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Supporting Information

Microwave-Assisted Preparation of Nucleoside-Phosphoramidites

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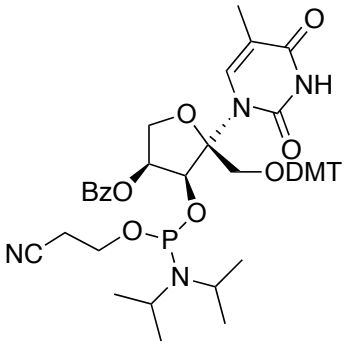
Experimental

Materials & Methods: Anhydrous CH_2Cl_2 was purchased from EMD chemicals and stored over 4Å molecular sieves, prior to use. Anhydrous toluene was obtained from Alfa Aesar and used as received. $(iPr)_2EtN$ (Hünig's base) was obtained from Sigma-Aldrich. All protected DNA and RNA monomers were obtained from Chem-Impex International, Inc. and used as received. 2-cyanoethyl-*N*,*N*,-diisopropyl chlorophosphoramidite **5** was obtained from Rasayn Inc. 2-cyanoethyl-*N*,*N*,*N*',*N*'-tetraisopropyl phosphorodiamidite **6** was obtained from ChemGenes Corporation. Pre-coated flexible silica gel TLC F254 plates were obtained from Whatman Ltd. Flash column chromatography was performed using silica gel 60 (40–60 μm) from Fisher Scientific. ^{1}H NMRs were recorded in parts per million (ppm) using Bruker DRX-600 at 600 MHz for proton referenced to $CDCl_3$ at 7.26 ppm. ^{13}C NMRs were recorded using Bruker DRX-600 equipped with a 5 mm DCH cryoprobe at 150 MHz for carbon referenced to $CDCl_3$ at 77.23 ppm. ^{31}P NMR chemical shifts were recorded in ppm relative to an external probe (85% H_3PO_4) referenced at 0.0 ppm. Mass analysis was performed using Agilent ESI-TOF mass spectrometer at an ESI voltage of 4000V and a flow rate of 200 μL/minute. Microwave assisted synthesis was performed on a Microwave synthesizer Biotage *initiator EXP US* (in K. B. Sharpless lab at TSRI).

Table S1. Phosphitylation attempts on ribulose-thymidine derivative **3** with reagents **5** or **6**.

Entry	Reagent	Activator	Base	Conditions	Observations
1.	**5** (1.5 eq.)	-	Hünig's base	rt, 18 h	Product with a lot of SM (TLC)
2.	**5** (1.5 eq.)	-	Hünig's base	rt, 48 h	Multiple peaks (^{31}P NMR)
3.	**5** (4 eq.)	-	DBU	rt, 6–20 h	Two new & clean spots; does not correspond to the product. (TLC).
4.	**6** (3 eq.)	5-Ethylthiotetrazole	-	rt, 28 h	Product spot together with a lot of SM (TLC)
5.	**6** (3 eq.)	Imidazolium triflate	-	rt, 28 h	Very faint product spot (TLC)
6.	**6** (3 eq.)	Benzimidazolium triflate	-	rt, 28 h	Very faint product spot (TLC)
7.	**6** (3 eq.)	Dicyanoimidazole	-	rt, 18 h	Very faint product spot (TLC)
8.	**6** (3 eq.)	*N,N,N′,N′*-tetraisopropyl-ammonium tetrazolide	-	rt, 18 h	No product (TLC)

4′-O-benzoyl-1′-O-(4,4'-dimethoxytrityl)-β-L-ribulofuranosylthymine-2′-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (**7**): 4′-*O*-benzoyl-1′-*O*-(4,4′-dimethoxytrityl)-β-L-



ribulofuranosylthymine **3** (0.318 g, 0.48 mmol) was azeotroped with anhydrous toluene (3 x 20 mL) and dried under high vacuum overnight. It was further dissolved in 5 mL of anhydrous CH_2Cl_2, followed by the addition of $(iPr)_2EtN$ (0.47 mL, 2.87 mmol) under argon atmosphere. The solution was cooled to 0 °C, followed by the addition of 2-cyanoethyl-*N*,*N*,-diisopropyl chlorophosphoramidite **5 (**0.21 mL, 0.95 mmol). The reaction mixture was brought to room temperature and stirred overnight. The reaction mixture was quenched by the addition of saturated aq. $NaHCO_3$ (5 mL) solution, extracted with CH_2Cl_2 (3 x 50 mL), washed with water (20 mL) followed by brine (50 mL). The combined organic extract was dried over anhydrous $MgSO_4$, filtered, concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (30% EtOAc/hexanes containing 2% Et_3N) to afford 0.144 g (35%) of phosphoramidite **7** (containing traces of H-phosphonate) as a mixture of diastereomers. R_f, 0.40, 0.55 (60% EtOAc/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$, δ (ppm)): 0.80–1.11 (m, 12 H), 2.00–2.11(m, 3H), 2.35–2.35 (m, 1H), 2.42–2.62 (m, 1H), 3.42–3.80 (m, 11H), 4.42–4.44 (m, 2H), 5.25–5.40 (m, 1H), 6.75–6.85 (m, 4H), 7.10–7.45 (m, 15H), 7.55–7.65 (m, 1H), 7.80 (s, 1H), 7.95–8.05 (m, 2H); ^{31}P NMR (243 MHz, $CDCl_3$, δ (ppm)): 153.1, 152.3. HRMS (ESI-TOF high-acc) calcd for $C_{47}H_{53}N_4O_{10}PSi$ $(M+H)^+$: 865.3572, found: 865.3589.

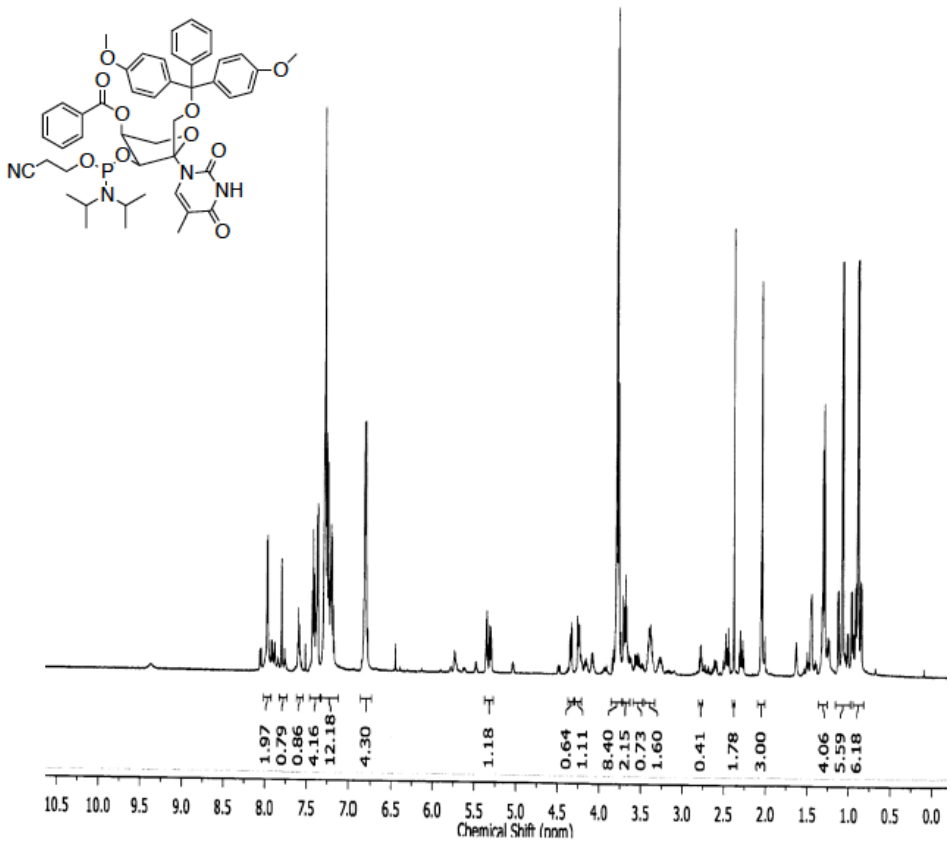


Figure S1. ^{1}H NMR of protected β-L-ribulo-T-phosphoramidite derivative **7** in $CDCl_3$

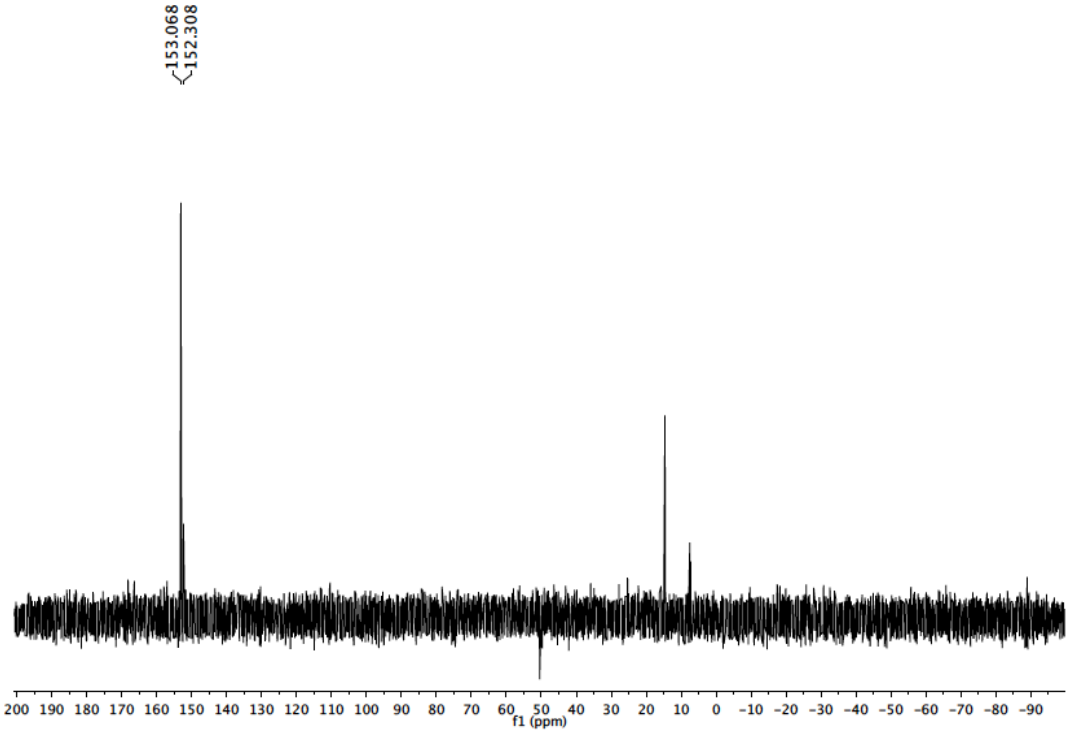


Figure S2. ^{31}P NMR of protected L-ribulo-T-phosphoramidite **7** in $CDCl_3$

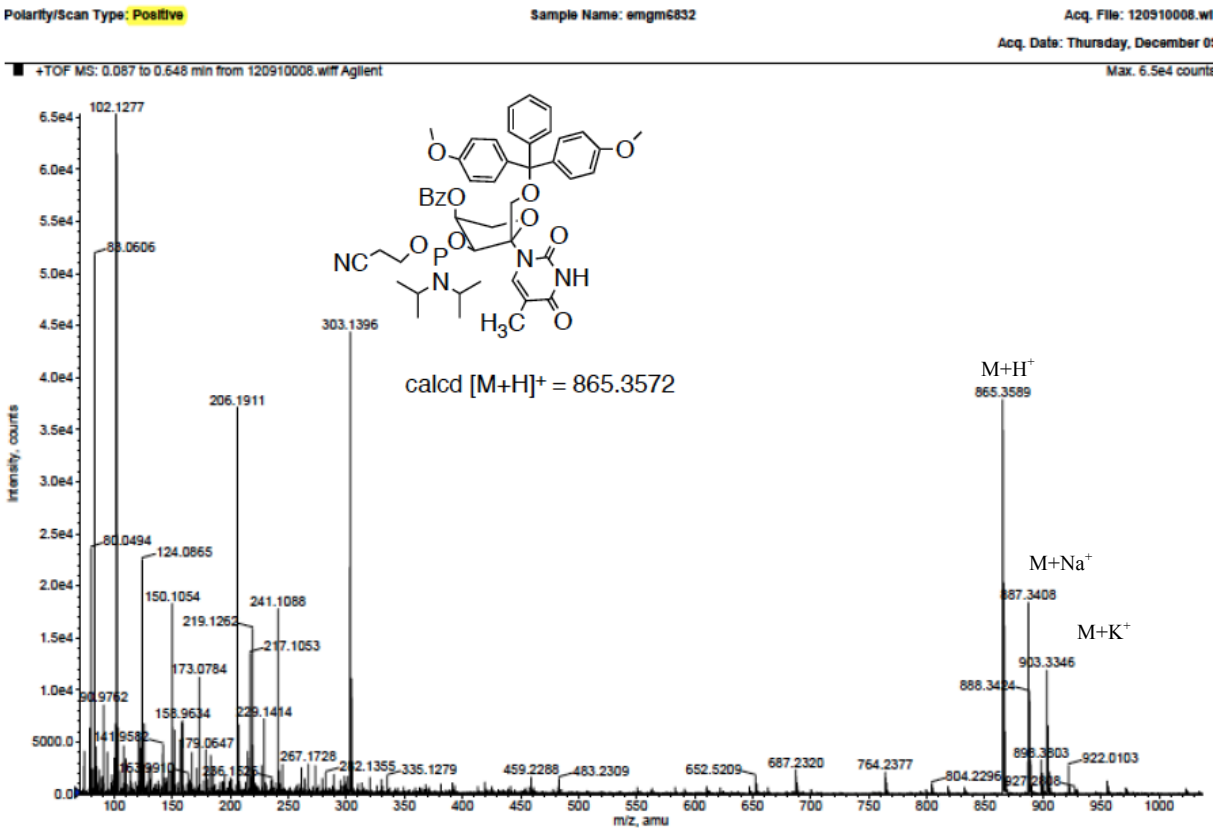


Figure S3. HRMS (ESI-TOF high-accu) of protected L-ribulo-T-phosphoramidite **7**

1′-O-(4,4′-dimethoxytrityl)-4′-O-tri-isopropylsilyloxymethyl-β-L-ribulofuranosylthymine-3′-O-[O-(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (**8**): 1′-dimethoxytrityl-4′-*O*-tri-isopropylsilyloxymethyl-L-ribulofuranosylthymine **4** (0.550 g, 0.74 mmol) was azeotroped with anhydrous toluene (3 x 20 mL), dried under high vacuum overnight, and dissolved in 3 mL of anhydrous CH_2Cl_2, followed by the addition of $(iPr)_2EtN$ (0.48 mL, 2.96 mmol) under argon atmosphere. The resulting solution was transferred to a microwave tube (2–5 mL) containing 2-cyanoethyl-*N*,*N*,-diisopropyl chlorophosphoramidite **5** (0.33 mL, 1.48 mmol). Microwave tube was sealed and irradiated at 65 °C for 20 min. The reaction mixture was quenched by the addition of saturated aq. $NaHCO_3$ (5 mL) solution, extracted with CH_2Cl_2 (3 x 50 mL), washed with water (20 mL) followed by brine (50 mL), and the combined organic extract was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (30% EtOAc/hexanes containing 2% Et_3N) to afford 0.526 g (75%) of pure phosphoramidite **8** as a white amorphous solid (mixture of diastereomers in ratio of 2:1). **8**: R_f, 0.61 and 0.54 (20% Et_2O/CH_2Cl_2); R_f: 0.62 and 0.42 (45% EtOAc/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$ δ (ppm)): 0.90–1.20 (m, 33H, $Si(CHCH_3)_3$, $Si(CHCH_3)_3$ and $N(CHCH_3)_2$), 1.98 and 2.03 (2s, 3H, CH_3(C–5)), 2.14–2.57 (m, 2H,

OCH$_2$*CH$_2$*CN), 3.34–3.50 (m, 2H, 2 Me$_2$*CH*), 3.53–4.28 (m, 13 H, H-1′, H-1″, 2 O*CH$_3$*, H-5′, H-5″, H-4′ and O*CH$_2$*CH$_2$CN), 4.82, 4.87 (2d, *J* = 4.8, 5.4 Hz, 1H, O*CH$_2$*O), 4.95, 5.00 (2d, *J* = 5.4, 4.8 Hz, 1H, O*CH$_2$*O), 5.04, 5.16 (2dd, *J* = 10.8, 4.8 Hz and *J* = 11.4, 5.4 Hz, H-3′), 6.66–6.86 (m, 4H, arom.), 7.15–7.33 (m, 9H, arom.), 7.69–7.76 (m, 1H, H-6), 7.84 (brs, 1H, NH). ^{13}C NMR (150 MHz, CDCl$_3$): 12.1, 12.1 (*C*H$_3$-C5) 12.8, 12.9, 18.0, 18.0 (Si(CH*C*H$_3$)$_3$), 19.9, 19.9 (Si(*C*HCH$_3$)$_3$, 20.5, 20.5 (*CH$_2$*CN), 24.4, 24.5, 24.6, 24.7, 24.9 (NCH(*C*H$_3$)$_2$), 43.4, 43.4, 43.6, 43.7 (N*C*H(CH$_3$)$_2$), 55.4, 55.4 (O*C*H$_3$), 58.6, 58.8, 58.9 (O*CH$_2$*CH$_2$CN), 63.1, 63.5 (C-1′), 70.2, 70.7 (C-5′), 74.8, 75.0, 75.1, 76.3 (C-3′/C-4′), 86.1, 86.2 (quaternery C-DMTr), 89.8, 90.1 (O*C*H$_2$O), 99.8, 99.9 (C-2′), 100.1, 100.2, 108.9, 109.3 (C-5), 113.2, 113.2, 113.2, 113.3 (arom.), 117.8, 117.9 (*C*N), 127.0, 127.9, 128.2, 128.3, 130.1, 130.1, 130.2, 130.2, 135.9, 135.9, 136.1 (arom.), 137.7, 138.0 (C-6), 144.8, 144.9, 149.8, 149.8 (C-2) (only 1 of each on carbon spectrum), 158.6, 158.6 (arom.) 164.1, 164.2 (C-4); ^{31}P NMR (243 MHz, CDCl$_3$ δ (ppm)): 152.5, 152.7; HRMS (ESI-TOF high-acc) calcd for C$_{50}$H$_{71}$N$_4$O$_{10}$PSi (M+H)$^+$: 947.4750, found: 947.4750.

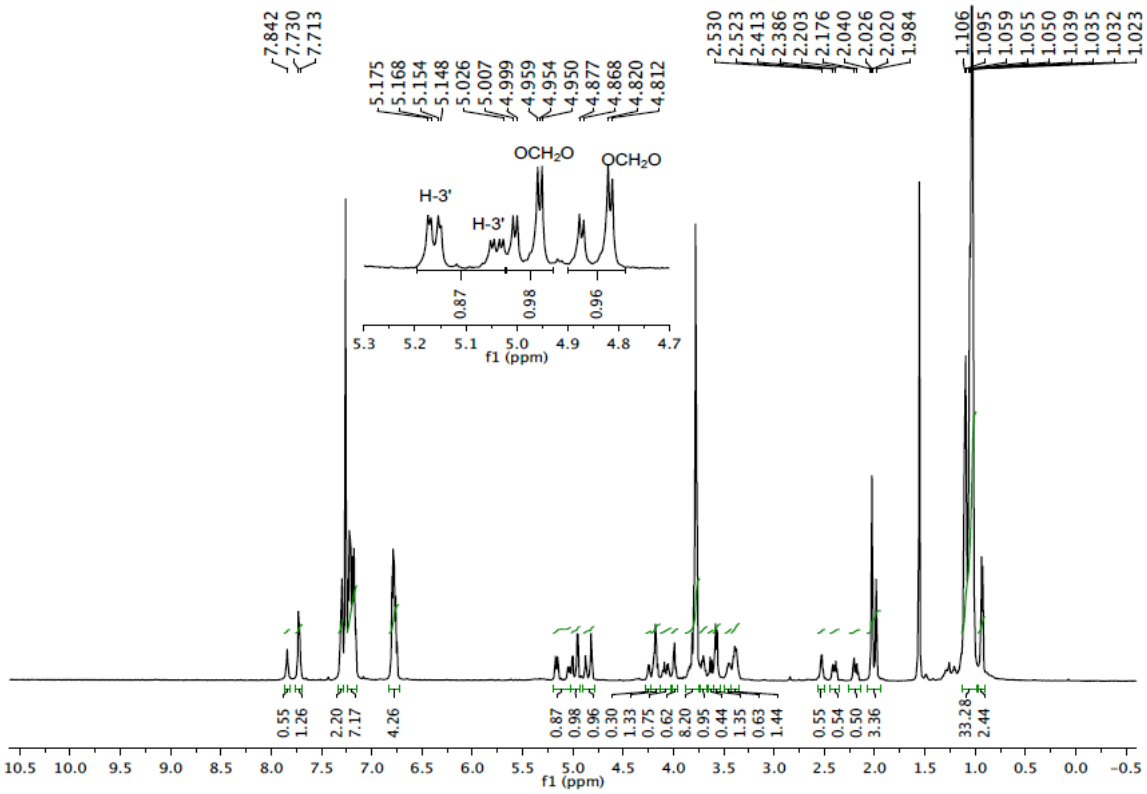


Figure S4. ^{1}H NMR of protected β-L-ribuloT-phosphoramidite **8** in CDCl$_3$

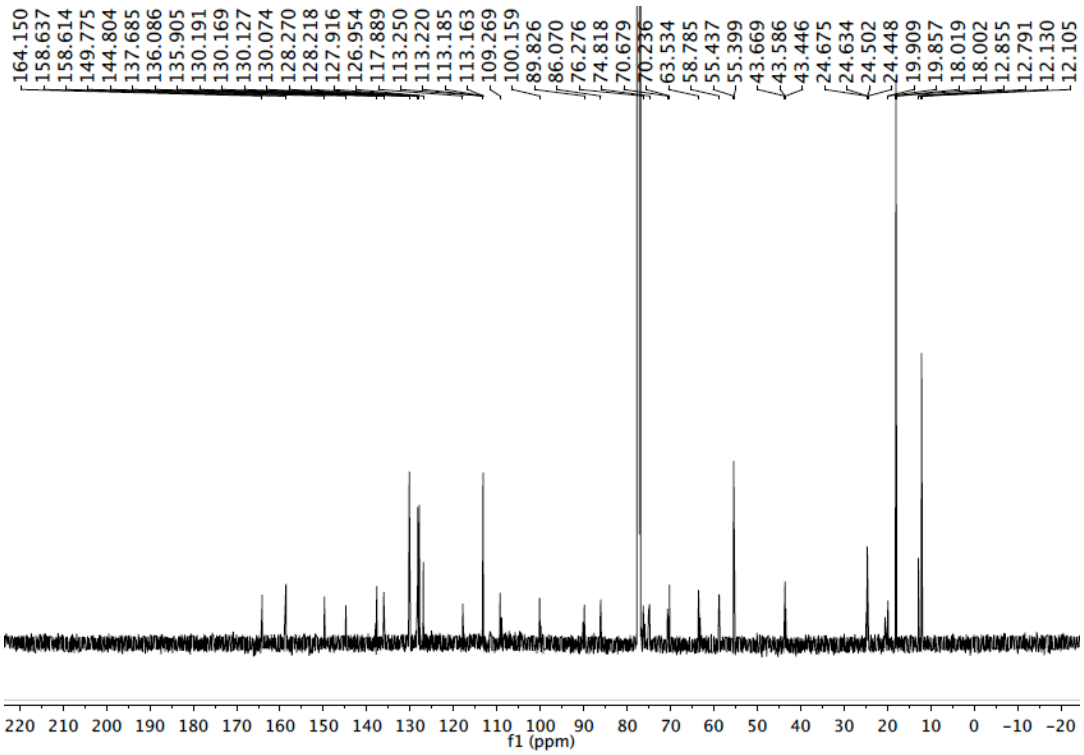


Figure S5. ^{13}C NMR of protected-β-L-ribulo-T-phosphoramidite **8** in $CDCl_3$

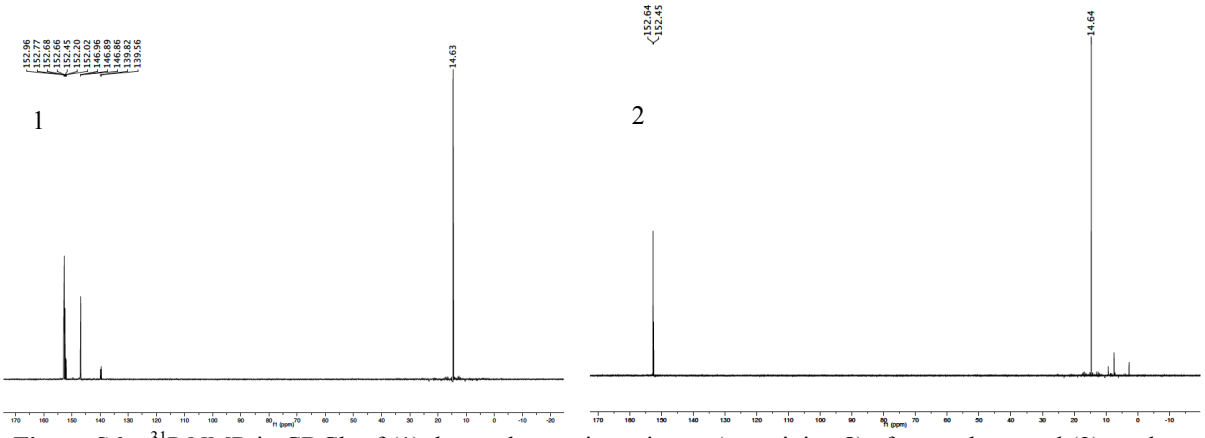


Figure S6a. ^{31}P NMR in $CDCl_3$ of (1) the crude reaction mixture (containing **8**) after work-up and (2) crude reaction mixture (containing **8**) after precipitation from CH_2Cl_2/hexanes. The presence of the excess reagent **5** resulted in the corresponding H-phosphonate (derived from **5**) after work-up. While attempts to remove H-phosphonate from the crude product by dissolving in minimum amount of CH_2Cl_2 and precipitating in hexanes were unsuccessful, other side products came out of solution as a sticky liquid. The solution was decanted and concentrated; the resulting residue, which showed a clean ^{31}P NMR (peaks at 152.7 and 152.4 ppm for product **8** and 14 ppm for H-phosphonate, Figure S6a-2), was further purified by short silica gel column chromatography to afford 75% yield of pure **8** free of H-phosphonate (Figure S6b).

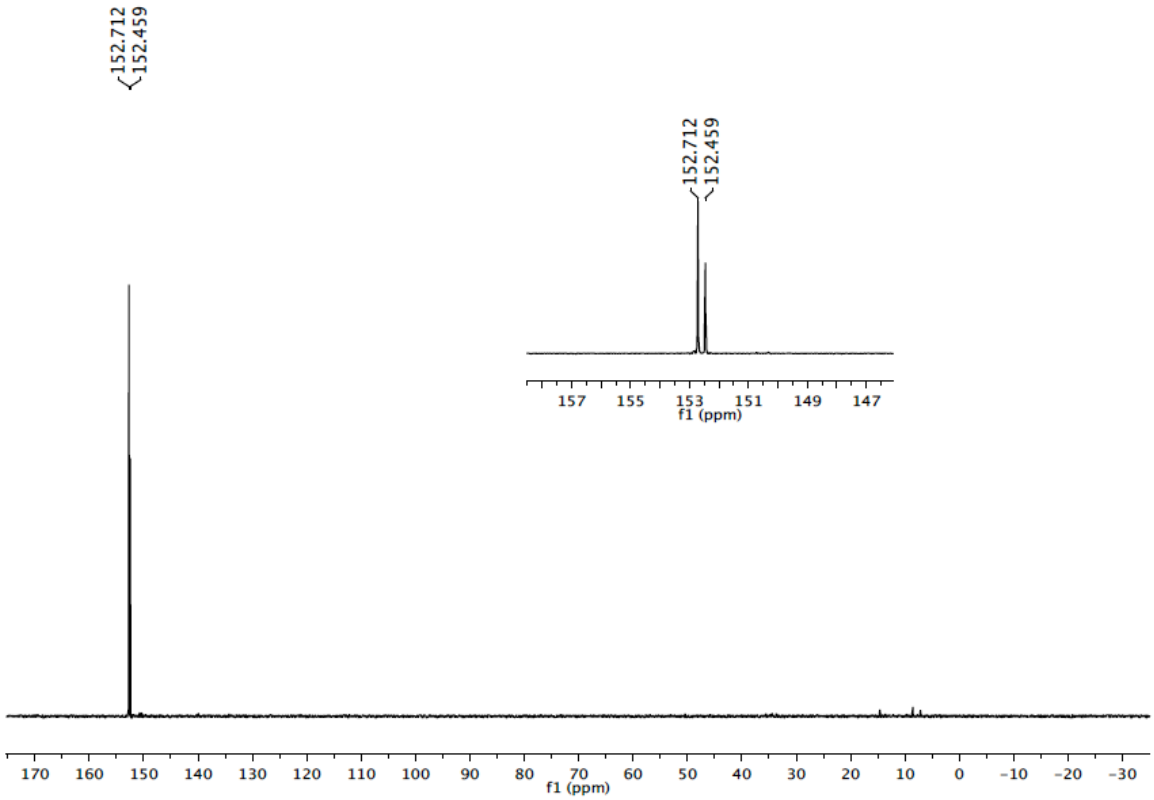


Figure S6b. ^{31}P NMR of protected-β-L-ribuloT-phosphoramidite **8** in $CDCl_3$

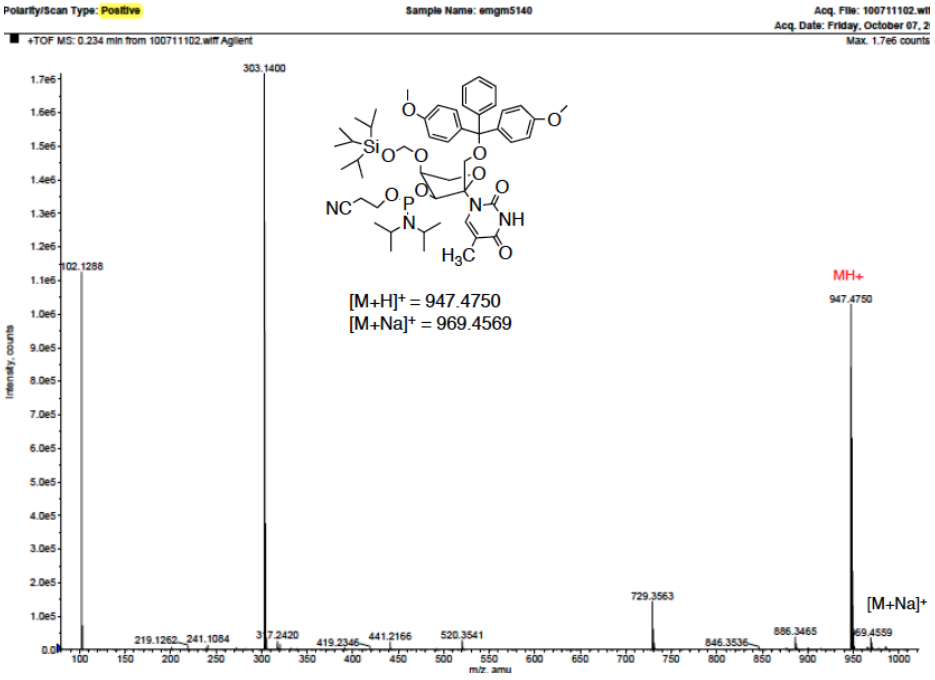


Figure S7. HRMS (ESI-TOF high-accu) of protected-β-L-ribuloT-phosphoramidite **8**

N6-benzoyl-1'-O-dimethoxytrityl-4'-O-triisopropylsilyloxymethyl-β-L-ribulofuranosyl-adenine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (**12**): DMT-protected nucleoside **9** (300 mg, 0.35 mmol) was dried by coevaporation with toluene (3 x 20 mL) and further dissolved in anhydrous CH_2Cl_2 (2.57 mL), followed by the addition of $(i\text{Pr})_2\text{EtN}$ (238 µL, 1.40 mmol, 4 eq.). 2-cyanoethyl-*N*,*N*,-diisopropyl chlorophosphoramidite **5** (157 µL, 0.70 mmol, 2.0 eq.) was added to the stirred solution and irradiated in a microwave reactor at 65 °C for 1 h. The reaction mixture was cooled to 0 °C and quenched by the addition of saturated aqueous $NaHCO_3$ (3 mL) solution. The reaction mixture was partitioned between CH_2Cl_2 (50 mL) and saturated aqueous $NaHCO_3$ (30 mL). Then, the aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL). The organic extracts were combined and washed with brine (20 mL) and dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography (hexanes/EtOAc 2:1 (2% Et_3N) → hexanes/EtOAc 3:2 (2% Et_3N)) to afford compound **12** as a white foam (256 mg, 0.24 mmol, 69% yield). **12**: R_f: 0.44 (hexanes/EtOAc 2:1). ^{1}H NMR (600 MHz, $CDCl_3$, δ (ppm)): 0.93–1.06 (*m*, 29H), 1.11–1.13 (*m*, 6H), 2.28–2.44 (*m*, 1H), 2.55–2.56 (*m*, 1H), 3.41–3.50 (*m*, 2H), 3.62–3.65 (*m*, 1H), 3.75–3.76 (*m*, 6H, 2 x OMe (DMT)), 3.79–4.29 (*m*, 5H), 4.46–4.51 (*m*, 1H), 4.76–4.81 (2*d*, *J* = 5.1, 5.0 Hz, 1H (2 diastereomers)), 4.92–4.96 (2*d*, *J* = 5.0, 5.1 Hz, 1H (2 diastereomers)), 5.45–5.51 (*m*, 1H), 6.64–6.72 (*m*, 4H), 7.01–7.20 (*m*, 9H), 7.52–7.55 (*m*, 2H), 7.60–7.61 (*m*, 1H), 8.03–8.06 (*m*, 2H), 8.33–8.40 (*m*, 1H), 8.55–8.61 (*m*, 1H), 9.04–9.07 (*m*, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, δ (ppm)): 12.0, 12.0, 17.9, 17.9, 17.9, 20.4, 20.4, 20.5, 24.4, 24.5, 24.5, 24.6, 24.6, 24.8, 24.8, 43.3, 43.5, 55.3, 59.0, 59.2, 63.4, 64.2, 71.4, 75.8, 86.1 (2*s*, quaternary C (DMT) (2 diastereomers)), 86.2, 89.8 (2*t*, OCH_2O (2 diastereomers)), 89.9, 98.4 (2*s*, C2' (2 diastereomers)), 98.6, 113.0 (arom. C (DMT)), 113.1, 113.1, 117.6 (2*s*, CN (2 diastereomers)), 117.7, 123.9 (arom. C), 126.8, 126.8, 127.8, 127.8, 128.0, 128.0, 128.1, 129.0, 129.8, 130.0, 130.0, 132.9, 132.9, 133.9, 133.9, 135.3, 135.7, 135.7, 142.9, 143.1, 144.3, 144.4, 149.2, 149.4, 150.5, 150.6, 151.9, 151.9, 158.5, 158.5, 164.6 (s, CO (Bz)); ^{31}P NMR (243 MHz, $CDCl_3$, δ (ppm)): 153.15, 153.31; HRMS (ESI-TOF high-acc.): calcd 1060.5127 ($[M+H]^+$); found 1060.5127 ($[M+H]^+$).

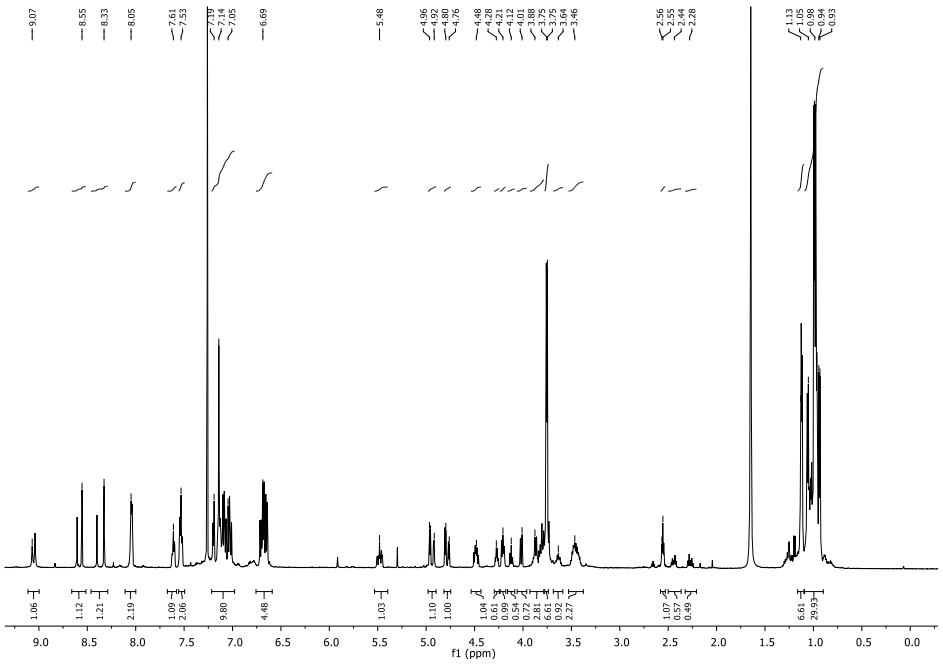


Figure S8. ^{1}H NMR of **12** in $CDCl_3$

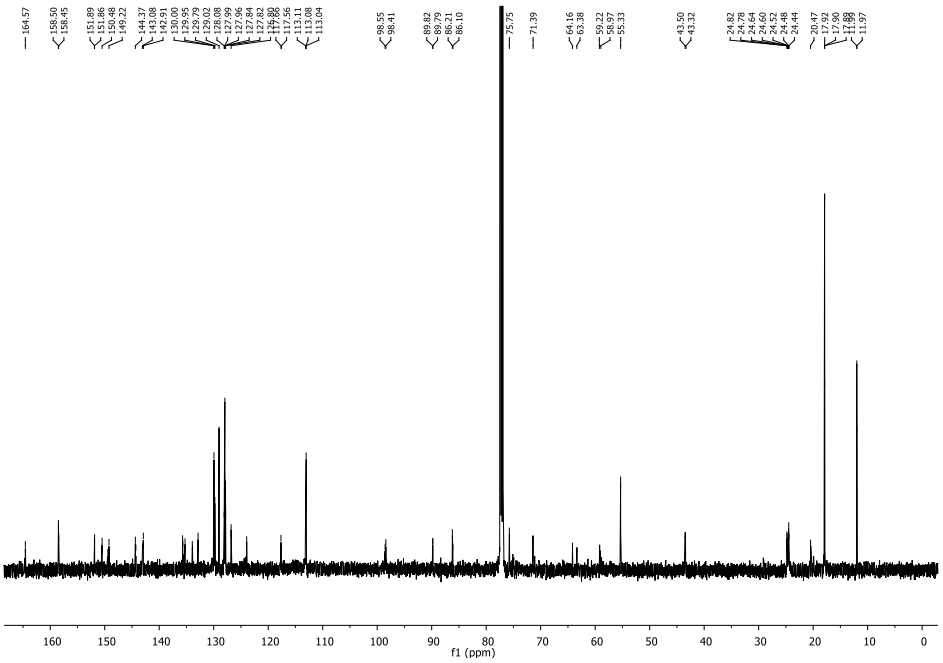


Figure S9. ^{13}C NMR of **12** in $CDCl_3$

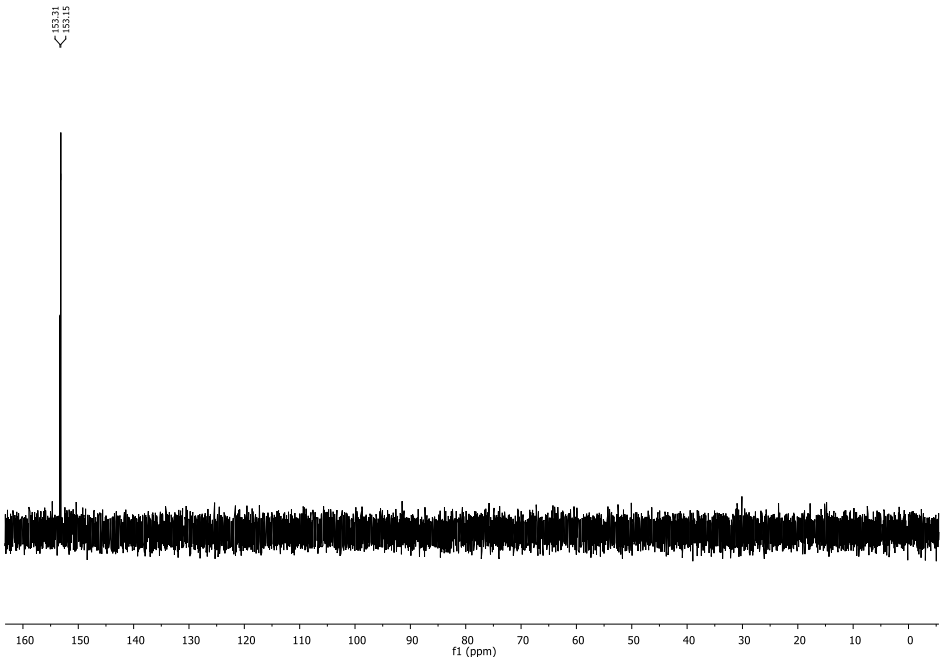


Figure S10. ^{31}P NMR of **12** in $CDCl_3$

N4-benzoyl-1'-O-dimethoxytrityl-4'-O-triisopropylsilyloxymethyl-β-L-ribulofuranosyl-cytosine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (**13**). DMT-protected nucleoside **10** (287 mg, 0.34 mmol) was dried by co-evaporation with toluene (3 x 10 mL) and dissolved in anhydrous CH_2Cl_2 (2.5 mL) followed by the addition of $(iPr)_2EtN$ (231.5 µL, 4 eq.). 2-cyanoethyl-*N*,*N*,-diisopropyl chlorophosphoramidite **5** (152.2 µL, 2.0 eq.) was added to the stirred solution and irradiated in a microwave reactor at 65 °C for 1 h. Saturated aqueous $NaHCO_3$ (3 mL) solution was added to quench the reaction at room temperature. The mixture was partitioned between CH_2Cl_2 (50 mL) and saturated aqueous $NaHCO_3$ (30 mL). Then, the aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic extract was washed with brine (20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by silica gel flash chromatography (hexanes/EtOAc 2:1 (2% Et_3N) → hexanes/EtOAc 3:2 (2% Et_3N)) to yield compound **13** as white foam (261 mg, 74% yield, 2:1 diasteromers). Data for **13**: R_f: 0.53 (AcOEt:hexanes, 1:1 containing 2%Et_3N); ^{1}H NMR (600 MHz, $CDCl_3$, δ (ppm)): 1.01–1.15 (m, 33H, Si(CHC*H*$_3$)$_3$, Si(C*H*CH$_3$)$_3$ and N(CHC*H*$_3$)$_2$), 2.28 (2t, *J* = 7.4 Hz, 0.3H, OCH$_2$*CH*$_2$CN), 2.52–2.60 (m, 0.7H, OCH$_2$*CH*$_2$CN), 3.38–3.51 (m, 2H, N(C*H*CH$_3$)$_2$), 3.59–4.25 (m, 13H, H-1′, H-1′′, 2 O*CH*$_3$, H-4′, H-5′, H-5′′ and O*CH*$_2$CH$_2$CN), 4.80–4.85 (m, 1H, O*CH*$_2$O), 4.96–5.00 (m, 1H, O*CH*$_2$O), 5.22–5.33 (m, 1H, H-3′), 6.76–6.84 (m, 4H, arom.), 7.16–7.32 (m, 12H, arom., H-5), 7.52–7.66 (m, 4H, arom., H-6), 7.94 (bs, NH); ^{13}C NMR (150 MHz, $CDCl_3$): 12.2–12.3 (Si(CH*C*H$_3$)$_3$, 18.2 (Si(*C*HCH$_3$)$_3$), 19.9–20.4 (OCH$_2$*CH*$_2$CN), 24.6–25.1 (NCH(*C*H$_3$)$_2$), 43.6–43.9 (N*C*H(CH$_3$)$_2$), 55.5, 55.6 (O*CH*$_3$), 59.1–59.5 (O*C*H$_2$CH$_2$CN), 63.54 (C-1′), 70.5, 70.7 (C-5′), 74.4-74.8 (C-3′/C-4′), 86.3 (quaternary C-DMTr), 89.9 (O*CH*$_2$O), 101.3 (C-2′), 113.4 113.3, 113.3, 113.4, 118.1, 118.3, 127.1, 128.1, 128.5, 129.5, 130.3, 130.4, 133.6, 136.3, 145.1, 145.2, 155.0, 158.7, 162.6, 163.4 (arom. C, C-4, C-5, CO); ^{31}P NMR (243 MHz, $CDCl_3$ δ (ppm)): 153.1, 153.3; HRMS (ESI-TOF high-acc) calcd for $C_{56}H_{74}N_5O_{10}PSi$ $(M+H)^+$: 1036.5015, found: 1036.4998. Calcd for $(M+MeOH+H)^+$: 1068.5283, found 1068.5267. Additional peaks: $(M+H_2O+H, 100\%)^+$: 1054.5126/1054.5106, $(M+Et_3NH)^+$: 1137.6225/1137.6202. The peak at 967 is attributed to $(M-N(CHCH_3)_2+MeOH)^+$.

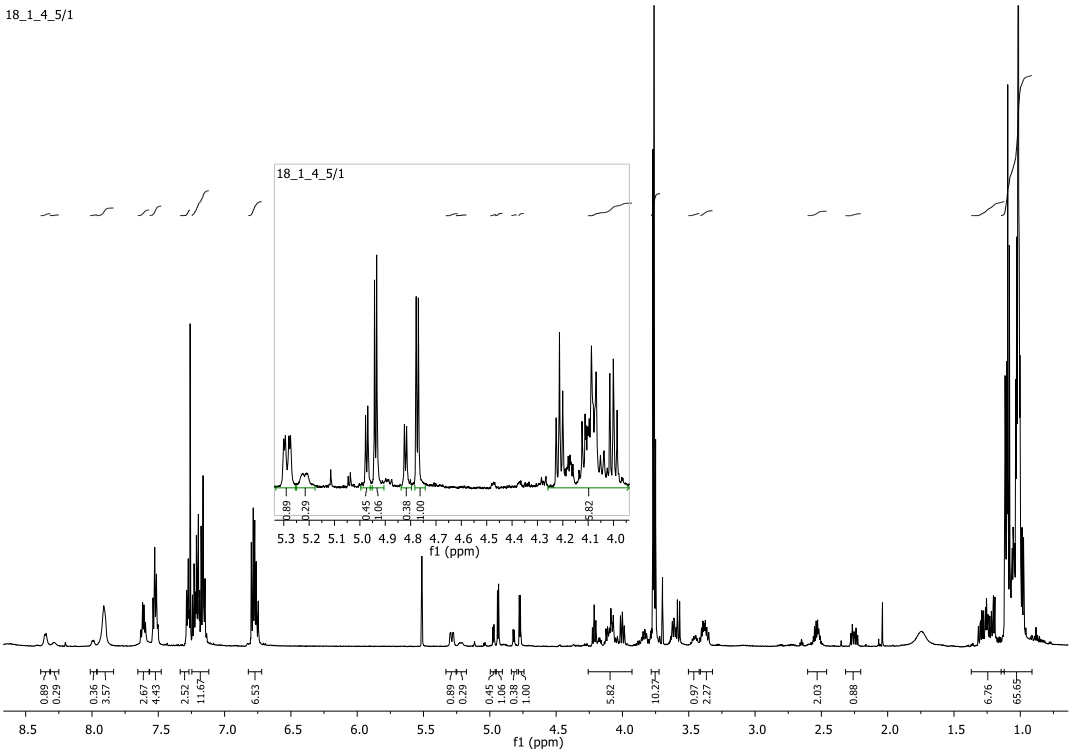


Figure S11. ^{1}H NMR of **13** in $CDCl_3$

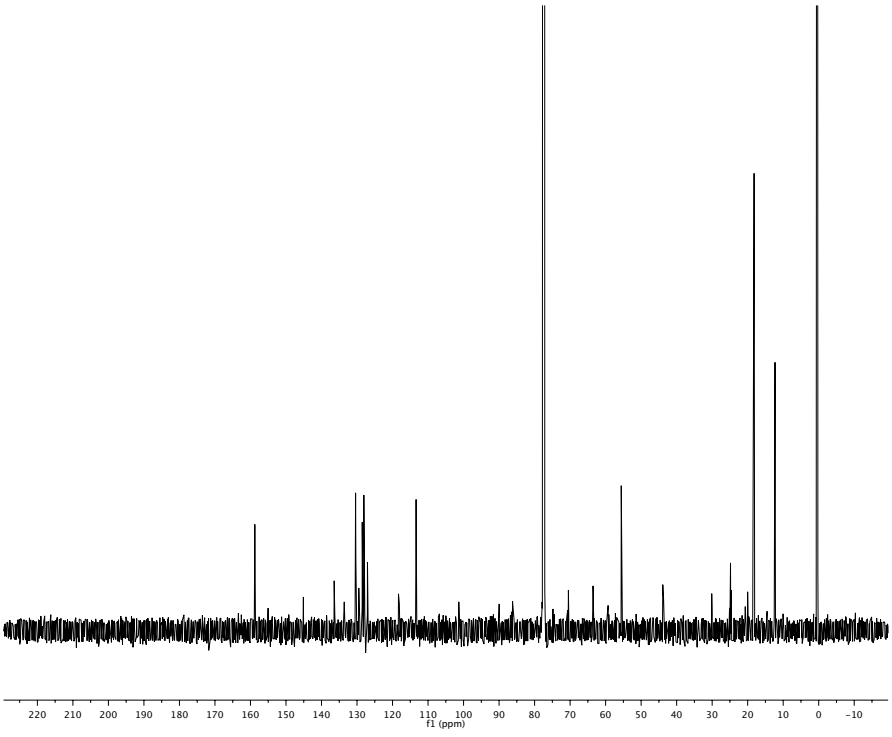


Figure S12. ^{13}C NMR of **13** in $CDCl_3$

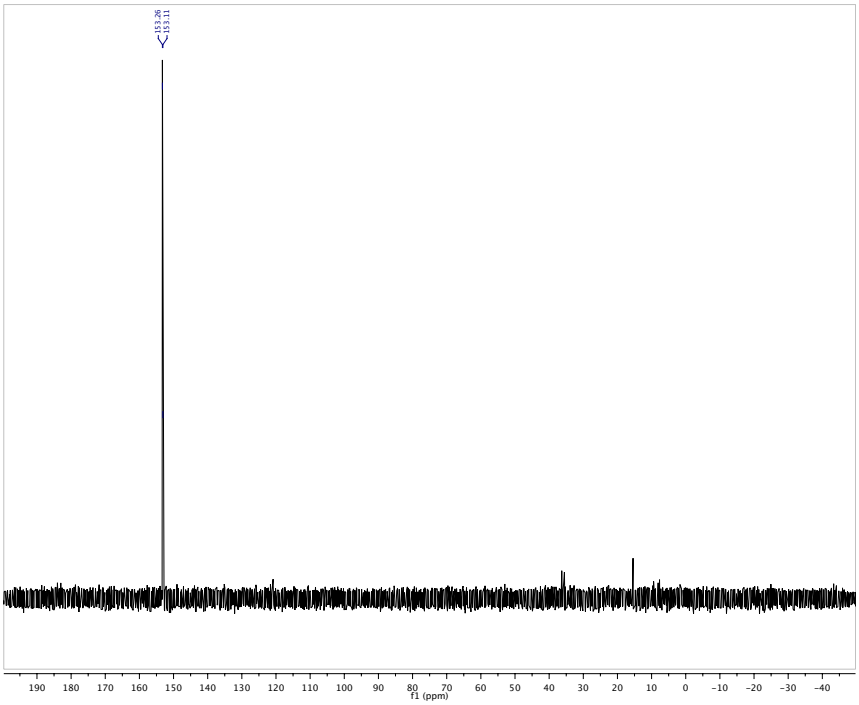


Figure S13. ^{31}P NMR of **13** in $CDCl_3$

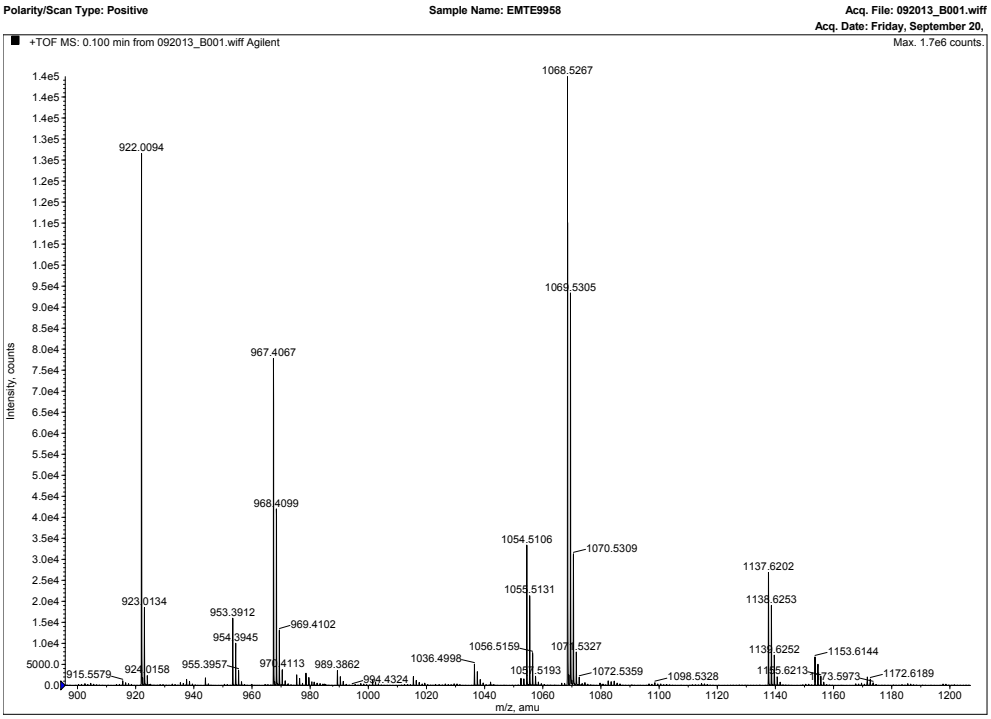


Figure S14. HRMS (ESI-TOF high-accu) of phosphoramidite **13**

N2-acetyl-1'-O-dimethoxytrityl-4'-O-triisopropylsilyloxymethyl-β-L-ribulofuranosyl-guanine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (**14**). DMT-protected nucleoside **11** (50 mg, 49.5 µmol) was dried by co-evaporation with toluene (3 x 10 mL) and dissolved in anhydrous CH_2Cl_2 (500 µL) followed by the addition of $(iPr)_2EtN$ (34.5 µL, 198.2 µmol). To this solution was added 2-cyanoethyl-*N*,*N*,-diisopropyl chlorophosphoramidite **5** (22.1 µL, 99.1 µmol), followed by a catalytic amount of dimethylaminopyridine (DMAP) (3 mg, 24.8 µmol), and this solution was irradiated in a microwave reactor at 65 °C for 1 h. After cooling the reaction on ice, it was quenched with saturated aqueous $NaHCO_3$ (1 mL). This mixture was partitioned between CH_2Cl_2 (10 mL) and saturated aqueous $NaHCO_3$ (5 mL). Then, the aqueous layer was back-extracted with CH_2Cl_2 (2 x 5 mL). The combined organic extracts were then washed with brine (5 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by silica gel flash chromatography using a gradient of 3:7 → 6:4 EtOAc/hex containing 2% Et_3N to yield compound **14** as a white foam (30 mg, 50% yield) and a mixture of diastereomers. Data for **14**: R_f: 0.64 (EtOAc:hexanes, 5:5 containing 2%Et_3N); ^{1}H NMR (600 MHz, $CDCl_3$, δ (ppm)): 0.92–1.04 (*m*, 23H), 1.08-1.15 (*m*, 10H), 2.29 (*t*, J = 6.3 Hz, 1H), 2.40, 2.48 (*2s*, 3H), 2.55 (*t*, *J* = 6.3 Hz, 1H), 3.16–3.56 (*m*, 4H), 3.64 (*d*, *J* = 10.5 Hz, 0.5H, H-1'), 3.69–3.71 (*4s*, 6H, 2 x OMe (DMT)), 3.74 (*d*, *J* = 10.5 Hz, 0.5H, H-1'), 3.77-3.88 (m, 1H), 3.93, 4.06 (*2d*, *J* = 10.5 Hz, 1H, H-1''), 4.09-4.24 (m, 2H), 4.40–4.44 (*m*, 1H), 4.74, 4.77, 4.91, 4.96 (4d, J = 5.0 Hz, 1.5H, SiOCH_2O), 5.26, 5.54 (2dd, J = 11.0, 3.9 Hz, 0.5H, SiOCH_2O), 6.59-6.69 (m, 4H), 7.00-7.17 (m, 8H), 7.20-7.25 (m, 4H), 7.34 (t, J = 7.7 Hz, 4H), 7.44 (bs, 4H), 8.20, 8.23 (2s, 1H, H-8), 8.28 (bs, 1H, N2-N*H*); ^{13}C NMR (150 MHz, $CDCl_3$, δ (ppm)): 12.0, 12.0, 17.9, 17.9, 17.9, 20.1, 20.1, 20.4, 20.4, 20.5, 24.5, 24.5, 24.6, 24.7, 29.2, 29.8, 31.7, 34.8, 43.3, 43.4, 43.5, 43.5, 55.2, 55.3, 55.3, 58.4, 58.6, 62.7, 63.4, 64.2, 71.1, 71.3, 75.4, 75.5, 75.7, 75.9, 86.2, 86.2, 89.8, 89.9, 98.3, 98.3, 98.5, 98.5, 113.0, 113.0, 113.1, 117.6, 117.7, 121.9, 122.0, 126.7, 126.8, 127.8, 127.9, 128.0, 129.3, 129.7, 129.9, 130.1, 134.7, 135.1, 135.6, 135.7, 142.0, 143.7, 144.4, 150.4, 150.5, 151.3, 151.5, 153.1, 153.4, 155.8, 155.9, 158.4, 158.5, 158.5, 158.5; ^{31}P NMR (243 MHz, $CDCl_3$, δ (ppm)): 150.83, 151.23; HRMS (ESI-TOF high-acc) calcd for $C_{65}H_{81}N_8O_{11}PSi$: calcd 1209.5604 $[M+H]^+$; found 1209.5602 $[M+H]^+$; peak at 1310.6808 corresponds to $[M+Et_3N^+H]^+$.

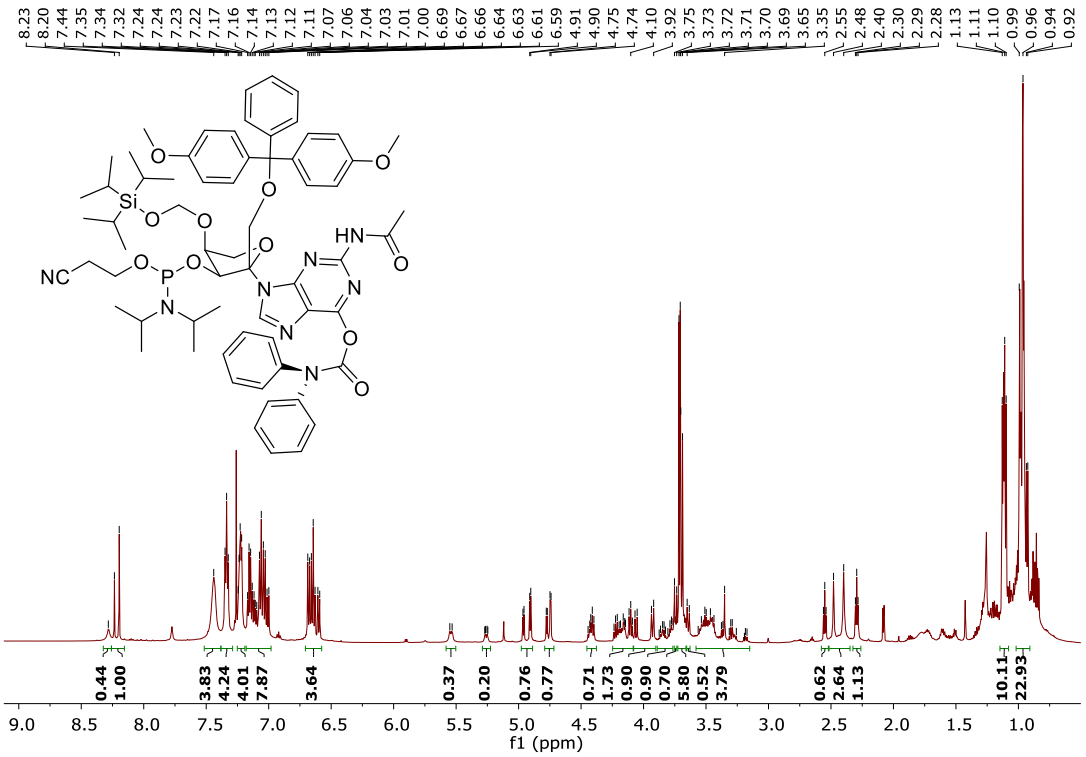


Figure **S15**. 1HNMR of phosphoramidite **14** in $CDCl_3$

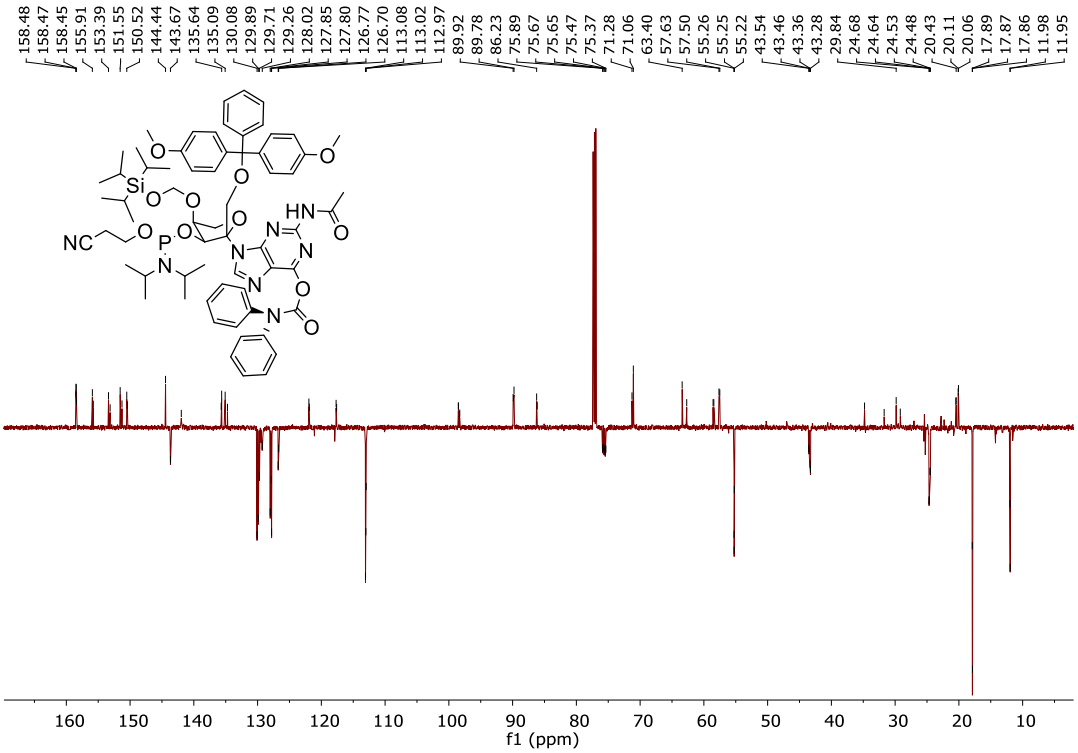


Figure **S16**. 13CNMR of phosphoramidite **14** in $CDCl_3$

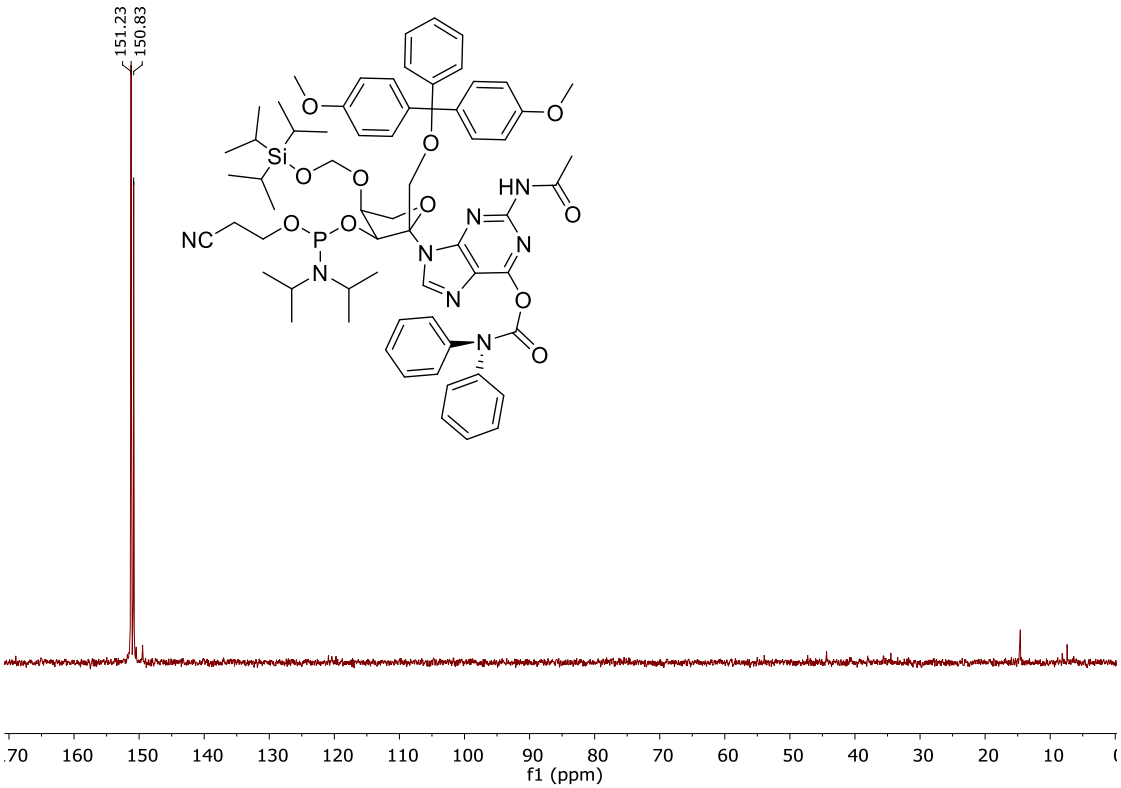


Figure **S17**. 31PNMR of phosphoramidite **14** in $CDCl_3$

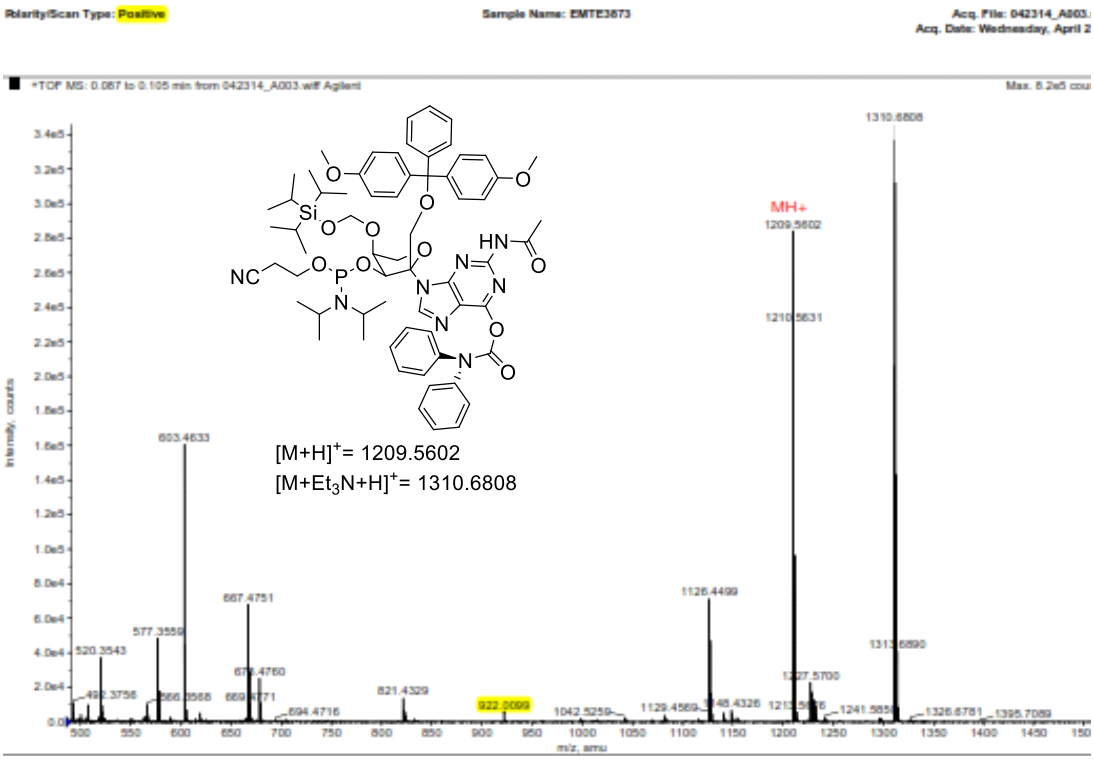
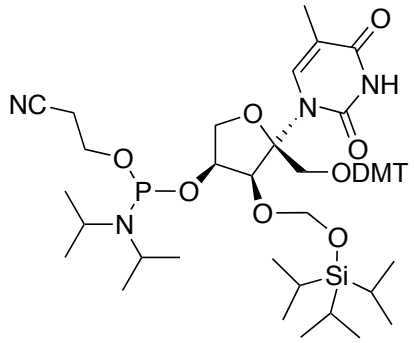


Figure **S18**. HRMS (ESI-TOF high-accu) of phosphoramidite **14**

1′O-(4,4′-dimethoxytrityl)-3′-O-tri-isopropylsilyloxymethyl-L-ribulofuranosylthymine-4′-O-[O-(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (**16**): Ribulofuranosylthymine **15** (0.070 g, 0.094 mmol) was azeotroped with anhydrous toluene (3 x 5 mL),dried under high vacuum overnight, and further taken up in of anhydrous CH_2Cl_2 (1 mL), followed by the addition of $(iPr)_2EtN$ (0.08 mL, 0.47 mmol) under argon atmosphere. This solution was transferred to a microwave tube (2–5 mL) containing 2-cyanoethyl-*N*,*N*,-diisopropyl chlorophosphoramidite **5** (0.04 mL, 0.19 mmol). The microwave tube was sealed and irradiated at 65 °C for 15 min. The reaction mixture was quenched by the addition of saturated $NaHCO_3$ (5 mL) solution, extracted with CH_2Cl_2 (3 x 50 mL), washed with water (20 mL) followed by brine (50 mL) and dried over anhydrous $MgSO_4$. The resulting solution was filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (30% EtOAc/hexanes containing 2% Et_3N) to afford 0.069 g (78%) of pure phosphoramidite **16** as a white amorphous solid (mixture of diastereomers in ratio of 2:1). Data for **16**: R_f: 0.62 and 0.55 (50% EtOAc/heaxnes); ^{1}H NMR (600 MHz, $CDCl_3$, δ (ppm)): 0.91–1.21 (m, 33H, Si(CHCH_3)$_3$, Si(CHCH$_3$)$_3$ and N(CHCH_3)$_2$), 1.98 and 1.99 (2s, 3H, CH_3(C-5)), 2.49 (t, J = 6.6 Hz, 1H, OCH$_2$CH_2CN), 2.60 (t, J = 6.0 Hz, 1H, OCH$_2$CH_2CN), 3.34–3.49 (m, 2H, N(CHCH$_3$)$_2$), 3.63–4.15 (m, 12H, H-1′, H-1′′, 2 OCH_3, H-5′, H-5′′ and OCH_2CH$_2$CN), 4.35–4.45 (m, H-4′); 4.88–4.93 (m, 1H, OCH_2O), 4.94–4.99 (m, 1H, OCH_2O), 4.99–5.04 (m, H-3′), 6.68–6.89 (m, 4H, arom.), 7.11–7.37 (m, 9H, arom.), 7.62–7.72 (m, 1H, H-6); ^{13}C NMR (150 MHz, $CDCl_3$ δ (ppm)): 12.0, 12.0 12.1, 12.7 (Si(CHCH$_3$)$_3$, CH$_3$(C-5)), 18.0, 18.0, 18.0, 18.0 (Si(CHCH$_3$)$_3$), 20.4, 20.5, 20.6, 20.7 (OCH$_2$CH_2CN), 24.6, 24.7, 24.7, 24.8, 24.8 (NCH(CH$_3$)$_2$), 43.4, 43.4, 43.4, 43.5 (NCH(CH$_3$)$_2$), 55.3, 55.4 (OCH_3), 57.7, 57.8, 58.7, 58.8 (OCH$_2$CH$_2$CN), 62.9, 63.0 (C-1′), 70.8, 70.8 (C-5′), 71.3, 71.3, 72.5, 72.6, 73.0, 73.1, 79.0, 79.1, 80.5, 80.5 (C-3′/C-4′), 86.1, 86.2 (quaternary C-DMTr), 90.1, 90.6 (OCH_2O), 99.1, 99.4 (C-2′), 108.7, 108.8 (C-5), 113.2, 113.2, 113.2, (arom. C), 117.8, 117.8 (CN), 126.9, 126.9, 127.0, 127.9, 127.9, 128.2, 128.2, 128.3, 130.1, 130.1, 130.2, 135.7, 135.8, 135.9, 136.0 (arom. C), 138.0, 138.3 (C-6), 144.8, 144.9, 149.7, 149.8 (C-2), 158.5, 158.6 (arom. C), 164.3, 164.4 (C-4); ^{31}P NMR (243 MHz, $CDCl_3$): δ 150.4, 150.7; HRMS (ESI-TOF high-acc) calcd for $C_{50}H_{71}N_4O_{10}PSi$ $(M+H)^+$: 947.4750, found: 947.4732.



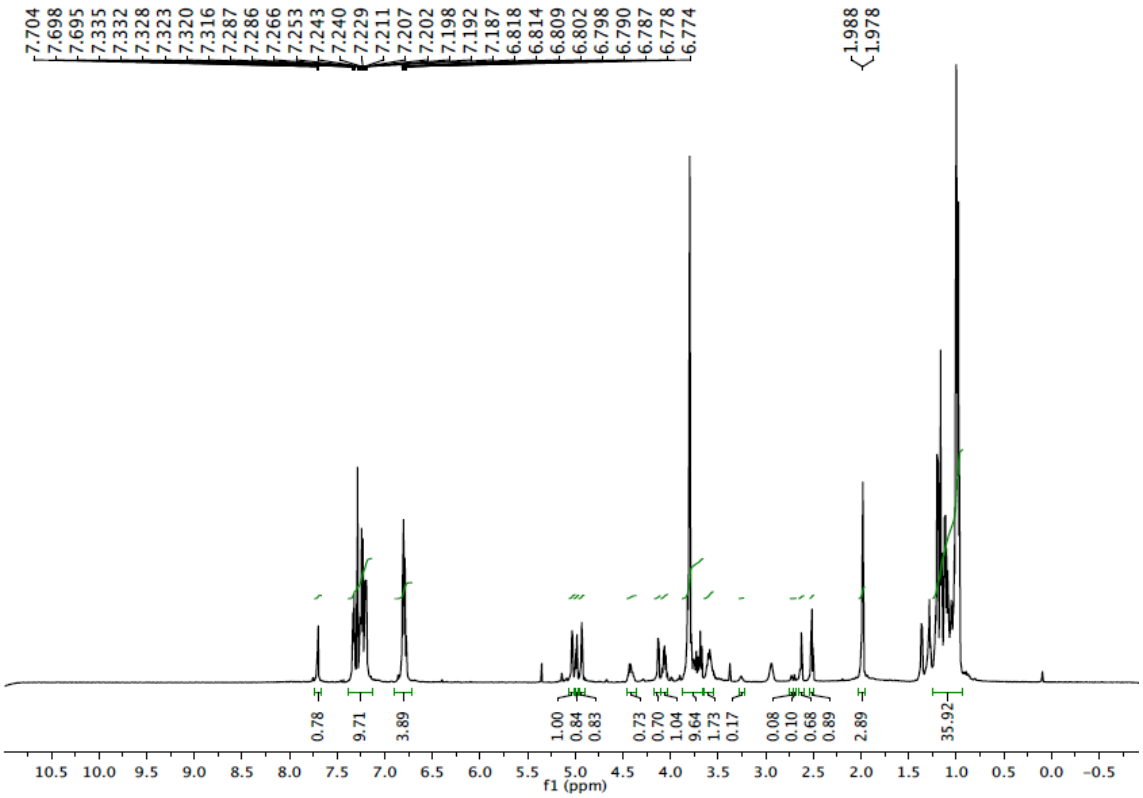


Figure S19. [1]H NMR of protected L-ribuloT-phosphoramidite **16** in $CDCl_3$

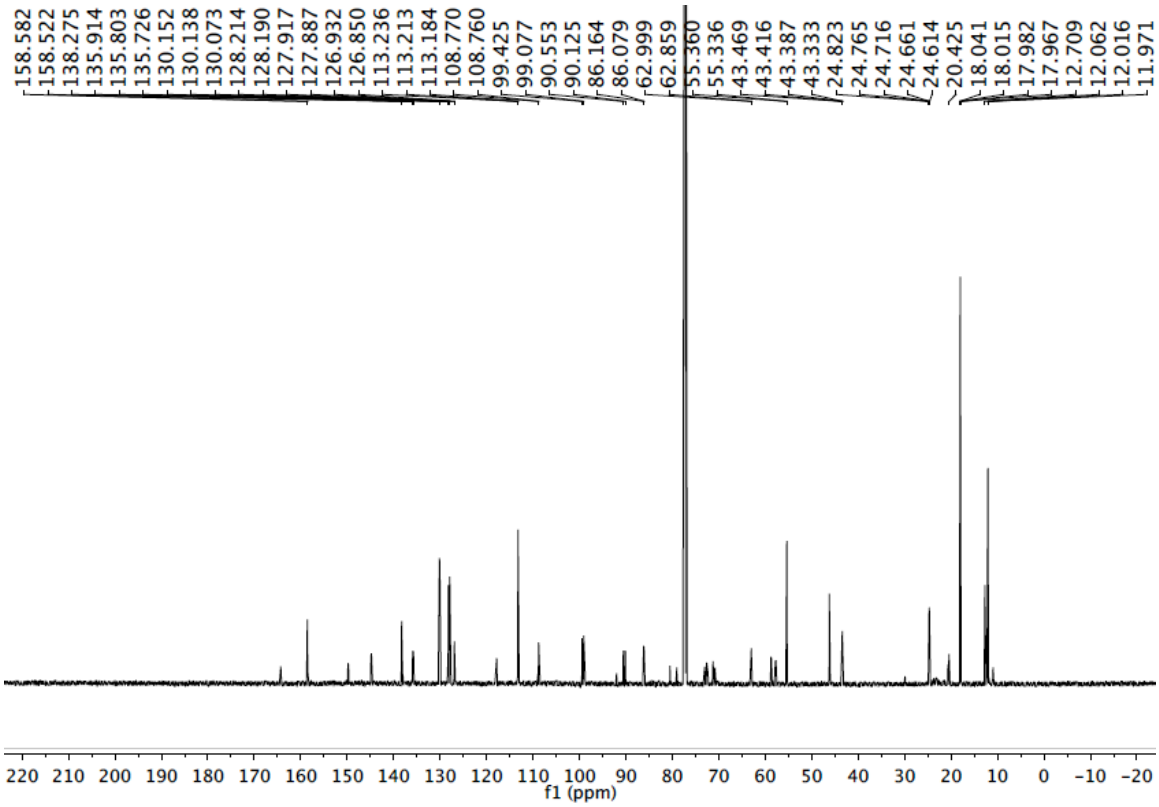


Figure S20. [13]C NMR of protected L-ribuloT-phosphoramidite **16** in $CDCl_3$

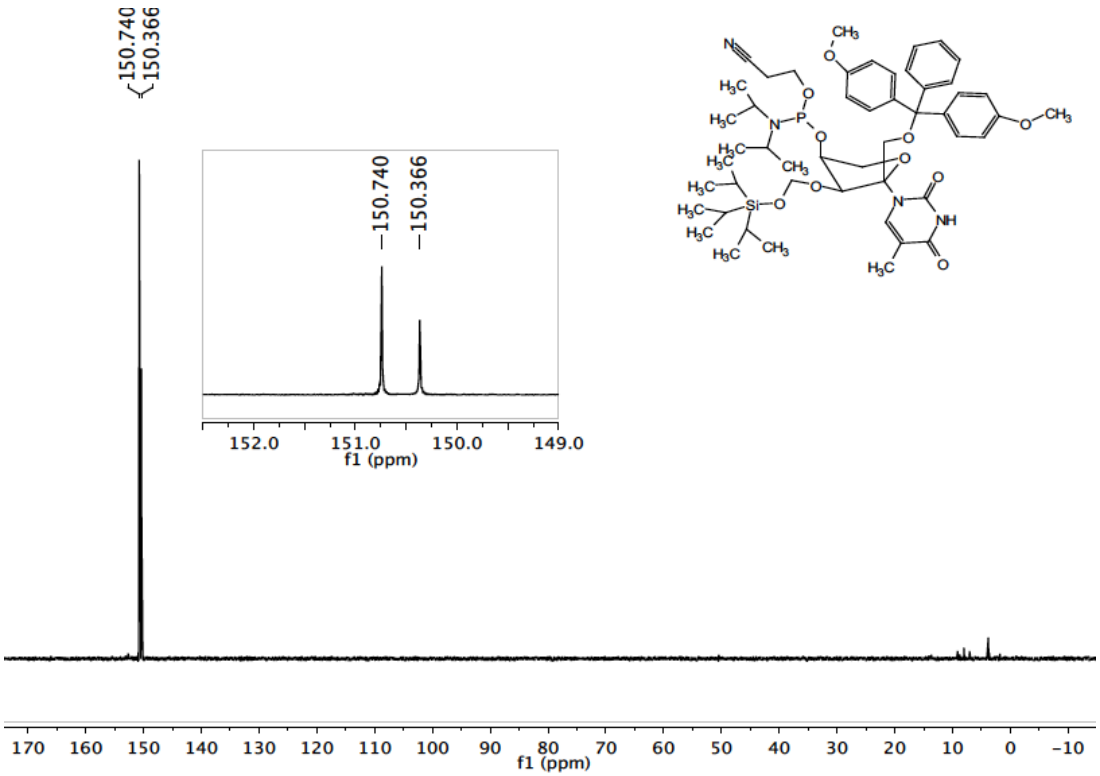


Figure S21.[31]P NMR of protected L-ribulo-T-phosphoramidite **14** in $CDCl_3$

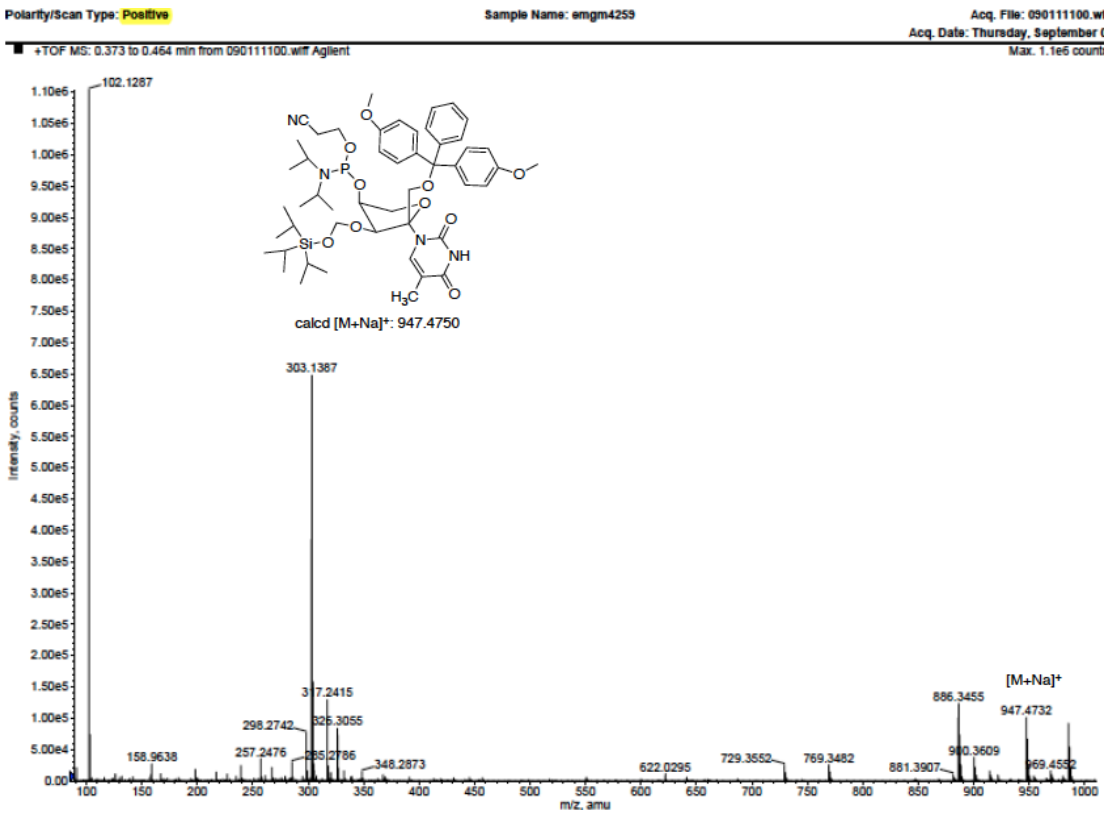


Figure S22. HRMS (ESI-TOF high-accu) of protected L-ribulo-T-phosphoramidite **16**

DNA and RNA phosphoramidites: The optimized procedures developed for the ribulo-nucleoside series (see above) was used for the synthesis of the DNA and RNA phosphoramidites. Since the reaction temperature, reaction time and solvent was optimized with the ribulose-nucleosides, and worked well in the DNA/RNA series, we did not have the need to optimize these parameters further, except with respect to the different activators as noted in Table S2. Only in the case of guanosine **24** (RNA) 10 min at 60 °C was explored.

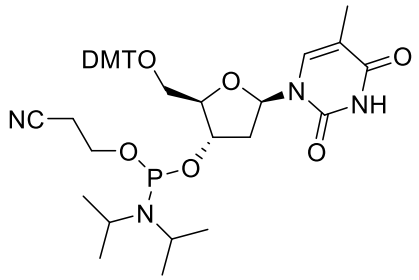
General procedure for synthesizing both DNA and RNA phosphoramidites via microwave using reagents 5 or 6: DNA and RNA monomers **17**-**20** and **21**-**24** (0.5 mmol) were dissolved in anhydrous CH_2Cl_2 (3 mL), followed by the addition of 2-cyanoethyl-*N*,*N*-diisopropyl chlorophosphoramidite **5** (0.65 mmol) with diisopropylethylamine (1.30 mmol), or 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropyl phosphorodiamidite **6** (0.65 mmol) with activators 5-ethylthiotetrazole (0.65 mmol), or pyridinium chloride (0.65 mmol) under argon atmosphere in a microwave tube. The tube was sealed and irradiated at 65 °C while stirring for 15 min. The reaction mixture was concentrated to dryness and directly loaded on silica gel for purification by column chromatography (elution with 30%–80% EtOAc/hexanes containing 2% Et_3N) to afford the corresponding DNA and RNA phosphoramidites, **25**–**28** and **29**-**32** respectively.

Table S2. Microwave phosphitylation of protected ribose and deoxyribose nucleosides **23**–**30**

Entry	Nucleoside	Reagent	Phosphoramidites	Time	Yield (%)
1.	**18**	**5**	**26**	15 min	75
2.	**17**	**6**	**25**	15 min	83
3.	**18**	**6**	**26**	15 min	89
4.	**19**	**6**	**27**	15 min	90
5.	**20**	**6**	**28**	15 min	47
6.	**20**	**5**	**28**	15 min	70
7.	**21**	**6**	**29**	15 min	62
8.	**22**	**6**	**30**	15 min	88
9.	**23**	**6**	**31**	15 min	50
10.	**24**	**5**	**32**	15 min	40
11.	**24**	**6**	**32**	15 min	40
12.	**24**	**6***	**32**	15 min	44

***Pyridinium chloride activator used for phosphitylating nucleoside **24**. Phosphitylations were performed in triplicate with reagent **6**, and at least once with reagent **5**.

5′-O-(4,4′-dimethoxytrityl)-2′-deoxythymidine-3′-O-[O-(2-cyanoethyl)-N,N-diisopropylphosphoramidite (**25**): 83% yield; ^{31}P NMR (243 MHz, $CDCl_3$ δ (ppm)): 149.04, 149.47.



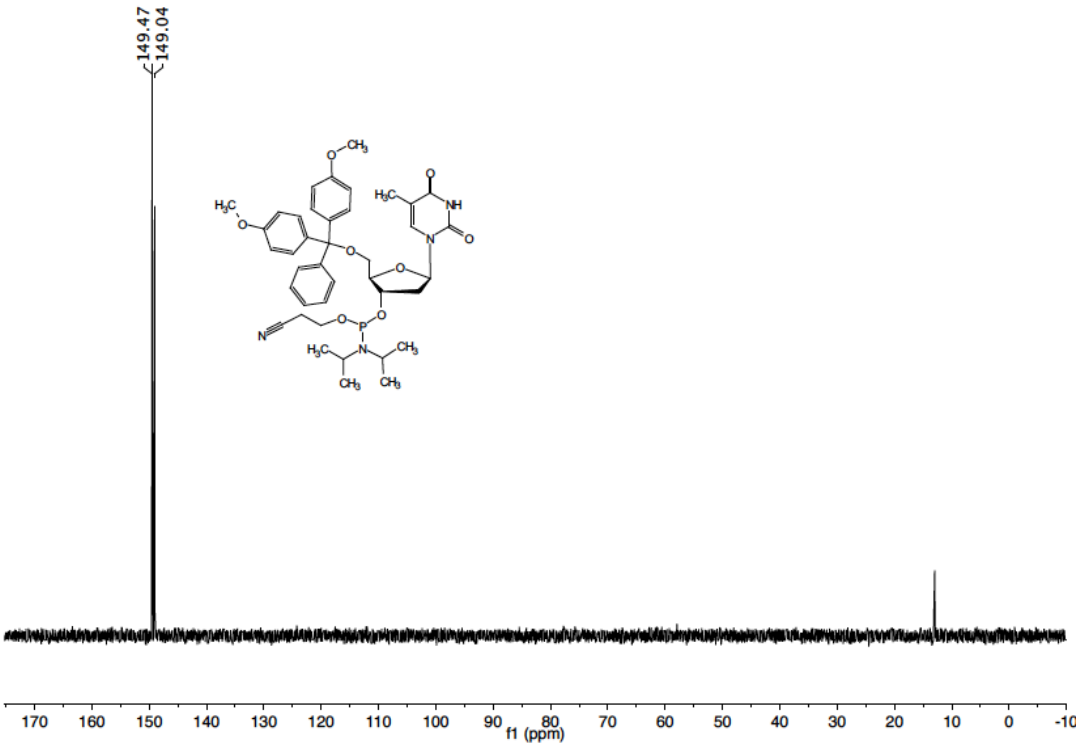


Figure S23. ^{31}P NMR of DNA phosphoramidite dT **25** in $CDCl_3$

N6-benzoyl-5′-O-(4,4′-dimethoxytrityl)-2′-deoxyadenosine-3′-O-[O-(2-cyanoethyl)-N,N-diisopropylphosphoramidite (**26**): 89% yield; ^{31}P NMR (243 MHz, $CDCl_3$ δ (ppm)): 149.31, 149.46.

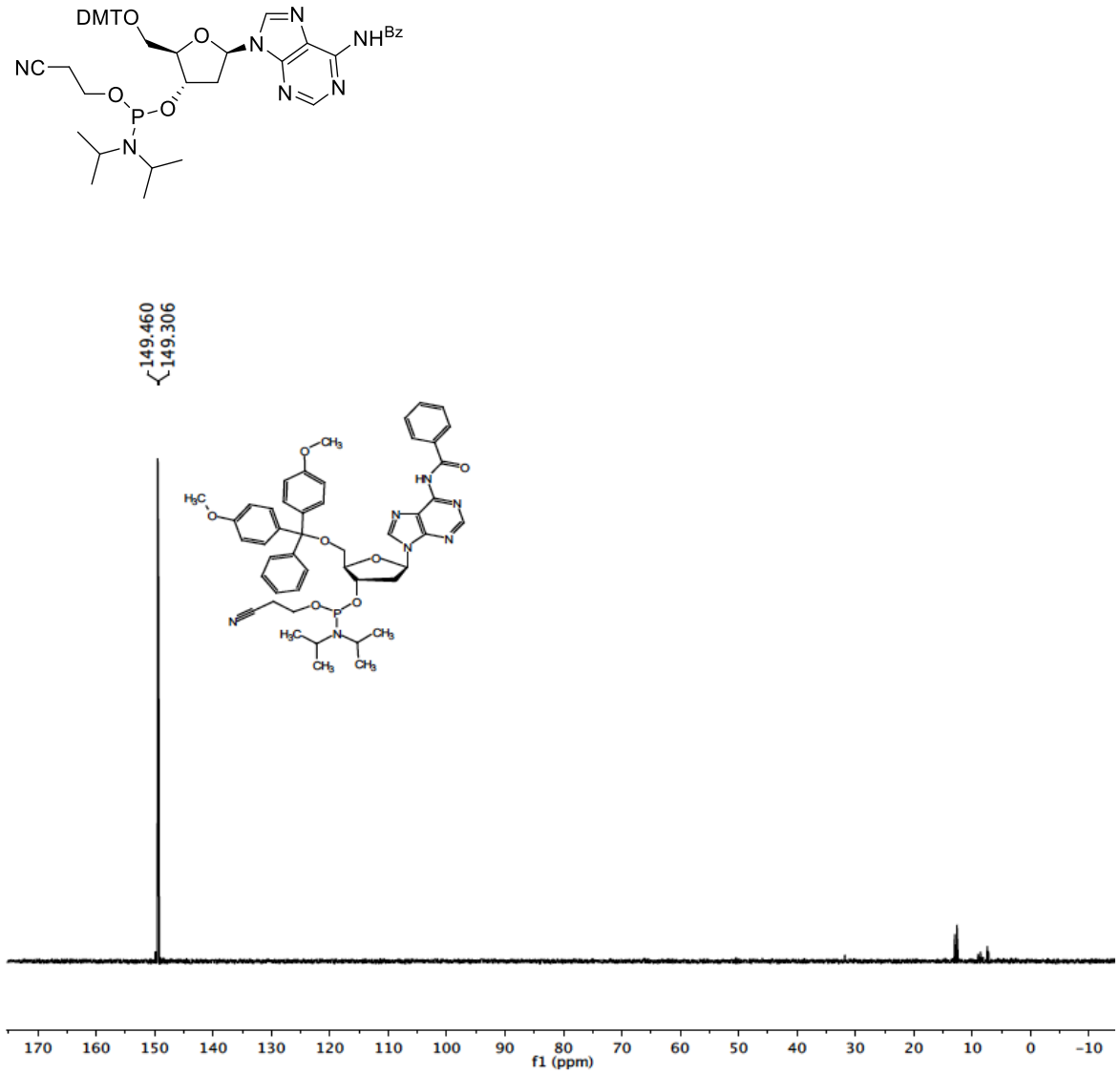
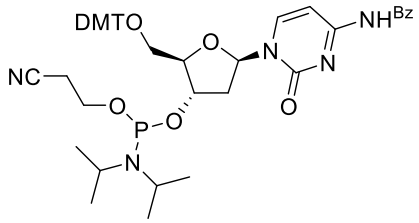


Figure S24. ^{31}P NMR of DNA phosphoramidite dA^{Bz} **26** in $CDCl_3$

N4-benzoyl-5′-O-(4,4′-dimethoxytrityl)-2′-deoxycytidine-3′-O-[O-(2-cyanoethyl)-N,N-diisopropylphosphoramidite (**27**): 90% yield; ^{31}P NMR (243 MHz, $CDCl_3$ δ (ppm)): 149.28, 149.86.



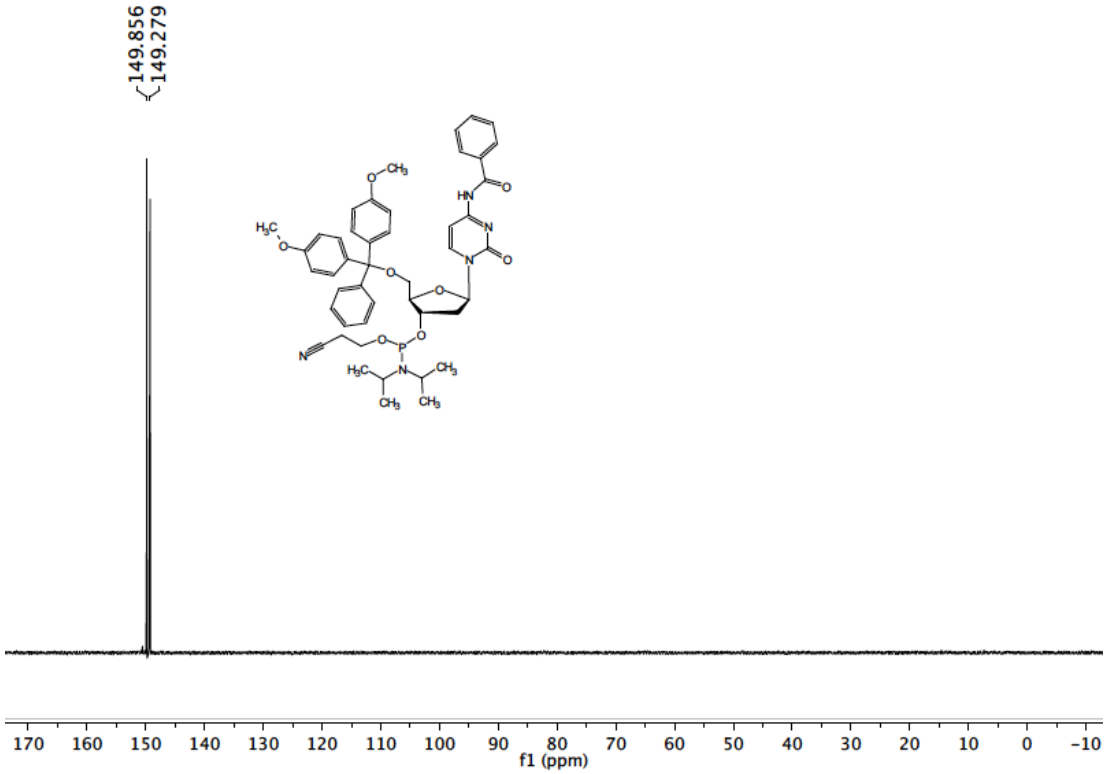


Figure S25. ^{31}P NMR of DNA phosphoramidite dC^{Bz} **27** in $CDCl_3$

N2–isobutyryl–5′–O–(4,4′–dimethoxytrityl)–2′–deoxyguanine–3′–O–[O–(2–cyanoethyl)–N,N–diisopropylphosphoramidite (**28**): 70% yield (with H-phosphonate impurity); ^{31}P NMR (243 MHz, $CDCl_3$ δ (ppm)): 148.28, 148.95.

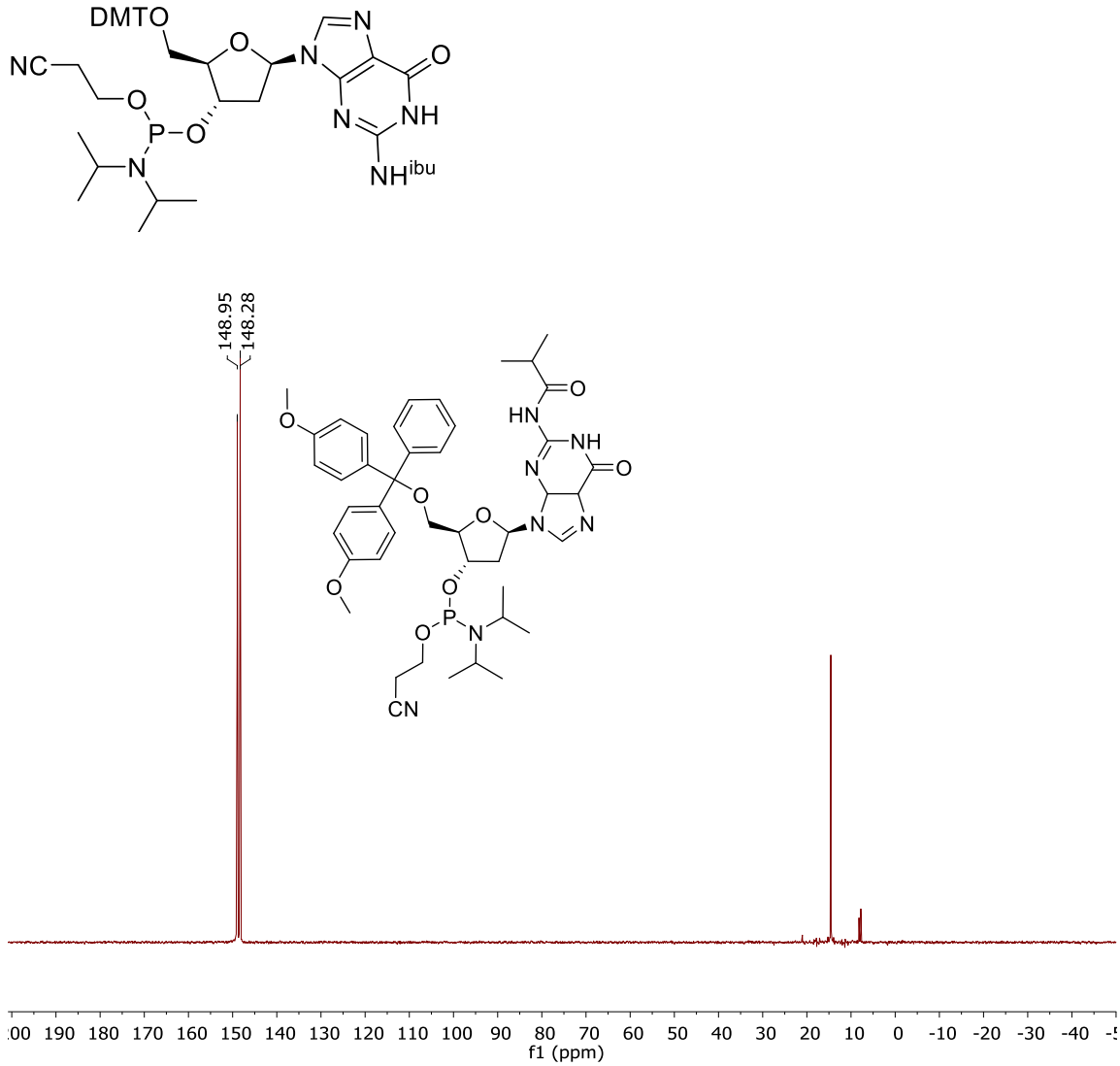
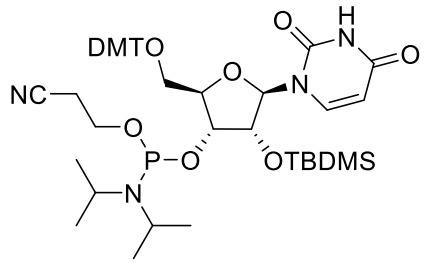


Figure **S26**. ^{31}P NMR of DNA phosphoramidite dG^{ibu} **28** in $CDCl_3$

5′–O–(4,4′–dimethoxytrityl)–2′–O–tertbutyl(dimethylsilyl)uracil–3′–O–[O–(2–cyanoethyl)–N,N–diisopropylphosphoramidite (**29**): 62% yield; ^{31}P NMR (243 MHz, $CDCl_3$ δ (ppm)): 149.95, 150.29.



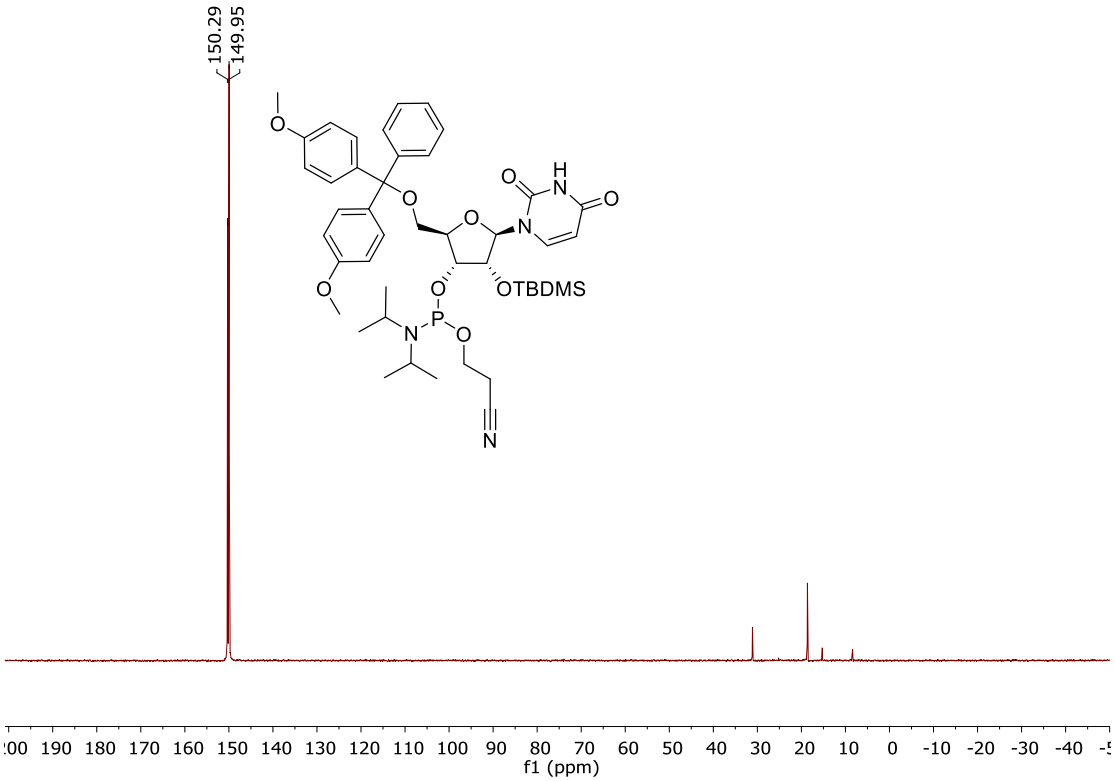
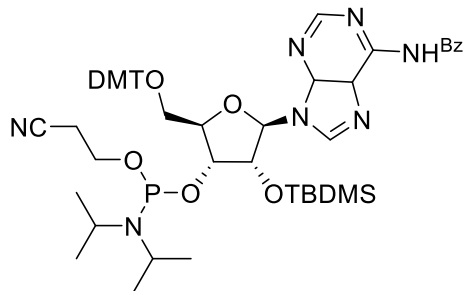


Figure **S27**. ^{31}P NMR of DNA phosphoramidite U **29** in $CDCl_3$

N6–benzoyl–5′–O–(4,4′–dimethoxytrityl)–2′–O–tertbutyl(dimethylsilyl)adenine–3′–O–[O–(2–cyanoethyl)–N,N–diisopropylphosphoramidite (**30**): 50% yield; ^{31}P NMR (243 MHz, $CDCl_3$ δ (ppm)): 149.45, 151.45.



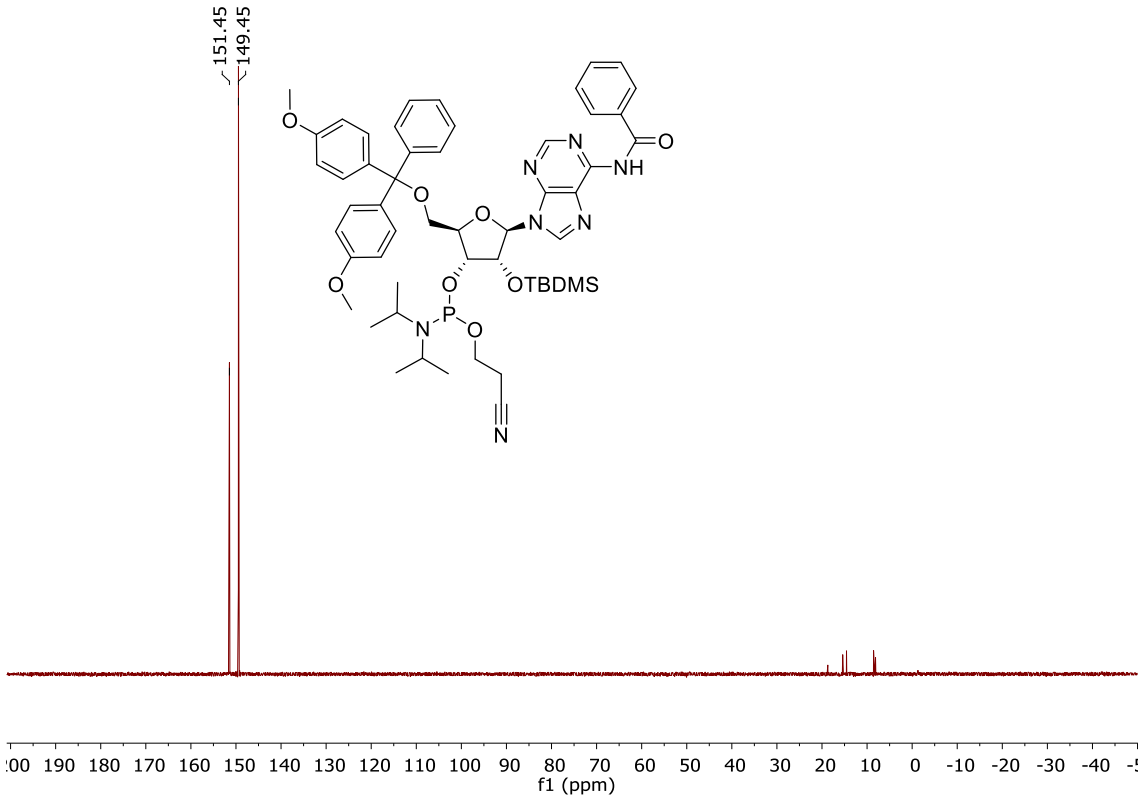


Figure **S28**. ^{31}P NMR of RNA phosphoramidite A^{Bz} **30** in $CDCl_3$

N4–acetyl–5′–O–(4,4′–dimethoxytrityl)–2′–O–tertbutyl(dimethylsilyl)cytidine–3′–O–[O–(2–cyanoethyl)–N,N–diisopropylphosphoramidite (**31**): 88% yield (with slight H-phosphonate impurity); ^{31}P NMR (243 MHz, $CDCl_3$ δ (ppm)): 149.51, 150.51.

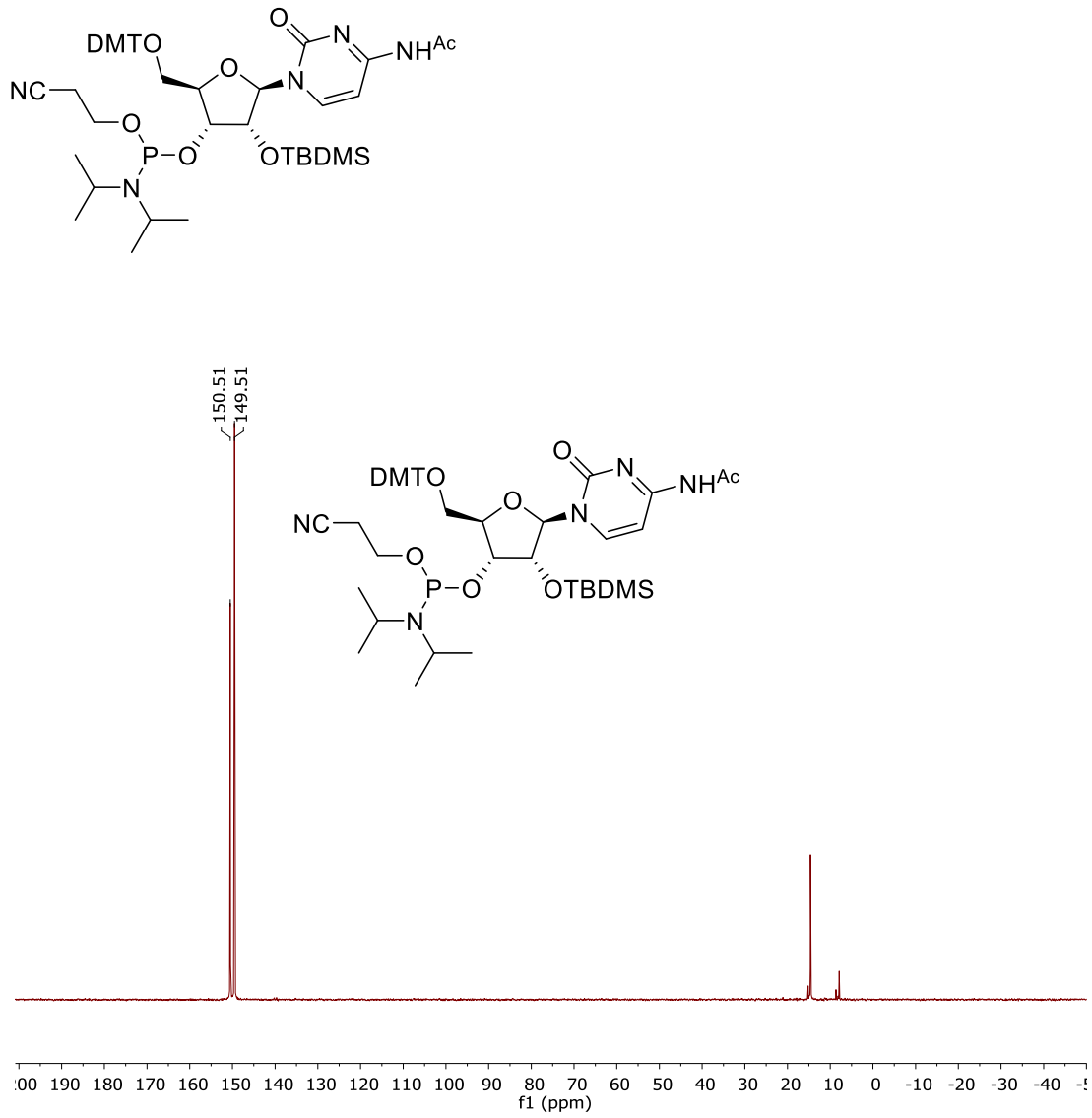
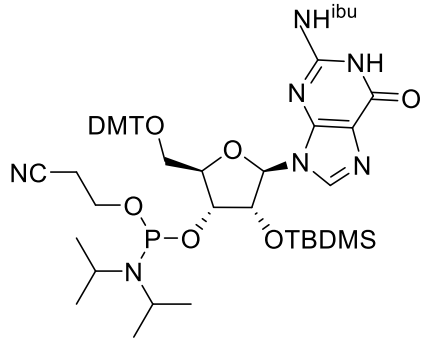


Figure **S29**. ^{31}P NMR of DNA phosphoramidite C^{Ac} **31** in $CDCl_3$

N2–isobutyryl–5′–O–(4,4′–dimethoxytrityl)–2′–O–tertbutyl(dimethylsilyl)guanine–3′–O–[O–(2–cyanoethyl)–N,N–diisopropylphosphoramidite (**32**): 44 % yield (with substantial H–phosphonate impurity); ^{31}P NMR (243 MHz, $CDCl_3$ δ (ppm)): 149.21, 151.18.



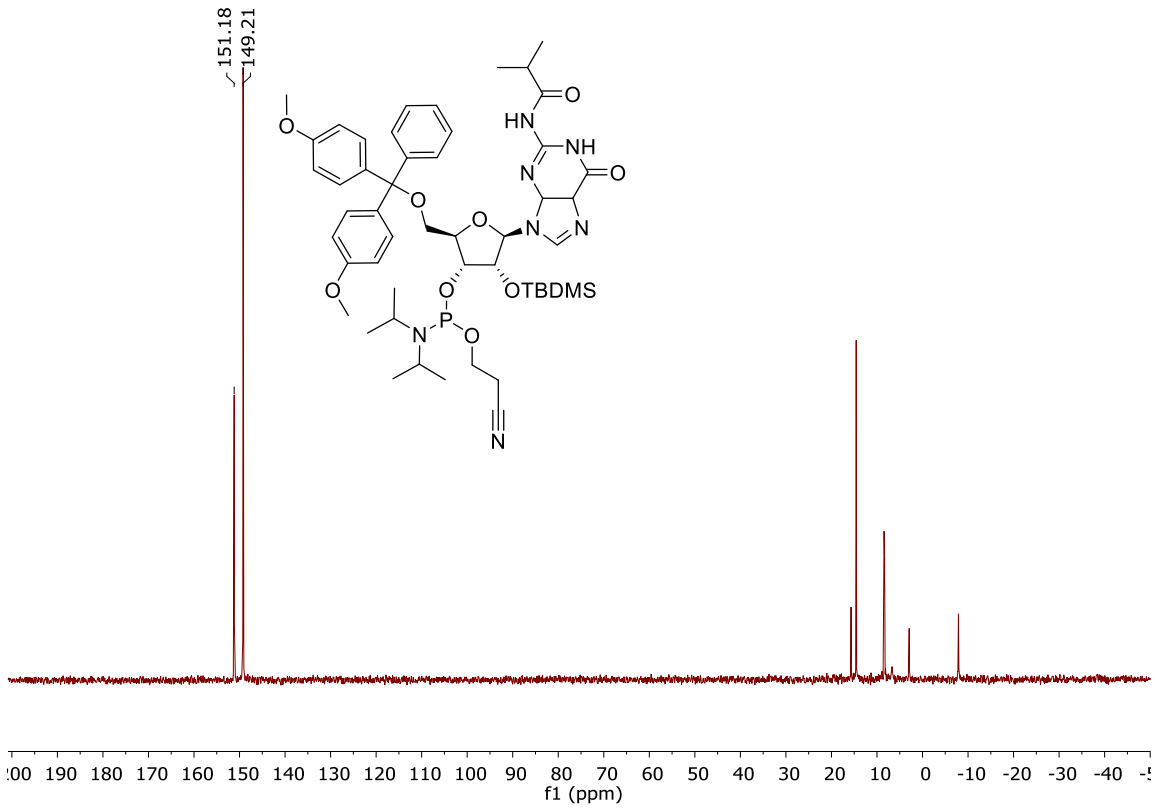


Figure **S30**. ^{31}P NMR of RNA phosphoramidite G^{ibu} **32** in $CDCl_3$