Supporting Information

Synthesis of 5′-Methylene-Phosphonate Furanonucleoside Prodrugs: Application to D-2′-Deoxy-2′-α-Fluoro-2′-β-*C*-Methyl Nucleosides

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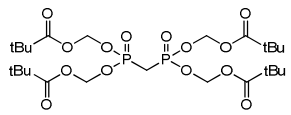
1. General Experimental Information

Nuclear magnetic resonance (NMR) spectra (^{1}H, ^{13}C, ^{19}F and ^{31}P) were recorded on a Varian Unity Plus 400 MHz Fourier transform spectrometer at ambient temperature, with tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm), and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad), dd (doublet of doublets), or ddd (doublet of doublets of doublets). Low-resolution mass spectra (MS) were measured on a time-of-flight (TOF) mass spectrometry with electrospray ionization (ESI). High-resolution mass spectra (HRMS) were recorded on a Micromass Autospec high-resolution mass spectrometer with ESI. Thin-layer chromatography (TLC) was performed on 0.25 mm silica gel. Purifications were carried out on silica gel column chromatography (60 Å, 63-200 μm, or 40-75 μm).

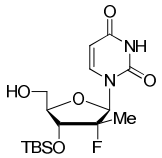
2. Experimental Procedures and Spectral Characterization:



Tetra(pivaloyloxymethyl)-bis-phosphonate (5)

Compound **5** was prepared according to the procedure described by Vepsäläinen[1] from commercially available tetramethyl bisphosphonate **4** in 67% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82–5.59 (m, 8H), 2.60 (t, J = 21.7 Hz, 2H), 1.16 (s, 36H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.6, 81.7, 38.5, 26.6. ^{31}P NMR (162 MHz, CD_3OD) δ –32.98.



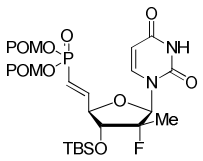
3′-*O*-*tert*-Butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl uridine (7)[2]

To a solution of compound **6**[3] (1.0 g, 2.67 mmol) in 5 mL of DMF was added imidazole (0.91 g, 13.35 mmol) and *t*-butyldimethylsilyl chloride (2.02 g, 13.35 mmol) under a N_2 atmosphere. The reaction mixture was stirred

[1] Vepsäläinen, J.J. *Tetrahedron Lett.* **1999**, *40*, 8491.

[2] Chang, W.; Du, J.; Rachakonda, S.; Ross, B.S.; Convers-Reignier, S.; Yau, W.T.; Pons, J.-F.; Murakami, E.; Bao, H.; Micolochick Steuer, H.; Furman, P.A.; Otto, M.J.; Sofia, M.J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4539–4543.

overnight at room temperature and treated with 25 mL of a saturated aqueous solution of $NaHCO_3$. After stirring for 10 min, the mixture was extracted with CH_2Cl_2 (3 x 15 mL). The combined organic fractions were washed with water (3 x 15 mL) and brine (15 mL), dried over Na_2SO_4 and filtered. The volatiles were removed under reduced pressure and the residue was purified on silica gel column chromatography (EtOAc:hexane = 30:70 v/v) to give 3′,5′-bis-O-*tert*-butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl uridine (1.27 g, 2.64 mmol). Subsequently, to a solution of 3′,5′-bis-O-*tert*-butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl uridine (1.27 g, 2.64 mmol) in 13 mL of THF was added, at 0 °C, a solution of trichloroacetic acid (6.92 g, 42.35 mmol) in 4 mL of water. After stirring for 3 h at 0 °C, the solution was neutralized with solid $NaHCO_3$ till gas evolution ceased. 40 mL of water were then added and the solution was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and the volatiles were concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc:hexane = 1:1 v/v) to give compound **7** (0.85 g, 2.26 mmol) in 85% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD) δ 8.12 (d, *J* = 8.1 Hz, 1H), 6.11 (d, *J* = 18.3 Hz, 1H), 5.72 (d, *J* = 8.1 Hz, 1H), 4.14 (dd, *J* = 22.2, 9.1 Hz, 1H), 4.01 (dd, *J* = 12.5, 1.9 Hz, 1H), 3.95 (dd, *J* = 9.0, 1.5 Hz, 1H), 3.74 (dd, *J* = 12.5, 2.2 Hz, 1H), 1.33 (d, *J* = 22.0 Hz, 3H), 0.94 (s, 9H), 0.18 (s, 3H), 0.15 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.5, 165.9, 152.3, 141.9, 103.0, 102.3, 100.5, 90.8, 90.4, 83.5, 73.1, 72.9, 59.5, 26.2, 18.9, 17.7, 17.4, -4.1, -4.3; MS-ESI+ *m/z* 375 ($M+H^+$); HRMS-ESI+: *m/z* calcd. for $C_{16}H_{28}N_2O_5FSi$ ($M+H^+$) 375.1746, found 375.1738.



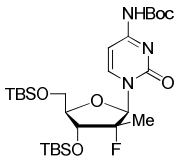
9-[5′,6′-Vinyl-6′-(bispivaloyloxymethylphosphinyl)-2′-deoxy-2′-α-fluoro-2′-β-C-methyl-D-ribo-hexofuranosyl] uridine (8)

Oxidation - Method A: To a solution of compound **7** (0.10 g, 0.26 mmol) in 2 mL of ACN was added 2-iodoxybenzoic acid (IBX) (0.02 g, 0.53 mmol). After stirring for 2 h at 80 ºC, the reaction mixture was concentrated under reduced pressure. The residue was then dissolved in a 1:1 AcOEt:THF mixture (5 mL) and filtrated through a short silica gel pad using 20 mL of a AcOEt:THF (1:1 v/v) mixture. The collected fractions were concentrated under reduced pressure and dried under high vacuum for 1 h to afford the crude aldehyde.

[3] Clark, J. L.; Hollecker, L.; Mason, J. C.; Stuyver, L. J.; Tharnish, P. M.; Lostia, S.; McBrayer, T. R.; Schinazi, R.; Watanabe, K. A.; Otto,M. J.; Furman, P.A.; Stec,W. J.; Patterson, S. E.; Pankiewicz, K. W. *J. Med. Chem.* **2005**, *48*, 5504–5508.

Oxidation - Method B: To a solution of compound **7** (0.10 g, 0.26 mmol) in 2.5 mL of CH_2Cl_2 was added Dess-Martin periodinane (DMP) (0.215 g, 0.50 mmol). After stirring at room temperature for 4 h, the reaction mixture was concentrated under reduced pressure. The residue was then dissolved in a 1:1 AcOEt:THF mixture (5 mL) and filtrated through a short silica gel pad using 20 mL of a AcOEt:THF (1:1 v/v) mixture. The collected fractions were concentrated under reduced pressure and dried under high vacuum for 1 h to afford the crude aldehyde.

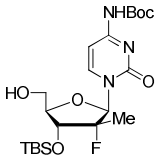
Subsequently, to a solution of the aldehyde in 2 mL of THF was added dropwise, at 0 ^oC, a freshly prepared solution of tetra(POM)-bisphophonate sodium salt (prepared by addition, at 0 ^oC, of bisphosphonate **5** (0.42 g, 0.67 mmol) to a suspension of NaH (0.03 g, 0.64 mmol) in 1.5 mL of THF and stirring for 10 min). The reaction mixture was then stirred at room temperature for 2 h before addition of 15 mL of a saturated solution of NH_4Cl. The mixture was then extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with water (2 x 10 mL) and brine (10 mL) and the resulting solution was dried over Na_2SO_4. The collected solution was concentrated under reduced pressure and the residue was purified on silica gel column chromatography (Hexane: EtOAc = 70:30 v/v) to give the title compound **8** (0.14 g, 0.21 mmol) as a mixture of E/Z isomers (95/5) in 84% yield over 2 steps. An analytic sample of pure *E* isomer was isolated and characterized: ^1H NMR (400 MHz, $CDCl_3$) δ 8.96 (s, 1H), 7.21 (d, *J* = 8.2 Hz, 1H), 6.90 (ddd, *J* = 23.6, 17.2, 5.1 Hz, 1H), 6.16–6.06 (m, 2H), 5.82 (dd, *J* = 8.2, 1.0 Hz, 1H), 5.72–5.63 (m, 5H), 4.52–4.42 (m, 1H), 3.71 (dd, *J* = 19.6, 8.9 Hz, 1H), 1.70 (s, 3H), 1.33 (d, *J* = 22.0 Hz, 3H), 1.22 (s, 18H), 0.92 (s, 9H), 0.10 (s, 3H), 0.09 (s,3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.1, 178.0, 175.5, 165.7, 152.0, 150.3, 150.2, 121.2, 119.2, 103.6, 101.7, 99.9, 83.2, 83.1 (2 signals), 83.00, 82.8, 82.5, 79.2, 79.1, 39.8, 37.6, 27.3 (2 signals), 26.2, 18.9, 17.9, 17.6, -3.7, -3.9. ^{31}P NMR (162 MHz, CD_3OD) δ -35.11. MS-ESI+ *m/z* 679 ($M+H^+$); HRMS-ESI+: *m/z* calcd. for $C_{29}H_{48}N_2O_{11}FNaPSi$ ($M+Na^+$) 701.2647, found 701.2654.



3′,5′-bis-*O*-*tert*-Butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-N^4-*tert*-butylcarbonatecytidine (10)

To a solution of compound **9**[3] (3 g, 11.58 mmol) in 15 mL of DMF was added imidazole (3.93 g, 57.9 mmol) and *t*-butyldimethylsilyl chloride (8.74 g, 57.9 mmol) at room temperature under a N_2 atmosphere. The reaction mixture was stirred overnight at room temperature and treated with 50 mL of a saturated aqueous solution of $NaHCO_3$. After stirring for 10 min, the mixture was extracted with CH_2Cl_2 (3 x 25 mL). The combined organic

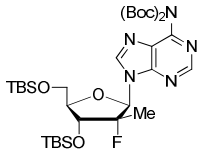
fractions were washed with water (3 x 25 mL) and then dried over Na_2SO_4. The volatiles were removed under reduced pressure and the residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 95:5 v/v) to give 3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl cytidine[4] (5.63 g, 11.56 mmol). Subsequently, to a solution of 3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl cytidine (5.63 g, 11.56 mmol) in 110 mL of THF was added Boc_2O (5.07 g, 23.19 mmol). After stirring at 60 °C overnight, the solvent was removed under pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 97:3 v/v) to give the title compound **12** (6.25g, 10.65 mmol) in 92% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD) δ 8.28 (d, *J* = 7.6 Hz, 1H), 7.31 (d, *J* = 7.6 Hz, 1H), 6.24 (d, *J* = 18.2 Hz, 1H), 4.15–4.00 (m, 3H), 3.86 (d, *J* = 11.3 Hz, 1H), 1.52 (s, 9H), 1.26 (d, *J* = 21.9 Hz, 3H), 0.98 (s, 9H), 0.92 (s, 9H), 0.17 (s, 6H), 0.15 (s, 3H), 0.13 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 165.0, 157.6, 153.3, 144.4, 102.2, 100.4, 97.1, 91.1, 90.7, 83.1, 72.7, 72.5, 61.2, 28.5, 26.7, 26.3, 19.4, 18.9, 17.7, 17.5, -3.8 (2 signals), -4.9, -5.0. MS-ESI+ *m/z* 588 ($M+H^+$); HRMS-ESI+: *m/z* calcd. for $C_{27}H_{50}N_3O_6FNaSi_2$ ($M+Na^+$) 610.3114, found 610.3102.



3′-*O*-*tert*-Butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-*N*^{4}-*tert*-butylcarbonatecytidine (11)

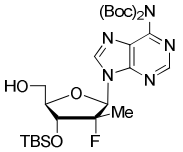
To a solution of compound **10** (0.50 g, 1.04 mmol) in 9 mL of THF was added, at 0 °C, a solution of trichloroacetic acid (4.5 g, 28 mmol) in 2.5 mL of water. After stirring for 5 h at 15 °C, the solution was neutralized with solid $NaHCO_3$ till gas evolution ceased. 40 mL of water were then added and the solution was extracted with AcOEt (3 x 15 mL). The combined organic layers were dried over Na_2SO_4. The volatiles were concentrated under reduced pressure and the residue was purified by silica gel column chromatography (Hexane:EtOAc = 60:40 v/v) to give compound the title compound **11** (0.40 g, 0.84 mmol) in 88% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 8.53 (d, *J* = 7.6 Hz, 1H), 7.30 (d, *J* = 7.6 Hz, 1H), 6.22 (d, *J* = 18.1 Hz, 1H), 4.16 (dd, *J* = 22.1, 9.1 Hz, 1H), 4.05 (dd, *J* = 12.5, 1.7 Hz, 1H), 4.00 (dd, *J* = 9.0, 1.3 Hz, 1H), 3.77 (dd, *J* = 12.5, 1.9 Hz, 1H), 1.52 (s, 9H), 1.29 (d, *J* = 22.0 Hz, 3H), 0.93 (s, 9H), 0.17 (s, 3H), 0.13 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 165.2, 157.9, 153.4, 145.5, 102.5, 100.6, 97.1, 83.6, 83.2, 72.9, 72.7, 59.4, 28.4, 26.2, 18.9, 17.7, 17.4, -4.1, -4.3. MS-ESI+ *m/z* 474 ($M+H^+$); HRMS-ESI+: *m/z* calcd. for $C_{21}H_{36}FN_3O_6NaSi$ (M+Na+) 496.2245, found 496.2242.

[4] Hecker, S. J.; Reddy, K. R. WO2009073506.



3′,5′-bis-*O*-*tert*-Butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-N^6-bis-*tert*-butylcarbonate adenine (13)

To a solution of compound **12**[5] (0.52 g, 1.82 mmol) in 5 mL of DMF was added imidazole (0.62 g, 9.10 mmol) and *t*-butyldimethylsilyl chloride (1.37 g, 9.10 mmol) at room temperature under a N_2 atmosphere. The reaction mixture was stirred overnight at room temperature and treated with 25 mL of a saturated solution of $NaHCO_3$. After stirring for 10 min, the mixture was extracted with CH_2Cl_2 (3 x 15 mL). The combined organic fractions were washed with water (3 x 15 mL) and then dried over Na_2SO_4. The volatiles were removed under reduced pressure and the residue was purified by silica gel column chromatography (Hexane:AcOEt = 50:50 v/v) to give 3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl adenine (0.86 g, 1.68 mmol). Subsequently, to a solution of 3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl adenine (0.86 g, 1.68 mmol) in 25 mL of THF was added DMAP (0.61 g, 5.04 mmol) and Boc_2O (2.23 g, 10.23 mmol). The reaction mixture was stirred at room temperature for 5 h and treated with 55 mL of a saturated solution of $NaHCO_3$, till gas evolution ceased. The mixture was extracted with CH_2Cl_2 (3 x 25 mL) and the combined organic layers dried over Na_2SO_4. The volatiles were removed under reduced pressure and the residue was purified by silica gel column chromatography (Hexane:EtOAc = 9:1 v/v) to give the title compound **13** (1.07 g, 1.51 mmol) in 83% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (s, 1H), 8.67 (s, 1H), 6.37 (d, *J* = 15.6 Hz, 1H), 4.36 (dd, *J* = 22.4, 8.9 Hz, 1H), 4.16–4.08 (m, 2H), 3.86 (d, *J* = 11.90 Hz, 1H), 1.41 (s, 18H), 1.06 (d, *J* = 21.9 Hz, 3H), 0.89 (s, 9H), 0.95 (s, 9H), 0.14 (s, 6H), 0.11 (s, 3H), 0.06 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.6, 152.1, 150.4, 150.2, 142.5, 128.6, 101.0, 99.2, 88.6, 88.2, 83.6, 82.1, 71.2, 71.0, 60.3, 27.6, 26.1, 25.5, 18.5, 17.9, 16.9, 16.6, -4.4, -4.5, -5.3, -5.5. MS-ESI+ *m/z* 711 ($M+H^+$); HRMS-ESI+: *m/z* calcd. for $C_{33}H_{58}N_5O_7FNaSi_2$ ($M+Na^+$) 734.3751, found 734.3740.

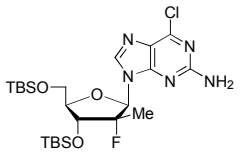


3′ -*O*-*tert*-Butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-N^6-bis-*tert*-butylcarbonate adenine (14)

Compound **14** was prepared using the same procedure as for compound **11**: yield 89%; ^{1}H NMR (400 MHz, CD_3OD) δ 9.05 (s, 1H), 8.89 (s, 1H), 6.45 (d, *J* = 16.3 Hz, 1H), 4.60 (dd, *J* = 22.5, 8.9 Hz, 1H), 4.14 (d, *J* = 9.1

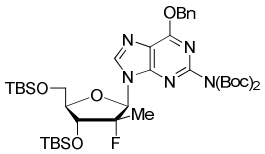
[5] Reddy, P.G.; Chun, B.-K.; Zhang, H.-R.; Rachakonda, S.; Ross, B.S.; Sofia, M.J. *J. Org. Chem.*, **2011**, *76* (10), 3782–3790

Hz, 1H), 4.09 (dd, *J* = 12.4, 1.9 Hz, 1H), 3.85 (dd, *J* = 12.5, 2.7 Hz, 1H), 1.39 (s, 18H), 1.13 (d, *J* = 21.9 Hz, 3H), 0.95 (s, 9H), 0.21 (s, 3H), 0.14 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 154.2, 153.4, 151.4, 151.3, 146.1, 130.4, 102.5, 100.7, 90.8, 90.4, 85.4, 84.3, 73.0, 72.8, 60.1, 28.0, 26.2, 18.9, 17.6, 17.3, -4.1, -4.3. MS-ESI+ *m/z* 598 ($M+H^+$); HRMS-ESI+: *m/z* calcd. for $C_{27}H_{44}N_5O_7FNaSi$ ($M+Na^+$) 620.2886, found 620.2876.



3′,5′-bis-*O-tert*-Butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-2-amino-6-chloro-purine ribonucleoside (16)

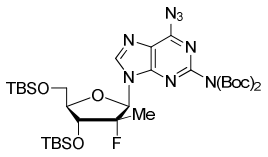
To a solution of compound **15**[5] (1.62 g, 5.14 mmol) in 10 mL of DMF was added imidazole (1.71 g, 25.17 mmol) and *t*-butyldimethylsilyl chloride (3.88 g, 25.17 mmol) at under a N_2 atmosphere. The reaction mixture was stirred overnight at room temperature and treated with 50 mL of a saturated solution of $NaHCO_3$. After stirring for 10 min, the mixture was extracted with CH_2Cl_2 (3 x 25 mL). The combined organic fractions were washed with water (3 x 25 mL) and brine (25 mL) and then dried over Na_2SO_4. The volatiles were removed under reduced pressure and the residue was purified by silica gel column chromatography (Hexane:AcOEt = 8:2 v/v) to give title compound **16** (2.51 g, 4.6 mmol) in 89% yield. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.30 (s, 1H), 6.15 (d, *J* = 16.8 Hz, 1H), 4.36 (dd, *J* = 22.3, 8.9 Hz, 1H), 4.07 (ddd, *J* = 10.6, 10.1, 1.4 Hz, 2H), 3.88 (dd, *J* = 12.1, 2.0 Hz, 1H), 1.15 (d, *J* = 22.0 Hz, 3H), 0.95 (s, 9H), 0.90 (s, 9H), 0.16 (s, 3H), 0.15 (s, 6H), 0.10 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 161.7, 154.6, 152.0, 141.1, 125.0, 102.3, 100.5, 89.8, 89.4, 83.5, 72.8, 72.7, 61.7, 26.8, 26.3, 19.5, 18.9, 17.8, 17.6, -3.9, -4.9, -5.0. MS-ESI+ *m/z* 546 ($M+H^+$); HRMS-ESI+: *m/z* calcd. for $C_{23}H_{42}N_5O_3ClSi_2$ ($M+H^+$) 546.2493, found 546.2495.



3′,5′-bis-*O-tert*-Butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-6-benzyloxy-N^2-bis-*tert*-butylcarbonatepurine ribonucleoside (17)

To a solution of compound **16** (0.60 g, 1.10 mmol) in 10 mL of THF was added 0.90 mL of a sodium benzyloxide solution in benzyl alcohol (freshly prepared by addition of NaH (0.13 g, 3.30 mmol) in 0.90 mL of butanol and stirring for 10 minutes). The reaction mixture was stirred at room temperature for 1 h and treated with 50 mL of a saturated solution of NH_4Cl. The mixture was extracted with CH_2Cl_2 (3 x 25 mL) and the

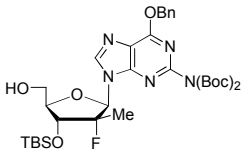
combined organic layers dried over Na_2SO_4. The volatiles were removed under reduced pressure to give the crude 3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-2-amino-6-benzyloxy-purine ribonucleoside. Subsequently, to a solution of 3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-2-amino-6-benzyloxy-purine ribonucleoside (0.68 g, 1.10 mmol) in 20 mL of THF was added DMAP (0.40 g, 3.30 mmol) and Boc_2O (1.43 g, 6.56 mmol). The reaction mixture was stirred at room temperature for 3 h and treated with 50 mL of a saturated aqueous solution of $NaHCO_3$. The mixture was extracted with CH_2Cl_2 (3 x 25 mL) and the combined organic layers dried over Na_2SO_4. The volatiles were removed under reduced pressure and the residue was purified by silica gel column chromatography (Hexane:EtOAc = 85:15 v/v) to give the title compound **17** (0.85 g, 1.04 mmol) in 87% yield over 2 steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (s, 1H), 7.50 (d, *J* = 7.0 Hz, 2H), 7.36–7.26 (m, 3H), 6.31 (d, *J* = 16.0 Hz, 1H), 5.67 (d, *J* = 12.1 Hz, 1H), 5.57 (d, *J* = 12.2 Hz, 1H), 4.36 (dd, *J* = 22.5, 8.8 Hz, 1H), 4.13 (t, *J* = 11.2 Hz, 2H), 3.87 (d, *J* = 11.7 Hz, 1H); 1.38 (s, 18H), 1.07 (d, *J* = 21.9 Hz, 3H) , 0.97 (s, 9H), 0.90 (s, 9H), 0.17 (s, 3H), 0.16 (s, 3H), 0.12 (s, 3H), 0.07 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.1, 160.8, 152.3, 151.7, 150.5, 141.0, 135.7, 128.6, 128.4, 128.2, 120.1, 101.0, 99.2, 88.7, 88.3, 83.0, 82.1, 71.0, 70.9, 68.7, 60.3, 27.8, 26.2, 25.6, 18.6, 17.9, 16.8, 16.5, -4.3, -4.5, -5.3 (2 signals). MS-ESI+ *m/z* 818 ($M+H^+$); HRMS-ESI+: *m/z* calcd. for $C_{40}H_{64}N_5O_8FNaSi_2$ ($M+Na^+$) 840.4170, found 840.4157.



3′,5′-bis-*O*-*tert*-Butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-6-azido-N^2-bis-*tert*-butylcarbonatepurine ribonucleoside (20)

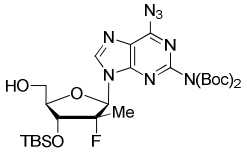
To a solution of **18** (0.69 g, 1.26 mmol) in 20 mL of DMF was added NaN_3 (0.41 g, 6.33 mmol). The reaction mixture was stirred at 60 ^{o}C for 12 h and treated with 50 mL of a saturated solution of $NaHCO_3$. The mixture was extracted with AcOEt (3 x 25 mL) and the combined organic layers washed with water (2x 25 mL), brine (25 mL) and dried over Na_2SO_4. The volatiles were removed under reduced pressure and the residue was purified by silica gel column chromatography (Hexane:EtOAc = 70:30 v/v) to give the 3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-6-azido-2-amino-purine ribonucleoside (0.63 g, 1.20 mmol). Subsequently, to a solution of 3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-6-azido-2-amino-purine ribonucleoside (0.63 g, 1.20 mmol) in 20 mL of THF was added DMAP (0.44 g, 3.60 mmol) and Boc_2O (1.57 g, 7.2 mmol). The reaction mixture was stirred at room temperature for 3 h and treated with 50 mL of a saturated solution of $NaHCO_3$. The mixture was extracted with CH_2Cl_2 (3 x 25 mL) and the

combined organic layers dried over Na_2SO_4. The volatiles were removed under reduced pressure and the residue was purified by silica gel column chromatography (Hexane:EtOAc = 90:10 v/v) to give the title compound **20** (0.80 g, 1.07 mmol) in 94% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 6.23 (d, *J* = 15.8 Hz, 1H), 4.27 (dd, *J* = 22.3, 8.9 Hz, 1H), 4.07 (t, *J* = 9.8 Hz, 2H), 3.82 (d, *J* = 10.7 Hz, 1H), 1.38 (s, 18H), 1.03 (d, *J* = 21.8 Hz, 3H), 0.91 (s, 9H), 0.84 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H), 0.06 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 153.4, 152.5, 152.1, 150.4, 142.5, 122.3, 100.8, 98.9, 88.6, 88.2, 83.1, 82.1, 70.9, 70.8, 60.2, 27.6, 26.0, 25.4, 18.4, 17.7, 16.7, 16.4, -4.5, -4.6, -5.5, -5.6. MS-ESI+ *m/z* 753 ($M+H^+$); HRMS-ESI+: *m/z* calcd. for $C_{33}H_{57}N_8O_7FNaSi_2$ ($M+Na^+$) 775.3765, found 775.3749.



3′ -*O*-*tert*-Butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-6-benzyloxy-*N*^{2}-bis-*tert*-butylcarbonatepurine ribonucleoside (19)

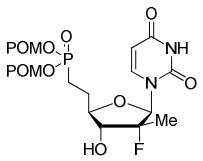
Compound **19** was prepared using the same procedure as for compound **11**: yield 88%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.81 (s, 1H), 7.53–7.49 (m, 2H), 7.38–7.28 (m, 3H), 6.29 (d, *J* = 17.0 Hz, 1H), 5.67 (s, 2H), 4.70 (dd, *J* = 22.6, 8.9 Hz, 1H), 4.13–4.08 (m, 1H), 4.02 (dd, *J* = 12.4, 1.8 Hz, 1H), 3.81 (dd, *J* = 12.4, 3.69 Hz, 1H), 1.40 (s, 18H), 1.17 (d, *J* = 22.1 Hz, 3H), 0.94 (s, 9H), 0.19 (s, 3H), 0.14 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 162.3, 153.3, 152.9, 152.0, 144.6, 137.3, 129.7, 129.5, 129.4, 121.2, 102.5, 100.6, 91.2, 90.8, 84.8, 84.4, 73.4, 73.3, 70.1, 60.9, 28.2, 26.3, 18.9, 17.7, 17.5, -3.9, -4.1. MS-ESI+ *m/z* 704 ($M+H^+$); HRMS-ESI+: *m/z* calcd. for $C_{34}H_{50}N_5O_8FNaSi$ ($M+Na^+$) 726.3305, found 726.3293.



3′-*O*-*tert*-Butyldimethylsilyl-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-6-azido-*N*^{2}-bis-*tert*-butylcarbonatepurine ribonucleoside (20)

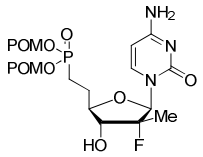
Compound **20** was prepared using the same procedure as for compound **11**: yield 88%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.89 (s, 1H), 6.30 (d, *J* = 16.7 Hz, 1H), 4.64 (dd, *J* = 22.4, 8.9 Hz, 1H), 4.12 (d, *J* = 8.8 Hz, 1H), 4.04 (dd, *J* = 12.4, 1.7 Hz, 1H), 3.82 (dd, *J* = 12.4, 3.4 Hz, 1H), 1.46 (s, 18H), 1.18 (d, *J* = 22.1 Hz, 3H), 0.94 (s, 9H), 0.20 (s, 3H), 0.14 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 155.1, 153.8, 153.2, 152.0, 145.9, 123.5,

102.4, 100.6, 91.1, 90.7, 85.0, 84.4, 73.3, 73.1, 60.6, 28.2, 26.3, 18.9, 17.7, 17.5, -3.9, -4.1. MS-ESI+ *m/z* 639 ($M+H^+$); HRMS-ESI+: *m/z* calcd. for $C_{27}H_{43}N_8O_7FNaSi$ ($M+Na^+$) 661.2900, found 661.2890.



9-[5′,6′-Dideoxy-6′-(bispivaloyloxymethylphosphinyl)-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-D-ribo-hexofuranosyl] uridine (21)

A solution of **8** (0.05 g, 0.07 mmol) in 5 mL of $HCOOH/H_2O$ (1:1, v:v) was stirred at room temperature for 48 h. After evaporation of the volatiles under vacuum at 30 °C, the residue was dissolved in 5 mL of EtOH/AcOEt (1:1, v:v) and Pd/C (0.01 g, 10% Pd on activated carbon) was added. The mixture was stirred for 1 minute under an atmosphere of H_2 (1 atm, balloon) and then filtered on short pad of celite and washed with EtOH (10 mL x 3). The collected solution was concentrated under reduced pressure and the residue was purified on silica gel column chromatography ($MeOH:CH_2Cl_2$ = 7:93 v/v) to give the title compound **21** (0.04 g, 0.07 mmol) in 84% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.48 (d, *J* = 8.1 Hz, 1H), 6.15–5.93 (br s, 1H), 5.77–5.64 (m, 5H), 3.88 (t, *J* = 8.0,1H), 3.82–3.57 (br s, 1H), 2.24–1.91 (m, 4H), 1.33 (d, *J* = 22.5 Hz, 3H), 1.23 (s, 18H); ^{13}C NMR (100 MHz, CD_3OD) δ 178.2, 165.7, 152.0, 103.4, 102.6, 100.8, 83.0 (2 signals), 81.6, 81.4, 78.4, 78.2, 39.8, 27.3, 26.3 (2 signals), 24.4, 23.0, 17.2, 17.0; ^{31}P NMR (162 MHz, CD_3OD) δ -17.90. MS-ESI+ *m/z* 567 ($M+H^+$); HRMS-ESI+: *m/z* calcd. for $C_{23}H_{36}N_2O_{11}FNaP$ ($M+Na^+$) 589.1933, found 589.1923.



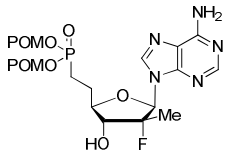
9-[5′,6′-Dideoxy-6′-(bispivaloyloxymethylphosphinyl)-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-D-ribo-hexofuranosyl] cytidine (22)

To a solution of compound **11** (0.05 g, 0.11 mmol) in 2 mL of ACN was added 2-iodoxybenzoic acid (IBX) (0.6 g, 0.21 mmol). After stirring for 2 h at 80 ºC, the reaction mixture was concentrated under reduced pressure. The residue was then dissolved in 5 mL of a AcOEt:THF mixture (1:1 v/v) and filtrated through a short silica gel pad using 30 mL of a AcOEt:THF mixture (1:1 v/v). The collected fractions were concentrated under reduced pressure and dried under high vacuum for 1 h to afford the crude aldehyde.

Subsequently, to a solution of the aldehyde in 5 mL of THF was added dropwise, at 0^{o}C, a freshly prepared solution of bisphophonate sodium salt (prepared by addition, at 0^{o}C, of bisphosphonate **5** (0.17 g, 0.26 mmol) to

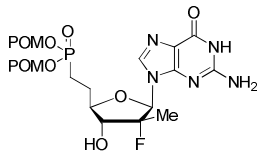
a suspension of NaH (0.01 g, 0.25 mmol) in 1.3 mL of THF and stirring for 10 min). The reaction mixture was then stirred at room temperature for 2 h before addition of 25 mL of a saturated solution of NH_4Cl. The mixture was then extracted with EtOAc (3 x 25 mL). The combined organic layers were washed with water (2 x 25 mL) and brine (25 mL) and the resulting solution was dried over Na_2SO_4. The collected solution was concentrated under reduced pressure and the residue was partially purified on silica gel column chromatography (MeOH: EtOAc = 1:10 v/v) to give the desired bis(POM)-vinylphosphonate cytidine derivative as a mixture with the excess of bisphosphonate **5**.

A solution of crude bis(POM)-vinylphosphonate cytidine in 5 mL of HCOOH/H_2O (1:1, v:v) was stirred at room temperature for 48 h. After evaporation of the volatiles, the residue was dissolved in 5 mL of EtOH/AcOEt (1:1, v:v) and Pd/C (0.10 g, 10% Pd on activated carbon) was added. The mixture was stirred for 1 h under an atmosphere of H_2 (1 atm, balloon) and then filtered on short pad of celite and washed with EtOH (10 mL x 3). The collected solution was concentrated under reduced pressure and the residue was purified on silica gel column chromatography (MeOH:CH_2Cl_2 = 7:93 v/v) to give the title compound **22** (0.04 g, 0.07 mmol) in 60% yield over 4 steps. ^{1}H NMR (400 MHz, CD_3OD) δ 7.47 (d, *J* = 7.5 Hz, 1H), 6.34–6.06 (br s, 1H), 5.95 (d, *J* = 7.5 Hz, 1H), 5.76–5.61 (m, 4H), 3.88 (t, *J* = 8.0 Hz, 1H), 3.72–3.42 (br s, 1H), 2.21–1.94 (m, 4H), 1.33–1.20 (m, 21H); ^{13}C NMR (100 MHz, CD_3OD) δ 178.2, 167.6, 158.1, 102.8, 101.0, 96.8, 83.1, 83.0 (2 signals), 81.2 (3 signals), 81.1, 78.6, 78.4, 39.8, 27.3, 26.4 (2 signals), 24.5, 23.1, 17.3, 17.0; ^{31}P NMR (162 MHz, CD_3OD) δ ppm -17.84. MS-ESI+ *m/z* 566 ($M+H^+$); HRMS-ESI+: *m/z* calcd. for $C_{23}H_{37}N_3O_{10}FNaP$ ($M+Na^+$) 588.2093, found 588.2087.



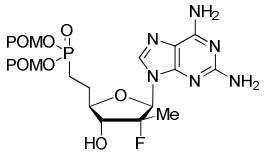
9-[5′,6′-Dideoxy-6′-(bispivaloyloxymethylphosphinyl)-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-D-ribo-hexofuranosyl]adenine (23)

Compound **23** was prepared using the same procedure as for compound **22**: yield 64% over 4 steps; ^{1}H NMR (400 MHz, CD_3OD) δ 8.22 (s, 1H), 8.19 (s, 1H), 6.20 (d, *J* = 19.6 Hz, 1H), 5.72–5.62 (m, 4H), 4.48 (dd, *J* = 23.6, 9.1 Hz, 1H), 4.02–3.98 (m, 1H), 2.23–2.04 (m, 4H), 1.22–1.16 (m, 21H); ^{13}C NMR (100 MHz, CD_3OD) δ 178.2, 178.1, 157.5, 154.1, 150.2, 141.7, 120.8, 103.1, 101.3, 91.5, 91.1, 83.0 (3 signals), 82.9, 82.1, 81.9, 77.7, 77.5, 39.8, 39.7, 27.3, 27.2, 26.21 (2 signals), 24.4, 23.0, 17.1, 16.9; ^{31}P NMR (162 MHz, CD_3OD) δ -17.55. MS-ESI+ *m/z* 590 ($M+H^+$); HRMS-ESI+: *m/z* calcd. for $C_{24}H_{37}N_5O_9FNaP$ ($M+Na^+$) 612.2205, found 612.2213.



9-[5′,6′-Dideoxy-6′-(bispivaloyloxymethylphosphinyl)-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-D-ribo-hexofuranosyl]guanosine (24)

Compound **26** was prepared using the same procedure as for compound **22**: Reaction time 6 h; :yield 67% over 4 steps; ^{1}H NMR (400 MHz, CD_3OD) δ 7.79 (s, 1H), 6.03 (d, *J* = 20.1 Hz, 1H), 5.72–5.63 (m, 4H), 4.33 (dd, *J* = 23.1, 7.6 Hz, 1H), 3.96 (t, *J* = 7.6 Hz, 1H), 2.25–2.01 (m, 4H), 1.28–1.17 (m, 21H); ^{13}C NMR (100 MHz, CD_3OD) δ 178.2, 159.4, 155.3, 152.5, 138.5, 118.4, 103.1, 101.3, 91.2, 90.8, 83.1, 83.0, 81.9, 81.7, 77.7, 77.5, 39.8, 27.3, 26.1 (2 signals), 24.2, 22.8, 17.3, 17.0; ^{31}P NMR (162 MHz, CD_3OD) δ -17.31. MS-ESI+ *m/z* 606 ($M+H^+$); HRMS-ESI+: *m/z* calcd. for $C_{24}H_{37}N_5O_{10}FNaP$ ($M+Na^+$) 628.2154, found 628.2145.



9-[5′,6′-Dideoxy-6′-(bispivaloyloxymethylphosphinyl)-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyl-D-ribo-hexofuranosyl]-2,6-diaminopurine nucleoside (25)

Compound **27** was prepared using the same procedure as for compound **22**: Reaction time 5 h; yield 60% over 4 steps; ^{1}H NMR (400 MHz, CD_3OD) δ 7.82 (s, 1H), 6.04 (d, *J* = 19.9 Hz, 1H), 5.72–5.63 (m, 4H), 4.40 (dd, *J* = 23.5, 8.8 Hz, 1H), 3.99–3.95 (m, 1H), 2.23–2.05 (m, 4H), 1.25–1.17 (m, 21H); ^{13}C NMR (100 MHz, CD_3OD) δ 178.2, 161.7, 157.6, 152.5, 138.5, 114.7, 103.2, 101.4, 91.0, 90.6, 83.0 (2 signals), 81.8, 81.6, 77.7, 77.6, 39.8, 27.3, 26.1 (2 signals), 24.2, 22.8, 17.2, 16.9; ^{31}P NMR (162 MHz, CD_3OD) δ -17.28. MS-ESI+ *m/z* 605 ($M+H^+$); HRMS-ESI+: *m/z* calcd. for $C_{24}H_{38}N_6O_9FNaP$ ($M+Na^+$) 627.2314, found 627.2307.