SYNTHESIS AND ANTIFUNGAL ACTIVITIES OF PRADIMICIN DERIVATIVES, MODIFICATION AT C4'-POSITION

HAJIME KAMACHI, SEIJI IIMURA, SATSUKI OKUYAMA, HIDEAKI HOSHI, SACHINO TAMURA, MIEKO SHINODA, KYOICHIRO SAITOH, MASATAKA KONISHI and TOSHIKAZU OKI

Bristol-Myers Squibb Research Institute, Bristol-Myers Squibb K.K., 2-9-3, Shimo-meguro, Meguro-ku, Tokyo 153, Japan

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The 4'-*N*-alkyl (**1**~**10**) and 4'-*N*-acyl derivatives (**11**~**21**) of pradimicins (PRMs) were synthesized by trimethylsilylation of PRMs A, C and FA-1 followed by condensation with appropriate alkylating and acylating agents. The 4'-hydroxy derivatives (**23** and **24**) were synthesized from PRM FA-2 in a 3-step sequence. Among these compounds, the 4'-*N*-carboxylsubstituted alkyl (**1**, **5**, **8** and **10**), 4'-*N*-formyl (**11**) and 4'-axial-hydroxy (**23**) derivatives retained the antifungal activity of the parent compounds and showed great improvement in water solubility.

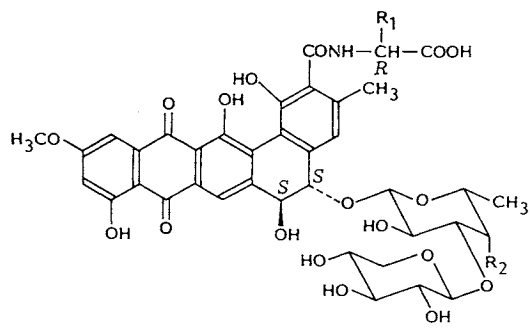
The pradimicins (PRMs) are a new family of antibiotics (Fig. 1)[1~5] that exhibited broad-spectrum antifungal activity both *in vitro* and *in vivo*. Although they are relatively non-toxic, their limited solubility in aqueous media prompted us to initiate chemical modification of PRMs focused on the C4'-position[6,7] in order to improve water-solubility. The objectives in this program were to determine the limitations of modification, establish structure-activity relationships and identify a compound for development. Herein, we describe the syntheses and *in vitro* activities of the PRM derivatives prepared.

Synthesis

4'-*N*-Alkyl Derivatives

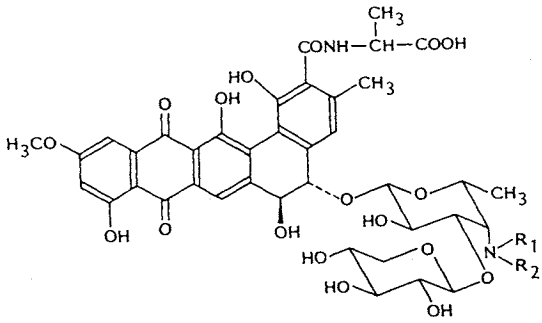
PRMs A, C and FA-1 have many functional groups that may interfere with *N*-alkylation. However, when they were alkylated with a variety of alkyl halides including carboxyalkyl halides in the presence of excess amounts of *N*,*O*-bis(trimethylsilyl)-acetamide (BSA) in dichloromethane, *N*-alkylation took place smoothly and the 4'-*N*-alkyl derivatives were obtained in excellent yields, after treatment with hydrochloric acid or tetrabutylammonium fluoride. Apparently the nucleophilicity of the nitrogen atom was retained[8], while the reactive hydroxyl and carboxyl groups present in the molecule were protected as trimethylsilyl ethers and esters, respectively. The 4'-*N*-alkyl derivatives (**1**~**4**, **6** and **7**) were prepared by the *N*-alkylation of PRM A with iodoacetic acid, iodoacetamide, ethyl bromoacetate, methyl 4-bromocrotonate, *p*-bromomethylphenylacetic acid, 2-(bromomethyl)acrylic

Fig. 1. Structures of natural pradimicins.



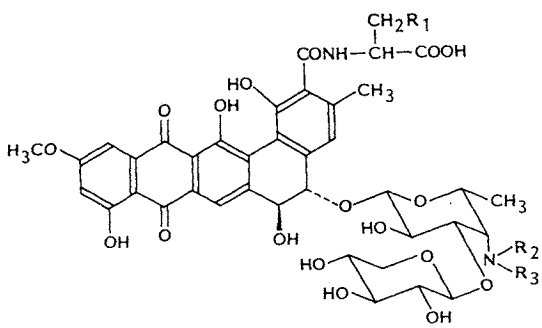
	R_1	R_2
Pradimicin A	CH_3	$NHCH_3$
Pradimicin C	CH_3	NH_2
Pradimicin FA-1	CH_2OH	$NHCH_3$
Pradimicin FA-2	CH_2OH	NH_2

Fig. 2. Structures of 4′-*N*-alkyl pradimicins (**1**~**10**).



No.	R_1	R_2
1	CH_3	CH_2CO_2H
2	CH_3	CH_2CONH_2
3	CH_3	$CH_2CO_2C_2H_5$
4	CH_3	$CH_2CH^{(E)}{=}CHCO_2CH_3$
5	CH_3	$CH_2CH^{(E)}{=}CHCO_2H$
6	CH_3	CH_2-(1,4-C$_6$H$_4$)-CH_2CO_2H
7	CH_3	$CH_2C(=CH_2)CO_2H$
8	H	CH_2CO_2H
9	CH_2CO_2H	CH_2CO_2H
10	H	$CH_2CH^{(E)}{=}CHCO_2H$

Fig. 3. Structures of 4′-*N*-acyl pradimicins (**11**~**21**).



No.	R_1	R_2	R_3
11	H	CH_3	CHO
12	H	CH_3	$COCH_3$
13	H	CH_3	COC_6H_5
14	H	CH_3	$COC(CH_2OH)_3$
15	H	CH_3	$COCH_2CH_2CO_2H$
16	H	CH_3	$COCH_2N(CH_3)_2$
17	H	CH_3	$COCH_2NH_2$
18	H	CH_3	$COCH_2CH_2NH_2$ (*S*)
19	H	CH_3	$COCH(CH_3)NH_2$
20	H	H	CHO
21	OH	CH_3	CHO

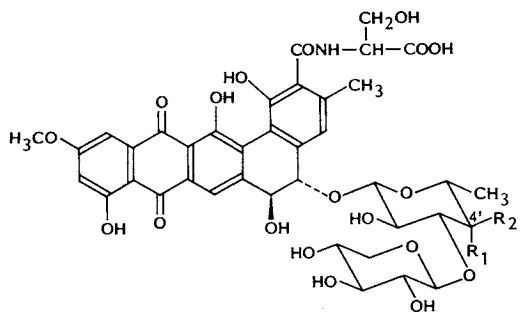
acid, respectively in 66~86% yields. The derivative **5** was prepared by alkaline hydrolysis of **4**. The derivative **8** was prepared by alkylation of PRM C with iodoacetic acid as a major product and **9** was obtained as a minor product. The derivative **10** was prepared by alkylation of PRM C with methyl 4-bromocrotonate, followed by alkaline hydrolysis. The structure of **1** was established by standard MS and ^{1}H NMR analysis, and the position of the newly introduced carboxymethyl group was confirmed by the NOESY experiment in which a correlation was demonstrated between the *N*-methyl protons at δ 2.61 and the methylene protons of the carboxymethyl group at δ 3.62. The structures of the other 4′-*N*-alkyl derivatives were determined analogously.

4′-*N*-Acyl Derivatives (Fig. 3)

The *N*-formyl derivatives (**11**, **20** and **21**) were prepared by acylation of TMS-PRMs A, C and FA-1 with acetic formic anhydride in the presence of BSA in dichloromethane followed by removal of the trimethylsilyl groups. The derivatives (**12**, **13**, **15** and **16**) were prepared similarly by reacting PRM A with acetic anhydride, benzoyl chloride, succinic anhydride and *N*,*N*-dimethylglycyl chloride, respectively. Compound **14** was prepared by acylating PRM A with 2,2,2-tri(acetoxymethyl)acetyl chloride and subsequent hydrolysis in 1 N NaOH - MeOH (1 : 1). The α- and β-amino acid substituted derivatives (**17**~**19**) were prepared by reacting PRM A with 1-hydroxybenzotriazol esters of *N*-Boc glycine, *N*-Boc β-alanine and *N*-Cbz-*S*-alanine, respectively, and subsequent deprotection. Analysis of the ^{1}H NMR spectra of these *N*-acyl derivatives indicated that they may exist as pairs of rotational isomers. In the case of the *N*-formyl

derivative **11**, two doublets at δ 0.94 (0.6H) and 1.04 (2.4H) assignable to the 5'-methyl protons and two singlets at δ 7.90 (0.8H) and 7.95 (0.2H) assignable to the 4'-*N*-formyl proton were observed at room temperature. These coalesced into one doublet and one singlet, respectively, when the spectrum was measured at 145°C.

Fig. 4. Structures of 4'-oxo and 4'-hydroxy derivatives (**22**~**24**).



22 R_1, R_2=O
23 R_1=OH, R_2=H
24 R_1=H, R_2=OH

4'-Oxo and Hydroxy Derivatives

The 4'-oxo and hydroxy derivatives (Fig. 4) were synthesized from PRM FA-2 following the method of COREY[9]. PRM FA-2 was reacted with 3,5-di-*tert*-butyl-1,2-benzoquinone in the presence of triethylamine to afford the corresponding 4'-SCHIFF's base, which was then treated with formic acid - MeOH (1 : 1) at 60°C for 1.5 hours. The resulting 4'-deamino-4'-oxo derivative **22** was reduced with sodium borohydride in water at 5°C for 1 hour to give a mixture (~1 : 1) of 4'-hydroxy epimers (the axial epimer **23** and the equatorial epimer **24**) which were separated by preparative HPLC. Their stereochemical assignments were based on analysis of the ^{1}H-^{1}H shift correlation spectra (COSY) in which **23** and **24** displayed $J_{4'\text{-}5'}$ of $<$1.0 and 8.6 Hz and $J_{3'\text{-}4'}$ of 2.9 and 8.6 Hz, respectively.

Water-solubility and *In Vitro* Activity

Table 1 summarizes the water-solubility and antifungal activity of the compounds synthesized in this study. The solubility was determined in phosphate-buffered saline containing Ca^{2+} and Mg^{2+} at pH 7[6]. The 4'-*N*-alkyl derivatives were more soluble than PRM A (0.02 mg/ml). Especially, **1** and **5**~**10**, which possess a carboxyl functionality in the *N*-alkyl substituent, were highly soluble in aqueous media. Among the *N*-acyl derivatives, the compounds having a small neutral acyl group (**11**, **20** and **21**) and a carboxyl-substituted acyl group (**15**) were highly water-soluble, while the compound having a lipophilic acyl group (**13**) was hardly soluble in water. The amino acid-substituted derivatives (**16**~**19**), were not soluble. The 4'-hydroxyl derivative **23** was highly soluble in aqueous media.

The antifungal activity was determined by the two-fold agar dilution method on yeast morphology agar buffered with 0.067 M phosphate, pH 7.0. The PRM derivatives having a small 4'-*N*-alkyl substituent (**1**~**5**, **8** and **10**) retained the antifungal activity of the PRMs, but those having a bulky substituent (**6** and **7**) showed much reduced activity against *Aspergillus fumigatus*, while the *N*,*N*-di-carboxymethyl derivative (**9**) had marginal activity only against *Candida albicans*. Among the 4'-*N*-acyl derivatives, the 4'-*N*-formyl derivatives (**11**, **20** and **21**) retained the antifungal activity of PRMs, but those having a large acyl substituent (**13**~**15**) were inactive against all the fungi tested. The α-amino-substituted acyl derivatives (**16**, **17** and **19**) showed activity against yeasts but were marginally active or inactive against *A. fumigatus*, while the β-amino-substituted acyl derivative **18** retained the activity. Among the 4'-hydroxy derivatives, the axial-hydroxy derivative (**23**) retained the antifungal activity of PRMs, while the equatorial isomer **24** was only marginally active against *C. albicans*. The 4'-oxo derivative (**22**) showed reduced activity against *Cryptococcus neoformans* and no activity against *A. fumigatus*.

In summary, among the 4'-modified derivatives of pradimicins synthesized in this study, the

Table 1. *In vitro* activity and water solubility of pradimicin derivatives.

Compound	Water solubility (mg/ml)[a]	MIC (μg/ml)[b]		
		Candida albicans A9540	*Cryptococcus neoformans* IAM 4514	*Aspergillus fumigatus* IAM 2034
1	>30	6.3	1.6	6.3
2	11	3.1	1.6	6.3
3	11	6.3	6.3	12.5
4	7.8	6.3	3.1	6.3
5	>20	6.3	3.1	6.3
6	>20	6.3	6.3	>100
7	>20	6.3	6.3	50
8	>20	6.3	3.1	6.3
9	>20	50	>100	>100
10	>20	12.5	3.1	3.1
11	>32	3.1	6.3	6.3
12	24	6.3	12.5	>50
13	0.04	>100	>100	>100
14	NT	>100	>100	>100
15	>28	>100	>100	>100
16	1.8	6.3	6.3	50
17	1.0	6.3	3.1	>100
18	0.25	6.3	3.1	12.5
19	0.30	12.5	50	>100
20	>20	6.3	6.3	12.5
21	>20	6.3	3.1	25
22	NT	6.3	25	>100
23	>40	6.3	3.1	3.1
24	NT	25	>100	>100
PRM A	0.02	12.5	1.6	1.6
PRM C	0.02	3.1	1.6	1.6
PRM FA-1	0.26	6.3	1.6	1.6
PRM FA-2	0.03	3.1	1.6	1.6

[a] Solubility in DULBECCO's phosphate buffered saline containing Ca^{2+} and Mg^{2+}.
[b] MIC's were determined on yeast morphology agar buffered at pH 7.0 (Incubation, 28°C, 48 hours).
NT: Not tested.

4'-*N*-carboxyl-substituted alkyl derivatives (**1**, **5**, **8** and **10**), the 4'-*N*-formyl derivatives (**11**) and the 4'-axial-hydroxy derivative **23** showed antifungal activity comparable to the PRMs with greatly improved water solubility.

Experimental

MPs were determined using a Yanagimoto micro hot-stage apparatus and are uncorrected. NMR spectra were recorded on a Jeol GX-400 (400 MHz). Mass spectra were recorded on a Jeol JMS-AX505H (FAB) mass spectrometer.

4'-Alkyl Derivatives of Pradimicins (**1**~**10**)

4'-*N*-Carboxymethylpradimicin A (General Procedure for **1**~**4** and **6**~**9**)

Iodoacetic acid (75 mg, 0.4 mmol) was added to a mixture of PRM A·HCl (50 mg, 0.057 mmol) and BSA (0.25 ml, 1 mmol) in dry dichloromethane (1 ml), and the mixture was refluxed overnight. After cooling, the reaction mixture was mixed with MeOH (3 ml) and 1 N HCl (1 ml) and then concentrated under reduced pressure. The oily residue was chromatographed on a reversed phase silica gel column (Nacalai, Cosmosil 75C_{18}-OPN, 20 i.d. × 250 mm), eluting with water and then 30% acetonitrile in water. The eluate was monitored by HPLC. The fractions containing the desired product were combined, concentrated, and

freeze-dried to provide 43 mg (84%) of **1** as an amorphous powder. MP 232°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 1.18 (3H, d, J=6 Hz, 5'-CH_3), 2.61 (3H, s, 4'-N-CH_3), 3.62 (2H, s, CH_2CO_2H), 4.60 (1H, d, J=7 Hz, 1'-H); FAB-MS m/z 899 $(M+H)^+$.

Compounds, **2**~**4**, **6** and **7** were prepared by coupling PRM A with iodoacetamide, ethyl bromoacetate, methyl 4-bromocrotonate, *p*-bromomethylphenylacetic acid, 2-(bromomethyl)acrylic acid, respectively, by a procedure similar to the above. Compounds **8** (major product) and **9** (minor product) were prepared by coupling PRM C with iodoacetic acid. Compound **5** was prepared by alkaline hydrolysis of **4** (in 1 N NaOH - MeOH (1 : 1), stirred overnight at room temperature). Compound **10** was prepared by a similar alkylation of PRM C, followed by the alkaline hydrolysis. The yield, MP and ^{1}H NMR spectral data of these compounds were as follows:

2: Yield 82%; MP 225°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 1.20 (3H, d, J=6 Hz, 5'-CH_3), 2.56 (3, s, 4'-N-CH_3), 3.72 (2H, br, CH_2CONH_2), 4.61 (1H, d, J=7 Hz, 1'-H); FAB-MS m/z 898 $(M+H)^+$.

3: Yield 74%; MP 225°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 1.17 (3H, d, J=6 Hz, 5'-CH_3), 1.18 (3H, t, J=7 Hz, $CH_2CO_2CH_2CH_3$), 2.61 (3, s, 4'-N-CH_3) 3.68 (2H, br, $CH_2CO_2CH_2CH_3$), 4.06 (2H, m, $CH_2CO_2CH_2CH_3$), 4.59 (1H, d, J=7 Hz, 1'-H); FAB-MS m/z 927 $(M+H)^+$.

4: Yield 66%; MP 180°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 1.13 (3H, d, J=6 Hz, 5'-CH_3), 2.56 (3, s, 4'-N-CH_3), 3.65 (3H, s, CH_3), 4.61 (1H, d, J=7 Hz, 1'-H), 6.00 (1H, d, J=16 Hz, vinyl proton), 6.83 (1H, dt, J=16 and 5.5 Hz, vinyl proton); FAB-MS m/z 939 $(M+H)^+$.

5: Yield 85%; MP 180°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 1.13 (3H, d, J=6 Hz, 5'-CH_3), 2.56 (3, s, 4'-N-CH_3), 4.60 (1H, d, J=7 Hz, 1'-H), 5.90 (1H, d, J=16 Hz, vinyl proton), 6.73 (1H, dt, J=16 and 5.5 Hz, vinyl proton); FAB-MS m/z 925 $(M+H)^+$.

6: Yield 62%; MP 200°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 1.17 (3H, d, J=6 Hz, 5'-CH_3), 2.52 (3, s, 4'-N-CH_3), 3.52 (2H, s, CH_2CO_2H), 4.60 (1H, d, J=7 Hz, 1'-H), 7.16 (2H, d, J=8 Hz, phenyl protons), 7.27 (2H, d, J=8 Hz, phenyl protons); FAB-MS m/z 989 $(M+H)^+$.

7: Yield 86%; MP 220°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 1.07 (3H, d, J=6 Hz, 5'-CH_3), 2.54 (3H, s, 4'-N-CH_3), 4.59 (1H, d, J=7 Hz, 1'-H), 5.63 (1H, s, vinyl proton), 5.95 (1H, s, vinyl proton); FAB-MS m/z 925 $(M+H)^+$.

8: Yield 45%; MP 230°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 1.18 (3H, d, J=6 Hz, 5'-CH_3), 4.68 (1H, d, J=7 Hz, 1'-H); FAB-MS m/z 885 $(M+H)^+$.

9: Yield 6%; MP 220°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 1.18 (3H, d, J=6 Hz, 5'-CH_3), 3.65 (2H, s, CH_2CO_2H), 3.70 (2H, s, CH_2CO_2H), 4.61 (1H, d, J=7 Hz, 1'-H); FAB-MS m/z 943 $(M+H)^+$.

10: Yield 36%; MP 230°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, d, J=6 Hz, 5'-CH_3), 4.49 (1H, d, J=7 Hz, 1'-H), 5.90 (1H, d, J=16 Hz, vinyl proton), 6.82 (1H, dt, J=16 and 5 Hz, vinyl proton); FAB-MS m/z 911 $(M+H)^+$.

4'-*N*-Acyl Derivatives of Pradimicin (**11**~**21**)

4'-*N*-Formylpradimicin A (**11**) (General Procedure for **11**~**16** and **20**~**21**)

To a mixture of PRM A·HCl (100 mg, 0.11 mmol) and BSA (0.50 ml, 2 mmol) in dichloromethane (2 ml) was added acetic formic anhydride (0.1 ml) and the mixture was stirred at room temperature overnight. To the reaction mixture were added 1 N HCl (1 ml) and MeOH (3 ml) and the organic solvents were removed under reduced pressure. The residue was chromatographed on a reversed phase silica gel column (20 i.d. × 150 mm), which was eluted with water and then 10%~25% acetonitrile in water. The eluate was monitored by HPLC and desired fractions were combined, concentrated and freeze-dried to obtain 78 mg (82%) of **11** as an amorphous powder. MP 250°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 0.94 (0.6H, d, J=6 Hz, 5'-CH_3), 1.04 (2.4H, d, J=6 Hz, 5'-CH_3), 2.97 (3H, s, 4'-N-CH_3), 4.74 (1H, d, J=7 Hz, 1'-H), 7.90 (0.8H, s, CHO), 7.95 (0.2H, s, CHO); FAB-MS m/z 869 $(M+H)^+$.

The *N*-formyl derivative (**11**, **20** and **21**) were prepared by formylation of PRMs A, C and FA-1 by a similar procedure to the above. The derivatives (**12**, **13**, **15** and **16**) were prepared by acylation of PRM A with acetic anhydride, benzoyl chloride, succinic anhydride and *N*,*N*-dimethylglycyl chloride, respectively. Compound **14** was prepared by acylating PRM A with 2,2,2-tri(acetoxymethyl)acetyl chloride and

subsequent hydrolysis in 1 N NaOH - MeOH (1 : 1). The yield, MP and ^{1}H NMR spectral data of these compounds were as follows.

12: Yield 74%; MP 230°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 0.97 (1H, d, J=6 Hz, 5'-CH_3), 1.06 (2H, d, J=6 Hz, 5'-CH_3), 2.03 (3H, s, $COCH_3$), 3.00 (3H, s, 4'-N-CH_3), 4.66 (0.3H, d, J=7 Hz, 1'-H), 4.70 (0.7H, d J=7 Hz, 1'-H); FAB-MS m/z 883 $(M+H)^+$.

13: Yield 93%; MP 230°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 1.04 (1.5H, d, J=6 Hz, 5'-CH_3), 1.11 (1.5H, d, J=6 Hz, 5'-CH_3), 4.71 (0.5H, d, J=7 Hz, 1'-H), 7.33~7.43 (5H, m, phenyl protons); FAB-MS m/z 947 $(M+3H)^+$.

14: Yield 20%; MP 200°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 1.01 (0.9H, d, J=6 Hz, 5'-CH_3), 1.10 (2.1H, d, J=6 Hz, 5'-CH_3), 2.54 (3H, s, 4'-N-CH_3), 3.32 (6H, s, CH_2OH), 4.66 (0.7H, d, J=7 Hz, 1'-H), 4.71 (0.3H, d, J=7 Hz, 1'-H); FAB-MS m/z 973 $(M+H)^+$.

15: Yield 80%; MP 220°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 0.95 (1H, d, J=6 Hz, 5'-CH_3), 1.06 (2H, d, J=6 Hz, 5'-CH_3), 4.67 (0.3H, d, J=7 Hz, 1'-H), 4.71 (0.7H, d, J=7 Hz, 1'-H); FAB-MS m/z 941 $(M+H)^+$.

16: Yield 65%; MP 19°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 1.00 (1H, d, J=6 Hz, 5'-CH_3), 1.11 (2H, d, J=6 Hz, 5'-CH_3), 2.67 (3H, s, 4'-N-CH_3), 2.67 (3H, s, glycine-N-CH_3), 2.72 (3H, s, glycine-N-CH_3), 4.70 (0.3H, d, J=7 Hz, 1'-H), 4.74 (0.7H, d, J=7 Hz, 1'-H); FAB-MS m/z 926 $(M+H)^+$.

20: Yield 55%; MP 230°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 0.98 (2H, d, J=6 Hz, 5'-CH_3), 1.04 (1H, d, J=6 Hz, 5'-CH_3), 4.67 (1H, d, J=7 Hz, 1'-H), 8.13 (1H, s, CHO); FAB-MS m/z 855 $(M+H)^+$.

21: Yield 28%; MP 200°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 0.94 (0.5H, d, J=6 Hz, 5'-CH_3), 1.05 (2.5H, d, J=6 Hz, 5'-CH_3), 2.97 (3H, s, 4'-N-CH_3), 4.68 (0.2H, d, J=7 Hz, 1'-H), 4.74 (0.8H, d, J=7 Hz, 1'-H); FAB-MS m/z 885 $(M+H)^+$.

4'-N-Glycylpradimicin A (**17**) (General Procedure for **17**~**19**)

To a solution of PRM A · HCl (105 mg, 0.12 mmol) and BSA (0.6 ml, 2.4 mmol) in dry dichloromethane (3 ml) was added N-Boc-glycine 1-hydroxybenzotriazole ester (350 mg, 1.2 mmol). The mixture was heated under reflux overnight and then cooled to room temperature. To the reaction mixture was added a 1 M solution of tetrabutylammonium fluoride in THF (2.5 ml) and the mixture was stirred for 30 minutes at room temperature. After concentration, the residue was diluted with H_2O and acidified with 1 N HCl to pH 5. The resulting solid was collected by filtration and the filtrate extracted with EtOAc. The organic layer was concentrated and combined with the solid and the mixture was chromatographed on a reversed phase silica gel column eluting with 40% acetonitrile - pH 3.5 phosphate buffer. The fractions containing the desired product were evaporated under reduced pressure. The residue was dissolved in EtOAc, washed with water and evaporated to give 52 mg of 4'-N-(N-Boc-glycyl)pradimicin A. A solution of the N-Boc intermediate in TFA (1 ml) was stirred for 30 minutes at room temperature. After TFA was evaporated, isopropyl ether was added to the residue and the resulting crude product was collected by filtration. The solid was purified by preparative HPLC (column, Nihon Seimitsu NS-20250: solvent, 30% acetonitrile in pH 3.5 phosphate buffer), followed by chromatography on a reversed phase silica gel column using 25~30% acetonitrile to give 23 mg (21%) of **17**. MP 220°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 1.00 (0.9H, d, J=6 Hz, 5'-CH_3), 1.09 (2.1H, d, J=6 Hz, 5'-CH_3), 4.70 (0.3H, d, J=7 Hz, 1'-H), 4.75 (0.7H, d, J=7 Hz, 1'-H); FAB-MS m/z 900 $(M+3H)^+$.

Compounds **18** and **19** were synthesized by coupling PRM A with N-Cbz-β-alanine and N-Cbz-S-alanine, respectively, by a procedure as above, followed by hydrogenolysis of the N-Cbz-products in dioxane - acetic acid in the presence of 10% palladium on charcoal. The yield, MP and ^{1}H NMR spectral data of these compounds were as follows.

18: Yield 22%; MP 230°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 0.99 (0.9H, d, J=6 Hz, 5'-CH_3), 1.07 (2.1H, d, J=6 Hz, 5'-CH_3), 4.68 (0.3H, d, J=7 Hz, 1'-H), 4.73 (0.7H, d, J=7 Hz, 1'-H); FAB-MS m/z 912 $(M+H)^+$.

19: Yield 38%; MP 190°C (gradual dec.); ^{1}H NMR (DMSO-d_6) δ 0.97 (1.5H, d, J=6 Hz, 5'-CH_3), 1.10 (1.5H, d, J=6 Hz, 5'-CH_3), 1.15 (1.5H, d, J=7 Hz, alanyl methyl), 1.24 (1.5H, d, J=7 Hz,

alanyl methyl), 4.67 (0.5H, d, $J=17$ Hz, 1'-H), 4.74 (0.5H, d, $J=7$ Hz, 1'-H); FAB-MS m/z 912 $(M+H)^+$.

4'-Deamino-4'-oxo and 4'-Hydroxy Derivatives of Pradimicins (**22**~**24**)

4'-Deamino-4'-oxo Pradimicin (**22**)

Triethylamine (0.20 ml, 1.43 mmol) was added to a mixture of PRM FA-2 (150 mg, 0.16 mmol), 3,5-di-*tert*-butyl-1,2-benzoquinone (150 mg, 0.68 mmol) in dry MeOH (2.5 ml) and the mixture was stirred overnight and concentrated under reduced pressure. To the residue were added EtOAc (5 ml) and aq saturated $NaHCO_3$ (2 ml) and the mixture was stirred for 30 minutes at room temperature. The precipitated SCHIFF's base (164 mg) was collected by filtration, washed with a small amount of EtOAc and dried. A mixture of the SCHIFF's base (160 mg), formic acid (3 ml) and MeOH (3 ml) was heated at 60°C for 1.5 hours. The mixture was concentrated *in vacuo* and the residue was chromatographed on a column of reversed phase silica gel (20 i.d. × 200 mm). The column was eluted with water, and then with 30% acetonitrile. Fractions were monitored by HPLC and the appropriate fractions were combined, concentrated *in vacuo* and freeze-dried, to give 147 mg (overall yield 83%) of **22**. ^{1}H NMR (DMSO-d_6) δ 3.94 (3H, s, 11-OCH_3), 6.88 (1H, s, 10-H), 7.25 (1H, s, 12-H), 7.95 (1H, s, 7-H); FAB-MS m/z 842 $(M+H)^+$.

4'-Hydroxy Derivatives (**23** and **24**)

To a stirred mixture of **22** (121 mg, 0.15 mmol) and 1 N-NaOH (0.3 ml) in water (12 ml) was added an aqueous solution of 0.1 M $NaBH_4$ (0.7 ml) at 5°C. The mixture was stirred for 1 hour at the same temperature, acidified by addition of 1 N H_2SO_4 to quench the reaction and then adjusted to pH 8 by $NaHCO_3$. HPLC analysis indicated that the reaction product contained a 1 : 1 mixture of two components. They were separated by preparative HPLC (System 500, Waters, solvent 7% aqueous acetonitrile). The appropriate fractions were acidified by addition of 1 N-H_2SO_4 and subjected to a short column of reversed phase silica gel, eluting sequentially with water and 80% acetonitrile. Concentration of these eluates, followed by freeze-drying afforded 3.0 mg (2.5%) of **23** and 5.4 mg (4.4%) of **24**.

23: MP >220°C (gradual dec.); ^{1}H NMR (DMSO-d_6 + D_2O) δ 1.10 (3H, d, $J=6.6$ Hz, 5'-CH_3), 3.53 (1H, dd, $J=9.5$ and 2.9, 3'-H), 3.59 (1H, br-q, $J=6.6$ and <1.0 Hz, 5'-H), 3.60 (1H, dd, $J=2.9$ and <1.0 Hz, 4'-H), 3.72 (1H, dd, $J=7.7$ and 9.5 Hz, 2'-H), 4.63 (1H, d, $J=7.7$ Hz, 1'-H); FAB-MS m/z 844 $(M+H)^+$.

24: MP >200°C (dec.); ^{1}H NMR (DMSO-d_6 + D_2O) δ 1.15 (3H, d, $J=6.0$ Hz, 5'-CH_3), 3.07 (1H, dd, $J=8.6$ and 9.0 Hz, 3'-H), 3.13 (1H, dq, $J=8.6$ and 6.0 Hz, 5'-H), 3.26 (1H, dd, $J=8.6$ and 8.6 Hz, 4'-H), 3.48 (1H, dd, $J=7.7$ and 9.0 Hz, 2'-H), 4.70 (1H, d, $J=7.7$ Hz, 1'-H); FAB-MS m/z 844 $(M+H)^+$.

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