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Serendipitous Synthesis of Novel Dehydro- and Dechloro-Pseudomycin B (PSB) Derivatives

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Abstract—The syntheses and preliminary investigation of antifungal activities of two dehydro PSB derivatives **8** and **10** as well as one 3-imido-9-dechloro PSB analogue **13** are described.

Introduction

The broad-spectrum antifungal activity, the unique mode of action and the increasing clinical need for safe and effective antifungal agents have made pseudomycins attractive potential development candidates for the treatment of life-threatening systemic fungal infections.[1,2] Depending on the nature of side-chain, pseudomycins[3] are subdivided into pseudomycin A (PSA), B (PSB) and C′(PSC′), etc. When tested against *Candida albicans* and *Cryptococcus neoformans*, two major fungi responsible for systemic fungal infections, PSB **1** showed better in vitro and in vivo activity than that achieved by amphotericin B (AMB),[4] the most often used antifungal drug in the clinic. Besides its exciting activity against *Candida* and *Cryptococcus*, PSB **1** also demonstrated modest activity against *Aspergillus fumigatus*.[3]

Despite its promising biological activity discussed herein, the development of PSB as a new therapeutic agent was hindered by its local and end organ toxicity in rats. Evidently, in order to take advantage of its promising antifungal activity, the toxicity issues associated with PSB must be circumvented. Bearing these considerations in mind, we carried out rather extensive structure–activity relationship (SAR) modifications on the parent natural products in hopes of identifying pseudomycin analogues with more favorable biological and toxicity profiles.

Towards this end, we have synthesized various pseudomycin analogues containing modifications either at the side-chain or at the core cyclic peptide. During the course of modification of the acid function at residue 3, we discovered several dehydrated and dechlorinated novel pseudomycin derivatives **8**, **10**, and **13** (as shown in Schemes 1 and 2). In this communication, we wish to describe the serendipitous syntheses and antifungal activities of these structurally unique PSB derivatives (Fig. 1).

Synthesis and Structure Determination

The synthesis of bislactone **8** was achieved via a two-step sequence from ZPSB **4**, which was prepared from **1** via simple *N*-acylation. As depicted in Scheme 1, treatment of a DMF solution of **4** (1.0 equiv, 10 mg/mL) with 1.3 equiv of HOBt, EDCI and DMAP provided the 'unexpected' dehydrated PSB derivative **7** (14%), presumably via intermediate **5**.[5] It is worthwhile to mention that several unidentified by-products were also obtained along with **7** under these reaction conditions. We found that DMAP, HOBt and dilution were all essential for obtaining reasonable yield of **7**, suggesting that this dehydration was a base induced intramolecular reaction. Surprisingly, simply reacting ZPSB **4** with Burgess reagent or DEAD/PPh_3/THF did not give any expected dehydrated PSB derivatives. Finally, compound **7** was converted to the bislactone bearing analogue **8** (96%) under standard hydrogenolysis conditions (1 atm H_2, 10% Pd/C, 1% HOAc/MeOH).

The structure of **8** was secured on the basis of detailed analyses of its ^{1}H NMR and COSY spectra. Careful examination of the data listed in Table 1 revealed that, in comparison to PSB **1**, the largest down-field shifts observed in **8** were the H8β (0.95 ppm) and H8α (0.48 ppm) protons, indicating the formation of the

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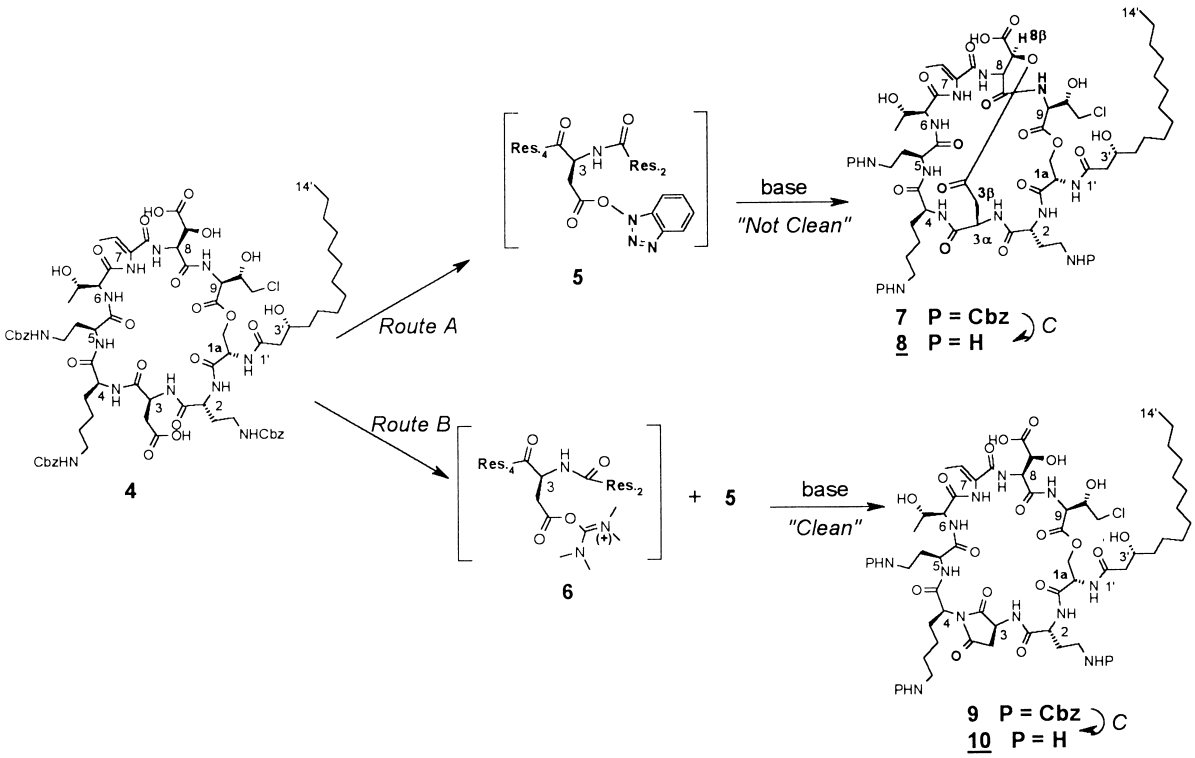
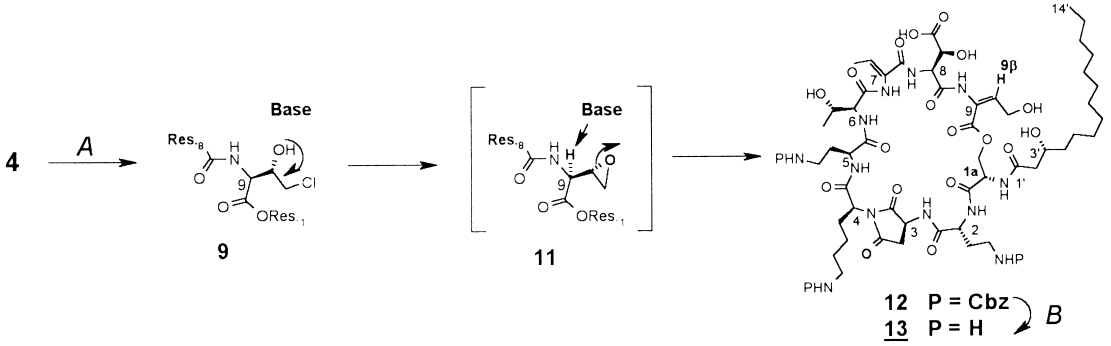


Scheme 1. Synthesis of novel dehydro-pseudomycin B analogues **8** and **10**. Conditions: (A) HOBt/EDCl/DMAP/DMF; (b) TBTU/Et(*i*-Pr)$_2$N/DMF; (C) H_2, 10% Pd/C in 1% HoAc/MeOH.



Scheme 2. Synthesis of dechloro-pseudomycin analogue **13**. Conditions: (A) K_2CO_3/NaI/*t*-BuC(O)OCH$_2$Cl/DMF/12 h; (B) H_2/10% Pd/C in 1% HOAc/MeOH.

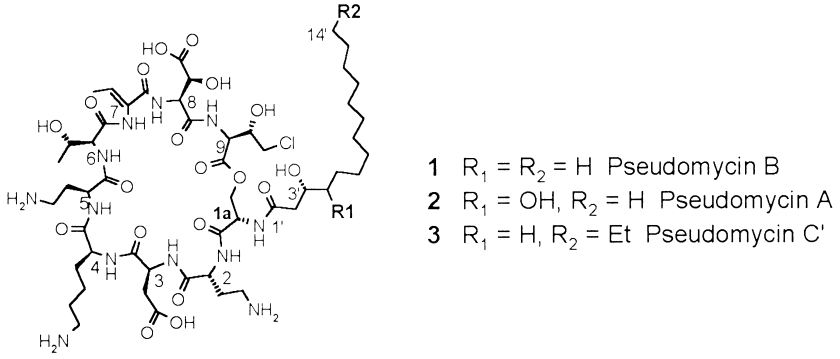


Figure 1. Structures of representive pseudomucins.

Table 1. Proton NMR assignments for pseudomycin B and its analogues **8**, **10** and **13**

Positions	PSB **1**	**8**	COSY of **8**	**10**	COSY of **10**	**13**	COSY of **13**
Residue 1							
α	4.59	4.78	1β1, 1β2	4.54	1β1, 1β2	4.60	1β1, 1β2
β1	4.38	4.23	1α1, 1β2	4.36	1α	4.29	1α1, 1β2
β2	4.53	4.36	1α1, 1β1	4.36	1α	4.48	1α1, 1β1
Residue 2							
α	4.13	4.43	2β1, 2β2	4.31	2β1, 2β2	4.34	2β1, 2β2
β1	2.01	1.77	2α1, 2β2, 2γ	1.95	2α1, 2β2, 2γ	1.95	2α, 2γ
β2	2.07	2.22	2α1, 2β1, 2γ	2.07	2α1, 2β1, 2γ	2.03	2α, 2γ
γ1	2.90	2.86	2β1, 2β2	2.92	2β1, 2β2	∼2.90	2β1, 2β2
γ2	2.97	2.86	2β1, 2β2	2.93	2β1, 2β2	∼2.90	2β1, 2β2
Residue 3							
α	4.55	4.67	3β1, 3β2	**4.44**	3β1, 3β2	**4.45**	3β1, 3β2
β1	2.82	2.98	3α, 3β2	**2.59**	3α, 3β2	**2.58**	3α, 3β2
β2	2.87	3.38	3α, 3β1	**3.11**	3α, 3β1	**3.12**	3α, 3β1
Residue 4							
α	4.13	4.26	4β1, 4β2	**4.61**	4β1, 4β2	**4.62**	4β1, 4β2
β1	1.75	1.64	4α, 4β2, 4γ2	**1.88**	4α, 4β2, 4γ	**1.89**	4α, 4γ
β2	1.78	1.77	4α, 4β1, 4γ1	**2.10**	4α, 4β1, 4γ	**2.09**	4α, 4γ
γ1	1.26	1.35	4β2, 4δ	1.24	4β1, 4β2, 4δ	1.25	4β1, 4β2, 4δ
γ2	1.32	1.41	4β1, 4δ	1.24	4β1, 4β2, 4δ	1.25	4β1, 4β2, 4δ
δ1	1.53	1.59	4γ, 4ϵ	1.57	4γ, 4ϵ	1.57	4γ, 4ϵ
δ2	1.56	1.59	4γ, 4ϵ	1.57	4γ, 4ϵ	1.57	4γ, 4ϵ
ϵ	2.84	2.88	4δ	2.83	4δ	2.85	4δ
Residue 5							
α	4.29	4.57	5β1, 5β2	4.57	5β1, 5β2	4.56	5β1, 5β2
β1	1.99	2.03	5α, 5β2, 5γ	2.04	5α, 5β2, 5γ	2.01	5α, 5β2, 5γ
β2	2.14	2.17	5α, 5β1, 5γ	2.15	5α, 5β1, 5γ	2.14	5α, 5β1, 5γ
γ1	2.89	2.92	5β1, 5β2	2.84	5β1, 5β2	∼2.90	5β1, 5β2
γ2	2.91	2.92	5β1, 5β2	2.84	5β1, 5β2	∼2.90	5β1, 5β2
Residue 6							
α	4.27	4.32	6β	4.11	6β	4.18	6β
β	3.92	4.14	6α, 6γ	4.01	6α, 6γ	3.99	6α, 6γ
γ	1.18	1.25	6β	1.22	6β	1.20	6β
Residue 7							
β	6.53	6.84	7γ	6.73	7γ	6.68	7γ
γ	1.70	1.63	7β	1.70	7β	1.71	7β
Residue 8							
α	4.96	**5.44**	8β	5.07	8β	5.04	8β
β	4.75	**5.70**	8α	4.73	8α	4.73	8α
Residue 9							
α	4.87	4.60	9β	4.77	9β	—	—
β	4.31	4.35	9α, 9γ1, 9γ2	4.31	9α, 9γ	**6.73**	9γ
γ1	3.44	3.59	9β	3.51	9β	**4.13**	9β
γ2	3.50	3.61	9β	3.51	9β	**4.13**	9β
Side chain							
2′α	2.25	2.27	SC2β, SC3	2.25	SC2β, SC3	2.26	SC2β, SC3
2′β	2.36	2.41	SC2α, SC3	2.37	SC2α, SC3	2.39	SC2α, SC3
3′	3.86	3.90	SC2α + 2β, SC4	3.84	SC2α + 2β, SC4	3.87	SC2α + 2β, SC4
4′	1.38	1.41	SC3	1.39	SC3	1.39	SC3
5′–13′	∼1.22	∼1.22	—	∼1.23	—	∼1.23	—
14′	0.83	0.83	SC13	0.83	SC13	0.83	SC13

intramolecular lactone bond between the 8α-hydroxyl group and the acid function at residue 3. In addition, due to the added ring strain imposed by the newly formed lactone bond, rather significant proton shifts were recorded for all of the cyclic core peptide residues.

As also shown in Scheme 1, reaction of ZPSB **4** with TBTU (1 equiv) and Et(*i*-Pr)$_2$N (6 equiv) afforded the presumed activated 3-esters **5** and **6**.[6] Remarkably, in the absence of external nucleophile, these reactive intermediates were trapped internally by the amide proton on residue 4 to yield the corresponding dehydro PSB derivative **9** (20%). Judging from the different product profiles observed under two sets of reaction conditions (HOBt/EDCI/DMAP versus TBTU/Et(*i*-Pr)$_2$N) shown in Scheme 1, we believed that the tetramethyl urea-bearing intermediate **6** was likely the species involved in the formation of the 3-imido derivative.

Upon standard hydrogenolysis, compound **9** was further converted to the 3-imido bearing PSB analogue **10** in 50% yield. The structure of **10** was confirmed again

Table 2. In vitro antifungal activity and tail vein toxicity of PSB analogues

Compound	MIC[a] (μg/mL)			Tail vein tox. (20 mg/kg)
	Candida albicans	*Crypteroccus neoformans*	*Aspergillus fumigatus*	
PSB	0.312	< 0.01	20	Positive
8	5	0.625	>20	Negative
10	>20	10	>20	Negative
13	>20	20	>20	—

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

with the assistance of the COSY data (shown in Table 1). Thus, the up-field shifts for 3α (0.11 ppm) and 3β (0.23 ppm) protons observed in **10** suggested the imido linkage at the residue 3. Large chemical shift changes for 4α, 4β protons were also noticed (see Table 1). Furthermore, the proton NMR spectrum taken in acetonitrile-d_3/water showed only eight amide resonances. No amide proton for residue 4 was found. This result further supported the proposed structure of **10**.

Scheme 2 shows the serendipitous synthesis of 3-imido-9-dechloro PSB analogue **13**. Treatment of a DMF solution of ZPSB **4** with iodomethyl pivalate (5 equiv formed in situ) and K_2CO_3 (3 equiv) for a few hours afforded unexpected 3-imido compound **9**, presumably via its respective 3-monoester intermediate. Prolonged base treatment of **9** led to the putative terminal epoxide **11**, which was further converted to the corresponding 9-allylic alcohol **12** in low yield, via base induced beta elimination. Removal of the Cbz protective group thus furnished the desired novel PSB analogue **13** in 33% yield. The structure of **13** was confirmed on the basis of careful proton NMR analysis. The up-field chemical shifts for 3α, 3β protons together with the down-field shifts for 4α, 4β protons confirmed the imido linkage at the residue 3. Moreover, a unique chemical shift was observed at 6.73 ppm, which indicated the presence of a double bond. This resonance also had a COSY correlation to a methylene at 4.13 ppm. The chemical shifts for normal residue 9 were absent. Based on these observations, we believed that the original chlorothreonine moiety at residue 9 was replaced by the allylic alcohol function in **13**.

Biological Evaluation

Pseudomycin B analogues (**8**, **10**, and **13**) were evaluated in vitro against the following three major pathogens responsible for systemic fungal infections: *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*. As listed in Table 2, all three PSB analogues exhibited reduced activity against *Candida* and *Cryptococcus*. Like PSB, all three structurally unique PSB analogues showed marginal activity towards *A. fumigatus* with MIC values around 20 μg/mL. Analogues **8** and **10** were also evaluated in the tail vein toxicity assay in mice. The testing result indicated that, in contrast to PSB, both analogues did not induce tail vein irritation at the dose tested.

In summary, we describe the serendipitous preparation of three structurally interesting PSB analogues (**8**, **10**, and **13**) under amidation/esterification conditions. Although improvements in toxicity profiles were obtained with the bislactone **8** and the 3-imido derivative **10**, all three new analogues showed much weaker antifungal activities in comparison to the parent pseudomycin B. All three new analogues adopt conformations that are different from PSB as judged by the proton shifts in 3α, and 3β at residue 3. Therefore we believe that the correct conformation of the acid at residue 3 is required for optimal antifungal activity.

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