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Synthesis and Antiviral Evaluation of 3'-Substituted Thymidine Analogues Derived from 3'-Amino-3'-Deoxythymidine

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Abstract : Based on the structure-activity relationship for antiviral activity, a series of 3'-deoxy-3'-*N*-functionalized thymidine analogues derived from 3'-amino-3'-deoxythymidine was synthesized. These compounds were evaluated for their antiviral activity. Three of the prepared molecules namely 3'-(1,2,4-triazol-1-yl)carbimidoylamino-3'-deoxythymidine **6**, 3'-(3-amino-1-methyl-1,2,4-triazol-5-yl)amino-3'-deoxythymidine **8b** and 3'-*N*-cyano-*O*-phenylisourea-3'-deoxythymidine 7 show moderate but selective *in vitro* activity against HIV-1 and HIV-2. These data demonstrate that some steric bulk in the 3'-position is compatible with anti-HIV activity

INTRODUCTION

Ever since the discovery of 3'-azido-3'-deoxythymidine (AZT)[1,2] as a highly active anti-HIV compound, much attention was paid to the synthesis of new 3'-substituted nucleosides[3]. Some of these compounds are very active against HIV replication e.g. 3'-fluoro-3'-deoxythymidine (FLT)[4], 2',3'-dideoxyinosine (DDI)[5], 2',3'-dideoxycytidine (DDC)[6] and didehydrodideoxythymidine (D4T)[7]. AZT, DDI, DDC and D4T have been approved for treatement of AIDS and AIDS-related complex (ARC). Unfortunately these drugs are not free of undesirable side effects. Moreover, the development of resistance limits the usefulness of these compounds[8]. As a result of intensive structure-activity relationship studies[3] the conclusion was made that steric bulk in the 3'-position is not compatible with anti-HIV activity. Most of the bulky substituents which were introduced in the 3'-position, however, are rather hydrophobic. Here we investigate the feasibility of having a hydrophilic small heterocyclic substituent on the nitrogen atom of 3'-amino-3'-deoxythymidine for anti-HIV activity.

In the past several small heterocycle groups were introduced in the 3'-position of 2'-deoxyribose nucleosides in order to mimic the three nitrogen system of AZT, but these compounds did not show appreciable activity against HIV[9,10,11]. One of the scarce series of antiviral molecules with a rather bulky group in 3'-position are the TSAOs[12].

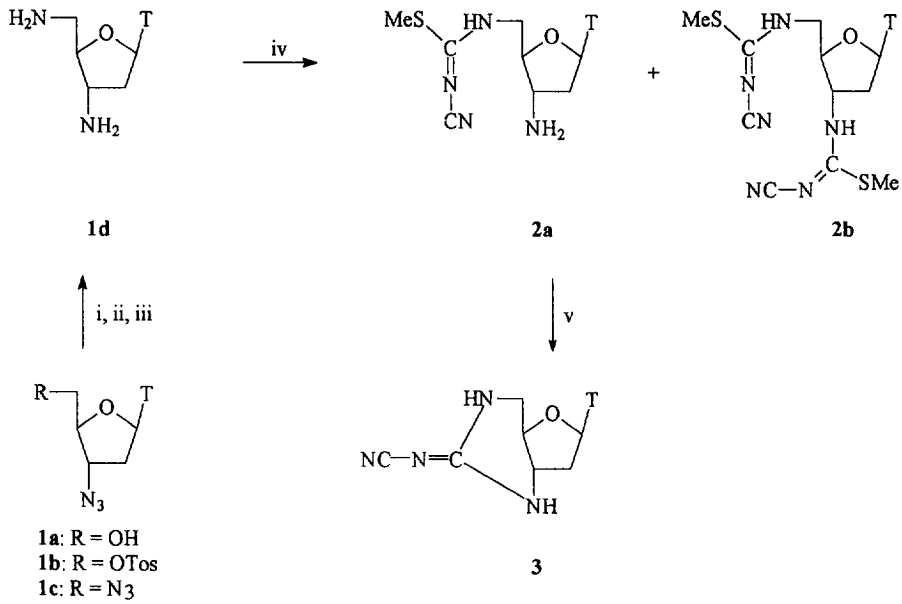
Three of the newly synthesized compounds exhibit moderate *in vitro* anti-HIV activity: 3'-(1,2,4-triazol-1-yl)carbimidoylamino-3'-deoxythymidine (**6**), 3'-(3-amino-1-methyl-1,2,4-triazol-5-yl)amino-3'-deoxythymidine (**8b**) and 3'-*N*-cyano-*O*-phenylisourea-3'-deoxythymidine (7)[13]. These data demonstrate that a certain degree of steric bulk in the 3'-position is compatible with anti-HIV activity.

CHEMISTRY

3'-Azido-3'-deoxythymidine (AZT) **1a** was used as starting material for the synthesis of all compounds. For the preparation of compounds **2a**, **2b** and **3**, the primary 5'-hydroxyl was tosylated **1b**, the tosyloxy was replaced by an azido function and the resulting 3',5'-diazido-3',5'-dideoxythymidine **1c** was reduced with hydrogen in the presence of palladium on charcoal according to the method described by Lin and Prusoff [14] yielding the diamino derivative **1d**.

Compound **1d** was reacted with 1.5 equivalents of *S,S*-dimethyl-*N*-cyanodithioimidocarbonate in ethanol at room temperature giving 1-cyano-3-(3'-amino-3',5'-dideoxythymidin-5'-yl)-2-methylisothiourea **2a** as the major compound and traces of the disubstituted compound **2b**.

Scheme 1



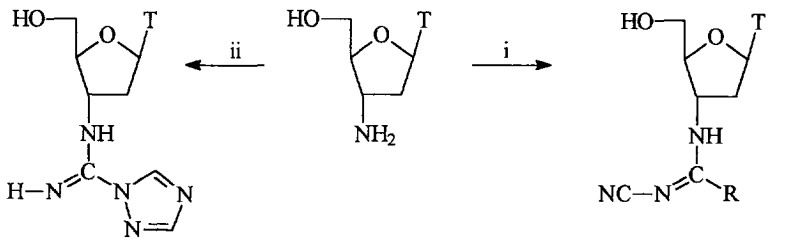
(i) 1.2 equivalents of TosCl in pyridine, RT; (ii) 3 equivalents of NaN_3 in DMF, 80°C; (iii) H2, 30 psi, Pd/ C, EtOH; (iv) 1.5 equivalents of $(SMe)_2C$=N-CN in EtOH, RT; (v) 1.2 equivalents of AgNO3, DMF:TEA (1:1),RT.

Using the method described for the formation of *N*-cyanoguanidines, an intramolecular cyclisation was performed leading to **3**. This reaction occurs *via* a carbodiimide intermediate which is formed by elimination of methylmercaptan by the action of silver nitrate[15]. The *N*-cyanoguanidine is not protonated at physiological *p*H because of the drop in pK_a caused by the presence of an electron-withdrawing cyano group.[16]

The 1-cyano-3-(3'-deoxythymidin-3'-yl)-2-methylisothiourea derivatives **5a** and **5b** were obtained by reaction of 3 equivalents of *S,S*-dimethyl-*N*-cyanodithioimidocarbonate with 3'-amino-3'-deoxythymidine **4** or its 5'-*O*-monomethoxytritylated congener, according to literature[17].

Compound **6**, 3'-(1,2,4-triazol-1-yl)carbimidoylamino-3'-deoxythymidine was obtained from the reaction of **4** with 1,1'-carbimidoyl-bis-(1,2,4-triazole)[18] in DMF (84%). Acid hydrolysis (50% aqueous acetic acid) of compound **6** resulted in the formation of 1-*H*-1,2,4-triazole and the ureum analogue **11** [13]

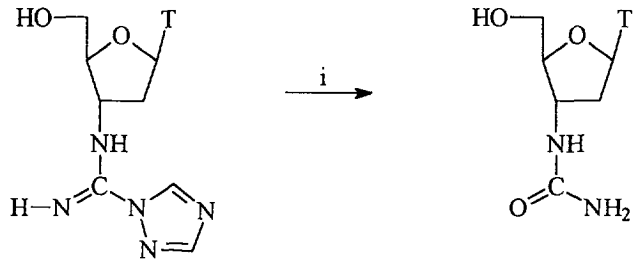
Scheme 2



6 **4** **5b**: R= SMe; **7** : R= OPh

(i) R_2C=NCN, EtOH ; (ii)1,1'-carbimidoyl-bis-(1,2,4-triazole), DMF

Scheme 3

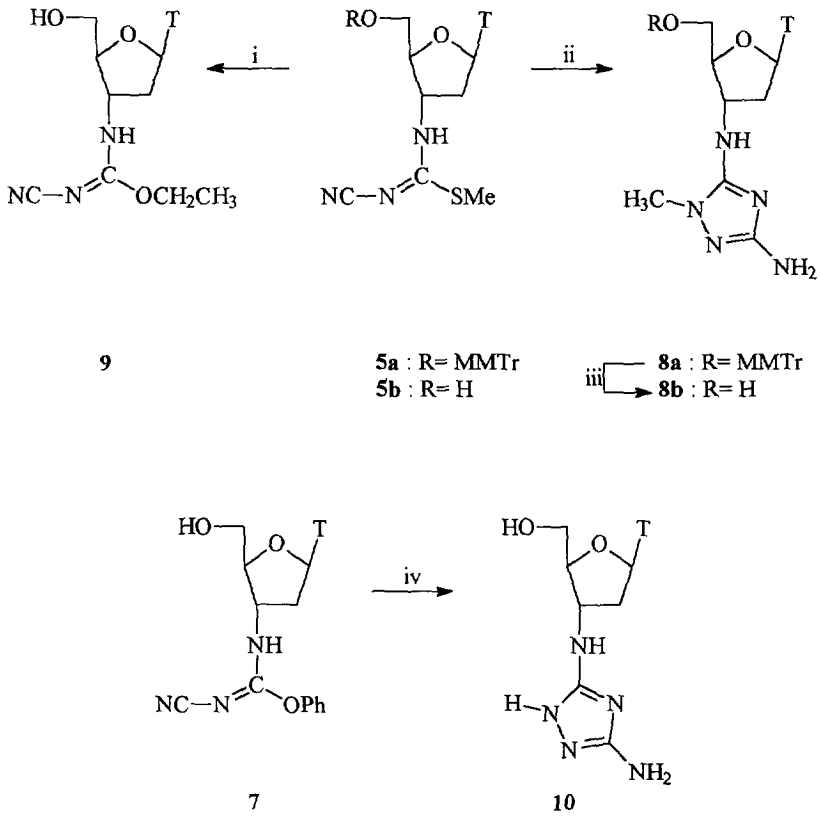


6 **11**

(i) 50% HOAc in water.

Compounds **5a** and **5b** were used as starting material for several cycloaddition reactions with different hydrazines.

Scheme 4



(i) EtOH, DBU (excess); (ii) Methyl hydrazine (excess), EtOH; (iii) 50% AcOH in water iv) hydrazine hydrate (excess), EtOH.

The cyclisation with methylhydrazine was performed with an excess of the hydrazine in ethanol. The reaction was repeated using the 5'-*O*-monomethoxytritylated analogue **5a** as starting material. The reaction is almost quantitative (94%). Routine mass and NMR analysis proved that the isolated compound is N^1-methyl positional isomer with respect to the methyl group in the triazole ring. From a HMQC-experiment (heteronuclear correlation through multiple quantum coherence) tuned for (two or three bonds) 7 Hz couplings, it was easily deduced that compound **8b** was the 1-methyl isomer. This follows from the clearly visible ^{3}J-interactions between triazole C-5 and H-3' and between C-5 and the 1-methyl protons. This reaction probably involves a preferential methylamino-attack on the carbodiimide intermediate, followed by an intramolecular reaction of the second amino function of the hydrazine on the cyano group and aromatisation of the triazoline.

The same reaction was performed with hydrazine and 3'-*N*-cyano-*O*-phenylisourea-3'-deoxythymidine **7** and afforded 89% of 3'-(3-amino-1,2,4-triazol-5-yl)amino-3'-deoxythymidine **10**. The compound with no substituent on the *N*-1 position of the triazole is very insoluble and precipitated from the reaction mixture. Its

NMR analysis was performed in CF_3COOD. The starting material **7** was obtained from **4** using three equivalents of diphenyl dicyanocarbonimidate in ethanol at room temperature.

When **5b** was dissolved in ethanol and an excess of DBU was added, compound **9** was formed (24%).

BIOLOGICAL ACTIVITY

Compounds **3**, **5**, **6**, **7**, **8b**, **9**, **10** and **11** were evaluated for their inhibitory effect on the cytopathogenicity of herpes simplex virus type 1 (HSV-1, strain KOS), thymidine kinase deficient (TK^-) HSV-1 (strains B 2006 and VMW 1837), herpes simplex virus type 2 (HSV-2, strain G), vaccinia virus (VV), vesicular stomatitis virus (VSV) in human embryonic skin muscle (ESM) fibroblast cell cultures according to previously published procedures.[19] No activity was observed against these viruses at concentrations up to 400 μg/mL. The above mentioned compounds were also evaluated for their inhibitory effect on the cytopathogenicity of HIV-1 and HIV-2 in CEM cells. Compound **6** was inhibitory to HIV-1 at a 50% effective concentration (EC_{50}) of 16 μg /mL and its EC_{50} for HIV-2 was >20 μg /mL. This concentration corresponded to the toxicity threshold of **6** [50% cytotoxic concentration (CC_{50}): 16 μg/ mL.] The compounds generated by hydrolysis of **6,** 1-*H*-1,2,4-triazole and compound **11,** were also evaluated to investigate whether or not the activity could be explained by an intracellular hydrolysis of the compound that would lead to the active product. However, no anti-HIV activity could be observed. Compound **6** might act as an alkylating agent as it is known that the 1-*H*-1,2,4-triazole is a very good leaving group especially after protonation of the *N*-1. Compound **7** was inhibitory to HIV-1 and HIV-2 at an EC_{50} of 25 and 35 μg/mL, respectively. Compound **8b** showed inhibition of HIV-1 and HIV-2 at 55 ± 7 and 65 ± 7 μg/mL, respectively. Neither compound **7** nor compound **8b** proved cytotoxic at 100 μg/mL.

Anti-HIV-1 and HIV-2 activity of the 3'-substituted thymidine analogues in CEM cells

compound	EC_{50} HIV-1 (III_b) ($\mu g.mL^{-1}$)	EC_{50} HIV-2 (ROD) ($\mu g.mL^{-1}$)	CC_{50} ($\mu g.mL^{-1}$)
3	>100	>100	>100
5	>100	>100	>100
6	16 ± 5.7	>20	16 ± 0.5
7	25 ± 7	35 ± 7	>100
8b	55 ± 7	65 ± 7	>100
10	>100	>100	>100
11	>100	>100	>100
1-*H*, 1,2,4-triazole	>100	>100	>100

CONCLUSION

Although this molecules (**6**, **7** and **8b**) display only moderate antiviral activity, they are interesting because their activity is delimited to the HIV-1 and HIV-2 virus. As there is no observed cytotoxicity for compound **7** and **8b**, it may be interesting to use this compounds as leads for further investigation in the field of structure-activity relation of 3'-modified nucleoside analogues.

EXPERIMENTAL SECTION

Melting points were determined with a Büchi-Tottoli apparatus and are uncorrected. Ultraviolet spectra were recorded with a Philips PU 8740 UV/Vis spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Gemini 200 spectrometer with tetramethylsilane as internal standard (s = singlet, d = doublet, t = triplet, m = multiplet, br = broad signal). The HMQC-experiments were performed on a Varian Unity 500 spectrometer.

Mass spectrometry measurements were obtained using a Kratos Concept 1H mass spectrometer. Precoated Machery-Nagel Alugram^{R} sil G/UV254 plates were used for TLC and the products were made visible with UV light and sulfuric acid-anisaldehyde spray. Column chromatography was performed on Janssen Chimica silica gel (0.060-200 nm).

Compounds **1a**[14], **1b**[14], **1c**[14], **1d**[14], **4**[2,14], **5a,b**[17] and **7**[13] were synthesized according to the procedure described in the litterature. The 1,1'-carbimidoyl-bis-(1,2,4-triazole) reagent was synthesized according to the procedure described by Becker and Eisenschmidt[18].

1-Cyan-3-(3'-amino-3',5'-dideoxythymidin-5'-yl)-2-methylisothiourea (2a) and 1-cyan-3-[1-cyan-3-(3',5'-dideoxythymidin-5'-yl)-2-methylisothiourea)-3'-yl]-2methylisothiourea (2b)

A solution of 360 mg (1.50mmol) of **1d** in 40mL of ethanol was added dropwise to a solution of 330 mg (2.26 mmol, 1.5 eq) of *S,S*-dimethyl-*N*-cyanodithioimidocarbonate in 20 mL of ethanol. The reaction was stirred at room temperature overnight. TLC evaluation (CH_2Cl_2: MeOH 85:15) revealed the formation of main compound **2a** and a less polar side product **2b**. The reaction mixture was evaporated to dryness on celite and chromatographically purified on silica gel, yielding 410 mg (1.21 mmol) of the title compound **2a** (81%). An analytical sample was obtained by crystallisation from methanol.

mp: 207°C

UV (MeOH): λ max= 251.5 nm (ε= 16,000)

Exact mass (thioglycerol) calculated for $C_{13}H_{19}N_6O_3S$ $[M+H]^+$ 339.1239, found 339.1248

^{13}C NMR (DMSO-d_6): δ 12.2 (CH_3); 14.2 ($S\underline{C}H_3$); 39.4 (C-2'); 45.3 (C-5'); 52.9 (C-3'); 83.6 and 83.7 (C-1' and C-4'); 109.7 (C-5); 115.9 (C≡N); 136.5 (C-6); 150.5 (C-2); 163.9 (C-4); 170.6 (C=N) ppm.

^{1}H NMR (DMSO-d_6): δ 1.81 (s, 3H, CH_3); 1.94-2.28 (m, 2H, H-2'); 2.59 (s, 3H, SCH_3); 3.34 (dd, ^{3}J= 6.7 Hz and 5.6 Hz, 1H, H-3'); 3.55-3.77 (m, 3H, H-4' and H-5'); 6.07 (dd, 1H, $^3J_{1',2'}$= 6.8 Hz and 6.9 Hz, H-1'); 7.45 (s, 1H, H-6) ppm.

The side product **2b** which eluted first was crystallised from methanol, yielding 60 mg (0.14 mmol, 9%)
mp: 191°C
UV (MeOH): λ_{max}= 227.3 nm (ε= 27,200); 250.2 nm (ε= 25,900)
Exact mass (thioglycerol) calculated for $C_{16}H_{21}N_8O_3S_2$ $[M+H]^+$ 437.1178, found 437.1170
^{13}C NMR (DMSO-d_6): δ 12.2 (CH_3); 14.2 and 14.4 (2xS<u>C</u>H$_3$); 35.2 (C-2'); 45.5 (C-5'); 54.5 (C-3'); 80.3 (C-1'); 84.2 (C-4'); 109.9 (C-5); 115.4 and 115.7 (2x C≡N); 136.8 (C-6); 150.5 (C-2); 163.5 (C-4);170.5 and 170.8 (2x C=N) ppm.
^{1}H NMR (DMSO-d_6): δ 1.81 (s, 3H, CH_3); 2.20-2.70 (m, 2H, H-2'); 2.57 (s, 3H, SCH_3); 2.64 (s, 3H, SCH_3); 3.57-3.63 (m, 2H, H-5'); 4.10 (dd, 1H, H-4'); 4.48-4.68 (m, 1H, H-3'); 6.19 (t, 1H, $^3J_{1',2'}$= 6.5 Hz, H-1'); 7.57 (s, 1H, H-6); 8.40-8.60 (br s, 2H, 2xNH) ppm.

3',5'-Diamino-3',5'-dideoxy-3'-*N*,5'-*N*-(*N*-cyanoiminocarbonyl)thymidine (3)

To a solution of 168 mg (0.5 mmol) of **2a** in 20 mL of a mixture DMF: TEA (1:1) which was protected from light, 125 mg (0.75 mmol, 1.5 eq) of silver nitrate was added. The reaction mixture was stirred at room temperature for 18 hours and evaporated to dryness on celite. Chromatographical purification on silica gel afforded 140 mg (0.48 mmol) of the title compound (96%). An analytical sample was obtained by crystallisation from methanol.
mp:>265°C
UV (MeOH): λ_{max} = 224.0 nm (ε= 21,600), 259.3 nm (ε= 10,200)
Exact mass (thioglycerol) calculated for $C_{12}H_{15}N_6O_3$ $[M+H]^+$ 291.1206, found 291.1235.
^{13}C NMR (DMSO-d_6): δ 12.1 (CH_3); 34.7 (C-2'); 43.6 (C-5'); 52.5 (C-3'); 74.6 (C-4'); 84.8 (C-1'); 110.4 (C-5); 118.0 (C≡N); 136.5 (C-6); 150.4 (C-2); 159.3 (C=N); 163.2 (C-4) ppm.
^{1}H NMR (DMSO-d_6): δ 1.80 (s, 3H, CH_3); 2.10-2.40 (m, 2H, H-2'); 3.35-3.80 (m, 4H, partly obscured by the HOD peak, H-3', H-4', H-5'); 6.31 (dd, $^3J_{1',2'A}$= 2.0 Hz, $^3J_{1',2'B}$= 8.2 Hz, H-1'); 7.42 (s, 1H, H-6); 7.65 (s, 1H, 5'-NH); 8.23 (s, 1H, 3'-NH); 11.35 (s, 1H, H-3) ppm.

3'-(1,2,4-triazol-1-yl)carbimidoylamino-3'-deoxythymidine (6)

To a solution of 1g (4.15 mmol) of **4** in 20 mL of DMF, 815 mg (5.0 mmol, 1.2 eq) of 1,1'-carbimidoyl-bis-(1,2,4-triazole) was added and the reaction mixture was stirred for one day at room temperature. The reaction was evaporated on celite and chromatographically purified yielding 1.13 g (3.37 mmol, 81%) of the title compound **6**.
An analytical sample was obtained by crystallisation from ethyl acetate.
mp: 167°C.
UV (MeOH): λ_{max} = 265.6 nm (ε= 11,300)

Exact mass (thioglycerol) calculated for $C_{13}H_{18}N_7O_4$ $[M+H]^+$ 336.1420, found 336.1409.
^{13}C NMR (DMSO-d_6): δ 12.5 (CH_3); 37.7 (C-2'); 53.9 (C-3'); 60.2 (C-5'); 84.0 (C-1'); 87.0 (C-4'); 108.7 (C-5); 136.8 (C-6); 142.3 (C-3"); 144.6 (C=N); 150.6 (C-2); 151.6 (C-5"); 164.0 (C-4) ppm.
^1H NMR (DMSO-d_6): δ 1.82 (s, 3H, CH_3); 1.95-2.14 (m, 1H, $^3J_{2'A,1'} = {}^3J_{2'A,3'}$ =6.9 Hz, $^2J_{2'A,2'B}$ =13.0 Hz, H-2'A); 2.35-2.60 (m, 1H, H-2'B); 3.50-3.63 (m, 1H, H-5'A); 3.63-3.86 (m, 2H, H-4' and H-5'B); 4.01 (dd, ^3J= 7.5 Hz and 6.9 Hz, 1H, H-3'); 5.18 (t, 1H, OH); 6.11 (dd, ^3J= 6.9 Hz and 4.0 Hz, 1H, H-1'); 7.02 (s, 2H, 2xNH); 7.86 (s, 1H, H-6); 8.18 (s, 1H, H-3"); 9.00 (s, 1H, H-5"); 11.27 (s, 1H, H-3) ppm

3'-(3-amino-1-methyl-1,2,4-triazol-5-yl)amino-3'-deoxythymidine (8b)

To a suspension of 1.00 g (1.63 mmol) of 1-cyano-3-(5'-monomethoxytrityl-3'-deoxythymidin-3'-yl)-2-methylisothiourea in 10 mL of ethanol 10 mL (187 mmol) of methylhydrazine were added. The reaction was stirred for 1 day at room temperature (TLC evaluation CH_2Cl_2-MeOH 90-10). The reaction mixture was evaporated to dryness and after chromatographical purification on a silica gel column the obtained product was deprotected by treatment of the sample with an 80% aqueous acetic acid solution for 5 minutes on a steam bath followed by extraction and evaporation of the aqueous layer 516 mg (1.53 mmol, 94 %) of **8b** was obtained. The obtained compound **8b** was crystallized from methanol.

Data for compound **8b**:
mp: 234°C (decomp)
Exact mass (thioglycerol) calculated for $C_{13}H_{20}N_7O_4$ $[M+H]^+$ 338.1577, found 338.1594.
UV (MeOH): λ_{max}:=267.2 nm (ε= 9,800)
^{13}C NMR (DMSO-d_6): δ 12.4 (CH_3 thym.); 32.6 (1"-CH_3); 37.7 (C-2'); 53.2 (C-3'); 61.3 (C-5'); 83.7 (C-1'); 85.3 (C-4'); 109.3 (C-5); 136.2 (C-6); 150.5 (C-2); 153.8 (C-3"); 160.1 (C-5"); 163.8 (C-4) ppm.
^1H NMR (DMSO-d_6): δ 1.78 (s, 3H, CH_3 thym.); 2.24 (m, 2H, H-2'); 3.30 (s, 3H, 1"-CH_3); 3.65 (m, 2H, H-5'); 3.84 (m, 1H, H-4'); 4.18 (m, 1H, H-3'); 4.80 (s, 2H, NH_2); 5.19 (s, 1H, OH); 6.18 (t, 1H, H-1'); 6.42 (d, 1H, 3'-NH); 7.80 (s, 1H, H-6); 11.25 (s, 1H, H-3) ppm.

1-Cyano-3-(3'-deoxythymidin-3'-yl)-2-ethylisourea (9)

To a solution of 540 mg (1.59 mmol) of 1-cyano-3-(3'-deoxythymidin-3'-yl)-2-methylisothiourea **5** in 20 mL of ethanol 4.5 mL of DBU were added and the solution was stirred at room temperature for 48 hours. The solvent was evaporated under vacuum, a 2N aqueous solution of HCl was added and the mixture was extracted three times with ethyl acetate. The combined organic layers were dried on anhydrous sodium sulphate and evaporated on celite. After chromatographical purification on silica gel 150 mg (0.45%, 28%) of the title compound were obtained. An analytical sample was prepared by crystallization from acetonitrile.
mp: 204°C.
UV (MeOH): λ_{max} = 217.1 nm (ε= 21,300); 265.2 nm (ε= 11,200)
MS: (thioglycerol) $[M+H]^+$ 338

^{13}C NMR (DMSO-d_6): δ 12.3 (CH_3); 14.1 ($\underline{C}H3$-CH2); 36.6 (C-2'); 51.8 (C-3'); 61.2 (C-5'); 65.3 (-$\underline{C}H_2$-CH_3); 83.5 (C-1'); 84.4 (C-4'); 109.6 (C-5); 115.1 (C≡N); 136.3 (C-6); 150.5 (C-2); 162.3 (C≡N); 163.8 (C-4) ppm.

^1H NMR (DMSO-d_6): δ 1.27 (t, 3H, $\underline{C}H_3$-CH_2); 1.79 (s, 3H, CH_3); 2.15-2.40 (m, 2H, H-2'); 3.45-3.75 (m, 2H, H-5'); 3.87-4.00 (m, 1H, H-4'); 4.10-4.40 (m, 3H, H-3' and O-CH_2-); 5.10 (t, 1H, OH); 6.26 (t, 1H, J= 6.4 Hz, H-1'); 7.75 (s, 1H, H-6); 8.65 (br, 1H, NH); 11.30 (s, 1H, H-3) ppm.

3'-Deoxy-3'-(3-amino-1,2,4-triazol-5-yl)aminothymidine (10)

To a solution of 192 mg (0.5 mmol) of 7[12] in ethanol, 2 mL of 85 % aqueous hydrazine hydrate was added and the reaction mixture was stirred overnight at room temperature. A white precipitate was formed. The very insoluble product was isolated by filtration and washed with diethyl ether, yielding 144 mg of the title compound **10** (0.45 mmol 89%)

mp: 246°C (decomp)

UV (MeOH): $\lambda_{max:}$ = 267.2 nm (No ε value determined due to the insolubility in methanol)

Exact mass (glycerol-TFA) calculated for $C_{12}H_{17}N_7O_4$ $[M+H]^+$ 324.1420, found 324.1445.

^{13}C NMR (CF_3COOD): δ 12.8 (CH_3); 39.0 (C-2'); 54.2 (C-3'); 62.8 (C-5'); 86.0 (C-1'); 89.8 (C-4'); 115.0 (C-5); 141.8 (C-6); 152.1 and 152.5 (C-3" and C-5"); 153.9 (C-2); 169.3 (C-4) ppm.

^1H NMR (CF_3COOD): δ 1.80 (br s, 3H, CH_3); 2.30-2.80 (m, 2H, H-2'); 3.80-4.20 (m, 3H, H-4' and H-5'); 4.20-4.40 (m, 1H, H-3'); 5.80-6.20 (br m, 1H, H-1'); 7.50 (br s, 1H, H-6'); 11.21 (br s, NH's) ppm.

3'-Ureido-3'-deoxythymidine (11)

A solution of 142 mg (0.5 mmol) of compound **6** in 25 mL of an 80% aqueous solution of acetic acid was stirred at room temperature for 1 day (TLC evaluation CH_2Cl_2: MeOH 90: 10). The reaction mixture was evaporated to dryness on celite and chromatographically purified on silica gel. This afforded 135 mg (0.475 mmol, 95% yield) of the title compound. An analytical sample was obtained by crystallisation from methanol and was found to be identical to the compound described before[12].

mp: 224°C.

UV (MeOH): λ_{max} = 211.3 nm (ε= 9,500); 267.2 nm (ε= 10,300)

Exact mass (thioglycerol) calculated for $C_{11}H_{17}N_4O_5$ $[M+H]^+$ 285.1199, found 285.1204.

^{13}C NMR (DMSO-d_6): δ 12.4 (CH_3); 37.8 (C-2'); 49.6 (C-3'); 61.3 (C-5'); 83.4 (C-1'); 85.5 (C-4'); 109.4 (C-5); 136.3 (C-6); 150.5 (C-2); 158.5 (NH-$\underline{C}O$-NH_2); 163.9 (C-4) ppm.

^1H NMR (DMSO-d_6): δ 1.79 (s, 3H, CH_3); 2.06 (dt, 1H, $J_{2'A,1'}$= $J_{2'A,3'}$= 6.5 Hz, $J_{2'A,2'B}$= 13.4 Hz, H-2'A); 2.20 (dt, 1H, $J_{2'B,1'}$= $J_{2'B,3'}$= 6.5 Hz, $J_{2'B,2'A}$= 13.4, H-2'B; 3.60 (m, 2H, H-5'); 3.69 (m, 1H, H-4'); 4.13 (q, 1H, J= 6.5 Hz, H-3'); 5.07 (t, 1H, $J_{NH,3'}$= 7.1Hz, 5'OH); 5.54 (s, 2H, NH_2); 6.12 (t, 1H,J= 6.5 Hz, H-1'); 6.44 (d, 1H, 3'-NH); 7.75 (s, 1H, H-6); 11.28 (s, 1H, H-3) ppm.

Antiviral assay procedures

Assays for activity against herpes viruses were performed as described previously[19]. The origin of the viruses, herpes simplex virus type 1 (HSV-1) (strain KOS, F, and McIntyre), thymidine kinase deficient TK$^-$ HSV-1 (strain B 2006), herpes simplex virus type 2 (HSV-2) (strains G, 196 and Lyons), varicella-zoster virus (VZV, strains Oka and YS), TK$^-$ VZV (strains 07-1 and YS-R), vaccinia virus (VV), vesicular stomatitis virus (VSV) and cytomegalovirus (CMV, strains AD169 and Davis) has been described[20]. Cytotoxicity measurements were based on either microscopically detectable alteration of normal cell morphology or inhibition of cell growth. The antiviral activity and cytotoxicity assays were performed in human embryonic skin-muscle (E_6SM) or human embryonic lung (HEL) cells seeded in 96-well microtiter trays. Assays for activity against human immuno deficiency virus type 1 (HIV-1) (strain III_B) and type 2 (HIV-2) (strain ROD), were performed as described previously[21].

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