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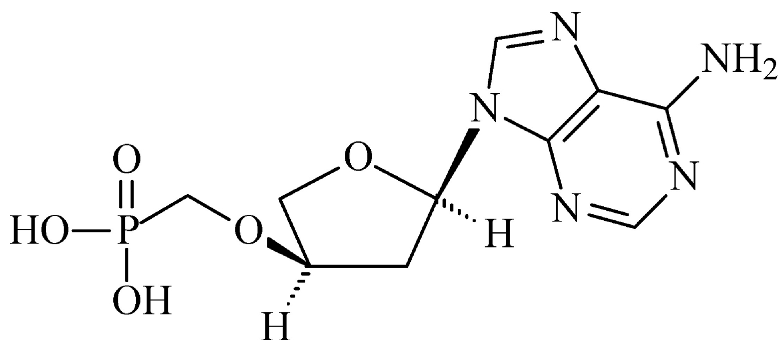

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Deoxythreosyl Phosphonate Nucleosides as Selective Anti-HIV Agents

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Abstract: Out of a series of eight new phosphonate nucleosides with an L-threose and an L-2-deoxythreose sugar moiety, two new compounds were identified (PMDTA and PMDTT) that showed potent anti-HIV-1 (HIV-2) activity [EC_{50} = 2.53 μM (PMDTA) and 6.59 μM (PMDTT)], while no cytoxicity was observed at the highest concentration tested [CC_{50} > 316 μM (PMDTA) and > 343 μM (PMDTT)]. The kinetics of incorporation of PMDTA into DNA (using the diphosphate of PMDTA as substrate and HIV-1 reverse transcriptase as catalyst) was similar to the kinetics observed for dATP, while the diphosphate of PMDTA was a very poor substrate for DNA polymerase α. The incorporated PMDTA fits very well in the active site pocket of HIV-1 reverse transcriptase.

Introduction

Pioneering work on the chemistry of phosphonate nucleosides has been carried out by A. Burger,[1,2] while the work of D. Rammler,[3,4] A. Holý,[5] and J. Moffatt[6] has led to important new reaction schemes to synthesize phosphonate nucleosides. This research has opened the way to biological applications of phosphonate nucleosides.

A first category of phosphonate nucleosides (**1**; Figure 1) are real nucleoside analogues as they contain a nucleobase and a sugar moiety.[3−6] A second series of phosphonate nucleosides represented by PMEA (**2a**; Figure 1) and PMPA (**2b**; Figure 1) can better be considered as alkylated nucleobases as the sugar moiety is replaced by an alkoxyalkyl moiety.[7]

Up to now, potent antiviral activity (HSV, CMV, HBV, HIV) has been associated with phosphonoalkoxyalkyl nucleobases and exceptionally with sugar containing phosphonate nucleosides. Several attempts to discover antiviral nucleoside phosphonates have led to synthetic schemes for the preparation of furanose,[8] pyranose,[9] and carbocyclic phosphonate nucleosides,[10] lacking, however, potent antiviral activity. Exceptions are the isosteric d_4T and d_4A analogues[11] and a 3,4-disubstituted tetrahydrofuran derived cytosine nucleoside. These compounds do not have a 4′-hydroxymethyl substituent (as in regular nucleosides), and the phosphonoalkoxy moiety is connected directly to the five-membered ring.

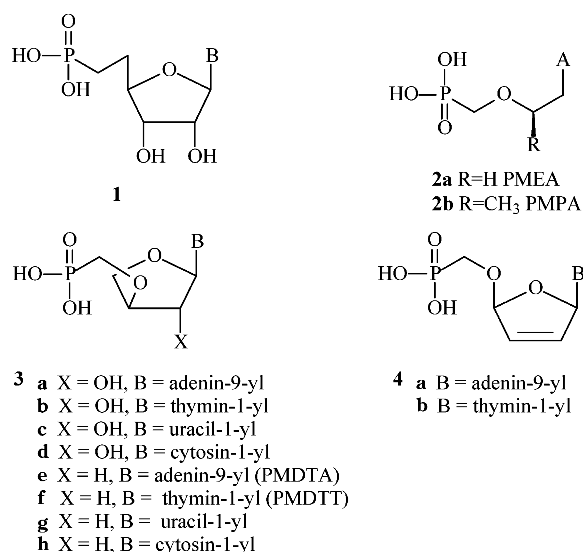

Figure 1. Structure of a phosphonate nucleoside **1**, phosphonomethoxyethyladenine (PMEA) and phosphonylmethoxypropyladenine (PMPA) **2**, threosyl nucleoside phosphonates **3**, and phosphonomethoxydihydrofuranyl nucleosides **4**.

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Phosphorylation by kinases and incorporation into nucleic acids (eventually leading to chain termination) is considered as an important mechanism to explain the antiviral activity of nucleosides. The lack of antiviral activity of nucleoside phosphonates is generally explained by their poor substrate properties for cellular and viral kinases. On the other hand, the potent antiviral activity of phosphonylated alkylated nucleobases is ascribed to their intracellular phosphorylation to their diphosphates and to a refractory incorporation of the modified nucleosides in nucleic acids.[12] The enzymatic incorporation of phosphonate nucleosides in nucleic acids is almost irreversible, which is not so for regular nucleotides. A disadvantage of the acyclic nucleoside phosphonates is their low selectivity index in cellular screening systems.[7,13] However, this drawback is compensated by their long half-life in the cell, resulting in a once a day (or even once a week) treatment schedule at low drug concentrations. The selectivity for HIV reverse transcriptase versus mitochondrial DNA polymerases of the triphosphates of anti-HIV nucleosides is an important factor determining in vivo toxicity.[14] In search for potent and selective anti-HIV nucleoside phosphonates, we decided to synthesize and evaluate nucleosides with a threose sugar moiety and a phosphonalkoxy substituent (**3a**−**h**).

Threose nucleosides have been previously synthesized because they can be assembled from natural precursor molecules.[15] It has been demonstrated that threose nucleic acids (TNA) form duplexes with DNA and RNA of thermal stability, similar to that of the natural nucleic acid association.[15] Triphosphates of threose nucleosides are accepted as substrates by several polymerases, and they can be enzymatically incorporated into DNA.[16,17] These nucleosides are accepted as substitutes for ribonucleosides in the catalytic site of a hammerhead ribozyme, although the catalytic efficiency of the ribozyme is significantly reduced.[18]

The phosphonoalkoxy group of the proposed threose nucleoside phosphonates **3a**−**h** (Figure 1) is bound at the 3′-position, bringing the phosphorus atom and the nucleobase closer to each other than in previously synthesized nucleoside phosphonates where the phosphonate group is bound to the primary hydroxyl group of the nucleoside.[8h] The presence of an anomeric center in threose nucleoside phosphonates gives them stereoelectronic properties similar to those of natural nucleosides. The phosphonate group is stable against enzymatic degradation (hydrolysis), and the absence of a hydroxymethyl substituent in the 4′-position of the proposed molecules **3a**−**h** (Figure 1) may avoid steric hindrance during enzymatic phosphorylation reaction (to obtain the diphosphate). The structure of the proposed compounds resembles somewhat the structure of the 5-phosphonomethoxy-2,5-dihydrofuranyl nucleosides **4a** and **4b** (Figure 1), previously synthesized by Kim et al.,[11] and of the apiose family of nucleoside analogues.[19]

Here, we describe the synthesis and selective anti-HIV activity of two series of threosyl nucleoside phosphonates, abbreviated as PMT (phosphonomethoxythreosyl) and PMDT (phosphonomethoxydeoxythreosyl), with an adenine, a thymine, a uracil, and a cytosine base moiety.

Chemistry

The nucleosides **3a**−**h** were synthesized starting from (*R*,*S*)-2,3-dihydroxy-dihydrofuran-1-one (**5**) (Scheme 1).[15] The hydroxyl group at C-2 of compound **5** can be selectively protected with a TBDMS group. The free hydroxyl group of **6** is then protected by benzoylation, and the lactone is reduced to the hemiketal using Dibal-H in THF. The anomeric hydroxyl group is protected with a TBDMS group, and the *O*-benzoyl group is removed with ammonia in methanol. At the stage of **9**, the phosphonate function is introduced using the triflate of diisopropylphosphonomethyl alcohol and NaH in THF. The two silyl protecting groups of **10** are removed and replaced by benzoyl protecting groups. The presence of a 2-*O*-benzoyl group allows selective introduction of the base moiety in the α-configuration. The nucleobases uracil, thymine, and N^4-acetylcytosine are introduced after silylation and using $SnCl_4$ as Lewis catalyst. Deprotection of **12**−**15** is done in two steps, first, removal of the benzoyl protecting groups with ammonia in methanol (yielding **16**−**19**) and, second, hydrolysis of the diisopropyl protecting groups with (TMS)Br at room temperature (giving **3a**−**d**). To obtain the 2′-deoxygenated analogues, the 2′-OH group of **16**−**18** is removed by Barton deoxygenation,[20,21] giving **20**−**22**. Compound **23** is obtained from **22**. Hydrolysis of the phosphonate ester function of **20** was carried out with (TMS)-Br at room temperature. However, for compounds **21**−**23**, (TMS)Br rapidly cleaved the nucleobase from the sugar even at 0 °C. For this reason, (TMS)I was used for hydrolysis of the diisopropyl esters of **21**−**23**. After purification by silica gel chromatography, Sephadex-DEAE A-25 resin and Dowex-sodium ion-exchange resin, nucleoside phosphonate acids **3e**−**h** were obtained.

The most active congener **3e** was converted to its diphosphate **24** to be able to study its interaction with reverse transcriptase and DNA polymerase α. Therefore, the amino group of PMDTA (**3e**, as triethylammonium salt) was protected with a dimethylaminomethylene group, and the free hydroxyl group was reacted with pyrophosphate in the presence of carbonyldiimidazole (Scheme 2). Deprotection with ammonia yielded the diphosphate **24**, which was isolated by ion-exchange chromatography and reversed-phase HPLC.

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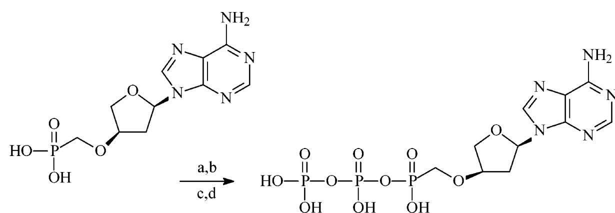
Scheme 1 [a]

[a] Conditions and reagents: (a) (TBDMS)Cl, imidazole, MeCN; (b) BzCl, pyridine; (c) Dibal-H, THF; (d) saturated NH_3 in MeOH; (e) trifluoromethanesulfonate of diisopropylphosphonylmethanol, NaH, THF; (f) TFA/H_2O; (g) $SnCl_4$, MeCN; (h) (1) PhOC(S)Cl, DMAP, MeCN; (2) Bu_3SnH, AIBN; (i) (1) $P(O)Cl_3$, 1,2,4-triazole, DCM; (2) NH_3; (j) (1) (TMS)Br, DCM; (2) Sephadex-DEAE, Dowex-Na^+; (k) (1) (TMS)I, DCM; (2) Sephadex-DEAE, Dowex-Na^+.

Scheme 2 [a]



[a] Conditions and reagents: (a) dimethylformamide dimethyl acetal, DMF, room temperature, 16 h; (b) $(Im)_2CO$, DMF, 12 h, dibutylammonium pyrophosphate, DMF, 2 h; (c) NH_3, H_2O, 1 h; (d) DEAE cellulose, LiChroprep C-18.

Antiviral Activity

Compounds **3a**−**h** were evaluated for their potential to inhibit the replication of HIV in a cell culture model for acute infection (Table 1). The cytotoxicity of the compounds was determined in parallel. The origin of the HIV-1 (IIIB) virus stock[22] and the HIV-2 (ROD)[23] stock has been described. They were obtained from the culture supernatant of HIV-1- or HIV-2-infected MT-4 cells, respectively. The inhibitory effect of the compounds on HIV-1 and HIV-2 replication were monitored by measuring the viability of MT-4 cells 5 days after infection.[24] The cytotoxicity of the compounds was determined in parallel by measuring the viability of mock-infected cells on day 5, using a tetrazolium-

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Table 1. Anti-HIV-1 Activity and Cytotoxicity of the Phosphonate Nucleosides **3a**−**h**

compd (base)	EC_{50}[a] (μM)	CC_{50}[b] (μM)	SI
PMTA, **3a**	>268	>268	
PMTT, **3b**	>328	>328	
PMTU, **3c**	>155	155	<1
PMTC, **3d**	>308	>308	
PMDTA, **3e**	2.53	>316	>125
PMDTT, **3f**	6.59	>343	>51
PMDTU, **3g**	>336	>336	
PMDTC, **3h**	>321	>321	
PMEA	5.50 (a)	246	44.9
	14.00 (b)	246	17.6
PMPA	3.35 (a)	>350	>104
	3.48 (b)	>350	>100

[a] EC_{50} = 50% effective concentration, or concentration required to protect 50% of the cells against viral cytopathicity [HIV-1(III_B), HIV-2(ROD)] in MT-4 cells. For PMEA and PMPA, (a) data for HIV-1, (b) data for HIV-2. [b] CC_{50} = 50% cytotoxic concentration, or concentration reducing the number of viable cells by 50%.

based colorimetric method to determine the number of viable cells.

PMDTA showed an EC_{50} value of 2.53 μM against both HIV-1 and HIV-2. PMDTT had an EC_{50} value of 6.59 μM against HIV-1 and HIV-2. No cytotoxicity was observed for PMDTA or PMDTT at the highest concentration tested (316 and 343 μM, respectively), giving the compounds an SI of >125 (PMDTA) and >51 (PMDTT) in these cellular systems. In this cellular test system, the anti-HIV activity of both compounds is in the same range as the activity of PMEA and PMPA, while no cellular toxicity has been observed (Table 1).

Incorporation of PMDTA into DNA Using Reverse Transcriptase

The antiviral activity of phosphonate nucleosides is mostly explained by their intracellular metabolism to their diphosphates followed by incorporation into the viral genome and chain termination.[7] We used a primer/extension assay to compare the ability of the HIV reverse transcriptase and the human DNA polymerase α to accept PMDTApp as a substrate in comparison to dATP. This should give us an idea about the selectivity of the anti-HIV compound since the human DNA polymerase α is mainly involved in replication of the nuclear genome while the HIV reverse transcriptase plays a key role in the replication of the viral genome. The incorporation studies were done with a DNA template and a DNA primer, as DNA polymerase α and reverse transcriptase are both able to synthesize double-stranded DNA (although reverse transcriptase is also able to synthesize a DNA strand using RNA as template). Both enzymes were able to extend a DNA primer with the phosphonate nucleotide, but the modified nucleotide was only a very poor substrate for the human DNA polymerase α. Only a high enzyme concentration (0.4 U/μL) resulted in the incorporation of PMDTApp, a concentration more than 100 times higher than the concentration used to incorporate the natural dATP substrate (data not shown).

The HIV reverse transcriptase, on the other hand, accepted PMDTApp as easily as the natural building block. These results from primer/extension assays were confirmed by our kinetic data. Using a steady-state method, values for K_m and K_{cat} were determined for dATP and PMDTApp insertion opposite a single T in the DNA template (Table 2). These data show for the

Table 2. Kinetic Parameters for dATP and PMDTApp Incorporation into a DNA Hybrid for HIV Reverse Transcriptase[a]

```
P1  5'  CAGGAAACAGCTATGAC       3'  + dATP or PMDTApp
                                     ——→
T1  3'  GTCCTTTGTCGATACTGTCCCC  5'
```

	K_m (μM)	K_{cat} (min^{-1})	K_{cat}/K_m (min^{-1} M^{-1})
dATP	0.10 ± 0.018	0.66 ± 0.019	6.6
PMDTApp	0.29 ± 0.026	0.79 ± 0.016	2.72

[a] For the assay conditions, see the Experimental Section.

incorporation of PMDTApp by the HIV reverse transcriptase a small increase in the K_m value, but also a slight increase in the k_{cat} value, compared to those for dATP. This indicates that, although the affinity of the enzyme for the phosphonate nucleotide might be a little lower, the overall catalytic efficiency differs only by a factor of 2.5.

Since PMDTApp was such a poor substrate for the DNA polymerase α, kinetic parameters could not be determined under steady-state conditions.

Now that we proved that PMDTA can be incorporated into DNA, functioning as a chain terminator, a model was built to analyze the interactions between the incorporated nucleotide and reverse transcriptase. Therefore, the adenine phosphonate nucleoside was built at the 3′-end of the primer and paired with a thymidine nucleotide in the template strand (Figure 2). This model revealed that the sugar ring is puckered in the C3′-endo conformation. Hydrophobic interactions between the phosphonate nucleotide and reverse transcriptase are occurring at Leu74, Tyr115, and Gln151, while no steric hindrance with Met184 is expected to occur during translocation. This model visualizes the experimental results of the incorporation study of PMDTA into DNA using the reverse transcriptase.

Conclusion

A new series of phosphonate nucleosides have been discovered with a deoxythreosyl sugar moiety. The compounds with a thymine and adenine base moiety showed potent anti-HIV-1,2 activity while showing no cellular toxicity at the highest concentration tested. These data demonstrate that considerable improvement in the anti-HIV phosphonate field is an attainable goal. The diphosphate of the adenine congener is an efficient substrate for HIV-1 reverse transcriptase and a very poor substrate for human DNA polymerase α. A 100-fold higher DNA polymerase α concentration was needed to observe incorporation of PMDTA into DNA. This makes PMDTA a good candidate for further evaluation as an anti-HIV-1 compound. It also shows that the TNA-scaffold[15] is a useful handle to develop new antiviral compounds.

Experimental Section

For all reactions, analytical grade solvents were used. All moisture-sensitive reactions were carried out in oven-dried glassware (135 °C) under a nitrogen atmosphere. Anhydrous THF was refluxed over sodium/benzophenone and distilled. A Varian Unity 500 MHz spectrometer and a 200 MHz Varian Gemini apparatus were used for ^1H NMR and ^{13}C NMR. Exact mass measurements were performed on a quadrupole time-of-flight mass spectrometer (Q-Tof-2, Micromass,

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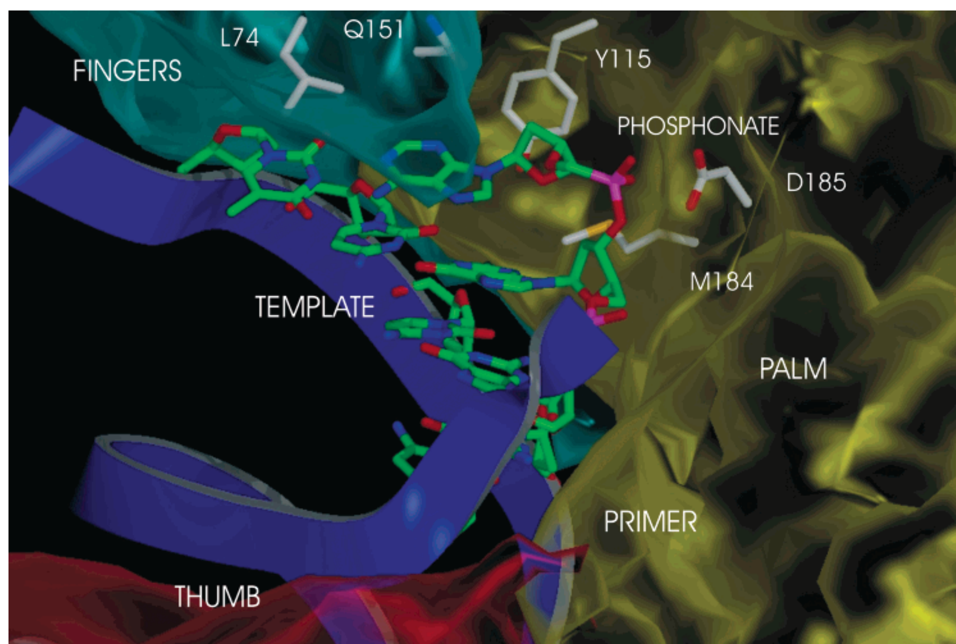

Figure 2. Close-up of the adenine phosphonate molecule (at the right) built in at the 3′-side of the primer and pairing with base T5 of the template (at the left). The reverse transcriptase domains are colored as blue (fingers, A2−A88, A121−A146), yellow (palm, A89−A120, A147−A242), red (thumb, A234−A311), and white (rest, A312−A539, B6−B433). The picture was generated using Bobscript, Molscript, and Raster3d.[25−27]

Manchester, U.K.) equipped with a standard electrospray-ionization (ESI) interface; samples were infused in *i*-PrOH/H_2O (1:1) at 3 μL/min. Precoated aluminum sheets (Fluka Silica gel/TLC-cards, 254 nm) were used for TLC; the spots were examined with UV light. Column chromatography was performed on ICN silica gel 63-200, 60 Å. For sake of clarity, NMR signals of sugar protons and carbons are indicated with a prime, and signals of base protons and carbons are given without a prime.

2-*O*-Tertbutyldimethylsilyl-L-threosolactone (6). To a solution of (*R*,*S*)-2,3-dihydroxydihydrofuran-1-one (**5**) (10.8 g, 92 mmol) and imidazole (12.5 g, 184 mmol) in 250 mL of MeCN was added (TBDMS)Cl (16.6 g, 110 mmol) at 0 °C in one portion. The reaction mixture was slowly warmed to room temperature and stirred overnight. The reaction mixture was concentrated. The residue was partitioned between H_2O and EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (*n*-hexane:EtOAC = 6:1) to afford **6** (15.2 g, 65.4 mmol, 71%) as a colorless solid: mp 99−100 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ_H 0.12 (s, 6H, $SiCH_3$), 0.90 (s, 9H, CH_3), 3.86 (dd, J_1 = 6.96 Hz, J_2 = 7.70 Hz, 1H, C(4′)H_a), 4.11−4.36 (m, 3H, OH, C(3′)H, C(4′)H_b), 5.82 (d, J = 5.13 Hz, 1H, C(2′)H); ^{13}C NMR (200 MHz, DMSO-d_6) δ_C −4.93 ($SiCH_3$), 17.99 ($C(CH_3)_3$), 25.61 ($C(CH_3)_3$), 69.62 (C-4′), 72.62 (C-2′), 74.59 (C-3′), 174.60 (C-1′); exact mass calcd for $C_{10}H_{20}O_4Si_1Na_1$ $[M + Na]^+$ 255.1028, found 255.1010.

2-*O*-Tertbutyldimethylsilyl-3-*O*-benzoyl-L-threosolactone (7). To a solution of **6** (18.00 g, 77.5 mmol) in 200 mL of pyridine was added dropwise BzCl (11.2 mL, 96.9 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was concentrated and coevaporated with 20 mL of toluene two times in vacuo. The residue was partitioned between H_2O (100 mL) and EtOAc (350 mL). The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (*n*-hexane:EtOAc = 8:1) to afford **7** (25.9 g, 77.0 mmol) as a colorless solid in 99% yield: mp 52−53 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 0.14 (d, J_1 = 13.2 Hz, 6H, $SiCH_3$), 0.87 (s, 9H, CH_3), 4.23 (dd, J_1 = 6.8 Hz, J_2 = 9.3 Hz, 1H, C(4′)H_a), 4.68 (dd, J_1 = 7.3 Hz, J_2 = 9.3 Hz, 1H, C(4′)H_b), 4.96 (d, J = 6.8 Hz, 1H, C(2′)H), 5.48 (dd, J_1 = 7.3 Hz, J_2 = 13.0 Hz, 1H, C(3′)H), 7.57−8.01 (m, 5H, Ar H); ^{13}C NMR (500 MHz, DMSO-d_6) δ_C −5.16 ($SiCH_3$), −4.84 ($SiCH_3$), 17.84 ($C(CH_3)_3$), 25.42 ($C(CH_3)_3$), 67.20 (C-4′), 71.67 (C-2′), 75.46 (C-3′), 128.65 (aroma-C), 128.90 (aroma-C), 129.36 (aroma-C), 133.94 (aroma-C), 165.08 (Bz CO), 172.76 (C-1′); exact mass calcd for $C_{17}H_{25}O_5Si_1$ $[M + H]^+$ 337.1471, found 337.1465.

2-*O*-Tertbutyldimethylsilyl-3-*O*-benzoyl-L-threose (8). To a solution of **7** (10.0 g, 29.7 mmol) in 100 mL of dry THF was slowly added dropwise 1.0 M diisopropyl aluminum hydride (37.1 mL, 37.1 mmol) in toluene at −78 °C. The reaction mixture was stirred at −78 °C, and as soon as the starting material was completely consumed (TLC, 4−10 h), methanol (10 mL) was added over a period of 5 min to quench the reaction. The cooling bath was removed, 100 mL of a saturated aqueous sodium potassium tartrate solution and 200 mL of EtOAc were added, and the mixture was stirred vigorously for 3 h. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (*n*-hexane:EtOAc = 8:1) to afford **8** (7.40 g, 21.8 mmol) as a colorless solid in 73% yield: mp 70−71 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ_H 0.10 (s, 6H, $SiCH_3$), 0.87 (s, 9H, CH_3), 3.93 (dd, J_1 = 9.89 Hz, J_2 = 3.66 Hz, 1H, C(4′)H_a), 4.16 (br s, 1H, OH), 4.24 (dd, J_1 = 10.26 Hz, J_2 = 5.86 Hz, 1H, C(4′)H_b), 5.02−5.07 (m, 2H, C(2′)H, C(3′)H), 6.54 (d, J = 4.76 Hz, 1H, C(1′)H), 7.51−8.00 (m, 5H, Ar H); ^{13}C NMR (200 MHz, DMSO-d_6) δ_C −7.39 ($SiCH_3$), −7.30 ($SiCH_3$), 15.41 ($C(CH_3)_3$), 23.27 ($C(CH_3)_3$), 67.06 (C-4′), 77.14 (C-2′), 78.87 (C-3′), 100.23 (C-1′), 126.58 (aroma-C), 127.09 (aroma-C), 131.40 (aroma-C), 163.18 (Bz CO); exact mass calcd for $C_{17}H_{26}O_5Si_1Na_1$ $[M + Na]^+$ 361.1447, found 361.1452.

1α,2-Di-*O*-tertbutyldimethylsilyl-L-threose (9a) and 1*β*,2-Di-*O*-tertbutyldimethylsilyl-L-threose (9b). To a solution of **8** (7.30 g, 21.6 mmol) and imidazole (2.94 g, 43.1 mmol) in 100 mL of MeCN was added (TBDMS)Cl (0.98 g, 23.8 mmol) at 0 °C in one portion. The reaction mixture was slowly warmed to room temperature and stirred overnight. The reaction mixture was concentrated. The residue was partitioned between H_2O and EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in MeOH saturated with ammonia (100 mL), and the reaction mixture was stirred at room temperature overnight. The mixture was concentrated, and the residue was purified by column

chromatography (*n*-hexane:EtOAc = 20:1, 10:1) to give compound **9a** (3.15 g, 9.06 mmol) as a colorless oil in 42% yield and **9b** (1.43 g, 4.10 mmol) as a colorless oil in 19% yield. The stereochemistry at position 1′ is tentatively assigned using ^{1}H NMR. The compound with the small coupling constant of H-1′ (J = 1.1 Hz) was characterized as **9a**, while the compound with a coupling constant of 3.6 Hz was characterized as **9b**. Data for **9a**: ^{1}H NMR (200 MHz, DMSO-d_6) δ_H 0.06−0.08 (m, 12H, SiCH$_3$), 0.87 (s, 18H, CH$_3$), 3.59−3.65 (m, 1H, C(2′)H), 3.87−3.99 (m, 3H, C(4′), C(3′), C(4′)H$_b$), 5.00 (d, J = 1.1 Hz, 1H C(1′)H), 5.07−5.10 (m, 1H, OH); ^{13}C NMR (200 MHz, DMSO-d_6) δ_C −5.14 (SiCH$_3$), −4.92 (SiCH$_3$), −4.65 (SiCH$_3$), −4.38 (SiCH$_3$), 17.66 (C(*C*H$_3$)$_3$), 17.81 (C(*C*H$_3$)$_3$), 25.61 (C(*C*H$_3$)$_3$), 25.73 (C(*C*H$_3$)$_3$), 71.92 (C-4′), 76.66 (C-2′), 85.58 (C-3′), 103.91 (C-1′); exact mass calcd for $C_{16}H_{36}O_4Si_2Na_1$ 371.2050, found 371.2059. Data for **9b**: ^{1}H NMR (200 MHz, DMSO-d_6) δ_H 0.05 (s, 6H, SiCH$_3$), 0.06 (d, J_1 = 5.2 Hz, 6H, SiCH$_3$), 0.86 (s, 9H, CH$_3$), 0.87 (s, 9H, CH$_3$), 3.41 (dd, J_1 = 8.0 Hz, J_2 = 3.7 Hz, 1H, C(2′)H); 3.81 (dd, J_1 = 5.2 Hz, J_2 = 3.7 Hz, C(3′)H), 3.94−4.07 (m, 2H, C(4′)H$_a$, C(4′)H$_b$), 5.12−5.15 (m, 2H, OH, C(1′)H); ^{1}H NMR 200 MHz (DMSO-d_6 + 1D D$_2$O) δ_H 0.02 (s, 6H, SiCH$_3$), 0.04 (d, J_2 = 4.4 Hz, 6H, SiCH$_3$), 0.84 (s, 18H, CH$_3$), 3.39 (dd, J_1 = 7.7 Hz, J_2 = 3.6 Hz, 1H, C(2′)H); 3.79 (dd, J_1 = 4.4 Hz, J_2 = 4.4 Hz, 1H, C(3′)H), 3.92−4.07 (m, 2H, C(4′)H$_a$ C(4′)H$_b$) 5.10 (d, 1H, J_2 = 3.6 Hz, C(1′)H); ^{13}C NMR (200 MHz, DMSO-d_6) δ_C −4.95 (SiCH$_3$), −4.74 (SiCH$_3$), −4.67 (SiCH$_3$), 17.45 (*C*(CH$_3$)$_3$), 25.64 (C(*C*H$_3$)$_3$), 25.79 (C(*C*H$_3$)$_3$), 70.80 (C-4′), 74.17 (C-2′), 79.45 (C-3′), 97.11 (C-1′); exact mass calcd for $C_{16}H_{36}O_4Si_2Na_1$ 371.2050, found 371.2052.

1α,2-Di-*O*-tertbutyldimethylsilyl-3-*O*-(diisopropylphosphonomethyl)-L-threose (10a) and 1*β*,2-Di-*O*-tertbutyldimethylsilyl-3-*O*-(diisopropylphosphonomethyl)-L-threose (10b). To a solution of **9a** (3.41 g, 9.8 mmol) in dried THF (25 mL) was added sodium hydride (80% dispersion in mineral oil, 0.56 mg, 19.6 mmol) at −78 °C. Then the solution of the triflate of diisopropylphosphonomethanol (5.80 g, 19.6 mmol) in dried THF (10 mL) was dropwise added, and the reaction mixture was slowly warmed to room temperature. The reaction was quenched with saturated NaHCO$_3$ and concentrated. The residue was partitioned between H$_2$O and EtOAc. The organic layer was washed with water and brine, dried over Na$_2$SO$_4$, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (*n*-hexane:EtOAC = 2:1) to afford **10a** (4.75 g, 9.0 mmol, 92%) as a colorless oil: ^{1}H NMR (200 MHz, DMSO-d_6) δ_H 0.06−0.10 (m, 12H, SiCH$_3$), 0.86 (s, 9H, C(CH$_3$)$_3$), 0.87 (s, 9H, C(CH$_3$)$_3$), 1.22−1.26 (m, 12H, C(CH$_3$)$_2$), 3.75 (d, J = 9.2 Hz, 2H, CH$_2$), 3.78 (d, J = 9.2 Hz, 1H, C(4′)H$_a$), 3.88−3.95 (m, 1H, C(3′)H), 3.99 (s, 1H, C(2′)H), 4.10 (dd, J_1 = 9.2 Hz, J_2 = 8.6 Hz, 1H, C(4′)H$_b$), 4.52−4.68 (m, 2H, CH), 5.02(s, 1H, C(1′)H); ^{13}C NMR (200 MHz, DMSO-d_6) δ_C −4.92 (SiCH$_3$), −4.80 (SiCH$_3$), −4.40 (SiCH$_3$), −4.26 (SiCH$_3$), 17.90 (SiC), 23.94 (OCH(*C*H$_3$)$_2$), 25.73 (C(*C*H$_3$)$_3$), 64.07 (d, $J_{P,C}$ = 165.5 Hz, PCH$_2$), 69.71 (C-4′), 70.43 (POCH), 83.12 (C-2′), 87.03 (d, $J_{P,C}$ = 12.0 Hz, C-3′), 103.8 (C-1′); exact mass calcd for $C_{23}H_{52}O_7P_1Si_2$ [M + H]$^+$ 527.2989, found 527.2988.

The synthesis of **10b** started from **9b** (2.00 g, 5.7 mmol), followed the same procedure as for the synthesis of **10a** from **9a**, and offered **10b** (2.70 g, 5.1 mmol, 90%) as a colorless oil: ^{1}H NMR (200 MHz, CDCl$_3$) δ_H 0.08−0.11 (m, 12H, SiCH$_3$), 0.93 (br s, 18H, C(CH$_3$)$_3$), 1.33 (d, J = 6.2 Hz, 12H, C(CH$_3$)$_2$), 3.66−3.94 (m, 3H, C(4′)H$_a$, PCH$_2$), 4.02−4.22 (m, 3H, C(2′)H, C(3′)H, C(4′)H$_b$), 4.67−4.83 (m, 2H, C*H*(CH$_3$)$_2$), 5.13 (d, J = 3.7 Hz, 1H, C(1′)H); ^{1}H NMR (200 MHz, DMSO-d_6) δ_H 0.06−0.93 (m, 12H, SiCH$_3$), 0.87 (s, 18H, C(CH$_3$)$_3$), 1.22−1.26 (m, 12H, C(CH$_3$)$_2$), 3.58−3.65 (m, 1H, C(4′)H$_a$), 3.78 (d, J = 9.2 Hz, PCH$_2$), 3.96−4.08 (m, 3H, C(2′)H, C(3′)H, C(4′)H$_b$), 4.51−4.67 (m, 2H, C*H*(CH$_3$)$_2$), 5.15 (d, J = 3.7 Hz, 1H, C(1′)H); ^{13}C NMR (200 MHz, DMSO-d_6) δ_C −5.22 (SiCH$_3$), −5.07 (SiCH$_3$), −4.58 (SiCH$_3$), 17.88 (*C*(CH$_3$)$_3$), 23.98 (OCH(*C*H$_3$)$_2$), 25.62 (C(*C*H$_3$)$_3$), 25.71 (C(*C*H$_3$)$_3$), 65.12 (d, $J_{P,C}$ = 173.6 Hz, PCH$_2$), 68.38 (C-4′), 70.87 (O*C*H-(CH$_3$)$_2$), 70.96 (O*C*H(CH$_3$)$_2$), 78.88 (C-2′), 85.68 (d, $J_{P,C}$ = 12.0 Hz, C-3′), 97.3 (C-1′); exact mass calcd for $C_{23}H_{52}O_7P_1Si_2$ [M + H]$^+$ 527.2989, found 527.2972.

1α,2-*O*-Benzoyl-3-*O*-(diisopropylphosphonomethyl)-L-threose (11a) and 1*β*,2-*O*-Benzoyl-3-*O*-(diisopropylphosphonomethyl)-L-threose (11b). A solution of **10a** (4.25 g, 8.1 mmol) in TFA−H$_2$O (3:1, 20 mL) was allowed to stand at room temperature for 2 h. The reaction mixture was neutralized with saturated NaHCO$_3$ solution. Then the mixture was partitioned between DCM (400 mL) and water (20 mL). The organic layer was washed with water and brine, dried over Na$_2$SO$_4$, and then concentrated in vacuo. The residue was purified by chromatography on silica gel (DCM:MeOH = 20:1) to give 3-*O*-diisopropylphosphonomethyl-L-threose (2.20 g, 7.3 mmol) as a colorless amorphous solid in 92% yield. To the solution of 3-*O*-(diisopropylphosphonomethyl)-L-threose (687 mg, 2.3 mmol) in 100 mL of pyridine was added dropwise BzCl (0.67 mL, 5.8 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was concentrated and coevaporated with 20 mL of toluene two times in vacuo. The residue was partitioned between H$_2$O (20 mL) and EtOAc (150 mL). The organic layer was washed with water and brine, dried over Na$_2$SO$_4$, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (*n*-hexane:EtOAc = 1:1) to afford **11a** and **11b** (1.0 g, 2.0 mmol) as a colorless oil in 87% yield. The H-1′ proton of **11a** appeared as a singlet, while the H-1′ proton of **11b** appeared as a doublet (J = 4.4 Hz). Data for **11a**: ^{1}H NMR (200 MHz, DMSO-d_6) δ_H 1.20−1.26 (m, 12H, C(CH$_3$)$_2$), 3.40−4.11 (m, 3H, PCH$_2$, C(4′)H$_a$), 4.40−4.54 (m, 2H, C(3′)H, C(4′)H$_b$), 4.56−4.71 (m, 2H, OC*H*(CH$_3$)$_2$), 5.51 (s, 1H, C(2′)H), 6.47 (s, 1H, C(1′)H), 7.43−8.07 (m, 10H, Ar H); ^{13}C NMR (200 MHz, DMSO-d_6) δ_C 23.82 (CH$_3$), 64.45 (d, J = 155.4 Hz, PCH$_2$), 70.59 (*C*H-(CH$_3$)), 73.23 (C-4′), 80.12 (C-2′), 80.30 (C-3′), 99.78 (C-1′), 129.04 (aroma-C), 129.83 (aroma-C), 134.14 (aroma-C), 164.61 (Bz CO), 165.07 (Bz CO); exact mass calcd for $C_{25}H_{31}O_9P_1Na_1$ [M + Na]$^+$ 529.1603, found 529.1601. Data for **11b**: ^{1}H NMR (200 MHz, CDCl$_3$) δ_H 1.30−1.36 (m, 12H, C(CH$_3$)$_2$), 3.84 (dAB, J = 13.9 Hz, $J_{P,H}$ = 8.8 Hz, 1H, PCH$_a$), 3.96 (dAB, J = 13.9 Hz, $J_{P,H}$ = 8.8 Hz, PCH$_b$), 4.09 (dd, J = 3.5 and 9.9 Hz, 1H, C(4′)H$_a$), 4.43 (dd, J = 6.2 and 9.9 Hz, 1H, C(4′)H$_b$), 4.67−4.86 (m, 3H, C(3′)H, POCH), 5.56 (t, J = 4.4, 1H, C(2′)H), 6.76 (d, J = 4.4, 1H, C(1′)H), 7.31−7.61 (m, 6H, Bz H), 7.89−8.02 (m, 4H, Bz H); ^{13}C NMR (200 MHz, CDCl$_3$) δ_C 23.94 (CH$_3$), 64.66 (d, J = 169.4 Hz, PCH$_2$), 70.72 (C-4′), 71.42 (POCH), 82.83 (C-3′), 95.67 (C-1′) 128.39 (aroma-C), 128.51 (aroma-C), 128.90 (aroma-C), 129.51 (aroma-C), 129.78 (aroma-C), 133.39 (Bz CO), 133.61 (Bz CO); C-2′ was hidden by the peak of CDCl$_3$; exact mass calcd for $C_{25}H_{31}O_9PNa$ [M + Na]$^+$ 529.1603, found 529.1594.

1-(N^6-Benzoyladenin-9-yl)-2-*O*-benzoyl-3-*O*-(diisopropylphosphonomethyl)-L-threose (12). To a mixture of **11a** and **11b** (425 mg, 0.83 mmol) and silylated N^6-benzoyladenine (401 mg, 1.6 mmol) in dry MeCN (30 mL) was dropwise added SnCl$_4$ (0.3 mL, 2.5 mmol) under N$_2$ at room temperature. The reaction mixture was stirred at room temperature for 4−5 h. Then the reaction was quenched with saturated NaHCO$_3$ and concentrated. The residue was partitioned between H$_2$O (20 mL) and EtOAc (100 mL). The organic layer was washed with water and brine, dried over Na$_2$SO$_4$, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (DCM: MeOH = 40:1) to afford **12** (431 mg, 0.69 mmol) as a colorless amorphous solid in 83% yield: ^{1}H NMR (500 MHz, CDCl$_3$) δ_H 1.31−1.36 (m, 12H, CH$_3$), 3.94 (dd, J_1 = 14.0 Hz, J_2 = 8.6 Hz, 1H, PCH$_a$), 4.01 (dd, J_1 = 14.0 Hz, J_2 = 8.6 Hz, 1H, PCH$_b$), 4.38 (dd, J_1 = 11.0 Hz, J_2 = 4.6 Hz, 1H, C(4′)H$_a$), 4.50−4.52 (m, 2H, C(3′)H, C(4′)H$_b$), 4.73−4.80 (m, 2H, OCH), 5.08 (s, 1H, C(2′)H), 6.56 (s, 1H, C(1′)H), 7.48−7.65 (m, 6H, Ar H), 8.02−8.08 (m, 4H, Ar H), 8.50 (s, 1H, A C(8)H), 8.82 (s, 1H, A C(2)H), 9.07 (br s, 1H, NH); ^{13}C NMR (500 MHz, CDCl$_3$) δ_C 23.97 (CH$_3$), 24.01 (CH$_3$), 24.03 (CH$_3$), 24.06 (CH$_3$), 65.36 (d, $J_{P,C}$ = 168.9 Hz, PCH$_2$), 71.45 (POCH), 71.51(POCH), 73.55-(C-4′), 80.27 (C-2′), 83.74 ($J_{P,C}$ = 9.8 Hz, C-3′), 87.86 (C-1′), 122.72 (A C(5)), 127.80 (aroma-C), 128.65 (aroma-C), 128.67 (aroma-C),

128.86 (aroma-C), 129.93 (aroma-C), 132.31 (aroma-C), 133.99 (aroma-C), 141.98 (A C(8)), 149.45 (A C(6), 151.59 (A C(4)), 152.93 (A C(2)), 164.44 (OBz CO), 165.17 (NBz CO); exact mass calcd for $C_{30}H_{35}N_5O_8P_1$ $[M + H]^+$ 624.2223, found 624.2222.

1-(Thymin-1-yl)-2-*O*-benzoyl-3-*O*-(diisopropylphosphonomethyl)-L-threose (13). Thymine (0.34 g, 2.7 mmol), ammonia sulfate (10 mg, 0.07 mmol), and 6 mL of HMDS were added to a dried flask. The mixture was refluxed overnight under nitrogen. HDMS was removed in vacuo. To the flask with the residue was added the solution of compound **11a**,**b** (0.92 g, 1.8 mmol) in 10 mL of dry MeCN followed by dropwise addition of $SnCl_4$ (640 μL 5.4 mmol) under N_2 at room temperature. The reaction mixture was stirred for 4 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and concentrated to a small volume. The residue was partitioned between H_2O (30 mL) and EtOAc (150 mL). The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (*n*-hexane:EtOAc = 1:1) to afford **13** (0.76 g, 1.4 mmol) as a colorless amorphous solid in 78% yield: ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.35 (d, J = 6.2 Hz, 12H, CH_3), 1.99 (d, J = 1.5 Hz, 3H, T CH_3), 3.86−4.05 (m, 2H, PCH_2), 4.11−4.16 (m, 1H, C(4′)H_a), 4.26 (br t, 1H, C(3′)H), 4.40 (d, J = 10.6 Hz, 1H, C(4′)H_b), 4.70−4.86 (m, 2H, OC*H*(CH_3)$_2$), 5.38 (s, 1H, C(2′)H), 6.29 (t, J = 2.2 Hz, 1H, C(1′)H), 7.43−7.66 (m, 4H, Ar H, T C(6)H), 8.02−8.07 (m, 2H, Ar H), 9.13 (s, 1H, NH); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 12.42 (T CH_3), 23.83 (CH(*C*H_3)$_3$), 23.92 (CH(*C*H_3)$_3$), 64.48 (d, $J_{P,C}$ = 168.5 Hz, PCH_2), 71.29 (*C*H(CH_3)$_3$), 71.45 (*C*H(CH_3)$_3$), 72.72 (C-4′), 80.28 (C-2′), 83.70 ($J_{P,C}$ = 10.6 Hz, C-3′), 89.02 (C-1′), 111.39 (T C(5)), 128.60 (aroma-C), 129.90 (aroma-C), 133.84 (T C(6)), 136.12 (aroma-C), 150.42 (T C(2)), 163.86 (T C(4)), 165.32 (Bz CO); exact mass calcd for $C_{23}H_{31}N_2O_9P_1$ $[M + H]^+$ 511.1845, found 511.1831.

1-(Uracil-1-yl)-2-*O*-benzoyl-3-*O*-(diisopropylphosphonomethyl)-L-threose (14). Uracil (0.81 g, 7.2 mmol), ammonia sulfate (10 mg, 0.07 mmol), and 20 mL of HMDS were added to a dried flask. The mixture was refluxed overnight under nitrogen. HDMS was removed in vacuo. To the residue was added the solution of compound **11a**,**b** (2.43 g, 4.8 mmol) in 50 mL of dry MeCN followed by a dropwise addition of $SnCl_4$ (1.7 mL, 14.4 mmol). The reaction mixture was stirred for 4 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and concentrated to a small volume. The residue was partitioned between H_2O (30 mL) and EtOAc (100 mL). The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (DCM:MeOH = 25:1) to afford **14** (2.09 g, 4.2 mmol) as a colorless amorphous solid in 84%: ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.23−1.26 (m, 12H, CH_3), 3.97 (d, J = 9.0 Hz, 2H, PCH_2), 4.16 (dd, J_1 = 10.7 Hz, J_2 = 4.2 Hz, 1H, C(4′)H_a), 4.36 (d, J = 10.7 Hz, 1H, C(4′)H_b), 4.39−4.40 (m, 1H, C(3′)H), 4.58−4.64 (m, 2H, OC*H*(CH_3)$_2$), 5.41 (s, 1H, C(2′)H), 5.61 (d, J = 8.1 Hz, 1H, U C(5)H), 6.02 (d, J = 2.0 Hz, 1H, C(1′)H), 7.55−7.60 (m, 2H, Ar H), 7.63 (d, J = 8.1 Hz, 1H, U C(5)H), 7.70−7.73 (m, 1H, Ar H), 8.02−8.04 (m, 2H, Ar H), 11.4 (s, 1H, NH); ^{13}C NMR (500 MHz, DMSO-d_6) δ_C 23.74 (CH(*C*H_3)$_3$), 23.84 (CH(*C*H_3)$_3$), 63.10 (d, $J_{P,C}$ = 168.5 Hz, PCH_2), 70.53 (*C*H(CH_3)$_3$), 72.32 (C-4′), 79.83 (C-2′), 82.78 (C-3′), 89.06 (C-1′), 101.91 (U C(5)), 128.95 (aroma-C), 129.63 (aroma-C), 134.07 (aroma-C), 140.72 (U C(6)), 150.39 (U C(2)), 163.19 (U C(4)), 164.73 (Bz CO); exact mass calcd for $C_{22}H_{29}N_2O_9P_1Na_1$ $[M + Na]^+$ 519.1508, found 519.1506.

1-(N^4-Acetylcytosin-1-yl)-2-*O*-benzoyl-3-*O*-(diisopropylphosphonomethyl)-L-threose (15). N^4-Acetylcytosine (0.41 g, 2.7 mmol), ammonia sulfate (10 mg, 0.07 mmol), and 6 mL of HMDS were added to a dried flask. The mixture was refluxed overnight under nitrogen. HDMS was removed in vacuo. To the residue was added a solution of compound **11a,b** (0.92 g, 1.8 mmol) in 10 mL of dry MeCN followed by a dropwise addition of stannic chloride (640 μL 5.4 mmol). The reaction mixture was stirred for 4 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and concentrated to a small volume. The residue was partitioned between H_2O (30 mL) and EtOAc (150 mL). The organic layer was washed with water and brine, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (*n*-hexane:EtOAc = 2:1) to afford **15** (0.51 g, 0.94 mmol) as a colorless amorphous solid in 52% yield: ^{1}H NMR (200 MHz, DMSO-d_6) δ_H 1.17−1.23 (m, 12H, CH(C*H*$_3$)$_2$), 2.10 (s, 3H, CH_3), 3.80−4.00 (m, 2H, PCH_2), 4.24−4.36 (m, 2H, C(4′)H_a, C(3′)H), 4.48−4.63 (m, 3H, C(4′)H_b, OC*H*(CH_3)$_2$), 5.44 (s, 1H, C(2′)H), 6.04 (s, 1H, C(1′)H), 7.27 (d, J = 7.7 Hz, 1H, C C(5)H), 7.54−7.77 (m, 3H, Ar H), 8.03−8.07 (m, 3H, Ar_o H, C C(6)H), 10.95 (s, 1H, NH); ^{13}C NMR (200 MHz, DMSO-d_6) δ_C 23.76 (CH(*C*H_3)$_3$), 24.39 (Ac CH_3), 63.77 (d, $J_{P,C}$ = 166.4 Hz, PCH_2), 70.44 (*C*H(CH_3)$_3$), 70.59 (*C*H(CH_3)$_3$), 73.56 (C-4′), 79.75 (C-3′) 82.83 (d, $J_{P,C}$ = 13.7 Hz,C-3′), 90.74 (C-1′), 94.74 (C C(5)), 128.86 (aroma-C), 129.14 (aroma-C), 134.07 (aroma-C), 129.77 (aroma-C), 134.23 (aroma-C), 145.40 (C C(6)), 154.69 (C C(2)), 162.95 (Bz CO), 164.77 (C C(4)), 171.26 (Ac CO); exact mass calcd for $C_{24}H_{33}N_3O_9P_1$ $[M + H]^+$ 538.1954, found 538.1956.

1-(Adenin-9-yl)-3-*O*-(diisopropylphosphonomethyl)-L-threose (16). A solution of **12** (431 mg, 0.80 mmol) in MeOH saturated with ammonia (100 mL) was stirred at room temperature overnight. The mixture was concentrated, and the residue was purified by column chromatography (CH_2Cl_2:MeOH = 9:1) to give compound **16** (278 mg, 0.67 mmol) as a white powder in 84% yield: ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.21−1.26 (m, 12H, CH_3), 3.85−3.94 (m, 2H, PCH_2), 4.10−4.13 (m, 2H, C(4′)H_a, C(3′)H), 4.24−4.27 (m, 1H, C(4′)H_b), 4.57−4.63 (m, 3H, C*H*(CH_3), C(2′)H), 5.93 (d, J = 2.1 Hz, 1H, C(1′)H), 6.05 (br s, 1H, OH), 7.24 (s, 2H, NH_2), 8.15 (s, 1H, C(2)H), 8.18 (s, 1H, C(8)H); ^{13}C NMR (200 MHz, DMSO-d_6): δ_C 23.82 (CH_3), 63.5 ($J_{P,C}$ = 164.6 Hz, PCH_2), 70.41 (OCH), 70.53 (OCH), 71.65 (C-4′), 78.27 (C-2′), 85.62 ($J_{P,C}$ = 13.6 Hz, C-3′), 89.53 (C-1′), 118.79 (A C(5)), 139.39 (A C(8)), 149.47 (A C(6)), 152.90 (A C(4)), 156.24 (A C(2)); exact mass calcd for $C_{16}H_{27}N_5O_6P_1$ $[M + H]^+$ 416.1699, found 416.1681.

1-(Thymin-1-yl)-3-*O*-(diisopropylphosphonomethyl)-L-threose (17). A solution of **13** (715 mg, 1.7 mmol) in MeOH saturated with ammonia (100 mL) was stirred at room temperature overnight. The mixture was concentrated, and the residue was purified by column chromatography (CH_2Cl_2:MeOH = 10:1) to give compound **17** (515 mg, 1.2 mmol) as a white powder in 71% yield: ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.27−1.33 (m, 12H, CH_3), 1.93 (d, J = 1.7 Hz, 3H, T CH_3), 3.75 (d, J = 8.8 Hz, 2H, PCH_2), 4.13 (br t, 1H, C(3′)H), 4.24−4.31 (m, 2H, C(4′)H_2), 4.38 (s, 1H, C(2′)H), 4.61−4.80 (m, 2H, OC*H*(CH_3)$_2$), 5.81 (s, 1H, C(1′)H), 7.41 (d, J = 1.46 Hz, 1H, T C(6)H), 10.27 (br s, 1H, NH); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 2.42 (T CH_3), 23.89 (CH(*C*H_3)$_3$), 64.46 (d, $J_{P,C}$ = 168.5 Hz, PCH_2), 71.26 (*C*H(CH_3)$_3$), 73.54 (C-4′), 78.94 (C-2′), 85.33 (d, $J_{P,C}$ = 10.6 Hz, C-3′), 93.12 (C-1′), 110.12 (T C(5)), 136.40 (T C(6)), 151.08 (T C(2)), 164.56 (T C(4)); exact mass calcd for $C_{16}H_{28}N_2O_8P_1$ $[M + H]^+$ 407.1583, found 407.1568.

1-(Uracil-1-yl)-3-*O*-(diisopropylphosphonomethyl)-L-threose (18). A solution of **14** (2.03 g, 4.0 mmol) in MeOH saturated with ammonia (300 mL) was stirred at room temperature overnight. The mixture was concentrated, and the residue was purified by column chromatography (CH_2Cl_2:MeOH = 20:1) to give compound **18** (1.52 g, 3.8 mmol) as a white powder in 96% yield: ^{1}H NMR (200 MHz, DMSO-d_6) δ_H 1.19−1.25 (m, 12H, CH_3), 3.78 (dd, J_1 = 13.9 Hz, J_2 = 9.2 Hz, 1H, PCH_a) 3.85 (dd, J_1 = 13.9 Hz, J_2 = 9.2 Hz, 1H, PCH_b), 3.98−4.28 (m, 4H, C(2′)H, C(3′)H, C(4′)H_2), 4.50−4.66 (m, 2H, C*H*(CH_3), 5.50 (d, J = 8.0 Hz, 1H, U C(5)H), 5.66 (d, J = 1.5 Hz, 1H, OH), 5.93 (d, J = 4.4 Hz, 1H, C(1′)H), 7.54(d, J = 8.0 Hz, 1H, U C(6)H); ^{13}C NMR (200 MHz, DMSO-d_6) δ_c 23.70 (CH(*C*H_3)$_3$), 23.79 (CH(*C*H_3)$_3$), 63.29 (d, $J_{P,C}$ = 166.3 Hz, PCH_2), 70.34 (*C*H(CH_3)$_3$), 70.47 (*C*H(CH_3)$_3$), 72.29 (C-4′), 77.84 (C-2′), 85.23 ($J_{P,C}$ = 10.7 Hz, C-3′), 91.68 (C-1′), 101.12 (U C(5)), 141.12 (U C(6)), 150.72 (U C(2)), 163.46 (U C(4)); exact mass calcd for $C_{15}H_{26}N_2O_8P_1$ $[M + H]^+$ 393.1427, found 393.1425.

1-(Cytosin-1-yl)-3-*O*-(diisopropylphosphonomethyl)-L-threose (19). A solution of **15** (450 mg, 0.84 mmol) in MeOH saturated with

ammonia (100 mL) was stirred at room temperature overnight. The mixture was concentrated, and the residue was purified by column chromatography (CH_2Cl_2:MeOH = 20:1) to give compound **19** (281 mg, 0.72 mmol) as a white powder in 86% yield. ^{1}H NMR (200 MHz, DMSO-d_6) δ_H 1.18−1.25 (m, 12H, CH_3), 3.72 (dd, J_1 = 13.6 Hz, J_2 = 8.8 Hz, 1H, PCH_a) 3.84 (dd, J_1 = 13.6 Hz, J_2 = 8.8 Hz, 1H, PCH_b), 3.95−4.05 (m, 3H, C(2′)H, C(3′)H, C(4′)H_a), 4.25 (d, J = 9.5 Hz, 1H, C(4′)H_b), 4.48−4.64 (m, 2H, C*H*(CH_3), 5.65 (d, J = 7.6 Hz, 1H, C C(5)H), 5.70 (d, J = 1.5 Hz, 1H, OH), 5.85 (d, J = 15.4 Hz, 1H, C(1′)H), 7.04 (br s, 1H, N_aH), 7.14(br s, 1H, N_bH), 7.50 (d, J = 7.6 Hz, 1H, C C(6)H); ^{13}C NMR (200 MHz, DMSO-d_6) δ_C 23.68 (CH-(*C*H_3)$_3$), 23.78 (CH(*C*H_3)$_3$), 64.46 (d, $J_{P,C}$ = 164.8 Hz, PCH_2), 70.30 (*C*H(CH_3)$_3$), 70.42 (*C*H(CH_3)$_3$), 72.00 (C-4′), 78.19 (C-2′), 85.66 (d, $J_{P,C}$ = 12.2 Hz, C-3′), 92.30 (C-1′), 93.46 (C C(5)), 141.63 (C C(6)), 155.47 (C C(2)), 165.94 (C C(4)); exact mass calcd for $C_{15}H_{27}N_3O_7P_1$ [M + H]$^+$ 392.1586, found 392.1577.

1-(Adenin-9-yl)-2-deoxy-3-*O*-(diisopropylphosphonomethyl)-L-threose (20). To a solution of phenyl(chloro)thiocarbonate (0.25 mL, 1.8 mmol) and DMAP (426 mg, 3.5 mmol) in dried MeCN (25 mL) was added compound **16** (483 mg, 1.2 mmol) at room temperature. The reaction mixture was stirred for 12 h. The mixture was concentrated, and the residue was purified by column chromatography (CH_2Cl_2:MeOH = 10:1) to give 1-(adenin-9-yl)-2-*O*-phenoxythiocarbonyl-3-*O*-diisopropylphosphonomethyl-L-threose as a colorless oil. To a solution of 1-(adenin-9-yl)-2-*O*-phenoxythiocarbonyl-3-*O*-diisopropylphosphonomethyl-L-threose in dried toluene (50 mL) were added tributytin hydride (339 μL, 1.2 mmol) and AIBN (48 mg, 0.3 mmol). The reaction mixture was refluxed for 6 h and concentrated in vacuo. The residue was purified by column chromatography (CH_2Cl_2:MeOH = 10:1) to give compound **20** (110 mg, 0.27 mmol) as a colorless oil in 23% yield: ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.27−1.34 (m, 12H, CH_3), 2.54−2.75 (m, 2H, C(2′)H_2), 3.62−3.82 (m, 2H, PCH_2), 4.04 (dd, J_1 = 10.3 Hz, J_2 = 4.0 Hz, 1H, C(4′)H_a), 4.35 (d, J = 10.3 Hz, 1H, C(4′)H_b), 4.43−4.48 (m, 1H, C(3′)H), 4.62−4.84 (m, 2H, OC*H*(CH_3)$_2$), 6.21 (br s, 2H, NH_2), 6.47 (dd, J_1 = 7.2 Hz, J_2 = 2.7 Hz, 1H, C(1′)H), 8.31(s, 1H, A C(2)H), 8.33(s, 1H, A C(8)H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.89 (CH(*C*H_3)$_3$), 38.05 (C-2′), 64.10 (d, $J_{P,C}$ = 169.4 Hz, PCH_2), 71.31 (*C*H(CH_3)$_3$), 71.46 (*C*H(CH_3)$_3$), 73.68 (C-4′), 80.49 (d, $J_{P,C}$ = 10.7 Hz, C-3′), 83.42 (C-1′), 119.50 (A C(5)), 136.63 (A C(8)), 149.73 (A C(6)), 153.07 (A C(4)), 155.62 (A C(2)); exact mass calcd for $C_{16}H_{27}N_5O_5P_1$ [M + H]$^+$ 400.1750, found 400.1740.

1-(Thymin-1-yl)-2-deoxy-3-*O*-(diisopropylphosphonomethyl)-L-threose (21). This compound was prepared as described for **20**, using **17** (450 mg, 1.1 mmol) as starting material. Column chromatographic purification (CH_2Cl_2:MeOH = 10:1) gave compound **21** (275 mg, 0.70 mmol) as a colorless oil in 64% yield: ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.31 (s, 6H, CH_3), 1.34 (s, 6H, CH_3), 1.97 (d, J = 1.1 Hz, 3H, T CH_3), 2.16 (d, J = 15.0 Hz, 1H, C(2′)H_a), 2.46−2.62 (m, 1H, C(2′)-H_b), 3.72(d, J = 9.2 Hz, 2H, PCH_2), 3.84 (dd, J_1 = 10.6 Hz, J_2 = 3.7 Hz, 1H, C(4′)H_a), 4.29−4.37(m, 2H, C(4′)H_b, C(3′)H), 4.66−4.84 (m, 2H, OC*H*(CH_3)$_2$), 6.24 (dd, J_1 = 8.0 Hz, J_2 = 2.6 Hz, 1H, C(1′)H), 7.55 (d, J = 1.1 Hz, 1H, T C(6)H), 8.48 (s, 1H, NH); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 12.45 (T CH_3), 23.92 (CH(CH_3)$_3$), 38.27 (C-2′), 63.99 (d, J = 169.2 Hz, PCH_2), 71.26 (CH(CH_3)$_3$, 73.36 (C-4′), 80.23 (d, J = 10.5 Hz, C-3′), 84.83 (C-1′), 110.72 (T C(5)), 136.55 (T C(6)), 150.57 (T C(2)), 163.80 (T C(4)); exact mass calcd for $C_{16}H_{27}N_2O_7P_1Na_1$ [M + Na]$^+$ 413.1454, found 413.1447.

1-(Uracil-1-yl)-2-deoxy-3-*O*-(diisopropylphosphonomethyl)-L-threose (22). This compound was prepared as described for **20**, using **18** (1.1 g, 2.8 mmol) as starting material. Column chromatographic purification (CH_2Cl_2:MeOH = 40:1) gave compound **22** (500 mg, 1.3 mmol) as a colorless oil in 46% yield: ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.29−1.34 (m, 12H, CH_3), 2.21 (d, J = 15.4, 1H, C(2′)H_a), 2.44−2.60 (m, 1H, C(2′)H_b), 3.69 (d, J = 9.2 Hz, 2H, PCH_2), 3.86 (dd, J_1 = 10.6 Hz, J_2 = 3.3 Hz, 1H, C(4′)H_a), 4.30−4.38 (m, 2H, C(4′)H_b, C(3′)H), 4.65−4.81 (m, 2H, OC*H*(CH_3)$_2$), 5.74 (d, J = 8.1 Hz, 1H, U C(5)H), 6.21 (dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 1H, C(1′)H), 7.71 (d, J = 8.0 Hz, 1H, U C(6)H), 9.16 (s, 1H, NH); ^{13}C NMR (200 MHz, $CDCl_3$) δ_P 23.98 (CH(*C*H_3)$_3$)), 38.42 (C-2′), 63.86 (d, $J_{P,C}$ = 170.7 Hz, PCH_2), 71.26 (*C*H(CH_3)$_3$), 71.36 (*C*H(CH_3)$_3$), 73.94 (C-4′), 80.11 (d, $J_{P,C}$ = 11.2 Hz, C-3′), 85.44 (C-1′), 101.95 (U C(5)), 140.92 (U C(6)), 150.63 (U C(2)), 163.47 (U C(4)); exact mass calcd for $C_{15}H_{26}N_2O_7P_1$ [M + H]$^+$ 377.1478, found 377.1479.

1-(Cytosin-1-yl)-2-deoxy-3-*O*-(diisopropylphosphonomethyl)-L-threose (23). To a solution of 1,2,4-triazole (662 mg, 9.6 mmol) in 15 mL of pyridine was added phosphorus oxychloride (223 μL, 2.4 mmol) at room temperature. The mixture was stirred for 10 min. Then the solution of **22** (289 mg, 0.80 mmol) was added to the mixture. The reaction mixture was stirred for 4 h. Then ammonia gas was bubbled into the reaction mixture for 1−3 h, and the reaction mixture was concentrated in vacuo. The residue was purified by column chromatography (CH_2Cl_2:MeOH = 12:1) to give compound **23** (220 mg, 0.58 mmol) as a colorless foam in 73% yield: ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.22−1.30 (m, 12H, CH_3), 2.27 (d, J = 15.0, 1H, C(2′)H_a), 2.41−2.55 (m, 1H, C(2′)H_b), 3.63 (d, J = 9.5 Hz, 2H, PCH_2), 3.91 (dd, J_1 = 10.3, J_2 = 3.5, 1H, C(4′)H_a), 4.22−4.36 (m, 2H, C(4′)H_b, C(3′)H), 4.56−4.76 (m, 2H, OC*H*(CH_3)$_2$), 5.77 (d, J = 7.3 Hz, 1H, C C(5)H), 6.17 (dd, J_1 = 7.3, J_2 = 1.8, 1H, C(1′)H), 7.67 (d, J = 7.3 Hz, 1H, C C(6)H), 8.18 (s, 2H, NH_2); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.80 (CH(*C*H_3)$_3$), 38.46 (C-2′), 63.66 (d, $J_{P,C}$ = 172.2 Hz, PCH_2), 71.48 (*C*H-(CH_3)$_3$), 71.60 (*C*H(CH_3)$_3$), 71.75 (*C*H(CH_3)$_3$), 74.12 (C-4′), 80.40 (d, $J_{P,C}$ = 11.2 Hz, C-3′), 86.68 (C-1′), 94.21 (C C(5)), 141.9 (C C(6)), 156.58 (C C(2)), 165.83 (C C(1)); exact mass calcd for $C_{15}H_{26}N_3O_6P_1$-Na_1 [M + H]$^+$ 376.1637, found 376.1638.

1-(Adenin-9-yl)-3-*O*-(phosphonomethyl)-L-threose Sodium Salt (3a). To a solution of **16** (220 mg, 0.55 mmol) and Et_3N (1 mL) in DCM (9 mL) was added bromotrimethylsilane (290 μL, 2.2 mmol) at room temperature. The reaction mixture was stirred for 48 h. The reaction was quenched with 1.0 M TEAB solution. The mixture was concentrated, and the residue was purified by column chromatography (CH_2Cl_2:MeOH = 2:1, 1:1, 1:2) to give the crude title compound. Purification using Sephadex-DEAE A-25 with gradient TEAB solution from 0.01 to 0.5 M and ion exchanges by the Dowex-Na+ resin offered **3a** (96 mg, 0.25 mmol) as a colorless solid after precipitation from diethyl ether in 45% yield: ^{1}H NMR (500 MHz, D_2O) δ_H 3.54−3.62 (m, 2H, PCH_2), 4.32−4.39 (m, 3H, C(4′)H_2, C(3′)H), 4.82 (dd, J_1 = 2.4 Hz, J_2 = 2.0 Hz, 1H, C(2′)H), 6.09 (d, J = 2.4 Hz, 1H, C(1′)H), 8.23 (s, 1H, A C(8)H), 8.45 (s, 1H, A C(2)H); ^{13}C NMR (500 MHz, D_2O) δ_C 70.1 (d, $J_{P,C}$ = 164.6 Hz, PCH_2), 75.38 (C-4′), 80.70 (C-2′), 87.56 ($J_{P,C}$ = 9.8 Hz, C-3′), 91.93 (C-1′), 121.21 (A C(5)), 143.74 (A C(8)), 151.49 (A C(6)), 155.48 (A C(4)), 158.30 (A C(2)); ^{31}P NMR (500 MHz, D_2O) δ_P 13.64; exact mass calcd for $C_{10}H_{13}N_5O_6P_1$ [M − H]$^-$ 330.0603, found 330.0602.

1-(Thymin-1-yl)-3-*O*-(phosphonomethyl)-L-threose Sodium Salt (3b). This compound was prepared as described for **3a**, using **17** (220 mg, 0.58 mmol) as starting material. Compound **3b** (90 mg, 0.24 mmol) was obtained as a colorless solid after precipitation from diethyl ether in 42% yield: ^{1}H NMR (500 MHz, D_2O) δ_H 1.89 (s, 3H, T CH_3), 3.60−3.68 (m, 2H, PCH_2), 4.16 (d, J = 4.1 Hz, 1H, C(3′)H), 4.24 (dd, J_1 = 10.7 Hz, J_2 = 4.1 Hz, 1H, C(4′)H_a), 4.42 (d, J = 10.7 Hz, 1H, C(4′)-H_b), 4.45 (s, 1H, C(2′)H), 5.85 (d, J = 1.2 Hz,1H, C(1′)H), 7.59−7.60 (m, 1H, T C(6)H); ^{13}C NMR (500 MHz, D_2O) δ_C 14.28 (T CH_3), 67.95 (d, $J_{P,C}$ = 157.2 Hz, PCH_2), 75.78 (C-4′), 80.17 (C-2′), 87.27 (d, $J_{P,C}$ = 11.7 Hz, C-3′), 94.22 (C-1′), 113.36 (T C(5)), 140.66 (T C(6)), 154.30 (T C(2)), 169.39 (T C(4)); ^{31}P NMR (500 MHz, D_2O) δ_P 15.68; exact mass calcd for $C_{10}H_{14}N_2O_8P_1$ [M − H]$^-$ 321.0488, found 321.0474.

1-(Uracil-1-yl)-3-*O*-(phosphonomethyl)-L-threose Sodium Salt (3c). This compound was prepared as described for **3a**, using **18** (200 mg, 0.53 mmol) as starting material and (TBMS)Br (200 mL, 2.1 mmol). Compound **3c** (93 mg, 0.26 mmol) was obtained as a colorless solid after precipitation from diethyl ether in 49% yield: ^{1}H NMR (500 MHz, D_2O) δ_H 3.58−3.67 (m, 2H, PCH_2), 4.16 (d, J = 3.3 Hz, 1H,

C(3′)H), 4.26 (dd, J_1 = 10.7 Hz, J_2 = 3.9 Hz, 1H, C(4′)H_a), 4.45 (d, J = 10.7 Hz, 1H, C(4′)H_b), 4.47 (s, 1H, C(2′)H), 5.85 (d, J = 8.0 Hz, 1H, U C(5)H), 5.85 (s, 1H, C(1′)H), 7.80 (d, J = 8.1 Hz, 1H, U C(6)H); ^{13}C NMR (500 MHz, D_2O) δ_C 67.98 (d, J = 156.2 Hz, PCH_2), 76.22 (C-4′), 80.09 (C-2′), 87.15 (d, J = 11.7 Hz, C-3′), 94.63 (C-1′), 104.09 (U C(5)), 145.23 (U C(6)), 154.26 (U C(2)), 169.22 (U C(4)); ^{31}P NMR (500 MHz, D_2O) δ 15.37; exact mass calcd for $C_9H_{12}N_2O_8P_1$ [M − H]$^-$ 307.0331, found 307.0325.

1-(Cytosin-1-yl)-3-*O*-(phosphonomethyl)-L-threose Sodium Salt (3d). This compound was prepared as described for **3a**, using **19** (150 mg, 0.38 mmol) as starting material. Compound **3d** (58 mg, 0.16 mmol) was obtained as a colorless solid after precipitation from diethyl ether in 43% yield: ^{1}H NMR (500 MHz, D_2O) δ_H 3.53−3.62 (m, 2H, PCH_2), 4.15 (d, J = 3.7 Hz, 1H, C(3′)H), 4.27 (dd, J_1 = 10.7 Hz, J_2 = 3.7 Hz, 1H, C(4′)H_a), 4.42 (s, 1H, C(2′)H), 4.44 (d, J = 10.7 Hz, 1H, C(4′)H_b), 5.86 (s, 1H, C(1′)H), 6.01 (d, J = 7.6 Hz, 1H, C C(5)H), 7.77 (d, J = 7.6 Hz, 1H, C C(6)H); ^{13}C NMR (500 MHz, D_2O) δ_C 68.0 (d, $J_{P,C}$ = 156.2 Hz, PCH_2), 76.17 (C-4′), 80.13 (C-2′), 87.27 (d, $J_{P,C}$ = 11.8 Hz, C-3′), 95.16 (C-1′), 98.23 (C C(5)), 145.04 (C C(6)), 160.06 (C C(2)), 168.84 (C C(4)); ^{31}P NMR (500 MHz, D_2O) δ_P 15.28; exact mass calcd for $C_9H_{13}N_3O_7P_1$ [M − H]$^-$ 306.0491, found 306.0481.

1-(Adenin-1-yl)-2-deoxy-3-*O*-(phosphonomethyl)-L-threose Sodium Salt (3e). This compound was prepared as described for **3a**, using **20** (70 mg, 0.23 mmol) as starting material. Compound **3e** (38 mg, 0.11 mmol) was obtained as a colorless solid after precipitation from diethyl ether in 43% yield: ^{1}H NMR (500 MHz, D_2O, 60 °C) δ_H 2.63- (dd, J_1 = 15.5 Hz, J_2 = 1.3 Hz, 1H, C(2′)H_a), 2.75−2.81 (m, 1H, C(2′)H_b), 3.55−3.64 (m, 2H, PCH_2), 4.09 (dd, J_1 = 10.0 Hz, J_2 = 4.0 Hz, 1H, C(4′)H_a), 4.33 (d, J = 10.0 Hz, 1H, C(4′)H_b), 4.51 (dd, J_1 = 5.5 Hz, J_2 = 4.5 Hz, 1H, C(3′)H), 6.39 (dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 1H, C(1′)H), 8.22 (s, 1H, A C(2)H), 8.49 (s, 1H, A C(8)H); ^{13}C NMR (500 MHz, D_2O) δ_C 39.78 (C-2′), 68.34 (d, $J_{P,C}$ = 155.2 Hz, PCH_2), 76.53 (C-4′), 82.79 (d, $J_{P,C}$ = 11.9 Hz, C-3′), 86.32 (C-1′), 121.13 (A C(5)), 143.89 (A C(8)), 151.33 (A C(6)), 155.25 (A C(4)), 158.18 (A C(2)); ^{31}P NMR (500 MHz, D_2O) δ_P 154.46; exact mass calcd for $C_{10}H_{13}N_5O_5P_1$ [M − H]$^-$ 314.0654, found 314.0632. Anal. Calcd for $C_{10}H_{12}N_5Na_2O_5P$·$2H_2O$: C, 30.39; H, 4.08; N, 17.72. Found: C, 30.39; H, 4.77; N, 17.42.

1-(Thymin-1-yl)-2-deoxy-3-*O*-(phosphonomethyl)-L-threose Sodium Salt (3f). To a solution of **21** (260 mg, 0.67 mmol) and Et_3N (1 mL) in DCM (25 mL) was added iodotrimethylsilane (0.73 mL, 5.36 mmol) at 0 °C. The reaction mixture was stirred for 2 h. The reaction was quenched with 1.0 M TEAB solution. The mixture was concentrated, and the residue was purified by column chromatography (CH_2Cl_2:MeOH = 2:1, 1:1, 1:2) to give crude **3f**. Purification using Sephadex-DEAE A-25 with gradient TEAB solution from 0.01 to 0.5 M and ion exchanges by the Dowex-Na^+ resin offered **3f** (95 mg, 0.27 mmol) as a colorless solid after precipitation from diethyl ether in 40% yield: ^{1}H NMR (500 MHz, D_2O) δ_H 1.91 (s, 3H, T CH_3), 2.29 (d, J = 15.4 Hz, 1H, C(2′)H_a), 2.58−2.64 (m, 1H, C(2′)H_a), 3.57−3.65 (m, 2H, PCH_2), 3.95 (dd, J_1 = 10.5 Hz, J_2 = 3.4 Hz, 1H, C(4′)H_a), 4.38−4.41 (m, 2H, C(4′)H_b, C(3′)H), 6.20 (dd, J_1 = 8.3 Hz, J_2 = 2.4 Hz, 1H, C(1′)H), 7.78 (d, J = 1.0 Hz, 1H, T C(6)H); ^{13}C NMR (500 MHz, D_2O) δ_C 14.50 (T CH_3), 39.62 (C-2′), 67.81 (d, J = 158.1 Hz, PCH_2), 76.63 (C-4′), 82.66 (d, J = 11.3 Hz, C-3′), 88.41 (C-1′), 113.94 (T (C(5)), 141.32 (T (C(6)), 154.68 (T (C(2)), 169.51 (T C(4)); ^{31}P NMR (500 MHz, D_2O) δ_P 16.02; exact mass calcd for $C_{10}H_{14}N_2O_7P_1$ [M − H]$^-$ 305.0538, found 305.0537. Anal. Calcd for $C_{10}H_{14}N_2Na_1O_7P$·$2H_2O$: C, 32.98; H, 4.98; N, 7.69. Found: C, 32.84; H, 4.94; N, 7.80.

1-(Uracil-1-yl)-2-deoxy-3-*O*-(phosphonomethyl)-L-threose Sodium Salt (3g). This compound was prepared as described for **3f**, using **22** (154 mg, 0.41 mmol) as starting material and iodotrimethylsilane (0.47 mL, 3.3 mmol). Compound **3g** (50 mg, 0.14 mmol) was obtained as a colorless solid after precipitation from diethyl ether in 34% yield: ^{1}H NMR (500 MHz, D_2O) δ_H 2.31−2.35 (m, 1H, C(2′)H_a), 2.57−2.62 (m, 1H, C(2′)H_b), 3.54−3.62 (m, 2H, PCH_2), 3.97 (dd, J_1 = 10.5 Hz, J_2 = 3.7 Hz, 1H, C(4′)H_a), 4.38−4.40 (m, 1H, C(3′)H), 4.42 (dd, J_1 = 10.5 Hz, J_2 = 2.0 Hz, 1H, C(4′)H_b), 5.88 (d, J = 8.3 Hz, 1H, U C(5)H), 6.21 (dd, J_1 = 8.2 Hz, J_2 = 2.0 Hz, 1H, C(1′)H), 7.99 (d, J = 8.2 Hz, 1H, U C(6)H); ^{13}C NMR (500 MHz, D_2O) δ_C 39.46 (C-2′), 67.56 (d, J = 156.9 Hz, PCH_2), 76.77 (C-4′), 82.31 (d, J = 13.8 Hz, C-3′), 88.57 (C-1′), 101.45 (U C(5)), 145.81 (U C(6), 169.23 (U C(4)); ^{31}P NMR (500 MHz, D_2O) δ_P 15.72; exact mass calcd for $C_9H_{12}N_2O_7P_1$ [M − H]$^-$ 291.0382, found 291.0391.

1-(Cytidin-1-yl)-2-deoxy-3-*O*-(phosphonomethyl)-L-threose Sodium Salt (3h). This compound was prepared as described for **3f**, using **23** (200 mg, 0.53 mmol) as starting material and iodotrimethylsilane (0.6 mL, 4.2 mmol). Compound **3h** (130 mg, 0.38 mmol) was obtained as a colorless solid after precipitation from diethyl ether in 73% yield: ^{1}H NMR (500 MHz, D_2O) δ_H 2.32 (d, J = 15.3, 1H, C(2′)H_a), 2.56−2.61 (m, 1H, C(2′)H_b), 3.52−3.61 (m, 2H, PCH_2), 4.01 (dd, J_1 = 10.5 Hz, J_2 = 3.6 Hz, 1H, C(4′)H_a), 4.39−4.40 (m, 1H, C(3′)H), 4.44 (dd, J_1 = 10.7 Hz, J_2 = 1.7 Hz, 1H, C(4′)H_b), 6.06 (d, J = 7.6 Hz, 1H, C C(5)H), 6.20 (dd, J_1 = 7.8 Hz, J_2 = 2.0 Hz,1H, C(1′)H), 7.95 (d, J = 7.6 Hz, 1H, C C(6)H); ^{13}C NMR (500 MHz, D_2O) δ_C 37.22 (C-2′), 64.88 (d, J = 157.2 Hz, PCH_2), 74.37 (C-4′), 79.99 (d, J = 11.7 Hz, C-3′), 86.71 (C-1′), 95.99 (C C(5)), 143.02 (C C(6), 157.56 (C C(2)), 169.23 (C C(4)); ^{31}P NMR (500 MHz, D_2O) δ_P 15.96; exact mass calcd for $C_9H_{13}N_3O_6P_1$ [M − H]$^-$ 290.0535, found 290.0542.

1-(Adenin-9-yl)-2-deoxy-3-*O*-(diphosphorylphosphonomethyl)-L-threose (24). A solution of the phosphonate triethylamine salt **3e** (52 mg, 0.12 mmol) in DMF (12 mL) was treated with dimethylformamide dimethyl acetal (0.72 mL) and kept at room temperature overnight. After evaporation of the solvent, the residue was dissolved in DMF (7.2 mL) and treated with *N*,*N*′-carbonyldiimidazole (58 mg, 0.36 mmol). After 12 h a 1 M solution of dibutylammonium pyrophosphate in DMF (2.0 mL) was added, and the mixture was kept at room temperature for 2 h. Then the mixture was treated with NH_4OH (2.4 mL) and subsequently concentrated under reduced pressure for 1 h. The residue was purified by chromatography on DEAE with a linear gradient of triethylammonium bicarbonate (0−0.8 M) as eluent. Further purification was carried out by reversed-phase chromatography on a LiChroprep C-18 column (2 × 30 cm). **3e** (5 mg, 0.01 mmol) as colorless oil was obtained in 8% yield. The ^{1}H NMR spectrum of **24** was identical to that of **3e**. Other data: ^{31}P NMR (D_2O) δ_P −22.73 (P-β), −10.28 (P-γ), 9.05 (P-α); exact mass calcd for $C_{10}H_{15}N_5O_{11}P_3$ [M − H]$^-$ 473.9950.

Kinetic Measurements. Determination of K_m and k_{cat} values for the incorporation of dATP and PMDTApp into a DNA hybrid was carried out under steady-state conditions as described by Creighton et al.[28] For each K_m and k_{cat} determination eight different substrate concentrations in the range of 0.1−12.5 μM were used. Results are the average of three independent determinations. The reaction mixture contained 250 nM primer/template complex and 0.025 U/μL HIV reverse transcriptase. The reaction was quenched after 1, 2, and 3 min by adding a double volume of stop solution (90% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol, and 50 mM EDTA). Samples were analyzed by gel electrophoresis on a 16% polyacrylamide ureum gel in TBE buffer (89 mM Tris−borate, 2 mM EDTA buffer, pH 8.3) after they were heated for 5 min at 70 °C. Products were visualized by phosphorimaging. The amount of radioactivity in the bands corresponding to the products of the enzymatic reactions was determined using the Optiquant image analysis software (Packard). Rate profiles were determined using GraphPad Prism software.

Construction of a Model. 1. Electrostatic Charges. Atomic electrostatic charges to be used in the Amber software package were calculated from the electrostatic potential at the 6-31G* level using the package Gamess[29] and the two-stage RESP fitting procedure keeping

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account of the phosphate linkages.[30] Also, the adenine base atomic charges were kept the same as those of DNA adenine in the Amber 94 topology files.[31]

2. Amber Parameters. The force field parameters used in the Amber simulations are those from the parm99 dataset.[32] However, they were modified for polyphosphate by contributions developed by H. A. Carlson[33] and for P−C linkage by M. Baaden.[34]

3. Model Building. The modeling is based on the PDB structure file 1rtd.[35] An initial model of the phosphonate nucleotide with a thymine base was constructed in Macromodel.[36] The geometry was optimized in Gamess in the AM1 force field.[29]

A locally developed software (Dancer) was used to fit the structure on the entering triphosphate in the 1rtd structure. This software performs a flexible fit by optimizing some defined dihedral angles and using a fitting procedure similar to the one used in the SEAL package.[37] In this modeling work the variable angles were the χ angle and the angles in the 3′-phosphonate linker. The molecule in the resulting conformation was then brought into the enzyme. The base was modified to an adenine (by fitting onto an adenine base nucleotide by Quatfit (Quatfit, CCL). The complementary adenine base (E5) was changed into a thymine by a similar procedure.

A phosphodiester bond was created between the terminal residue of the primer and the introduced phosphonate nucleotide. The Amber molecular mechanics energy of the system was minimized in the sander module of Amber 7 for 5000 steps.[38]

4. Analysis. The phosphonate nucleotide fits into the enzyme. The puckering of the sugar ring is between O4′-endo and C3′-endo. A combined Ligplot/HBPlus analysis[39,40] reveals hydrophobic contact with Asp185, Tyr115, and Gln151. However, no sterical hindrance with Met184 or Asp185 is to be expected to occur during translocation (the closest contact is for Asp185 OD2 999.O1P, 2.5 Å).

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