# Chemo-Enzymatic Synthesis of 3-Deoxy-β-D-ribofuranosyl Purines

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Dedicated to Prof. Dr. *Wolfgang Pfleiderer* on the occasion of his 75th birthday

9-(3-Deoxy-β-D-*erythro*-pentofuranosyl)-2,6-diaminopurine (**6**) was synthesized by an enzymatic transglycosylation of 2,6-diaminopurine (**2**) with 3′-deoxycytidine (**1**) as a donor of 3-deoxy-D-*erythro*-pentofuranose moiety. This transformation comprises *i*) deamination of **1** to 3′-deoxyuridine (**3**) under the action of whole cell (*E. coli* BM-11) cytidine deaminase (CDase), *ii*) the phosphorolytic cleavage of **3** by uridine phosphorylase (UPase) giving rise to the formation of uracil (**4**) and 3-deoxy-α-D-*erythro*-pentofuranose-1-*O*-phosphate (**5**), and *iii*) coupling of the latter with **2** catalyzed by whole cell (*E. coli* BMT-4D/1A) purine nucleoside phosphorylase (PNPase). Deamination of **6** by adenosine deaminase (ADase) gave 3′-deoxyguanosine (**7**). Treatment of **6** with $NaNO_2$ afforded 9-(3-deoxy-β-D-*erythro*-pentofuranosyl)-2-amino-6-oxopurine (3′-deoxyisoguanosine; **8**). *Schiemann* reaction of **6** ($HF/HBF_4 + NaNO_2$) gave 9-(3-deoxy-β-D-*erythro*-pentofuranosyl)-2-fluoroadenine (**9**).

**Introduction.** – The pentofuranosyl nucleosides of 2,6-diaminopurine are valuable precursors for the preparation of a broad range of base (guanine, isoguanine, 2-haloadenines *etc.*) and sugar-modified analogues of natural nucleosides. Among these analogues, some are recognized as very effective agents against lymphoproliferative disorders and hematologic malignances [1][2] (*e.g.*, 2-chloro-2′-deoxyadenosine (*cladribine*), 9-(β-D-arabinofuranosyl)-2-fluoroadenine (*fludarabine*), 9-(β-D-arabinofuranosyl)guanine (ara-G)), the others display highly promising antiviral activities (*e.g.*, 2′-deoxy-2′-fluoroguanosine [3][4]), and many of them are rather valuable tools for molecular biological studies (*cf.*, *e.g.*, [5–7]).

Chemical syntheses of 2,6-diaminopurine nucleosides are based on a convergent approach (for a comprehensive review, see [8]), and the transformations of purine bases of natural nucleosides [9–11] are multistep processes and rather laborious. On the contrary, enzymatic syntheses of purine nucleosides with pyrimidine nucleosides as donors of the carbohydrate moiety in the reaction of an enzymatic transglycosylation of purine bases have been shown to represent an expedient alternative to chemical methods. Thus, we have earlier shown that a combination of chemical and enzyme-catalyzed procedures is very efficient in the synthesis of ara-G [12]. This approach offers obvious advantages over chemical procedures and consists in the concerted biochemical reactions catalyzed by cytidine deaminase (CDase), uridine phosphorylase (UPase), and purine nucleoside phosphorylase (PNPase). The readily available

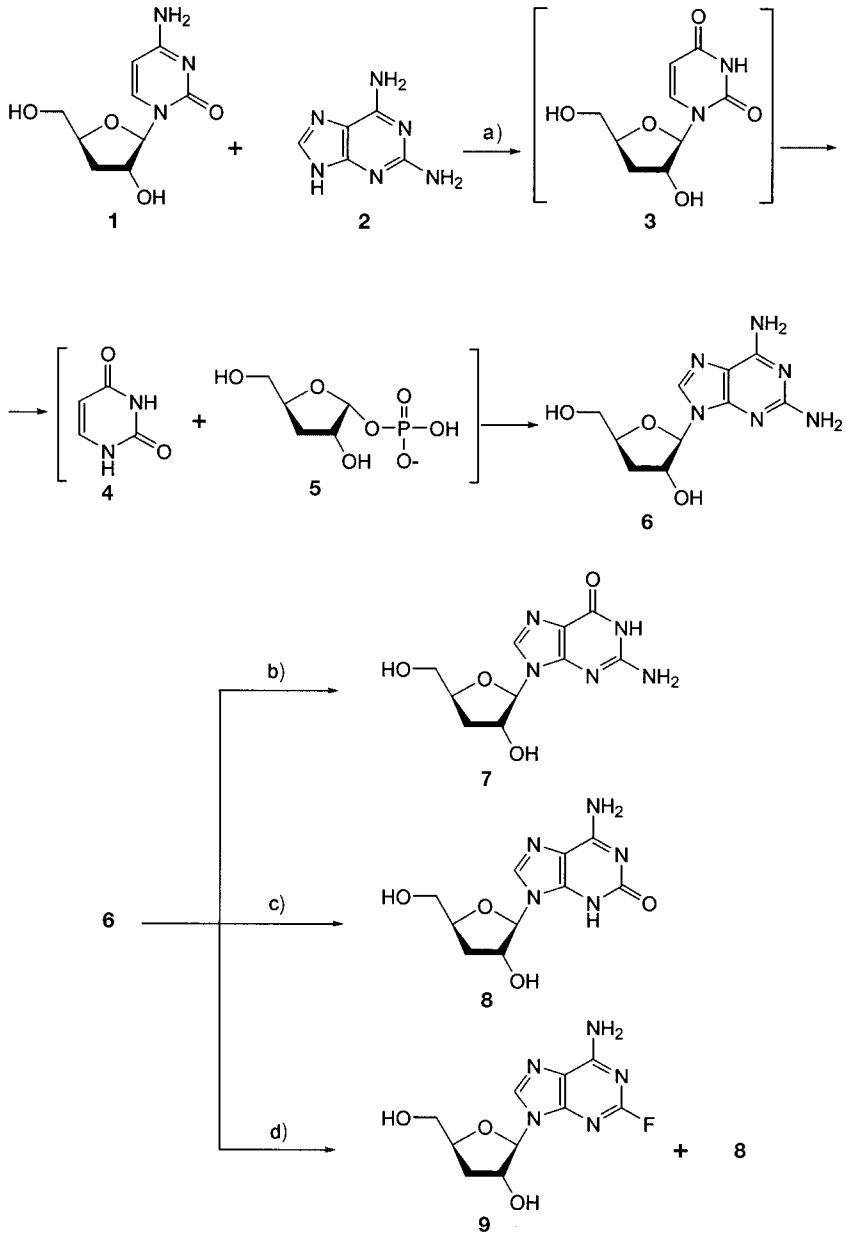
9-(β-D-arabinofuranosyl)cytosine (ara-C) [13] was employed as a donor of the β-D-arabinofuranose moiety and guanine as an acceptor. The ara-C is not a substrate for UPase and cannot be phosphorolyzed to arabinofuranose-1-*O*-phosphate (ara-1-α-P) and cytosine. To conduct an enzymatic transfer of the β-D-arabinofuranose moiety from ara-C to the purine base, the former should be first deaminated to 9-(β-D-arabinofuranosyl)uracil (ara-U). Therefore, the *Escherichia coli* BM-11 cells containing highly active CDase along with UPase and PNPase were selected. The CDase converted ara-C to ara-U. The latter, in the presence of inorganic phosphate, is subjected to phosphorolysis to give uracil and ara-1-α-P under the action of UPase. Guanosine (Guo) or 2′-deoxyguanosine (2′d-Guo) were employed as a soluble source of guanine. The latter was formed *in situ* under the action of PNPase and subsequently coupled stereo- and regioselectively with the ara-1-α-P to afford ara-G.

Recently, we have selected a new strain of *E. coli* BMT-4D/1A that was found to be more efficient for an enzymatic synthesis of purine nucleosides with pyrimidine nucleosides as donors of the carbohydrate moiety in the reaction of an enzymatic transglycosylation of purine bases [4]. We now report a convenient preparative-scale synthesis of 9-(3-deoxy-β-D-*erythro*-pentofuranosyl)-2,6-diaminopurine (**6**) and describe its use for the preparation of related nucleosides. Note that such compounds are of interest from a viewpoint of their biological activity and as constituent elements of oligonucleotides with 2′,5′-phosphodiester linkages [7][14][15].

**Results and Discussion.** – *Chemo-Enzymatic Transformations.* We initially studied the synthesis of nucleoside **6** from 1-(3-deoxy-β-D-*erythro*-pentofuranosyl)cytosine (3′-deoxycytidine; **1**; 3′d-Cyd) [16][17] and 2,6-diaminopurine (**2**) catalyzed by the intact cells of *E. coli* BMT-4D/1A under the experimental conditions optimized earlier for the preparation of 9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-2,6-diaminopurine [4]. Incubation of **1** and **2** (molar ratio 1.5 : 1.0) in the presence of the intact *E. coli* BMT-4D/1A cells in 60 mM K-phosphate buffer (pH 7.0) at 52° resulted in the slow deamination of 3′d-Cyd (**1**) and the formation of the desired **6**. An addition to the reaction mixture of the intact cells of *E. coli* BM-11 [18][19], which display much higher CDase activity, led to an acceleration of deamination of **1** and, as a consequence, to the formation of nucleoside **6**. The pathways for an enzymatic synthesis of nucleoside **6** from **1** and **2** are illustrated in the *Scheme*. An intermediary formation of 3′-deoxyuridine (**3**) and uracil (**4**) was observed during the TLC monitoring of the reaction course. Under optimized conditions (incubation of the substrates at 52° for 26 h), the yield of nucleoside **6** attained 72% relative to the initial amount of base **2**. The reaction was terminated by the boiling of the reaction mixture for 3 min, the cells were removed by centrifugation and washed with hot water with subsequent withdrawal of the cells by centrifugation. The combined supernatant and washings were evaporated, and the residue was chromatographed on the silica gel to afford the desired **6** in 64% yield. The enzymatic regio- and stereospecific glycosylation of purine **2** offers obvious advantages over the related chemical glycosylation of the 2,6-diaminopurine derivatives (*e.g.*, [20][21]).

Treatment of the supernatant, which was obtained after termination of the synthesis of nucleoside **6** and removal of the cells by centrifugation, with ADase gave rise to the crystallization of 3′-deoxyguanosine (**7**; 3′d-Guo) that was obtained in 57% yield. Silica-gel column chromatography of the residue afforded an additional quantity of

*Scheme*




a) **1**/**2** (molar ratio 1.5 : 1.0), the intact *E. coli* BMT-4D/1A and BM-11 cells, K-phosphate buffer (60 mM; pH 7.0), 52°, 26 h (**6**, 64%). b) ADase, r.t., 16 h (a + b, **7**, 68%; from **6**, **7**, 85%). c) **6**/$NaNO_2$ (molar ratio 1.0 : 4.5), AcOH, 50°, 6 min (71%). d) HF/HBF 4/$H_2O$/THF, $NaNO_2$, −10–12°, 1 h (**9**, 43%; **8**, 7%).

3′d-Guo, the combined yield of which amounted to 68%. Again, comparison of the enzymatic preparation of **7** as described here with recently published chemical methods [7] [14] is unambiguously in favor of the former. Deamination of pure nucleoside **6** with ADase afforded **7** in 85% isolated yield.

Chemical deamination of nucleoside **6** with $NaNO_2$ in an $AcOH/H_2O$ mixture gave, after ion-exchange chromatograpy on *Dowex 50W* × *4*, 3′-deoxyisoguanosine (**8**; 3′d-*i*Guo) in 71% yield. *Schiemann* reaction of nucleoside **6** (aq. $HF/HBF_4$ + aq. $NaNO_2$) conducted under slightly modified (*vs.* previously described [10][22]) conditions gave 9-(3-deoxy-β-D-*erythro*-pentofuranosyl)-2-fluoroadenine (**9**; 3′d-2FAdo) and **8** in 43 and 7% yield, respectively.

*Spectroscopic Studies.* All compounds synthesized were characterized by $^1$H-NMR (*Tables 1* and *2*), as well as by UV and CD (*Fig.*) spectroscopy. The values of the coupling constants of the pentofuranose ring of nucleosides **6**–**9** clearly point to the predominant population of the (*N*)-conformer similar to that of 3′-deoxyadenosine [23]. This is in contrast to the β-D-ribonucleosides, for which an equal population of (*N*)- and (*S*)-conformers was observed (for a comprehensive review, see [24]).

The CD spectrum of nucleoside **6** was similar in shape to that of 2,6-diamino-9-(β-D-ribofuranosyl)purine (DAP-Rib) [25] in the 230–300-nm region, but did not show a positive *Cotton* effect at *ca.* 220 nm as opposed to the latter. Minor molar-ellipticity changes of the $B_{2u}$ and $B_{1u}$ CD bands *vs.* DAP-Rib, as well as the absence of the $E_{1u}$ CD band and slight batochromic shifts of both negative *Cotton* effects can be attributed to the conformational changes about the glycosidic bond induced by the changes of the $(S) \rightleftharpoons (N)$ equilibrium of a pentofuranose ring [25][26]. It is noteworthy that the

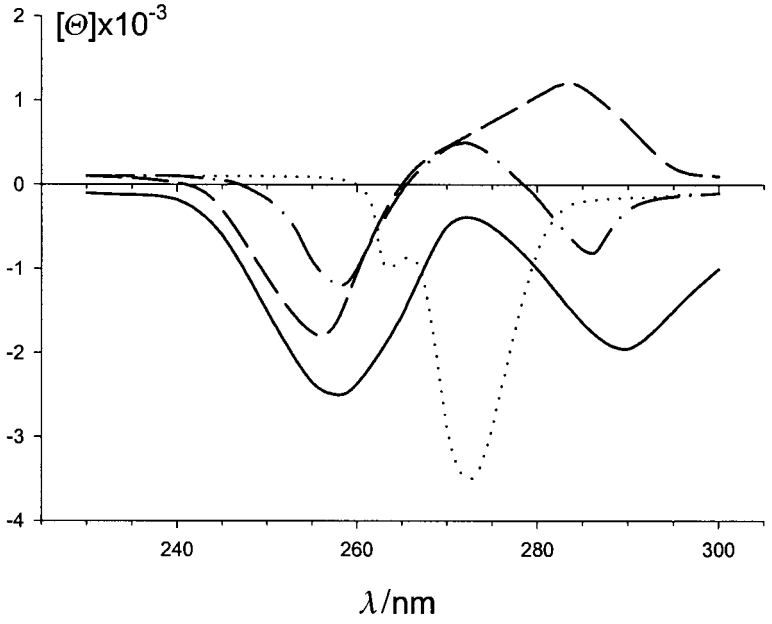



Figure. *The CD spectra of compounds* **6** (——), **7** (– · – · – · ), **8** (– – –), *and* **9** (· ·· ) *in* $H_2O$ *solutions*

Table 1. *$^1$H-NMR Chemical Shifts* ((D$_6$)DMSO) *of 9-(3-Deoxy-β-*D*-*erythro*-pentofuranosyl)purines* ($\delta$(H) in ppm)

| | H–C(1′) | H–C(2′) | H–C(3′) | H–C(3″) | H–C(4′) | H–C(5′) | H–C(5″) | Others |
|---|---|---|---|---|---|---|---|---|
| **6** | 5.70 (*d*) | 4.50 (br. *s*) | 2.24 (*m*) | 1.90 (*m*) | 4.30 (*m*) | 3.64 (*dd*) | 3.50 (*dd*) | 7.94 (*s*, H–C(8)); 6.76, 5.78 (2 br. *s*, NH$_2$–C(2), NH$_2$–C(6)). 5.56 (br. *d*, $^3J$(H–C(2′), OH–C(2′)) = 3.75; OH–C(2′)); 5.18 (br. *s*, OH–C(5′)) |
| **7** | 5.70 (*d*) | 4.46 (br. *m*) | 2.22 (*ddd*) | 1.90 (*ddd*) | 4.30 (*m*) | *ca.* 3.50[a]) | *ca.* 3.50[a]) | 10.76 (br. *s*, NH); 7.96 (*s*, H–C(8)); 6.52 (br. *s*, NH$_2$); 5.81 (*d*, $^3J$(H–C(2′), OH–C(2′)) = 4.0, OH–C(2′)); 5.20 (*t*, $^3J$(H–C(5′)), OH–C(5′) = $^3J$(H–C(5″), OH–C(5″)) = 5.0, OH–C(5′)) |
| **8** | 5.64 (br. *s*) | 4.50 (br. *s*) | 2.18 (*m*) | 1.90 (*m*) | 4.30 (br. *m*) | 3.64 (br. *m*) | 3.42 (br. *m*) | 8.00 (*s*, H–C(8)); 6.52 (br. *s*, NH$_2$); 5.64 (br. *s*, OH–C(2′), OH–C(5′)) |
| **9** | 5.78 (br. *s*) | 4.54 (br. *s*) | 2.22 (*ddd*) | 1.90 (*ddd*) | 4.34 (*m*) | *ca.* 3.70 (br. *m*) | *ca.* 3.50 (br. *m*) | 8.38 (*s*, H–C(8)); 7.84 (br. *s*, NH$_2$); 5.74 (*d*, $^3J$(H–C(2′), OH–C(2′) = 4.0, OH–C(2′)); 5.08 (*t*, $^3J$(H–C(5′, OH–C(5′)) = $^3J$(H–C(5″), OH–C(5″)) = 5.25, OH–C(5′)) |

[a]) The values were not determined owing to overlap of the resonances of H–C(5′) and H–C(5″) by an intense HOD line.

Table 2. *Coupling Constants $^3$J(H,H) of 9-(3-Deoxy-β-*D*-*erythro*-pentofuranosyl)purines* (*J* in Hz)

| | *J*(1′,2′) | *J*(2′,3′) | *J*(2′,3″) | *J*(3′,4′) | *J*(3″,4′) | *J*(4′,5′) | *J*(4′,5″) | *J*(5′,5″) | Others |
|---|---|---|---|---|---|---|---|---|---|
| **6** | 1.8 | 6.5 | 3.0 | 7.5 | 6.25 | 2.5 | 3.75 | 12.5 | $^2J$(3′,3″) = 13.0 |
| **7** | 2.0 | 5.5 | 2.75 | 9.0 | 6.0 | 20.5 | n.d. | n.d. | $^2J$(3′,3″) = 13.0 |
| **8** | n.d. | *ca.* 6.0 | *ca.* 3.0 | *ca.* 8.0 | *ca.* 6.0 | n.d. | n.d. | *ca.* 13.0 | |
| **9** | *ca.* 1.2 | 5.5 | 2.5 | 9.0 | 6.0 | n.d. | n.d. | n.d. | $^2J$(3′,3″) = 13.0 |

theoretical considerations also revealed a strong dependence of the rotational strength on base conformation about the glycosidic bond and changes in the puckered conformations of the ribofuranose residue [27].

A comprehensive investigation of the CD spectra of guanine nucleosides revealed that the *Cotton* effects are controlled by many factors, *viz*., the *syn*/*anti* base orientation, the (*S*) ⇌ (*N*) equilibrium of the pentofuranose ring, and display a rather broad diversity [25][26]. The CD spectrum of 3′d-Guo (**7**) resembles that of *i*) 2′d-Guo in trifluoroethanol (positive *Cotton* effect at *ca*. 275 nm ($B_{2u}$), and negative at *ca.* 248 nm ($B_{1u}$)), and *ii*) 2′,3′-*O*-isopropylidene-$N^3$,5′-anhydroguanosine at pH 7.0 (the respective positive and negative *Cotton* effects at 263 nm ($B_{2u}$) and 242 nm ($B_{1u}$)); the CD spectrum of 3′d-Guo (**7**) displayed, however, no *Cotton* effect in the 210–230-nm region, and showed a small negative *Cotton* effect at 287 nm.

The CD spectrum of 3′d-*i*Guo (**8**) is similar in shape to that of 2′,3′-*O*-isopropylidene-$N^3$,5′-anhydroxantosine [25], displaying the correspondence of the CD bands with the UV absorption bands; again, no *Cotton* effect(s) was observed in the 210–230-nm region.

The UV polarized absorption studies led to the conclusion that the 260-nm band contains both the $B_{2u}$ and $B_{1u}$ transitions and an n → $\pi^*$ transition as well [28]. Two $\pi \rightarrow \pi^*$ transitions exhibited by the adenine chromophore in the 260-nm region have an

intensity ratio of *ca.* 10:1 [28], and the negative *Cotton* effect of adenosine at 261 nm [29] was assigned to the $B_{2u}$ transition. Taken these data into account, the large negative CD band at 272.5 nm in the CD spectrum of 3′d-2FAdo (**9**) can be assigned to an $B_{2u}$ transition, whereas the small negative *Cotton* effect at *ca.* 263 nm to the $B_{1u}$ transition. The electronegative F-atom causes, most probably, the resolution of the $B_{2u}$ and $B_{1u}$ bands in the CD spectrum.

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## Experimental Part

1. *General*. Column chromatography (CC): silica gel *60 H* (70–230 mesh ASTM; *Merck*, Germany). TLC: *Silufol UV$_{254}$* (*Serva*, Germany); solvent systems for TLC (*v*/*v*): $CHCl_3$/MeOH/$H_2O$, 30:10:1 (*A*); $CHCl_3$/MeOH/$H_2O$, 10:6:1 (*B*). Except when otherwise indicated, the reactions were carried out at 20°. M.p.: *Boetius* apparatus (Germany); uncorrected. UV Spectra were measured with a *Specord M-400* spectrometer (*Carl Zeiss*, Germany). CD Spectra and the $[\alpha]_D^{20}$ values were obtained on a *J-20* (*JASCO*, Japan) spectropolarimeter. $^1$H- and $^{13}$C-NMR spectra were measured at 200.13 and 50.325 MHz, resp., at 23° on an *AC-200* spectrometer, equipped with an *Aspect 3000* data system (*Bruker*, Germany); $\delta$ values are in ppm downfield from internal $Me_4Si$ ($^1$H, $^{13}$C); coupling constants *J* are given in Hz; assignments of proton resonances were confirmed, when possible, by selective homonuclear decoupling experiments; the solvent employed for recording the NMR spectra was ($D_6$)DMSO, unless otherwise stated. Adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) from calf intestinal mucosa, specific activity 161 units/mg protein, type VI, was purchased from *Sigma*. All new crystalline compounds gave satisfactory microanalyses, C ±0.38, H ±0.40.

2. *Microorganisms. E. coli* BM-11 and *E. coli* BMT-4D/1A cells were used as the sources of CDase and nucleoside phosphorylases, resp. The genetically uncharacterized *E. coli* BM-11 strain was selected as reported in [18]. The cells of *E. coli* BMT-4D/1A were selected according to the technique offered by *Munch-Petersen et al.* [30]. They carry two regulatory mutations (cytR and deoR) and are able to synthesize constitutively nucleoside-catabolizing enzymes.

The biomasses of *E. coli* BM-11 cells possessing high CDase activity and of *E. coli* BMT-4D/1A cells possessing high UPase and PNPase activities were prepared as described in [12].

3. *9-(3-Deoxy-β-*D*-*erythro*-pentofuranosyl)-2,6-diaminopurine* (**6**). The reaction mixture (318 ml) containing *3′-deoxycytidine* (**1**; 6.50 g, 28.61 mmol; 90 mM concentration), *2,6-diaminopurine* (**2**; 2.865 g, 19.08 mmol; 60 mM concentration), K-phosphate buffer (60 mM; pH 7.0), and wet pastes of the intact *E. coli* BMT-4D/1A and BM-11 cells (0.4 and 0.6%, resp.; calculated as abs. dry weight) was incubated at 52° for 26 h with gentle stirring. The formation of the products was monitored by TLC on *Silufol UV$_{254}$* plates. The solvent used was distilled $H_2O$ cooled to 4°. Products were eluted from the plates with 5 mM K-phosphate buffer (pH 7.0) and quantified spectrophotometrically with a molar extinction coefficient, $\varepsilon$, of 9560 at 255 nm. Under these conditions, the yield of 2,6-diamino-3′-deoxypurine riboside attained 72% relative to the initial 2,6-diaminopurine amount. The reaction was terminated by boiling the reaction mixture for 3 min, then the mixture was allowed to cool to 40°, the cells were removed by centrifugation (10000 × g, 5 min) and washed with hot $H_2O$ (50–60°; 30 ml) with subsequent withdrawal of the cells by centrifugation. Two supernatants were combined and evaporated, and CC (silica gel (4.5 × 50 cm); AcOEt/MeOH/$H_2O$ first 100:10:1 (*v*/*v*, 2.5 l) and then 100:20:1 (*v*/*v*, 2.5 l)) gave **6** (3.26 g; 64% based on **2**). M.p. 126–128° (from MeOH). $[\alpha]_D^{15} = -57.6$ (c = 0.05 g/l, $H_2O$). TLC (*A*): $R_f$ 0.39. UV ($H_2O$): $\lambda_{max}$ 255 (9560), 279 (9620); $\lambda_{min}$ 236 (5700), 265 (8090). CD ($H_2O$; $\lambda$ [nm] ($[\Theta] \times 10^{-3}$)): 258.0 (−2.5), 290.0 (−2.0).

4. *3′-Deoxyguanosine* (**7**). *a*) To the combined supernatants, obtained similarly as described above, starting from 15.9 ml of the reaction mixture containing **1** (325 mg, 1.43 mmol) and **2** (143 mg, 0.95 mmol), ADase (2.5 units) was added, and the mixture was incubated for 16 h at r.t. The progress of the reaction was monitored by TLC on *Silufol UV$_{254}$* (*A*): $R_f$ 0.39 for **6** and $R_f$ 0.21 for **7**. The precipitate of **7** formed in the course of the reaction was filtered off, washed with cold $H_2O$ and then with EtOH, collected, and dried at 50° overnight to give **7** (144 mg, 57% based on **2**). Combined filtrate and washings were evaporated, and CC (silica gel

($1.5 \times 50$ cm); $CHCl_3$/MeOH 20:1 (*v*/*v*, 0.5 l) and then of 10:1 (0.5 l)) gave 30 mg of **7** (combined yield 68%). M.p. 237–239° (from MeOH/$H_2O$) ([31]): m.p. 240°; [32]: m.p. >300°). TLC (*A*): $R_f$ 0.21. $[\alpha]_D^{15} = -45.3$ ($c = 0.05$ g/l, $H_2O$). UV ($H_2O$): $\lambda_{max}$ 252 (11730), 274 (sh, 8150); $\lambda_{min}$ 224 (3090). CD ($H_2O$; $\lambda$ [nm] ($[\Theta]) \times 10^{-3}$)): 258.0 (−1.2), 272.0 (+0.5), 280.0 (−0.8), 247.0, 265.5, 278.5, and 300.0 (0).

*b*) Deamination of **6** (49 mg, 1.84 mmol) with ADase (0.5 units) in K-phosphate buffer (50 mM; pH 7.4; 3.0 ml) overnight resulted in precipitation of **7** that was filtered off, washed with cold $H_2O$ ($2 \times 2.0$ ml), cold EtOH (2 ml), and dried to give 34 mg (69%) of the product, which was identical (TLC, m.p., UV) with the sample from the aforementioned experiment. Combined filtrate and washings were evaporated, CC (silica gel ($1.5 \times 15$ cm); $CHCl_3$/MeOH 10:1 (*v*/*v*, 0.4 l)) gave additional 8 mg of **7** (combined yield 42 mg, 85%).

5. *6-Amino-9-(3-deoxy-β-*D*-*erythro*-pentofuranosyl)oxopurine* (**8**). To a soln. of **6** (0.10 g, 0.376 mmol) and $NaNO_2$ (0.10 g, 1.67 mmol) in $H_2O$ (2.5 ml) at 50°, AcOH (0.15 ml, 2.6 mmol) was added dropwise under stirring during 1 min, the mixture was further stirred for 5 min and diluted with $H_2O$ (4 ml). Conc. 25% aq. $NH_3$ (3 ml) was added, the mixture was stirred for 10 min and evaporated to dryness. The residue was applied onto a column ($1.5 \times 10$ cm) packed with *Dowex 50W* × *4* (200–400 mesh; $H^+$-form), washed with $H_2O$ (150 ml), and the product was eluted with a 10% aq. $NH_3$ (150 ml). The eluate was evaporated to dryness to afford **8** as an amorphous powder (71 mg, 71%). Crystallization from a mixture MeOH/$H_2O$ gave a product with m.p. 237–239°. TLC (*B*): $R_f$ 0.32. $[\alpha]_D^{15} = -277.0$ ($c = 0.04$ g/l, $H_2O$). UV ($H_2O$): $\lambda_{max}$ 247 (8390), 292 (10600); $\lambda_{min}$ 229 (4650), 265 (3500). CD ($H_2O$; $\lambda$ [nm]($[\Theta]) \times 10^{-3}$): 256.0 (−1.8), 283.0 (+1.2); 241.0, 265.0, 300.0 (0).

6. *6-Amino-9-(3-deoxy-β-*D*-*erythro*-pentofuranosyl)-2-fluoropurine* (**9**). To a soln. of **6** (97 mg, 0.364 mmol) in a mixture of aq. 45% HF (0.25 ml), aq. 50% $HBF_4$ (0.5 ml), and THF (3 ml) at −10°, a 14.5 mM aq. soln. of $NaNO_2$ (0.25 ml) was added, and the mixture was stirred at −10–12° for 30 min, monitoring the course of the reaction by TLC. Then, 0.3 ml of aq. HF/aq. $HBF_4$/THF 0.4:2.0:1.0, followed by 0.2 ml of the aforementioned $NaNO_2$ soln., were added, and the mixture was further stirred at −10–12° for 30 min. After consumption of **6**, powdered $CaCO_3$ (0.4 g) was added, the mixture was stirred at r.t. for 30 min, aq. 25% $NH_3$ was added, and, after stirring at r.t for 10 min, the precipitate formed was filtered off, washed with MeOH (20 ml), and combined filtrates and washing were evaporated to dryness. The residue was chromatographed (silica gel ($1.5 \times 30$ cm); $CHCl_3$/MeOH 10:1 (*v*/*v*; 300 ml) and then $CHCl_3$/MeOH/$H_2O$ 20:10:1 (*v*/*v*; 200 ml)) to give, in order of elution, **9** (42 mg; 43%) and **8** (7 mg; 7%).

*Data of* **9**: m.p. 254–256° (EtOH). TLC (*A*): $R_f$ 0.56. $[\alpha]_D^{15} = +65.0$ ($c = 0.04$ g/l, $H_2O$). UV ($H_2O$): $\lambda_{max}$ 260 (13500); $\lambda_{min}$ 223 (2850). CD ($H_2O$; $\lambda$ [nm] ($[\Theta]) \times 10^{-3}$)): *ca.* 263 (sh, −1.2), 272.5 (−3.5); 260.0, 300.0 (0). $^{13}$C-NMR ((D$_6$)DMSO): 162.61 (*d*, $^1J = 203.82$, C(2)); 161.52 (*d*, $^3J = 21.04$, C(6)); 154.17 (*d*, $^3J = 19.93$, C(4)); 143.33 (br. *s*, C(8)); 121.40 (br. s, C(5)); 94.76 (*s*, C(1′)), 85.00 (*s*, C(4′)); 78.84 (*s*, C(2′)); 66.39 (*s*, C(5′)); 37.91 (*s*, C(3′)).

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