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Total Synthesis and Structure Revision of Boholamide A

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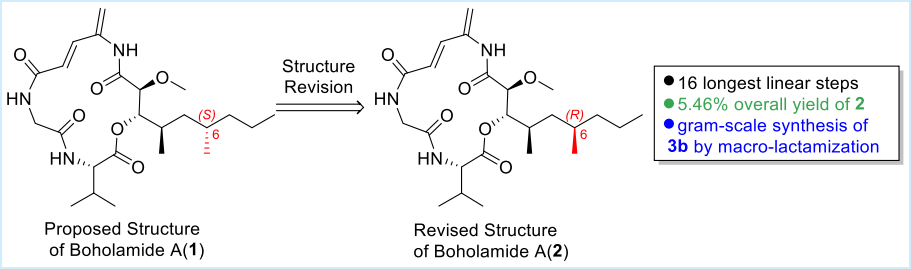

ABSTRACT: The 15-membered cyclic depsipeptide boholamide A and an epimer were prepared by total synthesis for the first time, thus leading to a revision of C6 stereochemistry in the originally proposed structure of natural boholamide A. This convergent route features achievement of a macro-lactamization step in a gram scale. The revised boholamide A was sythesized with 16 linear steps in 5.46% overall yield. This work facilitates the investigations of boholamide A as a potential hypoxia-selective anticancer agent.

The anticancer natural product, boholamide A, was originally isolated from a *Truncatella* sp. mollusk. It was named after the source island in the Philippines.[1]

Boholamide A, a macrocyclic depsipeptide, features a 4-amino-2,4-pentadienolate (APD) moiety. The unique APD moiety is present in a few natural products, such as rakicidin (A, B, C, D, E, F, G, H and I),[2−7] vinylamycin,[8] microtermolide A,[9] and BE-43547[10] (Figure 1). Because of their novel structure, selective inhibition activity against a variety of cancer cells under hypoxia conditions, and the lethal effect on cancer stem cells, they drew our attention[11−17] and that of other research groups[18−24] in recent years.

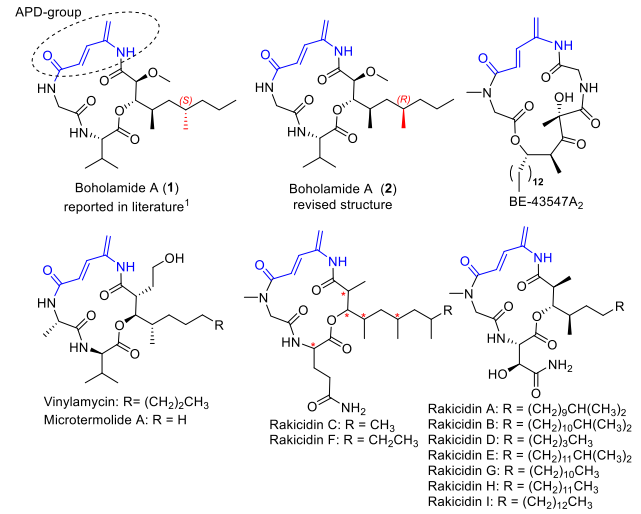

Figure 1. Structures of cyclodepsipeptides containing APD moiety.

The isolation of boholamide A was very difficult, and the amount was too little to accomplish more biological evaluation (2.2 mg from Marine Broth 15 L).[1] The cytotoxicity of boholamide A with IC_{50} values ranging from 100 to 400 nM[1] was comparable with rakicidin A (IC_{50} = 200−700 nM).[11] Boholamide A has better inhibition activity structure compared to other APD nature products, such as rakicidin A, vinylamycin, and BE-43547A_2. It is worth noting that boholamide A is the first reported cyclic depsipeptide containing APD moiety with a methoxy group at C-2, and it still maintains hypoxia-selectivity against many cancer cells. The unique structure and interesting biological activity prompted us to achieve its total synthesis for exploring its potential biological activity.

The retrosynthetic analysis of boholamide A is shown in Scheme 1. Compound **1**/**2** would be derived from alcohol **3a**/**3b**. Coupling of Boc-L-valine **6**, ester **5a**/**5b**, and amine **7** was proposed for the synthesis of alcohol **3a**/**3b**. Ester **5a**/**5b** may be prepared from the chiral aldehyde **9a**/**9b** and D-camphorsultam derivative **8**. Amine **7** would be prepared from L-serine derivative **20**.

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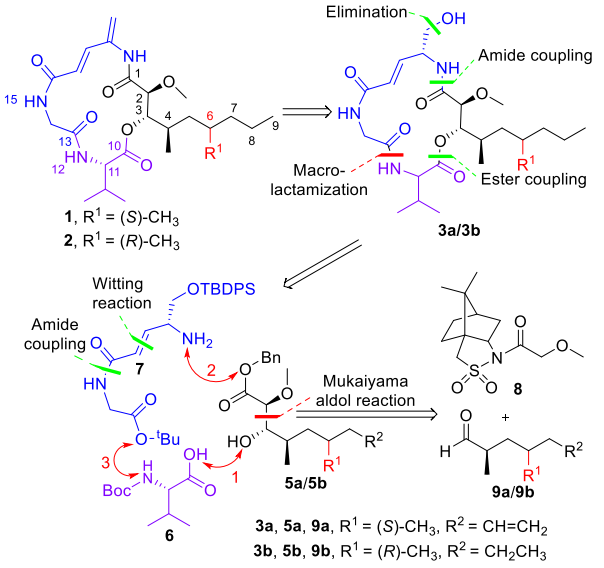
Scheme 1. Retrosynthetic Analysis of Proposed and Revised Structure of Boholamide A



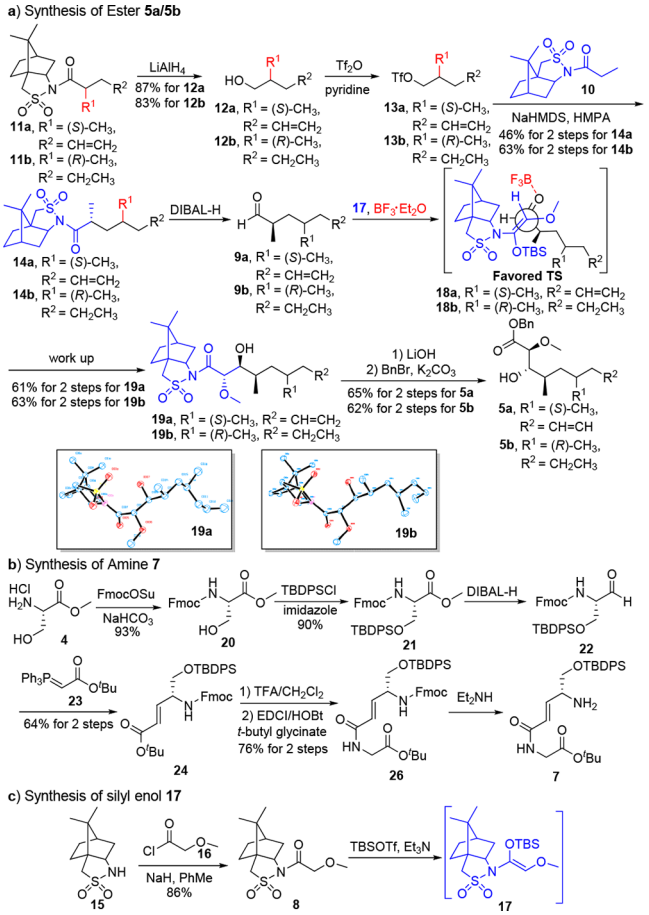
Synthesis of aldehyde **9a**/**9b** started with known camphorsultam derivatives **11a**/**11b**.[14,25] **11a**/**11b** was reduced with $LiAlH_4$ followed by treatment with Tf_2O to afford triflate **13a**/**13b**, which was subjected to asymmetric alkylation with the enolate generated from camphorsultam derivative **10** and gave rise to **14a**/**14b** (*dr* > 30:1, according to ^1H NMR) in two steps, installing the required 2,4-dimethyl stereochemistry.[26] Treatment of **14a**/**14b** with DIBAL-H produced aldehyde **9a**/**9b**. With aldehyde **9a**/**9b** in hand, we started the construction of the key chirality of boholamide A. Different from the structure of compounds such as vinylamycin and rakicidins, the methoxy group in C-2 may affect the transition state of the chiral configuration of C-2 and C-3.[27] Silyl enol ether **17** (Scheme 2c) and BF_3-mediated aldehyde **9** were used to synthesize via a Mukaiyama aldol reaction.[28] In the case of silyl enol ether **17**, the BF_3-mediated reaction was proposed to proceed through open transition state **18a**/**18b** and the absolute configuration of **19a**/**19b** was confirmed by X-ray analysis (CCDC 2080746−2080747). The aldol product **19a**/**19b** was hydrolyzed with LiOH, and the resulting carboxylic acid was then treated with K_2CO_3 and BnBr to achieve the ester **5a**/**5b** (Scheme 2a).[18]

Synthesis of amine **7** started with known L-serine derivative **4**. The TBDPS group was used to protect the hydroxyl in **20**. The aldehyde **22** produced by reduction of **21** with DIBAL-H was used directly for a Witting reaction to obtain the fragment **24** in a yield of 64% in two steps. Deprotection of fragment **24** produced carboxylic acid, followed by amide coupling with *tert*-butyl glycinate to obtain compound **26** in a yield of 76% in two steps. Removing the Fmoc group by Et_2NH afforded amine **7** (Scheme 2b), which was used in the next step without further purification.

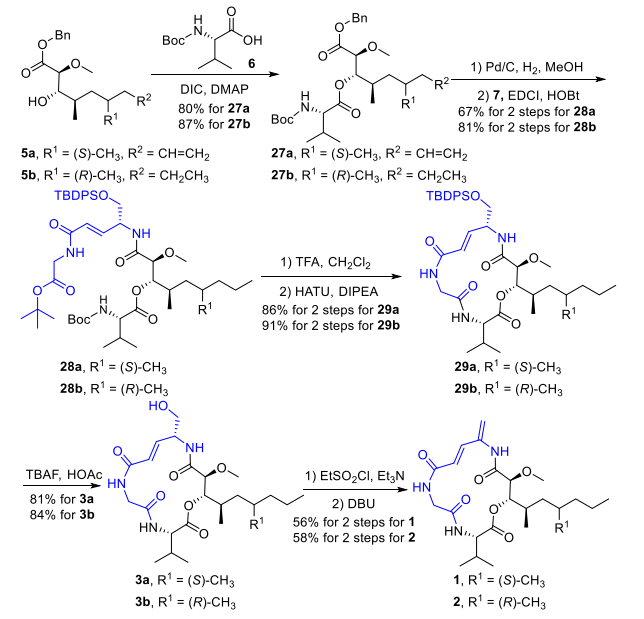
With ester **5a**/**5b** and amine **7** in hand, fragment coupling was performed to synthesize compound **1**/**2** (Scheme 3). Ester **5a**/**5b** was coupled with Boc-L-valine **6** under DIC and DMAP condition in high yield.[16] **27a**/**27b** was subjected to Pd/C hydrogenation, followed by coupling with amine **7** produced amides **28a**/**28b** in yields of 67% and 81%, respectively.

Compared with other syntheses of APD products, such as compounds **31** and **33**,[12,17] our strategy of macro-lactamization

Scheme 2. Synthesis of Ester 5a/5b and Amine 7 (Inset: ORTEP of 19a/19b)



Scheme 3. Forward Synthesis of Boholamide A



was performed at C-12 and N-13 (Scheme 4). The *tert*-butyl and Boc groups in compound **28a**/**28b** were removed with TFA, and the resulting key intermediate was used directly in the next macro-lactamization under HATU and DIPEA conditions to produce **29a**/**29b** in yields of 86% and 91%,

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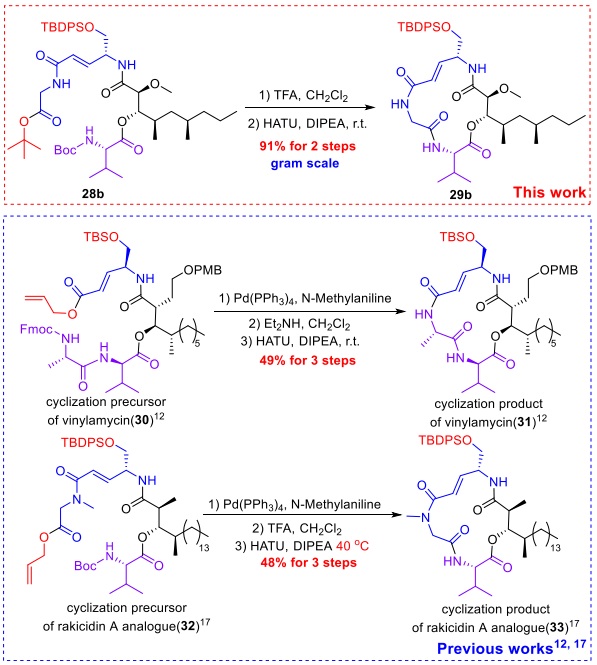
Scheme 4. Optimization of Macro-Lactamization



respectively, in two steps in gram scale, which were much higher than those from our previous work (Scheme 4).[12,17] TBS also can be removed with TFA; however, if we switched TBDPS to the TBS group in **28a**/**28b**, complete removal with TFA would not be possible, and the prolonged reaction time may led to decomposition of **28a**/**28b**.[11] Thus, we use TBDPS for the total synthesis of boholamide A. Deprotection of TBDPS with TBAF/HOAc yielded alcohol **3a**/**3b**. The hydroxyl group was activated by ethanesulfonyl chloride, followed by treatment with DBU to afford target **1**/**2** (Scheme 3).

After we finished the total synthesis of proposed structure **1**, the NMR data of **1** were compared with those of natural boholamide A reported in the literature (Supporting Information).[1] The spectra of compound **1** did not match that of natural boholamide A, with great differences involving ^{13}C NMR peaks at C- 2, C-3, C-4, C-6, C-7, C-9 and ^{1}H NMR data at the C-6 proton. Accordingly, we speculated that the difference between the putative structure and real structure would be the chirality of C-6 and the configuration of boholamide A at C-6 may be 6*R*. Compound **2** with 6*R* chirality at C-6 was synthesized following the strategy for the syntheis of compound **1**. To our delight, the ^{1}H NMR data for compound **2** matched with those of reported natural boholamide A. For ^{13}C NMR data, only one methyl of valine part was different from the literature.[1] In the literature, the two methyls of valine part were overlapped at 19.4 ppm in ^{13}C NMR.[1] The chemical shift values of two methyls in the valine part were 19.1 and 20.1 ppm in synthesized compound **2**. Vinylamycin and microtermolide A also containned a valine moiety. The two methyls in the valine part of vinylamycin and microtermolide A showed different chemical shift values, 18.1 and 18.3 ppm, 18.5 and 19.7 ppm, respectively.[8,9] Consequently, the chiral centers of boholamide A were tentatively assigned as 2*S*, 3*S*, 4*R*, 6*R*, and 11*S*. The normoxia and hypoxia cytotoxicity of compounds **1** and **2** were tested and compared with those of natural boholamide A reported by Torres and coworkers (Figure 2).[1] As is shown in Figure 2a, compound **2** showed similar potent anticancer activity compared with

(a)

cell line	natural boholamide A IC_{50}(μM) [1]	synthetic boholamide A IC_{50} (μM)[a]	
		proposed structure (**1**)	revised structure (**2**)
Huh-7	0.19±0.0[b]	6.41±0.03	3.97±1.18
A549	0.39±0.06[b]	2.00±0.13	2.60±0.02
U87MG	0.41±0.02[b]	2.63±0.38	1.92±0.21

(b)

compound	IC_{50} (μM)[a]		HSI[c]
	normoxia	hypoxia	
natural boholamide A[1]	0.41±0.04[b]	0.12±0.02[b]	3.6[b]
doxorubicin[1]	0.45±0.02[b]	0.98±0.08[b]	0.45[b]
proposed structure(**1**)	2.63±0.38	2.38±0.03	1.10
revised structure(**2**)	1.92±0.21	0.96±0.09	1.66
doxorubicin	0.014±0.006	0.027±0.014	0.54

Figure 2. Biological evaluation of boholamide A. (a) Cytotoxicity of boholamide A. (b) Hypoxia-selectivity of boholamide A against U87MG cells. [a]All values are the mean of three independent experiments and reported as mean ± SD. [b]Data from reported literature.[1] [c]Hypoxia selectivity Index (HSI) was calculated from IC_{50} (normoxia)/IC_{50} (hypoxia).

compound **1**, which indicated that the configuration of C-6 showed no evident effect on their anticancer activity in normoxia conditions. It is worth noting that the compound with the revised structure (**2**) showed better hypoxia activity than the proposed structure (**1**) in U87MG cells. Accordingly, more modifications based on the present methodology should be explored to study their structure−activity relationships.

In conclusion, the proposed structure of boholamide A (**1**) and the revised structure of boholamide A (**2**) were totally synthesized for the first time. Our convergent route features achievement of the macro-lactamization step in a gram scale. Both compounds **1** and **2** were synthesized in a highly efficient manner with 16 linear steps in 2.80% and 5.46% overall yield, respectively. Moreover, The chiral center at C-6 of boholamide A was tentatively revised as 6*R*. The total synthesis and structure revision of boholamide A provide the opportunity for further development of boholamide A as a potential hypoxia-selective anticancer agent.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.1c01382.

Experimental details, characterization data, and copies of ^{1}H NMR, ^{13}C NMR, COSY, HSQC spectra, and X-ray crystallography data. (PDF)

Accession Codes

CCDC 2080746−2080747 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by

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Notes

The authors declare no competing financial interest.

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