A SYNTHESIS AND AN X-RAY ANALYSIS OF 2'-*C*-, 3'-*C*- AND 5'-*C*-METHYLSANGIVAMYCINS

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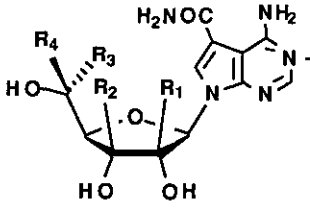
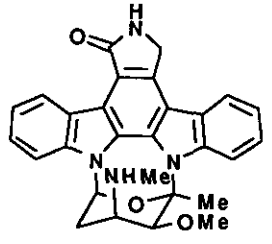
Abstract- 3'-*C*-, 5'(*R*)-*C*- and 5'(*S*)-*C*-Methylsangivamycins (**3**-**5**) were synthesized by the trimethylsilyl triflate mediated coupling reaction of the methyl substituted ribose derivatives (**7**), (**9**) and (**10**) with the base moiety (**11**) and the successive functional group manipulation. 2'-*C*-Methylsangivamycin (**2**) was synthesized by the condensation of the sodium salt of **23** with the chlorosugar (**28**) as the key step. The crystalline structures of *C*-methylsangivamycins (**2**-**4**) were determined by X-ray studies.

In the process of developing an inhibitor of protein kinase C (PKC), we were interested in the relation between the conformational property and the bioactivity of sangivamycin (**1**), a weak but rather selective inhibitor of PKC.[1] The weak activity of **1** may be related to its conformational flexibility: namely, the mobility, especially around the glycosyl bond, is presumed to disfavor a requisite interaction of **1** with the enzyme PKC entropically. In fact, staurosporine which is structurally related to cyclic nucleosides but possesses an extremely rigid structure exhibits a much stronger PKC inhibitory activity than **1**.[1] Thus, we intended to clarify the active conformation of sangivamycin by restricting the rotation of the glycosyl bond by a minimum structural modification.



	R_1	R_2	R_3	R_4
1	H	H	H	H
2	Me	H	H	H
3	H	Me	H	H
4	H	H	Me	H
5	H	H	H	Me



Staurosporine

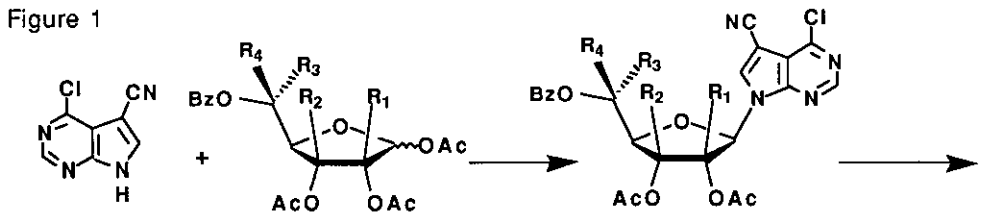
It was presumed by molecular-mechanics procedures that the introduction of even the simple methyl group into ribose portion of **1** should significantly influence the population of the *syn* and

anti conformers.[2] In the present paper, we report the synthesis of the methyl substituted analogues (**2-5**) and the conformational analysis of these compounds by X-ray diffraction studies.

Initially, we applied a trimethylsilyl triflate-mediated coupling reaction between methyl substituted ribose derivatives and the base moiety for the syntheses of methyl substituted sangivamycins. The requisite 2-*C*- and 3-*C*-methylribose derivatives (**6**) and (**7**) were prepared from D-glucose.[3] 5(*R*)-*C*-Methylribose derivative (**9**) was prepared from methyl 2,3-*O*-Isopropylidene-5(*R*)-*C*-methyl-D-ribofuranoside (**8**)[4] by simple benzoylation and 5(*S*)-*C*-methylribose derivative (**10**) was synthesized by inversion of the configuration of the C5-hydroxyl group in **8** by the Mitsunobu reaction. The requisite base moiety (**11**) was prepared according to the literature, in which the total synthesis of sangivamycin has been reported.[5]

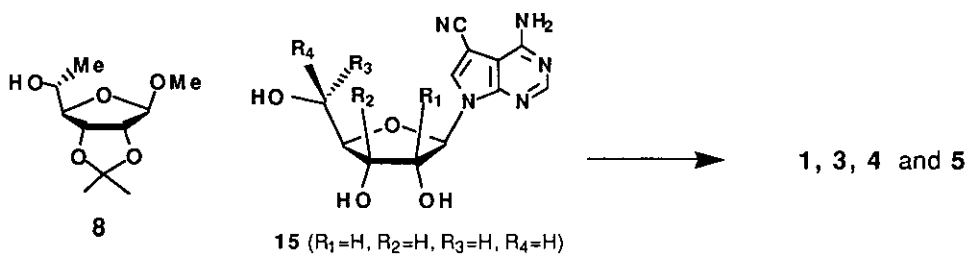
The trimethylsilyl triflate-mediated coupling reaction[6] of 3-*C*-methylribose derivative (**7**) with **11** afforded **12** (31%) with recovery of the starting material (**11**) (44%). As the purification of **12** by a silica gel column chromatography was difficult, identification of the structure was achieved after conversion into **16** [liq. NH_3 / MeOH (51%)]. Finally, hydrolysis of **16** [H_2O_2, conc. NH_4OH (90%)] afforded the methyl substituted sangivamycin (**3**) (Figure 1). Sangivamycin itself, 5'-(*R*)-*C*- and 5'-(*S*)-*C*-methylsangivamycins were prepared in the same way as used for **3** from ribofuranose tetraacetate, (**9**) and (**10**) (Table 1).

Figure 1



6 (R_1=Me, R_2=H, R_3=H, R_4=H)
7 (R_1=H, R_2=Me, R_3=H, R_4=H)
9 (R_1=H, R_2=H, R_3=Me, R_4=H)
10 (R_1=H, R_2=H, R_3=H, R_4=Me)

12 (R_1=H, R_2=Me, R_3=H, R_4=H)
13 (R_1=H, R_2=H, R_3=Me, R_4=H)
14 (R_1=H, R_2=H, R_3=H, R_4=Me)



15 (R_1=H, R_2=H, R_3=H, R_4=H)
16 (R_1=H, R_2=Me, R_3=H, R_4=H)
17 (R_1=H, R_2=H, R_3=Me, R_4=H)
18 (R_1=H, R_2=H, R_3=H, R_4=Me)

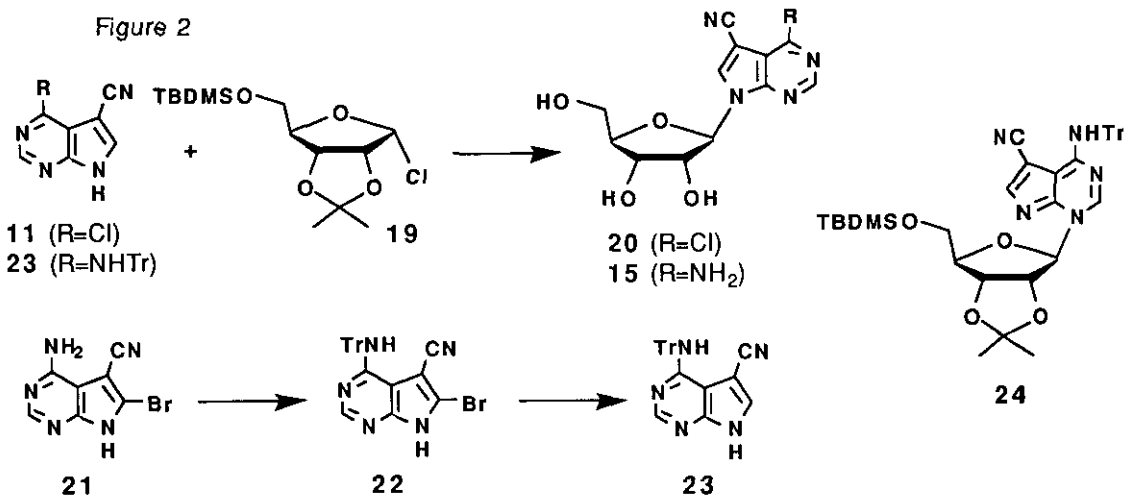
Table 1

conditions	TMSOTf, HMDS, TMSCl MeCN	liq. NH_3, MeOH	H_2O_2, conc. NH_4OH
sangivamycin (**1**)	31%, recovery (**11**): 44%	**15**: 53%	**1**: 87%
3'-*C* methyl (**3**)	**12**: 28%, **11**: 44%	**16**: 51%	**3**: 90%
5'(*R*)-*C* methyl (**4**)	**13**: 47%, **11**: 34%	**17**: 70%	**4**: 95%
5'(*S*)-*C* methyl (**5**)	**14**: 32%, **11**: 50%	**18**: 55%	**5**: quant.

However, the coupling reaction of 2-*C*-methylribose derivative (**6**) with **11** did not take place even at the elevated temperature. In this reaction only several unidentified carbohydrate derivatives were obtained with recovery of **11**. This low reactivity would be ascribed to the *steric hindrance* due to the β-methyl group at the C-2 position of ribofuranose which closely located to the reaction site and the rapid formation of a carbocation from the tertiary alcohol under Lewis acidic conditions.[3b] Since the reaction of **6** with benzoyladenine provides the condensation product under the same reaction conditions,[3b] the present failure would also be ascribed to the diminished nucleophilicity at the 9-nitrogen atom (purine numbering has been used throughout this paper) due to the presence of the cyano group at the C-7 position.

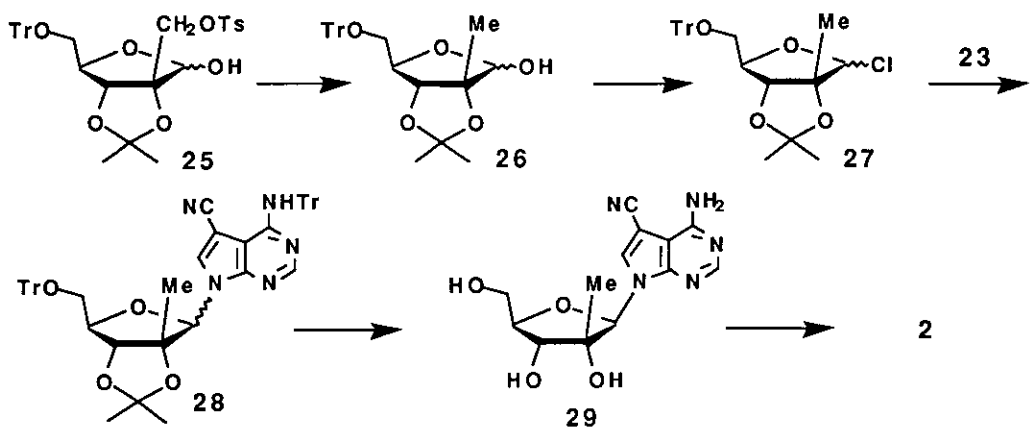
In order to overcome this difficulty, we then examined a coupling reaction under basic conditions to prevent the formation of the carbocation species and to increase the nucleophilicity at the 9-nitrogen atom. The coupling reaction[7] between the sodium salt of **11** and the properly protected α-chlorosugar (**19**)[8] was carried out as a model experiment and the desired β-nucleoside (**20**) was obtained after removal of the protective groups (Figure 2). Unfortunately during the optimization of the reaction conditions, we encountered the poor reproducibility of this reaction.

Figure 2



Since the low solubility of the base moiety (**11**) was presumed to be responsible for this low yield and poor reproducibility, modification of the base moiety was undertaken to increase the solubility. Trityl protecting group seemed to be a good candidate for this purpose, and thus **23** was prepared from **21**[5] by tritylation of the amino group [TrCl, DMAP / pyridine (84%)] and the successive debromination [H_2, Pd-C / EtOH-NH_4OH (76%)]. The reaction of this tritylated base (**23**) with α-chlorosugar (**19**) smoothly occurred to afford the desired nucleoside (**15**) in 62% after deprotection (1. NaH / MeCN-THF. 2. aq. 90% TFA). In this coupling reaction, the 3-glycosyl isomer (**24**) was also obtained in 29% yield (Figure 2). Since 3-glycosyl isomer of sangivamycin is an interesting molecule from a biological point of view, deprotection of **24** under acidic conditions was examined. However, the glycosyl bond in **24** was cleaved much faster under acidic conditions (aq. 90% TFA, 0 °C) than the trityl protecting group. 3-Glycosyl adenosine has also been reported to be acid labile.[9] The present method was then successfully applied for the synthesis of the desired 2'-*C*-methylsangivamycin (Figure 3). The 2-*C*-methylribose derivative (**27**) was prepared from tosylate (**25**).[3b] 1-Hydroxyl group of tosylate (**25**) was protected by the ethoxyethyl group (ethyl vinyl ether, PPTS / CH_2Cl_2). Reduction of the tosylate into the methyl group by Super-Hydride® in refluxing THF and the successive deprotection of the ethoxyethyl group (PPTS / MeOH) afforded the properly protected 2-*C*-methylribose derivative (**26**) in 38% overall yield. The coupling reaction between the tritylated base (**23**) and (**27**) prepared from **26** [$(Me_2N)_3P$, CCl_4 / THF][8,10] took place successfully to afford the desired β-nucleoside (**28β**) as a mixture with **26** (*ca.* 25% estimated by ^1H nmr), α-nucleoside (**28α**) (8%) accompanied by **23** (28%) and 3-glycosyl isomers (**30**) (21%). Removal of all protective groups from **28β** (aq. 90% TFA) and the successive conversion of the cyano group to the amide group (H_2O_2, conc. NH_4OH) afforded 2'-*C*-methylsangivamycin (**2**).

Figure 3



The conformational properties of sangivamycin, 2'-*C*-, 3'-*C*- and 5'-(*R*)-*C*-methylsangivamycins (**1**, **2**, **3** and **4**) were studied by X-ray diffraction analysis. Although the crystalline structure may not be the stable conformation in solution, it is expected to play an important role in the conformational spaces in solution. The results of the X-ray diffraction studies were shown in Figures 4-7 (stereo-view). These structures in crystal consisted with the major conformation in solution by our ^{1}H nmr analysis including NOE experiments.[2] Sangivamycin (**1**) shows the *anti C3'-endo gg* conformation, which is in accord with the data already reported by Palczewski.[11] The stereochemistry found here is a typical conformation of purine type nucleoside.[12] 2'-*C*-Methylsangivamycin (**2**) also possesses the same *anti C3'-endo* conformation as **1** as expected from our molecular mechanics calculations.[2] The analogous X-ray crystalline structure of 2'-*C*-methylcytidine (pyrimidine nucleoside) has been reported by Mikahailov.[3b] On the other hand, 3'-*C*- and 5'(*R*)-*C*-methylsangivamycins (**3** and **4**) were found to exhibit *syn C2'-endo* conformation. Although introduction of the bulky group into the C-8 position in the base part has been known to shift the *syn/anti* equilibrium toward *syn*,[12] enhancement of the *syn* conformation by modification of the ribose moiety is a rare case to our knowledge.[3a] Since the methyl group introduced is far from the base part in 5'(*R*)-*C*-methylsangivamycin (**4**), the preference of *syn* conformation is an interesting observation. In these cases, the hydrogen bonding (N3 - O5') was found to assist in stabilizing the *syn C2'-endo* conformation. It is interesting that the *anti* conformers (**1** and **2**) were crystallized with one molecule of water as solvate, and the syn conformers (**3** and **4**) with methanol. It is note worthy that the preference of the *syn C2'-endo* conformation in **3** and **4** is hard to predict by simple inspection of molecular model but can be estimated by our molecular mechanics calculations.[2]

In summary, we were able to prepare two conformers having different conformations (*anti C3'-endo* and *syn C2'-endo*) in crystal form, which might be the major ones in solution and thus have the different bioactivities.[2] Detailed studies of the bioactivities of the compounds obtained here are under investigation and will be reported separately.

Figure 4 Sangivamycin (**1**)

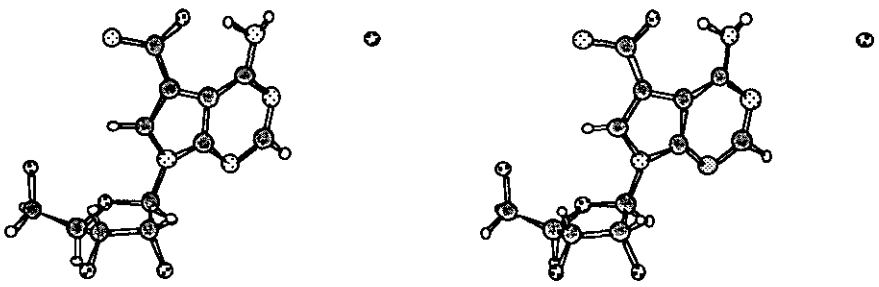


Figure 5 2'-*C*-Methylsangivamycin (**2**)

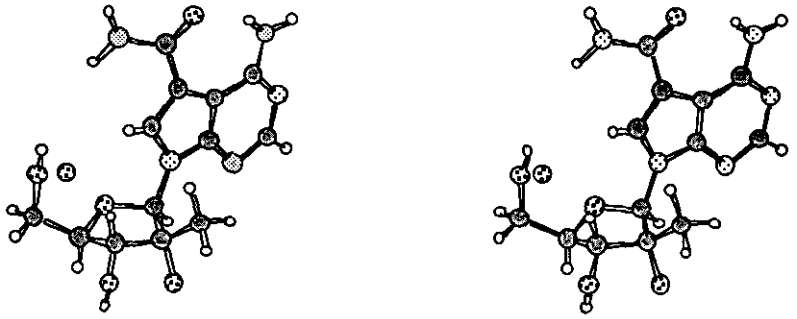


Figure 6 3'-*C*-Methylsangivamycin (**3**)

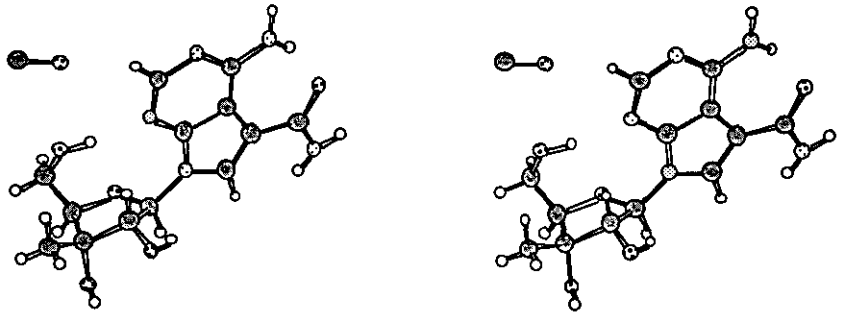
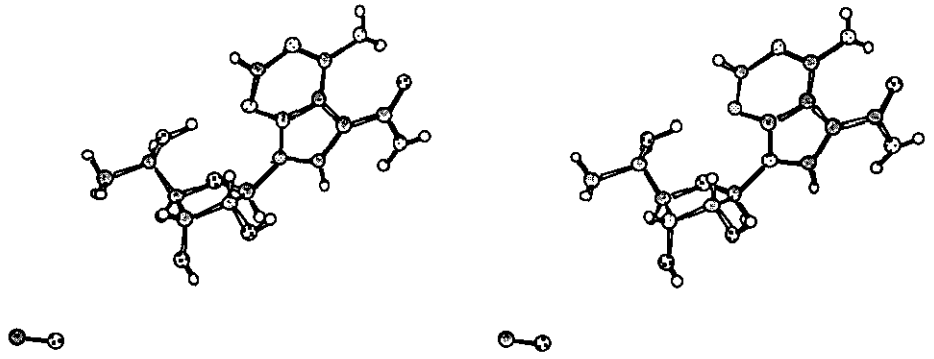


Figure 7 5'(*R*)-*C*-Methylsangivamycin (**4**)



EXPERIMENTAL SECTION

General Procedures. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from potassium prior to use. Acetonitrile (MeCN) and acetone were distilled and stored over 4Å molecular sieves. Methylene chloride (CH_2Cl_2), dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were dried by storing over 4Å molecular sieves. All other solvents and reagents were of the best commercial grade available and used without further purification. Column chromatography was performed by using Wakogel C-200 or C-300, and thin-layer chromatography (tlc) was carried

out on 0.25-mm E. Merck precoated silica-gel glass plate (60 F_{254}). Melting points were determined on a Büchi 520 and are uncorrected. Ir spectra were recorded on a Hitachi 260-10 spectrometer. ^{1}H Nmr spectra were recorded on a Jeol FX 270 spectrometer (270 MHz). High and low mass spectra were recorded on a Hitachi M-80 spectrometer.

X-Ray Studies. The crystals for X-ray diffraction studies were obtained by recrystallization from methanol-water. All measurements were made on a Rigaku AFC5R diffractometer with graphite monochromated Cu-Kα radiation at room temperature and were summarized in Table 2. In the case of **4**, an empirical absorption correction, based on azimuthal scans of several reflection, was applied which resulted in transmission factors ranging from 0.97 to 1.00. The data were corrected for Lorentz and polaization effects. The structure were solved by direct methods. All calculations were performed using TEXSAN crystallographic software package of Molecular Structure Corporation. Crystallographic data has been deposited with the Cambridge Crystallographic Data Center, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, England.

Table 2

	2'-*C*-Methyl **2**	3'-*C*-Methyl **3**	5'(*R*)-*C*-Methyl **4**
Empirical Formula	$C_{13}H_{17}N_5O_5$	$C_{13}H_{17}N_5O_5$	$C_{13}H_{17}N_5O_5$
Formula Weight	323.31	323.31	323.31
Crystal Size (mm)	0.15 x 0.1 x 0.2	0.05 x 0.1 x 0.15	0.05 x 0.05 x 0.1
Crystal System	orthorhombic	monoclinic	monoclinic
No. of Reflections used Unit Cell Determination	25 (81.1 - 96.3°)	24 (32.1 - 56.4)	24 (61.2 - 98.1)
Lattice Parameters:	*a* = 11.6580 (9) Å *b* = 17.210 (2) Å *c* = 7.508 (1) Å *V* = 1506.2 (3) Å	*a* = 18.426 (2) *b* = 6.6247 (5) *c* = 14.106 (2) β = 118.346 (7) ° *V* = 1515.4 (3)	*a* = 18.384 (2) *b* = 6.658 (2) *c* = 14.008 (2) β = 117.875 (8) *V* = 1515.7 (8)
Space Group	$P2_12_12_1$	C2	C2
Z value	4	4	4
D calcd	1.426 g/cm^3	1.417	1.417
F(000)	680	680	680
μ (CuKα)	9.03 cm^{-1}	8.98	8.97
2θ max	119.9°	120.2	120.0
No. of Reflections Measured	Total: 1339	Total: 1302 Unique: 1246 (R_{int} = 0.038)	Total: 1304 Unique: 1248 (R_{int} = 0.020)
No. Observations (I>3.00σ(I))	1121	1012	824
No. Variables	285	289	281
Residuals: R; R_W	0.044; 0.057	0.037; 0.044	0.052; 0.058

1,2,3-Tri-*O*-acetyl-5-*O*-benzoyl-5(*R*)-*C*-methyl-β-D-ribofuranose (9). To a solution of **8**[4] (1.3 g, 6 mmol) in pyridine (10 ml) was added benzoyl chloride (1.5 ml, 13 mmol) with ice cooling. The mixture was stirred for 24 h at 0 °C, and partitioned between AcOEt and water. The organic layer was separated, washed with brine, dried over $MgSO_4$ and concentrated. The residue was chromatographed on silica gel with hexane-AcOEt (4:1 to 3:1) to give methyl 2,3-*O*-isopropylidene-5-*O*-benzoyl-5(*R*)-*C*-methyl-D-ribofuranoside (1.62 g, 84%). To methyl 2,3-*O*-isopropylidene-5-*O*-benzoyl-5(*R*)-*C*-methyl-D-ribofuranoside (635 mg, 2.0 mmol) was added 90% trifluoroacetic acid (5 ml) at 25 °C. The reaction mixture was stirred for 4 h at 25 °C, then concentrated. The residue was dissolved in toluene and concentrated to dryness, then dissolved in pyridine (4 ml) and acetic anhydride (2 ml). The mixture was stirred for 20 h at 25 °C, and partitioned between AcOEt and ice-water. The organic layer was separated, washed with brine, dried over $MgSO_4$ and concentrated. The crude product was purified by silica gel column chromatography with hexane-AcOEt (2:1) to afford **9** (710 mg, 76 %): Ir (neat) 1740, 1720 cm^{-1}; ^1H nmr ($CDCl_3$) δ 1.43 (3H, d, J = 6.6 Hz), 2.00 (3H, s), 2.05 (3H, s), 2.12 (3H, s), 4.13 (1H, dd, J = 6.9, 7.4 Hz), 4.39 (1H, dd, J = 3.0, 7.4 Hz), 5.25-5.41 (3H, m), 7.45 (2H, t, J = 8.3 Hz), 7.58 (1H, t, J = 8.3 Hz), 8.08 (2H, d, J = 8.3 Hz); SIms *m/z* 335 (M^+-AcO).

1,2,3-Tri-*O*-acetyl-5-*O*-benzoyl-5(*S*)-*C*-methyl-β-D-ribofuranose (10). To a solution of **8** (1.09 g, 5 mmol) in THF (20 ml) was added diethyl azodicarboxylate (2.6 ml, 16 mmol), benzoic acid (1.5 g, 8 mmol) and triphenylphosphine (3.2 g, 12 mmol) at 5 °C. The reaction mixture was stirred for 24 h at 25 °C and concentrated. The residue was chromatographed on silica gel with hexane-AcOEt (4:1 to 3:1) to give methyl 2,3-*O*-isopropylidene-5-*O*-benzoyl-5(*S*)-*C*-methyl-D-ribofuranoside (850 mg, 53%). Triacetate **10** was prepared in the same way as used for **9** in 91% yield. **10**: Ir (neat) 1750, 1720 cm^{-1}; ^1H nmr ($CDCl_3$) δ 1.38 (3H, d, J = 6.6 Hz), 1.77 (3H, s), 2.02 (3H, s), 2.13 (3H, s), 4.32 (1H, dd, J = 4.3, 6.9 Hz), 5.30-5.46 (3H, m), 5.61 (1H, dd, J = 2.3, 5.0 Hz), 7.43 (2H, dd, J = 7.3, 8.3 Hz), 7.67 (1H, t, J = 7.3 Hz), 8.05 (2H, d, J = 8.3 Hz); SIms *m/z* 335 (M^+-AcO).

General Procedure for TMSOTf Mediated Nucleoside Formation. To a mixture of 1,2,3-Tri-*O*-acetyl-5-*O*-benzoyl-D-ribofuranose derivative (**6**, **7**, **9** or **10**) (1.3 mmol), 4-chloro-5-cyanopyrrolo[2,3-*d*]pyrimidine[5] (**11**) (178 mg, 1 mmol), HMDS (0.11 ml, 0.5 mmol), and TMSCl (0.06 ml, 0.5 mmol) in MeCN (14 ml) was added TMSOTf (0.34 ml, 1.7 mmol) and the reaction mixture was refluxed for 20-24 h. After being cooled, the reaction mixture was partitioned between AcOEt and ice-water containing $NaHCO_3$. The organic layer was

separated, washed with water, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography with hexane-AcOEt (1:1) as an eluant.

4-Chloro-5-cyano-7-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*] pyrimidine: ^{1}H Nmr ($CDCl_3$) δ 2.08 (3H, s), 2.16 (3H, s), 2.17 (3H, s), 4.41-4.51 (3H, m), 5.50-5.55 (1H, m), 5.70-5.75 (1H, m), 6.40 (1H, d, *J* = 5.3 Hz), 8.07 (1H, s), 8.78 (1H, s).

4-Chloro-5-cyano-7-(2',3'-di-*O*-acetyl-5'-*O*-benzoyl-3'-*C*-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (12): ^{1}H Nmr ($CDCl_3$) δ 1.81 (3H, s), 4.56 and 4.84 (2H, dq, *J* = 12.5, 2.0 Hz), 5.03 (1H, t, *J* = 3.6 Hz), 5.84 (1H, d, *J* = 7.6 Hz), 6.48 (1H, d, *J* = 7.6 Hz), 7.50 (2H, t, *J* = 7.3 Hz), 7.65 (1H, t, *J* = 7.3 Hz), 7.90 (1H, s), 8.06 (2H, d, *J* = 7.3 Hz), 8.70 (1H, s).

4-Chloro-5-cyano-7-(2',3'-di-*O*-acetyl-5'-*O*-benzoyl-5'(*R*)-*C*-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (13): ^{1}H Nmr ($CDCl_3$) δ 1.47 (3H, d, *J* = 6.9 Hz), 2.05 (3H, s), 2.18 (3H, s), 4.37 (1H, t, *J* = 3.0 Hz), 5.54 (1H, dq, *J* = 3.0, 6.6 Hz), 5.84-5.92 (2H, m), 6.35 (1H, d, *J* = 5.9 Hz), 7.52 (2H, t, *J* = 7.6 Hz), 7.59 (1H, s), 7.65 (1H, t, *J* = 7.6 Hz), 8.10 (1H, s), 8.52 (1H, s).

4-Chloro-5-cyano-7-(2',3'-di-*O*-acetyl-5'-*O*-benzoyl-5'(*S*)-*C*-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (14): ^{1}H Nmr ($CDCl_3$) δ 1.53 (3H, d, *J* = 6.6 Hz), 2.06 (3H, s), 2.15 (3H, s), 4.40 (1H, dd, *J* = 3.6, 5.0 Hz), 5.48-5.56 (3H, m), 5.70 (1H, t, *J* = 5.3 Hz), 6.45 (1H, d, *J* = 5.0 Hz), 7.50 (2H, t, *J* = 8.2 Hz), 7.63 (1H, t, *J* = 8.2 Hz), 8.00 (2H, d, *J* = 8.2 Hz), 8.18 (1H, s), 8.71 (1H, s).

General Procedure for Ammonolysis and Amination with Methanolic Ammonia: The protected nucleoside obtained above (1.0 mmol) in MeOH-NH_3 (saturated at 0 °C; 60 ml) was heated at 110 °C for 8 h in a steel reaction vessel. The steel vessel was cooled, opened, and contents were concentrated. The residue was chromatographed on Diaion HP-20 (50 ml). After being washed with water (100 ml), the product was eluted with 50 % aq. MeOH. The product was recrystallized from MeOH-H_2O.

4-Amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (toyocamycin) (15) : mp 211-212 °C (from MeOH-H_2O) (lit.,[5] mp 243 °C); ir (nujol) 3300, 3100, 2230, 1660, 1595 cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 3.56 (1H, ddd, *J* = 4.0, 6.0, 12.2 Hz), 3.63-3.74 (1H, m), 3.92 (1H, dt, *J* = 3.9, 4.0 Hz), 4.07-4.12 (1H, m), 4.34-4.40 (1H, m), 5.21 (1H, *J* = 5.0 Hz), 5.23 (1H, dd, *J* = 4.6, 5.0 Hz), 5.49 (1H, d, *J* = 5.9 Hz), 6.06 (1H, d, *J* = 5.9 Hz), 6.93 (2H, br s), 8.22 (1H, s), 8.45 (1H, s).

4-Amino-5-cyano-7-(3'-*C*-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (16): mp 259-260 °C (from MeOH-H_2O); ir (nujol) 3450, 3300, 3200, 2230, 1630, 1595

cm^{-1}; ^{1}H nmr (DMSO-d$_6$) δ 1.27 (3H, s), 3.54 (1H, ddd, *J* = 3.0, 6.3, 12.2 Hz), 3.59-3.74 (1H, m), 3.86 (1H, dd, *J* = 2.6, 3.0 Hz), 4.28 (1H, dd, *J* = 6.9, 7.9 Hz), 4.87 (1H, s), 5.40 (1H, d, *J* = 6.9 Hz), 5.43 (1H, dd, *J* = 5.6, 6.3 Hz), 6.03 (1H, d, *J* = 7.9 Hz), 6.93 (2H, br s), 8.21 (1H, s), 8.44 (1H, s); HRms Calcd for $C_{13}H_{15}N_5O_4$: 305.1122. Found: 305.1089.

4-Amino-5-cyano-7-(5'(*R*)-*C*-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (17): mp 189-190 °C (from MeOH-H$_2$O); ir (nujol) 3350, 3100, 2230, 1660, 1600 cm^{-1}; ^{1}H nmr (DMSO-d$_6$) δ 1.08 (3H, d, *J* = 6.3 Hz), 3.73 (1H, dd, *J* = 1.7, 4.3 Hz), 3.77-3.87 (1H, m), 4.12-4.16 (1H, m), 4.43 (1H, dd, *J* = 5.3, 6.9 Hz), 5.18 (1H, d, *J* = 4.3 Hz), 5.35 (1H, d, *J* = 4.3 Hz), 5.39 (1H, d, *J* = 6.5 Hz), 6.00 (1H, d, *J* = 6.9 Hz), 6.94 (2H, br s), 8.21 (1H, s), 8.45 (1H, s); HRms Calcd for $C_{13}H_{15}N_5O_4$: 305.1122. Found: 305.1082.

4-Amino-5-cyano-7-(5'(*S*)-*C*-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (18): mp 200-201 °C (from MeOH-H$_2$O); ir (nujol) 3400, 3300, 3100, 2220, 1640, 1590 cm^{-1}; ^{1}H nmr (DMSO-d$_6$) δ 1.12 (3H, d, *J* = 6.6 Hz), 3.73-3.83 (2H, m), 4.03-4.09 (1H, m), 4.28-4.34 (1H, m), 4.39-4.46 (1H, m), 5.12 (1H, d, *J* = 5.0 Hz), 5.18 (1H, d, *J* = 5.9 Hz), 5.43 (1H, d, *J* = 5.9 Hz), 6.04 (1H, d, *J* = 5.6 Hz), 6.91 (2H, br s), 8.21 (1H, s), 8.47 (1H, s); HRms Calcd for $C_{13}H_{15}N_5O_4$: 305.1122. Found: 305.1092.

6-Bromo-5-cyano-4-tritylaminopyrrolo[2,3-*d*]pyrimidine (22). A mixture of trityl chloride (2.39 g, 8.58 mmol), **21**[5] (817 mg, 3.43 mmol) and *N*,*N*-dimethylaminopyridine (1.05 g, 8.58 mmol) in pyridine (8 ml) was heated at 90 °C for 36 h. After being cooled, the reaction mixture was diluted with benzene (40 ml) and filtered. The filtrate was concentrated (*ca.* 4 ml) and chromatographed on silica gel with hexane-AcOEt (3:2) to give **22** (1.38 g, 84 %): mp 249-250 °C (from EtOH); ir (KBr) 3420, 2230, 1590, 1580 cm^{-1}; ^{1}H nmr (CDCl$_3$) δ 7.05 (1H, s), 7.24-7.35 (15H, m), 7.98 (1H, s). Anal. Calcd for $C_{26}H_{18}N_5Br$: C, 65.01; H, 3.77; N, 14.58. Found: C, 65.37; H, 4.05; N, 14.35.

5-Cyano-4-tritylaminopyrrolo[2,3-*d*]pyrimidine (23). A solution of **22** (712 mg, 1.5 mmol) in conc. ammonium hydroxide-ethanol (1:2, 60 ml) was stirred with 10% Pd/C (127 mg) under an atmosphere of hydrogen for 27 h at 25 °C. The mixture was filtered through celite and the catalyst was washed with conc. ammonium hydroxide-ethanol (1:2, 30 ml). The filtrate was evaporated, purified with silica gel column chromatography with Et$_2$O-CH$_2$Cl$_2$ (1:2) to give **23** (453 mg, 76 %): mp 272-273 °C (from AcOEt); ir (CHCl$_3$) 3450, 2235, 1595 cm^{-1}; ^{1}H nmr (CDCl$_3$) δ 7.04 (1H, s), 7.25-7.37 (15H, m), 7.58 (1H, s), 8.04 (1H, s). Anal. Calcd for $C_{26}H_{19}N_5$-0.25H$_2$O: C, 76.92; H, 4.84; N, 17.25. Found: C, 77.00; H, 4.74; N, 17.40.

Nucleoside Formation under the Basic Conditions. A mixture of **23** (60 mg, 0.15 mmol), NaH (60% oil dispersion, 8 mg, 0.17mmol) and MS 4Å (60 mg) in MeCN (1.5 ml) was stirred for 1 h at 25 °C. To this reaction mixture was added a solution of **19** (97 mg, 0.3 mmol) in THF (1.5 ml). After being stirred for 17 h at 50 °C, the reaction mixture was diluted with AcOEt (9 ml), filtered and concentrated. The residue was chromatographed on silica gel with hexane-AcOEt (3:1) to give the crude protected nucleoside of **15** (114 mg) and **24** (40 mg, 29%). The protected nucleoside of **15**: ^{1}H Nmr ($CDCl_3$) δ 0.06 (6H, s), 0.89 (9H, s), 1.27 (3H, s), 1.53 (3H, s), 4.25-4.30 (1H, m), 6.21 (1H, s), 6.84 (1H, s), 7.10-7.30 (15H, m), 7.85 (1H, s), 7.97 (1H, s). **24**: ^{1}H Nmr ($CDCl_3$) δ 0.06 (6H, s), 0.86 (9H, s), 1.42 (3H, s), 1.67 (3H, s), 3,82 (1H, dd, J = 3.3, 11.0 Hz), 3.96 (1H, dd, J = 2.3, 11.0 Hz), 4.40-4.45 (1H, m), 4.87-4.93 (1H, m), 5.02-5.07 (1H, m), 6.49 (1H, s), 7.20-7.35 (15H, m), 7.51 (1H, m), 7.99 (1H, s), 8.29 (1H, s); FDms *m/z* 688 (M^+).

To the crude protected nucleoside of **15** obtained above (114 mg) was added 90% trifluoroacetic acid (1.2 ml) at 25 °C. The reaction mixture was stirred for 2 h, then concentrated to dryness. The residue was dissolved in water and neutralized with $NaHCO_3$ and choromatographed on Diaion HP-20 (10 ml). After being washed with water (20 ml), the crude product was eluted with 50% aq. MeOH. The crude **15** was purified by silica gel column chromatography with $CHCl_3$-MeOH (5:1) as an eluent. The spectral data were identical with **15** obtained by the TMSOTf mediated coupling reaction.

2,3-*O*-Isopropylidene-1-hydroxy-2-*C*-methyl-5-*O*-trityl-D-ribofuranose (26). To a solution of **25**[3b] (5.55 g, 8.76 mmol) in CH_2Cl_2 (50 ml) was added ethyl vinyl ether (35 ml) and PPTS (350 mg). The reaction mixture was stirred for 48 h at 25 °C and partitioned between AcOEt and water. The organic layer was separated, washed with brine, dried over $MgSO_4$ and concentrated to give an oily residue (6.5 g). To a solution of this oily residue (623 mg, 0.86 mmol) in THF (4 ml) was added Super-Hydride® (1.0 M THF solution, 4.3 ml). The reaction mixture was refluxed for 2 h and cooled. To the reaction mixture was added 4N NaOH (4 ml) and H_2O_2 (aq. 35% solution, 6 ml) with ice cooling. After being stirred for 30 min, the reaction mixture was washed with 10% $Na_2S_2O_3$ and brine, dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography with hexane-ether (2:1) to afford the ethoxyethyl derivative of **26** (290 mg, 62%). To a solution of the ethoxyethyl derivative of **26** (670 mg, 1.26 mmol) in methanol (14 ml) was added PPTS (30 mg). The reaction mixture was stirred for 2 h at 25 °C, then methanol was evaporated (1/2 volume). This methanol solution was partitioned between AcOEt and water contained $NaHCO_3$. The organic layer was separated, washed with brine, dried over $MgSO_4$ and

concentrated to give an oily residue. The residue was purified by silica gel column chromatography with hexane-AcOEt (4:1) to give **26** (340 mg, 58%): mp 120-121 °C (from hexane-Et_2O); ir (KBr) 3480, 1595 cm^{-1}; ^1H nmr ($CDCl_3$) δ 1.45 (3H, s), 1.52 (3H, s), 1.64 (3H, s), 3.07-3.39 (2H, m), 3.57 (1H, d, *J* = 7.9 Hz), 3.84 (1H, d, *J* = 9.6 Hz), 4.15-4.40 (2H, m), 5.13-5.29 (1H, m), 7.20-7.50 (15H, m); FDms *m/z* 446 (M^+). Anal. Calcd for $C_{28}H_{30}O_5$: C, 75.34; H, 6.73. Found: C, 75.19; H, 6.77.

2,3-*O*-Isopropylidene-2-*C*-methyl-5-*O*-trityl-D-ribofuranosyl chloride (27). Phosphorous trisdimethylamide (0.18 ml, 1.05 mmol) was added to a solution of **26** (434 mg, 0.97 mmol) and CCl_4 (0.87 ml) in Et_2O (8.6 ml) at -70 °C. After being stirred in an ice bath, the reaction mixture was partitioned between ether and water. The organic layer was separated, washed with water, dried over $MgSO_4$ and concentrated to give **27** as an oil (453 mg, 100%). The product was used without further purification for the next step.

4-Amino-5-cyano-7-(2'-*C*-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (29): A mixture of **23** (60 mg, 0.15 mmol), NaH (60% oil dispersion, 8 mg, 0.17 mmol) and MS 4Å (60 mg) in MeCN (1.5 ml) was stirred for 1 h at 25 °C. Then the solution of **27** (171 mg, 0.3 mmol) in THF (1.5 ml) was added to the reaction mixture. After being stirred for 5 day at 50 °C, the reaction mixture was diluted with AcOEt (9 ml) , filtered and concentrated. The residue was chromatographed on silica gel with hexane-AcOEt (4:1) to give the protected nucleoside (**28β**) as a mixture with **27** (85 mg, *ca.* 25% yield estimated by integration of ^1H nmr), **28α** (12 mg, 8%), **30** (less polar 9 mg, 6% and more polar 21 mg, 15 %). **28α:** ^1H Nmr ($CDCl_3$) δ 1.24 (3H, s), 1.39 (3H, s), 1.52 (3H, s), 3.15-3.45 (2H, m), 4.10-4.50 (2H, m), 6.63 (1H, s), 6.93 (1H, s), 7.20-7.50 (30H, m), 7.87 (1H, s), 8.03 (1H, s). **28β:** ^1H Nmr ($CDCl_3$) δ 6.45 (1H, s), 6.90 (1H, s), 7.10-7.50 (30H, m), 7.57 (1H, s), 8.03 (1H, s). **30** (less polar): ^1H Nmr ($CDCl_3$) δ 0.85 (3H, s), 1.20 (3H, s), 1.60 (3H, s), 3.30 (2H, d, *J* = 5.6 Hz), 4.30 (1H, s), 4.54 (1H, t, *J* = 5.6 Hz), 6.55 (1H, s), 7.1-7.5 (31H, m), 7.87 (1H, s), 7.98 (1H, s). **30** (more polar): ^1H Nmr ($CDCl_3$) δ 0.92 (3H, s), 1.41 (3H, s), 1.65 (3H, s), 3.37 (2H, s), 4.39 (2H, s), 6.70 (1H, s), 7.1-7.4 (30H, m), 7.50 (1H, s), 7.93 (1H, s), 7.94 (1H, s).

To the crude nucleoside (**28β**) obtained above (85 mg, *ca.* 50% purity) was added 90% trifluoroacetic acid (0.85 ml) at 25 °C. The reaction mixture was stirred for 2 h, then concentrated to dryness. The residue was dissolved in water and neutralized with $NaHCO_3$ (pH 7-8) and chromatographed on Diaion HP-20 (5 ml). After being washed with water (10 ml), the crude product was eluted with 50 % aq. MeOH. The crude **29** was purified by silica gel column chromatography with $CHCl_3$-MeOH (5:1) as an eluant: mp 115-125 °C (from

MeOH) (decomp.); ir (nujol) 3320, 2220, 1620, 1590 cm^{-1}; ^1H nmr (DMSO-d_6) : 0.72 (3H, s), 3.67-3.98 (4H, m), 5.18 (1H, d, J = 6.3 Hz), 5.22-5.28 (1H, m), 5.28 (1H, s), 6.14 (1H, s), 6.88 (2H, br s), 8.23 (1H, s), 8.54 (1H, s); HRms Calcd for $C_{13}H_{15}N_5O_4$: 305.1122. Found: 305.1205.

General Procedure for Conversion of Cyano Group into Carboxamide Group. A mixture of 5-cyano derivatives (0.1 mmol), H_2O_2 (aq. 35% solution, 0.2 ml), and conc. NH_4OH (2 ml) was stirred at room temperature for 1 h. The reaction mixture was concentrated and the product was crystallized from MeOH-H_2O.

4-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (sangivamycin) (1): mp 252-253 °C (from MeOH-H_2O) (lit.,[5] mp 260 °C); ir (nujol) 3450, 3230, 1630 cm^{-1}; ^1H nmr (DMSO-d_6) δ 3.62 (1H, ddd, J = 4.0, 5.3, 12.2 Hz), 3.71 (1H, ddd, J = 4.0, 6.3, 12.2 Hz), 3.90 (1H, dt, J = 3.9, 4.0 Hz), 4.08 (1H, dd, J = 3.9, 5.0 Hz), 4.34 (1H, dd, J = 5.9, 6.3 Hz), 5.14 (1H, dd, J = 5.3, 6.3 Hz), 5.20 (1H, d, J = 5.0 Hz), 5.43 (1H, d, J = 6.3 Hz), 6.01 (1H, d, J = 5.9 Hz), 7.40 (2H, br s), 7.90 (2H, br s), 8.06 (1H, s), 8.10 (1H, s).

4-Amino-7-(2'-*C*-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (2): mp 182-183 °C (from MeOH-H_2O); ir (nujol) 3350, 3120, 1600 cm^{-1}; ^1H nmr (DMSO-d_6) δ 0.72 (3H, s), 3.60-3.95 (4H, m), 4.89 (1H, s), 5.14 (1H, d, J = 4.6 Hz), 6.18 (1H, s), 7.35 (2H, br s), 7.90 (2H, br s), 7.96 (1H, s), 8.08 (1H, s); HRms Calcd for $C_{13}H_{17}N_5O_5$: 323.1227. Found: 323.1247. Anal. Calcd for $C_{13}H_{17}N_5O_5$-H_2O: C, 45.74; H, 5.61; N, 20.52. Found: C, 45.39; H, 5.54; N, 20.30.

4-Amino-7-(3'-*C*-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (3): mp 234-235 °C (from MeOH-H_2O); ir (nujol) 3350, 3170, 1615 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.28 (3H, s), 3.50 (1H, ddd, J = 3.3, 6.9, 12.2 Hz), 3.63 (1H, ddd, J = 3.3, 4.3, 12.2 Hz), 3.84 (1H, t, J = 3.3 Hz), 4.26 (1H, dd, J = 6.9, 7.9 Hz), 4.82 (1H, s), 5.37 (1H, d, J = 6.9 Hz), 5.55 (1H, dd, J = 4.3, 6.9 Hz), 5.89 (1H, d, J = 7.9 Hz), 7.39 (2H, br s), 7.88 (2H, br s), 8.06 (1H, s), 8.12 (1H, s); HRms Calcd for $C_{13}H_{17}N_5O_5$: 323.1227. Found: 323.1222. Anal. Calcd for $C_{13}H_{17}N_5O_5$-0.7CH_3OH: C, 47.59; H, 5.77; N, 20.25. Found: C, 47.37; H, 5.56; N, 20.41.

4-Amino-7-(5'(*R*)-*C*-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (4): mp 197-198 °C (from MeOH-H_2O); ir (nujol) 3350, 3200, 1655, 1620 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.09 (3H, d, J = 6.3 Hz), 3.71 (1H, dd, J = 1.3, 4.0 Hz), 3.73-3.80 (1H, m), 4.09-4.15 (1H, m), 4.43 (1H, dd, J = 5.3, 6.9 Hz), 5.15 (1H, d, J = 4.3 Hz), 5.35 (1H, d, J = 6.9 Hz), 5.43 (1H, d, J = 4.0 Hz), 5.93 (1H, d, J = 7.6 Hz), 7.40 (2H, br s), 7.91 (2H, br s) 8.06 (1H, s), 8.12 (1H, s); HRms Calcd for $C_{13}H_{17}N_5O_5$: 323.1227. Found: 323.1218.

Anal. Calcd for $C_{13}H_{17}N_5O_5$-0.7CH_3OH: C, 47.59; H, 5.77; N, 20.25. Found: C, 47.29; H, 5.61; N, 20.08.

4-Amino-7-(5'(*S*)-*C*-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (5): mp 140-150 °C (from MeOH-H_2O) (decomp.); ir (nujol) 3300, 3150, 1635, 1615 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.12 (3H, d, *J* = 6.3 Hz), 3.73-3.78 (2H, m), 4.08 (1H, dd, *J* = 3.0, 5.0 Hz), 4.37 (1H, dd, *J* = 5.9, 6.3 Hz), 5.11 (1H, d, *J* = 6.6 Hz), 5.15 (1H, d, *J* = 5.0 Hz), 5.40 (1H, d, *J* = 6.3 Hz), 5.98 (1H, d, *J* = 6.3 Hz), 7.40 (2H, br s), 7.93 (2H, br s), 8.06 (1H, s), 8.11 (1H, s); HRms Calcd for $C_{13}H_{17}N_5O_5$: 323.1227. Found: 323.1257.

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