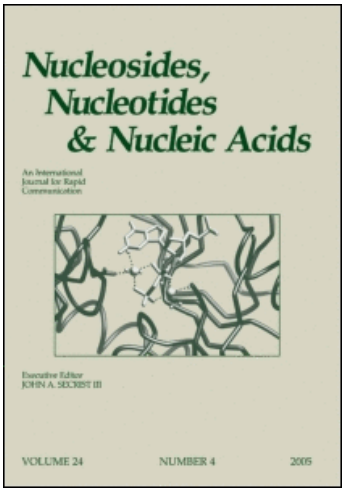


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Anti-HIV-1 Activity of Base Modified Analogues of 3'-Azido-2',3'-Dideoxythymidine (AZT)

S. Czernecki[a]; T. Le Diguarher[a]; J. M. Valéry[a]
[a] Laboratoire de Chimie des Glucides T 74, Université Pierre et Marie Curie, PARIS, FRANCE

To cite this Article Czernecki, S. , Diguarher, T. Le and Valéry, J. M.(1993) 'Synthesis and Anti-HIV-1 Activity of Base Modified Analogues of 3'-Azido-2',3'-Dideoxythymidine (AZT)', Nucleosides, Nucleotides and Nucleic Acids, 12: 3, 369 — 380
To link to this Article: DOI: 10.1080/07328319308017833
URL: http://dx.doi.org/10.1080/07328319308017833

NUCLEOSIDES & NUCLEOTIDES, 12(3&4), 369-380 (1993)

SYNTHESIS AND ANTI-HIV-1 ACTIVITY OF BASE MODIFIED ANALOGUES OF 3'-AZIDO-2',3'-DIDEOXYTHYMIDINE (AZT).

S. Czernecki*, T. Le Diguarher and J.M. Valéry
Université Pierre et Marie Curie
Laboratoire de Chimie des Glucides
T 74, E6. 4 Place JUSSIEU. 75005 PARIS. FRANCE

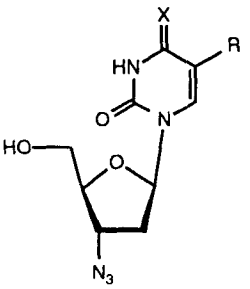
Abstract. Analogues of AZT have been synthesized by modifications at the 4-position of the base. Two synthetic routes are described. Among the new compounds, 3'-azido-2',3'-dideoxy-4-thiouridine **2b** exhibited an unexpectedly marked anti-HIV activity on CEM-Cl13 cell lines.

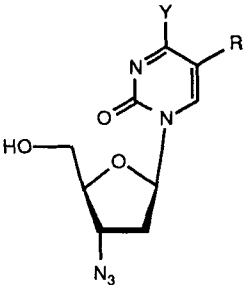
Since the discovery of Human Immuno-Deficiency Virus (HIV) as the causative agent of AIDS,[1] intense effort was developed to find drugs that can selectively inhibit the replication of HIV. Among such compounds 3'-azido-2',3'-dideoxythymidine **1a** (AZT)[2] was approved for AIDS therapy,[3] in spite of serious side effects.[4]

In order to obtain an improved selectivity many nucleoside analogues have been designed and synthesized, including modifications of the carbohydrate moiety [5] as well as of the base.[6] In this last domain recent work by Palomino *et al* [7] about some modified 4-thiopyrimidine nucleosides concluded to weakened activity of the sulfur analogues despite their enhanced lipophilicity. This was particularly the case for 3'-azido-2',3'-dideoxy-4-thiothymidine **2a** relative to AZT.[7]

Recently, the introduction of a methoxy group on the NH_2 position of both cytidine and 5-methylcytidine was shown to result in interesting biological properties due to increased stability of the derived oligodeoxynucleotides duplexes with DNA,[8] consequently we thought that compounds **3a** and **3b** could be valuable targets (Scheme I).

Many of the synthetic routes allowing chemical transformation at the C-4 position of pyrimidine nucleosides involve 4-thiomethyl intermediates (e.g. **4**). Such compounds are easily obtained from 4-thiopyrimidyl derivatives (e.g. **2**). Further nucleophilic attack on the thiated position commonly affords the base modified compounds.[9]





1a R= Me, X= O
1b R= H, X= O
2a R= Me, X= S
2b R= H, X= S

3a R= Me, Y= NHOMe
3b R= H, Y= NHOMe
4a R= Me, Y= SMe
4b R= H, Y= SMe

SCHEME I

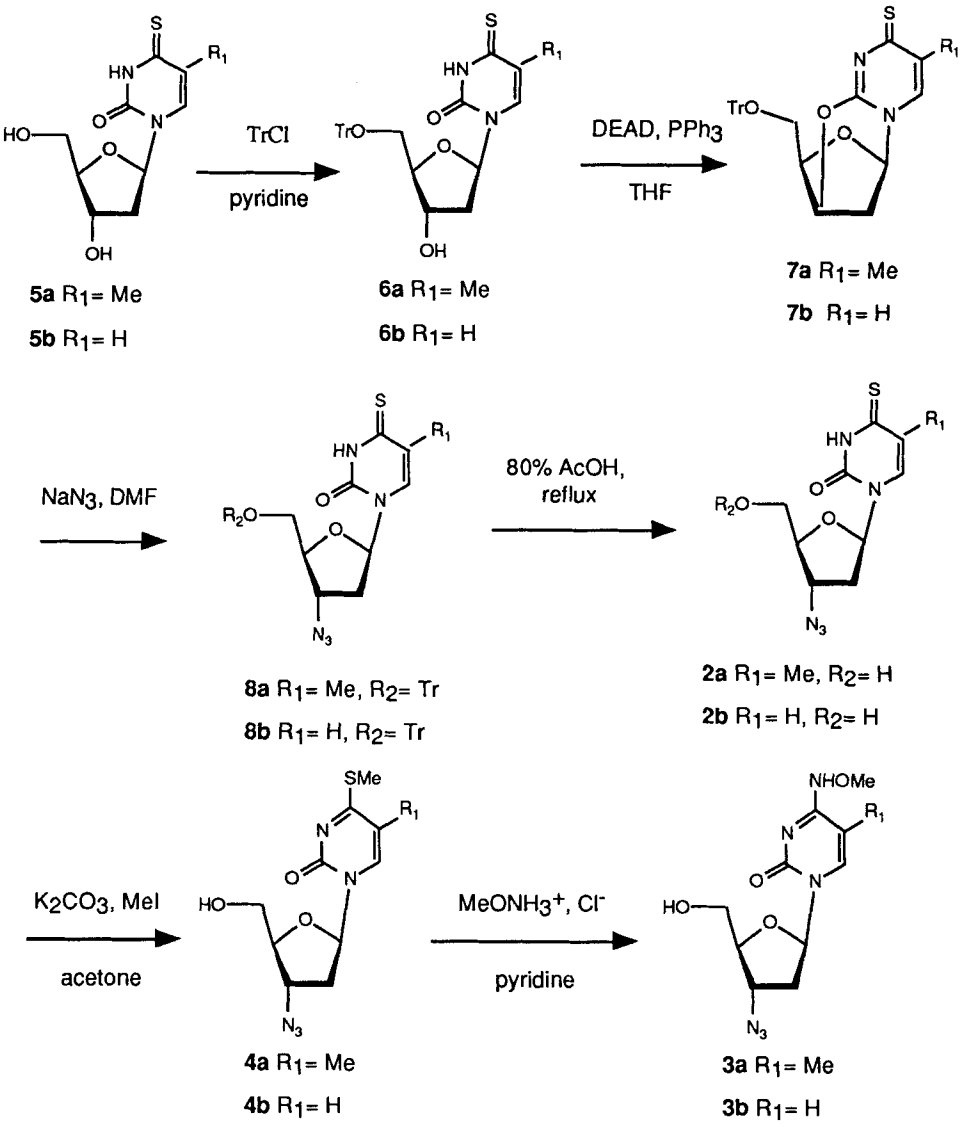
Attempts of thiation at the C-4 position of 3'-azido-2',3'-dideoxy nucleosides **1** resulted in important decomposition of the substrates whatever were the reagents and conditions that we have used.

So the azidation step had to be realized on previously thiated 2'-deoxynucleosides **5** which were obtained from 3',5'-di-O-benzoyl-2'-deoxynucleosides by reaction between Lawesson's reagent followed by usual deprotection.[10] The correlated reaction sequence is depicted in Scheme II.

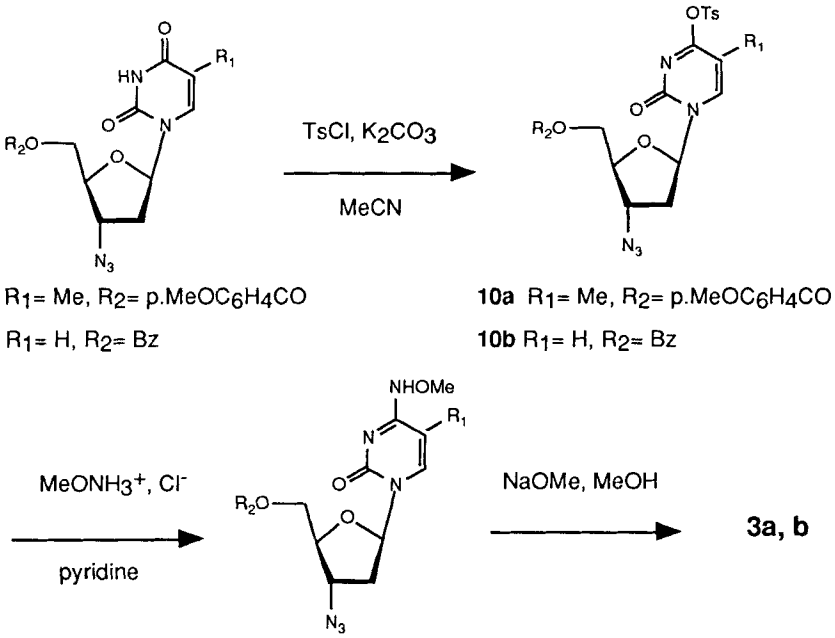
The tandem one-pot Mitsunobu reaction which allowed us to prepare 5'-O-aroyl-O^2,3'-anhydrothymidine in high yields,[11] proved to be unsatisfactory when applied in the 4-thio-series. In order to circumvent this difficulty, compound **5** were first reacted with triphenylmethylchloride in pyridine. The 5'-O-trityl derivatives **6** were readily converted (diethylazodicarboxylate, triphenylphosphine in THF solution) into the O^2,3'-anhydro-5'-O-trityl nucleosides **7**.

Further nucleophilic opening of the anhydro ring by means of either lithium or sodium azide proceeded easily albeit considerable loss of products **8** occurred during the purification process. The following steps are straightforward proceeding via the 4-S-methyl derivatives **4**,[10a] which were reacted with methoxyamine at room temperature to afford the target nucleosides **3**.

Although easy to perform this synthetic route may appear somewhat lengthy. Furthermore we were not able to improve significantly the yield of the key azidation step, despite of the numerous attempts which were conducted in this intent. So we undertook the reexamination of



SCHEME II



9a R_1= Me, R_2= p.$MeOC_6H_4CO$
9b R_1= H, R_2= Bz

10a R_1= Me, R_2= p.$MeOC_6H_4CO$
10b R_1= H, R_2= Bz

11a R_1= Me, R_2= p.$MeOC_6H_4CO$
11b R_1= H, R_2= Bz

SCHEME III

modifications at the C-4 position of the pyrimidine moiety which would be compatible with an azido group. We thought that the high-yielding synthesis of cytosine described by Kawada *et al* [12] could be easily fitted to our purpose (Scheme III).

The readily available 5'-O-aroyl-3'-azido-2',3'-dideoxy nucleosides **9**[13] were heated with tosyl chloride in the presence of potassium carbonate in acetonitrile solution yielding the unstable tosylates **10** which were not isolated but directly reacted with methoxyamine . Conventional base-catalyzed trans esterification in methanol afforded the free nucleosides **3** in fairly good overall yield.

Antiviral activity.

The antiviral activity of the new 3'-azido-2',3'-dideoxy base modified nucleosides **3a** and **3b** was measured on lymphocytic (CEM-Cl13) cell lines infected with HIV-1 cell-free supernatants. Although compound **2a** is known to be devoid of any significant activity,[7] the 4-thio- and 4-thiomethyl-uracil derivatives **2b** and **4b** were also evaluated and compared to the parent 3'-azido-2',3'-dideoxy-uridine **1b** which showed significant anti-HIV activity.[14]

Table I: Activity of base-modified nucleosides on cytopathic effect and HIV production in CEM-Cl 13 cells infected with HIV-1.[a]

compound	CD_{50}[b]	ED_{50CPE}[b]	ED_{50RT}[b]	SI_{CPE}[b]	SI_{RT}[b]
AZT: **1a**	>19	<0.007	0.15	> 2,715	> 127
AZddU: **1b**	197	< 0.2	< 0.2	> 1,000	> 1,000
2b	> 1,120	1.5	0.07	> 745	> 16,000
3a	> 330	23.6	169	14	1.95
3b	> 350	30	177	11.6	1.98
4b	> 350	10.6	176	33	1.99

[a] See the Experimental Section (Antiviral Assay on Cells). [b] SI (selectivity index): ratio of CD_{50} to ED_{50}. CD_{50} (50% cytotoxic dose)= concentration required to reduce the viability of uninfected cells by 50% at 7 days of incubation in the presence of the compound. Results are expressed in μM. ED_{50CPE} (50% antiviral effective dose)= concentration (in μM) that reduced by 50% the HIV induced CPE (cytopathic effect). ED_{50RT}= concentration (in μM) that reduced by 50% the RT production in supernatants after 7 days.

Two parameters were studied to evaluate the antiviral activity of these compounds: inhibition of HIV-1 induced cytopathic effect using the MTT viability assay and the inhibition of the reverse transcriptase production in culture supernatants (Table I).

These modified nucleosides showed a very low toxicity for CEM-Cl13 cells. Among them **2b** was totally devoid of toxicity at doses equal or up to 1100 μM.

For compounds **3a**,**3b** and **4b**, a small anti-HIV activity was observed, but the selectivity index was low. A similar effect of mercaptomethyl substitution on the base was reported by Chu *et al.*[10] in the 2',3'-dideoxypurine series.

Interestingly, a noticeable anti-HIV activity was measured for compound **2b**. Although the EC_{50} of this compound was slightly higher than the EC_{50} of the parent nucleoside **1b**, the therapeutic index was in the same range because of a lower cytotoxicity.

The efficiency of these compounds was also evaluated for their ability to inhibit the production of reverse transcriptase (RT) in culture supernatant after 7 days of incubation. Among the new nucleosides, only **2b** was able to significantly inhibit the RT production at low concentrations. It was even more efficient that the parent 2'-azido-2',3'-dideoxyuridine **1b**.

EXPERIMENTAL SECTION

General methods and procedures are the same as previously described.[13]

2'-Deoxy-4-thiothymidine (5a). This compound was prepared by reaction between

Lawesson's reagent (3.3 g, 8.16 mmol) and 3',5'-di-O-benzoyl-2'-deoxythymidine[15] (3.06 g, 6.8 mmol) in hot toluene (182 mL) at reflux for 4 hours under N_2. Conventional deprotection (NaOMe, MeOH and work up according to reference 16) of the crude thiated dibenzoate afforded compound **5a** (1.12g, 64% overall yield): mp 123-125°C (crystallized from EtOH/AcOEt) (Litt.[7] mp 125-127°C); $[\alpha]_D^{20}$ + 73.4° (*c* 1, MeOH); UV (H2O) λ max (ε) 332 (20,000), 243 (5,300), 202 nm (16,200) ; ^{1}H NMR (D_2O) δ 1.87 (s, 3 H, CH), 2.2 (m, 2 H, *J* = 14.1, 6.5 and 5.3Hz, 2'α-H, 2'-β–H), 3.65 (m, 2 H, *J* = 12.4, 4.8 and 3.5Hz, 5'-Ha, 5'-Hb), 3.9 (m, 1H, *J* = 4.1Hz, 4'-H), 4.3 (m, 1 H, *J* = 5.6 and 4.6Hz, 3'-H), 6.05 (t, 1 H, *J* = 6.5Hz, 1'-H), 7.58 (s, 1 H, 6-H); CIMS m/z (relative intensity) 276 $[M+NH_4]^+$ (25), 259 $[M+H]^+$ (100), 143 (16). Anal. ($C_{10}H_{14}N_2O_4S$): calculated C: 46.50, H: 5.42, N: 10.85; found C: 46.33, H: 5.41, N: 10.71

2'-Deoxy-4-thiouridine (5b). This compound was obtained as above from 2',5'-di-O-benzoyl-2'-deoxyuridine in 88% overall yield: mp 159-160°C; $[\alpha]_D^{20}$ + 87.2°(*c* 0.55,MeOH) ; UV(H2O) λ max (ε) 330 (22,300), 242 (4,300), 202 nm (18,800) ; ^{1}H NMR (D2O) δ 2.25 (m, 2 H, *J*= 14.2, 6.5 and 4.4Hz, 2'α-H,2'β-H), 3.6 (m, 2 H, *J*= 12.5, 5.15 and 3.5Hz, 5'-Ha, 5'-Hb), 3.9 (m, 1 H, *J*= 8.7 and 3.8Hz, 4'-H), 4.3 (m, 1 H, *J*= 8.45, 6.4 and 4.35Hz, 3'-H), 6.05 (t, 1 H, *J*= 6.5Hz, 1'-H), 6.4 (d, 1 H, *J*= 7.6Hz, 5-H), 7.56 (d, 1 H, *J*= 7.6Hz, 6-H); CIMS m/z (relative intensity) 262 $[M+NH_4]^+$ (5.4), 245 $[M+H]^+$ (73), 129 (100). Anal. ($C_9H_{12}N_2O_4S$):calculated C: 44.26, H: 4.92, N: 11.47; found C: 44.50, H: 5.06, N: 11.12.

2'-Deoxy-5'-O-triphenylmethyl-4-thiothymidine (6a). This compound was obtained from **5a** by standard tritylation procedure and work up: yield 75%; mp 109-111°C; $[\alpha]_D^{20}$ +63.6° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.65 (s, 3 H, CH_3), 2.4 (m, 2 H, J= 13.4, 7.15 and 3.35 Hz, 2'α-H, 2'β-H), 3.4 (m, 2 H, *J*= 10.8 and 3.05 Hz, 5'-Ha, 5'-Hb), 4.1 (m, 1 H, *J*= 6.4 and 3.35 Hz, 4'-H), 4.6 (m, 1 H, *J*= 6.2 and 3.25 Hz, 3'-H), 6.33 (t, 1 H, *J*= 6.6 Hz, 1'-H), 7.3 (m, 15 H, arom), 7.6 (s, 1 H, 6-H), 9.7 (br s, 1 H, 3-H); CIMS m/z (relative intensity) 501 $[M+H]^+$ (10.5), 243 (100), 143 (5.1). Anal. ($C_{29}H_{28}N_2O_4S$): calculated C: 69.58, H: 5.63, N: 5.60; found C: 69.27, H: 5.90, N: 5.24.

2'-Deoxy-5'-O-triphenylmethyl-4-thiouridine (6b). Obtained in a similar way from **5b** :total yield 70.3% ; mp 112°C; $[\alpha]_D^{20}$ + 71.4 (*c* 0.5, $CHCl_3$) ; ^{1}H NMR ($CDCl_3$) δ 2.3 (m, 1 H, *J*= 13.7 and 6.25 Hz, 2'α-H), 2.48 (m, 1 H, *J* = 13.5, 4.95 and 2.05 Hz, 2'β-H), 3.5 (m, 2 H, *J*= 11.0, 4.75 and 3.2 Hz, 5'-Ha, 5'-Hb), 4 (m, 1 H, *J*= 6.05 and 3.25 Hz, 4'-H), 4.5 (m, 1 H, *J*= 5.97 and 4.65 Hz, 3'-H), 6.1 (dd, 1 H, *J*= 7.65 and 2.05 Hz, 5-H), 6.2 (t, 1 H, *J*= 6.1 Hz, 1'-H), 7.3 (m, 15 H, arom), 7.65 (d, 1 H, *J*= 7.7 Hz, 6-H), 9.4 (br s, 1 H, 3 H); CIMS m/z (relative intensity) 487 $[M+H]^+$ (8.1), 243 (100), 129 (3.1). Anal. ($C_{28}H_{26}N_2O_4S$): calculated C: 69.13, H: 5.35, N: 5.76; found C: 69.43, H: 5.43, N: 5.80.

O^2,3'-Anhydro-2'-deoxy-5'-O-triphenylmethyl-4-thiouridine (7b): To a solution of **6b** (500 mg, 1.02 mmol) and triphenylphosphine (402 mg, 1.53 mmol) in dry THF (2.5 mL), a solution of DEAD (0.24 mL, 1.53 mmol) in dry THF (2.5 mL) was added dropwise. The mixture was stirred at room temperature until completion of the reaction (c.a. 10 min.) and then concentrated to dryness. The residual syrup was purified by silica gel column chromatography (3 x 15 cm) eluting with petroleum ether/EtOAc (1:2). The appropriate fractions were combined and evaporated to afford **7b** (407 mg, 85%) as a yellow powder: mp 145-150°C; $[\alpha]_D^{20}$ + 99.3 (*c* 0.59, $CHCl_3$); UV (EtOH) λ max (ε) 327 (24,800), 265 (3,000), 212 (31,400), 207 nm (28,500); ^{1}H NMR δ 2.4 (m, 1 H, *J*= 13.3, 3.50 and 3.1 Hz, 2'α–H), 2.9 (m, 1 H, *J*= 13.3 Hz, 2'β-H) 3.4 (m, 2 H, *J*= 10.45, 7.30 and 6.0 Hz, 5'-Ha, 5'-Hb), 4.2 (m, 1 H, *J*= 6.95 and 3.85 Hz, 4'-H), 5.2 (br s, 1 H, 3'-H), 5.5 (d, 1 H, *J*= 3.8 Hz, 1'-H), 6.75 (d, 1 H, *J*= 7.15 Hz, 5-H), 6.8 (d, 1 H, *J*= 7.05 Hz, 6-H), 7.3 (m, 15 H, arom); CIMS m/z (relative intensity) 469 $[M+H]^+$ (5.1), 243 (100), 129 (18). Anal. ($C_{28}H_{24}N_2O_3S$): calculated C: 71.77, H: 5.16, N: 5.98;found C: 71.65, H: 5.20, N: 5.90.

O^2,3'Anhydro-2'-deoxy-5'-O-triphenylmethyl-4-thiothymidine (7a): was obtained from **6a** as above in 77% yield: mp 170-172°C;;$[\alpha]_D^{20}$ +170° (*c* 0.5, $CHCl_3$); UV (EtOH) λ max (ε) 329 (18,300), 237 (4,300), 208 nm (32,400); ^{1}H NMR ($CDCl_3$) δ 2.1 (s, 3 H, CH_3), 2.4 (m, 1H, *J*= 13.1, 3.8 and 2.9 Hz, 2'α-H), 3.05 (d, 1 H, *J*= 13.2 Hz, 2'β-H), 3.4 (m, 2 H, *J*= 13.25 and 6.6 Hz, 5'-Ha, 5'-Hb), 4.2 (m, 1 H, *J* = 6.5 and 2.4 Hz, 4'-H), 5.2 (br s, 1 H, 3'-H), 5.5 (d, 1 H, *J*= 3.75 Hz, 1'-H), 6.9 (s, 1 H, 6-H), 7.3 (m, 15 H, arom); CIMS m/z (relative intensity) 483 $[M+H]^+$ (9.5), 243 (100), 143 (15.4). Anal.($C_{29}H_{26}N_2O_3S$): calculated C: 72.18, H: 5.43, N: 5.80; found C: 72.50, H: 5.20, N: 5.55.

3'-Azido-2',3'-dideoxy-5'-O-triphenylmethyl-4-thiouridine (8b): A solution of **7b** (191 mg, 0.41 mmol) and sodium azide (79 mg, 1.22 mmol) in dry DMF (2.5 mL) was heated at 135°C (bath temperature) for 1 h. Dilute hydrochloric acid ($1.37x10^{-2}$ M, 30 mL) was added and the mixture extracted with EtOAc (3x10 mL). The combined extracts were washed with brine (2x10 mL), dried ($MgSO_4$) and concentrated to dryness. The solid residue (190 mg) was dissolved in toluene and applied to a silica gel column (2x15 cm) which was eluted with petroleum ether/EtOAc mixtures of respective amounts of EtOAc : 5%, 10%, 15% and 20% (100 mL each). Appropriate fractions were combined and evaporated to yield **8b** (42 mg, 21%): $[\alpha]_D^{20}$ + 145.2° (*c* 0.5, $CHCl_3$); UV (EtOH) λ max (ε) 330 (18,200), 242 (4,000), 210nm (28,000); ^{1}H NMR ($CDCl_3$) δ 2.5 (m, 2H, *J*= 13.7, 6.4, 2.33 and 1.1 Hz, 2'α-H, 2'β-H), 3.5 (m, 2H, *J*= 11.1 and 2.8 Hz, 5'-Ha, 5'-Hb), 4 (m, 1H, *J*= 6.6 and 2.8 Hz, 4'-H), 4.3 (m, 1H, *J*= 6.5 Hz, 3'-H), 6.1 (d, 1H, *J* = 7.8 Hz , 5-H), 6.1 (dd, 1H, *J* = 6.2 and 5.0 Hz, 1'-H), 7.3 (m, 15H, arom), 7.7 (d, 1H, *J*= 7.9 Hz, 6-H), 9.5 (br s, 1H, 3-H); CIMS

m/z (relative intensity) 512 $[M+H]^+$ (2.5), 243 (100), 129 (5). Anal. ($C_{28}H_{25}N_5O_3S$): calculated C: 65.75, H: 4.92, N: 13.70; found C: 65.62, H: 4.80, N: 13.56.

3'-Azido-2',3'-dideoxy-5'-O-triphenylmethyl-4-thiothymidine (8a): this compound was obtained from **7a** as above. Yield 28.5% : mp 89-91°C; $[\alpha]_D^{20}$ + 59° (*c* 0.5, $CHCl_3$) ; UV (EtOH) λ max (ε) 332 (18,600), 208 nm (30,600); ^1H NMR (CDCl3) δ 1.6 (s, 3H, CH3), 2.4 (m, 2H, *J*= 12.9 and 6.75 Hz, 2'α-H, 2'β-H), 3.4 (m, 2H, *J*= 11.0, 6.95 and 3.2 Hz, 5'-Ha, 5'-Hb), 3.9 (m, 1H, *J*= 5.05 and 2.95 Hz, 4'-H), 4.3 (m, 1H, *J*= 5.1 Hz, 3'-H), 6.12 (t, 1H, *J*= 6.25 Hz, 1'-H), 7.3 (m, 15H, arom), 7.6 (s, 1H, 6-H), 9.5 (br s, 1H, 3-H) ; CIMS m/z (relative intensity) 526 $[M+H]^+$ (10.1), 243 (100), 143 (5). Anal. ($C_{29}H_{27}N_5O_3S$): calculated C: 66.33, H: 5.17, N: 13.32; found C: 66.55, H: 5.05, N: 13.10.

3'-Azido-2',3'-dideoxy-4-thiouridine (2b): A suspension of **8b** (42mg, 0.082 mmol) in 80% AcOH (1.5 mL) was heated under reflux for 15 min. The reaction mixture was concentrated to dryness and the resulting solid was purified by silica gel column chromatography (2x10 cm) eluting with petroleum ether/EtOAc (1:1) to give **2b** (15 mg, 68%) as a yellow powder : mp 110-112°C; $[\alpha]_D^{20}$ + 100° (*c* 0.4, $CHCl_3$); UV (EtOH) λ max (ε) 329 (19,400) 247 (4,400), 202 (13,500) ; ^1H NMR ($CDCl_3$) δ 2.5 (pseudo t, 2H, *J*= 6. 3 Hz, 2'α-H, 2'β-H), 3.9 (m, 3H, *J*= 9.6 and 7.05 Hz, 4'-H, 5'-H_a, 5'-H_b) 4.38 (m, 1H, *J*= 6.2 Hz, 3'-H), 6.05 (t, 1H, *J*= 6.2 Hz, 1'-H), 6.4 (d, 1H, *J*= 7.55 Hz, 5-H), 7.6 (d, 1H, *J*= 7.55 Hz, 6-H), 9.4 (br s, 1H, 3-H); CIMS m/z (relative intensity) 287 $[M+NH_4]^+$ (7.6), 270 $[M+H]^+$ (100), 129 (6.6). Anal. ($C_9H_{11}N_5O_3S$): calculated C: 40.15, H: 4.12, N: 25.99; found C: 40.23, H: 4.09, N: 26.07.

3'-Azido-2',3'-dideoxy-4-thiothymidine (2a): was prepared from **8a** in the same way, yield 63%: mp 83°C (Litt.[7] 82-85°C); $[\alpha]_D^{20}$ + 97.6° (*c* 1.1, $CHCl_3$); UV (EtOH) λ max (ε) 334 (16,500), 242 (4,000), 202 nm (30,000); CIMS m/z (relative intensity) 301 $[M+NH_4]^+$ (10.5), 284 $[M+H]^+$ (100), 143 (5.7).

3'-Azido-2',3'-dideoxy-4-methylthiouridine (4b): To a solution of **2b** (60 mg, 0.22 mmol) in dry acetone was added K_2CO_3 (40.5 mg, 0.29 mmol) and MeI (38 µL, 0.6 mmol). The mixture was stirred at room temperature until completion of the reaction (c.a. 1h.) and then concentrated to dryness.The resulting residue was purified by preparative TLC eluting with petroleum ether/EtOAc (1:2) to give **4b** (38 mg, 60%): mp 95-97°C; $[\alpha]_D^{20}$ + 94.2° (*c* 0.35, $CHCl_3$); UV (EtOH) λ max (ε) 300 (17,300), 278 (12,200), 202 nm (15,300); CIMS m/z (relative intensity) 284 $[M+H]^+$ (100), 143 (19.3). Anal. ($C_{10}H_{13}N_5O_3S$): calculated C: 42.39, H: 4.63, N: 24.72; found C: 42.44, H: 4.59, N: 24.72.

3'-Azido-2',3'-dideoxy-4-methylthiothymidine (4a): This compound was prepared from **2a** as above in 83% yield: mp 134-136°C; $[\alpha]_D^{20}$ + 148.8° (*c* 0.5, $CHCl_3$) ; UV

(EtOH) λ max (ε) 308 (10,900), 271 (9,500), 206 nm (16,500); CIMS m/z (relative intensity) 298 (100), 157 (21.5). Anal. ($C_{11}H_{15}N_5O_3S$): calculated C: 44.43,H: 5.08,N: 23.55; found C: 44.49, H: 5.12, N: 23.51.

3'-Azido-2',3'-dideoxy-4-N-methoxycytidine (3b): To a solution of **4b** (22mg, 0.077 mmol) in dry pyridine methoxyamine hydrochloride (129mg, 1.54 mmol) was added and the resulting mixture was stirred at room temperature overnight and then concentrated to dryness. The oily residue was dissolved in $CHCl_3$ (50mL) and this solution washed with water (2x20mL), dried ($MgSO_4$) and evaporated to afford crude **3b** as a white solid which was purified by preparative TLC eluting with petroleum ether/EtOAc (1:3) to give **3b** (13mg, 60%): mp 121-123°C; $[\alpha]_D^{20}$ + 54° (c 0.5, $CHCl_3$); UV (EtOH) λ max (ε) 276 (7,900), 236 (13,470), 200nm (8,520); ^{1}H NMR (D_2O) δ 2.3 (m, 2H, *J*= 6.85 Hz, 2'α-H, 2'β-H), 3.6 (m + s, 5 H, *J*= 12.45, 4.8 and 3.7 Hz, 5'-Ha, 5'-Hb, OCH_3), 3.8 (m, 1H, *J*= 4.9 and 4.1 Hz, 4'-H), 4.2 (m, 1H, *J*= 6.75 and 5.6 Hz, 3'-H), 5.55 (d, 1H, *J*= 8.2 Hz, 5-H), 6.05 (t, 1H, *J*= 6.7 Hz, 1'-H), 6.9 (d, 1H, *J*= 8.3 Hz, 6-H). CIMS m/z (relative intensity) 283 $[M+H]^+$ (100), 141 (27.4). Anal.($C_{10}H_{14}N_6O_4$): calculated C: 42.55, H: 4.96, N: 29.78; found C: 42.75, H: 4.85, N: 29.90.

3'-Azido-2',3'-dideoxy-4-N-methoxy-5-methylcytidine (3a): This compound was obtained from **4a** as above in 67% yield: mp 134-135°C; $[\alpha]_D^{20}$ + 39° (*c* 0.5, $CHCl_3$); UV (EtOH) λ max (ε) 272 (7,700) ; 238 (10,800), 202 nm (12,000); ^{1}H NMR (D_2O) δ 1.6 (d, *J*= 0.95 Hz, CH_3), 2.25 (m, 2H, *J*= 12.8, 7.7 and 6.4 Hz, 2'α-H, 2'β-H), 3.65 (m, 2H, 5'-Ha, 5'-Hb and s, 3H, OCH_3), 4.2 (m, 1H, *J* = 7.05 Hz, 4'-H), 4.7 (m, 1H, 3'-H), 6.0 (t, 1H, *J*= 6.7 Hz, 1'-H), 6.7 (d, 1H, *J*= 1.0 Hz, 6-H); CIMS m/z (relative intensity) 297 $[M+H]^+$ (100), 155 (20.7). Anal. ($C_{11}H_{16}N_6O_4$): calculated C: 44.59, H: 5.45, N: 28.37; found C: 44.77, H: 5.45, N: 28.19.

3'-Azido-5'-O-benzoyl-2',3'-dideoxy-4-N-methoxycytidine (11b): To a solution of compound **9b** (1.0 g, 2.8 mmol) in dry acetonitrile (50 mL), K_2CO_3 (570 mg, 4.8 mmol) and TsCl (640 mg, 3.36 mmol) were added. The mixture was stirred for 2h at 90°C (bath temperature) and then concentrated to dryness. To the residue a solution of methoxyamine hydrochloride (4.68 g, 56 mmol) in dry pyridine (50 mL) was readily added. The resulting mixture was stirred at room temperature for 15 min. and then concentrated to dryness. Water (30 mL) was added to the residue and the whole was extracted with EtOAc (2x50 mL). The combined extracts were washed with water (2x30 mL), dried ($MgSO_4$) and the solvent evaporated. The oily residue was purified by silica gel column chromatography (4x15 cm) eluting with petroleum ether/EtOAc (3:1) to give **11b** (594 mg, 55%): mp 68-70°C; $[\alpha]_D^{20}$ + 22° (*c* 0.5, $CHCl_3$) ; ^{1}H NMR ($CDCl_3$) δ 2.3 (m, 2H, *J*= 13.9, 7.5, 6.5 and 5.40 Hz, 2'α-H, 2'β-H), 3.8 (s, 3H, OCH_3), 4.15 (m, 1H, *J*= 5.4 and 3.7 Hz, 4'-H), 4.3

(m, 1H, *J*= 7.5 and 5.35 Hz, 3'-H), 4.6 (m, 2H, *J*= 12.25, 3.65 and 3.35 Hz, 5'-Ha, 5'-Hb), 5.5 (dd, 1H, *J*= 8.3 and 2.05 Hz, 5-H), 6.15 (t, 1H, *J*= 6.4 Hz, 1'-H), 6.75 (d, 1H, *J*= 8.3 Hz, 6-H), 7.55 and 8 (2 m, 5H, arom); CIMS m/z (relative intensity) 387 [M+H]$^+$ (100), 141 (60). Anal. ($C_3H_3N_3O_3$): calculated C: 52.80, H: 4.66, N: 21.76; found C: 52.50, H: 4.60, N:21.50.

3'-Azido-2',3'-dideoxy-5'-O-*p*.methoxybenzoyl-4-N-methoxy-5-methyl-cytidine (11a): was obtained starting from **9a** in the same way. Yield 66.5%; mp 99-101°C; $[\alpha]_D^{20}$ + 7.2° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.6 (s, 3H, CH_3), 2.3 (m, 2H, *J*= 13.9 and 6.95 Hz, 2'α-H, 2'β-H), 3.85 and 3.9 (2s, 2x3H, OCH_3), 4.15 (m, 1H, *J*= 3.6 Hz, 4'-H), 4.3 (m, 2H, *J*= 6.45 Hz, 3' -H), 4.6 (m, 2H, *J*= 12.2, 3.15 and 2.85 Hz, 5'-Ha, 5'-Hb), 6.2 (t, 1H, *J*= 6.55 Hz, 1'-H), 6.5 (s, 1H, 6-H), 6.95 and 8.0 (2d, 2x2H, arom), 8.1 (br s, 1H, NH); CIMS m/z (relative intensity) 431 [M+H]$^+$ (100) 155 (53). Anal. ($C_{19}H_{22}N_6O_6$): calculated C: 53.02, H: 5.11, N: 19.53; found C: 53.21, H: 5.16, N: 19.45.

Debenzoylation of 4-N-methoxycytosine derivatives 11 a, b was conventionally realized and worked up according to reference 16. **3b** was obtained in crude form with mp 120-123°C (yield 74%). Purification by preparative TLC eluting with petroleum ether/EtOAc (1 : 3) afforded pure **3b** in 55% yield: mp 121-123°C; and the other analytical data were the same as described above. Same procedure afforded **3a** (58% yield): mp 134-136°C; and the other analytical data were the same as above.

Cells and Virus. The CEM-Cl13, a subclone enriched in CD4 receptors, was obtained from the CEM T-lymphoblastoid tumor cell line.[17] Cells were grown as in reference 18. The LAV-Bru strain of HIV-1, isolated at the Pasteur Institute from lymphocytes of a patient with lymphadenopathy,[19] was employed for infection of the cells as described in reference 18.

Antiviral assay on cells. Compounds were tested and compared with AZT (**1a**) and CS 87 (**1b**) (prepared in our laboratory) for cytotoxicity and for their ability to inhibit HIV replication. The protocol was the same as in reference 18. The HIV-1-induced CPE was monitored by the MTT viability assay [20,21] after 7 days of incubation. Reverse transcriptase activity in supernatants of cell cultures was also measured as in reference 18 to follow HIV replication.

Aknowledgments: The bio-assays were run at Rhône-Poulenc Rorer (Vitry, France) by Dr. M. Lemaître and Dr. Y. Henin who are aknowledged. We also thank Dr. Y. Henin for her helpful and critical comments.

REFERENCES.

1) F. Barre-Sinoussi, J. C. Chermann, R. Rey, M. T. Nugeyre, S. Chamaret, C.Gruest,

C. Dauguet, C. Axler-Blin, C. Rouzioux, F. Vezinet-Brun, W. Rozenbaum and L. Montagnier, *Science* **220**, 868 (1983).

2) H. Mitsuya, K. J Weinhold, P. A. Furman, M. H. Saint-Clair, S. NusinoffLehrman, R. C. Gallo, D. Bolognesi, D. W. Barry and S. Broder, *Proc. Natl. Acad. Sci. USA.* **82**, 7096 (1985).

3) M. A. Fischl, D. D. Richman, M. H. Grieco, M. S. Gottlieb, P. A. Volberding, O. L. Laskin, J. M. Leedom, J. E. Groopman, D. Mildvan, R. T. Schooley, G. G. Jackson, D. T. Durack and D. King, *N. Engl. J. Med.* **317**, 185 (1987).

4) (a) D. D. Richman, M. A. Fischl, M. H. Grieco, M. S. Gottlieb, P. A. Volberding, O. L. Laskin, J. M. Leedom, J. E. Groopman, D. Mildvan, M. S. Hirsch, G. G. Jackson, D. T Durack and S. NusinoffLehrman, *N. Engl. J. Med.* **317**, 192 (1987). (b) J.P. Sommadossi and R. Carlisle, *Antimicrob. Agents Chemother.* **31**, 452 (1987).

5) P. Herdewijn and E. De Clercq, *Design of Anti-AIDS Drugs*; E. De Clercq, Editor; Pharmacochemistry Library Vol 14, pp. 141- 174, Elsevier. **1990**.

6) M. Nasr, C. Litterst and J. McGowan, *Antiviral Res.* **14**, 125 (1990).

7) E. Palomino, B. R. Meltsner, D. Kessel and J. P. Horwitz, *J. Med. Chem.* **33**, 258 (1990).

8) N. N. Anand, D. M. Brown and S. A. Salisbury, *Nucl. Acids Res.* **15**, 8167 (1987).

9) (a) I. Wempen, N. Miller, E. A. Falco and J. J. Fox, *J. Med. Chem.* **11**, 144 (1968). (b) A. Holy, *Collect. Czech. Chem. Commun.* **37**, 4072 (1972). (c) A. Kraszewsky, A. M. Delort and R. Teoule, *Tetrahedron Lett.* **27**, 861 (1986).

10) (a) J. E. Starrett, D. R. Tortolani, D. C. Baker, M. T. Omar, A. K. Hebbler, J. A. Wos, J. C. Martin and M. M. Mansuri, *Nucleosides & Nucleotides* **9**, 885 (1990). (b) C. K. Chu, G. V. Ullas, L. S. Jeong, S. K. Ahn, B. Doboszewski, Z. X. Lin, J. W. Beach and R. F. Schinazi, *J. Med. Chem.* **33**, 1553 (1990).

11) S. Czernecki and J. M. Valéry, *J. Chem. Soc. Chem. Commun.* 801 (1990).

12) M. Kawada, K. Matsumoto and M. Tsurushima, M., *Eur.Pat.Appl.* 204,264 (Cl. C 07 H 19/06, 10 Dec 86). *Chem. Abstr.* **106**, 214307m (1987).

13) S. Czernecki and J. M. Valéry, *Synthesis,* 239(1991).

14) R. F. Schinazi, B. F. Eriksson and C. K. Chu, *Antiviral Res.* **9**, 84 (1988).

15) A. Holy and M. Soucek, *Tetrahedron Lett.* 185(1971).

16) A. Holy, *Collect. Czech. Chem. Commun.* **38**, 100 (1973).

17) L. Montagnier, F. Clavel, B. Krust, S. Chamaret, F. Rey, F. Barre-Sinoussi and J. C. Chermann, *Virology,* **144**, 283 (1985).

18) Y. Henin, C. Gouyette, O. Schwartz, J. C. Debouzy, J. M. Neumann and T. Huynh-Dinh, *J. Med. Chem.* **34**, 1830 (1991).

19) F. Barre-Sinoussi, J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet- Brun, C. Rouzioux, W. Rozenbaum and L. Montagnier, *Science,* **220**, 868 (1983).

20) O. Schwartz, Y. Henin, V. Marechal and L. Montagnier, *AIDS Res. Hum. Retroviruses,* **4**, 441 (1988).

21) R. Pauwels, J. Balzarini, M. Baba, R. Snoeck, D. Schols, P. Herdewijn, J. Desmyter and E. De Clercq, *J. Virol. Methods,,***20**, 309 (1988).

Received 10/13/92
Accepted 2/4/93