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Synthesis and Antiherpetic Activity of (*Z*)- and (*E*)-9-(3-Phosphonomethoxyprop-1-en-yl)adenines

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Abstract—The isomeric (*Z*)- and (*E*)-9-(3-phosphonomethoxyprop-1-en-yl)adenines were synthesized. The stereoselectivity of double bond formation was studied by variation of sulfonyl groups. The resulting phosphonates exhibited a moderate antiherpetic activity in a culture of Vero cells infected with herpes simplex type 1 virus. The *Z*-isomer was shown to be a more effective inhibitor of virus reproduction in the case of both wild and acyclovir-resistant strains.

Key words: herpes simplex virus, nucleotides, phosphonates

INTRODUCTION

Acyclic nucleoside and nucleotide analogues constitute a relatively new group of antiviral agents. Both a variety of structures and a wide range of antiviral activities are characteristic of them.[2] For example, synadenol (**I**) and relative compounds inhibit the reproduction of HSV, HIV, and hepatitis B viruses [1–3]. Compounds containing the phosphonomethoxy fragment, e.g., PMEA (**II**) and (*S*)-9-(3-hydroxy)-2-phosphonomethoxypropyl)adenine (**III**), which inhibit both the HIV and HSV replication, belong to another group of analogues [4]. Moreover, a number of such acyclic analogues are already used in clinical practice as drugs, e.g., acyclovir (Zovirax®), famcyclovir (Famvir®), penciclovir (Denavir®), etc. [5].

RESULTS AND DISCUSSION

We herein report the synthesis of two new acyclic nucleotide analogues containing the heterocyclic base-conjugated double bond like that in synadenol (**I**) and the phosphonomethoxy fragment like that in PMEA (**II**). The *Z*- and *E*-isomers of 9-(3-phosphonomethoxyprop-1-en-yl)adenine (**X**) and (**XII**) were actually synthesized (Scheme 1) and their antiherpetic activities were studied.

The synthesis of (**X**) and (**XII**) consisted in obtaining a glycerol-based alkylating agent (**IVa**) and its further coupling with adenine sodium salt. The resulting mixture of alkenes (**Va**) and (**VIIa**) was separated, and the individual isomers were treated with acetic acid to remove trityl groups. Phosphonomethylation of alcohols (**VI**) and (**VIII**) and subsequent deprotection resulted in the target *Z*- and *E*-phosphonates (**X**) and (**XII**).

Several approaches to the synthesis of alkenes bearing the double bond conjugated with heterocyclic aromatic system have been reported in literature. One of the them involves the obtaining of substance with a nonconjugated double bond and its further isomerization by heating with a base. For example, (*Z*)- and (*E*)-isomers of 9-(4-hydroxybut-1-en-1-yl)adenine were obtained by heating of the corresponding but-2-en-1-yl derivatives in the presence of potassium *tert*-butylate [6]. Note that this method is inappropriate for obtaining propene derivatives. Another strategy includes the alkylation of a heterocyclic base with synthons containing vicinal bromine atoms and subsequent HBr elimination [1]. We chose this approach for the synthesis of target alkoxypropenyladenines (**V**) and (**VII**) (Scheme 2). Alkylating agents (**IVa**)–(**IVc**) were synthesized from glycerol through the protection of one of the primary hydroxyl groups with trityl or TBDMS group and subsequent introduction of two sulfonyl groups. The resulting synthons (**IVa**)–(**IVc**) were used for the alkylation of adenine in the presence of a base, sodium hydroxide or potassium bis(trimethylsilyl)amide.

The reaction of (**IVa**) with adenine in the presence of sodium hydride yielded the mixture of *Z*- and *E*-isomers of 9-(3-trityloxyprop-1-en-1-yl)adenine (**Va**) and (**VIIa**). Note that the reaction in the presence of insufficient quantity of sodium hydride resulted in 9-(2-

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[2] Abbreviations: HSV-1/L_2, herpes simplex virus type 1 strain L_2; HSV-1/L_2/R, acyclovir-resistant herpes simplex virus type 1 strain L_2; ID_{50} and ID_{95}, doses inhibiting virus cytopathogenic activity by 50 and 95%, respectively; IMP, ethyl iodometylphosphonate; Ms, mesyl (methanesulfonyl); PMEA, 9-(2-phosphonomethoxyethyl)adenine; SI, selectivity index (the TCD_{50} : ID_{50} ratio); TBDMS, *tert*-butyldimethylsilyl; TCD_{50}, tissue cytotoxicity dose responsible for the change in 50% of cell monolayer; Tr, trityl (triphenylmethyl); and Ts, tosyl (*p*-toluenesulfonyl).

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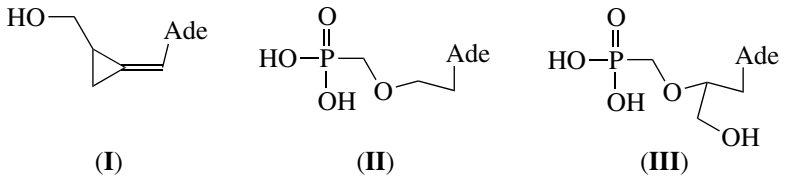


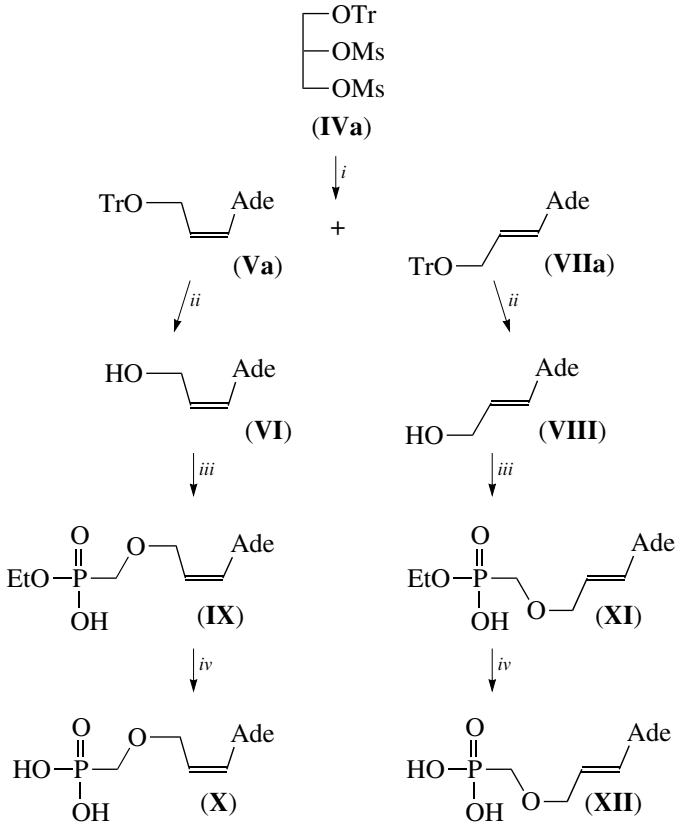
(**I**) (**II**) (**III**)

mesyloxy-3-trityloxypropyl)adenine (**XIIIa**) as the main product, whereas the use of excess sodium hydride excludes the intermediate (**XIIIa**). It seems likely that the alkylation of heterocyclic base rather than elimination of 2'-mesyl group is a rate-limiting stage under these conditions.

The separation of *Z*- and *E*-isomers of trityl derivatives (**Va**) and (**VIIa**) was achieved by crystallization from chloroform. *E*-Alkene (**VIIa**) is much worse soluble in chloroform and can be obtained as pure substance even after a single crystallization. To achieve a more complete separation of the *Z*-isomer, the mother liquors were evaporated, and the residue was repeatedly crystallized from chloroform. Thus, the final mother liquor contained approximately 90% of the *Z*-isomer (**Va**). After the removal of the protecting group, *Z*-isomer (**VI**) was purified by chromatography on silica gel.

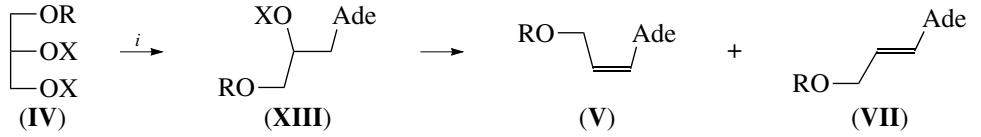
The configuration of the double bond was determined by ^{1}H NMR spectroscopy. In the case of *Z*-alkenes (**Va**) and (**VI**), the spin–spin coupling constant $^3J_{1',2'}$ was ~8.5 Hz, whereas *E*-isomers (**VIIa**) and (**VIII**) had the constant exceeded 14 Hz.

The ratio of *Z*- and *E*-isomers (**Va**) and (**VIIa**) in the reaction mixture was 1 : 3.5 when using dimesyltrilyl glycerol (**IVa**). To alter this ratio, we have substituted ditosyltrityl glycerol (**IVb**) or ditosylsilyl derivative (**IVc**) for dimesyltrityl glycerol (**IVa**). However, the transition from mesyl to the more hydrophobic and bulky tosyl groups did not change the ratio of isomers (**Va**) and (**VIIa**), although a similar yield was achieved at a decreased reaction time (from 8 to 6 h). The ratio of



(*i*) AdeH, NaH/DMF; (*ii*) CH_3COOH; (*iii*) IMP, NaH/DMF; (*iv*) Me_3SiBr/DMF.

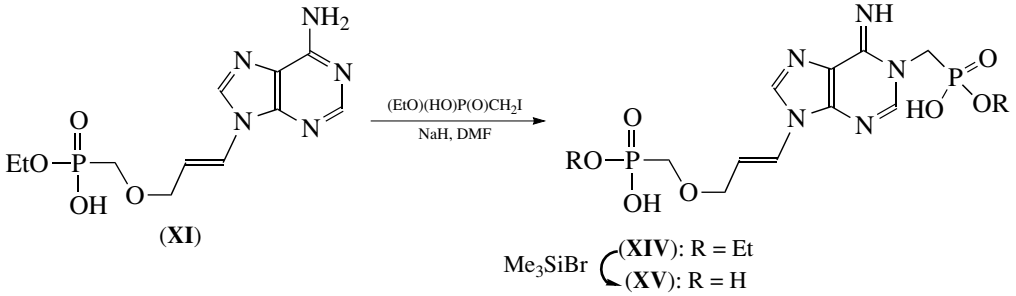
Scheme 1.



(**IVa**): R = Tr, X = Ms (**XIIIa**): R = Tr, X = Ms
(**IVb**): R = Tr, X = Ts (**XIIIb**): R = Tr, X = Ts (**Va**): R = Tr (**VIIa**): R = Tr
(**IVc**): R = TBDMS, X = Ts (**XIIIc**): R = TBDMS, X = Ts (**Vb**): R = TBDMS (**VIIb**): R = TBDMS

(*i*) AdeH, NaH or $(Me_3Si)_2NK$/DMF

Scheme 2.



Scheme 3.

Z- and *E*-isomers also remained unchanged when the TBDMS protecting group was substituted for trityl. The use of potassium hexamethyldisilylamide instead of NaH also caused no change in the ratio of *Z*/*E*-isomers.

The traditional method of the phosphonomethyl fragment introduction into a molecule consists in the reaction of the corresponding alcohol with mono- or dialkyl esters of iodo-, tosyloxy-, or trifluoromethanesulfonyloxymethylphosphonic acids in the presence of sodium hydride [7–12]. We herein used ethyl iodomethylphosphonate or ethyl tosyloxymethylphosphonate, which are readily available and stable substances. Furthermore, their use enables the isolation of the reaction product by ion-exchange or reversed-phase chromatography.

Alcohols (**VI**) and (**VII**) were alkylated with IMP in DMF in the presence of NaH (Scheme 1). The products were separated by reversed-phase chromatography under elution with ammonium bicarbonate buffer. This allowed us to obtain the target phosphonates in the form of ammonium salts. The structures of the resulting compounds were confirmed by the methods of UV and NMR spectroscopy. The absorption maxima in the UV spectra of products (**IX**) and (**XI**) were the same as those in the UV spectra of starting (**VI**) and (**VII**), which supports the absence of the heterocyclic base modifications.

The ^{1}H NMR spectra of phosphonates (**IX**) and (**XI**) exhibit the doublet at ~3.8 ppm corresponding to the methylene group at the phosphorus atom. In addition, in ^{31}P NMR spectra, these compounds exhibited a signal with a chemical shift at approximately 15–18 ppm, which is characteristic of alkyloxymethylphosphonic acids [10]. In the case of the *E*-isomer, the by-product (**XIV**) was isolated besides the target phosphonate (**XI**) (Scheme 3). The signals of two ethyl groups were observed in ^{1}H NMR spectrum of (**XIV**), whereas only one broadened singlet was observed in its ^{31}P NMR spectrum. After the removal of the ethyl groups by the treatment of (**XIV**) with trimethylbromosilane, ^{31}P NMR spectrum of (**XV**) contained two signals at 16.2 and 16.4 ppm, whereas the ^{1}H NMR spectrum contained well-resolved signals of two methylene groups at phosphorus atoms. It should be mentioned that, in the case of phosphonomethyl group at the oxygen atom, the value of $^2J_{CH_2,P}$ was 8.7 Hz, whereas the coupling constant value for another signal reached 11 Hz, which is characteristic of phosphonomethyl group at the nitrogen atom of heterocyclic base [13]. Furthermore, the transition from N^1-nonsubstituted phosphonates (**IX**) and (**XII**) to N^1-substituted phosphonates (**XIV**) and (**XV**) resulted in the long-wave shift of absorption maximum in UV spectrum by 7 nm.

Antiherpetic activity of (*Z*)- and (*E*)-9-(3-phosphonomethoxyprop-1-en-1-yl)adenine

Compound	TCD_{50}, mM	HSV-1/L_2			HSV-1/ACV^R		
		ID_{50}, mM	ID_{95}, mM	SI	ID_{50}, mM	ID_{95}, mM	SI
(**X**)	>3.5	0.095	1.53	>34	0.11	0.86	>34
(**XII**)	>3.5	0.39	1.57	>8	0.44	1.57	>8
Acyclovir	>2.2	0.0017	0.0036	>1300	0.53	0.89	>2.5
(**II**)*	>0.36	0.08		>4.5	0.08		>4.5

* The data are taken from [12]. The authors used BWS HSV-1 strain in the Vero cell culture. The infection multiplicity was the same as described in the Experimental section.

The complex of these data allowed us to assign the structure of (*E*)-1-(phosphonomethyl)-9-(phosphonomethoxyprop-1-en-1-yl)adenine (**XV**) to the resulting compound. The aforementioned information correlate well with that reported in literature for a similar compound in the series of nucleoside carbocyclic analogues [9].

The by-product (**XIV**) became main reaction product at 2–3-fold excess of IMP as alkylating agent, whereas the yield of target monophosphonate (**XI**) reached 38% when using 1.5 equiv of IMP per (**VIII**) and the portion of diester (**XIV**) did not exceed 15%. According to literature, the protection of exocyclic amino group of nucleic base helps avoid its modification including the N^1-alkylation [14]. However, in our case, the protection of the adenine residue in (**VIII**) using dimethylformamide diethyl acetal with the aim to treat the resulting (**XVI**) with IMP had no effect on the ratio of the mono- and bisalkylation products. The substitution of tosyloxymethylphophonate for IMP also failed to decrease the proportion of the by-product (**XIV**).

Monoesters (**IX**) and (**XI**) were transformed into the corresponding acids (**X**) and (**XII**) by the treatment with trimethylbromosilane in DMF. The reaction products were isolated by reversed-phase chromatography.

Thus, we have obtained the target *Z*- and *E*-isomers of 9-(3-phosphonomethoxyprop-1-en-1-yl)adenine.

The antiviral activity of phosphonates (**X**) and (**XII**) was studied using Vero cell culture infected with HSV-1. Both compounds displayed a moderate antiherpetic activity for both the wild and the acyclovir-resistant strain (see the table). The *Z*-isomer (**X**) was four times more active than the *E*-isomer (**XII**) in respect of inhibition of HSV replication. The activity was therewith compatible with that of 9-(2-phosphonomethoxyethyl)adenine (**II**). The synthesized phosphonates (**X**) and (**XII**) appeared to be less toxic than (**II**) and, therefore, exceeded it in SI.

The retention of antiherpetic activity of (**X**) and (**XII**) for the acyclovir-resistant virus strain may be connected with the fact that, in 95% cases, the resistance to acyclovir is due to the mutation of the viral thymidine kinase, which resulted in the enzyme that does not phosphorylate modified nucleosides [15]. However, (**X**) and (**XII**) do not require the first act of kination for their transformation into the corresponding dNMP analogues and, therefore, retain their activity against the acyclovir-resistant strain.

EXPERIMENTAL

Adenine was from Reanal (Hungary) and methanesulfonyl chloride, *p*-toluenesulfonyl chloride, chlorotriphenylmethane, bromotrimethylsilane, glycerol, NaH (80% suspension in oil), *tert*-butylchlorodimethylsilane, and potassium bis(trimethylsilyl)amide were from Aldrich (United States). Ethyl iodomethylphosphonate and ethyl tosyloxymethylphosphonate were synthesized as described in [11, 16].

Column chromatography was carried out on silica gel 60 (63–100 μm), LiChroprep RP-8 (40–63 μm), and LiChroprep RP-18 (25–40 μm) (Merck, Germany).

NMR spectra were registered on a Bruker AMX III-400 (United States) spectrometer at a working frequency of 400 MHz for ^{1}H NMR (using Me_4Si for $CDCl_3$ solutions and sodium 3-(trimethylsilyl)-1-propanesulfonate for D_2O solutions as internal references), 162 MHz for ^{31}P NMR (with phosphorus–proton spin decoupling using 85% phosphoric acid as the external reference), and 100 MHz for ^{13}C NMR. Chemical shifts are given in ppm (δ scale) and spin coupling constants in Hz. UV spectra were registered on a Shimadzu UV-2401 P (Japan) spectrophotometer.

3-Trityloxypropane-1,2-diol was synthesized as described in [17]; yield 81%; mp 90–92°C (lit. 92–94°C); ^{1}H NMR ($CDCl_3$): 7.46 (6 H, d, $^3J_{o,m}$ 7.2, *o*-CH, Tr), 7.32 (6 H, t, $^3J_{m,p}$ 7.8, *m*-CH, Tr), 7.3 (3 H, t, *p*-CH, Tr), 3.87 (1 H, m, H2), 3.67 (1 H, dd, $^2J_{1a,1б}$ 11.2, $^3J_{1a,2}$ 3.4, $H1_a$), 3.58 (1 H, dd, $^3J_{1б,2}$ 6.2, $H1_b$), and 3.23 (2 H, m, H3).

1,2-Bis(mesyloxy)-3-trityloxypropane (IVa). Methanesulfonyl chloride (1.70 ml, 22.4 mmol) was added to a solution of 3-trityloxypropan-1,2-diol (3.00 g, 8.97 mmol) in pyridine (50 ml) at 0°C. The reaction mixture was stirred for 1 h at +4°C and left at room temperature for 20 h. The mixture was evaporated

in a vacuum; the residue was dissolved in CCl_4 (50 ml); and the resulting solution was washed with aqueous $NaHCO_3$ (50 ml) and water (2 × 50 ml), dried over Na_2SO_4, and evaporated in a vacuum. The oily residue was crystallized from CCl_4 and air-dried to yield 3.24 g (74%) of (**IVa**); mp 107–110°C (decomp.); ^{1}H NMR ($CDCl_3$): 7.41 (6 H, d, $^3J_{o,m}$ 7.8, *o*-CH), 7.32 (6 H, dd, $^3J_{m,p}$ 7.2, *m*-CH), 7.26 (3 H, t, *p*-CH), 4.85 (1 H, m, $^3J_{2,3a}$ 4.7, $^3J_{2,3b}$ 5.3 H2), 4.40 (2 H, m, H1), 3.47 (1 H, dd, $^2J_{3a,3b}$ 10.6, $H3_a$), 3.40 (1 H, dd, $H3_b$), 3.04 and 3.01 (2 × 3 H, 2 s, 2 CH_3).

1,2-Bis(tosyloxy)-3-trityloxypropane (IVb). A solution of 3-trityloxypropane-1,2-diol (3.00 g, 8.97 mmol) and *p*-toluenesulfonyl chloride (5.13 g, 26.9 mmol) in pyridine (50 ml) was kept for 20 h at room temperature and evaporated in a vacuum. The residue was dissolved in CCl_4 (70 ml), successively washed with aqueous citric acid (30 ml), water (2 × 30 ml), aqueous $NaHCO_3$ (20 ml), and water (2 × 30 ml), and dried with Na_2SO_4. The oily residue obtained after evaporation was crystallized from CCl_4 (25 ml) and air-dried to give 4.16 g (72%) of (**IVb**); mp 104–106°C (decomp.); ^{1}H NMR ($CDCl_3$): 7.68 and 7.64 (2 × 2 H, 2 d, $^3J_{o,m}$ 8.4, *o*-CH, Ts), 7.29–7.23 (19 H, m, Tr and *m*-CH of Ts), 4.55 (1 H, m, H2), 4.14 (2 H, m, H1), 3.26 (2 H, m, H3), 2.43 and 2.42 (2 × 3 H, 2 s, 2 CH_3, Ts).

1,2-Bis(tosyloxy)-3-(*tert*-butyldimethylsilyloxy)-propane (IVc). A solution of *tert*-butylchlorodimethylsilane (1.62 g, 10.8 mmol) and glycerol (12.5 ml, 200 mmol) in pyridine (80 ml) was kept for 18 h at room temperature and then evaporated in a vacuum. The residue was coevaporated with toluene (2 × 15 ml) and dissolved in chloroform (50 ml). The solution was washed with aqueous $NaHCO_3$ (40 ml) and water (2 × 40 ml) and dried with Na_2SO_4. The oily residue was dissolved in pyridine (70 ml), *p*-toluenesulfonyl chloride (5.13 g, 26.9 mmol) was added, and the reaction mixture was kept for 18 h at room temperature. Then the reaction mixture was evaporated in a vacuum, the residue was dissolved in chloroform (50 ml), and the solution was washed with aqueous $NaHCO_3$ (30 ml) and water (2 × 30 ml) and dried with Na_2SO_4. The solvents were removed under reduced pressure to give 2.82 g (51%) of oily product (**IVc**); ^{1}H NMR ($CDCl_3$): 7.71 (4 H, d, $^3J_{o,m}$ 8.4, *o*-CH), 7.28 (4 H, d, *m*-CH), 4.57 (1 H, m, H2), 4.17 (2 H, m, H1), 3.30 (2 H, m, H3), 2.45 and 2.43 (2 × 3 H, 2 s, 2 CH_3, Ts), 0.90 (9 H, s, Me_3C), 0.11 (6 H, s, Me_2Si).

(*Z*)- and (*E*)-9-(3-Trityloxyprop-1-en-1-yl)adenine (Va) and (VIIa). Method A. A suspension of adenine (827 g, 6.12 mmol) and NaH (230 mg, 7.67 mmol) in DMF (30 ml) was stirred until the hydrogen liberation ceased and kept for another 1 h at 60°C. Then a solution of 1,2-bis(mesyloxy)-3-trityloxypropane (**IVa**) (1.50 g, 3.06 mmol) was added in a DMF (15 ml) and acetonitrile (5 ml) mixture. The reaction mixture was refluxed for 8 h, cooled, and evaporated in a vacuum. The residue was dissolved in chloroform (40 ml), washed with water (2 × 50 ml), dried with Na_2SO_4, and evaporated in a vacuum. The residue was dissolved in chloroform (4 ml) and chromatographed on a silica gel column (3.8 × 10 cm) eluted with chloroform (200 ml) and then 2% EtOH in chloroform (400 ml). The fractions containing the mixture of *Z*- and *E*-alkenes (**Va**) and (**VIIa**) was evaporated in a vacuum to get 710 mg (54%) of 1 : 3.5 *Z*-/*E*-isomer mixture (according to ^{1}H NMR). Crystallization from chloroform resulted in homogeneous *E*-isomer (**VIIa**). An additional quantity of (**VIIa**) was obtained by concentrating the filtrates and repeated crystallization. The total yield of pure *E*-isomer (**VIIa**) after air-drying was 451 mg (34%). The mother liquor after two crystallizations was evaporated in a vacuum to give 93 mg (7%) of oily *Z*-isomer (**Va**) (purity >90%).

(*Z*)-9-(3-Trityloxyprop-1-en-1-yl)adenine (**Va**): UV (CH_3OH): λ_{max} 261 nm; ^{1}H NMR ($NDCl_3$): 8.33 (1 I, s, I8), 7.89 (1 I, s, I2), 7.42 (6 H, d, $^3J_{o,m}$ 8.4, *o*-CH), 7.32–7.13 (9 H, m, *m*-CH and *p*-CH), 6.96 (1 H, d, $^3J_{1',2'}$ 9.0, H1'), 5.60 (2 H, br. s, 6-NH_2), 5.88 (1 H, dt, $^3J_{2',3'}$ 6.9, H2'), 3.78 (2 H, dd, $^4J_{1',3'}$ 0.9, H3').

(*E*)-9-(3-Trityloxyprop-1-en-1-yl)adenine (**VIIa**): UV (CH_3OH): λ_{max} 261 nm; mp 192–195°C; ^{1}H NMR ($NDCl_3$): 8.39 (1 H, s, H8), 7.96 (1 H, s, H2), 7.49 (6 H, d, $^3J_{o,m}$ 7.5, *o*-CH), 7.33 (6 H, t, $^3J_{m,p}$ 7.5, *m*-CH), 7.25 (4 H, m, *p*-CH and H1'), 6.53 (1 H, dt, $^3J_{1',2'}$ 14.3, $^3J_{2',3'}$ 5.6, H2'), 5.56 (2 H, br. s, 6-NH_2), 3.86 (2 H, dd, $^4J_{1',3'}$ 1.6, H3').

Methods B and C. A suspension of adenine (1.5 g, 11 mmol) and NaH (467 mg, 15.6 mmol) in DMF (50 ml) was stirred until the hydrogen liberation ceased and heated for another 1 h at 60°C. Then a solution of 1,2-bis(tosyloxy)-3-trityloxypropane (**IVb**) (method B) (5.7 g, 5.6 mmol) or (method C) 1,2-bis(tosyloxy)-3-(*tert*-butylmethylsilyloxy)propane (**IVc**) (2.8 g, 5.5 mmol) in a DMF (25 ml) and acetonitrile (10 ml) mixture was added. The mixture was refluxed for 6 h, cooled, and treated as described in method A. The yield of the mixture of target (**Va**) and (**VIIa**) by the method B was 1.64 g (68%). The yield of the oily mixture of (**Vb**) and (**VIIb**) was 0.98 g (58%) according to method C. The *Z*/*E* isomer ratio was 1 : 3.5 in both cases (according to ^{1}H NMR spectroscopy).

Method D. A suspension of adenine (1.5 g, 11 mmol) and potassium bis(trimethylsilyl)amide (3.11 g, 15.6 mmol) in DMF (50 ml) was stirred for 1 h and treated with a solution of 1,2-bis(mesyloxy)-3-trityloxypropane (**IVa**) (5.7 g, 5.6 mmol) in a DMF (25 ml) and acetonitrile (10 ml) mixture. The mixture was refluxed for 6 h, cooled and treated as described in method A. Yield of the mixture of target (**Va**) and

(**VIIa**) was 1.44 g (60%). The *Z*/*E* isomer ratio was 1 : 3.5 (according to ^{1}H NMR).

(*Z*)-9-[3-(*tert*-Butyldimethylsilyloxy)prop-1-en-1-yl]adenine (**Vb**): UV (CH_3OH): λ_{max} 261 nm; ^{1}H NMR ($NDCl_3$): 8.38 and 8.10 (2 H, 2 s, H8 and H2), 6.97 (1 H, d, $^3J_{1',2'}$ 9.0, H1'), 5.86 (1 H, dt, $^3J_{2',3'}$ 6.9, H'), 5.86 (2 H, br. s, 6-NH_2), 4.32 (2 H, dd, $^4J_{1',3'}$ 1.2, H3'), 0.88 (9 H, s, Me_3C), 0.06 (6 H, s, Me_2Si).

(*E*)-9-[3-(*tert*-Butyldimethylsilyloxy)prop-1-en-1-yl]adenine (**VIIb**): UV (CH_3OH): λ_{max} 261 nm; ^{1}H NMR ($CDCl_3$): 8.39 and 7.94 (2 H, 2 s, H8 and H2), 7.17 (1 H, dt, $^3J_{1',2'}$ 14.3, $^4J_{1',3'}$ 1.9, H1'), 6.60 (1 H, dt, $^3J_{2',3'}$ 5.0, H2'), 5.86 (2 H, br. s, 6-NH_2), 4.40 (2 H, dd, H3'), 0.92 (9 H, s, Me_3C), 0.13 (6 H, s, Me_2Si).

(*E*)-9-(3-Hydroxyprop-1-en-1-yl)adenine (VIII). Method A. A solution of (*E*)-9-(3-trityloxyprop-1-en-1-yl)adenine (**VIIa**) (267 mg, 0.62 mmol) in 80% aqueous acetic acid (25 ml) was refluxed for 2 h, cooled, and evaporated. The residue was coevaporated with toluene (2 × 25 ml) and dissolved in 50% aqueous methanol. The solution was washed with chloroform (2 × 15 ml) and evaporated in a vacuum; yield of (**VIII**) 98 mg (83%); UV (CH_3OH): λ_{max} 261 nm; mp 224–227°C; ^{1}H NMR (D_2O): 8.33 (1 H, s, H8), 8.26 (1 H, s, H2), 7.18 (1 H, dt, $^3J_{1',2'}$ 14.3, $^4J_{1',3'}$ 1.2, H1'), 6.54 (1 H, dt, $^3J_{2',3'}$ 5.9, H2'), 4.40 (2 H, dd, H3').

Method B. A solution of (*E*)-9-[3-(*tert*-butyldimethylsilyloxy)prop-1-en-1-yl]adenine (**VIIb**) (150 mg, 0.49 mmol) in 80% aqueous acetic acid (25 ml) was refluxed for 2 h, cooled, and evaporated in a vacuum. The residue was coevaporated with toluene (2 × 25 ml), crystallized from aqueous methanol, and dried in a vacuum to give 71 mg (76%) of (**VIII**).

(*Z*)-9-(3-Hydroxyprop-1-an-1-yl)adenine (VI). A solution of (*Z*)-9-(3-trityloxyprop-1-en-1-yl)adenine (**Va**) (100 mg, 0.23 mmol) in 80% aqueous acetic acid (20 ml) was refluxed for 2 h, cooled, and evaporated in a vacuum. The residue was coevaporated with toluene (2 × 15 ml), dissolved in 50% aqueous methanol (30 ml), washed with chloroform (2 × 15 ml), and evaporated in a vacuum. The residue was dissolved in 93 : 7 chloroform–methanol and chromatographed on a silica gel column (1 × 15.5 cm). Fractions absorbing in UV light were concentrated and dried in a vacuum to give 34 mg (76%) of (**VI**); UV (CH_3OH): λ_{max} 261 nm; mp 195–198°C; ^{1}H NMR (D_2O): 8.28 (1 H, s, H8), 8.16 (1 H, s, H2), 6.77 (1 H, dt, $^3J_{1',2'}$ 8.4, $^4J_{1',3'}$ 1.6, H1'), 6.02 (1 H, dt, $^3J_{2',3'}$ 6.5, H2'), 4.06 (2 H, dd, H3').

***N*^{6}-Dimethylaminomethylidene-9-(3-hydroxyprop-1-en-1-yl)adenine (XVI)** A solution of (*E*)-9-(3-hydroxyprop-1-en-1-yl)adenine (**VIII**) (25 mg, 0.13 mmol) and dimethylformamide diethylacetal (400 µl, 2.33 mmol) in DMF (10 ml) was kept for 18 h at room temperature and evaporated in a vacuum. The residue was coevaporated with water (2 × 10 ml), dissolved in chloroform (1 ml), and chromatographed on a silica gel column (1 × 15.5 cm) eluted with $CHCl_3$ and then with 95 : 5 $CHCl_3$–EtOH. Fractions containing the target alcohol were evaporated in a vacuum, and the residue was air-dried to give 30 mg (92%) of (**XVI**). ^{1}H NMR ($NDCl_3$): 8.92 (1 H, s, N=C<u>H</u>–N), 8.53 (1 H, s, H2), 8.02 (1 H, s, H8), 7.21 (1 H, br. d, $^3J_{1',2'}$ 14.3, H1'), 6.58 (1 H, dt, $^3J_{2',3'}$ 5.6, H2'), 4.37 (2 H, dd, $^4J_{1',3'}$ 1.6, H3'), 3.24 and 3.19 (6 I, 2 s, Me_2N).

(*E*)-9-[3-(O-Ethylphosphonomethoxyprop-1-en-1-yl]adenine (XI). Method A. Sodium hydride (30 mg, 1.0 mmol) was added to a solution of (*E*)-9-(3-hydroxyprop-1-en-1-yl)adenine (**VIII**) (50 mg, 0.26 mmol) and monoethyl iodomethylphosphonate (90 mg, 0.36 mmol) in DMF (40 ml). The mixture was stirred for 18 h at room temperature, quenched with acetic acid (60 µl), and evaporated in a vacuum. The residue was dissolved in water (1 ml) and chromatographed on a LiChroprep RP-8 column (1.8 × 22 cm) eluted with 0.05 M aqueous NH_4HCO_3. The fractions containing the target monoester were evaporated in a vacuum; the residue was coevaporated with water (4 × 15 ml) and dried in a vacuum to give 32 mg (38%) of (**XI**); UV (H_2O, pH 7): λ_{max} 261 nm; ^{1}H NMR (D_2O): 8.02 (1 H, s, H8), 7.87 (1 H, s, H2), 6.90 (1 H, d, $^3J_{1',2'}$ 14.3, H1'), 6.24 (1 H, dt, $^3J_{2',3'}$ 6.5, H2'), 4.22 (2 H, d, H3'), 3.92 (2 H, dq, $^3J_{CH_2,CH_3} \approx {}^3J_{CH_2,P}$ 7.2, $CH_3C\underline{H}_2$), 3.67 (2 H, d, $^2J_{CH_2,P}$ 8.7, PCH_2), 1.20 (3 H, t, $C\underline{H}_3CH_2$). ^{31}P NMR (D_2O): 18.17 s.

Method B. A mixture of (*E*)-9-(3-hydroxyprop-1-en-1-yl)adenine (**VIII**) (50 mg, 0.26 mmol), monoethyl tosyloxymethylphosphonate (90 mg, 0.5 mmol) and NaH (50 mg, 1.7 mmol) in DMF (50 ml) was stirred for 18 h at room temperature, quenched with acetic acid (100 µl), and evaporated in a vacuum. The residue was dissolved in water (1 ml) and chromatographed on a LiChroprep RP-8 column (1.8 × 22 cm) eluted with 0.05 M aqueous NH_4HCO_3. The fractions containing monoester (**XI**) were evaporated in a vacuum; the residue was coevaporated with water (4 × 15 ml) and dried in a vacuum to give 35 mg (40%) of ester (**XI**).

Method C. Sodium hydride (25 mg, 0.83 mmol) was added to a solution of *N*^{6}-dimethylaminomethylidene-9-(3-hydroxyprop-1-en-1-yl)adenine (**XVI**) (30 mg, 0.12 mmol) and ethyl iodomethylphosphonate (60 mg, 0.24 mmol) in DMF (40 ml), and the mixture was stirred for 18 h at room temperature, quenched with acetic acid (60 µl), and evaporated in a vacuum. The residue was dissolved in 5% ammonia, the resulting solution was kept for 18 h at room temperature, and evaporated in a vacuum. The residue was dissolved in water (1 ml) and chromatographed on a LiChroprep RP-8 column (1.8 × 22 cm) eluted with 0.05 M aqueous NH_4HCO_3. The fractions containing monoester (**XI**)

were evaporated in a vacuum, coevaporated with water (4 × 15 ml), and lyophilized. Yield 5 g (22%).

(*E*)-1-(*O*-Ethylphosphonomethyl)-9-(3-*O*-ethylphosphonomethoxyprop-1-en-1-yl)adenine (XIV). Sodium hydride (30 mg, 1.0 mmol) was added to a solution of (*E*)-9-(3-hydroxyprop-1-en-1-yl)adenine (**VIII**) (50 mg, 0.26 mmol) and monoethyl iodomethylphosphonate (150 mg, 0.60 mmol) in DMF (5 ml). The mixture was stirred for 18 h at room temperature, quenched with acetic acid (60 μl), and evaporated in a vacuum. The residue was dissolved in water (1 ml) and chromatographed on a LiChroprep RP-8 column (1.8 × 22 cm) eluted with 0.05 M aqueous NH_4HCO_3. The fractions containing diester (**XIV**) were evaporated in a vacuum. Yield of (**XIV**) 40 mg (35%); UV (H_2O, pH 7): λ_{max} 268 nm. ^{1}H NMR (D_2O): 8.21 and 8.14 (2 H, 2 s, H8 and H2), 7.11 (1 H, d, $^3J_{1',2'}$ 14.3, H1'), 6.33 (1 H, dt, $^3J_{2',3'}$ 6.5, H2'), 4.18 (2 H, d, H3'), 3.86–3.58 (6 H, m, 2 C$\underline{H}_3$CH$_2$ and PCH$_2$N), 3.58 (2 H, d, $^2J_{CH_2,P}$ 8.4, PCH$_2$O), 1.12 (3 H, t, $^3J_{CH_2,CH_3}$ 6.9, C$\underline{H}_3$CH$_2$ at 3'-O]), 0.98 (3 H, t, $^3J_{CH_2,CH_3}$ 7.2, C$\underline{H}_3$CH$_2$ at N^1]); ^{31}P NMR (D_2O): 18.17 br. s.

(*Z*)-9-(3-I-Ethylphosphonomethoxyprop-1-en-1-yl)adenine (IX) was synthesized by the method described for phosphonate (**XI**) in 37% yield; UV (H_2O, pH 7): λ_{max} 261 nm; ^{1}H NMR (D_2O): 8.04 and 8.02 (2 H, 2 s, H8 and H2), 6.82 (1 H, d, $^3J_{1',2'}$ 8.4, H1'), 5.96 (1 H, dt, $^3J_{2',3'}$ 6.5, H2'), 4.11 (2 H, d, H3'), 3.96 (2 H, dq, $^3J_{CH_2,CH_3} \approx {}^3J_{CH_2,P}$ 7.0, CH$_3$C$\underline{H}_2$), 3.46 (2 H, d, $^2J_{CH_2,P}$ 8.7, PCH$_2$), 1.22 (3 H, t, C$\underline{H}_3$CH$_2$); ^{31}P NMR (D_2O): 15.82 s.

(*E*)-9-(3-Phosphonomethoxyprop-1-en-1-yl)adenine (XII). A solution of ethyl ester of (*E*)-9-(3-phophonomethoxyprop-1-en-1-yl)adenine (**XI**) (30 mg, 0.095 mmol) in a mixture of water (20 ml), DMF (10 ml), and tributylamine (200 μl) was evaporated in a vacuum to a volume of ~3 ml and coevaporated with DMF (4 × 10 ml). DMF (15 ml) and Me_3SiBr (51 μl, 0.38 mmol) were added to the residue, and the mixture was kept for 18 h at room temperature and evaporated in a vacuum. The residue was dissolved in water (1 ml) and chromatographed on a LiChroprep RP-18 column (2 × 17 cm) eluted with 0.05 M aqueous NH_4HCO_3. The fractions containing phosphonate (**XII**) were evaporated in a vacuum to give 22 mg (81%) of the product (**XII**); UV (H_2O, pH 7): λ_{max} 261 nm; ^{1}H NMR (D_2O): 7.99 (1 H, s, H8), 7.85 (1 H, s, H2), 6.85 (1 H, d, $^3J_{1',2'}$ 14.3, H1'), 6.18 (1 H, dt, $^3J_{2',3'}$ 6.2, H2'), 4.14 (2 H, d, H3'), 3.51 (2 H, d, $^2J_{CH_2,P}$ 8.7, PCH$_2$). ^{31}P NMR (D_2O): 15.66 s; ^{13}C NMR (D_2O): 157.25 (s, C6), 154.49 (s, C2), 150.00 (s, C4), 142.37 (s, C8), 125.30 (s, C1'), 120.88 (s, C2'), 120.71 (s, C5), 72.73 (d, $^3J_{C,P}$ 12.1, C3'), 68.57 (d, $^1J_{C,P}$ 156.6, CP).

(*Z*)-9-(3-Phosphonomethoxyprop-1-en-1-yl)adenine (X) was obtained from ester (**IX**) by the method used for the synthesis of (**XII**) in 84% yield; UV (H_2O, pH 7): λ_{max} 261 nm; ^{1}H NMR (D_2O): 8.00 and 7.99 (2 H, 2 s, H8 and H2), 6.79 (1 H, d, $^3J_{1',2'}$ 8.4, H1'), 5.95 (1 H, dt, $^3J_{2',3'}$ 6.5, H2'), 4.09 (2 H, d, H3'), 3.45 (2 H, d, $^2J_{CH_2,P}$ 8.7, PCH$_2$); ^{31}P NMR (D_2O): 15.88 s; ^{13}C NMR (D_2O): 156.64 (s, C6), 153.81 (s, C2), 150.00 (s, C4), 143.07 (s, C8), 126.34 (s, C1'), 123.10 (s, C2'), 119.19 (s, C5), 68.39 (d, $^3J_{C,P}$ 11.1, C3'), 66.96 (d, $^1J_{C,P}$ 156.6, CP).

(*E*)-1-(Phosphonomethyl)-9-(3-phosphonomethoxyprop-1-en-1-yl)adenine (XV) was obtained from diethyl ester (**XIV**) by the method described for (**XII**); yield 76%; UV (H_2O, pH 7): λ_{max} 269 nm; ^{1}H NMR (D_2O): 8.34 and 8.32 (2 H, 2 s, H8 and H2), 7.28 (1 H, d, $^3J_{1',2'}$ 14.0, H1'), 6.50 (1 H, dt, $^3J_{2',3'}$ 6.5, H2'), 4.38 (2 H, d, H3'), 3.83 (2 H, d, $^2J_{CH_2,P}$ 11.8, PCH$_2$N), 3.73 (2 H, d, $^2J_{CH_2,P}$ 8.7, PCH$_2$O); ^{31}P NMR (D_2O): 16.40 (s, 1P) + 16.20 (s, 1P).

Experiments in cell systems. The Vero cell culture (kidney cell culture from African green monkeys) obtained from the Laboratory of Cell Cultures (Ivanovskii Institute of Virology, Russian Academy of Medical Sciences) was used. Cells were maintained in the Eagle MEM nutrition medium (PANEKO, Moscow) containing 7% fetal calf serum.

The HSV-1/L_2 wild strain from the Virus Museum (Ivanovskii Institute of Virology, Russian Academy of Medical Sciences) was used. The mutant acyclovir-resistant strain HSV-1/ACV^R was obtained by serial passages in the presence of increasing acyclovir concentrations. The resulting viral strain was used for obtaining the acyclovir-resistant virus clone [18]. The viral strains were passaged in an Eagle mixture and 199 media (1 : 1) containing 2% of fetal calf serum. The 96-well plates (Linbro, Flow Lab., UK) were used for cell culturing and subsequent infection.

Antiviral activity was estimated according to the ability of the compounds under study to inhibit the development of virus-induced cytopathogenic response by 50 and 95% as compared with total cell death in reference infected cultures [19]. The infection multiplicity was 0.1 TCD_{50}/cell. The cells were incubated for 48 h, until 95–100% cytotoxic effect developed.

The Trypane Blue test was used for the detection of dead cells [20]. The concentration that resulted in 50% cell survival after 72-h contact with the compound under study, was taken as TCD_{50}.

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