Synthesis of Dinucleotide Thiophosphoramidates as Anti-HIV New Prodrugs

Changxue Lin,[a] Hua Fu,*[a] Guangzhong Tu,[b] Yufen Zhao*[a]

[a] The Key Laboratory of Bioorganic Phosphorus Chemistry, Ministry of Education, Department of Chemistry, School of Life Sciences and Engineering, Tsinghua University, Beijing 100084, P. R. China
Fax +86(10)62781695; E-mail: fuhua@mail.tsinghua.edu.cn

[b] Beijing Institute of Microchemistry, Beijing 100091, P. R. China

Received 6 May 2003; revised 16 June 2003

Abstract: Sequential transesterification of diphenyl phosphite with 5′-*O*-(4,4′-dimethoxytrityl)thymidine (**1**) and hydrogen sulfide gave *O*-[5′-*O*-(4,4′-dimethoxytrityl)thymidine-3′-yl] *H*-thiophosphonate (**2**), and subsequent condensation of **2** with AZT or d4T in the presence of diphenyl chlorophosphoate provided the dinucleotide *H*-thiophosphonates **3** or **3′**. The Antherton–Todd reaction of **3** or **3′** with L-amino acid methyl ester in a solution of CCl_4–Et_3N–H_2O–MeCN gave the dinuleotide thiophosphoramidates **4** or **4′**, and removal of the dimethoxytrityl protecting group in formic acid yielded the target products AZT/d4T thiophosphoramidates **5** and **5′**.

Key words: nucleotides, phosphorus, antiviral agents, esterification, drugs

In combating acquired immunodeficiency syndrome (AIDS) and its related complex, the search for therapeutic agents possessing activity against human immunodeficiency virus (HIV) has yielded a number of compounds demonstrating potent and selective antiviral activity. Despite the recent introduction of HIV protease[1] and integrase,[2] reverse transcriptase is an attractive target for the chemotherapy of human immunodeficiency virus (HIV).[3] Among the current diversity of compounds against HIV, the 2′,3′-dideoxynucleosides (ddNs) remain by far the most potent,[4] and 3′-azido-2′,3′-dideoxythymidine (AZT, zidovudine) and 2′,3′-dideoxythymidine (d4T, stavudine) are prime (Figure 1).

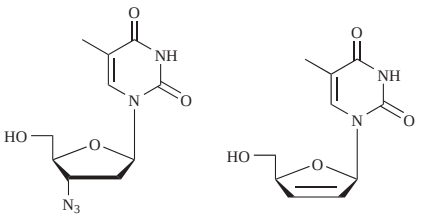


Figure 1

It has been proven that ddNs must be converted into their 5′-*O*-triphosphate analogues (ddNs-TP) by a cellular enzyme to inhibit HIV reverse transcriptase, by competitive inhibition of the viral reverse transcriptase and/or incorporation and subsequent chain termination of growing viral DNA strands.[5] The major limitations of AZT/d4T are due to clinical toxicity[6] and to the development of AZT/d4T resistance by HIV.[7] In an attempt to overcome these problems, numerous chemical strategies have been developed by medicinal chemists for designing AZT/d4T prodrugs, which mainly included 5′-*O*-carboxylic ester derivatives and 5′-*O*-masked phosphonates.[8] The expected advantages of these 5′-substituted prodrugs can be multiple improvement in anti-HIV activity, synergistic drug interaction, enhancement of intracellular uptake and decrease of toxicity.

Among the prodrugs, dinucleotide phosphate derivatives have attracted great attention. Various homo- and heterodinucleotides, such as AZT-P-ddI, AZT-P-ddZ, AZT-P-AZT, have been synthesized and tested for HIV-infected MT-2 cells[9]. It was found that dinucletide analogues showed enhanced anti-HIV potency relative to monomers. In addition, AZT-P-ddI was 10 times less toxic than AZT to human granulocyte macrophage progenitor cells.[10] Almost all the dinucleotide prodrugs contain two unnatural nucleosides, namely 2′,3′-dideoxynucleside (ddNs). Because the cellular kinases involved in activating the nucleoside prodrugs are usually specific,[11] it is thought that the replacement of ddNs with natural nucleosides, such as thymidine, could improve the rate of phosphorylation and inhibit the HIV-RT. On the other hand, the substitution of a single non-bridging oxygen atom with a sulfur atom renders the internucleotide linkage nuclease resistant and ensures that thymidine is not hydrolyzed.[12] Therefore, the dinucleotide analogues could be phosphorylated at the 5′-position of thymidine and directly linked to the DNA chains. In order to improve the lipophilicity and member-penetration, we introduced amino acid methyl esters in the molecules. McGuigan et al.[13] suggested that HIV-aspartate proteinase could recognize phosphonoamidate derivatives of certain nucleosides and thus specifically hydrolyze these membrane-soluble prodrugs. The resultant bioactive nucleotides would then be trapped inside the infected cells and act as potent inhibitors of viral proliferation. Taking into account these findings, we synthesized dinucleotide thiophosphoramidates containing AZT or d4T as new anti-HIV prodrugs, as described in this paper.

Dinucleotide thiophosphoramidates were prepared as shown in Scheme 1. Diphenyl phosphite reacted with 5′-*O*-dimethoxytritylthymidine (**1**) in anhyd pyridine for 20

Synthesis 2003, No. 13, Print: 18 09 2003. Web: 28 08 2003.
Art Id.1437-210X,E;2003,0,13,1989,1994,ftx,en;F04103SS.pdf.
DOI: 10.1055/s-2003-41039

minutes, and then mixtures of H_2S and triethylamine in dioxane were added to the resulting solution at room temperature under a nitrogen atmosphere to give the product **2**. The coupling of **2** with AZT or d4T by diphenyl chlorophosphoate led to dinucleotide *H*-thiophosphate **3**. Atherton–Todd reaction[14] of **3** with L-amino acid methyl ester in CCl_4–Et_3N–H_2O–MeCN solution at room temperature gave product **4** or **4′**. Product **5** or **5′** was obtained as a white foam after 5′-deprotection of **4** or **4′** in formic acid and purification on silica gel column chromatography.

In conclusion, dinucleotide thiophosphoramidates [(2*R*,4*S*,5*R*)-1-(4-Azidotetrahydro -5-{[(3′-*O*-thymidinyl)(methoxy-L-alaninyl)phosphoryl]methyl-2-furyl}thymine (diastereoisomeric mixture) (**5**) and (2*R*,4*S*,5*R*)-1-(2,5-dihydro-5-{[(3′-*O*-thymidinyl) (methoxy-L-alaninyl)phosphoryl]methyl-2-furyl}thymine (diastereoisomeric mixture) (**5′**)] were synthesized through Atherton–Todd reaction of dinucleotide *H*-thiophosphonates with amino acid methyl esters. Compounds **5** and **5′** were obtained as mixtures of diastereoisomers due to the chirality of phosphorus. The ratio of the two isomers was almost 1:1, as judged from ^{31}P and ^{1}H NMR. Their structures were confirmed by ^{31}P, ^{1}H, ^{13}C NMR and ESI-MS. Anti-HIV activity of these compounds is in progress. The details of the pharmacological properties will be described elsewhere.

Column chromatography was performed using silica gel 300–400 mesh. Pyridine was dried over CaH_2 by refluxing for 4–5 hours. ^{1}H and ^{13}C NMR spectra were recorded (tetramethylsilane as internal standard) on a Bruker AM 500 spectrometer using CD_3OD as the solvent. ^{31}P NMR spectra were taken on a Bruker AC 200 spectrometer at 81 MHz under ^{1}H decoupled conditions. ^{31}P NMR chemical shifts were reported in ppm downfield (+) or upfield (–) from external 85% H_3PO_4 as reference. Mass spectra were obtained using a Bruker Esquire ion-trap mass spectrometer in positive ion mode.

5′-*O*-Dimethoxytritylthymidine (1)

The starting material 5′-dimethoxytritylthymidine was prepared according to the published procedure.[15]

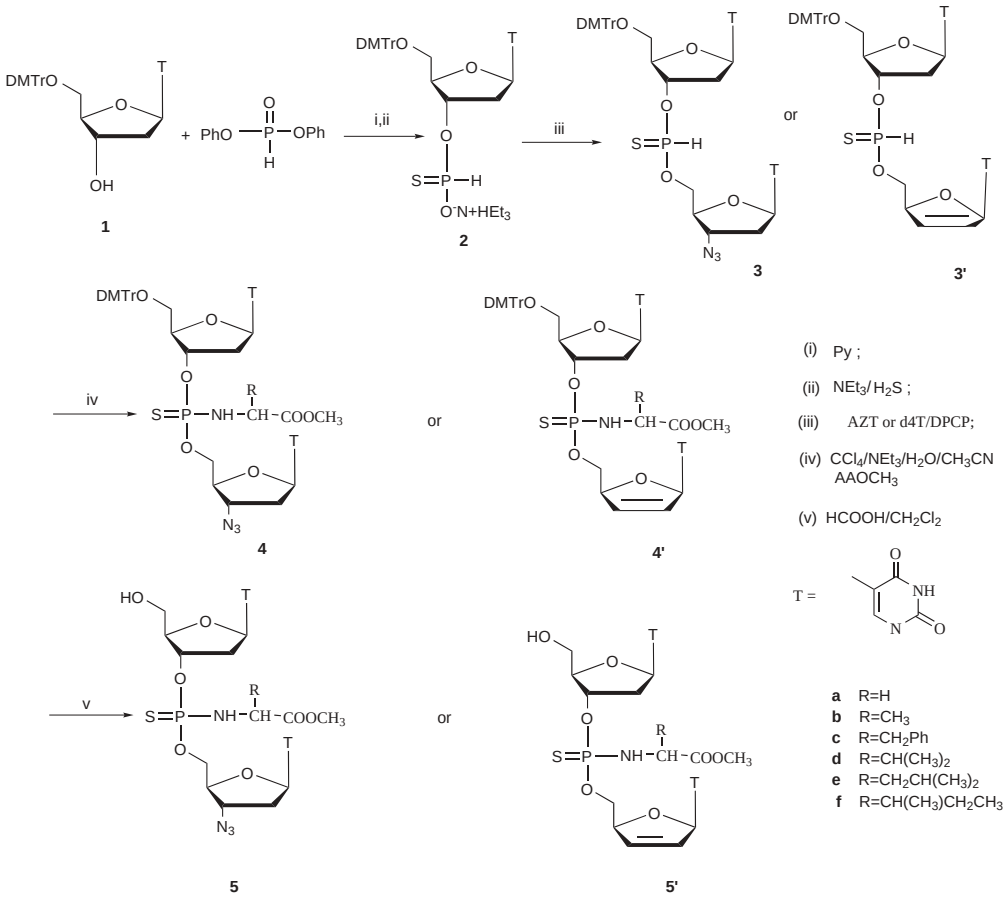
Yield: 95%; mp 126–129 °C

***O*-[5′-*O*-(4,4′-Dimethoxytrityl)thymidine-3′-yl] *H*-Thiophosphonate (2)**

According to the literature,[16] 5′-dimethoxytritylthymidine in anhyd pyridine was added to diphenyl phosphite in pyridine solution at room temperature, and the reaction lasted for 20 min. Et_3N and H_2S (1:1) dioxane solution (10 mL) was added to the reaction solution, and the reaction continued for a further 20 minutes. After evaporation of the solvent, **2** was obtained after column chromatography.

Yield: 84%; brittle yellow foam.

^{1}H NMR ($CDCl_3$): δ = 9.23 (d, *J* = 581 Hz, 1 H, PH), 6.96–7.86 (m, 14 H, H-6), 6.10–6.20 (m, 1 H, H-1′), 4.82 (m, 1 H, H-3′), 3.71–3.74 (m, 3 H, H4′, 2 × H-5′), 3.68 (d, 6 H, *J* = 1.2 Hz, OCH_3), 2.78–2.82 (m, 2 H, H-2′), 1.68 (d, 3 H, *J* = 8.06 Hz, C5-CH_3).



Scheme 1

^{13}C NMR ($CDCl_3$): δ = 165.0, 159.4 (C-4), 151.9 (C-2), 135.7 (C-6), 130.9–114.1 (Ph), 111.4–111.3 (C-5), 85.5 (C-4′), 85.1(C-1′), 76.2(C-3′), 64.8 (C-5′), 55.2 (OCH_3), 40.2 (C-2′), 12.3 (CH_3).

^{31}P NMR ($CDCl_3$): δ = 55.8, 55.2.

ESI-MS: *m*/*z* = 726 $[M + H]^+$.

Dinucleotide *H*-Thiophosphonate; General Procedure

5′-*O*-Dimethoxytritylthymidine 3′-phosphonothioate (**2**) (0.8g, 1.1mmol) and AZT/d4T (1 mmol) were dissolved in anhyd pyridine and co-evaporated twice. The residue was then dissolved in anhyd pyridine (10 mL), diphenyl chlorophosphoate (684 L, 3.3 mmol) was added dropwise and the reaction mixture was stirred at r.t. for 10 min. After addition of a few drops of H_2O, and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (25 mL) and washed with sat. aq NaCl. The organic phase was dried (Na_2SO_4). The crude product was purified with column chromatography. After concentration the product was obtained as white foam.

***O*-(5′-Dimethoxytrityl-2′-deoxythymidin-3′-yl)-*O*-(3′-azido-2′-deoxythymidin-5′-yl) *H*-Thiophosphonate (3)**

Yield: 0.635g (72.7%); R_f 0.4 ($CHCl_3$–MeOH, 40:1).

^{1}H NMR (CD_3COCD_3): δ = 10.27–10.26 (m, 2 H, ^{3}NH), 7.63 (d, 1 H, PH, J_{PH} = 673 Hz), 7.58–6.79 (m, 15 H, H-6, Ph), 6.42–6.35 (m, 1 H, H-1′), 6.19–6.17 (m, 1 H, H-1′), 5.23–5.19 (m, 1 H, H-3′), 4.52–4.49 (m, 1 H, H-3′), 4.42–4.32 (m, 4 H, 2 × H-4′, H-5′), 3.76–3.72 (m, 2 H, H-5′), 2.54–2.36 (m, 4 H, 2 × H-2′), 1.49–1.46 (m, 6 H, 2 × C5-CH_3).

^{13}C NMR (CD_3COCD_3): δ = 166.53 (2 × C-4), 152.48, 152.37 (2 × C-2), 137.96, 137.89, 137.81 (2 × C-6), 130.6–114.9 (Ph), 111.88–111.83 (2 × C-5), 87.52–86.08 (T-C-4′, 2 × C-1′), 83.86, 83.78 (AZT-C-4′), 79.85, 79.42 (T-C-3′), 67.06, 66.79 (AZT-C-5′), 62.93, 62.85 (T-C-5′), 62.18, 61.98 (AZT-C-3′) 55.8 (OCH_3), 39.85, 39.66 (T-C-2′), 37.91 (AZT-C-2′), 12.66, 2.47 (2 × C5-CH_3).

^{31}P NMR (CD_3COCD_3): δ = 72.17, 71.01 (J_{PH} = 671 Hz) (mixture of diastereoisomers).

ESI-MS: *m*/*z* =874 $[M + H]^+$, 896 $[M + Na]^+$.

***O*-(5′-Dimethoxytrityl-2′-deoxythymidin-3′-yl)-*O*-(2′,3′-dideoxythymidin-5′-yl) *H*-Thiophosphonate (3′)**

Yield: 0.575g (69.3%); R_f = 0.4 ($CHCl_3$–MeOH, 30:1).

^{1}H NMR (CD_3COCD_3): δ = 10.26–10.24 (m, 2 H, ^{3}NH), 7.65 (d, 1 H, PH, J_{PH} = 682 Hz), 7.82 (s, 1 H, H-6), 7.59–6.82 (m, 15 H, H-6, d4T-H-1′, Ph), 6.43–6.41 (m, 1 H, d4T-H-2′), 6.30–6.28 (m, 1 H, H-1′), 5.98, 5.97 (s, 1 H, d4T-H-3′), 5.19–5.16 (m, 1 H, T-H-3′), 5.03 (s, 1 H, d4T-H-4′), 4.28–4.19 (m, 3 H, 2 × d4T-H-5′, T-H-4′), 3.80–3.73 (m, 2 H, T-H-5′), 2.52–2.28 (m, 2 H, T-H-2′), 1.93–1.87 (m, 6 H, 2 × 5-CH_3).

^{13}C NMR (CD_3COCD_3): δ = 166.45, 166.37 (2 × C-4), 152.84, 152.37, 152.34 (2 × C-2), 138.02, 137.92, 137.88 (2 × C-6), 134.77 (d4T-C-2′), 130.6–114.2 (Ph, d4T-C-3′, 2 × C-5), 91.34, 91.11 (d4T-C-1′), 87.54, 87.29 (T-C-4′), 86.36–86.06 (T-C-1′, d4T-C-4′), 79.78, 79.12 (T-C-3′), 68.48, 68.17 (d4T-C-5′), 62.89, 62.75 (T-C-5′), 55.9 (OCH_3), 39.87, 39.67 (T-C-2′), 12.74, 12.67, 12.45 (5-CH_3).

^{31}P NMR (CD_3COCD_3): δ = 73.29, 72.13 (J_{PH} = 682 Hz, mixture of diastereoisomers).

ESI-MS: *m*/*z* = 831 $[M + H]^+$, 853 $[M + Na]^+$.

Thiophosphoramidates 5 and 5′; General Procedure

Dinucleotide *H*-thiophosphonate (0.2 mmol) in MeCN (2 mL) was added dropwise to L-amino acid methyl ester hydrochloride (0.22mmol) in solution [H_2O (100 μL), Et_3N (70 μL), CCl_4 (100 μL) and MeCN (5mL)] and stirred at r.t. until the *H*-thiophosphonate disappeared (^{31}P NMR determination). The crude product **4** or **4′** was obtained after the solvents were removed by rotary evaporation, and then treated with HCO_2H and CH_2Cl_2 until complete deprotection (TLC determination). The solution was neutralized with a few drops of sat. aq. $NaHCO_3$, and then concentrated under reduced pressure. The product **5** or **5′** was purified by column chromatography (CH_2Cl_2–MeOH, from 30:1 to 15:1).

(2*R*,4*S*,5*R*)-1-{4-Azidotetrahydro-5-[(3′-*O*-thymidinyl)(methoxy-L-glycinyl)phosphoryl]methyl-2-furyl}thymine (Diastereoisomeric Mixture) (5a)

Yield: 76.7%; R_f = 0.4 (CH_2Cl_2–MeOH, 30:1).

^{1}H NMR (CD_3OD, 500 MHz): δ = 7.83, 7.82 (s, 1 H, T-H-6), 7.60, 7.58 (s, 1 H, AZT-H-6), 6.31–6.27 (m, 1 H, T-H-1′), 6.18–6.15 (m, 1 H, AZT-H-1′), 5.19–5.16 (m, 1 H, T-H-3′), 4.48–4.45 (m, 1 H, AZT-H-3′), 4.28–4.22 (m, 4 H, 2 × H-4′, 2 × AZT-H-5′), 3.86–3.78 (m, 4 H, 2 × T-H-5′, CH_2), 3.71, 3.70 (s, 3 H, OCH_3), 2.53–2.30 (m, 4 H, 2 × H-2′), 1.93–1.87 (m, 6 H, 2 × C5-CH_3).

^{13}C NMR (CD_3OD, 125 MHz): δ = 175.32, 173.22 (COO), 166.33 (2 × C-4), 152.38, 152.22 (2 × C-2), 137.88, 137.82, 137.72 (2 × C-6), 111.96–111.85 (2 × C-5), 87.54–86.04 (T-C-4′, 2 × C-1′), 83.92, 83.79 (AZT-C-4′), 79.83, 79.36 (T-C-3′), 67.06, 66.75 (AZT-C-5′), 62.87, 62.75 (T-C-5′), 62.17, 61.94 (AZT-C-3′), 52.65 (OCH_3), 44.45, 44.15 (CH_2), 39.84, 39.65 (T-C-2′), 37.95 (AZT-C-2′), 12.67, 2.49 (2 × C5-CH_3).

^{31}P NMR (CD_3OD, 81 MHz): δ = 76.19, 75.21.

ESI-MS: *m*/*z* = 659 $[M + H]^+$, 681 $[M + Na]^+$.

(2*R*,4*S*,5*R*)-1-{4-Azidotetrahydro-5-[(3′-O-thymidinyl)(methoxy-L-alaninyl)phosphoryl]methyl-2-furyl}thymine (Diastereoisomeric Mixture) (5b)

Yield: 62.8%; R_f 0.4 (CH_2Cl_2–MeOH, 30:1).

^{1}H NMR (CD_3OD, 500 MHz): δ = 7.83, 7.82 (s, 1 H, T-H-6), 7.60, 7.58 (s, 1 H, AZT-H-6), 6.31–6.27 (m, 1 H, T-H-1′), 6.18–6.15 (m, 1 H, AZT-H-1′), 5.19–5.16 (m, 1 H, T-H-3′), 4.48–4.45 (m, 1 H, AZT-H-3′), 4.28–4.02 (m, 5 H, 2 × H-4′, 2 × AZT-H-5′, CH), 3.80–3.78 (m, 2 H, 2 × T-H-5′), 3.71, 3.70 (s, 3 H, OCH_3), 2.56–2.27 (m, 4 H, 2 × H-2′), 1.92–1.87 (m, 6 H, 2 × C5-CH_3), 0.92–0.88 (m, 3 H, CH_3).

^{13}C NMR (CD_3OD, 125 MHz): δ = 175.82 (COO), 166.33 (2 × C-4), 152.39, 150.18 (2 × C-2), 137.89, 137.78, 137.67 (2 × C-6), 112.00–111.86 (2 × C-5), 87.47–86.02 (T-C-4′, 2 × C-1′), 83.83, 83.75 (AZT-C-4′), 79.94, 79.24 (T-C-3′), 67.03, 66.55 (AZT-C-5′), 62.88, 62.72 (T-C-5′), 62.13, 61.80 (AZT-C-3′) 52.78 (OCH_3), 52.40, 51.99 (CH), 39.71 (T-C-2′), 37.87, 37.81 (AZT-C-2′), 20.35, 20.29 (CH_3), 12.68, 12.64, 12.48 (2 × C5-CH_3).

^{31}P NMR (CD_3OD, 81 MHz): δ = 74.29, 73.24.

ESI-MS: *m*/*z* = 673 $[M + H]^+$, 695 $[M + Na]^+$.

(2*R*,4*S*,5*R*)-1-{4-Azidotetrahydro-5-[(3′-*O*-thymidinyl)(methoxy-L-phenylalaninyl)phosphoryl]methyl-2-furyl}thymine (Diastereoisomeric Mixture) (5c)

Yield: 63.9%; R_f 0.4 (CH_2Cl_2–MeOH, 30:1).

^{1}H NMR (CD_3OD, 500 MHz): δ = 7.83 (s, 1 H, T-H-6), 7.58, 7.53 (s, 1 H, AZT-H-6), 6.31–6.29 (m, 1 H, T-H-1′), 6.15–6.12 (m, 1 H, AZT-H-1′), 5.15–5.10 (m, 1 H, T-H-3′), 4.48–4.42 (m, 1 H, AZT-H-3′), 4.27–4.07 (m, 4 H, 2 × H-4′, 2 × AZT-H-5′), 3.92–3.79 (m, 3 H, 2 × T-H-5′, NHC*H*), 3.72, 3.71 (s, 3 H, OCH_3), 2.56–2.28 (m, 4 H, 2 × H-2′), 2.09–2.01 [m, C*H*$(CH_3)_2$], 1.98, 1.87 (s, 6 H, 2 × C5-CH_3), 0.98–0.92 (m, 6 H, 2 × CH_3).

^{13}C NMR (CD_3OD, 125 MHz): δ = 175.10, 175.05 (COO), 166.33 (2 × C-4), 152.39, 150.17 (2 × C-2), 137.88, 137.78, 137.71 (2 × C-

6), 112.02–111.92, 111.72 (2 × C-5), 87.50–86.05 (T-C-4′, 2 × C-1′), 83.77, 83.67 (AZT-C-4′), 80.25, 79.33 (T-C-3′), 67.33, 66.67 (AZT-C-5′), 62.91, 62.75 (T-C-5′), 62.16, 62.10 (AZT-C-3′), 61.79 (NHC*H*), 52.53, 52.51 (OCH_3), 39.78 (T-C-2′), 37.78 (AZT-C-2′), 33.04, 32.98 [*C*H(CH_3)$_2$], 19.70, 18.63 (CH_3), 12.69, 12.51 (2 × C5-CH_3).

^{31}P NMR (CD_3OD, 81 MHz): δ = 74.47, 72.71.

ESI-MS: *m*/*z* = 749 $[M + H]^+$, 771 $[M + Na]^+$.

(2*R*,4*S*,5*R*)-L-{4-Azidotetrahydro-5-[(3′-*O*-thymidinyl)(methoxy-L-valinyl)phosphoryl]methyl-2-furyl}thymine (Diastereoisomeric Mixture) (5d)

Yield: 65.1%; R_f 0.4 (CH_2Cl_2–MeOH, 30:1).

^{1}H NMR (CD_3OD, 500 MHz): δ = 7.78 (s, 1 H, T-H-6), 7.47, 7.43 (s, 1 H, AZT-H-6), 7.28–7.17 (m, 5 H, Ph), 6.24–6.20 (m, 1 H, T-H-1′), 6.13–6.07 (m, 1 H, AZT-H-1′), 5.03–4.96 (m, 1 H, T-H-3′), 4.29–4.27 (m, 1 H, AZT-H-3′), 4.22–3.87 (m, 4 H, 2 × H-4′, 2 × AZT-H-5′), 3.76–3.74 (m, 3 H, 2 × T-H-5′, NHC*H*), 3.70, 3.69 (s, 3 H, OCH_3), 3.18–2.81 (m, 2 H, CH_2), 2.46–2.14 (m, 4 H, 2 × H-2′), 1.89–1.85 (m, 6 H, 2 × C5-CH_3).

^{13}C NMR (CD_3OD, 125 MHz): δ = 175.07, 174.96 (COO), 166.32 (2 × C-4), 152.35, 152.16 (2 × C-2), 138.60–127.96 (2 × C-6, Ph), 112.05–111.99, 111.87 (2 × C-5), 87.40–85.97 (T-C-4′, 2 × C-1′), 83.69, 83.63 (AZT-C-4′), 80.37, 79.34 (T-C-3′), 66.85, 66.48 (AZT-C-5′), 62.93, 62.68 (T-C-5′), 62.23, 61.82 (AZT-C-3′), 58.82, 58.25 (CH) 52.91 (OCH_3), 40.77, 40.65 (CH_2), 39.83, 39.66 (T-C-2′), 38.03, 37.96 (AZT-C-2′), 12.87, 12.60 (2 × C5-CH_3).

^{31}P NMR (CD_3OD, 81 MHz): δ = 75.79, 74.68.

ESI-MS: *m*/*z* = 701 $[M + H]^+$, 723 $[M + Na]^+$.

(2*R*,4*S*,5*R*)-1-{4-Azidotetrahydro-5-[(3′-*O*-thymidinyl)(methoxy-L-leucinyl)phosphoryl]methyl-2-furyl}thymine (Diastereoisomeric Mixture) (5e)

Yield: 61.5%; R_f 0.4 (CH_2Cl_2–MeOH, 30:1).

^{1}H NMR (CD_3OD, 500 MHz): δ = 7.83, 7.82 (s, 1 H, T-H-6), 7.59, 7.54 (s, 1 H, AZT-H-6), 6.31–6.29 (m, 1 H, T-H-1′), 6.17–6.13 (m, 1 H, AZT-H-1′), 5.17–5.11 (m, 1 H, T-H-3′), 4.48–4.43 (m, 1 H, AZT-H-3′), 4.32–4.08 (m, 5 H, 2 × H-4′, 2 × AZT-H-5′ , NHC*H*), 3.80–3.71 (m, 2 H, 2 × T-H-5′), 3.71, 3.70 (s, 3 H, OCH_3), 2.56–2.26 (m, 4 H, 2 × H-2′), 1.92, 1.86 (s, 6 H, 2 × C5-CH_3), 1.79–1.72 [m, 1 H, C*H*(CH_3)$_2$], 1.57–1.53 (m, 2 H, CH_2), 0.95–0.92 (m, 6 H, 2 × CH_3).

^{13}C NMR (CD_3OD, 125 MHz): δ = 176.03 (COO), 166.35, 166.32 (2 × C-4), 152.36, 152.17 (2 × C-2), 137.89, 137.78, 137.69 (2 × C-6), 112.01–111.98, 111.81 (2 × C-5), 87.48–86.05 (T-C-4′, 2 × C-1′), 83.79, 83.72 (AZT-C-4′), 80.16, 80.13 (T-C-3′), 67.24, 66.9 (AZT-C-5′), 62.91, 62.75 (T-C-5′), 62.16, 62.10 (AZT-C-3′), 55.47, 55.09 (NHC*H*), 52.69, 52.66 (OCH_3), 43.83, 43.70 (CH_2), 39.78, 39.74 (T-C-2′), 37.94, 37.84 (AZT-C-2′), 25.72, 25.67 [*C*H(CH_3)$_2$], 23.20, 21.99(CH_3), 12.70, 12.50 (2 × C5-CH_3).

^{31}P NMR (CD_3OD, 81 MHz): δ = 75.08, 74.16.

ESI-MS: *m*/*z* = 715 $[M + H]^+$, 737 $[M + Na]^+$.

(2*R*,4*S*,5*R*)-1-{4-Azidotetrahydro-5-[(3′-*O*-thymidinyl)(methoxy-L-isoleucinyl)phosphoryl]methyl-2-furyl}thymine (Diastereoisomeric Mixture) (5f)

Yield: 62.3%; R_f 0.4 (CH_2Cl_2–MeOH, 30:1).

^{1}H NMR (CD_3OD, 500 MHz): δ = 7.82 (s, 1 H, T-H-6), 7.58, 7.53 (s, 1 H, AZT-H-6), 6.31–6.29 (m, 1 H, T-H-1′), 6.16–6.13 (m, 1 H, AZT-H-1′), 5.15–5.12 (m, 1 H, T-H-3′), 4.48–4.40 (m, 1 H, AZT-H-3′), 4.28–4.08 (m, 4 H, 2 × H-4′, 2 × AZT-H-5′), 3.98–3.84 (m, 1 H, NHC*H*), 3.79 (s, 2 H, 2 × T-H-5′), 3.71, 3.70 (s, 3 H, OCH_3), 2.55–2.28 (m, 4 H, 2 × H-2′), 1.92, 1.88 (s, 6 H, 2 × C5-CH_3), 1.83–1.80 [m, 1 H, C*H*(CH_3)CH_2CH_3], 1.53–1.15 (m, 2 H, CH_2), 0.96–0.93 (m, 6 H, 2 × CH_3).

^{13}C NMR (CD_3OD, 125 MHz): δ = 175.06 (COO), 166.34 (2 × C-4), 152.39, 152.17 (2 × C-2), 137.89, 137.82, 137.71 (2 × C-6), 112.03–111.92, 111.83 (2 × C-5), 87.48–86.06 (T-C-4′, 2 × C-1′), 83.70 (AZT-C-4′), 80.25, 79.34 (T-C-3′), 67.33, 66.76 (AZT-C-5′), 62.91, 62.75 (T-C-5′), 62.10, 61.84 (AZT-C-3′), 61.66, 61.22 (NHC*H*), 52.52, 52.48 (OCH_3), 48.51 [*C*H(CH_3)CH_2CH_3], 39.84 (T-C-2′), 37.84, 37.80 (AZT-C-2′), 26.29, 26.26 (CH_2), 16.13, 16.06 [CH(*C*H$_3$)CH_2CH_3], 12.71, 12.52 (5-CH_3), 11.79, 11.76 [CH(CH_3)CH_2*C*H$_3$].

^{31}P NMR (CD_3OD, 81 MHz): δ = 75.66, 74.61.

ESI-MS: *m*/*z* = 715 $[M + H]^+$, 737 $[M + Na]^+$.

(2*R*,4*S*,5*R*)-1-{2,5-Dihydro-5-[(3′-*O*-thymidinyl)(methoxy-L-glycinyl)phosphoryl]methyl-2-furyl}thymine (Diastereoisomeric Mixture) (5′a)

Yield: 71.8%; R_f 0.4 (CH_2Cl_2–MeOH, 20:1).

^{1}H NMR (CD_3OD, 500 MHz): δ = 7.82 (s, 1 H, T-H-6), 7.47, 7.41 (s, 1 H, d4T-H-6), 6.93 (s, 1 H, d4T-H-1′), 6.43–6.41 (m, 1 H, d4T-H-2′), 6.30–6.26 (m, 1 H, T-H-1′), 5.98, 5.97 (d, 1 H, d4T-H-3′), 5.19–5.16 (m, 1 H, T-H-3′), 5.03 (s, 1 H, d4T-H-4′), 4.28–4.19 (m, 3 H, 2 × d4T-H-5′, T-H-4′), 3.80–3.73 (m, 4 H, T-H-5′, CH_2), 3.70 (s, 3 H, OCH_3), 2.52–2.28 (m, 2 H, T-H-2′), 1.93–1.87 (m, 6 H, 2 × 5-CH_3).

^{13}C NMR (CD_3OD, 125 MHz): δ = 173.19 (COO), 166.47, 166.35 (2 × C-4), 152.80, 152.38, 152.34 (2 × C-2), 138.02, 137.92, 137.88 (2 × C-6), 134.77 (d4T-C-2′), 128.05, 127.90 (d4T-C-3′), 111.98, 111.92, 111.80 (2 × C-5), 91.30, 91.11 (d4T-C-1′), 87.54, 87.29 (T-C-4′), 86.36–86.06 (T-C-1′,d4T-C-4′), 79.78, 79.12 (T-C-3′), 68.48, 68.17 (d4T-C-5′), 62.89, 62.75 (T-C-5′), 52.61 (OCH_3), 44.32, 43.90 (CH_2), 39.87, 39.67 (T-C-2′), 12.74, 12.68, 12.47 (5-CH_3).

^{31}P NMR (CD_3OD, 81 MHz): δ = 76.13, 75.28.

ESI-MS: *m*/*z* = 644 $[M + H]^+$, 666 $[M + Na]^+$.

(2*R*,4*S*,5*R*)-1-{2,5-Dihydro-5-[(3′-*O*-thymidinyl)(methoxy-L-alaninyl)phosphorylmethyl-2-furyl}thymine (Diastereoisomeric Mixture) (5′b)

Yield: 72.3%; R_f 0.4 (CH_2Cl_2–MeOH, 20:1).

^{1}H NMR (CD_3OD, 500 MHz): δ = 7.84, 7.82 (s, 1 H, T-H-6), 7.47, 7.39 (s, 1 H, d4T-H-6), 6.92 (s, 1 H, d4T-H-1′), 6.44–6.41 (s, 1 H, d4T-H-2′), 6.30–6.27 (m, 1 H, T-H-1′), 6.00–5.97 (m, 1 H, d4T-H-3′), 5.18–5.15 (m, 1 H, T-H-3′), 5.05–5.02 (s, 1 H, d4T-H-4′), 4.30–4.17 (m, 3 H, 2 × d4T-H-5′, T-H-4′), 4.07–3.95 (m, 1 H, NHC*H*), 3.80–3.77 (m, 2 H, T-H-5′), 3.70 (s, 3 H, OCH_3), 2.54–2.25 (m, 2 H, T-H-2′), 1.92, 1.90 (s, 6 H, 2 × 5-CH_3), 1.35–1.28 (m, 3 H, CH_3).

^{13}C NMR (CD_3OD, 125 MHz): δ = 175.87, 175.71 (COO), 166.47, 166.35 (2 × C-4), 152.79, 152.39, 152.32 (2 × C-2), 137.91, 137.85 (2 × C-6), 134.77, 134.72 (d4T-C-2′), 128.04, 127.93 (d4T-C-3′), 112.04, 111.97, 111.79 (2 × C-5), 91.37, 91.28 (d4T-C-1′), 87.48, 87.27 (T-C-4′), 86.40-86.04 (T-C-1′,d4T-C-4′), 79.91, 79.15 (T-C-3′), 68.88, 68.54, 68.15 (d4T-C-5′), 62.91, 62.73 (T-C-5′), 52.79–51.73 (NH*C*H, OCH_3), 39.84, 39.69 (T-C-2′), 20.36, 20.29, 20.21 (CH_3), 12.78, 12.74, 12.47 (5-CH_3).

^{31}P NMR (CD_3OD, 81 MHz): δ = 74.54, 73.36.

ESI-MS: *m*/*z* = 658 $[M + H]^+$, 680 $[M + Na]^+$.

(2*R*,4*S*,5R)-1-{2,5-Dihydro-5-[(3′-*O*-thymidinyl)(methoxy-L-phenylalaninyl)phosphoryl]methyl-2-furyl}thymine (Diastereoisomeric Mixture) (5′c)

Yield: 76.2%; R_f 0.4 (CH_2Cl_2–MeOH, 15:1).

^{1}H NMR (CD_3OD, 500 MHz): δ = 7.80 (s, 1 H, T-H-6), 7.39–7.17 (m, 6 H, d4T-H-6, Ph), 6.91, 6.88 (s, 1 H, d4T-H-1′), 6.31–6.28 (s, 1 H, d4T-H-2′), 6.24–6.20 (m, 1 H, T-H-1′), 5.96–5.93 (m, 1 H, d4T-H-3′), 5.05–5.00 (m, 2 H, T-H-3′, d4T-H-4′), 4.27–4.04 (m, 3 H, 2 × d4T-H-5′, T-H-4′), 4.00–3.83 (m, 1 H, NHC*H*), 3.77–3.75 (s, 2 H, T-H-5′), 3.70–3.67 (m, 3 H, OCH_3), 3.16–3.03 (m, 2 H, CH_2), 2.42–2.30 (m, 2 H, T-H-2′), 1.90, 1.86 (s, 6 H, 2 × 5-CH_3).

^{13}C NMR (CD_3OD, 125 MHz): δ = 174.98, 174.77 (COO), 166.45, 166.35 (2 × C-4), 152.75, 152.35, 152.29 (2 × C-2), 138.52, 137.81(2 × C-6), 134.66, 134.53 (d4T-C-2′), 130.63–127.89 (m, d4T-C-3′, Ph), 111.99, 111.76(2 × C-5), 91.26, 91.19 (d4T-C-1′), 87.43, 87.20 (T-C-4′), 86.28–85.96 (T-C-1′,d4T-C-4′), 80.15, 78.96 (T-C-3′), 68.13, 68.06 (d4T-C-5′), 62.90, 62.65 (T-C-5′), 58.73, 58.06 (NH*C*H), 53.17, 52.71 (OCH_3), 40.82, 40.76 (CH), 39.79, 39.62 (T-C-2′), 12.86, 12.77, 12.48 (5-CH_3).

^{31}P NMR (CD_3OD, 81 MHz): δ = 74.60, 73.04.

ESI-MS: *m*/*z* = 706 $[M + H]^+$, 728 $[M + Na]^+$.

(2*R*,4*S*,5*R*)-1-{2,5-Dihydro-5-[(3′-*O*-thymidinyl)(methoxy-L-valinyl)phosphoryl]methyl-2-furyl}thymine (Diastereoisomeric Mixture) (5′d)

Yield: 68.4%; R_f 0.4 (CH_2Cl_2–MeOH, 15:1).

^{1}H NMR (CD_3OD, 500MHz): δ = 7.83 (s, 1 H, T-H-6), 7.46, 7.37 (s, 1 H, d4T-H-6), 6.93–6.91 (m, 1 H, d4T-H-1′), 6.44–6.41 (s, 1 H, d4T-H-2′), 6.30–6.26 (m, 1 H, T-H-1′), 6.00–5.97 (m, 1 H, d4T-H-3′), 5.12–5.10 (m, 1 H, T-H-3′), 5.04–5.03 (m, 1 H, d4T-H-4′), 4.29–4.11 (m, 3 H, 2 × d4T-H-5′, T-H-4′), 3.85–3.80 (m, 1 H, NHC*H*), 3.80–3.77 (s, 2 H, T-H-5′), 3.71, 3.68 (s, 3 H, OCH_3), 2.53–2.26 (m, 2 H, T-H-2′), 2.05–1.92 (m, 1 H, CH), 1.92, 1.88 (s, 6 H, 2 × 5-CH_3), 0.92, 0.90 (s, 6 H, 2 × CH_3).

^{13}C NMR (CD_3OD, 125 MHz): δ = 175.03 (COO), 166.48, 166.35 (2 × C-4), 152.79, 152.37, 152.30 (2 × C-2), 137.91, 137.81 (2 × C-6), 134.76, 134.65 (d4T-C-2′), 128.15, 127.89 (d4T-C-3′), 112.06, 111.98, 111.78 (2 × C-5), 91.44, 91.34 (d4T-C-1′), 87.50, 87.31(T-C-4′), 86.40–86.05 (T-C-1′,d4T-C-4′), 80.16, 79.27 (T-C-3′), 68.88, 68.38 (d4T-C-5′), 62.91, 62.75 (T-C-5′), 62.59, 62.04 (NH*C*H), 52.50, 52.46 (OCH_3), 39.81, 39.71 (T-C-2′), 33.07, 33.01 (CH), 19.63, 19.59, 18.66 (2 × CH_3), 12.82, 12.75, 12.47 (5-CH_3).

^{31}P NMR (CD_3OD, 81 MHz): δ = 75.61, 74.68.

ESI-MS: *m*/*z* = 658 $[M + H]^+$, 670 $[M + Na]^+$.

(2*R*,4*S*,5*R*)-1-{2,5-Dihydro-5-[(3′-*O*-thymidinyl)(methoxy-L-leucinyl)phosphoryl]methyl-2-furyl}thymine (Diastereoisomeric Mixture) (5′e)

Yield: 73.6%; R_f 0.4 (CH_2Cl_2–MeOH, 15:1).

^{1}H NMR (CD_3OD, 500MHz): δ = 7.83 (s, 1 H, T-H-6), 7.48, 7.39 (s, 1 H, d4T-H-6), 6.92 (s, 1 H, d4T-H-1′), 6.42, 6.41 (s, 1 H, d4T-H-2′), 6.30–6.25 (m, 1 H, T-H-1′), 6.00–5.98 (m, 1 H, d4T-H-3′), 5.16–5.10 (m, 1 H, T-H-3′), 5.05, 5.02 (s, 1 H, d4T-H-4′), 4.34–4.10 (m, 3 H, 2 × d4T-H-5′, T-H-4′), 4.05–4.01 (m, 1 H, NHC*H*), 3.79, 3.77 (s, 2 H, T-H-5′), 3.71, 3.70 (s, 3 H, OCH_3), 2.53–2.23 (m, 2 H, T-H-2′), 1.91, 1.87 (s, 6 H, 2 × 5-CH_3), 1.76–1.70 [m, 1 H, C*H*(CH_3)$_2$], 1.56–1.50 (m, 2 H, CH_2), 0.91, 0.89 (s, 6 H, 2 × CH_3).

^{13}C NMR (CD_3OD, 125 MHz): δ = 175.94 (COO), 166.48, 166.35 (2 × C-4), 152.76, 152.37 (2 × C-2), 137.91, 137.84, 137.80 (2 × C-6), 134.71, 134.59 (d4T-C-2′), 128.23, 127.98 (d4T-C-3′), 112.06, 111.97, 111.77 (2 × C-5), 91.38, 91.26 (d4T-C-1′), 87.47, 87.26 (T-C-4′), 86.46-86.07 (T-C-1′,d4T-C-4′), 80.12, 79.22 (T-C-3′), 68.68, 68.35 (d4T-C-5′), 62.92, 62.75 (T-C-5′), 55.32, 54.82 (NH*C*H), 52.65, 52.62 (OCH_3), 43.82 (CH_2), 39.88, 39.75 (T-C-2′), 25.65 [*C*H(CH_3)$_2$], 23.25, 22.03 (2 × CH_3), 12.82, 12.77, 12.48 (5-CH_3).

^{31}P NMR (CD_3OD, 81 MHz): δ = 75.01, 74.12.

ESI-MS: *m*/*z* = 672 $[M + H]^+$, 694 $[M + Na]^+$.

(2*R*,4*S*,5*R*)-1-{2,5-Dihydro-5-[(3′-*O*-thymidinyl)(methoxy-L-isoleucinyl)phosphoryl]methyl-2-furyl]thymine (Diastereoisomeric Mixture) (5′f)

Yield: 74.2%; R_f 0.4 (CH_2Cl_2–MeOH, 15:1).

^{1}H NMR (CD_3OD, 500 MHz): δ = 7.83 (s, 1 H, T-H-6), 7.46, 7.37 (s, 1 H, d4T-H-6), 6.92–6.91 (m, 1 H, d4T-H-1′), 6.43, 6.41 (s, 1 H, d4T-H-2′), 6.30–6.26 (m, 1 H, T-H-1′), 6.00–5.98 (m, 1 H, d4T-H-3′), 5.12–5.09 (m, 1 H, T-H-3′), 5.04 (s, 1 H, d4T-H-4′), 4.29–4.10 (m, 3 H, 2 × d4T-H-5′, T-H-4′), 3.91–3.88 (m, 1 H, NHC*H*), 3.79–3.77 (m, 2 H, T-H-5′), 3.74, 3.72 (s, 3 H, OCH_3), 2.52–2.26 (m, 2 H, T-H-2′), 1.92–1.87 (m, 6 H, 2 × 5-CH_3), 1.77–1.73 [m, 1 H, C*H*(CH_3)CH_2CH_3], 1.51–1.14 (m, 2 H, CH_2), 0.91–0.87 (m, 6 H, 2 × CH_3).

^{13}C NMR (CD_3OD, 125 MHz): δ = 175.02 (COO), 166.47, 166.35 (2 × C-4), 152.77, 152.37, 152.31 (2 × C-2), 137.92, 137.85 (2 × C-6), 134.74, 134.63 (d4T-C-2′), 128.20, 127.94 (d4T-C-3′), 112.06, 111.98, 111.79 (2 × C-5), 91.43, 91.32 (d4T-C-1′), 87.49, 87.25 (T-C-4′), 86.41–86.07 (T-C-1′,d4T-C-4′), 80.20, 79.29 (T-C-3′), 68.92, 68.39 (d4T-C-5′), 62.92, 62.76 (T-C-5′), 61.45, 60.92 (NH*C*H), 52.44 (OCH_3), 48.51 [*C*H(CH_3)CH_2CH_3], 39.88, 39.83 (T-C-2′), 26.28 (CH_2), 16.03, 15.93 [CH(*C*H_3)CH_2CH_3], 12.85, 12.77 (5-CH_3), 11.73, 11.67 [CH(CH_3)CH_2*C*H_3].

^{31}P NMR (CD_3OD, 81 MHz): δ = 75.47, 74.50.

ESI-MS: *m*/*z* = 672 $[M + H]^+$, 694 $[M + Na]^+$.

Acknowledgments

The authors would like to thank the financial supports from the National Natural Science Foundation of China (No. 29902003, 20132020 and 20175026), the Ministry of Science and Technology, the Chinese Ministry of Education and Tsinghua University.

References

(1) Ueno, T.; Mitsuya, H. *Clin. Immunother*. **1995**, *4*, 451.
(2) Boussard, C.; Doyle, V. E.; Mahmood, N.; Klimkait, T.; Pritchard, M.; Gilbert, I. H. *Eur. J. Med. Chem.* **2002**, *37*, 883.
(3) (a) Vlieghe, P.; Clerc, T.; Pannecouque, C.; Witvrouw, M.; De Clercq, E.; Salles, J. P.; Kraus, J. L. *J. Med. Chem.* **2002**, *45*, 1275. (b) Wong, A.; Toth, I. *Curr. Med. Chem.* **2001**, *8*, 1123. (c) Gallo, J. M. *Adv. Drug Deliver Rev.* **1994**, *14*, 199.
(4) (a) Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; Clair, M. H. *Proc. Natl. Acad. Sci. U. S. A.* **1985**, *82*, 7096. (b) Rando, R. F.; Nguyen-Ba, N. *Drug Discovery Today* **2000**, *5*, 465. (c) Fischl, M. A.; Richman, D. D.; Grieco, M. H.; Gottlieb, M. S. *N. Engl. J. Med.* **1987**, *317*, 185.
(5) Wanger, C. R.; Iyer, V. V.; McIntee, E. *J. Med. Res. Rev.* **2000**, *20*, 417.
(6) (a) Richman, D. D.; Fischl, M. A.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. L.; Leedon, J. M.; Groopman, J. E.; Mildvan, D.; Hirsch, M. S.; Jackson, G. G.; Durack, D. T.; Nusinoff-Lehrman, S. *N. Engl. J. Med.* **1987**, *317*, 192. (b) Pan-Zhou, X. R.; Cretton-Scott, E.; Zhou, X. J.; Yang, M. X.; Lasker, J. M.; Sommadossi, J. P. *Biochem. Pharmacol.* **1998**, *55*, 757. (c) Trapnell, C. B.; Klecker, R. W.; Jamisdow, C.; Collins, J. M. *Antimicrob. Agents. Chemother.* **1998**, *42*, 1592.

(7) (a) Larder, B. A.; Darby, G.; Richman, D. D. *Science* **1989**, *243*, 1731. (b) Kallam, P.; Boucher, C. A. B.; Larde, B. A. *Proc. Natl. Acad. Sci. U. S. A.* **1992**, *89*, 1934. (c) Rando, R. F.; Nguyen-Ba, N. *Drug Discovery Today* **2000**, *5*, 465.
(8) (a) Parang, K.; Wiebe, L. I.; Knaus, E. E. *Curr. Med. Chem.* **2000**, *7*, 995. (b) Sergheraert, C.; Pierlot, C.; Tartar, A.; Henin, Y.; Lemaitre, M. *J. Med. Chem.* **1993**, *36*, 826. (c) Hostetler, K. Y.; Stuhmiller, L. M.; Lenting, H. B. M.; van den Bosch, H.; Richman, D. D. *J. Biol. Chem.* **1990**, *265*, 6112.
(9) Busso, M.; Mian, A. M.; Hahn, E. F.; Resnick, L. *AIDS Res. Hum. Retroviruses* **1988**, *4*, 449.
(10) Shinazi, R. F.; Sommadossi, J. P.; Saalmann, V.; Cannon, D. L.; Xie, M. Y.; Hart, G. C.; Smith, G. A.; Hahn, E. F. *Antimicrob. Agents Chemother.* **1990**, *34*, 1061.
(11) De Clerq, E. *Nucleosides Nucleotides Nucleic Acids* **1994**, *13*, 1271.
(12) Stein, C. A. *Nucleic Acids Res.* **1988**, *16*, 3209.
(13) (a) McGuigan, C.; Devine, K. G.; O'Connor, T. J.; Galpin, S. A.; Jeffries, J. D.; Kinchington, D. *Antiviral Chem. Chemother.* **1990**, *1*, 107. (b) McGuigan, C.; Devine, K. G.; O'Connor, T. J.; Kinchington, D. *Antiviral Res.* **1991**, *15*, 255.
(14) (a) Atherton, F. R.; Openshaw, H. T.; Todd, A. R. *J. Chem. Soc.* **1945**, 660. (b) Atherton, F. R.; Todd, A. R. *J. Chem. Soc.* **1947**, 674.
(15) Peyser, J. R.; Ferris, J. P. *Orig. Life Evol. Biosph.* **2001**, *31*, 363.
(16) Kers, I.; Kers, A.; Stawinski, J. *Tetrahedron Lett.* **1999**, *40*, 3945.