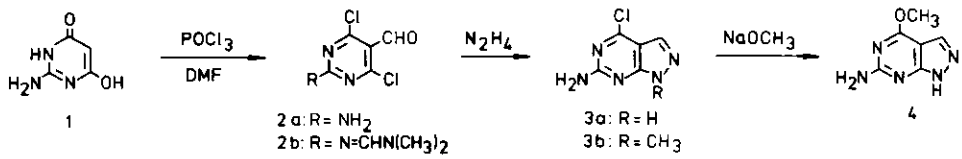
SYNTHESIS OF THE ß-D-DEOXYRIBOFURANOSIDE OF 6-AMINO-1H-PYRAZOLO[3,4-d]-PYRIMIDIN-4(5H)-ONE — A NEW ISOSTER OF 2'-DEOXYGUANOSINE

Frank Seela* and Herbert Steker
Laboratorium für Bioorganische Chemie, Fachbereich Chemie,
Universität Paderborn, D-4790 Paderborn, Bundesrepublik Deutschland

Abstract —— 6-Amino-1-(2-deoxy-ß-D-erythro-pentofuranosyl)-1H-pyrazolo-[3,4-d]pyrimidin-4(5H)-one (8) has been synthesized via regio- and diastereo-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)-α-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 [1]. 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 [2]. 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 [3] 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-oxopyrimidine (1). This was employed in a Vilsmeier-Haack reaction ($POCl_3$-DMF) to yield the aldehyde 2a. According to the procedure of Klötzer [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 $POCl_3$) 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 $POCl_3$ treatment in an ice bath, the reaction intermediate 2b precipitated. Chromatographic separation (silica gel, CH_2Cl_2-EtOAc, 1:1) yielded pure 2b, which crystallized from methanol [Anal. Calcd. for $C_8H_8Cl_2N_4O$: 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 λ_{max} 335 nm; ^{1}H-nmr δ 3.14, 3.28 (2s, $2CH_3$), 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.



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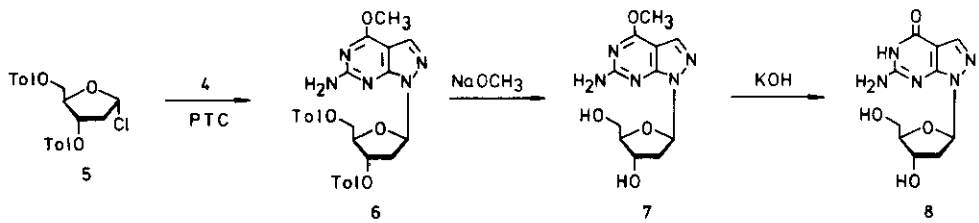

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Reaction of 2a [^{13}C-nmr δ 112.9 (C-5), 161.6 - 163.1 (C-2, C-4, C-6), 184.5 (CHO)] with aqueous hydrazine-1,2-dimethoxyethane afforded 6-amino-4-chloro-1H-pyrazolo-[3,4-d]pyrimidine (3a) in 80 % yield. [uv λ_{max} 227, 305 nm; Anal. Calcd. for $C_5H_4ClN_5$: 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 ($CHCl_3$-MeOH, 9:1, silica gel) of the condensation reaction allowed the detection of an intermediate, where hydrazine was only monofunctionalized.

In order to test the utility of compound 3a in phase-transfer catalyzed reactions it was methylated (bi-phasic mixture, dichloromethane-50 % aq. NaOH, CH_3I) in the

presence of benzyltriethylammonium chloride to yield the N-1 isomer 3b [uv λ_{max} 306 nm; ^{1}H-nmr δ 3.80 (s, CH_3), 7.28 (s, NH_2), 7.93 (s, 3-H)] and the N-2 isomer [uv λ_{max} 282, 312 nm; ^{1}H-nmr δ 3.99 (s, CH_3), 6.87 (s, NH_2), 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 [uv λ_{max} 276 nm; ^{1}H-nmr δ 3.95 (s, OCH_3), 6.61 (s, NH_2), 7.78 (s, 3-H), 12.81 (s, NH); Anal. Calcd. for $C_6H_7N_5O$: 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_2Cl_2-50 % aq. NaOH, benzyltriethylammonium chloride, ambient temperature) gave after vigorous mixing for 2 min and purification on silica gel(CH_2Cl_2-EtOAc, 4:1, v/v) compound 6 in 37 % yield. [^{1}H-nmr δ 2.35, 2.38 (2s, Me), 2.70 (m, 2'-H_b), 3.32 (m, 2'-H_a), 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), 7.94 (s, 3-H)]. The N-2 isomer and the α-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 [uv λ_{max} 252, 276 nm; Anal. Calcd. for $C_{11}H_{15}N_5O_4$: C, 46.97; H, 5.38; N, 24.90. Found: C, 46.71; H, 5.38; N, 24.98; ^{1}H-nmr δ 3.97 (s, MeO), 6.40 (pt, J = 6.5 Hz, 1'-H), 7.88 (s, NH_2), 7.94 (s, 3-H)]

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

	3a	3b	4	6	7	8
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_3			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 ($CHCl_3$-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 $C_{10}H_{13}N_5O_4$: 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'-deoxyadenosine [6]. 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 $k = 1/t \ln(E_o-E_\infty)/(E-E_\infty)$. The data [k(8) = 14 x 10^{-2} min^{-1}, $\tau_{1/2}$ = 4.95 min; k(2'-deoxyguanosine) = 6.5 x 10^{-2} min^{-1}, $\tau_{1/2}$ = 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 [6].

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