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Synthesis and Structure-Activity Relationship of Ribofuranosyl Echiguanine Analogs as Inhibitors of Phosphatidylinositol 4-Kinase

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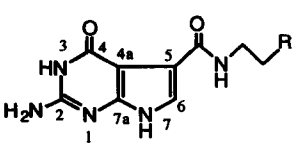
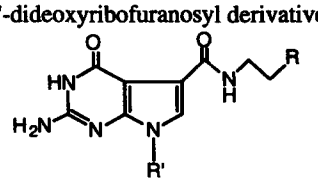
Abstract: *N*-Substituted-2-amino-4(3*H*)-7*H*-oxopyrrolo[2,3-*d*]pyrimidine-5-carboxamides and their ribofuranosyl and 2',3'-dideoxyribofuranosyl derivatives were prepared as membrane permeable echiguanine analogs and tested for their ability to inhibit phosphatidylinositol (PI) 4-kinase. Compounds **5** and **6** were found to inhibit the enzyme approximately at the same level as echiguanines A and B. It is noteworthy that ribofuranosides **18**, **19**, and **20** and dideoxyribofuranoside **29** effectively inhibited PI 4-kinase. Thus, the terminal amide and related structures may be preferable for inhibition of the enzyme in echiguanine analogs with or without ribofuranoside.

Phosphatidylinositol turnover is considered to be important for regulation of cell growth and differentiation.[1-3] Enzymic degradation of phosphatidylinositol 4,5-bisphosphate (PI 4,5-P_2) produces two signal transducing molecules, inositol triphosphate and diacylglycerol. Phosphatidylinositol 4-kinase (PI 4-kinase) is involved in the phosphatidylinositol turnover and may have a role in the regulation of PI 4,5-P_2 levels. In particular, the PI 4-kinase activity was reported to be elevated in rat hepatoma[4] and human ovarian carcinoma[5,6] cells. One of us reported the isolation of two novel and potent inhibitors of PI 4-kinase derived from the A431 cell membrane, echiguanines A **1** and B **2**, from the fermentation broth of *Streptomyces*.[7] However, echiguanines did not inhibit PI 4-kinase in cultured cells possibly because of their poor permeability to the cell membrane. They also did not inhibit the growth of cultured cells. To prepare effective and permeable inhibitors of PI 4-kinase, we became interested in the modification of the terminal functional moieties of echiguanines and in the preparation of their glycosides. In the present study we now report the synthesis and evaluation for inhibition of PI 4-kinase of a diverse class of *N*-substituted-2-amino-4(3*H*)-7*H*-oxopyrrolo[2,3-*d*]pyrimidine-5-carboxamides and their ribofuranosyl and 2',3'-dideoxyribofuranosyl derivatives.



1. R = C(=NH)NH_2 Echiguanine A

2. R = CH_2NH_2 Echiguanine B



R' = H, ribose or 2,3,-dideoxyribose

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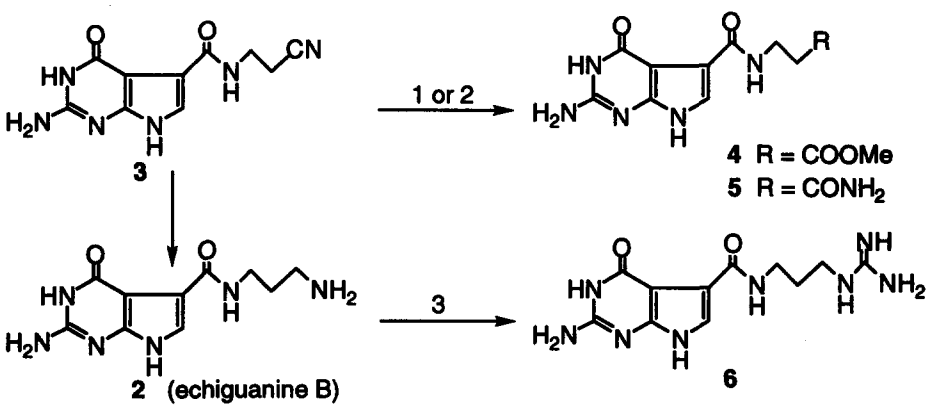
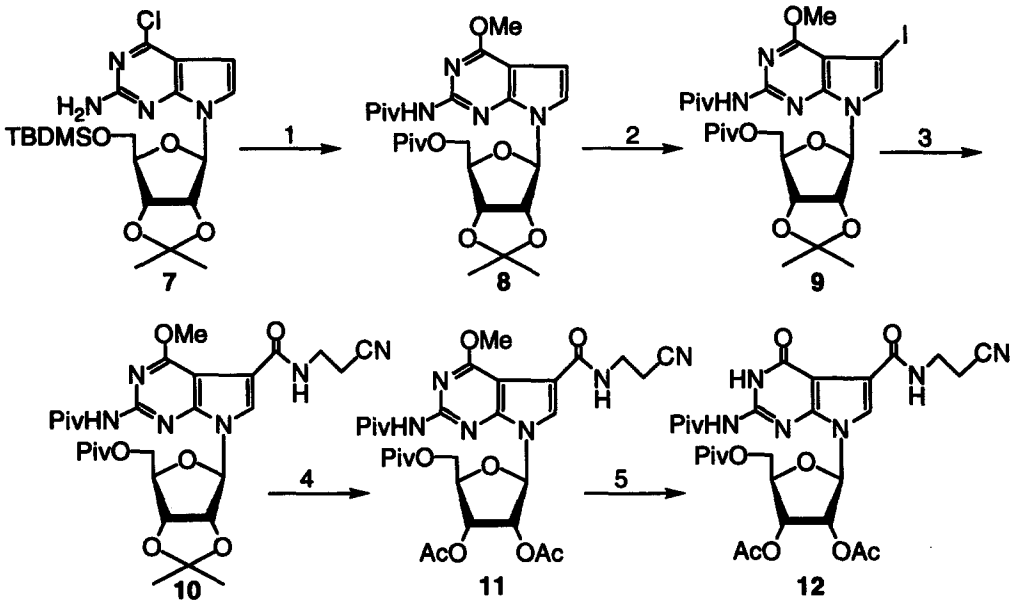
The scheme for the synthesis of target compounds having a carboxyester, carboxamide, and guanidine group, respectively, is outlined in Scheme 1. Cyanoethylamide-deazaguanine **3**, which served as a starting material, was prepared from 7-iodo-7-deazaguanine and 3-aminopropionitrile under a carbon monoxide atmosphere in the presence of bis-(triphenylphosphine)palladium (II) chloride according to the protocol of Shih and Hu.[8,9] The cyano group of **3** was treated with saturated methanolic hydrogen chloride followed by hydrolysis of the imidate with water to give methyl ester **4** in 96 % yield. Initial attempts to obtain amide **5** from compound **4** by treatment with ammonia under various conditions were unsuccessful due to the low solubility of **4**. Alternatively, compound **3** was converted to **5** by use of 35 % H_2O_2 in aqueous base, resulting in a 66 % yield.[10] Compound **2** (echiguanine B) was obtained from **3** by hydrogenation on PtO_2 in acetic acid, and **2** was treated with aminoiminomethanesulfonic acid (AIMSA) in the presence of aqueous potassium carbonate to give guanidine **6** in 64 % yield.[11]



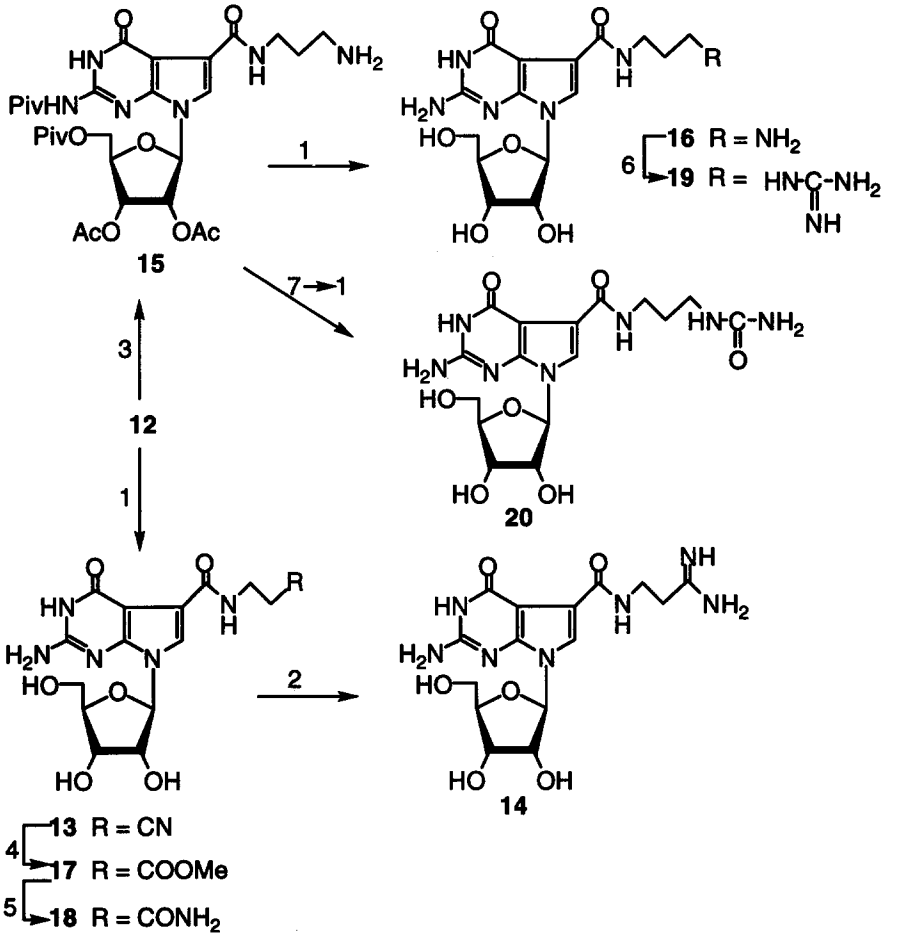
Scheme 1 *Reagents and conditions*: 1) a: HCl gas, MeOH, 0 °C, 2 h, b: H_2O, 0 °C, 1.5 h; 2) 35 % H_2O_2, NH_4OH, H_2O, MeOH, rt, 2 h; 3) AIMSA, K_2CO_3, H_2O, 35 °C, 5 h.

The synthetic route used to prepare the ribofuranosyl analogs of **1** and **2** is illustrated in Scheme 2. Compound **7** was readily prepared from 2-amino-4-chloropyrrolo[2,3-*d*]pyrimidine and 2,3-*O*-isopropylidene-5-*O*-(*t*-butyl)dimethylsilyl-α-D-ribofuranosyl chloride.[12] Compound **7** was first converted to dipivalate **8** *via* a three step sequence for an overall 78 % yield: 1) treatment with NaOMe in MeOH to change the C-4 chloro group into a methoxy group, 2) desilylation with nBu_4NF, 3) acylation with pivaloyl chloride. Reaction of **8** with *N*-iodosuccinimide in DMF afforded 7-iodo-7-deazapurine **9** in 81 % yield as the sole regioisomer. When **9** was treated with cyanoethylamine under a carbon monoxide atmosphere in the presence of bis-(triphenylphosphine)palladium (II) chloride, cyanoethylamide **10** was obtained in 95 % yield. At this stage, the isopropylidene protecting group of **10** was altered into two acetyl groups for the sake of future reactions. Hydrolysis of **10** with 90 % trifluoroacetic acid followed by acetylation with acetic anhydride gave diacetate **11** in 70 % overall yield. The key compound **12** as a common intermediate for the synthesis of glycosylated analogs could be obtained in 95 % yield by cleavage of the ether linkage of **11** with trimethylsilyl iodide[13] in refluxing CH_3CN.



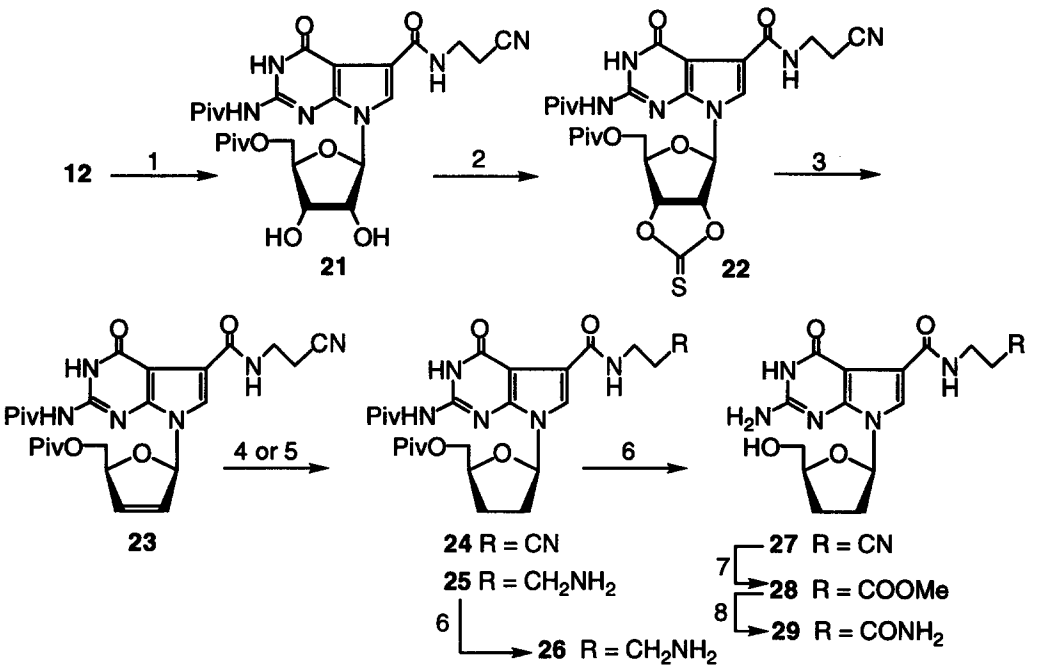
Scheme 2 *Reagents and conditions*: 1) a: 1N-NaOMe, MeOH, 70 °C, 4.5 h; b: nBu_4NF, THF, rt, 1 h; c: pivaloyl chloride, pyridine, rt, 14 h; 2) NIS, DMF, rt, 25 h; 3) $H_2NCH_2CH_2CN$, CO, $PdCl_2(Ph_3P)_2$, DMF, 80 °C, 2 h; 4) a: CF_3COOH-H_2O (9:1), 0 °C, 1.5 h; b: Ac_2O, pyridine, rt, 20 h; 5) TMSI, CH_3CN, rt, 1 h, then reflux, 4 h.

In the preparation of ribofuranosyl echiguanine A **14**, compound **12** was first transformed into the deprotected cyanoethylamide **13** with 77 % yield by heating in methanolic ammonia at 70 °C for 72 h. Then, the cyano group of **13** was converted to the amidinoethyl side chain by treatment with anhydrous ethanolic hydrogen chloride at 0 °C to give the corresponding imino ethyl ether, which was then subjected to ammonolysis with anhydrous ethanolic ammonia to afford the target compound **14** in 39 % overall yield. For the preparation of ribofuranosyl echiguanine B **16**, compound **12** was hydrogenated over PtO_2 as a catalyst in acetic acid to 3-aminopropylamide **15** in 74 % yield, which was subsequently deprotected by heating in methanolic ammonia to provide the target compound **16** in 47 % yield. By employing a reaction similar to that described for compound **4** from **3**, we obtained ribo-methylester **17** in 92 % yield from **13**. Treatment of **17** with methanolic ammonia provided ribo-amide **18** in 99 % yield. Guanidination of compound **16** with AIMSA in the presence of aqueous potassium carbonate provided ribo-guanidine **19** in 64 % yield. Ribo-urea **20** could be prepared from compound **15** by treatment with trimethylsilyl isocyanate in CH_2Cl_2 followed by deprotection with methanolic ammonia in 52 % overall yield.



Scheme 3 *Reagents and Conditions*: 1) NH_3, MeOH, sealed tube, 70 °C, 72 h; 2) a: HCl gas, anhydrous EtOH, 0 °C, 20 h; b: NH_3, anhydrous EtOH, sealed tube, rt, 20 h; 3) PtO_2, H_2, AcOH, rt, 20 h; 4) a) HCl gas, MeOH, 0 °C, 2 h, b) H_2O, 0 °C, 2h; 5) NH_3 gas, MeOH, rt, 14 h; 6) AIMSA, K_2CO_3, H_2O, rt, 5h; 7) TMS-N=C=O, CH_2Cl_2, rt, 14 h.

Deoxygenation of the 2',3' vicinal diol function of **21** to the corresponding olefin **23** was effected by the method of Crisp and Flynn.[14] To begin with, compound **12** was deacetylated with 29 % NH_4OH at room temperature to furnish diol **21** in 98 % yield. Treatment of **21** with thiocarbonyldiimidazole in dichloroethane gave thiocarbonate **22**, which was then refluxed with trimethyl phosphite to provide olefin **23** in 45 % overall yield. Under neutral hydrogenation conditions with 10 % Pd/C in MeOH **23** afforded the nitrile compound **24** in 88 % yield. Dideoxyribofuranosyl analogs **26**, **28**, and **29** were prepared from **24** by the method described for **16**, **17**, and **18**, respectively, as shown in Scheme 4.

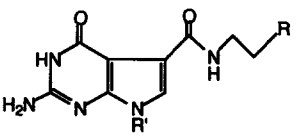


Scheme 4 *Reagents and conditions*: 1) 29 % NH_4OH, MeOH, rt, 30 min; 2) TCDI, $ClCH_2CH_2Cl$, rt, 4 h; 3) $P(OMe)_3$, 120 °C, 3 h; 4) 10 % Pd-C, MeOH, H_2, rt, 16 h; 5) PtO_2, AcOH, H_2, rt, 20 h; 6) NH_3, MeOH, 70 °C, 72 h; 7) a: HCl, MeOH, 0 °C, 2 h, b: H_2O, rt,1 h; 8) NH_3, MeOH, rt, 14 h.

Biological Results and Discussion

7-Substituted echiguanine analogs with or without ribose or dideoxyribose were evaluated for their ability to inhibit PI 4-kinase. As shown in Table 1, compounds **5** and **6** were found to inhibit the enzyme approximately at the same level as echiguanines A and B, having IC_{50} values of 0.02 and 0.15 μg/ml, respectively. Although ribofuranosyl echiguanines **14** and **16** did not inhibit the enzyme, dideoxyribofuranosyl echiguanine B **26** showed moderate activity. It is noteworthy that ribofuranosides **18**, **19**, and **20** and dideoxyribofuranoside **29** effectively inhibited PI 4-kinase. Thus, compounds having a terminal amide inhibited PI 4-kinase, even when they are ribosylated or dideoxyribosylated as in **18**, **20** and **29**. Echiguanines A and B did not inhibit the growth of A431 cells, even at 200 μg/ml. Echiguanine analogs **3-6**, **13**, **14**, **16**, **17**, **19** and **26-29** all did not inhibit the growth of the A431 cells at 100 μg/mL. However, **18** and **20** were shown to inhibit the growth with IC_{50} values of 41 and 82 μg/ml, respectively, suggesting that both compounds are permeable to the membrane. Thus, the biological effect in cultured cells was observed only in ribosylated compounds. Ribosylated echiguanine analogs may mimic nucleosides on the transporter proteins. Inhibition of PI 4-kinase by ribosylated or dideoxyribosylated echiguanines in cultured mammalian cells remains to be studied.

Table 1. Inhibitory Activity of echiguanine analogs on PI 4-kinase



	IC_{50} μg/ml					
R	R' = H		R' = ribose		R' = dideoxyribose	
$C(=NH)NH_2$	**1**	0.03	**14**	100	n. p.	
CH_2NH_2	**2**	0.18	**16**	>100	**26**	12
CN	**3**	0.45	**13**	>100	**27**	>100
COOMe	**4**	0.30	**17**	25	**28**	10
$CONH_2$	**5**	0.02	**18**	2.4	**29**	3.8
$CH_2NHC(=NH)NH_2$	**6**	0.15	**19**	7.5	n. p.	
$CH_2NHCONH_2$	n. p.		**20**	1.0	n. p.	

n. p. : not prepared

In conclusion, the design, synthesis, and evaluation of inhibition against PI 4-kinase of several pyrrolo[2.3-*d*]pyrimidines and their glycosides were performed. The inhibitory activity of several compounds reported herein may represent significant improvements over the biological properties compared with the natural products. The data reported in the present study have unequivocally established that the terminal carbamoyl group at the 5 position plays an important part in the inhibition of PI 4-kinase.

Experimental

^1H NMR and ^{13}C NMR spectra were recorded with JEOL JNM GSX-270, JEOL JNM Lambda-300 or JNM ALPHA-400. Chemical shifts (δ) are expressed in ppm from Me_4Si as an internal standard. Mass spectra were recorded in the EI mode with Hitachi M-80 or JEOL JMS-DX302 mass spectrometer at the ionization energy of 70 eV. UV spectra were recorded on SHIMADZU UV-2400 PC UV spectrophotometer. Infrared spectra were recorded on Bio-Rad FTS-165 or JASCO A-202 infrared spectrophotometer. Optical rotations were recorded at the sodium D line and the ambient temperatures with JASCO DIP-360. Melting points were measured on Yanaco MP-S3 and uncorrected. Only the strongest and/or structurally important peaks are reported for the IR spectra. Silica gel column chromatography was carried out using Silica Gel 60 (E. Merck, Darmstadt). Thin-layer chromatography (TLC) was carried out on 0.25 mm precoated plates of Silica Gel 60 F_{254} (E. Merck, Darmstadt). Reaction progress was monitored by either UV (254 nm) or stained with 5 % phosphomolybdic acid in ethanol as developing agent, followed in the latter case by heating on an electric plate. Preparative-layer chromatography (PLC) was performed on 0.25, 0.5, and 1 mm x 20 cm x 20 cm (E. Merck, Darmstadt) precoated silica gel-60 (60F-254) and 0.25 mm x 20 cm x 20 cm RP-$18F_{254s}$ (E. Merck, Darmstadt) plates. Dichloromethane (CH_2Cl_2), *N,N*-dimethylformamide (DMF), and acetonitrile (CH_3CN) were distilled over calcium hydride. Methanol (MeOH) and ethanol (EtOH) were distilled over magnesium. THF was distilled over sodium benzophenone ketyl prior to use. Reactions were carried out under an argon atmosphere unless

otherwise stated. All evaporations were carried out on a rotary evaporator under reduced pressure. Reaction temperatures were measured externally. Yields refer to chromatographycally and spectroscopically pure compounds.

N-(2-methoxycarbonylethyl)-2-amino-4(3H)-7H-oxopyrrolo[2,3-d]pyrimidine-5-carboxamide (4).

A solution of **3** (62.4 mg, 0.25 mmol) in dry MeOH saturated with HCl gas (1.5 mL) was stirred at 0 °C for 2 h. The reaction mixture was concentrated and the residue was dissolved with H_2O (1.0 mL) at 0 °C. After the mixture was stirred for 1 h, the solution was evaporated to dryness and the residue was purified by PLC on C-18 reversed phase silica gel (MeOH/H_2O 1:1) to afford **4** (67.5 mg, 96 %) as a colorless powder: mp 229-235 °C (dec); IR (KBr) ν_{max} 3420, 1730, 1630, 1580, 1510 cm^{-1}; ^1H NMR (DMSO-d_6, 270MHz) δ 2.54 (t, 2H, J=6.6Hz), 3.46 (dt, 2H, J=5.9, 6.6Hz), 3.60 (s, 3H), 6.36 (bs, 2H), 7.22 (s, 1H), 10.2 (t, 1H, J=5.9Hz), 10.8 (bs, 1H), 11.6 (bs, 1H); ^{13}C NMR (DMSO-d_6, 67.5MHz) δ 32.8, 33.3, 50.1, 94.5, 113.1, 121.5, 151.3, 151.4, 159.1, 161.4, 170.5; HRMS m/z 264.0733 calcd for $C_{10}H_{10}N_5O_4$ (M^+-Me), found m/z 264.0727.

N-(2-carbamoylethyl)-2-amino-4(3H)-7H-oxopyrrolo[2,3-d]pyrimidine-5-carboxamide (5).

To a solution of **3** (13.0 mg, 0.056 mmol) in MeOH (0.5 mL) and H_2O (0.5 mL) was added 29% NH_4OH (0.04 mL) and 35% H_2O_2 (0.04 mL). The reaction mixture was stirred at room temperature for 2h. After concentration of the solution to dryness, the residue was purified by PLC on C-18 reversed phase silica gel (MeOH/H_2O 1:1) to afford **5** (9.2 mg, 66%) as a colorless powder: mp 225-229 °C (dec); IR (KBr) ν_{max} 3420, 1650, 1618, 1580, 1510 cm^{-1}; ^1H NMR (DMSO-d_6, 270MHz) δ 2.30 (t, 2H, J=6.6Hz), 3.41 (dt, 2H, J=5.9, 6.6Hz), 6.42 (bs, 2H), 6.78 (bs, 1H), 7.32 (s, 1H), 10.1 (t, 1H, J=5.9Hz); ^{13}C NMR (DMSO-d_6, 67.5MHz) δ 35.2, 35.6, 104.7, 114.7, 122.6, 152.6, 152.8, 160.4, 162.7, 172.5; HRMS m/z 248.0783 calcd for $C_{10}H_{10}N_5O_3$ (M^+-NH_2), found m/z 248.0800.

N-(2-guanidinoethyl)-2-amino-4(3H)-7H-oxopyrrolo[2,3-d]pyrimidine-5-carboxamide (6).

To a mixture of **2** (32.6 mg, 0.13 mmol) and potassium carbonate (18.0 mg, 0.13 mmol) in H_2O (3.0 mL) was added aminoiminomethanesulfonic acid (16.1 mg, 0.13 mmol). The reaction mixture was stirred at room temperature for 14 h. After concentration of the solution to dryness, the residue was purified by PLC on C-18 reversed phase silica gel (MeOH/H_2O 1:1) to afford **6** (22.4 mg, 64 %) as a colorless powder: mp 235-241°C (dec); IR (nujol) ν_{max} 3350, 1660, 1590, 1515 cm^{-1}; ^1H NMR (D_2O, 270MHz) δ 1.92 (tt, 2H, J=6.6, 6.6Hz), 3.27 (t, 2H, J=6.6Hz), 3.43 (t, 2H, J=6.6Hz), 7.45 (s, 1H). ^{13}C NMR (D_2O+CD_3OD, 67.5MHz) δ 26.4, 35.8, 38.0, 101.2, 112.6, 123.3, 151.4, 152.1, 155.9, 160.2, 163.4; HRMS m/z 292.1396 calcd for $C_{11}H_{16}N_8O_2$ (M^+), found m/z 292.1398.

7-(2'3'-O-isopropylidene-5'-O-trimethylacetyl-β-D-ribofuranosyl)-4-(methoxy)-2-(trimethylacetoamino)pyrrolo[2,3-d]pyrimidine (8).

A solution of **7** (699 mg, 1.47 mmol) and NaOMe (2.16 g) in dry MeOH (40 mL) was refluxed for 4.5 h. The reaction mixture was cooled and then neutralized (pH 6) with AcOH. After evaporation of the solvent, the residue was suspended in H_2O (15 mL). The aqueous solution was extracted with EtOAc (20 mL x2) and the

combined organic layers were dried over anhydrous Na_2SO_4, filtered, concentrated to give an oily compound. Then, to the solution of the oily compound in dry THF (25 mL) was added n-TBAF (1M in THF, 2.6 mL) at 0 °C and the reaction mixture was stirred at room temperature for 14 h. The reaction mixture was concentrated to dryness and the residue was dissolved in H_2O (15 mL). The aqueous solution was extracted with EtOAc (20 mL x2) and the organic extract was dried over anhydrous Na_2SO_4, filtered, concentrated to afford the crude material (701 mg). To the solution of the crude material in pyridine (5.5 mL) was added trimethylacetyl chloride (0.14 mL, 1.16 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 14 h. The reaction was quenched by addition of MeOH (1.0 mL) and stirred for further 2 h. The mixture was evaporated to dryness and H_2O (15 mL) was added to the residue. The aqueous layer was extracted with EtOAc (20 mL x2) and the combined organic layers were washed with 1N HCl (20 mL), saturated aqueous $NaHCO_3$ (20 mL), and brine (20 mL). The organic solution was dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica gel column chromatography (n-hexane/EtOAc 2:1) to afford **8** (580 mg, 78 %) as a colorless foam: $[\alpha]^{22}_D$ +40° ($CHCl_3$, *c* 1.0); IR (film) ν_{max} 3450, 1715, 1615, 1575, 1505 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz) δ 1.19 (s, 9H), 1.36 (s, 9H), 1.41 (s, 3H), 1.62 (s, 3H), 4.09 (s, 3H), 4.10 (dd, 1H, J=5.9, 11.0Hz), 4.37 (dd, 1H, J=3.3, 5.9, 5.9Hz), 4.44 (dd, 1H, J=5.9, 11.0Hz), 5.37 (dd, 1H, J=3.3, 6.2Hz), 5.46 (dd, 1H, J=1.5, 6.2Hz), 6.09 (d, 1H, J=1.5Hz), 6.46 (d, 1H, J=3.7Hz), 6.95 (d, 1H, J=3.7Hz), 8.04 (s, 1H): ^{13}C NMR ($CDCl_3$, 75.5MHz) δ 25.5, 27.1, 27.1 (x3), 27.5 (x3), 38.8, 40.2, 53.9, 64.1, 81.9, 84.7, 84.9, 91.7, 99.7, 103.1, 113.9, 124.4, 151.5, 151.8, 163.4, 175.3, 178.2; HRMS m/z 504.2582 calcd for $C_{25}H_{36}N_4O_7$ (M^+), found m/z 504.2592.

5-Iodo-7-(2'3'-*O*-isopropylidene-5'-*O*-trimethylacetyl-β-D-ribofuranosyl)-4-(methoxy)-2-(trimethylacetoamino)pyrrolo[2,3-*d*]pyrimidine (9).

A solution of **8** (580 mg, 1.15 mmol) and *N*-iodosuccinimide (221 mg, 0.98 mmol) in dry DMF (11.5 mL) was stirred at room temperature for 25 h. The reaction mixture was diluted with H_2O (250 mL) and extracted with EtOAc (150 mL x2). The combined organic layers were washed with brine (80 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. Silica gel column chromatography (n-hexane/EtOAc 2:1) afford **9** (499 mg, 81 %, recovery 109 mg) as a colorless foam: $[\alpha]^{22}_D$ +14° ($CHCl_3$, *c* 0.5); IR (film) ν_{max} 3430, 1720, 1600, 1565, 1500 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz) δ 1.19 (s, 9H), 1.34 (s, 9H), 1.40 (s, 3H), 1.60 (s, 3H), 4.09 (m, 1H), 4.10 (s, 3H), 4.34-4.41 (complex, 2H), 5.32 (dd, 1H, J=3.3, 6.2Hz), 5.41 (dd, 1H, J=1.8, 6.2Hz), 6.04 (d, 1H, J=1.8Hz), 7.07 (s, 1H), 8.00 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.5MHz) δ 25.4, 27.1, 27.2 (x3), 27.5 (x3), 38.8, 40.2, 50.8, 54.0, 64.0, 81.6, 84.6, 85.0, 91.5. 104.7, 114.0, 129.1, 151.6, 151.7, 163.5, 175.3, 178.2; HRMS m/z 630.1550 calcd for $C_{25}H_{35}N_4O_7I$ (M^+), found m/z 630.1580.

***N*-(2-Cyanoethyl)-7-(2'3'-*O*-isopropylidene-5'-*O*-trimethylacetyl-β-D-ribofuranosyl)-4-(methoxy)-2-(trimethylacetoamino)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (10).**

To a solution of **9** (499 mg, 0.79 mmol) and bis(triphenylphosphine)palladium (II) chloride (28.2 mg, 0.036mmol) in dry DMF (5.0 mL) was added 3-aminopropionitrile (0.12 mL, 1.58 mmol) under CO atmosphere at room temperature. The resulting mixture was heated at 80 °C for 2 h under a CO balloon and was then evaporated to dryness. The residure was purified by silica gel column chromatography ($CHCl_3$/MeOH 15:1) to give **10** (456 mg, 95 %) as a pale yellow foam: $[\alpha]^{21}_D$ +14° ($CHCl_3$, *c* 0.7); IR (film) ν_{max} 3370, 2250, 1725, 1645, 1605, 1510 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.19 (s, 9H), 1.26 (s, 3H), 1.36 (s,

9H), 1.41 (s, 3H), 2.70 (t, 2H, J=6.2Hz), 3.75 (dt, 2H, J=6.2, 6.2Hz), 4.11 (dd, 1H, J=5.6, 11.7Hz), 4.38 (ddd, 1H, J=3.3, 5.6, 5.9Hz), 4.41 (dd, 1H, J=5.9, 11.7Hz). 5.33 (dd, 1H, J=3.3, 6.6Hz), 5.41 (dd, 1H, J=1.8, 6.6Hz), 6.11 (d, 1H, J=1.8Hz), 7.85 (s, 1H), 8.10 (s, 1H), 8.50 (t, 1H, J=6.2Hz); ^{13}C NMR ($CDCl_3$, 75.5MHz) δ 18.9, 25.4, 27.2 (x3), 27.5 (x3), 29.7, 35.6, 38.8, 40.3, 55.1, 63.9, 81.7, 84.6, 85.1, 92.0, 98.8, 111.0, 114.2, 118.5, 131.3, 151.9, 152.8, 162.2, 162.6, 175.4, 178.2; HRMS m/z 600.2908 calcd for $C_{29}H_{40}N_6O_8$ (M^+), found m/z 600.2905.

_N_-(2-Cyanoethyl)-7-(2'3'-di-_O_-acetyl-5'-_O_-trimethylacetyl-β-D-ribofuranosyl)-4-(methoxy)-2-(trimethylacetoamino)pyrrolo[2,3-_d_]pyrimidine-5-carboxamide (11).

A solution of **10** (451 mg, 0.76 mmol) in 90 % aqueous trifluoroacetic acid (11.6 mL) was stirred at 0 °C for 1.5 h. The reaction mixture was concentrated and the residue was dissolved in pyridine (1.5 mL) and acetic anhydride (1.5 mL) at 0 °C. Then, the reaction mixture was stirred at room temperature for 20 h. After concentration of the reaction mixture to dryness, purification of the residue by silica gel column chromatography ($CHCl_3$/MeOH 20:1) gave **11** (353 mg, 70 %) as a colorless powder: mp 156-157 °C; $[\alpha]^{24}_D$ -14° ($CHCl_3$, *c* 0.5); UV λ_{max} (MeOH) 234 nm (ε = 19600), 245 nm (ε = 21500), 276 nm (ε = 26800); IR (film) ν_{max} 3370, 2245, 1745, 1650, 1605, 1545 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.21 (s, 9H), 1.37 (s, 9H), 2.08 (s, 3H), 2.16 (s, 3H), 2.70 (t, 2H, J = 6.2 Hz), 3.75 (dt, 2H, J = 5.9, 6.2 Hz), 4.30 (s, 3H), 4.33 (m, 1H), 4.38 (dd, 1H, J = 3.3, 3.6 Hz), 4.41 (dd, 1H, J = 2.2, 3.3Hz), 5.84 (dd, 1H, J = 5.1, 5.5 Hz), 5.94 (dd, 1H, J = 5.1, 5.5 Hz), 6.21 (d, 1H, J = 5.1 Hz), 7.90 (s, 1H), 8.16 (s, 1H), 8.52 (t, 1H, J = 5.9 Hz); ^{13}C NMR ($CDCl_3$, 75.5MHz) d 18.9, 20.4, 20.6, 27.1 (x3), 27.4 (x3), 35.6, 38.8, 40.3, 55.2, 63.1, 70.6, 73.1, 79.6, 87.2, 111.7 (x2), 118.4, 130.1, 152.2, 153.5, 162.2, 162.4, 169.4, 169.6, 175.6, 178.1; HRMS m/z 587.2099 calcd for $C_{26}H_{31}N_6O_{10}$ (M^+ - ^{t}Bu), found m/z 587.2079.

_N_-(2-Cyanoethyl)-7-(2'3'-di-_O_-acetyl-5'-_O_-trimethylacetyl-β-D-ribofuranosyl)-2-(trimethylacetoamino)-4(3H)-oxopyrrolo[2,3-_d_]pyrimidine-5-carboxamide (12).

A solution of **11** (343 mg, 0.53 mmol) and iodotrimethylsilane (110 mg, 0.55 mmol) in dry acetonitrile (5.5 mL) was stirred at room temperature for 1 h, and at 110 °C for 4 h. The reaction solution was evaporated to dryness and the residue was purified by silica gel column chromatography (n-hexane/EtOAc 1:3) to give **12** (321 mg, 95 %) as a colorless foam: $[\alpha]^{22}_D$ -37° ($CHCl_3$, *c* 1.0); UV λ_{max} (MeOH) 279 nm (ε = 7400); IR (film) ν_{max} 3200, 2250, 1735, 1665, 1605, 1545 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz) δ 1.16 (s, 9H), 1.36 s, 9H), 2.10 (s, 3H), 2.14 (s, 3H), 2.74 (t, 2H, J = 6.6 Hz), 3.71 (dt, 2H, J = 5.9, 6.6 Hz), 4.24 (dd, 1H, J = 4.4, 12.1 Hz), 4.40 (m, 1H), 4.49 (dd, 1H, J = 4.0, 12.1 Hz), 5.90-6.03 (complex, 3H), 7.67 (s, 1H), 8.98 (s, 1H), 10.4 (t, 1H, J = 5.9 Hz), 12.2 (s, 1H); ^{13}C NMR ($CDCl_3$, 75.5MHz) δ 17.9, 20.2, 20.4, 26.5 (x3), 26.7 (x3), 35.4, 38.5, 40.1, 62.2, 70.2, 72.8, 78.7, 87.8, 102.0, 115.6, 117.9, 127.2, 146.7, 147.8, 158.5, 162.5, 169.2, 169.7, 178.0, 180.5; HRMS m/z 630.2649 calcd for $C_{29}H_{38}O_{10}N_6$ (M^+), found m/z 630.2648.

_N_-(2-Cyanoethyl)-2-(amino)-7-(β-D-ribofuranosyl)-4(3_H_)-oxopyrrolo[2,3-_d_]pyrimidine-5-carboxamide (13).

A solution of **12** (61.5 mg, 0.098 mmol) in dry MeOH (2.0 mL) and liq.NH_3 (2.0 mL) was heated at 70 °C for 72 h in a sealed tube. The MeOH and excess NH_3 were evaporated, and the residue was washed with Et_2O to

afford **13** (28.3 mg, 77 %) as a colorless powder: mp 220-226°C (dec); $[\alpha]^{21}_D$ -43° (DMSO, *c* 0.67); IR (KBr) ν_{max} 3420, 2250, 1660, 1510 cm^{-1}; ^1H NMR (DMSO-d_6, 300MHz) δ 2.75 (t, 2H, J=2.9Hz), 3.40-3.46 (complex, 4H), 3.85 (m, 1H), 4.05 (dd, 1H, J=2.9, 5.1Hz), 4.29 (dd, 1H, J=5.1, 6.6Hz), 5.06 (bs, 2H), 5.27 (bs, 1H), 5.91 (d, 1H, J=6.6Hz), 6.57 (bs, 2H), 7.61 (s, 1H), 10.57 (t, 1H, J=5.1Hz); ^{13}C NMR (DMSO-d_6, 75.5MHz) δ 17.8, 34.8, 61.4, 70.6, 73.9, 85.0, 85.8, 95.8, 114.4, 119.2, 123.2, 152.5, 152.9, 160.3, 162.6; HRMS m/z 379.1366 calcd for $C_{15}H_{19}N_6O_6$ (M^++H), found m/z 379.1361.

N-(2-Amidinoethyl)-2-(amino)-7-β-D-ribofuranosyl-4(3*H*)-oxopyrrolo[2,3-*d*]pyrimidine-5-carboxamide (14, ribo-echiguanine A)

A solution of **13** (25.1 mg, 0.066 mmol) in dry EtOH saturated with HCl gas (1.5 mL) was stirred at 0 °C for 20 h. The reaction mixture was concentrated and the residue was treated with anhydrous ethanolic ammonia (2.0 mL) at room temperature for 20 h. After evaporation of the solvent and the excess ammonia, the residue was purified by PLC (BuOAc/BuOH/MeOH/H_2O 2:4:1:1) to afford **14** (10.3 mg, 39 %) as a colorless foam; $[\alpha]^{25}_D$ -21° (H_2O, *c* 0.1). IR (nujol) ν_{max} 3400, 1665, 1640, 1585, 1505 cm^{-1}; ^1H NMR (D_2O, 400MHz) δ 2.64 (t, 2H, J=6.6Hz), 3.62 (t, 2H, J=6.6Hz), 3.68 (dd, 1H, J=4.4, 12.6Hz), 3.76 (dd, 1H, J=3.1, 12.6Hz), 4.07 (ddd, 1H, J=3.1, 3.7, 4.4Hz), 4.23 (dd, 1H, J=3.7, 4.5Hz), 4.46 (dd, 1H, J=4.5, 5.8Hz), 5.86 (d, 1H, J=5.8Hz), 7.52 (s, 1H). ^{13}C NMR (D_2O, 100.5MHz) δ 33.7, 37.4, 62.4, 71.3, 74.8, 85.8, 88.4, 97.9, 114.5, 125.8, 153.3, 154.0, 162.1, 166.1, 170.0; HRMS m/z 431.1320 calcd for $C_{15}H_{22}N_7O_6Cl$ (M^++HCl), found m/z 431.1340.

N-(3-Aminopropyl)-7-(2',3'-di-*O*-acetyl-5'-*O*-trimethylacetyl-β-D-ribofuranosyl)-2-(trimethylacetoamino)-4(3*H*)-oxopyrrolo[2,3-*d*]pyrimidine-5-carboxamide (15).

A mixture of **12** (50.5 mg, 0.080 mmol) and PtO_2 (2.5 mg) in AcOH (1.5 mL) was stirred under hydrogen atmosphere for 20 h. The reaction mixture was filtered through a Celite pad and washed with MeOH (2 mL x2), the filtrate and the washings were combined. The organic solution was concentrated, and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH/29% NH_4OH 100:20:1) to afford **15** (38.0 mg 74 %) as a colorless foam: $[\alpha]^{22}_D$ -42° ($CHCl_3$, *c* 0.5); IR (film) ν_{max} 3380, 1735, 1655, 1590, 1550 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz) δ 1.14 (s, 9H), 1.37 (s, 9H), 1.79 (tt, 2H, J=6.2, 6.6Hz), 2.11 (s, 3H), 2.14 (s, 3H), 2.83 (t, 2H, J=6.2Hz), 3.51 (dt, 2H, J=5.9, 6.6Hz), 4.23 (dd, 1H, J=4.8, 12.1Hz), 4.41 (ddd, 1H, J=4.0, 4.8, 5.5Hz), 4.50 (dd, 1H, J=4.0, 12.1Hz), 5.55 (bs, 3H), 5.90 (d, 1H, J=1.5Hz), 5.99 (dd, 1H, J=5.5, 5.5Hz), 6.07 (dd, 1H, J=1.5, 5.5Hz), 7.61 (s, 1H), 10.0 (d, 1H, J=5.9Hz); ^{13}C NMR ($CDCl_3$, 75.5MHz) δ 20.5, 20.6, 26.8 (x3), 27.0 (x3), 32.8, 36.4, 38.8, 39.1, 40.3, 62.4, 70.5, 73.0, 78.7, 88.6, 102.5, 116.4, 127.6, 146.9, 147.9, 159.2, 162.6, 169.5, 170.0, 178.4, 180.5; HRMS m/z 634.2959 calcd for $C_{29}H_{42}N_6O_{10}$ (M^+), found m/z 634.2932.

N-(3-Aminoethyl)-2-(amino)-7-β-D-ribofuranosyl-4(3*H*)-oxopyrrolo[2,3-*d*]pyrimidine-5-carboxamide (16, ribo-echiguanine B)

A solution of **15** (38.0 mg, 0.060 mmol) in MeOH (1.0 mL) and liq. NH_3 (2.0 mL) was heated at 70°C for 72 h in a sealed tube. The volatiles were removed under reduced pressure, and the residue was purified by PLC (BuOH/AcOH/H_2O 4:1:1) to afford **16** as a salt of acetic acid, which was then dissolved in 29% NH_4OH (2.0

mL). The solution was evaporated to dryness, and the residue was purified on an HW-40 column chromatography eluted with MeOH to afford **16** (10.8 mg, 47 %) as a colorless foam (free amine form): $[\alpha]^{20}_D$ -3.4° (DMSO, *c* 0.1); IR (nujol) ν_{max} 3400, 1660, 1590, 1505 cm^{-1}; ^1H NMR (D_2O, 400MHz) δ 1.67 (m, 2H), 2.85 (t, 2H, J=7.7Hz), 3.17 (t, 2H, J=6.4Hz), 3.58 (dd, 1H, J=4.0, 12.0Hz), 3.67 (dd, 1H, J=3.2, 12.0Hz), 3.96 (m, 1H), 4.11 (dd, 1H, J=4.0, 5.1Hz), 4.31 (dd, 1H, J=5.1, 5.5Hz), 5.86 (d, 1H, J=5.5Hz), 7.31 (s, 1H); ^{13}C NMR (D_2O+DCl, 100.5MHz) δ 27.4, 37.2, 38.0, 62.0, 71.4, 75.0, 86.6, 90.8, 99.1, 115.3, 126.4, 151.7 (x2), 160.4, 165.2; HRMS m/z 419.1446 calcd for $C_{15}H_{24}N_6O_6Cl$ (M^++H+HCl), found 419.1447.

_N_-(2-Carbomethoxyethyl)-2-(amino)-7-β-D-ribofuranosyl-4(3_H_)-oxopyrrolo[2,3-_d_]pyrimidine-5-carboxamide (17).

Compound **17** (16.5 mg, 92 %) was prepared from **13** (16.5 mg, 0.044 mmol) in dry MeOH saturated with HCl gas (1.0 mL) by the method described for **4**. Purification by PLC on C-18 reversed phase silica gel (MeOH/H_2O 1:1) gave **17** as a colorless powder: mp 225-229°C (dec); $[\alpha]^{23}_D$ -23° (MeOH, *c* 0.7); IR (KBr) ν_{max} 3420, 1730, 1640, 1600, 1520 cm^{-1}; ^1H NMR (CD_3OD, 270MHz) δ 2.61 (t, 1H, J=6.5Hz), 3.62 (t, 1H, J=6.5Hz), 3.71 (dd, 1H, J=3.8, 12.6Hz), 3.81 (dd, 1H, J=3.5, 12.6Hz), 4.03 (ddd, 1H, J=3.5, 3.8, 3.8Hz), 4.22 (dd, 1H, J=3.8, 5.6Hz), 4.43 (dd, 1H, J=5.6, 5.6Hz), 5.97 (d, 1H, J=5.6Hz), 7.61 (s, 1H); ^{13}C NMR (CD_3OD, 67.5MHz) δ 35.0, 36.2, 52.3, 63.3, 72.2, 75.9, 86.7, 89.7, 98.2, 115.5, 125.7, 153.8, 154.4, 162.2, 165.7, 173.9; HRMS m/z 412.1468 calcd for $C_{16}H_{22}N_5O_8$ (M^++H), found m/z 412.1448.

_N_-(2-Carbamoylethyl)-2-(amino)-7-β-D-ribofuranosyl-4(3_H_)-oxopyrrolo[2,3-_d_]pyrimidine-5-carboxamide (18).

A solution of **17** (32.1 mg, 0.078 mmol) in MeOH (2.0 mL) and liq. NH_3 (2.0 mL) in a sealed tube was stirred at room temperature for 14 h . After evaporation of the volatiles, the residue was purified by PLC on C-18 reversed phase silica gel (MeOH/H_2O 1:1) to afford **18** (25.2 mg, 99 %, recovery 5.7 mg) as a colorless powder: mp 225-231°C (dec); $[\alpha]^{22}_D$ -24° (DMSO, *c* 0.6); IR (KBr) ν_{max} 3420, 1660, 1600, 1560, 1520 cm^{-1}; ^1H NMR (D_2O, 400MHz) δ 2.42 (t, 2H, J=6.6Hz), 3.46 (t, 2H, J=6.6Hz), 3.62 (dd, 1H, J=4.4, 12.8Hz), 3.69 (dd, 1H, J=3.1, 12.8Hz), 4.02 (ddd, 1H, J=3.1, 3.8, 4.4Hz), 4.18 (dd, 1H, J=3.8, 5.1Hz), 4.45 (dd, 1H, J=5.1, 5.9Hz), 5.82 (d, 1H, J=5.9), 7.49 (s, 1H); ^{13}C NMR (DMSO-d_6, 100.5MHz) δ 35.3, 35.5, 61.6, 70.6, 73.9, 85.1, 85.8, 96.0, 115.1, 122.8, 152.5, 153.0, 160.2, 162.3, 172.4; HRMS m/z 379.1366 calcd for $C_{15}H_{19}N_6O_6$ (M^+-OH), found 379.1396.

_N_-(2-Guadininoethyl)-2-(amino)-7-β-D-ribofuranosyl-4(3_H_)-oxopyrrolo[2,3-_d_]pyrimidine-5-carboxamide (19).

To a mixture of **16** (16.4 mg, 0.043 mmol) and potassium carbonate (6.0 mg, 0.043 mmol) in H_2O (1.5 mL) was added aminoiminomethanesulfonic acid (5.3 mg, 0.043 mmol). The reaction mixture was stirred at room temperature for 14 h. The solution was concentrated, and the residue was purified by PLC on C-18 reversed phase silica gel (MeOH/H_2O 1:1) to give **19** (11.7 mg, 64 %) as a colorless powder: mp 239-247 °C (dec); $[\alpha]^{22}_D$ -1.0° (H_2O, *c* 0.2); IR (KBr) ν_{max} 3320, 1680, 1660, 1520 cm^{-1}; ^1H NMR (D_2O, 270MHz) δ 1.76 (m, 2H), 3.23 (t, 2H, J=6.6Hz), 3.34 (t, 2H, J=6.6Hz), 3.76 (dd, 1H, J=4.4, 12.5Hz), 3.84 (dd, 1H, J=2.9, 12.5Hz), 4.14 (ddd, 1H, J=2.9, 4.3, 4.4Hz), 4.30 (dd, 1H, J=4.3, 5.1Hz), 4.52 (dd, 1H, J=5.1, 5.9Hz),

5.90 (d, 1H, J=5.9Hz), 7.52 (s, 1H); ^{13}C NMR (D_2O+CD_3OD, 67.5MHz) δ 26.6, 35.7, 37.9, 60.5, 69.4, 73.0, 83.8, 86.5, 96.0, 112.9, 123.4, 151.3, 152.0, 155.8, 160.1, 163.8; HRMS m/z 424.1819 calcd for $C_{16}H_{24}N_8O_6$ (M^+), found 424.1820.

_N_-(2-Ureidoethyl)-2-(amino)-7-β-D-ribofuranosyl-4(3_H_)-oxopyrrolo[2,3-_d_]pyrimidine-5-carboxamide(20).

A solution of **15** (26.0 mg, 0.041 mmol) and trimethylsilyl isocyanate (6.6 mg, 0.049 mmol) in dichloromethane (4.0 mL) was stirred at room temperature for 14 h. The reaction mixture was concentrated to dryness and the residure was purified by PLC ($CHCl_3$/MeOH 4:1) to afford the Piv-protected urea compound (17.5 mg, 63 %) as a waxy solid: $[\alpha]^{22}_D$ -40° ($CHCl_3$, *c* 0.5); IR (film) ν_{max} 3350, 1740, 1660, 1595, 1540 cm^{-1}; ^{1}H NMR (CD_3OD, 300MHz) δ 1.23 (s, 9H), 1.32 (s, 9H), 1.79 (tt, 2H, J=6.6, 7.0Hz), 2.01 (s, 3H), 2.13 (s, 3H), 3.22 (t, 2H, J=7.0Hz), 3.43 (dt, 2H, J=5.5, 6.5Hz), 4.36-4.37 (complex, 2H), 4.43 (m, 1H), 5.60 (dd, 1H, J=3.7, 5.9Hz), 5.72 (dd, 1H, J=5.9, 6.2Hz), 6.36 (d, 1H, J=6.2Hz), 7.76 (s, 1H), 10.3 (t, 1H, J=5.5Hz); ^{13}C NMR (CD_3OD, 75.5MHz) δ 18.3, 18.6, 25.1 (x3), 25.8 (x3), 29.1, 35.9, 38.0, 39.6 (x2), 62.7, 70.3, 73.0, 79.6, 84.8, 100.4, 115.5, 124.5, 147.5, 149.2, 158.6, 160.3, 162.8, 169.2, 169.5, 177.7, 181.1; HRMS m/z 678.3096 calcd for $C_{30}H_{44}N_7O_{11}$ (M^++H), found m/z 678.3097.

Then, the solution of the Piv-protected urea compound (13.4 mg, 0.020 mmol) in dry MeOH (2.5 mL), and liq. NH_3 (2.0 mL) was heated at 70 °C for 72 h in a sealed tube. After evaporation of the volatility the residue was purified by PLC on C-18 reversed phase silica gel (MeOH/H_2O 1:1) to afford **20** (6.9 mg, 81 %) as a colorless powder: mp 211-215°C (dec); $[\alpha]^{22}_D$ -18° (H_2O, *c* 0.6); IR (KBr) ν_{max} 3380, 1690, 1650, 1600, 1560, 1510 cm^{-1}; ^{1}H NMR (D_2O, 270MHz) δ 1.76 (m, 2H), 3.16 (t, 2H, J=6.6Hz), 3.32 (t, 2H, J=6.6Hz), 3.77 (dd, 1H, J=4.4, 12.5Hz), 3.85 (dd, 1H, J=12.5Hz), 4.14 (ddd, 1H, J=3.7, 3.7, 4.4Hz), 4.32 (dd, 1H, J=3.7, 5.1Hz), 4.53 (dd, 1H, J=5.1, 5.9Hz), 5.92 (d, 1H, J=5.9Hz), 7.64 (s, 1H); ^{13}C NMR (D_2O+CD_3OD, 100.5MHz) δ 27.6, 36.0, 36.6, 60.5, 69.4, 72.9, 83.7, 86.5, 96.0, 113.0, 123.3, 151.3, 152.0, 160.1, 163.7, 165.5; HRMS m/z 425.1658 calcd for $C_{16}H_{23}N_7O_7$ (M^+), found 425.1650.

_N_-(2-Cyanoethyl)-7-(5'-_O_-trimethylacetyl-β-D-ribofuranosyl)-2-(trimethylacetoamino)-4(3_H_)-oxopyrrolo[2,3-_d_]pyrimidine-5-carboxamide (21).

A mixture of **12** (116 mg, 0.18 mmol), MeOH (4.0 mL) and 29% NH_4OH (2.0 mL) was stirred at room temperature for 30 min. The reaction solution was concentrated to dryness, and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH 10:1) to afford **21** (98.9 mg, 98 %) as a colorless powder: mp 196-204°C (dec); $[\alpha]^{23}_D$ -19° (MeOH, *c* 1.0); IR (film) ν_{max} 3250, 2250, 1720, 1655, 1595, 1540 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ 1.21 (s, 9H), 1.32 (s, 9H), 2.78 (t, 2H, J=6.4Hz), 3.65 (dt, 2H, J=6.0, 6.4Hz), 4.20-4.23 (complex, 2H), 4.31-4.39 (complex, 3H), 6.13 (d, 1H, J=5.2Hz), 7.73 (s, 1H), 10.59 (t, 1H, J=6.0Hz); ^{13}C NMR ($CDCl_3$, 100.5MHz) δ 18.7, 27.0 (x3), 27.6 (x3), 36.7, 39.9, 41.5, 65.2, 72.0, 76.2, 83.4, 89.2, 102.0, 116.2, 119.5, 126.4, 149.2, 151.0, 160.5, 165.2, 179.8, 183.0; HRMS m/z 528.2332 calcd for $C_{25}H_{32}N_6O_7$ (M^+-H_2O), found m/z 528.2313.

_N_-(2-Cyanoethyl)-7-(2',3'-_O_-thiocarbonylene-5'-_O_-trimethylacetyl-β-D-ribofuranosyl)-2-(trimethylacetoamino)-4(3_H_)-oxopyrrolo[2,3-_d_]pyrimidine-5-carboxamide (22).

A mixture of **21** (92.6 mg, 0.17 mmol) and 1,1'-thiocarbonyl diimidazole (75.7 mg, 0.43 mmol) in 1,2-dichloroethane (2.5 mL) was stirred at room temperature for 4 h. After evaporation of the solvent, the resulting solid was dissolved in chloroform (2.0 mL) and adsorbed onto silica gel (1.0 g), and the solvent was removed *in vacuo*. The silica gel powder was placed on the top of a prepacked silica gel column. Elution of the column with n-hexane/EtOAc (1:2) gave **22** (83.5 mg, 83 %) as a colorless solid: mp 215-219°C (dec); $[\alpha]^{23}_D$ -43° ($CHCl_3$, *c* 1.0); IR (film) ν_{max} 3170, 2250, 1725, 1660, 1595, 1545 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz) δ 1.28 (s, 9H), 1.39 (s, 9H), 2.73 (t, 2H, J=6.6Hz), 3.71 (dt, 2H, J=5.9, 6.6Hz), 3.89 (dd, 1H, J=4.8, 11.0Hz), 4.61 (ddd, 1H, J=2.9, 4.8, 10.8Hz), 5.21 (dd, 1H, J=10.8, 11.0Hz), 5.82 (dd, 1H, J=2.9, 7.3Hz), 5.88 (dd, 1H, J=1.1, 7.3Hz), 6.23 (d, 1H, J=1.1Hz), 7.67 (s, 1H), 9.22 (s, 1H), 10.3 (t, 1H, J=5.9Hz), 12.5 (bs, 1H); ^{13}C NMR ($CDCl_3$, 75.5MHz) δ 18.3, 26.7(x3), 21.2(x3), 35.6, 39.3, 40.6, 61.6, 85.2, 85.9, 88.9, 91.9, 102.9, 115.8, 118.2, 128.1, 146.8, 147.7, 158.4, 162.5, 180.4, 181.1, 188.7; HRMS m/z 589.2077 calcd for $C_{26}H_{33}N_6O_8S$ (M^++H), found m/z 589.2047.

_N_-(2-Cyanoethyl)-7-(2',3'-didehydro-2',3'-dideoxy-5'-_O_-trimethylacetyl-β-D-ribofuranosyl)-2-(trimethylacetoamino)-4(_3H_)-oxopyrrolo[2,3-_d_]pyrimidine-5-carboxamide (23).

A solution of **22** (77.2 mg, 0.13 mmol) in trimethyl phosphite (2.0 mL) was refluxed for 3 h. After completion of the reaction, the excess trimethyl phosphite was removed *in vacuo*. The resulting solid was dissolved in MeOH (2.0 mL) and adsorbed onto silica gel (1.0 g), and the solvent was removed. The silica gel powder was placed on the top of a prepacked silica gel column. Elution of the column with $CHCl_3$/MeOH (15:1) provided **23** (36.2 mg, 54 %) as a waxy solid: $[\alpha]^{23}_D$ -33° ($CHCl_3$, *c* 0.5); IR (film) ν_{max} 3200, 2250, 1730, 1660, 1605, 1535 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz) δ 1.21 (s, 9H), 1.35 (s, 9H), 2.72 (t, 2H, J=7.0Hz), 3.70 (dt, 2H, J=5.9, 7.0Hz), 4.16 (dd, 1H, J=5.5, 11.4Hz), 4.27 (dd, 1H, J=5.1, 11.4Hz), 5.07 (ddd, 1H, J=1.4, 5.1, 5.5Hz), 6.01 (dd, 1H, J=1.4, 5.9Hz), 6.39 (dd, 1H, J=1.1, 5.9 Hz), 6.96 (d, 1H, J=1.1 Hz), 7.59 (s, 1H), 8.38 (s, 1H), 10.4 (t, 1H, J=5.9Hz), 12.1 (s, 1H); ^{13}C NMR ($CDCl_3$, 75.5MHz) δ 18.2, 26.9 (x3), 27.1 (x3), 35.7, 38.8, 40.3, 65.2, 84.7, 89.0, 101.9, 115.7, 118.1, 125.3, 125.6, 133.9, 146.8, 148.4, 158.8, 163.0, 178.3, 180.1; HRMS m/z 512.2381 calcd for $C_{25}H_{32}N_6O_6$ (M^+), found m/z 512.2370.

_N_-(2-Cyanoethyl)-7-(2',3'-dideoxy-5'-_O_-trimethylacetyl-β-D-ribofuranosyl)-2-(trimethyl acetoamino)-4(3_H_)-oxopyrrolo[2,3-_d_]pyrimidine-5-carboxamide (24).

A mixture of **23** (34.5 mg, 0.067 mmol) and 10% Pd/C (2.5 mg) in MeOH (1.5 mL) was stirred under hydrogen atmosphere for 16 h. The reaction mixture was filtered through a Celite pad and washed with MeOH (2 mL x2), the filtrate and the washings were combined. After evaporation of the solvent, the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH 15:1) to afford **24** (30.5 mg, 88 %) as a waxy soid: $[\alpha]^{22}_D$ -22° ($CHCl_3$, *c* 0.5); IR (film) ν_{max} 3195, 2250, 1725, 1655, 1595, 1535 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz) δ 1.20 (s, 9H), 1.36 (s, 9H), 2.15 (complex, 2H), 2.44 (m, 1H), 2.73 (t, 2H, J=6.6Hz), 3.71 (dt, 2H, J=5.9, 6.6Hz), 4.18 (dd, 1H, J=4.8, 11.0Hz), 4.35 (m, 1H), 4.41 (dd, 1H, J=4.4, 11.0Hz), 6.22 (dd, 1H, J=4.0, 6.5Hz), 7.64 (s, 1H), 8.36 (s, 1H), 10.4 (t, 1H, J=5.9Hz), 12.1 (s, 1H); ^{13}C NMR ($CDCl_3$, 75.5MHz) δ 18.2, 26.9 (x3), 27.0, 27.1 (x3), 31.7, 35.7, 38.8, 40.3, 65.0, 78.6, 85.2, 101.9,

115.4, 118.1, 125.2, 146.5, 147.9, 158.7, 163.2, 178.6, 180.1. HRMS m/z 514.2536 calcd for $C_{25}H_{34}N_6O_6$ (M^+), found 514.2517.

N-(3-Aminopropyl)-7-(2',3'-dideoxy-5'-O-trimethylacetyl-β-D-ribofuranosyl)-2-(trimethylacetoamino)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxamide (25).

Compound **25** (16.1 mg, 72 %) was prepared from **24** (22.1 mg, 0.043 mmol) by the method described for **15**. Purification by silica gel column chromatography ($CHCl_3$/MeOH/29 % NH_4OH 100:25:1) gave **25** as a waxy solid; IR (nujol) ν_{max} 3210, 1660, 1595 cm^{-1}; $[\alpha]^{22}_D$ -2.8° (MeOH, *c* 0.2); ^1H NMR ($CDCl_3$, 270MHz) δ 1.19 (s, 9H), 1.35 (s, 9H), 1.83 (tt, 2H, J=6.6, 6.6Hz), 2.08-2.22 (complex, 2H), 2.37-2.49 (complex, 2H), 2.86 (t, 2H, J=6.6Hz), 3.53 (dt, 2H, J=5.6, 6.6Hz), 4.18 (dd, 2H, J=4.6, 10.9Hz), 4.30-4.43 (complex, 2H), 4.66 (bs, 2H), 6.23 (dd, 1H, J=4.6, 6.3Hz), 7.67 (s, 1H), 10.02 (t, 1H, J=5.6Hz); ^{13}C NMR (CD_3OD, 100.5MHz) δ 24.9, 25.7, 27.0 (x3), 27.6 (x3), 30.8 (x2), 33.2, 39.9, 41.5, 66.8, 80.2, 86.2, 102.1, 118.1, 126.3, 150.5 (x2), 161.0, 163.3, 170.0, 174.3; HRMS m/z 518.2853 calcd for $C_{25}H_{38}N_6O_6$ (M+), found m/z 518.2875.

N-(3-Aminopropyl)-7-(2',3'-dideoxy-β-D-ribofuranosyl)-2-(amino)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxamide (26).

Compound **26** (8.4 mg, 89 %) was prepared from **25** (14.0 mg, 0.027 mmol) by the method described for **16**. Purification by silica gel column chromatography ($CHCl_3$/MeOH/29 % NH_4OH 100:20:1) gave **26** as a colorless powder: mp 180-185 °C; IR (nujol) ν_{max} 3350, 1680, 1615, 1540 cm^{-1}; $[\alpha]^{21}_D$ -45° (H_2O, *c* 0.6); ^1H NMR (D_2O, 400MHz) δ 1.82 (tt, 2H, J=6.4, 7.6Hz), 1.85 (m, 1H), 2.05 (m, 1H), 2.19 (m, 1H), 2.34 (m, 1H), 2.91 (t, 2H, J=7.6Hz), 3.31 (t, 2H, J=6.4Hz), 3.49 (dd, 1H, J=5.2, 12.4Hz), 3.64 (dd, 1H, J=3.2, 12.4Hz), 4.12 (m, 1H), 6.04 (dd, 1H, J=3.6, 7.2Hz), 7.41 (s, 1H); ^{13}C NMR (D_2O+CD_3OD, 100.5MHz) δ 26.8, 28.4, 32.0, 37.2, 38.3, 64.5, 82.4, 85.2, 98.5, 113.6, 124.3, 153.3 (x2), 158.1, 166.8; HRMS m/z 349.1624 calcd for $C_{15}H_{21}N_6O_4$ (M^+-H), found m/z 349.1609.

N-(2-Cyanoethyl)-7-(2',3'-dideoxy-β-D-ribofuranosyl)-2-(amino)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxamide (27).

Compound **27** (15.7 mg, 93 %) was prepared from **24** (25.0 mg, 0.049 mmol), dry MeOH (3.0 mL), and liq. NH_3 (2.0 mL) by the method described for **13**. Purification by silica gel column chromatography ($CHCl_3$/MeOH 4:1) gave **27** as a colorless powder: mp 210-219 °C (dec); $[\alpha]^{22}_D$ -13° ($CHCl_3$, *c* 0.3); IR (film) ν_{max} 3325, 2245, 1680, 1655, 1615, 1510 cm^{-1}; ^1H NMR (CD_3OD, 300MHz) δ 2.01-2.12 (complex, 2H), 2.26 (m, 1H), 2.42 (m, 1H), 2.76 (t, 2H, J=6.6Hz), 3.64 (dt, 2H, J=1.5, 6.6Hz), 3.67 (dd, 1H, J=5.1, 11.7Hz), 3.76 (dd, 1H, J=3.6, 11.7Hz), 4.17 (m, 1H), 6.26 (dd, 1H, J=3.7, 6.6Hz), 7.69 (s, 1H), 10.8 (t, 1H, J=1.5Hz); ^{13}C NMR (CD_3OD, 75.5MHz) δ 18.7, 27.3, 33.4, 36.6, 65.1, 83.0, 86.1, 97.9, 114.8, 119.5, 125.2, 153.3, 154.4, 162.3, 166.2; HRMS m/z 346.1389 calcd for $C_{15}H_{18}N_6O_4$ (M^+), found m/z 346.1392.

N-(2-methoxycarbonylethyl)-7-(2',3'-dideoxy-β-D-ribofuranosyl)-2-(amino)-4(3H)-oxopyrrolo[2,3-d]pyrimidine-5-carboxamide (28).

Compound **28** (27.1 mg, 77 %) was prepared from **27** (32.2 mg, 0.093 mmol) by the method described for **17**. Purification by silica gel column chromatography ($CHCl_3$/MeOH 5:1) gave **28** as a colorless powder: mp 184-189 °C; $[\alpha]^{22}_D$ -16° (MeOH, *c* 0.5); IR (film) ν_{max} 3345, 1725, 1640, 1510 cm^{-1}; ^1H NMR (CD_3OD, 300MHz) δ 1.98-2.16 (complex, 2H), 2.28 (m, 1H), 2.42 (m, 1H), 2.65 (t, 2H, J=6.6Hz), 3.64 (t, 2H, J=6.6Hz), 3.68 (s, 3H), 3.73 (dd, 1H, J=1.5, 12.1Hz), 3.77 (dd, 1H, J=3.7, 12.1Hz), 4.19 (m, 1H), 6.24 (dd, 1H, J=4.0, 6.5Hz), 7.69 (s, 1H); ^{13}C NMR (CD_3OD, 100.5MHz) δ 27.1, 33.4, 34.9, 36.5, 52.3, 64.9, 83.1, 86.8, 98.1, 114.7, 125.5, 151.6, 154.1, 162.0, 165.8, 173.8; HRMS m/z 379.1492 calcd for $C_{16}H_{21}N_5O_6$ (M^+), found m/z 379.1487.

_N_-(2-Carbamoylethyl)-7-(2',3'-dideoxy-β-D-ribofuranosyl)-2-(amino)-4(_3H_)-oxopyrrolo[2,3-_d_]pyrimidine-5-carboxamide (29).

Compound **29** (14.8 mg, 85 %) was prepared from **28** (18.1 mg, 0.048 mmol) by the method described for **18**. Purification by silica gel column chromatography ($CHCl_3$/MeOH 4:1) gave **29** as a colorless powder: mp 162-169 °C; $[\alpha]^{21}_D$ -7.6° (MeOH, *c* 0.5). IR (film) ν_{max} 3350, 1685, 1650, 1590 cm^{-1}; ^1H NMR (CD_3OD, 400MHz) δ 1.98-2.16 (complex, 2H), 2.26 (m, 1H), 2.42 (m, 1H), 2.53 (t, 2H, J=7.3Hz), 3.64 (t, 2H, J=7.3Hz), 3.67 (dd, 1H, J=5.3, 11.6Hz), 3.76 (dd, 1H, J=3.6, 11.6Hz), 4.17 (m, 1H), 4.60 (s, 1H), 6.26 (dd, 1H, J=4.0, 6.9Hz), 7.66 (s, 1H); ^{13}C NMR (CD_3OD, 100.5MHz) δ 23.7, 33.3, 36.4, 36.9, 65.2, 82.9, 86.1, 95.8, 115.2, 124.9, 152.4, 153.3, 162.7, 163.9, 170.0; HRMS m/z 347.1468 calcd for $C_{15}H_{19}N_6O_4$ (M^+-OH), found m/z 347.1482.

Cell culture and growth assay

A431 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 5 % calf serum. The cells were cultured at 37 °C in a 5 % CO_2-95 % air atmosphere. The cells (10^4) in 48-well plate were incubated for 3 days with a test chemical, and the cell number was counted by the coulter counter.

Phosphatidylinositol kinase assay

Preparation of the membrane fraction and the enzyme assay were carried out as described by Nishioka *et al.*.[15] The reaction mixture containing phosphatidylinositol, the membrane fraction of A431 cells, [γ-^{32}P]ATP (5 μCi/mL), and the inhibitor was incubated in 100 μL of 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.2) at 20 °C. The reaction was stopped by addition of 500 μL of mixture of $CHCl_3$, MeOH and 1N HCl (2:1:2). After vortexing, the lower organic phase was taken and applied on packed silica gel column to separate phosphorylated lipid and unreacted [γ-^{32}P]ATP. Then, the phosphorylated lipid was eluted with a mixture of $CHCl_3$, MeOH and 4N NH_4OH (9:7:2) and counted for radioactivity.

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