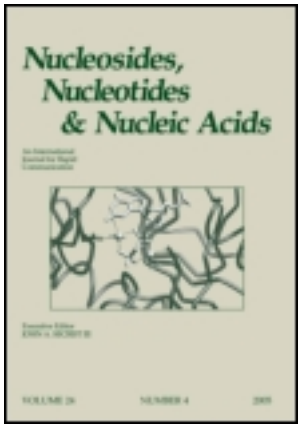


Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

A Novel Synthesis of Antiviral Nucleoside Phosphoramidate and Thiophosphoramidate Prodrugs via Nucleoside H-Phosphonamidates

Qi Sun [a], Xingjian Li [a], Shanshan Gong [a], Gang Liu [a], Liang Shen [a] & Liang Peng [b]

[a] Jiangxi Key Laboratory of Organic Chemistry, Jiangxi Science and Technology Normal University, Nanchang, Jiangxi, PR China

[b] School of Pharmacy, Jiangxi Science and Technology Normal University, Nanchang, Jiangxi, PR China

Published online: 18 Oct 2013.

To cite this article: Qi Sun, Xingjian Li, Shanshan Gong, Gang Liu, Liang Shen & Liang Peng (2013) A Novel Synthesis of Antiviral Nucleoside Phosphoramidate and Thiophosphoramidate Prodrugs via Nucleoside H-Phosphonamidates, Nucleosides, Nucleotides and Nucleic Acids, 32:11, 617-638, DOI: 10.1080/15257770.2013.838262

To link to this article: http://dx.doi.org/10.1080/15257770.2013.838262

Nucleosides, Nucleotides and Nucleic Acids, 32:617–638, 2013

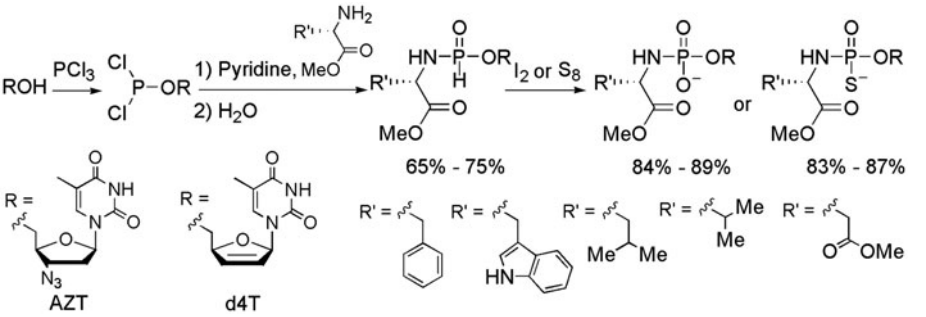
ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770.2013.838262

A NOVEL SYNTHESIS OF ANTIVIRAL NUCLEOSIDE PHOSPHORAMIDATE AND THIOPHOSPHORAMIDATE PRODRUGS VIA NUCLEOSIDE *H*-PHOSPHONAMIDATES

Qi Sun,[1] Xingjian Li,[1] Shanshan Gong,[1] Gang Liu,[1] Liang Shen,[1] and Liang Peng[2]

[1]*Jiangxi Key Laboratory of Organic Chemistry, Jiangxi Science and Technology Normal University, Nanchang, Jiangxi, PR China*
[2]*School of Pharmacy, Jiangxi Science and Technology Normal University, Nanchang, Jiangxi, PR China*

GRAPHICAL ABSTRACT



□ *A novel and efficient method for the preparation of antiviral nucleoside 5′-H-phosphonamidates has been developed. The oxidization of the H-phosphonamidate intermediates with iodine and sulfur afforded nucleoside 5′-phosphoramidates and 5′-thiophosphoramidates in high yields.*

Keywords *H*-Phosphonamidate; phosphoramidate; thiophosphoramidate; antiviral nucleoside; prodrug

Received 23 July 2013; accepted 22 August 2013.

We thank the National Natural Science Foundation of China (Nos. 21002041, 21262014), Natural Science Foundation of Jiangxi Province (No. 20114BAB203008), Project of the Science Funds of Jiangxi Education Office (No. GJJ12589), Key Project of Chinese Ministry of Education (No. 212092) for financial support to Qi Sun

Address correspondence to Professor Qi Sun, Jiangxi Key Laboratory of Organic Chemistry, Jiangxi Science and Technology Normal University, 605 Fenglin Avenue, Nanchang, China. E-mail: sunqi96@tsinghua.org.cn

INTRODUCTION

Synthesis of proviral DNA by reverse transcriptase (RT) plays a pivotal role in the replication of retroviruses. Currently, six nucleoside analog reverse transcriptase inhibitors (NRTIs), including zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), and abacavir (ABC), have been licensed for HIV therapy.[1] To exert their antiviral activity, all of these NRTIs must be consecutively phosphorylated to the corresponding 5′-triphosphates in the host cells. AZT and ddC-induced decrease in nucleoside kinase activity has been ascribed as a major cause for the reduced therapeutic efficacy and development of drug resistance in HIV treatment. To overcome the clinical limitations of NTRIs, a variety of "pronucleotide" strategies have been explored to improve their therapeutic activity by promoting the intracellular uptake of monophosphorylated nucleoside drugs.[2]

Among the numerous 5′-phosphorylated nucleoside prodrugs, amino acid conjugated phosphoramidates of NTRIs have been proved effective in enhancing the antiviral activity of the parent nucleoside analogs.[2] For instance, McGuigan et al. reported that the amino acid methyl ester phosphoramidate diesters of AZT, d4T, and ddA exhibited significantly enhanced potency against HIV via sequential metabolic activation.[3] In comparision, aromatic amino acid methyl ester conjugated phosphoramidate monoesters of AZT synthesized by Wagner et al. also showed enhanced antiviral activity and low cytotoxicity via a direct P–N hydrolysis mechanism. While the phosphoramidate monoester prodrugs maintained the membrane permeability of the parent AZT, their solubility in water, plasma half-life, and volume of distribution were remarkably improved.[4] In 2007, Herdewijn et al. reported that amino acid (L-Asp and L-His) phosphoramidate monoesters of 2′-deoxyadenosine could be recognized as 2′-deoxyadenosine triphosphate (dATP) mimetics for viral DNA synthesis by HIV RT.[5] This result indicated that amino acid phosphoramidate monoester prodrugs may circumvent all three kinase activation steps and directly serve as substrates of RT.

Currently, several methods are available for the synthesis of amino acid phosphoramidate monoesters and related compounds. While the DCC coupling method condenses nucleoside 5′-monophosphate and amino acid methyl esters under refluxing condition in 50–60% yields,[5a,6] amino acid methyl esters could be coupled with nucleoside *H*-phosphonates by in situ oxidation of nucleoside 5′-*H*-phosphonates with I_2[7] or CCl_4/Et_3N in moderate yields.[5b] Alternatively, aryl phosphoramidate diesters could be synthesized by tandem substitution reactions on phenylphosphodichloridate with L-alanine methyl ester and nucleosides,[8] or by phosphoramidite methodology followed by in situ oxidation.[4a,b] Removal of the phosphoester protecting groups under basic conditions afforded the desired nucleoside phosphoramidate monoesters in only low to moderate overall yields.

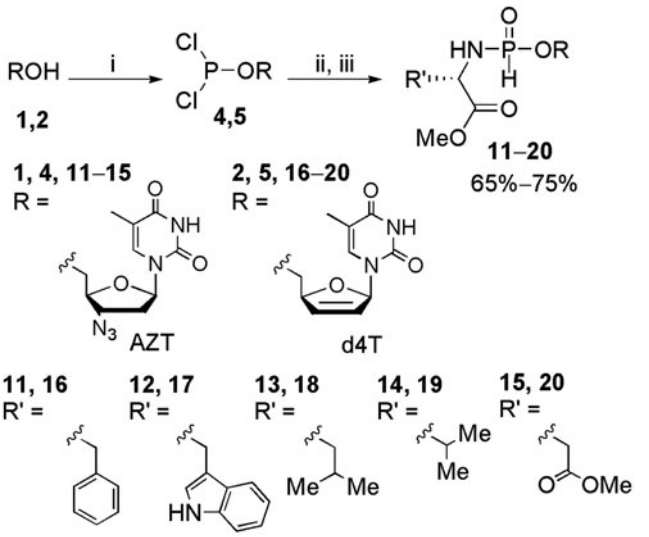
Nucleoside 5′-thiophosphoramidates have been synthesized in 63–80% yields by treating the highly toxic and smelly thiophosphoryl chloride with amino acid methyl esters, nucleosides, and aqueous ammonia sequentially in a one-pot manner.[9] The sequential *N*-phosphorylation of amino acid methyl esters with a specific phosphitylating reagent, 1,3,2-oxathiaphospholane, oxidation with sulfur[10] or borane,[11] and coupling with nucleosides provided another route to nucleoside thiophosphoramidates and boranophosphoramidates. For the synthesis of nucleoside boranophosphoramidates, Shaw et al. also attempted the *H*-phosphonamidate approach[11] based on the precedent report[12] of dinucleoside P3′→N5′ phosphoramidates. However, the low yields of nucleoside 5′-*H*-phosphonamidates by aminolysis of aryl *H*-phosphonate diesters[13] (produced in situ by condensing *H*-phosphonate monoesters with trichlorophenol) and lack of an efficient purification method greatly limited its application as versatile precursors for the synthesis of nucleoside phosphoramidates and heteroatom-substituted phosphoramidates.

In this paper, we report a novel and efficient method for the synthesis and isolation of amino acid *H*-phosphonamidates of AZT and d4T based on the controlled tandem substitution reactions on PCl_3. The subsequent oxidation of *H*-phosphonamidates with I_2 and S_8 afforded the phosphoramidates and thiophosphoramidates of AZT and d4T in excellent yields.

RESULTS AND DISCUSSION

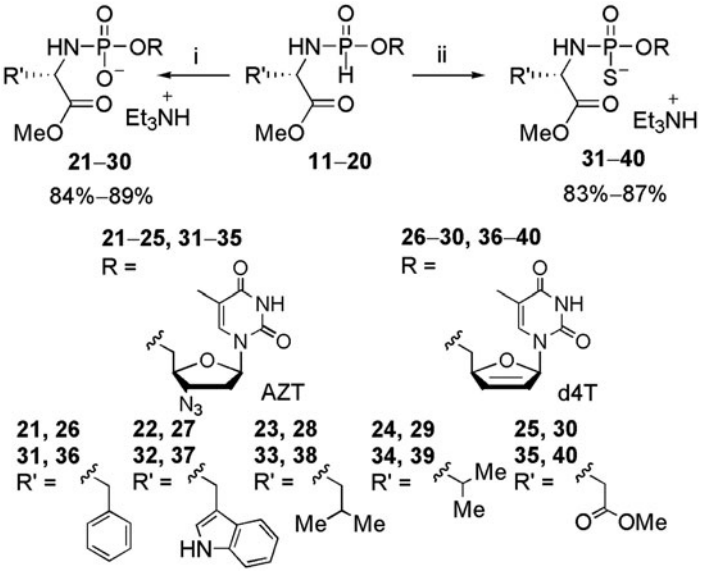
As shown in Scheme 1, monosubsituted nucleoside 5′-dichlorophos phites (**4**−**5**) were prepared by treating AZT (**1**) and d4T (**2**) with 10-fold excess of PCl_3 (**3**). Concentration under reduced pressure afforded the desired **4**−**5** in quantitative yields. To minimize the formation of the undesired phosphorodiamidite byproduct, the solutions of amino acid methyl esters in CH_2Cl_2 were added to those of **4**−**5** at −20°C over 3 hours with 3-fold excess of pyridine as base. Upon completion, hydrolysis of the chlorophosphoramidites gave the *H*-phosphonamidates of AZT and d4T (**11**−**20**) instantly. Flash chromatography on silica gel with neat ethyl acetate containing 0.05% triethylamine (TEA) afforded **11**−**20** in good yields ranging from 65−75%.

The nucleoside 5′-phosphoramidates (**21**−**30**) were obtained by oxidizing *H*-phosphonamidates **11**−**20** with I_2/TEA in aqueous pyridine. Alternatively, **11**−**20** could be oxidized with elemental sulfur/TEA in pyridine to afford the corresponding nucleoside 5′-thiophosphoramidates (**31**−**40**). Column chromatography on silica gel afforded the phosphoramidates and thiophosphoramidates of AZT and d4T in excellent yields (83−89%) and high purity (Scheme 2).



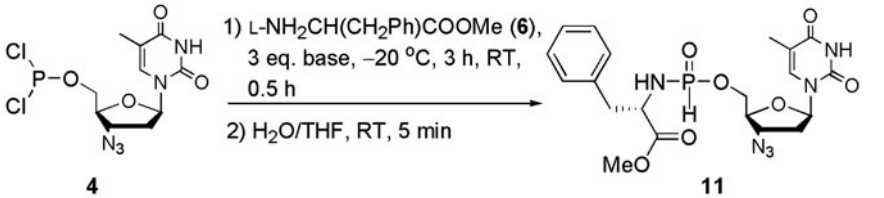
SCHEME 1 Synthesis of amino acid *H*-phosphonamidates of AZT and d4T (**11–20**). Reagents and conditions: (i) PCl_3 (**3**, 10.0 equiv), CH_2Cl_2, −20°C, 1 hour, RT, 2 hours; (ii) NH_2-CHR′-COOMe (**6–10**, 1.0 equiv), pyridine, CH_2Cl_2, −20°C, 3 hours, RT, 30 minutes; (iii) H_2O/THF, RT, 5 minutes.

Though nucleoside 5′-*H*-phosphonate diesters have long been recognized and utilized as versatile precursors for the synthesis of nucleoside 5′-phosphates and heteroatom-substituted phosphates,[14] however, the application of nucleoside 5′-*H*-phosphonamidates is still very limited due



SCHEME 2 Synthesis of 5′-phosphoramidates (**21**−**30**) and 5′-thiophosphoramidates (**31**−**40**) of AZT and d4T. Reagents and conditions: (i) I_2 (1.5 equiv), TEA (5.0 equiv), pyridine/H_2O (98:2, *v*/*v*), RT, 1 hour; (ii) S_8 (6.0 equiv), TEA (5.0 equiv), pyridine, RT, 4 hours.

TABLE 1 Effect of base on the yield of *H*-phosphonamidate **11**



Entry	Base	Yield (%)[a]
1	Imidazole	No product
2	TEA	18
3	DIEA	No product
4	Pyridine	85
5	2,6-Lutidine	71

[a] ^{31}P NMR yield.

to the lack of efficient synthetic methods for this type of compounds. To solve this problem, we attempted to synthesize nucleoside 5′-*H*-phosphonamidates directly from PCl_3 based on the tandem substitution method that has been applied for the synthesis of chlorophosphoramidite reagents.[15] First, AZT and d4T were phosphitylated with large excess of PCl_3 according to the procedure reported by Zhao et al. avoided the formation of multisubstituted byproducts.[16] After removal of excess PCl_3, nucleoside 5′-dichlorophosphites (**4**–**5**) could be obtained quantitatively. To obtain the disubstituted nucleoside 5′-chlorophosphoramidites via tandem substitution, phenylalanine methyl ester (**6**) was slowly added to the solution of highly reactive **4** at –20°C over 3 hours. Meanwhile, a variety of organic bases with different pK_as were tested as the deacid reagents. After in situ hydrolysis, the crude yield of desired AZT 5′-*H*-phosphonamidate of phenylalanine methyl ester (**11**) was determined by ^{31}P NMR. The data in Table 1 showed that imidazole, which has been widely used to react with PCl_3 to form a less reactive phosphitylating reagent, tri(imidazolyl)phosphite (PIm_3),[14b,17] gave no **11**. Similarly, TEA and diisopropylethylamine (DIEA), which have been commonly used in the preparation of chlorophosphoramidite reagents, afforded either low yield (18%) of **11** or no **11** at all. In contrast, when weakly basic pyridine and 2,6-lutidine were used, **11** was obtained in 85% and 71%, respectively.

As shown in Figure 1, ^{31}P NMR tracing experiment showed that when pyridine was added to dichlorophosphite **4**, it caused no change to the peak of 4 at δ_P 181.1 ppm. Upon completion of the addition of phenylalanine methyl ester, the major product (~60%) was the desired chlorophosphoramidite (**41**, δ_P 163.3 ppm). Due to its highly hydroscopic nature, ~35% of **41** had been hydrolyzed to the *H*-phosphonamidate (**11**, δ_P 12.5, 11.9 ppm) during the sample transfer to the NMR tube. After H_2O was added, **11**

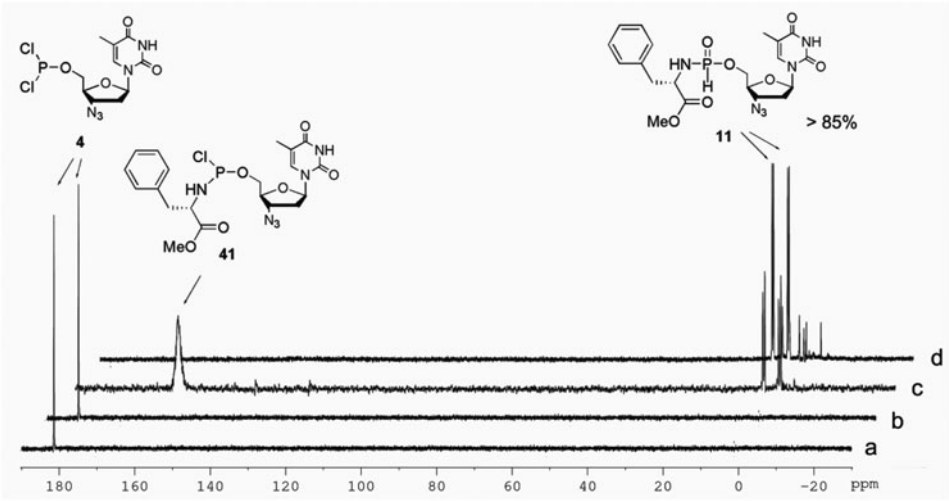


FIGURE 1 Analysis of sequential conversion of AZT-5′-dichlorophosphite (**4**) to AZT-5′-*H*-phosphonamidate (**11**) by ^{31}P NMR. (a) **4**; (b) 5 minutes after addition of pyridine (3.0 equiv); (c) 30 minutes after addition of phenylalanine methyl ester (**6**, 1.0 equiv); (d) 5 minutes after addition of H_2O (20.0 equiv).

was obtained in over 85% yield with small amounts of nucleoside 5′-*H*-phosphonate monoester and diester as the byproducts. Due to the instability of *H*-phosphonamidate on weakly acidic silica gel, trace amount of TEA (0.05%, *v*/*v*) was added to the ethyl acetate eluent and flash chromatography was applied to the purification of compounds **11–20**. The *H*-phosphonamidate products were flushed out within 5 minutes and obtained in 65–75% yields. Compounds **11–20** were fairly stable in solid form and could be kept for at least 6 months without decomposition at –20°C.

Compared to *H*-phosphonate diesters (δ_p = 8∼9 ppm, $^1J_{P,H}$ = ∼700 Hz),[14c] *H*-phosphonamidates showed up at lower magnetic field ranging from 10 to 14 ppm on ^{31}P NMR spectra with significantly smaller $^1J_{P,H}$ values around 660 Hz (Table 2).[12,13] The lowered electron density of the P atom in *H*-phosphonamidates revealed by NMR data predicted that these P–N compounds were less prone to be oxidized than their P–O counterparts.

When *H*-phosphonamidates **11–20** were treated with I_2/TEA in pyridine/H_2O (98:2, *v*/*v*), the conversion to the corresponding phosphoramidates proceeded smoothly in high yields. Compared to the oxidation reactions of *H*-phosphonate diesters, which typically finished within 5 minutes, complete consumption of **11–20** was much slower and required 1 hour.

The thiophosphoramidates could also be obtained in excellent yields by conventional sulfur oxidation in the presence of TEA. Similar to the oxidation with I_2, the reactions of *H*-phosphonamidates with sulfur (4 hours) were also significantly slower than those of *H*-phosphonate diesters (1–2 hours).[14c] It is worth noting that if a stronger base DBU was

TABLE 2 ^{31}P NMR data of amino acid *H*-phosphonamidates of AZT and d4T (**11–20**)

Compound	δ_P (ppm)	$^1J_{P,H}$ (Hz)
11	12.5, 11.9	667
12	12.6, 11.8	665
13	13.0, 12.3	663
14	12.8, 12.0	663
15	14.0, 11.5	666
16	11.2, 10.4	664
17	12.0, 10.8	666
18	11.8, 10.8	656
19	13.0, 12.2	657
20	13.1, 11.3	673

used instead of TEA, the reaction rate could be dramatically accelerated. All reactions went to completion in only 5 minutes with only slightly lowered yields. This result suggested that deprotonation of the less acidic P–H in phosphonamidate is crucial for the generation of the reactive phosphite anion. All of the above observations about the oxidation of nucleoside 5′-*H*-phosphonamidates of amino acid methyl esters were in good accordance with the results reported for the dinucleoside P3′→N5′ phosphoramidates and thiophosphoramidates.[12]

CONCLUSIONS

In summary, a series of amino acid methyl ester *H*-phosphonamidates of AZT and d4T were prepared in excellent yields from the controlled tandem substitution reactions on PCl_3 with pyridine as a suitable base. Due to the lowered electron density on the P atom of *H*-phosphonamidates, their oxidation reactions with iodine and sulfur were slower than those of *H*-phosphonate diesters, but afforded the desired amino acid phosphoramidates and thiophosphoramidates of AZT and d4T in high yields.

EXPERIMENTAL SECTION

General Methods

Chemical reagents and solvents were obtained from commercial suppliers. All reactions were performed under an atmosphere of dry argon and monitored by analytical thin-layer chromatography on plates coated with 0.25 mm silica gel 60 F254. TLC plates were visualized by UV irradiation (254 nm). Flash column chromatography employed silica gel (particle size 32–63 μm). All NMR spectra were obtained with a 400 MHz instrument with chemical shifts reported in parts per million (ppm, δ) and referenced to $CDCl_3$ or D_2O. IR spectra were recorded on a FT-IR spectrometer. Low- and

high-resolution mass spectra were obtained with an ion trap and a TOFQ mass spectrometer and reported as *m/z*.

Synthesis of Nucleoside-5′-*H*-phosphonamidates (11–20)

General Procedure

To a solution of PCl_3 (0.87 mL, 10.0 mmol) in CH_2Cl_2 (10 mL) was added nucleoside (1.0 mmol) and stirred at –20°C for 1 hour. The reaction was warmed up to ambient temperature and stirred for 2 hours. Concentration in vacuo afforded nucleoside-5′-dichlorophosphite as white foam. To a solution of dichlorophosphite and pyridine (0.24 mL, 3.0 mmol) in CH_2Cl_2 (10 mL) was added a solution of amino acid methyl ester hydrochloride (1.0 mmol) and TEA (0.14 mL, 1.0 mmol) in CH_2Cl_2 (10 mL) dropwise at –20°C over 3 hours. The reaction was warmed up to ambient temperature and stirred for 30 minutes. H_2O (0.36 mL) was added to the solution. The reaction was stirred for 5 minutes and diluted with CH_2Cl_2 (80 mL). The organic phase was washed with saturated $NaHCO_3$ aqueous solution (30 mL), HCl aqueous solution (0.5 M, 30 mL), and saturated NaCl aqueous solution (30 mL). The organic phase was dried with anhydrous Na_2SO_4, and concentrated in vacuo to give the crude product. Flash column chromatography on silica gel (Eluent: ethyl acetate with 0.05% TEA) afforded the diastereomeric mixture of *H*-phosphonamidate as white foam.

3′-Deoxy-3′-azidothymidin-5′-yl-L-phenylpropionyl-*H*-phosphonamidate (11). Starting from AZT (267 mg, 1.0 mmol) and L-phenylalanine methyl ester hydrochloride (216 mg, 1.0 mmol), the diastereomeric mixture of compound **11** was synthesized according to the general procedure. Flash column chromatography afforded **11** (335 mg, 68%) as white foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.81, 9.77 (s, 1H, NH-3), 7.42–7.29 (m, 5H, aromatic protons), 7.26, 7.24 (s, 1H, H-6), 6.88, 6.81 (d, $J_{P,H}$ = 667 Hz, 1H, PH), 6.21, 6.13 (t, J = 6.2 Hz, 1H, H-1′), 4.40–4.10 (m, 3H, H-3′, H-5′), 4.01–3.87 (m, 3H, H-4′, NH, H-a), 3.84 (s, 3H, OCH_3), 3.28–2.93 (m, 2H, CH_2Ph), 2.50–2.30 (m, 2H, H-2′), 1.96 (s, 3H, CH_3-5) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.1, 163.9, 150.4, 136.1, 135.9, 135.6, 129.6, 129.5, 128.9, 127.4, 111.5, 111.4, 85.7, 85.1, 82.3, 62.2, 60.2, 60.0, 54.9, 54.7, 52.7, 40.4, 40.3, 37.3, 37.2, 12.6 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 12.49, 11.88 ppm; IR (KBr): ν_{max} 3452, 3065, 2955, 2823, 2419, 2109, 1740, 1466, 1274, 1115, 967, 752, 558 cm^{-1}; HRMS (ESI+): *m/z* Calcd. for $C_{20}H_{26}N_6O_7P$ $[M+H]^+$ 493.1595; found 493.1604.

3′-Deoxy-3′-azidothymidin-5′-yl-L-tryptophanyl-*H*-phosphonamidate (12). Starting from AZT (267 mg, 1.0 mmol) and L-tryptophan methyl ester hydrochloride (255 mg, 1.0 mmol), the diastereomeric mixture of compound **12** was synthesized according to the general procedure. Flash column chromatography afforded **12** (345 mg, 65%) as white foam; ^{1}H

NMR (400 MHz, $CDCl_3$): δ 9.37 (s, 1H, indole NH), 8.69, 8.65 (s, 1H, NH-3), 7.55, 7.53 (s, 1H, H-6), 7.34 (d, $J = 8.1$ Hz, 1H, indole H-4), 7.23–7.13 (m, 2H, indole H-7, indole H-2), 7.10 (m, 1H, indole H-6), 7.04 (s, 1H, indole H-5), 6.87, 6.74 (d, $J_{P,H} = 665$ Hz, 1H, PH), 6.01, 5.95 (t, $J = 6.4$ Hz, 1H, H-1′), 4.39–4.21 (m, 1H, H-3′), 4.20–3.97 (m, 2H, H-5′), 3.96–3.70 (m, 6H, H-4′, NH, H-α, OCH_3), 3.35–3.11 (m, 2H, indole CH_2), 2.36–2.12 (m, 2H, H-2′), 1.83, 1.80 (s, 3H, CH_3-5) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.5, 164.0, 150.3, 136.3, 136.1, 135.9, 127.4, 123.6, 122.4, 119.9, 119.8, 118.5, 111.7, 111.6, 111.4, 111.3, 109.8, 109.7, 86.2, 85.4, 82.3, 62.4, 60.1, 60.0, 54.2, 52.8, 37.2, 30.3, 30.1, 12.6, 12.5 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 12.58, 11.81 ppm; IR (KBr): ν_{max} 3446, 2374, 2109, 1657, 1274, 1108, 968, 738, 558 cm^{-1}; HRMS (ESI+): *m/z* Calcd. for $C_{22}H_{27}N_7O_7P$ $[M+H]^+$ 532.1704; found 532.1715.

3′-Deoxy-3′-azidothymidin-5′-yl-L-leucyl-*H*-phosphonamidate (13). Starting from AZT (267 mg, 1.0 mmol) and L-leucine methyl ester hydrochloride (182 mg, 1.0 mmol), the diastereomeric mixture of compound **13** was synthesized according to the general procedure. Flash column chromatography afforded **13** (321 mg, 70%) as white foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.71 (s, 1H, NH-3), 7.35, 7.32 (s, 1H, H-6), 7.06, 7.04 (d, $J_{P,H} = 663$ Hz, 1H, PH), 6.16, 6.05 (t, $J = 6.4$ Hz, 1H, H-1′), 4.44–4.35 (m, 1H, H-3′), 4.34–4.10 (m, 2H, H-5′), 4.02–3.87 (m, 2H, H-4′, NH), 3.85–3.74 (m, 1H, H-α), 3.72 (s, 3H, OCH_3), 2.47–2.31 (m, 2H, H-2′), 1.90 (s, 3H, CH_3-5), 1.82–1.66 (m, 1H, $CH(CH_3)_2$), 1.65–1.45 (m, 2H, $CHCH_2(CH_3)_2$), 0.92 (d, $J =$ 6.5 Hz, 6H, $CHCH_2(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.5, 163.9, 150.4, 136.1, 135.7, 111.6, 111.5, 86.0, 85.2, 82.4, 62.7, 62.5, 60.2, 60.1, 52.6, 51.9, 43.4, 37.3, 37.2, 24.7, 24.6, 22.9, 21.6, 12.5 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 13.01, 12.32 ppm; IR (KBr): ν_{max} 3434, 2107, 1639, 1405, 1206, 1111, 612 cm^{-1}; HRMS (ESI+): *m/z* Calcd. for $C_{17}H_{28}N_6O_7P$ $[M+H]^+$ 459.1752; found 459.1763.

3′-Deoxy-3′-azidothymidin-5′-yl-L-valyl-*H*-phosphonamidate (14). Starting from AZT (267 mg, 1.0 mmol) and L-valine methyl ester hydrochloride (168 mg, 1.0 mmol), the diastereomeric mixture of compound **14** was synthesized according to the general procedure. Flash column chromatography afforded **14** (289 mg, 72%) as white foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.46 (s, 1H, NH-3), 7.35, 7.30 (s, 1H, H-6), 7.07, 7.03 (d, $J_{P,H} = 663$ Hz, 1H, PH), 6.16, 6.04 (t, $J = 6.1$ Hz, 1H, H-1′), 4.40 (m, 1H, H-3′), 4.37–4.10 (m, 2H, H-5′), 3.99 (s, 1H, H-4′), 3.92–3.78 (m, 1H, H-α), 3.74 (s, 3H, OCH_3), 2.42 (m, 2H, H-2′), 2.15 (m, 1H, $CH(CH_3)_2$), 1.91 (s, 3H, CH_3-5), 0.98 (d, $J = 4.8$ Hz, 3H, $CH(CH_3)_2$), 0.91–0.80 (m, 3H, $CH(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.5, 163.8, 150.4, 136.1, 135.7, 111.6, 111.5, 86.1, 85.3, 82.4, 62.6, 60.2, 60.1, 58.7, 52.6, 37.4, 37.3, 32.0, 31.8, 19.4, 19.3, 17.2, 17.0, 12.6 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 12.75, 11.97 ppm; IR (KBr): ν_{max} 3857, 3733, 3182, 2966, 2826, 2423, 2110, 1469, 1273, 1107, 773,

560 cm^{-1}; HRMS (ESI+): *m/z* Calcd. for $C_{16}H_{26}N_6O_7P$ $[M+H]^+$ 445.1595; found 445.1585.

3′-Deoxy-3′-azidothymidin-5′-yl-L-aspartyl-*H*-phosphonamidate (15). Starting from AZT (267 mg, 1.0 mmol) and L-aspartic acid dimethyl ester hydrochloride (198 mg, 1.0 mmol), the diastereomeric mixture of compound **15** was synthesized according to the general procedure. Flash column chromatography afforded **15** (313 mg, 66%) as white foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.61, 9.57 (s, 1H, NH-3), 7.33, 7.28 (s, 1H, H-6), 7.17, 7.07 (d, $J_{P,H} = 666$ Hz, 1H, PH), 6.15, 5.98 (t, $J = 6.4$ Hz, 1H, H-1′), 4.52–4.13 (m, 5H, H-3′, H-5′, H-4′, NH), 4.04–3.90 (m, 1H, H-α), 3.73 (s, 3H, OCH_3-α), 3.69 (s, 3H, OCH_3-γ), 3.07–2.75 (m, 2H, CH_2-β), 2.52–2.34 (m, 2H, H-2′), 1.89, 1.88 (s, 3H, CH_3-5) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.4, 172.3, 171.7, 171.4, 163.9, 150.4, 136.4, 135.8, 111.5, 86.5, 85.4, 82.4, 62.7, 62.4, 60.2, 59.9, 53.1, 52.3, 50.3, 49.9, 38.2, 37.3, 37.2, 12.6, 12.5 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 14.07, 11.50 ppm; IR (KBr): ν_{max} 3428, 2958, 2898, 2824, 2359, 1442, 1276, 1111, 997, 675, 556 cm^{-1}; HRMS (ESI+): *m/z* Calcd. for $C_{16}H_{24}N_6O_9P$ $[M+H]^+$ 475.1337; found 475.1329.

2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl-L-phenylpropionyl-*H*-phosphonamidate (16). Starting from d4T (224 mg, 1.0 mmol) and L-phenylalanine methyl ester hydrochloride (216 mg, 1.0 mmol), the diastereomeric mixture of compound **16** was synthesized according to the general procedure. Flash column chromatography afforded **16** (314 mg, 70%) as white foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.06 (s, 1H, NH-3), 7.33–7.09 (m, 6H, aromatic protons, H-6), 7.00, 6.97 (s, 1H, H-1′), 6.69 (d, $J_{P,H} = 664$ Hz, 1H, PH), 6.28, 6.21 (d, $J = 5.7$ Hz, 1H, H-2′), 5.86 (m, 1H, H-3′), 4.90, 4.85 (s, 1H, H-4′), 4.27–3.98 (m, 2H, H-5′), 3.92–3.79 (m, 1H, NH), 3.75, 3.74 (s, 3H, OCH_3), 3.59–3.40 (m, 1H, H-α), 3.18–2.80 (m, 2H, CH_2Ph), 1.82, 1.81 (s, 3H, CH_3-5) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.0, 163.8, 150.9, 136.0, 135.6, 133.8, 133.1, 129.6, 129.5, 128.9, 127.7, 127.5 127.4, 127.2, 111.3, 111.0, 90.0, 89.8, 84.7, 84.6, 63.8, 62.8, 54.8, 54.7, 52.7, 40.6, 40.4, 12.6 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 11.19, 10.39 ppm; IR (KBr): ν_{max} 3731, 3422, 3032, 2952, 2823, 2417, 2318, 1743, 1464, 1223, 1116, 965, 910, 779, 574 cm^{-1}; HRMS (ESI+): *m/z* Calcd. for $C_{20}H_{25}N_3O_7P$ $[M+H]^+$ 450.1425; found 450.1437.

2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl-L-tryptophanyl-*H*-phosphonamidate (17). Starting from d4T (224 mg, 1.0 mmol) and L-tryptophan methyl ester hydrochloride (255 mg, 1.0 mmol), the diastereomeric mixture of compound **17** was synthesized according to the general procedure. Flash column chromatography afforded **17** (322 mg, 66%) as white foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.41 (br, 1H, indole NH), 8.75 (s, 1H, NH-3), 7.52, 7.50 (s, 1H, H-6), 7.32 (d, $J = 7.8$ Hz, 1H, indole H-4), 7.20–6.99 (m, 4H, indole H-7, indole H-6, indole H-2, indole H-5), 6.96–6.88 (m, 1H, H-1′), 6.76, 6.69 (d, $J_{P,H} = 666$ Hz, 1H, PH), 6.21–6.04

(m, 1H, H-2′), 5.78 (d, J = 5.8 Hz, 1H, H-3′), 4.79 (s, 1H, H-4′), 4.33–4.15 (m, 1H, H-5′), 4.08–3.79 (m, 2H, H-5′, NH), 3.78–3.66 (m, 4H, OCH_3, H-α), 3.32–3.11 (m, 2H, indole CH_2), 1.77, 1.73 (s, 3H, CH_3-5) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.6, 173.4, 164.1, 151.1, 151.0, 136.4, 136.1, 135.8, 133.8, 133.1, 129.1, 128.3, 127.4, 127.0, 125.4, 123.7, 123.6, 122.4, 122.3, 119.7, 118.5, 118.4, 111.7, 111.6, 111.2, 111.0, 109.6, 90.0, 89.8, 84.7, 63.8, 63.1, 54.3, 54.1, 52.7, 30.3, 30.1, 12.5 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 12.05, 10.78 ppm; IR (KBr): ν_{max} 3732, 3382, 3256, 2952, 2419, 1463, 1224, 1113, 1087, 983, 738, 576 cm^{-1}; HRMS (ESI+): *m/z* Calcd. for $C_{22}H_{26}N_4O_7P$ $[M+H]^+$ 489.1534; found 489.1547.

2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl-L-leucyl-*H*-phosphonamidate (18). Starting from d4T (224 mg, 1.0 mmol) and L-leucine methyl ester hydrochloride (182 mg, 1.0 mmol), the diastereomeric mixture of compound **18** was synthesized according to the general procedure. Flash column chromatography afforded **18** (299 mg, 72%) as white foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.55 (s, 1H, NH-3), 7.25–7.17 (m, 1H, H-6), 7.03–6.96 (m, 1H, H-1′), 7.00, 6.95 (d, $J_{P,H}$ = 656 Hz, 1H, PH), 6.35, 6.31 (d, J = 6.0 Hz, 1H, H-2′), 5.87 (m, 1H, H-3′), 4.99 (s, 1H, H-4′), 4.33–4.08 (m, 2H, H-5′), 3.97–3.81 (m, 1H, NH), 3.75–3.61 (m, 4H, OCH_3, H-α), 1.87, 1.84 (s, 3H, CH_3-5), 1.77–1.42 (m, 3H, $C\underline{H}CH_2(CH_3)_2$, $CHC\underline{H}_2(CH_3)_2$), 0.90 (t, J = 5.7 Hz, 6H, $CHCH_2(C\underline{H}_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.4, 163.9, 151.0, 135.9, 135.6, 133.7, 133.2, 127.6, 127.3, 111.3, 111.1, 90.0, 84.8, 84.7, 64.1, 63.3, 52.5, 51.9, 43.5, 43.4, 24.8, 24.6, 22.8, 21.7, 12.5 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 11.79, 10.77 ppm; IR (KBr): ν_{max} 3730, 3594, 3167, 2959, 2875, 2412, 1743, 1468, 1223, 1113, 988, 783, 576 cm^{-1}; HRMS (ESI+): *m/z* Calcd. for $C_{17}H_{27}N_3O_7P$ $[M+H]^+$ 416.1581; found 416.1589.

2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl-L-valyl-*H*-phosphonamidate (19). Starting from d4T (224 mg, 1.0 mmol) and L-valine methyl ester hydrochloride (168 mg, 1.0 mmol), the diastereomeric mixture of compound **19** was synthesized according to the general procedure. Flash column chromatography afforded **19** (301 mg, 75%) as white foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.68 (s, 1H, NH-3), 7.24–7.14 (m, 1H, H-6), 7.02–6.90 (m, 1H, H-1′), 6.97, 6.94 (d, $J_{P,H}$ = 657 Hz, 1H, PH), 6.34, 6.30 (d, J = 5.9 Hz, 1H, H-2′), 5.87 (m, 1H, H-3′), 4.98 (s, 1H, H-4′), 4.30–4.05 (m, 2H, H-5′), 3.82–3.63 (m, 5H, NH, OCH_3, H-α), 2.11 (m, 1H, $C\underline{H}(CH_3)_2$), 1.86, 1.83 (s, 3H, CH_3-5), 0.93 (t, J = 6.8 Hz, 3H, $CH(C\underline{H}_3)_2$), 0.84, 0.80 (d, J = 6.8 Hz, 3H, $CH(C\underline{H}_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.4, 164.0, 151.1, 151.0, 135.9, 135.6, 133.7, 133.2, 127.6, 127.3, 111.3, 111.0, 90.0, 89.7, 84.7, 64.1, 63.1, 58.6, 52.4, 31.9, 31.7, 19.3, 19.2, 17.2, 17.0, 12.5 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 13.00, 12.24 ppm; IR (KBr): ν_{max} 3731, 3450, 3222, 3065, 2965, 2881, 2414, 1468, 1223, 1086, 984, 781, 576 cm^{-1}; HRMS (ESI+): *m/z* Calcd. for $C_{16}H_{25}N_3O_7P$ $[M+H]^+$ 402.1425; found 402.1415.

2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl-L-aspartyl-*H*-phosphonamidate (20). Starting from d4T (224 mg, 1.0 mmol) and L-aspartic acid dimethyl ester hydrochloride (198 mg, 1.0 mmol), the diastereomeric mixture of compound **20** was synthesized according to the general procedure. Flash column chromatography afforded **20** (293 mg, 68%) as white foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.61 (s, 1H, NH-3), 7.23, 7.20 (s, 1H, H-6), 7.11, 6.96 (d, $J_{P,H} = 673$ Hz, 1H, PH), 7.00, 6.97 (s, 1H, H-1′), 6.38–6.20 (m, 1H, H-2′), 5.86 (m, 1H, H-3′), 4.99 (s, 1H, H-4′), 4.39–4.06 (m, 4H, H-5′, NH, H-α), 3.75–3.56 (m, 6H, $OCH_3 \times 2$), 3.02–2.68 (m, 2H, CH_2-β), 1.85, 1.82 (s, 3H, CH_3-5) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.3, 171.5, 171.3, 164.0, 151.1, 135.9, 135.7, 133.8, 133.2, 127.6, 127.2, 111.3, 111.0, 89.9, 89.7, 84.7, 64.0, 63.2, 53.1, 52.3, 50.2, 49.8, 38.2, 12.5 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 13.13, 11.25 ppm; IR (KBr): ν_{max} 3731, 3447, 2958, 2892, 2429, 1696, 1443, 1223, 1086, 987, 782, 579 cm^{-1}; HRMS (ESI+): *m/z* Calcd. for $C_{16}H_{23}N_3O_9P$ $[M+H]^+$ 432.1166; found 432.1154.

Synthesis of Nucleoside-5′-Phosphoramidates (21–30)

General Procedure

To a solution of *H*-phosphonamidate (0.25 mmol) in pyridine (2.5 mL) was added TEA (0.17 mL, 1.25 mmol), H_2O (51 μL), and I_2 (95 mg, 0.375 mmol). The reaction was stirred at ambient temperature for 1 hour and concentrated in vacuo to give the crude product. Flash column chromatography on silica gel (CH_2Cl_2/MeOH 20:1 with 0.5% TEA) afforded nucleoside-5′-phosphoramidate as light yellow oil.

2(*S*)-[3′-Deoxy-3′-azidothymidin-5′-yl(hydroxy)phosphorylamino]-3-phenylproprionic acid methyl ester, triethylammonium salt (21). Starting from **11** (123 mg, 0.25 mmol) and I_2 (95 mg, 0.375 mmol), compound **21** was synthesized according to the general procedure. Flash column chromatography afforded **21** (131 mg, 86%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.44 (br, 1H, Et_3NH^+), 9.93 (s, 1H, NH-3), 7.72 (s, 1H, H-6), 7.23–7.13 (m, 5H, aromatic protons), 6.23 (t, $J = 6.5$ Hz, 1H, H-1′), 4.33 (s, 1H, H-3′), 4.13–4.03 (m, 1H, H-4′), 3.91 (s, 1H, H-α), 3.87–3.68 (m, 2H, H-5′), 3.61 (s, 3H, OCH_3), 3.11 (m, 1H, NH), 3.04–2.84 (m, 8H, $N(\underline{CH_2}CH_3)_3$, CH_2Ph), 2.36–2.16 (m, 2H, H-2′), 1.93 (s, 3H, CH_3-5), 1.24 (t, 9H, $N(CH_2\underline{CH_3})_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.1, 164.3, 150.7, 137.4, 136.1, 129.5, 128.4, 126.7, 111.2, 84.5, 83.7, 64.0, 61.2, 56.6, 51.8, 45.4, 41.2, 37.6, 12.5, 8.6 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 4.97 ppm; IR (KBr): ν_{max} 3779, 3425, 2940, 2886, 2742, 2495, 2356, 2107, 1473, 1276, 1113, 1077, 701, 556 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{20}H_{24}N_6O_8P$ $[M-H]^-$ 507.1; found 507.3.

2(*S*)-[Hydroxy(3′-deoxy-3′-azidothymidin-5′-yl)phosphorylamino]-3-(3-indolyl)proprionic acid methyl ester, triethylammonium salt (22). Starting from **12** (133 mg, 0.25 mmol) and I_2 (95 mg, 0.375 mmol), compound **22** was synthesized according to the general procedure. Flash column chromatography afforded **22** (136 mg, 84%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.93 (br, 1H, indole NH), 9.42 (s, 1H, NH-3), 7.64 (s, 1H, H-6), 7.53 (d, J = 7.7 Hz, 1H, indole H-4), 7.33 (d, J = 8.1 Hz, 1H, indole H-7), 7.12–6.96 (m, 3H, indole H-2, indole H-6, indole H-5), 6.15 (t, J = 6.7 Hz, 1H, H-1′), 4.26 (m, 1H, H-3′), 4.18 (m, 1H, H-4′), 3.94–3.82 (m, 3H, H-5′, H-α), 3.60 (s, 3H, OCH_3), 3.24 (m, 1H, NH), 3.20–3.01 (m, 2H, indole CH_2), 2.85 (q, 6H, $N(CH_2CH_3)_3$), 2.20–2.07 (m, 2H, H-2′), 1.90 (s, 3H, CH_3-5), 1.15 (t, 9H, $N(CH_2CH_3)_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.6, 164.4, 150.7, 136.5, 136.3, 127.7, 123.6, 121.7, 119.1, 118.6, 111.5, 111.1, 110.6, 84.8, 83.7, 64.1, 61.3, 55.7, 51.9, 45.4, 37.3, 30.9, 12.6, 8.5 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 4.42 ppm; IR (KBr): ν_{max} 3775, 3410, 2978, 2869, 1403, 1152, 683, 618, 540 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{22}H_{25}N_7O_8P$ $[M-H]^-$ 546.2; found 546.3.

2(*S*)-[3′-Deoxy-3′-azidothymidin-5′-yl(hydroxy)phosphorylamino]-4-methylvaleric acid methyl ester, triethylammonium salt (23). Starting from **13** (115 mg, 0.25 mmol) and I_2 (95 mg, 0.375 mmol), compound **23** was synthesized according to the general procedure. Flash column chromatography afforded **23** (125 mg, 87%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.93 (s, 1H, NH-3), 7.76 (s, 1H, H-6), 6.26 (t, J = 6.5 Hz, 1H, H-1′), 4.45 (s, 1H, H-3′), 4.01 (m, 3H, H-5′, H-4′), 3.81 (m, 1H, H-α), 3.63 (s, 3H, OCH_3), 3.03 (q, 6H, $N(CH_2CH_3)_3$), 2.29 (m, 2H, H-2′), 1.94 (s, 3H, CH_3-5), 1.81–1.64 (m, 1H, $CHCH_2(CH_3)_2$), 1.51–1.36 (m, 2H, $CHCH_2(CH_3)_2$), 1.27 (t, 9H, $N(CH_2CH_3)_3$), 0.87 (t, J = 5.5 Hz, 6H, $CHCH_2(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.5, 164.3, 150.8, 136.1, 111.3, 84.5, 83.8, 64.1, 61.3, 53.6, 51.7, 45.4, 44.4, 37.6, 24.7, 22.9, 22.2, 12.6, 8.6 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 5.37 ppm; IR (KBr): ν_{max} 3416, 2955, 2486, 2356, 2104, 1465, 1275, 1110, 1077, 927, 819, 555 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{17}H_{26}N_6O_8P$ $[M-H]^-$ 473.2; found 473.3.

2(*S*)-[3′-Deoxy-3′-azidothymidin-5′-yl(hydroxy)phosphorylamino]-3-methylbutyric acid methyl ester, triethylammonium salt (24). Starting from **14** (111 mg, 0.25 mmol) and I_2 (95 mg, 0.375 mmol), compound **24** was synthesized according to the general procedure. Flash column chromatography afforded **24** (123 mg, 88%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.44 (br, 1H, Et_3NH^+), 10.16 (s, 1H, NH-3), 7.69 (s, 1H, H-6), 6.23 (m, 1H, H-1′), 4.42 (s, 1H, H-3′), 4.01 (m, 3H, H-5′, H-4′), 3.62 (m, 4H, H-α, OCH_3), 3.03 (q, 6H, $N(CH_2CH_3)_3$), 2.28 (s, 2H, H-2′), 2.00–1.84 (m, 4H, CH_3-5, $CH(CH_3)_2$), 1.26 (t, 9H, $N(CH_2CH_3)_3$), 0.90 (d, J = 4.4 Hz, 3H, $CH(CH_3)_2$), 0.83 (d, J = 4.4 Hz, 3H, $CH(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.2, 164.4, 150.8, 136.0, 111.2, 84.5, 83.6,

64.2, 61.2, 60.4, 51.6, 45.4, 37.5, 32.3, 19.2, 17.8, 12.5, 8.5 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 4.82 ppm; IR (KBr): ν_{max} 3383, 2938, 2743, 2678, 2110, 1472, 1368, 1275, 1072, 962, 768, 559 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{16}H_{24}N_6O_8P$ $[M-H]^-$ 459.1; found 459.3.

2(*S*)-[3′-Deoxy-3′-azidothymidin-5′-yl(hydroxy)phosphorylamino]-succinic acid dimethyl ester, triethylammonium salt (25). Starting from **15** (119 mg, 0.25 mmol) and I_2 (95 mg, 0.375 mmol), compound **25** was synthesized according to the general procedure. Flash column chromatography afforded **25** (126 mg, 85%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.28 (br, 1H, Et_3NH^+), 9.53 (s, 1H, NH-3), 7.73 (s, 1H, H-6), 6.25 (t, $J = 6.2$ Hz, 1H, H-1′), 4.46 (s, 1H, H-3′), 4.18 (m, 1H, H-4′), 4.02 (s, 3H, H-5′, H-α), 3.69 (s, 3H, OCH_3-α), 3.62 (s, 3H, OCH_3-γ), 3.54–3.35 (m, 1H, NH), 3.06 (q, 6H, $N(C\underline{H}_2CH_3)_3$), 2.90–2.74 (m, 2H, CH_2-β), 2.33 (m, 2H, H-2′), 1.94 (s, 3H, CH_3-5), 1.30 (t, 9H, $N(CH_2C\underline{H}_3)_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 171.5, 164.1, 150.6, 136.1, 111.3, 84.7, 83.7, 64.1, 61.2, 52.4, 51.9, 45.6, 39.4, 37.6, 12.5, 8.6 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 4.61 ppm; IR (KBr): ν_{max} 3428, 2939, 2742, 2677, 2493, 2357, 2109, 1693, 1438, 1278, 1110, 853, 556 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{16}H_{22}N_6O_{10}P$ $[M-H]^-$ 489.1; found 489.2.

2(*S*)-[(2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl)(hydroxy)phosphoryl amino]-3-phenylproprionic acid methyl ester, triethylammonium salt (26). Starting from **16** (112 mg, 0.25 mmol) and I_2 (95 mg, 0.375 mmol), compound **26** was synthesized according to the general procedure. Flash column chromatography afforded **26** (123 mg, 87%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.58 (br, 1H, Et_3NH^+), 9.81 (s, 1H, NH-3), 7.62 (s, 1H, H-6), 7.22–7.07 (m, 5H, aromatic protons), 6.97 (m, 1H, H-1′), 6.24 (d, $J = 5.4$ Hz, 1H, H-2′), 5.68 (d, $J = 5.4$ Hz, 1H, H-3′), 4.80 (s, 1H, H-4′), 4.01 (m, 1H, H-5′), 3.93–3.84 (m, 1H, H-5′), 3.82–3.73 (m, 1H, H-α), 3.56 (s, 3H, OCH_3), 2.96 (q, 6H, $N(C\underline{H}_2CH_3)_3$), 2.90 (d, $J = 6.3$ Hz, 2H, CH_2Ph), 1.92 (s, 3H, CH_3-5), 1.22 (t, 9H, $N(CH_2C\underline{H}_3)_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.0, 164.4, 151.2, 137.3, 137.1, 134.8, 129.5, 128.3, 126.6, 126.0, 111.1, 89.5, 86.1, 65.1, 56.4, 51.6, 45.4, 41.2, 12.3, 8.5 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 4.87 ppm; IR (KBr): ν_{max} 3730, 3417, 2936, 2743, 2677, 2493, 2350, 1470, 1207, 1071, 949, 775, 576 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{20}H_{23}N_3O_8P$ $[M-H]^-$ 464.1; found 464.3.

2(*S*)-[Hydroxy(2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl)phosphoryl amino]-3-(3-indolyl) proprionic acid methyl ester, triethylammonium salt (27). Starting from **17** (122 mg, 0.25 mmol) and I_2 (95 mg, 0.375 mmol), compound **27** was synthesized according to the general procedure. Flash column chromatography afforded **27** (130 mg, 86%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.47 (br, 1H, Et_3NH^+), 9.69 (s, 1H, indole NH), 9.60 (s, 1H, NH-3), 7.60 (s, 1H, H-6), 7.49 (d, $J = 7.8$ Hz, 1H, indole H-4), 7.33 (d, $J = 8.0$ Hz, 1H, indole H-7), 7.11–6.94 (m, 4H, indole H-6, indole

H-2, indole H-5, H-1′), 6.20 (d, $J = 5.8$ Hz, 1H, H-2′), 5.66 (d, $J = 5.8$ Hz, 1H, H-3′), 4.81 (s, 1H, H-4′), 4.10 (m, 1H, H-5′), 4.01–3.83 (m, 2H, H-5′, H-α), 3.54 (s, 3H, OCH_3), 3.29–3.02 (m, 3H, NH, indole CH_2), 2.81 (q, 6H, $N(CH_2CH_3)_3$), 1.91 (s, 3H, CH_3-5), 1.12 (t, 9H, $N(CH_2CH_3)_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.6, 164.4, 151.2, 137.1, 136.5, 134.9, 127.8, 126.0, 123.6, 121.6, 119.0, 118.6, 111.5, 111.1, 110.6, 89.6, 86.2, 65.3, 55.7, 51.8, 45.3, 30.9, 12.4, 8.5 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 4.86 ppm; IR (KBr): ν_{max} 3731, 3384, 2947, 2681, 2487, 2317, 1462, 1205, 1116, 1071, 912, 745, 520 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{22}H_{24}N_4O_8P$ $[M-H]^-$ 503.1; found 503.3.

2(*S*)-[(2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl)(hydroxy)phosphoryl amino]-4-methylvaleric acid methyl ester, triethylammonium salt (28). Starting from **18** (104 mg, 0.25 mmol) and I_2 (95 mg, 0.375 mmol), compound **28** was synthesized according to the general procedure. Flash column chromatography afforded **28** (116 mg, 87%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.70 (br, 1H, Et_3NH^+), 9.83 (s, 1H, NH-3), 7.63 (s, 1H, H-6), 6.98 (m, 1H, H-1′), 6.33 (d, $J = 5.6$ Hz, 1H, H-2′), 5.69 (d, $J = 5.6$ Hz, 1H, H-3′), 4.90 (s, 1H, H-4′), 4.09–3.91 (m, 2H, H-5′), 3.76–3.67 (m, 1H, H-α), 3.61 (s, 3H, OCH_3), 3.00 (q, 6H, $N(CH_2CH_3)_3$), 1.92 (s, 3H, CH_3-5), 1.75–1.60 (m, 1H, $CHCH_2(CH_3)_2$), 1.45–1.33 (m, 2H, $CHCH_2(CH_3)_2$), 1.24 (t, 9H, $N(CH_2CH_3)_3$), 0.82 (d, $J = 5.5$ Hz, 6H, $CHCH_2(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.3, 164.5, 151.2, 137.0, 134.8, 126.1, 111.1, 89.5, 86.1, 65.2, 53.4, 51.6, 45.3, 44.4, 24.6, 22.8, 22.2, 12.3, 8.5 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 4.87 ppm; IR (KBr): ν_{max} 3730, 3383, 2939, 2743, 2677, 2492, 2350, 1471, 1257, 1209, 1073, 1042, 991, 737, 577 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{17}H_{25}N_3O_8P$ $[M-H]^-$ 430.1; found 430.3.

2(*S*)-[(2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl)(hydroxy)phosphoryl amino]-3-methylbutyric acid methyl ester, triethylammonium salt (29). Starting from **19** (100 mg, 0.25 mmol) and I_2 (95 mg, 0.375 mmol), compound **29** was synthesized according to the general procedure. Flash column chromatography afforded **29** (115 mg, 89%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.70 (br, 1H, Et_3NH^+), 9.77 (s, 1H, NH-3), 7.65 (s, 1H, H-6), 6.98 (s, 1H, H-1′), 6.31 (d, $J = 5.8$ Hz, 1H, H-2′), 5.69 (d, $J = 5.8$ Hz, 1H, H-3′), 4.89 (s, 1H, H-4′), 4.08–3.92 (m, 2H, H-5′), 3.61 (s, 3H, OCH_3), 3.56 (m, 1H, H-α), 3.00 (q, 6H, $N(CH_2CH_3)_3$), 1.93 (s, 3H, CH_3-5), 1.91–1.86 (m, 1H, $CH(CH_3)_2$), 1.24 (t, 9H, $N(CH_2CH_3)_3$), 0.87 (d, $J = 6.8$ Hz, 3H, $CH(CH_3)_2$), 0.81 (d, $J = 6.8$ Hz, 3H, $CH(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.4, 164.5, 151.2, 137.1, 134.9, 126.0, 111.1, 89.5, 86.2, 65.2, 60.3, 51.4, 45.4, 32.4, 19.1, 17.9, 12.3, 8.5 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 5.94 ppm; IR (KBr): ν_{max} 3730, 3382, 2938, 2742, 2677, 2491, 1471, 1257, 1209, 1073, 913, 779, 581 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{16}H_{23}N_3O_8P$ $[M-H]^-$ 416.1; found 416.2.

2(*S*)-[(2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl)(hydroxy)phosphoryl amino]-succinic acid dimethyl ester, triethylammonium salt (30). Starting from **20** (108 mg, 0.25 mmol) and I_2 (95 mg, 0.375 mmol), compound **30** was synthesized according to the general procedure. Flash column chromatography afforded **30** (116 mg, 85%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.56 (br, 1H, Et_3NH^+), 9.55 (s, 1H, NH-3), 7.66 (s, 1H, H-6), 7.00 (s, 1H, H-1′), 6.32 (d, J = 5.4 Hz, 1H, H-2′), 5.72 (d, J = 5.4 Hz, 1H, H-3′), 4.92 (s, 1H, H-4′), 4.06 (m, 2H, H-5′), 4.00 (m, 1H, H-α), 3.66 (s, 3H, OCH_3-α), 3.60 (s, 3H, OCH_3-γ), 3.54–3.32 (m, 1H, NH), 3.02 (q, 6H, $N(C\underline{H}_2CH_3)_3$), 2.88–2.70 (m, 2H, CH_2-β), 1.93 (s, 3H, CH_3-5), 1.26 (t, 9H, $N(C\underline{H}_2CH_3)_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.7, 171.5, 164.4, 151.1, 137.1, 134.7, 126.2, 111.1, 89.5, 86.1, 65.2, 52.3, 51.8, 51.6, 45.4, 39.1, 12.3, 8.6 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 4.72 ppm; IR (KBr): ν_{max} 3730, 3441, 2939, 2678, 1697, 1440, 1214, 1076, 993, 781, 584 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{16}H_{21}N_3O_{10}P$ $[M-H]^-$ 446.1; found 446.2.

Synthesis of Nucleoside-5′-thiophosphoramidates (31–40)

General Procedure

To a solution of *H*-phosphonamidate (0.25 mmol) in pyridine (2.5 mL) was added elemental sulfur (48 mg, 1.5 mmol) and TEA (0.17 mL, 1.25 mmol). The reaction was stirred at ambient temperature for 4 hours and concentrated in vacuo. The residue was dissolved in MeOH (2.5 mL). The sulfur was removed by filtration, and the filtrate was concentrated to give the crude product. Flash column chromatography on silica gel (CH_2Cl_2/MeOH 20:1 with 0.5% TEA) afforded the diastereomeric mixture of nucleoside-5′-thiophosphoramidate as light yellow oil.

2(*S*)-[(3′-Deoxy-3′-azidothymidin-5′-yl)thiophosphorylamino]-3-phenylproprionic acid methyl ester, triethylammonium salt (31). Starting from **11** (123 mg, 0.25 mmol) and sulfur (48 mg, 1.5 mmol), the diastereomeric mixture of compound **31** was synthesized according to the general procedure. Flash column chromatography afforded **31** (133 mg, 85%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.04 (br, 1H, Et_3NH^+), 9.38 (s, 1H, NH-3), 7.82, 7.68 (s, 1H, H-6), 7.24–7.11 (m, 5H, aromatic protons), 6.27, 6.23 (t, J = 6.6 Hz, 1H, H-1′), 4.34 (s, 1H, H-3′), 4.29–4.07 (m, 1H, H-4′), 3.98–3.79 (m, 2H, H-5′), 3.62, 3.60 (s, 3H, OCH_3), 3.57–3.48 (m, 1H, NH), 3.41–3.28 (m, 1H, H-α), 3.07 (q, 6H, $N(C\underline{H}_2CH_3)_3$), 3.00–2.80 (m, 2H, CH_2Ph), 2.31–2.11 (m, 2H, H-2′), 1.98 (s, 3H, CH_3-5), 1.26 (t, 9H, $N(CH_2C\underline{H}_3)_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.7, 164.2, 150.6, 137.3, 137.2, 136.3, 136.1, 129.5, 128.5, 128.4, 126.8, 126.7, 111.4, 84.7, 84.6, 83.5, 64.6, 64.5, 61.6, 61.5, 57.0, 56.5, 51.9, 51.7, 45.5, 40.9, 37.7, 37.6, 12.6, 12.4, 8.6 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 59.58, 58.87 ppm; IR (KBr):

ν_{max} 3434, 2677, 2108, 1640, 1475, 1274, 1109, 562 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{20}H_{24}N_6O_7PS$ $[M-H]^-$ 523.1; found 523.3.

2(*S*)-[(3′-Deoxy-3′-azidothymidin-5′-yl)thiophosphorylamino]-3-(3-indolyl)proprionic acid methyl ester, triethylammonium salt (32). Starting from **12** (133 mg, 0.25 mmol) and sulfur (48 mg, 1.5 mmol), the diastereomeric mixture of compound **32** was synthesized according to the general procedure. Flash column chromatography afforded **32** (138 mg, 83%) as light yellow oil; ^{1}H NMR (400 MHz, D_2O): δ 7.52, 7.50 (s, 1H, H-6), 7.31 (m, 2H, indole H-4, indole H-7), 7.13 (m, 1H, indole H-2), 7.08 (t, $J = 7.3$ Hz, 1H, indole H-6), 6.97 (m, 1H, indole H-5), 5.94 (t, $J = 6.7$ Hz, 1H, H-1′), 4.21–4.10 (m, 1H, H-3′), 4.07–3.93 (m, 2H, H-5′), 3.84–3.73 (m, 2H, H-4′, H-α), 3.68 (s, 3H, OCH_3), 3.20–2.93 (m, 8H, $N(CH_2CH_3)_3$, indole CH_2), 2.13–2.02 (m, 1H, H-2′), 1.86–1.76 (m, 1H, H-2′), 1.73, 1.68 (s, 3H, CH_3-5), 1.23 (t, 9H, $N(CH_2CH_3)_3$) ppm; ^{13}C NMR (100 MHz, D_2O): δ 177.4, 177.0, 166.3, 151.4, 136.7, 136.2, 126.9, 126.8, 124.2, 124.1, 121.9, 119.1, 118.2, 111.6, 109.9, 109.7, 84.6, 83.1, 82.9, 64.3, 63.9, 60.7, 60.6, 56.9, 55.6, 52.6, 52.5, 46.8, 36.5, 36.3, 29.7, 29.6, 11.8, 11.7, 8.3 ppm; ^{31}P NMR (162 MHz, D_2O): δ 58.80, 58.31 ppm; IR (KBr): ν_{max} 3786, 3416, 2934, 2347, 2106, 1686, 1457, 1276, 1106, 845, 654, 556 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{22}H_{25}N_7O_7PS$ $[M-H]^-$ 562.1; found 562.3.

2(*S*)-[(3′-Deoxy-3′-azidothymidin-5′-yl)thiophosphorylamino]-4-methylvaleric acid methyl ester, triethylammonium salt (33). Starting from **13** (115 mg, 0.25 mmol) and sulfur (48 mg, 1.5 mmol), the diastereomeric mixture of compound **33** was synthesized according to the general procedure. Flash column chromatography afforded **33** (124 mg, 84%) as light yellow oil; ^{1}H NMR (400 MHz, D_2O): δ 7.73 (s, 1H, H-6), 6.20 (m, 1H, H-1′), 4.44 (s, 1H, H-3′), 4.14 (s, 1H, H-4′), 4.09–3.93 (m, 2H, H-5′), 3.86–3.70 (m, 1H, H-α), 3.66 (s, 3H, OCH_3), 3.14 (q, 6H, $N(CH_2CH_3)_3$), 2.51–2.34 (m, 2H, H-2′), 1.92 (s, 3H, CH_3-5), 1.57 (m, 1H, $CHCH_2(CH_3)_2$), 1.43 (m, 2H, $CHCH_2(CH_3)_2$), 1.22 (t, 9H, $N(CH_2CH_3)_3$), 0.84–0.74 (m, 6H, $CHCH_2(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, D_2O): δ 180.6, 180.4, 169.8, 154.7, 139.8, 114.2, 87.5, 85.8, 85.7, 67.0, 66.6, 63.3, 56.4, 56.0, 55.0, 54.9, 49.2, 45.5, 39.0, 26.7, 24.3, 24.0, 23.9, 14.4, 10.8 ppm; ^{31}P NMR (162 MHz, D_2O): δ 57.43, 56.84 ppm; IR (KBr): ν_{max} 3791, 3428, 2859, 2677, 2492, 2358, 1463, 1375, 1274, 1106, 665, 559 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{17}H_{26}N_6O_7PS$ $[M-H]^-$ 489.1; found 489.3.

2(*S*)-[(3′-Deoxy-3′-azidothymidin-5′-yl)thiophosphorylamino]-3-methylbutyric acid methyl ester, triethylammonium salt (34). Starting from **14** (111 mg, 0.25 mmol) and sulfur (48 mg, 1.5 mmol), the diastereomeric mixture of compound **34** was synthesized according to the general procedure. Flash column chromatography afforded **34** (124 mg, 86%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.00 (br, 1H, Et_3NH^+), 9.67 (s, 1H, NH-3), 7.86, 7.69 (s, 1H, H-6), 6.28, 6.24 (t, $J = 6.2$ Hz, 1H,

H-1′), 4.46 (s, 1H, H-3′), 4.18–3.92 (m, 3H, H-5′, H-4′), 3.82–3.67 (m, 1H, H-α), 3.63, 3.61 (s, 3H, OCH_3), 3.44–3.23 (m, 1H, NH), 3.09 (q, 6H, $N(CH_2CH_3)_3$), 2.35–2.18 (m, 2H, H-2′), 1.96 (m, 4H, CH_3-5, $CH(CH_3)_2$), 1.28 (t, 9H, $N(CH_2CH_3)_3$), 0.89 (m, 3H, $CH(CH_3)_2$), 0.83 (d, $J = 6.4$ Hz, 3H, $CH(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.0, 174.9, 164.3, 164.2, 150.7, 150.6, 136.2, 135.9, 111.3, 84.5, 83.6, 64.8, 64.5, 61.6, 61.5, 60.7, 60.4, 51.6, 51.5, 45.5, 37.6, 37.5, 32.3, 32.2, 19.3, 19.2, 18.0, 12.5, 12.3, 8.6 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 60.67 ppm; IR (KBr): ν_{max} 3447, 2964, 2814, 2677, 2491, 1472, 1273, 1105, 998, 886, 732, 560 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{16}H_{24}N_6O_7PS$ $[M-H]^-$ 475.1; found 475.3.

2(*S*)-[(3′-Deoxy-3′-azidothymidin-5′-yl)thiophosphorylamino]-succinic acid dimethyl ester, triethylammonium salt (35). Starting from **15** (119 mg, 0.25 mmol) and sulfur (48 mg, 1.5 mmol), the diastereomeric mixture of compound **35** was synthesized according to the general procedure. Flash column chromatography afforded **35** (126 mg, 83%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.95 (br, 1H, Et_3NH^+), 9.08 (s, 1H, NH-3), 7.82, 7.71 (s, 1H, H-6), 6.29, 6.26 (t, $J = 6.7$ Hz, 1H, H-1′), 4.47 (s, 1H, H-3′), 4.43–4.24 (m, 1H, H-4′), 4.20–3.98 (m, 3H, H-5′, H-α), 3.70, 3.67 (s, 3H, OCH_3-α), 3.62, 3.61 (s, 3H, OCH_3-γ), 3.10 (q, 6H, $N(CH_2CH_3)_3$), 2.93–2.71 (m, 2H, CH_2-β), 2.32 (m, 2H, H-2′), 1.97 (s, 3H, CH_3-5), 1.31 (t, 9H, $N(CH_2CH_3)_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.5, 171.4, 164.1, 150.6, 136.2, 136.0, 111.3, 84.6, 83.6, 64.7, 64.5, 61.6, 61.5, 52.5, 52.3, 52.0, 51.8, 45.7, 39.1, 37.6, 12.5, 12.4, 8.6 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 60.10, 59.29 ppm; IR (KBr): ν_{max} 3774, 3416, 2982, 2877, 2494, 2356, 1439, 1277, 1105, 673, 556 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{16}H_{22}N_6O_9PS$ $[M-H]^-$ 505.1; found 505.2.

2(*S*)-[(2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl)thiophosphoryl amino]-3-phenylproprionic acid methyl ester, triethylammonium salt (36). Starting from **16** (112 mg, 0.25 mmol) and sulfur (48 mg, 1.5 mmol), the diastereomeric mixture of compound **36** was synthesized according to the general procedure. Flash column chromatography afforded **36** (124 mg, 85%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.22 (s, 1H, NH-3), 7.66, 7.48 (s, 1H, H-6), 7.24–7.08 (m, 5H, aromatic protons), 6.99, 6.95 (s, 1H, H-1′), 6.30–6.21 (m, 1H, H-2′), 5.71 (d, $J = 5.3$ Hz, 1H, H-3′), 4.87, 4.83 (s, 1H, H-4′), 4.31–3.62 (m, 3H, H-5′, H-α), 3.59, 3.55 (s, 3H, OCH_3), 3.33 (m, 1H, NH), 3.04 (q, 6H, $N(CH_2CH_3)_3$), 2.96–2.86 (m, 2H, CH_2Ph), 1.93 (s, 3H, CH_3-5), 1.25 (t, 9H, $N(CH_2CH_3)_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.7, 164.3, 151.1, 137.3, 137.2, 137.1, 136.8, 134.8, 134.7, 129.5, 128.4, 128.3, 126.7, 126.1, 126.0, 111.2, 89.8, 89.6, 85.9, 65.8, 56.9, 56.2, 51.8, 51.6, 45.6, 40.9, 12.5, 12.2, 8.6 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 59.51, 58.18 ppm; IR (KBr): ν_{max} 3749, 3416, 2948, 2623, 2376, 1463, 1251, 1113, 1087, 906, 738, 619 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{20}H_{23}N_3O_7PS$ $[M-H]^-$ 480.1; found 480.3.

2(*S*)-[(2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl)thiophosphoryl amino]-3-(3-indolyl)proprionic acid methyl ester, triethylammonium salt (37). Starting from **17** (122 mg, 0.25 mmol) and sulfur (48 mg, 1.5 mmol), the diastereomeric mixture of compound **37** was synthesized according to the general procedure. Flash column chromatography afforded **37** (130 mg, 84%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.08 (br, 1H, Et_3NH^+), 9.17, 9.13 (s, 1H, indole NH), 8.62 (s, 1H, NH-3), 7.67–7.44 (m, 2H, H-6, indole H-4), 7.31 (t, $J = 7.5$ Hz, 1H, indole H-7), 7.13–6.91 (m, 4H, indole H-6, indole H-2, indole H-5, H-1′), 6.20 (m, 1H, H-2′), 5.66 (m, 1H, H-3′), 4.87, 4.81 (s, 1H, H-4′), 4.35–3.75 (m, 3H, H-5′, H-α), 3.55, 3.50 (s, 3H, OCH_3), 3.42 (m, 1H, NH), 3.16–3.05 (m, 2H, indole CH_2), 2.98 (q, 6H, $N(CH_2CH_3)_3$), 1.93 (s, 3H, CH_3-5), 1.21 (t, 9H, $N(CH_2CH_3)_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.2, 175.0, 164.3, 164.2, 151.0, 137.1, 136.8, 136.3, 134.8, 134.7, 127.8, 127.7, 126.0, 125.9, 123.3, 123.1, 121.9, 119.3, 118.8, 118.7, 111.3, 111.2, 110.9, 89.8, 89.6, 85.9, 66.0, 55.9, 55.5, 51.9, 51.8, 45.5, 30.7, 12.5, 12.3, 8.6 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 58.08, 57.32 ppm; IR (KBr): ν_{max} 3730, 3384, 2930, 2675, 2484, 1462, 1252, 1112, 1039, 908, 741, 616 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{22}H_{24}N_4O_7PS$ $[M-H]^-$ 519.1; found 519.3.

2(*S*)-[(2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl)thiophosphoryl amino]-4-methylvaleric acid methyl ester, triethylammonium salt (38). Starting from **18** (104 mg, 0.25 mmol) and sulfur (48 mg, 1.5 mmol), the diastereomeric mixture of compound **38** was synthesized according to the general procedure. Flash column chromatography afforded **38** (118 mg, 86%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.16 (br, 1H, Et_3NH^+), 9.22 (s, 1H, NH-3), 7.73, 7.49 (s, 1H, H-6), 6.98 (d, $J = 8.0$ Hz, 1H, H-1′), 6.33 (s, 1H, H-2′), 5.71 (s, 1H, H-3′), 4.94 (s, 1H, H-4′), 4.32–3.70 (m, 3H, H-5′, H-α), 3.63, 3.60 (s, 3H, OCH_3), 3.30–2.94 (m, 7H, $N(CH_2CH_3)_3$, NH), 1.94 (s, 3H, CH_3-5), 1.67 (m, 1H, $CHCH_2(CH_3)_2$), 1.48–1.34 (m, 2H, $CHCH_2(CH_3)_2$), 1.28 (t, 9H, $N(CH_2CH_3)_3$), 0.82 (m, 6H, $CHCH_2(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.0, 164.3, 164.2, 151.0, 137.2, 136.8, 134.9, 134.8, 126.1, 125.9, 111.1, 89.7, 89.5, 86.0, 85.8, 65.8, 53.6, 53.2, 51.8, 51.6, 45.6, 44.2, 24.6, 24.5, 22.8, 22.7, 22.2, 12.5, 12.2, 8.6 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 59.53, 58.87 ppm; IR (KBr): ν_{max} 3729, 3384, 2951, 2875, 2677, 2493, 1470, 1253, 1089, 1039, 907, 736, 618 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{17}H_{25}N_3O_7PS$ $[M-H]^-$ 446.1; found 446.3.

2(*S*)-[(2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl)thiophosphoryl amino]-3-methylbutyric acid methyl ester, triethylammonium salt (39). Starting from **19** (100 mg, 0.25 mmol) and sulfur (48 mg, 1.5 mmol), the diastereomeric mixture of compound **39** was synthesized according to the general procedure. Flash column chromatography afforded **39** (116 mg, 87%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.12 (br, 1H, Et_3NH^+), 9.14 (s, 1H, NH-3), 7.68, 7.51 (s, 1H, H-6), 6.98, 6.95 (s, 1H,

H-1′), 6.35 (m, 1H, H-2′), 5.74 (s, 1H, H-3′), 4.95 (s, 1H, H-4′), 4.31–3.94 (m, 2H, H-5′), 3.71 (m, 1H, H-α), 3.68–3.58 (m, 4H, OCH_3, NH), 3.08 (q, 6H, $N(CH_2CH_3)_3$), 1.98–1.84 (m, 4H, CH_3-5, $CH(CH_3)_2$), 1.31 (t, 9H, $N(CH_2CH_3)_3$), 0.87 (m, 3H, $CH(CH_3)_2$), 0.83 (d, J = 6.7 Hz, 3H, $CH(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.4, 175.1, 164.3, 151.1, 151.0, 137.3, 137.0, 134.8, 126.0, 111.3, 89.9, 89.7, 86.0, 65.9, 61.0, 60.2, 51.8, 51.5, 45.7, 32.3, 32.2, 19.2, 18.2, 18.1, 12.4, 12.2, 8.7 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 60.10, 59.41 ppm; IR (KBr): ν_{max} 3731, 3595, 2936, 2742, 2677, 2492, 1470, 1254, 1089, 909, 780, 621 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{16}H_{23}N_3O_7PS$ $[M-H]^-$ 432.1; found 432.3.

2(*S*)-[(2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl)thiophosphoryl amino]succinic acid dimethyl ester, triethylammonium salt (40). Starting from **20** (108 mg, 0.25 mmol) and sulfur (48 mg, 1.5 mmol), the diastereomeric mixture of compound **40** was synthesized according to the general procedure. Flash column chromatography afforded **40** (119 mg, 84%) as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.54 (br, 1H, NH-3), 7.60, 7.48 (s, 1H, H-6), 7.05–6.89 (m, 1H, H-1′), 6.34 (m, 1H, H-2′), 5.77 (m, 1H, H-3′), 4.98 (s, 1H, H-4′), 4.40–3.93 (m, 3H, H-5′, H-α), 3.84–3.73 (m, 1H, NH), 3.68, 3.67 (s, 3H, OCH_3-α), 3.61, 3.59 (s, 3H, OCH_3-γ), 3.10 (q, 6H, $N(CH_2CH_3)_3$), 2.91–2.68 (m, 2H, CH_2-β), 1.93 (s, 3H, CH_3-5), 1.28 (t, 9H, $N(CH_2CH_3)_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.9, 173.7, 171.6, 164.5, 151.3, 137.2, 136.9, 134.7, 126.1, 111.4, 89.9, 89.8, 86.1, 86.0, 65.9, 52.6, 52.4, 51.9, 51.8, 45.6, 38.8, 12.5, 12.4, 8.7 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 59.77, 58.42 ppm; IR (KBr): ν_{max} 3730, 3424, 2954, 2680, 2487, 2377, 1738, 1470, 1225, 1088, 991, 839, 784, 617 cm^{-1}; LRMS (ESI−): *m/z* Calcd. for $C_{16}H_{21}N_3O_9PS$ $[M-H]^-$ 462.1; found 462.2.

REFERENCES

1. (a) De Clercq, E. Molecular targets for antiviral agents. *J. Pharmacol. Exp. Ther.* **2001**, 297, 1–10. (b) De Clercq, E. Strategies in the design of antiviral drugs. *Nat. Rev. Drug Discov.* **2002**, 1, 13–25.
2. (a) Parang, K.; Wiebe, L.I.; Knaus, E.E. Novel approaches for designing 5′-*O*-ester prodrugs of 3′-azido-2′,3′-dideoxythymidine (AZT). *Curr. Med. Chem.* **2000**, 7, 995–1039. (b) Schultz, C. Prodrugs of biologically active phosphate esters. *Bioorg. Med. Chem.* **2005**, 11, 885–898. (c) Hecker, S.J.; Erion, M.D. Prodrugs of phosphates and phosphonates. *J. Med. Chem.* **2008**, 51, 2328–2345. (d) Bobeck, D.R.; Schinazi, R.F.; Coats, S.J. Advances in nucleoside monophosphate prodrugs as anti-HCV agents. *Antivir. Ther.* **2010**, 15, 935–950.
3. (a) McGuigan, C.; Pathirana, R. N.; Balzarini, J.; De Clercq, E. Intracellular delivery of bioactive AZT nucleotides by aryl phosphate derivatives of AZT. *J. Med. Chem.* **1993**, 36, 1048–1052. (b) McGuigan, C.; Cahard, D.; Sheeka, H. M.; De Clercq, E.; Balzarini, J. Phosphoramidate derivatives of d4T with improved anti-HIV efficacy retain full activity in thymidine kinase-deficient cells. *Bioorg. Med. Chem. Lett.* **1996**, 6, 1183–1186. (c) McGuigan, C.; Cahard, D.; Sheeka, H. M.; De Clercq, E.; Balzarini, J. Aryl phosphoramidate derivatives of d4T have improved anti-HIV efficacy in tissue culture and may act by the generation of a novel intracellular metabolite. *J. Med. Chem.* **1996**, 39, 1748–1753. (d) Siddiqui, A. Q.; McGuigan, C.; Ballatore, C.; Zuccotto, F.; Gilbert, I. H.; De Clercq, E.; Balzarini, J. Design and synthesis of lipophilic phosphoramidate d4T-MP prodrugs expressing high potency against HIV in cell culture: structural determinants for in vitro activity and QSAR. *J. Med. Chem.* **1999**,

42, 4122–4128. (e) Mehellou, Y.; Balzarini, J.; McGuigan, C. Aryloxy phosphoramidate triesters: a technology for delivering monophosphorylated nucleosides and sugars into cells. *Chem. Med. Chem.* **2009**, 4, 1779–1791.

4. (a) Abraham, T. W.; Wagner, C. R. A phosphoramidite-based synthesis of phosphoramidate amino acid diester of antiviral nucleosides. *Nucleos. Nucleot.* **1994**, 13, 1891–1903. (b) McIntee, E. J.; Remmel, R. P.; Schinazi, R. F.; Abraham, T. W.; Wagner, C. R. Probing the mechanism of action and decomposition of amino acid phosphomonoester amidates of antiviral nucleoside prodrugs. *J. Med. Chem.* **1997**, 40, 3323–3331. (c) Wagner, C. R.; Chang, S. L.; Griesgraber, G. W.; Song, H.; McIntee, E. J.; Zimmerman, C. L. Antiviral nucleoside drug delivery via amino acid phosphoramidates. *Nucleos. Nucleot.* **1999**, 18, 913–919.
5. (a) Adelfinskaya, O.; Herdewijn, P. Amino acid phosphoramidate nucleotides as alternative substrates for HIV-reverse transcriptase. *Angew. Chem. Int. Ed.* **2007**, 46, 4356–4358. (b) Zlatev, I.; Giraut, A.; Morvan, F.; Herdewijin, P.; Vasseur, J. δ-Dicarboxybutyl phosphoramidate of 2′-deoxycytidine-5′-monophosphate as substrate for DNA polymerization by HIV-1 reverse transcriptase. *Bioorg. Med. Chem.* **2009**, 17, 7008–7014.
6. (a) Abraham, T. W.; Kalman, T. I.; McIntee, E. J.; Wagner, C. R. Synthesis and biological activity of aromatic amino acid phosphoramidates of 5-fluoro-2′-deoxyuridine and 1-β-arabinofuranosylcytosine: evidence of phosphoramidase activity. *J. Med. Chem.* **1996**, 39, 4569–4575. (b) Maiti, M.; Michielssens, S.; Dyubankova, N.; Maiti, M.; Lescrinier, E.; Ceulemans, A.; Herdewijn, P. Influence of the nucleobase and anchimeric assistance of the carboxyl acid groups in the hydrolysis of amino acid nucleoside phosphoramidates. *Chem. Eur. J.* **2012**, 18, 857–868.
7. Iyer, V. V.; Griesgraber, G. W.; Radmer, M. R.; McIntee, E. J.; Wagner, C. R. Synthesis, in vitro anti-breast cancer activity, and intracellular decomposition of amino acid methyl ester and alkyl amide phosphoramidate monoesters of 3′-azido-3′-deoxythymidine (AZT). *J. Med. Chem.* **2000**, 43, 2266–2274.
8. (a) Cho, J. H.; Amblard, F.; Coats, J. S.; Schinazi, R. F. Efficient synthesis of nucleoside aryloxy phosphoramidate prodrugs utilizing benyloxycarbonyl protection. *Tetrahedron* **2011**, 67, 5487–5493. (b) Bao, D. H.; Chang, W.; Nagarathnam, D.; Sofia, M. J. Nucleoside analogs. US Patent 20100286083, 2010.
9. (a) Miao, Z. W.; Fu, H.; Han, B.; Zhao, Y. F. One pot synthesis of nucleoside 5′-thiophosphoramidates. *Synth. Commun.* **2002**, 32, 1069–1076. (b) Miao, Z. W.; Fu, H.; Han, B.; Chen, Y.; Zhao, Y. F. A stepwise one pot synthesis of alkyl thiophosphoramidate derivatives of nucleosides. *Synth. Commun.* **2002**, 32, 1159–1167. (c) Liu, W.; Zhang, L.; Zhou, H.; Yang, C.; Miao, Z. W.; Zhao, Y. F. Synthesis of novel nucleoside analogue phosphorothioamidate prodrugs and in vitro anticancer evaluation against RKO human colon carcinoma cells. *Nucleos. Nucleot. Nucl.* **2013**, 32, 161–173.
10. (a) Baraniak, J.; Kaczmarek, R.; Stec, W. J. Conjugation of amino acid *O*-methyl esters with AZT-5′-*O*-phosphorothioate and phosphorodithioate. *Tetrahedron Lett.* **2000**, 41, 9139–9142. (b) Baraniak, J.; Kaczmarek, R.; Korczynski, D.; Wasilewska, E. Oxathiaphospholane approach to *N*- and *O*-phosphorothioylation of amino acids. *J. Org. Chem.* **2002**, 67, 7267–7274.
11. (a) Li, P.; Shaw, B. R. Synthesis of nucleoside boranophosphoramidate prodrugs conjugated with amino acids. *J. Org. Chem.* **2005**, 70, 2171–2183. (b) Baraniak, J.; Kaczmarek, R.; Wasilewska, E. Synthesis of nucleoside–amino acid conjugates containing boranephosphate, boranephosphorothioate and boranephosphoramidate linkages. *Tetrahedron Lett.* **2004**, 45, 671–675. (c) Li, P.; Shaw, B. R. Model synthesis of nculeoside boranophosphoramidate with amino acid for prodrug purpose. *Nucleos. Nucleot. Nucl.* **2005**, 24, 675–678.
12. Kers, I.; Stawinki, J. Aryl *H*-phosphonates. 10. Synthesis of nucleoside phosphoramidate and nucleoside phosphoramidothioate analogues via *H*-phosphonamidate intermediates. *Tetrahedron Lett.* **1999**, 55, 11579–11588.
13. Sobkowska, A.; Sobkowski, M.; Cieslak, J.; Kraszewski, A. Aryl *H*-phosphonates. 6. Synthetic studies on the preparation of nucleoside *N*-alkyl-*H*-phosphonamidates. *J. Org. Chem.* **1997**, 62, 4791–4794.
14. (a) Garegg, P. J.; Regberg, T.; Stawinski, J.; Stromberg, R. Nucleoside phosphonates: Part 7. Studies on the oxidation of nucleoside phosphonate esters. *J. Chem. Soc. Perkin Trans. 1* **1987**, 1269–1273. (b) Lindh, I.; Stawinski, J. A general method for the synthesis of glycerophospholipids and their analogs via *H*-phosphonate intermediates. *J. Org. Chem.* **1989**, 54, 1338–1342. (c) Xiao, Q.; Sun, J.; Sun, Q.; Ju, Y.; Zhao, Y. F.; Cui, Y. X. Synthesis of AZT 5′-*O*-hydrogen phospholipids and their derivatives. *Synthesis* **2003**, 1, 107–111.

15. (a) Hecker, S. J.; Minich, M. L.; Lackey, K. Synthesis of compounds designed to inhibit bacterial cell wall transglycosylation. *J. Org. Chem.* **1990**, 55, 4904–4911. (b) Sinha, N. D.; Biernat, J.; Koester, H. β-Cyanoethyl *N*,*N*-dialkylamino/*N*-morpholinomonochloro phosphoamidites, new phosphitylating agents facilitating ease of deprotection and work-up of synthesized oligonucleotides. *Tetrahedron Lett.* **1983**, 24, 5843–5846.
16. Sun, X. B.; Kang, J. X.; Zhao, Y. F. One-pot synthesis of hydrogen phosphonate derivatives of d4T and AZT. *Chem. Commun.* **2002**, 2414–2415.
17. (a) Garegg, P. J.; Regberg, T.; Stawinski, J.; Stromberg, R. Nucleoside hydrogenphosphonates in oligonucleotide synthesis. *Chem. Scr.* **1986**, 26, 59–62. (b) Nikolaev, A. V.; Rutherford, T. J.; Ferguson, M. A. J.; Brimacombe, J. S. Parasite glycoconjugates. Part 5. Blockwise approach to oligo(glycosyl phosphates): chemical synthesis of a terminal tris(glycobiosyl phosphate) fragment of *Leishmania donovani* antigenic lipophosphoglycan. *J. Chem. Soc. Perkin Trans. 1* **1996**, 1559–1566.