Pergamon

BIOORGANIC & MEDICINAL CHEMISTRY

Bioorganic & Medicinal Chemistry 9 (2001) 163–170

Synthesis and Cytotoxicity of 4′-*C*- and 5′-*C*-Substituted Toyocamycins

Esmir Gunic,[a] Jean-Luc Girardet,[a] Zbigniew Pietrzkowski,[b] Cathey Esler[b] and Guangyi Wang[a,*]

[a]*Chemistry Laboratories, ICN Pharmaceuticals, Inc., 3300 Hyland Avenue, Costa Mesa, California 92626, USA*
[b]*Cancer Biology Laboratories, ICN Pharmaceuticals, Inc., 3300 Hyland Avenue, Costa Mesa, California 92626, USA*

Received 21 June 2000; accepted 15 August 2000

Abstract—Toyocamycin and some analogues have shown potent antitumor activities; however, none of them could be used clinically primarily owing to their cytotoxicity to normal human cells. In order to overcome the weakness of these nucleoside analogues, substitution of a variety of modified sugars for the ribofuranose was explored in our laboratories with expectation that certain sugar-modified toyocamycin analogues may be selectively cytotoxic to cancer cells. In this article, we report synthesis and cytotoxicity of 4′-*C*- and 5′-*C*-substituted toyocamycins, which were prepared via the condensations of 4-*C*- and 5-*C*-substituted ribofuranose derivatives **11**, **12**, **13**, **20**, **21**, and **26** with the silylated form of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**27**) and subsequent debromination and debenzoylation. When compared to the parent toyocamycin, all these analogues showed much lower cytotoxicity to human prostate cancer cells (HTB-81), mouse melanoma cancer cells (B16) as well as normal human fibroblasts. Compound **1e** showed a significant cytotoxicity to the prostate cancer cells and a moderate selectivity. The results suggested that sugar modifications, especially those that may affect phosphorylation of nucleosides, could alter cytotoxicity profile significantly.

Introduction

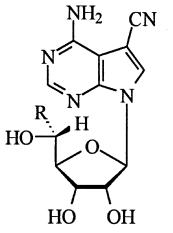
Many toyocamycin analogues have been synthesized and evaluated for antitumor and antiviral activities since toyocamycin was isolated four decades ago.[1–9] Although some toyocamycin analogues are potent inhibitors of many cancer cells, they are also toxic to normal human cells. Many efforts have been made to improve their selectivity profiles. Unfortunately, none of the toyocamycin analogues are used clinically. It was reported that the toxicity profile of neplanocin A, another antibiotic nucleoside, was improved when a methyl group was introduced at its 6′-carbon.[10] 5′-*C*-Methyl toyocamycin and sangivamycin were also reported,[11] but no detailed biological studies were given. A few nonphosphorylatable, 5′-modified analogues of toyocamycin and sangivamycin designed as protein kinase inhibitors were reported.[12,13] Apparently, 5′-*C*-substituted toyocamycin analogues as anticancer and antiviral agents have not been intensively explored. In other words, it was not known whether C-branched nucleoside analogues can be selectively cytotoxic to cancer cells. If a compound can be selectively phosphorylated in cancer cells, a selective inhibition of cancer cells might be achieved.[14,15] In order to search for such a nucleoside analogue, we synthesized a series of sugar-modified toyocamycin analogues having a lower alkyl, alkenyl, or alkynyl substituent on the ribose moiety of toyocamycin. In this article we report the synthesis and in vitro cytotoxicity of 4′-*C*- and 5′-*C*-substituted toyocamycins (**1b–g**, **2a–d**, and **3a–c**), as shown in Chart 1.

Chemistry

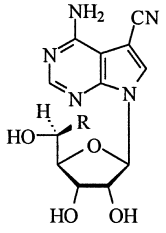
The 4′-*C*- and 5′-*C*-substituted toyocamycins were synthesized via the condensations of 4-*C*- and 5-*C*-substituted ribofuranose derivatives with the silylated form of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine by a similar procedure as those used for preparation of toyocamycin.[16,17] Synthesis of the modified ribofuranoses is shown in Scheme 1. The 5(*R*)-allylribofuranose derivative **5** was prepared according to a previously published procedure.[18] Reaction of **4**[19] with ethynylmagnesium bromide afforded **6** in good yield, which was a mixture of the 5(*R*)- and 5(*S*)-isomers (1:1). A controlled catalytic

*Corresponding author. Tel.: +714-545-0100 ext. 4165; fax: +714-668-3141; e-mail: gwang@icnpharm.com

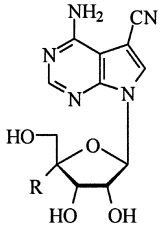
0968-0896/01/$ - see front matter
PII: S0968-0896(00)00221-2



1a R = H (toyocamycin)
1b R = methyl **1c** R = ethynyl
1d R = vinyl **1e** R = ethyl
1f R = allyl **1g** R = propyl



2a R = methyl
2b R = ethynyl
2c R = vinyl
2d R = ethyl



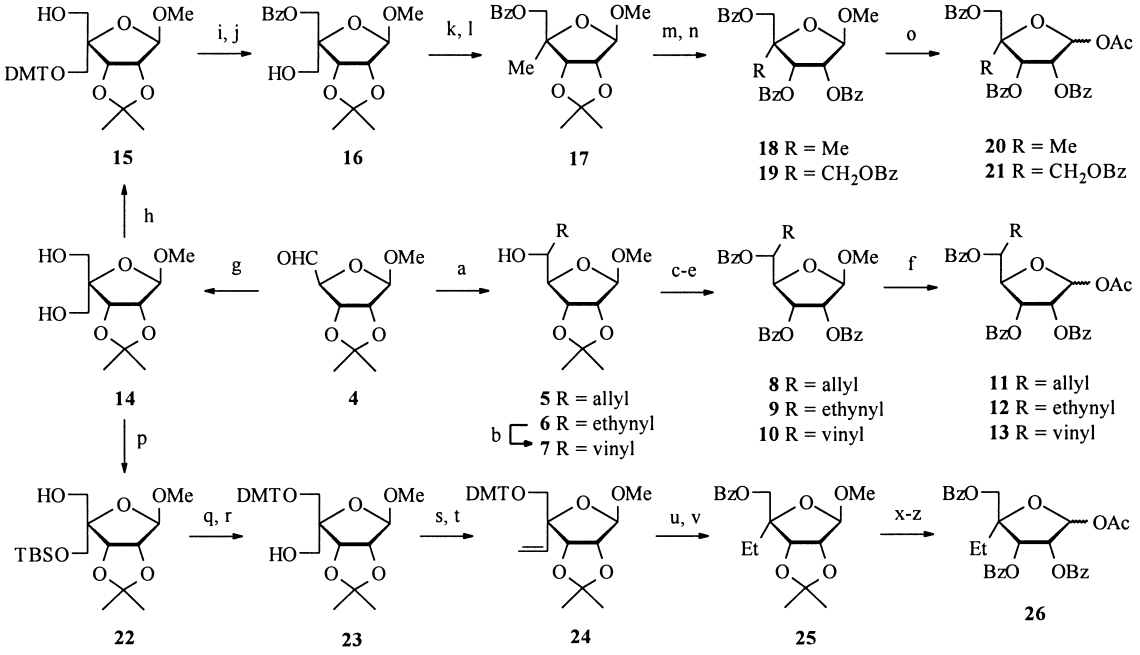
3a R = methyl
3b R = ethyl
3c R = CH_2OH

Chart 1.

hydrogenation of **6** over nickel boride[20] afforded **7** (*R*/*S* ratio, 1:1). The 5(*R*)-isomer (**7a**) of **7** was also prepared following a published procedure.[21] Compounds **5**–**7** were converted to **11**–**13** in moderate to good yields, respectively, through a sequence of reactions: benzoylation at O-5; removal of 2,3-*O*-isopropylidene; benzoylation at O-2 and O-3; and acetylation at O-1.

Treatment of **4** with formaldehyde in the presence of sodium hydroxide yielded **14**, which was selectively protected with 4,4′-dimethoxytrityl (DMT) at the 4α-hydroxymethyl to give **15**. The benzoylation of **15** and the subsequent removal of DMT gave **16**, which was converted to the 4-*C*-methyl derivative **17** by a Barton type deoxygenation.[22,23] Compounds **17** and the 4,5-*O*-dibenzoylated form of **14** (not shown) were converted to **18** and **19**, respectively, by treatment with TFA at low temperature and the subsequent benzoylation. Compounds **18** and **19** were acetylated in the presence of sulfuric acid to give the 1-*O*-acetylribofuranoses **20** and **21**, respectively. Compound **14** was selectively protected with *t*-butyldimethylsilyl (TBS) at the 4α-hydroxymethyl to give **22** in good yield. Tritylation of **22** at the 5-hydroxyl and the subsequent removal of the TBS group afforded **23**. Compound **23** was converted to an aldehyde, which was subjected to a Wittig reaction to give **24** in very good yield. After hydrogenation of **24** over palladium, the resulting product was subjected to the same sequence of reactions as **5**–**7** to give the 4-*C*-ethyl derivative **26**.

The Vorbrüggen condensations (Scheme 2)[16,17,24] of the 1-*O*-acetylated ribofuranose derivatives **11**, **12**, **13**, **20**, **21**, and **26** with the fully silylated form of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**27**)[25] in the presence of trimethylsilyl triflate yielded compounds **28**–**35**, respectively. The removal of bromine in compounds **28**–**32** was accomplished by treatment with zinc in acetic acid,[26]



Scheme 1. (a) HC≡CMgBr, THF, −40 to 0 °C, 1.50 h, 77% for **6**; (b) H_2, Ni_2B, $(CH_2NH_2)_2$, EtOH, rt, 10 days, 63%; (c) BzCl, pyridine, rt, overnight; (d) TFA/H_2O, 0 °C, 1.5 h; (e) same as c; (f) Ac_2O, AcOH, H_2SO_4, rt, overnight, 37% for **11**, 55% for **12**, 63% for **13** (4 steps); (g) CH_2O, NaOH, dioxane/H_2O, rt, overnight, 85%; (h) DMT-Cl, pyridine, rt, overnight; (i) same as c; (j) 80% AcOH, rt, 2 h; (k) PHOC(S)Cl, DMAP, CH_3CN, rt, 2 h; (l) $(TMS)_3SiH$, ACCN, toluene, 100 °C, 15 h; (m–o) same as d–f, 28% for **20** (8 steps), 73% for **21** (4 steps); (p) TBDMS-Cl, pyridine, rt, 24 h; (q) same as h; (r) TBAF, THF, 3 days, 65% (3 steps); (s) DMSO, DCC, TFA, pyridine, rt, 8 h, 89%; (t) $Ph_3P{=}CH_2$, ether, rt, 6 h, 99% (2 steps); (u) H_2, Pd/C, rt, 6 h; (v) same as c; (x–z) same as d–f, 58% (5 steps).

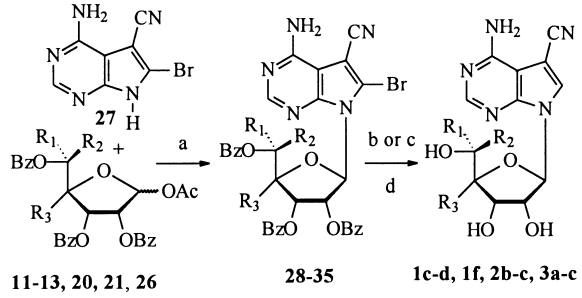
followed by debenzoylation, to afford compounds **1c**, **1d**, **1f**, **2b** and **2c**, respectively, in moderate to good yields, with **1c** and **2b** as an isomeric mixture and **1d** and **2c** as another isomeric mixture. Compounds **1d** and **2c** could be separated by chromatography, and compounds **1c** and **2b** were only partially separated. Compounds **33**–**35** were subjected to catalytic hydrogenolysis over Pd/C, followed by debenzoylation, to give **3a**–**c**, respectively, in good yields. The mixture of **1c** and **2b** was hydrogenated over palladium to give a mixture of **1e** and **2d**, which was separated by chromatography. Compound **1g** was obtained from the catalytic hydrogenation of **28** over palladium and the subsequent debenzoylation. Compounds **1b** and **2a** were reported previously[11] and synthesized recently in our laboratory by a different synthetic route.[24]

Assignments of the selective protection site of 14

From previous publications,[27,28] we could anticipate that the 4α-hydroxymethyl of **14** would be selectively protected. As can be seen from Scheme 1, compounds **15** and **22** were prepared in good yields from such selective protections of **14**. In order to make certain that the selective protection took place at the 4α-hydroxymethyl rather than at the O-5 position of **14**, we have investigated the stereochemical assignments. The major product (77%) and the minor product (8%) from the tritylation of **14** with DMT-Cl were methylated, respectively. The methylated products were separately subjected to treatment with TFA at 0 °C to remove DMT and isopropylidene protecting groups. The reactions of the deprotected products with *p*-tosyl chloride gave the tosyl derivatives **36** (from the major tritylation product) and **37** (from the minor tritylation product), respectively. The tosylates **36** and **37** were treated separately with sodium hydride in THF at 50 °C. One reaction was complete within 2 h with formation of two products while the other reaction did not proceed at all even after 24 h. The two products formed from the first reaction were tentatively assigned as 3-*O*,4-*C*-methylene and 2-*O*,4-*C*-methylene ribofuranose derivatives **38** and **39**, respectively (Scheme 3).[29] As can be seen from a ball–stick model, the ring formation could only occur to compound **36** since its tosyl group is at the 4α-hydroxymethyl and adjacent to the 2- and 3-hydroxyls, which allowed us to determine, without ambiguity, that the major tritylation product which led to the formation of **36** is compound **15** as shown in Scheme 1 and the minor tritylation product is compound **23** (Scheme 1). By comparison of the two NMR spectra (identical) of **23** from the two routes, it is evident that the selective silylation also took place at the 4α-*C*-hydroxymethyl of **14**.

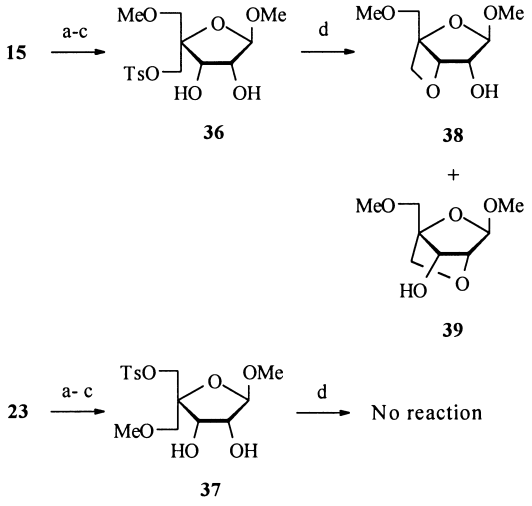
Assignments of configurations of the C-5′ positions

In the 5′-*C*-substituted toyocamycins, the C-5′ can have either *R* or *S* configuration, which depends on the orientation of the substituents at the C-5′. Since the condensations of the 1-*O*-acetylated ribofuranose derivatives with the silylated base and the subsequent reactions did not change the configurations at the C-5, we can determine the configurations at the C-5′ of the



28 R_1 = allyl, R_2, R_3 = H
29 R_1 = ethynyl, R_2 , R_3 = H
30 R_2 = ethynyl, R_1, R_3 = H
31 R_1 = vinyl, R_2, R_3 = H
32 R_2 = vinyl, R_1, R_3 = H
33 R_1, R_2 = H, R_3 = methyl
34 R_1, R_2 = H, R_3 = ethyl
35 R_1, R_2 = H, R_3 = CH_2OBz

Scheme 2. (a) 1. HMDS, $(NH_4)_2SO_4$, reflux, overnight; 2. TMSOTf, $ClCH_2CH_2Cl$, reflux, 3 days; (b) Zn, AcOH, rt, overnight (for **28**–**32**); (c) H_2, Pd/C, rt, 6 h (for **33**–**35**); (d) 1. NH_3/MeOH, rt, overnight; 2. NaOAc/DMF, 120 °C, 5 h, 51% for **1f**, 62% for **1c** and **2b**, 29% for **1d** and **2c**, 69% for **3a**, 62% for **3b** and 54% for **3c** (3 steps).



Scheme 3. (a) MeI, NaH, THF, rt; (b) TFA/H_2O, 0 °C; (c) TsCl, pyridine, rt; (d) NaH, THF, 50 °C.

nucleosides simply through the assignments of the 5-*C*-substituted ribofuranose intermediates. The *R* configuration at the C-5 of **5** was assigned in a previous publication.[18] The 5(*R*)-isomer (**7a**) of **7** prepared according to a published procedure[21] was converted to **1d**. Comparison of the proton NMR spectrum of **1d** with those of the two separated products from **7** (~1:1 mixture) could clearly distinguish **1d** and **2c**. 5′(*R*)-*C*-Ethyltoyocamycin (**1e**), the fully hydrogenated product of **1d**, has identical proton NMR spectrum to that of one of the hydrogenated products of **1c** and **2b** mixture.

In vitro cytotoxicity

Compounds **1c**–**g**, **2b**–**d** and **3a**–**c** as well as known compounds **1a**,[16] **1b** and **2a**[11,24] were evaluated for their cytotoxicity to human prostate cancer cells, mouse melanoma cancer cells, and normal human fibroblasts. The results (Table 1) indicated that all the compounds tested showed a dramatic reduction in cytotoxicity to both

Table 1. Cytotoxicity (EC_{50}[a] μM) of 4′-*C*- and 5′-*C*-substituted toyocamycins

Compound	HTB-81[b]	B-16[c]	NHF[d]
1a	0.012	0.005	0.004
1b	17.3	45.3	40.5
1c	17.2	> 100	59.5
1d	19.1	85.6	64.6
1e	10.3	98.5	68.1
1f	> 100	> 100	> 100
1g	52.1	> 100	> 100
2a	44.4	19.6	41.7
2b	5.8	23.6	11.5
2c	9.9	23.7	14.9
2d	11.7	15.2	19.8
3a	22.1	82.5	53.6
3b	30.8	> 100	95.7
3c	> 100	44.4	> 100

[a]EC_{50} is the concentration of compound that caused 50% reduction in absorbance at 490 nm relative to untreated cells using MTS assay.
[b]HTB-81, human prostate cancer cells.
[c]B-16, mouse melanoma cells.
[d]NHF, normal human fibroblasts.

cancer and normal cells when compared with toyocamycin (**1a**). Compounds **1b**, **1c**, **1d**, **1e**, **2b**, **2c**, and **2d** still retain moderate to significant cytotoxicity to the prostate cancer cells (HTB-81), but less cytotoxicity to normal cells (NHF). It was noted that three 5′(*R*)-substituted compounds (**1c**, **1d**, **1e**) have less cytotoxicity to NHF cells than their 5′(*S*)-isomers (**2b**, **2c**, **2d**) although both types of isomers have comparable cytotoxicity to HTB-81 cells. These results indicated a moderate selectivity in cytotoxicity for the 5′(*R*)-isomers, which implicated that there might be a possibility to find more selectively cytotoxic compounds through sugar modifications.

In summary, we have reported synthesis and in vitro cytotoxicity evaluation of a number of new toyocamycin analogues containing 4′-*C*- and 5′-*C*-substituted ribofuranoses. The cytotoxicity of these compounds to human prostate cancer (HTB-81) and mouse melanoma cancer cells (B-16) as well as normal human fibroblasts was dramatically reduced compared to that of toyocamycin. Several compounds (**1c**, **1d**, **1e**, **2b**, **2c**, **2d**) retained moderate to significant cytotoxicity to the cancer cells, with compound **1e** demonstrating a moderate selectivity. Further evaluation of these compounds for cytotoxicity to other types of cancer cells and their antiviral activity are under way.

Experimental

^{1}H NMR spectra were recorded on a Varian 300 spectrometer and tetramethylsilane was used as the internal standard. Elemental analysis was conducted by NuMega Resonance, Inc., San Diego, CA. Melting points were measured on a capillary Melting Point Measurement Apparatus and are uncorrected. Anhydrous solvents were purchased from Aldrich or Fluka without further treatment unless noted. Thin layer chromatography plates and silica gel for column chromatography were supplied by ICN Biomedicals. Solvent ratios are based on volume in cases where a solvent mixture was used.

A usual work-up procedure was used for most of the reactions in the Experimental section: the mixture was diluted with ethyl acetate (or methylene chloride), washed sequentially with water (or brine), dilute sodium bicarbonate, and water (or brine), dried (Na_2SO_4), filtered, and concentrated to dryness at reduced pressure. The crude product was purified by flash chromatography on silica gel.

5(*R,S*)-*C*-Ethynyl-2,3-*O*-isopropylidene-1-*O*-methyl-β-D-ribofuranoses (6). A solution of **4**[19] (4.0 g, 19.8 mmol) in anhydrous THF (20 mL) was added to a stirred solution of ethynylmagnesium bromide (0.5 M in THF, 80 mL) at −40 °C under argon. The mixture was warmed up to 0 °C during 1.5 h, poured into ice/water, and neutralized with acetic acid. After the usual work-up, the residue was chromatographed (ethyl acetate:hexanes, 1:3) to give 3.48 g (77%) of **6** as a colorless solid (ratio of the two isomers, 1:1); ^{1}H NMR ($CDCl_3$) δ 1.31, 1.33 (2 s, 3H, Me), 1.48, 1.49 (2 s, 3H, Me), 2.51 (dd, J = 2.4, 0.6 Hz, 0.5 H, =CH), 2.54 (dd, J = 2.1, 0.6 Hz, 0.5H, =CH), 3.45, 3.50 (2 s, 3H, OMe), 3.90–4.02 (m, 1H, OH), 4.32–4.48 (m, 2H), 4.59 (d, J = 6.0 Hz, 1H), 4.81 (d, J = 5.7 Hz, 0.5H), 5.01 (d, J = 5.7 Hz, 1H), 5.02 (d, J = 6.3 Hz, 0.5H).

2,3-*O*-Isopropylidene-1-*O*-methyl-5(*R,S*)-*C*-vinyl-β-D-ribofuranoses (7). A mixture of **6** (two isomers, 6.1 g, 26.73 mmol) and nickel boride (3.5 g) in ethanol (200 ml) containing ethylenediamine (5.8 mL) was shaken in a hydrogenation apparatus (10 psi hydrogen) for 10 days. The catalysts were filtered and washed with ethanol, and the filtrate was concentrated to dryness. Chromatography (ethyl acetate:hexanes, 1:2) gave 4.2 g (63%) of **7** (two isomers, 1:1) as a syrup and 1.1 g of recovered **5**. ^{1}H NMR ($CDCl_3$) δ 1.30, 1.32 (2 s, 3H, Me), 1.48 (s, 3H, Me), 3.40, 3.46 (2 s, 3H, OMe), 3.94, 4.10 (2 br s, 1H, OH), 4.25–4.42 (m, 2H), 4.56–4.61 (m, 1H), 4.79–4.87 (m, 1H), 4.95, 4.98 (2 s, 1H), 5.22–5.48 (m, 2H, vinyl), 5.80–5.96 (m, 1H, vinyl).

2,3-*O*-Isopropylidene-1-*O*-methyl-5(*R*)-*C*-vinyl-β-D-ribofuranose (7a). A solution of **4**[19] (1.0 g, 5.0 mmol) in anhydrous THF (25 mL) was added to a stirred solution of vinylmagnesium bromide (1.0 M in THF, 14.8 ml) in THF (115 mL) at −20 °C. The reaction mixture was stirred at −10 °C for 2 h. Similar work-up as described for **5** and subsequent chromatography (1% methanol in dichloromethane) gave 0.41 g (36%) of **7a** as a syrup; ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 3H, Me), 1.47 (s, 3H, Me), 3.46 (s, 3H, OMe), 3.94 (s, 1H, OH), 4.27–4.34 (m, 2 H), 4.58 (d, J = 6.0 Hz, 1H), 4.80 (d, J = 6.0 Hz, 1H), 5.00 (s, 1H, 1-H), 5.26–5.31 (m, 1H, -C=CH_2), 5.41–5.48 (m, 1H, -C=CH_2), 5.81–5.92 (m, 1H, -CH=C).

1-*O*-Acetyl-5(*R*)-*C*-allyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (11). A solution of **5**[18] (4.49 g, 18.38 mmol) and benzoyl chloride (2.7 mL, 23.2 mmol) in anhydrous pyridine (40 ml) was stirred at room temperature overnight and quenched with water. After the usual work-up, the residue was purified by chromatography (15% ethyl acetate in hexanes) to give 6.26 g of the benzoylated product, which was dissolved in cold TFA:water (9:1, 60 mL) and stood at 0 °C for 1.5 h. Solvent was evaporated at 0 °C,

and the residue was dissolved in methanol/toluene and concentrated to dryness. The crude was subjected to benzoylation as described above to give 5.30 g of **8**, which was dissolved in a mixture of acetic acid (18 ml) and acetic anhydride (2.3 mL). Under cooling with ice, sulfuric acid (96%, 250 μL) in acetic acid (5 mL) was added, and the resulting mixture was stirred at room temperature overnight. After the usual work-up, the crude was purified by chromatography (ethyl acetate:hexanes, 1:4) to give 2.82 g (37%, 4 steps) of **11** as a colorless solid; ^{1}H NMR ($CDCl_3$) δ 2.14 and 2.35 (2 s, 3H, Ac), 2.50–2.70 (m, 2H), 4.68–4.75 (m, 1H), 5.06–5.21 (m, 2H), 5.48–5.92 (2 m, 3H), 6.02–6.12 (m, 1H), 6.38 (d, J = 0.9 Hz, 2/3H), 6.60 (d, J = 4.5 Hz, 1/3H), 7.10–8.18 (m, 15H).

1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-5(*R*,*S*)-*C*-ethynyl-β-D-ribofuranoses (12). A colorless syrup (*R*:*S*, 1:1; α:β, 1:2) was prepared in 55% yield from **6** by the same procedure as described for **11**. ^{1}H NMR ($CDCl_3$) δ 1.75, 2.13, 2.14 and 2.19 (4 s, 3H), 2.48, 2.54, 2.63, and 2.64 (4 d, J = 2.1 Hz, 1H), 4.79–4.87 (m, 1H), 5.69–5.82 (m, 1H), 5.93-6.19 (m, 2H), 6.39 (s, 1/3H), 6.43 (s, 1/3H), 6.68 (d, J = 4.2 Hz, 1/6H), 6.78 (d, J = 4.8 Hz, 1/6H), 7.28–8.16 (m, 15H).

1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-5(*R*,*S*)-*C*-vinyl-β-D-ribofuranoses (13). A colorless syrup (*R*:*S*, 1:1; α:β, 1:2) was prepared in 63% yield from **7** by the same procedure as described for **11**. ^{1}H NMR ($CDCl_3$) δ 1.77, 2.12, 2.13 and 2.19 (4 s, 3H), 4.71–4.78 (m, 1H), 5.30–5.61 (m, 3H), 5.73–6.03 (m, 3H), 6.38 (s, 1/3H), 6.42 (s, 1/3H), 6.63 (d, J = 4.5 Hz, 1/6H), 6.76 (d, J = 4.5 Hz, 1/6H), 7.28–8.15 (m, 15H).

4-*C*-Hydroxymethyl-2,3-*O*-isopropylidene-1-*O*-methyl-β-D-ribofuranose (14). To a stirred solution of **4**[19] (20.22 g, 0.1 mol) and formaldehyde (37% aq., 76 mL) in 1,4-dioxane (380 mL) at 0 °C was added aqueous NaOH (2.0 M, 188 mL). The reaction mixture was stirred at room temperature overnight, neutralized with 10% AcOH, and concentrated to half the volume. After the usual work-up, the residue was purified by chromatography (4% methanol in chloroform) to give 20.2 g (85%) of **14** as a colorless solid; ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 3H, Me), 1.51 (s, 3H, Me), 2.37 (t, 1H, OH), 3.4 (m, 1H), 3.44 (s, 3H), 3.60–3.82 (m, 4H), 4.67 (d, J = 6.0 Hz, 1H), 4.86 (d, J = 6.3 Hz, 1H), 5.00 (s, 1H).

5-*O*-Benzoyl-4-*C*-hydroxymethyl-2,3-*O*-isopropylidene-1-*O*-methyl-β-D-ribofuranose (16). A solution of **14** (3.5 g, 15 mmol) and 4,4′-dimethoxytrityl chloride (6.0 g, 18 mmol) in anhydrous pyridine (78 mL) was stirred at room temperature overnight, quenched with methanol (6 mL) at 0 °C, and concentrated. After the usual work-up, the residue was purified by chromatography (20–25% ethyl acetate in hexanes) to give 6.2 g (77%) of **15** as a colorless foam, which was subjected to benzoylation as described before. The crude product was dissolved in toluene and concentrated to dryness. The residue was dissolved in 80% acetic acid (174 mL), and the mixture stood at room temperature for 2 h and concentrated to dryness. Chromatography (1–2% methanol in dichloromethane) gave **16** as a white foam, which was used directly in the next reaction. ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 3H, Me), 1.53 (s, 3H, Me), 3.34 (s, 3H, OMe), 3.87 (m, 2H), 4.41–4.54 (m, 2H), 4.74 (m, 2H), 5.04 (s, 1H), 7.43–7.62 (m, 3H, Bz), 8.07 (m, 2H, Bz).

1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-4-*C*-methyl-β-D-ribofuranose (20). A solution of **16** (the whole product obtained above), DMAP (4.3 g, 35 mmol), and phenoxythiocarbonyl chloride (2.4 mL, 17 mmol) in acetonitrile (174 ml) was stirred at room temperature for 2 h, concentrated to dryness, dissolved in methylene chloride, and washed with 0.5 M hydrochloric acid. After the usual work-up, the residue was dried and dissolved in toluene (93 mL), and tris(trimethylsilyl)silane (9.0 ml, 29 mmol) and 1,1′-azobis(cyclohexanecarbonitrile) (0.71 g, 2.9 mmol) were added. The reaction mixture was stirred at 100 °C for 15 h, cooled, and concentrated. The residue was purified by chromatography (1–2% methanol in dichloromethane) to give **17** as a syrup, which was converted to **20** (1.70 g, 28%, 8 steps) by the same procedure as described for **11**. Recrystallization from ethyl acetate/hexanes gave a colorless, crystalline solid (β-isomer); ^{1}H NMR ($CDCl_3$) δ 1.61 (s, 3H, Me), 1.96 (s, 3H, Ac), 4.49 (dd, J = 58.5 Hz, 11.4 Hz, 2H), 5.84 (d, J = 5.4 Hz, 1H), 5.98 (d, J = 5.1 Hz, 1H), 6.43 (s, 1H), 7.26–7.64 (m, 9H, Bz), 7.81–8.14 (m, 6H, Bz).

1-*O*-Acetyl-4-*C*-benzoyloxymethyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (21). Prepared from **14** (3.0 g, 12.8 mmol) by the same procedure as described for **11**. Total yield: 5.82 g (73%) as a syrup (α:β ratio, 1:2). ^{1}H NMR ($CDCl_3$) δ 2.05 and 2.18 (2 s, 3H, Ac), 4.69–4.92 (m, 3H), 5.83–5.88 (m, H), 6.18 (d, J = 5.4 Hz, 2/3H), 6.24 (d, J = 6.3 Hz, 1/3H), 6.51 (s, 2/3H), 6.74 (d, J = 4.5 Hz, 1/3H), 7.20–8.20 (m, 20H, Bz).

5-*O*-(4,4′-Dimethoxytrityl)-4-*C*-hydroxymethyl-2,3-*O*-isopropylidene-1-*O*-methyl-β-D-ribofuranose (23). A solution of **14** (4.5 g, 19 mmol) and *tert*-butyldimethylsilyl chloride (3.4 g, 23 mmol) in anhydrous pyridine (96 mL) was stirred at room temperature for 24 h, quenched with water (5 mL), and concentrated. After the usual work-up, the residue (**22**) was dried and dissolved in anhydrous pyridine, and 4,4′-dimethoxytrityl chloride (8.4 g, 25 mmol) was added. The reaction mixture was stirred at room temperature overnight, quenched with methanol, and concentrated. After the usual work-up, the residue was dried and dissolved in THF (57 mL), and TBAF (1.0 M in THF, 23 mL) was added. After 24 h at room temperature, more TBAF (3.8 mL) was added, and the mixture was stirred for an additional 36 h. The solvent was evaporated and the residue was chromatographed (ethyl acetate:hexanes, 1:1) to give 6.6 g (65%, 3 steps) of **23** as a colorless foam; ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 3H, Me), 1.49 (s, 3H, Me), 2.15 (m, 1H, OH), 3.17 (s, 3H, OMe), 3.27 (m, 2H), 3.79 (s, 6H, OMe), 3.86–4.01 (m, 2H), 4.45 (m, 2H), 4.91 (s, 1H), 6.84 (m, 4H, DMT), 7.20–7.46 (m, 9H, DMT).

5-*O*-(4,4′-Dimethoxytrityl)-2,3-*O*-isopropylidene-1-*O*-methyl-4-*C*-vinyl-β-D-ribofuranose (24). A solution of TFA (0.49 mL, 6.4 mmol) and pyridine (1.6 mL, 19 mmol) in DMSO (11 mL) was added to a stirred solution of **23**

(6.9 g, 13 mmol) and DCC (6.6 g, 32 mmol) in a mixture of toluene (26 mL) and DMSO (66 mL) at 5 °C. The reaction mixture was stirred at ambient temperature for 8 h and cooled to 0 °C. Ethyl acetate (80 mL) and a solution of oxalic acid (1.8 g, 19 mmol) in methanol (10 mL) were added, and the mixture was stirred at room temperature overnight. The precipitate was filtered and washed with a 1:1 mixture of hexanes and ethyl acetate. After the usual work-up, the residue was purified by chromatography (ethyl acetate:hexanes, 1:3) to give 6.1 g (89%) of the 4-*C*-formyl product as a colorless foam.

A solution of sodium pentoxide (2.5 g, 22 mmol) in benzene (34 mL) was added to a stirred suspension of methylphosphonium bromide (8.8 g, 25 mmol) in ether (250 mL) under argon. The mixture was stirred at room temperature for 6 h, and a solution of the 4-*C*-formyl product (6.0 g, 11 mmol) in ether (30 mL) was added. The resulting mixture was stirred at room temperature overnight, quenched with brine at 0 °C. After the usual work-up, the residue was chromatographed (ethyl acetate: hexanes, 1:3) to give 5.9 g (99%) of **24** as a colorless foam; ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 3H, Me), 1.42 (s, 3H, Me), 3.20 (m, 5H), 3.79 (s, 6H, OMe), 4.38 (d, J = 6.0 Hz, 1H), 4.50 (d, J = 6.0 Hz, 1H), 4.90 (s, 1H), 5.30 (dd, J = 11.1 Hz, 1.8 Hz), 5.45 (dd, J = 17.4 Hz, 1.8 Hz), 6.20 (dd, J = 17.4 Hz, 11.1 Hz), 6.82 (m, 4H, DMT), 7.20–7.46 (m, 9H, DMT).

1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-4-*C*-ethyl-β-D-ribofuranose (25). A suspension of 10% Pd/C (50% water, 508 mg) and **24** (5.0 g, 9.4 mmol) in methanol (254 mL) was shaken in a hydrogenation apparatus (5 psi hydrogen) at room temperature for 6 h. The catalyst was filtered and washed with methanol. The solvent was evaporated, and the residue was dried by evaporation with anhydrous pyridine and dissolved in pyridine (75 mL). Benzoyl chloride (1.2 ml, 10 mmol) was added, the reaction mixture was stirred at room temperature for 15 h, then cooled to 0 °C. Methanol (5 mL) was added, and the solvents were evaporated under reduced pressure. Ethyl acetate, hexane and brine were added, and the organic extract was washed with brine, dried over sodium sulfate, filtered and evaporated to dryness. The crude product **25** was converted to **26** by the same procedure as described for **11**. Total yield (ratio of α:β, 1:2): 58% (5 steps) as a colorless syrup; ^{1}H NMR ($CDCl_3$) δ 1.05 and 1.11 (2 t, J = 7.6 Hz, 3H, CH_3), 1.94 and 2.10 (2 m, 2H, CH_2), 1.98 and 2.14 (2 s, 3H, Ac), 4.40–4.58 (m, 2H), 5.85 (m, 1H), 6.02 and 6.06 (2 d, J = 6.3 Hz and 5.7 Hz, 1H), 6.45 and 6.66 (2 d, J = 1.2 Hz and 4.8 Hz, 1H), 7.34–8.15 (m, 15H, Bz).

4-Amino-5-cyano-7-(5(*R*)-*C*-allyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (1f). A mixture of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (1.05 g, 4.41 mmol), HMDS (75 ml), anhydrous *m*-xylene (25 mL), and ammonium sulfate (50 mg) was refluxed under argon overnight, concentrated to dryness, and dried under vacuum for 1 h. The residue together with **11** (2.0 g, 3.67 mmol) was dissolved in anhydrous 1,2-dichloroethane (80 mL), the resulting solution was cooled to 10 °C, and TMSOTf (1.3 ml, 7.3 mmol) in 1,2-dichloroethane (5 mL) was added. The solution was refluxed under argon for 3 days, poured into ice-water (50 mL) containing sodium bicarbonate (3 g), extracted with chloroform, and the combined organic layer was washed with water, dried over sodium sulfate, and concentrated. Chromatography on silica gel (ethyl acetate: hexanes, 2:3) gave 1.8 g (66%) of **28** as a colorless solid.

A mixture of **28** (750 mg 1.02 mmol) and zinc dust (0.53 g, 8.16 mmol) in acetic acid (25 mL) was stirred at room temperature for 4 h, then more zinc dust (0.53 g, 8.16 mmol) was added, and the mixture was stirred for another 7 h. The solid was filtered, and the filtrate was concentrated to dryness. After the usual work-up, the residue was dissolved in ammonia-saturated methanol (40 mL), and the resulting solution stood at ambient temperature overnight. Solvent was evaporated, and the resulting residue and sodium acetate (20 mg) in anhydrous DMF (20 mL) was stirred at 120°C for 5 h. After evaporation, the residue was purified by chromatography (4% methanol in ethyl acetate) to give 145 mg (77%, last two steps) of **1f** as a colorless solid: mp 165–167 °C; ^{1}H NMR (DMSO-d_6) δ 2.05–2.27 (m, 2H), 3.67–3.74 (m, 1H), 3.79 (m, 1H), 4.16 (m, 1H), 4.42 (dd, J = 12.0 and 5.7 Hz, 1H), 4.97–5.09 (m, 2H), 5.17 (d, J = 4.5 Hz, 1H, OH), 5.39 (d, J = 6.6 Hz, 1H, OH), 5.46 (d, J = 4.8 Hz, 1H, OH), 5.76–5.92 (m, 1H), 5.97 (d, 1H, J = 6.9 Hz), 6.92 (br s, 2 H, NH_2), 8.19 (s, 1H), 8.42 (s, 1H). Anal. calcd for $C_{15}H_{17}N_5O_4$: C, 54.38; H, 5.17; N, 21.14. Found: C, 54.37; H, 5.04; N, 21.08.

4-Amino-5-cyano-7-(5(*R*)-*C*-ethynyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (1c) and 4-amino-5-cyano-7-(5(*S*)-*C*-ethynyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (2b). Prepared as a mixture (1:1) from **12** by the same procedure as described for **1f**. Yield: 62% (3 steps). The two isomers were partially separated by a flash chromatography on silica (4% methanol in ethyl acetate). **1c** (5′(*R*)-isomer): ^{1}H NMR (DMSO-d_6) δ 3.42 (s, 1H), 3.91 (d, J = 4.2 Hz, 1H), 4.16 (t, J = 4.5 Hz, 1H) 4.40–4.45 (m, 2H), 5.36 (d, J = 4.2 Hz, 1H), 5.45 (d, J = 6.6 Hz, 1H), 6.07 (d, J = 7.5 Hz, 1H), 6.24 (d, J = 5.4 Hz, 1H), 6.95 (br s, 2H), 8.21 (s, 1H), 8.34 (s, 1H). **2b** (5′(*S*)-isomer): ^{1}H NMR (DMSO-d_6) δ 3.39 (s, 1H), 3.90 (m, 1H), 4.10 (m, 1H) 4.39–4.45 (m, 2H), 5.34 (d, J = 4.8 Hz, 1H), 5.50 (d, J = 6.6 Hz, 1H), 6.09 (m,), 6.95 (br, s, 2H), 8.22 (s, 1H), 8.38 (s, 1H). Anal. calcd for $C_{14}H_{13}N_5O_4$: C, 53.33; H, 4.16; N, 22.21. Found: C, 53.04; H, 4.06; N, 22.15.

4-Amino-5-cyano-7-(5(*R*)-*C*-vinyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (1d) and 4-amino-5-cyano-7-(5(*S*)-*C*-vinyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (2c). Prepared as a mixture (1:1) from **13** by the same procedure as described for **1f**. Chromatography (3% methanol in ethyl acetate) gave **1d** (17%, 3 steps) and **2c** (12%, 3 steps), both as a colorless solid. Compound **1d** was also prepared starting from **7a** in 20% yield (3 steps). **1d** (5′(*R*)-isomer): mp 228–230 °C (recrystallized from water); ^{1}H NMR (DMSO-d_6) δ 3.90 (m, 1H), 4.04 (m, 1H), 4.23 (m, 1H), 4.46 (m, 1H), 5.15 (d, J = 10.8 Hz, 1H), 5.20 (d, J = 4.2 Hz, 1H, OH), 5.34 (d, J = 17.1 Hz, 1H), 5.40 (d, J = 6.6 Hz, 1H, OH), 5.83–5.94 (m, 1H,), 5.87 (d, J = 3.3 Hz, 1H, OH), 6.01 (d, J = 7.5 Hz,

1H), 6.96 (br s, 2H, NH_2), 8.21 (s, 1H), 8.43 (s, 1H). Anal. calcd for $C_{14}H_{15}N_5O_4$: C, 52.99; H, 4.76; N, 22.07. Found: C, 53.02; H, 4.69; N, 21.88. **2c** (5′(*S*)-isomer): mp 165–167 °C (recrystallized from methanol and water); ^{1}H NMR (DMSO-d_6) δ 3.90 (t, J = 3.0 Hz, 1H), 4.10 (dd, J = 8.4 and 3.6 Hz, 1H), 4.18 (m, 1H), 4.34 (dd, J = 11.1 and 4.8 Hz, 1H), 5.08 (dt, J = 9.0 and 1.5 Hz, 1H) 5.20 (d, J = 4.8 Hz, 1H, OH), 5.27 (dt, J = 15.3 and 1.8 Hz, 1H), 5.45 (d, J = 6.0 Hz, 1H, OH), 5.62 (d, J = 6.3 Hz, 1H, OH), 5.85–5.98 (m, 1H,), 6.00 (d, J = 6.0 Hz, 1H), 6.92 (br s, 2H, NH_2), 8.21 (s, 1H), 8.44 (s, 1H). Anal. calcd for $C_{14}H_{15}N_5O_4$: C, 52.99; H, 4.76; N, 22.07. Found: C, 53.08; H, 4.67; N, 21.91.

4-Amino-5-cyano-7-(5(*R*)-*C*-ethyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (1e) and 4-amino-5-cyano-7-(5(*S*)-*C*-ethyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (2d). A mixture of **1c** and **2b** (1:1, 150 mg, 0.476 mmol) and 10% Pd/C (100 mg) in methanol (50 mL) was shaken in a hydrogenation apparatus (20 psi hydrogen) for 3 h. The catalysts were filtered and washed with methanol, and the filtrate was concentrated to dryness. Chromatography (4% methanol in ethyl acetate) gave 60 mg (39%) of **1e** and 70 mg (46%) of **2d**, both as a colorless solid. **1e** (5′(*R*)-isomer): mp 187–188 °C (recrystallized from methanol); ^{1}H NMR (DMSO-d_6) δ 0.89 (t, J = 7.2 Hz, 3H), 1.20–1.50 (m, 2H), 3.54 (m, 1H) 3.77 (dd, J = 4.2 and 2.4 Hz, 1H), 4.12 (m, 1H), 4.41 (m, 1H), 5.16 (d, J = 4.2 Hz, 1H, OH), 5.33 (d, J = 4.8 Hz, 1H, OH), 5.38 (d, J = 5.7 Hz, 1H, OH), 5.97 (d, J = 7.2 Hz, 1H,), 6.91 (bs, 2H, NH_2), 8.19 (s, 1H), 8.43 (s, 1H). Anal. calcd for $C_{14}H_{17}N_5O_4$: C, 52.66; H, 5.37; N, 21.93. Found: C, 52.84; H, 5.38; N, 21.99. **2d** (5′(*S*)-isomer): mp 163–165 °C (methanol); ^{1}H NMR (DMSO-d_6) δ 0.88 (t, J = 7.8 Hz, 3H), 1.45 (m, 2H), 3.48 (m, 1H) 3.86 (t, J = 3.3 Hz, 1H), 4.08 (dd, J = 8.7 and 3.9 Hz, 1H), 4.30 (dd, J = 11.4 and 5.7 Hz, 1H), 5.12 (d, J = 4.8 Hz, 1H, OH), 5.20 (d, J = 6.6 Hz, 1H, OH), 5.44 (d, J = 6.0 Hz, 1H, OH), 6.02 (d, J = 5.4 Hz, 1H), 6.91 (br s, 2H, NH_2), 8.20 (s, 1H), 8.46 (s, 1H). Anal. calcd for $C_{14}H_{17}N_5O_4$: C, 52.66; H, 5.37; N, 21.93. Found: C, 52.58; H, 5.35; N, 21.77.

4-Amino-5-cyano-7-(4-*C*-hydroxymethyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (3c). The same condensation procedure as described for **1f** gave 3.70 g of **35** as a colorless solid from **21** (4.32 g, 7.07 mmol).

A mixture of **35** (3.6 g, 4.41 mmol) and 10% Pd/C (500 mg) in anhydrous dioxane (150 mL) containing triethylamine (1.5 mL) was shaken in a hydrogenation apparatus (20 psi hydrogen) for 5 h. The catalysts were filtered and washed with chloroform, and the filtrate was concentrated to dryness. After the usual work-up, the residue was dissolved in ammonia-saturated methanol (200 mL), and the resulting solution stood at room temperature overnight. Solvent was evaporated, and the residue and sodium acetate (50 mg) were heated in DMF (50 mL) at 120 °C for 5 h and then concentrated to dryness. Chromatography (12% methanol in ethyl acetate) gave 1.17 g (54%, 3 steps) of **3c** as a colorless solid: mp 216–218 °C (recrystallized from methanol); ^{1}H NMR (DMSO-d_6) δ 3.55 (m, 4H) 4.14 (t, J = 4.8 Hz, 1H), 4.58 (m, 2H, including 1 OH), 5.17 (d, J = 5.1 Hz, 1H, OH), 5.21 (t, J = 5.7 Hz, 1H, OH), 5.36 (d, 1H, J = 7.2 Hz, OH), 6.06 (d, 1H, J = 7.2 Hz), 6.90 (br s, 2H, NH_2), 8.20 (s, 1H), 8.42 (s, 1H). Anal. calcd for $C_{13}H_{15}N_5O_5 \cdot H_2O$: C, 46.02; H, 5.05; N, 20.64. Found: C, 46.25; H, 4.69; N 20.67.

4-Amino-5-cyano-7-(5(*R*)-*C*-propyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (1g). A colorless solid was prepared from **1f** by the same hydrogenation and deprotection procedure as described for **3c**. Yield: 23% (3 steps): mp 184–186 °C (recrystallized from methanol); ^{1}H NMR (DMSO-d_6) δ 0.86 (t, J = 6.3 Hz, 3H), 1.20–1.50 (m, 4 H), 3.62 (m, 1H) 3.76 (m, 1H), 4.11 (br s, 1H), 4.41 (dd, J = 11.4 and 5.4 Hz, 1H), 5.15 (d, J = 4.2 Hz, 1H, OH), 5.32 (d, J = 4.8 Hz, 1H, OH), 5.37 (d, J = 6.3 Hz, 1H, OH), 5.96 (d, J = 6.9 Hz, 1H), 6.92 (br s, 2H, NH_2), 8.19 (s, 1H), 8.42 (s, 1H).

4-Amino-5-cyano-7-(4-*C*-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (3a). A colorless solid was prepared from **20** by the same procedure as described for **3c**. Yield: 69% (3 steps); mp 179–180 °C (recrystallized from water); ^{1}H NMR (DMSO-d_6) δ 1.28 (s, 3H, Me), 3.32–3.51 (m, 2H), 4.01 (t, J = 4.8 Hz, 1H), 4.60 (dd, J = 12.9 and 6.3 Hz, 1H), 5.14 (d, J = 4.8 Hz, 1H, OH), 5.32 (m, 2H, OH), 6.02 (d, J = 6.9 Hz, 1H), 6.91 (br s, 2H, NH_2), 8.20 (s, 1H), 8.44 (s, 1H). Anal. calcd for $C_{13}H_{15}N_5O_4$: C, 51.15; H, 4.95; N, 22.94. Found: C, 50.92; H, 4.85; N, 22.85.

4-Amino-5-cyano-7-(4-*C*-ethyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (3b). Prepared from **26** by the same procedure as described for **3c**. Yield: 62% (3 steps) as a colorless solid (recrystallized from water): mp 207–209 °C; ^{1}H NMR (DMS-d_6) δ 0.83 (t, J = 12.5 Hz, 3H, $C\underline{H}_3CH_2$), 1.63 (m, 2H, $CH_3C\underline{H}_2$), 3.47 (m, 2H), 4.02 (t, J = 4.8 Hz, 1H), 4.66 (dd, J = 12.9 and 7.2 Hz, 1H), 5.11 (d, J = 4.8 Hz, 1H, OH), 5.27 (m, 2H, OH), 6.00 (d, J = 7.8 Hz, 1H), 6.91 (br s, 2H, NH_2), 8.20 (s, 1H), 8.44 (s, 1H). Anal. calcd for $C_{14}H_{17}N_5O_4$: C, 52.66; H, 5.37; N, 21.93. Found: C, 52.53; H, 5.29; N, 21.77.

Assignment of the tritylation and silylation site of compound 14

The major tritylation product **15** was added to a stirred solution of NaH (2 equiv) in THF, followed by addition of MeI (1.2 equiv). The reaction mixture was stirred at room temperature overnight, cooled, and quenched with dilute acetic acid until pH 7 was reached. After the usual work-up, the crude was purified by a short chromatography (EtOAc/hexane). The resulting product was dissolved in cold TFA:water (9:1) and stirred at 0 °C for 3 h. Methanol was added, and the mixture was concentrated at 0 °C in vacuo. After a quick chromatography (0–5% methanol in methylene chloride), the product was dissolved in pyridine, and *p*-tosyl chloride (2 equiv) was added. The mixture was stirred at room temperature for 24 h, cooled to 0 °C, and quenched with water. After the usual work-up and purification by flash chromatography, the dried tosyl derivative (**36**) was added to a suspension of NaH (3 equiv) in THF. The

resulting mixture was stirred at 50 °C for 2 h. After the usual work-up, chromatography (20% acetone in methylene chloride) gave the 4-*C*,3-*O*-methylene ribofuranose derivative (**38**) and 4-*C*,2-*O*-methylene ribofuranose derivative (**39**) in a ratio of 1:2, both as colorless solid; ^{1}H NMR ($CDCl_3$) of **39**: δ 2.54 (d, J = 5.7 Hz, OH), 3.38 (s, 3H, OMe), 3.41 (s, 3 H, OMe), 3.73 (d, 1H, J = 7.8 Hz, 4′-H), 3.74 (s, 2H, 5-H), 3.98 (d, J = 7.8 Hz, 1H, 4′-H), 4.01 (s, 1H, 2-H), 4.23 (d, J = 5.4 Hz, 1H, 3-H), 4.77 (s, 1H, 1-H). ^{1}H NMR ($CDCl_3$) of **38**: δ 3.39 (s, 3H, OMe), 3.44 (s, 3H, OMe), 3.48, 3.55 (AB, J = 9.9 Hz, 2H, 5-H), 4.06 (dd, J = 1.5, 5.7 Hz, 1H, 2-H), 4.37 (d, J = 7.2 Hz, 1H, 4′-H), 4.84 (d, J = 7.2 Hz, 1H, 4′-H), 5.05 (d, J = 1.5 Hz, 1H, 1-H), 5.07 (d, J = 5.7 Hz, 1H, 3-H).

The minor tritylation product **23** was subjected to the same reactions as **15** to give **37**, which was intact after it was treated with sodium hydride in THF at 50 °C for 24 h.

MTS cytotoxicity assay

All compounds tested in vitro were dissolved in DMSO (100 mM solution) and subsequently diluted in the culture medium before treatment of the cultured cells. Tested cells were plated in 96-well plates at a density 4×10^3 cells/well/200 μL of the proper culture medium and treated with the compounds at concentration 0.78 to 100 μM. In parallel, the cells were treated with 0.1% of DMSO as control.

MTS Assay (Promega, G5430) was performed 72 h later according to instruction provided by Promega. This assay is based on the cellular conversion of the tetrazolium salt, MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt], into a formazan that is soluble in cell culture medium and is measured at 490 nm directly in 96-well assay plates without additional processing. Absorbance is directly proportional to the number of living cells in culture. Three types of cells were used in these studies: 1. normal human dermal fibroblasts (NHF) from Clonetics, Inc., #CC-2509, cultivated in FGM Medium provided by Clonetics, #CC-3132; 2. human prostate cancer cells DU-145 (HTB-81) provided by ATCC and cultivated in MEM (ICN, #1210254) supplemented with 10% fetal bovine serum (Hyclone, #SH30070.03) and 2 mM of L-glutamine (ICN, #1680149); and 3. mouse melanoma B16 from ATCC cultivated in the same type of medium as HTB-81 cells.

References and Notes

1. Revankar, G. R.; Robins, R. K. In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum: New York, 1991; Vol. 2, pp 161–398.
2. De Clercq, E.; Balzarini, J.; Madej, D.; Hansske, F.; Robins, M. J. *J. Med. Chem.* **1987**, *30*, 481–486.
3. Gupta, P. K.; Nassiri, M. R.; Coleman, L. A.; Wotring, L. L.; Drach, J. C.; Townsend, L. B. *J. Med. Chem.* **1989**, *32*, 402.
4. Bobek, M. and Bloch, A. *Nucleosides Nucleotides* **1994**, *13*, 429.
5. Renau, T. E.; Lee, J. S.; Kim, H.; Young, C. G.; Wotring, L. L.; Townsend, L. B.; Drach, J. C. *Biochem. Pharmacol.* **1994**, *48*, 801.
6. Bhattacharya, B. K.; Rao, T. S.; Revankar, G. R. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1543.
7. Krawczyk, S. H.; Nassiri, M. R.; Kucera, L. S.; Kern, E. R.; Ptak, R. G.; Wotring, L. L.; Drach, J. C.; Townsend, L. B. *J. Med. Chem.* **1995**, *38*, 4106.
8. Krawczyk, S. H.; Renau, T. E.; Nassiri, M. R.; Westerman, A. C.; Wotring, L. L.; Drach, J. C.; Townsend, L. B. *J. Med. Chem.* **1995**, *38*, 4115.
9. Finch, R. A.; Revankar, G. R.; Chan, P. K. *Anti-Cancer Drug Des.* **1997**, *12*, 205.
10. Shuto, S.; Obara, T.; Kosugi, Y.; Saito, Y.; Toriya, M.; Yaginuma, S.; Shigeta, S.; Matsuda, A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 605.
11. Murai, Y.; Shitoto, H.; Ishizaki, T.; Limori, T.; Kodama, Y.; Ohtsuka, Y.; Oishi, T. *Heterocycles* **1992**, *33*, 391.
12. Sharma, M.; Li, Y. X.; Ledvina, M.; Bobek, M. *Nucleosides Nucleotides* **1995**, *14*, 1831.
13. Huang, B. G.; Bober, M. *Carbohydr. Res.* **1998**, *308*, 319.
14. Hattori, H.; Nozawa, E.; Iino, T.; Yoshimura, Y.; Shuto, S.; Shimamoto, Y.; Nomura, M.; Fukushima, M.; Tanaka, M.; Sasaki, T.; Matsuda, A. *J. Med. Chem.* **1998**, *41*, 2892.
15. Takatori, S.; Kanda, H.; Takenaka, K.; Wataya, Y.; Matsuda, A.; Fukushima, M.; Shimamoto, Y.; Tanaka, M.; Sasaki, T. *Cancer Chemother. Pharmacol.* **1999**, *44*, 97.
16. Sharma, M.; Bloch, A.; Bobek, M. *Nucleosides Nucleotides* **1993**, *12*, 643.
17. Porcari, A. R.; Townsend, L. B. *Nucleosides Nucleotides* **1999**, *18*, 153.
18. Danishefsky, S.; DeNinno, M. P.; Phillips, G. B.; Zelle, R. E.; Lartey, P. A. *Tetrahedron* **1986**, *42*, 2809.
19. Jones, G. H.; Moffatt, J. G. In *Methods in Carbohydrate Chemistry*; Whistler, R. L.; Moffat, J. L., Eds.; Academic Press: New York, 1972; pp 315–322.
20. Brown, C. A.; Ahuja, V. K. *J. Chem. Soc., Chem. Commun.* **1973**, 553.
21. Kato, K.; Chen, C. Y.; Akita, H. *Synthesis* **1998**, 1527.
22. Robins, M. J.; Wilson, J. S. In *Nucleic Acid Chemistry*; Townsend, L. B.; Tipson, R. S., Eds.; John Wiley and Sons: New York, 1991; Part 4, pp 194–200.
23. Chatgilialoglu, C.; Driller, D.; Lesage, M. *J. Org. Chem.* **1988**, *53*, 3641.
24. Wang, G.; Tam, R.; Gunic, E.; Du, J.; Bard, J.; Pai, B. *J. Med. Chem.* **2000**, *43*, 2566.
25. Tolman, R. L.; Robins, R. K.; Townsend, L. B. *J. Am. Chem. Soc.* **1969**, *91*, 2102.
26. Renau, T. E.; Wotring, L. L.; Drach, J. C.; Townsend, L. B. *J. Med. Chem.* **1996**, *39*, 873.
27. Wang, G.; Seifert, W. E. *Tetrahedron Lett.* **1996**, *37*, 6515.
28. Nomura, M.; Shuto, S.; Tanaka, M.; Sasaki, T.; Mori, S.; Shigeta, S.; Matsuda, A. *J. Med. Chem.* **1999**, *42*, 2901.
29. Obika, S.; Hari, Y.; Morio, K-i; Imanishi, T. *Tetrahetron Lett.* **2000**, *41*, 215.