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# Total Synthesis and Stereochemical Reassignment of Bisebromoamide

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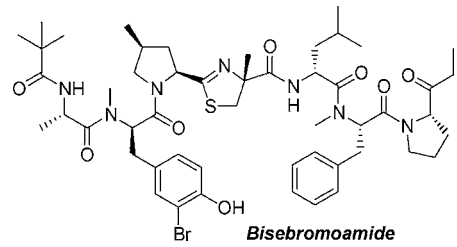
## ABSTRACT




**A revised configurational assignment for the thiazoline moiety of the marine peptide bisebromoamide is proposed and validated by total synthesis.**

Marine cyanobacteria have produced a wide variety of natural products, many of which have shown striking biological activity.[1] Consequently, they are key synthetic targets in the quest for new leads in the pharmaceutical industry. One particular species, *Lyngbya*, is responsible for the production of many varied metabolites, the biological activity of which ranges from anticancer and antiviral to antifungal activity. Unusual effects include immunosuppression and antifeedant properties.[1d−f] These compounds have potential applications as therapeutic agents, and this has driven our group to synthesize a range of these metabolites, with the aim of structure modification for fine-tuning biological activity. We have been interested for some time in marine secondary metabolites and view their syntheses as a key route to structural confirmation, structural modification, and subsequent activity control.[2] Here we report the first total synthesis and revised stereochemical assignment of bisebromoamide.

Bisebromoamide is a thiazoline-containing marine peptide isolated by Suenaga and co-workers[3] in 2009 from the marine cyanobacterium *Lyngbya* sp. harvested in the Okinawa

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Prefecture. On the basis of extensive spectroscopic investigations, chemical degradation, and derivatization studies, planar structure **1**, depicted in Figure 1, was assigned as bisebromoamide. In addition to the amino acid L-alanine, bisebromoamide contains a significant number of D-amino acids and *N*-methylated amino acids along with several other modified amino acid residues that include D-leucine, *N*-methyl-3-bromotyrosine, modified 4-methylproline, a 2-substituted thiazoline-4-methyl-4-carboxylic acid unit, *N*-methylphenylalanine, and a rare 2-(1-oxopropyl)pyrrolidine moiety which has not been previously reported in any natural product. Furthermore, and to the best of our knowledge, there is no report on the total synthesis of bisebromoamide.

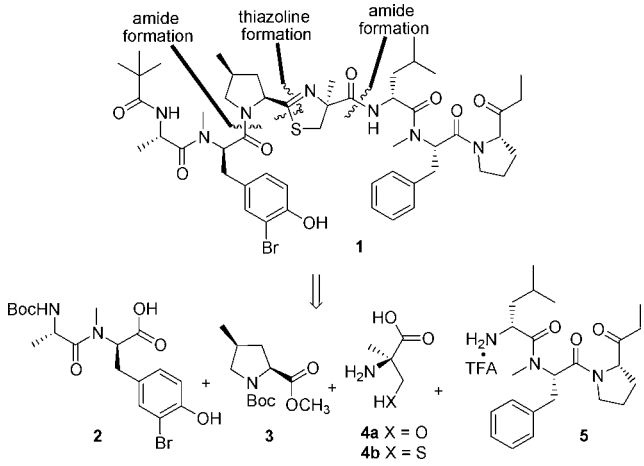
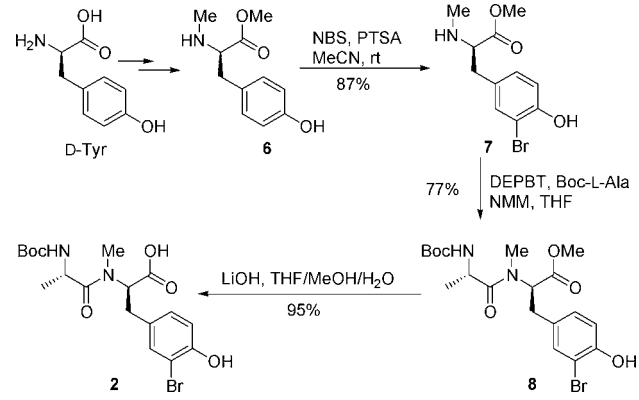



**Figure 1.** Retrosynthetic analysis of **1**.

The principal synthetic challenge associated with the preparation of bisebromoamide is the efficient formation and assembly of the sensitive thiazoline heterocycle.[4] From the retrosynthetic perspective, we envisioned that the thiazoline-ring moiety could arise from either 2-methylserine or 2-methylcysteine derivatives and be installed at a late stage in the synthesis. Bearing this in mind, we anticipated that **1** might be conveniently constructed by the assembly of four fragments: dipeptide **2**, *N*-Boc-4-methylproline methyl ester **3**, 2-methylserine **4a** (or 2-methylcysteine **4b**), and tripeptidyl ketone **5**.

While subunits *N*-Boc-4-methylproline methyl ester **3**[5] and 2-methylserine **4a**[6] (or 2-methylcysteine **4b**[7]) could be fully synthesized according to literature procedures, peptide-based fragments **2** and **5** were unknown and had to be elaborated from amino acids as shown in Schemes 1 and 2. The synthesis of fragment **2** (Scheme 1) began with the known *N*-methyl D-tyrosine methyl ester **6**, which was prepared according to the procedure of Boger[8] from D-tyrosine. *Ortho*-selective monobromination of **6** was effected with *N*-bromosuccinimide and *p*-toluenesulfonic acid in acetonitrile[9] to afford **7** in 87% yield. Coupling of **7** with Boc-L-Ala-OH was achieved through carboxyl activation with 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT)[10] and provided dipeptide **8** in 77% yield. Saponification of the methyl ester of **8** gave the corresponding acid **2** in 95% yield.

**Scheme 1.** Synthesis of Dipeptide **2**




The synthesis of fragment **5** commenced with the condensation of the known *N*-methyl L-phenylalanine methyl ester **9**[11] with Boc-D-leucine (Scheme 2). Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl)[12] was employed to effect the condensation process, and dipeptide **10** was obtained in 86% yield. Amino ethyl ketone **11** was easily prepared by addition of ethylmagnesium bromide to the Boc-protected proline-derived Weinreb amide according to published procedures.[13] Hydrolysis of the methyl ester of **10** followed by a DEPBT-mediated condensation with amine **11** afforded the corresponding tripeptide. Next, cleavage of the Boc group with trifluoroacetic acid provided fragment **5** as the corresponding ammonium trifluoroacetate salt in 74% yield (over three steps).

At this juncture, the time had arrived to explore a suitable strategy for the assembly of fragments and installation of the sensitive thiazoline heterocycle. In the initial phases of our study, Cbz-protected 2-methylserine (**15**) and dipeptide **10** were employed to examine the feasibility and efficiency of the important thiazoline-forming reaction from the corresponding

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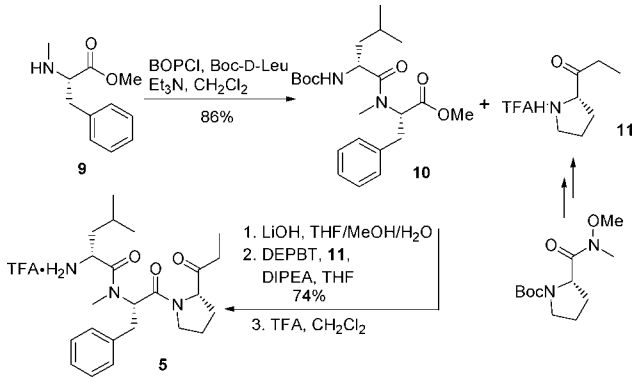
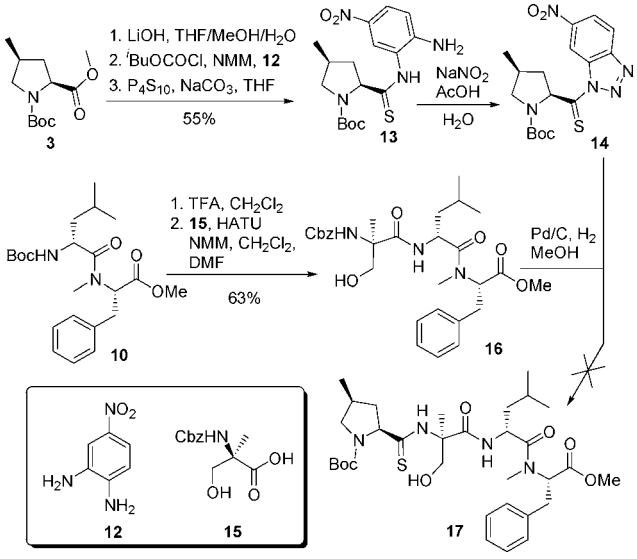
**Scheme 2.** Synthesis of Tripeptide **5**



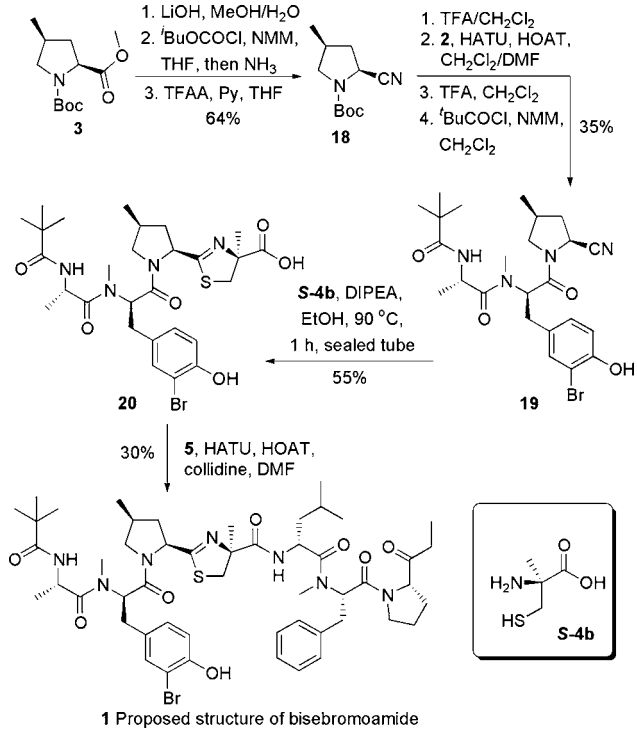

**Scheme 3.** Attempted Synthesis Toward Thiopeptide **17**




$\beta$-hydroxy thiopeptide (Scheme 3). Thus, saponification of the methyl ester of **3** was followed by coupling with diamine **12** via a mixed anhydride to afford an amide which was then converted into a corresponding thioamide **13** in 55% yield over three steps. Thioamide **13** was then converted into the corresponding α-amino thionoacid derivative of nitrobenzotriazole (**14**) via diazotization as described by Rapoport.[14] Treatment of dipeptide **10** with trifluoroacetic acid effected the removal of the Boc protecting group to give the corresponding amine that underwent a HATU[15]-mediated coupling reaction with Cbz-protected 2-methylserine (**15**) to provide **16** in 63% yield. Hydrogenolysis of the Cbz protecting group of **16** cleanly afforded the amine, which was immediately treated with excess thioacylating agent **14** to effect the formation of thioamide **17**.

To our disappointment and surprise, the coupling conditions failed to produce the desired thioamide **17**. To rationalize this result, we postulate that there exists a severe steric interaction between the thioacylating agent and the sterically demanding amine-bearing quaternary center.

**Scheme 4.** Synthesis of Proposed Structure (**1**)




Due to the difficulties in forming the thiazoline heterocycle using cyclodehydration of a $\beta$-hydroxy thioamide-based approach, we altered our synthetic route by constructing the thiazoline moiety via cyclocondensation of peptide-derived nitrile **19** and 2-methylcysteine **4b**. Although the revised strategy was designed to circumvent the problem encountered in the formation of the thiazoline ring from $\beta$-hydroxy thioamide, this strategy might have a potential drawback as we do not know whether the cyclocondensation process and/or the coupling of thiazoline-containing acid **20** with tripeptide **5** would result in any epimerization of stereogenic centers presented at two pyrrolidine moieties. In the event, *N*-Boc-4-methylproline methyl ester **3** was converted into the corresponding nitrile **18** according to a published procedure.[2f,16] Removal of the Boc group of **18** followed by a HATU-mediated condensation with dipeptide **2** furnished the corresponding peptide that was then resubjected to a TFA-mediated Boc deprotection, and the resulting amine was acylated with pivaloyl chloride to afford nitrile **19** in 35% yield (over four steps, Scheme

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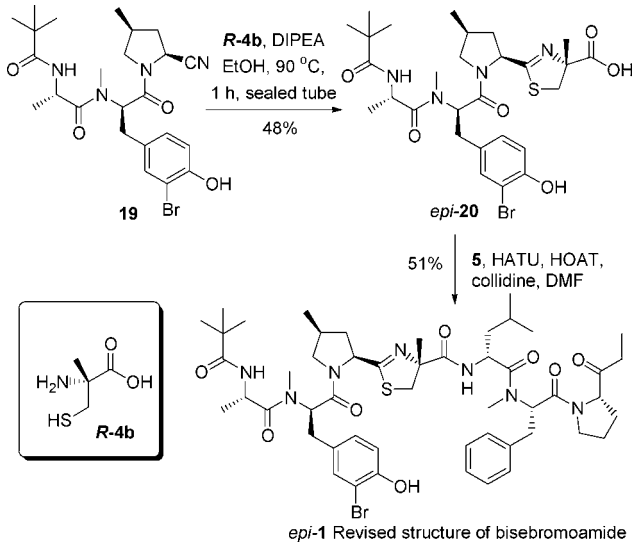
4). This set the stage for the cyclocondensation. Gratifyingly, condensation reaction between the nitrile **19** and the (*S*)-2-methylcysteine (***S*-4b**) in a sealed tube at 90 °C led to the key intermediate **20** as a single isomer in 55% yield. Finally, carboxyl activation of **20** with a HATU/collidine system and coupling with fragment **5** produced the proposed structure (**1**) for bisebromoamide in 30% yield. Unfortunately, neither $^{1}$H nor $^{13}$C NMR spectra for **1** were identical with those of the natural product. In addition, the optical rotation was of equal magnitude but of opposite sign [natural bisebromoamide $[\alpha]_D^{22}$ +17.8 (*c* 1.00, $CHCl_3$); synthetic sample $[\alpha]_D^{20}$ − 19.0 (*c* 0.31, $CHCl_3$)]. Taken together, these data suggested that the reported structure (**1**) must be incorrect.

A thorough examination of $^{1}$H and $^{13}$C NMR spectra of **1** and comparison of reported spectra for natural bisebromoamide revealed that the discrepancies of chemical shifts are mostly located in the thiazoline ring region, particularly $^{1}$H NMR chemical shifts of the $CH_2$ at C-5 of the thiazoline ring and the $^{13}$C NMR chemical shifts at carbons at and adjacent to the thiazoline ring. The actual structure of bisebromoamide appeared to be epimeric to the proposed structure at one or more stereocenters in or adjacent to the thiazoline ring system.

**Scheme 5.** Synthesis of Revised Structure (*epi*-**1**)




On the basis of these chemical shift variations, we hypothesized that the stereogenic center at the thiazoline ring of the originally proposed bisebromoamide structure might require revision. We therefore elected to synthesize the epimer (with *R*-configuration of the stereogenic center at the thiazoline ring) of the proposed structure **1**. As shown in Scheme 5, we synthesized *epi*-**1** following the same synthesis as for **1**, but incorporating (*R*)-2-methylcysteine (***R*-4b**) to the thiazoline moiety. This was readily achieved, and *epi*-**20** was incorporated into the final synthesis as previously performed to afford *epi*-**1** in 51% yield. To our delight, the synthetic bisebromoamide's (*epi*-**1**, $[\alpha]_D^{20}$ + 15.9 (*c* 0.48, $CHCl_3$)) spectral data ($^{1}$H and $^{13}$C NMR) are identical to those of the natural bisebromoamide.

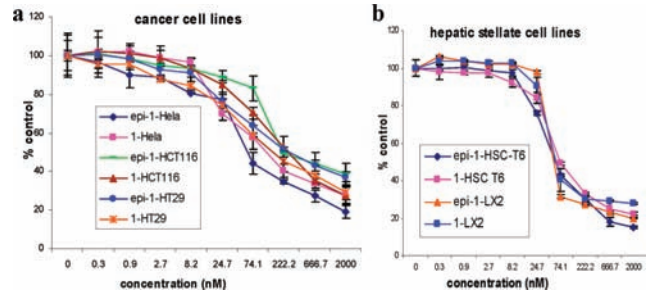



**Figure 2.** Effect of compounds **1** and *epi*-**1** on cancer cell and hepatic stellate cell proliferation.

The initial cytotoxicity evaluation of **1** and *epi*-**1** was performed across a panel of three cancer cell lines of different histological origins (HeLa, cervix; HT29, colon; and HCT-116, colon), and cell proliferation was measured by MTS assay. The $IC_{50}$ values of **1** were 43.1 nM (Hela), 62.9 nM (HT29), and 134.2 nM (HCT116), and the $IC_{50}$ values of *epi*-**1** were 48.8 nM (Hela), 55.1 nM (HT29), and 115 nM (HCT116). These data indicated that both **1** and *epi*-**1** inhibited the proliferation of cancer cells at comparable levels of potency, with $IC_{50}$ values slightly higher than these reported for natural bisebromoamide. These results led us to believe that stereochemistry at the thiazoline moiety of bisebromoamide may not be an important factor for its bioactivities. We also expanded the scope of our tests by screening the effect of **1** and *epi*-**1** on hepatic stellate cells (LX-2 and HSCT6) (Figure 2). The $IC_{50}$ values of **1** were 42.3 nM (LX-2) and 53.4 nM (HSCT6), which were also comparable to the $IC_{50}$ values of *epi*-**1** [45.4 nM (LX-2) and 46.4 nM (HSCT6)].

In summary, the first total synthesis of bisebromoamide was completed, leading to a revision of the reported stereochemistry from structure **1** to *epi*-**1**.

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**Note Added after ASAP Publication.** Scheme 4 contained an error in the version published ASAP June 8, 2010; the correct version reposted June 11, 2010.

**Supporting Information Available:** Full details for experimental procedures for compounds **1**, *epi*-**1**, **2**, **5**, **7**, **8**, **10**, **11**, **13**, **14**, **16**, **19**, **20**, *epi*-**20**, and **S1**−**S3** and $^{1}$H and $^{13}$C NMR spectra for compounds **1**, *epi*-**1**, **7**, **8**, **10**, **13**, **16**, **19**, **20**, *epi*-**20**, and **S1**−**S3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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