Conformation matters: siRNAs with antisense strands with 5'-(*E*)-vinyl-phosphonate-α-L-LNA elicit stronger RNAi-mediated gene silencing than those with 5'-(*E*)-vinyl-phosphonate-LNA

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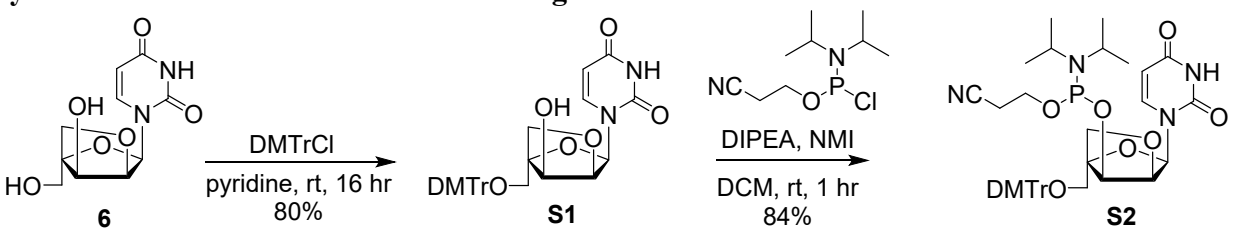
Supporting Information

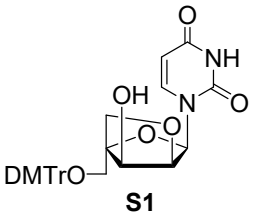
Experimental Section

General conditions: TLC was performed on Merck silica gel 60 plates coated with F254. Compounds were visualized under UV light (254 nm) or after spraying with the p-anisaldehyde staining solution followed by heating. Flash column chromatography was performed using a Teledyne ISCO Combi Flash system with pre-packed RediSep Flash-Prep-HPLC (IntelFlash-1) C18 Column packed with silica gel. All moisture-sensitive reactions were carried out under anhydrous conditions using dry glassware, anhydrous solvents, and argon atmosphere. All commercially available reagents and solvents were purchased from Sigma-Aldrich unless otherwise stated and were used as received. ESI-MS spectra were recorded on a Waters QTof Premier instrument using the direct flow injection mode. ^{1}H NMR spectra were recorded at 300, 400, 500 and 600 MHz. ^{13}C NMR spectra were recorded at 101 and 151 MHz. ^{31}P NMR spectra were recorded at 161, 202 and 243 MHz. Chemical shifts are given in ppm referenced to the solvent residual peak (DMSO-d_6 – ^{1}H: δ at 2.50 ppm and ^{13}C δ at 39.5 ppm; $CDCl_3$ – ^{1}H: δ at 7.26 ppm and ^{13}C δ at 77.16 ppm; CD_3CN – ^{1}H: δ at 1.94 ppm and ^{13}C δ at 1.32 ppm respectively). Coupling constants are given in Hertz. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), septet (sept), broad signal (brs), or multiplet (m). ^{31}P NMR spectra were recorded under proton-decoupled mode.

Synthesis and characterization of building blocks

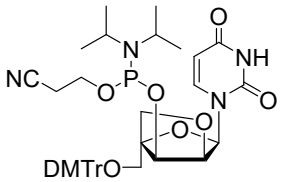


Scheme S1: Synthesis of phosphoramidite **S2** for building block **VIII**



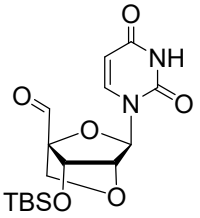
1-((1S,3R,4S,7R)-1-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptan-3-yl)pyrimidine-2,4(1H,3H)-dione **S1**: To a clear solution of **6**[1] (0.4 g, 1.56 mmol) in pyridine (10 mL) was added 4,4′-dimethoxytrityl chloride (634.78 mg, 1.87 mmol) in two portions. Reaction mixture was stirred at for 16 hr, diluted with DCM (20 mL) and then quenched with 10% $NaHCO_3$ (20 mL). Organic layer was washed with brine (2 x 20 mL), separated, dried over anhydrous Na_2SO_4 and filtered. Filtrate was evaporated under high vacuum pump and crude mass obtained, was purified by flash column chromatography (gradient: 0-5% MeOH in DCM) to afford **S1** (0.7 g, 80% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.40 (s, 1H), 7.81 (d, J = 8.1 Hz, 1H), 7.42 (dd, J = 8.2, 1.4 Hz, 2H), 7.36 – 7.18 (m, 7H), 6.95 – 6.88 (m, 4H), 5.99 (s, 1H), 5.95 (d, J = 4.4 Hz, 1H), 5.69 (dd, J = 8.1, 1.4 Hz, 1H), 4.41 (d, J = 4.4 Hz, 1H), 4.23 (s, 1H), 4.12 (d, J = 8.4 Hz, 1H), 3.94 (d, J = 8.4 Hz, 1H), 3.74 (s, 6H), 3.35 – 3.27 (m, 2H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.3, 158.1, 150.4, 144.7, 140.5, 135.3, 135.2, 129.8, 129.8,

127.9, 127.7, 126.7, 113.3, 100.5, 89.2, 86.6, 85.4, 78.7, 72.9, 72.2, 60.1, 59.8, 55.0 ppm. HRMS (ESI^+) m/z calcd for $C_{31}H_{31}N_2O_8$ $[M + H]^+$ 559.2080, found 559.2089.



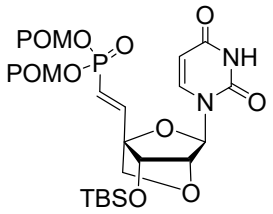
S2

3-[[(4S,6R)-4-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-6-(2,4-dioxopyrimidin-1-yl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl]oxy-(diisopropylamino)phosphanyl]oxypropanenitrile:
Compound **S1** (0.7 g, 1.12 mmol) was dissolved in dichloromethane (DCM) (10 mL) and the resultant clear solution was added N-methyl imidazole (NMI) (256.35 mg, 3.12 mmol, 248.88 µL) and diisopropylethylamine (DIPEA) (1.01 g, 7.81 mmol, 1.36 mL) in single portions. After stirring the reaction mixture for 5 minutes at 22 °C, 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (739.01 mg, 3.12 mmol, 697.18 µL) was added and continued stirring for 1 hr and TLC was checked. Starting material was consumed and reaction mixture was diluted with DCM (15 mL). DCM layer was washed with 10% $NaHCO_3$ (2 x 25 mL) solution, and brine (30 mL). Organic layer was separated, dried over anhydrous Na_2SO_4, filtered and filtrate was evaporated at 36°C to afford crude compound which was purified by flash chromatography (gradient: 35-80% EtOAc in hexane) to obtain **S2** (0.8 g, 84%) as white foam. ^1H NMR (500 MHz, CD_3CN) δ 9.16 (s, 1H), 7.80 (dd, *J* = 8.2, 3.4 Hz, 1H), 7.50 – 7.43 (m, 2H), 7.38 – 7.27 (m, 7H), 7.23 (ddt, *J* = 9.3, 7.1, 2.9 Hz, 1H), 6.91 – 6.82 (m, 4H), 6.03 (t, *J* = 1.1 Hz, 1H), 5.68 (dd, *J* = 8.2, 2.7 Hz, 1H), 4.70 – 4.49 (m, 1H), 4.48 (t, *J* = 1.3 Hz, 1H), 4.22 – 4.11 (m, 1H), 4.01 – 3.87 (m, 1H), 3.82 – 3.68 (m, 8H), 3.65 – 3.46 (m, 3H), 3.44 – 3.35 (m, 2H), 2.62 (t, *J* = 6.0 Hz, 1H), 2.47 (t, *J* = 6.0 Hz, 1H), 1.15 – 1.06 (m, 11H), 0.98 (d, *J* = 6.8 Hz, 2H) ppm. ^{13}C NMR (101 MHz, CD_3CN) δ 164.1, 159.7, 159.7, 151.4, 145.9, 145.9, 141.6, 141.5, 136.6, 136.6, 136.5, 136.4, 131.1, 131.1, 131.0, 129.0, 128.9, 128.9, 127.9, 127.9, 119.5, 119.3, 114.1, 114.1, 90.6, 90.6, 90.4, 90.38, 88.2, 88.2, 87.0, 86.9, 79.4, 79.4, 79.0, 78.9, 75.8, 75.7, 75.3, 75.1, 74.1, 74.0, 61.2, 61.0, 61.0, 59.68, 59.5, 59.5, 59.3, 55.9, 55.9, 55.3, 44.2, 44.2, 44.1, 44.1, 25.0, 24.9, 24.9, 24.8, 24.7, 21.1, 21.0, 20.9, 20.9, 20.9 ppm. ^{31}P NMR (202 MHz, CD_3CN) δ 151.02, 150.40 ppm. HRMS (ESI^+) m/z calcd for $C_{40}H_{48}N_4O_9P$ $[M + H]^+$ 759.3159, found 759.3164.

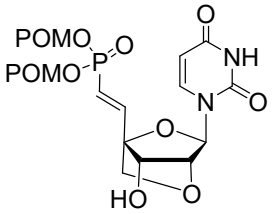


***(1S,3R)-7-[(tert-butyldimethylsilyl)oxy]-3-(2,4-dioxo-3H-pyrimidin-1-yl)-2,5-dioxabicyclo [2.2.1]heptane-1-carbaldehyde* 9**: To a clear solution of commercially available **1**[2] (20 g, 53.984 mmol) in EtOAc (600.0 mL) at 0 °C was added a solution of Dess-Martin periodinane (114.49 g, 269.922 mmol) in DMSO (300 mL) slowly. The resulting solution was stirred for 8 hr at 22 °C. The reaction mixture was then added to 10% sodium hyposulfite solution (1500 mL). The resulting mixture was extracted with ethyl acetate (3 x 1000 mL). The combined organic layers were washed with water (2 x 1000 mL) and brine (2 x1000 mL). The organic layer was separated, dried over

anhydrous Na_2SO_4 and concentrated to afford **2** (20 g, crude, quantitative) of as a white solid which was used for the next step without further purification.



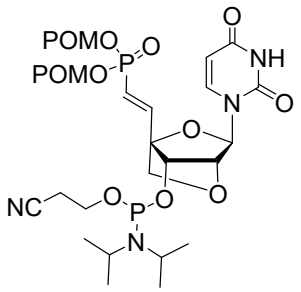
[[(E)-2-[(1S,3R)-7-[(tert-butyldimethylsilyl)oxy]-3-(2,4-dioxo-3H-pyrimidin-1-yl)-2,5-dioxabicyclo[2.2.1]heptan-1-yl]ethenyl([(2,2-dimethylpropanoyl)oxy]methoxy)phosphoryl] oxy]methyl 2,2-dimethylpropanoate **3:** To a mixture of **2** (20.0 g, 54.28 mmol) and potassium carbonate (22.51 g, 162.839 mmol) in DMF (200 mL) was added bis-POM reagent (24.01 g, 37.991 mmol) at 0°C. The resulting solution was stirred overnight at 22 °C. LCMS analysis confirmed the formation of the product (E/Z=4:1). The reaction was then quenched by the addition of saturated NH_4Cl (500 mL) at 0°C and then extracted with EtOAc (3 x 200 mL). Combined organic layer was washed with water (2 x 200 mL) and brine (2 x 200 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated. The residue was purified by flash-prep-HPLC [(CombiFlash-1): Column, C18 silica gel; mobile phase, ACN/H_2O=50/50 increasing to ACN/H_2O=95/5 within 30 min] to afford **3** (8.5 g, 23% yield) of as a oil and corresponding Z-isomer **3Z** (1.5g, 4% yield).
Data for **3**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.39 (s, 1H), 7.65 (d, J = 8.1 Hz, 1H), 6.87 (dd, J = 24.6, 17.5 Hz, 1H), 6.27 (dd, J = 20.9, 17.5 Hz, 1H), 5.69 – 5.40 (m, 6H), 4.40 (s, 1H), 4.16 – 3.98 (m, 2H), 3.73 (d, J = 8.1 Hz, 1H), 1.16 (d, J = 1.8 Hz, 18H), 0.85 (s, 9H), 0.07 (d, J = 3.5 Hz, 6H) ppm. HRMS (ESI$^+$) m/z calcd for $C_{29}H_{48}N_2O_{12}PSi$ [M + H]$^+$ 675.2714, found 675.2709.
HRMS (ESI$^+$) m/z calcd for $C_{24}H_{40}N_7O_4Si$ [M + H]$^+$ 518.2911, found 518.2923.
Data for **3Z**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.38 (d, J = 2.2 Hz, 1H), 7.86 (d, J = 8.1 Hz, 1H), 6.61 (dd, J = 52.7, 14.5 Hz, 1H), 6.18 (dd, J = 18.3, 14.4 Hz, 1H), 5.70 – 5.50 (m, 6H), 4.39 (s, 1H), 4.12 (s, 1H), 4.02 (dd, J = 14.8, 7.6 Hz, 1H), 3.89 (d, J = 8.0 Hz, 1H), 1.17 (d, J = 1.4 Hz, 18H), 0.87 (s, 9H), 0.10 (d, J = 2.4 Hz, 6H) ppm. HRMS (ESI$^+$) m/z calcd for $C_{29}H_{48}N_2O_{12}PSi$ [M + H]$^+$ 675.2714, found 675.2711.



([[(2,2-dimethylpropanoyl)oxy]methoxy((E)-2-[(1S,3R)-3-(2,4-dioxo-3H-pyrimidin-1-yl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptan-1-yl]ethenyl)phosphoryl]oxy)methyl-2,2-dimethylpropanoate **4:** Compound **3** (10.30 g)was suspended in 50% aqueous formic acid (500.00 mL) at 0°C. The resulting solution was stirred for 36 h at 35°C in an oil bath. The pH of the solution was adjusted to 7 with saturated aqueous sodium bicarbonate at 0°C. The resulting solution was extracted with ethyl acetate (3 x 800 mL) and the combined organic layer washed with water (2 x 200 mL) and brine (2 x 200 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated. The residue was purified by flash-prep-HPLC [(CombiFlash-1): Column, C18 silica gel; mobile phase, ACN/H_2O=15/85 increasing to

ACN/H_2O=95/5 within 30 min] to afford **4** (4.7 g, 55% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.40 (s, 1H), 7.62 (d, *J* = 8.1 Hz, 1H), 6.92 (dd, *J* = 24.6, 17.5 Hz, 1H), 6.41 – 6.09 (m, 2H), 5.77 – 5.47 (m, 6H), 4.37 (s, 1H), 4.20 – 4.04 (m,1H), 3.92 (d, *J* = 4.6 Hz, 1H), 3.72 (d, *J* = 8.0 Hz, 1H), 1.18 (s, 18H) ppm. HRMS (ESI$^+$) m/z calcd for $C_{23}H_{34}N_2O_{12}P$ [M + H]$^+$ 561.1849, found 561.1851.

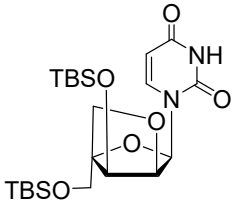
***[[[(2,2-dimethylpropanoyl)oxy]methoxy((Z)-2-[(1S,3R)-3-(2,4-dioxo-3H-pyrimidin-1-yl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptan-1-yl]ethenyl)phosphoryl]oxy)methyl-2,2-dimethylpropanoate* 4Z**: Into a 250-mL round-bottom flask purged and maintained with an inert atmosphere of argon, was placed **3Z** (1.70 g).This was followed by the addition of 50% formic acid(80.00 mL) at 22 °C. The resulting solution was stirred for 40 h at 35 °C in an oil bath. The pH was adjusted to 7 with saturated aqueous sodium bicarbonate at 0°C. The resulting solution was extracted with 3x150 mL of ethylacetate and organic layers combined. The organic solution was washed with 2x100 ml of water and 2x100 mL of brine. The solution was dried over anhydrous sodium sulfate and concentrated. The residue was purified by Flash-Prep-HPLC [(CombiFlash-1): Column, C18 silica gel; mobile phase, ACN/H2O=5/95 increasing to ACN/H_2O=95/5 within 30 min] to afford **4Z** (0.52 g, % yield) of as a solid. ^{1}H NMR (300 MHz, CD_3CN) δ 9.11 (s, 1H), 8.13 – 7.84 (m, 1H), 6.79 – 6.46 (m, 1H), 6.09 (m, 1H), 5.63 (m, 6H), 4.39 (s, 1H), 4.24 – 4.08 (m, 1H), 4.03 (s, 1H), 3.95 (d, *J* = 8.5 Hz, 1H), 1.23 (p, *J* = 1.9 Hz, 18H) ppm. HRMS (ESI$^+$) m/z calcd for $C_{23}H_{34}N_2O_{12}P$ [M + H]$^+$ 561.1849, found 561.1844.



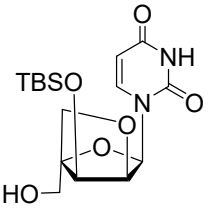
[[(E)-2-[(1S,3R)-7-[[(2-cyanoethoxy)(diisopropylamino)phosphanyl]oxy]-3-(2,4-dioxo-3H-pyrimidin-1-yl)-2,5-dioxabicyclo[2.2.1]heptan-1-yl]ethenyl([(2,2-dimethylpropanoyl)oxy]methoxy)phosphoryl]oxy]methyl-2,2-dimethylpropanoate **5:** To a solution of 3-[[bis(diisopropylamino)phosphanyl]oxy]propanenitrile (4.62 g, 15.344 mmol) in DCM (43.00 mL) was added a solution of *1H*-imidazole-4,5-dicarbonitrile (DCI) (1.36 g, 11.508 mmol) at 22 °C. After stirring for 10 min, a solution of **4** (4.30 g, 7.672 mmol) was added at 22 °C. The resulting solution was stirred for 1 hr at 22 °C. The reacting solution was diluted with DCM (300 mL) and washed with saturated aqueous $NaHCO_3$ (100 mL) and brine (200 mL). The organic layer was dried over anhydrous Na_2SO_4 filtered and the filtrate was concentrated. The residue was purified by flash-prep-HPLC [(CombiFlash-1): Column, C18 silica gel; mobile phase, ACN/H_2O=20/80 increasing to ACN/H_2O=95/5 within 40 min] to afford **5** (5.4 g, 93% yield) as a solid. ^{1}H NMR (600 MHz, CD_3CN) δ 9.04 (s, 1H), 7.51 (dd, *J* = 8.2, 4.3 Hz, 1H), 7.04 – 6.85 (m, 1H), 6.34 – 6.23 (m, 1H), 5.68 – 5.56 (m, 6H), 4.52 (d, *J* = 11.7 Hz, 1H), 4.18 – 4.04 (m, 2H), 3.87 – 3.77 (m, 2H), 3.74 (dtd, *J* = 10.7, 5.4, 2.4 Hz, 1H), 3.62 (tdd, *J* = 11.3, 8.9, 5.7 Hz, 2H), 2.77 – 2.61 (m, 2H), 1.26 – 1.12 (m, 31H) ppm. ^{13}C NMR (151 MHz, CD_3CN) δ 177.6, 177.6, 177.5, 163.9, 150.9, 143.7, 143.7, 143.3, 143.2, 139.9, 139.8, 122.0, 121.6, 120.7, 120.4, 119.6, 119.5, 102.4, 102.3, 88.7, 88.6, 87.6, 87.6, 87.5, 87.4, 87.4, 87.4, 87.2, 87.2, 82.8, 82.7, 82.7, 82.7, 80.9, 80.9, 80.6, 80.6, 76.4, 76.4, 76.2, 76.1, 74.5, 74.3, 59.6, 59.5, 59.4, 59.4, 44.2, 44.2, 44.2, 44.1, 39.4, 39.4,

27.1, 27.1, 25.0, 24.9, 24.8, 24.8, 24.8, 24.7, 21.0, 20.9 ppm. ^{31}P NMR (243 MHz, CD_3CN) δ 149.45, 149.31, 15.80, 15.49 ppm. HRMS (ESI^+) m/z calcd for $C_{32}H_{51}N_4O_{13}P_2$ $[M + H]^+$ 761.2928, found 761.2916.

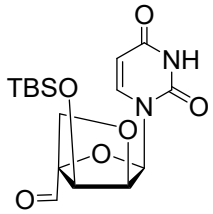
[[(Z)-2-[(1S,3R)-7-[[(2-cyanoethoxy)(diisopropylamino)phosphanyl]oxy]-3-(2,4-dioxo-3H-pyrimidin-1-yl)-2,5-dioxabicyclo[2.2.1]heptan-1-yl]ethenyl([(2,2-dimethylpropanoyl)oxy]methoxy)phosphoryl]oxy]methyl 2,2-dimethylpropanoate* 5Z*: To round-bottom under an inert atmosphere of argon, was placed 3-[[bis(diisopropylamino)phosphanyl]oxy]propanenitrile (537.03 mg, 1.784 mmol) and DCI (126.32 mg, 1.070 mmol) in DCM (5.00 mL) at 22 °C. After 10 min to this reaction mixture was added **4Z** (500.00 mg, 0.892 mmol, 1.00 equiv) at 22 °C. The resulting solution was stirred for 1 hr at 22 °C. The reacting solution was diluted with 300 mL of DCM and washed with 2x150 ml of water and 2x150 mL of brine. The solution was dried over anhydrous sodium sulfate and concentrated. The residue was purified by Flash-Prep-HPLC [(CombiFlash-1): Column, C18 silica gel; mobile phase, ACN/H_2O=20/80 increasing to ACN/H_2O=95/5 within 48 min] to afford **5Z** (0.230 g, 34.0% yield) as a white solid. ^{1}H NMR (600 MHz, CD_3CN) δ 9.07 – 8.89 (m, 1H), 7.97 (dd, *J* = 9.3, 8.2 Hz, 1H), 6.82 – 6.49 (m, 1H), 6.09 (ddt, *J* = 17.4, 14.5, 1.4 Hz, 1H), 5.66 – 5.53 (m, 6H), 4.54 (d, *J* = 17.5 Hz, 1H), 4.13 – 4.07 (m, 1H), 4.05 (dd, *J* = 8.2, 2.6 Hz, 1H), 3.99 (dd, *J* = 8.2, 4.4 Hz, 1H), 3.62 (dh, *J* = 10.4, 6.7 Hz, 2H), 2.64 (dt, *J* = 9.7, 5.9 Hz, 2H), 1.22 – 1.18 (m, 19H), 1.18 – 1.13 (m, 14H) ppm. ^{13}C NMR (151 MHz, CD_3CN) δ 177.7, 177.7, 177.7, 163.9, 151.0, 141.7, 141.35, 141.0, 140.9, 119.5, 119.5, 102.0, 102.0, 89.4, 89.3, 87.7, 87.6, 87.6, 87.4, 87.4, 87.4, 83.2, 83.2, 82.7, 82.7, 82.7, 80.0, 80.0, 79.9, 79.8, 76.2, 76.1, 76.0, 75.9, 74.1, 74.0, 59.7, 59.6, 59.5, 59.5, 44.2, 44.2, 44.2, 44.1, 39.4, 27.10, 24.85, 24.83, 24.80, 24.78, 24.75, 24.74, 24.68, 21.03, 20.98, 20.93 ppm. ^{31}P NMR (243 MHz, CD_3CN) δ 149.60, 149.16, 13.96, 13.76 ppm. HRMS (ESI^+) m/z calcd for $C_{32}H_{51}N_4O_{13}P_2$ $[M + H]^+$ 761.2928, found 761.2922.



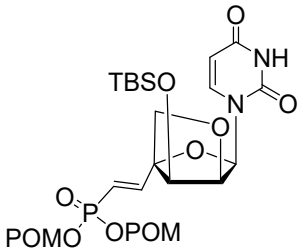
***1-[(1S,3R)-7-[(tert-butyldimethylsilyl)oxy]-1-[[(tert-butyldimethylsilyl)oxy]methyl]-2,5-dioxabicyclo[2.2.1]heptan-3-yl]-3H-pyrimidine-2,4-dione* 7**: To a clear solution of **6**[1] (1.90 g, 7.416 mmol) in DMF (4.0 mL) was added imidazole (1.77 g, 25.955 mmol) at 22 °C. After stirring for 5 min, *tert*-butyldimethylsilyl chloride (TBSCl) (2.79 g, 18.51 mmol) was added at 22 °C. The resulting solution was stirred overnight at 22 °C. The reaction was then quenched by the addition of saturated $NaHCO_3$ solution (100 mL). After stirring for 10 min, the resulting solution was extracted with EtOAc (2 x 100 mL). The organic layer was washed with water (100 mL) and brine (100 mL). The organic solution was separated, dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under high vacuum pump. The residue thus obtained was purified by flash column chromatography (gradient: 30-75% EtOAc in hexane) to afford **7** (3.11 g, 87% yield) of as a white foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.36 (d, *J* = 2.3 Hz, 1H), 7.73 (d, *J* = 8.1 Hz, 1H), 5.93 (s, 1H), 5.62 (dd, *J* = 8.1, 2.1 Hz, 1H), 4.49 (s, 1H), 4.21 (s, 1H), 3.87 (d, *J* = 9.3 Hz, 4H), 0.87 (d, *J* = 1.5 Hz, 18H), 0.13 (d, *J* = 3.7 Hz, 6H), 0.05 (s, 6H) ppm. HRMS (ESI^+) m/z calcd for $C_{22}H_{41}N_2O_6Si_2$ $[M + H]^+$ 485.2503, found 485.2500.



***1-[(1R,3R)-7-[(tert-butyldimethylsilyl)oxy]-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]heptan-3-yl]-3H-pyrimidine-2,4-dione* 8:** To a clear solution of **7** (2.80 g, 5.776 mmol) in THF (30 mL) under inert atmosphere was added 50% aqueous TFA (15 mL) dropwise with stirring at 0°C. The resulting solution was stirred for 2 hr at 0 °C. The reaction was then quenched by the addition of saturated $NaHCO_3$ solution (200 mL) at 0 °C. The resulting mixture was extracted with EtOAc (3 x 200 mL). The organic layer was washed with water (100 mL) and brine (100 mL). The organic solution was separated, dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under high vacuum pump. The residue thus obtained was purified by flash column chromatography (gradient: 0-5% MeOH in DCM) to afford **8** (1.68 g, 79% yield) of as a white foam. ^{1}H NMR: (300 MHz, DMSO-d_6) δ 11.40 (d, J = 2.3 Hz, 1H), 7.81 (d, J = 8.1 Hz, 1H), 5.95 (s, 1H), 5.65 (dd, J = 8.1, 2.1 Hz, 1H), 5.02 (s, 1H), 4.50 (s, 1H), 4.22 (s, 1H), 4.02 – 3.86 (m, 2H), 3.70 (s, 2H), 0.90 (s, 9H), 0.15 (d, J = 2.3 Hz, 6H) ppm. HRMS (ESI$^+$) m/z calcd for $C_{16}H_{27}N_2O_6Si$ $[M + H]^+$ 371.1638, found 371.1641.

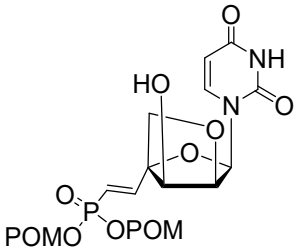


***(1R,3R)-7-[(tert-butyldimethylsilyl)oxy]-3-(2,4-dioxo-3H-pyrimidin-1-yl)-2,5dioxabicyclo [2.2.1]heptane-1-carbaldehyde* 9**: To a solution of **8** (1.70 g, 4.589 mmol) in anhydrous EtOAc (75 mL) under inert atmosphere was added Dess-Martin periodinane (5.84 g, 13.769 mmol) in DMSO (15 mL) dropwise at 0 °C .The resulting solution was stirred for 3 hr at 0 °C. To the reaction mixture was then added 10% aqueous sodium hyposulfite (200 mL) and was extracted with EtOAc (3 x 200 mL). The organic solution was separated, dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under high vacuum pump to afford **9** (1.7 g, crude, quantitative) as white solid which was used for the next step without further purification.



***[(E)-2-[(1R,3R)-7-[(tert-butyldimethylsilyl)oxy]-3-(2,4-dioxo-3H-pyrimidin-1-yl)-2,5dioxabicyclo[2.2.1]heptan-1-yl]ethenyl([(2,2-dimethylpropanoyl)oxy]methoxy)phosphoryl]oxy]methyl 2,2-dimethylpropanoate* 10**: To a suspension of **9** (1.80 g, 4.885 mmol) in DMF (30 mL) at 0 °C was added potassium carbonate (2.03 g, 14.656 mmol). To this reaction mixture was added bis-POM reagent (3.71 g, 5.862 mmol) at 0°C. The resulting solution was stirred overnight at 22 °C.

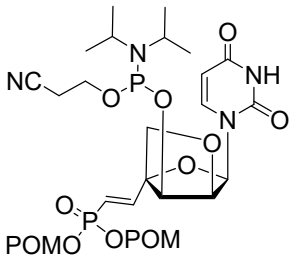
LCMS analysis confirmed the formation of the product (E/Z=4:1). The reaction was then quenched by the addition of saturated ammonium chloride (200 mL) at 0 °C. The resulting solution was extracted with of ethyl acetate (200 mL). The organic layer was washed with water (100 mL) and brine (100 mL). The organic solution was separated, dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under high vacuum pump. The residue was purified by Flash-Prep-HPLC with the following conditions (IntelFlash-1): Column, C18 silica gel; mobile phase, ACN/H_2O=10:90 increasing to ACN/H_2O=95:5 within 60 min. This resulted in major E-isomer **10** (1.5 g, 45% yield) as a white solid along with the Z-isomer **10Z** (0.3 g, 9% yield) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.44 (s, 1H), 7.81 (d, J = 8.2 Hz, 1H), 6.74 (dd, J = 24.4, 17.4 Hz, 1H), 6.23 (dd, J = 21.4, 17.4 Hz, 1H), 6.10 (s, 1H), 5.70 – 5.57 (m, 5H), 4.62 (s, 1H), 4.37 (s, 1H), 4.06 (dd, J = 42.2, 8.6 Hz, 2H), 1.19 (d, J = 5.6 Hz, 18H), 0.89 (s, 9H), 0.14 (d, J = 3.1 Hz, 6H) ppm. HRMS (ESI$^+$) m/z calcd for $C_{29}H_{48}N_2O_{12}PSi$ [M + H]$^+$ 675.2714, found 675.2724.
Data for **10Z**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.41 (d, J = 2.2 Hz, 1H), 8.25 (d, J = 8.1 Hz, 1H), 6.68 (dd, J = 52.8, 14.1 Hz, 1H), 6.20 (dd, J = 17.9, 14.1 Hz, 1H), 6.00 (s, 1H), 5.66 – 5.51 (m, 5H), 4.74 (s, 1H), 4.33 (s, 1H), 4.16 (d, J = 8.4 Hz, 1H), 3.98 (d, J = 8.4 Hz, 1H), 1.15 (d, J = 1.1 Hz, 18H), 0.88 (s, 9H), 0.15 (d, J = 3.1 Hz, 6H) ppm. HRMS (ESI$^+$) m/z calcd for $C_{29}H_{48}N_2O_{12}PSi$ [M + H]$^+$ 675.2714, found 675.2734.



(2,2-dimethylpropanoyl)oxy]methoxy((E)-2-[(1R,3R)-3-(2,4-dioxo-3H-pyrimidin-1-yl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptan-1-yl]ethenyl)phosphoryl]oxy)methyl-2,2-dimethylpropanoate **11**: A solution containing mixture of **10** (1.60 g, 2.371 mmol) in 120 mL of 50% aqueous formic acid was stirred for 2 days at 35°C. The reaction was then quenched by the addition of 200 mL of saturated sodium bicarbonate at 0 °C. The resulting solution was extracted with EtOAc (2 x 300 mL). The organic layer was washed with water (100 mL) and brine (100 mL). The organic solution was separated, dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under high vacuum pump. The residue was purified by Flash-Prep-HPLC to afford **11** (0.72 g, 54% yield) as white solid. Purification conditions (IntelFlash-1): Column, C18 silica gel; mobile phase, ACN/H_2O=10:90 increasing to ACN/H_2O=95:5 within 60 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.44 – 11.38 (m, 1H), 7.81 (d, J = 8.1 Hz, 1H), 6.81 (dd, J = 24.4, 17.4 Hz, 1H), 6.41 – 6.11 (m, 2H), 6.02 (s, 1H), 5.70 – 5.57 (m, 5H), 4.38 (d, J = 9.0 Hz, 2H), 4.12 (d, J = 8.5 Hz, 1H), 3.94 (d, J = 8.5 Hz, 1H), 1.19 (s, 18H) ppm. HRMS (ESI$^+$) m/z calcd for $C_{23}H_{33}N_2O_{12}PNa$ [M + Na]$^+$ 583.1669, found 583.1688.

([[(2,2-dimethylpropanoyl)oxy]methoxy((Z)-2-[(1R,3R)-3-(2,4-dioxo-3H-pyrimidin-1-yl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptan-1-yl]ethenyl)phosphoryl]oxy)methyl-2,2-dimethylpropanoate **11Z:** A solution containing mixture of **10Z** (300.00 mg, 0.445 mmol, 1.00 equiv), in 120 mL of 50% formic acid. The resulting solution was stirred for 2 days at 35 °C. The reaction was then quenched by the addition of 200 mL of ice water. The resulting solution was extracted with 2 x 300 mL of ethyl acetate. The organic solution was washed with 2 x 200 mL of water and 2 x 200 mL of brine. The solution was dried over anhydrous sodium sulfate and

concentrated under vacuum. The residue was purified by Flash-Prep-HPLC [(IntelFlash-1): Column, C18 silica gel; mobile phase, ACN/H_2O=10:90 increasing to ACN/H_2O=95:5 within 60 min] to afford **11Z** (0.14 g, 56% yield) as a white solid. ^{1}H NMR (300 MHz, CD_3CN) δ 8.34 (d, J = 8.2 Hz, 1H), 6.72 (dd, J = 52.7, 14.2 Hz, 1H), 6.10 (dd, J = 17.2, 14.2 Hz, 1H), 5.96 (s, 1H), 5.65 – 5.50 (m, 5H), 4.53 (s, 1H), 4.43 (s, 1H), 4.19 (d, J = 8.7 Hz, 1H), 4.07 (d, J = 8.7 Hz, 1H), 1.21 (d, J = 2.7 Hz, 18H) ppm. HRMS (ESI$^+$) m/z calcd for $C_{23}H_{34}N_2O_{12}P$ $[M + H]^+$ 561.1849, found 561.1855.



[[(E)-2-[(1R,3R)-7-[[(2-cyanoethoxy)(diisopropylamino)phosphanyl]oxy]-3-(2,4-dioxo-3H-pyrimidin-1-yl)-2,5-dioxabicyclo[2.2.1]heptan-1-yl]ethenyl([(2,2-dimethylpropanoyl)oxy]methoxy)phosphoryl]oxy]methyl 2,2-dimethylpropanoate **12**: To a well stirred reaction mixture of DCI (227.56 mg, 1.927 mmol) and 3-[[bis(diisopropylamino)phosphanyl]oxy]propanenitrile (774.39 mg, 2.569 mmol) in DCM (5 mL) under an inert atmosphere was added **11** (720.00 mg, 1.285 mmol) dissolved in DCM (2 mL). The resulting solution was stirred for 1 hr at 22 °C. The reaction was then quenched by the addition of ice water (50 mL). The resulting solution was extracted with ethyl acetate (20 mL). The organic layer was washed with water (50 mL), separated, dried over anhydrous Na_2SO_4, then concentrated. The residue was purified by Flash-Prep-HPLC to afford **12** (0.7 g, 72% yield) as a white solid. Purification conditions (IntelFlash-1): Column, C18 silica gel; mobile phase, ACN/H_2O=10:90 increasing to ACN/H_2O=95:5 within 50 min. ^{1}H NMR (600 MHz, CD_3CN) δ 8.98 (s, 1H), 7.69 (dd, *J* = 8.2, 5.7 Hz, 1H), 6.95 – 6.77 (m, 1H), 6.26 – 6.16 (m, 1H), 6.01 (d, *J* = 8.8 Hz, 1H), 5.67 – 5.57 (m, 5H), 4.59 (d, *J* = 10.8 Hz, 1H), 4.55 – 4.47 (m, 1H), 4.18 (dd, *J* = 8.8, 5.4 Hz, 1H), 3.93 (dd, *J* = 8.8, 6.6 Hz, 1H), 3.91 – 3.75 (m, 2H), 3.65 (dqt, *J* = 13.7, 6.8, 3.8 Hz, 2H), 2.75 – 2.64 (m, 2H), 1.25 – 1.15 (m, 33H) ppm. ^{13}C NMR (151 MHz, CD_3CN) δ 177.6, 177.5, 163.8, 151.2, 143.7, 143.6, 143.3, 143.2, 141.2, 141.2, 122.0, 121.7, 120.7, 120.4, 119.5, 101.5, 101.5, 89.2, 89.2, 89.0, 89.0, 88.9, 88.9, 88.8, 88.8, 88.2, 88.1, 82.8, 82.7, 82.7, 82.7, 82.7, 82.7, 82.7, 80.5, 80.5, 80.2, 80.2, 79.1, 78.9, 78.9, 78.8, 75.2, 75.0, 59.9, 59.7, 59.6, 44.3, 44.3, 44.3, 44.2, 39.4, 27.1, 27.1, 25.0, 25.0, 24.9, 24.8, 24.8, 24.8, 24.7, 21.0, 20.9, 20.9 ppm. ^{31}P NMR (243 MHz, CD_3CN) δ 149.66, 149.51, 15.99, 15.75 ppm. HRMS (ESI$^+$) m/z calcd for $C_{32}H_{51}N_4O_{13}P_2$ $[M + H]^+$ 761.2928, found 761.2932.

[[(Z)-2-[(1R,3R)-7-[[(2-cyanoethoxy)(diisopropylamino)phosphanyl]oxy]-3-(2,4-dioxo-3H-pyrimidin-1-yl)-2,5-dioxabicyclo[2.2.1]heptan-1-yl]ethenyl([(2,2-dimethylpropanoyl)oxy]methoxy)phosphoryl]oxy]methyl-2,2-dimethylpropanoate **12Z:** Under inert atmosphere of argon, to a solution of DCI (35.40 mg, 0.300 mmol) in DCM (0.7 mL) was added 3-[[bis(diisopropylamino)phosphanyl]oxy]propanenitrile (150.57 mg, 0.500 mmol) and stirred for 10 min. To this reaction mixture was added **11Z** (140.00 mg, 0.250 mmol) in DCM (2 mL). The resulting solution was stirred for 2 hr at 22 °C. The reaction was then quenched by the addition of 20 mL of ice water. The resulting solution was extracted with 3 x 20 mL of ethyl acetate. The organic solution was washed with 2 x 50mL of water. Then the solution was dried over anhydrous

sodium sulfate and concentrated. The residue was purified by Flash-Prep-HPLC [(IntelFlash-1): Column, C18 silica gel; mobile phase, ACN/H_2O=10:90 increasing to ACN/H_2O=95:5 within 60 min] to afford **12Z** (86 mg, 45% yield) as a white solid. ^{1}H NMR (600 MHz, CD_3CN) δ 9.12 – 8.81 (m, 1H), 8.30 (dd, *J* = 15.3, 8.2 Hz, 1H), 6.90 – 6.60 (m, 1H), 6.10 (ddd, *J* = 17.5, 14.1, 3.6 Hz, 1H), 6.02 – 5.97 (m, 1H), 5.64 – 5.52 (m, 6H), 4.70 (t, *J* = 9.1 Hz, 1H), 4.62 – 4.50 (m, 1H), 4.24 (d, *J* = 8.7 Hz, 1H), 4.05 (dd, *J* = 8.7, 2.8 Hz, 1H), 3.92 – 3.75 (m, 2H), 3.65 (dh, *J* = 10.4, 6.7 Hz, 2H), 2.68 (q, *J* = 6.0 Hz, 2H), 1.26 – 1.17 (m, 35H) ppm. ^{13}C NMR (151 MHz, CD_3CN) δ 177.6, 177.6, 164.0, 151.2, 142.4, 142.2, 142.2, 142.1, 124.7, 124.3, 123.5, 123.1, 119.5, 119.5, 101.2, 101.2, 89.2, 89.2, 88.4, 88.3, 88.3, 88.3, 88.1, 88.1, 88.1, 88.0, 82.9, 82.9, 82.8, 82.8, 82.8, 82.7, 82.7, 79.7, 79.7, 79.4, 79.4, 78.4, 78.3, 78.1, 78.0, 74.4, 74.2, 59.8, 59.7, 59.7, 59.6, 44.3, 44.3, 44.2, 44.2, 39.4, 39.4, 27.1, 24.9, 24.9, 24.9, 24.8, 24.8, 24.7, 21.0, 21.0, 20.9 ppm. ^{31}P NMR (243 MHz, CD_3CN) δ 149.68, 149.24, 13.76, 13.55 ppm. HRMS (ESI^+) m/z calcd for $C_{32}H_{51}N_4O_{13}P_2$ $[M + H]^+$ 761.2928, found 761.2940.

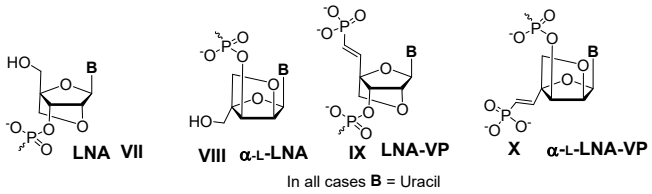
Oligonucleotide synthesis and purification: Sense strands were synthesized on L96-tri-*N*-acetylgalactosamine (GalNAc)-cluster[3] immobilized on controlled pore glass (CPG) solid support. On the other hand, antisense strands were assembled on 2′-*O*-methyl-uridine loaded CPG-solid support, available from LGC Biosearch Technologies (Petaluma, CA, USA) (porosity 616 Å, loading 87 µmol/g). The single strands were synthesized on K&A H-8-SE synthesizer. A solution of 0.5 M 5-(*S*-ethylthio)-1*H*-tetrazole in acetonitrile (CH_3CN) was used as the activator. The solutions of commercially available phosphoramidites and synthesized phosphoramidities were used at 0.1 M in anhydrous CH_3CN or CH_2Cl_2.The oxidizing reagent was 0.05 M I_2 in THF/pyridine/H_2O. 100 mM solution of 3-Amino-1,2,4-dithiazole-5-thione (or Xanthane hydride obtained from TCI Chemicals, Germany) dissolved in acetonitrile-pyridine (2:3 *v/v*) was employed as sulfurizing agent . The detritylation reagent was 3% trichloroacetic acid in CH_2Cl_2. Waiting times for coupling, capping, oxidation, and sulfurization step were 450 s, 25 s, 80 s, and 300 s, respectively. After completion of the automated synthesis, the oligonucleotide was manually released from the solid support and deprotected using AMA (1:1 (*v/v*) mixture of concentrated aqueous ammonia and 40% aqueous methylamine, both available from Sigma Aldrich). For 5'-VP-modified antisense strands, cleavage from solid support and quantitative deprotection was achieved using 3% DEA in concentrated aqueous NH_3, following published protocol (*Tetrahedron* **2018**, *74*, 6182)

After filtration through a 0.45-µm nylon filter, oligonucleotides were purified by ion exchange HPLC using a Dionex DNA Pac100 (9 x 250 mm) (ThermoFisher, Dreieich, Germany). Appropriate gradients of mobile phase (eluent A: 20 mM TRIS buffer, 20% CH_3CN, pH 7.4; eluent B: 500 mM $NaClO_4$ in eluent A) were employed. Oligonucleotides were desalted using size-exclusion chromatography using a column packed with Sephadex G25 (GE Healthcare) and water as an eluent. Oligonucleotides were then quantified by measuring the absorbance at 260 nm. Extinction coefficients were calculated using the following extinction coefficients for each residue: A, 13.86; T/U, 7.92; C, 6.57; and G, 10.53 $M^{-1}cm^{-1}$. The identities of modified oligonucleotides were verified by mass spectrometry. Sequences and mass spectroscopy data are shown in Table S1. Purities were evaluated by analytical reverse-phase HPLC. For reverse-phase HPLC, a C-18 column was used with a gradient of 2-29% buffer B (eluent A: 95 mM hexafluoroisopropanol, 16.3 mM TEA, 0.05 mM EDTA; eluent B: MeOH) over 39 min.

Table S1: Table showing characterization of synthesized oligonucleotides

Entry	Sequence (5'-3')[a]	Sense / Antisense	Purity [%]		MS	
			IEX	RP	calc.	observed
ON1	a•a•*C*a*G*u*G*u*UCU*u*G*c*U*c*U*a*U*a*AL	S	94.6	89.9	8602.2	8600.6
ON2	**VII***U*•a*U*a*G*a*G*c*A*aga*A*c*A*c*U*g*U*u•u•u	AS	95.1	92.3	7577.9	7577.4
ON3	**VIII***U*•a*U*a*G*a*G*c*A*aga*A*c*A*c*U*g*U*u•u•u	AS	94.5	87.7	7577.9	7577.5
ON4	u*U*•a*U*a*G*a*G*c*A*aga*A*c*A*c*U*g*U*u•u•u	AS	94.9	93.0	7579.9	7579.5
ON5	u•*U*•a*U*a*G*a*G*c*A*aga*A*c*A*c*U*g*U*u•u•u	AS	99.4	94.2	7595.9	7595.5
ON6	**IX***U*•a*U*a*G*a*G*c*A*aga*A*c*A*c*U*g*U*u•u•u	AS	91.3	92.4	7654.9	7653.5
ON7	**X***U*•a*U*a*G*a*G*c*A*aga*A*c*A*c*U*g*U*u•u•u	AS	95.0	90.8	7654.9	7653.5
ON8	VPu*U*•a*U*a*G*a*G*c*A*aga*A*c*A*c*U*g*U*u•u•u	AS	96.3	90.0	7655.9	7655.5
ON9	VPu•*U*•a*U*a*G*a*G*c*A*aga*A*c*A*c*U*g*U*u•u•u	AS	95.7	93.2	7671.9	7671.5

[a]Upper case italics indicate 2'-F RNA; lower case indicates 2'-OMe modification; VP indicates 5'-(*E*)-vinylphosphonate; • indicates phosphorothioate modification; **VII-X** are modifications shown below.



***In vitro* siRNA activity:** The *in vitro* activity of modified and control duplexes was evaluated for gene silencing in cell culture by targeting the target *TTR*. Primary mouse hepatocytes (PMH) were plated in 96 well format for free uptake with hepatocyte plating medium (Primacyt, Germany). 10x stocks were prepared to create free uptake final concentrations of 100, 10, 1nM. Each duplex was tested in quadruplicate. Cells were incubated 48 hours for free uptake at 37°C and subsequently lysed for mRNA quantification using the Quantigene Singleplex assay system (Thermo), according to the manufacurers protocol. Probesets for murine *TTR* and GAPDH were custom designed by Thermo. Luciferase signal was read on a Victor light plate luminometer (Perkin Elmer) For each well, TTR mRNA level was normalized to the respective GAPDH mRNA level. The activity of a given siRNA was expressed as percent of *TTR* mRNA concentration (normalized to GAPDH mRNA) in treated cells, relative to the *TTR* mRNA concentration averaged across control wells.

Evaluation of silencing in mice. All studies were conducted following the animal welfare regulations of the state of Bavaria (Germany) and the European Union. Protocols were approved by the government of lower Franconia. Mice received a single subcutaneous injection of 1 mg/kg siRNA, prepared in an injection volume of 5 µL/g body weight in PBS. At the indicated time pre- or post-dosing, blood was obtained by puncturing the facial vein. TTR protein was quantified by ELISA from serum isolated from whole blood. The ELISA was performed according to the manufacturer's protocol (ALPCO, 41-PALMS-E01) after a 4000-fold dilution of the serum samples. TTR protein concentration was calculated from a standard curve, and each data point is the average of all the mice within each cohort (n = 3).

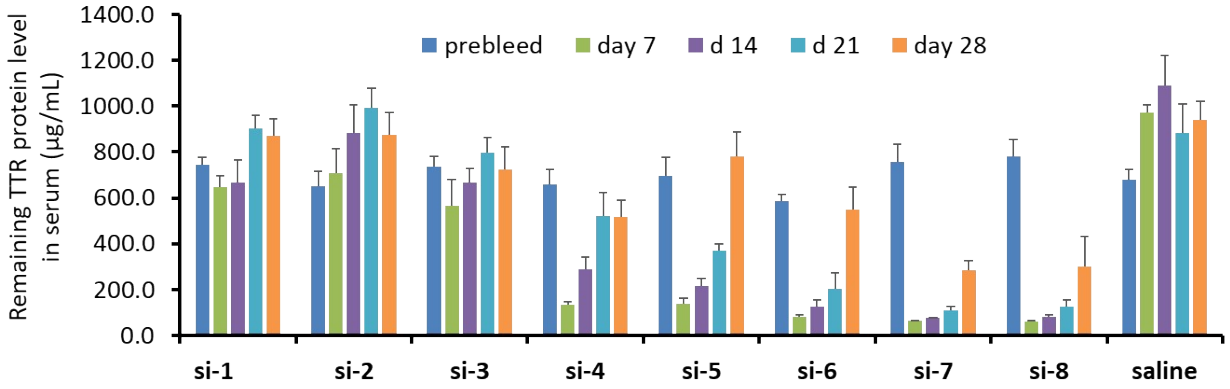


Figure S1: (B) *TTR* protein amounts in serum at indicated days after mice were dosed subcutaneously with 1 mg/kg indicated siRNA. Plotted are averages ± standard deviations (n=3).

Modeling studies:
In Fig 5 of the main text, models were built based on the crystal structure of the complex between miR-20a and human Ago2, PDB ID 4f3t.[4] The build/modify options in UCSF Chimera[5] were used to install the modified residues. All models were energy-minimized with the AMBER ff14 force field[6] as implemented in UCSF Chimera until conversion. The 5'-VP-2'-OMe nucleotide adopts the C2′-*endo* sugar pucker as does the uridine in the parent structure.[4] This computational model was overlaid on the crystal structure of an RNA carrying the 5'-VP bound to Ago2, PDB ID 5t7b.[7]

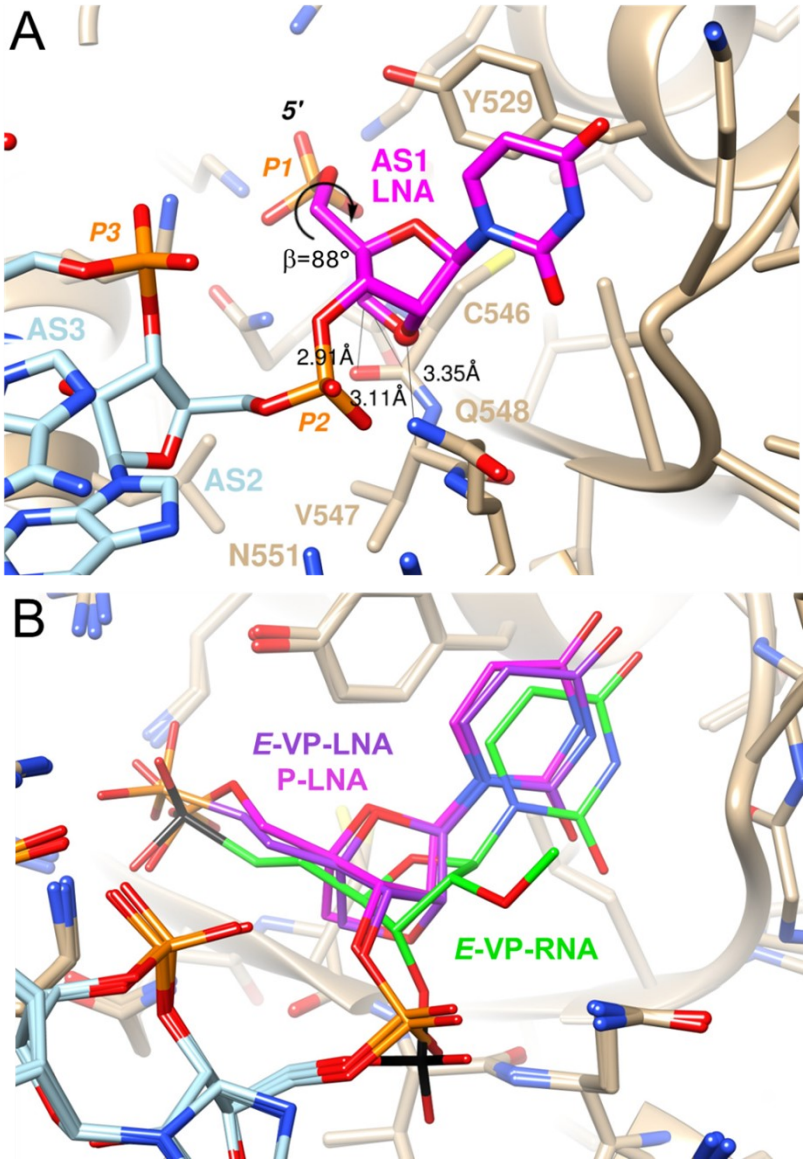
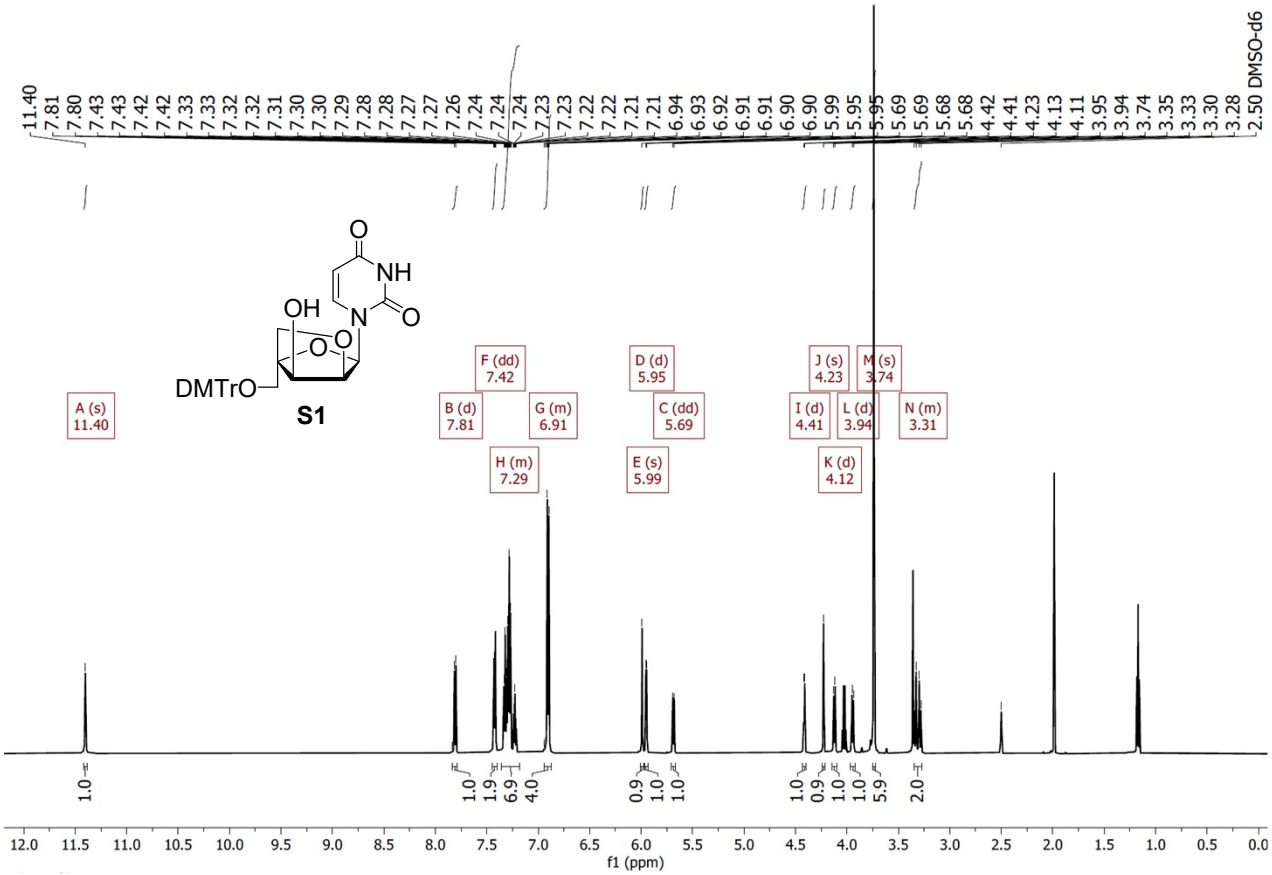
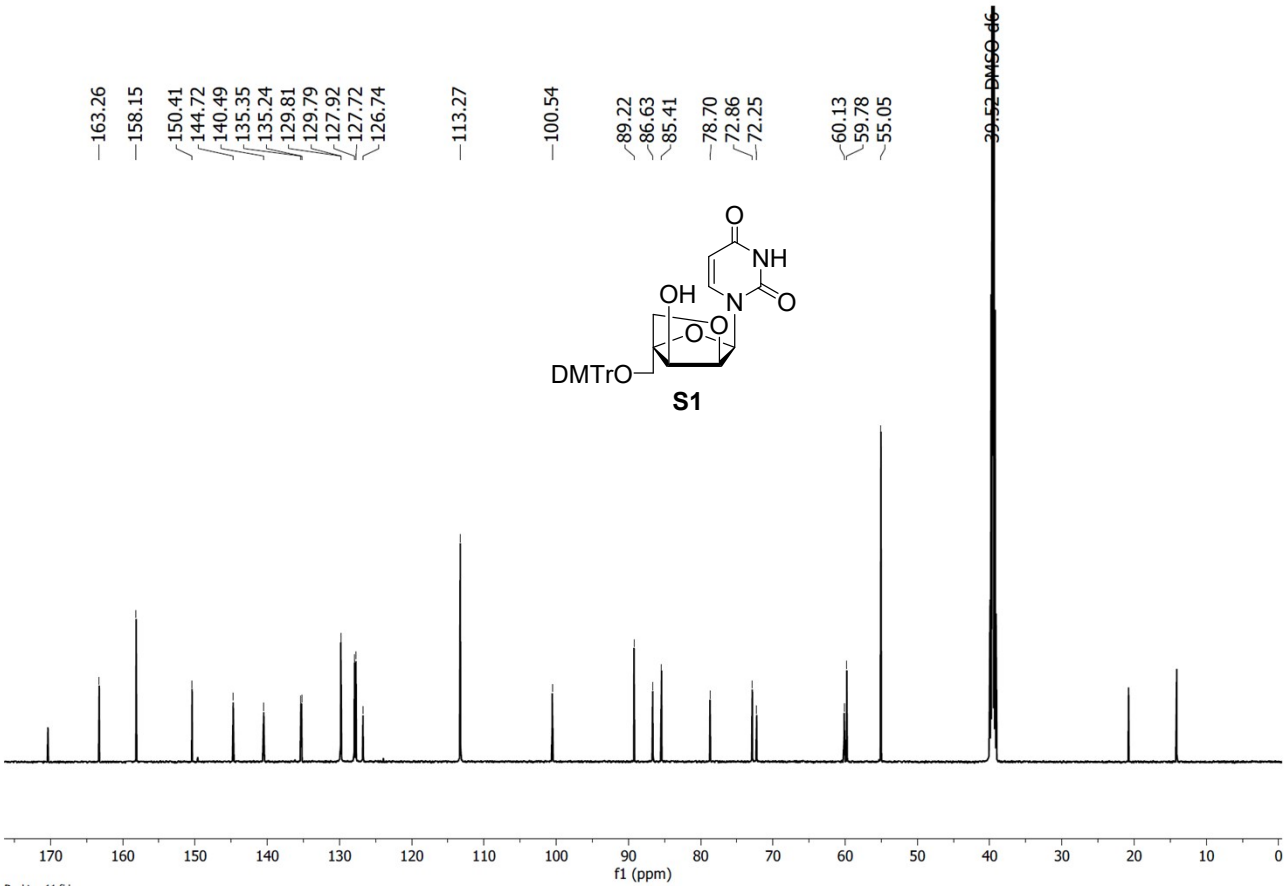


Figure S2: Models of 5′-terminal guide strand nucleotides lodged at the MID domain of RISC Ago2. (A) LNA with a 5′-phosphate, and (B) Overlay of the LNA model shown in panel A, LNA with an *E*-VP moiety, and *E*-VP-RNA. Carbon atoms of AS1 residues are colored in magenta, purple and green for P-LNA (5′-phosphate LNA), *E*-VP-LNA and *E*-VP-RNA, respectively, and the AS1 and AS2 phosphorus atoms of RNA are highlighted in black. The phosphorus position of P-LNA virtually matches the position of the *E*-VP-RNA phosphorus (panel B), but the β torsion angle of the former nucleotide is in a *gauche* conformation to do so (panel A). Combining LNA with an *E*-VP moiety at AS1 results in a shift by the phosphorus of almost 1 Å relative to *E*-VP-RNA in addition to a shift by the entire LNA nucleoside (panel B).

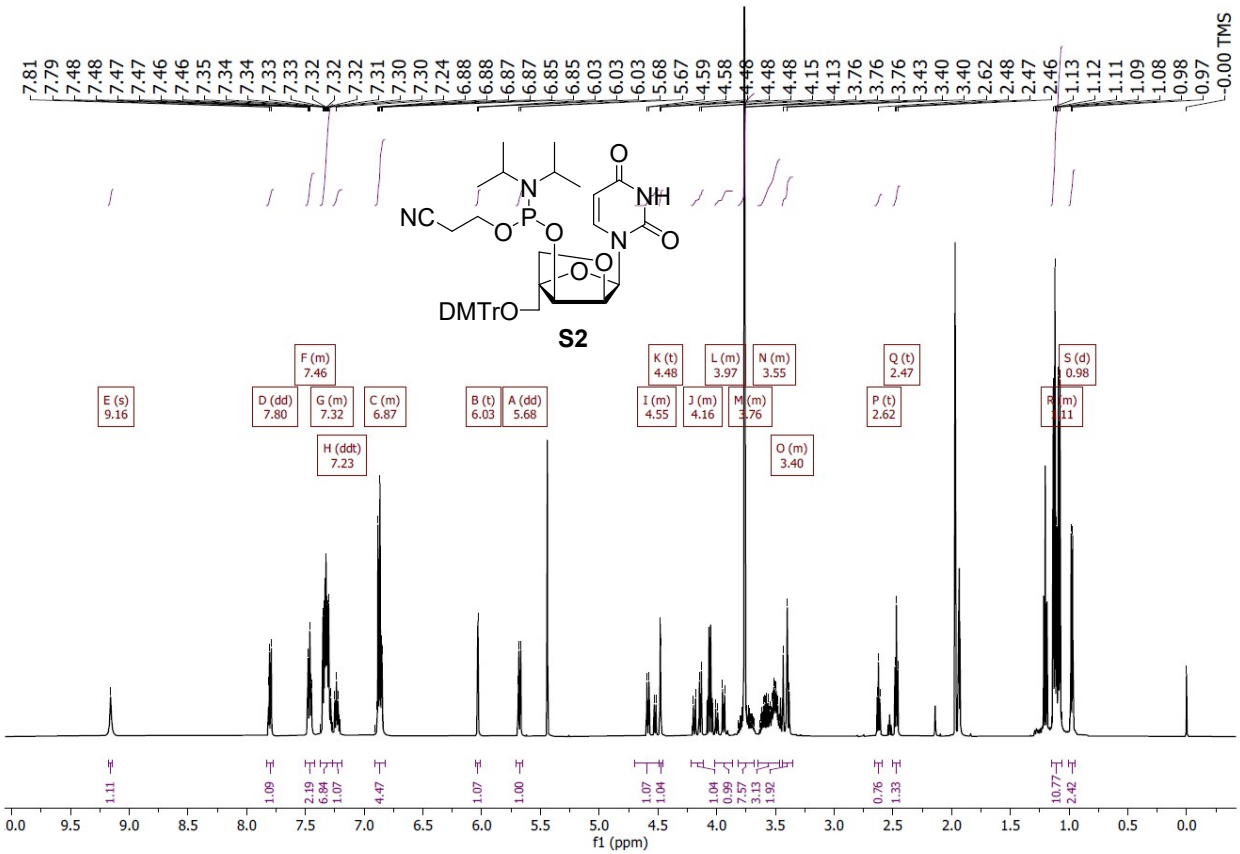
NMR Spectra



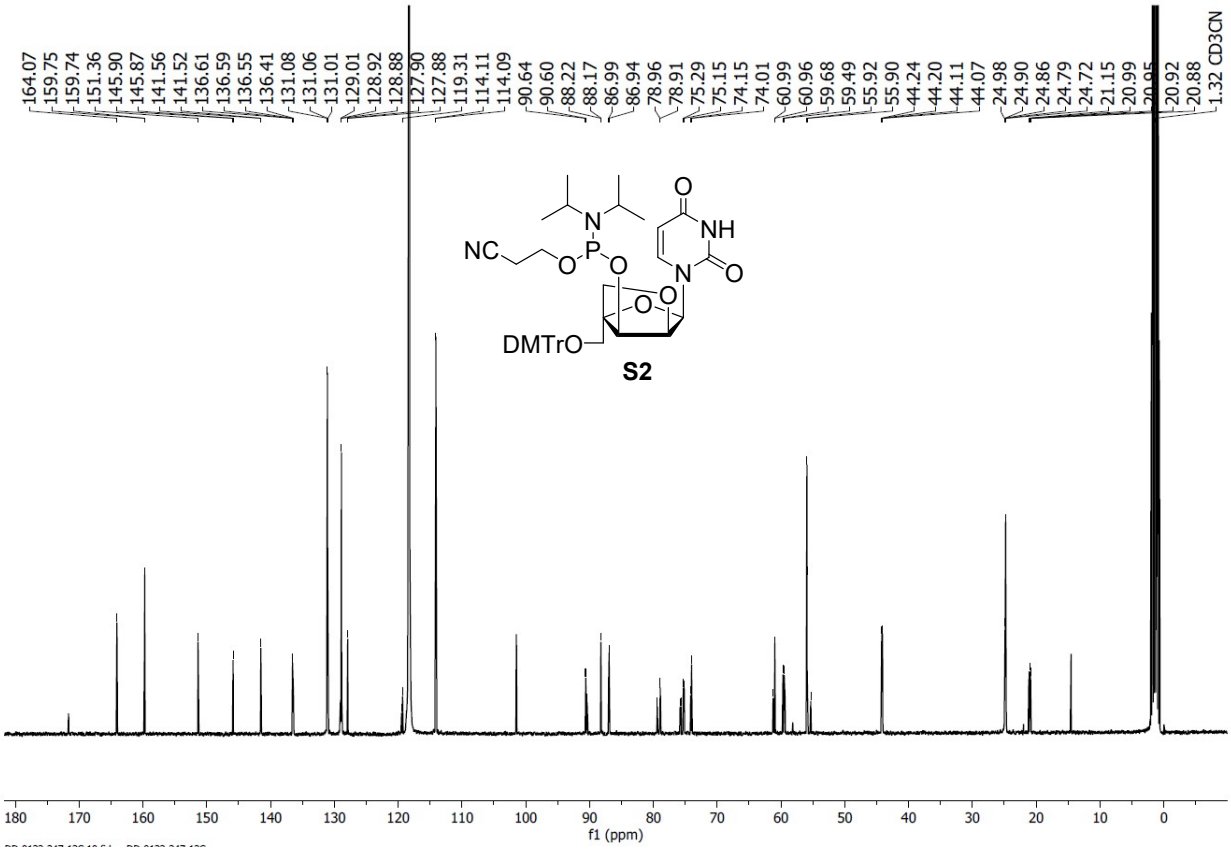
^{1}H NMR (600 MHz, DMSO-d_6) of **S1**



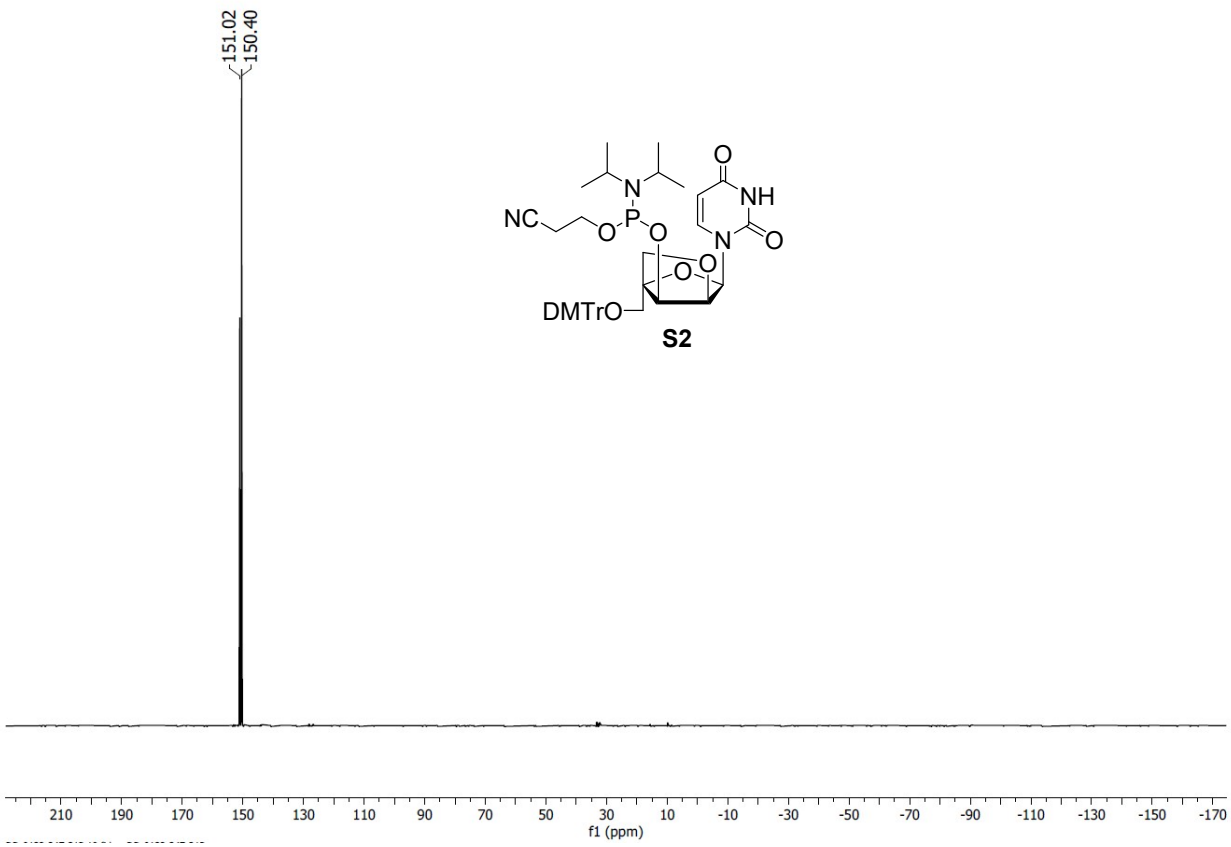
^{13}C NMR (151 MHz, DMSO-d_6) of **S1**



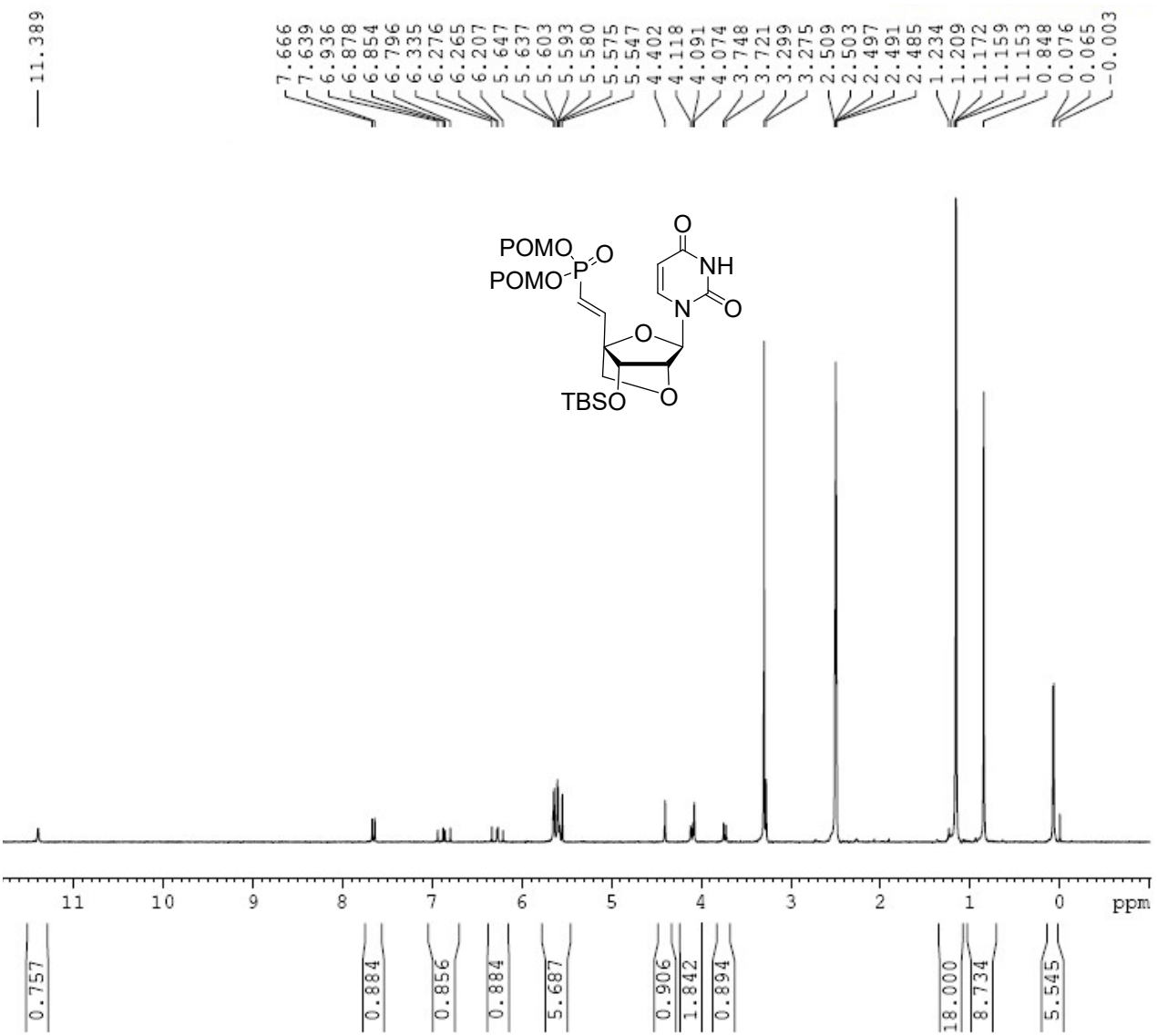
^{1}H NMR (500 MHz, CD_3CN) of **S2**



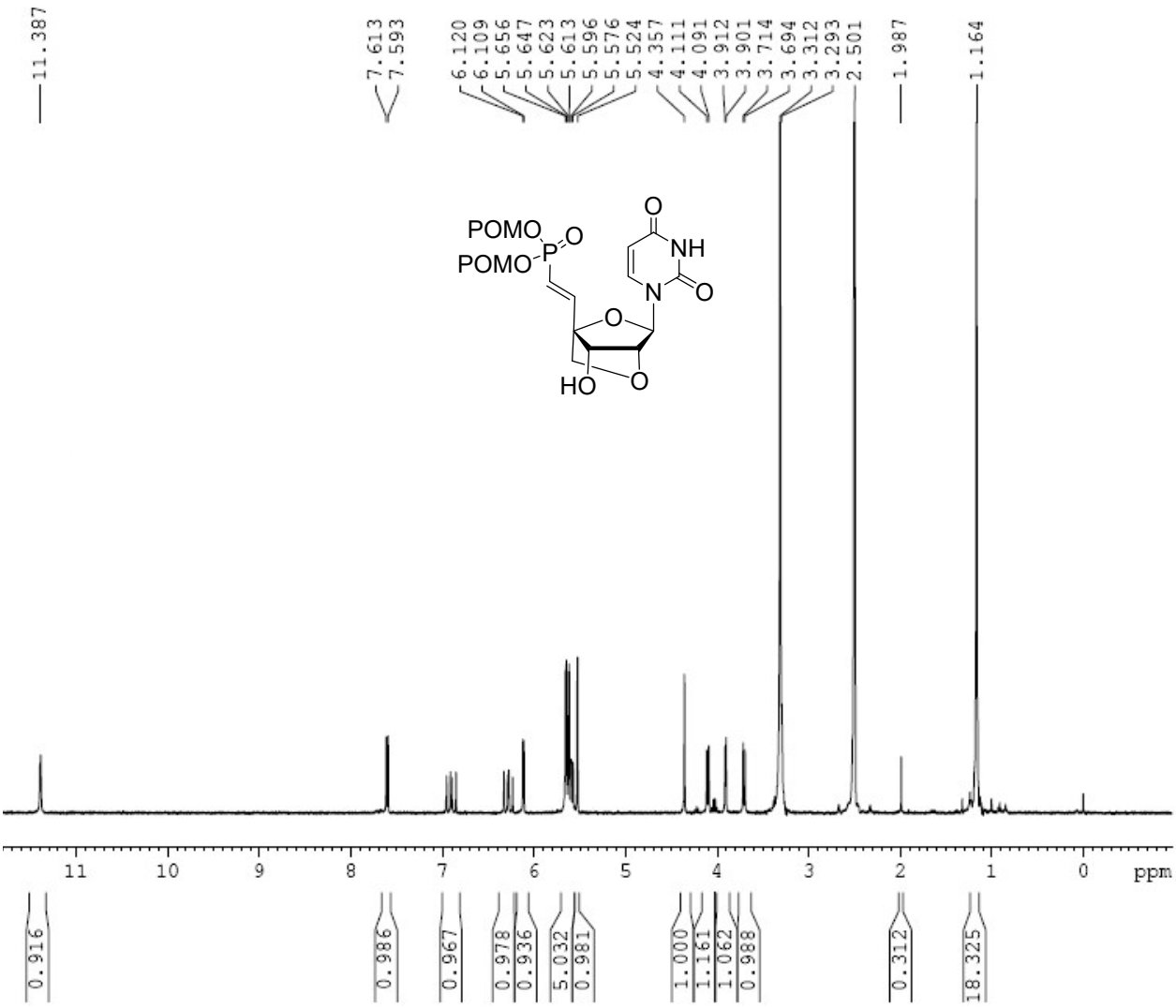
^{13}C NMR (101 MHz, CD_3CN) of **S2**



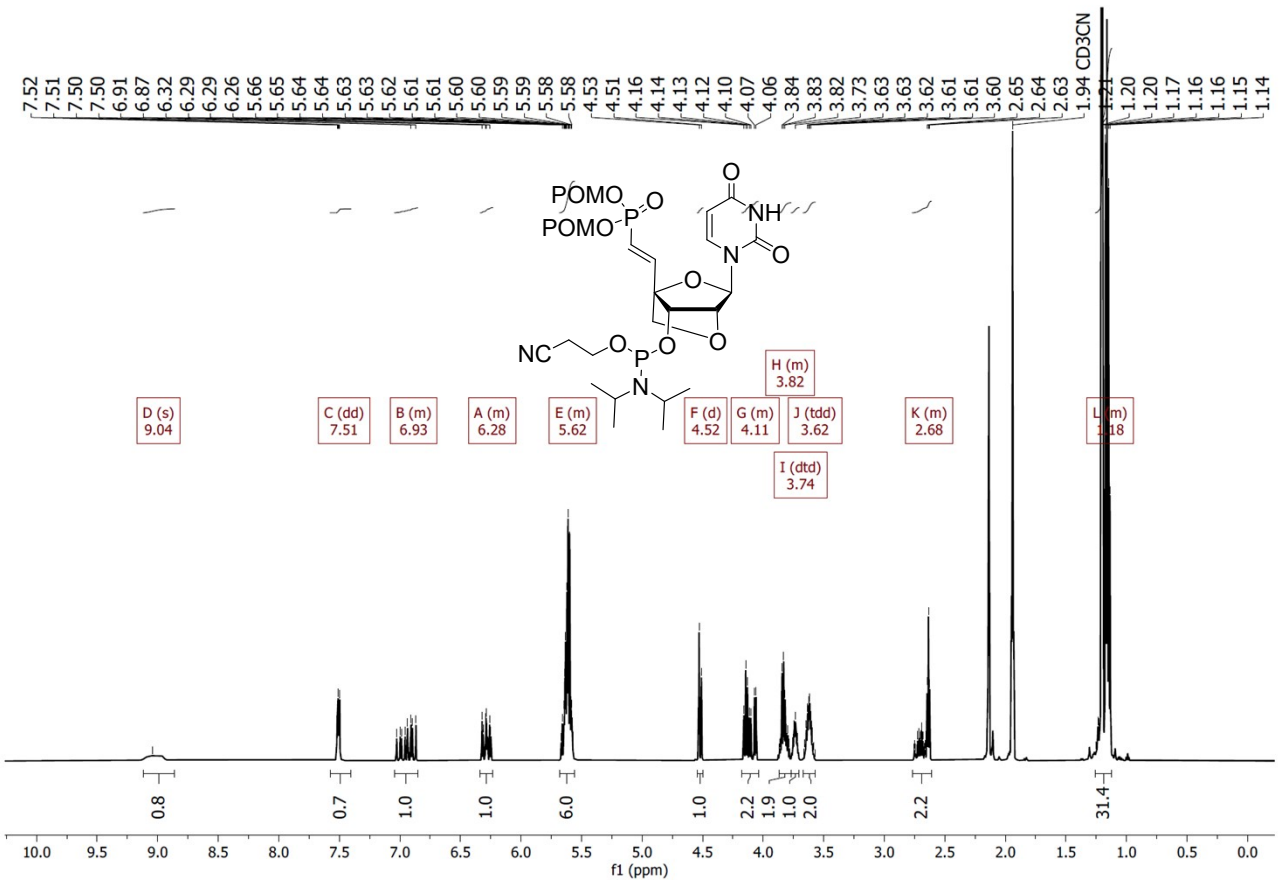
^{31}P NMR (202 MHz, CD_3CN) of **S2**



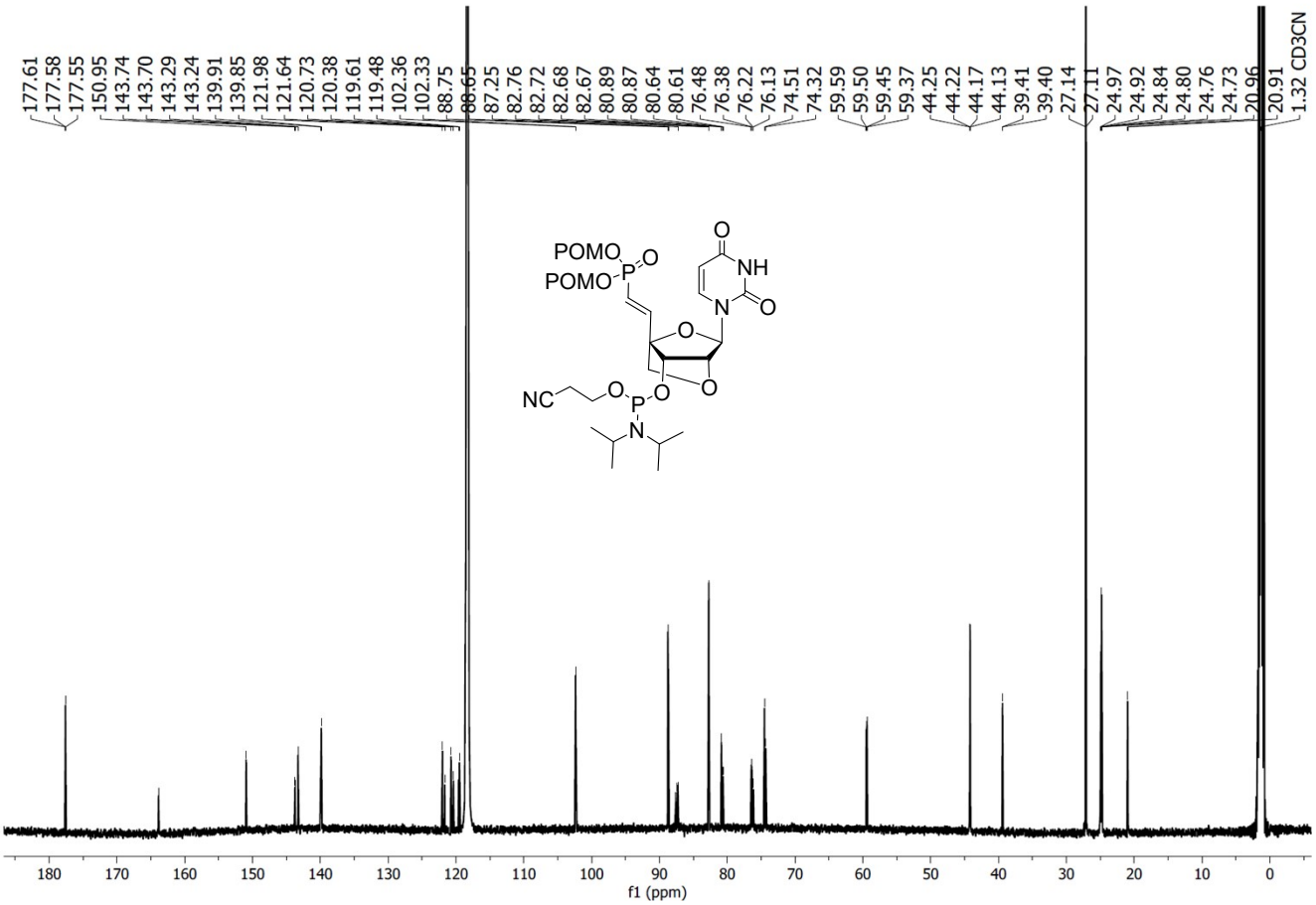
^{1}H NMR (300 MHz, DMSO-d_6) of **3**



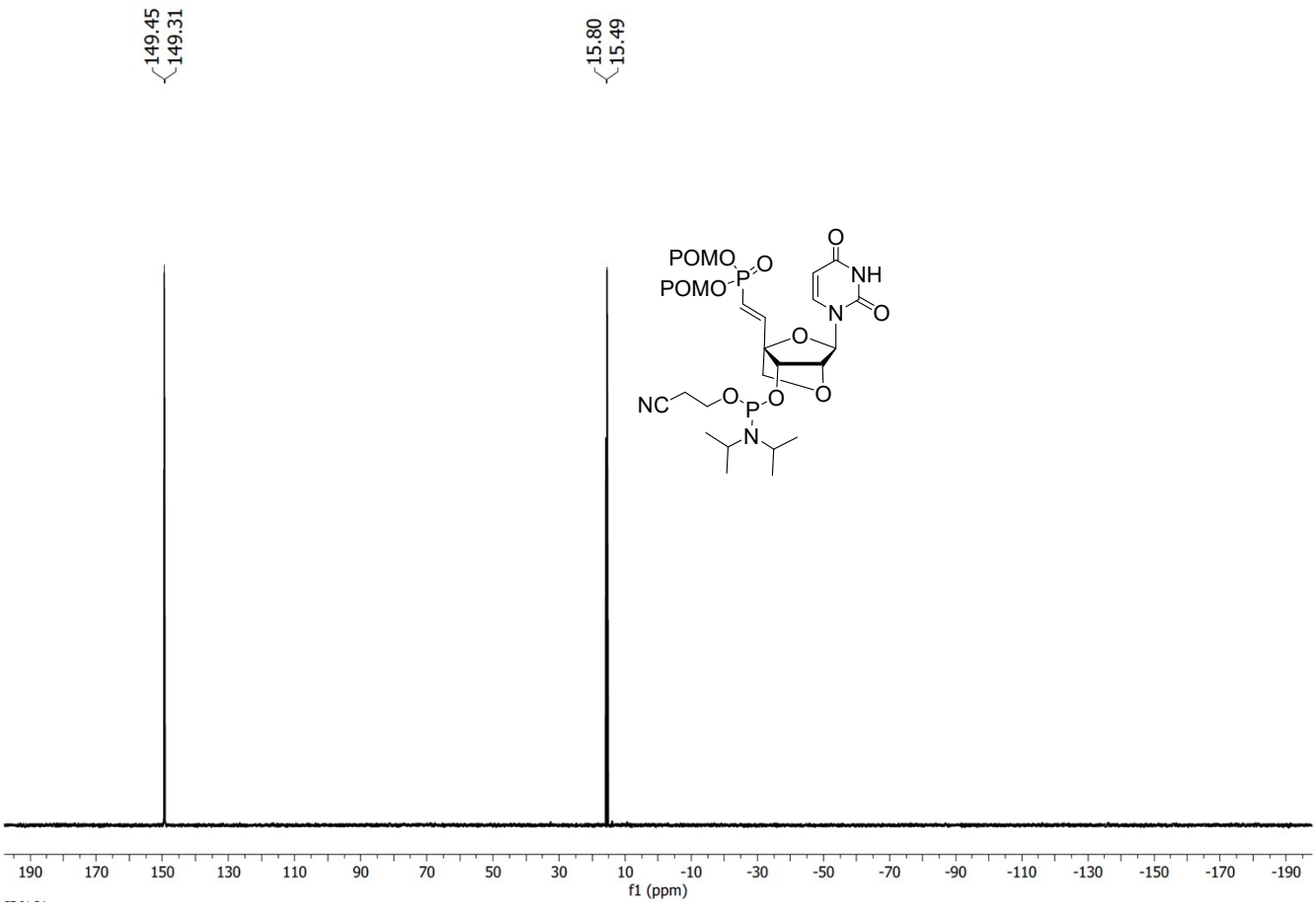
^{1}H NMR (400 MHz, DMSO-d_6) of **4**



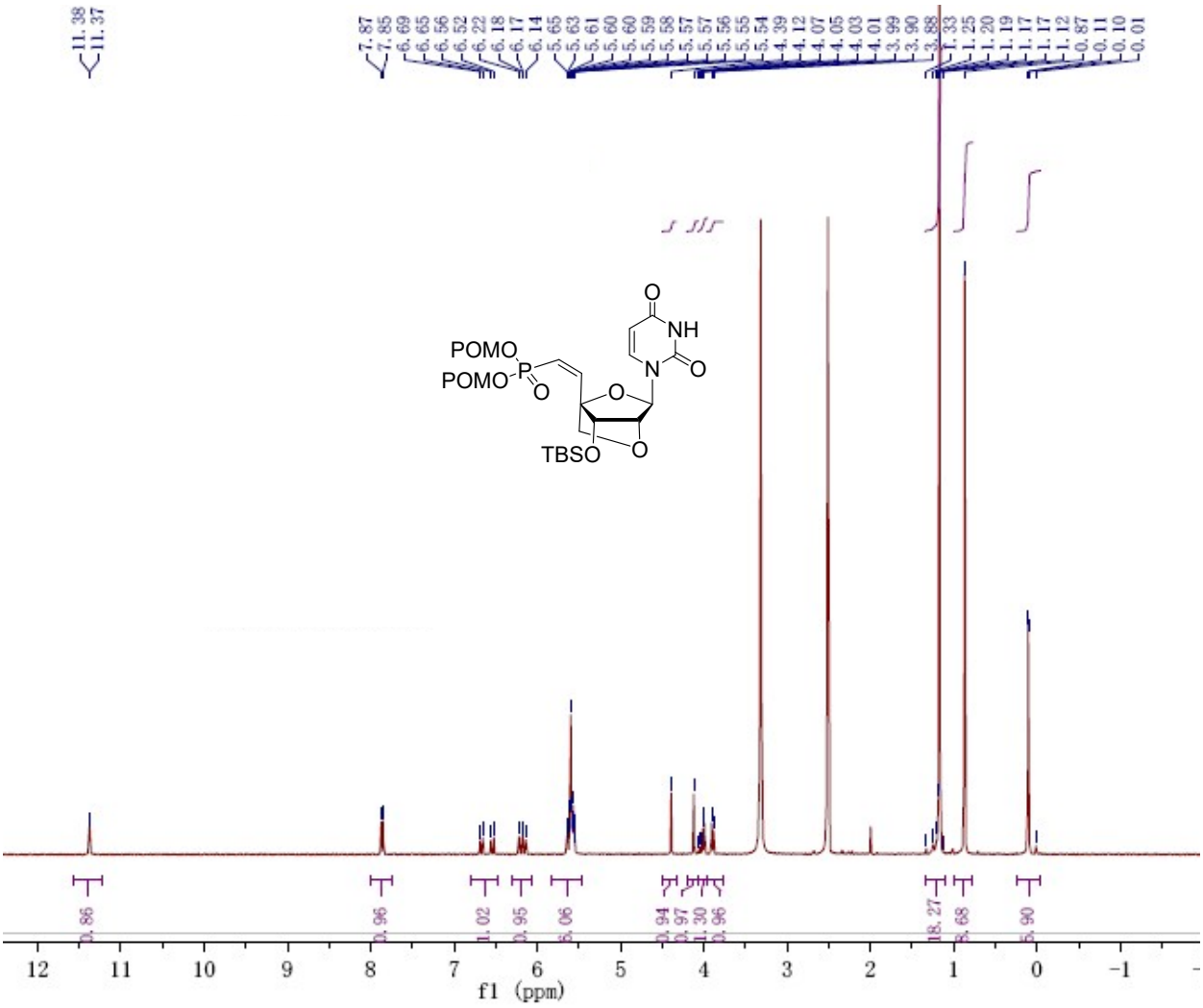
^{1}H NMR (600 MHz, CD_3CN) of **5**



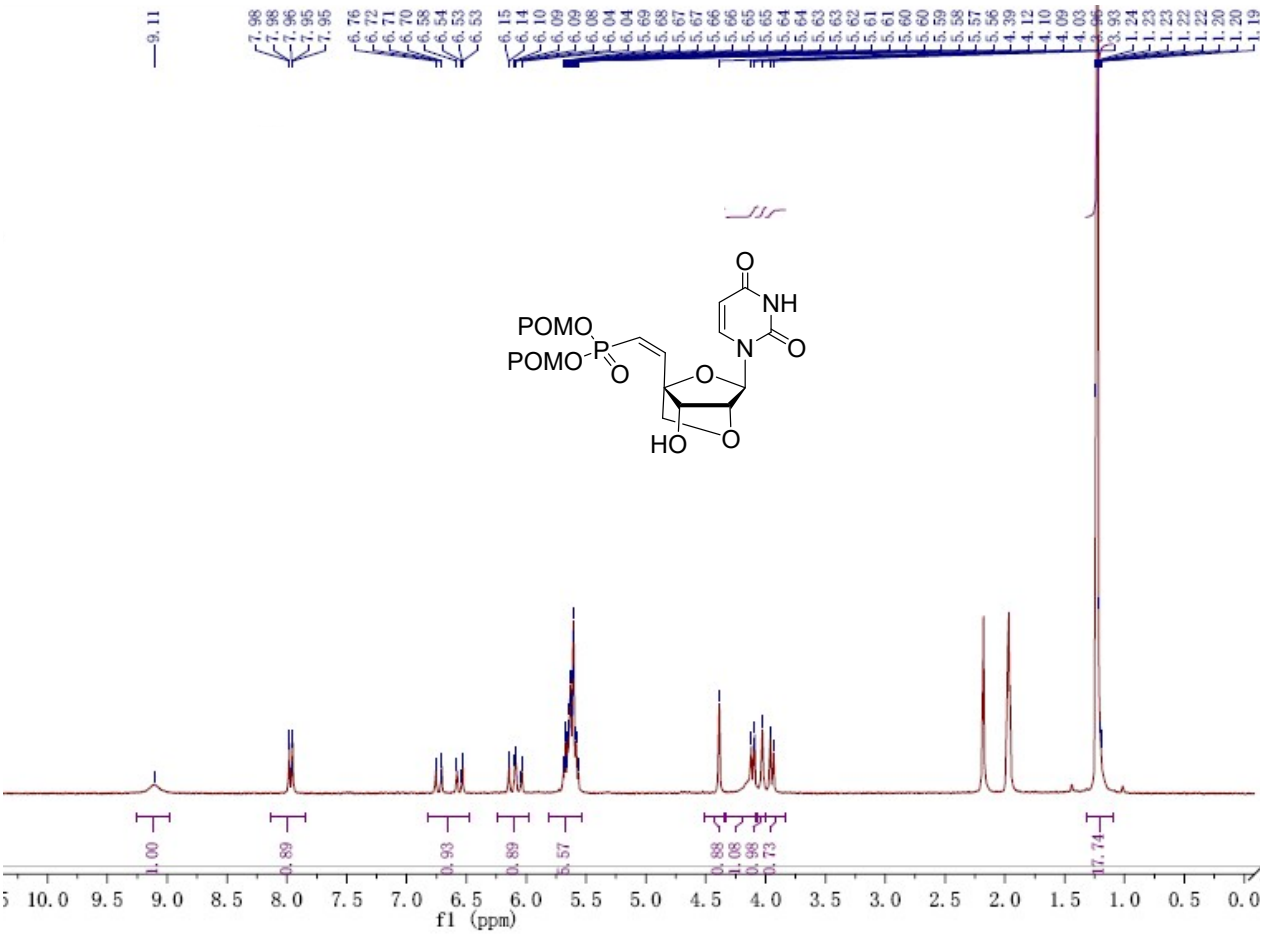
^{13}C NMR (151 MHz, CD_3CN) of **5**



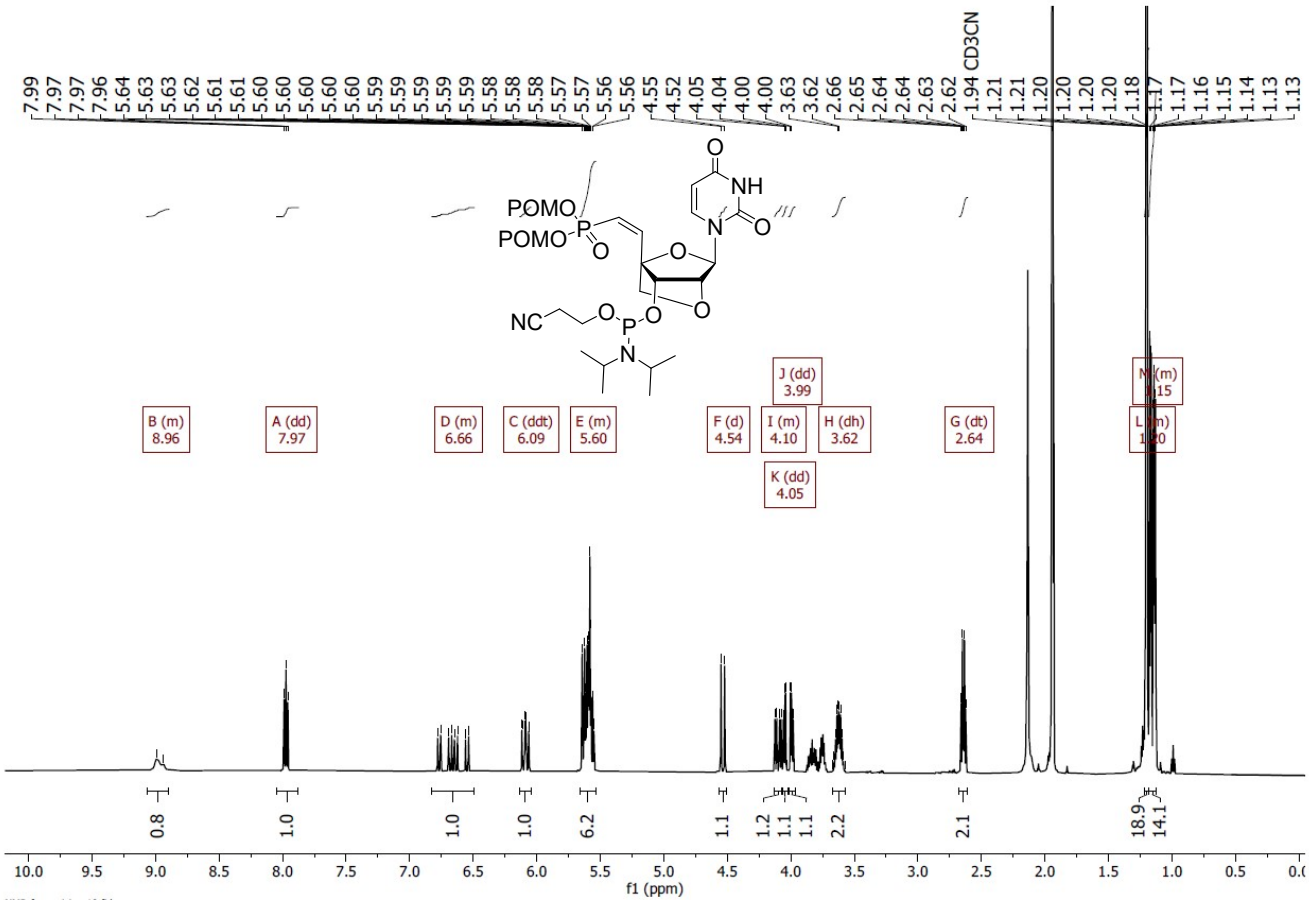
^{31}P NMR (243 MHz, CD_3CN) of **5**



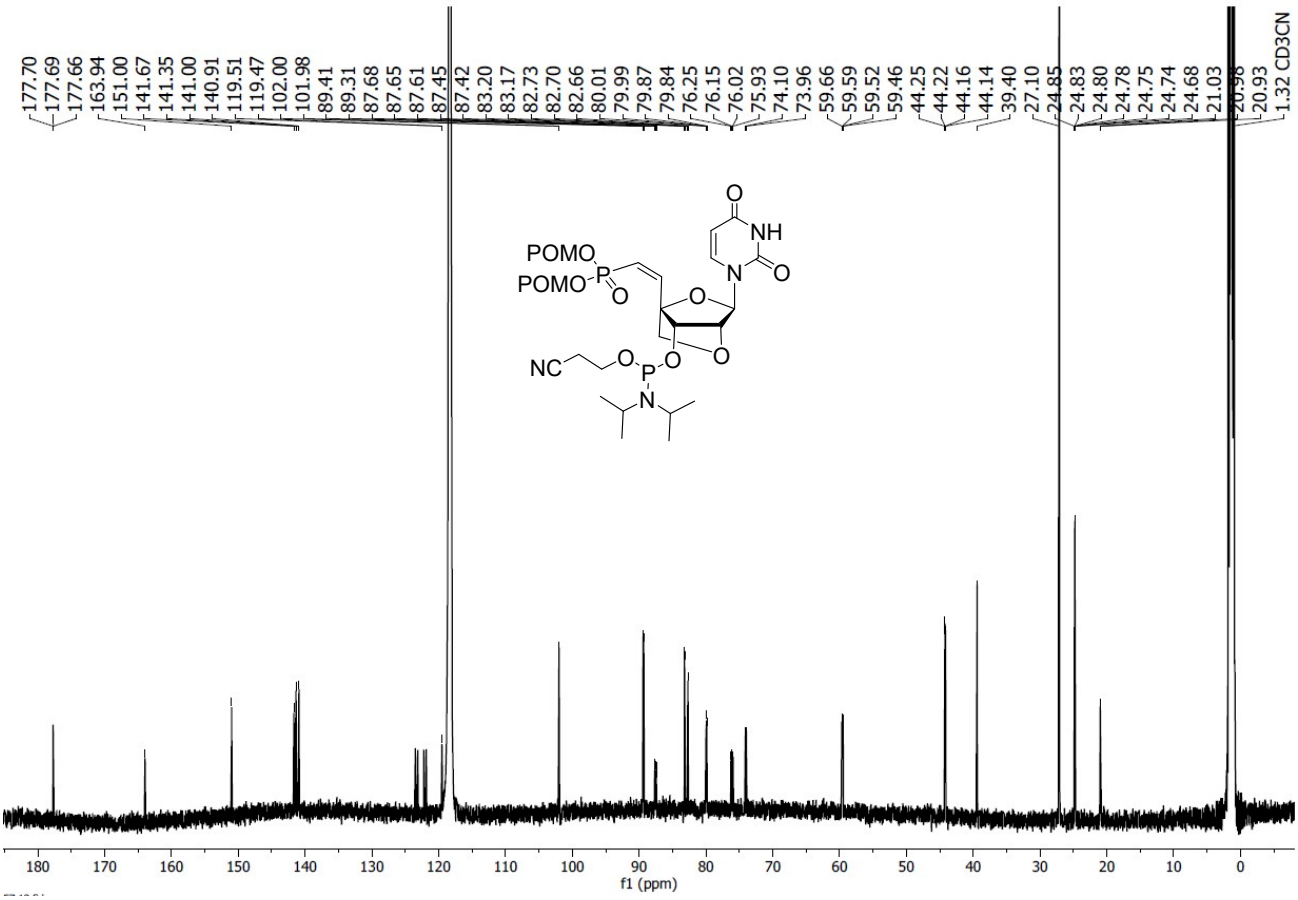
^{1}H NMR (300 MHz, DMSO-d_6) of **3Z**



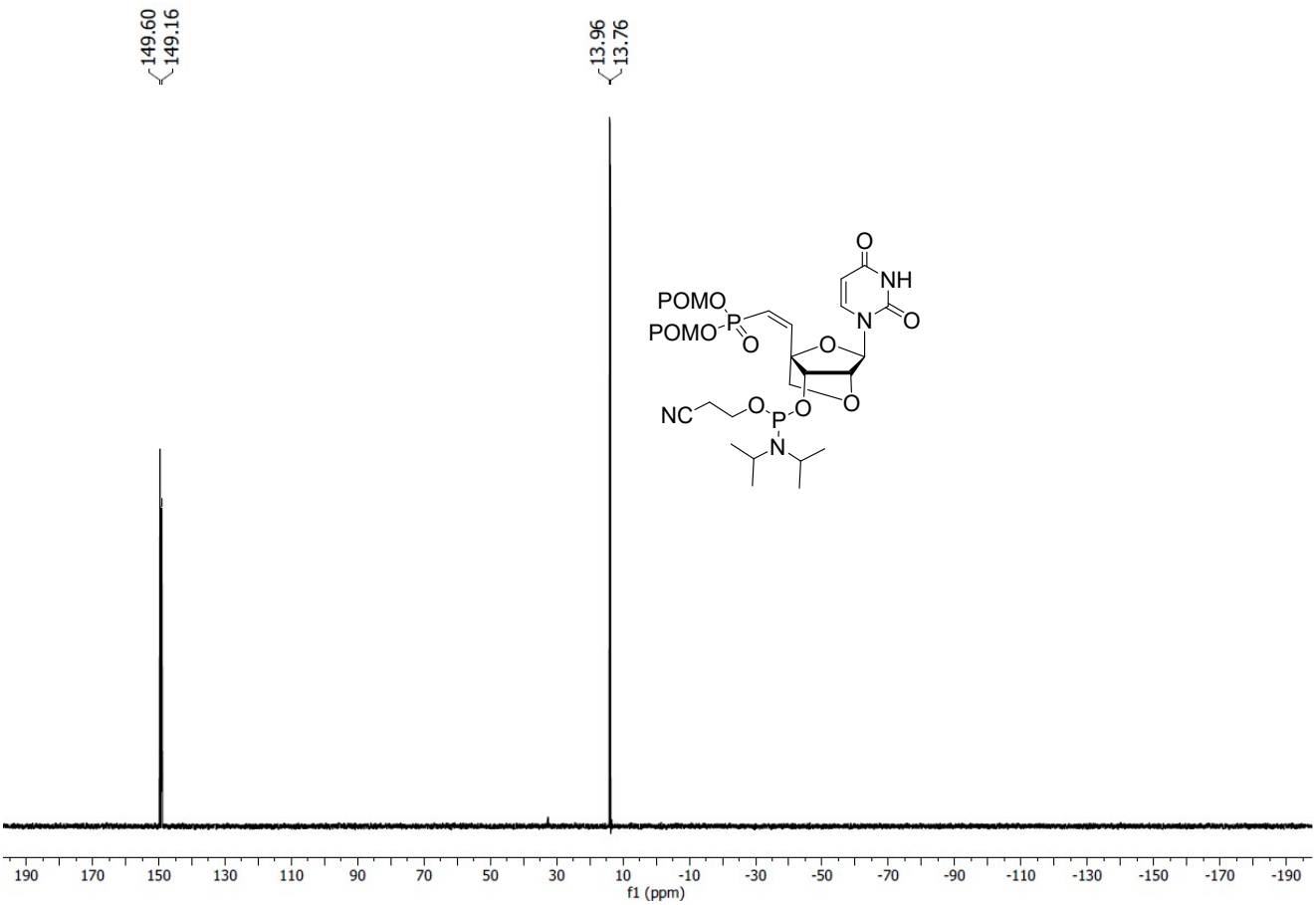
^{1}H NMR (300 MHz, CD_3CN) of **4Z**



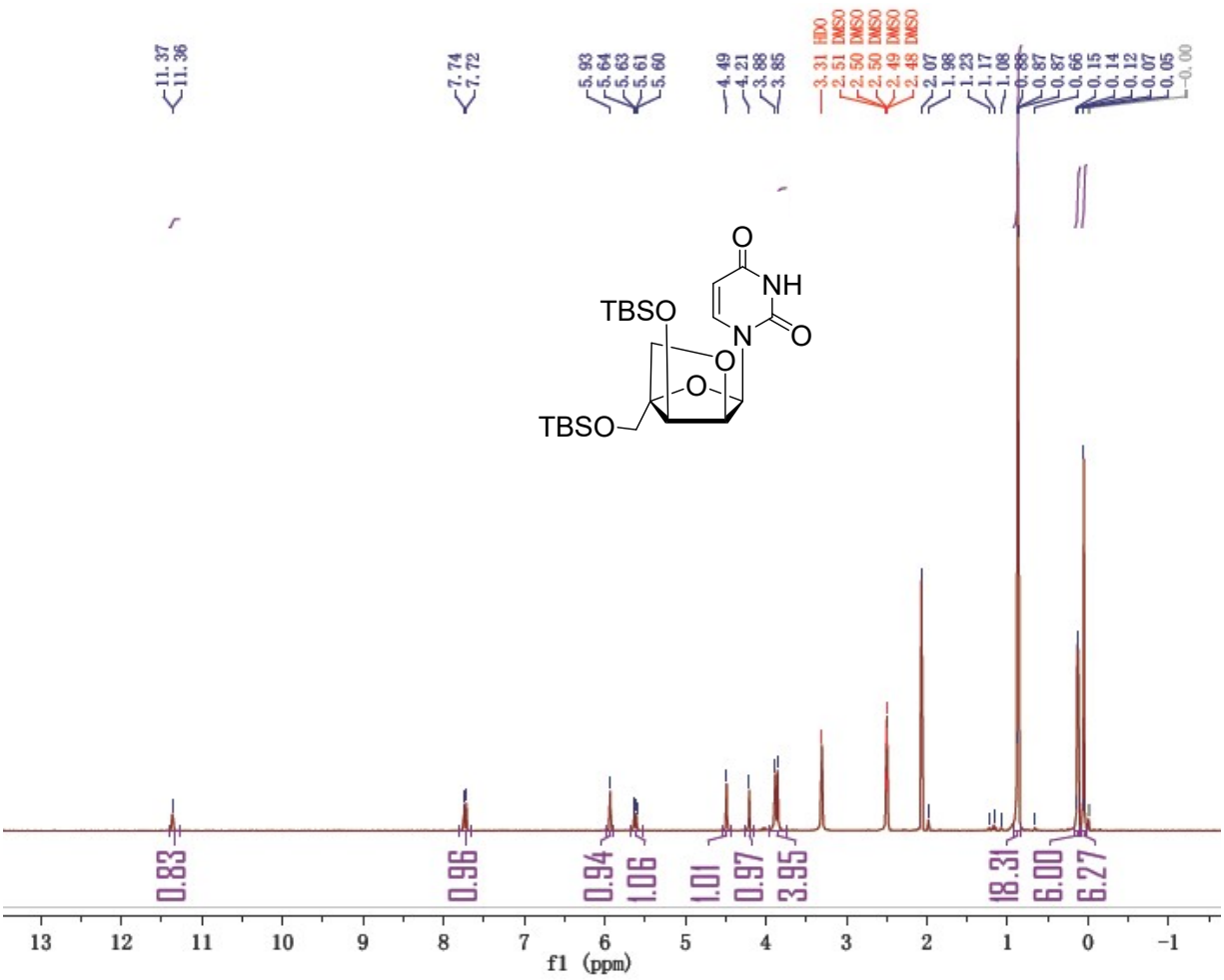
^{1}H NMR (600 MHz, CD_3CN) of **5Z**



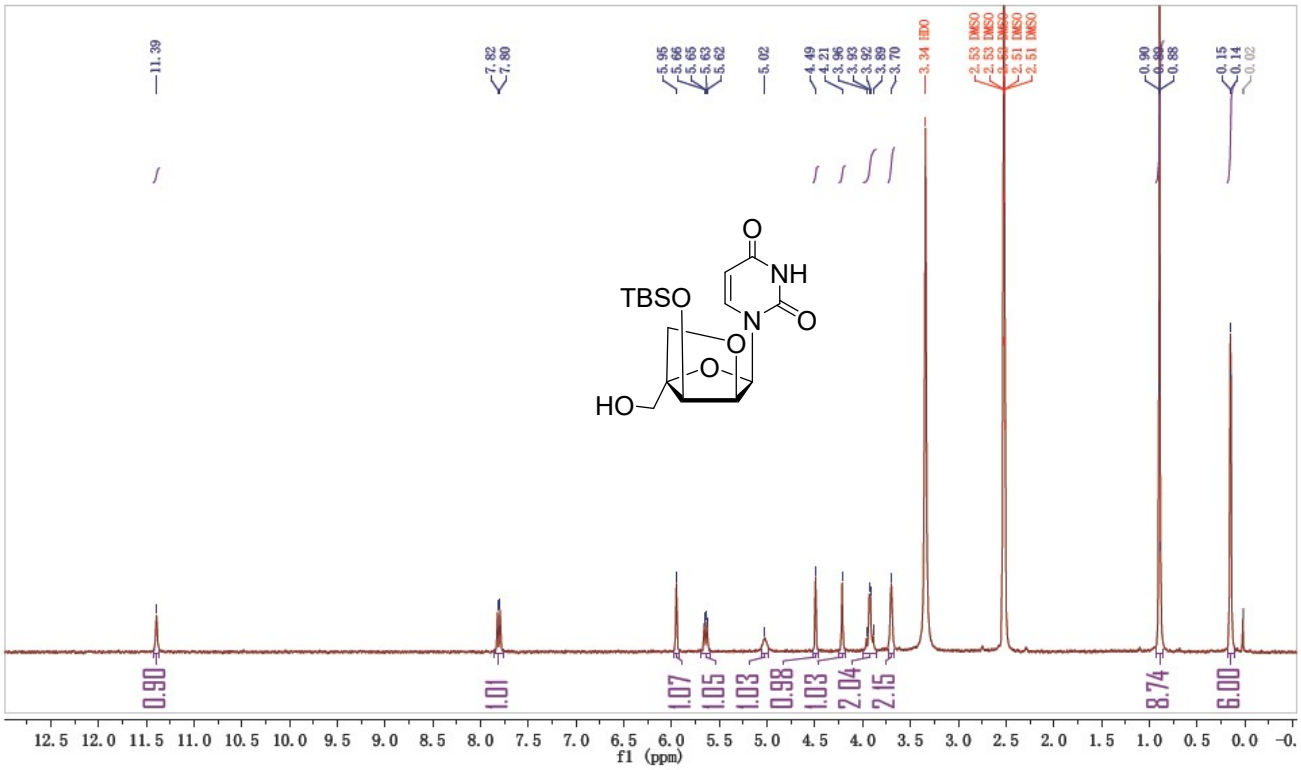
^{13}C NMR (151 MHz, CD_3CN) of **5Z**



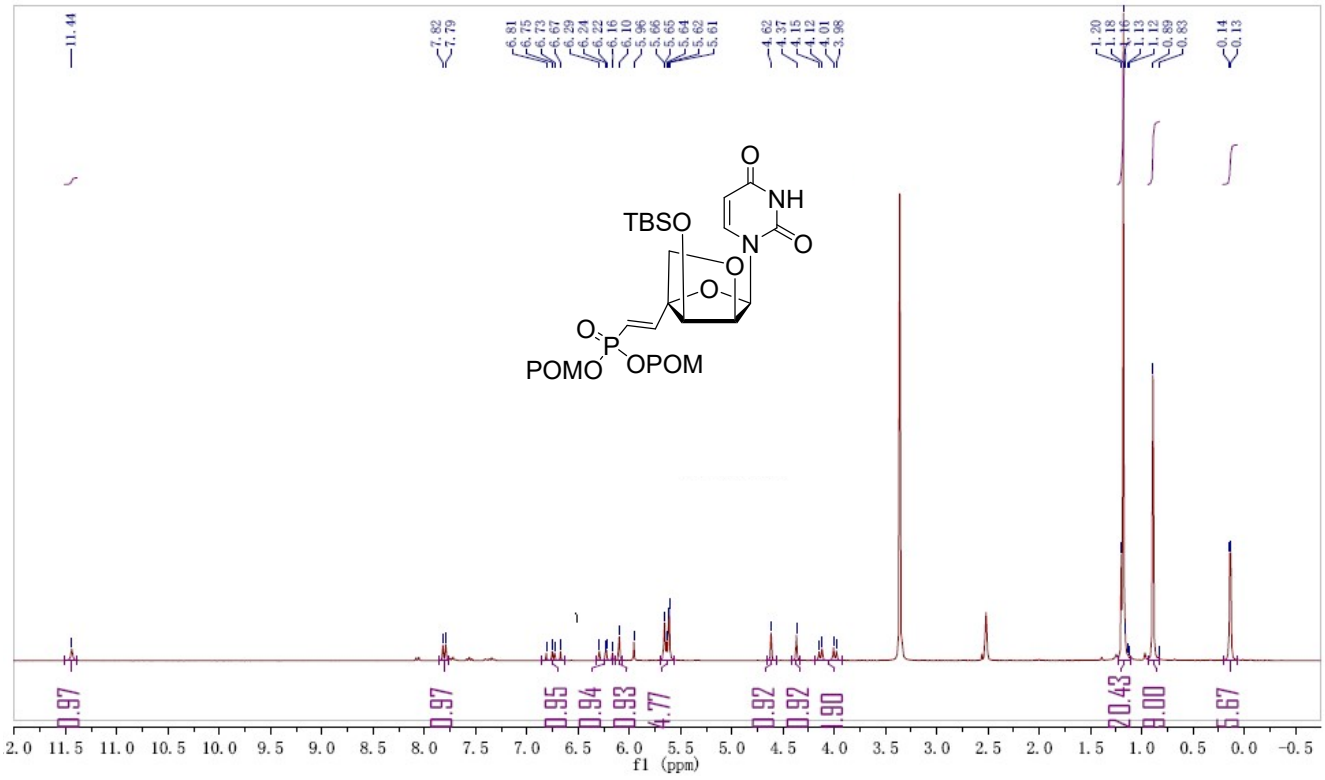
^{31}P NMR (243 MHz, CD_3CN) of **5Z**



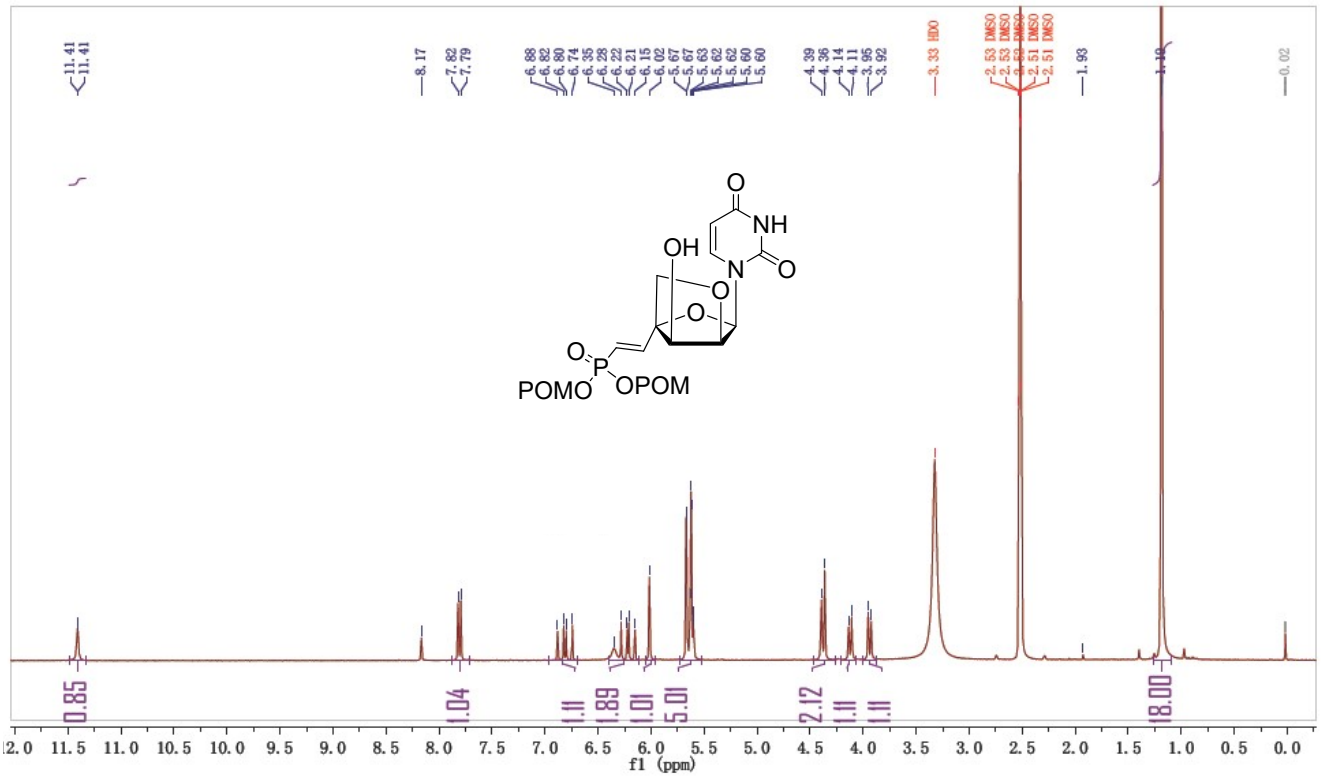
^{1}H NMR (300 MHz, DMSO-d_6) of **7**



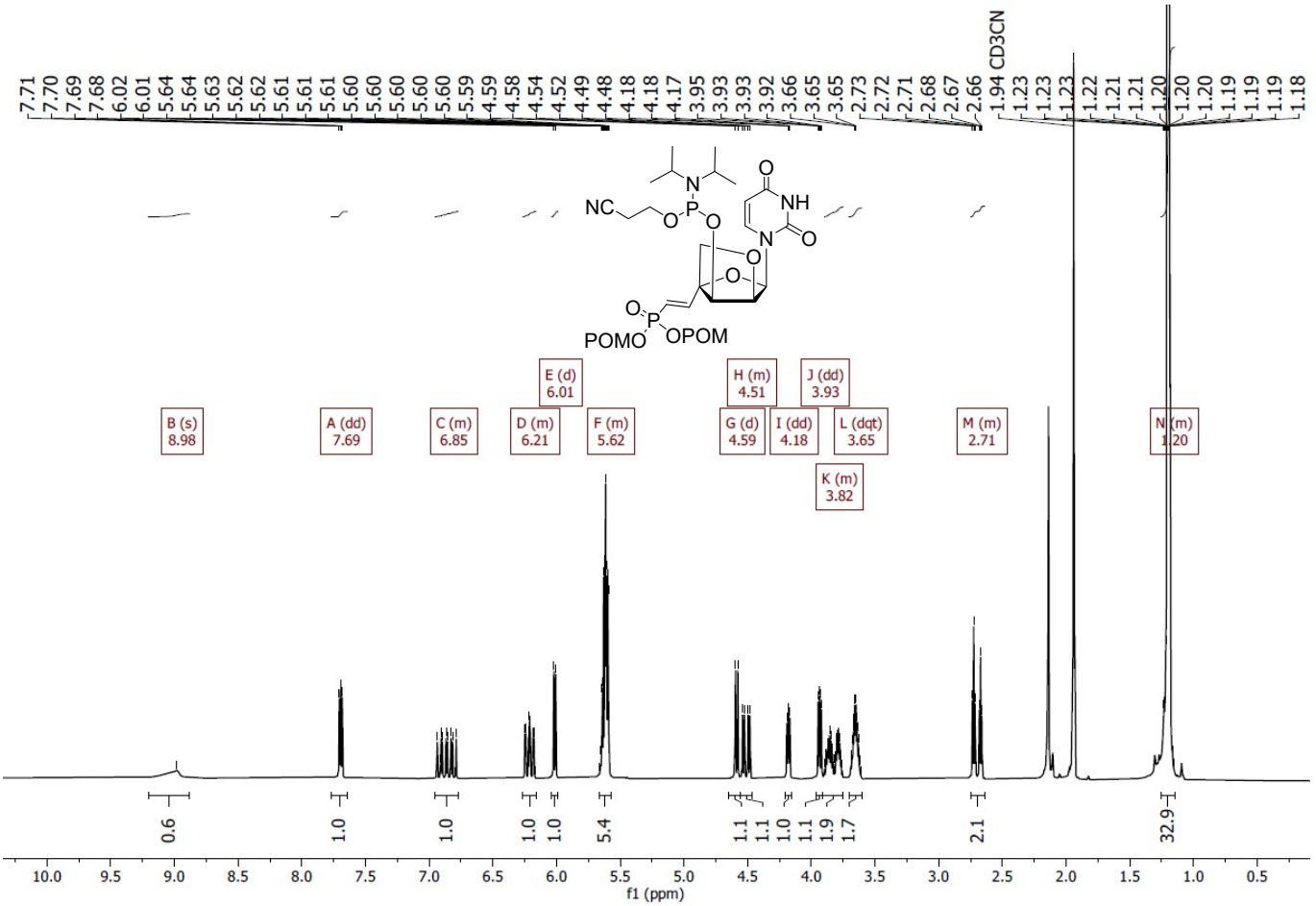
^{1}H NMR (300 MHz, DMSO-d_6) of **8**



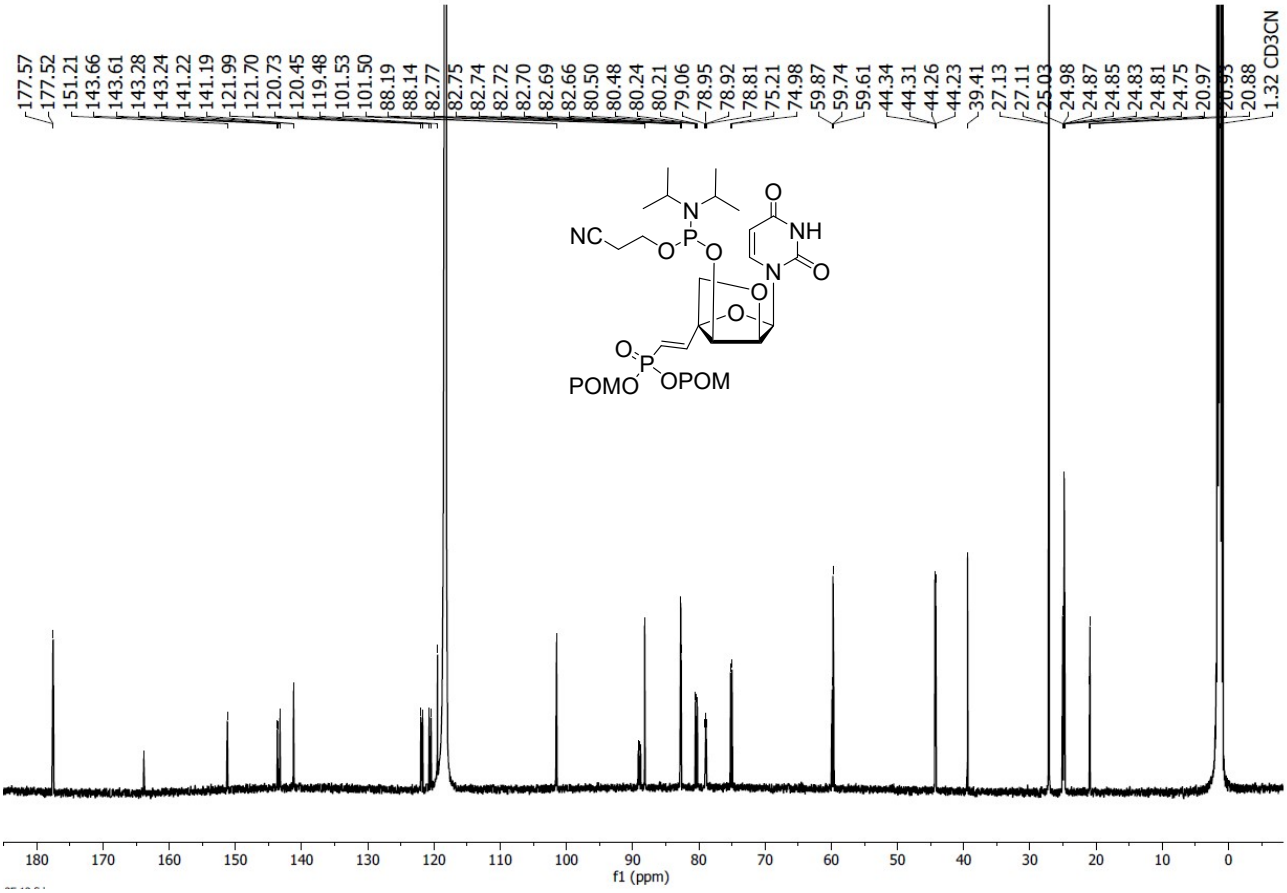
^{1}H-NMR (300 MHz, DMSO-d_6) of **10**



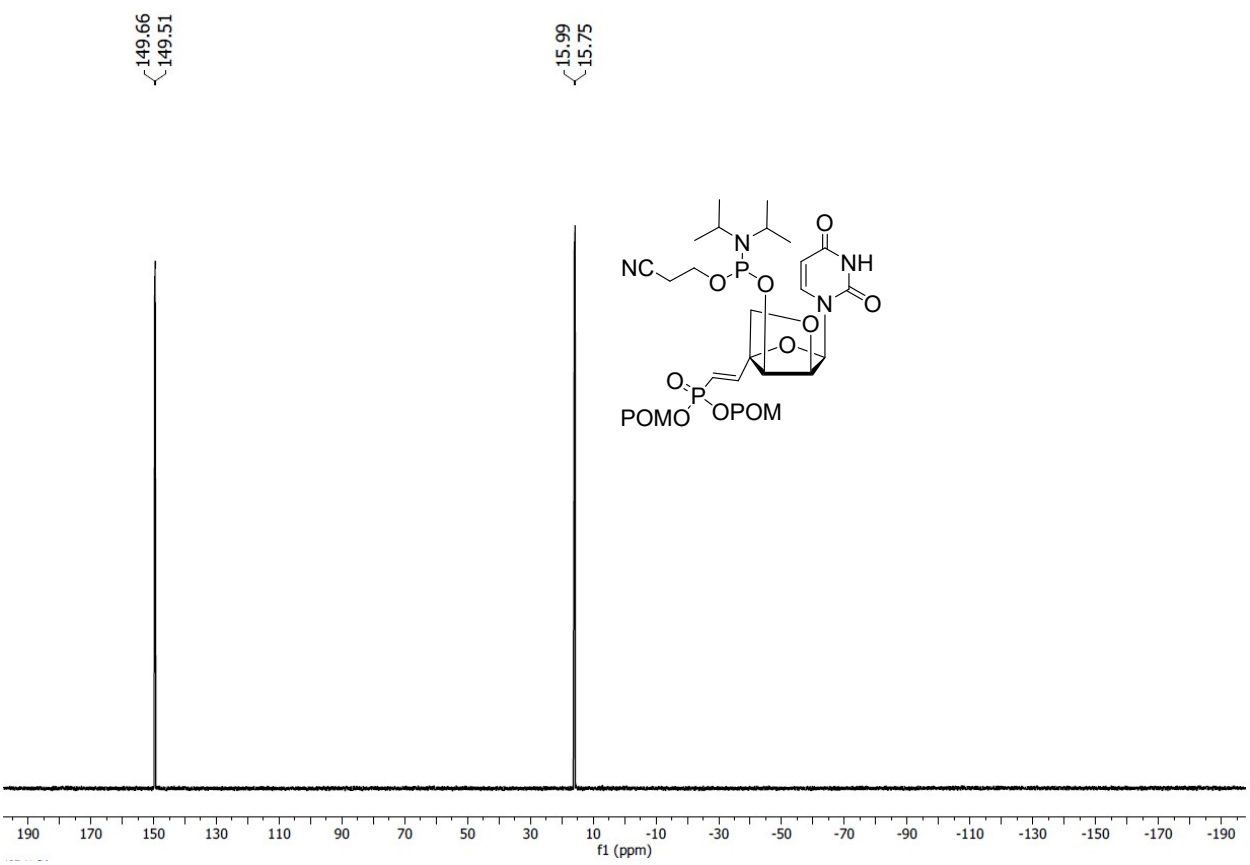
^{1}H NMR (300 MHz, DMSO-d_6) of **11**



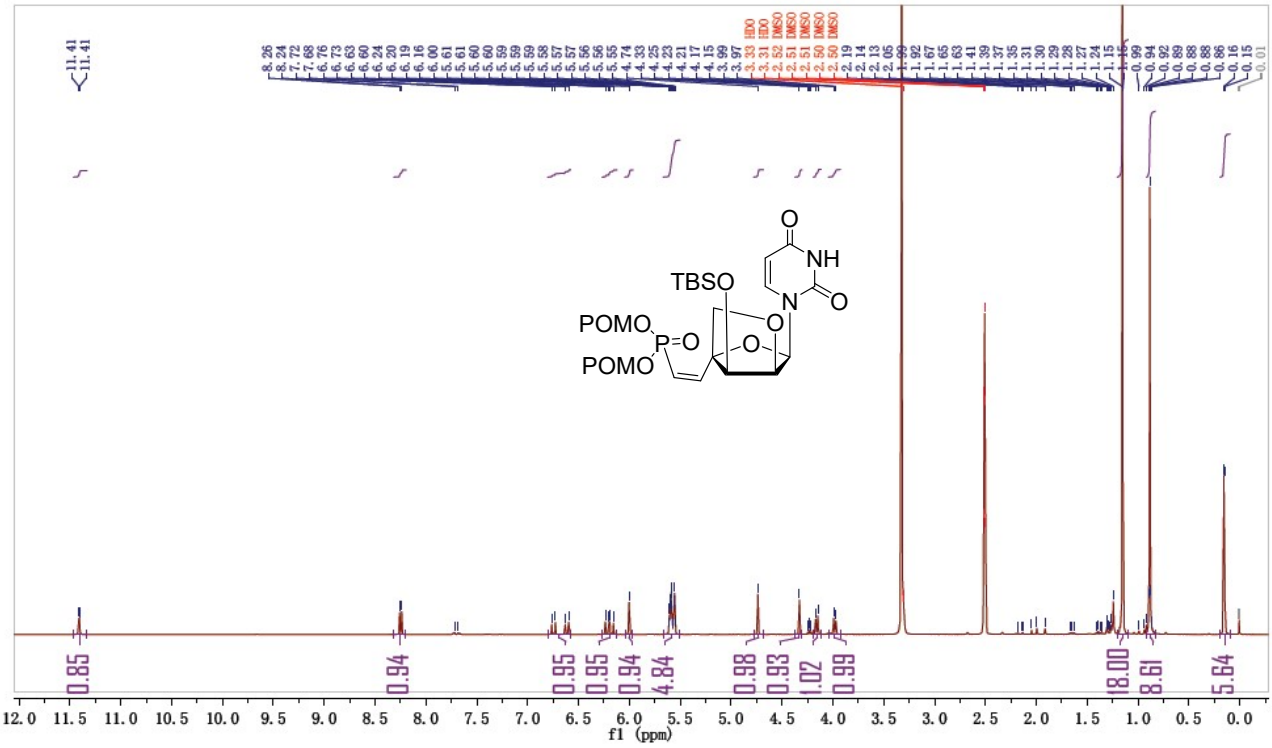
^{1}H NMR (600 MHz, CD_3CN) of **12**



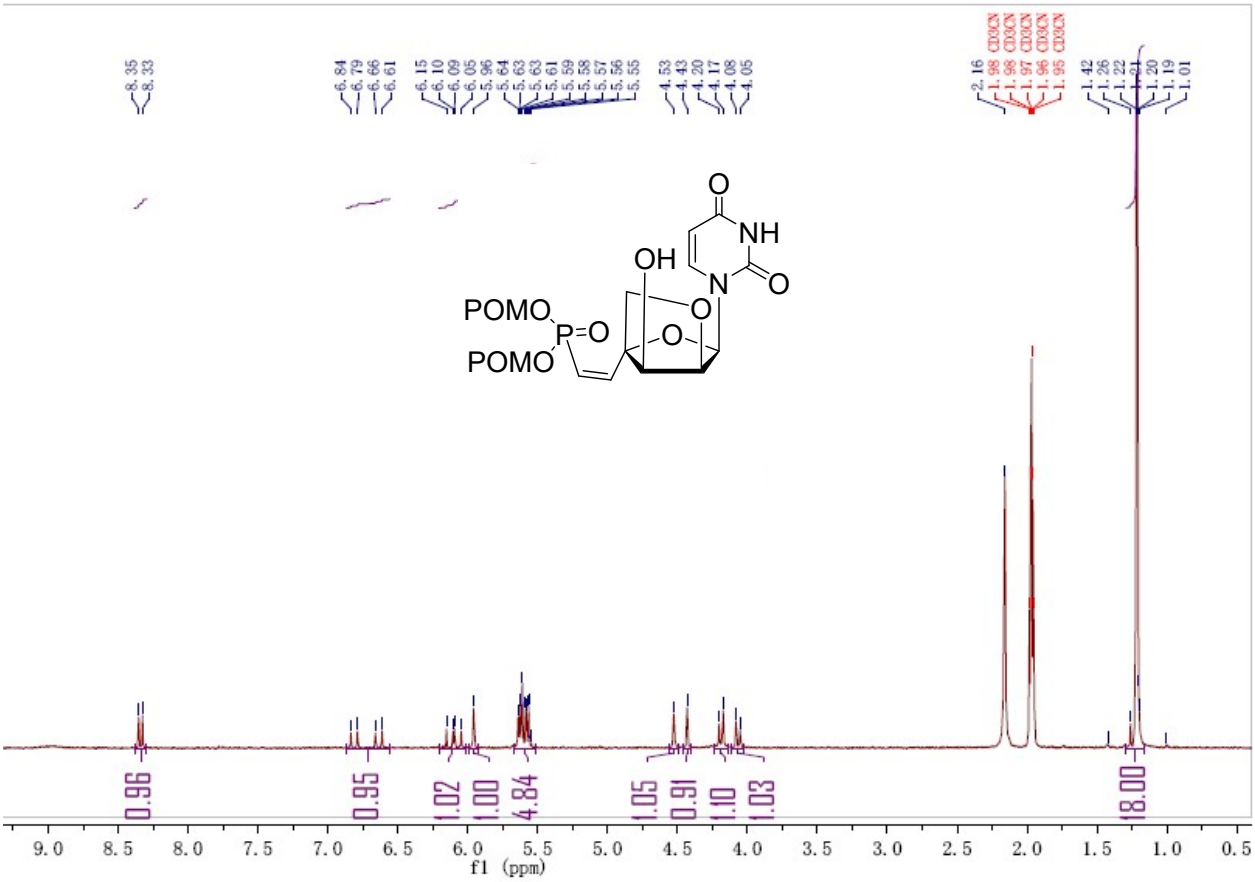
^{13}C NMR (151 MHz, CD_3CN) of **12**



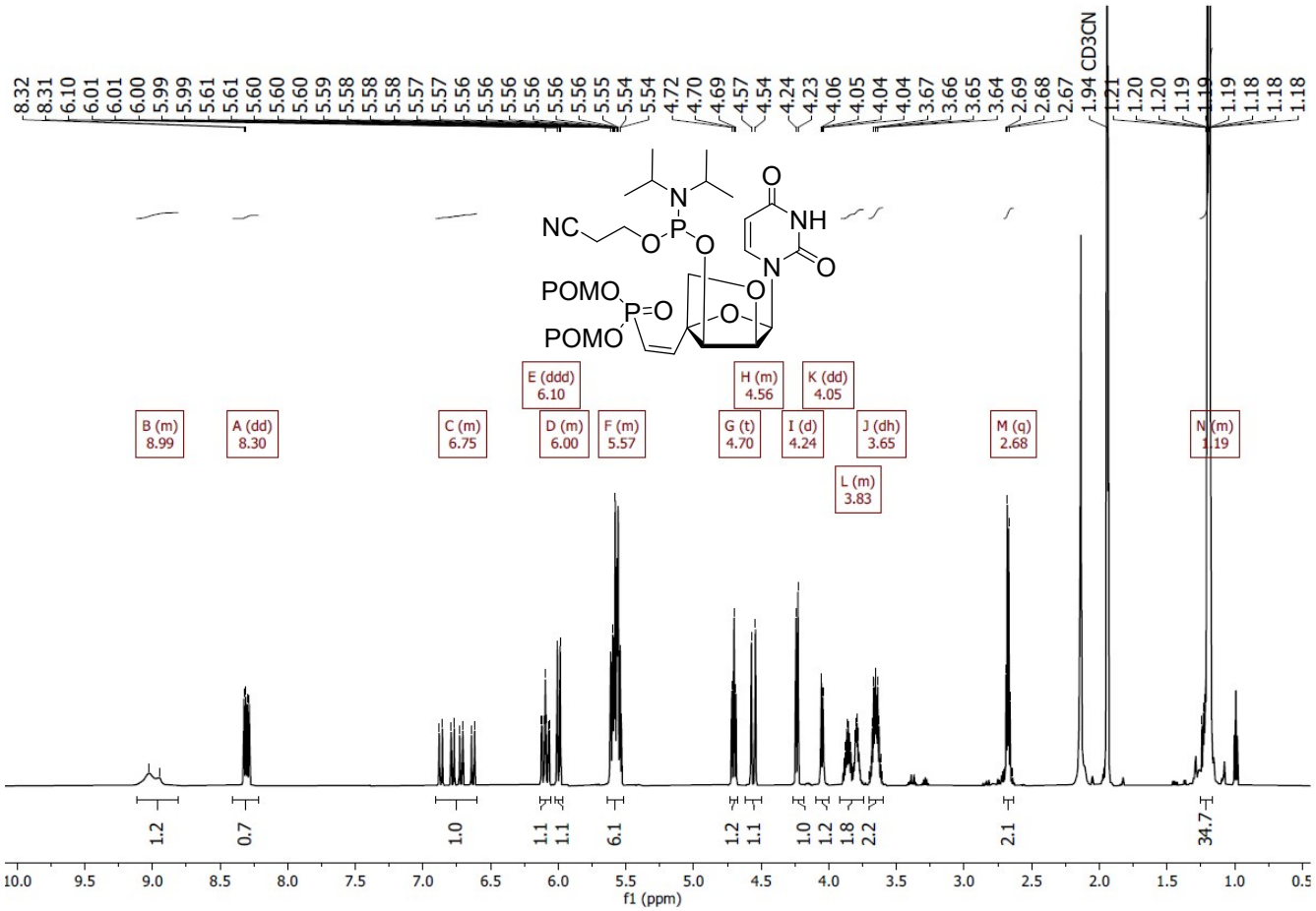
^{31}P NMR (243 MHz, CD_3CN) of **12**



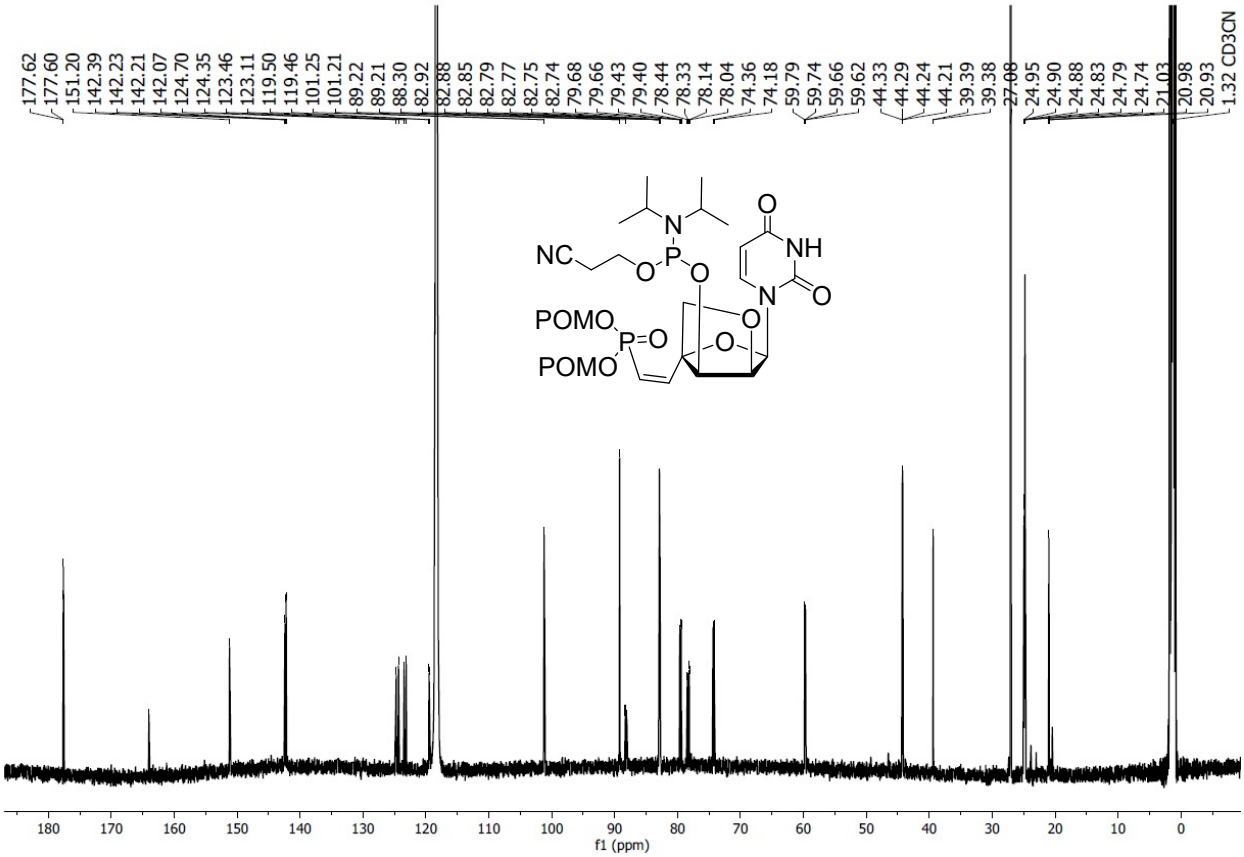
^{1}H NMR (400 MHz, DMSO-d_6) of **10Z**



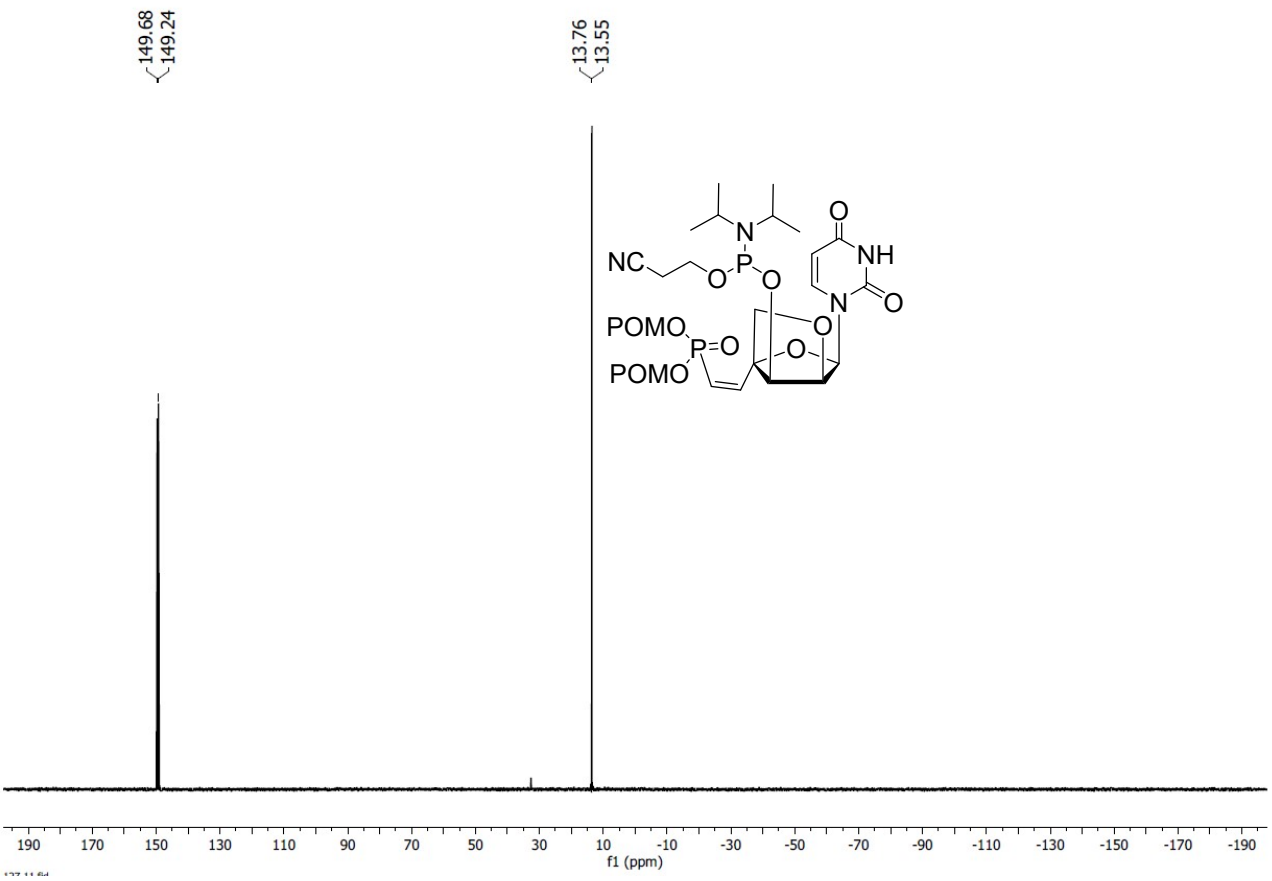
^{1}H NMR (300 MHz, CD_3CN) of **11Z**



^{1}H NMR (600 MHz, CD_3CN) of **12Z**



^{13}C NMR (151 MHz, CD_3CN) of **12Z**



^{31}P NMR (243 MHz, CD_3CN) of **12Z**

Summary of ^{31}P NMR for the new compounds

The following table shows the ^{31}P signals for VP-amidites **5**, **5Z**, **12**, and **12Z** for the phosphoramidite and vinyl phosphonate phosphorus atom, respectively:

Compound #	Phosphoramidite signal (ppm)	vinyl phosphonate signal (ppm)
5	149.45, 149.31	15.80, 15.49
5Z	149.60, 149.16	13.96, 13.76
12	149.66, 149.51	15.99, 15.75
12Z	149.68, 149.24	13.76, 13.55

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