JOC*Article*

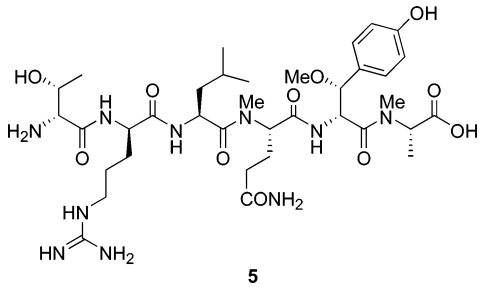
Solid-Phase Synthesis and Configurational Reassigment of Callipeltin E. Implications for the Structures of Callipeltins A and B

Selçuk Çalimsiz, Ángel I. Morales Ramos, and Mark A. Lipton*

Department of Chemistry and Cancer Center, Purdue University, 560 Oval Drive, West Lafayette, Indiana 47907-2084

lipton@purdue.edu

Received February 20, 2006



5

Two possible isomers of the natural product callipeltin E (**1**, **5**) were synthesized by using an Fmoc-based solid-phase strategy in 7 steps, in 20% and 26% overall yields, respectively. The ^{1}H NMR spectrum of synthetic **5** correlated closely with that of the natural product, whereas that of **1** did not, providing confirmation of the configurational reassignment of the N-terminal residue of callipeltin E as D-allothreonine. This result strongly implies that the corresponding residue in the closely related cyclic depsipeptides callipeltins A and B should also be considered a D-allothreonine residue.

Introduction

Callipeltin E (**1**, Figure 1), an acyclic hexapeptide member of the callipeltin family, was isolated from the marine sponge *Latrunculia* sp. by D'Auria and co-workers in 2002.[1] **1** was shown to be a truncated, open-chain derivative of callipeltins A and B (**2** and **3**, Figure 1), previously isolated cyclic depsipeptides that possess anti-HIV and antifungal activity and cytotoxicity against several human carcinoma cell lines.[2,3] Due to the acid-sensitive nature of the β-OMeTyr residue present in **1**−**3**, its configuration was only determined recently as the 2*R*,3*R* isomer by D'Auria and co-workers using chemical degradation of callipeltin A and derivatization of the resulting amino acids.[4] By extension, the β-OMeTyr residue present in **1** is now believed to be the 2*R*,3*R* isomer. Recently, by employing quantum mechanical calculation of coupling constants, it was suggested by Bifulco et al. that both of the threonine residues in callipeltin A have the D-allo configuration,[5] consistent with what was independently found in the structurally related cyclic depsipeptide neamphamide A (**4**, Figure 1).[6] Since the configuration of the threonine residue in callipeltin E corresponds to the revised D-allothreonine in callipeltin A, the revision of configuration of **2** calls into question the original configurational assignment of callipeltin E. To resolve this question and definitively establish the configuration of callipeltin E, we decided to synthesize the two possible isomers of callipeltin E: the originally reported structure (**1**) and its D-allothreonine isomer (**5**). Herein we report the efficient solid-phase syntheses of **1** and **5**, and their spectral correlation with the natural product.

Results and Discussion

Our approach to the synthesis of callipeltin E employed an Fmoc-based, solid-phase strategy. It was envisaged that, due to

* Address correspondence to this author. Phone: +765-494-0132. Fax: +765-494-0239.

(1) Zampella, A.; Randazzo, A.; Borbone, N.; Luciani, S.; Trevisi, L.; Debitus, U.; D'Auria, M. V. *Tetrahedron Lett.* **2002**, *43*, 6163−6166.

(2) Zampella, A.; D'Auria, M. V.; Paloma, L. G.; Casapullo, A.; Minale, L.; Debitus, C.; Henin, Y. *J. Am. Chem.* Soc. **1996**, *118*, 6202−6209.

(3) D'Auria, M. V.; Zampella, A.; Paloma, L. G.; Minale, L.; Debitus, C.; Roussakis, C.; LeBert, V. *Tetrahedron* **1996**, *52*, 9589−9596.

(4) Zampella, A.; D'Orsi, R.; Sepe, V.; Casapullo, A.; Monti, M. C.; D'Auria, M. V. *Org. Lett.* **2005**, *7*, 3585−3588.

(5) Bassarello, C.; Zampella, A.; Monti, M. C.; Gomez-Paloma, L.; D'Auria, M. V.; Riccio, R.; Bifulco, G. *Eur. J. Org. Chem.* **2006**, 604−609.

(6) Oku, N.; Krishnamoorthy, R.; Benson, A. G.; Ferguson, R. L.; Lipton, M. A.; Phillips, L. R.; Gustafson, K. R.; McMahon, J. B. *J. Org. Chem.* **2005**, *70*, 6842−6847.

10.1021/jo060351h CCC: $33.50
Published on Web 07/21/2006

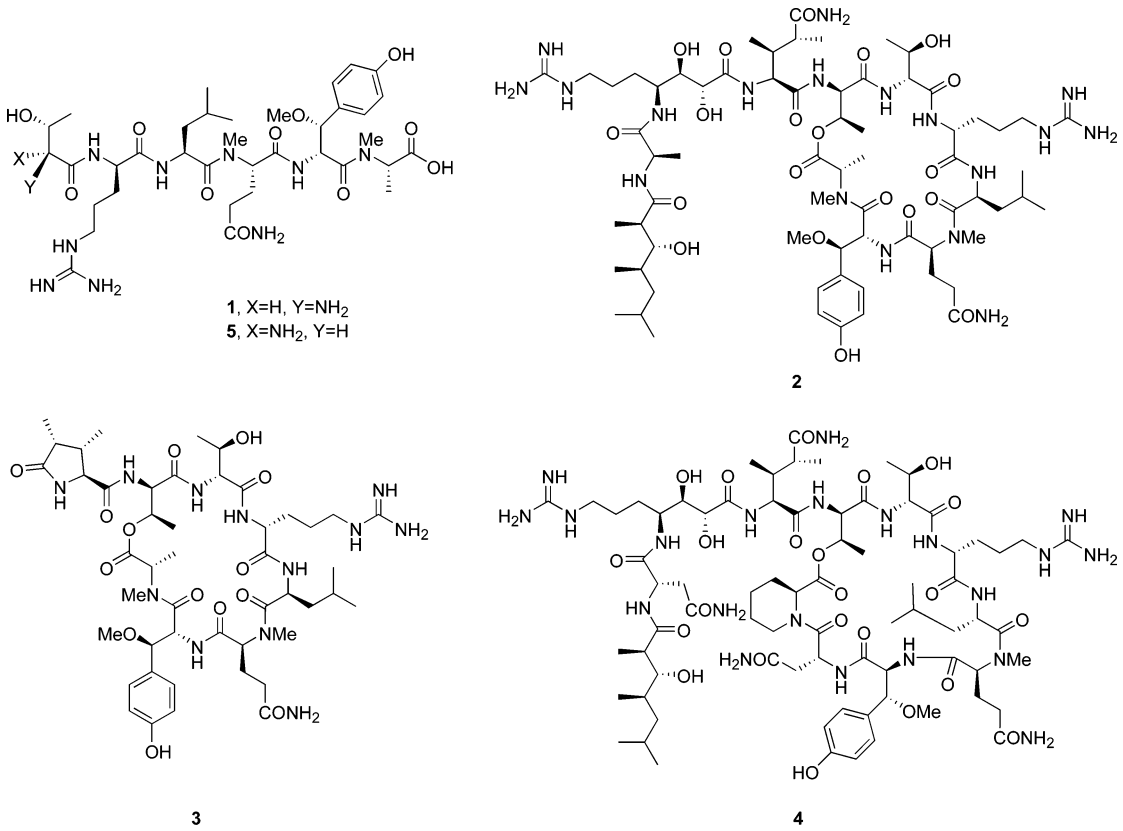


FIGURE 1. Two possible isomers of callipeltin E (**1** and **5**), callipeltin A (**2**), callipeltin B (**3**), and neamphamide A (**4**).

the acid-labile nature of the (2*R*,3*R*)-*β*-methoxytyrosine (*β*-MeOTyr) residue, the coupling conditions, cleavage from the resin, and removal of protecting groups would be performed under either mildly acidic or nonacidic conditions. Our initial strategy followed closely the synthetic plan we had developed for callipeltins A and B. Thus, anchoring the side chain of the *N*-methylglutamine residue to the Sieber amide resin (**10**)[7] was initially explored rather than a linear C to N strategy. Additionally, the protecting groups used in the synthesis were chosen for their facile removal by hydrogenolysis.

The initial retrosynthetic analysis for the synthesis of callipeltin E is shown in Scheme 1. It was envisaged that both isomers **1** and **5** could be obtained from pentapeptide **6** via coupling of either D-allo or L-threonine, followed by resin cleavage and hydrogenolytic deprotection. Pentapeptide **6** would be obtained by sequential N to C coupling of *N*-methylalanine to the resin-bound dipeptide **7**, followed by standard C to N coupling of the leucine and D-arginine residues. **7** could be derived in turn from the regioselective ring opening of anhydride **8** with deprotected Sieber Resin **10**, followed by N to C coupling of *β*-methoxytyrosine **9**.

Cyclic anhydride **8** was synthesized from Fmoc-Glu(O*t*Bu) in 88% overall yield (Scheme 2) by acid deprotection followed by treatment with neat acetic anhydride, giving **8**. *N*-Methylalanine benzyl ester (**11**) was prepared in 67% overall yield from Boc-*N*-methylalanine by esterification followed by Boc deprotection.

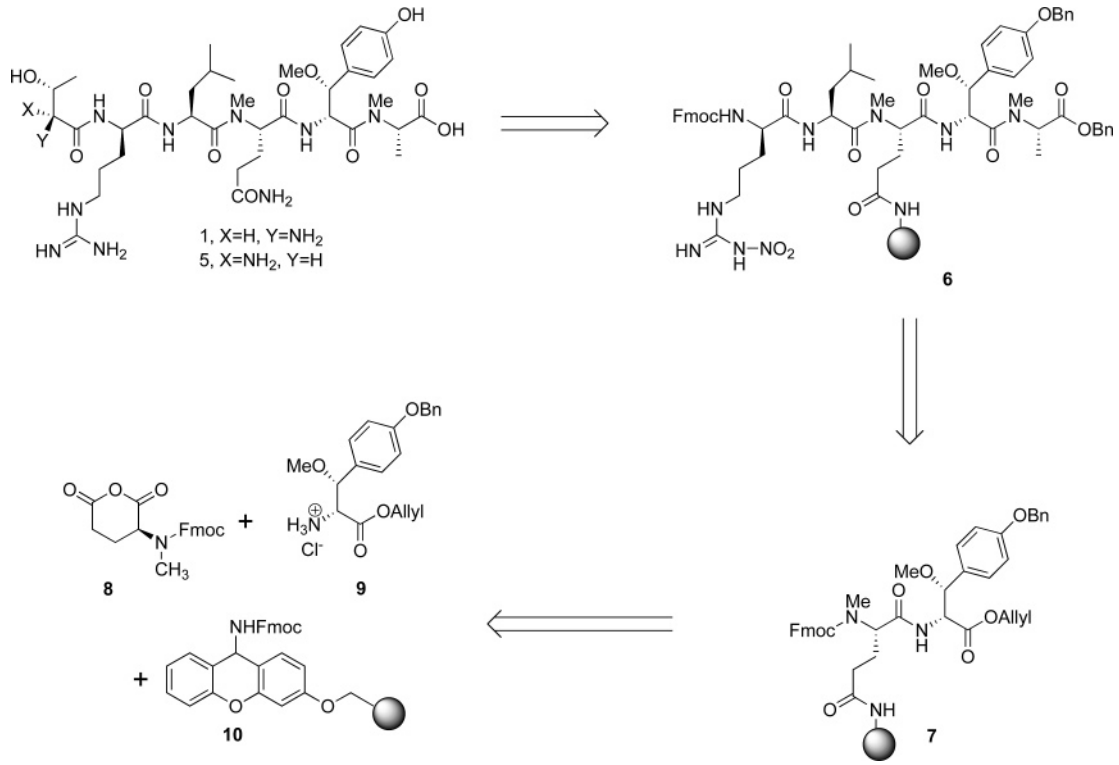
The peptide coupling conditions were screened by employing a model system in which L-tyrosine was used in lieu of the *β*-methoxytyrosine residue. All reactions were monitored by cleavage of a small aliquot of resin and analysis of the crude cleavage product by reverse-phase HPLC and MALDI-MS. Thus, Fmoc deprotection of the Sieber resin linker followed by the regioselective addition of cyclic anhydride **8** provided resin-bound Fmoc-MeGln (**12**). Activation of **12** with PyBOP, followed by introduction of Tyr(OBn)-OAllyl·HCl provided dipeptide **13** without any evidence of epimerization as indicated by HPLC (Scheme 3).

Palladium-catalyzed deprotection of the allyl ester in **13** (Scheme 4) was followed by activation of the resin-bound carboxylic acid and coupling with *N*-methylalanine benzyl ester (**11**). Fmoc removal followed by peptide coupling of Fmoc-leucine yielded resin-bound tetrapeptide **14**. Attempts to couple N^{ω}-nitro-N^{α}-Fmoc-D-arginine to **15** produced a single product 14 amu lighter than the expected pentapeptide **6**. Similar results were observed when N^{ω}-N^{ω}-bis(benzyloxycarbonyl)-N^{α}-Fmoc-D-arginine or N^{ω}-N^{ω}-bis(allyoxycarbonyl)-N^{α}-Fmoc-D-arginine were coupled to **14**. ^{1}H NMR comparison of the unexpected product and callipeltin E showed a loss of the *N*-methylalanine *N*-methyl group, suggesting the formation of pentapeptide **15**. To confirm the site of demethylation, authentic **15** was synthesized by using Fmoc-alanine in place of Fmoc-*N*-methylalanine. The product of this synthesis was the expected one with no loss of the glutamine *N*-methyl group.

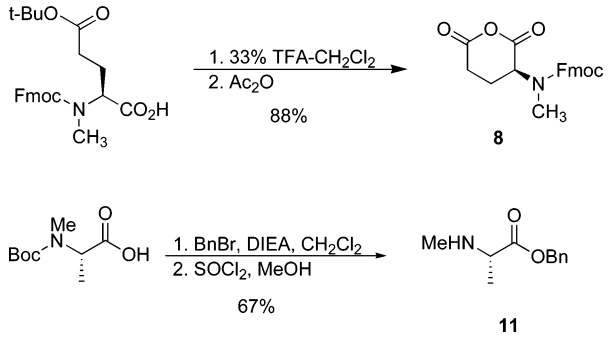
Changing the addition sequence by performing the C to N couplings first and adding the *N*-methylalanine residue last again afforded the demethylated product **15**. At this stage, it was concluded that demethylation resulted when the *N*-methylalanine and D-arginine were both present in the growing peptide. It was proposed that the anchoring point of peptide **6** to the solid support might have an effect on the conformation of the peptide, promoting a pseudocyclic conformation containing a strong

(7) Sieber, P. *Tetrahedron Lett.* **1987**, *28*, 2107−2110.

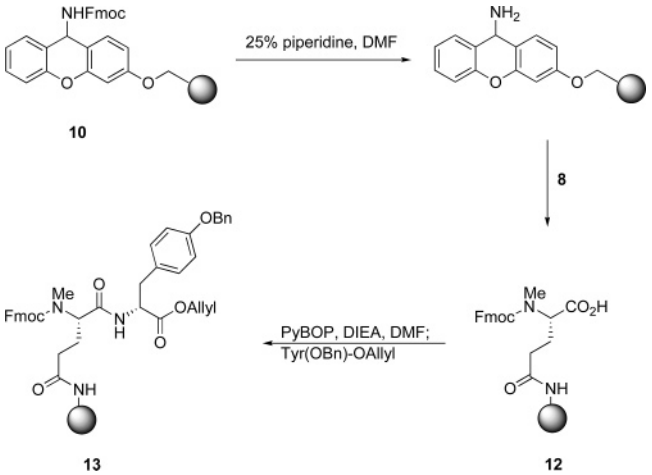
SCHEME 1. Initial Retrosynthetic Analysis



SCHEME 2



SCHEME 3



hydrogen bond between the protected guanidine and the alanine carbonyl (Figure 2). This hydrogen bond might weaken the methyl−nitrogen bond, making the CH_3 a labile electrophile. It has been previously reported that cleavage of *N*-methylated peptides from resins with use of acidic conditions can result in acid-promoted demethylation.[8] On the basis of these results, our synthetic strategy was revised.

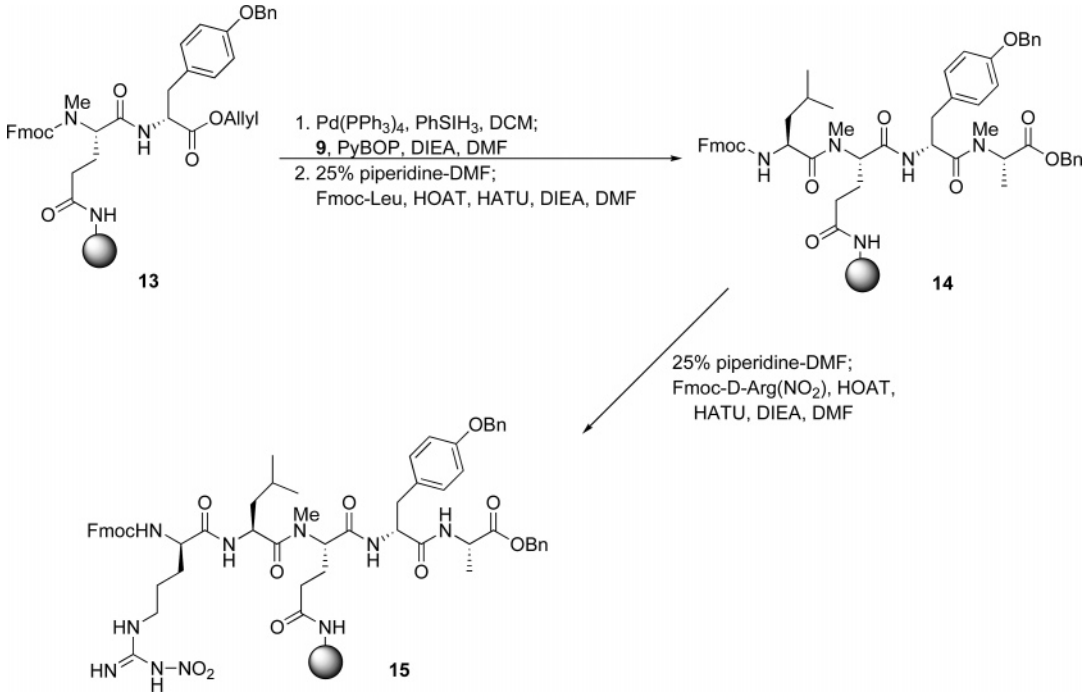
On the basis of the proposed demethylation process, it was decided to change the anchoring point of the solid support to the peptide backbone. It was envisaged that placing a bulky linker on the carboxylate terminus of the *N*-methylalanine might hinder its demethylation. By employing this strategy, not only the pseudocyclic conformation of the hexapeptide backbone would be avoided, but also only C to N couplings would be needed. For these reasons the acid-labile 2-chlorotrityl chloride resin (**16**)[9] was chosen.

The required residue Fmoc-(2*R*,3*R*)-β-methoxytyrosine (**19**) was synthesized from the previously reported (2*R*,3*R*)-β-methoxytyrosine allyl ester (**17**)[6] in two steps in 56% overall yield (Scheme 5). Also, Fmoc-N^{α}-methylglutamine (**22**) was prepared from commercially available N^{δ}-trityl-N^{α}-Fmoc-glutamine (**20**) via the oxazolidinone intermediate **21** employing Freidinger's procedure [10,11] in 82% yield (Scheme 5).

In the synthesis of **1**, each peptide coupling was complete in 30 min, using 1.5 equiv of HOAt/HATU and 1.5 equiv of the Fmoc-protected amino acid in the presence of 3 equiv of Hunig's base. 2-Chlorotrityl chloride resin (**16**) activation, followed by coupling with Fmoc-*N*-methylalanine, **19**, **22**, Fmoc-leucine, N^{ω},N^{ω}-bis(benzyloxycarbonyl)-N^{α}-Fmoc-D-arginine, and Fmoc-D-allothreonine, respectively, afforded the resin-bound hexapeptide **23** in 71% purity as judged by HPLC analysis (Scheme 6).

(8) Teixido, M.; Albericio, F.; Giralt, E. *J. Pept. Res.* **2005**, *65*, 153−166.
(9) Barlos, K.; Chatzi, O.; Dimitrios, G.; Stavropoulos, G. *Int. J. Pept. Protein Res.* **1991**, *38*, 513−520.
(10) Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. *J. Org. Chem.* **1983**, *48*, 77−81.
(11) Aurelio, L.; Box, J. S.; Brownlee, R. T. C.; Hughes, A. B.; Sleebs, M. M. *J. Org. Chem.* **2003**, *68*, 2652−2667.

SCHEME 4



No demethylation of the MeAla was observed after coupling of the Fmoc-arginine residue to the growing peptide, supporting our hypothesis about the cause of demethylation. Removal of the Fmoc group and cleavage of the hexapeptide from the resin, followed by reverse-phase HPLC purification, yielded a partially deprotected hexapeptide that was fully deprotected by hydrogenation in 2-propanol.[12] Final purification by reverse-phase HPLC afforded **1** in 20% overall yield. The second isomer **5** was prepared in the same manner, using Fmoc-D-allothreonine in place of Fmoc-threonine, in 26% overall yield.

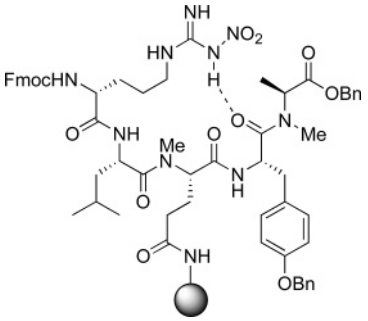
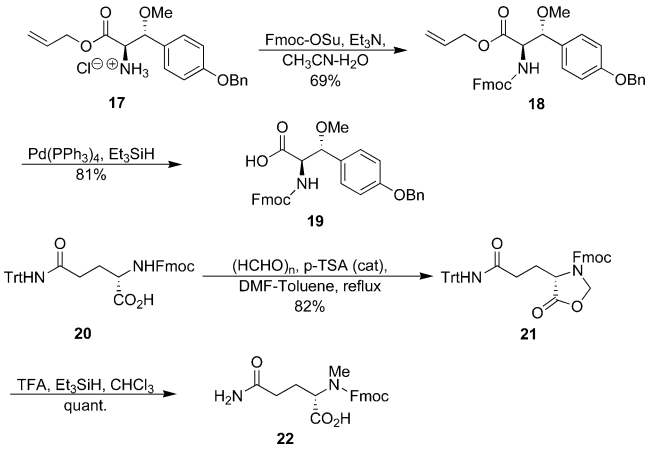


FIGURE 2. Proposed conformation of **6** leading to acid-promoted demethylation.

SCHEME 5



Visual comparison of the ^{1}H NMR spectra of callipeltin E, **1**, and **5** revealed several important differences. In particular, there were no significant differences between the spectra of callipeltin E and **5** in the region from 3.8 to 4.5 ppm, whereas the spectrum of **1** was substantially different in that region (Figure 3[13]). This distinction is especially noteworthy since the signals for the N-terminal Thr/D-alloThr residue lie within that region of the ^{1}H NMR. On the basis of these results we conclude that **5** is identical with callipeltin E, thereby confirming that D-allothreonine is the N-terminal residue.[14]

Conclusion

In summary, a reliable, solid-phase synthesis for both isomers of callipeltin E was developed. Callipeltin E was synthesized in 7 steps in 26% overall yield. The ^{1}H NMR of synthetic **5** correlated closely with that of the natural product, confirming the reassignment of the configuration of the N-terminal residue in callipeltin E as D-allothreonine. This finding also reinforces the recent configurational reassignment of the corresponding residue in callipeltin A and, by extension, in callipeltin B. Combined with the recent confirmation of the configurational assignment of the AGDHE residue in callipeltin A,[15] we now believe that the structures of **2** and **3** have been firmly established pending their confirmation by total synthesis.

(12) Hydrogenation in methanol and ethanol afforded *N*-methylated and *N*-ethylated byproducts, respectively.

(13) NMR spectrum obtained from Prof. M. V. D'Auria and reproduced with permission.

(14) Tabulated ^{1}H NMR signals of callipeltin E; **1** and **5** are compared in Table 1 in the Supporting Information.

(15) Cranfill, D. C.; Morales-Ramos, A. I.; Lipton, M. A. *Org. Lett.* **2005**, *7*, 5881−5883.

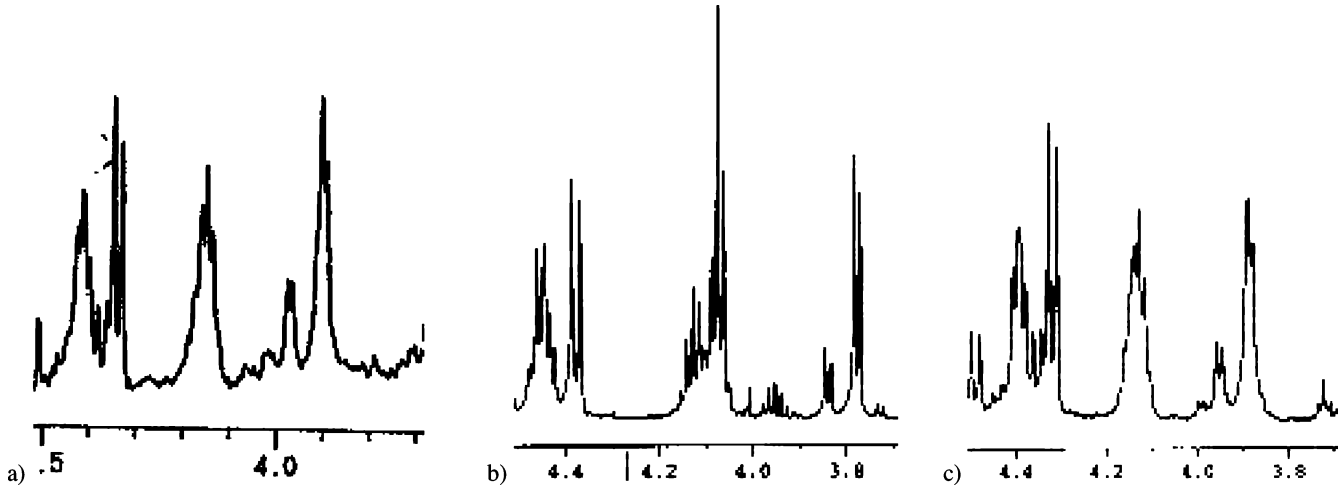
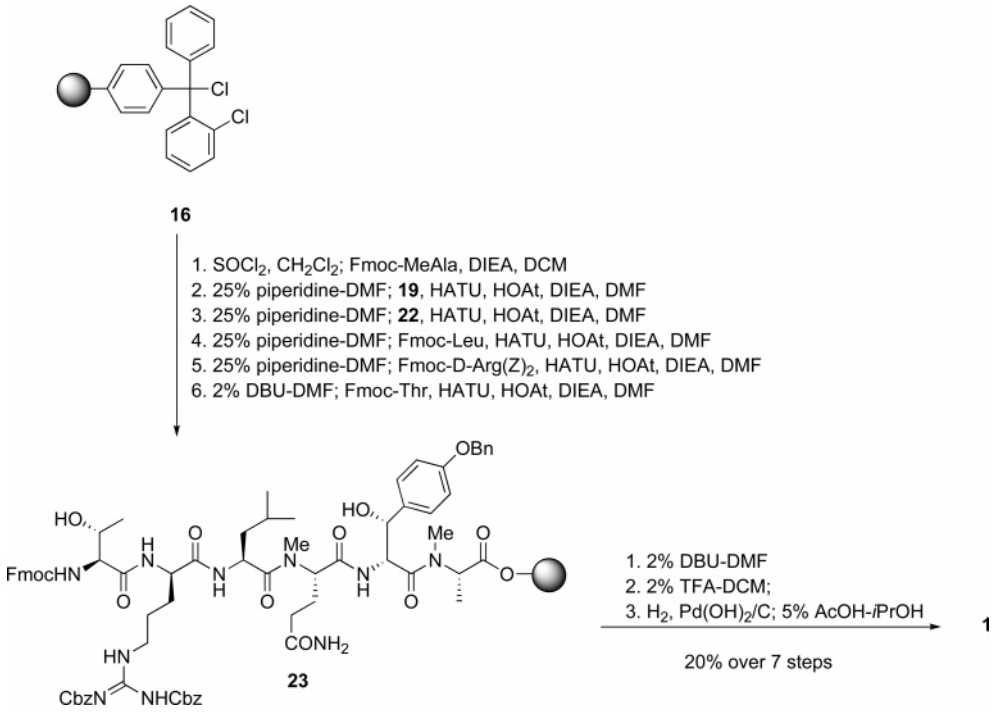


FIGURE 3. Comparison of the 3.8−4.5 ppm region of the ^{1}H NMR spectra of (a) callipeltin E, (b) **1**, and (c) **5**.

SCHEME 6



Experimental Section

All reactions were monitored by cleaving the growing peptide from a small quantity of resin and analysis of the crude cleavage product by reverse-phase HPLC and MALDI-TOF mass spectrometry.

Callipeltin E (D-Allothreonine Isomer, 5). 2-Chlorotrityl chloride resin (**16**, 50 mg, 100−200 mesh, 1% DVB, 1.2 mmol/g) was washed with dry DMF and dry CH_2Cl_2 (3×) under a positive N_2 atmosphere. In a flame-dried vial, $SOCl_2$ (6.5 μL, 0.090 mmol) was dissolved in CH_2Cl_2 (0.5 mL). The resulting solution was transferred to the resin and the mixture was agitated by bubbling N_2 through it for 1 h. The solvent was removed by filtration and the resin was washed with DMF (2×) and CH_2Cl_2 (3×). In another flame -dried vial, Fmoc-*N*-methylalanine (12 mg, 0.037 mmol) and DIEA (25.6 μL, 0.147 mmol) were dissolved in CH_2Cl_2 (0.5 mL) and added to the preactivated resin. N_2 was bubbled through the reaction for 1.5 h, after which the excess sites on the resin were quenched with a solution of 9:1 MeOH−DIEA (0.5 mL) and N_2 was bubbled through the reaction for an additional 5 min. The solvents were removed by filtration and the resin washed sequentially with the following solvents: 9:1 MeOH−DIEA (2×), DMF (3×), *i*-PrOH (2×), DMF, *i*-PrOH, CH_2Cl_2, and Et_2O.

To the washed resin was added CH_2Cl_2 (0.35 mL) and a 25% piperidine/DMF solution (0.35 mL), and N_2 was bubbled through the reaction for 10 min. The solvents were removed by filtration and the resin was treated again with a 25% piperidine/DMF solution (0.7 mL) for 15 min. The solvent was removed by filtration and the resin washed with DMF (3×), *i*-PrOH (2×), and DMF (2×). In a separate vial, HATU (20 mg, 0.052 mmol), HOAt (7 mg, 0.052 mmol), and Fmoc-(2*R*,3*R*)-β-methoxytyrosine (**19**) (27 mg, 0.052 mmol) were dissolved in DMF (0.4 mL). DIEA (27 μL, 0.16 mmol) was added and the mixture was stirred for 1.5 min and added to the resin. N_2 was bubbled through the reaction for 30 min, the solvent was removed by filtration, and the resin was washed with DMF (3×), *i*-PrOH (2×), DMF (2×), *i*-PrOH, CH_2Cl_2, and Et_2O.

To the washed resin was added CH_2Cl_2 (0.35 mL) and a 25% piperidine/DMF solution (0.35 mL), and N_2 was bubbled through

the reaction for 10 min. The solvents were removed by filtration and the resin was treated again with a 25% piperidine/DMF solution (0.7 mL) for 15 min. The solvent was removed by filtration and the resin washed with DMF (3×), *i*-PrOH (2×), and DMF (2×). In a separate vial, HATU (20 mg, 0.052 mmol), HOAt (7 mg, 0.052 mmol), and Fmoc-N^{α}-methylglutamine (**22**) (20 mg, 0.052 mmol) were dissolved in 0.4 mL of DMF. DIEA (30 μL, 0.173 mmol) was added and the mixture stirred for 1.5 min and added to the resin. N_2 was bubbled through the reaction for 30 min, the solvent was removed by filtration, and the resin was washed with DMF (3×), *i*-PrOH (2×), DMF (2×), *i*-PrOH, CH_2Cl_2, and Et_2O.

To the washed resin was added CH_2Cl_2 (0.35 mL) and a 25% piperidine/DMF solution (0.35 mL), and N_2 was bubbled through the reaction for 10 min. The solvents were removed by filtration and the resin was treated again with a 25% piperidine/DMF solution (0.7 mL) for 15 min. The solvent was removed by filtration and the resin washed with DMF (3×), *i*-PrOH (2×), and DMF (2×). In a separate vial, HATU (20 mg, 0.052 mmol), HOAt (7 mg, 0.052 mmol), and Fmoc-leucine (18 mg, 0.052 mmol) were dissolved in 0.4 mL of DMF. DIEA (30 μL, 0.173 mmol) was added and the mixture was stirred for 1.5 min and added to the resin. N_2 was bubbled through the reaction for 30 min, the solvent was removed by filtration, and the resin was washed with DMF (3×), *i*-PrOH (2×), DMF (2×), *i*-PrOH, CH_2Cl_2, and Et_2O.

To the washed resin was added CH_2Cl_2 (0.35 mL) and a 25% piperidine/DMF solution (0.35 mL), and N_2 was bubbled through the reaction for 10 min. The solvents were removed by filtration and the resin was treated again with a 25% piperidine/DMF solution (0.7 mL) for 15 min. The solvent was removed by filtration and the resin was washed with DMF (3×), *i*-PrOH (2×), and DMF (2×). In a separate vial, HATU (20 mg, 0.052 mmol), HOAt (7 mg, 0.052 mmol), and N^{ω}-N^{ω}-bis(benzyloxycarbonyl)-N^{α}-Fmoc-D-arginine (34.8 mg, 0.052 mmol) were dissolved in 0.4 mL of DMF. DIEA (30 μL, 0.173 mmol) was added and the mixture was stirred for 1.5 min and added to the resin. N_2 was bubbled through the reaction for 30 min, the solvent was removed by filtration, and the resin was washed with DMF (3×), *i*-PrOH (2×), DMF (2×), *i*-PrOH, CH_2Cl_2, and Et_2O.

To the washed resin was added a 2% DBU/DMF solution (0.4 mL), and N_2 was bubbled through the reaction for 20 min. The solvents were removed by filtration and the resin was bubbled again with a 2% DBU/DMF solution (0.4 mL) for 20 min. The solvent was removed by filtration and the resin washed with DMF (3×), *i*-PrOH (2×), and DMF (2×). In a separate vial, HATU (20 mg, 0.052 mmol), HOAt (7 mg, 0.052 mmol), and Fmoc-D-allothreonine (18 mg, 0.052 mmol) were dissolved in 0.4 mL of DMF. DIEA (30 μL, 0.173 mmol) was added and the mixture was stirred for 1.5 min and added to the resin. N_2 was bubbled through the reaction for 30 min, the solvent was removed by filtration, and the resin was washed with DMF (3×), *i*-PrOH (2×), DMF (2×), *i*-PrOH, CH_2Cl_2, and Et_2O.

To **13** was added a 2% DBU/DMF solution (0.4 mL), and N_2 was bubbled through the reaction for 20 min. The solvents were removed by filtration and the resin was bubbled again with a 2% DBU/DMF solution (0.4 mL) for 20 min. The solvent was removed by filtration and the resin was washed with DMF (3×), *i*-PrOH (2×), DMF (2×), *i*-PrOH, CH_2Cl_2, and Et_2O. The product was cleaved from the resin by treatment with a 2% TFA/CH_2Cl_2 solution for 15 min, followed by removal of the resin by filtration. Evaporation of the solvent under reduced pressure afforded 47 mg of crude product. The crude product was purified with reverse-phase HPLC (C8, 40−60% gradient of CH_3CN-H_2O over 60 min, retention time 29.5 min), yielding protected **5** (13.6 mg) as a colorless, glassy solid.

In a 250-mL round-bottom flask **5** (4 mg) was dissolved in AcOH−*i*PrOH (2.0 mL) and 10% $Pd(OH)_2$-C (∼1 mg) was added. The system was flushed and evacuated three times with argon, and then flushed with H_2 twice and maintained under a positive pressure of H_2. The reaction proceeded for 140 min, with the balloon being refilled with hydrogen hourly. The catalyst was removed by filtration and the solution was concentrated under reduced pressure. This procedure was repeated three times until all of **5** was deprotected.

The combined crude product (14 mg) was purified with reverse-phase HPLC (C8, 5−40% gradient of CH_3CN-H_2O over 50 min, retention time 33 min), yielding **5** (7.7 mg, 26%) as a colorless foam. ^{1}H NMR (500 MHz, CD_3OD) δ 7.19 (d, 2H, J = 8.5 Hz), 6.78 (d, 2H, J = 8.5 Hz), 5.22 (t, d, 1H, J = 7, 2 Hz), 5.11 (q, 1H, J = 7.3 Hz), 4.83 (overlapped, 1H), 4.74 (d, d, 1H, J = 10.4, 3.8 Hz), 4.39 (d, d, 1H, J = 7.9, 5.7 Hz), 4.32 (d, 1H, J = 8.9 Hz), 4.13 (m, 1H), 3.89 (d, 1H, J = 5.5 Hz), 3.2 (m, 2H), 3.13 (s, 3H), 3.08 (s, 3H), 2.86 (s, 3H), 1.99 (overlapped, 2H), 1.98, 1.72 (overlapped, 2H), 1.97, 1.65 (overlapped, 2H), 1.63 (overlapped, 2H), 1.6 (overlapped, 2H), 1.4 (d, 3H, J = 6.9 Hz), 1.28 (d, 2H, J = 6.3 Hz), 0.95 (d, 3H, J = 6.7 Hz), 0.93 (d, 3H, J = 6.7 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ 129.1, 129, 114.8, 83.9, 65.8, 57.9 56.3, 55.7, 53.2, 52.8, 48.4, 40.5, 39.6, 31.5, 30.1, 28.5, 24.9, 24.6, 22.2, 17.4,13.3. HRMS (ESI) calcd for $C_{36}H_{60}N_{10}O_{11}$ (H^+) 809.4521, found 809.4535.

Callipeltin E (L-Threonine Isomer, 1). 1 was prepared in the same manner as **5**. The product was cleaved from the resin by treatment with a 2% TFA/CH_2Cl_2 solution for 15 min, followed by removal of the resin. Evaporation of solvent under reduced pressure afforded crude product (40 mg), which was purified with reverse-phase HPLC (C8, 40−60% gradient of CH_3CN-H_2O over 60 min, retention time 29.5 min), yielding protected **1** (15.1 mg) as a colorless, glassy solid. After hydrogenation (4 mg at a time) the combined crude product was purified with reverse-phase HPLC (C8, 5−40% gradient of CH_3CN-H_2O over 50 min, retention time 32.5 min), yielding **1** (5.8 mg, 20%) as a colorless foam. ^{1}H NMR (500 MHz, CD_3OD) δ 7.24 (d, 2H, J = 8.5 Hz), 6.81 (d, 2H, J = 8.5 Hz), 5.23 (q, 1H, J = 9.2 Hz), 5.13 (q, 1H, J = 7.2 Hz), 4.9 (overlapped, 1H), 4.74−4.8 (m, 1H), 4.45 (d, d, 1H, J = 7.8, 6.6 Hz), 4.38 (d, 1H, J = 9.1 Hz), 4.08 (p, 1H, J = 6.5 Hz), 3.78 (d, 1H, J = 6.5 Hz), 3.24 (m, 2H), 3.16 (s, 3H), 3.14 (s, 3H), 2.87 (s, 3H), 1.99 (overlapped, 2H), 1.98, 1.72 (overlapped, 2H), 1.97, 1.65 (overlapped, 2H), 1.63 (overlapped, 2H), 1.6 (overlapped, 2H), 1.44 (d, d, 3H, J = 7.26, 1.77 Hz), 1.31 (d, 2H, J = 6.3 Hz), 1.01 (d, 3H, J = 5.7 Hz), 0.93 (d, 3H, J = 3.6 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ 129.1, 129, 128.9, 114.8, 83.8, 65.8, 58.7, 55.7, 55.4, 52.9, 52.7, 48.4, 40.5, 39.6, 31.5, 31.2, 28, 25, 24.6, 22.2, 18.9, 13.3. HRMS (ESI) calcd for $C_{36}H_{60}N_{10}O_{11}$ (H^+) 809.4521, found 809.4532.

Acknowledgment. We gratefully acknowledge the assistance of Prof. M. V. D'Auria for her provision of NMR spectra, a manuscript preprint, and helpful discussions. We thank Dr. Huaping Mo for assistance with the variable-temperature experiments. We also thank the National Institutes of Health (AI-50888) for support of this work.

Supporting Information Available: Full experimental details and tabulated NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

JO060351H