Synthesis and Hydrolytic Stability of 4-Substituted Pyrazolo[3,4-*d*]pyrimidine 2'-Deoxyribofuranosides

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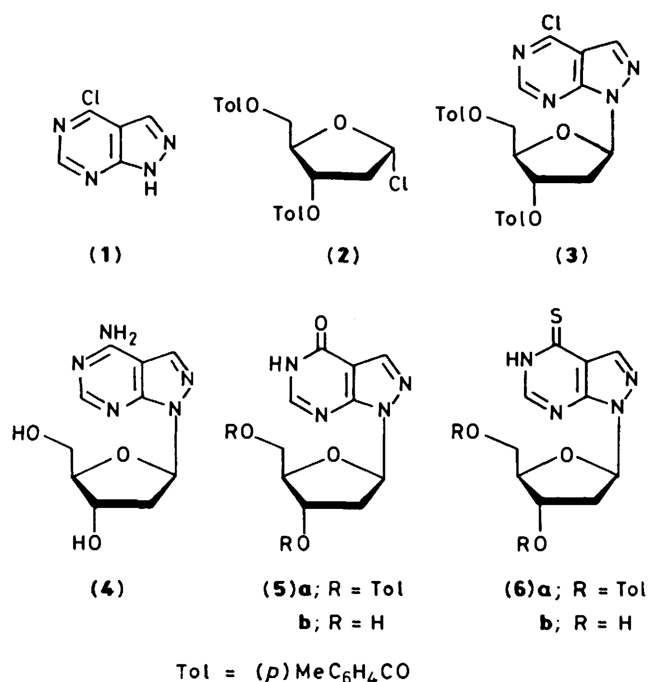
Phase-transfer glycosylation of 4-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidine (**1**) with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride (**2**) yielded the N-1 glycosylation product (**3**) in 42%. The protected intermediate (**3**) was converted into pyrazolo[3,4-*d*]pyrimidine 2'-deoxyribofuranosides with amino, oxo, and thioxo substituents at C-4. Kinetic data of proton-catalysed hydrolysis showed that pyrazolo[3,4-*d*]pyrimidine 2'-deoxyribofuranosides are more stable at the *N*-glycosylic bond than are the parent purine nucleosides.

In vitro mutagenesis experiments by incorporation of modified 2'-deoxyribonucleosides into oligonucleotides[1] have directed our interest towards the synthesis of monomeric DNA-constituents with modified heterocyclic bases.[2] In particular, aza- and deaza-purine 2'-deoxyribofuranosides with an altered imidazole moiety are useful tools to elucidate the influence of modified nucleosides on DNA structure and function.[3,4]

Recently we have reported on the synthesis of allopurinol 2'-deoxyribofuranoside (**5b**) as well as 4-methoxy and 4-amino derivatives thereof, employing 4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine as nucleobase intermediate.[5,6] Parallel to these experiments, 4-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidine (**1**)[7] was used in glycosylation reactions. In contrast to a recently reported procedure,[8] we decided to employ phase-transfer conditions;[9] these are easily attained and result generally in high



yields of glycosylation products. We now report on the phase-transfer glycosylation of the relatively labile chromophore (**1**) with the halogenose (**2**). Furthermore, the conversion of the protected glycosylation product (**3**) into thioallopurinol 2'-deoxyribonucleoside (**6b**) and other 4-substituted pyrazolo[3,4-*d*]pyrimidine 2'-deoxyribonucleosides, including a dimethyl sulphoxide (DMSO)-mediated hydroxylation, is described. In order to test the stability of these nucleosides *vs.* hydrolytic cleavage at the *N*-glycosylic bond, kinetic parameters of the hydrolysis reaction are elaborated and compared with those of 2'-deoxyribofuranosides of both purine and pyrrolo[2,3-*d*]-pyrimidine.

It has been reported that the aglycone (**1**) is a rather labile molecule.[7] As a consequence special conditions of glycosylation have to be chosen in order to obtain the N-1 deoxyribofuranoside in high yield. Application of regular conditions of phase-transfer glycosylation[10] by using a biphasic mixture of dichloromethane and 50% sodium hydroxide in the presence of tetrabutylammonium hydrogen sulphate failed. If solid potassium carbonate was used instead of aqueous NaOH in the absence of a phase-transfer catalyst the N-1 and N-2 glycosylation products of compound (**3**) were formed, which was confirmed by their conversion into the β-D-2'-deoxyribofuranosides of 4-methoxypyrazolo[3,4-*d*]pyrimidine.[6] However, the long reaction time (16 h) of this procedure led to a partial decomposition of the aglycone and resulted in low yields of the glycosylation products. In order to shorten the reaction time a biphasic mixture was used again but now in the absence of a phase-transfer catalyst. In a mixture of tetrahydrofuran(THF)–50% aqueous sodium hydroxide the reaction was rapid (3 min) and led to one main glycosylation product. This was isolated in crystalline form, after purification, in 42% yield. Its structure was confirmed by elemental analysis and n.m.r. spectra and was in agreement with formula (**3**). The N-2 glycosylation product was detected in only trace amounts.

Investigations of the stability of the protected 4-chloro compound (**3**) under glycosylation conditions as described above revealed that the material did not decompose significantly in the biphasic system if the phase-transfer catalyst was absent. Consequently, the phase-transfer catalyst used in experiments described above is the source which generates side-reactions with the reactive halogen of compound (**1**) or (**3**).

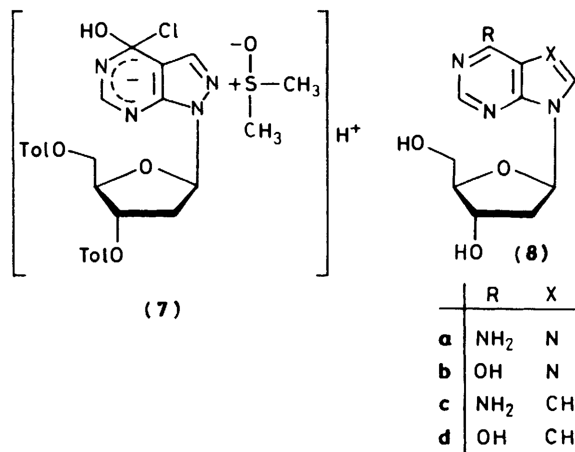
In contrast to the phase-transfer glycosylation of 4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine[5,6] the reaction of compound (**1**) with the halogenose (**2**) did not lead to an α-anomer as a glycosylation side-product. Since it has been shown[11,12] that catalysts can equilibrate the halogenose (**2**), thus resulting in an anomeric mixture of nucleosides *via* an S_N2 mechanism or by switching the mechanism from S_N2 to S_N1,[2,13] the absence of an α-anomer is a direct consequence of the absence of catalyst.

The protected 4-chloronucleoside (**3**) was then converted into several pyrazolo[3,4-*d*]pyrimidine 2'-deoxynucleosides by one- or two-step reactions. Treatment of compound (**3**) with methanolic ammonia in an autoclaved reaction gave the crystalline aminonucleoside (**4**) in 77% yield.[5,8] A similar reaction with thiourea in methanol solution resulted in the formation of the

protected thionucleoside (**6a**), which precipitated directly from the reaction mixture. Deprotection of compound (**6a**) with sodium methoxide yielded the free thionucleoside (**6b**). This compound, which was characterized by elemental analysis and spectroscopic methods, possesses a u.v. absorption maximum at 322 nm, which is well separated from those of regular 2′-deoxyribofuranosides of both purine and pyrazolo[3,4-*d*]-pyrimidine. Since it is documented that thio substituents stabilize nucleic acid structures, the long-wavelength absorption of compound (**6b**) makes it suitable to be used as a probe for DNA secondary structure and recognition.

In contrast to the conversion of compound (3) into (**6b**), nucleophilic displacement of the 4-halogen with sodium hydroxide did not result in a clean conversion into the protected allopurinol 2′-deoxyribofuranoside (**5a**); instead a mixture of reaction products was formed. However, we observed a clean reaction during n.m.r. experiments on compound (3) in DMSO solution. This reaction, which takes place due to traces of water in the solvent, was then attempted in a preparative-scale experiment in which we obtained compound (**5a**) in 68% yield. Deprotection of compound (**5a**) with sodium methoxide gave the nucleoside (**5b**), which was identical in all spectroscopic and chromatographic properties with an authentic sample synthesized by another route.[5,6]

It has been reported that DMSO is able to enhance nucleophilic displacement reactions by several orders of magnitude.[14,15] If one agrees that during nucleophilic displacement in compound (3) a Meisenheimer complex[16] as depicted in formula (7) is formed, then DMSO as a dipolar solvent can stabilize this complex and decrease the activation energy.[17]



Moreover, DMSO solvates protons through its oxygen which increases the nucleophilicity of water. Both phenomena can account for the acceleration of chlorine displacement in compound (3). The hydrogen chloride formed under these conditions has a low capacity to act as hydrolysing agent on the *N*-glycosylic bond due to the solvent acting as proton scavenger

The glycosyl hydrolysis of purine 2′-deoxynucleosides is still a problem in the synthesis of oligonucleotides. Since we started a programme to study structural and functional alterations of DNA structure by pyrazolo[3,4-*d*]pyrimidine 2′-deoxyribonucleosides we have become interested in the stability of the *N*-glycosylic bond of these compounds compared with that in purine 2′-deoxyribofuranosides. For this purpose the hydrolytic stability of compounds (**4**), (**5b**), and (**6b**) was studied and compared with that of the parent purine nucleosides (**8a**) and (**8b**). Hydrolysis experiments were carried out in 1M-hydrochloric acid at 25 °C (Figure). Under these conditions, the intact nucleobases were released from the nucleosides as was

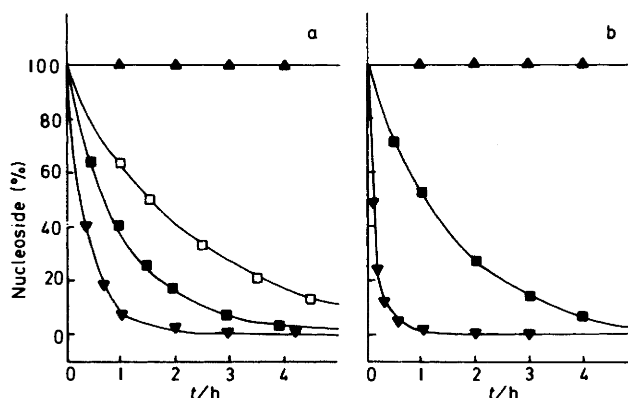
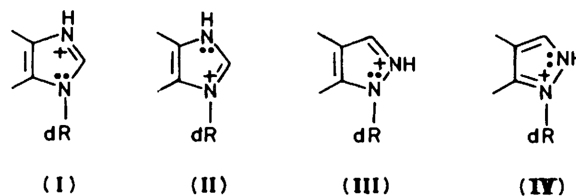


Figure. Hydrolysis rates of isosteric 2′-deoxyribofuranosides in 1M-hydrochloric acid at 25 °C. Data are taken from the decrease of the u.v. absorption at the wavelength indicated. (a) (**8d**) [260 nm, ▲], (**6b**) [321 nm, □], (**5b**) [253 nm, ■], (**8b**) [251 nm, ▼]; (b) (**8c**) [260 nm, ▲], (**4**) [258 nm, ■], (**8a**) [258 nm, ▼]

confirmed by comparison of the pH-dependent u.v. spectra of the hydrolysis products with authentic samples of the aglycones. Data were obtained from the decrease of the u.v. absorbance differences between the nucleosides and the corresponding aglycones, and are given in the Table. The measurements were carried out at a wavelength where this difference was most pronounced. As the Figure shows, the pyrazolo[3,4-*d*]pyrimidine 2′-deoxyribofuranosides (**4**) and (**5b**) are definitely more stable than the parent purine compounds (**8a**) and (**8b**). On the other hand, they do not reach the stability of the corresponding pyrrolo[2,3-*d*]pyrimidine nucleosides (**8c**) and (**8d**). The differences in stability between purine nucleosides and pyrazolo[3,4-*d*]pyrimidine nucleosides are more pronounced in the 4-amino than in the 4-oxo series. A thioxo group as in compound (**6b**), compared to the oxo group in compound (**5b**), contributes a stabilizing effect to the glycosylic bond.

An interpretation of the different hydrolytic stabilities can be given as follows: the generation of a positive charge at the nitrogen of the *N*-glycosylic bond is a prerequisite for its cleavage under acidic conditions. Since, in pyrrolo[2,3-*d*]pyrimidine nucleosides, protonation of the five-membered ring cannot take place without affecting the aromatic state,[18] the hydrolytic stability of these nucleosides is a direct consequence. Purine nucleosides as well as pyrazolo[3,4-*d*]pyrimidine nucleosides have a free pair of electrons which does not participate with the aromatic system and can be protonated easily [formulaes (**I**)—(**IV**)]. However, the activation energy for



imidazole protonation in purine nucleosides should be lower than that of the pyrazole moiety of pyrazolo[3,4-*d*]pyrimidine nucleosides. This is due to better delocalization of the positive charge at the imidazole [(**I**)—(**II**)] than at the pyrazole system [(**III**)—(**IV**)]. Only in the latter case does the delocalization affect the aromaticity of the fused pyrimine ring. As a consequence, pyrazolo[3,4-*d*]pyrimidine 2′-deoxyribonucleosides are less easily hydrolysed than are purine nucleosides.

Table. Kinetic data of *N*-glycosyl bond hydrolysis of nucleobase-modified 2′-deoxyribonucleosides:[a] data are taken from the curves of the Figure

	(4)	**(5b)**	**(6b)**	**(8a)**	**(8b)**
10^2k/min^{-1}	1.1	1.6	0.9	20	4.5
$\tau_{\frac{1}{2}}$/min	63	43	77	3.5	15

[a] Measured in 1×10^{-4}M solution at 25 °C; k was calculated according to the equation $k = 1/t \cdot \ln(E_0 - E_\infty)/(E_t - E_\infty)$.

Experimental

General. M.p.s were determined on a Linström apparatus (Wagner & Munz, W. Germany) and are not corrected. U.v. spectra were measured on a Uvicon 810 spectrophotometer (Kontron, Switzerland), and reaction-kinetic measurements were carried out with a Shimadzu UV 210 A spectrophotometer (Shimadzu, Japan) equipped with a thermostatted cell holder. ^{1}H N.m.r. spectra were recorded at 250.0 MHz and ^{13}C n.m.r. spectra were recorded at 62.9 MHz on a Bruker WM 250 spectrometer; δ values are relative to Me_4Si as internal standard for ^{1}H and ^{13}C nuclei. Elemental analyses were performed by Mikroanalytisches Labor Beller (Göttingen, W. Germany). Thin-layer chromatography (t.l.c.) was carried out on silica gel SIL G-25 UV_{254} plates (Macherey-Nagel & Co, Düren, W. Germany). Silica gel 60 (230—400 mesh ASTM; Merck, Darmstadt, W. Germany) was used for column chromatography. The columns were connected to a Uvicord S detector and an UltroRac fraction collector (LKB Instruments, Bromma, Sweden). T.l.c. was performed with the following solvent systems: A, CH_2Cl_2–ethyl acetate (19:1 v/v); B, cyclohexane–ethyl acetate (4:1 v/v); C, $CHCl_3$–MeOH (19:1 v/v); D, $CHCl_3$–MeOH (9:1 v/v), D, $CHCl_3$–MeOH (9:1 v/v); E, $CHCl_3$–MeOH (4:1 v/v). Allopurinol was purchased from Sigma Chemical Co. (St. Louis, U.S.A.).

4-Chloro-1-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]-1H-pyrazolo[3,4-d]pyrimidine **(3)**.—A solution of freshly prepared compound **(1)**[7] (400 mg, 2.6 mmol) and 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride **(2)**[19] (1.45 g, 3.7 mmol) in THF (50 ml) was added to 50% aqueous sodium hydroxide (15 ml) and the resulting two phases were thoroughly mixed (vibromixer) for 3 min at ambient temperature. The organic layer was decanted and the aqueous layer was extracted with THF (3 × 50 ml). The combined extracts were filtered and evaporated to dryness. The amorphous residue was chromatographed on a silica gel column (15 × 5 cm; solvent A). Pooling the fast migrating main zone and work-up yielded crystals of the nucleoside **(3)** (548 mg, 42%), m.p. 135 °C (from propan-2-ol) (lit.,[8] m.p. 130—132 °C) (Found: C, 61.65; H, 4.7; Cl, 6.9; N, 11.1. Calc. for $C_{26}H_{23}ClN_4O_5$: C, 61.60; H, 4.57; Cl, 6.99; N, 11.05%); R_F (silica gel, solvent B) 0.71; $\lambda_{max.}$(MeOH) 240 nm (ε 34 600) (lit.,[8] ε 17 600); δ_H[$(CD_3)_2SO$] 2.37 and 2.40 (6 H, 2 s, 2 Me), 2.85 (1 H, m, 2′-H_b), 3.30 (1 H, m, 2′-H_a), 4.47 (3 H, m, 4′-H + 5′-H_2), 5.87 (1 H, m, 3′-H), 6.92 (1 H, pseudo-t, *J* 6.0 Hz, 1′-H), 7.31 7.35, 7.82, and 7.94 (8 H, 4 d, ArH), 8.60 (1 H, s, 3-H), and 8.92 (1 H, s, 6-H); δ_C[$(CD_3)_2SO$] 20.9 (2 Me), 35.3 C-2′), 63.6 (C-5′), 74.4 (C-3′), 81.5 (C-4′), 84.7 (C-1′), 113.6 (C-3a), 126.3—129.1 (10 arom. C), 133.3 (C-3), 143.4 and 143.7 (2 arom. C), 153.3/153.7 (C-4/C-7a), 154.8 (C-6), and 165.1 p.p.m. (2 C=O).

4-Amino-1-(2-deoxy-β-D-erythro-pentofuranosyl)-1H-pyrazolo[3,4-d]pyrimidine **(4)**.—A solution of compound **(3)** (253 mg, 0.5 mmol) in methanolic ammonia (dry methanol saturated with dry ammonia at 0 °C (100 ml) was autoclaved at 140 °C for 4 h. After being cooled, the solution was evaporated and the residue was adsorbed onto silica gel (2 g). Chromatography was performed on a silica gel column (20 × 2 cm) with solvent E. The content of the slow migrating zone gave compound **(4)** as crystals (from propan-2-ol) (97 mg, 77%), m.p. 245—246 °C (lit.,[20] 245—246 °C); R_F (silica gel, solvent E) 0.39; $\lambda_{max.}$ 260 and 275 nm (ε 9 500 and 10 900).

1-[2-Deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidine-4-thione **(6a)**.—Compound **(3)** (507 mg, 1.0 mmol) and thiourea (240 mg, 3.2 mmol) were dissolved in methanol (50 ml) and the solution was refluxed for 1 h. After the mixture had cooled (ice-bath), compound **(6a)** had precipitated out and was filtered off and washed with ice-cold methanol to give *needles* (420 mg, 83%), m.p. 200 °C (Found: C, 61.8; H, 4.7; N, 11.2; S, 6.3. $C_{26}H_{24}N_4O_5S$ requires C, 61.89; H, 4.79; N, 11.10; S, 6.35%); R_F (silica gel, solvent C) 0.4; $\lambda_{max.}$(MeOH) 239 and 322 nm (ε 39 400 and 23 100); δ_H[$(CD_3)_2SO$] 2.36 and 2.38 (6 H, 2 s, 2 Me), 2.78 (1 H, pseudo-quin, *J* 6.3 Hz, 2′-H_b), 3.20 (1 H, pseudo-quin, *J* 6.5 Hz, 2′-H_a), 4.38 (3 H, m, 4′-H + 5′-H_2), 5.85 (1 H, m, 3′-H), 6.71 (1 H, pseudo-t, *J* 5.9 Hz, 1′-H), 7.31 (4 H, pseudo-t, *J* 9.4 Hz, ArH), and 7.84 and 7.92 (4 H, 2 d, *J* 8.0 and 8.0 Hz, ArH); δ_C[$(CD_3)_2SO$] 20.8 (2 Me), 35.3 (C-2′), 63.7 (C-5′), 74.5 (C-3′), 81.5 (C-4′), 84.3 (C-1′), 117.7 (C-3a), 137.2 (C-3), 126.4, 129.0, and 143.5 (12 arom. C), 146.9 (C-6), 147.2 (C-7a), 165.1 (2 C=O), and 178.7 p.p.m. (C-4).

1-(2-Deoxy-β-D-erythro-pentofuranosyl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidine-4-thione **(6b)**.—A solution of compound **(6a)** (356 mg, 0.71 mmol) in 0.01M-sodium methoxide–methanol (25 ml) was stirred for 3 h at ambient temperature. After neutralization with 0.1M-aqueous hydrochloric acid (2.5 ml), the solution was evaporated and the residue was adsorbed onto silica gel (1.5 g). Chromatography on a silica gel column (11 × 2 cm; solvent C) yielded the *deprotected product* **(6b)** as needles (130 mg, 69%), m.p. 176 °C (from propan-2-ol) (Found: C, 44.7; H, 4.7; N, 20.8; S, 11.8. $C_{10}H_{12}N_4O_3S$ requires C, 44.77; H, 4.51; N, 20.88; S, 11.95%); R_F (silica gel, solvent D) 0.3; $\lambda_{max.}$(MeOH) 237 and 322 nm (ε 8 500 and 23 100); δ_H[$(CD_3)_2SO$] 2.30 (1 H, pq, *J* 6.1 Hz, 2′-H_b), 2.77 (1 H, pseudo-quin, *J* 6.3 Hz, 2′-H_a), 3.43 (2 H, m, 5′-H_2), 3.82 (1 H, dt, *J* 3.9 and 5.6 Hz, 4′-H), 4.44 (1 H, m, 3′-H), 4.70 (1 H, s, 5′-OH), 5.30 (1 H, s, 3′-OH), 6.52 (1 H, pseudo-t, *J* 6.2 Hz, 1′-H), 8.23 and 8.27 (2 H, 2 s, 3- + 6-H), and 13.71 (1 H, s, 5-H); δ_C[$(CD_3)_2SO$] 38.0 (C-2′), 62.1 (C-5′), 70.8 (C-3′), 84.1 (C-1′), 87.7 (C-4′), 117.5 (C-3a), 136.9 (C-3), 146.8 (C-6), 147.0 (C-7a), and 178.7 p.p.m. (C-4).

1-[2-Deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one **(5a)**.—A solution of compound **(3)** (530 mg, 1.05 mmol) in DMSO (10 ml, analytical grade) was kept at 100 °C for 90 min. After having cooled, the solution was stirred for 20 min at ambient temperature in the presence of sodium hydrogen carbonate (2.0 g). Inorganic salt was removed and the solution was diluted with dichloromethane (100 ml). The solution was then washed with water (3 × 100 ml). The dried organic phase was evaporated to give an oily residue which was dissolved in hot methanol (50 ml). As it was being stirred the solution was brought to ambient temperature and was then cooled in an ice-bath to yield the *ketone* **(5a)** as needles (355 mg, 70%), m.p. 223—225 °C (Found: C, 63.9; H, 4.9; N, 11.6. $C_{26}H_{24}N_4O_6$ requires C, 63.93; H, 4.95; N, 11.47%); R_F (silica gel, solvent C) 0.3; $\lambda_{max.}$(MeOH) 240 nm (ε 39 600); δ_H[$(CD_3)_2SO$] 2.37 and 2.39 (6 H, 2 s, 2 Me), 2.77 (1 H, pseudo-quin, *J* 6.3 Hz, 2′-H_b), 3.24 (1 H, pseudo-quin, *J* 6.5 Hz, 1′-H_a), 4.48 (3 H, m, 4′-H + 5′-H_2), 5.85 (1 H, m, 3′-H), 6.72 (1 H, pseudo-t, *J* 6.0 Hz, 1′-H), 7.28, 7.34, 7.86, and 7.92 (8 H, 4 d, *J* 8.0 Hz, ArH), 8.14 and 8.21

(2 H, 2 s, 3- + 6-H), and 12.34 (1 H, s, 5-H); δ_C[$(CD_3)_2SO$] 20.9 (2 Me), 35.3 (C-2′), 63.8 (C-5′), 74.6 (C-3′), 81.4 (C-4′), 84.1 (C-1′), 106.4 (C-3a), 126.5 and 129.0 (10 arom. C), 135.4 (C-3), 143.6 (2 arom. C), 148.4 (C-6), 152.6 (C-7a), 156.4 (C-4), and 165.2 (2 C=O).

1-(*2-Deoxy*-β-D-erythro-*pentofuranosyl*)-1,5-*dihydro*-4H-*pyrazolo*[3,4-d]*pyrimidin*-4-*one* (**5b**).—A solution of compound (**5a**) (300 mg, 0.61 mmol) in 0.01M-sodium methoxide–methanol (25 ml) was stirred for 3 h at ambient temperature. After neutralization with 0.1M-hydrochloric acid (2.5 ml), the solution was evaporated and the residue was adsorbed onto silica gel (1.2 g). Chromatography on a silica gel column (10 × 2 cm, solvent C) yielded the deprotected product (**5b**) as needles (103 mg, 68%), m.p. 202—203 °C (from propan-2-ol) (lit.,[6] 202—203 °C); R_F (silica gel, solvent D) 0.2; $\lambda_{max.}$(MeOH) 251 nm (ε 7 500).

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References

1 C. L. Greene, E. L. Loechler, C. W. Fowler, and J. M. Essigmann, *Proc. Natl. Acad. Sci. USA*, 1984, **81**, 13.
2 F. Seela, H. Driller, A. Kehne, S. Menkhoff, J. Ott, and H.-D. Winkeler, in 'Chemical Synthesis in Molecular Biology,' eds. H. Blöcker, R. Frank, and H.-J. Fritz, Verlag Chemie, Weinheim, 1985, in the press.
3 B. L. Gaffney, L. A. Marky, and R. A. Jones, *Biochemistry*, 1984, **23**, 5686.
4 F. Seela and H. Driller, *Nucleic Acids Res.*, 1985, **13**, 911.
5 F. Seela and H. Steker, *Tetrahedron Lett.*, 1984, **25**, 5017.
6 F. Seela and H. Steker, *Helv. Chim. Acta*, 1985, **68**, 563.
7 R. K. Robins, *J. Am. Chem. Soc.*, 1956, **78**, 784.
8 Z. Kazimierczuk, H. G. Cottam, G. R. Revankar, and R. K. Robins, *J. Am. Chem. Soc.*, 1984, **106**, 6379.
9 E. V. Dehmlow and S. S. Dehmlow, 'Phase Transfer Catalysis,' Verlag Chemie, Weinheim, 1983.
10 F. Seela and H.-D. Winkeler, *Angew. Chem., Int. Ed. Engl.*, 1979, **18**, 536.
11 M. P. Kotick, C. Szantay, and T. J. Bardos, *J. Org. Chem.*, 1969, **34**, 3806.
12 A. J. Hubbard, A. S. Jones, and R. T. Walker, *Nucleic Acids Res.*, 1984, **12**, 6827.
13 H.-D. Winkeler and F. Seela, *J. Org. Chem.*, 1983, **48**, 3119.
14 E. Tommila and M.-L. Murto, *Acta Chem. Scand.*, 1963, **17**, 1947.
15 H. Rosemeyer, M. Anders, and F. Seela, manuscript in preparation.
16 J. Meisenheimer, *Justus Liebigs Ann. Chem.*, 1902, **323**, 205.
17 D. Martin and H. G. Hauthal, 'Dimethylsulfoxid,' Akademie-Verlag, Berlin 1971.
18 F. Seela and U. Liman, *Liebigs Ann. Chem.*, 1984, 273.
19 M. Hoffer, *Chem. Ber.*, 1960, **93**, 2777.
20 M. G. Stout, D. E. Hoard, M. J. Holman, E. S. Wu, and J. M. Siegel, in 'Methods in Carbohydrate Chemistry,' eds. R. L. Whistler and J. N. BeMiller, Academic Press, New York, 1976, vol. VII, p. 19.

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