91. Pyrazolo[3,4-*d*]pyrimidine 2′-Deoxy- and 2′,3′-Dideoxyribonucleosides: Studies on the Glycosylation of 4-Methoxypyrazolo[3,4-*d*]pyrimidine

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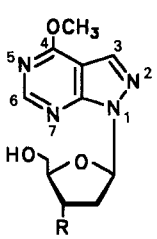
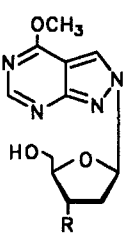
(25.II.93)

The glycosylation of the 4-methoxypyrazolo[3,4-*d*]pyrimidine (**5**) anion with 1-halo-2-deoxyribose **6** in MeCN/TDA-1 gives N^2-deoxynucleoside **9** (29%) together with N^1-isomer **7** (48%) and its anomer **8** (6%) [7]. The α-D-anomer **8** is not formed and the yield of the β-D-anomer **7** increased to 62% when dimethoxyethane is used as solvent and [18]crown-6 as catalyst. Employing 1-halo-2,3-dideoxyribose **10** instead of halogenose **6**, the 2′,3′-dideoxynucleosides **12** and **14** were formed which were desilylated (→ **1b** and **2b**) and converted into the ddI and ddA derivatives **3b** ($c^7z^8I_{dd}$), **15b** ($c^7z^8A_{dd}$), and **17** ($c^7z^8A'_{dd}$). Contrary to 7-deazapurine nucleotides, the triphosphates of **3b** and **15b** showed no appreciable activity against HIV-reverse transcriptase.

Introduction. – Pyrazolo[3,4-*d*]pyrimidines as well as their nucleosides exhibit therapeutic activity against several diseases [1–4]. The synthesis of pyrazolo[3,4-*d*]pyrimidine 2′-deoxyribonucleosides such as **1a**, **2a**, and **3a** and 2′,3′-dideoxyribonucleosides, *e.g.* **3b** or **15b**, were described by our laboratory [5–8]. The synthesis of other pyrazolo[3,4-*d*]pyrimidine nucleosides were reported [9–11]. During our work, it appeared that anion



1a R = OH (systematic numbering)
b R = H



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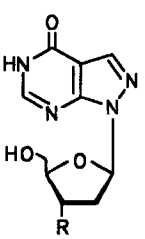
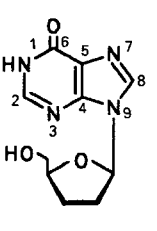
b R = H



3a R = OH
b R = H



4 (purine numbering)

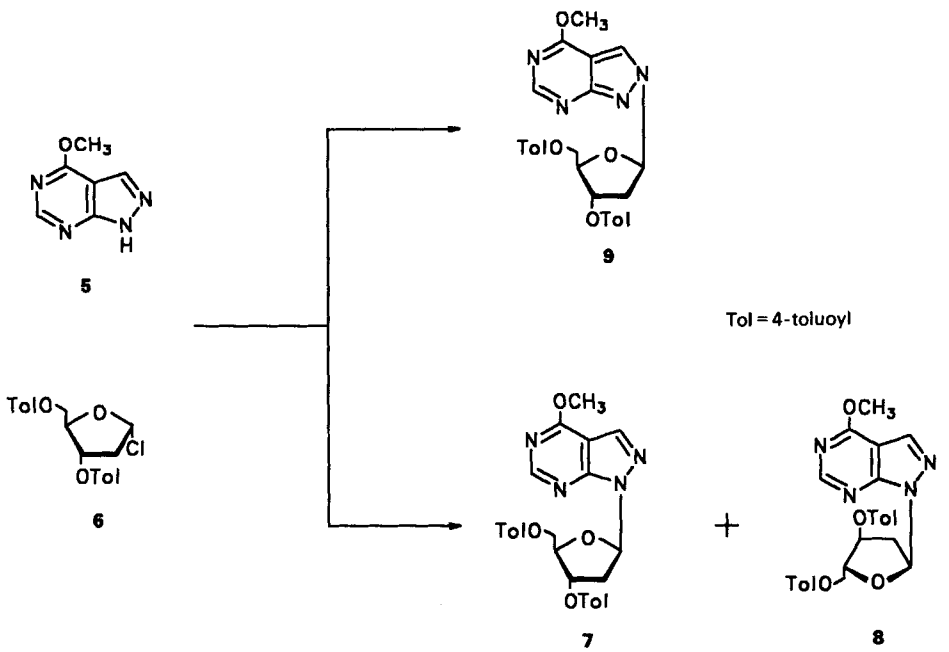
glycosylation of 4-methoxypyrazolo[3,4-*d*]pyrimidine (**5**) yields the N^1-isomers as the major and the N^2-isomers as the minor products [5] [7]. The deoxynucleoside **1a** was later used as the starting material for the synthesis of the 2′,3′-dideoxyribonucleosides such as **3b** *via* deoxygenation [8]. Compound **3b** is related to ddI (**4**) which is a clinically used drug against AIDS [12].

In the present work, the reaction of **5** with halogenose **6** was studied with respect to the ratio and yields of N^1- *vs.* N^2-glycosylation giving pyrazolo[3,4-*d*]pyrimidine 2′-de-

oxyribonucleosides. Reaction conditions and catalysts were changed. Additionally, the synthesis of pyrazolo[3,4-*d*]pyrimidine 2′,3′-dideoxynucleosides was carried out by nucleobase-anion glycosylation of **5** with 5-*O*-[(*tert*-butyl)dimethylsilyl]-2,3-dideoxy-D-*glycero*-pentofuranosyl chloride (**10**) [13] [14]. Triphosphates **19** and **20** of the 2′,3′-dideoxynucleosides were prepared and tested as inhibitors of HIV-reverse transcriptase.

Results and Discussion. – It was already reported [7] that the anion glycosylation of the 4-methoxypyrazolo[3,4-*d*]pyrimidine (**5**) with halogenose **6** resulted in the formation of **7** in 48% yield besides the isomers **8** (6%) and **9** (29%; *Scheme 1*); in this case, MeCN was used as solvent, KOH (5 equiv.) as base, and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) as catalyst [7]. We have now altered the reaction parameters such as tempera-

Scheme 1



ture, catalyst, and solvent. If 1,2-dimethoxyethane ($MeOCH_2CH_2OMe$) was used instead of MeCN, the overall yield decreased (*Table 1*). However, smaller amounts of powdered KOH (1.5 equiv. instead of 5) could be used, and no α-D-anomer **8** was formed. The use of CsOH in $MeOCH_2CH_2OMe$ in the absence of catalyst afforded longer reaction times and gave lower yields. The yield of glycosylation products increased, if TDA-1 was replaced by [18]crown-6 in $MeOCH_2CH_2OMe$; no α-D-anomer was detected also in this case, the total yield was more than 90%, the use of THF instead of $MeOCH_2CH_2OMe$ gave similar results. From the results of *Table 1*, it can be concluded that [18]crown-6 is a more powerful catalyst than TDA-1, gives higher yields, and avoids the formation of α-D-anomers. The N^1/N^2 ratio is only slightly changed using different reaction conditions.

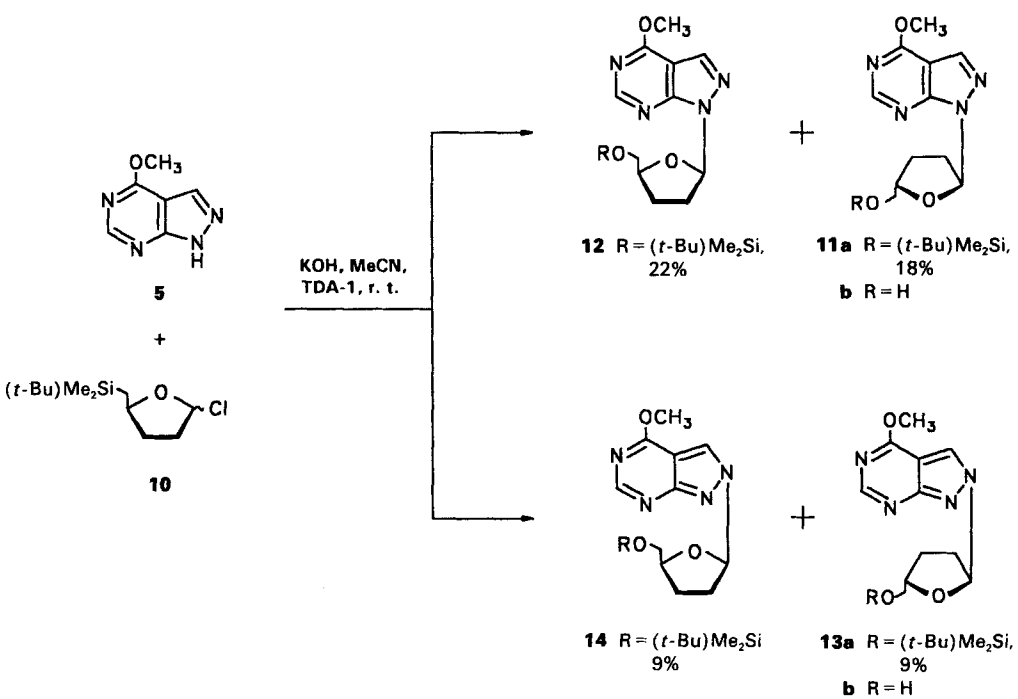
Table 1. *Yields* [%] *of the Isomers* **7**–**9** *Formed upon Glycosylation of* **5** *with Halogenose* **6**[a])

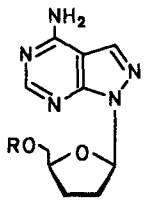
Base (equiv. [mmol])	Solvent	Catalyst	Temp. [°C]	Time [min]	**7** [%]	**8** [%]	**9** [%]
KOH (5)	MeCN	TDA-1	20	20	48	6	29
KOH (1.5)	$MeOCH_2CH_2OMe$	TDA-1	20	20	38	–	18
CsOH (1.8)	$MeOCH_2CH_2OMe$	–	20	45	53	–	22
KOH (1.8)	$MeOCH_2CH_2OMe$	[18]crown-6	20	10	62	–	31
KOH (1.8)	THF	[18]crown-6	20	10	61	–	28

[a]) Conditions, see *Exper. Part.*

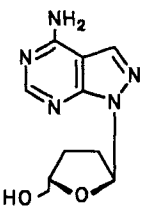
Earlier, pyrazolo[3,4-*d*]pyrimidine 2′,3′-dideoxynucleosides such as **3b** or **15b** were obtained from the 2′-deoxynucleoside **1a** by nucleophilic displacement of the 4-MeO group followed by deoxygenation. As an alternative route, we have now employed the standard glycosylation conditions (KOH, TDA-1, MeCN) and treated compound **5** with 5-*O*-[(*tert*-butyl)dimethylsilyl]-2,3-dideoxy-D-*glycero*-pentofuranosyl chloride (**10**) under conditions described previously [15] [16] (*Scheme 2*). The mixture of the four glycosylation products was separated by FC and isolated in 58% overall yield, the ratio of the two faster migrating N^1 derivatives **11a**/**12** being *ca.* 1:1. The same ratio was found for the slower migrating N^2 compounds **13a**/**14** which were obtained in lower yield and the ratio N^1- *vs.* N^2-glycosylation (2:1) was almost the same as in the case of 2′-deoxyribonucleosides [7]. The N^1 compounds **11a** and **12** were desilylated with 80% $AcOH/H_2O$ to give the 4-MeO compounds **11b** or **1b**, respectively. The corresponding N^2-isomers **13a**

Scheme 2





15a R = (*t*-Bu)Me_2Si
b R = H



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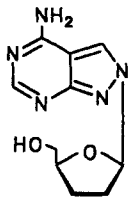

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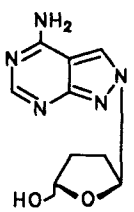


18

and **14** were deblocked with Bu_4NF (→ **13b** and **2b**, resp.) because of the more labile N^2-glycosylic bond [5]. Nucleophilic displacement of the 4-MeO group of **1b** or **2b** as well as of **11b** or **13b** with aqueous ammonia afforded the ddA derivatives **15b** or **17**, respectively, as well as **16** or **18**. Compound **15b** was also obtained from **12** by treatment with NH_3/MeOH *via* **15a** which was desilylated with Bu_4NF. The allopurinol derivative **3b** was obtained from the 4-MeO compound **1b** upon treatment with NaOH.

The position of glycosylation of the N^2-(2′,3′-dideoxynucleosides) **2b** and **18** was determined by NOE-difference spectroscopy [17]. Upon irradiation of H–C(1′), NOE's were observed on H–C(3) (**2b**: 6.8%; **18**: 6.8%) confirming N(2) as glycosylation position. The NOE of H–C(4′) was used to assign the anomeric configuration in other cases [17]. Here, both compounds (**2b** and **18**) showed NOE's on H–C(4′) upon irradiation of H–C(1′) (**2b**: 1.1%; **18**: 0.8%). The NOE in case of **18** was not expected but can be explained by a three-spin effect [18]. As a consequence, another method had to be used. If the ^{13}C-NMR chemical shifts of C(4′) of the β-D-anomers **15b** and **17** are compared to that of the α-D-anomers **16** and **18**, respectively (*Table 2*), the signal of the β-D-anomers is 0.9–1.4 ppm downfield shifted compared to the α-D-compounds. This was already observed in other cases [19] and established the anomeric configuration. This assignment was transferred to all N^2 compounds obtained within a reaction sequence, *e.g.* **2b**, **14**, and **17**, or **13a**, **b** and **18**. The structure of the N^1 compounds **3b** and **15b** was already established [8]. The corresponding α-D-compounds (**11a**, **b** and **16**) were also assigned on the basis of ^{13}C-NMR chemical-shift differences of C(4′), as reported for the N^2 compounds. Characteristic differences were also found in the case of H–C(4′) signals. Also the β-D-anomers migrated faster on TLC than the α-D-anomers [19].

Table 2. *NMR Chemical Shifts of Anomeric Pyrazolo[3,4-*d*]pyrimidine* N^1- *and* N^2-*Nucleosides*

	δ(C(4′))[a]	δ(H–C(4′))[a]	δ(C(1′))[a]	R_f[b]
15b (N^1,β-D)	81.7	4.09	84.4	0.41
16 (N^1,α-D)	80.8	4.24	84.6	0.35
17 (N^2,β-D)	83.1	4.19	91.1	0.17
18 (N^2,α-D)	81.7	4.37	91.3	0.11

[a]) Measured in (D_6)DMSO. [b]) TLC (silica gel, CH_2Cl_2/MeOH 9:1).

As shown in *Table 3*, the N^1 compounds **1b**, **3b**, **11b**, **15b**, and **16** give *ca.* 10-ppm downfield shifts of C(3) and similar upfield shifts of C(7a) compared to the N^2-isomers **2b**, **13b**, **17**, and **18**, also indicating the glycosylation position. An additional downfield shift of C(1′) is observed in the case of the N^2-isomers. The assignment of the signal of the sugar moiety was established by ^{13}C-NMR-INAPT spectroscopy [15].

It was shown that the sign of the *Cotton* effect can be used to assign the anomeric configuration of nucleosides [20]. Pyrimidine β-D-ribonucleosides show a positive *Cotton* effect at 260 nm, while the α-D-anomers show a negative lobe. The opposite situation holds in the purine nucleoside case, where the β-D-anomer exhibit a negtive *Cotton* effect

Table 3. *^{13}C-NMR Chemical Shifts* ((D$_6$)DMSO) *of Pyrazolo[3,4-d]pyrimidine 2′,3′-Dideoxyribofuranosides at 23°*[a][b])

	C(3)	C(3a)	C(4)	C(6)	C(7a)	MeO
1a [5]	131.9	102.4	163.3	155.2	154.5	54.0
b	132.1	102.3	163.5	155.4	154.8	54.0
2a [5]	123.1	102.0	164.9	154.9	160.6	53.9
b	122.9	101.8	165.0	155.1	160.9	53.9
3b [8]	135.2	106.1	157.3	148.4	152.3	–
11a	132.0	102.3	163.5	155.5	154.7	54.4
b	132.0	102.3	163.5	155.4	154.7	54.1
12	131.9	102.2	163.3	155.3	154.7	54.4
13a	123.4	102.1	165.2	155.3	161.1	54.4
b	123.2	102.0	165.1	155.1	161.0	54.4
14	122.8	102.0	165.1	155.2	161.0	54.2
15a	132.9	100.3	158.1	156.1	153.7	–
b [8]	133.0	100.3	158.1	156.1	153.6	–
16	132.9	100.4	158.1	156.1	153.6	–
17[c])	123.4	101.1	159.5	156.4	159.7	–
18	123.3	101.2	159.5	156.4	159.8	–

	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
1a [5]	84.2	38.0	70.9	87.6	62.2
b[b])	84.8	30.6	27.4	82.1	64.2
2a [5]	90.9	40.5	69.9	88.4	61.3
b	91.6	33.1	24.7	83.5	64.9
3b [8]	84.6	30.7	27.3	82.2	64.2
11a	85.3	30.2	26.6	80.7	65.1
b	85.2	30.0	26.9	81.2	63.5
12	84.7	30.3	27.0	81.4	65.5
13a	92.0	32.0	25.5	81.6	65.0
b	91.8	31.8	25.5	82.0	63.2
14	91.6	33.0	24.5	83.0	64.1
15a	84.3	30.2	27.2	81.1	65.9
b [8]	84.4	30.4	27.4	81.7	64.3
16	84.6	29.8	27.0	80.8	63.5
17	91.1	32.5	25.9	83.1	63.5
18	91.3	31.5	25.5	81.7	63.2

[a]) The assignment of the aglycone signals was made on the basis of J(C,H) coupling constants [5].
[b]) The sugar signals were assigned by ^{13}C-NMR-INAPT spectroscopy [15].
[c]) Analogous to the 2′-deoxyribonucleoside [5].

at 260 nm and α-D-anomers have positive *Cotton* effects. The ring puckering of the furanose and the OH substituents have only a minor influence on this phenomenon. However, it was already recognized that the sign of the *Cotton* effect depends on the electronic state and the torsion angle of the nucleobase [21]. We measured the CD spectra of the N^1 compounds **15b** and **16** and of the N^2 regioisomers **17** and **18** (*Fig.*). The anomeric pairs nearly bear mirror image relationship. However, the sign is opposite for the pairs of the N^2- *vs.* the N^1-nucleosides. Due to the 'high-*anti*'-conformation of the base in the case of pyrazolo[3,4-*d*]pyrimidine nucleosides [22], it is not surprising that they do not obey the purine rules.

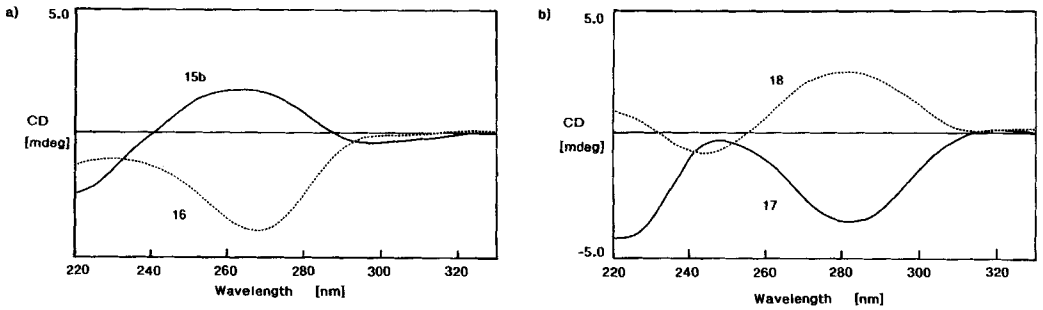
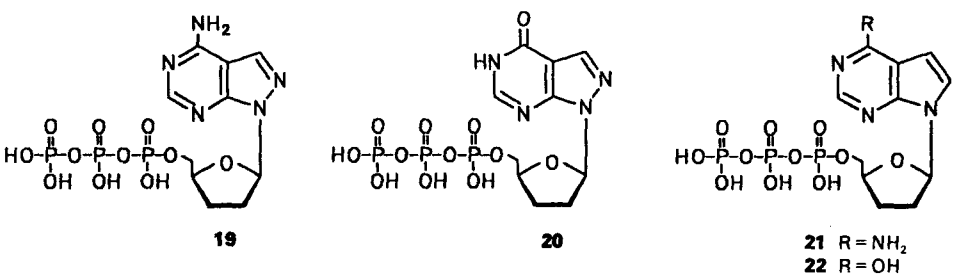


Figure. *CD Spectra* (H_2O) *of 8-aza-7-deazaadenine 2',3'-dideoxyribonucleosides* **15b** *and* **16**, *and* **17** *and* **18**

Earlier, compound **15b** was tested against HIV-infected MT-2 cells [8]. However, only a very low activity was found. It was not clear whether cellular phosphorylation of **15b** is restricted or its triphosphate is not able to inhibit the HIV-reverse transcriptase. Therefore, **15b** was converted into the triphosphate **19** in a one-pot reaction [23]. The same phosphorylation conditions were used for **3b** (→ **20**). The triphosphates **19** and **20** were purified by DEAE-cellulose column chromatography and isolated as triethylammonium salts. A second purification step on HPLC was necessary to obtain pure **20**. None of the triphosphates showed inhibitory activity (IS_{50}) below 100 μM against HIV-reverse transcriptase [16] [24]. This is surprising as 7-deazapurine 2',3'-dideoxyribonucleoside triphosphates such as **21** or **22** are highly active reverse-transcriptase inhibitors [16].



We thank Dr. *B. König* from *Boehringer Mannheim GmbH* for the inhibitory studies on HIV-reverse transcriptase. Financial support by the *Deutsche Forschungsgemeinschaft* and the *Fonds der Chemischen Industrie* is gratefully acknowledged.

Experimental Part

General. See [16]. Powdered KOH was a commercial product of *Fluka* (Switzerland). MeCN was distilled from CaH_2. HIV-Reverse transcriptase inhibitory studies were carried out according to [16]. TLC: for nucleoside quantification, a TLC scanner (*Shimadzu*, Japan) was used. Flash chromatography (FC): at 0.8 bar with silica gel *60* (*Merck*, Germany). Solvent systems: light petroleum ether/Et_2O 4:1 (*A*), light petroleum ether/Et_2O 1:1 (*B*), light petroleum ether/Et_2O 1:4 (*C*), CH_2Cl_2/MeOH 95:5 (*D*), CH_2Cl_2/MeOH 9:1 (*E*), CH_2Cl_2/MeOH 4:1 (*F*), CH_2Cl_2/AcOEt 95:5 (*G*). M.p.: *Büchi-SMP-20* apparatus (*Büchi*, Switzerland) and are not corrected. CD Spectra: *Jasco-600* spectropolarimeter; thermostatically controlled 1-cm cuvettes.

*Glycosylation of 4-Methoxypyrazolo[3,4-*d*]pyrimidine* (**5**) *with 2-Deoxy-3,5-di-*O*-(4-toluoyl)-*α*-*D*-erythro-pentofuranosyl Chloride* (**6**). *General Method:* Compound **5** [25] (900 mg, 6 mmol) was dissolved in the particular solvent (MeCN, $MeOCH_2CH_2OMe$, or THF, 80 ml) under warming and then cooled to r.t. Powdered KOH or CsOH and the catalyst were added under stirring at r.t. (amounts, see *Table 1*). After 15 min, the halogenose **6** [26] (2.33 g, 6 mmol) was introduced in portions. Stirring was continued (see *Table 1*). Insoluble material was filtered off and the solvent evaporated. The oily residue was dissolved in CH_2Cl_2 and applied to FC (column 20 × 4 cm). A pre-run with CH_2Cl_2 eluted non-nucleoside material, CH_2Cl_2/AcOEt 95:5 gave 2 zones. The N^1-(*β*-D-isomer) **7** migrated somewhat faster than the N^1-(α-D-isomer) **8**. The N^2-isomer **9** was eluted with CH_2Cl_2/AcOEt 9:1. Compounds were isolated as colourless foams showing identical ^{1}H-NMR spectra as authentic samples [5].

Glycosylation of **5** *with 2,3-Dideoxy-5-*O*-[(1,1-dimethylethyl)dimethylsilyl]-α/β-*D*-glycero-pentofuranosyl Chloride* (**10**). To a soln. of **5** (300 mg, 2.00 mmol) in dry MeCN (50 ml), powdered KOH (500 mg, 8.9 mmol) and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1; 20 µl, 0.06 mmol) were added while stirring. After 10 min, a soln. of the freshly prepared halogenose **10** (1.1 g, 4.4 mmol) [13] [14] was added in portions, and stirring was continued for another 30 min. Insoluble material was filtered off and aq. $NaHCO_3$ soln. (100 ml) added. The soln. was extracted with AcOEt (30 ml, 3 times), the org. layer combined, dried (Na_2SO_4), filtered, and evaporated, and the oily residue applied to FC (column, 20 × 4 cm) resulting in 4 nucleoside-containing (UV-active) zones.

*1-{2,3-Dideoxy-5-*O*-[(1,1-dimethylethyl)dimethylsilyl]-α-*D*-glycero-pentofuranosyl}-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine* (**11a**). From the fast migrating zone (eluent *A*), a colourless oil (130 mg, 18%) was obtained TLC (*B*): R_f 0.61. UV (MeOH): 247 (7700), 263 (sh, 4700). ^{1}H-NMR ((D_6)DMSO): −0.05, −0.03 (2*s*, Me_2Si); 0.86 (*s*, *t*-Bu); 1.87 (*m*, CH_2(3′)); 2.39, 2.50 (2*m*, CH_2(2′)); 3.54, 3.61 (2*m*, CH_2(5′)); 4.11 (*s*, MeO); 4.29 (*m*, H−C(4′)); 6.62 (*dd*, *J* = 3.7, 6.5, H−C(1′)); 8.30 (*s*, H−C(3)); 8.63 (*s*, H−C(6)). Anal. calc. for $C_{17}H_{28}N_4O_3Si$: C 56.02, H 7.74, N 15.37; found: C 56.14, H 7.81, N 15.50.

*1-{2,3-Dideoxy-5-*O*-[(1,1-dimethylethyl)dimethylsilyl]-β-*D*-glycero-pentofuranosyl}-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine* (**12**). The second zone (eluent *A*) yielded colourless amorphous **12** (160 mg, 22%). TLC (*B*): R_f 0.51. UV (MeOH): 247 (7800), 263 (sh, 4800). ^{1}H-NMR ((D_6)DMSO): −0.11, −0.14 (2*s*, Me_2Si); 0.76 (*s*, *t*-Bu); 2.15 (*m*, CH_2(3′)); 2.48 (*m*, CH_2(2′)); 3.57 (*m*, CH_2(5′)); 4.11 (*s*, MeO); 4.13 (*m*, H−C(4′)); 6.59 (*dd*, *J* = 2.6, 6.9, H−C(1′)); 8.28 (*s*, H−C(3)); 8.62 (*s*, H−C(6)). Anal. calc. for $C_{17}H_{28}N_4O_3Si$: C 56.02, H 7.74, N 15.37; found: C 55.95, H 7.80, N 15.42.

*2-{2,3-Dideoxy-5-*O*-[(1,1-dimethylethyl)dimethylsilyl]-α-*D*-glycero-pentofuranosyl}-4-methoxy-2*H*-pyrazolo[3,4-*d*]pyrimidine* (**13a**). From the third migrating zone (eluent *C*), colourless amorphous **13a** was isolated. Crystallisation from hexane gave colourless crystals (65 mg, 9%). M.p. 109–111°. TLC (*B*): R_f 0.12. UV (MeOH): 259 (9600). ^{1}H-NMR ((D_6)DMSO): −0.05, −0.00 (2*s*, Me_2Si); 0.81 (*s*, *t*-Bu); 1.81 (*m*, CH_2(3′)); 2.20 (*m*, CH_2(2′)); 3.59 (*m*, CH_2(5′)); 4.02 (*s*, MeO); 4.44 (*m*, H−C(4′)); 6.30 (*dd*, *J* = 2.4, 6.3, H−C(1′)); 8.49 (*s*, H−C(6)); 8.69 (*s*, H−C(3)). Anal. calc. for $C_{17}H_{28}N_4O_3Si$: C 56.02, H 7.74, N 15.37; found: C 56.32, H 7.72, N 15.10.

*2-{2,3-Dideoxy-5-*O*-[(1,1-dimethylethyl)dimethylsilyl]-β-*D*-glycero-pentofuranosyl}-4-methoxy-2*H*-pyrazolo[3,4-*d*]pyrimidine* (**14**). The slowest migrating zone (eluent *E*) yielded **14** as a colourless oil (65 mg, 9%). TLC (*B*): R_f 0.05. UV (MeOH): 259 (9600). ^{1}H-NMR ((D_6)DMSO): −0.00, −0.01 (2*s*, Me_2Si); 0.82 (*s*, *t*-Bu); 1.95 (*m*, CH_2(3′)); 2.41 (*m*, CH_2(2′)); 3.72, 3.89 (2*m*, CH_2(5′)); 4.06 (*s*, MeO); 4.24 (*m*, H−C(4′)); 6.28 (*'d'*, *J* = 5.1, H−C(1′)); 8.54 (*s*, H−C(6)); 8.77 (*s*, H−C(3)). Anal. calc. for $C_{17}H_{28}N_4O_3Si$: C 56.02, H 7.74, N 15.37; found: C 56.25, H 7.84, N 15.31.

*4-Amino-1-{2,3-dideoxy-5-*O*-[(1,1-dimethylethyl)dimethylsilyl]-β-*D*-glycero-pentofuranosyl}-1*H*-pyrazolo[3,4-*d*]pyrimidine* (**15a**). Compound **12** (280 mg, 0.77 mmol) in MeOH (50 ml, sat. with ammonia at 0°) was stirred for 60 h at 50°. The soln. was evaporated and the residue purified on silica gel *60* (column 10 × 3 cm, *E*). From the main zone, a colourless solid (210 mg, 78%) was obtained. TLC (*E*): R_f 0.47. UV (MeOH): 260 (9200), 275 (10600). ^{1}H-NMR ((D_6)DMSO): −0.09, −0.10 (2*s*, Me_2Si); 0.78 (*s*, *t*-Bu); 2.18 (*m*, CH_2(3′)); 2.37 (*m*, CH_2(2′)); 3.57 (*m*, CH_2(5′)); 4.10 (*m*, H−C(4′)); 6.46 (*dd*, *J* = 2.6, 6.9, H−C(1′)); 7.68 (*s*, NH_2); 8.13 (*s*, H−C(3)); 8.18 (*s*, H−C(6)). Anal. calc. for $C_{16}H_{27}N_5O_2Si$: C 54.98, H 7.79, N 20.04; found: C 55.25, H 7.92, N 19.82.

*1-(2,3-Dideoxy-α-*D*-glycero-pentofuranosyl)-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine* (**11b**). A soln. of **11a** (286 mg, 0.78 mmol) in 80% AcOH/H_2O (15 ml) was stirred at r.t. for 1 h. The soln. was neutralized with 5% $NaHCO_3$ soln. (50 ml) and extracted 3 times with CH_2Cl_2 (50 ml). The combined org. phases were dried (Na_2SO_4), filtered, and evaporated. FC (silica gel, column 20 × 4 cm, *D*) yielded **11b** which crystallized upon storing (150 mg, 77%). TLC (*D*): R_f 0.55. UV (MeOH): 247 (8000), 263 (sh, 4700). ^{1}H-NMR ((D_6)DMSO): 1.86 (*m*, CH_2(3′)); 2.38, 2.49 (*m*, H−C(2′)); 3.44 (*m*, CH_2(5′)); 4.11 (*s*, MeO); 4.25 (*m*, H−C(4′)); 4.75 (*t*, *J* = 5.7, OH−C(5′)); 6.64 (*dd*, *J* = 3.8, 6.5, H−C(1′)); 8.30 (*s*, H−C(3)); 8.63 (*s*, H−C(6)). Anal. calc. for $C_{11}H_{14}N_4O_3$: C 52.79, H 5.64, N 22.39; found: C 52.60, H 5.90, N 22.10.

*1-(2,3-Dideoxy-β-*D*-glycero-pentofuranosyl)-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine* (**1b**). Compound **12** (300 mg, 0.82 mmol) was treated with 80% $AcOH/H_2O$ and worked up as described for **11a**. Crystallisation from H_2O afforded colourless crystals (160 mg, 78%). M.p. 103°. TLC (*D*): R_f 0.61. UV (MeOH): 247 (7900), 263 (sh, 4600). ^{1}H-NMR ((D_6)DMSO): 2.15 (*m*, CH_2(3′)); 2.44 (*m*, H–C(2′)); 3.40 (*m*, CH_2(5′)); 4.11 (*s*, MeO); 4.12 (*m*, H–C(4′)); 4.68 (*t*, *J* = 5.7, OH–C(5′)); 6.58 (*dd*, *J* = 3.4, 6.6, H–C(1′)); 8.30 (*s*, H–C(3)); 8.62 (*s*, H–C(6)). Anal. calc. for $C_{11}H_{14}N_4O_3$: C 52.79, H 5.64, N 22.39; found: C 52.87, H 5.62, N 22.42.

*2-(2,3-Dideoxy-α-*D*-glycero-pentofuranosyl)-4-methoxy-2*H*-pyrazolo[3,4-*d*]pyrimidine* (**13b**). To a soln. of **13a** (330 mg, 0.91 mmol) in THF (20 ml), 1M Bu_4NF in THF (0.5 ml) was added. The mixture was stirred for 30 min at r.t. and the solvent evaporated. FC (silica gel, column 20 × 4 cm, *D*) gave a colourless oil (170 mg, 75%). TLC (*E*): R_f 0.63. UV (MeOH): 259 (9500). ^{1}H-NMR ((D_6)DMSO): 1.88 (*m*, CH_2(3′)); 2.21, 2.42 (*m*, H–C(2′)); 3.45 (*m*, CH_2(5′)); 4.09 (*s*, MeO); 4.48 (*m*, H–C(4′)); 4.84 (*t*, *J* = 5.8, OH–C(5′)); 6.36 (*dd*, *J* = 2.7, 6.4, H–C(1′)); 8.57 (*s*, H–C(6)); 8.77 (*s*, H–C(3)). Anal. calc. for $C_{11}H_{14}N_4O_3$: C 52.79, H 5.64, N 22.39; found: C 52.65, H 5.75, N 22.35.

*2-(2,3-Dideoxy-β-*D*-glycero-pentofuranosyl)-4-methoxy-2*H*-pyrazolo[3,4-*d*]pyrimidine* (**2b**). To a soln. of **14** (280 mg, 0.77 mmol) in THF (20 ml), 1M Bu_4NF in THF (1 ml) was added. The mixture was treated and worked up as described for **13b**. FC (column, 20 × 4 cm, *D*) gave a colourless oil (140 mg, 73%) which crystallized upon storing. TLC (*E*): R_f 0.71. UV (MeOH): 259 (9500). ^{1}H-NMR ((D_6)DMSO): 1.99 (*m*, CH_2(3′)); 2.41, 2.50 (2*m*, H–C(2′)); 3.56, 3.66 (2*m*, CH_2(5′)); 4.12 (*s*, MeO); 4.24 (*m*, H–C(4′)); 5.09 (*t*, *J* = 5.7, OH–C(5′)); 6.32 (*dd*, *J* = 3.8, 6.5, H–C(1′)); 8.59 (*s*, H–C(6)); 8.95 (*s*, H–C(3)). Anal. calc. for $C_{11}H_{14}N_4O_3$: C 52.79, H 5.64, N 22.39; found: C 52.90, H 5.71, N 22.33.

*4-Amino-1-(2,3-dideoxy-α-*D*-glycero-pentofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine* (**16**). Compound **11b** (160 mg, 0.64 mmol) was stirred in 25% aq. NH_3 soln. (50 ml) at r.t. for 3 days. The solvent was evaporated and the residue applied to FC (column 20 × 4 cm, *E*). Evaporation of the main zone and crystallisation from H_2O afforded colourless crystals (115 mg, 76%). M.p. 210–211°. TLC (*E*): R_f 0.35. UV (MeOH): 230 (8900), 275 (10100). ^{1}H-NMR ((D_6)DMSO): 1.80 (*m*, CH_2(3′)); 2.26, 2.50 (2*m*, H–C(2′)); 3.40 (*m*, CH_2(5′)); 4.24 (*m*, H–C(4′)); 4.75 (*t*, *J* = 5.7, OH–C(5′)); 6.50 (*dd*, *J* = 3.5, 6.5, H–C(1′)); 7.37 (*s*, NH_2); 8.09 (*s*, H–C(3)); 8.18 (*s*, H–C(6)). Anal. calc. for $C_{10}H_{13}N_5O_2$: C 51.06, H 5.57, N 29.77; found: C 50.90, H 5.69, N 29.43.

*4-Amino-1-(2,3-dideoxy-β-*D*-glycero-pentofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine* (**15b**). From **1b**: Compound **1b** (150 mg, 0.60 mmol) was treated with 25% aq. NH_3 soln. (50 ml) and worked up as described for **11b**: colourless solid (110 mg, 78%), identical with an authentic sample [11].

From **15a**: A soln. of **15a** (140 mg, 0.40 mmol) in THF (20 ml) was treated and worked up as described for **13b**: colourless solid (70 mg, 74%). TLC (*E*): R_f 0.41. UV (MeOH): 260 (8900), 275 (10000). ^{1}H-NMR ((D_6)DMSO): 2.14 (*m*, CH_2(3′)); 2.42 (*m*, H–C(2′)); 3.40 (*m*, CH_2(5′)); 4.09 (*m*, H–C(4′)); 4.74 (*t*, *J* = 5.7, OH–C(5′)); 6.46 (*dd*, *J* = 3.5, 6.9, H–C(1′)); 7.71 (*s*, NH_2); 8.15 (*s*, H–C(3)); 8.19 (*s*, H–C(6)).

*4-Amino-2-(2,3-dideoxy-β-*D*-glycero-pentofuranosyl)-2*H*-pyrazolo[3,4-*d*]pyrimidine* (**17**). Compound **2b** (140 mg, 0.56 mmol) was treated with 25% aq. NH_3 soln. (50 ml) as described for **16**. From the main zone **17** was obtained as colourless solid (110 mg, 83%). TLC (*E*): R_f 0.17. UV (MeOH): 231 (7600), 261 (6800), 268 (7600), 291 (10100). ^{1}H-NMR ((D_6)DMSO): 1.88, 2.02 (2*m*, CH_2(3′)); 2.40 (*m*, H–C(2′)); 3.57 (*m*, CH_2(5′)); 4.19 (*m*, H–C(4′)); 4.91 (*t*, *J* = 5.2, OH–C(5′)); 6.21 (*dd*, *J* = 3.1, 6.7, H–C(1′)); 7.70 (*s*, NH_2); 8.13 (*s*, H–C(6)); 8.53 (*s*, H–C(3)). Anal. calc. for $C_{10}H_{13}N_5O_2$: C 51.06, H 5.57, N 29.77; found: C 50.64, H 5.91, N 29.14.

*4-Amino-2-(2,3-dideoxy-α-*D*-glycero-pentofuranosyl)-2*H*-pyrazolo[3,4-*d*]pyrimidine* (**18**). Compound **13b** (130 mg, 0.52 mmol) was treated with 25% aq. NH_3 soln. (50 ml) as described for **16**. From the main zone a colourless oil (95 mg, 81%) was isolated. TLC (*E*): R_f 0.11. UV (MeOH): 231 (8000), 261 (7000), 268 (8000), 291 (10300). ^{1}H-NMR ((D_6)DMSO): 1.86, 2.10 (2*m*, CH_2(3′)); 2.41 (*m*, H–C(2′)); 3.45 (*m*, CH_2(5′)); 4.37 (*m*, H–C(4′)); 4.90 (*t*, *J* = 5.7, OH–C(5′)); 6.27 (*dd*, *J* = 3.3, 5.4, H–C(1′)); 7.70 (*s*, NH_2); 8.13 (*s*, H–C(6)); 8.57 (*s*, H–C(3)). Anal. calc. for $C_{10}H_{13}N_5O_2$: C 51.06, H 5.57, N 29.77; found: C 51.19, H 5.57, N 29.74.

*1-(2,3-Dideoxy-β-*D*-glycero-pentofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-(5*H*)-one* (**3b**). Compound **1b** (80 mg, 0.32 mmol) was stirred in 2N NaOH (20 ml) at r.t. for 3 h. The soln. was neutralized with 2N HCl and then applied to an *Amberlite-XAD-4* resin (column 20 × 2 cm). Inorg. salt was washed out with H_2O and **3b** eluted with H_2O/i-PrOH 9:1. After evaporation, the solid residue was chromatographed (silica gel, column 10 × 3 cm, *E*). The main zone yielded **3b** as colourless solid (55 mg, 73%), which crystallized from H_2O as colourless needles. M.p. 170° ([11]: 171°).

*4-Amino-1-(2,3-dideoxy-β-*D*-glycero-pentofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine 5′-[Tetrakis(triethylammonium) Triphosphate]* (**19** · 4 Et_3N). A soln. of **15b** (21 mg, 0.089 mmol) in trimethyl phosphate (0.5 ml, 2.12 mmol) was cooled to 0°. Freshly distilled $POCl_3$ (30 µl, 0.32 mmol) was added and the mixture stored at 4° for 3.5 h. A soln. of tributylammonium disphosphate (0.5M in anh. DMF, 1 ml) and Bu_3N (200 µl, 0.84 mmol) were added. After 3 min, the mixture was neutralized with 1M aq. $(Et_3NH)HCO_3$. The residue obtained upon evaporation was

chromatographed on *DEAE Sephadex* (column 30 × 2.5 cm, HCO_3^-) with a linear gradient of 0.7M TBK (1 l) and H_2O (1 l). From the main zone, amorphous **19** (172 A_{275} units, 17.2 μmol, 19.3%) was isolated. ^{31}P-NMR (0.1M *Tris*-HCl, pH 7.5, 100 mM EDTA/D_2O): −7.04 (*d*, *J* = 19, P(γ)); −10.17 (*d*, *J* = 19, P(α)); −21.60 (*t*, *J* = 19, P(β)).

*1-(2,3-Dideoxy-β-*D*-glycero-pentofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-(5*H*)-one 5′-[Tetrakis(triethylammonium)(Triphosphate]* (**20** · 4 Et_3N). Compound **20** was prepared from **3b** (18 mg, 0.076 mmol) as described for **19**. After CC, a second purification step on HPLC (*LiChrosorb RP-18*, 0.1N (Et_3NH)OAc/5% MeCN) yielded a colourless solid (7.2 μmol, 9.6%). ^{31}P-NMR (0.1M *Tris*-HCl, pH 7.5, 100 mM EDTA/D_2O): −10.01 (*d*, *J* = 19.4, P(γ)); −10.4 (*d*, *J* = 19.3, P(α)); −22.48 (*t*, *J* = 19, P(β)).

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