-1}cm^{-1}$); IR (film): $\tilde{\nu}_{max}$ = 3600–3200, 3438, 2930, 1722, 1684, 1624, 1460, 1380, 1319, 1247, 1093, 953 $cm^{-1}$; $^1$H NMR (600 MHz, $CDCl_3$): $\delta=0.77$ (d, $J=6.6$ Hz, 16-$H_3$), 0.88 (t, $J=6.6$ Hz, 14′-$H_3$), 1.10 (d, $J=7.2$ Hz, 17-$H_3$), 1.14 (d, $J=7.2$ Hz, 9-$H_3$), 1.21 (d, $J=7.2$ Hz, 6-$H_3$), 1.23–1.33 (m, 9′-$H_2$–13′-$H_2$), 1.35 (d, $J=7.2$ Hz, 3-$H_3$), 1.44 (m, 8′-$H_2$), 2.19 (t, $J=6.0$ Hz, 6′-$H_2$), 2.37 (m, 3′-$H_2$), 2.43 (m, 2′-$H_2$), 2.63 (m, 15-H), 2.95 (s, 1-$H_3$), 3.15 (quint, $J=6.0$ Hz, 7′-H), 3.32 (s, 15′-$H_3$), 3.62 (dd, $J=9.0$, 2.4 Hz, 7-H), 3.85 (s, 18-$H_3$), 3.88 (dq, $J=9.0$, 7.2 Hz, 8-H), 4.06 (m, 5-H), 4.53 (d, $J=2.4$ Hz, 14-H), 5.07 (s, 12-H), 5.23 (q, $J=7.2$ Hz, 2-H), 5.48 (dt, $J=15.6$, 6.0 Hz, 5′-H), 5.53 (dt, $J=15.6$, 6.0 Hz, 4′-H), 6.45 ppm (d, $J=7.8$ Hz, NH); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 13.7 (C3), 14.2 (C9), 14.1 (C14′), 15.4 (C16), 18.2 (C6), 18.7 (C17), 22.7 (C13′), 25.3 (C9′), 28.1 (C3′), 29.0 (C15), 29.3 (C10′), 29.8 (C11′), 30.6 (C1), 31.8 (C12′), 33.4 (C8′), 33.8 (C2′), 36.4 (C6′), 42.4 (C8), 46.7 (C5), 51.9 (C2), 56.5 (C15′), 58.5 (C18), 64.5 (C14), 77.4 (C7), 80.8 (C7′), 94.6 (C12), 127.3 (C4′), 131.1 (C5′), 171.0 (C11), 171.4 (C4), 173.5 (C1′), 175.7 (C10), 179.9 ppm (C13); MS (HR FAB): *m/z* calcd for $C_{33}H_{58}N_3O_7$: 608.4275 $[M+H]^+$; found: 608.4272.

**1a**: A solution of **1** (0.6 mg, 0.99 μmol), 4-dimethylaminopyridine (DMAP; 1.22 mg, 10 μmol), and triethylamine (100 μL, 720 μmol) in $CH_2Cl_2$ (1 mL) was treated with (+)-(*S*)-MTPA-Cl (30 μL, 160 μmol),

  

and the mixture was vigorously stirred at room temperature for 37 h. 3-[(Dimethylamino)propyl]amine (20 μL, 160 μmol) was added, and the residue after evaporation of the solvent was partitioned between 20% aqueous MeOH and EtOAc (2:1). The EtOAc extract was evaporated and purified by preparative TLC (hexane/EtOAc = 1:3); this gave pure **1a** ($R_f$ = 0.3, 0.6 mg, 73%).

**1a**: $^1$H NMR ($CDCl_3$, 600 MHz, $CDCl_3$): $\delta$ = 0.52 (d, $J$ = 7.2 Hz, 16-$H_3$), 0.88 (t, $J$ = 6.4 Hz, 14′-$H_3$), 1.05 (d, $J$ = 7.2 Hz, 6-$H_3$), 1.08 (d, $J$ = 7.2 Hz, 17-$H_3$), 1.21 (d, $J$ = 7.2 Hz, 9-$H_3$), 1.25 (brs, 9′-$H_2$–13′-H), 1.35 (d, $J$ = 7.2 Hz, 3-$H_3$), 1.43 (m, 8′-$H_2$), 2.19 (dd, $J$ = 5.6, 5.6 Hz, 6′-$H_2$), 2.31 (m, 3′-$H_2$), 2.35 (m, 2′-$H_2$), 2.46 (m, 15-H), 2.90 (s, 1-$H_3$), 3.15 (m, 7′-H), 3.50 (s, 15′-$H_3$), 3.83 (s, 18-$H_3$), 4.22 (m, 8-H), 4.48 (d, $J$ = 2.4 Hz, 14-H), 4.49 (m, 5-H), 5.04 (s, 12H), 5.16 (q, $J$ = 7.2 Hz, 2-H), 5.45 (m, 5′-H), 5.52 (m, 4′-H), 5.62 (dd, $J$ = 9.6, 1.6 Hz, 7-H), 6.04 ppm (d, $J$ = 9.6 Hz, NH); MS (ESI/Q/TOF): $m/z$ calcd for $C_{43}H_{65}O_9N_3F_3$: 824.47; found: 824.52.

**1b**: By the same method described for **1a**, **1b** was prepared from (−)-(*R*)-MTPA-Cl instead of (+)-(*S*)-MTPA-Cl. The reaction mixture was vigorously stirred at room temperature for 56 h. The workup and purification method was the same as that used for **1a**; this gave **1b** ($R_f$ = 0.3, 0.6 mg, 73%). $^1$H NMR ($CDCl_3$, 600 MHz, $CDCl_3$): $\delta$ = 0.35 (d, $J$ = 7.2 Hz, 16-$H_3$), 0.88 (t, $J$ = 6.4 Hz, 14′-$H_3$), 0.98 (d, $J$ = 7.2 Hz, 17-$H_3$), 1.13 (d, $J$ = 7.2 Hz, 6-$H_3$), 1.19 (d, $J$ = 7.2 Hz, 9-$H_3$), 1.25 (brs, 9′-$H_2$–13′-H), 1.37 (d, $J$ = 7.2 Hz, 3-$H_3$), 1.43 (m, 8′-$H_2$), 2.11 (m, 15-H), 2.19 (dd, $J$ = 5.6, 5.6 Hz, 6′-$H_2$), 2.37 (m, 3′-$H_2$), 2.39 (m, 2′-$H_2$), 2.92 (s, 1-$H_3$), 3.15 (m, 7′-H), 3.40 (s, 15′-$H_3$), 3.80 (s, 18-$H_3$), 4.20 (m, 8-H), 4.38 (d, $J$ = 2.4 Hz, 14-H), 4.50 (m, 5-H), 5.01 (s, 12-H), 5.20 (q, $J$ = 7.2 Hz, 2-H), 5.46 (m, 5′-H), 5.53 (m, 4′-H), 5.63 (dd, $J$ = 9.6, 1.6 Hz, 7-H), 6.19 ppm (d, $J$ = 9.6 Hz, NH); MS (ESI/Q/TOF): $m/z$ calcd for $C_{43}H_{65}O_9N_3F_3$: 824.47; found: 824.52.

*NMR Chiral Solvation Experiments*

Samples for NMR chiral solvation experiments were typically prepared by separately mixing a sample with the desired amount of the CSA TFAE (Pirkle alcohol) in a very clean NMR tube with $CDCl_3$ as the solvent. If not indicated otherwise, $^1$H NMR spectra were recorded at 273 K on a 600-MHz NMR spectrometer. Chemical shifts were reported in ppm relative to the chemical shift of tetramethylsilane (TMS) as internal standard. The digital resolution of the final spectra was 0.386 Hz/point, that is, 0.000643 ppm/point. The chemical shifts of the proton signals were assigned from the $^1$H,$^1$H-COSY data.

*Synthesis of Model Compounds*

Model compounds were designed to serve the correlation of the configurations of the CSA–**1** diastereomeric complexes about the C14 and C7′ stereogenic centers. Syntheses of model compounds for models of the 5-isopropyl-4-methoxy-$\Delta^3$-pyrrolin-2-one system in **1** (C14) started with the coupling of the pure enantiomer of *N*-Boc-valine (L-valine for the 5*S* isomer and D-valine for the 5*R*) and Meldrum acid by the method of Akaji et al.[19] After deprotection and N-propionylation of each isomer, the model compounds **7**–**9** were afforded. By the Lewis acid mediated aldol reaction developed by Walker and Heathcock,[28] the N-protected C1–C18 fragment of **1** (model **10**) was prepared from the boron enolate of **9a** and *N*-Boc-L-alaninal in the presence of $Et_2AlCl$. Syntheses of model compounds **11** and **12** as models of the chiral methoxylated homoallyl ether in the fatty acid part of **1** (C7′) were achieved by the coupling of the pure enantiomer of glycidyl tosylate (2*S* isomer for 7′*S* model compounds and 2*R* isomer for 7′*R* model compounds) and hexyl organocuprate; this was followed by coupling to pent-4-ynol, $\Delta^{4',5'}$-*trans* reduction, and O-methylation.

**7a**: Pale-yellow solid; $[\alpha]_D^{28}$ = +83.5 ($c$ = 0.20, $CH_2Cl_2$); $^1$H NMR ($CDCl_3$, 400 MHz, $CDCl_3$): $\delta$ = 0.80 (d, $J$ = 7.2 Hz, 7-$H_3$), 1.09 (d, $J$ = 7.2 Hz, 8-$H_3$), 1.54 (s, 9H, *t*Bu), 2.42 (m, 6-H), 3.81 (s, 9-$H_3$), 4.37 (d, $J$ = 2.4 Hz, 5-H), 5.06 ppm (s, 3-H); MS (ESI/Q/TOF): $m/z$ calcd for $C_{13}H_{21}NO_4Na$: 278.1368 $[M+Na]^+$; found: 278.1373.

**8a**: White solid; $[\alpha]_D^{28}$ = +5.6 ($c$ = 1.00, $CH_2Cl_2$); $^1$H NMR ($CDCl_3$, 400 MHz, $CDCl_3$): $\delta$ = 0.80 (d, $J$ = 6.8 Hz, 7-$H_3$), 1.00 (d, $J$ = 6.8 Hz, 8-$H_3$), 2.08 (m, 6-H), 3.79 (s, 9-$H_3$), 3.98 (dd, $J$ = 1.2, 2.8 Hz, 5-H), 5.03 (d, $J$ = 1.2 Hz, 3-H), 5.40 ppm (brs, NH); MS (ESI/Q/TOF): $m/z$ calcd for $C_8H_{14}NO_2$: 156.1024 $[M+H]^+$; found: 156.1016.

**9a**: Pale-yellow solid; $[\alpha]_D^{28}$ = +75.3 ($c$ = 0.60, $CH_2Cl_2$); $^1$H NMR ($CDCl_3$, 400 MHz, $CDCl_3$): $\delta$ = 0.74 (d, $J$ = 7.2 Hz, 7-$H_3$), 1.11 (d, $J$ = 7.2 Hz, 8-$H_3$), 1.15 (t, $J$ = 7.2 Hz, 3′-$H_3$), 2.55 (m, 6-H), 2.94 (q, $J$ = 7.2 Hz, 2′-$H_2$), 3.83 (s, 9-$H_3$), 4.37 (d, $J$ = 2.4 Hz, 5-H), 5.06 ppm (s, 3-H); MS (ESI/Q/TOF): $m/z$ calcd for $C_{11}H_{17}NO_3Na$: 234.1106 $[M+Na]^+$, found: 234.1108.

**8b**: White solid; $[\alpha]_D^{28}$ = −6.5 ($c$ = 1.00, $CH_2Cl_2$); $^1$H NMR ($CDCl_3$, 400 MHz): spectrum was identical to that of **8a**; MS (ESI/Q/TOF): $m/z$ calcd for $C_8H_{14}NO_2$: 156.10 $[M+H]^+$; found: 156.44.

**9b**: Pale-yellow solid; $[\alpha]_D^{28}$ = −60.9 ($c$ = 0.60, $CH_2Cl_2$); $^1$H NMR ($CDCl_3$, 400 MHz): spectrum was identical to that of **9a**; MS (ESI/Q/TOF): $m/z$ calcd for $C_{11}H_{17}NO_3Na$: 234.11 $[M + Na]^+$; found: 234.51.

**10**: Yellow oil; $[\alpha]_D^{29}$ = +8.15 ($c$ = 0.10, $CHCl_3$); $^1$H NMR ($CDCl_3$, 400 MHz, $CDCl_3$): $\delta$ = 0.76 (d, $J$ = 6.8 Hz, 8-$H_3$), 1.10 (d, $J$ = 6.8 Hz, 7-$H_3$), 1.19 (d, $J$ = 6.8 Hz, 14-$H_3$), 1.21 (d, $J$ = 6.8 Hz, 15-$H_3$), 2.61 (m, 6-H), 3.59 (dd, $J$ = 8.0, 2.4 Hz, 12-H), 3.82 (m, 13-H), 3.85 (s, 9-$OCH_3$), 3.98 (dq, $J$ = 8.0, 6.8 Hz, 11-H), 4.54 (d, $J$ = 2.8 Hz, 5-H), 4.80 (brs, NH), 5.09 ppm (s, 3-H); MS (ESI/Q/TOF): $m/z$ calcd for $C_{19}H_{32}N_2O_6Na$: 407.2168 $[M+Na]^+$; found: 407.2167.

**11a**: Yellow oil; $[\alpha]_D^{27}$ = −10.3 ($c$ = 0.50, $CHCl_3$); $^1$H NMR ($CDCl_3$, 400 MHz, $CDCl_3$): $\delta$ = 0.88 (t, $J$ = 7.2 Hz, 14-$H_3$), 1.10–1.40 (brs, 10H, 9-$H_2$–13-H), 1.14–1.90 (m,10H, 2-$H_2$, 8-$H_2$, 2′-$H_2$–4′-H), 2.0–2.4 (m, 4H, 3-$H_2$, 6-$H_2$), 3.14 (m, 1a-$H_2$), 3.32 (s, 7-$OCH_3$), 3.39 (m, 5′a-$H_2$), 3.50 (m, 7-H), 3.73 (m, 1b-$H_2$), 3.88 (m, 5b-$H_2$), 4.58 (t, $J$ = 4.4 Hz, 1′-H), 5.42 (dt, $J$ = 15.2, 6.4 Hz, 5-H), 5.49 ppm (dt, $J$ = 15.2, 6.4 Hz, 4-H); MS (EI): $m/z$ calcd for $C_{20}H_{38}O_3$: 326.2821 $[M]^+$; found: 326.2802.

**12a**: Yellow oil; $[\alpha]_D^{27}$ = −13.8 ($c$ = 0.12, $CHCl_3$); $^1$H NMR ($CDCl_3$, 400 MHz, $CDCl_3$): $\delta$ = 0.88 (t, $J$ = 6.8 Hz, 14-$H_3$), 1.15–1.25 (brs, 9-$H_2$–13, 2′-$H_3$), 1.40 (m, 8-$H_2$), 2.20 (m, 6′-$H_2$), 2.25–2.45 (m, 2-$H_2$, 3-$H_2$), 3.13 (m, 7-H), 3.32 (s, 7-$OCH_3$), 4.14 (q, $J$ = 7.2 Hz, 1′-H), 5.40–5.51 ppm (m, 4-H, 5-H); MS (EI): $m/z$ calcd for $C_{17}H_{32}O_3$: 284.2351 $[M]^+$; found: 284.2388.

**11b**: Yellow oil; $[\alpha]_D^{27}$ = +13.3 ($c$ = 0.50, $CHCl_3$); $^1$H NMR ($CDCl_3$, 400 MHz): spectrum was identical to that of **11a**; MS (EI): $m/z$ calcd for $C_{20}H_{38}O_3$: 326.28 $[M]^+$; found: 326.28.

**12b**: Yellow oil; $[\alpha]_D^{27}$ = +9.4 ($c$ = 0.12, $CHCl_3$); $^1$H NMR ($CDCl_3$, 400 MHz): spectrum was identical to that of **12a**; MS (EI MS): $m/z$ calcd for $C_{17}H_{32}O_3$: 284.24 $[M]^+$; found: 284.24.

(7′*S*)-**1**:[23] Pale-yellow oil; $[\alpha]_D^{27}$ = −15.4 ($c$ = 0.8, MeOH); $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ = 0.77 (d, $J$ = 7.2 Hz, 16-$H_3$), 0.88 (t, $J$ = 6.6 Hz, 14′-$H_3$), 1.10 (d, $J$ = 6.6 Hz, 17-$H_3$), 1.14 (d, $J$ = 6.6 Hz, 9-$H_3$), 1.21 (d, $J$ = 6.6 Hz, 6-$H_3$), 1.23–1.33 (m, 9′-$H_2$–13′-$H_2$), 1.35 (d, $J$ = 7.2 Hz, 3-$H_3$), 1.44 (m, 8′-$H_2$), 2.19 (t, $J$ = 6.0 Hz, 6′-$H_2$), 2.37 (m, 3′-$H_2$), 2.43 (m, 2′-$H_2$), 2.63 (m, 15-H), 2.95 (s, 1-$H_3$), 3.15 (quint, $J$ = 6.0 Hz, 7′-H), 3.32 (s, 15′-$H_3$), 3.63 (dd, $J$ = 9.0, 2.4 Hz, 7-H), 3.85 (s, 18-$H_3$), 3.88 (dq, $J$ = 9.0, 6.6 Hz, 8-H), 4.07 (m, 5-H), 4.53 (d, $J$ = 2.4 Hz, 14-H), 5.08 (s, 12-H), 5.23 (q, $J$ = 7.2 Hz, 2-H), 5.48 (dt, $J$ = 15.6, 6.0 Hz, 5′-H), 5.53 (dt, $J$ = 15.6, 6.0 Hz, 4′-H), 6.48 ppm (d, $J$ = 9.0 Hz, NH); $^{13}$C NMR (150 MHz, $CDCl_3$): $\delta$ = 13.7 (C3), 14.2 (C9), 14.1 (C14′), 15.4 (C16), 18.1 (C6), 18.7 (C17), 22.6 (C13′), 25.3 (C9′), 28.1 (C3′), 29.0 (C15), 29.3 (C10′), 29.7 (C11′), 30.6 (C1), 31.8 (C12′), 33.4 (C8′), 33.8 (C2′), 36.4 (C6′), 42.4 (C8), 46.7 (C5), 51.9 (C2), 56.5 (C15′), 58.5 (C18), 64.5 (C14), 77.4 (C7), 80.8 (C7′), 94.6 (C12), 127.3 (C4′), 131.1 (C5′), 171.0 (C11), 171.4 (C4), 173.5 (C1′), 175.7 (C10), 179.9 ppm (C13); MS (FAB): $m/z$ calcd for $C_{33}H_{58}N_3O_7$: 608.4275 $[M+H]^+$; found: 608.4305.

*Cytotoxicity Test*[29]

The cytotoxicity against breast cancer (BC), oral human epidermoid carcinoma of the nasopharynx (KB), and human small cell lung cancer (NCI-H187) cell lines was examined by employing the colorimetric method described by Collins and Franzblau. Ellipticine was used as a reference substance, with activity toward BC, KB, and NCI-H187 cell lines in the $IC_{50}$ range 0.4 ± 0.1 μg mL$^{-1}$.

*Antimalarial Test*[30]

The parasite *Plasmodium falciparum* (K1, multidrug-resistant strain) was cultured continuously according to the method of Trager and Jensen.

  

Quantitative assessment of antimalarial activity in vitro was determined by means of the microculture radioisotope technique based on the method described by Desjardins et al. The inhibitory concentration ($IC_{50}$) represents the concentration that causes 50% reduction in parasite growth as indicated by the in vitro uptake of [$^3$H]hypoxanthine by *P. falciparum*. An $IC_{50}$ value of 0.001 $\mu g\,mL^{-1}$ was observed for the standard sample, artemisinin, in the same test system.

*Antitubercular Test*[31]

Antitubercular activity was assessed against *Mycobacterium tuberculosis* H37Ra with Microplate Alamar Blue (MABA). The mycobacterium was cultured in Middle-brook 7H9 broth. The standard drugs isoniazid and kanamycin sulfate showed MIC values of 0.040–0.090 and 2.0–5.0 $\mu g\,mL^{-1}$, respectively. The MIC values of the compounds were determined in the same experiment as experimental samples.

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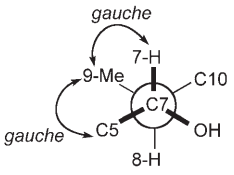
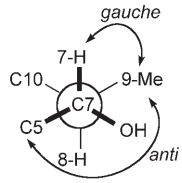
[1] A. M. Burja, B. Banaigs, E. Abou-Mansour, J. G. Burgess, P. C. Wright, *Tetrahedron* **2001**, *57*, 9347–9377.
[2] K. L. McPhail, W. H. Gerwick, *J. Nat. Prod.* **2003**, *66*, 132–135.
[3] D. R. Appleton, M. A. Sewell, M. V. Berridge, B. R. Copp, *J. Nat. Prod.* **2002**, *65*, 630–631.
[4] Compound **3** is the major fatty acid substructure in malyngamides; for a review, see reference [1]; a) L. T. Tan, T. Okino, W. H. Gerwick, *J. Nat. Prod.* **2000**, *63*, 952–955; b) L. M. Nogle, W. H. Gerwick, *J. Nat. Prod.* **2003**, *66*, 217–220; c) K. E. Milligan, B. Márquez, R. T. Williamson, M. Davies-Coleman, W. H. Gerwick, *J. Nat. Prod.* **2000**, *63*, 965–968; d) Y. Kan, B. Sakamoto, T. Fujita, H. Nagai, *J. Nat. Prod.* **2000**, *63*, 1599–1602.
[5] a) J. S. Mynderse, R. E. Moore, *J. Org. Chem.* **1978**, *43*, 4359–4363; b) W. H. Gerwick, S. Reyes, B. Alvarado, *Phytochemistry* **1978**, *17*, 1701–1704.
[6] F. Wan, K. L. Erickson, *J. Nat. Prod.* **1999**, *62*, 1696–1699.
[7] W. Gallimore, P. J. Scheuer, *J. Nat. Prod.* **2000**, *63*, 1422–1424.
[8] S. Suntornchashwej, N. Chaichit, M. Isobe, K. Suwanborirux, *J. Nat. Prod.* **2005**, *68*, 951–955.
[9] The tripeptide moiety **1** was numbered with respect to the neurotoxin janolusimide (**6**), a very similar tripeptide; a) G. Sodano, A. Spinella, *Tetrahedron Lett.* **1986**, *27*, 2505–2508; b) A. Giordano, A. Spinella, G. Sodano, *Tetrahedron: Asymmetry* **1999**, *10*, 1851–1854; c) A. Giordano, C. D. Monica, F. Landi, A. Spinella, G. Sodano, *Tetrahedron Lett.* **2000**, *41*, 3979–3982.
[10] J. H. Cardellina, D. Dalietos, F.-J. Marner, J. S. Mynderse, R. E. Moore, *Phytochemistry* **1978**, *17*, 2091–2095.
[11] D. L. Boger, H. Cai, *Angew. Chem.* **1999**, *111*, 470–500; *Angew. Chem. Int. Ed.* **1999**, *38*, 448–476.
[12] For a review of naturally occurring 4-methoxy-$\Delta^3$-pyrrolin-2-one, see: B. J. L. Royles, *Chem. Rev.* **1995**, *95*, 1981–2001.
[13] For α-methyl-β-hydroxy carbonyl compounds, the observed vicinal coupling constants for *anti* $C_\alpha/C_\beta$ protons is large ($^3J_{H,H}$=7–12 Hz), whereas that for the *gauche* $C_\alpha/C_\beta$ protons is small ($^3J_{H,H}$=0–4 Hz); a) C. H. Heathcock, M. C. Pirrung, J. E. Sohn, *J. Org. Chem.* **1979**, *44*, 4294–4299; b) S. Paik, S. Carmeli, J. Cullingham, R. E. Moore, G. M. L. Patterson, M. A. Tius, *J. Am. Chem. Soc.* **1994**, *116*, 8116–8125.
[14] If $^3J_{H,H}$ is a value typical for an *anti* configuration, the NOE analysis should be reliable, as no significant conformational change takes place; N. Matsumori, D. Kaneno, M. Murata, H. Nakamura, K. Tachibana, *J. Org. Chem.* **1999**, *64*, 866–876.




H/H *anti* configuration of 7*S**,8*R**




H/H *anti* configuration of 7*S**,8*S**

[15] I. Ohtani, T. Kusumi, Y. Kashman, H. Kakisawa, *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
[16] The method pioneered by Pirkle and Beare follows from the possibility of relating the sense of chemical shift nonequivalence (upfield vs. downfield of a signal) for one enantiomer of a compound dissolved in an optically active solvent; see, for instance: a) W. H. Pirkle, S. D. Beare, *J. Am. Chem. Soc.* **1969**, *91*, 5150–5155; b) W. H. Pirkle, C. W. Boeder, *J. Org. Chem.* **1977**, *42*, 3697–3700; c) D. Parker, *Chem. Rev.* **1991**, *91*, 1441–1457; d) T. J. Wenzel, J. D. Wilcox, *Chirality* **2003**, *15*, 256–270.
[17] There are very few reports about the use of CSAs for absolute configuration assignment; a) S. Latypov, X. Franck, J.-C. Jullian, R. Hocquemiller, B. Figadére, *Chem. Eur. J.* **2002**, *8*, 5662–5666; b) J.-C. Jullian, X. Franck, S. Latypov, R. Hocquemiller, B. Figadére, *Tetrahedron: Asymmetry* **2003**, *14*, 963–966; c) R. Stipanovic, J. P. McCormick, E. O. Schlemper, B. C. Hamper, T. Shinmyozu, W. H. Pirkle, *J. Org. Chem.* **1986**, *51*, 2500–2504.
[18] The interaction between TFAE and the substrate was not as strong as the covalent bond of the MTPA ester in the modified Mosher method. The observed $\Delta\delta_{RS}$ values were thus the average shifts of the signals of the diastereomeric complexes that rapidly equilibrate with the bulk of cosolvent on the NMR timescale.
[19] K. Akaji, Y. Hayashi, Y. Kiso, N. Kuriyama, *J. Org. Chem.* **1999**, *64*, 405–411.
[20] For absolute configuration assignment with aid from conformational calculations, see, for example: a) M. Ubutaka, X.-C. Cheng, M. Isobe, K. Isono, *J. Chem. Soc. Perkin Trans. 1* **1993**, 617–624; b) H. Luesch, W. Y. Yoshida, R. E. Moore, V. J. Paul, T. H. Corbett, *J. Am. Chem. Soc.* **2001**, *123*, 5418–5423; c) R. T. Williamson, A. Boulanger, A. Vulpanovici, M. A. Roberts, W. H. Gerwick, *J. Org. Chem.* **2002**, *67*, 7927–7936; d) P. G. Williams, W. Y. Yoshida, M. K. Quon, R. E. Moore, V. J. Paul, *J. Nat. Prod.* **2003**, *66*, 651–654.
[21] One conformer of the 2*R* isomer was found that showed an NOE of 3.5 Å between 1-$H_3$ and 8-H.
[22] The biosynthetic considerations have recently been employed to solve the stereochemistry of C7′; see: Y. Kan, T. Fujita, H. Nagai, B. Sakamoto, Y. Hokama, *J. Nat. Prod.* **1998**, *61*, 152–155, and reference [7].
[23] We recently completed the total synthesis of (7′*R*)-**1**. The specific rotation of natural **1**, $[\alpha]_D=-6.2$ ($c$=0.8, MeOH), matched that of (7′*R*)-**1**, $[\alpha]_D=-5.9$ (c=0.8, MeOH), but differed from that of (7′*S*)-**1**, $[\alpha]_D=-15.4$ ($c$=0.8, MeOH). This difference must be due to the large distance between the C7′ stereogenic center and the other five stereogenic centers in the tripeptide portion. The consistent result in the NMR chiral solvation experiments was obtained with synthetic (7′*R*)-**1**. The details for the total synthesis of both (7′*R*)- and (7′*S*)-

malyngamide X and the NMR chiral solvation experiments will be reported elsewhere.
[24] V. Mesguiche, R. Valls, L. Piovetti, G. Peiffer, *Tetrahedron Lett.* **1999**, *40*, 7473–7476.
[25] M. Wu, K. E. Milligan, W. H. Gerwick, *Tetrahedron* **1997**, *53*, 15983–15990.
[26] About 10–20 mg quantities of the malyngamides were used for hydrolysis to provide a proper amount of the hydrolyzed products for $[\alpha]_D$ measurements.
[27] N. M. Carballeira, M. Pagán, *J. Nat. Prod.* **2001**, *64*, 620–623.
[28] M. A. Walker, C. H. Heathcock, *J. Org. Chem.* **1991**, *56*, 5747–5750.
[29] L. Collins, S. G. Franzblau, *Antimicrob. Agents Chemother.* **1997**, *41*, 1004–1009.
[30] a) W. Trager, J. B. Jensen, *Science* **1976**, *193*, 673–675; b) R. E. Desjardins, C. J. Canfield, J. D. Haynes, J. D. Chulay, *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.
[31] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.

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