Supporting Information

Site-specific incorporation of 5’-methyl DNA enhances the therapeutic profile of gapmer ASOs

Guillermo Vasquez, Graeme Freestone, W. Brad Wan, Audrey Low, Cheryl Li De Hoyos, Jinghua Yu, Thazha P. Prakash, Michael E. Oestergaard, Xue-hai Liang, Stanley T. Crooke, Eric E. Swayze, Michael T. Migawa*, Punit P. Seth*

Ionis Pharmaceuticals, 2855 Gazelle Court, Carlsbad, CA, 92010, USA
*mmigawa@ionisph.com, tel 760-603-2713
*pseth@ionisph.com, tel 760-760-2587

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1) Synthesis of Monomers

**General.** All reagents were purchased from commercial vendors and used without any further purification. Unless specified otherwise, glassware was dried in an oven and the reactions were carried out under an atmosphere of nitrogen. $^1$H-NMR spectra were obtained on a Bruker 300 MHz instrument. Low resolution mass spectrometry analysis were carried out on an Agilent 1100 series LCMS system equipped with a S.E.D.E.R.E. (France) Sedex 75 Evaporative Light Scattering detector.

**Scheme S1.** Synthesis of 5’-(R)- and (S)-Methyl Thymidine Nucleosides.

**Compound 1:** 1,2:5,6-Di-O-isopropylidene-alpha-D-allofuranose (100 g, 0.385 mol) was dissolved in dry N,N-dimethylformamide (1L) and the solution was cooled in an ice bath. NaH (23 g, 0.577 mol) was added to the above solution carefully in portions over 15-20 minutes. The reaction mixture was stirred in an ice bath for 30 minutes and BnBr (50.25 mL, 0.423 mol) was introduced slowly. The reaction was warmed to room temperature and stirred for 12 hours. The
reaction was quenched with ice water (2 L) and extracted with ethyl acetate (2 x 800 mL). The resulting ethyl acetate solution was dried over Na₂SO₄ and concentrated to dryness. The crude product was purified by silica gel column chromatography and eluted with 20% ethyl acetate in hexanes. Desired product was obtained as white solid (109 g, 81%). ¹H NMR (300 MHz, CDCl₃) δ 7.29-7.47 (m, 5H), 5.76 (d, J=3.77 Hz, 1H), 4.55-4.84 (m, 3H), 4.38 (dt, J=3.11, 7.02 Hz, 1H), 4.15 (dd, J=3.20, 8.67 Hz, 1H), 3.94-4.06 (m, 2H), 3.89 (dd, J=4.62, 8.57 Hz, 1H), 1.60 (s, 3H), 1.29-1.42 (m, 9H); ¹³C NMR (75 MHz, CDCl₃) δ 137.5, 128.4, 128.2, 128.0, 112.9, 109.7, 103.9, 78.0, 77.5, 74.8, 72.2, 65.0, 26.8, 26.6, 26.2, 25.1; LRMS (ES, positive): m/z calcd for C₁₉H₂₆O₆: 350.2, found 373.1 [m+23]+

Water (55 mL) was added to a solution of 1,2:5,6-Di-O-isopropylidene-3-OBn-alpha-D-allofuranose (109 g, 0.31 mol) in acetic acid (500 mL). The mixture was warmed to 40 ºC and stirred for 12 hours. Ethyl acetate (1 L) was added to the reaction and the organic phase was washed with brine, saturated NaHCO₃, brine, dried over Na₂SO₄ and concentrated to dryness. The crude product was obtained as light-yellow goo and used without any further purification (56.46 g, 59%). ¹H NMR (300 MHz, CDCl₃) δ 7.29-7.42 (m, 5H), 5.77 (d, J=3.77 Hz, 1H), 4.79 (d, J=11.30 Hz, 1H), 4.62 (t, J=4.05 Hz, 1H), 4.56 (d, J=11.30 Hz, 1H), 4.09-4.15 (m, 1H), 3.98-4.06 (m, 1H), 3.89-3.97 (m, 1H), 3.65-3.73 (m, 2H), 2.57 (br d, J=2.83 Hz, 1H), 2.49 (br s, 1H), 1.60 (s, 3H), 1.37 (s, 3H); LRMS (ES, positive): m/z calcd for C₁₆H₂₂O₆: 310.1, found 333.1 [m+23]+

The bis-alcohol (56.10 g, 0.18 mol) obtained above was dissolved in a mixture of dichloromethane (270 mL) and pyridine (147 mL) and the reaction was cooled in an ice bath. To this solution, a solution of TsCl (42 g, 0.22 mol) in dichloromethane (160 mL) was added slowly. The reaction was stirred in an ice bath for 3 hours and carefully quenched with saturated NaHCO₃. The mixture was extracted with dichloromethane, washed with water, brine, dried over Na₂SO₄ and concentrated to dryness (105 g brown oil). The tosylated sugar (105 g, 0.18 mol) obtained above was dissolved in THF (800 mL) and the reaction was cooled in an ice bath. Lithium aluminum hydride (25 g, 0.66 mol) was added slowly to the reaction over 15-20 minutes. The reaction was stirred in an ice bath for 2 hours and water (18 mL) was carefully added to quench the unreacted hydride. After 20 minutes of stirring, 4 N NaOH (18 mL) was
added and the reaction mixture was stirred for another 20 minutes. To the mixture, more water (54 mL) was added and the reaction was stirred for another 15 minutes. Diethyl ether (1.5 L) was added to the reaction and mixture was stirred for an hour. The mixture was filtered through a pad of celite and rinsed sequentially with diethyl ether, dichloromethane, ethyl acetate. The organic solution was concentrated to dryness. The crude product was purified by column chromatography (silica gel, eluting with ethyl acetate/hexanes and MeOH/dichloromethane). Desired product was obtained as light-yellow oil (37.4 g, 72%).

\[1^H NMR \ (300 MHz, CDCl_3) \delta \]

\[7.29-7.48 \ (m, 5H), \ 5.74 \ (d, J=3.77 \ Hz, 1H), \ 4.52-4.82 \ (m, 3H), \ 3.98-4.18 \ (m, 2H), \ 3.82-3.96 \ (m, 1H), \ 2.15 \ (d, J=2.45 \ Hz, 1H), \ 1.61 \ (s, 4H), \ 1.37 \ (s, 3H), \ 1.23 \ (d, J=6.59 \ Hz, 3H); \ \text{LRMS (ES, positive): } m/z \ \text{calcd for C}_{16}H_{22}O_5: 294.1, \ \text{found 317.1 [m+23]^+} \]

The 5'-methyl sugar (35.4 g, 0.12 mol) obtained above was dissolved in dichloromethane (700 mL) and triethylamine (33.6 mL, 0.24 mol) and the reaction was cooled in an ice bath. BzCl (16.7 mL, 0.14 mol) was added slowly and the reaction mixture was warmed to room temperature and stirred for 12 hours. The reaction was quenched with MeOH (10 mL) and stirred for 30 minutes. The dichloromethane solution was washed with 1 N HCl aqueous solution, saturated sodium bicarbonate, brine, dried over Na_2SO_4 and concentrated to dryness. The crude product was purified by column chromatography (silica gel, eluting with ethyl acetate/hexanes). Desired product was obtained as white solid (38.9 g, 81%).

\[1^H NMR \ (300 MHz, CDCl_3) \delta \]

\[7.90-7.98 \ (m, 2H), \ 7.50-7.59 \ (m, 1H), \ 7.34-7.44 \ (m, 2H), \ 7.31 \ (s, 5H), \ 5.65-5.84 \ (m, 1H), \ 5.26-5.51 \ (m, 1H), \ 4.78 \ (dd, J=1.04, 11.77 Hz, 1H), \ 4.63 \ (t, J=3.96 Hz, 1H), \ 4.52 \ (dd, J=1.32, 11.68 Hz, 1H), \ 4.21 \ (ddd, J=1.13, 4.10, 8.71 Hz, 1H), \ 3.92 \ (ddd, J=1.32, 4.43, 8.76 Hz, 1H), \ 1.51-1.70 \ (m, \ 3H), \ 1.31-1.43 \ (m, 6H); \ \text{LRMS (ES, positive): } m/z \ \text{calcd for C}_{23}H_{26}O_6: 398.2, \ \text{found 421.1 [m+23]^+} \]

**Compound 2:** Sugar (1) (150 g, 376 mmol) was dissolved in ethyl acetate (1.0 L) and the solution was stirred under nitrogen. Acetic anhydride (116 g, 107 mL g, 113 mmol, 3 eq.) was added followed by dropwise addition of sulfuric acid (7.4 g, 8.0 mL 125 mmol, 0.4eq). The reaction continued to stir for 3 hours at which time, TLC analysis (40% EtOAc in hexanes)

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indicated that the reaction was completed. The reaction was concentrated to a reduced volume under reduced pressure, diluted with ethyl acetate and the organic layer was carefully washed with aqueous saturated sodium bicarbonate. The organic layer was separated, washed with brine, dried over Na$_2$SO$_4$, filtered, concentrated to a crude oil and evaporated three times with acetonitrile at 40°C and then dried under high vacuum over P$_2$O$_5$ for 16 hours to afford the desired product as a colorless oil (153 g, 92 % yield). This material was split into portions and used as is without further purification. $^1$H NMR (300MHz, CHLOROFORM-d) $\delta$ = 8.10 - 7.87 (m, 2H), 7.60 - 7.31 (m, 4H), 7.27 - 7.13 (m, 2H), 5.43 - 5.06 (m, 2H), 4.65 - 4.36 (m, 2H), 4.33 - 4.25 (m, 1H), 4.23 - 4.08 (m, 1H), 4.04 (dd, $J$=3.5, 5.7 Hz, 1H), 2.19 - 1.98 (m, 4H), 1.52 - 1.18 (m, 3H). $^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta$ = 176.78, 166.05, 165.77, 137.02, 136.63, 133.23, 133.16, 133.10, 133.08, 130.20, 130.14, 130.00, 129.87, 129.80, 129.78, 129.66, 128.66, 128.59, 129.03 (br dd, $J$=9.0, 95.6 Hz, 1C), 128.46 (q, $J$=8.4 Hz, 1C), 128.28, 128.13 (q, $J$=5.6 Hz, 1C), 127.91, 101.97, 99.90, 96.87, 83.40, 82.80, 82.51, 79.16, 73.50, 73.13, 73.10, 73.05, 72.95, 72.42, 71.81, 71.31, 70.98, 70.87, 70.64, 20.86, 16.54, 16.48, 16.36, 20.74.

Thymine (31.9 g, 133 mmol, 1.3 eq) and N,O-Bis(trimethylsilyl)acetamide (339.0 mL, 1.387 mol, 4.0 eq) were added to a solution of the crude sugar above (153 g, 0.347mol) in acetonitrile (1.0 L). The reaction was heated at 80°C for 20 minutes to obtain a clear solution, after which the solution was cooled in an ice bath and trimethylsilyl trifluoromethanesulphonate (123 g, 0.555mol, 1.6 eq) was added slowly over 10 minutes. The reaction was warmed to 80°C and stirred for 3 hours at which time TLC analysis (50% ethyl acetate in hexane/EtOAc) indicated that the reaction was complete. The solvent was evaporated under reduced pressure to obtain a crude oil which was dissolved in ethyl acetate (500 mL) and the organic layer was sequentially washed with water, saturated sodium bicarbonate, brine, dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure to obtain a crude oil. Purification by column chromatography (silica gel, eluting with 0-10% dichloromethane/acetone) afforded the desired product as a white solid. 146 g, 83 % yield. $^1$H NMR (300MHz, CHLOROFORM-d) $\delta$ = 8.79 (br s, 1H), 8.04 (d, $J$=7.4 Hz, 3H), 7.64 - 7.54 (m, 1H), 7.53 - 7.41 (m, 3H), 7.41 - 7.23 (m, 7H), 6.93 - 6.77 (m, 1H), 6.09 (d, $J$=5.6 Hz, 1H), 5.46 (br dd, $J$=3.0, 6.7 Hz, 2H), 5.32 (t, $J$=5.6 Hz, 1H), 4.71 - 4.39 (m, 4H), 4.21 (dd, $J$=3.1, 4.4 Hz, 1H), 2.20 - 2.02 (m, 4H), 1.52 (s, 1H), 1.45 - 1.26 (m, 7H). $^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta$ = 170.29, 165.44, 163.38, 150.16,
Compound 3. A solution of methanolic ammonia (7.0 M, 229 mL, 1.606 mol, 6.0 eq) was added to a solution of Compound 2 (139.30 g, 0.273 mol) in methanol (150.0 mL) that was previously cooled in an ice bath. The reaction was stirred at 0°C for 16 hours, and then evaporated under reduce pressure to an oil while maintaining the temperature around 0°C. The crude oil was purified by flash chromatography to afford the desired product as a white solid (126 g, 94 % yield). $^1$H NMR (300MHz, CHLOROFORM-d) $\delta$ = 9.53 (br s, 1H), 8.00 (d, $J$=7.3 Hz, 2H), 7.65 - 7.54 (m, 1H), 7.52 - 7.41 (m, 2H), 7.41 - 7.26 (m, 6H), 6.85 (s, 1H), 5.98 (d, $J$=5.3 Hz, 1H), 5.42 (br dd, $J$=3.0, 6.6 Hz, 1H), 4.82 - 4.60 (m, 2H), 4.30 - 4.20 (m, 3H), 2.13 (s, 1H), 1.52 (s, 1H), 1.42 - 1.23 (m, 7H). $^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta$ = 165.51, 163.64, 150.93, 136.69, 134.82, 133.64, 129.56, 128.88, 128.68, 128.36, 128.19, 111.53, 88.40, 84.41, 75.94, 74.03, 72.69, 70.51, 16.33, 11.70; LCMS (ESI) m/z: [M+H] calcd, 466.2; found, 467.2

Compound 4. Compound 3 (120 g, 0.257mol) and 4-dimethylaminopyridine (62.9 g, 0.514 mol, 2.0 eq) were dissolved in anhydrous acetonitrile (360 mL) and the solution was cooled in an ice bath. O-4-methylphenyl chlorothioformate (46.70 mL, 0.309 mol, 1.2 eq) was added dropwise to the solution and the ice bath was removed. The reaction was stirred for 2 hours during which the reaction gradually warmed up to room temperature. The reaction was monitored by TLC (70% ethyl acetate in hexanes) to ensure conversion of the starting material. The solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate and water. The aqueous layer was removed, and the organic layer washed successively with 10% HCl, saturated sodium bicarbonate, and brine. The organic layer was dried over Na$_2$SO$_4$, filtered and evaporated under reduce pressure. Purification by flash chromatography (silica gel, eluting with 70% diethyl ether/hexanes, followed by 100% ethyl acetate) afforded the desired product as a white solid. 143 g, 90 % yield. $^1$H NMR (300MHz, CHLOROFORM-d) $\delta$ = 8.90 (s, 1H), 8.15 - 7.98 (m, 2H), 7.63 - 7.52 (m, 1H), 7.47 - 7.30 (m, 7H), 7.29 - 7.12 (m, 2H), 6.99 - 6.89 (m, 2H), 6.84 (d, $J$=1.1 Hz, 1H), 6.25 (d, $J$=6.0 Hz, 1H), 5.83 (t, $J$=5.8 Hz, 1H), 5.46 (dd, $J$=2.8, 6.7 Hz,
Compound 5. A solution of azobisisobutyronitrile (AIBN) (4.36 g, 0.292 mol) and tributyltin hydride (156.40 mL, 0.583 mol) in toluene (200 mL) was added slowly to a degassed (with nitrogen) solution of compound 4 (144 g, 0.233 mol) in toluene (1L) at 80°C. After the addition was complete, the reaction was heated at 80°C for 1 hour and cooled to room temperature and the solvent was removed under reduced pressure. Purification by flash chromatography (silica gel, eluting with 0-40% ethyl acetate/hexanes) afforded 116g of 2’-deoxy nucleoside contaminated with some Bu3SnH. 

1H NMR (300MHz, CHLOROFORM-d) δ = 8.71 (s, 1H), 8.09 - 7.88 (m, 2H), 7.66 - 7.53 (m, 1H), 7.51 - 7.42 (m, 2H), 7.39 - 7.25 (m, 6H), 7.10 - 6.87 (m, 2H), 6.86 - 6.61 (m, 1H), 6.32 (dd, J=5.5, 8.8 Hz, 1H), 5.57 - 5.32 (m, 2H), 4.69 - 4.48 (m, 2H), 4.42 - 4.28 (m, 1H), 4.19 (dd, J=2.5, 3.6 Hz, 1H), 2.57 (ddd, J=1.4, 5.5, 13.7 Hz, 1H), 2.26 (s, 1H), 1.99 (ddd, J=6.4, 8.6, 13.8 Hz, 1H), 1.75 (s, 1H), 1.64 - 1.47 (m, 1H), 1.46 - 1.31 (m, 6H), 1.11 - 0.83 (m, 1H); LCMS (ESI) m/z: [M+H] calcd, 450.2; found, 451.2.

Compound 6. Boron trichloride (BCl3) (644 mL, 0.644 mol, 2.5 eq) was added slowly to a solution of compound 5 (110.6 g, 36.40 mmol) dissolved in anhydrous dichloromethane (500 mL) at -78°C. After the addition, the reaction was continued to stir at -78°C for 30 minutes. The reaction was quenched by slow addition of a mixture of Et3N/MeOH/DCM/ (300 mL/300 mL/300 mL), always maintaining the temperature below -60°C. The mixture was then allowed to stir for an additional hour. This solution was slowly added to a cold saturated NaHCO3 solution and stirred for 20 minutes. After separating the aqueous layer and washing the organics successively with saturated NaHCO3 and brine, the organic layer was dried over Na2SO4, filtered and evaporated under reduced pressure. The crude material was purified by flash chromatography (silica gel, eluting with 0-5% methanol/dichloromethane) to afford the desired product as a white solid. 68 g, 77 % yield. 1H NMR (300MHz, DMSO-d6) δ = 11.31 (s, 1H),
8.07 - 7.98 (m, 2H), 7.74 - 7.65 (m, 1H), 7.61 - 7.49 (m, 2H), 7.08 (d, J=1.1 Hz, 1H), 6.20 (t, J=7.0 Hz, 1H), 5.50 (d, J=4.7 Hz, 1H), 5.29 (dd, J=3.8, 6.6 Hz, 1H), 4.60 - 4.45 (m, 1H), 3.90 - 3.88 (m, 1H), 3.34 (s, 1H), 2.25 - 2.08 (m, 2H), 1.41 - 1.26 (m, 7H), 1.19 (t, J=7.3 Hz, 1H).

13C NMR (75MHz, DMSO-d6) \( \delta = 165.52, 163.88, 150.80, 135.44, 134.11, 130.04, 129.73, 129.44, 110.23, 87.95, 83.71, 71.08, 69.88, 16.46, 11.94 \); LCMS (ESI) m/z: [M+H] calcd, 360.1; found, 361.1

**Compound 7.** Compound 6 (62 g, 0.172 mol,) was dissolved in DMF (500 mL) and the solution was cooled in an ice bath and imidazole (23.4 g, 0.344 mol, 2eq) was added as a solution of DMF (50 mL) followed by tert-butyl-chlorodimethylsilane (31.6 g, 0.210 mol 1.2 eq). The reaction was stirred overnight, and then slowly poured on ice. Hexanes (800 mL) were added and the mixture was stirred for 90 minutes to affect a precipitate. The solvents were then decanted and the white solid was washed with water and hexanes twice. The white solid was co-evaporated with toluene under reduced pressure at 60°C twice to give the desired product as a white solid. 60 g, 74 % yield. \(^1\)H NMR (300MHz, CHLOROFORM-d) \( \delta = 8.20 \) (br s, 1H), 7.98 - 7.85 (m, 2H), 7.54 - 7.45 (m, 1H), 7.43 - 7.31 (m, 2H), 7.15 (s, 1H), 6.86 (s, 1H), 6.19 (dd, J=5.6, 8.4 Hz, 1H), 5.30 (br dd, J=3.8, 6.6 Hz, 1H), 4.55 - 4.44 (m, 1H), 3.95 - 3.88 (m, 1H), 2.26 - 2.12 (m, 1H), 2.04 - 1.85 (m, 1H), 1.53 (br s, 2H), 1.38 - 1.26 (m, 6H), 1.14 (s, 1H), 0.79 (s, 9H). \(^{13}\)C NMR (75MHz, CHLOROFORM-d) \( \delta = 165.56, 163.27, 150.04, 134.65, 133.64, 129.75, 129.48, 128.78, 111.19, 89.19, 84.63, 71.34, 70.64, 41.17, 25.67, 17.85, 16.75, 11.83 \); LCMS (ESI) m/z: [M+H] calcd, 474.2; found, 475.2

**Compound 8.** Compound 7 (60 g, 0.126 mol) was dissolved in methanol (1L). K\(_2\)CO\(_3\) (70 g, 0.506 mol) was added and the reaction was stirred overnight. The solids were then removed by filtration and rinsed with DCM/MeOH (8/2). The filtrate was concentrated under reduced pressure and the resultant crude solid was suspend in ethyl acetate (500 mL), and washed successively with water, saturated NaHCO\(_3\) and brine. The organics were dried over Na\(_2\)SO\(_4\), filtered and evaporated under reduced pressure to obtained crude material that was purified by flash chromatography (silica gel, eluting with 40-60 % ethyl acetate/hexanes) afforded the desired product as a white solid. 32 g, 68 % yield. \(^1\)H NMR (300MHz, CHLOROFORM-d) \( \delta = \)
8.68 (br s, 1H), 7.24 (d, J=1.1 Hz, 1H), 7.17 (s, 1H), 5.97 (dd, J=6.0, 8.3 Hz, 1H), 4.48 - 4.41 (m, 1H), 4.01 - 3.91 (m, 1H), 3.69 (t, J=2.6 Hz, 1H), 2.80 (d, J=2.8 Hz, 1H), 2.36 - 2.23 (m, 1H), 2.03 (ddd, J=2.4, 6.0, 13.2 Hz, 1H), 1.81 (d, J=0.9 Hz, 3H), 1.66 (s, 1H), 1.17 (d, J=6.6 Hz, 3H), 0.80 (s, 9H). 13C NMR (75MHz, CHLOROFORM-d) δ = 163.65, 150.37, 137.55, 111.04, 91.40, 87.60, 70.62, 67.62, 40.34, 25.70, 19.30, 17.83, 12.51; LCMS (ESI) m/z: [M+H] calcd, 370.2; found, 371.2

**Compound 9.** DMTriCl (52.2 g, 154 mmol) was added to a solution of compound 8 (16.3 g, 4.40 mmol) and 2,6-lutidine (20.20 mL, 176 mmol, 4 eq), in pyridine (250 mL) at room temperature and the solution was stirred at 45°C for 14 hours. The reaction was then quenched by the addition of methanol (10 mL), followed by water and ethyl acetate. The resulting suspension was extracted with ethyl acetate and the combined organics were washed with saturated NaHCO₃ and brine. After the removal of the solvents under reduced pressure, purification by flash chromatography (silica gel, eluting with 40-60% diethyl ether/hexanes) afforded the desired product as a white solid. 29 g, 97 % yield. 1H NMR (300MHz, CHLOROFORM-d) δ = 8.66 (s, 1H), 7.42 - 7.34 (m, 2H), 7.33 - 7.24 (m, 4H), 7.20 - 7.05 (m, 4H), 7.00 - 6.82 (m, 1H), 6.71 (dd, J=2.2, 8.9 Hz, 4H), 6.15 (dd, J=5.7, 8.6 Hz, 1H), 4.57 - 4.43 (m, 1H), 3.73 - 3.62 (m, 7H), 3.33 (s, 1H), 2.41 (s, 1H), 1.97 (s, 1H), 1.81 (s, 2H), 1.40 (s, 3H), 0.82 - 0.76 (m, 12H), 0.01 (d, J=3.2 Hz, 6H). 13C NMR (75MHz, CHLOROFORM-d) δ = 163.67, 158.62, 158.60, 150.24, 146.28, 136.85, 136.59, 135.49, 130.29, 130.24, 129.16, 128.12, 127.85, 127.78, 126.89, 120.22, 113.17, 113.12, 113.07, 110.97, 90.81, 86.57, 84.07, 71.46, 69.93, 55.23, 40.68, 25.71, 18.04, 17.86, 11.98. LCMS (ESI) m/z: [M+H] calcd, 672.3; found, 673.2

**Compound 10.** Triethylamine (7.77 mL, 55.7 mmol) was added to a solution of compound 9 (15 g, 22.3 mmol) in THF (60 mL). The reaction was then cooled in an ice bath and triethylamine trihydrofluoride (18.2 mL, 111 mmol, 5 eq) was added slowly. The reaction was warmed to room temperature and stirred overnight. The solvents were then removed under reduced pressure and the resultant material was purified by flash chromatography (silica gel, eluting with 50-70% ethyl acetate/hexanes) to afford the desired product as a white solid. 11.6 g, 93 % yield; LCMS (ESI) m/z: [M-H] calcd, 555.2; found, 554.2
**Compound 11.** 1H-Tetrazole (1.14 g, 16.4 mmol, 0.8 eq) and 1-methylimidazole (0.408 mL, 5.14 mmol, 0.25 eq) were added to a solution of nucleoside 10 (15.0 g, 20.60 mmol) in DMF (200 mL) at room temperature under an atmosphere of nitrogen. 3-bis(diisopropylamino)-phosphanyloxypropanenitrile (9.80 mL, 30.8 mmol, 1.5 eq) was then added drop-wise and the reaction was stirred at room temperature for 3 hours. Water (10.0 mL) was added to quench the reaction. A 3:1 mixture of toluene/hexanes (80 mL) was added and the organic layer was washed (4x50 mL) with a 3:2 mixture of DMF/H2O. The organics were then washed with saturated sodium bicarbonate solution, brine, dried over solid sodium sulfate and concentrated under reduced pressure to afford a white foam. Purification by flash chromatography (silica gel, 50g column, eluting with 50-60% ethyl acetate/hexanes + 1 % triethylamine) afforded the desired product as a white solid. 12.8 g, 82 % yield. 1H NMR (300MHz, CHLOROFORM-d) δ = 7.54 - 7.46 (m, 2H), 7.44 - 7.35 (m, 4H), 7.30 - 7.17 (m, 4H), 7.03 (d, J=8.7 Hz, 1H), 6.86 - 6.78 (m, 4H), 3.91 - 3.62 (m, 10H), 2.67 - 2.35 (m, 3H), 1.61 - 1.36 (m, 4H), 1.33 - 1.12 (m, 16H), 1.00 - 0.82 (m, 7H). 31P NMR (121MHz, CHLOROFORM-d) δ = 148.94 (s, 1P), 148.49 (s, 1P); LCMS (ESI) m/z: [M-H] calcd, 758.3; found, 757.3

**Compound 12.** Triphenylphosphine (Ph3P) (16.7 g, 63.6 mmol, 4.0eq) and p-nitrobenzoic acid (10.6 g, 63.6 mmol, 4 eq) were added to a solution of compound 11 in anhydrous THF (50 mL). The reaction was cooled in an ice bath and diisopropyl azodicarboxylate (DIAD) (12.2 mL, 62.1 mmol, 4.0 eq) was added dropwise. The reaction was stirred for 4 hours, at which time the solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate and water. The water layer was extracted twice more with ethyl acetate, and the organics were combined and washed successively with saturated NaHCO3 and brine. The organic layer was then dried over Na2SO4, filtered and the solvent was removed under reduced pressure to a crude oil. Purification by flash chromatography (silica gel, eluting with 0-60% diethyl ether/hexanes) afforded the inverted ester as a white solid. 8.12 g, 98 % yield. 1H NMR (300MHz, CHLOROFORM-d) δ = 8.27 - 8.20 (m, J=8.9 Hz, 2H), 8.19 - 8.07 (m, J=8.8 Hz, 2H), 6.17 (t, J=6.6 Hz, 2H), 5.43 - 5.23 (m, 1H), 4.98 - 4.86 (m, 1H), 4.35 - 4.11 (m, 1H), 3.91 (dd, J=4.0, 5.8 Hz, 1H), 2.25 (br dd, J=3.9, 6.2 Hz, 1H), 2.14 - 1.95 (m, 1H), 1.88 (s, 3H), 1.44 (d,
$J=6.5 \text{ Hz}, 4\text{H}$), 1.21 (d, $J=6.3 \text{ Hz}, 5\text{H}$), 0.84 (s, 8H). $^{13}$C NMR (75MHz, CHLOROFORM-d) $d = 164.21, 163.59, 150.74, 150.04, 135.39, 135.34, 130.69, 123.71, 111.11, 88.52, 85.14, 72.01, 71.42, 70.12, 41.01, 25.70, 25.65, 21.96, 17.84, 17.17, 12.59; LCMS (ESI) m/z: [M+H] calcd, 519.2; found, 520.2

**Compound 13.** Ester 12 (8.67 g, 16.70 mmol) obtained above was dissolved in methanol (200 mL) and K$_2$CO$_3$ (9.22 g, 6.67 mmol, 4.0 eq) was added. The reaction was stirred for 14 hours and the solids were filtered and rinsed with DCM/MeOH (8/2) and the filtrate was evaporated under reduced pressure. The crude solid was suspended in ethyl acetate (100 mL), and washed successively with water (2x100 mL), saturated NaHCO$_3$, and brine. The organic layer was dried over Na$_2$SO$_4$, filtered and evaporated under reduced pressure to obtain the desired product as a white solid. 4.68 g, 76 % yield. $^1$H NMR (300MHz, CHLOROFORM-d) $d = 8.42$ (br s, 1H), 7.32 - 7.27 (m, 1H), 7.18 (s, 1H), 6.05 (t, $J=6.9 \text{ Hz}, 1\text{H}$), 4.38 - 4.31 (m, 1H), 3.83 (br d, $J=3.0 \text{ Hz}, 1\text{H}$), 3.63 (t, $J=3.2 \text{ Hz}, 1\text{H}$), 2.39 - 2.22 (m, 2H), 2.11 (br dd, $J=3.4, 6.3 \text{ Hz}, 1\text{H}$), 1.87 - 1.81 (m, 3H), 1.58 (s, 3H), 1.26 - 1.15 (m, 4H), 0.81 (s, 9H). $^{13}$C NMR (75MHz, CHLOROFORM-d) $d = 163.53, 150.22, 137.17, 111.05, 90.65, 87.09, 72.73, 67.42, 40.12, 25.73, 20.54, 17.94, 12.56; LCMS (ESI) m/z: [M+H] calcd, 370.1; found, 371.1

**Compound 14.** DMTrCl (17.1 g, 50.5 mmol, 4 eq.) was added to a solution of compound 13 (4.68 g, 12.6 mmol), 2,6-Lutidine (5.88 mL, 50.5 mmol, 4 eq) and pyridine (150 mL) and the reaction was stirred for 14 hours at 45$^\circ$C. The reaction was quenched by the addition of methanol (5 mL) and diluted with water and ethyl acetate. The organic layer was separated, and the aqueous layer was further extracted with ethyl acetate. The organic layers were combined and washed with saturated NaHCO$_3$ and brine. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel, eluting with 40-60% diethyl ether/hexanes) afforded the desired product as a white solid. 7.64 g, 90 % yield. $^1$H NMR (300MHz, CHLOROFORM-d) $d = 8.44$ (s, 1H), 7.90 (s, 1H), 7.51 - 7.20 (m, 11H), 6.86 (dd, $J=3.3, 8.8 \text{ Hz}, 4\text{H}$), 6.35 (dd, $J=5.7, 7.8 \text{ Hz}, 1\text{H}$), 4.36 - 4.28 (m, 1H), 3.86 - 3.73 (m, 7H), 3.63 (br dd, $J=2.4, 6.4 \text{ Hz}, 1\text{H}$), 2.57 (s, 1H), 2.38 - 2.02 (m, 2H), 1.81 (s, 3H), 1.69 (br s, 2H), 1.31
(d, J=6.3 Hz, 1H), 1.03 (d, J=6.4 Hz, 3H), 0.94 - 0.82 (m, 9H). $^{13}$C NMR (75MHz, CHLOROFORM-d) δ = 163.64, 158.67, 158.65, 150.11, 145.90, 136.57, 136.29, 135.69, 130.48, 130.35, 128.32, 127.71, 126.99, 113.04, 113.03, 111.01, 91.84, 86.92, 84.62, 72.48, 69.93, 55.25, 41.85, 25.70, 18.91, 17.79, 12.30; LCMS (ESI) m/z: [M-H] calcd, 672.3; found, 671.2

**Compound 15.** Triethylamine (1.86 mL, 13.4 mmol 2.5eq) was added to a solution of compound 14 (3.60 g, 5.35 mmol) in THF (60 mL). The reaction was cooled in an ice bath and triethylamine trihydrofluoride (4.32 mL, 26.8 mmol, 5 eq) was added slowly. The reaction was warmed to room temperature and stirred for 14 hours after which the solvents were removed under reduced pressure and the crude residue was purified by flash chromatography (silica gel, eluting with 50-70% ethyl acetate/hexanes) to afford the desired product as a white solid. 2.24 g, 75 % yield. $^1$H NMR (300MHz, DMSO-d6) δ = 11.36 (s, 1H), 7.88 (s, 1H), 7.56 (s, 1H), 7.44 (d, J=7.5 Hz, 2H), 7.35 - 7.17 (m, 10H), 7.07 (d, J=8.7 Hz, 2H), 6.92 - 6.80 (m, 6H), 6.23 - 6.18 (m, 1H), 6.14 (br d, J=6.6 Hz, 1H), 5.21 (br d, J=4.8 Hz, 1H), 4.23 (br s, 1H), 3.80 - 3.51 (m, 12H), 2.50 (s, 10H), 1.76 (s, 1H), 1.67 (s, 3H), 1.13 (d, J=6.4 Hz, 2H), 0.70 (d, J=6.2 Hz, 3H). $^{13}$C NMR (75MHz, DMSO-d6) δ = 164.22, 164.16, 158.62, 158.57, 158.28, 150.91, 150.85, 146.71, 140.70, 136.98, 136.78, 136.73, 135.99, 130.64, 130.51, 129.38, 128.36, 128.11, 127.88, 127.13, 113.49, 113.23, 110.10, 109.67, 90.80, 89.74, 86.38, 84.15, 83.83, 80.37, 71.70, 70.56, 70.20, 66.47, 55.49, 20.69, 18.06, 12.83, 12.56; LCMS (ESI) m/z: [M-H] calcd, 558.2; found, 557.4

**Compound 16.** 1H-Tetrazole (221 mg, 3.21 mmol,0.8 eq) and 1-methylimidazole (0.80 µL, 1.0 mmol, 0.25 eq) were added to a solution of compound 15 (2.24 g, 3.21 mmol) in DMF (40 mL) at room temperature. 3-bis(diisopropylamino)phosphanyloxypropanenitrile (1.91 mL, 6.01 mmol, 1.5 eq) was then added drop wise and the reaction was stirred at room temperature for 3 hours. Water (3.0 mL) was added to quench the reaction. A 3:1 mixture of toluene/hexanes (50 mL) was added and the organic layer was washed (4x50 mL) with a 3:2 mixture of DMF/H2O. The organics were then washed with saturated sodium bicarbonate solution, brine, dried over solid sodium sulfate and concentrated under reduced pressure to a white foam. Purification by flash chromatography (silica gel, 50-60% ethyl acetate/hexanes + 1 % triethylamine) afforded the desired product as a white solid, 1.76 g, 91 % yield. $^1$H NMR (300MHz, CHLOROFORM-d) δ = 8.06 (s, 1H), 7.52 - 7.23 (m, 11H), 6.90 - 6.82 (m, 4H), 6.33 (t, J=6.2 Hz, 1H), 4.33 (br d,
Scheme S2. Synthesis of 5’-(R)-Methyl Cytidine Nucleosides.

Compound 17. POCl₃ (4.88 mL, 53.3 mmol, 8 eq) was added dropwise to a suspension of 1,2,4-1H-triazole (20.7 g, 299 mmol, 34 eq.) in acetonitrile (150 mL) at room temperature and the reaction was stirred for 20 minutes. The reaction was cooled down in an ice bath and triethylamine (49.10 mL, 352 mmol, 40 eq.) added dropwise, after the addition was complete, the ice bath was removed and the reaction continued to stir for an additional 30 minutes at room temperature. The reaction was then cooled down to 0°C and a solution of compound 10 (4.48 g, 6.66 mmol) in acetonitrile (30 mL) was added dropwise. This was stirred at room temperature overnight. The next day, the reaction was concentrated to small volume under reduced pressure,
diluted with ethyl acetate and the organic layer was washed with aqueous saturated sodium bicarbonate (2x), water, brine and concentrated to a yellow oil. Without any further purification, the crude material was suspended in dioxane/NH\textsubscript{4}OH (30 mL/10 mL) solution and stirred at room temperature for 2 hours. TLC in EtOAc/hexanes (8/2) indicated reaction was completed. The solvent was concentrated under reduced pressure and the resulting oil was diluted with ethyl acetate and washed with 1x200 ml water, 1x200 ml sat. NaHCO\textsubscript{3}, 1x200 ml brine, and dried over Na\textsubscript{2}SO\textsubscript{4}, filtered and concentrated under reduce pressure to obtain a crude oil. The crude material was dissolved in DCM and purified by silica gel chromatography (silica gel, 40-100% EtOAc/hexanes) to afford the desired product as a white solid. 3.37 g, 73 % yield. \textsuperscript{1}H NMR (300MHz, CHLOROFORM-d) \textit{d} = 7.41 (d, \textit{J}=7.2 Hz, 2H), 7.35 - 7.26 (m, 4H), 7.24 - 7.05 (m, 5H), 6.72 (dd, \textit{J}=3.1, 8.9 Hz, 4H), 6.17 (dd, \textit{J}=5.9, 7.6 Hz, 1H), 4.54 - 4.47 (m, 1H), 3.69 (d, \textit{J}=2.2 Hz, 7H), 3.42 (br dd, \textit{J}=3.4, 6.4 Hz, 1H), 2.29 - 2.03 (m, 1H), 1.76 (td, \textit{J}=7.0, 13.8 Hz, 3H), 1.43 (s, 3H), 0.84 - 0.73 (m, 11H). \textsuperscript{13}C NMR (75MHz, CHLOROFORM-d) \textit{d} = 165.31, 158.56, 155.84, 146.37, 138.22, 136.96, 136.72, 130.35, 130.27, 128.18, 127.73, 126.81,113.07, 113.04, 101.35, 90.52, 86.46, 85.13, 71.38, 69.79, 55.23, 41.81, 25.73, 17.92, 17.85, 12.74; LCMS (ESI) m/z: [M-H] calcd, 671.3; found, 670.3

**Compound 18. (11a)** 4-amino-1-((2R,4S)-5-((R)-1-(bis(4-methoxyphenyl)(phenyl)ethoxy)ethyl)-4-((tert-butyldimethylsilyl)oxy)tetrahydrofuran-2-y1)-5-methylpyrimidin-2(1H)-one (3.24 g, 4.81 mmol) was dissolved in anhydrous pyridine (20 mL) and the reaction was cooled in an ice bath. Benzoyl chloride (0.61 mL, 5.29 mmol, 1.1 3q.) was added dropwise and the reaction was stirred for room temperature for 14 hours at which point TLC analysis in (EtOAc/hexanes (6/4)) indicated that the reaction was complete. The reaction was cooled in an ice bath and 20 ml of water was slowly added followed by EtOAc (50 mL). The organic layer was washed with water, saturated NaHCO\textsubscript{3}, brine and dried over Na\textsubscript{2}SO\textsubscript{4} for 10 minutes, filtered and solvent was concentrated under reduce pressure to obtained crude oil. The crude material was dissolved in DCM and purified by chromatography (silica gel, 40-70% EtOAc/hexanes) afforded the desired product as a white solid. 3.57 g, 95 % yield.\textsuperscript{1}H NMR (300MHz, CHLOROFORM-d) \textit{d} = 8.23 - 8.16 (m, 5H), 7.45 - 7.26 (m, 18H), 7.21 - 7.04 (m, 19H), 6.79 - 6.65 (m, 11H), 6.16 (dd, \textit{J}=5.7, 8.3 Hz, 2H), 5.99 (dd, \textit{J}=6.0, 8.1 Hz, 1H), 4.60 - 4.52 (m, 1H), 4.51 - 4.42 (m, 1H), 3.98 (dd, \textit{J}=2.8, 6.6 Hz, 1H), 3.73 - 3.64 (m, 17H), 3.36 (dd,
$J$=3.6, 6.4 Hz, 2H), 2.29 (br dd, $J$=6.0, 7.9 Hz, 2H), 2.21 - 2.05 (m, 3H), 2.05 - 1.95 (m, 4H), 1.92 - 1.68 (m, 4H), 1.59 (s, 5H), 1.51 (s, 2H), 1.35 -1.0 (m, 5H)$^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta$ = 179.59, 179.57, 159.67, 159.63, 158.65, 147.90, 147.33, 146.28, 139.47, 138.48, 137.20, 137.12, 136.85, 136.72, 136.56, 132.48, 132.42, 130.09 (dd, $J$=3.5, 26.4 Hz, 1C), 129.14, 128.13, 127.86, 127.80, 127.78, 127.10, 126.92, 113.18, 113.15,113.10, 111.99, 111.85, 91.58, 91.09, 88.16, 86.62, 84.72, 81.45, 71.46, 70.67, 69.91, 67.73, 55.25, 41.08, 40.65, 25.71, 19.49, 18.12, 17.86, 13.64,13.06; LCMS (ESI) m/z: [M-H] calcd, 775.3; found, 774.3

**Compound 19.** Triethylamine (1.60 mL, 11.5 mmol, 2.5 eq.) was added to a solution of compound (12b) N-(1-((2R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((tert-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)-5-methyl-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide (3.57 g, 4.60 mmol) in tetrahydrofuran (45.0 mL). The reaction was cooled down in an ice bath and triethylamine trihydrofluoride (3.74 mL, 22.9 mmol, 5 eq.) was added slowly. The reaction was stirred for 16 hours at room temperature after which triethylamine (1.60 ml) was added to the reaction. The solvent was removed under reduced pressure to obtained crude solid that was dissolved in EtOAc and the organic layer was washed with water, sat. NaHCO$_3$, brine, dried over Na$_2$SO$_4$, filtered and concentrated under reduce pressure to obtained crude oil. Purification by flash chromatography (silica gel, 20-100 % EtOAc/hexanes) afforded the desired product as a white solid. 2.90 g, 95 % yield. $^1$H NMR (300MHz, DMSO-d6) $\delta$ = 12.99 (br s, 1H), 8.25 - 8.12 (m, 3H), 8.07 (br s, 2H), 7.73 - 7.43 (m, 8H), 7.40 - 7.16 (m, 13H), 7.11 - 7.03 (m, 6H), 6.93 - 6.79 (m, 9H), 6.29 - 6.12 (m, 3H), 5.34 (d, $J$=4.7 Hz, 1H), 5.23 (d, $J$=4.3 Hz, 1H), 5.08 (d, $J$=4.8 Hz, 1H), 4.41 - 4.05 (m, 1H), 3.83 - 3.63 (m, 14H), 2.32 - 2.08 (m, 3H), 2.07 - 1.93 (m, 4H), 1.83 (s, 1H), 1.63 (s, 2H), 1.24 - 1.09 (m, 4H), 0.80 (br d, $J$=6.2 Hz, 2H).$^{13}$C NMR (75MHz, DMSO-d6) $\delta$ = 158.62, 158.28, 148.83, 140.71, 130.53, 129.83, 129.39, 128.80, 128.22, 128.12, 127.88, 126.90, 113.61, 113.23, 80.38, 69.68, 66.79, 55.52, 55.47, 20.35; LCMS (ESI) m/z: [M-H] calcd, 661.2; found, 660.2

**Compound 20.** $^1$H-Tetrazole (0.23 g, 16.4 mmol,0.8 eq) and 1-methylimidazole (0.085 mL, 1.0 mmol, 0.25 eq) were added to a solution of compound (13c) N-(1-((2R,4S)-5-((R)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)-4-hydroxytetrahydrofuran-2-yl)-5-methyl-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide (15.0 g, 20.60 mmol) in DMF (51 mL) at room temperature.
3-bis(diisopropylamino)phosphanyloxypropanenitrile (2.06 mL, 6.49 mmol, 1.5 eq) was added drop wise and the reaction was stirred at room temperature for 3 hours. Water (5.0 mL) was added to quench the reaction. A 3:1 mixture of toluene/hexanes (80 mL) was added and the organic layer was washed (4x50 mL) with a 3:2 mixture of DMF/H₂O. The organics were then washed with saturated sodium bicarbonate solution, brine, dried over solid sodium sulfate and concentrated under reduced pressure to a white foam. Purification by flash chromatography (Silica gel, eluting with 40% ethyl acetate/hexanes + 1% triethylamine) afforded the desired product (14d) as a white solid. 3.14 g, 84% yield. ¹H NMR (300MHz, CHLOROFORM-d) δ = 8.33 - 8.27 (m, 2H), 7.55 - 7.37 (m, 9H), 7.31 - 7.19 (m, 5H), 6.87 - 6.80 (m, 4H), 4.12 (d, J=7.0 Hz, 1H), 3.93 (br s, 1H), 3.78 (d, J=2.8 Hz, 9H), 2.69 - 2.44 (m, 3H), 2.04 (s, 2H), 1.63 (s, 4H), 1.32 - 1.13 (m, 14H), 1.05 - 0.89 (m, 3H) ³¹P NMR (121MHz, CHLOROFORM-d) δ = 149.00 (s, 1P), 148.57 (s, 1P); LCMS (ESI) m/z: [M-H] calcd, 861.4; found, 860.4

**Scheme S3.** Synthesis of 5’-(S)-Methyl Cytidine Nucleosides.

**Compound 21.** POCl₃ (4.53 mL, 49.5 mmol, 8 eq) was added dropwise to a suspension of 1,2,4-1H-Triazole (14.5 g, 210 mmol, 34 eq.) in acetonitrile (200 mL) and the reaction was stirred for 20 minutes at room temperature. The reaction was cooled in an ice bath and triethylamine (34.5
mL, 247 mmol, 40 eq.) was added. The ice bath was removed and the reaction was stirred at room temperature for another 30 minutes. The reaction was cooled in an ice bath and a solution of compound (12c) 1-((2R,4S)-5-((S)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)-4-((tert-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (4.16 g, 6.18 mmol) in acetonitrile (30 mL) was added dropwise to reaction. This was stirred at room temperature for 16 hours and concentrated under reduced pressure and diluted with ethyl acetate. The organic layer was washed with aqueous saturated sodium bicarbonate (2x), water, brine and concentrated to a yellow oil and used without any further purification. The crude material obtained above was suspended in dioxane/NH₄OH (30 mL/10 mL) solution and stirred at room temperature for 2 hours. TLC in EtOAc/hexanes (8/2) indicated reaction was completed. Solvent was concentrated under reduce pressure and remaining oil was diluted with ethyl acetate and the organic layer was washed with water, sat. NaHCO₃, brine and dried over sodium sulfate, filtered and concentrated under reduce pressure to obtain a crude oil. The crude material was dissolved in purified by column chromatography (silica gel, 40-60% EtOAc/hexanes) to afford the desired product as a white solid. 3.77 g, 91 % yield. 

**1H NMR (300MHz, CHLOROFORM-d) δ = 8.06 (s, 1H), 7.52 - 7.22 (m, 10H), 6.86 (dd, J=6.0, 8.1 Hz, 4H), 6.32 (t, J=6.1 Hz, 1H), 3.86 - 3.82 (m, 6H), 3.80 - 3.60 (m, 2H), 1.75 (s, 4H), 1.05 (d, J=6.3 Hz, 3H), 0.88 - 0.80 (m, 9H) 

**13C NMR (75MHz, CHLOROFORM-d) δ = 165.53, 158.63, 158.60, 155.93, 146.08, 138.54, 136.76, 136.46, 130.56, 130.40, 128.40, 127.66, 126.92, 112.98, 101.21, 91.50, 86.70, 85.76, 71.77, 69.64, 55.25, 42.59, 25.71, 19.13, 17.76, 12.93, -4.41, -4.93; LCMS (ESI) m/z: [M-H] calcd, 671.3; found, 670.3

**Compound 22.** Compound (13a) 4-amino-1-((2R,4S)-5-((R)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)-4-((tert-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)-5-methylpyrimidin-2(1H)-one (3.77 g, 5.59 mmol) was dissolved in anhydrous pyridine (50 mL) and the reaction was cooled in an ice bath. Benzoyl chloride (0.71 mL, 6.51 mmol, 1.1 3q.) was added dropwise and the reaction was stirred at room temperature for 16 hours at which time TLC analysis in (EtOAc/hexanes (6/4)) indicated that the reaction was completed. The reaction was diluted with ethyl acetate and the organic layer was washed with water, sat. NaHCO₃, brine, dried over sodium sulfate, filtered and concentrated under reduce pressure to obtain a crude oil. The crude material was dissolved in DCM and purified by column chromatography (silica gel,
40-70% EtOAc/hexanes) afforded the desired product as a white solid. 3.35 g, 99 % yield. 

\[^1\text{H}\] NMR (300MHz, CHLOROFORM-d) \(\delta = 7.35 \text{ (br d, } J=7.8 \text{ Hz, 5H)\), 7.27 - 7.14 (m, 5H), 7.13 - 7.02 (m, 3H), 6.73 (d, } J=8.8 \text{ Hz, 4H), 3.69 (s, 6H), 1.98 (d, } J=18.5 \text{ Hz, 3H), 1.31 - 1.11 (m, 3H), 0.87 - 0.76 (m, 7H), 0.71 (s, 2H), 0.03 - 0.05 (m, 5H), -0.11 (d, } J=11.7 \text{ Hz, 1H)\)

\[^13\text{C}\] NMR (75MHz, CHLOROFORM-d) \(\delta =\) 158.62, 147.36, 139.50, 132.48, 130.04, 129.92, 128.64, 128.35, 128.31, 128.14, 127.86, 127.80, 129.16, 127.39, 127.08, 113.17, 113.06, 111.93, 90.85, 87.41, 81.45, 72.64, 67.30, 55.26, 40.49, 25.75, 25.70, 20.60, 17.95, 13.68;

LCMS (ESI) m/z: [M-H] calcd, 775.3; found, 744.

**Compound 23.** Triethylamine (1.95 mL, 14.0 mmol, 2.5 eq.) was added to a solution of compound (14b) N-(1-((2R,4S)-5-((S)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)-4-((tert-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)-5-methyl-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide (4.35 g, 5.59 mmol) in tetrahydrofuran (50.0 mL). The reaction was cooled in an ice bath. Triethylamine trihydrofluoride (4.56 mL, 28.0 mmol, 5 eq.) was slowly added and the reaction was stirred for 16 hours at room temperature after which triethylamine (1.95 ml) was added to the reaction and the solvent was removed under reduced pressure. The crude material was dissolved in ethyl acetate and the organic layer was washed with water, saturated NaHCO3, brine, dried over Na2SO4, filtered and the solvent was concentrated under reduced pressure. Purification by flash chromatography (silica gel, 20-50% EtOAc/hexanes) afforded the desired product as a white solid. 2.45 g, 66 % yield. 

1H NMR (300MHz, DMSO-d6) \(\delta = 13.02 \text{ (br s, 1H), 8.32 - 8.12 (m, 3H), 7.69 - 7.39 (m, 4H), 7.35 - 7.16 (m, 7H), 7.11 - 7.03 (m, 4H), 6.92 - 6.77(m, 5H), 6.24 - 6.15 (m, 2H), 5.25 (d, } J=4.4 \text{ Hz, 1H), 5.10 (d, } J=4.6 \text{ Hz, 1H), 4.38 - 4.07 (m, 1H), 4.03 - 3.76 (m, 1H), 3.70 - 3.53 (m, 2H), 3.33 (s, 4H), 2.48 - 2.10 (m, 2H), 2.07 - 1.94 (m, 3H), 1.90 (s, 1H), 1.83 (s, 1H)\)

13C NMR (75MHz, DMSO-d6) \(\delta =\) 158.28, 148.83, 140.71, 132.97, 129.83, 129.38, 128.79, 128.12, 127.88, 126.90, 113.23, 91.23, 80.38, 71.29, 66.18, 55.47, 20.85; LCMS (ESI) m/z: [M-H] calcd, 661.2; found, 660.2

**Compound 24.** 1H-Tetrazole (0.22 g, 3.21 mmol,0.8 eq) and 1-Methylimidazole (0.080 mL, 1.0 mmol, 0.25 eq) were added to a solution of compound (15c) N-(1-((2R,4S)-5-((S)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)-4-hydroxytetrahydrofuran-2-yl)-5-methyl-2-oxo-1,2-
dihydropyrimidin-4-yl)benzamide (2.24 g, 4.0 mmol) in DMF (40 mL) at room temperature under an atmosphere of nitrogen. 3-bis(Diisopropylamino)phosphanyloxypropanenitrile (1.91 mL, 6.0 mmol, 1.5 eq) was then added drop wise and the reaction was stirred at room temperature for 3 hrs. Water (5.0 mL) was added to quench the reaction. A 3:1 mixture of toluene/hexanes (50 mL) was added and the organic layer was washed (4x50 mL) with a 3:2 mixture of DMF/H$_2$O. The organics were then washed with saturated sodium bicarbonate solution, brine, dried over solid sodium sulfate and concentrated under reduced pressure to a white foam. Purification by flash chromatography (Si, 50g col, 40-60% ethyl acetate/hexanes + 1 % triethylamine) afforded the desired product (16d) as a white solid.

2.76 g, 91 % yield. 1H NMR (300MHz, CHLOROFORM-d) $\delta = 7.55 - 7.33$ (m, 9H), 7.26 (s, 6H), 6.84 (br d, $J=4.4$ Hz, 4H), 3.83 - 3.51 (m, 12H), 2.35 (s, 3H), 2.04 -1.93 (m, 3H), 1.22 - 1.11 (m, 14H), 1.05 (br d, $J=6.8$ Hz, 6H). 31P NMR (121MHz, CHLOROFORM-d) $\delta = 149.35$ (s, 1P), 149.13 (s, 1P); LCMS (ESI) m/z: [M-H] calcd, 861.3; found, 860.3

**Scheme S4.** Synthesis of 5’-(R)-Methyl Adenosine Nucleosides.
Compound 26. N-(9H-purin-6-yl)benzamide (31.9 g, 133 mmol, 1.3 eq) and N,O-Bis(trimethylsilyl)acetamide (83.30 g, 100 mL, 410 mmol, 4.0 eq) were added to a solution of (3R,4R)-5-((S)-1-(benzoyloxy)ethyl)-4-(benzylloxy)tetrahydrofuran-2,3-diyl diacetate (2) (45.3 g, 102 mmol) in dichloroethane (600 mL). After heating at 80°C for 1 hour to obtain a clear solution, the reaction was cooled in an ice bath and trimethylsilyl trifluoromethanesulfonate (36.40 g, 164 mmol, 1.6 eq) was added dropwise. The reaction was then warmed to 80 °C and stirred for 14 hours after which it was concentrated under reduced pressure to obtain a crude oil. The crude material was dissolved in ethyl acetate (500 mL) and the organic layer was washed with water, saturated sodium bicarbonate solution, brine, dried over Na₂SO₄ for 15 minutes, filtered and concentrated under reduced pressure to obtain a crude oil. Purification by flash chromatography (silica gel, 0-5% dichloromethane/methanol) afforded the desired product as a white solid. 35.18 g, 58 % yield. ¹H NMR (300MHz, CHLOROFORM-d) δ = 8.84 (s, 1H), 8.54
(s, 1H), 8.01 - 7.87 (m, 5H), 7.64 - 7.47 (m, 4H), 7.44 - 7.26 (m, 9H), 6.19 - 6.13 (m, 2H), 5.50 (dd, J=3.3, 6.7 Hz, 1H), 4.88 (t, J=4.9 Hz, 1H), 4.76 - 4.57 (m, 2H), 4.33 (dd, J=3.3, 5.4 Hz, 1H), 2.14 (s, 3H), 2.04 (s, 1H), 1.79 (s, 1H), 1.41 - 1.20 (m, 4H).

13C NMR (75MHz, CHLOROFORM-d) δ = 170.00, 165.47, 164.40, 152.77, 151.22, 149.56, 142.08, 136.87, 133.57, 133.22, 132.82, 129.72, 129.53, 128.89, 128.58, 128.44, 128.38, 128.34, 127.83, 123.30, 87.05, 84.61, 75.46, 73.42, 73.10, 69.66, 20.72, 15.96.

LCMS (ESI) m/z: [M-H] calcd, 671.3; found, 670.4.

LCMS (ESI) m/z: [M+H] calcd, 621.2; found, 622.2.

**Compound 27.** Methanolic ammonia (7.00 M, 45 mL, 319 mmol, 6.0 eq) was added to a solution of (1S)-1-((3R,4R,5R)-4-acetoxy-5-(6-benzamido-9H-purin-9-yl)-3-(benzyloxy)tetrahydrofuran-2-yl)ethyl benzoate (3) (35 g, 56.30 mmol) in methanol (150.0 mL). The reaction was stirred in an ice bath for 16 hours and concentrated under reduced pressure while maintaining the bath temperature close to 0 °C. Purification by flash chromatography (silica gel, 0 - 3% dichloromethane/methanol) afforded the desired product as a white solid. 26.30 g, 81 % yield. 1H NMR (300MHz, CHLOROFORM-d) δ = 9.24 (s, 1H), 8.54 (s, 1H), 7.99 - 7.87 (m, 5H), 7.58 - 7.27 (m, 12H), 5.97 (d, J=5.4 Hz, 1H), 5.48 (dd, J=4.5, 6.6 Hz, 1H), 5.04 (br d, J=5.6 Hz, 1H), 4.80 - 4.70 (m, 2H), 4.57 (dd, J=3.9, 5.3 Hz, 1H), 4.32 (t, J=4.2 Hz, 1H), 4.21 (d, J=6.3 Hz, 1H), 2.26 (s, 2H), 1.34 (d, J=6.5 Hz, 3H). 13C NMR (75MHz, CHLOROFORM-d) δ = 165.51, 164.44, 152.58, 151.20, 149.57, 141.92, 136.50, 133.61, 133.31, 132.81, 129.65, 129.47, 128.88, 128.76, 128.57, 128.45, 128.35, 127.83, 123.19, 89.69, 84.88, 73.27, 72.90, 69.87, 16.34; LCMS (ESI) m/z: [M+H] calcd, 579.2; found, 579.1

**Compound 28.** (1S)-1-((3S,4R,5R)-5-(6-benzamido-9H-purin-9-yl)-3-(benzyloxy)-4-hydroxytetrahydrofuran-2-yl)ethyl benzoate (4) (26.3 g, 45.50 mmol) and 4-Dimethylaminopyridine (11.10 g, 90.80 mmol, 2.0 eq) were dissolved in anhydrous acetonitrile (360 mL) followed by slow addition of O-4-methylphenyl chlorothioformate (10.20 g, 8.33 mL, 54.50 mmol, 1.20 eq). The reaction was stirred at room temperature for 2 hours. The solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate and the combined organics were washed with
10% aqueous HCl, water, saturated sodium bicarbonate solution, and brine. The organic was dried over magnesium sulfate and concentrated. Purification by flash chromatography (350 g col, 0-3% dichloromethane/methanol) afforded the desired product as a white solid. 25.30 g, 77 % yield. $^1$H NMR (300MHz, CHLOROFORM-d) $\delta = 9.02$ (s, 1H), 8.39 (s, 1H), 8.03 - 7.94 (m, 5H), 7.61 - 7.34 (m, 11H), 7.26 - 7.16 (m, 2H), 6.91 (d, $J$=7.7 Hz, 2H), 6.58 (t, $J$=5.2 Hz, 1H), 6.35 (d, $J$=4.9 Hz, 1H), 5.53 (dd, $J$=3.0, 6.7 Hz, 1H), 5.11 (t, $J$=5.1 Hz, 1H), 4.83 - 4.72 (m, 2H), 4.42 (dd, $J$=3.0, 4.7 Hz, 1H), 4.11 (q, $J$=7.2 Hz, 1H), 2.36 (s, 3H), 2.04 (s, 1H), 1.38 - 1.22 (m, 4H) 

$^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta = 194.43, 165.52, 151.36, 151.24, 142.28, 136.84, 136.77, 133.56, 133.27, 132.83, 130.05, 129.68, 129.50, 128.64, 128.57, 128.50, 129.29 (dd, $J$=37.6, 99.6 Hz, 1C), 127.86, 123.40, 121.21, 120.50, 86.36, 85.22, 81.01, 74.81, 73.39, 69.68, 20.98, 16.13; LCMS (ESI) m/z: [M+H] calcd, 729.2; found, 730.3 

**Compound 29.** Azobisisobutyronitrile (AIBN) (0.72 g, 0.70 mL 4.38 mmol, 0.12 eq) and tributyltin hydride (26 g, 23.60 mL, 87.60 mmol, 2.5 eq) in toluene (30 mL) were added dropwise to a degassed (with nitrogen) solution of (1S)-1-((3R,4R,5R)-5-((6-benzamido-9H-purin-9-yl)-3-(benzyloxy)-4-((phenoxycarbonothioyl)oxy)tetrahydrofuran-2-yl)ethyl benzoate (5) (25 g, 35.10 mmol) in toluene (300 mL) and the solution was refluxed at 110°C for two hours. The solvents were concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-2% dichloromethane/methanol) afforded the desired product as a white solid. 19.5 g, 99 % yield. $^1$H NMR (300MHz, CHLOROFORM-d) $\delta = 8.66$ (s, 1H), 8.05 - 7.91 (m, 6H), 7.63 - 7.25 (m, 14H), 6.45 (dd, $J$=5.8, 8.1 Hz, 1H), 5.48 (dd, $J$=5.3, 6.5 Hz, 1H), 4.63 (s, 2H), 4.59 - 4.44 (m, 1H), 4.31 (dd, $J$=2.6, 5.2 Hz, 1H), 3.00 (dd, $J$=6.0, 7.9, 13.7 Hz, 1H), 2.69 (ddd, $J$=2.3, 5.8, 13.5 Hz, 1H), 2.14 (s, 1H), 2.04 (s, 1H), 1.91 (br s, 3H), 1.38 (d, $J$=6.5 Hz, 4H), 1.26 (s, 1H), $^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta = 165.59, 141.45, 137.17, 133.28, 132.78, 129.82, 128.57, 128.08, 128.67 (dd, $J$=46.9, 77.1 Hz, 1C), 127.82, 123.40, 86.92, 85.22, 78.33, 71.50, 70.32, 37.25, 16.72; LCMS (ESI) m/z: [M+H] calcd, 563.2; found, 564.2 

**Compound 30.** Borontrichloride (BCl$_3$) 1.0 M in DCM (90.90 mL 90.9 mmol, 2.5 eq) was added slowly to a solution of (1S)-1-((3S,5R)-5-((6-benzamido-9H-purin-9-yl)-3-
(benzylxy)tetrahydrofuran-2-yl)ethyl benzoate (6) (20.50 g, 36.40 mmol) in anhydrous dichloromethane (350 mL) at -78°C. After the addition, the reaction was stirred at -78°C for 30 minutes. Quenched reaction by adding slowly a mixture solution of Et₃N/MeOH/DCM/ (180 mL/180 mL/180 mL) while keeping temperature at -70°C continue stir reaction for one hour. This solution was slowly added to a cold saturated NaHCO₃ solution and stir for 20 minutes. The aqueous layer was removed and the organic layer was washed for second time with saturated NaHCO₃ solution, dried over Na₂SO₄ and filtered. The solvent was concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-5% methanol/dichloromethane) afforded the desired product as a white solid. 14.80 g, 81 % yield. ¹H NMR (300MHz, CHLOROFORM-d) δ = 8.96 (s, 1H), 8.68 (s, 1H), 8.06 - 7.94 (m, 6H), 7.64 - 7.37 (m, 7H), 6.47 (t, J=6.7 Hz, 1H), 5.48 (dd, J=5.1, 6.5 Hz, 1H), 4.92 (td, J=3.2, 6.1 Hz, 1H), 4.19 - 4.08 (m, 2H), 3.16 - 2.83 (m, 4H), 2.60 (dddd, J=3.3, 6.1, 13.6 Hz, 1H), 2.05 (s, 1H), 1.47 - 1.37 (m, 7H), 1.26 (t, J=7.2 Hz, 2H). ¹³C NMR (75MHz, CHLOROFORM-d) δ = 165.69, 152.65, 141.45, 133.30, 132.81, 129.81, 129.50, 129.25, 128.87, 128.49, 128.45, 127.87, 127.74, 88.87, 84.77, 71.71, 70.51, 45.84, 40.21, 16.63, 8.62; LCMS (ESI) m/z: [M+H] calcd, 473.2; found, 473.1

**Compound 31.** (1S)-1-((3S,5R)-5-(6-benzamido-9H-purin-9-yl)-3-hydroxytetrahydrofuran-2-yl)ethyl benzoate (7) (14.0 g, 31.20 mmol) was dissolved in anhydrous DMF (300 mL) under nitrogen. Imidazole (8.28 g, 122 mmol, 2.20 eq) was added and the reaction was cooled in an ice bath and tert-butyl-chloro-dimethyl-silane (11.0 g, 73.0 mmol) was slowly added as a solution in anhydrous DMF (20 ml). The reaction was stirred for 16 hours at room temperature and the reaction was slowly poured onto 400 mL of ice. Then hexanes (800 mL) were added. The mixture was stirred and let stand for 90 minutes to precipitate. The solvents were then decanted. The white solid was washed with water and hexanes two more times. The white solid was then co-evaporated with toluene under reduced pressure at 60°C twice to give the desired product as a white foam 16.80 g, 92 % yield. ¹H NMR (300MHz, CHLOROFORM-d) δ = 8.99 (br s, 1H), 8.58 (s, 1H), 7.95 - 7.81 (m, 6H), 7.51 - 7.26 (m, 7H), 7.14 (s, 1H), 6.31 (dd, J=5.9, 7.8 Hz, 1H), 5.34 (dd, J=5.6, 6.4 Hz, 1H), 4.67 - 4.63 (m, 1H), 4.00 (dd, J=2.5, 5.4 Hz, 1H), 2.92 (s, 1H), 2.03 - 1.67 (m, 3H), 1.26 (d, J=6.5 Hz, 4H), 0.83 - 0.73 (m, 14H), ¹³C NMR (75MHz, CHLOROFORM-d) δ = 165.67, 164.57, 152.64, 151.42, 149.53, 141.62, 133.68, 133.26, 132.76,
Compound 32. (1S)-1-((3S,5R)-5-(6-benzamido-9H-purin-9-yl)-3-((tert-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)ethyl benzoate (8) (16.0 g, 27.20 mmol) was dissolved in methanol (200 mL). K$_2$CO$_3$ (15 g, 109 mmol, 4 eq.) was added, to the reaction and stirred for 16 hours after which the solid was filtered and rinsed with DCM/MeOH (8/2) the filtrate was concentrated under reduced pressure to half of volume. The solution was let sit at room temperature and product crystalized out from solution within 30 minutes. The solid was collected and rinsed with water (2x10 mL), suspend the solid in diethyl ether (50 mL) stir mixture for 30 minutes, filtered the solid to obtained 9.11 g, 88 % yield. $^1$H NMR (300MHz, CHLOROFORM-d) δ = 8.32 (s, 1H), 7.87 (s, 1H), 6.91 (s, 1H), 6.25 (dd, $J$=5.2, 9.9 Hz, 1H), 5.77 (br s, 2H), 4.68 (d, $J$=4.6 Hz, 1H), 4.10 (d, $J$=6.7 Hz, 1H), 4.03 - 3.96 (m, 1H), 3.04 (ddd, $J$=5.0, 10.0, 13.0 Hz, 1H), 2.15 (dd, $J$=5.4, 13.0 Hz, 1H), 1.75 (br s, 7H), 1.35 - 1.21 (m, 5H), 0.98 - 0.87 (m, 11H), $^{13}$C NMR (75MHz, CHLOROFORM-d) δ = 155.89, 152.34, 148.65, 140.39, 121.36, 94.15, 87.60, 71.66, 67.64, 41.34, 25.74, 18.62, 17.89; LCMS (ESI) m/z: [M+H] calcd, 587.3; found, 588.3

Compound 33. (1S)-1-(((3S,5R)-5-(6-amino-9H-purin-9-yl)-3-((tert-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)ethan-1-ol (9) (9.0 g, 24.0 mmol) was dissolved in pyridine (100 mL) under nitrogen. Cooled solution with ice bath and added trimethylsilyl chloride (13.50 mL, 108 mmol, 5 eq.) dropwise. Removed the ice bath and let reaction stir for 1 hr at room temperature. Cooled reaction again with ice bath, added dropwise benzoyl chloride (13.9 mL, 120 mmol, 5 eq.) after the addition, continue stir reaction for 16 hours by letting reaction warm up slowly to room temperature. The reaction was cooled in an ice bath and water (60 ml) was added dropwise while keeping temperature below 7°C. Continued to stir reaction at room temperature for 1 hour. Cooled reaction, NH$_4$OH (60 mL) was added dropwise and continue stir reaction for another 30 minutes. Evaporated most of the aqueous NH$_4$OH at room temperature. The remaining solution was diluted with EtOAc and the organics were washed with water 100 (ml). The aqueous layer was removed, and organic layer was washed with
saturated NaHCO₃, brine, dried over Na₂SO₄ filtered and filtrate was concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-5% methanol/dichloromethane) afforded the desired product as a white solid. 10.5 g, 90 % yield. ¹H NMR (300MHz, CHLOROFORM-d) δ = 9.11 (br s, 1H), 8.66 - 8.63 (m, 1H), 7.97 - 7.88 (m, 3H), 7.69 (s, 1H), 7.68 - 7.66 (m, 1H), 7.51 - 7.28 (m, 6H), 7.13 (s, 1H), 6.19 (dd, J=5.2, 9.9 Hz, 2H), 4.56 (d, J=4.7 Hz, 1H), 3.99 (dd, J=1.5, 6.8 Hz, 1H), 3.92 - 3.87 (m, 1H), 2.91 (ddd, J=5.0, 9.9, 13.0 Hz, 1H), 2.07 (dd, J=5.3, 13.0 Hz, 1H), 1.94 - 1.58 (m, 3H), 1.16 (d, J=6.8 Hz, 3H), 0.83 - 0.76 (m, 10H). ¹³C NMR (75MHz, CHLOROFORM-d) δ = 152.02, 150.68, 150.36, 142.70, 133.48, 133.28, 132.93, 132.05, 128.90, 128.64, 127.96, 127.35, 124.66, 94.12, 87.65, 71.53, 67.67, 41.43, 25.75, 18.54, 17.90; LCMS (ESI) m/z: [M+H] calcd, 483.2; found, 484.2

**Compound 34.** N-((9-((2R,4S)-4-((tert-butyldimethylsilyl)oxy)-5-((S)-1-hydroxyethyl)tetrahydrofuran-2-yl)-9H-purin-6-yl)benzamid (10) (6 g, 12.40 mmol) was co-evaporated with toluene (2x40 mL) under reduced pressure at 60°C. Dissolved material into dry pyridine (100 mL) added 2,6-lutidine (3.60 mL, 30.9 mmol 4 eq.). To this was added DMTetrCl (16.8 g, 30.9 mmol 4 eq.) and silver nitrate (3.50 g, 20.6 mmol 3.8 eq.) and the reaction was heated to 45°C for 16 hours. The next day, after cooling to 0°C and ethyl acetate was added, followed by water. The aqueous layer was removed and the organic layer was washed with saturated sodium bicarbonate solution, brine, dried over Na₂SO₃ filter and concentrated to a crude yellow oil under reduced pressure. Purification by flash chromatography (silica gel, 0-60% ethyl acetate/hexanes) gave the desired product as yellow foam 7.76 g, 80 % yield. ¹H NMR (300MHz, CHLOROFORM-d) δ = 8.95 (s, 1H), 8.73 (s, 1H), 8.01 (d, J=7.2 Hz, 2H), 7.89 (s, 1H), 7.81 (d, J=7.5 Hz, 1H), 7.64 - 7.34 (m, 11H), 7.32 - 7.27 (m, 1H), 7.24 - 7.06 (m, 3H), 6.82 (s, 2H), 6.80 - 6.78 (m, 2H), 6.37 (dd, J=6.0, 7.9 Hz, 1H), 4.61 - 4.56 (m, 1H), 4.12 (q, J=7.1 Hz, 1H), 3.97 (dd, J=2.6, 5.7 Hz, 1H), 3.82 - 3.74 (m, 7H), 3.57 (t, J=6.0 Hz, 1H), 2.04 (s, 2H), 1.72 (s, 1H), 1.26 (t, J=7.1 Hz, 3H), 0.95 - 0.85 (m, 11H). ¹³C NMR (75MHz, CHLOROFORM-d) δ = 158.64, 158.53, 139.49, 136.82, 132.76, 132.05, 130.40, 128.94, 127.91, 129.14 (t, J=96.9 Hz, 1C), 128.27 (dd, J=16.5, 74.6 Hz, 1C), 128.11 (dd, J=32.9, 81.2 Hz, 1C), 127.08, 126.84, 113.18, 113.00, 112.98, 91.30, 86.49, 72.35, 71.54, 55.26, 55.25, 39.79, 25.74, 17.89, 17.71; LCMS (ESI) m/z: [M-H] calcd, 785.4; found, 784.3
Compound 35. Triethylamine (3.46 mL, mmol, 2.5 eq) was added to a solution of 9-((2R,4S)-5-((S)-1-(bis(4-methoxyphenyl)(phenyl) methoxy)ethyl)-4-((tert-butyl(dimethyl)silyl)oxy)tetrahydrofuran-2-yl)-2-(isobutylamino)-1,9-dihydro-6H-purin-6-one (11) (7.80 g, 9.92 mmol) in dry THF (100 mL). The reaction was cooled in an ice bath and triethylamine trihydrofluoride (8.09 mL, 49.6 mmol, 5 eq.) was added. The reaction was warmed up slowly to room temperature and stirred for another 16 hours. The reaction was quenched by adding triethylamine (3.5 mL) and concentrated under reduced pressure to obtain a crude yellow oil. Purification by flash chromatography (silica gel, 0-3% methanol/dichloromethane + 1% Et3N) gave the product as a white solid 4.38 g, 67 % yield. 1H NMR (300MHz, CHLOROFORM-d) δ = 9.09 (br s, 1H), 8.70 (s, 1H), 8.02 (d, J=7.2 Hz, 2H), 7.81 (s, 1H), 7.82 (d, J=8.1 Hz, 2H), 7.63 - 7.35 (m, 11H), 7.30 - 7.17 (m, 4H), 6.85 - 6.78 (m, 4H), 6.38 (t, J=6.5 Hz, 1H), 4.54 - 4.47 (m, 1H), 4.12 (q, J=7.2 Hz, 1H), 3.88 (dd, J=3.9, 6.7 Hz, 1H), 3.78 (d, J=1.4 Hz, 6H), 3.59 (d, J=7.3 Hz, 1H), 3.48 (t, J=6.4 Hz, 1H), 3.09 (q, J=7.3 Hz, 1H), 2.58 (td, J=6.6, 13.4 Hz, 2H), 2.43 (ddd, J=4.4, 6.4, 13.6 Hz, 1H), 2.07 - 2.03 (m, 2H), 1.48 - 1.36 (m, 2H), 1.26 (t, J=7.1 Hz, 2H), 0.98 (d, J=6.1 Hz, 3H), 13C NMR (75MHz, CHLOROFORM-d) δ = 158.62, 142.57, 139.49, 132.94, 132.06, 130.30, 130.24, 129.15, 128.90, 128.85, 127.97, 127.82, 127.79, 127.36, 127.85 (t, J=59.0 Hz, 1C), 113.16, 93.14, 87.31, 81.45, 70.88, 67.65, 55.26, 45.85, 40.93, 18.38, 8.66; LCMS (ESI) m/z: [M-H] calcd, 671.3; found, 670.3

Compound 36. Benzamide 35 (2.38 g, 3.54 mmol) was dissolved in dry DMF (30 mL) under an atmosphere of nitrogen. To this was added 1H-tetrazole (0.196 g, 2.83 mmol, 0.8 eq) and 1-methylimidazole (72 µL, 0.88 mmol, 0.25 eq), followed by dropwise addition of 3-bis(diisopropylamino)phosphanyloxypropanenitrile (1.69 mL, 5.31 mmol, 1.5 eq). This was stirred at room temperature for 90 minutes. Water (1 mL) was added to quench the reaction. The reaction was diluted with a mixture of toluene/hexanes (3:1) The organics were washed four times with a mixture of DMF/H2O (3:2). The organics were then washed with saturated sodium bicarbonate solution, brine, dried over sodium sulfate and concentrated to a white foam. Purification by flash chromatography (silica gel, 50 g col, 0-5% methanol/dichloromethane + 1 % triethyl amine) gave the desired product as a white solid 2.70
g, 87 % yield. $^1$H NMR (300MHz, CHLOROFORM-d) δ = 9.06 - 8.90 (m, 1H), 8.72 (s, 1H), 8.01 (d, $J$=7.3 Hz, 2H), 7.90 - 7.85 (m, 1H), 7.63 - 7.35 (m, 9H), 7.30 - 7.17 (m, 4H), 6.84 - 6.77 (m, 4H), 6.40 (t, $J$=6.9 Hz, 1H), 4.84 (ddd, $J$=2.6, 5.5, 8.0 Hz, 1H), 4.17 - 4.07 (m, 2H), 3.78 (d, $J$=1.4 Hz, 7H), 2.68 - 2.45 (m, 4H), 2.04 (s, 2H), 1.93 - 1.78 (m, 1H), 1.33 - 1.14 (m, 15H), 0.92 - 0.84 (m, 3H), $^{31}$P NMR (121MHz, CHLOROFORM-d) δ = 149.05 (s, 1P), 148.54 (s, 1P), LCMS (ESI) m/z: [M-H] calcd, 871.4; found, 870.3

**Scheme S5.** Synthesis of 5’-(S) Methyl Adenosine Nucleosides.

**Compound 37.** Benzamide 33 (7.60 g, 15.7 mmol), triphenylphosphine (16.5 g, 4eq, 69.9 mmol) and p-nitrobenzoic acid (10.5 g, 4 eq, 62.9 mmol) were dissolved in anhydrous THF (50 mL). Diisopropyl azodicarboxylate (DIAD) (12.4 g, 3.9 eq, 12.2 mL, 61.4 mmol) was added dropwise and the reaction was stirred for 4 hours at room temperature. The solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate and water. The aqueous layer was washed twice with ethyl acetate and the organic layers were combined and washed with saturate NaHCO$_3$, brine, dried over Na$_2$SO$_4$, filtered and the solvent was removed under reduced pressure to give a crude oil. Purification by flash chromatography (silica gel, 0-
50% ethyl acetate/hexanes) afforded the desired product as a white solid. 8.0 g, 81 % yield. $^1$H NMR (300MHz, CHLOROFORM-d) $\delta = 8.98$ (s, 1H), 8.59 - 8.56 (m, 1H), 8.15 - 8.10 (m, 3H), 7.97 - 7.90 (m, 4H), 7.74 - 7.66 (m, 1H), 7.62 - 7.28 (m, 84H), 7.15 (s, 2H), 6.31 (t, $J=6.3$ Hz, 1H), 5.34 (dd, $J=4.6$, 6.5 Hz, 1H), 4.58 (q, $J=5.4$ Hz, 1H), 3.97 (t, $J=4.7$ Hz, 1H), 2.83 (td, $J=5.9$, 13.0 Hz, 1H), 2.41 (ddd, $J=5.5$, 6.6, 13.2 Hz, 1H), 1.93 (br d, $J=0.9$ Hz, 1H), 1.88 - 1.68 (m, 8H).

$^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta = 164.07$, 152.50, 150.62, 141.98, 133.20, 131.98, 131.82, 130.59, 132.17, 128.44, 132.04, 131.94, 128.60, 88.69, 84.64, 71.84, 70.80, 40.56, 25.70, 17.89, 17.19. LCMS (ESI) m/z: [M+H] calcd, 632.2; found, 633.2

**Compound 38.** 4-Nitrobenzoate 37 (8.0 g, 12.10 mmol) was dissolved in methanol (150 mL). K$_2$CO$_3$ (6.67 g, 4 eq, 48.4 mmol) was added to the reaction and it was stirred for 16 hours. The solids was removed by filtration and rinsed with DCM/MeOH (8/2). The filtrate was reduced under reduced pressure to half volume. The solution was let sit at room temperature and the product crystalized out from solution within 30 minutes. The product was collected, suspended in diethyl ether (50 mL) and stirred for 30 minutes, filtered to obtain the product as a solid 2.0 g, 44 % yield. $^1$H NMR (300MHz, CHLOROFORM-d) $\delta = 8.21$ (s, 1H), 7.75 (s, 1H), 7.16 (s, 1H), 6.21 - 6.07 (m, 2H), 5.81 (br s, 2H), 4.53 (d, $J=4.7$ Hz, 1H), 3.87 - 3.75 (m, 2H), 2.93 (ddd, $J=5.0$, 9.7, 13.0 Hz, 1H), 2.09 - 1.91 (m, 1H), 1.13 (d, $J=6.5$ Hz, 3H), 0.86 - 0.77 (m, 9H). $^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta = 155.90$, 152.32, 148.73, 140.34, 121.35, 93.08, 87.83, 75.16, 68.23, 40.57, 25.81, 20.38, 18.02. LCMS (ESI) m/z: [M+H] calcd, 379.2; found, 380.2

**Compound 39.** Alcohol 38 (9.0 g, 24.0 mmol) was dissolved in pyridine (80 mL) and the reaction was cooled in an ice bath. Trimethylsilyl chloride (3.34 mL, 26.3 mmol, 5 eq.) was added dropwise after which the ice bath was removed, and the reaction was stirred at room temperature for 1 hour. The reaction was cooled again with an ice bath and benzoyl chloride (3.0 mL, 26.3 mmol, 5 eq.) was added dropwise. After the addition, the reaction was warmed to room temperature and continued to stir for 16 hours. The reaction was cooled reaction with an ice bath, and water (63 ml) was added dropwise while keeping temperature below 7°C. The reaction was stirred at room temperature for 1 hour before cooling with an ice bath and quenched by dropwise
addition of aqueous NH₄OH (15 mL) and further stirring for another 30 minutes. The volume of water was reduced under pressure and diluted with EtOAc. The organic layer was washed with water 100 (ml), saturated aqueous NaHCO₃, brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure. Purification by flash chromatography (silica gel, 1-5% methanol/dichloromethane) afforded the desired product as a white solid. 2.2 g, 86 % yield. ¹H NMR (300MHz, CHLOROFORM-d) δ = 9.03 (s, 1H), 8.67 - 8.63 (m, 1H), 8.00 - 7.88 (m, 3H), 7.71 (s, 1H), 7.68 (s, 1H), 7.58 - 7.29 (m, 10H), 7.15 (s, 1H), 6.25 (dd, J=5.3, 9.5 Hz, 1H), 5.36 (d, J=10.9 Hz, 1H), 4.53 (d, J=4.9 Hz, 1H), 3.88 - 3.77 (m, 2H), 2.92 (ddd, J=5.1, 9.5, 13.0 Hz, 1H), 2.10 (ddd, J=0.8, 5.4, 13.0 Hz, 1H), 1.79 (s, 1H), 1.16 - 1.10 (m, 3H), 0.84 - 0.78 (m, 10H).

Compounds 40. Alcohol 39 (2.2 g, 4.5 mmol) was co-evaporated with toluene (40 mL) under reduced pressure at 60°C three times before being dissolved into dry pyridine (100 mL) and 2,6-lutidine (2.88 mL, 24.8 mmol, 4eq.). To this was added DMT-Cl (8.41 g, 24.80 mmol, 4 eq.) and silver nitrate (1.2 g, 20.6 mmol, 2.5 eq.) and the reaction was heated to 45°C for 16 hours. After cooling to 0°C, ethyl acetate was added, followed by water. The aqueous layer was removed, and the organic layer was washed with saturated sodium bicarbonate solution, brine, dried over Na₂SO₃ filter and concentrated under reduced pressure to a crude yellow oil. Purification product by flash chromatography (silica gel, 100g col, 0-60% ethyl acetate/hexanes) gave the desired product as yellow foam 2.90 g, 83 % yield. ¹H NMR (300MHz, CHLOROFORM-d) δ = 9.00 (br s, 1H), 8.80 (s, 1H), 8.29 (s, 1H), 8.07 - 8.00 (m, 2H), 7.64 - 7.15 (m, 15H), 6.81 - 6.73 (m, 4H), 6.41 (dd, J=5.2, 8.7 Hz, 1H), 4.51 - 4.46 (m, 1H), 4.12 (q, J=7.2 Hz, 1H), 3.88 (dd, J=2.0, 4.0 Hz, 1H), 3.78 (d, J=2.6 Hz, 6H), 2.77 (ddd, J=5.7, 8.6, 13.1 Hz, 1H), 2.41 (ddd, J=1.7, 5.3, 13.0 Hz, 1H), 2.04 (s, 1H), 1.81 - 1.68 (m, 1H), 1.26 (t, J=7.2 Hz, 2H), 0.94 - 0.84 (m, 13H). ¹³C NMR (75MHz, CHLOROFORM-d) δ = 164.55, 158.53, 158.47, 145.90, 141.48, 136.71, 136.50, 133.73, 132.78, 130.56, 130.43, 129.15, 128.89, 128.44, 127.86, 127.61, 126.80, 113.18, 112.94, 91.54, 86.58, 84.58, 72.50, 69.64, 55.21, 41.57, 25.81, 25.75, 17.86, 17.84; LCMS (ESI) m/z: [M-H] calcd, 785.4; found, 784.3
Compound 41. Triethylamine (1.29 mL, 9.22 mmol, 2.5 eq.) was added to a solution of benzamide 40 (2.90 g, 2.91 mmol) in THF (30 mL). The reaction was cooled with an ice bath under an atmosphere of nitrogen. Triethylamine trihydrofluoride (3.1 mL, 18.4 mmol, 5 eq.) was added slowly and then the reaction warmed to room temperature and stirred for 16 hours. Triethylamine (1.3 mL) was added, and the solvents were removed to give a pale yellow solid. Purification by flash chromatography (silica gel, 100 g col, 0-3% methanol/dichloromethane + 1% Et₃N) gave the product as a white solid 2.0 g, 87% yield. $^1$H NMR (300 MHz, CHLOROFORM-d) $\delta$ = 9.09 (br s, 1H), 8.77 (s, 1H), 8.12 (s, 1H), 8.02 (d, $J=7.3$ Hz, 2H), 7.63 - 7.43 (m, 5H), 7.39 - 7.20 (m, 8H), 6.81 (dd, $J=2.8$, 8.8 Hz, 4H), 6.30 (t, $J=6.5$ Hz, 1H), 4.80 (br d, $J=6.4$ Hz, 1H), 4.12 (q, $J=7.2$ Hz, 1H), 3.98 - 3.86 (m, 1H), 3.79 (s, 6H), 3.55 (t, $J=4.7$ Hz, 1H), 3.09 (q, $J=7.3$ Hz, 1H), 2.78 (br d, $J=6.7$ Hz, 1H), 2.48 (br dd, $J=6.0$, 13.3 Hz, 2H), 2.27 (s, 1H), 2.04 (s, 2H), 1.47 - 1.36 (m, 3H), 1.26 (t, $J=7.2$ Hz, 3H), 0.93 (d, $J=6.3$ Hz, 3H). $^{13}$C NMR (75 MHz, CHLOROFORM-d) $\delta$ = 158.65, 147.34, 139.48, 129.26, 129.15, 127.86, 127.78, 127.74, 127.09, 113.18, 81.47, 55.27, 8.63; LCMS (ESI) m/z: [M-H] calcd 671.3; found, 670.3

Compound 42. Alcohol 41 (2.0 g, 3.20 mmol) was dissolved in dry DMF (30 mL) under an atmosphere of nitrogen. To this was added 1H-tetrazole (0.18 g, 2.5 mmol, 0.8 eq.) and 1-methylimidazole (0.063 mL, 0.80 mmol, 0.25 eq.), followed by dropwise addition of 3-bis(diisopropylamino)phosphanyloxypropanenitrile (1.52 mL, 4.80 mmol, 1.5 eq). This was stirred at room temperature for 90 minutes. Water (1 mL) was added to quench the reaction. The reaction was diluted with a mixture of toluene/hexanes (3:1). The organics were washed four times with a mixture of DMF/H₂O (3:2). The organics were then washed with saturated sodium bicarbonate solution, brine, dried over sodium sulfate and concentrated to a white foam. Purification by flash chromatography (silica gel, 50 g col, 0-5% methanol/dichloromethane + 1% triethyl amine) gave the desired product as a white solid 2.55 g, 91% yield. $^1$H NMR (300 MHz, CHLOROFORM-d) $\delta$ = 8.99 - 8.74 (m, 2H), 8.26 - 8.19 (m, 1H), 8.06 - 7.99 (m, 2H), 7.63 - 7.33 (m, 10H), 7.29 - 7.17 (m, 4H), 6.83 - 6.72 (m, 4H), 6.44 - 6.36 (m, 1H), 4.78 (br d, $J=2.3$ Hz, 1H), 4.20 - 4.00 (m, 2H), 3.89 - 3.59 (m, 12H), 2.81 (s, 2H), 2.72 - 2.58 (m, 2H), 2.40 (t, $J=6.4$ Hz, 1H), 2.04 (s, 2H), 1.43 (s, 1H), 1.31 - 1.13 (m, 19H). $^{31}$P
NMR (121MHz, CHLOROFORM-d) δ = 149.52 (s, 1P), 149.02 (s, 1P), LCMS (ESI) m/z: [M-H] calcd, 871.4; found, 870.3

Scheme S6. Synthesis of (R)-5′-Methyl Guanosine Nucleosides.

Compound 43. 2-(iso-Butylamino)-1,9-dihydro-6H-purin-6-one (33.8 g, 153 mmol, 1.3 eq.) and N,O-Bis(trimethylsilyl)acetamide (115 mL, 410 mmol, 4.0 eq.) were added to a solution of diacetate 25 (52 g, 118 mmol) in dichloroethane (600 mL). After heating at 80°C for 1 hour a clear solution was obtained, this was then cooled with ice bath. Trimethylsilyl trifluoromethanesulfonate (41.8g, 180 mmol, 1.6 eq.) was added and continued to stir overnight at 80°C. The reaction was concentrated under reduced pressure to obtain a crude oil. The crude
material was dissolved in ethyl acetate (500 mL) and washed with water, saturated sodium bicarbonate solution, brine. The organics were dried over Na$_2$SO$_4$ for 15 minutes, filtered and concentrated under reduced pressure to obtain a crude oil. Purification by flash chromatography (silica gel, 350g col, 0-5% dichloromethane/methanol) afforded the desired product as a white solid. 47 g, 66 % yield. $^1$H NMR (300MHz, DMSO-d$_6$) δ = 12.07 (s, 1H), 11.57 (s, 1H), 8.09 - 8.05 (m, 1H), 7.99 - 7.93 (m, 2H), 7.73 - 7.64 (m, 1H), 7.57 - 7.48 (m, 2H), 7.39 - 7.28 (m, 5H), 6.10 (d, J=5.0 Hz, 1H), 5.74 (t, J=5.3 Hz, 1H), 5.36 (dd, J=4.7, 6.5 Hz, 1H), 4.74 - 4.66 (m, 2H), 4.63 - 4.54 (m, 1H), 4.30 (t, J=4.8 Hz, 1H), 3.42 - 3.32 (m, 1H), 2.82 - 2.70 (m, 1H), 2.08 (s, 3H), 1.33 (d, J=6.5 Hz, 3H), 1.18 - 1.07 (m, 7H).

Compound 44. To a three neck round flask, with a mechanical stirrer, was added anhydrous THF (250 mL) and 3-hydroxypropionitrile (4.09 mL, 59.9 mmol, 1.4 eq) under an atmosphere of nitrogen. NaH (60 % mineral oil) (2.46 g, 64.2 mmol, 1.5 eq.) was added portion-wise at room temperature and stirred for 1 hour. Acetate 43 (18.3, 31.0 mmol) in anhydrous THF (100 mL) was added to the reaction using a cannula keeping reaction between -10 to -1 °C. After addition the reaction was stirred for 2 hours. The reaction was quenched by adding slow addition of water (50 mL), keeping temperature below 0°C. Stirring was continued for 30 minutes and the reaction was transferred to a separatory funnel. The aqueous layer was extracted with EtOAc and the combined organics were washed with saturated NaHCO$_3$, brine, dried organic over Na$_2$SO$_3$, filtered and concentrated under reduced pressure to obtain a crude oil. Purification by flash chromatography (silica gel, 200g col, 0-3% dichloromethane/methanol) afforded the desired product as a white solid. 23 g, 98 % yield. $^1$H NMR (300MHz, CHLOROFORM-d) δ = 12.09 (s, 1H), 9.96 (s, 1H), 8.02 - 7.87 (m, 2H), 7.66 - 7.38 (m, 4H), 7.33 - 7.14 (m, 6H), 6.15 - 5.94 (m, 1H), 5.86 (d, J=4.4 Hz, 1H), 4.92 (q, J=4.7 Hz, 1H), 4.76 - 4.47 (m, 4H), 4.40 - 4.27 (m, 1H), 4.27 - 4.13 (m, 1H), 3.90 (br d, J=1.0 Hz, 1H), 2.87 (quin, J=6.9 Hz, 1H), 2.61 (t, J=6.3 Hz, 1H), 1.89 (s, 3H), 1.47 - 1.23 (m, 10H). $^{13}$C NMR (75MHz, CHLOROFORM-d) δ = 179.08, 166.98, 155.47, 147.44, 147.29, 139.00, 136.81, 133.74, 129.78, 129.58, 128.70, 128.65, 128.53, 128.30,
Compound 45. Alcohol 44 (20.3 g, 36.1 mmol) and 4-dimethylaminopyridine (8.81 g, 72.2 mmol, 2.0 eq) were dissolved in anhydrous acetonitrile (360 mL), followed by slow addition of O-4-methylphenyl chlorothioformate (6.62 mL, 43.3 mmol, 1.2 eq). The reaction was stirred at room temperature for 2 hours. The solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate and the combined organics were washed with 10% HCl (aq.), water, saturated sodium bicarbonate solution, water and brine. The organics were dried over magnesium sulfate for 15 minutes, filtered and concentrated under reduced pressure to obtain a crude oil. Purification by flash chromatography (200 g col, 0-3% dichloromethane/methanol) afforded the desired product as a white solid. 20.3 g, 79 % yield. \(^1\)H NMR (300MHz, CHLOROFORM-d) \(\delta = 12.10\) (s, 1H), 9.80 (s, 1H), 7.68 - 7.60 (m, 2H), 7.46 (t, \(J=7.5\) Hz, 2H), 7.27 - 7.15 (m, 7H), 7.03 (d, \(J=6.5\) Hz, 2H), 6.90 (d, \(J=7.7\) Hz, 2H), 6.21 - 6.11 (m, 2H), 5.90 (dd, \(J=2.8, 5.4\) Hz, 1H), 5.29 (t, \(J=5.6\) Hz, 1H), 4.47 - 4.30 (m, 3H), 2.85 (quin, \(J=6.9\) Hz, 1H), 2.36 (s, 3H), 1.69 (s, 5H), 1.49 - 1.25 (m, 10H). \(^13\)C NMR (75MHz, CHLOROFORM-d) \(\delta = 194.50, 179.00, 167.29, 155.40, 151.21, 147.82, 147.18, 138.42, 136.84, 136.43, 133.87, 130.21, 129.87, 129.48, 128.75, 128.41, 128.18, 122.66, 121.15, 88.45, 85.35, 83.17, 73.51, 70.61, 36.50, 20.96, 19.06, 18.88, 18.54; LCMS (ESI) m/z: [M+H] calcd, 681.3; found, 682.3

Compound 46. Azobisisobutyronitrile (AIBN) (0.58 g, 3.5 mmol, 0.12 eq) and tributyltin hydride (19.3 mL, 71.7 mmol, 2.5 eq) in toluene (30 mL) was added dropwise to a degassed (with nitrogen) solution of 4-methylbenzothioate 45 (20 g, 28.7 mmol) in toluene (250 mL) at reflux. After addition the reaction was stirred for 1 hour and the solvent was removed under reduced pressure. Purification by flash chromatography (silica gel, 220g col, 0-5% dichloromethane/methanol) afforded the desired product as a white solid. 14 g, 90 % yield. \(^1\)H NMR (300MHz, CHLOROFORM-d) \(\delta = 12.04\) (br s, 1H), 9.65 (s, 1H), 8.02 - 7.99 (m, 1H), 7.98 (s, 1H), 7.69 - 7.60 (m, 2H), 7.55 - 7.42 (m, 2H), 7.30 - 7.14 (m, 6H), 7.02 (d, \(J=8.1\) Hz, 1H), 6.81 - 6.75 (m, 1H), 6.33 - 6.15 (m, 2H), 4.57 (td, \(J=2.9, 6.0\) Hz, 1H), 4.48 (s, 2H), 4.25 (dd,
\[ J=2.6, 9.2 \text{ Hz}, 1\text{H}), \ 3.02 - 2.80 \text{ (m, 2H)}, \ 2.53 \text{ (ddd, } J=3.1, 7.2, 14.0 \text{ Hz, 1H)}, \ 2.26 \text{ (s, 1H)}, \ 1.54 \text{ (s, 1H), 1.45 - 1.25 \text{ (m, 10H)}, 0.86 \text{ (s, 1H)}. \]  

\[ ^{13}\text{C NMR (75MHz, CHLOROFORM-d) } \delta = 179.07, \ 166.99, \ 155.62, \ 153.86, \ 147.41, \ 147.20, \ 138.66, \ 137.14, \ 133.75, \ 129.94, \ 129.70, \ 129.65, \ 128.71, \ 128.47, \ 127.95, \ 127.79, \ 122.69, \ 115.23, \ 87.50, \ 86.57, \ 79.30, \ 71.55, \ 70.33, \ 36.45, \ 20.47, \ 19.04, \ 18.83, \ 17.97; \text{LCMS (ESI) m/z: } [\text{M+H}] \text{ calcd, 531.3; found, 532.2} \]

**Compound 47.** Boron trichloride (BCl3) 1.0 M in DCM (65.5 mL, 65.5 mmol, 2.5 eq) added slowly to a solution of benzoate 46 (18.6 g, 34.20 mmol) in anhydrous dichloromethane (350 mL) at -78°C under nitrogen. After the addition the reaction was stirred at -78°C for 30 minutes and then quenched by slowly adding a mixture of Et3N/MeOH/DCM (180 mL/180 mL/180 mL), keeping temperature at -70°C. This was then stirred for one hour. The reaction was slowly added to a cold solution of saturated aqueous NaHCO3 and stirred for 20 minutes. The aqueous layer was discarded, and the organics were washed a second time with saturated aqueous NaHCO3 solution. The organic layer was dried over Na2SO4, filtered and concentrated under reduced pressure. Purification by flash chromatography (silica gel, 350g col, 0-5% methanol/dichloromethane) afforded the desired product as a white solid. 11 g crude, 70 % yield. Note: product mixt with boron salt hard to remove, product was taking to the next step as crude.  

\[ ^{1}H \text{ NMR (300MHz, CHLOROFORM-d) } \delta = 12.08 \text{ (br s, 1H), 9.78 (s, 1H), 8.07 - 7.98 (m, 3H), 7.70 (s, 1H), 7.61 (br d, } J=7.3 \text{ Hz, 1H), 7.55 - 7.42 (m, 3H), 7.27 (d, } J=0.6 \text{ Hz, 3H), 6.27 - 6.13 (m, 2H), 5.03 - 4.97 (m, 1H), 4.11 (dd, } J=3.1, 8.6 \text{ Hz, 1H), 3.01 - 2.82 (m, 4H), 1.88 - 1.50 (m, 8H), 1.50 - 1.19 (m, 12H). \]  

\[ ^{13}\text{C NMR (75MHz, CHLOROFORM-d) } \delta = 179.20, \ 166.96, \ 155.65, \ 147.45, \ 147.26, \ 138.74, \ 129.71, \ 129.66, \ 128.69, \ 122.57, \ 89.60, \ 86.10, \ 77.81, \ 72.49, \ 70.75, \ 39.66, \ 36.45, \ 19.05, \ 18.85, \ 17.91; \text{LCMS (ESI) m/z: } [\text{M+H}] \text{ calcd, 441.2; found, 442.2} \]

**Compound 48.** Alcohol 47 (1.0 g,24.2 mmol) was dissolved in anhydrous DMF (250 mL) under nitrogen. Imidazole (3.62 g, 53.0 mmol, 2.20 eq) was added and the solution was cooled with an ice bath. tert-Butyl-chloro-dimethyl-silane (6.12 g, 40.6 mmol) in anhydrous DMF (20 mL) was slowly added and the reaction was stirred for 16 hours. The reaction was slowly poured onto 400 mL of ice and stirred. The aqueous layer was extracted with EtOAc (2x100 mL). The combined
organics were washed with saturated NaHCO3 solution, brine, dried over, filtered and concentrated under reduce pressure. Purification by flash chromatography (silica gel, 220g col, 0-5% methanol/dichloromethane) afforded the desired product as a white solid. 13 g, 100% yield crude. Without any further purification the crude material was taking to the next step, LCMS (ESI) m/z: [M+H] calcd, 555.3; found, 556.2

**Compound 49.** Benzoate 48 (16.0 g, 27.20 mmol) was dissolved in methanol (200 mL). K2CO3 (13.4 g, 96.90 mmol, 4 eq.) was added and the reaction was stirred for 16 hours. The solvent was concentrated under reduced pressure to obtain a white solid. The solid was suspended in water (50 ml), filtered, and washed with diethyl ether (50 mL) to obtain the desired product. 7.13 g, 74% yield. 1H NMR (300MHz, DMSO-d6) δ = 10.79 (br s, 1H), 7.92 (s, 1H), 6.60 (s, 2H), 6.13 - 6.06 (m, 1H), 5.04 (d, J=5.0 Hz, 1H), 4.58 (d, J=4.7 Hz, 1H), 3.73 - 3.53 (m, 2H), 3.34 (s, 1H), 1.06 (d, J=6.3 Hz, 3H), 0.92 - 0.83 (m, 11H), 0.13 - 0.08 (m, 7H). 13C NMR (75MHz, METHANOL-d4) δ = 155.21, 152.27, 138.54, 101.42, 93.94, 86.44, 73.54, 73.42, 72.06, 68.88, 62.27, 50.98, 41.86, 26.26, 19.47, 18.78; LCMS (ESI) m/z: [M+H] calcd, 395.2; found, 396.2

**Compound 50.** Alcohol 49 (3.50 g, 8.85 mmol) was dissolved in pyridine (100 mL) under an atmosphere of nitrogen. The reaction was cooled with an ice bath, trimethylsilyl chloride (7.86 mL, 62 mmol, 7 eq.) was added dropwise. The ice bath was removed, and the reaction was stirred for 1 hour at room temperature. The reaction was cooled with an ice bath, isobutyl chloride (5.71 mL, 58.40 mmol, 6 eq.) was added dropwise stirred for 16 hours while warming up to room temperature. The reaction was cooled reaction with ice bath and quenched by dropwise addition of water (60 mL), keeping temperature below 5°C. The reaction was stirred at room temperature for 1 hour, cooled with an ice bath, followed by dropwise addition of aqueous NH4OH (20 mL) and stirred reaction for a further 30 minutes. The reaction was concentrated under reduced, diluted with EtOAc and the organics were washed with water (100 ml), saturated NaHCO3, brine, dried over Na2SO4 and concentrated under reduced pressure. Purification by flash chromatography (silica gel, 100g col, 0-5% methanol/dichloromethane) afforded the desired product as a white solid. 4.0 g, 98+% yield. 1H NMR (300MHz, CHLOROFORM-d) δ = 12.08 (s, 1H), 9.69 (s, 1H), 8.07 - 7.90 (m, 3H), 7.72 - 7.57 (m, 2H), 7.56 - 7.38 (m, 3H), 7.34 -
7.14 (m, 8H), 6.34 - 6.13 (m, 2H), 4.80 - 4.52 (m, 2H), 4.48 (s, 2H), 4.25 (dd, J=2.4, 9.2 Hz, 2H), 3.14 - 2.92 (m, 1H), 2.91 - 2.78 (m, 2H), 2.10 (s, 1H), 1.89 (s, 3H). \(^{13}\)C NMR (75MHz, CHLOROFORM-d) δ = 178.89, 166.96, 155.66, 147.34, 147.18, 138.68, 137.16, 133.72, 129.70, 129.67, 129.53, 128.75, 128.69, 128.57, 128.52, 128.46, 127.98, 127.94, 127.79, 122.75, 87.50, 86.56, 79.32, 71.55, 70.35, 37.04, 36.45, 19.03, 18.82, 17.97; LCMS (ESI) m/z: [M+H] calcd, 451.3; found, 452.3

**Compound 51.** Alcohol 50 (2.5 g, 5.37 mmol) was co-evaporated with toluene (2x40 mL) under reduced pressure at 60°C and dissolved into dry pyridine (50 mL) added 2,6-lutidine (2.49 mL, 21.5 mmol, 4 eq.). To this was added DMT-Cl (7.28 g, 21.5 mmol 4 eq.) and silver nitrate (3.50 g, 20.6 mmol 3.8 eq.), and the reaction was heated to 45°C for 16 hours. The reaction was cooled down with an ice bath and ethyl acetate was added, followed by water. The aqueous layer was discarded and the organics were washed with saturated sodium bicarbonate solution, brine, dried organic over Na\(_2\)SO\(_3\), filtered and concentrated under reduced pressure to a crude yellow oil. Purification product by flash chromatography (silica gel, 100g col, 0-60% ethyl acetate/hexanes (1L), 0-3% methanol/dichloromethane) gave the desired product as yellow foam 1.5 g, 37% yield. \(^1\)H NMR (300MHz, CHLOROFORM-d) δ = 12.07 (s, 1H), 8.26 (br s, 1H), 7.52 - 7.33 (m, 8H), 7.32 - 7.16 (m, 6H), 6.86 - 6.74 (m, 5H), 6.14 (t, J=7.1 Hz, 1H), 4.56 (br d, J=2.0 Hz, 1H), 4.12 (q, J=7.1 Hz, 1H), 3.90 (dd, J=2.2, 5.5 Hz, 1H), 3.82 - 3.74 (m, 8H), 3.45 - 3.36 (m, 1H), 2.53 - 2.41 (m, 1H), 2.16 - 2.03 (m, 3H), 1.72 (s, 1H), 1.31 - 1.12 (m, 10H), 0.95 - 0.72 (m, 14H), 0.14 - 0.01 (m, 8H). \(^{13}\)C NMR (75MHz, CHLOROFORM-d) δ = 177.90, 158.65, 158.62, 158.59, 155.67, 148.25, 147.19, 146.05, 136.75, 136.65, 136.52, 130.47, 130.43, 130.31, 129.15, 128.41, 128.36, 127.86, 127.78, 127.70, 126.92, 121.59, 113.18, 113.02, 113.01, 91.56, 86.51, 86.49, 83.19, 72.15, 69.79, 55.30, 55.28, 55.25, 39.84, 36.52, 25.53, 25.73, 19.06, 19.04, 18.96, 18.91, 18.22, 18.12, 17.86; LCMS (ESI) m/z: [M-H] calcd, 753.4; found, 752.3

**Compound 52.** Triethylamine (0.65 mL, 4.69 mmol, 2.5 eq) was added to a solution of compound 51(1.4 g, 1.88 mmol) in dry THF (20 mL). The reaction was cooled in an ice bath. Triethylamine trihydrofluoride (1.53 mL, 9.38 mmol, 5 eq.) was added dropwise and continued to stir reaction for 16 hours, letting the reaction warm up slowly to room temperature.
The reaction was quenched by addition of 0.65 mL of triethylamine, and then concentrated under reduced pressure to obtained a crude yellow oil. Purification by flash chromatography (silica gel, 5 g col, 0-3% methanol/dichloromethane + 1% Et$_3$N) gave the product as a white solid 0.89 g, 65% yield. Used as in in the next step, LCMS (ESI) m/z: [M-H] calcd, 639.3; found, 638.3

**Compound 53.** Alcohol 52 (0.80 g, 1.22 mmol) was dissolved in dry DMF (5 mL) under an atmosphere of nitrogen. To this was added 1H-tetrazole (67 mg, 0.97 mmol, 0.8 eq) and 1-methylimidazole (4.3 µL, 0.30 mmol, 0.25 eq), followed by dropwise addition of 3-bis(diisopropylamino)phosphonylpropanenitrile (0.56 mL, 1.84 mmol, 1.5 eq). This was stirred at room temperature for 90 minutes. Water (1 mL) was added to quench the reaction. The reaction was diluted with a mixture of toluene/hexanes (3:1). The organics were washed four times with a mixture of DMF/H$_2$O (3:2). The organics were then washed with saturated sodium bicarbonate solution, brine, dried over sodium sulfate and concentrated to a white foam. Purification by flash chromatography (silica gel, 5 g col, ethyl acetate + 1% triethyl amine) gave the desired product as a white solid 0.75 g, 72% yield. $^1$H NMR (300MHz, CHLOROFORM-d) δ = 12.06 - 11.88 (m, 1H), 8.87 (br s, 1H), 7.53 - 7.15 (m, 12H), 6.85 - 6.74 (m, 4H), 6.17 - 6.04 (m, 1H), 4.86 - 4.75 (m, 1H), 4.18 - 4.00 (m, 2H), 3.91 - 3.53 (m, 12H), 3.47 - 3.38 (m, 1H), 2.79 - 2.67 (m, 2H), 2.63 - 2.35 (m, 3H), 2.14 - 2.03 (m, 2H), 1.93 - 1.60 (m, 2H), 1.37 - 1.09 (m, 24H), 0.97 - 0.75 (m, 3H). $^{31}$P NMR (121MHz, CHLOROFORM-d) δ = 149.08 (s, 1P), 147.39 (s, 1P), LCMS (ESI) m/z: [M-H] calcd, 839.4; found, 838.3

**Scheme S7.** Synthesis of (S)-5’-Methyl Guanosine Nucleosides.
Compound 54. Alcohol 50 (5.27 g, 11.30 mmol, ) was dissolved in anhydrous THF (50 mL) under nitrogen. Triphenylphosphine (11.9 g, 45.3 mmol, 4 eq.) and p-nitrobenzoic acid (7.57 g, 45.3 mmol, 4 eq.) were added, followed by dropwise addition of diisopropyl azodicarboxylate (8.77 mL, 44.2 mmol, 3.9 eq.). The reaction was stirred reaction for 4 hours. The solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate (2x) and the combined organics were washed with saturate NaHCO₃ solution, brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure to a crude oil. Purification by flash chromatography (silica gel, 220g col, 0-2% dichloromethane/methanol) afforded the desired product as a white solid. 7.8 g, 100 % yield. Note: Product contaminated with a small amount of Ph₃P. Product used in the next step without further purification. ¹H NMR (300MHz, CHLOROFORM-d) δ = 12.01 (br s, 1H), 9.19 (br s, 1H), 8.30 - 8.16 (m, 1H), 8.12 - 8.06 (m, 1H), 7.78 - 7.43 (m, 16H), 7.27 (s, 1H), 6.23 - 6.09 (m, 1H), 5.55 (t, J=6.5 Hz, 1H), 5.29 - 5.21 (m, 1H), 4.49 - 4.44 (m, 1H), 4.31 - 4.26 (m, 1H), 4.14 - 4.06 (m, 1H), 2.89 - 2.69 (m, 1H), 2.66 - 2.56 (m, 1H), 2.28 (ddd, J=3.6, 6.1, 13.3 Hz, 1H), 2.09
- 2.02 (m, 1H), 1.48 (d, J=6.4 Hz, 1H), 1.38 - 1.12 (m, 12H), 0.94 - 0.84 (m, 8H); LCMS (ESI) m/z: [M+H] calcd, 600.3; found, 601.3

**Compound 55.** 4-Nitrobenzoate 54 (6.90 g, 11.20 mmol) was dissolved in methanol (100 mL), followed by addition of K₂CO₃ (3.88 g, 28.1 mmol, 2.5). The reaction was stirred for 16 hours at room temperature. The solvent was removed under reduced pressure to obtained a white solid. The solid was suspended in water (50 ml), filtered and rinsed with diethyl ether (50 mL) to obtained 3.24 g, 73 % yield. Used as is in the next step, LCMS (ESI) m/z: [M+H] calcd, 395.2; found, 396.2

**Compound 56.** Alcohol 55 (3.34 g, 8.40 mmol) was dissolved in pyridine (60 mL) under an atmosphere of nitrogen and cooled with an ice bath. Trimethylsilyl chloride (7.50 mL, 59 mmol, 7 eq.) was added dropwise. The ice bath was removed and the reaction was stirred for 1 hr at room temperature. The reaction again cooled with an ice bath, followed by dropwise addition of isobutyryl chloride (5.44 mL, 55.7 mmol, 7 eq.) and stirred for a further 16 hours letting reaction warm up slowly to room temperature. The reaction was cooled with an ice bath, and water (30 mL) was added dropwise while keeping temperature below 5°C. Stirring was continued at room temperature for 1 hour. The reaction was cooled in an ice bath, followed by dropwise addition of aqueous NH₄OH (20 mL) and stirred for another 30 minutes. Evaporation of most of the aqueous NH₄OH at room temperature was achieved under reduce pressure. The remaining solution was diluted with EtOAc and washed with water 100 (ml). The aqueous layer was discarded, and the organics were washed with saturated NaHCO₃, brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure. Purification by flash chromatography (silica gel, 100g col, 0-5% Methanol/Dichloromethane) afforded the desired product as a white solid. 1.30 g, 33 % yield. ¹H NMR (300MHz, CHLOROFORM-d) δ = 12.14 (br dd, J=2.2, 3.5 Hz, 1H), 8.78 (s, 1H), 7.75 - 7.72 (m, 1H), 6.19 (dd, J=5.4, 9.4 Hz, 1H), 4.55 (d, J=5.4 Hz, 1H), 3.97 - 3.89 (m, 2H), 2.85 - 2.62 (m, 2H), 2.20 (ddd, J=1.4, 5.6, 13.1 Hz, 1H), 1.99 - 1.65 (m, 10H), 1.60 (s, 1H), 1.38 - 1.13 (m, 12H), 0.96 - 0.85 (m, 9H), 0.15 - 0.01 (m, 7H). ¹³C NMR (75MHz, CHLOROFORM-d) δ = 147.50, 138.65, 92.03, 86.96, 74.52, 68.36, 40.79, 36.36, 25.75, 25.69, 25.65, 20.89, 18.91, 17.99; LCMS (ESI) m/z: [M+H] calcd, 451.3; found, 452.3
**Compound 57.** Alcohol 56 (1.20 g, 2.58 mmol) was co-evaporated with toluene (2x40 mL) under reduced pressure at 60°C. This was then dissolved into dry pyridine (50 mL) and 2,6-lutidine (1.50 mL, 10.30 mmol, 4 eq.) was added. DMT-Cl (3.49 g, 10.3 mmol 4 eq.) and silver nitrate (1.7 g, 10 mmol 3.8 eq.) were then added and the reaction was heated to 45°C for 16 hours. The reaction was cooled in an ice bath and ethyl acetate (50 mL) was added, followed by water (50 mL) and stirred for 10 minutes. The aqueous layer was removed, and the organics were washed with saturated sodium bicarbonate solution, brine, dried organic over Na$_2$SO$_3$, filtered and concentrated to a crude yellow oil under reduced pressure. Purification product by flash chromatography (silica gel, 100g col, 0-60% ethyl acetate/hexanes (1L), 0-3% methanol/dichloromethane) afforded the desired product as yellow foam 1.5 g, 37% yield. $^1$H NMR (300MHz, CHLOROFORM-d) δ = 11.94 (s, 1H), 7.84 (s, 1H), 7.73 (br s, 1H), 7.59 - 7.51 (m, 2H), 7.50 - 7.35 (m, 4H), 7.32 - 7.27 (m, 1H), 7.24 - 7.15 (m, 2H), 6.85 - 6.76 (m, 4H), 6.12 (dd, J=4.9, 9.7 Hz, 1H), 4.51 (d, J=5.8 Hz, 1H), 4.12 (q, J=7.2 Hz, 1H), 3.82 - 3.76 (m, 7H), 3.51 (dd, J=3.3, 6.4 Hz, 1H), 2.23 (dd, J=4.9, 12.7 Hz, 1H), 2.05 (s, 1H), 1.90 - 1.76 (m, 1H), 1.72 - 1.60 (m, 2H), 1.32 - 1.13 (m, 4H), 0.99 - 0.77 (m, 18H). $^{13}$C NMR (75MHz, CHLOROFORM-d) δ = 158.63, 146.47, 136.79, 130.28, 129.14, 128.21, 127.78, 126.92, 113.18, 113.08, 113.05, 91.74, 86.12, 84.51, 72.78, 69.88, 55.27, 55.26, 40.82, 36.33, 35.76, 18.69, 18.41, 17.88, 17.85; LCMS (ESI) m/z: [M-H] calcd, 753.4; found, 752.3

**Compound 58.** Triethylamine (0.69 mL, 4.98 mmol, 2.5 eq) was added to a solution of compound 57 (1.53 g, 1.99 mmol) in dry THF (20 mL). The reaction was cooled in an ice bath. Triethylamine trihydrofluoride (1.62 mL, 9.9 mmol, 5 eq.) was added dropwise and the reaction was stirred for 16 hours and slowly warmed to room temperature. The reaction was quenched by adding triethylamine (0.65 mL) and concentrated under reduced pressure to obtained a crude yellow oil. Purification by flash chromatography (silica gel, 5g col, 0-10% methanol/dichloromethane + 1% Et$_3$N) afforded the product as a white solid 1.0 g, 77 % yield. $^1$H NMR (300MHz, CHLOROFORM-d) δ = 12.11 (s, 1H), 8.82 (br s, 1H), 7.80 (s, 1H), 7.52 - 7.43 (m, 2H), 7.41 - 7.32 (m, 5H), 7.28 - 7.18 (m, 5H), 6.83 - 6.75 (m, 5H), 6.07 (t, J=6.6 Hz, 1H), 4.77 - 4.70 (m, 1H), 4.12 (q, J=7.2 Hz, 1H), 3.80 - 3.73 (m, 8H), 3.63 (t, J=4.2 Hz, 1H), 2.57 - 2.52 (m, 2H), 2.32 - 2.22 (m, 1H), 2.19 - 1.90 (m, 1H), 1.89 - 1.71 (m, 1H), 0.90 - 0.75 (m, 18H). $^{13}$C NMR (75MHz, CHLOROFORM-d) δ = 157.72, 146.47, 136.69, 130.28, 129.14, 128.21, 127.77, 126.92, 113.18, 113.08, 113.05, 91.74, 86.12, 84.51, 72.78, 69.88, 55.27, 55.26, 40.82, 36.33, 35.76, 18.69, 18.41, 17.88, 17.85; LCMS (ESI) m/z: [M-H] calcd, 753.4; found, 752.3
2.99 (br s, 1H), 2.77 - 2.60 (m, 1H), 2.48 - 2.30 (m, 2H), 2.05 (s, 1H), 1.80 (s, 1H), 1.29 - 1.07 (m, 9H), 0.86 (d, J=6.3 Hz, 4H). $^{13}$C NMR (75MHz, METHANOL-$d_4$) $\delta$ = 181.78, 160.05, 149.30, 141.25, 139.72, 130.47, 129.17, 128.56, 127.83, 113.88, 92.38, 85.33, 82.43, 73.55, 73.15, 68.34, 62.28, 55.70, 42.11, 36.97, 20.20, 19.33; LCMS (ESI) m/z: [M-H] calcd, 639.3; found, 638.3

**Compound 59.** Alcohol 58 (1.0 g, 1.50 mmol) was dissolved in dry DMF (5 mL) under an atmosphere of nitrogen. To this was added 1H-tetrazole (82 mg, 1.20 mmol, 0.8 eq) and 1-methylimidazole (29 µL, 37 mmol, 0.25 eq), followed by dropwise addition of 3-bis(diisopropylamino)phosphonyloxypropanenitrile (0.713 mL, 2.25 mmol, 1.5 eq.) This was stirred at room temperature for 90 minutes. Water (1 mL) was added to quench the reaction. The reaction was diluted with a mixture of toluene/hexanes (3:1) The organics were washed four times with a mixture of DMF/H$_2$O (3:2). The organics were then washed with saturated sodium bicarbonate solution, brine, dried over sodium sulfate and concentrated to a white foam. Purification by flash chromatography (silica gel, 5 g col, ethyl acetate + 1 % triethyl amine) gave the desired product as a white solid 1.20 g, 94 % yield. $^1$H NMR (300MHz, CHLOROFORM-d) $\delta$ = 7.82 - 7.77 (m, 1H), 7.56 - 7.33 (m, 2H), 7.29 - 7.16 (m, 1H), 6.83 - 6.75 (m, 1H), 6.13 (dd, J=5.7, 8.6 Hz, 1H), 5.63 (s, 1H), 4.84 - 4.75 (m, 1H), 4.09 - 3.97 (m, 1H), 3.89 - 3.56 (m, 3H), 3.06 - 2.89 (m, 1H), 2.79 - 2.65 (m, 1H), 2.64 - 2.34 (m, 1H), 2.24 - 2.08 (m, 1H), 2.05 (s, 1H), 1.58 - 1.36 (m, 1H), 1.30 - 0.95 (m, 6H), 0.91 - 0.77 (m, 1H). $^{31}$P NMR (121MHz, CHLOROFORM-d) $\delta$ = 149.44 (s, 1P), 148.41 (s, 1P), 147.36 (s, 1P). LCMS (ESI) m/z: [M-H] calcd, 839.4; found, 838.3

**Scheme S8.** Synthesis of (R)- and (S)-5'-Ethyl Thymidine Nucleosides.
Synthesis of (R)- and (S)-5'-Ethyl Thymidine nucleosides were prepared using EtMgBr in a manner identical to the procedure reported in Kel'in, et al J Org Chem 2016, 81, 2261-79.

**Compound 61.** Starting material was dried over P₂O₅ under high vacuum for 16 hours prior to use. 3'-OTBDMS thymidine 60 (2 g, 5.61 mmol) was dissolved in dry DCM (50 ml) and the reaction was cooled in an ice bath with stirring under nitrogen. Dess-Martin periodane (3.57 g, 8.42 mmol, 1.5 eq.) was added and the reaction was allowed to stir for 3 hour at 0 °C. The ice bath was removed, and the reaction was allowed to warm to room temperature. Excess Dess-Martin reagent was quenched by pouring the cold reaction mixture into a mixture of 5% aq. sodium thiosulfate (20 mL) and saturated aq. NaHCO₃ (20 mL). The reaction was concentrated under reduced pressure and then diluted with EtOAc (75 ml). The organic layer was washed with brine (100 ml) and dried over MgSO₄. The crude product was filtered through a small plug of silica gel, and the solvent was removed under reduced pressure. The crude material was used
without purification in the next step. Yield: 1.4 grams (74%). \(^1\)H NMR (300MHz, CHLOROFORM-d) \(\delta = 9.77\) (s, 1H), 8.58 (br s, 1H), 7.58 - 7.28 (m, 1H), 6.32 (dd, \(J=5.8, 8.1\) Hz, 1H), 4.68 (td, \(J=1.9, 5.4\) Hz, 1H), 4.49 (d, \(J=1.9\) Hz, 1H), 4.13 (d, \(J=7.2\) Hz, 1H), 2.38 - 2.20 (m, 1H), 2.13 - 1.84 (m, 5H), 1.27 (t, \(J=7.1\) Hz, 1H), 1.01 - 0.83 (m, 11H), 0.19 - 0.00 (m, 8H).

\(^{13}\)C NMR (75MHz, DMSO-d\(_6\)) \(\delta = 200.92, 163.72, 150.52, 150.43, 137.20, 109.68, 109.53, 90.92, 88.87, 86.83, 72.97, 25.80, 25.70, 25.62, 17.67, 12.11, -3.21, -4.89, -4.94, -4.96. LCMS (ESI) m/z: [M+H] calcd, 354.5; found, 355.2.

**Compound 62 and 63.** Starting material was dried over P\(_2\)O\(_5\) overnight prior to use. Aldehyde 61 (1.48 g, 4.02 mmol) was dissolved in dry THF 20 ml) and was cooled to -20 °C with stirring under nitrogen. Ethylmagnesium bromide (3M in Et\(_2\)O, 5.6 ml, 17 mmol, 4 eq.) was added dropwise via syringe and the reaction was stirred for 6 hours at 0 °C under nitrogen. The reaction was quenched by slow addition of saturated aqueous NH\(_4\)Cl (100 ml). The reaction was diluted with EtOAc (70 ml) and was transferred to a separatory funnel. The reaction mixture was washed with a mixture of water and saturated aqueous NH\(_4\)Cl (1:1, 3 x 200 ml), followed by brine (1 x 200 ml). The organics were collected, dried over MgSO\(_4\), filtered, and concentrated under reduced pressure. Yield: 850 mg (55%). LCMS suggests ~ 1:1 ratio of R- and S-isomers. The isomers were separated by silica gel chromatography. Solvent A = 1% triethylamine in hexanes and solvent B = Et\(_2\)O. Gradient from 50% B to 100% B over 15 column volumes and monitor by TLC (75% Et\(_2\)O in hexanes with 1% triethylamine). Consistent with the literature protocol, the S-isomer eluted first. Yield: 300 mg S-isomer (14 %), and 450 mg R-isomer (21%).

(S)-5’-ethyl T TBDMS - \(^1\)H NMR (300MHz, CHLOROFORM-d) \(\delta = 7.42\) (d, \(J=1.2\) Hz, 1H), 6.13 (t, \(J=6.9\) Hz, 1H), 4.56 - 4.46 (m, 1H), 3.83 - 3.78 (m, 1H), 3.61 (br d, \(J=2.3\) Hz, 1H), 3.26 (q, \(J=7.3\) Hz, 1H), 2.57 (d, \(J=7.2\) Hz, 1H), 2.44 - 2.29 (m, 1H), 2.28 - 2.08 (m, 1H), 1.92 (d, \(J=1.2\) Hz, 3H), 1.71 - 1.49 (m, 3H), 1.32 (t, \(J=7.2\) Hz, 3H), 1.07 - 0.83 (m, 9H), 0.11 - -0.03 (m, 6H). LCMS (ESI) m/z: [M-H] calcd, 384.5; found, 385.2.

(R)-5’-ethyl T TBDMS - \(^1\)H NMR (300MHz, CHLOROFORM-d) \(\delta = 8.35\) (br s, 1H), 7.41 - 7.32 (m, 1H), 6.15 - 6.05 (m, 1H), 4.56 - 4.46 (m, 1H), 3.95 - 3.73 (m, 2H), 2.74 (br s, 1H), 2.44 - 2.32 (m, 1H), 2.28 - 2.09 (m, 1H), 1.95 - 1.90 (m, 3H), 1.78 - 1.43 (m, 4H), 1.11 - 0.88 (m, 6H).
13H, 0.14 - 0.07 (m, 6H). \(^{13}\)C NMR (75MHz, CHLOROFORM-d) \(\delta = 163.38, 150.21, 137.38, 111.00, 90.23, 87.30, 73.39, 70.78, 40.52, 26.39, 25.72, 25.67, 17.79, 12.51, 10.44, -4.43, -4.88. LCMS (ESI) m/z: [M-H] calcd, 384.5; found, 385.2.

**Compound 64.** Starting material was dried over P$_2$O$_5$ for 16 hours under high vacuum prior to use. Alcohol 62 (300 mg, 0.78 mmol) was dissolved in pyridine (2.5 ml) and lutidine (0.275 ml, 2.34 mmol, 3 eq.). DMT-Cl (800 mg, 2.34 mmol, 3 eq.) was added to the reaction followed by AgNO$_3$ (100 mg, 0.59 mmol, 0.75 eq.). The reaction was purged with argon, capped tightly, and allowed to stir at 40 °C for 4 days. Upon completion, the reaction was cooled to room temperature, diluted with EtOAc (50 ml), and filtered through a plug of celite to remove silver salts. The filtrate was transferred to a separatory funnel and washed with saturated aqueous NaHCO$_3$ (2 x 25 ml) and brine (1 x 25 ml). The organics were collected, dried over MgSO$_4$, filtered, and concentrated under reduced pressure. The crude material was purified by silica gel chromatography, neutralize with 5% triethylamine in hexanes, and purified using an EtOAc/hexanes gradient. Yield: 507 mg (94.6 %). \(^1\)H NMR (300MHz, CHLOROFORM-d) \(\delta = 8.40 - 8.23 (m, 1H), 7.50 - 7.28 (m, 8H), 7.26 - 7.04 (m, 3H), 6.92 - 6.74 (m, 5H), 6.41 - 6.21 (m, 1H), 4.88 - 4.82 (m, 1H), 3.98 - 3.76 (m, 9H), 3.17 (td, \(J=3.0, 9.2\) Hz, 1H), 2.32 - 2.00 (m, 2H), 1.65 - 1.21 (m, 6H), 0.94 - 0.71 (m, 11H), 0.64 - 0.51 (m, 3H). \(^{13}\)C NMR (75MHz, CHLOROFORM-d) \(\delta = 163.42, 158.60, 158.56, 150.08, 146.60, 136.98, 136.63, 135.57, 130.18, 130.13, 129.11, 127.97, 127.83, 127.76, 126.85, 113.11, 113.07, 113.02, 110.89, 87.67, 86.51, 83.61, 75.88, 71.22, 55.21, 41.13, 25.69, 24.20, 17.83, 11.78, 10.09, -4.24, -4.86. LCMS (ESI) m/z: [M-H] calcd, 686.6; found, 686.3.

**Compound 65.** Compound 64 (500 mg, 0.73 mmol) was dissolved in dry THF (20 ml). A solution of TBAF was added (1 M in THF, 3.50 ml, 3.50 mmol, 1.5 eq.) and the reaction was stirred at room temperature overnight under nitrogen. Upon completion, the solvent was removed under reduced pressure. The crude material was dissolved in EtOAc (100 ml) and was transferred to a separatory funnel. The reaction was washed with saturated aqueous NaHCO$_3$ (50 ml), water (50 ml), and then brine (50 ml). The organics were collected, dried over MgSO$_4$, filtered, and concentrated under reduced pressure. The crude material was purified by silica gel
chromatography using a MeOH/DCM gradient. Yield: 417 mg (84%). $^1$H NMR (300MHz, CHLOROFORM-d) $\delta = 8.79$ (s, 1H), 7.51 - 7.28 (m, 7H), 7.26 - 7.12 (m, 2H), 6.94 (d, $J=1.1$ Hz, 1H), 6.84 (dd, $J=1.9$, 8.9 Hz, 5H), 6.37 - 6.15 (m, 1H), 4.68 - 4.57 (m, 1H), 3.92 - 3.72 (m, 8H), 3.26 - 3.14 (m, 1H), 2.92 - 2.76 (m, 1H), 2.57 - 2.25 (m, 2H), 2.19 - 2.01 (m, 1H), 1.94 - 1.83 (m, 1H), 1.75 - 1.39 (m, 4H), 1.33 - 1.20 (m, 1H), 1.10 - 0.81 (m, 1H), 0.75 - 0.57 (m, 3H). $^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta = 163.52$, 158.63, 158.60, 150.25, 146.44, 136.86, 136.54, 135.26, 130.33, 130.20, 130.15, 129.11, 128.10, 127.94, 127.74, 127.69, 126.91, 113.16, 113.13, 113.11, 111.11, 86.87, 86.75, 83.51, 76.16, 71.46, 55.21, 40.61, 24.39, 11.92, 9.77. LCMS (ESI) m/z: [M-H] calcd, 572.6; found, 571.3.

**Compound 66.** Alcohol 65 (350 mg, 0.611 mmol) and tetrazole (3.43 mg, 0.49 mmol, 0.8 eq.) were dried over P$_2$O$_5$ for 16 hours under high vacuum. The reagents were dissolved in dry DMF (9 ml) with stirring under nitrogen. N-Methylimidazole (12.1 µl, 0.497 mmol, 0.25 eq.) and 3-bis(diisopropylamino)phosphanyloxypropanenitrile (291 µl, 0.917 mmol, 1.5 eq.) were added via syringe and the reaction was allowed to stir at room temperature under nitrogen for 4 hours. The reaction was diluted with EtOAc (50 ml) and triethylamine (1 ml) and was transferred to a separatory funnel. The reaction was washed with saturated aqueous NaHCO$_3$ (50 ml), water (50 ml) and brine (50 ml). The organics were collected, dried over MgSO$_4$, filtered, and concentrated under reduced pressure. The crude material was purified by dissolving in a minimum amount of DCM and precipitating by slow addition into hexanes. The product was dried under high vacuum to give the desired product as a brittle foam. Yield: 399 mg (85%). $^1$H NMR (300MHz, CHLOROFORM-d) $\delta = 9.00$ - 8.62 (m, 1H), 7.96 - 7.86 (m, 1H), 7.47 - 7.13 (m, 10H), 6.91 - 6.75 (m, 4H), 6.51 - 6.25 (m, 1H), 4.55 - 4.32 (m, 1H), 4.28 - 3.98 (m, 2H), 3.98 - 3.86 (m, 1H), 3.83 - 3.48 (m, 9H), 3.35 - 3.07 (m, 1H), 2.78 - 2.71 (m, 1H), 2.66 - 2.29 (m, 6H), 1.89 - 1.58 (m, 4H), 1.47 - 1.34 (m, 1H), 1.31 - 1.05 (m, 12H), 0.54 (q, $J=7.2$ Hz, 3H). $^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta = 163.78$, 158.63, 158.61, 150.29, 146.18, 137.82, 136.69, 136.63, 136.39, 136.28, 135.58, 135.53, 130.34, 130.29, 130.21, 130.15, 128.21, 128.18, 128.01, 127.94, 130.41, 127.66, 128.99, 126.93, 125.25, 117.49, 117.21, 113.19, 113.15, 84.20, 58.08, 55.24, 55.19, 43.26, 24.60, 24.54 (2C), 24.39, 21.41, 20.34, 20.24, 20.02, 19.92, 12.23, 9.90. $^{31}$P
NMR (121MHz, CHLOROFORM-d) δ = 149.33 (s, 1P), 148.88 (s, 1P). LCMS (ESI) m/z: [M-H] calcd, 772.9; found, 771.4.

**Compound 67.** Starting material was dried over P₂O₅ for 16 hours under high vacuum prior to use. Alcohol 63 (300 mg, 0.78 mmol) was dissolved in pyridine (2.5 ml) and lutidine (0.3 ml, 2.58 mmol, 3.31 eq.). DMT-Cl (1 g, 2.95 mmol, 3.78 eq.) was added to the reaction followed by AgNO₃ (100 mg, 0.59 mmol, 0.75 eq.). The reaction was purged with argon, capped tightly, and was allowed to stir at 40 °C for 4 days. Upon completion, the reaction was cooled to room temperature, diluted with EtOAc (50 ml), and filtered through a plug of celite to remove silver salts. The filtrate was transferred to a separatory funnel and was washed with saturated aqueous NaHCO₃ (2 x 25 ml) and brine (1 x 25 ml). The organics were collected, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude material was purified by silica gel chromatography, neutralize with 5% triethylamine in hexanes, and purified using an EtOAc/hexanes gradient. Yield: 368 g (69 %). ¹H NMR (300MHz, CHLOROFORM-d) δ = 7.93 (s, 1H), 7.53 - 7.43 (m, 2H), 7.42 - 7.28 (m, 6H), 7.26 - 7.17 (m, 2H), 7.08 - 7.04 (m, 1H), 6.92 - 6.79 (m, 5H), 6.24 (dd, J=5.4, 8.9 Hz, 1H), 4.88 - 4.83 (m, 1H), 3.90 (t, J=2.8 Hz, 1H), 3.82 - 3.77 (m, 7H), 3.17 (br d, J=9.1 Hz, 1H), 2.18 (br d, J=1.7 Hz, 1H), 1.78 (d, J=1.2 Hz, 1H), 1.57 (s, 4H), 1.42 (d, J=1.2 Hz, 6H), 0.97 - 0.87 (m, 9H), 0.80 (s, 2H), 0.59 (t, J=7.4 Hz, 4H), 0.17 (d, J=5.9 Hz, 6H). ¹³C NMR (75MHz, CHLOROFORM-d) δ = 163.24, 158.62, 158.58, 149.97, 146.60, 137.00, 136.65, 135.58, 130.20, 130.14, 127.99, 127.77, 126.86, 113.13, 113.08, 113.05, 110.88, 87.70, 86.53, 83.63, 77.20, 75.89, 71.24, 55.22, 41.13, 25.70, 24.21, 17.84, 11.78, 10.09, -4.24, -4.84. LCMS (ESI) m/z: [M-H] calcd, 686.9; found, 686.3.

**Compound 68.** Compound 67 (450 mg, 0.655 mmol) was dissolved in dry THF (4 ml). A solution of TBAF was added (1 M in THF, 0.6 ml, 6.0 mmol, 9 eq.) and the reaction was stirred at room temperature overnight under nitrogen. Upon completion, the solvent was removed under reduced pressure. The crude material was dissolved in EtOAc (100 ml) and was transferred to a separatory funnel. The reaction was washed with saturated aqueous NaHCO₃ (50 ml), water (50 ml), and brine (50 ml). The organics were collected, dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude material was purified by silica gel
chromatography using a MeOH/DCM gradient. Yield: 311 mg (83 %). $^1$H NMR (300MHz, CHLOROFORM-d) δ = 8.28 (s, 1H), 7.51 - 7.28 (m, 8H), 7.26 - 7.16 (m, 2H), 6.92 - 6.80 (m, 5H), 6.27 (dd, J=5.8, 8.0 Hz, 1H), 4.63 - 4.55 (m, 1H), 4.17 - 4.09 (m, 1H), 3.90 (t, J=3.8 Hz, 1H), 3.82 - 3.78 (m, 6H), 3.27 - 3.17 (m, 1H), 2.39 - 2.22 (m, 1H), 2.18 - 2.04 (m, 3H), 1.86 (d, J=1.2 Hz, 1H), 1.61 (s, 2H), 1.51 (d, J=1.2 Hz, 4H), 1.27 (t, J=7.1 Hz, 1H), 0.70 (t, J=7.4 Hz, 3H). $^{13}$C NMR (75MHz, CHLOROFORM-d) δ = 163.28, 158.69, 158.65, 150.03, 146.42, 136.85, 136.54, 135.22, 130.35, 130.22, 130.16, 128.12, 128.04, 127.96, 127.85, 126.95, 113.20, 113.14, 111.04, 86.81, 86.72, 83.51, 77.20, 76.13, 71.57, 60.39, 55.22, 40.56, 24.43, 21.04, 14.19, 11.96, 9.70. LCMS (ESI) m/z: [M-H] calcd, 572.7; found, 571.3.

**Compound 69.** Alcohol 68 (310 mg, 0.54 mmol) and tetrazole (3.03 mg, 0.43 mmol, 0.8 eq.) were dried over P$_2$O$_5$ for 16 hours under high vacuum. The reagents were dissolved in dry DMF (9 ml) with stirring under nitrogen. N-Methylimidazole (11 µl, 0.135 mmol, 0.25 eq.) and 3-bis(Diisopropylamino)phosphanyloxypropanenitrile (258 µl, 0.812 mmol, 1.5 eq.) were added via syringe and the reaction was allowed to stir at room temperature under nitrogen for 4 hours. The reaction was diluted with EtOAc (50 ml) and triethylamine (1 ml) and was transferred to a separatory funnel. The reaction was washed with saturated aqueous NaHCO$_3$ (50 ml), water (50 ml), and brine (50 ml). The organics were collected, dried over MgSO$_4$, filtered, and concentrated under reduced pressure. The crude material was purified by dissolving in a minimum amount of DCM and precipitating by slow addition into hexanes. The product was dried under high vacuum to give the desired product as a brittle foam. Yield: 370 mg (89%). $^1$H NMR (300MHz, CHLOROFORM-d) δ = 7.50 - 7.13 (m, 10H), 7.08 - 6.90 (m, 1H), 6.82 (dd, J=2.6, 8.9 Hz, 4H), 6.42 - 6.16 (m, 1H), 5.10 - 5.00 (m, 1H), 4.23 - 4.01 (m, 1H), 3.91 - 3.57 (m, 9H), 3.52 (dt, J=4.0, 7.1 Hz, 1H), 3.19 - 3.08 (m, 1H), 2.85 - 2.61 (m, 2H), 2.59 - 2.29 (m, 2H), 2.25 - 1.96 (m, 1H), 1.82 - 1.55 (m, 1H), 1.52 - 1.45 (m, 1H), 1.41 - 1.11 (m, 17H), 0.69 - 0.48 (m, 3H). $^{13}$C NMR (75MHz, CHLOROFORM-d) δ = 163.44, 158.61, 150.19, 150.12, 146.57, 136.97, 136.94, 136.61, 136.49, 135.53, 135.43, 130.42, 130.20, 130.16, 130.13, 129.01, 128.19, 128.00, 127.94, 127.79, 127.67, 126.85, 125.27, 11113.10, 112.97, 111.01, 83.36, 58.43, 58.20, 55.20 (2C), 43.36, 24.71, 24.46 (2C), 24.34, 22.97, 22.89, 22.85, 21.43, 20.39, 11.66, 10.17. $^{31}$P
NMR (121MHz, CHLOROFORM-d) δ = 148.62 (s, 1P), 147.96 (s, 1P). LCMS (ESI) m/z: [M-H] calcd, 772.9; found, 771.4.

Scheme S9. Synthesis of 5’-(R) Ethyl Cytidine Nucleosides.

Compound 70. POCl₃ (5.5 mL, 60.2 mmol, 8 eq) was added dropwise to a suspension of 1,2,4-1H-triazole (17.70 g, 256 mmol, 34 eq.) in acetonitrile (200 mL) under an atmosphere of nitrogen at room temperature and stirred reaction for 20 minutes. The reaction was cooled down with ice bath at 0°C, triethylamine (42 mL, 301 mmol, 40 eq.) was added dropwise, the ice bath was removed and continued to stir reaction for 30 minutes at room temperature. The reaction was cooled down with an ice bath and a solution of Compound (1) 1-((2R,4S,5R)-5-((R)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)propyl)-4-((tert-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (5.17 g, 7.53 mmol) in acetonitrile (30 mL) was added dropwise to the reaction. This was stirred at room temperature for 16 hours. The reaction was concentrated to small volume under reduced pressure, diluted with ethyl acetate and the organic layer was washed with aqueous saturated sodium bicarbonate (2x), water, brine and concentrated to afford the desired crude material as a yellow oil.
Without any further purification, the crude material was suspended in dioxane/NH$_4$OH (40 mL/10 mL) and stirred at room temperature for 2 hours. TLC in EtOAc/hexane (6/4) indicated reaction was completed. The solvents were concentrated under reduce pressure and the remaining oil was diluted with Ethyl acetate and washed with water (1x200 ml), sat. NaHCO$_3$ (1x200 ml), brine (1x200 ml), dried over Na$_2$SO$_4$, filtered and concentrated under reduce pressure to obtain a crude oil. The crude material was dissolved in DCM and purified by silica gel chromatography (silica gel, 50g col, 40-100% EtOAc/Hexane) afforded the desired product as a white solid. 4.10 g, 77 % yield. $^1$H NMR (300MHz, CHLOROFORM-d) $\delta = 7.33$ (br d, $J$=8.1 Hz, 3H), 7.28 - 7.00 (m, 14H), 6.71 - 6.59 (m, 6H), 6.09 (br t, $J$=6.8 Hz, 2H), 4.71 - 4.61 (m, 1H), 3.74 (br s, 1H), 3.67 - 3.60 (m, 9H), 3.11 - 2.83 (m, 2H), 2.31 - 2.02 (m, 2H), 1.99 - 1.68 (m, 3H), 1.55 (s, 1H), 1.38 - 1.15 (m, 9H), 1.10 (s, 1H), 0.77 - 0.72 (m, 12H), 0.70 - 0.56 (m, 2H), 0.43 (br t, $J$=7.2 Hz, 4H). $^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta = 165.14, 158.54, 155.74, 146.74, 138.52, 137.18, 136.83, 130.28, 130.19, 128.07, 127.74, 126.78, 113.09, 113.05, 101.20, 87.30, 86.38, 84.72, 75.81, 71.10, 55.24, 42.37, 25.74, 24.21, 17.85, 12.62, 10.26; LCMS (ESI) m/z: [M-H] calcd, 685.4; found, 684.3

**Compound 71.** Compound 70 (3.27 g, 4.81 mmol) was dissolved in anhydrous pyridine (20 mL) under nitrogen at 0 °C. Benzoyl chloride ( 0.61 mL, 5.29 mmol, 1.13 eq.) was added dropwise and continued to stir the reaction for 16 hours at room temperature. TLC analysis in EtOAc/Hexane (6/4) indicated reaction was completed. The reaction was cooled ice bath and H$_2$O (20 ml ) was added slowly followed by EtOAc (50 mL). The solution was transferred to a separatory funnel and was washed with water (50 mL). The aqueous layer was discarded and the organics were washed with sat. NaHCO$_3$ and brine. The organics were dried over Na$_2$SO$_4$, filtered and concentrated under reduce pressure to obtained crude oil. The crude material was purified by silica gel chromatography (silica gel, 50g col, 40-70% EtOAc/hexanes) to afford the desired product as a white solid. Yield: 3.20 g (85 %). $^1$H NMR (300MHz, CHLOROFORM-d) $\delta = 8.31$ (d, $J$=7.3 Hz, 1H), 8.12 (d, $J$=7.7 Hz, 4H), 7.64 - 7.54 (m, 3H), 7.53 - 7.37 (m, 7H), 7.31 - 7.24 (m, 9H), 7.17 (d, $J$=8.8 Hz, 5H), 6.83 (d, $J$=8.8 Hz, 4H), 3.80 (s, 8H), 2.11 (s, 3H), 1.93 (s, 1H), 1.25 (s, 3H), 1.12 - 0.97 (m, 4H), 0.90 (s, 10H). $^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta = 170.62, 158.64, 147.31, 139.45, 138.38, 133.63, 132.18, 130.18, 129.92, 129.34, 129.13, 128.68, 128.47, 128.13, 127.86, 127.77, 127.36, 127.09, 113.17, 111.95, 90.39, 87.81, 81.45, 73.47,
Compound 72. TEA (0.90 mL, 6.32 mmol, 2.5 eq.) was added to a solution of compound 71 (2.0 g, 2.5 mmol) in tetrahydrofuran (20.0 mL). The reaction was cooled down with an ice bath under an atmosphere of nitrogen. Triethylamine trihydrofluoride (2.0 mL, 12.65 mmol, 5 eq.) was slowly added and continued to stir reaction for 16 hours at room temperature. TEA (0.90 ml) was added to the reaction and the solvent was removed under reduced pressure to obtained crude solid. This material was dissolved in EtOAc (30 ml) and washed with water (50 ml). The aqueous layer was discarded and the organics were washed with saturated NaHCO₃ and brine. The organics were dried over Na₂SO₄, filtered and concentrated under reduce pressure to obtained a crude oil. The crude product was purified by flash chromatography (silica gel, 20g col, 20-100 % EtOAc/hexanes) to afford the desired product as a white solid. Yield. 1.71 g (79 %) ¹H NMR (300MHz, CHLOROFORM-d) δ = 8.35 - 8.26 (m, 3H), 7.57 - 7.35 (m, 9H), 7.33 - 7.22 (m, 10H), 7.22 - 7.11 (m, 6H), 6.87 - 6.79 (m, 7H), 6.18 (s, 2H), 3.86 - 3.75 (m, 13H), 2.39 (s, 5H), 2.11 (s, 6H), 1.69 (s, 5H), 1.25 (s, 2H), 1.05 (t, J=7.4 Hz, 4H), ¹³C NMR (75MHz, CHLOROFORM-d) δ = 159.62, 158.65, 147.33, 139.47, 137.86, 132.52, 130.26, 130.17, 129.93, 129.15, 128.15, 127.99, 127.86, 127.78, 127.10, 113.23, 113.18, 112.10, 89.04, 86.83, 81.45, 73.72, 70.88, 55.27, 40.46, 26.73, 13.70, 10.11; LCMS (ESI) m/z: [M-H] calcd, 675.3; found, 674.2

Compound 73. 1H-Tetrazole (0.110 g, 1.60 mol, 0.8 eq) and 1-Methylimidazole (40 µL, 0.50 mmol, 0.25 eq) were added to a solution of compound 72 (1.35 g, 2.0 mmol) in DMF (15 mL) at room temperature under an atmosphere of nitrogen. 2-Cyanoethyl-N,N,N',N'-tetraisopropylphosphorodiamidite (0.90 mL, 3.0 mmol, 1.5 eq) was then added drop-wise and the reaction was stirred at room temperature for 3 hrs. Water (0.5.0 mL) was added to quench the reaction. A 3:1 mixture of toluene/hexanes (30 mL) was added and the organic layer was washed (4x 20 mL) with a 3:2 mixture of DMF/H₂O. The organics were washed with saturated NaHCO₃ and brine. The organics were dried over Na₂SO₄, filtered and concentrated under reduce pressure to give a white foam. Purification by flash chromatography (silica gel, 25g col, 40% ethyl acetate/hexanes
+ 1 % triethylamine) afforded the desired white solid. Yield: 1.6 g (90 %) \(^1\)H NMR (300MHz, CHLOROFORM-d) \(\delta = 8.34 - 8.21\) (m, 2H), 7.55 - 7.34 (m, 9H), 7.32 - 7.17 (m, 6H), 6.83 (dd, \(J=2.3, 8.9\) Hz, 4H), 3.78 (d, \(J=3.2\) Hz, 10H), 2.64 (s, 3H), 1.55 (d, \(J=5.3\) Hz, 5H), 1.36 - 1.11 (m, 15H), 0.56 (s, 3H). \(^{31}\)P NMR (121MHz, CHLOROFORM-d) \(\delta = 148.68\) (s, 1P), 148.02 (s, 1P), LCMS (ESI) m/z: [M-H] calcd, 875.4; found, 874.0

**Scheme S10.** Synthesis of 5’-(R/S) Ethyl Adenosine Nucleosides.

**Compound 75.** 5’-O-DMT-N6-Benzoyl-2’- deoxyadenosine (40.0 g, 60.8 mmol) was dissolved in DMF (304 mL). Imidazole (8.28 g, 122 mmol) was added followed by tert-butyl-chloro-dimethyl-silane (11.0 g, 73.0 mmol). The reaction was stirred for 16 hours. The reaction was slowly poured onto 400 mL of ice. Hexanes was added (800 mL), and the mixture was stirred 90 minutes to precipitate. The solvent was removed (decant). The white solid was washed with hexanes (2 x 200 ml). The white solid was then co-evaporated with toluene under reduced pressure at 60 °C to give the desired product as a white foam. Yield: 45.9 grams (97.7 %) \(^1\)H NMR (300 MHz, CHLOROFORM-d) \(\delta = 9.03\) (s, 1H), 8.76 (s, 1H), 8.23 (s, 1H), 7.92-8.11 (m, 2H), 7.48-7.67 (m, 3H), 7.37-7.44 (m, 2H), 7.27-7.33 (m, 4H), 7.15-7.25 (m, 3H), 6.73-6.90 (m, 4H), 6.49 (t, \(J=6.53\) Hz, 1H), 4.56-4.73 (m, 1H), 4.06-4.21 (m, 1H), 3.78 (s, 6H), 3.24-3.48 (m, 2H), 2.80 (td, \(J=6.42, 13.15\) Hz, 1H), 2.47 (ddd, \(J=3.71, 6.05, 13.03\) Hz, 1H), 0.88 (s, 9H), 0.07
(s, 3H), 0.03 (s, 3H) $^{13}$C NMR (75 MHz, CHLOROFORM-d) δ 171.1, 164.6, 158.5, 152.6, 151.5, 149.5, 144.5, 141.6, 135.7, 133.7, 132.7, 130.0, 128.8, 128.1, 127.9, 126.9, 123.5, 113.2, 87.0, 86.5, 84.9, 72.5, 63.2, 60.4, 55.2, 40.7, 25.8, 18.0, 14.2, -4.7, -4.8. LCMS (ESI) m/z: [M+H] calcd, 772.4; found, 772.4.

**Compound 76.** Intermediate 75 (1.00 g, 1.30 mmol) was dissolved in 3:1 DCM/methanol (129 mL) and was cooled in an ice bath. Toluene-4-sulfonic acid (0.296 g, 1.55 mmol) was added and the reaction was stirred at 0 °C for 30 minutes. Sodium carbonate (206 mg, 1.5 eq) was added at 0 °C and the reaction was stirred until the orange color has disappeared. The solvents were removed under reduced pressure. Purification by flash chromatography (silica gel, 50g col, 0-5% methanol/dichloromethane) gave the desired product as a white solid. Yield: 0.545 g (89.6 %)

$^1$H NMR (300 MHz, CHLOROFORM-d) δ 9.01 (s, 1H), 8.79 (s, 1H), 8.10 (s, 1H), 7.98-8.07 (m, 2H), 7.47-7.71 (m, 3H), 6.37 (dd, J=5.38, 9.47 Hz, 1H), 5.75 (dd, J=1.98, 11.46 Hz, 1H), 4.73 (d, J=4.86 Hz, 1H), 4.18 (s, 1H), 3.99 (br d, J=12.93 Hz, 1H), 3.70-3.86 (m, 1H), 3.06 (ddd, J=5.12, 9.50, 13.02 Hz, 1H), 2.19-2.38 (m, 1H), 0.92-1.02 (m, 9H), 0.14 (s, 6H) $^{13}$C NMR (75 MHz, CHLOROFORM-d) δ 164.7, 152.1, 150.8, 150.3, 142.6, 133.5, 132.9, 128.8, 128.0, 124.5, 90.3, 87.7, 73.8, 63.1, 41.4, 25.8, 18.0, -4.7, -4.8. LCMS (ESI) m/z: [M+H] calcd, 470.2; found, 470.2.

**Compound 77.** 2-Iodoxybenzoic acid (IBX) (11.9 g, 42.6 mmol) was added to a solution of intermediate 76 (10.0 g, 21.3 mmol) in acetonitrile (213 mL). The reaction was heated at 70 °C for 4 hours. After cooling to room temperature, the reaction mixture was filtered through a plug of celite. The solvent was removed under reduced pressure to give the crude reaction mixture as a yellow foam. The crude material was dissolved in ethyl acetate and washed with water. The organics were dried over magnesium sulfate and concentrated under reduced pressure to give the desired product as a pale yellow foam. Compound 77 was used in the next step without further purification. Yield: 10.3 g (quantitative)

**Compound 78.** A solution of 77 (10.3 g, 22.0 mmol) in tetrahydrofuran (220 mL) was cooled to -20 °C under an atmosphere of nitrogen. EtMgBr in diethyl ether (3.00 M, 14.7 mL, 44.1 mmol) was added and the reaction was maintained at -20 °C for 2.5 hours. The reaction was quenched
with saturated ammonium chloride solution. The aqueous layer was extracted with ethyl acetate and the combined organics were concentrated under reduced pressure to give a thick oil. The crude material was purified by flash chromatography (silica gel, 220g col, 0-60% ethyl acetate/hexanes) to give 77 as a white foam. Yield: 2.46 g (22%).  

$^1H$ NMR (300 MHz, CHLOROFORM-d) $\delta$ 9.08 (s, 1H), 8.79 (d, $J$=6.53 Hz, 1H), 8.10 (d, $J$=2.56 Hz, 1H), 8.03 (d, $J$=7.04 Hz, 2H), 7.59-7.71 (m, 1H), 7.49-7.58 (m, 2H), 6.34 (ddd, $J$=5.18, 9.76, 12.58 Hz, 1H), 5.27-6.17 (m, 1H), 4.68 (t, $J$=4.48 Hz, 1H), 4.09 (s, 1H), 3.53-3.90 (m, 1H), 2.94-3.17 (m, 1H), 2.14-2.31 (m, 1H), 1.49-1.60 (m, 2H), 0.97-1.15 (m, 3H), 0.93 (d, $J$=1.41 Hz, 9H), 0.13 (s, 6H).

LCMS (ESI) m/z: [M+H] calcd, 498.3; found, 498.3.

Compound 79. Intermediate 78 (5.12 g, 10.3 mmol) was co-evaporated with toluene (40 mL) under reduced pressure at 60 °C three times before being dissolved in dry pyridine (100 mL) and 2,6-lutidine (3.60 mL, 30.9 mmol). To this was added DMT-Cl (10.5 g, 30.9 mmol) and silver nitrate (3.50 g, 20.6 mmol) and the reaction was heated to 40 °C for 20 hours. After cooling in an ice bath, ethyl acetate and water was added, and the reaction mixture was transferred to a separatory funnel. The aqueous layer was extracted with ethyl acetate, and the organics were washed with saturated sodium bicarbonate solution and brine. The organics were concentrated to a crude yellow oil under reduced pressure. The crude product was purified by flash chromatography (silica gel, 220g col, 0-40% ethyl acetate/hexanes) to give 79 as a yellow foam. Yield: 5.53 g, (67.1 %). $^1H$ NMR (300 MHz, CHLOROFORM-d) $\delta$ 9.02 (d, $J$=17.03 Hz, 1H), 8.81 (d, $J$=19.07 Hz, 1H), 7.83-8.61 (m, 2H), 7.27-7.66 (m, 9H), 7.11-7.25 (m, 3H), 6.69-6.87 (m, 4H), 6.31-6.57 (m, 1H), 4.31-4.75 (m, 1H), 4.00-4.08 (m, 1H), 3.73-3.81 (m, 6H), 3.19-3.55 (m, 1H), 2.17-2.92 (m, 2H), 1.33-1.59 (m, 2H), 0.81-0.97 (m, 9H), 0.51-0.78 (m, 3H), 0.13 (d, $J$=12.93 Hz, 3H), -0.13--0.02 (m, 3H) $^{13}C$ NMR (75 MHz, CHLOROFORM-d) $\delta$ 171.2, 164.6, 158.6, 158.8, 158.5, 158.4, 152.6, 152.6, 151.6, 149.5, 149.4, 146.3, 146.2, 141.6, 141.5, 136.9, 136.9, 136.8, 136.7, 133.7, 132.7, 130.5, 130.5, 130.4, 130.3, 128.9, 128.8, 128.4, 128.3, 127.9, 127.6, 126.8, 126.7, 123.3, 113.0, 112.9, 88.6, 88.2, 86.6, 86.6, 84.6, 83.9, 75.7, 74.3,
Compound 80. TEA (2.41 mL, 17.3 mmol) was added to a solution of 79 (5.53 g, 6.91 mmol) in THF (34.5 mL). The reaction was cooled in an ice bath under an atmosphere of nitrogen. Triethylamine trihydrofluoride (5.63 mL, 34.6 mmol) was added slowly and then the reaction warmed to room temperature and stirred for 14 hours. Further portions of triethylamine (1 mL) and triethylamine trihydrofluoride (2.25 mL) were added and after 4 more hours until no starting material remained (TLC). Triethylamine (5 ml) was added and the solvents were removed under reduced pressure to give a pale yellow solid. The crude material was purified by flash chromatography (silica gel, 220g col, 0-100% ethyl acetate/hexanes) gave the product as a white solid. Yield: 4.44 g (93.7 %) \(^1\)H NMR (300 MHz, CHLOROFORM-d) \(\delta\) 9.05 (d, \(J=17.03\) Hz, 1H), 8.69-8.87 (m, 1H), 7.71 8.45 (m, 3H), 7.30-7.69 (m, 9H), 7.15-7.26 (m, 3H), 6.73-6.88 (m, 4H), 6.39 (td, \(J=6.70, 15.01\) Hz, 1H), 4.39-4.75 (m, 1H), 3.84-4.06 (m, 1H), 3.74-3.83 (m, 6H), 3.41 (br s, 1H), 2.31-2.95 (m, 2H), 1.84-2.29 (m, 1H), 1.34-1.56 (m, 2H), 0.56-0.85 (m, 3H). \(^13\)C NMR (75 MHz, CHLOROFORM-d) \(\delta\) 171.2, 164.8, 158.6, 152.7, 152.6, 151.5, 151.4, 149.6, 149.5, 146.3, 146.2, 141.5, 141.3, 139.5, 136.8, 136.7, 136.5, 133.7, 132.8, 130.4, 129.2, 128.8, 128.3, 128.0, 127.9, 127.8, 127.7, 127.7, 130.4, 126.9, 126.9, 123.3, 123.1, 113.1, 113.1, 113.0, 87.5, 87.1, 86.8, 84.0, 83.9, 75.1, 74.7, 72.2, 71.2, 64.4, 60.4, 55.3, 55.2, 40.8, 39.9, 30.6, 24.3, 24.2, 21.1, 19.1, 14.2, 13.7, 10.3, 9.2. LCMS (ESI) m/z: [M+H] calcd, 686.3; found, 686.3.

Compound 81. Intermediate 80 (2.00 g, 2.92 mmol) was dissolved in dry DMF (29.2 mL) under an atmosphere of nitrogen. To this was added 1H-tetrazole (0.163 g, 2.33 mmol) and 1-methylimidazole (0.0578 mL, 0.729 mmol), followed by dropwise addition of 2-cyanoethyl-N,N,N',N'-tetraisopropyl-phosphorodiamidite (1.39 mL, 4.37 mmol). The reaction was stirred at room temperature for 90 minutes. Water (1 mL) was added to quench the reaction, and the reaction was diluted with a mixture of toluene/hexanes (3:1) The organics were washed four times with a mixture of toluene/hexanes (3:1) The organics were concentrated, dried over sodium sulfate and concentrated to a white foam. The crude product was purified by flash chromatography (silica gel, 50g col, 80% ethyl acetate/hexanes) to give 81 as a white solid. Yield: 1.99 g (76.9 %) \(^1\)H NMR (300 MHz,
Scheme S11. Synthesis of 5'--(S)-Allyl Thymidine Nucleosides.

Compound 82. Starting material 60 was dried over P₂O₅ for 16 hours prior to use. The starting material (12.8 g, 35.9 mmol) was dissolved in dry DCM (350 ml) and was cooled in an ice bath with stirring under nitrogen. TMS allyl(trimethyl)silane (28.5 ml, 180 mmol, 5 eq.) and BF₃-Et₂O (22.5 ml, 182 mmol, 5 eq.) was added dropwise via syringe and was allowed to stir for 1 hr at 0 °C under nitrogen. The reaction was monitored by TLC (2:1 EtOAc/hexanes. Aldehyde: Rf
= 0.30; product: Rf = 0.63). The reaction was quenched by slow addition of saturated aqueous NaHCO₃ (100 ml). The reaction was diluted with EtOAc (70 ml) and was transferred to a separatory funnel. The reaction mixture was washed with 1: 1 water and saturated aqueous NaHCO₃ (3 x 200 ml) followed by brine (1 x 200 ml). The organics were collected, dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography, using a gradient of 2% EtOAc in hexanes up to 70% EtOAc over 10 CV. Product fractions were pooled and concentrated to a white brittle foam. Yield: 9.1 g (64%). ¹H NMR (300MHz, CHLOROFORM-d) δ = 8.69 (s, 1H), 7.57 - 7.43 (m, 1H), 6.18 (dd, J=6.5, 7.2 Hz, 1H), 5.93 - 5.78 (m, 1H), 5.25 - 5.13 (m, 2H), 4.50 (td, J=3.2, 6.2 Hz, 1H), 3.90 - 3.70 (m, 2H), 2.49 (d, J=5.2 Hz, 1H), 2.42 - 2.28 (m, 3H), 2.26 - 2.11 (m, 1H), 2.05 (s, 1H), 1.97 - 1.85 (m, 3H), 1.26 (t, J=7.1 Hz, 1H), 0.92 - 0.83 (m, 10H), 0.16 - -0.01 (m, 6H). LCMS (ESI) m/z: [M+H] calcd, 396.6; found, 397.2.

Note: (S)-5’-allyl T 3’-O-TBDMS has been reported in the Escudier paper: Catana, D. A., Renard, B. L., Maturano, M., Payrastre, C., Tarrat, N., & Escudier, J. M. (2012). Dioxaphosphorinane-constrained nucleic Acid dinucleotides as tools for structural tuning of nucleic acids. Journal of nucleic acids, 2012, 215876. https://doi.org/10.1155/2012/215876

Compound 83. Intermediate 82 was dried over P₂O₅ for 16 hours under high vacuum prior to use. 5’-allyl dT 3’-O-TBDPS (1.35 g, 2.59 mmol) was dissolved in pyridine (20 ml) and lutidine (0.9 ml, 7.78 mmol, 3 eq.). DMT-Cl (2.70 g, 8 mmol, 3 eq.) was added to the reaction, followed by AgNO₃ (132 mg, 0.78 mmol, 0.3 eq.). The reaction was purged with argon, capped tightly, and was allowed to stir at 40 °C for 4 days. The reaction was monitored by TLC (5% MeOH in DCM). Upon completion, the reaction was cooled to room temperature, diluted with EtOAc (50 ml), and was filtered through a plug of celite to remove silver salts. The filtrate was transferred to a separatory funnel and washed with saturated aqueous NaHCO₃ (2 x 25 ml) and brine (1 x 25 ml). The organics were collected, dried over MgSO₄, filtered, and concentrated under reduced pressure. The crudes material was purified by silica gel chromatography, neutralize with 5% triethylamine in hexanes, and purified using an EtOAc/hexanes gradient. Yield: 7.0 g (87 %)

¹H NMR (300MHz, CHLOROFORM-d) δ = 8.46 (s, 1H), 7.93 (d, J=1.2 Hz, 1H), 7.54 - 7.30 (m, 9H), 7.26 - 7.06 (m, 12H), 6.86 - 6.60 (m, 5H), 6.51 (dd, J=5.4, 8.9 Hz, 1H), 5.09 - 4.95 (m, 1H), 5.00 - 4.76 (m, 1H), 4.61 - 4.47 (m, 1H), 4.36 (dd, J=3.9, 6.2 Hz, 1H), 4.16 - 4.02 (m, 1H), 3.62 - 3.48 (m, 1H), 3.45 - 3.32 (m, 1H), 3.30 - 3.17 (m, 1H), 3.16 - 3.03 (m, 1H), 3.02 - 2.89 (m, 1H), 2.88 - 2.75 (m, 1H), 2.74 - 2.61 (m, 1H), 2.60 - 2.47 (m, 1H), 2.46 - 2.33 (m, 1H), 2.32 - 2.19 (m, 1H), 2.18 - 2.05 (m, 1H), 2.05 - 1.92 (m, 1H), 1.91 - 1.78 (m, 1H), 1.77 - 1.64 (m, 1H), 1.63 - 1.50 (m, 1H), 1.50 - 1.37 (m, 1H), 1.36 - 1.23 (m, 1H), 1.23 - 1.10 (m, 1H), 1.10 - 0.97 (m, 1H), 0.97 - 0.84 (m, 1H), 0.84 - 0.71 (m, 1H), 0.71 - 0.58 (m, 1H), 0.58 - 0.45 (m, 1H), 0.45 - 0.32 (m, 1H), 0.32 - 0.19 (m, 1H), 0.19 - 0.06 (m, 1H), 0.06 - -0.03 (m, 1H).
4.85 - 4.74 (m, 2H), 4.37 (d, J=5.9 Hz, 1H), 4.13 (q, J=7.1 Hz, 1H), 3.96 (t, J=1.5 Hz, 1H), 3.78 (d, J=1.5 Hz, 7H), 3.04 - 2.97 (m, 1H), 2.37 - 1.96 (m, 6H), 1.74 (d, J=1.0 Hz, 3H), 1.70 - 1.57 (m, 1H), 1.32 - 0.98 (m, 12H), 0.95 - 0.73 (m, 1H), 0.03 - -0.02 (m, 1H). \(^{13}\)C NMR (75MHz, CHLOROFORM-d) δ = 171.10, 163.60, 158.64, 158.57, 150.15, 145.72, 136.25, 135.84, 135.82, 135.63, 135.47, 133.51, 133.21, 133.15, 130.32, 130.16, 129.82, 129.74, 128.12, 127.71, 127.61, 126.97, 117.77, 112.97, 111.15, 87.73, 87.19, 84.62, 77.20, 75.02, 74.77, 60.36, 55.20, 41.70, 36.24, 26.79.

LC MS (ESI) m/z: [M+Na] calcd, 823.1; found, 846.0

**Compound 84.** Intermediate 83 (1.88 g, 2.28 mmol) was dissolved in dry THF (20 ml). A solution of TBAF was added (1 M in THF, 3.43 ml, 3.43 mmol, 1.5 eq.) and the reaction was stirred at room temperature for 16 hours under nitrogen. Upon completion, the solvent was removed under reduced pressure. The crude material was dissolved in EtOAc (100 ml) and was transferred to a separatory funnel. The reaction was washed with saturated aqueous NaHCO\(_3\) (50 ml), water (50 ml) and brine (50 ml). The organics were collected, dried over MgSO\(_4\), filtered, and concentrated under reduced pressure. The crude material was purified by silica gel chromatography using a MeOH/DCM gradient. Yield: 1.27 grams (95 %). \(^{1}\)H NMR (300MHz, CHLOROFORM-d) δ = 8.93 (br s, 1H), 7.87 (d, J=1.2 Hz, 1H), 7.48 - 7.19 (m, 10H), 6.91 - 6.75 (m, 4H), 6.33 (t, J=6.8 Hz, 1H), 5.48 - 5.33 (m, 1H), 4.95 - 4.84 (m, 2H), 4.42 - 4.34 (m, 1H), 4.12 (q, J=7.2 Hz, 1H), 3.80 (d, J=1.8 Hz, 7H), 3.48 - 3.34 (m, 1H), 2.45 - 2.11 (m, 5H), 2.09 - 2.00 (m, 1H), 1.83 (d, J=1.0 Hz, 4H), 1.26 (t, J=7.1 Hz, 1H), 0.02 - -0.08 (m, 1H). \(^{13}\)C NMR (75MHz, DMSO-d\(_6\)) δ = 169.38, 140.62, 140.51, 139.27, 139.10, 139.08, 139.02, 135.92, 135.84, 135.80, 132.62, 132.58, 131.76, 131.32, 128.39, 126.62, 59.68, 45.94, 38.44, 38.32, 38.15, 30.70, 30.57, 32.37, 29.47, 28.56, 28.03, 27.77, 24.05, 23.73, 21.84, 20.65, 14.00, 13.76, 13.73. LCMS (ESI) m/z: [M-H] calcd, 584.7; found, 584.0.

**Compound 85.** Intermediate 84 (4.14 g, 7.08 mmol) and tetrazole (400 mg, 5.66 mmol, 0.8 eq.) were dried over P\(_2\)O\(_5\) for 16 hours under high vacuum. The reagents were dissolved in dry DMF (710 ml) with stirring under nitrogen. N-methylimidazole (140 µl, 1.77 mmol, 0.25 eq.) and 2-cyanoethyl-N,N,N',N'-tetraisopropyl-phosphorodiamidite (3.37 ml, 10.6 mmol, 1.5 eq.) were added via syringe and the reaction was allowed to stir at room temperature under nitrogen for 4
hours. The reaction was diluted with EtOAc (200 ml) and triethylamine (1 ml) and was transferred to a separatory funnel. The reaction was washed with saturated aqueous NaHCO₃ (200 ml), water (200 ml) and brine (200 ml). The organics were collected, dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude material was purified by dissolving in a minimum amount of DCM and precipitating by slow addition into hexanes. The product was dried under high vacuum to give the desired product as a brittle foam. Yield: 4.01 g (74%).

^1H NMR (300MHz, CHLOROFORM-d) δ = 8.65 (br s, 1H), 7.97 (dd, J=1.2, 12.9 Hz, 1H), 7.48 - 7.28 (m, 7H), 7.26 - 7.20 (m, 1H), 6.84 (td, J=3.3, 8.9 Hz, 4H), 6.43 - 6.29 (m, 1H), 5.43 - 5.23 (m, 1H), 4.96 - 4.81 (m, 2H), 4.49 (dquin, J=3.0, 7.0 Hz, 1H), 4.01 (br d, J=9.9 Hz, 1H), 3.83 - 3.65 (m, 6H), 3.63 - 3.44 (m, 3H), 3.40 - 3.31 (m, 1H), 2.62 - 2.26 (m, 5H), 2.24 - 2.13 (m, 1H), 1.80 - 1.74 (m, 3H), 1.68 (s, 1H), 1.37 - 1.09 (m, 10H), 0.97 (d, J=6.8 Hz, 3H), 0.10 - -0.06 (m, 1H).

^13C NMR (75MHz, CHLOROFORM-d) δ = 163.66, 163.64, 158.78, 158.77, 158.75, 150.25, 150.15, 145.93, 145.86, 136.46, 136.41, 136.19, 136.07, 135.60, 135.52, 133.69, 130.45, 130.41, 128.27, 127.77, 130.35, 117.98, 117.46, 117.20, 113.11, 113.08, 111.34, 111.21, 87.31, 87.25, 86.47, 86.45, 86.42, 86.37, 84.30, 84.23, 77.20, 74.30, 73.95, 73.99 (br dd, J=18.4, 83.7 Hz, 1C), 58.14, 55.28, 55.23, 43.25, 40.49, 40.43, 36.23, 24.61, 24.56, 24.44 (dd, J=2.2, 7.7 Hz, 1C), 20.35, 20.26, 20.02, 19.93, 12.21.

^31P NMR (121MHz, CHLOROFORM-d) δ = 149.54 (s, 1P), 149.10 (s, 1P). LCMS (ESI) m/z: [M-H] calcd, 784.9; found, 783.5.

**Scheme S12.** Synthesis of 5’-(R)-Allyl Thymidine Nucleosides.
**Compound 86.** (S) 5'-allyl dT 3'-O-TBDPS (82, 1.83 g, 3.51 mmol) and PPh₃ (3.69 grams, 14.1 mmol, 4 eq.) was dissolved in dry THF (46 ml) with stirring under nitrogen. DIAD (2.72 ml, 14.1 mmol, 4 eq.) was added dropwise via syringe, and the reaction was allowed to stir at room temperature for 16 hours. Upon completion, the solvent was removed under reduced pressure. The residue was resuspended in DCM (50 ml) and was transferred to a separatory funnel. The reaction mixture was washed with saturated aqueous NaHCO₃, dried over MgSO₄, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (flash chromatography) using a DCM/EtOAc gradient. Yield: 2.3 grams (98\%).

\[ ^1H \text{NMR} (300MHz, \text{CHLOROFORM-d}) \delta = 8.39 - 8.18 \text{ (m, 3H)}, 8.13 - 7.79 \text{ (m, 3H)}, 7.69 - 7.57 \text{ (m, 5H)}, 7.49 - 7.30 \text{ (m, 8H)}, 6.55 - 6.24 \text{ (m, 6H)}, 5.10 - 4.85 \text{ (m, 8H)}, 4.33 - 4.07 \text{ (m, 2H)}, 2.43 - 2.16 \text{ (m, 4H)}, 1.93 - 1.67 \text{ (m, 7H)}, 1.35 - 0.98 \text{ (m, 47H)}, 0.07 - 0.07 \text{ (m, 2H)}. \]

LCMS (ESI) m/z: [M+H] calcd, 669.8; found, 670.2.

The purified intermediate (2.3 grams, 3.43 mmol) was dissolved in MeOH (35 ml). K₂CO₃ was added (1.42 grams, 10.3 mmol, 3 eq.), and the reaction was stirred vigorously overnight at room temperature. The solvent was removed under reduced pressure. The residue was resuspended in EtOAc (50 ml) and was transferred to a separatory funnel. The reaction was washed with water, followed by brine (50 ml each), dried over MgSO₄, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography using a DCM/MeOH gradient. Product fractions were concentrated and dried under high vacuum to give 86 as a white brittle foam. Yield: 1.42 grams (79\%). LCMS (ESI) m/z: [M+H] calcd, 396.6; found, 395.2.

**Compound 87.** Intermediate 86 (1.42 g, 2.73 mmol) was dissolved in pyridine (15 ml) and lutidine (0.95 ml, 8.18 mmol, 3 eq.). DMT-Cl (2.77 g, 8.18 mmol, 3 eq.) was added to the reaction followed by AgNO₃ (100 mg, 0.59 mmol, 0.072 eq.). The reaction was purged with argon, capped tightly, and was allowed to stir at 45 °C for 4 days. The reaction was monitored by TLC (5% MeOH in DCM). Upon completion, the reaction was cooled to room temperature, diluted with EtOAc (50 ml), and was filtered through a plug of celite to remove silver salts. The filtrate was transferred to a separatory funnel and washed with saturated aqueous NaHCO₃ (2 x 25 ml) and brine (1 x 25 ml). The organics were collected, dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude material was purified by silica gel
chromatography (flash chromatography) and purified using an EtOAc/hexanes gradient. Product fractions were pooled and dried under high vacuum to give the desired product as a yellow brittle foam. Yield: 1.33 g (60 %).  

$^1$H NMR (300MHz, CHLOROFORM-d) $\delta = 8.27$ (s, 1H), 7.78 - 7.64 (m, 4H), 7.55 - 7.33 (m, 8H), 7.33 - 7.28 (m, 1H), 7.26 - 7.06 (m, 10H), 6.82 (d, J=1.2 Hz, 2H), 6.70 (dd, J=6.1, 9.0 Hz, 5H), 4.13 (d, J=7.0 Hz, 2H), 3.81 - 3.72 (m, 9H), 2.05 (s, 4H), 1.73 (d, J=1.0 Hz, 2H), 1.65 (s, 3H), 1.42 (d, J=1.0 Hz, 3H), 1.31 - 1.23 (m, 6H), 1.10 (s, 9H), 0.99 (s, 3H), 0.01 (s, 2H).  

$^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta = 163.32$, 158.58, 158.55, 150.04, 146.06, 136.62, 136.21, 136.01, 135.97, 135.63, 135.47, 135.39, 133.97, 133.28, 133.13, 130.20, 130.15, 130.11, 130.02, 128.12, 128.01, 127.85, 127.73, 127.61, 126.82, 117.45, 113.10, 113.07, 112.97, 110.80, 87.95, 86.83, 83.80, 77.20, 74.27, 72.91, 70.19, 60.36, 55.20, 40.08, 35.40, 26.90, 26.79, 21.93, 21.01, 19.03, 18.87, 14.18, 11.76.  

LCMS (ESI) m/z: [M-H] calcd, 823.1; found, 821.9.

**Compound 88.** Starting material 87 (1.33 g, 1.62 mmol) was dissolved in dry THF (16 ml). A solution of TBAF was added (1 M in THF, 2.42 ml, 2.42 mmol, 1.5 eq.) and the reaction was stirred at room temperature overnight under nitrogen. Upon completion, the solvent was removed under reduced pressure. The crude material was dissolved in EtOAc (100 ml) and was transferred to a separatory funnel. The reaction was washed with saturated aqueous NaHCO₃ (150 ml), water (150 ml), and brine (150 ml). The organics were collected, dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude material was purified by silica gel chromatography using an EtOAc/hexanes gradient. Yield: 760 mg (80 %). $^1$H NMR (300MHz, CHLOROFORM-d) $\delta = 8.90$ (s, 1H), 7.52 - 7.35 (m, 6H), 7.33 - 7.28 (m, 1H), 7.26 - 7.17 (m, 2H), 6.97 (d, J=1.2 Hz, 1H), 6.89 - 6.79 (m, 4H), 6.28 (dd, J=5.6, 8.4 Hz, 1H), 5.30 (s, 1H), 4.99 - 4.70 (m, 3H), 3.91 (t, J=3.4 Hz, 1H), 3.83 - 3.75 (m, 6H), 3.36 (s, 1H), 2.37 - 2.12 (m, 6H), 2.05 (s, 1H), 1.79 (s, 1H), 1.47 (d, J=1.0 Hz, 3H), 1.38 - 1.15 (m, 1H), 0.01 (s, 1H).  

$^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta = 163.54$, 158.76, 158.72, 150.26, 146.22, 136.63, 136.35, 135.31, 133.95, 130.25, 130.21, 128.00, 127.87, 127.02, 117.88, 113.24, 113.21, 113.16, 111.07, 87.20, 86.96, 83.55, 74.13, 71.08, 55.22, 40.61, 36.35, 11.86.  

LCMS (ESI) m/z: [M-H] calcd, 584.7; found, 584.0.
**Compound 89.** Starting material (88, 260 mg, 0.54 mmol) and tetrazole (24.9 mg, 0.356 mmol, 0.8 eq.) were dried over P₂O₅ for 16 hours under high vacuum. The reagents were dissolved in dry DMF (9 ml) with stirring under nitrogen. N-methylimidazole (8.8 µl, 0.111 mmol, 0.25 eq.) and 2-cyanoethyl-N,N,N',N'-tetraisopropyl-phosphorodiamidite (212 µl, 0.667 mmol, 1.5 eq.) were added via syringe and the reaction was allowed to stir at room temperature under nitrogen for 12 hours. The reaction was diluted with EtOAc (50 ml) and triethylamine (1 ml) and was transferred to a separatory funnel. The reaction was washed with saturated aqueous NaHCO₃ (50 ml), water (50 ml), and brine (50 ml). The organics were collected, dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude material was purified by silica gel chromatography (flash chromatography) using an EtOAc/hexanes gradient. The product was dried under high vacuum to give the desired product as a brittle foam. Yield: 276 mg (79%).

**¹H NMR** (300MHz, CHLOROFORM-d)  δ = 7.60 - 7.13 (m, 7H), 7.03 (dd, J=1.2, 6.9 Hz, 1H), 6.89 - 6.74 (m, 1H), 8.54 - 6.59 (m, 7H), 6.35 - 6.19 (m, 1H), 6.40 - 4.59 (m, 1H), 4.45 - 3.14 (m, 1H), 2.96 - 0.88 (m, 2H).

**¹³C NMR** (75MHz, CHLOROFORM-d)  δ = 163.41, 158.74, 158.71, 158.68, 150.19, 150.12, 146.30, 136.76, 136.72, 136.41, 136.25, 135.55, 135.43, 133.90, 133.81, 130.27, 130.22, 130.15, 128.06, 127.97, 127.87, 127.85, 126.98, 126.93, 117.71, 117.60, 117.39, 113.22 (t, J=2.7 Hz, 1C), 113.10, 113.07, 111.04, 111.01, 86.96, 86.91, 86.44, 86.33, 83.57, 83.41, 76.26, 74.37, 74.00, 72.65 (br dd, J=16.5, 73.0 Hz, 1C), 58.52, 58.28, 57.88, 57.62, 55.22, 43.48, 43.36, 43.31, 43.20, 39.51, 39.47, 36.02, 35.96, 35.93, 24.73, 24.66, 24.60, 24.57 (dd, J=3.3, 6.0 Hz, 1C), 24.47, 24.45, 20.53, 20.41, 20.31, 14.17, 11.67, 11.62.

**³¹P NMR** (121MHz, CHLOROFORM-d)  δ = 148.71 (s, 1P), 147.79 (s, 1P).

**LCMS (ESI) m/z:** [M-H] calcd, 784.9; found, 783.5
Scheme S13. Synthesis of 5’-(R/S)-Allyl Adenosine Nucleosides.

**Compound 90.** Protected nucleoside 74 (20 g, 29.10 mmol,) and imidazole (8.0 g, 118 mmol, 4 eq) were dissolved in anhydrous DMF (50 mL), and the solution was cooled with ice bath under an atmosphere of nitrogen. A solution of tert-butyl-chloro-dimethyl-silane (8.0 g, 29.10 mol 1.0 eq) in DMF (20 mL) was added dropwise. The reaction was stirred for 3 hours at room temperature. TLC in EtOAc/hexanes (7/3) indicated reaction was completed. The reaction was slowly poured in 100 mL of ice with stirring to obtain a white precipitate, stirring was continued until all the ice melted. The solid was collected by filtration, and washed with water (3x50 mL) and hexanes (3x50 mL). The solid was collected and dried under high vacuum at 35°C to obtain the desired product (90) as a white solid. Yield: 29 g (99 %) 1H NMR (300MHz, CHLOROFORM-d) δ = 9.03 (s, 1H), 8.71 (s, 1H), 8.07 - 7.97 (m, 3H), 7.72 - 7.56 (m, 6H), 7.53 - 7.46 (m, 2H), 7.44 - 7.11 (m, 18H), 6.70 (dd, J=1.6, 8.8 Hz, 4H), 6.55 (t, J=6.6 Hz, 1H), 4.68 - 4.61 (m, 1H), 4.28 (br d, J=2.7 Hz, 1H), 3.75 (s, 6H), 3.20 (dd, J=4.1, 10.3 Hz, 1H), 3.03 (dd, J=4.5, 10.4 Hz, 1H), 2.60 (br d, J=7.4 Hz, 1H), 2.51 (br s, 1H), 1.75 (br s, 2H), 1.19 - 0.97 (m, ...
12H) $^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta = 164.52, 158.43, 152.60, 149.39, 144.44,$
$141.42, 135.72, 135.58, 135.25, 134.81, 133.75, 133.14, 132.99, 132.73, 130.04, 129.98, 129.96,$
$129.62, 128.86, 128.08, 127.88, 127.84, 127.79, 127.71, 126.82, 123.25, 113.07, 87.11, 86.40,$
$84.83, 73.95, 63.44, 55.22, 40.56, 26.91, 26.58, 19.08, 19.03.$

LCMS (ESI) m/z: [M-H] calcd, 771.4; found, 771.3

**Compound 91.** Intermediate 90 (26 g, 29.20 mmol,) was dissolved in 80% acetic acid/water
(156 mL) and stirred for 1 hour at room temperature. TLC in EtOAc/hexanes (9/1) indicated the
reaction was completed. The solvents were evaporated under reduce pressure to obtain an oil.
The crude material was dissolved in ethyl acetate (100 mL), washed with saturated NaHCO$_3$ an d
brine. The organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure.
The crude material was purified by flash chromatography (silica gel, 350g col, 60 % diethyl
ether/hexanes, 50-100 % EtOAc/hexanes) to afford the desired product (91) as a white solid.
Yield: 13.6 g (78 %). $^1$H NMR (300MHz, CHLOROFORM-d) $\delta = 9.20$ (s, 1H), 8.69 (s, 1H),
8.10 - 7.96 (m, 3H), 7.69 - 7.37 (m, 13H), 7.26 (s, 1H), 6.43 (dd, $J=5.3, 9.5$ Hz, 1H), 5.62 (br d,
$J=10.9$ Hz, 1H), 4.74 (d, $J=4.8$ Hz, 1H), 4.16 (s, 1H), 3.72 (br d, $J=12.7$ Hz, 1H), 3.21 (br t,
$J=11.6$ Hz, 1H), 3.00 - 2.82 (m, 1H), 2.31 (dd, $J=5.3, 13.1$ Hz, 1H), 2.04 (br s, 1H), 1.26 (s, 1H),
1.13 (s, 9H) $^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta = 164.57, 152.03, 150.70, 150.24,$
142.59, 135.73, 135.68, 133.47, 133.24, 133.10, 132.90, 130.19, 130.15, 128.87, 127.96, 127.92,
124.56, 90.28, 88.00, 74.94, 62.95, 41.14, 26.98, 19.08. LCMS (ESI) m/z: [M-H] calcd, 469.3;
found, 468.1

**Compound 92.** A suspension of 91 (13.2 g, 22.3 mmol,) in anhydrous dichloromethane (220
mL) was cooled with an ice bath under an atmosphere of nitrogen. To this was added Dess-
Martin periodinane (14.20 g, 33.4 mmol, 1.5 eq) and the reaction was stirred for 3 hours. TLC in
EtOAc/hexanes indicated reaction was complete. The reaction was quenched by slow addition of
1.0 M Na$_2$S$_2$O$_3$ (175.0 mL) and 1.0 M NaHSO$_4$ (175 mL) and then stirred for a further 10
minutes. The reaction mixture was filtered through celite and rinsed with dichloromethane. The
solution was transferred to a separatory funnel and the aqueous layer was discarded. The
organics were dried over Na$_2$SO$_4$ for 10 minutes, filtered and concentrated under reduce pressure
to obtain a colorless oil. The oil was dissolved in a minimum amount of EtOAc and slowly added to hexanes (500 mL) with stirring to obtained white precipitate. The solid was filtered and dried under high vacuum over P₂O₅ to give the product. Yield: 8.40 g (64 %)

\[ \text{H NMR} (300MHz, \text{CHLOROFORM-d}) \delta = 9.39 \text{ (s, 1H), 8.80 - 8.65 (m, 1H), 8.35 (s, 1H), 8.12 (s, 1H), 8.08 - 7.94 (m, 4H), 7.73 - 7.37 (m, 21H), 6.70 (s, 1H), 4.93 - 4.74 (m, 1H), 4.60 (s, 1H), 4.56 - 4.49 (m, 1H), 4.25 \text{ (s, 1H), 2.57 (td, } J = 3.3, 5.5 \text{ Hz, 1H), 1.26 (s, 1H), 1.21 - 1.06 (m, 15H)} \]

\[ \text{C NMR} (75MHz, \text{CHLOROFORM-d}) \delta = 198.95, 141.97, 135.83, 135.78, 135.69, 132.84, 132.52, 130.43, 130.37, 130.30, 128.93, 128.88, 128.13, 128.09, 128.05, 128.02, 127.95, 91.50, 86.49, 73.90, 39.82, 26.96, 26.88, 19.10, 19.08 \]

\[ \text{LCMS (ESI) m/z: } [\text{M-H}] \text{ calcd, 467.2}; \text{ found, 466.0} \]

**Compound 93.** Intermediate 92 (8.0 g, 13.51 mmol) was dissolved in anhydrous dichloromethane (130 mL) under nitrogen and was cooled in an ice bath. Allyltrimethylsilane (10.6 mL, 67.60 mmol, 5 eq) was added, followed by boron trifluoride diethyl etherate (8.33 mL, 67.5 mmol, 5 eq). The reaction was stirred at 0°C for 2 hours and was monitored by TLC (EtOAc/hexanes 9:1). Upon completion the reaction was quenched by slowly adding saturated NaHCO₃ solution (50 mL). The reaction was transferred to a separatory funnel and the aqueous layer was discarded. The organics were washed with water (100 mL), and brine. The organics were collected, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (50 g col, 30-60% hexanes/EtOAc) afforded the desired product. Yield: 4.5 g (53 %)

\[ \text{H NMR} (300MHz, \text{CHLOROFORM-d}) \delta = 8.95 \text{ (s, 1H), 8.72 (s, 1H), 8.11 (s, 1H), 8.01 (d, } J = 7.4 \text{ Hz, 2H), 7.70 - 7.37 (m, 17H), 4.04 (s, 1H), 1.55 (s, 29H), 1.25 (s, 5H), 1.21 - 1.00 (m, 13H)} \]

\[ \text{LCMS (ESI) m/z: } [\text{M-H}] \text{ calcd, 509.3}; \text{ found, 508.2} \]

**Compound 94.** Intermediate 93 (1 g, 1.6 mmol) was coevaporated with toluene (3x20 mL) under reduced pressure at 60°C before being dissolved in dry pyridine (10 mL) and 2,6-lutidine (0.52 mL, 4.40 mmol, 3 eq). To this was added DMT-Cl (2.0 g, 3 mmol, 4 eq) and silver nitrate (0.54 g, 20.62, 2.8 mmol, 2 eq) and the reaction was heated to 45°C for 16 hours. The reaction was monitored by TLC (EtOAc/hexanes 6:4). Upon completion, the reaction was cooled to 0 °C and was quenched by the addition of ethyl acetate and water. The aqueous layer was removed
and the organics were washed with saturated sodium bicarbonate solution and brine. The organics were dried over Na$_2$SO$_3$, filtered, and concentrated to a crude yellow oil under reduced pressure. The crude product was purified by flash chromatography (silica gel, 220g col, 0-50% ethyl acetate/hexanes) to give 94 as yellow foam. Yield: 1.50 g (99 %) \textsuperscript{1}H NMR (300MHz, CHLOROFORM-d) $\delta = 9.01$ (s, 1H), 8.85 (d, $J$=3.1 Hz, 1H), 8.54 (d, $J$=11.8 Hz, 1H), 8.05 (br d, $J$=8.3 Hz, 2H), 7.55 (br d, $J$=7.5 Hz, 4H), 7.47 - 7.28 (m, 7H), 7.25 - 7.04 (m, 6H), 6.91 - 6.71 (m, 3H), 6.61 (s, 3H), 3.81 - 3.71 (m, 7H), 1.60 (s, 6H), 1.26 (s, 2H), 1.03 (s, 4H), 0.93 (s, 2H), 0.83 (s, 4H), 0.19 - 0.05 (m, 2H) \textsuperscript{13}C NMR (75MHz, CHLOROFORM-d) $\delta = 158.57$, 151.68, 135.68, 135.57, 134.05, 132.79, 130.40, 130.32, 130.24, 129.86, 128.91, 128.26, 128.14, 127.84, 127.80, 127.70, 127.57, 113.17, 113.04, 113.00, 112.92, 112.88, 73.77, 55.23, 55.19, 26.87, 25.74, 17.84. LCMS (ESI) m/z: [M-H] calcd, 811.4; found, 810.3

\textbf{Compound 95.} TEA (0.372 mL, 2.5 eq, 2.67 mmol, 2.5 eq) was added to a solution of intermediate 94 (1.0 g, 1.07 mmol) in THF (10 mL). The reaction was cooled in an ice bath under an atmosphere of nitrogen. Triethylamine trihydrofluoride (0.871 mL, 5.34 mmol, 5.0 eq) was added slowly and then stirred at room temperature for 16 hours. Triethylamine (0.40 mL) was added and the solvent was removed under reduced pressure to give a pale yellow solid. The crude product was purified by flash chromatography (silica gel, 220g col, 50-100% ethyl acetate/hexanes) to give 95 as a white solid. Yield: 0.72 g (97 %) \textsuperscript{1}H NMR (300MHz, CHLOROFORM-d) $\delta = 8.97$ (s, 1H), 8.81 (s, 1H), 8.35 (s, 1H), 8.04 (br d, $J$=7.8 Hz, 2H), 7.55 (br d, $J$=7.7 Hz, 3H), 7.48 - 7.30(m, 6H), 7.24 - 7.15 (m, 2H), 6.86 - 6.76 (m, 4H), 4.94 (br s, 2H), 3.84 - 3.60 (m, 8H), 1.58 (s, 9H), 1.26 (s, 2H). LCMS (ESI) m/z: [M-H] calcd, 697.3; found, 696.2

\textbf{Compound 96.} Precursor 95 (0.70 g, 1.0 mmol) was dissolved in dry DMF (20 mL) under an atmosphere of nitrogen. To this was added 1H-tetrazole (55 mg, 0.80 mmol, 0.8 eq) and 1-methylimidazole (19 µL, 0.251 mmol, 0.25 eq), followed by dropwise addition of 2-cyanoethyl-N,N,N',N'-tetraisopropyl-phosphorodiamidite (0.478 mL, 1.5 mmol, 1.5 eq). The reaction was stirred at room temperature for 2 hours. Water (1 mL) was added to quench the reaction. The reaction was diluted with a mixture of toluene/hexanes (3:1). The organics were washed four times...
times with a mixture of DMF/H\textsubscript{2}O (3:2). The organics were then washed with saturated sodium bicarbonate solution and brine. The organics were collected and dried over sodium sulfate, filtered, and concentrated to a white foam. The crude product was purified by flash chromatography (silica gel, 25 g col, 50-80% EtOAc/hexanes + 1 % triethyl amine) to give the desired product (96) as a white solid. Yield: 0.70 g (78 %).  

\[ \text{H NMR (300MHz, CHLOROFORM-d)} \delta = 8.82 (s, 1H), 8.51 - 8.46 (m, 1H), 8.05 (d, J=7.6 Hz, 2H), 7.62 - 7.17 (m, 15H), 6.84 - 6.74 (m, 4H), 4.90 (s, 2H), 4.16 (br s, 1H), 3.88 - 3.68 (m, 8H), 3.68 - 3.45 (m, 5H), 2.77 - 2.52 (m, 3H), 2.36 - 2.18 (m, 2H), 2.05 (s, 2H), 1.73 - 1.64 (m, 3H), 1.29 - 1.12 (m, 19H), 1.05 (d, J=6.8 Hz, 3H) \]

\[ \text{31P NMR (121MHz, CHLOROFORM-d)} \delta = 149.43 (s, 1P), 149.34 (s, 1P), 148.83 (s, 1P), 148.02 (s, 1P), 123.65 (s, 1P). \]

\[ \text{LCMS (ESI) m/z: [M-H] calcd, 897.4; found, 896.4.} \]

**Scheme S14.** Synthesis of 5'-(R/S)-Allyl Guanosine Nucleosides.

**Compound 98.**

Starting material 97 (5 grams, 7.82 mmol) and imidazole (1.33 grams, 19.5 mmol, 2.5 equiv.) was suspended in dry DMF (80 ml) in a 250 ml RBF. TBDMS-Cl was added (1.77 grams, 11.7 mmol, 1.5 equiv.), and the reaction was stirred at room temperature under argon.
overnight. Upon completion (TLC, 1:1 EtOAC/hexanes), the reaction mixture was diluted with EtOAc (200 ml) and was transferred to a separatory funnel. The reaction was washed with saturated aqueous NaHCO$_3$ (2 x 100 ml) and brine (1 x 200 ml). The organics were collected, dried over MgSO$_4$, filtered, and concentrated to a syrup. The crude material was purified by silica gel chromatography (EtOAC/hexanes). The product was dried under high vacuum to give 98 as brittle foam. Yield: 5.31 grams (90 %).

$^1$H NMR (300MHz, CHLOROFORM-d) $\delta =$ 11.92 (s, 1H), 7.80 (s, 1H), 7.69 (s, 1H), 7.52 - 7.44 (m, 2H), 7.39 - 7.28 (m, 5H), 7.26 - 7.18 (m, 2H), 6.85 - 6.77 (m, 4H), 6.20 (dd, $J=$5.4, 8.3 Hz, 1H), 4.61 - 4.55 (m, 1H), 4.09 - 4.02 (m, 1H), 3.78 (d, $J=$2.5 Hz, 6H), 3.38 (dd, $J=$3.5, 10.5 Hz, 1H), 3.15 (dd, $J=$3.9, 10.5 Hz, 1H), 2.90 - 2.76 (m, 1H), 2.30 (ddd, $J=$2.5, 5.4, 12.9 Hz, 1H), 2.01 (spt, $J=$6.9 Hz, 1H), 1.63 (s, 1H), 1.08 (d, $J=$6.9 Hz, 3H), 0.98 - 0.81 (m, 12H), 0.12 - -0.02 (m, 6H).

$^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta =$ 177.93, 158.67, 155.43, 147.99, 146.98, 144.78, 137.57, 135.96, 135.75, 129.96, 128.06, 127.99, 127.05, 122.34, 113.25, 87.15, 86.28, 84.51, 72.53, 63.31, 55.27, 40.63, 36.40, 25.76, 25.66, 18.73, 18.67, 17.98, -4.72, -4.80. LCMS (ESI) m/z: [M-H] calcd, 753.9; found, 752.4.

**Compound 99.**

98 (5.31 grams, 7.04 mmol) was dissolved in 80% acetic acid (20 ml). The reaction was heated to 40 °C for 60 min, and was monitored by TLC (100% EtOAc, Rf = 0.5). Upon completion, the reaction was cooled and diluted with EtOAc (150 ml) and was transferred to a separatory funnel. Saturated aqueous NaHCO$_3$ (50 ml) was carefully added. The organics were collected and were washed with NaHCO$_3$ (50 ml) and brine (50 ml). The organics were dried over MgSO$_4$, filtered, and the solvent was removed under reduced pressure. The crude material was purified by silica gel chromatography (10% MeOH in DCM). The desired product was dried under high vacuum to give 99 as pale yellow brittle foam. Yield: 1.92 grams (60%).

$^1$H NMR (300MHz, CHLOROFORM-d) $\delta =$ 12.22 - 11.85 (m, 1H), 8.51 (br s, 1H), 7.84 - 7.74 (m, 1H), 7.37 - 7.28 (m, 1H), 6.19 (dd, $J=$5.7, 8.8 Hz, 1H), 5.32 - 4.95 (m, 1H), 4.61 (br d, $J=$5.4 Hz, 1H), 4.18 - 4.04 (m, 1H), 3.95 (dd, $J=$1.9, 12.4 Hz, 1H), 3.87 - 3.57 (m, 2H), 2.93 - 2.47 (m, 2H), 2.43 - 1.73 (m, 3H), 1.31 - 1.07 (m, 6H), 0.98 - 0.82 (m, 9H), 0.17 - -0.01 (m, 6H).

$^{13}$C NMR (75MHz, CHLOROFORM-d) $\delta =$ 178.46, 155.15, 147.40, 146.90, 138.46, 122.77, 89.36, 86.71,
Oxalyl chloride (480 µl, 5.60 mmol, 1.33 equiv.) was dissolved in dry DCM (40 ml) and the reaction was cooled to -78 °C with stirring under nitrogen. DMSO was added via syringe (780 µl, 11.0 mmol, 2.61 equiv.) and the reaction was allowed to stir for 10 min. In a separate vessel, intermediate 99 (1.9 grams, 4.21 mmol) was dissolved in dry DCM (5 ml) and was added to the reaction via syringe. The reaction was stirred at – 78 °C for 15 minutes. Et₃N (2.31 ml, 16.6 mmol, 3.94 equiv.) was added and the reaction was allowed to warm to room temperature. The reaction was diluted with EtOAc (70 ml) and was transferred to a separatory funnel. The reaction mixture was washed with 1: 1 water and saturated aqueous NaHCO₃ (3 x 100 ml) followed by brine (1 x 100 ml). The organics were collected, dried over MgSO₄, filtered, and concentrated under reduced pressure (rotovap). The aldehyde intermediate 100 was used immediately without further purification.

The crude aldehyde 100 was dissolved in dry DCM (100 ml), and the reaction was cooled in an ice bath with stirring under nitrogen. TMS allylsilane was added via syringe (3.34 ml, 21.0 mmol, 5 equiv.), followed by BF₃-Et₂O (2.64 ml, 2.14 mmol, 5 equiv.). The reaction was stirred at 0 °C for 30 min. The reaction was diluted with EtOAc (50 ml) and was transferred to a separatory funnel. The reaction mixture was washed with 1: 1 water and saturated aqueous NaHCO₃ (3 x 100 ml) followed by brine (1 x 100 ml). The organics were collected, dried over MgSO₄, filtered, and concentrated under reduced pressure (rotovap). The crude product was purified by silica gel chromatography (DCM/MeOH) to give the desired product 101 as an inseparable mixture of isomers. 1.59 grams (77% as a brittle yellow foam) ¹H NMR (300MHz, CHLOROFORM-d) δ = 12.32 - 12.05 (m, 1H), 9.26 - 9.15 (m, 1H), 7.99 - 7.74 (m, 1H), 6.37 - 6.10 (m, 1H), 5.98 - 5.72 (m, 1H), 5.26 - 5.02 (m, 2H), 4.75 - 4.39 (m, 1H), 4.13 - 3.86 (m, 2H), 3.82 - 3.66 (m, 1H), 2.99 - 2.61 (m, 3H), 2.50 - 2.16 (m, 3H), 1.47 - 1.19 (m, 7H), 1.04 - 0.69 (m, 13H), 0.17 - -0.20 (m, 9H). ¹³C NMR (75MHz, CHLOROFORM-d) δ = 179.00, 155.31, 147.55, 147.39, 147.22, 138.94, 134.72, 134.34, 122.45, 117.57, 91.55, 86.19, 74.49, 71.55, 71.48, 71.28,
Compound 102. Precursor 101 (3.26 g, 5.29 mmol) was coevaporated with toluene (3x 20 mL) under reduced pressure at 60°C before being dissolved into dry pyridine (10 mL) and 2,6-lutidine (1.84 mL, 15.9 mmol, 3 eq). To this was added DMT-Cl (7.18 g, 21.20 mmol, 4 eq) and silver nitrate (1.80 g, 10.6 mmol, 2 eq) and the reaction was heated to 45°C for 16 hours. TLC in EtOAc/hexanes (6/4) indicated reaction was complete. The reaction mixture was cooled in an ice bath and was quenched by addition of ethyl acetate followed by water. The aqueous layer was removed and the organics were washed with saturated sodium bicarbonate solution and brine. The organics were collected, dried over Na₂SO₃, filtered and concentrated to a crude yellow oil under reduced pressure. The crude product was purified by flash chromatography (silica gel, 220g col, 0-50% ethyl acetate/hexanes) to give 102 as yellow foam. Yield: 2.20 g (45 %).

'H NMR (300MHz, CHLOROFORM-d) δ = 11.90 (s, 1H), 7.96 - 7.86 (m, 1H), 7.71 (d, J=6.6 Hz, 2H), 7.64 - 7.50 (m, 3H), 7.47 - 7.09 (m, 18H), 6.85 - 6.57 (m, 6H), 3.80 - 3.70 (m, 6H), 1.92 (br s, 2H), 1.66 (br s, 3H), 1.25 (t, J=6.8 Hz, 5H), 1.14 - 1.01 (m, 9H), 0.71 (d, J=6.9 Hz, 1H), 0.55(d, J=6.8 Hz, 1H). 

'13C NMR (75MHz, CHLOROFORM-d) δ = 177.92, 177.84, 158.55, 158.52, 155.57, 155.35, 148.22, 147.17, 146.60, 145.86, 136.35, 136.19, 136.02, 135.90 (br t, J=18.8 Hz, 1C), 135.49, 133.94, 133.75, 133.21, 133.18, 130.50, 130.41, 130.07, 130.04, 129.99, 129.93, 129.83, 129.14, 128.23, 127.92, 127.86, 127.79, 127.66, 126.89, 126.84, 121.63, 117.64, 117.51, 113.18, 113.03, 112.95, 88.62, 87.83, 86.83, 85.84, 85.63, 82.88, 74.87, 74.68, 73.59, 73.15, 55.31, 55.28, 55.24, 39.17, 36.68, 36.00, 35.35, 26.95, 26.88, 19.05, 18.98, 18.95, 18.48, 17.93. LCMS (ESI) m/z: [M+H] calcd, 491.7; found, 492.2

Compound 103. TEA (0.911 mL, 6.53 mmol, 2.5 eq) was added to a solution of 102 (2.40 g, 2.61 mmol) in THF (25 mL). The reaction was cooled in an ice bath under an atmosphere of nitrogen. Triethylamine trihydrofluoride (2.13 mL, 13.1 mmol, 5.0 eq) was added, and the reaction was stirred at room temperature for 16 hours. Triethylamine (1.0 mL) was added and the solvent was removed to give a pale yellow solid. The crude product was purified by flash chromatography (silica gel, 220g col, 0-5% MeOH/DCM) to give compound 103 as a white
solid. Yield: 1.40 g (79 %) \(1^\text{H} \text{NMR} \) (300MHz, CHLOROFORM-d) \(\delta = 8.00 \) (s, 1H), 7.57 - 7.33 (m, 7H), 7.30 - 7.13 (m, 4H), 6.85 - 6.72 (m, 4H), 6.09 (t, \(J=6.9 \) Hz, 1H), 5.61 (br d, \(J=6.9 \) Hz, 1H), 4.93 - 4.76 (m, 2H), 4.76 - 4.61 (m, 1H), 4.05 - 3.96 (m, 1H), 3.90 (s, 1H), 3.80 - 3.70 (m, 6H), 3.58 - 3.47 (m, 1H), 2.90 (br d, \(J=7.3 \) Hz, 1H), 2.76 - 2.54 (m, 1H), 2.52 - 2.23 (m, 3H), 2.22 - 2.03 (m, 1H), 1.96 (br dd, \(J=6.6, 13.7 \) Hz, 1H), 1.29 - 1.07 (m, 6H), 0.94 (d, \(J=6.8 \) Hz, 1H), 0.85 (d, \(J=6.8 \) Hz, 1H) \(13^\text{C} \text{NMR} \) (75MHz, CHLOROFORM-d) \(\delta = 179.19, 178.74, 158.69, 158.67, 155.72, 148.36, 148.32, 147.64, 147.20, 146.53, 146.03, 138.86, 137.18, 136.91, 136.64, 136.58, 136.46, 134.38, 134.31, 130.52, 130.47, 130.26, 129.14, 128.33, 128.32, 128.10, 127.74, 127.01, 122.10, 121.25, 117.57, 117.50, 113.14, 113.06, 87.98, 87.41, 86.83, 86.39, 84.61, 83.14, 73.94, 73.47, 72.00, 71.08, 55.26, 45.89, 39.96, 36.23, 36.12, 35.99, 35.62, 18.96, 18.92, 18.68, 18.38, 9.69.

\(\text{LCMS (ESI) m/z: [M-H] calcd, 665.3; found, 664.2} \)

**Compound 104.** Intermediate 103 (1.40 g, 2.06 mmol) was dissolved in dry DMF (20 mL) under an atmosphere of nitrogen. To this was added 1H-tetrazole (114 mg, 165 mmol, 0.8 eq) and 1-methylimidazole (41 µL, 0.515 mmol, 0.25 eq), followed by dropwise addition of 2-cyanoethyl-N,N,N',N'-tetraisopropyl-phosphorodiamidite (0.981 mL, 3.10 mmol, 1.5 eq). The reaction was stirred at room temperature for 2 hours, and was monitored by TLC (EtOAc/hexanes, 7:3). Upon completion, the reaction was quenched reaction by addition of water (1 mL). The reaction was diluted with a mixture of toluene/hexanes (3:1) and was transferred to a separatory funnel. The organics were washed four times with a mixture of DMF/H\(_2\)O (3:2). The organics were washed with saturated sodium bicarbonate solution and brine. The organic layer was collected, dried over sodium sulfate, and concentrated to a white foam. The crude product was purified by flash chromatography (silica gel, 25 g col, 50-80% EtOAc/Hexane + 1 % triethyl amine) to give 104 as a white solid. Yield: 1.24 g (68 %). \(1^\text{H} \text{NMR} \) (300MHz, CHLOROFORM-d) \(\delta = 11.96 \) (br s, 1H), 7.63 - 7.56 (m, 1H), 7.51 - 7.17 (m, 6H), 6.84 - 6.70 (m, 2H), 6.15 - 5.98 (m, 1H), 5.03 - 4.68 (m, 2H), 4.18 - 4.05 (m, 1H), 3.81 - 3.52 (m, 6H), 2.87 - 2.62 (m, 1H), 2.48 - 2.20 (m, 1H), 2.05 (s, 2H), 1.76 (br s, 1H), 1.29 - 1.11 (m, 10H), 1.01 (dd, \(J=6.8, 15.4 \) Hz, 1H), 0.93 - 0.80 (m, 1H), 0.69 (d, \(J=6.8 \) Hz, 1H) \(31^\text{P} \text{NMR} \) (121MHz, CHLOROFORM-d) \(\delta = 149.12 \) (s, 1P), 148.99 (s, 1P), 148.87 (s, 1P), 147.09 (s, 1P)\(\text{LCMS (ESI) m/z: [M-H] calcd, 865.4; found, 864.3} \)

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Figure S1. Determination of the absolute configuration of 5’-Me group.

Table S1. Crystal data and structure refinement.

| Property                        | Value                                      |
|---------------------------------|--------------------------------------------|
| Identification code             | isis19                                     |
| Empirical formula               | C18 H20 N2 O6                              |
| Formula weight                  | 360.36                                     |
| Temperature                     | 100(2) K                                   |
| Wavelength                      | 0.71073 Å                                  |
| Crystal system                  | Orthorhombic                               |
| Space group                     | P2(1)2(1)2(1)                              |
| Unit cell dimensions            | a = 10.8056(14) Å  a= 90°                  |
|                                 | b = 16.240(2) Å   b= 90°                   |
|                                 | c = 19.613(3) Å  g = 90°                   |
| Volume                          | 3441.6(8) Å³                                 |
| Z, Z’                           | 8, 2                                       |
| Density (calculated)            | 1.391 g/cm³                                 |
| Property                                             | Value                                      |
|------------------------------------------------------|--------------------------------------------|
| Absorption coefficient                               | 0.105 mm$^{-1}$                            |
| F(000)                                               | 1520                                       |
| Crystal size                                         | 0.40 x 0.20 x 0.10 mm$^3$                  |
| Theta range for data collection                      | 1.63 to 25.54°                            |
| Index ranges                                         | -13$\leq h \leq$12, -16$\leq k \leq$19, -23$\leq l \leq$23 |
| Reflections collected                                | 14565                                     |
| Independent reflections                              | 6132 [R(int) = 0.0529]                     |
| Completeness to theta = 25.00°                       | 98.6 %                                    |
| Absorption correction                                | Multi-scan                                 |
| Refinement method                                    | Full-matrix least-squares on $F^2$         |
| Data / restraints / parameters                        | 6132 / 0 / 473                            |
| Goodness-of-fit on $F^2$                             | 1.029                                     |
| Final R indices [I>2sigma(I)]                        | $R_1 = 0.0515$, $wR_2 = 0.1041$            |
| R indices (all data)                                 | $R_1 = 0.0792$, $wR_2 = 0.1190$            |
| Absolute structure parameter                         | 0.3(11)                                   |
| Largest diff. peak and hole                          | 0.279 and -0.298 e Å$^{-3}$                |
Table S2. Atomic coordinates (x $10^4$) and equivalent isotropic displacement parameters ($\AA^2$ x $10^3$). U(eq) is defined as one third of the trace of the orthogonalized $U_{ij}$ tensor.

|     | x      | y      | z      | U(eq) |
|-----|--------|--------|--------|-------|
| O(1) | 5798(2) | 8555(1) | 10274(1) | 26(1) |
| O(1')| 6197(2) | 1448(1) | 10340(1) | 29(1) |
| O(2) | 5959(2) | 7952(1) | 9241(1) | 19(1) |
| O(2')| 6049(2) | 2050(1) | 9307(1) | 20(1) |
| O(3) | 7069(2) | 5866(1) | 8062(1) | 24(1) |
| O(3')| 4590(2) | 4046(1) | 8175(1) | 31(1) |
| O(4) | 4912(2) | 6361(1) | 9116(1) | 22(1) |
| O(4')| 7016(2) | 3645(1) | 9151(1) | 22(1) |
| O(5) | -352(2) | 7342(2) | 8278(1) | 30(1) |
| O(5')| 12294(2) | 3059(1) | 8162(1) | 29(1) |
| O(6) | 2451(2) | 5251(1) | 8315(1) | 26(1) |
| O(6')| 9260(2) | 4979(1) | 8318(1) | 26(1) |
| N(1) | 3176(2) | 6562(2) | 8418(1) | 19(1) |
| N(1')| 8681(2) | 3612(2) | 8388(2) | 20(1) |
| N(2) | 1081(2) | 6319(2) | 8257(1) | 22(1) |
| N(2')| 10729(2) | 3988(2) | 8210(1) | 20(1) |
| C(1) | 4936(3) | 9300(2) | 8596(2) | 18(1) |
| C(1')| 7141(3) | 707(2) | 8677(2) | 22(1) |
| C(2) | 4290(3) | 9931(2) | 8278(2) | 22(1) |
| C(2')| 7762(3) | 56(2) | 8371(2) | 25(1) |
| C(3) | 3611(3) | 10484(2) | 8674(2) | 24(1) |
| C(3')| 8381(3) | -513(2) | 8777(2) | 28(1) |
| C(4)  | 3590(3) | 10414(2) | 9377(2) | 28(1) |
|-------|---------|----------|---------|------|
| C(4') | 8388(3) | -432(2)  | 9480(2) | 27(1) |
| C(5)  | 4253(3) | 9788(2)  | 9697(2) | 25(1) |
| C(5') | 7741(3) | 211(2)   | 9786(2) | 24(1) |
| C(6)  | 4932(3) | 9230(2)  | 9299(2) | 19(1) |
| C(6') | 7108(3) | 778(2)   | 9378(2) | 19(1) |
| C(7)  | 5616(3) | 8563(2)  | 9669(2) | 19(1) |
| C(7') | 6397(3) | 1438(2)  | 9737(2) | 19(1) |
| C(8)  | 6513(3) | 7225(2)  | 9568(2) | 20(1) |
| C(8') | 5472(3) | 2763(2)  | 9636(2) | 22(1) |
| C(9)  | 7883(3) | 7349(2)  | 9690(2) | 25(1) |
| C(9') | 4106(3) | 2616(2)  | 9760(2) | 28(1) |
| C(10) | 6242(3) | 6509(2)  | 9096(2) | 20(1) |
| C(10')| 5699(3) | 3480(2)  | 9161(2) | 21(1) |
| C(11) | 6592(3) | 6620(2)  | 8340(2) | 19(1) |
| C(11')| 5287(3) | 3357(2)  | 8416(2) | 20(1) |
| C(12) | 5352(3) | 6785(2)  | 7994(2) | 19(1) |
| C(12')| 6500(3) | 3310(2)  | 8024(2) | 20(1) |
| C(13) | 4457(3) | 6279(2)  | 8431(2) | 20(1) |
| C(13')| 7374(3) | 3813(2)  | 8461(2) | 20(1) |
| C(14) | 2904(3) | 7389(2)  | 8524(2) | 21(1) |
| C(14')| 9074(3) | 2793(2)  | 8427(2) | 21(1) |
| C(15) | 1753(3) | 7694(2)  | 8484(2) | 22(1) |
| C(15')| 10250(3)| 2557(2)  | 8347(2) | 20(1) |
| C(16) | 1471(3) | 8597(2)  | 8598(2) | 30(1) |
| C(16')| 10645(3)| 1671(2)  | 8392(2) | 29(1) |
| C(17) | 734(3)  | 7139(2)  | 8336(2) | 23(1) |
| C(17')| 11186(3)| 3187(2)  | 8234(2) | 23(1) |
| C(18) | 2240(3) | 5997(2)  | 8334(2) | 21(1) |
| C(18')| 9539(3) | 4237(2)  | 8311(2) | 22(1)|
| Bond          | Length [Å] | Bond          | Length [Å] | Bond          | Length [Å] |
|---------------|------------|---------------|------------|---------------|------------|
| O(1)-C(7)     | 1.201(4)   | C(1)-C(2)     | 1.387(4)   |               |            |
| O(1')-C(7')   | 1.203(4)   | C(1')-C(6')   | 1.381(4)   |               |            |
| O(2)-C(7)     | 1.352(4)   | C(1')-C(2')   | 1.388(5)   |               |            |
| O(2)-C(8)     | 1.471(4)   | C(2)-C(3)     | 1.397(5)   |               |            |
| O(2')-C(7')   | 1.357(4)   | C(2')-C(3')   | 1.391(5)   |               |            |
| O(2')-C(8')   | 1.465(4)   | C(3)-C(4)     | 1.384(5)   |               |            |
| O(3)-C(11)    | 1.436(4)   | C(3')-C(4')   | 1.385(5)   |               |            |
| O(3')-C(11')  | 1.430(4)   | C(4)-C(5)     | 1.393(5)   |               |            |
| O(4)-C(13)    | 1.437(4)   | C(4')-C(5')   | 1.393(5)   |               |            |
| O(4)-C(10)    | 1.458(4)   | C(5)-C(6)     | 1.402(5)   |               |            |
| O(4')-C(13')  | 1.433(4)   | C(5')-C(6')   | 1.397(4)   |               |            |
| O(4')-C(10')  | 1.449(4)   | C(6)-C(7)     | 1.500(5)   |               |            |
| O(5)-C(17)    | 1.224(4)   | C(6')-C(7')   | 1.495(5)   |               |            |
| O(5')-C(17')  | 1.223(4)   | C(8)-C(9)     | 1.513(4)   |               |            |
| O(6)-C(18)    | 1.234(4)   | C(8)-C(10)    | 1.515(4)   |               |            |
| O(6')-C(18')  | 1.241(4)   | C(8')-C(10')  | 1.510(4)   |               |            |
| N(1)-C(18)    | 1.376(4)   | C(8')-C(9')   | 1.516(5)   |               |            |
| N(1)-C(14)    | 1.389(4)   | C(10)-C(11)   | 1.540(5)   |               |            |
| N(1)-C(13)    | 1.459(4)   | C(10')-C(11') | 1.541(5)   |               |            |
| N(1')-C(18')  | 1.383(4)   | C(11)-C(12)   | 1.525(4)   |               |            |
| N(1')-C(14')  | 1.398(4)   | C(11')-C(12') | 1.522(4)   |               |            |
| N(1')-C(13')  | 1.457(4)   | C(12)-C(13)   | 1.530(4)   |               |            |
| N(2)-C(18)    | 1.366(4)   | C(12')-C(13') | 1.515(4)   |               |            |
| N(2)-C(17)    | 1.392(4)   | C(14)-C(15)   | 1.342(5)   |               |            |
| N(2')-C(18')  | 1.362(4)   | C(14')-C(15') | 1.336(4)   |               |            |
| N(2')-C(17')  | 1.393(4)   | C(15)-C(17)   | 1.453(5)   |               |            |
| C(1)-C(6)     | 1.385(4)   | C(15)-C(16)   | 1.514(5)   |               |            |
| Bond                  | Length (Å) | Bond                  | Length (Å) |
|-----------------------|------------|-----------------------|------------|
| C(15')-C(17')         | 1.456(4)   | C(15')-C(16')         | 1.503(4)   |
| C(7)-O(2)-C(8)        | 115.4(2)   | O(1)-C(7)-O(2)        | 124.1(3)   |
| C(7')-O(2')-C(8')     | 115.1(3)   | O(1)-C(7)-C(6)        | 124.5(3)   |
| C(13)-O(4)-C(10)      | 109.0(2)   | O(2)-C(7)-C(6)        | 111.4(3)   |
| C(13')-O(4')-C(10')   | 108.2(2)   | O(1')-C(7')-O(2')     | 123.5(3)   |
| C(18)-N(1)-C(14)      | 120.5(3)   | O(1')-C(7')-C(6')     | 124.4(3)   |
| C(18)-N(1)-C(13)      | 119.3(3)   | O(2')-C(7')-C(6')     | 112.1(3)   |
| C(14)-N(1)-C(13)      | 120.2(3)   | O(2)-C(8)-C(9)        | 111.2(3)   |
| C(18')-N(1')-C(14')   | 120.0(3)   | O(2)-C(8)-C(10)       | 105.7(2)   |
| C(18')-N(1')-C(13')   | 119.8(3)   | C(9)-C(8)-C(10)       | 112.8(3)   |
| C(14')-N(1')-C(13')   | 120.1(3)   | O(2')-C(8')-C(10')    | 105.6(3)   |
| C(18)-N(2)-C(17')     | 126.9(3)   | O(2')-C(8')-C(9')     | 111.1(3)   |
| C(18')-N(2')-C(17')   | 127.4(3)   | C(10')-C(8')-C(9')    | 112.3(3)   |
| C(6)-C(1)-C(2)        | 120.5(3)   | O(4)-C(10)-C(8)       | 107.5(3)   |
| C(6')-C(1')-C(2')     | 120.4(3)   | O(4)-C(10)-C(11)      | 106.8(2)   |
| C(1)-C(2)-C(3)        | 119.3(3)   | C(8)-C(10)-C(11)      | 116.9(3)   |
| C(1')-C(2')-C(3')     | 119.4(3)   | O(4')-C(10')-C(8')    | 108.1(3)   |
| C(4)-C(3)-C(2)        | 120.7(3)   | O(4')-C(10')-C(11')   | 107.1(3)   |
| C(4')-C(3')-C(2')     | 120.7(3)   | C(8')-C(10')-C(11')   | 116.0(3)   |
| C(3)-C(4)-C(5)        | 120.0(3)   | O(3)-C(11)-C(12)      | 107.3(3)   |
| C(3')-C(4')-C(5')     | 119.8(3)   | O(3)-C(11)-C(10)      | 110.7(3)   |
| C(4)-C(5)-C(6)        | 119.4(3)   | C(12)-C(11)-C(10)     | 103.4(3)   |
| C(4')-C(5')-C(6')     | 119.5(3)   | O(3')-C(11')-C(12')   | 109.0(3)   |
| C(1)-C(6)-C(5)        | 120.2(3)   | O(3')-C(11')-C(10')   | 111.4(3)   |
| C(1)-C(6)-C(7)        | 122.7(3)   | C(12')-C(11')-C(10')  | 103.7(3)   |
| C(5)-C(6)-C(7)        | 117.1(3)   | C(11)-C(12)-C(13)     | 102.3(3)   |
| C(1')-C(6')-C(5')     | 120.2(3)   | C(13')-C(12')-C(11')  | 102.9(3)   |
| C(1')-C(6')-C(7')     | 122.8(3)   | O(4)-C(13)-N(1)       | 108.1(3)   |
| C(5')-C(6')-C(7')     | 117.0(3)   | O(4)-C(13)-C(12)      | 105.0(3)   |
N(1)-C(13)-C(12)  114.9(3)
O(4')-C(13')-N(1')  108.2(3)
O(4')-C(13')-C(12')  105.3(3)
N(1')-C(13')-C(12')  115.3(3)
C(15)-C(14)-N(1)  123.0(3)
C(15')-C(14')-N(1')  123.7(3)
C(14)-C(15)-C(17)  119.0(3)
C(14)-C(15)-C(16)  122.4(3)
C(17)-C(15)-C(16)  118.6(3)
C(14')-C(15')-C(17')  118.5(3)
C(14')-C(15')-C(16')  122.6(3)
C(17')-C(15')-C(16')  118.9(3)
O(5)-C(17)-N(2)  120.4(3)
O(5)-C(17)-C(15)  125.3(3)
N(2)-C(17)-C(15)  114.3(3)
O(5')-C(17')-N(2')  120.1(3)
O(5')-C(17')-C(15')  125.4(3)
N(2')-C(17')-C(15')  114.5(3)
O(6)-C(18)-N(2)  122.8(3)
O(6)-C(18)-N(1)  121.6(3)
N(2)-C(18)-N(1)  115.6(3)
O(6')-C(18')-N(2')  121.3(3)
O(6')-C(18')-N(1')  123.2(3)
N(2')-C(18')-N(1')  115.5(3)
Table S4. Anisotropic displacement parameters (Å² × 10³). The anisotropic displacement factor exponent takes the form: -2p²[a*h²b*U11 + ... + 2 h k a*b*U12]

|     | U₁₁ | U₂₂ | U₃₃ | U₁₂ | U₁₃ | U₂₃ |
|-----|-----|-----|-----|-----|-----|-----|
| O(1) | 26(1) | 26(1) | 27(2) | 1(1) | -3(1) | 1(1) |
| O(1') | 33(2) | 30(2) | 24(2) | 3(1) | 3(1) | 2(1) |
| O(2) | 20(1) | 15(1) | 23(1) | 0(1) | -1(1) | 3(1) |
| O(2') | 21(1) | 17(1) | 23(1) | -1(1) | 1(1) | 3(1) |
| O(3) | 24(1) | 21(1) | 29(1) | -5(1) | 6(1) | 3(1) |
| O(3') | 16(1) | 31(1) | 46(2) | 10(1) | -5(1) | 5(1) |
| O(4) | 15(1) | 26(1) | 25(1) | 4(1) | 0(1) | -3(1) |
| O(4') | 15(1) | 27(1) | 23(1) | -3(1) | -1(1) | -4(1) |
| O(5) | 15(1) | 44(2) | 30(1) | 3(1) | -3(1) | 6(1) |
| O(5') | 13(1) | 35(2) | 40(2) | -7(1) | 1(1) | 3(1) |
| O(6) | 17(1) | 22(1) | 38(2) | -3(1) | 0(1) | -6(1) |
| O(6') | 20(1) | 18(1) | 41(2) | 1(1) | -3(1) | -5(1) |
| N(1) | 11(1) | 17(2) | 29(2) | -1(1) | -3(1) | -2(1) |
| N(1') | 10(1) | 13(1) | 35(2) | 2(1) | 0(1) | 1(1) |
| N(2) | 12(2) | 28(2) | 25(2) | -4(1) | -3(1) | -4(1) |
| N(2') | 13(2) | 21(2) | 27(2) | 4(1) | 2(1) | -1(1) |
| C(1) | 14(2) | 14(2) | 26(2) | -1(1) | -2(1) | -3(2) |
| C(1') | 19(2) | 16(2) | 29(2) | 2(2) | -1(2) | -2(2) |
| C(2) | 20(2) | 19(2) | 27(2) | 3(2) | -1(2) | -5(2) |
| C(2') | 23(2) | 23(2) | 30(2) | 1(2) | 3(2) | 3(2) |
| C(3) | 17(2) | 18(2) | 38(2) | 3(2) | -2(2) | 1(2) |
| C(3') | 18(2) | 19(2) | 46(3) | -3(2) | 2(2) | 1(2) |
| C(4) | 19(2) | 17(2) | 48(3) | -5(2) | 2(2) | 1(2) |
|      |      |      |      |      |      |      |      |      |      |
|------|------|------|------|------|------|------|------|------|------|
| C(4') | 18(2) | 24(2) | 39(2) | 7(2)  | -6(2) | 1(2)  |
| C(5)  | 18(2) | 28(2) | 28(2) | -4(2) | 2(2)  | -8(2) |
| C(5') | 22(2) | 24(2) | 26(2) | 4(2)  | 0(2)  | -4(2) |
| C(6)  | 13(2) | 16(2) | 28(2) | 0(2)  | -2(1) | -2(1) |
| C(6') | 14(2) | 14(2) | 29(2) | -1(2) | 0(2)  | -4(2) |
| C(7)  | 15(2) | 20(2) | 23(2) | 0(2)  | 2(2)  | -5(2) |
| C(7') | 17(2) | 15(2) | 25(2) | 4(2)  | -2(2) | -8(2) |
| C(8)  | 21(2) | 16(2) | 24(2) | 6(2)  | -3(2) | 1(2)  |
| C(8') | 18(2) | 19(2) | 28(2) | -4(2) | 4(2)  | 0(2)  |
| C(9)  | 17(2) | 22(2) | 34(2) | -3(2) | -3(2) | 3(2)  |
| C(9') | 23(2) | 25(2) | 36(2) | -1(2) | 8(2)  | -1(2) |
| C(10) | 13(2) | 18(2) | 29(2) | 5(2)  | -4(2) | 1(2)  |
| C(10')| 10(2) | 20(2) | 32(2) | 2(2)  | 5(2)  | -3(2) |
| C(11) | 15(2) | 14(2) | 28(2) | -1(2) | 4(2)  | -1(1) |
| C(11')| 12(2) | 17(2) | 30(2) | 1(2)  | 1(2)  | 2(1)  |
| C(12) | 18(2) | 16(2) | 24(2) | 0(1)  | 3(2)  | 0(2)  |
| C(12')| 10(2) | 20(2) | 31(2) | 2(2)  | -2(2) | -2(2) |
| C(13) | 13(2) | 20(2) | 26(2) | -2(2) | -3(2) | 2(2)  |
| C(13')| 15(2) | 18(2) | 28(2) | 2(2)  | 2(2)  | 1(2)  |
| C(14) | 18(2) | 20(2) | 26(2) | 1(1)  | 2(1)  | 0(2)  |
| C(14')| 19(2) | 19(2) | 25(2) | -1(2) | 0(1)  | -3(2) |
| C(15) | 18(2) | 23(2) | 24(2) | 3(2)  | 3(1)  | 1(2)  |
| C(15')| 15(2) | 22(2) | 24(2) | -2(2) | -1(2) | 4(2)  |
| C(16) | 20(2) | 29(2) | 39(2) | 3(2)  | 1(2)  | 6(2)  |
| C(16')| 22(2) | 24(2) | 42(2) | -3(2) | -4(2) | 3(2)  |
| C(17) | 19(2) | 28(2) | 22(2) | 4(2)  | 1(2)  | 3(2)  |
| C(17')| 17(2) | 28(2) | 22(2) | -3(2) | -1(2) | -1(2) |
| C(18) | 14(2) | 28(2) | 21(2) | 2(2)  | -1(2) | -2(2) |
| C(18')| 15(2) | 27(2) | 23(2) | -3(2) | -2(2) | -    |
|      |      |      |      |      |      |      |      |      |      |
Table S5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^2 x 10^3).

|  | x  | y  | z   | U(eq) |
|---|----|----|-----|-------|
| H(3B) | 7255 | 5936 | 7650 | 37    |
| H(3'B) | 3846 | 3909 | 8127 | 47    |
| H(2B) | 490  | 5971 | 8145 | 26    |
| H(2'B) | 11270| 4378 | 8119 | 24    |
| H(1A) | 5383 | 8913 | 8329 | 22    |
| H(1'A) | 6737 | 1106 | 8401 | 26    |
| H(2A) | 4309 | 9985 | 7795 | 26    |
| H(2'A) | 7764 | -1   | 7889 | 30    |
| H(3A) | 3158 | 10913| 8459 | 29    |
| H(3'A) | 8802 | -961 | 8571 | 33    |
| H(4B) | 3124 | 10793| 9642 | 34    |
| H(4'A) | 8833 | -814 | 9753 | 32    |
| H(5A) | 4246 | 9739 | 10179| 30    |
| H(5'A) | 7729 | 264  | 10268| 29    |
| H(8A) | 6093 | 7127 | 10015| 24    |
| H(8'A) | 5893 | 2871 | 10081| 26    |
| H(9A) | 8006 | 7815 | 10000| 37    |
| H(9B) | 8297 | 7461 | 9255 | 37    |
| H(9C) | 8234 | 6850 | 9894 | 37    |
| H(9'A) | 4003 | 2152 | 10075| 42    |
| H(9'B) | 3696 | 2489 | 9327 | 42    |
| H(9'C) | 3734 | 3111 | 9959 | 42    |
| H(10A) | 6675 | 6010 | 9274 | 24    |
| H(10B) | 5265 | 3974 | 9348 | 25    |
| H(11A) | 7182 | 7087 | 8274 | 23    |
| H(11B) | 4804 | 2835 | 8364 | 23 |
|--------|------|------|------|----|
| H(12A) | 5354 | 6592 | 7515 | 23 |
| H(12B) | 5140 | 7378 | 8006 | 23 |
| H(12C) | 6788 | 2733 | 7981 | 24 |
| H(12D) | 6414 | 3552 | 7563 | 24 |
| H(13A) | 4494 | 5688 | 8289 | 24 |
| H(13B) | 7248 | 4412 | 8363 | 24 |
| H(14A) | 3564 | 7754 | 8630 | 26 |
| H(14B) | 8470 | 2381 | 8515 | 25 |
| H(16A) | 2233 | 8886 | 8725 | 44 |
| H(16B) | 861  | 8653 | 8965 | 44 |
| H(16C) | 1137 | 8836 | 8178 | 44 |
| H(16D) | 9915 | 1322 | 8458 | 44 |
| H(16E) | 11211| 1602 | 8779 | 44 |
| H(16F) | 11067| 1513 | 7970 | 44 |
Supplementary Table S6 – Sequence, design and analytical data on synthesized ASOs, blue letters represent cEt, black DNA and red represent modifications.

| Ion #   | Sequence (5’ to 3’)           | Modification | UV purity | Mass calcd | Mass found |
|---------|--------------------------------|--------------|-----------|------------|------------|
| 558807  | GCA TGTTCACATTA               | DNA          | 96.0      | 5380.5     | 5379.6     |
| 942943  | GCA TGTTCACATTA               | 5’S’-Me DNA  | 94.3      | 5394.5     | 5392.8     |
| 942944  | GCA TGTTCACATTA               | 5’S’-Me DNA  | 93.8      | 5394.5     | 5392.9     |
| 957908  | GCA TGTTCACATTA               | S’-5’-Me DNA | 96.4      | 5394.5     | 5393.7     |
| 957909  | GCA TGTTCACATTA               | S’-5’-Me DNA | 98.5      | 5394.5     | 5394.1     |
| 957910  | GCA TGTTCACATTA               | 5’S’-allyl DNA | 93.5     | 5420.6     | 5419.6     |
| 957911  | GCA TGTTCACATTA               | 5’S’-allyl DNA | 96.1     | 5420.6     | 5419.6     |
| 957912  | GCA TGTTCACATTA               | 5’S’-allyl DNA | 93.1     | 5420.6     | 5420.0     |
| 957913  | GCA TGTTCACATTA               | 5’S’-allyl DNA | 95.3     | 5420.6     | 5419.9     |
| 989027  | GCA TGTTCACATTA               | bcDNA        | 96.8      | 5406.6     | 5405.9     |
| 989028  | GCA TGTTCACATTA               | bcDNA        | 96.1      | 5406.6     | 5405.9     |
| 1318996 | GCA TGTTCACATTA               | S’-5’-Me DNA | 83.7      | 5394.5     | 5394.2     |
| 1123318 | GCA TGTTCACATTA               | S’-5’-Me DNA | 93.0      | 5394.5     | 5394.3     |
| 957908  | GCA TGTTCACATTA               | S’-5’-Me DNA | 96.4      | 5394.5     | 5393.67    |
| 957909  | GCA TGTTCACATTA               | S’-5’-Me DNA | 98.5      | 5394.5     | 5394.1     |
| 1318997 | GCA TGTTCACATTA               | S’-5’-Me DNA | 91.9      | 5394.5     | 5394.4     |
| 1318998 | GCA TGTTCACATTA               | S’-5’-Me DNA | 85.8      | 5394.5     | 5394.3     |
| 1318999 | GCA TGTTCACATTA               | S’-5’-Me DNA | 92.7      | 5394.5     | 5394.3     |
| 1319000 | GCA TGTTCACATTA               | S’-5’-Me DNA | 95.5      | 5394.5     | 5394.3     |
| 1319001 | GCA TGTTCACATTA               | S’-5’-Me DNA | 92.0      | 5394.5     | 5394.3     |
| 1319002 | GCA TGTTCACATTA               | S’-5’-Me DNA | 85.1      | 5394.5     | 5394.2     |
| 1319011 | GCA TGTTCACATTA               | 2’-OMe; S’-5’-Me DNA | 91.6 | 5424.6 | 5424.4 |
| 1319003 | GCA TGTTCACATTA               | 5’S’-Me DNA  | 83.1      | 5394.5     | 5394.3     |
| 1123322 | GCA TGTTCACATTA               | 5’S’-Me DNA  | 95.6      | 5394.5     | 5394.4     |
| 942943  | GCA TGTTCACATTA               | 5’S’-Me DNA  | 94.3      | 5394.5     | 5392.8     |
| 942944  | GCA TGTTCACATTA               | 5’S’-Me DNA  | 93.8      | 5394.5     | 5392.9     |
| 1319004 | GCA TGTTCACATTA               | 5’S’-Me DNA  | 85.6      | 5394.5     | 5394.3     |
| 1319005 | GCA TGTTCACATTA               | 5’S’-Me DNA  | 84.0      | 5394.5     | 5394.4     |
| 1319006 | GCA TGTTCACATTA               | 5’S’-Me DNA  | 85.9      | 5394.5     | 5394.4     |
| 1319007 | GCA TGTTCACATTA               | 5’S’-Me DNA  | 95.6      | 5394.5     | 5394.1     |
| 1319008 | GCA TGTTCACATTA               | 5’S’-Me DNA  | 86.2      | 5394.5     | 5394.3     |
| 1319009 | GCA TGTTCACATTA               | 5’S’-Me DNA  | 92.4      | 5394.5     | 5394.3     |
| 1319010 | GCA TGTTCACATTA               | 2’-OMe; R’-5’-Me DNA | 86.0 | 5424.6 | 5424.3 |
| 1123320 | GCA TGTTCACATTA               | 5’S’-Me DNA  | 96.0      | 5394.5     | 5394.5     |
| 1123322 | GCA TGTTCACATTA               | 5’S’-Me DNA  | 95.6      | 5394.5     | 5394.4     |
| 1123479 | GCA TGTTCACATTA               | 5’-Allyl DNA | 94.0      | 5420.6     | 5420.6     |
| 1175782 | GCA TGTTCACATTA               | 5’-Et DNA    | 93.1      | 5408.5     | 5408.2     |
| 1175785 | GCA TGTTCACATTA               | 5’-Et DNA    | 94.7      | 5408.5     | 5408.3     |
| 1175786 | GCA TGTTCACATTA               | 5’-Et DNA    | 94.4      | 5408.5     | 5408.4     |
| Ion #   | Sequence (5' to 3') | Modification | UV purity | Mass calcd | Mass found |
|--------|---------------------|--------------|-----------|------------|------------|
| 1175787 | GCATGTCTCACATT      | 5'-Et DNA    | 93.6      | 5408.5     | 5408.4     |
| 1133122 | TAGTCTCTGTGAGTTA    | OMe          | 94.3      | 5413.5     | 5412.6     |
| 1280765 | TCTCTCTGTCAGTTA     | MOP          | 92.9      | 5437.5     | 5436.6     |
| 1280766 | TAGTCTCTGTCAGTTA    | R-5'-Me DNA  | 94.3      | 5411.5     | 5411.3     |
| 1280767 | TAGTCTCTGTCAGTTA    | R-5'-Me DNA  | 83.9      | 5411.5     | 5411.6     |
| 1280768 | TAGTCTCTGTCAGTTA    | R-5'-Et DNA  | 86.4      | 5425.5     | 5425.7     |
| 1280769 | TAGTCTCTGTCAGTTA    | R-5'-Et DNA  | 86.7      | 5425.5     | 5425.6     |
| 1133201 | TCTGCTACCTATAGTC    | OMe          | 95.7      | 5410.5     | 5410.2     |
| 1280770 | TCTGCTACCTATAGTC    | OMe          | 93.6      | 5420.5     | 5419.5     |
| 1280779 | TCTGCTACCTATAGTC    | R-5'-Me DNA  | 93.6      | 5394.5     | 5394.2     |
| 1280789 | TCTGCTACCTATAGTC    | R-5'-Me DNA  | 92.9      | 5394.5     | 5394.2     |
| 1280798 | TCTGCTACCTATAGTC    | R-5'-Et DNA  | 86.3      | 5408.5     | 5409.1     |
| 1280804 | TCTGCTACCTATAGTC    | 5'-Et DNA    | 84.8      | 5408.5     | 5409.2     |
| 1133332 | GTCAGTATCCCATGTTG   | OMe          | 92.6      | 5451.5     | 5450.6     |
| 1280775 | GTCAGTATCCCATGTTG   | MOP          | 93.7      | 5461.5     | 5460.5     |
| 1280784 | GTCAGTATCCCATGTTG   | R-5'-Me DNA  | 83.8      | 5435.5     | 5435.8     |
| 1280794 | GTCAGTATCCCATGTTG   | R-5'-Me DNA  | 90.6      | 5435.5     | 5435.5     |
| 1280803 | GTCAGTATCCCATGTTG   | R-5'-Et DNA  | 83.5      | 5449.6     | 5449.9     |
| 1281809 | GTCAGTATCCCATGTTG   | R-5'-Et DNA  | 84.6      | 5449.6     | 5449.8     |
| 1133247 | GATATGTTGTTGGCGC    | OMe          | 97.4      | 5497.5     | 5496.7     |
| 1280774 | GATATGTTGTTGGCGC    | MOP          | 96.6      | 5521.5     | 5520.8     |
| 1280783 | GATATGTTGTTGGCGC    | R-5'-Me DNA  | 92.8      | 5495.5     | 5495.9     |
| 1280793 | GATATGTTGTTGGCGC    | R-5'-Me DNA  | 90.5      | 5495.5     | 5495.8     |
| NT     | GATATGTTGTTGGCGC    | R-5'-Et DNA  | --        | --         | --         |
| NT     | GATATGTTGTTGGCGC    | R-5'-Et DNA  | --        | --         | --         |
| 1202810 | AGACTCTCGGTTCCGA    | OMe          | 94.3      | 5436.5     | 5435.6     |
| 1280776 | AGACTCTCGGTTCCGA    | MOP          | 96.8      | 5460.6     | 5459.8     |
| 1280785 | AGACTCTCGGTTCCGA    | R-5'-Me DNA  | 90.5      | 5434.5     | 5434.6     |
| 1280795 | AGACTCTCGGTTCCGA    | R-5'-Me DNA  | 94.9      | 5434.6     | 5434.5     |
| 1280804 | AGACTCTCGGTTCCGA    | R-5'-Et DNA  | 93.1      | 5448.8     | 5448.6     |
| 1280810 | AGACTCTCGGTTCCGA    | R-5'-Et DNA  | 92.1      | 5448.8     | 5448.7     |
Supplementary Figure S2. Immunofluorescent staining of P54nr in HeLa cells transected with 200 nM of (A) S-5′Me DNA and (B) R-5′-Me DNA ASOs for 2 h. Mis-localized P54nr is exemplified with arrows. Scale bar: 20 μm.
Supplementary Figure S3. 5'-allyl DNA modification that reduce toxicity also reduces protein binding and nucleolar mislocalization. A) 5'-allyl DNA modification can reduce toxicity and protein mislocalization. ASO toxicity is illustrated by increased caspase activity and ALT elevation. P54nrb mislocalization was detected by immunofluorescence staining. The percentage of cells containing mis-localized P54nrb protein was calculated based on manual counting of approximately 100 cells in each condition. B) Silver staining of proteins co-isolated with ASOs of different modifications. The toxic ASOs are marked in red. The proteins in green dotted box were quantified and the levels relative to parental ASO (558807) were calculated and presented below the green box. C) western analyses for the levels of RNase H1 and P54nrb co-isolated with ASOs, as described in panel B. Ku70 protein was probed as a control for loading as the binding of Ku70 is less affected by 2'-modifications. Data indicated by asterisk were obtained from a different experiment. (D) Structures of modifications used in the ASOs.
(A) 5’ allyl modification but not bcDNA can reduce toxicity and protein mislocalization. ASO toxicity is illustrated by increased caspase activity and ALT elevation. (B) Structures of 5’-allyl DNA and bicycloDNA (bcDNA). (C) P54nrb mislocalization was detected by immunofluorescence staining. The percentage of cells containing mis-localized P54nrb protein was calculated based on manual counting of approximately 100 cells in each condition. Immunofluorescent staining of P54nrb in HeLa cells transected with 200 nM of R- and S-5’-allyl DNA, and bicycloDNA modified ASOs for 2 h. Mis-localized P54nrb is exemplified with arrows. Scale bar: 20 µm.

Supplementary Figure S4.