

SYNTHESIS OF THE  $\beta$ -D-DEOXYRIBOFURANOSIDE OF 6-AMINO-1H-PYRAZOLO[3,4-d]-  
PYRIMIDIN-4(5H)-ONE -- A NEW ISOSTER OF 2'-DEOXYGUANOSINE

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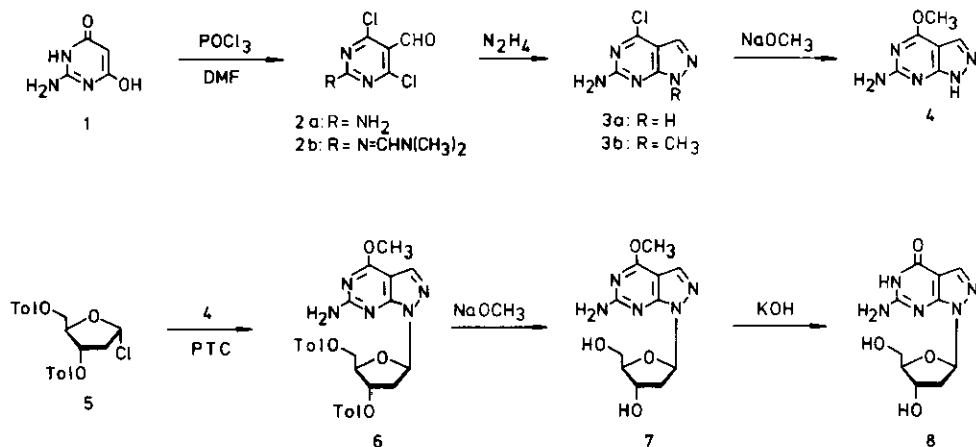
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**Abstract** — 6-Amino-1-(2-deoxy- $\beta$ -D-erythro-pentofuranosyl)-1H-pyrazolo-  
[3,4-d]pyrimidin-4(5H)-one (**8**) has been synthesized via regio- and dia-  
stereo-selective phase-transfer glycosylation of 6-amino-4-methoxy-1H-  
pyrazolo[3,4-d]pyrimidine (**4**) with 2-deoxy-3,5-di-O-(p-toluoyl)- $\alpha$ -D-ery-  
thro-pentofuranosyl chloride (**5**). Compound **4** was obtained from 2-amino-  
4,6-dichloro-5-pyrimidinecarboxaldehyde (**2a**). Hydrolysis experiments  
under acidic conditions showed that the N-glycosylic bond of **8** is more  
labile than that of 2'-deoxyguanosine.

Pyrazolo[3,4-d]pyrimidine ribofuranosides exhibit significant activities in vari-  
ous parasitic systems<sup>1</sup>. This is due to the fact that protozoan parasites such as  
Leishmania cannot synthesize purines *de novo* and depend exclusively on salvage  
pathways for their purine supply. As a result they utilize also pyrazolo[3,4-d]py-  
rimidine ribonucleosides and incorporate them into RNA. In contrast to these ribo-  
nucleosides very little is known about pyrazolo[3,4-d]pyrimidine 2'-deoxyribonu-  
cleosides<sup>2</sup>. In the following we report on the synthesis of 8-aza-7-deaza-2'-de-  
oxyguanosine (**8**), which is isosteric to 2'-deoxyguanosine. Due to the interchange  
of the nitrogen at position 7 with the carbon at position 8 (purine numbering)  
which is one of the smallest modifications of the 2'-deoxyguanosine molecule, al-  
tered physicochemical and biological properties are expected.

Earlier results from the regio- and diastereo-selective synthesis of pyrrolo[2,3-d]-  
pyrimidine 2'-deoxyribofuranosides<sup>3</sup> suggest that 6-amino-4-methoxy-1H-pyrazolo-  
[3,4-d]pyrimidine (**4**) would be an appropriately protected nucleobase intermediate,  
which can be employed in phase-transfer catalyzed glycosylation. It possesses a  
C-4 substituent which can be nucleophilically replaced by a hydroxyl group.

Starting material for the synthesis of compound 4 was the commercially available 2-amino-1,4-dihydro-6-hydroxy-5-oxypyrimidine (1). This was employed in a Vilsmeier-Haack reaction ( $\text{POCl}_3$ -DMF) to yield the aldehyde 2a. According to the procedure of Klötzer <sup>4</sup> a yield of 28 % was reported. L. Bell et al. <sup>5</sup> who used the same protocol obtained 2a in 51 % yield. We employed more vigorous reaction conditions (1.5 h of heating under reflux, more  $\text{POCl}_3$ ) and isolated 2a in 80 % yield. When the reaction mixture was not stored for 12 h at ambient temperature under acidic conditions but was neutralized immediately after  $\text{POCl}_3$  treatment in an ice bath, the reaction intermediate 2b precipitated. Chromatographic separation (silica gel,  $\text{CH}_2\text{Cl}_2$ -EtOAc, 1:1) yielded pure 2b, which crystallized from methanol [Anal. Calcd. for  $\text{C}_8\text{H}_8\text{Cl}_2\text{N}_4\text{O}$ : C, 38.99; H, 3.26; Cl, 28.70; N, 22.68. Found: C, 39.19; H, 3.10; Cl, 28.94; N, 22.89; uv  $\lambda_{\text{max}}$  335 nm;  $^1\text{H}$ -nmr  $\delta$  3.14, 3.28 (2s, 2 $\text{CH}_3$ ), 8.78 (s, CHN), 10.15 (s, CHO)]. The isolation of the reaction intermediate 2b shows that DMF takes part in transient protection of the 2-amino function avoiding intermolecular condensation.



Reaction of 2a [ $^{13}\text{C}$ -nmr  $\delta$  112.9 (C-5), 161.6 - 163.1 (C-2, C-4, C-6), 184.5 (CHO)] with aqueous hydrazine-1,2-dimethoxyethane afforded 6-amino-4-chloro-1H-pyrazolo[3,4-d]pyrimidine (3a) in 80 % yield. [uv  $\lambda_{\text{max}}$  227, 305 nm; Anal. Calcd. for  $\text{C}_5\text{H}_4\text{ClN}_5$ : C, 35.42; H, 2.38; Cl, 20.91; N, 41.30. Found: C, 35.37; H, 2.42; Cl, 21.01; N, 41.38]. TLC monitoring ( $\text{CHCl}_3$ -MeOH, 9:1, silica gel) of the condensation reaction allowed the detection of an intermediate, where hydrazine was only mono-functionalized.

In order to test the utility of compound 3a in phase-transfer catalyzed reactions it was methylated (bi-phasic mixture, dichloromethane-50 % aq. NaOH,  $\text{CH}_3\text{I}$ ) in the

presence of benzyltriethylammonium chloride to yield the N-1 isomer 3b [ $\text{uv } \lambda_{\text{max}} 306 \text{ nm}$ ;  $^1\text{H-nmr } \delta 3.80 \text{ (s, CH}_3\text{)}, 7.28 \text{ (s, NH}_2\text{)}, 7.93 \text{ (s, 3-H)}$ ] and the N-2 isomer [ $\text{uv } \lambda_{\text{max}} 282, 312 \text{ nm}$ ;  $^1\text{H-nmr } \delta 3.99 \text{ (s, CH}_3\text{)}, 6.87 \text{ (s, NH}_2\text{)}, 8.41 \text{ (s, 3-H)}$ ] in a 2:1 ratio. In contrast to this methylation reaction, which furnished a total yield of isomers of 70 %, the glycosylation of compound 3a with the halogenose 5 was not successful under phase-transfer conditions. This problem was overcome by employing the methoxy compound 4 [ $\text{uv } \lambda_{\text{max}} 276 \text{ nm}$ ;  $^1\text{H-nmr } \delta 3.95 \text{ (s, OCH}_3\text{)}, 6.61 \text{ (s, NH}_2\text{)}, 7.78 \text{ (s, 3-H)}, 12.81 \text{ (s, NH)}$ ; Anal. Calcd. for  $\text{C}_6\text{H}_7\text{N}_5\text{O}$ : C, 43.63; H, 4.27; N, 42.41. Found: C, 43.52; H, 4.28; N, 42.50] which was obtained from 3a by treatment with sodium methoxide in 80 % yield. Phase-transfer glycosylation of 4 with the halogenose 5 in a bi-phasic mixture ( $\text{CH}_2\text{Cl}_2$ -50 % aq. NaOH, benzyltriethylammonium chloride, ambient temperature) gave after vigorous mixing for 2 min and purification on silica gel ( $\text{CH}_2\text{Cl}_2$ -EtOAc, 4:1, v/v) compound 6 in 37 % yield. [ $^1\text{H-nmr } \delta 2.35, 2.38 \text{ (2s, Me)}, 2.70 \text{ (m, 2'-H}_b\text{)}, 3.32 \text{ (m, 2'-H}_a\text{)}, 3.97 \text{ (s, MeO)}, 4.43 \text{ (m, 4'-H, 5'-H)}, 5.80 \text{ (m, 3'-H)}, 6.60 \text{ (pt, J = 6.0 Hz, 1'-H)}, 6.93 \text{ (s, NH}_2\text{)}, 7.94 \text{ (s, 3-H)}$ ]. The N-2 isomer and the  $\alpha$ -anomer (not shown) were only formed to a small extent. Deprotection of compound 6 was accomplished with sodium methoxide at ambient temperature to give the nucleoside 7 [ $\text{uv } \lambda_{\text{max}} 252, 276 \text{ nm}$ ; Anal. Calcd. for  $\text{C}_{11}\text{H}_{15}\text{N}_5\text{O}_4$ : C, 46.97; H, 5.38; N, 24.90. Found: C, 46.71; H, 5.38; N, 24.98;  $^1\text{H-nmr } \delta 3.97 \text{ (s, MeO)}, 6.40 \text{ (pt, J = 6.5 Hz, 1'-H)}, 7.88 \text{ (s, NH}_2\text{)}, 7.94 \text{ (s, 3-H)}$ ]

 Tab.  $^{13}\text{C-nmr}$  Chemical Shifts of Pyrazolo[3,4-d]pyrimidine 2'-Deoxyribofuranosides

	<u>3a</u>	<u>3b</u>	<u>4</u>	<u>6</u>	<u>7</u>	<u>8</u>
C-3	132.5	131.6	131.3	132.5	131.9	134.9
C-3a	105.7	106.0	95.4	96.2	96.1	99.7
C-4	153.1	153.3	163.4	163.5	163.4	157.4
C-6	161.4	161.3	161.8	162.1	161.9	154.6
C-7a	157.4	155.6	158.7	158.0	157.6	155.2
CH <sub>3</sub>			53.0	53.2	53.2	
C-1'				81.0	83.3	83.1
C-2'				34.9	37.8	37.9
C-3'				74.9	71.0	71.0
C-4'				83.4	87.3	87.3
C-5'				64.0	62.4	62.4

in 70 % yield after silica gel chromatography (CHCl<sub>3</sub>-MeOH, 9:1, v/v). Nucleophilic displacement of the methoxy group of 7 (2 N KOH, 24 h, ambient temperature) gave the nucleoside 8. Chromatographic purification on Amberlite XAD-4 (water-propanol-2, 9:1) resulted in crystalline material [dioxane, mp 196° C (decomp); Anal. Calcd. for C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub>: C, 44.94; H, 4.90; N, 26.21. Found: C, 44.81; H, 5.00; N, 26.11; uv λ<sub>max</sub> 252 nm (MeOH), 250 nm (1 N HCl), 265 nm (2 N KOH)].

Hydrolysis experiments of 4-amino-1H-pyrazolo[3,4-d]pyrimidine N-1-β-D-2'-deoxy-ribofuranoside have shown that this compound was more stable at its N-glycosylic bond than 2'-deoxyadenosine <sup>6</sup>. Therefore, it was of interest to compare the hydrolytic stability of 8 vs. 2'-deoxyguanosine. Hydrolysis experiments were carried out in 0.5 N hydrochloric acid at 25° C. Under these conditions the nucleobases were released from both compounds. In order to get quantitative data the decrease of the uv absorbance was monitored at 251 nm for 8 and 260 nm for 2'-deoxyguanosine. From time-absorbance plots the pseudo first order hydrolysis constants (k) were determined according to the equation  $k = 1/t \ln(E_0 - E_\infty) / (E - E_\infty)$ . The data [k(8) = 14 x 10<sup>-2</sup> min<sup>-1</sup>, τ<sub>1/2</sub> = 4.95 min; k(2'-deoxyguanosine) = 6.5 x 10<sup>-2</sup> min<sup>-1</sup>, τ<sub>1/2</sub> = 10.6 min] indicate that the nucleoside 8 is less stable under acidic conditions than the parent 2'-deoxyguanosine, which is in contrast to other pyrazolo[3,4-d]pyrimidine 2'-deoxyribofuranosides <sup>6</sup>.

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