Synthesis and Properties of Conformationally Locked 1,3-Bisalkyl-7-deazaxanthine 2'-Deoxy-D-ribofuranosides

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1,3-Dimethyl-7-deazaxanthine 2'-deoxyribofuranosides **1a** and **6a** and their N-3 isopropyl congeners **1b** and **6b** have been prepared employing the nucleobase anions **7a** or **7b** and 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-erythropentofuranosyl chloride (**8**) upon glycosylation. The reaction was not stereoselective as found in case of other pyrrolo[2,3-*d*]pyrimidine nucleosides induced by the bulky N-3 substituent. Configuration of anomers was established by ^{1}H-nmr nOe difference spectroscopy. Those data also indicated that the conformation around the *N*-glycosylic bond was locked by the bulky N-3 substituent. Contrary to the purine nucleoside such as wyosine (**2a**) the hydrolytic stability of the *N*-glycosylic bond of the pyrrolo[2,3-*d*]pyrimidine nucleosides was increased by N-3 alkylation. Moreover, it was shown by ^{15}N-nmr spectroscopy that different to purine nucleosides the aglycon was not protonated in acidic medium. As a result the *N*-glycosylic bond hydrolysis did not follow an A-1 but an A-2 mechanism.

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Introduction.

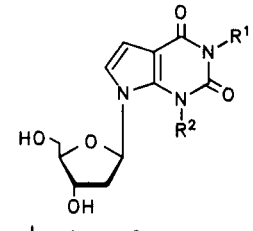
Methylated nucleosides are naturally occurring constituents of DNA and RNA [1]. Methylation can protect DNA against phosphodiester hydrolysis by endodeoxyribonucleases [2]. Messenger-RNA uses the 7-methylguanine moiety as recognition signal in cap structures [3]. The rare nucleoside wyosine (**2a**) which was found adjacent to the 3'-end of the anticodon of several tRNAs contains a N^3-methylated guanine moiety inducing an extremely hydrolytically labile *N*-glycosylic bond [4].

Recently, we have synthesized and assigned *N*-methyl isomers of pyrrolo[2,3-*d*]pyrimidines, in particular those of 7-deazaadenine [5], 7-deazahypoxanthine [6], and 7-deazaguanine [7]. Moreover, the 1-deaza analogue of 2'-deoxywyosine (**2b**) has been prepared [8]. In the following we describe the synthesis of 1,3-bisalkylated 7-deazaxanthines (purine numbering is used throughout the Results and Discussion section) carrying a bulky substituent close to the glycosylation site. Moreover, the hydrolytic stability of the *N*-glycosylic bond of those nucleosides conformationally locked by the bulky substituent is studied.

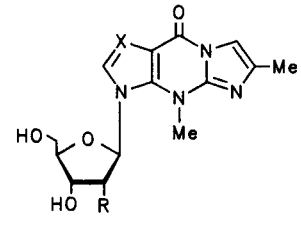
Results and Discussion.

Earlier investigations have shown that glycosylation of the pyrrolo[2,3-*d*]pyrimidine moiety with the halogenose **8** employing the nucleobase anion is stereoselective and β-nucleosides are obtained almost exclusively [9]. However, it has been reported within the group of other heterocyclic systems, such as imidazo[1,2-*a*]-*s*-triazines, that anomeric mixtures are formed if the nucleophilicity of the nitrogen anion is low [10]. α-Anomeric 2'-deoxy-D-ribofuranosides can be the sole glycosylation products as shown on 2(1*H*)-pyrimidinone as a result of a repeated inversion of configuration upon glycosylation of the lactam-oxygen [11].

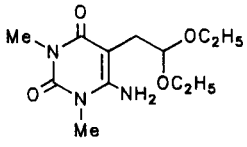
For the glycosylation experiments described within this manuscript we have chosen the bis-alkylated pyrrolo-[2,3-*d*]pyrimidines **7a** and **7b**. The theophylline analogue **7a** was synthesized by two different routes. (i) Starting with ethyl 2-cyano-4,4-diethoxybutyrate and condensation



	R^1	R^2
1a	Me	Me
1b	Me	iPro
1c	H	H



	R	X
2a	OH	N
2b	H	CH



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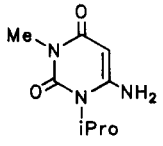

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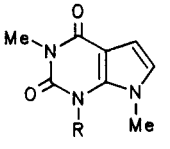
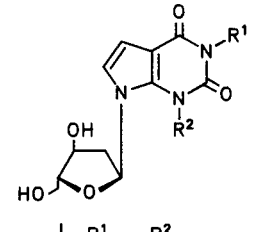


5a: R = Me
5b: R = iPro



	R^1	R^2
6a	Me	Me
6b	Me	iPro

with *N,N'*-dimethylurea the pyrimidine derivative **3** was formed in 76% yield. Its structure was confirmed by ^{1}H- and ^{13}C-nmr spectroscopy (see Experimental and Table 2). Compound **3** was then cyclized to yield the pyrrolo-[2,3-*d*]pyrimidine **7a**. On another route (ii) 6-amino-1,3-dimethyluracil was reacted with chloroacetic aldehyde according to Senda and Hirota [12] to yield compound **7a** in 51%. The ^{13}C-nmr-spectra (Table 1) confirmed the identity of both compounds synthesized by the different routes. The assignment of ^{13}C-nmr chemical shifts was made by proton coupled ^{1}H/^{13}C-nmr spectra. However, assignment of C-2 and C-6 is still tentative.

To obtain compound **7b** *N*-isopropyl-*N'*-methylurea was reacted with cyanoacetic acid in acetic anhydride. Upon treatment with sodium hydroxide the uracil derivative **4** was formed. Although compound **4** has been already described in the literature [13], there was no unambiguous proof for its structure, as the condensation reaction can lead to regioisomers with the isopropyl group either at N-1 or N-3. As structural proof was difficult on that stage but tlc and nmr-spectra (Table 1) confirmed that the condensation product was a single pure compound it was reacted with chloroacetic aldehyde to yield the pyrrolo[2,3-*d*]pyrimidine **7b** or its regioisomer **7c**. To identify this compound ^{1}H-nmr nOe-difference spectra were measured. Irradiation of H-(N-9) at 11.5 ppm resulted in nOe's of the secondary H-(C) as well as of the CH_3 of the isopropyl group (Table 2). In a second experiment the secondary proton of the isopropyl group was irradiated resulting in nOe's of the NH and the CH_3-signals (Table 2). According to this compound **7b** was the sole condensation products and **4** was its precursor.

It has been reported earlier that methylation of **7a** under alkaline condition yields **5a** [12] in 68% yield. We have employed phase-transfer conditions (50% sodium hydroxide, dichloromethane, benzyltriethylammonium chloride, methyl iodide) and obtained **5a** in a similar yield (78%). Also **5b** was obtained from **7b** in 90% yield. The introduction of the 9-CH_3 group caused a downfield shift of the methyl- or isopropyl signal at N-3 (see Experimental and Table 1) which was an additional proof of chemical shift assignment. Recently, the downfield shift of those signals has been reported also by Pfleiderer [14]. As methylation was selective for N-9 we have carried out glycosylation experiments with the halogenose **8**. Different reaction conditions have been used in case of the glycosylation of **7a**: (i) Liquid-liquid conditions (dichloromethane-50% aqueous sodium hydroxide, benzyltriethylammonium chloride), (ii) solid-liquid conditions (acetonitrile, powdered potassium hydroxide, TDA-1). Both reaction conditions afforded two glycosylation products in a total yield of 71% yield (i) and 52% (ii). The anomeric ratio was determined from the original reaction mixture either by integration of the H-1'-signals (i) or by tlc-scanning at 250 nm (ii). The content of the faster vs. the slower migrating isomers was 7:3 (i), 8:2 (ii). The crude mixture of anomers obtained by method (ii) was then separated by preparative scale flash-chromatography resulting in colourless crystals

Table 1
^{13}C-NMR Chemical Shifts of Pyrimidine and Pyrrolo[2,3-*d*]pyrimidine Deriviatives in DMSO-d_6

Compound	C-2 [a,b] [C-2] (C-2)	C-4 [b] [C-6] (C-4)	C-4a [C-5] (C-5)	C-5 [C-7]	C-6 [C-8]	C-7a [C-4] (C-6)
1a	151.5	158.1	101.0	103.5	117.7	138.0
1b	151.1	158.5	101.6	103.9	118.2	138.8
3 [b]	161.6	152.6	81.7	–	–	150.8
4 [b]	161.5	154.6	76.0	–	–	151.1
5a	151.3	158.0	100.7	102.3	123.8	137.6
5b	150.8	158.3	101.3	102.5	124.0	137.9
6a	151.5	158.1	101.0	103.0	119.2	138.0
6b	151.3	158.7	101.5	103.6	119.9	138.9
7a	150.8	158.4	98.5	103.8	116.5	138.8
7b	150.1	158.5	99.2	103.6	116.7	137.2
9a	151.5	158.0	101.2	104.2	117.3	138.4
9b	151.0	158.4	101.9	104.6	117.8	139.3
10a	151.5	158.1	101.3	103.2	118.2	137.7
10b	151.1	158.5	102.0	103.8	118.5	138.6

Compound	Me-N(3) [Me-N(1)] (Me-N(3))	Me-N(1) [Me-N(3)] (Me-N(1))	CH	CH_3	Me-N(7) [Me-N(9)] (CH_2/OCH_2)
1a	27.8	32.3	–	–	–
1b	27.1	–	53.2	20.5/19.7	–
3 [b]	27.3	29.6	102.8	15.1	29.5
4 [b]	26.5	–	47.0	19.6	–
5a	27.7	31.3	–	–	36.4
5b	27.0	–	50.7	20.0	36.7
6a	27.8	32.7	–	–	–
6b	27.3	–	53.4	20.7/19.8	–
7a	27.4	30.3	–	–	–
7b	27.3	–	48.4	19.1	–
9a	27.8	32.2	–	–	–
9b	27.1	–	53.3	20.4/19.7	–
10a	27.8	32.5	–	–	–
10b	27.1	–	53.1	21.1/20.3	–

Table 1 (continued)

Compound	C-1'	C-2'	C-3'	C-4'	C-5'	Toluoyl
1a	84.7	DMSO	70.2	87.4	61.4	–
1b	85.3	DMSO	70.3	87.5	61.5	–
6a	85.5	DMSO	70.5	88.0	61.4	–
6b	86.2	DMSO	70.5	88.2	61.6	–
9a	81.2	36.7	74.1	84.8	63.6	165.3/143.8/129.2 126.4/21.1
9b	81.2	36.8	74.4	85.4	63.8	165.2/144.0/131.6 126.5/21.1
10a	82.8	37.8	74.5	86.2	63.9	165.4/143.8/129.2 126.5/21.1
10b	82.6	37.8	74.5	86.5	63.9	165.4/143.8/129.2 126.6/19.5

[a] Pyrrolo[2,3-*d*]pyrimidine numbering; purine numbering in brackets; pyrimidine numbering in parenthesis. [b] Tentative assignment.

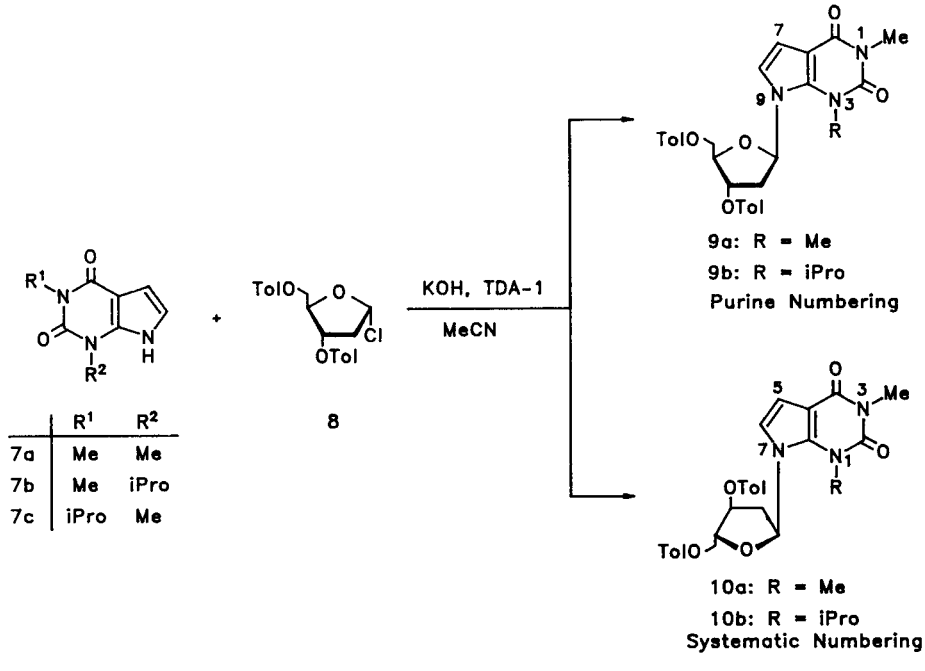
Table 2
^{1}H-NMR NOE Data (%) of Pyrrolo[2,3-*d*]pyrimidine Deriviatives in DMSO-d_6 [a]

Compound	Irradiated Proton	Observed n. O. e.
1a	H-1'	CH_3-N(3) (13.5), H-4' (3.5), H_α-2' (2.8), 3'-OH (1.0)
1b	H-1'	CH-N(3) (12.6), H-4' (2.0), H_α-2' (4.7), 3'-OH (0.8)
5a	CH_3-N(9)	CH_3-N(3) (2.2), H-8 (5.0)
	CH_3-N(3)	CH_3-N(9) (5.0)
5b	CH_3-N(9)	CH_3-N(3) (10.0), H-8 (3.1)
	H-8	H-7 (8.7)
6b	H-1'	CH-N(3) (14.5), H-3' (1.1), H_β-2' (6.5) CH_3 (4.1 and 1.9)
	CH	H-1' (13.8), CH_3 (7.0 and 7.6)
7a	HN	CH_3-N(3) (9.5), H-8 (8.9)
7b	HN	$(CH_3)_2CH$-N(3) (14.9), H-8 (9.8),
	$(CH_3)_2CH$-N(3)	CH_3 (5.0) CH_3 (14.6), NH (11.6)

[a] purine numbering.

(50%, faster migrating zone) and a second crystalline material (10%, slower zone). Due to the very similar R_f values an anomeric mixture was left from the overlapping zones which could be separated by repeated column chromatography. According to the chemical shift differences of H-4' and H-5' [15] the faster migrating main isomer was considered to be the β-anomer **9a**. The slower migrating zone should therefore contain the α-anomer **10a**. This demonstrated that the glycosylation was not stereoselective as found for glycosylation of other pyrrolo[2,3-*d*]pyrimidines [16].

We have also carried out glycosylation of **7b**. Liquid-liquid PTC failed and we were not able to isolate the reaction products. This may be due to the unfortunate partition of the aglycone between the organic and the inorganic phase. Therefore, sodium hydride-mediated reaction (i) as well as the solid-liquid phase-transfer glycosylation (ii) was applied. Similar yields of glycosylated materials were obtained in both cases [48% (i) and 56% (ii)]. Again, anomeric mixtures were obtained with anomeric ratios of 6.5:3.5 (i) and 7.5:2.5 (ii) determined from the integration of the H-1'-signal. As the time of glycosylation of



sterically hindered pyrrolo[2,3-*d*]pyrimidines had to be increased from 5 minutes to 30 minutes in case of **7a** or **7b** equilibration of the halogenose **8** before glycosylation may explain the loss of stereoselectivity. However, steric interference of the N-3 substituent within the transition complex of **7a** or **7b** with the halogenose **8** may be also taken into account.

Separation of **9a/10a**- and **9b/10b**-mixtures proved to be difficult due to their very similar R_f values. Nevertheless, it was possible to separate the anomeric mixtures in both cases by repeated chromatography on silica gel. Due to the zone overlap this process was not quantitative. As the separation of **9b/10b** was particularly difficult we have deprotected a 500 mg mixture of anomers to yield a **1b/6b** mixture. Chromatography on Dowex 1 × 2 (OH^-) removed by-products. Recrystallization from water or methanol afforded crystalline **1b** in a 70% yield based on the amount of the mixture of anomers. The mother liquor contained about equal amounts of both anomers from which **6b** could not be obtained. This was only accessible after separation of **10b**, from **9b** followed by deprotection.

The anomeric configuration of **1a**, **1b** and **6b** was assigned by ^{1}H-nmr nOe difference spectroscopy (Table 2). According to the spatial proximity irradiation of the anomeric proton gave an nOe of H-4′, H_α-2′ and OH-3′ of **1a** and **1b** which confirmed β-configuration [17]. On the other hand **6b** did not show an nOe of H-4′ and H_α-2′ but gave a nOe of H-3′ and H_β-2′ confirming α-configuration. The ^{13}C-nmr spectra of the pairs of anomers **1a/6a** and **1b/6b** were almost identical with the exemption of the H-1′- and H-8 signals, which were downfield shifted (1-2 ppm) in case of the α-anomers (Table 2). Assignment was in agreement with the empirical ^{1}H nmr triplet/pseudo-quartet rule of Srivastava and Robins [18]. From the CPK-models of **1a** and **1b** and their α-anomeric counterparts **6a** and **6b** it is apparent that the bulky N-3 alkyl group locks the sugar part *vs* the aglycon in a special conformation. This conformation can be described with high-syn [19]. To confirm this ^{1}H-nmr nOe data were used. Upon irradiation of the anomeric proton compound **1b** shows a strong nOe for the secondary CH, but not for the methyl residues of the isopropyl group (Table 2). A similar nOe is found for ipro-CH of the α-anomer **6b**. But contrary to the β-configurated **1b** the α-anomer **6b** gave a nOe for the $(CH_3)_2C$ groups but with a different intensity (Table 2). The latter indicates that not only the rotation of the nucleobase is restricted around the glycosylic bond but also the rotation of the isopropyl group is frozen in case of **1b** and **6b**. As irradiation of H-8 of **1b** gives an nOe of H-7 but does not result in a nOe of any other proton-signal including that of H-1′ being different to other pyrrolo[2,3-*d*]-pyrimidine nucleosides [20] the high-syn conformation is proved additionally.

From hydrolysis experiments on 3-methylguanosine Itaya and coworkers have concluded that the N-3 alkyl group destabilizes the N-9-glycosylic bond by steric interference [21]. To find out whether the N-3 substituent of **1a** or **1b** shows the same behaviour both compounds were hydrolyzed in 2 *N* hydrochloric acid within a temperature range of 50° and 80°. The hydrolysis was followed uv-spectrophotometrically (for wavelength see Table 3). As the same experiments were carried out with the aglycons **7a** or **7b** and no uv-change was observed, the decrease indicated glycosylic bond hydrolysis. As can be seen from table 3 the bis-methylnucleoside **1a** is six times more stable than the parent nucleoside **1c**, and the isopropyl compound **1b** is even 9 times more stable than **1c**. This is in contrary to purine ribonucleosides *e.g.* wyosine (**2a**) in which N-3 alkylation destabilizes the *N*-glycosylic bond upon proton-catalyzed hydrolysis. To explain these findings it was of interest to find differences between protonation of N-3 alkylated pyrrolo[2,3-*d*]pyrimidine and purine nucleosides.

Table 3
Kinetic and Thermodynamic Data of Proton-catalyzed Hydrolysis of Compounds **1a**, **1b**, **1c** and Xanthosine in 2*N* Hydrochloric acid at Various Temperatures

Compound	k x 10^4 sec^{-1}				
	50°C	60°C	70°C	75°C	80°C
1a	–	1.3	3.1	6.4	–
1b	–	–	2.1	3.1	5.1
1c	3.1	8.0	19.3	–	–
Xanthosine	5.0	18.4	56.0	–	–

Compound	$t_1/_2$ [min]			
	60°C	70°C	ΔH [kJ/mol]	ΔS [J/mol•K]
1a	90	37	87	-59
1b	–	55	83	-74
1c	14.5	6	81	-61
Xanthosine	6.3	2	108	+19

Measurements were carried out at 249 nm (**1a**), 277 nm (**1b**), 270 nm (**1c**) and 240 nm (xanthosine)

The pK_a of protonation of wyosine triacetate is 2.36 and the protonation position in DMSO-d_6 was found to be N-5 [22]. We have investigated the protonation of **7b** in DMSO-d_6. At first ^{15}N-nmr spectra were measured in the absence of acid under neutral conditions. Three signals of **7b** were observed. According to the coupling constants obtained from the ^{15}N-nmr INEPT spectra the signal at −242.7 ppm (J(NH) = 92 Hz and ^{2}J(HN) = 5 Hz) was identified as N-9. The signal at −233.3 ppm (^{2}J(HN) = 3 Hz) was assigned as that of N-1, as a similar chemical shift

was reported for N-1 of methylated 8-azaxanthines [23]. The remaining signal (−250.0 ppm; $^2J(NH)$ = 7 Hz) was that of N-3. An isopropyl group (compound **7b**) shifted the N-3 signal upfield (−271.7 ppm; ^2J = 3 Hz) compared to the N-3 methylated compound **7a**. When the spectrum of **7b** was measured in the presence of even 5 mole equivalents of trifluoroacetic acid no differences in ^{15}N-nmr shift were observed, indicating that protonation of the nucleobases does not take place under those strong acidic conditions.

The use of ΔS has been proposed as a criterion for distinguishing between an A-1 and A-2 mechanism [24]. From kinetic data of Table 3 those values can be calculated using the Eyring approach [25]. Schaleger reported entropy data of ΔS = 0-10 cal/mol·K for the A-1 mechanism and −15 to −30 cal/mol·K for the A-2 mechanism [24]. Panzica observed values from −2.9 to +11.6 cal/mol·K for the proton-catalyzed *N*-glycosylic bond hydrolysis of purine and pyrimidine nucleosides suggesting the A-1 mechanism [26]. According to the values of Table 3 the activation entropy of hydrolysis is −14 cal/mol·K in case of **1a**, −18 cal/mol·K for **1b** and −15 cal/mol K for **1c**. These values fit well with the A-2 mechanism. In opposite to these findings the ΔS value of the purine nucleoside xanthosine is + 4.5 cal/mol·K (Table 3). The change in the mechanism from N-3 methylated purine (A-1) to N-3 alkylated pyrrolo-[2,3-*d*]pyrimidine nucleosides (A-2) can be understood from the fact that purine nucleosides, *e.g.* wyosine (**2a**) [27] are protonated at the aglycone, whereas protonation of **1a** or **1b** occurs at the sugar moiety. As a consequence, a water molecule is required for Schiff base hydrolysis [28]. However, it has to be considered, that the extraordinary stability of the pyrrolo[2,3-*d*]pyrimidine *N*-glycosylic bond allows sugar isomerization before hydrolysis which makes the mechanism more complex. Nevertheless, this process does not cause an uv change but includes the possibility that different species (anomeric furanosides and pyranosides [28]) vary in their hydrolysis rate and the hydrolysis kinetics represent just the overall process.

EXPERIMENTAL

Melting points were determined on a Linström apparatus (Wagner-Munz, West-Germany) and are not corrected. Liquid-liquid PTC was performed with an UltraTurrax TP 18/10 stirrer (20 000 U/min) (Janke & Kunkel, West-Germany). The uv spectra were measured on an Uvicon-810 spectrometer (Kontron, Switzerland); reaction kinetics were assayed on a SuperScan-3 spectrophotometer (Varian, Australia). The tlc-scanning was performed on a Shimadzu CS-920 Dual Wavelength tlc-scanner (Shimadzu, Japan). The nmr spectra were recorded on Bruker WM 250 - and AC 250 spectrometers (Bruker, West-Germany) (δ-values are relative to tetramethylsilane for ^{1}H- and ^{13}C-nmr and relative to nitromethane for ^{15}N-nmr spectra). Tlc was performed on silica gel G-25 UV_{254} plates (Macherey-Nagel, West-Germany); hptlc was performed on silica gel 60 F_{254} precoated plates (Merck, West-Germany) with solvent systems A (chloroform-methanol, 9:1); B (dichloromethane-methanol, 95:5); C (dichloromethane-methanol 9:1); D (dichloromethane-acetone, 97:3); E (dichloromethane-acetone, 95:5); F (toluene-ethyl acetate 8:2). Elemental analyses were performed by the Institut für Organische Chemie, Biochemie und Isotopenforschung der Universität Stuttgart (West-Germany) and by Mikroanalytisches Labor Beller (Göttingen, West-Germany). 6-Amino-1,3-dimethyluracil is commercially available from Aldrich Chemical Corporation (USA), *N*-isopropyl-*N'*-methyl urea was synthesized according to Papesch *et. al.* [13].

6-Amino-5-(2,2-diethoxyethyl)-1,3-dimethylpyrimidine-2,4-dione (**3**).

To a solution of ethyl 2-cyano-4,4-diethoxyethylbutyrate (4.6 g, 20 mmoles) and *N,N'*-dimethylurea (1.85 g, 21 mmoles) in methanol (20 ml) 1 *M* sodium methoxide (25 ml) was added. After refluxing for 4 hours, the solvent was evaporated and the residue was dissolved in water (40 ml). Upon storing in an ice-bath for 5 hours colourless crystals precipitated. They were filtered off, washed with cold water and recrystallized from water yielding colourless needles (4.1 g, 76%), mp 143°; tlc (silica gel, solvent A) R_f 0.63; uv (methanol): λ max 273, 229 (δ 16 200, 4 300); ^{1}H-nmr (DMSO-d_6): δ 6.41 (s, 2H, NH_2), 4.45 (t, 1H, CH_2CH, J = 4.5 Hz), 3.60 (m, 1H, OCH_3CH_3), 3.40 (m, 1H, OCH_2CH_3), 3.30 (s, 3H, N1-CH_3), 3.12 (s, H, N3-CH_3), 2.55 (d, 2H, CH_2CH, J = 5.5 Hz), 1.08 (t, 3H, OCH_2CH_3).

Anal. Calcd. for $C_{12}H_{21}N_3O_4$ (271.3): C, 53.12; H, 7.80; N, 15.49. Found: C, 53.50; H, 7.77; N, 15.68.

1,3-Dimethylpyrrolo[2,3-*d*]pyrimidine-2,4-dione (**7a**).

Method (i).

Compound **3** (1.62 g, 6 mmoles) in 1 *N* hydrochloric acid (10 ml) was stirred at room temperature for 1 hour. The precipitate was filtered off and washed with water. Recrystallization from water yielded colourless crystals (0.8 g, 75%), mp 295° (lit 300°); tlc (silica gel, solvent A) R_f 0.3.

Method (ii).

To a solution of sodium acetate (8.2 g, 0.1 mole) in water (15 ml) chloracetaldehyde (50% in water) (23.5 g, 0.30 mole) was added. A suspension of 6-amino-1,3-dimethyluracil (15.5 g, 0.1 mmole) and sodium acetate (8.2 g, 0.1 mole) in water (50 ml) was prepared simultaneously and combined with the solution upon stirring at 70°. Within 15 minutes crystals precipitated which were recrystallized from water, colourless needles (9.1 g, 51%), mp 285° dec; tlc (silica gel, solvent A) R_f 0.3; uv (methanol): λ max 273, 243 nm (ε 9 300, 8 600); ^{1}H-nmr (DMSO-d_6): δ 6.76 (d, 1H, 6-H, J = 3 Hz), 6.35 (d, 1H, 5-H, J = 3 Hz), 3.43 (s, 3H, N1-CH_3), 3.20 (s, 3H, N3-CH_3).

1,3,7-Trimethylpyrrolo[2,3-*d*]pyrimidine-2,4-dione (**5a**).

Compound **5a** was prepared as described for **5b** (Method i). The starting material **7a** (100 mg, 0.56 mmole) yielded 91 mg of **5a** (78%), mp 264° (lit 265-266°); tlc (silica gel, solvent C) R_f 0.77; ^{1}H-nmr (DMSO-d_6): σ 6.69 (d, 1H, 6-H), 6.32 (d, 1H, 5-H), 3.90 (s, 3H, N7-CH_3), 3.32 (s, 3H, N1-CH_3), 3.21 (s, 3H, N3-CH_3).

6-Amino-1-isopropyl-3-methylpyrimidine-2,4-dione (**4**).

N-Isopropyl-*N'*-methylurea (25 g, 0.22 mole) and cyanoacetic acid (21.0 g, 0.25 mole) were dissolved in acetic anhydride (70 ml) and stirred for 4 hours at 85-90°. Excess of solvent was removed

by coevaporation with water (200 ml). The residue was dissolved in water (150 ml), cooled (10°) and treated with sodium hydroxide in water (12 g each) under vigourous stirring. The solution was allowed to come to room temperature and was extracted with ethyl acetate (three times, 250 ml each). The combined organic layers were washed with water, dried over sodium sulfate and filtered. After removal of the solvent the residue was recrystallized from water. Colourless crystals (31.5 g, 79%), mp 208-210° (lit 210-212°); tlc (silica gel, solvent C) R_f 0.53; uv (methanol): λ max 268 nm (ε 7 500); ^{1}H-nmr (DMSO-d_6): δ 6.70 (s, 2H, NH_2), 4.68 (s, 1H, 5-H), 4.49 (m, 1H, CH), 3.03 (s, 3H, N3-CH_3), 1.40 (d, 3H, CH_3).

1-Isopropyl-3-methylpyrrolo[2,3-*d*]pyrimidine-2,4-dione (**7b**).

6-Amino-1-isopropyl-3-methylpyrimidine-2,4-dione (**4**) (5.5 g, 30 mmoles) and sodium acetate (2.5 g) were dissolved in water (50 ml) at 60°. Then a solution of chloracetaldehyde (50% in water) (4.5 ml, 35 mmoles) and sodium acetate (2.5 g) in water (10 ml) was added and stirred for 30 minutes at this temperature. The solution was cooled and extracted with ethyl acetate (three times, 150 ml each). The combined organic layers were washed with water, dried over sodium sulfate and filtered. After evaporation of the solvent recrystallization of the oily residue from water yielded colourless needles (3.3 g, 53%), mp 211°; tlc (silica gel, solvent B) R_f 0.40; uv (methanol): λ max 243, 274 nm (ε 7 000, 7 200); ^{1}H-nmr (DMSO-d_6): δ 11.51 (s, 1H, ring-NH), 6.74 (d, 1H, H-6, J = 3 Hz), 3.36 (d, 1H, H-5, J = 3 Hz), 4.75 (m, 1H, CH, J = 6.6 Hz), 3.18 (s, 3H, N3-CH_3), 1.36 (d, 3H, CH_3).

Anal. Calcd. for $C_{10}H_{13}N_3O_2$ (207.23): C, 57.96; H, 6.32; N, 20.28. Found: C, 57.83; H, 6.26; N, 20.04.

3,7-Dimethyl-1-isopropylpyrrolo[2,3-*d*]pyrimidine-2,4-dione (**5b**).

Method (i).

To compound **7b** (400 mg, 1.9 mmoles) suspended in dichloromethane (30 ml) benzyltriethylammonium chloride (280 mg, 1.0 mmole) and 50% aqueous sodium hydroxide (2 ml) were added and stirred for 10 minutes (UltraTurrax). Within this time methyl iodide (0.6 ml, 10 mmoles) was added in small portions. The organic layer was separated the aqueous layer extracted with dichloromethane (two times, 50 ml each). The combined layers were dried over sodium sulfate filtered and the solvent was evaporated. After recrystallization from water colourless crystals (380 mg, 90%) were obtained, mp 239-240°.

Method (ii).

Compound **7b** (200 mg, 0.95 mmole) was dissolved in dry DMF (5 ml). After addition of sodium hydride (70 mg, 50% in oil) the suspension was stirred under nitrogen atmosphere for 10 minutes then methyl iodide (0.2 ml, 3 mmoles) was added. After stirring for 30 minutes at room temperature the solvent was evaporated and the residue was dissolved in water (5 ml) and then extracted with ethyl acetate (50 ml). The organic layer was dried over sodium sulfate and then evaporated. Recrystallization from water yielded 160 mg (76%) colourless crystals, mp 240°; hptlc (silica gel, solvent B) R_f 0.55; uv (methanol): λ max 249, 276 nm (ε 8 600, 8 300); ^{1}H-nmr (DMSO-d_6): δ 6.72 (d, 1H, H-6, J = 3 Hz), 6.31 (d, 1H, 5-H, J = 3 Hz), 4.68 (m, 1H, CH, J = 6.6 Hz), 3.83 (s, 3H, N7-CH_3), 3.16 (s, 3H, N3-CH_3), 1.54 (d, 6H, 2 CH_3, J = 6.6 Hz).

Anal. Calcd. for $C_{11}H_{15}N_3O_2$ (221.26): C, 59.71; H, 6.83; N, 18.99. Found: C, 59.70; H, 6.92; N, 18.82.

Glycosylation of **7a** with the Halogenose **8**.

Method (i).

To a suspension of **7a** (500 mg, 2.8 mmoles) and benzyltriethylammonium chloride (50 mg, 0.2 mmoles) in dichloromethane (25 ml), 50% aqueous sodium hydroxide (1 ml) was added. The mixture was stirred for 30 minutes with an UltraTurrax stirrer. During this period the halogenose **8** [29] (1.25 g, 3.2 mmoles) was added in small portions. The organic layer was separated and the aqueous phase extracted with dichloromethane (twice, 50 ml each). The combined organic layers were dried over sodium sulfate and evaporated to yield an amorphous foam (1.05 g, 71%) containing **10a** (30%) and **9a** (70%) according to the integration of the anomeric signals of the ^{1}H-nmr spectra.

Method (ii).

Compound **7a** (1.0 g, 5.5 mmoles) was dissolved in anhydrous acetonitrile (200 ml) at 50°. Powdered potassium hydroxide (1.5 g, 26 mmoles) and then tris 2-(2-methoxyethoxy)ethylamine (TDA-1) (0.15 ml, 0.55 mmole) were added and the stirring was continued for another 15 minutes. The solution was filtered, dried over sodium sulfate and evaporated to yield an amorphous solid (1.4 g, 52%) containing **10a** (20%) and **9a** (80%) according to uv-quantification by tlc-scanning.

7-[2′-Deoxy-3′,5′-di-*O*-(*p*-toluoyl)-β-D-erythropentofuranosyl]-1,3-dimethylpyrrolo[2,3-*d*]pyrimidine-2,4-dione (**9a**).

The diastereomeric mixture of compounds **9a**/**10a** (500 mg, 0.95 mmoles) synthesized by method (ii) was applied on the top of a silica gel column (30 × 4 cm). Flash-chromatography with solvent E gave two zones. From the fast migrating zone **9a** (350 mg) was obtained as a colourless foam. Crystallization from methanol yielded colourless crystals (250 mg), mp 190-192°; hptlc (silica gel, solvent D) R_f 0.58; uv (methanol): λ max 272, 241 nm (ε 18 500, 10 500); ^{1}H-nmr (DMSO-d_6): δ 7.87 (dd, toluene-H), 7.33 (dd, toluene-H), 7.10 (d, 1H, 6-H, J = 3 Hz), 6.63 (t, 1H, 1′-H, J = 6 Hz), 6.45 (d, 1H, 5-H, J = 3 Hz), 5.65 (t, 1H, 3′-H, J = 3 Hz), 4.54-4.50 (m, 1H, 5′-H), 4.43-4.40 (m, 1H, 4′-H), 3.75 (s, 3H, N1-CH_3), 3.20 (s, 3H, N3-CH_3), 3.1-3.0 (m, 1H, 2′-H_α), 2.8-2.7 (m, 1H, 2′-H_β).

Anal. Calcd. for $C_{29}H_{29}N_3O_7$ (531.6): C, 65.63; H, 5.50; N, 7.91. Found: C, 65.85; H, 5.61; N, 8.02.

7-[2′-Deoxy-3′,5′-di-*O*-(*p*-toluoyl)-α-D-erythropentofuranosyl]-1,3-dimethylpyrrolo[2,3-*d*]pyrimidine-2,4-dione (**10a**).

From the slow migrating zone compound **10a** (50 mg) was isolated as colourless foam. Crystallization from methanol gave colourless crystals (40 mg), mp 187-188°; hptlc (solvent D) R_f 0.56; uv (methanol): λ max 272, 241 nm (ε 18 500, 10 500); ^{1}H-nmr (DMSO-d_6): δ 7.88 (dd, toluene-H), 7.33 (dd, toluene-H), 7.15 (d, 1H, 6-H, J = 3.5 Hz), 6.75 (dd, 1H, 1′-H, J = 5 Hz), 6.45 (d, 1H, 5-H, J = 3.5 Hz), 5.65 (d, 1H, 3′-H, J = 2 Hz), 4.81 (m, 1H, 4′-H), 4.50 (m, 1H, 5′-H), 3.68 (s, 3H, N1-CH_3), 3.20 (s, 3H, N3-CH_3), 3.13-3.07 (m, 1H, 2′-H_α), 2.83-2.77 (m, 1H, 2′-H_β).

Anal. Calcd. for $C_{29}H_{29}N_3O_7$ (531.6): C 65.63; H, 5.50; N, 7.91. Found: C, 65.78; H, 5.51; N, 7.80.

7-(2′-Deoxy-β-D-erythropentofuranosyl)-1,3-dimethylpyrrolo-[2,3-*d*]pyrimidine-2,4-dione (**1a**).

A solution of **9a** (200 mg, 0.38 mmole) in methanol-25% aqueous ammonia (1:1, 200 ml) was stirred at 50° for 10 hours. After evaporation to dryness the residue was partitioned between water (50 ml) and ethyl acetate (50 ml). The aqueous layer was

separated and evaporated. The colourless residue was crystallized from methanol to yield **1a** (106 mg, 95%) as colourless crystals, mp 198°; hptlc (silica gel, solvent B) R_f 0.34; uv (methanol): λ max 276, 245 nm (ε 5 600, 6 800); ^{1}H-nmr (DMSO-d_6): δ 7.08 (d, 1H, 6-H, J = 3.5 Hz), 6.38 (m, 2H, 5-H and 1'-H), 5.44 (d, 1H, 3'-OH, J = 4.5 Hz), 4.87 (t, 1H, 5'-OH, J = 5 Hz), 4.30 (m, 1H, 3'-H), 3.80 (m, 1H, 4'-H), 3.37 (s, 3H, N1-CH_3), 3.55 (m, 1H, 5'-H), 3.22 (s, 3H, N3-CH_3), 3.51 (m, 1H, 2'-H_α), 2.24 (m, 1H, 2'-H_β).

Anal. Calcd. for $C_{13}H_{17}N_3O_5$ (295.3): C, 52.88; H, 5.80; N, 14.23. Found: C, 52.74; H, 5.80; N, 14.19.

7-(2'-Deoxy-α-D-erythropentofuranosyl)-1,3-dimethylpyrrolo-[2,3-*d*]pyrimidine-2,4-dione (**6a**).

Compound **10a** (40 mg, 0.08 mmoles) was treated as described for **1a**. Crystallization from methanol yielded colourless crystals (20 mg, 89%), mp 234°; hptlc (silica gel, solvent B) R_f 0.33; uv (methanol): λ max 276, 245 nm (ε 5 400, 6 900); ^{1}H-nmr (DMSO-d_6): δ 7.25 (d, 1H, 6-H, J = 3.5 Hz), 6.45 (m, 1H, 1'-H), 6.41 (d, 1H, 5-H, J = 3.5 Hz), 5.48 (d, 1H, 3'-OH, J = 4.5 Hz), 4.83 (t, 1H, 5'-OH, J = 5 Hz), 4.30 (m, 1H, 3'-H), 3.97 (q, 1H, 4'-H, J = 5 Hz), 3.72 (s, 3H, N1-CH_3), 3.47 (m, 1H, 5'-H), 3.22 (s, 3H, N3-CH_3), 2.76-2.65 (m, 1H, 2'-H_α), 2.58-2.51 (m, 1H, 2'-H_β).

Anal. Calcd. for $C_{13}H_{17}N_3O_5$ (295.3): C, 52.88; H, 5.80; N, 14.23. Found: C, 52.74; H, 5.80; N, 14.19.

Glycosylation of **7b** with the Halogenose **8**.

Method (i).

To a solution of **7b** (600 mg, 2.9 mmoles) in anhydrous acetonitrile (25 ml) sodium hydride (50% in oil, 200 mg, 4.1 mmoles) was added and stirred under nitrogen atmosphere for 30 minutes at 70°. Then the halogenose **8** (1.4 g, 3.5 mmoles) was added and stirring was continued for one hour (70-80°). After evaporation to dryness the residue was dissolved in ethyl acetate (250 ml) and filtered. The filtrate was washed with water, dried over sodium sulfate and evaporated. The oily residue was disolved in dichloromethane and applied to flash chromatography on a silica gel column (15 × 4 cm, solvent D) to yield a mixture of **9b/10b** (750 mg, 48%) as colourless foam. This contains compound **9b** (65%) and **10b** (35%) according to the integration of the 1'-H signal.

Method (ii).

Compound **7b** (1.0 g, 4.8 mmoles) in anhydrous acetonitrile (50 ml) was stirred at room temperature. Powdered potassium hydroxide (1.5 g, 27 mmoles) and TDA-1 (0.15 ml, 0.5 mmole) were added and the mixture was stirred for another 5 minutes. The halogenose **8** (2.15 g, 5.5 mmoles) was introduced under stirring and stirring was continued for 15 minutes. Solid material was filtered off and the filtrate was evaporated to dryness. Flash-chromatography on silica gel (column 15 × 4 cm) yielded a colourless foam (1.5 g, 56%) containing **9b** (75%) and **10b** (25%) according to ^{1}H-nmr analysis by quantification of the 1'-H signal; hptlc (silica gel, solvent F) R_f 0.30, 0.31; uv (methanol): λ max 242, 272 (ε 22 700, 11 800).

7-[2'-Deoxy-3',5'-di-*O*-(*p*-toluoyl)-α-D-erythropentofuranosyl]-1-isopropyl-3-methylpyrrolo[2,3-*d*]pyrimidine-2,4-dione (**10b**).

The anomeric mixture of **9b/10b** (300 mg) was separated by repeated flash chromatographie (silica gel, 5 × 40 cm, solvent F) to give colourless amorphous **9b** (50 mg) from the slower migrating zone. This material was crystallized from methanol to yield colourless crystals, mp 189°; ^{1}H-nmr (DMSO-d_6): δ 7.92-7.84 (toluene-H), 7.35-7.32 (toluene-H), 7.14 (d, 1H, 6-H), 6.44 (d, 1H, 5'-H), 6.33 (d, 1H, 1'-H), 5.63 (d, 1H, 3'-H), 4.82 (m, 1H, 4'-H), 4.62-4.43 (d, 2H, CH and 5'-H), 3.35 (s, 3H, N3-CH_3), 3.39-3.20 (m, 1H, 2'-H_α), 2.80-2.74 (m, 1H, 2'-H_β), 2.38 (toluene-CH_3), 1.62 (d, 3H, CH_3, J = 6.5 Hz), 1.37 (d, 3H, CH_3, J = 6.5 Hz).

7-[2'-Deoxy-3',5'-di-*O*-(*p*-toluoyl)-β-D-erythropentofuranosyl]-1-isopropyl-3-methylpyrrolo[2,3-*d*]pyrimidine-2,4-dione (**9b**).

By repeated chromatographic separation compound **9b** was isolated from the faster migrating zone (silica gel, solvent F) to give colourless amorphous **9b**; ^{1}H-nmr (DMSO-d_6): δ 7.94-7.84 (m, toluene-H), 7.36-7.30 (m, toluene-H), 7.07 (d, 1H, 6-H), 6.44 (d, 1H, 5-H), 6.22 (t, 1H, 1'-H), 5.66 (m, 1H, 3'-H), 4.64-4.41 (m, 2H, CH and 5'-H), 4.20 (m, 1H, 4'-H), 3.34 (s, 3H, N3-CH_3), 2.99-2.91 (m, 1H, 2'-H_α), 2.79-2.74 (m, 1H, 2'-H_β), 2.38 (toluene-CH_3), 1.61 (d, 3H, CH_3, J = 6.5 Hz), 1.48 (d, 3H, CH_3, J = 6.5 Hz).

Anal. Calcd. for $C_{31}H_{33}N_3O_7$ (559.6): C, 66.63; H, 5.94; N, 7.51. Found: C, 66.46; H, 5.91; N, 7.55.

7-(2'-Deoxy-α-D-erythropentofuranosyl)-1-isopropyl-3-methylpyrrolo[2,3-*d*]pyrimidine-2,4-dione (**6b**).

Compound **10b** (45 mg) was dissolved in a solution of 25% aqueous ammonia/methanol (50 ml, 1:1) and stored at 50° overnight in a stoppered flask. After evaporation the residue was applied on a silica gel plate (methanol) and developed with solvent B. Colouless amorphous **6b** (15 mg) was isolated from the main zone; hptlc (silica gel, solvent B), R_f 0.73; uv (methanol): λ max 245, 275 nm (ε 7 400, 6 500); ^{1}H-nmr (DMSO-d_6): δ 7.28 (d, 1H, 6-H, J = 3.5 Hz), 6.39 (d, 1H, 5-H, J = 3.5 Hz), 6.02 (q, 1H, 1'-H, J = 4.2 Hz), 5.75 (m, 1H, 3'-OH), 5.10 (m, 1H, 5'-OH), 4.65 (m, 1H, CH, J = 6.5 Hz), 4.33 (t, 1H, 3'-H, J = 3.7 Hz), 4.05 (m, 1H, 4'-H), 3.47 (m, 2H, 5'-H), 3.16 (s, 3H, N-CH_3), 2.77 (m, 1H, 2'-H_α), 2.20 (m, 1H, 2'-H_β), 1.60 (d, 3H, CH_3, J = 6.5 Hz), 1.46 (d, 3H, CH_3, J = 6.5 Hz).

7-(2'-Deoxy-β-D-erythropentofuranosyl)-1-isopropyl-3-methylpyrrolo[2,3-*d*]pyrimidine-2,4-dione (**1b**).

The mixture of **9b/10b** (500 mg, 0.89 mmole) obtained by method (ii) was dissolved in a solution of 25% aqueous ammonia/methanol (200 ml, 1:1) and stored at 55° for 5 hours in a stoppered flask. After evaporation to dryness the residue was dissolved in water (50 ml) and applied on an ion-exchanger (DOWEX 1×2, OH^-, 15 × 2 cm). Elution with water-methanol (gradient 0-20% methanol) furnished an anomeric mixture of **1b/6b** (260 mg) from which the pure β-anomer **1b** was separated by fractionalized crystallization (water). Colourless crystals (180 mg, 62%), mp 180° dec; hptlc (silica gel, solvent B) R_f 0.73; uv (methanol): λ max 245, 276 nm (ε 7 700, 6 200); ^{1}H-nmr (DMSO-d_6): δ 7.07 (d, 1H, 6-H, J = 3.5 Hz), 6.40 (d, 1H, 5-H, J = 3.5 Hz), 6.02 (t, 1H, 1'-H, J = 6.3 Hz), 5.32 (d, 1H, 3'-OH, J = 4 Hz), 4.93 (t, 1H, 5'-OH, J = 5 Hz), 4.58 (m, 1H, CH, J = 6.5 Hz), 4.31 (m, 1H, 3'-H), 3.83 (d, 1H, 4'-H, J = 3 Hz), 3.51 (m, 1H, 5'-H), 3.17 (s, 3H, N-CH_3), 2.55-2.25 (m, 2H, 2'-H), 1.60 (d, 3H, CH_3, J = 6.5 Hz), 1.48 (d, 3H, CH_3, J = 6.5 Hz).

Anal. Calcd. for $C_{15}H_{21}N_3O_5$ (323.35): C, 55.72; H, 6.55; N, 13.00. Found: C, 55.57; H, 6.47; N, 12.80.

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