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The Synthesis of Pseudomycin C′ via a Novel Acid Promoted Side-Chain Deacylation of Pseudomycin A

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Abstract—The γ hydroxyl present in the aliphatic side chain of the natural products pseudomycin A and C′ provided a unique handle for the pH dependent side-chain deacylation. Low pH reaction conditions were used to cleave the side chain with minimal degradation of the peptide core. The pseudomycin nucleus intermediate obtained from the deacylation of pseudomycin A was pivotal in the synthesis of novel side-chain analogues. A practical synthesis of a minor fermentation factor pseudomycin C′ and related analogues is reported.

Introduction

Fungal infections are a significant cause of disease, degradation of quality of life, and mortality among humans, particularly for immune compromised patients. The increased incidence in fungal infections is in part due to increased numbers of people with immune systems weakened or devastated by organ transplants, cancer chemotherapy, AIDS, age, and other similar disorders. Such patients are prone to attack by fungal pathogens that are prevalent throughout the population but are kept in check by a functioning immune system.[1]

The pseudomycins A–C′(PSA–C′) are part of a new class of promising fungicidal agents derived from isolates of *Pseudomonas syringae*. Isolates of *P. syringae* that have been first associated with plant diseases are part of a large family of plant bacteria that have been the source of several interesting bioactive metabolites with potent antifungal activity against human pathogens.[2] The pseudomycins belong to the nonadepsipeptide family of compounds, which consists of a cyclic peptide including one or more uncommon amino acids with a long, hydroxylated, aliphatic side chain. The amino acid residues on the cyclic peptide are identical for each factor. The significant structural differences among the factors reside on the length and degree of the hydroxylation state of the side chain (Fig. 1). The side chain is thought to be partly responsible for the observed antifungal activity. PSC′, a minor component of the fermentation with the C-16 side chain having the highest degree of lipophilicity exhibits the best overall in vitro and in vivo activity against the three major fungal pathogens of interest: *Candida albicans, Cryptococcus neoformans,* and *Aspergillus fumigatus*. As a potential clinical candidate, a semisynthetic approach was developed to obtain suitable quantities of this minor component for extended biological testing by converting PSA, the major component of the fermentation, into PSC′.

Results and Discussion

The synthesis of PSC′ from PSA took place in three distinct stages: (1) conversion of PSA to CBZ-protected pseudomycin A (ZPSA), (2) deacylation of ZPSA to Z protected nucleus (ZPSN), synthesis of pseudomycin side chain, and (3) coupling of the side chain to the ZPSN followed by Z deprotection. As shown in Scheme 1, pseudomycin A was converted to ZPSN by first protecting the free amine groups with benzyl carbamate by treating ZPSA with excess (6 equiv) of *N*-(benzyloxycarbonyloxyl)-succinimide. The use of *Actinoplanes utahensis*, a common deacylase for side-chain cleavage was not practical under optimum catalytic conditions.[3,4] Under the biocatalytic conditions (pH >7), the desired ZPSN rapidly degraded. Several attempts to lower the

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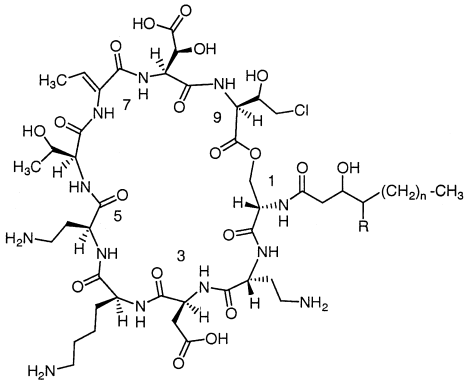
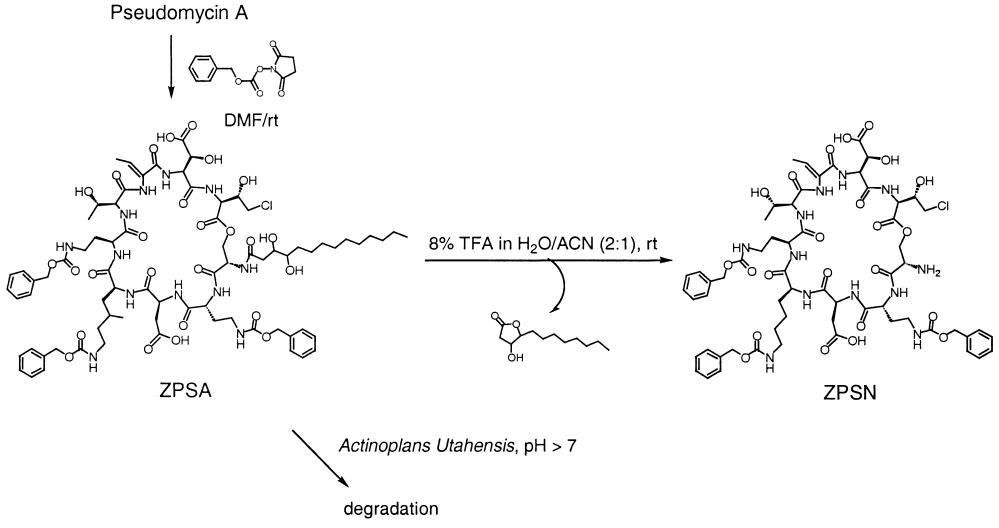
pH below 6 failed to produce ZPSN. It was noted during the large production scale work up of PSA that the percent yield recoveries varied with low pH. It was determined that pseudomycin A was not stable under strongly acidic work up conditions (pH $\leq$2). From the complex decomposition mixture, one of the by-products was identified as the desired pseudomycin nucleus. By varying the acid and organic solvent concentrations, the production of the CBZ-protected pseudomycin nucleus was optimized.

Typically, the deacylation was promoted with an 8% aq TFA solution at room temperature, 24–48 h. An organic solvent such as acetonitrile was used to enhance the



Factor			MIC (μg/mL)			IP ED_{50} (mg/kg)
PS-	R	n	*Candida albicans*	*Cryptococcus neoformans*	*Aspergillus fumigatus*	*Candida albicans*
A	OH	9	0.625	0.078	10	7.1
A′	OH	10	2.5	1.25	>20	Not determined
B	H	9	0.156	0.02	10	3.5
B′	H	7	10.0	1.25	>20	Not determined
C	OH	11	0.078	0.02	1.25	≥5
C′	H	11	0.156	0.005	0.625	2.5

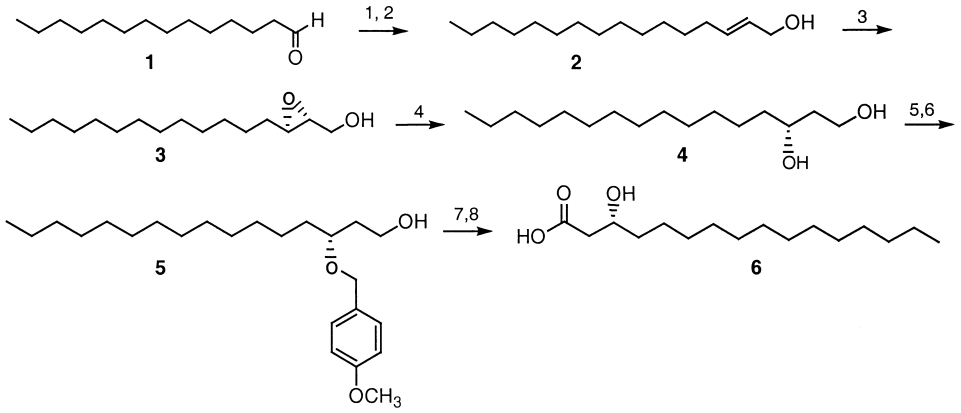
Figure 1. Pseudomycin natural product factors and broad spectrum activity.



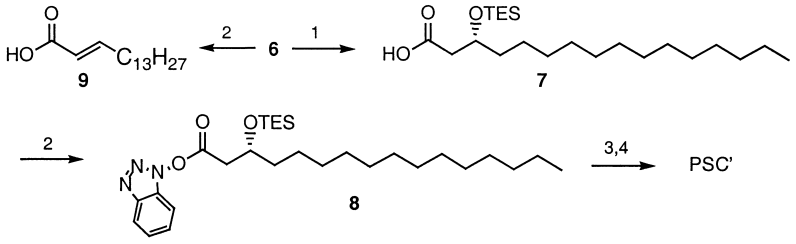
Scheme 1. Synthesis of Z-protected pseudomycin nucleus (ZPSN).

solubility of ZPSA in the reaction mixture. Under these conditions, the deacylation occurred readily (75–90%) and the degradation of the cyclic peptide core was kept to a minimum. The acid promoted deacylation occurs with the participation of the γ hydroxy alcohol. Without the γ hydroxy as present in PSB and PSC, no side-chain cleavage was observed. The synthesis of the PSC′ side chain used a multistep approach that started with tetradecylaldehyde and featured a Sharpless epoxidation step (**1**–**2**, 70%) to introduce the *R* β-OH stereochemistry (Scheme 2).

The regioselective epoxide ring opening (**3**–**4**, 80–90%) using DIBAL followed by potassium permanganate

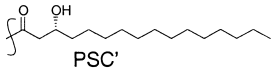
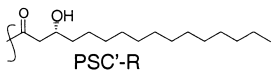
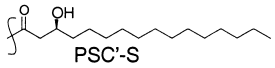
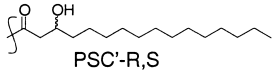
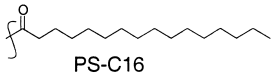


Scheme 2. Pseudomycin C′ side chain, *R* β-OH palmitic acid. (1) (>-O_2POCH_2COOEt, *t*BuOK, THF; (2) Dibal, CH_2Cl_2/hexanes; (3) Ti(O-<)$_4$, DET (−), CH_2Cl_2; (4) Dibal, CH_2Cl_2/hexanes; (5) *p*$CH_3OC_6H_5CH(OCH_3)_2$, *p*TsOH, DMF; (6) Red-Al; (7) $KMnO_4$, KH_2PO_4, *t*BuOH; (8) H_2/Pd–C, *t*BuOH/H_2O.



Scheme 3. Side-chain coupling and final deprotection. (1) TES-Cl, imidazole, DMAP, CH_2Cl_2; (2) HOBT-Ms, TEA, THF; (3) ZPSN, DMF/THF, rt; (4) H_2 10%Pd/C, MeOH/AcOH.

Table 1. Antifungal activity of PSC′ and related analogues

Side chain	MIC (μg/mL)				
	Candida albicans	*Cryptococcus neoformans*	*Aspergillus fumigatus*	*Candida parapsilosis*	*Histoplasma capsulatum*
 PSC'	0.039	<0.01	0.312	0.039	0.156
 PSC'-R synthetic	0.039	<0.01	0.625	0.02	0.156
 PSC'-S synthetic	5.0	5.0	20	5.0	5.0
 PSC'-R,S synthetic	0.156	<0.01	1.25	0.156	0.312
 PS-C16	10.0	1.25	NT	10.0	10.0

oxidation provided the pCH_3O benzyl ether protected palmitic acid (86%). The reduction step performed under aqueous conditions provided the desired *R* β-hydroxypalmitic acid (**5**–**6**, 70–80%) in gram quantities (by NMR >99% ee).[5] The direct coupling of the β hydroxyl side chain using HOBT-Ms resulted in the formation of the dehydration product **9** (Scheme 3).

By protecting the β-hydroxy with TES, the HOBT activated ester was prepared without decomposition and used without further purification (**6**–**8**). The final coupling of the activated ester to ZPSN proceeded by using a slight excess of the acid in a 1:1 mixture of DMF/THF at rt for 48 h (30%). The removal of the CBZ groups to obtain final product PSC′ required acetic acid. A 10% acetic acid/methanol solution with 10% Pd/C was used for complete removal of the Z groups (65%). There was no reduction of the double bond (residue 7) under these conditions. Additional analogues were prepared with *S* β-hydroxy palmitic acid (obtained from DET(+)) and with commercially available racemic hydroxyl palmitic acid and palmitic acid. All analogues were submitted for broad spectrum antifungal analysis.[6] The minimum inhibitory concentration data (MICs) reported in Table 1 indicate that the synthetic side chain with the β hydroxy in the *R* configuration had identical broad spectrum activity as shown for authentic PSC′ obtained by biosynthesis. The analogue with the *S* β-hydroxy side-chain configuration was 7-fold (100×) less active against *C. albicans* than PSC′. The non-hydroxylated C16 side chain significantly reduced the antifungal spectrum and activity. In summary, we have shown a practical synthesis to obtain novel pseudomycin analogues from a relatively abundant source of ZPSN. From this study, the β hydroxyl group appears to be essential to retain antifungal activity. Further elaboration of the side-chain SAR is currently in progress.

Acknowledgements

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References and Notes

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4. Debono, M.; Turner, W. W.; LaGrandeur, L.; Burkjardt, F. J.; Nissen, J. S.; Nichols, K. K.; Rodriguez, M. J.; Zweifel, M. J.; Zeckner, D. J.; Gordee, R. S.; Tang, J.; Parr, T. R., Jr. *J. Med. Chem.* **1995**, *38*, 3271.
5. The *R*-OH palmitic acid was alternatively obtained in mg quantitites via the Ru-(+)-BINAP catalyzed reduction of β-ketopalmitic acid methyl ester which was commercially available (note: β-keto acids readily decarboxylate and could not be used as starting materials). The reduction produced the desired alcohol in 42% yield with >99% ee as determined by NMR studies doped with (*S*)-(+) 2,2,2-trifluoro-1-(9-anthryl)ethanol chiral shift reagent.
6. **In vitro susceptibility studies**. A robotic microdilution microtiter testing procedure was used to determine MICs. Pseudomycin analogues were dissolved in 100% dimethyl sulfoxide. Compound solutions were diluted in broth to yield a starting concentration of 20 μg/mL in the first wells following the addition of inoculum. Serial 2-fold dilutions in 100 μL aliquots were made in 96-well microtiter plates using a QuadFlex automatic pipetting, liquid delivery instrument (Titertek Instruments Inc., Huntsville, AL). Fungal yeast isolates were grown on Sabouraud dextrose agar slants at 35 °C for 24 h. *C. albicans, C. parapsilosis, C. neoformans*, and *H. capsulatum* conidia were suspended in saline and adjusted to 1×10^5 conidia/mL in Sabouraud dextrose broth (DIFCO, Detroit, MI). *A. fumigatus* spores were gently teased from mycelia grown on potato dextrose agar (DIFCO, Detroit, MI) in 0.1% Tween 80 and saline. Spores were adjusted to 1×10^5 spores/mL in Sabouraud broth. Cell suspensions were counted using a hemacytometer under phase contrast microscopy. Aliquots containing 100 μL of the above were added to each well of a 96-well microtiter plate with a Titertex Multidrop Dispenser (Titertek Instruments Inc., Huntsville, AL). Plates were incubated for 48 h at 35 °C in ambient air. MICs were defined as the lowest concentrations of drugs which inhibited 90–100% of visible growth compared to untreated controls.

Survival studies. Mice were infected by an intravenous (iv) injection of 0.1 mL in the lateral tail vein. Mice received 2×10^6 blastoconidia per mouse. Untreated controls were moribund within 3–4 days post-infection. Mice were treated at 0, 4, 24, and 48 h post-infection. Mice were treated intraperitoneally (ip) with 0.2 mL. Experimental compounds were tested at titrated concentrations (serial 2-fold dilutions) of 20, 10, and 5.0 mg/kg. Six mice were tested at each level. Compounds were formulated in 4.0% hydroxypropyl β cyclodextrin and sodium acetate, pH 7.0 and 1.75% dextrose. Infected shamtreated mice (10 animals) were dosed with the vehicle alone. Morbidity and mortality were recorded for 7 days. The 50% effective doses (ED_{500}'s) were determined using the method of Reed and Muench. Statistical differences in treated groups compared to untreated infection controls were determined using the Student's *t*-test.