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# Synthesis and biological evaluation of the [D-MeAla$^{11}$]-epimer of coibamide A

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ABSTRACT

Coibamide A is a highly potent antiproliferative cyclic depsipeptide, which was originally isolated from a Panamanian marine cyanobacterium. In this study, the synthesis of coibamide A has been investigated using Fmoc-based solid-phase peptide synthesis followed by the cleavage of the resulting linear peptide from the resin and its subsequent macrolactonization. The peptide sequence of the linear coibamide A precursor was constructed on a solid-support following the optimization of the coupling conditions, where numerous coupling agents were evaluated. The macrocyclization of the resulting linear peptide provided the [D-MeAla$^{11}$]-epimer of coibamide A, which exhibited nanomolar cytotoxic activity towards a number of human cancer cell lines.



Coibamide A (**1**) is a cyclic depsipeptide, which was originally isolated from a Panamanian marine cyanobacterium (Fig. 1).[1] This compound has been reported to exhibit potent antiproliferative activity against a number of human cell lines, where it operates according to a unique mechanism of action. For this reason, there has been considerable interest in the use of coibamide A as a potential lead in cancer drug discovery. The recent results also revealed that coibamide A induces macroautophagy and can trigger apoptosis and non-apoptotic forms of cell death in human cancer cells.[2] The production and isolation of coibamide A via the cultivation of coibamide A-producing cyanobacterium is a laborious process, and the development of new methods for the chemical synthesis of coibamide A and its derivatives is therefore highly desired to allow for further pharmaceutical research and development.[1] The proposed structure of coibamide A was recently synthesized by He et al.,[3] but the analytical and biological activity data for the synthetic sample were inconsistent with those reported for natural coibamide A.

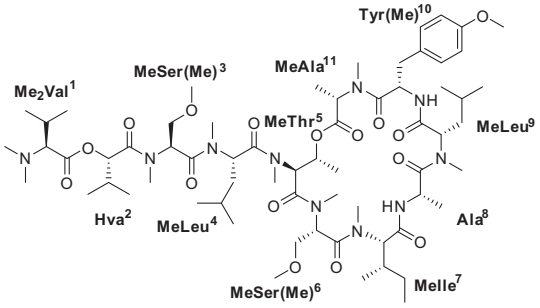


**Figure 1.** Structure of coibamide A.

Coibamide A has eight *N*-methylamino acids and two ester bonds. The coupling of an amino acid onto the *N*-methylamino terminus of a peptidyl resin proceeds slowly because of steric hindrance, and the synthesis of *N*-methyl-rich peptides can be made difficult by the sluggish nature of this reaction.[4] The synthesis of *N*-methyl-rich peptides on a solid-support therefore requires the optimization of the reagent used for each coupling step, as exemplified by the syntheses of [MeLeu$^1$]–cyclosporin A,[5] the potent nematicide omphalotin A,[6] the antitumor agent IB-01212[7] and the immunosuppressive antifungal compound petriellin A.[8] The coupling conditions developed for the synthesis of [MeLeu$^1$]–cyclosporin A[5] involved the use a highly reactive

*Abbreviations:* Hva, α-hydroxyisovaleric acid; HATU, *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; BTC, bis(trichloromethyl) carbonate; DIC, 1,3-diisopropylcarbodiimide; HOBt, 1-hydroxybenzotriazole; HOAt, 1-hydroxy-7-azabenzotriazole; TFA, trifluoroacetic acid; MSNT, 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole; NMI, *N*-methylimidazole; DCM, dichloromethane.

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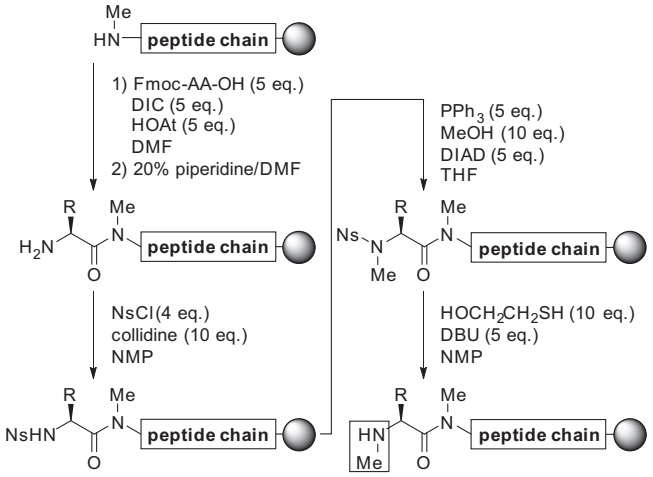
DIC/1-hydroxy-7-azabenzotriazole (HOAt) system[9] and *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU),[10] which led to significant improvements in the yields of the coupling products. Bis(trichloromethyl)carbonate (BTC) has also been used to improve the coupling efficiency of amidation reactions by converting Fmoc-amino acids into highly reactive and sterically unhindered acid chlorides in situ.[6]

It was envisaged in the current study that coibamide A could be synthesized via the Fmoc-based solid-phase peptide synthesis (SPPS) of a linear peptidyl resin, followed by the cleavage of the resulting linear peptide from the resin and a subsequent macrocyclization reaction between its MeThr[5] β-hydroxy group and its C-terminal carboxylic acid moiety (Scheme 1). A series of different coupling protocols were investigated in the current study, including the use of DIC/HOAt, HATU and BTC systems to optimize the coupling conditions required for all seven of the *N*-methylamino acid-termini on the solid-support. The progress of each coupling reaction was monitored by HPLC analysis following the cleavage of the peptide product from the peptidyl resin with a 5:95 (v/v) mixture of TFA/DCM.

Tyr(Me)[10] and Ala[8] were efficiently coupled to *N*-methylamino acids using a HATU/$(i\text{-Pr})_2$NEt system. In contrast, MeLeu[9] and MeIle[7] were successfully coupled onto unmethylated amino acids using the standard protocol for Fmoc-based SPPS (i.e., DIC/HOBt). The coupling of MeSer(Me)[6] onto the *N*-methylamino group of MeIle[7] in the presence of HATU/$(i\text{-Pr})_2$NEt led to the partial epimerization of the product (dr = 72:28), which was attributed to slow reaction of the two coupling partners under the basic conditions. When the same coupling reaction was conducted with DIC/HOAt, which produces an HOAt ester as the active species without the addition of base, the reaction did not proceed to completion, although no epimerization was observed. In contrast, the coupling of Fmoc-Ser(Me)-OH proceeded smoothly to completion using the same DIC/HOAt reagents. Subsequent deprotection of the Fmoc group followed by an on-resin N-methylation protocol using an *o*-nitrobenzenesulfonyl (Ns)-strategy (Scheme 2)[11] provided the desired hexapeptide containing MeSer(Me)[6] in sufficient purity. Overall, this N-methylation protocol involved the nosylation of the Ser(Me) α-amino group, N-methylation of the resulting nosylated amine using a Mitsunobu reaction and the subsequent deprotection of the Ns-group. The coupling of Fmoc-Thr(Trt)-OH was also followed by an on-resin N-methylation process using the Ns-strategy described above to give the MeThr(Trt)[5] residue.

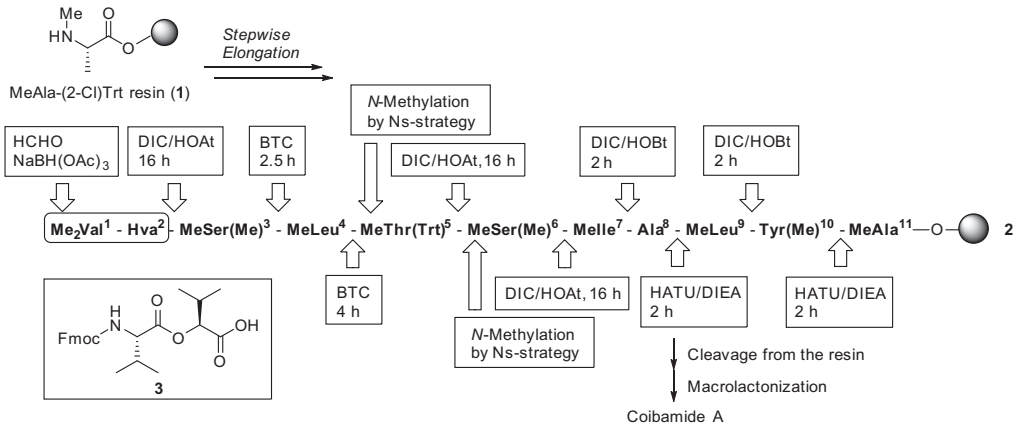
As was the case for MeSer(Me)[6], the HATU-mediated coupling of MeLeu[4] did not proceed to completion (61% conversion). The failure of this reaction to reach completion was most likely due to steric hindrance of the *N*-methylamino group and the protected




**Scheme 2.** Preparation of *N*-methylamino acids [i.e., MeSer(Me)[6] and MeThr(Trt)[5]] using the Ns-strategy on a solid support.

bulky side chain of MeThr(Trt)[5]. Among the various coupling conditions investigated, BTC was found to be the only coupling agent capable of driving the MeLeu[4] coupling to completion to obtain the desired octapeptide in good purity (83% purity). The second MeSer(Me)[3] residue was also incorporated into the linear peptide by the BTC-mediated coupling of Fmoc-MeSer(Me)-OH, which was synthesized according to the Freidinger method.[12] Finally, to allow for the incorporation of the N-terminal esterified dimer ($Me_2$Val[1]-Hva[2]) into the linear peptide resin, the resulting nonapeptide was conjugated to ester **3** using DIC/HOAt; ester **3** was synthesized in advance of this particular step using solution phase chemistry. The resulting peptide was then *N*,*N*-dimethylated under reductive amination conditions with formalin and $NaBH(OAc)_3$ to afford the desired linear undecapeptidyl resin **2** in 52% purity.

The final deprotection and cleavage of peptidyl resin **2** was achieved by the treatment of the resin-bound peptide with a 5:95 (v/v) mixture of TFA/DCM, which led to the formation of two products with identical molecular weights (Fig. 2). The treatment of the minor product with $Et_3N$ provided only the major product, while the major product underwent partial conversion to the minor product by treatment with TFA/DCM (5:95) (accompanied by the unchanged major product). These suggested that the major product was the desired linear peptide **4**, and that the minor product was the *O*-acyl peptide **5**, which was most likely formed by the rearrangement of the N-terminal tetrapeptide from the MeThr[5]




**Scheme 1.** Overview of synthetic strategy used for the construction of the coibamide A.