Phosphodiester Amidates of Unsaturated Nucleoside Analogues: Synthesis and Anti-HIV Activity[1]

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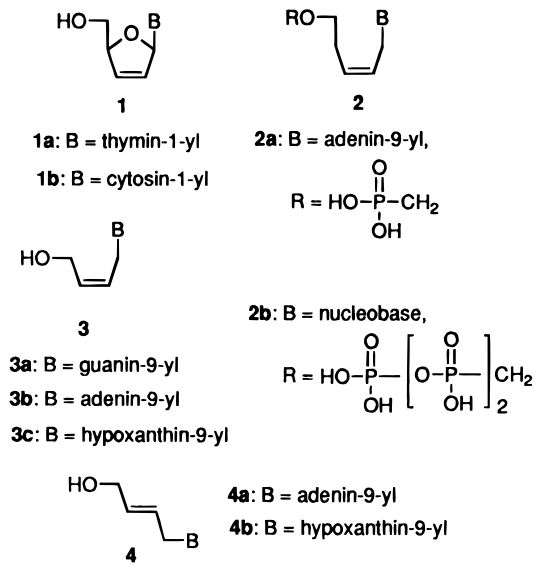
The effect of introduction of a lipophilic phosphodiester amidate moiety on the HIV activity of inactive unsaturated nucleoside analogues was investigated. Phosphodiester alaninates **5a**, **5b**, and **6** derived from unsaturated nucleoside analogues **3b**, **3c**, and **4a** were synthesized and investigated as inhibitors of cytopathic effect and replication of HIV-1 in ATH-8 cells. Compound **5a** is an inhibitor of HIV-1 whereas analogue **6** is inactive with cytotoxicity appearing above 10 μM and **5b** is both inactive and nontoxic. Alkaline or enzymic hydrolysis of **5a** gave phosphomonoester alaninate **14**, a putative product of intracellular metabolism. Compound **14** as well as adenallene derivative **15c** were devoid of anti-HIV activity, and they also failed to inhibit HIV reverse transcriptase. A new regioselective method for preparation of (*Z*)-4-(benzoyloxy)-1-hydroxy-2-butene, **7**, a key intermediate for the synthesis of unsaturated nucleoside analogues of *cis* configuration such as **3a**, **3b**, and **3c**, is also described.

Unsaturated nucleosides **1** are important anti-HIV agents (Chart 1). A recently approved[2] drug for AIDS, d4T (**1a**, stavudine, Zerit), belongs to this class of analogues. The corresponding cytosine analogue **1b** is also a strong inhibitor of replication[3] of HIV. In the acyclic series, two groups of analogues were studied as potential antiviral agents. Compounds of the type **2** (R = H), formally derived from **1** by deletion of the ribofuranose oxygen, did not exhibit antiviral (anti-HIV) activity[4] *per se*, but phosphonate analogue **2a** was an anti-HIV agent (EC_{50} 9 μM). The corresponding diphosphate phosphonates **2b** inhibited viral DNA polymerases and HIV-1 reverse transcriptase.[4] Foreshortened acyclic analogues of unsaturated nucleosides **1** such as **3a** and **3b** were also investigated as potential antiviral agents. Compound **3b** can be regarded[5] as an acyclic analogue of antibiotic neplanocin A. Analogues of type **3** as well as the respective *E*-isomers **4** were devoid of antiviral activity,[5,6] and only guanine analogue **3a** exhibited a moderate antiherpetic effect.[5,7−9]

Recent results have indicated[10,11] that lipophilic phosphodiester amidates of anti-HIV agents such as AZT and d4T can be successfully employed as delivery forms of the corresponding 5′-phosphorylated intermediates to overcome kinase deficiency of particular host cells. Similar derivatives have also been shown to impart anti-HIV activity on inactive nucleoside analogues.[12] In addition, anti-HIV activity of allenic nucleoside analogues such as adenallene[6,13] was significantly enhanced by introduction of a lipophilic phenylphosphoralaninate moiety.[14] It was therefore of interest to study additional phosphoramidates derived from other inactive acyclic and unsaturated nucleoside analogues. In this paper we describe results obtained with phenylphosphoralaninates **5a**, **5b**, and **6**.

Chart 1



Synthesis. The 2-butenol **3b**, a starting material for synthesis of **5a**, was obtained by the known procedure.[9] The previous method[15] for preparation of 1-(benzoyloxy)-4-hydroxy-2-butene, **7**, a key intermediate in this synthesis, suffers from a lack of regioselectivity. This drawback was removed by transformation of butene-1,4-diol **8** to the cyclic orthobenzoate **9** using trimethyl orthobenzoate and *p*-toluenesulfonic acid in DMF (Scheme 1) in 68% yield. Hydrolysis of **9** with 1% acetic acid in THF afforded smoothly compound **7** (66% yield). The latter was converted as described[15] to the respective 1-(benzoyloxy)-4-bromo-2-butene, **10**.

Although *E*-olefin **4a** was readily deaminated with adenosine deaminase[5] on a preparative scale to give the corresponding hypoxanthine derivative **4b** in 79% yield, the reaction of the *Z*-isomer **3b** was sluggish. Therefore, 6-chloropurine was alkylated with 1-(benzoyloxy)-4-bromo-2-butene, **10**, to furnish intermediate **11** (51% yield). Hydrolysis with 80% formic acid[16] followed by debenzoylation with methanolic ammonia afforded the requisite hypoxanthine derivative **3c** in 60% yield.

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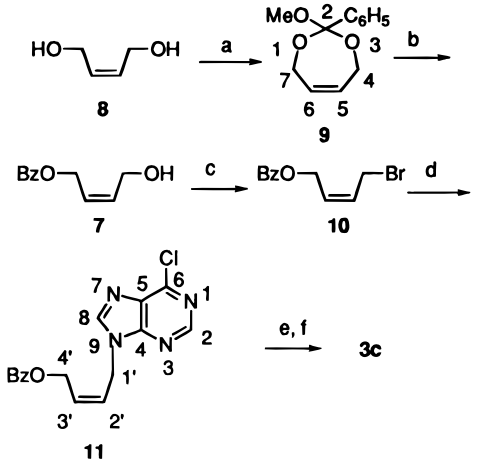
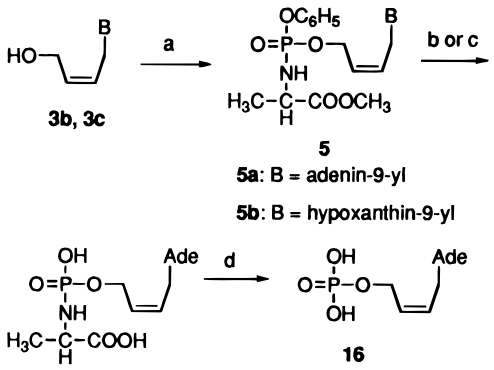
Scheme 1



a. $C_6H_5C(OMe)_3$, TsOH, DMF.
b. 1 % CH_3COOH, THF.
c. PBr_3, C_6H_6.
d. 6-Chloropurine, NaH, DMF.
e. 80 % HCOOH, 80°C.
f. NH_3, MeOH.

Scheme 2

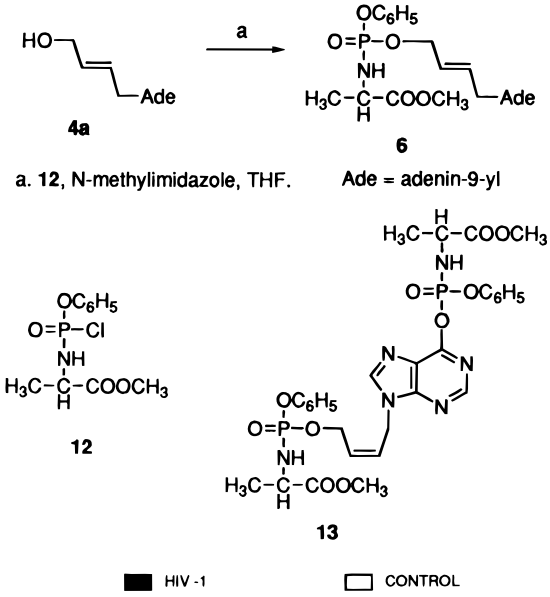


a. **12**, N-methylimidazole, THF.
b. $N(Et)_3$, H_2O.
c. PLE or intracellular esterase.
d. Phosphoramidase (?).

Analogues **5a**, **5b**, and **6** were obtained by phosphorylation of the corresponding 2-butenols **3b**, **3c**, and **4a** with phenyl chlorophosphoralaninate **12** and *N*-methylimidazole[17] in yields ranging from 53 to 75% (Schemes 2 and 3). In the case of hypoxanthine derivative **3c** the crude product contained, in addition to **5b**, a significant amount of a faster moving component, presumably intermediate **13**. A similar byproduct was observed earlier[14] during reaction of hypoxallene with **12**. The workup of the reaction mixture with 80% acetic acid led to a disappearance of **13**, and phosphodiester amidate **5b** was obtained in 53% yield.

Phosphodiester amidates **5a**, **5b**, and **6** are each a mixture of two diastereoisomers which is reflected in their HPLC profiles and NMR spectra. A similar differentiation was observed previously in the case of phosphodiester amidates derived from AZT[10] and allenic analogues.[14] Thus, adenine derivatives **5a** and **6** were resolved into two peaks whereas hypoxanthine amidate **5b** gave only a single one. The CH_3O signals in the ^1H NMR and phosphorus in ^{31}P NMR appeared in all cases as two singlets. It is also interesting that the H_8 signals of ^1H NMR of the *Z*-derivatives **5a** and **5b** formed two

Scheme 3



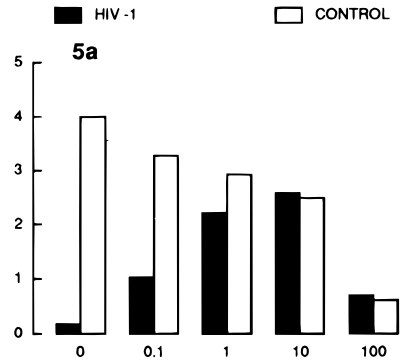


Figure 1. Inhibition of infectivity and cytopathic effect of HIV-1 in ATH-8 cells by analogue **5a**. For details, see the Experimental Section.

singlets, but both diastereoisomers of the *E*-amidate **6** gave only a single peak.

Biological Activity. Analogues **5a**, **5b**, and **6** were tested as inhibitors of replication and cytopathic effect of HIV-1 in ATH-8 cells. The results are given in Figure 1. Phosphoramidate **5a** derived from *Z*-alkene **3b** was the most potent compound. The protective effect against HIV infection was seen in the range of 1−10 μM with a relatively low cytotoxicity. The corresponding hypoxanthine derivative **5b** was noncytotoxic in the tested concentration region with a negligible anti-HIV effect. Analogue **6** derived from *E*-alkene **4a** was devoid of anti-HIV activity, but a significant cytotoxicity was apparent at a concentration of 1 μM and higher (data not shown). Results with compound **5a** provide additional evidence that inactive unsaturated acyclic nucleoside analogues can be activated by a transformation to the corresponding lipophilic phosphoralaninates. In this respect, the present findings extend our previous observations with allenic phosphoralaninates[14] to structurally more simplified analogues such as **5a**, **5b**, and **6**.

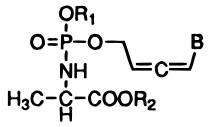
Hydrolysis of analogue **5a** with aqueous triethylamine[11] afforded phosphomonoester alaninate **14** in 77% yield. Pig liver esterase (PLE)-catalyzed reaction at pH 7.4 led to the same product. A similar transformation was observed with d4T and allenic phosphodiester alaninates.[11,14] Kinetics of PLE-catalyzed hydrolysis of phosphoralaninates **5a**, **5b**, and **6** indicated a significant retardation relative to allenic derivatives **15a**

Table 1. Kinetics of Hydrolysis of Phenylphosphoralaninate Analogues **5a**, **5b**, and **6** Catalyzed by Pig Liver Esterase at 38 °C and pH 7.4[a]

compd	half-life ($t_{1/2}$, h)	compd	half-life ($t_{1/2}$, h)
5a	43.5	**15a**	8.5[b]
5b	58.3	**15b**	7.3[b]
6	57.1		

[a] For details, see the Experimental Section. [b] Reference 14.

and **15b** (Table 1). It is then possible that differences



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15a: B = Hyp, $R_1 = C_6H_5$, $R_2 = CH_3$

15b: B = Ade, $R_1 = C_6H_5$, $R_2 = CH_3$

15c: B = Ade, $R_1 = R_2 = H$

in affinity toward intracellular esterase(s) can also influence the biological activity of prodrugs containing a carboxylic ester group. Phosphoralaninates **14** and **15c** are inactive against HIV, and they are not inhibitors of HIV reverse transcriptase. Interestingly, less polar amino acid phosphoramidate *esters* derived from AZT were found to exhibit antiviral activity surpassing that of parent compound, but they were inactive toward reverse transcriptase.[18] It is recognized that the less polar phosphoramidate esters may penetrate cellular membrane easier than phosphoramidate acids. At any rate, our results show that intermediates **14** and **15c** cannot be directly responsible for antiviral effect of phosphoralaninates **5a** and **15a**. Additional intracellular activation appears to be necessary. Previously, we suggested that intermediates such as **15c** were further processed to monophosphates by an intracellular phosphoramidase.[14] Therefore, a similar transformation of **14** to phosphate **16** is also likely. This reaction course was also postulated for phosphomonoester amidates derived from 2′-deoxy-5-fluorouridine.[19] Nevertheless, a detailed mechanism of intracellular transformation of P → N-amino acid amidates to phosphates remains to be elucidated. Our results with compound **5a** and previous findings[14] indicate that lipophilic nucleotide analogues lacking an intact ribofuranose ring can be activated by such a mechanism.

Experimental Section

General Methods. See ref 14. The NMR spectra were determined in $CDCl_3$ at 300.095 (^{1}H), 74.47 (^{13}C), and 121.47 MHz (^{31}P) unless stated otherwise.

1,3-Dioxa-2-methoxy-2-phenyl-5-cycloheptene (9). A mixture of 2-butene-1,4-diol (**8**) (2 mL, 0.024 mol), trimethyl orthobenzoate (5 mL, 0.30 mol), and *p*-toluenesulfonic acid (200 mg, 1.1 mmol) in DMF (15 mL) was stirred at room temperature for 8 h. Triethylamine (1 mL) was added, followed by water (150 mL), and the solution was extracted with petroleum ether (5 × 150 mL). The organic phase was dried (Na_2SO_4) and evaporated. The crude product was chromatographed on a silica gel column using petroleum ether−triethylamine (98:2) to give compound **9** as a colorless oil (3.34 g, 68.0%): ^{1}H NMR ($CDCl_3$) ∂ 7.65 and 7.36 (2 m, 5, phenyl), 5.70 (apparent t, 2, H_5 and H_6), 4.45 and 4.07 (2d, 4, J = 15.6 Hz, H_4 and H_7), 3.05 (s, 3, OCH_3); ^{13}C NMR 136.63, 128.87, 127.97, 127.40 (phenyl, C_5 and C_6), 116.01 (C_2), 61.41 (C_4 and C_7), 50.87 (OCH_3); EI-MS 206 (M, 1.9), 175 (50.6, M − CH_3O), 105 (100.0), 77 (phenyl, 40.2); HRMS calcd M 206.09428, found 206.0946. Anal. ($C_{12}H_{14}O_3$) C, H.

(*Z*)-4-(Benzoyloxy)-1-hydroxy-2-butene (7). 1,3-Dioxa-2-methoxy-2-phenyl-5-cycloheptene (**9**) (600 mg, 2.91 mmol) was dissolved in THF (50 mL), and aqueous acetic acid (1%, 100 mL) was added. The resultant mixture was stirred at room temperature for 5 h, whereupon it was extracted with CH_2Cl_2 (2 × 100 mL). The organic phase was dried (Na_2SO_4), and it was evaporated to yield **7** as a colorless oil (370 mg, 66.2%): ^{1}H NMR ∂ 7.99 and 7.45 (2 m, 5, benzoyl), 5.85 and 5.70 (2m, 2, H_2 and H_3), 4.87 and 4.30 (2d, J = 6.9 and 6.3 Hz, H_1 and H_4); ^{13}C NMR 166.72 (CO), 133.68, 133.14, 129.60, 128.41, 125.27 (phenyl, C_2 and C_3), 60.72 (C_4), 58.30 (C_1). This product was used for the preparation of (*Z*)-4-(benzoyloxy)-1-bromo-2-butene[15] (**10**).

9-[(*Z*)-4-(Benzoyloxy)-2-buten-1-yl]-6-chloropurine (11). 6-Chloropurine (2.00 g, 12.9 mmol) was dissolved in DMF (100 mL), and NaH (60%, 600 mg, 0.016 mol) was added in portions. The mixture was stirred at room temperature for 30 min. 1-(Benzoyloxy)-4-bromo-2-butene[15] (**10**) (4.0 g, 0.016 mol) was added over a period of 20 min. The solution was stirred at room temperature for 3 h. The solvent was evaporated, and the residue was chromatographed on a silica gel column using petroleum ether−ethyl acetate (1:1) to give **11** as a colorless solid (2.18 g, 51.4%), which was recrystallized from methanol: mp 105−106 °C; R_f (petroleum ether−ethyl acetate, 2:3) 0.5; UV max (EtOH) 265 nm (9800), 230 (15 200), 209 (18 700); ^{1}H NMR ($CDCl_3$) ∂ 8.73 (s, 1, H_8), 8.28 (s, 1, H_2), 8.03 (dd), 7.58 (split t) and 7.44 (t, total 5 protons, benzoyl), 6.05 and 5.90 (2 m, 2, $H_{2'}$ and $H_{3'}$), 5.14 and 5.07 (2d, 4, J = 6.9 and J = 7.2 Hz, $H_{1'}$ and $H_{4'}$); FAB MS 437 (M + thioglycerol + H, 7.8), 329.0 (M + H, 53.1). Anal. ($C_{16}H_{13}N_4O_2Cl$) C, H, N, Cl.

9-[(*Z*)-4-Hydroxy-2-buten-1-yl]hypoxanthine (3c). A solution of 9-[(*Z*)-4-(benzoyloxy)-2-buten-1-yl]-6-chloropurine (**11**, 1.00 g, 3.05 mmol) in 80% formic acid (100 mL) was stirred at 80 °C for 5 h. After cooling, the volatile components were removed *in vacuo* and water (20 mL) was evaporated from the residue, which was then dissolved in methanolic ammonia (20%, 100 mL). The solution was stirred at room temperature for 36 h. The solvent was evaporated, and the residue was chromatographed on a silica gel column using CH_2Cl_2−MeOH (9:1) to give a colorless solid (380 mg, 60.3%), which was recrystallized from methanol: mp 215−216 °C; R_f (CH_2Cl_2−MeOH, 9:1) 0.1; UV max (EtOH) 207 nm (18 100), 250 (10 800); ^{1}H NMR (CD_3SOCD_3, 500 MHz) ∂ 12.26 (s, 1, NH), 8.04 and 8.02 (2s, 2, H_8 and H_2), 5.70 and 5.58 (2m, 2, $H_{2'}$ and $H_{3'}$), 4.80 (m, 3, $H_{1'}$ and OH), 4.16 (m, 2, $H_{4'}$); ^{13}C NMR (125 MHz) 134.72, 124.00 ($C_{2'}$, $C_{3'}$), 57.01 ($C_{4'}$), $C_{1'}$ is overlapped with solvent signal; purine 156.61 (C_6), 148.12 (C_2), 145.50 (C_4), 139.89 (C_8), 124.25 (C_5); FAB MS 207 (M + H, 61.4), 137 (hypoxanthine + H, 24.4). Anal. ($C_9H_{10}N_4O_3$) C, H, N.

9-[(*E*)-4-Hydroxy-2-buten-1-yl]hypoxanthine (4b). 9-[(*E*)-4-Hydroxy-2-buten-1-yl]adenine[20] (**4a**) (200 mg, 0.98 mmol) was dissolved in 0.02 M Na_2HPO_4 (30 mL, pH 7.5). Adenosine deaminase (Sigma Chemical Co., St. Louis, MO, type II, 60 mg) was added, and the solution was stirred at room temperature for 22 h. The solvent was evaporated, and the residue was extracted with CH_2Cl_2−MeOH (1:1, 2 × 200 mL). The organic phase was evaporated, and the residue was chromatographed on a silica gel column using CH_2Cl_2−MeOH (9:1) to give the title compound **4b** as a colorless solid (158 mg, 79.1%), which was recrystallized from methanol: mp 216−218 °C; R_f (CH_2Cl_2−MeOH, 9:1) 0.1; UV max (EtOH) 209 nm (13 400), 250 (12 000); ^{1}H NMR (CD_3SOCD_3) ∂ 12.28 (s, 1, NH), 8.04 and 8.01 (2s, 2, H_8 and H_2), 5.75 and 5.65 (2 m, 2, $H_{2'}$ and $H_{3'}$), 4.74 (m, 3, $H_{1'}$ and OH), 3.89 (bs, 2, $H_{4'}$); ^{13}C NMR 134.95, 123.99 ($C_{2'}$, $C_{3'}$), 60.86 ($C_{4'}$), 44.84 ($C_{1'}$); purine 157.10 (C_6), 148.63 (C_4), 146.03 (C_2), 140.50 (C_8), 124.32 (C_5); FAB MS 413 (2M + H, 4.8), 315 (M + thioglycerol + H, 5.0), 207 (M + H, 61.4), 137 (hypoxanthine + H, 24.4). Anal. ($C_9H_{10}N_4O_3$) C, H, N.

9-[(*Z*)-4-Hydroxy-2-buten-1-yl]adenine (*R*,*S*)-4′-(Methyl phenyl phosphoryl-/*P*→*N*/-L-alaninate) (5a). 9-[(*Z*)-4-Hydroxy-2-buten-1-yl]adenine[20] (**3b**, 120 mg, 0.58 mmol) was added to a solution of phenyl methoxyalaninylphosphorochloridate[17] (**12**, 400 mg, 1.35 mmol) in THF (10 mL). After

addition of *N*-methylimidazole (0.25 mL, 3 mmol), the mixture was stirred at room temperature for 2.5 h. The solvent was removed and the residue dissolved in CH_2Cl_2 (50 mL). The solution was washed with water (3 × 15 mL), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica gel column using CH_2Cl_2−MeOH (95:5) to yield the title compound **5a** as a colorless foam (195 mg, 75.3%): R_f (CH_2Cl_2−MeOH, 9:1) 0.4; HPLC, retention time 10.25 and 10.85 min; purity 97.8%; UV max (EtOH) 209 nm (25 700), 261 (14 900 nm); ^{1}H NMR ($CDCl_3$) ∂ 8.31, 8.23 (2s, 1, H_8), 7.85 (s, 1, H_2), 7.21 (m, 5, phenyl), 6.33 (s, 2, NH_2), 5.80 (m, 2, $H_{2'}$ and $H_{3'}$), 5.02 - 4.73 (m, 5, $H_{1'}$, $H_{4'}$ and NH of Ala), 4.10 (m, 1, CH of Ala), 3.68, 3.65 (2s, 3, OCH_3 of Ala), 1.37 (t, 3, CH_3 of Ala); ^{31}P NMR 3.73, 3.41; ^{13}C NMR 128.53, 128.44, 128.09, 127.94 ($C_{3'}$, $C_{2'}$), 61.76 ($C_{4'}$), 40.38, 40.28 ($C_{1'}$); purine 155.61 (C_6), 152.87, 152.77 (C_2), 149.67 (C_4), 140.18 (C_8), 119.45, 119.40 (C_5); alanine 174.06 (split peak, CO, ester), 52.36, 52.32 (OMe); 50.25, 50.16 (CH); 20.79, 20.82 (Me); phenyl 150.61 (C-ipso), 129.68 (C-para), 124.93 (C-ortho); 120.17 (C-meta); FAB MS 555 (M + thioglycerol + H, 2.5), 447 (M + H, 17.2), 296.0 (17.8), 188 (100), 136 (adenine + H, 24.5). Anal. ($C_{19}H_{23}N_6O_5P$) C, H, N.

9-[(*E*)-4-Hydroxy-2-buten-1-yl]adenine (*R,S*)-4′-(Methyl phenyl phosphoryl-/*P*→*N*/-L-alaninate) (6). 9-[(*E*)-4-Hydroxy-2-buten-1-yl]adenine[20] (**4a**, 100 mg, 0.486 mmol) was added to a solution of phenyl methoxyalaninylphosphorochloridate (**12**, 340 mg, 1.13 mmol) in dry THF (10 mL). After addition of *N*-methylimidazole (0.213 mL, 2.6 mmol), the reaction mixture was stirred at room temperature for 3 h. The solvent was removed and the residue dissolved in CH_2Cl_2 (50 mL). The solution was washed with water (3 × 15 mL), dried (Na_2SO_4), and evaporated. The residue was purified by chromatography on a silica gel column using CH_2Cl_2−MeOH (95:5) to yield compound **6** as a colorless foam (131 mg, 60.5%): R_f (CH_2Cl_2−MeOH, 9:1) 0.4; HPLC, retention time 9.58 and 10.53 min; purity 98.7%; UV max (EtOH) 210 nm (24 500), 261 (14 700 nm); ^{1}H NMR ($CDCl_3$) ∂ 8.32 (s, 1, H_8), 7.78 (s, 1, H_2), 7.26 and 7.14 (t and m, 5, phenyl), 6.34 (s, 2, NH_2), 5.90 and 5.70 (m, 2, $H_{2'}$ and $H_{3'}$), 4.78 (d, 2, J = 5.4 Hz, $H_{1'}$), 4.57 and 4.46 (2 m, 3 H, $H_{4'}$ and NH of Ala); 4.00 (m, 1, CH of Ala), 3.66, 3.64 (2s, 3, OCH_3 of Ala), 1.33 (t, 3, CH_3 of Ala); ^{31}P NMR 3.03, 2.92; ^{13}C NMR 129.01, 128.91, 127.32, 127.22 ($C_{3'}$, $C_{2'}$), 65.77 (split peak, $C_{4'}$), 44.38 ($C_{1'}$); purine 155.65 (C_6), 153.03 (C_2), 149.78 (C_4), 140.10 (C_8), 119.40 (C_5); alanine 173.93 (CO, ester), 52.36 (OMe); 50.07 (CH); 20.84, 20.77 (Me); phenyl 150.66 (C-ipso), 129.56 (C-para), 124.79 (C-ortho); 120.04 (split peak, C-meta); FAB MS 555 (M + thioglycerol + H, 16.4), 447 (M + H, 72.0), 296 (42.1), 188 (100.0), 136 (adenine + H, 51.9). Anal. ($C_{19}H_{23}N_6O_5P$) C, H, N.

9-[(*Z*)-4-Hydroxy-2-buten-1-yl]hypoxanthine (*R,S*)-4′-(Methyl phenyl phosphoryl-/*P*→*N*/-L-alaninate) (5b). 9-[(*Z*)-4-Hydroxy-2-buten-1-yl]hypoxanthine (**3c**, 100 mg, 0.486 mmol) was added to a solution of phenyl methoxyalaninylphosphorochloridate (**12**, 340 mg, 1.23 mmol) in dry THF (10 mL). After addition of *N*-methylimidazole (0.213 mL, 2.59 mmol), the reaction mixture was stirred at room temperature for 4 h. The solvent was evaporated, the residue was dissolved in 80% acetic acid (40 mL), and the mixture was stirred at room temperature for 16 h. The solvent was evaporated and the residue purified by chromatography on a silica gel column using CH_2Cl_2−MeOH (9:1) to give compound **5b** as a colorless foam (115 mg, 53%): R_f (CH_2Cl_2−MeOH, 9:1) 0.3; HPLC, retention time 9.96 min; purity 98.7%; UV max (EtOH) 209 nm (17 300), 250 (11 100 nm); ^{1}H NMR ($CDCl_3$) ∂ 8.16 and 8.12 (2s, 1, H_8), 7.86 (s, 1, H_2), 7.34−7.12 (m, 5, phenyl), 5.90 and 5.80 (2m, 2, $H_{2'}$ and $H_{3'}$), 4.90 (m, 4, $H_{1'}$ and $H_{4'}$), 4.26 and 4.12 (2m, 2, NH of Ala and CH of Ala), 3.71, 3.68 (2s, 3, OCH_3 of Ala), 1.39 (t, 3, CH_3 of Ala); ^{31}P NMR 3.45, 3.25; ^{13}C NMR 128.93, 128.84, 127.71, 127.59 ($C_{3'}$, $C_{2'}$), 61.95 (split peak, $C_{4'}$), 40.69 ($C_{1'}$); purine 158.71 (C_6), 148.77 (C_2), 145.18 (C_4), 139.81 (C_8), 124.43 (C_5); alanine 173.90 (CO, ester), 52.44 (OMe); 50.23 (split peak, CH); 20.76 (Me); phenyl 150.64 (C-ipso), 129.62 (C-para), 124.93 (C-ortho); 120.10 (split peak, C-meta); FAB MS 556 (M + thioglycerol + H, 6.9), 448 (M + H, 52.0), 297 (33.1), 189 (97.1), 137 (hypoxanthine + H, 35.0). Anal. ($C_{19}H_{22}N_5O_6P$) C, H, N.

9-[(*Z*)-4-Hydroxy-2-buten-1-yl]adenine 4′-(Phosphoryl-/*P*→*N*/-L-alaninate) (14). Analogue **5a** (180 mg, 0.41 mmol) was dissolved in H_2O−Et_3N (1:1, 15 mL), and the reaction mixture was stirred at room temperature for 2 h. The triethylamine phase was removed and the aqueous phase evaporated *in vacuo* at room temperature. The resulting crude product was chromatographed on silica gel column using CH_2Cl_2−MeOH−H_2O−NH_3 (120:70:10:1) to give compound **14** as a colorless foam (121.5 mg, 77.4%): R_f 0.35; HPLC, retention time 4.6 min; purity 100%; UV max (EtOH) 262 nm (ϵ 11 800), 211 (ϵ 15 500); ^{1}H NMR (D_2O + 1 drop of Et_3N) ∂ 8.06 and 8.04 (2s, 2, H_8 and H_2), 5.80 and 5.70 (2m, 2, $H_{2'}$ and $H_{3'}$), 4.82 and 4.44 (m and t, 4, $H_{4'}$ and $H_{1'}$), 3.52 (m, 1, CH-Ala), 1.18 (d, 3, CH_3-Ala); ^{31}P NMR 7.65.

Enzyme Studies. A. Pig Liver Esterase. Analogues **5a, 5b**, and **6** were dissolved in 0.02 M Na_2HPO_4 (pH 7.4) at a concentration of 2.2 × 10^{-3} M. To each solution (1 mL) was added pig liver esterase (PLE, 200 units), and the mixtures were stirred at 38 °C. At selected time intervals aliquots (20 μL) were analyzed by HPLC as described above using water −CH_3CN (9:1, 0−3 min) and water −CH_3CN (7:3, 3−15 min). The half-lives $t_{1/2}$ are listed in Table 1.

B. HIV Reverse Transcriptase. Inhibitory activity of phosphoramidates **14** and **15c** against reverse transcriptase (RT) was determined as previously described.[21,22] Briefly, various concentrations of tested analogues were added to reaction mixtures (50 μL) containing poly(rA)·$(dT)_{12-18}$, Tris·HCl (50 mM, pH 7.8), $MgCl_2$ (6 mM), Triton X-100 (0.01%), [^{3}H]TTP, and RT (2 nM) at 0 °C. Reaction was initiated by raising the temperature of the reaction mixtures from 0 to 37 °C. The mixtures were incubated for 30 min at 37 °C, and they were quenched by the addition of EDTA (0.5 M, 25 μL). Products were then examined by a DE81 filter binding assay.[22] A portion of the reaction mixture (40 μL) was spotted on DE81 filters, and in order to remove unincorporated [^{3}H]TTP, filters were washed twice with sodium citrate (0.3 M, pH 7.0), twice with ethanol, and once with acetone before they were air-dried. The amount of incorporated [^{3}H] nucleotides was determined by a scintillation counter. The results were expressed as counts per minute of [^{3}H]TMP (40−60 Ci/mmol; 1 Ci = 37 GBq) incorporated per 40 μL of the reaction mixture. Analogues **14** and **15c** did not inhibit RT at 20 μM. In a control experiment, AZTTP blocked 90% of the activity of RT at 1 μM.

Inhibition of HIV-1 Cytopathic Effect. The assay was performed with CD_4^+ ATH8 cells as described.[14] The ATH-8 cells (2 × 10^5) were exposed to HIV-1/HIV_{LAI} (500 50% tissue culture infectious dose) for 45 min, and they were cultured in the presence of compounds **5a**, **5b**, and **6**. Total viable cells were counted on day 7. Percent protective effect of a compound on survival and growth of ATH-8 cells exposed to the virus was determined by the following formula: 100 × [(number of viable cells exposed to HIV-1 and cultured in the presence of the compound) − (number of viable cells exposed exposed to HIV-1 and cultured in the absence of of the compound)]/[(number of viable cells cultured alone) − (number of viable cells exposed to HIV-1 and cultured in the absence of the compound)]. Percent cytotoxicity of a compound was determined as follows: 100 × [1 − (number of total viable cells cultured in the presence of the compound)/(number of total viable cells cultured alone)]. The data obtained for **5a** are given in Figure 1. Positive controls performed with ddI as well as results with less effective compounds **5b** and **6** are not shown.

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