Supporting Information: Direct Strand Scission in Double Stranded RNA via a C5-Pyrimidine Radical.

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Contents:

1. General Methods (S2)
2. Experimental Procedures. (S2-S14)
3. **Figure S1.** ^{1}H (top) and ^{13}C NMR (bottom) spectra of **8**. (S15)
4. **Figure S2.** ^{1}H (top) and ^{13}C NMR (bottom) spectra of **9**. (S16)
5. **Figure S3.** ^{1}H (top) and ^{13}C NMR (bottom) spectra of **10**. (S17)
6. **Figure S4.** ^{1}H (top) and ^{13}C NMR (bottom) spectra of **30**. (S18)
7. **Figure S5.** ^{1}H (top) and ^{31}P NMR (bottom) spectra of **11**. (S19)
8. **Figure S6.** ^{1}H (top) and ^{13}C NMR (bottom) spectra of **31**. (S20)
9. **Figure S7.** ^{1}H (top) and ^{31}P NMR (bottom) spectra of **32**. (S21)
10. **Figure S8.** ^{1}H (top) and ^{13}C NMR (bottom) spectra of **33**. (S22)
11. **Figure S9.** ^{31}P NMR spectra of **33** (top) and ^{1}H NMR (bottom) spectra of **14**. (S23)
12. **Figure S10.** ^{13}C (top) and ^{31}P NMR (bottom) spectra of **14**. (S24)
13. **Figure S11.** ^{1}H (top) and ^{13}C NMR (bottom) spectra of **15**. (S25)
14. **Figure S12.** MALDI-TOF MS of **18**. (S26)
15. **Figure S13.** MALDI-TOF MS of **19**. (S26)
16. **Figure S14.** MALDI-TOF MS of **20**. (S27)
17. **Figure S15.** MALDI-TOF MS of **21**. (S27)
18. **Figure S16.** Effect of BME on strand cleavage from 5,6-dihydrouridin-5-yl (**1**) in **16**. (S28)
19. **Figure S17.** Effect of BME on 3'-end group from 5,6-dihydrouridin-6-yl (**2**). (S28)
20. **Figure S18.** Deuterium incorporation in recovered **14** following photolysis. (S29)
21. **Figure S19.** Detection of **26a**,**b** via gel electrophoresis using $NaBH_4$. (S30)
22. **Figure S20.** 3'-Enzymatic end group analysis. (S31)
23. **Figure S21.** 5'-Enzymatic end group analysis. (S32)

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General Methods. Oligonucleotides were synthesized on an Applied Biosystems Incorporated 394 oligonucleotide synthesizer. Oligonucleotide synthesis reagents were purchased from Glen Research (Sterling, VA). 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 LCQDeca. MALDI-TOF MS was carried out on a Bruker Autoflex instrument. Samples for MALDI-TOF analysis were prepared as described using a 3-hydroxypicolinic acid (HPA) or dihydroxybenzoic acid (DHB) 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. C18-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. UPLC was carried out on an Agilent 1290 Infinity.

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 16º 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 37º 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 10 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-**16** or **17** 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 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 37º 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 37º C for 11 h, 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.

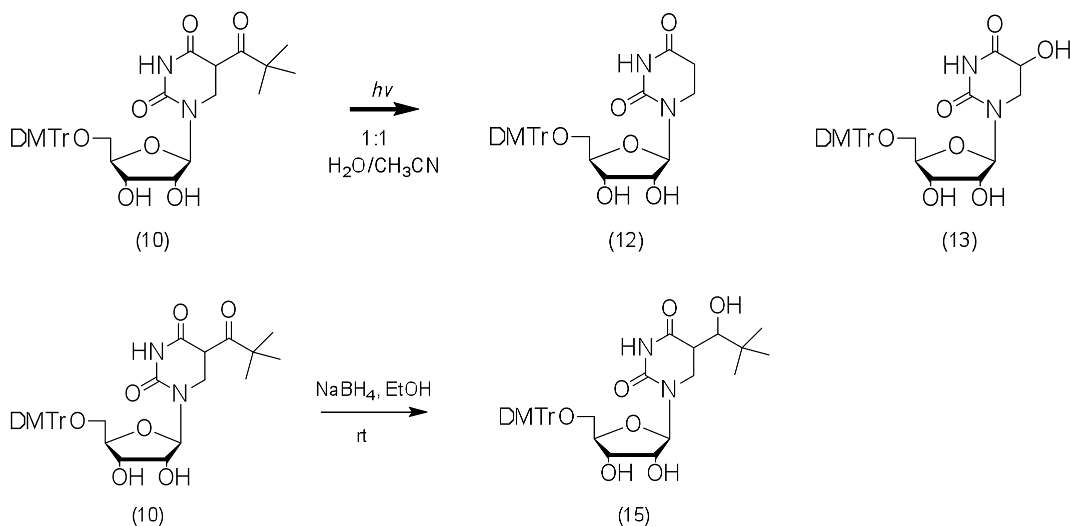
Probing for Ketone Using $NaBH_4$. Aliquots (9 μL) of photolyzed RNA (20 nM) were incubated with 1 μL $NaBH_4$ (1 M) or 1 μL water (control) at room temperature for 5 min, then diluted to 100 μL with 300 mM NaOAc buffer (pH 5.6) and precipitated with 300 μL ethanol. Samples were resuspended in 10 μL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

Thiol Competition. 5'-^{32}P-**16** was diluted to a final concentration of 5 nM. β-Mercaptoethanol (BME) added to a final concentration of 0, 25 μM, 100 μM, 250 μM, 500, μM, 750 μM, 1.1 mM, 1.5 mM, 2.5 mM or 5 mM and volume of 50 μL. All samples were photolyzed for 10 h, diluted

with loading buffer, and separated by 20% denaturing PAGE. All reactions were carried out in triplicate. The percentage of cleaved products in samples containing maximum BME was subtracted from the percentage of cleaved products in the samples with no BME control, 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.

Photolyses of Monomer 10. All photolyses were carried out in Pyrex tubes using a Rayonet photoreactor 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).

UPLC samples were analyzed with a ZORBAX Eclipse Plus C18 Rapid Resolution HD 1.8 μm column (2.1 × 50 mm). Samples were detected at 235 nm using one of the following three gradients. Hold for 1 min (97.7 % water / 0.3 % CH_3CN); then ramp to 35% CH_3CN linearly in 2 min; then hold for 15 min (35 % CH_3CN); then ramp to 50% CH_3CN linearly in 3 min; hold at 50% CH_3CN. Samples containing large amounts o BME or isopropanol were concentrated under reduced pressure and redissolved in water prior to analysis.

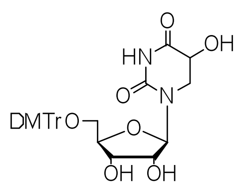


Determination of response factors. Response factors for **10**, and **12** were calculated using 3'-TBDMS-thymidine (3'-OSi-T) as an internal standard. Response factors were calculated using the following formula: ([X]/[S])=R_f(A(X)/A(S), where [X] is the concentration of the compound of interest and [S] is the concentration of the internal standard. A(X) and A(S) are the areas under the peaks corresponding to the compound of interest and the internal standard, respectively (Supporting Information Table 1).

The Response factor obtained for **12** was also used for hydrate **13**. Characterization of alcohol **13** was carried out by collection of the peak of interest (10.5 min, obtained upon aerobic photolysis in the presence of BME) followed by concentration of the sample under reduced pressure and ESI-MS analysis.

Supporting Information Table 1. Response Factors and retention times using 3'-TBDMS-T as internal standard.

Compound	Retention time (min)	Response Factor
10	22.7/23.5	8.94
12	15.3	9.21
13	10.5	—
15	19.5/20.8	—
3'-Si-T	11.4	—

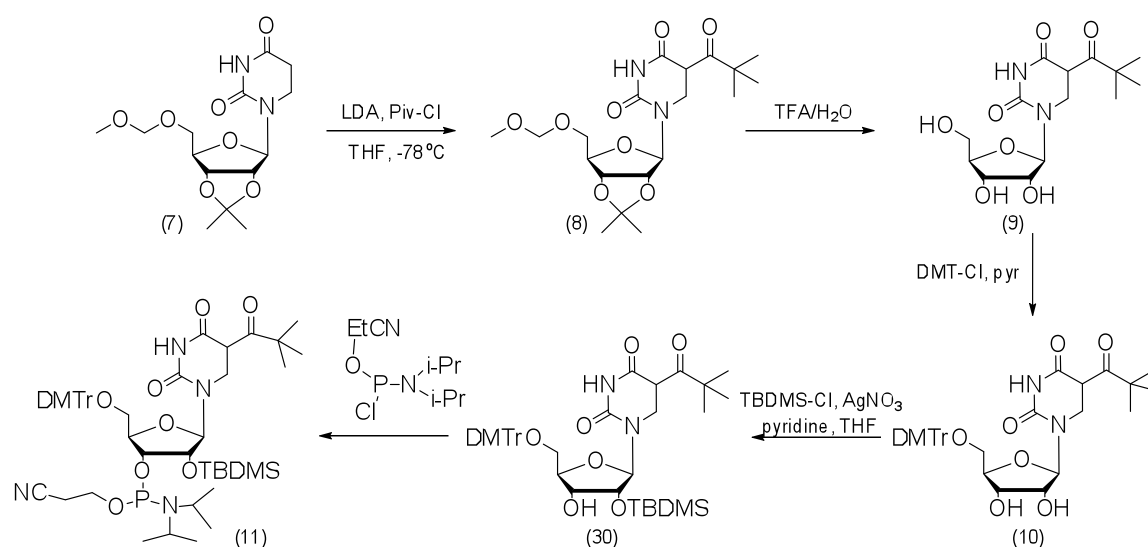


ESI/MS
Obtained: (-) = 563.1
(+) = 586.9 (+ Na)

$C_{30}H_{32}N_2O_9$
Exact Mass: 564.2108

Photolyses of Dinucleotide 14. A solution containing a known concentration of **14** was concentrated to dryness under reduced pressure, redissolved in D_2O (D, 99.96 %, Cambridge Isotope Laboratories, Inc.) to a desired concentration (75 µM, 80 µL) and transferred to a Pyrex

tube. The tubes were immediately degassed and sealed as discussed above. All photolyses were carried out using a Rayonet photoreactor fitted with 16 lamps having an output maximum at 350 nm. Following irradiation, the samples were concentrated to dryness and redissolved in H_2O (250 μL). The samples were vortexed (5 min) and concentrated to dryness. This washing procedure was repeated 2 more times. The residues were redissolved in a DHB matrix (5 μL, 10 mg DHB in 1:1 CH_3CN/ 0.2 % aq. TFA) and transferred onto a MALDI plate for analysis.



Preparation of 8.[2] A solution of dihydrouridine **7** (0.7g, 2.12 mmol in THF, 5 ml) was added dropwise to a cooled solution (-78° C) containing lithium diisopropyl amide (4.66 mmol in THF, 35 ml) and stirred for 30 min. Pivaloyl chloride (0.28 g, 2.33 mmol) was then added dropwise and stirred for 30 min. A solution of ammonium chloride (60 ml, sat) was added at once and extracted with EtOAc (35 ml × 2). The organic layers were combined and dried over brine (35 mL) and Na_2SO_4. The solution was then concentrated under reduced pressure to dryness and purified by column chromatography (3% MeOH in DCM) to yield **8** as a mixture of diastereomers in the form of a white foam (0.69 g, 1.66 mmol, 79 %). ^{1}H NMR ($CDCl_3$) δ 7.97 (br, 2H), 5.78 (d, J = 3.2, 1H), 5.65 (d, J = 3.2, 1H), 4.76-4.58 (m, 8H), 4.20-4.16 (m, 4H), 3.69-

3.67 (m, 6 H), 3.52-3.46 (m, 2H), 3.38-3.32 (m, 8H), 1.56 (s, 6H), 1.34 (s, 6H), 1.21 (s, 9H) and 1.20 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 208.6, 208.0, 167.4, 167.3, 151.7, 151.3, 114.6, 114.4, 96.7, 96.6, 91.9, 91.0, 83.4, 83.1, 82.5, 81.9, 81.0, 80.5, 77.2, 67.8, 67.5, 55.5, 55.4, 47.3, 45.5, 45.4, 42.7, 41.8, 27.3, 25.6 and 25.5; IR (NaCl) 3452, 3055, 2985, 1703, 1478, 1262 and 1302 cm^{-1}; HRMS *m/z* calculated for $C_{19}H_{30}N_2O_8Na$ (M^+ + Na), 437.1901, observed *m/z* = 437.1900.

Preparation of 9. Nucleoside **8**[2] (0.27g, 0.65 mmol) was dissolved in a 1:1 TFA/H_2O solution and stirred for 4 h. The solution was then concentrated under reduced pressure to dryness and purified by column chromatography (15% MeOH in DCM) to yield **9** as a mixture of diastereomers in the form of a white foam (0.2 g, 0.61 mmol, 93 %). ^{1}H NMR (DMF) δ 8.03 (br, 1H), 5.89-5.84 (m, 1H), 5.26-5.24 (m, 1H), 5.04-4.94 (m, 1H), 4.54-4.46 (m, 1H), 4.16-4.09 (m, 2 H), 3.85-3.84 (m, 1H), 3.67-3.64 (m, 2H), 3.54-3.52 (m, 1H) and 1.19 (s, 9H); ^{13}C NMR (DMF) δ 210.2, 209.6, 169.4, 153.3, 96.7, 88.4, 88.0, 84.7, 84.5, 71.7, 71.4, 62.4, 62.3, 47.9, 47.6, 45.3, 45.2, 42.7, 40.0, 39.9, 25.2 and 25.1; IR (NaCl) 3534, 3452, 3280, 3057, 2959, 1714, 1059 and 1264 cm^{-1}; HRMS *m/z* calculated for $C_{14}H_{23}N_2O_7$ (M^+ + H), 331.1499, observed *m/z* = 331.1503.

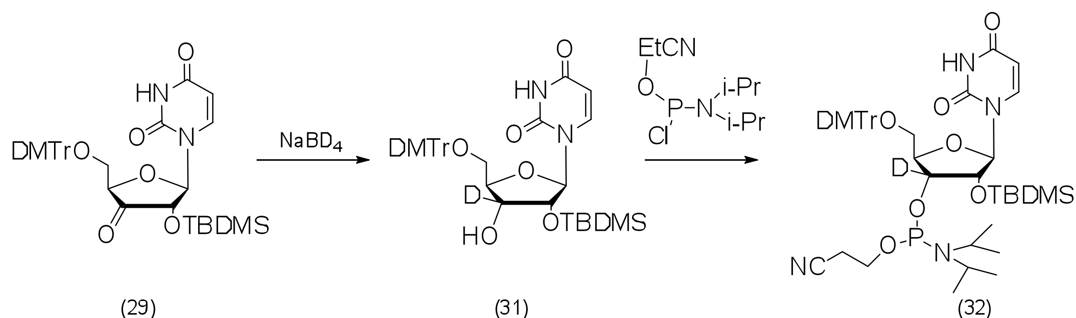
Preparation of 10. Ketone **9** (2.34 g, 7.08 mmol) was azeotropically dried over pyridine, dissolved in pyridine (45 mL), and cooled to 0 °C. DMT-Cl (2.64 g, 7.8 mmol) was added at once and the solution was allowed to warm to room temperature over a 16 h period. A 20 % $NaHCO_3$ solution (80 mL) was added and the organic layer was extracted with DCM (2 × 60 mL). The organic layer was dried with brine (70 mL) and evaporated to dryness. Flash column chromatography (5 % MeOH, 1% Et_3N in DCM) provided **10** as a diastereomeric mixture in the

form of a white foam (2.0 g, 3.2 mmol, 45%). ^{1}H NMR ($CDCl_3$) δ 9.24 (br, 1H, NH), 9.10 (br, 1H, NH), 7.36-7.35 (m, 4H), 7.32-7.25 (m, 12H), 7.17-7.15 (m, 2H), 6.82-6.78 (m, 8H), 5.95 (d, *J* = 5.6 Hz, 1H), 5.87 (d, *J* = 4.4 Hz, 1H), 4.81-4.56 (m, 2H), 4.26-4.17 (m, 6H), 4.08-4.02 (m, 2H), 3.75-3.69 (m, 14H), 3.46-3.44 (m, 2H), 3.26-3.17 (m, 2H), 0.99(s, 9H) and 0.94 (s, 9H); ^{13}C NMR ($CDCl_3$) 209.1, 208.4, 168.3, 168.2, 158.5, 158.4, 153.4, 152.7, 144.5, 144.4, 135.7, 135.6, 135.5, 135.4, 130.0, 129.9, 128.1, 128.0, 127.8, 126.8, 113.2, 87.8, 87.2, 86.4, 86.3, 82.9, 82.6, 72.0, 71.5, 71.0, 70.9, 63.5, 62.9, 55.1,53.4, 46.8, 46.5, 45.3, 40.0, 39.6, 25.3 and 25.2; IR (NaCl plate) 3055, 2984, 1701, 1509 and 1033 cm^{-1}; UV (H_2O/CH_3CN): λ_{max} = 235 nm (ε = 21,976), λ_{max} = 274 nm (ε = 6.973); HRMS *m/z* calculated for $C_{35}H_{40}N_2O_9Na$ (M^+ + Na), 655.2631, observed *m/z* = 655.2625.

Preparation of 30. A mixture of pyridine (0.93 g, 11.8 mmol) and silver nitrate (0.64 g, 3.8 mmol) was dissolved in THF (70 mL) and sonicated (30 min). Addition of TBDMSCl (0.56 g, 3.7 mmol) with stirring over 5 min yielded a milky solution. Ketone **10** (2.0 g, 3.2 mmol) was added at once and stirred for an additional 8 h. The solution was filtered into a stirring 20 % $NaHCO_3$ solution (60 mL) and extracted with ethyl acetate (3 × 40 mL). The organics were dried over brine (20 mL), Na_2SO_4, and concentrated under reduced pressure. Purification through column chromatography (1:1, EtOAc:hexanes) provided ketone **30** as a diastereomeric mixture in the form of a white foam (0.78 g, 1.04 mmol, 33 %). ^{1}H NMR ($CDCl_3$) δ 7.51 (d, *J* = 8.4, 2H), 7.37-7.34 (m, 4H), 7.29-7.20 (m, 14H), 6.83-6.80 (m, 8H), 5.91 (d, *J* = 4.8, 1H), 5.85 (d, *J* = 6, 1H), 4.36 (t, *J* = 6, 1H), 4.24 (t, *J* = 5.2, 1H), 4.19-4.06 (m, 6H), 3.99-3.94 (m, 2H), 3.80 (s, 12H), 3.76-3.72, (m, 2H), 3.66-3.62 (m, 1H), 3.51-3.49 (m, 1H), 3.42-3.37 (m, 1H), 3.31-3.30 (m, 2H), 3.22-3.19 (m, 1H), 2.74 (d, *J* = 2.8, 1H), 2.64 (d, *J* = 5.6, 1H), 1.01 (s, 9H),

0.99 (s, 9H), 0.96 (s, 9H), 0.92 (s, 9H), 0.20 (s, 3H), 0.19 (s, 3H), 0.18 (s, 3H) and 0.14 (s, 3H); ^{13}C NMR ($CDCl_3$) 208.2, 207.7, 171.0, 167.1, 167.0, 158.6, 151.9, 150.9, 144.6, 144.4, 135.7, 135.5, 135.3, 130.0, 129.9, 129.8, 128.1, 128.0, 127.9, 127.8, 126.9, 113.2, 113.1, 88.4. 86.5, 86.4, 82.9, 82.3, 77.2, 72.9, 72.7, 71.2, 70.8, 60.3, 55.2, 46.9, 46.8, 45.4, 39.9, 25.6, 25.4, 21.0, 18.0, 14.2, -4.6, -4.7, -4.8 and -5.1; IR (NaCl plate) 3382, 3053, 2958, 1704 and 1508 cm^{-1}; HRMS *m/z* calculated for $C_{41}H_{54}N_2O_9SiNa$ (M^+ + Na), 769.3496, observed *m/z* = 769.3486.

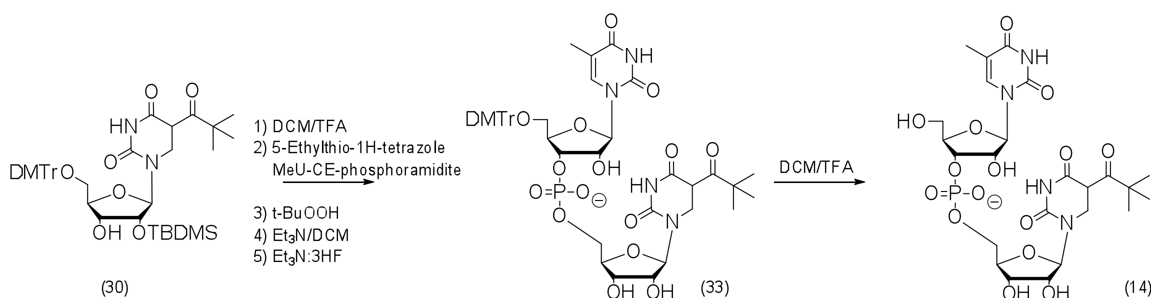
Preparation of 11. Nucleoside **30** (0.24 g, 0.33 mmol) was dissolved in a solution of diisopropylethylamine (0.17 g, 1.31 mmol) and DCM (1 mL). 2-Cyanoethyl-N,N-diisopropyl-chlorophosphoramidite (0.1 g, 0.4 mmol) was added and the solution was stirred for 1 h, followed by addition of a sat. $NaHCO_3$ solution (2 mL). The organics were extracted with DCM (2 × 3 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Column chromatography (2:3, EtOAc/hexanes) yielded a mixture of diastereomers of **11** as a white foam (0.22 g, 0.24 mmol, 73 %). ^{1}H NMR ($CDCl_3$) δ 7.78 (br, 1H), 7.34-7.36 (m, 2H), 7.28-7.23 (m, 8H), 6.83-6.79 (m, 4H), 5.99-5.89 (m, 1H), 4.41-4.11 (m, 4H), 4.03-3.92 (m, 2H), 3.85-3.76 (m, 10H), 3.59-3.43 (m, 4H), 3.24-3.11 (m, 1H), 2.64-2.63 (m, 1H), 2.27-2.24 (m, 1H), 1.17-1.12 (m, 9H), 1.01-0.84 (m, 26H) and 0.21-0.09 (m, 6H); ^{31}P NMR ($CDCl_3$) δ 150.9, 150.8 and 148.7. IR (NaCl plate) 3375, 3049, 2988, 1697 and 1364 cm^{-1}; HRMS *m/z* calculated for $C_{50}H_{71}N_4O_{10}PsiNa$ (M^+ + Na), 969.4575, observed *m/z* = 969.4569.



Preparation of 31. To a solution containing ketone **29**[3] (0.65 g, 0.99 mmol) dissolved in ethanol (40 mL) was added $NaBD_4$ (0.06 g, 1.4 mmol) at once and the mixture was stirred for 10 min. Water was added (60 mL) and the milky suspension was washed with EtOAc (3 × 70 mL), followed by concentration of the organics under reduced pressure. Column chromatography (4:1, EtOAc:hexanes) afforded **31** as a white foam (0.065 g, 0.1 mmol, 10 %). ^{1}H NMR ($CDCl_3$) δ 8.20 (s, 1H, NH), 7.93 (d, J = 8, 1H), 7.38-7.26 (m, 2H), 7.32-7.26 (m, 7H), 6.86-6.83 (m, 4H), 5.94 (d, J = 3.6, 1H), 5.28 (d, J = 8, 1H), 4.34 (d, J = 3.6, 1H), 4.13-4.09 (m, 1H), 3.80 (s, 6H), 3.51-3.49 (m, 2H), 2.56 (s, 1H, OH), 0.93 (s, 9H), 0.18 (s, 3H) and 0.16 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 163.3, 158.7, 150.3, 144.3, 140.2, 135.1, 134.9, 130.1, 130.0, 128.1, 128.0, 127.2, 113.3, 102.2, 88.7, 87.1, 83.4, 76.2, 62.2, 55.2, 25.6, 18.0, -4.7, -5.3; IR (NaCl plate) 3060, 2989, 1695, 1509 and 1276 cm^{-1}; HRMS m/z calculated for $C_{36}H_{43}DN_2O_8SiNa$ (M^+ + Na), 684.2827, observed m/z = 684.2847.

Preparation of 32. Nucleoside **31** (0.075 g, 0.114 mmol) was dissolved in a solution of diisopropylethylamine (0.06 g, 0.46 mmol) and DCM (1 mL). 2-Cyanoethyl-N,N-diisopropyl-chlorophosphoramidite (0.031 g, 0.13 mmol) was added and stirred for 2 h, followed by addition of a sat. $NaHCO_3$ solution (2 mL). The organics were extracted with DCM (2 × 3 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash column chromatography (2:3, EtOAc/hexanes) yielded a mixture of diastereomers of phosphoramidite **32** as a white foam (0.07

g, 0.081 mmol, 71 %). ^{1}H NMR ($CDCl_3$) δ 9.02 (br, 2H, NH), 8.02 (d, *J* = 8.4, 1H), 7.91 (d, *J* = 8, 1H), 7.43-7.37 (m, 4H), 7.31-7.23 (m, 14H), 6.86-6.82 (m, 8H), 5.99 (d, *J* = 4.8, 1H), 5.90 (d, *J* = 3.6, 1H), 5.33-5.28 (m, 2H), 4.44 (d, *J* = 4.8, 1H), 4.35 (d, *J* = 4, 1H), 4.31 (s, 1H), 4.22 (s, 1H), 3.98-3.93 (m, 1H), 3.83-3.79 (m, 14 H), 3.73-3.71 (m, 1H), 3.68-3.51 (m, 9H), 3.41-3.37 (m, 3H), 2.67-2.63, (m, 2H), 2.42-2.39 (m, 2H), 1.28-1.24 (m, 18H), 1.05 (s, 6H), 0.91 (s, 9H), 0.89 (s, 9H), 0.14-0.11 (m, 12H); ^{31}P NMR ($CDCl_3$) 149.9 and 149.5. IR (NaCl plate) 3272. 3059, 2956, 2860, 1690, 1574 and 1264 cm^{-1}; HRMS *m/z* calculated for $C_{45}H_{60}DN_4O_9PSiNa$ (M^+ + Na), 884.3906, observed *m/z* = 884.3902.

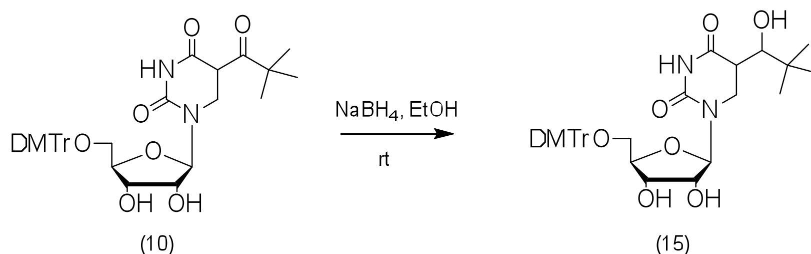


Preparation of 33. To a flask containing ketone **30** (0.1g, 0.134 mmol) dissolved in DCM (15 mL) was added TFA (1 mL) at once. The bright orange solution was stirred for 75 min and concentrated under reduced pressure. The residues were washed over diethyl ether (10 mL × 5) to yield a white solid, which was used for the next step. The detritylated material was azeotropically dried over pyridine (3 mL) and mixed in a flask with MeU-CE phosphoramidite (0.13g, 0.147 mmol) and CH_3CN (0.5 mL). 5-Ethylthio-1H-tetrazole (0.59 mL, 0.25 M in CH_3CN) was added to the clear solution and stirred for 1.25 h, at which time a solution of *t*-BuOOH in decane (0.1 ml, 5 M) was added. The reaction was stirred for an additional 2 h and concentrated to dryness under reduced pressure. The residue was redissolved in DCM/Et_3N (2:2 mL), stirred for 12 h and concentrated under reduced pressure. The obtained solid was redissolved in THF (3 mL) and stirred in the presence of Et_3N•3HF (0.06 mL, 0.368 mmol) for

6h. The yellow solution was concentrated under reduced pressure and the final product was purified via flash column chromatography (1% Et_3N : 20% MeOH : 80% DCM) to yield **33** as a diastereomeric mixture in the form of a white foam (0.025 g, 0.019 mmol, 14%). ^{1}H NMR (CD_3CN) δ 7.45-7.43 (m, 3H), 7.32-7.29 (m, 5H), 7.24-7.22 (m, 1H), 6.87 (d, J = 8.8 Hz, 4H), 5.88-5.86 (m, 1H), 5.68-5.65 (m, 1H), 4.68-4.63 (m, 1H), 4.42-4.39 (m, 1H), 4.30-4.26 (m, 1H), 4.21-4.16 (m, 1H), 4.09-4.01 (m, 3H), 3.92-3.85 (m, 3H), 3.76 (s, 6H), 3.59-3.42 (m, 2H), 3.30-3.28 (m, 2H), 2.94-2.89 (m, 6H, ET_3N), 1.45 (s, 3H), 1.22-1.15 (m, 9H, ET_3N) and 1.12-1.04 (m, 9H); ^{31}P NMR (CD_3CN) δ -0.33; ^{13}C NMR ($CDCl_3$) δ 210.9, 210.6, 172.1, 170.0, 169.9, 165.1, 160.2, 154.0, 153.6, 152.3, 146.2, 137.2, 137.1, 137.0, 131.5, 129.4, 128.4, 118.7, 114.6, 112.1, 89.9, 88.0, 75.8, 75.0, 72.5, 64.8, 56.4, 56.3, 47.3, 46.5, 46.4, 26.4, 26.1, 12.6, 10.0 and 9.9; HRMS *m/z* calculated for $C_{45}H_{52}N_4O_{17}P^-$, 951.3065, observed *m/z* = 951.3062.

Preparation of 14. Dinucleotide **33** (0.015 g, 0.0142 mmol) was stirred in solution containing DCM and TFA (3:1, 2 mL) for 10 min. The solution was concentrated to dryness under reduced pressure and the residues were partitioned in a DCM/H_2O (7 mL) mixture. The aqueous layer was concentrated to dryness to yield **14** as a diastereomeric mixture in the form of a white film (7 mg, 0.0108 mmol, 76 %). ^{1}H NMR (D_2O) δ 7.76-7.73 (m, 1H), 5.96-5.95 (m, 1H), 5.88-5.82 (m, 1H), 4.61-4.55 (m, 2H), 4.44-4.41 (m, 1H), 4.31-4.29 (m, 1H), 4.24-4.21 (m, 2H), 4.18-4.16 (m, 1H), 4.13-4.12 (m, 1H), 4.08-4.02 (m, 2H), 3.93-3.89 (m, 1H), 3.86-3.63 (m, 3H), 1.91-1.90 (m, 3H) and 1.21 (m, 9H); ^{1}P NMR (D_2O) δ -0.72 and -0.84; ^{13}C NMR (D_2O) δ 213.2, 212.6, 170.6, 170.4, 166.3, 154.1, 153.9, 151.8, 111.2, 111.1, 88.4, 87.9, 86.8, 86.2, 83.4, 82.3, 82.2, 82.0, 81.8, 73.4, 73.3, 72.4, 72.3, 70.4, 69.9, 69.8, 65.4, 65.3, 60.4, 60.3, 47.1, 46.8, 45.4, 45.2, 39.8,

38.7, 24.8, 24.5 and 11.4; HRMS *m/z* calculated for $C_{24}H_{34}N_4O_{15}P^-$, 649.1764, observed *m/z* = 649.1806.



Preparation of 15. Sodium borohydride (0.02 g, 0.53 mmol) was added to a cooled (0° C) solution of ketone **10** (0.09 g, 0.142 mmol) in ethanol (10 mL) and warmed to rt over a 10 min period. The reaction mixture was stirred for 1 h followed by addition of NH_4Cl sat. (ca. 0.1 mL / neutral pH). The organics were evaporated under reduced pressure. Column chromatography (15% MeOH in DCM) yielded nucleoside **15** as a mixture of diastereomers in the form of a white foam (0.02 g, 0.031 mmol, 22 %). ^{1}H NMR ($CDCl_3$) δ 7.38-7.18 (m, 9H), 6.82-6.78 (m, 4H), 6.01-5.96 (m, 1H), 4.33-4.05 (m, 3H), 3.76-3.56 (m, 7H), 3.31-3.11 (m, 5H), and 0.87 (s, 9H); ^{13}C NMR ($CDCl_3$) 171.2, 158.5, 153.8, 144.4, 135.6, 130.0, 128.1, 127.8, 126.9, 113.1, 86.7, 86.5, 82.7, 71.3, 70.9, 63.8, 55.1, 45.9, 41.4, 36.1, 29.7, 25.6 and 10.3 ppm; IR (NaCl plate) 3335 (br), 3048, 2760, 1696 and 1265 cm^{-1}; HRMS *m/z* calculated for $C_{35}H_{42}N_2O_9Na$ (M^+ + Na), 657.2788, observed *m/z* = 657.2789.

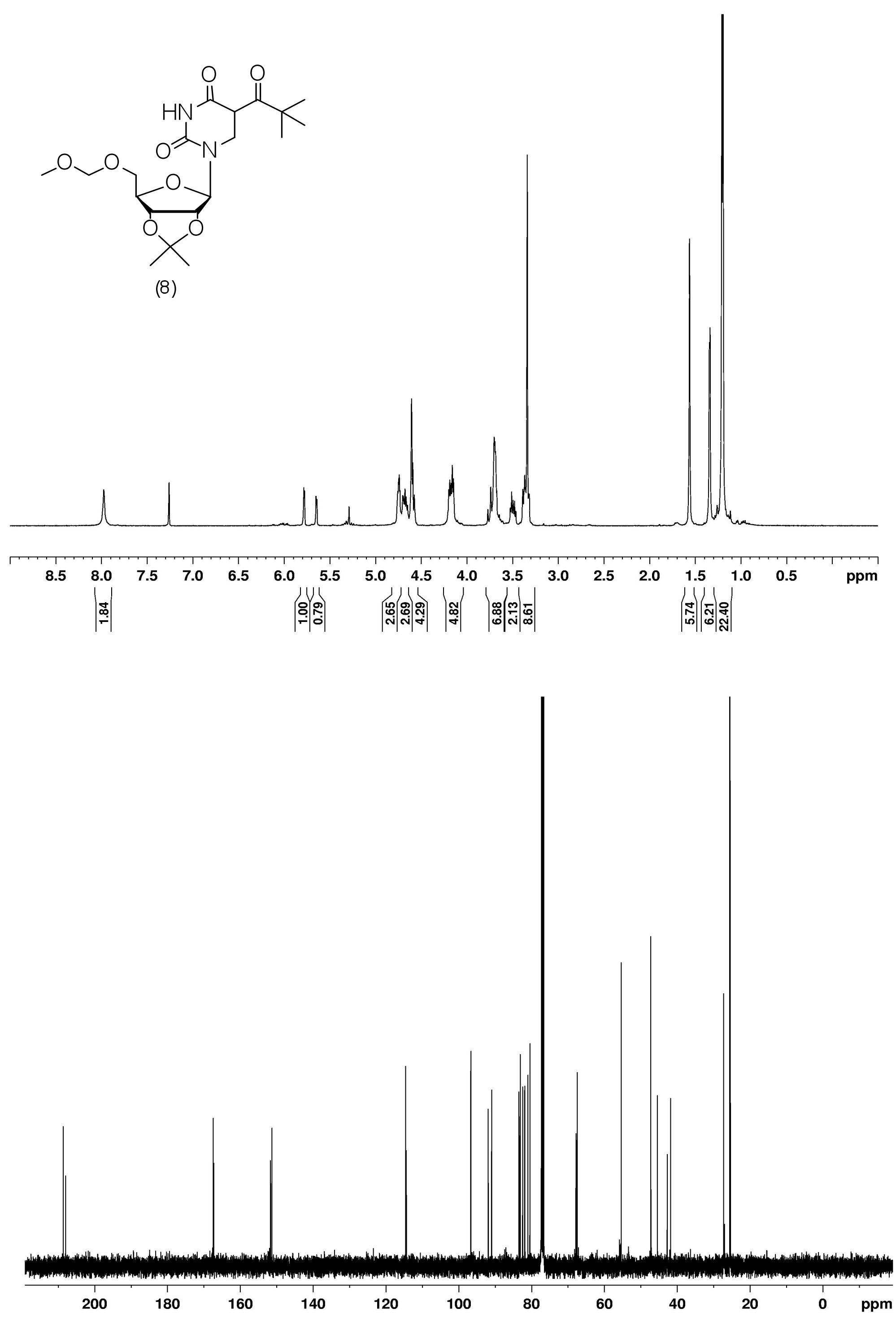


Figure S1. ^{1}H (top) and ^{13}C NMR (bottom) spectra of **8**.

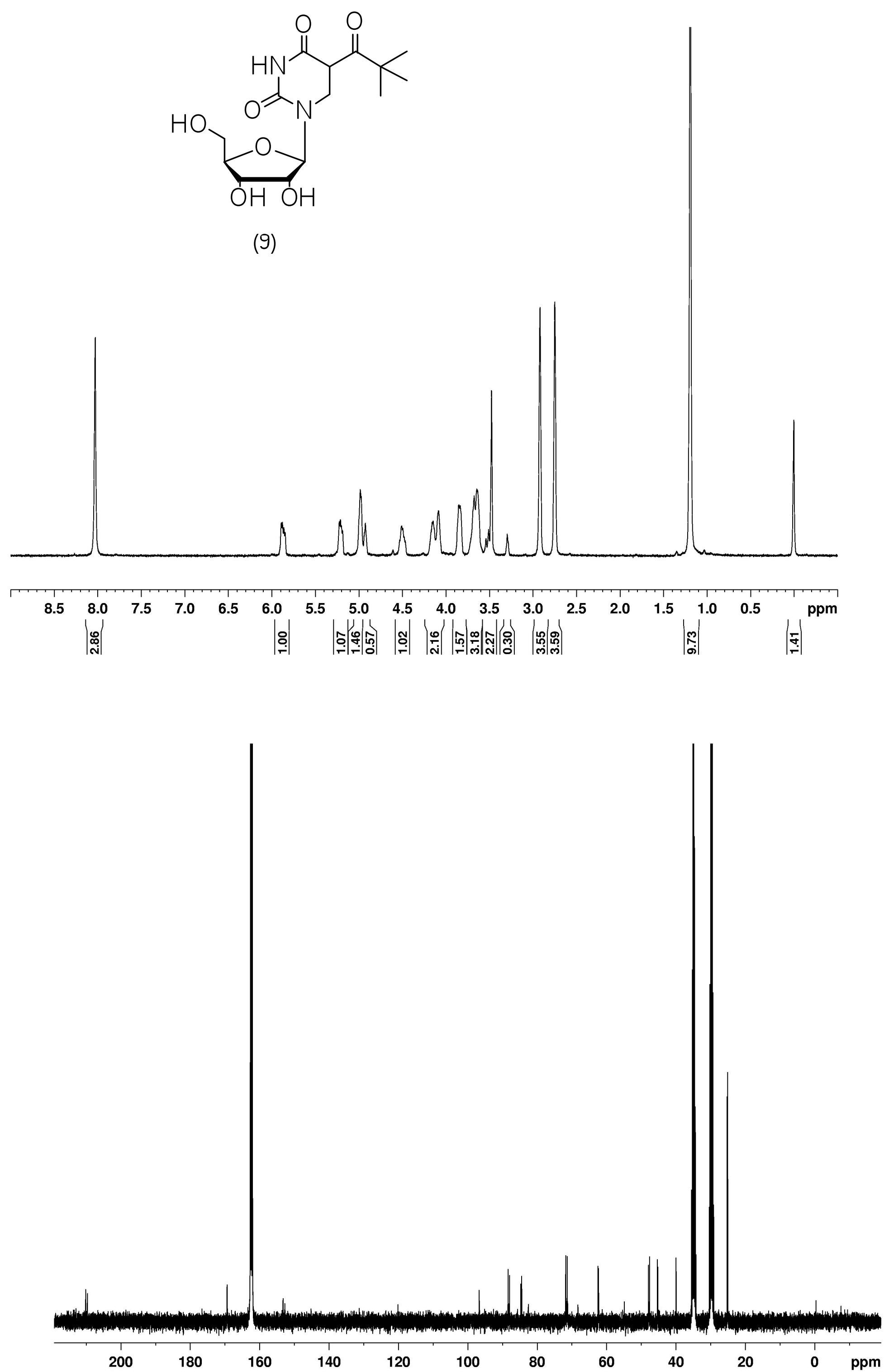


Figure S2. ^{1}H (top) and ^{13}C NMR (bottom) spectra of **9**.

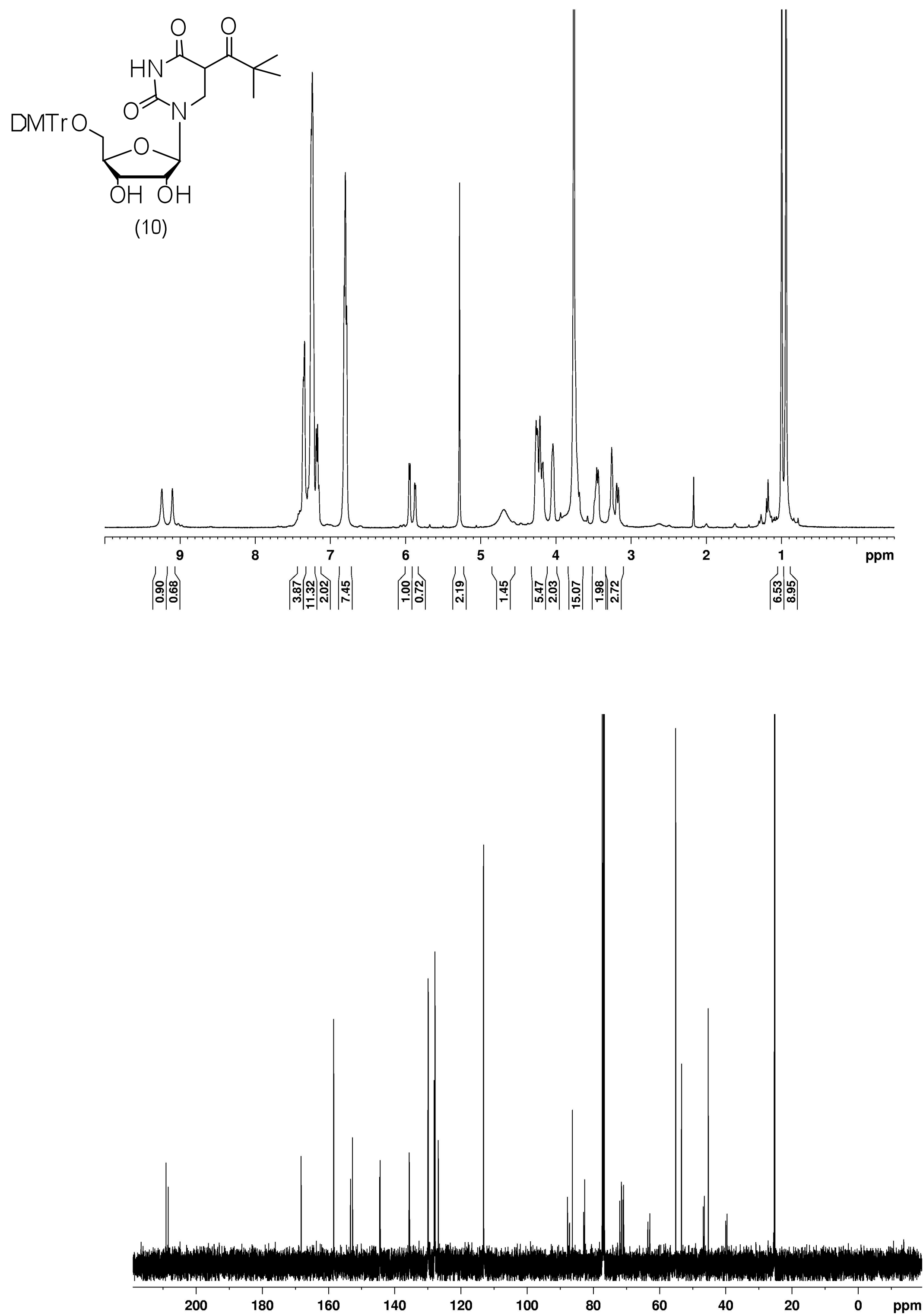
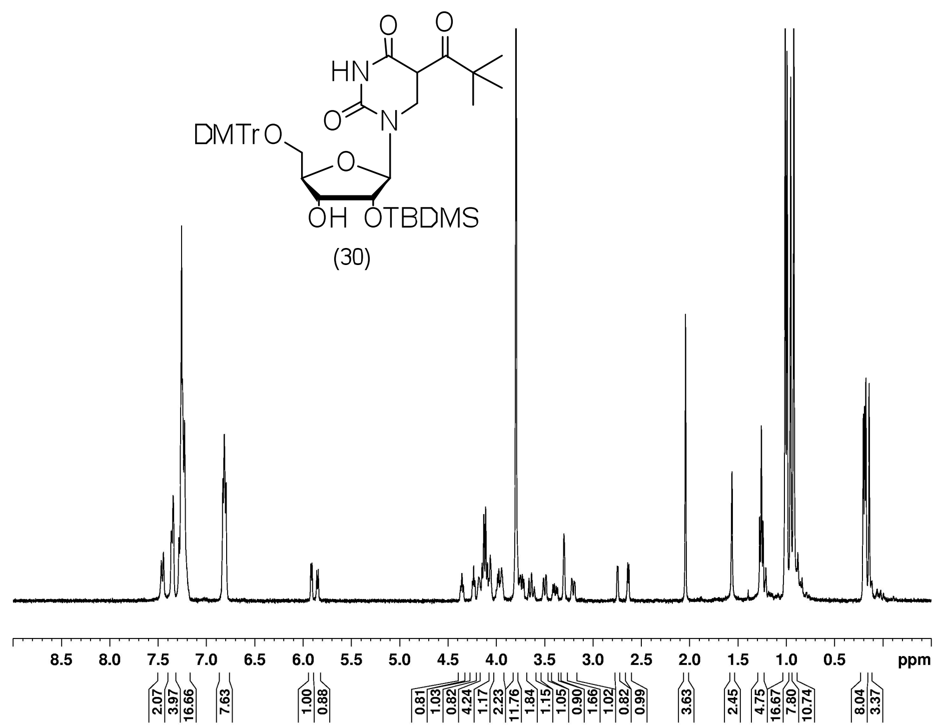


Figure S3. ^{1}H (top) and ^{13}C NMR (bottom) spectra of **10**.



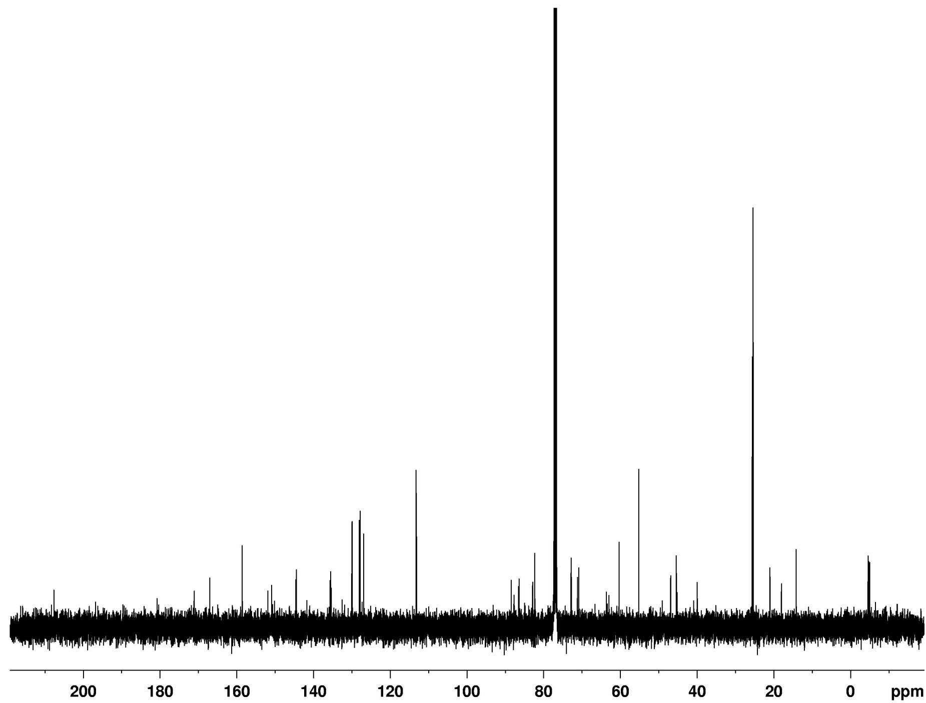


Figure S4. ^{1}H (top) and ^{13}C NMR (bottom) spectra of **30**.

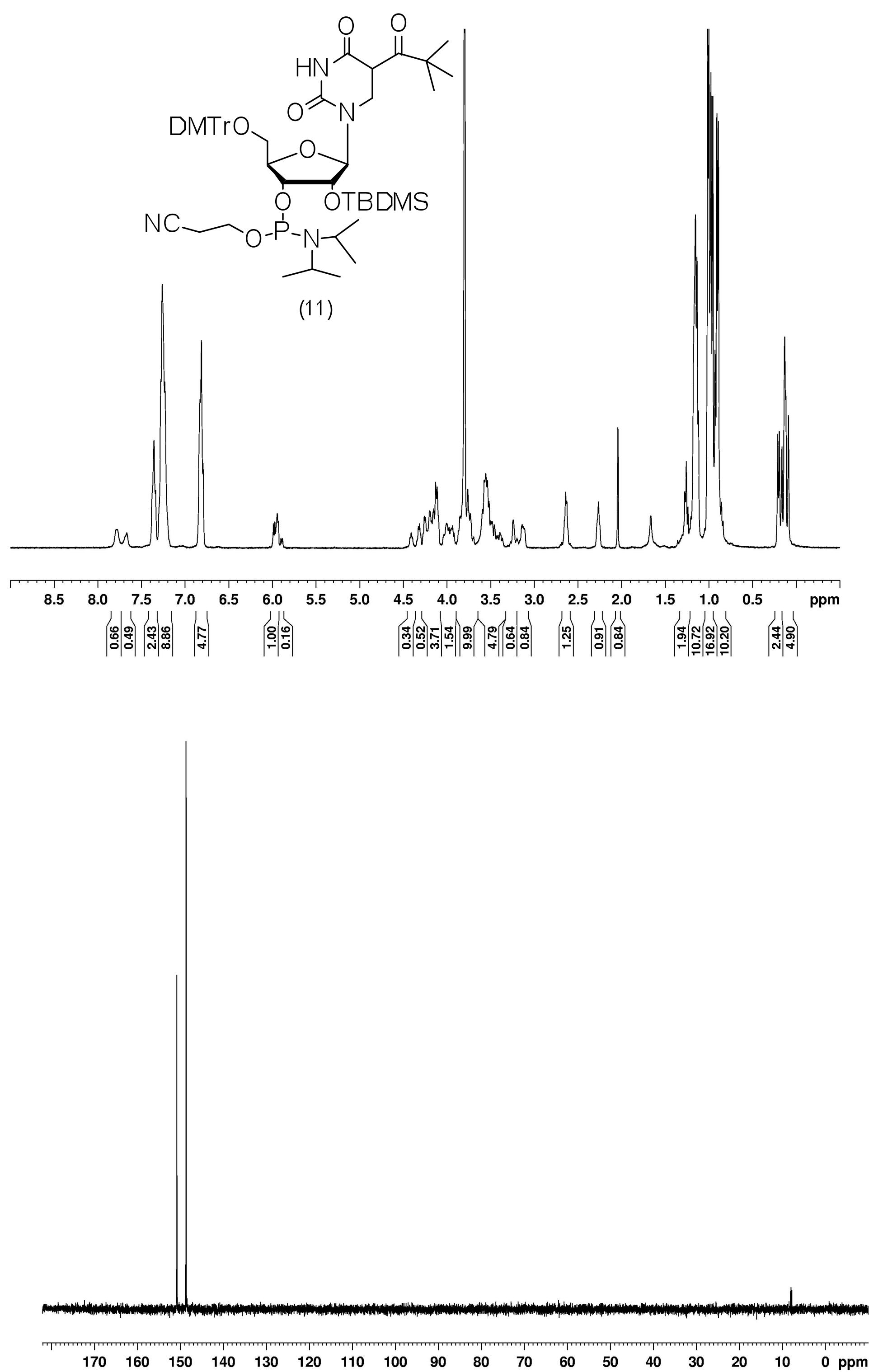


Figure S5. ^{1}H (top) and ^{31}P NMR (bottom) spectra of **11**.

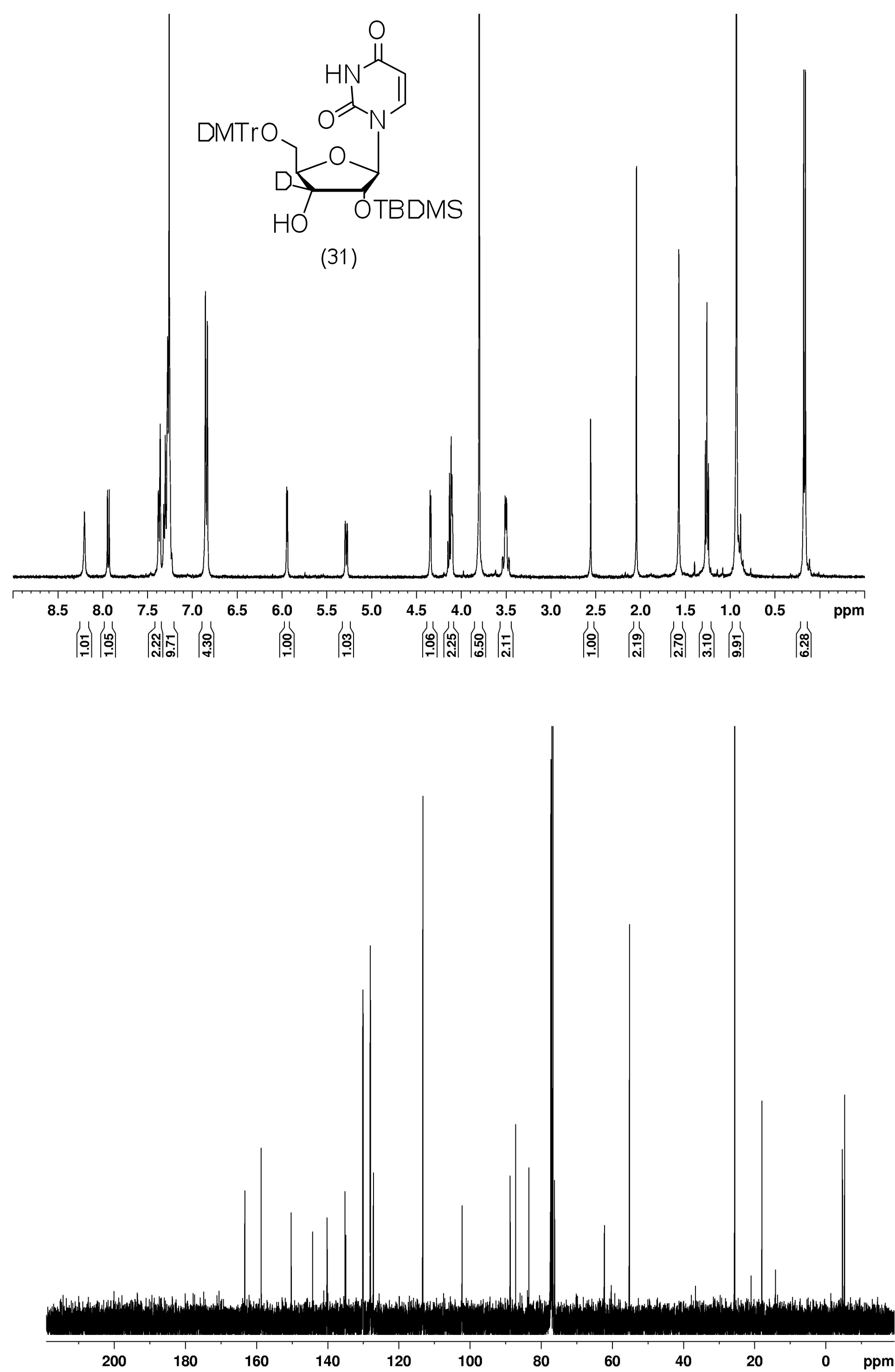


Figure S6. ^{1}H (top) and ^{13}C NMR (bottom) spectra of **31**.

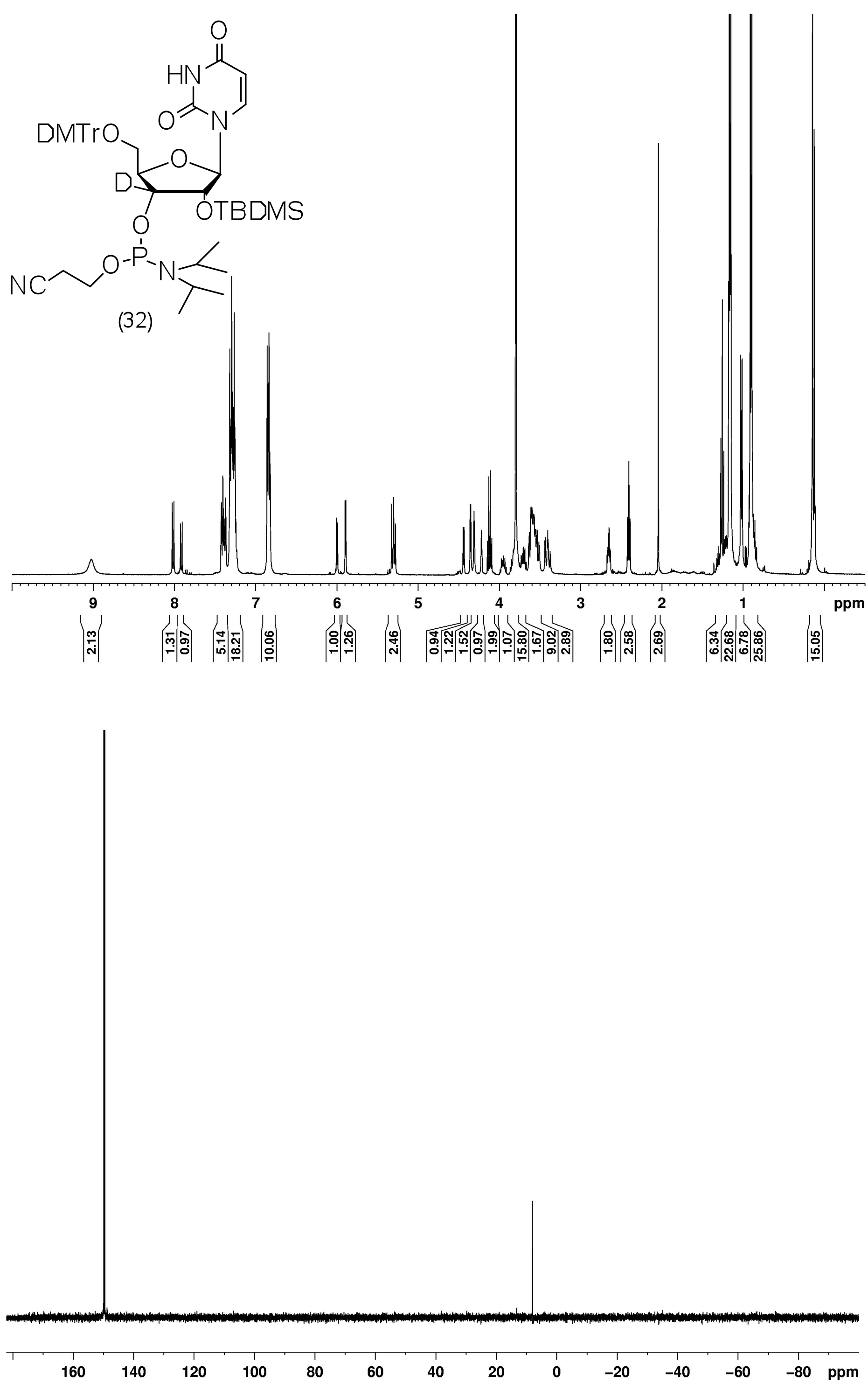


Figure S7. ^{1}H (top) and ^{31}P NMR (bottom) spectra of **32**.

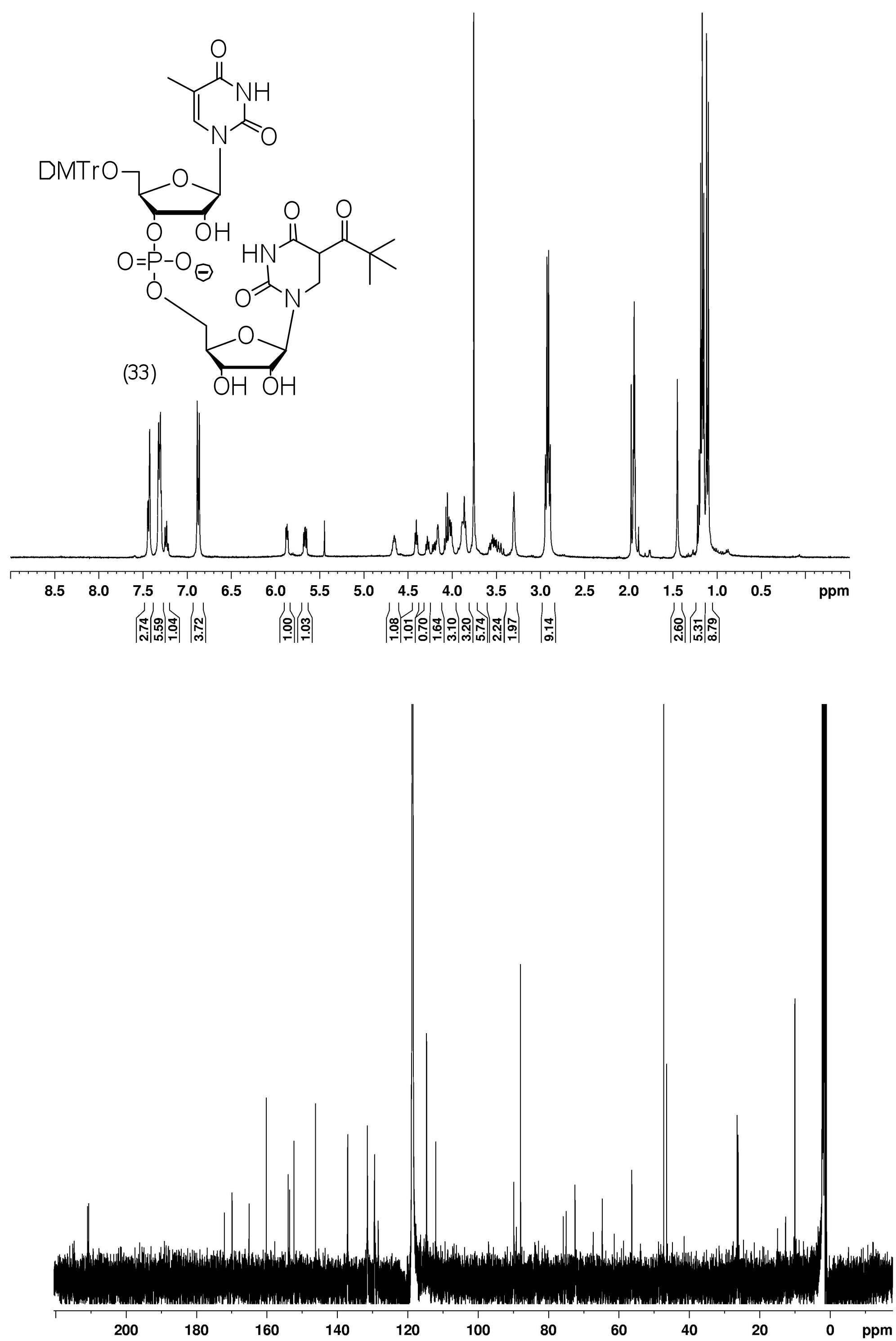
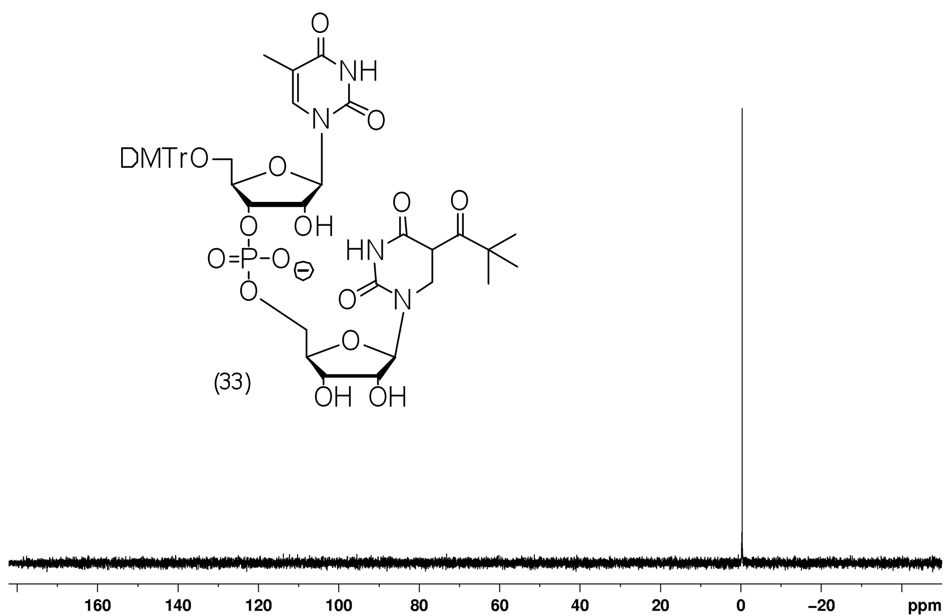


Figure S8. ^{1}H (top) and ^{13}C NMR (bottom) spectra of **33**.



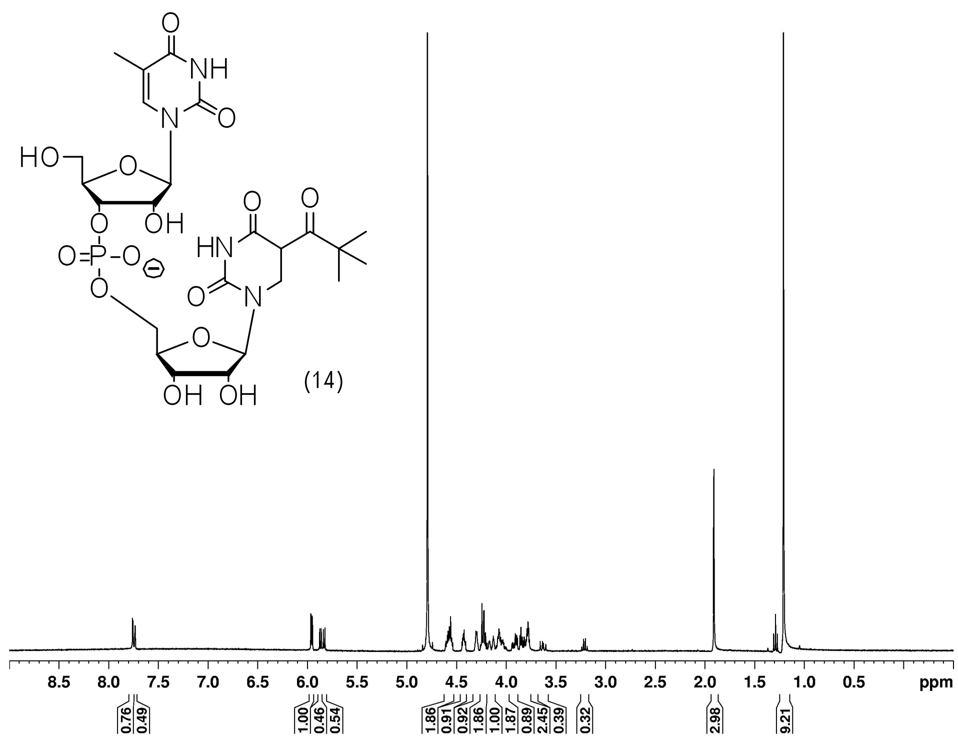


Figure S9. ^{31}P NMR spectra of **33** (top) and ^{1}H NMR (bottom) spectra of **14**.

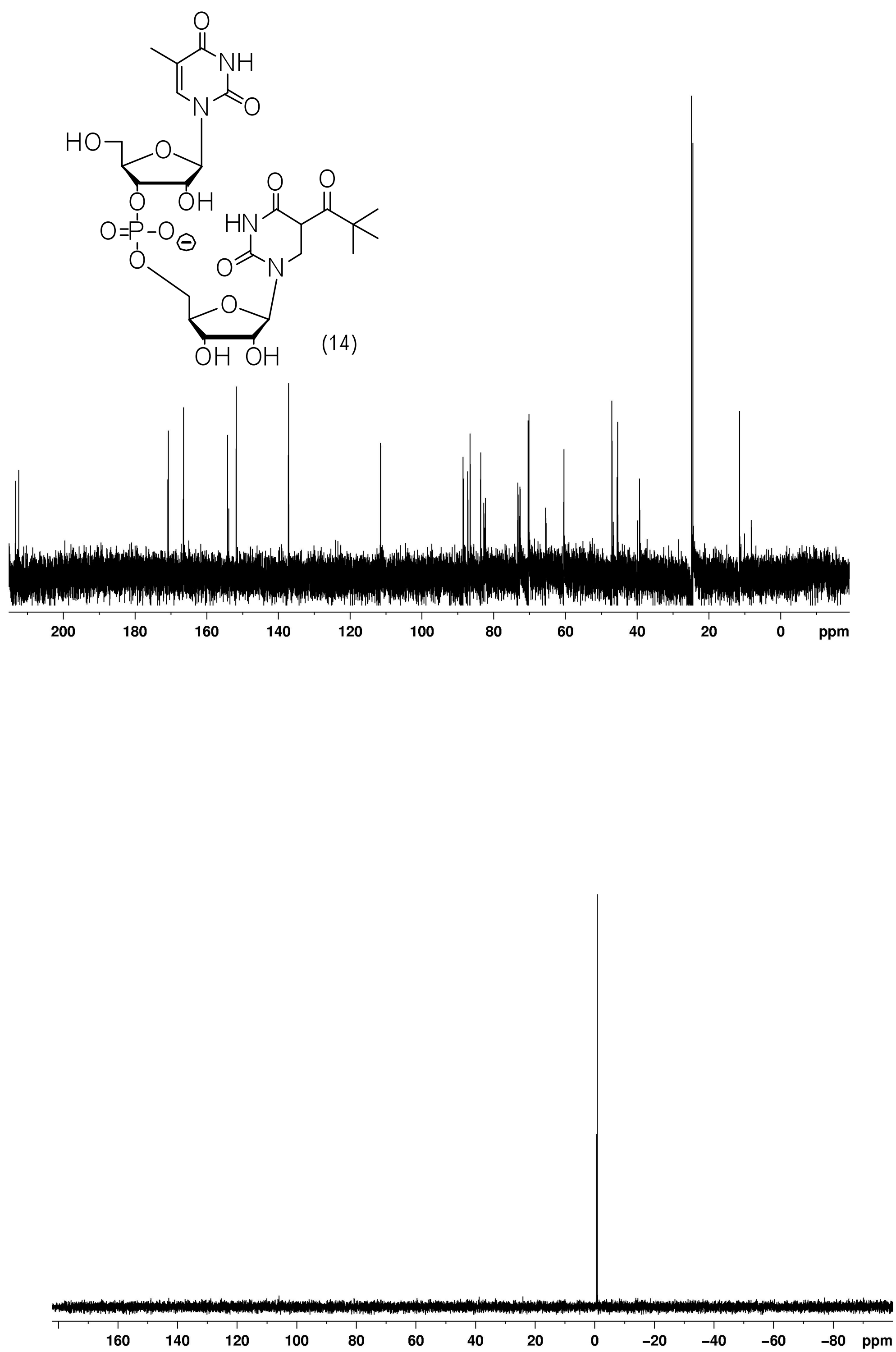
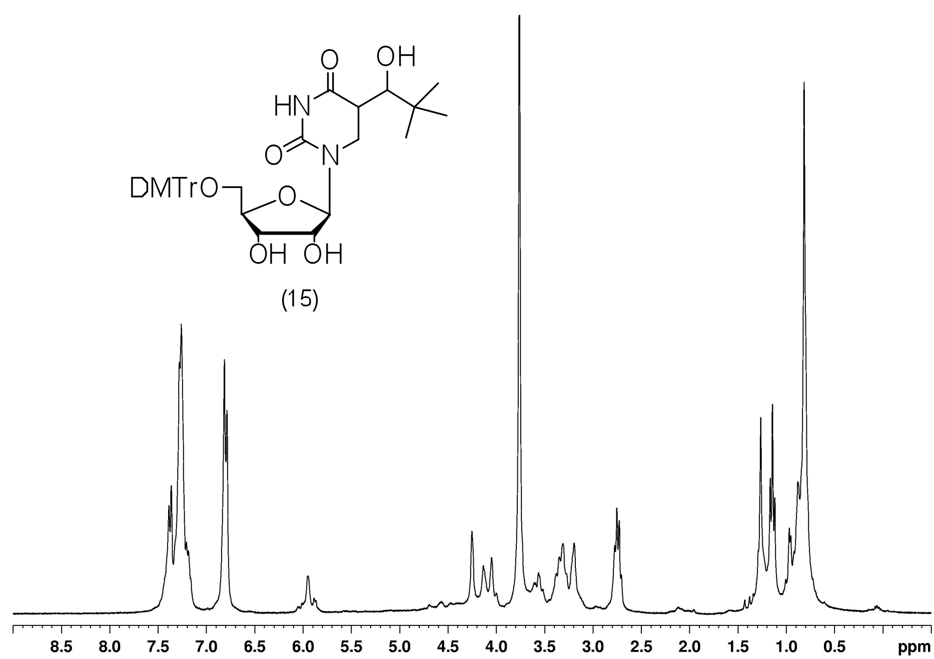


Figure S10. ^{13}C (top) and ^{31}P NMR (bottom) spectra of **14**.



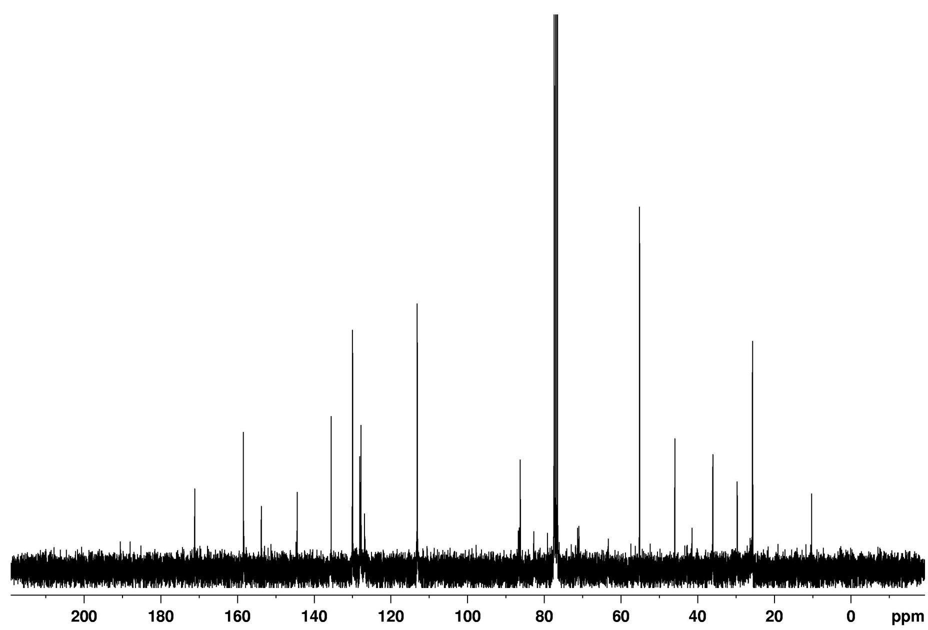


Figure S11. ^{1}H (top) and ^{13}C NMR (bottom) spectra of **15**.

(18) = 5'-GAU CAG GCU **6**UG CCA UCG C-3'

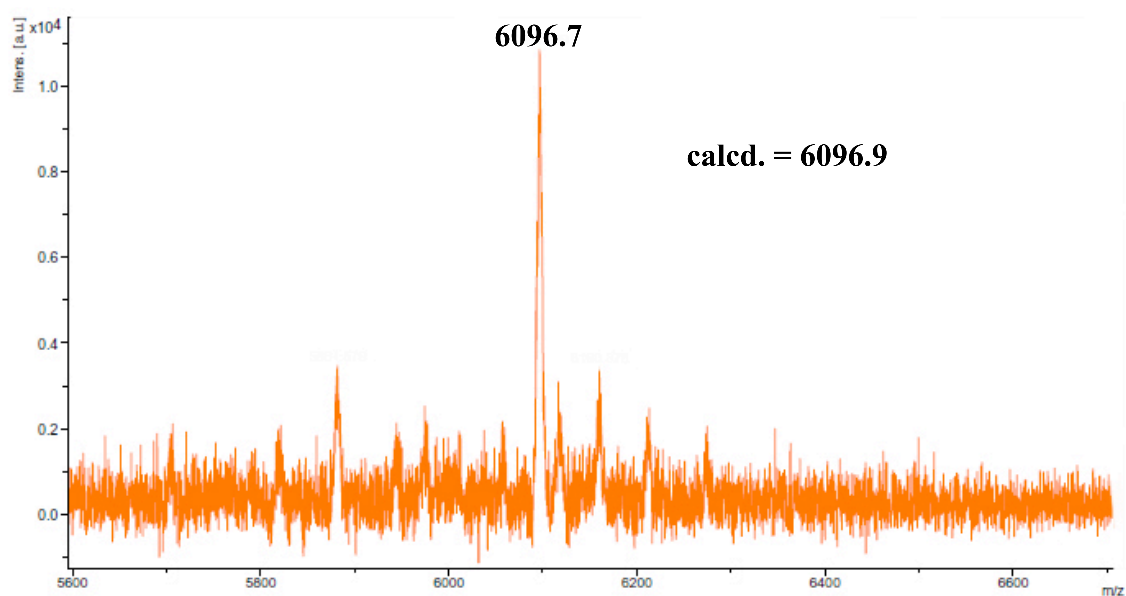


Figure S12. MALDI-TOF MS of **18**.

(19) = 5'-GAU CAG GCA **6**AG CCA UCG C-3'

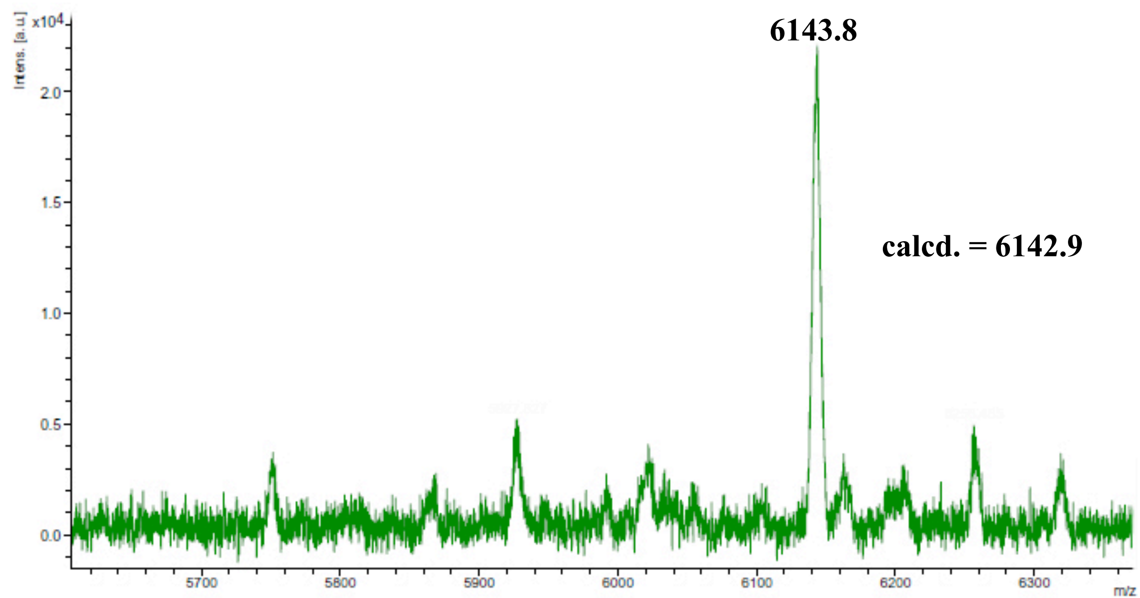


Figure S13. MALDI-TOF MS of **19**.

(20) = 5’-GAU CAG GC(**2’-^{2}H-U**) **6**UG CCA UCG C-3’

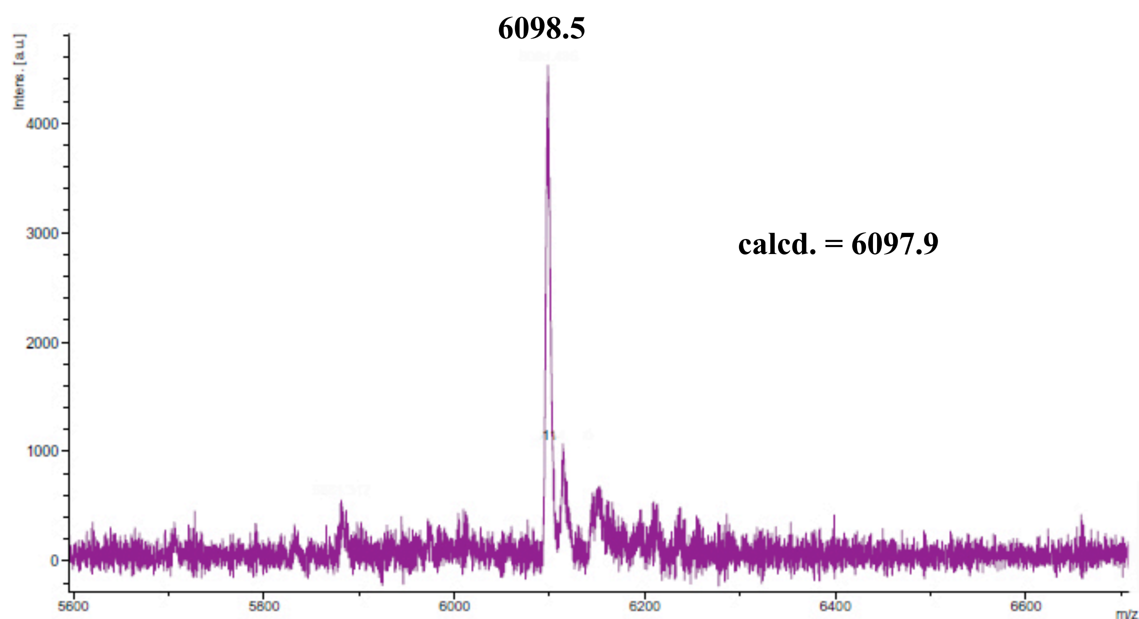


Figure S14. MALDI-TOF MS of **20**.

(21) = 5’-GAU CAG GC(**3’-^{2}H-U**) **6**UG CCA UCG C-3’

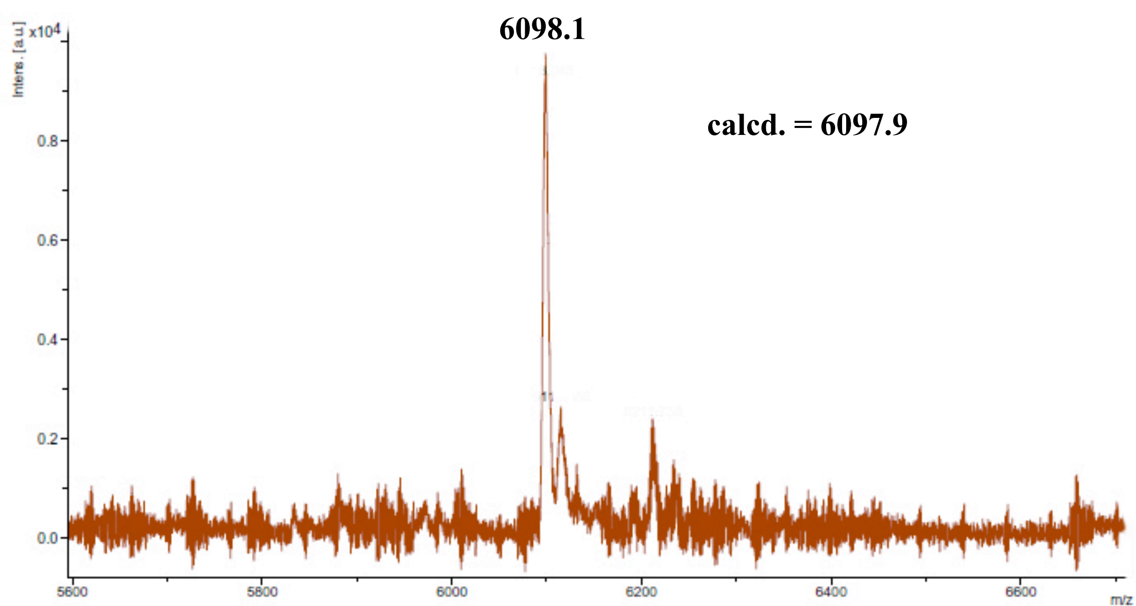


Figure S15. MALDI-TOF MS of **21**.

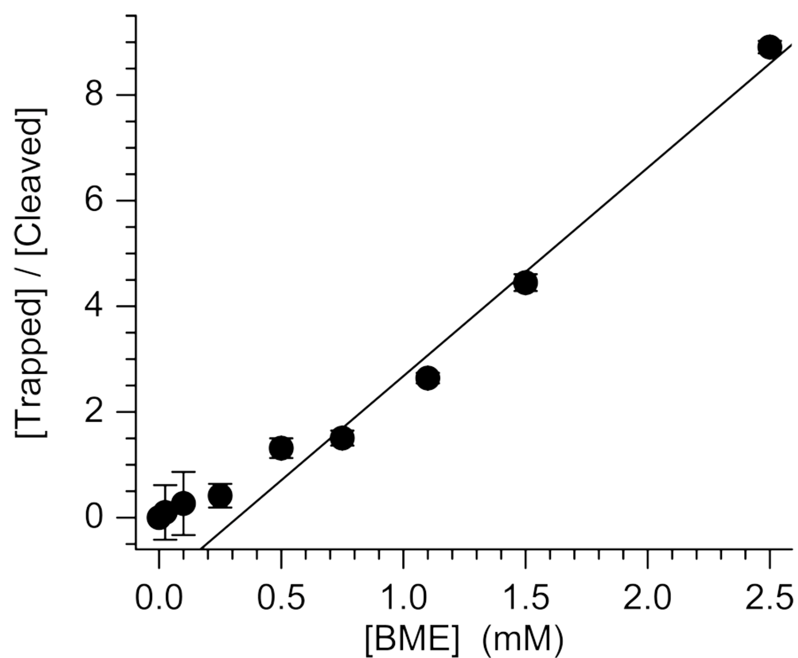


Figure S16. Effect of BME on strand cleavage from 5,6-dihydrouridin-5-yl (**1**) in **16**.

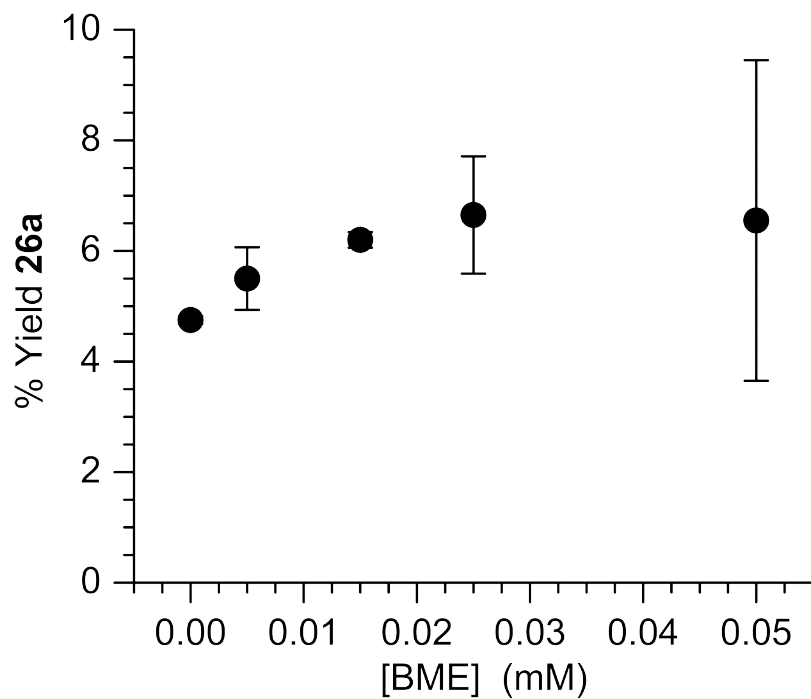


Figure S17. Effect of BME on 3'-end group from 5,6-dihydrouridin-6-yl (**2**).

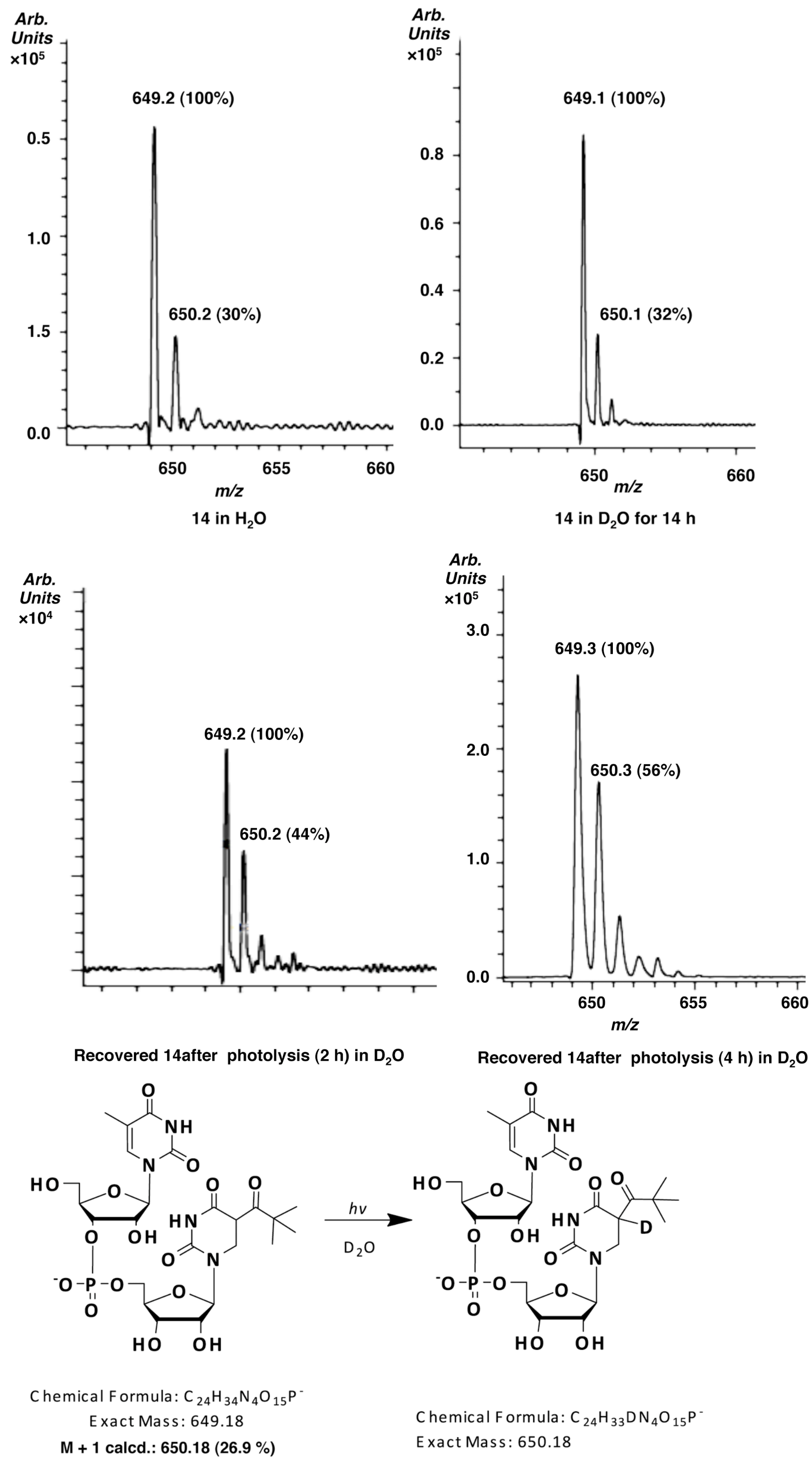


Figure S18. Deuterium incorporation in recovered **14** following photolysis.

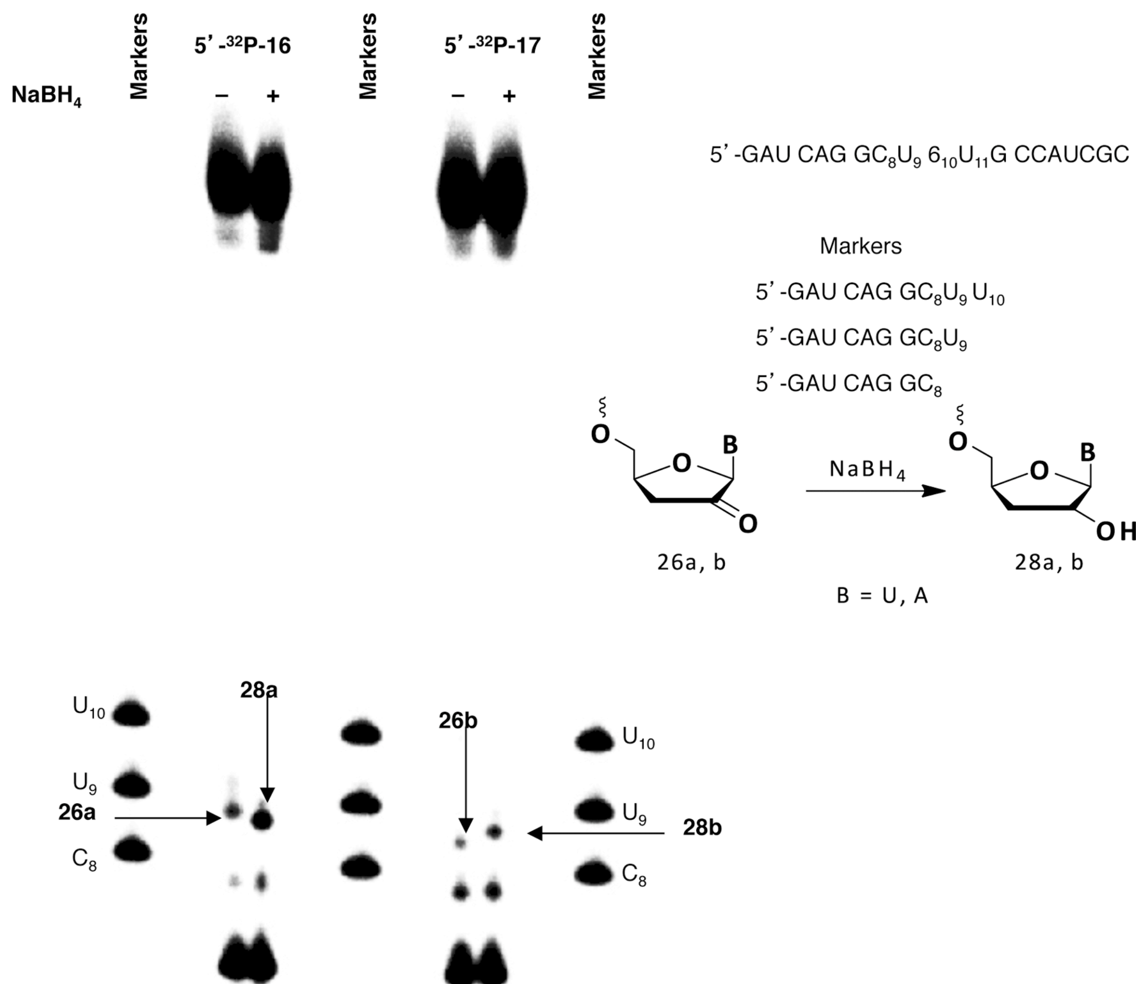


Figure S19. Detection of **26a**,**b** via gel electrophoresis using $NaBH_4$.

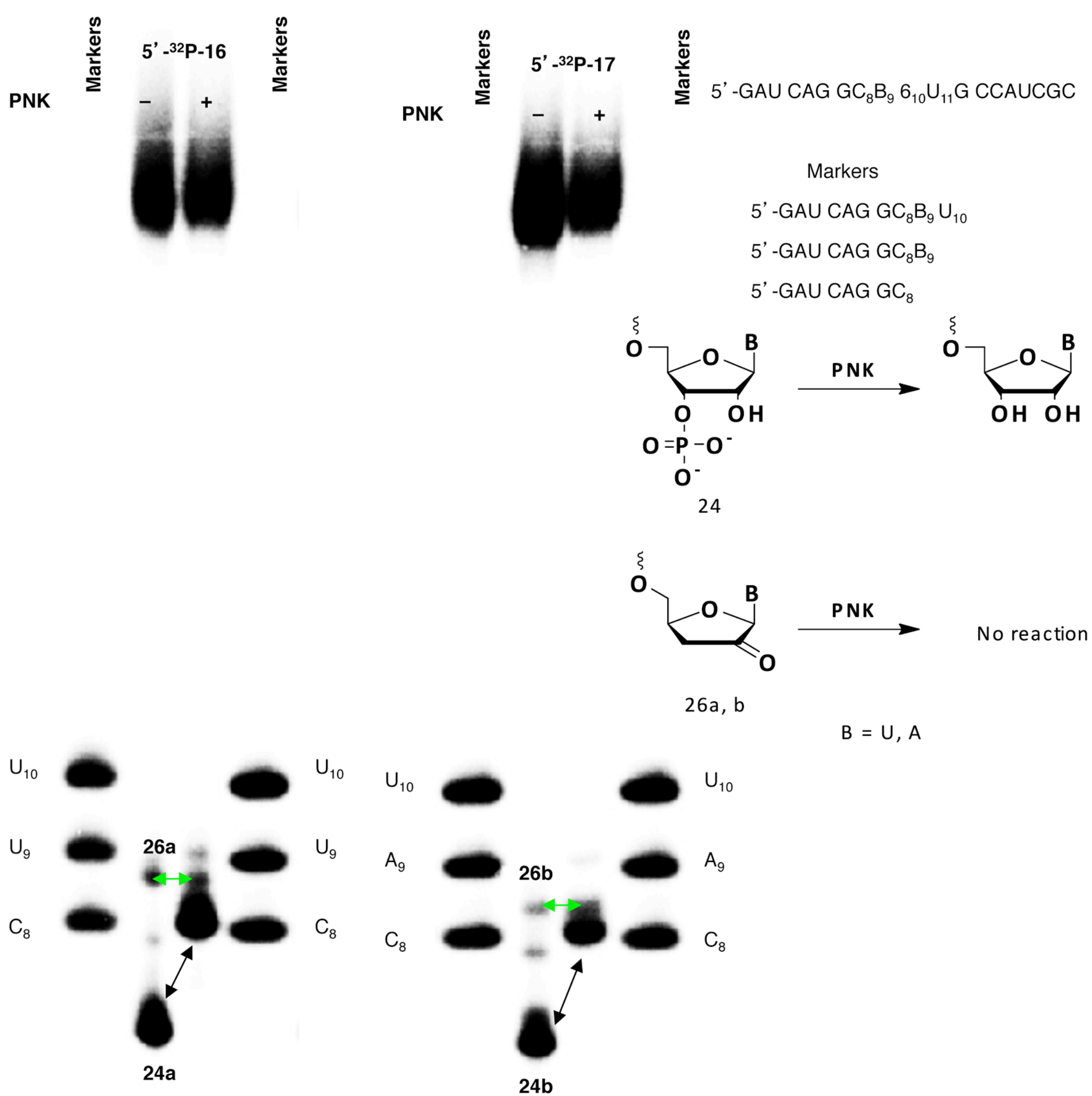


Figure S20. 3'-Enzymatic end group analysis.

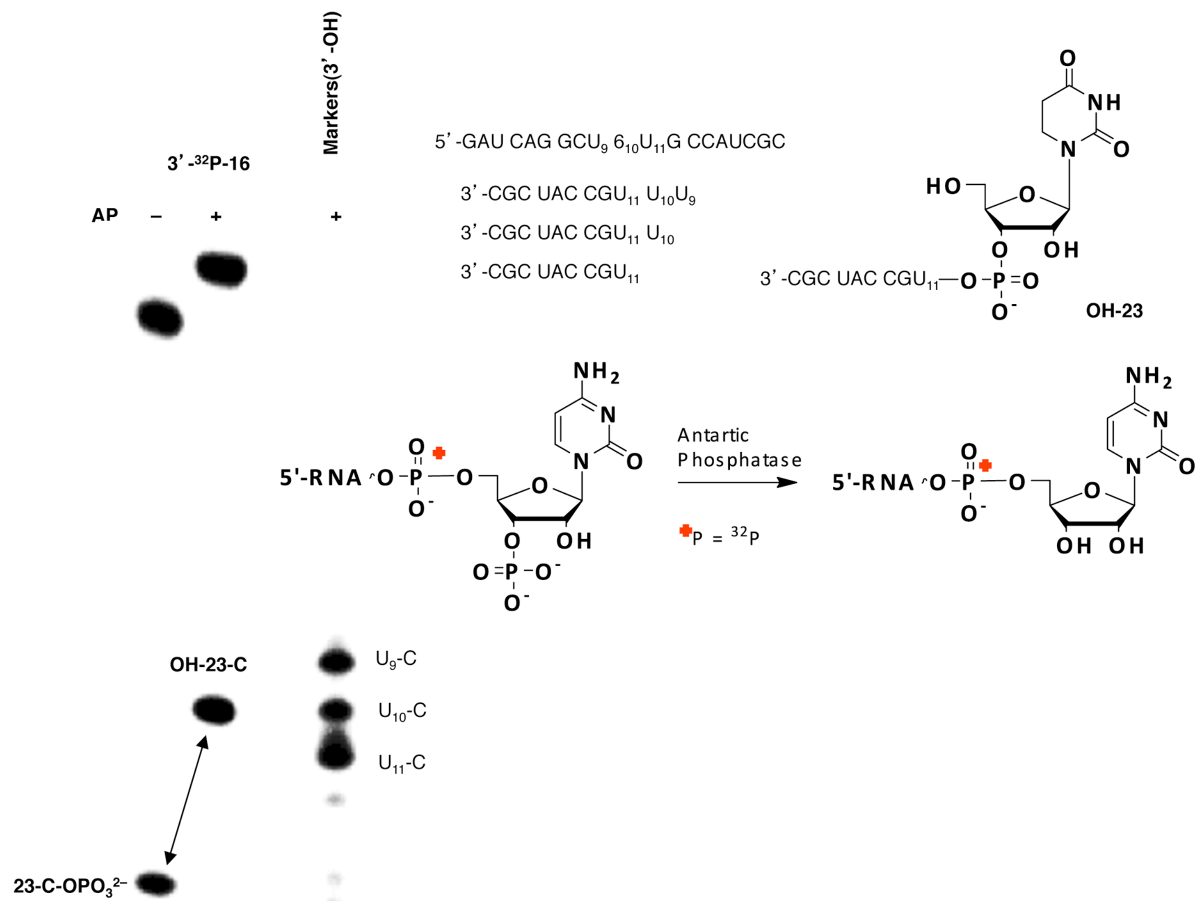


Figure S21. 5'-Enzymatic end group analysis.

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[2] Hayakawa, H.; Tanaka, H.; Miyasaka, T. *Tetrahedron,* **1985**, 41, 1675-1683.

[3] Serafinowski, P. J.; Barnes, C. L. *Tetrahedron*, **1996**, 52, 7929-7938.