Synthesis of *N*-Substituted 3'-Amino-3'-deoxythymidines and their Biological Evaluation against HIV

Mohammed S. Motawia and Erik B. Pedersen

Department of Chemistry, Odense University, Campusvej 55, 5230 Odense M, Denmark

Carsten M. Nielsen

Retrovirus Laboratory, Enterovirus Department, Statens Seruminstitut, Amager Boulevard 80, DK-2300 Copenhagen, Denmark

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Treatment of 3'-amino-3'-deoxythymidine (**1**) with carboxylic acid anhydrides afforded the corresponding acylamino derivatives **2a-f**. Reaction of **1** whith a variety of isothiocyanates led to the corresponding thioureido derivatives **3a-i**. Also, conversion of **1** into 3'-carbylamino-3'-deoxythymidine (**7**) is reported. The compounds **2**, **3**, and **8** were evaluated for their anti-HIV activity in MT-4 cells, but did not show sufficient efficacy.

Synthese *N*-substituierter 3'-Amino-3'-desoxythymidin-Derivate und ihre biologische Prüfung gegen HIV

Umsetzung von 3'-Amino-3'-desoxythymidin (**1**) mit Carbonsäureanhydriden führt zu den entspr. Acylamin-Derivaten **2a-f**. Aus **1** und verschiedenen Isothiocyanaten entstehen die Thioharnstoffe **3a-i**. Auch die Herstellung des Isonitrils **7** wird beschrieben. Die Verbindungen **2**, **3** und **8** wurden auf Aktivität gegen HIV geprüft, sie erwiesen sich jedoch als nicht ausreichend wirksam.

The retrovirus, human immunodeficiency virus (HIV)[1], has been recognized as the etiologic agent of AIDS[2,3]. An essential step in the replicative cycle of all retroviruses is the synthesis of DNA from a viral RNA by using the viral enzyme, reverse transcriptase. Inhibition of this enzyme is the mechanism of the action of certain antiretroviral nucleoside analogues, such as 3'-azido-3'-deoxythymidine (AZT)[4] and 2',3'-dideoxycytidine[5]. These compounds are able to enter cells and are converted by cellular enzymes to their triphosphates which are potent competitive inhibitors of reverse transcriptase. Since these analogues do not have the 3'-OH group of the natural substrates, DNA chain elongation is precluded. In general, inhibitors of cellular processes will often limit viral replication, but these agents are usually quite toxic for the host as well.

Since 3'-azido-3'-deoxythymidine (AZT), the drug currently advocated for treatment of AIDS patients, exhibits toxicity in a clinical setting[6], there is still an urgent need for new antiviral agents with low toxicity to normal cells. As it has been reported[7,8] that the ability of AZT to diffuse across cell membranes is due to the considerable lipophilicity imparted to this molecule by replacement of the 3'-OH group of thymidine by an azido substituent, one can speculate that replacement of the azido group of AZT by different substituents with variable lipophilic parameters might produce biologically active compounds with moderate toxicity. Acutally, the lipophilic parameter (π) has been firmly established as the parameter of choice for correlating both binding to biological macromolecules and transport through a biological system[9-12]. Therefore, it would be of interest to synthesize several thymidine derivatives having various 3'-substituents, which, like azide, would block DNA chain elongation.

Recently, it has been reported[13] that the triphosphate of 3'-amino-3'-deoxythymidine is a potent inhibitor of isolated reverse transcriptase, but the nucleoside itself has only slight antiretroviral activity. The inactivity of 3'-amino-3'-deoxythymidine (**1**) could be attributed to the considerably reduced lipophilicity of **1** by replacement of the azido group of AZT by an amino substituent (the lipophilic parameter π values[14]: $N_3 = 0.46$; $NH_2 = -1.23$). Thus, one could suggest that the considerably reduced lipophilicity of **1** has precluded its diffusion across cell membranes and the nucleoside **1** loses its activity. However, we thought it might be possible to enhance the lipophilicity of **1** by acylation of 3'-amino group or its conversion into thioureido derivatives. For both classes of derivatives the electronic parameter σ_m of the 3'-substituent will be modified to values close to those of fluoro and azido groups. With proper choices of acyl or thioureido groups, both lipophilic and electronic parameters can thus be adjusted to be very close to those of active compounds. Actually, fluorine has σ_m and π values close to those of the azido group and the activity against HIV was increased when the azido group in AZT was replaced with fluorine. On the other hand, it has been of interest to synthesize 3'-cyanamino-3'-deoxythymidine[15], not only because of its structural similarity to AZT but also for the close electronic parameters σ_m and σ_p of both the azido and the cyanamino groups[14]. Further, in the search of other linear substituents to replace the azido group in AZT, the isothiocyanate and the thiocyanate groups resulted in active compounds with activity against the RF strain of HIV-1 in 8166 cells[16]. Inhibition of HIV replication in MT-4 cells has also been reported for the latter group[17]. Therefore, conversion of the amino group of **1** into the isonitrile group was similarly found interesting[15].

Chemistry

The starting material 3'-amino-3'-dioxythymidine (**1**) was prepared as reported[18].

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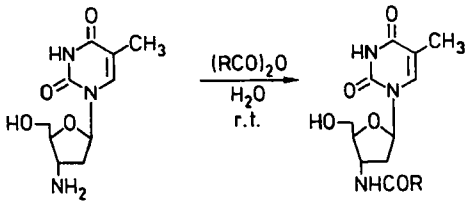
The acylamino derivatives **2a-f** were prepared in 47-65% yield by treatment of **1** with the corresponding acid anhydride at room temp. (Scheme 1).

Scheme 1

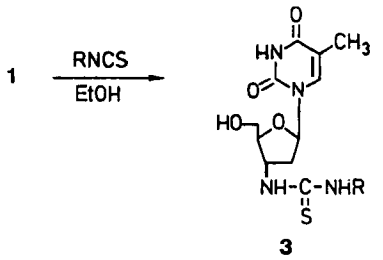


1 2

2	R
a	CH_3
b	CF_3
c	C_2H_5
d	C_3H_7
e	$CH(CH_3)_2$
f	C_4H_9

The thioureido derivatives **3a-i** were prepared in 67-96% yield through reaction of **1** with the corresponding isothiocyanate derivative (Scheme 2).

Scheme 2

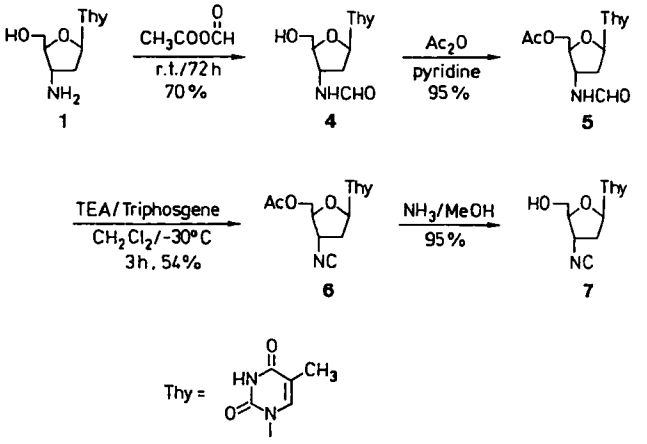


1 3

3	R
a	C_2H_5
b	C_4H_9
c	$CH_2CH_2CH(CH_3)_2$
d	$C(CH_3)_2CH_2C(CH_3)_3$
e	cyclohexyl
f	1-adamantyl
g	$CH_2CH{=}CH_2$
h	$4\text{-}CH_3OC_6H_4$
i	$4\text{-}ClC_6H_4$

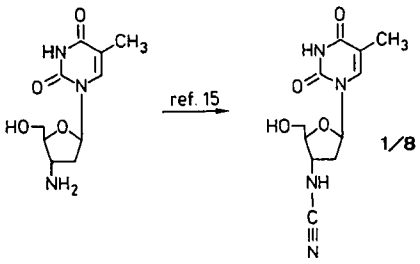
The carbylamino derivative **7** was prepared in 34% overall yield as shown in Scheme 3. Thus, treatment of **1** with acetic-formic anhydride[19] afforded the *N*-formyl derivative **4** in 70% yield. Acetylation of **4** with acetic anhydride produced the protected *N*-formyl derivative **5** in 95% yield which was reacted with triphosgene using *Ugi's* method[20] to give the protected isonitrile **6** in 54%. Treatment of **6** with saturated methanolic ammonia solution resulted in complete deprotection of the 5-hydroxy group to give the carbylamino derivative **7** in 95% yield.

Scheme 3



1 4 5 6 7

3'-Cyanamino-3'-deoxythymidine (**8**) was prepared as recently described[15].



Biological Evaluation

Compounds **2a-f, 3a-i**, and **8** were evaluated on their inhibitory effects of the replication of HIV in human MT-4 cells. None of the compounds showed marked anti-HIV activity at concentrations that were significantly below their toxicity threshold. The biological properties of compounds **2** and **3** were disappointing; the 3'-substituent was selected as acylamino ($\sigma_m = 0.21$) or thioureido ($\sigma_m = 0.30$) groups with electron attracting properties close to those of azido ($\sigma_m = 0.27$) and fluoro ($\sigma_m = 0.34$)[14] which are typical substituents in potent anti-HIV compounds[4,17]. For both acylamino and thioureido, the substituent lipophilicity parameter π has 0.54 increments for each CH_2 added to the substituent. In this way the 3'-substituent can be adjusted to have nearly the same π values[14] as azido ($\pi = 0.46$) or fluoro ($\pi = 0.14$). Although the lipophilicities of $NHCOC_3H_7$ ($\pi = 0.11$) or $NHCSNHC_4H_9$ ($\pi \sim 0.37$), were close to the latter groups, no anti-HIV activity was observed for **2d** or **3b**. $NHCOCF_3$ ($\sigma_m = 0.30$, $\pi = 0.08$) has both electronegativity and lipophilicity close to fluoro, but no anti-HIV activity was observed for **2b**.

Since both electronic and lipophilic properties of the 3'-substituent in the compounds **2** and **3** have been adjusted to

be close to those in biologically active substances, the lack of anti-HIV activity is best ascribed to the bulkiness of the 3'-substituent. On the other hand the sterical requirements for biological activity seem to be fulfilled for the isocyano and cyanamino groups in **7** and **8**, respectively, when compared with the azido group in AZT. Nevertheless, these compounds have no anti-HIV activity in CEM cells[15] and we have confirmed the lacking anti-HIV activity when they are tested on MT-4 cells. The lack of anti-HIV activity with NHCN (π = -0.24) as 3'-substituent may be due to reduced lipophilicity of this substituent.

In conclusion, non-linear 3'-substituents with lipophilicity and electronegativity comparable to azido cannot secure potent anti-HIV activity for new nucleosides.

Experimental Part

3'-Acylamino-3'-deoxythymidines **2b-f**, *General Procedure*

1 (0.5 g, 2.1 mmol), was added to 3 ml of water and stirred with the appropriate acid anhydride (4.2 mmol) for 3 h at room temp. The precipitate was filtered off and dried.

3'-Trifluoroacetylamino-3'-deoxythymidine (**2b**)

White solid (from EtOH), yield 0.45 g (65%). M.P. 228-230°C. - ^{1}H-NMR (250 MHz, d_6-DMSO): 1.79 (s, 3H, CH_3), 2.35 (m, 2H, H-2'), 3.68 (m, 2H, H-5'), 3.87 (m, 1H, H-4'), 4.01 (m, 1H, H-3'), 5.36 (s, 1H, OH), 6.32 (t, 1H, J = 6.58 Hz, H-1'), 7.73 (s, 1H, H-6), 8.48 (s, 1H, $NHCOCF_3$), 11.33 (s, 1H, NH). - ^{13}C-NMR (62.5 MHz, d_6-DMSO): 163.73 (C-4), 158.74 (d, J = 32 Hz, $\underline{C}OCF_3$), 150.40 (C-2), 136.02 (C-6), 117.05 (q, J = 291 Hz, CF_3), 109.64 (C-5), 83.43 (C-1'), 82.78 (C-4'), 60.95 (C-5'), 50.27 (C-3'), 35.36 (C-2'), 12.17 (CH_3). - $C_{12}H_{14}F_3N_3O_5 \cdot H_2O$ (355.3). Calcd. C 40.6 H 4.54 N 11.8 Found C 40.5 H 4.54 N 11.8.

3'-Propionylamino-3'-deoxythymidine (**2c**)

White solid (from EtOH), yield 0.41 g (67%). M.p. 202-204°C. - ^{1}H-NMR (250 MHz, d_6-DMSO): 1.01 (t, 3H, J = 7.50 Hz, $C\underline{H}_3CH_2CO$), 1.79 (s, 3H, CH_3), 2.06-2.28 (m, 4H, $CH_3C\underline{H}_2CO$ + H-2'), 3.59-3.69 (m, 2H, H-5'), 3.77 (m, 1H, H-4'), 4.33 (m, 1H, H-3'), 5.08 (s, 1H, OH), 6.20 (t, 1H, J = 6.44 Hz, H-1'), 7.78 (s, 1H, H-6), 8.21 (d, 1H, J = 7.07 Hz, NHCO), 11.27 (s, 1H, NH). - ^{13}C-NMR (62.5 MHz, d_6-DMSO): 172.89 ($\underline{C}OCH_2CH_3$), 163.67 (C-4), 150.37 (C-2), 136.07 (C-6), 109.33 (C-5), 85.16 (C-4'), 83.51 (C-1'), 61.40 (C-5'), 49.02 (C-3'), 37.01 (C-2'), 28.32 ($CH_3\underline{C}H_2CO$), 12.15 (CH_3), 9.70 ($\underline{C}H_3CH_2CO$). - $C_{13}H_{19}N_3O_5 \cdot 1/2H_2O$ (306.3). Calcd. C 51.0 H 6.58 N 13.7 Found C 50.8 H 6.22 N 13.6.

3'-Butyrylamino-3'-deoxythymidine (**2d**)

White solid (from EtOH), yield 0.3 g (47%). M.P. 220-222°C. - ^{1}H-NMR (250 MHz, d_6-DMSO): 0.86 (t, 3H, J = 7.24 Hz, $C\underline{H}_3(CH_2)_2CO$), 1.53 (m, 2H, $CH_3C\underline{H}_2CH_2CO$), 1.79 (s, 3H, CH_3), 2.07 (t, 2H, J = 7.05 Hz, $CH_3CH_2C\underline{H}_2CO$), 2.17-2.28 (m, 2H, H-2'), 3.58-3.70 (m, 2H, H-5'), 3.76 (m, 1H, H-4'), 4.33 (m, 1H, H-3'), 5.09 (s, 1H, OH), 6.20 (t, 1H, J = 6.20 Hz, H-1'), 7.79 (s, 1H, H-6), 8.25 (d, 1H, J = 6.96 Hz, $N\underline{H}CO$), 11.20 (s, 1H, NH). - ^{13}C-NMR (62.5 MHz, d_6-DMSO): 172.02 ($\underline{C}OC_3H_7$), 163.66 (C-4), 150.37 (C-2), 136.08 (C-6), 109.33 (C-5), 85.19 (C-4'), 83.50 (C-1'), 61.37 (C-5'), 48.97 (C-3'), 37.15 ($COC\underline{H}_2CH_2CH_3$), 37.02 (C-2'), 18.56 ($CH_3C\underline{H}_2CH_2CO$), 13.48 ($\underline{C}H_3(CH_2)_2CO$), 12.17 ($CH_3$). - $C_{14}H_{21}N_3O_5$ (311.3) Calcd. C 54.0 H 6.80 N 13.5 Found C 54.0 H 6.78 N 13.4.

3'-Isobutyrylamino-3'-deoxythymidine (**2e**)

White solid (from EtOH) yield 0.36 g (57%). M.p. 198-200°C. - ^{1}H-NMR (250 MHz, d_6-DMSO): 1.02 (d, 6H, J = 6.57 Hz, $CH(C\underline{H}_3)_2$), 1.79 (s, 3H, CH_3), 2.06-2.42 (m, 3H, H-2' + CH), 3.54-3.67 (m, 2H, H-5'), 3.75 (m, 1H, H-4'), 4.32 (m, 1H, H-3'), 5.10 (s, 1H, OH), 6.21 (t, 1H, J = 6.06 Hz, H-1'), 7.79 (s, 1H, H-6), 8.19 (d, 1H, J = 6.91 Hz, NHCO), 11.13 (s, 1H, NH). - ^{13}C-NMR (62.5 MHz, d_6-DMSO): 176.10 ($\underline{C}OCH(CH_3)_2$), 163.62 (C-4), 150.32 (C-2), 136.05 (C-6), 109.29 (C-5), 85.17 (C-4'), 83.46 (C-1'), 61.37 (C-5'), 48.90 (C-3'), 36.91 (C-2'), 34.04 ($\underline{C}H$), 19.39, 19.27 (CH_3), 12.10 (CH_3). $C_{14}H_{21}N_3O_5 \cdot 1/4H_2O$ (315.8). Calcd. C 53.24, H 6.86, N 13.30. Found C 53.04, H 6.79, N 13.18.

3'-Valerylamino-3'-deoxythyimidine (**2f**)

White solid [after chromatography on silica (30 g, 40-63 µ) with $CHCl_3$/MeOH (9:1)] yield 0.32 g (47%). M.p. 178-180°C. - ^{1}H-NMR (250 MHz, d_6-DMSO): 0.87 (t, 3H, J = 7.24 Hz, CH_3), 1.27 (m, 2H, $CH_3C\underline{H}_2CH_2CH_2CO$), 1.49 (m, 2H, $CH_3CH_2C\underline{H}_2CH_2CO$), 1.79 (s, 3H, CH_3), 2.03-2.27 (m, 4H, H-2' + $-CH_2CO$), 3.38-3.67 (m, 2H, H-5'), 3.75 (m, 1H, H-4'), 4.27-4.37 (m, 1H, H-3'), 5.10 (s, 1H, OH), 6.20 (t, 1H, J = 6.57 Hz, H-1'), 7.79 (s, 1H, H-6), 8.27 (d, 1H, J = 7.27, NHCO), 11.30 (s, 1H, NH). - ^{13}C-NMR (62.5 MHz, d_6-DMSO): 172.16 ($\underline{C}OC_4H_9$), 163.66 (C-4), 150.35 (C-2), 136.07 (C-6), 109.30 (C-5), 85.16 (C-4'), 83.49 (C-1'), 61.35 (C-5'), 48.96 (C-3'), 36.99 (C-2'), 34.93 ($-\underline{C}H_2C$), 27.28 (CH_2), 21.69 (CH_2), 13.61 (CH_3), 12.15 (CH_3). - $C_{15}H_{23}N_3O_5 \cdot 1/2H_2O$ (334.4). Calcd. C 53.9 H 7.23 N 12.6 Found C 53.8 H 7.04 N 12.1.

3'-[(3-Substituted)thioureido]-3'-deoxythymidines **3a-i**, *General Procedure*

The appropriate isothiocyanate derivative (1.4 mmol) was added to a suspension of **1** (0.3 g, 1.24 mmol) in 10 ml of EtOH and the mixture was refluxed for 2 h. The solvent was evaporated and the residue was chromatographed on silica (20 g, 40-63 µ) with $CHCl_3$/MeOH (9:1). In some cases, the precipitate formed was recrystallized from EtOH.

3'-(3-Ethylthioureido)-3'-deoxythymidine (**3a**)

Yield 0.35 g (86%). M.p. 132-134°C. - ^{1}H-NMR (250 MHz, d_6-DMSO): 1.08 (t, 3H, J = 7.15 Hz, CH_3), 1.80 (s, 3H, CH_3), 2.12-2.36 (m, 4H, H-2' + CH_2), 3.67 (m, 2H, H-5'), 3.88 (m, 1H, H-4'), 4.72 (m, 1H, H-3'), 5.15 (s, 1H, OH), 6.20 (t, 1H, J = 6.53 Hz, H-1'), 7.36 (m, 1H, $N\underline{H}CH_2$-), 7.80 (s, 2H, H-6 + NH), 11.31 (s, 1H, NH). - ^{13}C-NMR (62.5 MHz, d_6-DMSO): 163.70 (C-4), 150.41 (C-2), 136.01 (C-6), 109.33 (C-5), 85.30 (C-4'), 83.57 (C-1'), 61.40 (C-5'), 53.86 (C-3'), 38.23 ($NHCH_2$), 37.24 (C-2'), 14.31 (CH_3), 12.27 (CH_3). - $C_{13}H_{20}N_4O_4S \cdot 1/2H_2O$ (337.4). Calcd C 46.3 H 6.27 N 16.6 Found C 46.3 H 6.34 N 16.3.

3'-(3-n-Butylthioureido)-3'-deoxythymidine (**3b**)

Yield 0.42 g (95%). M.p. 206-208°C. - ^{1}H-NMR (250 MHz, d_6-DMSO): 0.89 (t, 3H, J = 7.25 Hz, CH_3), 1.30 (m, 2H, CH_2), 1.46 (m, 1H, CH_2), 1.79 (s, 3H, CH_3), 2.11-2.36 (m, 4H, H-2' + CH_2), 3.64 (m, 2H, H-5'), 3.88 (m, 1H, H-4'), 4.71 (m, 1H, H-3'), 5.15 (s, 1H, OH), 6.20 (t, 1H, J = 6.58 Hz, H-1'), 7.37 (s, 1H, NH), 7.80 (s, 2H, H-6, NH), 11.31 (NH). - ^{13}C-NMR (62.5 MHz, d_6-DMSO): 181.83 (C=S), 163.67 (C-4), 150.38 (C-2), 135.95 (C-6), 109.31 (C-5), 85.35 (C-4'), 83.56 (C-1'), 61.44 (C-5'), 53.86 (C-3'), 43.20 ($NHCH_2$), 37.21 (C-2'), 30.71 (CH_2), 19.49 (CH_2), 13.63 (CH_3), 12.23 (CH_3). - $C_{15}H_{24}N_4O_4S \cdot 1/4H_2O$ (360.9). Calcd C 49.9 H 6.84 N 15.5 Found C 49.9 H 6.89 N 15.1.

3'-(3-Isoamylthioureido)-3'-deoxythymidine (**3c**)

Yield 0.44 g (96%). M.p. 202-204°C. - ^{1}H-NMR (250 MHz, d_6-DMSO): 0.88 (d, 6H, J = 6.56, 2 x CH_3), 1.38 (m, 2H, CH_2), 1.57 (m, 1H, CH), 1.79 (s, 3H, CH_3), 2.11-2.36 (m, 4H, H-2' + CH_2), 3.64 (m, 2H, H-5'), 3.88 (m,

1H, H-4'), 4.70 (m, 1H, H-3'), 5.15 (s, 1H, OH), 6.20 (t, 1H, J = 6.59 Hz, H-1'), 7.34 (m, 1H, NH), 7.80 (s, 2H, H-6, NH), 11.32 (s, 1H, NH). - ^{13}C-NMR (62.5 MHz, d_6-DMSO). 163.69 (C-4), 150.41 (C-2), 135.97 (C-6), 109.34 (C-5), 85.41 (C-4'), 83.57 (C-1'), 61.46 (C-5'), 53.97 (C-3'), 41.83 ($NHCH_2$), 37.55 (CH), 37.23 (C-2'), 25.22 (CH_2), 22.36 (CH_3), 12.26 (CH_3). - $C_{16}H_{26}N_4O_4S \cdot 1/4H_2O$ (375.0). Calcd. C 51.3 H 7.12 N 14.9. Found C 51.4 H 7.25 N 14.7.

3'-[3-(1,1,3,3-Tetramethylbutyl)thioureido]-3'-deoxythymidine (**3d**)

Yield 0.45 g (88%). M.p. 208-210°C. - ^{1}H-NMR (250 MHz, d_6-DMSO): 0.96 (s, 6H, 2 x CH_3), 1.02 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.46 (s, 2H, CH_2), 1.79 (s, 3H, CH_3), 1.97-2.35 (m, 2H, H-2'), 3.68 (m, 2H, H-5'), 3.83 (m, 1H, H-4'), 4.72 (m, 1H, H-3'), 5.12 (s, 1H, OH), 6.18 (t, 1H, J = 6.83 Hz, H-1'), 6.98 (s, 1H, NH), 7.67 (d, 1H, J = 6.78 Hz, NH), 7.81 (s, 1H, H-6), 11.29 (s, 1H, NH). - ^{13}C-NMR (62.5 MHz, d_6-DMSO): 180.71 (C=S), 163.59 (C-4), 150.37 (C-2), 135.78 (C-6), 109.35 (C-5), 85.99 (C-4'), 83.59 (C-1'), 61.67 (C-5'), 53.44 (C-3'), 48.44 (NHC), 37.22 (C-2'), 31.01 ($C(CH_3)_3$), 30.86 ($\underline{C}(CH_3)_3$), 30.58 ($-CH_2-$), 29.84 ($C(C\underline{H}_3)_2CH_2$), 12.18 ($CH_3$). - $C_{19}H_{32}N_4O_4S$ (412.6). Calcd. C 55.3 H 7.82 N 13.6 Found C 55.6 H 8.21 N 12.6.

3'-(3-Cyclohexylthioureido)-3'-deoxythymidine (**3e**)

White crystals (from EtOH), yield 0.38 g (80%). M.p. 218-220°C. - ^{1}H-NMR (250 MHz, d_6-DMSO): 1.03-2.36 (m, 16H, H-2', CH_3 and H-cyclohex.), 3.67 (m, 2H, H-5'), 3.86 (m, 1H, H-4'), 4.71 (m, 1H, H-3'), 5.17 (s, 1H, OH), 6.19 (t, 1H, J = 6.58 Hz, H-1'), 7.28 (br s, 1H, NH), 7.75 (d, 1H, J = 6.4 Hz, NH), 7.80 (s, 1H, H-6), 11.30 (s, 1H, NH). - ^{13}C-NMR (62.5 MHz, d_6-DMSO): 180.66 (C=S), 163.68 (C-4), 150.42 (C-2), 135.93 (C-6), 85.51 (C-4'), 83.58 (C-1'), 61.55 (C-5'), 53.96 (C-3'), 51.70 (C-1"), 37.18 (C-2'), 32.10 (C-2"), 25.10 (C-4"), 24.36 (C-3"), 12.25 (CH_3). - $C_{17}H_{26}N_4O_4S \cdot H_2O$ (400.5) Calcd. C 51.0 H 7.05 N 14.0 Found C 50.6 H 6.87 N 13.9.

3'-(3-Adamantylthioureido)-3'-deoxythymidine (**3f**)

White crystals (from EtOH) yield 0.37 g (67%). M.p. 216-218°C. - ^{1}H-NMR (250 MHz, d_6-DMSO): 1.62 (s, 6H, $(CH_2)_3$-adam.), 1.79 (s, 3H, CH_3), 2.03-2.34 (m, 11H, H-2' + H-adam.), 3.67 (m, 2H, H-5'), 3.84 (m, 1H, H-4'), 4.68 (m, 1H, H-3'), 5.15 (s, 1H, OH), 6.18 (t, 1H, J = 6.78 Hz, H-1'), 6.93 (s, 1H, NH), 7.75 (d, 1H, J = 6.6 Hz, NH), 7.81 (s, 1H, H-6), 11.32 (s, 1H, NH). - ^{13}C-NMR (62.5 MHz, d_6-DMSO): 180.18 (C=S), 163.53 (C-4), 150.33 (C-2), 135.73 (C-6), 109.31 (C-5), 85.81 (C-4'), 83.60 (C-1'), 61.67 (C-5'), 53.44 (C-3'), 52.70 (C-1", adam.), 40.97 (C-2", C-8", C-9", adam.), 37.10 (C-2'), 35.86 (C-3", C-5", C-7", adam.), 28.90 (C-4", C-6", C-10", adam.), 12.11 (CH_3). - $C_{21}H_{30}N_4O_4S$ (434.5). Calcd. C 58.0 H 6.96 N 12.9 Found C 58.2 H 7.25 N 12.5.

3'-(3-Allylthioureido)-3'-deoxythymidine (**3g**)

Yield 0.38 g (90%). M.p. 202-204°C. - ^{1}H-NMR (250 MHz, d_6-DMSO): 1.79 (s, 3H, CH_3), 2.10-2.38 (m, 2H, H-2'), 3.67 (m, 2H, H-5'), 3.89 (m, 1H, H-4'), 4.06 (m, 2H, $NHC\underline{H}_2$), 4.73 (m, 1H, H-3'), 5.12 (m, 3H, $C\underline{H}_2$=CH + OH), 5.87 (m, 1H, $C\underline{H}$=CH_2), 6.20 (t, 1H, J = 6.68 Hz, H-1'), 7.50 (s, 1H, NH), 7.80 (s, 1H, H-6), 7.93 (s, 1H, NH), 11.29 (s, 1H, NH). - ^{13}C-NMR (62.5 MHz, d_6-DMSO): 182.04 (C=S), 163.65 (C-4), 150.37 (C-2), 135.94 (C-6), 134.85 (-$\underline{C}$H=CH_2), 115.40 (-CH=$\underline{C}H_2$), 109.30 (C-5), 85.28 (C-4'), 83.58 (C-1'), 61.42 (C-5'), 54.01 (C-3'), 45.78 ($NH\underline{C}H_2$), 37.21 (C-2'), 12.20 (CH_3). - $C_{14}H_{20}N_4O_4S \cdot H_2O$ (358.4). Calcd. C 46.9 H 6.19 N 15.6 Found C 47.5 H 5.89 N 15.4.

3'-[3-(4-Methoxyphenyl)thioureido]-3'-deoxythymidine (**3h**)

White crystals (from EtOH) yield 0.44 g (87%). M.p. 224-226°C. - ^{1}H-NMR (250 MHz, d_6-DMSO): 1.79 (s, 3H, CH_3), 2.15-2.38 (m, 2H, H-2'), 3.68 (m, 2H, H-5'), 3.74 (s, 3H, OCH_3), 3.93 (m, 1H, H-4'), 4.87 (m, 1H, H-3'), 5.12 (s, 1H, OH), 6.21 (t, 1H, J = 6.71 Hz, H-1'), 6.91 (d, 2H, J = 8.95 Hz, H-aromat.), 7.26 (d, 2H, J = 8.90 Hz, H-aromat.), 7.80 (s, 1H, H-6), 8.09 (d, 1H, J = 6.92 Hz, NH), 9.34 (s, 1H, NH), 11.31 (s, 1H, NH). - ^{13}C-NMR (62.5 MHz, d_6-DMSO): 180.63 (C=S), 163.69 (C-4), 156.44 (C-4"), 150.39 (C-2), 136.06 (C-6), 131.76 (C-1"), 125.74 (C-3"), 113.76 (C-2"), 109.35 (C-5), 84.88 (C-4'), 83.50 (C-1'), 61.52 (C-5'), 55.16 (OCH_3), 54.27 (C-3'), 37.05 (C-2'), 12.23 (CH_3). - $C_{18}H_{22}N_4O_5S$ (406.5). Calcd. C 53.2 H 5.46 N 13.8 Found C 53.3 H 5.57 N 13.7.

3'-[3-(4-Chlorophenylthioureido)]-3'-deoxythymidine (**3i**)

White Crystals (from EtOH) yield 0.38 g (75%). M.p. 222-224°C. - ^{1}H-NMR (250 MHz, d_6-DMSO): 1.81 (s, 3H, CH_3), 2.18-2.42 (m, 2H, H-2'), 3.72 (m, 2H, H-5'), 3.97 (m, 1H, H-4'), 4.87 (m, 1H, H-3'), 5.16 (s, 1H, OH), 6.25 (t, 1H, J = 6.76 Hz, H-1'), 7.37 (d, 2H, J = 8.76 Hz, H-aromat.), 7.49 (d, 2H, J = 8.77 Hz, H-aromat.), 7.82 (s, 1H, H-6), 8.38 (d, 1H, J = 6.94 Hz, NH), 9.57 (s, 1H, NH), 11.31 (s, 1H, NH). - ^{13}C-NMR (62.5 MHz, d_6-DMSO): 180.39 (C=S), 163.66 (C-4), 150.38 (C-2), 138.24 (C-1"), 135.97 (C-6), 128.29 (C-3"), 127.91 (C-4"), 124.62 (C-2"), 109.39 (C-5), 84.94 (C-4'), 83.60 (C-1'), 61.60 (C-5'), 54.38 (C-3'), 37.0 (C-2'), 12.20 (CH_3). - $C_{17}H_{19}ClN_4O_4S$ (410.9). Calcd. C 49.7 H 4.66 N 13.6 Found C 49.9 H 4.77 N 13.8.

3'-Formylamino-3'-deoxythymidine (**4**)

Acetic-formic anhydride[19] (6.44 g, 7.31 mmol) was added dropwise to a stirred solution of **1** (2g, 8.3 mmol) in 30 ml of dry pyridine at 0°C. The mixture was stirred for 72 h at room temp. The solvent was evaporated and the residue was dissolved in 30 ml of MeOH and stirred under reflux for 30 min. Methanol was evaporated and the residue was chromatographed on silica (40 g, 40-63 µ) with $CHCl_3$/MeOH (9:1) to give pure **4**, yield 1.57 g (70%). M.p. 198-200°C (soften at 182°C). (lit.[15]: 194°C). - ^{1}H-NMR (250 MHz, d_6-DMSO): 1.80 (s, 3H, CH_3), 2.08-2.32 (m, 2H, H-2'), 3.54-3.80 (m, 3H, H-4' + H-5'), 4.39-4.46 (m, 1H, H-3'), 5.12 (s, 1H, OH), 6.19 (t, 1H, J = 6.50 Hz, H-1'), 7.78 (s, 1H, CHO), 8.04 (s, 1H, H-6), 8.51 (d, 1H, J = 7.43 Hz, N$\underline{H}$CHO). - ^{13}C-NMR (62.5 MHz, d_6-DMSO): 163.67 (C-4), 161.0 (NHCHO), 150.33 (C-2), 136.07 (C-6), 109.34 (C-5), 84.77 (C-4'), 83.46 (C-1'), 61.11 (C-5'), 47.59 (C-3'), 36.95 (C-2'), 12.15 (CH_3).

5'-O-Acetyl-3'-formylamino-3'-deoxythymidine (**5**)

4 ml of acetic anhydride was added to a solution of **4** (1.33 g, 4.94 mmol) in 15 ml of dry pyridine and the mixture was stirred for 1 h at room temp. The solvent was evaporated and the residue was coevaporated twice with 20 ml of dry toluene. The residue was chromatographed on silica (40 g, 40-63 µ) with $CHCl_3$/MeOH (9:1) to give pure **5**, yield 1.5 g (98%). M.p. 98-100°C. - ^{1}H-NMR (250 MHz, d_6-DMSO): 1.81 (s, 3H, CH_3), 2.05 (s, 3H, $COCH_3$), 2.14-2.41 (m, 2H, H-2'), 3.87-3.94 (m, 1H, H-4'), 4.14-4.30 (m, 2H, H-5'), 4.38-4.49 (m, 1H, H-3'), 6.19 (t, 1H, J = 6.58 Hz, H-1'), 7.53 (s, 1H, -CHO), 8.05 (s, 1H, H-6), 8.48 (d, 1H, J = 7.57 Hz, N$\underline{H}$-CHO), 11.34 (s, 1H, NH). - ^{13}C-NMR (62.5 MHz, d_6-DMSO): 170.01 ($COCH_3$), 163.58 (C-4), 161.0 (NH-CHO), 150.25 (C-2), 135.97 (C-6), 109.71 (C-5), 83.53 (C-1'), 81.14 (C-4'), 63.84 (C-5'), 47.68 (C-3'), 35.77 (C-2'), 20.46 ($COCH_3$), 11.97 (CH_3).

5'-O-Acetyl-3'-Carbylamino-3'-deoxythymidine (**6**)

A solution of triphosgene (0.45 g, 1.52 mmol) in 5 ml of dry CH_2Cl_2 was added dropwise to a stirred solution of **5** (1.21 g, 3.9 mmol) in 1.5 ml of triethylamine and 10 ml of dry CH_2Cl_2 at -30°C. Stirring was continued for 3 h at -30°C. After the addition of 20 ml of conc. aqueous solution of Na_2CO_3, the org. phase was separated and dried over Na_2SO_4. The solvent was evaporated and the residue was chromatographed on silica (40 g, 40-63 µ) with $CHCl_3$/MeOH (99:1) yield 0.62 g (54%). M.p. 128-130°C. - ^{1}H-

NMR (250 MHz, d_6-DMSO): 1.79 (s, 3H, CH_3), 2.06 (s, 3H, $COCH_3$), 2.50-2.69 (m, 2H, H-2'), 4.22-4.32 (m, 3H, H-4' + H-5'), 4.52-4.60 (m, 1H, H-3'), 6.22 (t, 1H, J = 6.45 Hz, H-1'), 7.43 (s, 1H, H-6), 11.38 (s, 1H, NH). - ^{13}C-NMR (62.5 MHz, d_6-DMSO): 169.92 ($COCH_3$), 163.51 (C-4), 158.01 (-NC), 150.19 (C-2), 136.16 (C-6), 109.91 (C-5), 83.61 (C-1'), 80.64 (C-4'), 62.24 (C-5'), 51.34 (C-3'), 36.50 (C-2'), 20.39 ($COCH_3$), 11.92 (CH_3). IR (KBr): δ_{NC} 2146 cm^{-1}.

3'-Carbylamino-3'-deoxythymidine (**7**)

0.5 g of **6** was stirred in 50 ml of saturated solution of ammonia in MeOH for 2 h at room temp. The solvent was evaporated at room temp. The residue was chromatographed on silica (20 g, 40-63 µ) with $CHCl_3$/MeOH (95:5) yield 0.4 g (95%). M.p. 154-156°C (lit.[15]: 150°C). - ^{1}H-NMR (250 MHz, d_6-DMSO): 1.77 (s, 3H, CH_3), 2.43-2.62 (m, 2H, H-2'), 3.57-3.71 (m, 2H, H-5'), 4.04-4.09 (m, 1H, H-4'), 4.41-4.49 (m, 1H, H-3'), 5.28 (s, 1H, OH), 6.20 (t, 1H, J = 6.43 Hz, H-1'), 7.61 (s, 1H, H-6), 11.35 (s, 1H, NH). - ^{13}C-NMR (62.5 MHz, d_6-DMSO): 163.54 (C-4), 157.53 (-NC), 150.24 (C-2), 135.96 (C-6), 109.56 (C-5), 84.27 (C-4'), 83.21 (C-1'), 59.91 (C-5'), 51.24 (C-3'), 37.07 (C-2'), 12.04 (CH_3). - IR (KBr) δ_{NC} 2147 cm^{-1}.

Anti-HIV Assay Procedure

Nucleoside analogues were examined for possible antiviral activity using HIV-1 (strain HTLV IIIB)-infected MT-4 cells as target system. For screening studies MT-4 cells were incubated with virus for 2 h, washed and thereafter added in a proportion of 1:10 to uninfected MT-4 cells, which had been preincubated in drug-containing growth medium for 2 h. Compounds were screened using a concentration in the medium of 0.33 mM. In case of low solubility a 1:3 dilution of saturated solution was used. Cultures were maintained for one week in parallel with virus-infected control cultures without compound added. Expression of HIV in the culture medium was quantified by HIV antigen detection ELISA. Compounds mediating less than 20% reduction of antigen expression were considered without bioligical activity. Compounds mediating a reduction of 20% or more were examined for cytotoxic potential using concentration-dependent inhibition of MT-4 cell proliferation as measure of cytotoxicity. A 20% inhibition of cell growth relative to control cultures was considered significant. Cytotoxicity TD_{50}: **2a**, 10 µM; **2b**, 1 µM; **2d**, 1000 µM; 3d, 0.1 x saturated; 3f, 0.1 x saturated; **8**, 100 µM. The compounds were finally reexamined for antiviral activity in the highest sub-toxic concentration observed.

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References

1 J. Coffin, A. Haase, J.A. Levy, L. Montagnier, S. Oroszlan, N. Teich, H. Temin, K. Toyoshima, H. Varmus, P. Vogt, and R. Weiss, Science (Washington, D.C.) *232*, 697 (1986).

2 F. Barré-Sinoussi, J.C. Chermann, R. Rey, M.T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vézinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier, Science (Washington, D.C.) *220*, 868 (1983).

3 R.C. Gallo, P.S. Sarin, E.P. Gelmann, M. Robert-Guroff, E. Richardson, V.S. Kalyanaraman, D.L. Mann, G. Sidhu, R.E. Stahl, S. Zolla-Pazner, J. Leibowitch, and M. Popovic, Science (Washington, D.C.) *220*, 865 (1983).

4 H. Mitsuya, K.J. Weinhold, P.A. Furman, M.H.St. Clair, S.N. Lehrman, R.C. Gallo, D. Bolognesi, D.W. Barry, and S. Broder, Proc. Natl. Acad. Sci. U.S.A. *82*, 7096 (1985).

5 H. Mitsuya and S. Broder, Proc. Natl. Acad. Sci. U.S.A. *83*, 1911 (1986).

6 R. Yarchoan, R.W. Klecker, K.J. Weinhold, P.D. Markham, H.K. Lyerly, D.T. Durack, E. Gelmann, S.N. Lehrmann, R.M. Blum, D. Barry, G. Shearer, M.A. Fischl, H. Mitsuya, R.C. Gallo, J.M. Collins, D.P. Bolognesi, C.E. Myers, and S. Broder, Lancet *1*, 575 (1986).

7 E. Palomino, D. Kessel, and J.P. Horwitz, J. Med. Chem. *32*, 622 (1989).

8 T.P. Zimmermann, W.B. Mahoney, and K.L. Prus, J. Biol. Chem. *262*, 574 (1987).

9 T. Fujita, J. Iwasa, and C. Hansch, J. Am. Chem. Soc. *86*, 5175 (1964).

10 C. Hansch and W.J. Dunn, J. Pharm. Sci. *61*, 1 (1972).

11 C. Hansch and J.M. Clayton, J. Pharm. Sci. *62*, 1 (1972).

12 A.E. Bird and A.C. Marshall, Biochem. Pharmacol. *16*, 2275 (1967).

13 Y.-C. Cheng, G.E. Dutschman, K.F. Bastow, M.G. Sarnyadharan, and R.Y.C. Ting, J. Biol. Chem. *262*, 2187 (1987).

14 C. Hansch and A. Leo, Substitution Constants for Correlation Analysis in Chemistry and Biology, John Wiley & Sons, New York 1979.

15 M. Maillard, A. Faraj, F. Frapier, J.-C. Florent, D.S. Grierson, and C. Monneret, Tetrahedron Lett. *30*, 1955 (1989).

16 S.L. Schreiber and N. Ikemoto, Tetrahedron Lett. *29*, 3211 (1988).

17 P. Herdewijn, J. Balzarini, E. De Clercq, R. Pauwels, M. Baba, S. Broder, and H. Vanderhaege, J. Med. Chem. *30*, 1270 (1987).

18 M.S. Motawia, J. Wengel, A.E.-S. Abdel-Megied, and E.B. Pedersen, Synthesis *1989*, 384.

19 L.I. Krimen, Org. Synth. Coll. Vol. *4*, 8 (1963).

20 I. Ugi, W. Betz, V. Fetzer, and K. Offermann, Chem. Ber. *94*, 2814 (1961); R. Karl, P. Lemmen, and I. Ugi, Synthesis *1989*, 718.

21 N. Miller and J.J. Fox, J. Org. Chem. *29*, 1772 (1964).

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