Synthesis and Cytostatic Activity of Novel 6-(Difluoromethyl)purine Bases and Nucleosides

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Abstract: An expeditious synthesis of modified purine bases and nucleosides bearing a difluoromethyl group in position 6 is described starting from the corresponding 6-(hydroxymethyl)purine derivatives. Their oxidation using Dess–Martin reagent gave 6-formylpurines that were converted to the title compounds by reaction with Deoxofluor. 6-(Difluoromethyl)purine ribonucleoside exerted significant cytostatic effect against leukemia cell lines.

Key words: purines, nucleobases, nucleosides, fluorination, antineoplastic activity

Several types of purines bearing C-substituents in position 6 are biologically active (Figure 1). 6-Aryl and 6-hetarylpurine,[1] 6-trifluoromethylpurine,[2] as well as 6-(hydroxymethyl)purine[3] ribonucleosides display significant cytostatic activity and some 6-hetarylpurine ribonucleosides exert[4] also potent antiviral activity against HCV. 6-Methylpurine and its ribonucleoside are highly cytotoxic[5] and its liberation by purine nucleoside phosphorylases from its non-toxic deoxyribonucleoside was proposed as a novel principle in the gene therapy of cancer.[6] Fluoromethyl and difluoromethyl groups are isosteric to methyl groups and fluorine is often used as surrogate of oxygen in biologically active compounds. Hydrophobic nucleobase surrogates, including fluorinated and trifluoromethylated (het)arenes, are[7] very promising tools in chemical biology (e.g. extension of the genetic alphabet etc.). The biological activities and other applications of the above-mentioned important classes of compounds imply that further logical members of the series, 6-(fluoromethyl)purines and 6-(difluoromethyl)purines, are of great interest both in medicinal chemistry and chemical biology. Surprisingly, these simple compounds have not been reported until very recently when we[8] and others[9] have published a synthesis of 6-(fluoromethyl)purines and the corresponding ribonucleoside was found to possess significant cytostatic effect.[8] Here we report the synthesis and biological activity of 6-(difluoromethyl)purine bases and nucleosides as the last missing members of this interesting class of compounds.

Purines bearing C-substituents in position 6 are generally prepared by cross-coupling reactions[10] of 6-halopurines with organometallics. However, in the case of fluoromethyl and difluoromethyl groups the corresponding organometallics are hardly accessible. Therefore, purine derivatives containing these groups must be prepared by functional group transformations of other types of 6-[(substituted)methyl]purines. Protected 6-methylpurines were reported to be deprotonated by NaHMDS at the methyl group. Quenching of the anion with NFSI gave 6-(fluoromethyl)purines in moderate yields [trace amounts of 6-(difluoromethyl)purines were formed as by-products]. We have recently reported[8] a facile synthesis of 6-(fluoromethyl)purines via direct deoxyfluorination or by multistep functional group transformation starting from now easily available 6-(hydroxymethyl)purines. Apparently, the hydroxymethyl group is a suitable precursor for many other transformations.

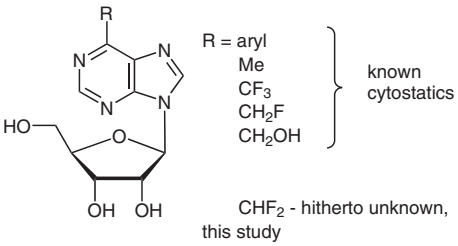

Figure 1 Biologically active substituted 6-methylpurine nucleosides

Our strategy for the preparation of the desired 6-(difluoromethyl)purines was to perform a deoxofluorination of the corresponding 6-formylpurines. 6-Formylpurines were reported[11] several times in the past but their syntheses based on multistep or direct oxidations of 6-methylpurines were rather low-yielding procedures with limited applicability. We have tried to prepare these key intermediates by oxidation of 6-(hydroxymethyl)purines **1a–d** readily available by cross-coupling of 6-halopurines with (acetoxymethyl)zinc iodide[3] followed by chemoselective deacetylation.[8] The oxidation was performed using Dess–Martin periodinane [1,1,1-tris(acetyloxy)-1,1-dihydro-1,2-benziodoxol-3-(1*H*)-one] which is a general reagent for oxidation of alcohols to aldehydes.[12] Thus the protected 6-(hydroxymethyl)purine bases and nucleosides **1a–d** were treated with Dess–Martin reagent at ambient temperature to give quantitative conversion to 6-formylpurines **2a–d**. These compounds occur in the form of the aldehydes **2** in absence of water but in presence of even traces of water (e.g. NMR measurement in DMSO-d_6) they oc-

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cur as mixtures of aldehydes **2** and hydrates **3**. Analytically pure hydrate **3a** was obtained by crystallization of **2a** from acetone–water mixture. For the synthetic purposes the aldehydes **2** were directly used in the next step after column chromatography and drying.

The deoxyfluorinations were performed using Deoxofluor {[bis(2-methoxyethyl)amino]sulfur trifluoride}[13] that was also previously found to be the best reagent for deoxyfluorinations of 6-(hydroxymethyl)purines.[8] Thus the formylpurines **2** were treated with Deoxofluor at –20 °C and the reaction mixture was then stirred at ambient temperature for 10–16 hours until the consumption of the aldehydes **2**. The desired protected 6-(difluoromethyl)purine bases and nucleosides **4a–d** were easily isolated in acceptable yields of ca. 50% (Table 1) as the only products besides tarry, chromatographically immobile mixture of side-products of decomposition and/or polymerization of starting compounds.

Standard deprotection of 6-(difluoromethyl)purine intermediates **4b–d** was used to prepare the final free 6-(difluoromethyl)purine base and nucleosides **4e–g** (Scheme 1). The THP protective group in position 9 of purine is easily cleavable under mild acidic conditions. Thus compound **4b** was deprotected using Dowex 50X8 (H^+ form)[14] in ethanol to afford free base **4e** in 74% of yield. The ester groups in **4c**,**d** were cleaved by sodium methoxide in methanol at room temperature[15] to give free nucleosides **4f**,**g** in about 83–90% of yield (Table 1).

Table 1 Yields of the Particular Steps in the Synthesis

Entry	Starting compound	Reagent	Product yield (%)
1	**1a**	Dess–Martin	**2a** (93)[a]
2	**1b**	Dess–Martin	**2b** (95)
3	**1c**	Dess–Martin	**2c** (98)
4	**1d**	Dess–Martin	**2d** (93)
5	**2a**	Deoxofluor	**4a** (49)
6	**2b**	Deoxofluor	**4b** (52)
7	**2c**	Deoxofluor	**4c** (48)
8	**2d**	Deoxofluor	**4d** (49)
9	**4b**	Dowex (H^+)	**4e** (74)
10	**4c**	MeONa	**4f** (83)
11	**4d**	MeONa	**4g** (90)

[a] Isolated in the form of a hydrate **3a** after crystallization from water–acetone.

Title 6-(difluoromethyl)purine bases and nucleosides **4a**,**e**,**f**,**g** were screened for biological activity. In vitro cytostatic activity tests (inhibition of cell growth) were performed using the following cell cultures: mouse leukemia L1210 cells (ATCC CCL 219), human promyelocytic leukemia HL60 cells (ATCC CCL 240), human cervix carcinoma HeLa S3 cells (ATCC CCL 2.2), and human T lymphoblastoid CCRF-CEM cell line (ATCC CCL 119). The results are summarized in Table 2. 6-(Difluoromethyl)purine ribonucleoside (**4f**) exerted significant cytostatic effect (IC_{50} in micromolar range) against leukemia cell lines HL-60 and CCRF-CEM but virtually no effect against solid tumor (HeLa S3) while the deoxyribonucleoside as well as the purine bases were inactive in all these cell lines.

The 6-substituted purine nucleosides could be considered as transition state analogues for adenosine deaminase (ADA), an important catabolic enzyme. In previous studies, 6-(hydroxymethyl)purine ribonucleoside was found[3] to be a moderate inhibitor of ADA while the fluoromethyl derivative[8] was inactive. Adenosine deaminase (ADA) inhibition was studied[16] on calf intestinal adenosine aminohydrolase (EC 3.5.4.4.). Both riboside **4f** and deoxyriboside **4g** exerted only very low inhibitory effect to ADA.

It could be concluded, that the activity of 6-(difluoromethyl)purine derivatives is comparable to that of 6-(fluoromethyl)purines[8] and 6-(trifluoromethyl)purines;[2] their ribonucleosides are active against leukemia cell lines while other derivatives are inactive. In addition, they do not significantly inhibit ADA.

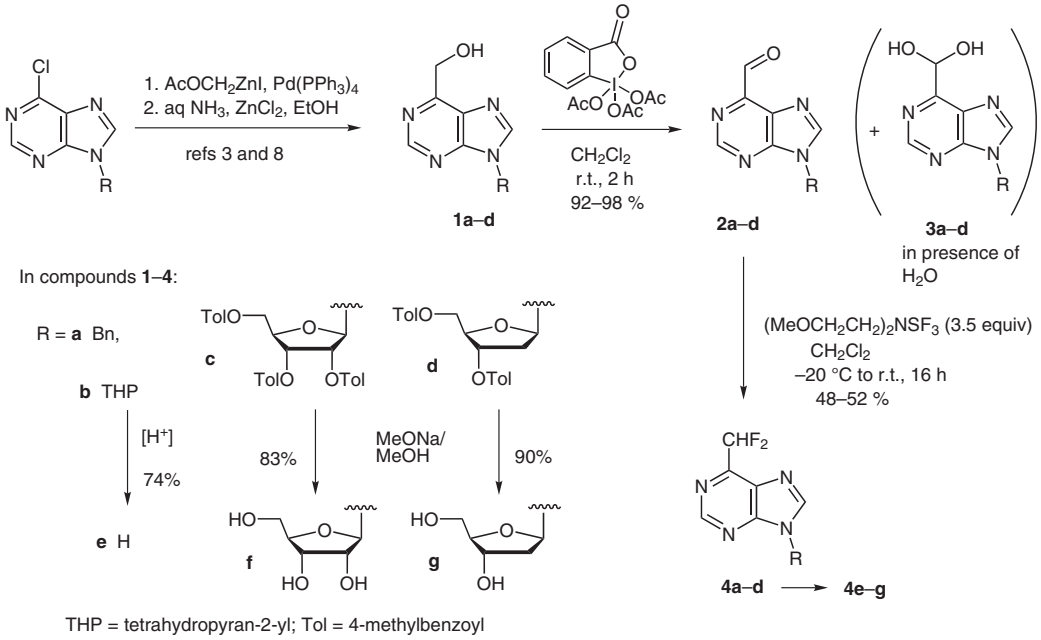
Table 2 Cytostatic and ADA Inhibitory Activity of Title Compounds

Compound	IC_{50} (μmol/L)[a]		
	HL60	CCRF-CEM	ADA
4a	NA[b]	NA	NA
4e	NA	NA	NA
4f	1.05 ± 0.001	1.02 ± 0.001	31.5 ± 2.1
4g	NA	NA	18.0 ± 0.9

[a] Values are means of four experiments, standard deviation is given in parentheses.
[b] NA = not active (inhibition of cell growth at 10 μM was lower than 30%).

In conclusion, 6-(difluoromethyl)purine bases and nucleosides can be prepared by this expeditious methodology in reasonable yields from 6-(hydroxymethyl)purines. They complement the series of cytostatic 6-methylpurine derivatives.

NMR spectra were recorded on Bruker Avance 400 (^{1}H NMR at 400 MHz, ^{13}C NMR at 100.6 MHz) or Bruker Avance 500 (500 MHz for ^{1}H NMR and 125.8 MHz for ^{13}C NMR) spectrometers. Chemical shifts (in ppm, δ scale) were referenced to TMS as internal standard. Complete assignment of all NMR signals was performed using a combination of H,H-COSY, H,C-HSQC and H,C-HMBC experiments. Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured at 25 °C on a Autopol IV (Rudolph Research Analytical) polarimeter and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Mass spectra were mea-



Scheme 1 Synthesis of 6-(difluoromethyl)purine bases and nucleosides

sured on a ZAB-EQ (VG Analytical) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix). Cytostatic activity tests were performed as described in the literature.[1a] ADA inhibition assay was performed by standard technique given in the literature.[16]

Preparation of 6-Formylpurines (2); General Procedure

Dess–Martin periodinane (510 mg, 1.2 mmol) was added to a solution of 6-(hydroxymethyl)purine **1** (1 mmol) in CH_2Cl_2 (15 mL). After consumption of starting material (2 h), the reaction mixture was quenched with sat. aq $NaHCO_3$ (15 mL) and extracted with $CHCl_3$ (4 × 20 mL). The collected organic layers were dried over $MgSO_4$, evaporated and chromatographed on a column of silica gel (hexanes–acetone, 3:1–2:1).

9-Benzyl-6-formylpurine (2a)

Yield: 93%. Crystallization from acetone–H_2O afforded an analytically pure hydrate **3a**; mp 119–120 °C.

3a

IR (KBr): 3381, 3087, 2935, 1605, 1590, 1510, 1405, 1341, 1211, 1099, 1043 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 5.53 (s, 2 H, CH_2Ph), 6.27 (t, J = 7.8 Hz, 1 H, CH), 6.55 (d, J = 7.8 Hz, 2 H, 2 × OH), 7.26–7.37 (m, 5 H, Ph), 8.74 (s, 1 H, H-8), 8.92 (s, 1 H, H-2).

^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 46.7 (CH_2Ph), 87.0 (CH), 127.8, 128.1, 128.9 (CHPh), 129.6 (C-5), 136.7 (C*i*-Ph), 146.7 (CH-8), 151.9 (C-4), 151.9 (CH-2), 158.7 (C-6).

MS (FAB): *m*/*z* (%) = 257 (10) [MH^+], 239 (5), 149 (6), 91 (43).

HRMS (FAB): *m*/*z* calcd for $C_{13}H_{12}N_4O_2$: 257.1039; found: 257.1028.

Anal. Calcd for $C_{13}H_{12}N_4O_2$: C, 60.93; H, 4.72; N, 21.86. Found: C, 60.76; H, 4.71; N, 21.58.

6-Formyl-9-(tetrahydropyran-2-yl)purine (2b)

Yield: 95%.

IR ($CHCl_3$): 3372, 2951, 2861, 1602, 1496, 1407, 1334, 1087, 1045, 959 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 1.67–1.89, 2.02–2.24 [m, 6 H, $CH_{2(THP)}$], 3.81 [td, J = 2.6, 11.7 Hz, 1 H, b$CH_2O_{(THP)}$], 4.22 [ddt, J = 1.8, 4.3, 11.7 Hz, 1 H, a$CH_2O_{(THP)}$], 5.88 [dd, J = 2.5, 10.4 Hz, 1 H, $CHO_{(THP)}$], 8.53 (s, 1 H, H-8), 9.19 (s, 1 H, H-2), 10.50 (s, 1 H, CHO).

^{13}C NMR (125.8 MHz, $CDCl_3$): δ = 22.6, 24.8, 31.9 [$CH_{2(THP)}$], 68.9 [$CH_2O_{(THP)}$], 82.4 [$CHO_{(THP)}$], 131.5 (C-5), 146.4 (CH-8), 146.8 (C-6), 152.7 (CH-2), 153.7 (C-4), 191.6 (CHO).

MS (FAB): *m*/*z* (%) = 233 (5) [MH^+], 149 (44), 85 (52).

HRMS (FAB): *m*/*z* calcd for $C_{11}H_{13}N_4O_2$: 233.1033; found: 233.1042.

6-Formyl-9-(2,3,5-tri-*O*-toluoyl-β-D-ribofuranosyl)purine (2c)

Yield: 98%.

IR ($CHCl_3$): 1726, 1612, 1591, 1495, 1408, 1331, 1267, 1180, 1093, 1020, 840 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 2.38, 2.42, 2.43 [3 × s, 9 H, 3 × $CH_{3(Tol)}$], 4.67 (dd, $J_{5'b,4'}$ = 4.0 Hz, J_{gem} = 12.3 Hz, 1 H, H-5′b), 4.85 (td, $J_{4',5'}$ = 3.1, 4.0 Hz, $J_{4',3'}$ = 4.6 Hz, 1 H, H-4′), 4.93 (dd, $J_{5'a,4'}$ = 3.1 Hz, J_{gem} = 12.3 Hz, 1 H, H-5′a), 6.21 (dd, $J_{3',4'}$ = 4.6 Hz, $J_{3',2'}$ = 5.8 Hz, 1 H, H-3′), 6.41 (t, $J_{2',1'}$ = 5.4 Hz, $J_{2',3'}$ = 5.8 Hz, 1 H, H-2′), 6.52 (d, $J_{1',2'}$ = 5.4 Hz, 1 H, H-1′), 7.16, 7.24, 7.25 [3 × m, 6 H, 3 × 2 $H_{(m\text{-Tol})}$], 7.80, 7.93, 7.97 [3 × m, 6 H, 3 × 2 $H_{(o\text{-Tol})}$], 8.46 (s, 1 H, H-8), 9.05 (s, 1 H, H-2), 10.45 (s, 1 H, CHO).

^{13}C NMR (125.8 MHz, $CDCl_3$): δ = 21.7, 21.8 [$CH_{3(Tol)}$], 63.2 (CH_2-5′), 71.4 (CH-3′), 73.7 (CH-2′), 81.3 (CH-4′), 87.2 (CH-1′), 125.5, 125.9, 126.4 [$C_{(i\text{-Tol})}$], 129.3, 129.3, 129.4 [$CH_{(m\text{-Tol})}$], 129.73, 129.87 [$CH_{(o\text{-Tol})}$], 131.97 (C-5), 144.4, 144.7, 144.8 [$C_{(p\text{-Tol})}$], 147.1 (CH-8), 147.2 (C-6), 152.9 (CH-2), 154.2 (C-4), 165.2, 165.4, 166.1 (CO), 191.3 (CHO).

MS (FAB): *m*/*z* (%) = 635 (1) [MH^+], 487 (42), 369 (5), 149 (7), 119 (100).

HRMS (APCI): *m*/*z* calcd for $C_{35}H_{31}N_4O_8$: 635.2142; found: 635.2175.

9-(2-Deoxy-3,5-di-*O*-toluoyl-β-D-*erythro*-pentofuranosyl)-6-formylpurine (2d)
Yield: 93%.

IR ($CHCl_3$): 1719, 1612, 1589, 1492, 1406, 1326, 1269, 1179, 1102, 1020, 939, 841 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 2.40, 2.45 [2 × s, 6 H, 2 × $CH_{3(Tol)}$], 2.91 (ddd, $J_{2'b,3'}$ = 2.3 Hz, $J_{2'b,1'}$ = 5.9 Hz, J_{gem} = 14.4 Hz, 1 H, H-2′b), 3.21 (ddd, $J_{2'a,3'}$ = 6.4 Hz, $J_{2'a,1'}$ = 8.2 Hz, J_{gem} = 14.4 Hz, 1 H, H-2′a), 4.64–4.71 (m, 2 H, H-5′b, H-4′), 4.82 (m, 1 H, H-5′a), 5.86 (dt, $J_{3',4'}$ = 2.3 Hz, $J_{3',2'}$ = 2.3, 6.4 Hz, 1 H, H-3′), 6.65 (dd, $J_{1',2'}$ = 5.9, 8.2 Hz, 1 H, H-1′), 7.21, 7.30 [2 × m, 4 H, 2 × $H_{(m\text{-}Tol)}$], 7.87, 7.98 [2 × m, 4 H, 2 × $H_{(o\text{-}Tol)}$], 8.48 (s, 1 H, H-8), 9.12 (s, 1 H, H-2), 10.46 (s, 1 H, CHO).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 21.7, 21.8 [$CH_{3(Tol)}$], 37.9 (CH_2-2′), 63.7 (CH_2-5′), 75.0 (CH-3′), 83.4 (CH-4′), 85.2 (CH-1′), 126.3, 126.5 [$C_{(i\text{-}Tol)}$], 129.31, 129.33 [$CH_{(m\text{-}Tol)}$], 129.6, 129.8 [$CH_{(o\text{-}Tol)}$], 132.0 (C-5), 144.3, 144.7 [$C_{(p\text{-}Tol)}$], 146.8 (CH-8), 146.9 (C-6), 152.7 (CH-2), 154.0 (C-4), 165.9, 166.1 [$CO_{(Tol)}$], 191.4 (CHO).

MS (FAB): *m*/*z* (%) = 501 (1) [MH^+], 353 (2), 149 (40), 119 (82).

HRMS (APCI): *m*/*z* calcd for $C_{27}H_{25}N_4O_6$: 501.1774; found: 501.1786.

Deoxyfluorinations with Deoxofluor; General Procedure
A 6-formylpurine **2** (0.5 mmol) was dissolved in CH_2Cl_2 (10 mL) under an Ar atmosphere and cooled to –20 °C. Deoxofluor (0.32 mL, 1.75 mmol) was then added dropwise through septum and the reaction mixture was allowed to warm to r.t. and stirred for 10–16 h. Then the reaction was quenched with 5% $NaHCO_3$ and extracted with $CHCl_3$. Organic phase was dried over $MgSO_4$, concentrated and chromatographed on silica gel (hexanes–EtOAc, 2:1–1:1).

9-Benzyl-6-(difluoromethyl)purine (4a)
Yield: 49%; white crystals; mp 78–79 °C.

IR (CCl_4): 3070, 3036, 1598, 1501, 1408, 1330, 1209, 1109, 1066 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 5.50 (s, 2 H, CH_2Ph), 7.05 (t, $J_{H,F}$ = 53.9 Hz, 1 H, CHF_2), 7.31–7.42 (m, 5 H, Ph), 8.20 (s, 1 H, H-8), 9.10 (t, $J_{H,F}$ = 0.7 Hz, 1 H, H-2).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 47.6 (CH_2Ph), 112.1 (t, $J_{C,F}$ = 242 Hz, CHF_2), 127.9, 128.9, 129.3 (CHPh), 130.6 (C-5), 134.5 (C*i*-Ph), 146.4 (CH-8), 149.8 (t, $J_{C,F}$ = 26 Hz, C-6), 152.5 (CH-2), 153.2 (C-4).

MS (FAB): *m*/*z* (%) = 261 (32) [MH^+], 242 (3), 171 (4), 91 (100).

HRMS (FAB): *m*/*z* calcd for $C_{13}H_{11}F_2N_4$: 261.0952; found: 261.0946.

Anal. Calcd for $C_{13}H_{10}F_2N_4$: C, 60.00; H, 3.87; F, 14.60; N, 21.53. Found: C, 59.80; H, 3.83; F, 14.31; N, 21.19.

6-(Difluoromethyl)-9-(tetrahydropyran-2-yl)purine (4b)
Yield: 52%; white foam.

IR (CCl_4): 2950, 2858, 1598, 1592, 1498, 1410, 1329, 1210, 1109, 1088, 1066 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 1.66–1.88, 2.04–2.22 [m, 6 H, $CH_{2(THP)}$], 3.81 [td, *J* = 2.6, 11.8 Hz, 1 H, b$CH_2O_{(THP)}$], 4.21 [ddt, *J* = 1.8, 4.4, 11.8 Hz, 1 H, a$CH_2O_{(THP)}$], 5.86 [dd, *J* = 2.5, 10.5 Hz, 1 H, $CHO_{(THP)}$], 7.05 (t, $J_{H,F}$ = 53.9 Hz, 1 H, CHF_2), 8.43 (s, 1 H, H-8), 9.06 (s, 1 H, H-2).

^{13}C NMR (125.8 MHz, $CDCl_3$): δ = 22.6, 24.8, 31.9 (CH_2THP), 68.9 (CH_2OTHP), 82.3 (CHOTHP), 112.0 (t, $J_{C,F}$ = 242 Hz, CHF_2), 130.9 (C-5), 144.5 (CH-8), 149.7 (d, $J_{C,F}$ = 26 Hz, C-6), 152.25 (CH-2), 152.34 (C-4).

MS (FAB): *m*/*z* (%) = 255 (11) [MH^+], 171 (100), 152 (9), 85 (64).

HRMS (FAB): *m*/*z* calcd for $C_{11}H_{13}F_2N_4O$: 255.1052; found: 255.1061.

6-(Difluoromethyl)-9-(2,3,5-tri-*O*-toluoyl-β-D-ribofuranosyl)purine (4c)
Yield: 48%; white foam.

IR (CCl_4): 3039, 1732, 1612, 1600, 1499, 1411, 1331, 1266, 1114, 1068, 1020 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 2.38, 2.42, 2.43 [3 × s, 9 H, 3 × $CH_{3(Tol)}$], 4.67 (dd, $J_{5'b,4'}$ = 4.1 Hz, J_{gem} = 12.4 Hz, 1 H, H-5′b), 4.86 (td, $J_{4',5'}$ = 3.1, 4.1 Hz, $J_{4',3'}$ = 4.6 Hz, 1 H, H-4′), 4.92 (dd, $J_{5'a,4'}$ = 3.1 Hz, J_{gem} = 12.4 Hz, 1 H, H-5′a), 6.20 (dd, $J_{3',4'}$ = 4.6 Hz, $J_{3',2'}$ = 5.8 Hz, 1 H, H-3′), 6.40 (t, $J_{2',1'}$ = 5.4 Hz, $J_{2',3'}$ = 5.8 Hz, 1 H, H-2′), 6.50 (d, $J_{1',2'}$ = 5.4 Hz, 1 H, H-1′), 7.01 (t, $J_{H,F}$ = 53.8 Hz, 1 H, CHF_2), 7.16, 7.23, 7.25 [3 × m, 6 H, 3 × $H_{(m\text{-}Tol)}$], 7.80, 7.92, 7.98 [3 × m, 6 H, 3 × $H_{(o\text{-}Tol)}$], 8.36 (s, 1 H, H-8), 8.92 (s, 1 H, H-2).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 21.7, 21.8 [$CH_{3(Tol)}$], 63.2 (CH_2-5′), 71.3 (CH-3′), 73.6 (CH-2′), 81.2 (CH-4′), 87.1 (CH-1′), 111.8 (t, $J_{C,F}$ = 242 Hz, CHF_2), 125.5, 125.9, 126.5 [$C_{(i\text{-}Tol)}$], 129.25, 129.30, 129.37 [$CH_{(m\text{-}Tol)}$], 129.7, 129.9 [$CH_{(o\text{-}Tol)}$], 131.5 (C-5), 144.4, 144.7, 144.8 [$C_{(p\text{-}Tol)}$], 145.3 (CH-8), 150.1 (t, $J_{C,F}$ = 26 Hz, C-6), 152.5 (CH-2), 152.7 (C-4), 165.2, 165.4, 166.2 [$CO_{(Tol)}$].

MS (FAB): *m*/*z* (%) = 657 (0.5) [MH^+], 487 (16), 119 (100).

HRMS (APCI): *m*/*z* calcd for $C_{35}H_{31}F_2N_4O_7$: 657.2161; found: 657.2145.

9-(2-Deoxy-3,5-di-*O*-toluoyl-β-D-*erythro*-pentofuranosyl)-6-(difluoromethyl)purine (4d)
Yield: 49%; white foam.

IR (CCl_4): 3040, 1727, 1613, 1600, 1496, 1409, 1329, 1267, 1209, 1178,1101, 1068, 1021 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 2.40, 2.45 [2 × s, 6 H, 2 × $CH_{3(Tol)}$], 2.90 (ddd, $J_{2'b,3'}$ = 2.2 Hz, $J_{2'b,1'}$ = 5.9 Hz, J_{gem} = 14.2 Hz, 1 H, H-2′b), 3.19 (ddd, $J_{2'a,3'}$ = 6.4 Hz, $J_{2'a,1'}$ = 8.2 Hz, J_{gem} = 14.2 Hz, 1 H, H-2′a), 4.65–4.70 (m, 2 H, H-5′b, H-4′), 4.80 (m, 1 H, H-5′a), 5.85 (dt, $J_{3',4'}$ = 2.2 Hz, $J_{3',2'}$ = 2.2, 6.4 Hz, 1 H, H-3′), 6.63 (dd, $J_{1',2'}$ = 5.9, 8.2 Hz, 1 H, H-1′), 7.02 (t, $J_{H,F}$ = 53.9 Hz, 1 H CHF_2), 7.21, 7.30 [2 × m, 4 H, 2 × $H_{(m\text{-}Tol)}$], 7.87, 7.98 [2 × m, 4 H, 2 × $H_{(o\text{-}Tol)}$], 8.38 (s, 1 H, H-8), 8.98 (s, 1 H, H-2).

^{13}C NMR (125.8 MHz, $CDCl_3$): δ = 21.7, 21.8 [$CH_{3(Tol)}$], 37.9 (CH_2-2′), 63.8 (CH_2-5′), 75.0 (CH-3′), 83.4 (CH-4′), 85.2 (CH-1′), 111.9 (t, $J_{C,F}$ = 242 Hz, CHF_2), 126.3, 126.5 [$C_{(i\text{-}Tol)}$], 129.3, 129.3 [$CH_{(m\text{-}Tol)}$], 129.6, 129.8 [$CH_{(o\text{-}Tol)}$], 131.5 (C-5), 144.3, 144.7 [$C_{(p\text{-}Tol)}$], 144.9 (CH-8), 150.0 (t, $J_{C,F}$ = 26 Hz, C-6), 152.3 (CH-2), 152.5 (C-4), 165.9, 166.1 [$CO_{(Tol)}$].

^{19}F NMR (188.2 MHz, $CDCl_3$): δ = –118.41 (d, $J_{F,H}$ = 53.9 Hz).

MS (FAB): *m*/*z* (%) = 523 (1) [MH^+], 353 (5), 170 (16), 119 (75).

HRMS (FAB): *m*/*z* calcd for $C_{27}H_{25}F_2N_4O_5$: 523.1793; found: 523.1803.

6-(Difluoromethyl)-9*H*-purine (4e)
6-(Difluoromethyl)-9-(tetrahydropyran-2-yl)purine (**4b**; 144 mg, 0.566 mmol) was dissolved in EtOH (20 mL) and Dowex 50 WX8 (H^+ form; ca. 30 mg) was added. This suspension was stirred for 1.5 h at 75 °C. After a complete deprotection of starting material, the reaction mixture was filtered and Dowex was washed with NH_3–EtOH. Volatiles were evaporated in vacuo. Crude product was chromatographed on silica gel (hexanes–EtOAc, 1:2–0:1) and crystallized from EtOAc–hexanes to afford a white solid (71 mg, 74%; mp 244–245 °C).

IR (KBr): 3081, 1621, 1607, 1479, 1382, 1325, 1229, 1103, 1075, 1041 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32 (t, $J_{H,F}$ = 53.6 Hz, 1 H, CHF_2), 8.78 (s, 1 H, H-8), 9.03 (s, 1 H, H-2).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 112.6 (t, $J_{C,F}$ = 239 Hz, CHF_2), 126.5 (C-5), 146.5 (t, $J_{C,F}$ = 26 Hz, C-6), 148.3 (CH-8), 151.8 (CH-2), 157.4 (C-4).

MS (FAB): *m/z* (%) = 171 (100) [MH$^+$], 152 (12), 134 (17).

HRMS (FAB): *m/z* calcd for $C_6H_5F_2N_4$: 171.0482; found: 171.0477.

6-(Difluoromethyl)-9-(β-D-ribofuranosyl)purine (4f)

A methanolic solution of MeONa (*c* = 1 M; 0.1 mL, 0.1 mmol) was added to a solution of 6-(difluoromethyl)purine ribonucleoside **4c** (230 mg, 0.35 mmol) in MeOH (20 mL). After a complete deprotection of starting material (16 h, monitoring by TLC), the reaction mixture was adsorbed on silica gel and chromatographed (EtOAc–MeOH, 1:0–9:1) to give a white solid (88 mg, 83%); crystallization from MeOH–EtOAc–heptane; mp 181–182 °C; $[\alpha]_D^{20}$ –30.5 (*c* = 2.75, MeOH).

IR (KBr): 3401, 3327, 1607, 1590, 1499, 1405, 1332, 1212, 1118, 1089, 1048 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.59 (ddd, $J_{5'b,4'}$ = 4.0 Hz, $J_{5'b,OH}$ = 5.7 Hz, J_{gem} = 12.1 Hz, 1 H, H-5′b), 3.71 (dt, $J_{5'a,4'}$ = 4.0 Hz, $J_{5'a,OH}$ = 5.3 Hz, J_{gem} = 12.1 Hz, 1 H, H-5′a), 4.00 (q, $J_{4',3'}$ = 3.8 Hz, $J_{4',5'}$ = 4.0 Hz, 1 H, H-4′), 4.21 (br q, $J_{3',4'}$ = 3.8 Hz, $J_{3',2'}$ = 4.9 Hz, $J_{3',OH}$ = 5.2 Hz, 1 H, H-3′), 4.62 (q, $J_{2',3'}$ = 4.9 Hz, $J_{2',1'}$ = 5.4 Hz, $J_{2',OH}$ = 5.7 Hz, 1 H, H-3′), 5.11 (t, $J_{OH,5'}$ = 5.3, 5.7 Hz, 1 H, OH-5′), 5.28 (d, $J_{OH,3'}$ = 5.2 Hz, 1 H, OH-3′), 5.60 (d, $J_{OH,2'}$ = 5.7 Hz, 1 H, OH-2′), 6.10 (d, $J_{1',2'}$ = 5.4 Hz, 1 H, H-1′), 7.36 (t, $J_{H,F}$ = 53.3 Hz, 1 H, CHF_2), 9.01 (s, 1 H, H-8), 9.09 (s, 1 H, H-2).

^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 61.3 (CH_2-5′), 70.4 (CH-3′), 74.1 (CH_2-2′), 85.9 (CH-4′), 88.1 (CH-1′), 112.3 (t, $J_{C,F}$ = 240 Hz, CHF_2), 131.0 (C-5), 147.1 (CH-8), 148.9 (t, $J_{C,F}$ = 25 Hz, C-6), 152.1 (CH-2), 153.0 (C-4).

^{19}F NMR (188.2 MHz, DMSO-d_6): δ = –118.80 (t, $J_{F,H}$ = 53.3 Hz).

MS (FAB): *m/z* (%) = 303 (30) [MH$^+$], 171 (100), 152 (16).

HRMS (FAB): *m/z* calcd for $C_{11}H_{13}F_2N_4O_4$: 303.0905; found: 303.0908.

Anal. Calcd for $C_{11}H_{12}F_2N_4O_4$: C, 43.71; H, 4.00; F, 12.57; N, 18.54. Found: C, 43.68; H, 3.95; F, 12.78; N, 18.19.

9-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-6-(difluoromethyl)purine (4g)

Prepared from 6-(difluoromethyl)purine 2′-deoxynucleoside **4d** (150 mg, 0.287 mmol) by the procedure described for **4f**. Yield: 74 mg (90%); white solid; crystallization from MeOH–EtOAc–heptane; mp 122–123 °C; $[\alpha]_D^{20}$ +28.0 (*c* = 2.54, MeOH).

IR (KBr): 3343, 3264, 1596, 1501, 1339, 1326, 1208, 1101, 1061, 1029 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.39 (ddd, $J_{2'b,3'}$ = 3.9 Hz, $J_{2'b,1'}$ = 6.4 Hz, J_{gem} = 13.4 Hz, 1 H, H-2′b), 2.80 (ddd, $J_{2'a,3'}$ = 6.0 Hz, $J_{2'a,1'}$ = 6.9 Hz, J_{gem} = 13.4 Hz, 1 H, H-2′a), 3.54 (ddd, $J_{5'b,4'}$ = 4.7 Hz, $J_{5'b,OH}$ = 5.5 Hz, J_{gem} = 11.8 Hz, 1 H, H-5′b), 3.63 (dt, $J_{5'a,4'}$ = 5.1 Hz, $J_{5'a,OH}$ = 5.5 Hz, J_{gem} = 11.8 Hz, 1 H, H-5′a), 3.90 (td, $J_{4',3'}$ = 3.2 Hz, $J_{4',5'}$ = 4.7, 5.1 Hz, 1 H, H-4′), 4.46 (dq, $J_{3',4'}$ = 3.2 Hz, $J_{3',OH}$ = 4.1 Hz, $J_{3',2'}$ = 3.9, 6.0 Hz, 1 H, H-3′), 4.98 (t, $J_{OH,5'}$ = 5.5 Hz, 1 H, OH-5′), 5.39 (d, $J_{OH,3'}$ = 4.1 Hz, 1 H, OH-3′), 6.52 (t, $J_{1',2'}$ = 6.4, 6.9 Hz, 1 H, H-1′), 7.35 (t, $J_{H,F}$ = 53.3 Hz, 1 H, CHF_2), 8.96 (s, 1 H, H-8), 9.08 (s, 1 H, H-2).

^{13}C NMR (125.8 MHz, DMSO-d_6): δ = 39.5 (CH_2-2′), 61.6 (CH_2-5′), 70.6 (CH-3′), 84.1 (CH-1′), 88.28 (CH-4′), 112.3 (t, $J_{C,F}$ = 240 Hz, CHF_2), 131.0 (C-5), 147.1 (CH-8), 148.7 (t, $J_{C,F}$ = 25 Hz, C-6), 151.9 (CH-2), 152.7 (C-4).

^{19}F NMR (188.2 MHz, DMSO-d_6): δ = –118.78 (d, $J_{F,H}$ = 53.3 Hz).

MS (FAB): *m/z* (%) = 287 (18) [MH$^+$], 171 (63), 152 (5), 117 (10).

HRMS (FAB): *m/z* calcd for $C_{11}H_{13}F_2N_4O_3$: 287.0956; found: 287.0950.

Anal. Calcd for $C_{11}H_{12}F_2N_4O_3$: C, 46.16; H, 4.23; F, 13.27; N, 19.57. Found: C, 46.00; H, 4.30; F, 13.23; N, 19.56.

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