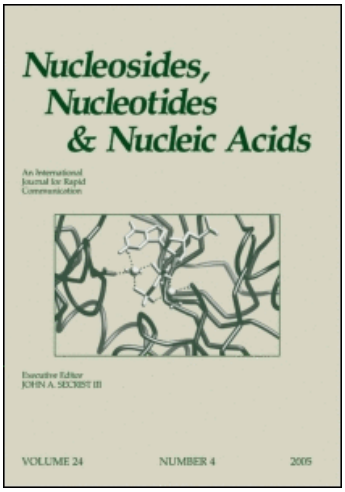

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SYNTHESIS OF THYMIDINE DERIVATIVES 3'-MODIFIED WITH A POLAR THREE-ATOM GROUP AS POTENTIAL ANTI-HIV-1 AGENTS

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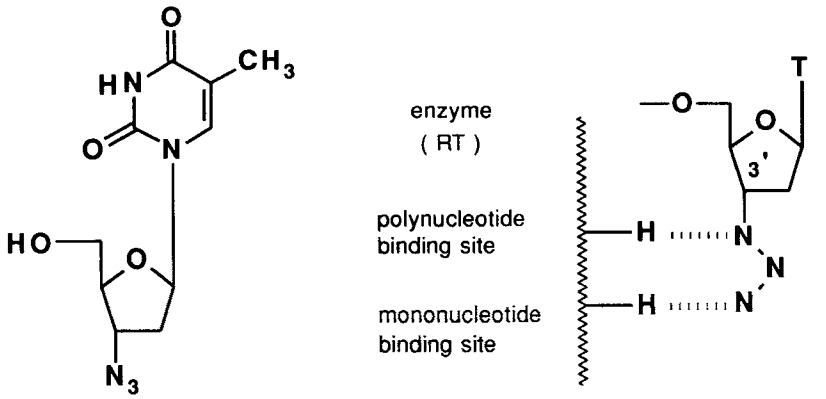
Abstract: 3'-Modified thymidine analogs, such as 3'-*O*-[(methylthio)thiocarbonyl]thymidine (**4**), 3'-*O*-thiocarbamoylthymidine (**7**), and *N*-(3'-deoxythymidin-3'-yl)phosphoramidates (**9a**, **b**), were synthesized from thymidine derivatives (**1**), (**5**), and (**8**), respectively, as potential anti-human immunodeficiency virus type 1 (HIV-1) agents. No significant activity against HIV-1 was, however, observed with any of these compounds.

3'-Azido-3'-deoxythymidine (AZT, zidovudine) is a drug approved for the treatment of acquired immunodeficiency syndrome (AIDS) resulting from infection with human immunodeficiency virus type 1 (HIV-1).[1] The mode of action of AZT involves conversion to the 5'-triphosphate and its inhibition against the HIV-1 reverse transcriptase (RT).[2] It has been suggested that the role of the 3'-azido group for the inhibition of RT might involve binding to the poly- and/or mono-nucleotide binding site of the enzyme[3] because the charge distribution in the azido group mimics the charge distribution in O-P-O bond (3'-phosphate moiety) of the nucleotide[3,4] (Scheme 1). Therefore, a number of 3'-deoxythymidines 3'-modified with a polar three-atom group have been synthesized and their anti-HIV-1 activity has been evaluated.[5]



AZT

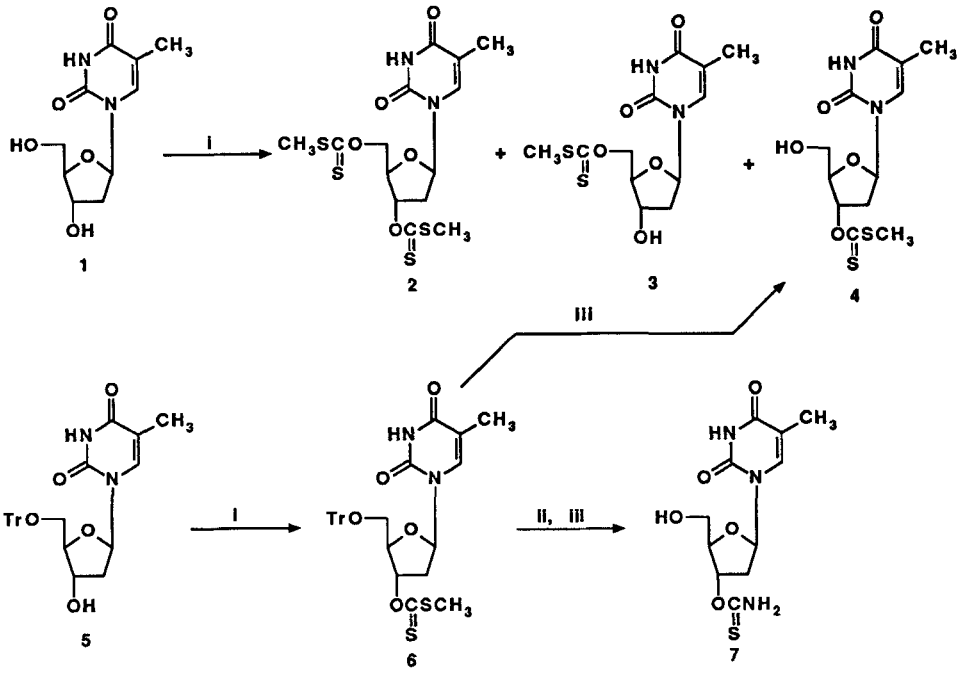
Scheme 1

This paper describes synthesis of thymidine derivatives (**4**, **7**, and **9**) equipped at the 3'-position with functional groups such as -OCSSCH$_3$, -OCSNH$_2$, and -NHPO(OR)$_2$, which are expected to have interaction with HIV-1 RT.

3'-Xanthates of 2'-deoxynucleosides have been synthesized as intermediates for the preparation of anti-HIV-1 active 2',3'-dideoxynucleosides.[6] In these cases 5'-protected 2'-deoxynucleosides have been employed. We tried *S*-methyldithiocarbonylation (xanthation) of 5'-unprotected thymidine. Thus, thymidine was treated with carbon disulfide (2.4 eq.) in the presence of sodium hydride (3.0 eq.) and then allowed to react with methyl iodide (2 eq.) to give 3',5'-bis-xanthate (**2**), 5'-xanthate (**3**), and 3'-xanthate (**4**) in 46%, 15%, and 12% yields, respectively. The structure of the expected 3'-xanthate (**4**) was confirmed by comparison with an authentic sample prepared by the xanthation of 5'-*O*-tritylthymidine and subsequent deprotection of the resulting xanthate (**6**). Although direct xanthation of thymidine gave the desired product (**4**) in only 15% yield, this is a convenient method on small scale.

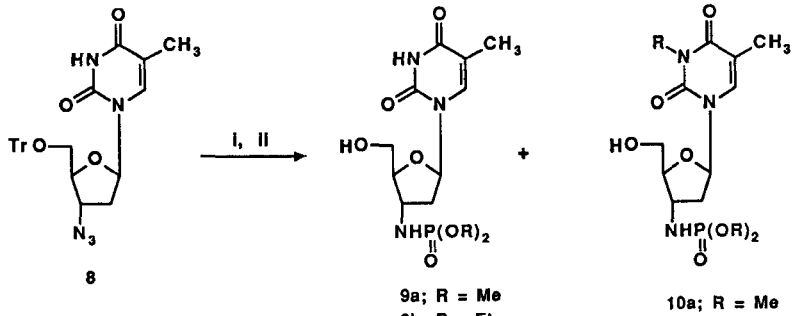
Ammonolysis of **6** followed by deprotection in 80% acetic acid afforded 3'-*O*-thiocarbamoylthymidine (**7**) in 71% yield.

Synthesis of 3'-thymidylamidates (**9**) could be achieved according to the method for the preparation of 5'-thymidylamidates.[7] Treatment of 3'-azido-3'-deoxy-5'-tritylthymidine (**8**) with trimethyl phosphite in toluene at room



i NaH, CS_2 then CH_3I. ii NH_3. iii 80% AcOH, 100°C.

Scheme 2



i $P(OR)_3$. ii 80%AcOH.

Scheme 3

temperature followed by detritylation in 80% acetic acid gave the thymidyl-3'-phosphoramidate (**9a**) in 67% yield together with its N^3-methyl derivative (**10a**) in 26% yield. Analogous treatment of **8** with triethyl phosphite at room temperature and subsequent deprotection led to the formation of diethyl amidate (**9b**) as the sole product in 86% yield. When the 3'-azidothymidine (**8**) was, however, refluxed with triethyl phosphite in toluene and subsequently detritylated, the N^3-ethyl derivative (**10b**) was formed.

The anti-HIV-1 activity of compounds **4**, **7**, and **9a** was evaluated using HIV-1 infected human peripheral blood mononuclear (PBM) cells. No appreciable antiviral activity (EC_{50} > 100μM , AZT = 0.006μM) was observed with these compounds.

EXPERIMENTAL

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded at 270 MHz on a JEOL JNX-270 spectrometer in $CDCl_3$ or $(CD_3)_2SO$ (DMSO-d_6). Chemical shifts are quoted in parts per million (s=singlet, d=doublet, dd=double doublet, t=triplet, m=multiplet, br=broad, brs=broad singlet). Mass spectra (MS) were measured at 70 eV with a JEOL JMS-D300 spectrometer. Fast atom bombardment (FAB)-MS were measured on a JEOL JMS-HX100 spectrometer with a xenon FAB beam at 6 kV energy. The matrix was glycerol and calibration was performed using a mixture of CsI and NaI (5:1). Infrared (IR) spectra were obtained with Perkin-Elmer 1600 FT-IR instrument for KBr pellets. Column chromatography was carried out on silica gel (Wako gel C-300).

3',5'-*O*-Bis[(methylthio)thiocarbonyl]thymidine (2), 5'-*O*-[(Methylthio)thiocarbonyl]thymidine (3), and 3'-*O*-[(Methylthio)-thiocarbonyl]thymidine (4) Thymidine (1) (242 mg, 1 mmol) was added to a mixture of 50% NaH (120 mg, 3 mmol) and CS_2 (183 mg, 2.4 mmol) in dry DMF (5 ml) and the mixture was stirred at room temperature for 30 min. And then methyl iodide (284 mg, 2 mmol) was added thereto and the reaction mixture was stirred for 1 h. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel column with benzene-ethyl acetate (1:1) as eluents to give **2** (195 mg, 46%), **4** (40 mg, 12%), and **3** (48 mg, 15%) in the order.

2: Isolated as a foam. ^{1}H-NMR ($CDCl_3$) δ: 8.10 (1H, br, NH), 7.35 (1H, s, 6-H), 6.48 (1H, dd, J=9.4 and 5.6 Hz, 1'-H), 6.01 (1H, d, J=6.0 Hz, 3'-H), 5.03 and 4.91 (each 1H, each dd, 5'-H), 4.57 (1H, s, 4'-H), 2.66 and 2.60 (each 3H, each s, SMe x 2), 2.38 (2H, m, 2'-H), 1.94 (3H, s, 5-Me). MS m/z: 206 (M^+ - 2 x MeCSSOH). Exact FAB-MS Calcd for $C_{14}H_{18}N_2O_5S_4$ ($[MH]^+$) m/z: 423.0177. Found m/z: 423.0157.

3: mp 178-179 °C (AcOEt). ^{1}H-NMR ($CDCl_3$) δ: 8.05 (1H, br, NH), 7.33 (1H, s, 6-H), 6.38 (1H, t, 6.4 Hz, 1'-H), 4.96 and 4.82 (each 1H, each dd, 5'-H), 4.54 (1H, m, 3'-H), 4.27 (1H, m, 4'-H), 2.64 (3H, s, SMe), 2.38-2.47 and 2.17-2.27 (each 1H, each m, 2'-H), 1.95 (3H, s, 5-Me). MS m/z: 332 (M^+). Anal. Calcd for $C_{12}H_{16}N_2O_5S_2$: C, 43.36; H, 4.85; N, 8.43. Found: C, 43.39; H, 4.86; N, 8.46.

4: mp 208-210 °C (AcOEt). ^{1}H-NMR ($CDCl_3$) δ: 8.03 (1H, br, NH), 7.51 (1H, s, 6-H), 6.26 (1H, dd, J=9.0 and 6.4 Hz, 1'-H), 6.06 (1H, m, 3'-H), 4.33 (1H, m, 4'-H), 4.05 and 3.97 (each 1H, each dd, each J=11.5 and 2.1 Hz, 5'-H), 2.60 (3H, s, SMe), 2.57 (2H, m, 2'-H), 1.94 (3H, s, 5-Me). MS m/z: 222 (M^+ - MeCSSOH). Anal. Calcd for $C_{12}H_{16}N_2O_5S_2$: C, 43.36; H, 4.85; N, 8.43. Found: C, 43.23; H, 4.79; N, 8.38.

3'-*O*-[(Methylthio)thiocarbonyl]thymidine (4) A solution of **6** (57 mg, 1 mmol) in 80% acetic acid (3 ml) was heated at 100 °C for 30 min. The reaction solution was evaporated under reduced pressure and water (10 ml) was added to the residue. An insoluble material was removed by filtration, the filtrate was evaporated under reduced pressure, and the residue was recrystallized from ethyl acetate to give **4** (26 mg, 78%), which was identical with the product prepared by xanthation of **1**.

3'-*O*-[(Methylthio)thiocarbonyl]-5'-*O*-tritylthymidine (6) 5'-Tritylthymidine (**5**) (242 mg, 0.5 mmol) was added to a mixture of 50% NaH (24 mg, 0.5 mmol) and CS_2 (46 mg, 0.6 mmol) in dry THF (5 ml) and the mixture was stirred at room temperature for 15 min. And then methyl iodide was added thereto and the solution was stirred for 24 h. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with benzene-ethyl acetate (1:1) to give the 3'-xanthate (**6**) (176 mg, 61%), which was employed directly in the next steps. Mp 197-198 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.04 (1H, brs, NH), 7.65 (1H, s, 6-H), 7.25-7.44 (15H, m, trityl-H), 6.49 (1H, dd, J=9.3 and 5.4 Hz, 1'-H), 6.22 (1H, d, J=6.4 Hz, 3'-H), 4.29 (1H, s, 4'-H), 3.64 and 3.46 (each 1H, each dd, each J=10.4 and 2.4 Hz, 5'-H), 2.67-2.74 and 2.50-2.60 (each 1H, each m, 2'-H), 2.57 (3H, s, SMe), 1.44 (3H, s, 5-Me).

3'-*O*-Thiocarbamoylthymidine (7) A solution of **6** (114 mg, 0.2 mmol) in 11% methanolic ammonia (3 ml) was stirred at room temperature for 2 h. The reaction solution was evaporated under reduced pressure to give crude 3'-*O*-thiocarbamoyl-5'-*O*-tritylthymidine (107 mg, 99%) as a foam. ^{1}H-NMR ($CDCl_3$) δ: 8.12 (1H, br, N^3-H), 7.63 (1H, s, 6-H), 7.45-7.28 (15H, m, trityl-H), 6.45 (2H, m, 1'-H and NH), 6.17 (1H, br, NH), 6.07 (1H, m, 3'-H), 4.26 (1H, m, 4'-H), 3.70 and 3.41 (each 1H, each m, 5'-H), 2.59 (2H, m, 2'-H), 1.43 (3H, s, 5-Me).

A solution of the obtained thioamide (54 mg, 0.1 mmol) in 80% AcOH was heated at 100 °C for 30 min. The reaction solution was evaporated under reduced pressure, water (10 ml) was added to the residue, and an insoluble material was removed by filtration. The filtrate was evaporated under reduced pressure and the residue was recrystallized from ethyl acetate to give **7** (22 mg, 72%): mp 200-203 °C. ^{1}H-NMR (DMSO-d_6) δ: 10.84 (1H, br, N^3-H), 9.05 and 8.74 (each 1H, each br, NH_2), 7.88 (1H, s, 6-H), 6.32 (1H, t, J=6.8 Hz, 1'-H), 5.76 (1H, brs, 3'-H), 5.36 (1H, t, J=4.7 Hz, OH), 4.15 (1H, s, 4'-H), 3.75 (2H, m, 5'-H), 2.37 (2H, m, 2'-H), 1.88 (3H, s, 5-Me). MS m/z: 284 (M^+ - NH_3). Exact FAB-MS Calcd for $C_{11}H_{16}N_3O_5S$ ($[MH]^+$) m/z: 302.0811. Found m/z: 302.0785.

Di-*O*-methyl *N*-(3'-deoxythymidin-3'-yl)phosphoramidate (9a) and Di-*O*-methyl *N*-(3'-deoxy-3-methylthymid-3'-yl)phosphoramidate (10a) A solution of **8** (127 mg, 0.25 mmol) and trimethyl phosphite (310 mg, 2.5 mmol) in dry toluene (3 ml) was stirred at room temperature for 10 h. The reaction solution was evaporated under reduced pressure and the residue was heated in 80% AcOH (3 ml) at 100 °C for 30 min. The reaction mixture was evaporated under reduced pressure, water (10 ml) was added to the residue, and an insoluble material was removed by filtration. The filtrate was evaporated under reduced pressure and the residue was chromatographed on silica gel column with $CHCl_3$-MeOH (10:1) to give **10a** (24 mg, 26%) and **9a** (58 mg, 67%) as a foam. **10a**: ^{1}H-NMR (DMSO-d_6) δ: 7.93 (1H, s, 6-H), 6.20 (1H, t, J=6.0 Hz, 1'-H), 5.51 (1H, t, J=10.3 Hz, 3'-H), 5.19 (1H, t, J=4.7 Hz, OH), 3.80 (4H, m, 4'-H, 5'-H, and NH), 3.64 (6H, d, 11.1 Hz, OMe x 2), 3.24 (3H, s, NMe), 2.28 (2H, m, 2'-H), 1.91 (3H, s, 5-Me). MS m/z: 363 (M^+). Exact MS Calcd for $C_{13}H_{23}N_3O_7P$ ($[MH]^+$) m/z: 364.1273. Found m/z: 364.1292. **9a:** ^{1}H-NMR (DMSO-d_6) δ: 11.38 (1H, brs, N^3-H), 7.83 (1H, s, 6-H), 6.18 (1H, t, J=6.4 Hz, 1'-H), 5.52 (1H, t, 3'-H), 5.19 (1H, t, J=5.1 Hz, OH), 3.78 (4H, m, 4'-H, 5'-H, and NH), 3.64 (6H, d, 11.1 Hz, OMe x 2), 2.25 (2H, m, 2'-H), 1.85 (3H, s, 5-

Me). MS m/z: 224 (sugar moiety, M^+ - thymine). Exact MS Calcd for $C_7H_{15}NO_5P$ (sugar moiety): 224.0688. Found: 224.0690.

Di-*O*-ethyl *N*-(3'-deoxythymidin-3'-yl)phosphoramidate (9b) A mixture of **8** (255 mg, 0.5 mmol) and triethyl phosphite (831 mg, 5 mmol) in dry toluene (5 ml) was allowed to react and work up as described above to give **9b** (164 mg, 87%): mp 169-171 °C. ^{1}H-NMR (DMSO-d_6) δ: 11.37 (1H, brs, N^3-H), 7.83 (1H, s, 6-H), 6.18 (1H, t, J=6.0 Hz, 1'-H), 5.43 (1H, br t, 3'-H), 5.15 (1H, t, J=5.1 Hz, OH), 3.99 (4H, m, J=7.3 Hz, $\underline{CH_2}CH_3$ x 2), 3.77-3.60 (4H, m, 4'-H, 5'-H, and NH), 2.23 (2H, m, 2'-H), 1.85 (3H, s, 5-Me), 1.30 (6H, t, J=6.8 Hz, $CH_2\underline{CH_3}$ x 2). MS m/z: 377 (M^+), 252 (sugar moiety). Exact FAB-MS Calcd for $C_{14}H_{25}N_3O_7P$ ($[MH]^+$) m/z: 378.1430. Found m/z: 378.1441.

Di-*O*-ethyl *N*-(3'-deoxy-3-ethylthymidin-3'-yl)phosphoramidate (10b) A solution of **8** (255 mg, 0.5 mmol) and triethyl phosphite (831 mg, 5 mmol) in dry toluene (5 ml) was refluxed for 30 m. The reaction solution was evaporated under reduced pressure and the residue was chromatographed on silica gel column with benzene-ethyl acetate (1:3). The obtained 5'-trityl-phosphoramidate was heated in 80% AcOH (40 ml) at 100 °C for 30 min. The reaction mixture was worked up as described above. A mixture of $CHCl_3$-MeOH (20:1) was used for column chromatography as eluents to give **10b** (105 mg, 51%), mp 146-147 °C. ^{1}H-NMR (DMSO-d_6) δ: 7.90 (1H, s, 6-H), 6.22 (1H, t, J=6.0 Hz, 1'-H), 5.42 (1H, t, J=10.0 Hz, 3'-H), 5.16 (1H, t, J=5.1 Hz, OH), 3.87-4.05 (6H, m, $O\underline{CH_2}CH_3$ x 2 and $N\underline{CH_2}CH_3$), 3.80-3.62 (4H, m, 4'-H, 5'-H, and NH), 2.23-2.33 (2H, m, 2'-H), 1.90 (3H, s, 5-Me), 1.30 (6H, t, J=6.8 Hz, $OCH_2\underline{CH_3}$ x 2), 1.16 (3H, t, J=6.8 Hz, $NCH_2\underline{CH_3}$). MS m/z: 405 (M^+). Anal. Calcd for $C_{14}H_{17}N_3O_7P$: C, 47.41; H, 6.96; N, 10.37. Found: C, 47.31; H, 6.88; N, 10.25.

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