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Solid Phase Total Synthesis and Structure Proof of Callipeltin B

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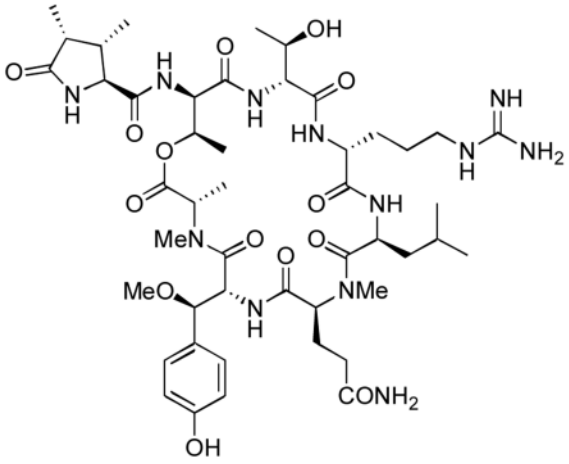
Abstract



1c

The cytotoxic, cyclic heptadepsipeptide natural product callipeltin B was synthesized on a solid-phase support in 15% overall yield. Comparison of the ^{1}H NMR spectra of three synthetic isomers with those of callipeltin B confirmed the configurational reassignment of its threonine residues as D-allothreonine and the assignment of the configuration of its β-methoxytyrosine residue as (2*R*, 3*R*).

The cyclic depsipeptide callipeltin B (**1**) was isolated from the Lithistid sponge *callipelta sp.* and structurally characterized by Minale and coworkers in 1996.[1] Along with the related cyclic depsipeptide callipeltin A,[2] **1** possesses a 22-membered macrolactone composed of the unique, non-proteinogenic amino acids β-methoxytyrosine (β-MeOTyr) and (3*S*,4*R*)-3,4-dimethyl-L-pyroglutamic acid (DiMePyroGlu) as well as several D- and *N*-methylated amino acids. Both callipeltins A and B showed broad-spectrum cytotoxicity against a number of tumor cell lines, including several drug-resistant cell lines,[1] and callipeltin A shows potent anti-HIV activity.[2]

Although the initially published structure of callipeltin B (**1a**) has never been revised, revisions to the structure of the closely related cyclic depsipeptide callipeltin A have strongly implied a need for a structural revision of **1**. Specifically, D'Auria and coworkers, after isolating and

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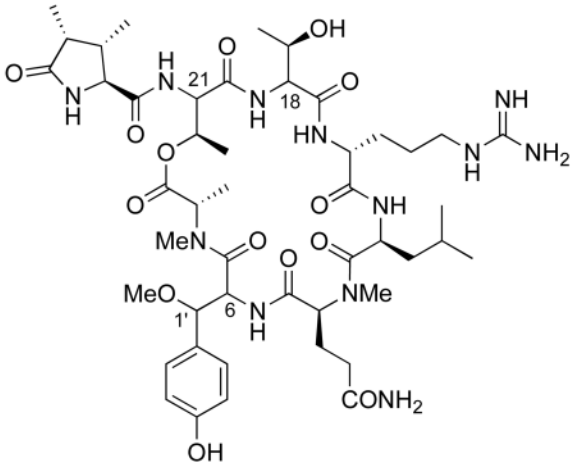
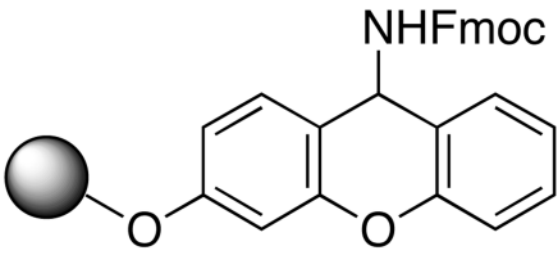
characterizing two smaller, linear peptidic fragments of callipeltin A that they termed callipeltins D and E, suggested that one of the residues initially identified as L-threonine should be reassigned as D-allothreonine.[3] Such a change thus implied that the structure of callipeltin B should be reassigned as **1b**, in which the configuration of C-21 is *R*.



1a, 18-*S*, 21-*S*
1b, 18-*S*, 21-*R*
1c, 1'-*R*, 6-*R*, 18-*R*, 21-*R*

More recently, Bifulco and D'Auria have published computational studies that suggest that both of the residues in callipeltin A initially assigned as L-threonine by Minale and coworkers are actually D-allothreonine.[4] At the same time, the configuration of the β-MeOTyr residue of callipeltin A, which could not be determined in Minale's original structural study, was determined to be (2*R*, 3*R*) by D'Auria and co-workers, using a combination of synthesis and degradation studies.[5] Both of these structural reassignments were confirmed in our laboratory by the synthesis and spectral correlation of two diastereomers of callipeltin E.[6] Taken together, these studies implied that the structure of callipeltin B should be further revised to **1c**, in which the configuration of C-18 has been changed and those of C-1′ and C-6 are assigned as *R*. Herein we wish to report the synthesis of the three candidate structures **1a-c** and the confirmation of **1c** as the structure of callipeltin B by ^{1}H NMR correlation.

A solid phase synthetic strategy was chosen to expedite the synthesis of **1**, its analogues and related depsipeptides. To permit macrocyclization on a solid support, it was decided to anchor the sidechain of the N-methylglutamine residue to a peptide amide synthesis resin, thereby leaving available both N- and C-termini for macrocyclization by amide bond formation. The Tentagel-based TG Sieber amide resin[7] (**2**) was chosen for the relatively low amounts of TFA (1-5% in CH_2Cl_2) needed for efficient cleavage of the peptide, thereby minimizing the possibility of acid-catalyzed β-MeOTyr decomposition during cleavage from the resin. A standard Fmoc-based, C-to-N peptide synthesis approach thus required the use of allyl-based protecting groups for N- and C- termini immediately prior to macrocyclization. All sidechain protecting groups were selected for their ability to be removed either during the mildly acidic cleavage from the Sieber resin or a subsequent catalytic hydrogenation.



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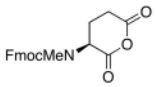
The initial plan to close the macrocycle at the MeGln/β-MeOTyr peptide bond, permitting a C-to-N synthesis from the MeGln residue, had to be abandoned because of spontaneous diketopiperazine formation upon deprotection of the Leu-MeGln dipeptide; instead, it was decided to close the macrocycle at the β-MeOTyr/*N*-methylalanine peptide bond, despite the known problem of slower acylation of secondary amines. Additionally, an initial plan to install the N-terminal (3*S*,4*R*)-3,4-dimethyl-L-pyroglutamic acid after macrocyclization was revised because of rapid O to N transacylation upon deprotection of the N-terminal Fmoc group. As a result, it was decided to install the (3*S*,4*R*)-3,4-dimethyl-L-pyroglutamic acid residue prior to forming the ester bond to prevent transacylation.

All of the constituent residues needed to make **1a-c** were either commercially available or had been previously synthesized. In addition to D'Auria's synthesis of β-MeOTyr,[5] several others had been published;[8] several different syntheses of DiMePyroGlu had also been reported.[9] The syntheses of **1a-c** began with the deprotection and acylation of **2** (Scheme 1) with the cyclic anhydride of Fmoc-*N*-methylglutamic acid (**3**),[10] followed by activation of the resin-bound acid with HATU and coupling with (2*R*, 3*R*)-*O*-benzyl-β-methoxytyrosine allyl ester (**4**)[7c] to afford a resin-bound dipeptide. All deprotection and coupling reactions were monitored by removal of an aliquot of 1-2 mg of resin, cleavage of the peptide from the resin and analysis of the crude cleavage mixture by reverse phase HPLC and MALDI-MS. It was found that use of HATU with no added HOAt afforded complete consumption of starting material with no epimerization evident by HPLC analysis. Sequential installation of Fmoc-Leu, Fmoc-D-Arg(NO_2), Fmoc-D-*a*Thr(THP) and Fmoc-D-*a*Thr using coupling conditions optimized for each step proceeded without incident to afford the resin-bound hexapeptide **5** in >95% purity as determined by HPLC analysis. For the syntheses of **1a** and **1b**, protected L-threonine was substituted in this sequence for the second, or both, D-allothreonine residues. Additionally, in the syntheses of **1a** and **1b**, Fmoc-D-Arg(Z,Z) was substituted for Fmoc-D-Arg(NO_2).

Deprotection of the N-terminal Fmoc afforded a β-amino alcohol that could be selectively N-acylated with the *N*-hydroxysuccinimide ester of (3*S*,4*R*)-3,4-dimethyl-L-pyroglutamic acid (**6**). Esterification of the hydroxyl with alloc-*N*-methylalanine using the sulfonyl nitrotriazole reagent MSNT[11] in conjunction with *N*-methylimidazole afforded a resin-bound, protected heptadepsipeptide in high purity. Palladium-catalyzed deprotection of N- and C-termini followed by macrolactamization using PyAOP to minimize epimerization of the activated β-methoxytyrosine afforded the resin-bound macrocycle in roughly 80% purity as judged by HPLC analysis of the crude deprotection mixture. Removal of the cyclized product from the resin with 2% TFA-CH_2Cl_2 and complete deprotection by catalytic transfer hydrogenation afforded **1c**, after purification by reverse phase HPLC, in 15% overall yield. The isomeric depsipeptides **1a** and **1b** were obtained in 15% and 14% overall yields, respectively.



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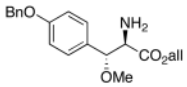

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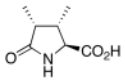

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Because of the unavailability of a sample of natural callipeltin B, the correlation of synthetic **1a-c** with callipeltin B involved the comparison of spectroscopic data. Comparison of the ^{1}H NMR of **1c** with that of callipeltin B showed no significant differences between the two apart from the absence of several exchangeable amide protons in CD_3OD.[12] On the other hand, the ^{1}H NMR spectra of **1a** and **1b** showed considerable differences from that of callipeltin B; in fact, the spectra of **1a** and **1b** showed evidence of conformational heterogeneity at ambient temperature. On the basis of these comparisons, we now conclude that the correct structure of callipeltin B is **1c**. Current studies are underway to synthesize analogues of **1c** and several related cyclic depsipeptides using the methodology described herein.

In summary, the synthesis of the cyclic depsipeptide natural product callipeltin B has been accomplished, thereby providing confirmation of the recent configurational reassignment of the previously assigned L-threonine residues as D-allothreonine and the recent configurational assignment of the β-methoxytyrosine residue as (2*R*, 3*R*). This synthesis illustrates the facility with which even complex natural products can be synthesized on solid supports. In addition, the expeditious solid-phase synthesis of **1** opens the door for the rapid synthesis of analogues to explore structure-function relationships and for the synthesis of other, more complex cyclic depsipeptides.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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10. The regioselectivity of opening of **3** was established by cleavage of the acylation product from the resin and HPLC correlation with authentic samples of Fmoc-N^{α}-methylglutamine and the C-terminal carboxamide of Fmoc-N^{α}-methylglutamic acid. Exclusive attack on the less hindered carbonyl by the sterically hindered nitrogen of the Sieber linker was found by reverse phase HPLC analysis.
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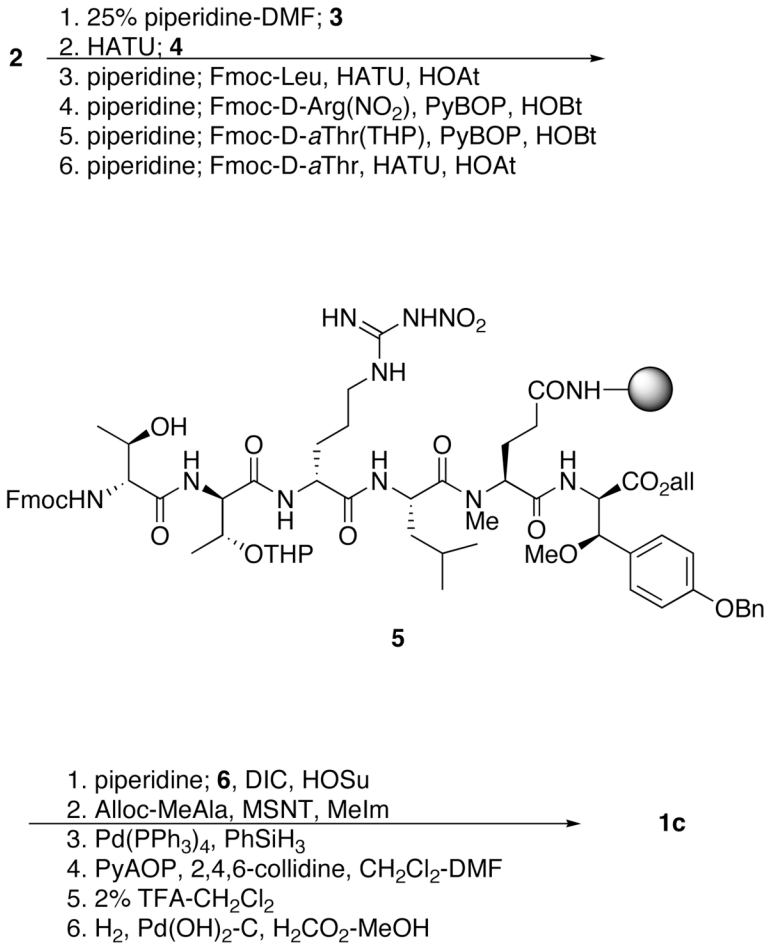
12. The only differences outside of the amide proton region noted in the comparison were three singlets present in the ^{1}H NMR of the natural product (at δ ~2.08, 2.1 and 2.2) that were not found in the spectrum of **1c**, nor were included in the tabulated data in Ref. 1; additionally, a singlet at δ 2.85 was found in the ^{1}H NMR of **1c** that didn't correspond to any resonance in callipeltin B and might possibly result from a small amount of DMF present in the sample.



Scheme 1.
Solid Phase Synthesis of **1c**