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Syntheses and Antifungal Activity of Pseudomycin Side-Chain Analogues. Part 1

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Abstract—We have described herein the syntheses of three novel series of aromatic ring containing pseudomycin side-chain analogues. Preliminary biological evaluations of these analogues clearly indicate that it is possible to synthesize rigid pseudomycin side-chain analogues without compromising in vitro antifungal activity.

Introduction

Pseudomycins, produced as metabolites by *Pseudomonas syringae* in plants,[1] represent a novel series of natural products possessing antifungal activities against a number of fungi. Structurally, the pseudomycins share similarity with several other lipodepsinonapeptides generated by *P. syringae* in plants including syringomycins,[2] syringotoxin[3] and syringostatins.[4] Depending on the nature of side chain, pseudomycins are subdivided into pseudomycin A, B, and C′, etc. In 1994 the gross structure of pseudomycin A (PSA) was reported by Ballio et al.[5] The structures of pseudomycin B and C′ (PSB and PSC′) were fully established through extensive NMR analysis and later confirmed by a pseudomycin semisynthetic effort.[6] When evaluated against *Candida albicans* and *Cryptococcus neoformans,* two major fungal pathogens responsible for systemic fungal infection, PSB exhibited superior activity to that achieved by amphotericin B (AMB), the commonly used antifungal drug.

Previous work from this institution demonstrated that certain pseudomycin side-chain analogues can be prepared via *N*-acylation of the amino function of Residue 1 within the pseudomycin core **5a** or **5b** with various β-hydroxyacids (see Fig. 1 for structures).[6] It was further demonstrated by Rodriguez et al. that the 3′-hydroxyl group was essential for the optimal antifungal activity of PSC′.[6] Additionally, a recent publication by Debono et al.[7] documented that many rigid side-chain bearing analogues of cyclic lipopeptide echinocandin B (ECB) (e.g., aromatic, unsaturated, etc.) exhibited improved antifungal potency in comparison to the parent compound. Bearing these considerations in mind, we decided to prepare a series of aromatic ring containing pseudomycin side-chain analogues in search for compounds possessing improved antifungal potency. In this paper, we wish to report the synthesis and our preliminary biological evaluation of three types of aromatic ring bearing side-chain analogues as exemplified by **6**, **7**, and **8** shown in Figure 2.

Chemical Synthesis

The synthetic scheme employed for the preparation of **6a–c** is shown in Scheme 1. Palladium mediated coupling[8] of 3-bromobenzaldehyde **9** with 1-octyne or 1-dodecyne afforded the desired acetylene bearing products **10a** (51%) and **10b** (51%), respectively. Saturation of the triple bond moieties in **10a** and **10b** was affected by standard hydrogenolysis, leading to **11a** and **11b** in good yields. Treatment of **11a** and **11b** with the lithium enolate generated in situ from *t*-butylacetate provided the corresponding β-hydroxyesters **12a** (67%) and **12b** (33%), respectively. Alternatively, *O*-alkylation of 3-hydroxybenyaldehyde with dodecyl bromide afforded **10c** (15%), which was further converted to the requisite β-hydroxyester **12c** (77%) using the same chemistry as described for **12a**. Treatment of **12a–c** with TFA yielded the desired β-hydroxyacids, all of which were further coupled with ZPSB (**5a**) to provide the corresponding three pairs of 3′-diastereomers **13a–c**. The resulting diastereomers **13a–c** were separated using reverse-phase

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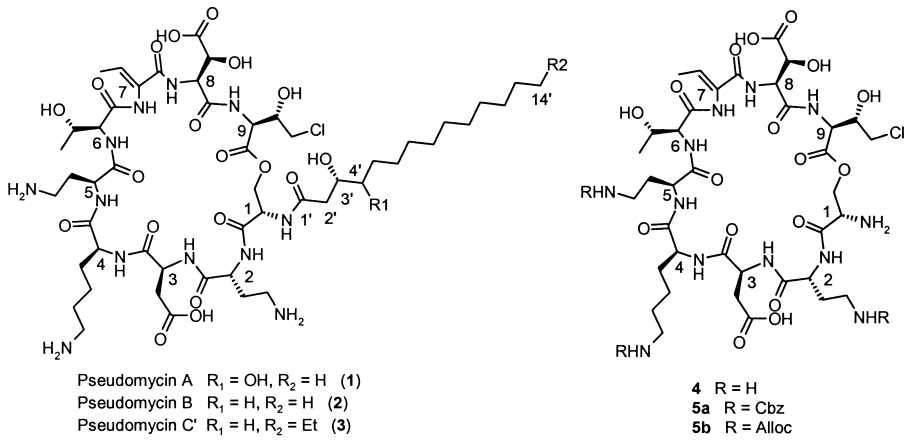


Figure 1. Representative pseudomycin structures.

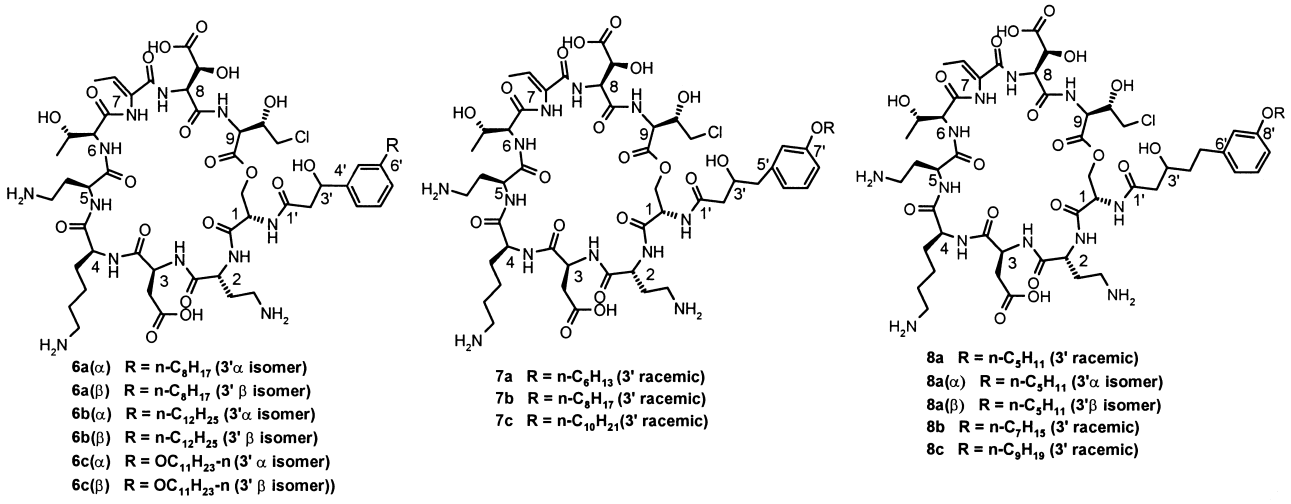
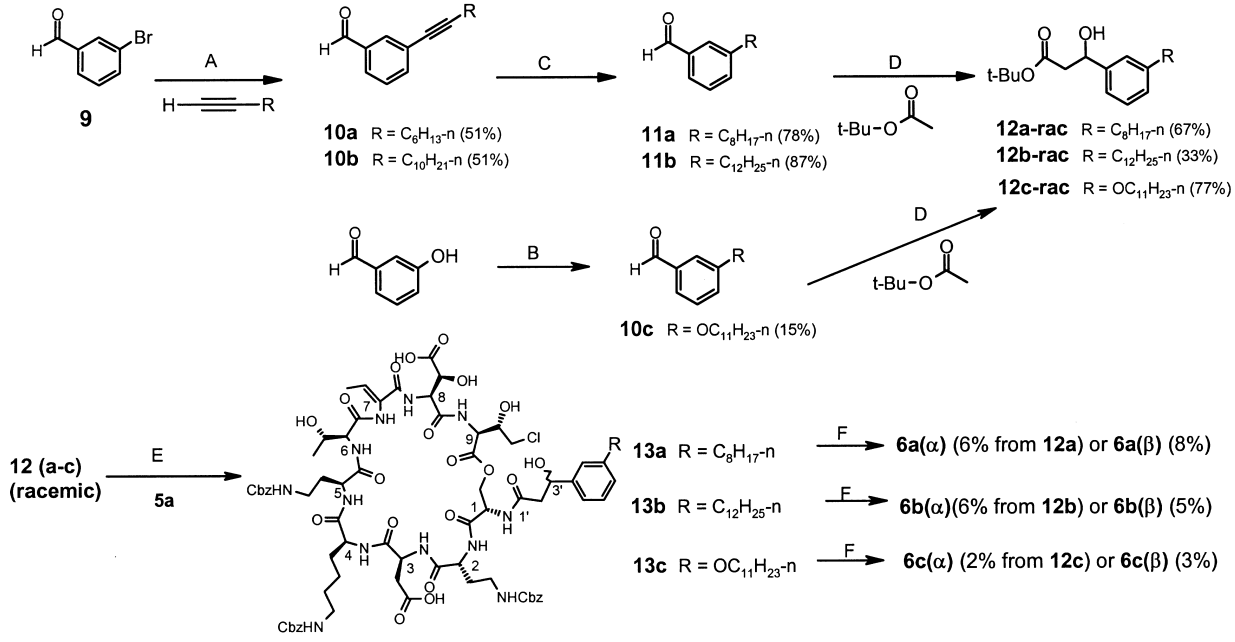


Figure 2. Pseudomycin side-chain analogues.



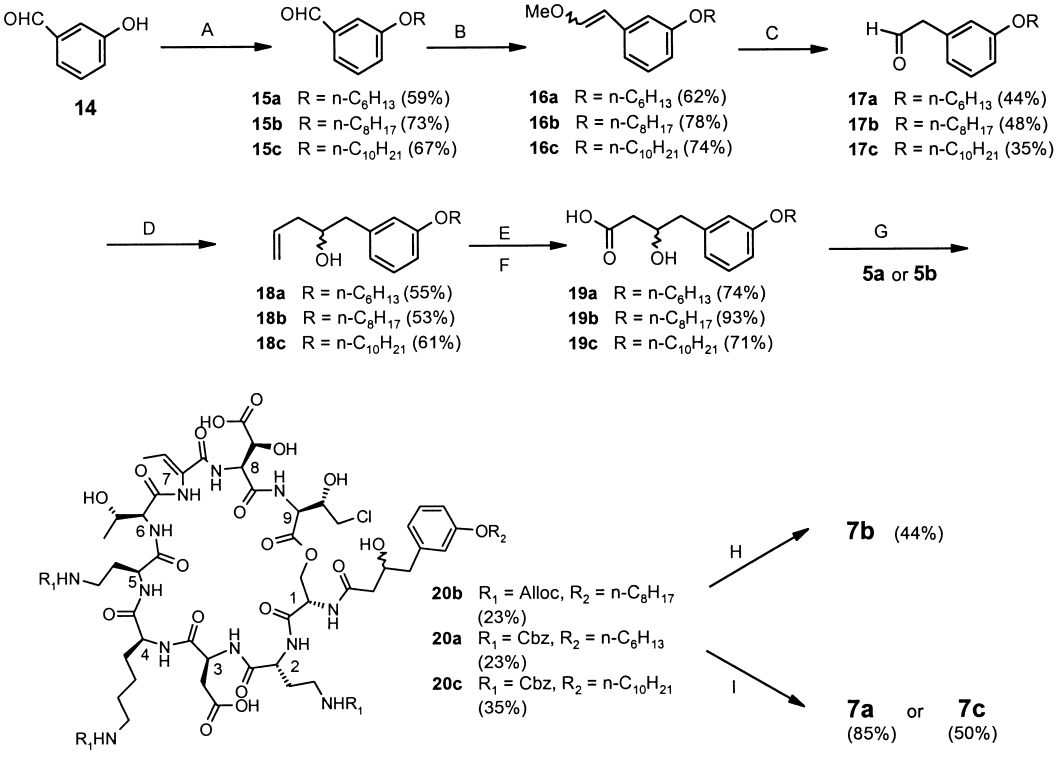
Reagents and Conditions: (A) $PdCl_2/PPh_3/CuI/TEA/ACN$ reflux; (B) Dodecyl bromide/K_2CO_3/acetone reflux; (C) H_2/5%Pd/Al_2O_3/EtOAc; (D) LDA/THF -78°C; (E) i. TFA/0°C ii. HOBT/EDC/DMF then **5a**; (F) H_2/Pd/C/10% HOAc/MeOH.

Scheme 1. Synthesis of pseudomycin side-chain analogues **6a**–**6c**.

HPLC to give the respective 3′-enantiomers. As expected, the 3′α-hydroxyl group bearing isomer had a relatively shorter retention time than that observed with their 3′β-hydroxyl bearing counterpart.[6] Final removal of the *N*-protective groups (Cbz) in **13a–c** (from either 3′α or 3′β enantiomers) thus completed the syntheses of pseudomycin side-chain analogues **6a–c**. The structures of final products were confirmed by spectroscopic NMR analyses and mass spectra.

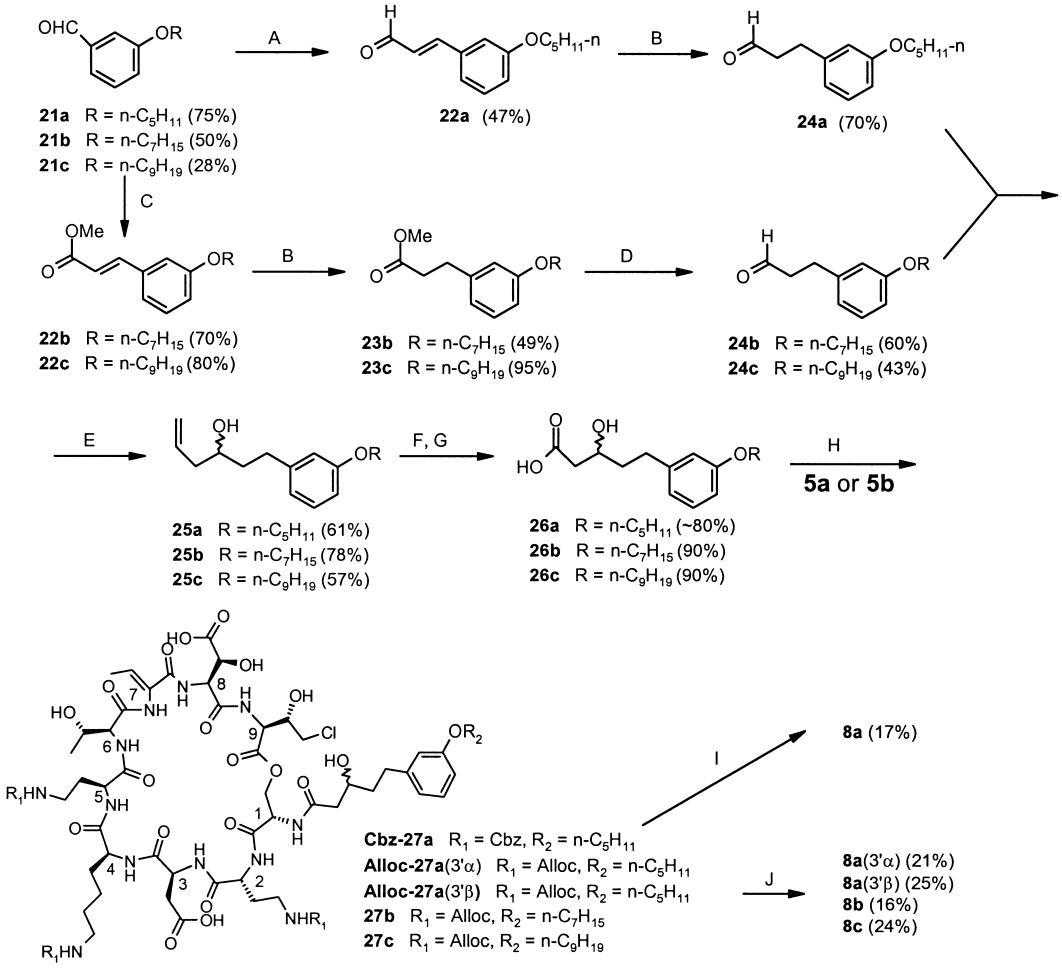
As shown in Scheme 2, the synthesis of **7a–7c** began with conversion of 3-hydroxybenzaldehyde **14** to the ethers **15a–c** under Mitsunobu conditions. Compounds **15a–c** were then transformed, via the Wittig reaction, into the corresponding enolethers **16a–c**, and thereafter, the desired aldehydes **17a–c**, upon exposure to aqueous acid. Allylmagnesium bromide addition to **17a–c** furnished the carbinols **18a–c** in modest yields, which were converted to the requisite β-hydroxyl acids **19(a–c)** via a sequence consisting of osmylation, vicinol diol cleavage ($NaIO_4$) and subsequent oxidation ($NaClO_2$). The acids thus obtained were activated as their HOBT esters, and then coupled with either **5a** (for **19a** and **19c**) or **5b** (for **19b**) to afford the expected products **20a–c** in 23–35% yield. Finally, **20b** was deallylated using tributyltin hydride and $Pd(PPh_3)_2Cl_2$ to provide **7b** in 44% yield.[9] Intermediates **20a** and **20c** were deprotected under standard hydrogenation conditions,[10] to the desired products **7a** (85%) and **7c** (50%). The structures of **7a–c**, obtained through semipreparative HPLC purification and lyophilization, were secured by their mass spectrometry and ^1H NMR spectroscopic analyses.

The synthetic sequence employed for pseudomycin analogues **8a–c** is outlined in Scheme 3. The starting materials **21a–c** used in this case were obtained via standard Mitsunobu reaction from 3-hydroxy-benzaldehyde. The reaction of **21a** with triphenylphosphoranylidene-acetaldehyde gave the unsaturated aldehyde **22a** (47%), which was then converted to **24a** (70%) via the usual palladium catalyzed hydrogenation. Alternatively, **21b** and **21c** were first converted to their unsaturated esters **22b** (70%) and **22c** (80%), and were in turn converted to the corresponding aldehydes **24b** (60%) and **24c** (43%) via intermediates **23b** and **23c**, respectively. Titanium tetrachloride promoted allylsilane addition to **24a–c** afforded the homoallylic alcohols **25a–c** in yields ranging from 61–78%. The carbinols **25a–25c** were next transformed, via the same three-step sequence as described for **19a–c** in Scheme 2, to the requisite acids **26a–c** in excellent overall yields. Standard coupling of **26a–c** with **5a** or **5b** provided the expected 3′-racemic products **27a–c**. Compound **27a** was deprotected to give **8a** via catalytic hydrogenation in 17% overall yield from **26a**. The alloc-protected 3′-racemic adducts **27a** were separated by semipreparative HPLC to yield the corresponding two stereoisomers **27a** (3′α) and **27a** (3′β). Alloc deprotection[9] gave the desired products **8a** (3′α) and **8a** (3′β) in 21 and 25%, respectively. Similarly, the alloc protecting groups in **27b** and **27c** were removed to give **8b** and **8c** in



Reagents and Conditions: (A) $ROH/PPh_3/DEAD/THF$; (B) $MeOCH_2PPh_3^+Cl^-$/n-BuLi/THF; (C)6N HCl/dioxane; (D) Allylmagnesium bromide/THF; (E) cat. OsO_4/NMO/aq THF; (F) $NaIO_4$/aq MeOH, then $NaClO_2/KH_2PO_4$/cyclohexene/t-BuOH; (G) HOBt/EDCI/THF+DMF, then **5a** or **5b**; (H) n-$Bu_3SnH/PdCl_2(PPh_3)_2$/HOAc; (I) H_2/Pd/C/10% HOAc/MeOH.

Scheme 2. Synthesis of side-chain analogues **7a–7c**.



Reagents and Conditions: (A) Ph_3P=CHCHO/benzene; (B) H_2/Pd/C/EtOAc; (C) Ph_3P=$CHCO_2Me$/benzene; (D)DIBAL-H, then Swern oxidation; (E) trimethylallylsilane/$TiCl_4$/CH_2Cl_2; (F) cat. OsO_4/NMO/aq THF; (G) $NaIO_4$/aq MeOH; then $NaClO_2$/KH_2PO_4/cyclohexene+t-BuOH; (H) HOBt/EDCI/THF+DMF, then **5a** or **5b**;(I) H_2/Pd/C/10% HOAc/MeOH; (J) n-Bu_3SnH/$PdCl_2(PPh_3)_2$/HOAc/THF.

Scheme 3. Syntheses of side-chain analogues **8a**–**8c**.

low overall yields (16–24%). Again, the structures of these pseudomycin side-chain analogues were confirmed by their mass spectrometry and proton NMR spectroscopy.

Biological Evaluation

The newly synthesized pseudomycin analogues (**6**, **7**, and **8**) were subjected to a robotic microdilution microtiter testing system to determine their MICs. *Candida albicans, Cryptococuus neoformans* and *Aspergillus fumigatus* account for about 90% of systemic fungal infections in immunocompromised patients. Therefore, all new pseudomycin side-chain analogues described herein were tested against these fungi. Pseudomycin B was included as a positive control.

The samples used for this in vitro assay were prepared according to the following procedure: Pseudomycin analogues (**6**–**8**) were dissolved in 100% DMSO. Compound solutions were diluted in Sabourauds 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 pipeting liquid delivery instrument (Titertek Instruments Inc., Huntville, AL).

The fungal yeast isolates used for in vitro assay were grown on Sabouraud dextrose agar slants at 35 °C for 24 h. *C. albicans* and *C. neoformans* were suspended in saline and adjusted to 2×10^5 conidia/mL in Sabourauds dextrose broth (DIFCO, Detroit, MI). *A. fumigatus* spores were gently teased from mycelia grown on Potato dextrose agar (DIFCO, Detroit, MI) and suspended in 0.1% Tween 80 and saline.

The results of in vitro evaluation of **6**–**8** are detailed in Table 1. Pseudomycin B (PSB), the positive control, exhibited excellent MIC's against *C. albicans* (0.312–0.625 μg/mL) and *C. neoformans* (0.039 μg/mL). When tested against *A. fumigates*, PSB showed only weak activity (MIC >20 μg/mL).

Table 1. Biological evaluation of pseudomycin analogues **6–8**

Compounds	3′-OH configuration	MIC[a] (In vitro) (μg/mL) *C. albicans*	*C. neoformans*	*A. fumigates*
PSB	α	0.312–0.625	0.039	>20
6a (α)	α	5.0	0.312	>20
6a(β)	β	>20	5.0	>20
6b(α)	α	1.25	0.01	2.5
6b(β)	β	5.0	1.25	>20
6c(α)	α	0.625	0.02	>20
6c(β)	β	10	0.625	>20
7a	Racemic	>20	>20	>20
7b	Racemic	>20	>20	>20
7c	Racemic	15	1.25	>20
8a	Racemic	>20	>20	>20
8a(α)	α	>20	>20	>20
8a(β)	β	>20	>20	>20
8b	Racemic	10	20	>20
8c	Racemic	20	2.5	>20

[a]MIC, Lowest drug concentration required to inhibit 90–100% of visible growth compared to controls.

In vitro evaluation of **6a–c** revealed the following trends: (1) 3′-α stereochemistry is required for the optimal antifungal activity. Thus, **6a** (α) was found to be more potent than **6a** (β) when evaluated against *Candida* and *Cryptococcus*. Likewise, **6b** (α) exhibited greater activity against all three major fungi than its 3′-β counterpart **6b** (β). This observation is in agreement with that discovered with PSC′.[6] (2) C-18 side-chain bearing analogues, **6b** and **6c**, proved to be more potent than the C-14 side-chain counterpart **6a** and (3) generally speaking, all analogues within this subgroup demonstrated excellent activity against *Cryptococcus*. (4) With the exception of **6b** (α), the remaining analogues within this class exhibited weak activity towards *Aspergillus*. (5) All six analogues within this class were tested in the mice tail vein irritation/toxicity assay.[11] Although two C-14 side-chain bearing analogues, **6a** (α) and **6a** (β), were devoid of tail vein irritation, four remaining analogues (including more potent analogues **6c** (α) and **6b** (α)) were all capable of inducing irritation in this assay.

We next examined the antifungal activity of the novel side-chain analogues **7a–c** as their 3′-racemates. None of these analogues exhibited good activity against *Candida* and *Aspergillus*. Furthermore, **7a** and **7b** were totally devoid of activity against *Cryptococcus*. The C-18 side-chain bearing analogue, **7c**, showed relatively weaker activity against this *Cryptococcus* in comparison to PSB.

When side-chain analogues **8a–c** were evaluated, we observed the following trends: (1) all analogues within this subset exhibited marginal activities against *Candida* and *Cryptococcus*. (2) The best analogue within this class, **8c,** showed relatively weak activity against *Cryptococcus*, with a MIC value of 2.5 μg/mL. All other analogues failed to inhibit the growth of *Cryptococcus* at the dose of up to 20 μg/mL.

In conclusion, we have described herein the syntheses of three types of rigid side-chain containing pseudomycin analogues. In light of our testing results, it is clear that incorporation of an aromatic ring, located one or two carbons away from 3′-hydroxyl group on the side chain (e.g., **7a–c** and **8a–c**), is detrimental to the antifungal activity associated with these pseudomycin analogues. Furthermore, in view of the promising antifungal activities displayed by **6b** (α) and **6c** (α), it is evident that it is possible to synthesize novel rigid pseudomycin side-chain analogues without compromising in vitro potencies. Being mindful of the fact that compounds **6b** (α) and **6c** (α) were also capable of inducing irritation, we continue our search for novel pseudomycin side-chain analogues endowed with good antifungal activities and acceptable toxicity profiles. The results of these investigations will be detailed in the near future.

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9. General deallylation procedure: Dangles, O.; Guibe, F.; Balavoine, G.; Lavielle, S.; Marquet, A. *J. Org. Chem*. **1987**, *52*, 4984.
10. The hydrogenation reactions were conducted in 10% HOAc/MeOH for about 40 min. Prolonged hydrogenation may lead to saturation of the double bond presented on pseudomycin core.
11. General procedure for performing tail vein toxicity assay: Mice (Outbred, male ICR mice about 18–20 g; harlan Sprangue Dawley, Indianapolis, IN) were treated intravenously (IV) through the lateral tail vein with 0.1 mL of testing compounds (20 mg/kg) at 0, 24, 48, and 72 h. Two mice were included in each group. Compounds were formulated in 5.0% dextrose and sterile water for injection. Mice were monitored closely for signs of irritation including erythema, swelling, discoloration, necrosis and tail loss, etc., for a total of 7 days following first treatment. Mice were also observed for any other signs of adverse effects that are indicative of toxicity.