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Synthesis and Antifungal Activity of Rhodopeptin Analogues. 2. Modification of the West Amino Acid Moiety

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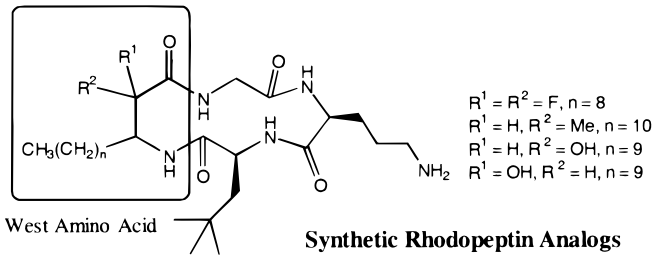
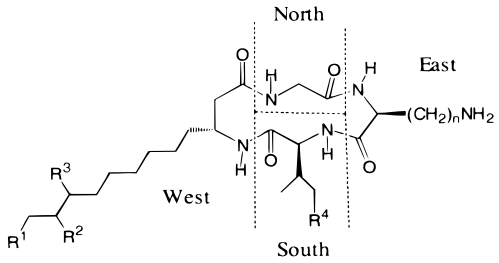
ABSTRACT



Structure–activity relationships of the west amino acid modified analogues of rhodopeptins, novel antifungal tetrapeptide isolated from *Rhodococcus* species Mer-N1033, have been investigated. Among the analogues synthesized, 2,2-difluoro and 2-hydroxy derivatives retained the antifungal activity with better physical properties, i.e., solubility or acute toxicity.

Rhodopeptins, a new family of antifungal cyclic lipopeptides, were isolated from *Rhodococcus* species Mer-N1033.[1] They are composed of three α-amino acids and one *β*-amino acid with a lipophilic side chain as shown in Figure 1.

Fascinated by the attractive fungicidal activity of the rhodopeptins along with the unique structural characteristics, we executed a structure−activity relationship (SAR) study on the molecule. The SAR of the south and the east amino acid moieties has been reported in the preceding paper.[2] The result of this SAR study revealed that a hydrophobic and bulky neutral amino acid (i.e., *γ*-methylleucine) as the south amino acid and a basic amino acid moiety (lysine or ornithine) as the east amino acid were indispensable structural motifs for antifungal activity. We have also found that the structure of the lipophilic side chain does not have a crucial



	R1	R2	R3	R4	n
Rhodopeptin C1	H	H	Me	H	3
Rhodopeptin C2	H	H	Me	Me	3
Rhodopeptin C3	H	Me	H	H	3
Rhodopeptin C4	H	Me	H	H	3
Rhodopeptin B5	Me	Me	H	H	4

Figure 1.

(1) (a) Chiba, H.; Agematu, H.; Kaneto, R.; Terasawa, T.; Sakai, K.; Dobashi, K.; Yoshioka, T. *J. Antibiot.* **1999**, *52*, 695. (b) Chiba, H.; Agematu, H.; Dobashi, K.; Yoshioka, T. *J. Antibiot.* **1999**, *52*, 700. (c) Chiba, H.; Agematu, H.; Sakai, K.; Dobashi, K.; Yoshioka, T. *J. Antibiot.* **1999**, *52*, 710.

(2) Kawato, H.; Nakayama, K.; Inagaki, H.; Nakajima, R.; Kitamura, A.; Someya, K.; Ohta, T. *Org. Lett.* **2000**, *2*, 973−976.

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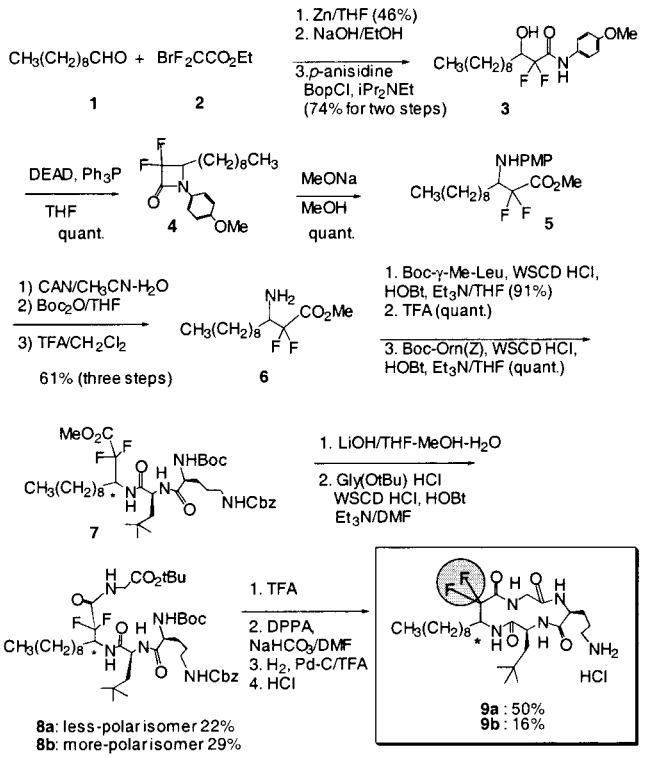
effect on the activity as long as the total number of carbons ranges from 9 to 11.[1] The compounds that fulfilled these structural requirement exhibited antifungal activities with the same magnitude as amphotericin B (AMPH) against *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*, the main fungi that cause systemic antifungal infections. However, these analogues also displayed acute toxicity and poor water solubility. Our next concern, therefore, was to identify new rhodopeptin analogues with decreased toxicity and improved physicochemical properties.

To accomplish these objectives, our effort was focused on the introduction of hydroxy and difluoro groups[3] to the west amino acid moiety. We also targeted an alkylated analogue as a means of further exploring the SAR of the β-amino acid moiety. In this Letter, we report the syntheses and resultant antifungal activities of these analogues.

Our initial targets were difluoro analogues **9a**,**b**, which were synthesized by utilizing β-lactams as intermediates[4] for the β-amino acid moiety (Scheme 1). Reformatsky reaction

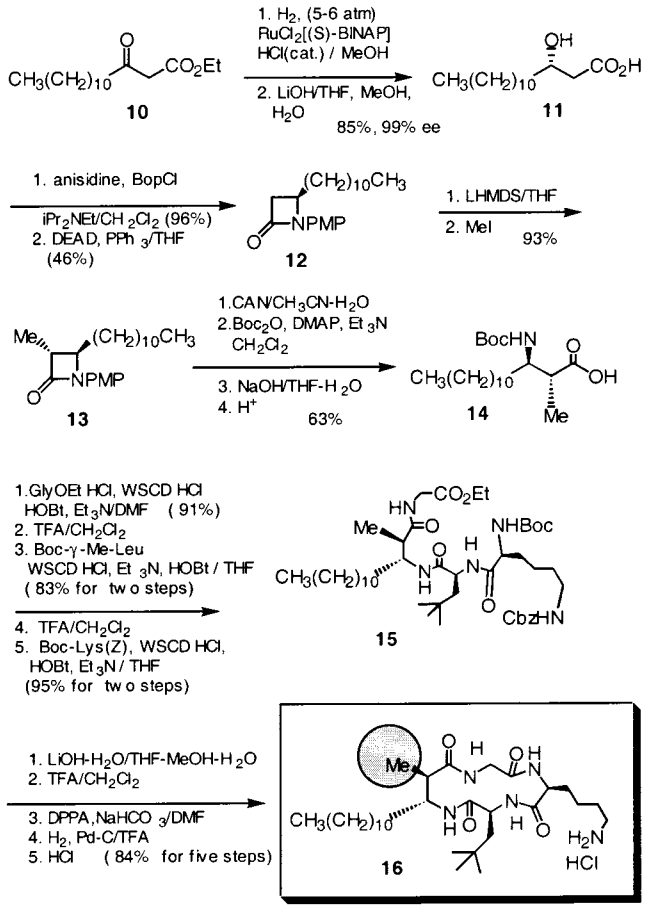
Scheme 1



between **1** and **2** was followed by formation of the *p*-methoxyphenyl (PMP) amide and intramolecular Mitsunobu reaction to provide racemic β-lactam **4**. Following ring opening with sodium methoxide to yield **5**, a protecting group conversion accomplished by treatment with ceric(IV) ammonium nitrate (CAN) and di-*tert*-butyl carbonate (Boc_2O) was necessary for purification of the intermediate. After removal of the Boc group to provide **6**, conventional methods for peptide synthesis were used to obtain tetrapeptides **8a**,**b**, which were separated by flash chromatography. Cyclization of each diastereomer with diphenylphosphoryl azide (DPPA)[5] and hydrogenolysis in TFA furnished the desired difluoro analogues **9a**,**b**.

Introduction of a methyl group onto the β-amino acid was next examined (Scheme 2). Condensation of commercially

Scheme 2



available (*S*)-3-hydroxytetradecanoic acid[6] with *p*-anisidine, followed by Mitsunobu reaction, provided optically pure β-lactam **12**. Alternatively **12** could be obtained in 99% ee via reduction of compound **10** with Ru−(*S*)-BINAP complex.[7] Formation of **12** was complicated by competitive

(3) Uoto, K.; Ohsuki, S.; Takenoshita H.; Ishiyama T.; Iimura S.; Hirota Y.; Mitsui I.; Terasawa H.; Soga T. *Chem. Pharm. Bull.* **1997**, *45*, 1793.

(4) (a) Thaisrivongs, S.; Pals, D. T.; Kati, W. M.; Turner, S. R.; Thomasco, L. M.; Watt, W. *J. Med. Chem.* **1986**, *29*, 2080. (b) Thaisrivongs, S.; Schostarez, H. J.; Pals, D. T.; Turner, S. R. *J. Med. Chem.* **1987**, *30*, 1837. Typical coupling constants for trans isomer ca. 2 Hz, for cis isomer, ca. 5 Hz.

(5) Shioiri, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203.

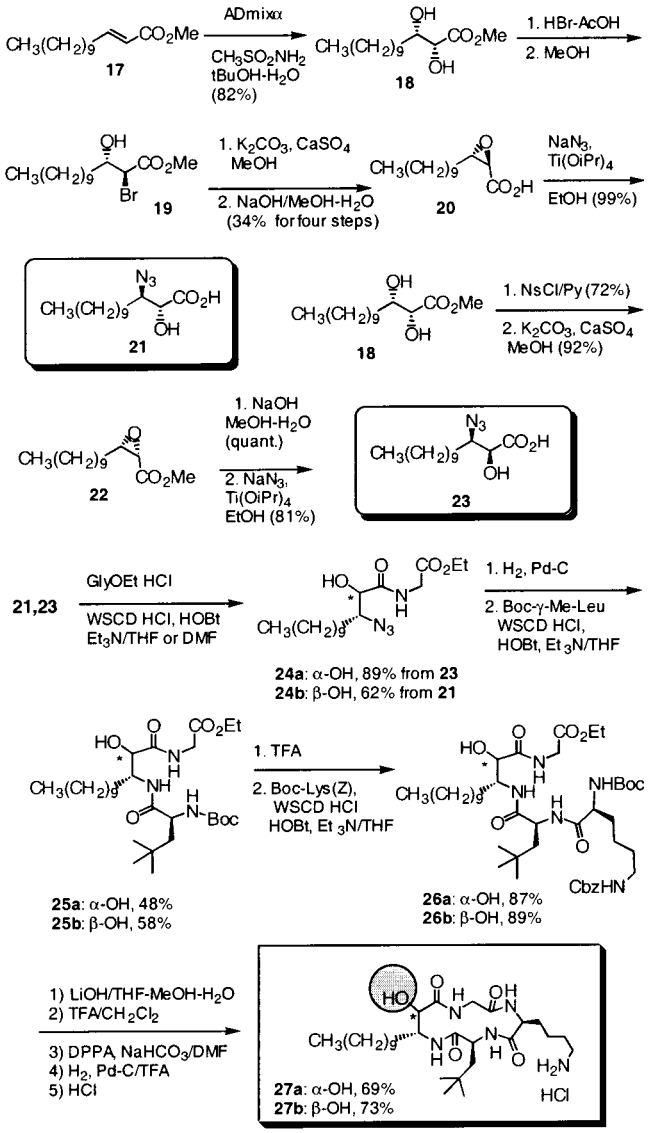
(6) (*S*)-3-Hydroxytetradecanoic acid was kindly provided by Daiichi Pure Chemical Co. Ltd. The enantiomeric excess was determined on compound **12** by comparison of the $[\alpha]_D$ value. The $[\alpha]_D$ values of compound **12** are from BINAP reduction, −67.6°, and from the authentic sample, −67.9°, respectively.

(7) (a) Taber, D. F.; Silverberg, L. J. *Tetrahedron Lett.* **1991**, *32*, 4227. (b) King, S. A.; Thompson, A. S.; King, A. O.; Verhoeven, T. R. J. *Org. Chem.* **1992**, *57*, 6689.

β-elimination, resulting in a diminished yield in comparison to that of the corresponding difluoro analogue. Methylation of **12** furnished a single product, whose structure was confirmed to be the trans isomer **13** by the coupling constant of the protons on the β-lactam ring.[5] Subsequent removal of the PMP group, protection with a Boc group, and hydrolysis of the lactam gave the β-amino acid component **14**. Conventional methods for peptide synthesis were used to provide linear tetrapeptide **15**, which was cyclized with DPPA, followed by removal of the Cbz group to complete compound **16** in good yield.

Finally, the synthesis of analogues containing a hydroxyl group was investigated (Scheme 3). The 2-hydroxy-β-amino

Scheme 3



acid moieties **21** and **23** were synthesized according to the Shioiri protocol.[8,9] Thus, after Sharpless asymmetric dihydroxylation[9] of alkene **17**, bromination and methanolysis provided *anti*-3-hydroxy-2-bromo ester **19**. Subsequent epoxide formation and hydrolysis gave epoxy acid **20**, which furnished *anti*-α-hydroxy-β-amino acid moiety **21** upon treatment with sodium azide. The corresponding *syn*-α-hydroxy-β-amino acid **23** was prepared as follows. Treatment of diol **18** with *p*-nitrobenzenesulfonyl chloride (NsCl) selectively provided the 2-*O*-sulfonated compound, which was then cyclized to afford epoxide **22**. Hydrolysis of **22**, followed by treatment with sodium azide, completed **23**. Compounds **21** and **23** were independently converted to the linear tetrapeptides **26a**,**b** by conventional methods, followed by cyclization with DPPA and deprotection of the Cbz group complete compounds **27a**,**b**.

The antifungal activity of our new analogues are summarized in Table 1. The activity of amphotericin B (AMPH),[10] fluconazole (FLCZ),[10] and the nonsubstituted rhodopeptin analogues **28**−**30** (Figure 2) are cited as references. The difluoro analogue **9b** showed moderate activity against all

Table 1. Antifungal Activity[11,12] (MIC, μg/mL), Acute Toxicity (MTD, maximum tolerance dose, mg/kg), and Solubility in Water (mg/mL) of Rhodopeptin Analogues

	antifungal activity				
compd	*Candida albicans*[12]	*Cryptococcus neoformans*[12]	*Aspergillus fumigatus*[12]	acute toxicity	solubility
9a	64	8	>128		0.2
9b	16	4	16	>40	1.9
16	128	8	128		
27a	16	4	32	20	>4
27b	32	8	32		
28	4	4	>128	5	0.9
29	16	8	32	10	3.1
30	4	4	8	20	2.6
AMPH	4	2	4	3	
FLCZ	>128	8	>128		

(8) Matsuura, F.; Hamada, Y.; Shioiri, T. *Tetrahedron* **1994**, *50*, 11303.

(9) (a) Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-M.; Xu, D.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768. (b) Sharpless, K. B.; Fleming, P. R. *J. Org. Chem.* **1991**, *56*, 2869. (c) Chong, J. M.; Sharpless, K. B. *J. Org. Chem.* **1985**, *50*, 2869.

(10) Drugs used: amphotericin B (AMB; Fungizon, Bristol Myers Squibb, New Brunswick, NJ) and fluconazole (FCZ; Diflucan, Pfizer, New York, NY) were obtained commercially. Cyclic peptides were initially dissolved in dimethyl sulfoxide (DMSO; Nakarai Chemicals, Kyoto, Japan) and further diluted with distilled water. The final concentration of DMSO contained in culture in wells was 2% at maximum.

(11) The in vitro susceptibility testings were done by a microdilution method, using 96-well microplates (flat bottom). Synthetic amino acid medium fungal (SAAMF; pH 7.0 at 30 °C, Nippon Bio-Supp. Center, Tokyo, Japan) was used throughout in this study. Freshly grown yeasts on slopes of sabouraud dextrose agar (SDA; Difco Laboratories, Detroit, MI) were suspended with physiological saline and counted in a hemacytometer, and cell concentration was adjusted to 1×10^6 cells per mL. When *A. fumigatus* was studied, subcultured organisms were suspended with saline containing 0.1% Tween 80 (monooleate polyoxyethylenesorbitan, Sigma Chemicals, St. Louis, MO), and then conidia were collected by passing the culture through a glass filter. Serial 2-fold dilutions of test compounds (256 to 0.125 μg/mL, 100 μL per well) were dispensed with an aid of an automatic dispenser (Model, Dinatech, Kyoto, Japan) and 2× concentrated medium was added to each well (100 μL per well). After inoculation (5 μL per well) by use of an automatic inoculator (Model, Dinatech), plates were gently but thoroughly shaken and were incubated at 30 °C for 48 h (*C. albicans*) or 72 h (*C. neoformans* and *A. fumigatus*). The inoculum size was 5×10^3 cells/mL. Minimal inhibitory concentration (MIC) was defined as the lowest concentration of a compound which gave no visibly detectable fungal growth.

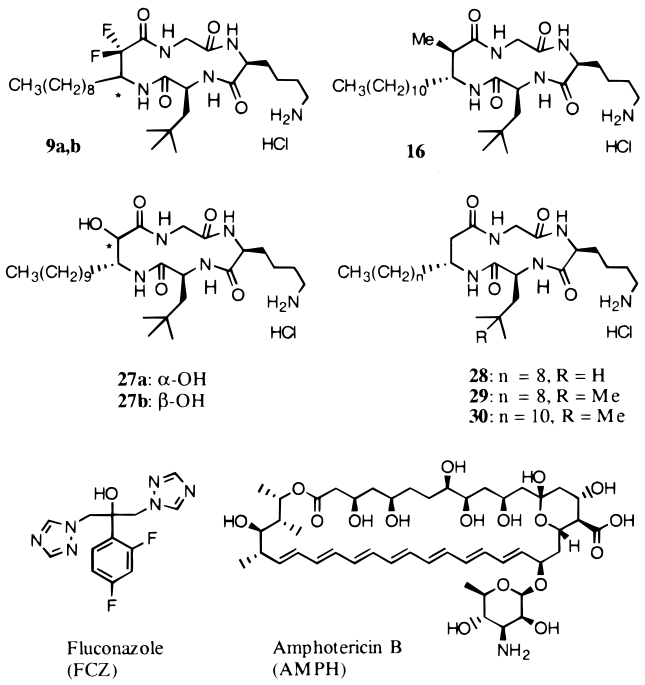


Figure 2.

fungi tested, while the C3 stereoisomer **9a** was devoid of activity against *Candida albicans* and *Aspergillus fumigatus*. The methylated analogue **16** displayed the same loss of activity as **9a**. Both of the hydroxyl analogues **27a**,**b** showed moderate activity, with the α-hydroxy analogue **27a** being slightly more active.

The acute toxicity (MTD; maximum tolerance dose) and solubility in water of the new analogues were also measured. The nonsubstituted analogue **30** was safe up to the dose of 20 mg/kg on iv injection in mice (no lethal toxicity). The hydroxyl analogue **27a** displayed similar toxicity to that of **30**, while the difluoro analogue **9b** was found to be at least 2 times safer. As for the solubility in water, analogue **9b** displays solubility at 1.9 mg/mL, which is comparable to compound **30** (2.6 mg/mL), while hydroxy analogue **27a** has an increased solubility (above 4 mg/mL).

In summary, introduction of difluoro, methyl, and hydroxyl groups to the β-amino acid moiety of the 13-membered rhodopeptin peptide ring has been achieved. The requsite β-amino acid fragments were synthesized via the corresponding β-lactam compounds or through the use of Sharpless asymmetric dihydroxylations. The difluoro and hydroxy analogues retained the antifungal activity of the nonderivatized rhodopeptins and displayed improved physical properties. The synthesis and biological evaluation of additional derivatives are currently underway in our laboratory.

Acknowledgment. We thank Mr. Hiroshi Kuga, Ms. Chie Makino for measuring the solubility, and Prof. William Moser of IUPUI for critiquing the manuscript. We also thank Mr. Chiba (Mercian Co. Ltd.) for useful discussions.

Supporting Information Available: Spectroscopic and analytical data for compounds **3**−**9**, **11**−**16**, and **18**−**27**. Protocol of the acute toxicity (MTD) assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Fungal strains: *C. albicans* (ATCC 24433), *A. fumigatus* (TIMM 0063), and *C. neoformans* (IFO 1420) were employed.