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A TOTAL SYNTHESIS OF CADEGUOMYCIN, A NUCLEOSIDE ANTIBIOTIC PRODUCED BY STREPTOMYCES HYGROSCOPICUS

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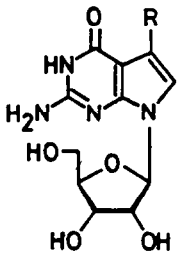
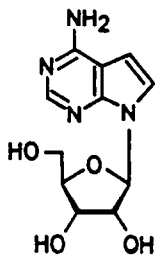
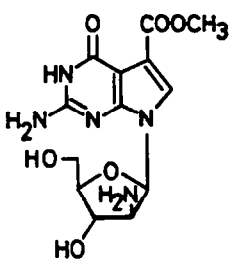
Abstract - The nucleoside antibiotic cadeguomycin, 2-amino-3,4-dihydro-4-oxo-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine-5-carboxylic acid (**1**) was synthesized from 3,4-dihydro-3-methoxymethyl-5-methyl-2-methylthio-7-(2,3-O-isopropylidene-5-O-triphenylmethyl-β-D-ribofuranosyl)-7H-pyrrolo-[2,3-d]pyrimidin-4-one (**10β**), which was effectively prepared by glycosylation of 3,4-dihydro-3-methoxymethyl-5-methyl-2-methylthio-7H-pyrrolo-[2,3-d]pyrimidin-4-one (**8**) with 2,3-O-isopropylidene-5-O-triphenylmethyl-β-D-ribofuranosyl chloride (**9**).

In 1982, a novel nucleoside antibiotic, cadeguomycin (**1**), was isolated from the culture broth of Streptomyces hygroscopicus IM7912T as a minor component concomitant with tubercidin (**2**).[1] Cadeguomycin (**1**) displays a property of enhancing incorporation of (^{3}H)thymidine into DNA of K 562 human leukemic cells and exhibits inhibitory effects on transplantable animal tumors, but shows no significant antimicrobial activity against bacteria and fungi in contrast to tubercidin (**2**).[1] It has a guanosine-like pyrrolo[2,3-d]pyrimidine nucleus, which has been found in nucleoside Q (**3**),[2] preQ$_0$ (**4**),[3] preQ$_1$ (**5**),[4] and kanagawamicin (**6**).[5] In our previous paper,[6] we reported a total synthesis of cadeguomycin (**1**) involving glycosylation with ribosyl bromide protected with benzyl groups, which were replaced with isopropylidene group in a later stage.







Cadeguomycin (**1**), R=COOH

Nucleoside Q (**3**), R=CH$_2$NH- 

preQ$_0$ (**4**), R=CN

preQ$_1$ (**5**), R=CH$_2$NH$_2$

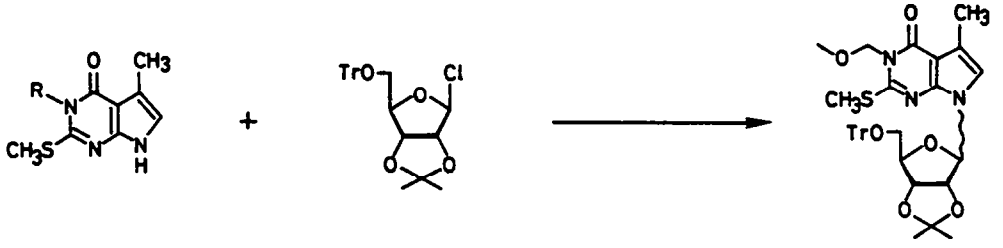
Tubercidin (**2**)

Kanagawamicin (**6**)

Fig. 1.

We now report a total synthesis of cadeguomycin (**1**) using a much improved method of glycosylation. In this case, replacement of protecting groups is unnecessary. The new synthetic route reduced the number of reaction steps and improved the α:β ratio, thus raising the yield of the desired nucleoside. 3,4-Dihydro-5-methyl-2-methylthio-7H-pyrrolo[2,3-d]pyrimidin-4-one (**7**) was synthesized according to the previously reported procedure.[8] Direct methoxymethylation of **7** was

accomplished by using n-BuLi (1.0 eq) and chloromethyl methyl ether in 1,2-dimethoxyethane (DME) to give **8** in 72% yield. In this case, 4-O-methoxymethyl isomer was formed in trace amounts, whereas more polar solvents such as N,N-dimethylformamide (DMF) were used formation of the O-protected compound increased. 2,3-O-Isopropylidene-5-O-triphenylmethyl-β-D-ribofuranosyl chloride **9** was obtained in three steps from D-(-)-ribose according to the literature.[9] Assuming that condensation reaction of the base **8** with **9** proceeds in S_N2 type, it is predicted that the α isomer is formed in preference to the β isomer. Indeed, as shown in table 1, glycosylation of **8** with **9** in DMF afforded **10αβ** in 98% yield as a 3:1 mixture of α and β isomers. It is expected that the addition of halide ion in the reaction system would cause conversion of the β-chloro ribose to the more active α form to raise the ratio of the β isomer **10β**. The best result (α:β = 1:2) was obtained when the reaction was carried out in the presence of powdered sodium bromide at room temperature.



7, R=H
8, R=CH_2OCH_3

9

10αβ

Fig. 2.

Table. 1. Glycosylation of the base **8** with the sugar **9**

Base	Additive	Solv.	Reaction		Yield (%)	**10** Ratio (α:β)
			Temp.	Time (h)		
NaH	Non	DMF	rt	4	98	3:1
NaH	NaBr	DMF	0°C-rt	10	64	1:1
NaH	NaBr	DMF	rt	4	81	1:2
NaH	NaI	DMF	rt	4	70	3:2
NaH	$MgBr_2OEt_2$	DMF	rt	12	no reaction	
NaH	$(n\text{-Bu})_4NBr$	DMF	rt	4	90	2:1

The 1:2 mixture of nucleosides **10α** and **10β** was used in the following reaction without separation. Treatment of the mixture with acetamide anion generated by fusion of fleshly sublimed acetamide and sodium hydride at 135 °C gave the corresponding acetylamino derivative **11αβ** as an inseparable mixture in 81% yield. Detritylation of **11αβ** was accomplished with hydrogen bromide in dichloromethane at 0 °C to give a mixture of **12α** and **12β** in 69% yield. The anomeric mixture **12αβ** was separated into β (44% yield) and α isomer (25% yield) by silica gel column chromatography. Acetylation of **12β** with acetic anhydride and pyridine at room temperature gave 5'-O-acetyl derivative **13** in quantitative yield, which was identical with the authentic nucleoside.[7] In order to convert **13** into cadeguomycin (**1**), the methyl group at five position must be oxidized to carboxylic acid. Prior to oxidation of the methyl group, **13** was treated with N-bromosuccinimide (NBS) in benzene to give the bromide **14** (93% yield). Further treatment of the bromide **14** with NBS in carbon tetrachloride in the presence of benzoyl peroxide (BPO) as a radical initiator afforded the dibromide, which was hydrolyzed immediately with silver carbonate to give the bromo-alcohol **15** in 81% yield. Oxidation of the alcohol **15** with active manganese(IV) oxide was accompanied partial deacetylation of N-diacetyl group to give an aldehyde, which was reacetylated with acetic anhydride and pyridine to afford the bromo-aldehyde **16** (66% yield). Conversion of the aldehyde **16** into the

carboxylic acid **17** was accomplished by the use of NBS.[10] Thus, **16** was treated with NBS in carbon tetrachloride under irradiation by a reflecting photo-lamp at room temperature to give the corresponding acid bromide, which was subsequently hydrolyzed to afford the bromo-carboxylic acid **17** in 74% overall yield from **16**. Debromination of **17** in the presence of 10% Pd-C and potassium acetate gave the corresponding carboxylic acid **18** in 74% yield. Treatment of **18** with ammonium hydroxide followed by trifluoroacetic acid (TFA) afforded almost pure cadeguomycin (**1**) in 84% yield. Further purification was carried out by ODS HPLC. The synthetic cadeguomycin was identified by comparing its physical data with that of the natural cadeguomycin (**1**).[1]

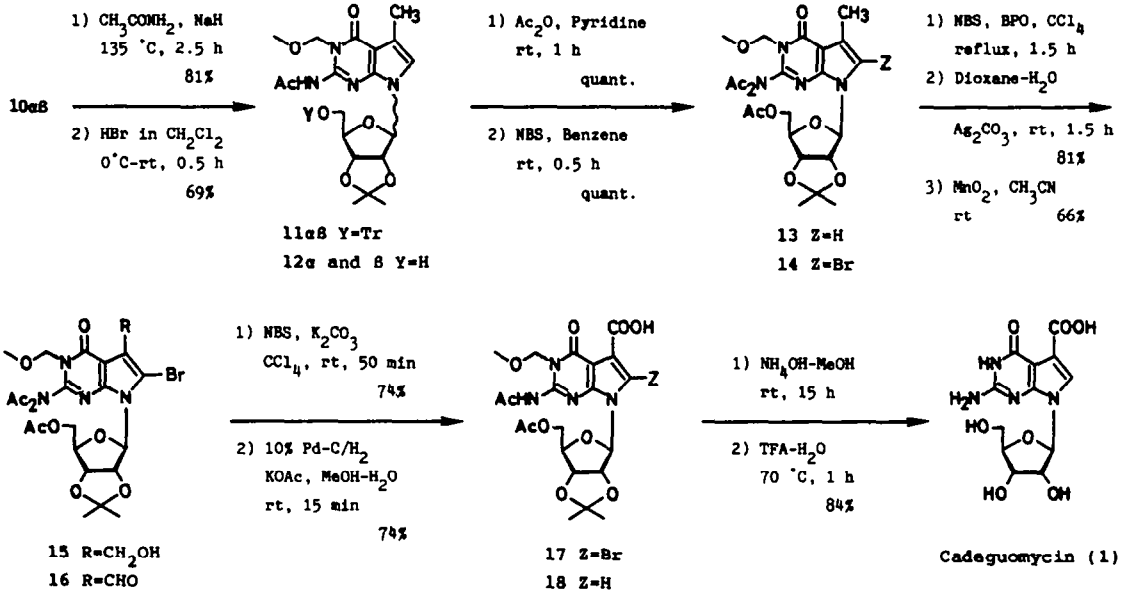


Fig. 3.

EXPERIMENTAL

General. Melting points were taken on a Mitamura Riken flat-bulb thermometer with a heating metal block and uncorrected. Elemental analyses were done on a Perkin-Elmer 240C elemental analyzer. Nuclear magnetic resonance spectra (NMR) were obtained with a JEOL FX-100 or a FX-200 instrument in the FT mode. Chemical shifts were expressed in parts per million from internal tetramethylsilane (δ) unless otherwise noted. Coupling constants are in hertz (Hz) and splitting pattern abbreviations are: s, singlet; d, doublet; q, quartet; dd, doublet of doublets; ddd, doublet of double doublets; br, broad. Mass spectra (MS) were obtained on a JEOL D-100 (EI) or DX-300 (high-resolution and FAB) spectrometer. Infrared spectra (IR) were recorded on either a Shimadzu IR 435 or a JASCO A-3 spectrophotometer. Ultraviolet spectra (UV) were measured on a Hitachi 228 spectrophotometer. Optical rotations $[\alpha]_D$ were recorded on a JASCO DIP-181 digital polarimeter.

Analytical thin-layer chromatography (TLC) was conducted on precoated TLC glass sheets (silica gel 60F-254, layer thickness 0.25 mm) manufactured by E. Merck. Preparative silica gel thick layer chromatography was performed on 20 X 20 cm glass plates coated with silica gel PF-254 (E. Merck, Darmstadt). Silica gel columns for chromatography were prepared with Merck silica gel 60 (70-230 mesh).

3,4-Dihydro-3-methoxymethyl-5-methyl-2-methylthio-7H-pyrrolo[2,3-d]pyrimidin-4-one (**8**). To a stirred solution of **7**[8] (6.0 g, 30.7 mmol) in anhydrous 1,2-dimethoxyethane (DME) was added dropwise a 1.70 M hexane solution of n-BuLi (18.0 ml, 30.6 mmol) at -40 °C under argon atmosphere. After 20 min the mixture was cooled to -60 °C and chloromethyl methyl ether (4.8 ml, 63.8 mmol) was added to it. The mixture was allowed to warm to room temperature and stirred for further 4 h. Anhydrous potassium carbonate (5g) was added to it,[11] the resulting mixture was evaporated *in vacuo*, and the residue was partitioned between CH_2Cl_2 and water. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and evaporated to give a crystalline mass, which was washed with ether to yield **8** (5.3 g, 72%) as white crystals: mp 193-194 °C; UV(MeOH) 302 (ε 11080) and 280 nm (sh, 9220); ^{1}H-NMR ($CDCl_3$) δ 2.38 (3H, d, J=1.0Hz, CH_3), 2.57 (3H, s, SCH_3), 3.46 (3H, s, OCH_3), 5.59 (2H, s, OCH_2N), and 6.53 (1H, q, J=1.0Hz, H-6); IR(KBr) 3210 (NH), 1658 (amide I), and 1585 (amide II) cm^{-1}; MS(EI) m/z 239 (M^+). Anal. Calcd for $C_{10}H_{13}N_3O_2S$: C, 50.19; H, 5.48; N, 17.56. Found: C, 50.25; H, 5.29; N, 17.55. The washings were evaporated *in vacuo* and the residue was chromatographed on a silica gel column (benzene-ethyl acetate, 6:1) to give a trace amount of *4-O-methoxymethyl-5-methyl-2-methylthio-7H-pyrrolo[2,3-d]pyrimidine*: mp 126-127 °C; ^{1}H-NMR($CDCl_3$) δ 2.40 (3H, d, J=1.0Hz, CH_3), 2.60 (3H, s, SCH_3), 3.60 (3H, s, OCH_3), 5.75 (2H, s, OCH_2O), 6.78 (1H, q, J=1.0Hz, H-6), and 9.50 (1H, br, NH); MS(EI) m/z 239 (M^+). Anal. Calcd for $C_{10}H_{13}N_3O_2S$: C, 50.19; H, 5.48; N, 17.56. Found: C, 50.33; H, 5.45; N, 17.33.

Glycosylation of the base **8** with **9**. To a mixture of **8** (4.2 g, 17.6 mmol), **9**[9] (12.0 g, 26.6 mmol), powdered sodium bromide (7.0 g, 68.0 mmol), and sodium hydride (50% oil suspension, 1.1 g, 22.9 mmol) at room temperature was added at once anhydrous DMF (60 ml) with stirring under argon atmosphere. The suspended mixture was stirred for 4 h, and poured into cold water, then extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, and the solvent was removed *in vacuo*. The crude material was purified by chromatography on a silica gel column (hexane-ethyl acetate, gradient elution from 2:1 to 1:2) to give 3,4-dihydro-3-methoxymethyl-5-methyl-2-methylthio-7-(2,3-O-isopropylidene-5-O-triphenylmethyl-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidin-4-one (**10αß**) (9.5 g, 81%) as a 1:2 mixture of α and ß isomer, which showed single spot on TLC, and could not be separated: UV(MeOH) 307 and 271 nm; ^{1}H-NMR ($CDCl_3$) δ 1.36 and 1.62 (2 X s, 2 X CH_3), 1.94 (s, α-SCH_3), 2.27 (d, J=1.0Hz, ß-CH_3-5), 2.44 (d, J=1.0Hz, α-CH_3-5), 2.53 (s, ß-SCH_3), 3.15 (dd, $J_{5',4'}$=2.4Hz, $J_{5',5'}$=10.3Hz, α-H-5'), 3.33 (dd, $J_{5',4'}$=2.4Hz, $J_{5',5'}$=10.5Hz, ß-H-5'), 3.35 (dd, $J_{5',4'}$=2.4Hz, $J_{5',5'}$=10.5Hz, ß-H-5'), 3.44 (s, ß-OCH_3), 3.45 (s, α-OCH_3), 3.62 (dd, $J_{5',4'}$=2.2Hz, $J_{5',5'}$=10.3Hz, α-H-5'), 4.3-4.4 (m, α,ß-H-4'), 4.84 (dd, $J_{3',2'}$=3.2Hz, $J_{3',4'}$=6.4Hz, ß-H-3'), 4.95 (d, J=6.1Hz, α-H-3'), 5.0-5.1 (m, α,ß-H-2'), 5.56 and 5.58 (AB quartet, J=12.0Hz, OCH_2N), 6.26 (d, $J_{1',2'}$=3.4Hz, ß-H-1'), 6.60 (q, J=1.0Hz, ß-H-6), 6.97 (q, J=1.0Hz, α-H-6), 7.08 (d, $J_{1',2'}$=3.9Hz, α-H-1'), and 7.1-7.6 (m, ArH); IR(neat) 2925, 1690 (amide I),and 1517 cm^{-1}; MS(EI) m/z 653 (M^+).

A 1:2 mixture of 2-Acetylamino-3,4-dihydro-3-methoxymethyl-5-methyl-7-(2,3-O-isopropylidene-5-O-triphenylmethyl-α and ß-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidin-4-one (**11αß**). A mixture of sodium hydride (3.2 g), acetamide (32 g, sublimed just before use), and **10αß** (6.4 g, 9.8 mmol) was heated at 135 °C for 2.5 h under argon atmosphere. After cooling, acetamide was largely removed *in vacuo*, and the residue was then carefully neutralized with 50% acetic acid under ice cooling, and extracted with benzene. The combined extracts were washed with water, saturated $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to afford a yellow syrup, which was chromatographed on a silica gel column (hexane-ethyl acetate, gradient elution from 2:1 to 1:2) to give **11αß** (5.3 g, 81%) as a pale yellow syrup: UV(MeOH) 303 and 270 nm; ^{1}H-NMR($CDCl_3$) δ 1.33 (s, α-CH_3), 1.35 (s, ß-CH_3), 1.53 (s, α-CH_3), 1.59 (s, ß-CH_3), 1.99 (s, α-CH_3CON), 2.24 (d, J=1.0Hz, ß-CH_3-5), 2.41 (d, J=1.0Hz, α-CH_3-5), 2.46 (s, ß-CH_3CON), 3.15 (dd, $J_{5',4'}$=2.4Hz, $J_{5',5'}$=10.5Hz, α-H-5'), 3.3-3.4 (m, ß-H-5'), 3.57 (dd, $J_{5',4'}$=2.2Hz, $J_{5',5'}$=10.5Hz, α-H-5'), 4.3-4.5 (m, α,ß-H-4'), 4.8-4.9 (m, α,ß-H-3'), 4.9-5.1 (m, α,ß-H-2'), 5.4-5.6 (m, OCH_2N), 6.14 (d, $J_{1',2'}$=3.2Hz, ß-H-1'), 6.67 (q, J=1.0Hz, ß-H-6), 6.81 (d, $J_{1',2'}$=4.4Hz, α-H-1'), 6.95 (q, J=1.0Hz, α-H-6), 7.1-7.5 (m, ArH), 8.34 (br. s, α-NH), and 8.40 (br. s, ß-NH); IR(neat) 3320 (NH), 1688 (amide I), and 1575 (amide II) cm^{-1}; MS(EI) m/z 664 (M^+).

2-Acetylamino-3,4-dihydro-3-methoxymethyl-5-methyl-7-(2,3-O-isopropylidene-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidin-4-one (**12β**) and its α isomer (**12α**). To a solution of **11αβ** (5.0 g, 7.5 mmol) in anhydrous CH_2Cl_2 (50 ml) was treated with a CH_2Cl_2 solution of hydrogen bromide (0.035 M, 310 ml, 10.9 mmol) at 0 °C under argon atmosphere. After stirring for 10 min at the same temperature, the mixture was allowed to warm to room temperature, and stirred for further 20 min. Saturated $NaHCO_3$ was added to it and the organic layer was separated, dried over anhydrous Na_2SO_4, and evaporated in vacuo to give a syrup, which was chromatographed on a silica gel column (ethyl acetate-acetone, 7:1). The first fraction contained β isomer **12β** (1.4 g, 44%) and the second, α isomer **12α** (0.8 g, 25%), both of which were crystallized from hexane-benzene: **12β**; mp 182-184 °C; UV(MeOH) 302 nm (ε 9840); $[\alpha]_D^{26}$ -30.5° (c 0.2, $CHCl_3$); ^{1}H-NMR($CDCl_3$) δ 1.37 (3H, s, CH_3), 1.60 (3H, s, CH_3), 2.36 (3H, d, J=1.0Hz, CH_3-5), 2.37 (3H, s, CH_3CON), 3.20 (1H, br, OH), 3.45 (3H, s, CH_3O), 3.7-3.95 (m, 2H, H-5'), 4.30 (1H, q, J=3.0Hz, H-4'), 5.06 (1H, dd, $J_{3',2'}$=6.5Hz, $J_{3',4'}$=2.4Hz, H-3'), 5.15 (1H, dd, $J_{2',1'}$=3.6Hz, $J_{2',3'}$=6.5Hz, H-2'), 5.46 and 5.61 (2H, AB quartet, J=11.0Hz, OCH_2N), 5.84 (1H, d, $J_{1',2'}$=3.6Hz, H-1'), 6.63 (1H, q, J=1.0Hz, H-6), and 8.38 (1H, br. s, NH); IR(KBr) 3478, 3299 (NH, OH), 1702, 1661 (amide I), and 1590 (amide II) cm^{-1}; MS(EI) 422 (M^+). Anal. Calcd for $C_{19}H_{26}N_4O_7$: C, 54.02; H, 6.21; N, 13.26. Found: C, 53.95; H, 6.24; N, 13.46. **12α**: mp 145-147 °C; UV(MeOH) 304 nm (ε 9570); $[\alpha]_D^{11}$ -71.3° (c 0.1, $CHCl_3$); ^{1}H-NMR($CDCl_3$) δ 1.32 (3H, s, CH_3), 1.51 (3H, s, CH_3), 1.7 (1H, br, OH), 2.38 (3H, d, J=1.5Hz, CH_3), 2.42 (3H, s, CH_3CON), 3.42 (3H, s, CH_3O), 3.7-3.9 (2H, m, H-5'), 4.3-4.4 (1H, m, H-4'), 4.7-5.0 (2H, m, H-2'and 3'), 5.51 (2H, br. s, OCH_2N), 6.48 (1H, d, $J_{1',2'}$=7.5Hz, H-1'), 6.88 (1H, q, J=1.5Hz, H-6), 8.39 (1H, br. s, NH); IR(KBr) 3440, 3300 (NH, OH), 1693, 1675 (amide I), and 1588 (amide II) cm^{-1}; MS(EI) m/z 422 (M^+). Anal. Calcd for $C_{19}H_{26}N_4O_7$: C, 54.02; H, 6.21; N, 13.26. Found: C, 54.03; H, 6.45; N, 13.08.

2-Diacetylamino-3,4-dihydro-3-methoxymethyl-5-methyl-7-(5-O-acetyl-2,3-O-isopropylidene-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidin-4-one (**13**). A mixture of **12β** (1.0 g, 2.4 mmol), acetic anhydride (2.5 ml), and anhydrous pyridine (7.0 ml) was stirred at room temperature for 1 h and evaporated in vacuo to give a syrup, which was chromatographed on a silica gel column (hexane-ethyl acetate, gradient elution from 2:1 to 2:3) and then crystallized from hexane-benzene to give **13** (1.1 g, 100%) as a white powder: mp 56-57 °C; $[\alpha]_D^{11}$ -40.0° (c 0.1, $CHCl_3$); UV(MeOH) 304 nm (ε 8480); ^{1}H-NMR($CDCl_3$) δ 1.36 (3H, s, CH_3), 1.60 (3H, s, CH_3), 2.11 (3H, s, CH_3COO), 2.36 (3H, s, CH_3CON), 2.38 (3H, s, CH_3CON), 2.41 (3H, d, J=0.7Hz, CH_3-5), 3.41 (3H, s, CH_3O), 4.18 (1H, dd, $J_{5',4'}$=6.6Hz, $J_{5',5'}$=12.7Hz, H-5'), 4.33 (1H, dd, $J_{5',4'}$=4.4Hz, $J_{5',5'}$=12.7Hz, H-5'), 4.34 (1H, ddd, $J_{4',3'}$=3.9Hz, $J_{4',5'}$=4.4 and 6.6Hz, H-4'), 4.81 (1H, dd, $J_{3',2'}$=6.3Hz, $J_{3',4'}$=3.9Hz, H-3'), 4.96 (1H, dd, $J_{2',1'}$=2.7Hz, $J_{2',3'}$=6.3Hz, H-2'), 5.29 and 5.32 (2H, AB quartet, J=10.5Hz, OCH_2N), 6.17 (1H, d, $J_{1',2'}$=2.7Hz, H-1'), and 6.75 (1H, q, J=0.7Hz, H-6); IR(KBr) 2940, 1733 (COO), 1690 (amide I), and 1575 (amide II) cm^{-1}; MS(EI) m/z 506 (M^+). Anal. Calcd for $C_{23}H_{30}N_4O_9$: C, 54.54; H, 5.97; N, 11.06. Found: C, 54.81; H, 6.11; N, 10.85.

6-Bromo-2-diacetylamino-3,4-dihydro-3-methoxymethyl-5-methyl-7-(5-O-acetyl-2,3-O-isopropylidene-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidin-4-one (**14**). To a solution of **13** (1.2 g, 2.4 mmol) in benzene (30 ml) was added NBS (465 mg, 2.6 mmol) and the mixture was stirred at room temperature for 30 min. After filtration, the filtrate was evaporated in vacuo to give a syrup, which was chromatographed on a silica gel column (hexane-ethyl acetate, gradient elution from 2:1 to 1:1) and triturated with hexane to give **14** (1.38g, 100%) as a pale yellow powder: mp 64-66 °C; UV(MeOH) 310 (ε 10730), 271 nm (6700); $[\alpha]_D^{15}$ -7.8° (c 0.1, $CHCl_3$); ^{1}H-NMR($CDCl_3$) δ 1.36 (3H, s, CH_3), 1.61 (3H, s, CH_3), 2.07 (3H, s, CH_3COO), 2.37 (3H, s, CH_3-5), 2.38 (3H, s, CH_3CON), 2.39 (3H, s, CH_3CON), 3.41 (3H, s, CH_3O), 4.11 (1H, dd, $J_{5',4'}$=6.0Hz, $J_{5',5'}$=10.3Hz, H-5'), 4.28 (1H, ddd, $J_{4',3'}$=4.4Hz, $J_{4',5'}$=4.9 and 6.0Hz, H-4'), 4.36 (1H, dd, $J_{5',4'}$=4.9Hz, $J_{5',5'}$=10.3Hz, H-5'), 4.88 (1H, dd, $J_{3',2}$=6.3Hz, $J_{3',4'}$=4.4Hz, H-3'), 5.30 (2H, s, 2H, OCH_2N), 5.31 (1H, dd, $J_{2',1'}$=2.4Hz, $J_{2',3'}$=6.3Hz, H-2'), and 6.30 (1H, d, $J_{1',2'}$=2.4Hz, H-1'); IR(KBr) 2930, 1735 (COO), 1695 (amide I), 1575, and 1560 (amide II) cm^{-1}; exact MS m/z 584.1096 (calcd for $C_{23}H_{29}N_4O_9{}^{79}Br$, 584.1118). Anal. calcd for $C_{23}H_{29}N_4O_9Br$: C, 47.19; H, 4.99; N, 9.57. Found: C, 47.34; H, 5.21; N, 9.83.

6-Bromo-2-diacetylamino-3,4-dihydro-5-hydroxymethyl-3-methoxymethyl-7-(5-O-acetyl-2,3-O-

isopropylidene-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidin-4-one (**15**). To a solution of **14** (655 mg, 1.12 mmol) in carbon tetrachloride (15 ml) were added NBS (260 mg, 1.46 mmol), anhydrous potassium carbonate (550 mg, 3.98 mmol), and benzoyl peroxide (BPO, 30 mg). The mixture was refluxed for 1.5 h with stirring under argon atmosphere. After cooling, the precipitate was removed through a glass filter and the filtrate was condensed to afford a dibromide. The crude dibromide was dissolved in dioxane-water (3/1 v/v, 60 ml) and silver carbonate (500 mg, 1.81 mmol) was added to it. The heterogeneous mixture was stirred for 1.5 h at room temperature, filtered, and evaporated *in vacuo* to give a syrup, which was chromatographed on a silica gel column (benzene-ethyl acetate, gradient elution from 2:1 to 1:1) and triturated with hexane to give **15** (545 mg, 81%) as a pale yellow powder: mp 65-67 °C; UV(MeOH) 306 (ε 10500) and 269 nm (8310); $[\alpha]_D^{10}$ -16.7° (c 0.1, $CHCl_3$); ^{1}H-NMR($CDCl_3$) δ 1.36 (3H, s, CH_3), 1.61 (3H, s, CH_3), 2.07 (3H, s, CH_3COO), 2.37 (3H, s, CH_3CON), 2.39 (3H, s, CH_3CON), 3.42 (3H, s, CH_3O), 4.09 (1H, dd, $J_{5',4'}$=6.0Hz, $J_{5',5'}$=10.3Hz, H-5'), 4.29 (1H, ddd, $J_{4',3'}$=4.4Hz, $J_{4',5'}$=4.9 and 6.0Hz, H-4'), 4.37 (1H, dd, $J_{5',4'}$=4.9Hz, $J_{5',5'}$=10.3Hz, H-5'), 4.58 (1H, br. t, J=4.2Hz, OH), 4.74 (2H, br. d, J=4.2Hz, CH_2O-5), 4.87 (1H, dd, $J_{3',2'}$=6.3Hz, $J_{3',4'}$=4.4Hz, H-3'), 5.28 (1H, dd, $J_{2',1'}$=2.4Hz, $J_{2',3'}$=6.3Hz, H-2'), 5.34 (2H, s, OCH_2N), and 6.29 (1H, d, $J_{1',2'}$=2.4Hz, H-1'); IR(KBr) 3430 (OH), 1735 (COO), 1670 (amide I), and 1570 (amide II) cm^{-1}; MS(EI) 600 and 602 (M^+). Anal. Calcd for $C_{23}H_{29}O_{10}Br$: C, 45.93; H, 4.86; N, 9.32. Found: C, 46.08; H, 4.85; N, 9.46.

6-Bromo-2-diacetylamino-3,4-dihydro-5-formyl-3-methoxymethyl-7-(5-O-acetyl-2,3-O-isopropylidene-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidin-4-one (**16**). To a solution of **15** (610 mg, 1.01 mmol) in acetonitrile was added active MnO_2 (4.0 g) in four portions every 30 min with stirring. After stirring for further 2 h at room temperature, the mixture was filtered and the solid was washed well with acetone. The filtrate was evaporated and the residual syrup, which was deacetylated partially, was acetylated with acetic anhydride (1.5 ml) and anhydrous pyridine (3.0 ml) at room temperature for 1 h. The mixture was evaporated *in vacuo* to give a syrup, which was chromatographed on a silica gel column (hexane-ethyl acetate, gradient elution from 2:1 to 1:1) and crystallized with hexane to afford **16** (402 mg, 66%): mp 57-59 °C; UV(MeOH) 296 (ε 13890) and 239 nm (15140); $[\alpha]_D^{12}$ -28.8° (c 0.1, $CHCl_3$); ^{1}H-NMR($CDCl_3$) δ 1.37 (3H, s, CH_3), 1.62 (3H, s, CH_3), 2.07 (3H, s, CH_3COO), 2.39 (3H, s, CH_3CON), 2.42 (3H, s, CH_3CON), 3.44 (3H, s, CH_3O), 4.0-4.5 (3H, m, H-4' and 5'), 4.88 (1H, dd, $J_{3',2'}$=6.8Hz, $J_{3',4'}$=3.9Hz, H-3'), 5.32 (1H, dd, $J_{2',1'}$=2.4Hz, $J_{2',3'}$=6.8Hz, H-2'), 5.37 (2H, s, OCH_2N), 6.40 (1H, d, $J_{1',2'}$=2.4Hz, H-1'), and 10.51 (1H, s, CHO); IR(KBr) 1740 (COO), 1700 (CHO), 1680 (amide I), and 1570 (amide II) cm^{-1}; MS(EI) m/z 598 and 600 (M^+). Anal. Calcd for $C_{23}H_{27}N_4O_{10}Br$: C, 46.09; H, 4.54; N, 9.35. Found: C, 45.72; H, 4.37; N, 9.66.

2-Acetylamino-6-bromo-3,4-dihydro-3-methoxymethyl-4-oxo-7-(5-O-acetyl-2,3-O-isopropylidene-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine-5-carboxylic acid (**17**). To a solution of **16** (240 mg, 0.40 mmol) in carbon tetrachloride (60 ml) were added NBS (85 mg, 0.48 mmol) and anhydrous potassium carbonate (360 mg, 2.60 mmol). The mixture was stirred with irradiation by a 500 W reflecting photo-lamp at room temperature for 50 min under argon atmosphere. The pale yellow color of the solution turned to dark brown and decolorized at the end of the reaction. The mixture was ice-cooled and dioxane-water (3/1 v/v, 4 ml) was added to it. The resulting mixture was stirred for 30 min and acidified (pH ca 2) with 2N HCl, and then partitioned with CH_2Cl_2 and water. The organic layer was separated and washed with water and brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to give a crude product, which was treated with 28% ammonium hydroxide (2 drops) in methanol (60 ml) to hydrolyze the remaining diacetate. After evaporation, the residue was purified by preparative silica gel TLC (CH_2Cl_2-MeOH, 20:1) and triturated with hexane to give the carboxylic acid **17** (170 mg, 74%) as a white powder: mp 73-75 °C; UV(MeOH) 305 (sh, ε 9930), 287 (12300), and 223 nm (18030); $[\alpha]_D^{14}$ +34.1° (c 0.1, $CHCl_3$); ^{1}H-NMR($CDCl_3$) δ 1.41 (3H, s, CH_3), 1.62 (3H, s, CH_3), 2.04 (3H, s, CH_3COO), 2.30 (3H, s, CH_3CON), 3.54 (3H, s, CH_3O), 4.0-4.5 (3H, m, H-4' and 5'), 5.22 (1H, dd, $J_{3',2'}$=6.8Hz, $J_{3',4'}$=4.0Hz, H-3'), 5.44 and 5.84 (2H, AB quartet, J=11.0Hz, OCH_2N), 5.56 (1H, dd, $J_{2',1'}$=2.1Hz, $J_{2',3'}$=6.8Hz, H-2'), 6.40 (1H, d, $J_{1',2'}$=2.1Hz, H-1'), 8.75 (1H, br. s, NH), and 13.77 (1H, br. s, COOH); IR(KBr) 3400 (NH), 1736 (COO), 1638 (amide I), and 1580 (amide II)cm^{-1}; MS(EI) m/z 572 and 574 (M^+). Anal. Calcd for $C_{21}H_{25}N_4O_{10}Br$: C, 43.99; H, 4.40;

N, 9.77. Found: C, 44.23; H, 4.27; N, 9.88.

2-Acetylamino-3,4-dihydro-3-methoxymethyl-4-oxo-7-(5-O-acetyl-2,3-O-isopropylidene-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine-5-carboxylic acid (**18**). A mixture of **17** (150 mg, 0.26 mmol), potassium acetate (1.2 g), and 10% Pd-C (450 mg) in methanol (45 ml) and water (6 ml) was stirred vigorously at room temperature for 15 min under hydrogen atmosphere. After removal of the catalyst by filtration, the filtrate was evaporated in vacuo and partitioned between CH_2Cl_2 and 1N HCl. The organic layer was separated and washed with water and brine, dried over anhydrous Na_2SO_4 and evaporated in vacuo to give a syrup, which was purified by preparative silica gel TLC (chloroform-methanol, 7:1) and crystallized from carbon tetrachloride to give **18** (96 mg, 74%) as white crystals: mp 60-62 °C; UV(MeOH) 298 (ε 8170), 281 (8840), and 226 nm (14640); $[\alpha]_D^{13}$ -11.8° (c 0.1, $CHCl_3$); ^{1}H-NMR($CDCl_3$) δ 1.37 (3H, s, CH_3), 1.61 (3H, s, CH_3), 2.14 (3H, s, CH_3COO), 2.50 (3H, s, CH_3CON), 3.52 (3H, s, CH_3O), 4.1-4.6 (3H, m, H-4' and 5'), 4.8-5.1 (2H, m, H-2' and 3'), 5.60 and 5.65 (2H, AB quartet, J=11.0Hz, OCH_2N), 6.13 (1H, d, J=2.5Hz, H-1'), 7.81 (1H, s, H-6), 8.60 (1H, br. s, NH), and 13.28 (1H, br. s, COOH); IR(KBr) 3430 (NH), 1738 (COO), 1720 (sh, COO), 1652 (amide I), and 1538 (amide II) cm^{-1}; MS(FAB) m/z 495 (M+H). Anal. Calcd for $C_{21}H_{26}N_4O_{10}$: C, 51.01; H, 5.30; N, 11.33. Found: C, 50.98; H, 5.18; N, 11.47.

2-Amino-3,4-dihydro-4-oxo-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine-5-carboxylic acid (cadeguomycin) (**1**). A solution of **18** (70 mg, 0.14 mmol) in 28% ammonium hydroxide (4 ml) and methanol (4 ml) was stirred at room temperature for 15 h and evaporated in vacuo. The residue was dissolved in trifluoroacetic acid-water (2/1 v/v, 9 ml) and heated at 70 °C for 1 h under argon atmosphere. The reaction mixture was evaporated in vacuo to give a powder, which was washed with ether to give almost pure cadeguomycin (**1**) (39 mg, 84%). A sample for analysis was obtained by purification with ODS HPLC (JASCO Finepak SIL C_{18}, at 40 °C; eluent: 20% MeOH containing 1% acetic acid): mp 230-240 °C (dec); UV(H_2O) 299 (ε 7630), 272 (6890), and 232 nm (19710); $[\alpha]_D^{12}$ -44.6° (c 0.1, Me_2SO); ^{1}H-NMR(D_2O-CF_3COOD, 4:1; internal standard: t-BuOH as 1.23 ppm; at 60 °C) δ 3.89 (1H, dd, $J_{5',4'}$=3.7Hz, $J_{5',5'}$=12.5Hz, H-5'), 3.93 (1H, dd, $J_{5',4'}$=3.0Hz, $J_{5',5'}$=12.5Hz, H-5'), 4.26 (1H, ddd, $J_{4',3'}$=3.9Hz, $J_{4',5'}$=3.0 and 3.7Hz, H-4'), 4.36 (1H, dd, $J_{3',2'}$=5.6Hz, $J_{3',4'}$=3.9Hz, H-3'), 4.51 (1H, t, $J_{2',1'}$=5.6Hz, $J_{2',3'}$=5.6Hz, H-2'), 5.93 (1H, d, $J_{1',2'}$=5.6Hz, H-1'), and 7.83 (1H, s, H-6); IR(KBr) 3450 (NH, OH) and 1655 (COO) cm^{-1}; MS(FAB) m/z 327 (M+H).

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