

SYNTHESIS AND ANTIFUNGAL ACTIVITY OF PRADIMICIN DERIVATIVES

MODIFICATIONS ON THE AGLYCONE PART

S. ABURAKI, S. OKUYAMA, H. HOSHI, H. KAMACHI,
M. NISHIO, T. HASEGAWA, S. MASUYOSHI, S. IIMURA,
M. KONISHI and T. OKI

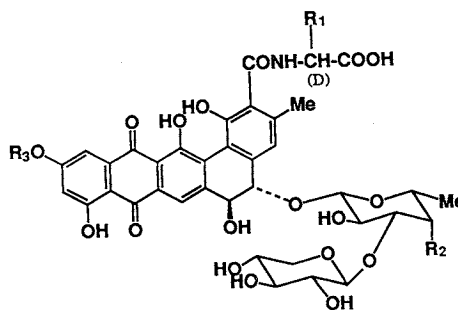
Bristol-Myers Squibb Research Institute, Bristol-Myers Squibb K. K.,
2-9-3 Shimo-meguro, Meguro-ku, Tokyo 153, Japan

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Synthesis and antifungal activity of pradimicin analogs modified on the aglycone part is described. Upon modification studies at various sites of the aglycone part using pradimicin A (PRM A), C-11 position was found to be the sole site to be modified without losing antifungal activity. Further modification studies at C-11 position were carried out with 11-OH derivative of pradimicin T1 (PRM T1) because of its easy availability. Among the compounds prepared, 11-demethoxy derivative of PRM A (**12**) and 11-*O*-ethyl (**13**) and 11-*O*-fluoroethyl (**14**) derivatives of PRM T1 showed promising antifungal activity comparable to that of PRM A.

Pradimicin A (PRM A)¹⁾ and benanomycin A²⁾ are a new family of antifungal antibiotics exhibiting broad spectrum activity against a variety of fungi and yeasts. Both antibiotics have the common aglycone of [(5*S*,6*S*)-5,6,8,13-tetrahydro-1,5,6,9,14-pentahydroxy-11-methoxy-3-methyl-8,13-dioxobenzo- $[a]$ naphthacene-2-yl]-carbonyl nucleus coupled with a D-alanine and a disaccharide. Chemical modifications of PRM A have been extensively carried out to produce a new compound possessing more potent activity and/or to improve its water solubility, specifically focusing on the D-alanine and the sugar part of the molecule.^{3,4)} These studies indicated that both parts of amino acid and sugar moiety played an important role for the antifungal activity. Meanwhile, there has recently been reported a new member of pradimicins, pradimicin T1 (PRM T1) having 11-*O*-L-xylosyl group from fermentation and preparation of the 11-hydroxy derivative by selective 11-*O*-detylosylation of PRM T1.⁵⁾ These compounds are the first examples of the pradimicin and benanomycin family antibiotics possessing a substituent other than OMe group at C-11 position. These compounds were reported to be as active as PRM A. This encouraged us to modify the aglycone part of PRMs, specifically at C-11 position to obtain a compound more potent than PRM A. Herein, we describe the synthesis and *in vitro* antifungal activity of pradimicin derivatives modified on the aglycone part of PRM A and T1.

Fig. 1. Chemical structures of pradimicins.



| | R ₁ | R ₂ | R ₃ |
|------------------------|----------------|----------------|----------------|
| Pradimicin A (PRM A) | Me | NHMe | Me |
| Benanomycin A | Me | OH | Me |
| Pradimicin T1 (PRM T1) | H | OH | β -L-Xyl |
| 11-OH PRM T1 | H | OH | H |

Chemistry

Modifications on the Aglycone Part of PRM A

Various kinds of reactions were performed to introduce a functional group on the aglycone part of PRM A and the results are summarized in Scheme 1.

Phenolic-*O*-methylations of 4'-NBoc derivative of PRM A (**B**) with MeI-K₂CO₃ in DMSO or Me₂SO₄-NaOH in dioxane in the presence of phase transfer catalyst, followed by deprotections and purification by C₁₈ reversed phase column chromatography afforded 9-OMe (**1**) and 1-OMe (**2**) derivatives of PRM A in 4.7 and 1.7% yield, respectively. The structure of compound **1** was definitely determined by the strong NOE observed between 9-OMe and 10-H. In compound **2**, the unusually high field resonance of 1-OMe protons (δ 3.55, presumably due to the anisotropic effect of non-coplanar aromatic ring system) and lower field shift of 4-H (δ 7.34, the para position of C-1) in ¹H NMR, along with the characteristic shift in UV absorption (λ_{\max} 523 nm) were observed. These data strongly indicated the methylation site of compound **2** to be the 1-OH position of PRM A. Similar spectroscopic data were reported in the 1-*O*-glucoside derivative of PRM L.⁴⁾

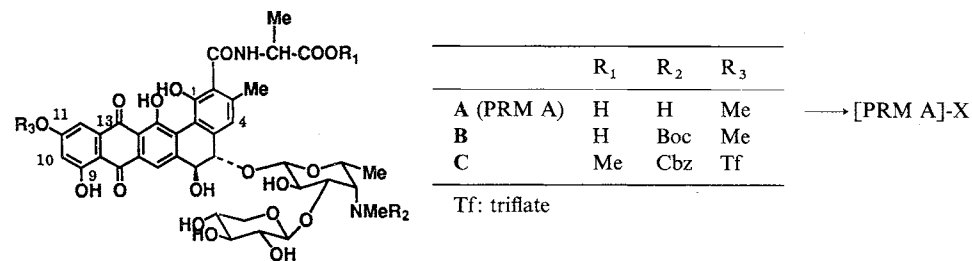
Halogenation of PRM A with pyridinium bromide perbromide (Py-HBr₃) or I₂-NaI-NaOH gave different two types of substitution products, 4-bromo (**3**) and 10-iodo (**4**) derivatives of PRM A, respectively, whose structures were determined by comparative NMR analysis of the aromatic protons as shown in Scheme 1. These results suggested that substitution reactions such as halogenation occurred at 4-position under acidic conditions and at 10-position under alkaline conditions. This observation was supported by the fact of introduction of nitro group at 4-position (compound **6**) under acidic condition and at 10-position (compound **8**) by basic Mannich reaction described below. 10-Iodo derivative **4** was further converted to the corresponding 10-carboxy derivative **5** by lithiation with *tert*-BuLi, followed by treatment with carbon dioxide.

Treatment of PRM A with NOBF₄⁶⁾ in MeCN afforded 4-nitro derivative **6** in an excellent yield. Although the hydrogenolysis of **6** on Pd-C under hydrogen atmosphere did not proceed at all, reduction with NaSH in MeOH was found to smoothly afford the desired 4-amino derivative **7** in 58% yield. All C-4 substituted derivatives (**3**, **6** and **7**) were found to have a different conformation of 5,6-dihydro-ring from that of PRM A, by judging from coupling constants ($J_{5,6} = ca. 3$ Hz for **3**, **6** and **7** and $J = ca. 10$ Hz for PRM A). This was also supported by the alteration of CD spectra between **3** [λ_{extreme} (MeOH) nm ($\Delta\epsilon$) 210 (-26.5), 232 (+53.0)] and PRM A [λ_{extreme} (MeOH) nm ($\Delta\epsilon$) 206 (+22.7), 226 (-30.0), 239 (-24.0)]. Such a change of the ring conformation was presumably caused by the repulsion between substituent at C-4 and bulky C-5-*O*-sugar group. A similar observation has been reported in the case of 7-hydroxy derivative of PRM A.⁷⁾

The Mannich reaction of PRM A with Me₂NH-HCHO at 90°C overnight unexpectedly afforded 4'-NMe₂-10-CH₂N⁺Me₃ derivative **8** in 54% yield, which might be formed *via* the normal 10-CH₂NMe₂ Mannich type derivative by over *N*-methylation with HCHO at an elevated temperature. The structure of **8** was also confirmed by the formation of the corresponding 10-Me derivative **9** by the hydrogenolysis of 10-trimethyl ammonium group with H₂-Pd-C.

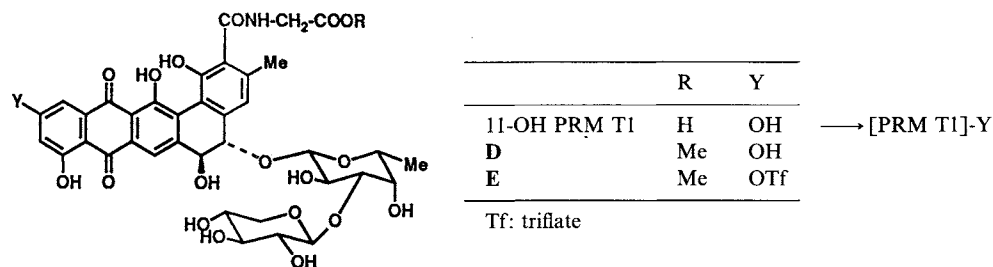
Reduction of quinone group of PRM A with zinc-powder in AcOH gave 13-deoxo derivative **10**, whose structure was confirmed by NOE observed between 12-H and newly appeared 13-methylene protons (δ 4.18) in ¹H NMR.

Scheme 1. Modifications on the aglycone part of pradimicin A.



| Compound | Structure (X) | Starting material | Reaction conditions | Yield (%) | FAB-MS <i>m/z</i> (M+H) | UV λ _{max} nm (ε) | ¹ H NMR (δ (ppm) in DMSO- <i>d</i> ₆) | | | | | | | |
|----------|--|-------------------|--|-----------|-------------------------|----------------------------|--|------------|------------------------------|------|------|-----------|-----------|--------------------------------|
| | | | | | | | 5-H | 6-H | <i>J</i> _{5,6} (Hz) | 4-H | 7-H | 10-H (d) | 12-H (d) | Others |
| 1 | 9-OMe | B | MeI - K ₂ CO ₃ | 4.4 | 855 | 492 (8,600) | 4.35 | 4.41 | 12.0 | 6.92 | 7.49 | 6.84 | 7.28 | 3.89 (s, 9-OMe) |
| 2 | 1-OMe | B | Me ₂ SO ₄ - NaOH - Bu ₄ NHSO ₄ | 1.7 | 855 | 523 (10,100) | 4.48 | 4.56 | 10.0 | 7.34 | 8.01 | 6.92 | 7.28 | 3.55 (s, 1-OMe) |
| 3 | 4-Br | A | Py-HBr ₃ | 69 | 921 (919) | 493 (15,400) | 5.23 | 4.68 | 3.0 | — | 7.34 | 6.71 | 7.12 | |
| 4 | 10-I | A | I ₂ - NaI - NaOH | 60 | 967 | 511 (15,000) | | 4.60 (brs) | | 6.84 | 7.68 | — | 7.23 (s) | |
| 5 | 10-COOH | 4 | <i>tert</i> -BuLi/CO ₂ | 7.3 | 885 | 504 (13,300) | | 4.60 (brs) | | 7.15 | 8.07 | — | 7.49 (s) | |
| 6 | 4-NO ₂ | A | NOBF ₄ | 79 | 886 | 495 (7,900) | 4.67 | 4.70 | 3.6 | — | 7.40 | 6.74 | 7.12 | |
| 7 | 4-NH ₂ | 6 | NaSH | 58 | 856 | 496 (9,900) | 4.85 | 4.92 | 3.0 | — | 7.80 | 6.96 | 7.32 | |
| 8 | 10-CH ₂ NMe ₃ (4'-NMe ₂) | A | Me ₂ NH - HCHO | 54 | 926 | 506 (11,200) | | 4.53 (brs) | | 7.06 | 8.10 | — | 7.53 (s) | 3.00 (s, 4'-NMe ₂) |
| 9 | 10-Me (4'-NMe ₂) | 8 | H ₂ - Pd-C | 9.0 | 869 | 502 (14,200) | 4.41 | 4.46 | 11.5 | 6.90 | 7.82 | — | 7.30 (s) | 2.10 (s, 10-Me) |
| 10 | 13-Deoxo | A | Zn-powder - AcOH | 35 | 827 | 407 (10,000) | 4.47 | 4.54 | 8.6 | 6.75 | 7.70 | 6.40 | 6.71 | 4.18 (s, 13-CH ₂) |
| 11 | 11-OH | A | LiI - Collidine | 9.4 | 827 | 502 (15,500) | | 4.53 (brs) | | 6.95 | 7.79 | 6.55 | 7.14 | |
| 12 | 11-H | C | HCOOH - NEt ₃ /Pd(0) | 25 | 811 | 500 (11,800) | | 4.49 (brs) | | 6.85 | 7.71 | 7.21 (dd) | 7.62 (dd) | 7.73 (t, <i>J</i> =7.7, 11-H) |

Scheme 2. Modification at C-11 position of pradimicin T1.



| Compound | Structure (Y) | Starting material | Reaction conditions | Yield (%) | FAB-MS <i>m/z</i> | UV λ_{\max} nm (ϵ) | $^1\text{H NMR}$ (δ (ppm) in DMSO- d_6) | | | | |
|----------|-------------------------------------|-------------------|---------------------------------------|-----------|-------------------|---------------------------------------|--|------|------|------|---|
| | | | | | | | 5-H | 6-H | 10-H | 12-H | Others |
| 13 | OEt | D | EtBr | 52 | 827 (M) | 497 (14,500) | 4.42 | 4.49 | 6.75 | 7.16 | 1.38 (t, $J=7.3$ Hz, CH_2CH_3), 4.22 (q, 11-OCH ₂) |
| 14 | OCH ₂ CH ₂ F | D | FCH ₂ CH ₂ Br | 39 | 845 (M) | 497 (14,700) | 4.40 | 4.45 | 6.76 | 7.16 | 4.79 (dt, $J=47.4, 3.4$ Hz, -CH ₂ F) |
| 15 | OCH ₂ CH=CH ₂ | D | CH ₂ =CHCH ₂ Br | 28 | 839 (M) | 497 (15,200) | 4.45 | 4.54 | 6.83 | 7.24 | 4.79 (d, $J=5.1$ Hz, 11-OCH ₂), 5.33, 5.45 and 6.08 ($J=16.1, 10.7$ and 1.7 Hz, vinyl-H) |
| 16 | OCH ₂ COOH | D | BrCH ₂ COOMe | 66 | 858 (M+H) | 497 (14,800) | 4.44 | 4.51 | 6.99 | 7.19 | 4.93 (s, 11-OCH ₂) |
| 17 | OCH ₂ CONH ₂ | D | BrCH ₂ CONH ₂ | 43 | 856 (M) | 496 (15,100) | 4.46 | 4.55 | 6.87 | 7.25 | 4.96 (s, 11-OCH ₂) |
| 18 | H | E | HCOOH - NEt ₃ /Pd(0) | 14 | 784 (M+H) | 499 (13,000) | 4.40 | 4.45 | 7.21 | 7.61 | 7.71 (t, $J=7.7$ Hz, 11-H) |
| 19 | NMe ₂ | E | Me ₂ NH | 34 | 827 (M+H) | 522 (12,600) | 4.38 | 4.45 | 6.27 | 7.02 | 3.11 (s, NMe ₂) |
| 20 | CN | E | Bu ₃ SnCN/Pd(0) | 5.8 | 809 (M+H) | 506 (13,200) | 4.41 | 4.47 | 7.72 | 7.87 | |
| 21 | CONH ₂ | E | Bu ₃ SnCN/Pd(0) | 9.5 | 826 (M) | 508 (11,800) | 4.40 | 4.46 | 7.62 | 8.09 | 7.20 (br s, CONH ₂) |

Finally, de-*O*-methylation at C-11 position of PRM A was successfully performed using LiI-collidine at reflux temperature to afford the 11-OH derivative of PRM A (**11**)[†] in 9.4% yield. When an other aryloether cleavage reagent⁸⁾ such as TMS-I, BBr₃, BF₃-Et₂O, PhSNa or EtSNa was used, the reaction did not proceed at all or resulted in decompositions. 11-Hydroxy derivative **11** was deoxygenated by reduction of the corresponding 11-*O*-triflate intermediate **C** (Scheme 1) with HCOOH-NEt₃ in the presence of Pd(0) catalyst to afford the desired 11-deoxy derivative of PRM A (**12**) in 25% yield.

Modification at C-11 Position of PRM T1

The *in vitro* antifungal activity of a series of the aglycone modified derivatives of PRM A (details see below) indicated that the C-11 position was the only site to be modified without losing activity. Thus we carried out further modifications at C-11 position starting from 11-hydroxy pradimicin T1, because of its easy availability from PRM T1 by 11-*O*-detylosylation. The modification results are summarized in Scheme 2.

11-Hydroxy PRM T1 methyl ester (**D**) was treated with an appropriate (substituted)alkyl halide in DMSO in the presence of K₂CO₃ to afford 11-*O*-(substituted)alkyl derivatives of PRM T1 (**13**~**17**) in 28~66% yield, after deblocking and purification by C₁₈ column chromatography. The alkylation site of the products was confirmed to be at 11-OH position by NOE observed between 11-*O*-methylene protons and 10-/12-protons.

11-Deoxy derivative of PRM T1 (**18**) was also prepared by the same procedure as described in the preparation of **12** *via* the corresponding 11-*O*-triflate derivative (**E**). Treatments of compound **E** with Me₂NH in DMF gave 11-NMe₂ derivative (**19**) and with Bu₃SnCN in the presence of Pd(0) catalyst gave 11-CN (**20**) and 11-CONH₂ (**21**) derivatives after deblocking with 1 N NaOH.

Antifungal Activity

The *in vitro* antifungal activity of the aglycone modified derivatives of pradimicins was determined by the agar dilution method on yeast morphology agar containing 1/15 M phosphate buffer (pH 7.0) and the results are summarized in Table 1.

Among the derivatives modified on the aglycone part of PRM A, most of the compounds (**1**, **3**~**10**) obtained by the modifications at C-4, 9, 10 and 13 positions, were found to be completely inactive against all fungi tested. It has been noted that compounds (**3**, **6** and **7** and 7-hydroxy PRM A⁷⁾), having different conformation from that of PRM A showed no antifungal activity. Although the 1-OMe derivative (**2**) showed apparently decreased activity, 11-*O*-demethyl (**11**) and 11-demethoxy (**12**) derivatives showed

Table 1. *In vitro* antifungal activity: MIC (μg/ml).

| | Compound | | | | | | | | | | | | PRM A | 11-OH PRM T1 |
|-------|----------|-----|------|------|-----|------|------|------|------|------|------|------|-------|-----------------|
| | 2 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | | |
| Ca-4 | 25 | 6.3 | 6.3 | 3.1 | 3.1 | 6.3 | 50 | 50 | 6.3 | 12.5 | 12.5 | 12.5 | 6.3 | 6.3 |
| Ct-12 | >100 | 25 | 12.5 | 12.5 | 6.3 | 12.5 | 100 | 100 | 12.5 | >100 | 25 | 25 | 12.5 | 12.5 |
| Cn-2 | 12.5 | 3.1 | 1.6 | 1.6 | 1.6 | 3.1 | 100 | >100 | 6.3 | 50 | 6.3 | 6.3 | 1.6 | 12.5 |
| Af-3 | 50 | 6.3 | 6.3 | 6.3 | 6.3 | 25 | >100 | >100 | 50 | >100 | 50 | 25 | 3.1 | 100 |

Ca-4: *Candida albicans* A9540, Ct-12: *Candida tropicalis* IFO 10241.

Cn-2: *Cryptococcus neoformans* IAM 4514, Af-3: *Aspergillus fumigatus* IAM 2034.

Compounds **1** and **3**~**10** showed no growth inhibition over 100 μg/ml against all strains tested.

[†] The same compound **11** was recently isolated by fermentation in our laboratory and designated pradimicin H.

significant activity comparable to that of PRM A. This indicated that the substituents on the aglycone of PRM A are strictly required to be as they are to reveal antifungal activity except for C-11 position.

In a series of the C-11 modified derivatives of PRM T1, 11-OEt (**13**) and 11-OCH₂CH₂F (**14**) derivatives were found to be more active than the corresponding 11-OH derivative and are as active as PRM A. Although the 11-OCH₂COOH (**16**) and 11-OCH₂CONH₂ (**17**) derivatives of PRM T1 were nearly inactive, the C-11 directly substituted 11-CN (**20**) and 11-CONH₂ (**21**) derivatives showed fairly good activity. 11-Deoxy derivative of PRM T1 (**18**) also showed excellent level of activity as in the case of PRM A, except against *Aspergillus fumigatus*.

Experimental

MPs were determined on a Yanagimoto micro hot-stage apparatus and are uncorrected. IR spectra (KBr) were measured on a JASCO IR Report-100 spectrometer and UV spectra (in 0.01 N NaOH) on a Shimadzu UV-260 spectrometer. ¹H NMR spectra (in DMSO-*d*₆) were recorded on a JEOL GX-400 (400 MHz) and mass spectra on a JEOL JMS-AX505H mass spectrometer. CD spectra were measured in MeOH on a JASCO J-600 spectrometer.

4'-N-Boc-PRM A (B)

Title compound **B** (896 mg, 70% yield) was prepared from PRM A (1.20 g, 1.36 mmol) by the analogous method preparing the corresponding 4'-N-Cbz derivative⁴⁾: MP 195~205°C; IR ν_{\max} cm⁻¹ 1720, 1660, 1620, 1600; UV λ_{\max} nm (ϵ) 499 (11,600); ¹H NMR δ (ppm) 1.02 (3H, d, *J*=6.4 Hz, 5'-Me), 1.35 (3H, d, *J*=7.3 Hz, 17-Me), 1.38 (9H, s, *tert*-Bu), 3.94 (3H, s, OMe), 6.85 (1H, brs, 10-H), 6.96 (1H, s, 4-H), 7.23 (1H, brs, 12-H), 7.93 (1H, s, 7-H); FAB(+)-MS *m/z* 941 (M+H).

9-O-Methyl-PRM A (1)

To a solution of compound **B** (300 mg, 0.32 mmol) in dry DMSO (10 ml) were added K₂CO₃ (440 mg, 3.2 mmol) and MeI (0.40 ml, 6.4 mmol) and the mixture was stirred at room temperature for 5 days. After being poured into water (100 ml), the mixture was extracted with EtOAc (3 × 100 ml). The combined extracts were washed with brine, dried over Na₂SO₄ and evaporated. The residue was chromatographed on a silica gel (Wako-gel C-200, 150 g) by eluting with CH₂Cl₂-MeOH = 20 : 1 ~ 3 : 1 to give a fraction (72 mg), showing R_f = 0.22 on TLC (Kieselgel 60 F₂₅₄, CH₂Cl₂-MeOH = 20 : 1). The compound obtained above in MeOH (5 ml) was treated with 1 N NaOH (0.1 ml) at room temperature for 30 minutes, neutralized with 1 N HCl to pH 5.0 and evaporated to dryness. The residue was then treated with trifluoroacetic acid (2 ml) at room temperature for 5 minutes and evaporated *in vacuo* below 30°C. The residue was purified on a C₁₈ column (Prep C₁₈, 55 ~ 105 μ m, Waters, 50 ml), which was washed with water and eluted with 25% acetonitrile (MeCN)-phosphate buffer (pH 3.5). The fractions showing R_t = 3.7 minutes on HPLC (SSC-ODS-262, 6 × 100 mm, 27.5% MeCN-phosphate buffer (pH 3.5)) were collected, desalted on a C₁₈ column by washing with water and eluting with 50% aq MeCN, evaporated and lyophilized to afford the title compound **1** as red powder (12 mg, 4.4% yield from **B**): MP 210°C; IR ν_{\max} cm⁻¹ 1640, 1590; ¹H NMR (see also Scheme 1) δ 1.13 (3H, d, *J*=6.4 Hz, 5'-Me), 1.28 (3H, d, *J*=7.3 Hz, 17-Me), 2.20 (3H, s, 3-Me), 2.37 (3H, s, 4'-NMe), 3.70 (1H, dd, *J*=5.1 and 11.5 Hz, 5'-Heq), 3.93 (3H, s, 11-OMe), 4.36 (1H, d, *J*=7.3 Hz, 1''-H), 4.58 (1H, d, *J*=6.8 Hz, 1'-H).

1-O-Methyl-PRM A (2)

To a solution of **B** (370 mg, 0.39 mmol) in dry dioxane (37 ml) were sequentially added Bu₄NHSO₄ (132 mg, 0.39 mmol), powdered NaOH (109 mg, 2.71 mmol) and Me₂SO₄ (0.13 ml, 1.36 mmol) and the mixture was stirred at room temperature overnight and the insoluble matters were removed by filtration and washed with dioxane. The combined filtrate and washings were evaporated and the residual syrup was chromatographed on a silica gel (50 g) by eluting with CH₂Cl₂-MeOH = 20 : 1. The fractions containing the product showing R_f = 0.73 (TLC, same as above) were evaporated and the residue (159 mg) was

deblocked and purified by a similar procedure as described above to afford the title compound **2** (5.5 mg, 1.7% from **B**) as pale orange powder: MP 230°C; IR ν_{\max} cm^{-1} 1680, 1600; $^1\text{H NMR}$ δ 1.16 (3H, d, $J=6.4$ Hz, 5'-Me), 1.33 (3H, d, $J=6.8$ Hz, 17-Me), 2.30 (3H, s, 3-Me), 2.43 (3H, s, 4'-NMe), 3.70 (1H, dd, $J=5.1$ and 11.1 Hz, 5''-Heq), 3.95 (3H, s, 11-OMe), 4.27 (1H, q, 17-H), 4.36 (1H, d, $J=7.3$ Hz, 1''-H), 4.62 (1H, br d, 1'-H).

4-Bromo-PRM A (**3**)

To a solution of PRM A (86 mg, 0.1 mmol) in dry pyridine (2 ml) was added pyridinium bromide perbromide (32 mg, 0.1 mmol) and the mixture was stirred at room temperature for 30 minutes. To the mixture was added 1 N HCl and the mixture was charged on a C_{18} column (80 ml), which was washed with water and eluted with 20% MeCN - buffer (pH 3.5). The desired fractions were collected, concentrated, desalted and lyophilized to give the title compound **3** (63 mg, 69%): MP 200°C; IR ν_{\max} cm^{-1} 1620, 1600; $^1\text{H NMR}$ δ 1.16 (3H, d, $J=6.4$ Hz, 5'-Me), 1.35 (3H, d, $J=7.3$ Hz, 17-Me), 2.29 (3H, s, 3-Me), 2.47 (3H, s, 4'-NMe), 3.06 (1H, t, $J=11.1$ Hz, 5''-Hax), 3.25 (1H, m, $J=5.1, 9.0$ and 11.1 Hz, 4''-H), 3.69 (1H, dd, $J=5.1$ and 11.1 Hz, 5''-Heq), 3.90 (3H, s, 11-OMe), 4.36 (1H, d, 17-H), 4.37 (1H, d, $J=7.3$ Hz, 1''-H), 4.69 (1H, br d, 1'-H).

10-Iodo-PRM A (**4**)

A solution of iodine (2.5 g, 12 mmol) and NaI (1.92 g, 13.2 mmol) in water (20 ml) was added dropwise to a stirred solution of PRM A (1.0 g, 1.2 mmol) in water (100 ml) under keeping the pH of the solution between 8.5~9.0 by the addition of 0.1 N NaOH (45 ml) and the mixture was stirred at room temperature for 1.5 hours. After being acidified with 1 N HCl to pH 4, the mixture was desalted by HP-20 column (Diaion, 600 ml) and the crude solid of **4** obtained (1.14 g) was purified by a C_{18} column (400 ml) with elution of 35~40% MeCN - buffer (pH 3.5). The desired fractions were combined, desalted and lyophilized to afford the title compound **4** (700 mg, 60%): MP 187~195°C; IR ν_{\max} cm^{-1} 1620, 1575; $^1\text{H NMR}$ δ 1.28 (3H, d, $J=6.8$ Hz, 5'-Me), 1.34 (3H, d, $J=7.3$ Hz, 17-Me), 2.32 (3H, s, 3-Me), 2.67 (3H, s, 4'-NMe), 3.75 (1H, dd, $J=5.1$ and 11.1 Hz, 5''-Heq), 3.90 (1H, q, 5'-H), 4.11 (3H, s, 11-OMe), 4.40 (1H, q, 17-H), 4.49 (1H, d, $J=7.3$ Hz, 1''-H), 4.79 (1H, br d, 1'-H).

10-Carboxy-PRM A (**5**)

A mixture of **4** (75 mg, 0.078 mmol) and hexamethyldisilazane (10 ml) was refluxed for 16 hours and the resulting clear solution was evaporated to dryness. To a solution of the residue in dry THF (10 ml) was added *tert*-BuLi (1.7 M solution in pentane, 0.2 ml) at -70°C and the mixture was stirred at the same temperature for 2 hours. Carbon dioxide was bubbled to the mixture for 30 minutes and then quenched with 1 N HCl. The mixture was purified by a C_{18} column (80 ml), eluting with 10~20% MeCN - buffer (pH 3.5). The desired fractions were collected, desalted, concentrated and lyophilized to afford the title compound **5** (5 mg, 7.3%): MP 200°C; $^1\text{H NMR}$ δ 1.28 (3H, d, $J=6.8$ Hz, 5'-Me), 1.34 (3H, d, $J=7.3$ Hz, 17-Me), 2.32 (3H, s, 3-Me), 2.67 (3H, s, 4'-NMe), 3.75 (1H, dd, $J=5.1$ and 11.1 Hz, 5''-Heq), 3.90 (1H, q, 5'-H), 4.05 (3H, s, 11-OMe), 4.40 (1H, q, 17-H), 4.47 (1H, d, $J=6.8$ Hz, 1''-H), 4.80 (1H, br d, 1'-H).

4-Nitro-PRM A (**6**)

To a suspension of PRM A (500 mg, 0.57 mmol) in MeCN (20 ml) was added nitrosonium tetrafluoroborate (NOBF_4 , 197 mg, 1.69 mmol) and the mixture was stirred at room temperature for 5 minutes. Insoluble solids were removed by filtration and the filtrate was added to cold 2 N NaOH (20 ml). After evaporation of the organic solvent, the aqueous residue was acidified with 3 N HCl and chromatographed on a C_{18} column, which was eluted with 15~20% aq MeCN containing 0.001 N HCl. The desired fractions were combined, concentrated and lyophilized to give 398 mg (79%) of **6**: MP 210°C; IR ν_{\max} cm^{-1} 1720, 1620; $^1\text{H NMR}$ δ 1.19 (3H, d, $J=6.8$ Hz, 5'-Me), 1.36 (3H, d, $J=7.3$ Hz, 17-Me), 2.13 (3H, s, 3-Me), 2.48 (3H, s, 4'-NMe), 3.69 (1H, dd, $J=5.1$ and 11.1 Hz, 5''-Heq), 3.91 (3H, s, 11-OMe), 4.33 and 4.34 (1H each, d, $J=7.7$ Hz, 1'- and 1''-H), 4.37 (1H, q, 17-H).

4-Amino-PRM A (**7**)

A mixture of **6** (200 mg, 0.23 mmol) and 17% aqueous solution of NaSH (4.5 ml) in MeOH (20 ml)

was heated at 80°C for 6 hours. After evaporation of the organic solvent, the aqueous residue was acidified with 3 N HCl and charged on a C₁₈ column, which was washed with water and then eluted with 10~15% aq MeCN containing 0.001 N HCl. The desired fractions were combined, evaporated and lyophilized to afford the title compound **7** (113 mg, 58%): MP 170°C; IR ν_{\max} cm⁻¹ 1720, 1620; ¹H NMR δ 1.35 (3H, d, $J=6.8$ Hz, 5'-Me), 1.37 (3H, d, $J=7.3$ Hz, 17-Me), 2.16 (3H, s, 3-Me), 2.59 (3H, s, 4'-NMe), 3.72 (1H, dd, $J=5.1$ and 11.1 Hz, 5''-Heq), 3.97 (3H, s, 11-OMe), 4.38 and 4.39 (1H each, d, $J=7.3$ Hz, 1'- and 1''-H).

4'-N,N-Dimethyl-10-trimethylammoniummethyl-PRM A (8)

A mixture of PRM A (177 mg, 0.20 mmol), dimethylamine (50% aq soln, 1.4 ml) and formaldehyde (37% aq soln, 2.8 ml) in water (1.4 ml) was heated at 90°C overnight. After concentration, the residue was dissolved in 3 N HCl and chromatographed on a C₁₈ column by eluting with 10~20% aq MeCN containing 0.001 N HCl. The desired fractions were combined, concentrated and lyophilized to give the title compound **8** (101 mg, 54%): MP 170°C; IR ν_{\max} cm⁻¹ 1720, 1620; ¹H NMR δ 1.33 (3H, d, $J=6.8$ Hz, 5'-Me), 1.38 (3H, d, $J=7.3$ Hz, 17-Me), 2.31 (3H, s, 3-Me), 3.77 (1H, dd, $J=5.1$ and 11.1 Hz, 5''-Heq), 4.12 (3H, s, 11-OMe), 4.40 (1H, q, 17-H), 4.52 (1H, br d, 1''-H), 4.83 (1H, br d, 1'-H).

4'-N,N-Dimethyl-10-methyl-PRM A (9)

Compound **8** (55 mg, 0.06 mmol) in a mixture of MeOH (0.5 ml) and 1 N HCl (0.1 ml) was subjected to hydrogenation in the presence of 5% Pd-C (10 mg) under hydrogen atmosphere at room temperature for 1 hour. The catalyst was removed by filtration and the filtrate was evaporated. The residue was chromatographed on a C₁₈ column eluting with 25% aq MeCN containing 0.001 N HCl to afford the title compound **9** (4.5 mg, 9%): MP 220°C; IR ν_{\max} cm⁻¹ 1720, 1620; ¹H NMR δ 1.21 (3H, d, $J=6.8$ Hz, 5'-Me), 1.34 (3H, d, $J=7.3$ Hz, 17-Me), 2.28 (3H, s, 3-Me), 3.72 (1H, dd, $J=5.1$ and 11.1 Hz, 5''-Heq), 4.02 (3H, s, 11-OMe), 4.37 (1H, d, $J=7.3$ Hz, 1''-H), 4.65 (1H, br d, 1'-H).

13-Deoxo-PRM A (10)

A mixture of PRM A (150 mg, 0.18 mmol) and zinc-powder (150 mg, 0.18 mmol) in acetic acid (3 ml) was heated under reflux for one hour. After cooling, water (10 ml) was added and the mixture was filtered to remove insoluble materials. The filtrate was chromatographed on a C₁₈ column (50 ml), which was washed with water and eluted with 30% aq MeCN. The fractions containing the desired product were combined, concentrated and freeze-dried to give 51 mg (35%) of the product **10**: MP 200°C (dec); IR ν_{\max} cm⁻¹ 1720, 1620, 1590; ¹H NMR δ 1.26 (3H, d, $J=6.4$ Hz, 5'-Me), 1.35 (3H, d, $J=7.3$ Hz, 17-Me), 2.35 (3H, s, 3-Me), 2.64 (3H, s, 4'-NMe), 3.74 (1H, dd, $J=5.1$ and 11.1 Hz, 5''-Heq), 3.86 (3H, s, OMe), 4.40 (1H, q, $J=7.3$ Hz, 17-H), 4.45 (1H, d, $J=7.3$ Hz, 1''-H), 4.75 (1H, d, $J=7.3$ Hz, 1'-H).

11-O-Demethyl-PRM A (11)

A mixture of pradimicin A (420 mg, 0.5 mmol) and anhydrous lithium iodide (1.34 g, 10 mmol) in collidine (50 ml) was refluxed for 2 hours with stirring, diluted with ether (200 ml) after cooling and filtered. The solid was dissolved in water (100 ml) and the solution was acidified with 1 N HCl and charged on a C₁₈ column (400 ml), which was eluted with 25~30% MeCN - buffer (pH 3.5). The desired fractions showing retention time 4.1 minutes [25% MeCN - buffer (pH 3.5)] were combined, concentrated, desalted and freeze-dried to give 39 mg (9.4%) of the title compound **11**: MP >200°C; IR ν_{\max} cm⁻¹ 1720, 1610; ¹H NMR δ 1.27 (3H, d, $J=6.8$ Hz, 5'-Me), 1.34 (3H, d, $J=7.3$ Hz, 17-Me), 2.30 (3H, s, 3-Me), 2.65 (3H, s, N-Me), 3.12 (1H, t, $J=11.1$ Hz, 5''-Hax), 3.14 (1H, dd, $J=8.1$ and 6.8 Hz, 2''-H), 3.16 (1H, dd, $J=9.0$ and 8.1 Hz, 3''-H), 3.32 (1H, m, 4''-H), 3.43 (1H, m, 4'-H), 3.75 (1H, dd, $J=11.1$ and 5.1 Hz, 5''-Heq), 3.90 (1H, q, $J=6.8$ Hz, 5'-H), 3.95 (1H, dd, $J=9.4$ and 5.1 Hz, 3'-H), 4.40 (1H, q, $J=7.3$ Hz, 17-H), 4.46 (1H, d, $J=6.8$ Hz, 1''-H), 4.79 (1H, d, $J=8.1$ Hz, 1'-H).

4'-N-Cbz-11-O-trifluoromethanesulfonyl-PRM A Methyl Ester (C)

A mixture of 4'-N-Cbz-11-hydroxy-PRM A methyl ester [17 mg, 0.018 mmol, prepared from compound **11** by the conventional method reported before⁴⁾], N-phenyltrifluoromethanesulfonimide (9.4 mg, 0.026 mmol) and N,N-dimethylaminopyridine (3.2 mg, 0.026 mmol) in dry pyridine (1.7 ml) was stirred at room temperature for one hour. The mixture was diluted with pH 3.5 phosphate buffer (20 ml) and charged

on a C_{18} column (80 ml), which was eluted with 50~60% MeCN - buffer (pH 3.5). The desired fractions were combined, concentrated, desalted and freeze-dried to give 16 mg (82.5%) of the title compound **C**: FAB(+)-MS m/z 1,107 (M+H), 1,129 (M+Na).

11-Demethoxy-PRM A (12)

To a solution of the triflate **C** obtained above (23 mg, 0.02 mmol) in dry DMF (2 ml) were sequentially added palladium acetate (9 mg, 0.04 mmol), triphenylphosphine (21 mg, 0.08 mmol), triethylamine (28 μ l, 0.2 mmol) and 98% formic acid (8 μ l, 0.21 mmol) and the mixture was stirred at room temperature for 4 hours. The mixture was diluted with pH 3.5 phosphate buffer and charged on a C_{18} column (80 ml), which was eluted with 40~50% MeCN - buffer (pH 3.5). The desired fractions were combined, concentrated, desalted and freeze-dried to give 14 mg (73%) of the 11-deoxy derivative of compound **C**: MP > 200°C; FAB(+)-MS m/z 959 (M+H), 981 (M+Na).

A mixture of the compound obtained above (14 mg, 0.015 mmol) and 10% Pd-C (10 mg) in a mixture of MeOH (3 ml), water (1 ml) and AcOH (1 ml) was hydrogenated at room temperature for 3 hours under hydrogen atmosphere. The catalyst was removed by filtration and the filtrate was charged on a C_{18} column (80 ml), which was washed with water and eluted with 75% aq dioxane. The desired fractions were concentrated, diluted with MeOH (5 ml) and treated with 1 N NaOH (1 ml). The mixture was stirred at room temperature for 30 minutes and adjusted to pH 6 with 1 N HCl and purified by a C_{18} column (80 ml), eluting with 30% MeCN - buffer (pH 3.5). The desired fractions were combined, concentrated, desalted and freeze-dried to give 4 mg (34%) of the title compound **12**: MP > 200°C; $^1\text{H NMR } \delta$ 1.25 (3H, d, $J=6.8$ Hz, 5'-Me), 1.35 (3H, d, $J=7.3$ Hz, 17-Me), 2.30 (3H, s, 3-Me), 2.60 (3H, s, 4'-Me), 3.11 (1H, t, $J=11.1$ Hz, 5''-Hax), 3.12 (1H, m, 2''-H), 3.16 (1H, t, $J=9.0$ Hz, 3''-H), 3.3 (1H, m, 4''-H), 3.49 (1H, m, 4'-H), 3.51 (1H, t, $J=8.1$ Hz, 2'-H), 3.74 (1H, dd, $J=11.1$ and 5.1 Hz, 5''-Heq), 3.85 (1H, m, 5'-H), 3.89 (1H, dd, $J=9.8$ and 4.7 Hz, 3'-H), 4.38 (1H, q, $J=7.3$ Hz, 17-H), 4.43 (1H, d, $J=7.3$ Hz, 1''-H), 4.75 (1H, d, $J=8.1$ Hz, 1'-H).

11-Hydroxy-PRM T1 Methyl Ester (D)

To a suspension of 11-*O*-dexylosylpradimicin T1⁵⁾ (300 mg, 0.375 mmol) in MeOH (30 ml) was added thionyl chloride (96 μ l, 1.32 mmol) and the mixture was stirred at room temperature overnight. After evaporation of the solvent, the residue was precipitated from MeOH - ether to give the title compound **D** (316 mg, quantitative yield): $^1\text{H NMR } \delta$ 1.10 (3H, d, $J=6.4$ Hz, 5'-Me), 2.34 (3H, s, 3-Me), 3.06 (1H, t, $J=11.5$ Hz, 5''-Hax), 3.11 (1H, t, $J=8.6$ Hz, 2''-H), 3.13 (1H, t, $J=8.6$ Hz, 3''-H), 3.28 (1H, m, 4''-H), 3.45 (1H, m, 4'-H), 3.53 (1H, m, 3'-H), 3.59 (2H, m, 2'- and 5'-H), 3.68 (3H, s, COOMe), 3.69 (1H, dd, $J=11.5$ and 5.1 Hz, 5''-Heq), 3.99 (2H, d, $J=6.4$ Hz, 17-CH₂), 4.40 (1H, d, $J=6.8$ Hz, 1''-H), 4.48 (1H, d, $J=10.2$ Hz, 5-H), 4.56 (1H, d, 6-H), 4.63 (1H, d, $J=7.3$ Hz, 1'-H), 6.65 (1H, d, $J=2.1$ Hz, 10-H), 7.15 (1H, s, 4-H), 7.22 (1H, d, 12-H), 8.01 (1H, s, 7-H); FAB(+)-MS m/z 814 (M+H).

11-*O*-Ethyl-PRM T1 (13)

Ethyl bromide (10 μ l, 0.14 mmol) was added to a mixture of compound **D** (25 mg, 0.030 mmol) and K₂CO₃ (8 mg, 0.058 mmol) in DMSO (0.3 ml) and the mixture was stirred overnight at room temperature. To the mixture was added 1 N NaOH (3 ml) and the mixture was stirred overnight at room temperature. After acidification with dil HCl, the mixture was chromatographed on a C_{18} column, which was washed with water and then eluted with 10~15% aq MeCN. The fractions containing the desired product were combined, concentrated *in vacuo* and freeze-dried to give 13 mg (52%) of the product **13**: MP 200~210°C (dec); $^1\text{H NMR}$ (see also Scheme 2) δ 1.12 (3H, d, $J=6.4$ Hz, 5'-Me), 2.31 (3H, s, 3-Me), 3.85 (2H, d, $J=6.4$ Hz, 17-CH₂), 4.40 (1H, d, $J=6.8$ Hz, 1''-H), 4.63 (1H, d, $J=7.7$ Hz, 1'-H), 7.00 (1H, s, 4-H), 7.82 (1H, s, 7-H).

By the similar procedures described above, treatment of the ester **D** with 1-bromo-2-fluoroethane, allyl bromide, ethyl bromoacetate or iodoacetamide, followed by deblocking and purification afforded compounds **14**~**17**, respectively.

11-*O*-(2-Fluoroethyl)-PRM T1 (14)

Compound **14**: 10 mg (39%); MP 200~210°C (dec); $^1\text{H NMR } \delta$ 1.11 (3H, d, $J=6.4$ Hz, 5'-Me), 2.31

(3H, s, 3-Me), 3.90 (2H, d, $J=6.0$ Hz, 17-CH₂), 4.40 (1H, d, $J=7.7$ Hz, 1''-H), 4.64 (1H, d, $J=7.3$ Hz, 1'-H), 6.95 (1H, s, 4-H), 7.74 (1H, s, 7-H).

11-*O*-Allyl-PRM T1 (15)

Compound **15**: 7 mg (28%); MP 220~230°C (dec); ¹H NMR δ 1.11 (3H, d, $J=6.4$ Hz, 5'-Me), 2.33 (3H, s, 3-Me), 3.91 (2H, d, $J=6.0$ Hz, 17-CH₂), 4.40 (1H, d, $J=6.8$ Hz, 1''-H), 4.63 (1H, d, $J=7.3$ Hz, 1'-H), 7.03 (1H, s, 4-H), 7.87 (1H, s, 7-H).

11-*O*-Carboxymethyl-PRM T1 (16)

Compound **16**: 17 mg (66%); MP 210~220°C (dec); ¹H NMR δ 1.11 (3H, d, $J=6.4$ Hz, 5'-Me), 2.32 (3H, s, 3-Me), 3.90 (2H, d, $J=6.0$ Hz, 17-CH₂), 4.40 (1H, d, $J=6.8$ Hz, 1''-H), 4.64 (1H, d, $J=8.1$ Hz, 1'-H), 4.93 (2H, s, 11-OCH₂), 7.04 (1H, s, 4-H), 7.88 (1H, s, 7-H).

11-*O*-Carbamoylmethyl-PRM T1 (17)

Compound **17**: 11 mg (43%); MP >230°C (dec); ¹H NMR δ 1.11 (3H, d, $J=6.4$ Hz, 5'-Me), 2.33 (3H, s, 3-Me), 3.90 (2H, d, $J=6.0$ Hz, 17-CH₂), 4.40 (1H, d, $J=7.3$ Hz, 1''-H), 4.64 (1H, d, $J=7.7$ Hz, 1'-H), 7.11 (1H, s, 4-H), 7.97 (1H, s, 7-H).

11-*O*-Trifluoromethanesulfonyl-PRM T1 Methyl Ester (E)

By the similar procedure described in the preparation of **C**, compound **D** (157 mg, 0.193 mmol) was converted to the 11-*O*-triflate **E** (155 mg, 85%); ¹H NMR δ 1.10 (3H, d, $J=6.0$ Hz, 5'-Me), 2.34 (3H, s, 3-Me), 3.06 (1H, t, $J=10.7$ Hz, 5''-Hax), 3.11 (1H, t, $J=8.1$ Hz, 2''-H), 3.13 (1H, t, $J=8.1$ Hz, 3''-H), 3.28 (1H, m, 4''-H), 3.46 (1H, m, 4'-H), 3.52 (1H, m, 3'-H), 3.60 (2H, m, 2'- and 5'-H), 3.68 (3H, s, COOMe), 3.70 (1H, dd, $J=11.5$ and 5.6 Hz, 5''-Heq), 3.99 (2H, d, $J=6.0$ Hz, 17-CH₂), 4.40 (1H, d, $J=6.8$ Hz, 1''-H), 4.49 (1H, d, $J=9.8$ Hz, 5-H), 4.58 (1H, d, 6-H), 4.64 (1H, d, $J=7.3$ Hz, 1'-H), 7.16 (1H, s, 4-H), 7.66 (1H, d, $J=2.6$ Hz, 10-H), 7.73 (1H, d, 12-H), 8.03 (1H, s, 7-H); FAB(+)-MS m/z 946 (M+H).

11-Deoxy-PRM T1 (18)

Compound **E** (43 mg, 0.046 mmol) was converted to the corresponding 11-deoxy derivative **18** by a similar procedure as described in the preparation of **12** (5 mg, 14%); MP >230°C; IR ν_{\max} cm⁻¹ 3400, 1720, 1620; ¹H NMR δ 1.12 (3H, d, $J=6.4$ Hz, 5'-Me), 2.28 (3H, s, 3-Me), 3.08 (1H, t, $J=11.1$ Hz, 5''-Hax), 3.10 (1H, t, $J=9.0$ Hz, 2''-H), 3.15 (1H, t, $J=9.0$ Hz, 3''-H), 3.29 (1H, m, 4''-H), 3.55 (1H, m, 3'-H), 3.61 (1H, q, $J=6.4$ Hz, 5'-H), 3.70 (1H, dd, $J=11.1$ and 5.5 Hz, 5''-Heq), 3.71 (1H, t, $J=7.7$ Hz, 2''-H), 3.86 and 3.91 (2H, ABq, $J=17.1$ Hz, 17-CH₂), 4.41 (1H, d, $J=8.1$ Hz, 1''-H), 4.64 (1H, d, $J=7.7$ Hz, 1'-H), 6.96 (1H, s, 4-H), 7.76 (1H, s, 7-H).

11-Dimethylamino-PRM T1 (19)

To a solution of compound **E** (19 mg, 0.020 mmol) in DMF (1.0 ml) was added a 2 M solution of dimethylamine in EtOAc [0.4 ml; prepared from Me₂NH-HCl (164 mg, 2.0 mmol) by treating with excess NaOH in water (1 ml)-EtOAc (1 ml), followed by trapping gaseous Me₂NH in upper EtOAc layer] and the mixture was stirred at room temperature overnight. After being poured into pH 3.5 buffer, the mixture was charged on a C₁₈ column, which was washed with water and then eluted with 40% MeCN-buffer (pH 3.5). The appropriate fractions were evaporated, desalted and lyophilized to afford the methyl ester of title compound (10 mg). The sample in MeOH (2 ml) was treated with 1 N NaOH (0.5 ml) at room temperature for 1 hour. After evaporation of the solvent, the residue was charged on a C₁₈ column, which was washed with water and then eluted with 15% MeCN-buffer (pH 7.0). The desired fractions were evaporated, desalted and lyophilized to afford the title compound **19** (6 mg, 34% from **D**); MP >230°C; IR ν_{\max} cm⁻¹ 3400, 1730, 1600; ¹H NMR δ 1.12 (3H, d, $J=6.4$ Hz, 5'-Me), 2.30 (3H, s, 3-Me), 3.07 (1H, t, $J=11.5$ Hz, 5''-Hax), 3.12 (1H, t, $J=9.0$ Hz, 2''-H), 3.14 (1H, t, $J=9.0$ Hz, 3''-H), 3.70 (1H, dd, $J=11.5$ and 5.1 Hz, 5''-Heq), 3.71 (1H, q, $J=6.4$ Hz, 5'-H), 3.90 (2H, d, $J=6.0$ Hz, 17-CH₂), 4.41 (1H, d, $J=6.8$ Hz, 1''-H), 4.63 (1H, d, $J=7.7$ Hz, 1'-H), 6.97 (1H, s, 4-H), 7.76 (1H, s, 7-H).

11-Cyano (**20**) and 11-Carbamoyl (**21**)-PRM T1

To a solution of compound **E** (60 mg, 0.063 mmol) in DMF (4 ml) were sequentially added Pd(OAc)₂ (28 mg, 0.126 mmol), PPh₃ (66 mg, 0.254 mmol) and tributyltin cyanide (200 mg, 0.64 mmol) and the whole mixture was heated at 70°C for 3 days. The solution was diluted with MeOH (20 ml) and treated with 1 N NaOH (5 ml) at room temperature for 1 hour. After evaporation of the solvent, the residue was charged on a C₁₈ column (50 ml), which was washed with water and then eluted with 5, 10 and 15% aq MeCN to give the following two fractions after evaporation and lyophilization.

Fraction 1; 5 mg (9.5% from **E**) of **21**: Rt 1.9 minutes (30% MeCN - buffer (pH 3.5)); MP >230°C; IR ν_{\max} cm⁻¹ 3400, 1720, 1620; ¹H NMR δ 1.11 (3H, d, $J=6.4$ Hz, 5'-Me), 2.29 (3H, s, 3-Me), 3.07 (1H, t, $J=10.7$ Hz, 5''-Hax), 3.10 (1H, t, $J=7.3$ Hz, 2''-H), 3.14 (1H, dd, $J=7.3$ and 9.0 Hz, 3''-H), 3.29 (1H, m, 4''-H), 3.54 (1H, dd, $J=2.6$ and 9.8 Hz, 3'-H), 3.59 (1H, d, $J=2.6$ Hz, 4'-H), 3.70 (1H, dd, $J=10.7$ and 5.1 Hz, 5''-Heq), 3.71 (1H, t, $J=7.7$ Hz, 2'-H), 3.89 (2H, d, $J=6.0$ Hz, 17-CH₂), 4.40 (1H, d, $J=7.3$ Hz, 1''-H), 4.64 (1H, d, $J=7.7$ Hz, 1'-H), 6.95 (1H, s, 4-H), 7.76 (1H, s, 7-H).

Fraction 2; 3 mg (5.8% from **E**) of **20**: Rt 5.9 minutes (same as above); MP >230°C; IR ν_{\max} cm⁻¹ 3400, 1720, 1620; ¹H NMR δ 1.11 (3H, d, $J=6.4$ Hz, 5'-Me), 2.30 (3H, s, 3-Me), 3.07 (1H, t, $J=10.7$ Hz, 5''-Hax), 3.10 (1H, t, $J=7.3$ Hz, 2''-H), 3.14 (1H, dd, $J=7.3$ and 8.6 Hz, 3''-H), 3.29 (1H, m, 4''-H), 3.54 (1H, dd, $J=3.4$ and 9.8 Hz, 3'-H), 3.59 (1H, d, $J=3.4$ Hz, 4'-H), 3.70 (1H, dd, $J=11.1$ and 5.1 Hz, 5''-Heq), 3.70 (1H, t, $J=7.7$ Hz, 2'-H), 3.90 (2H, d, $J=5.6$ Hz, 17-CH₂), 4.40 (1H, d, $J=7.3$ Hz, 1''-H), 4.63 (1H, d, $J=7.7$ Hz, 1'-H), 6.95 (1H, s, 4-H), 7.74 (1H, s, 7-H).

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