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Total Synthesis of Callipeltin B and M, Peptidyl Marine Natural Products

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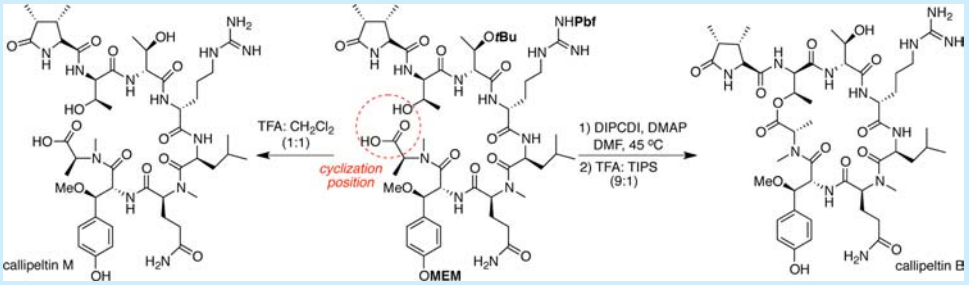
Supporting Information



ABSTRACT: Total synthesis of callipeltins B and M, peptidyl cytotoxic agents isolated from marine sponges, by the combination of Fmoc solid-phase peptide synthesis and cyclization and global deprotection in the solution phase is described. Eight amino acids, including several unusual amino acids, were assembled on a solid support, and effective TFA-mediated deprotection was employed to reach callipeltin M. Callipeltin B was accomplished via the macrolactonization between the side chain of D-*a*Thr and the C-terminus carboxylic acid of protected callipeltin M.

Callipeltin A (**1**) has potent cytotoxicity and anti-HIV activity and is the first marine natural peptide isolated from the marine sponge *Callipelta* sp.[1] The molecule is composed of unique amino acids: L-*N*-methylalanine (MeAla), (2*R*,3*R*)-*β*-methoxytyrosine (*β*MeOTyr), L-*N*-methylglutamine (MeGln), L-leucine (Leu), D-arginine (D-Arg), D-allothreonine (D-*a*Thr), (2*S*,3*S*,4*R*)-dimethylglutamine (DMQ), (2*R*,3*R*,4*S*)-4-amino-7-guanidino-2,3-dihydroxypentanoic acid (AGDHE), and (2*R*,3*R*,4*R*)-3-hydroxy-2,4,6-trimethylheptanoic acid (HTAHA). Though the total synthesis of the cyclic depsipeptides, namely, papuamide B,[2] pipecolidepsin A,[3] and stellatolide A[4] isolated from marine sponges, was achieved by Ma's,[5] Albericio's,[6] and Cuevas's groups,[4] callipeltin A (**1**) has never been reported in the literature. Callipeltin B (**2**), a truncated analogue cleaved between the AGDHE moiety and DMQ moiety which was directly transformed to (2*S*,3*S*,4*R*)-dimethylpyroglutamic acid (pDME) residue, was discovered with callipeltin A (**1**) from the same marine sponge by Minale's group.[7] Callipeltins E (**3**)[8] and M (**4**),[9] truncated linear peptides of callipeltin B (**2**), were isolated from the marine sponge *Latrunculia* sp. by D'Auria and co-workers. Callipeltins E (**3**) and M (**4**) used the same amino acids as callipeltin B (**2**). Since the stereochemistry of *β*MeOTyr and D-*a*Thr could not be determined in the isolation and structural analysis of callipeltin A (**1**), D'Auria et al. reported the stereochemistry of *β*MeOTyr as 2*R*,3*R* using chemical degradation of callipeltin A (**1**) and derivatization of the resulting amino acids.[10] We also reported the confirmation to be 2*R*,3*R* by a comparison of ^{1}H and ^{13}C NMR spectral data of all four diastereoisomeric tripeptides, H-L-Gln-*β*MeOTyr-L-MeAla-OH.[11] In contrast, the stereochemistry of Thr residues in callipeltin A (**1**) was revised as a D-*a*Thr by Bifulco and co-workers.[12] Based on these reports, callipeltin E (**3**) was determined to have the structure H-D-*a*Thr-D-Arg-L-Leu-L-MeGln-(2*R*,3*R*)-*β*MeOTyr-L-MeAla-OH. Lipton's[13] and our groups[14] reported the solid-phase total synthesis of callipeltin E (**3**) starting from the C-terminus and proof of its structure independently. Callipeltin M (**4**) was characterized to elongate two amino acids, D-*a*Thr and pDME (**5**) on the N-terminus of callipeltin E (**3**), namely, acyclic callipeltin B (**2**). Synthesis of pDME (**5**) was reported by Lipton's[15] and Hamada's[16] groups, and the stereochemistry of dimethyl groups of **5** was determined as 3*S*,4*R*. Recently, we also confirmed the stereochemistry of the natural **5** form by comparison of ^{1}H NMR and CD spectral data of four diastereoisomers and a computational chemical method.[17] However, total synthesis of callipeltin M (**4**) has never been reported, and its detailed biological activity is unknown. As a continuous work, Lipton et al. reported the solid-phase total synthesis of callipeltin B (**2**) and complete assignment of its chemical structure.[18] They selected Tentagel-based TG Sieber amide resin to afford callipeltin B (**2**) from the resin by mild acidic conditions and decided to anchor the side chain of MeGln residue to the resin for peptide amide synthesis. In addition, benzyl and nitro groups were used for side chain protections of *β*MeOTyr and D-Arg because it is commonly understood that *β*MeOTyr residue is labile to acidic conditions.

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As mentioned above, Lipton's group synthesized callipeltin E (**3**) starting from MeAla of the C-terminus, and in contrast, synthesis of callipeltin B (**2**) was commenced with *β*MeOTyr to avoid macrolactonization as much as an inefficient deprotection process. These approaches were unfavorable to prepare a variety of callipeltin analogues for structure−activity relationship studies to research antitumor and/or anti-HIV agents. Herein, we report the total syntheses of the cyclodepsipeptide callipeltin B (**2**) and the linear octapeptide callipeltin M (**4**). Our synthetic strategies are summarized as follows: (a) conventional Fmoc-SPPS starting from the C-terminus, (b) use of MEM, Pbf, and *t*-Bu groups for protection, (c) macrolactonization between D-*a*Thr and MeAla residues. Although it is generally difficult to obtain the cyclic depsipeptide by macrolactonization of the corresponding linear precursor peptide, we assumed that the conformation of callipeltin M (**4**) in nature would be similar to those of callipeltin B (**2**), and therefore, it is likely to directly convert callipeltin M (**4**) to callipeltin B (**2**) (Figure 1).

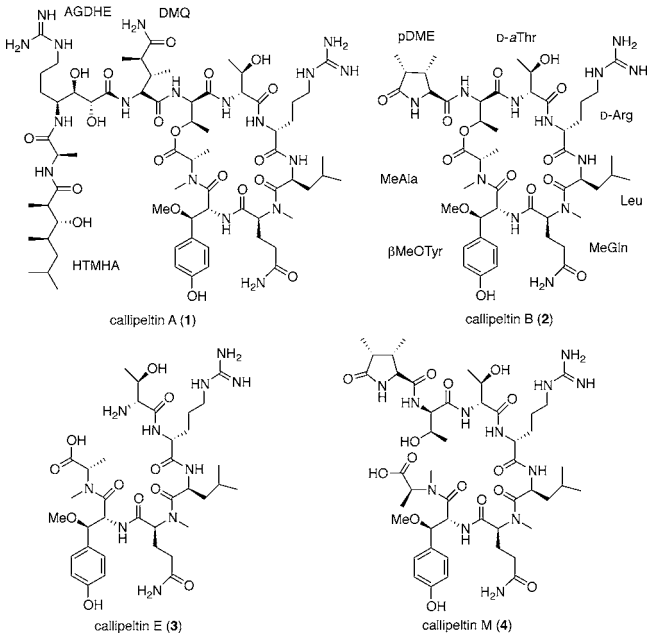

Figure 1. Callipeltins A (**1**), B (**2**), E (**3**), and M (**4**).

Prior to the total synthesis of callipeltins B (**2**) and M (**4**), the necessary six unusual amino acids with protecting groups for the Fmoc-SPPS, Fmoc-MeAla-OH (**6**), Fmoc-*β*MeOTyr (MEM)-OH (**7**), Fmoc-MeGln-OH (**8**), Fmoc-D-*a*Thr-OH (**9**), Fmoc-D-*a*Thr(*t*Bu)-OH (**10**), and pDME (**5**) were synthesized as in our previous reports.[11,14,17,19] Fmoc-Leu-OH (**11**) and Fmoc-D-Arg(Pbf)-OH (**12**) were from commercially available materials (Figure 2). To use these building blocks, we attempted the solid-phase synthesis of callipeltin M (**4**). As a solid support, 2-chlorotrityl chloride resin was selected. After loading **6** on the resin, the Fmoc group of the resulting resin (**13**) was removed with 20% piperidine/DMF. The amino acids **7** and **8** were introduced using PyBOP/HOBt/*i*-Pr_2NEt in DMF for 4 h to give the tripeptide (**14**). Because of the difficulty with introducing **11** to usual conditions, several coupling reagents were attempted. As a result, we found the optimized condition using COMU/HOAt/*i*-Pr_2NEt in DMF for 5 h to give the tetrapeptide **15**. Fmoc-D-

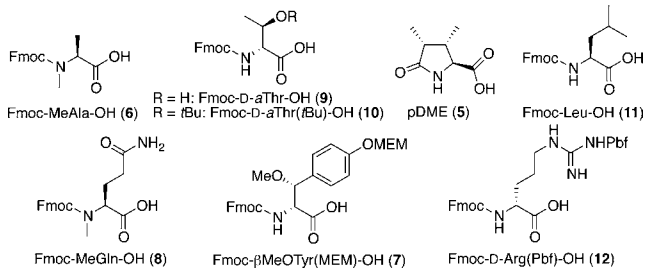

Figure 2. Constituent amino acids of callipeltins.

Arg(Pbf)-OH (**12**) was coupled with PyBOP/HOBt/*i*-Pr_2NEt for 3 h to give the pentapeptide **16**, and subsequent elongation of Fmoc-D-*a*Thr(*t*Bu)-OH (**10**) was successful to give the hexapeptide **17** with the same conditions for 2 h without any problems. The coupling of **10** with the resin-bound **17** followed by the smooth coupling of **5** (1.5 equiv) proceeded to complete the elongation of the desired peptide chain. Cleavage from the resin was performed using 20% HFIP/CH_2Cl_2 for 2.5 h to give a crude protected octapeptide, which was purified by RP-HPLC to give pure protected callipeltin M (**18**) in 4% overall yield (16 steps) from the resin loading. Finally, protected callipeltin M (**18**) was treated with 50% TFA/CH_2Cl_2 to remove the protecting groups to obtain the desired callipeltin M (**4**) in a yield of 29%. The ^{1}H NMR spectrum of synthesized callipeltin M (**4**) was identical to those of natural callipeltin M. Therefore, we briefly achieved the first total synthesis of callipeltin M (**4**) (Scheme 1).

Scheme 2 shows the synthesis of callipeltin B (**2**). For the macrolactonization between the hydroxy group of the D-*a*Thr residue adjacent to pDME and carboxylic acid of the MeAla residue, each hydroxy group of two D-*a*Thr residues was distinguished using Fmoc-D-*a*Thr-OH (**9**). Resin-bound **17** was coupled with **9** and **5** continuously in the presence of PyBOP/HOBt/*i*-Pr_2NEt in DMF, and subsequently, the resin-cleaving reaction was performed with 20% HFIP/CH_2Cl_2 to obtain the crude material. Despite a single peak on HPLC analysis, its ^{1}H NMR spectrum showed a few products. Structural analysis by ^{1}H NMR and MS spectra revealed that **5** was introduced on the hydroxy group of the D-*a*Thr residue. Saponification of the mixture by 1 M aqueous NaOH for 20 min was guided to desired linear peptide **19** without epimerization in 7% overall yield from the resin loading.

Next, the cyclization reaction using protected callipeltin M (**19**) was optimized. By the primary screening of coupling reagents and bases, we found efficient conditions, MSNT/MeIm and DIPCDI/DMAP, to proceed with the macrolactonization between the hydroxy group of the D-*a*Thr residue and the C-terminus carboxylic acid. As depicted in Table 1, reagents and conditions were optimized in detail. Treatment of the linear peptide **19** with MSNT/MeIm in DMF reacted quickly to form a sulfonate intermediate; however, chemical yields of the cyclized product **20** were unsatisfactory (entries 1 and 2). The linear peptide **19** activated by DIPCDI/DMAP was formed over a long time (∼10 h), in which it gradually disappeared, and formation of the desired cyclic depsipeptide **20** was confirmed. The excess reagents, especially 20 equiv of DMAP, may have accelerated this process (entries 4 and 5). Macrolactonization of **19** in approximately ∼10 mM substrate concentrations was enabled efficiently (entries 6 and 7). As a result, cyclic peptide **20** was provided using DIPCDI (5 equiv)/DMAP (20 equiv) in DMF (7.5 mM) at 45 °C in 44% yield

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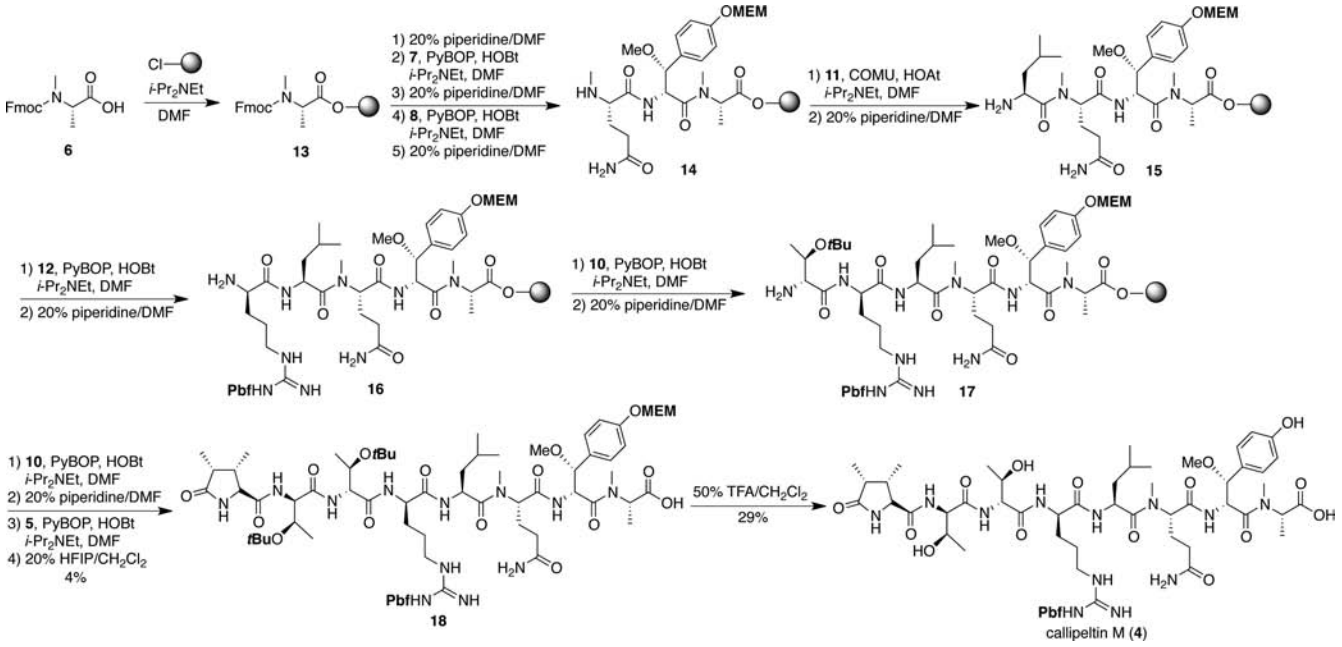
Scheme 1. Total Synthesis of Callipeltin M (4)



Scheme 2. Synthesis of Callipeltin B (2) via Macrolactonization

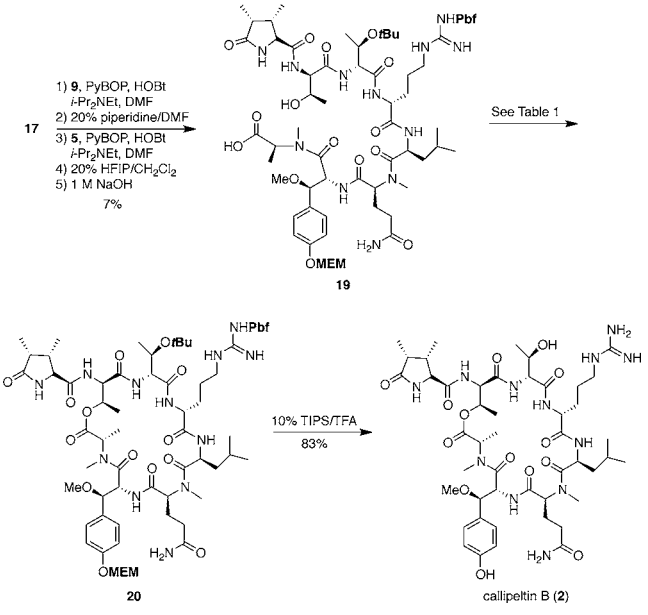


Table 1. Cyclization Conditions for 20

	reagents[a] (equiv)	concn (mM)	time (h)	yield (%)
1	MSNT (7), MeIm (12)	11	1	13
2	MSNT (7), MeIm (12)	111	2	25
3	DIPCDI (7), DMAP (0.5)	7.2	11	27
4	DIPCDI (5), DMAP (5)	7.0	19	18
5	DIPCDI (5), DMAP (20)	7.5	18	44
6	DIPCDI (10), DMAP (20)	20	10	41
7	DIPCDI (10), DMAP (20)	111	10	13

[a]The reactions were performed at 45 °C.

(entry 5). Though the final deprotection for **2** was carried out with 25% TFA in CH_2Cl_2, the Pbf group of the Arg residue of the **20** remained. We considered that the cyclic structure was difficult to remove from the protecting group as compared with the linear peptides. To convert completely, treatment of **20** with 10% TIPS in TFA was successfully accomplished to afford callipeltin B (**2**) in 83% yield after purification by preparative HPLC. The ^{1}H NMR spectrum of synthetic **2** was identical to those of the natural product from Minale's group[7] and the synthetic product of Lipton's group.[18] We achieved the total synthesis of **2** via macrolactonization (Scheme 2). It was noted that **2** was not labile to acidic conditions in the absence of water for 2 h, as shown in the HPLC profile. As expected, moist acidic conditions led to decomposition of the callipeltins.

In summary, we accomplished the total synthesis of callipeltins B (**2**) and M (**4**) by a combination of standard Fmoc-SPPS and cyclization and deprotection in the solution phase. The key macrolactonization between MeAla and D-*a*Thr residues of protected callipeltin M (**19**) proceeded fortunately to generate protected callipeltin B **20**. Final deprotection by TFA-mediated conditions was successful to give cyclic depsipeptide callipeltin B (**2**). Synthetic and structure−activity relationship studies of callipeltin A (**1**) and its derivatives are now underway.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedure, ^{1}H and ^{13}C NMR and MS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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