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Carbohydrate Research 308 (1998) 319–328

Synthesis and in vitro antitumor activity of some amino-deoxy 7-hexofuranosylpyrrolo[2,3-*d*] pyrimidines

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Received 20 January 1998; accepted 30 March 1998

Abstract

7-(6-amino-6-deoxy-β-D-glucofuranosyl)-5-cyanopyrrolo[2,3-*d*]pyrimidine (**22**) and 7-(3-aminomethyl-3-deoxy-β-D-allofuranosyl)-5-cyanopyrrolo[2,3-*d*]pyrimidine (**28**) were synthesized by sequentially coupling silylated 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine with the corresponding protected sugars **9** and **17**, followed by deblocking and catalytic hydrogenation. Conversion of the 5-nitrile in **22** and **28** into a carboxamide gave the corresponding sangivamycin derivatives **23** and **29**. Whereas 5′-aminomethyl nucleosides **22** and **23** inhibited the growth of four different human tumor cell lines at μM concentrations, the 3′-aminomethyl analogs **28** and **29** were much less active against these cells.

Keywords: 3′-Branched-3′-deoxy nucleosides; Aminomethyl nucleosides; Pyrrolo[2,3-*d*]pyrimidines; Synthesis; Antitumor activity

1. Introduction

Inhibition of signal-transducing protein kinases has recently emerged as a promising approach to anticancer design. As part of our effort to develop ATP-competitive inhibitors of such kinases, we prepared a series of analoges of sangivamycin (**1a**) and toyocamycin (**1b**) that were resistant to phosphorylation and found that substituting certain groups for the 5′-hydroxyl in these nucleosides gave rise to more potent inhibitors [1,2] of protein kinase C (PKC) and/or protein kinase A (PKA). Because, among these agents, 5′-amino-5′-deoxysangivamycin (**2a**) inhibited PKC more potently than **1a**, and because of the suggested presence of an amine-binding site at the ATP-binding domain of PKC [3], it was of interest to examine the effect on biological activity of the aminomethyl functionality attached to other positions of **1a** and/or **1b**. In this report, we describe the synthesis of 3′- and 5′-aminomethyl analogs of **1a** and **1b**. Taking into consideration that xylofuranosyl sangivamycin (**2b**) was a better inhibitor [4] of PKC than (**1a**), we incorporated this structural feature into the design of the 5′-aminomethyl analogs.

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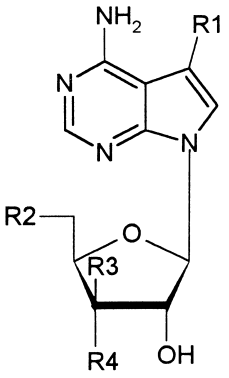
0008-6215/98/$19.00
PII S0008-6215(98)00098-6



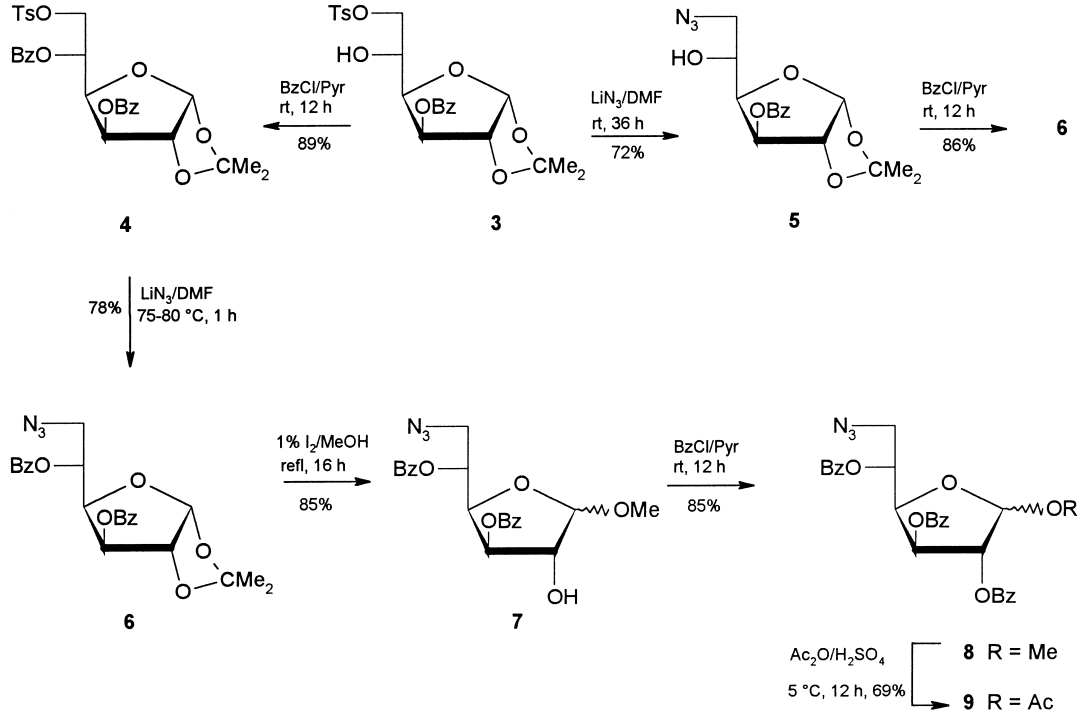
1a: R1 = $CONH_2$, R2 = OH, R3 = H, R4 = OH
1b: R1 = CN, R2 = OH, R3 = H, R4 = OH
2a: R1 = $CONH_2$, R2 = NH_2, R3 = H, R4 = OH
2b: R1 = $CONH_2$, R2 = OH, R3 = OH, R4 = H

2. Results and discussion

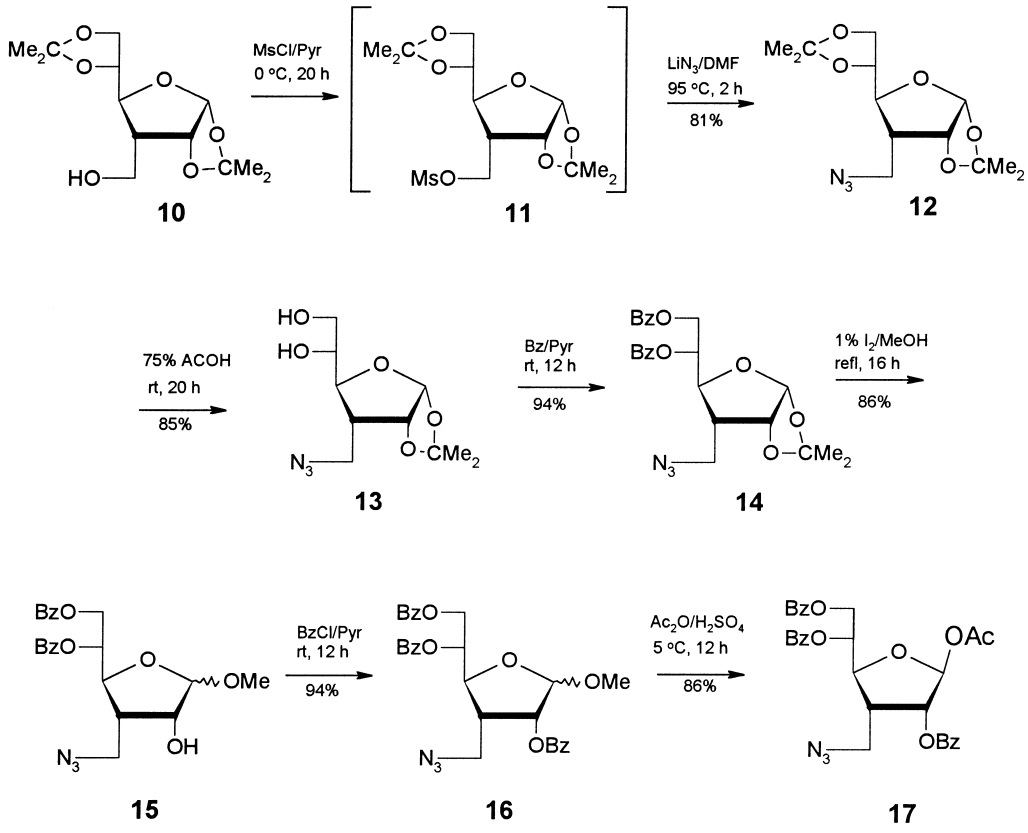
For the synthesis of the target 7-glycosylated pyrrolo[2,3-*d*]pyrimidine nucleosides, we chose the coupling [5] of the silylated 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine with the appropriate blocked sugar in the presence of trimethylsilyl trifluoromethanesulfonate (Me_3SiOTf) [6], which involved the preparation of 1-*O*-acetyl-6-azido-2,3,5-tri-*O*-benzoyl-6-deoxy-α,β-D-glucofuranose (**9**) (Scheme 1) and 1-*O*-acetyl-3-azidomethyl-2,5,6-tri-*O*-benzoyl-3-deoxy-β-D-allofuranose (**17**) (Scheme 2).

Thus, benzoylation of 3-*O*-benzoyl-1,2-*O*-isopropylidene-6-*O*-*p*-toluenesulfonyl-α-D-glucofuranose (**3**) [7] (Scheme 1) furnished the dibenzoyl derivative **4** [8], which gave the 6-azido-6-deoxy derivative **6** [9] on treatment with lithium azide in DMF. Alternatively, **6** was prepared by treating **3** with lithium azide in DMF at room temperature to give **5** [7], followed by benzoylation. Compound **6** had previously been prepared by benzoylation of 6-azido-6-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose and purified by silica gel chromatography in benzene–EtOH and crystallization from EtOH [9]. In our hands, silica gel chromatography of **6** in petroleum ether–EtOAc gave a sufficiently pure product to be used in the subsequent methanolysis step. In the present study, we found it more convenient to use 1% iodine in methanol [10] in place of methanolic HCl followed by neutralization with solid lead carbonate [9] to convert **6** into an anomeric mixture of methyl glucofuranosides **7** [9], which afforded the sugar acetates **9** by benzoylation (to give **8**), followed by acetolysis.

The 3-azidomethyl-3-deoxy derivative **17** was prepared in several steps starting from 3-*C*-hydroxy-



Scheme 1.

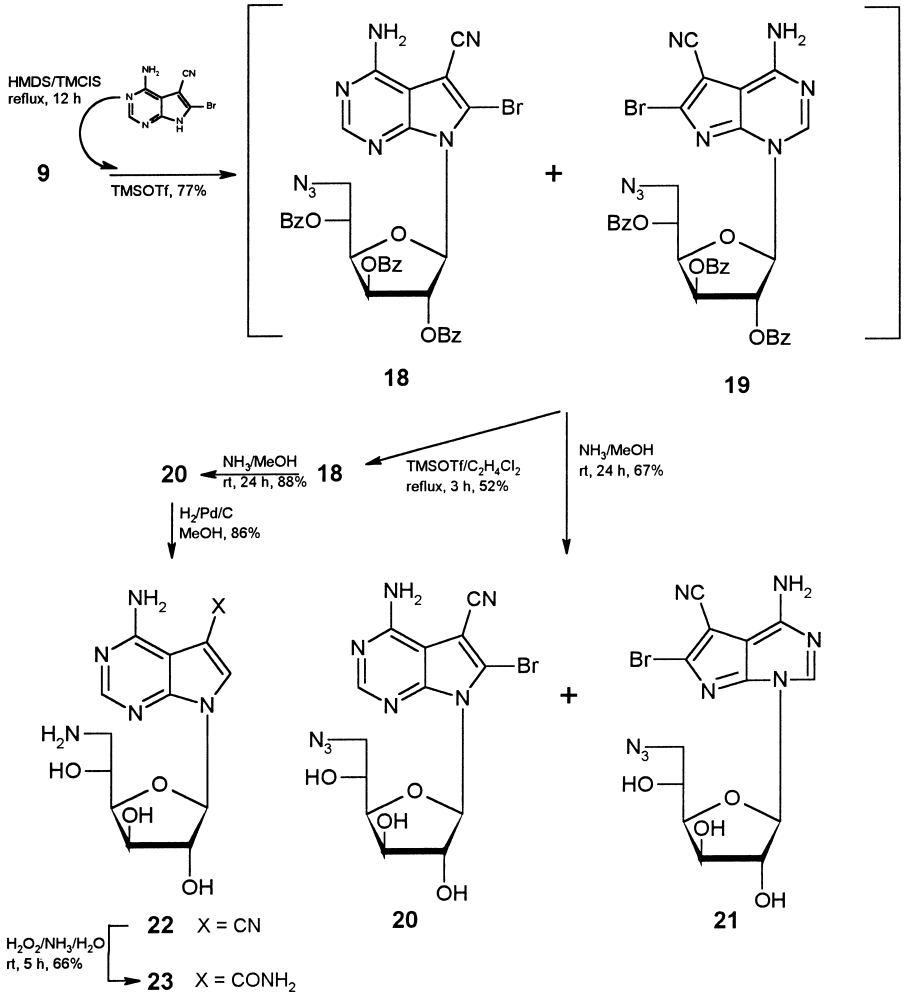


Scheme 2.

methyl-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**10**) [11] (Scheme 2). Treatment of **10** with methanesulfonyl chloride in pyridine gave 3-deoxy-1,2:5,6-di-*O*-isopropylidene-3-*C*-methanesulfonyloxymethyl-α-D-allofuranose (**11**) that was converted to 3-azidomethyl-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**12**) by displacement of the methanesulfonyloxy group with lithium azide in DMF. Selective deblocking [12] of the 5,6-*O*-isopropylidene group in **12** gave **13**, which was benzoylated with benzoyl chloride in pyridine to furnish a 5,6-di-*O*-benzoyl derivative **14**. Methanolysis of **14**, followed by benzoylation of the intermediate anomeric mixture of methyl allosides **15**, gave methyl 3-*C*-azidomethyl-2,5,6-tri-*O*-benzoyl-3-deoxy-α,β-D-allofuranoside (**16**), and acetolysis of **16** with acetic anhydride and sulfuric acid afforded an azidomethyl sugar **17**. The structure of **17**, which was isolated as a single β-anomer, was confirmed by its ^{1}H NMR spectrum where the signal for the anomeric proton appeared as a singlet at δ 6.25.

Coupling of **9** with silylated 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine [13,14] (Scheme 3) in 1,2-dichloroethane and in the presence of Me_3SiOTf yielded an inseparable mixture (77% yield) of N-7 and N-1 nucleosides **18** and **19**, respectively. Treatment of this mixture with Me_3SiOTf in 1,2-dichloroethane at reflux temperature resulted in an isomerization of **19** to give **18** as a single product in 52% yield. Compound **18** and the mixture of **18** and **19** were deblocked with methanolic ammonia to afford **20** and a mixture of nucleosides **20** and **21**, which was separated by silica gel chromatography. Debromination of **20** by catalytic hydrogenation over 10% Pd/C was accompanied by the reduction of the azido group to give an amino nucleoside **22**. Treatment of **22** with hydrogen peroxide in concentrated ammonium hydroxide produced a 5′-aminomethyl analog of sangivamycin **23**.

Similarly, coupling of 3-azidomethyl-3-deoxy sugar **17** with the silylated pyrrolo[2,3-*d*]pyrimidine (Scheme 4) afforded an inseparable mixture of **24** and its N-1 isomer **25** in 68% yield. Treatment of this mixture with Me_3SiOTf in 1,2-dichloroethane at reflux temperature gave **24** as the sole product, which was deprotected by treatment with methanolic ammonia to provide **26**. Similar deprotection of the mixture of **24** and **25** gave a mixture of **26**

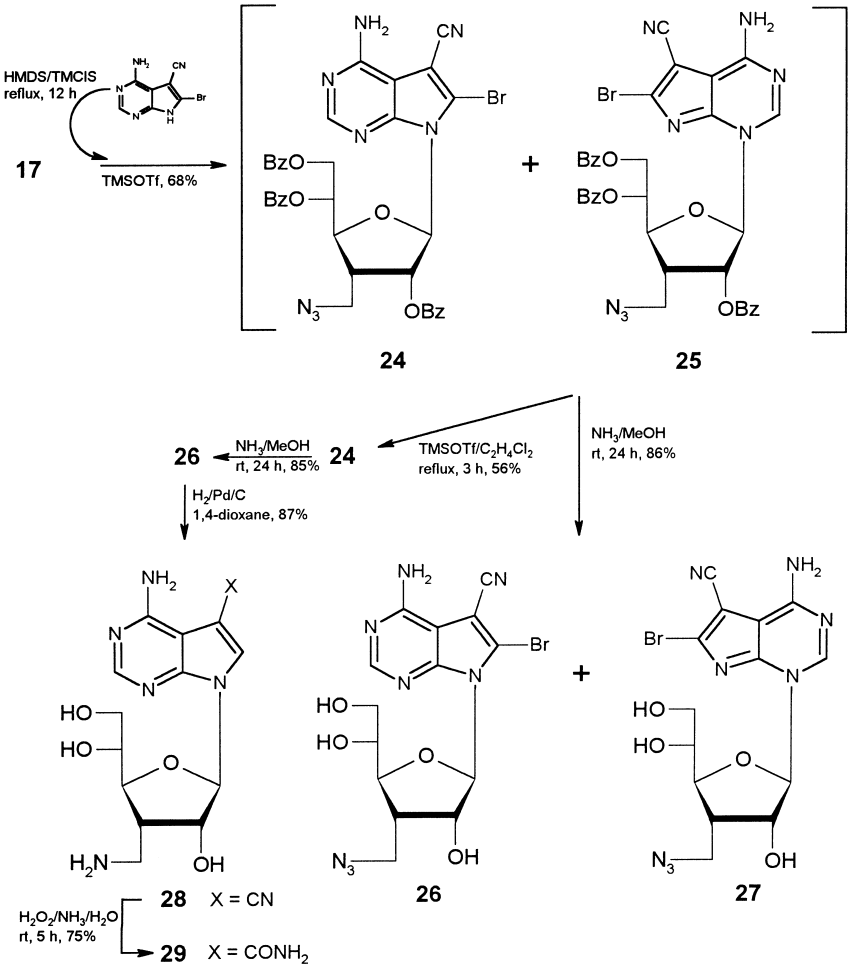


Scheme 3.

and **27** that was separated by chromatography. Catalytic hydrogenation converted **26** to a branched-chain aminomethyl analog of toyocamycin **28**, which when treated with hydrogen peroxide in concentrated ammonium hydroxide, gave the corresponding sangivamycin analog **29**.

Assignment of the site of ribosylation in **20** and **26** as N-7, and in **21** and **27** as N-1, was made on the basis of their ultraviolet spectra [14,15]. The anomeric configuration of the newly prepared nucleosides was assigned as β-based on the basis of ^{1}H NMR studies. In the 5′-aminomethyl series of nucleosides, a singlet corresponding to the anomeric proton was observed in the ^{1}H NMR spectra of **21**, **22** and **23**. For the 3′-aminomethyl nucleoside **29**, a doublet appeared at δ 6.02 with a coupling constant $J_{1'2'}$ 2.43 Hz, which is within the acceptable limits for β-ribonucleosides as predicted by the Karplus equation [16]. This assignment was in harmony with stereocontrol exercised by the 2′-OBz group in the glycosylation reaction [17] under Lewis acid conditions and with the results of our previous studies [5,15]. The IR spectra confirmed the presence of the azido and/or nitrile groups in the sugar precursors and in the target nucleosides by the sharp absorbance observed in the 2100–2150 and 2250–2280 cm^{-1} regions, respectively.

The newly synthesized compounds were evaluated for their in vitro anticancer activity by growth-inhibition studies using four human tumor-cell lines: human ovarian A121, human lung NSCLC A549, human colon HT-29, and human breast MCF7. As shown in Table 1, the 7-(6-amino-6-deoxy-β-D-glucofuranosyl)pyrrolo[2,3-*d*]-pyrimidines (**22**, **23**) showed inhibitory activity against these cell lines ranging from 3.9 to 11.5 μM; the other compounds tested were much less active. In contrast, sangivamycin itself inhibited cell growth at 0.006 μM. That greater inhibition



Scheme 4.

Table 1
The effect of newly synthesized aminonucleosides on the growth of some tumor cells in vitro during continuous exposure [a]

	IC_{50} [μM]			
Compound	A121	A549	HT-29	MCF7
22	3.9	4.8	5.0	4.0
23	4.2	11.5	7.8	8.5
26	33	53	154	59
28	> 100	> 100	> 100	> 100
29	51	NT [b]	67	64
Sangivamycin	0.004	0.006	0.009	0.005

[a] The tumor cells were maintained in complete RPMI 1640 medium. Cell growth inhibition was determined, and these results are the average of at least two separate determinations performed with duplicate cultures.
[b] Not tested.

derived from the fact that phosphorylation in the intact cell allows this compound to interfere with nucleic acids as well as with other metabolic sites [18,19]. The effects of the newly prepared compounds on PKA, PKC, and cyclin-dependent kinase activity will be determined and reported elsewhere.

3. Experimental

General methods.—Thin-layer chromatography was performed on EM Separations silica gel aluminum-backed plates F254. Solvent A: 3:1 petroleum ether (PE)–EtOAc, solvent B: 1:1 PE–EtOAc, solvent C: 20:2:2:1 EtOAc–EtOH–Me_2O–H_2O, solvent D: 8:2:1 MeOH–EtOAc–concd NH_3–H_2O. Column chromatography was carried out on silica gel (230–400 mesh) from E. Merck Co. Melting points were taken on a Mel-Temp capillary point block and are uncorrected. Elemental analyses were performed by Robertson Laboratories, Madison, NJ. ^{1}H NMR spectra were recorded on either a Varian 390 (90 MHz) or a Bruker AM 400 (400 MHz) spectrometer. IR spectra were recorded

on Perkin–Elmer Models 457 and 710B spectrometers. Evaporations were carried out under reduced pressure at water bath temperature below 35 °C.

3,5-Di-O-*benzoyl-1,2*-O-*isopropylidene-6*-O-p-*toluenesulfonyl*-α-D-*glucofuranose* (**4**).—To an ice-cooled solution of **3** [7] (52.58 g, 0.11 mol) in CH_2Cl_2 (400 mL) and pyridine (44 mL) was added dropwise benzoyl chloride (15.6 mL, 0.13 mol), and the mixture was stirred at rt overnight and then poured into a vigorously stirred mixture of ice–water (300 mL) and $NaHCO_3$ (21.8 g). The organic layer was washed with H_2O (3×200 mL), dried ($MgSO_4$), evaporated, and chromatographed to give **4** (57.3 g, 89%); R_f 0.36 (solvent A). ^{1}H NMR ($CDCl_3$) δ 1.23, 1.46 (2 s, 6 H, CMe_2), 2.20 (s, 3 H, CH_3), 4.33–4.69 (m, 4 H), 5.34–5.43 (m, 2 H), 5.83 (d, 1 H, H-1, *J* 3.0 Hz), 7.0–7.86 (m, 14 H, aromatic); IR (neat) ν 1780 (C = O) cm^{-1}.

6-Azido-3-O-*benzoyl-6-deoxy-1,2*-O-*isopropylidene*-α-D-*glucofuranose* (**5**).—A mixture of **3** (3.60 g, 7.53 mmol) and LiN_3 (3.69 g, 75.4 mmol) in DMF (40 mL) was stirred at rt for 36 h and evaporated. The residue was triturated with ether (50 mL), and the ether solution was washed with H_2O (3×20 mL), dried ($MgSO_4$), evaporated, and chromatographed to give **5** as a syrup (1.88 g, 72%); R_f 0.55 (solvent A). ^{1}H NMR ($CDCl_3$) δ 1.27, 1.49 (2 s, 6 H, CMe_2), 3.30–3.43 (m, 3 H, H-6, OH), 3.60–4.30 (m, 2 H, H-4,5), 4.59 (d, 1 H, *J* 3.0 Hz, H-2), 5.46 (d, 1 H, H-3), 5.86 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 7.30–7.53 (m, 3 H, aromatic), 7.89–8.05 (m, 2 H, aromatic); lit. [7] ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.43, 1.57 (2 s, 6 H, CMe_2), 3.43–3.6 (m, H-6, OH), 4.7 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-2), 5.52 (d, 1 H, $J_{3,4}$ 2.5 Hz, H-3), 5.97 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1); IR (neat) ν 3200–3600 (OH), 2160 (N_3), 1760 (C = O) cm^{-1}.

6-Azido-3,5-di-O-*benzoyl-6-deoxy-1,2*-O-*isopropylidene*-α-D-*glucofuranose* (**6**).—*Method A:* A mixture of **4** (56.7 g, 0.097 mol) and LiN_3 (21.2 g, 0.43 mol) in DMF (250 mL) was stirred at 75–80°C for 1 h and evaporated. The residue was sequentially taken up into ether (250 mL), washed with H_2O (3×100 mL), dried ($MgSO_4$), evaporated, and chromatographed to yield **6** as a yellow syrup (34.6 g, 78.3%); R_f 0.68 (solvent A). ^{1}H NMR ($CDCl_3$) δ 1.26, 1.51 (2 s, 6 H, CMe_2), 3.66 (m, 2 H, H-6), 4.50–4.73 (m, 2 H, H-2,4), 5.34–5.50 (m, 2 H, H-3,5), 5.87 (d, 1 H, H-1, *J* 3.0 Hz), 7.20–7.46 (m, 6 H, aromatic), 7.76–7.89 (m, 4 H, aromatic); lit. [9] ^{1}H NMR ($CDCl_3$) δ 1.34 and 1.61 (2 s, CMe_2), 5.98 (d, 1 H, H-1, *J* 3.5 Hz); IR (neat) ν 2150 (N_3), 1750 (C = O) cm^{-1}.

Method B. To an ice-cooled solution of **5** (2.20 g, 6.30 mol) in CH_2Cl_2 (25 mL) and pyridine (2.50 mL) was added dropwise benzoyl chloride (0.95 mL, 7.92 mmol), and the mixture was stirred at rt overnight and then poured into a vigorously stirred mixture of ice–water (25 mL) and $NaHCO_3$ (1.40 g). The organic phase was washed with H_2O (3×20 mL), dried ($MgSO_4$), evaporated, and chromatographed to give **6** (2.45 g, 86%), which was identical by TLC and NMR spectrum with the product of Method A.

Methyl 6-azido-3,5-di-O-*benzoyl-6-deoxy*-α,β-D-*glucofuranoside* (**7**).—A solution of **6** (34.6 g, 0.076 mol) in 1% I_2 in MeOH (400 mL) was heated at reflux for 16 h. The solution was then concentrated to half of the volume and poured into a stirred aqueous solution of $Na_2S_2O_3$ (1%, 600 mL). The mixture was extracted with ethyl acetate (3×200 mL), and the extract was washed with H_2O (3×200 mL), dried ($MgSO_4$), evaporated and chromatographed to give **7** (27.7 g, 85%); R_f 0.24 (solvent A). ^{1}H NMR ($CDCl_3$) β:α ~2:1, δ 3.35 (s, 3 H, OCH_3), 3.66 (m, 2 H, H-6), 4.25 (s, 1 H, OH), 4.87 (m, 1 H), 5.17–5.51 (m, 4 H), 7.15–7.41 (m, 6 H, aromatic), 7.73–7.80 (m, 4 H, aromatic); IR (neat) ν 3200–3600 (OH), 2180 (N_3), 1770 (C = O) cm^{-1}.

Methyl 6-azido-2,3,5-tri-O-*benzoyl-6-deoxy*-α,β-D-*glucofuranoside* (**8**).—The title compounds were prepared by benzoylation of **7** as previously reported [9]: R_f 0.64 (solvent A); ^{1}H NMR ($CDCl_3$) δ 3.46 (s, 3 H, OCH_3), 3.76–4.86 (m, 3 H, H-5,6), 5.10–5.59 (m, 3 H, H-2,3,4), 5.83 (d, 1 H, H-1, *J* 6.0 Hz), 7.15–7.41 (m, 9 H, aromatic), 7.73–7.80 (m, 6 H, aromatic); IR (neat) ν 2160 (N_3), 1760 (C = O) cm^{-1}.

1-O-*Acetyl-6-azido-2,3,5-tri*-O-*benzoyl-6-deoxy*-α,β-D-*glucofuranose* (**9**).—To an ice-cooled solution of **8** (29.3 g, 0.055 mol) in CH_2Cl_2 (200 mL) and acetic anhydride (33.5 mL) was added dropwise sulfuric acid (1.35 mL), and the mixture was kept at 5 °C overnight. Sodium acetate (11 g) was added at rt, and the mixture was then stirred for 20 min, filtered, and the filtrate was evaporated. The residue was dissolved in ethyl acetate (200 mL), and the solution was washed with saturated $NaHCO_3$ solution (3×100 mL), H_2O (3×100 mL), dried ($MgSO_4$), evaporated, and chromatographed to provide **9** (21.3 g, 69%) as an oil which solidified on standing; R_f 0.52 (solvent A). ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3 H, CH_3), 3.73–3.86 (m, 2 H, H-6), 3.92–

5.10 (m, 1 H, H-5), 5.54–5.66 (m, 2 H, H-3,4), 5.86 (d, 1 H, H-2, *J* 4.5 Hz), 6.40 (d, 1 H, H-1, *J* 4.5 Hz), 7.26–7.50 (m, 9 H, aromatic), 7.76–8.07 (m, 6 H, aromatic); IR (neat) ν 2125 (N_3), 1750 (C=O) cm^{-1}. Anal. Calcd for $C_{29}H_{25}N_3O_9$ (559.5): C, 62.25; H, 4.47; N, 7.51. Found: C, 61.98; H, 4.43; N, 7.48.

3-Azidomethyl-3-deoxy-1,2,5,6-di-O-*isopropylidene*-α-D-*allofuranose* (**12**).—Methanesulfonyl chloride (27.5 g, 18.6 mL, 0.24 mol) was added dropwise at 0 °C to a solution of **10** [11] (25.5 g, 0.093 mol) in pyridine (350 mL), and the mixture was stirred at 0 °C for 20 h. The solvent was removed by evaporation, and the residue was dissolved in CH_2Cl_2 (500 mL). The solution was washed with saturated $NaHCO_3$ (3×200 mL), H_2O (3×200 mL), dried ($MgSO_4$), and evaporated to give a yellowish solid (**11**), which was used in the next step without purification. A solution of **11** and lithium azide (45.5 g, 0.93 mol) in DMF (600 mL) was stirred at 95 °C for 2 h and evaporated. The residue was sequentially dissolved in CH_2Cl_2 (500 mL), washed with H_2O (3×200 mL), dried ($MgSO_4$), and evaporated to give **12** (24.2 g, 81%). An analytical sample was obtained by chromatography of **12**; R_f 0.57 (solvent A). ^1H NMR ($CDCl_3$) δ 1.36, 1.41, 1.53 (3 s, 12 H, 2 CMe_2), 2.17 (m, 1 H, H-3), 3.53–4.13 (m, 6 H, H-4,5,6, CH_2), 4.73 (dd, 1 H, H-2, $J_{1,2}$ 3.0 Hz, $J_{2,3}$ 3.5 Hz), 5.74 (d, 1 H, H-1, *J* 3.0 Hz); IR (neat) ν 2150 (N_3) cm^{-1}. Anal. Calcd for $C_{13}H_{21}N_3O_5$ (299.3): C, 52.17; H, 7.02; N, 14.04. Found: C, 52.42; H, 7.02; N, 13.98.

3-Azidomethyl-3-deoxy-1,2-O-*isopropylidene*-α-D-*allofuranose* (**13**).—A solution of **12** (24.2 g, 0.08 mol) in 200 mL of 75% acetic acid was stirred at rt for 20 h and evaporated. The residue was co-evaporated with toluene (3×100 mL) and then chromatographed to give **13** (17.8 g, 85%); R_f 0.23 (solvent B). ^1H NMR ($CDCl_3$) δ 1.26, 1.45 (2 s, 6 H, CMe_2), 2.13 (m, 1 H, H-3), 3.07 (br s, 1 H, OH), 3.36 (br s, 1 H, OH), 3.61 (m, 6 H, H-4,5,6, CH_2), 4.63 (t, 1 H, H-2), 5.69 (d, 1 H, H-1, *J* 3.0 Hz); IR (neat) ν 3200–3600 (OH), 2150 (N_3) cm^{-1}. Anal. Calcd for $C_{10}H_{17}N_3O_5$ (259.3): C, 46.33; H, 6.56; N, 16.22. Found: C, 46.32; H, 6.62; N, 15.99.

3-Azidomethyl-5,6-di-O-*benzoyl-3-deoxy-1,2*-O-*isopropylidene*-α-D-*allofuranose* (**14**).—To a stirred and ice-cooled solution of **13** (11.83 g, 45.6 mmol) in CH_2Cl_2 (200 mL) and pyridine (13 mL) was added dropwise benzoyl chloride (11.7 mL, 0.10 mol), and the mixture was kept at rt overnight. It was then poured into a vigorously stirred mixture of ice–water (300 mL) and $NaHCO_3$ (16.8 g), and the organic phase was washed with H_2O (3×200 mL), dried ($MgSO_4$), and evaporated. The residue was chromatographed to give **14** (20.1 g, 94%), which was crystallized from petroleum ether–ether: mp 68–69 °C; R_f 0.48 (solvent A). ^1H NMR ($CDCl_3$) δ 1.38, 1.54 (2 s, 6 H, CMe_2), 2.40 (m, 1 H, H-3), 3.26 (m, 1 H), 4.16 (m, 2 H), 4.80 (m, 3 H), 5.53 (m, 1 H), 5.86 (d, 1 H, H-1, *J* 3.0 Hz), 7.46 (m, 6 H, aromatic), 8.0 (m, 4 H, aromatic); IR (neat) ν 2150 (N_3), 1720 (C=O) cm^{-1}. Anal. Calcd for $C_{24}H_{25}N_3O_7$ (467.5): C, 61.67; H, 5.35; N, 8.99. Found: C, 61.62; H, 5.36; N, 9.07.

Methyl 3-azidomethyl-5,6-di-O-*benzoyl-3-deoxy*-α,β-D-*allofuranoside* (**15**).—A solution of **14** (19.92 g, 42.6 mmol) in I_2–MeOH (1% w/v, 300 mL) was heated at reflux for 16 h and evaporated to half of the volume, and then poured into a stirred solution of $Na_2S_2O_3$ (1%, 450 mL). The mixture was extracted with ethyl acetate (3×200 mL), and the extract was washed with H_2O (3×200 mL), dried ($MgSO_4$), evaporated, and chromatographed to give **15** (16.1 g, 86%); R_f 0.21, (solvent A). ^1H NMR ($CDCl_3$) δ 2.56 (m, 1 H, H-3), 3.02 (s, 1 H, OH), 3.33 (s, 3 H, Me), 3.46 (m, 2 H), 4.20 (m, 2 H), 4.53–4.80 (m, 3 H), 5.41 (m, 1 H, H-1), 7.40 (m, 6 H, aromatic), 7.96 (m, 4 H, aromatic); IR (neat) ν 3200–3600 (OH), 2150 (N_3), 1750 (C=O) cm^{-1}. Anal. Calcd for $C_{22}H_{23}N_3O_7$ (441.4): C, 59.86; H, 5.21; N, 9.52. Found: C, 59.89; H, 5.22; N, 9.51.

Methyl 3-azidomethyl-2,5,6-tri-O-*benzoyl-3-deoxy*-α,β-D-*allofuranoside* (**16**).—To an ice-cooled solution of **15** (15.8 g, 35.8 mmol) in CH_2Cl_2 (100 mL) and pyridine (8 mL) was added dropwise benzoyl chloride (4.5 mL, 37.5 mmol), and the mixture was stirred at rt overnight, and then poured into a vigorously stirred mixture of ice–water (100 mL) and $NaHCO_3$ (6.6 g). The organic phase was washed with H_2O (3×100 mL), dried ($MgSO_4$), evaporated, and chromatographed to give **16** (18.3 g, 94%); R_f 0.48 (solvent A). ^1H NMR ($CDCl_3$) δ 3.0 (m, 1 H, H-3), 3.46 (s, 3 H, Me), 3.60 (m, 2 H, CH_2), 4.30–4.83 (m, 5 H), 5.46 (m, 1 H, H-1), 7.46 (m, 6 H, aromatic), 8.0 (m, 4 H, aromatic); IR (neat) ν 2150 (N_3), 1740 (C=O) cm^{-1}. Anal. Calcd for $C_{29}H_{27}N_3O_8 \cdot H_2O$ (563.5): C, 61.75; H, 4.79; N, 7.45. Found: C, 62.06; H, 4.81; N, 7.37.

1-O-*Acetyl-3-azidomethyl-2,5,6-tri*-O-*benzoyl-3-deoxy*-β-D-*allofuranose* (**17**).—To an ice-cooled solution of **16** (14.2 g, 26.0 mmol) in CH_2Cl_2

(150 mL) and acetic anhydride (15.6 mL) was added dropwise and with stirring sulfuric acid (0.63 mL), and the mixture was kept at 5 °C overnight. Sodium acetate (6.3 g) was added to the mixture with stirring, and after 20 min the mixture was filtered and evaporated. The residue was dissolved in ethyl acetate (200 mL), washed with saturated $NaHCO_3$ solution (3×100 mL), H_2O (3×100 mL), dried ($MgSO_4$), and evaporated. The residue was purified by chromatography to give **17** (12.9 g, 86%), which was then crystallized from petroleum ether–ether: mp 105–106 °C; R_f 0.43 (solvent A). ^1H NMR ($CDCl_3$) δ 2.0 (s, 3 H, COMe), 2.92 (m, 1 H, H-3), 3.56 (m, 2 H, CH_2), 4.33–4.83 (m, 3 H), 5.50 (m, 2 H), 6.25 (s, 1 H, H-1), 7.34 (m, 6 H, aromatic), 7.95 (m, 4 H, aromatic); IR (neat) ν 2130 (N_3), 1740 (C=O) cm^{-1}. Anal. Calcd for $C_{30}H_{27}N_3O_9$ (573.5): C, 62.83; H, 4.71; N, 7.33. Found: C, 62.64; H, 4.89; N, 7.33.

4-Amino-7-(6-azido-2,3,5-tri-O-*benzoyl-6-deoxy*-β-D-*glucofuranosyl)-6-bromo-5-cyanopyrrolo[2,3*-d*]pyrimidine* (**18**).—4-Amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (2.50 g, 10.5 mmol) was dried at 50 °C and 0.1 Torr for 3 h. The apparatus was flushed with N_2 and hexamethyldisilazane (HMDS, 35 mL), followed by chlorotrimethylsilane (0.5 mL), were added through a septum, and the mixture was heated at reflux for 12 h. The solution was evaporated, and the residue was co-evaporated with dry toluene (3×10 mL) at 0.1 mmHg to give a yellow solid. A solution of **9** (4.90 g, 8.54 mmol) in 1,2-dichloroethane (100 mL) was added to this solid, and the mixture was cooled (0 °C), and then trimethylsilyl trifluoromethanesulfonate (Me_3SiOTf) (2.03 mL, 10.5 mmol) in 1,2-dichloroethane (10 mL) was added dropwise. The mixture was sequentially stirred at 0 °C for 30 min, at rt for 1 h, at 50 °C overnight, and then at 75–80 °C for 3 h. It was then chilled, and diluted with CH_2Cl_2 (200 mL), and poured into a stirred mixture of ice–water and solid $NaHCO_3$ (4.0 g). The organic phase was washed with H_2O (3×100 mL), dried ($MgSO_4$), evaporated, and chromatographed to give a mixture of **18** and its N-1 isomer (**19**) (4.99 g, 79.2%). A portion of this mixture (0.74 g, 1.0 mmol) was co-evaporated with toluene (3×10 mL) and dried at 50 °C and 0.1 Torr for 3 h. HMDS (20 mL), xylene (20 mL), and chlorotrimethylsilane (50 μL) were added, and the mixture was heated at reflux for 12 h. The solvent was evaporated, and the residue was co-evaporated with toluene (3×20 mL) to give a crystalline residue of silylated nucleosides. To this residue were added 1,2-dichloroethane (20 mL), followed by Me_3SiOTf (0.39 mL, 2.0 mmol), and the mixture was heated at reflux for 3 h. Workup as above gave the N-7 isomer **18** (385 mg, 52%); R_f 0.47 (solvent B). ^1H NMR ($CDCl_3$) δ 3.65–4.10 (m, 2H), 5.0 (m, 1 H), 6.05 (m, 2 H), 6.33 (d, 1 H, H-1′), 6.80 (m, 1 H), 7.46 (m, 9 H, aromatic), 8.0 (m, 7 H, aromatic, H-2); IR (KBr) ν 3200–3600 (NH), 2280 (CN), 2150 (N_3), 1750 (C=O) cm^{-1}. Anal. Calcd for $C_{34}H_{25}BrN_8O_7$ (737.5): C, 55.37; H, 3.42; N, 15.19. Found: C, 55.36; H, 3.60; N, 14.78.

4-Amino-7-(6-azido-6-deoxy-β-D-*glucofuranosyl)-6-bromo-5-cyanopyrrolo[2,3*-d*]pyrimidine* (**20**).—*Method A*: A solution of **18** (770 mg, 1.04 mmol) in methanolic ammonia (35 mL) was kept at rt for 24 h and evaporated. The residue was triturated with ether (2×20 mL) and filtered, and the solid was dried and recrystallized from ethanol to give 390 mg (88%) of **20**; R_f 0.59 (solvent C); mp 205–206 °C, dec. ^1H NMR (Me_2SO-d_6) δ 3.20 (dd, 1 H, H-6a′, $J_{5',6a'}$ 6.26 Hz, $J_{6a',6b'}$ 12.85 Hz), 3.30 (dd, 1 H, H-6b′, $J_{5',6b'}$ 2.61 Hz, $J_{6a',6b'}$ 12.94 Hz), 3.90 (dd, 1 H, H-4′, $J_{4',5'}$ 3.61 Hz, $J_{4',3'}$ 8.37 Hz), 4.02–4.07 (m, 2 H, H-3′, 5′), 4.66 (dd, H-2′, $J_{1',2'}$ 3.12 Hz, $J_{2',3'}$ 1.50 Hz), 5.40 (bs, 1 H, OH), 5.71 (d, 1 H, H-1′, $J_{1',2'}$ 3.05 Hz), 5.95 (bs, 1 H, OH), 6.78 (d, 1 H, OH), 7.20 (bs, 2 H, NH_2), 8.22 (s, 1 H, H-2); IR (KBr) ν 3200–3600 (NH), 2250 (CN), 2120 (N_3) cm^{-1}; UV λ_{max} pH 1, 282, 234, EtOH, 286, 218, pH 11, 285, 215 nm. Anal. Calcd for $C_{13}H_{13}BrN_8O_4$ (425.1): C, 36.70; H, 3.08; N, 26.36. Found: C, 36.56; H, 2.96; N, 25.65.

Method B.—The mixture of **18** and **19** (7.7 g, 10.4 mmol) was kept in methanolic ammonia (350 mL) at rt for 24 h and evaporated. The residue was crystallized from water to give an isomeric mixture (3.0 g, 67%) of **20** and 4-amino-1-(6-azido-6-deoxy-β-D-glucofuranosyl)-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**21**). Recrystallization of this mixture from ethanol gave pure **20** (1.2 g). Evaporation of the mother liquor and chromatography of the residue gave **21** (1.8 g); R_f 0.65 (solvent C); mp 136–138 °C. ^1H NMR (Me_2SO-d_6) δ 4.02–4.30 (m, 3 H, H-2′,3′,5′), 5.52 (bs, 1 H, OH), 6.06 (s, 1 H, H-1′), 7.82 (bs, 2 H, NH_2), 8.41 (s, 1 H, H-2); UV λ_{max} pH 1, 289, 261, 221, EtOH, 285, 259, 215, pH 11, 285, 259, 214 nm. Anal. Calcd For $C_{13}H_{13}BrN_8O_4$ (425.1): C, 36.70; H, 3.08; N, 26.36. Found: C, 36.79; H, 2.92; N, 26.10.

4-Amino-7-(6-amino-6-deoxy-β-D-glucofuranosyl)-5-cyanopyrrolo[2,3-d]pyrimidine (**22**).—A suspension of **20** (160 mg, 0.38 mmol) in methanol (30 mL) was warmed to obtain a solution and then cooled to rt. Triethylamine (0.5 mL) followed by 10% Pd/C (100 mg) (Caution: Extreme fire hazard) were added to this solution, and the mixture was stirred under atmospheric pressure H_2 for 20 h. It was then filtered and the catalyst was washed with hot methanol. The combined filtrate was evaporated and chromatographed to give 104 mg (86.3%) of **22**; R_f 0.42 (solvent D). ^{1}H NMR (Me_2SO-d_6) δ 2.60, 2.82, 4.00 (3 m, 5 H, H-3′,4′,5′,6a′,6b′), 4.16 (s, 1 H, H-2′), 6.05 (s, 1 H, H-1′), 6.84 (bs, 2 H, NH_2), 8.20 (s, 1 H, H-6), 8.22 (s, 1 H, H-2); IR (neat) ν 3200–3600 (OH, NH), 2280 (CN) cm^{-1}; UV λ_{max} pH 1, 276, 234, EtOH, 280, 231, 205, pH 11, 278, 210 nm. Anal. Calcd for $C_{13}H_{16}N_6O_4$ (320.3): C, 48.75; H, 5.00; N, 26.25. Found: C, 48.63, H, 4.89, N, 26.02.

4-Amino-7-(6-amino-6-deoxy-β-D-glucofuranosyl)-5-carboxamidopyrrolo[2,3-d]pyrimidine (**23**).—A solution of **22** (200 mg, 0.6 mmol) in concd ammonium hydroxide (30 mL) was treated with 30% H_2O_2 (3.0 mL) at rt for 5 h and evaporated. The residue was co-evaporated with ethanol and chromatographed to give 140 mg (66.4%) of **23**; R_f 0.27 (solvent D). ^{1}H NMR (Me_2SO-d_6) δ 2.70–4.16 (m, 5 H, H-3′,4′,5′,6a′, 6b′), 4.21 (s, 1 H, H-2′), 5.93 (s, 1 H, H-1′), 8.07 (s, 1 H, H-6), 8.11 (s, 1 H, H-2); IR (KBr) ν 3200–3600 (OH, NH), 1630 ($CONH_2$) cm^{-1}; UV λ_{max} pH 1, 277, 231, EtOH, 281, 229, 205, pH 11, 279, 228, 210 nm. Anal. Calcd for $C_{13}H_{18}N_6O_5$ (338.3): C, 46.15; H, 5.32; N, 24.85. Found: C, 45.78, H, 5.29, N, 24.76.

4-Amino-7-(3-azidomethyl-2,5,6-tri-O-benzoyl-3-deoxy-β-D-allofuranosyl)-6-bromo-5-cyanopyrrolo-[2,3-d]pyrimidine (**24**).—4-Amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (3.94 g, 16.5 mmol) was silylated with HMDS (50 mL) and chlorotrimethylsilane (0.5 mL) as described above for **18**. To the silylated base was added a solution of **17** (8.6 g, 15.0 mmol) in 1,2-dichloroethane (200 mL), and the solution was cooled in ice–water. Me_3SiOTf (3.45 mL, 18.0 mmol) was added dropwise to this solution, which was then stirred sequentially at 0 °C for 30 min, rt for 1 h, 50 °C overnight, and 75–80 °C for 3 h. The mixture was chilled and diluted with CH_2Cl_2 (200 mL) and then poured into a stirred mixture of ice–water and solid $NaHCO_3$ (7.0 g). The organic phase was washed with H_2O (3×100 mL), dried ($MgSO_4$), evaporated, and chromatographed to give 7.47 g (68%) of an isomeric mixture of **24** and **25**. A portion (0.50 g, 0.67 mmol) of this mixture was co-evaporated with toluene (3×10 mL) and dried at 50 °C/0.1 Torr for 3 h. HMDS (15 mL), xylene (15 mL) and chlorotrimethylsilane (50 (L) were added to the residue, and the mixture was heated at reflux for 12 h. The solvent was evaporated and the residue was co-evaporated with toluene (3×10 mL) to give a crystalline mixture of silylated nucleosides. To this mixture were added 1,2-dichloroethane (15 mL) followed by Me_3SiOTf (0.26 mL, 1.34 mmol), and the mixture was heated at reflux for 3 h. Workup as above gave **24** (280 mg, 56%); R_f 0.57 (solvent B). ^{1}H NMR ($CDCl_3$) δ 3.83 (m, 3 H), 4.33–4.92 (m, 3 H), 5.80–6.0 (m, 3 H), 6.13 (d, 1 H, H-1′, *J* 3.0 Hz), 6.33 (m, 1 H), 7.50 (m, 9 H, aromatic), 8.0 (m, 6 H, aromatic), 8.20 (s, 1 H, H-2); IR (KBr) ν 2250 (CN), 2130 (N_3), 1740 (C=O) cm^{-1}. Anal. Calcd for $C_{35}H_{27}BrN_8O_7 \cdot H_2O$ (769.6): C, 54.57; H, 3.77; N, 14.55. Found: C, 54.61; H, 3.52; N, 14.49.

4-Amino-7-(3-azidomethyl-3-deoxy-β-D-allofuranosyl)-6-bromo-5-cyanopyrrolo[2,3-d]pyrimidine (**26**).—*Method A*: A solution of **24** (800 mg, 1.06 mmol) in methanolic ammonia (35 mL) was kept at rt for 24 h and evaporated. The residue was triturated with ether (2×20 mL), filtered, and washed with ethanol (3×10 mL) to give a white solid (400 mg, 85.5%). An analytical sample was obtained by recrystallizing this solid from 1,4-dioxane; R_f 0.45 (solvent C); mp 213–216 °C, dec. ^{1}H NMR (Me_2SO-d_6) δ 2.90, 3.20 (2 m, 6 H, H-3′,5′,6′$_{a,b}$, CH_2), 3.56 (t, 1 H, H-2′, *J* 5.15 Hz), 4.09 (t, 1 H, OH), 4.66 (m, 1 H, H-4′), 5.12, 5.38 (2 d, 2 OH), 5.48 (d, 1 H, H-1′, *J* 5.36 Hz), 6.62 (bs, 2 H, NH_2), 7.75 (s, 1 H, H-2); IR (KBr) ν 3200–3600 (NH), 2225 (CN), 2100 (N_3) cm^{-1}; UV λ_{max} pH 1, 282, 231, 207, EtOH, 286, 219, 204, pH 11, 285, 216 nm. Anal. Calcd for $C_{14}H_{15}BrN_8O_4$ (439.2): C, 38.29; H, 3.44; N, 25.51. Found: C, 38.45; H, 3.42; N, 25.50.

Method B.—The mixture of **24** and **25** (8.0 g, 11 mmol) was kept in methanolic ammonia (400 mL) at rt for 24 h and evaporated. The residue was triturated with ether to give a mixture of **26** and **27** (4.0 g, 85.5%). Recrystallization of this mixture from 1,4-dioxane gave pure **26** (2.4 g). The isomeric **27** (1.5 g) was obtained from the mother liquor by chromatography. For **27**: R_f 0.50 (solvent C); ^{1}H NMR (Me_2SO-d_6) δ 4.02–4.07 (m, 2 H, H-3′,5′), 5.24 (bs, 1 H, OH), 5.78 (d, 1 H, H-1′, $J_{1',2'}$ 3.3 Hz), 7.15 (bs, 2 H, NH_2), 7.95 (s, 1 H, H-2); UV λ_{max} pH 1, 288, 260, 222, EtOH, 286, 260, 214, pH

11, 285, 258, 215 nm. Anal. Calcd for $C_{14}H_{15}BrN_8O_4$ (439.2): C, 38.29; H, 3.44; N, 25.51. Found: C, 38.10; H, 3.40; N, 25.45.

*4-Amino-7-(3-aminomethyl-3-deoxy-*β*-*D*-allofuranosyl)-5-cyanopyrrolo[2,3-*d*]pyrimidine* (**28**).—A suspension of **26** (380 mg, 0.87 mmol) in 1,4-dioxane (60 mL) was warmed to obtain a solution which was then cooled to rt. Triethylamine (1.0 mL), followed by 10% Pd/C (300 mg), were added and the mixture was hydrogenated at rt for 24 h. It was then filtered and the catalyst was washed with hot 1,4-dioxane. The combined filtrate was evaporated and chromatographed to give 250 mg (86.5%) of **28**; R_f 0.52 (solvent D). ^{1}H NMR (Me_2SO-d_6) δ 2.65 (m, 1 H, H-3′), 3.00–4.57 (m, 7 H), 6.07 (d, 1 H, H-1′, *J* 2.87 Hz), 6.88 (bs, 2 H, NH_2), 8.34 (s, 1 H, H-6), 8.37 (s, 1 H, H-2); IR (neat) ν 3200–3600 (OH, NH), 2210 (CN) cm^{-1}; UV λ_{max} pH 1, 274, 232, 201, EtOH, 281, 204, pH 11, 278, 210 nm. Anal. Calcd for $C_{14}H_{18}N_6O_4$ (334.3): C, 50.30; H, 5.43; N, 25.14. Found: C, 50.05; H, 5.39; N, 24.82.

*4-Amino-7-(3-aminomethyl-3-deoxy-*β*-*D*-allofuranosyl)-5-carboxamidopyrrolo[2,3-*d*]pyrimidine* (**29**).—A solution of **28** (150 mg, 0.45 mmol) in concd ammonium hydroxide (21 mL) was treated with 30% H_2O_2 (2.1 mL) at rt for 5 h. The solvent was evaporated, and the residue was co-evaporated with ethanol and chromatographed to give 118 mg (75%) of **29**; R_f 0.29 (solvent D). ^{1}H NMR (Me_2SO-d_6) δ 2.42 (m, 1 H, H-3′), 2.76–3.60 (m, 4 H), 3.93 (t, 1 H), 4.42 (m, 1 H, H-2′), 6.02 (d, 1 H, H-1′, *J* 2.43 Hz), 6.63 (s, 1 H, OH), 7.32, 7.25 (2 s, 2 H, NH_2), 8.04 (s, 1 H, H-6), 8.07 (s, 1 H, H-2); IR (KBr) ν 3200–3600 (OH, NH), 1630 ($CONH_2$) cm^{-1}; UV λ_{max} pH 1, 274, 232, EtOH, 280, 230, 205, pH 11, 274, 231, 210 nm. Anal. Calcd for $C_{14}H_{20}N_6O_5$ (352.3): C, 47.73; H, 5.72; N, 23.86. Found: C, 47.60; H, 5.65; N, 23.75.

Acknowledgements

We thank Dr Bernacki and his co-workers for performing the cytotoxicity studies. This study was aided in part by grant CA 13038 from the National Cancer Institute.

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