148. 8-Azaguanine 2′,3′-Dideoxyribonucleosides: Glycosylation of the 5-Amino-7-methoxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidinyl Anion with 2,3-Dideoxy-D-*glycero*-pentofuranosyl Chloride

by **Frank Seela*** and **Karin Mersmann**

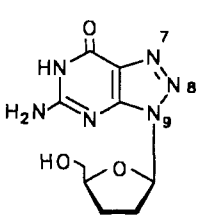
Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie, Universität Osnabrück, Barbarastr. 7, D–49069 Osnabrück

(30.IV.93)

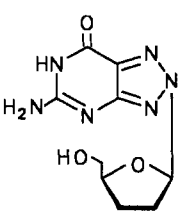
The synthesis of the regioisomeric 8-azaguanine N^7-, N^8-, and N^9-(β-D-2′,3′-dideoxyribonucleosides) (**1**, **2**, and **3**, respectively) and of the diamino derivative **13** is described. The anion of 5-amino-7-methoxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (**5**) was glycosylated with 5-*O*-[(*tert*-butyl)dimethylsilyl]-2,3-dideoxy-D-*glycero*-pentofuranosyl chloride (**6**; anomeric mixture), yielding the regioisomeric 2′,3′-dideoxyrihofuranosides as anomeric mixtures **7a**/**10a**, **8a**/**11a**, and **9a**/**12a**. They were desilylated with Bu_4NF in THF affording the 5-amino-7-methoxy-nucleosides **7b**–**12b**. Treatment with aqueous NaOH gave the 8-azaguanine β-D-2′,3′-dideoxynucleosides **1**–**3** and their α-D-anomers **14**–**16**. The reaction of **7b** with NH_3/MeOH yielded the diamino compound **13**. The N-glycosylic bond of 8-aza-2′,3′-dideoxyguanosine (**1**) is four-times more stable against acid than that of 2′,3′-dideoxyguanosine. Compounds **1**, **2**, and **13** were converted to their 5′-triphosphates **17**–**19** which showed only modest inhibitory activity against HIV-reverse transcriptase.

Introduction. – The antiviral, antifungal, and anticancer activity of 8-azaguanine (= 5-amino-1,4-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one; purine numbering is used throughout the *General Part*) [1] [2] has generated much interest in 8-azapurines in general as purine antimetabolites [3–5]. The 8-azaguanine is naturally occurring and was isolated from microbial fermentation as pathocidin [6]. The chemistry of 8-azapurines (= 1*H*-1,2,3-triazolo[4,5-*d*]pyrimidines) was reviewed by *Albert* [7], and reports on the biochemistry and pharmacology of this class of compounds have appeared [8]. Ribofuranosides [9] [10] and 2′-deoxyribofuranosides [11] of 8-azaguanine were already prepared, and 8-aza-2′,3′-dideoxyguanosine (**1**) was obtained from the ribonucleoside by the *Mattocks* reaction [12].

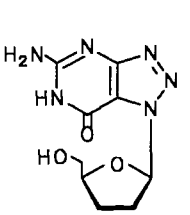
Recently, 8-azaadenine 2′,3′-dideoxyribonucleosides were synthesized in our laboratory and their triphosphates tested as inhibitors of HIV-reverse transcriptase [13]. In the



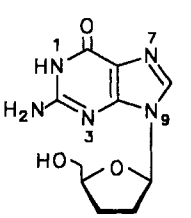
1
purine numbering



2



3

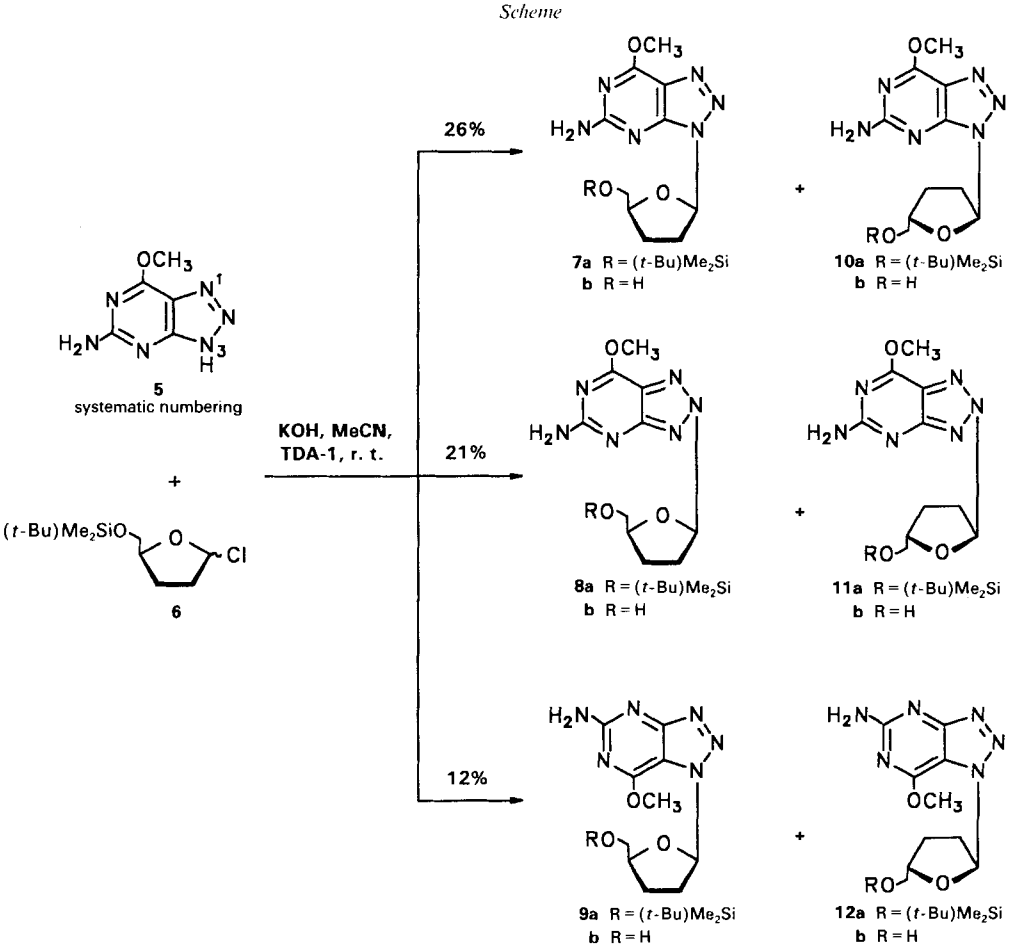


4
purine numbering

following, we report on the synthesis by nucleobase-anion glycosylation [15], on the stability, and on the activity of the regioisomeric 8-azaguanine 2′,3′-dideoxyribofuranosides **1**–**3** [14] which are related to $G_{d2'3'}$ (**4**).

Results and Discussion. – As mentioned above, 8-azapurine 2′,3′-dideoxyribonucleosides were previously obtained from corresponding ribonucleosides [12]. The synthesis of the latter can be avoided using the direct glycosylation of an 8-azapurinyl anion by the dideoxyhalogenose mixture **6** (see *Scheme*). As 8-azapurinyl anion, we chose 5-amino-7-methoxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (**5**) [16], since the corresponding 7-methoxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine was already successfully used in the synthesis of 8-azaadenine 2′,3′-dideoxynucleosides and 2′-deoxynucleosides [13] [17]. We prepared **5** from the commercially available 2,4,5-triaminopyrimidin-6-ol by $POCl_3$ treatment (→2,4,5-triamino-6-chloropyrimidine), reaction with pentyl nitrite (→5-amino-7-chloro-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine), and finally NaOMe treatment (→**5**). The intermediate 2,4,5-triamino-6-chloropyrimidine was already described by

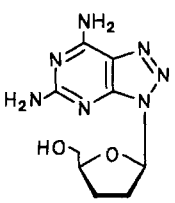
Scheme



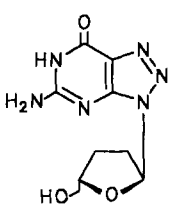
Montgomery and coworkers [16] but was synthesized by a different route. The halogenose mixture **6** [15] was prepared from the corresponding lactol and generated *in situ* [18].

Thus, the glycosylation of **5** with chlorides **6** was carried out in MeCN in the presence of a three-fold excess of powdered KOH and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) as described for the synthesis of 8-azaadenine 2′,3′-dideoxyribonucleosides [13]. The products were separated by flash chromatography (FC; CH_2Cl_2/acetone) to give the six glycosides **7a**–**12a** in 60% overall yield. Isomers **10a** (zone *1*), **7a** (zone *2*), **9a** (zone *4*), and **12a** (zone *5*) were obtained pure after a single FC. Zone *3* contained the anomeric mixture **8a**/**11a**, which could not be separated at this stage. The regioisomer ratio $N^9/N^8/N^7$ was close to 2:2:1. Compounds **7a**, **9a**, **10a**, and **12a** and the mixture **8a**/**11a** were desilylated separately with Bu_4NF in THF, and the desilylated mixture **8b**/**11b** was successfully separated by chromatography. The 7-amino-5-methoxynucleosides **7b**–**12b** were crystallized and their structures assigned.

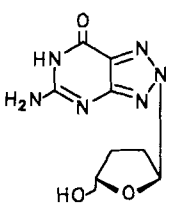
Displacement of the MeO group of **7b**–**12b** with 0.25N NaOH furnished the regioisomers **1**–**3** and their α-D-anomers **14**–**16** as crystalline compounds. Moreover, **7b** was converted into the diamino derivative **13** upon treatment with NH_3/MeOH. Compound **13** could be deaminated with adenosine deaminase to yield 8-aza-2′,3′-dideoxyguanosine (**1**). As adenosine deaminase is able to select only the N^9-(β-D-isomer) as substrate and cannot use other regioisomers or anomers (see 8-aza-2′,3′-dideoxyadenosine [13]), the enzyme-catalyzed reaction is a structural proof for N(9) as glycosylation site and β-D-configuration of **13**.



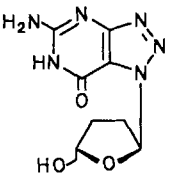
13



14



15



16

Earlier, the structures of 8-azaguanine ribonucleosides were assigned on the basis of their UV spectra and comparison with those of methylated derivatives [10]: UV spectra of the N^9-substituted derivatives (systematic numbering: N^3) differ strongly from those of the N^7- or N^8-isomers (systematic numbering: N^1 and N^2, resp.). Similar observations are made for the dideoxyribofuranosides **1**–**3** (*Table 1*). A good agreement of the UV data of the regioisomeric 7-methoxy-dideoxynucleosides **7b**–**9b** with the corresponding ribonucleosides is also found. Again the N^9-compounds show different UV spectra compared to the N^7- or N^8-regioisomers (*Table 1*). From these observation, the assignment of the N^9-compounds is conclusive.

The N^7- and N^8-regioisomers can be distinguished by their ^{13}C-NMR spectra (*Table 2*). The ^{13}C-NMR data of *N*-unsubstituted 8-azaguanine in aqueous alkaline solution was reported and assigned by comparison of chemical-shift differences with guanine [19]. However, this assignment was made for the anion and is not unambiguous. The ^{13}C-NMR data of *Table 2* allow the selection of the anomeric pairs of the regioisomeric silylated nucleosides **7a**/**10a**, **8a**/**11a**, and **9a**/**12a** as well as of the corresponding deblocked compounds (**b** series).

Table 1. *UV Data of* N*-Substituted 5-Amino-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one* (= 8-Azaguanine) *and 5-Amino-7-methoxy-1,2,3-triazolo[4,5-*d*]pyrimidine Derivatives* (systematic numbering)

N-Substitution	λ_{max} (H_2O)	*N*-Substitution	λ_{max} (MeOH)
5-Amino-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-ones			
N^2-Methyl [10]	241 (6700), 292 (6300)		
N^1-Methyl [10]	240 (7000), 296 (5200)		
N^3-Ribosyl [10]	256 (12900), 275 (sh)	N^3-(2′,3′-Dideoxyribosyl) (see **1**)	256 (13100), 270 (9000)
N^2-Ribosyl [10]	240 (sh), 304	N^2-(2′,3′-Dideoxyribosyl) (see **2**)	241 (9300), 294 (6000)
N^1-Ribosyl [10]	240 (sh), 300	N^1-(2′,3′-Dideoxyribosyl) (see **3**)	240 (sh), 301 (4600)
5-Amino-7-methoxy-1,2,3-triazolo[4,5-*d*]pyrimidines			
N^3-Ribosyl [10]	246 (5400), 287 (10600)	N^3-(2′,3′-Dideoxyribosyl) (see **7b**)	246 (5900), 287 (9800)
N^2-Ribosyl [10]	311 (8300)	N^2-(2′,3′-Dideoxyribosyl) (see **8b**)	234 (sh), 311 (8000)
N^1-Ribosyl [10]	313 (4500)	N^1-(2′,3′-Dideoxyribosyl) (see **9b**)	234 (sh), 314 (4100)

Table 2. *^{13}C-NMR Chemical Shifts of 8-Aza-2′,3′-dideoxyguanosine* (**1**) *and Related 1,2,3-Triazolo-[4,5-*d*]pyrimidine 2′,3′-Dideoxyribofuranosides in (D_6)DMSO at 23°*

	C(3a)[a][b]	C(5)[a][b]	C(7a)[a]	C(7)[a][b]	C(1′)[c]	C(2′)[c]	C(3′)[c]	C(4′)[c]	C(5′)[c]
z^{8}Gua	153.8	155.1	123.8	156.3	–	–	–	–	–
1	151.2	155.7	124.2	155.6	84.6	30.6	27.1	82.5	63.9
14	151.7	155.8	124.6	155.7	85.5	30.0	26.2	80.8	64.9
2	156.9	154.4	126.7	159.4	93.3	31.5	26.5	83.2	63.9
15	156.9	154.4	126.9	159.5	93.8	30.9	25.9	82.0	63.2
3	161.1	154.1	113.1	154.1	88.2	31.3	27.0	83.0	63.9
16	161.3	153.7	112.8	153.8	88.9	30.8	26.4	81.7	63.2
5	154.8	162.2	119.7	161.1	–	–	–	–	–
7a	153.1[d]	162.4[e]	120.6	161.4[d]	85.0	30.5	26.8	82.1	65.5
b	153.0[d]	162.4[e]	120.6	161.3[d]	85.0	30.6	27.2	82.6	64.0
8a	161.5	161.6	122.4	162.0[d]	93.9	31.6	26.2	83.0	65.4
b	161.6	161.6	122.3	162.0[b]	93.9	31.9	26.6	83.6	64.0
9a	164.8	160.8	109.2	157.2[d]	88.4	30.5	26.4	82.5	65.3
b	164.6	161.0	109.3	157.3[b]	88.7	31.0	27.0	83.1	63.8
10a	152.8	162.3	120.6	161.2	85.5	30.0	26.2	80.8	64.9
b	153.9	162.4	120.7	161.4	85.5	29.9	26.6	81.4	63.3
11a	161.5	161.6	122.4	162.0	94.2	31.2	25.6	81.6	65.4
b	161.6	161.6	122.3	162.0	94.2	31.2	25.9	82.1	63.2
12a	164.8	161.0	109.0	157.3	89.4	30.5	26.1	81.0	64.9
b	164.8	161.0	109.0	157.3	89.4	30.6	26.3	81.6	63.2
13	151.5	162.9	120.4	156.2	84.8	30.5	27.3	82.3	64.1

[a]) Systematic numbering.
[b]) Tentative.
[c]) According to [20].
[d]) According to gated-decoupled spectra.
[e]) According to the COLOC spectrum.

^{1}H-NMR NOE difference spectroscopy is also used for structure determination (see *Table 3*). Unfortunately, the assignment of the ^{13}C-NMR chemical shifts of the nucleobases of the various glycosylation products is difficult because of few couplings. In the case of the 7-MeO derivatives **7b**–**12b**, the chemical shift of C(7) can be determined from the ^3J(C,H) coupling constants to the MeO group in the gated-decoupled spectra. The assignment of C(7a) is possible on the basis of its upfield location compared to other C-atoms. In the case of **7b** and **10b**, the assignment of C(5) is accomplished by COLOC spectra. The sugar ^{13}C-signals are easily identified as unambiguous data are

Table 3. *NMR Data and Chromatographic Mobilities of Anomeric 8-Azaguanine 2′,3′-Dideoxyribonucleosides*

	NOE [%] at H–C(4′)[a])[b])	δ (C(4′)) [ppm]	R_f[c])		Δδ (CH_3Si) [ppm]		δ (C(4′)) [ppm]
7b (N^9, β-D)	1.5	82.6	0.55	**7a**	0.044	**1** (N^9, β-D)	82.5
8b (N^8, β-D)	1.8	83.6	0.45	**8a**	0.052	**2** (N^8, β-D)	83.2
9b (N^7, β-D)	1.9[d])	83.1	0.35	**9a**	0.040	**3** (N^7, β-D)	83.0
10b (N^9, α-D)	0	81.4	0.40	**10a**	0.016	**14** (N^9, α-D)	81.2
11b (N^8, α-D)	*ca.* 1	82.1	0.35	**11a**	0.017	**15** (N^8, α-D)	82.0
12b (N^7, α-D)	1.5[d])	81.6	0.35	**12a**	0.015	**16** (N^7, α-D)	81.7

[a]) Upon irradiation of H–C(1′).
[b]) Measured in (D_6)DMSO.
[c]) TLC (silica gel, CH_2Cl_2/MeOH 9:1).
[d]) Together with the NOE of MeO.

available from other dideoxynucleosides [20]. The position of the C(1′) *vs.* C(4′) signals is determined by a ^1J(C,H) coupling constant of *ca.* 170 (C(1′)) *vs.* 150 Hz (C(4′)). As the assignment of the bridgehead C(7a) is unambiguous in the three series of regioisomers, the upfield shift of 10 ppm on this C-atom occurring between the series of N^9- and N^7-nucleosides [21] is conclusive for these glycosylation sites.

It was already shown that the NOE of H–C(4′) upon irradiation of H–C(1′) can be used for the assignment of β-D-configuration [22]. Corresponding data for **7b**–**12b** are given in *Table 3*. In the case of β-D-2′,3′-dideoxynucleosides **7b**–**9b**, a NOE of *ca.* 1–1.5% is observed, apart from a smaller NOE in the case of the α-D-isomers, which is due to the three-spin effect [23]. The assignment of the N^8-isomers results from the absence of NOE's at NH_2, which should be present if the sugar is attached to N(1) or N(3). The anomeric assignments are also supported by the chemical shifts of C(4′) of the MeO compounds **7b**–**12b** and of **1**–**3** and **14**–**16**: the C(4′) signal is shifted by 1.2–1.5 ppm in the case of the α-D-anomers compared to the β-D-compounds (*Table 3*). Furthermore, the ^{1}H-NMR chemical-shift differences of the Me signals of the (*t*-Bu)Me_2Si groups of **7b**–**12b** are *ca.* 0.04 for the β-D-anomers and 0.02 for the α-D-anomers.

The correlation of the anomeric configuration of nucleosides with the *Cotton* effect in their CD spectra as proposed by *Ulbricht* and coworkers [24] (negative *Cotton* effect for purine β-D-nucleosides at *ca.* 260 nm and positive *Cotton* effect for corresponding α-D-anomers) did not work in the case of 8-azaadenine 2′,3′-dideoxynucleosides [13] (this behaviour depends on the electronic state and the torsion angle of the nucleobases [25]). In contrast, all 8-azaguanine β-D-nucleosides **1**–**3** show negative *Cotton* effects in the CD spectra, while the α-D-anomers **14**–**16** have positive values (see *Fig.*), in accordance with the proposed correlation for purine nucleosides.

As discussed in [13], 2′,3′-dideoxyribonucleosides are extremely labile towards hydrolysis at the N-glycosylic bond induced by phosphorylase within cells [26]. In the series of 8-azaadenine 2′,3′-dideoxyribofuranosides, the N^7-regioisomers are the most labile and the N^9-isomers the most stable under acid-catalysed hydrolysis conditions [13]. In the series of 8-azaguanine 2′,3′-dideoxyribonucleosides, again the N^7-isomers are the most labile compounds, whereas the N^8- and the N^9-isomers are comparatively stable (see *Table 4*). The β-D-anomers are hydrolysed slightly faster than the α-D-compounds by a factor of *ca.* 1.5, which is in line with the 8-azaadenine 2′,3′-dideoxyribonucleosides [13]. In comparison to $G_{d_2'_{,3'}}$ (**4**) [20], compound **1** is much more stable. The replacement of C(8) by N(8) reduces the basicity and enhances the acidity of 8-azapurine nucleosides compared to purine nucleosides. Thus, 8-aza-2′,3′-dideoxyguanosine is easier to deprotonate and more difficult to protonate (*Table 4*) as 2′-deoxyguanosine (pK_{a1} 2.5, pK_{a2} 9.5) [27].

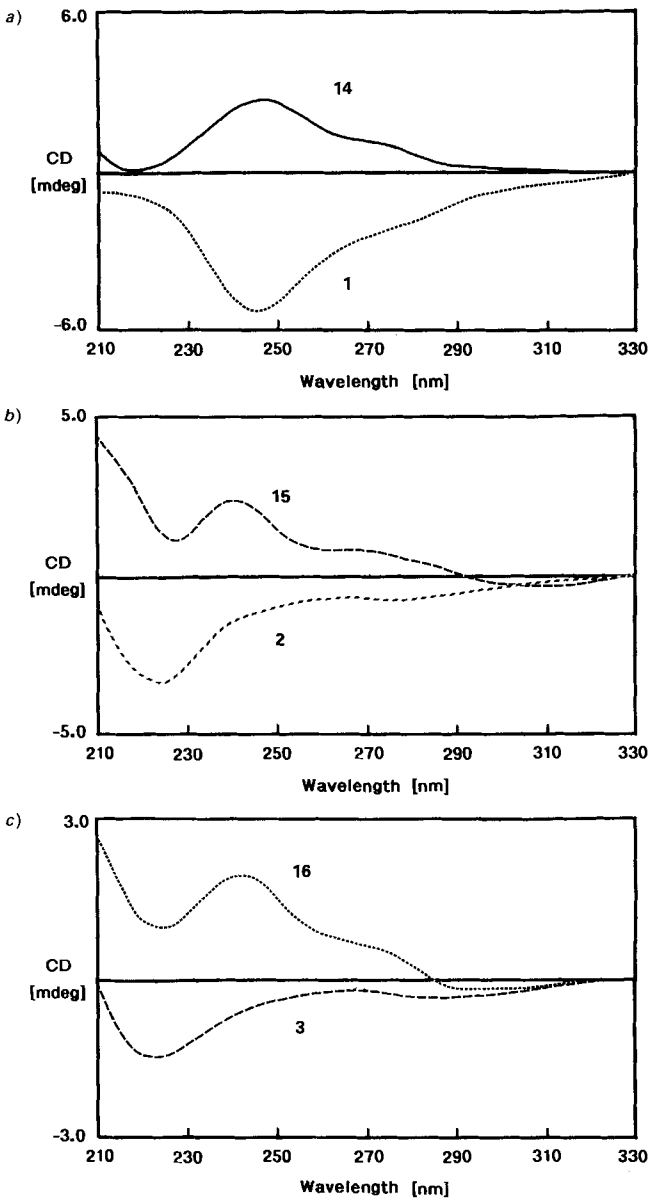


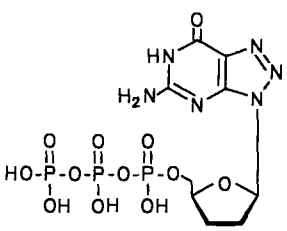
Figure. *CD Spectra of 8-azaguanine 2′,3′-dideoxynucleosides* **1**–**3** *and* **14**–**16** *measured in* H_2O *at 5°*

Finally, the dideoxynucleosides **1**, **2**, and **13** were phosphorylated in a one-pot reaction with $PO(MeO)_3/POCl_3/(Bu_4N)P_2O_7$ (see also [13]) following a protocol originally developed for phosphorylation of purine nucleosides [29]. The triphosphates **17**–**19** were purified by *DEAE*-cellulose column chromatography and isolated as solid triethylammonium salts. Their inhibitory activity was tested against HIV-reverse transcriptase.

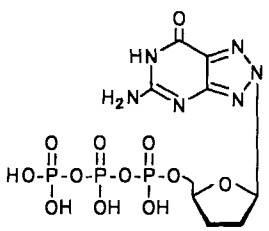
Table 4. *Half-lifes of N-Glycosylic Bond on Hydrolysis and pK_a Values of 8-Azaguanine 2′,3′-Dideoxyribofuranosides*

	T [°C]	$t_{1/2}$[a]) [min]	c_{HCl} [mol l^{-1}]	pK_a[b])
1 (N^9, β-D)	25	15.7	0.1	< 1, 8.5
2 (N^8, β-D)	25	8.0	0.1	2.1, 8.6
3 (N^7, β-D)	25	2.2	0.001	6.6, 10.1
4 (N^9, β-D) [20]	25	37	0.01	
13 (N^9, β-D)	25	17.9	0.1	
14 (N^9, α-D)	25	21.6	0.1	
15 (N^8, α-D)	25	9.8	0.1	
16 (N^7, α-D)	25	2.8	0.001	

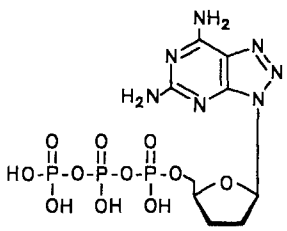
[a]) Measured at 250 nm. [b]) In *Teorell-Stenhagen* buffer [28].



17



18



19

Diamino compound **19** showed an IC_{50} value of 27 μM against HIV-reverse transcriptase, whereas the 8-azaguanosine derivative **17** was not as active (93 μM) and the regioisomeric **18** was inactive (> 100 μM).

We thank Dr. *B. König* from *Boehringer Mannheim GmbH* for the inhibitory studies on HIV-reverse transcriptase and the *Fonds der Chemischen Industrie* for financial support.

Experimental Part

1. *General.* See [13] [17]. Deamination of **13** was carried out with adenosine deaminase from calf intestine mucosa (*Boehringer Mannheim*, EC 3.5.4.4) in 1/15M Na-phosphate buffer (pH 7.0) at r.t. Solvent systems: *A* = CH_2Cl_2/acetone 8:2, *B* = light petroleum ether/AcOEt 9:1, *C* = CH_2Cl_2/MeOH 9:1, *D* = CH_2Cl_2/MeOH 8:2, *E* = i-PrOH/NH_3/H_2O 3:1:1.

2. *Glycosylation of 5-Amino-7-methoxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine* [16] (**5**) *with 2,3-Dideoxy-5-O-[(1,1-dimethylethyl)dimethylsilyl]-*D-glycero-*pentofuranosyl Chloride* (**6**; anomeric mixture). Compound **5** (0.66 g, 4 mmol) was dissolved in hot MeCN (100 ml) and cooled to r.t. Powdered KOH (0.5 g, 9 mmol) and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1; 26 μl, 0.08 mmol) were added. The suspension was stirred for 10 min at r.t., and a freshly prepared cold (−80°) THF soln. (30 ml) of **6** prepared *in situ* from the corresponding lactol [15] [18] was added in portions (5 ml each) within 30 min. Stirring was continued for 30 min, insoluble material filtered off, and the filtrate poured into aq. 5% $NaHCO_3$ soln. (100 ml). The aq. layer was extracted twice with CH_2Cl_2, the combined org. layer dried (Na_2SO_4), evaporated, and the residue applied to FC (column 50 × 3 cm, *A*). Five zones were separated.

*5-Amino-3-{2,3-dideoxy-5-O-[(1,1-dimethylethyl)dimethylsilyl]-*α-D-glycero-*pentofuranosyl}-7-methoxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine* (**10a**). The fast-migrating zone *1* gave **10a** (180 mg, 12%). Colourless syrup. TLC (*B*): R_f 0.6. UV (MeOH): 286 (9600), 245 (5600). ^{1}H-NMR ((D_6)DMSO): 0.04, 0.05 (2*s*, Me_2Si); 0.86 (*s*, *t*-Bu); 1.91 (*m*, 2 H–C(3′)); 2.59 (*m*, H–C(2′)); 3.63 (‘*t*’, *J* = 4.7, 2 H–C(5′)); 4.05 (*s*, MeO); 4.31 (*m*, H–C(4′)); 6.39 (*dd*,

J = 6.7, 3.1, H–C(1′)); 7.19 (*s*, NH_2). Anal. calc. for $C_{16}H_{28}N_6O_3Si$ (380.5): C 50.51, H 7.42, N 22.09; found: C 50.69, H 7.42, N 21.99.

*5-Amino-3-{2,3-dideoxy-5-O-[(1,1-dimethylethyl)dimethylsilyl]-β-*D-glycero-*pentofuranosyl}-7-methoxy-3*H-*1,2,3-triazolo[4,5-*d*]pyrimidine* (**7a**). Zone *2* yielded a colourless solid (205 mg, 14%). TLC (*B*): R_f 0.4. UV (MeOH): 286 (9700), 245 (5700). ^{1}H-NMR ((D$_6$)DMSO): −0.15, −0.12 (2*s*, Me_2Si); 0.76 (*s*, *t*-Bu); 2.17 (*m*, 2 H–C(3′)); 2.64 (*m*, H–C(2′)); 3.58 (*m*, 2 H–C(5′)); 4.05 (*s*, MeO); 4.18 (*m*, H–C(4′)); 6.32 (*dd*, J = 7.2, 2.1, H–C(1′)); 7.17 (*s*, NH_2). Anal. calc. for $C_{16}H_{28}N_6O_3Si$ (380.5): C 50.51, H 7.42, N 22.09; found: C 50.69, H 7.38, N 22.11.

*5-Amino-2-{2,3-dideoxy-5-O-[(1,1-dimethylethyl)dimethylsilyl]-β-*D- *and* -α-D-glycero-*pentofuranosyl}-7-methoxy-2*H-*1,2,3-triazolo[4,5-*d*]pyrimidine* (**8a** and **11a**; resp.). Zone *3* contained the anomeric mixture **8a**/**11a** (310 mg, 21%) which could not be separated at this stage. Separation was achieved after desilylation, see **8b** and **11b**.

*5-Amino-1-{2,3-dideoxy-5-O-[(1,1-dimethylethyl)dimethylsilyl]-β-*D-glycero-*pentofuranosyl}-7-methoxy-1*H-*1,2,3-triazolo[4,5-*d*]pyrimidine* (**9a**). Zone *4* yielded colourless crystals from light petroleum ether/Et_2O 1:1 (110 mg, 7%). M.p. 146°. TLC (*C*): R_f 0.55. UV (MeOH): 235 (sh), 314 (4300). ^{1}H-NMR ((D$_6$)DMSO): −0.20, −0.16 (2*s*, Me_2Si); 0.73 (*s*, *t*-Bu); 2.08 (*m*, 2 H–C(3′)); 2.76 (*m*, H–C(2′)); 3.39, 3.54 (2*m*, 2 H–C(5′)); 4.05 (*s*, MeO); 4.21 (*m*, H–C(4′)); 6.48 (*d*, J = 6.7, H–C(1′)); 6.74 (*s*, NH_2). Anal. calc. for $C_{16}H_{28}N_6O_3Si$ (380.5): C 50.51, H 7.42, N 22.09; found: C 50.69, H 7.42, N 22.25.

*5-Amino-1-{2,3-dideoxy-5-O-[(1,1-dimethylethyl)dimethylsilyl]-α-*D-glycero-*pentofuranosyl}-7-methoxy-1*H-*1,2,3-triazolo[4,5-*d*]pyrimidine* (**12a**). Zone *5* yielded, after crystallization from light petroleum ether/Et_2O 1:1, colourless crystals of **12a** (80 mg, 5%). M.p. 126°. TLC (*C*): R_f 0.55. UV (MeOH): 235 (sh), 312 (4200). ^{1}H-NMR ((D$_6$)DMSO): 0.04, 0.05 (2*s*, Me_2Si); 0.87 (*s*, *t*-Bu); 1.91 (*m*, 2 H–C(3′)); 2.66 (*m*, H–C(2′)); 3.64 (*m*, 2 H–C(5′)); 4.06 (*s*, MeO); 4.25 (*m*, H–C(4′)); 6.56 (*dd*, J = 6.9, 3.0, H–C(1′)); 6.76 (*s*, NH_2). Anal. calc. for $C_{16}H_{28}N_6O_3Si$ (380.5): C 50.51, H 7.42, N 22.09; found: C 50.62, H 7.42, N 22.15.

3. *Desilylations. 5-Amino-3-(2,3-dideoxy-β-*D-glycero-*pentofuranosyl)-7-methoxy-3*H-*1,2,3-triazolo[4,5-*d*]pyrimidine* (**7b**). Bu_4NF (1.1M in THF, 2 ml) was added to a soln. of **7a** (380 mg, 1 mmol) in THF (15 ml), and the mixture was stirred for 15 min at r.t. FC (column 10 × 3 cm, *C*) and crystallization from acetone afforded **7b** (255 mg, 96%). Colourless crystals. M.p. 168–170°. TLC (*C*): R_f 0.55. UV (MeOH): 287 (9800), 246 (5900). ^{1}H-NMR ((D$_6$)DMSO): 2.17 (*m*, 2 H–C(3′), 1 H–C(2′)); 2.59 (*m*, 1 H–C(2′)); 3.40 (*m*, 2 H–C(5′)); 4.05 (*s*, MeO); 4.14 (*m*, H–C(4′)); 4.72 (*t*, J = 5.4, OH–C(5′)); 6.31 (*d*, J = 5.6, H–C(1′)); 7.18 (*s*, NH_2). Anal. calc. for $C_{10}H_{14}N_6O_3$ (266.3): C 45.10, H 5.30, N 31.56; found: C 45.14, H 5.33, N 31.46.

*5-Amino-2-(2,3-dideoxy-β-*D-glycero-*pentofuranosyl)-7-methoxy-2*H-*1,2,3-triazolo[4,5-*d*]pyrimidine* (**8b**). The mixture **8a**/**11a** (380 mg, 1.0 mmol) was treated (30 min) with Bu_4NF/THF as described for **7b**. After chromatography (column 30 × 3 cm, *C*) of **8b**/**11b**, the faster migrating zone gave **8b** as a colourless solid (130 mg, 49%), which crystallized from acetone. M.p. 141°. TLC (*C*): R_f 0.45. UV (MeOH): 311 (8000), 234 (sh). ^{1}H-NMR ((D$_6$)DMSO): 2.14 (*m*, 2 H–C(3′)); 2.42 (*m*, 2 H–C(2′)); 3.45 ('*t*', 2 H–C(5′)); 4.05 (*s*, MeO); 4.20 (*m*, H–C(4′)); 4.75 (*t*, J = 5.6, OH–C(5′)); 6.34 ('*t*', J = 4.2, H–C(1′)); 6.87 (*s*, NH_2). Anal. calc. for $C_{10}H_{14}N_6O_3$ (266.3): C 45.10, H 5.30, N 31.56; found: C 45.19, H 5.24, N 31.56.

*5-Amino-2-(2,3-dideoxy-α-*D-glycero-*pentofuranosyl)-7-methoxy-2*H-*1,2,3-triazolo[4,5-*d*]pyrimidine* (**11b**). From the slower migrating zone of the **8b**/**11b** chromatography, colourless crystals were obtained after crystallization from acetone (120 mg, 45%). M.p. 174°. TLC (*C*): R_f 0.35. UV (MeOH): 311 (7900), 234 (sh). ^{1}H-NMR ((D$_6$)DMSO): 1.88, 2.3 (*m*, 2 H–C(3′)); 3.44 (*m*, 2 H–C(5′)); 4.05 (*s*, MeO); 4.34 (*m*, H–C(4′)); 4.80 (*t*, J = 5.8, OH–C(5′)); 6.41 (*dd*, J = 5.6, 3.6, H–C(1′)); 6.87 (*s*, NH_2). Anal. calc. for $C_{10}H_{14}N_6O_3$ (266.3): C 45.10, H 5.30, N 31.56; found: C 45.28, H 5.33, N 31.40.

*5-Amino-1-(2,3-dideoxy-β-*D-glycero-*pentofuranosyl)-7-methoxy-1*H-*1,2,3-triazolo[4,5-*d*]pyrimidine* (**9b**). Deprotection of **9a** (190 mg, 0.5 mmol) was carried out as described for **7b** within 45 min. FC (column 30 × 3 cm, *C*) and crystallization from AcOEt afforded colourless crystals (90 mg, 68%). M.p. 125°. TLC (*C*): R_f 0.35. UV (MeOH): 314 (4100), 234 (sh). ^{1}H-NMR ((D$_6$)DMSO): 2.12 (*m*, 2 H–C(3′)); 2.70 (*m*, H–C(2′)); 3.35 (*m*, 2 H–C(5′)); 4.06 (*s*, MeO); 4.17 (*m*, H–C(4′)); 4.69 (*t*, J = 5.3, OH–C(5′)); 6.49 (*d*, J = 6.4, H–C(1′)); 6.75 (*s*, NH_2). Anal. calc. for $C_{10}H_{14}N_6O_3$ (266.3): C 45.10, H 5.30, N 31.56; found: C 45.26, H 5.36, N 31.43.

*5-Amino-3-(2,3-dideoxy-α-*D-glycero-*pentofuranosyl)-7-methoxy-3*H-*1,2,3-triazolo[4,5-*d*]pyrimidine* (**10b**). Compound **10a** (380 mg, 1.0 mmol) was treated for 15 min with Bu_4N/THF as described for **7b**. FC (column 10 × 3 cm, *C*) yielded **10b** (255 mg, 96%), which crystallized from acetone. M.p. 181°. TLC (*C*): R_f 0.40. UV (MeOH): 287 (9700), 246 (5800). ^{1}H-NMR ((D$_6$)DMSO): 1.88 (*m*, 1 H–C(3′)); 2.63 (*m*, 1 H–C(2′)); 3.44 (*m*, 2 H–C(5′)); 4.06 (*s*, MeO); 4.27 (*m*, H–C(4′)); 4.78 (*t*, J = 5.7, OH–C(5′)); 6.40 (*dd*, J = 7.0, 3.4, H–C(1′)); 7.18 (*s*, NH_2). Anal. calc. for $C_{10}H_{14}N_6O_3$ (266.3): C 45.10, H 5.30, N 31.56; found: C 45.38, H 5.34, N 31.37.

*5-Amino-1-(2,3-dideoxy-α-*D*-glycero-pentofuranosyl)-7-methoxy-1*H*-1,2,3-triazolo[4,5-*d*]pyrimidine* (**12b**). Desilylation of **12a** (190 mg, 0.5 mmol) as described for **7b** (30 min) followed by FC (column 30 × 3 cm, *C*) afforded colourless crystals of **12b** (100 mg, 75%), after crystallization from AcOEt. M.p. 136°. TLC (*C*): R_f 0.35. UV (MeOH): 312 (4000), 234 (sh). ^{1}H-NMR ((D$_6$)DMSO): 1.90, 2.28 (2*m*, 2 H–C(3′)); 2.64 (*m*, H–C(2′)); 3.44 (*m*, 2 H–C(5′)); 4.07 (*s*, MeO); 4.21 (*m*, H–C(4′)); 4.79 (*t*, *J* = 5.6, OH–C(5′)); 6.59 (*dd*, *J* = 6.8, 3.0, H–C(1′)); 6.77 (*s*, NH_2). Anal. calc. for $C_{10}H_{14}N_6O_3$ (266.3): C 45.10, H 5.30, N 31.56; found: C 45.11, H 5.40, N 31.43.

4. *De-*O*-methylations. 5-Amino-3-(2,3-dideoxy-β-*D*-glycero-pentofuranosyl)-3,6-dihydro-7*H*-1,2,3-triazolo-[4,5-*d*]pyrimidin-7-one* (**1**). To a soln. of **7b** (266 mg, 1 mmol) in 1,4-dioxane (10 ml), 0.25N NaOH (40 ml) was added under stirring. After 30 h, the mixture was neutralized with 2N AcOH and evaporated, the residue dissolved in MeOH and adsorbed on silica gel, and the soln. filtered over a 5-cm layer of silica gel (*D*). The filtrate was evaporated and the residue crystallized from MeOH. Colourless needles (220 mg, 87%). M.p. > 240° (dec.). TLC (*D*): R_f 0.7. UV (MeOH): 256 (13100), 270 (9000). ^{1}H-NMR ((D$_6$)DMSO): 2.14 (*m*, 2 H–C(3′)); 2.5 (*m*, 2 H–C(2′)); 3.40 (*m*, 2 H–C(5′)); 4.13 (*m*, H–C(4′)); 4.73 (*s*, OH–C(5′)); 6.20 (*dd*, *J* = 7.1, 2.4, H–C(1′)); 6.98 (*s*, NH_2); 11.1 (*s*, NH). Anal. calc. for $C_9H_{12}N_6O_3$ (252.3): C 42.85, H 4.79, N 33.31; found: C 42.73, H 4.91, N 33.28.

*5-Amino-2-(2,3-dideoxy-β-*D*-glycero-pentofuranosyl)-2,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one* (**2**). As described for **1**, **8b** (133 mg, 0.5 mmol) was treated with 0.25N NaOH (48 h). Crystallization from H_2O yielded **2** (100 mg, 79%). Colourless crystals. M.p. > 240° (dec.). TLC (*D*): R_f 0.65. UV (MeOH): 294 (6000), 241 (9300). ^{1}H-NMR ((D$_6$)DMSO): 2.10 (*m*, 2 H–C(3′)); 2.5 (*m*, 2 H–C(2′)); 3.44 (*d*, *J* = 5.4, 2 H–C(5′)); 4.17 (*m*, H–C(4′)); 4.75 (*s*, OH–C(5′)); 6.26 (‘*t*’, *J* = 3.1, H–C(1′)); 6.54 (*s*, NH_2); 11.0 (*s*, NH). Anal. calc. for $C_9H_{12}N_6O_3$ (252.3): C 42.85, H 4.79, N 33.31; found: C 42.68, H 4.85, N 33.05.

*5-Amino-1-(2,3-dideoxy-β-*D*-glycero-pentofuranosyl)-7*H*-1,6-dihydro-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one* (**3**). As described for **1**, **9b** (133 mg, 0.5 mmol) was treated with 0.25N NaOH (10 h). Colourless crystals (95 mg, 75%) of **3** from H_2O. M.p. > 230° (dec.). TLC (*D*): R_f 0.50. UV (MeOH): 240 (sh), 301 (4600). ^{1}H-NMR ((D$_6$)DMSO): 2.11 (*m*, 2 H–C(3′)); 2.5 (*m*, 2 H–C(2′)); 3.39 (*d*, *J* = 5.3, 2 H–C(5′)); 4.17 (*m*, H–C(4′)); 4.72 (*s*, OH–C(5′)); 6.55 (*s*, NH_2); 6.61 (*dd*, *J* = 6.8, 2.1, H–C(1′)); 11.4 (*s*, NH). Anal. calc. for $C_9H_{12}N_6O_3$ (252.3): C 42.85, H 4.79, N 33.31; found: C 43.00, H 4.82, N 33.15.

*5-Amino-3-(2,3-dideoxy-α-*D*-glycero-pentofuranosyl)-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one* (**14**). As described for **1**, **10b** (133 mg, 0.5 mmol) was treated with NaOH (48 h). After FC (column 5 × 3 cm, *D*) and crystallization from MeOH, colourless needles were obtained (90 mg, 71%). M.p. > 230° (dec.) TLC (*D*): R_f 0.55. UV (MeOH): 256 (13000), 270 (9000). ^{1}H-NMR ((D$_6$)DMSO): 1.85, 2.3 (*m*, 2 H–C(3′)); 2.5 (*m*, 2 H–C(2′)); 3.43 (*m*, 2 H–C(5′)); 4.26 (*m*, H–C(4′)); 4.8 (*s*, OH–C(5′)); 6.28 (*dd*, *J* = 6.9, 3.5, H–C(1′)); 7.24 (*s*, NH_2). Anal. calc. for $C_9H_{12}N_6O_3$ (252.3): C 42.85, H 4.79, N 33.31; found: C 42.61, H 4.77, N 33.25.

*5-Amino-2-(2,3-dideoxy-α-*D*-glycero-pentofuranosyl)-2,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one* (**15**). As described for **1**, **11b** (133 mg, 0.5 mmol) was treated with NaOH (24 h). Crystallization from H_2O yielded **15** (88 mg, 70%). Colourless crystals. M.p. > 240° (dec.). TLC (*D*): R_f 0.2. UV (MeOH): 294 (6100), 241 (9200). ^{1}H-NMR ((D$_6$)DMSO): 1.86, 2.28 (2*m*, 2 H–C(3′)); 2.4 (*m*, 2 H–C(2′)); 3.44 (*m*, 2 H–C(5′)); 4.30 (*m*, H–C(4′)); 4.79 (*s*, OH–C(5′)); 6.32 (‘*t*’, *J* = 4.3, H–C(1′)); 6.45 (*s*, NH_2); 11.0 (*s*, NH). Anal. calc. for $C_9H_{12}N_6O_3$ (252.3): C 42.85, H 4.79, N 33.31; found: C 42.67, H 4.81, N 33.01.

*5-Amino-1-(2,3-dideoxy-α-*D*-glycero-pentofuranosyl)-1,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one* (**16**). As described for **1**, **12b** (133 mg, 0.5 mmol) was treated with NaOH (8 h). Neutralization with AcOH and crystallization from H_2O afforded colourless crystals (86 mg, 68%). M.p. > 230° (dec.). TLC (*D*): R_f 0.45. UV (MeOH): 240 (sh), 301 (4500). ^{1}H-NMR ((D$_6$)DMSO): 1.89, 2.30 (2*m*, 2 H–C(3′)); 2.5 (*m*, 2 H–C(2′)); 3.46 (*m*, 2 H–C(5′)); 4.31 (*m*, H–C(4′)); 4.80 (*s*, OH–C(5′)); 6.54 (*s*, NH_2); 6.69 (*dd*, *J* = 6.6, 3.3, H–C(1′)); 11.36 (*s*, NH). Anal. calc. for $C_9H_{12}N_6O_3$ (252.3): C 42.85, H 4.80, N 33.31; found: C 42.77, H 4.80, N 33.12.

5. *3-(2,3-Dideoxy-β-*D*-glycero-pentofuranosyl)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine-5,7-diamine* (**13**). A soln. of **7b** (266 mg, 1 mmol) in MeOH (100 ml; saturated previously with 10 ml of liq. NH_3 at 0°) was stirred at 60° for 48 h. The mixture was evaporated, the residue dissolved in MeOH and adsorbed on silica gel filtered over a 5-cm layer of silica gel (*C*). The filtrate was evaporated. Crystallization from MeOH afforded **13** (200 mg, 80%). Colourless crystals. M.p. 192°. TLC (*D*): R_f 0.4. UV (MeOH): 286 (10400), 265 (sh), 231 (23700). ^{1}H-NMR ((D$_6$)DMSO): 2.19 (*m*, 2 H–C(3′)); 2.5 (*m*, 2 H–C(2′)); 3.44 (*m*, 2 H–C(5′)); 4.13 (*m*, H–C(4′)); 4.79 (*t*, *J* = 5.6, OH–C(5′)); 6.25 (*dd*, *J* = 7.2, 2.9, H–C(1′)); 6.41 (*s*, NH_2); 7.57 (*s*, NH_2). Anal. calc. for $C_9H_{13}N_7O_2$ (251.2): C 43.03, H 5.22, N 39.03; found: C 42.98, H 5.24, N 38.86.

6. *Phosphorylations. 5-Amino-3-(2,3-dideoxy-β-*D*-glycero-pentofuranosyl)-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one 5′-[Tetrakis(triethylammonium) Triphosphate]* (**17**·4 Et_3N). Compound **1** (50 mg, 0.2 mmol) and *N*,*N*,*N*′,*N*′-tetramethylnaphthalene-1,8-diamine (65 mg, 0.3 mmol) were dissolved in $PO(MeO)_3$ (2 ml) under

warming. The soln. was cooled to 0°, freshly distilled $POCl_3$ (50 µl, 0.54 mmol) added, and the mixture allowed to stand at 4° for 2 h and then treated with 0.5N tributylammonium diphosphate/DMF (2 ml) and Bu_3N (200 µl, 0.84 mmol). After being stirred for 3 min at 0°, 1M aq. $(Et_3NH)HCO_3$ (TBK, 20 ml) was added. Evaporation resulted in a semi-solid, which was chromatographed on a *DEAE-Sephadex* column (30 × 1.5 cm, HCO_3^- form; linear gradient of 1.0M TBK buffer (1 l) and H_2O (1 l)). The main zone was eluted at 0.7M TBK yielding **17** as a colourless solid (44 µmol, 22%). TLC (*H*): R_f 0.15. UV (H_2O): 256 (13000). ^{31}P-NMR (0.1M *Tris*-HCl, pH 7.5, 100 nM EDTA/D_2O): −8.36 (*d*, *J* = 19.2, P(γ)); −10.29 (*d*, *J* = 19.7, P(α)); −21.65 (*t*, *J* = 19.1, P(β)).

*5-Amino-2-(2,3-dideoxy-β-*D-glycero-*pentofuranosyl)-2,6-dihydro-7*H-*1,2,3-triazolo[4,5-*d*]pyrimidin-7-one 5'-[Tetrakis(triethylammonium) Triphosphate]* (**18**·4 Et_3N). As described for **17**, from **2** (50 mg, 0.2 mmol). On *DEAE-Sephadex* chromatography, **18** was eluted at 0.7M TBK: colourless solid (30 µmol, 15%). TLC (*H*): R_f 0.16. UV (H_2O): 294 (9700). ^{31}P-NMR (0.1M *Tris*-HCl, pH 7.5, 100 nM EDTA/D_2O): −6.95 (*d*, *J* = 19.4, P(γ)); −10.23 (*d*, *J* = 19.0, P(α)); −21.54 (*t*, *J* = 19.9, P(β)).

*3-(2,3-Dideoxy-β-*D-glycero-*pentofuranosyl)-3*H-*1,2,3-triazolo[4,5-*d*]pyrimidin-5,7-diamine 5'-[Tetrakis(triethylammonium) Triphosphate]* (**19**·4 Et_3N). As described for **17**, including workup, from **13** (50 mg, 0.2 mmol): colourless solid **19** (71 µmol, 36%). TLC (*H*): R_f 0.20. UV (H_2O): 286 (10400). ^{31}P-NMR (0.1M *Tris*-HCl, pH 7.5, 100 nM EDTA/D_2O): −10.0 (*d*, *J* = 20.0, P(γ)); −10.45 (*d*, *J* = 19.2, P(α)); −22.36 (*t*, *J* = 19.4, P(β)).

REFERENCES

[1] G.W. Kidder, V.C. Dewey, R.E. Parks, G.L. Woodside, *Cancer Res.* **1951,** *11,* 204.
[2] R.E.F. Matthews, J.D. Smith, *Adv. Virus Res.* **1955,** *3,* 49.
[3] J.A. Montgomery, in 'Handbuch der experimentellen Pharmakologie', Eds. O. Eichler, A. Farha, H. Herken, and A.D. Welch, Springer, Heidelberg, 1974, Vol.28/1, p.76.
[4] 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, 1972, Vol.IV, p.446.
[5] Y. Mizuno, T. Itoh, A. Nomura, *Heterocycles* **1982,** *17,* 61.
[6] K. Anzai, J. Nagatsu, S. Suzuki, *J. Antibiot. Ser. A* **1961,** *14,* 340; K. Anzai, S. Suzuki, *ibid.* **1961,** *14,* 253.
[7] A. Albert, in 'Advances in Heterocyclic Chemistry', Ed. A.R. Katrizky, Academic Press, New York, 1986, Vol.39, p.117.
[8] R.E. Parks, Jr., K.C. Agarwal, in 'Handbook Exp. Pharmacol.' 1975, Vol.38, pp.458.
[9] J. Davoll, *J. Chem. Soc.* **1958,** 1593.
[10] R.D. Elliott, J.A. Montgomery, *J. Med. Chem.* **1976,** *19,* 1186.
[11] W. Hutzenlaub, R.L. Tolmann, R.K. Robins, *J. Med. Chem.* **1972,** *15,* 879.
[12] J.P.H. Verheyden, J.G. Moffat, to *Synthex Inc.* (USA), US 3.817.982, June 18, 1974 (*CA:* **1974,** *81,* 63942b).
[13] F. Seela, K. Mersmann, *Helv. Chim. Acta* **1992,** *75,* 1885.
[14] F.Seela, H.Rosemeyer, R.Gumbiowski, K.Mersmann, H.-P.Muth, A.Röling, *Nucleos. Nucleot.* **1991,** *10,* 409.
[15] F. Seela, H. Rosemeyer, S. Fischer, *Helv. Chim. Acta* **1990,** *73,* 1602.
[16] Y.F. Shealy, J.D. Clayton, C.A. O'Dell, J.A. Montgomery, *J. Org. Chem.* **1962,** *27,* 4518.
[17] Z. Kazimierczuk, U. Bindig, F. Seela, *Helv. Chim. Acta* **1989,** *72,* 1527.
[18] M. Okabe, R.-C. Sun, S.Y.-K. Tam, L.B. Todaro, D.L. Coffen, *J. Org. Chem.* **1988,** *53,* 4780.
[19] L.G. Purnell, D.J. Hodgson, *Org. Magn. Reson.* **1977,** *10,* 1
[20] F. Seela, H.-P. Muth, *Liebigs Ann. Chem.* **1990,** 215.
[21] Z. Kazimierczuk, F. Seela, *Liebigs Ann. Chem.* **1990,** 647.
[22] H. Rosemeyer, G. Toth, B. Golankiewicz, Z. Kazimierczuk, W. Bourgeois, U. Kretschmer, H.-P. Muth, F. Seela, *J. Org. Chem.* **1990,** *55,* 5784.
[23] D. Neuhaus, M. Williamson, in 'The Nuclear Overhauser Effect, Structural and Conformational Analysis', Verlag Chemie, Weinheim, 1989, p.150.
[24] T.R. Emerson, R.J. Swan, T.L.V. Ulbricht, *Biochemistry* **1967,** *6,* 843.
[25] C.A. Bush, in 'Basic Principles in Nucleic Acid Chemistry', Eds. P.O.P. Ts'o, Academic Press, New York, 1974, Vol.2, p.129.
[26] J.L. York, *J. Org. Chem.* **1981,** *46,* 2171.
[27] 'Handbook of Biochemistry and Molecular Biology, Nucleic Acids', Ed.G.D. Fasman, CRC Press, 1979, Vol.1, p.144.
[28] T. Teorell, E. Stenhagen, *Biochem. Z.* **1938,** *299,* 416.
[29] J. Ludwig, *Acta Biochem. Biophys. Acad. Sci. Hung.* **1981,** *16,* 131.