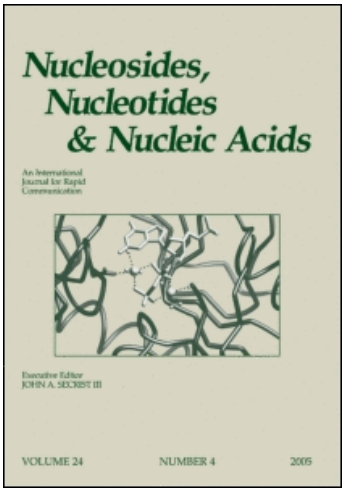

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SYNTHESIS OF 2',3'-DIDEHYDRO-2',3'-DIDEOXYISOINOSINE AND OXIDATION OF FLUORESCENT 2-HYDROXYPURINE NUCLEOSIDES BY XANTHINE OXIDASE

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ABSTRACT: The syntheses of 2',3'-didehydro-2',3'-dideoxyisoinosine (d4isoI, **4**) as well as 7-deaza-2',3'-didehydro-2',3'-dideoxyisoinosine (d4c^{7}isoI, **5**) are described. Compounds **4** and **5** show both strong fluorescence. Compound **4** is oxidized by xanthine oxidase to give the corresponding xanthine 2',3'-dideoxy-2',3'-didehydronucleosides. A preparative chemo-enzymatic synthesis of 2'-deoxyxanthosine (**3**) is described.

2'-Deoxyinosine (**1a**) is widely used as ambiguous nucleoside in oligonucleotide chemistry.[1-3] The synthesis of the isomeric 2'-deoxyisoinosine (**2a**) as well as of 7-deaza-2'-deoxyisoinosine (**2b**) has been described previously.[4-5] Both compounds are highly fluorescent. The nucleoside **1a**, as well as the compound **2a** were incorporated into oligonucleotides and were found to show ambiguous base pairing with the four conventional nucleosides.[1-3, 6]

Since 2',3'-dideoxynucleosides such as ddI (**1b**) as well as 2',3'-unsaturated nucleosides, e.g. d4T and d4C show antiviral activity [7] the synthesis of compound **4** as

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The manuscript is dedicated to the memory of Professor Tsujiaki Hata

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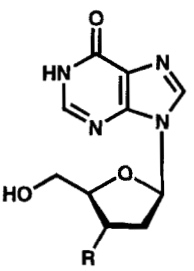
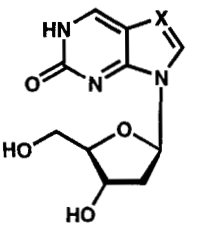
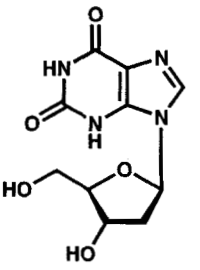


1a: R = OH
b: R = H



2a: X = N
b: X = CH



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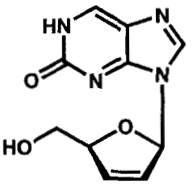

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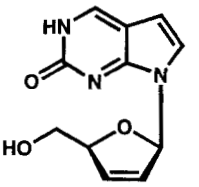

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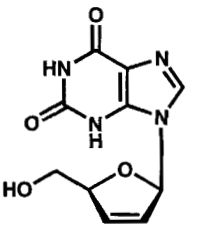
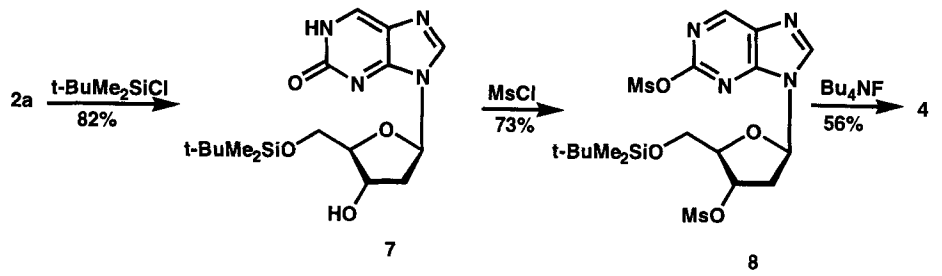


6

SCHEME 1

well as of its 7-deazapurine derivative **5** was undertaken. Moreover, the oxidation of these nucleosides and of the parent 2'-deoxyribo compounds **2a** and **2b** by xanthine oxidase was studied. In this regard an efficiently combined chemical and enzymatic synthesis of 2'-deoxyxanthosine (**3**) will be presented.

Synthesis. The base-promoted elimination of 2'-deoxyribonucleoside mesylates has been reported for the synthesis of a number of 2',3'-didehydro-2',3'-dideoxy nucleosides such as d4G or d4T.[8-10] This reaction sequence was now performed on 2'-deoxyisoinosine (**2a**). Thus, compound **2a** was silylated with *tert*-butyldimethylsilyl chloride (TBDMS-Cl) to give the 5'-protected derivative **7** in 82% yield. Mesylation of **7** afforded the bis-mesylate **8** (73% yield) with one mesyl at the 3'-hydroxyl group and the other one at the 2-oxo function. The bis-mesylate **8** was then treated with 1.1 M Bu_4NF in THF to give the 2',3'-didehydro-2',3'-dideoxynucleoside **4** in 56% yield. The elimination and hydrolysis of the second mesyl residue and deprotection of 5'-hydroxyl group occurred simultaneously in an one-pot reaction (**SCHEME 2**).

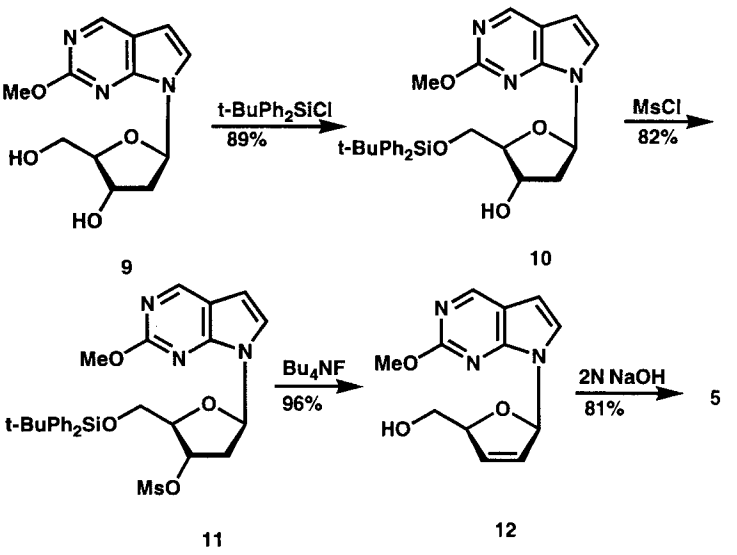


SCHEME 2

For the preparation of the related 2',3'-didehydro-2',3'-dideoxy-7-deazaisoinosine (**5**), the 2-methoxy nucleoside **9** [4] was used as starting material. The nucleoside **9** was treated as it was already described for other 7-deazapurine 2',3'-dideoxy-2',3' -didehydronucleosides.[11] The methoxy nucleoside **9** was silylated at the 5-hydroxyl group with *tert*-butyldiphenylsilyl chloride (TBDPS-Cl) and then mesylated at OH-3' to form compound **11**. The elimination and deprotection of mesylate **11** occurred simultaneously with 1.1 M Bu_4NF/THF to give the 2',3'-didehydro-2',3'-dideoxy compound (**12**) in 96% yield. This was treated with 2 N NaOH (reflux) to afford the target nucleoside **5** in 81% yield (**SCHEME 3**).

The structures of the final 2',3'-dideoxy-2',3'-didehydronucleosides and of the intermediates were proven by ^{1}H-NMR and ^{13}C-NMR spectroscopy (**TABLE 1**). Gated-decoupled ^{13}C-NMR spectra were used to assign ^{13}C-NMR resonances. The ^{13}C-NMR chemical shifts of the unsaturated sugar moiety of compounds **4**, **5**, and **12** were found to be similar to those of related nucleosides.[11] Also the ^{1}H-NMR spectra showed two dublets at ~6.0 and ~6.4 ppm representing the two olefinic protons (H-(C2') and H-(C3')). The bis-mesylated intermediate **8** shows a 10 ppm downfield shift of both C-5 and C-6 compared to those of the parent **2a** as well as an upfield shift (~6 ppm) of C-4. Finally, the UV maximum of compound **8** (λ_{max} at 268 nm in MeOH) was hypsochromically shifted (54 nm) over that of parent 2'-deoxyisoinosine (322 nm).[4] Moreover, compound **8** is no longer fluorescent.

According to the ^{1}H-NOE data (**TABLE 2**) the N-glycosylic bond conformation of compound **2a** was found to be anti. The anti conformer population is 63%. However,



SCHEME 3

due to the flattening of the sugar moiety 2', 3'-didehydro-2', 3'-dideoxyisoinosine shows an increase of the population to 80%. The 7-deazapurine dideoxy-didehydro nucleoside **5** gives an even higher value (90% anti-conformer). In the case of 2'-deoxy-7-deazaisoinosine (**2b**), the situation is not clear due the an overlap of the H-(C1') and H-(C7) signals.

Fluorescence. All of the 2-hydroxypurine nucleosides and analogs show strong fluorescence. As it can be seen from the data in **TABLE 3** the absorption spectra of the substances exhibit their long wavelength maximum between 314 and 331 nm in bi-distilled water. The 2-amino-9-[2-deoxy-ß-D-erythro-pentofuranosyl]-9H-purine ($2NH_2P_d$ λ_{max} = 304 nm) was used for comparison. Upon excitation at 300 nm all compounds have strong fluorescence with emission maxima between 360 and 444 nm (**FIG. 1**). No photobleaching was observed on exposing the samples for over 24 h at r.t. or during the spectrophotometric measurements (data not shown). The 2-amino nucleoside $2NH_2P_d$ has 0.8 of the quantum yield (quinine sulfate in 0.1 N H_2SO_4 with a quantum yield of 0.7 as standard). Replacement of the amino group by hydroxy (**2a** and **4**) results in a bathochromic shift in absorption and emission as well as a decreased fluorescence quantum yield. The Stokes' shifts of 2-hydroxypurine compounds (**2a** and

TABLE 1. ^{13}C-NMR Chemical Shifts of 2'-Deoxyisoinosine and Derivatives. [a]

Compd.	C-2[b] C-2[c]	C-6 C-4	C-5 C-4a	- C-5	C-8 C-6	C-4 C-7a	Me
2a	156.1	139.4	123.6	-	145.5	158.8	
2b	155.7	140.0	108.1	101.4	126.6	158.1	
4	157.5	142.5	123.2	-	142.7	159.5	
5	155.6	139.9	107.9	101.1	126.3[d]	158.0	
7	155.9	139.1	123.5	-	145.3	158.9	
8	154.1	150.2	133.5	-	146.9	152.6	
9	161.4	151.3	114.7	100.7	125.7	152.5	54.4
10	161.3	151.2	114.6	100.5	125.3	152.3	54.2
11	161.3	151.4	114.8	100.8	125.7	152.4	54.3
12	161.4	151.2	114.5	100.5	125.7[d]	152.4	54.2

	C-1'	C-2'	C-3'	C-4'	C-5'
2a	82.9	[e]	70.9	87.9	61.7
2b	81.9	[e]	70.9	87.1	61.9
4	87.9	125.6	134.4	87.1	62.9
5	87.1[d]	125.9[d]	134.0	86.7[d]	63.2
7	82.5	[e]	70.2	87.1	63.2
8	83.9	37.9	80.2	84.8	62.3
9	82.6	39.4	71.1	87.4	62.1
10	82.5	[e]	70.4	86.4	64.3
11	82.8	37.9	80.2	83.5	63.3
12	87.3[d]	125.4[d]	134.1	87.2[d]	63.2

[a] Measured in (D_6)DMSO at 23°C. [b] Purine numbering. [c] Systematic numbering. [d] Tentative. [e] Superimposed by DMSO

4) are 60 nm similar to $2NH_2P_d$, but the fluorescence lifetimes (~4 ns) are shorter over that of 2-amino nucleoside (τ = 10.27 ns). In the absence of N-7 the Stokes' shifts increase to 110 nm and the fluorescence lifetimes become up to ~10 ns. The χ^2 reprents the quality of decay fits and all are around a value of 0.9.

Oxidation by Xanthine oxidase. Xanthine oxidase catalyzes the oxidation of hypoxanthine at the C-2 position yielding xanthine which then undergoes further reaction at C-8. On the other hand the corresponding 9-methyl hypoxanthine [12] as well as inosine are resistant against the enzyme.[13] Similarly, 2-hydroxypurine is a substrate of

TABLE 2. ^{1}H-NMR NOE Data of Compounds **2a, 2b, 4,** and **5** [a, b]

Comp.	Proton irradiated	NOE
2a	H-(C8)	H-(C1') (4.2%), H_{β}-(C2') (3.2%), H-(C3') (1.1%).
	H-(C1')	H-(C8) (4.8%), H_{α}-(C2') (7.4%), H-(C4') (1.2%).
2b	H-(C8)	H-(C1') and H-(C7) (11.8%), H_{β}-(C2') (5.4%), H-(C3') (1.5%).
4	H-(C8)	H-(C1') (2.3%), H-(C3') (0.9%), H-(C2') (2.3%), H-(C5') (1.1%).
	H-(C1')	H-(C8) (2.7), H-(C2') (5.4%), H-(C4') (1.5%).
5	H-(C8)	H-(C1') (1.2%), H-(C7) (10.0%), H-(C3') (1.0%), H-(C2') (3.0%), OH-(C5') (1.4%), H-(C5') (2.4%).
	H-(C1')	H-(C8) (0.9%), H-(C2') (7.1%), OH-(C5') (0.8%), H-(C4') (2.0).

[a]) Taken from spectra measured in (D_6)DMSO at 23°C. [b]) Purine numbering.

TABLE 3. Fluorescent Properties of 2'-Deoxyisoinosine and Derivatives [a]

Compound	λ_{max} [nm]	λ_{em} [nm]	Φ_f	τ [ns]	χ^2
$2NH_2P_d$	304	360	0.80	10.27	0.898
$isoI_d$ (**2a**)	316	374	0.23	3.95	0.997
c^7isoI_d (**2b**)	331	444	0.22	9.11	1.085
d4isoI (**4**)	314	374	0.21	4.43	0.910
$d4c^7isoI$ (**5**)	331	443	0.30	11.09	0.953

[a] Measured in bi-distilled water.

xanthine oxidase giving also uric acid upon enzymic oxidation. But, contrary to hypoxanthine, 2-hydroxy-9-methylpurine can also be oxidized to 2,6-dihydroxy-9-methylpurine.[12-14] However, further oxidation to uric acid derivative does not take place. As it would be interesting to investigate the behavior of 2-hydroxypurine- and 7-deaza-2-hydroxypurine 2'-deoxyribo (**2a,b**) as well as 2',3'-unsaturated nucleosides (**4** and **5**) they were tested against enzymatic conversion by xanthine oxidase.

It has already been reported that 2-aminopurine nucleosides are substrates of xanthine oxidase which can be oxidized to guanosine derivatives. [14, 15] A further oxidation at C-8 does not take place. When the nucleosides **2a** or **4** were treated with

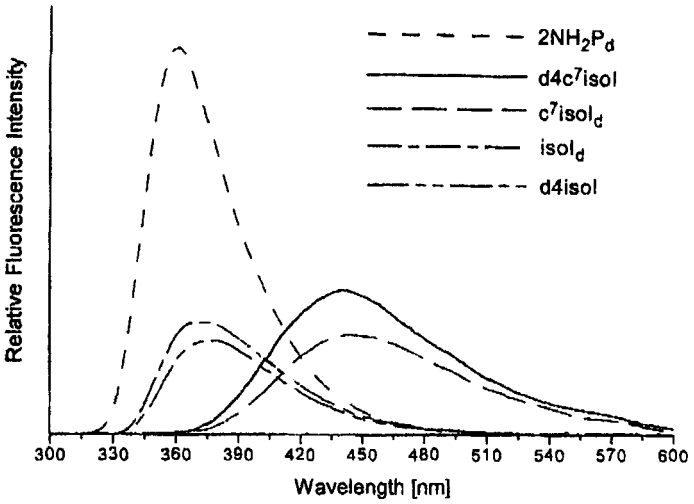


FIG. 1 Relative emission spectra of $2NH_2P_d$, 2-hydroxypurine derivatives in bi-distilled water excited at 300 nm.

xanthine oxidase (EC 1.1.3.22) the corresponding 2,6-dihydroxypurine nucleosides were obtained which are not further oxidized to 2,6,8-trihydroxypurine nucleoside (**SCHEME 4**). This oxidation is shown in the following figures which represent a serial overlay of the UV-spectra. According to **FIG. 2a,b** a continuous shift of the UV-maximum from 315 nm to 276 and 247 nm takes place. The final spectrum is identical with that of 9-substituted xanthines (λ_{max} : 278 and 248 nm) [16] and not with the corresponding uric acid derivatives (λ_{max} : 293 and 238 nm).[17]

Earlier, it has been shown by our laboratory [18-20] and by others [13] that xanthine oxidase requires an intact imidazole ring in its purine substrate. An alteration of the structure results in inhibitors or suicide substrates like allopurinol which can only be oxidized at the C-2 position.[21] The enzymatic oxidation at this position is also observed in the case of 7-deazahypoxanthine. On the other hand, the 9-methyl-7-deazahypo xanthine is not a substrate but develops inhibitory activity.[18] Consequently, the 7-deazapurine nucleoside **2b** or 2',3'-didehydro-2',3'-dideoxy-7-deazaisoinosine (**5**) are also resistant against enzymic oxidation. Obviously, the 7-deazapurine nucleosides cannot bind the molybdenium enzyme effectively.

2a: R = 2'-deoxyribofuranosyl
4: R = 2',3'-didehydro-2',3'-dideoxyribofuranosyl

3: R = 2'-deoxyribofuranosyl
6: R = 2',3'-didehydro-2',3'-dideoxyribofuranosyl

SCHEME 4

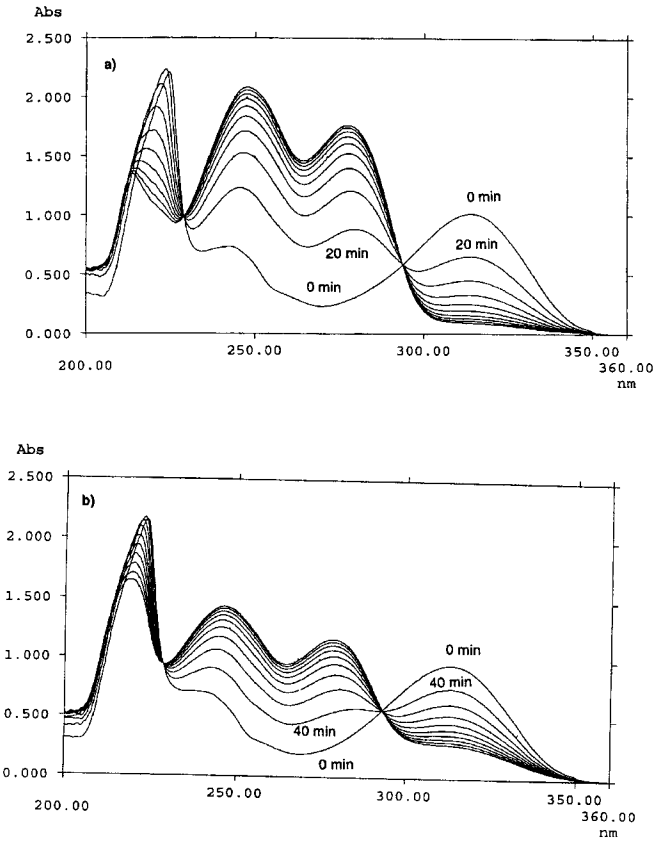


FIG. 2 Time-dependent UV spectra of the enzymatic conversion of 2'-deoxyisoinosine (**2a**) a) and 2'3'-didehydro-2'3'-dideoxyisoinosine (**4**) b) to xanthine nucleosides catalyzed by xanthine oxidase (phosphate buffer, pH 7.4, at 25°C).

Compound **3** is extremely labile towards acid and the cleavage of the glycosylic bond occurs below pH 4.[16] This makes the common route of nitrous acid deamination of 2'-deoxyguanosine (dG) problematic. Small quantities of compound **3** have been prepared by enzymatic transglycosylation, [22, 23] and a nitrosative deamination of dG under alkaline conditions was reported,[16] yet 2'-deoxyxanthosine remains commercially unavailable and seldom mentioned in the literature.

According to the points discussed above 2'-deoxyxanthosine (**3**) can be synthesized from 2'-deoxyisoinosine (**2a**) enzymatically using xanthine oxidase. The preparative scale experiment described in the Experimental leads to a 75% yield of compound **3** which was characterized by UV and ^{1}H-NMR spectra. The same preparative scale conditions should also be applicable to the 2',3'-dideoxy-2',3'-didehydronucleoside **4** and other 2-hydroxypurine nucleosides, compounds which are difficult to prepare otherwise.

EXPERIMENTAL

General. TLC: Aluminium sheets coated with 0.2-mm layer of silica gel 60 F_{254} (Merck, Germany). Flash chromatography (FC) was carried out at 0.5 bar (silica gel 60 (Merck, Germany)). An Uvicord S (LKB Instruments, Sweden) was used for detection. UV Spectra: Hitachi-150-20 spectrometer (Hitachi, Japan). M.p.: Büchi-SMP-20 apparatus (Büchi, Switzerland). NMR Spectra: Bruker-AC-250 and AMX-500 spectrometers; δ values in ppm rel. to Me_4Si as internal standard (^{1}H and ^{13}C) or to external phosphoric acid (^{31}P). Elemental analyses were performed by Mikroanalytisches Laboratorium Beller, Göttingen, Germany. Xanthine oxidase from cow's milk (EC 1.1.3.22) was a generous gift from the Boehringer Mannheim GmbH, Germany.

Fluorescence spectra measurements. All measurements were done in bi-distilled water at 20°C. Absorption spectra were measured with a Perkin Elmer Lambda 18 UV/VIS spectrophotometer. In order to avoid inner filter effects the sample was not allowed to exceed 0.05 at the excitation wavelength using standard quartz cuvettes with a pathlength of 1 cm. Fluorescence spectra were recorded in the wavelength range between 320 and 600 nm using the fluorescence spectrophotometer LS100 (PTI). For all

calculations the water background was substracted from the sample. The fluorescence quantum yields were determined using quinine sulfate in 0.1 N H_2SO_4 (Fluorescence quantum yield 0.70) [24] as a standard with the following relation:

$\Phi_{f,\,sample} = \Phi_{f,\,standard}$ x $(F_{sample}/F_{standard})$ x $(A_{standard\ at\ 300\ nm}/A_{unknown\ at\ 300\ nm})$

where $\Phi_{f,\,sample}$ is the unknown fluorescence quantum yield of the florophore, F is the integrated fluorescence intensity between 320 and 600 nm after excitation at 300 nm, and A is the absorbance at 300 nm in 1 cm cuvettes.

The fluorescence lifetimes were measured with a hydrogen-filled flashlamp (1.5 ns FWHM) using a time-correlated single-photon counting technique with an instrument from PTI (Model LS100). The samples were excited at the long-wavelength maximum. The fluorescence decay was monitored at the emission maximum. The instrument response function needed for deconvolution was obtained from a scattering solution. The quality of the decay fits were controlled by the reduced chi-squared statistical parameter. Most of the decays could be described satisfactorily by a mono-exponential model. In the cases where a second component was necessary a bi-exponential model was used to fit the decay:

$I(t) = a_1\ exp(-t/\tau_1) + a_2\ exp(-t/\tau_2)$

Where a_1 and a_2 are the preexponential factors, which describe the ratio of the excited species ($a_1 + a_2 = 1$), and τ_1 and τ_1 denote their lifetimes.

Enzymatic oxidation of nucleosides by xanthine oxidase (analytical scale experiments). The compounds **2a, 2b, 4,** and **5** (~1.0 A_{315} units) dissolved in 1 ml of phosphate buffer (pH = 7.4) were treated with xanthine oxidase (0.2 units) at 25°C. The reaction was monitored UV-spectrophotometrically (wavelength scans from 380-200 nm).

9-[2-Deoxy-5-*O*-*tert*-butyldimethylsilyl-ß-D-*erythro*-pentofuranosyl]- 1,9-dihydro-2H-purin-2-one (7). To a suspension of **2a** (1.0 g, 4.0 mmol) [4] dried by coevaporation with dry pyridine (3 x 10 ml), and dry pyridine (40 ml), 1.46 g of TBDMS-Cl (1.46 g, 9.7 mmol) was added. The reaction mixture was stirred at r.t. for 12 h, MeOH (10 ml) was added, and the stirring was continued for 30 min. The mixture was evaporated to an oil, then 5% aq. $NaHCO_3$ (100 ml) was introduced, and the solution was extracted with CH_2Cl_2 (3 x 80ml). The organic layer was dried with

anhydrous Na_2SO_4, and then evaporated. The residue was supplied to FC (4 x 15 cm, CH_2Cl_2/MeOH 95:5). White powder (1.2 g, 82%): TLC (CH_2Cl_2/MeOH 9:1): R_f [CY1]0.51; ^1H NMR (DMSO-d_6): δ 0.00 (s, 6H, $2SiCH_3$), 0.83 (s, 9H, $3CCH_3$), 2.26, 2.58 (2m, 2H, H-(C2')), 3.69, 3.76 (2m, 2H, H-(C5')), 3.81 (m, 1H, H-(C4')), 4.34 (br, 1H, H-(C3')), 5.35 (m, 1H, OH-(C3')), 6.14 (t, J = 6.8 Hz, 1H, H-(C1')), 8.31 (s, 1H, H-(C8)), 8.44 (s, 1H, H-(C6)), 11.96 (br, 1H HN); Anal. calcd. for $C_{16}H_{26}N_4O_4Si$ (366.5): C 52.44, H 7.15, N 15.29. Found: C 52.52, H 7.23, N 15.21.

9-[2-Deoxy-5-*O*-*tert*-butyldimethylsilyl-3-*O*-methylsulfonyl-ß-D-*erythro*-pentofuranosyl]-2-*O*- methylsulfonyl -9H-purine (8). To a solution of **7** (500 mg, 1.36 mmol) and dry pyridine (5 ml) in CH_2Cl_2 (20 ml), methylsulfonyl chloride (1.0 ml, 13.5 mmol) was added dropwise, and the solution was stirred at r.t. for 5 h. The mixture was then poured into 5% aq. $NaHCO_3$ (80 ml) and extracted with CH_2Cl_2 (3 x 40 ml). The combined organic layers were dried with anhydrous Na_2SO_4, evaporated, and submitted to FC (silica gel, column 3 x 7 cm, CH_2Cl_2/MeOH 98:2); colorless solid (520 mg, 73%): TLC (CH_2Cl_2/MeOH 95:5): R_f 0.60; UV(MeOH): λ_{max} 268 nm (ε = 7800); ^1H NMR (DMSO-d_6): δ 0.02 (s, 6H, $2SiCH_3$), 0.80 (s, 9H, $3CCH_3$), 2.83, 3.15 (2m, 2H, H-(C2')), 3.84, 3.88 (2m, 2H, H-(C5')), 3.31, 3.73 (2s, 6H, $2CH_3S$), 4.29 (br, 1H, H-(C4')), 5.41 (br, 1H, H-(C3')), 6.49 (t, J = 6.4 Hz, 1H, H-(C1')), 8.83, 9.20 (2s, 2H, H-(C6), H-(C8)); Anal. calc. for $C_{18}H_{30}N_4O_8S_2Si$ (522.7): C 41.36, H 5.79, N 10.72, S 12.27. Found: C 41.42, H 5.70, N 10.92, S 12.35.

9-[2,3-Didehydro-2,3-dideoxy-ß-D-*erythro*-pentofuranosyl]-1,9-dihydro-2H-purin-2-one (4). To a solution of **8** (100 mg, 0.19 mmol) in THF (3 ml), 1.1 M Bu_4NF/THF (2.0 ml) was added at 50°C with stirring. After 5h, the mixture was evaporated to an oil, and applied to FC (silica gel, collum 2 x 10 cm, CH_2Cl_2 / MeOH 95:5). Crystallization from MeOH gave a light yellow solid (25 mg, 56%): mp 170-172°C (dec.); TLC (CH_2Cl_2/MeOH 3:1): R_f 0.57; UV (H_2O): λ_{max} 314, 241, 218 nm (ε = 4700, 3000, 21700); ^1H NMR (DMSO-d_6): δ 3.58 (m, 2H, H-(C5')), 4.85 (br, 1H, H-(C4')), 6.06 (d, J = 5.9 Hz, 1H, H-(C2')), 6.42 (d, J = 5.9 Hz, 1H, H-(C3')), 6.74 (br, 1H, H-(C1')), 8.04 (s, 1H, H-(C8)), 8.45 (s, 1H, H-(C6)); Anal. calcd. for $C_{10}H_{10}N_4O_3$ (234.2): C 51.28, H 4.30. Found: C 51.40, H 4.48.

7-[2-Deoxy-5-*O*-*tert*-butyldiphenylsilyl)-ß-D-*erythro*-pentofuranosyl]-2-methoxy-7H-pyrrolo[2,3-d]pyrimidine (10). As described for **7**, with **9** (520 mg, 2.0 mmol), [4] TBDPS-Cl (0.6 ml, 2.3 mmol) and dry pyridine (15 ml). FC (3 × 10 cm, CH_2Cl_2/MeOH 98:2) and white powder (900 mg, 89%): TLC (CH_2Cl_2/MeOH 95:5): R_f 0.57. ^{1}H NMR (DMSO-d_6): δ 1.00 (s, 9H, 3 CCH_3), 2.30, 2.61 (2m, 2H, H-(C2')), 3.76, 3.85 (2m, 2H, H-(C5')), 3.90 (s, 3H, OCH_3), 3.95 (m, 1H, H-(C4')), 4.51 (br, 1H, H-(C3')), 5.40 (br, 1H, OH-(C3')), 6.55 (m, 2H, H-(C1'), H-(C5)), 7.3-7.6 (H-(C6) and aromatic H), 8.76 (s, 1H, H-(C4)).

7-[2-Deoxy-5-*O*-*tert*-butyldiphenylsilyl-3-*O*- methylsulfonyl -ß-D-*erythro*-pentofuranosyl]-2-methoxy-7H-pyrrolo[2,3-d]pyrimidine (11). According to the preparation of **8** using **10** (850 mg, 1.69 mmol), methansulfonyl chloride (0.7 ml, 9.0 mmol), pyridine (6 ml) and CH_2Cl_2 (25 ml). FC (3 × 12 cm, CH_2Cl_2 / MeOH 98:2) and colorless foam (810 mg, 82%): TLC (CH_2Cl_2/MeOH 95:5%): R_f 0.60. ^{1}H NMR (DMSO-d_6): δ 1.00 (s, 9H, 3 CCH_3), 2.73, 3.08 (2m, 2H, H-(C2')), 3.81, 3.92 (2m, 2H, H-(C5')), 3.90 (s, 3H, OCH_3), 4.30 (m, 1H, H-(C4')), 5.54 (m, 1H, H-(C3')), 6.55 (m, 2H, H-(C1'), H-(C5)), 7.3-7.6 (m, H-(C6) and aromatic H), 8.78 (s, 1H, H-(C4)).

7-[2,3-Didehydro-2,3-dideoxy-ß-D-*erythro*-pentofuranosyl]-2-methoxy-7H-pyrrolo[2,3-d]pyrimidine (12). Analogously to compound **4**, with **11** (440 mg, 0.76 mmol), 1.1 M Bu_4NF / THF (3.0 ml) and THF (4.0 ml). FC (4 × 15 cm, CH_2Cl_2/MeOH 95:5) yields colorless crystals from MeOH (180 mg, 96%): mp 168-169°C; TLC (CH_2Cl_2/MeOH 9:1): R_f 0.70; ^{1}H NMR (DMSO-d_6): δ 3.57 (m, 2H, H-(C5')), 3.95 (s, 3H, OCH_3), 4.85 (br, 1H, H-(C4')), 4.89 (t, J = 5.3 Hz, 1H, OH-(C5')), 6.08 (d, J = 5.5 Hz, 1H, H-(C2')), 6.48 (d, J = 5.7 Hz, 1H, H-(C3')), 6.56 (d, J = 3.5 Hz, 1H, H-(C5)), 7.18 (br, 1H, H-(C1')), 7.42 (d, J = 3.5 Hz, 1H, H-(C6)), 8.78 (s, 1H, H-(C4)); Anal. calcd. for $C_{12}H_{13}N_3O_3$ (247.3): C 58.29, H 5.30, N 17.00. Found: C 58.38, H 5.27, N 16.90.

7-[2,3-Didehydro-2,3-dideoxy-ß-D-*erythro*-pentofuranosyl]-3,7-dihydro-2H-pyrrolo[2,3-d]pyrimidine-2-one (5). A solution of **12** (100 mg, 0.4 mmol) in 2N NaOH (4 ml) and DMSO (50 µl) was heated under reflux for 5 h. The solution was cooled and neutralized with AcOH. The solution was kept in a refrigerator forming colorless needles (76 mg, 81%): mp 217-219°C; TLC (CH_2Cl_2/MeOH 4:1): R_f 0.54; ^{1}H

NMR (DMSO-d_6): δ 3.56 (m, 2H, H-(C5')), 4.81 (br, 1H, H-(C4')), 4.91 (t, J = 5.3 Hz, 1H, OH-(C5')), 6.00 (d, J = 5.7 Hz, 1H, H-(C2')), 6.29 (d, J = 4.0 Hz, 1H, H-(C5)), 6.43 (d, J = 5.4 Hz, 1H, H-(C3')), 6.94 (d, J = 7.0 Hz, 1H, H-(C1')), 7.19 (d, J = 4.0 Hz, 1H, H-(C6)), 8,21 (s, 1H, H-(C4)), 11.66 (s, 1H, NH). Anal. calc. for $C_{11}H_{11}N_3O_3$ (233.2): C 56.65, H 4.75, N 18.02. Found: C 56.50, H 4.94, N 17.84.

2'-Deoxyxanthosine (3). To a solution of compound **2a** (10 mg, 0.04 mmol) in water (10 ml), xanthine oxidase (EC 1.1.3.22, Boehringer Mannheim, 10 units) was added at r.t. with stirring. The stirring was continued for 10 h at r.t. The reaction mixture was diluted with water (20 ml) and supplied to a *Serdolit AR-4* column (200-400 mesh, 3 x 8 cm). The salts were removed by elution with water. The material of the main zone was eluted with H_2O/ i-PrOH 95:5 and isolated by evaporation. Colorless powder (8.0 mg, 75%): UV (MeOH): λ_{max} 276, 247 nm. ^{1}H NMR (DMSO-d_6): δ 2.16 (m, 1H, H-(C2')), 3.58 (m, 2H, H-(C5')), 3.79 (m, 1H, H-(C4')), 4.35 (m, 1H, H-(C3')), 6.14 (t, J = 6.3 Hz, 1H, H-(C1')), 7.74 (s, 1H, H-(C8)).

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REFERENCES

1. Martin, F.H.; Castro, M.M.; Aboul-ela, F.; Tinoco Jr., I. *Nucleic Acids Res.* **1985**, ***13***, 8927-8838.
2. Ohtsuka, E.; Matsuki, S.; Ikehara, M.; Takahashi, Y.; Matsubara, K. *J. Biol. Chem.* **1985, *260***, 2605-2608.
3. Kawase, Y.; Iwai, S.; Inoue, H.; Miura, K.; Ohtsuka, E. *Nucleic Acids Res.* **1986, *14***, 7727-7736.
4. Seela, F.; Chen, Y.; Bindig, U.; Kazimierczuk, Z. *Helv. Chim. Acta* **1994, *77***, 194-202.
5. Seela, F.; Chen, Y. *Nucleosides Nucleotides* **1995, *14***, 863-866.
6. Seela, F.; Chen, Y. *Nucleic Acids Res.* **1995, *23***, 2499-2505.
7. Mitsuya, H.; Yarchoan, R.; Broder, S. *Science* **1990, *249***, 1533-1544.
8. Huryn, D.M.; Okabe, M. *Chem. Rev.* **1992, *92***, 1745-1768.
9. Herdewijn, P.; Balzarini, J.; Baba, M.; Pauwels, R; van Aerschot, A.; Janssen, G.; De Clercq, E.; *J. Med. Chem.* **1988, *31***, 2040-2048.
10. Chu, C.K.; Bhadti, V.S.; Doboszewski, B.; Gu, Z.P.; Kosugi, Y.; Pullaiah, K.C.; Van Roey, P. *J. Org. Chem.* **1989, *54***, 2217-2225.

11. Rosemeyer, H.; Seela, F. *Helv. Chim. Acta* **1989**, *72*, 1084-1094.
12. Bergmann, F.; Kwietny, H.; Levin, G.; Brown, D.J. *J. Am. Chem. Soc.* **1960**, *82*, 598-605.
13. Krenitsky, T.A.; Neil, S.M.; Elion, G.B.; Hitchings, G.H. *Arch. Biochem. Biophys.* **1972**, *150*, 585-599.
14. Krenitsky, T.A.; Spector, T.; Hall, W.W. *Arch. Biochem. Biophys.* **1986**, *247*, 108-119.
15. Krenitsky, T.A.; Hall, W.W.; De Miranda, P.; Beauchamp, L.M.; Schaeffer, H.J.; Whiteman, P.D. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 3209-3213.
16. Moschel, R.C.; Keefer, L.K. *Tetrahedron Lett.* **1989**, *30*, 1467-1468.
17. Schulz, B.S.; Pfleiderer, W. *Helv. Chim. Acta* **1987**, *70*, 210-218.
18. Rosemeyer, H.; Seela, F. *Eur. J. Biochem.* **1983**, *134*, 513-515.
19. Seela, F.; Bussmann, W.; Götze, A.; Rosemeyer, H. *J. Med. Chem.* **1984**, *27*, 981-985.
20. Rosemeyer, H.; Kaiser, K.; Seela, F. *Int. J. Biol. Macromol.* **1987**, *9*, 205-210.
21. Massey, V.; Komai, H.; Palmer, G.; Elion, G.B. *J. Biol. Chem.* **1970**, *245*, 2837-2844.
22. Stefano, C.M.D.S.; Magri, E. *Enzymologia* **1972**, *43*, 423-429.
23. Holguin-Hueso, J.; Cardinaud, R. *FEBS Lett.* **1972**, *20*, 171-173.
24. Scott, T.G.; Spencer, R.D.; Leonard, N.J.; Weber, G. *J. Am. Chem. Soc.* **1970**, *92*, 687-695.