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An expedient synthesis of the amide analog of the potent antifungal lipopeptidolactone FR901469

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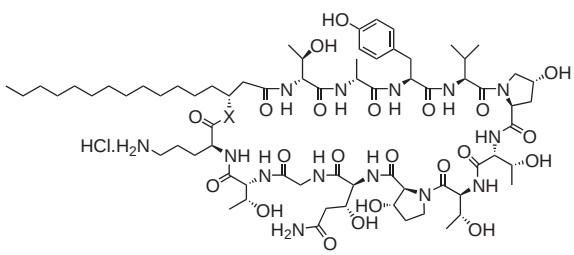
Abstract—An expedient synthesis of the lactam analog (**2**) of the 40-membered lipopeptidolactone antifungal antibiotic, FR901469 (**1**), is described. The key steps in this synthesis are a novel biotransformation of the natural product to produce the highly versatile linear peptide building block **3**, and efficient formation of the 40-membered ring by macrolactamization under high-dilution conditions.

The last decades of the twentieth century have seen an increase in the incidence of severe, life-threatening invasive fungal diseases. This can be attributed to the increase in the population of immunosuppressed individuals, resulting from improved outcomes in transplantation surgery, aggressive anticancer regimens, and the HIV epidemic.[1] During this period, the polyene Amphotericin B (AmpB) has remained the primary agent for first-line treatment of these infections.[2] However, this drug is well known to produce a variety of dose-limiting toxic side effects that result in discontinuation of treatment and clinical failure.[2] The introduction of various lipid formulations of AmpB has gone some way to reducing the toxicity associated with this agent,[3] however, the need clearly exists for a novel, broad spectrum, fungicidal, antifungal agent, with a superior safety profile to AmpB.

We recently reported the isolation and biological activity of the novel water-soluble natural products, FR901379 (WF11899A) and FR901469 (**1**), which inhibit the synthesis of 1,3-β-glucan, a key component of the fungal cell wall.[4,5] FR901379 is a member of the echinocandin class of natural products[6] and a semi-synthetic derivative, FK463, is currently in phase III clinical trials.[7] FR901469 shows potent antifungal activity and excellent water solubility, however the presence of an ornithine amine group adjacent to the ester moiety results in a pH-dependent ring opening process leading to a biologically inactive linear lipopeptide. In order to remove this propensity for ring opening, we designed the amide analog **2** of the natural product and herein disclose efficient methodology for the synthesis of this compound.



1 X = O **(FR901469)**

2 X = NH

Keywords: antifungals; macrocycles; microbial reactions; peptides and polypeptides.

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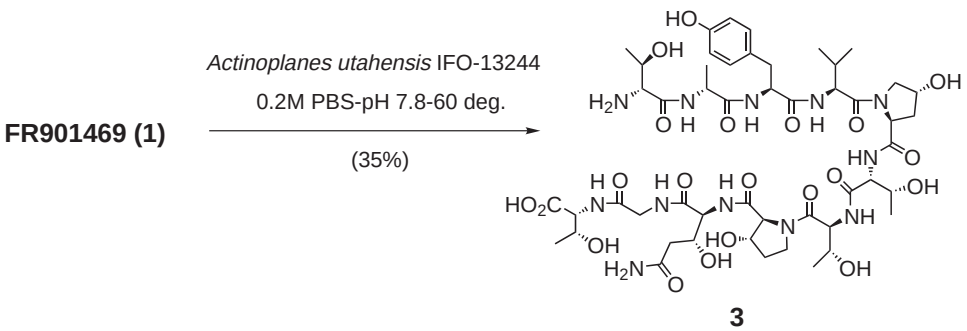
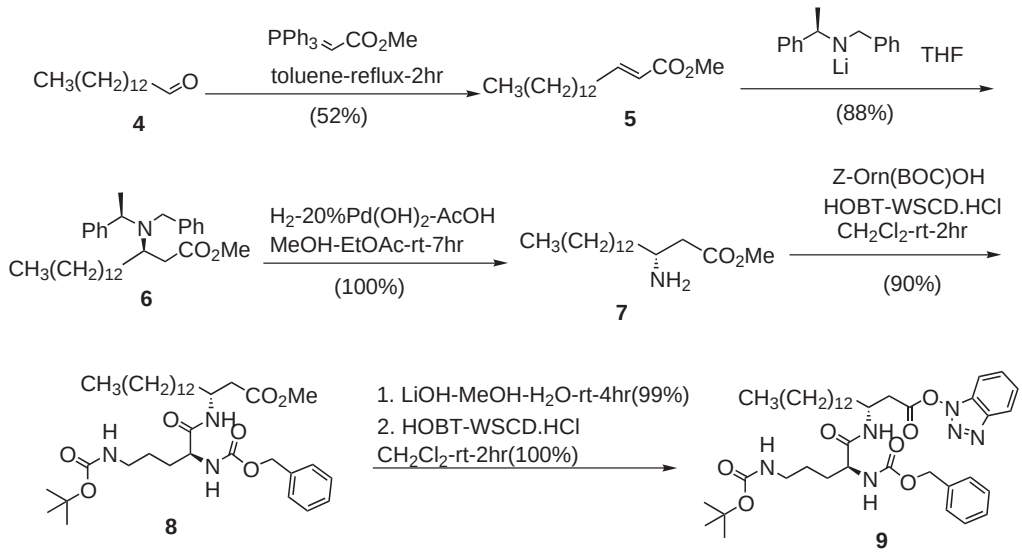
Our approach to the synthesis of **2** is based on deacylation of the lipophilic side chain of the natural product, introduction of a pre-formed amide-substituted side chain moiety, macrolactamization and ornithine amino group deprotection. The key intermediate for the synthesis of **2**, the linear peptide **3**, was produced in 35% yield by direct incubation of FR901469 with *Actinoplanes utahensis* IFO-13244 in 0.2 M phosphate at pH 7.8 and 60°C (Scheme 1).[8] In general, this organism has been employed for deacylation of various lipopeptide compounds and cleaves only the amide bond connecting the lipophilic side chain to the peptide skeleton. For example, removal of the side chain from echinocandin B.[9] In this work, we have demonstrated another facet of this organism, whereby simultaneous removal of a β-hydroxyacid side chain and an ornithine moiety is also possible.

For the synthesis of **2**, the appropriate side-chain fragment **9** was prepared as shown in Scheme 2. Aldehyde **4** was converted to the *E*-olefin **5** in 52% yield by Wittig reaction with methyl(triphenylphosphoranilidene)-acetate. A trace amount of the *Z*-isomer was removed by column chromatography. Application of the Davies asymmetric β-amino acid synthesis methodology[10] involving reaction of **5** with the lithium amide derived from (*R*)-*N*-(α-methylbenzyl)benzylamine afforded the adduct **6** in 88% yield as an oil. Removal of the benzyl groups from **6** under hydrogenation conditions produced the amine **7** in 100% yield. The enantiomeric excess of this amine was 93.1% by conversion to the Mosher amide ((*S*)-(+)-MTPACl-Et_3N-CH_2Cl_2) and GC analysis. Completion of the synthesis of **9** involved coupling of **7** with Z-Orn(BOC)OH using a water soluble carbodiimide derivative (1-ethyl-3-dimethylaminopropylcarbodiimide hydrochloride, WSCD.HCl) to afford the β-amino acid derivative **8** in 90% yield. Ester hydrolysis (LiOH-MeOH-H_2O, 99%) gave the corresponding carboxylic acid, which was then smoothly converted to the activated ester **9** (HOBT-WSCD.HCl-CH_2Cl_2-rt, 100%).

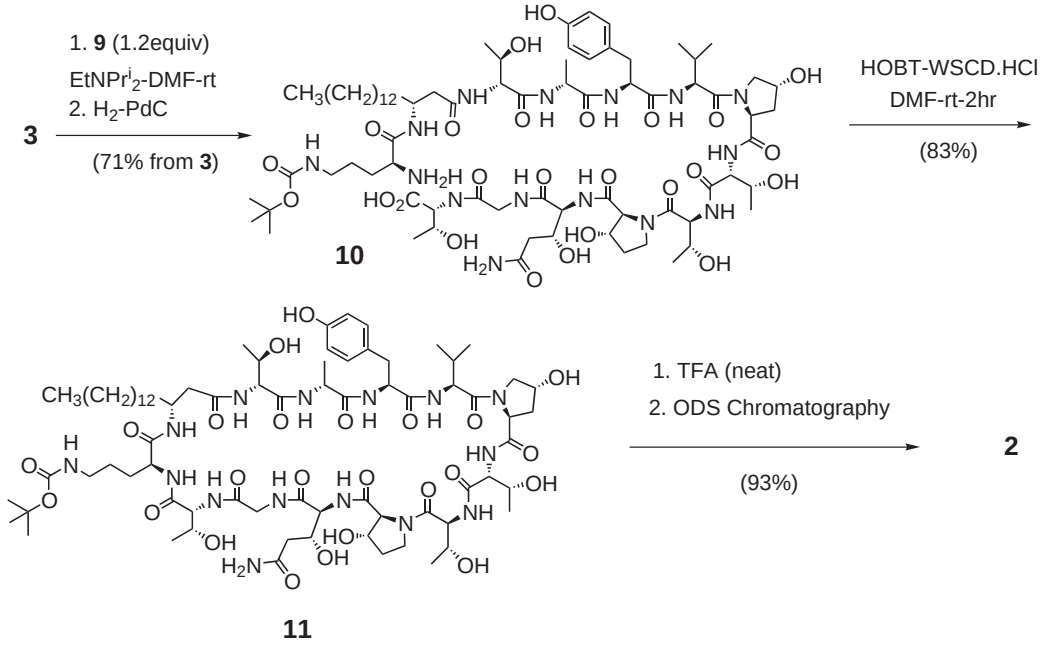
The final steps in the synthesis of **2** are shown in Scheme 3. Selective acylation of the amine group of **3** with activated ester **9** proceeded smoothly in DMF in the presence of diisopropylethylamine. Since the resulting acylated intermediate had poor water solubility, impeding purification by ODS column chromatography, the crude material was directly subjected to catalytic hydrogenation to remove the benzyloxycarbonyl protecting group. Purification afforded the cyclization precursor **10** in 71% yield from **3**. Macrocyclization under high dilution conditions (1.2 mM) employing WSCD.HCl-HOBT in DMF resulted in smooth cycliza-



Scheme 1. Biotransformation of FR901469.



Scheme 2. Synthesis of the activated ester fragment.



Scheme 3. Synthesis of lactam (**2**).

tion of **10** to afford the cyclic peptide **11** in 83% yield as an amorphous white solid after purification by ODS column chromatography and lyophilization. Removal of the BOC protecting group using neat TFA afforded **2** as the hydrochloride salt after ion-exchange chromatography and freeze-drying.[11]

In summary, we disclose novel methodology whereby the lactone moiety of the natural product FR901469 can be modified to an amide group, thereby removing the propensity for ring opening whilst maintaining potent antifungal activity.[12] The key intermediate linear peptide **3** was readily produced by a novel biotransformation of the natural product. This methodology is applicable to the synthesis of a variety of novel lipophilic side chain-modified analogs, the synthesis and biological activity of which will be reported in future publications.

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8. Selected data for **3**: $[\alpha]_D^{22}=-33.7$ (*c* 1.0, MeOH); IR (KBr) 3300, 2980, 1655, 1540, 1455, 1235 cm^{-1}; FAB-MS (*m*/*z*): 1183 $(M+H)^+$.
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11. Selected data for **2**: $[\alpha]_D^{28}=+17.4$ (*c* 0.67, MeOH); IR(KBr) 3307, 2854, 1659, 1522, 1450, 1240 cm^{-1}; FAB-MS (*m*/*z*): 1532 (MH^+–HCl); ^{1}H NMR (200 MHz, CD_3OD, δ): 0.76–1.00 (9H, m), 1.15 (3H, d, $J=6.3$ Hz), 1.18–1.65 (40H, m), 1.65–2.60 (9H, m), 2.80–3.05 (4H, m), 3.58–4.90 (24H, m), 5.04 (1H, d, $J=3$ Hz), 6.90 (2H, d, $J=8.5$ Hz), 7.06 (2H, d, $J=8.5$ Hz).
12. The amide analog **2** showed comparable antifungal activity to the natural product **1** and the clinically used AmpB. For example, MIC values against *A. niger* ATCC6275 were 0.5, 0.25, and 0.25 μg/ml for **2**, **1**, and AmpB, respectively.