Novel Acyclic Nucleotides and Nucleoside 5′-Triphosphates Imitating 2′,3′-Dideoxy-2′,3′-didehydronucleotides: Synthesis and Biological Properties

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A series of pyrophosphoryl (*Z*)-(phosphonomethoxy)but-2-enyl derivatives of pyrimidines and purines **9a–d** and the corresponding phosphonates **10a–d** were synthesized. The prepared compounds contain the phosphonate group as an α-phosphate mimic as well as an acyclic residue emulating the sugar moiety in 2′,3′-dideoxy-2′,3′-didehydronucleoside 5′-triphosphates known as highly potent chain terminators of DNA polymerases. Phosphonates **10a–d** were obtained by alternative alkylations of the nucleic bases followed by condensation with ethyl [[(*p*-tolylsulfonyl)oxy]methyl]phosphonate. Pyrophosphorylation of **10a–d** afforded phosphonate diphosphates **9a–d**. Their substrate properties were evaluated in cell-free systems containing various DNA polymerases including viral reverse transcriptases. Compounds **9a–d** manifested good terminating substrate properties toward HIV-1 and AMV reverse transcriptases. They exhibited high selectivity and were not recognized by human DNA polymerases α and ϵ, DNA polymerase β from rat liver, *Escherichia coli* DNA polymerase I, and HSV-1 and CMV DNA polymerases. Phosphonates **10b–d** displayed no activity in HIV-1-infected MT-4 cells cultures; **10a** was moderately effective (ED_{50} = 9 μM).

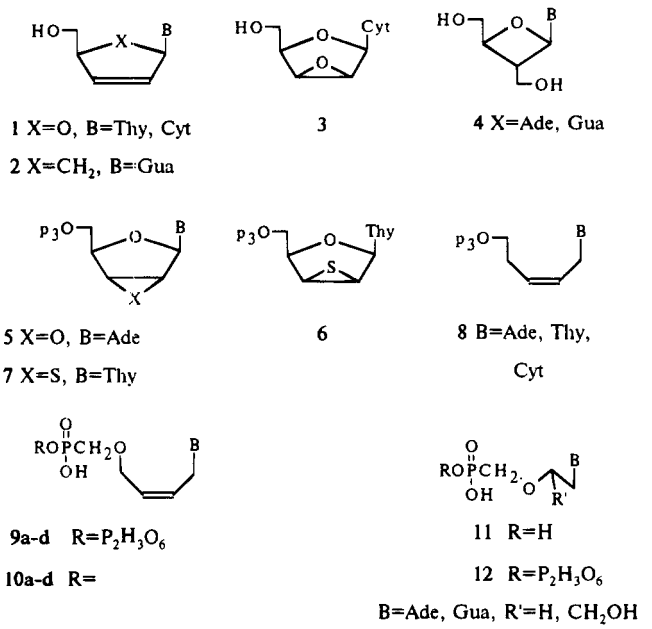
Among the numerous modified nucleosides evaluated for anti-HIV activity, one can recognize a group of compounds with restricted conformational flexibility of the sugar residue. The best known of this group are 2′,3′-didehydro-2′,3′-dideoxy nucleosides **1**,[1] their carbocyclic analog, carbovir (**2**),[2] 2′,3′-lyxoanhydrocytidine (**3**)[3], and oxetanocines **4**[4] (Chart 1). All these nucleoside analogs pass through the triphosphorylation cascade and, after conversion to the corresponding 5′-triphosphates,[5–7] inhibit the synthesis of proviral DNA catalyzed by human immunodeficiency virus (HIV) reverse transcriptase.[8–11] Several nucleoside 5′-triphosphates with the conformationally rigid carbohydrate residue terminate DNA synthesis catalyzed by different DNA polymerases including HIV reverse transcriptase. Among them are 5′-triphosphates of 2′,3′-riboanhydroadenosine (**5**),[9] 1-(2′,3′-dideoxy-2′,3′-epithio-β-D-lyxofuranosyl)thymine (**6**), and 1-(2′,3′-dideoxy-2′,3′-epithio-β-D-ribofuranosyl)thymine (**7**)[12] and some acyclic analogs **8**.[13] Compounds **8** are known to be very efficient as chain terminators of a large set of DNA polymerases including herpes simplex virus type I (HSV-1), cytomegalovirus (CMV), and adenovirus DNA polymerases,[14,15] HIV and avian myeloblastosis virus (AMV) reverse transcriptases, and even such highly specific enzymes as human DNA polymerases α and ϵ.

These data prompted us to synthesize and examine in cell-free systems a novel series of nucleoside 5′-triphosphate analogs, **9a–d**, which, being isosters of **8**, also reveal conformational rigidity of the pseudosugar residue due to the presence of a *cis* double bond but contain a nonhydrolyzable bond between the triphosphate residue and the acyclic fragment.

However, the nucleosides corresponding to **8** at concentrations up to 100 μM have shown no inhibitory effect on HIV reproduction in both MT-4 and peripheral blood lymphocyte (PBL) cell cultures (Dr. B. Polsky, Memorial Sloan Kettering Cancer Center, New York, personal communication). We attribute this evidence to the lack of their phosphorylation in the cell. Precusors of **9**, 1-[(*Z*)-4-(phosphonomethoxy)but-2-enyl]-pyrimidines **10b,c** and 9-[(Z)-4-(phosphonomethoxy)but-2-enyl]purines **10a,d** unlike **8**, contain a hydrolytically stable phosphonate fragment instead of α-phosphate. Such replacement has been performed earlier: isosteric nucleoside derivatives **11** and **12** displayed pronounced inhibitory activity both in cell cultures and in cell-free systems with purified enzymes[16–19].

In this paper, we report the synthesis and substrate properties of compounds **9a–d**. They were shown to be highly specific terminating substrates for HIV and AMV reverse transcriptases. However, compounds **10b–d** showed no inhibitory effect on HIV-I reproduction in the

Chart 1



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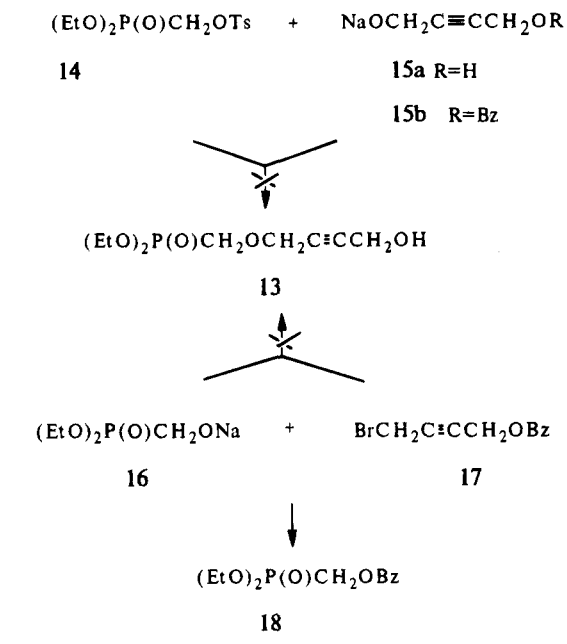
Scheme 1



MT-4 lymphoid cells culture at concentrations up to 100 μM, whereas **10a** inhibited HIV-I reproduction by 50% at a concentration of 9 μM (Dr. B. Polsky).

Chemistry

Originally we planned to use diethyl [[(4-hydroxybut-2-ynyl)oxy]methyl]phosphonate (**13**) as the key intermediate in the synthesis of **9a–d**. However, the reaction of phosphonate **14** with sodium but-2-yne-1,4-diol (**15a**) or its benzoyl derivative, **15b**, that has been successfully performed with various alkanols[20] did not yield **13** (Scheme 1). Reaction of sodium (hydroxymethyl)phosphonate **16** with bromide **17**, according to the literature procedure,[21] resulted in *trans* esterification affording not **13** but diethyl [(benzoyloxy)methyl]-phosphonate (**18**).

The above-mentioned results prompted us to modify the strategy and first synthesize the known compounds $BCH_2C{=}CCH_2OH$ **19a–d**[22,23] (where B is the nucleic base) and then condense them with the phosphonate component (Scheme 2).

For alkylation of adenine, we used the hydride method.[22] Bromide **17** was obtained with the overall yield of 60% by mesylation of 4-(benzoyloxy)but-2-ynol (**20**) with MsCl in dioxane in the presence of NEt_3 followed by treatment of mesylate **21** with KBr and dibenzo-18-crown-6 under reflux in acetone. The resulting bromide **17** was TLC homogeneous and could be used in further transformations without purification by chromatography or distillation. We obtained **17** according to a widely cited procedure[24] with a yield of only 30–35%. It should be mentioned that in our hands mesylate **21** proved to be a more convenient alkylating agent than the bromide **17** in preparation of **22a**, resulting in a 75% versus 49% yield, respectively. Similar condensation occurred when bromide **17** was affected by the potassium salt of adenine prepared from adenine and potassium bis(trimethylsilyl)amide. The yield of **22a** was 64%. Alkaline deprotection afforded the target compound **19a** which was found to be identical to that reported earlier.[22] Compound **19a** was also synthesized with a yield of 59% by the reaction of the sodium salt of adenine with mesylate **24** obtained from but-2-yne-1,4-diol as described above for **21**. Our attempts to obtain **27** by treating **19a** with phosphonate **14** resulted in a complex mixture of products as revealed by TLC.

A route involving activation of the hydroxyl group in **19a** followed by condensation with phosphonate **16** was also unsuccessful. Mesylation of **19a** yielded only traces of the desired **25**. However, **25** proved to be easily available when the adenine sodium salt was treated with 1,4-bis[(methylsulfonyl)oxy]but-2-yne (**26**). The prepared **25** manifested unusual properties in routine reactions of nucleophilic substitution. When **25**, KBr or KI and dibenzo-18-crown-6 were refluxed in acetone as described for **17**, TLC revealed only the starting compounds in the reaction mixture. An increase of temperature up to 80–90 °C (in the presence of a small amount of DMF) resulted in degradation of **25**. Utilization of PBr_3 also was not successful. In the reaction with phosphonate **16** under the described conditions,[20] mesylate **25** remained intact.

For alkylation of pyrimidines with bromide **17**, we used a routine procedure.[25] Mesylate **21** also proved to be convenient for this reaction, but the difference in the yields of **22b** was not as significant as with adenine (48% versus 43%).

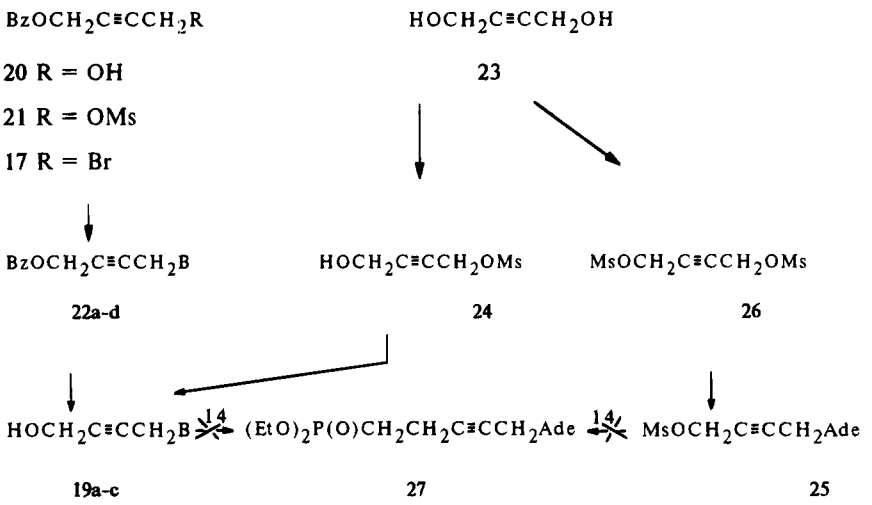
Deprotection of the obtained **22a–c** with aqueous ammonia afforded **19a–c** which were identical to those reported.[22,26] All our attempts to condense **19a–c** with phosphonate **14** were unsuccessful. Therefore **19a–c** were hydrogenated on the Lindlar catalyst to the corresponding (*Z*)-alkenols **28a–c**.[26] These compounds were also prepared by an alternative synthetic route (Scheme 3) starting from (*Z*)-4-(benzoyloxy)but-2-enol (**29**).

Several approaches were used to obtain a guanine derivative, **31d**. Condensation of persilylated guanine with mesylate **21** in CH_2Cl_2 or MeCN[24] yielded a complex mixture from which **22d** was isolated in 15% yield (Scheme 2). Replacement of **21** with bromide **30** resulted in isolation of the 9-substituted guanine **31d** (10%) and the 7,9-disubstituted guanine **32** (22%) (Scheme 3). Treatment of persilylated guanine with **30** in DMSO in the presence of K_2CO_3 increased the yield of **31d** to 25%. The best yields were achieved in the reaction of 2-amino-6-chloropurine with **30** as described[23] followed by deblocking. The overall yield of **28d** was 27%.

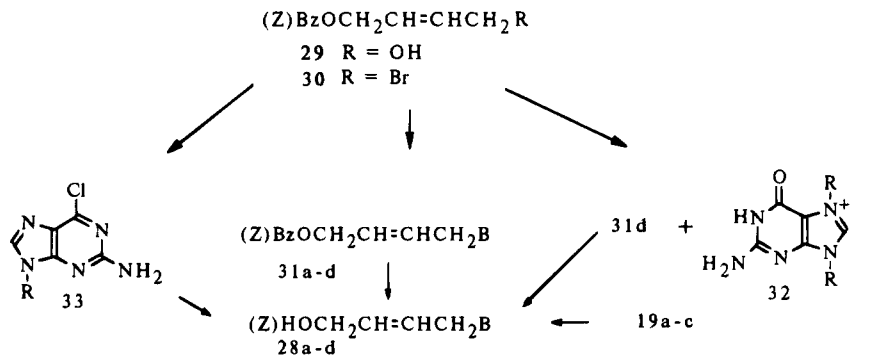
To verify the *Z* configuration of the double bond in **28a–d**, the comparison of ^1H NMR spectra for **28a** and the corresponding *E* isomer[22] was made. The signals of the vinyl protons in the *E* isomer appeared as two clear doublets of triplets at 5.64 and 5.89 ppm (J = 15.6 Hz). For the *Z* isomer, the signals of H2′ and H3′ were observed as two overlapped multiplets at 5.64 and 5.74 ppm. The spectrum registered with protons spin decoupling revealed their transformation into doublets (J = 11 Hz). A similar pattern was observed for **28b–d**.

Butenols **28b,d** were converted to 1-[(*Z*)-4-[(ethylphosphonyl)methoxy]but-2-enyl]thymine (**37b**) and 9-[(*Z*)-4-[(ethylphosphonyl)methoxy]but-2-enyl]guanine (**37d**), respectively, by the reaction with ethyl [[(*p*-tolylsulfonyl)oxy]methyl]phosphonate (**35**) as described[20] (Scheme 4). Compounds **28a,c** were first N-benzoylated, and the obtained **34a,c** were condensed with **35** providing **36a,c**. We used ester **35** as the phosphonate component for

Scheme 2

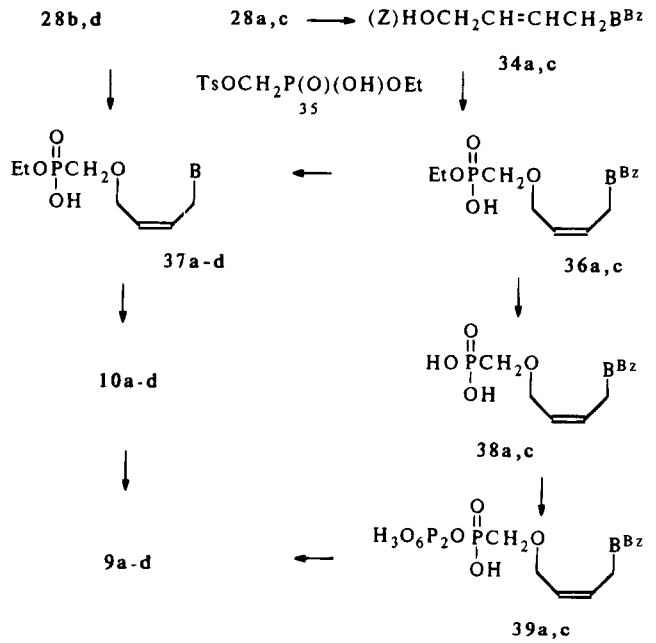


Scheme 3[a]



[a] R = (Z)BzOCH$_2$CH=CHCH$_2$.

Scheme 4[a]



[a] (**a**) B^{Bz} = N^6-benzoyladenin-9-yl; (**c**) B^{Bz} = N^4-benzoylcytosin-1-yl.

these condensation reactions because of its advantages over diester **14**.[27] The synthesized phosphonates **36a,c** and **37b,d** were deblocked by treating with Me_3SiBr. As a result, **10b,d** and *N*-benzoyl derivatives **38a,c**, respectively, were obtained. They were then converted to the corresponding diphosphates **9b,d** and **39a,c** by reaction with *N,N'*-carbonyldiimidazole followed by treatment with bis(tributylammonium) pyrophosphate. Deprotection of the benzoyl group of **39a,c** with aqueous ammonia gave the target compounds **9a,c**. It should be mentioned that pyrophosphorylation proceeded with a higher yield if **10a** was replaced by the N-protected derivative **38a** (51% versus 30%).

The ^{1}H NMR spectra of phosphonates **36a,c**, **37b,d**, and **10a–d** revealed a clear doublet of the CH_2-P group at 3.6–3.8 ppm (J = 8.5–9.5 Hz) characteristic of compounds containing an (oxymethyl)phosphonate group.[20] In the ^{31}P NMR spectra of **10a–d** decoupled with ^{1}H, a phosphorus singlet was observed at 16.0–17.0 ppm. This evidence is consistent with the data earlier obtained for similar phosphonates.[27] The coupling patterns of signals of the vinylic protons in **36b,d**, **37a,c**, and **10a–d** were similar. In the ^{1}H NMR spectra, the proton signals for the phosphonate diphosphates **9a–d** coincided with those for **10a–d**. The ^{31}P NMR spectra were as expected.

Enzymology

Substrate properties of diphosphates **9** were evaluated using two different systems. In both systems, phage M13mp10 single-stranded DNA annealed with a synthetic tetradecadeoxynucleotide was used as the template–primer (Chart 2). At the first stage, we employed the system containing one of the analogs or its natural counterpart (as a control) and a reduced number of dNTPs, sufficient to elongate the chain prior to incorporation of the diphosphates **9**. Substrate properties

Chart 2

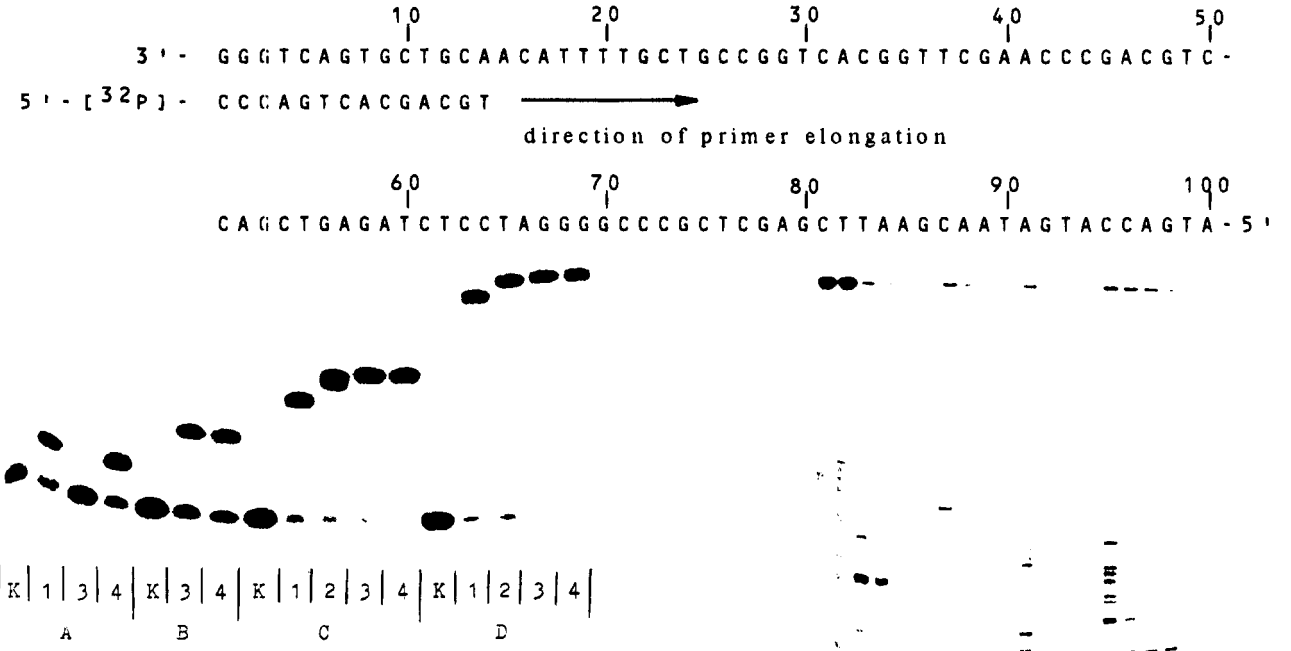


Figure 1. Extension of 5'-^{32}P-labeled primer catalyzed by HIV reverse transcriptase. K—template–primer complex + enzyme. Series A—as in K + one substrate, (1) 10 μM dTTP, (3) 10 μM **9b**, (4) 100 μM **9b**; series B—as in K + two substrates, (3) 10 μM dTTP and 10 μM **9d**, (4) 10 μM dTTP and 100 μM **9d**; series C—(1) as in K + 10 μM dTTP + 10 μM dGTP, (2) as in 1 + 10 μM ddATP, (3) as in 1 + 10 μM **9a**, (4) as in 1 + 100 μM **9a**; series D—(1) as in K + 10 μM dTTP + 10 μM dGTP + 10 μM dATP, (2) as in 1 + 10 μM ddCTP, (3) as in 1 + 10 μM **9c**, (4) as in 1 + 100 μM **9c**.

in competition with the natural substrates were studied at the next stage by using the standard DNA-sequencing procedures.

Figure 1 presents the electrophoregram of the products of primer elongation catalyzed by HIV-1 reverse transcriptase. Clearly, compounds **9a–d** were incorporated into the DNA chain according to the template context. Compound **9b** elongated the primer by one residue (series A, lanes 3 and 4), revealing concentration dependency of the efficiency of incorporation; the control assay employed dTTP (lane 2). The incorporation was also observed for **9a,c,d**; ddATP and ddCTP served as the controls (lanes 3 of series C and D, respectively).

Figure 2 presents the results of a DNA-sequencing assay using **9a–d** as terminating substrates. The sequence is easily determined from the pattern, revealing pronounced substrate-terminating properties of the analogs.

Similar termination patterns were observed with AMV reverse transcriptase. Meanwhile, no incorporation of **9a–d** was observed with DNA polymerases α from human placenta and β from rat liver, DNA polymerase I (Klenow fragment) from *Escherichia coli*, and HSV-1 and CMV DNA polymerases (data not shown).

These data imply a lower substrate specificity of reverse transcriptases as compared to mammalian and viral DNA polymerases. The difference in specificity of these enzymes toward nucleotide substrates has been earlier shown with various nucleoside 5'-triphosphate analogs.[11]

With the ultimate aim of evaluating the affinity of **9** to the HIV reverse transcriptase + template–primer

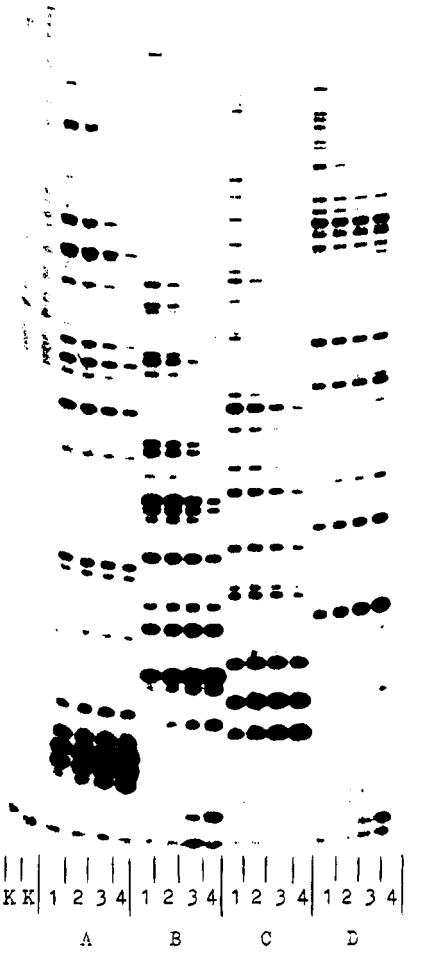
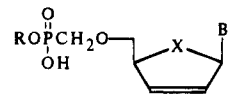


Figure 2. Termination pattern obtained with compounds **9a–d**. Lanes K—DNA synthesis in the absence of terminators (controls). Series A—DNA synthesis in the presence of **9a**; series B—in the presence of **9d**; series C—in the presence of **9c**; series D—in the presence of **9b**. Concentrations of the terminators were as follows: 5 μM (1), 10 μM (2), 20 μM (3), and 50 μM (4). In series D, the concentrations were higher: 20 μM (1), 50 μM (2), 100 μM (3), and 300 μM (4). The concentration of the base analog natural substrate was 3 μM in all series; the concentration of other substrates was 20 μM.

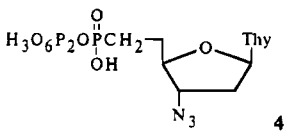
complex, we determined the [**9c**]/[dTTP] concentration ratio at which DNA synthesis is inhibited by 50% under the established conditions.[13] We observed 50% inhibition of DNA synthesis at a **9c**/dCTP mole ratio of 1.2–1.3, i.e., 1.3–1.4-fold higher than that for **8**.[13]

Compounds **10** and **28** were evaluated as inhibitors of HIV reproduction in the cultures of MT-4 and PBL

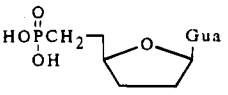
Chart 3



40 X=CH_2, B=Gua, R=$P_2O_6H_3$



41

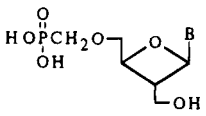


42 X=O, B=Ade, R=H

46

43 X=O, B=Thy, R=H

44 X=CH_2, B=Gua, R=H



45 X=CH_2, B=Thy, R=H

47

cells and showed no activity at concentrations up to 100 μM. The only exception was **10a** which inhibited HIV reproduction by 50% at a concentration of 9 μM.

Discussion

Proceeding to the synthesis and examination of **9** and **10,** we posed the following tasks.

It has been previously shown that compounds containing the conformationally rigid sugar residue manifest good substrate properties toward DNA polymerases.[9,12,13] At the same time, in veiw of the lower substrate specificity of HIV reverse transcriptase relative to human DNA polymerases,[11] we attempted to obtain selective compounds inhibiting only viral reverse transcriptases (compounds **8**[13] showed marked inhibitory activity toward human DNA polymerases α, β, and ϵ). For this purpose, we replaced the natural α-phosphate group by an isosteric phosphonate. Indeed, this modification yielded selective analogs. Compounds **9** were substrates of only HIV and AMV reverse transcriptases and did not display substrate properties toward mammalian DNA polymerases α, β, and ϵ. Furthermore, they were not incorporated into the DNA chain by HSV and CMV DNA polymerases.

The [**9c**]/[dCTP] concentration ratio at which DNA synthesis is inhibited by 50% was 1.3-fold higher than that for **8**,[13] implying a slightly lower affinity of **9** to the DNA synthesizing complex. Moreover, appreciable termination is observed for **9a**–**d** (Figure 1) and **8**[13] at the same [analog]/[dNTP] concentration ratios. These findings are consistent with the similar affinity of the triphosphate of **2** and isosteric **40** (Chart 3) to HIV reverse transcriptase.[28] Meanwhile, replacement of the 5'-O atom by the methylene group inactivates the substrates. The K_M value for **41** in the polymerization reaction catalyzed by HIV reverse transcriptases is 320-fold higher than that for ddTTP(3'N_3).[29] Besides, the k_{cat}/K_M ratio differs 3000-fold for these compounds.

Several studies on anti-HIV activity of isosteric nucleotides have been published; the data obtained are rather diverse. 2',3'-Dideoxy-2',3'-didehydro derivatives **42** and **43** exhibited activity close to that of nucleosides **1**,[30,31] whereas phosphonates **44**–**46**, unlike the corresponding nucleosides, were practically inactive in HIV infected cell cultures.[32–34] Phosphonate **47** showed no activity against HIV, HSV-1, and CMV, while the corresponding nucleoside **4** inhibited the reproduction of these viruses.[35] As mentioned above, compounds **8** have shown no activity in cell cultures.[13] The preliminary results for isosteric **10** indicated that only one of the examined compounds was active in cell cultures. It may be connected with the problems of intracellular phosphorylation and could imply a special mechanism of action for **10a**. Further studies will be reported later.

Experimental Section

Pyridine, MeCN, and DMF (Aldrich) were distilled over CaH_2. LichroPrep RP-8 (40–63 μm), RP-18 (25–40 μm) and Kieselgel 60 F_{254} were from Merck; DEAE Toyopearl was from Toyosoda. UV spectra were registered on a Specord-M10 spectrophotometer and ^{1}H NMR spectra on Varian XL 100 15 and Bruker 250 spectrometers. ^{31}P NMR spectra with P–H decoupling were recorded on a MS-200 spectrometer (external standard—85% H_3PO_3, solvent D_2O). FAB-mass spectra were registered on a Kratos MS 50TC mass spectrometer. Samples were mixed with glycerol in the probe tip. Xenon was used for the fast atom gun at 8 keV.

1-(Benzoyloxy)-4-[(methylsulfonyl)oxy]but-2-yne (21). To a solution of 4-benzoyloxybut-2-ynol (20,0.96 g, 5 mmol) and anhydrous triethylamine (0.77 mL, 5.5 mmol) in ether (50 mL) was added MsCl (0.49 mL, 6.3 mmol) at 2–4 °C and the reaction mixture was stirred at 20 °C for 2 h. The resulting precipitate was removed and the filtrate washed with a saturated $NaHCO_3$ solution (2 × 10 mL), water (2 × 10 mL), and brine (1 × 10 mL) and evaporated. The obtained oil was diluted with toluene (7 mL) and filtered; the solution was concentrated to dryness and twice reevaporated with toluene. The afforded 21 (1.13 g, 84%), TLC homogeneous, as a yellowish oil was used without further purification.

4-(Benzoyloxy)-1-bromobut-2-yne (17). A suspension of **21** (1.34 g, 5 mmol), dibenzo-18-crown-6 (3.6 g, 10 mmol), and KBr (1.19g, 10 mmol) in acetone (20 mL) was refluxed for 2 h and filtered. The filtrate was concentrated to dryness, and the residue was washed with hexane (4 × 10 mL). The hexane solutions were combined and concentrated to dryness affording **17** (0.91 g, 72%) as an oil: n^{18}_D 1.5760; ^{1}H NMR ($CDCl_3$) δ 8.10–7.95, 7.60–7.45 (2 m, 5 H, C_6H_5CO), 4.96 (br s, 2 H, CH_2O), 3.94 (br s, 2 H, CH_2Br).

9-[1-(Benzoyloxy)but-2-yn-1-yl]adenine (22a). Method a. A suspension of adenine (0.63 g, 4.7 mmol) in 10 mL of anhydrous DMF was treated with 80% NaH (0.2 g, 6.7 mmol). After the mixture was stirred for 0.5 h, a solution of **17** (1.42 g, 5.6 mmol) in DMF (5 mL) was added and stirring was continued for 7 h. Then the reaction was quenched with AcOH (0.45 mL), and the solvent was evaporated. Pure **22a** (0.7 g, 49%) was obtained as white crystals by chromatography over Kieselgel eluting with chloroform–EtOH, 9:1(system A): mp 186–188 °C dec; UV (water) λ_{max} 261 nm (ϵ 11 700); ^{1}H NMR (DMSO-d_6) δ 8.16, 8.11 (2 s, 2 H, H-2, H-8), 7.95–7.85, 7.65–7.50 (m, 5 H, C_6H_5CO), 7.2 (br s, 2 H, NH_2), 5.11 (s, 2 H, CH_2O), 4.98 (s, 2 H, CH_2-Ade).

Method b. A suspension of adenine (0.7 g, 5.2 mmol) in DMF (5 mL) was heated to 45 °C, and then 80% NaH (0.23 g, 7.7 mmol) was added while stirring. After 15 min, mesylate **21** (2.15 g, 8 mmol) was added. Stirring was continued for 0.5 h without heating. To the reaction solution was added AcOH (0.45 mL), and it was concentrated to dryness. Purification was carried out as described above to obtain **22a** (1.2 g, 75%).

Method c. To a suspension of adenine (0.2 g, 1.48 mmol) in DMF (10 mL) under N_2 was added potassium bis(trimethylsilyl)amide (0.36 g, 1.8 mmol). After stirring for 15 min, a clear solution was formed. A solution of **17** (0.4 g, 1.5 mmol) in DMF (5 mL) was added, and the reaction mixture was stirred under N_2 for 3 h at 20 °C and then for 2 h at 40 °C. After cooling to 23 °C, AcOH (0.15 mL) was added and the solvent was evaporated. After purification of the residue as described above, **22a** (0.29 g, 64%) was obtained.

1-[4-(Benzoyloxy)but-2-yn-1-yl]thymine (22b). To a suspension of thymine (0.126 g, 1 mmol) in hexamethyldisilazane (5 mL) was added Me_3SiCl (0.75 mL), and the mixture was refluxed for 10 h. When a clear solution was

formed, the solvent was evaporated and the residue was dissolved in MeCN (5 mL). Mesylate **21** or bromide **17** (0.35 or 0.33 g, respectively, 1.3 mmol) was added, and the mixture was refluxed for 4 h. After cooling, the mixture was diluted with ethanol (5 mL) and filtered. Pure **22b** (1.28 g, 43% from **17**, and 1.42 g, 48% from **21**) as white crystals was isolated by chromatography over Kieselgel (elution with chloroform): mp 162–164 °C; UV (water) λ_{max} 266 nm (ϵ 9700); ^{1}H NMR ($CDCl_3$) δ 8.10–7.95, 7.50–7.35 (m, 5 H, C_6H_5CO), 7.23 (d, J = 2.0 Hz, 1 H, 6-H), 4.97 (t, J = 2.0 Hz, 2 H, CH_2O), 4.60 (d, J = 2.0 Hz, 2 H, CH_2-Thy), 1.98 (d, J = 1.5 Hz, 3 H, CH_3); MS *m/e* 299 (MH^+).

1-[4-(Benzoyloxy)-2-butyn-1-yl]cytosine (22c) was obtained as described for **22b** from cytosine (0.17 g, 1.5 mmol) and **17** (0.56 g, 2.2 mmol) in 45% yield (0.19 g): mp 189–191 °C dec; UV (water) λ_{max} 271 nm (ϵ = 12 700); ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 8.00–7.85, 7.45–7.25 (m, 5 H, C_6H_5CO), 7.41 (d, J = 7 Hz, 1H, H-6), 5.72 (d, J = 7 Hz, 1H, H-5), 4.98 (br t, 2H, CH_2O), 4.57 (br t, 2 H, CH_2-Cyt); MS *m/e* 284 (MH^+).

9-[4-(Benzoyloxy)-2-butyn-1-yl]guanine (22d). To a suspension of guanine (0.76 g, 5 mmol) in hexamethyldisilazane (15 mL) was added $(NH_4)_2SO_4$ (0.11 g, 0.8 mmol), and the mixture was refluxed until a solution was formed (20 h). The solvent was evaporated, and a solution of **21** (1.6 g, 6 mmol) in MeCN (5 mL) was added. The mixture was refluxed for 6 h, the reaction quenched with ethanol (10 mL), the mixture filtered, and the filtrate concentrated. The title compound (0.24 g, 15%) as white crystals was isolated by chromatography over Kieselgel (elution with system A): mp 185–188 °C dec; UV (water) λ_{max} 256 (ϵ 9000) (at pH 2.0), 229 (15 000), 253, 276 nm (br shoulders) (at pH 7.0); ^{1}H NMR (DMSO-d_6) δ 7.95–7.85, 7.60–7.45 (m, 5 H, C_6H_5CO), 7.69 (s, 1 H, H-8), 6.46 (br s, 2 H, NH_2), 4.96 (br t, J = 1.5 Hz, 2 H, CH_2O), 4.87 (br t, J = 1.5 Hz, 2 H, CH_2-Gua); MS *m/e* 324 (MH^+).

9-(4-Hydroxybut-2-yn-1-yl)adenine (19a). Method a. A suspension of 22a (0.7 g, 2.3 mmol) in MeOH saturated with ammonia (5 mL) was stirred overnight at 18 °C, and the resulting solution was concentrated *in vacuo*. Pure 19a (0.34 g, 73%) was obtained by chromatography over LicroPrep RP-8 (elution with a linear gradient of EtOH in water, 0 → 12%). It was eluted at 6–9% EtOH and isolated as white crystals: mp 229–232 °C dec; UV (water) λ_{max} 260 nm (ϵ 14 900); ^{1}H NMR (DMSO-d_6) δ 8.19, 8.16 (s, 2 H, H-2, H-8), 7.25 (br s, 2 H, NH_2), 5.01 (br s, 1 H, OH), 4.96 (br s, 2 H, CH_2-Ade), 4.31 (br s, 2 H, CH_2O); MS *m/e* 204 (MH^+).

Method b. A solution of adenine (0.135 g, 1 mmol) and potassium bis(trimethylsilyl)amide (0.36 g, 1.8 mmol) in DMF (3 mL) was stirred for 30 min under N_2 at 18 °C. A solution of **24** (0.18 g, 1.1 mmol) in DMF (3 mL) was added, and the mixture was stirred for 5 h at 18 °C under N_2. It was diluted with MeOH (10 mL) and filtered, and the solvents were evaporated. Purification was performed as described above and afforded **19a** (0.12 g, 59%).

1-(4-Hydroxybut-2-yn-1-yl)thymine (19b) was obtained as described for **19a** (method a) from **22b** (0.18 g, 0.6 mmol) as white crystals in 71% yield: mp 173–175 °C; UV (water) λ_{max} 267 nm (ϵ 9800); ^{1}H NMR (DMSO-d_6) δ 7.25 (d, J = 1.5 Hz, 1 H, 6-H), 5.23 (br s, 1 H, OH), 4.49 (d, J = 1.5 Hz, 2 H, CH_2-Thy), 4.06 (br t, J = 1.5 Hz, 2 H, CH_2O), 1.78 (d, J = 1.0 Hz, 3 H, 5-CH_3); MS *m/e* 195 (MH^+).

1-(4-Hydroxybut-2-yn-1-yl)cytosine (19c) was obtained as described for **19a** (method a) from **22c** (0.19 g, 0.67 mmol) as white crystals in 75% yield: mp >250 °C dec; UV (water) λ_{max} 271 nm (ϵ 12 500); ^{1}H NMR (D_2O) δ 7.34 (d, J = 7 Hz, 1 H, H-6), 5.74 (d, J = 7 Hz, 1 H, H-5), 4.54 (br s, 2 H, CH_2-Cyt), 4.19 (br s, 2 H, CH_2-O); MS *m/e* 180 (MH^+).

1-((*Z*)-4-Hydroxybut-2-en-1-yl)adenine (28a). Method a. A suspension of **19a** (0.20 g, 1 mmol) in methanol (10 mL) containing Lindlar catalyst was hydrogenated under H_2 for 5 h. The solution was filtered, concentrated to dryness, and purified by chromatography on LicroPrep RP-8 (elution with a linear gradient of 0 → 10% EtOH in water). The title compound eluted at 5–7% EtOH and was isolated as white crystals (0.18 g, 88%): mp 197–200 °C; UV (water) λ_{max} 262 nm (ϵ 14 600); ^{1}H NMR (DMSO-d_6) δ 8.10, 8.07 (s, 2 H, H-2, H-8), 7.14 (br s, 2 H, NH_2), 5.71–5.59 (m, 2H, CH=CH), 4.93 (br s, 1 H, OH), 4.81 (d, J = 5 Hz, 2 H, CH_2-Ade), 4.19 (d, J = 5 Hz, 2 H, CH_2O); MS *m/e* 206 (MH^+).

Method b. The title compound was prepared as described for **19a** from **31a** (0.14 g, 0.45 mmol) in a 76% yield.

1-((*Z*)-4-Hydroxybut-2-en-1-yl)thymine (28b). Method a. 28b was prepared as described for **28a** from **19b** (0.12 g, 0.62 mmol) in 86% yield as white crystals: mp 167–169 °C; UV (water) λ_{max} 271 nm (ϵ 9700); ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 7.21 (br s, 1 H, 6-H), 5.90–5.77, 5.42–5.30 (2 m, 2 H, CH=CH), 4.44 (d, J = 6.0 Hz, 2 H, CH_2-Thy), 4.25 (d, J = 6.0 Hz, 2 H, CH_2O), 1.92 (d, J = 1.0 Hz, 3 H, 5-CH_3); MS *m/e* 197 (MH^+).

Method b. The title compound was prepared as described for **19a** from **31b** (0.28 g, 0.97 mmol) as white crystals in 74% yield.

1-((*Z*)-4-Hydroxybut-2-en-1-yl)cytosine (28c). Method a. 28c was prepared as described for **28a** from **19c** (0.1 g, 0.57 mmol) as white crystals in 84% yield: mp >250 °C; UV (water) λ_{max} 273 nm (ϵ 12 100). ^{1}H NMR (D_2O) δ 7.46 (d, J = 7 Hz, 1 H, H-6), 5.86 (d, J = 7 Hz, 1 H, H-5), 5.82–5.69, 5.55–5.42 (2 m, 2 H, CH=CH), 4.34 (d, J = 6.5 Hz, 2 H, CH_2-Cyt), 4.17 (d, J = 6.5 Hz, 2 H, CH_2O); MS *m/e* 182 (MH^+).

Method b. The title compound was prepared as described for **19a** from **31c** (0.27 g, 0.95 mmol) as white crystals in 76% yield (0.13 g).

9-((*Z*)-4-Hydroxybut-2-en-1-yl)guanine (28d). Method a. The title compound was prepared as described for **19a** (method a) from **31d** (0.16 g, 0.5 mmol) in a 70% yield as white crystals: mp >250 °C; UV (water) λ_{max} 254 (ϵ 9700), 273 nm (br shoulder); ^{1}H NMR (DMSO-d_6) δ 7.56 (s, 1 H, H-8), 7.35 (br s, 2 H, NH_2), 5.76–5.64, 5.62–5.50 (2 m, J = 11.5 Hz, 2 H, CH=CH), 4.60 (d, J = 5.5 Hz, 2H, CH_2-Gua), 4.14 (d, J = 5.5 Hz, 2 H, CH_2O); MS *m/e* 222 (MH^+).

Method b. A solution of **33** (0.17 g, 0.5 mmol) in 90% EtOH (5 mL) and aqueous ammonia (0.4 mL) was kept at 20 °C overnight and concentrated *in vacuo*. The residue was dissolved in 0.1 M HCl (3 mL) and refluxed for 3 h. The title compound (0.06 g, 50%) was purified as described for **28a**.

(*Z*)-4-(Benzoyloxy)-1-bromobut-2-ene (30) was prepared as described for 17 from 4-(benzoyloxy)but-2-enol (29; 0.38 g, 2 mmol) through intermediate 1-(benzoyloxy)-4-(mesyloxy)but-2-ene in 74% yield (0.38 g) as a yellowish oil: ^{1}H NMR ($CDCl_3$) δ 8.10–7.95, 7.60–7.45 (2 m, 5 H, C_6H_5CO), 5.90–5.75 (m, 2 H, CH=CH), 4.87 (d, J = 5.5 Hz, 2 H, CH_2O), 3.99 (d, J = 5.5 Hz, 2 H, CH_2Br).

9-[(*Z*)-4-(Benzoyloxy)but-2-en-1-yl]adenine (31a) was prepared as described for **17a** (method a) from adenine (0.135 g, 1 mmol) and bromide **30** (0.38 g, 1.5 mmol) in 58% yield; UV (water) λ_{max} 263 nm (ϵ 11 700); ^{1}H NMR (DMSO-d_6) δ 8.10 (s, 2 H, H-2, H-8), 8.00–7.85, 7.60–7.45 (m, 5 H, C_6H_5CO), 7.15 (br s, 2 H, NH_2), 5.91–5.81 (m, 2 H, CH=CH), 5.06 (d, J = 5 Hz, 2 H, CH_2O), 4.93 (d, J = 5 Hz, 2 H, CH_2-Ade); MS *m/e* 310 (MH^+).

1-[(*Z*)-4-(Benzoyloxy)but-2-en-1-yl]thymine (31b) was obtained as described for **31a** from thymine (0.25 g, 2 mmol) and **30** (0.64 g, 2.5 mmol) in 48% yield: mp 154–157 °C; UV (water) λ_{max} 271 nm (ϵ 9000); ^{1}H NMR ($CDCl_3$) δ 8.05–7.90, 7.50–7.35 (m, 5 H, C_6H_5CO), 7.23 (d, J = 2.0 Hz, 1 H, 6-H), 5.81–5.64 (m, 2H, CH_2O), 4.94 (t, J = 6.5 Hz, 2 H, CH=CH), 4.47 (d, J = 6.5 Hz, 2 H, CH_2-Thy), 1.96 (d, J = 1.5 Hz, 3 H, CH_3); MS *m/e* 301 (MH^+).

1-[(*Z*)-4-(Benzoyloxy)but-2-en-1-yl]cytosine (31c) was obtained as described for 31a from cytosine (0.2 g, 1.8 mmol) and 30 (0.54 g, 2.1 mmol) in 52% yield: mp 181–184 °C; UV (water) λ_{max} 271 nm (ϵ 12 700); ^{1}H NMR (DMSO-d_6) δ 8.00–7.85, 7.45–7.25 (m, 5 H, C_6H_5CO), 7.46 (d, J = 7.0 Hz, 1 H, H-6), 5.86 (d, J = 7.0 Hz, 1 H, H-5), 5.82–5.69, 5.70–5.57 (2 m, 2 H, CH=CH), 4.92 (d, J = 6.5 Hz, 2 H, CH_2O), 4.56 (d, J= 6.5 Hz, 2 H, CH_2-Cyt); MS *m/e* 286 (MH^+).

9-[(*Z*)-4-(Benzoyloxy)but-2-en-1-yl]guanine (31d). Method a. To persilylated guanine, obtained from guanine (0.3 g, 2 mmol) as described for **22d** were added anhydrous DMSO (3 mL), K_2CO_3 (0.44 g, 3 mmol), and **30** (0.78 g, 3 mmol). The mixture was stirred at 20 °C for 16 h, the reaction quenched with ethanol (10 mL), and the mixture filtrated. The title

compound (0.16 g, 25%) as white crystals was isolated by chromatography on Kieselgel (elution with ethyl acetate–ethanol, 9:1, v/v): mp 176–179 °C; UV (water) λ_{max} 257 (ϵ 9200) (at pH 2.0), 229 (15 400), 256, 280 nm (br shoulders) (at pH 7.0); ^{1}H NMR (DMSO-d_6) δ 7.95–7.85, 7.60–7.45 (m, 5 H, C_6H_5CO), 7.67 (s, 1 H, H-8), 5.85–5.73, 5.71–5.59 (2 m, 2 H, CH=CH), 4.86 (d, J = 5.0 Hz, 2 H, CH_2O), 4.79 (d, J = 5.0 Hz, 2 H, CH_2-Gua); MS m/e 326 (MH^+).

7,9-Bis[(*Z*)-4-(benzoyloxy)but-2-en-1-yl]guanine (32) was obtained as described for **22d** from guanine (0.15 g, 1 mmol) and **30** (0.39 g, 1.5 mmol) as white crystals in 22% yield (0.11 g): mp >250 °C; UV (water) λ_{max} 257 (ϵ 9300) (at pH 2.0), 228 (15 600), 276 nm (7200) (at pH 12.0); ^{1}H NMR (CD_3OD) δ 7.95–7.85, 7.60–7.45 (m, 10 H, C_6H_5CO), 6.03–5.90, 5.92–5.77 (2 m, 4 H, CH=CH), 5.31 (d, J = 5.5 Hz, 2 H, CH_2-Gua$^{N\text{-}7}$), 5.05, 5.01 (2 d, J = 5.5, 6.9 Hz, 4 H, CH_2O), 4.96 (d, J = 6.0 Hz, 2 H, CH_2-Gua$^{N\text{-}9}$); MS m/e 500 (MH^+).

2-Amino-6-chloro-9-[(*Z*)-4-(benzoyloxy)but-2-en-1-yl]-purine (33) was prepared as described for **31d** from 2-amino-6-chloropurine (0.12 g, 0.7 mmol), K_2CO_3 (0.15 g, 1 mmol), and **30** (0.26 g, 1 mmol) as a foam in 54% yield: UV (MeOH) λ_{max} 322 (ϵ 6800) (at pH 2.0), 228 (15 600), 311 nm (7200) (at pH 7.0); ^{1}H NMR ($CDCl_3$) δ 7.86 (s, 1 H, H-8), 8.05–7.95, 7.60–7.45 (m, 5 H, C_6H_5CO), 5.99–5.87, 5.88–5.76 (2 m, 2 H, CH=CH), 5.27 (br s, 2 H, NH_2), 5.07 (d, J = 6.0 Hz, 2 H, CH_2-purine), 4.87 (d, J = 6.0 Hz, 2 H, CH_2O); MS m/e 344, 346 (MH^+).

***N*^{6}-Benzoyl-9-((*Z*)-4-hydroxybut-2-en-1-yl)adenine (34a).** To a stirred suspension of **22a** (0.07 g, 0.3 mmol) in pyridine (5 mL) was added Me_3SiCl (0.190 g, 0.22 mL, 1.75 mmol). After stirring for 30 min, the mixture was cooled to 4 °C and BzCl (0.145 g, 0.175 mL, 1.0 mmol) was added. Stirring was continued for 2 h, and then the reaction was quenched with water (0.2 mL) and in 15 min with 25% aqueous ammonia (0.2 mL). The solution was concentrated to dryness, diluted with water (1 mL), and extracted with ether (3 × 0.5 mL). The aqueous fraction was concentrated to dryness, the residue was dissolved in dioxane–$CHCl_3$, 1:1 (1 mL), and purified on Kieselgel (elution with $CHCl_3$–EtOH, 95:5, v/v). The product (0.06 g, 57%) was afforded as a foam: UV (MeOH) λ_{max} 280 nm (ϵ 20 600); ^{1}H NMR (DMSO-d_6) δ 8.68, 8.48 (2 s, 2 H, H-2, H-8), 8.00–7.90, 7.60–7.45 (m, 5 H, C_6H_5CO), 5.78–5.54 (m, 2 H, CH=CH), 4.95 (d, J = 6 Hz, 2 H, CH_2-Ade), 4.89 (poorly resolved t, 1 H, OH), 4.22 (d, J = 6 Hz, 2 H, CH_2O); MS m/e 310 (MH^+).

***N*^{4}-Benzoyl-1-((*Z*)-4-hydroxybut-2-en-1-yl)cytosine (34c)** was prepared as a foam as described for 34a from 28c (0.09 g, 0.5 mmol) in 63% yield: UV (MeOH) λ_{max} 260 nm (ϵ 16 100), 302 nm (ϵ 8200); ^{1}H NMR ($CDCl_3$) 7.95–7.85, 7.60–7.45 (m, 5 H, C_6H_5CO), 7.72 (d, J = 5.0 Hz, 1 H, H-6), 7.47 (d, J = 5.0 Hz, 1 H, H-5, overlapped with C_6H_5CO signals), 5.78–5.64 (m, 2 H, CH=CH), 4.86 (poorly resolved t, 1 H, OH), 4.63 (d, J = 6.0 Hz, 2 H, CH_2-Cyt), 4.30 (d, J = 6.0 Hz, 2 H, CH_2O); MS m/e 286 (MH^+).

***N*^{6}-Benzoyl-9-[(*Z*)-4-[(ethylphosphonyl)methoxy]but-2-en-1-yl]adenine (36a).** To a solution of 34a (0.1 g, 0.3 mmol) coevaporated previously twice with DMF in absolute DMF (3 mL) was added sodium hydride (0.031 g, 1 mmol), and the mixture was stirred at 20 °C for 30 min. To the resulting suspension was added phosphonate 35 (0.14 g, 0.45 mmol), and the mixture was stirred for 12 h (TLC control, dioxane–25% aqueous NH_3, 4:1). The reaction was quenched with glacial AcOH (0.085 mL) and the mixture concentrated to dryness. Compound 36a was isolated by chromatography on DEAE Toyopearl (HCO_3^-); a linear gradient of 0 → 0.1 M ammonium bicarbonate in water (pH 7.5, total volume 500 mL) was used. The fractions collected at 0.05–0.08 M were evaporated, reevaporated with water (2 × 5 mL) and ethanol (2 × 5 mL), and freeze-dried to give pure 36a (0.064 g, 46%) as a white lyophiliate: UV (MeOH) λ_{max} 280 nm (ϵ 20 800); ^{1}H NMR (D_2O) δ 8.00–7.90, 7.60–7.45 (m, 5 H, C_6H_5CO), 7.78, 7.76 (2 s, 2 H, H-2, H-8), 5.69–5.55 (m, 2 H, CH=CH), 4.60 (d, J = 6 Hz, 2 H, CH_2-Ade), 4.05 (d, J = 6 Hz, 2 H, CH_2O), 3.73 (q, J = 7 Hz, 2 H, CH_2-CH_3), 3.48 (d, J = 8.5 Hz, 2 H, CH_2P), 1.04 (t, J = 7 Hz, 3 H, CH_3-CH_2); MS m/e 433 (MH^+).

***N*^{4}-Benzoyl-1-[(*Z*)-4-[(ethylphosphonyl)methoxy]but-2-en-1-yl]cytosine (36c)** was obtained as a white lyophiliate as described for 36a from 34c (0.1 g, 0.35 mmol), NaH (0.034 g, 1.1 mmol), and 35 (0.16 g, 0.5 mmol) in 38% yield: UV (water) λ_{max} 259 (ϵ 16 600), 303 nm (8900); ^{1}H NMR (D_2O) δ 7.95–7.85, 7.7–7.50 (m, 5 H, C_6H_5CO), 7.2, 7.1 (2 br s, 2 H, H-5, H-6), 5.8–5.5 (m, 2 H, CH=CH), 4.42 (d, J = 7 Hz, 2 H, CH_2-Cyt), 4.19 (d, J = 6 Hz, 2 H, CH_2O), 3.90 (dt, J = 7 Hz, 2 H, CH_2-CH_3), 3.62 (d, J = 9 Hz, CH_2-P), 1.21 (t, 3 H, J = 7 Hz, CH_3-CH_2); MS m/e 409 (MH^+).

9-[(*Z*)-4-[(Ethylphosphonyl)methoxy]but-2-en-1-yl]-adenine (37a). Phosphonate 36a (0.04 g, 0.01 mmol) was kept at 20 °C in 25% aqueous ammonia (10 mL) for 30 h. The solution was concentrated to dryness. The title compound (0.022 g, 72%) as a white lyophiliate was purified as described for 36a: UV (water) λ_{max} 259 nm (ϵ 14 400); ^{1}H NMR (D_2O) δ 8.06, 8.03 (2 s, 2 H, H-2, H-8), 5.92–5.78 (m, 2 H, CH=CH), 4.84 (d, J = 6 Hz, 2 H, CH_2-Ade), 4.31 (d, J = 6 Hz, 2 H, CH_2O), 4.00 (q, J = 7 Hz, 2 H, CH_2-CH_3), 3.40 (d, J = 8.5 Hz, CH_2P), 1.27 (t, J = 7 Hz, 3 H, CH_3-CH_2); ^{31}P NMR δ 18.3 (d, J = 8.5 Hz, CH_2-P); MS m/e 328 (MH^+).

1-[(*Z*)-4-[(Ethylphosphonyl)methoxy]but-2-en-1-yl]-thymine (37b) was obtained as described for 36a as a white lyophiliate from 28b (0.06 g, 0.3 mmol), NaH (0.03 g, 1 mmol), and 35 (0.14 g, 0.45 mmol) in 38% yield: UV (water) λ_{max} 267 nm (ϵ 9400); ^{1}H NMR (D_2O) δ 7.47 (d, J = 1 Hz, 1 H, H-6), 5.92–5.79, 5.76–5.63 (2 m, 2 H, CH=CH), 4.46 (d, J = 6.5 Hz, 2 H, CH_2-Thy), 4.27 (d, J = 6.5 Hz, 2 H, CH_2O), 3.98 (m, 2 H, CH_2-CH_3), 3.71 (d, J = 9.0 Hz, CH_2-P), 1.88 (d, J = 1.0 Hz, 3 H, CH_3-5), 1.25 (t, J = 7.0 Hz, 3 H, CH_3-CH_2); ^{31}P NMR δ 17.87 (d, J = 9.0 Hz, CH_2-P); MS m/e 319 (MH^+).

1-[(*Z*)-4-[(Ethylphosphonyl)methoxy]but-2-en-1-yl]-cytosine (37c) as a white lyophiliate was prepared as described for **37a** from **36c** (0.1 g, 0.24 mmol) in 66% yield: UV (water) λ_{max} 272 nm (ϵ 12 700); ^{1}H NMR (D_2O) δ 7.55 (d, J = 7.0 Hz, 1 H, H-6), 5.91 (d, J = 7.0 Hz, 1H, H-5), 5.82–5.69, 5.70–5.57 (2 m, 2 H, CH=CH), 4.51 (d, J = 6.0 Hz, 2 H, CH_2-Cyt), 4.22 (d, J = 6.0 Hz, 2 H, CH_2O), 3.92 (m, 2 H, CH_2-CH_3), 3.59 (d, J = 9.5 Hz, CH_2-P), 1.22 (t, J = 7.0 Hz, 3 H, CH_3-CH_2); ^{31}P NMR δ 18.11 (d, J = 9.2 Hz, CH_2-P); MS m/e 304 (MH^+).

9-[(*Z*)-4-[(Ethylphosphonyl)methoxy]but-2-en-1-yl]guanine (37d) as a white lyophiliate was prepared as described for **36a** from **28d** (0.088 g, 0.4 mmol), NaH (0.04 g, 1.3 mmol) and **35** (0.19 g, 0.6 mmol), in 36% yield; UV (water) λ_{max} 254 (ϵ 8800), 279 nm (shoulder); ^{1}H NMR (D_2O) 7.71 (s, 1 H, H-8), 5.87–5.75, 5.78–5.66 (2 m, 2 H, CH=CH), 4.64 (d, J = 6.5 Hz, 2 H, CH_2-Gua), 4.23 (d, J = 6.5 Hz, 2 H, CH_2O), 3.90 (m, 2 H, CH_2-CH_3), 3.66 (d, J = 9.0 Hz, CH_2-P), 1.19 (t, J = 7.0 Hz, 3 H, CH_3-CH_2); ^{31}P NMR δ 18.45 (d, J = 8.5 Hz, CH_2-P); MS m/e 344 (MH^+).

9-[(*Z*)-4-(Phosphonomethoxy)but-2-en-1-yl]adenine, Ammonium Salt (10a). To a solution of 9-[(*Z*)-4-[(ethylphosphonyl)methoxy]but-2-enyl]adenine (0.044 g, 0.13 mmol) in DMF (1 mL) was added Me_3SiBr (0.12 mL), and the resulting solution was kept at 20 °C for 45 h. The mixture was concentrated to dryness and reevaporated with DMF (1 mL) and toluene (1 mL). Crude **10a** was purified on DEAE Toyopearl as described for **24a** (linear gradient of 0 → 0.15 M NH_4HCO_3). Fractions collected at 0.09–0.11 M NH_4HCO_3 were concentrated *in vacuo* and reevaporated with water (3 × 5 mL) and ethanol (2 × 5 mL). The obtained solid was diluted with water (1 mL) and put onto a LichroPrep RP-18 column (1 × 10 cm). Elution with water followed by freeze-drying afforded pure **10a** (0.026 g, 65%) as a white lyophiliate; UV (water) λ_{max} 261 nm (ϵ 14 800); ^{1}H NMR (D_2O) δ 7.97, 7.95 (2 s, 2H, H-2, H-8), 5.85–5.74 (m, 2 H, CH=CH), 4.60 (d, overlapped with water, 2 H, CH_2-Ade), 4.10 (d, J = 6 Hz, 2 H, CH_2O), 3.45 (d, J = 8.5 Hz, CH_2-P); ^{31}P NMR δ 16.43 (d, J = 8.5 Hz, CH_2-P); MS m/e 300 (MH^+), 317 ($MH + NH_3^+$).

1-[(*Z*)-4-(Phosphonomethoxy)but-2-en-1-yl]thymine, ammonium salt (10b) as a white lyophiliate was obtained as described for **10a** from **37b** (0.04 g, 0.12 mmol) and Me_3SiBr (0.11 mL) in 58% yield: UV (water) λ_{max} 268 nm (ϵ 9700); ^{1}H NMR (D_2O) δ 7.41 (s, 1 H, H-6), 5.90–5.78, 5.74–5.61 (2 m, 2 H, CH=CH), 4.46 (d, J = 6.5 Hz, 2 H, CH_2-Thy), 4.29 (d, J = 6.5 Hz, 2 H, CH_2O), 3.71 (d, J = 9.0 Hz, CH_2-P), 1.90 (s, 3 H,

CH_3-5); ^{31}P NMR δ 16.71 (d, J = 8.9 Hz, CH_2-P); MS *m/e* 291 (MH^+), 308 ($MH + NH_3$)$^+$.

1-[(Z)-4-(Phosphonomethoxy)but-2-en-1-yl]cytosine, ammonium salt (10c) as a white lyophiliate was obtained as described for **10a** from **37c** (0.032 g, 0.1 mmol) and Me_3SiBr (0.1 mL) in 67% yield: UV (water) λ_{max} 272 nm (ϵ 12 800); ^1H NMR (D_2O) δ 7.59 (d, J = 7.0 Hz, 1 H, H-6), 5.93 (d, J = 7.0 Hz, 1 H, H-5), 5.80–5.67, 5.69–5.57 (2 m, 2 H, CH=CH), 4.51 (d, J = 6.0 Hz, 2 H, CH_2-Cyt), 4.24 (d, J = 6.0 Hz, 2 H, CH_2O), 3.64 (d, J = 9.5 Hz, CH_2-P); ^{31}P NMR δ 16.2 (d, J = 9.3 Hz, CH_2-P); MS *m/e* 276 (MH^+), 293 ($MH + NH_3$)$^+$.

9-[(Z)-4-(Phosphonomethoxy)but-2-enyl]guanine, ammonium salt (10d) as a white lyophiliate was obtained as described for **10a** from **37c** (0.033 g, 0.09 mmol) and Me_3SiBr (0.1 mL) in 57% yield: UV (water) λ_{max} 252 (ϵ 11 000), 272 nm (shoulder); ^1H NMR (D_2O) 7.76 (s, 1 H, H-8), 5.87–5.75, 5.80–5.68 (2 m, 2 H, CH=CH), 4.70 (d, J = 6.5 Hz, 2 H, CH_2-Gua), 4.29 (d, J = 6.5 Hz, 2 H, CH_2O), 3.66 (d, J = 9.0 Hz, CH_2-P); ^{31}P NMR δ 16.49 (d, J = 9.2 Hz, CH_2-P); MS *m/e* 316 (MH^+), 333 ($MH + NH_3$)$^+$.

9-[(Z)-4-[(Pyrophosphorylphosphonyl)methoxy]but-2-en-1-yl]adenine, Bis(ammonium salt) (9a). Method a. Compound **10a** (0.021 g, 0.066 mmol) freshly evaporated with DMF (2 × 1 mL) was dissolved in a mixture of DMF (5 mL) and Bu_3N (0.015 mL). *N,N'*-Carbonyldiimidazole (0.055 g, 0.34 mmol) was added, and the reaction mixture was stirred at 22 °C for 10 h. Then the reaction was quenched with methanol (0.015 mL), and a 0.5 M solution of bis(tributylammonium) pyrophosphate (1 mL) was added while stirring. In 2 h, the reaction mixture was diluted with water (300 mL) and put onto a DEAE Toyopearl column. Compound **9a** (0.01 g, 30%) was isolated by chromatography on DEAE Toyopearl (HCO_3^-). Elution was with a linear gradient of 0 → 0.35 M NH_4HCO_3 in water (pH 7.5, total volume 300 mL). The fractions collected at 0.19–0.22 M buffer were concentrated *in vacuo*, reevaporated with water (4 × 4 mL) and ethanol (2 × 5 mL), and freeze-dried to give a white lyophiliate: UV (MeOH) λ_{max} 261 nm (ϵ 14 800); ^1H NMR (D_2O) δ 8.07, 8.03 (2 s, 2 H, H-2, H-8), 5.89–5.77, 5.83–5.70 (m, 2 H, CH=CH), 4.26 (d, J = 6.0 Hz, 2 H, CH_2O), 3.70 (d, J = 8.5 Hz, 2 H, CH_2-P), CH_2-Ade shift signal overlapped with water signal; ^{31}P NMR δ 9.69 (d, $J_{\alpha,\beta}$ = 26.8 Hz, P_α), −9.96 (d, $J_{\gamma,\beta}$ = 19.54 Hz, P_γ), −22.55 (m, P_β).

Method b. The title compound was obtained as described for **37a** from **39a** (0.02 g, 0.033 mmol) in 85% yield.

1-[(Z)-4-[(Pyrophosphorylphosphonyl)methoxy]but-2-en-1-yl]thymine, bis(ammonium salt) (9b) as a white lyophiliate was obtained as described for 9a from 10b (0.02 g, 0.065 mmol) (method a) in 44% yield: UV (water) λ_{max} 268 nm (ϵ 9700); ^1H NMR (D_2O) δ 7.41 (s, 1 H, H-6), 5.90–5.78, 5.74–5.61 (2 m, 2 H, CH=CH), 4.46 (d, J = 6.5 Hz, 2 H, CH_2-Thy), 4.27 (d, J = 6.5 Hz, 2 H, CH_2O), 3.70 (d, J = 9.0 Hz, CH_2-P), 1.90 (s, 3 H, CH_3-5); ^{31}P NMR δ 10.06 (d, $J_{\alpha,\beta}$ = 26.7 Hz, P_α), −9.99 (d, $J_{\gamma,\beta}$ = 19.5 Hz, P_γ), −22.03 (m, P_β).

1-[(Z)-4-[(Pyrophosphorylphosphonyl)methoxy]but-2-en-1-yl]cytosine, bis(ammonium salt) (9c) as a white lyophiliate was obtained as described for 9a (method b) from 39c (0.012 g, 0.02 mmol) in 81% yield: UV (water) λ_{max} 271 nm (ϵ 12 400); ^1H NMR (D_2O) δ 7.63 (d, J = 7.0 Hz, 1 H, H-6), 5.99 (d, J = 7.0 Hz, 1 H, H-5), 5.93–5.77, 5.75–5.62 (2 m, 2 H, CH=CH), 4.48 (d, J = 6.0 Hz, 2 H, CH_2-Cyt), 4.27 (d, J = 6.0 Hz, 2 H, CH_2O), 3.71 (d, J = 9.5 Hz, CH_2-P); ^{31}P NMR δ 10.56 (d, $J_{\alpha,\beta}$ = 26.1 Hz, P_α), −9.16 (d, $J_{\gamma,\beta}$ = 19.7 Hz, P_γ), −22.35 (m, P_β).

9-[(Z)-4-[(Pyrophosphorylphosphonyl)methoxy]but-2-en-1-yl]guanine, bis(ammonium salt) (9d) as a white lyophiliate was obtained as described for 9a from 10d (method a) in 34% yield; UV (water) λ_{max} 252 (ϵ 10 300), 272 nm (shoulder); ^1H NMR (D_2O) 7.76 (s, 1 H, H-8), 5.87–5.75, 5.80–5.68 (2 m, 2 H, CH=CH), 4.70 (d, J = 6.5 Hz, 2 H, CH_2-Gua), 4.29 (d, J = 6.5 Hz, 2 H, CH_2O), 3.66 (d, J = 9.0 Hz, CH_2-P); ^{31}P NMR δ 10.11 (d, $J_{\alpha,\beta}$ = 26.8 Hz, P_α), −9.81 (d, $J_{\gamma,\beta}$ = 19.62 Hz, P_γ), −22.45 (m, P_β).

***N*⁶-Benzoyl-9-[(Z)-4-(phosphonomethoxy)but-2-en-1-yl]adenine, ammonium salt (38a)** as a white lyophiliate was prepared as described for **10a** from **36a** (0.064 g, 0.143 mmol) and Me_3SiBr (0.13 mL) in 68% yield: UV (water) λ_{max} 281 nm (ϵ 19 500); ^1H NMR (D_2O) δ 8.05–7.94, 7.60–7.45 (m, 5 H, C_6H_5CO), 7.81, 7.78 (2 s, 2 H, H-2, H-8),5.80–5.75 (m, 2 H, CH=CH), 4.58 (d, J = 6 Hz, 2 H, CH_2-Ade), 4.10 (d, J = 6 Hz, 2 H, CH_2O), 3.53 (d, J = 8.5 Hz, CH_2-P); MS *m/e* 404 (MH^+), 421 ($MH + NH_3$)$^+$.

***N*⁴-Benzoyl-1-[(Z)-4-(phosphonomethoxy)but-2-en-1-yl]cytosine, ammonium salt (38c)** as a white lyophiliate was prepared as described for **10a** from **36c** (0.076 g, 0.18 mmol) and Me_3SiBr (0.16 mL) in 71% yield: UV (water) λ_{max} 258 (ϵ 15 700), 302 nm (8600); ^1H NMR (D_2O) δ 8.00–7.85, 7.7–7.55 (m, 5 H, C_6H_5CO), 7.24, 7.11 (2 br s, 2 H, H-5, H-6), 5.78–5.66, 5.63–5.50 (m, 2 H, CH=CH), 4.40 (d, J = 7.0 Hz, 2 H, CH_2-Cyt), 4.21 (d, J = 6.5 Hz, 2 H, CH_2O), 3.59 (d, J = 9 Hz, CH_2-P); MS *m/e* 380 (MH^+), 397 ($MH + NH_3$)$^+$.

***N*⁶-Benzoyl-9-[(Z)-4-[(pyrophosphorylphosphonyl)methoxy]but-2-en-1-yl]adenine, bis(ammonium salt) (39a)** as a white lyophiliate was obtained as described for 9a (method a) from 38a (0.04 g, 0.1 mmol), NBu_3 (0.024 mL, 0.1 mmol), KDI (0.085 g, 0.52 mmol), and a 0.5 M solution of bis(tributylammonium) pyrophosphate (1 mL) in 51% yield: UV (water) λ_{max} 281 nm (ϵ 19 200); ^1H NMR (D_2O) δ 8.05–7.94, 7.60–7.45 (m, 5 H, C_6H_5CO), 7.81, 7.78 (2 s, 2 H, H-2, H-8), 5.71–5.59, 5.65–5.53 (2 m, 2 H, CH=CH), 4.60 (d, J = 6.0 Hz, 2 H, CH_2-Ade), 4.05 (d, J = 6.0 Hz, 2 H, CH_2O), 3.48 (d, J = 8.5 Hz, 2 H, CH_2P); MS *m/e* 566 (MH^+), 582 ($MH + NH_3$)$^+$).

***N*⁴-Benzoyl-1-[(Z)-4-[(pyrophosphorylphosphonyl)methoxy]but-2-en-1-yl]cytosine, bis(ammonium salt) (39c)** as a white lyophiliate was obtained as described for **9a** (method a) from **38c** (0.015 g, 0.038 mmol), NBu_3 (0.01 mL, 0.042 mmol), KDI (0.031 g, 0.19 mmol), and a 0.5 M solution of bis(tributylammonium) pyrophosphate (0.4 mL) in 55% yield: UV (water) λ_{max} 259 (ϵ 15 200), 303 nm (8100); ^1H NMR (D_2O) δ 7.85–7.65, 7.7–7.35 (m, 5 H, C_6H_5CO), 7.26, 7.09 (2 br s, 2 H, H-5, H-6), 5.78–5.65, 5.63–5.51 (m, 2 H, CH=CH), 4.46 (d, J = 7.0 Hz, 2 H, CH_2-Cyt), 4.23 (d, J = 6.0 Hz, 2 H, CH_2O), 3.62 (d, J = 9.0 Hz, CH_2-P); MS *m/e* 541 (MH^+), 558 ($MH + NH_3$)$^+$.

4-[(Methylsulfonyl)oxy]but-2-yn-1-ol (25). A solution of anhydrous triethylamine (1.1 g, 11 mmol) in anhydrous dioxane (5 mL) was added dropwise to a cold (10 °C) solution of but-2-yne-1,4-diol (0.85 g, 10 mmol) and MsCl (1.26 g, 11 mmol) in dioxane (20 mL) under vigorous stirring. After 0.5 h, the reaction mixture was filtered and the solvent was removed *in vacuo*. The residue was diluted with EtOH–water (6:1, 10 mL), the precipitate was filtrated, and the filtrate was concentrated *in vacuo*. The crude product was purified by flash chromatography on neutral Al_2O_3. Elution was with hexane–$CHCl_3$ (1:3), and **25** (0.92 g, 56%) was obtained as an oil: ^1H NMR ($CDCl_3$ + CD_3OD) δ 5.08 (br s, 2 H, CH_2OMs), 4.45 (br s, 2 H, CH_2OH), 3.18 (s, 3 H, CH_3SO_2).

9-[4-[(Methylsulfonyl)oxy]but-2-ynyl)adenine (26). To a suspension of the sodium salt of adenine in DMF (7 mL), obtained from adenine (0.34 g, 2.5 mmol) and NaH (0.12 g, 3.75 mmol), was added 1,4-bis(mesyloxybut-2-yne (0.726 g, 3 mmol) under vigorous stirring. The reaction was stirred for 1 h until the solution was formed. Glacial AcOH (0.2 mL) was added, and the mixture was concentrated *in vacuo*. Purification was over Kieselgel. Elution with $CHCl_3$–MeOH (9:1) gave pure **26** (0.38 g, 45%) as white crystals that decomposed at >210 °C: UV (water) λ_{max} 261 nm (ϵ 13 800); ^1H NMR (DMSO-d_6) δ 8.18, 8.15 (2 s, 2 H, H-2, H-8), 7.3 (br s, 2 H, NH_2), 5.15 (br t, J = 1.5 Hz, 2 H, CH_2O), 4.99 (br t, J = 1.5 Hz, 2 H, CH_2-Ade), 3.22 (s, 3 H, CH_3SO_2).

1,4-Bis[(methylsulfonyl)oxy]but-2-yne (27). To a cold (10 °C) solution of 2-butyne-1,4-diol (0.43 g, 5 mmol) and MsCl (1.15 g, 10 mmol) in dioxane (20 mL) was added a solution of NEt_3 (1.01 g, 10 mmol) in dioxane (5 mL) dropwise under stirring. After stirring for 1 h, the reaction mixture was filtered and concentrated *in vacuo*. The residue was diluted with EtOH–water (9:1, 12 mL), and the formed precipitate was filtered and dried *in vacuo* to afford the title compound (0.88 g, 73%) as white crystals: mp 95–97 °C (EtOH); ^1H NMR ($CDCl_3$) δ 4.94 (s, 4 H, 2 CH_2O), 3.16 (s, 6H, 2 CH_3SO_2).

Enzymatic Experiments. Deoxynucleoside triphosphates were purchased from Fluka; [γ-^{32}P]dATP with a specific activity of 1500 Ci/mmol was from Izotop (Russia). Other chemicals were of the highest grade available. Phage M13mp10

single-stranded DNA was isolated from the cultural fluid of the recipient *E. coli* K12xL1 strain according to the reported procedure.[35] A synthetic tetradecanucleotide (Chart 2) was labeled at the 5' terminus using phage T4 polynucleotide kinase and annealed with the template by heating to 65 °C and subsequent slow cooling. DNA polymerases α and β were isolated from human placenta[36] and rat liver,[37] respectively. Recombinant HIV-1 reverse transcriptase was isolated as described.[38] Avian myeloblastosis virus reverse transcriptase was obtained from Omutninsk Chemicals (Russia); *E. coli* DNA polymerase was from Amersham. Herpes simplex virus type I and human cytomegalovirus DNA polymerases were isolated according to the reported procedures,[14,15] respectively.

Substrate properties of nucleoside analogs were evaluated in the assay mixture (volume 6 μL) containing 0.01 μM 5'-^{32}P-labeled template–primer complex, nucleoside analog (or dNTP in control assays), enzyme, and the corresponding buffer. The enzyme amounts and buffer compositions have been reported.[38,19,15] The reaction was carried out for 20 min at 37 °C (10 min at 20 °C for DNA polymerase I) and terminated by adding 3 μL of deionized formamide containing EDTA and dyes. Reaction products were separated by electrophoresis in 20% PAAG.

To evaluate the termination properties of the compounds, standard DNA sequencing assays were used. Reaction products were separated in 8% PAAG. Autoradiographic assays were carried out using XRP-5 X-ray film (Kodak).

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