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Syntheses and Biological Evaluation of Novel Pseudomycin Side-Chain Analogues. Part 2

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Abstract—A series of aliphatic side-chain analogues of pseudomycin was synthesized and evaluated during the course of our side-chain SAR effort. We found that several of the pseudomycin side-chain analogues (e.g., **10**) exhibited good in vitro activity against all three major fungi responsible for systemic fungal infections.

Introduction

Systemic fungal infections (SFI) are those infections that involve deep viscera such as liver and lung. Clinically, it has been shown that such SFI can cause serious life-threatening diseases in normal healthy humans. *Candida albican, Cryptococcus neoformans,* and *Aspergillus fumigatus* are the major opportunistic pathogens responsible for systemic infections.[1,2] In recent years, there has been a significant increase in the incidence of SFI. This is mainly due to the growing population of immunocompromised individuals including those suffering from HIV, patients receiving chemotherapy, and patients undergoing heart surgery and organ transplantation, etc. Although amphotericin B and several azole based drugs (e.g., fluconazole) are used to treat systemic fungal infections, the usefulness of these drugs are limited either by severe toxicity (e.g., amphotericin B)[3] or by the lacking of broad spectrum of activity against all three major fungi (e.g., fluconazole).[4] Therefore, there is an urgent need for the development of new antifungal agents that can be used safely for the treatment of systemic fungal infections caused by all of the three major fungi mentioned above.

Recently, we and others reported the structure and preliminary biological profiles of three novel antifungal agents: pseudomycin A,[5] pseudomycin B (PSB, **7**),[6] and pseudomycin C′(PSC′, **1**).[7] When tested against *Candida* and *Cryptococcus*, PSB and PSC′ displayed better activity than that of amphoterin B (AMB). However, when both PSB and PSC′ were evaluated against *Aspergillus*, they exhibited only modest activity, with MIC values in the range of 10–20 μg/mL. In order to further extend the therapeutic utility of natural occurring pseudomycins, it would be desirable to synthesize analogues that possess balanced activity against all three major fungi that are responsible for systemic fungal infections. Bearing these considerations in mind, we synthesized several series of ridged side-chain bearing pseudomycin analogues.[6] A recent report by Rodriguez et al.[7] demonstrated that PSC′ **1** was more potent than its 3′-epimer **2** and the 3′-racemate **3**. Furthermore, all three pseudomycin analogues (**1**–**3**) displayed better activity than the 3′-deoxy analogue **4**. Taking all this information into account, it became evident that the presence of the 3′α hydroxyl group is required for optimal anti-fungal activity for PSC′.[7] Encouraged by this important finding, we decided to extend the side-chain SAR by preparing additional side-chain analogues as shown in Figure 1. Compounds **8** and **9** contain C-14 side-chain. Three C-18 and three C-20 aliphatic side-chain analogues (**10**–**15**) were also synthesized. In this paper, we wish to report the synthesis and preliminary evaluation of these novel pseudomycin side-chain analogues.

Chemical Syntheses

The synthetic scheme employed for the preparation of new side-chain analogues is briefly outlined in Scheme 1. Three requisite 3′-racemic acids (**19a**–**19c**) were prepared from their corresponding aldehydes (**16a**–**16c**) via a sequence consisting of allylation, osmylation, vicinal

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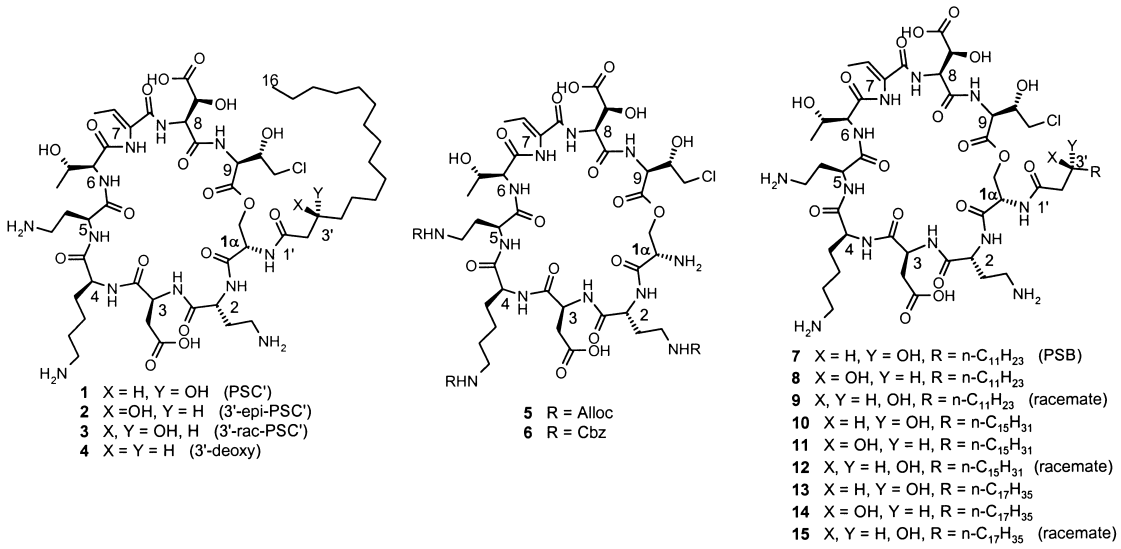
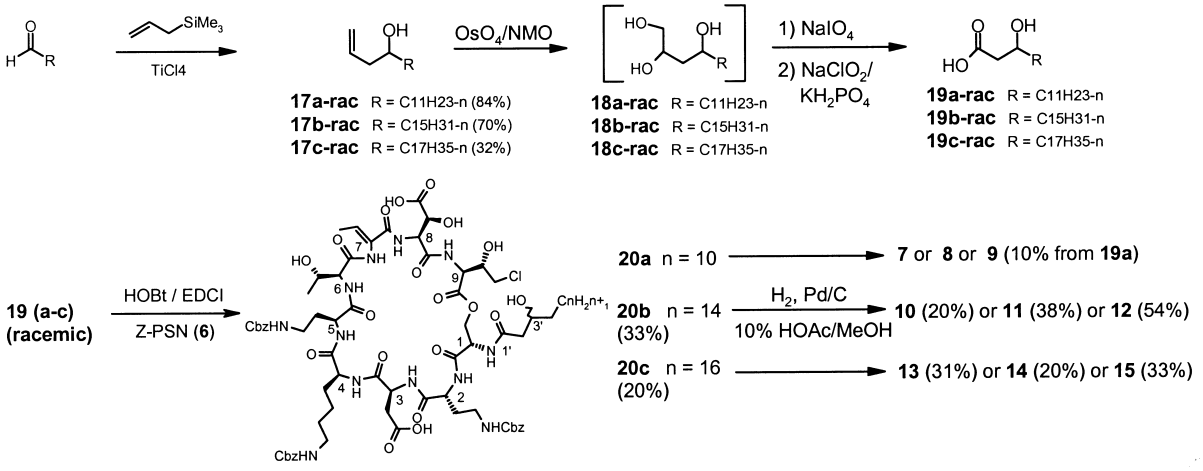


Figure 1. Structures of pseudomycin side-chain analogues.



Scheme 1. Synthesis of pseudomycin side-chain analogues **10–15**.

diol cleavage and $NaClO_2$ mediated aldehyde oxidation as shown in Scheme 1. Most of the chemistry described in this sequence was done without purification of the intermediates. Thus, the crude acids (**19a–19c**) were coupled with ZPSN **6** in the presence of HOBt and EDCI in DMF to afford three pairs of the 3′-diastereomers (**20a–20c**) in low yields. Direct hydrogenolysis on (**20a–20c**) provided three 3′-diastereomers **9** (10%), **12** (18%), and **15** (7%), respectively. Satisfactory mass spectra for all final products were obtained.

Alternatively, reverse-phase HPLC separation of the 3′-diastereomers **20a** afforded two pure 3′-distereomers **20a**(α) and **20a**(β). In accordance with our previous experience obtained with PSC′ analogues, the 3′α-OH bearing isomer **20a**(α) had a relatively shorter retention time than that observed with the 3′β-OH bearing counterpart **20a**(β).[7] Both **20a**(α) and **20a**(β) thus obtained were converted to the desired products **7** and **8**, via hydrogenolysis, in good yield. The proton NMR spectra of **7** and **8** showed only one small apparent difference in the vicinity of 4.5–4.7 ppm. A COSY experiment was used to assign the resonances of that region. The biggest chemical shift difference between **7** and **8** was noted for the 1α proton (see Figure 1) with 4.59 ppm for PSB **7** and 4.63 ppm for 3′-*epi* PSB **8**. To further confirm the structural assignments, additional spiking studies were performed on both samples. The results from these investigations indicated that proton NMR spectrum of synthetic PSB **7** (TFA salt) was identical to that obtained from standard PSB-TFA adduct. Whereas compound **8** was indeed the 3′-epimer of PSB.

Following the same procedure described for **7** and **8**, four pure 3′-diastereomers, **10** and **11** (C-18 side chain) along with **13** and **14** (C-20 side chain), were obtained from either **20b** or **20c** in yields ranging from 20–38%. The proton NMR spectra of **10**, **11**, **13,** and **14** were assigned according to the method described for PSB **7** and its 3′-epimer **8.** The detailed peak assignments for these new analogues are listed in Table 1. It should also be mentioned that all of the pseudomycin side-chain analogues with same side chain length (e.g., C-14 side-chain analogues: **7**–**9**), regardless of the stereochemistry at C-3′ position, displayed identical retention time on our HPLC system. All of the new pseudomycin side-

analogues discussed herein (**7**, **8**, **10**, **11**, **13**, and **14**) exhibited satisfactory mass spectra.

Although the chemistry shown in Scheme 1 permitted the synthesis of each 3′-diastereomers of interest, the HPLC separation of the intermediates (**20a**–**c**) was very tedious and time consuming. Furthermore, this procedure proved to be more troublesome for C-18 or C-20 side-chain analogues. In view of this difficulty, we devised a chiral acetal based stereoselective route for side-chain analogues.[8] Following the new route shown in Scheme 2, we resynthesized the C-18 side-chain analogue **10**. Thus, the homoallylic ether **23** was prepared from the chiral acetal **22** with good yield and stereoselectivity (>6:1 versus the undesired 3′-epimer). Compound **23** was then converted to the homoallylic alcohol **17b(α)** via a one-pot procedure. From this point on, the C-18 side-chain precursor **17b(α)** was processed as that shown in Scheme 1 to provide the final product **10** (~17% overall yield). Presumably, the same procedure shown in Scheme 2 can be used for the preparation of other related side-chain bearing analogues.

Table 1. Proton NMR data of pseudomycin side-chain analogues **7**–**11** and **13**–**14**

Positions	7	8	10	11	13	14
Residue 1						
α	4.59	4.63	4.59	4.62	4.59	4.63
β1	4.38	4.38	4.39	4.38	4.38	4.38
β2	4.53	4.53	4.53	4.52	4.53	4.52
Residue 2						
α	4.13	4.14	4.13	4.13	4.13	4.13
β1	2.01	2.01	2.01	2.00	2.01	2.00
β2	2.07	2.07	2.07	2.07	2.08	2.06
γ1	2.90	2.90	2.91	2.90	2.91	2.91
γ2	2.97	2.97	2.98	2.96	2.97	2.96
Residue 3						
α	4.55	4.55	4.54	4.55	4.54	4.55
β1	2.82	2.82	2.82	2.82	2.83	2.82
β2	2.87	2.87	2.86	2.86	2.86	2.86
Residue 4						
α	4.13	4.13	4.12	4.15	4.12	4.15
β1	1.75	1.75	1.74	1.73	1.73	1.74
β2	1.78	1.78	1.78	1.77	1.78	1.78
γ1	1.26	1.26	1.26	1.28	1.26	1.25
γ2	1.32	1.32	1.33	1.34	1.32	1.32
δ1	1.53	~1.55	1.55	1.55	1.55	1.55
δ2	1.56	1.55	1.55	1.55	1.55	1.55
ε	2.84	2.84	2.84	2.84	2.84	2.85
Residue 5						
α	4.29	4.29	4.29	4.30	4.29	4.30
β1	1.99	1.99	1.99	1.99	2.00	2.00
β2	2.14	2.14	2.14	2.15	2.14	2.14
γ1	2.89	2.90	2.90	2.90	2.90	2.90
γ2	2.91	2.90	2.90	2.90	2.90	2.90
Residue 6						
α	4.27	4.28	4.27	4.28	4.27	4.28
β	3.92	3.92	3.91	3.91	3.91	3.91
γ	1.18	1.18	1.18	1.18	1.18	1.18
Residue 7						
β	6.53	6.51	6.53	6.51	6.53	6.51
γ	1.70	1.70	1.70	1.70	1.70	1.70
Residue 8						
α	4.96	4.95	4.96	4.95	4.96	4.95
β	4.75	4.75	4.75	4.75	4.75	4.75
Residue 9						
α	4.87	4.89	4.87	4.88	4.87	4.88
β	4.31	4.32	4.31	4.30	4.31	4.30
γ1	3.44	3.44	3.44	3.44	3.44	3.44
γ2	3.50	3.51	3.51	3.51	3.51	3.51
Side chain						
2′α	2.25	2.25	2.25	2.25	2.25	2.25
2′β	2.36	2.35	2.36	2.35	2.36	2.36
3′	3.86	3.90	3.86	3.89	3.86	3.90
4′	1.38	1.38	1.38	1.38	1.38	1.38
5–13,17,19	~1.22	~1.22	~1.22	~1.22	~1.22	~1.22
14/18/20	0.83	0.83	0.82	0.83	0.82	0.82

Biological Evaluation

All eight newly synthesized pseudomycin analogues (**8**–**15**) were evaluated in vitro against *Candida albican*, *Cryptococcus neoformans* and *Aspergillus fumigatus*. Pseudomycin B **7** was used as positive control. As shown in Table 2, when tested against *Candida* and *Cryptococcus*, 3′-*epi* PSB **8** was found to be less potent than 3′-racemate **9**, with the latter being 2-fold less potent than PSB **7**. None of these C-14 side-chain analogues showed significant activity against *Aspergillus*. These results are in good agreement with that found with PSC′ series analogues reported by Rodriguez et al.[7]

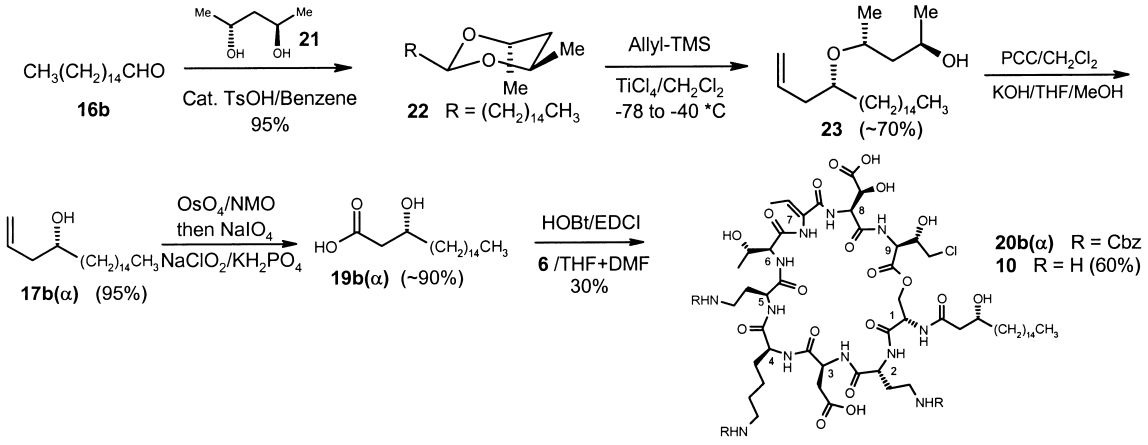
When three C-18 side-chain bearing analogues (**10**–**12**) were evaluated, it was found that in vitro antifungal potencies (against all three fungi) decrease according to the following order: **10** > **12** > **11**. From the data shown in Table 2, it is again evident that 3′α-hydroxyl group is required for optimal antifungal activity. It is encouraging to note that, when compared with PSB **7**, the C-18 side chain analogue **10** showed improved potency against *Cryptococcus* (4-fold) and *Aspergillus* (8-fold). Furthermore, better activity (vs **7**) towards *Aspergillus* were also observed with compounds **11** and **12**.

Further side-chain elongation by 2-carbons led to three C-20 analogues **13** through **15**, all displayed reduced activity against *Candida* and *Cryptococcus* in comparison to PSB **7**. However, compounds **13** and **15** showed improved activity towards *Aspergillus*, (ranging from 2.5–5.0 μg/mL), which was ~5-fold more potent than

Table 2. In vitro antifungal activity of pseudomycin derivatives **7**–**15**

Compounds	3′ Configuration	MIC (μg/mL)[a]		
		C. albicans	*C. neoformans*	*A. fumigatus*
7 (PSB)	α	0.312–1.25	0.039–0.312	>20
8	β	5.0–10	1.25–2.5	>20
9	Racemates	0.625–1.25	0.078–0.312	>20
10	α	0.625	0.01	2.5
11	β	2.5	0.312	10
12	Racemates	1.25	0.02–0.078	5.0
13	α	5.0	1.25	5.0
14	β	5.0	5.0	>20
15	Racemates	10	0.625	2.5

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



Scheme 2. Stereoselective synthesis of **10** via coupling of ZPSN **6** with 3′α-hydroxyacid **19b**(α).

PSB **7**. It is also interesting to note that the activity against *Candida* for **13** and **14** is independent of the 3′-stereochemistry on the side chain.

In conclusion, we have synthesized eight new pseudomycin analogues (**8**–**15**) during the course of our side-chain SAR studies. When compared with PSB **7**, some of these side-chain analogues (e.g., **10**, **12**, **13**, and **15**) showed improved activity against *Aspergillus*. In particular, the C-18 side-chain analogue **10** demonstrated balanced activity against all three major fungi responsible for systemic fungal infections. In light of this encouraging result, further in vivo evaluation of **10** is clearly warranted.

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