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# SYNTHESIS AND CRYSTAL STRUCTURE OF 3'-FLUORO-3'-METHYL-2',3'-DIDEOXYTHYMIDINE. INHIBITORY PROPERTIES OF 3'-FLUORO-3'-METHYL-2',3'-DIDEOXYTHYMIDINE-5'-TRIPHOSPHATE IN THE SYNTHESIS OF DNA IN CELL-FREE MEDIA

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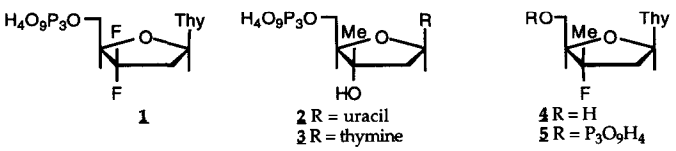
**Abstract** The synthesis, physicochemical properties and chain-termination properties of 3'-fluoro-3'-methyl-2',3'-dideoxythymidine is described. The synthesis was accomplished by the coupling of bis(TMS)thymine with 3-fluoro-3-methyl-2,3-dideoxy-D-erythro-pentose which is obtained from non-carbohydrate precursors.

Select 3'-substituted-2',3'-dideoxynucleoside-5'-triphosphates have been shown to be effective inhibitors of HIV reverse transcriptase; notably, the 5'-triphosphates of 3'-azido-2',3'-dideoxythymidine, 3'-fluoro-2',3'-dideoxythymidine, 2',3'-dideoxycytidine and 2',3'-dideoxy-2',3'-didehydrothymidine are the most potent.[1-3] However, reports on the activity of 3',3'-disubstituted-2',3'-dideoxynucleoside-5'-triphosphates in DNA-synthesis are scarce. It was reported[4] that 3',3'-difluoro-2',3'-dideoxythymidine-5'-triphosphate (**1**) is a weak inhibitor of human γ-DNA polymerase and HIV reverse transcriptase, but the molecular mechanism of inhibition was not described. Similarly, 3'-methyl-2'-deoxyuridine-5'-triphosphate (**2**)[5] and 3'-methylthymidine-5'-triphosphate (**3**)[6] showed weak inhibitory activity in DNA synthesis catalyzed by various DNA polymerases and reverse transcriptases, by the virtue there was no incorporation into the DNA chain. A strict interpretation for the absence of substrate **2** (*i.e.*, terminator) incorporation is complicated by the fact that it contains uracil, a nucleic base which is not a constituent of DNA. In contrast, X-ray crystallographic analysis of triphosphate **3** had shown it to have a significantly different conformation from that of known chain terminators.[6] Thus, the absence of chain-terminator properties for **3** may be conformational in nature.

In furtherance of the structure-inhibitor investigations, we synthesized 3'-fluoro-3'-methyl-2',3'-dideoxythymidine (3'-methyl-FLT, **4**) and investigated its crystal structure, as well as the properties of 3'-methyl-FLT-5'-triphosphate (**5**) in some cell-free DNA-polymerase systems.

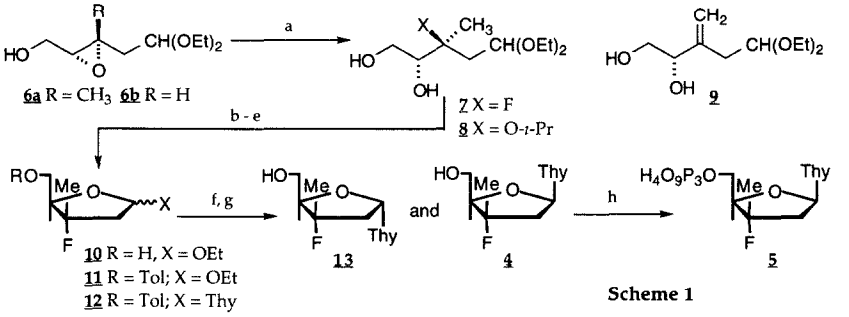



**1**

**2** R = uracil
**3** R = thymine

**4** R = H
**5** R = $P_3O_9H_4$

**Chemistry**. The synthesis of nucleoside **4** commences with (3*R*,4*R*)-5-hydroxy-3-methyl-3,4-epoxypentanal diethyl acetal (**6a**), which was obtained in 4 steps from 3-methyl-2-butenal.[7,8] We envisioned that regioselective epoxide cleavage of **6a** with bis(*iso*-propoxy)titanium difluoride[9] could afford 3-fluoro-3-methyl-2,3-dideoxy-D-*erythro*-pentose diethyl acetal (**7**). We have previously reported that bis(*iso*-propoxy)titanium difluoride [Ti(O-*i*-Pr)$_2$F$_2$] effectively converts 2,3-epoxyalcohols to fluorohydrins.[9] However, with acetal **6a**, three concommitant reactions occurred with two of the products due to nucleophilic ring cleavage: a) 3-fluoro-3-methyl-2,3-dideoxy-D-*erythro*-pentose diethyl acetal (**7**) and b) 3-methyl-3-O-isopropyl-2-deoxy-D-*erythro*-pentose diethyl acetal (**8**). The other product, 2,3-dideoxy-3-C-methylene-D-*glycero*-pentose diethyl acetal (**9**)[7], is due to epoxide-allylic rearrangement (Scheme 1). As shown in Table 1, the yield of acetal **9** is almost independent of reaction conditions; however, the relative ratio of acetals **7** and **8** is dependent on reaction temperature. Thus, optimal yield (40%) of fluorohydrin **7** occurs at high temperatures. These results are similar to that for the cleavage of (3*R*,4*R*)-5-hydroxy-3,4-epoxypentanal diethyl acetal (**6b**),[9] but there was complete absence of C-4 addition products with epoxide **6a**. Thus, the methyl group enhances the regioselectivity of the oxirane cleavage.




Scheme 1

a) Ti(O-*i*-Pr)$_2$F$_2$, $C_6H_6$; b) 1% EtOH - HCl; c) *p*-$CH_3C_6H_4COCl$ - $C_5H_5N$, d) $CH_3COOH$ - HCl;
e) bis(TMS)thymine, f) $CH_3ONa$ - $CH_3OH$; g) silica gel chromatography; h) $POCl_3$ - pyrophosphate

**Table 1. Ring Cleavage of Epoxy Alcohol 6 by Ti(O-*i*-Pr)$_2$F$_2$ in Benzene**

| Reaction Conditions | | | | Composition of mixture, % | | |
|---|---|---|---|---|---|---|
| Acetal 6 -Ti-Reag. Ratio | Temp (C°) | Time (hrs) | Conversion (%) | (7) | (8) | (9) |
| 1 | 10 | 1 5 | 94 | 14 | 70 | 16 |
| 1.5 | 15 | 1 0 | 96 | 19 | 52 | 29 |
| 2 | 20 | 1 0 | >98 | 25 | 45 | 30 |
| 2 | 30 | 1.0 | >98 | 28 | 40 | 31 |
| 2 | 80 | 0.5 | >98 | 38 | 42 | 20 |

The cyclization of acetal **7** to the anomeric furanosides **10** (90%) was achieved in dichloromethane at 20°C in the presence of 0.001% ethanolic hydrogen chloride with formation of minimal amounts (2-3%) of the corresponding pyranoside. Coupling of the p-toluoyloxy furanoside **11** with bis(trimethylsilyl)thymine was performed in the presence of trimethylsilyl triflate in acetonitrile. The anomeric mixture **12** (35:65, *per* NMR) was deprotected with methanolic sodium methoxide (83%) to give nucleoside **4** and the α-anomer **13**, separable by column chromatography on silica gel. Trisphosphorylation of **4** was accomplished with phosphorus oxychloride - bis(tributylammonium)pyrophosphate, according to the procedure of Ludvig to afford **5**.[10]

**NMR studies.** The composition of oxirane cleavage products was determined by $^{13}$C-NMR spectroscopy by using the resonances for C-1, C-2 and C-4. Data for allylic alcohol **9** were from an earlier publication[7], while $^1$H and $^{13}$C NMR resonance assignments for **7** and **8** were determined on isolated compounds. The location of the

fluoro- or isopropoxy- substituent was determined by comparison of the chemical shifts in $^{13}C$ NMR spectra with calculated values based on the additivity factors obtained from analogous compounds with C-3 methyl group.[11] The incremental shift values used for the substitution OH → F and OH → O-*i*-Pr are based on literature substituent value increments for aliphatic compounds.[12] Typically, the experimental values of the chemical shifts deviated from the calculated values by 1-3 ppm. For acetal **7**, the site of fluorination was also aided by the presence of additional spin-spin couplings (SSCC) in the $^{1}H$ and $^{13}C$ NMR spectra.[13]

Cyclization of **7** to the furanosides **10** was readily identified by the carbon resonances for C-4 (85-88 ppm) and C-2 (45-47 ppm).[14] The corresponding signals for the pyranosides, which were obtained in significant amount at higher concentration of HCl (>0.1%), have chemical shifts at 75-77 ppm (C-4) and 38-40 ppm (C-2). The anomeric configuration of the furanosides was determined on the basis of NMR spectra correlations which were previously reported from these laboratories.[14]

The anomeric configuration of **4**, as determined by NMR spectroscopy, is based on spin-spin coupling analyses and correlations between SSCC and torsional angles based on the Karplus equation, with correction for the electronegativity of the substituents.[15,16] Significant are the low SSCC values (<2 Hz) observed in a narrow range of torsional angles (80° to 100°). The presence of SSCC values in the spectra of the nucleosides infers a low conformational mobility for these molecules and the probable existence of one conformer. Since $J_{C(1'),F} \leq 1°$ for the nucleosides **4**, **12** and **13**, as well for the glycosides **10** and **11**, it infers a torsional angle (C1'-C2'-C3'-F) *ca.* 90°, which points definitely to *S*-conformation of the furanose ring with an *exo*-orientation of the C(3') atom (for N-C(2)-*endo* conformation this angle is 140-150°). The values of all possible torsion angles was obtained by conformational analyses. The correlation of experimental SSCC with the calculated values, based on the Karplus equations for α and β anomers in various conformations, further confirmed the *S*-conformation of the furanose ring in nucleosides **13** and **4**. The $J_{1,2}$ and $J_{1,2'}$ values lead to assigning α and β configuration to the nucleosides **4** and **13**, respectively. The results were further confirmed by X-ray studies.

**X-ray studies** confirmed the *anti*-orientation of the N-glycoside bond of 3'-methyl-FLT **4** with the torsional angle X (C2-N1-C1'-O4') at -127.3°. This value is similar to those observed with analogs containing a C(3')-$CH_3$ unit; for example, 1-(3'-methyl-2'-deoxyribofuranosyl)thymine [dT(3'-Me)] (X = -116.6°)[10] and 1-(3'-methyl-β-D-ribofuranosyl)cytosine [C(3'-Me)] (X = -130.9°).[11] The *gauche* conformation of the C4'-C5' exocyclic bond is consistent with the torsional angle γ (O5'-C5'-C4'-C3') in **4** at 56.8°, while in dT(3'-Me) and C(3'-Me) it is 50.1° and 61.7°, respectively.

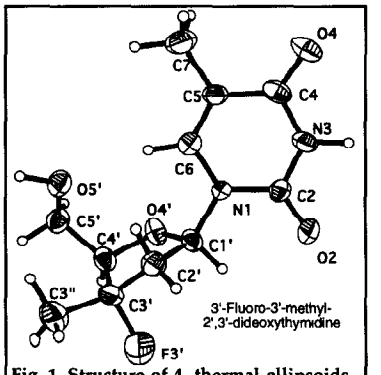


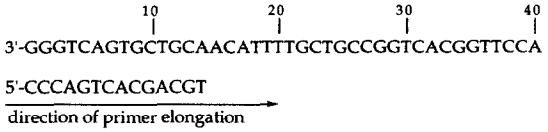
**Fig. 1. Structure of 4, thermal ellipsoids correspond to 50% probability.**

The conformation of the furanose ring of **4** can be described by the phase angle of pseudo-rotation P = 173.0° and degree of pucker $\psi_m$ = 38.4°. This corresponds to the C2'-*endo*-C3'-*exo*-($^{2}T_3$) conformation, with C-2' and C-3' deviating from the plane defined by C1'-C4'-O4' at a distance of 0.414Å and 0.192Å, respectively. For comparative purposes, the conformation of the sugar unit in dT(3'-Me) - C2-*endo*-C1'-*exo* ($^{2}T_1$, P = 157.5°, $\psi_m$ = 38.0°) and C(3'-Me) - C2'-*endo*-C3'-*exo* ($^{2}T_3$, P = 167.0°, $\psi_m$ = 37.8°). Thus, in all the examined compounds conformation of the sugar rings are in the *S*-configuration.

These results are similar to the literature data[17,18] which demonstrate that pyrimidine nucleosides having C3'-methyl groups, irrespective of the base, have the same *anti* conformation in relation to the N-glycoside bond. That

is, *gauche* conformation around the exocyclic C4'-C5' bond and *endo* orientation of C2' carbon atom (*S*-population) in the furanoside unit. In the nucleosides, which have *S*-conformation of the carbohydrate moiety, either nucleic base or the C(3')-methyl carbon exist in the equatorial position, which minimizes steric hinderance between the bulky substituents.

**Biological Results and Discussion.** DNA polymerase inhibitory activity of 3'-fluoro-3'-methyl-2',3'-dideoxythymidine-5'-triphosphate **5** was determined in cell-free systems using various DNA polymerases in a system with an incomplete set of substrates. A model consisting of the DNA template of the M13 mp 10 phage and synthetic deoxytetradecanucleotide was used (Scheme 2). To this assay system was added nucleoside **5** and the appropriate DNA polymerase. The template-primer was chosen so that select links in the growing DNA chain should incorporate thymidine-5'-triphosphate residues (the controls were carried out in the presence of thymidine-5'-triphosphate (**3**) - Tracks 7, 11, 15 and 19) (Fig. 2). The absence of incorporation of **5** into the DNA chain is illustrated by the absence of the appropriate zone in tracks 4-5, 8-9, 12-13, 16-17, 20-21.




**Scheme 2. Primer-Template Complex**

As shown in Figure 2, the gel illustrates the comparative evaluation of **5** in five DNA preparations: the reverse transcriptase from the avian myeloblastosis virus (AMV) (tracks 2-5), and human immunodeficiency virus (tracks 6-9), DNA polymerases α and ε from human placenta (tracks 10-13 and 14-17, correspondingly) and DNA polymerase I (Klenow fragment) (track 18-21). It is clear from the gels, that none of these preparations incorporate **5** into the primer. The presence of additional zones of tridecanucleotide and dodecanucleotide in the tracks 14, 16-18 and 20-21 can be explained by the adventitious 3'→ 5'-exonuclease activity of DNA polymerases ε and I, which occurs in the absence of substrates in the reaction medium. The tridecanucleotide band in track 13 is explained by the mix activity of DNA polymerase α. The DNA polymerases selected include high specificity enzymes (DNA polymerases α and ε), as well as low specificity enzymes (the reverse transcriptases).[19,20] DNA-polymerase I was used in the control preparation. The lack of elongation of the oligonucleotide chain for all five preparations demonstrated clearly that **5** is not a terminator substrate for these enzymes. Thus, the polymerase activity of nucleotide **5** is similar to that for nucleosides **2-3**.

## EXPERIMENTAL

NMR spectra were recorded with either Bruker AM-360 (360.13 MHz, $^1$H; 90.56 MHz, $^{13}$C) or Varian Gemini-300 (300 MHz, $^1$H; 75 MHz, $^{13}$C) spectrometers in either acetone-$d_6$, methanol-$d_4$ or deuterium oxide at 20°C ($^1$H) and 30°C ($^{13}$C). Optical rotations were determined with a Jasko polarimeter in methanol. Thin layer chromatography was accomplished on Silufol-254 plates (Czechoslovakia) with spot detection by UV or by heating and column chromatography was carried out on silica gel 60 (63-100μ, Merck).

**3-Fluoro-3-methyl-2,3-dideoxy-D-*erythro*-pentose diethyl acetal (7).** A suspension of 7.44 g (60 mmol) of titanium (IV) fluoride and 24 g of powdered potassium carbonate in 200 ml of dry benzene was treated with 17.04 g (60 mmol) of titanium (IV) isopropoxide and stirred at ambient temperature for 0.5 h. The resultant mixture was heated to reflux and a solution of 6.12 g (30 mmol) of (3*R*,4*R*)-5-hydroxy-3-methyl-3,4-

epoxypentanal diethyl acetal (**6a**) in 20 ml of dry benzene was added dropwise to the reaction. After stirring for 0.5 h, the reaction was cooled to 5-10°C and quenched with sat. aqueous potassium carbonate (100 ml). The precipitate was filtered and the filtercake washed thoroughly with diethyl ether (5 x 100 ml). The organic phase was dried ($K_2CO_3$) and concentrated *in vacuo*. NMR analysis of the crude mixture showed a composition of **7** (38%), **8** (42%) and **9** (20%)[7], which was separated by column chromatography ($CH_2Cl_2$). Fluorohydrin **7** (the last spot) (2.3 g, 35%): $R_f$ 0.14 (chloroform-methanol, 10:1), $[\alpha]_D^{20}$ +15.2° (*c* 1.0, $CH_3OH$); $^{13}C$ NMR (90 MHz, $CD_3COCD_3$) δ 15.50 ($OCH_2CH_3$), 20.81 ($^2J_{CF}$ = 24.1 Hz, C(3)-$CH_3$), 41.50 ($^2J_{CF}$ = 21.6 Hz, C-2), 61.16 and 61.38 ($OCH_2$'s), 62.70 ($^3J_{CF}$ = 5.0 Hz, C-5), 76.63 ($^2J_{CF}$ = 26.2 Hz, C-4), 97.13 ($^1J_{CF}$ = 170.6 Hz, C-3), 100.04 ($^3J_{CF}$ = 6.0 Hz, C-1). Anal. Calc. for $C_{10}H_{21}O_4F$: C, 53.56; H, 9.44; F, 8.47. Found: C, 53.38; H, 9.40; F, 8.55.

**Ethyl 3-fluoro-3-methyl-2,3-dideoxy-D-*erythro*-pentofuranosides (10).** A solution of 0.63 g (2.8 mmol) of acetal **7** in dichloromethane (20 ml) was treated with 1% ethanolic hydrogen chloride (0.01 ml). The reaction, which was monitored by TLC, was complete in 0.5 h and neutralized with solid potassium bicarbonate. The precipitate was filtered and the filtrate was concentrated *in vacuo* and purified by column chromatography ($CHCl_3$). Furanoside **10** was obtained as an anomeric mixture (0.45 g, 90%): $R_f$ 0.65 and 0.80 (chloroform-methanol, 10:1). Alpha anomer **10a**: $^{13}C$ NMR (90 MHz, $CD_3COCD_3$) δ 15.5 ($OCH_2CH_3$), 20.5 ($^2J_{CF}$ = 26.6 Hz, C(3)-$CH_3$), 46.6 ($^2J_{CF}$ = 21.4 Hz, C-2), 61.9 ($^3J_{CF}$ = 8.8 Hz, C-5), 63.3 ($OCH_2$), 86.5 ($^2J_{CF}$ = 27.3 Hz, C-4), 101.5 ($^2J_{CF}$ = 175.5 Hz, C-3), 103.6 ($^3J_{CF}$ = 0.9 Hz, C-1). Beta anomer **10b**: $^{13}C$ NMR (90 MHz, $CD_3COCD_3$) δ 15.5 ($OCH_2CH_3$), 19.6 ($^2J_{CF}$ = 25.4 Hz, C(3)-$CH_3$), 46.3 ($^2J_{CF}$ = 22.0 Hz, C-2), 62.8 ($^3J_{CF}$ = 10.3 Hz, C-5), 64.3 ($OCH_2$), 87.8 ($^2J_{CF}$ = 24.1 Hz, C-4), 103.4 ($^2J_{CF}$ = 172.3 Hz, C-3), 104.5 ($^3J_{CF}$ = 0.9 Hz, C-1). Anal. Calc. for $C_8H_{15}O_3F$: C, 53.92; H, 8.49; F, 10.66. Found: C, 54.03; H, 8.44; F, 10.58.

**Ethyl 3-fluoro-3-methyl-5-O-(p-toluoyl)-2,3-dideoxy-D-*erythro*-pentofuranosides (11).** A cold (-15°C) solution of 1.23g (6.9 mmol) of furanosides **10** in 10 ml of dry pyridine, was treated with 1.17 g (7.6 mmol) of p-toluoyl chloride. The resultant mixture was stirred at ambient temperature for 16 h and then concentrated *in vacuo*. The residue was dissolved in 60 ml of chloroform and the solution was washed sequentially with water (5 x 25 ml), sat. aqueous sodium bicarbonate (25 ml) and water (25 ml) and dried with anhydrous $Na_2SO_4$. After removal of solvent, the oily residue was purified by column chromatography over silica gel (50 g) (hexane-ethyl acetate, 25:1). There was obtained 0.92 g (45%) of **11**; alpha anomer **11a**: $^{13}C$ NMR (90 MHz, $CD_3COCD_3$) δ 15.41 ($OCH_2CH_3$), 20.7 ($^2J_{CF}$ = 27.5 Hz, C(3)-$CH_3$), 46.4 ($^2J_{CF}$ = 21.8 Hz, C-2), 63.7 ($^3J_{CF}$ = 7.1 Hz, C-5), 64.0 ($OCH_2$), 83.1 ($^2J_{CF}$ = 30.4 Hz, C-4), 101.3 ($^1J_{CF}$ = 180.0 Hz, C-3), 103.7 ($^3J_{CF} \leq$ 1.0 Hz, C-1). Beta anomer **11b**: $^{13}C$ NMR (90 MHz, $CD_3COCD_3$) δ 15.40 ($OCH_2CH_3$), 19.8 ($^2J_{CF}$ = 25.7 Hz, C(3)-$CH_3$), 45.8 ($^2J_{CF}$ = 22.5 Hz, C-2), 64.6 ($^3J_{CF}$ = 10.7 Hz, C-5), 64.1 ($OCH_2$), 84.3 ($^2J_{CF}$ = 27.2 Hz, C-4), 103.3 ($^1J_{CF}$ = 173.2 Hz, C-3), 104.8 ($^3J_{CF} \leq$1.0 Hz, C-1). Anal. Calc. for $C_{16}H_{21}O_4F$: C, 64.85; H, 7.14; F, 6.41. Found: C, 64.99; H, 7.12; F, 6.53.

**1-[3-Fluoro-3-methyl-5-O-(p-toluoyl)-2,3-dideoxy-D-*erythro*-pentofuranosyl]thymine (12).** A solution of 0.156 g (0.525 mmol) of **11** and 0.288 g (1.065 mmol) of bis(trimethylsilyl)thymine in 3 ml of dry acetonitrile was treated with 0.20 ml (1.04 mmol) trimethylsilyl triflate. The resultant solution was kept at ambient temperature for 2.5 h, cooled to 0° and quenched with sat. sodium bicarbonate (4 ml). The mixture was extracted with chloroform (3 x 5 ml), washed with water (2 x 5 ml) and dried over $Na_2SO_4$. After evaporation of the solvent, the residue was separated by column chromatography over silica gel (15 g) ($CHCl_3$), 0.125 g (63.3%);

alpha anomer **12a**: $^{13}C$ NMR (90 MHz, $CD_3COCD_3$) δ 19.4 ($^2J_{CF}$ = 25.0 Hz, C(3)-$CH_3$), 43.6 ($^2J_{CF}$ = 22.1 Hz, C-2), 64.2 (C-5), 84.8 ($^3J_{CF}$ ≤0.5 Hz, C-1), 86.5 (C-4), 103.0 ($^1J_{CF}$ = 171.8 Hz, C-3). Beta anomer **12b**: $^{13}C$ NMR (90 MHz, $CD_3COCD_3$) δ 19.3 ($^2J_{CF}$ = 25.0 Hz, C(3)-$CH_3$), 44.9 ($^2J_{CF}$ = 22.6 Hz, C-2), 64.3 (C-5), 84.5 ($^3J_{CF}$ ≤0.5 Hz, C-1), 86.5 (C-4), 102.7 ($^1J_{CF}$ = 172.0 Hz, C-3). Anal. Calc. for $C_{19}H_{21}N_2O_5F$: C, 60.63; H, 5.62; N, 7.44; F, 5.05. Found: C, 60.47; H, 5.66; N, 7.51; F, 5.11.

**3'-Fluoro-3'-methyl-2',3'-dideoxythymidine (4) and 1-[3'-fluoro-3'-methyl-2',3'-dideoxy-α-D-*erythro*-pentofuranosyl]thymine (13).** A solution of **12** (0.371 g) in absolute methanol (18 ml) was treated with 0.2 M methanolic sodium methoxide (10 ml). The mixture was maintained at 24° for 4 h, neutralized with DOWEX 50W x 1 ($H^+$-form) ion exchange resin, filtered and concentrated *in vacuo*. The residue was dissolved in chloroform and chromatographed over silica gel (20 g) using a gradient of acetone (100:0 → 8:1 → 6:1). There was obtained 0.136 g (53.5%) of **4**: mp 200-202° (dec.) (ethanol); $R_f$ 0.20 (hexane-ethyl acetate, 1:1); $[\alpha]_D^{20}$ +8.88° (*c* 0.83, methanol); $^1H$ NMR (300 MHz, $CD_3OD$) δ 1.58 (d, 3H, $^3J_{HF}$ = 22.2 Hz, C(3')-$CH_3$), 1.86 (s, 3H, =$CCH_3$), 2.19 (ddd, 1H, $^3J_{HF}$ = 40.6; $J_{2,2'}$ = 13.6 Hz, H-2), 2.43 (ddd, 1H, $^3J_{HF}$ = 16.1 Hz, H-2'), 3.70 (ddd, 1H, $J_{5,5'}$ = 12.1 Hz, H-5), 3.79 (ddd, 1H, $^4J_{HF}$ = 2.1 Hz, H-5'), 4.08 (ddd, 1H, $^3J_{HF}$ = 25.0; $J_{4,5}$ = 2.5; $J_{4,5'}$ = 2.1 Hz, H-4), 6.27 (dd, 1H, $^4J_{HF}$ ≤0.5; $J_{1,2}$ = 9.7; $J_{1,2'}$ = 5.3 Hz, H-1), 8.05 (s, 1H, H-6); $^{13}C$ NMR (90 MHz, $CD_3COCD_3$) δ 18.7 ($^2J_{CF}$ = 25.3 Hz, C(3')-$CH_3$), 45.7 ($^2J_{CF}$ = 21.3 Hz, C-2), 61.9 (C-5), 84.2 (C-1), 90.2 ($^2J_{CF}$ = 24.0 Hz, C-4), 102.9 ($^1J_{CF}$ = 170.2 Hz, C-3). Later fractions afforded nucleoside **13** (74 mg, 29%): $^1H$ NMR (300 MHz, $CD_3OD$) δ 1.56 (d, 3H, $^3J_{HF}$ = 22.3 Hz, C(3')-$CH_3$), 1.87 (s, 3H, =$CCH_3$), 2.31 (ddd, 1H, $^3J_{HF}$ = 21.9; $J_{2,2'}$ = 15.1 Hz, H-2), 2.43 (ddd, 1H, $^3J_{HF}$ = 41.2 Hz, H-2'), 3.61 (ddd, 1H, $^4J_{HF}$ = 2.1; $J_{5,5'}$ = 12.6 Hz, H-5), 3.71 (ddd, 1H, $^4J_{HF}$ = 2.9 Hz, H-5'), 4.44 (ddd, 1H, $^3J_{HF}$ = 21.8; $J_{4,5}$ = 1.1; $J_{4,5'}$ = 3.2 Hz, H-4), 6.25 (dd, 1H, $^4J_{HF}$ ≤0.5; $J_{1,2}$ = 1.6; $J_{1,2'}$ = 7.6 Hz, H-1), 7.55 (s, 1H, H-6); $^{13}C$ NMR (90 MHz, $CD_3COCD_3$) δ 19.8 ($^2J_{CF}$ = 24.7 Hz, C(3')-$CH_3$), 43.8 ($^2J_{CF}$ = 21.5 Hz, C-2), 61.5 ($^3J_{CF}$ = 12.1 Hz, C-5), 84.8 (C-1), 87.7 ($^2J_{CF}$ = 25.2 Hz, C-4), 103.4 ($^1J_{CF}$ = 170.7 Hz, C-3). Anal. Calc. for $C_{11}H_{15}N_2O_4F$: C, 51.16; H, 5.85; N, 10.85; F, 7.36. Found: C, 51.37; H, 5.92; N, 10.78; F, 7.22.

**3'-Fluoro-3'-methyl-2',3'-dideoxythymidine-5'-triphosphate (5).** A solution of 40 mg (0.16 mmol) of 3'-fluoro-3'-methyl-2',3'-dideoxythymidine (**4**) in 1 ml of triethyl phosphate was added, at 0°C, 50 μL (0.54 mmol) phosphorus oxychloride. The mixture was stirred for 2 days at +5°C, then a cold mixture (5°C) of 0.5 M bis(tributylammonium)pyrophosphate in DMF (2 ml) and tributylamine (130 μL, 0.54 mmol) was added. The reaction mixture was stirred for 40 min. at 20° and then neutralized with 1 M triethylammonium bicarbonate to pH 7.5, evaporated at 20°. The solid in 400 mL of water was applied onto a DEAE-column (DE-32, Whatman, $HCO_3^-$ form, 13 x 4 cm) and the column eluted with a linear gradient of ammonium bicarbonate, pH 7.5 (0 → 0.4 M; 1L). Triphosphate **5** was eluted with 0.25 M buffer. The fractions containing **5** were evaporated at 20°, and reevaporated with 10% EtOH (5 x 10 ml) and freeze-dried (23 mg, 26%): $^1H$ NMR (360 MHz, $D_2O$), δ 1.83 (d, 2H, $J_{Me,F}$ = 23 Hz, C(3')-Me), 1.94 (s, 3H, =CMe), 1.96-2.70 (m, 2H, H-2'), 4.17 (m, 2H, H-5'), 4.38 (dm, 1H, $J_{H,F}$ = 27 Hz, H-4'), 6.31 (dd, 1H, *J* = 9.5; 5.5 Hz, H-1') and 7.83 (s, 1H, H-6).

**X-ray studies.** Crystals for X-ray crystallographic analysis were grown from a saturated solution of **4** in ethanol by slow evaporation of the solvent at 4°C. The space group of crystals was P212121,with a = 5.579(1); b = 11.435(2); c = 19.186(4)Å; z = 4. The parameters of unit cell and the intensity of reflections were measured on the Syntax P1 difractometer (e/2e - scanning, MoKα radiation) and the data was corrected for Lorentz and polarization factors. Intensities of 625 independent reflections with $I > 3\sigma(I)$ were used for the structural

investigations. The structure was solved by direct methods and refined by full-matrix least squares with anisotropic approximation for nonhydrogen atoms. Coordinates of the hydrogen atoms were determined from difference Fourier syntheses and refined with isotropic temperature factors. The final value of R factor was 2.8%. All the calculations were made with the SHELXTL program complex.

**Cell-Free DNA Synthesis Studies.** The DNA from M13pm10 phage was freshly isolated.[21] Tetradecanucleotide primer (Scheme 2) was labeled at the 5'-position with T4 polynucleotide kinase (Amersham) by means of [$\gamma$-$^{32}$P]-ATP, 1500 Ci/mmol.[22] After phenol extraction and resedimentation in ethanol, the [$^{32}$P]-oligonucleotide was reconstituted in water to 1 nmol/mL concentration and used for the hybridization reactions[23], catalyzed with DNA polymerases. The following polymerases were used: a) *E. Coli* DNA polymerase I, Klenow fragment (Amersham), b) DNA polymerases from human placenta $\alpha$ (A. Atrazhev, Institute of Molecular Biology, Moscow) and $\varepsilon$ (D. Mozzerin, Institute of Molecular Biology, Moscow), c) avian myeloblastosis virus (AMV) reverse transcriptase (Omutninsk, Russia) and d) human immunodeficiency virus HIV-1 (T. Rosovskaya and R. Beabealashvili, Cardiology Center, Moscow).

The DNA M13pm10 was hybridized with [5'-$^{32}$P]-primer (0.75 $\mu$M) in the appropriate buffers: 10 $\mu$M tris-HCl, pH 7.9, 5 $\mu$M $MgCl_2$, 1 $\mu$M dithiothreitol (for DNA polymerase I); 10 $\mu$M tris-HCl, pH 8.2, 5 $\mu$M $MgCl_2$, 40 $\mu$M KCl, 1 $\mu$M dithiothreitol (for the reverse transcriptases); 10 $\mu$M tris-HCl, pH 7.4, 6 $\mu$M $MgCl_2$, 0.4 $\mu$M dithiothreitol (for DNA polymerases $\alpha$ and $\varepsilon$).

The addition of 2'-deoxythymidine-5'-phosphate to the 3'-position of [5'-$^{32}$P]-primer was conducted in 6 $\mu$L mixtures, containing the appropriate buffer, one of the enzymes (0.5 units of DNA polymerase I, 1 unit of both DNA polymerases $\alpha$ and $\varepsilon$, 3 units of reverse transcriptases), 3 nM primer-template complex, 10 $\mu$M dTTP and the triphosphate **5** at different concentrations, as shown in Figure 2. The mixture was incubated for 20 min. at 37°C (10 min. at 20°C for DNA polymerase I) and then quenched by the addition of 2 $\mu$L deionized formamide containing 0.1% Xylene Cyanole, 0.1% of Bromophenol Blue and 20 $\mu$M EDTA, pH 8.0. The products were separated by electrophoresis in denaturated 20% PAAG.

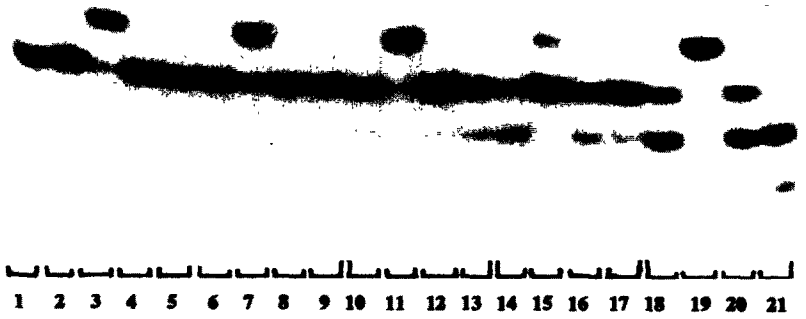



**Figure 2.** Autoradiography/PAGE analysis of [$^{32}$P]-tetradecanucleotide elongation catalyzed by DNA polymerases: avian myeloblastosis virus (AMV) reverse transcriptase (3 units) (2-5), and human immunodeficiency virus HIV-1 reverse transcriptase (3 units) (6-9), DNA polymerase $\alpha$ (2 units) (10-13), DNA polymerase $\varepsilon$ (1 unit) (14-17), DNA polymerase 1, Klenow fragment (0.4 units) (18-21). Tracks: [$^{32}$P]-primer template complex (control) (1); the same and emzyme (2, 6, 10, 14, 18); the same and enzyme and 10 $\mu$M dTTP (3, 7, 11, 15, 19); the same and enzyme and 10 $\mu$M IV (4, 8, 12, 16, 10); the same and enzyme and 100 $\mu$M IV (5, 9, 13, 17, 21).

## REFERENCES

† Microcide Pharmaceuticals, Inc., 850 Maude Avenue, Mountain View, California 94043

1. Nasr, M.; Litterst, C.; McGowan, J. *Antiviral Research*, **1990**, *14*, 125-148.
2. Herdewijn, P.; Bazarini, J.; De Clercq, E.; Pauwels, R.; Baba, M.; Broder, S.; Vanderhaege, H. *J. Med. Chem.*, **1987**, *30*, 1270-1278.
3. Balzarini, J.; Baba, M.; Pauwels, R.; Herdewijn, P.; De Clercq, E. *Biochem. Pharmacol.*, **1988**, *37*, 2847-2856.
4. Cheng, Y.-U.; Dutschman, G.E.; Bastow, K.F.; Sarngadharan, M.G.; Ting, R.Y.C. *J. Biol. Chem.*, **1987**, *262*, 2187-2189.
5. Mikhailov, S.N.; Padyakova, N. *Sh. Nucleosides and Nucleotides*, **1991**, *10*, 339-343.
6. Fedorov, I.I.; Kazmina, E.M.; Novicov, N.A.; Gurskaya, G.V.; Bochkarev, A.V.; Jasko, M.V.;Victorova, L.S.; Kukhanova, M.K.; Balzarini, J.; De Clercq, E.; Krayevsky, A.A. *J. Med. Chem.*, in press.
7. Raifeld, Yu.E.; Vid, G. Ya; Mikerin, I.E.; Arshava, B.M.; Nikitenko, A.A. *Carbohydrate Res.*, **1992**, *224*, 103-109.
8. The NMR spectra of **6** is given in Table 2. Its optical purity was determined by $^1$H NMR analysis of its diastereomeric esters with the Mosher reagent (*cf.*, Dale, J.A.; Dull, D.L.; Mosher, H.S. *J. Org. Chem.*, **1969**, *34*, 2543-2549), (+)-α-methoxy-α-(trifluoromethyl)phenyl acetic acid. Quantitative measurements of the integrals of the H-5 and H-5' resonances, which have the largest difference in the chemical shifts for the diastereomeric esters and the least overlap with other resonances. The chemical shifts (δ), in $CDCl_3$, of (3*R*,4*R*)-epoxy alcohol **6** were 4.326 (dd, $J$ = 7; 12 Hz) and 4.570 (dd, $J$ = 4.5; 12 Hz), while for (3*S*,4*S*)-enantiomer **6** they were 4.378 (dd, $J$ = 6.8; 12 Hz) and 4.508 (dd, $J$ = 4.4; 12 Hz). The *ee* was 94%.
9. Nikitenko, A.A.; Arshava, B.M.; Mikerin, I.E.; Raifeld, Yu. E.; Lee, V.J.; Lang, S.A. *Tetrahedron Lett.*, **1992**, *33*, 7087-7088.
10. Ludvig, I. *Acta Biochim. Biophys, Acad. Sci. Hung.*, **1981**, *16*, 131-133.
11. Raifeld, Yu. E.; Nikitenko, A.A.; Vid, G. Ya.; Arshava, B.M.; Mikerin, I.E.; Lee, V.J., submitted to *Tetrahedron* (1993).
12. Wehrly F.W.; Wirthlin, T. Interpretation of $^{13}$C-NMR Spectra, Heyden, London, **1976**.
13. Stothers, J.B. Carbon-13 NMR Spectroscopy, Chapter 10, *Academic Press*, NY, **1972**.
14. Arshava, B.M.; Raifeld, Yu. E.; Makin, S.M. *Zh. Org. Khim.*, **1990**, *26*, 778-785.
15. Karplus, M. *J. Am. Chem. Soc.* **1963**, *85*, 2870-2875.
16. Abraham, R.J.; Cavalli, L.; Pachler, K.G.R. *Mol. Phys.*, **1966**, *11*, 471-475.
17. Gurskaya, G.V.; Javadova, G.M.; Mikhailov, S.N. *Crystal Structure Communication*, **1982**, *11*(4/A), 1253-1258.
18. Bochkarev, A.V.; Gurskaya, G.V.; Zhdanov, A.S.; Kaz'mina, E.M.; Fedorov, I.I. *Bioorg. Khim.* **1991**, *17*, 1094-1100.
19. Krayevsky, A.A.; Kukhanova, M.K. *Sov. Sci. Rev. D. Physicochem. Biol.*, **1990**, *9*, 179-242.
20. Krayevsky, A.A. *Zh. Org. Khim.* **1982**, *18*, 27-37, .
21. Krayev, A.S.; *Mol. Biol.*, **1988**, *22*, 1164-1197.
22. Maniatis, T.; Fritch, E.; Sambruck, D. Molecularnoye klonirovanie, Moscow, Mir, **1984**.
23. Dyatkina, N.B.; Viktorova, L.S.; Mozzherin, D. Yu.; Atrazhev, A.M.; Rozenberg, S.G., Kukhanova, M.K.; Krayevsky, A.A. *Mol. Biol.*, **1991**, *25*, 1688-1700.

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