Furanoside–Pyranoside Isomerization of Tubercidin and its 2'-Deoxy Derivatives: Influence of Nucleobase and Sugar Structure on the Proton-catalysed Reaction

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2'-Deoxy-2-methoxytubercidin (**6a**) which was prepared from the nucleobase (**1a**) with the halogenose (**2**) *via* phase-transfer glycosylation isomerizes rapidly under acidic conditions. Two pyranosides [(**7a**) and (**8a**)] and the anomeric furanoside (**5a**) are formed. The isomerization process was followed kinetically, demonstrating that furanoside formation is kinetically controlled whereas the β-pyranoside (**7a**) is the thermodynamically most stable product. From 2'-deoxytubercidin (**6b**) similar results were obtained but isomerization was slow, compared with (**6a**). The ribonucleoside tubercidin (**6c**) did isomerize only under vigorous acid treatment leading to the α-furanoside (**5c**) and the nucleobase (**1c**) by cleavage of the *N*-glycosylic bond.

Pyrrolo[2,3-*d*]pyrimidine nucleosides are highly stable towards acidic hydrolysis. This is in contrast to purine nucleosides which are susceptible to hydrolytic cleavage of the *N*-glycosylic bond under acidic conditions. The extraordinary stability of pyrrolo[2,3-*d*]pyrimidine nucleosides has been documented in the series of D-ribo-[1], D-arabino-,[2] and 2'-deoxyribo-furanosides.[3] As a consequence, proton-catalysed reactions of the sugar moiety can be observed on pyrrolo[2,3-*d*]pyrimidine nucleosides which would normally lead to cleavage of the *N*-glycosylic bond in purine nucleosides.

In a previous communication we reported on the isolation of anomeric pyranosides and furanosides formed from a 7-deazaguanine 2'-deoxyribofuranoside after treatment with hydrochloric acid.[4] A study of the literature showed that there are no quantitative data on proton-catalysed nucleoside isomerizations although the isomerization process has been examined in more detail under alkaline conditions.[5,6] We now report on the proton-catalysed isomerization of three structurally related 7-deaza-adenine β-D-ribofuranosides. Two of them, tubercidin (**6c**)[7] and 2'-deoxytubercidin (**6b**),[8] are isosters of regular nucleic acid constituents. The isomerization process was followed kinetically and the structures of the reaction products were assigned on the basis of chemical and spectroscopic data. The influences of the nucleobase and sugar structure on the isomerization rate were also investigated. As a result of these studies we suggest that this proton-catalysed isomerization mechanism is different to that of *O*-glycosides.[9]

Whereas tubercidin is commercially available, 2'-deoxytubercidin had to be prepared by total synthesis following a procedure developed in our laboratory.[8] The synthesis of the 2-methoxy derivative (**6a**) employing a chemical transformation of 2,4-dichloro-7*H*-pyrrolo[2,3-*d*]pyrimidine 2'-deoxy-β-D-ribofuranoside[10] has been described recently. Since this synthesis requires a large number of reaction steps we decided to prepare compound (**6a**) by phase-transfer glycosylation[9] of the nucleobase (**1a**) with the halogenose (**2**). The nucleobase (**1a**) was synthesized according to a procedure of Davoll[11] but using sodium methoxide instead of sodium ethoxide to circumvent a side reaction yielding a mixture of compound (**1a**) and its 2-ethoxy derivative (**1b**). The structure of (**1a**) was confirmed by ^{1}H and ^{13}C n.m.r. spectroscopy (Table); acid hydrolysis furnished 7-deazaisoguanine (**3**). The methoxy compound (**1a**) was then employed in phase-transfer-catalysed glycosylation with the halogenose (**2**) yielding one glycosylation product. After detoluoylation with sodium methoxide the nucleoside (**6a**) was obtained in crystalline form and was identical with an authentic sample.[10] Demethylation of the nucleoside was accomplished in anhydrous *p*-dioxane–hydrogen chloride to give 7-deaza-2'-deoxyisoguanosine (**4**).

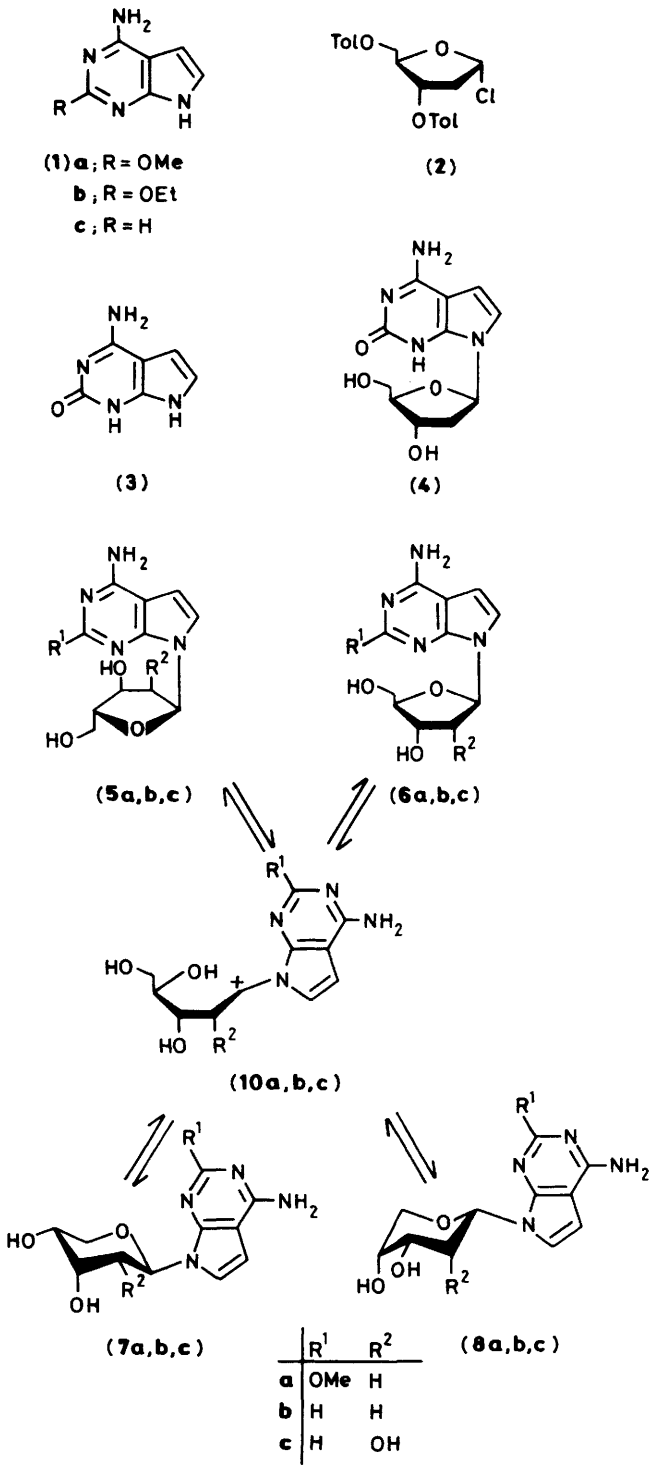
From the structure of (**4**) it is apparent that the lactam proton can be localized either at N-1 or N-3. The correct location was obtained from a comparison of the ^{13}C n.m.r. spectra in neutral and alkaline solutions (Table). Compound (**4**) has two p*K*-values, one of protonation (pK = 4.5) and one of deprotonation (pK = 11.0). It is well established that deprotonation of a heterocyclic nitrogen results in a strong downfield shift of the nitrogen α-carbon signals.[12,13] Since the greatest shifts were seen for the signals of C-2 and C-7a (Table), deprotonation must occur at N-1 and therefore this nitrogen carries the proton, as depicted in formula (**4**).

In contrast to acid treatment of compound (**6a**) under nonaqueous conditions which resulted in demethylation, treatment with 1M aqueous hydrochloric acid did not convert (**6a**) into (**4**); instead isomerization was observed. Qualitative isomerization experiments with the nucleosides (**6a**—**c**) indicated that all three nucleosides isomerize under proton catalysis.

Figures 1*a*—*c* show typical isomerization patterns obtained from compounds (**6a**—**c**). From these patterns it is apparent that in the series of 2'-deoxynucleosides (**6a**) and (**6b**), three new compounds were formed in addition to starting material, whereas compound (**6c**) yielded only two new products. Since the isomerization process of (**6a**) was faster than the others it was followed kinetically. For this purpose samples were taken at different time intervals, neutralized, and analysed by h.p.l.c. The isomerization products were quantified spectrophotometrically at 260 nm, since it can be assumed that they have almost identical extinction coefficients.

According to previous results,[4] the four isomerization products (Figure 1*a*) should be two anomeric furanosides (**5a**) and (**6a**) and the anomeric pyranosides (**7a**) and (**8a**). The assignment of the peaks (I—IV) was made on the basis of chemical transformations and spectroscopy.

Both pyranosides (**7a**) and (**8a**) contain a *cis*-glycol moiety which is not present in the anomeric furanosides (**5a**) and (**6a**). Since *cis*-glycol systems are susceptible to oxidation with sodium *m*-periodate, the isomerization mixture of (**6a**) was treated with this reagent. Excess of periodate was then destroyed with butane-2,3-diol and the resultant solution was analysed by h.p.l.c. In contrast to a regular isomerization mixture the mixture which was obtained from treatment with periodate gave rise to only two peaks. Their retention times correlated



with those of peaks III and IV of the mixture. Consequently, these peaks must be due to the anomeric furanosides (**5a**) and (**6a**), while peaks I and II can be assigned to the anomeric pyranosides (**7a**) and (**8a**). Peak III was that of the starting β-furanoside (**6a**), so that peak IV must be that of the α-anomer (**5a**).

Assignment of peaks I and II was accomplished by isolating the pyranosides from a preparative-scale isomerization in 1M hydrochloric acid at 70 °C for 200 min, followed by several chromatographic purification steps (see Experimental section).

According to the ^{13}C n.m.r. shifts of the anomeric carbons (Table)[4,13] the compound with the low-field signal (78.4 p.p.m.) was the β-pyranoside (**7a**). This material gave rise to peak I of the isomerization pattern (Figure 1*a*). The other pyranoside, which exhibited the high-field n.m.r. signal (75.7 p.p.m.) and yielded peak II of the isomerization pattern, was the α-anomer (**8a**).

After having assigned the structures of the isomerization products of compound (**6a**), the reaction was followed kinetically by taking samples at different intervals of time. The isomerization process was stopped by neutralization. Separation on h.p.l.c. using a reversed-phase column yielded the pattern which is depicted in Figure 2*a*. Attempts to fit the curves to first-order kinetics failed, in agreement with the complex reaction process. A detailed evaluation of the kinetic parameters of the isomerization was not made due to the fact that this process is different from the isomerization or mutarotation of sugars or *O*-glycosides. Moreover, the structure of the intermediates, important for such an evaluation, has yet to be established. However, it can be seen from Figure 2*a* that α-furanoside formation is controlled kinetically. The pyranosides (**7a**) and (**8a**) are formed in a thermodynamically controlled process. Equilibrium, not depicted in Figure 1*a*, was observed after a 10-day isomerization in M HCl at 50 °C. H.p.l.c. analysis revealed a distribution of 12% of the β-furanoside (**6a**), 13% of the α-furanoside (**5a**), 57% of the β-pyranoside (**7a**), and 18% of the α-pyranoside (**8a**). Small amounts of hydrolysis products are not considered in this calculation. Hence it follows that the β-pyranoside (**7b**) with the least non-bonded interactions represents the most stable conformer and is obtained in the greatest yield.

It has been reported for OCH_3-glycosides that furanoside anomerization may occur, without opening of the furanose ring, by release of the aglycone from the *O*-glycosylic bond.[9] As a consequence, a sugar carboxonium ion should be formed as an intermediate. This carboxonium ion which is more planar in a five-membered ring than that of a pyranoside system could then account for the rapid anomerization of furanosides. However, this mechanism cannot be accepted for the proton-catalysed anomerization of (**6a**) to (**5a**), since the heterocyclic base would have to be released from the nucleoside, which is unlikely. We favour a mechanism as depicted in the sequence (**9**)—(**13**).

According to the p*K*-value of (**6a**) (pK_a = 4.8) the nucleobase of (**6a**) is protonated under anomerization conditions (**9a**)—(**13a**). As found for purines, the protonation site is most likely to be N-3 (purine numbering N-1). This protonation can facilitate cleavage of the *N*-glycosylic bond but does not facilitate isomerization. As a consequence protonation at the furanose oxygen is required. By this means a mesomeric stabilized ion (**10a**/**11a**) can be formed, which accounts for the loss of stereochemistry at the anomeric centre. It has been shown by oxygen-exchange experiments on D-glucose and D-erythrose that ^{18}O from [^{18}O]H_2O is incorporated into furanose or pyranose ring systems.[14] If this finding is also considered for the isomerization of (**9a**), then intermediates such as (**12a**) or (**13a**) have to be taken into account. The formation of these intermediates is supported by the fact that anomerization did not occur under anhydrous conditions. The more rapid kinetically controlled formation of the α-furanoside (**5a**) could then be due to ring closure of the diastereomeric mixture of (**12a**) without rotation of the C–3′/C–4′ and C–4′/C–5′ bonds. However, this rotation *via* (**13a**), which could be rate limiting, is a prerequisite for the formation of the pyranosides, such (**7a**) and (**8a**).

Qualitative isomerization experiments with 2′-deoxytubercidin (**6b**), lacking the 2′-methoxy group, indicated a decreased isomerization rate (Figure 1*b*). Assignment of the reaction products (**5b**)—(**8b**) was made on the basis of the chemical shifts of the anomeric protons of preparatively isolated material.

Table. ^{13}C N.m.r. data of anomeric 7-deazapurine D-2′-deoxyribofuranosides and pyranosides in $(CD_3)_2SO$[a]

Compd.		C-2	C-4	C-4a	C-5	C-6	C-7a	C-1′	C-2′	C-3′	C-4′	C-5′	OMe
(**1a**)		161.8	158.3	97.9	98.9	118.7	152.2						53.3
(**1c**)		151.6	157.4	102.3	98.9	120.9	150.8						
(**4**)		152.6	156.0	92.9	100.8	119.0	153.9	83.3	39.5	71.2	87.3	62.3	
(**4**)[b]		160.0	155.5	96.1	100.7	118.4	168.2	83.7	39.5	72.3	87.1	63.1	
(**5a**)	Fα	161.9	158.4	98.6	99.7	120.1	151.6	82.6	39.3	70.7	87.0	61.7	53.3
(**6a**)	Fβ	161.9	158.4	98.6	99.7	119.2	151.6	82.8	39.3	71.0	87.1	62.0	53.3
(**6b**)	Fβ	151.3	157.3	102.8	99.4	121.4	149.5	83.2	39.6	71.0	87.1	62.0	
(**7a**)	Pβ	161.9	158.3	98.0	99.7	119.0	151.4	78.4	34.0	68.9	67.3	66.4	53.2
(**8a**)	Pα	161.9	158.4	98.6	99.7	119.6	151.4	75.5	36.4	66.4[c]	66.3[c]	65.4[c]	53.2

[a] Chemical shifts are given as δ-values relative to TMS as internal standard. [b] In D_2O–1M NaOD (1:1). [c] Tentative assignments.

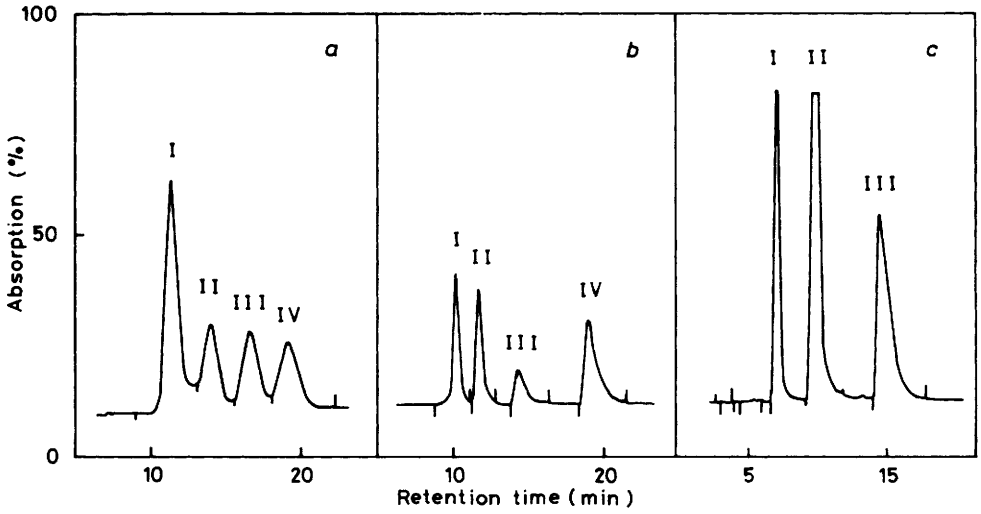


Figure 1. H.p.l.c. elution profiles of compounds (**6a**) (Figure 1*a*), (**6b**) (1*b*), and (**6c**) (1*c*). Peaks I—IV refer to increasing retention times. Conditions of isomerization and h.p.l.c. analysis are given in the Experimental section

From ^1H n.m.r. data the compounds exhibiting the small coupling constants for 1′-H were identified as the furanosides (**5b**) and (**6b**). Since one substance was identical with the starting material (**6b**) the other had to be the α-furanoside (**5b**). The anomeric pyranosides exhibited signals at δ = 5.75 and 6.09. Since it is established that α-pyranosides resonate at lower field,[4] the anomer exhibiting the signal at 6.09 is the α-anomer (**8b**), whereas the β-anomer (**7b**) was assigned on the basis of the anomeric proton signal located at 5.75. This assignment led also to the identification of the peaks I—IV of Figure 1*b*. In that case h.p.l.c. analysis was carried out on silica gel with chloroform–methanol as solvent.

Due to the change of the chromatographic system the pyranosides migrate more slowly than the furanosides (Figure 1*b*) whereas the opposite is true in Figure 1*a*. As can be seen from the isomerization conditions, 6M HCl for (**6b**) (Figure 2*b*) instead of 1M HCl for (**6a**) (Figure 2*a*) and the time scale of Figures 2*a*/*b*, 2′-deoxytubercidin isomerizes much more slowly than the 2-methoxy compound (**6a**). From the pK_a-values [(**6b**); pK_a = 5.3 and (**6a**); pK_a = 4.8] which were determined by u.v. spectroscopy, the nucleobases of these nucleosides are protonated under isomerization conditions. According to the formulae (**9a**/**b**)—(**11a**/**b**), sugar ring opening should be facilitated by anomeric substituents which release electrons to this carbon. As a consequence a protonated nucleobase is unfavourable for the anomerization reaction. However, the positively charged nucleobase should more readily stabilize the carbonium ion of the anomeric centre if it carries $+I$ or $+M$-substituents. This is true for the 2-methoxy compound (**10a**/**11a**) and may also be the reason for the rapid isomerization of a 7-deaza-2′-deoxy analogue of 7-methylguanosine[4] and indole nucleosides.[15,16] It is also in line with the finding that (**4**) did not isomerize but was cleaved hydrolytically at the *N*-glycosylic bond.

A further decrease in the isomerization rate was observed for the ribonucleoside tubercidin (**6c**). According to Figure 1*c* only two reaction products were detected besides starting material (peak II), even after prolongated treatment with acid. One of them (peak III) was identified as the nucleobase (**1c**). The other was compared with an authentic sample of the α-anomer of tubercidin (**5c**) and was found to be identical. Neither the pyranoside (**7c**) nor its α-anomer (**8c**) could be detected. These results show that in contrast to the 2′-deoxy compounds (**6a**) or (**6b**), cleavage of the *N*-glycosylic bond is preferred in (**6c**) over pyranoside formation. Similar findings have been observed on pyrrolo[2,3-*d*]pyrimidine D-arabinofuranosides.[17] This leads to the conclusion that $-I$ substituents such as hydroxy groups have a destabilizing effect on the formation of the carbonium ion (**10c**/**11c**) thus decreasing the isomerization rate. Under the vigorous reaction conditions, necessary for such a process, cleavage of the *N*-glycosylic bond is favoured over pyranoside formation.

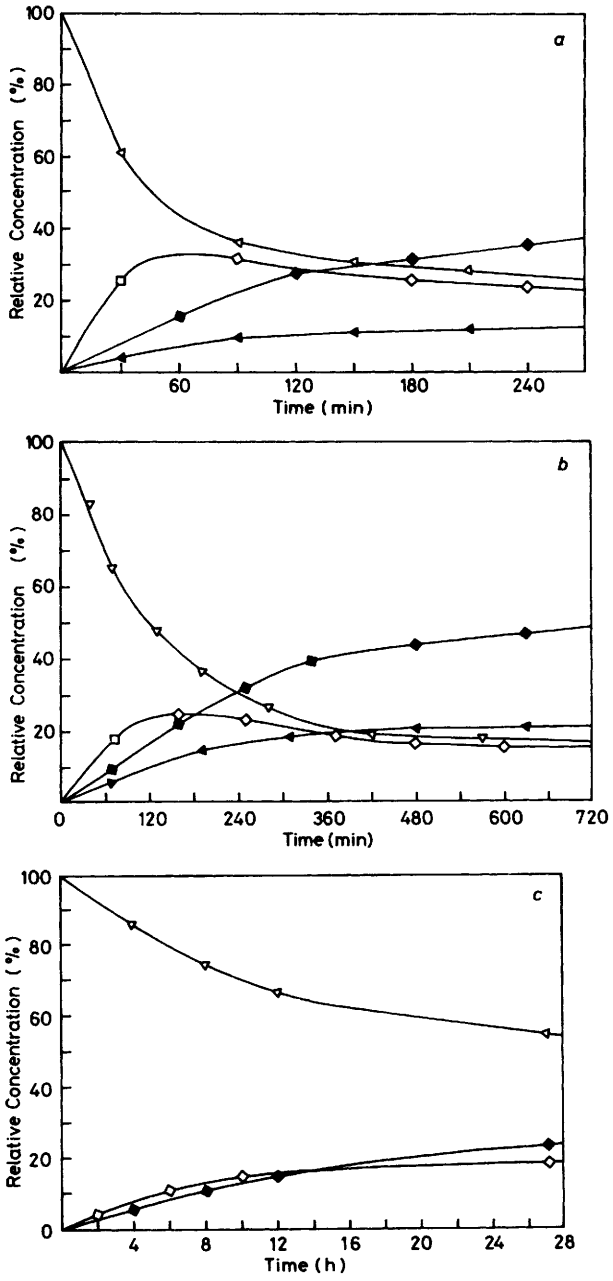
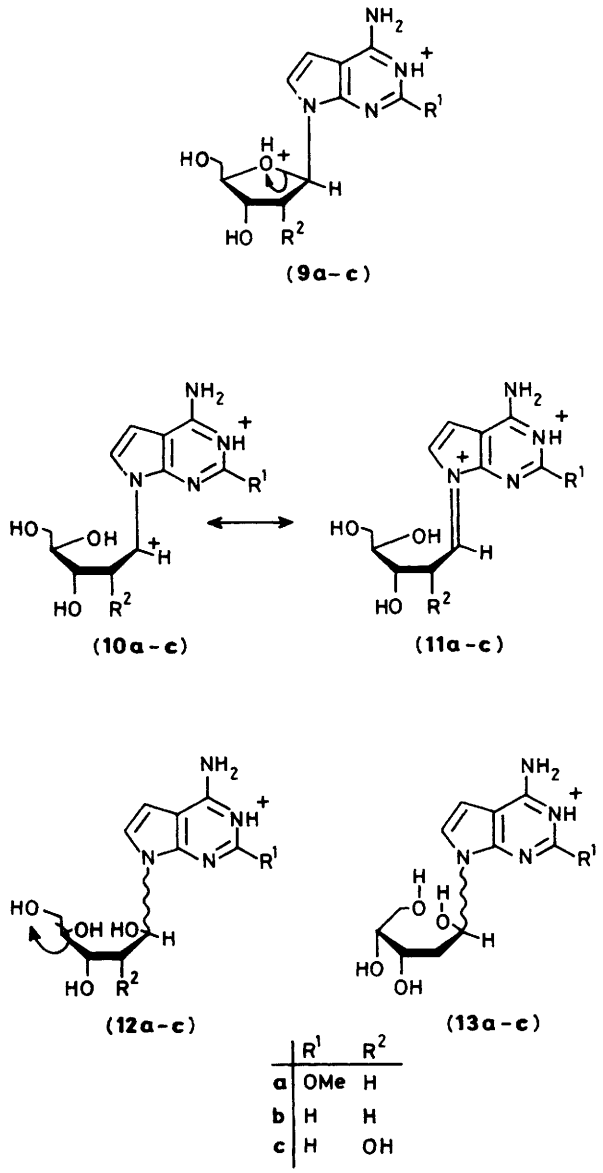


Figure 2. Isomerization kinetics of (**6a**) (Figure 2*a*), (**6b**) (2*b*), and (**6c**) (2*c*). Data were taken from h.p.l.c. elution profiles; concentration was determined at 260 nm and the sum of isomerization products was taken as 100%. For reaction conditions see Experimental section. Figure 2*a*: (**6a**, ◁—◁),(**5a**,□—□),(**8a**,◀—◀),(**7a**,◆—◆);Figure 2*b*: (**6b**, ◁—◁), (**5b**, □—□), (**8b**, ◀—◀), (**7b**, ◆—◆); Figure 2*c*: (**6c**, ◁—◁), (**5c**, □—□), (**1c**, ◆—◆)



	R^1	R^2
a	OMe	H
b	H	H
c	H	OH

Experimental

M.p.s were determined on a Linström apparatus (Wagner & Munz, Munich, FRG), and are not corrected. Elemental analyses were performed by Mikroanalytisches Labor Beller, Göttingen, FRG. ^1H and ^{13}C n.m.r. spectra were recorded on a Bruker WM 250 spectrometer, and δ-values are in p.p.m. relative to internal Me_4Si. U.v. spectra were measured on a Uvicon 810 spectrometer (Kontron, Switzerland) and mass spectra on a Varian MAT 311A spectrometer. The p*K*-values were determined spectrophotometrically at 20 °C in Teorell-Stenhagen[18] buffer solution. Thin layer chromatography (t.l.c.) was carried out on silica gel plates (Sil-G 25 UV_{254}, Macherey-Nagel, Düren, FRG) or h.p.t.l.c. plates with concentrating zone (Merck, Darmstadt, FRG). H.p.l.c. was performed on pre-packed columns [Merck, LiChrosorb RP-18, 4 × 250 (10 μm)] and Merck cartridge system (Si 60 standard, 4 × 250, 7 μm) using a LKB h.p.l.c. system with two pumps (model 2150), a variable-wavelength monitor (model 2152), and a controller (model 2151), connected to an integrator (Hewlett Packard 3390^A). H.p.l.c. was performed with a flow rate of 1.0 ml min^{-1}. Preparative column chromatography was performed on silica gel 60 (0.040—0.063 mm, Merck, FRG) and Amberlite XAD-4 resin (Serva, FRG). Solvent systems for chromatography were: (A) $CHCl_3$–MeOH (95:5), (B) 1M aqueous LiCl, (C) $CHCl_3$–MeOH (8:2), (D) 0.25M aqueous LiCl, (E) water–MeOH (3:2), (F) $CHCl_3$–MeOH (9:1), (G) 0.1M NH_4Ac–MeOH (8:2), and (H) $CHCl_3$–Pr^iOH (8:2).

4-*Amino*-2-*methoxy*-7H-*pyrrolo*[2,3-d]*pyrimidine* (**1a**).—To a solution of *O*-methylisourea hydrogen sulphate (3.44 g, 20

mmol) in methanol (15 ml) sodium methoxide (1.45 g Na, 163 mmol) in methanol (30 ml) was added. The mixture was refluxed with 2,2-diethoxyethylmalonitrile (3.65 g, 30 mmol) for 4 h, cooled, diluted with water (30 ml) and evaporated. The residue was suspended in water (50 ml) and the crystalline material isolated by filtration. To the crude reaction product 1M hydrochloric acid (80 ml, 80 mmol) was added and the mixture stirred for 3 h at room temperature. Addition of 1M ammonia precipitated (**1a**) which was filtered off and recrystallized from methanol–water to give colourless crystals (1.64 g, 50.0%), m.p. 275 °C;[11] t.l.c. (silica gel, solvent A), R_F 0.4; $\lambda_{max.}$ (MeOH) 275 (ε 8 700) and 259 nm (ε 7 300); δ_H [$(CD_3)_2SO$] 3.78 (3 H, s, OMe), 6.42 (1 H, m, 5-H), 6.88 (1 H, m, 6-H), 6.90 (2 H, s, NH_2), and 11.20 (1 H, s, NH). For ^{13}C n.m.r. data see Table.

4-*Amino*-1,7-*dihydro*-2H-*pyrrolo*[2,3-d]*pyrimidin*-4-*one* (**3**).—Compound (**1a**) (1.0 g, 6.1 mmol) in concentrated hydrochloric acid (10 ml) was heated at 100 °C (oil-bath) for 1 h. After cooling, the solution was diluted with a small amount of water, filtered, and the filtrate neutralized with ammonia. The precipitate was filtered and recrystallized from methanol–water as colourless crystals (687 mg, 75%) which did not melt[11] (Found: C, 47.8; H, 4.25; N, 37.2. $C_6H_6N_4O$ requires C, 48.00; H, 4.03; N, 37.32%); t.l.c. silica gel (solvent **B**), R_F 0.8; $\lambda_{max.}$ (1M HCl) 294 (ε 7 700); $\lambda_{max.}$ (0.1M NaOH) 291 (ε 7 100) and 254 nm (ε 6 800); δ_H [D_2O–NaOD, 1:1] 6.30 (d, H-5, *J* 3.5 Hz) and 6.60 (d, H-6, *J* 3.5 Hz).

4-*Amino*-7-(2′-*deoxy*-β-D-erythro-*pentofuranosyl*)-2-*methoxy*-7H-*pyrrolo*[2,3-d]*pyrimidine* (**6a**).—Compound (**1a**) (500 mg, 3.05 mmol) and benzyltriethylammonium chloride (50 mg, 0.22 mmol) dissolved in dichloromethane–1,2-dimethoxyethane (20:1, v/v, 20 ml) and an equal volume of 50% aqueous sodium hydroxide were stirred with a vibromixer for 1 min. The halogenose (**2**)[19] (1.2 g, 3.1 mmol) in dichloromethane (5 ml) was added and stirring was continued for another 45 min. The layers were separated, the aqueous phase was extracted with dichloromethane, and the combined organic phases washed with water. After evaporation of the organic solvent under reduced pressure, the residue was treated with 1M sodium methoxide in methanol (100 ml) at 25 °C for 24 h. Thereafter evaporation yielded a viscous solid which was dissolved in water (10 ml) and applied to an ion-exchange column (Dowex 1 × 2, OH^--form, column 10 × 3 cm). Elution with water gave a main zone which yielded, after evaporation and crystallization, *colourless needles* (269 mg, 30%) from methanol, m.p. 187 °C (Found: C, 51, 55; H, 5.7; N, 19.9. $C_{12}H_{16}N_4O_4$ requires C, 51.42; H, 5.75; N, 19.98%); t.l.c. (silica gel, solvent C) R_F 0.6; $\lambda_{max.}$ (MeOH) 261 (ε 9 600) and 271 nm (ε 9 600); δ_H[$(CD_3)_2SO$] 2.10 (1 H, m, 2′-H_b), 2.50 (1 H, m, 2′-H_a), 3.50 (2 H, m, 5′-H), 3.80 (4 H, s, OMe, m, 4′-H), 4.32 (1 H, m, 3′-H), 4.93 (1 H, t, 5′-OH, *J* 5.5 Hz), 5.22 (1 H, d, 3′-OH, *J* 4 Hz), 6.38 (1 H, dd, 1′-H, J_1 8 Hz), 6.48 (1 H, d, 5-H, *J* 4 Hz), 7.01 (2 H, s, NH_2), and 7.11 (1 H, 6-H, *J* 4 Hz).

4-*Amino*-7-(2′-*deoxy*-β-D-erythro-*pentofuranosyl*)-3,7-*dihydro*-2H-*pyrrolo*[2,3-d]*pyrimidin*-2-*one* (**4**).—Compound (**6a**) (300 mg, 1.07 mmol) in *p*-dioxane (30 ml) and 7M hydrogen chloride in *p*-dioxane (45 ml) were refluxed under nitrogen for 6 h. The solvent was evaporated off, the residue taken up in water, and excess of acid removed by repeated evaporation. After adjusting the pH to 9 with ammonia the solution (15 ml) was applied to a hydrophobic resin (Amberlite XAD-4, column 25 × 5 cm). A pre-run with water eluted inorganic salt, a mixture of methanol–water (2:3) the main material. Evaporation of the main fraction and recrystallization from water (15 ml) yielded *colourless needles* (167 mg, 59%), m.p. 229—231 °C (Found: C, 49.7; H, 5.4; N, 21.1. $C_{11}H_{14}N_4O_4$ requires C, 49.62; H, 5.30; N, 21.04%); t.l.c. (silica gel, solvent D) R_F 0.8; $\lambda_{max.}$ (MeOH) 255 (ε 7 600) and 305 nm (ε 7 200); δ_H[$(CD_3)_2SO$] 2.15 (m, 2′-H_a), 2.35 (m, 2′-H_b), 3.52 (m, 5′-H_2), 3.78 (m, 4′-H), 4.29 (m, 3′-H), 5.20 (3′- and 5′-OH), 6.22 (pt, 1′-H), 6.41 (d, 5-H, *J* 4 Hz), 6.90 (d, 6-H, *J* 4 Hz), 7.63 (s, NH_2), and 11.0 (s, NH).

Preparative-scale Isomerization of the Nucleoside (**6a**).—Compound (**6a**) (500 mg, 1.78 mmol) in 1M HCl (30 ml) was isomerized for 200 min at 70 °C (water-bath). After cooling, the solution was neutralized with 1M NaOH (30 ml), the pH-value was adjusted to 7.5 and the residue was applied to a hydrophobic resin column (Amberlite XAD, 30 × 3 cm). A pre-run with water (1500 ml) eluted inorganic material, methanol–water (2:3, v/v) the anomerization mixture. The pooled fractions were evaporated yielding a colourless residue. Analytical h.p.l.c. of a small probe (RP 18 column, solvent G) separated the mixture into the 4 isomerization products and a small amount of the chromophore (**1a**).

4-*Amino*-7-(2-*deoxy*-α-D-erythro-*pentopyranosyl*)-2-*methoxy*-7H-*pyrrolo*[2,3-d]*pyrimidine* (**7a**).—The desalted isomerization product of (**6a**) was dissolved in water and applied onto an ion-exchange column (Dowex 1 × 2, OH^-, 30 × 3.5 cm). The resin was washed with water (500 ml) and water–methanol (9:1) eluting three zones. The slowest migrating main zone contained the anomeric furanosides (**5a**) and (**6a**) together with the α-pyranoside (**8a**). The slightly faster migrating zone contained traces of the chromophore (**1a**). From the fast migrating zone colourless (**7a**) (100 mg, 20%) was isolated after evaporation, and crystallized upon storing; t.l.c. (silica gel, solvent C) R_F 0.52 [R_F(**6a**) 0.60]; $\lambda_{max.}$ 260 and 272 nm; for ^{13}C n.m.r. data see Table.

4-*Amino*-7-(2-*deoxy*-β-D-erythro-*pentopyranosyl*)-2-*methoxy*-7H-*pyrrolo*[2,3-d]*pyrimidine* (**8a**).—The content of the main zone from the Dowex 1 × 2 (OH^-) chromatography was chromatographed on a silica gel column (30 × 3.5 cm) with solvent E to give two zones. The slow migrating zone contained the anomeric mixture of the furanosides (**5a**)–(**6a**). From the fast migrating zone, colourless amorphous (**8a**) (50 mg, 10%) was isolated after evaporation; t.l.c. (silica gel, solvent C) R_F 0.53 [R_F(**6a**) 0.60]; $\lambda_{max.}$ 260 and 271 nm; for ^{13}C n.m.r. data see Table.

4-*Amino*-7-(2-*deoxy*-α-D-erythro-*pentofuranosyl*)-2-*methoxy*-7H-*pyrrolo*[2,3-d]*pyrimidine* (**5a**).—The content of the slow migrating zone of silica gel chromatography was again loaded onto a silica gel column (30 × 3.5 cm) and chromatographed with solvent H. From the slow migrating zone compound (**5a**) was isolated, after evaporation of the solvent, as a colourless solid; t.l.c. (silica gel, solvent H) R_F 0.34; $\lambda_{max.}$ 260 and 271 nm; for ^{13}C n.m.r. data see Table.

Preparative-scale Isomerization of 2′-*Deoxytubercidin* (**6b**).—Compound (**6b**) (500 mg, 2.0 mmol) in 6M HCl (30 ml) was isomerized for 3 h at 70 °C (water-bath). The solution was cooled, neutralized with 6M NaOH, and evaporated. Inorganic salt was separated over an Amberlite XAD resin (column 24 × 2 cm) by washing with water (500 ml). Methanol–water (1:9, 500 ml) eluted the anomerization mixture. The pooled fractions were evaporated and chromatographed on a silica gel column (20 × 3 cm, solvent A). The fast migrating zone contained a small amount of the chromophore (**1c**), the slow migrating zone the isomerization mixture. A second chromatography on silica gel (column 40 × 4.5 cm) first with the same solvent and then with solvent C separated the isomerization mixture into four zones (1—4, increasing mobility). They were separated and the ^1H n.m.r. spectra were measured. A sample of

each zone was injected into the h.p.l.c. apparatus (silica gel column, solvent F) allowing identification of the h.p.l.c. peaks (1—5) obtained from the analytical-scale isomerization.

4-*Amino*-7-(2-*deoxy*-β-D-erythro-*pentofuranosyl*)-7H-*pyrrolo*[2,3-d]*pyrimidine* (**6b**), *Zone* 1 *and Peak* I.—Colourless solid; $\lambda_{max.}$(MeOH) 270 nm; δ_H[$(CD_3)_2SO$–D_2O, 1:1] 8.00 (s, 2-H), 7.32 (d, 6-H, *J* 4 Hz), 6.57 (d, 5-H, *J* 4 Hz), and 6.42 (pt, 1′-H); the sample was identical with starting material.

4-*Amino*-7-(2-*deoxy*-α-D-erythro-*pentofuranosyl*)-7H-*pyrrolo*[2,3-d]*pyrimidine* (**5b**), *Zone* 2 *and Peak* II.—Colourless solid; $\lambda_{max.}$(MeOH) 270 nm; δ_H[$(CD_3)_2SO$–D_2O, 1:1] 8.02 (s, 2-H), 7.49 (d, 6-H, *J* 4 Hz), 6.56 (d, 5-H, *J* 4 Hz), and 6.43 (dd, 1′-H, *J* 4 and 8 Hz).

4-*Amino*-7-(2-*deoxy*-α-D-erythro-*pentopyranosyl*)-7H-*pyrrolo*[2,3-d]*pyrimidine* (**8b**), *Zone* 3 *and Peak* III.—Colourless solid; $\lambda_{max.}$(MeOH) 270 nm; δ_H[$(CD_3)_2SO$–D_2O, 1:1] 8.04 (s, 2-H), 7.28 (d, 6-H, *J* 4 Hz), 6.57 (d, 5-H, *J* 4 Hz), and 6.09 (d, 1′-H, *J* 12 Hz).

4-*Amino*-7-(2-*deoxy*-β-D-erythro-*pentopyranosyl*)-7*H*-*pyrrolo*[2,3-d]*pyrimidine* (**7b**), *Zone* 4 *and Peak* IV.—Colourless solid; $\lambda_{max.}$(MeOH) 270 nm; δ_H[$(CD_3)_2SO$–D_2O, 1:1] 8.05 (s, 2-H), 7.31 (d, 6-H, *J* 4 Hz), 6.60 (d, 5-H, *J* 4 Hz), and 5.75 (d, 1′-H, *J* 10 Hz).

Analytical-scale Isomerization of Compounds (**6a**—**c**).—Compounds (**6a**—**c**) (1 mg of each) were dissolved in hydrochloric acid (3 ml each) and stored at 70 °C in a water-bath. Samples were taken at time intervals and separated by h.p.l.c.

Compound (**6a**). This experiment was carried out in 1M HCl. Samples (250 μl) were taken at time intervals of 30 min, neutralized with 1M NaOH, and injected into the h.p.l.c. apparatus. Column: Lichrosorb RP 18, 10 μm, 4 × 250 mm; solvent G; for h.p.lc. pattern see Figure 1*a*, for kinetics see Figure 2*a*.

Compound (**6b**). 6M HCl was used instead of 1M HCl. After neutralization water was evaporated and the residue was extracted three times with ethanol. After evaporation the residue was dissolved in $CHCl_3$–MeOH. H.p.l.c. analysis was carried out on LiChrosorb Si 60, 7 μm, 4 × 250 mm; solvent F; for h.p.l.c. pattern see Figure 1*b*, for kinetics see Figure 2*b*.

Compound (**6c**). The isomerization followed the protocol of (**6a**) except that 6M HCl was employed and samples were taken at time intervals of 2 h. For elution a gradient of 0.1M NH_4Ac–MeOH was used in the following order: 12 min (10% MeOH), 6 min (10%—15% MeOH). Peak I was identical with that of α-tubercidin (**5c**),[20,21] peak II contained (**6c**), and peak III the nucleobase (**1c**).

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