PURINE AND PYRIMIDINE NUCLEOSIDE ANALOGS OF 3′-*C*-METHYLADENOSINE AS ANTITUMOR AGENTS

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Dedicated to Professor Antonín Holý on the occasion of his 70th birthday in recognition of his outstanding contribution to the area of nucleic acid chemistry.

3′-*C*-Methyladenosine (3′-Me-Ado) is a mechanism-based ribonucleotide reductase inhibitor endowed with antitumor activity against both human leukemia and carcinoma cell lines. In this paper, we report the synthesis and antitumor evaluation of a series of purine and pyrimidine 3′-*C*-methylribonucleoside analogs of 3′-Me-Ado. A stereoselective synthesis of the arabino analog of 3′-Me-Ado is also described. Among the tested compounds, only 3′-*C*-methyluridine showed moderate antitumor activity against human myelogenous leukemia K562 cell line.

Keywords: Antimetabolites; Purines; Nucleosides; Pyrimidines; C-Branched nucleosides; 3′-*C*-Methyl-β-D-ribonucleosides; Cytoxicity.

Nucleoside antimetabolites have been widely used as antitumor agents[1–3]. Several *C*-branched ribosyl nucleosides have recently been identified as promising new anticancer drugs[4]. In our continuous efforts in the development of nucleoside analogs as chemotherapeutic agents by their interaction with target enzymes in DNA/RNA biosynthesis, we were interested in the potential cytotoxicity of adenosine derivatives modified by introduction of a methyl group at 1′-, 2′-, or 3′-*C*-position of the ribofuranose ring. From structure–activity relationship studies, 3′-*C*-methyladenosine (3′-Me-Ado)

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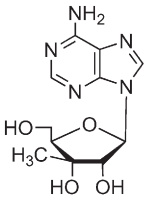
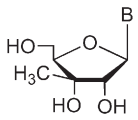
emerged as the most cytotoxic compound against a panel of human leukemia and carcinoma cell lines[5]. The antiproliferative activity of 3′-Me-Ado appears to be related to its ability to deplete the levels of both intracellular purine and pyrimidine deoxynucleotides mediated by mechanism-based inhibition of ribonucleotide reductase (RR). We found that the substitution of a hydrogen atom of the amino group at the 6-position of 3′-Me-Ado with a small alkyl or cycloalkyl group, the introduction of a chlorine atom at the 2-position, or the shifting of the methyl group from the 3′-position to the other positions of the ribose ring results into a decrease or loss of activity.

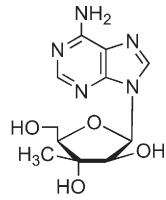
To further investigate the structural determinants of 3′-Me-Ado required for the antitumor activity, we prepared a number of 3′-*C*-methyl ribonucleosides containing different purine and pyrimidine nucleobases such as guanine (3′-Me-G, **1**), uracil (3′-Me-UR, **2**), thymine (3′-Me-T, **3**), and cytosine (3′-Me-C, **5**). The 3′-*C*-methyl-5-fluorouridine (3′-Me-5-FUR, **4**), as a novel ribose-modified analog of the antitumor agent 5-fluorouridine, and the 3′-*C*-methyl analog of the antiviral and antitumor agent 9-(β-D-arabinofuranosyl)adenine (3′-Me-ara-A, **6**), were also prepared.



3'-Me-Ado



1, B = Gu
2, B = U
3, B = Th
4, B = 5-FU
5, B = Cy



6, 3'-Me-ara-A

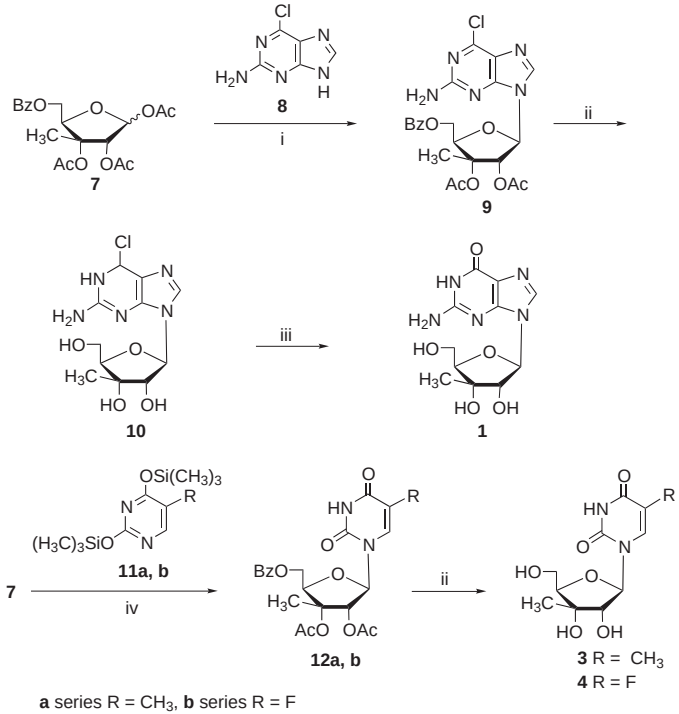
RESULTS AND DISCUSSION

3′-*C*-Methyluridine (**2**) and 3′-*C*-methylcytidine (**5**) were synthesized according to Mikhailov et al.[6]

The synthesis of ribose-modified nucleosides **1**, **3**, and **4** was carried out starting from 1,2,3-tri-*O*-acetyl-5-*O*-benzoyl-3-*C*-methyl-D-ribofuranose (**7**)[5] as described in Scheme 1. Stereoselective coupling of **7** with 2-amino-6-chloropurine (**8**) was performed by trimethylsilyl trifluoromethanesulfonate (TMSiOTf) mediated *N*-glycosylation in acetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to obtain the protected 2-amino-6-chloropurine derivative **9**. Deprotection of **9** gave the ribonucleoside **10** which was converted to 3′-*C*-methylguanosine (**1**) by substitution of the 6-chloro substituent with OH group. 3′-*C*-Methylguanosine

was previously synthesized by Beigelman et al.[7] using a different route and with a lower overall yield.

Glycosylation of silylated thymine **11a** and 5-fluorouracil **11b** with **7** in the presence of $SnCl_4$ in 1,2-dichloroethane furnished the nucleosides **12a** and **12b**, which were deacylated with ammonia in methanol to give nucleosides **3** and **4**.



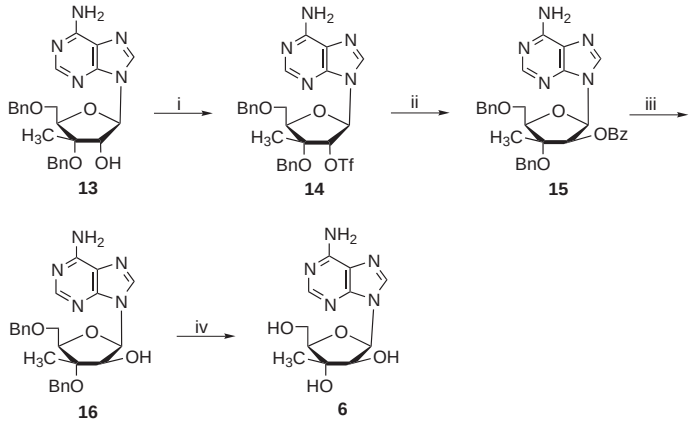
(i) DBU, $TMSiOTf$, CH_3CN; (ii) NH_3/CH_3OH; (iii) C_2H_5SH, CH_3OH, 0.5 M CH_3ONa; (iv) $SnCl_4$, $ClCH_2CH_2Cl$

SCHEME 1

The structure of the synthesized compounds was confirmed by ^1H NMR. Assignment of the β-configuration of nucleosides **1**–**5** was performed by proton NOE experiments. In particular, selective irradiation of the H-1′ signal increased the intensity of H-4′ signal; this indicates that H-1′ and H-4′ are located on the same face of the ribosyl ring[8].

The arabino analog of 3′-Me-Ado (3′-Me-ara-A, **6**) was stereoselectively synthesized as depicted in Scheme 2. Briefly, 9-(3,5-di-*O*-benzyl-3-*C*-methyl-β-D-ribofuranosyl)adenine (**13**), prepared as previously reported[9], was con-

verted into the corresponding 2′-*O*-triflate **14** with trifluoromethanesulfonyl chloride in dichloromethane in the presence of triethylamine and 4-(dimethylamino)pyridine (DMAP). SN2 displacement of the 2′-*O*-triflate group with tetrabutylammonium benzoate afforded the 2′-*O*-benzoyl arabino derivative **15**. Debenzoylation of intermediate **15** with methanolic ammonia, and then deprotection of **16** by catalytic transfer hydrogenation with ammonium formate in methanol in the presence of 10% Pd/C gave 3′-Me-ara-A (**6**).



(i) CF_3SO_2Cl, TEA, DMAP, CH_2Cl_2; (ii) $Bu_4NH^+BzO^-$, toluene/H_2O (1:0.4 v/v); (iii) NH_3/CH_3OH; iv) $HCOO^-NH_4^+$, 10% Pd/C,CH_3OH

SCHEME 2

The 3′-*C*-methyl-β-D-ribonucleosides **1**–**5** and 3′-Me-ara-A (**6**) were evaluated for their *in vitro* cytotoxicity against human myelogenous leukemia K562, and human colon carcinoma HT-29 and CaCo-2 cell lines (Table I). 3′-Me-Ado and ara-A [10] were used as reference compounds.

3′-Me-Ado confirmed to be cytotoxic against leukemia K562 and carcinoma HT-29 [5], and also proved to be active against CaCo-2 cells with an IC_{50} value of 24.3 μM. Ara-A was found to be moderately active against K562 cells and marginally active against CaCo-2 cells. Among 3′-Me-Ado analogs, only 3′-*C*-methyluridine (**2**) showed a cytotoxic effect against human leukemia K562 with an IC_{50} value of 43 μM, but not against the carcinoma cell lines at the highest tested concentration (100 μM).

The poor or no activity of these nucleosides might be due to one or more of the following reasons: (i) inefficient intracellular phosphorylation to the 5′-diphosphate derivatives, the metabolites that could act as mechanism-

based RR inhibitors; (ii) reduced inhibitory activity of the 5′-diphosphates against human RR; (iii) instability in cell culture medium. We have determined the stability of tested compounds in minimal essential medium-alpha (MEM) containing 10% fetal bovine serum in order to mimic their behavior under the experimental conditions of the *in vitro* assays. From the half-life values of the tested nucleosides, the stability of 3′-*C*-methyladenosine analogs appears not to be related to the cytotoxicity because 3′-Me-UR (**2**), active against K562 cells, resulted the less stable compound (Table I). On the contrary, the 5-fluoro analog **4**, which was found to be inactive against all tested cell lines, showed a half life of 31.5 h, the same half life of 3′-Me-ara-A and similar to that of 3′-Me-G. Under the same conditions, 3′-Me-Ado was found the most stable compound with a half life of 43.5 h [5].

TABLE I
In vitro activity of 3′-*C*-methyladenosine analogs **1**–**5** and **6** against human myelogenous leukemia K562 cells, and human colon carcinoma HT-29 and CaCo-2 cell lines, and stability in fetal calf serum

Compound	IC_{50}, μmol/l[a]			Half-life, h in serum
	K562	HT-62	CaCo-2	
1 (3′-Me-G)	>100	>100	>100	26.5
2 (3′-Me-UR)	43	>100	>100	4
3 (3′-Me-T)	>100	>100	>100	10
4 (3′-Me-5-FUR)	>100	>100	>100	31.5
5 (3′-Me-C)	>100	>100	>100	8
6 (3′-Me-ara-A)	>100	>100	>100	31.5
3′-Me-Ado	18.2	23.2	24.3	43.5
ara-A	184	>100	753	ND[b]

[a] IC_{50} values represent the drug concentration required to inhibit cancer cell replication by 50%. The compounds were tested up to a concentration of 100 μmol/l (1 mmol/l in the case of ara-A). [b] ND, not determined.

In conclusion, it was confirmed that the structure of 3′-Me-Ado is crucial for the antitumor activity of this type of ribose-modified nucleosides. The substitution of adenine in 3′-Me-Ado with other purine and pyrimidine nucleobases or the inversion of configuration of OH group at the 2′-posi-

tion (3′-Me-ara-A), induces the loss or a significant decrease in cytotoxicity in both human leukemia and carcinoma cell lines.

EXPERIMENTAL

Melting points were measured on a Buchi apparatus without correction. Elemental analyses were determined on an EA 1108 CHNS-O (Fisons Instruments) analyzer. Thin layer chromatography (TLC) was run on silica gel 60 F_{254} plates (Merck); silica gel 60 (70–230 and 230–400 mesh, Merck) for column chromatography was used. NMR spectra were determined with Varian VXR-200 (200 MHz for ^{1}H) or Varian Mercury AS400 (400 MHz for ^{1}H, and 100.6 MHz for ^{13}C) instruments. Chemical shift (δ-scale) are expressed in ppm; coupling constants (*J*) in Hz. TMS was used as internal standard for the ^{1}H and ^{13}C NMR spectra. The presence of all exchangeable protons was confirmed by addition of D_2O. Stationary NOE experiments were run on degassed solutions at 25 °C. A presaturation delay of 1 s was used, during which the decoupler low power was set at 20 dB attenuation. Mass spectroscopy was carried out on an HP 1100 series instrument. All measurements were performed in the negative ion mode using an atmospheric pressure electrospray ionization (API-ESI). Optical rotations were measured on a Perkin–Elmer 241 polarimeter at 20 °C; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. UV spectra (λ in nm) were recorded on a Varian Cary 1E spectrofotometer.

1,2,3-Tri-*O*-acetyl-5-*O*-benzoyl-3-*C*-methyl-D-ribofuranose (**7**)

This compound was prepared in four steps from 1,2-*O*-isopropylidene-α-D-xylofuranose in the same way as previously described[5].

6-Chloro-9-(2,3-di-*O*-acetyl-5-*O*-benzoyl-3-*C*-methyl-β-D-ribofuranosyl)-9*H*-purin-2-amine (**9**)

To a precooled (0 °C) solution of **7** (1 mmol), 2-amino-6-chloropurine (1.1 equiv.) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (3 equiv.) in anhydrous acetonitrile (12 ml) was added trimethylsilyl triflate (4 equiv.) dropwise. The reaction mixture was heated at 50 °C for 2 h, allowed to come to room temperature, poured into saturated aqueous sodium hydrogen carbonate (80 ml) and extracted with dichloromethane (4 × 40 ml). The combined organic phase was dried over anhydrous sodium sulfate and evaporated in vacuo. The residue was purified by chromatography on a silica gel column eluting with hexane–ethyl acetate (6:4) to give **9** as a foam (65%). ^{1}H NMR (200 MHz, DMSO-d_6): 1.75 s, 3 H (CH_3); 2.04 and 2.1 2 × s, 2 × 3 H (2 × CH_3CO); 4.60–4.80 m, 3 H (overlapped H-4′, H-5′a, H-5′b); 5.97 d, 1 H, *J*(2′,1′) = 7.0 (H-2′); 6.05 d, 1 H, *J*(1′2′) = 7.0 (H-1′); 7.06 brs, 2 H (NH_2); 7.52 m, 2 H (H-arom.); 7.66 m, 1 H (H-arom.); 7.96 m, 2 H (H-arom.); 8.32 s, 1 H (H-8). For $C_{22}H_{22}ClN_5O_7$ (503.9) calculated: 52.44% C, 4.40% H, 13.90% N; found: 52.35% C, 4.22% H, 14.0% N.

6-Chloro-9-(3-*C*-methyl-β-D-ribofuranosyl)-9*H*-purin-2-amine (**10**)

A solution of **9** (0.5 mmol) in saturated methanolic ammonia (30 ml) was stirred at room temperature for 4 h. The solvent was removed in vacuo and the residue was purified on a silica gel column eluting with $CHCl_3$–MeOH (92:8) to afford **10** as a foam (80%). ^{1}H NMR

(400 MHz, DMSO-d_6): 1.24 s, 3 H (CH_3); 3.56 m, 2 H (H-5′a, H-5′b); 3.82 t, 1 H, J(4′,5′a) = J(4′,5′b) = 3.2 (H-4′); 4.32 dd, 1 H, J(2′,1′) = 8.1, J(2′,OH) = 6.4 (H-2′); 4.9 s, 1 H (3′-OH); 5.2 t, 1 H, J(OH,5′a) = J(OH,5′b) = 4.9 (5′-OH); 5.52 d, 1 H, J(OH,2′) = 6.4 (2′-OH); 5.78 d, 1 H, J(1′,2′) = 8.1 (H-1′); 6.95 brs, 2 H (NH_2); 8.32 s, 1 H (H-8). For $C_{11}H_{14}ClN_5O_4$ (315.7) calculated: 41.85% C, 4.47% H, 22.18% N; found: 41.68% C, 4.38% H, 22.31% N.

9-(3-*C*-Methyl-β-D-ribofuranosyl)guanine (**1**)

To a solution of **10** (1 mmol) and 2-sulfanylethan-1-ol (5 equiv.) in methanol (30 ml) was added 0.5 M sodium methoxide (5 equiv.) in methanol and the mixture was heated under reflux for 7 h. The solution was cooled to room temperature and neutralized with acetic acid. The solvent was evaporated in vacuo and the residue was crystallized from water to afford **1** as a white solid (75%); m.p. 269–271 °C (dec.) ^{1}H NMR (200 MHz, DMSO-d_6): 1.24 s, 3 H (CH_3); 3.5 m, 2 H (H-5′a, H-5′b); 3.78 t, 1 H, J(4′,5′a) = J(4′,5′b) = 3.1 (H-4′); 4.26 d, 1 H, J(2′,1′) = 8.1 (H-2′); 4.80 brs, 1 H (3′-OH); 5.3 brs, 1 H (5′-OH); 5.44 brs, 1 H (2′-OH); 5.66 d, 1 H, J(1′,2′) = 8.1 (H-1′); 6.8 brs, 2 H (NH_2); 7.85 s, 1 H (H-8); 11.4 brs, 1 H (NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): 20.06 (CH_3); 61.18 (CH_2-5′); 76.21 and 77.22 (CH-2′ and CH-3′); 85.55 and 87.70 (CH-4′ and CH-1′); 116.82 (C-5); 135.56 (CH-8); 151.53 (C-4); 154.98 (C-2); 158.18 (C-6). UV, λ_{max} (log ε) (H_2O): (pH 2) 255 (4.10); 275 sh (3.93); (pH 7) 253 (4.10); 275 sh (3.92); (pH 12) 234 (3.78), 281 (3.76). MS: *m/z* 296.2 [M – H]. For $C_{11}H_{15}N_5O_5$ (297.3) calculated: 44.44% C, 5.08% H, 23.56% N; found: 44.60% C, 5.18% H, 23.40% N.

Glycosylation of Pyrimidine Bases with 2,3-Di-*O*-acetyl-5-*O*-benzoyl-3-*C*-methyl-D-ribofuranose (**7**). General Procedure

To thymine or 5-fluorouracil (1 mmol) in dry 1,2-dichloroethane (5 ml) under nitrogen was added hexamethyldisilazane (HMDS) (0.8 equiv.) and chlorotrimethylsilane (TMSCl) (0.8 equiv.). The reaction mixture was heated at 80 °C for 5 h. After cooling to room temperature, a solution of **7** (1 equiv.) in dry dichloroethane (5 ml) was added to the persilylated nucleobases **11a** and **11b** followed by 1 M $SnCl_4$ in dry dichloromethane (2 equiv.) dropwise. The mixture was stirred at room temperature or 85 °C as reported below, and then the solution was quenched by saturated solution of $NaHCO_3$ and extracted with chloroform. The organic phase was dried over anhydrous sodium sulfate and, after filtration, the solvent was evaporated to dryness and the residue was purified by chromatography to obtain compounds **12a** or **12b** as foams.

1-(2,3-Di-O-acetyl-5-O-benzoyl-3-C-methyl-β-D-ribofuranosyl)thymine (**12a**). The reaction of **7** with silylated thymine **11a** at room temperature for 1 h, followed by chromatography on a silica gel column (CH_2Cl_2–C_2H_5OH, 99.5:0.5) gave **12a** as a foam (39%). ^{1}H NMR (200 MHz, DMSO-d_6): 1.55 s, 3 H (CH_3); 1.70 s, 3 H (CH_3); 2.1 s, 2 × 3 H (2 × CH_3CO); 4.5 m, 2 H (H-5′a, H-5′b); 4.7 m, 1 H (H-4′); 5.45 d, 1 H, J(2′,1′) = 7.7 (H-2′); 6.05 d, 1 H, J(1′,2′) = 7.7 (H-1′); 7.52 m, 3 H (H-6 and H-arom.); 7.65 m, 1 H (H-arom.); 8.0 m, 2 H (H-arom.); 11.4 brs, 1 H (NH). For $C_{22}H_{24}N_2O_9$ (460.4) calculated: 57.39% C, 5.25% H, 6.08% N; found: 57.25% C, 5.10% H, 6.18% N.

5-Fluoro-1-(2,3-di-O-acetyl-5-O-benzoyl-3-C-methyl-β-D-ribofuranosyl)uracil (**12b**). The reaction of **7** with silylated 5-fluorouracil **11b** at room temperature for 2 h, followed by chromatography on a silica gel column (hexane–EtOAc, 6:4) gave **12b** as a foam (70%). ^{1}H NMR

(200 MHz, $CDCl_3$): 1.6 s, 3 H (CH_3); 2.10 s, 2 × 3 H (2 × CH_3CO); 4.63 and 4.75 2 m, 3 H (H-4′, H-5′a, H-5′b); 5.40 d, 1 H, *J*(2′,1′) = 7.7 (H-2′); 6.0 dd, 1 H, *J*(1′,OH) = 1.8, *J*(1′,2′) = 7.7 (H-1′); 7.54 m, 2 H (H-arom.); 7.65 m, 1 H (H-arom.); 7.97 m, 2 H (H-arom.); 8.1 d, 1 H, *J*(6,F) = 7.0 (H-6); 11.9 brs, 1 H (NH). For $C_{21}H_{21}FN_2O_9$ (464.4) calculated: 54.31% C, 4.56% H, 6.03% N; found: 54.48% C, 4.61% H, 5.92% N.

Deprotection of Nucleosides **12a** and **12b**. General Procedure

Protected nucleosides **12a** or **12b** were treated with methanolic ammonia (saturated at 0 °C) and the mixtures were stirred at room temperature overnight. Then the solvent was evaporated and the residue was purified by chromatography to give the deprotected nucleosides **3** and **4**.

*1-(3-C-Methyl-*β*-D-ribofuranosyl)thymine* (**3**). Compound **3** was obtained from **12a** and purified by chromatography on a silica gel column eluting with $CHCl_3$–MeOH (92:8), as a white solid (50%); m.p. 248–251 °C (dec.) ^{1}H NMR (200 MHz, DMSO-d_6): 1.20 s, 3 H (CH_3); 1.78 s, 3 H (CH_3); 3.54 m, 2 H (H-5′a, H-5′b); 3.74 t, 1 H, *J*(4′,5′a) = *J*(4′,5′b) = 2.7 (H-4′); 3.86 dd, 1 H, *J*(2′,OH) = 6.6, *J*(2′,1′) = 8.1 (H-2′); 4.74 s, 1 H (3′-OH); 5.16 t, 1 H, *J*(OH,5′a) = *J*(OH,5′b) = 4.0 (5′-OH); 5.30 d, 1 H, *J*(OH,2′) = 6.6 (2′-OH); 5.84 d, 1 H, *J*(1′,2′) = 8.1 (H-1′); 7.87 s, 1 H (H-6); 11.25 brs, 1 H (NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): 12.36 (CH_3-5); 20.08 (CH_3-3′); 60.81 (CH_2-5′); 76.09 and 76.65 (CH-2′ and CH-3′); 85.59 (CH-4′); 87.24 (CH-1′); 109.64 (CH-5); 136.92 (CH-6); 151.23 (C-2); 163.84 (C-4). UV, λ_{max} (log ε) (H_2O): (pH 2) 267 (4.01); (pH 7) 267 (4.01); (pH 12) 267 (3.90). MS: *m/z* 271.1 [M – H]. For $C_{11}H_{16}N_2O_6$ (272.3) calculated: 48.53% C, 5.92% H, 10.29% N; found: 48.70% C, 6.0% H, 10.15% N.

*5-Fluoro-1-(3-C-methyl-*β*-D-ribofuranosyl)uracil* (**4**). Compound **4** was obtained from **12b**. Chromatography on a silica gel column eluting with $CHCl_3$–MeOH (86:14) gave **4** as a white solid (52%); m.p. 241–244 °C (dec.) ^{1}H NMR (200 MHz, DMSO-d_6): 1.2 s, 3 H (CH_3); 3.55 m, 2 H (H-5′a, H-5′b); 3.76 t, 1 H, *J*(4′,5′a) = *J*(4′,5′b) = 2.6 (H-4′); 3.82 d, 1 H, *J*(2′,1′) = 8.1 (H-2′); 4.76 s, 1 H (3′-OH); 5.3 t, 1 H, *J*(OH,5′a) = *J*(OH,5′b) = 3.7 (5′-OH); 5.38 d, 1 H, *J*(OH,2′) = 6.2 (2′-OH); 5.85 dd, 1 H, *J*(1′,OH) = 2.2, *J*(1′,2′) = 8.1 (H-1′); 8.35 d, 1 H, *J*(6,F) = 7.3 (H-6); 11.75 brs, 1 H (NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): 19.94 (CH_3); 60.72 (CH_2-5′); 76.18 and 77.17 (CH-2′ and CH-3′); 86.17 (CH-4′); 87.49 (CH-1′); 125.10 (d, $^2J_{C,F}$ = 34.3, CH-6); 140.06 (d, $^1J_{C,F}$ = 231.2, CF-5); 149. 78 (C-2); 157.06 (d, $^2J_{C,F}$ = 25.8, C-4). UV, λ_{max} (log ε) (H_2O): (pH 2) 270.5 (3.95); (pH 7) 270.5 (3.82); (pH 12) 270.5 (3.96). MS: *m/z* 275.1 [M – H]. For $C_{10}H_{13}FN_2O_6$ (276.2) calculated: 43.48% C, 4.74% H, 10.14% N; found: 43.40% C, 4.61% H, 10.30% N.

9-(3,5-Di-*O*-benzyl-3-*C*-methyl-β-D-ribofuranosyl)adenine (**13**)

This compound was prepared in two steps starting from 1,2-di-*O*-acetyl-3,5-di-*O*-benzyl-D-ribofuranose[11] and 6-chloropurine as described[9].

9-[3,5-Di-*O*-benzyl-3-*C*-methyl-2-*O*-(trifluoromethylsulfonyl)-β-D-ribofuranosyl]-adenine (**14**)

A solution of **13** (1 mmol) in dry dichloromethane (18.5 ml) was treated with triethylamine (1.08 equiv.) and 4-dimethylaminopyridine (0.98 equiv.) and chilled in an ice bath. To the stirred solution was added trifluoromethanesulfonyl chloride (1.08 equiv.) with a syringe. The reaction mixture was stirred at room temperature for 1.5 h. Additional trifluoro-

methanesulfonyl chloride (0.3 equiv.) was added in two portions over the next 3 h with cooling prior to the addition. After 3 h the solvent was removed in vacuo, and the residue was chromatographed on a silica gel column eluting with $CHCl_3$. Evaporation of the appropriate fractions gave **14** as a foam (73%). ^{1}H NMR (400 MHz, DMSO-d_6): 1.6 s, 3 H (CH_3); 3.74 dd, 1 H, J(5′a,5′b) = 11.3, J(5′a,4′) = 3.1 (H-5′a); 3.86 dd, 1 H, J(5′b,5′a) = 11.3, J(5′b,4′) = 3.1 (H-5′b); 4.64 d, 2 H, J = 10.6 (C**H**$_2$C$_6$H$_5$); 4.67 t, 1 H, J(4′,5′a) = J(4′,5′b) = 3.3 (H-4′); 4.72 d, 2 H, J = 11.7 (C**H**$_2$C$_6$H$_5$); 5.88 d, 1 H, J(2′,1′) = 7.8 (H-2′); 6.42 d, 1 H, J(1′,2′) = 7.8 (H-1′); 7.28–7.42 m, 10 H (H-arom); 8.10 s, 1 H (H-2); 8.13 s, 1 H (H-8). For $C_{26}H_{26}F_3N_5O_6S$ (593.6) calculated: 52.61% C, 4.41% H, 11.80% N; found: 52.58% C, 4.55% H, 11.60% N.

9-(2-*O*-Benzoyl-3,5-di-*O*-benzyl-3-*C*-methyl-β-D-arabinofuranosyl)-adenine (**15**)

Compound **14** (2.5 mmol) was dissolved in 10 ml of toluene containing 10 mmol of tetrabutylammonium benzoate and water (4 ml). The mixture was heated under reflux and stirred rapidly for 6 h. The solvent was removed in vacuo and the residue was purified by chromatography on a silica gel column eluting with $CHCl_3$ to give **15** (73%) as a foam. ^{1}H NMR (200 MHz, DMSO-d_6): 1.42 s, 3 H (CH_3); 3.82 d, 2 H, J(5′a,4′) = J(5′b,4′) = 5.1 (H-5′a, H-5′b); 4.48 t, 1 H, J(4′,5′a) = J(4′,5′b) = 5.7 (H-4′); 4.65 m, 4 H (C**H**$_2$C$_6$H$_5$); 5.58 d, 1 H, J(2′,1′) = 4.0 (H-2′); 6.65 d, 1 H, J(1′,2′) = 4.4 (H-1′); 7.2–7.7 m, 15 H (H-arom); 8.0 s, 1 H (H-2); 8.08 s, 1 H (H-8). For $C_{32}H_{31}N_5O_5$ (565.6) calculated: 67.95% C, 5.52% H, 12.38% N; found: 67.80% C, 5.40% H, 12.48% N.

9-(3,5-Di-*O*-benzyl-3-*C*-methyl-β-D-arabinofuranosyl)adenine (**16**)

Compound **15** (2.0 mmol) was treated with methanolic ammonia (saturated at 0 °C; 20 ml) and stirred at room temperature overnight. The solvent was removed in vacuo, and the residue was purified by chromatography on a silica gel column eluting with $CHCl_3$–MeOH (99:1) to give **16** as a foam (54%). ^{1}H NMR (200 MHz, DMSO-d_6): 1.4 s, 3 H (CH_3); 3.7 m, 2 H (H-5′a, H-5′b); 3.95 dd, 1 H, J(2′,1′) = 5.9, J(2′,OH) = 3.7 (H-2′); 4.32 dd, 1 H, J(4′,5′a) = 5.12, J(4′,5′b) = 7.0 (H-4′); 4.58 m, 4 H (C**H**$_2$C$_6$H$_5$); 5.95 d, 1 H, J(OH,2′) = 5.9 (2′-OH); 6.42 d, 1 H, J(1′,2′) = 3.7 (H-1′); 7.2–7.4 m, 10 H (H-arom); 7.98 s, 1 H (H-2); 8.1 s, 1 H (H-8). For $C_{24}H_{27}N_5O_4$ (461.5) calculated: 65.06% C, 5.89% H, 15.17% N; found: 65.26% C, 5.96% H, 15.0% N.

9-(3-*C*-Methyl-β-D-arabinofuranosyl)adenine (**6**)

To a mixture of **16** (1 mmol) and ammonium formate (8 equiv.) in anhydrous methanol (11 ml) under nitrogen was added 10% Pd/C (1.2 g). The mixture was heated at reflux for 1 h, cooled to room temperature, and filtered. After evaporation, the crude material was purified by chromatography on a silica gel column eluting with $CHCl_3$–MeOH (87:13) to give **6** as a white solid (40%); m.p. 238–242 °C (dec.); $[\alpha]_D$ = +29.6 (*c* 0.25, CH_3OH). ^{1}H NMR (200 MHz, D_2O): 1.3 s, 3 H (CH_3); 3.71 d, 2 H, J(5′a,4′) = J(5′b,4′) = 5.1 (H-5′a, H-5′b); 3.95 d, 1 H, J(2′,1′) = 3.7 (H-2′); 4.03 t, 1 H, J(4′,5′a) = J(4′,5′b) = 4.9 (H-4′); 6.37 d, 1 H, J(1′,2′) = 3.7 (H-1′); 7.98 s, 1 H (H-2); 8.1 s, 1 H (H-8). ^{13}C NMR (100.69 MHz, D_2O): 17.45 (CH_3); 61.19 (CH_2-5′); 76.81 and 80.80 (CH-2′ and CH-3′); 86.33 and 88.04 (CH-4′ and CH-1′); 118.35 (C-5); 141.34 (CH-8); 148.28 (C-4); 152.33 (C-2); 155.34 (C-6). UV, λ_{max} (log ε) (H_2O): (pH 2)

257 (4.05); (pH 7) 258 (4.15); (pH 12) 259 (4.14). MS: *m/z* 280.1 [M – H]. For $C_{11}H_{15}N_5O_4$ (281.3) calculated: 46.97% C, 5.37% H, 24.90% N; found: 47.0% C, 5.47% H, 24.80% N.

Cells and Culture

The cell lines human myelogenous leukemia K562, human colon carcinoma HT-29 and human colon adenocarcinoma CaCo-2 were obtained from the American Type Culture Collection (ATCC, Manasses, VA, U.S.A.). K562 cells were maintained in RPMI 1640 medium (Gibco/Life Technologies, Gaithersburg, MD, U.S.A.) containing 10% heat-inactivated fetal bovine serum (FBS) (Atlanta Biologicals, Atlanta, GA, U.S.A.) and 10,000 U/l penicillin and 50 mg/l streptomycin[12]. HT-29 and CaCo-2 cells were maintained in MEM with Earl's balanced salts, 10% FBS, penicillin and streptomycin as above. Logarithmically growing HT-29 and CaCo-2 cells were incubated with 0.05% trypsin containing 1 mM EDTA at 37 °C for about 5 min until cells were non-adherent and formed a single cell suspension. Trypsin activity was neutralized by adding 20-fold excess of the serum-containing medium. Cells were cultured at 37 °C in an atmosphere of air and 5% CO_2.

Antitumor Activity

Antitumor activity of the compounds was determined by their cytotoxic action on cultured tumor cells. Cytotoxicity assays were conducted by tetrazolium reduction of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) with *N*-methylphenazonium methyl sulfate (PMS) (CellTiter Assay, Promega, Madison, WI, U.S.A.). Logarithmically growing cells were plated in 0.1 ml aliquots in 96-well microtiter plates. Cells were plated at an initial density of about 50,000 cells/ml and allowed to acclimatize for 24 h. Cell suspensions were treated with various dilutions of compounds in triplicate, mixed well and allowed to incubate at 37 °C for 48 h in an atmosphere of air and 5% CO_2. To the cell suspension was added 20 µl of tetrazolium reagent, the mixture was incubated at 37 °C for 3 h in an atmosphere of air and 5% CO_2 and absorbance at 490 nm was read by microplate reader. Control plates with serial dilutions of cell types were counted as a control for the assay. In all cases, controls indicated a linear response versus cell number, $R^2 \geq 0.99$.

Stability of Nucleosides **1**–**6** in Culture Medium

Stability of nucleosides **1**–**6** was evaluated by incubation of the compounds (50 µM) in MEM containing 10% fetal bovine serum (Sigma Chemical Co.) at 37 °C for 72 h. At various times, 200 µl of the medium was removed and frozen. The collected samples were thawed, passed through 0.22-µm syringe filters and then subjected to HPLC. Samples were eluted with a linear gradient from water to 100% methanol using a 4.6 × 250 mm Beckman Ultrasphere 5-mm C_{18} column. A HP1090 system was used.

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