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Total synthesis and cytotoxicity of bisebromoamide and its analogues

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ABSTRACT

A highly convergent route for assembling bisebromoamide, its stereoisomers and a simplified analogue has been accomplished, which features connecting its left part and right part via thiazoline ring formation at the final stage. Preliminary biological studies revealed that compounds with both proposed and revised structures, and a simplified analogue have similar potency against the proliferation of HeLa S_3 cell, indicating that the stereochemistry of methylthiazoline part, and methyl group at the 4-methylproline residue in bisebromoamide, have limited influence on its cytotoxicity.

Bisebromoamide (**1**, Fig. 1) is a potent cytotoxic linear peptide that was isolated from the marine cyanobacterium *Lyngbya* sp. by Suenaga and co-workers last year.[1] Biological studies revealed that bisebromoamide could selectively inhibit the phosphorylation of ERK in NRK cells by PDGF (platet-derived growth factor)-stimulation at 10 to 0.1 μM, without an effect on the phosphorylation of AKT, PKD, PLCγ1 or S6 ribosomal protein at the same concentration. Furthermore, this peptide exhibited cytotoxicity against HeLa S_3 cells with an IC_{50} value of 0.04 μg/mL, as well as a panel of 39 human cancer cell lines at the Japanese Foundation for Cancer Research with an average GI_{50} value of 40 nM.[1]

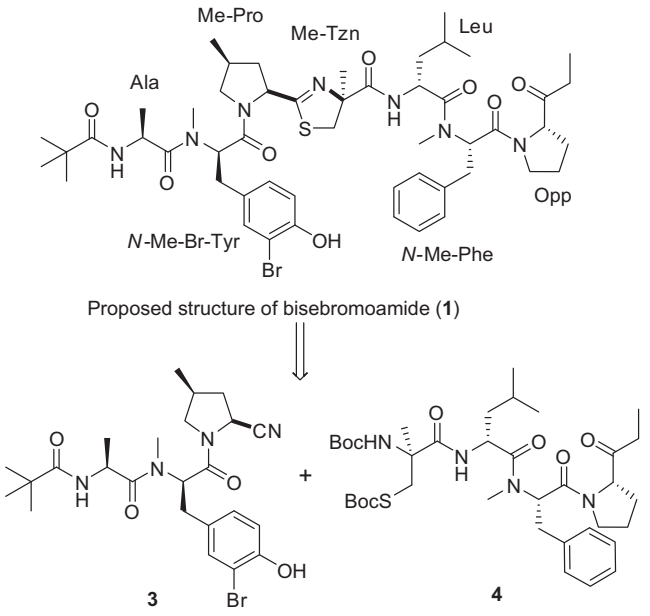

Figure 1. Proposed structure of bisebromoamide and its retrosynthetic analysis.

Structurally, bisebromoamide contains seven amino acid residues, namely, *N*-pivaloyl-alanine, *N*-methyl-3-bromo-tyrosine (*N*-Me-Br-Tyr), 4-methylproline (Me-Pro), 2-methylcystine (Me-Cys), leucine (Leu), *N*-methylphenyl-alanine (*N*-Me-Phe) and 2-(1-oxopropyl)pyrrolidine (Opp). Among them, 4-methylproline is connected with 2-methylcystine through methylthiazoline (Me-Tzn). The stereochemistry of bisebromoamide was assigned by chemical degradation and treatment with Marfey's reagent followed by comparison of HPLC retention times with the authentic samples. A foreshadowing of the problems regarding the structure was that the configuration of the methylthiazoline in the initial Letter was *S*, which was inconsistent with that observed in other methylthiazoline-containing natural products, such as thiangazole,[2] halipeptins[3,5a] and largazole.[4]

As a continuing effort on the total synthesis and structure–activity relationship (SAR) studies of complex natural peptides,[5] we wish to report here our synthesis and preliminary SAR results of bisebromoamide. Our studies confirm the structural revision of this natural peptide, which was made by Ye and co-workers in their recently disclosed total synthesis.[6]

Our synthetic strategy is outlined in Figure 1. Given that the methylthiazoline is sensitive to both acid and base,[7] we planned to construct this unit at the late stage of the synthesis. Accordingly, bisebromoamide was degraded to two units with similar complexity, cyanide **3** and protected aminothiol **4**. Further amide bond disconnection led to smaller amino acid fragments. This highly convergent and flexible route would allow the preparation of its analogues, thereby facilitating the subsequent studies on structure–activity relationship.

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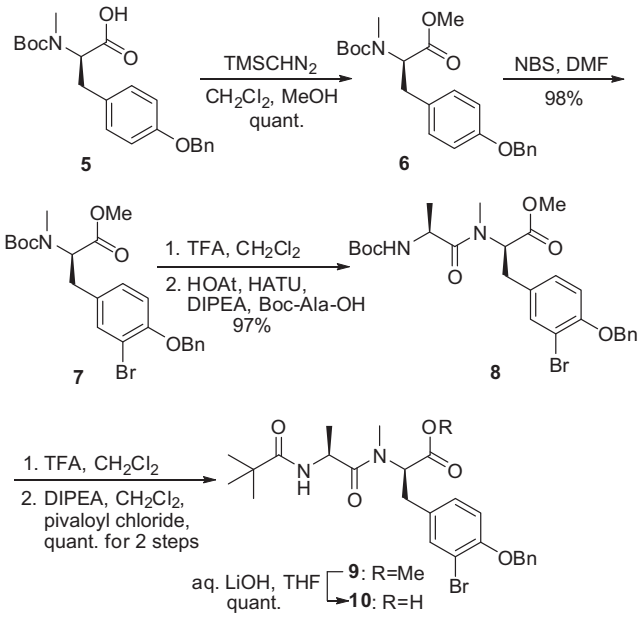
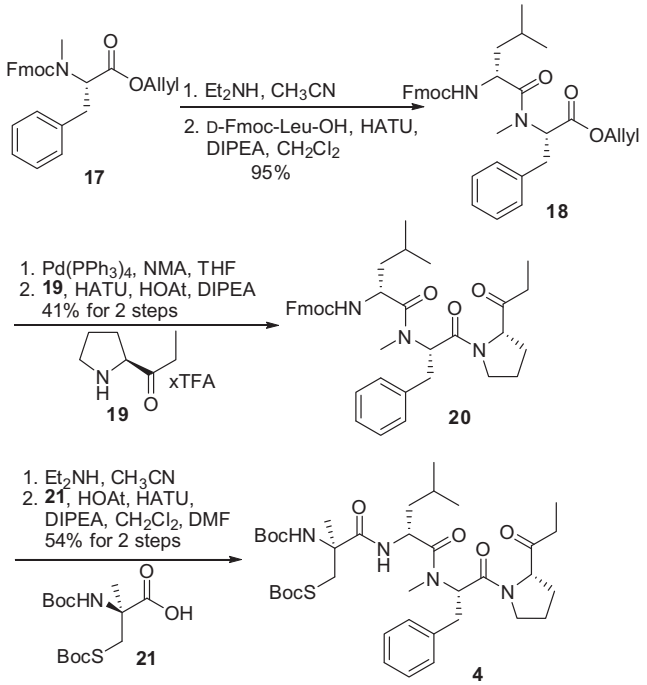


Scheme 1.



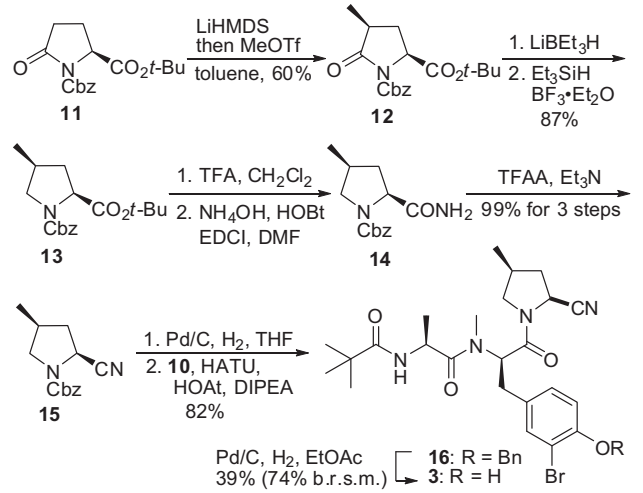
Scheme 3.

Our synthesis commenced with preparation of *N*-methyl-3-bromotyrosine (Scheme 1). Methylation of commercially available tyrosine derivative **5** followed by bromination with NBS provided fully protected *N*-methyl-3-bromo-tyrosine **7**. After removal of Boc group with TFA, the liberated amine was coupled with Boc-Ala-OH under the action of HATU and HOAt to give dipeptide **8**. Switching the protecting group from Boc to Piv delivered amide **9**, which was hydrolysed to give acid **10**.

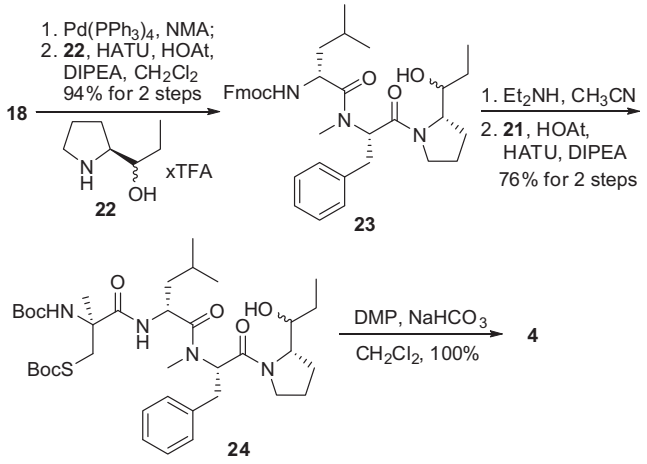
The assembly of cyanide **3** is illustrated in Scheme 2. Methylation of pyroglutamic acid derivative **11**[8] gave lactam **12** with *syn* configuration.[9] The lactam **12** was then reduced to cyclic amine **13** via treatment with $LiBEt_3H$ and Et_3SiH successively.[10] The *tert*-butyl ester **13** was converted to cyanide **15** via deprotection, primary amide formation and subsequent dehydration.[11] The *syn* configuration was secured by the X-ray analysis of a sulfonylamide derivative of **15**. Hydrogenation of **15** followed by condensation of the liberated amine with acid **10** afforded tripeptide **16**, which was hydrogenated carefully in ethyl acetate to generate the desired cyanide **3** with 39% yield (74% yield based on recovered starting material). Noteworthy is that hydrogenation in protic solvents led to formation of a debromination product.

We next turned our attention to the synthesis of the aminothiol fragment **4**. As depicted in Scheme 3, after Fmoc removal of phenylalanine derivative **17**, coupling with D-Fmoc-Leu-OH was conducted in the presence of HATU to produce dipeptide **18**. After deallylation via Pd chemistry, the liberated carboxylic acid was condensed with TFA salt **19** that was prepared from its corresponding Boc-protected precursor,[12] to provide tripeptide **20** in a moderate yield. The free amine liberated from tripeptide **20** with Et_2NH was condensed with the free acid (*S*)-**21**,[13] to generate the desired protected aminothiol **4** in 54% yield.

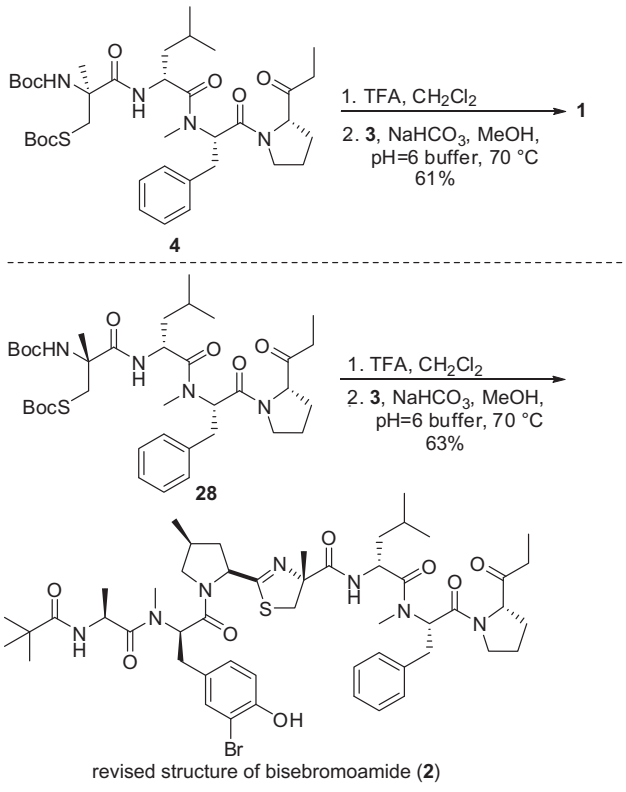
Although the protected aminothiol **4** was obtained successfully, the yields for last two condensation steps were not satisfactory. We believed that this problem should result from reactive ketone moiety. Therefore, an alternative route to **4** was developed, as shown in Scheme 4. At this stage we employed amino alcohol **22**



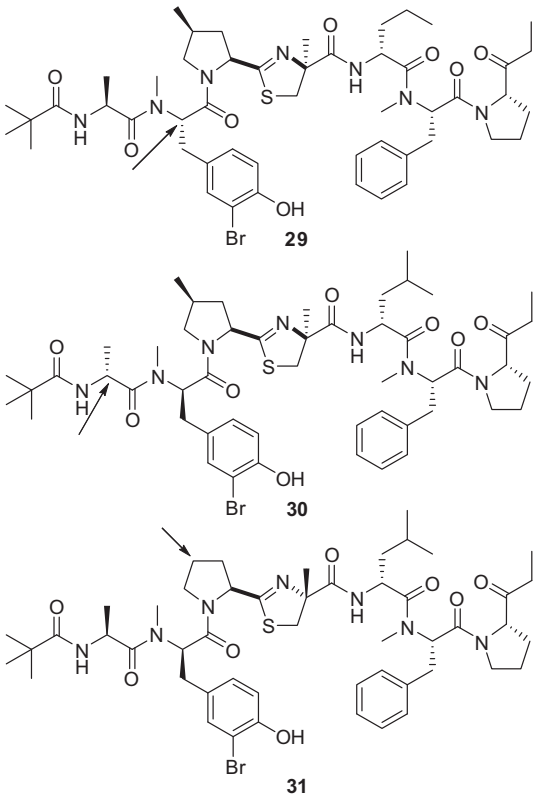
Scheme 2.



Scheme 4.



Scheme 5.



Scheme 6.

(prepared from **19** via $NaBH_4$ reduction) as a coupling partner. The same coupling sequence was carried out to produce tetrapeptide **24** with a good yield. The tetrapeptide **24** was then oxidized with Dess–Martin reagent to give **4** in quantitative yield.

With two key fragments in hand, we completed the synthesis of bisebromoamide as outlined in Scheme 5. Treatment of the protected aminothiol **4** with TFA gave free aminothiol, which was condensed with cyanide **3** in the presence of $NaHCO_3$ in a mixture of methanol and buffer to deliver the target molecule **1**.[14] However, spectroscopic data for **1** did not match the characterization data reported for natural bisebromoamide. After careful comparison of our data with those reported, we found disturbing discrepancies in regard to the positions of 4-methylproline, 2-methylcystine and leucine residues. The different configuration in the methylthiazoline part between **1** and other methylthiazoline-containing natural products[2–4] suggested that the proposed stereochemistry at this position for bisebromoamide might be wrong. Thus, peptide **28** was prepared from (*R*)-**21** following the same procedure, and reacted with the cyanide **3**, after deprotection, to afford **2**. To our delight, the spectroscopic data for **2** showed unambiguous agreement with those of nature one, which indicated that the structure of bisebromoamide should be revised to **2**.

In order to check if the stereochemistry of other amino acid residues, and the methyl group at the 4-methylproline residue play some roles for maintaining the cytotoxicity of bisebromoamide. Two other isomers **29** and **30**, and a simplified analogue **31** of bisebromoamide were assembled from suitable intermediates following a similar procedure as described above (Scheme 6). All our synthetic compounds were evaluated as inhibitors against the proliferation of HeLa S_3 cell, and the results were summarized in Table 1. Surprisingly, peptide **1** was even more potent than bisebromoamide **2** in our assay. This result demonstrated that the stereochemistry at the methylthiazoline part of bisebromoamide has limited influence to its cytotoxicity. Similar trend was seen by changing the stereochemistry of the *N*-Me–Br-Tyr residue, as evident from compound **29** that has an IC_{50} value of 0.296 μg/mL. However, changing the configuration of the alanine residue led to losing cytotoxicity because compound **30** was found inactive even at the concentration of 8 μg/mL, indicating that the stereochemistry of this residue plays a key role for maintaining the cytotoxicity. More interestingly, simplified analogue **31** was found as potent as bisebromoamide, and, thereby delivering a valuable lead for further SAR studies because its synthesis is easier than bisebromoamide.

Table 1
In vitro cytotoxicity data for synthetic bisebromoamide and its analogues towards HeLa S_3 cell lines

Compound	IC_{50} (μg/mL)
1	0.038
2	0.165
29	0.296
30	—[a]
31	0.134

[a] Inactive at 8 μg/mL.

In conclusion, we have achieved the total synthesis and structural revision of bisebromoamide by developing a highly convergent route. Accompanying the total synthesis its SAR was preliminarily explored. These results should be of benefit to the further SAR studies of this relatively simple but potent antitumor agent.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.11.058.

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