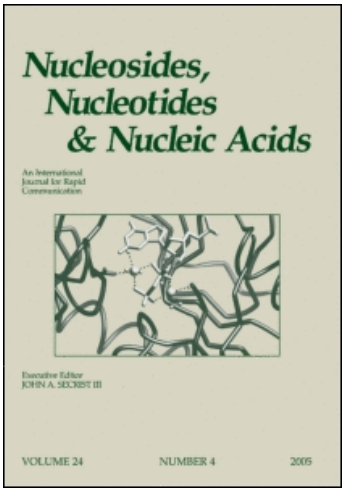

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8-Aza Analogues of Deaza Purine Nucleosides. Synthesis and Biological Evaluation of 8-Aza-1-deazaadenosine and 2'-Deoxy-8-aza-1-deazaadenosine

Palmarisa Franchetti[a]; Loredana Cappellacci[a]; Mario Grifantini[a]; Giulio Lupidi[b]; Giuseppe Nocentini[c]; Anna Barzi[c]
[a] Dipartimento di Scienze Chimiche, Università di Camerino, Camerino [b] Dipartimento di Biologia Cellulare, Università di Camerino, Perugia, Italy [c] Istituto di Farmacologia Medica, Università di Perugia, Perugia, Italy

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8-AZA ANALOGUES OF DEAZA PURINE NUCLEOSIDES. SYNTHESIS AND BIOLOGICAL EVALUATION OF 8-AZA-1-DEAZAADENOSINE AND 2'-DEOXY-8-AZA-1-DEAZAADENOSINE.

Palmarisa Franchetti,*+ Loredana Cappellacci,+ Mario Grifantini,+ Giulio Lupidi,° Giuseppe Nocentini§ and Anna Barzi§

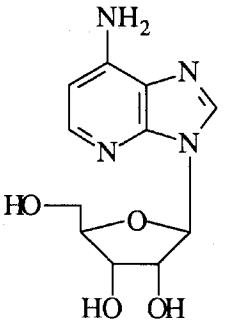
+*Dipartimento di Scienze Chimiche, Università di Camerino, 62032 Camerino,*
°*Dipartimento di Biologia Cellulare, Università di Camerino,*
§*Istituto di Farmacologia Medica, Università di Perugia, 06100 Perugia, Italy.*

Abstract-. The syntheses of 7-amino-3-(β-D-ribofuranosyl)-3*H*-1,2,3-triazolo[4,5-b]pyridine (8-aza-1-deazaadenosine) (**2**) and 7-amino-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3*H*-1,2,3-triazolo[4,5-b]pyridine (2'-deoxy-8-aza-1-deazaadenosine) (**3**) by glycosylation of the anion of 7-chloro-3*H*-1,2,3-triazolo[4,5-b]pyridine are described. The anomeric configuration as well as the position of glycosylation were determined by ^{1}H, ^{13}C NMR, UV and N.O.E. difference spectroscopy. The cytotoxicity of these nucleosides against several murine and human tumor cell lines is discussed. Compounds **2** and **3** proved to be good inhibitors of adenosine deaminase.

The 3*H*-1,2,3-triazolo[4,5-d]pyrimidine (8-azapurine) nucleosides exhibit extraordinary biochemical and pharmacological activities.[1] In many biological systems, they act as nucleoside antimetabolites. As they are isosteric to purine nucleosides, their incorporation into DNA or RNA fragment is of interest. The observance of *in vivo* activity for 8-azaadenosine, 8-azainosine and 8-azaguanine has served as the basis for the synthesis of a variety of congeners.[1] All three of these compounds are thought to be activated by conversion to the nucleotides of 8-azaadenosine or 8-azaguanosine, followed by incorporation into RNA. Furthermore it was observed that 8-aza-2'-deoxyadenosine has an antitumor activity slightly lower than that of 8-azaadenosine.[1]

8-Azaadenosine is rapidly deaminated to 8-azainosine by adenosine deaminase (ADA) and its cytotoxicity may be enhanced in several cell lines when these lines are pretreated with ADA inhibitors.[2]

We found that it is possible to prevent enzymatic deamination of adenosine derivative by substituting the nitrogen atom at 1 position of the purine moiety with CH group. 1-Deazaadenosine (**1**) proved to be not only resistent to ADA, but also able to inhibit the enzyme.[3] Furthermore 1-deazaadenosine showed *in vitro* antitumor activity against several human and murine cell lines.[4] These findings prompted us to synthesize and study the antitumor activity of 8-aza-analogues of 1-deazaadenosine (**2**) and 2'-deoxy-1-deazaadenosine (**3**) .



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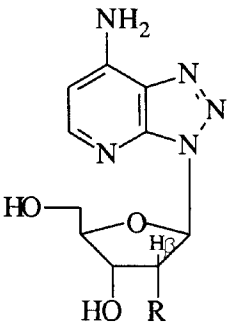
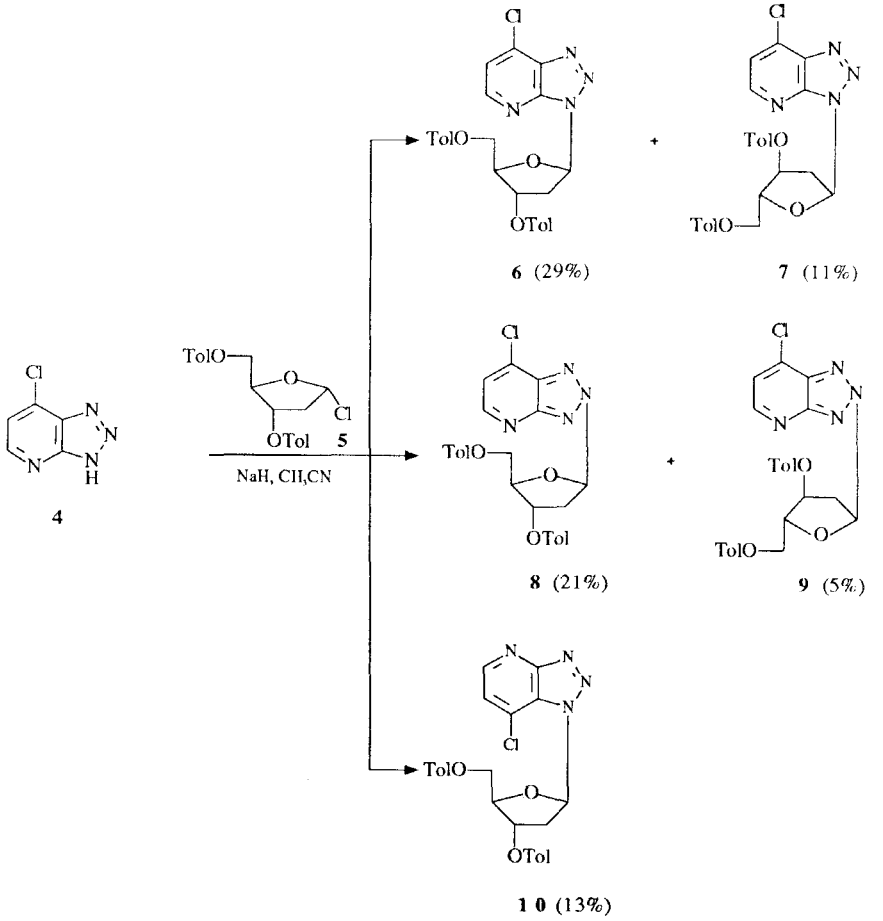


2: R = OH
3: R = H

CHEMISTRY

The synthesis of 7-amino-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3*H*-1,2,3-triazolo[4,5-b]pyridine (**3**) was carried out according to Schemes I-II. Glycosylation of 7-chloro-3*H*-1,2,3-triazolo[4,5-b]pyridine[5] (**4**) as sodium salt generated in situ by treatment of NaH in acetonitrile, with 2-deoxy-3,5-di-*O*-(p-toluoyl)-α-D-*erythro*-pentofuranosyl chloride[6] (**5**), afforded a mixture of N^3-, N^2 and N^1-glycosylated regioisomers (**6, 8** and **10**, respectively), together with the α-D-anomers **7** and **9**, which were separated by chromatographic column on silica gel (Scheme I). Structural assignment of all glycosylated products will be discussed below.

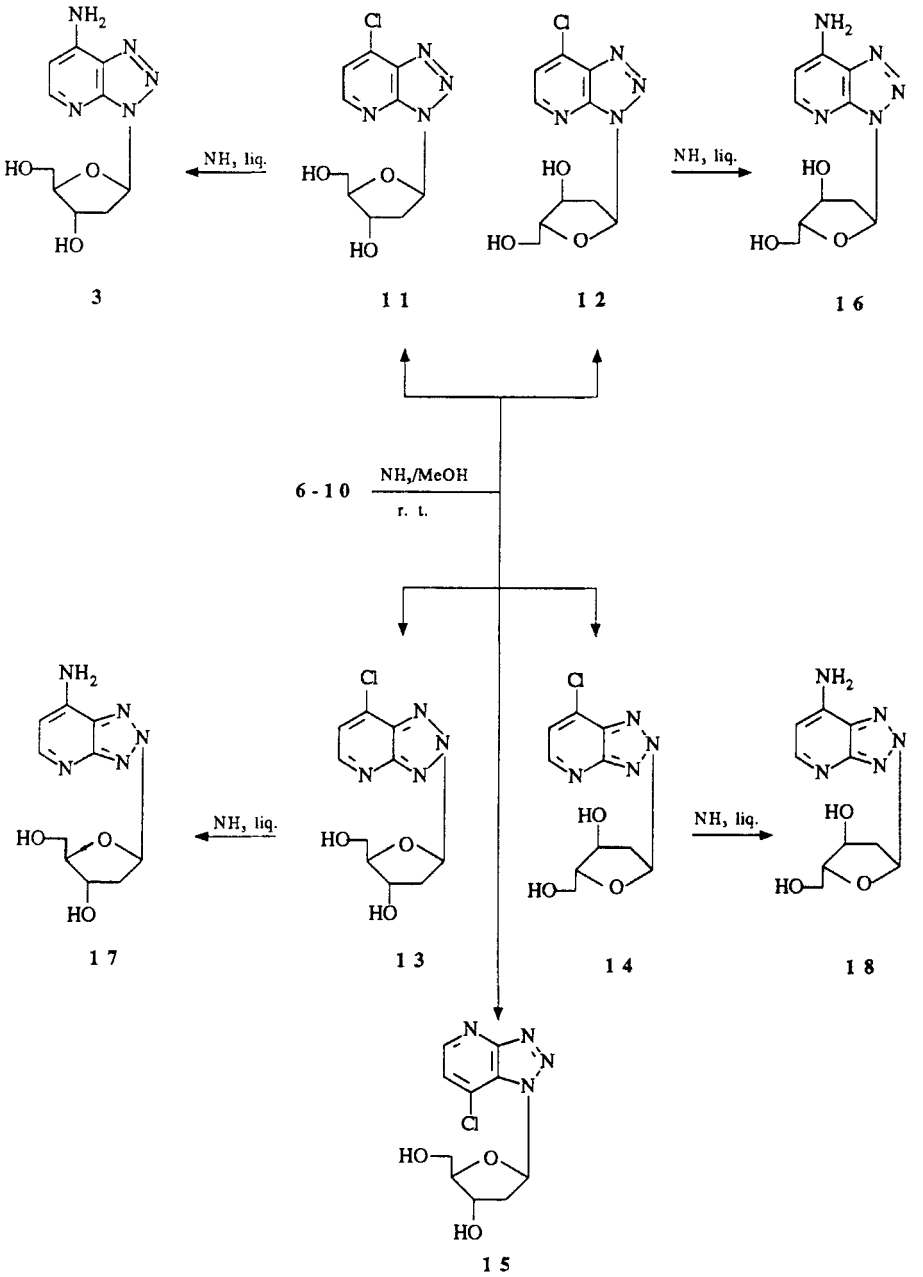
Deblocking of the five glycosylation products **6-10** with saturated NH_3/MeOH at room temperature, yielded the 2'-deoxy-nucleosides **11-15**.



SCHEME I

Their configuration has been established by ^{1}H-, ^{1}H N.O.E., and ^{13}C NMR difference spectroscopy. For this purpose, the H-(1') signal of the deprotected compounds was irradiated. The resulting N.O.E. effects on H_{α}-(2') and H-(4') indicated β-D-configuration for **11**, **13** and **15**, and the N.O.E. effects on H_{β}-(2') and H(3') confirmed α-D-configuration for **12** and **14** (Table I).[7]

Treatment of 7-chloro derivatives **11-14** with liquid ammonia at 80 °C, yielded 2'-deoxy-8-aza-1-deazaadenosine (**3**), 7-amino-2-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2*H*-1,2,3-triazolo[4,5-b]pyridine (**17**) and corresponding α-D-anomers **16** and **18**. Compound **15** could not be converted in amino-derivative owing to its instability under alkaline conditions.



SCHEME II

TABLE I. N.O.E. Data % upon Irradiation of H-(1') (DMSO-d_6, 25°C, 300 MHz)

	D-2'-deoxy-ribofuranosides								
	11	**12**	**13**	**14**	**15**	**3**	**16**	**17**	**18**
Hα-(2')	b		5.3		5.8	5.3		5.0	
Hβ-(2')		b	5.2		a	5.0		4.9	
H-(3')		a		a			0.7		0.6
H-(4')	0.8		a		1.4	0.7		a	

	D-ribofuranosides				
	23	**24**	**25**	**2**	**26**
H-(2')	1.5	2.06	2.1	1.9	2.0
H-(3')	a	0.54		a	a
H-(4')	1.0	0.8	1.0	1.5	1.6

a: no detectable intensity enhancement (< 0.5%)
b: overlapping with DMSO.

TABLE II. ^{13}C NMR Chemical Shifts of 1-Deaza-8-Azaadenine Derivatives

Compd	C(3a)	C(5)	C(6)	C(7)	C(7a)
2	147.6	150.1	101.8	146.3	127.8
3	146.3	150.2	101.8	147.7	127.9
11	146.1	151.5	120.6	134.8	134.9
12	146.0	151.4	120.5	134.8	135.0
13	155.5	152.9	122.5	133.4	134.7
14	155.5	152.6	122.3	133.3	134.6
15	158.2	148.8	123.6	127.2	123.2
16	145.8	149.9	101.8	147.9	128.0
17	153.9	150.4	100.7	148.4	128.9
18	155.2	151.7	100.7	147.6	129.1
23	146.4	151.7	120.8	134.9	135.0
24	155.6	153.0	122.6	133.4	134.8
25	158.0	149.0	123.8	126.9	128.5
26	155.0	151.6	100.6	147.6	129.2

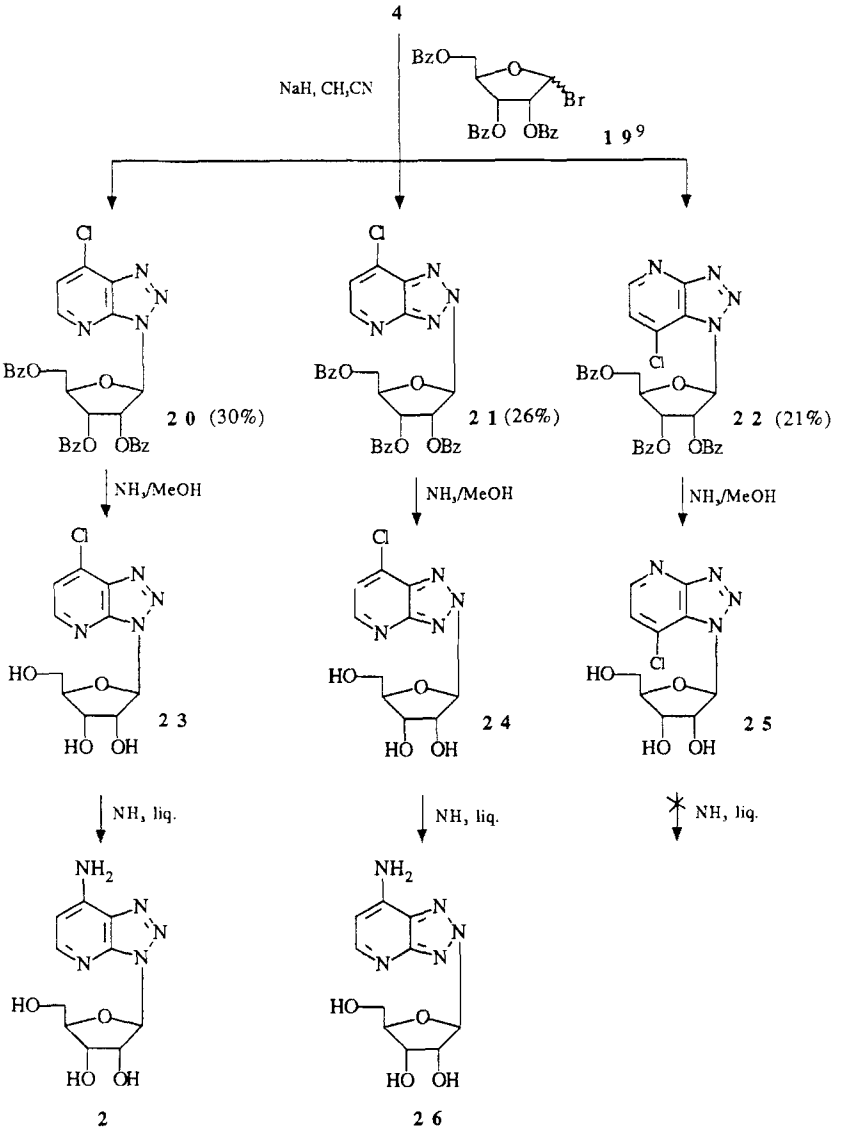
In order to establish the position of glycosylation, ^1H-, ^1H-NOE, ^{13}C-NMR and UV spectra of compounds **3**, **16**, **17** and **18** were measured. The ^{13}C NMR spectra of **3** and **16** were almost identical (Table II) indicating a pair of anomers.

The UV absorption spectra of **3** and **16** are very similar to that reported for the 7-amino-3-(β-D-ribofuranosyl)-3*H*-1,2,3-triazolo[4,5-b]pyridine (**2**) indicating that the position of glycosylation was N^3.[5]

The ^{13}C NMR spectra of the other glycosylated anomers **17** and **18** were similar but different from those of the N^3-substituted compounds. A downfield shift of C(3a) (9.3 and 7.6 ppm) of regioisomers **17** and **18**, respectively, as compared to that of **3** and **16** indicated that N^3 did not carry a substituent.[8] As C(7a) was unchanged, the position of glycosylation was N^2. The configuration of compounds **3**, **16**, **17**, and **18** was confirmed by N.O.E. experiments (Table I).[7] Having assigned the structure to the N^2- and N^3-glycosylated isomers (**11-14**) the glycosylation site of compound **15** could only be at N^1 or N^4. The chemical shift of C(7a) of **15** was upfield shifted as compared to the corresponding signal of the N^3-glycosylated isomer **11**, whereas the chemical shift of C(3a) was downfield shifted and the signal of C(5) proved only slightly upfield shifted.[8] Furthermore no N.O.E. effect was observed on H-(5) when H-(1') was irradiated. Therefore, N^1-glycosylation was established confirming the structure of **15**.

The next target was the synthesis of 8-aza-1-deazaadenosine. Up to now, only one report has appeared in the literature on the synthesis of this compound. In 1971 De Roos and Salemink reported on the glycosylation of **4** with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride in nitromethane in the presence of KCN.[5] Such a reaction yielded two regioisomeric blocked nucleosides which, after debenzoylation, were identified as 7-chloro-3-(β-D-ribofuranosyl)-3*H*-1,2,3-triazolo[4,5-b]pyridine (**23**) and its 2-ribosylated isomer (**24**). Replacement of the chloro group of **23** with hydrazine hydrate, followed by conversion of the 7-hydrazino- into 7-azido-derivative and then reduction of the azido group furnished the 8-aza-1-deazaadenosine (**2**).

We have considered a similar glycosylation of **4** with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**19**)[9]. Glycosylation of the anion of **4** in MeCN with the halogenose **19** afforded N^3-, N^2-, and N^1-glycosylation products (**20-22**, respectively) (Scheme III). Their ratio was determined by TLC and found to be comparable. Nucleosides **20-22** were separated by column chromatography on silica gel. Debenzoylation of **20** and **21** with NH_3/MeOH at room temperature yielded the chloro-nucleosides **23** and **24**. The UV and ^1H NMR spectra of these compounds were identical to those reported in the literature.[5] Therefore the glycosylation site of **22** could only be at N^1 or N^4. The glycosylation position was determined more easily on the corresponding deprotected nucleoside **25**. The anomeric configuration was established by N.O.E. difference spectroscopy.[7] Irradiation of the anomeric proton resulted in N.O.E.s on H-(4') (Table I) establishing β-D-configuration. The ^{13}C NMR spectra of **25** showed that the



SCHEME III

chemical shift of C(7a) was upfield shifted as compared to the corresponding signal of the N^3-glycosylated isomer **23**, whereas the chemical shift of C(3a) was downfield shifted and the signal of C(5) was only slightly upfield shifted.[8] Futhermore, no N.O.E. effect was observed on H-(5) when H-(1') was irradiated. Therefore, N^1-glycosylation was established.

Treatment of **23** and **24** with liquid ammonia at 80 °C afforded 8-aza-1-deazaadenosine (**2**) and 7-amino-2-(β-D-ribofuranosyl)-2*H*-1,2,3-triazolo[4,5-b]pyridine (**26**). Compound **25** could not be converted in 7-amino-derivative neither with liquid ammonia nor with hydrazine hydrate and Raney nichel, owing to its instability under alkaline conditions.

In conclusion, whereas the glycosylation of nucleobase-anion of **4** with the halogenose **5** in MeCN was neither regioselective nor diasteroselective, the reaction became stereoselective for the β-D-nucleosides if the anion of **4** was glycosylated with the ribofuranosyl bromide **19**. In this case the stereoselectivity might be favored by the participation of the benzoyloxy group at C(2') position.

Biological evaluation and Discussion

Antitumor activity. Compounds **2**, **3**, **11-18**, **23**, **24** and **26** have been evaluated *in vitro* for their ability to inhibit the growth of P388 murine lymphocytic leukemia, L1210 murine leukemia, B16 murine melanoma, HL60 human promyelocytic leukemia, and LoVo human colon adenocarcinoma. The ID_{50} (μM) values for these nucleosides, 1-dazaadenosine and 2'-deoxy-1-deazaadenosine are summarized in Table III. Among these the 8-aza-1-deazaadenosine (**2**) was 3 times less active than 1-deazaadenosine against L1210 and marginally active against P388 and LoVo cell lines. The 2'-deoxy-8-aza-1-deazaadenosine (**3**) as well as 1-deaza-2'-deoxyadenosine was completely inactive against all cellular lines up to 100 μM. The 7-chloro-nucleosides **11**, **12**, **23**, and **24** showed marginal activity against P388 murine leukemia and B16 murine melanoma. Interestingly the α-anomer of the 7-chloro-2'-deoxy-nucleoside **12** appeared to be more active against P388 murine lymphocytic leukemia, B16 murine melanoma and LoVo human colon adenocarcinoma than the β-anomer **11**. Compounds **23** and **24** were effective inhibitors of the growth of HL60 human promyelocytic leukemia being only 2-3 times less active than 1-deaza-adenosine.

In conclusion the substitution of CH group at 8-position with nitrogen in the 1-deaza purine moiety of 1-deazaadenosine is detrimental for the antitumor activity. The substitution of the 2'-hydroxyl group with hydrogen in the

TABLE III. Antitumor activity and kinetic constants for inhibition of calf intestinal adenosine deaminase (ADA).

Compd	ID_{50}[a] (μM) P388	L1210	B16	HL60	LoVo	ADA inhibition K_i, M
1-DeazaA	2.27	2.32	3.21	1.44	1.39	6.60×10^{-7}
1-Deaza-2'-deoxyA	>100	>100	>100	>100	>100	1.35×10^{-7}
2	83.82	6.95	>100	>100	27.10	9.03×10^{-7}
3	>100	>100	>100	>100	>100	1.90×10^{-7}
11	48.64	>100	26.65	>100	14.53	5.36×10^{-5}
12	20.77	>100	21.54	>100	7.45	inactive
13	>100	>100	22.76	>100	>100	inactive
14	>100	>100	17.10	>100	>100	
15	>100	>100	45.82	>100	>100	
16	>100	>100	>100	>100	>100	
17	>100	>100	>100	>100	>100	2.62×10^{-4}
18	>100	>100	>100	>100	>100	
23	54.6	>100	81.8	4.70	61.9	2.27×10^{-4}
24	53.8	31.3	50.7	3.30	>100	inactive
25	>100	>100	>100	>100	>100	
26	>100	>100	>100	67.6	>100	inactive

[a]ID_{50} (Inhibitory Dose 50): compound concentration required to reduce by 50% radioactive UdR incorporation. Values are the mean of at least three separate experiments.

ribosyl moiety of 8-aza-1-deazaadenosine and 1-deazaadenosine (compound **3** and 1-deaza-2'-deoxyadenosine) makes the compounds inactive as antitumor agents.

Adenosine deaminase inhibitory activity. Nucleosides **2, 3, 11, 12, 13, 17, 23, 24** and **26** have been tested as inhibitors of calf intestine ADA. As shown by results (Table III), the substitution of a CH group for the nitrogen atom in the 1-position of 8-azaadenosine turns a substrate[10] into a strong inhibitor of the enzyme; so 8-aza-1-deazaadenosine behaves as 1-deazaadenosine.[3]

The substitution of the hydroxy group with hydrogen at 2'-position (compounds **3, 11** and 1-deaza-2'-deoxyadenosine) increases by about 5 times the activity, confirming that 2'-deoxy-nucleosides have greater affinity for the enzyme.[11] The inhibitory activity drops dramatically when the sugar moiety is moved from N^3- to N^2-position or when the amino group at 7-position is replaced by Cl.

EXPERIMENTAL SECTION

Chemistry. Melting points were determined with 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 N.O.E. experiments 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 with 2-deoxy-3,5-di-*O*-(p-toluoyl)-α-D-*erythro*-pentofuranosyl chloride. To a stirred suspension of 7-chloro-3*H*-1,2,3-triazolo[4,5-b]pyridine[5] (**4**) (2 g, 12.94 mmol) in dry CH_3CN (100 ml), NaH (60% in oil, 0.55 g, 22.88 mmol) was added. The reaction mixture was strirred at room temperature under nitrogen

for 15 min. 2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride[6] (**5**) (5 g, 12.94 mmol) was added portionwise under stirring within 30 min. Stirring was continued at room temperature for 30 min. The reaction mixture was filtered through Celite, and the Celite pad washed further with $CHCl_3$ (20 mL). Evaporation of the filtrate to dryness gave a mixture which was chromatographed on silica gel column eluting with a mixture of CH_2Cl_2-EtOAc (98:2). Three zones (I, II, III) were separated, each containing the anomeric mixtures.

7-Chloro-3-[2-deoxy-3,5-di-*O*-(p-toluoyl)-β-D-*erythro*-pentofuranosyl]-3*H*-1,2,3-triazolo[4,5-b]pyridine (6). The fast migrating zone I was separated into two zones. From the faster migrating zone, **6** (1.92 g, 29 %) was obtained as white foam. TLC (silica gel , CH_2Cl_2-EtOAc, 98:2): Rf 0. 4. ^1H NMR (Me_2SO-d_6): δ 2.38, 2.42 (2 s, 6 H, CH_3); 2.98 (m, 1 H, Hα-2'); 3.62 (m, 1 H, Hβ-2'); 4.48, 4.59 (2 m, 2 H, H-5'); 4.71 (m, 1 H, H-4'); 5.99 (m, 1 H, H-3'); 7.03 (t, J = 6.6 Hz, 1 H, H-1'); 7.28, 7.39, 7.77, 7.98 (4 d, 8 H, arom.); 7.74 (d, J = 4.9, 1 H, H-6); 8.72 (d, J = 4.9 Hz, 1 H, H-5). Anal. Calcd. for $C_{26}H_{23}ClN_4O_5$: C 61.60; H 4.57; N 11.05. Found: C 61.73; H 4.48; N 10.98.

7-Chloro-3-[2-deoxy-3,5-di-*O*-(p-toluoyl)-α-D-*erythro*-pentofuranosyl]-3*H*-1,2,3-triazolo[4,5-b]pyridine (7). The slower migrating zone of zone I yielded **7** as a white foam (0.75 g, 11 %). TLC (silica gel CH_2Cl_2-EtOAc, 98:2): Rf 0.23. ^1H NMR (Me_2SO-d_6): δ 2.40, 2.51 (2 s, 6 H, CH_3); 3.30 (m, 2 H, Hα-2', Hβ-2'); 4.59 (m, 2 H, H-5'); 4.89 (m, 1 H, H-4'); 5.69 (m, 1 H, H-3'); 7.06 (q, J = 3.8, 6.6 Hz, 1 H, H-1'); 7.35 (dd, 4 H, arom.); 7.73 (d, J = 4.9 Hz, 1 H, H-6); 7.80, 7.92 (2 d, 4 H, arom.); 8.71 (d, J = 4.9 Hz, 1 H, H-5). Anal. Calcd. for $C_{26}H_{23}ClN_4O_5$: C 61.60; H 4.57; N 11.05. Found: C 61.82; H 4.60; N 11.18.

7-Chloro-2-[2-deoxy-3,5-di-*O*-(p-toluoyl)-β-D-*erythro*-pentofuranosyl]-2*H*-1,2,3-triazolo[4,5-b]pyridine (8). Zone II was separated by same silica gel column yielding the faster migrating **8** as a colorless foam (1.4 g, 21 %). TLC (silica gel CH_2Cl_2-EtOAc, 98-2): Rf 0.15. ^1H NMR (Me_2SO-d_6): δ 2.36, 2.40 (2 s, 6 H, CH_3); 2.57 (m, 1 H, Hα-2'); 3.02 (m, 1 H, Hβ-2')); 4.48, 4.62 (2 m, 2 H, H-5'); 4.77 (m, 1 H, H-4'); 5.98 (m, 1 H, H-3'); 6.93 (dd, J = 4.2, 6.9 Hz, 1 H, H-1'); 7.22, 7.39 (2 d, 4 H ,arom.); 7.74 (d, J = 4.6 Hz,1 H, H-6); 7.76, 7.98 (2 d, 4 H, arom.); 8.83 (d, J = 4.6 Hz, 1 H, H-5). Anal. Calcd. for $C_{26}H_{23}ClN_4O_5$: C 61.60; H 4.57; N 11.05. Found: C 61.78; H 4.52; N 10.95.

7-Chloro-2-[2-deoxy-3,5-di-*O*-(p-toluoyl)-α-D-*erythro*-pentofuranosyl]-2*H*-1,2,3-triazolo[4,5-b]pyridine (9). The slower migrating zone of II yielded **9** as a colorless foam (0.35 g, 5 %). TLC (silica gel CH_2Cl_2-EtOAc, 92: 8): Rf 0. 42. ^1H NMR (Me_2SO-d_6): δ 2.34, 2.42 (2 s, 6 H, CH_3); 3.22 (m, 1

H, H_{α}-2'), 3.43 (m, 1 H, H_{β}-2'); 4.60 (m, 2 H, H-5'); 5.02 (m, 1 H, H-4'); 5.59 (m, 1 H, H-3'); 7.03 (d, J = 6.4 Hz 1 H, H-1'); 7.14, 7.33, 7.58, 7.92 (4 d, 8 H, arom); 7.76 (d, J = 4.7 Hz, 1 H, H-6); 8.79 (d, J = 4.7 Hz, 1 H, H-5). Anal. Calcd for $C_{26}H_{23}ClN_4O_5$: C 61.60; H 4.57; N 11.05. Found: C 61.66; H 4.53; N 11.20.

7-Chloro-1-[2-deoxy-3,5-di-*O*-(p-toluoyl)-β-D-*erythro*-pentofuranosyl]-1*H*-1,2,3-triazolo[4,5-b]pyridine (10). Zone III, separated by same chromatographic silica gel column, was evaporated to give **10** as a colorless foam (0.84 g, 13 %). TLC (CH_2Cl_2-EtOAc, 92:8): Rf 0.35. ^1H NMR (Me_2SO-d_6): δ 2.38, 2.41 (2 s, 6 H, CH_3); 3.02, (m, 1 H, H_{α}-2'); 3.79 (m, 1 H, H_{β}-2'); 4.29, 4.43 (2 m, 2 H, H-5'); 4.73 (m, 1 H, H-4'); 5.94 (m, 1 H, H-3'); 7.20 (m, 1 H, H-1'); 7.29, 7.40, 7.68, 7.96 (4 d, 8 H, arom.); 7.84 (d, J = 5.0 Hz, 1 H, H-6); 8.70 (d, J = 5.0 Hz, 1 H, H-5). Anal. Calcd. for $C_{26}H_{23}ClN_4O_5$: C 61.60; H 4.57; N 11.05. Found: C 61.70; H 4.65; N 10.96.

7-Chloro-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3*H*-1,2,3-triazolo[4,5-b]pyridine (11). Compound **6** (1 g, 1.97 mmol) in dry MeOH (50 ml, saturated with NH_3 at 0 °C) was stirred for 48 h at room temperature. The solution was evaporated to dryness, the residue oil cromatographed on a silica gel column and eluted with $CHCl_3$-MeOH, (95:5) yielding **11** (0.32 g, 60%) as a colorless solid which was crystallized from CH_2Cl_2. M.p.135-137 °C. TLC (silica gel, $CHCl_3$-MeOH 95:5): Rf 0.31. UV (NaOH 0.1N): λ_{max} 216 nm (ε1900); 256 (ε 4900); 286 (ε 3600). ^1H NMR (Me_2SO-d_6): δ 2.51 (m, 1 H, H_{α}-2'); 3.13 (m, 1 H, H_{β}-2'); 3.40, 3.58 (2 m, 2 H, H-5'); 3.95 (m, 1 H, H-4'); 4.62 (m, 1 H, H-3'); 4.74 (t, J = 5.8 Hz,1 H, OH-5'); 5.43 (d, J = 4.7 Hz, 1 H, OH-3'); 6.82 (dd, J = 5.6, 6.6 Hz, 1 H, H-1'); 7.73 (d, J = 5.0 Hz, 1 H, H-6); 8.72 (d, J = 5.0 Hz, 1 H, H-5). Anal. Calcd. for $C_{10}H_{11}ClN_4O_3$: C 44.36; H 4.09; N 20.70. Found: C 44.21; H 4.18; N 20.63.

7-Chloro-3-(2-deoxy-α-D-*erythro*-pentofuranosyl)-3*H*-1,2,3-triazolo[4,5-b]pyridine (12). Compound **12** was prepared as described for **11**, but for 24 h, using **7** (0.7 g, 1.38 mmol); a yellow foam (0.26 g, 70%), was obtained. TLC (silica gel $CHCl_3$-MeOH, 95:5) Rf 0.29. UV (NaOH 0.1 N): λ_{max} 216 nm (ε 1900); 254 (ε 4800); 284 (ε 3700). ^1H NMR (Me_2SO-d_6): δ 2.93 (m, 2 H, H_{α}-2', H_{β}-2'); 3.49, 3.65 (2 m, 2 H, H-5'); 4.10 (m, 1 H, H-4'); 4.29 (m, 1 H, H-3'); 4.81 (t, J = 5.8 Hz, 1 H, OH-5'); 5.48 (d, J = 5.5 Hz, 1 H, OH-3'); 6.78 (t, J = 6.6 Hz, 1 H, , H-1'); 7.78 (d, J = 5.5 Hz, 1 H, H-6); 8.77 (d, J = 5.5 Hz, 1 H, H-5). Anal. Calcd. for $C_{10}H_{11}ClN_4O_3$: C 44.36; H 4.09; N 20.70. Found: C 44.25; H 4.19; N 20.76.

7-Chloro-2-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2*H*-1,2,3-triazolo[4,5-b]pyridine (13). Compound **13** was prepared as described for **11**, using **8** (1 g, 1.97 mmol). Upon evaporation of the ammonia, the residue

obtained was crystallized from CH_2Cl_2 to give **13** as a white solid (0.34 g, 64%). M.p. 143-145 °C. TLC (silica gel, $CHCl_3$-MeOH, 95:5) : Rf 0.24 UV (NaOH 0.1 N): λ_{max} 218 nm (ε 1600); 278 (ε 7000). ^1H NMR (Me_2SO-d_6): δ 2.57 (m, 1 H, H_α-2'); 2.94 (m, 1 H, Hβ-2'); 3.46, 3.60 (2 m, 2 H, H-5'); 3.98 (m, 1 H, H-4'); 4.60 (m, 1 H, H-3'); 4.78 (t, J = 5.6 Hz, 1 H, OH-5'); 5.59 (d, J = 4.1 Hz, 1 H, OH-3'); 6.70 (dd, J = 3.9 , 7.0 Hz, 1 H, H-1'); 7.77 (d, J = 4.6 Hz, 1 H, H-6); 8.80 (d, J = 4.6 Hz, 1 H, H-5). Anal. Calcd. for $C_{10}H_{11}ClN_4O_3$: C 44.36; H 4.09; N 20.70. Found: C 44.40; H 4.02; N 20.63.

7-Chloro-2-(2-deoxy-α-D-*erythro*-pentofuranosyl)-2*H*-1,2,3-tri-azolo[4,5-b]pyridine (14). Compound **14** was prepared as described for **12**, using **9** (0.3 g, 0.59 mmol). Crystallization from CH_2Cl_2 gave a white solid (0.10 g, 35%). M.p. 108-110 °C dec. TLC (silica gel, $CHCl_3$-MeOH-NH_4OH, 90:9:1) Rf 0.48. UV (NaOH 0.1 N): λ_{max} 218 nm (ε1100); 278 (ε 5700). ^1H NMR (Me_2SO-d_6): δ 2.73 (m, 1 H, H_α-2'); 2.93 (m, 1 H, Hβ-2'); 3.51, 3.65 (2 m, 2 H, H-5'); 4.18 (m, 1 H, H-4'); 4.22 (m, 1 H, H-3'); 4.88 (t, J = 5.8 Hz, 1 H, OH-5'); 5.30 (d, J = 4.9 Hz, 1 H, OH-3'); 6.67 (dd, J = 4.2, 7.2 Hz , 1 H, H-1'); 7.76 (d, J = 4.6 Hz, 1 H, H-6); 8.80 (d, J = 4.6 Hz, 1 H , H-5). Anal. Calcd. $C_{10}H_{11}ClN_4O_3$: C 44.36; H 4.09; N. 20.70. Found: C 44.45; H 4.03; N 20.75.

7-Chloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1*H*-1,2,3-tri-azolo[4,5-b]pyridine (15). Compound **15** was prepared from **10** (0.5 g, 0.98 mmol) as described for **12**. Upon evaporation and chromatography on a silica gel column using $CHCl_3$-MeOH (90:10), **15** as white solid (0.29 g, 59%) was obtained. M.p. 95-97 °C. UV (NaOH 0.1 N): λ_{max} 208 nm (ε 1100); 216 (ε 2000); 266 (ε 4500). ^1H NMR (Me_2SO-d_6): δ 2.55 (m, 1 H, H_α-2'); 3.29 (m, 1 H, Hβ-2'); 3.43, (m, 2 H, H-5'); 3.94 (m, 1 H, H-4'); 4.60 (m, 2 H, OH-5', H-C(3'); 5.39 (d, J = 4.6 Hz, 1 H, OH-3'); 7.03 (dd, J = 4.6, 6.9 Hz , 1 H, H-1'); 7.81 (d, J = 4.8 Hz, 1 H, H-6); 8.69 (d, J = 4.8 Hz, 1 H , H-5). Anal. Calcd. for $C_{10}H_{11}ClN_4O_3$: C 44.36; H 4.09; N 20.70. Found: C 44.29; H 4.11; N 20.68.

7-Amino-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3*H*-1,2,3-tri-azolo[4,5-b]pyridine (3). A solution of **11** (0.4 g, 1.58 mmol) in liquid ammonia (50 mL) was heated in a steel reaction vessel at 80 °C for 21 h. The excess ammonia was removed to afford product **3** which was crystallized from CH_2Cl_2: (0.35 g, 91% as white crystals). M.p. 219-222 °C. TLC (silica gel, $CHCl_3$-MeOH, 88:12): Rf 0.47. UV (NaOH O.1N): λ_{max} 228 (ε 6500); 262 (ε 5000); 304 (ε 7000). ^1H NMR (Me_2SO-d_6): δ 2.38 (m, 1 H, H_α-2'); 3.07 (m, 1 H, Hβ-2'); 3.43, 3.60 (2 m, 2 H, H-5'); 3.92 (m, 1 H, H-4'); 4.55 (m, 1 H, H-3'); 5.18 (t, J = 5.6 Hz, 1 H, OH-5'); 5.37 (d, J = 4.5 Hz, 1 H, OH-3'); 6.42 (d, J = 5.6 Hz, 1 H, H-6); 6.68 (t, J = 6.7 Hz , 1 H, H-1'); 7.39 (s, 2 H, NH_2); 8.05 (d, J = 5.6 Hz, 1 H, H-5). Anal. Calcd. for $C_{10}H_{13}N_5O_3$: C 47.80; H 5.22; N 27.88. Found: C 47.71; H 5.35; N 27.94.

7-Amino-3-(2-deoxy-α-D-*erythro*-pentofuranosyl)-3*H*-1,2,3-triazolo[4,5-b]pyridine (16). Compound **16** was prepared from **12** (0.25 g, 0.98 mmol) as described for **3** but for 8 h. The residue obtained after evaporation of ammonia was crystallized from CH_2Cl_2 to give **16** (0. 185 g, 75 %) as white crystals. M.p. 172-174 °C; TLC (silica gel, $CHCl_3$-MeOH, 90:10): Rf 0.46. UV (NaOH 0.1 N): λ_{max} 226 nm (ε 4800); 262 (ε 2800); 306 (ε 4400). ^{1}H NMR (Me_2SO-d_6): δ 2.75 (m, 1 H, H_α-2'); 2.40 (m, 1 H, Hβ-2'); 3.46-3.58, (2 m, 2 H, H-5'); 4.05 (m, 1 H, H-4'); 4.23 (m, 1 H, H-3'); 4.80 (t, J = 5.6 Hz, 1 H, OH-5'); 6.27 (d, J = 7.6 Hz, 1 H, OH-3'); 6.42 (d, J = 5.6 Hz, 1 H, , H-6); 6.63 (dd, J = 5.1, 7.5 Hz, 1 H, H-1'); 7.42 (s, 2 H, NH_2); 8.08 (d, J = 5.6 Hz, 1 H, H-5). Anal. Calcd. for $C_{10}H_{13}N_5O_3$: C 47.80; H 5.22; N 27.88. Found: C 47.93; H 5.08; N 27.80.

7-Amino-2-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2*H*-1,2,3-triazolo[4,5-b]pyridine (17). Compound **17** was prepared as described for **3**, but the reaction mixture was heated at 80 °C for 7 h. Using **13** (0.3 g, 1.10 mmol), a colorless solid was obtained (0.16 g, 54 %). M.p. 105-107 °C. dec. TLC (silica gel, $CHCl_3$-MeOH-NH_4OH, 82:17:1): Rf 0.44. UV (NaOH 0.1N): λ_{max} 226 nm (ε 6600); 272 (ε 3800); 278 (ε 3600); 320 (ε 3900). ^{1}H NMR (Me_2SO-d_6): δ 2.57 (m,1 H, H_α-2'); 2.95 (m, 1 H, Hβ-2'); 3.45-3.62, (2 m, 2 H, H-5'); 3.98 (m, 1 H, H-4'); 4.61 (m, 1 H, H-3'); 4.80 (t, J = 5.6 Hz, 1 H, OH-5'); 5.44 (d, J = 4.7 Hz, 1 H, OH-3'); 6.44 (d, J =5.5 Hz, 1 H, H-6); 6.56 (dd, J = 4.4, 6.8 Hz, 1 H, H-1'); 7.62 (s, 2 H, NH_2); 8.28 (d, J = 5.5 Hz, 1 H, H-5). Anal. Calcd. for $C_{10}H_{13}N_5O_3$: C 47.80; H 5.22; N 27.88. Found: C 47.78; H 5.34; N 27.96.

7-Amino-2-(2-deoxy-α-D-*erythro*-pentofuranosyl)-2*H*-1,2,3-triazolo[4,5-b]pyridine (18). Compound **18** was prepared as described for **17**. Using **14** (0.1 g, 0.36 mmol) colorless foam was obtained (25 mg, 25%). TLC (silica gel, $CHCl_3$-MeOH-NH_4OH, 82:17:1): Rf 0.4. UV (NaOH 0.1N): λ_{max} 226 nm (ε 4100); 272 (ε 3000); 278 (ε 2900), 320 (ε 3100). ^{1}H NMR (Me_2SO-d_6): δ 2.71 (m, 1 H, H_α-2'); 2.90 (m, 1 H, Hβ-2'); 3.49-3.64 (2 m, 2 H, H-5'); 4.12 (m, 1 H, H-4'); 4.23 (m, 1 H, H-3'); 4.82, 5.34 (2 s, 2 H, OH-5', OH-3'); 6.40 (d, J = 5.5 Hz, 1 H, H-6); 6.48 (dd, J = 4.6 7.2 Hz, 1 H, H-1'); 7.33 (s, 2 H, NH_2); 8.22 (d, J = 5.5 Hz, 1 H, H-5). Anal. Calcd. for $C_{10}H_{13}N_5O_3$: C 47.80; H 5.22; N 27.88. Found: C 47.78; H 5.40; N 27.79.

Glycosylation of 7-chloro-3*H*-1,2,3-triazolo[4,5-b]pyridine with 2,3,5-tri-*O*-benzoyl-α-D-ribofuranosyl bromide. To a stirred suspension of **4** (1.5 g, 9.7 mmol) in dry CH_3CN (70 mL), NaH (60% in oil, 0.45 g, 18.67 mmol) was added and the reaction mixture was stirred at room temperature for 0.5 h. The 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**19**)[9] (5.08 g, 9.7 mmol) in dry CH_3CN (10 mL) was added, and the stirring was continued for 24 h at room temperature. Evaporation to dryness of the filtrate gave a foam which

was chromatographed on a silica gel column eluting with CH_2Cl_2-EtOAc (98:2). Tree main zones were separed.

7-Chloro-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-3*H*-1,2,3-triazolo[4,5-b]pyridine (20).[5] From the fast migrating zone, **20** (1.7 g, 30%) as a white foam was obtained. TLC (silica gel, CH_2Cl_2-EtOAc, 98:2): Rf 0.4. ^1H NMR (Me_2SO-d_6): δ 4.60-4.76 (2 m, 2 H, H-5'); 5.03 (m, 1 H, H-4'); 6.37 (t, J = 6.0 Hz, 1 H, H-3'); 6.64 (dd, J = 3.1, 5.4 Hz, 1 H, H-2'); 7.1 (d, J = 5.2, 1 H, H-1'); 7.48, 7.67, 7.91 (3 m, 15 H, arom.), 7.76 (d, J = 5.2 Hz, 1 H, H-6); 8.70 (d, J = 5.2 Hz, 1 H, H-5). Anal. Calcd. for $C_{31}H_{23}ClN_4O_7$: C 62.15; H 3.87; N 9.35. Found: C 62.31; H 3.94; N 9.26.

7-Chloro-2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2*H*-1,2,3-triazolo[4,5-b]pyridine (21).[5] The following zone was eluted with a mixture of CH_2Cl_2-EtOAc (95:5) to give **21** as white foam (1.5 g, 26%). TLC (silica gel, CH_2Cl_2-EtOAc, 95:5): Rf 0.6. ^1H NMR (Me_2SO-d_6): δ 4.62-4.75 (2 m, 2 H, H-5'); 5.09 (m, 1 H, H-4'); 6.35 (dd, J = 5.1, 7.0 Hz, 1 H, H-3'); 6.47 (dd, J = 2.0, 5.1 Hz, 1 H H-2'); 7.12 (d, J = 2 Hz, 1 H, H-1'); 7.45, 7.67, 7.94 (3 m, 15 H, arom.); 7.79 (d, J = 4.6 Hz, 1 H , H-6); 8.86 (d, J = 4.6 Hz, 1 H, H-5). Anal. Calcd. for $C_{31}H_{23}ClN_4O_7$: C 62.15; H 3.87; N 9.35. Found: C 62.25; H 3.86; N 9.18.

7-Chloro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-1,2,3-triazolo[4,5-b]pyridine (22). From the same mixture (CH_2Cl_2-EtOAc, 95:5) the following compound (**22**) was eluted (1.1 g, 21%) as yellow foam. TLC (silica gel, CH_2Cl_2-EtOAc, 95:5): Rf 0.28. ^1H NMR (Me_2SO-d_6): δ 4.49-4.63 (2 m, 2 H, H-5'); 5.08 (m, 1 H, H-4'); 6.32 (dd, J = 5.1 , 7.6 Hz, 1 H, H-3'); 6.73 (dd, J = 1.9, 5.0 Hz, 1 H, H-2'); 7.21 (d, J = 1.8 Hz, 1 H, H-1'); 7.45, 7.68, 8.02 (3 m, 15 H, arom.); 7.88 (d, J = 4.9 Hz, 1 H, H-6); 8.72 (d, J = 4.9 Hz , 1 H, H-5). Anal. Calcd. for $C_{31}H_{23}ClN_4O_7$: C 62.15; H 3.87; N 9.35. Found: C 62.36; H 3.70; N 9.44.

7-Chloro-3-(β-D-ribofuranosyl)-3*H*-1,2,3-triazolo[4,5-b]pyridine (23).[5] To compound **20** (1 g, 1.66 mmol) in a stainless steel bomb was added MeOH (50 mL, saturated with NH_3 at 0 °C). The mixture was left at room temperature for 18 h; the solvent was then removed, and the resulting gummy solid was chromatographed on a silica gel column and eluted with $CHCl_3$-MeOH (90:10), yielding **23** (0.4 g, 84%) as a white solid. M.p. 151-153 °C (lit.[5] 153 °C). TLC (silica gel, $CHCl_3$-MeOH 90:10): Rf 0.47.

7-Chloro-2-(β-D-ribofuranosyl)-2*H*-1,2,3-triazolo[4,5-b]pyridine (24).[5] Compound **24** was prepared from **21** (1 g, 1.66 mmol) as described for **23** (reaction time 20 h). The solvent was removed, and the resulting solid was crystallized from CH_2Cl_2 to give 0.35 g (78%) of colorless crystals. M.p. 158-160 °C dec. [lit.[5] 130 °C dec.] TLC (silica gel, $CHCl_3$-MeOH, 90:10): Rf 0.41.

7-Chloro-1-(β-D-ribofuranosyl)-1*H*-1,2,3-triazolo[4,5-b]pyridine (25). Compound **25** was prepared from **22** (0.9 g, 1.5 mmol) as described for **23** but at 4 °C for 38 h The solvent was removed and the residue was chromatographed on a silica gel column eluting with a mixture of $CHCl_3$-MeOH (88:12) to yield **25** (0.58 g, 65 %) as a white crystals. M.p. 149-151 °C. TLC (silica gel, $CHCl_3$-MeOH, 88:12): Rf 0.38. UV (NaOH 0.1 N): λ_{max} 218 (ε 1900); 268 (ε 3500). ^1H NMR (Me_2SO-d_6): δ 3. 38, 3.55 (2 m, 2 H , H-5'); 4.01 (m, 1 H, H-4'); 4.40 (dd, J = 5.4, 10.5 Hz, 1 H, H-3'); 4.69 (t, J = 5.6 Hz, 1 H, OH-5'); 5.0 (dd, J = 4.4, 9.4 Hz, 1 H, H-2'); 5.35 (d, J = 5.8 Hz, 1 H, OH-3'); 5.71 (d, J = 5.4 Hz, 1 H, OH-2'); 6.58 (d, J = 3.6 Hz, 1 H, H-1'); 7.86 (d, J = 4.9 Hz, 1 H, H-6); 8.70 (d, J = 4.9 Hz, 1 H, H-5). Anal. Calcd. for $C_{10}H_{11}ClN_4O_4$: C 41.90; H 3.87; N 19.54. Found: C 41.81; H 3.79; N 19.63.

7-Amino-3-(β-D-ribofuranosyl)-3*H*-1,2,3-triazolo[4,5-b]pyridine (2)[5]. To compound **23** (0.4 g, 1.39 mmol) liquid ammonia (20 mL) was added, and the mixture was heated in a steel reaction vessel at 80 °C for 8 h. The mixture was evaporated and the residue was chromatographed on a silica gel column eluting with $CHCl_3$-MeOH (85:15); white needles (0.18 g, 45 %) were obtained. M.p. 205-207 °C [lit.[5] 194 °C] TLC (silica gel, $CHCl_3$-MeOH, 85:15): Rf 0.43. ^1H NMR (Me_2SO-d_6): δ 3.48-3.56 (2 m, 2 H, H-5'); 4.02 (dd, J = 4.2, 7.8 Hz, 1 H, H-4'); 4.29 (t, J = 4.1 Hz, 1 H, H-3'); 4.90 (t, J = 5.5 Hz, 1 H, H-2'); 5.25-5.41 (2 br s, 3 H, OH); 6.20 (d, J = 5.8 Hz, 1 H, H-6); 6.42 (d, J = 5.6 Hz, 1 H, H-1'); 7.40 (s, 2 H, NH_2); 8.04 (d, J = 5.8 Hz, 1 H, H-5). Anal. Calcd. for $C_{10}H_{13}N_5O_4$: C 44.94; H 4.90; N 26.21. Found: C 45.05; H 5.12; N 26.13.

7-Amino-2-(β-D-ribofuranosyl)-2*H*-1,2,3-triazolo[4,5-b]pyridine (26). Compound **26** was prepared from **24** (0.3 g, 1.04 mmol) as described for **2** (reaction time 18 h). After evaporation, the reaction mixture was chromatographed on a silica gel column eluting with a mixture of $CHCl_3$-MeOH-NH_4OH (75:24:1) yielding **26** (0.146 g, 52%) as a white solid. M.p. 130-132 °C. TLC (silica gel, $CHCl_3$-MeOH-NH_4OH, 75:24:1): Rf 0.3. UV (NaOH 0.1 N): λ_{max} 222 (ε 8500); 276 (ε 3500); 322 (ε 3300). ^1H NMR (Me_2SO-d_6): δ 3.56-3.65 (2 m, 2 H, H-5'); 4.04 (m, 1 H, H-4'); 4.35 (dd, J = 5.1, 10.0 Hz, 1 H, H-3'); 4.60 (dd, J = 4.8, 8.8 Hz, 1 H, H-2'); 4.81 (m, 1 H, OH-5'); 5.31 (d, J = 5.6 Hz, 1 H, OH-3'); 5.72 (d, J = 5.6 Hz, 1 H, OH-2'); 6.08 (d, J = 3.6 Hz, 1 H, H-1'); 6.39 (d, J = 5.3 Hz, 1 H, H-6); 7.43 (s, 2 H, NH_2); 8.23 (d, J = 5.3 Hz, 1 H, H-5). Anal. Calcd. for $C_{10}H_{13}N_5O_4$: C 44.94; H 4.90; N 26.21. Found: C 44.86; H 5.02; N 26.33.

Biological Determination

Antiproliferative assay. An assay developed for predictive evaluation of tumor chemosensitivity has been used.[12] The following cell lines were used:

P388 murine lymphocytic leukemia, L1210 murine leukemia, B16 murine melanoma, HL60 human promyelocytic leukemia and LoVo human colon adenocarcinoma. Cell lines were maintained *in vitro*, in exponential growth. The LoVo cell line was maintained in HAM'S F-12 medium containing antibiotics (penicillin 100 U/mL, streptomycin 100 μg/mL) supplemented with 10% fetal calf serum, 1% vitamin and 3 mM glutamine. The other cell lines were cultured in RPMI-1640 supplemented with antibiotics (penicillin 100 U/mL, streptomycin 100 μg/mL, gentamicin 50 μg/mL), 3 mM glutamine, 10 mM HEPES buffer and 15% (for P388 and L1210 cell lines) heat-inactivated new-born calf serum, or 10% (for B16 cell line), or 15% (for HL60 cell line) heat-inactivated FCS. Compounds were solubilized in DMSO and then water and culture medium were added; the highest DMSO concentration used (0.5%) did not have any cytotoxic effect in our testing system. Briefly, various concentrations of each drug were placed with tumor cell suspension (P388, $5x10^4$ cells/mL; L1210, 10^5 cells/mL; B16, $1.2x10^4$ cells/mL; HL60, $2.5x10^5$ cells/mL; LoVo, $5x10^4$ cells/mL). Forty eight hours later DNA synthesis was evaluated by adding 0.1 μg/well of [^{125}]I-5-iodo-2'-deoxyuridine along with 2'-deoxy-5-fluorouridine (0.01 μg/well) to the cultured cells for an additional 18 h. Harvesting was performed by a multiple suction filtration apparatus on a fiberglass filter. Immediately before harvesting, B16 and LoVo cells were treated with trypsin 0.05% plus EDTA 0.02%. Paper disks containing the aspirate cells were read in a gamma-scintillation counter. At each dose level of compounds tested, cell growth inhibition was expressed as a percentage of inhibition of radioisotope incorporation in the treated cultures with respect to control cultures. A dose resulting in 50% inhibition of radioisotope incorporation (ID_{50}) was determined as suggested by Chou[13]; the mean ID_{50} of at least three experiments was reported.

Enzyme assay. The method used for the determination of activity against adenosine deaminase has been described in a preceding paper.[14]

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