Supporting Information

An Exocyclic Methylene Group Acts As A Bio-isostere of the 2'-Oxygen Atom in LNA

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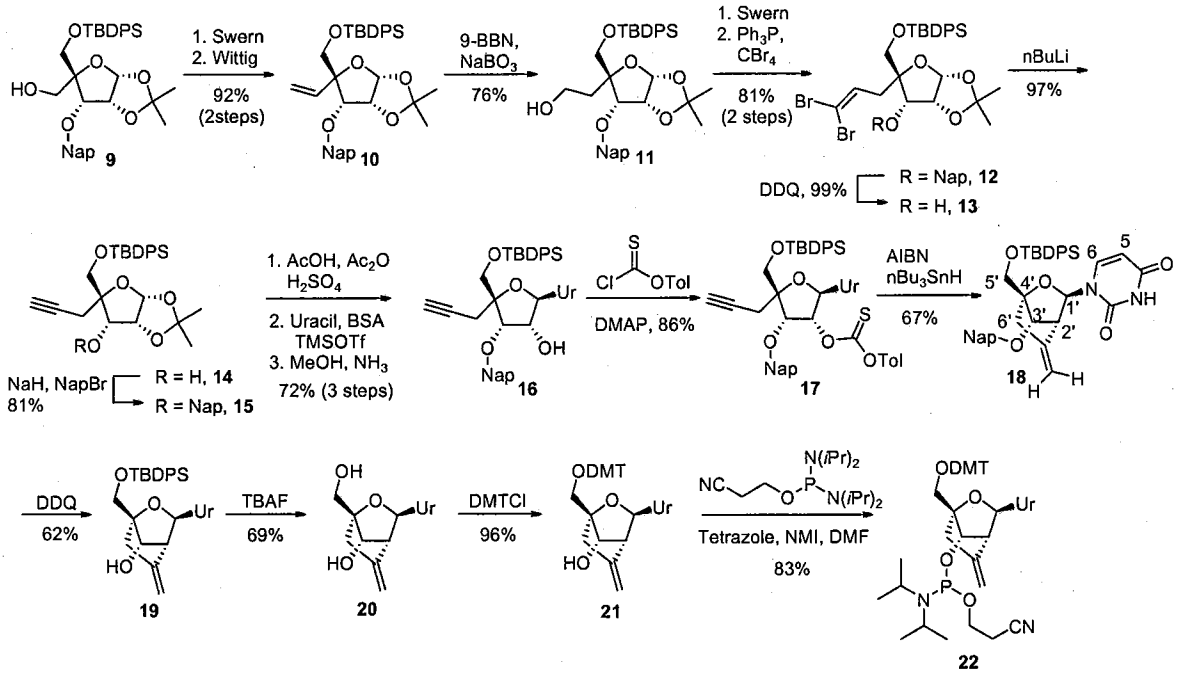
Supporting Information Content

General Experimental

All chemicals were purchased from commercial suppliers and used without any further purification. Oligonucleotides synthesis and purification and T_m measurements were carried out according to the general procedures described previously.[1] Animal studies, quantitation of liver mRNA by RT-PCR, measurement of plasma ALT levels were carried out according to the general procedures described previously.[2,3] Oligonucleotides were characterized by IP-HPLC – MS analysis using an Agilent 1100 MSD system. UV purities are reported as percent area of the full-length product by ion-pair analysis. Calculated masses are for the fully protonated form of the oligonucleotide, with the observed mass being calculated from either the -3 or -4 charge state as measured using ESI-MS. ED_{50} values for the in vivo experiments were calculated using GraphPad Prsim 4.0 software.

SI Scheme 1. Synthesis of Methylene-cLNA Uridine phsophoramidite 22



5-*O*-(*tert*-Butyldiphenylsilyl)-1,2-*O*-isopropylidene-3-*O*-(2-naphthyl)-4-*C*-vinyl-α-D-ribofuranose (10). Dimethylsulfoxide (188 mmol, 13.3 mL) was added drop-wise to a cold (–78 °C) solution of oxalyl chloride (94 mmol, 8.2 mL) in dichloromethane (600 mL). After stirring for 30 minutes, a solution of alcohol **9** (67 mmol, 40.0 g) in dichloromethane (100 mL) was added to the reaction and stirring was continued for 45 minutes at –78 °C. Triethylamine (281 mmol, 39.0 mL) was added and the reaction was removed from the cold bath and stirring was continued for another 30 minutes. The reaction was diluted with dichloromethane and washed sequentially with 5% HCl, saturated $NaHCO_3$, brine, dried (Na_2SO_4) and concentrated to provide the corresponding aldehyde which was used without any purification.

*n*BuLi (84 mmol, 33.5 mL of a 2.5 M solution) was added to a cold (0 °C) solution of methyltriphenyphosphonium bromide (84 mmol, 29.9 g) in THF (600 mL). After stirring for 2 hours in the ice bath, the deep red solution was cooled to –78 °C and a solution of the crude aldehyde from above in THF (70 mL) was added to the reaction over 20 minutes. The reaction was allowed to warm to room temperature gradually and stirred for 16 hours. Saturated ammonium chloride was added to the reaction and roughly 80% of the THF was evaporated under reduced pressure. The resulting oil was diluted with ethyl acetate and the organic layer was washed with water, brine, dried (Na_2SO_4) and concentrated. Purification by chromatography (silica gel, eluting with hexanes to 20% ethyl acetate in hexanes) provided **10** (36.4 g, 92% over two steps) as an oil. ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.87–7.73 (m, 4 H), 7.64–7.25 (m, 13 H), 6.27–6.12 (m, 1 H), 5.78 (d, J = 3.8 Hz, 1 H), 5.45 (dd, J = 1.9, 17.5 Hz, 1 H), 5.20 (dd, J = 1.9, 10.9 Hz, 1 H), 4.95 (d, J = 12.4 Hz, 1 H), 4.83 (d, J = 12.4 Hz, 1 H), 4.67–4.59 (m, 1 H), 4.49 (d, J = 4.9 Hz, 1 H), 3.52 (s, 2 H), 1.56 (s, 3 H), 1.32 (s, 3 H), 0.96–0.85 (m, 9 H). ^{13}C NMR (75MHz, $CDCl_3$) δ: 135.7, 135.6, 135.5, 135.4, 133.4, 133.2, 133.2, 133.0, 129.7, 129.6, 128.3, 127.9, 127.7, 127.7, 127.6, 126.8, 126.2, 126.0, 125.7, 116.3, 113.5, 104.0, 87.4, 78.8, 77.0, 72.7, 66.5, 26.7, 26.3, 25.9, 19.2. ESI-MS $[M+Na]^+$ calcd. 617.3; found 617.2. HRMS (QTOF), Calcd for $C_{37}H_{42}O_5SiNa$, 617.2699; Found 617.2709.

5-*O*-(*tert*-Butyldiphenylsilyl)-4-*C*-(2-hydroxyethyl)-1,2-*O*-isopropylidene-3-*O*-(2-naphthyl)-α-D-ribofuranose (11). A solution of **10** (57.5 mmol, 34.2 g) in THF (40 mL) was added to a solution of 9-BBN (0.5 M in THF, 172 mmol, 345 mL). After stirring at room temperature for 24 hours, the reaction was cooled in an ice bath and carefully quenched with EtOH. A suspension of sodium perborate tetrahydrate (340 mmol, 53.0 g)

in ethanol (340 mL) and water (340 mL) was added to the reaction and the mixture was heated at 50 °C for 4 hours. Roughly 80% of the solvent was evaporated under reduced pressure and the residue was suspended in ethyl acetate and washed sequentially with water, brine, dried (Na_2SO_4) and concentrated. Purification by chromatography (silica gel, eluting with 10 to 30% ethyl acetate in hexanes) provided **11** (27.7 g, 76%) as an oil. ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.87–7.75 (m, 4 H), 7.63–7.26 (m, 13 H), 5.78 (d, J = 3.8 Hz, 2 H), 4.93 (d, J = 12.2 Hz, 2 H), 4.75 (d, J = 12.2 Hz, 1 H), 4.65 (m, 1H), 4.33 (d, J = 5.1 Hz, 1 H), 3.80–3.61 (m, 3 H), 3.45 (d, J = 10.7 Hz, 1 H), 2.85–2.78 (m, 1 H), 2.58–2.42 (m, 1 H), 1.81–1.72 (m, 1 H), 1.70 (s, 3 H), 1.35 (s, 3 H), 0.92 (s, 9 H). ^{13}C NMR (75MHz, CDCl3) δ: 135.6, 135.5, 135.1, 133.2, 133.2, 133.0, 132.9, 129.8, 129.8, 128.4, 127.9, 127.8, 126.8, 126.2, 126.1, 125.7, 113.8, 104.3, 88.2, 79.3, 77.8, 72.7, 66.8, 58.9, 33.6, 26.7, 26.5, 26.2, 19.2. ESI-MS $[M+Na]^+$ calcd. 635.3; found 635.2. HRMS (QTOF), Calcd for $C_{37}H_{44}O_6SiNa$, 635.2805; Found 635.2804.

5-*O*-(*tert*-Butyldiphenylsilyl)-4-*C*-[3-(1,1-dibromo-allyl)]-1,2-*O*-isopropylidene-3-*O*-(2-naphthyl)-α-D-ribofuranose (12). Dimethylsulfoxide (68.6 mmol, 4.9 mL) was added drop-wise to a cold (–78 °C) solution of oxalyl chloride (34.3 mmol, 3.0 mL) in dichloromethane (170 mL). After stirring for 30 minutes, a solution of **11** (22.9 mmol, 14.0 g) in dichloromethane (50 mL) was added to the reaction and stirring was continued for another 45 minutes at –78 °C. Triethylamine (102.9 mmol, 14.4 mL) was added and the reaction was removed from the cold bath and stirring was continued for another 30 minutes. The reaction was then diluted with dichloromethane and washed sequentially with 5% HCl, saturated $NaHCO_3$, brine, dried (Na_2SO_4) and concentrated to provide the corresponding aldehyde which was used in the next step without any purification.

A solution of triphenylphosphine (91.3 mmol, 24.0 g) in dichloromethane (50 mL) was added to a cold (0 °C) solution of carbon tetrabromide (45.6 mmol, 15.0 g) in dichloromethane (200 mL). After stirring for 30 minutes, the reaction was cooled to –78 °C and a solution of the crude aldehyde (57.5 mmol) in dichloromethane (40 mL) was added to the reaction. After stirring at -78 °C for 2 hours, the reaction was quenched with saturated sodium bicarbonate solution and the organic layer was washed with brine, dried (Na_2SO_4) and concentrated. Purification by chromatography (silica gel, eluting with hexanes to 15% ethyl acetate in hexanes) provided **12** (14.1 g, 81% from **11**). ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.87–7.75 (m, 4 H), 7.62–7.28 (m, 13 H), 6.62 (dd, J = 5.3, 8.3 Hz, 1 H), 5.75 (d, J = 3.8 Hz, 1 H), 4.95 (d, J = 12.2 Hz, 1 H), 4.73 (d, J = 12.2 Hz, 1 H), 4.66 (dd, J = 4.0, 5.1 Hz, 1 H), 4.29 (d, J = 5.1 Hz, 1 H), 3.56 (d, J = 10.9 Hz, 1 H), 3.42 (d, J = 11.1 Hz, 1 H), 2.87 (dd, J = 5.2, 16.5 Hz, 1 H), 2.48 (dd, J = 8.2, 16.5 Hz, 1 H), 1.61 (s, 3 H), 1.35 (s, 3 H), 0.93 (s, 9 H). ^{13}C NMR (75MHz, $CDCl_3$) δ: 137.4, 137.3, 136.9, 136.8, 135.0, 134.9, 134.8, 134.7, 131.6, 131.5, 130.1, 129.7, 129.5, 129.5, 128.5, 128.0, 127.8, 127.5, 115.3, 106.0, 88.5, 81.1, 79.6, 79.2, 78.8, 78.4, 74.5, 68.5, 38.0, 28.7, 28.5, 28.0, 21.0. ESI-MS $[M+Na]^+$ calcd. 789.1; found 789.1. HRMS (QTOF), Calcd for $C_{38}H_{42}Br_2O_5SiNa$, 789.1045; Found 789.1034.

5-*O*-(*tert*-Butyldiphenylsilyl)-4-*C*-[3-(1,1-dibromo-allyl)]-1,2-*O*-isopropylidene-α-D-ribofuranose (13). DDQ (31.3 mmol, 7.1 g) was added to a solution of **12** (15.7 mmol, 12.0 g) in dichloromethane (150 mL) and water (7.5 mL). After stirring at room temperature for 6 hours, the reaction was evaporated to dryness under reduced pressure and the residue was redissolved in ethyl acetate. The organic layer was then washed with water, 10% sodium bisulfite, saturated sodium bicarbonate, brine, dried (Na_2SO_4) and

concentrated. Purification by chromatography (silica gel, 10 to 20% ethyl acetate in hexanes) provided **13** (9.7 g, 99%). ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.68–7.62 (m, 4 H), 7.49–7.35 (m, 6 H), 6.57 (dd, J = 5.7, 7.8 Hz, 1 H), 5.89 (d, J = 4.1 Hz, 1 H), 4.73 (dd, J = 4.1, 6.2 Hz, 1 H), 4.33 (dd, J = 6.3, 7.6 Hz, 1 H), 3.54 (s, 6 H), 2.68 (d, J = 7.5 Hz, 1 H), 2.60 (dd, J = 5.8, 16.0 Hz, 1 H), 2.45 (dd, J = 7.9, 16.0 Hz, 1 H), 1.60 (s, 3 H), 1.38 (s, 3 H), 1.06 (s, 9 H). ^{13}C NMR (75MHz, $CDCl_3$) δ: 135.6, 134.4, 132.7, 129.9, 129.9, 127.9, 127.8, 113.7, 104.9, 89.8, 88.4, 80.4, 72.4, 68.0, 35.7, 26.9, 26.6, 19.2. ESI-MS $[M+Na]^+$ calcd. 649.0; found 649.0.

5-*O*-(*tert*-Butyldiphenylsilyl)-1,2-*O*-isopropylidene-4-*C*-[3-propynyl]-α-D-ribofuranose (14). *n*BuLi (75 mmol, 30 mL of a 2.5 M solution in hexanes) was added to a cold (-78 °C) solution of **13** (15 mmol, 9.7 g) in THF (150 mL). After stirring at -78 °C for 30 minutes, the reaction was quenched using saturated ammonium chloride and diluted with ethyl acetate. The organic layer was then sequentially washed with water, brine, dried (Na_2SO_4) and concentrated to provide **14** (7.3 g, 97%). ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.71–7.64 (m, 4 H), 7.47–7.34 (m, 6 H), 5.93 (d, J = 4.1 Hz, 1 H), 4.76 (dd, J = 4.1, 6.1 Hz, 1 H), 4.38 (t, J = 6.6 Hz, 1 H), 3.83 (d, J = 10.5 Hz, 1 H), 3.76 (d, J = 10.5 Hz, 1 H), 2.74 (d, J = 7.0 Hz, 1 H), 2.69 (dd, J = 2.7, 5.0 Hz, 2 H), 1.95 (t, J = 2.7 Hz, 1 H), 1.62 (s, 3 H), 1.40 (s, 3 H), 1.06 (s, 9 H). ^{13}C NMR (75MHz, $CDCl_3$) δ: 133.7, 133.7, 130.9, 130.9, 128.0, 127.9, 125.9, 112.1, 103.1, 86.3, 78.8, 78.0, 75.3, 70.2, 68.7, 66.1, 25.0, 24.9, 24.9, 20.6, 17.3. ESI-MS $[M+Na]^+$ calcd. 489.2; found 489.1. HRMS (QTOF), Calcd for $C_{27}H_{34}O_5SiNa$, 489.2073; Found 489.2078.

5-*O*-(*tert*-Butyldiphenylsilyl)-1,2-*O*-isopropylidene-3-*O*-(2-naphthyl)-4-*C*-[3-propynyl]-α-D-ribofuranose (15). Sodium hydride (18.5 mmol, 0.74 g, 60% in mineral oil) was added in portions to a cold (0 °C) solution of **14** (14.8 mmol, 7.3 g) and 2-bromomethyl-naphthalene (18.5 mmol, 4.1 g) in DMF (100 mL). After stirring for 1 hour, additional sodium hydride (0.35 g) was added to the reaction and the stirring was continued for another 3 hours at room temperature. The reaction was then cooled in an ice bath and carefully quenched with saturated ammonium chloride and then diluted with ethyl acetate. The organic layer was sequentially washed with water, brine, dried (Na_2SO_4) and concentrated. Purification by column chromatography (silica gel, eluting with 10 to 15 % ethyl acetate in hexanes) provided **15** (7.6 g, 81%) and unreacted **14** (0.6 g, 8.3%). ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.87–7.74 (m, 4 H), 7.65–7.27 (m, 13 H), 5.77 (d, J = 3.8 Hz, 1 H), 4.92 (d, J = 12.4 Hz, 1 H), 4.74 (d, J = 12.1 Hz, 1 H), 4.66 (dd, J = 3.8, 5.1 Hz, 1 H), 4.33 (d, J = 5.1 Hz, 1 H), 3.89 (d, J = 10.7 Hz, 1 H), 3.70 (d, J = 10.9 Hz, 1 H), 3.08 (dd, J = 2.7, 17.4 Hz, 1 H), 2.67 (dd, J = 2.7, 17.4 Hz, 1 H), 1.91 (t, J = 2.7 Hz, 1 H), 1.65 (s, 3 H), 1.36 (s, 3 H), 0.95 (s, 9 H). ^{13}C NMR (75MHz, $CDCl_3$) δ: 135.6, 135.6, 135.2, 133.2, 133.1, 133.1, 129.7, 129.7, 128.3, 127.9, 127.7, 127.7, 126.6, 126.2, 126.0, 125.7, 113.7, 104.1, 86.1, 80.3, 79.5, 77.7, 72.8, 70.3, 66.4, 27.0, 26.8, 26.4, 23.0, 19.2. ESI-MS $[M+Na]^+$ calcd. 629.3; found 629.2. HRMS (QTOF), Calcd for $C_{38}H_{34}O_5SiNa$, 629.2699; Found 629.2702.

1-[5-*O*-(*tert*-Butyldiphenylsilyl)-3-*O*-(2-naphthyl)-4-*C*-(3-propynyl)-α-D-ribofuranosyl]-thymine (16). Concentrated sulfuric acid (2 drops) was added to a solution of compound **15** (12.0 mmol, 7.5 g) in acetic acid (36 mL) and acetic anhydride (7 mL). After stirring at room temperature for 15 minutes, the reaction was concentrated

under reduced pressure and the residue was diluted with ethyl acetate. The organic layer was washed with water, saturated sodium bicarbonate (until washings are pH>10), brine, dried (Na_2SO_4) and concentrated to provide the bis-acetate which was used in the next step without any purification.

N,O-bis-trimethylsilyl-acetamide (60.0 mmol, 14.8 mL) was added to a suspension of uracil (24 mmol, 2.7 g) and the crude anomeric diacetate from above in acetonitrile (60 mL). The suspension was heated until dissolution occurred after which it was cooled in an ice bath. TMSOTf (18.0 mmol, 3.3 mL) was added drop-wise to the reaction and the reaction was refluxed for 2 hours. The reaction was then cooled in an ice bath and carefully quenched with saturated sodium bicarbonate solution. The reaction was then diluted with ethyl acetate and the organic layer was washed with water, brine, dried (Na_2SO_4) and concentrated to provide the crude nucleoside which was used in the next step without any further purification.

The crude nucleoside obtained from above, was dissolved in a solution of ammonia in methanol (7 N, 36 mL). After stirring for 16 hours at room temperature, the reaction was concentrated under reduced pressure and the residue was purified by chromatography (silica gel, eulting with 35 to 50% ethyl acetate in hexanes) to provide **16** (5.9 g, 72%). ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.78 (s, 1 H), 7.88–7.75 (m, 4 H), 7.66–7.30 (m, 14 H), 5.97 (d, J = 5.8 Hz, 1 H), 5.38 (d, J = 8.1 Hz, 1 H), 4.83 (s, 2 H), 4.40–4.30 (m, 1 H), 4.22 (d, J = 5.8 Hz, 1 H), 3.99 (d, J = 11.1 Hz, 1 H), 3.90 (d, J = 10.9 Hz, 1 H), 3.35 (d, J = 8.1 Hz, 1 H), 2.69 (dd, J = 2.6, 8.9 Hz, 2 H), 1.97 (t, J = 2.5 Hz, 1 H), 1.08 (s, 9 H). ^{13}C NMR (75MHz, $CDCl_3$) δ: 162.7, 150.6, 140.0, 135.7, 135.4, 134.3, 133.2, 133.2, 132.6, 132.0, 130.3, 130.2, 128.6, 128.0, 128.0, 127.8, 127.2, 126.4, 126.3,

125.8, 102.6, 89.2, 87.5, 79.8, 78.5, 75.4, 75.3, 71.2, 66.9, 27.0, 23.1, 19.3. ESI-MS $[M+Na]^+$ calcd. 683.3; found 683.2. HRMS (QTOF), Calcd for $C_{39}H_{41}N_2O_6Si$, 661.2734; Found 661.2729.

1-[5-*O*-(*tert*-Butyldiphenylsilyl)-3-*O*-(2-naphthyl)-4-*C*-(3-propynyl)-2-*O*-toluneoxy-thiocarbonyl-α-D-ribofuranosyl]-thymine (17). Tolyl-chlorothionoformate (1.5 mL, 9.9 mmol) was added drop-side to a cold (0 °C) solution of **16** (8.9 mmol, 5.9 g) and dimethylaminopyridine (19.7 mmol, 2.4 g) in acetonitrile (90 mL). After stirring for 1 hour, the reaction was quenched with methanol. Roughly 50% of the solvent was evaporated under reduced pressure and the reaction was diluted with ethyl acetate and washed with 5% HCl, saturated sodium bicarbonate, brine, dried (Na_2SO_4) and concentrated. Purification by chromatography (silica gel, eluting with 30 to 40% ethyl acetate in hexanes) provided **17** (6.3 g, 86%). ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.56–8.46 (m, 1 H), 7.91–7.76 (m, 4 H), 7.69–7.60 (m, 5 H), 7.54–7.32 (m, 9 H), 7.11 (d, J = 8.7 Hz, 2 H), 6.84 (d, J = 8.5 Hz, 1 H), 6.42 (d, J = 6.0 Hz, 1 H), 5.95 (t, J = 5.9 Hz, 1 H), 5.38 (dd, J = 2.2, 8.2 Hz, 1 H), 4.88 (d, J = 10.7 Hz, 1 H), 4.74 (d, J = 11.1 Hz, 1 H), 4.68 (d, J = 5.8 Hz, 1 H), 4.06 (d, J = 10.9 Hz, 1 H), 3.96 (d, J = 11.7 Hz, 1 H), 2.67 (d, J = 2.4 Hz, 2 H), 2.32 (s, 3 H), 1.99–1.93 (m, 1 H), 1.10 (s, 9 H). ^{13}C NMR (75MHz, $CDCl_3$) δ: 194.6, 162.6, 151.3, 150.0, 140.0, 136.6, 135.7, 135.5, 134.5, 133.2, 132.6, 131.8, 130.3, 130.2, 130.1, 128.3, 128.0, 127.8, 127.0, 126.3, 126.2, 125.9, 121.2, 103.0, 87.6, 85.9, 82.7, 79.2, 76.9, 75.4, 71.2, 66.9, 27.1, 23.0, 20.9, 19.4. ESI-MS $[M+Na]^+$ calcd. 833.3; found 833.2.

(1*R*,3*R*,4*R*,7*S*)-1-(*tert*-Butyldiphenylsilyloxymethyl)-5-methylene-7-(2-naphthyloxy)-3-(uracil-1-yl)-2-oxabicyclo[2.2.1]heptane (18). A solution of **17** (7.7 mmol, 6.2 g) in

toluene (230 mL) was refluxed for 30 minutes. A solution of nBu$_3$SnH (15.4 mmol, 4.1 mL) in toluene (36 mL) was added drop-wise to the refluxing reaction. After about half (18 mL) of the nBu$_3$SnH solution was added to the reaction (30 minutes), a solution of AIBN (15.4 mmol, 2.5 g) in toluene (40 mL) was added drop-wise to the reaction over 90 minutes. After refluxing for another 2 hours, the reaction was cooled and the solvent was evaporated under reduced pressure. The residue was diluted with ether and washed with saturated KF solution, brine, dried (Na_2SO_4) and concentrated. Purification of the residue by chromatography (silica gel, eluting with 25 to 40% ethyl acetate in hexanes) provided **18** (3.3 g, 67%, product contaminated with tributyltin byproducts). ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.85–8.78 (m, 1 H, *N*-H), 8.07 (d, J = 8.3 Hz, 1 H, H6), 7.80 (d, J = 8.5 Hz, 3 H, aromatic), 7.71–7.58 (m, 5 H, aromatic), 7.53–7.27 (m, 10 H, aromatic), 5.55 (s, 1 H, H1'), 5.38 (s, 1 H, CH), 5.37 (d, overlapped, 1H, H5), 5.13 (s, 1 H, CH), 4.70 (d, J = 11.1 Hz, 1 H, CH_2Nap), 4.65 (d, J = 11.1 Hz, 1 H, CH_2Nap), 4.22 (s, 1 H, H3'), 4.02 (d, J = 11.9 Hz, 1 H, H5'), 3.96 (d, J = 11.9 Hz, 1 H, H5"), 3.40 (s, 1 H, H2'), 2.50 (d, J = 16.0 Hz, 1 H, H6'), 2.25 (d, J = 16.2 Hz, 1 H, H6"), 1.07 (s, 9 H, *t*-Bu). ^{13}C NMR (75MHz, $CDCl_3$) δ: 163.3, 149.9, 143.8, 140.1, 135.6, 135.4, 134.5, 133.1, 133.1, 132.8, 132.3, 130.0, 130.0, 128.3, 127.9, 127.8, 127.7, 126.6, 126.3, 126.2, 125.7, 111.6, 101.0, 90.8, 87.7, 72.2, 61.0, 52.8, 37.0, 26.8, 19.3. ESI-MS $[M+H]^+$ calcd. 645.3; found 645.2. HRMS (QTOF), Calcd for $C_{39}H_{42}N_2O_5Si$, 645.2785; Found 645.2788.

(1*R*,3*R*,4*R*,7*S*)-1-(*tert*-Butyldiphenylsilyloxymethyl)-7-hydroxy-5-methylene-3-(uracil-1-yl)-2-oxabicyclo[2.2.1]heptane (19). DDQ (9.3 mmol, 2.1 g) was added to a solution of **18** (4.7 mmol, 3.0 g) in dichloromethane (47 mL) and water (2.5 mL). After stirring at room temperature for 6 hours, the reaction was concentrated under reduced

pressure and re-dissolved in ethyl acetate. The organic layer was washed with water, 10% sodium bisulfite, saturated sodium bicarbonate, brine, dried (Na_2SO_4) and concentrated. Purification of the residue by chromatography (silica gel, eluting with 30 to 60% ethyl acetate in hexanes) provided **19** (1.46 g, 62%). ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.09 (d, J = 8.1 Hz, 1 H, H6), 7.78–7.69 (m, 4 H, aromatic), 7.54–7.40 (m, 6 H, aromatic), 5.51 (s, 1H, H1'), 5.50 (d, overlapped, 1H, H5), 5.45 (s, 1 H, C<u>H</u>), 5.20 (s, 1 H, C<u>H</u>), 4.30–4.25 (m, 1 H, H3'), 4.11 (d, J = 12.1 Hz, 1 H, H5'), 3.90 (d, J = 12.1 Hz, 1 H, H5"), 3.19 (s, 1 H, H2'), 2.39 (d, J = 16.2 Hz, 1 H, H6'), 2.26 (d, J = 15.8 Hz, 1 H, H6"), 1.57–1.47 (m, 1 H, O<u>H</u>), 1.14 (s, 9 H, *t*-Bu). ^{13}C NMR (75MHz, $CDCl_3$) δ: 163.0, 149.7, 143.6, 140.1, 135.6, 135.5, 132.6, 132.5, 130.3, 130.2, 128.1, 128.1, 112.7, 101.0, 90.8, 87.4, 71.3, 60.8, 55.4, 36.0, 26.9, 19.3. ESI-MS $[M+H]^+$ calcd. 505.2; found 505.1. HRMS (QTOF), Calcd for $C_{28}H_{33}N_2O_5Si$, 505.2159; Found 505.2143.

(1*R*,3*R*,4*R*,7*S*)-7-Hydroxy-1-(hydroxymethyl)-5-methylene-3-(uracil-1-yl)-2-oxabicyclo[2.2.1]heptane (20). Triethylamine trihydrofluoride (0.94 mL, 5.8 mmol) was added to a solution of **19** (2.9 mmol, 1.45 g) in THF (20 mL). After stirring at room temperature for 16 hours, additional triethylamine trihydrofluoride (0.9 mL) was added to the reaction. After stirring for 40 hours, the solvent was evaporated under reduced pressure and the residue was purified by chromatography (silica gel, eluting with 5 to 10% methanol in chloroform) provided **20** (0.53 g, 69%). ^{1}H NMR (300MHz, CD_3OD) δ: 7.98 (d, J = 8.1 Hz, 1 H, H6), 5.57 (d, J = 8.1 Hz, 1 H, H5), 5.28 (s, 1 H, H1'), 5.19 (s, 1 H, C<u>H</u>), 5.00 (s, 1 H, C<u>H</u>), 4.05 (s, 1 H, H3'), 3.75 (m, 2 H, H5'), 3.00 (s, 1 H, H2'), 2.38 (d, J = 15.6 Hz, 1 H, H6'), 2.15 (d, J = 15.3 Hz, 1 H, H6"). ^{13}C NMR (75MHz, CD_3OD) δ: 166.5, 151.9, 147.2, 142.0, 111.1, 101.2, 92.6, 89.1, 71.8, 59.9, 57.0, 37.2.

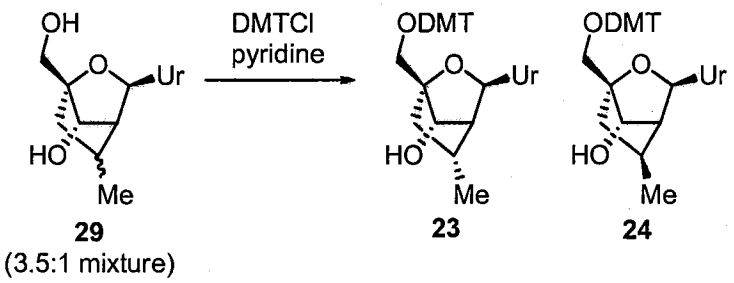
ESI-MS $[M+Na]^+$ calcd. 289.1; found 289.0. HRMS (QTOF), Calcd for $C_{12}H_{15}N_2O_5$, 267.0981; Found 267.0981.

(1*R*,3*R*,4*R*,7*S*)-1-(4,4'-Dimethoxytritryloxymethyl)-7-hydroxy-5-methylene-3-(uracil-1-yl)-2-oxabicyclo[2.2.1]heptane (21). Dimethoxytrityl chloride (3.0 mmol, 1.0 g) was added to a solution of **20** (2.5 mmol, 0.67 g) in pyridine (10 mL). After stirring at room temperature for 16 hours, the reaction was quenched with methanol and the pyridine was removed under reduced pressure. The residual oil was dissolved in ethyl acetate and the organic layer was washed with saturated sodium bicarbonate, brine, dried (Na_2SO_4) and concentrated. Purification by chromatography (silica gel, eluting with 70% ethyl acetate in hexanes containing 1% triethylamine) provided **21** (1.36 g, 96%). ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.53–8.44 (m, 1 H, *N*-H), 8.19 (d, J = 8.1 Hz, 1 H, H6), 7.49–7.42 (m, 2 H, DMTr), 7.40–7.24 (m, 9 H, DMTr), 6.86 (dd, J = 1.5, 8.9 Hz, 4 H, DMTr), 5.57 (d, J = 8.1 Hz, 1H, H5), 5.50 (s, 1 H, H1'), 5.39 (s, 1 H, CH), 5.14 (s, 1 H, CH), 4.27–4.21 (m, 1 H, H3'), 3.80 (s, 6 H, OCH_3), 3.68 (d, J = 11.3 Hz, 1 H, H5'), 3.42 (d, J = 11.5 Hz, 1 H, H5"), 3.17 (s, 1 H, H1'), 2.37 (d, J = 16.0 Hz, 1 H, H6'), 2.24 (d, J = 16.2 Hz, 1 H, H6"), 1.55 (d, J = 4.5 Hz, 1 H, OH). ^{13}C NMR (75MHz, $CDCl_3$) δ: 163.1, 158.8, 149.7, 144.5, 143.5, 140.4, 135.4, 135.4, 130.1, 130.0, 128.1, 127.2, 113.4, 112.5, 101.0, 90.0, 87.5, 87.1, 71.7, 60.0, 55.3, 55.2, 36.5. ESI-MS $[M+Na]^+$ calcd. 591.2; found 591.1. HRMS (QTOF), Calcd for $C_{33}H_{32}N_2O_7Na$, 591.2107; Found 591.2105.

(1*R*,3*R*,4*R*,7*S*)-7-(Cyanoethyl-diisopropylaminophosphinoxy)-1-(4,4'-dimethoxytritryloxymethyl)-5-methylene-3-(uracil-1-yl)-2-oxabicyclo[2.2.1]heptane (22). $(iPr_2N)_2POCH_2CH_2CN$ (1.7 mmol, 0.53 mL) was added to a solution of **21** (1.1 mmol, 0.63 g), NMI (0.28 mmol, 0.022 g) and tetrazole (0.88 mmol, 0.062 g) in DMF

(3.0 mL). After stirring at room temperature for 6 hours, the reaction was diluted with ethyl acetate and washed with saturated sodium bicarbonate, brine, dried (Na_2SO_4) and concentrated. Purification by chromatography (silica gel, eluting with 50 to 60% ethyl acetate in hexanes) provided **22** (0.67 g, 83%). ^{31}P NMR (121 MHz, $CDCl_3$) δ: 149.2, 148.6. ESI-MS $[M+Na]^+$ calcd. 769.3; found 769.3. HRMS (QTOF), Calcd for $C_{42}H_{50}N_4O_8P$, 769.3366; Found 769.3381.

SI Scheme 2. Synthesis of 5'-*O*-DMT nucleosides 23 and 24



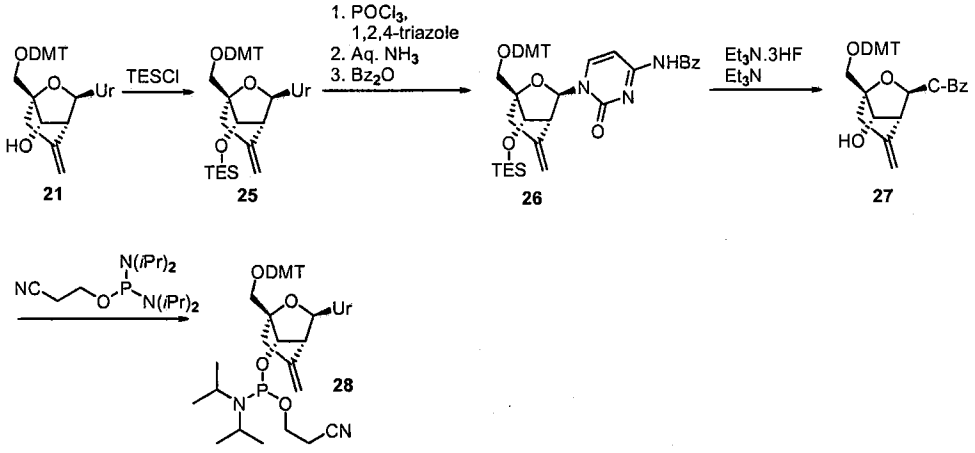
(1*R*,3*R*,4*R*,5*R*,7*S*)-1-(4,4'-Dimethoxytritryloxymethyl)-7-hydroxy-5-methyl-3-(uracil-1-yl)-2-oxabicyclo[2.2.1]heptane (23) and (1*R*,3*R*,4*R*,5*S*,7*S*)-1-(4,4'-Dimethoxytritryloxymethyl)-7-hydroxy-5-methyl-3-(uracil-1-yl)-2-oxabicyclo[2.2.1]heptane (24). Dimethoxytrityl chloride (4.3 mmol, 1.3 g) was added to a solution of a mixture of nucleoside **29** (1.05 g, 3.9 mmol, 3.5:1 *R*-Me:*S*-Me cLNA mixture prepared according to general procedures described previously[4,14]) in pyridine (20 mL). After stirring for 16 hours at room temperature, the reaction was diluted with ethyl acetate and the organic layer was washed with water and brine. The aqueous layers were combined and extracted with dichloromethane and the organic layers were combined, dried (Na_2SO_4) and concentrated. Purification by column chromatography

(silica gel, eluting with 15 to 25% acetone in chloroform) provided **23** (1.24 g, 56%), a mixture of compounds **23** and **24** (0.42 g, 18%) and unreacted starting material (0.27 g, 24%). Repurification of the mixture by column chromatography as above provided pure **23** (228 mg) and **24** (122 mg). **23** ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.96–8.86 (m, 1 H), 8.20 (d, J = 8.1 Hz, 1 H), 7.49–7.40 (m, 2 H), 7.38–7.22 (m, 9 H), 6.85 (dd, J = 1.5, 8.9 Hz, 4 H), 5.75 (s, 1 H), 5.52 (dd, J = 1.7, 8.3 Hz, 1 H), 4.20 (s, 1 H), 3.79 (s, 6 H), 3.60 (d, J = 11.5 Hz, 1 H), 3.31 (d, J = 11.3 Hz, 1 H), 2.72–2.54 (m, 1 H), 2.51–2.44 (m, 1 H), 1.96 - 1.83 (m, 1 H), 1.73 (s, 1 H), 1.65 (d, J = 3.6 Hz, 1 H), 1.23 (d, J = 7.3 Hz, 3 H), 1.06 (dd, J = 4.7, 12.6 Hz, 1 H). ^{13}C NMR (75MHz, $CDCl_3$) δ: 163.5, 158.7, 149.7, 144.6, 140.9, 135.6, 135.5, 130.0, 130.0, 129.1, 128.1, 128.0, 127.2, 113.3, 113.3, 113.2, 100.8, 89.6, 86.9, 84.3, 72.6, 60.3, 55.3, 50.1, 37.2, 28.5, 15.4. ESI-MS $[M+Na]^+$ calcd. 593.2; found 593.2. HRMS (QTOF), Calcd for $C_{33}H_{34}N_2O_7Na$, 593.2264; Found 593.2259. **24** ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.88–8.78 (m, 1 H), 8.22 (d, J = 8.1 Hz, 1 H), 7.49–7.40 (m, 2 H), 7.39–7.22 (m, 8 H), 6.86 (dd, J = 1.5, 8.9 Hz, 4 H), 5.53–5.45 (m, 2 H), 5.36 (s, 1 H), 4.12 (s, 1 H), 3.80 (s, 6 H), 3.66 (d, J = 11.5 Hz, 1 H), 3.39 (d, J = 11.3 Hz, 1 H), 2.38 (s, 1 H), 2.20–2.04 (m, 1 H), 1.88–1.76 (m, 1 H), 1.47 (d, J = 3.8 Hz, 1 H), 1.42 (dd, J = 5.7, 12.6 Hz, 1 H), 1.20 (d, J = 7.0 Hz, 3 H). ^{13}C NMR (75MHz, $CDCl_3$) δ: 161.3, 156.5, 147.8, 142.4, 138.6, 133.4, 133.3, 127.8, 126.9, 125.9, 125.8, 125.6, 125.6, 125.0, 111.1, 111.1, 111.0, 98.5, 87.4, 86.7, 84.8, 70.2, 58.0, 53.1, 48.1, 36.0, 31.4, 18.1. ESI-MS $[M+Na]^+$ calcd. 593.2; found 593.2. HRMS (QTOF), Calcd for $C_{33}H_{34}N_2O_7Na$, 593.2264; Found 593.2252.

Alternate preparation of 23 and 24. A solution of **21** (20 mg, 0.04 mmol) in methanol (3 mL) was hydrogenated using a hydrogen balloon and 10% palladium/charcoal (2 mg)

for 2 hours. The reaction was concentrated and purified by column chromatography (silica gel, eluting with 10 to 25% acetone in dichlorormethane) to provide **23** (8 mg, 40%) and **24** (10 mg, 50%). Analytical data was identical to that obtained above.

SI Scheme 3. Synthesis of Methylene-cLNA cytosine phosphoramidite



(1*R*,3*R*,4*R*,7*S*)-1-(4,4'-Dimethoxytritryloxymethyl)-5-methylene-7-(triethylsilyloxy)-3-(uracil-1-yl)-2-oxabicyclo[2.2.1]heptane (25). Triethylsilyl chloride (3.2 mmol, 0.5 mL) was added to a solution of **21** (1.6 mmol, 0.91 g) and imidazole (6.4 mmol, 0.44 g) in DMF (10 mL). After stirring at room temperature for 16 hours, the reaction was diluted with ethyl acetate and the organic layer was washed with water, brine, dried (Na_2SO_4) and concentrated. Purification by chromatography (silica gel, eluting with 40% ethyl acetate in hexanes) provided **25** (1.05 g, 96%). ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.79 (s, 1 H), 8.26 (d, J = 8.1 Hz, 1 H), 7.46–7.38 (m, 2 H), 7.36–7.23 (m, 8 H), 6.85 (dd, J = 2.9, 8.9 Hz, 4 H), 5.54 (dd, J = 1.9, 8.1 Hz, 1 H), 5.50 (s, 1 H), 5.29 (s, 1 H), 5.06 (s, 1 H), 4.41 (s, 1 H), 3.80 (s, 6 H), 3.53 (d, J = 11.1 Hz, 1 H), 3.31 (d, J = 10.7 Hz, 1 H), 3.09 (s, 1 H), 2.38 (d, J = 15.4 Hz, 1 H), 2.17 (d, J = 16.0 Hz, 1 H), 0.88–0.76 (m, 9 H), 0.55–0.44 (m, 6 H). ^{13}C NMR (75MHz, $CDCl_3$) δ: 163.4, 158.7, 158.7, 149.9, 144.4, 144.4,

140.5, 135.4, 135.3, 130.2, 130.2, 128.2, 127.9, 127.1, 113.2, 113.2, 111.1, 101.0, 90.5, 87.9, 86.7, 72.0, 60.1, 55.8, 55.3, 37.0, 6.6, 4.7. ESI-MS $[M+Na]^+$ calcd. 705.3; found 705.3.

(1*R*,3*R*,4*R*,7*S*)-3-(4-*N*-Benzoylcytosin-1-yl)-1-(4,4'-dimethoxytritryloxymethyl)-5-methylene-7-(triethylsilyloxy)-2-oxabicyclo[2.2.1]heptane (26). Phosphorus oxychloride (9.4 mmol, 0.9 mL) was added to a cold suspension of 1,2,4-triazole (39.7 mmol, 2.7 g) in acetonitrile (20 mL). After stirring for 15 minutes, triethylamine (46.7 mmol, 6.6 mL) was added to the reaction and the white suspension was stirred for another 30 minutes. A solution of **25** (1.2 mmol, 0.80 g) in acetonitrile (5 mL) was added to the reaction and the stirring was continued at room temperature for 5 hours. The reaction was then concentrated under reduced pressure and the residue was dissolved in ethyl acetate and the organic layer was washed with water, brine, dried (Na_2SO_4) and concentrated and the crude material was used in the next step without any purification.

Aqueous ammonia (0.2 mL) was added to a solution of the crude material obtained above in dioxane (5 mL). After stirring at room temperature for 4 hours, the reaction was evaporated to dryness and the residue was dried to provide the cytosine nucleoside.

Benzoic anhydride (1.6 mmol, 0.37 g) was added to a solution of the crude cytosine nucleoside from above in DMF (3.5 mL). After stirring at room temperature for 24 hours, the reaction was diluted with ethyl acetate and the organic layer was washed with water, brine, dried (Na_2SO_4) and concentrated. Purification by chromatography (silica gel, eluting with 3% methanol in dichloromethane) provided **26** (0.69, 75% over 3

steps). ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.72 (d, J = 7.7 Hz, 2 H), 7.91 (d, J = 7.3 Hz, 2 H), 7.65–7.28 (m, 14 H), 6.89 (d, J = 8.7 Hz, 4 H), 5.63 (s, 1 H), 5.42–5.34 (m, 1 H), 5.09 (s, 1 H), 4.39 (s, 1 H), 3.85 (s, 6 H), 3.58 (d, J = 10.0 Hz, 1 H), 3.37 (d, J = 10.7 Hz, 1 H), 3.28 (s, 1 H), 2.42 (d, J = 15.4 Hz, 1 H), 2.22 (d, J = 14.9 Hz, 1 H), 0.87–0.73 (m, 9 H), 0.56–0.38 (m, 6 H). ^{13}C NMR (75MHz, $CDCl_3$) δ: 162.3, 158.7, 144.5, 144.2, 135.6, 135.5, 133.1, 130.2, 130.1, 129.0, 128.4, 128.0, 127.5, 127.2, 113.3, 113.2, 111.3, 90.5, 88.7, 86.7, 71.8, 60.1, 55.3, 54.7, 37.1, 6.6, 4.7. ESI-MS $[M+Na]^+$ calcd. 808.3; found 808.3. HRMS (QTOF), Calcd for $C_{46}H_{52}N_3O_7Si$, 786.3575; Found 786.3577.

(1*R*,3*R*,4*R*,7*S*)-3-(4-*N*-Benzoylcytosin-1-yl)-1-(4,4'-dimethoxytritryloxymethyl)-7-hydroxy-5-methylene-2-oxabicyclo[2.2.1]heptane (27). Triethylamine trihydrofluoride (5.3 mmol, 0.9 mL) was added to a solution of **26** (0.9 mmol, 0.69 g) and triethylamine (2.2 mmol, 0.3 mL) in THF (9 mL). After stirring at room temperature for 16 hours the reaction was then diluted with ethyl acetate and the organic layer was washed with water, saturated sodium bicarbonate, brine, dried (Na_2SO_4) and concentrated. Purification by chromatography (silica gel, eluting with 10% methanol in dichloromethane) provided **27** (0.57 g, 96%). ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.68 (d, J = 7.5 Hz, 1 H), 7.73 (d, J = 7.3 Hz, 2 H), 7.60–7.47 (m, 3 H), 7.46–7.32 (m, 7 H), 6.89 (dd, J = 2.0, 8.9 Hz, 4 H), 5.58 (s, 1 H), 5.46 (s, 1 H), 5.16 (s, 1 H), 4.34 (s, 1 H), 3.83 (s, 6 H), 3.56 (d, J = 10.9 Hz, 1 H), 3.51 (d, J = 11.1 Hz, 1 H), 3.41 (s, 1 H), 2.43 (d, J = 15.8 Hz, 1 H), 2.27 (d, J = 16.2 Hz, 1 H). ^{13}C NMR (75MHz, $CDCl_3$) δ: 162.5, 158.8, 158.7, 145.6, 144.4, 143.9, 135.9, 135.7, 133.0, 130.1, 129.9, 128.9, 128.2, 128.1, 127.5, 127.2, 113.4, 112.5, 90.2, 88.5, 87.0, 71.4, 60.3, 55.3, 54.5, 36.8. ESI-MS $[M+H]^+$ calcd. 672.3; found 672.2. HRMS (QTOF), Calcd for $C_{40}H_{38}N_3O_7$, 672.2710; Found 672.2703.

(1*R*,3*R*,4*R*,7*S*)-3-(4-*N*-Benzoylcytosin-1-yl)-7-(Cyanoethyl-diisopropylaminophosphinoxy)-1-(4,4'-dimethoxytritryloxymethyl)-5-methylene-2-oxabicyclo[2.2.1]heptane (28). $(iPr_2N)_2POCH_2CH_2CN$ (1.2 mmol, 0.38 mL) was added to a solution of **27** (0.81 mmol, 0.54 g), *N*-methylimidazole (0.21 mmol, 0.016 g) and tetrazole (0.64 mmol, 0.045 g) in DMF (4 mL). After stirring at room temperature for 6 hours, the reaction was diluted with ethyl acetate and washed with saturated sodium bicarbonate, brine, dried (Na_2SO_4) and concentrated. Purification by chromatography (silica gel, eluting with 70 to 80% ethyl acetate in hexanes) provided **28** (0.62 g, 88%). ^{31}P NMR (121 MHz, $CDCl_3$) δ: 149.2, 148.9. ESI-MS $[M+Na]^+$ calcd. 872.4; found 872.3. HRMS (QTOF), Calcd for $C_{49}H_{56}N_5O_8P$, 873.3867; Found 873.3827.

SI Table 1. Analytical data for oligonucleotides

ASO	Modification	Backbone	Sequence (5' - 3')[a]	%UV Purity	Observed Mass (Da)	Calculated Mass (Da)
A1	DNA[a]	PO	d(GCGTTTTTTGCT)	>90	3632.9	3633.4
A2	Methylene-cLNA	PO	d(GCGTT**U**TTTGCT)	98.0	3656.7	3657.4
A3	*R*-Me-cLNA	PO	d(GCGTT**U**TTTGCT)	96.8	3658.8	3659.4
A4	*S*-Me-cLNA	PO	d(GCGTT**U**TTTGCT)	97.1	3658.8	3659.4
B1	DNA[a]	PO	d(CCAGTGATATGC)	>90	3645.2	3645.5
B2	Methylene-cLNA	PO	d(CCAG**U**GAUAUGC)	98.5	3717.0	3717.5
B3	*R*-Me-cLNA	PO	d(CCAG**U**GAUAUGC)	97.7	3723.0	3723.5
B4	LNA	PO	d(CCAG**U**GAUAUGC)	97.1	3686.8	3687.3
C1	Methylene-cLNA	PS	**CU**TAGCACTGGC**CU**	97.3	4547.5	4547.8
C2	*R*-Me-cLNA	PS	**CU**TAGCACTGGC**CU**	97.9	4554.9	4555.8
C3	LNA	PS	**^{m}CT**TAGCACTGGC**^{m}CT**	98.6	4563.0	4563.7
D1	Methylene-cLNA	PS	**CU**GCTAGCCTCTGGAT**UU**	97.2	5839.2	5839.8
D2	*R*-Me-cLNA	PS	**CU**GCTAGCCTCTGGAT**UU**	97.1	5846.8	5847.9
D3	LNA	PS	**^{m}CT**GCTAGCCTCTGGAT**TT**	97.4	5854.7	5855.7
E1	Methylene-cLNA	PO	GCGTA**U**ACGC	>95	3052.3	3051.6
E2	*R*-Me-cLNA	PO	GCGTA**U**ACGC	>95	3054.3	3053.4
E3	*S*-Me-cLNA	PO	GCGTA**U**ACGC	>95	3054.3	3053.3

[a] Oligonucleotides were purchased from commercial vendors and used as supplied.

Crystallization experiments

The three DNA decamers d(GCGTAU*ACGC) (**E1** U* = Methylene-cLNA-U **6**, **E2** *R*-Me-cLNA-U **4**, or **E3** *S*-Me-cLNA-U **5**) were synthesized as described previously[1] and purified by ion exchange chromatography. The stock concentrations for all three strands were adjusted to ca. 1.2 mM and crystallization trials were performed by the hanging drop vapor diffusion technique, using the 24 conditions of the Nucleic Acid Miniscreen (Hampton Research Inc., Aliso Viejo, CA).[5] The reservoir solution was 700 μL of a 35% v/v solution of 2-methyl-2,4-pentanediol (MPD). Droplets (1 μL) of modified DNA decamer solutions were mixed with droplets of equal volume of the individual sparse matrix screen solutions and equilibrated against MPD at room temperature. The optimal crystallization conditions for the three oligonucleotides were as follows. Vindec: condition 10, 40 mM sodium cacodylate, pH 6.0, 12 mM sodium chloride, 80 mM potassium chloride, 12 mM spermine tetrahydrochloride, and 10% v/v MPD. Rmedec: condition 15, 40 mM sodium cacodylate, pH 7.0, 80 mM potassium chloride, 12 mM spermine tetrahydrochloride, and 10% v/v MPD. Smedec: condition 10, 40 mM sodium cacodylate, pH 6.0, 12 mM sodium chloride, 80 mM potassium chloride, 12 mM spermine tetrahydrochloride, and 10% v/v MPD.

X-ray data collection, structure determination and refinement

Crystals were mounted in nylon loops, flash-frozen in liquid nitrogen without further cryoprotection and stored in liquid nitrogen prior to data collection. Data for Vindec and Smedec were collected on the 21-ID-F beam line of the Life Sciences Collaborative Access Team (LS-CAT) at the Advanced Photon Source (APS), located at Argonne National Laboratory (Argonne, IL) using a MARCCD 225 detector at a wavelength of 0.98 Å and the crystals were kept at 110

K during data collection. Data for Rmedec was collected on the 21-ID-D beamline of LS-CAT using a MARCCD 300 detector at a wavelength of 1.00 Å and the crystal was kept at 110 K during data collection. Diffraction data were integrated, scaled and merged with HKL2000[6]; selected crystal data and diffraction data statistics are listed in **Table 4**. The structures were determined by the molecular replacement method with the program Molrep[7] in the CCP4 suite of crystallographic software[8], using an A-form DNA as the search model (PDB ID 3EY2). Following initial positional and isotropic temperature factor refinement cycles with the program Refmac[9], the chemically modified uridines were built into Fourier ($2F_o$-F_c) sum and (F_o-F_c) difference electron density maps visualized either with Turbo-Frodo[10] or Coot.[11] Following adaptation of the dictionary files, refinement was continued and all nucleic acid atoms and solvent molecules (water as well as an MPD and a partial spermine molecule in the Vindec and Smedec structures, respectively) were treated with anisotropic B-factors.[12] Final refinement statistics are summarized in **Table 4**. Helical parameters were calculated with the program Curves[13] and the illustrations of the three base-pair steps shown in **Figure 3** were generated with the program UCSF Chimera.[14]

Data deposition

Atomic coordinates and structure factor data for the three crystal structures have been deposited in the Protein Data Bank (http://www.rcsb.org; awaiting entry code assignments).

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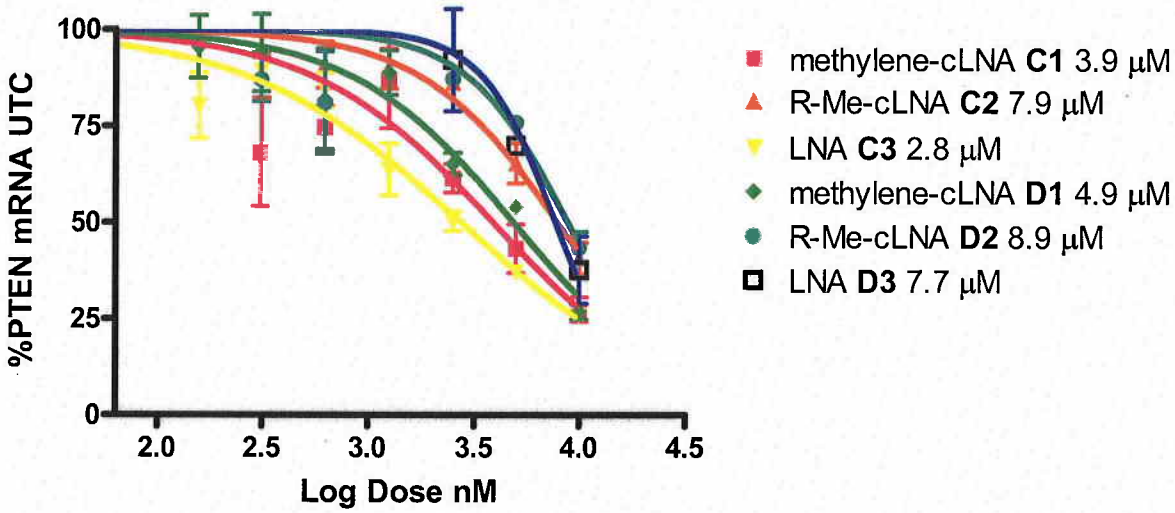
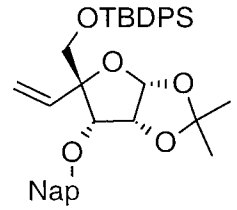
Dose response curves for reduction of PTEN mRNA using ASOs C1, C2, C3, D1, D2 and D3 in mouse brain endothelial (bEND) cells after electroporation.

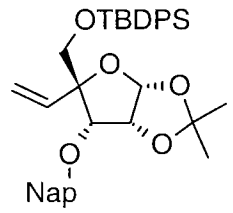


The activity of the lead oligonucleotides were confirmed with control oligonucleotides in the mouse bEND.3 cells line (ATCC) using electroporation mediated transfection. The cells were routinely cultured in DMEM high glucose medium supplemented with 10% FBS (growth medium) at 37°C and 5% CO_2. Cells were trypsinized for plating or subcultured when they reached 80% confluency. Breifly, cells were counted and diluted to 35,000 cells per ml in room temperature growth medium before adding 100μl of the cell suspension to the wells of a 2mm electroporation plate (Harvard Apparatus) which contained 11μl of 10X oligonucleotide in water. After shaking the plate for 30 seconds the cells were pulsed at 120V for 6mS with the ECM 830 instrument (Harvard Apparatus). After electroporating, the cells were transferred to a 96-well culture plate containing 50μl of growth medium and incubated at 37°C and 5% CO_2. After 16hrs, the cells were washed 1X with PBS before lysing for RNA isolation and target RNA quantitation. The RNA was purified with the Ambion Magmax kit and the target message was quantitated with real time RT-PCR on the StepOnePlus instrument (Applied Biosystems). The target message was normalized to total RNA (Ribogreen). Primers used for determination of pTEN RNA level are as follows: FP 5′ GCCACAGGCTCCCAGACAT3′, RP 5′ TCCATCCTCTTGATATCTCCTTTTG 3′, and PR 5′ 6FAM-ACAGCCATCATCAAAGAGATCGTTAGCAGAA-TAMRA 3′ (Eurogentec).


OTBDPS
O
O
O
O
O
Nap

10

3.9 13.0
0.9 0.9 1.0 1.0 0.9 0.9 0.9 0.9
1.9
3.4 2.9 8.9
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5
Chemical Shift (ppm)


OTBDPS
O
O
O
O
Nap

10

190
180
170
160
150
140
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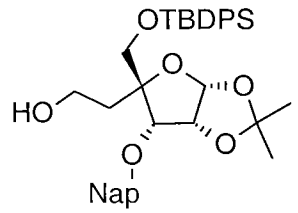
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)


OTBDPS
HO
O
O
O
O
Nap

11

3.9
13.0
0.9
0.9
1.9
0.9
2.9
0.9
0.9
0.9
1.2
3.2
2.9
8.8
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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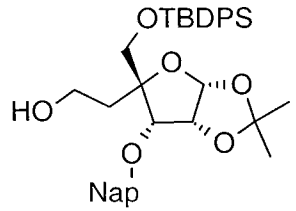
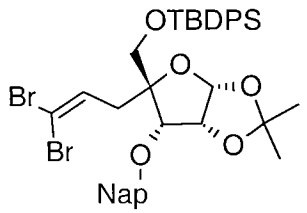
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
Chemical Shift (ppm)


OTBDPS
HO
O
O
O
O
Nap

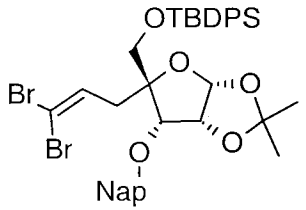
11

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)


OTBDPS
Br
Br
O
Nap
O
O
O

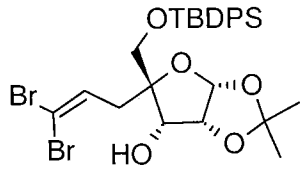
12

4.2
13.8
1.0
1.0
1.0
1.0
1.0
1.0
1.0
1.0
1.0
1.0
1.0
3.0
3.0
9.2
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
Chemical Shift (ppm)


OTBDPS
Br
Br
O
Nap
O
O
O

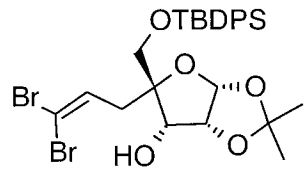
12

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)


OTBDPS
Br
Br
HO
O
O
O

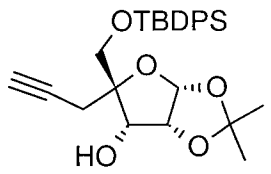
13

3.9 6.0
1.0
1.0
1.0
1.0
1.9
1.0 0.9 0.9
2.9 2.9
8.9
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5
Chemical Shift (ppm)


OTBDPS
Br
Br
HO
O
O
O

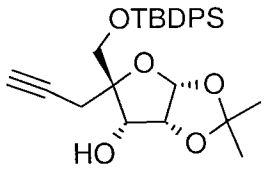
13

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)


OTBDPS
O
O
O
HO

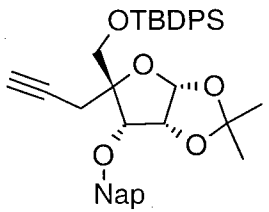
14

4.0 6.2
1.0
1.0 1.0
1.0 1.0
1.1 1.7
0.9 3.0 3.0 9.0
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5
Chemical Shift (ppm)


OTBDPS
O
O
O
HO

14

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)


OTBDPS
O
O
O
O
Nap

15

4.0
13.0
0.9
0.9
0.9
1.00
0.9
0.90
0.9
0.9
0.9
0.9
2.8
2.9
8.7
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
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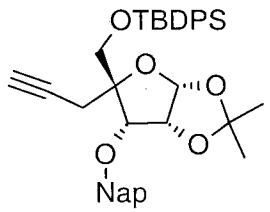
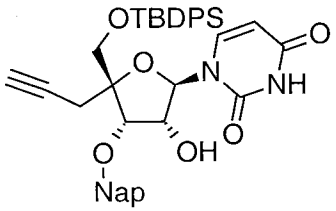
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
Chemical Shift (ppm)


OTBDPS
O
O
O
O
Nap

15

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)


OTBDPS
O
N
NH
O
O
OH
O
Nap

16

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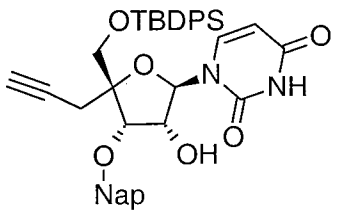
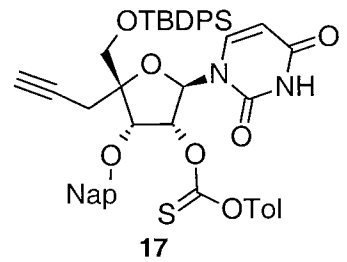
4.0
14.1
0.9
0.9
1.9
0.9
0.9
0.9
0.9
0.8
1.9
0.9
8.3
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5
Chemical Shift (ppm)


OTBDPS
O
N
NH
O
O
OH
O
Nap

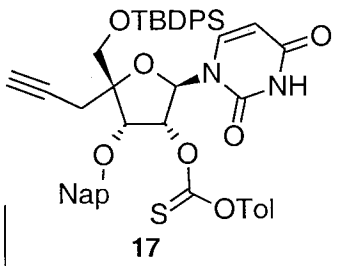
16

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)


OTBDPS
O
N
NH
O
O
O
Nap
O
S
OTol

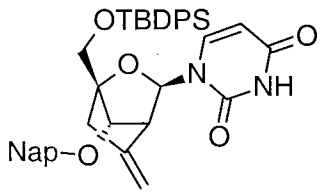
17

1.0
4.2
5.2
9.7
2.1
2.0
1.0
1.0
1.0
1.0
1.0
1.0
1.0
1.0
1.0
2.0
3.2
1.0
9.1
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
Chemical Shift (ppm)


OTBDPS
O
N
NH
O
O
Nap
O
S
OTol

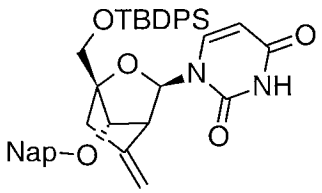
17

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)


OTBDPS
O
N
NH
O
O
Nap–O

18

0.7
0.8
3.2
5.4
10.0
0.8
1.7
0.8
0.9
1.0
0.8
0.9
1.0
0.8
0.9
1.0
9.1
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5
Chemical Shift (ppm)


OTBDPS
O
N
NH
O
O
Nap–O

18

210
200
190
180
170
160
150
140
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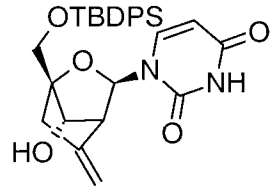
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)


OTBDPS
O
N
NH
O
O
HO

19

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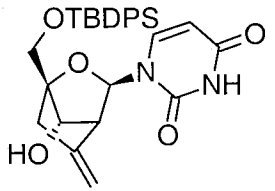
3.8
6.0
1.8
0.9
0.9
0.9
0.9
0.9
0.9
0.9
0.9
0.9
0.9
0.9
8.5
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5
Chemical Shift (ppm)


OTBDPS
O
N
NH
O
O
HO

19

210
200
190
180
170
160
150
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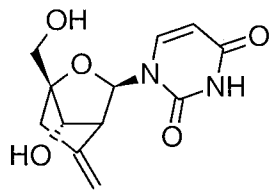
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)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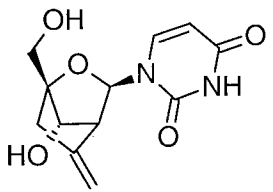
O
N
O
NH
O
HO

20

1.0
1.0 1.0 1.0 1.0
1.0 2.0
1.0
1.0 1.0
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5
Chemical Shift (ppm)


OH
O
N
NH
O
O
HO

20

210
200
190
180
170
160
150
140
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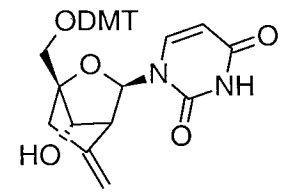
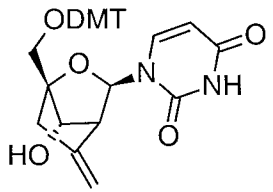
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)


ODMT
O
N
NH
O
O
HO

21

0.8 1.0 2.0 8.7 4.0 1.0 0.9 0.9 0.9 0.9 6.0 0.9 0.9 0.9 0.9 1.0 0.9
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5
Chemical Shift (ppm)


ODMT
O
N
NH
O
O
HO

21

210
200
190
180
170
160
150
140
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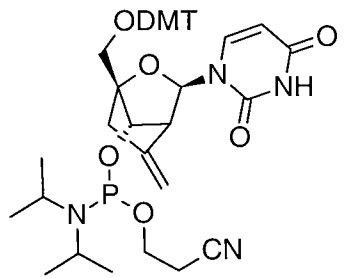
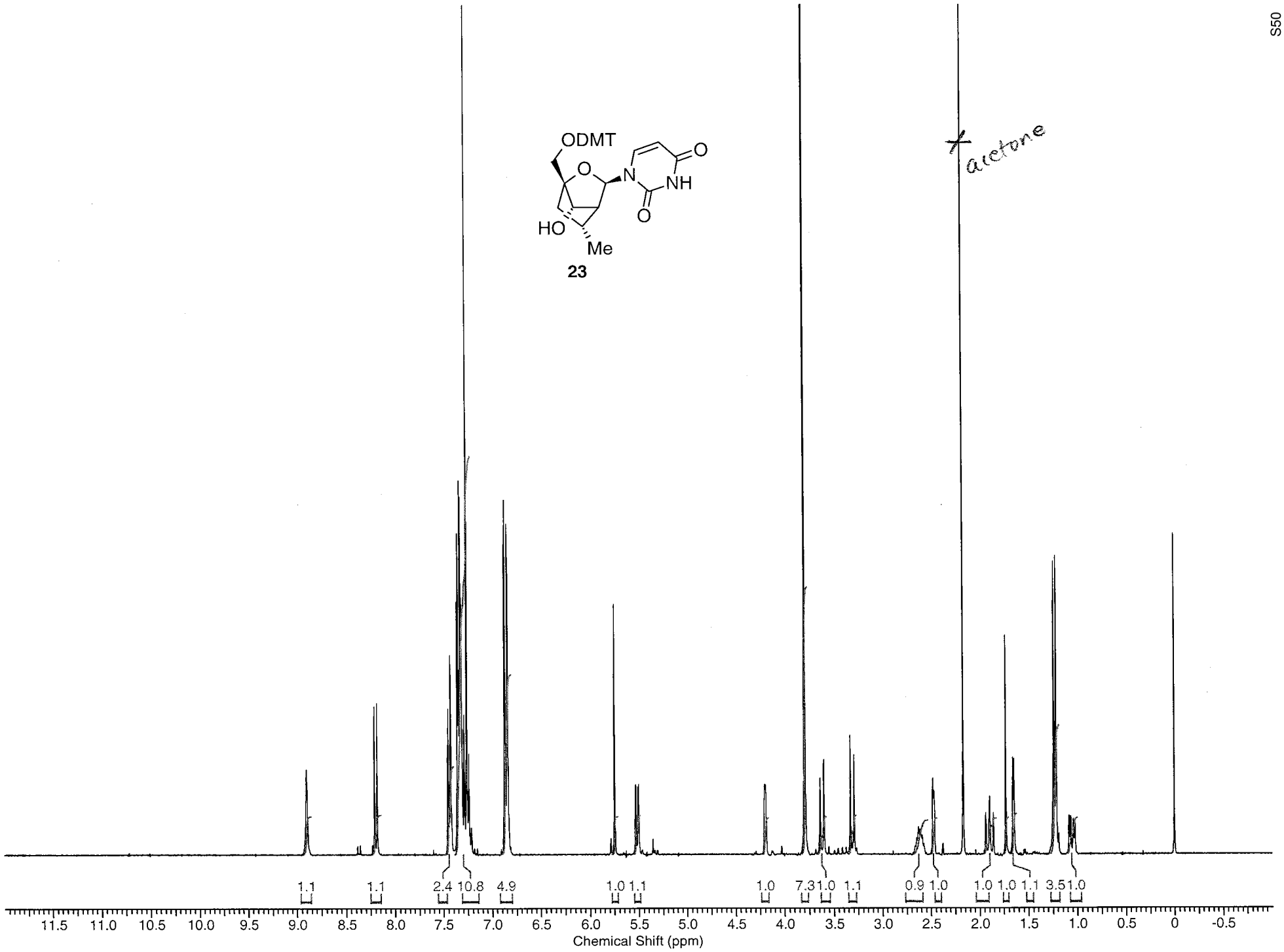
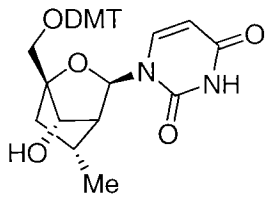
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)


ODMT
O
N
NH
O
O
O
P
N
O
CN

22

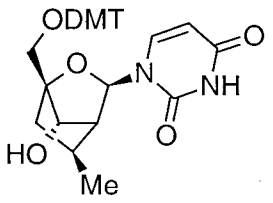
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)


ODMT
O
N
NH
O
O
HO
Me
23
acetone
1.1
1.1
2.4
10.8
4.9
1.0
1.1
1.0
7.3
1.0
1.1
0.9
1.0
1.0
1.0
1.1
3.5
1.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
Chemical Shift (ppm)


ODMT
O
N
NH
O
O
HO
Me

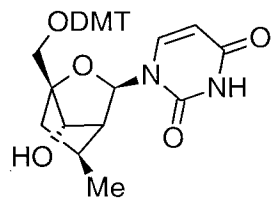
23

192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
-8
Chemical Shift (ppm)


ODMT
O
N
NH
O
O
HO
Me

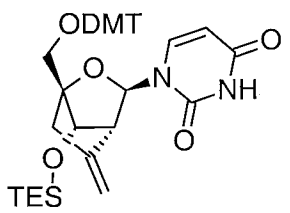
24

0.8 0.9 2.0 8.3 4.0 0.9 0.9 0.9 6.0 0.9 0.9 0.9 0.9 0.8 0.9 0.9 2.8
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5
Chemical Shift (ppm)


ODMT
O
N
NH
O
O
HO
Me

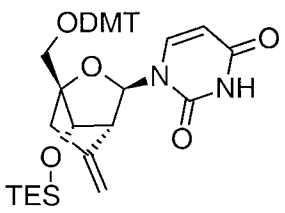
24

200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)


ODMT
O
N
NH
O
O
TES

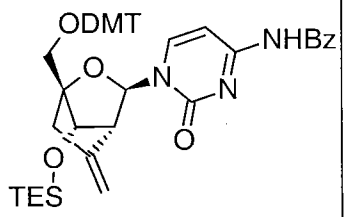
25

0.9
1.0
2.0
8.0
4.0
1.0
0.9
0.9
0.9
1.0
6.1
1.0
0.9
1.0
0.9
1.0
9.4
5.9
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
Chemical Shift (ppm)


ODMT
O
N
NH
O
O
TES

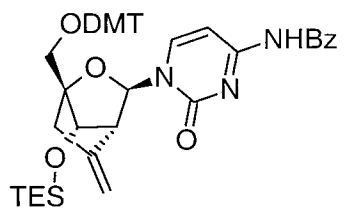
25

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)


ODMT
NHBz
O
N
N
O
O
TES

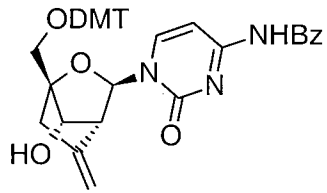
26

1.6
2.0
14.0
4.3
1.0
1.0
1.0
1.0
6.3
1.0
1.0
1.0
1.0
1.0
9.9
6.6
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5
Chemical Shift (ppm)


ODMT
NHBz
O
N
N
O
O
TES

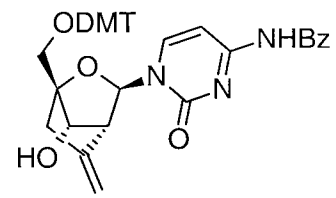
26

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)


ODMT
NHBz
O
N
N
O
HO

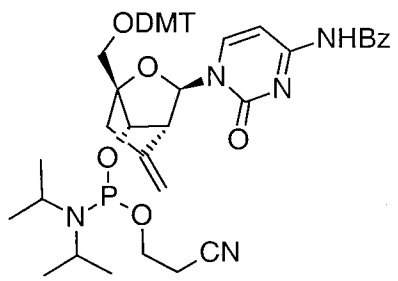
27

1.1
1.8
3.8
8.2
4.0
0.9
0.9
0.9
0.9
5.8
1.0
1.0
1.0
1.1
1.0
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5
Chemical Shift (ppm)


ODMT
NHBz
O
N
N
O
HO

27

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)


ODMT
NHBz
O
N
N
O
O
N
P
O
CN

28

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)