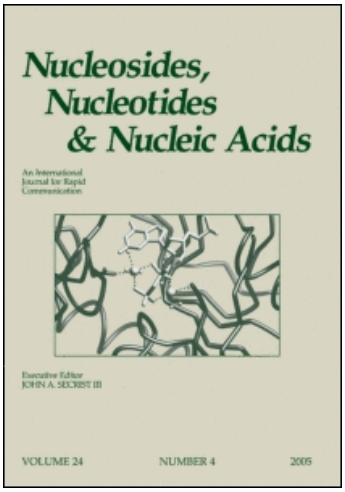

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Synthesis of Pyrrolo[2,3-*d*]pyrimidines that are Structurally Related to Methylated Guanosines from tRNA and the Nucleoside Q Analogs, $PreQ_0$ and $PreQ_1$

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Synthesis of Pyrrolo[2,3-*d*]pyrimidines that are Structurally Related to Methylated Guanosines from tRNA and the Nucleoside Q Analogs, PreQ0 and PreQ1

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ABSTRACT: The N-3- and N-2-methylated analogs of several 5-substituted 2-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-ones were synthesized from 5-cyano-2,4-dichloro-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**10**). These compounds are analogs of nucleoside Q that are methylated in a manner similar to some of the naturally occurring methylated guanosines.

INTRODUCTION

At least ninety-three modified nucleosides with assigned structures have been identified in various species of RNA[1]. These modified nucleosides include guanosines that have been methylated on the pyrimidine ring (e.g., 1-N-methyl-, **1**[2] , 2-N-methyl-, **2**[2] , and 2-N,N'-dimethylguanosine, **3**[3]) and the nucleosides related to queuosine.

The hypermodified nucleoside queuosine (Q, **4**) was discovered in the first position of the anticodon of *Escherichia coli* $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$, and $tRNA^{Asp}$[4] prior to the elucidation of its structure[5] and absolute stereochemistry[6]. Queuosine has been shown to be widely distributed in tRNA from plant as well as animal sources[7]. The presence or absence of the Q modification in the anticodon of tRNA has been found to correlate with the efficiency of aminoacylation[8], ribosomal frameshifting[9,10], translation of an amber stop codon[11,12], and cell proliferation[13].

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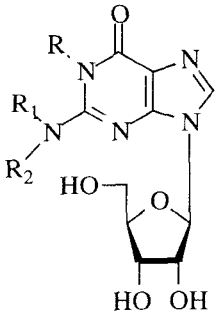
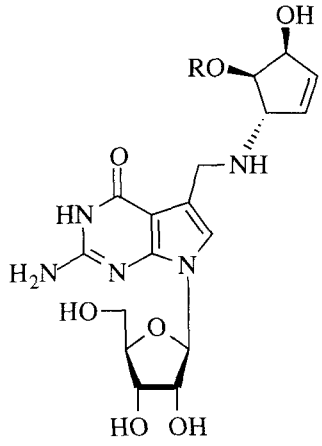


1 R = CH_3, R^1 = R^2 = H

2 R = R^1 = H, R^2 = CH_3

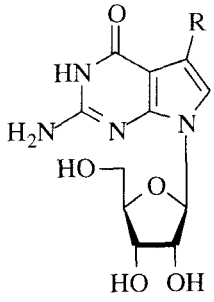
3 R = H, R^1 = R^2 = CH_3



4 R = H

5 R = mannosyl

6 R = galactosyl



7 R = $-CH_2NH_2$ $preQ_1$ Nucleoside

8 R = -CN $preQ_0$ Nucleoside

9 R = $-C(=NH)-NH_2$ Archaeosine

FIG. 1

The structurally related nucleosides manQ and galQ were also found in tRNA's from various organisms[7] and subsequently characterized[14] as **5** and **6**, respectively. $PreQ_1$ nucleoside (**7**), a biosynthetic precusor to Q in prokaryotes, was discovered[15] in methyl-deficient *E. coli* tRNA and subsequently characterized[16]. $PreQ_0$ nucleoside (**8**) was first prepared synthetically[17] and then subsequently isolated from the tRNA of an *E. coli* mutant selected for a deficiency of Q[18].

The structure of the modified nucleoside archaeosine (**9**) was recently elucidated[19] and found to be a 7-substituted-7-deazaguanosine, as are nucleoside Q and its derivatives.

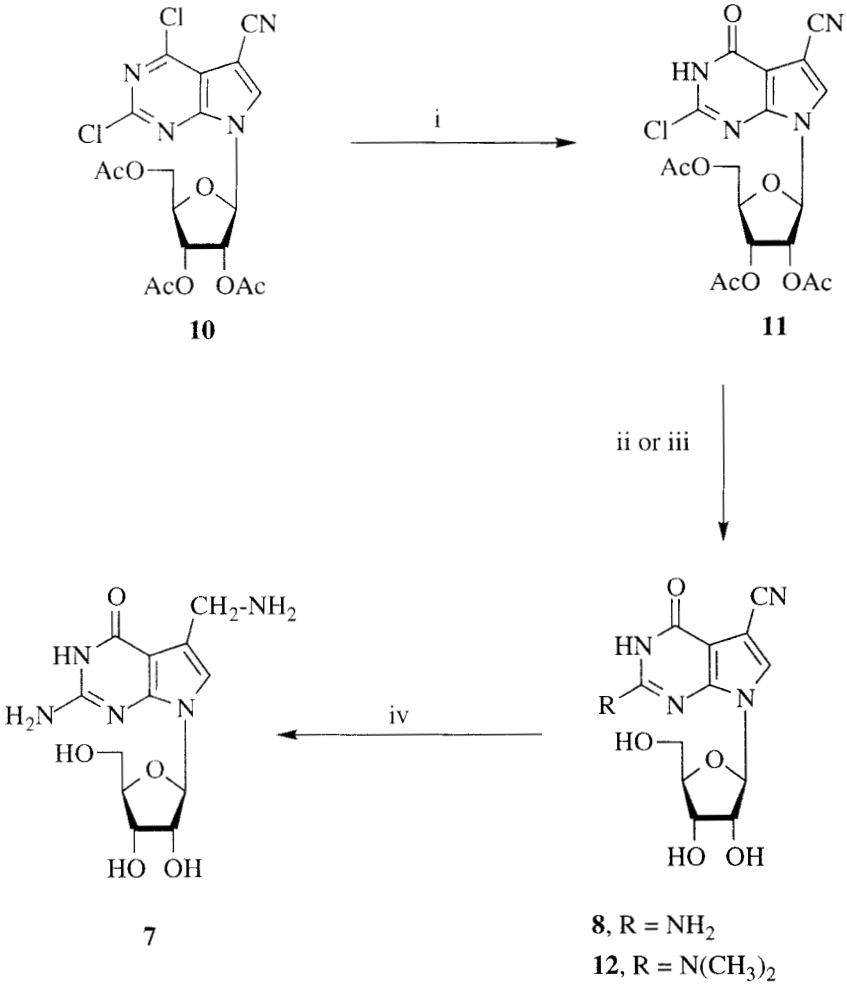
Conspicuously absent from the list of fully characterized modified nucleosides are the methylated analogs of the Q nucleoside family. The discovery of such compounds, if they exist in naturally occurring nucleic acids, would be facilitated by the availability of fully characterized standards. Thus, we report here the syntheses of methylated analogs of $preQ_0$ and $preQ_1$ as well as the corresponding 5-carboxamide derivatives.

CHEMISTRY

5-Cyano-2,4-dichloro-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**10**)[17], was synthesized, *via* a multi-step procedure, from the nucleoside antibiotic toyocamycin[20]. We considered a number of hydrolysis procedures involving **10**. However, most of these procedures resulted in not only a hydrolysis of the 4-chloro group but also a concomitant removal of all or at least some of the protecting groups from the ribosyl moiety. 2-Chloro-5-cyano-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidin-4-one (**11**) was produced in a good yield by the treatment of **10** with sodium nitrite in ethanol. This preparation of compound **11** posesses the distinct advantage of leaving all of the acetyl blocking groups intact. As a result, **11** is very soluble in acetonitrile and the blocking groups prevent methylation of the ribosyl hydroxyl groups. A retrosynthetic evaluation revealed that compound **11** could be used as a common intermediate for the synthesis of the methylated pyrrolo[2,3-*d*]pyrimidine nucleosides corresponding to the purine N1-, N2-, and N1,N2-methylated series of compounds.

5-Cyano-2-dimethylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**12**) was prepared by a nucleophilic displacement of the chloro group of **11** with ethanolic dimethylamine at 55 °C. Likewise, $preQ_0$ nucleoside (2-amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one, **8**) could be prepared *via* the treatment of **11** with liquid ammonia at 100 °C. We found that $PreQ_1$ nucleoside (**7**) could be prepared directly from **8** by hydrogenation at atmospheric pressure.

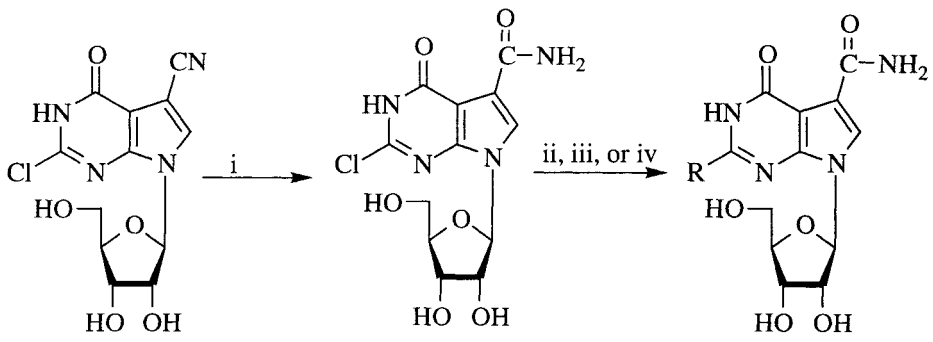
2-Amino-5-carboxamido-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**15**) was first prepared in a low yield (14%) by treating **8** with basic hydrogen peroxide[20] at <20 °C. This prompted us to investigate an alternative route for the preparation of **15**. We



(i) $NaNO_2$, 18-crown-6, EtOH. (ii) NH_3. (iii) $HN(CH_3)_2$, EtOH.

(iv) H_2, Pd/C

SCHEME 1



(i) H_2O_2, NH_2OH; (ii) NH_3; (iii) H_2NCH_3, EtOH; (iv) $HN(CH_3)_2$, EtOH

SCHEME 2

subsequently elected to use 5-carboxamido-2-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidin-4-one (**14**), prepared from 2-chloro-5-cyano-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidin-4-one[17] (**13**), as our starting material for the synthesis of the 2-amino- and all of the N2-methylated 2-amino-5-carboxamido-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidin-4-ones. We found that the treatment of **14** with liquid ammonia (at 100 °C), ethanolic monomethylamine (at 70 °C), and ethanolic dimethylamine (at 55 °C) furnished good yields of **15**, 5-carboxamido-2-monomethylamino-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidin-4-one (**16**), and 5-carboxamido-2-dimethylamino-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidin-4-one (**17**), respectively.

For the series of N3-methylated derivatives, we elected to use compound **11** as our starting material. This decision was based on the fact that methylation on the pyrimidine ring nitrogen of compound **11**, provided a common intermediate, 2-chloro-5-cyano-3-N-methyl-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**18**), for the subsequent amination reactions. Compound **11** was treated with methyl iodide to afford the N3-methylated derivative **18**. The assignment of N3 as the site of methylation for **18** was supported by an examination of the UV spectrum (λ_{max} in MeOH = 269 nm), which closely resembled that of **11** (λ_{max} in MeOH = 268 nm). Methylation at N-1 would have been expected[21] to produce a greater bathochromic shift (> 10 nm) in the UV spectrum of **11**. O-Methylation was ruled out by a comparison of the ^{1}H-NMR spectrum

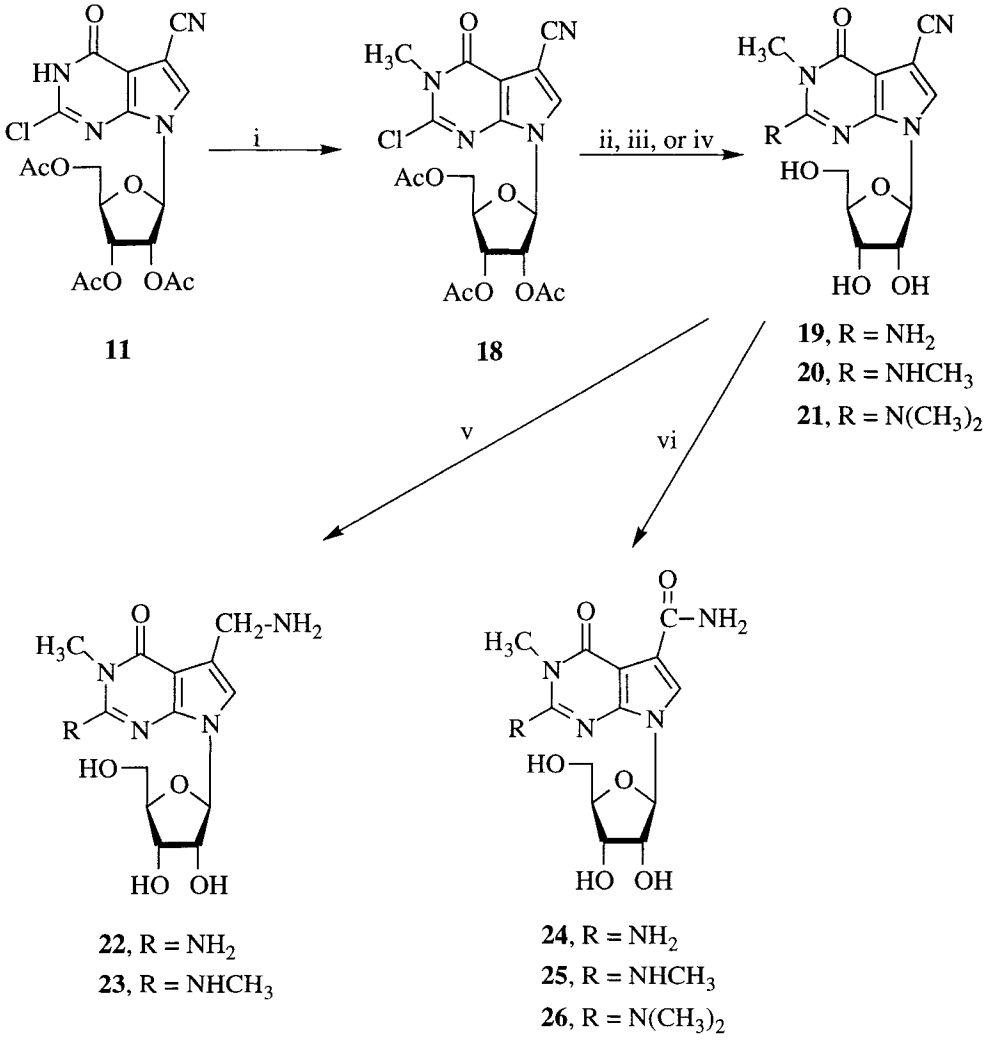
for 5-cyano-4-methoxy-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine[22] (δ = 4.10 ppm, s, 3H, -OCH_3) to that of 2-chloro-5-cyano-3-N-methyl-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**18**) (δ = 3.62 ppm, s, 3H, -NCH_3).

The reactivity of the 2-chloro group of **18** toward nucleophilic attack was markedly enhanced relative to that of the 2-chloro group of **11**. This increased reactivity was demonstrated by a reaction of the chloro group of **18** with saturated methanolic ammonia at room temperature. Unexpectedly, the chloro function was displaced by methoxide instead of ammonia to give 5-cyano-3-N-methyl-2-methoxy-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidin-4-one. As a result of this increased reactivity, 2-amino-5-cyano-3-N-methyl-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**19**), 5-cyano-3-N-methyl-2-methylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**20**), and 5-cyano-2-dimethylamino-3-N-methyl-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**21**) were prepared by treating the common precursor **18** with liquid ammonia, liquid monomethylamine and liquid dimethylamine, respectively. Heating was not required for these reactions to go to completion.

Compounds **19** and **20** were catalytically hydrogenated in saturated ethanolic ammonia to give the corresponding 2-amino-5-aminomethyl-3-N-methyl-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**22**) and 5-aminomethyl-3-methyl-2-methylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**23**) in good yield. The N-1 methylated compounds **19**, **20**, **21** were smoothly converted, with basic hydrogen peroxide at room temperature, to their corresponding carboxamido nucleosides, namely, 2-amino-5-carboxamido-3-methyl-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**24**), 5-carboxamido-3-methyl-2-methylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidin-4-one (**25**), and 5-carboxamido-2-dimethylamino-3-methyl-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one(**26**).

EXPERIMENTAL SECTION

Melting points were observed with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Ultraviolet spectra (UV, sh = shoulder) were recorded on Beckman DIC-2, Acta CIII, and Gilford RESPONSE spectrophotometers. Infrared (IR) spectra were recorded on Perkin-Elmer 281 and Beckman IR 10 spectrophotometer. Nuclear magnetic resonance (^{1}H-NMR) spectra were obtained by using Jeol C 60 H, Varian EM-360 and JNM-PS-100 spectrometers in DMSO-d_6 or $CDCl_3$ using tetramethylsilane as an internal standard. Elemental analyses were performed by M-H-W Laboratories, Phoenix, Arizona.



(i) CH_3I, KF on Celite, CH_3CN; (ii) NH_3; (iii) H_2NCH_3, EtOH; (iv) $HN(CH_3)_2$, EtOH; (v) H_2, Pd/C, NH_3, EtOH; (vi) H_2O_2, NH_4OH

SCHEME 3

Chromatography

Thin-layer chromatography (tlc) was run (ascending) on glass plates coated (0.25 mm thickness) with silica gel (SilicAR 7GF, Mallinckrodt). Commercially available precoated tlc plates were also used (SilicAR 7GF, Analtech). Solvent systems used for the development of tlc plates were varied in accordance with each individual compound. Most often, the mixture of methanol:methylene chloride (or ethyl acetate) (1~4:9~6, v/v) were used. Compounds of interest on tlc plates were detected with ultraviolet lamp (Mineralight, 254 nm) and charring the compound(s) with 10% aqueous sulfuric acid solution. Chromatographic separations were performed in glass columns packed with silica gel (J. T. Baker) or SilicAR (Mallinckrodt) under gravity flow unless otherwise described. Solvent removals during work-up were conducted with a water aspirator at 45 °C, while evaporations *in vacuo* were performed at 40 °C and 1 torr with a rotary evaporator unless otherwise stated.

2-Chloro-5-cyano-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidin-4-one (11). 5-Cyano-2,4-dichloro-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine[17] (**10**, 7.1 g, 15.07 mmol) was dissolved in 2 L of ethanol and to this solution was added sodium nitrite (2.1 g, 30.4 mmol), followed by a catalytic amount of dicyclohexyl-18-crown-6 ether (0.29 g, 0.78 mmol). The reaction mixture was heated, with stirring, at reflux temperature for 20 h. The solvent was removed under diminished pressure and the remaining residue was redissolved in 800 mL of ethyl acetate. This solution was washed with water (1 x 100 mL) and the organic layer was then collected and dried for 18 h over anhydrous magnesium sulfate. The solid was removed through a Celite pad and the filter-cake washed with ethyl acetate (2 x 50 mL). The combined filtrate and washings was evaporated under diminished pressure to afford 4.85 g (71.1%) of the desired product as a solid foam. A portion of the solid foam (0.1 g) was eluted through a short silica gel column (J. T. Baker, 1 x 2 cm) with a solution of acetone and chloroform (1:9, v/v). Fractions containing product as indicated by tlc (acetone:chloroform, 1:9, v/v) were collected and evaporated to give 91 mg of solid; mp >102° (gradual melting; 155-156° melted); ^{1}H-NMR ($CDCl_3$): δ 2.08 (s, 3H, $COCH_3$), 2.13 (s, 6H, 2 $COCH_3$), 6.23 (d, 1H, $H_{1'}$, $J_{1',2'}$ = 4.2 Hz), 7.53 (s, 1H, H_6); UV λ_{max} nm (ε x 10^3 $M^{-1}\cdot cm^{-1}$) (MeOH) 224 (15.3), 268 (12.2); (pH 1) 223 (17.1), 267 (12.2); (pH 11) 231 (10.2), 267 (13.0); Anal. Calcd. for $C_{18}H_{17}ClN_4O_8$: C, 47.75; H, 3.78; N, 12.37. Found: C, 47.58; H, 3.87; N, 12.14.

2-Amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (8). 2-Chloro-5-cyano-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidin-4-one, (**11**, 0.3 g, 0.92 mmol) was placed in a steel reaction vessel (50 mL) and liquid ammonia (25 mL) was added. The reaction vessel was sealed and heated in an oil

bath to 100 °C. The reaction mixture was heated with constant stirring for 96 h. The reaction was monitored by tlc (methanol : ethyl acetate, 1 : 9, v/v) by cooling in a dry ice - acetone bath and then opening the reaction vessel for sampling. The reaction was continued until a complete disappearence of starting material (**11**) was observed. The excess ammonia was then allowed to evaporate at room temperature and the gray residue was coevaporated with methanol (2 x 10 mL). The resulting powder was placed on a dry packed silica gel column (2.5 x 20 cm). The column was eluted with methanol : chloroform (3 : 7, v/v) and the fractions containing **8**, as indicated by tlc, were combined and evaporated to furnish 0.22 g (77 %) of **8**; mp 265.5-267 °C; ^{1}H-NMR ($CDCl_3$): δ 5.91 (d, 1H, $J_{1',2'}$ = 5.5 Hz, $H_{1'}$), 6.90 (br s, 2H, 2-NH_2), 7.97 (s, 1H, H_6), 11.1 (br s, 1H, CONH); UV λ_{max} nm (ε x 10^3 $M^{-1}\cdot cm^{-1}$) (MeOH) 228 (16.1), 269 (9.2), 295 (6.5); (pH 1) 227 (17.4), 268 (8.5), 288 (6.9); (pH 11) 226 (20.0), 268 (6.5), 286 (7.0); Anal. Calcd. for $C_{12}H_{13}N_5O_5$: C, 46.91; H, 4.24; N, 22.80. Found: C, 46.97; H, 4.29; N, 22.48.

5-Cyano-2-dimethylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidin-4-one (12). 2-Chloro-5-cyano-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**11**, 0.5 g, 1.10 mmol) was added to a steel reaction vessel (50 mL) containing 25 mL of cold (-20 °C) ethanolic dimethylamine (1 : 1, v/v). The steel reaction vessel was sealed and heated at 55 °C (oil-bath) with constant stirring for 18 h. The excess ethanolic dimethylamine was evaporated under diminished pressure at room temperature, then at 45 °C. The resulting residue was coevaporated with ethanol (2 x 10 mL). This crude powder was purified by silica gel column chromatography (J. T. Baker, 3.5 x 25 cm, dry packed) eluting with CH_2Cl_2/CH_3OH (9:1, v/v). Evaporation of the fractions containing **12** gave 0.24 g (65.5%) of the desired nucleoside; mp 296-297 °C (dec); ^{1}H-NMR (DMSO-d_6) δ 3.08 (s, 6H, -$N(CH_3)_2$), 5.89 (d, 1H, $H_{1'}$, $J_{1',2'}$ = 6.0 Hz), 7.95 (s, 1H, H_6), 10.8 (broad s, 1H, CONH-); UV λ_{max} nm (ε x 10^3 $M^{-1}\cdot cm^{-1}$) (MeOH) 230 (15.8), 273 (10.9), 291 (7.5); (pH 1) 234 (11.4), 274 (5.9), 302 (5.1); (pH 11) 235 (19.1), 270 (5.9), 300 (5.5); Anal. Calcd. for $C_{14}H_{17}N_5O_5$: C, 50.15; H, 5.11; N, 20.89 Found: C, 50.15; H, 5.05; N, 20.88.

2-Amino-5-aminomethyl-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidin-4-one (7). 2-Amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidin-4-one, (**8**, 200 mg, 0.65 mmol) was dissolved in aq HCl (10 mL, 1 M). This solution was purged with nitrogen gas and then 10 % palladium on carbon (80 mg) was added. The inert gas was displaced by hydrogen gas. The reaction mixture was stirred under hydrogen at atmospheric pressure and room temperature for 18 h. The reaction mixture was degassed and the insoluble solid removed by filtration. The filtered solid was

washed with water (2 x 2 mL) and the combined filtrate and washings evaporated under reduced presure at 40 °C. The residue was dissolved in 2 mL of distilled water and the aqueous solution applied to to the top of an ion-exchange resin column (1 x 5 mL, 6.4 meq of Dowex-50W-X4, H^+ form). The column was eluted with water (10 mL) followed by 0.15 M aq ammonium hydroxide. The fractions containing **7**, as determined by tlc (methanol : S.S.E.[A] , 3:7, v/v), were combined and evaporated under reduced pressure to one half of the volume. Upon standing, **7** crystallized out of solution. The crystalline compound was collected by filtration, rinsed with water (1 mL), air-dried, and then dried *in vacuo* (110 °C, 4 h) to give 97 mg (48 %) of **7**; mp >250 °C (dec); IR: absence of cyano stretching band in the 2200 cm^{-1} region; ^{1}H-NMR (DMSO-d_6): δ 5.81 (d, 1H, $H_{1'}$, $J_{1',2'}$ = 6.4 Hz), 6.39 (br s, 2H, $-NH_2$), 6.74 (s, 1H, H_6); UV λ_{max} nm (ε x 10^3 $M^{-1}\cdot cm^{-1}$) (MeOH) 219 (14.7), 261 (10.2), 282 (6.7); (pH 1) 221 (9.7), 258 (8.0), 280 (4.5); (pH 11) 261 (10.1), 275 (8.2); Anal. Calcd. for $C_{12}H_{15}N_5O_5 \cdot 1.5\ H_2O$: C, 42.86; H, 5.40; N, 20.82. Found: C, 42.84; H, 5.51; N, 20.67.

5-Carboxamido-2-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidin-4-one (14). 2-Chloro-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidin-4-one[17] (**13**, 1.2 g, 3.68 mmol) was dissolved in 100 mL of concentrated ammonium hydroxide. The solution was cooled to 10 °C and then 10 mL of 30% hydrogen peroxide was added. The solution was stirred continuously at 10 °C for 18 h and then evaporated to dryness under a stream of compressed air. The residue was dissolved in 100 mL of methanol and again evaporated to dryness under a stream of compressed air. This procedure was repeated twice and the solid residue was recrystallized from an acetone-methanol solution to give 1 g (78.8%) of the carboxamido nucleoside derivative **14**, mp >235 °C (dec); IR: absence of cyano stretching band in the 2200 cm^{-1} region; ^{1}H-NMR (DMSO-d_6): δ 5.96 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.3 Hz), 8.00 (s, 1, H_6), 7.42 and 9.29 (s and s, 1 and 1, $CONH_2$); Anal. Calcd. for $C_{12}H_{13}ClN_4O_6 \cdot 0.5\ H_2O$: C, 40.75; H, 3.99; N, 15.84. Found: C, 40.76; H, 4.01; N, 15.89.

2-Amino-5-carboxamido-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidin-4-one (15). Method 1. 2-Amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidin-4-one[17] (**8**, 200 mg, 0.65 mmol) was dissolved in 20 mL of concentrated ammonium hydroxide. This solution was then cooled to 15 °C and 2 mL of 30% hydrogen peroxide was added. The solution was stirred at 15 °C for two days and then stirred at 20 °C for an additional 18 h. Gaseous substances were removed under diminished pressure (at <20 °C), and then water was gradually removed *in vacuo* (room temperature) to afford a

[A] S.S.E. = solvent system E = the upper phase of ethyl acetate:*n*-propanol:water (4:1:2, v/v/v)

solid residue. This solid was crystallized from methanol (50 mL) containing a trace of water to give 30 mg (13.9%) of **15**; m.p.: 228 °C (dec), 260.5-261.5 °C (melted with dec.); ^{1}H-NMR (DMSO-d_6): δ 5.90 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.4 Hz), 6.50 (broad s, 2, C2-NH_2), 7.10 and 9.54 (two broad s, 2 x 1, $CONH_2$), 7.55 (s, 1, H_6), 10.95 (broad s, 1, cyclic amide proton); UV λ_{max} nm (ε x 10^3 $M^{-1}\cdot cm^{-1}$) (MeOH) 229 (13.7), 270 (6.8), 293 (6.8); (pH 1) 234 (7.6), 245 (sh,4.5), 295 (4.2); (pH 11) 232 (11.3), 253 (sh,6.2), 290 (4.6); Anal. Calcd. for $C_{12}H_{15}N_5O_6 \cdot 0.5\ H_2O$ (verified by ^{1}H-NMR): C, 43.15; H, 4.83; N, 20.95. Found: C, 42.67; H, 4.71; N, 20.55.

Method 2. 5-Carboxamido-2-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidin-4-one (**14**, 100 mg, 0.29 mmol) was placed in a steel reaction vessel (50 mL), and then liquid ammonia (20 mL) was added to the vessel. The reaction vessel was sealed and then immersed in an oil bath. The temperature of the oil bath was raised to 100 °C and the reaction was then heated at 100 °C for 96 h. The reaction mixture was cooled to room temperature and the excess ammonia was released from the steel reaction vessel. The solid residue was coevaporated with methanol (2 x 5 mL) and the resulting solid was dissolved in methanol (25 mL) containing a trace of water to furnish 82 mg (86.2%) of the nucleoside **15**. This compound was shown to be identical to the product obtained by Method 1 in all respects (UV, IR, ^{1}H-NMR, R_f, and mp).

5-Carboxamido-2-methylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidin-4-one (16). 5-Carboxamido-2-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidin-4-one (**14**, 200 mg, 0.58 mmol) was added to a steel reaction vessel (50 mL) containing 30 mL of an ethanolic methylamine solution (1: 1, v/v). The steel reaction vessel was sealed and placed into an oil bath. The oil bath temperature was raised to 70 °C and the reaction mixture was heated, with constant stirring, at that temperature for 18 h. The reaction mixture was cooled to room temperature and the excess methylamine and the solvent were removed under diminished pressure. The resulting residue was purified by silica gel column chromatography (J. T. Baker, 2 x 25 cm, dry packed), eluting with a solution of methanol and ethyl acetate (1 : 9, v/v) and the fractions containing **16**, as detected by tlc, were combined and the solvent removed to furnish 140 mg (70.7%) of nucleoside **16**; mp >152 °C (dec); ^{1}H-NMR (DMSO-d_6): δ 2.83 (s, 3, NCH_3), 5.95 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6 Hz), 6.65 (broad s, 1, C2-NH-), 7.57 (s, 1, H_6), 7.11 and 9.60 (two broad s, 1 and 1, $CONH_2$); UV λ_{max} nm (ε x 10^3 $M^{-1}\cdot cm^{-1}$) (MeOH) 222 (sh,13.7), 272 (8.1), 289 (sh,6.5), 305 (sh,4.0); (pH 1) 229 (9.5), 270 (5.5), 289 (sh,4.4); (pH 11) 231 (12.4), 269 (7.3), 287 (sh,5.5), 302 (sh,3.3); Anal. Calcd. for $C_{13}H_{17}N_5O_6 \cdot 1.5\ H_2O$: C, 42.62; H, 5.50; N, 19.12. Found: C, 42.63; H, 5.35; N, 18.86.

5-Carboxamido-2-dimethylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidin-4-one (17). 5-Carboxamido-2-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidin-4-one (**14**, 200 mg, 0.58 mmol) was placed in a steel reaction vessel (50 mL) to which 30 mL of an ethanolic dimethylamine solution (1:1, v/v) was then added. The sealed reaction vessel was heated at 55 °C (oil bath temperature) for 18 h. The reaction was cooled to room temperature and the excess dimethylamine and solvent were removed under diminished pressure. The resulting crude residue was placed on the top of a silica gel column (J. T. Baker, 2 x 25 cm, dry packed) and was eluted with a solvent mixture of methanol and chloroform (2:8, v/v). The progress of column chromatography was monitored by checking individual fractions using tlc. The fractions containing **17** were combined and the solvent removed under diminished pressure to furnish 105 mg (51.7%) of the nucleoside **17**; mp >171 °C (dec); ^{1}H-NMR (DMSO-d_6) δ 2.73 (s, 6, $N(CH_3)_2$), 5.89 (d, 1, $H_{1'}$, $J_{1',2'}$ = 5 Hz), 6.82 and 8.75 (two broad s, 2 x 1, $CONH_2$); UV λ_{max} nm (ε x 10^3 $M^{-1}\cdot cm^{-1}$) (MeOH) 228 (14.1), 280 (8.8), 300 (sh,7.8); (pH 1) 240 (18.4), 275 (sh,8.1), 305 (sh,6.3); (pH 11) 235 (15.7), 265 (sh,9.5), 305 (sh,6.4); Anal. Calcd. for $C_{14}H_{19}N_5O_6$: C, 47.59; H, 5.42; N, 19.82. Found: C, 47.57; H, 5.65; N, 19.60.

2-Chloro-5-cyano-3-N-methyl-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (18). 2-Chloro-5-cyano-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**11**, 6 g, 13.2 mmol) was dissolved in 0.5 L of acetonitrile. To this solution was added 7.7 g (5 equivalents) of Celite coated with potassium fluoride[B] and 2 g (13.2 mmol) of methyl iodide. The reaction suspension was then stirred at room temperature for 15 h. The solid was removed by filtration and the filter-cake was washed with 2 x 10 mL of acetonitrile. The combined filtrate and washings were evaporated to dryness under diminished pressure. The residue was recrystallized from methanol containing a trace of water to afford the methylated nucleoside **18** (4.1 g, 67%); mp 116-117 °C; ^{1}H-NMR ($CDCl_3$): δ 2.13 (s, 9, $COCH_3$), 3.62 (s, 3, NCH_3), 6.18 (d, 1, $H_{1'}$, $J_{1',2'}$ = 4.5 Hz), 7.63 (s, 1, H_6); UV λ_{max} nm (ε x 10^3 $M^{-1}\cdot cm^{-1}$) (MeOH) 219 (21.4), 269 (12.9), 286 (sh, 8.4); (pH 1) 221 (21.4), 269 (13.3); (pH 11) 228 (20.8), 269 (12.4) 287 (sh, 8.6); Anal. Calcd. for $C_{19}H_{19}ClN_4O_8$: C, 48.89; H, 4.10; N, 12.00. Found: C, 49.07; H, 4.19; N, 11.88.

2-Amino-5-cyano-3-N-methyl-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidin-4-one (19). 2-Chloro-5-cyano-3-N-methyl-7-(2,3,5-tri-*O*-acetyl-β-D-

[B] This reagent was prepared by mixing Celite (Johns-Manville, 3.85 g) with its equivalent weight of potassium fluoride in 100 mL water. The water was removed in vacuo at 50 - 60 °C in a rotatory evaporator. The resulting powder was then shaken in 20 mL acetonitrile, filtered, washed with two 10 mL portions of acetonitrile, and dried in a dessicator at room temperature.

ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**18**, 300 mg, 0.64 mmol) was added to a steel reaction vessel (50 mL) containing 20 mL of liquid ammonia. The reaction vessel was then sealed and allowed to stir at room temperature for 18 h. The excess ammonia was released from the vessel and the residue was coevaporated with methanol (2 x 10 mL). The resulting solid was recrystallized from methanol containing a trace amount of water. The crystalline product was collected by filtration, washed with 2 mL of methanol and air-dried to furnish 200 mg (96.9%) of nucleoside **19**; mp >255 °C (dec); ^{1}H-NMR (DMSO-d_6) δ 3.32 (s, 3, NCH_3), 5.91 (d, 1, $H_{1'}$, $J_{1',2'}$ = 5.2 Hz), 7.15 (broad s, 2, NH_2), 7.93 (s, 1, H_6); UV λ_{max} nm (ε x 10^3 $M^{-1}\cdot cm^{-1}$) (MeOH) 228 (19.3), 269 (11.6), 283 (sh,9.5); (pH 1) 228 (20.7), 268 (11.3), 283 (sh,9.2); (pH 11) 231 (18.0), 269 (11.1), 285 (sh,9.2); Anal. Calcd. for $C_{13}H_{15}N_5O_5$: C, 48.60; H, 4.71; N, 21.80. Found: C, 48.44; H, 4.82; N, 21.89.

5-Cyano-3-N-methyl-2-methylamino-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidin-4-one (20). 2-Chloro-5-cyano-3-N-methyl-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**18**, 900 mg, 1.93 mmol) was dissolved in 30 mL of ethanolic methylamine (1:1, v/v) in a steel reaction vessel (50 mL). The reaction vessel was sealed and the reaction mixture stirred at room temperature for 18 h. After a removal of the excess ethanolic methylamine, the resulting residue was coevaporated with methanol (2 x 10 mL) under diminished pressure. The solid residue was recrystallized from methanol to yield 550 mg (85.0%) of **20**: mp 276.5-278 °C (dec); ^{1}H-NMR (DMSO-d_6) δ 2.87 (s, 3, $NHCH_3$), 3.30 (s, 3, 3N-CH_3), 5.92 (d, 1, $H_{1'}$, $J_{1',2'}$ = 5.4 Hz), 7.18 (broad s, 1, -NH-), 7.94 (s, 1, H_6); UV λ_{max} nm (ε x 10^3 $M^{-1}\cdot cm^{-1}$) (MeOH) 227 (21.5), 270 (10.8), 286 (sh,8.4); (pH 1) 228 (23.0), 270 (11.2), 291 (9.0); (pH 11) 231 (19.8), 270 (9.5), 293 (7.6); Anal. Calcd. for $C_{14}H_{17}N_5O_5$: C, 50.15; H, 5.11; N, 20.89. Found: C, 49.99; H, 5.25; N, 20.83.

5-Cyano-2-dimethylamino-3-N-methyl-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidin-4-one (21). 2-Chloro-5-cyano-3-N-methyl-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**18**, 300 mg, 0.64 mmol) was placed in a steel reaction vessel (50 mL) and then 25 mL of liquid dimethylamine was added. The reaction vessel was sealed and the reaction mixture was stirred at room temperature for 18 h. Excess dimethylamine was released from the reaction vessel and the residue was coevaporated with methanol (2 x 10 mL). The resulting solid was placed on a silica gel column (J. T. Baker, 2.5 x 20 cm, dry packed). The column was eluted with methanol : chloroform (1 : 9, v/v) and the fractions containing **21**, as indicated by tlc, were collected and the solvent removed to afford 300 mg (84%) of the desired nucleoside **21** mp ~110 °C (partial melting started at >85 °C); ^{1}H-NMR (DMSO-d_6): δ 2.87 (s, 6, $N(CH_3)_2$), 3.42 (s, 3, 3N-CH_3), 5.95 (d, 1, $H_{1'}$, $J_{1',2'}$ = 5.5 Hz), 8.13 (s, 1, H_6); UV λ_{max} nm (ε x 10^3 M^{-}

^{1}·cm^{-1}) (MeOH) 232 (16.1), 276 (12.9), 297 (sh,8.3); (pH 1) 234 (16.4), 278 (12.6), 297 (sh,8.4); (pH 11) 235 (16.7), 276 (12.6), 297 (sh,8.4); Anal. Calcd. for $C_{15}H_{19}N_5O_5$: C, 51,57; H, 5.48; N, 20.05. Found: C, 51.63; H, 5.41; N, 19.96.

2-Amino-5-aminomethyl-3-methyl-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidin-4-one (22). 2-Amino-5-cyano-3-methyl-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidin-4-one (**19**, 100 mg, 0.31 mmol) was dissolved in 25 mL of ethanolic ammonia (prepared by saturation of ethanol with ammonia at room temperature) and then 100 mg of 10% palladium over carbon was added to the ethanolic solution under a nitrogen atmosphere. The inert gas in the reaction flask was then displaced with hydrogen gas. The catalytic hydrogenation reaction was allowed to proceed for 18 h, with constant stirring, under an atmospheric pressure of hydrogen. The solid material in the reaction mixture was removed by filtration. The filter-cake was washed with 5 mL of ethanol and the combined filtrate and washing were evaporated under reduced pressure. The desired aminomethyl nucleoside derivative **22** (25 mg, 26% yield) was isolated from a preparative silica gel plate (1 mm thickness, SilicAR 7GF, Analtech) by using methanol as the developing solvent. mp >135 °C (dec); ^{1}H-NMR (DMSO-d_6) δ 3.33 (s, 3, 3N-CH_3), 3.93 (s, 2, -CH_2-), 5.84 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.0 Hz), 6.99 (s, 2, aromatic NH_2), 6.99 (s, 1, H_6); IR: absence of cyano band in the 2200 cm^{-1} region; UV λ_{max} nm (ε x 10^3 M^{-1}·cm^{-1}) (MeOH) 217 (13.9), 263 (7.9), 282 (sh,5.9); (pH 1) 219 (11.8), 262 (7.6), 280 (sh, 5.4); (pH 11) 264 (10.4), 282 (sh,5.9); Anal. Calcd. for $C_{13}H_{19}N_5O_5$•0.75 CH_3OH: C, 47.27; H, 6.35; N, 20.05. Found: C, 47.40; H, 5.84; N, 19.66.

5-Aminomethyl-3-methyl-2-methylamino-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidin-4-one (23). 5-Cyano-3-methyl-2-methylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**20**, 100 mg, 0.30 mmol) was dissolved in 50 mL of ethanolic ammonia (prepared by saturation of ethanol with ammonia at room temperature). The reaction solution was purged with nitrogen gas and then 100 mg of 10% palladium over carbon was added. The inert gas in the reaction flask was then displaced with hydrogen gas. The catalytic hydrogenation was accomplished, with constant stirring, under an atomspheric pressure of hydrogen and at room temperature for 18 h. The solid material in the reaction mixture was removed by filtration. The filter-cake was washed with 5 mL of ethanol and the combined filtrate and washing were evaporated under reduced pressure. The desired product **23** (45 mg) was isolated from a preparative silica gel plate (1 mm thickness, SilicAR 7GF, Analtech) (S.S.E.: methanol = 1 : 2 mixture as developing solvent system) with the yield of 43%. mp 202-203 °C (dec); IR, absence of CN stretching band of 2200 cm^{-1} region; ^{1}H-NMR (DMSO-d_6) δ 2.85 (d, 3, NH-CH_3), 3.30 (s, 3, 3N-CH_3), 3.61 (s, 2, -CH_2-), 5.86 (d, 1, $H_{1'}$, $J_{1',2'}$ = 5.9 Hz), 6.73 (s, 1, H_6),

6.87 (s, 1, -NH-); UV λ_{max} nm (ε x 10^3 $M^{-1}\cdot cm^{-1}$) (MeOH) 217 (19.6), 261 (10.9), 282 (6.8); (pH 1) 220 (18.4), 260 (11.4), 282 (sh,6.8); (pH 11) 261 (10.5), 282 (sh,6.6); Anal. Calcd. for $C_{15}H_{23}N_5O_5 \bullet$ $0.5H_2O \bullet 5CH_3OH$ (verified by ^{1}H-NMR): C, 49.2; H, 6.93; N, 18.51. Found: C, 49.25; H, 6.51; N, 18.17.

2-Amino-5-carboxamido-3-methyl-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidin-4-one (24). 2-Amino-5-cyano-3-methyl-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidin-4-one (**19**, 100 mg, 0.31 mmol) was dissolved in 100 mL of conc. ammonium hydroxide and 0.4 mL of 30% hydrogen peroxide was then added. The reaction mixture was allowed to stir at room temperature in a closed round-bottom flask for 18 h. The reaction mixture was evaporated to dryness under reduced pressure at room temperature and then coevaporated with water (2 x 5 mL) at 40 °C. The resulting solid was crystallized from water to afford 90 mg (87%) of the carboxamido nucleoside **24**. mp 292-293 °C (dec); ^{1}H-NMR (DMSO-d_6) δ 3.33 (s, 3, 3N-CH_3), 5.95 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.0 Hz), 7.12 (broad s, 2, NH_2), 7.62 (s, 1, H_6) 7.2 and 9.67 (two broad s, 2 x 1, $CONH_2$); UV λ_{max} nm (ε x 10^3 $M^{-1}\cdot cm^{-1}$) (MeOH) 229 (16.6), 272 (9.5), 291 (8.5); (pH 1) 230 (14.1), 269 (6.6), 292 (6.3); (pH 11) 230 (15.1), 267 (7.4), 292 (7.4); Anal. Calcd. for $C_{13}H_{17}N_5O_6$: C, 46.02; H, 5.05; N, 20.64. Found: C, 45.85; H, 5.05; N, 20.46.

5-Carboxamido-3-methyl-2-methylamino-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidin-4-one (25). 5-Cyano-3-methyl-2-methylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**20**, 100 mg, 0.30 mmol) was dissolved in 20 mL of conc. ammonium hydroxide and 0.4 mL of 30% hydrogen peroxide was then added. The reaction mixture was allowed to stir at room temperature in a closed round-bottom flask for 18 h. The reaction mixture was evaporated to dryness under reduced pressure at room temperature. The residue was then coevaporated with water (2 x 5 mL) at 40 °C. The resulting white solid was added to 20 mL of refluxing methanol and then heated at reflux temperature for 1 h. The solid was collected by filtration from the hot methanolic mixture and air-dried to give 80 mg (76%) of the carboxamido nucleoside **25**, mp 325-326 °C (dec); IR: absence of CN stretching band at 2200 cm^{-1} region; ^{1}H-NMR (DMSO-d_6) δ 2.89 (s, 3, N-CH_3), 3.36 (s, 3, 3N-CH_3), 6.0 (d, 1, $H_{1'}$, $J_{1',2'}$ = 5.8 Hz), 7.17 broad s, 1, Me-N<u>H</u>-), 7.65 (s, 1, H_6), 7.17 and 9.72 (two broad s, 2 x 1, $CONH_2$); UV λ_{max} nm (ε x 10^3 $M^{-1}\cdot cm^{-1}$) (MeOH) 230 (16.6), 272 (7.4), 295 (7.2); (pH 1) 232 (14.3), 271 (sh,4.6), 298 (5.7); (pH 11) 232 (15.6) ,268 (5.7), 297 (6.4); Anal. Calcd. for $C_{14}H_{19}N_5O_6$: C, 47.59; H, 5.42; N, 19.82. Found: C, 47.40; H, 5.38; N, 19.54.

5-Carboxamido-2-dimethylamino-3-methyl-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidin-4-one (26). 5-Cyano-2-dimethylamino-3-methyl-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one (**21**, 100 mg, 0.29 mmol) was dissolved in 10 mL of conc. ammonium hydroxide and 1 mL of 30% hydrogen peroxide was then

added. The reaction mixture was allowed to stir at room temperature in a closed round-bottom flask for 18 h. The reaction mixture was evaporated to dryness under reduced pressure at room temperature and then coevaporated with water (2 x 5 mL) at 40 °C. The resulting solid residue was crystallized from ethanol to give 85 mg (80%) of the carboxamido nucleoside **26**. mp 213-214 °C; ^{1}H-NMR (DMSO-d_6): δ 2.83 (s, 6, $N(CH_3)_2$), 3.44 (s, 3, 3N-CH_3), 5.99 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.0 Hz), 7.23 and 9.62 (two broad s, 2 x 1, -$CONH_2$), 7.79 (s, 1, H_6); UV λ_{max} nm (ε x 10^3 $M^{-1}\cdot cm^{-1}$) (MeOH) 224 (sh,7.4), 278 (5.1), 293 (sh,4.6); (pH 1) 232 (8.1), 279 (5.6), 297 (sh,4.5); (pH 11) 235 (7.4), 279 (5.5) 297 (sh,4.4); Anal. Calcd. for $C_{15}H_{21}N_5O_6$: C, 49.04; H, 5.76; N, 19.06. Found: C, 48.93; H, 5.81; N, 19.21.

DISCUSSION

These 5-substituted 2-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4(3H)-ones contain the intact pyrimidine ring of guanosine fused to a pyrrole, rather than an imidazole. It is conceivable that those enzymes responsible for the methylation of the pyrimidine ring of guanosine residues in tRNA could methylate the pyrimidine ring of the Q nucleosides as well. These tRNA (guanine)-methyltransferases are generally considered to be highly specific. However, preparations of these enzymes have been found[23 24,25]to produce small amounts (relative to the preferred substrates) of unidentified methylated compounds.

Queuosine in tRNA arises *via* a post-transcriptional base exchange of queuine (the aglycone of queuosine) with the genetically encoded guanine in the anticodon of substrate tRNA. The enzyme responsible for this base exchange is tRNA-guanine transglycosylase[26] (TGT, E.C. 2.4.2.29). Higher organisms do not appear to be able to synthesize queuine. Furthermore, in these organisms, queuine is either obtained as a dietary factor or is salvaged from tRNA containing queuine [27]. In *E. coli*, however, the queuine modification occurs *via* a three-step process (see Figure 2): 1) TGT-catalyzed base exchange of guanosine-34 [28] (the wobble position of the anticodon) for $preQ_1$ (a queuine precursor), 2) addition of a cyclopentyl diol epoxide to the $preQ_1$-34 tRNA to yield oQ-34 tRNA, and 3) conversion of the epoxy-Q to queuosine.

The $preQ_1$ and $preQ_0$ nucleosides in tRNA arise *via* TGT-catalyzed base exchange of the corresponding aglycones for guanine. The aglycone of $preQ_1$ nucleoside is known to arise from guanine (most likely in the form of GTP). It is possible that N-methylated $preQ_1$ free base could be biosynthesized from the corresponding methylated guanines salvaged from tRNA.

The availability of these fully characterized nucleosides should facilitate the discovery of new modified nucleosides in naturally occurring nucleic acids. Furthermore,

the methylated preQ$_0$ analogs **12**, **20**, and **21** could be employed as synthetic precursors to the corresponding methylated archaeosine analogs *via* a conversion of the exocyclic nitrile to a formamidine group.

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