# SYNTHESIS OF 4'-*N*-CBZ-PRADIMIC ACID FROM PRADIMICIN A

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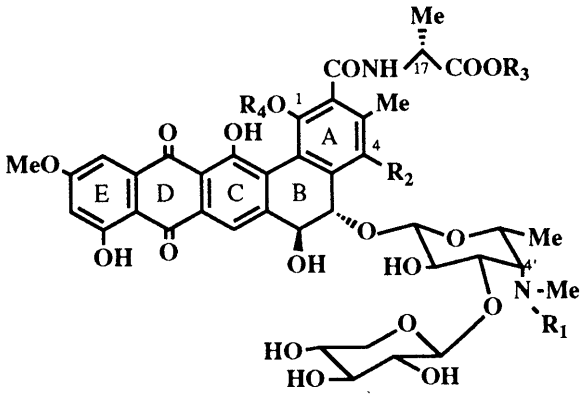
The sterically hindered alanine-amide bond of pradimicin A (PRM A, 1) was successfully cleaved via less hindered primary amide (11) by treatment with $NOBF_4$ in eight steps to afford practical yield of 4'-*N*-Cbz-pradimic acid (13), which is a useful intermediate for the synthesis of alanine-exchanged derivatives.

**KEYWORDS** pradimicin A; pradimic acid; antifungal antibiotics; D-alanine; amide-cleavage

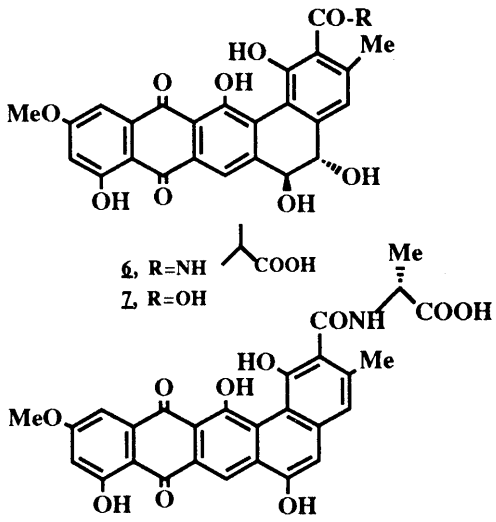
Pradimicin A[1,2](PRM A, 1), which is produced by *Actinomadura hibisca* P157-2(ATCC53557), belongs to a novel group of antifungal antibiotics possessing a glycosylated dihydrobenzo[*a*]napthacenequinonecarbonyl-D-alanine. Previously it was reported that the L-alanine isomer of 1 was inactive against various yeasts and fungi[3]. This suggested that the amino acid moiety in 1 plays an important role in expression of the antifungal activity. Therefore, in exploration of more potent derivatives, it is interesting to exchange the alanine moiety of 1 with other amino acids. Thus desalanyl-4'-*N*-Cbz-PRM A (4'-*N*-Cbz-pradimic acid, 13) is required for the synthesis of various kinds of alanine-exchanged analogs of 1. Recently D. Ikeda [4] et al. reported the cleavage of alanine-amide bond of benanomicin A, which has a very similar structure to pradimicin A, by use of Meerwein's reagent. This prompted us to publish our practical method for cleavage of the alanine-amide bond of 1 to prepare 13.

Since the PRM A (1) possesses many functional groups such as labile glycosyl groups, polyhydroxyquinone and dihydroaromatic moieties, selective cleavage of the amide bond is difficult. The earlier degradation studies[2] of 1 demonstrated that the amide bond was highly resistant to acid hydrolysis (in 6 N HCl at 115° C for 14 h), which resulted in the facile elimination of the sugar part to produce an aglycone retaining the alanine moiety (6), B-ring aromatized product (8) and only a trace amount



**1**, $R_1=R_2=R_3=R_4=H$, PRADIMICIN A
**2**, $R_1$=Cbz, $R_2=R_3=R_4=H$
**3**, $R_1$=Cbz, $R_2$=H, $R_3$=Me, $R_4=H$
**4**, $R_1$=Cbz, $R_2=NO_2$, $R_3$=Me, $R_4=H$
**5**, $R_1$=Cbz, $R_2$=H, $R_3$=Me, $R_4=COCH_3$

Fig 1



**6**, R=NH-CH(Me)-COOH
**7**, R=OH

**8**

of the alanine-cleaved aglycone (7). Alkaline hydrolysis (1 N NaOH at 115° C, overnight) of 1 also afforded the aglycone (8). To cleave the amide bond thermally after conversion to *N*17-nitrosoamide[5], *N*-nitrosation of 4'-*N*-Cbz-PRM A methyl ester (3) (prepared by 4'-*N*-benzyloxycarbonylation of 1 followed by esterificaion with thionyl chloride-methanol) was treated with $NOBF_4$ and organic bases[6]. But the product obtained was an unexpected 4-$NO_2$ compound (4), which was presumed to be generated by oxidative aromatic nitration at *C*-4 position[7]. In order to reduce the nucleophilicity of *C*-4 position, 1-hydroxy group of 3 was acetylated by the method of V. O. Illi[8](AcCl, NaOH, $Bu_4NHSO_4$/1,4-dioxane) to afford 1-acetoxy-4'-*N*-Cbz-PRM A methyl ester (5) where the position of the acetyl group was confirmed by $^1$H-NMR spectra; the spectra of 5 showed 4-H at 7.47 ppm (in DMSO-$d_6$), which was 0.45 ppm lower than that of 3. However, the reaction of 5 with $NOBF_4$ did not proceed, probably due to the heavy steric hindrance around the amide-linkage. Therefore, we tried first to convert the alanine amide to a less hindered primary amide (12) and then to convert it to the desired carboxylic acid by way of *N*-nitrosation.

Conversion of PRM A (1) to 4'-*N*-Cbz-Pradimic Acid Amide (11)

In order to convert the alanine residue of 1 to a primary amide group, the Curtius rearrangement of 4'-*N*-Cbz-PRM A (2) was attempted by treatment with diphenylphosphoryl azide(DPPA)[9]. When the mixture of 2, DPPA and triethylamine in tert-butyl alcohol was heated, two products were obtained in a ratio of 1:1, which were separated by column chromatography. These two compounds showed the same molecular weights in mass spectra (FAB-MS, m/z 929$(M+H)^+$) and very similar $^1$H-NMR spectra except 17-H (isomer A; 17-H 4.99 ppm, 17-methyl 1.45 ppm: isomer B; 17-H 5.52 ppm, 17-methyl 1.45 ppm). From these data, these two compounds were thought to be the oxazinone diastereoisomers (9) concerning the 17-methyl group. Because of severe steric hindrance around the amide bond, an isocyanate intermediate (2b) generated by the Curtius reaction was not considered to react with tert-butyl alcohol, but the isocyanate group was eliminated to produce an imino-intermediate (2c), concomitant cyclization of which was supposed to afford two diastereomeric oxazinones (9) (Fig 2). Mild acid hydrolysis of the isomeric mixture (9) (6 N HCl-MeOH, room temperature, 2 days) did not give the desired 4'-*N*-Cbz-pradimic acid amide (11), whereas treatment with 1 N NaOH-methanol (1:10) afforded methoxy compounds (10), which were easily hydrolyzed (3 N HCl/$CH_3CN$, room temperature, overnight) to give the desired desalanyl 4'-*N*-Cbz-pradimic acid amide (11) (FAB-MS, m/z 903$(M+H)^+$; $^1$H-NMR, 7.54 and 7.66 ppm ($CONH_2$)). The reaction could be conducted without isolation of 9 and 10 in each step, and the overall yield was highly practical (71% yield from 2).

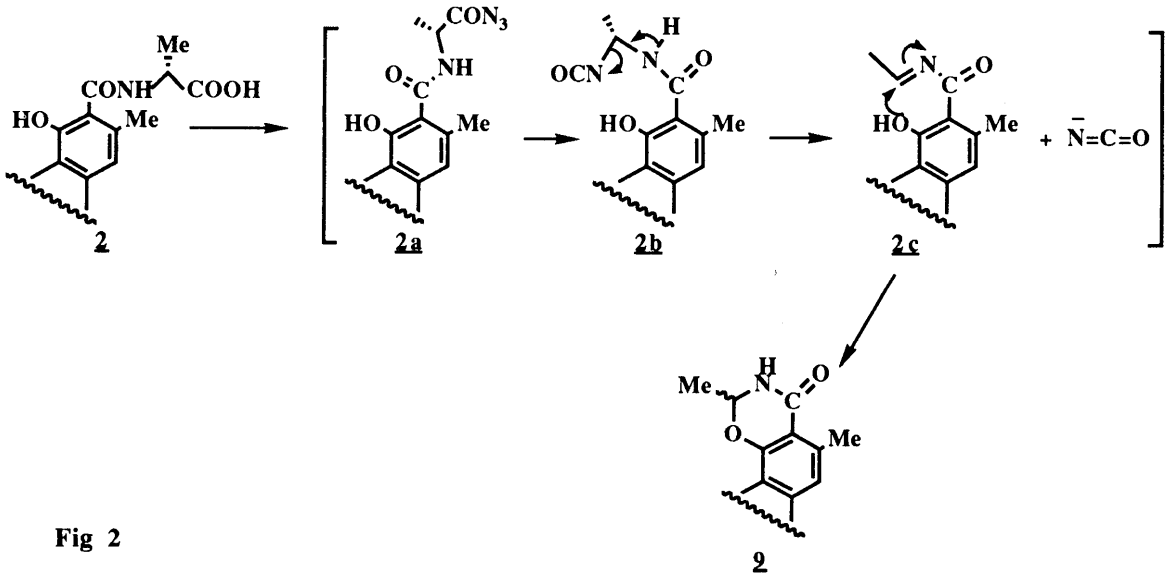



Fig 2

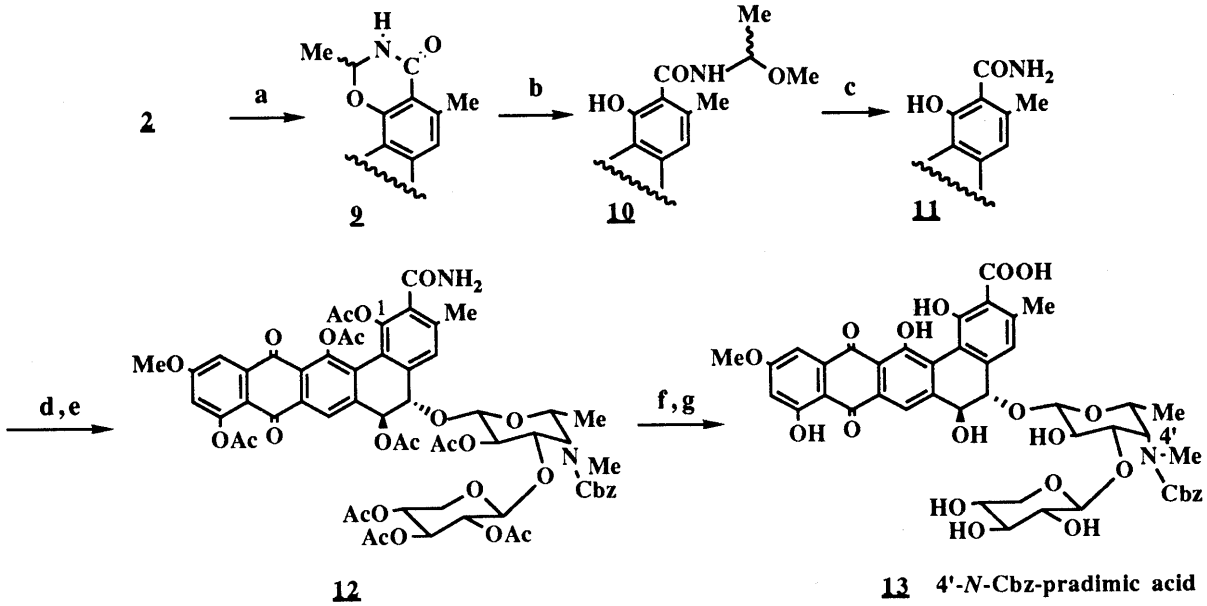



**Chart 1** (a) DPPA, $Et_3N$/t-BuOH, reflux; (b) 1N NaOH-MeOH; (c) 3N HCl/$CH_3CN$; (d) AcCl, NaOH, $Bu_4NHSO_4$/dioxane; (e) $Ac_2O$-Py; (f) $NOBF_4$, $Et_3N/CH_3CN$; (g) 1N NaOH.

Conversion of 11 to 4'-*N*-Cbz-Pradimic Acid (13)

In order to avoid the 4-nitration, the 1-hydroxy group of 11 was acetylated by the method to prepare 5 and then peracetylated by acetic anhydride-pyridine to increase the solubility in acetonitrile. Then the completely acetylated compound (12) was treated with $NOBF_4$[6a] and triethylamine in acetonitrile, followed by alkaline hydrolysis to afford the desired 4'-*N*-Cbz-pradimic acid (13) (FAB-MS, m/z 904$(M+H)^+$). This sequence of reactions could be conducted without isolation of 12, and the overall yield of 13 from 11 was also practical (51%).

For the confirmation of the structure of 4'-*N*-Cbz-pradimic acid (13), conversion of 13 to PRM A (1) was carried out. Acylation of D-alanine methyl ester with benzotriazol-1-yl ester of 13 afforded 4'-*N*-Cbz-PRM A methyl ester (3). Subsequent alkaline hydrolysis and catalytic hydrogenation of 3 gave 1 in 20% overall yield. The product was completely identical with the natural sample of PRM A in light of $^1$H-NMR, IR, UV, mass and HPLC mobilities.

In summary, the sterically hindered amide bond of PRM A (1) was successfully cleaved via less hindered amide (11) by treatment with NOBF4 to afford 4'-*N*-Cbz-pradimic acid (13) in practical yield.

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