Tetrahedron Letters 55 (2014) 6109–6112

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Total synthesis of proposed structure of coibamide A, a highly N- and O-methylated cytotoxic marine cyclodepsipeptide

Wei He [a], Hai-Bo Qiu [a], Yi-Jie Chen [b], Jie Xi [b], Zhu-Jun Yao [a,b,*]

[a] State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 345 Lingling Road, Shanghai 200032, China
[b] State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering, Nanjing University, 22 Hankou Road, Nanjing, Jiangsu 210093, China

ARTICLE INFO

Article history:
Received 12 August 2014
Accepted 9 September 2014
Available online 16 September 2014

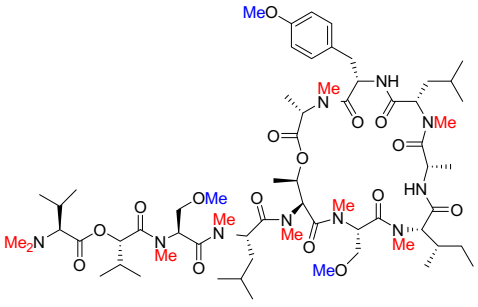
Keywords:
Coibamide A
Cyclodepsipeptide
Anticancer
N-Methylamino acid
Total synthesis

ABSTRACT

Total synthesis of the originally proposed structure of coibamide A, a highly N- and O-methylated cytotoxic marine cyclodepsipeptide, has been accomplished by using a [(4+1)+3+3]-peptide fragment-coupling strategy and careful examination and optimization of the multiple dense N-methylated amide-bond formations. The synthetic sample of the proposed coibamide A could not match the natural product in both ^{1}H and ^{13}C NMR spectra, but was found to exhibit low micromolar cytotoxicity against the proliferation of three tested cancer cells.

Marine cyanobacteria are a rich source of complex secondary metabolites, and some of them have shown striking biological activity[1–4] and have consequently become attractive synthetic targets in the quest for new leads for pharmaceutical development.[5] Coibamide A (**1**, Fig. 1) is a cyclodepsipeptide recently isolated by McPhail and co-workers from the marine cyanobacterium *Leptolyngbya* sp. collected from the Coiba National Park, Panama.[6] It was found to exhibit attractive low-nanomolar inhibitory activities against the proliferation of various cancer cells, including MDA-MB-231 (IC_{50} 2.8 nM), LOX IMVI (IC_{50} 7.4 nM), HL-60 (IC_{50} 7.4 nM), and SNB-75 (IC_{50} 7.6 nM). The significant inhibitory potencies and possible new mechanism of action make coibamide A to be a promising lead in anticancer drug discovery.[6–8] For the limited sources of marine natural products, establishment of a practical chemical synthesis of coibamide A is of extreme value to acquire sufficient sample for further pharmaceutical development and exploration of its structure–activity relationship.

Full assignment of natural coibamide A was carried out when its isolation by aid of NMRs and MS studies, as well as chiral HPLC analysis of the degraded amino acids (Fig. 1).[6] Among the amino-acid components, the absolute configuration of threonine was predicted by the MM2 method and was further confirmed with ROESY experiments. Coibamide A comprised a 22-membered macrocycle with a high content of *N*- and *O*-methylamino acids (eight of its eleven amino acids are N-methylated). Such a structural feature may help to improve the metabolic stability and enhance the hydrophobicity,[9] however, synthesis of a macrocyclic peptide containing multiple dense N-methylated amides is often full of challenges. Besides lower efficiency of the amide couplings with *N*-methylamino acids, common problems encountered in the synthesis also include the side reactions of forming diketopiperazine, epimerization, and the lability of N-alkylated peptides to acids.[10,11] Despite many reports have been addressed the development of proper reagents for the coupling of sterically hindered *N*-methylamino acids,[12] a satisfactory solution to resolve all the above problems has not yet been found.



Coibamide A (**1**)

Figure 1. Proposed structure of coibamide A.

* Corresponding author. Tel./fax: +86 21 54925123.
E-mail address: yaoz@sioc.ac.cn (Z.-J. Yao).

http://dx.doi.org/10.1016/j.tetlet.2014.09.047
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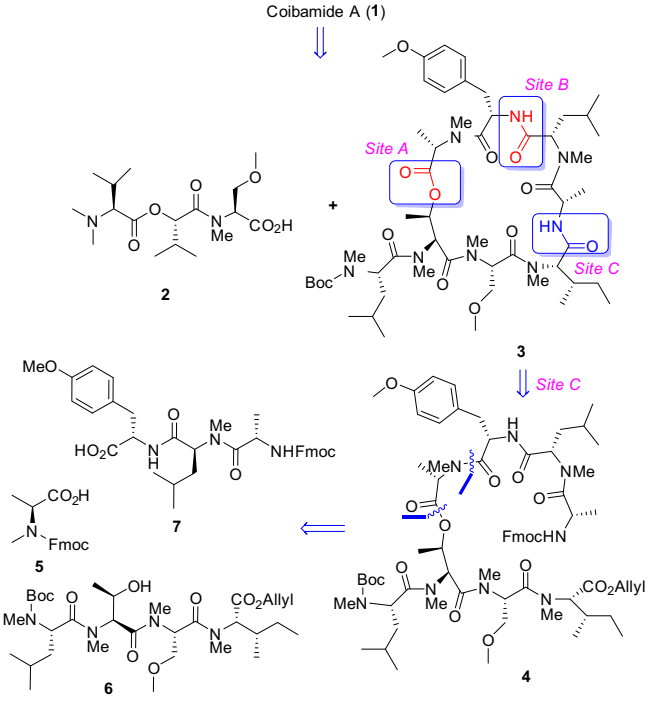
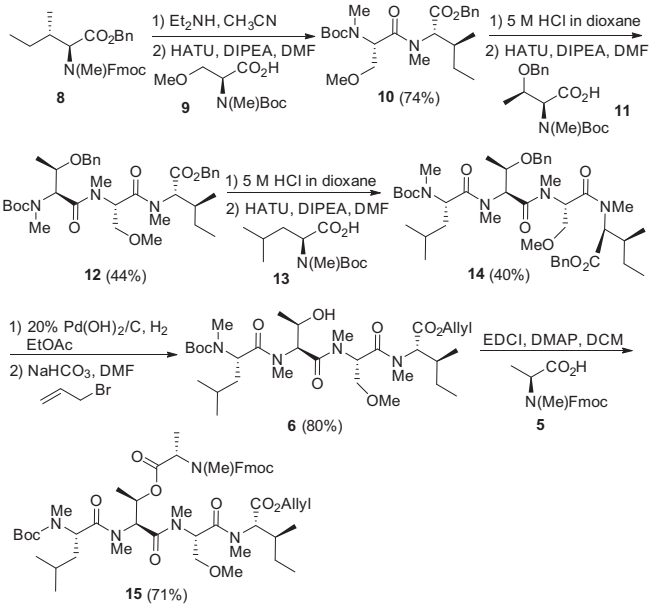
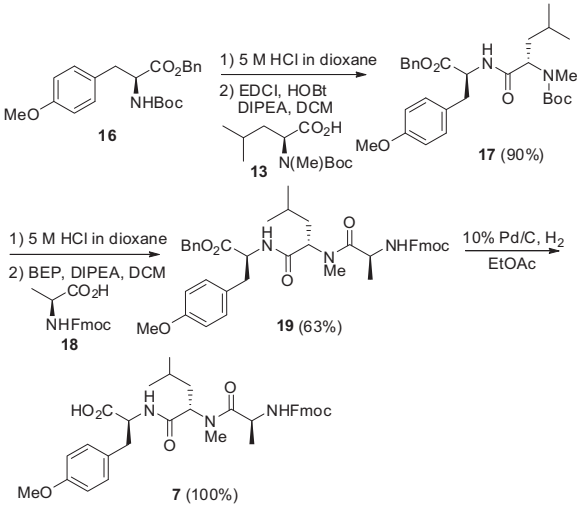


Figure 2. Retrosynthetic analysis of coibamide A.



Scheme 1. Synthesis of tetrapeptide **6**.



Scheme 2. Synthesis of tripeptide acid **7**.

Our retrosynthetic analysis of coibamide A is shown in Figure 2. At first, the side chain is disconnected from the macrocycle at the Ser(OMe)-*N*-MeLeu junction, affording fragment **2** and macrocycle **3** having a residue of *N*-MeLeu, which would reduce the difficulty of future segment-coupling with **2**. Further disconnection of the macrocycle **3** renders significant strategic importance and may dictate the success of the synthesis. According to the common guidelines of peptide synthesis, a proper cyclization position should avoid those sterically encumbered by the N-methylated amide bond.[10] Esterification is firstly excluded from the potential cyclization protocols, because unfavorable kinetics of lactone-formation based cyclization (at site A) may imply a major risk of racemization. For larger distribution of Z-amide conformer in the N-methylated peptides (compared to the non-N-methylated peptides), cyclization at *N*-MeLeu-Tyr(OMe) junction (site B) would be also unfavorable because of a high risk of 2,5-diketopiperizine formation resulting in the degradation of the linear peptide. Therefore, a potentially suitable macrocyclization position is determined at the *N*-MeIle-Ala amide bond (site C), affording the linear precursor **4**. Precursor **4** can be further broken into three fragments: Fmoc-*N*-Me-Ala-OH (**5**), tetrapeptide **6**, and tripeptide **7**.

The total synthesis began with the preparation of tetrapeptide **6** (Scheme 1). In order to overcome the possible epimerization of the active ester of N-methylated amino acid component (via oxazolone formation) during the couplings, stepwise coupling protocol was applied in the synthesis.[13] Coupling of *N*-Boc-*N*-Me-Ser(OMe) (**9**) with the *N*-Fomc deblocked product derived from **8** yielded dipeptide **10**. Removal of the *N*-Boc group of **10** with 5 M HCl in dioxane followed by condensation with the active ester of *N*-Boc-*N*-Me-Thr(OBzl) (**11**) afforded tripeptide**12**. Using a similar operation, tetrapeptide **14** was obtained after coupling with acid **13**. Considering the orthogonality of the protecting groups, the benzyl ester of **14** was altered with an allyl ester by hydrogenolysis of **14** and subsequent selective O-allylation with allyl bromide in the presence of $NaHCO_3$ in DMF. In order to reduce the steric hindrance of segments coupling, the resulting segment **6** was firstly coupled with the single *N*-methylamino acid **5**, providing the corresponding ester **15**. Coupling with a single *N*-methylamino acid is also advantageous in reducing the extent of epimerization during this ester-bond formation.

Tripeptide fragment **7** was synthesized in a fashion from the C-terminal to the N-terminal, beginning with the tyrosine derivative **16** (Scheme 2). Use of an *N*-Boc derivative **13** resulted in higher yield and minimized DKP formation in the second step of coupling. The benzyl ester protecting group of tripeptide **19** was then removed by hydrogenolysis, affording tripeptide acid **7** in quantitative yield.

To improve the coupling efficiency, we decided to synthesize the side-chain fragment **26** (a derivative of **2**, Fig. 2) from its N-terminal to C-terminal (Scheme 3). We reasoned that the presence of ester bond could be exploited to decrease the extent of oxazolone formation, which might otherwise lead to epimerization. The chiral α-hydroxy acid **21** was prepared readily from L-Val.[14] Esterification of alcohol **21** with acid **20** was carried out in the presence of EDCI and a catalytic amount of DMAP, affording ester **22** in quantitative yield. Removal of the *O*-allyl group of **22** with