170. 8-Aza-2′-deoxyguanosine and Related 1,2,3-Triazolo[4,5-*d*]pyrimidine 2′-Deoxyribofuranosides

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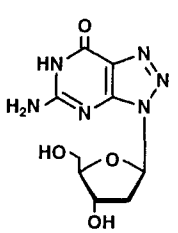
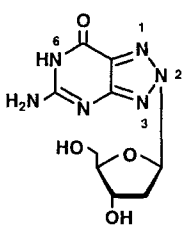
(1.VII.93)

The synthesis of 8-azaguanine N^9-, N^8-, and N^7-(2′-deoxyribonucleosides) **1–3**, related to 2′-deoxyguanosine (**4**), is described. Glycosylation of the anion of 5-amino-7-methoxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (**5**) with 2-deoxy-3,5-di-*O*-(4-toluoyl)-α-D-*erythro*-pentofuranosyl chloride (**6**) afforded the regioisomeric glycosylation products **7a**/**7b**, **8a**/**8b**, and **9** (*Scheme 1*) which were detoluoylated to give **10a**, **10b**, **11a**, **11b**, and **12a**. The anomeric configuration as well as the position of glycosylation were determined by combination of UV, ^{13}C-NMR, and ^{1}H-NMR NOE-difference spectroscopy. The 2-amino-8-aza-2′-deoxyadenosine (**13**), obtained from **7a**, was deaminated by adenosine deaminase to yield 8-aza-2′-deoxyguanosine (**1**), whereas the N^7- and N^8-regioisomers were no substrates of the enzyme. The N-glycosylic bond of compound **1** (0.1N HCl) is *ca.* 10 times more stable than that of 2′-deoxyguanosine (**4**).

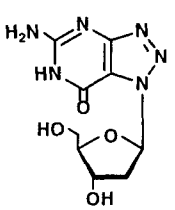
Introduction. – The 8-azaguanine (= pathocidin; **16**) [1] is a naturally occurring guanine derivative showing antifungal, antiviral, and anticancer activity [2–4]. Nucleosides of 8-azaguanine (purine numbering is used throughout the *General Part*) were prepared [5–7]. A non-stereoselective synthesis of the 2′-deoxyribonucleoside **1** was reported [8]. Recently, 8-azaguanine 2′,3′-dideoxyribonucleosides were synthesized [9]. It was observed that the glycosylation of 8-azaadenine anion proceeds stereoselectively [10]. According to the kinetic control of the reaction, the formation of three regioisomers is expected, as it was found in the case of the 2′,3′-dideoxynucleosides [9]. Consequently, nucleosides with unusual glycosylation sites, *e.g.* **2** or **3**, will become accessible. They can be used to study the recognition of unusual linked bases within oligonucleotide duplex structures. Similar work was already done in the case of N^7-linked adenine and guanine 2′-deoxyribonucleosides [11] [12]. In the following, we report on the synthesis and properties of regioisomeric 8-azaguanine 2′-deoxyribonucleosides **1–3** related to 2′-deoxyguanosine (**4**).



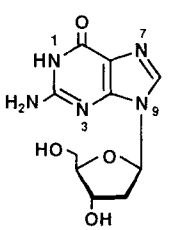
1



2
systematic numbering



3

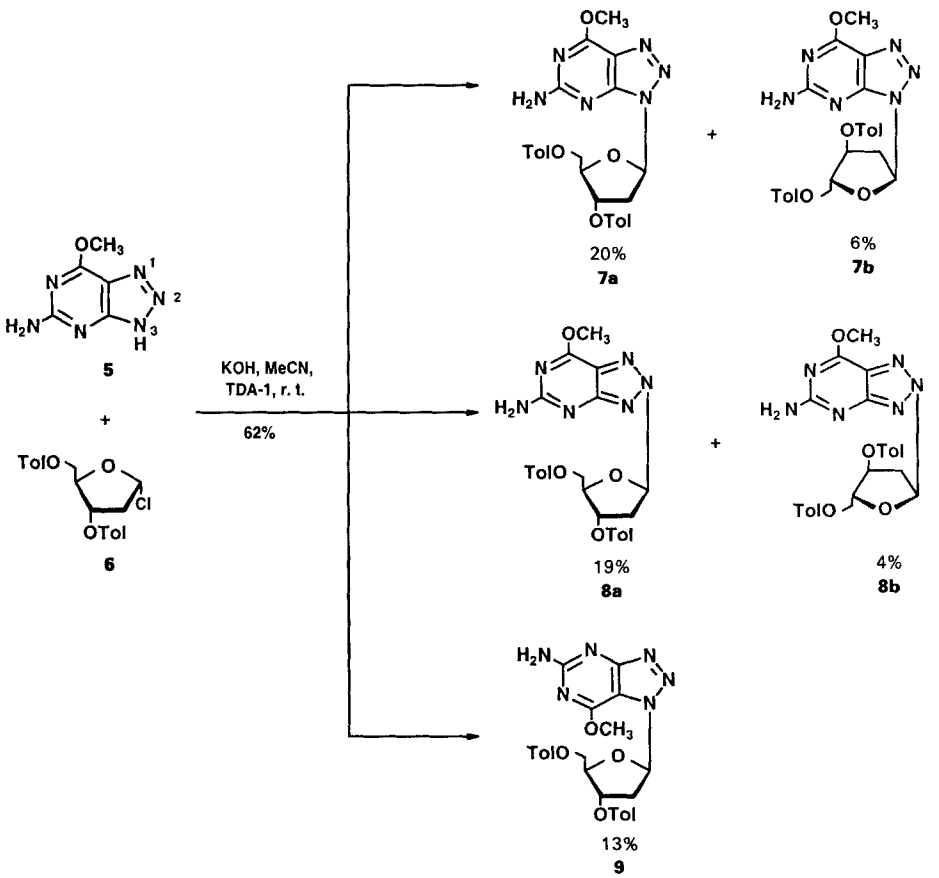


4
purine numbering

Results and Discussion. – The fusion of silylated 8-azaguanine with 2-deoxy-3,5-di-*O*-toluoyl-D-*erythro*-pentofuranosyl chloride yielded an anomeric mixture of N^9- glycosylation products [8]. The β-D-isomer was isolated in 15% yield and the α-D-compound in 9%, both yields based on 8-azaguanine as starting material. As the reaction was performed at elevated temperature, the N^9-isomer was formed exclusively which can be explained by thermodynamic control.

Other regioisomeric 8-azaguanine 2′-deoxyribonucleosides should be accessible, if the anion of **5** is used for glycosylation. The triazole ring of 8-azapurines is easier to deprotonate than the imidazole moiety of purines (pK_{BH} (**16**) = 6.54 [13]; pK_{BH}(guanine) = 9.42 [14]). Therefore, nucleobase-anion glycosylation should proceed smoothly at ambient temperature under stereoselective control [17]. For the glycosylation studies, we chose 5-amino-7-methoxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine [15] (**5**) as base. Preparation of **5** followed a route according to [9] using 2,4,5-triaminopyrimidin-6-ol as starting material. Treatment with $POCl_3$ gave the 6-chloro derivative [15], pentyl nitrite furnished the 5-amino-7-chloro-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine which was converted into **5**

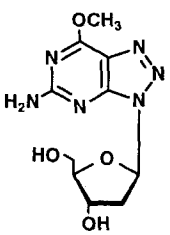
Scheme 1



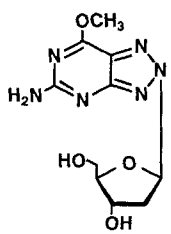
with NaOMe [15]. The MeO group of **5** protects the six-membered ring during glycosylation and allows a later conversion into the oxo function.

The glycosylation of **5** with 2-deoxy-3,5-di-*O*-(4-toluoyl)-α-D-*erythro*-pentofuranosyl chloride [16] (**6**) was carried out in MeCN in the presence of a five-fold excess of powdered KOH and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1), as described for the synthesis of 8-aza-2′-deoxyadenosine and other base-modified 2′-deoxyribonucleosides [17] [10]. The reaction mixture was separated by repeated flash chromatography (FC). The five glycosylation products **7a**/**7b**, **8a**/**8b**, and **9** were isolated after chromatographic separation in 62% overall yield. They gave three zones during the first chromatographical workup with AcOEt/cyclohexane 3:2 as eluent, *i.e.* **7a**/**7b**, **8a**/**8b**, and **9**. The anomers **7a**/**7b** and **8a**/**8b** were separated in a second purification step using the same eluent. The ratio of the regioisomers ($N^9/N^8/N^7$) was *ca.* 2:2:1.

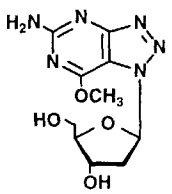
Next, the compounds **7a–9** were deprotected separately: Treatment with 0.1M NaOMe yielded the detoluoylated compounds **10a**, **10b**, **11a**, **11b**, and **12**, respectively, which were all isolated crystalline. The 8-azaguanine nucleosides **1–3** were obtained from the methoxy derivatives **10a**, **11a**, and **12**, respectively, by nucleophilic displacement with 0.1N NaOH.



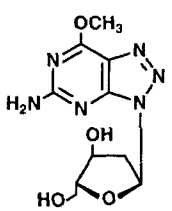
10a



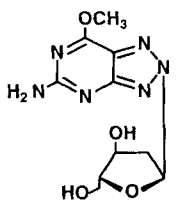
11a



12



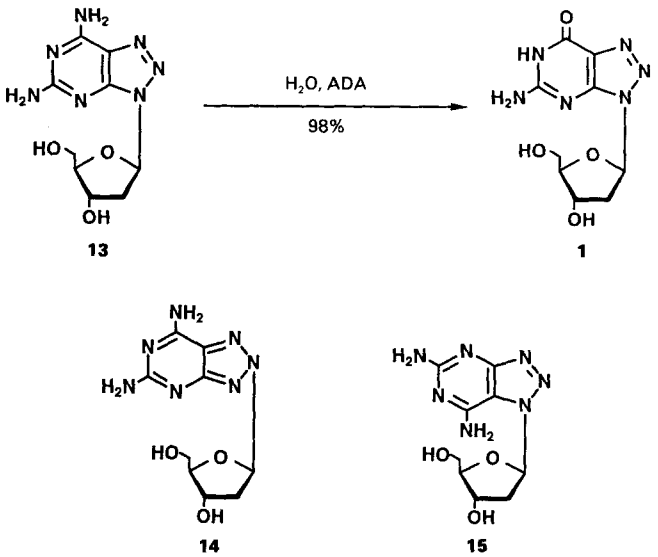
10b



11b

Treatment of **7a**, **8a**, and **9** with $MeOH/NH_3$ gave the diamino compounds **13–15**, respectively. The conversion of **13** into 8-aza-2′-deoxyguanosine (**1**) was best performed with adenosine deaminase in H_2O at 25° and was complete within a few h (*Scheme 2*); compounds **14** and **15** are no substrates for the enzyme. Kinetic data of the deamination of **13** were determined according to *Michaelis-Menten* [18]. Stock solutions (40–280 μM) were prepared and 0.02 mg of enzyme added. The initial velocities were taken from the increase of absorbance at 250 nm. A K_m value of 174 μM and a v_{max} of 11.5 $mM \cdot min^{-1} \cdot mg^{-1}$ was determined, indicating the good substrate properties of **13**. The 2-aminoadenosine and 8-azaadenosine show K_m values of 18 and 130 μM [19].

Scheme 2



The UV spectra of the regioisomeric 8-aza-O^6-methylguanine 2′-deoxyribonucleosides **10a**, **11a**, and **12** show the same spectroscopic characteristics as those of the ribo [6] (*Table 1*) and 2′,3′-dideoxyribo compounds [9]. The same is found for the regioisomeric 8-azaguanine nucleosides **1**–**3**. In both cases, the N^9-isomer exhibits UV maxima which are definitely different from those of the other isomers. We also used

Table 1. *UV Data of 8-Azapurine 2′-Deoxyribofuranosides* **10**–**15** *and* **1**–**3** *and Corresponding Ribofuranosides*

	λ_{max} (MeOH)	λ_{max} (0.1N HCl)	
10a (N^9, β-D)	246 (6100), 288 (9900)	243, 282	
b (N^9, β-D)	246 (5900), 288 (9600)	243, 282	
11a (N^8, β-D)	312 (8000)	243, 294	
b (N^8, α-D)	312 (8200)	243, 294	
12 (N^7, β-D)	315 (4000)	294	
N^9-Rib.[a])	246 (5400), 287 (10600)	244, 283	
N^8-Rib.[a])	311 (8300)	243, 292	
N^7-Rib.[a])	313 (4500)	294	
13 (N^9, β-D)	227 (23100), 286 (10300)	256, 330	
14 (N^8, β-D)	262 (6100), 323 (8000)	262, 285	
15 (N^7, β-D)	226 (16200), 315 (5700)	253, 274	
Compound	λ_{max} (MeOH)	λ_{max} (0.1N NaOH)	λ_{max} (0.1N HCl)
1 (N^9, β-D)	257 (12700)	279	255, 272 (sh)
2 (N^8, β-D)	240 (sh), 296 (5900)	303	274
3 (N^7, β-D)	240 (sh), 300 (4400)	245, 294	250 (sh), 272
N^9-Rib.[a])	256 (12900), 275 (sh)	278	255, 275
N^8-Rib.[a])	240 (sh), 304	308	277
N^7-Rib.[a])	240 (sh), 300	245, 298	273

[a]) Taken from [6]. Measured in H_2O (pH 7) instead of MeOH.

Table 2. *^{1}H-NMR-NOE Data and Chromatographic Mobilities of Anomeric 8-Azaguanine 2′-Deoxynucleosides*

	NOE [%] at H–C(4′)[a])[b])	NOE [%] at MeO[a])[b])	R_f[c])
10a (N^9, β-D)	2.2	0	0.62
11a (N^8, β-D)	1.5	0	0.53
12 (N^7, β-D)	2.3	1.7	0.40
10b (N^9, α-D)	*ca.* 1	0	0.59
11b (N^8, α-D)	0	0	0.46

[a]) Irradiation of H–C(1′). [b]) Measured in (D_6)DMSO. [c]) TLC (silica gel, $CHCl_3$/MeOH 9:1).

^{1}H-NMR NOE difference spectroscopy for structure determination (*Table 2*). A NOE at the MeO group is observed if H–C(1′) is irradiated in the case of the N^7-isomer **12**. The β-D-configuration of **10a**, **11a**, **12** is confirmed by NOE's at H–C(4′) upon irradiation of H–C(1′). A smaller NOE is observed in the case of α-D-anomer **10b**, which is due to the three-spin effect [20]. The regioisomeric nucleosides **1**–**3** can also be identified by HPLC and distinguished from their base **16** (*Fig.*).

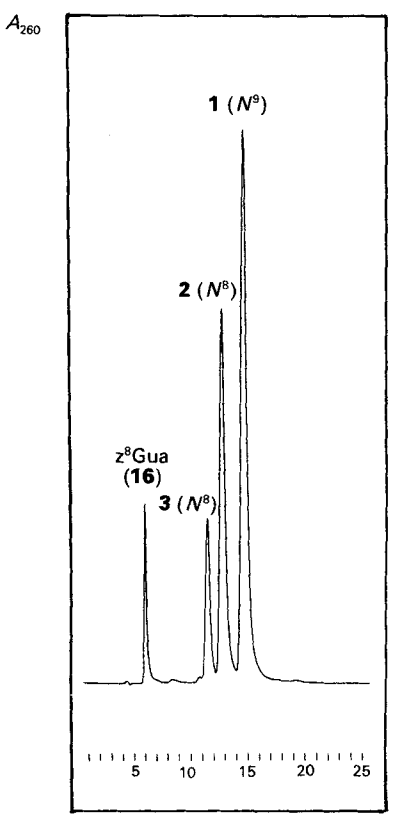


Figure. *HPLC Profile of the regioisomeric 8-azaguanine nucleosides* **1**–**3** *and 8-azaguanine* (**16**). *RP-18* Column, 5% MeCN in 0.1M (Et_3NH)OAc, flow rate 0.6 ml/min.

The assignment of ^{13}C-NMR chemical shifts of 8-azaguanine nucleosides is difficult. Gated-decoupled spectra do not give information regarding the ^{13}C assignment, because ring C-atoms do not carry H-atoms. Also the 'high-*anti*'-conformation around the N-glycosylic bond [21] prevents coupling between C(4) and the anomeric proton. A comparison of the ^{13}C-NMR spectra of **1** and of 8-azaguanine (**16**) in aqueous alkaline solution and in (D_6)DMSO allows a tentative assignment (*Table 3*).

All C-atoms of **16** are shifted downfield (6–11 ppm) in alkaline solution, except C(5). As the assignment of C(5) is unequivocal, these findings are in agreement with the structure of dianion **17** (*Scheme 3*). As already mentioned, the position of the glycosylation of **1** can be deduced from the UV spectra. Moreover, this nucleoside is the only compound among the regioisomeric 8-azaguanine nucleosides to be formed on deamination by adenosine deaminase. Earlier, ^{13}C-NMR spectra of **16** were measured in alkaline solution [22], and spectra of 8-azaguanine

Scheme 3

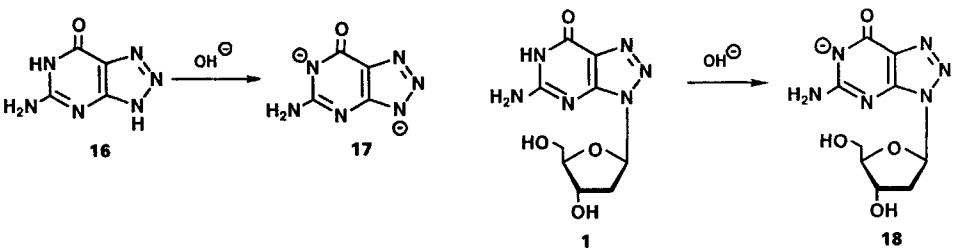


Table 3. *^{13}C-NMR Chemical Shifts* ((D_6)DMSO, 25°) *of 8-Aza-2′-deoxyguanosine and Related 1,2,3-Triazolo[4,5-d]pyrimidine 2′-Deoxyribofuranosides*

	C(7)[a])[b]) C(6)[a])[c])	C(5)[a])[b]) C(2)[a])[c])	C(3a)[b]) C(4)[c])	C(7a)[b]) C(5)[c])	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
16	156.3	155.1	153.8[a])	123.8					
16[d])	168.0	161.2	160.5[a])	125.0					
1	155.7	155.7	151.5	124.5	83.9	38.0	70.9	88.2	62.2
1[e])	157.5	155.4	150.9	124.1	84.6	–	70.7	87.1	61.4
1[d])	167.2	163.5	151.1	125.5	84.8	–	71.0	87.5	61.9
2	159.5	154.4	156.7[a])	127.2	92.8	DMSO	70.7	88.6	62.2
3	153.7	153.9	161.3[a])	113.1	87.4	DMSO	70.8	88.4	62.0
7a	161.4	162.6	153.3	120.9	84.3	35.2	74.8	82.1	63.9
b	161.3	162.5	153.3	120.8	84.7	35.8	74.2	81.9	64.0
8a	162.0	161.7	161.3[a])	123.0	92.9	36.6	74.0	82.4	63.6
b	162.0	161.7	161.6[a])	122.5	93.5	37.6	74.0	83.5	64.0
9	157.2	161.0	164.9[a])	109.2	87.9	35.9	74.5	82.2	63.7
10a	161.4	162.4	153.2	120.8	84.4	37.9	70.9	88.2	62.2
b	161.4	162.3	152.9	120.9	83.9	DMSO	70.0	86.3	60.8
11a	162.0	161.4	161.5[a])	122.6	93.2	DMSO	70.7	88.7	62.2
b	161.9	161.6	161.1[a])	122.4	92.7	DMSO	69.9	87.0	60.8
12	157.2	161.0	164.8[a])	109.4	87.8	38.4	70.7	88.5	60.8
13	156.1	162.7	151.5	120.5	84.2	37.8	70.9	88.0	61.0
14	162.8	160.2	156.8[a])	122.9	92.8	DMSO	70.6	88.6	62.3
15	151.7	161.6	164.3[a])	109.1	88.0	DMSO	70.3	88.6	61.2

[a]) Tentative. [b]) Systematic numbering. [c]) Purine numbering. [d]) Measured in 0.1N NaOH/(D_6)DMSO 9:1. [e]) Measured in H_2O/(D_6)DMSO 9:1.

dideoxyribonucleosides were reported in (D_6)DMSO [9]. The spectra of **16** and of the base moiety of **1** are similar, implying that the main tautomer of the base is the 3*H*-isomer (systematic numbering). In aqueous NaOH solution, nucleoside **1** is expected to be present as monoanion **18** (*Scheme 3*), resulting in downfield shifts of C(2) and C(6) which are actually observed. The signal of C(4) is not affected, in contrast to the situation of dianion **17** of 8-azaguanine (*Table 3*). Compound **1** and N^7-regioisomer **3** show the expected shift changes of C(4) and C(5). The assignment of the N^8-isomer **2** results from the absence of a NOE at the 2-NH_2 group, which should be observed, if the sugar moiety is attached to N^1 or N^3. Apart from these findings, typical chemical-shift differences are observed for the anomeric C-atoms of the regioisomeric 8-azaguanine nucleosides, with lowest value for the N^9- and the highest for the N^8-compounds, including the O^6-methylnucleosides **7a–9**, and their detoluoylated derivatives [9]. The assignment of C(2) *vs.* C(6) is still tentative.

It is known that ribo- and 2′-deoxyribonucleosides loose their activity in cells by N-glycosylic-bond hydrolysis catalyzed by phosphorylases. *Table 4* summarizes the half-life values of the nucleosides in 0.1N HCl. Within the series of N^8- and N^7-nucleosides, the diamino compounds are more stable than the amino/methoxy or amino/oxo derivatives.

Table 4. *Half-Life Values ($t_{1/2}$) of N-Glycosylic-Bond Hydrolysis of 8-Azaguanine 2′-Deoxyribofuranosides*[a])

	$t_{1/2}$ [min]	λ_{max}		$t_{1/2}$ [min]	λ_{max}
1 (N^9, β-D)	174	255	**12** (N^7, β-D)	7[b])	282
2 (N^8, β-D)	59	270	**13** (N^9, β-D)	220	270
3 (N^7, β-D)	1.8[b])	250	**14** (N^8, β-D)	247	285
dG	13	265	**15** (N^7, β-D)	12[b])	253
10a (N^9, β-D)	85	282	**10b** (N^9, β-D)	130	265
11a (N^8, β-D)	115	282	**11b** (N^8, α-D)	170	291

[a]) Measured at 40° in 0.1N HCl. [b]) 0.01N HCl.

The β-D-nucleosides are hydrolyzed slightly faster than the α-D-compounds which is in line with 8-azaadenine 2′-deoxyribonucleosides [10]. The N^7-isomers are extremely labile [23], whereas the N^8- and the N^9-isomers are comparably stable. Compared to 2′-deoxyguanosine (dG), the 8-aza derivative **1** is more stable in acid and does not show its unfavourable aggregation properties. The aggregation was already avoided on the monomeric level [24] as well as on oligonucleotides [25], if 7-deazaguanine was replacing guanine. The same is expected for oligonucleotides containing 8-azaguanine. These experiments are under current investigation and will be publised in the near future.

Experimental Part

General. See [10]. Adenosine deaminase from calf-intestine mucosa (EC 3.5.4.4) was purchased from *Boehringer*, Mannheim, Germany. TLC: glass plates coated with a 0.25-mm layer of silica gel *Sil G-25* with fluorescent indicator UV_{254} (*Merck*, Germany). Column flash chromatography (FC): silica gel *60 H* at 0.5 bar. TLC Scanning: *CS-930-TLC* scanner (*Shimadzu*, Japan). HPLC: *Merck-Hitachi*, model *655-12* with proportioning valve, model *665A* variable-wavelength monitor, model *L-5000* controller, and *D-2000* integrator; 4 × 25 cm *RP-18-LiChrosorb* column (*Merck*, Germany). M.p.: *Büchi-SMP-20* apparatus (*Büchi*, Switzerland); uncorrected. NMR Spectra: *AC-250-Bruker* and *500-Bruker* spectrometer.

*Glycosylation of 5-Amino-7-methoxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine* (**5**) *with 2-Deoxy-3,5-di-*O*-(4-toluoyl)-*α*-*D*-erythro-pentofuranosyl Chloride* (**6**). To a soln. of **5** (525 mg, 3.61 mmol) in dry MeCN (150 ml), powdered KOH (1.012 g, 18.04 mmol) and TDA-1 (20 µl) were added under stirring. After 30 min, **6** (2.10 g, 5.44 mmol) was added portionwise within 10 min. Stirring was continued for 20 min. The mixture was filtered over

Celite, the solvent evaporated, and the residue submitted to FC (silica gel *60 H*, column 20 × 4 cm, 0.5 bar, cyclohexane/AcOEt 2:3). Three zones I–III were separated, two of them containing an anomeric mixture.

*5-Amino-3-[2-deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-*erythro*-pentofuranosyl]-7-methoxy-3*H*-1,2,3-triazolo[4,5-*d*]-pyrimidine* (**7a**). The fast migrating zone I was separated by a second FC (silica gel, column 5 × 25 cm, cyclohexane/AcOEt 2:3). From the faster migrating part, **7a** (331 mg, 20%) was obtained. Colourless foam. TLC (cyclohexane/AcOEt 1:1): R_f 0.6. UV (MeOH): 284 (10680), 241 (36000). ^{1}H-NMR ((D$_6$)DMSO): 7.95–7.73, 7.38–7.27 (2*m*, NH_2, arom. H); 6.60 (*t*, *J* = 6.3, H–C(1′)); 5.86 (*m*, H–C(3′)); 4.57 (*m*, H–C(4′)); 4.45 (*m*, CH_2(5′)); 4.06 (*s*, MeO); 3.35 (*m*, H_β–C(2′)); 2.83 (*m*, H_α–C(2′)); 2.34, 2.37 (2*s*, 2 Me). Anal. calc. for $C_{26}H_{26}N_6O_6$ (518.5): C 60.23, H 5.05, N 16.21; found: C 60.20, H 5.14, N 16.21.

*5-Amino-3-[2-deoxy-3,5-di-*O*-(4-toluoyl)-α-*D*-*erythro*-pentofuranosyl]-7-methoxy-3*H*-1,2,3-triazolo[4,5-*d*]-pyrimidine* (**7b**). The slower migrating part of zone I yielded **7b** (105 mg, 6%). Colourless foam. TLC (cyclohexane/AcOEt 1:1): R_f 0.56. UV (MeOH): 283 (9100), 240 (33500). ^{1}H-NMR ((D$_6$)DMSO): 7.88–7.82, 7.30–7.19 (2*m*, NH_2, arom. H); 6.59 (*t*, *J* = 5.5, H–C(1′)); 5.60 (*m*, H–C(3′)); 4.75 (*m*, H–C(4′)); 4.51 (*m*, CH_2(5′)); 4.03 (*s*, MeO); 3.15 (*m*, H_β–C(2′)); 2.90 (*m*, H_α–C(2′)); 2.36 (*s*, 2 Me). Anal. calc. for $C_{26}H_{26}N_6O_6$ (518.5): C 60.23, H 5.05, N 16.21; found: C 60.03, H 5.13, N 16.13.

*5-Amino-2-[2-deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-*eyrythro*-pentofuranosyl]-7-methoxy-2*H*-1,2,3-triazolo[4,5-*d*]-pyrimidine* (**8a**). Zone II was separated on silica gel *60 H* (25 × 5 cm, cyclohexane/AcOEt 2:3) yielding as faster migrating part **8a** (308 mg, 19%). Colourless foam. TLC (cyclohexane/AcOEt 1:1): R_f 0.3. UV (MeOH): 311 (7600), 240 (35900). ^{1}H-NMR ((D$_6$)DMSO): 7.94–7.22, 7.37–7.24 (2*m*, arom. H); 6.96 (*s*, NH_2); 6.63 (*m*, H–C(1′)); 5.88 (*m*, H–C(3′)); 4.61 (*m*, H–C(4′)); 4.43 (*m*, CH_2(5′)); 4.04 (*s*, MeO); 3.20 (*m*, H_β–C(2′)); 2.87 (*m*, H_α–C(2′)); 2.39, 2.36 (2*s*, 2 Me). Anal. calc. for $C_{26}H_{26}N_6O_6$ (518.5): C 60.23, H 5.05, N 16.21; found: C 60.13, H 5.02, N 16.21.

*5-Amino-2-[2-deoxy-3,5-di-*O*-(4-toluoyl)-α-*D*-*erythro*-pentofuranosyl]-7-methoxy-2*H*-1,2,3-triazolo[4,5-*d*]-pyrimidine* (**8b**). The slower migrating part of II yielded **8b** (66 mg, 4%). Colourless foam. TLC (cyclohexane/AcOEt 2:3): R_f 0.26. UV (MeOH): 313 (7700), 240 (34800). ^{1}H-NMR ((D$_6$)DMSO): 7.94–7.17, 7.37–7.17 (2*m*, arom. H); 6.88 (*s*, NH_2); 6.68 (*d*, H–C(1′)); 5.58 (*m*, H–C(3′)); 4.81 (*m*, H–C(4′)); 4.53 (*m*, CH_2(5′)); 4.02 (*s*, MeO); 3.12 (*m*, H_β–C(2′)); 2.90 (*m*, H_α–C(2′)); 2.36 (*s*, Me). Anal. calc. for $C_{26}H_{26}N_6O_6$ (518.5): C 60.23, H 5.05, N 16.21; found: C 60.22, H 5.18, N 16.27.

*5-Amino-1-[2-deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-*erythro*-pentofuranosyl]-7-methoxy-1*H*-1,2,3-triazolo[4,5-*d*]-pyrimidine* (**9**). Zone III was pure **9** (221 mg, 13%). Colourless crystals from MeOH. M.p. 142°. TLC (cyclohexane/AcOEt 2:3): R_f 0.16. UV (MeOH): 317 (4100), 238 (40400). ^{1}H-NMR ((D$_6$)DMSO): 7.94–7.75, 7.37–7.25 (2*m*, arom. H); 6.83–6.75 (*m*, NH_2, H–C(1′)); 5.83 (*m*, *J* = 4.4, H–C(3′)); 4.64 (*m*, H–C(4′)); 4.33 (*m*, CH_2(5′)); 4.07 (*s*, MeO); 3.51 (*m*, H_β–C(2′)); 2.89 (*m*, H_α–C(2′)); 2.39, 2.36 (2*s*, 2 Me). Anal. calc. for $C_{26}H_{26}N_6O_6$ (518.5): C 60.23, H 5.05, N 16.21; found: C 60.03, H 5.21, N 16.29.

*5-Amino-3-(2-deoxy-β-*D*-erythro-pentofuranosyl)-7-methoxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine* (**10a**). A soln. of **7a** (540 mg, 1.04 mmol) in NaOMe (50 ml, 0.2M) was stirred for 2 h at r.t. The soln. was evaporated, the residue adsorbed on silica gel *60 H* and applied onto the top of a silica-gel column (15 × 2.5 cm, $CHCl_3$/MeOH 8:2). Colourless foam (188 mg, 64%), which gave colourless crystals from MeOH. M.p. 173°. TLC ($CHCl_3$/MeOH 8:2): R_f 0.62. UV (MeOH): 288 (10200), 246 (6100). ^{1}H-NMR ((D$_6$)DMSO): 7.19 (*s*, NH_2); 6.40 (*t*, *J* = 6.3, H–C(1′)); 5.33 (*d*, *J* = 4.5, OH–C(3′)); 4.76 (*t*, *J* = 5.7, OH–C(5′)); 4.48 (*m*, H–C(3′)); 4.05 (*s*, MeO); 3.85 (*m*, H–C(4′)); 3.42 (*m*, CH_2(5′)); 2.97 (*m*, H_β–C(2′)); 2.35 (*m*, H_α–C(2′)). Anal. calc. for $C_{10}H_{14}N_6O_4$ (282.3): C 42.55, H 4.99, N 29.77; found C 42.74, H 5.20, N 29.62.

*5-Amino-3-(2-deoxy-α-*D*-erythro-pentofuranosyl)-7-methoxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine* (**10b**). From **7b** (200 mg, 0.39 mmol) as described for **10a**: **10b** (75 mg, 68%). Colourless crystals from MeOH. M.p. 140°. TLC ($CHCl_3$/MeOH 8:2): R_f 0.59. UV (MeOH): 288 (9600), 246 (5900). ^{1}H-NMR ((D$_6$)DMSO): 7.20 (*s*, NH_2); 6.35 (*t*, *J* = 6.55, H–C(1′)); 5.49 (*d*, *J* = 5.80, OH–C(3′)); 4.79 (br. *s*, OH–C(5′)); 4.19 (*m*, H–C(3′)); 4.05 (*s*, MeO); 4.01 (*m*, H–C(4′)); 3.58 (*m*, CH_2(5′)); 2.79 (*m*, CH_2(2′)). Anal. calc. for $C_{10}H_{14}N_6O_4$ (282.3): C 42.55, H 4.99, N 29.77; found: C 42.63, H 4.93, N 29.62.

*5-Amino-2-(2-deoxy-β-*D*-erythro-pentofuranosyl)-7-methoxy-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidine* (**11a**). From **8a** (580 mg, 1.12 mmol) as described for **10a**: foam. Crystallization from MeOH gave colourless crystals (260 mg, 82%). M.p. 170°. TLC ($CHCl_3$/MeOH 8:2): R_f 0.53. UV (MeOH): 312 (8000). ^{1}H-NMR ((D$_6$)DMSO): 6.90 (*s*, NH_2); 6.40 (*m*, H–C(1′)); 5.39 (*d*, *J* = 3.2, OH–C(3′)); 4.76 (*t*, *J* = 5.4, OH–C(5′)); 4.36 (*m*, H–C(3′)); 4.07 (*s*, MeO); 3.89 (*m*, H–C(4′)); 3.44 (*m*, CH_2(5′)); 2.75 (*m*, H_β–C(2′)); 2.37 (*m*, H_α–C(2′)). Anal. calc. for $C_{10}H_{14}N_6O_4$ (282.3): C 42.55, H 4.99, N 29.77; found: C 42.69, H 5.04, N 29.76.

*5-Amino-2-(2-deoxy-α-*D*-erythro-pentofuranosyl)-7-methoxy-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidine* (**11b**). A soln. of **8b** (440 mg, 0.85 mmol) in NaOMe (40 ml, 0.2M) was stirred for 2 h at r.t. After evaporation, the residue was adsorbed on silica gel and applied to FC ($CHCl_3$/MeOH 8:2, 20 × 2.5 cm, silica gel *60 H*). The colourless foam

gave colourless crystals **11b** (180 mg, 75%) from MeOH. M.p. 200°. TLC ($CHCl_3$/MeOH): R_f 0.46. UV (MeOH): 312 (8200). ^{1}H-NMR ((D_6)DMSO): 6.90 (*s*, NH_2); 6.32 (*m*, H–C(1′)); 5.34 (*d*, *J* = 5.8, OH–C(3′)); 4.80 (*t*, *J* = 5.7, OH–C(5′)); 4.14 (*m*, H–C(3′)); 4.03 (*m*, H–C(4′), MeO); 3.44 (*m*, CH_2(5′)); 2.78 (*m*, H_β–C(2′)); 2.49 (*m*, H_α–C(2′)). Anal. calc. for $C_{10}H_{14}N_6O_4$ (282.3): C 42.55, H 4.99, N 29.77; found: C 42.69, H 5.10, N 29.73.

*5-Amino-1-(2-deoxy-β-*D-erythro-*pentofuranosyl)-7-methoxy-1*H-*1,2,3-triazolo[4,5-*d*]pyrimidine* (**12**). From **9** (310 mg, 0.6 mmol) as described above. Colourless crystals (140 mg, 83%) from MeOH. M.p. 168°. TLC ($CHCl_3$/MeOH 8:2): R_f 0.44. UV (MeOH): 315 (4000). ^{1}H-NMR ((D_6)DMSO): 6.67 (*s*, NH_2); 6.57 (*t*, *J* = 6.2, H–C(1′)); 5.39 (*d*, *J* = 4.3, OH–C(3′)); 4.71 (*t*, *J* = 8.4, OH–C(5′)); 4.48 (*m*, H–C(3′)); 4.07 (*s*, MeO); 3.87 (*m*, H–C(4′)); 3.45, 3.28 (2*m*, CH_2(5′)); 3.01 (*m*, H_β–C(2′)); 2.38 (*m*, H_α–C(2′)). Anal. calc. for $C_{10}H_{14}N_6O_4$ (282.3): C 42.55, H 4.99, N 29.77; found: C 42.66, H 5.12, N 29.82.

*5,7-Diamino-3-(2-deoxy-β-*D-erythro-*pentofuranosyl)-3*H-*1,2,3-triazolo[4,5-*d*]pyrimidine* (**13**). Compound **7a** (460 mg, 0.89 mmol) was treated with MeOH/NH_3 (saturated at 0°) for 72 h at 50° in a pressure bottle. The soln. was evaporated, the residue adsorbed on silica gel and applied to the top of a silica-gel column (20 × 2.5 cm). Chromatography with $CHCl_3$/MeOH 8:2 yielded a colourless foam which gave colourless crystals (200 mg, 84%) from MeOH. M.p. 217°. TLC ($CHCl_3$/MeOH 8:2): R_f 0.37. UV (MeOH): 286 (10300), 227 (23100). ^{1}H-NMR ((D_6)DMSO): 7.74 (br. *s*, NH_2); 6.48 (*s*, NH_2); 6.42 (*t*, *J* = 6.5, H–C(1′)); 5.37 (*d*, *J* = 4.3, OH–C(3′)); 4.96 (*t*, *J* = 5.7, OH–C(5′)); 4.53 (*m*, H–C(3′)); 3.91 (*m*, H–C(4′)); 3.60, 3.45 (2*m*, CH_2(5′)); 3.42 (*s*, MeO); 2.96 (*m*, H_β–C(2′)); 2.34 (*m*, H_α–C(2′)). Anal. calc. for $C_9H_{13}N_7O_3$ (267.3): C 40.45, H 4.90, N 36.69; found: C 40.38, H 4.98, N 36.64.

*5,7-Diamino-2-(2-deoxy-β-*D-erythro-*pentofuranosyl)-2*H-*1,2,3-triazolo[4,5-*d*]pyrimidine* (**14**). From **8a** (500 mg, 0.96 mmol) as described for **13**. After workup (column 20 × 2.5 cm, $CHCl_3$/MeOH 8:2), **14** was obtained as a foam. Colourless crystals (240 mg, 94%) from MeOH. M.p. 130°. TLC ($CHCl_3$/MeOH 8:2): R_f 0.3. UV (MeOH): 313 (8000), 262 (6100). ^{1}H-NMR ((D_6)DMSO): 7.61 (br. *s*, NH_2); 6.32 (*t*, *J* = 5.9, H–C(1′)); 6.14 (*s*, NH_2); 5.35 (*d*, *J* = 4.7, OH–C(3′)); 4.75 (*t*, *J* = 5.6, OH–C(5′)); 4.50 (*t*, *J* = 5.1, H–C(3′)); 3.80 (*m*, H–C(4′)); 3.54 (*m*, CH_2(5′)); 2.80 (*m*, H_β–C(2′)); 2.39 (*m*, H_α–C(2)). Anal. calc. for $C_9H_{13}N_7O_3$ (267.3): C 40.45, H 4.90, N 36.69; found: C 40.54, H 4.94, N 36.64.

*5,7-Diamino-1-(2-deoxy-β-*D-erythro-*pentofuranosyl)-1*H-*1,2,3-triazolo[4,5-*d*]pyrimidine* (**15**). From **9** (300 mg, 0.58 mmol) as described for **13**. Workup (column 20 × 2.5 cm, $CHCl_3$/MeOH 8:2) and crystallization from MeOH gave colourless crystals (110 mg, 71%). M.p. 169°. UV (MeOH): 315 (5200), 226 (16200). ^{1}H-NMR ((D_6)DMSO): 7.19 (*s*, NH_2); 6.57 (*t*, *J* = 5.8, H–C(1′)); 6.06 (*s*, NH_2); 5.48 (*d*, *J* = 4.7, OH–C(3′)); 4.95 (*t*, OH–C(5′)); 4.39 (*m*, H–C(3′)); 3.95 (*m*, H–C(4′)); 3.37 (*m*, CH_2(5′)); 2.97 (*m*, H_β–C(2′)); 2.35 (*m*, H_α–C(2′)). Anal. calc. for $C_9H_{13}N_7O_3$ (267.3): C 40.45, H 4.90, N 36.69; found: C 40.55, H 4.94, N 39.63.

*5-Amino-3-(2-deoxy-β-*D-erythro-*pentofuranosyl)-3*H-*1,2,3-triazolo[4,5-*d*]pyrimidin-7(6*H*)-one* (**1**). *Method A*: A soln. of **13** (120 mg, 0.45 mmol) in H_2O (10 ml) was treated with adenosine deaminase (ADA; 40 µl, 10 mg/2 ml) at r.t. and stirred overnight. After evaporation, **1** was crystallized from a small amount of H_2O: colourless crystals (118 mg, 98%). M.p. 198° ([8] 196°).

Method B: A soln. of **10a** (50 mg, 0.18 mmol) in dioxane (3 ml) and 0.5N NaOH (5 ml) was stirred for 7 h at r.t. The soln. was neutralized with AcOH and evaporated and the residue crystallized from H_2O: **1** (27 mg, 56%). Colourless crystals. TLC ($CHCl_3$/MeOH 8:2): R_f 0.4. UV (MeOH): 257 (12700). ^{1}H-NMR ((D_6)DMSO): 10.99 (*s*, NH); 6.92 (*s*, NH_2); 6.28 (*t*, *J* = 6.25, H–C(1′)); 5.31 (*d*, *J* = 3.35, OH–C(3′)); 4.73 (br. *s*, OH–C(5′)); 4.45 (br. *s*, H–C(3′)); 3.82 (*m*, *J* = 3.7, H–C(4′)); 3.50, 3.37 (2*m*, CH_2(5′)); 2.89 (*m*, H_β–C(2′)); 2.31 (*m*, H_α–C(2′)). Anal. calc. for $C_9H_{12}N_6O_4$ (268.2): C 40.30, H 4.51, N 31.33; found: C 40.23, H 4.40, N 31.16.

*5-Amino-2-(2-deoxy-β-*D-erythro-*pentofuranosyl)-2*H-*1,2,3-triazolo[4,5-*d*]pyrimidin-7(6*H*)-one* (**2**). A soln. of **11a** (195 mg, 0.69 mmol) in dioxane/0.5N NaOH 1:1 (20 ml) was stirred 7 h at r.t. and neutralized with AcOH. The mixture was evaporated and the residue crystallized from H_2O. Colourless crystals (150 mg, 81%). M.p. > 240°. TLC ($CHCl_3$/MeOH 8:2): R_f 0.35. UV (MeOH): 296 (5900). ^{1}H-NMR ((D_6)DMSO): 6.55 (*s*, NH_2); 6.30 (*t*, *J* = 6.0, H–C(1′)); 5.36 (*m*, OH–C(3′)); 4.75 (*m*, OH–C(5′)); 4.46 (*m*, H–C(3′)); 3.85 (*m*, H–C(4′)); 3.48 (*m*, CH_2(5′)); 2.73 (*m*, H_β–C(2′)); 2.36 (*m*, H_α–C(2′)). Anal. calc. for $C_9H_{12}N_6O_4$ (268.2): C 40.30, H 4.51, N 31.33; found: C 40.28, H 4.52, N 31.10.

*5-Amino-1-(2-deoxy-β-*D-erythro-*pentofuranosyl)-1*H-*1,2,3-triazolo[4,5-*d*]pyrimidin-7(6*H*)-one* (**3**). From **12** (152 mg, 0.54 mmol), as described for **2**. After neutralization and evaporation, colourless crystals (76 mg, 52.5%) were obtained from H_2O. M.p. > 250°. TLC ($CHCl_3$/MeOH 8:2, trace acid): R_f 0.3. UV (MeOH): 300 (4400), 240 (sh). ^{1}H-NMR ((D_6)DMSO): 6.67 (*t*, H–C(1′)); 6.54 (*s*, NH_2); 5.37 (*s*, OH–C(3′)); 4.74 (*s*, OH–C(5′)); 4.44 (*d*, H–C(3′)); 3.85 (*m*, H–C(4′)); 3.46 (*m*, CH_2(5′)); 2.74 (*m*, H_β–C(2′)); 2.33 (*m*, H_α–C(2′)). Anal. calc. for $C_9H_{12}N_6O_4$ (268.2): C 40.30, H 4.51, N 31.33; found: C 41.41, H 4.55, N 31.32.

REFERENCES

[1] K. Anzai, J. Nagatsu, S. Suzuki, *J. Antibiot., Ser. A* **1961,** *14,* 340; K. Anzai, S. Suzuki, *ibid.* **1961,** *14,* 253.
[2] R. O. Roblin, Jr., J. O. Lampen, J. P. English, Q. P. Cole, J. R. Vaughan, Jr., *J. Am. Chem. Soc.* **1945,** *67,* 290.
[3] a) J. A. Montgomery, 'Handbuch der experimentellen Pharmakologie', Eds. O. Eichler, A. Farha, H. Herken, and A. D. Welch, Springer, Heidelberg, 1974, Vol. 28/1, p. 76; b) J. A. Montgomery, H. J. Thomas, in 'The Purines – Theory and Experiment', 'The Jerusalem Symposia on Quantum Chemistry and Biochemistry', Eds. E. D. Bergmann and B. Pullmann, Academy of Sciences and Humanities, Jerusalem, Israel, 1972, Vol. IV, p. 446.
[4] Y. Mizuno, T. Itoh, A. Nomura, *Heterocycles* **1982,** *17,* 615.
[5] J. Davoll, *J. Chem. Soc.* **1958,** 1593.
[6] R. D. Elliott, J. A. Montgomery, *J. Med. Chem.* **1976,** *19,* 1186.
[7] R. Vince, R. H. Turakhia, W. M. Shannon, G. Arnett, *J. Med. Chem.* **1987,** *30,* 2026.
[8] W. Hutzenlaub, R. L. Tolmann, R. K. Robins, *J. Med. Chem.* **1972,** *15,* 879.
[9] F. Seela, K. Mersmann, *Helv. Chim. Acta* **1993,** *76,* in press.
[10] Z. Kazimierczuk, U. Bindig, F. Seela, *Helv. Chim. Acta* **1989,** *72,* 1527.
[11] F. Seela, H. Winter, in preparation.
[12] F. Seela, P. Leonard, in preparation.
[13] A. Albert, in 'Advances in Heterocyclic Chemistry', Ed. A. R. Katrizky, Academic Press, New York, 1986, Vol. 39, p. 117.
[14] N. K. Kochetkov, E. I. Budovskii, in 'Organic Chemistry of Nucleic Acids', Plenum Press, London–New York, 1971, p. 162.
[15] Y. F. Shealy, J. D. Clayton, C. A. O'Dell, J. A. Montgomery, *J. Org. Chem.* **1962,** *27,* 4518.
[16] M. Hoffer, *Chem. Ber.* **1960,** *93,* 3041.
[17] H.-D. Winkeler, F. Seela, *J. Org. Chem.* **1983,** *48,* 3119.
[18] L. Stryer, in 'Biochemie', Spektrum Akademischer Verlag GmbH, Heidelberg, 1990, p. 196.
[19] R. Franco, J. J. Centelles, *Drugs of Today* **1989,** *25,* 155.
[20] D. Neuhaus, M. Williamson, in 'The Nuclear Overhauser Effect, Structural and Conformational Analysis', Verlag Chemie, Weinheim, 1989, p. 150.
[21] S. Sprang, R. Scheller, D. Rohrer, M. Sundaralingam, *J. Am. Chem. Soc.* **1978,** *100,* 2867.
[22] L. G. Purnell, D. J. Hodgson, *Org. Magn. Reson.* **1977,** *10,* 1.
[23] R. Käppi, Z. Kazimierczuk, P. Järvinen, F. Seela, H. Lönnberg, *J. Chem. Soc., Perkin Trans. 2* **1991,** 595.
[24] J. R. Williamson, M. K. Raghuraman, T. R. Cech, *Cell* **1989,** *59,* 871.
[25] F. Seela, K. Mersmann, *Helv. Chim. Acta* **1993,** *76,* 1435.