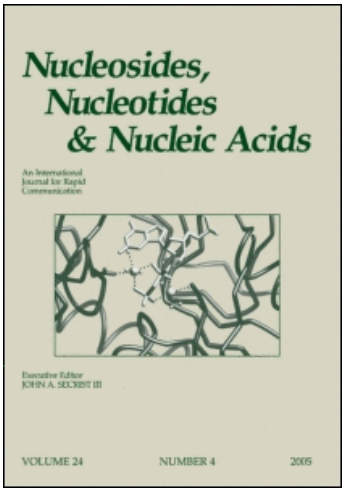

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Kobe, J. , Brdar, B. and Sorič, J.(1986) 'Formycin Analogs II. Antiviral and Cytotoxic s-Triazolo [4, 3-a] - and [1, 5-a] Pyridine Derivatives', Nucleosides, Nucleotides and Nucleic Acids, 5: 2, 135 — 151
To link to this Article: DOI: 10.1080/07328318608068668
URL: http://dx.doi.org/10.1080/07328318608068668

NUCLEOSIDES & NUCLEOTIDES, 5(2), 135-151 (1986)

FORMYCIN ANALOGS II. ANTIVIRAL AND CYTOTOXIC s-TRIAZOLO [4,3-a] - AND [1,5-a] PYRIDINE DERIVATIVES

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Abstract: The novel N-bridgehead formycin analog 3- β -D-ribofuranosyl--8-amino-s-triazolo [4,3-a] pyridine (8a-aza-4,6-dideaza formycin) has been prepared from 5- [2,3,5-tri-O-benzoyl- β -D-ribofuranosyl] -(2H)-tetrazole and 2-chloro-3-nitropyridine. The synthetic route used an initial condensation followed by deprotection and subsequent hydrogenation to afford 2a. 2-Hydroxyethoxymethyl group, an acyclic group, that mimics the ribofuranose unit was also introduced. These compounds were tested against type 1 herpes and poliovirus in tissue culture and their effect on cellular RNA and DNA synthesis was determined. All derivatives possess considerable cytotoxic effect which is expressed more with ribofuranosyl derivatives.

The diverse biological activity that has been reported[2] for the C-nucleoside adenosine analog formycin 1 and certain analogs of 1 has produced a continuing interest in C-nucleosides[3]. We have been involved with the synthesis of naturally occurring purine analogs possessing a bridgehead nitrogen[1]. Our novel approach leading to a stereoselective synthesis of various fused β-D-ribofuranosyl-s-triazolo[4,3-a] pyrimidines maintaining the beta configuration of the starting synthon 5 throughout the reaction scheme, prompted the synthesis of 3-β-D-ribofuranosyl-8-amino-s-triazolo[4,3-a]pyridine 2a, in order to mimic the formycin structure. Its isomer 8a has

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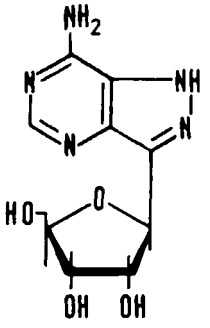

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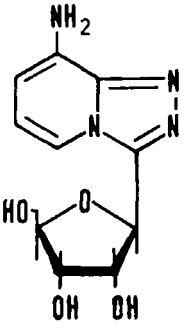
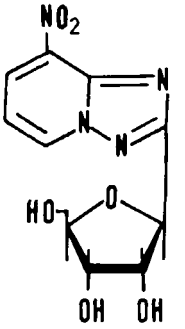


2a

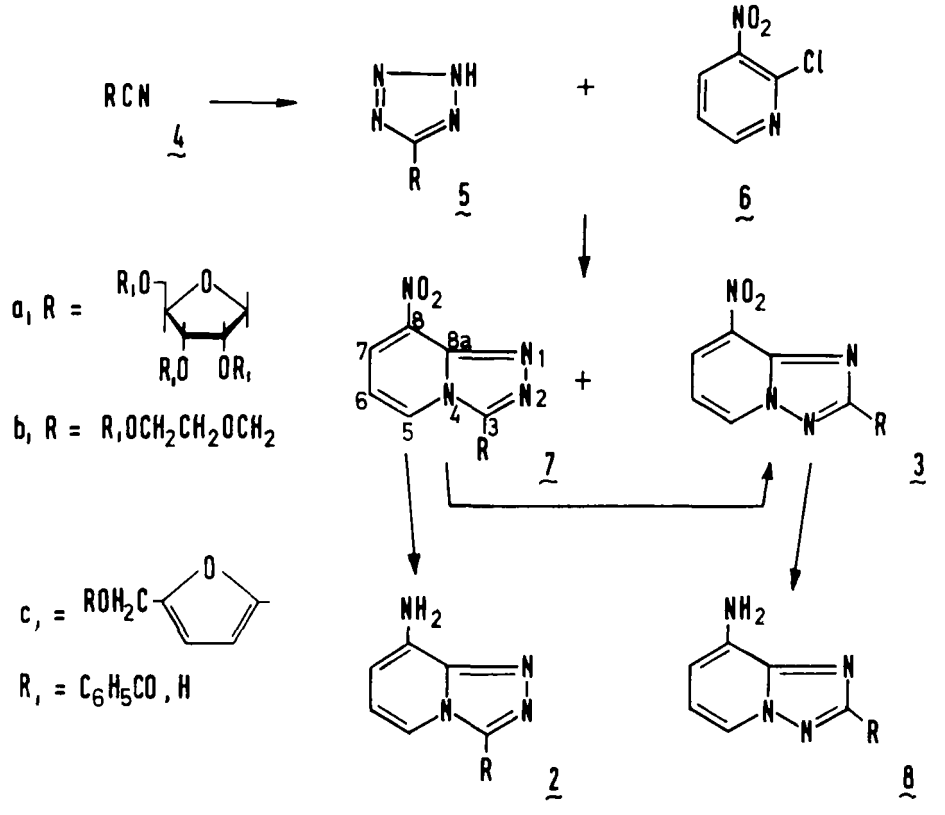


3a

already been reported[4], and its precursor 3a /CT 5394 in ref. 3b/ has shown remarkable cytotoxic and antiviral properties[3].

We would now like to report the detailed synthesis of 7a and 3a and to establish some structure-activity relationship regarding both heterocyclic systems. Additionally, the disclosure of the non-toxic antiviral properties of 9-(2-hydroxyethoxymethyl) guanine[5] and /S/-9-(2,3-hydroxypropyl)adenine[6] allow us to extend the idea of partial ribofuranose units[7] (acyclo substituents) to our studies. The synthesis of 2-β-D-ribofuranosyl-8-nitro-s-triazolo[1,5-a]pyridine 3a was first described by Tam Huynh-Dinh et al.[4]. However, this method utilizing 5-O-benzoyl-D-ribofuranosylthioformimidate provided an anomeric mixture of 3 and the ring closure with 3-nitro-2-pyridylhydrazine led exclusively to the s-triazolo[1,5-a]system. Therefore, an alternative route of synthesis was suggested.

Apparently, the condensation of 5-[2,3,5-tri-O-benzoyl-β-D-ribofuranosyl]-(2H)-tetrazole 5a, (R_1=benzoyl)[8] with 2-chloro-3-nitropyridine 6 in refluxing xylene, altered the reaction to yield 7a in a fair yield. Structure 3c was ascribed to a minor product, which has been identified by TLC and ^{1}H-nmr. That no anomerization occurred was proved by the synthesis of 3a, (R_1=H) which was obtained by deprotection with methanolic ammonia or sodium methoxide method of 7a, (R=benzoyl) or by rearrangement of 7a occurring during deprotection in methanolic ammonia. This product was identical in all respects to the compound described by Tam Huynh-Dinh et al[4].



Careful deprotection of 7a, (R=benzoyl) in sodium methoxide solution at room temperature afforded the desired 3-β-D-ribofuranosyl-8-nitro-s-triazolo [4,3-a]pyridine 7a, (R=H). The structure assigned to nucleoside 7a was confirmed by comparing the UV spectra with the UV spectral data reported[4]; by ^{1}H-nmr spectra (Table 2) and by mass spectral analysis. ^{13}C-nmr spectra support these conclusions (Table 1).

The structure assignment was made once again after reduction of the nitro heterocycles over palladium on charcoal. 7a and 3a were hydrogenated into 2a and 8a, the isomeric structures established by the ^{1}H-nmr methods and confirmed by UV data [14].

The acyclo derivatives 7b and 3b, as well as 2b and 8b were prepared by the same procedure. 5- 2-benzoyloxyethoxymethyl -(2H)-tetrazole (5b)was synthesized from benzoyloxyethoxymethyl nitrile 4b. 4b has been prepared from chloromethyleneglycol monobenzoate following the procedure described by Lingo et al[13].

TABLE 1. Carbon - 13 Chemical Shifts of -s-triazolo /4,3-a/ and /1,5-a/-pyridine (ppm from Me_4Si)

Comp.	CHEMICAL SHIFT, ppm											
	C_2	C_3	C_5	C_6	C_7	8	8a	C_1'	C_2'	C_3'	C_4'	$C_{8'5'}$
7a	-	146.7	131.8	112.0	128.2	135.0	145.9	85.9	75.8	72.9	70.9	61.2
7b	-	145.8	131.5	112.2	128.3	135.4	142.9					
3a	167.2	-	135.5	112.7	129.4	135.8	144.9	85.3	78.6	75.4	71.4	62.2
3b	165.1	-	135.4	112.5	129.2	135.9	144.8					
8a												
8b	161.6	-	119.0	111.4	116.7	135.6	144.7	86.9	77.1	75.0	73.0	63.4
2a	-	147.3	118.3	109.1	115.6	135.7						
2b	-	145.5	117.1	107.9	114.1	134.5	146.3					

TABLE 2. Proton - Chemical Shifts of -s-triazolo /4,3-a/ and /1,5-a/-pyridine (ppm from Me_4Si) in DMSO-d_6(a) and D_2O(b)

Comp.	H_5	H_6	H_7	H_1'	H_2'	H_3'	H_4'	$H_{5'5''}$	
7a[a]	9.05	7.22	8.51	5.35	4.64	4.19	4.04	3.5	3.7
7b[a]	8.98	7.35	8.60						
3a[a]	9.39	7.40	8.71	4.95	4.35	4.10	3.93	3.5	3.7
3b[a]	9.31	7.40	8.64						
8a*	8.07	6.88	6.59	4.81	4.31	4.04	3.86	3.5	3.7
8b[b]	8.09	7.01	6.91						
2a[b]	7.87	6.77	6.55	5.28		4.26	4.09	3.5	3.7
2b[b]	7.72	6.81	6.54						

* from Ref. 4

A similar reaction scheme was outlined in order to provide 4 possible derivatives e.g. 3-[2-hydroxyethoxymethyl]-8-nitro-s-triazolo[4,3-a]pyridine, 7b, 2-[2-hydroxyethoymethyl]-8-amino-s-triazolo[1,5-a]- pyridine 8b, 2-[2-Hydroxyethoxymethyl]-8-nitro-s-triazolo[1,5-a] pyridine, 3b, and 3-[2-hydroxyethoxymethyl]-8-amino-s-triazolo[4,3-a] pyridine 2b.

Biological Evaluation and Results

In vitro antiviral determination

The experiments were performed with monolayers of HeLa cells. Cells were maintained in Minimum Essential Medium (MEM) supplemented with 5 % /v/v/ foetal bovine serum at 37°C in 5 % CO_2. Inhibition of the virus induced cytopathic effect (CPE) was used as the initial indicator of antiviral activity. CPE was observed after infection with herpes simplex virus and poliovirus following the procedure utilized in ref.1. The degree of CPE inhibition and compound cytotoxicity were observed after 36-48 h of incubation at 37°C, and the effect expressed as a virus rating /VR/ as previously described[1,10].

HeLa cell survival determination

Cell cultures (Petri dish, 60 Ø) were pretreated with samples of different concentrations and incubated for 14 days. The colonies were scored numerically after being colored by a 10 % Giemse solution. The survival was determined toward the capability of the treated cells to form colonies, which possess 50 or more single cells, and was expressed as a percentage of the control.

The effect on Vero cells proliferation was determined as reported previously[12]. The test compound was added to actively growing cells and incubated for 48 h and the cell number and volume were measured by the use of an electronic cell counter (Analysinstrument A.B., Stockholm).

Macromolecule synthesis determinations

The synthesis of cellular macromolecules was measured in the cell cultures pretreated with samples studied for 24 h by labelling with pulses of ^3H thymidine (Ci/ml/0.5 g; spec. act. 2Ci/mmol) or ^3H uridine (Ci/ml/1 g; spec. act. 5Ci/mmol) and the incubation continued for 9 h.

Results

The antiviral effect against herpes simplex and poliovirus is shown in Table 3. None of the compounds exhibit any effect against herpes virus in comparison to Ribavirin. Derivatives 7a, 3a and 3b

slightly diminish the antiviral CPE of the poliovirus. 3a definitively possesses antiviral activity with a VR-0.7 in 3-100 μg/ml concentration limits. No toxic effect has been noticed up to these concentrations on uninfected cells but at 100 μg/ml morphological alterations of cells were determined together with the appearance of necrotic cells. These findings prompted the investigation of the influence of samples 7a and 7b, 3a and 3b on the survival and synthesis of cellular macromolecules in HeLa cells in the culture. In Figure 1 are shown the results of the experiment, where the cells were incubated with compound 7a and 7b, and 3a and 3b in a conc. of 133 μg/ml:1) for 3 days and 2) 2 or 6 h. They were washed afterwards and incubated in a fresh medium for an additional 3 days. It can be noticed that the compounds function cytotoxically. This effect is completely reversible in a 24 h period and after this time, the cells began to grow as in the untreated control. It must be emphasized that 2a is less effective in comparison to 7a, though it exhibits better reversibility. Compounds 7b and 3b possessing an acyclic substituent show a comparable effect on the cell growth. Table 4 presents the survival of HeLa and/or Vero cells treated with appropriate drugs in an exponential growth. The survival declines with a higher concentration of drugs. These data suggest a slightly stronger effect of 7a and 3a derivatives as compared to 7b and 3b. The same order was established with the effect on DNA and RNA synthesis (Figure 2). It is important to emphasize that 7a is a more potent inhibitor of the growth of DNA than 3a.

Conclusion

On the basis of these results, it may be concluded that 3- and/or 2-β-D-ribofuranosyl derivatives 7a and 3a possess a more potent antiviral and cytotoxic effect than their acyclo analogs 7b and 3b.

EXPERIMENTAL SECTION

(A) Chemistry

Melting points were determined on a Kofler microscope and are uncorrected. ^{1}H-nmr spectra were obtained at 200 MHz with a JEOL FX 200 spectrometer. ^{13}C-nmr spectra were determined with JEOL FX 90

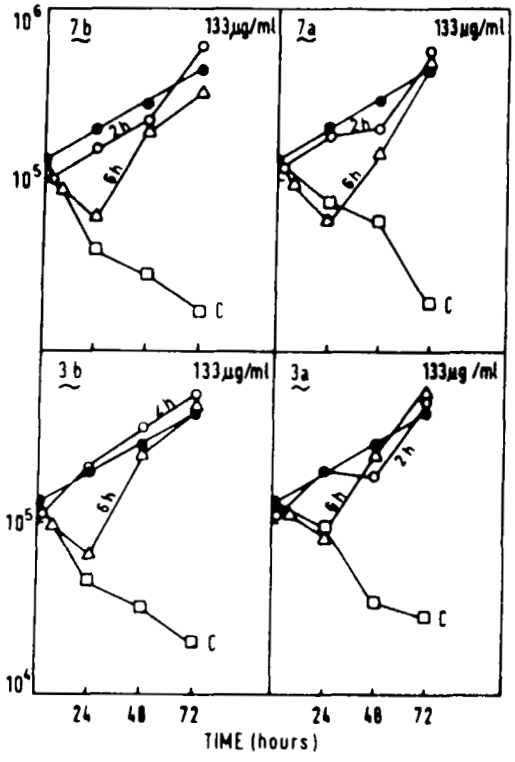


FIGURE 1. Effect of s-Triazolo [4,3-a] and [1,5-a] pyridine derivatives on Hela cell growth

Q and FX 200 instruments and the assignments made using the off resonance technique. Ultraviolet spectra were recorded with a Beckman Mo25 spectrophotometer.

Optical rotations were obtained on a Polatronic I, Schmidt/Haensch polarimeter. Mass spectra were run in a CEC 21-110B mass spectrometer.

Elemental analyses were performed by Dr.Tasovac at the Institute of Chemistry of the Faculty of Natural and Mathematical Sciences, Belgrade, Yugoslavia.

Thin layer chromatography (TLC) was performed with Merck silica gel 60 F_{254} plates; the spots were detected by irradiation with a Mineralight and by charring after spraying with 5 % H_2SO_4 in MeOH. Preparative chromatography was carried out with 20x20 plates covered

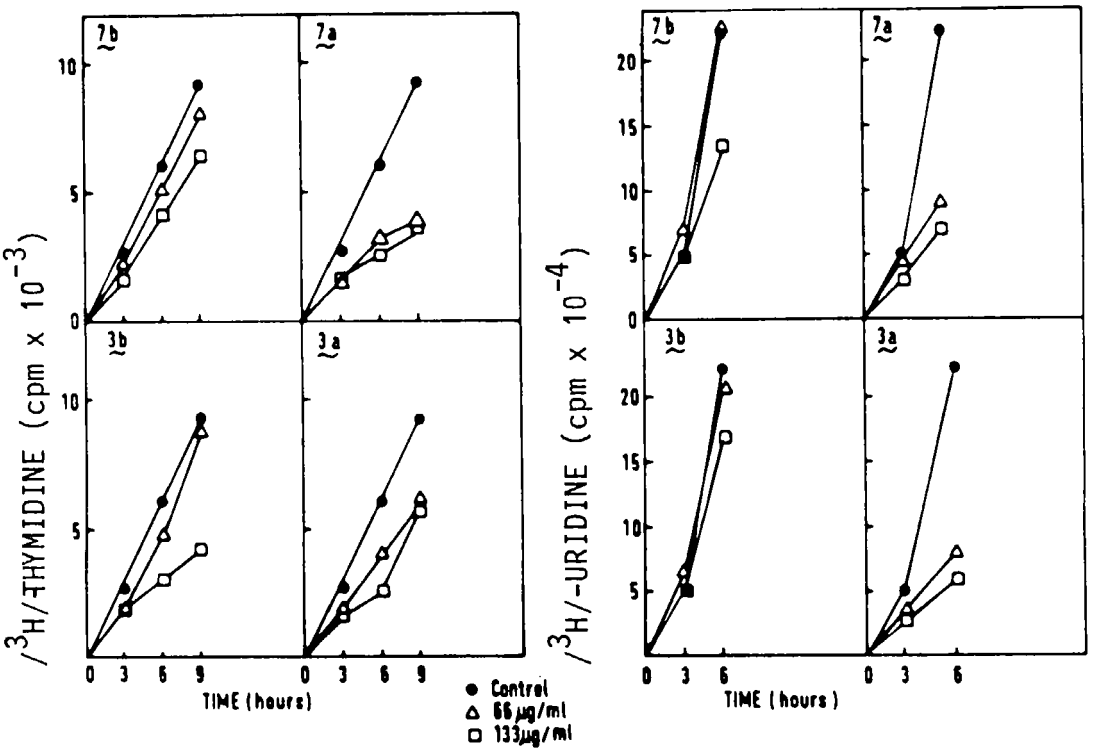


FIGURE 2. Effect of s-Triazolo [4,3-a] and [1,5-a] pyridine Derivatives on DNA(a) and RNA(b) synthesis

with a 2 mm layer of Merck silica gel PF_{254}. Merck silica gel (0.05-0.2 mm) was used for chromatographic separations. Purity of the products was determined by thin layer chromatography on silica gel.

Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within 0.4 % of the theoretical values.

3-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-8-nitro-s-triazolo [4,3-a]pyridine (7a, R_1=Benzoyl).

A mixture of 2.5 g (4.86 mmoles) of 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl-(2H)-tetrazole (5a, R_1=Benzoyl) and 4.5 g (30 moles) of 2-chloro-3-nitropyridine (6) in 40 ml of dry xylene was refluxed for

TABLE 3. Comparative in Vitro Antiherpes and Antipoliovirus Activity of Ribavirin and Certain -s-triazolo[4,3-a] and [1,5-a]pyridines

No.	Comp.	Virus ratings	
		HSV/I	POLIOVIRUS
7a (R_1=H)	3-β-D-ribofuranosyl-8-nitro-s-triazolo[4,3-a]pyridine	0	0.2
7b (R_1=H)	3-(2-hydroxyethoxy)methyl-8-nitro-s-triazolo[4,3-a]pyridine	0	0
3a (R_1=H)	2-β-D-ribofuranosyl-8-nitro-s-triazolo[1,5-a]pyridine	0.2	0.7
3b (R_1=H)	2- (2-hydroxyethoxy)methyl-8-nitro-s-triazolo[1,5-a]pyridine	0	0.3
2a (R_1=H)	3-β-D-ribofuranosyl-8-amino-s-triazolo[4,3-a]pyridine	0.15	0.3
2b (R_1=H)	3- (2-hydroxyethoxy)methyl-8-amino-s-triazolo[4,3-a]pyridine	0	0
8a /R_1=H)	2-β-D-ribofuranosyl-8-amino-s-triazolo[1,5-a]pyridine	0	0
8b (R_1=H)	2- (2-hydroxyethoxy)methyl-8-amino-s-triazolo [1,5-a]pyridine		
	Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide)	1.0	1.1

TABLE 4. a) The Effect of Certain s-triazolo[4,3-a] and [1,5-a]-pyridines on the Survival of HeLa Cells

Comp.	D_o (x10-5M)	D_q (x10-5M)	n
7a (R_1 = H)	11.2	2.60	1.35
7b (R_1 = H)	11.45	3.40	1.50
3a (R_1 = H)	9.8	3.85	1.90
3b (R_1 = H)	11.9	5.10	2.00

TABLE 4. b) The Effect of Certain s-triazolo[4,3-a] and [1,5-a]-pyridines on the Survival of Vero Cells

Comp.	μM	cell tox ($0\text{-}48^h$) % of control growth
7a (R = H)	20	45
3a (R = H)	20	60
7b (R = H)	50	45
3b (R = H)	50	45
2b (R = H)	100	65
8b (R = H)	100	60
2a (R = H)	100	80

18 h. Reaction was followed by TLC and was terminated after this time, in spite of 5 not being completely consumed. The solvent was removed and the residue dissolved in $CHCl_3$, washed with sodium hydrogen carbonate solution, the organic layer dried over anhydrous Na_2SO_4 and the conc. solution applied on a silica gel column (120 g/prepacked in $CHCl_3$/MeOH, 9.9:0.1) gave 1.20 g of 7a, which after crystallization from EtOAc-MeOH yielded 0.9 g (42 %, calculated on 6 consumed in the reaction). Unreacted 2-chloro-3-nitropyridine (3.9 g) was recovered from the column as a first fraction, and 150 mg of the 3-(5-benzoyloxymethylfuran)-8-nitro-s-triazolo[1,5-a]pyridine (3c), isolated as a foam, which followed the desired 7a. Final fractions consisted of a mixture (800 mg) of unidentified decomposition products of the starting nucleoside and 0.7 g of recovered 5.

m.p 198-199°C (from EtOAc-n-hexane), 7a ^1H NMR ($CDCl_3$) δ_{TMS}:8.70 (d,1,H_5,J_{56}=7H_2);5.96 (d,1,H_1,$J_{1',2'}$=3.5 H_2); Anal for ($C_{32}H_{24}N_4O_9$) Calc.: C, 63.16; H,3.98; N, 9.21, Found: C, 62.79; H, 4.34; 8.85

3c ^1H NMR ($DMSOd_6$) δ_{DSS}:9.23 (d,1,H_5,J_{56}=7Hz): 8,57 (d,1,H_7,J_{67}= 8Hz); 7.0 (d,1,H_3-furan ring, J_{34}=3.6Hz), 8.74 (d,1,H_4-furan ring); 5.5 (s,2,CH_2OBz); 7.38 (dd,1,H_6);m.p 207-210°C Anal for ($C_{18}H_{12}N_4O_5$); Calc: C,59.34; H,3.32; N,15.38 Found. 59.12;H,3.51;N,15.30. M^+abs.mass 364.081; Calc.364.080.

3-β-D-Ribofuranosyl-8-nitro-s-triazolo [4,3-a] pyridine (7a,R_1=H).

A mixture of 500 mg (0.82 mmoles) of 7a (R_1=benzoyl), 30 ml of MeOH and 2 ml of 2N methanolic sodium methoxide solution was kept at 30°C for 2 h. The solution was neutralized with Dowex 50WX3(H^+) resin. The resin was removed by filtration and thorougly washed with MeOH. The filtrate was concentrated and the residue adsorbed on 0.5 g of silica gel. This material was applied to a silica gel column (8 g) prepacked in $CHCl_3$. Elution with $CHCl_3$, followed by $CHCl_3$/MeOH 9.5: 0.5; 9:1; 8:2; and finally with a mixture of $CHCl_3$/MeOH 7:3 yielded 170 mg (48 %), m.p. 189-191°C (EtOH). ^1H NMR ($DMSOd_6$) δ_{TMS}: (H_5,J_{56}=7Hz); (H_7,J_{67}=7.5Hz); (H_6);($J_{1',2'}$=6Hz); (H_2'); (H_3'); (H_4'); ($H_{5'5''}$) UV max (EtOH: λ max=229 nm ε =16005; λ max=357 nm ε =4374. Anal for ($C_{11}H_{12}N_4O_6$) Calc: C,44.59, H,4.08; N, 18.91; Found: C,44.37; H, 4.54; N, 18.52. M^+: abs.mass 296.076; Calc. 296.076.

2- β -D-Ribofuranosyl-8-nitro-s-triazolo [1,5-a] pyridine (3a, R_1=H).

A solution of methanolic ammonia and 7a (R_1=H) 80 mg (0.27 mmoles) gave after 24 h 75 mg (94 %) of 3a. M.p 195-197°C (EtOH). All analytical and spectroscopic data are identical in all respects to the sample isolated by Tam Hynh-Dinh et al. (3). ^{1}H NMR (DMSO-d_6) δ_{TMS}: (H_5,J_{56}=7.5Hz); (H_7,J_{67}=8.5 Hz); (H_6); (H_1',$J_{1'2'}$=5.5Hz); (H_2'); (H_3'); (H_4'); ($H_{5'5''}$). UV max (EtOH): λ max=230 λ =12620. λ max=323 ε =4459. Anal for ($C_{11}H_{12}H_4O_6$) Calc. C,44.59; H,4.08;N, 18.91; Found C, 44.12; 4.38; N.18.62. M^+:abs.mass 296.075, Calc. 296.076.

3-(2-Benzoyloxyethoxy)methyl-8-nitro-s-triazolo [4,3-a] pyridine (7b,R_1=benzoyl) and 2-(2-benzoyloxyethoxy methyl-8-nitro-s-triazolo [1,5-a] pyridine (3b,R_1=benzoyl).

To a solution of 5-benzoyloxyethoxy/methyl-2(H)-tetrazole (5b, 2.48 g, 10 mmoles) and 5.5 g (35 mmoles) of 2-chloro-3-nitro-pyridine om 50 ml of dry xylene, 2 ml of dry xylene, saturated with dry HCl was added. The mixture was refluxed for 12 h. Solvent was removed, the residue was dissolved in $CHCl_3$ and the solution washed with saturated aqueous $NaHCO_3$ solution and dried over Na_2SO_4.

The excess solvent was removed and the residue was chromatographed on a silica gel column (60 g) prepacked in $CHCl_3$. Elution with $CHCl_3$, followed by $CHCl_3$/MeOH (9.8:0.2) and finally with $CHCl_3$/MeOH (9.6:0.4) gave 4.9 g of starting 2-chloro-3-nitro-pyridine and 400 mg (31 %) of 3b; m.p. 124.126°C (EtOAc: n-hexane), and 480 mg (37 %) of 7b; m.p 172-173°C (EtOAc: n-hexane:MeOH (percent): 7b ^{1}H NMR ($CDCl_3$) δ_{TMS}: 8.58 (d,1,H_5,J_{56}=6Hz); 8.20 (d,1,H_7,J_{67}; 6.84 (dd,1,H_6); 5.20 (s,2, OCH_2-Het); 4.46 (m2-CH_2OR); 3.93 (m,2,-CH_2CH_2). Anal for ($C_{16}H_{14}N_4O_5$) Calc. C. 56.14; H, 4.12; N.16.37, Found: C.56.41; H. 390; N. 16.73. 3b ^{1}H NMR ($CDCl_3$ δ_{TMS}: 8.94 (d,1,H_5,J_{56}=6Hz); 8.20 (d,1,H_7,J_{67}); 6.84 (dd,1,H_6);5,1 (s,2,OCH_2-Het); 4.55 (m,2,CH_2OR); 4.08 (m,2-CH_2CH_2). Anal. for ($C_{16}H_{14}N_4O_5$) Calc. C, 56.14; H, 4.12; N. 16.37; Found C. 55.92; H. 3.85; N. 15.94. M^+: abs. mass 342.097; Calc. 342.096.

3-(2-Hydroxyethoxy)methyl-8-nitro-s-triazolo [4,3] pyridine (7b, R_1=H)

The deprotection of 200 mg (0.58 mmoles) of 7b (R_1=benzoyl) was performed with a ml of 2N sodium methoxide solution in 40 ml MeOH at room temperature. The reaction was discontinued when the rearranged product 3b began to form (reaction was followed by TLC in $CHCl_3$/MeOH

9:1 mixture). The usual work up procedure, neutralization with Dowex and filtration column chromatography gave 120 mg (87 %) of 7b m.p 168-170°C (EtOAc-MeOH). ^{1}H NMR (DMSO-d_6) δ_{TMS} (H_5,J_{56}=6Hz); (H_7,J_{67}=7Hz); (H_6); 5.20 (s,2,OCH_2-Het); 3.60 (s,4,CH_2); UV max (EtOH); λmax=227 nm ε=13600, λmax=353 nm ε= 5236. Anal for ($C_9H_{10}N_4O_4$) Calc. C. 45.38; H. 4.23; N. 23.52; Found. C. 45.28; H,4.61, N, 23.16. M^+: Abs. mass 238.070. Calc. 238.070.

2-(-Hydroxyethoxy)methyl-8-nitro-s-triazolo[1,5-a]pyridine (3b, R_1=H).

Method A:

A solution of 7b, (R_1=benzoyl) 300 mg (0.88 mmoles) in methanolic ammonia (40 ml) yielded after 40 h, 170 mg (81 %) of 3b (R_1=H); m.p. 131-133°C (from MeOH). 3b ^{1}H NMR (DMSO-d_6) δ_{TMS}:(H_5,J_{56}=7Hz); (H_7,J_{67}=8Hz); (H_6); 4.80 (s,2,OCH_2-Het); 3.60 (s,4,CH_2); UV max (EtOH), λmax=228 ε=1437, λmax=325 ε=4900. Anal for ($C_9H_{10}N_4O_4$), Calc. C. 45.38; H. 4.23; N. 23.52; Found. C. 44.98; H. 4.76; N. 23.68. M^+: abs.mass 238.071, Calc. 238.070.

Method B:

300 mg (0.88 mmoles) 3b (R_1=benzoyl) in 30 ml of MeOH and 2 ml of 2N sodium methoxide solution was stirred for 2h. The usual work up procedure yielded 140 mg (67 %) of the product 3b (R_1=H), identical in all respects to the product obtained by method A.

2-Benzoyloxyethoxymethylcarbo-nitrile[13](4b, R_1=benzoyl).

Dry Cu/I/ cyanide (FLUKA) (17.5 g) in a 150 ml flask equipped with a 100 ml dropping funnel was heated to 80°C and 39 g of chloromethyl-ethylene-glycol-monobenzoate in 40 ml of dry benzene was added dropwise during a 1 h period. The mixture was then stirred and refluxed for 4 h, cooled to room temperature and the solid removed by filtration. Benzene was evaporated to yield 21 g (54 %) of 4b after distillation (1.2 torr b.p. 148°C).

Anal for ($C_{11}H_{11}N_1O_3$): C,H,N. Calc. C,64.36;H,5.40;N,6.82; Found:C,61.84, H,5.62;N,6.88. M^+: abs. mass 205.074, Calc. 205.074.

5-(Benzoyloxyethoxy)methyl-(2H)-tetrazole (5b, R_1=benzoyl)

To a solution of 20.5 g (100 mmoles) of 4b (R_1=benzoyl) and 5.8 g 108 mmoles of thoroughly dried NH_4Cl in freshly distilled DMF, (20 ml) 6.8 g (105 mmoles) of sodium azide was added. The suspension was

vigorously stirred for 3 h, under reflux. DMF was evaporated in vacuo at 70°C. The dark oily residue was chromatographed on a column (with 250 g) of silica gel prepacked in $CHCl_3$/MeOH (9.8:0.2) which were used for elution. Appropriate fractions were combined and evaporated to yield 14 g (56 %) of oily 6b, which had slowly crystallized in a refrigerator M.p 99-102°C. Anal for ($C_{11}H_{12}N_4O_3$), Calc: C. 53.22; H. 4.87; N. 2.57; Found: C. 53.29; H. 5.03; N. 22.71.

5-(2-Hydroxyethoxy)methyl-(2H)-tetrazole(5b,R_1=H).

4 ml of 1N sodium methoxide solution in MeOH was added to a solution of 5-(2-benzoyloxyethoxy)methyl-(2H)-tetrazole (992 mg, 4 mmoles) in 25 ml MeOH, and stirring was continued for 1 h at room temperature. TLC indicated that complete deprotection had occurred. The solution was then neutralized with Dowex 50 WX8/H^+/ resin, filtered and the resin washed with MeOH. The filtrate was evaporated and the oily residue slowly poured into 100 ml of diethyl ether. White oil separated. It was washed several times with 25 ml portions of diethyl ether, and this residual oily product was allowed to stand in a cool place. Several days afterwards, a white solid separated, which was further recrystallized from EtOAc to give 350 mg (60 %) of pure 5b with m.p. 155°C (decomp.). Anal for ($C_4H_8N_4O_2$) Calc. C. 33.33; H. 5.59; N. 38.87; Found: C. 32.90, H. 5.42, N. 38.51. M^+: Abs.mass 144.065, Calc. 144.065.

3-(2-Hydroxyethoxy)methyl-8-amino-s-triazolo- [4,3-a] pyridine (2b, R_1=H).

Method A:

320 mg (1.0 mmoles) of 7b, (R_1=benzoyl) was hydrogenated over Pd/C (10 %) in MeOH. After filtration, the solvent was removed and the residue crystallized from EtOAc to give 250 mg (80 %) of 3-(2-benzoyloxy-ethoxy) methyl-8-amino-s-triazolo[4,3-a]pyridine in the form of white crystals. m.p 151-153°C. ^{1}H-NMR ($CDCl_3$) δ_{TMS}: 7.95 (d,1,H_5,J_{56}=7Hz); 6.56 (dd,1,H_6); 6.30 (d,H_7,J_{67}=6.7Hz); 5.1 (s,2,OCH_2-Het); 4.48 (m, 2,OCH_2OR); 3.83 (m,2,CH_2CH_2). Anal. for ($C_{16}H_{16}N_4O_3$) Calc. C. 61.72; H. 5.16; N. 17.94 Found. C. 61.38; H. 5.53, N. 17.51. M^+: Abs. mass 312.122. Calc. 312.122.

80 mg (0,26 mmoles) of 2b (R_1=benzoyl) was deprotected in 0.5 ml 2N NaOMe in 10 ml MeOH (45 min). 80 WX 50 W (H^+)(0.7 g) was added, filtered and the resulting solution evaporated. Diethyl ether was

added, white crystals separated. m.p. = 82-84°C. UV max (EtOH). λ max = 297nm ε =9324 .

Method B:

A product indentical with 2b was obtained by hydrogenation of 7b, (R_1=H) in dioxane. The yield was 50 %. ^{1}H NMR (D_2O) δ_{TMS}(capillary): (H_5,J_{56}=6.5Hz); (H_6,$J_{6\,7}$=7Hz); (H_7); 4.90 (s,2,OCH_2-Het); 3.70 (m,4,-CH_2). Anal. for ($C_9H_{12}N_4O_2$).H_2O. Calc. C.47.78; H. 6.23, N. 24.76; Found. C. 48.05; H,5.82 N, 24.41. M^+: Abs. mass 208.096. Calc. 208.096.

2- (2-Hydroxyethoxy)methyl-8-amino-s-triazolo[1,5-a]pyridine 8b, R_1=H).

Method A:

320 mg (1.34 mmoles) of 3b (R_1=H) in 50 ml MeOH was hydrogenated over 30 mg Pd/C (10 %) at atmospheric pressure and room temperature. The catalyst was removed and the filtrate evaporated to dryness to yield on oily residue 8b (250 mg, 75 %).

Method B:

To a solution of 3b, (R_1=benzoyl 100 mg, 0.29 mmoles) in 10 ml of dioxane, 10 mg Pd/C (10 %) was added, and the mixture hydrogenated at room temperature and atm. pressure. 60 mg (72 %) of 2-(2-benzoyloxy ethoxy)methyl-8-amino-s-triazolo[1,5-a]pyridine was isolated as a dark foam. This derivative was submitted to deprotection with 1N sodium methoxide solution to give 8b with a 50 % yield, identical in all respects to the product obtained by procedure A,m.p.84-86°C.

^{1}H NMR (D_2O) δ_{DSS}: (H_5,J_{56}=6Hz); (H_6, from decoupled spectra); (H_7); 4.90 (s,2,0-CH_2-Het); 3.72 (s,4,CH_2CH_2)
Anal. for ($C_9H_{12}N_4O_3$) Calc: C. 51.91; H. 5.80; N. 26.91, Found: C. 51.62; 5.90; N. 26.78. M^+: Abs. mass 208.0955. Calc. 208.0960. UV max (EtOH). λmax=278 (ε=10.040).

3-(2,3,5-Tri-O.benzoyl-β-D-ribofuranosyl)-8-amino-s-triazolo [4,3-a]pyridine(8a, R_1=benzoyl)

7a (R_1=benzoyl, 12 mg was suspended in 10 ml EtOAc,12 mg Pd/C (10 %) was added and the reaction mixture hydrogenated at 50°C under low pressure with vigorous shaking. Hydrogen consumption was terminated within 2 h and the reaction mixture was allowed to stay under H_2 with shaking for additional 8 h. After filtration the solvent was evapora-

ted and the residue purified by column chromatography to yield 80 mg of white foam.

^{1}H NMR ($CDCl_3$) δ_{TMS}: 7.70 (d,1,H_5,J_{56}=6.8Hz); 6.2 (d,1,H_1',$J_{1'2'}$=7Hz) 6.1 (dd,1,H_6); 5.8 (d,1,H_7,J_{67}=5.4Hz). Anal. for ($C_{32}H_{24}N_4O_7$) Calc. C. 66.42; H. 4.53; N. 9.68. Found: C. 66.06; H. 4.56; N. 9.3. M^+ Abs. mass 578.

3-β-D-Ribofuranosyl-8-amino-s-triazolo[4,3-a]pyridine (2a, R_1=H)

Deblocking was achieved with NaOMe to yield a white solid; m.p 215-217°C. Analysis for ($C_{11}H_{14}N_4O_4.H_2O$). Calc. C. 46.47; H. 5.67; N. 19.71, Found: C. 46.24, H.6.01 N. 19.92. M^+: Abs mass. 266.1015. Calc. 266.1015.

^{1}H NMR (D_2O) δTMS (capillary): (H_5,J_{56}=6.8Hz); (H_6); (H_7,J_{67}=7.4Hz); ($H_{1'}$, $J_{1'2'}$=6.3Hz); (H_3'); (H_4'); ($H_{5'5''}$).

2-β-D-Ribofuranosyl-8-amino-s-triazolo[1,5-a]pyridine(8a,R_1=H).

A methanolic solution of 3a (80 mg, 0.27 mmoles) in 30 ml MeOH was hydrogenated over Pd/C (10 %) at room temperature and atm pressure. Hydrogen consumption was terminated in 45 minutes. After filtration, the solvent was evaporated and the residue chromatographed on a silica gel ($CHCl_3$-EtOH) to yield 60 mg (83 %) of white foam. Spectroscopic data were identical to compound 8a listed in ref.3. Anal. for ($C_{11}H_{14}N_4O_4$). Calc. C. 49.64; H. 5.30; N. 21.06. Found:C.49.31; H.5.65, N, 20.75. M^+: Abs. mass 266.101; Calc. 266.101.

Acknowledgements

This work was supported by NSF International Program YOR 81/035 and by Research Community of Slovenia. We thank Drs. L.B.Townsend and N.G. Johansson for many helpful suggestions and facilities, Mrs.Gunilla Brännström from ASTRA Läkemedel for cellular toxicity results and Mr.Thurin and Dr.B.Kralj for mass spectra determinations.

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14.

Note added in proof: 6 with 5-(5--O-benzoyl-2,3-isopropylidene-β-D-ribofuranosyl)-(2H)-tetrazole afforded 3-(5-O-benzoyl-2,3- isopropylidene-β-D-ribofuranosyl)-8-nitro-s-triazolo [4,3-a] pyridine as a major product, confirming the beta configuration according to $\Delta\delta$ (14.5 Hz) for ^1H signals of the isopropylidene methyl groups and 25.4 and 27.4 ppm in ^{13}C nmr spectra respectively. (^1H NMR ($CDCl_3$) δ_{TMS}: 8.56 (d, 1, H_5, J_{56} = 7 Hz); 8.14 (dd, 1, Hz; J_{67}=7.5 Hz); 6.80 (dd, 1, H_6); 5.93 (dd, 1, H_2'), 5.55 (d, 1, H_1', $J_{1'2'}$=2Hz). 4.91 (dd, 1, H_3'), 4.30 (m, 1, H_4'); 4.1 (m, 2, $H_{5'5''}$); 1.55 (s, 3, Me); 1.40 (s, 3, Me); ^{13}C NMR ($CDCl_3$) δ_{TMS} 1.46.12 (C_3) 114.38 ($\underline{C}(Me)_2$); 111.67 (C_6); 85.12 (C_1'); 83.61 (C_2'), 82.31 (C_3'), 78.84 (C_4'); 63.56 (C_5'); 27.16 (Me); 25.42 (Me).

Received April 15, 1985.