SYNTHESIS AND ANTIFUNGAL ACTIVITY OF PRADIMICIN DERIVATIVES

MODIFICATIONS OF THE SUGAR PART

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Modifications at the sugar part of pradimicins were carried out by glycosidations of the aglycones or chemical transformations of natural pradimicins and their antifungal activity was evaluated. Among them, some of the D-xylose-modified derivatives (**14**, **17**, **24**) showed activity comparable to that of pradimicin A. The structure-activity relationships obtained through there sutdies clarified the role of the sugar part in the manifestation of antifungal activity: The 5-*O*-(6-deoxy-*β*-D-sugar) is essential for activity and 2′-*epi*, 3′-oxo and 4′-deoxy sugar derivatives retain activity against yeasts.

Pradimicin A (PRM A) is a novel antifungal antibiotic produced by *Actinomadura hibisca* P157-2(ATCC 53557)[1]. It is active against a variety of fungi and yeasts *in vitro* and highly effective against systemic fungal infections *in vivo*[2]. The chemical structure of PRM A has been established to be composed of a 5,6-dihydrobenzo[*a*]naphthacenequinone substituted with D-alanine (pradimicinone, AG-2) and a disaccharide (D-xylosyl-*N*-methyl-D-thomosamine)[1,3]. One of the minor congeners, pradimicin B (PRM B) lacks the terminal D-xylose of PRM A but it shows similar antifungal activity to that of PRM A both *in vitro* and *in vivo*[2]. This prompted us to modify the sugar parts of pradimicins for preparation of compounds with more potent biological activity. In our previous paper, we reported modifications at C-4′ position of the D-thomosamine[4], and herein we present modification studies of the whole sugar part of pradimicins; preparations of neutral sugar derivatives of AG-2, D-xylose-exchanged sugar analogs of PRM A and chemical transformations of pradimicins. Structure-activity relationships are discussed to clarify the contribution of the sugar part to antifungal activity.

Chemistry

Neutral Sugar Derivatives of AG-2 (Scheme 1)

Selective acetylation of AG-2 methyl ester with AcOH and DCC afforded 1-*O*-acetate (**A**), whose structure was determined by deshielding of 4-H to δ 7.45 from δ 7.02 in AG-2 methyl ester in ^{1}H NMR. Glycosidation of **A** with *per*-*O*-acetylated glycosyl bromide (X=Br in Method A) under the modified Koenigs-Knorr conditions[5], followed by alkaline treatment and C_{18} reversed phase column chromatography afforded a series of neutral monosaccharide derivatives of AG-2 (**1**～**5**, **9**, **10**)†. The desired product **6** was not obtained upon glycosidation with acetyl-D-galactosyl bromide under the same conditions presumably due to the steric effect of a bulky 6-*O*-acetyl group. Glycosidations using the other activated sugars[7] such as X=-OPNB/TMS-OTf, -F/TMS-OTf or -*O*-imidate/$BF_3 \cdot Et_2O$ were not successful in

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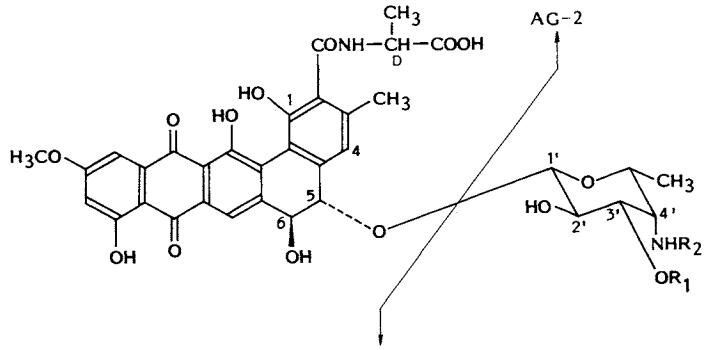
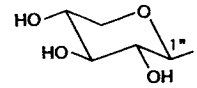
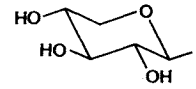
† Compound **1** was recently obtained from the fermentation or chemical degradation of benanomicin A by S. KONDO *et al.*[6].

Fig. 1. Chemical Structures of Pradimicins.



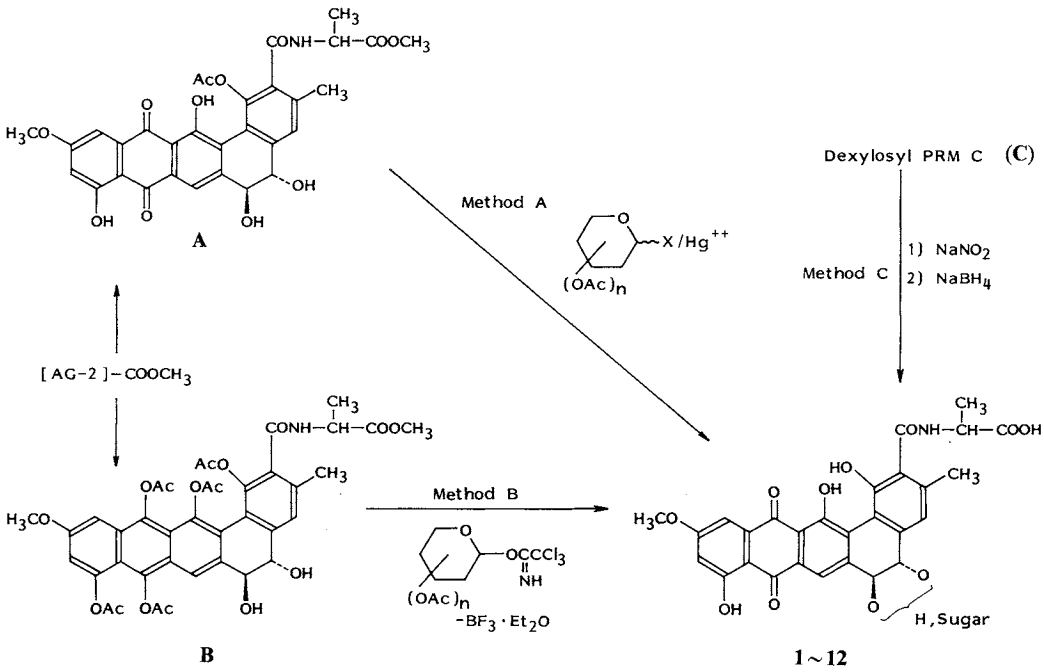
	R_1	R_2
Pradimicin A (PRM A)		CH_3
Pradimicin B (PRM B)	H	CH_3
Pradimicin C (PRM C)		H

galactosylation of compound **A**. However, using reductive *per-O*-acetylated aglycone (**B**) as a glycosyl acceptor, the imidate activating method catalyzed by $BF_3 \cdot Et_2O$[7] (Method B) was found to successfully proceed to afford the desired product **6**. The glycosidation sites (5- or 6-OH) of the products was established by NOEs observed between the anomeric proton (1'-H) and 5- or 6-H, and the coupling constant of $J_{1',2'}$ value defined the stereochemistry of the anomeric center. 4'-Deoxy monosaccharide derivatives (**7**, **8**) were derived from dexylosyl PRM C (**C**) by successive treatment with $NaNO_2$ and $NaBH_4$ (Method C). Stereochemistry of the 3'eq-OH in compound **8** was established by the coupling constants observed in ^1H NMR ($J_{2',3'}=9.0$, $J_{3',4'ax}=12.0$ Hz). 4'-Fluorobenanomicin A (**11**) was prepared by the Koenigs-Knorr glycosidation of the *O*-protected derivative of **9** with D-xylose, accompanying the formation of 2'-*O*-glycosidated product **12**. Their structures were established by NOEs observed between 1''-H and 3'- or 2'-H.

D-Xylose-exchanged Sugar Derivatives of PRM A (Scheme 2)

The D-xylose-exchanged disaccharide derivatives of PRM A (**13**~**19**) were prepared by glycosidation of 4'-*N*-Cbz-PRM B methyl ester (**D**) by the Koenigs-Knorr reaction and the products isolated are shown in Scheme 2. Although the glycosidation site of the products could not be identified by ^1H NMR due to overlapping of 2'- and 3'-H with other protons, it was determined to be at 3'-OH by the following chemical means. Treatment of the compound **D** with LiOH yielded cyclic carbamate (**F**) by participation of 4'-*N*-benzyloxycarbonyl group to the free 3'-OH, whereas no reaction occurred in the case of 4'-*N*-Cbz-PRM A[8] due to the presence of 3'-*O*-D-xylosyl substituent. Since all coupling intermediates (**E**) did not afford such a cyclic carbamate by a similar alkaline treatment, the attached sugars in compound **13**~**19** must be on the 3'-OH position. In glycosidation with acetylated D-glucose, the reaction did not proceed smoothly and required a prolonged period, presumably due to the steric effect described before. The desired compound

Scheme 1. Preparation of neutral sugar derivatives of AG-2.

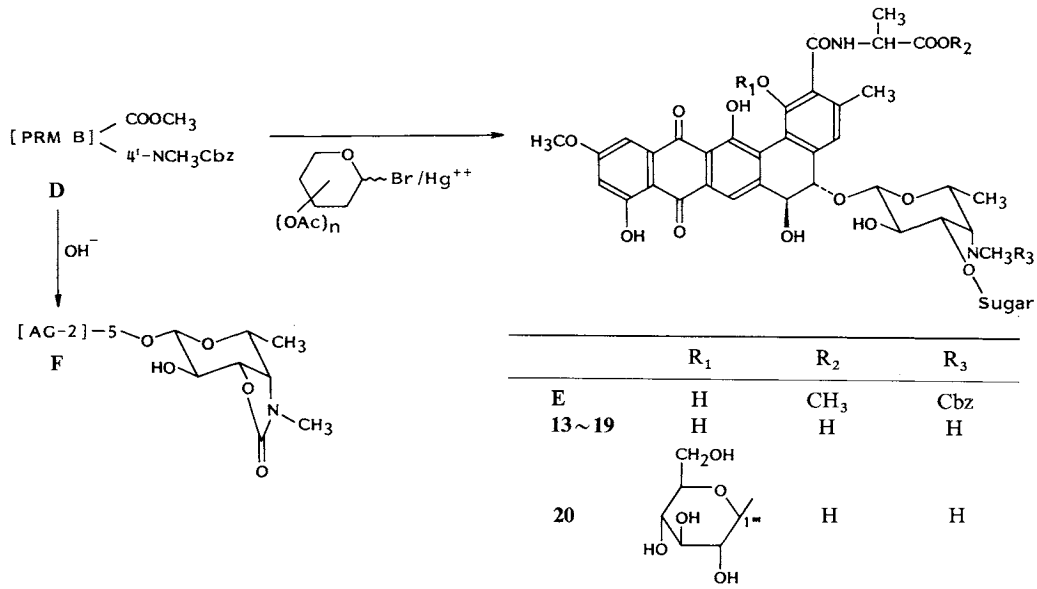


Compound	Sugar	Preparation method	Yield (%)	FAB-MS m/z (M+H)	^{1}H NMR (400 MHz, DMSO-d_6) δ (J in Hz) 5-H (d)	6-H (d)	($J_{5,6}$)	1'-H (d)	($J_{1',2'}$)
1	5-*O*-(β-D-Fucose)	A	2.5	696	4.37	4.46	(11.1)	4.53	(8.1)
2	6-*O*-(β-D-Fucose)	A	13	696	4.42	4.38	(11.1)	4.58	(7.3)
3	5-*O*-(α-D-Fucose)	A	7.8	696	4.30	4.43	(9.0)	4.81	(2.6)
4	5-*O*-(β-L-Fucose)	A	6.6	696	4.44	4.38	(9.4)	4.36	(7.7)
5	5-*O*-(α-L-Arabinose)	A	12	682	4.35	4.44	(11.1)	4.55	(6.8)
6	5-*O*-(β-D-Galactose)	B	8.0	712	4.42	4.43	(11.3)	4.62	(7.7)
7	5-*O*-(4-Deoxy-3-oxo-β-D-fucose)	C	15	678	4.56	4.51	(10.3)	4.84	(8.1)
8	5-*O*-(4-Deoxy-β-D-fucose)	C	10	680	4.39	4.45	(11.5)	4.53	(7.7)
9	5-*O*-(4-Deoxy-4-fluoro-β-D-fucose)	A	46	698	4.38	4.46	(11.5)	4.66	(7.3)
10	5-*O*-(5-Deoxy-β-D-xylofuranose)	A	13	666	4.34	4.30	(11.1)	5.17	(br s)
11	3'-*O*-(β-D-Xylosyl)-**9**		7.2	830	4.42	4.47	(9.8)	4.75	(8.1)
12	2'-*O*-(β-D-Xylosyl)-**9**		8.0	830	4.31	4.41	(11.1)	4.73	(7.7)

19† was obtained in poor yield along with the 1-*O*-glucoside (**20**). The unusually high field resonance of the 1'''-anomeric proton in **20** (δ 4.04, presumably due to the anisotropic effect of non-coplanar aromatic ring system), and lower field shift of 4-H (δ 7.42) in ^{1}H NMR and the characteristic shift of its UV absorption (λ_{max} 519 nm) suggested the structure of compound **20** to be the 1-*O*-glucoside.

† Compound **19** was also isolated from the fermentation broth of *Actinomadura verrucosospora* subsp. *neohibisca* R103-3 in our laboratory and designated as pradimicin L. The detailed physico-chemical properties will be reported in a separate paper.

Scheme 2. Preparation of D-xylose-exchanged sugar derivatives of PRM A.



	R_1	R_2	R_3
E	H	CH_3	Cbz
13～**19**	H	H	H
20		H	H

Compound	Sugar	Yield (%)	FAB-MS *m/z* (M+H)	^{1}H NMR (400 MHz, DMSO-d_6) δ (*J* in Hz) 1′-H (d)	($J_{1',2'}$)	1″-H (d)	($J_{1'',2''}$)
13	β-D-Ribose	3.9	841	4.68	(7.7)	4.89	(5.1)
14	α-L-Arabinose	5.9	841	4.58	(7.3)	4.37	(6.4)
15	β-L-Xylose	5.6	841	4.55	(7.7)	4.26	(7.3)
16	β-D-Chinovose	3.2	855	4.60	(7.3)	4.37	(7.3)
17	β-D-Fucose	4.6	855	4.59	(6.8)	4.29	(6.8)
18	2-Deoxy-2-fluoro-β-D-Glucose	2.0	873	4.52	(7.7)	4.79 (dd) ($J_{1'',F}$=4.0)	(7.3)
19	β-D-Glucose (PRM L)	1.1	871	4.79	(7.7)	4.50	(7.7)
20	1,3′-(β-D-Glucose)$_2$	1.6	1,033	4.86	(7.7)	4.49	(7.7)

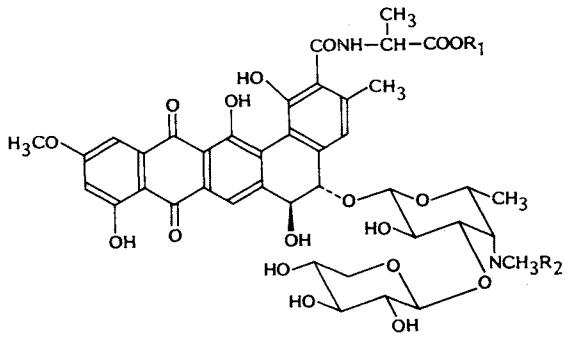
Chemical Transformations of PRM A Derivatives (Scheme 3)

A similar glycosidation of 4′-*N*-Cbz-PRM A methyl ester (**G**) with D-xylose afforded trisaccharide derivative (**21**), whose glycosidation site was elucidated to be at the 2″-OH by the NOE between 1‴- and 2″-protons. Treatment of compound **G** with diethylaminosulfur trifluoride (DAST) gave compound **22**, which was not the desired fluorinated derivative but was the 2′-*epi*-2′,4′-cyclic carbamate derivative of PRM A by the NMR and MS spectra. This was probably formed by participation of the 4′-*N*-benzyloxycarbonyl group with the 2′-*O*-SF_2NEt_2 intermediate, accompanying the inversion of configuration at C-2′. Successive treatment of PRM A with $NaIO_4$ and $NaBH_4$ gave the 3′-*O* and 4′-*N*-cyclized derivative (**23**), which might be formed by reductive alkylation of the 4′-NHMe group with the aldehyde generated by the IO_4 oxidation of the D-xylose moiety. Diol derivative (**24**) was obtained by a similar reaction of 4′-NMe_2-PRM A (**H**)[9].

Antifungal Activity

The *in vitro* and *in vivo* antifungal activity of the pradimicin derivatives were assessed by the previously

Scheme 3. Chemical transformations of PRM A.



	R_1	R_2
PRM A	H	H
G	CH_3	Cbz
H	H	CH_3

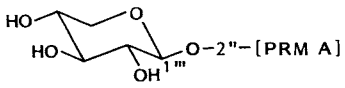
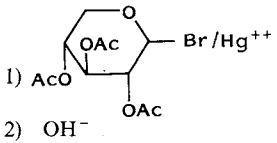
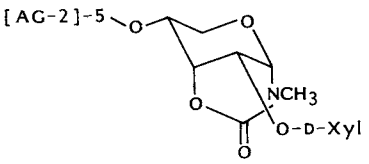
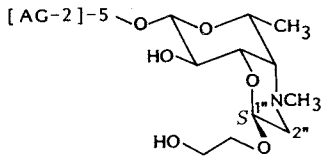
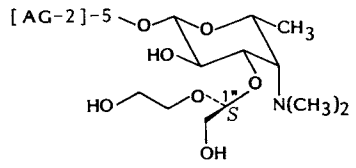
Compound	Structure	Starting material	Condition	Yield (%)	FAB-MS m/z (M+H)	^{1}H NMR (400 MHz, DMSO-d_6) δ (J in Hz)
21		**G**	 1) AcO, Br/Hg^{++}, OAc 2) OH^-	2.7	973	4.64 (d, J=7.1, 1′-H) 4.54 (d, J=7.4, 1″-H) 4.46 (d, J=7.3, 1‴-H)
22		**G**	1) DAST 2) OH^-	24	867	4.66 (br s, 1′-H) 4.97 (br s, 2′-H) 3.48 (s, 3′-H) 4.05 (br s, 4′-H)
23		PRM A	1) $NaIO_4$ 2) $NaBH_4$	35	795	4.63 (d, J=7.3, 1′-H) 4.94 (dd, J=2.6 and 8.1, 1″-H)
24		**H**	1) $NaIO_4$ 2) $NaBH_4$	33	827	4.72 (d, J=7.3, 1′-H) 4.81 (t, J=4.7, 1″-H)

Table 1. *In vitro* and *in vivo* antifungal activities of pradimicin derivatives.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	PRM B
Ca-4	3.1	>100	>100	25	25	>100	3.1	3.1	6.3	>100	12.5	>100	3.1
Cn-2	3.1	>100	>100	>100	>100	>100	6.3	6.3	12.5	>100	12.5	>100	1.6
Af-3	50	>100	>100	>100	>100	>100	>100	>100	100	>100	25	>100	3.1
PD_{50} (Ca-4)	>50							>50					11

	13	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	PRM A
Ca-4	50	6.3	>100	12.5	6.3	12.5	25	>100	25	6.3	6.3	6.3	6.3
Cn-2	6.3	3.1	3.1	3.1	3.1	3.1	0.8	25	100	25	3.1	1.6	1.6
Af-3	50	6.3	6.3	6.3	6.3	6.3	3.1	50	>100	>100	6.3	3.1	1.6
PD_{50} (Ca-4)		15			19							27	10

MIC (μg/ml, in YMA)[10]: Ca-4, *Candida albicans* A9540; Cn-2, *Cryptococcus neoformans* IAM 4514; Af-3, *Aspergillus fumigatus* IAM 2034.

PD_{50} (mg/kg, mice, iv)[10].

reported methods[10] and the results are summarized in Table 1. From the data obtained, the structure-activity relationships for the sugars of pradimicins are as follows:

1: In a series of fucose derivatives of AG-2 (**1**~**4**, **7**~**9**), only the 5-*O*-β-D-sugars are strictly required to exhibit *in vitro* antifungal activity. The derivatives are, however, inactive *in vivo*.

2: Although 5'-demethyl (**5**) and 5'-hydroxymethyl (**6**) derivatives of **1** are nearly inactive, 4'-deoxy (**8**) and 4'-fluoro (**9**) derivatives retain *in vitro* activity. This is also the case for the 3'-oxo compound **7**. Furanoside derivative **10** is totally inactive. As shown in compound **22**, the 2'-epimer shows good activity against *Candida albicans*.

3: All the neutral monosaccharide derivatives (**1**~**10**) are nearly inactive against *Aspergillus fumigatus* and some of them are active against *Candida* and *Cryptococcus* strains. The activity against *Aspergillus fumigatus* is improved by introduction of a second sugar (**9** *vs* **11** and also **1** *vs* benanomicin A[11]).

4: Most of D-xylose-replaced (**13**~**19**) and D-xylose modified derivatives (**23**, **24**) of PRM A are as active as the parent compound both *in vitro* and *in vivo*, with a few exceptions (**13** and **15**).

5: 2'-*O*-Sugar (**12**), 1-*O*-glycoside (**20**) and trisaccharide (**21**) derivatives are nearly inactive.

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 on a Shimadzu UV-260 spectrometer. NMR spectra were recorded on a JEOL GX-400 (400 MHz) and mass spectra on a JEOL JMS-AX505H mass spectrometer. Unless otherwise noted, UV spectra were taken in 0.01 N NaOH and ^{1}H NMR in DMSO-d_6.

1-*O*-Acetyl AG-2 Methyl Ester (**A**)

AG-2 methyl ester was prepared in a quantitative yield by refluxing AG-2 in 10% HCl-MeOH, followed by evaporation[12]; ^{1}H NMR δ 6.87 (1H, d, $J_{10,12}$=2.6 Hz, 10-H), 7.02 (1H, s, 4-H), 7.22 (1H, d, 12-H), 7.99 (1H, s, 7-H). To a suspension of AG-2 methyl ester (230 mg, 0.40 mmol) in dioxane (8 ml) were added AcOH (28 μl, 0.48 mmol), pyridine (39 μl, 0.48 mmol) and dicyclohexylcarbodiimide (DCC,

99 mg, 0.48 mmol) and the mixture was stirred at room temperature overnight. The insoluble materials were removed by filtration and the filtrate was evaporated to give the title compound 238 mg (98%): MP >250°C; IR ν_{max} 1745, 1610 cm^{-1}; UV λ_{max} (MeOH) nm (ε) 449 (13,200); ^1H NMR δ 2.02 (3H, s, Ac), 3.67 (3H, s, COOMe), 4.23 (1H, d, $J_{5,6}$=11.1 Hz, 5-H), 4.30 (1H, d, 6-H), 6.92 (1H, d, $J_{10,12}$=2.4 Hz, 10-H), 7.28 (1H, d, 12-H), 7.45 (1H, s, 4-H), 8.06 (1H, s, 7-H); FAB-MS *m/z* 606 (M+H).

1,2,3-Tri-*O*-acetyl-4-deoxy-4-fluoro-D-fucopyranose

The title compound was prepared from methyl 4-*O*-benzoyl-6-bromo-6-deoxy-α-D-glucopyranoside by sequential transformations under the conditions shown below: 1) 3,4-dihydro-2*H*-pyran (3 equiv), *p*-toluenesulfonic acid (TsOH, 0.1 equiv) in dioxane, room temperature (rt), overnight, 93%; 2) lithium aluminium hydride (2 equiv) in THF, rt, 2 hours, 100%; 3) methanesulfonyl chloride (2 equiv) in pyridine, rt, overnight, 82%; 4) Bu_4NF (10 equiv) in DMF, 150°C, 2 days, 25%; 5) TsOH (0.2 equiv) in MeOH, rt, 2 hours, 97% and 6) concd H_2SO_4 in acetic anhydride, rt, 1.5 hours, 86%: ^1H NMR ($CDCl_3$) of α-anomer δ 1.31 (1H, d, $J_{5,Me}$=6.4 Hz, 5-Me), 2.02, 2.12 and 2.13 (3H each, s, 3 × OAc), 4.16 (dq, $J_{5,F}$=28.6 Hz, 5-H), 4.73 (dd, $J_{4,F}$=50.0 Hz, 4-H), 5.26 (1H, ddd, $J_{3,4}$=2.6, $J_{3,F}$=26.1 Hz, 3-H), 5.37 (1H, ddd, $J_{2,3}$= 10.7, $J_{2,F}$=1.3 Hz, 2-H), 6.33 (1H, d, $J_{1,2}$=3.4 Hz, 1-H); EI-MS *m/z* 292 (M).

5-*O*-(4-Deoxy-4-fluoro-β-D-fucopyranosyl) AG-2 (**9**) (Typical Procedure for the Glycosidation by Method A in Scheme 1)

To a solution of compound **A** (303 mg, 0.50 mmol) in dry dichloroethane (DCE, 15 ml) were added molecular sieves 3A (3.0 g), $Hg(CN)_2$ (1.01 g, 4.0 mmol) and $HgBr_2$ (451 mg, 1.25 mmol) and the mixture was stirred at room temperature for 2 hours. To the mixture was added a solution of 2,3-di-*O*-acetyl-4-deoxy-4-fluoro-D-fucopyranosyl bromide [prepared *in situ* from the corresponding 1-*O*-acetate (439 mg, 1.50 mmol) by treating with 30% HBr-AcOH (1 ml) at room temperature for 2 hours, followed by evaporation] in DCE (1 ml) and the whole mixture was heated at 90°C overnight. After cooling to room temperature, the insoluble materials were removed by filtration and washed with DCE. The combined filtrate and washings were washed with saturated aq. $NaHCO_3$, dried over Na_2SO_4 and evaporated. The residual mass (470 mg) in MeOH (100 ml) was treated with 1 N NaOH (20 ml) at room temperature for 1 hour. After evaporation of the solvent, the residue was diluted with H_2O (100 ml) and charged on a C_{18} column (Prep C_{18}, 55~105 μm, Waters, 500 ml), which was washed with H_2O and then eluted with 35 and 40% acetonitrile (MeCN)-phosphate buffer (pH 3.5). The fractions showing retention time of 5.4 minutes on HPLC [SSC-ODS-262, 6, i.d. × 100 mm, 40% MeCN-buffer (pH 3.5)] were evaporated and desalted on a C_{18} column to afford the title compound (**9**, 160 mg, 46%): MP >230°C; IR ν_{max} 1730, 1620 cm^{-1}; UV λ_{max} nm (ε) 498 (11,200); ^1H NMR (see also Scheme 1) δ 1.17 (3H, d, $J_{5',Me}$=6.0 Hz, 5'-Me), 1.31 (3H, d, $J_{17,Me}$=6.8 Hz, 17-Me), 2.23 (3H, s, 3-Me), 3.72 (1H, dq, $J_{5',F}$=29.7 Hz, 5'-H), 3.90 (3H, s, 11-OMe), 3.96 (1H, q, 17-H), 4.42 (1H, dd, $J_{3',4'}$=2.1, $J_{4',F}$=50.4 Hz, 4'-H), 6.70 (1H, br d, 10-H), 6.90 (1H, s, 4-H), 7.11 (1H, br d, 12-H), 7.70 (1H, s, 7-H).

Aglycone **B**

To a mixture of AG-2 methyl ester (1.5 g, 2.66 mmol) in dry DMF (50 ml) were added 2,2-dimethoxypropane (4 ml) and TsOH (60 mg, 0.3 mmol) and the mixture was stirred at room temperature for 3 days. Extraction of the mixture with dichloromethane (CH_2Cl_2), followed by purification on a silica gel column (Wako-gel C-200, 100 g, CH_2Cl_2-MeOH=20:1 as an eluent) gave the corresponding 5,6-*O*-isopropylidene derivative of AG-2 methyl ester (607 mg, 39%); ^1H NMR ($CDCl_3$) δ 1.61 and 1.63 (3H each, s, $>CMe_2$), 4.54 (2H, s, 5- and 6-H); FAB-MS *m/z* 604 (M+H). The compound (423 mg, 0.70 mmol) was successively treated with acetic anhydride (2 ml) in pyridine (10 ml) and then with zinc powder (200 mg) at room temperature for 1 hour. The mixture was quenched with MeOH (1 ml) at 0°C and extracted with EtOAc (50 ml). Purification by a silica gel column (CH_2Cl_2-MeOH=50:1) gave 1,8,9,13,14-penta-*O*-acetyl derivative [518 mg, 90%, FAB-MS *m/z* 815 (M)]. Treatment of the sample (164 mg, 0.20 mmol) with TsOH (100 mg, 0.53 mmol) in 20% aq MeCN (5 ml) at room temperature for 20 minutes, followed by extraction with EtOAc and evaporation afforded the title compound **B** (115 mg, 74%) as a yellow powder: MP 180°C; IR ν_{max} 1770, 1730, 1660, 1640, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.27, 2.41, 2.43, 2.46 and 2.61 (3H each, s, 5 × OAc), 4.29 and 4.42 (1H each, d, $J_{5,6}$=9.8 Hz, 5- and

6-H), 6.92 (1H, d, $J_{10,12}=2.1$ Hz, 10-H), 7.06 (1H, s, 4-H), 7.46 (1H, d, 12-H), 8.06 (1H, s, 7-H); FAB-MS *m/z* 775 (M).

5-*O*-*β*-D-Galactopyranosyl AG-2 (**6**)

A mixture of **B** (82 mg, 0.11 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate[7] (224 mg, 0.46 mmol) in CH_2Cl_2 (10 ml) was treated at −30°C with $BF_3 \cdot Et_2O$ (57 μl, 0.46 mmol) for 1.5 hours and the solution was quenched by adding saturated aq. $NaHCO_3$ (0.5 ml). The organic layer was washed with brine and evaporated. The residue in MeOH (10 ml) was treated with 1 N NaOH (5 ml) and purified on a C_{18} column eluting with 25% MeCN-buffer (pH 3.5) to afford the title compound **6** (6 mg, 8.0%): MP 230°C; IR ν_{max} 1700, 1620, 1600 cm^{-1}; UV λ_{max} nm (ε) 501 (10,500); ^1H NMR see Scheme 1.

5-*O*-[4-Deoxy-3-oxo- (**7**) and 4-Deoxy- (**8**) *β*-D-fucopyranosyl] AG-2

To a solution of dexylosyl-PRM C[6] (840 mg, 1.21 mmol) in 0.1 M aq. AcOH (70 ml) was added dropwise 1 M sodium nitrite solution (30 ml) under ice-cooling and the mixture was stirred for 1 hour at room temperature. The resulting suspension was adjusted to pH 7 by addition of 0.02 N NaOH and chromatographed on a C_{18} column eluting with 30~40% MeCN-phosphate buffer (pH 7) to give compound **7** (120 mg, 15%): MP >210°C; IR ν_{max} 1720, 1620 cm^{-1}; ^1H NMR δ 2.35 (1H, dd, $J_{4',5'}=2.1$, $J_{gem}=14.1$ Hz, 4'-H), 2.54 (1H, d, 4'-H), 3.75 (1H, dq, $J_{5',Me}=6.4$ Hz, 5'-H), 4.12 (1H, br d, $J_{1',2'}=8.1$ Hz, 2'-H).

To a suspension of **7** (30 mg, 0.044 mmol) in 0.01 N NaOH (8 ml) was added 0.1 M sodium borohydride solution (100 μl) and the mixture was stirred for 1 hour at room temperature. The mixture was chromatographed on a C_{18} column eluting with 20~30%MeCN-buffer (pH 7) to give the title compound **8** (20 mg, 66%): MP >210°C; IR ν_{max} 1720, 1620 cm^{-1}; ^1H NMR δ 1.24 (1H, dt, $J_{4'ax,4'eq}=11.1$, $J_{3',4'ax}=J_{4'ax,5'}=12.0$ Hz, 4'ax-H), 1.82 (1H, dd, $J_{3',4'eq}=5.1$ Hz, 4'eq-H), 3.13 (1H, dd, $J_{1',2'}=7.7$, $J_{2',3'}=9.0$ Hz, 2'-H), 3.47 (1H, m, 3'-H), 3.54 (1H, dq, $J_{5',Me}=6.4$ Hz, 5'-H).

3'-*O*- (**11**) and 2'-*O*- (**12**) (*β*-D-xylopyranosyl)derivative of **9**

1-*O*-Acetyl derivative of compound **9** methyl ester (63 mg, 0.084 mmol), obtained by a similar selective 1-*O*-acetylation as described above, was glycosidated with per-*O*-acetylated-D-xylopyranosyl bromide under the Koenigs-Knorr conditions to afford two fractions, after deblocking and purifications: Fraction 1 (**11**, 4.5 mg, 7.2%); Rt=3.9 minutes [40% MeCN-buffer (pH 3.5)]; MP >230°C; ^1H NMR δ 1.16 (3H, d, $J_{5',Me}=6.0$ Hz, 5'-Me), 1.29 (3H, d, $J_{17,Me}=6.8$ Hz, 17-Me), 2.21 (3H, s, 3-Me), 3.07 (1H, dd, $J_{4'',5''ax}=9.0$, $J_{5''ax,5''eq}=12.4$ Hz, 5''ax-H), 3.63 (1H, t, $J_{1',2'}=8.1$ Hz, 2'-H), 3.70 (1H, dd, $J_{4'',5''eq}=5.6$ Hz, 5''$_{eq}$-H), 3.72 (1H, dd, $J_{2',3'}=11.1$, $J_{3',F}=28.0$ Hz, 3'-H), 3.90 (3H, s, 11-OMe), 4.40 (1H, d, $J_{1'',2''}=7.3$ Hz, 1''-H), 4.58 (1H, br d, $J_{4',F}=51.7$ Hz, 4'-H), 6.70 (1H, d, $J_{10,12}=2.6$ Hz, 10-H), 6.89 (1H, s, 4-H), 7.14 (1H, d, 12-H), 7.67 (1H, s, 7-H): Fraction 2 (**12**, 5.5 mg, 8.0%); Rt=4.5 minutes [same as above]; MP >230°C; ^1H NMR δ 4.49 (1H, br d, $J_{4',F}=48.0$ Hz, 4'-H), 4.57 (1H, d, $J_{1'',2''}=7.3$ Hz, 1''-H).

3'-*O*-(α-L-Arabinoryranosyl) PRM B (**14**) (Typical Procedure for the Preparation of Compounds **13**~**18** in Scheme 2)

4'-*N*-Cbz-PRM B methyl ester (**D**) was analogously prepared by the method reported for the corresponding PRM A derivative[8]. A solution of compound **D** (500 mg, 0.58 mmol) in DCE (50 ml) was treated with per-*O*-acetylated L-arabinopyranosyl bromide (prepared from the corresponding 1-*O*-acetate, 557 mg, 1.75 mmol) at 90°C overnight under the Koenigs-Knorr conditions. The usual work-up and purification of the reaction mixture gave a protected coupling product (**E**, 59 mg, 80% pure), which was successively deprotected by treatment with 1 N NaOH (2 ml) in MeOH (20 ml) and hydrogenation over Pd-C (10 mg) in a mixture of MeOH (10 ml) and H_2O (1 ml). Purification of the final product by a C_{18} column afforded the title compound **14** (29 mg, 5.9%): MP >230°C; IR ν_{max} 1610 cm^{-1}; UV λ_{max} nm (ε) 498 (15,500); ^1H NMR (see also Scheme 2) δ 1.15 (3H, d, $J_{5',Me}=6.4$ Hz, 5'-Me), 1.30 (3H, d, $J_{17,Me}=6.8$ Hz, 17-Me), 2.22 (3H, s, 3-Me), 2.42 (3H, s, 4'-NMe), 3.87 (3H, s, 11-OMe), 6.65 (1H, br s, 10-H), 6.91 (1H, s, 4-H), 7.07 (1H, br s, 12-H), 7.69 (1H, s, 7-H).

PRM L (**19**) and 1-*O*-(β-D-Glucopyranosyl) PRM L (**20**)

A similar glycosidation of compound **D** (1.03 g, 1.2 mmol) with tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1.48 g, 3.0 mmol) was repeated four times for 100 hours under heating at 90°C. The crude product was deblocked and purified to afford the following two fractions: Fraction 1 (**20**); 20 mg (1.6%); Rt=8.4 minutes [25% MeCN - buffer (pH 3.5)]; MP >205°C; IR ν_{max} 1620, 1600 cm^{-1}; UV λ_{max} nm (ε) 519 (13,400); ^1H NMR δ 4.04 (1H, d, $J_{1''',2'''}$=7.7 Hz, 1'''-H), 6.93 (1H, d, $J_{10,12}$=2.6 Hz, 10-H), 7.28 (1H, d, 12-H), 7.42 (1H, s, 4-H), 8.04 (1H, s, 7-H): Fraction 2 (**19**); 12 mg (1.1%); Rt=17.4 minutes (as above); to be identified as PRM L in all respect; UV λ_{max} nm (ε) 498 (13,900); ^1H NMR δ 6.91 (1H, d, $J_{10,12}$=2.6 Hz, 10-H), 7.13 (1H, s, 4-H), 7.31 (1H, d, 12-H), 8.02 (1H, s, 7-H).

Alkaline Hydrolysis of 4'-*N*-Cbz-PRM B Methyl Ester (**D**)

A solution of **D** (50 mg, 0.058 mmol) in MeOH (50 ml) containing 1 N LiOH (2.5 ml) was stirred at room temperature for 18 hours. After acidification with 1 N HCl at 5°C, the mixture was evaporated and diluted with water to give a precipitate, which was collected by filtration to afford 3',4'-cyclic carbamate derivative of PRM B (**F**, 32 mg, 74%); MP 218~221°C; IR ν_{max} 1742, 1607 cm^{-1}; UV λ_{max} (MeOH) nm (ε) 466 (9,600); ^1H NMR δ 2.88 (3H, s, 4'-NMe), 3.76 (1H, dd, $J_{3',4'}$=8.5, $J_{4',5'}$=1.7 Hz, 4'-H), 4.49 (2H, br s, 5- and 6-H), 4.56 (1H, dd, $J_{2',3'}$=5.6 Hz, 3'-H), 4.94 (1H, d, $J_{1',2'}$=5.6 Hz, 1'-H); FAB-MS *m/z* 735 (M+H).

4'-*N*-Cbz-PRM A Methyl Ester (**G**)

To a mixture of 4'-*N*-Cbz PRM A[8] (2.15 g, 2.2 mmol) in dry MeOH (50 ml) was added $SOCl_2$ (0.5 ml, 6.9 mmol) and the mixture was stirred at room temperature overnight and evaporated. The residue was triturated with ether to afford the title compound **G** (2.17 g, 100%): MP 180~185°C (dec); IR ν_{max} 1735, 1670, 1620 and 1600 cm^{-1}; UV λ_{max} (MeOH) nm (ε) 476 (10,800); ^1H NMR δ 3.66 (3H, s, COOMe), *ca.* 5.0 (2H, m, CH_2Ph), *ca.* 7.3 (5H, m, Ph); FAB-MS *m/z* 989 (M+H).

2''-*O*-β-D-Xylopyranosyl PRM A (**21**)

Compound **G** (494 mg, 0.5 mmol) was glycosidated with *per*-*O*-acetylated D-xylopyranosyl bromide by a similar way as in the preparation of compound **14**. Successive deprotections of the coupling product by alkaline hydrolysis and hydrogenolysis, followed by purification on a C_{18} column eluting with 15~25% MeCN - buffer (pH 3.5) afforded the title compound (13 mg, 2.7%): MP >247°C; IR ν_{max} 1610 cm^{-1}; UV λ_{max} nm (ε) 496 (13,200); ^1H NMR (see also Scheme 3) δ 1.18 (3H, d, $J_{5',Me}$=6.3 Hz, 5'-Me), 1.33 (3H, d, $J_{17,Me}$=7.3 Hz, 17-Me), 2.27 (3H, s, 3-Me), 2.44 (3H, s, 4'-NMe), 3.08 (1H, dd, $J_{1''',2'''}$=7.4, $J_{2''',3'''}$=9.0 Hz, 2'''-H), 3.17 (3H, m, 3'''-, 5''ax- and 5'''ax-H), 3.30 (1H, t, $J_{1'',2''}$=7.4, $J_{2'',3''}$=7.8 Hz, 2''-H), 3.40 (1H, m, 3''-H), 3.58 (1H, t, $J_{1',2'}$=7.1, $J_{2',3'}$=8.0 Hz, 2'-H), 3.90 (3H, s, 11-OMe), 4.34 (1H, q, 17-H), 4.43 (2H, s, 5- and 6-H), 6.70 (1H, d, $J_{10,12}$=2.3 Hz, 10-H), 6.92 (1H, s, 4-H), 7.12 (1H, d, 12-H), 7.70 (1H, s, 7-H).

Reaction of **G** with DAST [Formation of 2'-*O* and 4'-*N*-Cyclic Carbamate Derivative of PRM A (**22**)]

To a solution compound **G** (53 mg, 0.054 mmol) in dry CH_2Cl_2 (2.7 ml) were added at −15°C, pyridine (0.20 ml, 2.5 mmol) and DAST (66 μl, 0.50 mmol) and the mixture was stirred at the same temperature for 2.5 hours. After being quenched with MeOH (1 ml), the mixture was evaporated and purified on a C_{18} column eluting with 50% MeCN - buffer (pH 3.5) to give methyl ester of title compound (22 mg, 47%): FAB-MS *m/z* 881 (M+H). Alkaline hydrolysis of the compound, followed by purification afforded compound **22** (11 mg, 51%): MP 220°C (dec); IR ν_{max} 1680, 1610 cm^{-1}; UV λ_{max} nm (ε) 493 (16,100); ^1H NMR see Scheme 3.

Successive Treatment of (4'-NMe_2) PRM A with $NaIO_4$ and $NaBH_4$ (Formation of Compounds **23** and **24**)

A mixture of PRM A (432 mg, 0.5 mmol) and $NaIO_4$ (535 mg, 2.5 mmol) in H_2O (100 ml) was stirred at room temperature for 2.5 hours, to which was added a solution of $NaBH_4$ (95 mg, 2.5 mmol) in H_2O (2 ml) and the mixture was stirred for further 2 hours, acidified to pH 2.5 with 1 N HCl under ice-cooling and purified on a C_{18} column eluting with 35% MeCN - buffer (pH 3.5) to afford the 3'-*O*- and 4'-*N*-cyclic

product **23** (138 mg, 35%): MP 235°C; IR ν_{max} 1615, 1600 cm^{-1}; UV λ_{max} nm (ε) 500 (10,900); ^1H NMR δ 2.17 (1H, dd, $J_{2''ax,2''eq}=12.0$, $J_{1'',2''ax}=8.1$ Hz, 2''ax-H), 2.38 (3H, s, 4'-NMe), 2.81 (1H, dd, $J_{1'',2''eq}=2.6$ Hz, 2''eq-H), 3.51 (2H, m, 2'''-H), 3.71 (4H, m, 1'''-, 3'- and 5'-H).

A similar reaction was carried out for 4'-NMe_2-PRM A (446 mg) to afford 3'-*O*-[(*S*)-2-hydroxy-1-hydroxyethoxy-1-ethyl] derivative **24** (139 mg, 33%): MP 220°C; IR ν_{max} 1600 cm^{-1}; UV λ_{max} nm (ε) 500 (10,700); ^1H NMR δ 2.84 (6H, s, 4'-NMe_2), 3.59 and 3.73 (1H each, dt, $J_{1''',2'''}=4.7$, $J_{gem}=10.3$ Hz, 1'''-, 2'''-H).

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