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NUCLEOSIDE PEPTIDES - IX. SYNTHESIS OF PEPTIDE DERIVATIVES OF SANGIVAMYCIC ACID AND DEAMINOSANGIVAMYCIC ACID

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ABSTRACT - A number of amino acid and peptide conjugates of sangivamycic acid (4) and deaminosangivamycic acid (6) have been prepared in which the peptide linkage is on the carboxylic group of the aglycon moiety. The synthesis of these nucleoside peptides was accomplished, generally in excellent yields, via a two-step procedure involving HOBT/EDC mediated coupling of either 4 or 6 with an appropriately protected amino acid or peptide, followed by ammonolysis. Thus, condensation of 4 with glycine ethyl ester, L-phenylalanine methyl ester, L-glutamic acid diethyl ester, N^{ϵ}-Z-L-lysine methyl ester trifluoroacetate, and δ-benzyl-L-glutamic acid-N^{ϵ}-nitro-L-arginine methyl ester trifluoroacetate gave the corresponding protected linear nucleoside peptides (7, 11, 13, 19, and 22, respectively). Subsequent ammonolysis of 7, 11 and 13 furnished glycine amide (8), L-phenylalanine amide (12), and L-glutamic acid diamide (14) conjugates of sangivamycic acid, respectively. Catalytic hydrogenation of 19 and 22, followed by ammonolysis gave L-lysine amide (20) and L-glutamic acid-α-L-arginine amide (23) conjugates. Saponification of 13 and 16 gave the corresponding L-glutamic acid derivatives (15 and 18, respectively). A similar coupling of 6 with N^{ϵ}-nitro-L-arginine methyl ester, L-serine methyl ester and N^{ϵ}-Z-L-lysine-L-glutamic acid diethyl ester trifluoroacetate, and subsequent ammonolysis (after catalytic hydrogenation) gave L-arginine amide (28), L-serine amide (27), and L-lysine-L-glutamic acid diamide (30) conjugates of deaminosangivamycic acid. ^{1}H NMR spectral data of each of these novel nucleoside peptides were in good agreement with the assigned structures.

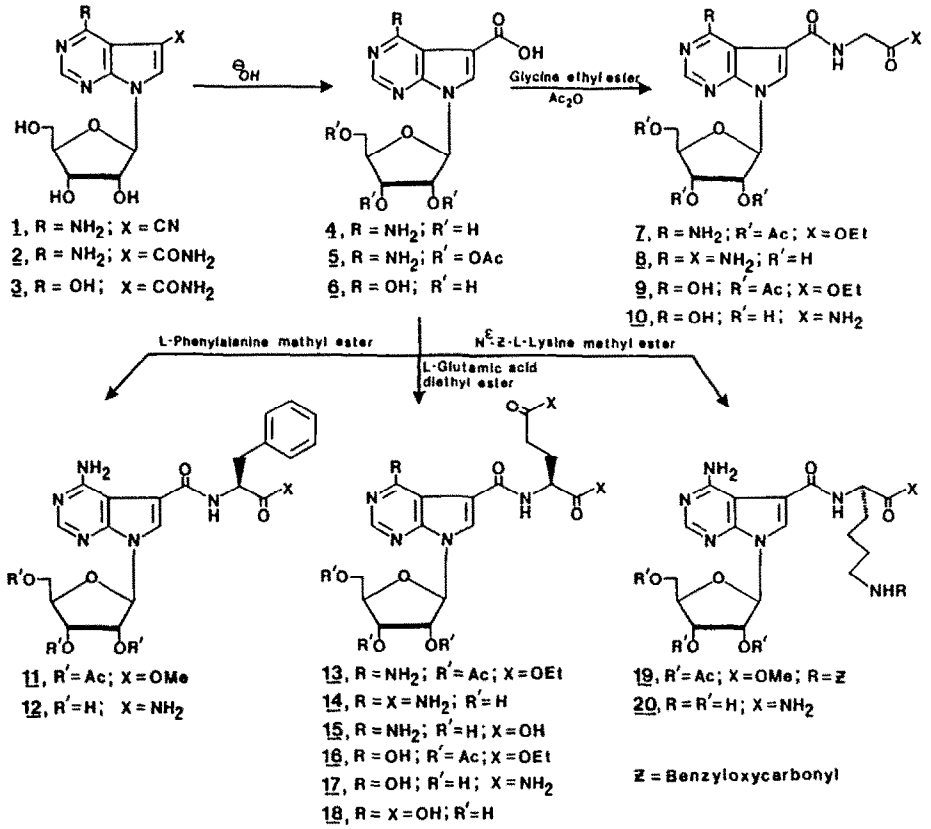
The interactions between proteins and nucleic acids, and the turning "on and off" of genes is one of the most important and vital events in life processes.[1,2] Not only are electrostatic, van der Waals, and hydrogen-bond interactions involved in these processes[3], but also covalent bonds other than those of the substrate may be formed and subsequently broken in order to facilitate these enzymatic processes.[4-7] Although the general concepts of these reactions are understood, very little is known about the details of the molecular interactions involved in such processes.[8]

An approach to understanding of such inter- and intramolecular interactions through relatively simple models of nucleoside peptides was delineated in our previous publication.[9] The importance of such models serving as a link between major fields in the study of proteins and nucleic acids has recently become much more apparent.[10] Moreover, the recent isolation of several new, naturally occurring peptidyl nucleoside antibiotics (e.g. arginomycin[11], chrysandin[12], and A201A[13]) have rekindled considerable interest in nucleoside peptides. The nucleoside and nucleotide peptides isolated from various sources differ considerably in structure, length of nucleotide and peptide chain, as well as in the nature of peptide linkage.[14-17] Such variance of type of linkage and position of peptide attachment may be correlated with different reactivity and biological function.[14] Certain nucleotide peptides readily bind to DNA and inhibit nucleic acid synthesis in vitro which is suggestive of a regulatory function.[18] Gabbay and coworkers[19] have shown that peptides containing aromatic amino acids readily interact with DNA and the aromatic residue of the peptide is partially inserted between base pairs. This intercalation is rather specific and shows an affinity for A:T binding sites.[20] Sequence specific DNA binding proteins regulate gene expression and also serve structural and catalytic functions in other cellular processes.[21,22] It is of particular interest that certain DNA binding oligopeptides exhibit remarkable antiviral activity[23], e.g. netropsin[24] and distamycin.[25,26] However, both netropsin and distamycin are too toxic for clinical use.[25,27] Another natural nucleotide peptide of interest is the transfer factor.[28,29] Evidence indicates that various peptides and proteins are linked to certain types of viral DNA[30] and RNA.[4,31]

In the present study we report the synthesis of amino acid and peptide derivatives of sangivamycic acid (4-amino-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine-5-carboxylic acid, 4) and deaminosangivamycic acid [7-β-D-ribofuranosyl-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxylic acid, 6], in which the peptide linkage is on the carboxylic acid group of the aglycon moiety. Sangivamycin (2) is a naturally occurring cytotoxic[11,32-35] nucleoside antibiotic, structurally related to tubercidin and toyocamycin (1). Sangivamycin was isolated from a strain of *Streptomyces rimosus* by Rao and Renn[36] in 1963 and the total synthesis was reported[37] from our laboratory in 1969. Sangivamycin is active against L1210 leukemia and Lewis Lung carcinoma in mice.[32] A phase I clinical trial with sangivamycin was first run in 1967 by Cavins et al.[38] and phase II studies sponsored by the National Cancer Institute are under consideration against colon cancer, gallbladder cancer and acute myelocytic leukemia (AML) in humans.[39] The carbamoyl group at position-5 of sangivamycin is of paramount importance to its biological activity.[40] We hoped that the $CONH_2$ group could be altered to a -CONH- function without loss of its antitumor activity by coupling amino acids or peptides with sangivamycic acid, which could impart greater selectivity of binding, thus lowering general toxicity observed with sangivamycin. The representative amino acids were selected on the basis of their ability to form hydrogen bonding either with A:T or G:C base pair.[21] Also the basic amino acids, such as lysine and arginine, may bind with the acidic portion of the enzyme. Aromatic amino acid was chosen because of its intercalation behavior with DNA.[19] These sangivamycic acid conjugates could permit an assessment of the role of the nature and size of the charged end group on binding to DNA. Such nucleoside peptide derivatives would offer a range of solubility, transport characteristics and lipophilic nature differing from sangivamycin itself.

The synthesis of these amino acid and peptide conjugates of sangivamycic acid (4) and deaminosangivamycic acid (6) was accomplished in excellent yields, via a two-step procedure

Scheme I



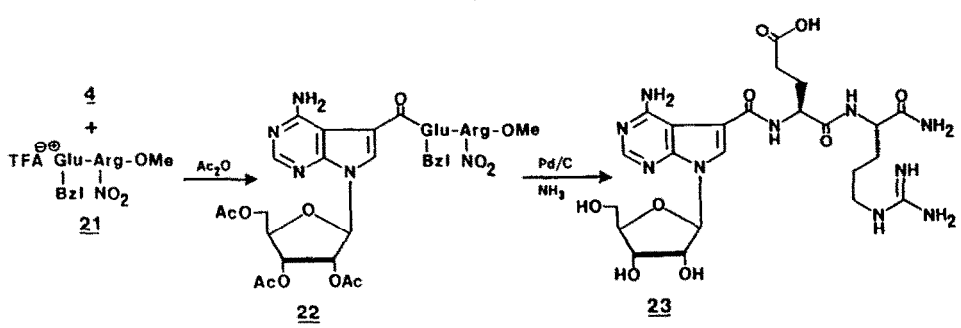
involving the coupling of either 4 or 6 with an appropriately protected amino acid or peptide ester. Since the purification of these coupling products was found to be difficult, due primarily to the coelution of unreacted nucleoside, acetylation of the reaction product was found to be beneficial. Saponification of toyocamycin[37] (1) with 6N KOH gave crystalline sangivamycic acid (4).[41] Acetylation of 4 with acetic anhydride in dry DMF/pyridine gave an 89% yield of 4-amino-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxylic acid (5) (Scheme I). A similar hydrolysis of 7-(β-D-ribofuranosyl)-4(3H)-oxopyrrolo-[2,3-d]pyrimidine-5-carboxamide[42] (3) with 6N NaOH gave 6. Compound 4 was coupled to glycine ethyl ester hydrochloride in anhydrous DMF using 1-hydroxybenzotriazole monohydrate (HOBT) and the water soluble carbodiimide 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC), in the presence of triethylamine (TEA).[43] Without extensive purification, the resulting reaction product was acetylated with acetic anhydride in DMF/pyridine to furnish 4-amino-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxyglycine ethyl ester (7) which was isolated as analytically pure material in 85% overall yeild. A similar coupling of 5 with glycine ethyl ester hydrochloride in DMF/CH_2Cl_2 in the presence of HOBT, EDC and N-methylmorpholine afforded 7 in 96% yield. Treatment of 7 with MeOH/NH_3 resulted in the ammonolysis of the ester function with concomitant deacetylation to give the desired 4-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxyglycine amide (8) in excellent yield.

This general synthetic procedure was found to be applicable equally well to the preparation of other linear nucleoside peptides. Thus, condensation of 4 with L-phenylalanine methyl ester hydrochloride or L-glutamic acid diethyl ester hydrochloride in the presence of HOBT, EDC and TEA gave the corresponding protected nucleoside peptide esters (11 and 13, respectively) in excellent yields. Although the carbodiimide mediated coupling of 4 with N^{ε}-Z-L-lysine methyl ester trifluoroacetate resulted in low yield of 19, use of the mixed anhydride (isobutyl carbonic anhydride) procedure[44] gave good yield of 19. Further ammonolysis of 11, 13 and 19 with MeOH/NH_3 gave the desired L-phenylalanine amide (12), L-glutamic acid diamide (14) and L-lysine amide (20) derivatives of sangivamycic acid. However, hydrolysis of the ester function of 13 with 1N NaOH in MeOH/acetone at room temperature gave 4-amino-7-(β-D-ribofuranosyl)pyrrolo-[2,3-d]pyrimidine-5-carboxy-L-glutamic acid (15), which was isolated in 81% yield.

These coupling reactions were further extended to 6. EDC/HOBT mediated condensation of 6 with glycine ethyl ester hydrochloride in anhydrous DMF in the presence of TEA, followed by the acetylation of the reaction product with acetic anhydride in pyridine resulted in the formation of the blocked nucleoside 7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxyglycine ethyl ester (9), which was isolated in 76% yield. Ammonolysis of 9 with MeOH/NH_3 furnished 7-(β-D-ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxyglycine amide (10). In a similar manner, condensation of 6 with L-glutamic acid diethyl ester hydrochloride led to the formation of the protected nucleoside peptide 16, which on subsequent ammonolysis afforded 7-(β-D-ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxy-L-glutamic acid diamide (17); whereas saponification of 16 by the treatment with 1N NaOH furnished 7-(β-D-ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxy-L-glutamic acid (18) in excellent yield.

Following the similar coupling procedure, several appropriately protected dipeptides were reacted with 4 and 6. When 4 was subjected to the above coupling conditions with the trifluoro-

Scheme II

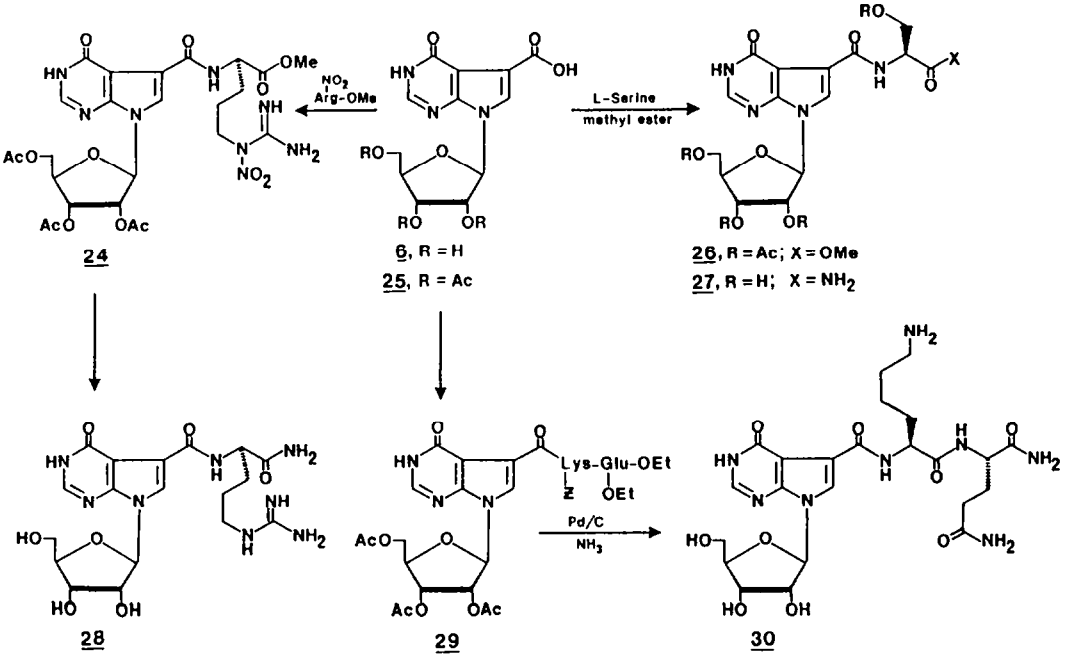


acetate salt of δ-benzyl-L-glutamic acid-N^{ε}-nitro-L-arginine methyl ester (21) in the presence of N-methylmorpholine, 4-amino-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxy-δ-benzyl-L-glutamic acid-N^{ε}-nitro-L-arginine methyl ester (22) was formed (Scheme II). Subsequent catalytic (Pd/C) hydrogenation of 22, followed by ammonolysis with MeOH/NH_3 gave 4-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxy-L-glutamic acid-α-L-arginine amide (23), in an overall yield of 45%.

Several 4(3H)-oxopyrrolo[2,3-d]pyrimidine nucleoside peptides were also prepared by employing this synthetic methodology (Scheme III). Thus, treatment of 6 with one molar equivalent of N^{ε}-nitro-L-arginine methyl ester hydrochloride in anhydrous DMF in the presence of HOBT, EDC and TEA, followed by acetylation of the condensation product gave 7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxy-N^{ε}-nitro-L-arginine methyl ester(24). Compound 24 was also prepared in 81% yield by the direct condensation of 7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxylic acid (25) with N^{ε}-nitro-L-arginine methyl ester hydrochloride. Hydrogenation of 24 in the presence of Pd/C at 50 psi, followed by ammmonolysis gave a 43% yield of 7-(β-D-ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]-pyrimidine-5-carboxy-L-arginine amide (28). Similarly, when an equimolar quantity of 6 was allowed to react with L-serine methyl ester hydrochloride, a 78% yield of 7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxy-L-serine-β-acetyl-α-methyl ester (26) was formed, which on treatment with MeOH/NH_3 furnished 7-(β-D-ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxy-L-serine amide (27) in 73% yield. The protected dipeptide N^{ε}-Z-L-lysine-L-glutamic acid diethyl ester trifluoroacetate was again coupled with either 6 or 25 by the standard procedure to give the blocked nucleoside dipeptide (29), which on catalytic (Pd/C) hydrogenation, followed by ammonolysis furnished crystalline 7-(β-D-ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxy-L-lysine-L-glutamic acid diamide (30) in good yield. All the nucleoside peptides synthesized during this study were fully characterized by spectroscopic and elemental analyses. Confirmation that little or no racemization of the amino acid moieties had occurred was ascertained by TLC, HPLC and GLC studies.[45]

In summary, a number of selected amino acid and peptide derivatives of sangivamycic acid, as well as deaminosangivamycic acid have been prepared in excellent yield. The peptide linkage is on the carboxylic group of the aglycon moiety, which mimics the substituted carbamoyl function at position-5 of sangivamycin. These nucleoside peptides may permit an assessment of the role of the nature and size of the end group on binding to DNA. Such studies are presently under investigation in our laboratory.

Scheme III



EXPERIMENTAL

General Procedures: Melting points (uncorrected) were determined in a Thomas-Hoover capillary melting-point apparatus. Elemental analyses were performed by Robertson Laboratory, Madison, NJ. Thin-layer chromatography (TLC) was performed on plates of silica gel 60F-254 (EM Reagents). Silica gel (E. Merck; 230-400 mesh) was used for flash column chromatography. All solvents used were reagent grade. Detection of nucleoside components in TLC was by UV light, and with 10% H_2SO_4 in MeOH spray followed by heating. Evaporations were conducted under diminished pressure with the bath temperature below 30°C. Infrared (IR) spectra were recorded in KBr with a Perkin-Elmer 1420-spectrophotometer and ultraviolet (UV) spectra with a Beckman DU-50 spectrophotometer (sh = shoulder). Nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 300 MHz with an IBM NR/300 spectrometer. The chemical-shift values were expressed in δ values (parts per million) relative to Me_4Si as the internal standard. The signals are described as s (singlet), d (doublet), t (triplet) and m (multiplet). The presence of H_2O and MeOH as indicated by elemental analysis was verified by ^{1}H NMR spectroscopy. Preparative HPLC was performed utilizing the Waters Delta prep 3000 system. The L-amino acids and coupling reagents used in this study were commercially available. The dipeptides were prepared by standard solution phase method. THF was distilled prior to use from sodium benzophenone ketyl. CH_2Cl_2 was distilled from P_2O_5 and stored over Linde 3A molecular sieves. DMF was distilled from CaH_2.

4-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxylic acid (Sangivamycic Acid, 4). A solution of toyocamycin[37] (1, 23.2 g, 79.7 mmol) in 6N KOH (100 ml) was heated under reflux for 3 h. The reaction mixture was cooled to 0°C and neutralized, with stirring, with acetic acid. The precipitated solid was collected by filtration, washed with cold water (2 x 25 ml) and dried under vacuum over P_2O_5. The dried solid on crystallization from hot water gave the title compound as light yellow crystals, 19.0 g (77%); mp 283-285°C; IR: ν_{max} 1650 (COOH), 3300-3400 (NH_2, OH) cm^{-1}; UV: λ_{max} (pH 1) 228 nm (ε 14,200), 274 (12,900); (pH 7) 225 nm (sh) (ε 10,800), 277 (14,300); (pH 11) 229 nm (sh) (ε 11,500), 277 (14,700); ^{1}H NMR (Me_2SO-d_6): δ 6.07 (d, 1, $J_{1',2'}$ = 5.8 Hz, C_1'-H), 7.48 (br s, 2, NH_2), 8.14 and 8.22 (2 s, 2, C_2-H and C_6-H). Anal. Calcd for $C_{12}H_{14}N_4O_6$ (310.23): C, 46.46; H, 4.55; N, 18.05. Found: C, 46.35; H, 4.52; N, 17.95.

4-Amino-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxylic acid (5). A solution of 4 (3.2 g, 10.3 mmol) in dry DMF (100 ml), anhydrous pyridine (20 ml) and acetic anhydride (5.1 ml, 50 mmol) was stirred at room temperature for 12 h and then evaporated to dryness. The residue was suspended in a mixture of water (50 ml) and EtOAc (75 ml) and stirred for 1 h. The aqueous phase was separated and again extracted with EtOAc (2 x 25 ml). The organic layers were combined and washed with saturated brine solution (2 x 25 ml), dried over Na_2SO_4 and evaporated to dryness to give the title compound as a homogeneous foam, 4.0 g (89%); IR: ν_{max} 1680 (COOH), 1750 (C=O), 3200-3300 (NH_2)cm^{-1}; UV: λ_{max} (MeOH) 230 nm (ε 12,100), 279 (14,300); ^{1}H NMR (Me_2SO-d_6): δ 2.02-2.11 (3 s, 9, 3COCH_3), 6.33 (d, 1, $J_{1',2'}$ = 5.7 Hz, C_1'-H), 7.50 (br s, 2, NH_2), 8.16 and 8.19 (2 s, 2, C_2-H and C_6-H), 11.78 (br s, 1, COOH). Anal. Calcd for $C_{18}H_{20}N_4O_9$ (436.34): C, 49.54; H, 4.62; N, 12.83. Found: C, 49.23; H, 4.36; N, 12.64.

7-(β-D-Ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxylic acid (6). A solution of 7-(β-D-ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxamide[42] (3, 9.6 g, 30.9 mmol) in 6N NaOH (100 ml) was heated under reflux for 1 h. The reaction mixture was cooled to 0°C and neutralized with acetic acid. The precipitated solid was collected by filtration, washed with cold water (2 x 20 ml) and the solid after crystallization from hot water gave 9.0 g (93%) of 6; mp 286-288°C; IR: ν_{max} 1650 (COOH), 1705 (C=O), 3200-3500 (NH_2, OH)cm^{-1}; UV: λ_{max} (pH 1) 222 nm (ε 18,400), 270 (14,600); (pH 7) 217 nm (ε 21,800), 266 (16,800); (pH 11) 270 nm (ε 15,500); ^{1}H NMR (Me_2SO-d_6): δ 6.09 (d, 1, $J_{1',2'}$ = 5.0 Hz, C_1'-H), 8.27 (m, 2, C_2-H and C_6-H), 14.23 (br s, 2, NH and COOH). Anal. Calcd for $C_{12}H_{13}N_3O_7$ (311.22): C, 46.31: H, 4.21; N, 13.49. Found: C, 46.02: H, 4.46; N, 13.40.

4-Amino-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxyglycine ethyl ester (7). Method A. A mixture of 4 (1.55 g, 5 mmol), glycine ethyl ester hydrochloride (0.83

g, 6 mmol) and 1-hydroxybenzotriazole monohydrate (HOBT, 0.83 g, 6.2 mmol) in dry DMF was cooled to 0°C. To this cold stirred solution was added 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC, 1.34 g, 7 mmol) and triethylamine (TEA, 0.88 ml, 6.3 mmol). The reaction mixture was stirred at 0°C for 3 h and then at room temperature overnight. The solvent was evaporated, and the oily residue was dissolved in MeOH. Dilution of the methanolic solution with ether, followed by cooling and filtration gave 4-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]-pyrimidine-5-carboxyglycine ethyl ester as an amorphous solid. Our attempts to crystallize the product were not successful. Yield 1.4 g (73%); mp 160-165°C; IR: ν_{max} 1640 (CONH), 1720 (COOEt), 3300-3400 (NH_2, OH) cm^{-1}; UV: λ_{max} (pH 1) 232 nm (ε 20,400), 275 (17,200); (pH 7) 204 nm (ε 38,500), 231 (15,600), 278 (20,600); (pH 11) 233 nm (ε 14,200), 279 (20,100); ^{1}H NMR (Me_2SO-d_6): δ 1.21 (t, 3, CH_2CH_3), 3.93 (m, 2, Gly-CH_2), 6.06 (d, 1, $J_{1',2'}$ = 6.0 Hz, C_1,H), 8.09 and 8.15 (2 s, 2, C_2H and C_6H), 8.91 (m, 1, NH).

A solution of the above solid (0.14 g, 0.35 mmol) in acetic anhydride (0.15 g, 1.42 mmol) and dry DMF/pyridine (1:1, 60 ml) was stirred at room temperature for 8 h. The reaction mixture was evaporated to dryness, the residue was triturated with EtOH (25 ml) and evaporated once again. The residue was purified by flash chromatography over silica gel using CH_2Cl_2:acetone (8:2) as the eluent. The homogeneous fractions were pooled together and evaporated to give 0.16 g (86%) of 7 as foam; IR: ν_{max} 1640-1650 (C=O), 1750 (COOEt), 3400-3450 (NH_2)cm^{-1}; UV: λ_{max} (MeOH) 218 nm (ε 21,900), 231 (15,100), 288 (14,600); ^{1}H NMR (Me_2SO-d_6): δ 1.20 (t, 3, CH_2CH_3), 2.03-2.12 (3 s, 9, $3COCH_3$), 4.02 (m, 2, Gly-CH_2), 6.36 (d, 1, $J_{1',2'}$ = 6.0 Hz, C_1,H), 8.17 and 8.22 (2 s, 2, C_2H and C_6H), 9.07 (t, 1, NH). Anal. Calcd for $C_{22}H_{27}N_5O_{10}\cdot 1/2CH_3OH$ (521.48): C, 50.28; H, 5.44; N, 13.02. Found: C, 50.05; H, 5.28; N, 12.74.

Method B. To a cold (0-5°C) solution of 5 (0.44 g, 1 mmol), glycine ethyl ester hydrochloride (0.14 g, 1 mmol) and HOBT (0.14 g, 1 mmol) in dry DMF:CH_2Cl_2 (1:4, 50 ml) was added N-methylmorpholine (0.11 ml, 1 mmol) and EDC (0.19 g, 1 mmol). The reaction mixture was stirred at 0°C for 3 h and then at room temperature for 8 h. The solution was evaporated to dryness and the residue was extracted with EtOAc (2 x 50 ml). The combined organic phase was washed with water (30 ml), followed by saturated brine solution (30 ml), dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product on purification by flash chromatography over silica gel (CH_2Cl_2:acetone, 8:2, as the solvent) gave 0.5 g (96%) of the title compound, which was identical to 7 prepared by method A.

4-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxyglycine amide (8). A solution of 7 (1.2 g, 2.3 mmol) in MeOH/NH_3 (100 ml, saturated at 0°C) was stirred at room temperature in a pressure bottle for 12 h. The bottle was cooled to 0°C, opened and the NH_3 allowed to evaporate. The MeOH was evaporated to dryness and the residue on crystallization from aqueous EtOH gave 0.7 g (83%) of 8; mp 179-181°C; IR: ν_{max} 1670 ($CONH_2$), 3200-3400 (NH_2, OH)cm^{-1}; UV: λ_{max} (pH 1) 232 nm (ε 13,900), 275 (11,400); (pH 7) 232 nm (ε 10,500), 278 (13,300); (pH 11) 233 nm (ε 9,400), 279 (ε 13,300); ^{1}H NMR (Me_2SO-d_6): δ 3.92 (d, 2, Gly-CH_2), 6.06 (d, 1, $J_{1',2'}$ = 6.0 Hz, C_1,H), 7.09 and 7.45 (2 s, 2, $CONH_2$), 8.09 and 8.16 (2 s, 2, C_2H and C_6H), 8.66 (m, 1, NH). Anal. Calcd for $C_{14}H_{18}N_6O_6$ (366.28): C,45.90; H,4.95; N,22.93. Found: C,45.62; H,5.21; N,22.60.

4-Amino-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxy-L-phenylalanine methyl ester (11). By following the procedure as described for the preparation of 7 (method A), the title compound was prepared by using 4 (0.93 g, 3 mmol), L-phenylalanine methyl ester hydrochloride (0.65 g, 3 mmol), HOBT (0.42 g, 3.1 mmol), EDC (0.67 g, 3.5 mmol) and TEA (0.42 ml, 3 mmol) in DMF (50 ml). Acetylation of the crude product with acetic anhydride (1.68 ml, 12 mmol) in DMF/pyridine (2:3, 50 ml) and purification by flash chromatography over silica gel using CH_2Cl_2:acetone (8:2) gave 1.0 g (56%) of 11 as a foam; IR: ν_{max} 1750 ($COOCH_3$), 3300-3400 (NH, NH_2)cm^{-1}; UV: λ_{max} (EtOH) 209 nm (ε 20,300), 229 (sh) (9,000), 279 (9,600); ^{1}H NMR (Me_2SO-d_6): δ 2.04-2.15 (3 s, 9, $3COCH_3$), 3.07-3.18 (m, 2, Phe-CH_2), 3.66 (s, 3, $COOCH_3$), 4.25 (m, 1, Phe-α-H), 6.37 (d, 1, $J_{1',2'}$ = 6.0 Hz, C_1,H), 7.19-7.29 (m, 5, Ph-H), 8.12 and 8.15 (2 s, 2, C_2H and C_6H), 8.90 (d, 1, NH). Anal. Calcd for $C_{28}H_{31}N_5O_{10}$ (597.53): C, 56.28; H, 5.23; N, 11.71. Found: C, 56.17; H, 5.41; N, 11.81

4-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxy-L-phenylalanine amide (12). The title compound was prepared in a similar manner as described for 8 using 11 (0.7 g, 1.17 mmol) and MeOH/NH_3 (75 ml). The product was crystallized from 95% EtOH/H_2O to yield 0.45 g (84%); mp 178-180°C; IR: ν_{max} 1670 ($CONH_2$), 3300-3400 (NH_2, OH)cm^{-1}; UV: λ_{max} (pH 1) 229nm (ε 15,000), 278 (12,300); (pH 7) 233 nm (ε 11,000), 280 (14,400); (pH 11) 233 nm (ε 11,300), 280 (14,600); ^1H NMR (Me_2SO-d_6): δ 2.93-3.15 (m, 2, Phe-CH_2) 4.62 (m, 1, Phe-α-H), 6.06 (d, 1, $J_{1',2'}$= 6.0 Hz, C_1,H), 7.13-7.36 (m, 5, Ph-H), 7.28 and 7.65 (2 s, 2, $CONH_2$), 8.07 and 8.20 (2 s, 2, C_2H and C_6H), 8.54 (d, 1, NH). Anal. Calcd for $C_{21}H_{24}N_6O_6.1/2H_2O$ (456.46): C, 54.19; H, 5.41; N, 18.05. Found: C, 54.45; H, 5.39; N, 17.98.

4-Amino-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxy-L-glutamic acid diethyl ester (13). In a similar manner as for 7 (method A), the title compound was prepared by using 4 (2.0 g, 6.4 mmol), L-glutamic acid diethyl ester hydrochloride (1.55 g, 6.5 mmol), HOBT (0.78 g, 6 mmol), EDC (1.33 g, 7 mmol) and TEA (1 ml, 7.2 mmol) in dry DMF (50 ml). Acetylation of the crude product with acetic anhydride (3.6 ml, 25.7 mmol) in dry DMF/pyridine (50 ml each) and purification by flash chromatography over silica gel using a CH_2Cl_2→ acetone gradient gave 2.5 g (62%) of 13 as an analytically pure foam; IR: ν_{max} 1630 ($COCH_3$), 1740 (COOEt), 3300-3400 (NH_2)cm^{-1}; UV: λ_{max} (MeOH) 209 nm (ε 31,200), 279 (19,000); ^1H NMR ($CDCl_3$): δ 1.22 (t, 3, CH_2CH_3), 1.29 (t, 3, CH_2CH_3), 2.07-2.17 (3 s, 9, $3COCH_3$), 2.20-2.54 (m, 4, Glu-CH_2), 4.35 (q, 4, $2CH_2CH_3$), 4.61 (m, 1, Glu-α-H), 6.47 (d, 1, $J_{1',2'}$= 6.0 Hz, C_1,H), 7.44-7.51 (m, 3, NH and NH_2), 7.75 and 8.24 (2 s, 2, C_2H and C_6H). Anal. Calcd for $C_{27}H_{35}N_5O_{12}$ (621.55): C, 52.17; H, 5.67; N, 11.26. Found: C, 52.23; H, 5.63; N, 11.41.

4-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxy-L-glutamic acid diamide (14). This compound was prepared by following the procedure as described for the synthesis of 8, using compound 13 (1.5 g, 2.4 mmol) and MeOH/NH_3 (100 ml). The residue after crystallization from aqueous MeOH gave the hygroscopic title compound; 0.8 g (76%); mp 147-150°C; IR: ν_{max} 1630 ($CONH_2$), 3200-3400 (NH_2, OH)cm^{-1}; UV: λ_{max} (pH 1) 234 nm (ε 15,100), 275 (14,000); (pH 7) 278 nm (ε 16,600); (pH 11) 278 nm (ε 15,900); ^1H NMR (Me_2SO-d_6): δ 1.70-2.18 (m, 4, Glu-CH_2), 3.40 (br s, 2, NH_2), 4.38 (m, 1, Glu-α-H), 6.06 (d, 1, $J_{1',2'}$= 6.0 Hz, C_1,H), 6.82 and 7.36 (2 s, 2, $CONH_2$), 7.10 and 7.49 (2 s, 2, $CONH_2$), 8.09 and 8.29 (2 s, 2, C_2H and C_6H), 8.43 (m, 1, NH); Anal. Calcd for $C_{17}H_{23}N_7O_7.1/2H_2O$ (437.41): C, 45.69; H, 5.42; N, 21.95. Found: C, 45.22; H, 5.41; N, 21.77.

4-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxy-L-glutamic acid (15). A solution of 13 (0.7 g, 1.13 mmol) in MeOH/acetone (20 ml each) and 1N NaOH (7 ml, 7 mmol) was stirred at ambient temperature for 12 h. MeOH/acetone was evaporated and the aqueous solution was diluted with water (50 ml). The pH of the aqueous solution was adjusted to 1 with Dowex-50 H^+ resin. The resin was removed by filtration, washed with water (2 x 20 ml) and the combined filtrates evaporated to dryness. The residue was crystallized from EtOH (95%) to give 15, 0.4 g (81%); mp 265-270°C; IR: ν_{max} 1670 (COOH), 3300-3400 (NH_2, OH)cm^{-1}; UV: λ_{max} (pH 1) 235 nm (ε 17,900), 277 (15,100); (pH 7) 235 nm (ε 13,300), 278 (17,300); (pH 11) 235 nm (ε 13,200), 278 (17,500); ^1H NMR (Me_2SO-d_6); δ 1.91-2.40 (m, 4, Glu-CH_2), 4.39 (m, 1, Glu-α-H), 6.05 (d, 1, $J_{1',2'}$= 6.0 Hz, C_1,H), 8.09 and 8.25 (2 s, 2, C_2H and C_6H), 8.58 (d, 1, NH), 12.52 (br s, 2, 2COOH). Anal. Calcd for $C_{17}H_{21}N_5O_9 \cdot H_2O$ (439.38): C, 44.64; H, 5.06; N, 15.31. Found: C, 44.66; H, 5.00; N, 15.35.

4-Amino-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxy-N^{ε}-Z-L-lysine methyl ester (19). Method A. In the same manner as for 7 (method A), reaction of 4 (0.87 g, 2.0 mmol), N^{ε}-Z-L-lysine methyl ester trifluoroacetate (1.0 g, 2.45 mmol), HOBT (0.39 g, 3 mmol), EDC (0.67 g, 3.5 mmol) and TEA (0.5 ml, 3.5 mmol) in dry DMF (50 ml) and subsequent acetylation of the reaction product with acetic anhydride (1.02 g, 10 mmol) in pyridine (50 ml) and DMF (20 ml) gave the crude product. Purification of the crude product by flash chromatography over silica gel using CH_2Cl_2→ acetone gradient gave 0.85 g (47%) of the title compound as a homogeneous foam; IR: ν_{max} 1750 (COOMe), 3300-3400 (NH_2)cm^{-1}; UV: λ_{max} (MeOH) 232 nm

(ε 15,500), 287 (14,300); ^{1}H NMR (Me_2SO-d_6): δ 1.45-1.95 (m, 6, Lys-C$\underline{H}_2$), 2.05-2.18 (3 s, 9, 3COC$\underline{H}_3$), 3.21 (m, 2, Lys-C$\underline{H}_2$), 3.78 (s, 3, OC$\underline{H}_3$), 4.65 (m, 1, Lys-α-$\underline{H}$), 4.99-5.01 (m, 1, N$\underline{H}$), 5.02 (s, 2, C$\underline{H}_2$Ph), 6.45 (d, 1, $J_{1',2'}$ = 6.0 Hz, C_1,$\underline{H}$), 7.15 (d, 1, N$\underline{H}$), 7.21-7.34 (m, 5, CH$_2$$\underline{Ph}$), 7.82 and 8.24 (2 s, 2, $C_2\underline{H}$ and $C_6\underline{H}$). Anal. Calcd for $C_{33}H_{40}N_6O_{12}$ (712.65): C, 55.61; H, 5.66; N, 11.78. Found: C, 55.34; H, 5.50; N, 11.91.

Method B. To a cold (-20°C) solution of 5 (2.18 g, 5 mmol) and N-methylmorpholine (0.54 ml, 5 mmol) in dry DMF (15 ml) was added isobutyl chloroformate (0.67 g, 5 mmol). The solution was stirred for 20 min before a solution of N^{ε}-Z-L-lysine methyl ester trifluoroacetate (1.9 g, 4.66 mmol) in dry THF (35 ml) was added, followed by N-methylmorpholine (0.54 ml, 5 mmol). The reaction mixture was stirred at -20°C for 2 h and then at ambient temperature overnight. The solvents were evaporated and the residue was partitioned between EtOAc (100 ml) and water (30 ml). The EtOAc phase was washed with 0.1 N HCl (50 ml), followed by 1N $NaHCO_3$ (30 ml), water (25 ml) and saturated brine solution (20 ml). The dried (Na_2SO_4) organic phase was evaporated to dryness. The residue was purified as described above in method A to yield 2.6 g (73%) of 19 as a foam, which was identical to the product prepared by method A.

4-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxy-L-lysine amide (20). To a solution of 19 (1.1 g, 1.54 mmol) in MeOH (50 ml) was added Pd/C (10%, 0.5 g) and the mixture was shaken under H_2 (50 psi) for 12 h. The catalyst was removed by filtration on a Celite pad, washed with aqueous EtOH (50 ml) and the combined filtrates evaporated to dryness. The residue (0.6 g) was used as such for the next step without characterization.

The above residue in MeOH/NH_3 (100 ml) was allowed to stir in a pressure bottle overnight at room temperature. The bottle was cooled, opened and the contents evaporated to dryness. The residue was crystallized from aqueous EtOH as an amorphous solid to yield 0.3 g (68% over-all yield) of 20 (after drying over P_2O_5 under vacuum overnight); mp >140°C; IR: ν_{max} 1630 ($CONH_2$) 3200-3400 (NH_2, OH)cm^{-1}; UV: λ_{max_1} (pH 1) 231 nm (ε 16,400), 275 (13,700); (pH 7) 279 nm (ε 15,700); (pH 11) 279 nm (ε 15,800); ^{1}H NMR (Me_2SO-d_6): δ 1.25-1.90 (m, 6, 3 Lys-C$\underline{H}_2$), 3.20-4.50 (m, 5, N$\underline{H}_2$, Lys-α-$\underline{H}$ and Lys-C$\underline{H}_2$), 6.06 (d, 1, $J_{1',2'}$ = 6.0 Hz, C_1,$\underline{H}$), 7.06 and 7.54 (2 s, 2, CON$\underline{H}_2$), 8.08-8.48 (m, 5, N$\underline{H}$, N$\underline{H}_2$, $C_2\underline{H}$ and $C_6\underline{H}$). Anal. Calcd for $C_{18}H_{27}N_7O_6$ (437.39): C, 49.42; H, 6.22; N, 22.40. Found: C, 49.28; H, 5.97; N, 22.21.

4-Amino-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxy-δ-benzyl-L-glutamic acid-N^{ε}-nitro-L-arginine methyl ester (22). The title compound was prepared by following the procedure employed for the synthesis of 7. The following quantities of the substrates were used: compound 4 (0.93 g, 3 mmol), δ-benzyl-L-glutamic acid-N^{ε}-nitro-L-arginine methyl ester trifluoroacetate (21, 1.69 g, 3 mmol), N-methylmorpholine (0.44 ml, 4 mmol), EDC (0.57 g, 3 mmol), HOBT (0.39 g, 3 mmol) and dry DMF (50 ml). The reaction product was acetylated with acetic anhydride (2.45 g, 24 mmol) in anhydrous pyridine (25 ml) and DMF (20 ml). The crude material was purified by flash chromatography over silica gel using $CHCl_3$→ acetone gradient to yield 1.5 g (58%) of analytically pure 22 as a foam; IR: ν_{max} 1610 (C=O), 3300-3400 (NH, NH_2)cm^{-1}; UV: λ_{max} (EtOH) 271 nm (ε 24,600); ^{1}H NMR (Me_2SO-d_6): δ 1.40-1.85 (m, 8, Glu- and Arg-C$\underline{H}_2$'s), 3.10-3.40 (m, 2, Arg-C$\underline{H}_2$), 3.61 (s, 3, OC$\underline{H}_3$), 4.25-4.43 (m, 2, Glu- and Arg-α-$\underline{H}$), 5.08 (s, 2, C$\underline{H}_2$Ph), 6.46 (d, 1, $J_{1',2'}$ = 6.0 Hz, C_1,$\underline{H}$), 7.32 (m, 5, CH$_2$$\underline{Ph}$), 8.59-8.83 (m, 6, 2 N$\underline{H}$ + N$\underline{H}_2$ + $C_2\underline{H}$ and $C_6\underline{H}$), 12.43 (s, 1, N$\underline{H}$). Anal. Calcd for $C_{37}H_{46}N_{10}O_{15}$·MeOH (870.83): C, 50.32; H, 5.41; N, 15.04. Found: C, 50.25; H, 5.30; N, 14.92.

4-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxy-L-glutamic acid-α-L-arginine amide (23). To a solution of 22 (1.5 g, 1.72 mmol) in 95% EtOH (100 ml) was added Pd/C (10%, 1.0 g) and the mixture was hydrogenated on a Parr hydrogenator at 50 psi for 12 h. The catalyst was removed by filtration on a celite pad, washed with aqueous EtOH (2 x 25 ml) and the combined filtrates evaporated to dryness. The residue was used as such for the next step without characterization.

The above residue was stirred with MeOH/NH_3 (saturated at 0°C, 100 ml) at ambient temperature overnight. The MeOH/NH_3 was evaporated and the residue was crystallized from 95% EtOH to yield 0.5 g (45%) of 23; mp >210°C (dec.); IR: ν_{max} 1640 ($CONH_2$), 3200-3400 (NH_2,OH)cm^{-1}; UV: λ_{max} (pH 1) 240 nm (ε 19,400), 271 (21,700); (pH 7) 204 nm (ε 33,100), 273 (23,100); (pH 11) 273 nm (ε 23,300); ^{1}H NMR (Me_2SO-d_6): δ 1.25-2.30 (m, 8, Glu- and Arg- C$\underline{H}_2$'s), 3.16 (m, 2, Arg-C$\underline{H}_2$),

3.93-4.46 (m, 2, Glu- and Arg-α-H), 6.09 (d, 1, $J_{1',2'}$ = 6.0 Hz, C_1,H), 7.10 and 7.35 (2 s, 2, $CONH_2$), 7.97-8.70 (m, 6, $2NH_2$, C_2H and C_6H), 9.85-10.10 (br d, 2, 2NH), 12.70 (br s, 2, NH and COOH). Anal. Calcd for $C_{23}H_{34}N_{10}O_9 \cdot 4H_2O$ (594.58): C, 41.43; H, 6.25; N, 21.00. Found: C, 41.09; H, 6.58; N, 20.94.

7-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxyglycine ethyl ester (9). In a similar manner as for 7 (method A), the title compound was prepared by using 6 (0.5 g, 1.6 mmol), glycine ethyl ester hydrochloride (0.23 g, 1.7 mmol), HOBT (0.26 g, 2 mmol), EDC (0.38 g, 2 mmol) and TEA (0.24 ml, 1.7 mmol) in dry DMF (30 ml). Acetylation of the crude reaction product with acetic anhydride (1.68 ml, 12 mmol) in DMF/pyridine (25 ml each) and purification by flash chromatography over silica gel using CH_2Cl_2/acetone (8:2) as the eluent gave 1.0 g (76%) of 9 as an amorphous solid; IR: ν_{max} 1650 (C=O), 1750 (COOEt)cm^{-1}; UV: λ_{max} (EtOH) 215 nm (ε 26,600), 267 (14,400); ^{1}H NMR ($CDCl_3$): δ 1.33 (t, 3, CH_2CH_3), 2.08-2.25 (3 s, 9, $3COCH_3$), 4.24-4.46 (m, 4, CH_2CH_3 and Gly-CH_2), 6.40 (d, 1, $J_{1',2'}$ = 6.0 Hz, C_1,H), 8.01 and 8.22 (2 s, 2, C_2H and C_6H), 10.77 (t, 1, NH), 12.86 (br s, 1, NH). Anal. Calcd for $C_{22}H_{26}N_4O_{11}$ (522.43): C, 50.57; H, 5.01; N, 10.72. Found: C, 50.64; H, 5.16; N, 11.00.

7-(β-D-Ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxyglycine amide (10). This compound was prepared by following the procedure as described for the synthesis of 8, using 9 (0.7 g, 1.4 mmol) and MeOH/NH_3 (60 ml). Crystallization from aqueous EtOH gave 0.3 g (61%) of 10; mp 152-155°C; IR: ν_{max} 1670 (C=O), 3300-3400 (NH, OH)cm^{-1}; UV: λ_{max} (pH 1) 270 nm (ε 13,500); (pH 7) 270 nm (ε 14,700); (pH 11) 229 nm (sh) (ε 13,600), 276 (17,000); ^{1}H NMR (Me_2SO-d_6): δ 3.60 (m, 2, Gly-CH_2), 6.08 (d, 1, $J_{1',2'}$ = 6.0 Hz, C_1,H), 7.04 and 7.39 (2 s, 2, $CONH_2$), 8.03 and 8.11 (2 s, 2, C_2H and C_6H), 10.37 (t, 1, NH), 12.64 (br s, 1, NH). Anal. Calcd for $C_{14}H_{17}N_5O_7 \cdot 1\ 1/2\ H_2O$ (367.31): C, 42.65; H, 5.11; N, 17.75. Found: C, 43.03; H, 5.03; N, 17.37.

7-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxy-L-glutamic acid diethyl ester (16). In a similar manner as for 7 (method A), compound 16 was prepared by using 6 (2.0 g, 6.4 mmol), L-glutamic acid diethyl ester hydrochloride (1.55 g, 6.5 mmol), HOBT (0.78 g, 6 mmol), EDC (1.33 g, 7 mmol) and TEA (1 ml, 7 mmol) in dry DMF (50 ml). Acetylation of the crude, dry (over P_2O_5 under vacuum) reaction product with acetic anhydride (2.61 g, 25.6 mmol) in pyridine (50 ml) and DMF (30 ml), and purification by flash chromatography using CH_2Cl_2→ acetone gradient as the eluent gave 2.5 g (62%) of the title compound; IR: ν_{max} 1650 (C=O), 1750 (COOEt)cm^{-1}; UV: λ_{max} (MeOH) 217nm (ε 24,500), 268 (12,900); ^{1}H NMR (Me_2SO-d_6): δ 1.18 (m, 6, $2CH_2CH_3$), 2.02, 2.08 and 2.12 (3 s, 9, $3COCH_3$), 1.94-2.47 (m, 4, Glu-CH_2s), 4.00-4.14 (m, 4, $2CH_2CH_3$), 4.46 (m, 1, Glu-α-H), 6.34 (d, 1, $J_{1',2'}$ = 6.0 Hz, C_1,H), 8.07 and 8.17 (2s, 2, C_2H and C_6H), 10.60 (d, 1, NH), 12.81 (br s, 1, NH). Anal. Calcd for $C_{27}H_{34}N_4O_{13}$ (622.58): C, 52.09; H, 5.51; N, 8.99. Found: C, 51.76; H, 5.66; N, 8.75.

7-(β-D-Ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxy-L-glutamic acid diamide (17). The title compound was prepared by following the procedure as described for the synthesis of 8, using 16 (1.2 g, 1.93 mmol) and MeOH/NH_3 (100 ml). The residue after crystallization from MeOH gave 0.7 g (83%) of 17; mp 267-270°C; IR: ν_{max} 1650 ($CONH_2$), 3200-3400 (NH_2, OH)cm^{-1}; UV: λ_{max} (pH 1) 219 nm (ε 32,100), 270 (25,000); (pH 7) 212 nm (ε 37,400), 269 (26,100); (pH 11) 227 nm (ε 25,000), 276 (28,000); ^{1}H NMR (Me_2SO-d_6): δ 1.76-2.04 (m, 4, Glu-CH_2s), 4.36 (m, 1, Glu-α-H), 6.08 (d, 1, $J_{1',2'}$ = 6.0 Hz, C_1,H), 6.75 and 7.29 (2 s, 2, $CONH_2$), 7.02 and 7.40 (2 s, 2, $CONH_2$), 8.01 and 8.11 (2 s, 2, C_2H and C_6H), 10.38 (d, 1, NH), 12.58 (d, 1, NH). Anal. Calcd for $C_{17}H_{22}N_6O_8 \cdot 1/2\ H_2O$ (438.39): C, 45.64; H, 5.18; N, 18.77. Found: C, 45.59; H, 4.92; N, 18.43.

7-(β-D-Ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxy-L-glutamic acid (18). In a similar manner as for 15, treatment of 16 (1.2 g, 1.93 mmol) with 1N NaOH (15 ml, 15 mmol) in MeOH (20 ml) and acetone (25 ml) gave 0.7 (82%) of the title compound after crystallization from aqueous EtOH; mp 209-212°C; IR: ν_{max} 1650 ($CONH_2$), 1730 (COOH), 3300-3400 (NH_2, OH)cm^{-1}; UV: λ_{max} (pH 1) 216 nm (ε 19,000), 269 (14,700); (pH 7) 215 nm (ε 22,400), 269 (15,800); (pH 11) 276 nm (ε 18,200); ^{1}H NMR (Me_2SO-d_6): δ 1.88-2.11 (m, 4, Glu-CH_2s), 4.41 (m, 1, Glu-α-H), 6.09 (d, 1, $J_{1',2'}$ = 6.0 Hz, C_1,H), 8.04 and 8.13 (2 s, 2, C_2H and C_6H), 10.54 (d, 1, NH), 12.66 (br s, 3, NH and 2COOH). Anal. Calcd for $C_{17}H_{20}N_4O_{10} \cdot 1/2\ H_2O$ (440.36): C, 45.43; H, 4.71; N, 12.46. Found: C, 45.66; H, 4.93; N, 12.38.

7-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxylic acid (25). The title compound was prepared by following the procedure employed for the synthesis of 5, using 6 (3.11 g, 10 mmol), acetic anhydride (4.08 g, 40 mmol) in dry DMF (30 ml) and pyridine (20 ml). The product was crystallized from CH_2Cl_2 as flakes to yield 3.9 g (89%); mp 184-186°C; IR: ν_{max} 1650 (C=O), 1750 (COOH), 3300 (OH)cm^{-1}; UV: λ_{max} (pH 1) 220 nm (ε 16,300), 268 (13,300); (pH 7) 264 nm (ε 13,100); (pH 11) 268 nm (ε 15,200); ^{1}H NMR (Me_2SO-d_6): δ 2.03-2.11 (3 s, 9, $3COCH_3$), 6.33 (d, 1, $J_{1',2'}$= 6.0 Hz, C_1,H), 8.27 and 8.28 (2 s, 2, C_2H and C_6H), 13.65 (br s, 1, NH), 14.14 (s, 1, COOH). Anal. Calcd for $C_{18}H_{19}N_3O_{10}$ (437.33): C, 49.43; H, 4.38; N, 9.60. Found: C, 49.31; H, 4.31; N, 9.46.

7-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxy-N^{ε}-nitro-L-arginine methyl ester (24). Method A. In a similar manner as for 7 (method A), the title compound was prepared by using 6 (1.0 g, 3.2 mmol), N^{ε}-nitro-L-arginine methyl ester hydrochloride (0.88 g, 3.3 mmol), HOBT (0.39 g, 3 mmol), EDC (0.67 g, 3.5 mmol) and TEA (0.5 ml, 3.5 mmol) in dry DMF (50 ml). The residue after evaporation of solvent was dried over P_2O_5 under vacuum overnight and used for acetylation without characterization.

Acetylation of the above dry residue with acetic anhydride (1.64 g, 16 mmol) in pyridine (50 ml) and DMF (20 ml), followed by purification of the reaction material by flash chromatography over silica gel using CH_2Cl_2→ MeOH gradient as the eluent gave 1.2 g (58%) of 24 (crystallized from MeOH); mp 152-153°C; IR: ν_{max} 1650 (C=O), 1750 ($COOCH_3$), 3200-3400 (NH, NH_2) cm^{-1}; UV: λ_{max} (pH 1) 267 nm (ε 26,500); (pH 7) 267 nm (ε 29,500); (pH 11) 271 nm (ε 30,100); ^{1}H NMR (Me_2SO-d_6): δ 1.50-1.92 (m, 4, Arg-CH_2s), 2.02-2.13 (3 s, 9, $3COCH_3$), 3.10-3.40 (m, 2, Arg-CH_2), 3.64 (s, 3, OCH_3), 4.37 (m, 1, Arg-α-H), 6.34 (d, 1, $J_{1',2'}$= 6.0 Hz, C_1,H), 7.80-8.65 (m, 3, NH and NH_2), 8.07 and 8.19 (2 s, 2, C_2H and C_6H), 10.63 (m, 1, NH), 12.81 (br s, 1, NH). Anal. Calcd for $C_{25}H_{32}N_8O_{13}$ (652.51): C, 46.01; H, 4.94; N, 17.16. Found: C, 46.11; H, 4.74; N, 16.90.

Method B. By following the procedure as described for the preparation of 7 (method B), the title compound was prepared by using 25 (0.66 g, 1.5 mmol), N^{ε}-nitro-L-arginine methyl ester hydrochloride (0.43 g, 1.6 mmol), N-methylmorpholine (0.16 g, 1.6 mmol), HOBT (0.19 g, 1.5 mmol), EDC (0.31 g, 1.6 mmol) and dry CH_2Cl_2 (150 ml). The reaction product was purified by flash chromatography using CH_2Cl_2→ MeOH gradient, and crystallized from MeOH to yield 0.8 g (81%) of 24; mp 150-153°C, and was found to be identical to 24 prepared by method A.

7-(β-D-Ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxy-L-arginine amide (28). To a solution of 24 (0.65 g, 1.0 mmol) in 95% EtOH (30 ml) was added Pd/C (10%, 0.5 g) and the mixture was hydrogenated on a Parr hydrogenator at 50 psi for 12 h. The catalyst was removed by filtration on a Celite pad, washed with EtOH (25 ml) and the combined filtrates evaporated to dryness. The residue was dissolved in MeOH/NH_3 (75 ml) and stirred at room temperature overnight. The MeOH/NH_3 was evaporated to dryness and the residue was purified by HPLC on a C-18 reverse phase column using 20% aqueous MeOH as the eluent. The homogeneous fractions were pooled and lyophilized to give 0.20 g (43%) of 28 as an amorphous solid; IR: ν_{max} 1650 (C=O), 3200-3400 (NH_2, OH)cm^{-1}; UV: λ_{max} (pH 1) 269 nm (ε 17,900); (pH 7) 268 nm (ε 18,500); (pH 11) 275 nm (ε 20,700); ^{1}H NMR (Me_2SO-d_6): δ 1.50-1.80 (m, 4, Arg-CH_2s), 3.10-3.30 (m, 2, Arg-CH_2), 4.25 (m, 1, Arg-α-H), 6.06 (d, 1, $J_{1',2'}$= 6.0 Hz, C_1,H), 7.50-7.80 (m, 6, $2NH_2$ and 2NH), 7.94 and 8.07 (2 s, 2, C_2H and C_6H), 8.97 (br s, 1, NH), 10.26 (d, 1, NH). Anal. Calcd for $C_{18}H_{26}N_8O_7 \cdot 1/4\ H_2O$ (466.39): C, 45.91; H, 5.67. Found: C, 45.88; H, 5.47.

7-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxy-L-serine-β-acetyl-α-methyl ester (26). In a similar manner as described for 7 (method A), the title compound was prepared by using 6 (0.5 g, 1.6 mmol), L-serine methyl ester hydrochloride (0.26 g, 1.7 mmol), HOBT (0.26 g, 2 mmol), EDC (0.38 g, 2 mmol), TEA (0.24 ml, 1.7 mmol) in dry DMF (30 ml). The residue, after evaporation of solvent, was dried over P_2O_5 under vacuum overnight and acetylated with acetic anhydride (1.68 ml, 12 mmol) in dry DMF/pyridine (25 ml each). The crude reaction product was purified by flash chromatography over silica gel using CH_2Cl_2→ acetone gradient to yield 1.1 g (78%) of 26 as a foam; IR: ν_{max} 1650 (C=O), 1750 ($COOCH_3$)cm^{-1}; UV: λ_{max} (EtOH) 216 nm (ε 22,700), 267 (12,900); ^{1}H NMR ($CDCl_3$): δ 2.05-2.25 (4 s, 12, $4COCH_3$) 3.83 (s, 3, OCH_3), 4.34-4.61 (m, 2, Ser-CH_2), 5.05 (m, 1, Ser-α-H), 6.40 (d, 1, $J_{1',2'}$= 6.0Hz, C_1,H),

8.02 and 8.26 (2 s, 2, $C_2\underline{H}$ and $C_6\underline{H}$), 10.96 (d, 1, $N\underline{H}$), 12.79 (s, 1, $N\underline{H}$). Anal. Calcd for $C_{24}H_{28}N_4O_{13}$ (580.46): C, 49.65; H, 4.86; N, 9.64. Found: C, 49.82; H, 4.90; N, 9.91.

7-(β-D-Ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxy-L-serine amide (27). The title compound was prepared by following the procedure employed for the preparation of 8. The following quantities of substrates were used: compound 26 (0.6 g, 1.03 mmol) and MeOH/NH_3 (60 ml). The material was crystallized from aqueous EtOH; 0.3 g (73%); mp 220-222°C; IR: ν_{max} 1670 ($CONH_2$), 3200-3400 (NH_2, OH)cm^{-1}; UV: λ_{max} (pH 1) 270 nm (ε 15,800); (pH 7) 214 nm (ε 23,800), 270 (16,900); (pH 11) 229 nm (sh) (ε 14,700), 276 (19,100); ^{1}H NMR (Me_2SO-$\underline{d}_6$): δ 4.35-4.45 (m, 3, Ser-α-$\underline{H}$ and $C\underline{H}_2$), 6.08 (d, 1, $J_{1',2'}$ = 6.0 Hz, $C_1\underline{H}$), 7.07 and 7.32 (2 s, 2, $CONH_2$), 8.05 and 8.09 (2 s, 2, $C_2\underline{H}$ and $C_6\underline{H}$), 10.38 (d, 1, $N\underline{H}$), 11.50 (br s, 1, $N\underline{H}$). Anal. Calcd for $C_{15}H_{19}N_5O_8 \cdot H_2O$ (397.34): C, 43.37; H, 5.09; N, 16.85. Found: C, 43.76; H, 5.05; N, 16.66.

7-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxy-N^{ε}-Z-L-lysine-L-glutamic acid diethyl ester (29). Method A. By following the procedure as described for the preparation of 7 (method A), the title compound was prepared by using 6 (0.93 g, 3 mmol), N^{ε}-Z-L-lysine-L-glutamic acid diethyl ester trifluoroacetate (1.74 g, 3 mmol), HOBT (0.39 g, 3 mmol), N-methylmorpholine (0.44 ml, 4 mmol), EDC (0.57 g, 3 mmol) and dry DMF (50 ml). After evaporation of the solvent the residue was dried over P_2O_5 under vacuum and then acetylated with acetic anhydride (2.45 g, 24 mmol) in DMF/pyridine (25 ml each). Purification of the crude product by flash chromatography over silica gel using $CHCl_3$→ acetone gradient, gave 1.1 g (42%) of 29; mp 158-161°C; IR: ν_{max} 1650 (C=O), 1750 (COOEt)cm^{-1}; UV: λ_{max} (MeOH) 212 nm (ε 34,800), 267 (21,100); ^{1}H NMR (Me_2SO-$\underline{d}_6$); δ 1.14 (m, 6, $2CH_2C\underline{H}_3$), 1.30-1.90 (m, 10, Lys- and Glu-$C\underline{H}_2$'s), 2.01-2.12 (3 s, 9, $3COC\underline{H}_3$), 2.97 (m, 2, Lys-$C\underline{H}_2$), 4.37 (m, 2, Lys-α-$\underline{H}$ and Glu-α-$\underline{H}$), 4.98 (s, 2, $C\underline{H}_2Ph$), 6.32 (d, 1, $J_{1',2'}$ = 6.0 Hz, $C_1\underline{H}$), 7.21-7.34 (m, 6, $CH_2\underline{Ph}$ + $N\underline{H}$), 8.02 and 8.14 (2 s, 2, $C_2\underline{H}$ and $C_6\underline{H}$), 8.39 (d, 1, $N\underline{H}$), 10.36 (d, 1, $N\underline{H}$), 12.72 (br s, 1, $N\underline{H}$). Anal. Calcd for $C_{41}H_{52}N_6O_{16}$ (884.89): C, 55.65; H, 5.92; N, 9.49. Found: C, 55.37; H, 5.89; N, 9.57.

Method B. Compound 29 was also synthesized according to the procedure described for 19, using 25 (0.52 g, 1.2 mmol), N-methylmorpholine (0.26 ml, 2.4 mmol), isobutyl chloroformate (0.16 g, 1.2 mmol), N^{ε}-Z-L-lysine-L-glutamic acid diethyl ester trifluoroacetate (0.62 g, 1.1 mmol) in dry DMF (10 ml) and THF (30 ml). The reaction product was purified by flash chromatography over silica gel using CH_2Cl_2/acetone as the eluent and crystallized from the same solvent mixture to yield 0.7 g (67%) of 29; mp 158-161°C, which was identical to the product prepared by method A.

7-(β-D-Ribofuranosyl)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxy-L-lysine-L-glutamic acid diamide (30). A solution of 29 (2.0 g, 2.26 mmol) in MeOH (100 ml) was hydrogenated at 40 psi in the presence of Pd/C (10%, 0.5 g) for 12 h, and worked up as described for 28. The crude reaction product was treated with MeOH/NH_3 (125 ml) and stirred in a pressure bottle for 24 h. MeOH/NH_3 was evaporated and the residue was crystallized from aqueous EtOH to yield 0.7 g (66%) of 30; mp >150°C (sinters); IR: ν_{max} 1650 (C=O), 3200-3400 (NH_2, OH)cm^{-1}; UV: λ_{max} (pH 1) 214 nm (ε 18,200), 268 (13,400); (pH 7) 214 nm (ε 17,700), 269 (13,600); (pH 11) 226 nm (sh) (ε 10,700), 275 (14,900); ^{1}H NMR (Me_2SO-$\underline{d}_6$): δ 1.50-2.30 (m, 10, Lys- and Glu-$C\underline{H}_2$'s), 2.50-2.72 (m, 2, Lys-$C\underline{H}_2$), 4.00-4.42 (m, 4, Lys- and Glu-α-$\underline{H}$s, $N\underline{H}_2$), 5.99 (d, 1, $J_{1',2'}$ = 6.0 Hz, $C_1\underline{H}$), 6.73 and 7.06 (2 s, 2, $CONH_2$), 7.33 (d, 2, $CONH_2$), 7.78-8.06 (m, 4, $2N\underline{H}$, $C_2\underline{H}$ and $C_6\underline{H}$), 11.52 (d, 1, $N\underline{H}$). Anal. Calcd for $C_{23}H_{34}N_8O_9$ (566.50): C, 48.76; H, 6.05; N, 19.77. Found: C, 48.48; H, 5.83; N, 19.46.

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* To whom correspondence should be addressed. For part 8 in this series, see K. Ramasamy, G.D. Kini, R.K. Robins and G.R. Revankar, Nucleosides Nucleotides, 6, --- (1987).

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