Antiviral Activity of C-Alkylated Purine Nucleosides Obtained by Cross-Coupling with Tetraalkyltin Reagents

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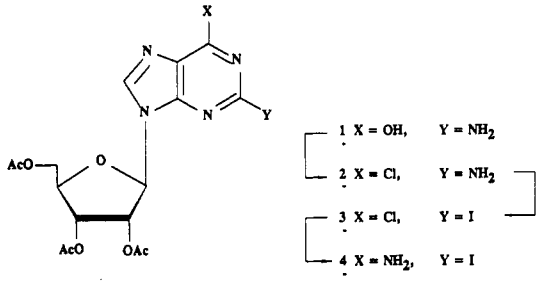
2-, 6-, And 8-alkylated (methyl, ethyl, and vinyl) adenosine analogues were synthesized by a palladium-catalyzed cross-coupling of a tetraalkyltin with the halogenated purine nucleosides. The synthesis of the 8-substituted analogues was accomplished using a transient protection procedure. The 6-alkylated-9-β-D-ribofuranosylpurines as well as 2-ethyladenosine were cytotoxic at relatively low concentrations (0.8–10 μg/mL). 8-Methyladenosine was a potent and selective inhibitor of vaccinia virus, whereas 8-ethyl- and 8-vinyladenosine were specifically inhibitory to respiratory syncytial virus. 8-Vinyladenosine displayed particular activity against herpes simplex virus (type 1).

Introduction

The palladium-catalyzed cross-coupling reaction using organotin reagents is of general use for the formation of C–C bonds in the 2-, 6-, and 8-position of purine nucleosides. These coupling reactions are normally achieved using organotin reagents in the presence of Pd(0) catalysts. Usually, alkenyl and alkynyl groups are introduced. Introduction of an alkyl group, however, is more difficult. 2-Substituted adenosine derivatives were prepared by Nair et al.,[1,2] and the 2-vinyladenosine was synthesized by the palladium-catalyzed cross-coupling reaction of 2-iodoadenosine and vinyl tri-*n*-butylstannane in the presence of $PdCl_2(CH_3CN)_2$.[3]



2-Vinyl-2′-deoxyadenosine and 2-(1-propen-3-yl)-2′-deoxyadenosine were obtained from 2-iodo-2′-deoxyadenosine using vinyl tri-*n*-butylstannane/$PdCl_2(CH_3CN)_2$ and allyl tri-*n*-butylstannane/$Pd(PhP_3)_4$ as reagents.[2] Careful control of the reaction temperature prevented isomerization of the 1-propen-3-yl group to the 1-propen-1-yl group.

Several publications describe the synthesis of 8-alkylated adenosine derivatives. Barton and co-workers were the first to describe the C-8 metalation and successive methylation of 6-(methylamino)-9-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)purine.[4] 8-Methyladenosine was obtained from 6-amino-8-(ethoxycarbonylmethyl)-9-(5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)purine by saponification, decarboxylation, and deprotection.[5] The same compound was also synthesized by lithiation of 2′,3′,5′-tris-*O*-(*tert*-butyldimethylsilyl)adenosine followed by reaction with methyl iodide and deprotection.[6]

Moriarty et al.[7] have described the allylation and vinylation of the 8-position of silyl-protected 8-iodoadenosine, 8-iodo-2′-deoxyadenosine, and 8-iodo-2′,3′-dideoxyadenosine with vinyltributyltin and allyltributyltin. No desilylation procedure was described. A disadvantage of this methodology is that it starts off from the 8-iodo derivatives, which have to be obtained by lithiation and reaction with iodine and which therefore are less readily available. The 8-bromo derivatives of adenine nucleosides, on the other hand, can be synthesized easily. Therefore, a simple alkylation procedure starting from this material is recommended. The recent publication of Kitade et al.,[8] using trimethylaluminum for the introduction of a methyl group in the 8-position of adenosine starting from 8-bromoadenosine, prompted us to report our results using symmetric organotin reagents.[9]

Methods for the preparation of 6-methyl substituted nucleosides are rather limited. These compounds can be obtained either by fusion of 6-methylpurine with the acetylated carbohydrates[10] or, starting from the 6-ethoxycarbonylmethyl derivative,[11] by treatment with alkali followed by decarboxylation under acidic conditions.

Here we describe a straightforward procedure for the synthesis of 2-, 6-, and 8-alkylated purine nucleosides using tetraalkyltin reagents. This procedure has been demonstrated previously to be useful also for the preparation of 5-substituted pyrimidine nucleosides.[12] Especially the methyl- and ethyl-substituted analogues are not easily available by other synthetic methodologies.

A report on C-alkylated purine nucleosides obtained by cross-coupling with trialkylaluminum derivatives has appeared,[13] but it should be pointed out that these reagents are more difficult to handle than the tetraorganotin reagents.

Montgomery, and Hewson reported that 6-methyl-β-D-ribofuranosylpurine (**10a**) has potent cytotoxic activity[10] and that this cytotoxicity seems to be related to phosphorylation of the compound by adenosine kinase. To our knowledge, few, if any, antiviral data have so far been reported for the alkylated purine nucleosides presented here.

Chemistry

The starting materials for the synthesis of the 2-, 6-, and 8-substituted purine nucleosides (**4**, **8**, and **12**,

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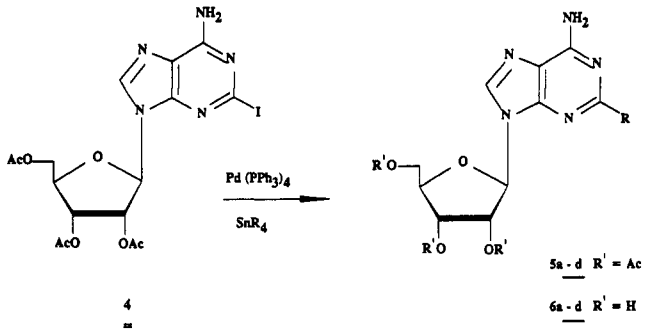
Scheme I



a, R = Me; b, R = Et; c, R = vinyl; d, R = allyl

respectively) were obtained as follows: 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-6-iodopurine (**8**) and 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2-iodo-6-chloropurine (**3**) were synthesized from 2′,3′,5′-tri-*O*-acetyladenosine[14] (**7**) and 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2-amino-6-chloropurine[15] (**2**), using isoamyl nitrite and diiodomethane in acetonitrile.[16] To convert the 2-iodo-6-chloropurine analogue **3** into 2′,3′,5′-tri-*O*-acetyl-2-iodoadenosine (**4**), 1,2-dimethoxyethane was used as a solvent[15] instead of ethanol.[16] 8-Bromoadenosine,[17] 8-bromo-2′-deoxyadenosine,[17] and 8-bromo-2′,3′-dideoxyadenosine[18] were obtained by bromination as originally described by Ikehara and Kaneko.[17]

Reaction of **4** with tetramethyltin in *N*-methylpyrrolidinone (NMP) in the presence of palladium(0) tetrakis(triphenylphosphine) [$Pd(Ph_3P)_4$] in an inert atmosphere afforded **5a** in about 80% yield. The reaction was carried out at 60° for 20 h. When the reaction temperature was raised to 80°, the reaction time could be reduced to 2 h without notable side reactions. The same reaction temperature was used for the synthesis of **5c** using tetravinyltin (4 h) and **5d** using tetraallyltin (14 h). The latter reaction proceeded in a much slower rate, and the temperature had to be raised to 110 °C in order to achieve a 2-h reaction time. These reaction circumstances did not give isomerization of the 1-propen-3-yl group to the 1-propen-1-yl group. Tetraethyltin is less reactive, and a temperature up to 140 °C was needed to complete the 2-alkylation reaction. This order of reactivity [Me_4Sn > $(CH_2{=}CH{-}CH_2)_4Sn$ > Et_4Sn] was also observed for the 6- and 8-alkylation reaction. All 2-substituted adenosine analogues (**5a–d**) could be deprotected with ammonia in methanol without yielding side reactions (**6a–d**). Compounds **6b** and **6c** were also obtained by Nair et al.[3]

Alkylation at the 6-position of adenosine was performed starting from 6-iodo-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**8**). Reaction of **8** with tetramethyltin in the presence of $Pd(Ph_3P)_4$ at 85 °C for 20 h gave a 53% yield of **9a** which was deprotected to give 6-methyl-9-(β-D-ribofuranosyl)purine (**10a**). This is the most straightforward synthesis of **10a** described in literature.[10,11] Vinylation of **8** afforded a 88% yield of **9b**, which was deprotected to give 6-vinyl-9-(β-D-ribofuranosyl)purine (**10b**). Allylation was hampered due to isomerization of the allyl moiety. Even at the low temperature of 85 °C, the reaction yielded an intractable mixture. Therefore, the synthesis of this compound was not pursued any further.

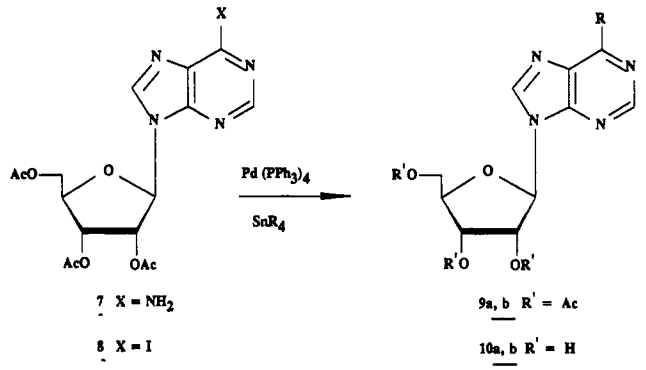
For the synthesis of the 8-alkylated analogues, a one pot procedure was worked out, using transient silylation followed by the cross-coupling reaction. This transient protection procedure avoids side reactions during the removal of the protecting groups. Usual protecting groups like *tert*-butyldimethylsilyl and acyl groups can be removed by nucleophiles (fluoride, ammonia, methanolate, etc). Yet, these reagents give rise to conjugate addition reactions, as noted during the synthesis of the 8-vinyl analogues starting from sugar-protected derivatives.

Table I. Reaction Conditions for Cross-Coupling

starting material	reagent (*a–d*)	temp (°C)	time (h)	reaction product	yield (%)
4	*a*	80	2	**5a**	80
4	*b*	140	5	**5b**	60
4	*c*	80	4	**5c**	95
4	*d*	110	2	**5d**	45[e]
8	*a*	85	20	**9a**	53
8	*c*	80	1	**9b**	88
12a	*a*	110	14	**13a**	74
12a	*b*	130	16	**14a**	80
12a	*c*	110	14	**15a**	70
12b	*a*	110	2	**13b**	92
12b	*b*	130	14	**14b**	87
12b	*c*	110	14	**15b**	75
12c	*a*	110	2	**13c**	72
12c	*c*	110	14	**15c**	65

[a] Me_4Sn. [b] Et_4Sn. [c] Tetravinyltin. [d] Tetraallyltin. [e] 4 h reaction at 100 °C for complete disappearance of starting material and 14 h at 80 °C.

Scheme II

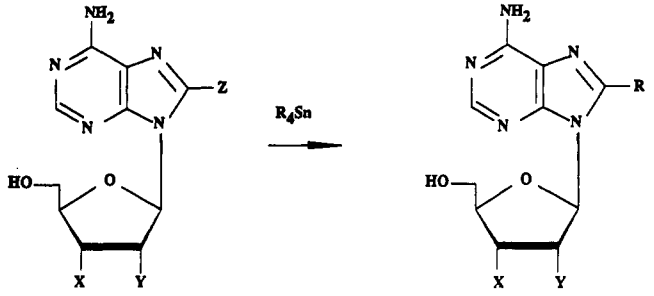


a R = Me; b, R = vinyl

Refluxing of **12a** with hexamethyldisilazane in dioxane for 8 h yielded persilylated 8-bromoadenosine. The residue was dissolved in NMP and $Pd(Ph_3P)_4$, the tetraorganotin was added, and the mixture was heated as indicated in Table I. The purification of the reaction products is facilitated because the presence of the trimethylsilyl ethers allows their extraction in ethyl acetate. The organic solvent (NMP) is removed by washing with water, after which the trimethylsilyl ethers can be removed with potassium carbonate or with ammonium chloride in methanol. Also 8-methyladenosine **13a**, 8-ethyladenosine **14a**, and 8-vinyladenosine **15a** were obtained by this procedure in high yield. By condensing persilylated 8-bromoadenosine with tetraallyltin in NMP in the presence of $Pd(Ph_3P)_4$, mainly isomerization to 8-(1-propen-1-yl)adenosine, besides reduction to adenosine, took place. Moriarty et al.[7] obtained higher yields of the protected 8-allyl derivative using shorter reaction time (30 min) at 145 °C starting from the more reactive 8-iodoadenosine.

To test the general applicability of this methodology, we repeated this reaction on more labile nucleosides, i.e., 2′-deoxyadenosine and 2′,3′-dideoxyadenosine, the latter because of its potential in the chemotherapy of human immunodeficiency virus (HIV) infections. As can be inferred from Table I, alkylation of the 8-position of these

Scheme III



11 a-c Z = H

12 a-c Z = Br

13 a-c R = Me

14 a-b R = Et

15 a-c R = vinyl

a, X = Y = OH; b, X = OH, Y = H; c, X = Y = H

labile nucleosides occurred quite readily. The reaction products were obtained in yields varying between 65% and 92% using the transient protection methodology.

In conclusion, the use of tetramethyltin for carrying out cross-coupling reaction on the 2-, 6-, and 8-position of purine nucleosides has allowed the preparation of 6-methyl-9-(β-D-ribofuranosyl)purine **10a**, 2-methyladenosine **6a**, and 8-methyladenosine **13a** in a straightforward manner. The reactions can be extended to 2′-deoxynucleosides and 2′,3′-dideoxynucleosides, as proven by the synthesis of **13b** and **13c**. This methodology was also used for the synthesis of the vinyl (**6c**, **10b**, **15a**, **15b**, and **15c**) and ethyl (**6b**, **14a**, and **14b**) analogues. The synthesis of the allyl analogues is hampered mainly due to isomerization reactions, in particular for the 6- and 8-position. Finally, 8-alkylated adenosine analogues can now be made available through a single one pot reaction starting from the 8-bromo analogues.

Antiviral Activity

All C-alkylated purine nucleoside analogues were examined for their activity against herpes simplex virus (HSV) type 1 and type 2, vaccinia virus (VV), and vesicular stomatitis virus (VSV) in ESM cell cultures; against reovirus type 1, parainfluenza virus type 3, Sindbis, Coxsackie B4 virus, and Semliki forest virus in Vero cells; against VSV, Coxsackie B4, polio type 1, and respiratory syncytial virus (RSV) in HeLa cell cultures; against influenza virus A and B in Madin–Darby canine kidney (MDCK) cells; and against HIV-1 and HIV-2 in CEM cells.

Only in a few instances was a selective antiviral effect noted, as shown in Tables II and III (results of other antiviral assays not shown). As was reported before,[10] 6-methyl-9-(β-D-ribofuranosyl)purine **10a** was quite cytotoxic. Therefore, the activity found with **10a** against HSV-1 and VSV [minimal inhibitory concentration (MIC): 0.4 μg/mL] cannot be considered as specific. However, against VV **10a** showed activity at 0.04 μg/mL, i.e., at a 25-fold lower concentration than its minimal cytotoxic concentration (MCC).

Much more striking is the highly selective inhibitory action of 8-methyladenosine **13a** against VV. The MIC of **13a** for VV was 0.2 μg/mL and as it was virtually not cytotoxic for the host (ESM) cells at a concentration of 200 μg/mL its selectivity index in this system could be estimated at ≥1000. The activity of **13a** against VV compared favorably with that of carbocyclic 3-deazaadenosine (C-c^{3}Ado, MIC: 2 μg/mL, Table II) and of 3′-deoxy-3′-fluoroadenosine (MIC: 1 μg/mL).[19] Moreover, compound **13a** was not active against any other of the viruses tested, except for RSV (MIC: 4 μg/mL). However, this was only 5-fold lower than the MCC for HeLa cells (Table III).

Table II. Inhibitory Effects of Selected Compounds on Virus-Induced Cytopathicity in E$_6$SM Cells

compd	MCC[a] (μg/mL)	HSV-1 (KOS strain)	MIC[b] (μg/mL) VV	MIC[b] (μg/mL) VSV
6b	≥10	>10	7	>10
10a	1	0.4	0.04	0.4
10b	10	2	>4	>4
13a	≥200	>100	0.2	>200
14a	≥100	>100	70	>100
15a	>400	2	7	>400
BVDU	>400	0.02	0.4	>400
ribavirin	>400	300	70	20
C-c^{3}Ado	>400	>400	2	0.7

[a] Minimum cytotoxic concentration required to cause a microscopically detectable alteration of normal cell morphology. [b] Minimum inhibitory concentration, required to reduce virus-induced cytopathicity by 50%.

Table III. Inhibitory Effects of Selected Compounds on RSV-Induced Cytopathicity in HeLa Cells

compd	MCC[a] (μg/mL)	MIC[b] (μg/mL)
6b	4	0.8
10a	0.8	0.4
10b	4	>4
13a	20	4
14a	100	10
15a	70	4

[a] Minimum cytotoxic concentration required to cause a microscopically detectable alteration of normal cell morphology. [b] Minimum inhibitory concentration, required to reduce virus-induced cytopathogenicity by 50%.

Introduction of an ethyl (**14a**) or vinyl (**15a**) group at the 8-position of adenosine left the inhibitory activity against RSV unaltered but lowered toxicity, so that the selectivity index of these compounds increased to about 15. Replacement of the 8-methyl group by 8-ethyl resulted in a virtual loss of anti-VV activity (compare **13a** with **14a**); **14a** was also inactive against HSV-1. The 8-vinyl derivative **15a**, however, gained marked activity against HSV-1 (MIC: 2 μg/mL), while it was less inhibitory to HSV-2 (MIC: 40 μg/mL). The selectivity index of **15a** for HSV-1 could be estimated at >200.

Within the series of C2-alkylated analogues (**6a–d**), 2-ethyladenosine **6b** was rather cytotoxic (MCC: 4 μg/mL for HeLa, MDCK, and Vero cells). Therefore, its activity against RSV (MIC = 0.8 μg/mL), influenza A and B (MIC = 0.8 μg/mL), and Sindbis virus (MIC = 2 μg/mL) cannot be considered as specific antiviral effects.

The 8-alkylated 2′-deoxynucleosides did not prove inhibitory to any of the viruses tested. Remarkable also is the total lack of activity of 2′-deoxy-8-methyladenosine (**13b**) against VV (MIC: >400 μg/mL).

The 2′,3′-dideoxynucleosides **13c** and **15c** did not show any inhibition of HIV-1 or HIV-2 in CEM cells (MIC: >100 and >20 μg/mL, respectively, data not shown). This absence of inhibitory activity is consistent with the previously reported inactivity of 8-bromodideoxyadenosine against HIV.[20]

Conclusion

From this study, the following compounds emerged as *particular* selective antiviral agents that should be worth

pursuing for their specific antiviral potential: 8-methyladenosine (**13a**) against vaccinia virus, 8-vinyladenosine (**15a**) against herpes simplex virus (type 1), and, albeit to a lesser extent, both 8-ethyladenosine (**14a**) and 8-vinyladenosine (**15a**) against respiratory syncytial virus.

Experimental Section

All methods and reagents were as previously described.[21] Tetraorganotin reagents and palladium derivatives were obtained from Aldrich, isoamyl nitrite and NMP were purchased from Janssen Chimica. Significant analytical data for new compounds and known compounds having no ^{1}H NMR data in the literature are given below.

The compounds **6a**,[22,23] **6b**,[23] **10a**,[10,24] **13a**,[6,8] **13b**,[25] **14a**,[5] and **14b**[13] have been fully described previously.

General Procedure for the Synthesis of 2-Alkyladenosine Derivatives. An amount of 1.56 g (3 mmol) of **4** and 250 mg (0.3 mmol) of $Pd(PPh_3)_4$ were dissolved in 7 mL of NMP, and 6 mmol of the respective tetraalkyltin reagents were added under nitrogen. The mixture was heated at the temperature and time indicated in Table I. Afterwards, this mixture was partitioned between 100 mL of EtOAc and 50 mL of water. The organic phase was filtered over Celite in order to remove small precipitates, washed with water (2 × 40 mL) and brine (40 mL), and dried (Na_2SO_4). Evaporation left an oil which was purified on silica gel eluted with hexane (200 mL) and CH_2Cl_2 (200 mL) to remove traces of NMP, followed by CH_2Cl_2–MeOH (98.5:1.5) to yield the protected 2-alkylated adenosine derivatives as amorphous powders. The acetyl groups were removed by treatment with either ammonia in MeOH or K_2CO_3 in MeOH (suspension). 6-Vinyl-9-β-D-ribofuranosylpurine (**10b**) is very prone to nucleophilic attack by ammonia. Deprotection was achieved by overnight treatment of 1 mmol of the protected derivatives with 50 mg of K_2CO_3 in 20 mL of methanol. The resulting mixture was adsorbed onto 1–2 g of silica gel and purified on a small silica gel column (20 g, elution CH_2Cl_2–MeOH from 98:2 to 92:8).

Synthesis of 6-Alkyl-9-(β-D-ribofuranosyl)purine Derivatives. An amount of 1.51 g (3 mmol) of **8** and 350 mg (0.3 mmol) of $Pd(PPh_3)_4$ were dissolved in 7 mL of NMP, and 6 mmol of the respective tetraalkyltin reagent were added. The mixture was heated as indicated in Table I and worked up as described for the 2-alkylated adenosines affording the title compounds.

General Synthesis of the 8-Alkyladenosine Derivatives. A mixture of 1.04 g (3 mmol) of 8-bromoadenosine (**12a**)[17] and 10 mL of HMDS was refluxed in 20 mL of anhydrous dioxane for 8 h, after which TLC on silica gel (CH_2Cl_2–MeOH, 95:5) indicated complete silylation. The volatiles were removed *in vacuo*, and the residue is coevaporated once with anhydrous toluene.

Under a N_2 stream, the residue was dissolved in 6 mL of NMP and 350 mg (0.3 mmol) of $Pd(PPh_3)_4$, and 6 mmol of the respective tetraalkyltin derivative was added. The mixture was heated at the indicated temperature. Aliquots can be taken at different time intervals for TLC analysis after partitioning between EtOAc and water (CH_2Cl_2–MeOH, 98:2).

After disappearance of the starting material, the mixture was partitioned between 100 mL of EtOAc and 50 mL of water. The organic phase was filtered over Celite to remove precipitates and was washed with water (3 × 50 mL) and with brine (50 mL). The organic layer was dried (Na_2SO_4) and evaporated, and the remaining oil was dissolved in 80 mL of MeOH to which 10 mL of water and 0.8 g of NH_4Cl were added. The mixture was refluxed till complete conversion to the deprotected nucleoside derivatives. After removal of the volatiles in vacuo and coevaporation with dioxane, the residue was dissolved in MeOH and adsorbed onto 3 g of silica gel. Chromatographic purification (20 g of silica gel, CH_2Cl_2–MeOH, 9:1) afforded the desired compound. Analytical samples were obtained after a second purification on preparative TLC plates.

Synthesis of 8-Alkyl-2′-deoxyadenosine Derivatives. The same modus operandi was followed starting with 0.99 g (3 mmol) of 8-bromo-2′-deoxyadenosine (**12b**).[17] Hydrolysis of the silyl ethers was accomplished by dissolving the residue, obtained after extraction with EtOAc, in 60 mL of MeOH and adding 0.5 g of K_2CO_3. The mixture was stirred overnight, 2 g of silica gel was added, and after removal of the solvent, the residue was put on top of a small silica gel column. Purification was accomplished with CH_2Cl_2–MeOH, 98:2 to 95:5.

8-Alkylation of 2′,3′-Dideoxyadenosine. 8-Bromo-2′,3′-dideoxyadenosine[18] (**12c**, 200 mg, 0.64 mmol) was refluxed in 10 mL of dioxane with 3 mL of HMDS for 6 h. After removal of the solvent and coevaporation with toluene, the residue was dissolved in 4 mL of NMP to which 60 mg of $Pd(PPh_3)_4$ and 0.5 g of the respective tetraalkyltin derivative were added. Workup was done as for the 2′-deoxyadenosine analogues.

2-Vinyladenosine (6c).[3] Mp (MeOH) 262–263 °C dec; ^{1}H NMR (DMSO-d_6) δ 5.56 (dd, J = 3.3 Hz and 9.5 Hz, vinylic-H), 5.89 (d, J = 6.2 Hz, H-1′), 6.43 (d, 1 H, J = 3.2 Hz), 6.55 (d, 1 H, J = 9.5 Hz) (vinylic-H), 7.25 (br s, NH_2), 8.33 (s, H-8) ppm; ^{13}C NMR (DMSO-d_6) δ 61.8 (C-5′), 70.8 (C-3′), 73.4 (C-2′), 85.9 (C-4′), 87.9 (C-1′), 118.6 (C-5), 121.0 (*C*H_2=CH-), 137.1 (CH_2=*C*H-), 140.3 (C-8), 149.8 (C-4), 155.7 (C-6), 157.9 (C-2) ppm; UV (MeOH) λ_{max} 239 (11 900), 265 (13 100), 270 (12 800), 290 (sh, 6500) nm; CIMS (iC_4H_{10}) m/z 294 (M + H), 162 (B + 2 H); Anal. ($C_{12}H_{15}N_5O_4$) C, H, N.

2-Allyladenosine (6d). The product was obtained as a hygroscopic foam: ^{1}H NMR (DMSO-d_6) δ 3.40 (dt, J = 6.5 Hz and J = 1.3 Hz, *C*H_2-C2), 4.99 (t, 1 H, J = 1 Hz), 5.15 (dt, 1 H, J = 7.6 Hz and 1 Hz) (*C*H_2=CH-), 5.84 (d, J = 6.6 Hz, H-1′), 5.8–6.1 (m, 1 H, allylic-H), 7.25 (br s, NH_2), 8.25 (s, H-8) ppm; ^{13}C NMR (DMSO-d_6) δ 43.1 (CH_2-C2), 61.9 (C-5′), 70.9 (C-3′), 73.4 (C-2′), 86.1 (C-4′), 88.0 (C-1′), 116.0 (*C*H_2=CH-), 117.8 (C-5), 135.7 (CH_2=*C*H-), 139.7 (C-8), 149.8 (C-4), 155.9 (C-6), 162.5 (C-2) ppm; UV (MeOH) λ_{max} 262 nm; CIMS (iC_4H_{10}) m/z 308 (M + H), 176 (B + 2 H); Anal. ($C_{13}H_{17}N_5O_4 \cdot 1.5H_2O$) C, H, N.

6-Vinyl-9-β-D-ribofuranosylpurine (10b). Mp 132 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.65 (m, H-5′, H-5″), 4.02 (q, H-4′), 4.22 (m, H-3′), 4.61 (t, J = 5 Hz after D_2O exchange, H-2′), 5.16 (br), 5.25 (br), 5.55 (br) (3 × OH), 5.98 (dd, J = 3 Hz and 9.8 Hz, *C*H=CH_2), 6.07 (d, J = 5 Hz, H-1′), 7.00 (dd, J = 3 Hz and 15 Hz, vinylic-H), 7.24 (dd, J = 9.8 Hz and 15 Hz, vinylic-H), 8.85 (s), 8.90 (s) (H-2, H-8) ppm; ^{13}C NMR (DMSO-d_6) δ 61.7 (C-5′), 70.7 (C-3′), 74.2 (C-2′), 86.1 (C-4′), 88.3 (C-1′), 127.4 (*C*H=CH_2), 131.3 (C-5), 132.1 (CH=CH_2), 145.5 (C-8), 152.1 (C-4), 152.4 (C-2), 153.0 (C-6) ppm; UV (MeOH) λ_{max} 238 (4700), 262 (sh), 272 (sh), 285 (10 100) nm; CIMS (iC_4H_{10}) m/z 279 (M + H), 147 (B + 2 H); Anal. ($C_{12}H_{14}N_4O_4$) C, H, N.

8-Methyl-2′,3′-dideoxyadenosine (13c). Mp (MeOH–acetone) 166–167 °C; ^{1}H NMR (DMSO-d_6) δ 2.56 (s, CH_3), 6.15 (t, H-1′), 7.05 (br s, NH_2), 8.06 (s, H-2) ppm; ^{13}C NMR (DMSO-d_6) δ 14.6 (CH_3), 26.6, 29.1 (C-2′, C-3′), 63.7 (C-5′), 80.5 (C-4′), 85.0 (C-1′), 118.0 (C-5), 148.8 (C-8), 149.9 (C-4), 151.3 (C-2), 155.2 (C-6) ppm; UV (MeOH) λ_{max} 261 (15 400) nm; CIMS (iC_4H_{10}) m/z 250 (M + H), 150 (B + 2 H); Anal. ($C_{11}H_{15}N_5O_2$) C, H, N.

8-Vinyladenosine (15a). Mp (H_2O–CH_3OH) 245 °C dec; ^{1}H NMR (DMSO-d_6) δ 5.75 (dd, J = 2 Hz and 11.4 Hz, vinylic-H), 6.00 (d, J = 6.8 Hz, H-1′), 6.37 (dd, J = 16.4 Hz and 2 Hz, vinylic-H), 7.10 (dd, J = 16.4 Hz and 11.4 Hz, vinylic-H), 7.35 (br s, NH_2), 8.15 (s, H-2) ppm; ^{13}C NMR (DMSO-d_6) δ 62.0 (C-5′), 70.7 (C-3′), 72.4 (C-2′), 86.5 (C-4′), 88.0 (C-1′), 119.0 (C-5), 123.2 (*C*H_2-vinyl), 124.3 (*C*H-vinyl), 148.0 (C-8), 150.0 (C-4), 152.1 (C-2), 155.9 (C-6) ppm; UV (MeOH) λ_{max} 229 (22 500), 294 (12 600) nm; CIMS (iC_4H_{10}) m/z 294 (M + H), 162 (B + 2 H); Anal. ($C_{12}H_{15}N_5O_4$) C, H, N.

8-Vinyl-2′-deoxyadenosine (15b). Mp (MeOH–acetone) 205 °C dec; ^{1}H NMR (DMSO-d_6) δ 5.67 (dd, J = 1.7 Hz and 11 Hz, vinylic-H), 6.34 (dd, J = 2 Hz and 17 Hz, vinylic-H), 6.42 (t, H-1′), 7.15 (dd, J = 11 Hz and 16.7 Hz, vinylic-H), 7.35 (s, NH_2), 8.11 (s, H-2) ppm; ^{13}C NMR (DMSO-d_6) δ 38.6 (C-2′), 61.9 (C-5′), 70.9 (C-3′), 83.8 (C-4′), 87.9 (C-1′), 118.9 (C-5), 122.7 and 124.4 (vinylic-C), 147.5 (C-8), 149.8 (C-4), 151.8 (C-2), 155.8 (C-6) ppm; UV (MeOH) λ_{max} 230 (23 000), 294 (12 600) nm; CIMS (iC_4H_{10}) m/z 278 (M + H), 162 (B + 2 H); Anal. ($C_{12}H_{15}N_5O_3$) C, H, N.

8-Vinyl-2′,3′-dideoxyadenosine (15c). Mp (MeOH–acetone) >280 °C; ^{1}H NMR (DMSO-d_6) δ 5.65 (dd, J = 2.2 Hz and 11 Hz, vinylic-H), 6.31 (t, J = 5.7 Hz, H-1′), 6.33 (dd, J = 2 Hz and 17 Hz, vinylic-H), 7.15 (dd, J = 11 Hz and 17 Hz, vinylic-H), 7.27 (s, NH_2), 8.11 (s, H-2) ppm; ^{13}C NMR (DMSO-d_6) δ 26.4, 29.6 (C-2′, C-3′), 63.5 (C-5′), 80.5 (C-4′) 84.5 (C-1′), 118.8 (C-5), 122.3, 124.6 (vinylic-C), 147.5 (C-8), 149.7 (C-4), 152.0 (C-2), 155.7 (C-

6) ppm; UV (MeOH) λ_{max} 229 (23 300), 294 (12 100) nm; CIMS (iC_4H_{10}) m/z 262 (M + H), 162 (B + 2 H); Anal. ($C_{12}H_{15}N_5O_2$) C, H, N.

Antiviral Assay Procedures. The virus stocks were prepared as described previously: influenza A virus (strain Ishikawa/7/82(H_3N_2)) and influenza B virus (strain Singapore/222/79);[26] RSV (strain Long);[27] parainfluenza virus type 3 (strain VR-93);[28] HSV-1 (strain KOS), HSV-2 (strain G) and HSV-1 TK$^-$ (strain Field C/37/101);[29] vaccinia virus, vesicular stomatitis virus, Coxsackie virus type B4, Sindbis virus and poliovirus type 1;[30] reovirus type 1 [American Type Culture Collection, Rockville, MD (ATCC) VR-230], Semliki forest virus (ATCC VR-67); HIV-1 (strain HTLV-III_B/LAI) and HIV-2 (strain LAV-2 ROD).[31] HIV-1 and HIV-2 were prepared from the culture supernatants of HIV-1- or HIV-2-infected cell lines (HUT-78/HTLV-IIIB and MT4/LAV-2 ROD, respectively).

The cell lines used for the antiviral assays were MDCK (Madin–Darby canine kidney) epithelial cells, Vero (an epithelial cell line derived from African green monkey), HeLa (a human epithelial cell line derived from a cervix carcinoma), ESM (human embryonic skin muscle) fibroblast, and MT-4 (a human T-cell line established by cocultivation of normal human cord blood leucocytes with T-lymphocytes from an adult T-cell leukemia patient). The cells were cultivated in Eagle's minimum essential medium (MEM) (Gibco) supplemented with 3 mM glutamine, 0.07% bicarbonate and 10% fetal calf serum, except for MT-4 cells which were cultivated in RPMI-1640 medium (Gibco).

Inhibition of virus-induced cytopathicity and cytotoxicity measurements were performed as described previously,[28] for anti-HIV assays, see ref 31.

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