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Solid-Phase Total Synthesis and Structure Proof of Callipeltin B

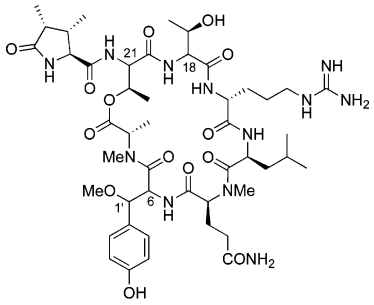
Ravi Krishnamoorthy, Leslie D. Vazquez-Serrano,† Jeffrey A. Turk,† Jennifer A. Kowalski,† Alan G. Benson,† Nneka T. Breaux,† and Mark A. Lipton*

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

Received September 13, 2006; E-mail: lipton@purdue.edu

The cyclic depsipeptide callipeltin B (**1**) was isolated from the Lithistid sponge *callipelta* sp. and structurally characterized by Minale and co-workers 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 show 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]

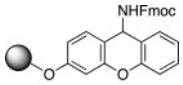
Although the initially published structure of callipeltin B (**1a**) has never been revised, revisions to the structure of the closely related cyclic depsipeptide callipeltin A have strongly implied a need for a structural revision of **1**. Specifically, D'Auria and co-workers, after isolating and characterizing 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 residues in callipeltin A initially assigned as L-threonine by Minale and co-workers 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 report the synthesis of **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 side chain 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 side-chain 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 DiMePyroGlu 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 DiMePyroGlu 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**)[8c] 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

† Present addresses: (L.D.V.S.) Owens Corning Composite Solutions, Granville, OH 43023; (J.A.T.) International Flavors and Fragrances, Union Beach, NJ 07735; (J.A.K.) Boehringer-Ingelheim Pharmaceuticals, Ridgefield, CT 06877; (A.G.B.) Mallinckrodt, St. Louis, MO 63134; (N.T.B.) Dow Agrosciences, Indianapolis, IN 46268.

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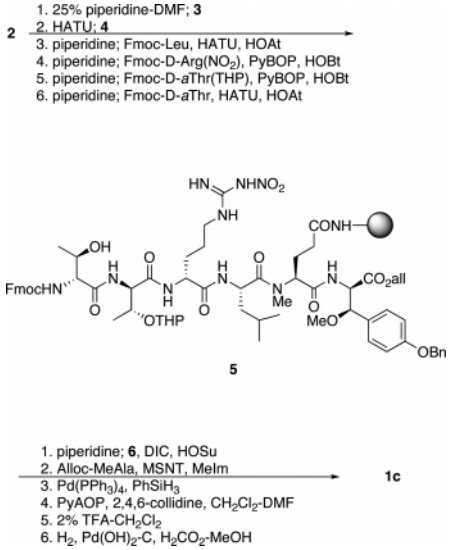
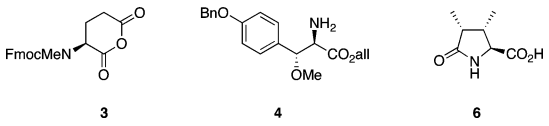
Scheme 1. Solid-Phase Synthesis of **1c**



mixture by reverse-phase HPLC (RP-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 DiMePyroGlu (**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 RP-HPLC, in 15% overall yield. The isomeric depsipeptides **1a** and **1b** were obtained in 15% and 14% overall yields, respectively.



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 spectra of **1c** with those 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 those 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.

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Supporting Information Available: Complete experimental details and spectroscopic details for **1** and the novel protected amino acids used in its synthesis; a tabulated comparison of ^{1}H NMR spectra of **1** and callipeltin B and reproduced spectra of both. This information is available free of charge via the Internet at http://pubs.acs.org.

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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 RP-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 they 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 have resulted from a small amount of DMF present in the sample.

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