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Efficient Synthesis and Stereochemical Revision of Coibamide A

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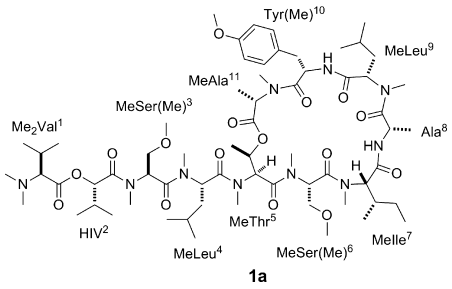
Supporting Information

ABSTRACT: Coibamide A is a highly potent antiproliferative cyclodepsipeptide originally isolated from a Panamanian marine cyanobacterium. Herein we report an efficient solid-phase strategy for assembly of highly *N*-methylated cyclodepsipeptides, which is invaluable in generating coibamide A derivatives for structure−activity relationship studies. As a consequence of our synthetic studies, two stereochemical assignments of coibamide A were revised and the total synthesis of this natural compound was achieved for the first time.

Cyclodepsipeptides are cyclic heterodetic peptides consisting of hydroxy and amino acids linked by ester and amide bonds.[1] Some of the cyclodepsipeptides isolated from both marine and terrestrial sources contain *N*-methylated amino acids. Such structural features may help to improve the receptor selectivity, metabolic stability, and hydrophobicity.[2] A range of biological activities, including anticancer, antiviral, antifungal, and immunosuppressive properties, have been observed for *N*-methylated cyclodepsipeptides.[3] The remarkable biological activities along with their complex molecular scaffolds make them interesting targets for chemical synthesis.[4] Coibamide A (Figure 1) is a highly *N*-methylated cyclodepsipeptides, which



1a

Figure 1. Originally proposed structure of coibamide A (**1a**).

was isolated by McPhail and co-workers from a Panamanian marine cyanobacterium in 2007.[5] Coibamide A exhibited low nanomolar inhibitory activities against the proliferation of a number of cancer cell lines with a potentially new mechanism of action.[6] As a promising lead agent, further exploration of this molecule in cancer drug discovery was however limited by the lack of availability from natural sources.

Coibamide A has been reported to be a side-to-tail cyclized depsipeptide possessing a 22-membered macrocycle with one hydroxy acid unit and ten L-amino acids where eight of the residues are *N*-methylated. The sequence and stereochemistry of the amino acids in this molecule were established by a combination of spectroscopic analysis, chemical degradation, and derivatization studies. The absolute configuration of threonine was determined using computational models of the two possible isomeric structures, constrained by ROESY correlations. The synthesis of a macrocyclic peptide containing multiple densely *N*-methylated amides are challenging as a consequence of the low inherent reactivity of *N*-methylated amino acids.[7] This problem is exacerbated by side reactions such as 2,5-diketopiperizine (DKP) formation, epimerization during the coupling steps, polymerization in the cyclization step, etc. In 2014, He et al. reported the first total synthesis of the proposed structure of coibamide A using a [(4 + 1) + 3 + 3]-peptide fragment-coupling strategy in solution phase.[8] But the analytical and biological activity data for the synthetic sample were inconsistent with those reported for natural coibamide A. Based on their results, further determination and reassignment of the full structure of coibamide A would be needed. Compared with the multistep and time-consuming synthesis in solution phase, a straightforward solid-phase peptide synthesis (SPPS) route would facilitate and speed up the synthesis of coibamide A. Recently, Nabika et al. reported their synthetic efforts toward coibamide A by using Fmoc-based SPPS followed by the cleavage of the resulting linear peptide from the resin and its subsequent macrolactonization.[9] However, although numerous coupling agents were evaluated, the macrolactonization between the hydroxyl group of MeThr[5] and C-terminal MeAla[11] failed to provide any of the desired product, and only the [D-MeAla[11]]-epimer of **1a** was formed in a low yield of 3.8%. In order to validate the configuration of this natural cyclodepsipeptide and to supply samples for further pharmaceutical development, we set out to establish a flexible and efficient solid-phase method for the total synthesis of coibamide A and its analogues.

First our efforts were focused on determining the cyclization point and optimizing the coupling methodology (Scheme 1). Besides the unfavorable late-stage macrolactonization, a proper cyclization position should also avoid those sterically encumbered by the *N*-methylated amide bonds. Considering the high risk of competing DKP (MeAla-Tyr(Me)) formation during the

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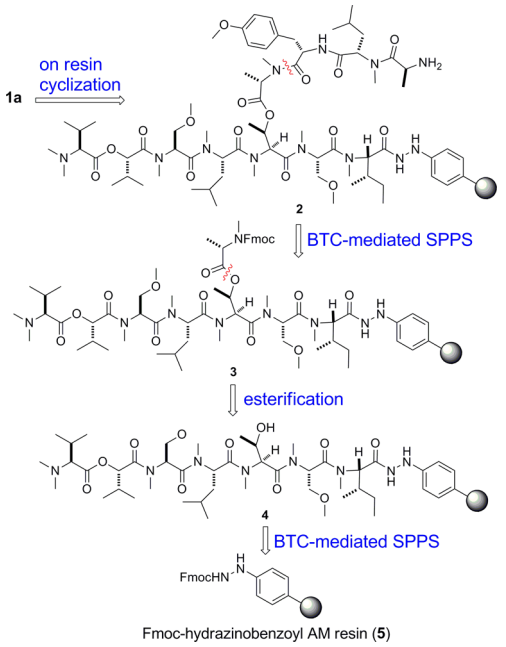
Scheme 1. Retrosynthetic Analysis of 1a

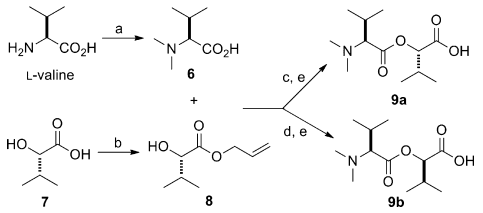


cyclization between *N*-terminal Tyr(Me)[10] and C-terminal MeLeu[9], the macrocyclization site was chosen at the MeIle[9]-Ala[8] junction, affording precursor **2**. Using orthogonally protected MeThr would allow successive solid-phase assembly of the main peptidyl chain of precursor **4** followed by introduction of MeAla via the ester bond formation and elongation of the side chain. Conveniently, Fmoc-*N*-methyl-*O*-*tert*-butyl-L-threonine is commercially available. However, the use of this *tert*-butyl (tBu) ether derivative would require establishing an Fmoc-based solid-phase method that also has to be compatible with trifluoroacetic acid (TFA) deprotection. Thus, aryl hydrazide resin was selected because the aryl hydrazide linker is stable in both strongly acidic and basic conditions during Boc- and Fmoc-based solid-phase synthesis, and yet it can be cleaved under very mild oxidative conditions.[10] The reactivity of the transient diazene species toward nucleophiles also allows cyclative cleavage, which releases the cyclodepsipeptide directly into solution without the need for an additional cyclization reaction.[11]

To overcome the difficulties associated with the synthesis of *N*-methylated amide bonds, the use of highly electrophilic species such as acid chlorides and acid anhydrides has attracted great attention. Triphosgene [bis(trichloromethyl) carbonate, BTC] converts carboxylic acids into acid chlorides, which readily acylate even sterically hindered and electronically deactivated amines.[12] It has shown superiority over benzotriazole-based reagents such as HATU or HOAt/DIC in the couplings of *N*-methylated or aromatic amino acids in both Fmoc-based and Boc-based SPPS.[13] However, it is commonly regarded that BTC does not work properly in the coupling of urethane-protected unmethylated amino acids, because the NH proton of the urethane function is susceptible to side reactions during strong activation with the BTC reagent. In the previous study, we demonstrated that increasing the molar ratio of BTC to the amino acids significantly improved the activation efficiency of unmethylated amino acids.[14] Further research revealed that the addition of 1 equiv of HOAt to the BTC activated intermediate could dramatically accelerate the coupling rate.[15] Therefore, the reagent set of BTC/collidine was expected to effect the coupling of both *N*-methylated amino acids and natural amino acids of coibamide A.

Among the nine building blocks, Fmoc-*N*-Me-Ser(Me)-OH and Me_2Val-HIV-OH are not commercially available and thus were stereoselectively prepared in solution phase. Fmoc-*N*-Me-Ser(Me)-OH was synthesized according to a previously reported procedure.[16] The synthesis of the Me_2Val-HIV-OH fragment is shown in Scheme 2. Reductive methylation of L-valine [aqueous

Scheme 2. Synthesis of Me_2Val-HIV-OH (9a) and Me_2Val-D-HIV-OH (9b)[a]



[a]Reagents and conditions: (a) H_2, Pd/C, aq. HCHO; (b) allyl bromide, Cs_2CO_3, DMF; (c) EDCI, DMAP, CH_2Cl_2; (d) PPh_3, DIAD, THF; (e) $Pd(PPh_3)_4$, morpholine, THF.

formaldehyde, H_2, Pd(C)] produced Me_2Val-OH (**6**).[17] Allyl ester **8** was prepared by selective *O*-allylation of (*S*)-(+)-2-hydroxy-3-methylbutyric acid (**7**) with allyl bromide and Cs_2CO_3. Me_2Val-HIV-OH (**9a**) was afforded by formation of the sterically hindered ester bond between **6** and **8** using EDCI/DMAP and subsequent removal of the allyl group with [$Pd(PPh_3)_4$] and morpholine.

With all of the building blocks in hand, the assembly of the precursor **13** on Fmoc-hydrazinobenzoyl AM resin could be initiated (Scheme 3). After removal of the Fmoc protecting group of the resin with 20% piperidine in DMF, the first amino acid, MeIle, was activated with BTC and attached onto the resin. A substitution level of 0.30 mmol/g was preferred to facilitate the tertiary amide bond formation. To avoid truncated products, acetic anhydride was then added to cap any of the remaining amino groups on the resin. As expected, the Fmoc-based solid-phase synthesis of the heptapeptide **11** proceeded smoothly employing BTC/collidine as the coupling reagents for *N*-methylated amino acids, MeSer(Me), MeThr(tBu), MeLeu, a second MeSer(Me), and Me_2Val-HIV. All couplings were carried out at room temperature with a single-coupling cycle for 1 h or less until a negative chloranil test showed the absence of free secondary amine on the resin.

To commence the side chain synthesis, the tBu protecting group on resin-bound **11** was successfully removed by treating the resin twice with TFA/TIS (triisopropylsilane)/water cocktail (95/2.5/2.5) for 2 and 5 min, respectively. HPLC analysis of a small sample cleaved from the resin revealed that TFA-induced amidolysis of the *N*-methylated peptide[18] could be suppressed using a minimized reaction time during deprotection of the *tert*-butyl ether. Esterification of the free secondary hydroxyl group of **4** with MeAla mediated by BTC/collidine was not efficient compared to amidation. An acceptable yield was achieved when symmetric anhydride formed by the reaction of Fmoc-*N*-Me-Ala-OH and DCC in a ratio of 2:1 was used, allowing the coupling reaction to be finished in 2.5 h with a single-coupling cycle.

Attachment of the second residue of the side chain, Fmoc-Tyr(Me)-OH, onto the peptidyl resin was carried out using the optimized protocol for BTC-mediated coupling of unmethylated

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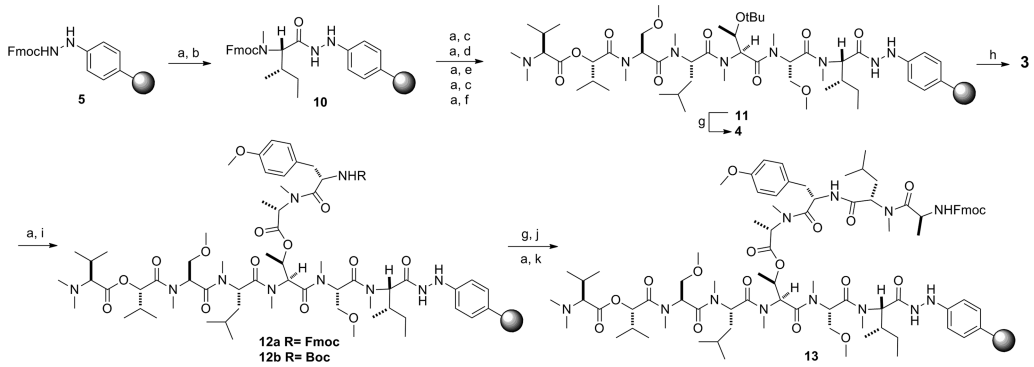
Scheme 3. Solid-phase Synthesis of Precursor 13[a]

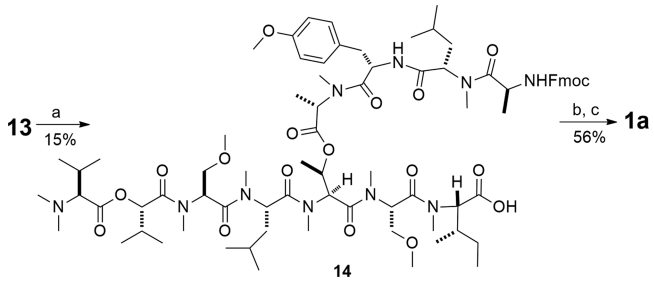


[a]Reagents and conditions: (a) 20% piperdine/DMF; (b) Fmoc-*N*-Me-Ile-OH; BTC, collidine, DIEA, THF; then acetic anhydride, DIEA, DMF; (c) Fmoc-*N*-Me-Ser(Me)-OH; BTC, collidine, DIEA, THF; (d) Fmoc-*N*-Me-Thr(tBu)-OH; BTC, collidine, DIEA, THF; (e) Fmoc-*N*-Me-Leu-OH; BTC, collidine, DIEA, THF; (f) Me_2Val-HIV−OH (**9a**), BTC, collidine, DIEA, THF; (g) TFA/TIS/H_2O (95/2.5/2.5); (h) Fmoc-*N*-Me-Ala-OH; DCC, DMAP, CH_2Cl_2, DMF; (i) Fmoc-Tyr(Me)-OH or Boc-Tyr(Me)-OH; BTC, collidine, THF, HOAt, DIEA, DMF; (j) Fmoc-*N*-Me-Leu-OH; BTC, collidine, DIEA, THF; (k) Fmoc-Ala-OH, BTC, collidine, THF, HOAt, DIEA, DMF.

amino acids with HOAt as an additive. However, removal of the Fmoc group on **12a** with 20% piperidine in DMF resulted in partial degradation of the linear peptide via DKP formation even when exposure to piperidine was minimized (3 min). It was envisioned that DKP formation could be avoided under acidic deprotection conditions. Therefore, Boc-Tyr(Me)-OH was attached to the resin as an alternative of Fmoc-Tyr(Me)-OH to form **12b**. Applying the aforementioned TFA-deprotection procedure used for the *tert*-butyl ether, Boc group was removed with no degraded linear peptide detected. The following two residues, MeLeu and Ala, were incorporated into the side peptidyl chain to give **13** by the coupling reactions mediated by BTC/collidine and BTC/collidine/HOAt, respectively.

After removal of the Fmoc group from **13**, cyclative cleavage of the cyclodepsipeptide from resin **2** was investigated under a variety of reaction conditions. The transient diazene species was formed by oxidation of the hydrazide with *N*-bromosuccinimide (NBS) or atmospheric O_2 in the presence of Cu(II) salts.[10] However, the following intramolecular nucleophilic attack by alanine amine did not occur possibly due to the steric hindrance of the C-terminal MeIle residue. Direct aminolysis of resin-bound peptide **2** under air at elevated temperatures (55 and 90 °C)[19] did not provide any of the cyclized product, either. Consequently, the solid-phase macrocyclization approach had to be adjusted and a solution-phase cyclization was conducted (Scheme 4). After cleavage from resin **13** using a mixture of

Scheme 4. Synthesis of the Proposed Structure of Coibamide A (1a)[a]



[a]Reagents and conditions: (a) $Cu(OAc)_2$, Py, CH_2Cl_2/H_2O; (b) Et_2NH, CH_3CN; (c) EDCI, HOAt, DIEA, CH_2Cl_2.

$Cu(OAc)_2$/Py/H_2O in CH_2Cl_2 and semipreparative HPLC purification, precursor **14** was obtained in 15% yield. The Fmoc group of **14** was removed, and the cyclization was carried out in the presence of EDCI/HOAt/DIEA in DCM. After purification, the proposed structure of coibamide A (**1a**) was obtained in 56% yield with spectroscopic data identical to those reported by He et al.[8] We also noted that ^{1}H and ^{13}C NMR spectral data of **1a** did not agree with those of natural coibamide A.[5] The significant difference in NMR spectral data once again brings into question the original structural assignment of the natural product.

The synthetic strategy was further explored in the synthesis of the [D-MeAla^{11}]-epimer of **1a** (**1b**), which was found to be slightly less potent than the natural one, but still exhibited nanomolar cytotoxicity toward a number of different cancer cell lines. Starting from resin-bound alcohol **4**, D-MeAla residue was incorporated to the resin by esterification. Following the same procedure for the side chain elongation, resin cleavage, and cyclization, **1b** was synthesized in an overall yield of 12%. Its spectroscopic data were consistent with those reported by Nabika et al.[9] Although X-ray structures of the synthetic samples were not available, possible epimerization during the coupling reactions and macrocyclization was excluded by synthesis of both the proposed structure **1a** and [D-MeAla^{11}]-epimer **1b**, which were reported by two individual groups previously.

Our next effort was focused on elucidation of the stereochemical structure of natural coibamide A. A detailed comparison of the ^{1}H NMR spectrum of the natural product with that of synthetic samples disclosed that **1b** was more similar to natural coibamide A than **1a**. The ^{1}H signals attributed to the seven amino acid residues belonging to the macrocycle of **1b** were almost identical to those of natural coibamide A, while significant differences were observed for the other four residues outside the macrocycle. This suggested that the figuration of MeAla^{11} residue in natural coibamide A should be revised from L to D, and at least one exocyclic residue also needs to be revised. Among the four exocyclic residues, misassignment of MeLeu^4 and MeSer(Me)^3 was less possible because their counterparts also exist in the macrocycle, the stereochemistry of which were believed to be correct. The figuration of HIV^2 was more suspicious in view of the limited influence on ^{1}H signals of the terminal Me_2Val^1. Therefore, [D-HIV^2]-[D-MeAla^{11}]-diastereomer **1c** was anticipated to be the real structure of natural coibamide A, thus becoming our next target (Figure 2).

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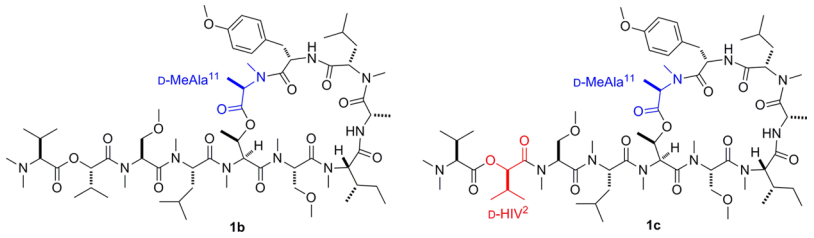

Figure 2. Chemical structures of **1b** and **1c**.

To convert the stereochemistry of HIV, a Mitsunobu reaction of Me_2Val-OH (**6**) and alcohol **8** was carried out and Me_2Val-D-HIV-OH (**9b**) was obtained after removal of the allyl group with $[Pd(PPh_3)_4]$ and morpholine. Following the sequence depicted in Schemes 3 and 4, we then performed the synthesis of **1c**, using **9b** and Fmoc-D-*N*-Me-Ala-OH instead of **9a** and Fmoc-*N*-Me-Ala-OH, respectively. The resulted synthetic compound and the natural product revealed a perfect overlap of the NMR spectra (see Supporting Information). Therefore, the absolute structure of coibamide A should be revised from **1a** to **1c**. Furthermore, a bioactivity assay was performed using the MTT method. As expected, **1c** displayed similar in vitro activity against MDA-MB-231 cells (IC_{50} 3.9 nM), corroborating the authenticity of our structural revisions.

In summary, the total synthesis and stereochemical revision of coibamide A were achieved for the first time by using an efficient and robust solid-phase method we have developed. The combined application of both the Boc- and Fmoc-based strategy on aryl hydrazide resin allows for successive assembly of the main and side peptidyl chains by solid-phase peptide synthesis. The use of BTC to generate all of the amide bonds on solid support is also featured. Establishment of the revised structure of coibamide A paves the way for structure−activity relationship studies and elucidation of the unknown mechanism of action, which are currently underway in our laboratory.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b09286.

Experimental procedures and analysis data (PDF)

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Notes

The authors declare no competing financial interest.

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