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Synthetic studies towards Pseudoxylallemycin B, an antibiotic active against gram-negative bacteria: Total synthesis of 3-*epi*-Pseudoxylallemycin B

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ABSTRACT

In an attempt towards the total synthesis of Pseudoxylallemycin B, a homo dimeric, *N*-methylated macrocyclic tetrapeptidic natural product, synthesis of its epimer at position 3 (D-Tyr instead of L-Tyr) is described here. During the course of synthesis we came across a striking yet unusual observation of complete epimerization which led to the formation of 3-*epi*-Pseudoxylallemycin B.

Infections caused by gram-negative bacteria have emerged as a serious problem owing to the gradual development of resistance against almost entire drug pipeline for the treatment of concerned diseases [1]. For instance, *Pseudomonas aeruginosa* has been a causative pathogen for approximately 8% of all healthcare-associated infections according to the reports in CDC's National Healthcare Safety Network [2]. Besides, about 13% of acute healthcare-associated infections caused by this gram-negative pathogen are multidrug resistant, thus leaving several classes of antibiotics ineffective towards its treatment [3]. *Pseudomonas aeruginosa* is a prevalent causative factor for a range of healthcare-associated infections which includes pneumonia, bloodstream infections, urinary tract infections, and surgical site infections [4–6]. There are a few drugs belonging to different chemical scaffolds which are being presently used or under clinical evaluation for treating infections caused by gram-negative bacteria. Among them, cyclic peptides occupy a special place as they possess broad range of biological properties [7]. In particular, cyclotetrapeptides are attractive pharmacological leads as compared to their larger ring size congeners, due to their close compliance to Lipinski's rules [8]. Pseudoxylallemycin A–F (Fig. 1), a group of macrocyclic peptide natural products, were isolated from termite associated fungus *Pseudoxylaria* sp. X802 by Beemelmanns' group in 2016 [9]. Among the six isolated Pseudoxylallemycin (A–F), Pseudoxylallemycin B (**1**) is a structurally symmetric molecule with a rare allenyl moiety whose structure was confirmed by NMR spectroscopy and Marfey's amino acid analysis. Besides, this macrocyclic natural product contains hydrophobic aromatic amino acids with an alternate pattern of *N*-methyl group present at L-leucine. It is found to exhibit antimicrobial activity against gram-negative human pathogen *Pseudomonas aeruginosa* (MIC: 12.5 μg/mL). In addition, this molecule is also found to possess antiproliferative activity (GI_{50}: 9.8 μg/mL and 25.5 μg/mL in HUVEC and K-562 cell lines, respectively). Our efforts towards the synthesis of Pseudoxylallemycin B are described here.

Our strategy to access the target natural product Pseudoxylallemycin B and its analogues is outlined in Scheme 1. We planned a dimerization approach using the dipeptide fragment to build the macrocyclic tetrapeptide. It is important to note that variations in the required alkylating agent will lead to the synthesis of a library of analogues around the scaffold (Scheme 1).

As per the plan, we have synthesized dipeptide **5** from **4** and *N*-Me-L-leucine methyl ester using solution phase peptide coupling. We tried several dimerization macrolactamization conditions on the dipeptide acid **6** but were unable to achieve the required macrocycle (Scheme 2).

Having failed in dimerization approach, we have decided to change the strategy to linear approach through a macrocyclization step at the end. While working on this project, we came across an elegant study by Brimble's group which reflects the feasibility of

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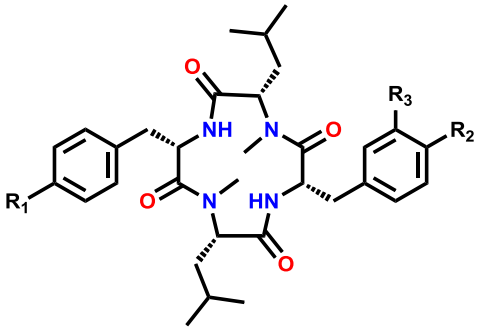
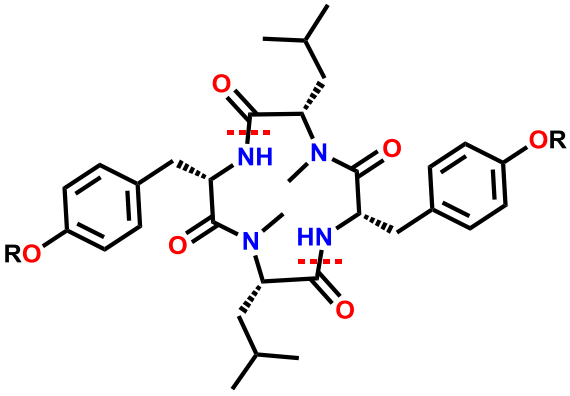
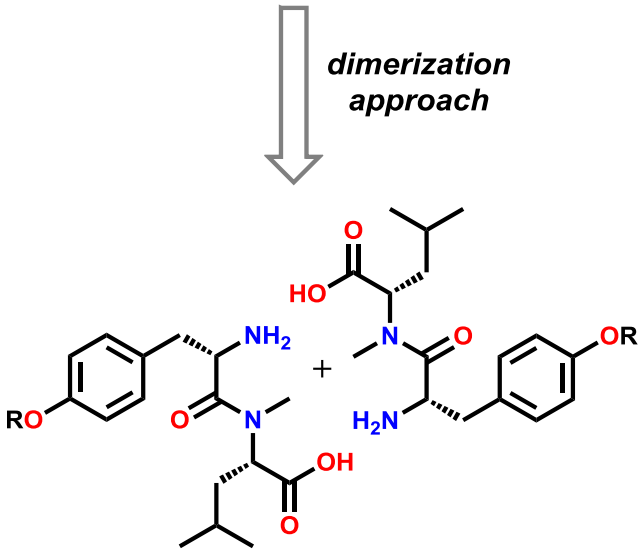


R_1, R_2, R_3= H, Pseudoxylallemycin A
R_1, R_2= OAllenyl, R_3= H, Pseudoxylallemycin B (1)
R_1= H & R_2= OAllenyl, R_3= H, Pseudoxylallemycin C
R_1= H & R_2= OAllenyl, R_3= OH, Pseudoxylallemycin D
R_1= H & R_2= oxybut-2-ene, R_3= H, Pseudoxylallemycin E
R_2= OAllenyl & R_2= oxybut-2-ene, R_3= H, Pseudoxylallemycin F

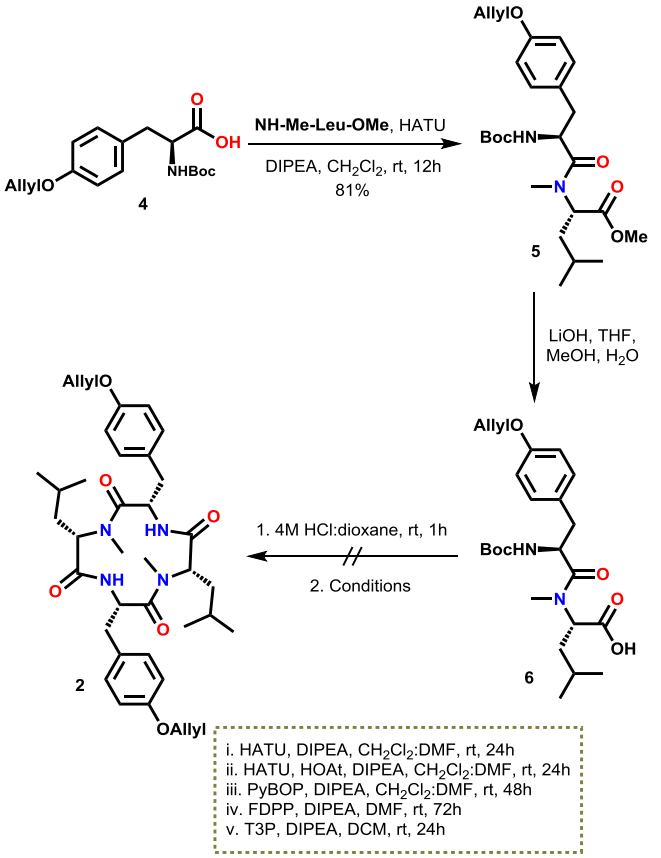
Fig. 1. Structures of Pseudoxylallemycin A–F.



R = Allenyl : Pseudoxylallemycin B (1)
R = Allyl : Pseudoxylallemycin B analogue (2)
R = H : Key fragment (3)



Scheme 1. Strategy to access target natural product and its analogues.



Scheme 2. Attempts for dimerization macrolactamization.

macrolactamization at *N*-Me position using propylphosphonic anhydride (T3P) to construct similar tetrapeptidic macrocycles [10]. We thus planned to synthesize our key macrocycle via macrolactamization at *N*-Me position. Our synthesis commenced with the synthesis of dipeptide **8** which we obtained from Boc deprotection of **7** using HCl in dioxane followed by coupling with Boc-*N*-Me-leu-OH using HATU and DIPEA in dichloromethane with a yield of 76%. Removal of Boc group in **8** and coupling the same with Boc-tyr(allyl)-OH using HATU gave tripeptide **9** in 70% yield which was then treated with 4 M HCl in dioxane and coupled with Boc-*N*-Me-leu-OH to obtain tetrapeptide **10** in 63% yield. Ester hydrolysis of **10** furnished the required tetrapeptide acid cyclization precursor **11**. After having the required cyclization precursor **11**, we treated the same with 4 M HCl in dioxane to afford the corresponding amino acid which on treatment with T3P and DIPEA in CH_2Cl_2 and DMF (9:1) mixture for 48 h resulted in the formation of the macrocyclic compound **12.** Macrocycle **12** on $Pd(PPh_3)_4$ catalyzed allyl deprotection afforded the compound **13** which upon coupling with allenyl bromide using potassium carbonate in DMF gave compound **14**. However ^1H and ^{13}C NMR of **14** was not in agreement with the reported NMR data of natural Pseudoxylallemycin B **(1)**. To decipher the reasons for such NMR discrepancies, we took X-ray crystal structure of **14** (CCDC No.: 1842533), which disclosed the surprising fact of complete epimerization of one of the L-tyrosine stereocenter to D-tyrosine (Scheme 3). To suppress or minimize the extent of racemization we also tried macrolactamization at room temperature. However, we did not observe any changes in the outcome of the reaction. Though epimerization during amino acid coupling is well-reported [11], complete epimerization during macrolactamization is unprecedented with