Design, Synthesis, and Structure–Activity Relationship of Novel Dinucleotide Analogs as Agents against Herpes and Human Immunodeficiency Viruses

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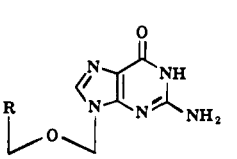
A new acyclic nucleoside phosphonate (**13**) containing an adenine moiety was synthesized, which acted as an excellent inhibitor of calf mucosal adenosine deaminase. This inhibitory property allows it to exert great synergistic effect on certain antiviral agents (e.g., ara-A, **37**). Phosphonate **13** was not phosphorylated by the bovine brain guanylate kinase nor by 5-phosphoribosyl 1-pyrophosphate synthetase. Syntheses of biologically active nucleotide phosphonate **40** and its phosphonoamidate derivative **42** were accomplished, which showed remarkable activity against herpes viruses and exhibited low host cell toxicity. 3′-Azido-nucleoside phosphonate **20** and 3′-fluoronucleoside phosphonate **32**, as well as the corresponding dinucleotide analogs **47** and **48**, and their respective phosphonoamidates **53–56** were also synthesized as new compounds, among which phosphonoamidates **53–56** showed potent activity against human immunodeficiency virus. Phosphonoamidates **55** and **56** bearing a methyl D-alaninate moiety exhibited less cellular toxicity than **53** and **54** bearing a methyl L-alaninate moiety. Nucleotide phosphonate **40** as well as dinucleotide phosphonates **47** and **48** were found susceptible to degradation by phosphodiesterases. Their respective phosphonoamidates **42** and **53–56**, however, were completely resistant to snake venom and spleen enzymes.

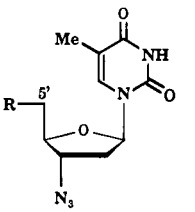
Introduction

9-[(2-Hydroxyethoxy)methyl]guanine (acyclovir, **1**)[1] and 3′-azido-3′-deoxythymidine (AZT, **4**)[2] exhibit remarkable activities against herpes simplex viruses (HSV) and human immunodeficiency virus type 1 (HIV-1), respectively. Viral inhibition resulting from **1**[1,3] and **4**[4,5] appears to require a selective phosphorylation of those compounds to give the corresponding monophosphates by thymidine kinase. Then host cell kinases convert these monophosphates to triphosphates. The triphosphate of acyclovir (**1**) inhibits viral-specified DNA polymerase and thus HSV replication.[6] The triphosphate anabolite of AZT (**4**) is an alternate substrate for HIV-1 reverse transcriptase, which terminates DNA synthesis after incorporation into the growing DNA strand.[4,5,7]

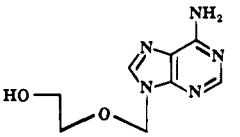
Phosphonate **2** exhibited remarkable anti-herpes virus activity.[8a] In addition, phosphonates of purines bearing a shorter length of the side chain have been utilized as antiviral agents. These included (*S*)-9-[3-hydroxy-2-(phosphonylmethoxy)propyl]adenine (HPMPA), 9-[2-(phosphonylmethoxy)ethyl]adenine (PMEA), and 9-[2-(phosphonylmethoxy)ethyl]guanine (PMEG).[9] Nevertheless, acyclic nucleoside phosphonate **3**, an isostere of PMEG, does not exhibit significant inhibitory effect against HSV.[8b] Thus the 3′-oxygen atom in PMEG plays a critical role in enzymic phosphorylation and on antiviral activity.[8] Kim et al.[8b] also reported that cellularly or virally induced kinases cannot catalyze the conversion of phosphonate **3** to the corresponding mono- and diphosphates.



1. R = CH_2OH
2. R = $OCH_2P(=O)(ONa)_2$
3. R = $CH_2PO(OH)_2$



4. R = OH
5. R = $CH_2PO(OH)_2$



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Recently, Freeman et al.[10a] have reported that the lack of activity of 3′-azidonucleoside phosphonate **5** toward HIV-1 in MT4 cells is due to the replacement of the 5′-oxygen atom of AZT 5′-phosphate with a methylene unit. This results in a 1800-fold decrease in the rate constant for the reverse transcriptase-catalyzed nucleoside phosphodiester bond formation.[10a] Herein, we report our design and synthesis of dinucleotide analogs **47** and **48**, which possess the 5′-hydroxyl group. Our results from biological tests indicate that their respective triphosphates are recognized as alternate substrates for HIV-1 reverse transcriptase.

Some acyclic nucleosides (e.g., **6**) and arabinosides (e.g., **37**) can inhibit viral replication but are not phosphorylated by HSV-thymidine kinase.[11–13] It appears that phosphorylation catalyzed by this enzyme is

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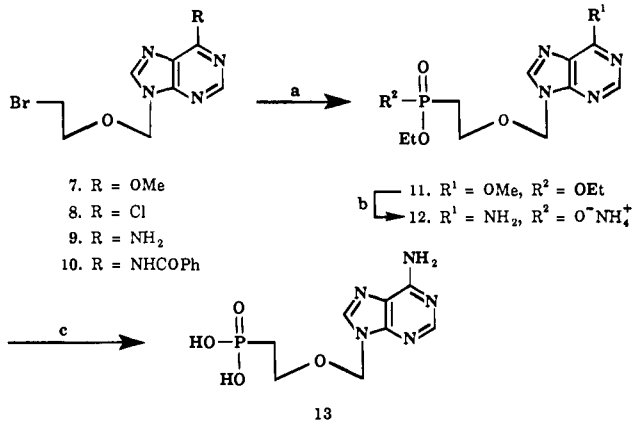
Scheme 1[a]



[a] Reagents: (a) $(EtO)_3P$, Δ; (b) NH_3, MeOH; (c) Me_3SiBr, DMF.

not always a prerequisite for activity. We have therefore investigated antiviral activity of the new acyclic nucleoside phosphonate **13**, which is structurally similar to compounds **3** and **6**, as well as a series of modified phosphonates of 3′-deoxythymidine (i.e., **20**, **29**, **32**, **34**, and **36**). We have also synthesized nucleotide analog **40**, a composite molecule of phosphonate **13** and arabinoside **37**, and tested the possibility of it being an alternate substrate for HSV DNA polymerase.

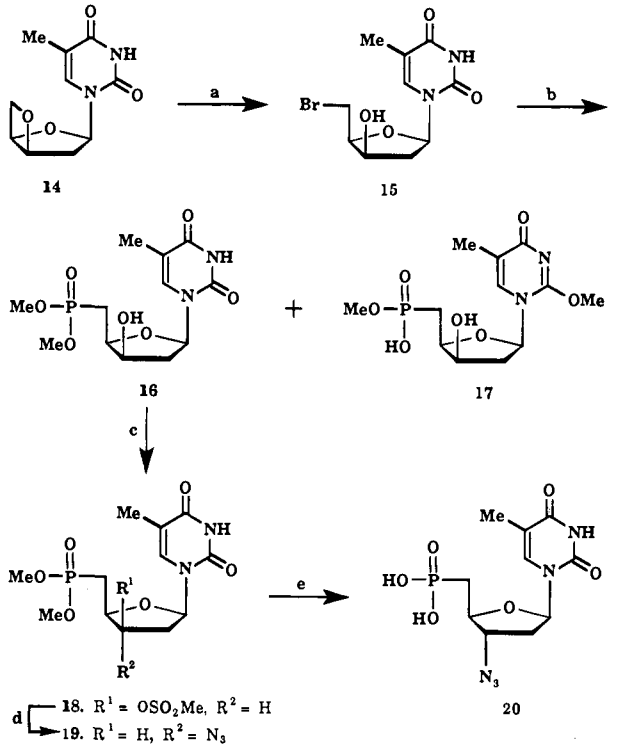
Low lipophilicity of nucleotide analog **40** and dinucleotide analogs **47** and **48** precludes their use for the treatment of viral infections.[14,15] On the other hand, McGuigan et al.[16] suggested that HIV-aspartate proteinase[17] may recognize phosphonoamidate derivatives of certain nucleosides and thus can specifically hydrolyze these membrane-soluble prodrugs. The resultant bioactive nucleotides would then be trapped inside the infected cells and act as potent inhibitors of viral proliferation. We have therefore synthesized the phosphonoamidates **42** and **53**–**56** and tested their antiviral activity.

Results

Synthesis of Acyclic Nucleoside Phosphonate 13 (Scheme 1). We carried out the Arbuzov reaction[18] by treatment of acyclonucleoside **7**[19] with triethyl phosphite to produce the desired diethyl phosphonate **11**. Arbuzov reaction of bromides **8**–**10**[19] afforded unidentifiable products. Reaction of **11** with NH_3 in MeOH gave monoammonium salt **12** in 40% yield. We then treated **12** with Me_3SiBr in DMF[10b] to provide a 45% yield of the desired phosphonate **13**, which may exist in its zwitterionic form.

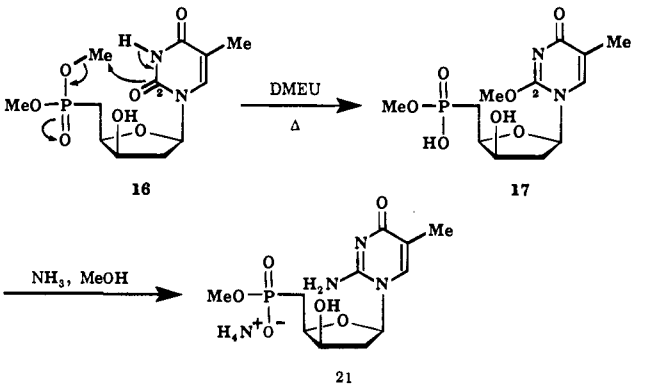
Syntheses of Cyclic Nucleoside Phosphonates 20, 26, 29, 32, 34, and 36 (Schemes 2 and 4–6). We treated 1-(3′,5′-anhydro-β-D-*threo*-pentofuranosyl)thymine (**14**)[20] with LiBr and $BF_3{\cdot}OEt_2$ to give bromide **15** in 70% yield (Scheme 2).[10b] Reaction of **15** with trimethyl phosphite at 140 °C afforded a mixture of phosphonates **16** and **17** (1:1) in 80% overall yield. Performance of the same reaction at 200–210 °C produced **17** exclusively in 90% yield. Furthermore, conversion of compound **16** to pyrimidine **17** in nearly quantitative yield was accomplished in 1,3-dimethyl-2-imidazolidinone at 200–210 °C. This reaction involves migration of a methyl group from the phosphonate functionality to the O^2-position of thymine, as shown in Scheme 3. For proof of the structure **17**, we converted

Scheme 2[a]



[a] Reagents: (a) LiBr, $BF_3{\cdot}OEt_2$, THF; (b) $(MeO)_3P$, Δ; (c) $MeSO_2Cl$, 4-(dimethylamino)pyridine, pyridine; (d) LiN_3, DMF; (e) Me_3SiBr, CH_2Cl_2.

Scheme 3



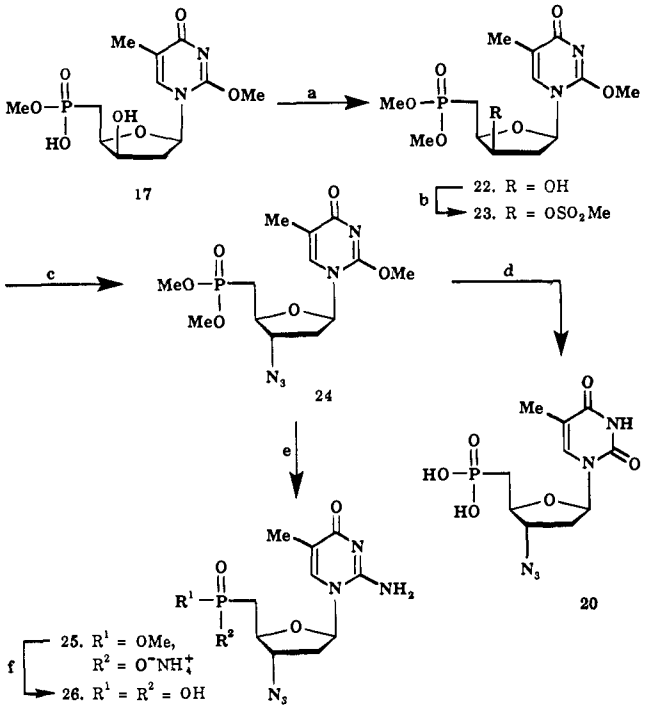
it to the expected isocytosine derivative **21** (55%) with NH_3 in MeOH by the procedure reported by Todd et al.[21] (Scheme 3).

We methylated monomethyl ester **17** with CH_2N_2 to give the corresponding dimethyl ester **22** in 98% yield (see Scheme 4). Treatment of alcohol **16** or **22** with methanesulfonyl chloride afforded mesylate **18** (90%) or **23** (95%), respectively. We then converted **18** → **19** (80%) and **23** → **24** (69%) by use of LiN_3 in DMF.[22] Demethylation of 3′-azidonucleoside phosphonate **19** or **24** with Me_3SiBr[10c] afforded phosphonic acid **20** in 75–80% yields.

For the preparation of phosphonic acid **26**, we treated dimethyl ester **24** with NH_3 in MeOH to give isocytosine derivative **25** in 35% yield (see Scheme 4). Reaction of **25** with Me_3SiBr afforded a 65% yield of phosphonic acid **26**, which may exist in its zwitterionic form.

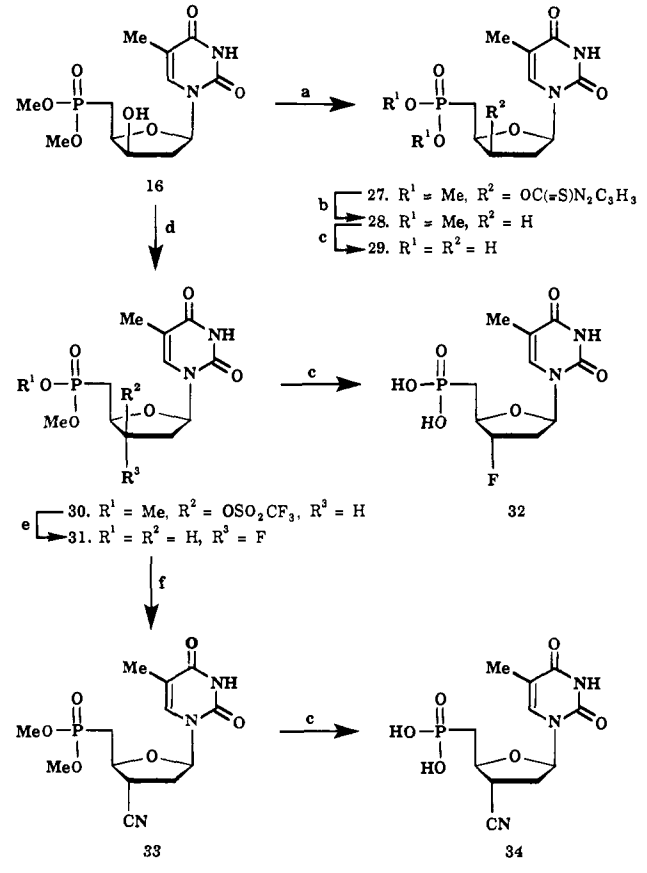
For the synthesis of 3′-deoxynucleoside phosphonate **29**, we treated alcohol **16** with thiocarbonyldiimidazole to give 3′-imidazole-1-carbothioate **27** in 40% yield (see

Scheme 4[a]



[a] Reagents: (a) CH_2N_2; (b) $MeSO_2Cl$, 4-(dimethylamino)pyridine, pyridine; (c) LiN_3, DMF; (d) Me_3SiBr, CH_2Cl_2; (e) NH_3, MeOH; (f) Me_3SiBr, DMF.

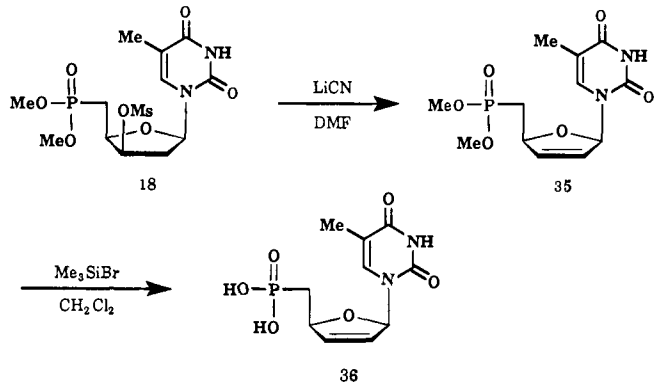
Scheme 5[a]



[a] Reagents: (a) thiocarbonyldiimidazole, DMF; (b) n-Bu_3SnH, 2,2′-azobis(2-methylpropionitrile), toluene, Δ; (c) Me_3SiBr, CH_2Cl_2; (d) CF_3SO_2Cl, pyridine; (e) n-Bu_4NF, THF; (f) n-Bu_4NCN, CH_3CN.

Scheme 5).[23] Reduction of **27** with n-Bu_3SnH and 2,2′-azobis(2-methylpropionitrile) in toluene at reflux gave

Scheme 6



3′-deoxynucleotide analog **28** (43%),[23] which was demethylated with Me_3SiBr to afford phosphonic acid **29** in 80% yield.

In another series of reactions, we treated alcohol **16** with trifluoromethanesulfonyl chloride in pyridine to give triflate **30**, as shown in Scheme 5. Reaction of **30** with n-Bu_4NF afforded 3′-fluoronucleoside phosphonate **31** in 50% overall yield.[24] The desired phosphonic acid **32** was then obtained in 85% yield by demethylation of **31** with Me_3SiBr. Moreover, we allowed triflate **30** to react with n-Bu_4NCN in CH_3CN.[24] The resultant 3′-cyano derivative **33** was demethylated with Me_3SiBr in CH_2Cl_2 to afford the desired phosphonic acid **34** in 65% yield.

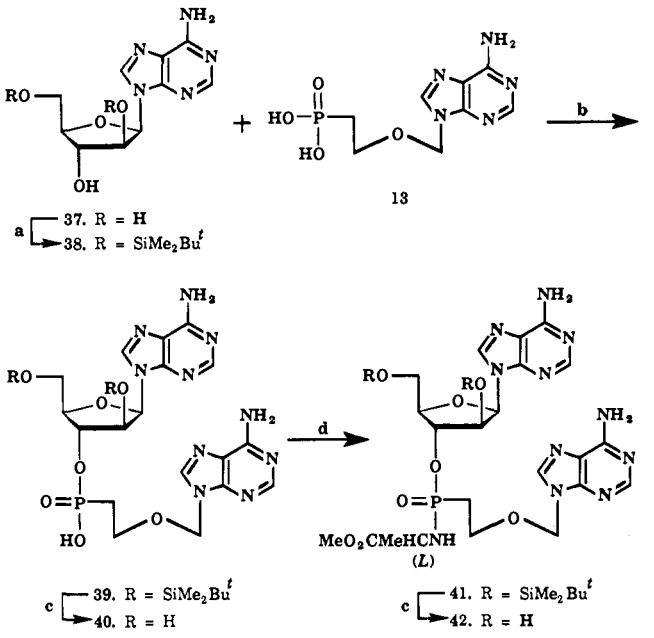
Furthermore, reaction of mesylate **18** with LiCN in DMF gave the elimination product **35** (see Scheme 6).[25] We then demethylated **35** with Me_3SiBr to afford the desired phosphonic acid **36** in 75% yield.

Syntheses of Phosphonates 40, 47, and 48 as Well as Phosphonoamidates 42 and 53–56 (Schemes 7 and 8). The nucleotide phosphonate **40** was readily obtained in three steps from arabinoadenosine **37**, which was first silylated with t-$BuMe_2SiCl$ in the presence of $AgNO_3$, pyridine, and THF (see Scheme 7).[26] We then condensed the resultant 2′,5′-disilyl ether **38** (97%) with phosphonic acid **13** by using trichloromethanesulfonyl chloride in collidine and THF to afford nucleotide phosphonate **39** in 55% yield. Desilylation of **39** with n-Bu_4NF at 25 °C gave the target molecule **40** in 78% yield.

We condensed nucleotide phosphonate **39** with methyl L-alaninate by using triisopropylbenzenesulfonyl chloride to give a diastereoisomeric mixture of phosphonoamidates **41** (1:1) in 97% yield. Compounds **41** showed two close signals at δ 38.71 and 38.80 in its ^{31}P NMR spectrum, resulting from the phosphonoamidate chiral center. Desilylation of **41** with n-Bu_4NF afforded the target phosphonoamidate **42** in 90% yield.

We synthesized dinucleotide analogs **47** and **48** as well as the corresponding phosphonoamidates **53–56** (see Scheme 8). Protection of thymidine (**43**) with t-$BuMe_2SiCl$ and $AgNO_3$[27] gave 5′-silyl ether **44** in 98% yield. Reaction of **44** with 3′-azidonucleoside phosphonate **20** or 3′-fluoronucleoside phosphonate **32** in the presence of trichloromethanesulfonyl chloride, collidine, and THF gave the corresponding dinucleotide analogs **45** (65%) and **46** (70%), respectively. Desilylation of **45** and **46** with n-Bu_4NF afforded the desired products **47** (90%) and **48** (95%), respectively. In a reaction similar to the conversion of **39** → **41**, we condensed dinucleotide

Scheme 7[a]



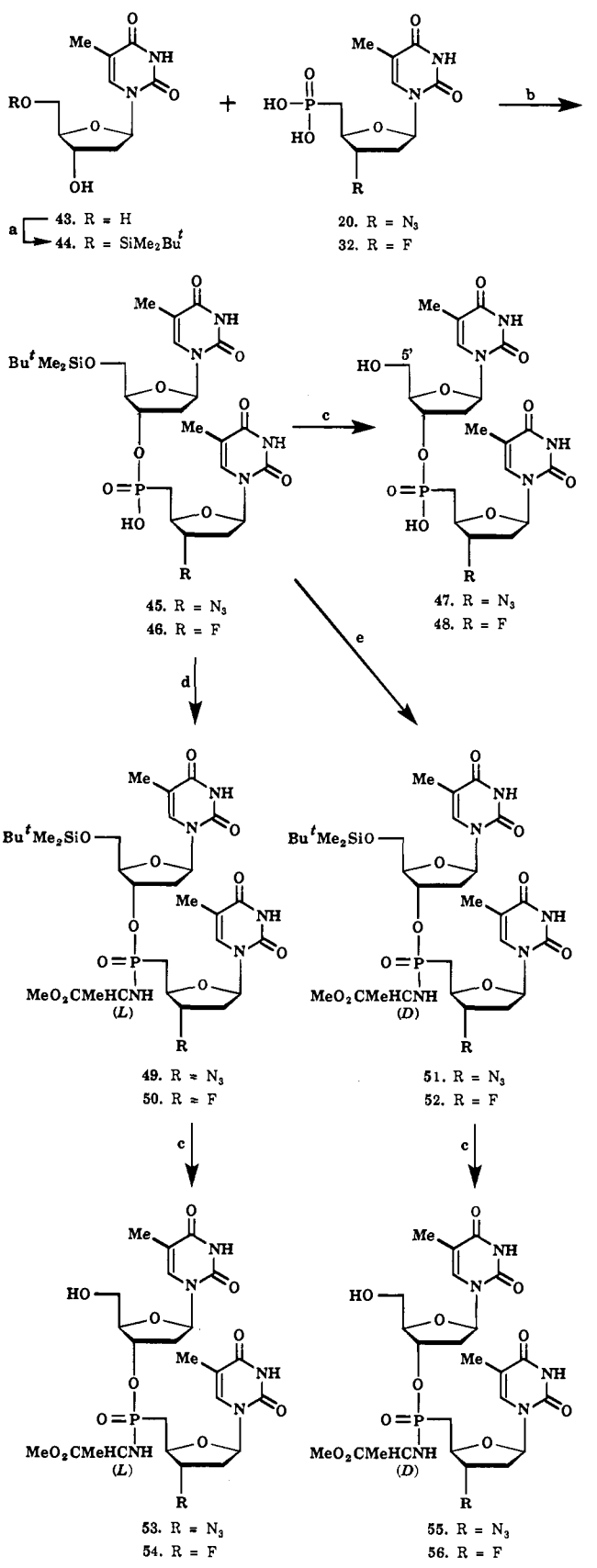
[a] Reagents: (a) *t*-BuMe$_2$SiCl, AgNO$_3$, pyridine, THF; (b) CCl$_3$SO$_2$Cl, collidine, THF; (c) *n*-Bu$_4$NF, THF; (d) 2,4,6-triisopropylbenzenesulfonyl chloride, methyl L-alaninate, pyridine.

phosphonate **45** or **46** with methyl L-alaninate by use of triisopropylbenzenesulfonyl chloride to give the diastereoisomeric phosphonoamidates **49** (95%) and **50** (90%). We also converted **45** → **51** (87%) and **46** → **52** (95%) with methyl D-alaninate by the same method. Desilylation of these compounds with *n*-Bu$_4$NF afforded the target phosphonoamidates **53** (88%), **54** (85%), **55** (86%), and **56** (90%).

Activity of Snake Venom and Spleen Phosphodiesterases against Nucleotide Analogs 40 and 42 as Well as Dinucleotide Analogs 47, 48, and 53–56. Studies of Bovine Brain Guanylate Kinase and 5-Phosphoribosyl 1-Pyrophosphate Synthetase on Phosphonate 13. Because compound **40** possessed a joint skeleton of acyclic nucleoside phosphonate **13** and arabinoside **37**, it was degraded by spleen phosphodiesterase in 70% yield after 8 h. Nevertheless, it was unaffected by snake venom phosphodiesterase.[28,29] These results indicate that the snake venom enzyme did not recognize the acyclic ether moiety of nucleotide analog **40** as a normal substrate. Moreover, we found that phosphonoamidate **42** was completely resistant to snake venom and spleen enzymes. By comparison, dinucleotide analogs **47** and **48** were susceptible to snake venom and spleen phosphodiesterases.[28,29] These dinucleotide analogs bear rigid furanosyl rings and exhibited complete degradation characteristics, similar to those of the natural nucleotides.[30] Phosphonoamidates **53**–**56** were resistant to both phosphodiesterases.

To investigate the anti-herpes activity of acyclic nucleoside phosphonate **13**, we studied its phosphorylation kinetics with bovine brain guanylate kinase[8b] and 5-phosphoribosyl 1-pyrophosphate synthetase.[9g] We found that phosphonate **13** was not phosphorylated by these enzymes. PMEG[8b] and PMEA[9g] are substrates of bovine brain guanylate kinase and 5-phosphoribosyl 1-pyrophosphate synthetase, respectively. Thus we conclude that the oxygen atom attached to the C-3′ of the ether moiety in the nucleoside phosphonates is essential for compounds to be substrates of the phosphorylating enzymes.

Scheme 8[a]



[a] Reagents: (a) *t*-BuMe$_2$SiCl, AgNO$_3$, THF; (b) CCl$_3$SO$_2$Cl, collidine, THF; (c) *n*-Bu$_4$NF, THF; (d) 2,4,6-triisopropylbenzenesulfonyl chloride, methyl L-alaninate, pyridine; (e) 2,4,6-triisopropylbenzenesulfonyl chloride, methyl D-alaninate, pyridine.

Table 1. Substrate Activities and Inhibitory Properties against Adenosine Deaminase

substrate	K_m (μM)	rel V_{max}	K_i (μM)
6	138.0	1.52×10^{-2}	142.5
13	247.5	1.49×10^{-6}	18.2
ara-A (**37**)	45.3	1	
40	166.2	7.64×10^{-2}	
42	*a*	*a*	

[a] No reaction at 1500 μM.

Table 2. Solubility in H_2O and Lipophilicity of Nucleoside and Nucleotide Analogs

compd	solubility in H_2O (mg/mL)	log *P* (1-pentanol/H_2O)[a]
AZT (**4**)	1.24	2.86
6	1.95	0.98
13	13.64	0.07
20	11.12	0.16
32	12.26	0.19
ara-A (**37**)	0.40	−0.47
40	2.46	−0.68
42	8.25	0.87
47	3.24	0.27
48	3.98	0.28
53	9.13	2.38
54	8.79	2.46
55	9.20	2.33
56	8.92	2.48

[a] Partition coefficients were calculated as $P = [\text{substrate}]_{1\text{-pentanol}}/[\text{substrate}]_{H_2O}$.

Kinetic Studies of Competitive Inhibition of Adenosine Deaminase by Nucleoside and Nucleotide Analogs. By following an established procedure,[29] we determined the rates of deamination of **6**, **13**, **37**, **40**, and **42** in the presence of calf mucosal adenosine deaminase (EC 3.5.4.4) in buffer solutions. Inhibition studies on these compounds were carried out on the basis of the Kaplan method.[31] The results are shown in Table 1.

We found that acyclonucleoside **6** and acyclic nucleoside phosphonate **13** were adenosine deaminase substrates. The V_{max} of **13** was, however, $\sim 10^{-4}$ times less than that of **6**. Compounds **6** and **13** showed competitive inhibition of adenosine deaminase when ara-A (**37**) was used as a substrate. Nevertheless, phosphonate **13** acted more efficiently than acyclonucleoside **6** as an inhibitor of adenosine deaminase. Nucleotide analog **40** was also a substrate of adenosine deaminase, but its V_{max} was 92% less than that of ara-A (**37**). The slow rate of deamination of compound **40** toward adenosine deaminase may reflect the inhibitory action of the acyclic nucleotide moiety therein at the active site of the enzyme. Subsequently, by assaying against calf mucosal adenosine deaminase *in vitro*, we found that phosphonoamidate **42** completely resisted deamination.

Lipophilicity and Solubility Tests. We found that phosphonoamidates **42** and **53**−**56** had higher lipophilicity than the corresponding nucleoside phosphonates **13**, **20**, and **32** as well as the nucleotide phosphonate **40** and dinucleotide phosphonates **47** and **48**. Their lipophilicity and solubility in water were determined by the distribution between pentanol and water according to the methods reported by Baker et al.[32] (Table 2).

Biological Activities. We tested the synthesized compounds for their inhibition of cytopathogenicity of herpes simplex type 1 virus (HSV-1), herpes simplex type 2 virus (HSV-2), and varicella-zoster virus (VZV) in Hela cell culture.[33,34] These compounds include **1**, **6**, **12**, **13**, **40**, **42**, a mixture of **6** and **13** (1:1), ara-A (**37**), and a mixture of **13** and **37** (1:1). Toxicity of these compounds was evaluated by their ability to cause morphological changes in cells at different concentrations. The minimum inhibitory concentrations (IC_{50}), measured by use of the linear regression method,[33,35] are summarized in Table 3.

Table 3. Anti-Herpes and Anticellular Activities of Nucleoside and Nucleotide Analogs in Tissue Culture

	IC_{50} (μg/mL)[a]			
compd	HSV-1 (KOS)	HSV-2 (G)	VZV (YS)	Hela cell[b]
acyclovir (**1**)	0.10	0.23	20.00	250.00
6	4.43	8.26	6.50	265.70
12	11.26	13.80	25.00	175.60
13	3.98	5.86	16.00	215.00
6 + **13** (1:1)	0.15	0.38	0.18	235.25
ara-A (**37**)	10.80	*c*	*c*	98.85
13 + **37** (1:1)	0.67	1.25	2.86	167.82
40	8.97	16.29	*c*	346.07
42	0.38	0.88	4.82	215.48

[a] Inhibitory concentrations (IC_{50}) represent the mean of triplicate determinations. [b] Concentration of the compound required to cause microscopically visible change or disruption in about 50% of the cell sheet. [c] Not active up to 128 μg/mL.

Table 4. Inhibitory Effects of Nucleoside and Nucleotide Analogs on the Cytopathogenicity of HIV-1 in MT4 Cells and Cellular Toxicity

	IC_{50} (μg/mL)[a]	
compd	HIV-1(IIIB)	MT4 cell[b]
AZT (**4**)	0.02	57.28
20	7.68	114.57
26	*c*	58.62
29	23.50	128.43
32	11.79	98.70
34	*c*	69.87
36	*c*	195.65
47	1.92	99.53
48	3.04	78.97
53	0.61	199.78
54	0.34	180.06
55	0.76	457.50
56	0.50	462.00

[a] Inhibitory concentrations (IC_{50}) represent the average of triplicate determinations. [b] Concentration of the compound required to reduce the number of viable uninfected cells by 50%. [c] Not active up to 128 μg/mL.

Furthermore, we screened compounds **4**, **20**, **26**, **29**, **32**, **34**, **36**, **47**, **48**, and **53**−**56** for their antiviral activity against HIV-1(IIIB) in MT4 cells in a cell-protection assay.[36] Most of the compounds demonstrated the ability to protect MT4 cells from HIV, which exhibits a cytopathic effect (Table 4).

Discussion

The potent antiviral activity of acyclovir (**1**) results from its monophosphorylation by HSV-specified thymidine kinase[1,3] and the sequential conversion to a triphosphate analog by cellular enzymes.[3,6,37] Adenine acyclonucleoside **6** and adenine arabinofuranoside **37** are however not phosphorylated in the presence of thymidine kinase,[13] and acyclic nucleoside phosphonate **13** is not phosphorylated by either bovine brain guanylate kinase[8b] or 5-phosphoribosyl 1-pyrophosphate synthetase.[9g] Therefore, phosphorylation of these compounds may not be essential for antiviral activity (Table 3), or phosphorylation may be performed by other enzymes.

Adenosine deaminase can form a complex with acyclonucleoside **6**[11] and arabinoside **37** (Table 1);[38] thus their antiviral activity is decreased. Compound **13** inhibits adenosine deaminase (Table 1). As a result of this inhibition, a synergistic effect on the antiviral activity of **6** and ara-A (**37**) was predicted and observed (Table 3).

Because lower dosage of two drugs in combination can be used to improve their efficacy in comparison with individual drug applied alone,[39] the combined modality may be beneficial in the treatment of patients who cannot tolerate high dosages of the drugs. One approach to combination therapy involves the linkage of two antiviral nucleosides with a phosphate[40] or a phosphonate bridge (e.g., **40**). The ability of a drug to penetrate a membrane and exhibit biological activity is correlated to its lipophilicity (Tables 2 and 3). Thus nucleotide phosphonate **40** in zwitterionic form is not expected to penetrate the cell membrane or the blood–brain barrier. Consequently, we prepared phosphonoamidate derivative **42** as a lipophilic prodrug, which displayed superior antiviral activity (Table 3). The activity increment of **42** over acyclic nucleoside phosphonate **13**, ara-A (**37**), and nucleotide phosphonate **40** may be due to a combination of increased lipophilicity and resistance to adenosine deaminase (Tables 1–3). We hypothesize that, as a masked membrane-soluble form of the bioactive nucleotide analog **40**, phosphonoamidate **42** may act as a proteinase substrate. With the aid of phosphodiesterases, the biologically active compounds **13** and **37** were then liberated as potential drugs and effective against infected cells (Table 3).

Dinucleotide phosphonates **47** and **48** possess anti-HIV activity. We believe that thymidine kinase could specifically monophosphorylate the 5′-hydroxyl group in **47** and **48** as in AZT.[4,5] After anabolization to their respective triphosphates, the resultant compounds can act as an alternate substrate for HIV-1 reverse transcriptase. Our results show that dinucleotide analogs **47** and **48** exhibited higher anti-HIV activity than their parent nucleoside phosphonates **20** and **32** (Table 4). We believe that the diphosphate analogs of **20** and **32** could not act as effective substrates[10a] as the triphosphate analogs of **47** and **48** for HIV-1 reverse transcriptase.

Furthermore, effective antiviral activity requires transport of the phosphonate analog across cell membranes; such a process is lipophilicity dependent.[41] Therefore, we prepared phosphonoamidates **53–56**, which exhibited superior lipophilicity (Table 2) and remarkable antiviral activity (Table 4). Nevertheless, HIV-aspartate proteinase[16] may hydrolyze phosphonoamidates **53–56** to the corresponding dinucleotide analogs **47** and **48** inside the infected cells. The thymidine moiety of each would then be converted sequentially to the respective triphosphates. The triphosphate anabolites of **47** and **48** could act as alternate substrates for HIV-1 reverse transcriptase, which terminates viral DNA synthesis after incorporation into the growing DNA strand. Therefore, phosphonoamidates **53–56** can be considered as potential prodrugs of the corresponding bioactive free dinucleotides **47** and **48** (Table 4).

Compounds **55** and **56** possess an unnatural methyl D-alaninate moiety, and compounds **53** and **54** possess a natural methyl L-alaninate moiety. These compounds showed similar anti-HIV activity but different cellular toxicity (Table 4). Thus HIV-aspartate proteinase may recognize phosphonates carrying an amino ester with the D- or L-configuration, whereas the cellular aspartyl proteases can only recognize and hydrolyze phosphonoamidates having a natural amino ester moiety. Consequently, compounds **55** and **56** are less toxic than compounds **53** and **54**.

Conclusions

A series of new compounds were synthesized, and their structure–activity relationship was studied. Those compounds include bioactive acyclic nucleoside phosphonate **13**, nucleoside phosphonates **20**, **29**, and **32**, and dinucleotide analogs **40**, **47**, and **48**, as well as phosphonoamidates **42** and **53–56**. Results from biological tests indicate that diphosphates of nucleoside phosphonates **20**, **29**, and **32** did not act as effective substrates for HIV-1 reverse transcriptase; thus these nucleoside phosphonates exhibited low anti-HIV activity. Dinucleotide phosphonates **40**, **47**, and **48**, possessing a 5′-free hydroxyl functionality, showed moderate antiviral activity. Lack of high activity is due to the inefficient transport of these dinucleotide analogs through cell membranes. Finally, phosphonoamidate derivatives **42** and **53–56** were designed to possess a methyl L- or D-alaninate moiety. These phosphonoamidates showed superior bioavailability and profound antiviral activity. Phosphonoamidates **55** and **56** bearing a D-amino ester moiety exhibited less cellular toxicity than phosphonoamidates **53** and **54** bearing an L-amino ester moiety.

Experimental Section

General. All reactions were carried out under a static atmosphere of nitrogen and mixtures stirred magnetically unless otherwise noted. For anhydrous reactions, glassware was dried overnight in an oven at 120 °C and cooled in a desiccator over anhydrous $CaSO_4$(s) or silica gel. Reagents were purchased from Fluka Chemical Co. Dry ether and tetrahydrofuran (THF) were obtained by distillation from the sodium ketyl of benzophenone under nitrogen. Other solvents, including chloroform, dichloromethane, ethyl acetate, and hexanes, were distilled over CaH_2 under nitrogen. Absolute methanol was purchased from Merck and used as received. Enzymes were purchased from Sigma Chemical Co. or Nutritional Biochemicals Corp.

Melting points were obtained with a Büchi 510 melting point apparatus. Ultraviolet (UV) spectra were recorded on a Cary 118 spectrophotometer, and λ_{max} was obtained in nm (ϵ) units. Infrared (IR) spectra were recorded on a Beckman IR 8 spectrophotometer. The wavenumbers reported are referenced to the 1601 cm^{-1} absorption of poly(styrene). Proton NMR spectra were obtained on a Varian XL-300 (300 MHz) spectrometer. Chloroform-*d* was used as solvent; Me_4Si (δ 0.00) was used as an internal standard. Phosphorus-31 NMR spectra were recorded on a Varian XL-300 spectrometer. Dimethyl sulfoxide-*d* was used as solvent. Phosphorus-31 chemical shifts are referenced to 85% phosphoric acid (δ 0.00). All NMR chemical shifts are reported as δ values in parts per million (ppm), and coupling constants (J) are given in hertz (Hz). The splitting pattern abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; br, broad; m, unresolved multiplet; dd, doublet of doublets; dm, doublet of multiplets. Microanalyses were performed on a Perkin-Elmer 240 B microanalyzer. Purification on silica gel refers to gravity column chromatography on Merck silica gel 60 (particle size 230–400 mesh). Analytical TLC was performed on precoated plates purchased from Merck (silica gel 60 F_{254}). Compounds were visualized by use of UV light, I_2 vapor, or 2.5% phosphomolybdic acid in ethanol with heating. Paper chromatography

was performed on Whatman 3-mm paper. A mixture of *i*-PrOH, concentrated NH_4OH, and H_2O (9:1:2) was used as eluant.

Enzyme Assays: A. Adenosine Deaminase. The reported procedures[29,31] were used for adenosine deaminase, and the results are summarized in Table 1.

B. Snake Venom Phosphodiesterase. Snake venom phosphodiesterase (200 units) was dissolved in tris(hydroxymethyl)aminomethane buffer (1.0 mL), which was adjusted to pH 9.2 with 0.1 N HCl. The enzyme solution (0.10 mL) was added to the nucleotide (0.50 mg), and the mixture was incubated at 37 °C for 8 h. The solution was then applied to Whatman 3-mm paper as a band, which was developed with a mixture of *i*-PrOH, concentrated NH_4OH, and H_2O (9:1:2). Degradation products or unreacted starting materials were separated. The isolated compounds were characterized by comparison with authentic samples.

C. Spleen Phosphodiesterase. Spleen phosphodiesterase (20 units) was dissolved in sodium pyrophosphate buffer (0.01 M, 1.0 mL), which was adjusted to pH 6.5 with phosphoric acid. Nucleotide (0.50 mg) was dissolved in ammonium acetate buffer (0.05 M, 0.20 mL), which was adjusted to pH 6.5 with acetic acid. An aliquot of the enzyme solution (0.1 mL) was added to the nucleotide solution, and the mixture was incubated at 37 °C for 8 h. The solution was then applied to Whatman 3-mm paper as a band and developed with a mixture of *i*-PrOH, concentrated NH_4OH, and H_2O (9:1:2). Bands containing nucleosides and nucleotides were cut out, which were eluted with H_2O, and the resultant mixture was freeze-dried. The isolated products were characterized by comparison with authentic samples.

Determination of Solubility. Each compound (70 mg) listed in Table 2 was agitated in a 25-mL volumetric flask with phosphate buffer (0.10 M, 5.0 mL) for 20 h. This solution was filtered from undissolved solid through a sintered glass funnel (4.0–5.5 mesh ASTM), and the concentration of the solution was determined by UV absorbance (Table 2).

Determination of Partition Coefficients (Lipophilicity). A solution of each compound (10 mL) in Table 2 in phosphate buffer (0.10 M) possessing an UV absorbance of 2.3–3.3 at 258–265 nm was shaken with 1-pentanol (10 mL) in a separatory funnel for 1.5 h. The layers were separated, and their concentrations were determined by an UV spectrophotometer. The partition coefficient was calculated as $P = [S]_{1\text{-pentanol}}/[S]_{H_2O}$ (Table 2).

Diethyl [2-[(6-Methoxypurin-9-yl)methoxy]ethyl]phosphonate (11). A mixture of **7** (2.87 g, 10.0 mmol) and triethyl phosphite (8.30 g, 50.0 mmol) was heated at 150 °C for 24 h. Ether (300 mL) was added to the solution at room temperature, and the resultant precipitate was filtered. Crystallization from a mixture of MeOH and Et_2O (1:4) gave **11** (1.01 g) in 30% yield: mp 140–141 °C; TLC R_f 0.17 (AcOEt/MeOH = 4:1); UV λ_{max} (EtOH) 249 nm (ϵ 12 250); ^{1}H NMR ($CDCl_3$) δ 1.10–1.54 (m, 8 H, 2 × CH_3 + CH_2P), 3.39–4.30 (m, 6 H, 2 × CH_2OP + CH_2O), 4.15 (s, 3 H, OCH_3), 5.61 (s, 2 H, OCH_2N), 8.12, 8.41 (2 s, 2 H, HC(2) + HC(8)). Anal. ($C_{13}H_{21}N_4O_5P$) C, H, N.

Ammonium Ethyl [2-(Adenin-9-ylmethoxy)ethyl]phosphonate (12), 1,4-Dihydro-2-amino-1-[2′,5′-dideoxy-5′-(ammoniumylmethylphosphono)-β-D-*threo*-pentofuranosyl]-4-oxo-5-methylpyrimidine (21), and 1,4-Dihydro-2-amino-1-[2′,3′,5′-trideoxy-3′-azido-5′-(ammoniumylmethylphosphono)-β-D-*erythro*-pentofuranosyl]-4-oxo-5-methylpyrimidine (25). A representative procedure is as follows for the conversions of **11** → **12** (40%), **17** → **21** (55%), and **24** → **25** (35%). To a solution of **11** (3.44 g, 10.0 mmol) in MeOH (40 mL) was added a saturated methanolic NH_3 solution (100 mL). The solution was heated in a sealed flask at 100 °C for 30 h. The solvent was evaporated, and the residue was crystallized from EtOH to give **12** (1.20 g) in 40% yield: mp 190–193 °C; TLC R_f 0.37 (AcOEt/MeOH = 1:1); UV λ_{max} (EtOH) 260 nm (ϵ, 14 000); ^{1}H NMR (DMSO-d_6/D_2O) δ 1.20–1.56 (m, 5 H, CH_3 + CH_2P), 3.40–4.10 (m, 2 H, CH_2OP), 4.30 (m, 2 H, CH_2O), 5.60 (s, 2 H, OCH_2N), 7.80, 8.12 (2 s, 2 H, HC(2) + HC(8)). Anal. ($C_{10}H_{19}N_6O_4P$) C, H, N.

21: mp 210–211 °C; TLC R_f 0.26 (AcOEt/MeOH = 1:1); UV λ_{max} (EtOH) 260 nm (ϵ 6400); ^{1}H NMR ($CDCl_3$/DMSO-d_6/D_2O) δ 1.93 (s, 3 H, CH_3), 1.91–2.62 (m, 4 H, $H_2C(2')$ + $H_2C(5')$), 3.58 (d, J = 11 Hz, 3 H, CH_3OP), 4.43 (m, 2 H, HC(4′) + HC(3′)), 5.65 (dd, J = 3.0, 6.0 Hz, 1 H, HC(1′)), 7.25 (s, 1 H, HC(6)). Anal. ($C_{11}H_{21}N_4O_6P$) C, H, N.

25: mp 184–185 °C; TLC R_f 0.34 (AcOEt/MeOH = 1:1); UV λ_{max} (EtOH) 260 nm (ϵ 6560); IR (Nujol) 3026–3545 (NH_2, NH_4^+), 2100 (N_3), 1705 cm^{-1} (2 C=O); ^{1}H NMR ($CDCl_3$/DMSO-d_6/D_2O) δ 1.94 (s, 3 H, CH_3), 1.95–2.55 (m, 4 H, $H_2C(2')$ + $H_2C(5')$), 3.60 (d, J = 11 Hz, 3 H, CH_3OP), 3.86–4.22 (m, 2 H, HC(4′) + HC(3′)), 5.85 (t, J = 6.4 Hz, 1 H, HC(1′)), 7.25 (s, 1 H, HC(6)). Anal. ($C_{11}H_{20}N_7O_5P$) C, H, N.

[2-(Adenin-9-ylmethoxy)ethyl]phosphonic Acid (13) and 3′-Azido-2′,3′,5′-trideoxy-5-methylisocytidine-5′-phosphonic Acid (26). A representative procedure is as follows for the conversions of **12** → **13** (45%) and **25** → **26** (65%). To a solution of **12** (0.32 g, 1.0 mmol) in DMF (7.0 mL) was added Me_3SiBr (1.07 g, 7.01 mmol). After the solution was stirred at 40 °C for 6 h, a mixture of MeOH and H_2O (5:1, 20 mL) was added, and the solvents were evaporated. The crude residue was purified by use of column chromatography (resin XAD-4, H_2O) to afford **13** (0.12 g): mp 296 °C dec; TLC R_f 0.32 (MeOH); UV λ_{max} (EtOH) 259 nm (ϵ 13 700); ^{1}H NMR (DMSO-d_6/D_2O) δ 1.49 (m, 2 H, CH_2P), 3.80 (m, 2 H, CH_2O), 5.55 (s, 2 H, OCH_2N), 7.90, 8.19 (2 s, 2 H, HC(2) + HC(8)). Anal. ($C_8H_{12}N_5O_4P$) C, H, N.

26: mp 269 °C dec; TLC R_f 0.36 (MeOH); UV λ_{max} (EtOH) 261 nm (ϵ 8270); IR (Nujol) 3150–3475 (NH_2, OH), 2110 (N_3), 1700 cm^{-1} (2 C=O); ^{1}H NMR (DMSO-d_6/D_2O) δ 1.93 (s, 3 H, CH_3), 1.84–2.60 (m, 4 H, $H_2C(2')$ + $H_2C(5')$), 3.85–4.22 (m, 2 H, HC(4′) + HC(3′)), 5.90 (t, J = 6.5 Hz, 1 H, HC(1′)), 7.21 (s, 1 H, HC(6)). Anal. ($C_{10}H_{15}N_6O_5P$) C, H, N.

1-(5′-Deoxy-5′-bromo-β-D-*threo*-pentofuranosyl)thymine (15). To a mixture of BF_3·OEt_2 (3.20 mL, 25.0 mmol) and LiBr (2.20 g, 25.0 mmol) in THF (45 mL) was added dropwise a THF solution (10 mL) of **14** (1.12 g, 5.00 mmol) under dry argon at −50 °C. The reaction mixture was stirred for 2 h and the reaction quenched with saturated $NaHCO_3$ (15 mL). The solvents were evaporated, and the residue was purified by use of column chromatography (SiO_2, AcOEt) to give **15** (1.01 g) as a foam in 70% yield: TLC R_f 0.75 (Et_2O/MeOH = 9:1); UV λ_{max} (EtOH) 265 nm (ϵ 9650); ^{1}H NMR ($CDCl_3$/D_2O) δ 1.89 (s, 3 H, CH_3), 2.32 (m, 2 H, $H_2C(2')$), 3.37 (dd, J = 3.0, 6.0 Hz, 2 H, $H_2C(5')$), 4.10 (m, 1 H, HC(4′)), 4.33 (m, 1 H, HC(3′)), 5.89 (dd, J = 3.3, 7.3 Hz, 1 H, HC(1′)), 7.32 (s, 1 H, HC(6)). Anal. ($C_{10}H_{13}N_2O_4Br$) C, H, N, Br.

1-[5′-Deoxy-5′-(dimethylphosphono)-β-D-*threo*-pentofuranosyl]thymine (16) and 1,4-Dihydro-2-methoxy-1-[2′,5′-dideoxy-5′-(methylphosphono)-β-D-*threo*-pentofuranosyl]-4-oxo-5-methylpyrimidine (17). A mixture of **15** (3.05 g, 10.0 mmol) and trimethyl phosphite (24.8 g, 0.200 mol) was heated at 140 °C. The reaction mixture was stirred for 20 h, cooled, and added to MeOH (10 mL). The resultant solution was poured into a stirred solution of Et_2O (300 mL) to afford a precipitate. The crude material was purified by use of column chromatography (SiO_2, AcOEt, and then AcOEt/acetone = 1:1) to afford **16** (1.30 g, 40% yield) and **17** (1.31 g, 40% yield), respectively.

16: mp 150–152 °C; TLC R_f 0.68 (Et_2O/MeOH = 9:1); UV λ_{max} (EtOH) 264 nm (ϵ 9170); ^{1}H NMR ($CDCl_3$) δ 1.92 (s, 3 H, CH_3), 2.01–2.65 (m, 4 H, $H_2C(2')$ + $H_2C(5')$), 3.85 (d, J = 11 Hz, 6 H, 2 × CH_3O), 4.45 (br m, 2 H, HC(4′) + HC(3′)), 4.91 (br, 1 H, HOC(3′)), 5.71 (dd, J = 3.1, 6.9 Hz, 1 H, HC(1′)), 7.35 (s, 1 H, HC(6)), 10.20 (br, 1 H, NH). Anal. ($C_{12}H_{19}N_2O_7P$) C, H, N.

17: mp 199–201 °C; TLC R_f 0.31 (Et_2O/MeOH = 9:1); UV λ_{max} (EtOH) 252 nm (ϵ 7080); ^{1}H NMR ($CDCl_3$/DMSO-d_6/D_2O) δ 1.91 (s, 3 H, CH_3), 1.90–2.60 (m, 4 H, $H_2C(2')$ + $H_2C(5')$), 3.30 (s, 3 H, CH_3O), 3.60 (d, J = 11 Hz, 3 H, CH_3OP), 4.44 (m, 2 H, HC(4′) + HC(3′)), 5.62 (dd, J = 3.0, 6.0 Hz, 1 H, HC(1′)), 7.40 (s, 1 H, HC(6)). Anal. ($C_{12}H_{19}N_2O_7P$) C, H, N.

1-[5′-Deoxy-5′-(dimethylphosphono)-3′-*O*-mesyl-β-D-*threo*-pentofuranosyl]thymine (18) and 1,4-Dihydro-2-methoxy-1-[2′,5′-dideoxy-5′-(dimethylphosphono)-3′-*O*-mesyl-β-D-*threo*-pentofuranosyl]-4-oxo-5-methylpyrimidine (23). To a pyridine (30 mL) solution containing compound **16** (3.34 g, 10.0 mmol) and 4-(dimethylamino)-

pyridine (0.10 g, 0.82 mmol) was added CH_3SO_2Cl (1.20 g, 10.0 mmol) dropwise at 20 °C. The mixture was stirred for 24 h and partitioned between AcOEt and H_2O. The organic layer was washed with 5% aqueous HCl solution (3 × 50 mL) and H_2O (50 mL), dried ($MgSO_4$), filtered, and condensed to give a syrup. The crude material was purified by use of column chromatography (SiO_2, AcOEt) to give **18** (3.70 g) as a foam in 90% yield: TLC R_f 0.71 (Et_2O/MeOH = 9:1); UV λ_{max} (EtOH) 265 nm (ϵ 9780); ^1H NMR ($CDCl_3$) δ 1.90 (s, 3 H, CH_3), 2.00–2.75 (m, 4 H, $H_2C(2') + H_2C(5')$), 3.15 (s, 3 H, CH_3SO_3), 3.75 (d, J = 11 Hz, 6 H, 2 × CH_3O), 4.51 (*m*, 1 H, HC(4')), 5.12 (m, 1 H, HC(3')), 6.08 (dd, J = 2.8, 7.8 Hz, 1 H, HC(1')), 7.31 (s, 1 H, HC(6)), 9.50 (br, 1 H, NH). Anal. ($C_{13}H_{21}N_2O_9PS$) C, H, N.

By the same method, **23** was obtained in 95% yield from **22**. **23:** TLC R_f 0.96 (Et_2O/MeOH = 9:1); UV λ_{max} (EtOH) 252 nm (ϵ 7500); ^1H NMR ($CDCl_3$) δ 1.90 (s, 3 H, CH_3), 1.95–2.70 (m, 4 H, $H_2C(2') + H_2C(5')$), 3.15 (s, 3 H, CH_3SO_3), 3.35 (s, 3 H, CH_3O), 3.69 (d, J = 11 Hz, 6 H, 2 × CH_3OP), 4.48 (m, 1 H, HC(4')), 5.20 (m, 1 H, HC(3')), 5.82 (dd, J = 3.0, 6.0 Hz, 1 H, HC(1')), 7.41 (s, 1 H, HC(6)). Anal. ($C_{14}H_{23}N_2O_9PS$) C, H, N.

1-[3',5'-Dideoxy-3'-azido-5'-(dimethylphosphono)-β-D-*erythro*-pentofuranosyl]thymine (19) and 1,4-Dihydro-2-methoxy-1-[2',3',5'-trideoxy-3'-azido-5'-(dimethylphosphono)-β-D-*erythro*-pentofuranosyl]-4-oxo-5-methylpyrimidine (24). A representative procedure is as follows. Compound **18** (2.06 g, 5.00 mmol) and LiN_3 (0.500 g, 10.0 mmol) were dissolved in dry DMF (20 mL) under N_2. The mixture was heated at 95 °C for 4 h; then it was partitioned between AcOEt (50 mL) and H_2O (60 mL). The organic layer was washed with H_2O (3 × 50 mL), dried ($MgSO_4$), filtered, and condensed. The residue was purified by use of column chromatography (SiO_2, $CHCl_3$/AcOEt = 1:1) to afford **19** (1.40 g) as a foam in 80% yield: TLC R_f 0.27 (Et_2O); UV λ_{max} (EtOH) 264 nm (ϵ 10 100); IR (CH_2Cl_2) 3410 (NH), 2100 (N_3), 1695 cm^{-1} (2 C=O); ^1H NMR ($CDCl_3$) δ 1.95 (s, 3 H, CH_3), 2.05–2.50 (m, 4 H, $H_2C(2') + H_2C(5')$), 3.80 (d, J = 12 Hz, 6 H, 2 × CH_3O), 3.82–4.03 (m, 2 H, HC(4') + HC(3')), 6.14 (**t**, J = 6.5 Hz, 1 H, HC(1')), 7.19 (s, 1 H, HC(6)), 9.15 (br, 1 H, NH). Anal. ($C_{12}H_{18}N_5O_6P$) C, H, N.

By the same method, **24** was obtain in 69% yield from **23**. **24:** TLC R_f 0.31 (Et_2O); UV λ_{max} (EtOH) 252 nm (ϵ 7110); IR (CH_2Cl_2) 2100 (N_3), 1720 cm^{-1} (2 C=O); ^1H NMR ($CDCl_3$) δ 1.95 (s, 3 H, CH_3), 2.00–2.52 (m, 4 H, $H_2C(2') + H_2C(5')$), 3.30 (s, 3 H, CH_3O), 3.75 (d, J = 11 Hz, 6 H, 2 × CH_3OP), 3.80–4.10 (m, 2 H, HC(4') + HC(3')), 5.95 (t, J = 6.5 Hz, 1 H, HC(1')), 7.20 (s, 1 H, HC(6)). Anal. ($C_{13}H_{20}N_5O_6P$) C, H, N.

3'-Azido-3',5'-dideoxythymidine-5'-phosphonic Acid (20), 3',5'-Dideoxythymidine-5'-phosphonic Acid (29), 3'-Fluoro-3',5'-dideoxythymidine-5'-phosphonic Acid (32), 3'-Cyano-3',5'-dideoxythymidine-5'-phosphonic Acid (34), and 3',5'-Dideoxythymidin-2'-ene-5'-phosphonic Acid (36). The following conversions were performed in the same manner: **19** → **20** (80%), **24** → **20** (75%), **28** → **29** (80%), **31** → **32** (85%), **33** → **34** (65%), and **35** → **36** (75%). All products were purified by use of column chromatography (SiO_2, AcOEt/MeOH = 4:1). A representative procedure is as follows. To a solution of **19** (3.60 g, 10.0 mmol) in CH_2Cl_2 (50 mL) was added Me_3SiBr (4.95 g, 30.0 mmol); then the solution was stirred at 25 °C for 7 h. A mixture of MeOH and H_2O (5:1, 50 mL) was added, and the solvents were evaporated to afford **20** (2.60 g) as a foam in 80% yield: TLC R_f 0.20 (AcOEt/MeOH = 6:1); UV λ_{max} (EtOH) 266 nm (ϵ 10 100); IR (Nujol) 3200–3460 (NH, OH), 2100 (N_3), 1690 cm^{-1} (2 C=O); ^1H NMR (D_2O) δ 1.75 (s, 3 H, CH_3), 1.80 (m, 2 H, $H_2C(5')$), 2.15–2.48 (m, 2 H, $H_2C(2')$), 3.78 (m, 1 H, HC(4')), 4.06 (m, 1 H, HC(3')), 6.01 (t, J = 6.2 Hz, 1 H, HC(1')), 7.10 (s, 1 H, HC(6)). Anal. ($C_{10}H_{14}N_5O_6P$) C, H, N.

29: TLC R_f 0.22 (AcOEt/MeOH = 6:1); UV λ_{max} (EtOH) 265 nm (ϵ 10 500); ^1H NMR (D_2O) δ 1.80 (s, 3 H, CH_3), 1.68–2.53 (m, 6 H, $H_2C(2') + H_2C(3') + H_2C(5')$), 3.75 (m, 1 H, HC(4')), 6.05 (t, J = 6.3 Hz, 1 H, HC(1')), 7.15 (s, 1 H, HC(6)). Anal. ($C_{10}H_{15}N_2O_6P$) C, H, N.

32: TLC R_f 0.23 (AcOEt/MeOH = 6:1); UV λ_{max} (EtOH) 263 nm (ϵ 8989); ^1H NMR (D_2O) δ 1.80 (br s, 3 H, CH_3), 1.79–2.68 (m, 4 H, $H_2C(2') + H_2C(5')$), 4.09 (m, $J_{4',5'}$ = 3.3 Hz, $J_{4',F}$ = 27.7 Hz, 1 H, HC(4')), 5.27 (m, $J_{3',F}$ = 54.2 Hz, 1 H, HC(3')), 6.32 (t, J = 7.0 Hz, 1 H, HC(1')), 7.52 (s, 1 H, HC(6)). Anal. ($C_{10}H_{14}N_2O_6FP$) C, H, N, F.

34: TLC R_f 0.18 (AcOEt/MeOH = 6:1); UV λ_{max} (EtOH) 265 nm (ϵ 10 100); IR (Nujol) 3200–3460 (NH, OH), 2245 (CN), 1690 cm^{-1} (2 C=O); ^1H NMR (DMSO-d_6/D_2O) δ 1.79 (s, 3 H, CH_3), 1.77–2.60 (m, 4 H, $H_2C(2') + H_2C(5')$), 3.50 (m, 1 H, HC(3')), 3.88 (m, 1 H, HC(4')), 6.12 (t, J = 6.5 Hz, 1 H, HC(1')), 7.59 (s, 1 H, HC(6)). Anal. ($C_{11}H_{14}N_3O_6P$) C, H, N.

36: TLC R_f 0.21 (AcOEt/MeOH = 6:1); UV λ_{max} (EtOH) 265 nm (ϵ 10 520); ^1H NMR (D_2O) 1.72 (br s, 3 H, CH_3), 1.81 (m, 2 H, H_2C (5')), 5.08 (m, 1 H, HC(4')), 5.90 (m, 1 H, HC(2')), 6.27 (m, 1 H, HC(3')), 7.08 (br s, 1 H, HC(1')), 7.29 (br s, 1 H, HC(6)). Anal. ($C_{10}H_{13}N_2O_6P$) C, H, N.

1,4-Dihydro-2-methoxy-1-[2',5'-dideoxy-5'-(dimethylphosphono)-β-D-*threo*-pentofuranosyl]-4-oxo-5-methylpyrimidine (22). Compound **17** (3.34 g, 9.99 mmol) was treated with ethereal CH_2N_2 (28.0 g, 662 mmol), and the resultant mixture was purified by use of column chromatography (SiO_2, AcOEt) to give **22** (3.41 g) as a foam in 98% yield: TLC R_f 0.85 (Et_2O/MeOH = 9:1); UV λ_{max} (EtOH) 252 nm (ϵ 7180); ^1H NMR ($CDCl_3$/DMSO-d_6/D_2O) δ 1.93 (s, 3 H, CH_3), 1.94–2.63 (m, 4 H, $H_2C(2') + H_2C(5')$), 3.30 (s, 3 H, CH_3O), 3.65 (d, J = 11 Hz, 6 H, 2 × CH_3OP), 4.45 (m, 2 H, HC(4') + HC(3')), 5.64 (dd, J = 3.0, 6.0 Hz, 1 H, HC(1')), 7.40 (s, 1 H, HC(6)). Anal. ($C_{13}H_{21}N_2O_7P$) C, H, N.

1-[5'-Deoxy-5'-(dimethylphosphono)-3'-*O*-(imidazol-1-ylthiocarbonyl)-β-D-*threo*-pentofuranosyl]thymine (27). To a solution of **16** (3.34 g, 10.0 mmol) in DMF (50 mL) was added thiocarbonyldiimidazole (5.34 g, 30.0 mmol). The solution was stirred at 25 °C for 8 h and then partitioned between AcOEt (250 mL) and H_2O (250 mL). The organic layer was separated, washed with H_2O (5 × 100 mL), dried ($MgSO_4$), and condensed. The crude material was purified by use of column chromatography (SiO_2, AcOEt) to afford **27** (1.80 g) as a foam in 40% yield: TLC R_f 0.81 (Et_2O/MeOH = 9:1); UV λ_{max} (EtOH) 264 nm (ϵ 10 100); ^1H NMR ($CDCl_3$) δ 1.92 (s, 3 H, CH_3), 1.83–2.61 (m, 4 H, $H_2C(2') + H_2C(5')$), 3.80 (d, J = 12 Hz, 6 H, 2 × CH_3O), 4.52 (m, 1 H, HC(4')), 4.81 (m, 1 H, HC(3')), 6.07 (dd, J = 3.4, 7.9 Hz, 1 H, HC(1')), 7.39 (s, 1 H, HC(6)), 7.35, 7.87 (2 br s, 2 H, NCH=CHN), 7.99 (s, 1 H, NCH=N), 9.51 (br, 1 H, NH). Anal. ($C_{16}H_{21}N_4O_7SP$) C, H, N, S.

1-[3',5'-Dideoxy-5'-(dimethylphosphono)-β-D-pentofuranosyl]thymine (28). A mixture of **27** (2.66 g, 6.01 mmol), 2,2'-azobis(2-methylpropionitrile) (0.20 g, 1.2 mmol), and *n*-Bu_3SnH (7.86 g, 27.0 mmol) in toluene (100 mL) was heated at reflux for 6 h. Solvent was removed at reduced pressure, and the residue was purified by use of column chromatography (SiO_2, $CHCl_3$/AcOEt = 1:1) to give **28** (0.82 g) as a foam in 43% yield: TLC R_f 0.68 (AcOEt); UV λ_{max} (EtOH) 264 nm (ϵ 11 000); ^1H NMR ($CDCl_3$) δ 1.91 (d, J = 1.1 Hz, 3 H, CH_3), 1.71–2.50 (m, 6 H, $H_2C(2') + H_2C(3') + H_2C(5')$), 3.79 (d, J = 11 Hz, 6 H, 2 × CH_3O), 4.18 (m, 1 H, HC(4')), 6.15 (t, J = 6.4 Hz, 1 H, HC(1')), 7.40 (q, J = 1.1 Hz, 1 H, HC(6)), 9.18 (br, 1 H, NH). Anal. ($C_{12}H_{19}N_2O_6P$) C, H, N.

1-[3',5'-Dideoxy-3'-fluoro-5'-(methylphosphono)-β-D-*erythro*-pentofuranosyl]thymine (31). To a solution of **16** (3.34 g, 10.0 mmol) in pyridine (30 mL) was added dropwise CF_3SO_2Cl (2.53 g, 15.0 mmol) at 0 °C, and the mixture was stirred at the same temperature for 5 h. The solution was partitioned between AcOEt (150 mL) and H_2O (200 mL). The organic layer was separated, washed with 2% aqueous HCl solution (3 × 60 mL) and H_2O (100 mL), dried ($MgSO_4$), filtered, and condensed to afford the crude triflate **30**, which was used without further purification. A solution of the crude **30** in THF (30 mL) was treated with *n*-Bu_4NF (1.0 M solution in THF, 2.00 mL, 20.0 mmol) at 0 °C. The reaction mixture was stirred at 25 °C for 13 h; the solvent was evaporated, and the residue was purified by use of column chromatography (SiO_2, AcOEt) to give **31** (1.60 g) as a foam in 50% yield: TLC R_f 0.18 (AcOEt); UV λ_{max} (EtOH) 265 nm (ϵ 9540); ^1H NMR (DMSO-d_6/D_2O) δ 1.87 (d, J = 1.1 Hz, 3 H, CH_3), 1.82–2.66 (m, 4 H, $H_2C(2') + H_2C(5')$), 3.75 (d, J = 11 Hz, 3 H, CH_3O), 4.25 (m, $J_{4',5'}$ = 3.3 Hz, $J_{4',F}$ = 28.08 Hz, 1 H, HC(4')), 5.29 (m, $J_{3',F}$ = 54.1 Hz, 1 H, HC(3')), 6.29 (dd, J = 3.9, 9.0 Hz, 1 H, HC(1')), 7.80 (q, J = 1.1 Hz, 1 H, HC(6)). Anal. ($C_{11}H_{16}N_2O_6FP$) C, H, N, F.

1-[3′,5′-Dideoxy-3′-cyano-5′-(dimethylphosphono)-β-D-*erythro*-pentofuranosyl]thymine (33). Compound **16** (1.67 g, 5.01 mmol) was converted to the crude triflate **30** as described above. To a solution of the crude **30** in dry CH_3CN (20 mL) was added *n*-Bu_4NCN (1.36 g, 5.02 mmol), and the mixture was stirred at 25 °C for 2 h. After the solvent was evaporated, the resultant syrup was dissolved in AcOEt (50 mL) and washed with H_2O (2 × 70 mL). The organic layer was dried ($MgSO_4$), filtered, and condensed. The crude material was purified by use of column chromatography (SiO_2, AcOEt/$CHCl_3$ = 2:1) to afford **33** (0.78 g) as an oil in 45% yield: TLC R_f 0.50 (AcOEt); UV λ_{max} (EtOH) 264 nm (ϵ 9685); IR (CH_2Cl_2) 3410 (NH), 2243 (CN), 1696 cm^{-1} (2 C=O); ^1H NMR ($CDCl_3$) δ 1.83 (br s, 3 H, CH_3), 1.79–2.52 (m, 4 H, H_2C-(2′) + H_2C(5′)), 3.49 (m, 1 H, HC(3′)), 3.78 (d, J = 11 Hz, 6 H, 2 × CH_3O), 4.13 (m, 1 H, HC(4′)), 6.13 (dd, J = 3.6, 8.6 Hz, 1 H, HC(1′)), 7.42 (br, 1 H, HC(6)), 10.56 (br s, 1 H, NH). Anal. ($C_{13}H_{18}N_3O_6P$) C, H, N.

Dimethyl 3′,5′-Dideoxythymidin-2′-ene-5′-phosphonate (35). Compound **18** (2.06 g, 5.01 mmol) and LiCN (0.660 g, 20.0 mmol) were dissolved in DMF (15 mL). The mixture was stirred at 45 °C for 3 h; then it was partitioned between AcOEt (80 mL) and H_2O (100 mL). The organic layer was washed with H_2O (4 × 50 mL), dried ($MgSO_4$), filtered, and condensed. The residue was purified by use of column chromatography (SiO_2, $CHCl_3$/AcOEt = 1:1) to afford **35** (0.47 g) as an oil in 30% yield: TLC R_f 0.59 (AcOEt); UV λ_{max} (EtOH) 265 nm (ϵ 10 835); ^1H NMR ($CDCl_3$) δ 1.69 (s, 3 H, CH_3), 1.85 (m, 2 H, H_2C(5′)), 3.78 (d, J = 12 Hz, 6 H, 2 × CH_3O), 5.16 (m, 1 H, HC(4′)), 5.94 (m, 1 H, HC(2′)), 6.29 (dt, J = 1.7, 6.0 Hz, 1 H, HC(3′)), 7.01 (br s, 1 H, HC(1′)), 7.33 (br s, 1 H, HC(6)), 8.69 (br, 1 H, NH). Anal. ($C_{12}H_{17}N_2O_6P$) C, H, N.

9-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine-3′-[[2-(adenin-9-ylmethoxy)ethyl]phosphonate] (39). Collidine (0.61 g, 5.0 mmol) was added to a solution of THF (2.0 mL) containing **13** (0.27 g, 0.99 mmol) at −10 °C. To this solution was added CCl_3SO_2Cl (0.22 g, 1.0 mmol) in THF (0.50 mL) dropwise. After **38** (0.50 g, 1.0 mmol) in THF (2.0 mL) was added to the mixture, it was stirred at 25 °C for 10 h. The solvents were removed, and the residue was dissolved in AcOEt (20 mL) and washed with H_2O (20 mL). The organic layer was concentrated, and the residue was purified by use of preparative TLC with a mixture of $CHCl_3$ and MeOH (6:1) as the eluant. The band at ca. R_f 0.50 was eluted with AcOEt to afford **39** (0.41 g) in 55% yield: mp 129–131 °C; TLC R_f 0.50 ($CHCl_3$/MeOH = 6:1); UV λ_{max} (EtOH) 259 nm (ϵ 17 500); ^1H NMR (DMSO-d_6/D_2O) δ 0.15 (br s, 12 H, 2 × $(CH_3)_2Si$), 0.90, 1.15 (2 s, 18 H, 2 × $(CH_3)_3C$), 1.68 (m, 2 H, CH_2P), 3.81–4.26 (m, 4 H, CH_2O + H_2C(5′)), 4.31–4.76 (m, 3 H, HC(2′) + HC(3′) + HC(4′)), 5.61 (s, 2 H, OCH_2N), 6.57 (d, J = 4.8 Hz, 1 H, HC(1′)), 8.01, 8.12, 8.52, 8.71 (4 s, 4 H, 2 × HC(2) + 2 × HC(8)); ^{31}P NMR (DMSO-d_6) δ 29.20. Anal. ($C_{30}H_{51}N_{10}O_7PSi_2$) C, H, N.

9-(β-D-Arabinofuranosyl)adenine-3′-[[2-(adenin-9-ylmethoxy)ethyl]phosphonate] (40). To a solution of **39** (0.37 g, 0.49 mmol) in THF (3.0 mL) was added *n*-Bu_4NF (1.0 M solution in THF, 0.31 g, 1.2 mmol). Acetic acid (0.50 mL) was added to the mixture after it was stirred at 25 °C for 30 min. The solvents were removed, and the residue was purified by use of Whatman 3-mm paper with a mixture of *i*-PrOH, NH_4OH, and H_2O (9:1:2) as the eluant. The band at ca. R_f 0.42 was eluted with H_2O and collected by lyophilization to give **40** (0.20 g) in 78% yield: mp >250 °C dec; UV λ_{max} (EtOH) 258 nm (ϵ 18 000); ^1H NMR (DMSO-d_6/D_2O) δ 1.59 (m, 2 H, CH_2P), 3.78–4.12 (m, 4 H, CH_2O + H_2C(5′)), 4.28–4.75 (m, 3 H, HC(2′) + HC(3′) + HC(4′)), 5.59 (s, 2 H, OCH_2N), 6.50 (d, J = 4.3 Hz, 1 H, HC(1′)), 7.99, 8.18, 8.60, 8.80 (4 s, 4 H, 2 × HC(2) + 2 × HC(8)); ^{31}P NMR (DMSO-d_6) δ 29.25. Anal. ($C_{18}H_{23}N_{10}O_7P$) C, H, N.

9-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine-3′-[(methoxyalaninyl)[2-(adenin-9-ylmethoxy)ethyl]phosphonate] (Diastereoisomeric mixture; 41). To a solution of **39** (0.75 g, 1.0 mmol) in pyridine (6.0 mL) was added 2,4,6-triisopropylbenzenesulfonyl chloride (0.54 g, 1.8 mmol). After the mixture was stirred at 25 °C for 13 h, methyl L-alaninate (0.26 g, 2.5 mmol) in pyridine (2.0 mL) was added, and the mixture was stirred at 25 °C for 4 h. The solvent was removed, and the residue was dissolved in AcOEt (30 mL). The organic layer was washed with H_2O (2 × 30 mL), dried, and concentrated. The residue was purified by use of preparative TLC with a mixture of $CHCl_3$ and MeOH (6:1) as the eluant. The band at ca. R_f 0.69 was eluted with a mixture of AcOEt and $CHCl_3$ (2:1) to afford **41** (0.81 g) in 97% yield: TLC R_f 0.69 ($CHCl_3$/MeOH = 6:1); UV λ_{max} (EtOH) 260 nm (ϵ 17 800); ^1H NMR ($CDCl_3$/DMSO-d_6/D_2O) δ 0.16, 0.18 (2 s, 12 H, 2 × $(CH_3)_2Si$), 0.83, 1.06 (2 s, 18 H, 2 × $(CH_3)_3C$), 1.42 (d, J = 5.8 Hz, 3 H, CH_3), 1.69 (m, 2 H, CH_2P), 3.68–4.25 (m, 8 H, CH_3O + CH_2O + CH + H_2C(5′)), 4.33–4.78 (m, 3 H, HC-(2′) + HC(3′) + HC(4′)), 5.62 (s, 2 H, OCH_2N), 6.56 (d, J = 4.9 Hz, 1 H, HC(1′)), 8.06, 8.13, 8.54, 8.73 (4 s, 4 H, 2 × HC(2) + 2 × HC(8)); ^{31}P NMR (DMSO-d_6) δ 38.71, 38.80. Anal. ($C_{34}H_{58}N_{11}O_8PSi_2$) C, H, N.

9-(β-D-Arabinofuranosyl)adenine-3′-[(methoxyalaninyl)-[2-(adenin-9-ylmethoxy)ethyl]phosphonate] (Diastereoisomeric mixture; 42). Compound **42** was obtained from 41 (1.20 g, 1.43 mmol) by following the procedure for preparation of **40** from **39**. The crude material was purified by use of TLC plates and eluted with a mixture of $CHCl_3$ and MeOH (6:1). The desired product **42** (0.78 g) was isolated in 90% yield: TLC R_f 0.16 ($CHCl_3$/MeOH = 6:1); paper chromatography R_f 0.62 (*i*-PrOH/NH_4OH/H_2O = 9:1:2); UV λ_{max} (EtOH) 259 nm (ϵ 18 760); ^1H NMR (DMSO-d_6/D_2O) δ 1.40 (d, J = 5.9 Hz, 3 H, CH_3), 1.66 (m, 2 H, CH_2P), 3.70–4.10 (m, 8 H, CH_3O + CH_2O + CH + H_2C(5′)), 4.32–4.76 (m, 3 H, HC(2′) + HC(3′) + HC(4′)), 5.59 (s, 2 H, OCH_2N), 6.51 (d, J = 4.3 Hz, 1 H, HC-(1′)), 8.05, 8.14, 8.56, 8.76 (4 s, 4 H, 2 × HC(2) + 2 × HC(8)); ^{31}P NMR (DMSO-d_6) δ 38.70, 38.82. Anal. ($C_{22}H_{30}N_{11}O_8P$) C, H, N.

5′-*O*-(*tert*-Butyldimethylsilyl)thymidine (44). Compound **44** was obtained from **43** in 98% yield as reported.[27] Reagents involved were **43** (2.42 g, 10.0 mmol), *tert*-butyldimethylsilyl chloride (1.90 g, 12.6 mmol), and $AgNO_3$ (2.21 g, 13.0 mmol). **44**: mp 126–128 °C; TLC R_f 0.42 (Et_2O); UV λ_{max} (EtOH) 264 nm (ϵ 10 600); ^1H NMR ($CDCl_3$/D_2O) δ 0.17 (s, 6 H, $(CH_3)_2Si$), 1.01 (s, 9 H, $(CH_3)_3C$), 1.90 (s, 3 H, CH_3), 2.36 (dd, J = 3.0, 6.0 Hz, 2 H, H_2C(2′)), 3.71–4.69 (m, 4 H, HC(3′) + HC(4′) + H_2C(5′)), 6.22 (t, J = 3.0 Hz, 1 H, HC(1′)), 7.46 (s, 1 H, HC(6)). Anal. ($C_{16}H_{28}N_2O_5Si$) C, H, N.

(2*R*,4*S*,5*R*)-1-[4-Azidotetrahydro-5-[[[3′-*O*-[5′-*O*-(*tert*-butyldimethylsilyl)thymidinyl]]phosphinico]methyl]-2-furyl]thymine (45). Compound **45** (0.435 g, 0.650 mmol) was prepared from **20** (0.330 g, 0.996 mmol) and **44** (0.356 g, 0.999 mmol) in 65% yield by following the procedure for the preparation of **39** from **13** and **38**. **45:** mp 113–114 °C; TLC R_f 0.67 ($CHCl_3$/MeOH = 6:1); UV λ_{max} (EtOH) 264 nm (ϵ 15 200); ^1H NMR ($CDCl_3$/DMSO-d_6/D_2O) δ 0.19 (s, 6 H, $(CH_3)_2Si$), 1.03 (s, 9 H, $(CH_3)_3C$), 1.85, 1.91 (2 s, 6 H, 2 × CH_3C(5)), 1.86–2.63 (m, 6 H, H_2C(2′) + H_2C(3) + CH_2P), 3.83–4.72 (m, 6 H, H_2C(5′) + HC(4′) + HC(5) + HC(3′) + HC(4)), 6.10, 6.30 (2 t, J = 6.2 Hz, 2 H, HC(1′) + HC(2)), 7.28, 7.42 (2 s, 2 H, 2 × HC(6)); ^{31}P NMR (DMSO-d_6) δ 29.30. Anal. ($C_{26}H_{40}N_7O_{10}PSi$) C, H, N.

(2*R*,4*S*,5*R*)-1-[4-Fluorotetrahydro-5-[[[3′-*O*-[5′-*O*-(*tert*-butyldimethylsilyl)thymidinyl]]phosphinico]methyl]-2-furyl]thymine (46). Compound **46** (0.453 g, 0.700 mmol) was prepared from **32** (0.31 g, 1.0 mmol) and 44 (0.358 g, 1.00 mmol) in 70% yield according to the procedure for the synthesis of **39** from **13** and **38**. **46:** mp 108–110 °C; TLC R_f 0.68 ($CHCl_3$/MeOH = 6:1); UV λ_{max} (EtOH) 264 nm (ϵ 15 500); ^1H NMR ($CDCl_3$/DMSO-d_6/D_2O) δ 0.17 (s, 6 H, $(CH_3)_2Si$), 1.01 (s, 9 H, $(CH_3)_3C$), 1.82, 1.92 (2 s, 6 H, 2 × CH_3C(5)), 1.83–2.62 (m, 6 H, H_2C(2′) + H_2C(3) + CH_2P), 3.85–4.72 (m, 5 H, H_2C(5′) + HC(4′) + HC(5) + HC(3′)), 5.24 (m, $J_{4,F}$ = 53.0 Hz, 1 H, HC(4)), 6.12–6.38 (m, 2 H, HC(1′) + HC(2)), 7.32, 7.48 (2 s, 2 H, 2 × HC(6)); ^{31}P NMR (DMSO-d_6) δ 29.30. Anal. ($C_{26}H_{40}N_4O_{10}FPSi$) C, H, N, F.

(2*R*,4*S*,5*R*)-1-[4-Azidotetrahydro-5-[(3′-*O*-thymidinylphosphinico)methyl]-2-furyl]thymine (47). Compound **47** (0.50 g, 0.90 mmol) was prepared from **45** (0.67 g, 1.0 mmol) in 90% yield as described for the synthesis of **40** from **39**. **47**: mp 165–167 °C; TLC R_f 0.20 ($CHCl_3$/MeOH = 6:1); paper chromatography R_f 0.75 (*i*-PrOH/NH_4OH/H_2O = 9:1:2); UV λ_{max}

(EtOH) 263 nm (ϵ 16 000); ^{1}H NMR (DMSO-d_6/D_2O) δ 1.88, 1.90 (2 s, 6 H, 2 × $CH_3C(5)$), 1.87–2.60 (m, 6 H, $H_2C(2')$ + $H_2C(3)$ + CH_2P), 3.55–4.71 (m, 6 H, $H_2C(5')$ + HC(4') + HC(5) + HC(3') + HC(4)), 6.08–6.26 (br m, 2 H, HC(1') + HC(2)), 7.30, 7.50 (2 s, 2 H, 2 × HC(6)); ^{31}P NMR (DMSO-d_6) δ 29.28. Anal. ($C_{20}H_{26}N_7O_{10}P$) C, H, N.

(2*R*,4*S*,5*R*)-1-[4-Fluorotetrahydro-5-[(3'-*O*-thymidinylphosphinico)methyl]-2-furyl]thymine (48). Compound **48** (0.51 g, 0.95 mmol) was obtained from **46** (0.65 g, 1.0 mmol) in 95% yield according to the procedure for the preparation of **40** from **39**. **48:** mp 159–161 °C; TLC R_f 0.21 ($CHCl_3$/MeOH = 6:1); paper chromatography R_f 0.80 (*i*-PrOH/NH_4OH/H_2O = 9:1:2); UV λ_{max} (EtOH) 263 nm (ϵ 15 600); ^{1}H NMR (DMSO-d_6/D_2O) δ 1.85, 1.91 (2 s, 6 H, 2 × $CH_3C(5)$), 1.87–2.64 (m, 6 H, $H_2C(2')$ + $H_2C(3)$ + CH_2P), 3.56–4.70 (m, 5 H, $H_2C(5')$ + HC(4') + HC(5) + HC(3')), 5.27 (m, $J_{4,F}$ = 50.5 Hz, 1 H, HC(4)), 6.10–6.42 (br m, 2 H, HC(1') + HC(2)), 7.39, 7.57 (2 s, 2 H, 2 × HC(6)); ^{31}P NMR (DMSO-d_6) δ 29.28. Anal. ($C_{20}H_{26}N_4O_{10}FP$) C, H, N, F.

(2*R*,4*S*,5*R*)-1-[4-Azidotetrahydro-5-[[[3'-*O*-[5'-*O*-(*tert*-butyldimethylsilyl)thymidinyl]](methoxy-L-alaninyl)-phosphinylidene]methyl]-2-furyl]thymine (Diastereoisomeric mixture; 49). Compound **49** (0.72 g, 0.95 mmol) was prepared from **45** (0.67 g, 1.0 mmol) in 95% yield according to the procedure for the preparation of **41** from **39**. **49:** TLC R_f 0.89 ($CHCl_3$/MeOH = 6:1); UV λ_{max} (EtOH) 265 nm (ϵ 16 100); ^{1}H NMR ($CDCl_3$/D_2O) δ 0.18 (s, 6 H, $(CH_3)_2Si$), 1.02 (s, 9 H, $(CH_3)_3C$), 1.40 (d, J = 5.8 Hz, 3 H, CH_3), 1.80, 1.90 (2 s, 6 H, 2 × $CH_3C(5)$), 1.82–2.59 (m, 6 H, $H_2C(2')$ + $H_2C(3)$ + CH_2P), 3.82–4.71 (m, 10 H, $H_2C(5')$ + CH + CH_3O + HC(4') + HC(5) + HC(3') + HC(4)), 6.12, 6.25 (2 t, J = 6.2 Hz, 2 H, HC(1') + HC(2)), 7.30, 7.46 (2 s, 2 H, 2 × HC(6)); ^{31}P NMR (DMSO-d_6) δ 38.56, 38.70. Anal. ($C_{30}H_{47}N_8O_{11}PSi$) C, H, N.

(2*R*,4*S*,5*R*)-1-[4-Fluorotetrahydro-5-[[[3'-*O*-[5'-*O*-(*tert*-butyldimethylsilyl)thymidinyl]](methoxy-L-alaninyl)-phosphinylidene]methyl]-2-furyl]thymine (Diastereoisomeric mixture; 50). Compound **50** (0.66 g, 0.90 mmol) was obtained from **46** (0.65 g, 1.0 mmol) in 90% yield according to the procedure for the synthesis of **41** from **39**. **50:** TLC R_f 0.92 ($CHCl_3$/MeOH = 6:1); UV λ_{max} (EtOH) 265 nm (ϵ 16 000); ^{1}H NMR ($CDCl_3$/D_2O) δ 0.19 (br s, 6 H, $(CH_3)_2Si$), 1.02 (s, 9 H, $(CH_3)_3C$), 1.41 (br d, J = 5.7 Hz, 3 H, CH_3), 1.79–1.91 (2 s, 6 H, 2 × $CH_3C(5)$), 1.81–2.60 (m, 6 H, $H_2C(2')$ + $H_2C(3)$ + CH_2P), 3.84–4.73 (m, 9 H, $H_2C(5')$ + CH + CH_3O + HC(4') + HC(5) + HC(3')), 5.23 (m, $J_{4,F}$ = 54.3 Hz, 1 H, HC(4)), 6.11–6.36 (m, 2 H, HC(1') + HC(2)), 7.40, 7.51 (2 s, 2 H, 2 × HC(6)); ^{31}P NMR (DMSO-d_6) δ 38.56, 38.70. Anal. ($C_{30}H_{47}N_5O_{11}FPSi$) C, H, N, F.

(2*R*,4*S*,5*R*)-1-[4-Azidotetrahydro-5-[[[3'-*O*-[5'-*O*-(*tert*-butyldimethylsilyl)thymidinyl]](methoxy-D-alaninyl)-phosphinylidene]methyl]-2-furyl]thymine (Diastereoisomeric mixture; 51). Compound **51** (0.66 g, 0.87 mmol) was prepared from **45** (0.67 g, 1.0 mmol) in 87% yield as described for the preparation of **41** from **39**. **51:** TLC R_f 0.89 ($CHCl_3$/MeOH = 6:1); UV λ_{max} (EtOH) 265 nm (ϵ 16 150); ^{1}H NMR ($CDCl_3$/D_2O) δ 0.18 (s, 6 H, $(CH_3)_2Si$), 1.02 (s, 9 H, $(CH_3)_3C$), 1.41 (d, J = 6.0 Hz, 3 H, CH_3), 1.80, 1.91 (2 s, 6 H, 2 × $CH_3C(5)$), 1.81–2.60 (m, 6 H, $H_2C(2')$ + $H_2C(3)$ + CH_2P), 3.81–4.70 (m, 10 H, $H_2C(5')$ + CH + CH_3O + HC(4') + HC(5) + HC(3') + HC(4)), 6.13, 6.26 (2 t, J = 6.0 Hz, 2 H, HC(1') + HC(2)), 7.30, 7.46 (2 s, 2 H, 2 × HC(6)); ^{31}P NMR (DMSO-d_6) δ 38.50, 38.69. Anal. ($C_{30}H_{47}N_8O_{11}PSi$) C, H, N.

(2*R*,4*S*,5*R*)-1-[4-Fluorotetrahydro-5-[[[3'-*O*-[5'-*O*-(*tert*-butyldimethylsilyl)thymidinyl]](methoxy-D-alaninyl)-phosphinylidene]methyl]-2-furyl]thymine (Diastereoisomeric mixture; 52). Compound **52** (0.70 g, 0.95 mmol) was obtained from **46** (0.65 g, 1.0 mmol) in 95% yield as described for the synthesis of **41** from **39**. **52:** TLC R_f 0.92 ($CHCl_3$/MeOH = 6:1); UV λ_{max} (EtOH) 265 nm (ϵ 16 080); ^{1}H NMR ($CDCl_3$/D_2O) δ 0.18 (br s, 6 H, $(CH_3)_2Si$), 1.01 (s, 9 H, $(CH_3)_3C$), 1.42 (br d, J = 5.8 Hz, 3 H, CH_3), 1.78, 1.91 (2 s, 6 H, 2 × $CH_3C(5)$), 1.81–2.61 (m, 6 H, $H_2C(2')$ + $H_2C(3)$ + CH_2P), 3.85–4.74 (m, 9 H, $H_2C(5')$ + CH + CH_3O + HC(4') + HC(5) + HC(3')), 5.24 (m, $J_{4,F}$ = 54.4 Hz, 1 H, HC(4)), 6.12–6.36 (m, 2 H, HC(1') + HC(2)), 7.40, 7.50 (2 s, 2 H, 2 × HC(6)); ^{31}P NMR (DMSO-d_6) δ 38.54, 38.68. Anal. ($C_{30}H_{47}N_5O_{11}FPSi$) C, H, N, F.

(2*R*,4*S*,5*R*)-1-[4-Azidotetrahydro-5-[[(3'-*O*-thymidinyl)-(methoxy-L-alaninyl)phosphinylidene]methyl]-2-furyl]-thymine (Diastereoisomeric mixture; 53). Compound **53** was prepared from **49** (0.75 g, 0.99 mmol) as described for the synthesis of **40** from **39**. The crude material was purified by use of TLC plates and eluted with a mixture of $CHCl_3$ and MeOH (6:1). The target molecule **53** (0.56 g) was isolated in 88% yield: TLC R_f 0.59 ($CHCl_3$/MeOH = 6:1); UV λ_{max} (EtOH) 264 nm (ϵ 14 960); ^{1}H NMR (DMSO-d_6/D_2O) δ 1.40 (d, J = 6.0 Hz, 3 H, CH_3), 1.79, 1.91 (2 s, 6 H, 2 × $CH_3C(5)$), 1.80–2.58 (m, 6 H, $H_2C(2')$ + $H_2C(3)$ + CH_2P), 3.54–4.65 (m, 10 H, $H_2C(5')$ + CH + CH_3O + HC(4') + HC(5) + HC(3') + HC(4)), 6.03, 6.15 (2 t, J = 6.8 Hz, 2 H, HC(1') + HC(2)), 7.25, 7.46 (2 s, 2 H, 2 × HC(6)); ^{31}P NMR (DMSO-d_6) δ 38.52, 38.69. Anal. ($C_{24}H_{33}N_8O_{11}P$) C, H, N.

(2*R*,4*S*,5*R*)-1-[4-Fluorotetrahydro-5-[[(3'-*O*-thymidinyl)(methoxy-L-alaninyl)phosphinylidene]methyl]-2-furyl]-thymine (Diastereoisomeric mixture; 54). Compound **54** was obtained from **50** (0.732 g, 1.00 mmol) as described for the preparation of **40** from **39**. The crude material was purified by use of TLC plates and eluted with a mixture of $CHCl_3$ and MeOH (6:1). The target compound **54** (0.52 g) was isolated in 85% yield: TLC R_f 0.62 ($CHCl_3$/MeOH = 6:1); UV λ_{max} (EtOH) 264 nm (ϵ 15 000); ^{1}H NMR (DMSO-d_6/D_2O) δ 1.42 (d, J = 5.8 Hz, 3 H, CH_3), 1.80, 1.92 (2 s, 6 H, 2 × $CH_3C(5)$), 1.81–2.63 (m, 6 H, $H_2C(2')$ + $H_2C(3)$ + CH_2P), 3.52–4.69 (m, 9 H, $H_2C(5')$ + CH + CH_3O + HC(4') + HC(5) + HC(3')), 5.25 (m, $J_{4,F}$ = 52.0 Hz, 1 H, HC(4)), 6.12–6.39 (br m, 2 H, HC(1') + HC(2)), 7.35, 7.56 (2 s, 2 H, 2 × HC(6)); ^{31}P NMR (DMSO-d_6) δ 38.52, 38.69. Anal. ($C_{24}H_{33}N_5O_{11}FP$) C, H, N, F.

(2*R*,4*S*,5*R*)-1-[4-Azidotetrahydro-5-[[(3'-*O*-thymidinyl)-(methoxy-D-alaninyl)phosphinylidene]methyl]-2-furyl]-thymine (Diastereoisomeric mixture; 55). Compound **55** was prepared from **51** (0.80 g, 1.0 mmol) as described for the synthesis of **40** from **39**. It was purified as described for **53** to give pure compound **55** (0.55 g) in 86% yield: TLC R_f 0.62 ($CHCl_3$/MeOH = 6:1); UV λ_{max} (EtOH) 264 nm (ϵ 15 000); ^{1}H NMR (DMSO-d_6/D_2O) δ 1.47 (d, J = 6.0 Hz, 3 H, CH_3), 1.80, 1.91 (2 s, 6 H, 2 × $CH_3C(5)$), 1.80–2.59 (m, 6 H, $H_2C(2')$ + $H_2C(3)$ + CH_2P), 3.55–4.66 (m, 10 H, $H_2C(5')$ + CH + CH_3O + HC(4') + HC(5) + HC(3') + HC(4)), 6.05, 6.14 (2 t, J = 6.8 Hz, 2 H, HC(1') + HC(2)), 7.25, 7.45 (2 br s, 2 H, 2 × HC(6)); ^{31}P NMR (DMSO-d_6) δ 38.51, 38.68. Anal. ($C_{24}H_{33}N_8O_{11}P$) C, H, N.

(2*R*,4*S*,5*R*)-1-[4-Fluorotetrahydro-5-[[(3'-*O*-thymidinyl)(methoxy-D-alaninyl)phosphinylidene]methyl]-2-furyl]-thymine (Diastereoisomeric mixture; 56). Compound **56** was obtained from **52** (0.732 g, 1.00 mmol) as described for the preparation of **40** from **39**. It was purified as described for **54** to give pure compound **56** (0.55 g) in 90% yield: TLC R_f 0.70 ($CHCl_3$/MeOH = 6:1); UV λ_{max} (EtOH) 264 nm (ϵ 15 050); ^{1}H NMR (DMSO-d_6/D_2O) δ 1.45 (d, J = 5.9 Hz, 3 H, CH_3), 1.81, 1.92 (2 s, 6 H, 2 × $CH_3C(5)$), 1.81–2.60 (m, 6 H, $H_2C(2')$ + $H_2C(3)$ + CH_2P), 3.52–4.67 (m, 9 H, $H_2C(5')$ + CH + CH_3O + HC(4') + HC(5) + HC(3')), 5.26 (m, $J_{4,F}$ = 52.0 Hz, 1 H, HC(4)), 6.12–6.40 (br m, 2 H, HC(1') + HC(2)), 7.31, 7.52 (2 br s, 2 H, 2 × HC(6)); ^{31}P NMR (DMSO-d_6) δ 38.51, 38.68. Anal. ($C_{24}H_{33}N_5O_{11}FP$) C, H, N, F.

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