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COMMUNICATIONS TO THE EDITOR

Site-specific Structural Transformation of the Novel Antifungal Cyclic Depsipeptide FR901469: Synthesis and Biological Activity of FR203903

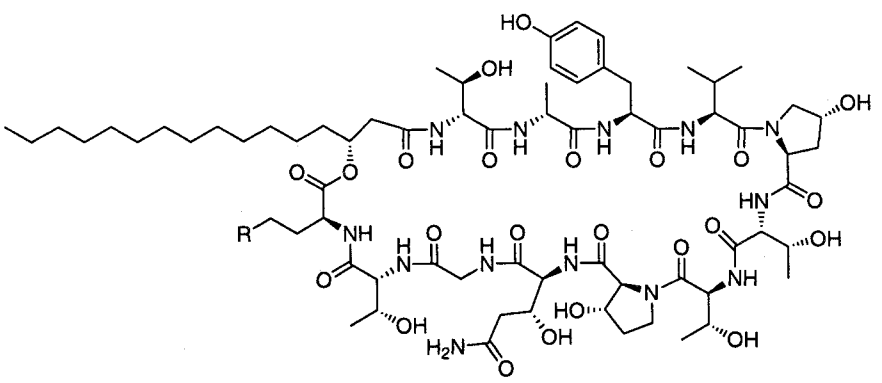
Sir:

The increased and widespread use of systemic antifungal agents has been accompanied by a growing number of patients with immune systems compromised by transplantation, cancer, or HIV infection.[1] Currently available antifungal therapy includes amphotericin B (AMPH-B) and azoles such as fluconazole (FLCZ) and itraconazole (ITCZ),[2,3] however, they have various problems, *i.e.* toxicity and rapid development of resistance. 1,3-β-Glucan synthase, the enzyme complex responsible for the synthesis of 1,3-β-glucan, a major component of the fungal cell wall, is an attractive antifungal target since it is essential to most pathogenic fungi, but is not present in mammalian cells.[4] Therefore, in recent years, echinocandin and pneumocandin types of antifungal lipopeptides have been studied intensively.[5,6]

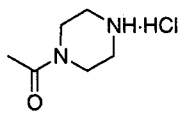
FR901469 (**1**) is a novel antifungal cyclic depsipeptide that was isolated from the culture broth of an unidentified fungus No. 11243 as a water-soluble 1,3-β-glucan synthase inhibitor, and is composed of twelve amino acids and a 3-hydroxypalmitoyl moiety (Figure 1).[7~9] Since intramolecular attack to the ester bond by the ornithine amino group leads to a pH-dependent ring opening process, we have investigated the preparation of novel analogs lacking the propensity to ring-open. In this paper, we describe the synthesis and antifungal activity of FR203903 (**2**), an ornithine-modified analog of FR901469, prepared by a novel semi-synthetic approach.

The synthetic routes to FR203903 (**2**) and the key coupling component **6** are outlined in Schemes 1 and 2. β-Ketoester **3**, easily obtained by coupling of myristoyl chloride with Meldrum's acid and subsequent methanolysis,[10] was subjected to Ru(II)-BINAP catalyzed asymmetric hydrogenation[11~13] to give β-hydroxy ketone **4**. The absolute configuration of **4** was shown to be (*R*) from the specific rotation ($[\alpha]_D^{19}$ −14.7° (*c* 1.00, $CHCl_3$) [lit.[14] $[\alpha]_D^{25}$ −14.3° (*c* 2.5, $CHCl_3$)]). The optical purity was determined to be over 99% ee by gas chromatography of the corresponding (*S*)-α-methoxy-α-(trifluoromethyl)-phenylacetate (Mosher ester). Transformation of the methyl

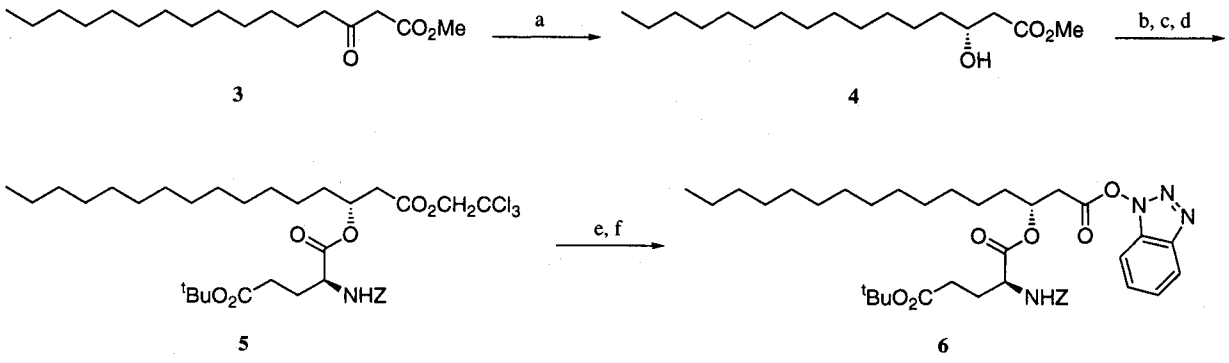
Fig. 1. Structures of FR901469 (**1**) and FR203903 (**2**).



FR901469 (**1**), R = $CH_2NH_2 \cdot HCl$

FR203903 (**2**), R = 

Scheme 1. Preparation of coupling component[a].



[a] Reagents: (a) H_2 (20 atm), $Ru_2Cl_4[(R)\text{-BINAP}]_2\cdot Et_3N$, (1*S*)-(+)-10-camphorsulfonic acid, MeOH (92%); (b) 1N NaOH, MeOH (94%); (c) CCl_3CH_2OH, WSC·HCl, 4-(dimethylamino)pyridine, CH_2Cl_2 (88%); (d) Z-Glu(O^{t}Bu), PyBOP, 4-(dimethylamino)pyridine, CH_2Cl_2 (90%); (e) Zn, 90% AcOH (99%); (f) HOBt, WSC·HCl, CH_2Cl_2 (100%).

ester **4** to the trichloroethyl ester, followed by condensation with Z-Glu(O^{t}Bu) using a powerful coupling reagent, benzotriazol-1-yloxytrispyrrolidinophosphonium hexafluorophosphate (PyBOP)[13,15] provided **5**. Cleavage of the trichloroethyl ester group was accomplished by zinc powder in AcOH, followed by condensation with 1-hydroxybenzotriazole (HOBt) to afford the corresponding activated ester **6**.

The synthesis of FR203903 (**2**) is summarized in Scheme 2. Condensation of **7**, a versatile key intermediate for various FR901469 derivatives, which was obtained by site-specific enzymatic ring cleavage of FR901469 (**1**),[16,17] with activated ester **6**, followed by deprotection of the carbobenzoxy group gave linear free intermediate **8**. The key macrocyclization step to afford compound **9** was achieved using HOBt and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (WSC·HCl) in DMF under high dilution conditions (1.0 mM). Transformation of **9** to FR203903 (**2**) was achieved *via* hydrolysis of the *tert*-butyl ester group, conversion to an amide, and final deprotection. The structure of FR203903 (**2**) was confirmed by elemental analysis and spectral data (Table 1).

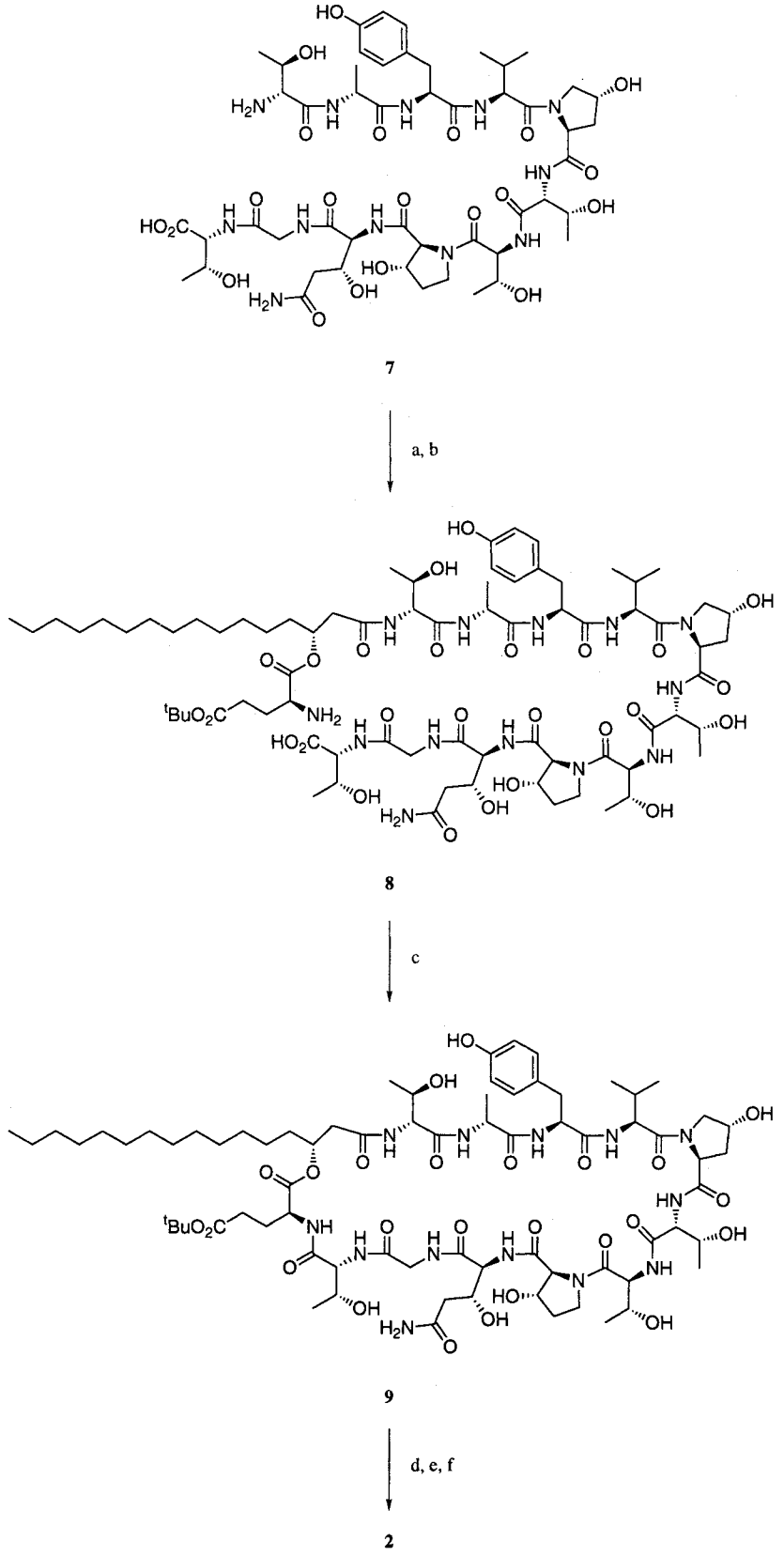
Antifungal activity of this newly synthesized FR203903 (**2**) is shown in Table 2 along with the parent FR901469 (**1**) and the clinically used AMPH-B and FLCZ. The MICs of FR203903 (**2**) were slightly greater compared with FR901469 (**1**), however both agents were effective against FLCZ-non-susceptible *Aspergillus* species. Furthermore, FR203903 (**2**) was comparable to AMPH-B especially against clinically relevant *Candida* and *Aspergillus* species. In accord with other 1,3-β-glucan synthase inhibitors, FR203903 (**2**) and FR901469 (**1**) did not display activity against *C. neoformans* and *T. asahii*.

FR203903 (**2**) showed excellent *in vivo* efficacy in normal mice against *C. albicans* FP633 in a murine systemic infection model by a single subcutaneous injection. The ED_{50} value, estimated on the basis of survival at day 14 after challenge, was 0.61 mg/kg, which was slightly superior to that of FR901469 (**1**) (ED_{50}= 0.88 mg/kg) in spite of slightly greater MIC for this organism. For comparison, in the same model AMPH-B and FLCZ showed ED_{50} values of 0.13 mg/kg and over 20 mg/kg, respectively.

The hemolytic property toward mouse red blood cells (RBCs) was also evaluated. LC_{30}, the concentration of a compound required to induce 30% hemolysis of RBCs, of FR203903 (**2**), FR901469 (**1**), and AMPH-B were 0.82, 0.47, and 0.036 mg/ml, respectively. It is thus clear that the hemolytic activity of FR203903 (**2**) was improved and it has comparable *in vivo* antifungal activity to FR901469 (**1**).

The structure-activity relationships and further pharmacological evaluation of FR203903 and other FR901469 derivatives synthesized by this novel microbial ring cleavage-macrocyclization methodology will be the subject of future publications from these laboratories.

Scheme 2. Preparation of FR203903 (**2**)[a].



[a] Reagents: (a) (i) *N*-(trimethylsilyl)acetamide, DMF; (ii) **6**; (iii) 1N HCl, THF (74%); (b) H_2, 10% Pd/C, MeOH (88%); (c) HOBt, WSC·HCl, DMF (64%); (d) TFA (87%); (e) 1-(*tert*-butoxycarbonyl)piperazine, HOBt, WSC·HCl, DMF (73%); (f) (i) TFA; (ii) Amberlyst A-26 (Cl^- type) ion-exchange resin (75%).

Table 1. Physical data for FR203903 (**2**).

$[\alpha]_D^{22}$	+18.1° (*c* 0.51, MeOH)
^{1}H NMR (200 MHz, CD_3OD) δ	0.70~1.00 (9H, m), 1,11 (3H, d, *J* = 6.5 Hz), 1.18~1.44 (34H, m), 1.44~2.65 (15H, m), 2.70~3.30 (6H, m), 3.30~4.80 (27H, m), 5.00~5.25 (2H, m), 6.95 (2H, d, *J* = 8.4 Hz), 7.12 (2H, d, *J* = 8.4 Hz)
IR (KBr) cm^{-1}	3338, 3074, 2927, 2854, 1734, 1653, 1535, 1522, 1450, 1240, 1093
FAB-MS *m/z*	1616.5 $(MH-HCl)^+$
Elemental Analysis	Calcd for $C_{75}H_{122}ClN_{15}O_{24}\cdot 8H_2O$: C 50.12, H 7.74, N 11.69 Found: C 50.23, H 7.68, N 11.60

Table 2. MIC of FR203903 (**2**) against clinical isolates of fungi[a~c].

Organism	FR203903 (**2**)	FR901469 (**1**)	AMPH-B	FLCZ
Candida albicans FP633	2	0.5	0.25	0.5
Candida albicans ATCC90028	1	0.5	0.5	0.5
Candida tropicalis TIMM0313	2	1	0.5	4
Candida glabrata ATCC90030	8	4	0.5	16
Candida krusei ATCC6258	2	1	1	32
Candida guilliermondii ATCC9390	1	0.5	0.5	4
Candida parapsilosis ATCC22019	4	2	0.5	2
Cryptococcus neoformans TIMM0354[d]	>64	64	0.25	0.5
Trichosporon asahii TIMM3144	>64	64	0.25	2
Aspergillus fumigatus TIMM0063[d]	1	0.5	0.5	>64
Aspergillus niger ATCC6275[d]	0.5	0.25	0.25	>64

[a] MICs were determined by the microdilution method according to the National Committee for Clinical Laboratory Standard M27-A.
[b] MIC (μg/ml): Concentration at which growth was inhibited.
[c] Culture: 35 °C, 2 days
[d] Culture: 35 °C, 3 days

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