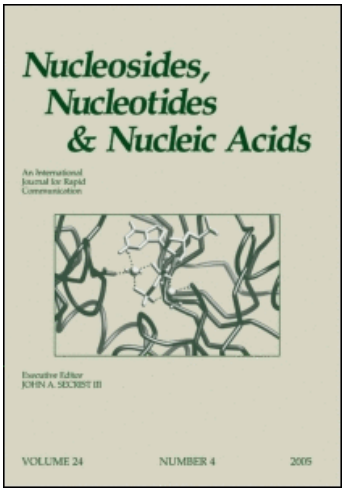

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8-SUBSTITUTED ADENINE ß-D-XYLOFURANOSIDES AND α-L-ARABINOFURANOSIDES

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Abstract 9-(β-D-Xylofuranosyl)adenine and 9-(α-L-arabinofuranosyl)-adenine have been modified to give a series of 8-amino-substituted nucleosides. Convenient methods of halogenation of these nucleosides and reactions of 8-halogenated nucleosides with various amines are described. No significant cytotoxic or antiviral activity was found.

Anticancer activity of 9-(β-D-xylofuranosyl)adenine (xylo-A, 1) was first reported in 1965,[1] and several reports have confirmed this activity. For example, xylo-A was reported to be cytotoxic against L1210 leukemia cells in combination with 2'-deoxycoformycin[2] and against wild type B-mix K-44/6 (Rous sarcoma virus-transformed) rat cells.[3] Considerable antiviral activity of xylo-A was also found - xylo-A was active against African Swine Fever Virus[4] and the Herpes simplex viruses HSV-1 and HSV-2 in vitro.[5,6] The main disadvantage of xylo-A as a therapeutic agent is its rapid deamination in vivo by adenosine deaminase. Introduction of bulky substituents at C-8 can prevent deamination of adenine nucleosides[7] as well as significantly modify the activity of the parent compound.

It is known that certain L-sugars are distributed in nature as the glycosidic moieties of several important antibiotics. Some synthetic analogs of L-daunosamine and L-acosamine also exhibit significant anticancer activity.[8] There is a structural similarity between β-D-xylofuranosyl and α-L-arabinofuranosyl derivatives of naturally occurring nucleoside bases, although the latter lack significant biological activity. 9-(α-L-Arabinofuranosyl)adenine (α-L-ara-A, 27) itself was synthesized as early as 1952.[9]

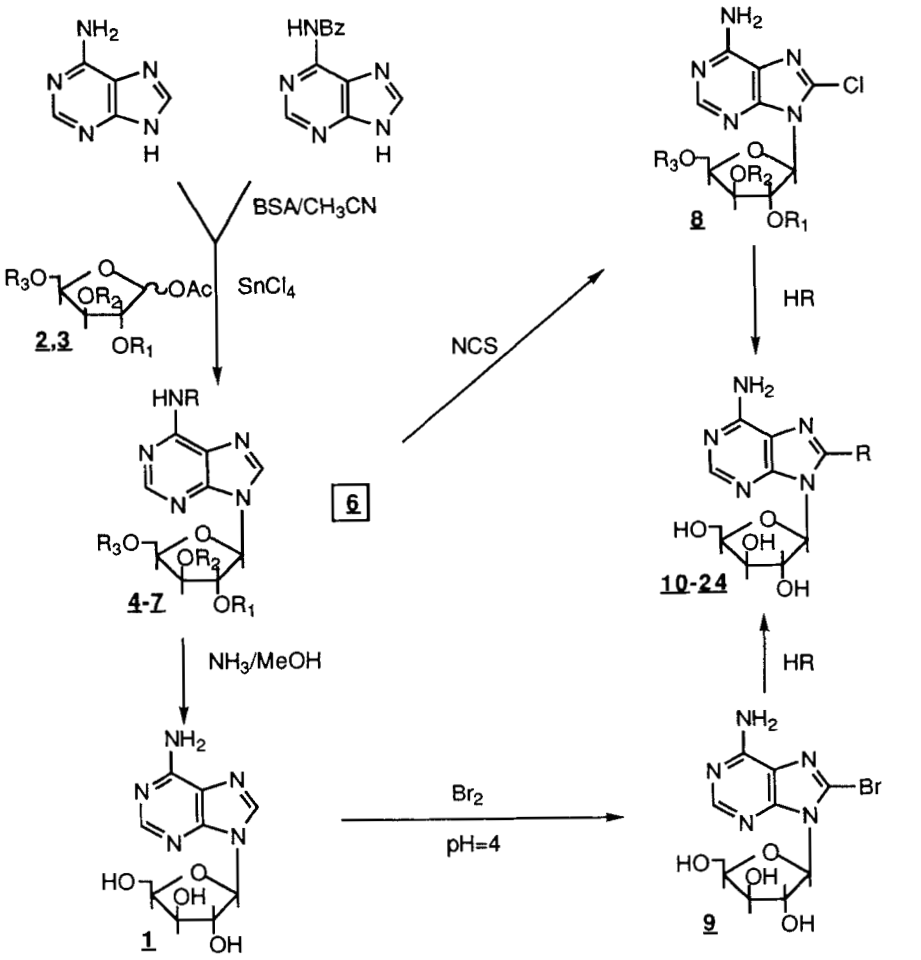
Synthesis of only five 8-substituted xylo-A derivatives has been reported,[10] without any data about their biological activity, and no α-L-

ara-A derivatives have been reported. These considerations as well as our earlier studies of 8-substituted purine nucleosides[11-13] led us to synthesize a variety of 8-substituted adenine β-D-xylofuranosides and α-L-arabinofuranosides in order to identify biologically active analogs.

To obtain xylo-A, the intermediate for the synthesis of 8-substituted derivatives, several possibilities were considered (SCHEME 1). According to Mikhailopulo et al.[14] xylo-A (1) can be synthesized from benzoyladenine and 1,2-di-O-acetyl-3,5-di-O-(p-nitrobenzoyl)-D-xylofuranose (2) using the silyl method with a yield of protected nucleoside of more than 80%. We employed 1-O-acetyl-2,3,5-tri-O-benzoyl-D-xylofuranose (3)[15] for the same reaction and obtained protected xylo-A 5 in 86% yield. Because this type of condensation reaction requires the use of protected adenine, and because benzoyladenine is obtained from adenine in only 70% yield, we investigated reactions where non-protected adenine could be condensed with the acylated sugar without silylation. Adenine reacted with 2 to give nucleoside 6 in 62% yield and with 3 to give nucleoside 7 in 77% yield (Neuman et al.[16] reported 80% yield for the latter reaction.) Therefore, most of the protected xylo-A used in this study was obtained by condensing adenine with 3.

Further reactions are shown in SCHEME 1. The most convenient route to 8-amino derivatives is through 8-halo derivatives of xylo-A. Again two possible routes were considered. According to the procedure developed in our laboratory[12,13] certain nucleosides can be chlorinated at C-8 with N-chlorosuccinimide (NCS). We tried to apply this procedure to the protected xylo-As, but only 6 appeared to be suitable for this reaction. In the next step the 8-chloronucleoside 8 reacted with a large excess of amine (at least 4-5-fold) to produce the desired product, purified on a silica gel column. 8-(Propylamino)-xylo-A (12) was synthesized using this approach.

Another approach consisted of deblocking xylo-A, bromination, and then reaction with amine. All protected xylo-As (4-7) could be easily deblocked with NH_3/MeOH to give xylo-A (1). Bromination with bromine in acetate buffer, according to Ikehara et al.[17] but with some improvements in work-up methods, gave 8-bromo-xylo-A (9) in 73% yield. This compound was easily converted in reactions with various amines to the corresponding 8-amino derivatives 10-24 (SCHEME 3). This second approach appeared to be more convenient, the products being obtained without column chromatography in most cases, and in good yields.



2, R_1=Ac, R_2=R_3=*p*-nitrobenzoyl
3, R_1=R_2=R_3=Bz
4, R=Bz, R_1=Ac, R_2=R_3=*p*-nitrobenzoyl
5, R=R_1=R_2=R_3=Bz

6, R=H, R_1=Ac, R_2=R_3=*p*-nitrobenzoyl
7, R=H, R_1=R_2=R_3=Bz
8, R_1=Ac, R_2=R_3=*p*-nitrobenzoyl
BSA=N,O-bis(trimethylsilyl)acetamide
NCS=N-chlorosuccinimide

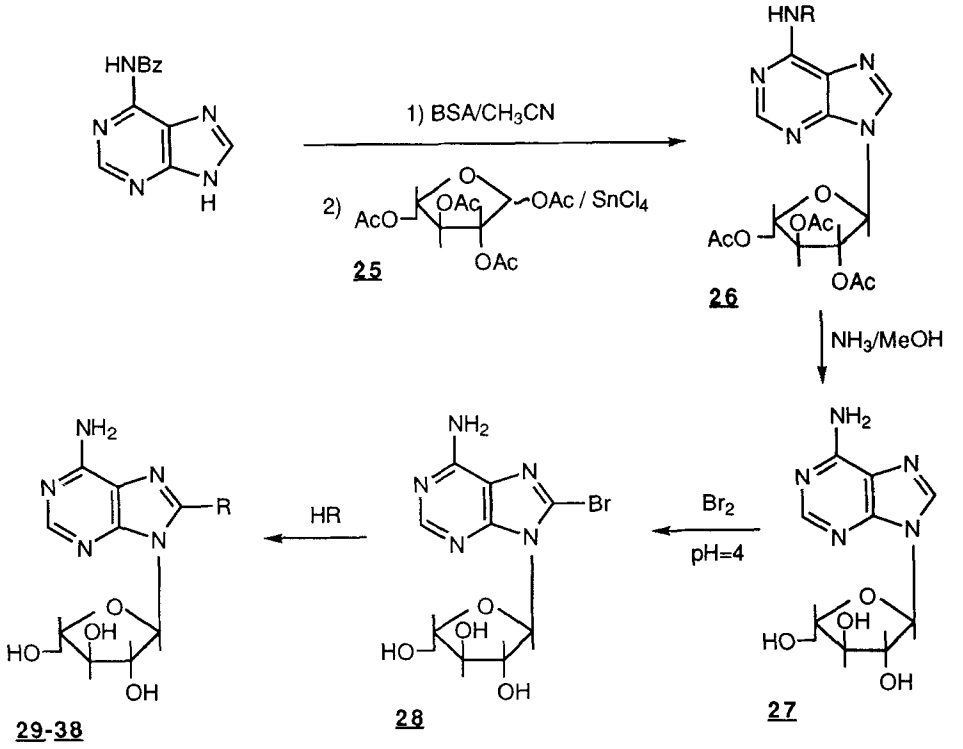
SCHEME 1

A similar approach was used to synthesize 8-substituted adenine-α-L-arabinofuranosides (SCHEME 2). We condensed benzoyladenine with 1,2,3,5-tetra-O-acetyl-L-arabinofuranose (25)[18] to obtain 9-(2,3,5-tri-O-acetyl-α-L-arabinofuranosyl)adenine (26), which, after treatment with NH_3/H_2O/MeOH, gave 9-(α-L-arabinofuranosyl)adenine (α-L-ara-A) (27). Purification of α-L-ara-A was performed using a Dowex 1x8 ion exchange column (OH^- form), which separated this product from the faster eluting β isomer, followed by crystallization of the crude product from methanol. Besides the desired α-L-ara-A obtained in 65% yield, 9-(β-L-arabinofuranosyl)adenine (27a) was isolated in 3-5% yields. Physical and spectroscopic properties of 27 were essentially identical with those previously reported for α-L-ara-A.[19] The structure of 27a was assigned as the β anomer based on the large value of $J_{1',2'}$ in the proton NMR spectrum, 8.8 Hz, relative to the lower value of 4.8 Hz for 27 (Table 1), typical of those in α anomers.[19]

α-L-Ara-A was brominated according to the method of Ikehara et al.[17] as described above for xylo-A, giving the 8-bromo derivative, 28, in 60% yield. In the reactions of 8-bromo-α-L-ara-A (28) with various amines a series of 8-substituted α-L-ara-As (29-38, SCHEME 3) was obtained. ^1H NMR data for all synthesized compounds are summarized in TABLE 1.

Compounds 19-24 were tested at 0.1 mM concentration for cytocidal effect on human ovary carcinoma CaOv cells growing in quiescent cell cultures (see Experimental Section). Interestingly these compounds initially stimulated cell proliferation by 2-3 fold, especially at 48 h after the addition of drugs to the cell cultures. After 72 h a marked decrease in stimulation was observed or, in the case of compound 24, reduction to control cell numbers. No reduction in cell numbers relative to control cultures was observed for compounds 19-24. Lack of cytotoxicity was supported by the results of testing compounds 10-13 and 21-24 on [^3H]thymidine incorporation into DNA of HeLa cells. None of the compounds showed significant inhibition of cellular DNA synthesis at 0.1 mM concentration.

Compounds 1,9,10,16-19,27,28,34 and 35 were tested for their antiviral activity against herpes viruses and respiratory viruses (see Experimental Section). Among xylo-A derivatives, xylo-A (1) itself was the most active compound with EC_{50} = 4.6 μg/mL in a cytopathic effect inhibition assay on Herpes simplex virus type 1 and EC_{50} = 12.7 μg/mL in a plaque reduction assay on Varicella zoster virus. All of its derivatives



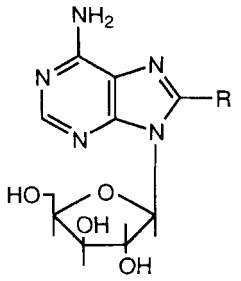
SCHEME 2

generally lacked antiviral activity at a concentration of 100 μg/mL. Consistent with a previous report,[19] α-L-ara-A had neither antiviral nor cytotoxic activity at 100 μg/mL, and none of the 8-substituted α-L-ara-A derivatives showed any noticeable antiviral or cytotoxic activity at the same concentration.

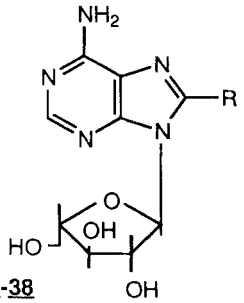
These results indicate that antiviral activity of xylo-A was reduced upon introduction of different alkylamino substituents at C-8, and that substitution with alkylamino groups at C-8 of α-L-ara-A did not result in cytotoxic or antiviral activity.

EXPERIMENTAL PROCEDURES

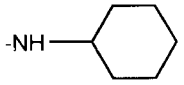
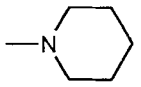
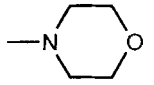
Melting points were determined on a Boëtius hot stage microscope and are uncorrected. Ultraviolet spectra were determined with a Specord UV VIS (Carl Zeiss) spectrophotometer. Nuclear magnetic resonance spectra were

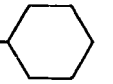


10-24



29-38

Cpd	R
10	-NHCH$_3$
11	-NHCH$_2$CH$_3$
12	-NH(CH$_2$)$_2$CH$_3$
13	-NH(CH$_2$)$_3$CH$_3$
14	-NHCH$_2$CH(CH$_3$)$_2$
15	-NH(CH$_2$)$_6$CH$_3$
16	
17	
18	
19	-NH(CH$_2$)$_2$OH
20	-NH(CH$_2$)$_3$OH
21	-NH(CH$_2$)$_2$NH$_2$
22	-NH(CH$_2$)$_4$NH$_2$
23	-NH(CH$_2$)$_5$NH$_2$
24	-NH(CH$_2$)$_6$NH$_2$

Cpd	R
29	-NHCH$_3$
30	-N(CH$_3$)$_2$
31	-NHCH$_2$CH$_3$
32	-NH(CH$_2$)$_2$CH$_3$
33	-NH(CH$_2$)$_3$CH$_3$
34	-NHCH$_2$CH(CH$_3$)$_2$
35	
36	-NH(CH$_2$)$_2$OH
37	-NH(CH$_2$)$_4$NH$_2$
38	-NH(CH$_2$)$_6$NH$_2$

SCHEME 3

obtained on a Bruker WH-90 (90 MHz) or Bruker WM-360 (360 MHz) spectrometer equipped with an Aspect-2000 computer, and tetramethylsilane was used as internal standard. Solvents were dried by standard methods. HPLC analysis for purity of final products was performed on a Gilson chromatograph using 4.6x100 or 4x150 mm columns with Silasorb SPH C18. All compounds were more than 98% pure. Elemental analysis of compounds (C,H,N) were within 0.4% of theoretical unless noted. Thin layer chromatography

TABLE 1. ^{1}H NMR Chemical Shifts in Me_2SO-d_6[1]

Cpd	H-1'	H-2'	H-2	6-NH_2	-NH-	substituent at C-8
10	5.72	4.40	7.85	6.55	7.07	2.87(CH_3)
11	5.73	4.32	7.83	6.45	7.03	0.88(CH_3);3.26(NCH_2)
12	5.74	4.33	7.84	6.47	7.07	0.89(CH_3);1.55(CH_2); 3.29(NCH_2)
13	5.74	4.31	7.83	6.45	7.00	0.87(CH_3);1.04-1.7 (2xCH_2);3.24(NCH_2)
14	5.75	4.32	7.84	6.40	7.03	0.88(2xCH_3);1.87(CH); 3.11(NCH_2)
15	5.73	4.31	7.83	6.45	7.02	0.78(CH_3);1.25(5xCH_2); 3.25(NCH_2)
16	5.78	4.29	7.83	6.43	6.90	0.91-2.09(CH and 5xCH_2)
17	5.44	4.51	7.98	7.07	-	1.61; 3.14(5xCH_2)
18	5.55	4.51	8.03	7.13	-	3.44-3.48(2xCH_2);3.09-3.55(2xCH_2)
19	5.69	4.38	7.83	6.51	7.08	4.74(ω-OH);3.20-3.69 (2xCH_2)
20	5.73	4.38	7.84	6.48	7.04	4.38(ω-OH);1.70(CH_2); 3.42(2xCH_2)
21	5.73	4.37	7.83	6.48	7.04	3.30(CH_2);3.49(CH_2)
22	5.79	4.39	7.90	6.50	-	1.43(CH_2);1.61(CH_2); 2.50(2xCH_2)
23	5.82	4.38	7.91	6.48	7.32	1.30-1.65(3xCH_2); 2.67(CH_2)
24	5.71	4.29	7.81	6.45	7.08	1.10-1.63(4xCH_2); 2.45(CH_2);3.27(CH_2)
27	5.81[2]	4.65	8.30	7.25	-	8.12(H-8)
27a	5.25[3]	4.30	8.07	7.15	-	8.16(H-8)
28	5.80	5.13	8.16	7.44	-	-
29	5.74	4.64	7.82	6.40	6.60	2.81(CH_3)
30	5.61	5.13	7.98	6.76	-	2.82(2xCH_3)
31	5.75	4.99	7.93	6.44	6.76	1.20(CH_3);3.39(CH_2)
32	5.78	4.96	7.92	6.43	6.77	0.95(CH_3);1.62(CH_2); 3.30(NCH_2)
33	5.78	4.97	7.92	6.41	6.73	0.91(CH_3);1.36;1.59 (2xCH_2);3.24(NCH_2)
34	5.85	4.88	7.93	6.42	6.78	0.91(2xCH_3);1.97(CH); 3.15(NCH_2)
35	5.83	4.90	8.00	6.41	6.59	1.06-2.04(5xCH_2);3.78(CH)
36	5.75	4.98	7.93	6.44	6.71	4.73(ω-OH);3.23-3.71 (2xCH_2)
37	5.78	4.96	7.94	6.42	6.73	1.40(CH_2);1.63(CH_2); 2.50(2xCH_2)
38	5.80	4.96	7.92	6.40	6.76	3.10-3.70(2xCH_2);1.10-1.60(4xCH_2)

[1] All other resonances as expected. [2] $J_{1',2'}$=4.8 Hz. [3] $J_{1',2'}$=8.8 Hz

was performed with Silufol UV-254 (Kavalier) analytical plates using the following systems: chloroform:methanol, 4:1 (A); chloroform:ethyl acetate, 1:1 (B); chloroform:methanol:water, 30:10:0.3 (C); 2-propanol:ammonia: water, 7:1:2 (D) and 7:1:4 (E). Adenine was purchased from Lachema, silica gel L 100/160 from Chemapol, and D-xylose from Serva.

9-(2,3,5-Tri-O-benzoyl-β-D-xylofuranosyl)adenine (7) was synthesized according to Neuman et al.[16] N-Benzoyl-9-[2-O-acetyl-3,5-di-O-(*p*-nitrobenzoyl)-β-D-xylofuranosyl]adenine (4) was synthesized according to Poopeiko et al.[14] N-Benzoyl-9-(2,3,5-tri-O-benzoyl-β-D-xylofuranosyl)-adenine (5) was synthesized using the same method as for (4), except that 1-O-acetyl-2,3,5-tri-O-benzoyl-D-xylose (3) was used.

9-[2-O-Acetyl-3,5-di-O-(*p*-nitrobenzoyl)-ß-D-xylofuranosyl]adenine (6). Adenine (1 g, 7.4 mmol) was suspended in 50 mL of dry acetonitrile, and a solution of 3.93 g (7.4 mmol) of 1 in acetonitrile (25 mL) was added. With vigorous stirring, 2 mL (17.1 mmol) of $SnCl_4$ was added, and the reaction mixture was kept at rt for 48 h. To the reaction mixture, 100 mL of chloroform and 5 mL of water were added; $NaHCO_3$ in small portions was then added with stirring until neutral pH was reached. The reaction mixture was filtered and evaporated to dryness, and the residue was dissolved in chloroform. The solution was dried (Na_2SO_4) for 24 h, and evaporated to a small volume, and the product was purified on a silica gel column (eluted with 200 mL each of $CHCl_3$ and $CHCl_3$:EtOH - 99:1; 98:2; 97:3). Fractions containing the desired product were combined and evaporated to dryness yielding 2.8 g (62.1 %) of 6 as a white foam. R_f(A)=0.6. Anal. ($C_{26}H_{21}N_7O_{11}$) C,H,N.

9-(ß-D-Xylofuranosyl)adenine (1) from 4, 5, 6 or 7. The protected nucleoside (1 g) was dissolved in 20 mL of methanolic ammonia (saturated at -10°C) and kept at rt for 24 h. The reaction mixture was evaporated to dryness, and the residue was dissolved in a mixture of 25 mL each of water and ethyl acetate. The aqueous phase was separated and washed with several more volumes of ethyl acetate until TLC (eluent C) of the aqueous phase showed no contamination. After evaporation of the aqueous phase to dryness the resulting residue was crystallized from methanol to yield 67-73% of 1 as a white powder. R_f(C)=0.32, mp 152-154°C (lit[14] mp 154-156°C). λ_{max}(pH=7) 259 nm (ε 14400). Anal. ($C_{10}H_{13}N_5O_4$) C,H,N.

9-(α-L-Arabinofuranosyl)adenine (27). N-Benzoyladenine (17.38 g, 72.6 mmol) was suspended in 150 mL of dry acetonitrile, and 44.3 mL (218 mmol)

of N,O-bis(trimethylsilyl)acetamide was added with stirring. After stirring at rt for 1 h, a solution of 22 g (69.1 mmol) of tetra-O-acetyl-L-arabinofuranose in acetonitrile (100 mL) was added with vigorous stirring, followed by 24.3 mL (208 mmol) of $SnCl_4$. The reaction mixture was heated at reflux for 45 min, then cooled to rt and worked-up as described for compound **6**. After column chromatography (silica gel, eluted with 500 mL of $CHCl_3$:EtOH - 98:2, then with 97:3 until no nucleoside (**26**) could be detected in the eluate), 31.6 g (87.4 %) of a mixture of α and β isomers was obtained as a white foam. This product was dissolved in a mixture of 100 mL of methanol and 100 mL of conc. ammonium hydroxide and heated at 60°C for 2h. The reaction mixture was evaporated to dryness, and the residue was dissolved in water and extracted with 4x150 mL of chloroform. The aqueous phase was mixed with charcoal and stirred for 10 min, then filtered and evaporated to a small volume. The solution was applied to a Dowex 1x8 (20-50 mesh, OH^- form) column, and the products were eluted with water. Fractions containing **27** were combined and evaporated to dryness. Crystallization from methanol gave 12.6 g (65%) of **27** as a white powder, mp 214-215°C (lit[19] mp 216°C). R_f(C)=0.28. λ_{max} (pH=1) 257 nm (ϵ 13733); (pH=7) 259.5 nm (ϵ 15731); (pH=11) 260 nm (ϵ 14728). Anal. ($C_{10}H_{13}N_5O_4$) H,N; C, calcd 44.94; found 45.36.

In several experiments 3-5% of 9-(β-L-arabinofuranosyl)adenine (**27a**) was isolated, mp 185-187°C. R_f(C)=0.13. λ_{max}(pH=1) 256 nm (ϵ 12780); (pH=7) 259 nm (ϵ 13291); (pH=11) 258 nm (ϵ 12575). Anal. ($C_{10}H_{13}N_5O_4 \cdot H_2O$) C,H; N, calcd 24.55; found 24.01.

8-Chloro-9-[2-O-acetyl-3,5-di-O-(p-nitrobenzoyl)-ß-D-xylofuranosyl]-adenine (**8**). Protected xylo-A **6** (5 g, 8.23 mmol) was dissolved in 30 mL of 1,2-dichloroethane, and 2.2 g (19 mmol) of N-chlorosuccinimide was added with stirring. After stirring at rt for 1h, the mixture was cooled at -5 °C for 1h. The cold reaction mixture was filtered and evaporated to dryness. The resulting oil was dissolved in a small amount of a mixture of chloroform:ethyl acetate, 1:1, and purified on a silica gel column (eluted with system B). Fractions containing **8** were collected and evaporated to dryness, yielding 3.3 g (62.4%) of **8** as a slightly yellow foam. R_f(B)=0.5. Anal.($C_{26}H_{20}N_7O_{11}Cl$) C,H,N.

8-Bromo-9-(ß-D-xylofuranosyl)adenine (**9**). Xylo-A, **1**, (1 g, 3.74 mmol) was dissolved in 16 mL of acetate buffer (0.17 M sodium acetate, pH 4). In another flask a mixture of 23 mL of water, 26 mL of the same buffer

and 0.65 mL (14.7 mmol) of bromine was prepared. With vigorous stirring the second mixture was added to the solution of xylo-A, and the reaction mixture was kept at rt for 48 h. The brown precipitate was filtered and dried to give 0.95 g (73%) of **9**, sufficiently pure for reactions with amines. An analytically pure sample of **9**, identical to that obtained by Ikehara et al.,[17] was obtained by crystallizing the crude product from water containing a small amount of ammonia, mp >200°C (dec.). R_f(C)=0.46. λ_{max}(pH=1) 263.5 nm (ϵ 18599); (pH=7) 266 nm (ϵ 17281); (pH=11) 266 nm (ϵ 16183). Anal.($C_{10}H_{12}N_5O_4Br$) C,H,N.

8-Bromo-9-(α-L-arabinofuranosyl)adenine (28). α-L-ara-A (**27**) (1.7 g, 6.36 mmol) was dissolved in 200 mL of acetate buffer (0.17 M sodium acetate, pH 4), and 300 mL of saturated bromine water was added with stirring. The reaction mixture was kept at rt for 24 h, then neutralized with 1N NaOH. The resulting solution was extracted with 6x250 mL of 1-butanol, and the butanolic fraction was separated and extracted with 200 mL portions of 10% Na_2SO_3 solution until the dark color disappeared, and then with 2x200 mL of water. The butanolic solution was evaporated to a small volume and cooled in a refrigerator. Precipitated material was filtered and crystallized from water to give 1.32 g (60%) of **28** as a white powder, mp 213-216°C (dec). R_f(C)=0.44. λ_{max}(pH=1) 263 nm (ϵ 17044); (pH=7) 265 nm (ϵ 16071); (pH=11) 265.5 nm (ϵ 17124). Anal. ($C_{10}H_{12}N_5O_4Br$) C,H,N.

8-(Propylamino)-9-(β-D-xylofuranosyl)adenine (12) from 8. Xylo-A derivative **8** (0.5 g, 0.78 mmol) was suspended in 3 mL of a mixture of 1-butanol:water (96:4), and 2 mL (1.43 g, 24.2 mmol) of propylamine was added. The reaction mixture was stirred at rt for 24 h, and then evaporated to dryness. The resulting oil was dissolved in 3 mL of water, and the solution was extracted with 4x5 mL of diethyl ether. The aqueous phase was evaporated to dryness with silica gel, loaded in dry form on a column of silica gel, and eluted with system C. Fractions containing **12** were collected and evaporated to a small volume and cooled in a refrigerator overnight. Precipitated material was filtered and crystallized from water to give 0.11 g (45.3%) of pure **12**, mp 206-207°C. R_f(C)=0.47. λ_{max}(pH=1) 274 nm (ϵ 16526); (pH=7) 280 nm (ϵ 19316); (pH=11) 281.5 nm (ϵ 18897). Anal.($C_{13}H_{20}N_6O_4 \cdot H_2O$) C,H,N.

General Method for the Synthesis of 8-Amino Nucleosides. Bromonucleoside **9** or **28** (0.5 g, 1.44 mmol) was suspended in 3 mL of 96% 1-butanol, and 2 mL or 2 g of the corresponding amine (12-20 fold excess) was added. The

reaction mixture was heated in a sealed glass vessel at 140°C for up to 2 h. The reaction mixture was cooled to rt and evaporated to dryness, and the residue was co-evaporated several times with ethanol. The resulting oil was dissoved in water and extracted with 6x10 mL of diethyl ether. The aqueous phase was separated and cooled in a refrigerator overnight, and the precipitated material was collected by filtration. The crude products were dissolved in hot water, decolorized with charcoal and allowed to crystallize. Products were dried over KOH.

8-(Propylamino)-9-(ß-D-xylofuranosyl)adenine (12). Yield 71.4%, identical with that obtained from 8 (above).

8-(Butylamino)-9-(ß-D-xylofuranosyl)adenine (13). Yield 58.6%. Mp 216-218°C. R_f(C)=0.49. λ_{max}(pH=1) 274 nm (ϵ 14109); (pH=7) 280 nm (ϵ 17896); (pH=11) 283 nm (ϵ 18761). Anal. ($C_{14}H_{22}N_6O_4 \cdot 2/3H_2O$) C,H,N.

8-(Isobutylamino)-9-(ß-D-xylofuranosyl)adenine (14). Yield 61.4%. Mp 130-132°C. R_f(C)=0.42. λ_{max}(pH=1) 273 nm (ϵ 14221); (pH=7) 280 nm (ϵ 17727); (pH=11) 282 nm (ϵ 18114). Anal. ($C_{14}H_{22}N_6O_4 \cdot 1/3H_2O$) C,H,N.

8-(Heptylamino)-9-(ß-D-xylofuranosyl)adenine (15). Yield 68.4%. Mp 224-226°C. R_f(C)=0.58. λ_{max}(pH=1) 273.5 nm (ϵ 15335); (pH=7) 281 nm (ϵ 20097); (pH=11) 282 nm (ϵ 17854). Anal. ($C_{17}H_{28}N_6O_4 \cdot 1/2H_2O$) C,H,N.

8-(Cyclohexylamino)-9-(ß-D-xylofuranosyl)adenine (16). Yield 67.7%. Mp 154-157°C. R_f(C)=0.61. λ_{max}(pH=1) 271 nm (ϵ 13901); (pH=7) 280.5 nm (ϵ 19461); (pH=11) 283 nm (ϵ 15888). Anal. ($C_{16}H_{24}N_6O_4 \cdot 1/3H_2O$) C,H,N.

8-(1-Piperidinyl)-9-(ß-D-xylofuranosyl)adenine (17). Yield 60.3%. Mp 143-145°C. R_f(C)=0.63. λ_{max}(pH=1) 284.5 nm (ϵ 14667); (pH=7) 276.5 (ϵ 18726); (pH=11) 277 nm (ϵ 16542). Anal. ($C_{15}H_{22}N_6O_4 \cdot 1/2H_2O$) C,N; H, calcd 6.45; found 6.90.

8-(4-Morpholinyl)-9-(ß-D-xylofuranosyl)adenine (18). Yield 58.4%. Mp 150-152°C. R_f(C)=0.64. λ_{max}(pH=1) 283 nm (ϵ 15070); (pH=7) 273 nm (ϵ 17707); (pH=11) 273 nm (ϵ 17355). Anal. ($C_{14}H_{20}N_6O_5 \cdot 1/3H_2O$) C,H; N, calcd 23.45; found 23.91.

8-(4-Aminobutyl)amino-9-(ß-D-xylofuranosyl)adenine (22). Yield 68.5%. Mp 192-194°C. R_f(E)=0.22. λ_{max}(pH=1) 277 nm (ϵ 14485); (pH=7) 279 nm (ϵ 14383); (pH=11) 281.5 nm (ϵ 17676). Anal. ($C_{14}H_{23}N_7O_4 \cdot 1/2H_2O$) C,H,N.

8-(6-Aminohexyl)amino-9-(ß-D-xylofuranosyl)adenine (24). Yield 69.3%. Mp 110-112°C. R_f(D)=0.33. λ_{max}(pH=1) 276 nm (ϵ 14188); (pH=7) 280 nm (ϵ 18394); (pH=11) 282 (ϵ 16426). Anal. ($C_{16}H_{27}N_7O_4 \cdot H_2O$) C,H,N.

8-(Propylamino)-9-(α-L-arabinofuranosyl)adenine (32). Yield 85%. Mp 118-121°C. R_f(C)=0.34. λ_{max}(pH=1) 271 nm (ε 12915); (pH=7) 278 nm (ε 16929); (pH=11) 280.5 nm (ε 16693). Anal. ($C_{13}H_{20}N_6O_4 \cdot 2H_2O$) C,H,N.

8-(Butylamino)-9-(α-L-arabinofuranosyl)adenine (33). Yield 63%. Mp 124-126°C. R_f(C)=0.44. λ_{max}(pH=1) 271 nm (ε 13221); (pH=7) 278 nm (ε 18093); (pH=11) 280.5 nm (ε 17090). Anal. ($C_{14}H_{22}N_6O_4 \cdot H_2O$) H,N; C, calcd 47.18; found 47.66.

8-(Isobutylamino)-9-(α-L-arabinofuranosyl)adenine (34). Yield 65.5%. Mp 108-110°C. R_f(C)=0.48. λ_{max}(pH=1) 270.5 nm (ε 12772); (pH=7) 278.5 nm (ε 16025); (pH=11) 280.5 nm (ε 15141). Anal. ($C_{14}H_{22}N_6O_4 \cdot H_2O$) C,H,N.

8-(Cyclohexylamino)-9-(α-L-arabinofuranosyl)adenine (35). Yield 66%. Mp 151-153°C. R_f(C)=0.51. λ_{max}(pH=1) 269.5 nm (ε 12979); (pH=7) 279.5 nm (ε 16951); (pH=11) 281.5 nm (ε 18715). Anal. ($C_{16}H_{24}N_6O_4 \cdot 1\ 1/2H_2O$) C,H,N.

8-(4-Aminobutyl)amino-9-(α-L-arabinofuranosyl)adenine (37). Yield 69.5%. Mp >200°C (dec.). R_f(D)=0.22. λ_{max}(pH=1) 276.5 nm (ε 12015); (pH=7) 277.5 nm (ε 14241); (pH=11) 280 nm (ε 14740). Anal. ($C_{14}H_{23}N_7O_4 \cdot 1/2H_2O$) C,H,N.

8-(6-Aminohexyl)amino-9-(α-L-arabinofuranosyl)adenine (38). Yield 66.5%. Mp 164-168°C. R_f(D)=0.34. λ_{max}(pH=1) 274.5 nm (ε 11985); (pH=7) 278 nm (ε 15684); (pH=11) 280 nm (ε 15755). Anal. ($C_{16}H_{27}N_7O_4 \cdot H_2O$) C,H,N.

General Method for the Synthesis of 11, 21 and 31. The same synthetic procedure as before was used, except that extraction with diethyl ether was not necessary.

8-(Ethylamino)-9-(ß-D-xylofuranosyl)adenine (11). Yield 70.8%. Mp 260-262°C. R_f(C)=0.26. λ_{max}(pH=1) 274 nm (ε 14021); (pH=7) 279 nm (ε 20155); (pH=11) 281 nm (ε 19962). Anal. ($C_{12}H_{18}N_6O_4 \cdot 1/2H_2O$) C,H,N.

8-(2-Aminoethyl)amino-9-(ß-D-xylofuranosyl)adenine (21). Yield 63.8%. Mp 221-224°C. R_f(E)=0.35. λ_{max}(pH=1) 277.5 nm (ε 18662); (pH=7) 277 nm (ε 18581); (pH=11) 281 nm (ε 16444). Anal. ($C_{12}H_{19}N_7O_4 \cdot 1/2H_2O$) C,H,N.

8-(Ethylamino)-9-(α-L-arabinofuranosyl)adenine (31). Yield 93%. Mp 232-234°C. R_f(C)=0.32. λ_{max}(pH=1) 271.5 nm (ε 14226); (pH=7) 277.5 nm (ε 16973); (pH=11) 280 nm (ε 17401). Anal. ($C_{12}H_{18}N_6O_4$) C,H,N.

General Method for the Synthesis of 19, 20, 23 and 36. Bromonucleoside 9 or 28 (0.5 g, 1.44 mmol) was suspended in 3 mL of 96% 1-butanol, and 2 mL of the corresponding amine was added. The reaction mixture was heated in a sealed glass vessel at 140°C for 2h and evaporated to a smaller volume. The residue was diluted with ethanol and co-evaporated with silica gel.

After purification on a silica gel column (dry loading, eluted with system C in the case of aminoalcohol derivatives and system D in the case of 1,5-diaminopentane), fractions containing the desired product were collected and evaporated to a small volume. The resulting solution was cooled in a refrigerator overnight, and the precipitated material was collected by filtration and crystallized from water. Products were dried over KOH.

8-(2-Hydroxyethyl)amino-9-(ß-D-xylofuranosyl)adenine (19). Yield 42.4%. Mp 126-128°C. R_f(C)=0.15. λ_{max}(pH=1) 276.5 nm (ϵ 16130); (pH=7) 278 nm (ϵ 17731); (pH=11) 280.5 nm (ϵ 16832). Anal. ($C_{12}H_{18}N_6O_5 \cdot 1/2H_2O$) C,H,N.

8-(3-Hydroxypropyl)amino-9-(ß-D-xylofuranosyl)adenine (20). Yield 48.4%. Mp 198-200°C. R_f(C)=0.2. λ_{max}(pH=1) 276 nm (ϵ 14812); (pH=7) 279 nm (ϵ 19015); (pH=11) 281 nm (ϵ 16214). Anal. ($C_{13}H_{20}N_6O_5$) H,N; C, calcd 45.88; found 45.45.

8-(5-Aminopentyl)amino-9-(ß-D-xylofuranosyl)adenine (23). Yield 45%. Mp 145-147°C. R_f(E)=0.27. λ_{max}(pH=1) 276.5 nm (ϵ 13890); (pH=7) 279 nm (ϵ 18320); (pH=11) 282 nm (ϵ 17315). Anal. ($C_{15}H_{25}N_7O_4$) C,H,N.

8-(2-Hydroxyethyl)amino-9-(α-L-arabinofuranosyl)adenine (36). Yield 47.4%. Mp >170°C (dec.). R_f(C)=0.19. λ_{max}(pH=1) 276 nm (ϵ 12722); (pH=7) 276.5 nm (ϵ 16725); (pH=11) 279 nm (ϵ 16746). Anal. ($C_{12}H_{18}N_6O_5 \cdot 1/2H_2O$) C,H,N.

General Method for the Synthesis of 10, 29 and 30. Bromonucleoside 9 or 28 (0.5 g, 1.44 mmol) was dissolved in 50 mL of a 20% aqueous solution of the corresponding amine, and the solution was stirred at rt for 24 h. The reaction mixture was evaporated to dryness, and the dry residue was dissolved in hot water, decolorized with charcoal, and allowed to crystallize. Products were dried over KOH.

8-(Methylamino)-9-(ß-D-xylofuranosyl)adenine (10). Yield 72.3%. Mp >275°C (dec.). R_f(C)=0.14. λ_{max}(pH=1) 274.5 nm (ϵ 14505); (pH=7) 279 nm (ϵ 17097); (pH=11) 281 nm (ϵ 20389). Anal. ($C_{11}H_{16}N_6O_4 \cdot H_2O$) C,H,N.

8-(Methylamino)-9-(α-L-arabinofuranosyl)adenine (29). Yield 47%. Mp 231-234°C. R_f(C)=0.24. λ_{max}(pH=1) 275 nm (ϵ 14590); (pH=7) 279 nm (ϵ 17312); (pH=11) 281.5 nm (ϵ 20292). Anal. ($C_{11}H_{16}N_6O_4 \cdot 1/2H_2O$) C,H,N.

8-(Dimethylamino)-9-(α-L-arabinofuranosyl)adenine (30). Yield 62%. Mp 181-183°C. R_f(C)=0.37. λ_{max}(pH=1) 285.5 nm (ϵ 11699); (pH=7) 275 nm (ϵ 16479); (pH=11) 276 nm (ϵ 16490). Anal. ($C_{12}H_{16}N_6O_4$) C,H,N.

Cytotoxicity Assays. Compounds 19-24 were tested for their influence on human ovary carcinoma (CaOv) cells in the Russian University of Friendship

of Nations, Department of Biochemistry, Faculty of Medicine, and in the Oncological Center of the Russian Academy of Sciences, Moscow. Human ovary carcinoma cells were maintained in monolayer culture at 37 °C in medium 199 with 10% fetal calf serum. Cells were distributed in flasks with 2 mL of medium 199, 10% fetal calf serum, and 160 μg/mL of gentamicin, and cell cultures were observed for 5 days. After the first day medium was replaced by Eagles medium containing 0.1 mM of compounds to be tested. Cells were collected in 3 parallel experiments after 0, 24, 48 and 72 h of cultivation vith compounds. Cells were washed with versene and counted in a Goryaev's camera. Control cell numbers were $1.9x10^5$, $2.1x10^5$, $1.8x10^5$, and $2.6x10^5$ cells/mL at 0, 24, 48 and 72 h, respectively.

Inhibition of [^{3}H]thymidine incorporation in HeLa cells by compounds 10-13 and 21-24 was tested. HeLa cells ($3x10^6$ cells/well) were plated in 24 well plates in Dulbecco's modified Eagles medium plus 10% fetal calf serum. After incubation at 37°C for 24 h, serial dilutions of compounds or diluent (DMSO) were added to duplicate wells. The cells were incubated a further 24 h at 37°C, and [^{3}H]thymidine (1 μCi/mL) was added to each well. After 3 h at 37°C the supernatants were removed, and the cells were lysed by addition of 0.4 M NaOH to each well and incubation for 30 min at 42°C. Macromolecules in the lysate were precipitated with 5% trichloroacetic acid, collected on GF/C filters, and placed in Omnifluor scintillation fluid for counting.

Antiviral Assays. These assays were performed through the National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland. Respiratory viruses. 1) Influenza A & B: virus strains Flu A/Taiwan/1/86 (H1N1), Beijing (H3N2), WSN (H1N1); Flu B/Panama. Cell line: Madin Darby canine kidney cells. 2) Respiratory syncytial virus: Wyde strain in HEp-2 cells. 3) Parainfluenza virus: Para-3 strain in HEp-2 cells. 4) Adenovirus: Adeno-5 strain (ATCC) in HEp-2 cells. 5) Measles virus: AC705 strain in Vero cells. Assays used: cythopathic effect (CPE) inhibition assay for antiviral activity; neutral red uptake assay for cell toxicity. Herpesviruses. 1) HSV-1: E-377 strain in human foreskin fibroblast (HFF) cells. 2) HSV-2: MS strain in HFF cells. 3) Human cytomegalovirus (HCMV): AD169 strain in HFF cells. 4) Varicella zoster virus (VZV): Ellen strain in HFF cells. 5) Epstein-Barr virus (EBV): P3HR strain in Daudi cells. Assays used: semiautomated CPE inhibition assay for HSV-1, HSV-2 and HCMV; plaque reduction assay for VZV; assay for early antigen and viral capsid production for EBV; neutral red uptake assay for cell toxicity.

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