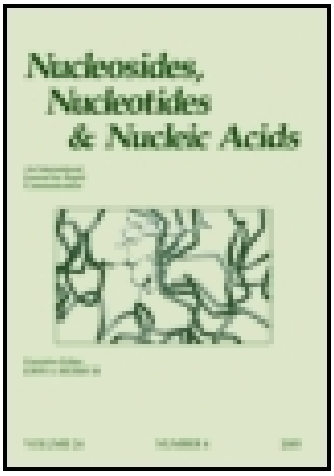

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8-AZA-1-DEAZAPURINE NUCLEOSIDES AS ANTIVIRAL AGENTS

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Abstract- 2',3'-Dideoxy-8-aza-1-deazaadenosine (**21**) and its α-anomer (**20**) were synthesized via glycosylation of 7-chloro-3*H*-1,2,3-triazolo[4,5-*b*]pyridine with 2,3-dideoxy-5-O-[(1,1)-dimethylethyl)diphenylsilyl]-D-*glycero*-pentofuranosyl chloride. The reaction gave a mixture of α- and β-anomers of N^3-, N^2- and N^1-glycosylated regioisomers (**12-15**). The α- and β-anomers of the N^4-glycosylated isomer **26** and **27** were also synthesized through the glycosylation of 8-aza-1-deazaadenine with 1-acetoxy-2,3-dideoxy-5-O-[(1,1-dimethylethyl)dimethylsilyl]-D-*glycero*-pentofuranose. These dideoxynucleosides and a series of previously synthesized 8-aza-1-deazapurine nucleosides were tested for activity against several DNA and RNA viruses, HIV-1 included. The α- and β-anomers of 7-chloro-3-(2-deoxy-D-*erythro*-pentofuranosyl)-3*H*-1,2,3-triazolo[4,5-*b*]pyridine (**3a** and **4**) showed activities against Sb-1 and Coxs viruses. The α- and β-anomers of 2',3'-dideoxy-8-aza-1-deazaadenosine (**20** and **21**) were found active as inhibitors of adenosine deaminase.

In vitro, 8-azapurine and 1-deazapurine nucleosides are endowed with a wide range of pharmacological activities. Following the discovery of the antibacterial and antitumor activity of 8-azaguanine, a variety of α and β anomers of 8-azaadenine nucleosides have been shown cytotoxic to tumor cells[1,2] or active as antiviral agents.[3]

Previous studies from our laboratory have pointed out the activity of 1-deazaadenosine as an inhibitor of adenosine deaminase (ADA)[4] and blood platelet aggregation,[5] as an adenosine receptor agonist,[6] and as an antitumor agent.[7]

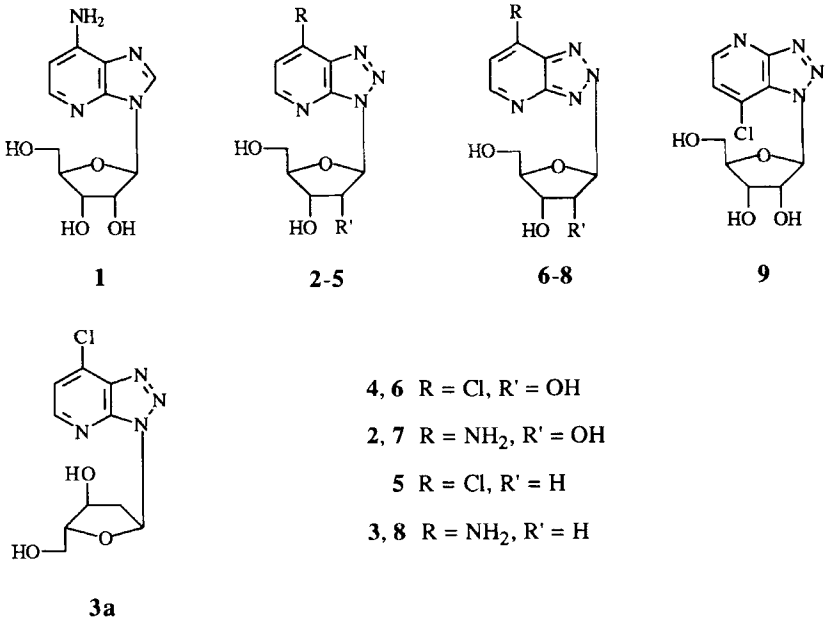

Fig. 1. Chemical structures of 1-deazaadenosine and 8-aza-1-deazapurine nucleosides

More recently, we have also synthesized α and β anomers of N^9-, N^8- and N^7-glycosylated isomers of 8-aza-1-deazapurines.[8] Among them, 8-aza-1-deazaadenosine and 2'-deoxy-8-aza-1-deazaadenosine turned out to be ADA inhibitors as potent as 1-deazaadenosine, but antitumor agents less potent than 8-aza or 1-deaza counterparts.

In this paper we present the results of the *in vitro* evaluation of the antiviral activity of the above 8-aza-1-deazapurine nucleosides (see structures in Fig. 1) and of newly synthesized 2',3'-dideoxy-8-aza-1-deazaadenosine and its N^3-glycosylated isomer.

CHEMISTRY

The synthesis of 2',3'-dideoxy-8-aza-1-deazaadenosine (**21**) was carried out according to Fig. 2. Glycosylation of the anion of 7-chloro-3*H*-1,2,3-triazolo-[4,5-*b*]pyridine (**10**)[9] with 2,3-dideoxy-5-*O*-[(1,1)-dimethylethyl)diphenylsilyl]-D-*glycero*-pentofuranosyl chloride (**11**)[10] in MeCN in the presence of powdered KOH and the cryptand tris[2(2-methoxyethoxy)-ethyl]amine (TDA-1),

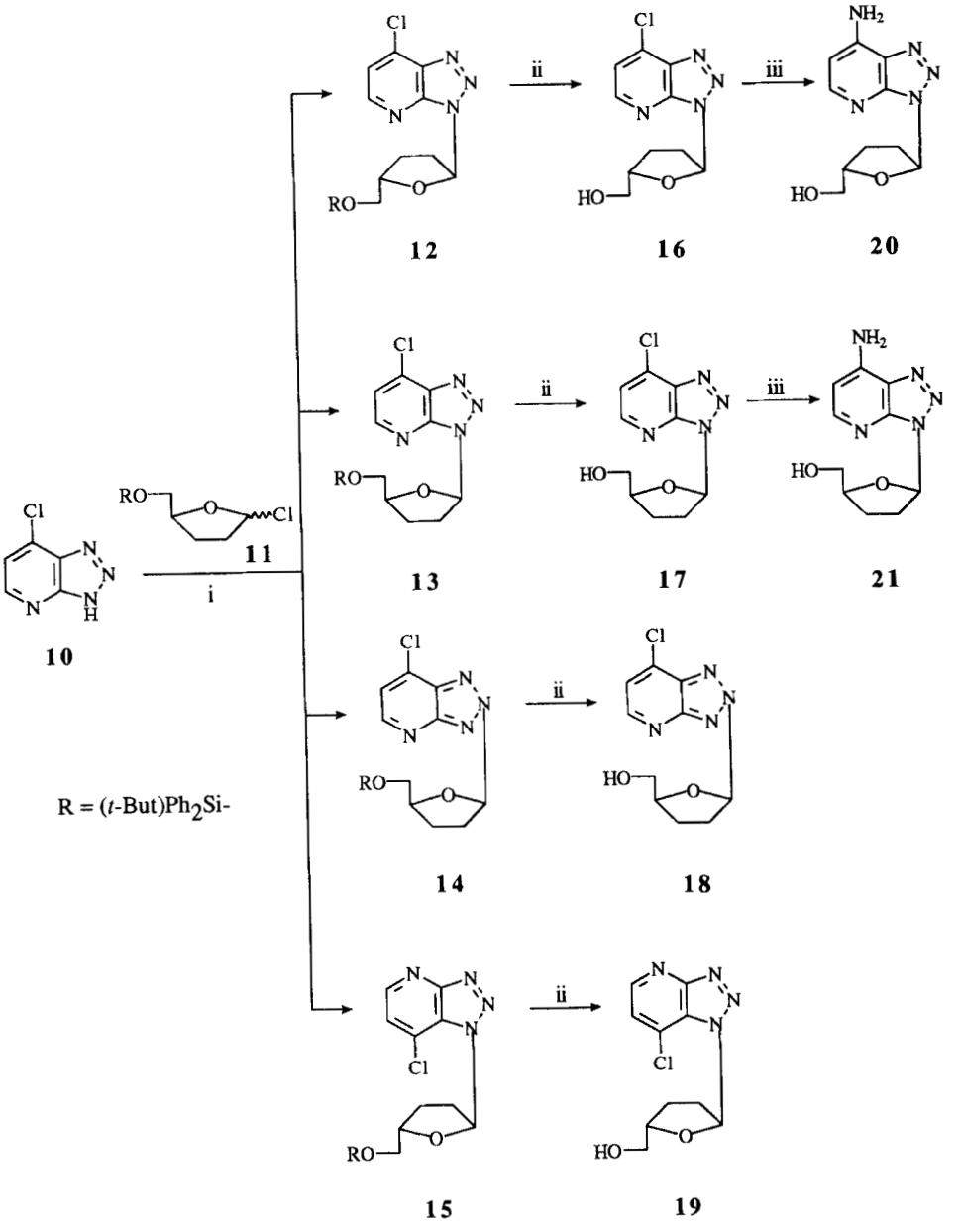

Fig. 2. Synthetic scheme for 2',3'-dideoxy-8-aza-1-deazaadenosine. (i) = KOH, TDA-1, MeCN; (ii) = Bu_4NF, THF; (iii) = NH_3 liq.

afforded a mixture of α- and β-D-anomers of N^3-, N^2-, and N^1-glycosylated regioisomers **12-15**, which were separated by chromatography on silica gel column.

Deblocking of silylated nucleosides with 1 M Bu_4NF in THF yielded the 2',3'-dideoxynucleosides **16-19**. Their structures and configurations have been established by ^{1}H-NOE difference experiments and ^{13}C-NMR spectroscopy.

In order to establish the position of glycosylation, ^{13}C-NMR and UV spectra of compounds **16** and **17** were measured. The ^{13}C-NMR spectra were almost identical indicating a pair of anomers. The UV absorption spectra of these compounds are similar and comparable to those reported for the corresponding ribo- and 2'-deoxyribo-derivatives **4** and **5**,[8] indicating that the position of glycosylation was N^3.

The ^{13}C-NMR of isomer **18** showed a downfield shift of C(3a) (9.4 ppm) as compared to that of **16** indicating that N^3 did not carry a substituent.[11] As C(7a) was unchanged, the glycosylation site of compound **18** was N^2. The structure was confirmed by the UV spectrum which was similar to that reported for 7-chloro-2-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2*H*-1,2,3-triazolo[4,5-*b*]pyridine.[8]

The ^{13}C-NMR of compound **19** showed that the chemical shift of C(7a) was downfield shifted and the signal of C(5) proved only slightly upfield shifted;[11] furthermore no NOE effect was observed on H-C(5) when H-C(1') was irradiated and the UV spectrum was similar to that reported for 7-chloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1*H*-1,2,3-triazolo[4,5-*b*]pyridine.[8] Therefore, N^1 glycosylation was established confirming the structure of **19**.

The assignment of the anomeric configuration of the deprotected 2',3'-dideoxynucleosides are based on NOE data (Table 1). The NOE of H-C(4') upon irradiation of H-C(1') can be used for the assignment of the β-D-configuration.[12]

In the case of **17**, a NOE of 1.0 % was observed, apart from a smaller NOE in the case of **16** (0.4 %), indicating that **17** was the β-anomer and **16** the α-anomer. The small NOE of the α-D-anomer **16** is due to the tree-spin effect.[13] In similar way, the β-configuration to compound **18** was assigned by the presence of NOE on H-C(4') (1.0 %), while the α-configuration of **19** was confirmed by the absence of NOE on H-C(4').

Nucleophilic displacement reaction with liquid ammonia of chloro nucleosides **16** and **17** afforded the α- and β-anomers of 2',3'-dideoxy-8-aza-1-deaza-adenosine (**20** and **21**).

Table 1. NOE Data % upon irradiation of H-C(1').[a]

	2',3'-dideoxyribofuranosides							
	16	**17**	**18**	**19**	**20**	**21**	**26**	**27**
Ha-C(2')	c	1.5	2.0	b	b	b	b	b
Hb-C(2')	b	c	c	b	1.6	c	c	1.3
H-C(4')	c	1.0	1.0	c		1.6	1.6	c
H-C(5)	c	1.5					5.6	7

[a] In DMSO-*d6*; [b] overlapping with DMSO; [c] no detectable intensity enhancement (< 0.5%)

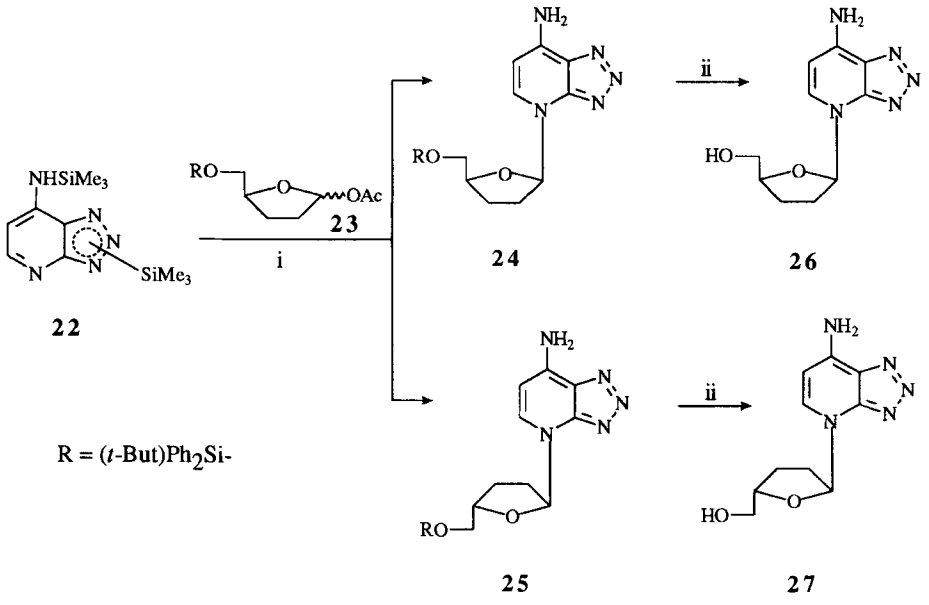

Fig. 3. Synthetic scheme for the synthesis of α and β anomers of 7-amino-4-(2,3-dideoxy-ribofuranosyl)-4*H*-triazolo[4,5-b]pyridine. (i) = $EtAlCl_2$, CH_2Cl_2; (ii) = Bu_4NF, THF.

As the precedent synthesis gave low yields of the desired compound, we decided to use the direct glycosylation of silylated 8-aza-1-deazaadenine[14] (**22**) with 1-acetoxy-2,3-dideoxy-5-*O*-[(1,1)-dimethylethyl)dimethylsilyl]-D-*glycero*-pentofuranose[15] (**23**) in CH_2Cl_2 in the presence of ethylaluminum dichloride (Fig. 3). The chromatographic separation of the reaction mixture gave two main fractions of impure isomeric dideoxynucleosides **24** and **25** which, by repeated chromatographies, were isolated as β- and α-anomers (1:1.5 ratio) of N^4-

glycosylated regioisomers. Structural assignment to two glycosylated products was made after desilylation with 1 M Bu_4NF in THF to yield the nucleosides **26** and **27**.

The ^{13}C-NMR spectra of these nucleosides were identical indicating a pair of anomers. Their configuration has been established by ^{1}H-NOE difference experiments. Only in the case of **26** a NOE was observed at H-C(4') (1.6%), upon irradiation of H-C(1'), attesting the β-D-configuration.[16] Because a NOE at H-C(5) was observed in both nucleosides **26** and **27** upon irradiation of H-C(1'), the glycosylation site should be at N^4.

The structure was confirmed by the ^{13}C-NMR spectra which showed that the chemical shift of C(5) was upfield shifted as compared to the corresponding signal of the N^3-glycosylated isomers **20** and **21**. So it was impossible to obtain the desired 2',3'-dideoxy-8-aza-1-deazaadenosine by this route.

BIOLOGICAL EVALUATION

Cytotoxicity and antiviral activity. Cytotoxicity and antiviral activity of the 8-aza-1-deazapurine nucleosides are shown in Table 2, together with those of the parent compounds 8-aza- and 1-deazaadenine nucleosides and of the reference drugs acyclovir and guanidine.

8-Azaadenosine (8-aza-A) and 1-deazaadenosine (1-deaza-A) (**1**) were the sole compounds cytotoxic to Vero cells at low concentrations. 8-Aza-A confirmed as an inhibitor of vaccinia (VV) and vesicular stomatitis virus (VSV) multiplication,[17] and 1-deaza-A resulted a potent, although not very selective inhibitor of both VV and african swine fever virus (ASFV). Neither 8-aza-dA nor 1-deaza-dA showed any antiviral activity. Among the 8-aza-1-deaza nucleosides, some 7-chloro-derivatives (**3a**, **4** and **6**) resulted active against polio (Sb-1) and coxsackie B1 (Coxs). The most potent was the α anomer of the 2'-deoxy nucleoside **3a**, which showed selectivity indices of 41 and 13 against Sb-1 and Coxs, respectively. The other 8-aza-1-deazapurine nucleosides were inactive.

The results of cytotoxicity and anti-HIV-1 activity of the test compounds are shown in Table 3; in this case 2',3'-dideoxyadenosine (ddA) was used as reference compound.

In MT-4 cells the most cytotoxic compounds were 8-aza-A, followed by 8-aza-dA and 1-deaza-A, which showed IC_{50}s in the range 0.3-6.4 μM. Among the 8-aza-1-deaza derivatives, **3a** was the most toxic compound (IC_{50} = 23 μM). When

Table 2. Cytotoxicity and antiviral activity of 8-aza-1-deaza-purine nucleosides in Vero cells.

Compd	$^{a}IC_{50}$ (μM)	$^{b}EC_{50}$					
	Vero	*HSV-1*	*VV*	*ASFV*	*Sb-1*	*Coxs*	*VSV*
8-aza-A	5.5	>5.5	1.0	>5.5	>5.5	>5.5	1.2
8-aza-dA	>396	>396	>396	>396	>396	>396	>396
1-deaza-A (**1**)	6	>6	1.5	3	>6	>6	>6
1-deaza-dA	>800	>800	>800	>800	>800	>800	>800
2	>740	>740	>740	>740	>740	>740	>740
3	>250	>250	>250	>250	>250	>250	>250
3 a	>370	>370	>370	>370	9	27	>370
4	>740	>740	>740	>740	140	50	>740
5	>250	>250	>250	>250	>250	>250	>250
6	>350	>350	>350	>350	140	50	>350
7	>370	>370	>370	>370	>370	>370	>370
8	>790	>790	>790	>790	>790	>790	>790
9	>700	>700	>700	>700	>700	>700	>700
acyclovir	>100	0.04	>10	>10	>10	>10	>10
guanidine	>500	>500	>500	>500	125	150	>500

[a] Compound dose required to reduce by 50% the number of viable mock-infected cells after three cell cycles. [b] Compound dose required to reduce the number of viral plaques by 50%. Plaque numbers in untreated cultures were: 125 (HSV-1), 110 (VV), 105 (ASFV), 120 (Sb-1), 115 (Coxs), 130 (VSV).

Table 3. Cytotoxicity and anti-HIV-1 effect of 8-aza-1-deaza-purine nucleosides in MT-4 cells.

Compd	[a]IC_{50} (μM) MT4	[b]EC_{50} (μM) HIV-1
8-aza-A	0.3	>0.3
8-aza-dA	1.2	>1.2
1-deaza-A (**1**)	6.4	>6.4
1-deaza-dA	>250	>250
2	64	>64
3	>250	>250
3a	23	>23
4	60	>60
5	174	>174
6	44	>44
7	67	>67
8	>250	>250
9	>250	>250
21	>425	>425
26	>425	>425
ddA	>500	10

[a] Compound dose required to reduce the viability of mock-infected cells by 50%.
[b] Compound dose required to achieve 50% protection of MT-4 cells against the HIV-1-induced cytopathogenicity.

evaluated for inhibition of the HIV-1-induced cytopathogenicity, none of the test compounds, 2',3'-dideoxy derivatives included, resulted effective. Under the same conditions, ddA showed an EC_{50} of 10 μM.

Overall, these results suggest that the substitution of nitrogen with a CH group at position 1 in 8-azaadenosine (or vice versa the substitution of a CH group with nitrogen at position 8 in 1-deazaadenosine) ablates both the cytotoxic and antiviral activities. On the other hand, the substitution of the NH_2 group with a chlorine atom at position 6 in 8-aza-1-deazaadenine nucleosides determines the appearance of a selective antiviral activity against polio and

coxsackie viruses. Finally, the substitution in ddA of both nitrogen with CH at position 1, and CH with nitrogen at position 8, is detrimental for anti-HIV activity.

Adenosine deaminase inhibitory activity. When 2',3'-dideoxy-nucleosides **20** and **21** were tested for inhibition of ADA, both were found inhibitory with K_i values of 1.1 x 10^{-5} M and 5.6 x 10^{-5} M respectively. Interestingly, the α-anomer was 5 times more potent than the β-anomer. Since we reported that 2'-deoxy-8-aza-1-deaza-A is a potent inhibitor of ADA (K_i = 1.9 x 10^{-7} M),[8] it can be concluded that the substitution of the hydroxy group with hydrogen at 3'-position in the 2'-deoxy-β-D-ribosyl moiety decreases of about 290 times the inhibitory potency, pointing out that the hydroxyl at 3'-position plays an important role in the interaction with the catalytic site of the enzyme.

EXPERIMENTAL SECTION

Melting points were determined on a Buchi apparatus and are uncorrected. Elemental analyses were determined on a Carlo Erba Model 1106 analyzer. Ultraviolet spectra were recorded with an HP 8452 A diode array spectrophotometer driven by an Olivetti M 24. Thin layer chromatography (TLC) was run on silica gel 60 F-254 plates (Merck); silica gel 60 (Merck) for column chromatography was used. Nuclear magnetic resonance ^1H and ^{13}C spectra were determined, respectively, at 300 and 75 MHz with a Varian VXR-300 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. All exchangeable protons were confirmed by addition of D_2O. Stationary NOE experiments were measured in (D_6)DMSO and were run on degassed solutions at 25°C. A presaturation delay of 1 sec was used, during which the decoupler low-power was set at 20 dB attenuation.

Glycosylation of 7-chloro-3*H*-1,2,3-triazolo[4,5-*b*]pyridine (10) with 2,3-dideoxy-5-O-[(1,1-dimethylethyl)diphenylsilyl]-D-*glycero*-pentofuranosyl chloride (11). To a stirred solution of **10**[9] (2 g, 12.8 mmol) in dry MeCN (120 mL) were added powdered KOH (1.9 g, 35.15 mmol) and TDA-1 (84 μl, 0.25 mmol). After 10 min the solution of **11**[10] (25.6 mmol corresponding to lactol) in THF was added portionwise (within 20 min). The mixture reaction was stirred at r.t. for 5 h, the insoluble material filtered and the filtrate poured into 5% aq. $NaHCO_3$ soln. (100 mL). The aqueous layer was extracted with EtOAc (3x100 mL) and the combined organic layer was washed with cold H_2O, dried and evaporated. From the residue chromatographed on silica gel column (cyclohexane-EtOAc 80:20) three fractions were separated.

7-Chloro-3-[2,3-dideoxy-5-O-[(dimethylethyl)diphenylsilyl]-α-D-*glycero*-pentofuranosyl]-3*H*-1,2,3-triazolo[4,5-*b*]pyridine (12). The

material isolated from third fraction was rechromatographed by flash column with cyclohexane-EtOAc (96:4) as eluent to give **12** as oil (660 mg, 11.5 %). TLC (cyclohexane-EtOAc 96:4): Rf 0.26. ^{1}H NMR (Me_2SO-d_6): δ 1.0 (s, t-Bu); 2.05, 2.52 (2m, 2H, H-3'); 2.63, 2.78 (2m, 2H, H-2'); 3.71 (m, 2H, H-5'); 4.48 (m, 1H, H-4'); 6.85 (dd, J = 2.9, 6.9 Hz, 1H, H-1'); 7.40, 7.65 (2m, arom. H); 7.72 (d, J = 5.0 Hz, 1H, H-6); 8.70 (d, J = 5.0 Hz, 1H, H-5). Anal. Calcd. for $C_{26}H_{29}ClN_4O_2Si$: C 63.33; H 5.93; N 11.36. Found; C 63.21; H 6.18; N 11.03.

7-Chloro-3-[2,3-dideoxy-5-O-[(dimethylethyl)diphenylsilyl]-β-D-*glycero*-pentofuranosyl]-3*H*-1,2,3-triazolo[4,5-*b*]pyridine (13). Rechromatography of fraction II by flash column with C_6H_6 gave **13**, as oil (710 mg, 12 %). TLC (C_6H_6): Rf 0.13. ^{1}H NMR (Me_2SO-d_6): δ 1.02 (s, t-Bu); 2.08 (m, 2H, H-3'); 2.45, 2.60 (2m, 2H, H-2'); 3.75 (m, 2H, H-5'); 4.60 (m, 1H, H-4'); 6.72 (dd, J = 2.5, 5.7 Hz, 1H, H-1'); 7.20-7.63 (m, arom. H); 7.73 (d, J = 4.6 Hz, 1H, H-6); 8.78 (d, J = 4.6 Hz, 1H, H-5). Anal. Calcd. for $C_{26}H_{29}ClN_4O_2Si$: C 63.33; H 5.93; N 11.36. Found: C 63.41; H 5.85; N 11.08.

7-Chloro-2-[2,3-dideoxy-5-O-[(dimethylethyl)diphenylsilyl]-β-D-*glycero*-pentofuranosyl]-2*H*-1,2,3-triazolo[4,5-*b*]pyridine (14). From the same flash chromatography of fraction II, **14** was obtained as oil (240 mg, 4 %). TLC (C_6H_6): Rf 0.07. ^{1}H NMR (Me_2SO-d_6): δ 0.95 (s, t-Bu); 2.20 (m, 2H, H-3'); 2.58, 2.70 (2m, 2H, H-2'); 3.78 (m, 2H, H-5'); 4.65 (m, 1H, H-4'); 6.65 (d, J = 5.8 Hz, 1H, H-1'); 7.15-7.45 (3m, arom. H); 7.68 (d, J = 4.7 Hz, 1H, H-6); 8.77 (d, J = 4.7 Hz, 1H, H-5). Anal. Calcd. for $C_{26}H_{29}ClN_4O_2Si$: C 63.33; H 5.93; N 11.36. Found: C 63.49; H. 6.05; N 11.55.

7-Chloro-1-(2,3-dideoxy-5-O-[(dimethylethyl)diphenylsilyl]-α-D-*glycero*-pentofuranosyl]-1*H*-1,2,3-triazolo[4,5-*b*]pyridine (15). By flash chromatography of fraction III with C_6H_6-EtOAc (99.5:0.5), compound **15** was separated as oil (94 mg, 1.6 %). TLC (C_6H_6-EtOAc 99:1): Rf 0.07. ^{1}H NMR (Me_2SO-d_6): δ 0.90 (s, t-Bu); 2.12, 2.60 (2m, 2H, H-3'); 3.12, 3.38 (2m, 2H, H-2'); 3.65 (m, 2H, H-5'); 4.40 (m, 1H, H-4'); 6.95 (d, J = 6.7 Hz, 1H, H-1'); 7.20-7.63 (m, arom. H); 7.83 (d, J = 4.9 Hz, 1H, H-6); 8.65 (d, J = 4.9 Hz, 1H, H-5). Anal. Calcd. for $C_{26}H_{29}ClN_4O_2Si$: C 63.33; H 5.93; N 11.36. Found: C 63.21; H 6.18; N 11.10.

7-Chloro-3-(2,3-dideoxy-α-D-*glycero*-pentofuranosyl)-3*H*-1,2,3-triazolo[4,5-*b*]pyridine (16). The protected **12** (600 mg, 1.21 mmol) dissolved in THF, was stirred with Bu_4NF 1.1 M in THF (17 mL) at r. t. for 30 min. The mixture was evaporated, and the residue was chromatographed on a silica gel column eluting with $CHCl_3$-MeOH (99:1); rechromatography with EtOAc-C_6H_6 (8:2) yielded **16** as oil (145 mg, 47 %). TLC ($CHCl_3$-MeOH 99:1): Rf 0.22. UV (NaOH

0.1 N): λ_{max} 256 nm (ε 4600), 284 (ε 2600). ^{1}H NMR (Me_2SO-d_6): δ 1.90, 2.45 (2m, 2H, H-3'); 2.65, 2.77 (2m, 2H, H-2'); 3.48 (m, 2H, H-5'); 4.38 (m, 1H, H-4'); 4.82 (t, J = 5.8 Hz, 1H, OH-5'); 6.85 (dd, J = 3.2, 6.7 Hz, 1H, H-1'); 7.77 (d, J = 4.9 Hz, 1H, H-6); 8.78 (d, J = 4.9 Hz, 1H, H-5). ^{13}C NMR (Me_2SO-d_6): 151.8 (C 5); 146.2 (C 3a); 134.8 (C 7); 134.6 (C 7a); 120.8 (C 6); 87.4 (C 1'); 82.2 (C 4'); 63.4 (C 5'); 30.6 (C 2'); 26.8 (C 3'). Anal. Calcd. for $C_{10}H_{11}ClN_4O_2$: C 54.79; H 5.06; N 25.56. Found: C 54.66; H 5.22; N 25.70.

7-Chloro-3-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl]-3*H*-1,2,3-triazolo[4,5-*b*]pyridine (**17**). This compound was obtained from **13** (700 mg, 1.42 mmol) as described for **16**. The reaction mixture was purified by flash chromatography with $CHCl_3$-MeOH (90:10) and then by silica gel column using $CHCl_3$-MeOH (99:1) as eluent, to give **17** as oil (220 mg, 61 %). TLC ($CHCl_3$-MeOH 99:1): Rf 0.34. UV (NaOH 0.1 N): λ_{max} 282 nm (ε 9500). ^{1}H NMR (Me_2SO-d_6): δ 1.95, 2.38 (2m, 2H, H-3'); 2.58 (m, 2H, H-2'); 3.52 (m, 2H, H-5'); 4.50 (m, 1H, H-4'); 4.88 (t, J = 5.6 Hz, 1H, OH-5'); 6.70 (dd, J = 3.0, 11.6 Hz, 1H, H-1'); 7.73 (d, J = 4.8 Hz, 1H, H-6); 8.78 (d, J = 4.8 Hz, 1H, H-5). ^{13}C NMR (Me_2SO-d_6): 151.9 (C 5); 146.6 (C 3a); 135.2 (C 7 and C 7a); 121.0 (C 6); 86.8 (C 1'); 83.5 (C 4'); 64.0 (C 5'); 31.3 (C 2'); 27.4 (C 3'). Anal. Calcd. for $C_{10}H_{11}ClN_4O_2$: C 54.79; H 5.06; N 25.56. Found: C 54.58; H 5.13; N 25.48.

7-Chloro-2-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl]-2*H*-1,2,3-triazolo[4,5-*b*]pyridine (18). Compound **18** was prepared as described for **17** starting from **14** (230 mg, 0.46 mmol) and purified by flash chromatography with $CHCl_3$-C_6H_{12} (85:15) to give an oil (70 mg, 60 %). TLC ($CHCl_3$-C_6H_{12} 85:15): Rf 0.15. UV (NaOH 0.1 N): λ_{max} 282 nm (ε 10500). ^{1}H NMR (Me_2SO-d_6): δ 2.20 (m, 2H, H-3'); 2.52, 2.65 (2m, 2H, H-2'); 3.57 (m, 2H, H-5'); 4.60 (m, 1H, H-4'); 4.80 (t, J = 5.7 Hz, 1H, OH-5'); 6.70 (dd, J = 2.4, 5.6 Hz, 1H, H-1'); 7.75 (d, J = 4.7 Hz, 1H, H-6); 8.81 (d, J = 4.7 Hz, 1H, H-5). ^{13}C NMR (Me_2SO-d_6): 155.9 (C 3a); 152.9 (C 5); 134.8 (C 7a); 133.8 (C 7); 122.7 (C 6); 95.6 (C 1'); 84.4 (C 4'); 64.0 (C 5'); 32.6 (C 2'); 26.6 (C 3'). Anal. Calcd. for $C_{10}H_{11}ClN_4O_2$: C 54.79; H 5.06; N 25.56. Found: C 54.68; H 5.00; N 25.67.

7-Chloro-1-(2,3-dideoxy-α-D-*glycero*-pentofuranosyl]-1*H*-1,2,3-triazolo[4,5-*b*]pyridine (19). Deprotection of **15** (90 mg, 0.18 mmol) yielded, after chromatographic separation on silica gel column with $CHCl_3$-MeOH (99:1), compound **19** as white solid (40 mg, 87 %); m.p. 114-115 °C. TLC ($CHCl_3$-MeOH 99:1): Rf 0.19. UV (NaOH 0.1 N): λ_{max} 264 nm (ε 12100). ^{1}H NMR (Me_2SO-d_6): δ 2.20 (m, 2H, H-3'); 2.62, 3.05 (2m, 2H, H-2'); 3.27 (m, 2H, H-5'); 4.37 (m, 1H, H-4'); 4.65 (t, J = 5.6 Hz, 1H, OH-5'); 6.98 (dd, J = 2.5, 7.0 Hz, 1H, H-1'); 7.82 (d, J = 4.9

Hz, 1H, H-6); 8.70 (d, J = 4.9 Hz, 1H, H-5). ^{13}C NMR (Me_2SO-d_6): 158.2 (C 3a); 149.7 (C 5), 127.6 (C 7); 123.8 (C 6); 123.2 (C 7a); 88.6 (C 1'); 83.5 (C 4'); 63.6 (C 5'); 31.1 (C 2'); 27.1 (C 3'). Anal. Calcd. for $C_{10}H_{11}ClN_4O_2$: C 54.79; H 5.06; N 25.56. Found: C 54.70; H 5.11; N 25.45.

7-Amino-3-(2,3-dideoxy-α-D-*glycero*-pentofuranosyl]-3*H*-1,2,3-triazolo[4,5-*b*]pyridine (20). To compound **16** (130 mg, 0.4 mmol) liquid ammonia (20 mL) was added, and the mixture was heated in a steel reaction vessel at 70 °C for 27 h. The excess ammonia was removed and the residue oil was cromatographed on a silica gel column with $CHCl_3$-MeOH (90:10) to give **20** as white solid (30 mg, 25 %); m.p. 170-172 °C. TLC ($CHCl_3$-MeOH 90:10): Rf 0.35. UV (MeOH): λ_{max} 258 nm (ε 4100); 266 (ε 3900); 306 (ε 7200); (NaOH 0.1 N): 256 (ε 8300); 304 (ε 6500). ^1H NMR (Me_2SO-d_6): δ 1.97, 2.40 (2m, 2H, H-3'); 2.60, 2.75 (2m, 2H, H-2'); 3.48 (m, 2H, H-5'); 4.32 (m, 1H, H-4'); 4.80 (t, J = 5.7 Hz, 1H, OH-5'); 6.42 (d, J = 5.5 Hz, 1H, H-6); 6.68 (dd, J = 3.7, 7.0 Hz, 1H, H-1'); 7.32 (bs, 2H, NH_2); 8.10 (d, J = 5.5 Hz, 1H, H-5). ^{13}C NMR (Me_2SO-d_6): 150.8 (C 5); 147.6 (C 3a); 146.7 (C 7); 125.7 (C 7a); 101.9 (C 6); 86.0 (C 1'); 81.6 (C 4'); 63.4 (C 5'); 30.2 (C 2'); 26.9 (C 3'). Anal. Calcd. for $C_{10}H_{13}N_5O_2$: C 51.06; H 5.57; N 29.77. Found: C 50.98; H 5.61; N 29.65.

7-Amino-3-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl]-3*H*-1,2,3-triazolo[4,5-*b*]pyridine (21). This compound was obtained from **17** (210 mg, 0.82 mmol) as described for **20**, but at 75 °C for 14 h. The excess ammonia was removed and the residue oil was chromatographed on a silica gel column with $CHCl_3$-MeOH, (90:10) to give **21** (65 mg, 34 %) as oil. TLC (silica gel, $CHCl_3$-MeOH 97:3): Rf 0.47. UV (MeOH): λ_{max} 266 (ε 4100); 304 (ε 7000); (NaOH 0.1 N): 264 (ε 5000); 304 (ε 7100). ^1H NMR (Me_2SO-d_6): δ 2.25 (m, 2H, H-3'); 2.55, 2.70 (2m, 2H, H-2'); 3.50 (m, 2H, H-5'); 4.22 (m, 1H, H-4'); 4.95 (t, J = 5.6 Hz, 1H, OH-5'); 6.40 (d, J = 5.5 Hz, 1H, H-6); 6.60 (dd, J = 3.5, 7.0 Hz, 1H, H-1'); 7.30 (bs, 2H, NH_2); 8.06 (d, J = 5.5 Hz, 1H, H-5). ^{13}C NMR (Me_2SO-d_6): 150.4 (C 5); 147.8 (C 3a); 145.7 (C 7); 127.6 (C 7a); 101.9 (C 6); 86.0 (C 1'); 82.5 (C 4'); 64.2 (C 5'); 30.8 (C 2'); 27.4 (C 3'). Anal. Calcd. for $C_{10}H_{13}N_5O_2$: C 51.06; H 5.57; N 29.77. Found: C 51.12; H 5.49; N 29.85.

Glycosylation of silylated 7-amino-3*H*-1,2,3-triazolo[4,5-*b*]pyridine (22) with 1-acetoxy-2,3-dideoxy-5-O-[(1,1-dimethylethyl)dimethylsilyl]-D-*glycero*-pentofuranose (23). To silylated amino derivative **22** [prepared by reaction of 7-amino-3*H*-1,2,3-triazolo[4,5-b]pyridine (1.3 g, 9.6 mmol) with hexamethyldisilazane and NH_4SO_4, at reflux for 1 h], was added portionwise a solution of **23** (2.3 g, 8.4 mmol) in anhydrous CH_2Cl_2 (25

mL) and then a 1.8 M solution of $EtAlCl_2$ in toluene (4.7 mL). After 1.5 h at room temperature, the reaction mixture was poured into an ice-cold mixture of CH_2Cl_2 and saturated $NaHCO_3$ solution, stirred for 10 min. and filtered through a Celite pad. The organic layer was washed with saturated $NaHCO_3$, brine and then dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel column with $CHCl_3$-MeOH (96:4) yielding two fractions.

7-Amino-4-[2,3-dideoxy-5-O-[(dimethylethyl)dimethylsilyl]-β-D-*glycero*-pentofuranosyl]-4*H*-1,2,3-triazolo[4,5-*b*]pyridine (24). From the first fraction, **24** was obtained as white solid (430 mg, 15 %); m.p. 146-148 °C. TLC ($CHCl_3$-MeOH 96:4): Rf 0.42. ^1H NMR (Me_2SO-d_6): δ 0.10, 0.93 (2s, t-Bu); 1.95 (m, 2H, H-3'); 2.23, 2.61 (2m, 2H, H-2'); 3.85, 4.05 (2m, 2H, H-5'); 4.31 (m, 1H, H-4'); 6.32 (d, J = 7.1 Hz, 1H, H-6); 6.47 (dd J = 2.1, 6.7 Hz, 1H, H-1'); 8.18, 8.40 (2s, 2H, NH_2); 8.30 (d, J = 7.1 Hz, 1H, H-5). Anal. Calcd. for $C_{26}H_{31}N_5O_2Si$: C 65.93; H 6.60; N 14.79. Found: C 65.87; H 6.68; N 14.92.

7-Amino-4-[2,3-dideoxy-5-O-[(dimethylethyl)dimethylsilyl]-α-D-*glycero*-pentofuranosyl]-4*H*-1,2,3-triazolo[4,5-*b*]pyridine (25). From the second fraction, **25** was obtained as yellow solid (460 mg, 16 %); m.p. 140-143 °C. TLC ($CHCl_3$-MeOH 96:4): Rf 0.38. ^1H NMR (Me_2SO-d_6): δ 0.12, 0.91 (2s, t-Bu); 1.95, 2.15 (m, 2H, H-3'); 2.32, 2.60 (2m, 2H, H-2'); 3.70 (m, 2H, H-5'); 4.71 (m, 1H, H-4'); 6.35 (d, J = 7.1 Hz, 1H, H-6); 6.31 (dd J = 2.3, 7.0 Hz, 1H, H-1'); 8.05 (d, J = 7.1 Hz, 1H, H-5); 8.20, 8.42 (2s, 2H, NH_2). Anal. Calcd. for $C_{26}H_{31}N_5O_2Si$: C 65.93; H 6.60; N 14.79. Found: C 65.73; H 6.78; N 14.80.

7-Amino-4-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl]-4*H*-1,2,3-triazolo[4,5-*b*]pyridine (26). Compound **26** was prepared from **24** (400 mg, 1.14 mmol) as described for **16**. After chromatography on silica gel eluting with CH_2Cl_2-MeOH (92:8), **26** was obtained as white solid (180 mg, 67 %); m.p. 160-163 °C. TLC (CH_2Cl_2-MeOH 98:2): Rf 0.25. UV (MeOH): λ_{max} 270 nm (ε 8500), 306 nm (ε 16500); (NaOH 0.1 N): 266 nm (ε 8100), 306 nm (ε 17200). ^1H NMR (Me_2SO-d_6): δ 2.01, 2.30 (2m, 2H, H-3'); 2.48, 3.20 (2m, 2H, H-2'); 3.65, 3.78 (2m, 2H, H-5'); 4.25 (m, 1H, H-4'); 5.47 (t, J = 5.5 Hz, 1H, OH-5'); 6.32 (d, J = 7.1 Hz, 1H, H-6); 6.40 (t, J = 3.2 Hz, 1H, H-1'); 8.20, 8.40 (2s, 2H, NH_2); 8.30 (d, J = 7.1 Hz, 1H, H-5). ^{13}C NMR (Me_2SO-d_6): 151.5 (C 7); 148.1 (C 3a); 134.5 (C 5); 128.2 (C 7a); 98.8 (C 6); 90.4 (C 1'); 82.9 (C 4'); 62.4 (C 5'); 32.8 (C 2'); 24.9 (C 3'). Anal. Calcd. for $C_{10}H_{13}N_5O_2$: C 51.06; H 5.57; N 29.77. Found: C 51.11; H 5.49; N 29.95.

7-Amino-4-(2,3-dideoxy-α-D-*glycero*-pentofuranosyl]-4*H*-1,2,3-triazolo[4,5-*b*]pyridine (27). Compound **27** was prepared from **25** (400 mg, 1.14 mmol) as described for **26**. The reaction mixture was purified by flash

chromatography with CH_2Cl_2-MeOH (92:8) and rechromatographed with CH_2Cl_2-MeOH-EtOAc 70:20:10) to give **27** as white solid (160 mg, 60 %); m.p. 65-70 °C. TLC (CH_2Cl_2-MeOH 98:2): Rf 0.21. UV (MeOH): λ_{max} 268 nm (ε 8100), 306 nm (ε 16100); (NaOH 0.1 N): 266 nm (ε 7300), 306 nm (ε 15700). ^1H NMR (Me_2SO-d_6): δ 1.90, 2.10 (2m, 2H, H-3'); 2.38, 2.55 (2m, 2H, H-2'); 3.50 (m, 2H, H-5'); 4.62 (m, 1H, H-4'); 4.90 (t, J = 5.7 Hz, 1H, OH-5'); 6.35 (d, J = 7.1 Hz, 1H, H-6); 6.45 (dd, J = 3.5, 6.2 Hz, 1H, H-1'); 8.00 (d, J = 7.1 Hz, 1H, H-5); 8.20, 8.40 (2s, 2H, NH_2).^{13}C NMR (Me_2SO-d_6): 151.1 (C 7); 147.8 (C 3a); 134.1 (C 5); 128.1 (C 7a); 98.7 (C 6); 90.9 (C 1'); 82.3 (C 4'); 63.3 (C 5'); 31.9 (C 2'); 25.6 (C 3'). Anal. Calcd. for $C_{10}H_{13}N_5O_2$: C 51.06; H 5.57; N 29.77. Found: C 51.22; H 5.69; N 29.65.

BIOLOGICAL DETERMINATION

Virology

Drugs

Test compounds were solubilized in DMSO at 100 mg/mL and then diluted in culture media.

Cells

The following cells were used: H9/IIIB cells, an H9 subline which is persistently infected with HIV-1; MT-4 and C8166, CD4+ T-cells; Vero, african green monkey kidney.

T-cell lines were grown in RPMI-1640 medium, supplemented with 10% fetal calf serum (FCS). Vero cells were grown in Dulbecco's modified minimal essential medium (MEM) supplemented with 10% new born calf serum (NCS). All cell culture media were also additioned of 100 U/mL penicillin G and 100 UI/mL streptomycin.

The cell lines were grown at 37 °C in a CO_2 incubator and were checked periodically for the absence of mycoplasma contamination with a MycoTect Kit (Gibco).

Viruses

Virus stocks of herpes simplex type 1 (HSV-1, ATCC VR 733), vaccinia (VV, ATCC VR 117), african swine fever (ASFV, Istituto Zooprofilattico of Sassari), coxsackie B1 (Coxs., ATCC VR 28), polio type 1 (Sabin strain, Sb-1), and vesicular stomatitis virus (VSV, ATCC VR 158) were obtained in Vero cells and had titres of $4x10^8$ plaque forming units (PFU)/mL, $5x10^5$ PFU/mL, 10^7PFU/mL, $2x10^8$ PFU/mL, $3x10^7$ PFU/mL, 10^8 PFU/mL, respectively. The human immuno-

deficiency virus type 1 (HIV-1, HTLV/IIIB strain) used in anti-HIV assays was obtained from supernatants of H9/IIIB cells. HIV-1 stocks were titrated in C8166 cells and stored at -80 °C until use. HIV-1 stocks had a titre of $2x10^6$ cell culture infective dose fifty ($CCID_{50}$)/mL.

Cytotoxicity assays

Cytotoxicity for Vero cells was carried out on cell monolayers seeded at a density of $1x10^5$ cells/mL in 24-multiwell plates and allowed to adhere overnight. Growth medium containing various concentrations of the compounds was then added. After a 4 day incubation at 37 °C, the number of viable cells was determined with a Coulter counter after trypsinization of the monolayers. Viability was determined by the trypan blue dye exclusion test. It should be noted that compound concentrations required to induce a 50% cytotoxic effect on confluent Vero monolayers were always considerably higher that those necessary to inhibit cell growth by 50%.

Cytotoxicity of test compounds for MT-4 cells was evaluated in parallel with their anti-HIV-1 activity and was based on viability of mock-infected cells as determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method.[18]

Antiviral assays

Antiviral assays against the RNA and DNA viruses were performed in Vero cells according to the Collins and Bauer[19] plaque reduction test.

Activity of the compounds against HIV-1 multiplication in acutely infected cells was based on the inhibition of virus-induced cytopathogenicity in MT-4 cells. Briefly, 50 µL of growth medium containing $1x10^4$ MT-4 cells were added to each well of flat-bottomed microtiter trays containing 50 µL of culture medium with or without various concentrations of test compounds. 20 µL of an HIV-1 suspension were then added, so as to obtain a multiplicity of infection of 0.01. After a 4-day incubation at 37 °C, the number of viable MT-4 cells was determined by the MTT method.

Enzyme assay. The method used for the determination of activity against adenosine deaminase has been described in a preceding paper.[20]

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