

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

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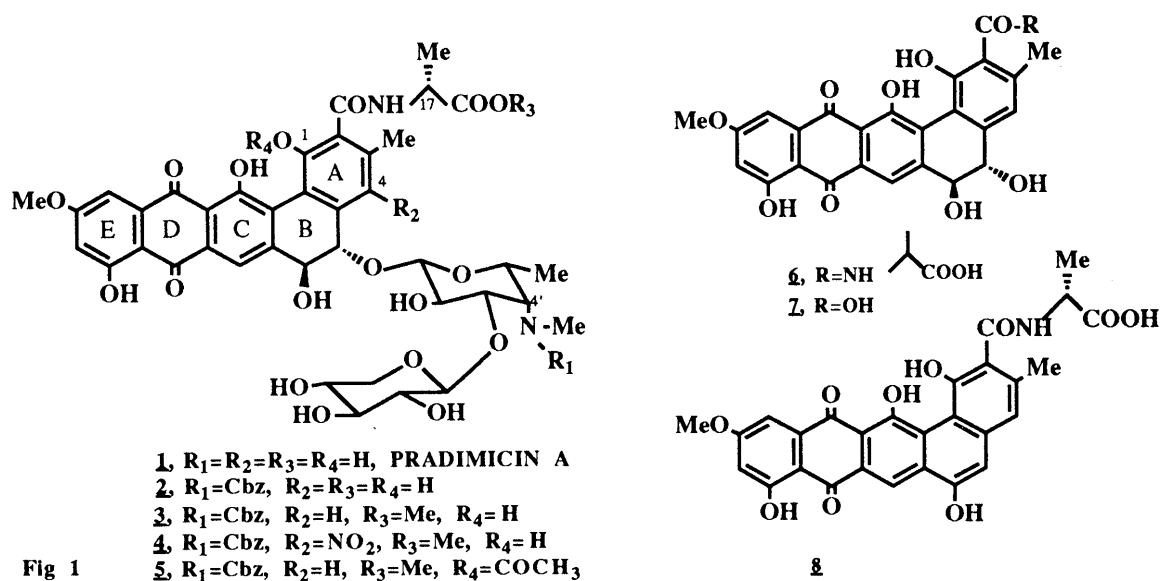
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The sterically hindered alanine-amide bond of pradimicin A (PRM A, **1**) was successfully cleaved via less hindered primary amide (**11**) by treatment with NOBF₄ in eight steps to afford practical yield of 4'-N-Cbz-pradimic acid (**13**), which is 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*]naphthacenequinonecarbonyl-D-alanine. Previously it was reported that the L-alanine isomer of **1** was inactive against various yeasts and fungi³⁾. 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. Ikeda⁴⁾ et al. reported the cleavage of alanine-amide bond of benanomycin 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 studies²⁾ 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



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-nitrosoamide⁵), *N*-nitrosation of 4'-*N*-Cbz-PRM A methyl ester (**3**) (prepared by 4'-*N*-benzyloxycarbonylation of **1** followed by esterification with thionyl chloride-methanol) was treated with NOBF₄ and organic bases⁶). But the product obtained was an unexpected 4-NO₂ compound (**4**), which was presumed to be generated by oxidative aromatic nitration at C-4 position⁷). In order to reduce the nucleophilicity of C-4 position, 1-hydroxy group of **3** was acetylated by the method of V. O. Illi⁸) (AcCl, NaOH, Bu₄NHSO₄/1,4-dioxane) to afford 1-acetoxy-4'-*N*-Cbz-PRM A methyl ester (**5**) where the position of the acetyl group was confirmed by ¹H-NMR spectra; the spectra of **5** showed 4-H at 7.47 ppm (in DMSO-d₆), which was 0.45 ppm lower than that of **3**. However, the reaction of **5** with NOBF₄ 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 ¹H-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/CH₃CN, room temperature, overnight) to give the desired desalanyl 4'-*N*-Cbz-pradimic acid amide (**11**) (FAB-MS, *m/z* 903(*M*+H)⁺; ¹H-NMR, 7.54 and 7.66 ppm (CONH₂)). 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**).

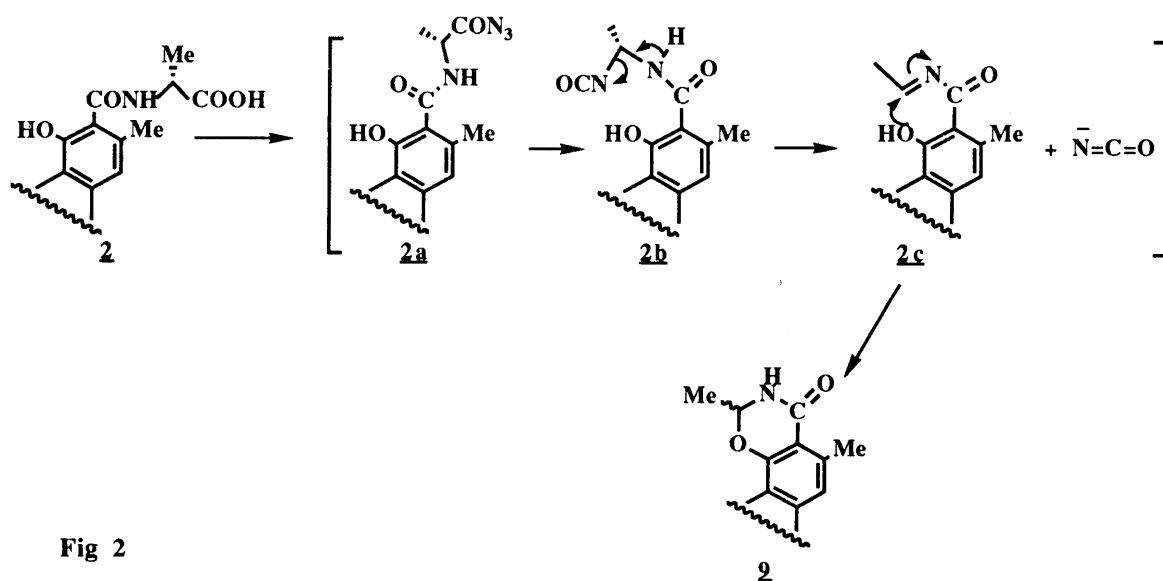


Fig 2

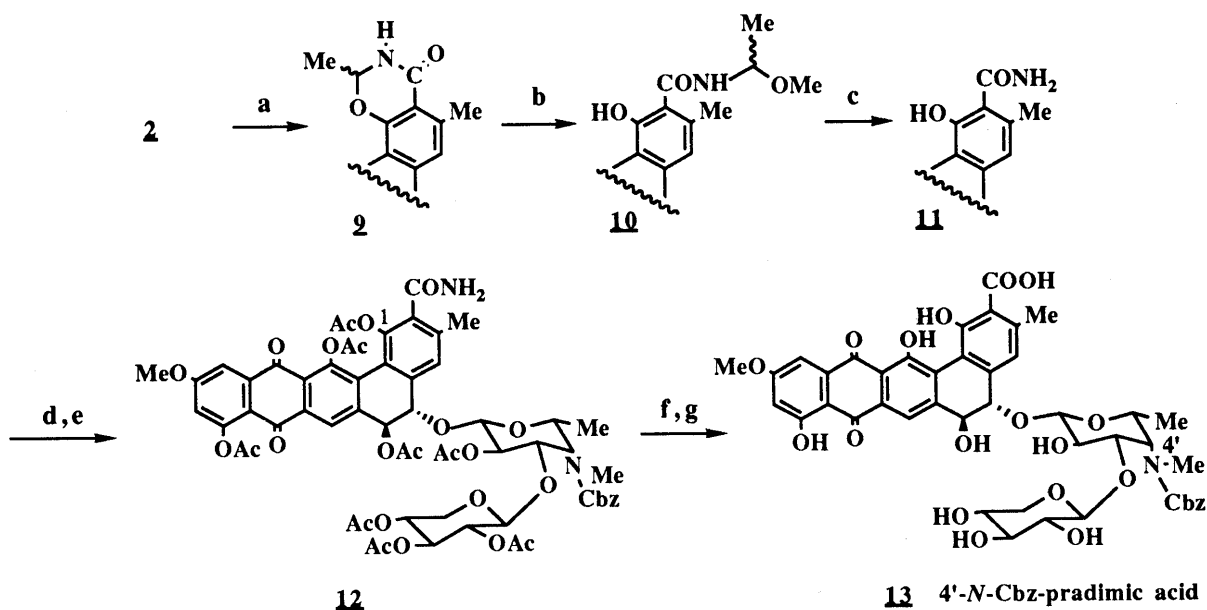


Chart 1 (a) DPPA, Et₃N/t-BuOH, reflux; (b) 1N NaOH-MeOH; (c) 3N HCl/CH₃CN; (d) AcCl, NaOH, Bu₄NHSO₄/dioxane; (e) Ac₂O-Py; (f) NOBF₄, Et₃N/CH₃CN; (g) 1N NaOH.

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 NOBF₄^{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 ¹H-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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