SYNTHESIS OF THE 8-D-DEOXYRIBOFURANOSIDE OF 6-AMINO-1H-PYRAZOL[3,4-d]-PYRIMIDIN-4(5H)-ONE — A NEW ISOSTER OF 2'-DEOXYGUANOSINE

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Abstract — 6-Amino-1-(2-deoxy-8-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)-D-erythro-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 various parasitic systems. 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]pyrimidine ribonucleosides and incorporate them into RNA. In contrast to these ribonucleosides very little is known about pyrazolo[3,4-d]pyrimidine 2'-deoxyribonucleosides. In the following we report on the synthesis of 8-aza-7-deaza-2'-deoxyguanosine (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, altered physicochemical and biological properties are expected.

Earlier results from the regio- and diastereo-selective synthesis of pyrrolo[2,3-d]pyrimidine 2'-deoxyribofuranosides 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.

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Starting material for the synthesis of compound 4 was the commercially available 2-amino-1,4-dihydro-6-hydroxy-5-oxopyrimidine (1). This was employed in a Vilsmeier-Haack reaction (POCl₃-DMF) to yield the aldehyde 2a. According to the procedure of Klützer ⁴ a yield of 28% was reported. L. Bell et al. ⁵ who used the same protocol obtained 2a in 51% yield. We employed more vigorous reaction conditions (1.5 h of heating under reflux, more POCl₃) 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 POCl₃ treatment in an ice bath, the reaction intermediate 2b precipitated. Chromatographic separation (silica gel, CH₂Cl₂-EtOAc, 1:1) yielded pure 2b, which crystallized from methanol. \[ \text{Anal. Calcd. for } \text{C}_6\text{H}_4\text{ClN}_2: \text{C}, 38.99; \text{H}, 3.26; \text{Cl}, 28.70; \text{N}, 22.68. \text{Found: } \text{C}, 39.19; \text{H}, 3.10; \text{Cl}, 28.94; \text{N}, 22.89; \text{uv } \lambda_{\text{max}} \text{ 335 nm; } ^1\text{H-nmr } 3.14, 3.28 (2s, 2\text{CH}_3), 8.78 (s, \text{C=N}), 10.15 (s, \text{CHO}). \]

The isolation of the reaction intermediate 2b shows that DMF takes part in transient protection of the 2-amino function avoiding intramolecular condensation.

\[ \begin{align*}
\text{POCl}_3 & \quad \text{DMF} & 2a: R = \text{NH}_2 \\
& & 2b: R = \text{N}\\n& & \text{HNN(CH}_3\text{)}_2
\end{align*} \]

\[ \begin{align*}
\text{N}_2\text{H}_4 & \quad \text{NaOCH}_3 & 3a: R = \text{H} \\
& & 3b: R = \text{CH}_3
\end{align*} \]

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

In order to test the utility of compound 3a in phase-transfer catalyzed reactions it was methylated (bi-phasic mixture, dichloromethane-50%aq. NaOH, CH₂I) in the
presence of benzyltriethylammonium chloride to yield the N-1 isomer 3b \( [\text{uv } \lambda_{\text{max}} \text{ 306 nm;} ^1\text{H-nmr } \delta \text{ 3.80 (s, CH}_3\text{), 7.28 (s, NH}_2\text{), 7.93 (s, 3-H)}] \) and the N-2 isomer \( [\text{uv } \lambda_{\text{max}} \text{ 282, 312 nm;} ^1\text{H-nmr } \delta \text{ 3.99 (s, CH}_3\text{), 6.87 (s, NH}_2\text{), 8.41 (s, 3-H)}] \) 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 \( [\text{uv } \lambda_{\text{max}} \text{ 276 nm;} ^1\text{H-nmr } \delta \text{ 3.95 (s, OCH}_3\text{), 6.61 (s, NH}_2\text{), 7.78 (s, 3-H), 12.81 (s, NH)}] \); Anal. Calcd. for C\text{6}H\text{7}N\text{5}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 (CH\text{2}Cl\text{2}-50 % aq. NaOH, benzyltriethylammonium chloride, ambient temperature) gave after vigorous mixing for 2 min and purification on silica gel(CH\text{2}Cl\text{2}-EtOAc, 4:1, v/v) compound 6 in 37 % yield. \( ^1\text{H-nmr } \delta \text{ 2.35, 2.38 (2s, Me), 2.70 (m, 2'-H}_p\text{), 3.32 (m, 2'-H}_a\text{), 3.97 (s, MeO), 4.43 (m, 4'-H, 5'-H), 5.80 (m, 3'-H), 6.60 (pt, J = 6.0 Hz, 1'-H), 6.93 (s, NH}_2\text{), 7.94 (s, 3-H)}] \). The N-2 isomer and the a-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 \( [\text{uv } \lambda_{\text{max}} \text{ 252, 276 nm;} \text{Anal. Calcd. for C}\text{11}H\text{15}N\text{5}O\text{4: C, 46.97; H, 5.38; N, 24.90. Found: C, 46.71; H, 5.38; N, 24.98; } ^1\text{H-nmr } \delta \text{ 3.97 (s, MeO), 6.40 (pt, J = 6.5 Hz, 1'-H), 7.88 (s, NH}_2\text{), 7.94 (s, 3-H)}] \)

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

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in 70% yield after silica gel chromatography (CHCl₃-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 C₁₀H₁₃N₅O₄: C, 44.94; H, 4.90; N, 26.21. Found: C, 44.81; H, 5.00; N, 26.11; uv λ max 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'-deoxyribofuranoside have shown that this compound was more stable at its N-glycosylic bond than 2'-deoxyguanosine. 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(E₀ - Eₗ)/(E - Eₗ). The data [k(8) = 14 x 10⁻² min⁻¹, t₁/2 = 4.95 min; k(2'-deoxyguanosine) = 6.5 x 10⁻² min⁻¹, t₁/2 = 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.

ACKNOWLEDGMENTS
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REFERENCES AND NOTES
7. Nmr spectra were recorded in (Me₂SO-d₆, uv spectra in MeOH solution.

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