Tetrahedron Vol. 42, No. 1, pp. 207 to 213, 1986
Printed in Great Britain.

0040-4020/86 $3.00 + .00

SYNTHESES OF HYPERMODIFIED NUCLEOSIDE Q, AND ITS BIOSYNTHETIC PRECURSORS $PREQ_0$ AND $PREQ_1$

TADAO KONDO,† KAORU OKAMOTO, TADAAKI OHGI, and TOSHIO GOTO*

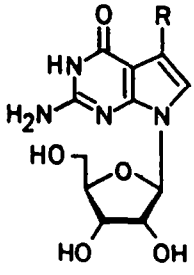
Laboratory of Organic Chemistry, Faculty of Agriculture; and †Chemical Instrument Center; Nagoya University, Chikusa, Nagoya 464, Japan

(*Received in UK 26 September 1985*)

Abstract - The nucleoside Q (1) and its biosynthetic precursors, $preQ_0$ (2) and $preQ_1$ (3) were synthesized from the key intermediate, 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 (6) by conversion of the methyl group on the base to 3S,4R,5S-4,5-dihydroxycyclopent-1-en-3-ylaminomethyl, cyano, and aminomethyl groups, respectively.

Nucleoside Q (1)[1] is a hypermodified nucleoside, which occurs in the first position of anticodon in some tRNAs, and whose structure was established by a total synthesis.[2] Nishimura et al. found that its biosynthesis is an unusual post-transcriptional modification[3] involving the precursors, $preQ_0$ (2) and/or $preQ_1$ (3). The former was isolated from $tRNA^{Tyr}$ of a mutant of Escherichia coli and the latter from E. coli methyl-deficient tRNA. The structures were determined to be 5-cyano-7-deazaguanosine (2)[4] and 5-aminomethyl-7-deazaguanosine (3)[5], respectively, by the syntheses described briefly in the previous papers.[4,5]

It seems that Q and the precursors have potential as biologically active compounds since deficiency of Q is related to tumor growth[6] and since an analogous antibiotic cadeguomycin (5-carboxy-7-deazaguanosine)(4) has activities of antitumor and immune response stimmulation.[7]



Nucleoside Q (1), R=CH_2NH- 

$preQ_0$ (2), R=CN

$preQ_1$ (3), R=CH_2NH_2

Cadeguomycin (4), R=COOH

Fig. 1.

We now report the efficient total syntheses of Q, $preQ_0$, and $preQ_1$, possessing a 7-deazaguanosine skeleton as a common structure. The protected 5-methyl-7-deazaguanosine (6) was chosen as the key intermediate because the synthesis of each nucleoside could be ideally achieved by conversion of the methyl group at 5 position on the base to the desired group such as cyano, aminomethyl, or 3S,4R,5S-4,5-dihydroxy-1-cyclopent-1-en-3-ylaminomethyl group. In turn, 6 was obtained from 5, which was prepared according to our newly developed method[8] for the large scale synthesis of cadeguomycin.

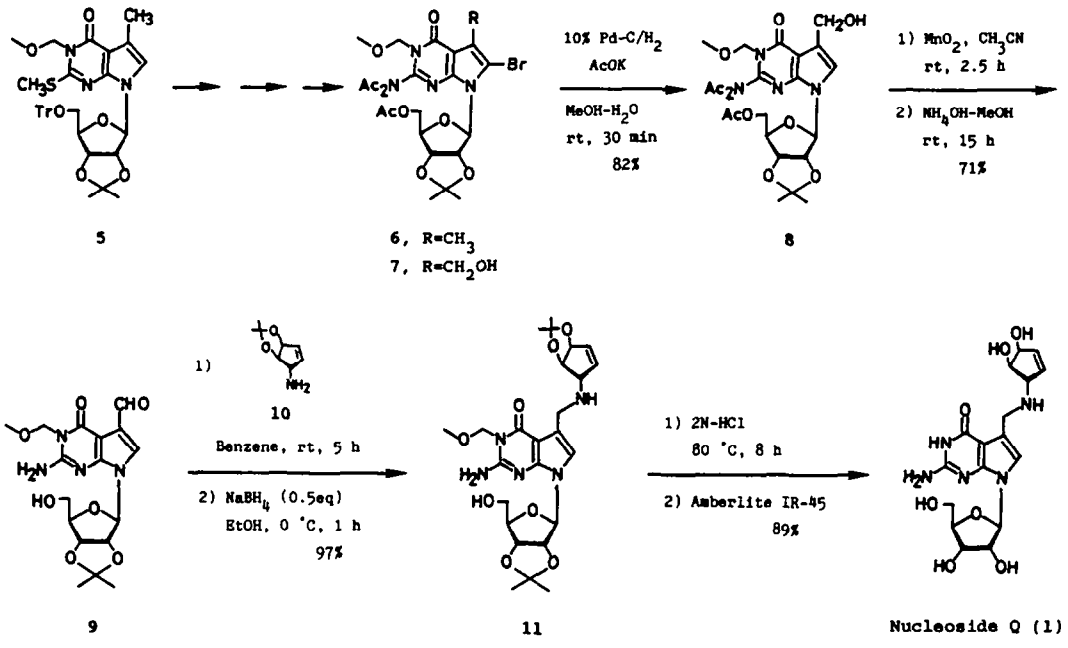


Fig. 2.

Synthesis of nucleoside Q (1)

In our total synthesis previously reported[2] there were two difficulties: (1) a large excess of the optically pure cyclopentenylamine (**10**) was necessary for condensation with **6** in order to avoid overalkylation, and (2) the subsequent debromination was unreproducible because of using a heterogeneous reagent, Zn-Cu couple.

Now we have overcome the above difficulties by adopting a new procedure involving a Shiff base of the aldehyde **9** as an intermediate. Nucleoside **6**, which was obtained from **5** by four steps,[8] was converted to the bromo-alcohol **7** by bromination and hydrolysis according to the previously reported method.[8] Prior to the oxidation of **7**, debromination at 6 position was carried out by hydrogenation in the presence of 10% Pd-C and potassium acetate to give **8** in 82% yield. Oxidation of **8** with active manganese(IV) oxide in acetonitrile gave an aldehyde, which was hydrolyzed with ammonium hydroxide in methanol to give **9** in 71% yield from **8**. The aldehyde **9** readily formed the Schiff base with 1.2 equivalents of the optically active cyclopentenylamine acetonide **10**[2] in benzene. In the ^{1}H-NMR spectrum, appearance of the signal at 8.84 ppm assignable to the imine proton and disappearance of the aldehyde proton at 10.25 ppm confirmed the structure of the Schiff base. Without purification the Schiff base was immediately reduced with sodium borohydride to afford the amine **11** in 97% yield. Deprotection of **11** with 2N HCl at 80 °C and neutralization by passing through an Amberlite IR-45 column gave nucleoside Q (1), which was further purified by use of a Sephadex G-10 column and by recrystallization from water to give pure nucleoside Q (1) as crystals in 89% yield. The physical data of the synthetic Q (1) was identical with that of natural necleoside Q.

Synthesis of preQ$_0$ (2)

PreQ$_0$ (2) was synthesized by transformation of the formyl group at the side chain of **9** to a cyano group. Thus, the treatment of the aldehyde **9** with hydroxylamine in ethanol gave an oxime **12** in 82% yield. The oxime **12** was refluxed in acetic anhydride to afford the acetylated nitrile **13** (90% yield), which exhibited a strong absorption band at 2230 cm^{-1} in IR spectrum indicating the

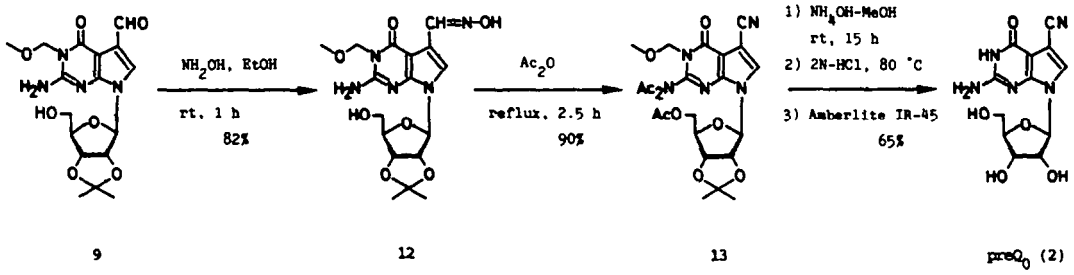


Fig. 3.

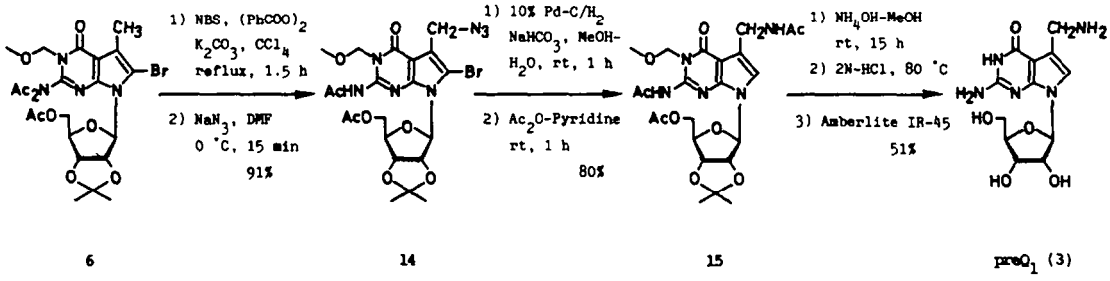


Fig. 4.

presence of a cyano group. Finally, deprotection of **13** was accomplished by treatment with ammonia-methanol followed by 2N HCl to obtain $preQ_0$ (2) hydrochloride, which was neutralized with an Amberlite IR-45 column. The synthetic $preQ_0$ (2) was identical with natural $preQ_0$ as reported in the previous paper.[4] Thus, strucure of natural $preQ_0$ was confirmed to be 2-Amino-5-cyano-7-ß-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidin-4-one.

Synthesis of $preQ_1$ (3)

We achieved a synthesis of $preQ_1$ (3) employing the key intermediate **6** as follows. Bromination of the allylic position in **6** with N-bromosuccinimide (NBS) gave a dibromide, which was treated immediately with sodium azide in N,N-dimethylformamide (DMF) at 0 °C to give azidomethyl derivative **14** in 91% yield. By catalytic hydrogenation of **14** were reduced both the azido and the bromo groups and the resulting compound was subsequently acetylated with acetic anhydride and pyridine (80% overall yield) to give **15**. The acetylation was carried out in order to make purification easier. Deacetylation of **15** was carried out by use of ammonia-methanol in the same manner as above. Reaction of removal of the remaining 2',3'-O-isopropylidene and 3-methoxymethyl protective groups with 2N HCl was monitored by ODS HPLC and stopped at the stage to afford $preQ_1$ in a maximum yield because the degradation simultaneously occurred. The crude product was purified by preparative HPLC. Natural $preQ_1$ (3) was identified by comparing its physical data with that of the synthetic $preQ_1$.[5] Thus, its structure was confirmed to be 2-Amino-5-aminomethyl-7-ß-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidin-4-one.

EXPERIMENTAL

General. Melting points were taken on a Mitamura Riken flat-bulb thermometer in 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-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 (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 60 F-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 made with Merck silica gel 60 (70-230 mesh).

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 (8). The bromo alcohol 7[8] (545 mg, 0.91 mmol) was dissolved in methanol (15 ml) and water (5 ml) containing potassium acetate (300 mg) and hydrogenated in the presence of 10% Pd-C (300 mg) at room temperature under hydrogen atmosphere for 30 min. After removal of the catalyst, the filtrate was evaporated in vacuo. The residue was partitioned between ethyl acetate and water and the organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo to give a syrup, which was chromatographed on a silica gel column (benzene-ethyl acetate, gradient elution from 1:1 to 1:2) and triturated with hexane to give 8 (390 mg, 82%) as a white powder: mp 57-59 °C, UV(MeOH) 300 (ε 8770) and 265 nm (5410); $[\alpha]_D^{12}$ -47.9° (c 0.2, $CHCl_3$); ^{1}H-NMR($CDCl_3$) δ 1.35 (3H, s, CH_3), 1.60 (3H, s, CH_3), 2.11 (3H, s, CH_3COO), 2.37 (3H, s, CH_3CON), 2.38 (3H, s, CH_3CON), 3.42 (3H, s, CH_3O), 4.20 (1H, dd, $J_{5',4'}$=6.6Hz, $J_{5',5'}$=12.7Hz, H-5'), 4.33 (1H, dd, $J_{5',4'}$=4.2Hz, $J_{5',5'}$=12.7Hz, H-5'), 4.36 (1H, ddd, $J_{4',3'}$=3.7Hz, $J_{4',5'}$=4.2 and 6.6Hz, H-4'), 4.3-4.5 (1H, br, OH), 4.75 (2H, br. s, OCH_2-5), 4.80 (1H, dd, $J_{3',2'}$=6.3Hz, $J_{3',4'}$=3.7Hz, H-3'), 4.93 (1H, dd, $J_{2',1'}$=2.9Hz, $J_{2',3'}$=6.3Hz, H-2'), 5.35 (2H, s, OCH_2N), 6.18 (1H, d, $J_{1',2'}$=2.9HZ, H-1'), and 6.94 (1H, s, H-6); IR(KBr) 3450 (OH), 1740 (COO), 1690, 1675 (amide I), and 1572 (amide II) cm^{-1}; MS(EI) m/z 522 (M^+). Anal. Calcd for $C_{23}H_{30}N_4O_{10}$: C, 52.87; H, 5.79; N, 10.72. Found: C, 52.97; H, 5.65; N, 10.93.

2-Amino-3,4-dihydro-5-formyl-3-methoxymethyl-7-(2,3-O-isopropylidene-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidin-4-one (9). To a solution of 8 (390 mg, 0.75 mmol) in acetonitrile (25 ml) was added active MnO_2 (3.0 g) in four portions every 30 min with stirring. After stirring for further 1 h at room temperature, the mixture was filtered and washed well with acetone. The filtrate was evaporated in vacuo to give a syrup, which was dissolved in a mixture of methanol (15 ml) and 28% ammonium hydroxide (15 ml) and stirred for 15 h at room temperature. The reaction mixture was dried up in vacuo to give a viscous syrup, which was chromatographed on a silica gel column (CH_2Cl_2-methanol, gradient elution from 20:1 to 10:1) and triturated with hexane to give 9 (208 mg, 71%) as a pale yellow powder: mp 89-91 °C; UV(MeOH) 317 (ε 6660), 290 (5190), and 249 nm (21300); $[\alpha]_D^{11}$ -77.4° (c 0.1, $CHCl_3$); ^{1}H-NMR($CDCl_3$) δ 1.37 (3H, s, CH_3), 1.61 (3H, s, CH_3), 3.44 (3H, s, CH_3O), 3.77 (1H, d, $J_{5',5'}$=12.2Hz, H-5'), 3.95 (1H, dd, $J_{5',4'}$=2.0Hz, $J_{5',5'}$=12.2Hz, H-5'), 4.43 (1H, t, $J_{4',3'}$=$J_{4',5'}$=2.0Hz, H-4'), 5.02 (1H, dd, $J_{3',2'}$=5.9Hz, $J_{3',4'}$=2.0Hz, H-3'), 5.13 (1H, dd, $J_{2',1'}$=4.4Hz, $J_{2',3'}$=5.9Hz, H-2'), 5.50 and 5.61 (2H, AB quartet J=10.8Hz, OCH_2N), 5.60 (2H, br. s, NH_2), 5.75 (1H, d, $J_{1',2'}$=4.4Hz, H-1'), 7.49 (1H, s, H-6), and 10.25 (1H, s, CHO); IR(KBr) 3440 (NH_2, OH), 1695 (CHO), 1662, 1630 (amide I), and 1530 (amide II) cm^{-1}; MS(FAB) m/z 395 (M+H). Anal. Calcd for $C_{17}H_{22}N_4O_7$: C, 51.77; H, 5.62; N, 14.21. Found: C, 51.47; H, 5.68; N, 14.06.

2-Amino-3,4-dihydro-5-[(3S,4R,5S)-4,5-dihydroxy-4,5-O-isopropylidenecyclopent-1-en-3-ylaminomethyl]-3-methoxymethyl-7-(2,3-O-isopropylidene-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]-

pyrimidin-4-one (11). 3S,4R,5S-4,5-Dihydroxycyclopent-1-en-3-ylamine 4,5-O-acetonide D(-)-mandelic acid salt[2] (190 mg, 0.62 mmol) was partitioned between 1N NaOH and CH_2Cl_2, and the organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residual free amine 10 was dissolved in benzene (10 ml) and this solution was added to a solution of 9 (208 mg, 0.53 mmol) in benzene (10 ml). The mixture was stirred at room temperature for 5 h and evaporated in vacuo to give the Shiff base, which was dissolved in ethanol (7 ml) and the solution was cooled to 0 °C. To this solution was added sodium borohydride (10 mg, 0.26 mmol) with stirring. After 1 h, the mixture was diluted with water (2 ml) and stirred at room temperature for further 30 min. The clear solution was dried up to give a residue, which was chromatographed on a silica gel column (CH_2Cl_2-methanol, 15:1) to give 11 (272 mg, 97%) as a yellow powder: mp 98-100 °C; UV(MeOH) 292 (ε 7100) and 264 nm (9950); $[\alpha]_D^{24}$ +11.8° (c 0.1, $CHCl_3$); ^{1}H-NMR($CDCl_3$) δ 1.35 (6H, s, CH_3), 1.39 (3H, s, CH_3), 1.60 (3H, s, CH_3), 3.41 (3H, s, OCH_3), 3.74 (1H, dd, $J_{5',4'}$=1.5Hz, $J_{5',5'}$=12.1Hz, H-5'), 3.85 (1H, d, $J_{3'',2''}$=1.5Hz, H-3"), 3.86 and 3.93 (2H, AB quartet, J=13.5Hz, CH_2N), 3.91 (1H, dd, $J_{5',4'}$=2.0Hz, $J_{5',5'}$=12.1Hz, H-5'), 4.38 (1H, dt, $J_{4',3'}$=2.0Hz, $J_{4',5'}$=1.5 and 2.0Hz, H-4'), 4.57 (1H, d, $J_{4'',5''}$=5.6Hz, H-4"), 4.99 (1H, dd, $J_{3',2'}$=6.0Hz, $J_{3',4'}$=2.0Hz, H-3'), 5.07 (1H, dd, $J_{2',1'}$=4.4Hz, $J_{2',3'}$=6.0Hz, H-2'), 5.28 (1H, dd, $J_{5'',4''}$=5.6Hz, $J_{5'',1''}$=1.5Hz, H-5"), 5.38 (2H, s, NH_2), 5.43 and 5.56 (2H, AB quartet, J=10.7Hz, OCH_2N), 5.63 (1H, d, $J_{1',2'}$=4.4Hz, H-1'), 5.85 (1H, dd, $J_{2'',1''}$=5.6Hz, $J_{2'',3''}$=1.5Hz, H-2"), 5.92 (1H, dd, $J_{1'',2''}$=5.6Hz, $J_{1'',5''}$=1.5Hz, H-1"), and 6.61 (1H, s, H-6); IR(KBr) 3430, 3330, 3200 (NH_2, OH), 1677 (amide I), and 1540 (amide II) cm^{-1}; MS(FAB) m/z 534 (M+H). Anal. Calcd for $C_{25}H_{35}N_5O_8$: C, 56.27; H, 6.61; N, 13.13. Found: C, 56.43; H, 6.84; N, 13.14.

2-Amino-3,4-dihydro-5-[(3S,4R,5S)-4,5-dihydroxycyclopent-1-en-3-ylaminomethyl]-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidin-4-one (nucleoside Q) (1). A solution of 11 (272 mg, 0.51 mmol) in 2N HCl (50 ml) was stirred at 80 °C for 8 h. The reaction was monitored by ODS HPLC (JASCO Finepak SIL C_{18}, at 40 °C; eluent: 20% methanol containing 1% acetic acid) and the mixture was dried up to afford nucleoside Q hydrochloride, which was applied to an Amberlite IR-45 column (eluent: 20% methancl). The almost pure nucleoside Q obtained was further purified by use of a Sephadex G-10 column (eluent: water) and crystallized from water to give free nucleoside Q (1) (185 mg, 89%) as pale yellow crystals: mp 225-230 °C (dec); UV(H_2O) 261 (ε 10020) and 220 nm (15450); $[\alpha]_D^{11}$ +43.4° (c 0.1, H_2O); ^{1}H-NMR(D_2O; internal standard: t-BuOH as 1.23 ppm) δ 3.82 (1H, dd, $J_{5',4'}$=4.0Hz, $J_{5',5'}$=12.5Hz, H-5'), 3.85 (1H, dd, $J_{5',4'}$=3.2Hz, $J_{5',5'}$=12.5Hz, H-5'), 4.20 (1H, ddd, $J_{4',3'}$=2.2Hz, $J_{4',5'}$=3.2 and 4.0Hz, H-4'), 4.22-4.31 (2H, m, H-3" and 4"), 4.34 (1H, dd, $J_{3',2'}$=5.4Hz, $J_{3',4'}$=2.2Hz, H-3'), 4.40 and 4.42 (2H, AB quartet, J=5.2Hz, CH_2N), 4.54 (1H, dd, $J_{2',1'}$=6.4Hz, $J_{2',3'}$=5.4Hz, H-2'), 4.67 (1H, ddd, $J_{5'',1''}$=1.5Hz, $J_{5'',4''}$=5.4Hz, $J_{5'',2''}$=2.2Hz, H-5"), 5.90 (1H, d, $J_{1',2'}$=6.4Hz, H-1'), 6.09 (1H, dd, $J_{1'',2''}$=6.4Hz, $J_{1'',5''}$=1.5Hz, H-1"), 6.27 (1H, ddd, $J_{2'',1''}$=6.4Hz, $J_{2'',5''}$=2.2Hz, $J_{2'',3''}$=2.0Hz, H-2"), and 7.18 (1H, s, H-6); IR(KBr) 3350 (NH_2, OH), 1660 (amide I), and 1635 cm^{-1} (amide II); MS(FAB) m/z 410 (M+H).

2-Amino-3,4-dihydro-5-hydroxyiminomethyl-3-methoxymethyl-7-(2,3-O-isopropylidene-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidin-4-one (12). To a stirred solution of 9 (100 mg, 0.25 mmol) in ethanol (5 ml) was added hydroxylamine (1.0 M ethanol solution, 0.3 ml, 0.30 mmol) at room temperature. The mixture was stirred for 1 h and evaporated in vacuo to give a syrup which was subjected to preparative silica gel TLC (CH_2Cl_2-methanol, 25:2) to afford 12 (85 mg, 82%) as pale yellow crystals: mp 213-215 °C; UV(MeOH) 306 (ε 9390), 284 (7730), and 245 nm (23870); $[\alpha]_D^{11}$ -45.9° (c 0.1, $CHCl_3$); ^{1}H-NMR(CD_3OD) δ 1.39 (3H, s, CH_3), 1.63 (3H, s, CH_3), 3.43 (3H, s, CH_3O), 3.77 (1H, dd, $J_{5',4'}$=3.4Hz, $J_{5',5'}$=12.2Hz, H-5'), 3.88 (1H, dd, $J_{5',4'}$=3.4Hz, $J_{5',5'}$=12.2Hz, H-5'), 4.33 (1H, ddd, $J_{4',3'}$=2.7Hz, $J_{4',5'}$=3.4 and 3.4Hz, H-4'), 5.03 (1H, dd, $J_{3',2'}$=6.4Hz, $J_{3',4'}$=2.7Hz, H-3'), 5.18 (1H, dd, $J_{2',1'}$=3.7Hz, $J_{2',3'}$=6.4Hz, H-2'), 5.49 and 5.54 (2H, AB quartet, J=10.8Hz, OCH_2N), 5.91 (1H, d, $J_{1',2'}$=3.7Hz, H-1'), 7.55 (1H, s, H-6), and 7.91 (1H, s, CH=N); IR(KBr) 3350 (NH_2, OH), 1696 (amide I), 1630, and 1550 (amide II) cm^{-1}; MS(FAB) m/z 410 (M+H). Anal. Calcd for $C_{17}H_{23}N_5O_7$: C, 49.87; H, 5.66; N, 17.11. Found: C, 50.02; H, 5.55; N, 16.93.

5-Cyano-2-diacetylamino-3,4-dihydro-3-methoxymethyl-7-(5-O-acetyl-2,3-O-isopropylidene-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidin-4-one (13). A solution of 12 (75 mg, 0.18 mmol) in

acetic anhydride (5 ml) was refluxed for 2.5 h under argon atmosphere. After evaporation, the residual oil was chromatographed on a silica gel column (hexane-ethyl acetate, 1:2) and triturated with hexane to give **13** (85 mg, 90%) as a white powder: mp 71-73 °C; UV(MeOH) 294 (ε 7150), 263 (9230), and 221 nm (18170); $[\alpha]_D^{11}$ -47.3° (c 0.1, $CHCl_3$); ^{1}H-NMR($CDCl_3$) δ 1.36 (3H, s, CH_3), 1.61 (3H, s, CH_3), 2.13 (3H, s, CH_3COO), 2.38 (3H, s, CH_3CON), 2.39 (3H, s, CH_3CON), 3.43 (3H, s, CH_3O), 4.25 (1H, dd, $J_{5',4'}$=4.9Hz, $J_{5',5'}$=12.2Hz, H-5'), 4.35 (1H, dd, $J_{5',4'}$=3.9Hz, $J_{5',5'}$=12.2Hz, H-5'), 4.44 (1H, ddd, $J_{4',3'}$=3.4Hz, $J_{4',5'}$=3.9 and 4.9Hz, H-4'), 4.81 (1H, dd, $J_{3',2'}$=6.1Hz, $J_{3',4'}$=3.4Hz, H-3'), 4.91 (1H, dd, $J_{2',1'}$=2.7Hz, $J_{2',3'}$=6.1Hz, H-2'), 5.35 (2H, s, OCH_2N), 6.18 (1H, d, $J_{1',2'}$=2.7Hz, H-1'), and 7.62 (1H, s, H-6); IR(KBr) 2230 (C≡N), 1738 (COO), 1705 (amide I), and 1580 (amide II) cm^{-1}; MS(EI) m/z 517 (M^+). Anal. Calcd for $C_{23}H_{27}N_5O_9$: C, 53.38; H, 5.26; N, 13.53. Found: C, 52.99; H, 5.26; N, 13.55.

2-Amino-5-cyano-3,4-dihydro-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidin-4-one (preQ$_0$) (**2**). A solution of **13** (80 mg, 0.16 mmol) in methanol (2 ml) and 28% ammonium hydroxide (2 ml) was stirred at room temperature for 15 h and evaporated to dryness. To the residual syrup was added 2N HCl (4 ml) and the resultant mixture was heated at 80 °C for 8 h and dried up to a syrup, which was applied to an Amberlite IR-45 column (eluent: 20% acetonitrile) to give free preQ$_0$ (**2**) (31 mg, 65%) as pale yellow crystals and showed a single peak on HPLC analysis (NOMURA ODS 5 μ, eluent: acetonitrile-water, 1:8): mp 260-270 °C (dec); UV(H_2O) 289 (ε 8750), 268 (10090), and 229 nm (19900); $[\alpha]_D^{15}$ -59.1° (c 0.1, DMSO); ^{1}H-NMR(D_2O-DMSO-D_6, 4:1; internal standard: t-BuOH as 1.23 ppm) δ 3.74 (1H, dd, $J_{5',4'}$=4.2Hz, $J_{5',5'}$=12.7Hz, H-5'), 3.83 (1H, dd, $J_{5',4'}$=3.4Hz, $J_{5',5'}$=12.7Hz, H-5'), 4.12 (1H, ddd, $J_{4',3'}$=3.9Hz, $J_{4',5'}$=3.4 and 4.2Hz, H-4'), 4.28 (1H, dd, $J_{3',4'}$=3.9Hz, $J_{3',2'}$=5.2Hz, H-3'), 4.49 (1H, dd, $J_{2',1'}$=5.6Hz, $J_{2',3'}$=5.2Hz, H-2'), 5.95 (1H, d, $J_{1',2'}$=5.6Hz, H-1'), and 7.82 (1H, s, H-6); IR(KBr) 3420 (NH_2, OH), 2230 (C≡N), 1680 (amide I), 1640, and 1598 (amide II) cm^{-1}; MS(FAB) m/z 308 (M+H).

2-Acetylamino-5-azidomethyl-6-bromo-3,4-dihydro-3-methoxymethyl-7-(5-O-acetyl-2,3-O-isopropylidene-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidin-4-one (**14**). To a solution of **6** (400 mg, 0.68 mmol) in carbon tetrachloride (8 ml) were added NBS (135 mg, 0.76 mmol), anhydrous potassium carbonate (320 mg, 2.32 mmol), and benzoyl peroxide (BPO, 10 mg). The mixture was purged with argon and refluxed for 1.5 h. After cooling, the precipitate was removed through a glass filter and the filtrate was evaporated *in vacuo* to give the dibromide. The crude dibromide in anhydrous DMF (2 ml) was dropped into a suspension of sodium azide (200 mg, 3.08 mmol) in anhydrous DMF (4 ml) at 0 °C under argon atmosphere. The mixture was stirred for 15 min, poured into water, and extracted with ethyl acetate. The organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was chromatographed on a silica gel column (benzene-ethyl acetate, gradient elution from 1:1 to 1:3) and triturated with hexane to give **14** (365 mg, 91%) as a pale yellow powder: mp 43-45 °C; UV(MeOH) 305 (ε 9690) and 272 nm (9270); $[\alpha]_D^{12}$ +44.1° (c 0.1, $CHCl_3$); ^{1}H-NMR($CDCl_3$) δ 1.42 (3H, s, CH_3), 1.61 (3H, s, CH_3), 2.06 (3H, s, CH_3COO), 2.30 (3H, s, CH_3CON), 3.48 (3H, s, CH_3O), 4.14 (1H, dd, $J_{5',4'}$=5.8Hz, $J_{5',5'}$=9.6Hz, H-5'), 4.35 (1H, ddd, $J_{4',3'}$=3.9Hz, $J_{4',5'}$=5.2 and 5.8Hz, H-4'), 4.42 (1H, dd, $J_{5',4'}$=5.2Hz, $J_{5',5'}$=9.6Hz, H-5'), 4.50 and 4.55 (2H, AB quartet, J=13.4Hz, CH_2N_3), 5.24 (1H, dd, $J_{3',2'}$=6.6Hz, $J_{3',4'}$=3.9Hz, H-3'), 5.36 and 5.77 (2H, AB quartet, J=11.0Hz, OCH_2N), 5.55 (1H, dd, $J_{2',1'}$=2.0Hz, $J_{2',3'}$=6.6Hz, H-2'), 6.21 (1H, d, $J_{1',2'}$=2.0Hz, H-1'), and 8.63 (1H, br. s, CONH); IR(KBr) 1735 (COO) and 1686 (amide I) cm^{-1}; MS(FAB) m/z 584 and 586 (M+H). Anal. Calcd for $C_{21}H_{26}N_7O_8Br$: C, 43.16; H, 4.49; N, 16.78. Found: C, 43.56; H, 4.68; N, 17.21.

5-N-Acetylaminomethyl-2-diacetylamino-3,4-dihydro-3-methoxymethyl-7-(5-O-acetyl-2,3-O-isopropylidene-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidin-4-one (**15**). A mixture of **14** (350 mg, 0.60 mmol), sodium hydrogen carbonate (350 mg) and 10% Pd-C (350 mg) in methanol (21 ml) and water (7 ml) was stirred vigorously at room temperature under hydrogen atmosphere for 45 min. After removal of the catalyst, the filtrate was evaporated and partitioned between chloroform and water. The chloroform layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was treated with anhydrous pyridine (4 ml) and acetic anhydride (2 ml) at room temperature for 1 h and concentrated. The resultant syrup was purified by preparative silica gel TLC (CH_2Cl_2-methanol,

20:1) and crystallized from hexane to give **15** (270 mg, 80%) as white crystals: mp 59-61 °C; UV(MeOH) 301 (ε 8520) and 265 nm (5490); $[\alpha]_D^{11}$ -61.8° (c 0.1, $CHCl_3$); ^{1}H-NMR($CDCl_3$) δ 1.35 (3H, s, CH_3), 1.60 (3H, s, CH_3), 1.97 (3H, s, CH_3CO), 2.13 (3H, s, CH_3CO), 2.37 (3H, s, CH_3CON), 2.40 (3H, s, CH_3CON), 3.43 (3H, s, CH_3O), 4.19 (1H, dd, $J_{5',4'}$=6.1Hz, $J_{5',5''}$=12.7Hz, H-5'), 4.33 (1H, dd, $J_{5'',4'}$=3.9Hz, $J_{5'',5'}$=12.7Hz, H-5''), 4.34 (1H, ddd, $J_{4',3'}$=3.9Hz, $J_{4',5''}$=3.9 and 6.1Hz, H-4'), 4.45 (1H, dd, J=5.9Hz, 14.9Hz, CH_2N), 4.58 (1H, dd, J=6.4Hz, 14.9Hz, CH_2N), 4.80 (1H, dd, $J_{3',2'}$=6.4Hz, $J_{3',4'}$=3.9Hz, H-3'), 4.92 (1H, dd, $J_{2',1'}$=2.7Hz, $J_{2',3'}$=6.4Hz, H-2'), 5.31 and 5.35 (2H, AB quartet, J=10.5Hz, OCH_2N), 6.18 (1H, d, $J_{1',2'}$=2.7Hz, H-1'), 7.01 (1H, s, H-6), and 7.20-7.23 (1H, m, NH); IR(KBr) 3400 (NH), 1735 (COO), and 1680 (amide I) cm^{-1}; MS(EI) m/z 563 (M^+). Anal. Calcd for $C_{23}H_{31}N_5O_9$: C, 52.97; H, 5.99; N, 13.43. Found: C, 53.04; H, 6.21; N, 13.22.

2-Amino-5-aminomethyl-3,4-dihydro-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidin-4-one ($preQ_1$) (**3**). A solution of **15** (110 mg, 0.20 mmol) in methanol (2 ml) and 28% ammonium hydroxide (2 ml) was stirred at room temperature for 15 h and concentrated. To the residue was added 2N HCl (4 ml) and the mixture was heated at 80 °C for 5 h. The reaction was monitored by HPLC (NOMURA ODS 5 μ, eluent: acetonitrile-acetic acid-water, 1:1:100). The mixture was evaporated *in vacuo* to give the $preQ_1$ hydrochloride, which was purified by preparative HPLC (NOMURA ODS 10-20 μ, 10 φ x 250 mm, eluent: acetonitrile-acetic acid-water, 1:1:100) and then applied to an Amberlite IR-45 column (eluent: water-acetonitrile, 10:1) to afford free $preQ_1$ (**3**) (31 mg, 51%) as a white powder: mp 145-150 °C (dec); UV(H_2O) 262 (ε 9270) and 219 nm (14030); $[\alpha]_D^{13}$ -52.0° (c 0.1, H_2O); ^{1}H-NMR(D_2O; internal standard: *t*-BuOH as 1.23 ppm) δ 3.76 (1H, dd, $J_{5',4'}$=4.2Hz, $J_{5',5''}$=12.7Hz, H-5'), 3.82 (1H, dd, $J_{5'',4'}$=3.4Hz, $J_{5'',5'}$=12.7Hz, H-5''), 4.16 (1H, ddd, $J_{4',3'}$=3.2Hz, $J_{4',5''}$=3.4 and 4.2Hz, H-4'), 4.21 (2H, s, 2H, CH_2N), 4.32 (1H, dd, $J_{3',2'}$=5.2Hz, $J_{3',4'}$=3.2Hz, H-3'), 4.57 (1H, dd, $J_{2',1'}$=6.6Hz, $J_{2',3'}$=5.2Hz, H-2'), 5.96 (1H, d, $J_{1',2'}$=6.6Hz, H-1'), and 7.08 (1H, s, H-6); IR(KBr) 3400 (NH_2, OH) and 1650 (amide I) cm^{-1}; MS(FAB) m/z 312 (M+H).

Acknowledgement: We thank Dr. S. Nishimura, National Cancer Center Research Institute, Tokyo, for valuable discussions. This work was supported by a Grant-in-Aid for Special Project Research from the Ministry of Education, Science and Culture, Japan, and a grant from the Naito Foundation.

REFERENCES

1. M. Kasai, K. Kuchino, and S. Nishimura, Nucleic Acids Res., **2**, 1931 (1975).
2. T. Ohgi, T. Kondo, and T. Goto, J. Am. Chem. Soc., **101**, 3629 (1979).
3. N. Okada, S. Noguchi, H. Kasai, N. Shindo-Okada, T. Ohgi, T. Goto, and S. Nishimura, J. Biol. Chem., **254**, 3067 (1979).
4. S. Noguchi, Z. Yamaizumi, T. Ohgi, T. Goto, Y. Nishimura, Y. Horita, and S. Nishimura, Nucleic Acids Res., **5**, 4215 (1978).
5. N. Okada, S. Noguchi, S. Nishimura, T. Ohgi, T. Goto, P. F. Crain, and J. A. McCloskey, Nucleic Acids Res., **5**, 2289 (1978); N. Okada, T. Yasuda, and S. Nishimura, ibid., **4**, 4063 (1977).
6. W. R. Farkas, J. Biol. Chem., **225**, 6832 (1980).
7. N. Tanaka, R.-T. Wu, T. Okabe, H. Yamashita, A. Shimazu, and T. Nishimura, J. Antibiotics, **35**, 272 (1982); R.-T. Wu, T. Okabe, M. Namikoshi, S. Okuda, T. Nishimura, and N. Tanaka, ibid., **35**, 279 (1982); B.-D. Yuan, R.-T. Wu, I. Sato, T. Okabe, H. Suzuki, T. Nishimura, and N. Tanaka, ibid., **38**, 642 (1985).
8. T. Kondo, K. Okamoto, M. Yamamoto, T. Goto, and N. Tanaka, preceding paper in this issue.