Supporting Information: Product and Mechanistic Analysis of the Reactivity of a C6-Pyrimidine Radical in RNA.

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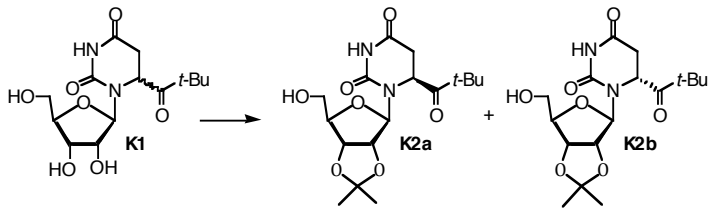
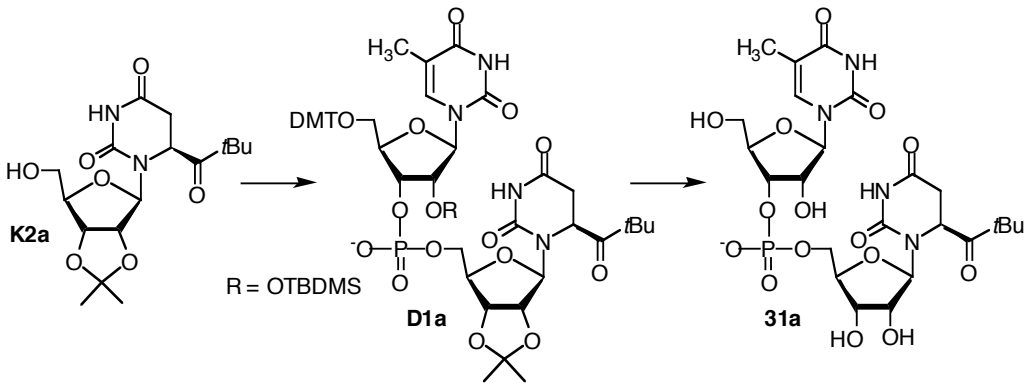
Contents:

General Methods. Oligonucleotides were synthesized on an Applied Biosystems Incorporated 394 oligonucleotide synthesizer. Oligonucleotide synthesis reagents were purchased from Glen Research (Sterling, VA). $^{18}O_2$ (97%) was purchased from Cambridge Isotope Laboratories. All other chemicals were purchased from either Sigma-Aldrich or Acros and were used without further purification. ESI-MS was carried out on a Thermoquest LCQ-Deca. MALDI-TOF MS was carried out on a Bruker Autoflex instrument. All Oligonucleotides were precipitated from 1.25 M ammonium acetate (pH 5.6) prior to ESI-MS analysis. Samples for MALDI-TOF analysis were prepared as described using a 2′,4′,6′-trihydroxyacetophenone (THAP) matrix.[1] T4 polynucleotide kinase and RNA ligase 1 were obtained from New England Biolabs. Absolve, γ-^{32}P-ATP and [5'-^{32}P]-cytidine-3',5'-bisphosphate were purchased from Perkin Elmer. C_{18}-Sep-Pak cartridges were obtained from Waters. Poly-Prep columns were obtained from Bio-Rad. Quantification of radiolabeled oligonucleotides was carried out using a Molecular Dynamics Phosphorimager 840 equipped with ImageQuant Version 5.1 software. All photolyses of oligonucleotides were carried out in RNase free Pyrex tubes in a Rayonet photoreactor (RPR-100) fitted with 16 lamps having an output maximum at 350 nm. All anaerobic photolyses were carried out in sealed Pyrex tubes, which were degassed and sealed using standard freeze-pump-thaw degassing techniques (three cycles, three minutes each). RNAse free tubes were prepared by washing open-ended Pyrex tubing (6 mm O.D, 4 mm I.D. x ~25 cm) with dilute Absolve (sodium hydroxide) solution, then rinsing with RNAse free water. Clean tubes were oven dried and flame sealed in the middle to create two shorter tubes with one sealed end, and were stored in a sealed RNAse free container until use. Reactions in the presence of $^{18}O_2$ were carried out in Pyrex tubes. The solutions were saturated (bubbling ~ 7 min) with commercially available labeled oxygen ($^{18}O_2$, 97%) followed by immediate sealing.



5,6-Dihydro-6-pivaloyl-2',3'-*O*-isopropylidene uridine (K2a, K2b). A 1 : 1.1 diastereomeric mixture of ketone **K1**[2] (0.25 g, 0.76 mmol) was added to a flask containing *p*-toluenesulfonic acid monohydrate (0.04 g, 0.21 mmol) and molecular sieves (4 Å, ~ 20). The contents were dissolved in DMF (10 mL) followed by addition of 2,2-dimethoxypropane (0.34 g, 3.25 mmol) and stirred for 2 h at 55° C. Amberlyst A21-ion exchange resin (0.15 g) was added at once with additional stirring (1 h). The slurry was filtered through a bed of celite 545 and concentrated under reduced pressure. Purification through column chromatography (10% MeOH in CH_2Cl_2) and collection of the first main fraction (R_f: 0.4) yielded **K2b** as a white solid (0.052 g, 0.14 mmol 18 %). ^{1}H NMR ($CDCl_3$) δ 8.37 (br, 1H), 5.18 (d, *J* = 8 Hz, 1H), 4.86-4.85 (m, 1H), 4.72 (d, *J* = 8 Hz, 1H), 4.12 (m, 1H), 3.06 (dd, *J* = 8, 8 Hz, 1H), 2.94 (br, 1H), 2.65 (d, 1H), 1.51 (s, 3H), 1.32 (s, 3H) and 1.21 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 212.7, 167.0, 153.0, 113.5, 97.4, 87.8, 83.4, 80.9, 62.9, 57.7, 44.1, 34.0, 27.2, 26.4 and 25.2; IR (NaCl plates) 2976, 1713, 1697, 1462 and 1233 cm^{-1}; HRMS *m/z* calculated for $C_{17}H_{26}N_2O_7$, 371.1740, observed *m/z* = 371.1809. Collection of a second eluted fraction resulted in diastereomer **K2a** (Rf: 0.35) as a white solid (0.064 g, 0.17 mmol, 23 %). ^{1}H NMR ($CDCl_3$) δ 8.34 (br, 1H), 5.02 (m, 1H), 4.93 (m, 1H), 4.85 (m, 1H), 4.17 (m, 1H), 3.82 (m, 1H), 3.70 (m, 1H), 3.42 (br, 1H), 3.10 (dd, *J* = 8, 8 Hz, 1H), 2.74 (d, 1H), 1.47 (s, 3H), 1.30 (s, 3H) and 1.21 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 200.8, 167.0, 153.4, 113.9, 96.9, 86.6, 83.7, 80.5, 63.0, 56.9, 43.8, 33.7, 27.3, 26.8 and 25.4; IR (NaCl plates)

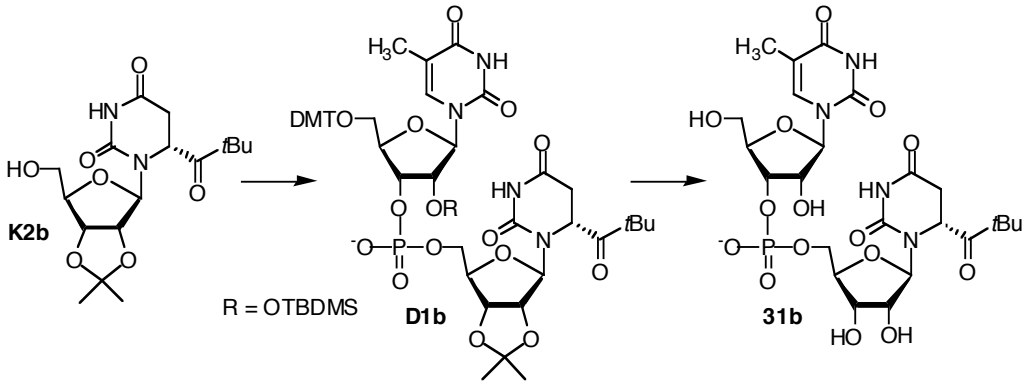
2982, 1722, 1690, 1462 and 1215 cm^{-1}; HRMS *m/z* calculated for $C_{17}H_{26}N_2O_7$, 371.1740, observed *m/z* = 371.1812.



Dinucleotide D1a. To a stirred solution of 5-MeU-CE-Phosphoramidite (0.25 g, 0.28 mmol) and 1*H*-tetrazole (0.36 mmol, 0.45 M in MeCN) in dry acetonitrile (6 mL), was added **K2a** (0.094 g, 0.25 mmol) dissolved in acetonitrile (15 mL). The reaction mixture was stirred for 2 h, followed by addition of *t*-BuOOH in hexanes (0.2 mL, 5 M) and stirred for an additional 45 min. The solution was concentrated under reduced pressure and stirred for 45 min in a 1:1/DCM:Et_3N mixture. The solution was concentrated under reduced pressure to dryness and dissolved in CH_2Cl_2 (40 mL). The resulting solution was washed with $NaHCO_3$ (10%, 2 × 10 mL), and brine (1 × 15 mL). The organic residues were concentrated to dryness under reduced pressure. Purification through column chromatography (1:3.5:95.5-Et_3N/MeOH/CH_2Cl_2 (×2), then apply gradient to 1:8:91) provided **D1a** in the form of a white powder (0.21 g, 0.17 mmol, 61 %). ^1H NMR (CD_3OD) δ 7.64 (s, 1H), 7.42-7.41 (m, 1H), 7.30-7.25 (m, 7H), 6.89-6.87 (m, 4H), 6.11 (d, *J* = 7.2 Hz, 1H), 5.18 (s, 1H), 5.03-4.99 (m, 1H), 4.86 (m, 1H), 4.80-4.77 (m, 1H), 4.69-4.67 (m, 1H), 4.63-4.60 (m, 1H), 4.56 (s, 1H), 4.19-4.18 (m, 1H), 4.01-3.97 (m, 1H), 3.95-3.91 (m, 1H), 3.78 (s, 6H), 3.54-3.31 (m, 3H), 3.14 (dd, *J* = 8.4, 8.4 Hz, 1H), 2.65 (m, 1H), 1.43 (s, 3H), 1.25 (s, 3H), 1.17 (s, 3H), 1.15 (s, 9H), 0.86 (s, 9H), 0.20 (s, 3H) and 0.13 (s, 3H); ^{13}C NMR

(CD_3OD) δ 213.5, 170.0, 166.0, 160.5, 160.4, 155.5, 152.7, 146.0, 137.2, 136.6, 136.5, 131.6, 131.4, 129.5, 129.1, 128.3, 114.4, 114.3, 112.1, 95.3, 88.7, 88.1, 87.2, 86.3, 84.8, 82.5, 76.9, 76.1, 66.9, 65.3, 56.1, 55.8, 47.5, 44.4, 34.6, 30.7, 27.5, 27.4, 26.3, 25.6, 19.1, 11.7, 9.6, -4.1 and -4.7; ^{31}P NMR (CD_3OD) δ -1.1; IR (NaCl plates) 3001, 2988, 1782, 1556 and 1215 cm^{-1}; HRMS *m/z* calculated for $C_{54}H_{70}N_4O_{17}PSi$, 1105.4248, observed *m/z* = 1105.4254.

Dinucleotide 31a. A solution of protected dinucleotide **D1a** (0.024 g, 19.88 μmol) was dissolved in a 1:4 TFA/H_2O mixture (7 mL). The bright orange solution was stirred for 45 min at room temperature. The solution was then concentrated under reduced pressure (~10 min) to obtain an orange solid. EtOH (2 × 5 mL) was added and evaporated under reduced pressure followed by addition of diethyl ether (8 mL). The orange solution was decanted to leave a white solid which was purified through column chromatography (1:20:79-Et_3N/MeOH/CH_2Cl_2 (×2), then apply gradient to 1:50:49) to yield ketone **31a** as a white solid with some minor impurities (11 mg, 14.0 μmol, 71 %). Further purification was achieved by dissolving the solid in H_2O, followed separation by reverse phase HPLC, (isocratic 5% CH_3CN in 0.05 M TEAA); λ_{max} = 267, H_2O, ε = 7616; ^1H NMR (CD_3OD) δ 7.73 (s, 1H), 5.93 (d, *J* = 6.8 Hz, 1H), 5.71 (d, *J* = 8 Hz, 1H), 5.16-5.14 (m, 1H), 4.58-4.52 (m, 1H), 4.39-4.36 (m, 1H), 4.29-4.24 (m, 1H), 4.20-4.16 (m, 2H), 4.09-3.96 (m, 3H), 3.95-3.79 (m, 2H), 3.10-2.99 (m, 2H), 2.92-2.90 (m, 1H), 1.88 (s, 3H) and 1.19 (s, 9H); ^{13}C NMR (D_2O) δ 215.3, 170.5, 155.1, 173.1, 87.0, 83.7, 83.6, 73.1, 72.8, 72.7, 71.7, 69.3, 64.9, 60.4, 52.8, 46.6, 42.9, 42.2, 26.6, 10.5 and 8.2; ^{31}P NMR (D_2O) δ -0.6; IR (NaCl plates) 2995, 1776, 1552 and 1215 cm^{-1}; HRMS *m/z* calculated for $C_{24}H_{34}N_4O_{15}P$, 649.1764, observed *m/z* = 649.1753.



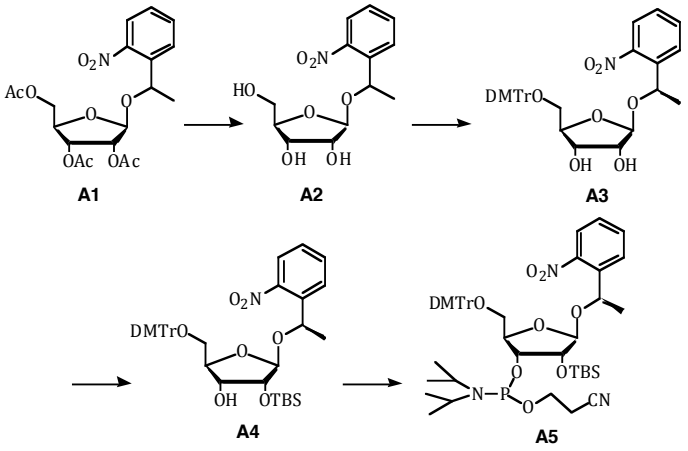
Dinucleotide D1b. To a stirred solution of MeU-CE-Phosphoramidite (0.51 g, 0.58 mmol) and 1*H*-tetrazole (0.58 mmol, 0.45 M in CH_3CN) was added a solution of **K2b** (0.2 g, 0.53 mmol) in acetonitrile (5 mL). The reaction mixture was stirred for 3 hours followed by addition of *t*-BuOOH (0.1 mL, 5 M in hexanes) and stirred for an additional 3 h. The solution was concentrated under reduced pressure and stirred for 45 min in a 1:1/DCM:Et_3N mixture. The solution was concentrated under reduced pressure to dryness and dissolved in CH_2Cl_2 (15 mL). The resulting solution was washed with $NaHCO_3$ (10%, 2 × 5 mL), H_2O (5 mL) and brine (2 × 5 mL). The organic residues were further dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. Purification through column chromatography (0.5:2:97.5-Et_3N/MeOH/CH_2Cl_2 (2 ×), then apply gradient to 1:15:84) provided dinucleotide **D1b** in the form of a white powder (0.15 g, 0.14 mmol, 70 %). ^{1}H NMR ($CDCl_3$) δ 7.59 (s, 1H), 7.36-7.34 (m, 2H), 7.27-7.21 (m, 7H), 6.80-6.78 (m, 4H), 6.11 (d, *J* = 7.2 Hz, 1H), 5.36 (s, 1H), 4.78-4.56 (m, 6H), 4.03-3.88 (m, 3H), 3.74 (s, 6H), 3.51-3.49 (m, 2H), 2.94-2.86 (m, 1H), 2.72-2.70 (m, 6H), 2.61-2.55 (m, 1H), 1.43 (s, 3H), 1.26-1.24 (s, 6H), 1.13-1.08 (m, 18H), 0.85 (s, 9H), 0.21 (s, 3H) and 0.08 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 210.8, 167.2, 163.9, 158.6, 158.5, 151.2, 144.3, 135.7, 135.3, 135.1, 130.2, 130.1, 128.2, 127.9, 127.1, 118.8, 113.4, 113.2, 111.2, 93.8, 87.2, 86.6, 83.5, 82.6, 81.2, 75.5, 74.5, 64.9, 63.9, 57.3, 55.1, 55.0, 53.3, 45.7, 43.7, 27.0, 26.3, 23.2, 21.3, 18.0, 14.7, 11.2, 10.1,

8.2, -4.7 and -5.1; ^{31}P NMR ($CDCl_3$) δ -1.1; IR (NaCl plates) 3000, 2992, 1779, 1545 and 1214 cm^{-1}; HRMS *m/z* calculated for $C_{54}H_{70}N_4O_{17}PSi$ + H, 1106.4321, observed *m/z* = 1106.4312.

Dinucleotide 31b. A solution of protected dinucleotide **D2b** (0.2 g, 0.17 mmol) was dissolved in a 1:4 TFA/H_2O mixture (10 mL) and the bright orange solution was stirred for 45 min. The solution was then concentrated under reduced pressure to obtain an orange solid. EtOH (2 × 5mL) was added and evaporated under reduced pressure followed by addition of diethyl ether (5 mL). The orange solution was decanted to leave a white solid which was purified through column chromatography (40:60-MeOH/$CHCl_3$) to yield ketone **31b** with minor impurities as a white solid (0.08 g, 0.12 mmol, 72 %). Further purification was achieved by dissolving the solid in H_2O followed by separation using a reverse phase HPLC, (isocratic 5% CH_3CN in 0.05 M ammonium formate);. ^{1}H NMR (CD_3OD) δ 8.5 (br, 4H), 7.88 (s, 1H), 5.94 (d, *J* = 5.6 Hz, 1H), 5.64 (d, *J* = 4.8 Hz, 1H), 5.07-5.05 (m, 1H), 4.64-4.60 (m, 1H), 4.34-4.31 (m, 1H), 4.25-4.24 (m, 1H), 4.16-4.11 (m, 1H), 4.01-3.98 (m, 2H), 3.93-3.90 (m, 2H), 3.78-3.77 (m, 2H), 3.20-3.14 (m, 1H), 2.82-2.79 (m, 1H), 1.87 (s, 3H) and 1.25 (s, 9H); ^{13}C NMR (CD_3OD) δ 213.6, 169.9, 166.3, 155.2, 152.7, 138.2, 111.6, 91.6, 89.7, 85.5, 85.4, 82.9, 82.8, 75.5, 75.4, 75.1, 72.4, 71.2, 66.3,66.3, 62.2, 55.4, 44.5, 35.2, 27.8 and 12.4; ^{31}P NMR ($CDCl_3$) δ 0.01; IR (NaCl plates) 2999, 1783, 1550 and 1212 cm^{-1}; HRMS *m/z* calculated for $C_{24}H_{34}N_4O_{15}P$, 649.1764, observed *m/z* = 649.1760.

Synthesis of the Phosphoramidite Precursor to a RNA Abasic Site.

(Compounds **A1**-**A3** were previously reported.[3])



Preparation of A1.[3] 1,2,3,5-Tetra-*O*-acetyl-ribofuranose (11.61 g, 36.5 mmol) was added under argon to a solution of (2-nitrophenyl)ethanol (5 g, 29.9 mmol) in dry acetonitrile (225 mL). The mixture was cooled to -20 °C and trimethylsilyl-trifluoromethanesulfonate (1.9 mL, 10.5 mmol) was added slowly in four equal portions every 30 min. After two hours the reaction mixture was taken up in ethyl acetate (300 mL) and washed with saturated $NaHCO_3$ (3 × 100 mL). The water layer was extracted with ethyl acetate (3 × 100 mL). The organic layers were dried with Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, 300 g, eluted with ethyl acetate/hexane 1:3) to give nucleoside **A1** (8.2 g, 19.3 mmol 65%) as two inseparable diastereomers, $R_f = 0.29$ (33% EtOAc in hexanes). ^{1}H NMR ($CDCl_3$) δ 7.86-7.88 (m, 1 H), 7.72-7.74 (m, 1 H), 7.60-7.64 (m, 1 H), 7.37-7.41 (m, 1 H), 5.35 (m, 1 H), 5.28-5.29 (m, 1 H), 5.15-5.20 (m, 2 H), 4.16-4.20 (m, 1 H), 3.98-4.02 (m, 1 H), 3.58-3.63 (m, 1 H), 2.09 (s, 3 H), 2.02 (s, 3 H), 1.90 (s, 3 H), 1.51 (m, 3 H).

Preparation of A2.[3] Na_2CO_3 (306 mg, 2.89 mmol) was added to a solution of **A1** (1.23 g, 2.89 mmol) in methanol (10 mL) under argon. The mixture was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate (150 mL) and washed with

saturated $NaHCO_3$. The water layer was then extracted with ethyl acetate (3 × 70 mL). The organic layers were dried with Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography (EtOAc) to give nucleoside **A2** (750 mg, 2.5 mmol, 87%) as two inseparable diastereomers, R_f = 0.31 (EtOAc). ^{1}H NMR (DMSO-d_6): δ 7.88-7.91 (m, 1 H), 7.68-7.72 (m, 2 H), 7.46-7.55 (m, 1 H,), 5.14 (m, 1 H), 5.07 (m, 1 H), 4.89 (s, 1 H), 4.80-4.82 (m, 1 H), 4.38 (m, 1 H), 3.73-3.78 (m, 2 H), 3.62-3.67 (m, 1 H), 3.21-3.28 (m, 1 H), 2.94-3.02 (m, 1 H), 1.40 (m, 3 H).

Preparation of A3.[3] Nucleoside **A2** (700 mg, 2.3 mmol) was azeotropically dried with pyridine (2 ×) and diluted with pyridine (5 mL). DMTCl (940 mg, 2.8 mmol) was added in four equal portions every 15 min. After 90 min. the solution was taken up in ethyl acetate (25 mL) and washed with saturated $NaHCO_3$ (3 × 15 mL). The combined aqueous phases were extracted with ethyl acetate (2 × 15 mL) and the combined organic layers were dried with Na_2SO_4 and concentrated under reduced pressure. The residue purified by column chromatography (40-60% EtOAc in hexanes) to give the separable (*R*)-isomer **A3** (1.51 g, 94 %) as a pale yellow foam, R_f = 0.3 (50% EtOAc in hexanes). ^{1}H NMR ($CDCl_3$) δ 7.82-7.85 (m, 1 H), 7.63-7.66 (m, 1 H), 7.19-7.36 (m, 11 H), 6.77-6.80 (m, 4 H), 5.32 (q, J = 6.3, 1 H), 5.09 (s, 1 H), 4.22-4.26 (m, 1 H), 4.14-4.16 (m, 1 H), 3.92-3.97 (m, 1 H), 3.79 (s, 6 H), 3.07-3.11 (m, 1 H), 2.92-2.98 (m, 1 H), 2.79 (s, 1 H), 2.53 (s, 1 H), 1.50 (d, J = 6.3, 3 H). ^{13}C NMR ($CDCl_3$): δ 158.4, 147.0, 144.6, 140.0, 135.8, 135.7, 133.2, 129.94, 129.92, 128.3, 128.0, 127.8, 127.6, 126.8, 124.0, 113.1, 106.0, 86.2, 81.6, 75.6, 73.2, 71.1, 64.3, 55.2, 22.7.

Preparation of A4. In a modification of the known 2'-silylation proceedure,[3] diol **A3** (1.58 g, 2.6 mmol) was azeotropically dried (2 ×) with pyridine and dissolved in THF (20 mL). Silver nitrate (0.54 g, 3.2 mmol, 1.2 eq.) and pyridine (0.8 mL, 9.7 mmol, 3.7 eq.) were added and the

flask sealed by rubber septum and purged with argon. The suspension was then sonicated for 20 min, and stirred an additional 30 min to break up large aggregates solvating as much solid as possible. Addition of *t*-butyldimethylsilyl chloride was followed by 4 h of stirring at room temperature. The solution was diluted with Et_2O (50 mL) and filtered, then washed (3 × 50 mL) with saturated bicarbonate solution. The organic layer was washed with brine (20 mL) then dried over anhydrous sodium sulfate and evaporated under reduced pressure. The 2'-silylated product **A4** (0.9 g, 1.3 mmol, 48%) was isolated by column chromatography (10% EtOAc in hexanes) as a yellow foam, R_f = 0.45 (4 x 10% EtOAc in hexanes). ^{1}H NMR ($CDCl_3$): δ 7.89 (dxd, J_1 = 1.2 Hz, J_2 = 8 Hz, 1 H), 7.79 (dd, J_1 = 1.2, J_2 = 7.6 Hz, 1 H), 7.41-7.20 (m, 11 H), 6.77 (m, 4 H), 5.41 (q, J = 6.4 Hz, 1 H), 5.07 (d, J = 2.8 Hz, 1 H), 4.31 (q, J = 2.4 Hz, 1 H), 4.06 (q, J = 5.6 Hz, 1 H), 3.95 (q, J = 4.4 Hz, 1 H), 3.79 (s, 3 H), 3.16 (dd, J_1 = 7.2 Hz, J_2 = 14.4 Hz, 1 H), 2.97 (dd, J_1 = 7.2 Hz, J_2 = 14.4 Hz, 1 H), 2.57 (d, J = 6 Hz, 1 H), 1.56 (d, J = 6.4, 3 H), 0.95 (s, 9 H), 0.20 (s, 3 H), 0.18 (s, 3 H). ^{13}C NMR ($CDCl_3$): δ 144.8, 140.2, 136.1, 130.1, 130.0, 128.6, 128.2, 127.7, 127.6, 124.1, 113.0, 106.9, 86.0, 83.4, 72.3, 72.0, 55.2, 25.7, 23.1, 18.1, -4.5, -4.8. IR 3450, 2930, 1608, 1526, 1510, 1463, 1348, 1300, 1249, 1176, 1097, 1034, 832 cm^{-1}. HRMS ($M+Na^+$) $C_{40}H_{49}NO_9NaSi$ calc = 738.3069, found = 738.3052. The 3'-silylated product is also produced (0.49 g, 7 mmol, 26%) and migrates slightly slower, R_f = 0.38 (4 x 10% EtOAc in hexanes).

Preparation of A5. Silylated **A4** (0.19 g, 0.27 mmol) was azeotropically dried (2 ×) with toluene, then dissolved in dry DCM (2 mL). Diisopropylethylamine (0.1 mL, 0.57 mmol, 2 eq.) and *N*,*N*-diisopropyl 2-cyanoethylchlorophosphoramidite (86 μL, 0.4 mmol, 1.5 eq.) were added, and the solution stirred for 1 h. The reaction was diluted with DCM (50 mL) and washed with bicarbonate solution (2 × 20 mL) and brine (20 mL), then dried over anhydrous sodium sulfate

and concentrated. Two columns (20-30% EtOAc in hexanes, packed with 1% TEA and oven dried silica gel) were required to obtain pure material (0.125 g, 0.14 mmol, 52%) as a light yellow foam. The TLC shows the phosphitylated product to be slightly more polar than starting material. TLC (20% EtOAc in hexanes): R_f = 0.48. ^{1}H NMR ($CDCl_3$): δ 7.89-7.80 (m, 2 H), 7.40-7.38 (m, 3 H), 7.30-7.15 (m, 9 H), 5.50-5.40 (m, 1 H), 5.08-5.01 (m, 1 H), 4.31-3.82 (m, 4 H), 3.79 (s, 3 H), 3.78 (s, 3 H), 3.65-3.45 (m, 2 H), 3.38-3.15 (m, 1 H), 2.95-2.85 (m, 1 H), 1.56 (m, 3 H), 1.12 (m, 6 H), 0.95 (m, 12 H), 0.15 (m, 6 H). ^{31}P NMR ($CDCl_3$): δ 149.0, 148.7. IR (film) 2930, 1608, 1526, 1510, 1462, 1363, 1301, 1250, 1177, 1034, 834, 782 cm^{-1}. HRMS ($M+Na^+$) $C_{49}H_{66}N_3O_{10}NaSiP$ calc = 938.4147, found = 938.4121.

Labeling and Purification of RNA. RNA oligonucleotides were 5'-labeled with T4-polynucleotide kinase (NEB) and [γ-^{32}P] ATP (Perkin Elmer) using standard methods. 3'-Labeling was carried out using RNA ligase 1 (NEB) and [5'-^{32}P]-cytidine-3',5'-bisphosphate, with 10% DMSO overnight at 16 ^{0}C. All labeling was done at 0.4 μM RNA concentration in reaction volumes of 50 μL. After labeling, excess radioactive nucleotide was removed from the solution using a Sephadex G-25 column, exchanging the buffer with RNase free water. Further purification by 20% denaturing PAGE reduced background cleavage. The radioactive band was excised from the gel and eluted in 1 × PBS buffer (10 mM sodium phosphate, 100 mM sodium chloride pH 7.2) overnight at 37 ^{0}C. The solution was filtered through a Poly-Prep column to remove gel fragments, evaporated to dryness, and resuspended in 300 mM sodium acetate (100 μL). This solution was precipitated twice with 200 μL EtOH, then washed with 70% EtOH (100 μL). Hybridization was performed with 2 equivalents of the complementary strand, with 100 nM labeled RNA, 1 × PBS, 5 mM $MgCl_2$ (200 μL).

General Photolysis Procedure for ^{32}P-Labeled Samples. Hybridized or single stranded

oligonucleotide solutions were diluted to 20 nM with PBS Buffer (10 mM sodium phosphate, 100 mM sodium chloride) pH 7.2, and 5 mM $MgCl_2$. Each sample (50 μL) was added to an RNAse free Pyrex tube sealed at one end. Anaerobic samples were subjected to 4 freeze-pump-thaw cycles at <10 μm Hg then flame sealed. Samples were irradiated 4 h at room temperature in a Rayonet photoreactor fitted with 16 lamps having an output maximum at 350 nm. The photoproducts were analyzed by gel electrophoresis (20% denaturing), and their migration was compared to synthetic oligonucleotides containing the sequence of possible fragments. Quantification was performed using a Molecular Dynamics Storm 840 Phosphorimager equipped with ImageQuant version 5.1 software.

pH Effect on 3'-End Group Products. Samples of 5'-^{32}P-**7** were prepared and photolyzed as above in 5 mM $MgCl_2$ and PBS Buffer (10 mM sodium phosphate, 100 mM sodium chloride) at pH 7.2, 6.2, 5.2 and 3.8.

3'-End Group Analysis. The 3'-termini of the photoproducts were examined by kinase treatment of 5'-labeled oligonucleotides. Reactions were performed on an 8 μL aliquot of photolyzed sample with T4-polynucleotide kinase (10 units), in the kinase buffer (70 mM Tris-HCl, 10 mM $MgCl_2$, 5 mM DTT, pH 7.6) supplied by the manufacturer. The reaction was incubated at 37 ^{0}C for 30 min, quenched with 10 μL formamide loading buffer, and separated by 20% denaturing PAGE.

5'-End Group Analysis. The 5'-termini of the photoproducts were examined by phosphatase treatment of 3'-labeled RNA. Reactions were performed on an 8 μL aliquot of photolyzed sample, with Antarctic phosphatase (10 units), buffer (50 mM Bis-Tris-Propane-HCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$ pH 6.0) provided by the manufacturer. The reaction was incubated at 37 ^{0}C for 30 min, quenched with 10 μL formamide loading buffer, and separated by 20% denaturing

PAGE. The markers were subjected to phosphatase treatment to remove the non-radioactive 3'-phosphate that is introduced in 3'-labeling with [5'-^{32}P]-cytidine 3', 5'-bisphosphate.

Probing for Ketone Using $NaBH_4$. Aliquots (9 μL) of photolyzed RNA (20 nM) were incubated with 1 μL $NaBH_4$ (1 M) or 1 μL water (control) at room temperature for 5 min, then diluted to 100 μL with 300 mM NaOAc buffer (pH 5.6) and precipitated with 300 μL ethanol. Samples were resuspended in 10 μL formamide loading buffer and separated by PAGE.

Thiol Competition. 5'-^{32}P-**9** was diluted to a final concentration of 5 nM. β-Mercaptoethanol (BME) added to a final concentration of 0, 2 μM, 5 μM, 10 μM, 15, μM, 20 μM, or 30 μM and volume of 50 μL. All samples were photolyzed for 4 h, diluted with loading buffer, and separated by 20% denaturing PAGE. All reactions were run in triplicate, with the exception of the no BME control, which was carried out with 6 replicates per experiment. The percentage of uncleaved products in the no BME control was subtracted from the percentage of uncleaved products in samples containing BME, with the remainder assigned as the percentage of thiol trapping product. The ratio of trapping product to cleavage product was plotted versus the BME concentration, and the slope of the data yielded the ratio of rate constants.

Analysis for abasic sites using aniline. The strand scission products resulting from photolysis were separated on 20% denaturing PAGE (0.4 mm gel thickness, bromophenyl blue run 20 cm), and the uncleaved material was excised. These products were eluted with 1× PBS buffer (200 μL), and then precipitated with ethanol. The material was resuspended in water (15 μL) and 50 μL aniline (0.5 M from distilled stock, pH 4.6 with HCl) was added. Samples were incubated 30 min at 37 ^{0}C, and precipitated from 300 mM sodium acetate (100 μM), with EtOH (200 μL). RNA abasic site control oligonucleotide (5'-^{32}P-**30**) was photolyzed 5 min at 350 nM, then subjected to the same conditions.

Dinucleotide Photolysis and Product Analysis by ESI-MS. Solutions containing dinucleotide **31** were prepared in water (75 μM) and transferred to Pyrex tubes. Samples were placed at an equal distance (ca. 10 cm) from the lamps (λ_{max}= 350 nm) in a Rayonet photoreactor and irradiated for 4 h. Reacted solutions were then diluted with MeOH (1:1 ratio) prior to ESI/MS analysis. All anaerobic photolyses were carried out in sealed Pyrex tubes, which were degassed and sealed using standard freeze-pump-thaw degassing techniques (three cycles, three minutes each). Reactions in the presence of $^{18}O_2$ were carried out in Pyrex tubes. The solutions were saturated (bubbling ~ 7 min) with commercially available labeled oxygen ($^{18}O_2$, 97%) followed by immediate sealing.

(1) Chapman, E. G.; DeRose, V. J. *J. Am. Chem. Soc.* **2010**, *132*, 1946-1952.
(2) Newman, C. A.; Resendiz, M. J. E.; Sczepanski, J. T.; Greenberg, M. M. *J. Org. Chem.* **2009**, *74*, 7007-7012.
(3) Kupfer, P. A.; Leumann, C. J. *Nucleic Acids Res.* **2007**, *35*, 58-68.

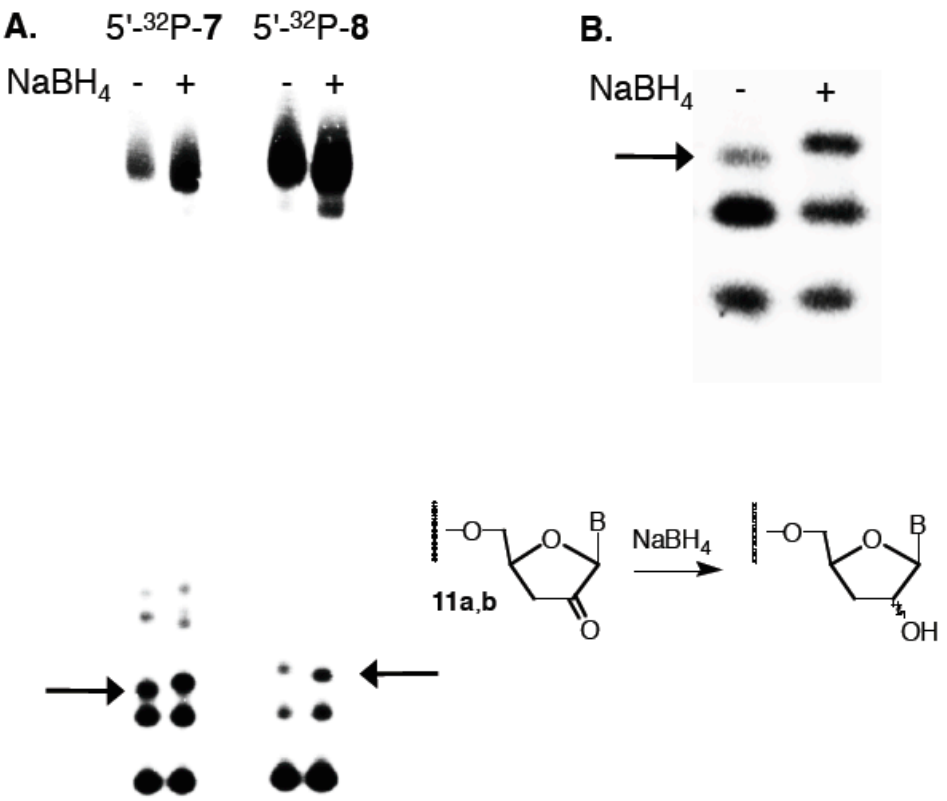


Figure S1. $NaBH_4$ (100 mM) reaction of 5'-^{32}P photoproducts produced under anaerobic conditions. A) Reaction of photolyzed 5'-^{32}-**7** and 5'-^{32}-**8**. B) Zoomed in region of photolyzed 5'-^{32}-**7** showing reaction of **11a**. Arrows indicate **11a**,**b**.

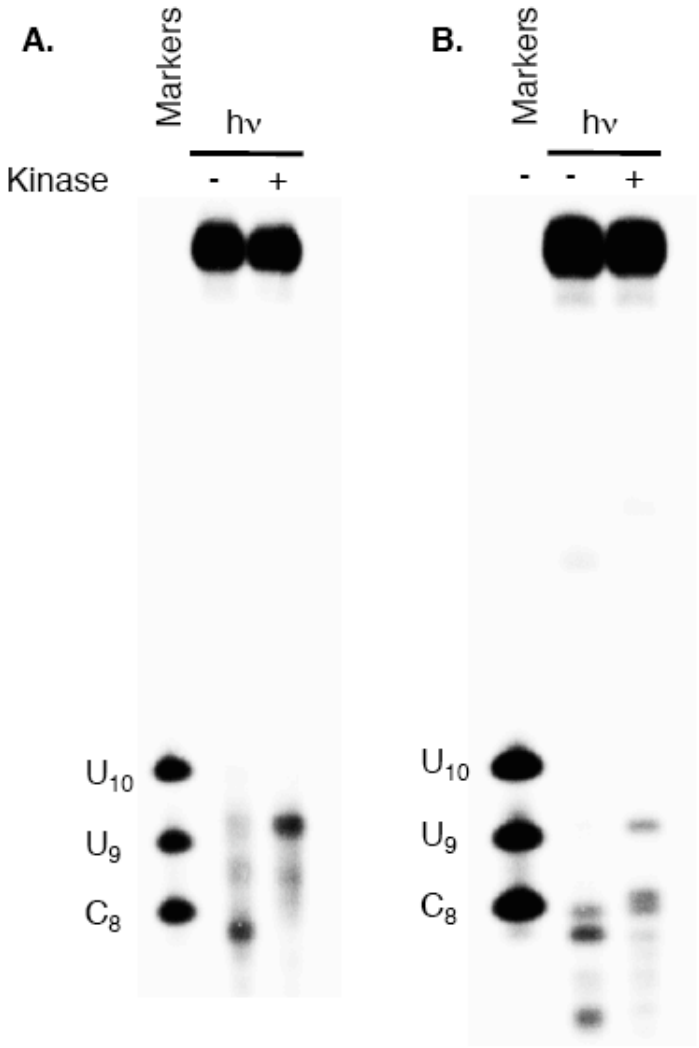


Figure S2. 3'-End group analysis of 5'-labeled photoproducts formed under aerobic conditions. Treatment of the product mixture with T4-polynucleotide kinase (PNK) caused dephosphorylation of any products that contained 3' terminal phosphates, resulting in slower migration of those products. The product mixtures were compared to synthetic oligonucleotide marker sequences. A) Photolyzed **9**; B) Photolyzed **7**.

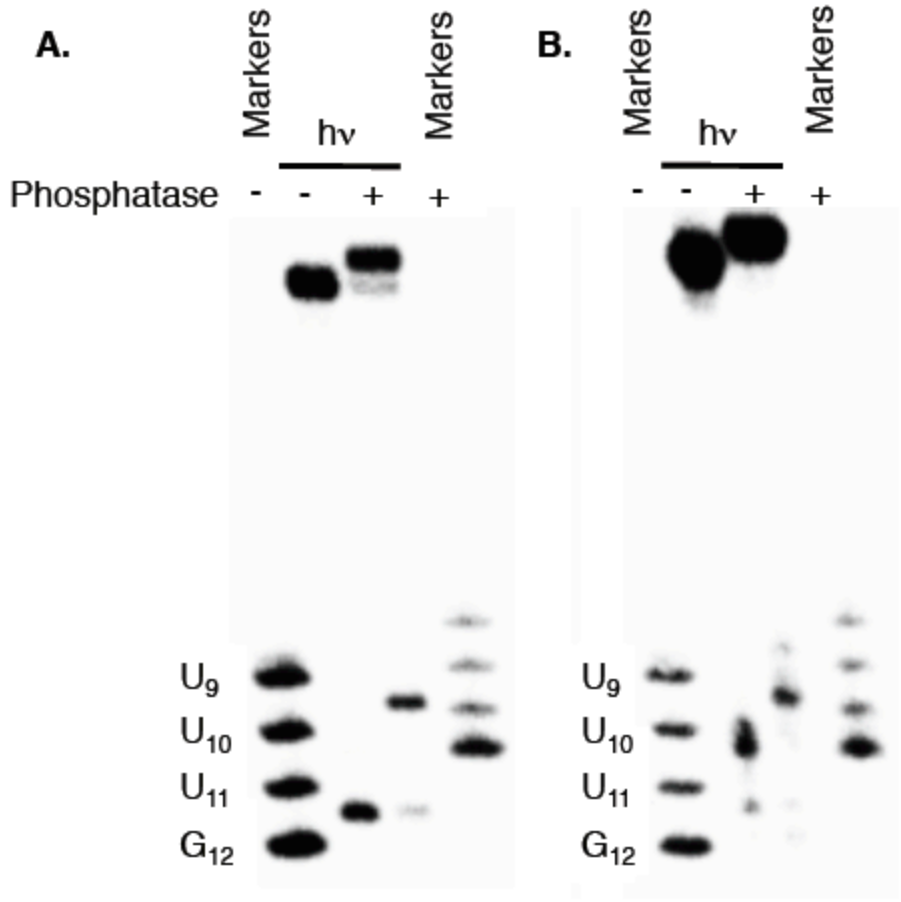


Figure S3. 5'-End group analysis of 3'-labeled photoproducts formed under aerobic conditions. Treatment of the product mixture with Antarctic phosphatase (AP) caused dephosphorylation of any products that contained 5'-terminal phosphates, resulting in slower migration of those products. A) Photolyzed **9**; B) Photolyzed **7**. 3'-Labeling introduces a 3'-terminal phosphate to all RNA oligonucleotides that is lost upon treatment with Antarctic phosphatase. Therefore, product mixtures were compared to two sets of synthetic oligonucleotide markers (with and without 3'-terminal phosphate).

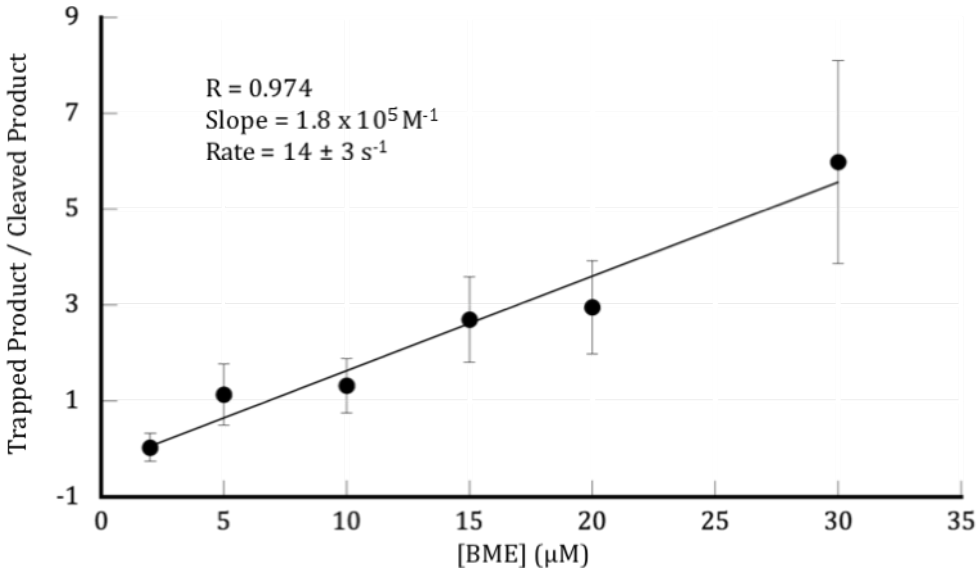


Figure S4. Sample thiol trapping experiment in single stranded RNA (5'-^{32}P-**9**). The ratio of trapped products (full length products at [BME] – full length in the absence of BME) to cleaved products (direct strand scission) plotted against concentration of BME. The slope is the ratio of rate constants for trapping and cleavage.

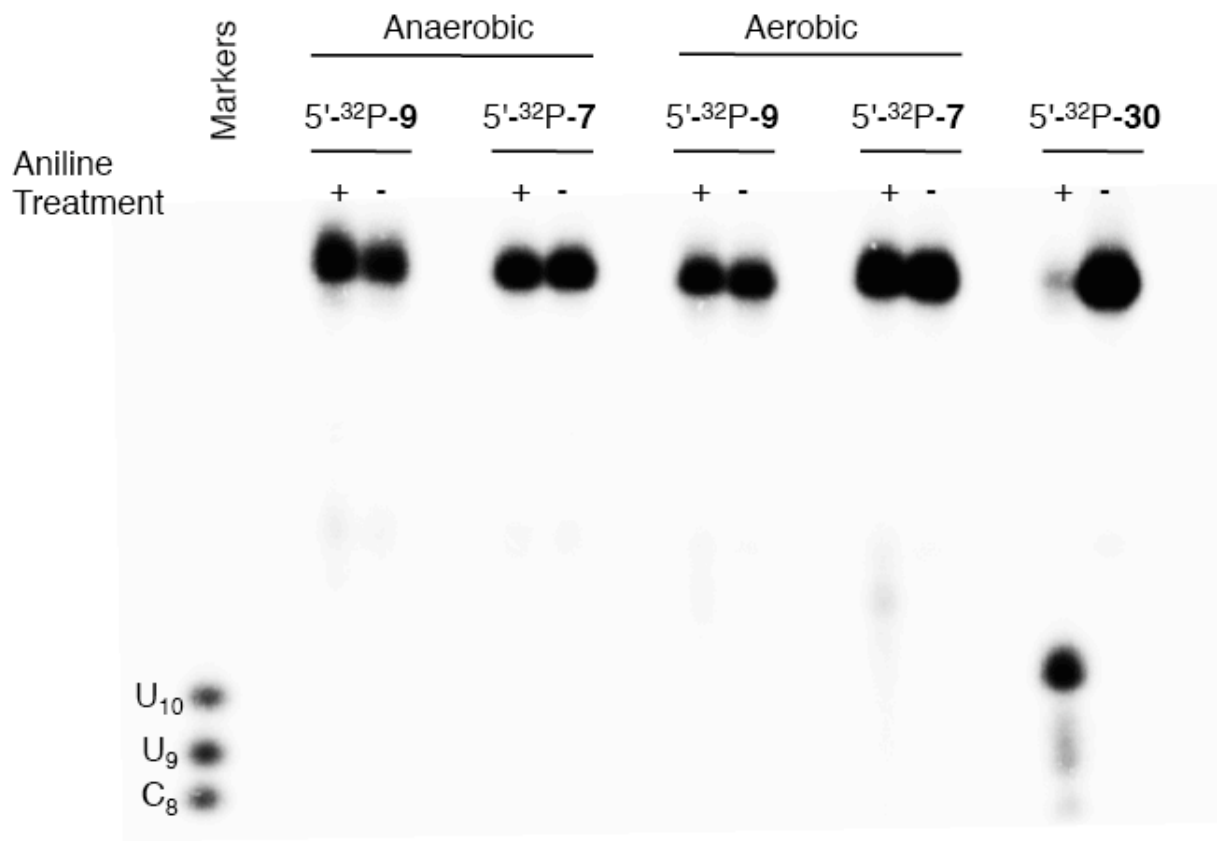


Figure S5. Aniline treatment for detecting RNA abasic sites in photolyzed 5'-^{32}P-**7** and 5'-^{32}P-**9**. The reaction is compared to that of independently synthesized abasic site in **30**.

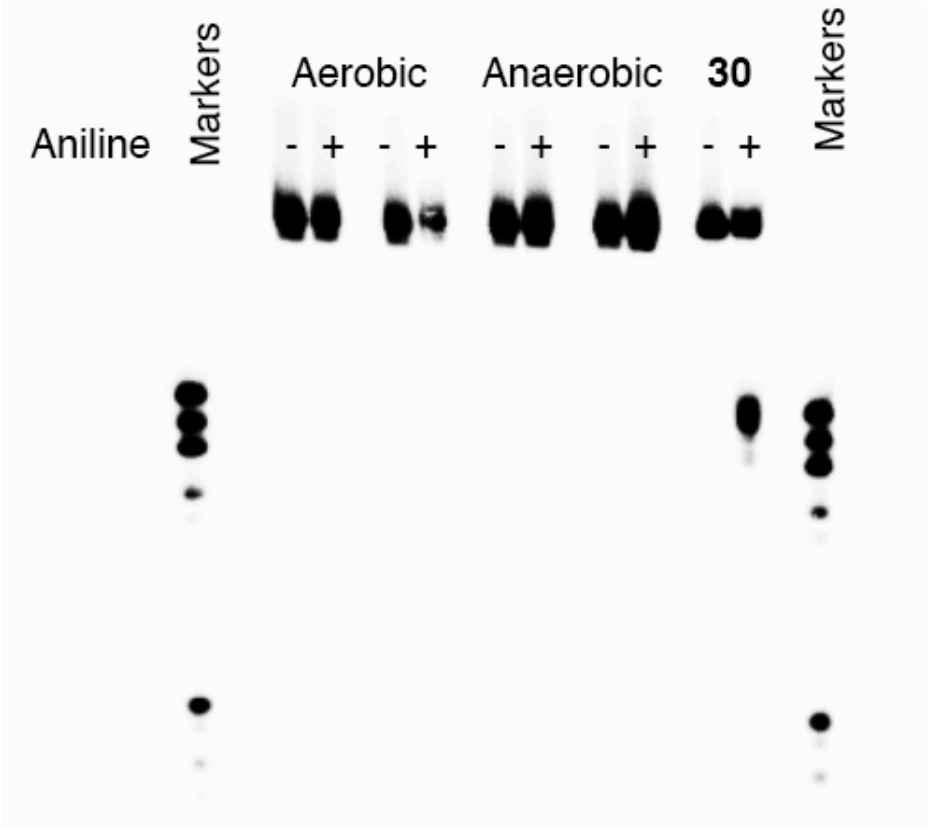


Figure S6. Aniline treatment for detecting RNA abasic sites in photolyzed 5'-^{32}P-**10**. The reaction is compared to that of independently synthesized abasic site (**29**) in **30**.

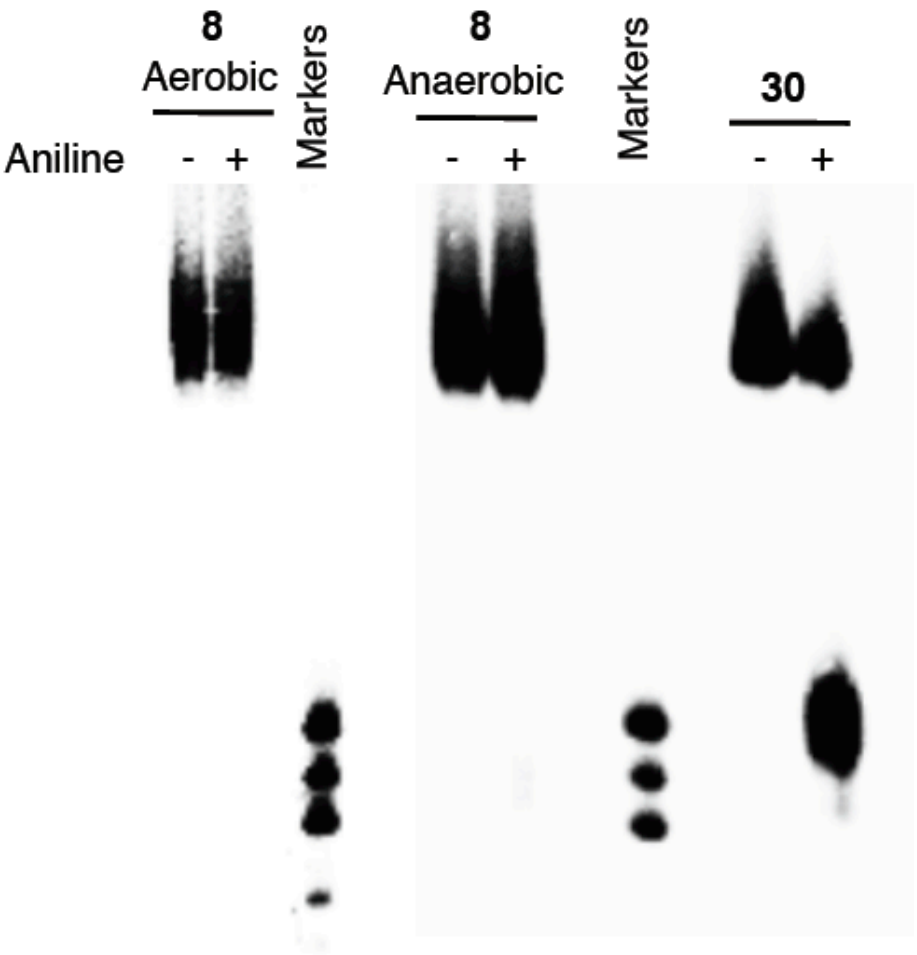


Figure S7. Aniline treatment for detecting RNA abasic sites in photolyzed 5'-^{32}P-**8**. The reaction is compared to that of independently synthesized abasic site (**29**) in **30**.

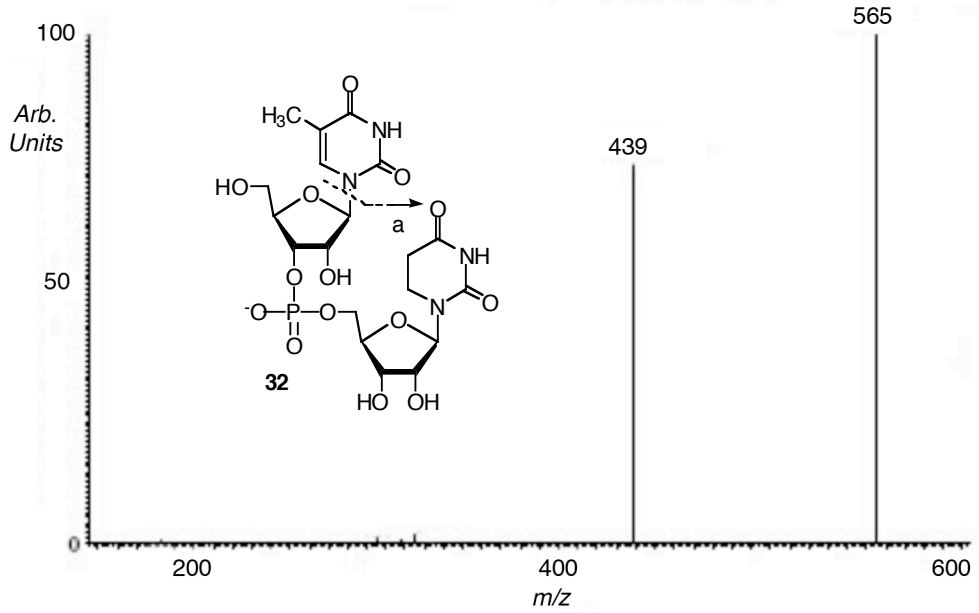


Figure S8. ESI-MS/MS of **32** (*calcd*. *m/z* = 565.1) obtained from photolysis of **31**.

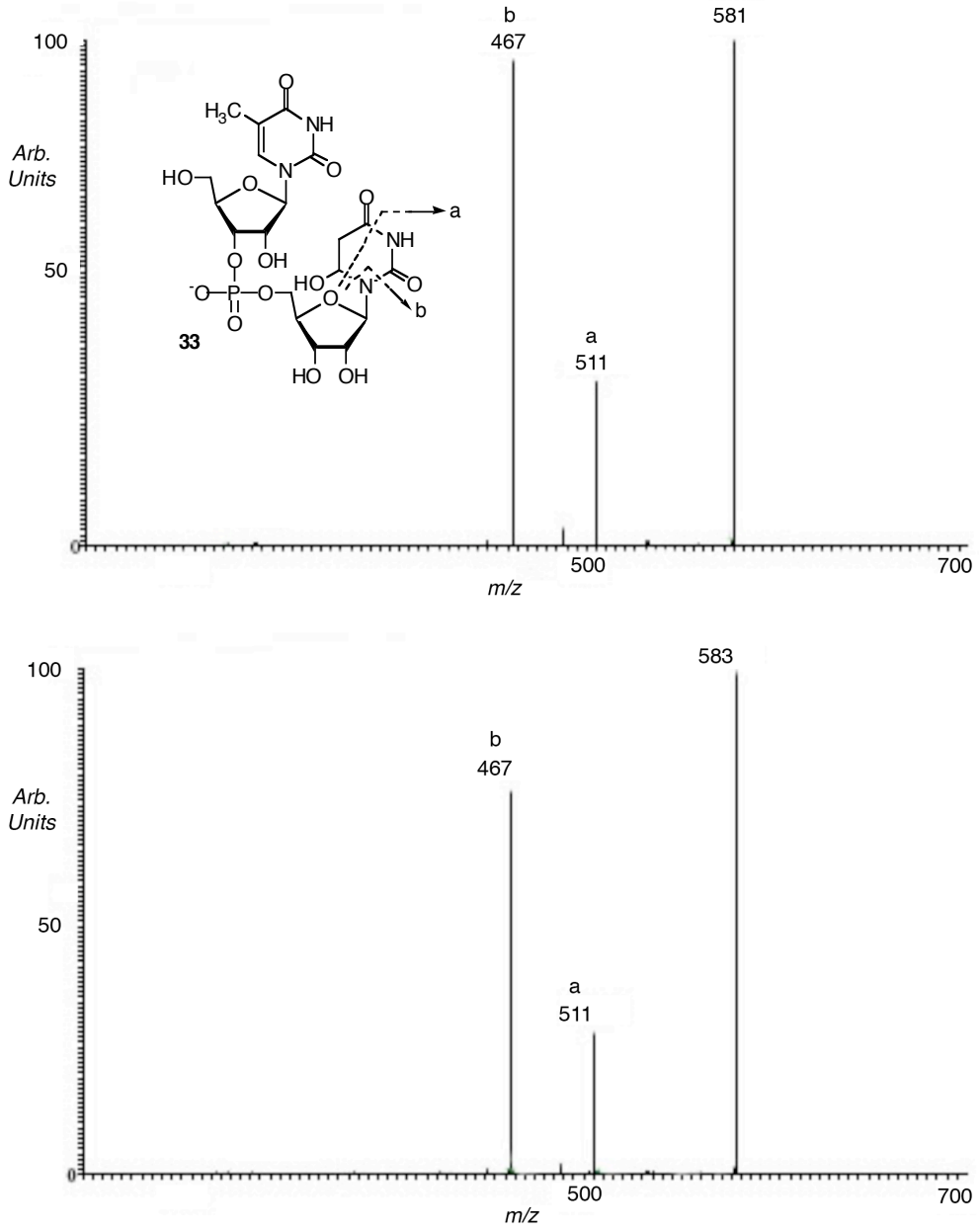


Figure S9. ESI-MS/MS of **33** (*calcd*. *m/z* = 581.1) obtained from photolysis of **31**. Top: $^{16}O_2$; Bottom: $^{18}O_2$.

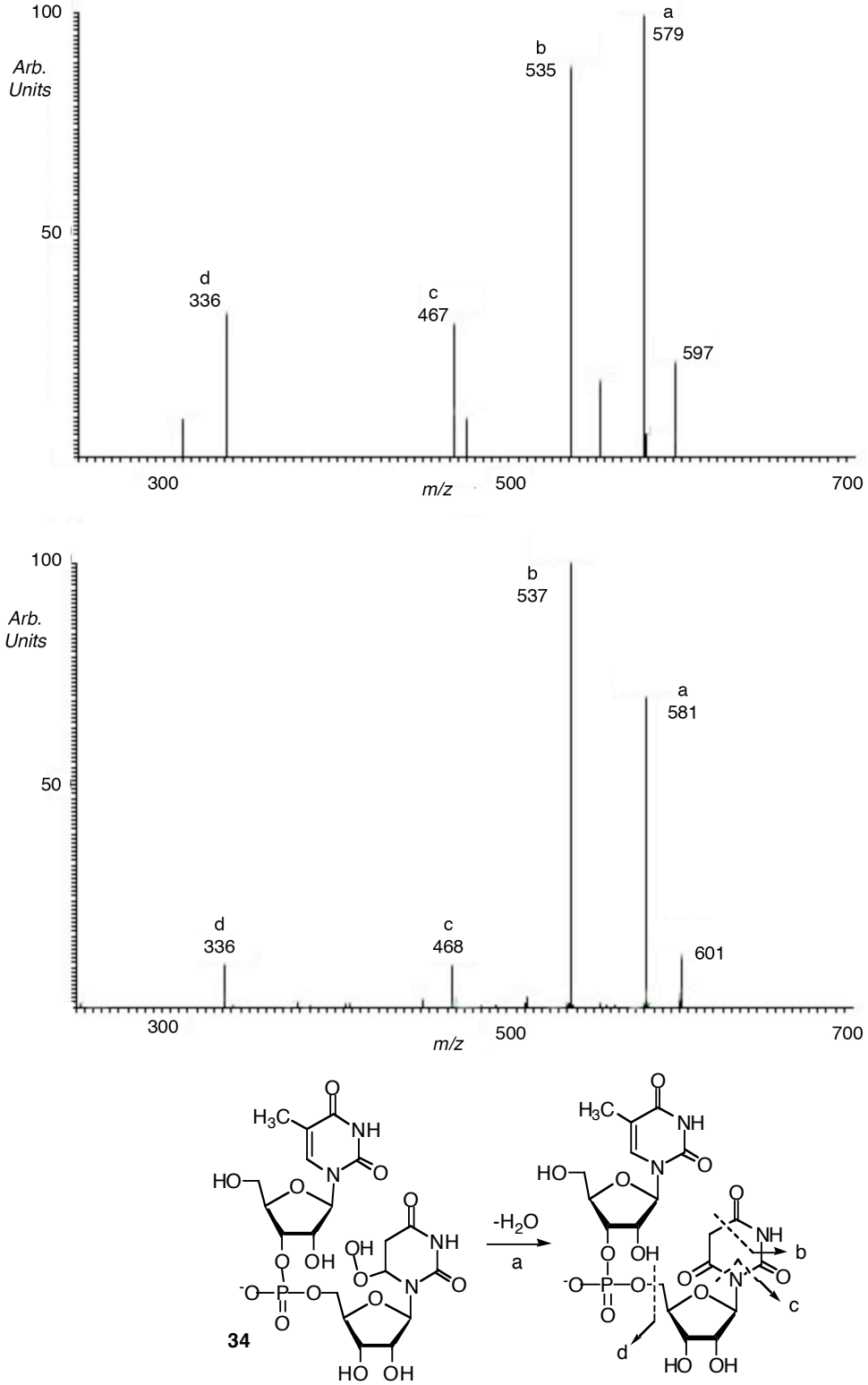


Figure S10. ESI-MS/MS of **34** (*calcd*. *m/z* = 597.1) obtained from photolysis of **31**. Top: $^{16}O_2$; Bottom: $^{18}O_2$.

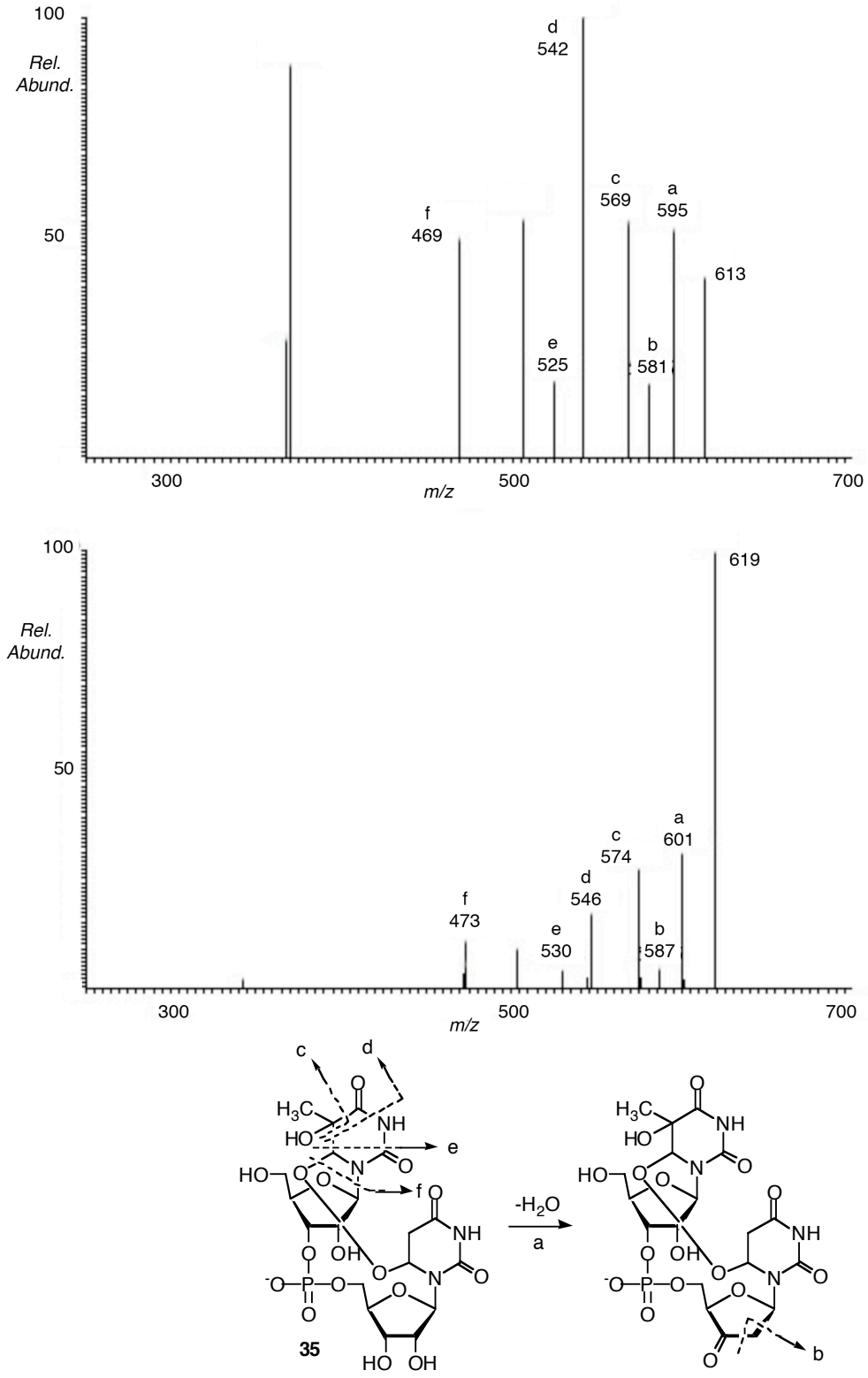


Figure S11. ESI-MS/MS of **35** (*calcd*. *m/z* = 613.1) obtained from photolysis of **31**. Top: $^{16}O_2$; Bottom: $^{18}O_2$.

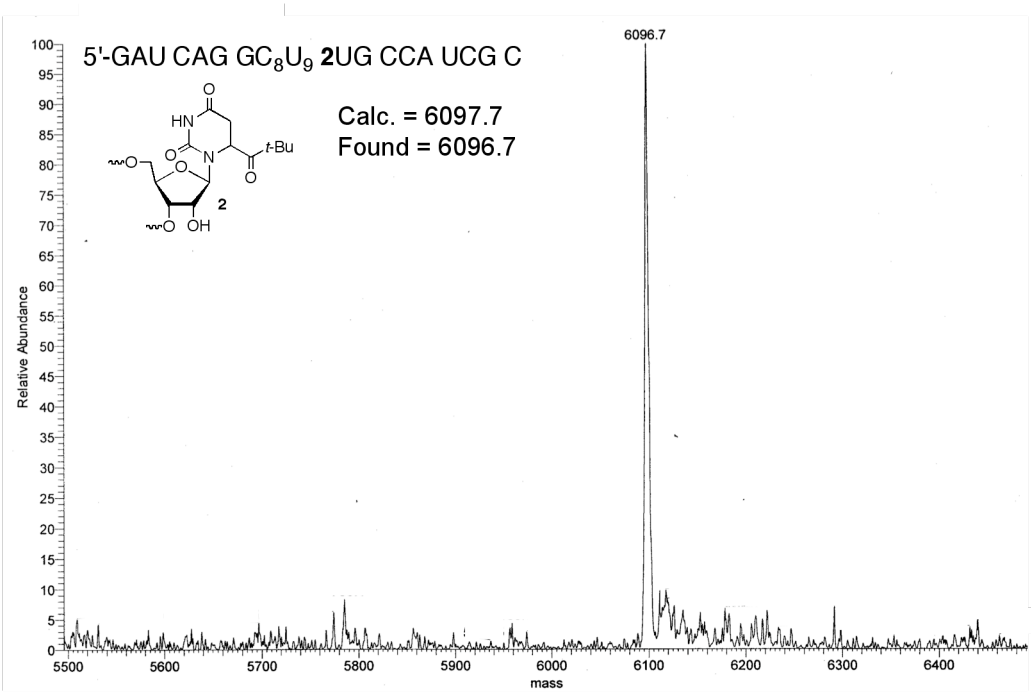


Figure S12. ESI-MS MS of oligonucleotide containing **2** used for **7** and **9**.

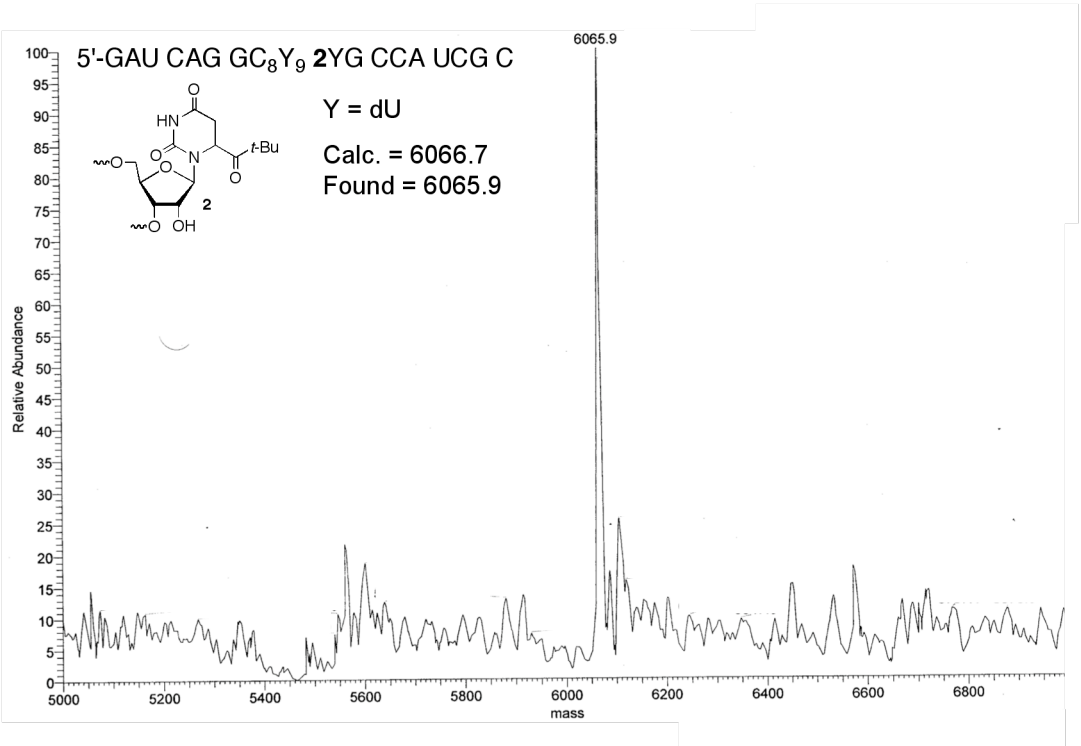


Figure S13. ESI-MS of oligonucleotide containing **2** used for **18** and **19**.

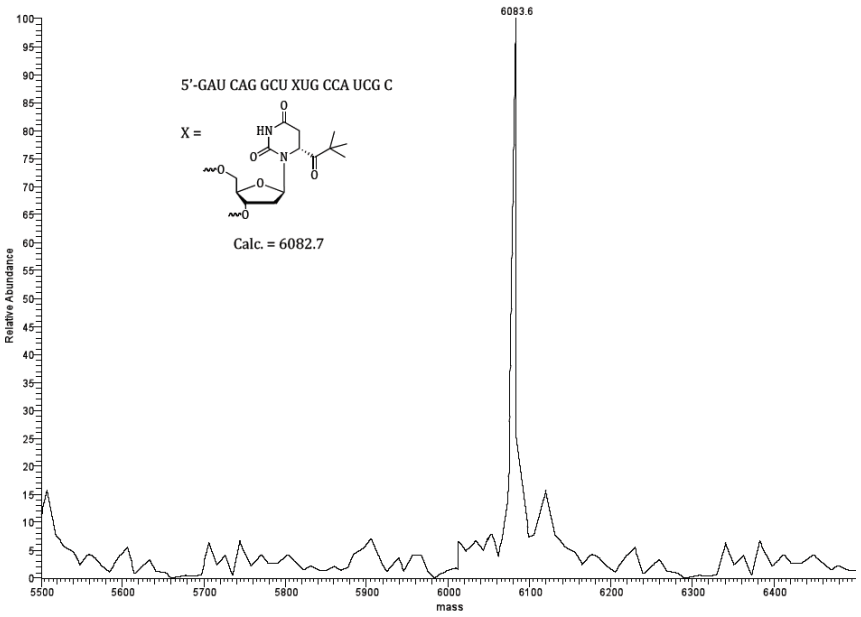


Figure S14. ESI-MS of oligonucleotide containing **2** used for **16** and **17**.

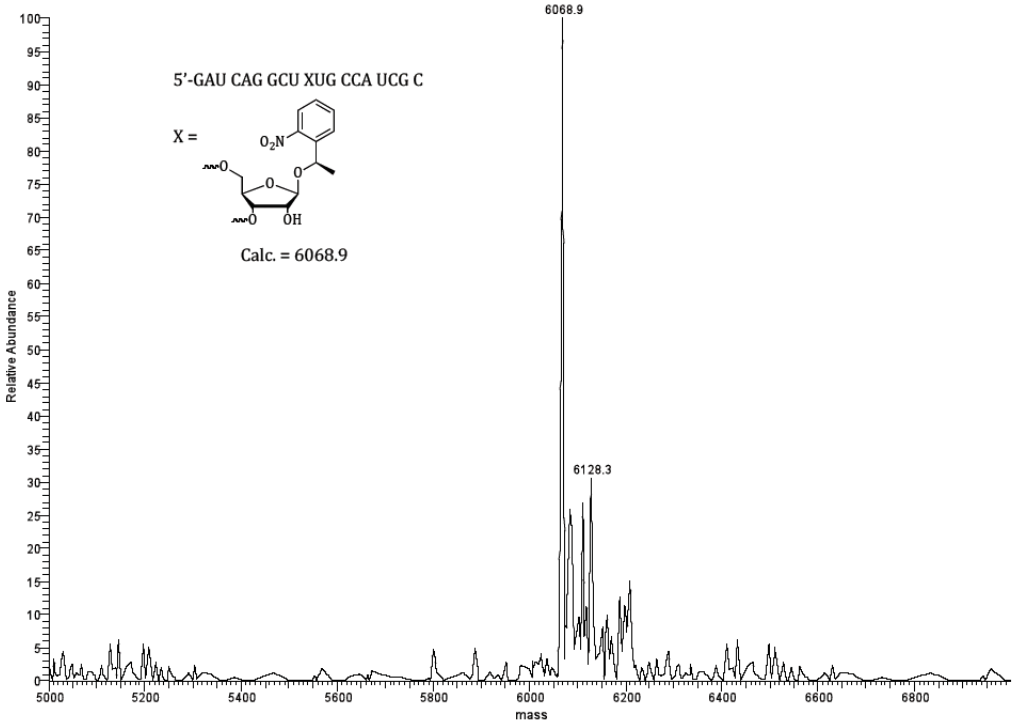


Figure S15. ESI-MS of oligonucleotide used to produce the abasic site (**29**) in **30**.

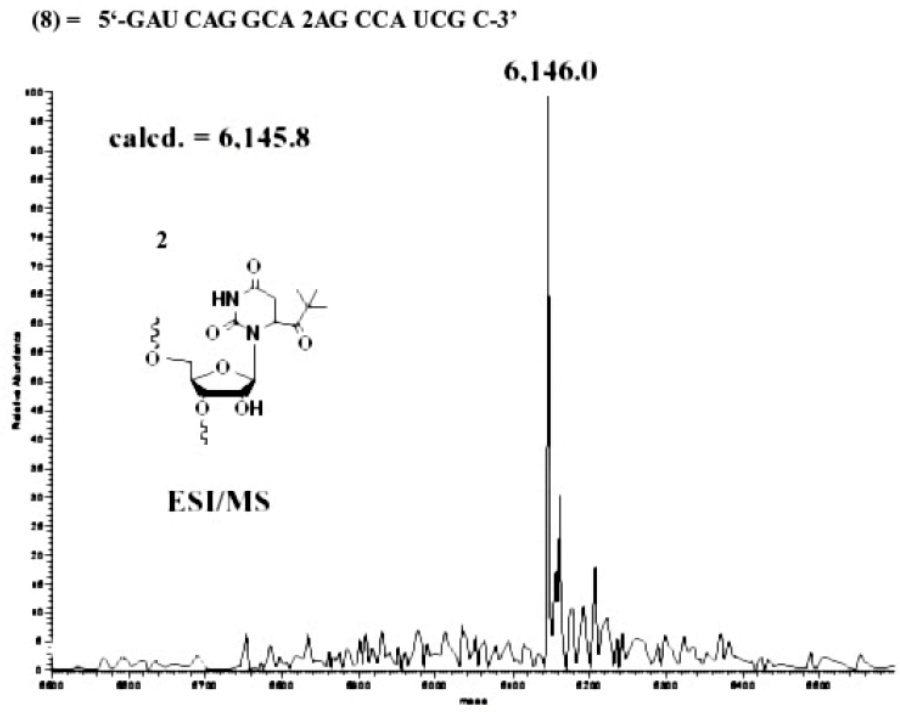


Figure S16. ESI- MS of oligonucleotide containing **2** used for **8** and **10**.

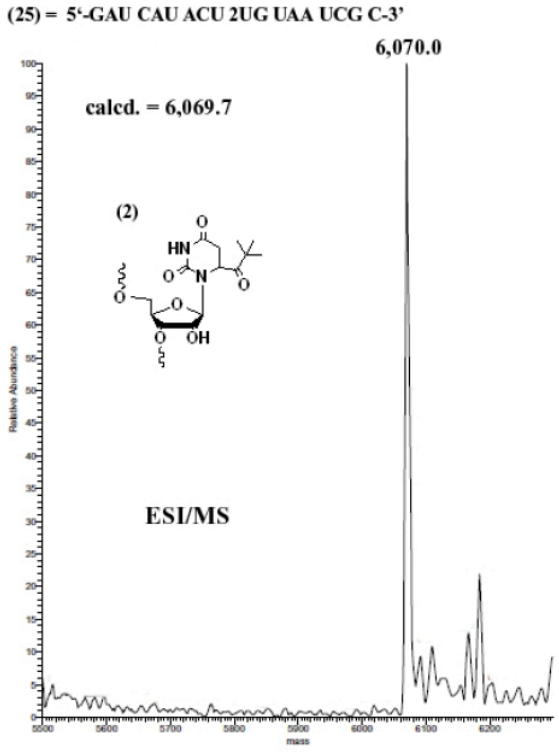


Figure S17. ESI-MS of oligonucleotide containing **2** used for **25**.

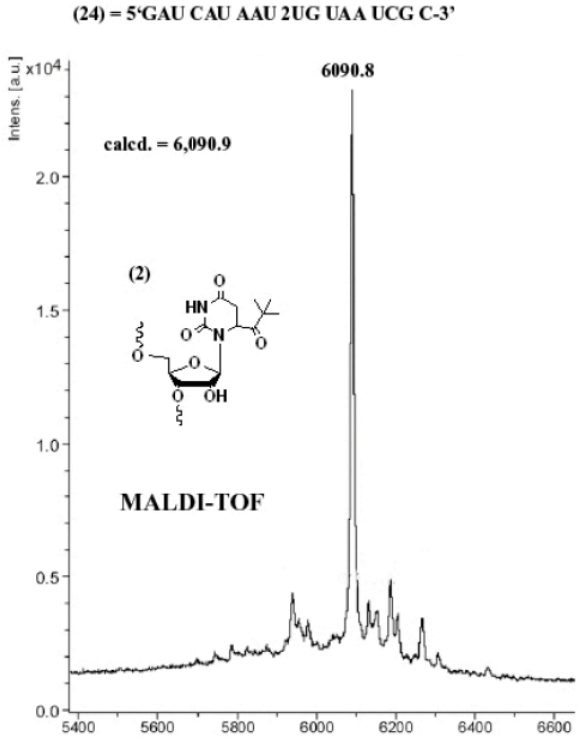


Figure S18. MALDI-TOF MS of oligonucleotide containing **2** used for **24**.

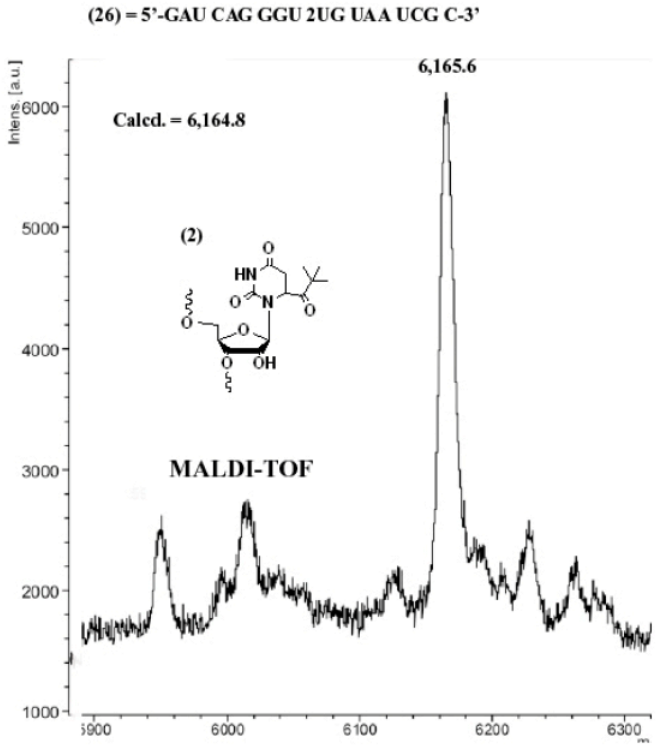


Figure S19. MALDI-TOF MS of oligonucleotide containing **2** used for **26**.

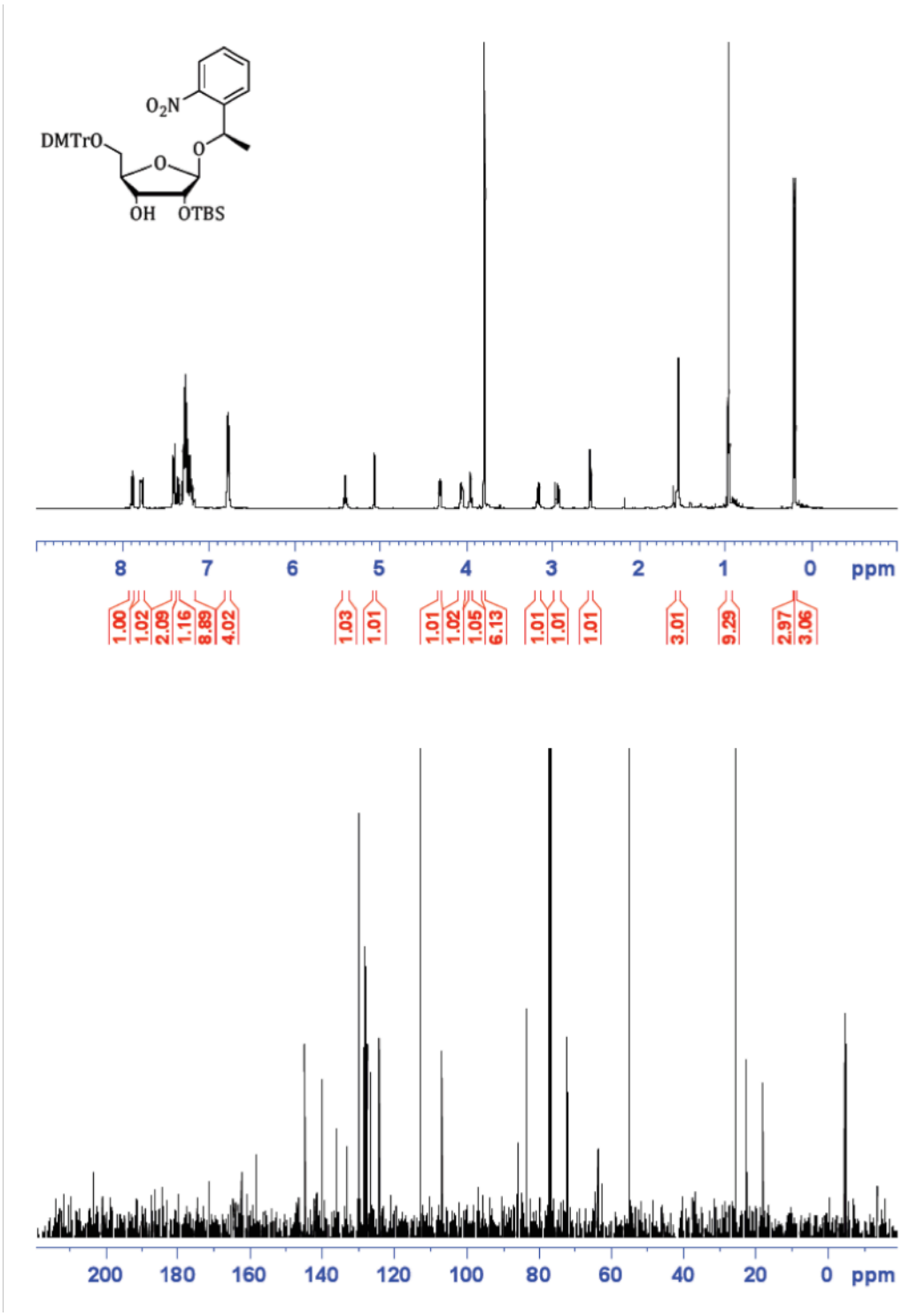


Figure S20. ^{1}H (top) and ^{13}C NMR (bottom) spectra of **A4**.

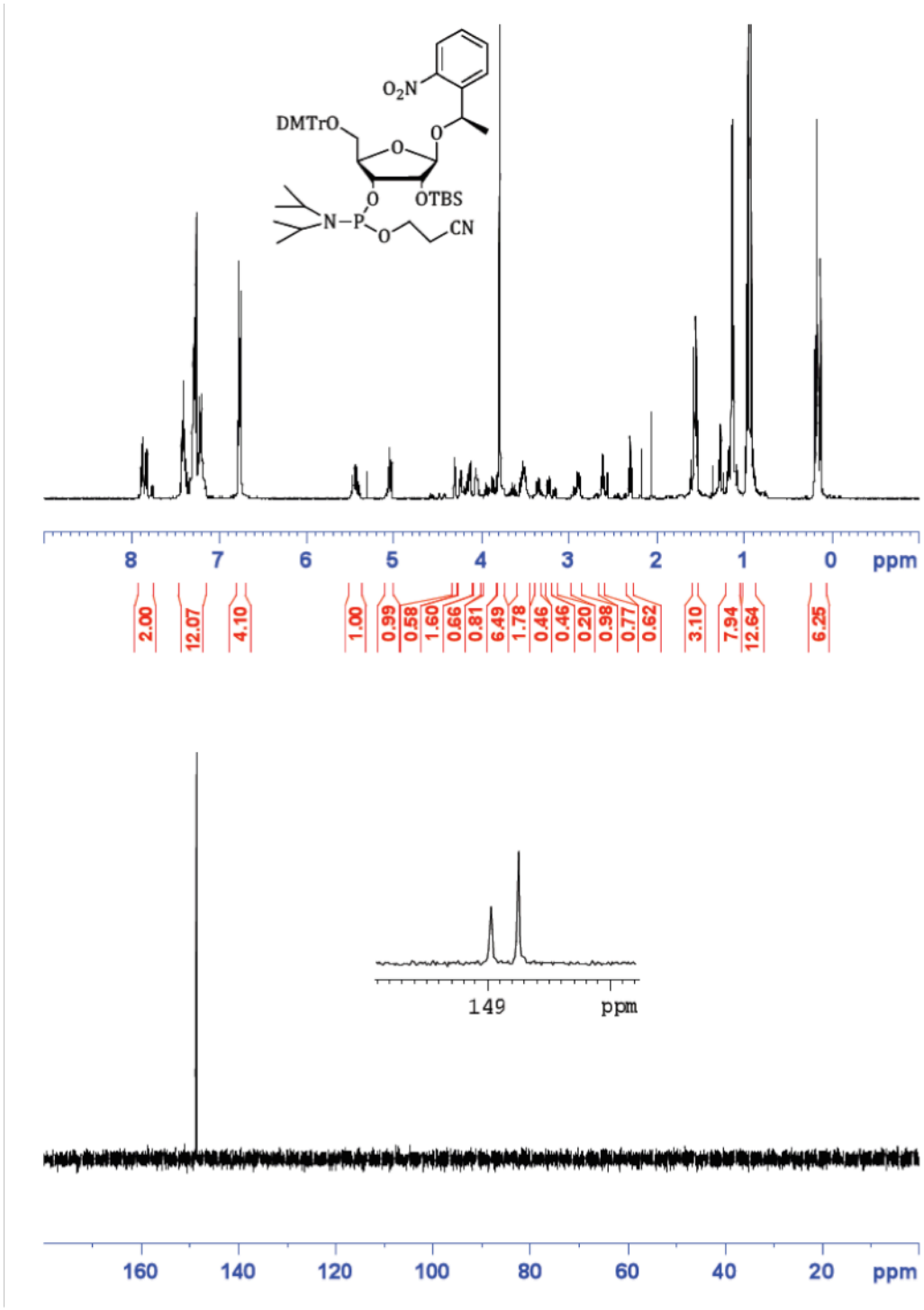


Figure S21. ^{1}H (top) and ^{31}P NMR (bottom) spectra of **A5**.

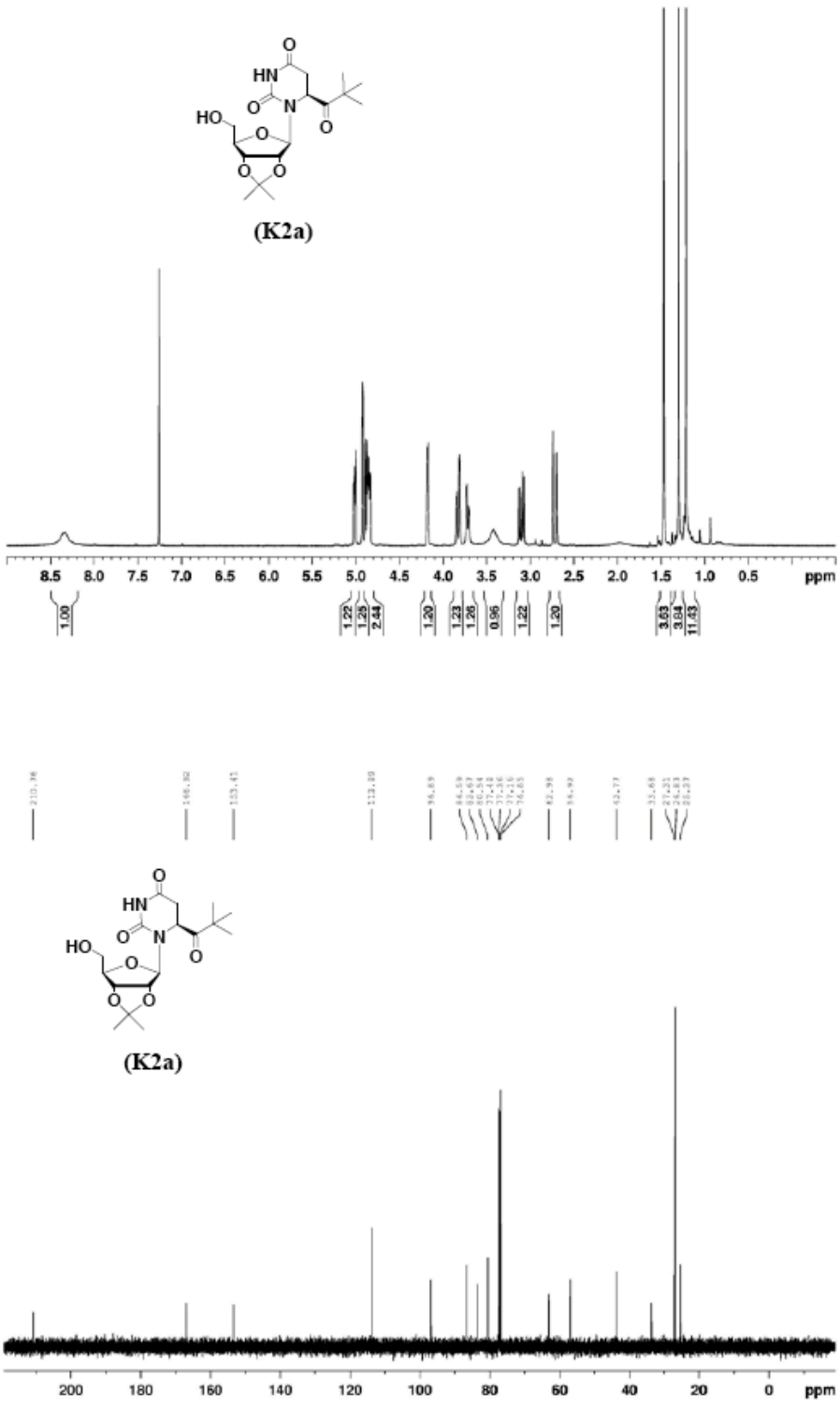


Figure S22. ^{1}H (top) and ^{13}C NMR (bottom) spectra of **K2a**.

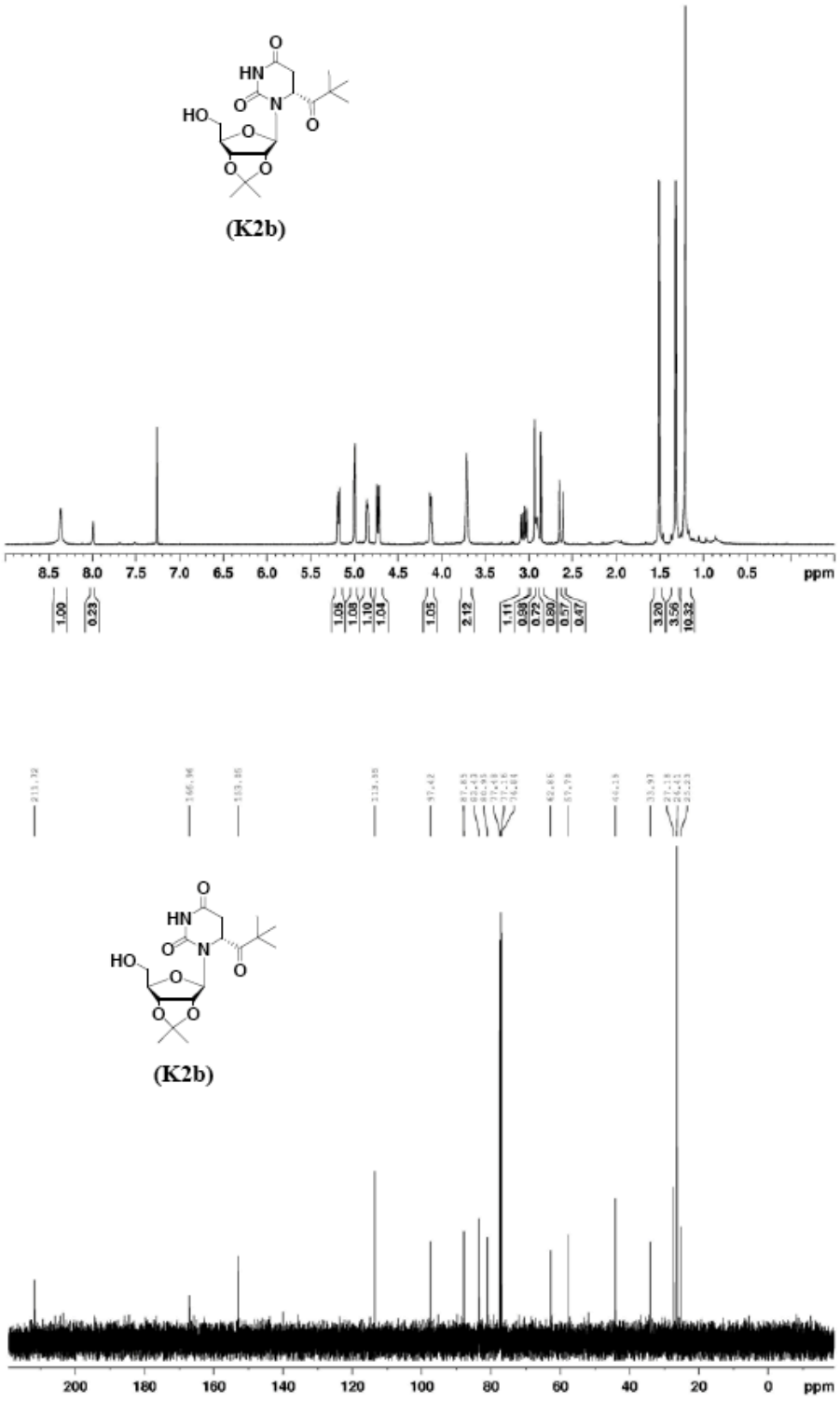


Figure S23. ^{1}H (top) and ^{13}C NMR (bottom) spectra of **K2b**.

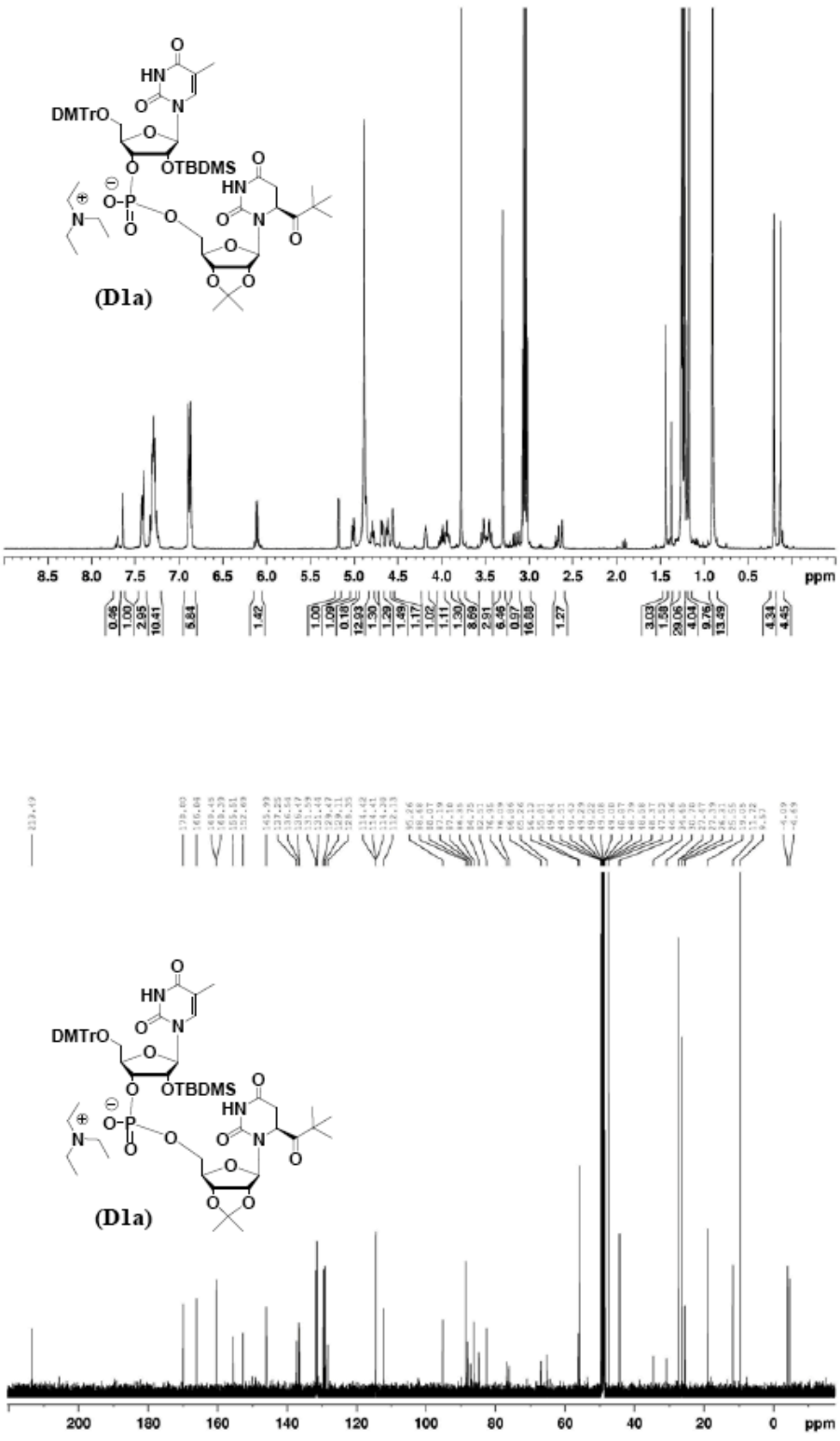


Figure S24. ^{1}H (top) and ^{13}C NMR (bottom) spectra of **D1a**.

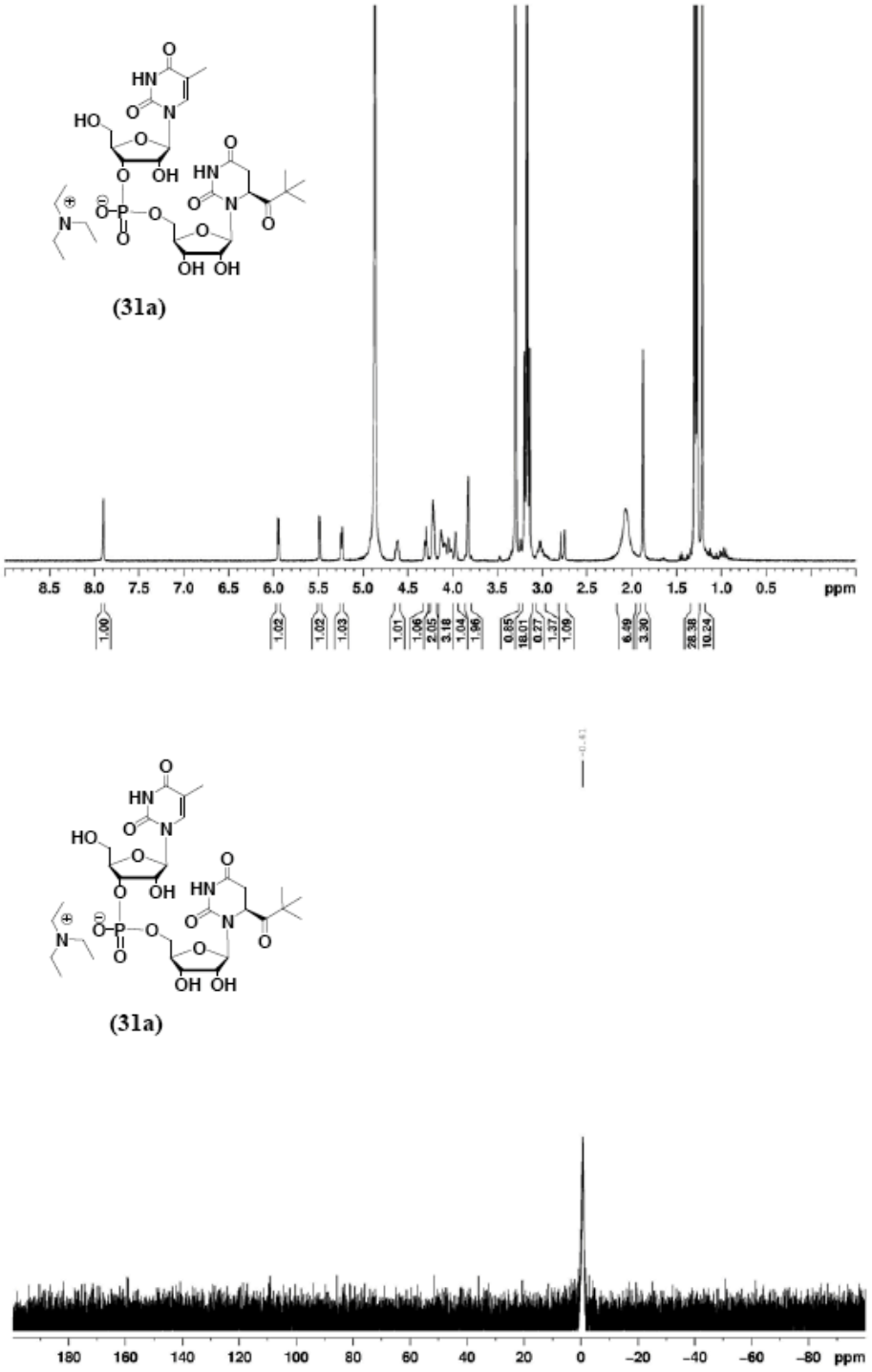


Figure S25. ^{1}H (top) and ^{31}P NMR (bottom) spectra of **31a**.

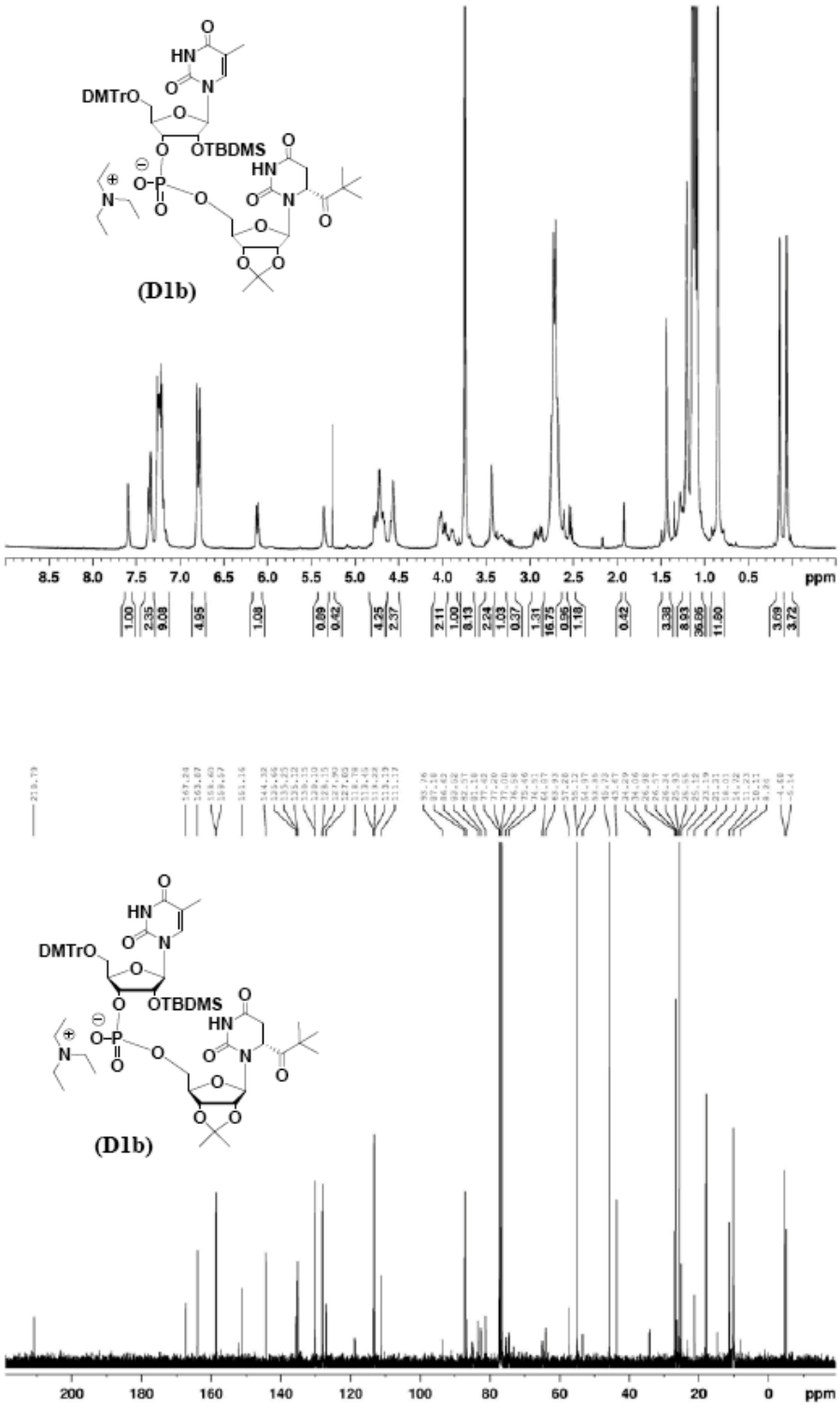


Figure S26. ^{1}H (top) and ^{13}C NMR (bottom) spectra of **D1b**.

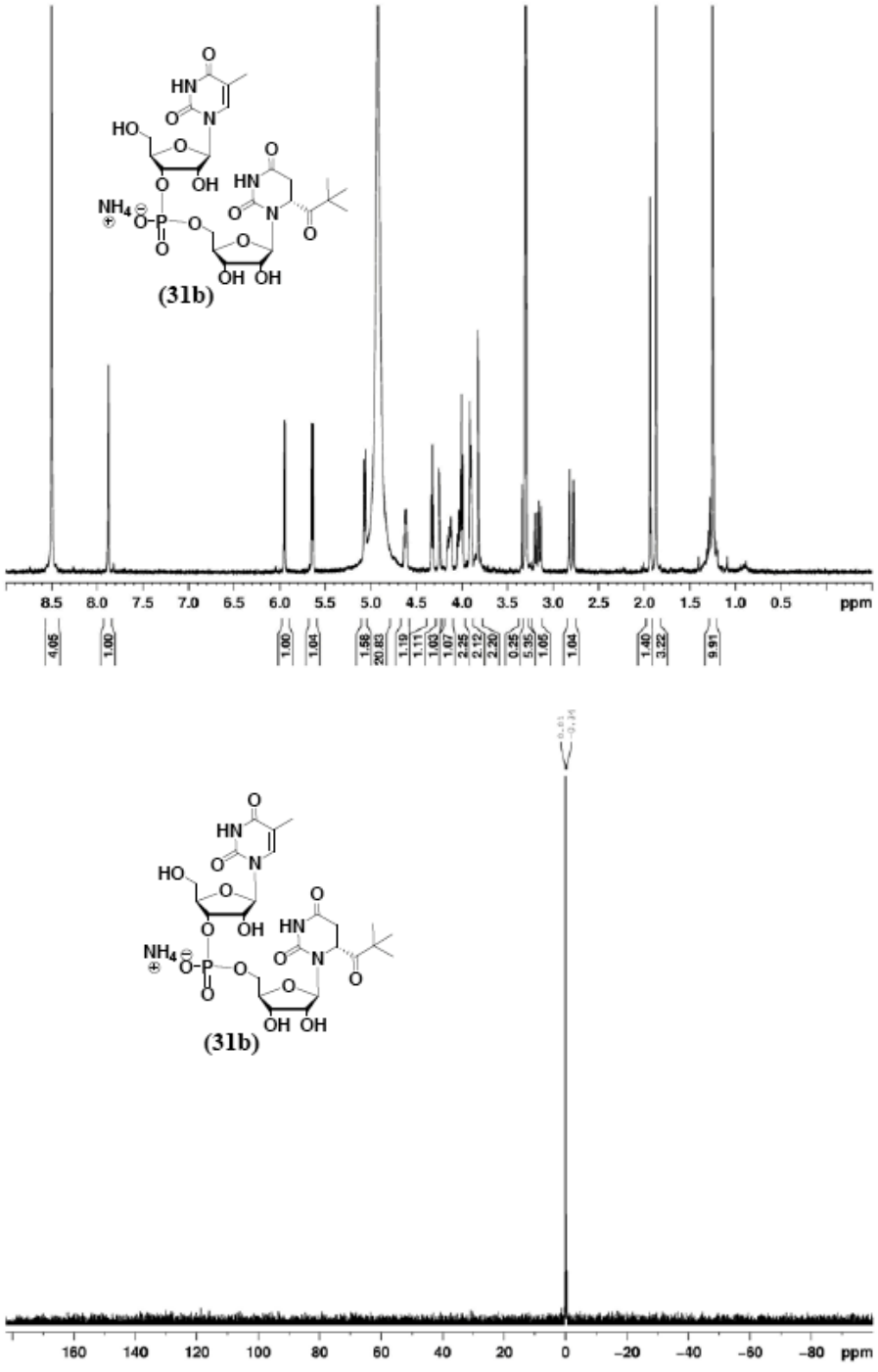


Figure S27. ^{1}H (top) and ^{31}P NMR (bottom) spectra of **31b**.