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# Optimized synthesis of 3′-*O*-aminothymidine and evaluation of its oxime derivative as an anti-HIV agent

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**Abstract:** The synthesis and isolation of 3′-*O*-aminothymidine oximes have been optimized. Synthesized compounds were characterized by NMR and UV spectral and analytical data. A mixture of previously not reported *syn* and *anti* isomers of acetaldoximes was assessed for anti-HIV activity and the prevention of syncytia formation caused by HIV-1 infection.

**Keywords:** anti-HIV properties; Mitsunobu reaction; nucleoside analogues; oxime derivatives; syncytium formation.

**Dedicated to** the memory of the late Professor Kyoichi A. Watanabe, a great medicinal chemist and our best friend.

## Introduction

3′-Modified nucleosides are used as inhibitors of HIV replication, and some of them have become efficient drugs. 3′-*O*-Aminothymidine is a nucleoside analogue of special interest as a cleavable primer extension terminator [1] and as a substrate for synthesis of antisense oligonucleotides [2, 3]. The reversible termination of oligonucleotides by 3′-*O*-aminothymidine takes place due to the -$ONH_2$ fragment capable of deamination with sodium nitrite. This ability may be potentially extended to the termination of DNA synthesis catalyzed by reverse transcriptase if a suitable oxime protection is applied for the 3′-blockage, as in typical nucleoside analogue inhibitors. Our attention was focused on previously non-reported oxime derivatives of 3′-*O*-aminothymidine [4]. The goal of this study was the optimization of 3′-*O*-aminothymidine synthesis as well as evaluation of anti-HIV properties of its acetaldoximes.

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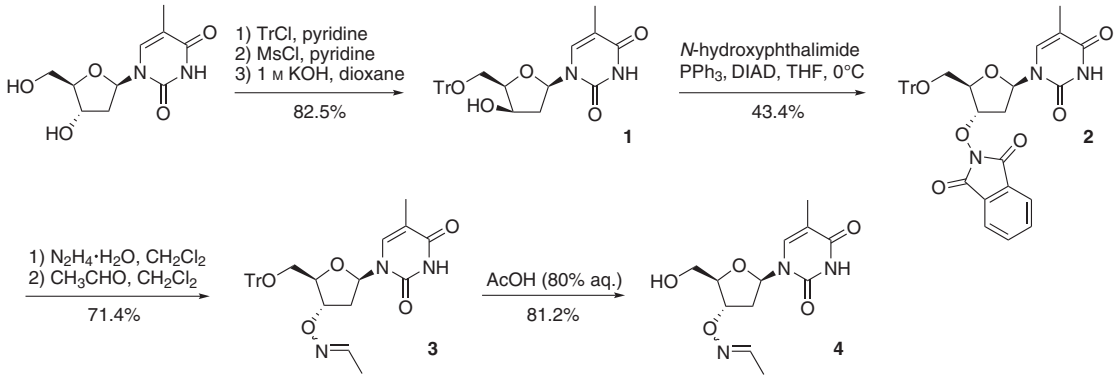
## Results and discussion

### Synthesis

Synthesis of 3′-*O*-aminothymidine ($T_{ONH_2}$) is usually conducted *via* the Mitsunobu reaction of xylothymidine with *N*-hydroxyphthalimide followed by the deblocking of the phthalimide moiety. Xylothymidine can be obtained either by 3′-keto-derivative reduction (non-selective for the resulting configuration) [3] or by the Mitsunobu procedure with reversible benzoic acid 3′-protection [1], but most frequently and successfully, xylothymidine is synthesized *via* 2,3′-anhydronucleoside formation [5]. Modifications usually take place after the introduction of the 5′-protecting group, but there is also a simplified ‘one-pot’ procedure [6] that was used here (Scheme 1). Thymidine was protected with trityl on the 5′-OH group and activated by mesylation at 3′-OH; at this point, the solvent was evaporated, and pure intermediate 5′-*O*-trityl-3′-*O*-mesylthymidine was crystallized from hexanes. Further treatment with aqueous KOH results in formation of a 2,3′-anhydrothymidine structure, and the use of excess of alkali causes ring opening into 5′-*O*-tritylxylothymidine **1**. After chromatographic purification of **1** on silica gel, we performed the Mitsunobu procedure under typical conditions, which proceeded smoothly. It was found that cooling the reaction mixture is required only at the moment the reagents are mixed, and an increase in temperature until 80°C does not affect the reaction. At 80°C, the elimination becomes a competitive reaction and




**Scheme 1** Synthesis of target 3′-*O*-aminothymidine *syn*/*anti*-acetaldoximes **4**.

formation of a small amount of 5′-*O*-trityl-2′,3′-dideoxy-2′,3′-didehydrothymidine can be observed.

The target Mitsunobu product **2** poses some difficulties in purification on a silica gel column using the previously described procedures [1–3]. Nevertheless, the earlier proposed eluting system, hexane/dichloromethane/ethyl acetate in a 3:2:2 ratio, may be used for convenient TLC detection [3]. We developed two-step column chromatography purification instead. In the first step, silica gel column chromatography in chloroform/ethanol system (linear gradient of ethanol 0→5%) permitted separation of the target product contaminated with $PPh_3O$. In the second step, re-purification using a fresh silica gel column in chloroform/hexane/ethanol system in a 7:3:1.5 ratio was conducted. This procedure allowed the isolation of pure 5′-*O*-trityl-3′-*O*-phthalimidothymidine. Removal of the phthalimidyl fragment of **2** was performed using excess hydrazine hydrate. The crude reaction mixture was then treated with acetaldehyde. Acetaldoxime structure **3** was the target product because it is more stable than formaldoxime and potentially less sterically hindered than acetoxime, hence more likely to be a substrate for enzymes. The trityl group was removed from the intermediate product **3** by treatment with acetic acid without any cleavage of the oxime bond. Both trityl-protected **3** and deprotected product **4** are mixtures of *syn* and *anti* isomers that could not be separated. Antiviral assays were carried out on a mixture of deprotected product **4** containing *syn* and *anti* isomers in a 1:1 ratio.

## Antiviral activity and toxicity

Antiviral activity was determined in MT-4 cells infected with HIV-1 (899A strain) at the multiplicity of infection 0.2–0.5 units/cell according to the previously published procedure [7]. Antiviral activity and toxicity were determined in the presence of the tested compounds (0.01–300 μM, three replicates for each dose) and were assessed in 4 days. The cell concentration and viability were measured by the trypan blue dye exclusion calorimetric assay, and the $EC_{50}$ and $CC_{50}$ values were calculated. 3′-Azido-3′-deoxythymidine (AZT or zidovudine, Retrovir® from ViiV Healthcare) was used as a control. Cytotoxicity was assessed in MT-4 cells, cultured in the presence of various doses of the compounds (0.01–1000 μM) for 4 days. The data in Table 1 show that the oxime product **4** possesses moderate activity against HIV-1.

## Syncytia formation

Infection of cell culture with HIV-1 results in the formation of multinucleated giant cells or syncytia. The ability to form syncytia may be a major contribution to the virulence of HIV and may play a role in the disease progression [8, 9]. It has been shown that exposure to AZT has a significant impact on syncytium formation and cell killing in an *in vitro* assay [10]. We compared the effects of AZT and compound **4** on syncytium development in lymphoblastoid cell culture MT-4 (Figure 1) according to the described procedure [10]. Figure 1B shows cells 3 days after infection with HIV-1, and Figure 1A shows uninfected control cells.

**Table 1** Antiviral activity and cytotoxicity *syn*+*anti* acetaldoximes of 3′-*O*-aminothymidine.

| Compound | $CC_{50}$[a] (μM) | $EC_{50}$[b] (μM) |
|---|---|---|
| **4** | 150 | 42 |
| AZT | >100 | 0.04 |

[a]Compound concentration required to reduce cell viability by 50%.
[b]Compound concentration required to inhibit HIV replication by 50%.

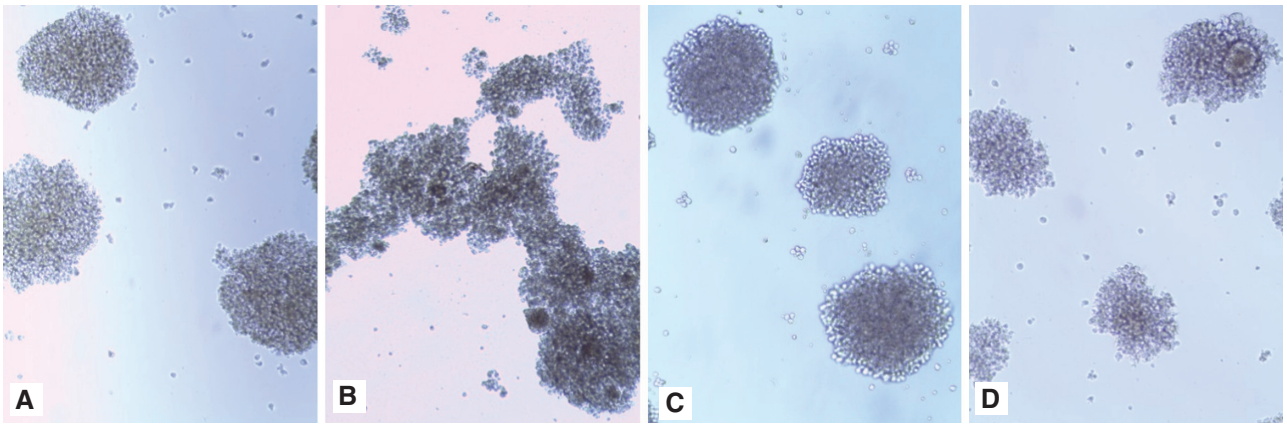



**Figure 1** Effects of compound **4** and AZT on syncytia formation (in 3 days after infection). (A) Control uninfected cells; (B) MT-4 cell culture after HIV-1 infection; (C) infected cell culture growing in the presence of 0.1 μM AZT; (D) infected cell culture growing in the presence of 10 μM **4**.

One can see that the presence of AZT (Figure 1C) or compound **4** (Figure 1D) in the assays results in a significant reduction in syncytia size. The action of both compounds is similar, although the effective concentration of **4** was 1–1.5 orders of magnitude higher. The effects were time (1–5 days) and dose dependent for AZT (0.01–1 μM) and for **4** (1–150 μM) (data not shown). These data correlate with anti-HIV activity of AZT and compound **4**.

## Conclusion

An optimized procedure for 3′-*O*-aminothymidine oximes synthesis and isolation of the intermediate products was developed. The synthesized *syn*-/*anti*-oximes possess anti-HIV activity and prevent cells from syncytia formation caused by HIV-1 infection.

## Experimental

Diethyl azodicarboxylate (Fluka), methanesulfonyl chloride (Acros), trityl chloride (Acros), hydrazine hydrate (Fluka), and thymidine (Fluka) were purchased from the indicated suppliers. THF was distilled over sodium before use. The reaction process was monitored using TLC on precoated Kieselgel 60$F_{254}$ plates (Merck, Germany), and column chromatography was performed on silica gel (40–63 μm; Merck, Germany). $^1$H and $^{13}$C NMR spectra were registered in DMSO-$d_6$ on a Bruker AMX III 400 spectrometer with the working frequency of 400 MHz for $^1$H NMR ($Me_4Si$ as an internal standard) and 100.6 MHz for $^{13}$C NMR (with carbon-proton decoupling, $Me_4Si$ as an internal standard). UV spectra were registered on a Shimadzu UV-2401PC spectrophotometer in $CH_3OH$ in the range of 220–300 nm. High-resolution mass spectra (HR-ESI-MS) were acquired in a positive ion mode on a Bruker maXis™ instrument; syringe injection was used for solutions in acetonitrile (flow rate 3 μL/min), nitrogen was applied as an inert gas, interface temperature was set at 180°C.

### Synthesis of 5′-*O*-trityl-xylothymidine (1)

The procedure was carried out with chromatographic purification of only small samples of the intermediate products for characterization. A solution of thymidine (5 g, 0.02 mol) in pyridine (30 mL) was stirred at room temperature and treated with trityl chloride (7.4 g, 0.024 mol). The mixture was stirred at 40°C for 16 h, then cooled, and the resulting intermediate product, 5′-*O*-tritylthymidine,[1] was treated with mesyl chloride (3.2 mL, 0.045 mol). The mixture was stirred for 8 h, then quenched with 5 mL of distilled water, concentrated, and extracted with chloroform and aqueous solution of $NaHCO_3$. The organic layer was dried with $Na_2SO_4$, concentrated, and the residue was crystallized at 0°C from a mixture of hexanes-chloroform. Crystals of pure 5′-*O*-trityl-3′-*O*-mesylthymidine[2] were separated and

**1** A sample of reaction mixture was collected to isolate intermediate 5′-*O*-tritylthymidine. An aliquot was diluted with 1 mL of distilled water, the solution was concentrated, and the residue was dissolved in chloroform and the solution was concentrated *in vacuo*. The residue was purified on a silica gel column eluting with ethanol (0→5%)/chloroform system; UV: $\lambda_{max}$ 267.2 nm; $^1$H NMR: δ 11.29 (s, 1H, 3-NH), 7.48 (q, 1H, $^4J_{6,\,5\text{-Me}}$ = 0.8 Hz, H-6) 7.28–7.41 (m, 15H, Tr), 6.22 (dd, 1H, $^3J_{1',\,2'b}$ = 6.74 Hz, $^3J_{1',\,2'a}$ = 6.94 Hz, H-1′), 5.30 (d, 1H, $^3J_{3'\text{-OH}}$, 3′ = 4.6 Hz, 3′-OH), 4.33 (m, 1H, H-3′), 3.89 (m, 1H, H-4′), 3.23–3.27 (dd, 1H, $^2J_{5'a,\,5'b}$ = 10.3 Hz, $^3J_{5'a,\,4'}$ = 4.5 Hz, H-5′a), 3.18–3.21 (dd, 1H, $^2J_{5'a,\,5'b}$ = 10.3 Hz, $^3J_{5'b,\,4'}$ = 3.0 Hz, H-5′b), 2.13–2.28 (m, 2H, H-2′), 1.48 (d, 3H, $^4J_{5\text{-Me},\,6}$ = 0.8 Hz, 5-Me); $^{13}$C NMR: δ 163.5 (C-4), 150.3 (C-2), 143.4 (*i*-C (Tr)), 135.5 (C-6), 128.2 (*m*-C (Tr)), 127.8 (*o*-C (Tr)), 127.1 (*p*-C (Tr)), 109.5 (C-5), 86.3 (C (Tr)), 85.3 (C-4′), 83.7 (C-1′), 70.4 (C-3′), 63.9 (C-5′), 39.4 (s, C-2′), 11.7 (s, 5-Me).

**2** A sample of reaction mixture was collected to isolate intermediate 5′-*O*-trityl-3′-*O*-mesylthymidine. An aliquot was diluted with 1 mL of distilled water, the solution was concentrated, the residue was

dissolved in dioxane, and the residual solvent was evaporated *in vacuo*. To a solution of 5′-*O*-trityl-3′-*O*-mesylthymidine in dioxane, a 1-M KOH aqueous solution (30 mL) was added in three portions, which resulted in a change of the color of the mixture from yellow to red, referring to the 5′-*O*-trityl-2,3′-anhydrothymidine[3] structure formation. The color turned back to yellow in 12 h, after which time the mixture was neutralized with aqueous acetic acid. The reaction mixture was concentrated and purified on a silica gel column eluting with ethanol (0→12%)/chloroform system. Target fractions were concentrated to give 8.98 g (82.5%) of pure compound **1** as a white powder; mp 231–232°C; UV: $\lambda_{max}$ 266.3 nm; $^1$H NMR: δ 11.24 (s, 1H, 3-NH), 7.61 (q, 1H, $^4J_{6,\ 5\text{-Me}}$ = 1.0 Hz, H-6) 7.22–7.38 (m, 15H, Tr), 6.12 (dd, 1H, $^3J_{1',\ 2'a}$ = 8.2 Hz, $^3J_{1',\ 2'b}$ = 2.2 Hz, H-1′), 5.19 (d, 1H, $^3J_{3'\text{-OH},\ 3'}$ = 3.4 Hz, 3′-OH), 4.21 (m, 1H, H-3′), 4.09 (m, 1H, H-4′), 3.38–3.43 (dd, 1H, $^2J_{5'a,\ 5'b}$ = 10.3 Hz, $^3J_{5'a,\ 4'}$ = 8.0 Hz, H-5′a), 3.20–3.24 (dd, 1H $^2J_{5'a,\ 5'b}$ = 10.3 Hz, $^3J_{5'b,\ 4'}$ = 2.9 Hz, H-5′b), 2.50–2.58 (m, 1H, H-2′a), 1.84–1.88 (dd, 1H, $^2J_{2'a,\ 2'b}$ = 14.6 Hz, $^3J_{2'b,\ 1'}$ = 2.2 Hz, H-2′b), 1.65 (d, 3H, $^4J_{5\text{-Me},\ 6}$ = 1.0 Hz, 5-Me); $^{13}$C NMR: δ 163.7 (C-4), 150.4 (C-2), 143.6 (*i*-C [Tr]), 136.7 (C-6), 128.3 (*m*-C [Tr]), 127.8 (*o*-C [Tr]), 126.9 (*p*-C [Tr]), 108.3 (C-5), 86.1 (C [Tr]), 84.1 (s, C-4′), 83.2 (C-1′), 68.9 (C-3′), 63.0 (C-5′), 40.7 (C-2′), 12.4 (5-Me).

## Synthesis of 5′-*O*-trityl-3′-phthalimidyloxythymidine (2)

A solution of 5′-*O*-trityl-xylothymidine (0.5 g, 1.04 mmol) in THF (15 mL) was cooled to 0°C and treated with *N*-hydroxyphthalimide (0.5 g, 1.56 mmol), triphenylphosphine (0.411 g, 1.23 mmol), and diisopropyl azodicarboxylate (0.244 mL, 1.23 mmol). After 6 h, the reaction mixture was concentrated *in vacuo* and partitioned between chloroform and water. Organic layer was dried with $Na_2SO_4$ and then concentrated. Chromatographic purification was performed in two steps using silica gel: first, eluting with chloroform/ethanol (95:5) to separate a mixture of the target product and triphenylphosphine oxide from other by-products, and second, using a new column and eluting with chloroform/hexane/ethyl acetate (7:3:1.5). Target fractions were concentrated *in vacuo*, resulting in 0.43 g (43.4%) of pure compound **2** as white powder; mp 109–110°C; UV: $\lambda_{max}$ 270.9 nm; $^1$H NMR: δ 11.36 (s, 1H, 3-NH), 7.88 (m, 4H, Phth), 7.48 (q, 1H, $^4J_{6,\ 5\text{-Me}}$ = 1.0 Hz, H-6) 7.23–7.35 (m, 15H, Tr), 6.34 (dd, 1H, $^3J_{1',\ 2'b}$ = 6.0 Hz, $^3J_{1',\ 2'a}$ = 6.1 Hz, H-1′), 5.15 (m, 1H, H-3′), 4.31 (m, 1H, H-4′), 3.30–3.34 (dd, 1H, $^2J_{5'a,\ 5'b}$ = 10.7 Hz, $^3J_{5'a,\ 4'}$ = 4.2 Hz, H-5′a), 3.24–3.28 (dd, 1H, $^2J_{5'a,\ 5'b}$ = 10.7 Hz, $^3J_{5'b,\ 4'}$ = 4.3 Hz, H-5′b), 2.13–2.28 (m, 2H, H-2′), 1.46 (d, 3H, $^4J_{5\text{-Me},\ 6}$ = 1.0 Hz, 5-Me); $^{13}$C NMR: δ 163.4 (C-4), 163.4 (CO [Phth]), 150.2 (C-2), 143.1 (*i*-C [Tr]), 135.3 (C-6), 134. 7 (4+5 [Phth]), 128.4 (1+2 [Phth]), 128.1 (*m*-C [Tr]), 127.8 (s, *o*-C [Tr]), 127.1 (*p*-C [Tr]), 123.3 (3+6 [Phth]), 109.8 (C-5), 87.5 (C [Tr]), 86.6 (C-4′), 83.6 (C-1′), 81.1 (C-3′), 63.6 (C-5′), 35.6 (C-2′), 11.6 (5-Me).

## Synthesis of 5′-*O*-trityl-3′-*O*-aminothymidine *syn*/*anti*-acetaldoximes (3)

A solution of 5′-*O*-trityl-3′-phthalimidyloxythymidine (56 mg, 0.088 mmol) in dichloromethane (2 mL) was cooled to 0°C and treated dropwise with a solution of hydrazine hydrate (0.03 mL) in dichloromethane (5 mL). The reaction mixture became cloudy as the phthalic hydrazide formed and precipitated. After 2 h, the solvent was evaporated, and the residue was treated with dichloromethane (5 mL) and acetaldehyde (0.04 mL). After stirring for 16 h, the mixture was filtered and the solution was applied to a silica gel column and eluted with ethanol (0→7%)/chloroform system. Target fractions were concentrated *in vacuo* to give 0.033 g (71.4%) of a mixture of *syn*/*anti* isomers of **3** as a white powder; UV: $\lambda_{max}$ 265.2 nm; $^1$H NMR: δ 11.34 (2s, 2H, 3-NH), 7.56 and 7.54 (2q, 2×1H, $^4J_{6,\ 5\text{-Me}}$ = 1.0 Hz, H-6), 7.49 (q, 1H, $^3J_{CH,\ Me}$ = 5.8 Hz, CH [*anti*]), 7.26–7.41 (m, 30H, 2Tr), 6.95 (q, 1H, $^3J_{CH,\ Me}$ = 5.5 Hz, CH [*syn*]), 6.17–6.23 (m, 2H, 2H-1′), 4.79–4.86 (m, 2H, 2H-3′), 4.08–4.14 (m, 2H, 2H-4′), 3.24–3.34 (m, 4H, 2H-5′), 2.37–2.43 (m, 4H, 2H-2′), 1.77 (d, 3H, $^3J_{Me,\ CH}$ = 5.5 Hz, Me [*syn*]), 1.76 (d, 3H, $^3J_{Me,\ CH}$ = 5.8 Hz, Me [*anti*]), 1.46 (br.s, 6H, 5-Me); $^{13}$C NMR: δ 163.5 (C-4), 150.3 (C-2), 149.1 and 148.8 (2s, 2CH), 143.3 (*i*-C [Tr]), 135.5 (C-6), 128.2 (*m*-C [Tr]), 127.94 (*o*-C [Tr]), 127.2 (*p*-C [Tr]), 109.8 (C-5), 86.5 (C [Tr]), 83.9 and 83.8 (2s, C-4′), 82.4 and 82.2 (2s, C-1′), 82.0 and 81.6 (2s, C-3′), 64.2 and 64.1 (2s, C-5′), 36.3 and 36.0 (2s, C-2′), 14.9 (Me [*anti*]), 11.8 (Me [*syn*]), 11.6 (5-Me). HRMS (ESI). Calcd for $C_{31}H_{31}N_3O_5$ $[M+H]^+$, $[M+Na]^+$): *m*/*z* 526.2336 and 548.2156. Found: *m*/*z* 526.2332 and 548.2156.

## Synthesis of 3′-*O*-aminothymidine *syn*/*anti*-acetaldoximes (4)

A solution of 5′-*O*-trityl-3′-*O*-aminothymidine *syn*/*anti*-acetaldoximes (0.033 g, 0.063 mmol) in 80% aqueous acetic acid (3 mL) was allowed to stand at 37°C for 16 h, then concentrated *in vacuo*. The residue was dissolved in toluene and the solution was concentrated to azeotropically remove water. The crude product **4** was purified on a silica gel column eluting with ethanol (0→20%)/chloroform system. Concentration of the target fractions gave 0.015 g (81.2%) of a mixture of *syn*/*anti*-isomers of **4** as white powder; UV: $\lambda_{max}$ 265.2 nm; $^1$H NMR: δ 11.28 (2s, 2H, 3-NH), 7.74 (q, 1H, $^4J_{6,\ 5\text{-Me}}$ = 1.0 Hz, H-6 [*anti*]), 7.73 (q, 1H, $^4J_{6,\ 5\text{-Me}}$ = 1.0 Hz, H-6 [*syn*]), 7.52 (q, 1H, $^3J_{CH,\ Me}$ = 5.8 Hz, CH [*anti*]), 6.97 (q, 1H, $^3J_{CH,\ Me}$ = 5.5 Hz, CH [*syn*]), 6.12–6.18 (m, 2H, 2H-1′), 5.13 (m, 2H, 2OH), 4.76 (m, 1H, H-3′ [*syn*]), 4.70 (m, 1H, H-3′ [*anti*]), 4.04 (m, 1H, H-4′ (*syn*]), 4.01 (m, 1H, H-4′ [*anti*]), 3.57–3.68 (m, 4H, H-5′), 2.16–2.33

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dissolved in chloroform and the solution was concentrated *in vacuo*. The residue was purified on a silica gel column eluting with ethanol (0→5%)/chloroform system; mp 129–131°C; UV: $\lambda_{max}$ 264.7 nm; $^1$H NMR: δ 11.35 (s, 1H, 3-NH), 7.49 (q, 1H, $^4J_{6,\ 5\text{-Me}}$ = 0.8 Hz, H-6) 7.26–7.43 (m, 15H, Tr), 6.24 (dd, 1H, $^3J_{1',\ 2'b}$ = 6.8 Hz, $^3J_{1',\ 2'a}$ = 7.4 Hz, H-1′), 5.38 (m, 1H, H-3′), 4.23 (m, 1H, H-4′), 3.38 (dd, 2H, $^2J_{5'a,\ 5'a}$ = 10.5 Hz, $^3J_{5'a,\ 4'}$ = 4.3 Hz, H-5′a), 3.32 (dd, 2H, $^2J_{5'a,\ 5'b}$ = 10.5 Hz, $J_{5'b,\ 4'}$ = 3.5 Hz, H-5′b), 3.22 (s, 3H, Me (Ms)), 2.58 (m, 2H, H-2′), 1.51 (d, 3H, $^4J_{5\text{-Me},\ 6}$ = 0.8 Hz, 5-Me); $^{13}$C NMR: δ 163.4 (C-4), 150.2 (C-2), 143.2 (*i*-C (Tr)), 135.4 (C-6), 128.20 (*m*-C (Tr)), 127.84 (*o*-C (Tr)), 127.13 (*p*-C (Tr)), 109.87 (C-5), 86.71 (C (Tr)), 83.6 (C-1′), 82.5 (C-4′), 80.0 (C-3′), 63.0 (C-5′), 37.4 (Me (Ms)), 36.7 (C-2′), 11.6 (5-Me).

**3** A sample of reaction mixture was collected during the reddening of the solution to isolate 5′-*O*-trityl-2,3′-anhydrothymidine. An aliquot was treated with acetic acid to neutralize the alkali, solvents were then evaporated, the residue was dissolved in chloroform and the solution was concentrated *in vacuo*. The residue was purified on a silica gel column eluting with ethanol (0→3%)/chloroform system; UV: $\lambda_{max}$ 216.0 nm, 252.0 nm; $^1$H NMR: δ 7.60 (q, 1H, $^4J_{6,\ 5\text{-Me}}$ = 0.9 Hz, H-6), 7.22–7.31 (m, 15H, Tr), 5.89 (d, 1H, $^3J_{1',\ 2'b}$ = 3.7 Hz, H-1′), 5.31 (m, 1H, H-3′), 4.43 (m, 1H, H-4′), 3.14 (m, 2H, H-5′), 2.55–2.59 (m, 2H, H-2′a), 2.43–2.50 (m, 2H, H-2′b), 1.78 (d, 3H, $^4J_{5\text{-Me},\ 6}$ = 0.9 Hz, 5-Me); $^{13}$C NMR: δ 170.5 (C-4), 153.2 (C-2), 143.2 (*i*-C (Tr)), 136.5 (C-6), 128.1 (*m*-C (Tr)), 127.8 (*o*-C (Tr)), 126.9 (*p*-C (Tr)), 116.1 (C-5), 86.7 (C-1′), 86.3 (C (Tr)), 83.4 (C-4′), 77.0 (C-3′), 62.4 (C-5′), 32.7 (C-2′), 12.9 (5-Me).

(m, 2×2H, 2×H-2′), 1.80 (2d, 6H, $^{3}J_{Me,\,CH}$ = 5.5 Hz, $^{3}J_{Me,\,CH}$ = 5.8 Hz, 2Me), 1.78 (d, 3H, $^{4}J_{5\text{-}Me,\,6}$ = 1.0 Hz, 5-Me); $^{13}$C NMR: δ 163.6 (C-4), 150.4 (C-2), 148.9 (CH [*anti*]), 148.6 (CH [*syn*]), 135.9 (C-6), 109.6 (C-5 [*syn*]), 109.5 (C-5 [*anti*]), 84.1 (C-4′ [*syn*]), 83.9 (bs, C-4′ [*anti*]+C-1′ [*syn*]), 83.8 (C-1′ [*anti*]), 82.5 (C-3′ [*syn*]), 82.1 (C-3′ [*anti*]), 61.7 (C-5′ [*syn*]), 61.7 (C-5′ [*anti*]), 36.3 (C-2′ [*syn*]), 36.1 (C-2′ [*anti*]), 15.0 (Me [*anti*]), 12.2 (5-Me), 11.8 (Me [*syn*]). HRMS (ESI). Calcd for $C_{12}H_{17}N_3O_5$ $[M+H]^+$ and $[M+Na]^+$): *m*/*z* 284.1241 and 306.1060. Found: *m*/*z* 284.1241 and 306.1055.

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## References

[1] Chen, F.; Gaucher, E. A.; Leal, N. A.; Hutter, D.; Havemann, S. A.; Govindarajan, S.; Ortlund, E. A.; Benner, S. A. Reconstructed evolutionary adaptive paths give polymerases accepting reversible terminators for sequencing and SNP detection. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 1948–1953.

[2] Burgess, K.; Gibbs, R.; Metzker, M.; Raghavachari, R. Synthesis of an oxyamide linked nucleotide dimer and incorporation into antisense oligonucleotide sequences. *J. Chem. Soc.* **1994**, 915–916.

[3] Yang, T.; Chien, F.; Chung, F. Synthesis of thymidine dimer containing novel (N-acetyl)imino linkage. *J. Chin. Chem. Soc.* **2001**, *48*, 949–952.

[4] De Clercq, E.; Inoue, I.; Kondo, K. *EP Patent* 0381335A1. Date of publication of application: August 8, 1990.

[5] Horwitz, J. P.; Chua, J.; Da Rooge, M. A.; Noel, M.; Klundt, I. L. Nucleosides. III. 1-(2′-Deoxy-3′,5′-epoxy-β-D-threo-pentofuranosyl) thymine. *J. Am. Chem. Soc.* **1963**, *28*, 942–944.

[6] Jung, M. E.; Toyota, A. Preparation of 4′-substituted thymidines by substitution of the thymidine 5′-esters. *J. Org. Chem.* **2001**, *66*, 2624–2635.

[7] Solyev, P. N.; Shipitsin, A. V.; Karpenko, I. L.; Nosik, D. N.; Kalnina, L. B.; Kochetkov, S. N.; Kukhanova, M. K.; Jasko, M. V. Synthesis and anti-HIV properties of new carbamate prodrugs of AZT. *Chem. Biol. Drug. Des.* **2012**, *80*, 947–952.

[8] Taleno, M.; Levy, J. A. MT-4 plaque formation can distinguish cytopathic subtypes of the human immunodeficiency virus (HIV). *Virology* **1988**, *167*, 299–301.

[9] Tersmette, M.; De Goede, R. E. Y.; Al, B. J. M.; Winkel, I. N.; Gruters, R. A.; Cuypers, H. T.; Huisman, H. G.; Miedema, F. Differential syncytium-inducing capacity of human immunodeficiency virus isolates: frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. *J. Virol.* **1988**, *62*, 2026–2032.

[10] Buckheit, R. W., Jr.; Germany-Decker, J.; Qualls-Goodwin, K.; Bowdon, B. J.; Shannon, W. M. 3′-Azido-3′-deoxythymidine-induced reduction in the ability of uninfected CD4-expressing cells to participate in syncytium formation. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 8361–8365.

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