Electronic Supplementary Material (ESI) for Chemical Communications

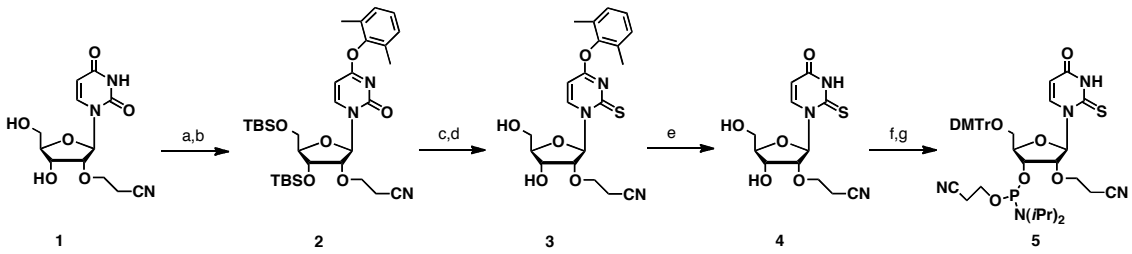
Prediction of the stability of modified RNA duplexes based on deformability analysis: Oligoribonucleotide derivatives modified with 2´-*O*-cyanoethyl-5-propynyl-2-thiouridine as a promising component

Yoshiaki Masaki, Ryuta Miyasaka, Kunihiro Hirai, Hirosuke Tsunoda, Akihiro Ohkubo, Kohji Seio, Mitsuo Sekine
Department of Life Science, Tokyo Institute of Technology, Japan

Supporting Information

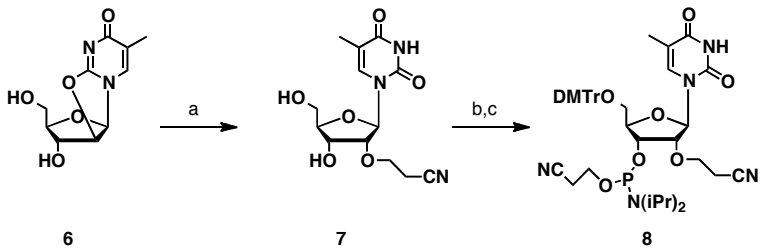
Contents

Synthesis of 2-thiouridine derivatives



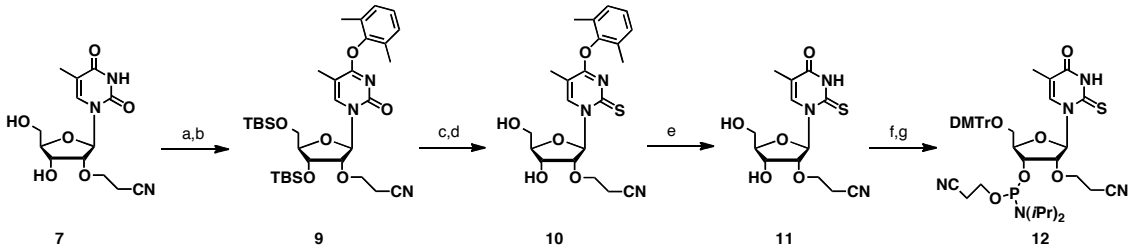
Scheme S1 Reagents and conditions: (a) TBSCl, imidazole, MeCN, rt, 13 h, 90%. (b) 1. TPSCl, TEA, DMAP, CH_2Cl_2, rt, 1 h; 2. dimethylphenol, DABCO, TEA, CH_2Cl_2, rt, 3h, 94% (2 steps). (c) Lawesson Reagent, PhMe, reflux, 1 h. (d) 3HF·TEA, TEA, THF, rt, 13 h, 85% (2 steps). (e) *syn*-*o*-nitrobenzoxime, TMG, MeCN, rt, 3 h, 90%. (f) DMTrCl, pyridine, rt, 4 h, 82%. (g) (CEO)P(N*i*Pr$_2$)$_2$, 1*H*-tetrazole, *i*Pr$_2$NH, CH_2Cl_2, rt, 13 h, 68%.

Synthesis of 5-methyl uridine derivatives



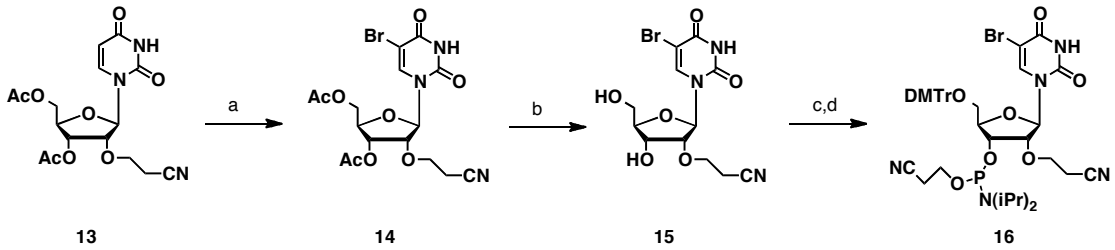
Scheme S2 Reagents and conditions: (a) TMScyanoehtanol, BF_3•Et_2O, DMA, reflux, 14 h, 62%. (b) DMTrCl, pyridine, rt, 4 h, 92%. (c) (CEO)P(N*i*Pr$_2$)$_2$, 1*H*-tetrazole, *i*Pr$_2$NH, CH_2Cl_2, rt, 12 h, 63%.

Synthesis of 5-methyl-2-thiouridine derivatives



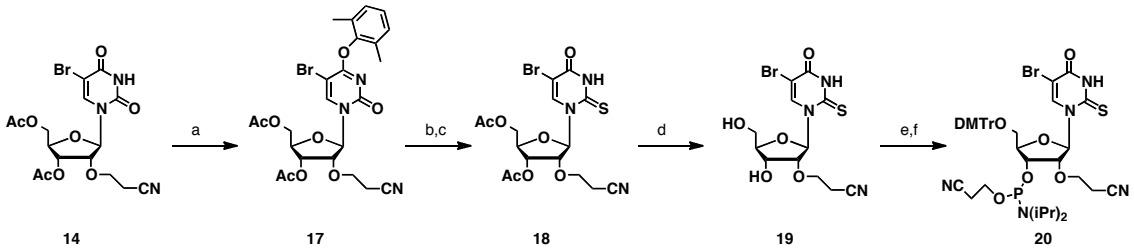
Scheme S3 Reagents and conditions: (a) TBSCl, Im, DMF, rt, 12 h, 94%. (b) 1. TPSCl, TBAB, CH_2Cl_2, aq. Na_2CO_3, rt, 24 h ; 2. dimethylphenol, DABCO, TEA, MeCN, rt, 5 h , 75% (2 steps). (c) Lawesson's Reagent, PhMe, reflux, 2 h, (d) 3HF•TEA, TEA, THF, rt, 15 h, 84% (2 steps). (e) *syn*-*o*-nitrobenzaldoxime, TMG, MeCN, rt, 5 h, 89%. (f) DMTrCl, pyridine, rt, 12 h, 93%. (g) (CEO)P(N*i*Pr$_2$)$_2$, 1*H*-tetrazole, *i*Pr$_2$NH, CH_2Cl_2, rt, 12 h, 62%.

Synthesis of 5-bromo uridine derivatives



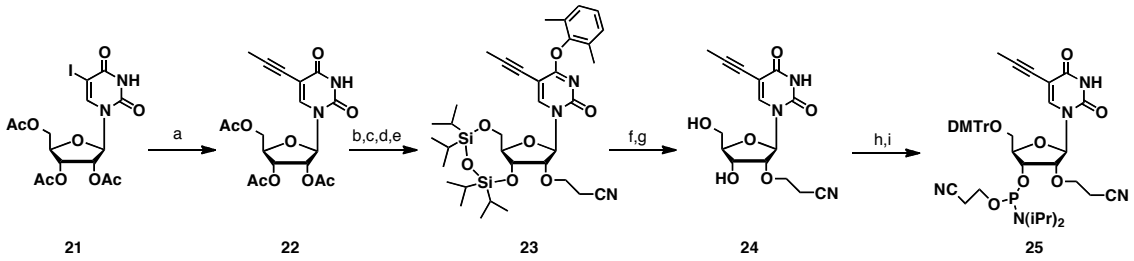
Scheme S4 Reagents and conditions: (a) LiBr, CAN, MeCN, 80 ˚C, 1 h, 92%. (b) NH_4OH, MeOH, THF, rt, 8 h, 69%. (c) DMTrCl, pyridine, rt, 14 h, 45%. (d) (CEO)P(N*i*Pr_2)$_2$, 1*H*-tetrazole, *i*Pr_2NH, CH_2Cl_2, rt, 23 h, 69%.

Synthesis of 5-bromo-2-thiouridine derivatives



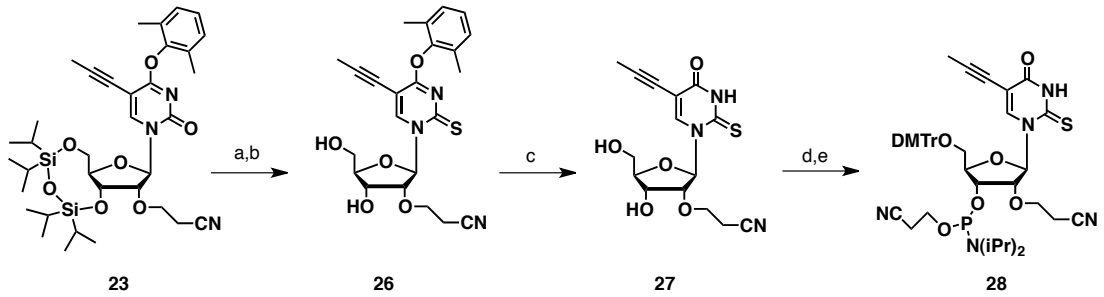
Scheme S5 Reagents and conditions: (a) 1. TPSCl, TBAB, CH_2Cl_2, aq. Na_2CO_3, rt, 10.5 h ; 2. dimethylphenol, DABCO, TEA, MeCN, rt, 2.5 h , 82% (2 steps). (b) Lawesson's Reagent, PhMe, reflux, 1 h , 90% (c) *syn-o*-nitrobenzaldoxime, TMG, MeCN, rt, 3.5 h, 72%. (d) NH_4OH, MeOH, THF, rt, 3 h, 79%. (e) DMTrCl, pyridine, rt, 11 h, 66%. (f) (CEO)P(N*i*Pr_2)$_2$, 1*H*-tetrazole, *i*Pr_2NH, CH_2Cl_2, rt, 23 h, 17%.

Synthesis of 5-propynyl uridine derivatives



Scheme S6 Reagents and conditions: (a)TMSpropyne, 3HF•TEA, TEA, CuI, $Pd(PPh_3)_4$, DMF, rt, 24 h, 83%.(b) NaOMe, MeOH, rt, 1 h, 83%. (c) $TIPDSCl_2$, pyridine, rt, 12 h, 91%. (d) 1. TPSCl, TBAB, CH_2Cl_2, aq. Na_2CO_3, rt, 17 h; 2. dimethylphenol, DABCO, TEA, MeCN, rt, 3 h , 82% (2 steps). (e) acrylonitrile, Cs_2CO_3, *t*BuOH, rt, 13 h, 97%. (f) 3HF•TEA, TEA, THF, rt, 3 h, 96%. (g) *syn-o*-nitrobenzaldoxime, TMG, MeCN, 50 ˚C, 3 h, 82%. (h) DMTrCl, pyridine, rt, 3.5 h, 70%. (i) $(CEO)P(N\textit{i}Pr_2)_2$, 1*H*-tetrazole, $\textit{i}Pr_2NH$, CH_2Cl_2, rt, 10.5 h, 60%.

Synthesis of 5-propynyl-2-thiouridine derivatives



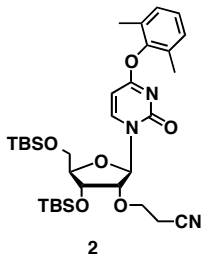
Scheme S7 Reagents and conditions: (a) Lawesson Reagent, PhMe, reflux, 2 h. (b) 3HF•TEA, TEA, THF, rt, 1 h, 49% (2 steps). (c) *syn-o*-nitrobenzaldoxime, TMG, MeCN, rt, 5 h, 84%. (d) DMTrCl, pyridine, rt, 4 h, 90%. (e) $(CEO)P(N\textit{i}Pr_2)_2$, 1*H*-tetrazole, $\textit{i}Pr_2NH$, MeCN, rt, 12 h, 62%.

Experimental Section

General Remarks

Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. NMR spectra (^{1}H and ^{13}C) were recorded on Varian inova-500 instruments and calibrated using residual undeuterated solvent as an internal reference ($CHCl_3$: 7.26 ppm ^{1}H NMR, 77.0 ppm ^{13}C NMR and DMSO-d_5: 2.49 ppm for ^{1}H NMR, 39.5 ppm ^{13}C NMR). NMR spectra (^{31}P) were recorded on Varian inova-500 instruments and calibrated using an external reference (85% orthophosphoric acid). The following abbreviations (or combinations thereof) were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. UV spectra were recorded with a U-2000 spectrometer. Column chromatography was performed with silica gel C-200 (purchased from Wako Co. Ltd.), C-300 (Wako Co. Ltd.), 60N (Kanto Chemical, Co., Inc.), and NH (Fuji Silysia Chemical Ltd.). High performance liquid chromatography (HPLC) was performed using the following systems: Reversed exchange HPLC was done on a Waters Alliance system with a Waters 3D UV detector and a Waters XTerra MS C18 column (4.6 × 150 mm); a linear gradient (0–30%) of solvent I [0.1 M ammonium acetate buffer (pH 7.0)] in solvent II (CH_3CN) was used at 50°C at a flow rate of 1.0 mL/min for 30 min; anion-exchange HPLC was done on a Shimadzu LC-10 AD VP with a Shimadzu 3D UV detector and a Gen-Pak FAX column (Waters, 4.6 × 100 mm); a linear gradient (10–67%) of solvent III [1 M NaCl in 25 mM phosphate buffer (pH 6.0)] in solvent IV [25 mM phosphate buffer (pH 6.0)] was used at 50°C at a flow rate of 1.0 mL/min for 40 min. The synthesis of modified oligonucleotides was carried out by use of a DNA/RNA synthesizer 392 (Applied Biosystem). ESI mass was performed by use of Mariner^{TM} (PerSeptive Biosystems Inc.) or microTOF II (Brüker Daltonics). MALDI-TOF mass was performed using a Brüker Daltonics [Matrix: 3-hydoroxypicolinic acid (100 mg/mL) in H_2O-diammoniumhydrogen citrate (100 mg/mL) in H_2O (10:1, v/v)].

2´-*O*-(2-Cyanoethyl)-3´,5´-*O*-bis-(*tert*-butyldimethylsilyl)-4-*O*-(2,6-dimethylphenyl)uridine (2)



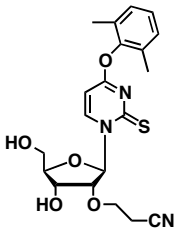
2

2´-*O*-(2-Cyanoethyl)-3´,5´-*O*-bis-(*tert*-butyldimethylsilyl)uridine (2.4 g, 4.56 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry CH_2Cl_2 (120 mL). To the solution were added triethylamine (1.0 mL, 6.85 mmol), 2,4,6-triisopropylbenzenesulfonyl chloride (2.1 g, 6.85 mmol) and dimethylaminopyridine

(0.14 g, 1.14 mmol). After being stirred at room temperature for 1 h, the reaction mixture was washed with sat. $NaHCO_3$ and brine. The organic phase was collected, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry MeCN (120 mL). To the solution were added 2,6-dimethylphenol (0.84 g, 6.8 mmol), triethylamine (4.8 mL, 34.2 mmol), 1,4-diazabicyclo[2,2,2]octane (51 mg, 0.46 mmol). After being stirred at room temperature for 3 h, the reaction mixture was concentrated, diluted with $CHCl_3$, washed with sat. $NaHCO_3$. The organic phase was concentrated and purified chromatography with hexane-ethylacetate to afford compound **2**. (2.7 g, 94%)

^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.07 (3H, s), 0.08 (3H, s), 0.14 (3H, s), 0.16 (3H, s), 0.90 (9H, s), 0.97 (9H, s), 2.13 (6H, s), 2.63-2.74 (2H, m), 3.77-3.82(2H, m), 3.88 (1H, td, *J* = 8.5, 10.0), 4.11-4.14 (2H, m), 4.17-4.25 (2H, m), 5.83 (1H, s), 6.06 (1H, d, *J* = 7.5), 7.04 (3H, s), 8.51 (1H, d, *J* = 7.5); ^{13}C NMR ($CDCl_3$, 126 MHz): δ -5.6, -5.4, -5.1, -4.5, 16.5, 18.0, 18.4, 18.9, 25.6, 26.0, 59.9, 65.1, 67.3, 82.8, 82.9, 89.1, 94.0, 117.6, 125.8, 128.7, 130.1, 144.1, 149.1, 155.5, 170.9; MS (ESI) calcd. for $C_{32}H_{52}N_3O_6Si_2$ $[M+H]^+$ 630.3389, found 630.3210.

2´-*O*-(2-Cyanoethyl)-4-*O*-(2,6-dimethylphenyl)-2-thiouridine (3)

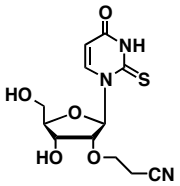


3

2´-*O*-(2-Cyanoethyl)-3´,5´-*O*-bis-(*tert*-butyldimethylsilyl)-4-*O*-(2,6-dimethylphenyl)uridine **2** (1.96 g, 3.11 mmol) was dissolved in dry PhMe (100 mL). To the solution, Lawesson Reagents (0.75 g, 1.89 mmol) was added. The resulting mixture was refluxed for 1 h and then cooled to room temperature. The mixture was concentrated under reduced pressure. The residue was dissolved in dry THF (60 mL). To the solution were added $3HF{\cdot}NEt_3$ (1.52 mL, 9.31 mmol) and triethylamine (1.29 mL, 9.31 mmol). After being stirred at room temperature for 13 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by chromatography with $CHCl_3$-MeOH to afford compound **3**. (1.11 g, 85%)

^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.08 (6H, s), 2.64-2.71 (2H, m), 3.01 (1H, d, *J* = 10.0), 3.43 (1H, t, *J* = 4.5), 3.85(1H, dd, *J* = 3.0, 7.0), 3.97-4.05 (3H, m), 4.10 (1H, d, *J* =4.5), 4.22-4.27 (1H, m), 4.46 (1H, td, *J* = 5.5, 11.0), 6.25 (1H, d, *J* = 7.5), 6.48 (1H, s), 7.05 (3H, s), 8.98 (1H, d, *J* = 7.5); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 16.3, 19.0, 58.5, 66.1, 66.6, 82.3, 83.8, 92.9, 98.3, 118.1, 126.3, 128.9, 130.0, 146.9, 148.7, 165.7, 180.6; MS (ESI) calcd. for $C_{20}H_{24}N_3O_5S$ $[M+H]^+$ 418.1431, found 418.1431.

2′-*O*-(2-Cyanoethyl)-2-thiouridine (4)

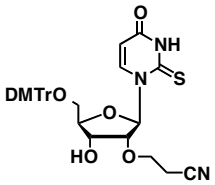


4

2′-*O*-(2-Cyanoethyl)- 4-*O*-(2,6-dimethylphenyl)-2-thiouridine **3** (1.11 g, 2.66 mmol) was dissolved in dry MeCN (27 mL). To the solution was added 1,1,3,3-tetramethylguanidine (0.9 mL, 8.0 mmol) and *syn*-*o*-nitrobenzaldoxime (1.3 g, 8.0 mmol), and the resulting mixture was stirred at room temperature for 3 h. The mixture was concentrated and purified by chromatography with $CHCl_3$-MeOH to afford compound **4**. (0.75 g, 90%)

^{1}H NMR (DMSO-d_6, 500 MHz): δ 2.52-2.84 (2H, m), 3.61 (1H, brd, *J* = 12.0), 3.75 (1H, brd, *J* = 12.0), 3.81-3.94 (3H, m), 3.97-4.03 (1H, brs), 4.07 (1H, dd, *J* = 7.0, 12.0), 5.21 (1H, d, *J* = 6.5), 5.30 (1H, t, *J* = 4.5), 5.97 (1H, d, *J* = 8.0), 6.50 (1H, d, *J* = 8.0), 8.23 (1H, d, *J* = 8.0), 12.68 (1H, s); ^{13}C NMR (DMSO-d_6, 126 MHz): δ 18.3, 58.9, 65.7, 67.5, 82.3, 84.2, 91.3, 106.5, 119.0, 140.6, 159.6, 175.7; MS (ESI) calcd. for $C_{12}H_{16}N_3O_5S$ $[M+H]^+$ 314.0805, found 314.0816.

2′-*O*-(2-Cyanoethyl)-5′-*O*-(4,4′-dimethoxytrityl)-2-thiouridine (4a)

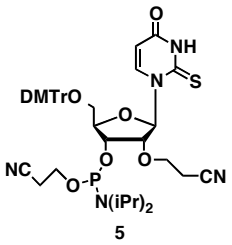


4a

2′-*O*-(2-Cyanoethyl)-2-thiouridine **4** (0.158 g, 0.504 mmol) was dissolved in dry pyridine (5 mL). To the solution was added 4,4′-dimethoxytrityl chloride (0.205 g, 0.6 mmol), and the resulting mixture was stirred at room temperature for 4 h. The mixture was concentrated under reduced pressure. The residue was diluted with $CHCl_3$ and washed with sat. $NaHCO_3$. The organic phase was concentrated and purified by chromatography with $CHCl_3$-MeOH containing 0.5% triethylamine to afford above compound **4a**. (0.255 g, 82%)

^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.62-2.78 (2H, m), 3.59-3.69 (2H, br s), 3.80 (6H, S) 3.97-4.05 (1H, m), 4.05-4.16 (2H, m), 4.44 (1H, td, *J* = 6.0, 9.5), 4.60 (1H, dd, *J* = 5.0, 9.5), 5.47 (1H, d, *J* = 8.0), 6.51 (1H, s), 6.83-6.91 (4H, m), 7.22-7.45 (9H,m), 8.44 (1H, d, *J* = 8.0); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 19.0, 55.1, 60.0, 66.1, 67.9, 82.8, 82.8, 87.1, 91.6, 106.8, 113.2, 117.6, 127.1, 127.9, 128.0, 130.0, 130.1, 134.7, 135.0, 140.7, 144.1, 157.6, 158.6, 160.2, 174.7; MS (ESI) calcd. for $C_{33}H_{34}N_3O_7S^+$ $[M+Na]^+$ 638.1931, found 638.1935.

2′-*O*-(2-Cyanoethyl)-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite)-2-thiouridine (5)

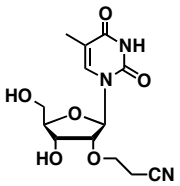


5

Compound **4a** (0.627 g, 1.02 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry CH_2Cl_2 (10 mL). To the solution were added diisopropylamine (95 µL, 0.67 mmol), 1*H*-tetrazole (47 mg, 0.67 mmol) and (2-cyanoethoxy)-bis-(*N*,*N*-diisopropylamino)phosphine (338 µL, 1.12mmol). The resulting mixture was stirred at room temperature for 13 h. The mixture was washed with sat. $NaHCO_3$. The organic phase was concentrated and purified by chromatography with hexane-ethylacetate containing 0.5% triethylamine to afford compound **5**. (0.573 g, 68%)

^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.89-1.30 (12H, m), 2.32-2.72 (4H, m), 3.39-4.31 (16H, m), 4.45-4.65 (1H, m), 5.30-5.45 (1H, m), 6.38-6.45 (1H, m), 6.72-6.89 (4H, m), 7.12-7.45 (9H, m), 8.34-8.47 (1H, m), 10.7-11.3 (1H, br s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 18.9, 19.0, 20.1, 20.3, 24.3, 24.5, 24.5, 24.6, 43.0, 43.1, 55.1, 57.5, 57.6, 57.7, 57.9, 59.4, 59.5, 66.0, 66.1, 68.6, 68.7, 69.5, 69.6, 81.4, 81.6, 82.3, 86.8, 87.0, 92.5, 92.7, 106.6, 113.0, 117.3, 117.4, 117.5, 127.1, 127.8, 128.0, 128.1, 130.1, 134.6, 134.7, 134.8, 140.9, 143.9, 144.1, 158.5, 160.4. 174.6; ^{31}P NMR ($CDCl_3$): δ 151.3, 149.4; MS (ESI) calcd. for $C_{42}H_{51}N_5O_8PS^+$ $[M+H]^+$ 816.3190, found 816.3138.

2′-*O*-(2-Cyanoethyl)-5-methyluridine (7)

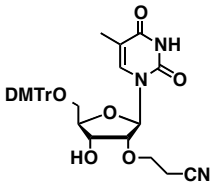


7

Compound **6** (5.0 g, 20.8 mmol) was dissolved in DMA (40 mL) and added 2-cyanoethanol trimethylsilyl ester (14.9 mL, 104 mmol) under Ar atmosphere. To the solution was slowly added $BF_3 \cdot OEt_2$ (6.5 mL, 52 mmol) at 0°C. The resulting mixture was warmed up to 120°C and stirred for 14 h. The mixture was concentrated and purified by chromatography with $CHCl_3$-MeOH containing 2.0% triethylamine to afford compound **11**. (4.0 g, 62%)

^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.76 (3H, d, *J* = 1.0), 2.71-2.84 (2H, m), 3.57 (1H, ddd, *J* = 3.2, 4.7, 13.5), 3.65-3.72 (2H, m), 3.78 (1H, dt, *J* = 9.9, 6.0), 3.84-3.88 (1H, m), 3.98 (1H, t, *J* =5.0), 4.12 (1H, dd, *J* = 5.0, 10.8), 5.15-5.20 (2H, m), 584 (1H, d, *J* =4.7), 7.79 (1H, d, *J* = 1.0), 11.34 (1H, s); ^{13}C NMR (DMSO-d_6, 126 MHz): δ 12.4, 18.2, 60.4, 64.8, 68.1, 81.2, 84.8, 86.1, 109.4, 119.1, 136.1, 150.6, 163.8; MS (ESI) calcd. for $C_{13}H_{18}N_3O_6^+$ $[M+H]^+$ 312.1190, found 312.1139.

2′-*O*-(2-Cyanoethyl)-5′-*O*-(4,4′-dimethoxytrityl)-5-methyluridine (7a)

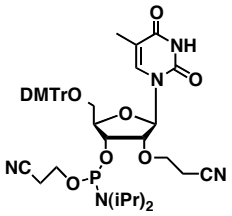


7a

Compound **7** (1.0 g, 3.2 mmol) was dissolved in dry pyridine (35 mL). To the solution was added 4,4′-dimethoxytrityl chloride (1.31 g, 3.9 mmol), and the resulting mixture was stirred at room temperature for 4 h. The mixture was diluted with $CHCl_3$ and washed with sat. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the mixture was concentrated under reduced pressure. The residue was purified by chromatography with hexane-$CHCl_3$ containing 2.0% triethylamine to afford compound **7a**. (1.82 g, 92%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.36 (3H, s), 2.51 (1H, d, *J* = 9.5), 2.66-2.80 (2H, m), 3.47 (1H, dd, *J* =2.5, 11.0), 3.58 (1H, d, *J* = 11.0), 3.79 (6H, s), 3.91-3.98 (1H, m), 4.02-4.10 (2H, m), 4.19-4.27 (1H, m), 4.48-4.58 (1H, m), 5.89 (1H, s), 6.84 (4H, d, *J* = 8.0), 7.21-7.44 (9H, m), 7.72 (1H, s), 8.97-9.08 (1H, br s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 11.9, 19.2, 55.2, 61.2, 65.4, 68.5, 82.9, 83.0, 86.8, 87.7, 111.1, 113.3, 117.5, 127.1, 128.0, 128.1, 130.1, 130.1, 134.8, 135.2, 135.3, 144.3, 150.4, 158.7, 158.7, 163.7; MS (ESI) calcd. for $C_{34}H_{35}N_3NaO_8^+$ $[M+Na]^+$ 636.2316, found 636.2319.

2′-*O*-(2-Cyanoethyl)-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite)-5-methyluridine (8)

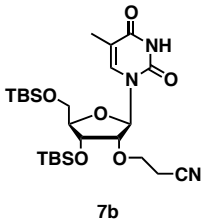


8

Compound **7a** (1.79 g, 2.9 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry CH_2Cl_2 (30 mL). To the solution were added diisopropylamine (250 μL, 1.8 mmol), 1*H*-tetrazole (120 mg, 1.8 mmol) and (2-cyanoethoxy)-bis-(*N*,*N*-diisopropylamino)phosphine (1.4 mL, 3.5 mmol). The resulting mixture was stirred at room temperature for 12 h. The mixture was washed with sat. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the mixture was concentrated and purified by chromatography with hexane-ethylacetate containing 2.0% triethylamine to afford compound **8**. (1.5 g, 63%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.95-1.34 (15H, m), 2.34-2.42 (1H, m), 2.58-2.76 (3H, m), 3.26-3.40 (1H, m) 3.44-4.31 (15H, m), 4.41-4.61 (1H, m), 5.82-5.94 (1H, m), 6.78-6.88 (4H, m), 7.18-7.45 (9H, m), 7.69-7.79 (1H, m), 9.81-9.99 (1H, m); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 11.6,11.7, 18.9, 18.9, 20.1, 10.2, 20.3, 22.9, 24.4, 24.4, 24.5, 24.5, 24.6, 24.6, 43.0, 43.1, 43.1, 43.2, 55.1, 55.2, 57.7, 57.8, 57.9, 58.0, 60.7, 61.2, 65.2, 65.5, 69.3, 69.4, 69.8, 69.9, 81.5, 81.6, 81.8, 81.8, 81.9, 82.0, 82.4, 86.6, 86.7, 88.4, 88.5, 110.8, 111.0, 113.1, 113.1, 113.1, 117.4, 117.5, 117.8, 117.8, 127.0, 127.1, 127.8, 128.2, 128.2, 130.1, 130.2, 134.9, 134.9, 135.1, 135.2, 144.1, 144.2, 150.6, 150.7, 158.6, 158.6, 164.2, 164.2; ^{31}P NMR ($CDCl_3$) δ 150.6, 151.5; MS (ESI) calcd. for $C_{43}H_{51}N_5O_9P^-$ $[M-H]^-$ 812.3430, found 812.3415.

2′-*O*-(2-Cyanoethyl)-3′,5′-*O*-bis-(*tert*-butyldimethylsilyl)-5-methyluridine (7b)

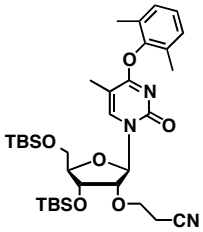


7b

Compound **7** (1.94 g, 6.2 mmol) was dissolved in dry DMF (6.5 mL). To the solution was added imidazole (3.4 g, 49.8 mmol) and *tert*-butyldimethylsiliy chloride (3.75 g, 24.9 mmol), and the resulting mixture was stirred at room temperature for 12 h. The mixture was diluted with $CHCl_3$ and washed with sat. $NaHCO_3$. The organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated and purified by chromatography with $CHCl_3$ to afford compound **7b**. (3.16 g, 94%)

^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.10 (3H, s), 0.12 (3H, s), 0.13 (3H, s), 0.14 (3H, s), 0.91 (9H, s), 0.95 (9H, s), 1.93 (3H, s), 2.59-2.69 (2H, m), 3.72-3.84 (3H, m), 3.94-4.06 (3H, m), 4.23 (1H, t, *J* = 5.5), 5.93 (1H, d, *J* =3.2), 7.53 (1H, s), 8.89-8.96 (1H, br s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ -5.4, -5.2, -4.9, -4.5, 12.6, 18.1, 18.6, 19.0, 25.7, 26.1, 61.4, 65.1, 69.3, 82.8, 84.2, 87.4, 111.0, 117.5, 135.0, 150.2, 163.6; MS (ESI) calcd. for $C_{25}H_{46}N_3O_6Si_2^+$ $[M+H]^+$ 540.2920, found 540.2752.

2´-*O*-(2-Cyanoethyl)-3´,5´-*O*-bis-(*tert*-butyldimethylsilyl)-4-*O*-(2,6-dimethylphenyl)-5-methyluridine (9)

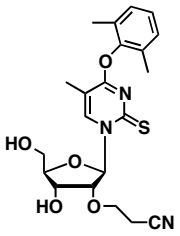


9

Compound **7a** (2.2 g, 4.1 mmol) was dissolved in CH_2Cl_2 (85 mL). To the solution were added 0.025M aq. Na_2CO_3 (165 mL), tetrabutylammonium bromide (0.53 g, 1.6 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (1.85 g, 6.1 mmol). The resulting two-phase solution was stirred vigorously at room temperature for 24 h. The organic phase was collected and the aqueous phase was extracted twice with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was rendered anhydrous by coevaporated with dry PhMe and dry MeCN. Then the residue was dissolved in dry MeCN (40 mL). Dimethylphenol (0.75 g, 6.1 mmol), 1,4-diazabicyclo[2,2,2]octane (46 mg, 0.41 mmol) and triethylamine (170 μL, 1.2 mmol) were added to the solution. After being stirred at room temperature for 5 h, the reaction mixture was concentrated. The residue was diluted with $CHCl_3$ and washed with sat. $NaHCO_3$. The organic phase was concentrated and purified by chromatography with hexane-ethylacetate to afford compound **17**. (2.0 g, 75%)

^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.08 (3H, s), 0.09 (3H, s), 0.16 (3H, s), 0.18 (3H, s), 0.90 (9H, s), 0.99 (9H, s), 2.06-2.12 (3H, br s), 2.13-2.16 (3H, br s), 2.17 (3H, s), 2.61- 2.75 (2H, m), 3.78-3.88 (3H, m), 4.07-4.15 (2H, m), 4.16-4.24 (2H, m), 5.83 (1H, s), 7.01-7.08 (3H, br s), 8.00 (1H, s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ -5.4, -5.0, -4.9, -4.4, 12.6, 16.5, 18.0, 18.7, 19.0, 25.7, 26.2, 60.5, 65.1, 68.0, 82.9, 83.1, 89.3, 103.8, 117.7, 125.7, 128.6, 128.7, 140.5, 149.3, 155.5, 169.7; MS (ESI) calcd. for $C_{33}H_{54}N_3O_6Si_2^+$ $[M+H]^+$ 644.3546, found 644.3302.

2′-*O*-(2-Cyanoethyl)-4-*O*-(2,6-dimethylphenyl)-5-methyl-2-thiouridine (10)

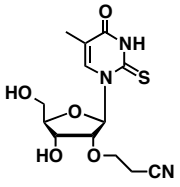


10

Compound **9** (1.24 g, 1.9 mmol) was dissolved in dry PhMe (25 mL). To the solution, Lawesson Reagents (0.70 g, 1.7 mmol) was added. The resulting mixture was refluxed for 2 h and then cooled to room temperature. The mixture was concentrated under reduced pressure. The residue was dissolved in THF (20 mL). $3HF \cdot NEt_3$ (0.65 mL, 4.0 mmol) and triethylamine (0.46 mL, 3.3 mmol) were added to the solution. After being stirred at room temperature for 15 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by chromatography with $CHCl_3$-MeOH to afford compound **10**. (0.74 g, 92%)

^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.06-2.15 (7H, m), 2.21 (3H, s), 2.56 (1H, d, *J* = 11.0), 2.66-2.79 (2H, m), 4.01 (1H, dd, *J* = 3.0, 12.0), 4.07-4.16 (3H, m), 4.17-4.23 (1H, m), 4.32 (1H, dt, *J* =5.0, 11.0), 4.64 (1H, dt, *J* =11.0, 5.5), 6.57 (1H, s), 7.06 (3H, s), 8.79 (1H, s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 12.9, 16.6, 19.3, 59.3, 66.1, 66.7, 82.3, 83.7, 92.6, 109.6, 117.9, 126.0, 128.8, 129.9, 143.5, 149.2, 164.3, 179.3; MS (ESI) calcd. for $C_{21}H_{26}N_3O_5S^+$ $[M+H]^+$ 432.1588, found 432.1580.

2′-*O*-(2-Cyanoethyl)-5-methyl-2-thiouridine (11)

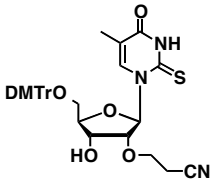


11

Compound **10** (0.86 g, 2.0 mmol) was dissolved in dry MeCN (20 mL). To the solution was added 1,1,3,3-tetramethylguanidine (750 μL, 6.0 mmol) and *syn*-*o*-nitrobenzaldoxime (1.0 g, 6.0 mmol), and the resulting mixture was stirred at room temperature for 5 h. The mixture was concentrated and purified by chromatography with $CHCl_3$-MeOH to afford compound **11**. (0.58 g, 89%)

^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.79 (3H, s), 2.72-2.84 (2H, m), 3.62 (1H, br d, *J* = 12.0), 3.76-4.00 (5H, m), 4.11 (1H, dd, *J* =6.5, 12.0), 5.18 (1H, d, *J* =6.4), 5.38 (1H, t, *J* =4.3), 6.50 (1H, d, *J* =2.0), 8.20 (1H, s), 12.55-12.69 (1H, br s); ^{13}C NMR (DMSO-d_6, 126 MHz): δ 12.6, 18.3, 58.9, 65.6, 67.4, 82.2, 84.1, 91.2, 114.8, 119.1, 136.8, 160.7, 174.4; MS (ESI) calcd. for $C_{13}H_{18}N_3O_5S^+$ $[M+H]^+$ 328.0962, found 328.0920.

2′-*O*-(2-Cyanoethyl)-5′-*O*-(4,4′-dimethoxytrityl)-5-methyl-2-thiouridine (11a)

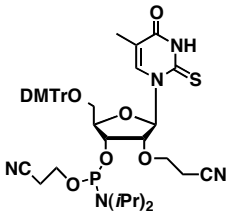


11a

Compound **11** (0.26 g, 0.84 mmol) was dissolved in dry pyridine (10 mL). To the solution was added 4,4′-dimethoxytrityl chloride (0.34 g, 1.0 mmol), and the resulting mixture was stirred at room temperature for 12 h. The reaction mixture was dilited with $CHCl_3$, washed with sat. $NaHCO_3$ and dried over Na_2SO_4. The filtrate was concentrated and purified by chromatography with $CHCl_3$-MeOH containing 1.5% triethylamine to afford compound **11a**. (0.49 g, 93%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.27 (3H, s), 2.64-2.82 (2H, m), 3.46-3.56 (1H, br d, *J* = 11.0), 3.61 (1H, d, *J* = 11.0), 3.79 (6H, s), 3.96-4.06 (1H, m), 4.07-4.20 (2H, m), 4.45-4.54 (1H, m), 4.54-4.63 (1H, m), 6.46 (1H, s), 6.84 (4H, d, *J* =7.5), 7.22-7.44 (9H, m), 7.98 (1H, s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 11.9, 19.2, 55.2, 60.7, 66.3, 68.4, 82.8, 83.0, 86.9, 92.0, 113.2, 116.7, 117.6, 127.1, 128.0, 128.1, 130.0, 130.1, 135.0, 135.1, 136.1, 144.1, 158.6, 158.7, 160.7, 173.5; MS (ESI) calcd. for $C_{34}H_{35}N_3NaO_7S^+$ $[M+Na]^+$ 652.2088, found 652.2073.

2′-*O*-(2-Cyanoethyl)-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite)-5-methyl-2-thiouridine (12)



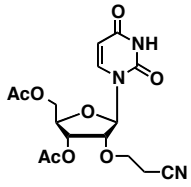
12

Compound **11a** (0.46 g, 0.74 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry CH_2Cl_2 (8 mL). To the solution were added diisopropylamine (63 μL, 0.44 mmol), 1*H*-tetrazole (31 mg, 0.44 mmol) and (2-cyanoethoxy)-bis-(*N*,*N*-diisopropylamino)phosphine (350 μL, 0.88 mmol). The resulting mixture was stirred at room temperature for 12 h. The mixture was diluted with Et_2O, washed with sat. $NaHCO_3$ and dried over Na_2SO_4. The filtrate was concentrated and purified by chromatography with $CHCl_3$-MeOH containing 1.0% triethylamine to afford compound **12**. (0.37 g, 62%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.91-1.35 (15H, m), 2.29-2.74 (4H, m), 3.30-3.42 (1H, m), 3.43-4.37 (15H, m), 4.50-4.61(1H, m), 6.40-6.47 (1H, m), 6.78-6.90 (4H, m), 7.21-7.49 (9H, m), 8.00-8.09 (1H, m), 9.20-9.42 (1H, br s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 11.6, 11.8, 19.1, 19.2, 20.2, 20.3, 24.4, 24.5, 24.5,

24.6, 24.6, 24.7, 29.4, 29.6, 29.6, 43.2, 43.3, 55.2, 55.3, 57.7, 57.9, 60.4, 66.2, 66.4, 69.3, 69.4, 81.7, 81.7, 82.0, 82.0, 86.8, 93.1, 93.3, 113.1, 113.2, 113.3, 116.6, 117.4, 117.4, 127.3, 127.9, 128.0, 128.5, 130.3, 130.4, 135.1, 136.2, 144.1, 158.8, 158.8, 160.3, 173.6; ^{31}P NMR ($CDCl_3$) δ 150.1, 152.1; MS (ESI) calcd. for $C_{43}H_{51}N_5O_8PS^-$ $[M-H]^-$ 828.3201, found 828.3190.

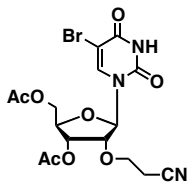
2´-*O*-(2-Cyanoethyl)-3´,5´-*O*-diacetyl-uridine (13)



13

^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.06 (3H, s), 2.11 (3H, s), 2.51-2.64 (2H, m), 3.73 (1H, dt, *J* = 10.0, 6.0), 3.94 (1H, dt, *J* = 10.0, 6.0), 4.25-4.36 (3H, m), 4.36-4.42 (1H, m), 5.70 (1H, d, *J* = 8.2), 5.78 (1H, s), 7.55 (1H, d, *J* = 8.2), 10.12-10.35 (1H, br s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 18.6, 20.4, 20.5, 61.9, 65.5, 69.4, 78.8, 80.1, 89.2, 102.3, 117.5, 138.9, 150.3, 163.3, 170.0, 170.2; MS (ESI) calcd. for $C_{16}H_{19}N_3NaO_8^+$ $[M+Na]^+$ 404.1064, found 404.1148.

2´-*O*-(2-Cyanoethyl)-3´,5´-*O*-diacetyl-5-bromouridine (14)

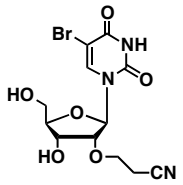


14

Compound **13** (1.0 g, 2.6 mmol) was dissolved in dry MeCN (39 mL). To the solution were added ceric ammonium nitrate (2.9g, 5.2mmol) and lithium bromide (0.27 g, 3.1 mmol). After being stirred at 80°C for 1 h, the reaction mixture was diluted with $CHCl_3$ and washed with aq. $Na_2S_2O_3$. The organic phase was concentrated under reduced pressure. The residue was purified by chromatography with hexane-$CHCl_3$ to $CHCl_3$-MeOH to afford compound 14. (1.11 g, 92%)

^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.20 (3H, s), 2.23 (3H, s), 2.57-2.70 (2H, m), 3.80 (1H, dt, *J* =9.5, 5.6), 4.06 (1H, dt, *J* =9.5, 5.6), 4.29 (1H, d, *J* = 5.0), 4.41-4.46 (2H, br s), 4.51 (1H, d, *J* =8.5), 4.81 (1H, dd, *J* = 5.5, 8.5), 5.85 (1H, s), 8.03 (1H, s), 8.66-8.83 (1H, br s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 18.7, 20.4, 20.9, 61.3, 65.6, 68.9, 79.0, 80.4, 89.1, 97.1, 117.6, 138.3, 149.8, 159.0, 170.2, 170.4; MS (ESI) calcd. for $C_{16}H_{19}BrN_3O_8^+$ $[M+H]^+$ 460.0350, found 460.0316.

2′-*O*-(2-Cyanoethyl)-5-bromouridine (15)

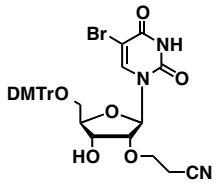


15

Compound **14** (0.2 g, 0.4 mmol) was dissolved in THF (6 mL) and MeOH (6 mL). To the solution were added 28% ammonium hydroxide (6 mL). After being stirred at room temperature for 6 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by chromatography with $CHCl_3$-MeOH to afford compound **15**. (0.11 g, 69%)

^{1}H NMR (DMSO-d_6, 500 MHz): δ 2.71-2.85 (2H, m), 3.54-3.62 (1H, br d, J = 12.0), 3.68-3.82 (3H, m), 3.83-3.89 (1H, m), 3.98 (1H, t, J = 4.5), 4.11 (1H, dd, J = 5.5, 12.0), 5.17 (1H, d, J = 6.3), 5.29-5.36 (1H, br s), 5.76 (1H, d, J =3.2), 8.51(1H, s), 11.75-11.91 (1H, br s); ^{13}C NMR (DMSO-d_6, 126 MHz): δ 18.2, 59.3, 64.9, 67.4, 81.7, 84.4, 87.1, 95.7, 119.0, 140.0, 149.8, 159.3; MS (ESI) calcd. for $C_{12}H_{15}BrN_3O_6^+$ $[M+H]^+$ 376.0139, found 376.0188.

2′-*O*-(2-Cyanoethyl)-5′-*O*-(4,4′-dimethoxytrityl)-5-bromo-uridine 15a

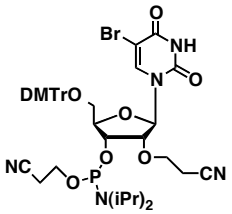


15a

Compound **15** (0.72 g, 1.91 mmol) was dissolved in dry pyridine (19 mL). To the solution was added 4,4′-dimethoxytrityl chloride (0.71 g, 2.11 mmol), and the resulting mixture was stirred at room temperature for 14 h. After the addition of MeOH, the mixture was concentrated under reduced pressure. The residue was purified by chromatography with hexane-$CHCl_3$ containing 1.0% triethylamine to afford compound **15a**. (0.58 g, 45%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.66-2.78 (2H, m), 3.46-3.57 (2H, m), 3.78 (6H, s), 3.91-3.98 (1H, m), 4.07-4.14 (2H, m), 4.20 (1H, dt, J = 10.0, 6.0), 4.53 (1H, dd, J = 6.0, 7.4), 5.84 (1H, s), 6.84 (4H, d, J = 8.0), 7.19-7.46 (9H, m), 8.15 (1H, s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 19.0, 55.2, 61.0, 65.6, 68.4, 82.7, 83.3, 86.9, 88.2, 97.5, 113.4, 117.6, 127.0, 127.9, 128.1, 130.0, 135.2, 135.4, 138.5, 144.3, 150.0, 158.6, 158.6, 159.1; MS (ESI) calcd. for $C_{33}H_{32}BrN_3NaO_8^+$ $[M+Na]^+$ 700.1265, found 700.1267.

2′-*O*-(2-Cyanoethyl)-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite)- 5-bromo-uridine (16)

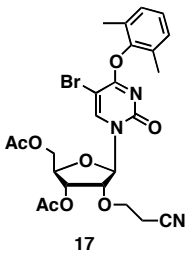


16

Compound **15a** (0.3 g, 0.4 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry CH_2Cl_2 (4.4 mL). To the solution were added diisopropylamine (38 µL, 0.3 mmol), 1*H*-tetrazole (19 mg, 0.3 mmol) and (2-cyanoethoxy)-bis-(*N*,*N*-diisopropylamino)phosphine (150 µL, 0.5 mmol). The resulting mixture was stirred at room temperature for 23 h. The mixture was washed with sat. $NaHCO_3$ and dried over Na_2SO_4. The organic phase was concentrated and purified by chromatography with $CHCl_3$-MeOH containing 1.0% triethylamine to afford compound **16**. (0.27 g, 69%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.03-1.17 (12H, m), 2.34-2.46 (1H, m), 2.54-2.76 (2H, m), 2.80-2.90 (1H, m), 3.31-4.61 (17H, m), 5.80-5.87 (1H, m), 6.75-6.90 (4H, m), 7.15-7.51 (9H, m), 8.05-8.15 (1H, m), 10.00-10.25 (1H, br s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 9.3, 18.8, 18.8, 20.1, 20.2, 20.2, 24.3, 24.4, 24.4, 24.5, 24.5, 24.6, 43.0, 43.0, 43.1, 43.1, 44.7, 55.0, 55.0, 57.5, 57.7, 57.7, 57.9, 60.5, 61.1, 65.3, 65.5, 69.2, 69.3, 69.7, 69.8, 70.3, 81.2, 81.8, 81.8, 82.1, 82.1, 82.3, 86.5, 86.6, 89.0, 89.0, 97.4, 97.5, 113.1, 113.2, 117.5, 117,6, 117.8, 117.9, 126.8, 127.8, 127.9, 130.0, 135.1, 135.2, 135.2, 135.3, 138.1, 138.2, 144.1, 144.2, 150.8, 150.8, 158.4, 158.4, 160.1, 160.2; ^{31}P NMR ($CDCl_3$) δ 150.2, 151.7; MS (ESI) calcd. for $C_{42}H_{48}BrN_5O_9P^-$ $[M-H]^-$ 876.2379, found 876.2392.

2′-*O*-(2-Cyanoethyl)-3′,5′-*O*-diacetyl-4-*O*-(2,6-dimethylphenyl)-5-bromouridine (17)



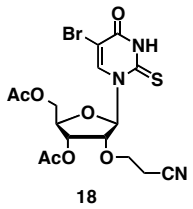
17

Compound **14** (1.1 g, 2.4 mmol) was dissolved in CH_2Cl_2 (48 mL). To the solution were added 0.025M aq. Na_2CO_3 (96 mL), tetrabutylammonium bromide (0.31 g, 1.0 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (1.09 g, 3.6 mmol). The resulting two-phase solution was stirred vigorously at room temperature for 10.5 h. The organic phase was collected and the aqueous phase was washed twice with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, filtered and

concentrated under reduced pressure. The residue was rendered anhydrous by coevaporated with dry PhMe and dry MeCN. Then the residue was dissolved in dry MeCN (24 mL). Dimethylphenol (0.44 g, 3.6 mmol), 1,4-diazabicyclo[2,2,2]octane (27 g, 0.2 mmol) and triethylamine (1.0 mL, 7.2 mmol) were added to the solution. After being stirred at room temperature for 2.5 h, the reaction mixture was concentrated. The residue was diluted with $CHCl_3$ and washed with sat. $NaHCO_3$. The organic phase was concentrated and purified by chromatography with hexane-ethylacetate to afford compound **17**. (1.1 g, 82%)

^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.05 (3H, s), 2.09 (3H, s), 2.12 (3H, s), 2.17 (3H, s), 2.43-2.51 (2H, m), 3.68-3.79 (1H, m), 3.98-4.08 (1H, m), 4.23 (1H, d, *J* = 5.0), 4.32-4.42 (2H, br s), 4.43-4.51 (1H, br d, *J* = 9.5, 4.62-4.75 (1H, m), 5.73 (1H, s), 6.91-7.08 (3H, br s), 8.27 (1H, s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 15.9, 16.0, 18.2, 20.1, 20.7, 60.6, 65.1, 68.2, 78.5, 79.6, 87.3, 90.0, 117.5, 125.8, 128.3, 129.4, 142.9, 148.8, 153.3, 165.7, 169.7, 170.1; MS (ESI) calcd. for $C_{24}H_{27}BrN_3O_8^+$ $[M+H]^+$ 564.0976, found 564.0907.

2´-*O*-(2-Cyanoethyl)-3´,5´-*O*-diacetyl-5-bromo-2-thiouridine (18)

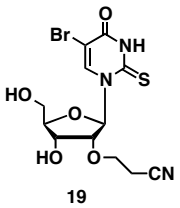


18

Compound **17** (1.0 g, 1.8 mmol) was dissolved in dry PhMe (18 mL). To the solution, Lawesson Reagents (0.86 g, 2.1 mmol) was added. The resulting mixture was refluxed for 2 h and then cooled to room temperature. The mixture was concentrated under reduced pressure. The residue was coevaporated with pyridine and toluene, then dissolved in dry MeCN (18 mL). To the solution was added 1,1,3,3-tetramethylguanidine (0.68 mL, 5.4 mmol) and *syn*-*o*-nitrobenzaldoxime (0.90 g, 5.4 mmol), and the resulting mixture was stirred at room temperature for 3.5 h. The mixture was concentrated, diluted with $CHCl_3$ and washed with sat. $NaHCO_3$. The organic phase was concentrated and purified by chromatography with hexane-$CHCl_3$ to afford compound **18**. (0.61 g, 72%)

^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.20 (3H, s), 2.27 (3H, s), 2.59-2.70 (2H, m), 3.79-3.95 (1H, m), 4.21-4.31 (1H, m), 4.39-4.61 (4H, m), 4.77 (1H, dd, *J* = 4.8, 9.6), 6.41 (1H, s), 8.21 (1H, s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 19.1, 20.6, 21.2, 60.7, 66.7, 68.7, 79.1, 80.7, 93.2, 102.7, 117.5, 139.1, 155.6, 170.2, 170.8, 173.4; MS (ESI) calcd. for $C_{16}H_{19}BrN_3O_7S^+$ $[M+H]^+$ 476.0122, found 476.0056.

2′-*O*-(2-Cyanoethyl)-5-bromo-2-thiouridine (19)

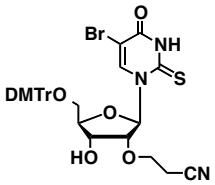


19

Compound **18a** (0.52 g, 1.1 mmol) was dissolved in THF (11 mL) and MeOH (11 mL). To the solution were added 28% ammonium hydroxide (0.7 mL). After being stirred at room temperature for 5 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by chromatography with $CHCl_3$-MeOH to afford compound **19**. (0.38 g, 89%)

^{1}H NMR (DMSO-d_6, 500 MHz): δ 2.73-2.85 (2H, m), 3.58-3.66 (1H, br d, J = 12.5), 3.78-3.98 (4H, m), 4.00-4.05 (1H, br d, J = 4.0), 4.07-4.14 (1H, m), 5.19 (1H, d, J = 6.5) 5.47-5.55 (1H, br s), 6.36 (1H, s), 8.87 (1H, s), 13.05-13.17 (1H, br s); ^{13}C NMR (DMSO-d_6, 126 MHz): δ 18.4, 58.0, 65.9, 66.7, 82.3, 83.9, 91.9, 101.7, 119.1, 140.6, 156.5, 174.2; MS (ESI) calcd. for $C_{12}H_{15}BrN_3O_5S^+$ $[M+H]^+$ 391.9910, found 391.9844.

2′-*O*-(2-Cyanoethyl)-5′-*O*-(4,4′-dimethoxytrityl)-5-bromo-2-thiouridine (19a)

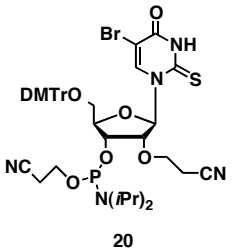


19a

Compound **19** (0.28 g, 0.71 mmol) was dissolved in dry pyridine (7.1 mL). To the solution was added 4,4′-dimethoxytrityl chloride (0.54 g, 2.2 mmol), and the resulting mixture was stirred at room temperature for 5 h. After the addition of MeOH, the mixture was concentrated under reduced pressure. The residue was purified by chromatography with hexane-$CHCl_3$ containing 1.0% triethylamine to afford compound **19a**. (0.44 g, 88%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.58 (1H, d, J = 10.0), 2.65-2.78 (2H, m), 3.53 (1H, dd, J = 1.5, 11.5), 3.57 (1H, dd, J =3.0, 11.5), 3.79 (6H, s), 3.96-4.04 (1H, m), 4.12-4.17 (1H, br d, J = 8.5), 4.19 (1H, d, J = 5.0), 4.42 (1H, dt, J = 5.5, 10.0), 4.55-4.62 (1H, m), 6.37 (1H, s), 6.84 (4H, d, J = 8.5), 7.22 (1H, t, J = 7.5), 7.29 (2H, t, J = 7.5, 7.34 (4H, d, J = 8.5), 7.43 (2H, br d, J = 8.5), 8.36 (1H, s), 9.91 (1H, s) ; ^{13}C NMR ($CDCl_3$, 126 MHz): δ 19.3, 55.2, 60.6, 66.5, 68.4, 82.8, 83.3, 87.1, 92.5, 103.2, 113.1, 113.4, 117.6, 127.1, 127.7, 127.8, 128.0, 128.1, 129.1, 130.1, 130.1, 135.1, 135.3, 139.6, 144.2, 155.9, 158.6, 158.7, 173.4; MS (ESI) calcd. for $C_{33}H_{32}BrN_3NaO_7S^+$ $[M+Na]^+$ 716.1037, found 716.0865.

2´-*O*-(2-Cyanoethyl)-5´-*O*-(4,4´-dimethoxytrityl)-3´-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite)-5-bromo-2-thiouridine 20

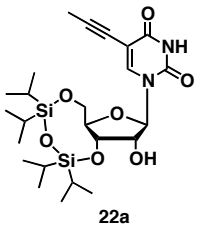


20

Compound **19a** (0.41 g, 0.6 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry CH_2Cl_2 (6.0 mL). To the solution were added diisopropylamine (50 µL, 0.4 mmol), 1*H*-tetrazole (25 mg, 0.4 mmol) and (2-cyanoethoxy)-bis-(*N*,*N*-diisopropylamino)phosphine (390 µL, 2.25 mmol). The resulting mixture was stirred at room temperature for 20 h. The mixture was washed with sat. $NaHCO_3$ and dried over Na_2SO_4. The organic phase was concentrated and purified by chromatography with hexane-$CHCl_3$ to $CHCl_3$-MeOH containing 1.0% triethylamine to afford compound **20**. (0.087 g, 17%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.00-1.23 (12H, m) 2.40-2.75 (4H, m), 3.38-4.62 (17H, m), 6.32-6.40 (1H, m), 6.79-6.91 (4H, m), 7.12- 7.50 (9H, m), 8.35-8.42 (1H, m); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 19.1, 19.2, 20.3, 20.4, 20,5, 20.5, 24.4, 24.5, 24.5, 24.6, 24.7, 24.7, 43.1, 43.3, 55.2, 55.2, 55.3, 57.7, 57.8, 60.3, 60.6, 66.3, 66.3, 66.4, 69.3, 69.4, 70.1, 70.2, 81.3, 81.3, 82.2, 82.3, 82.6, 86.9, 87.0, 93.5, 102.9, 102.9, 113.2, 113.2, 113.3, 117.3, 117.5, 117.6, 117.7, 127.0, 127.9, 128.2, 130.2, 130.2, 130.3, 130.3, 135.1, 135.2, 135.2, 135.3, 139.5, 139.6, 144.1, 144.2, 156.1, 158.6, 158.7, 173.6, 173.6; ^{31}P NMR ($CDCl_3$) δ 149.7, 152.2; MS (ESI) calcd. for $C_{42}H_{48}BrN_5O_8PS^-$ $[M-H]^-$ 892.2150, found 892.2158.

3´,5´-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-5-propynyluridine (22a)

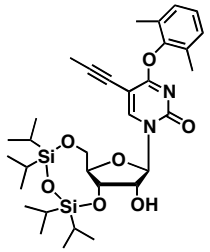


22a

5-propynyluridine (3.18 g, 11.3 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine and finally dissolved in dry pyridine (113 mL). To the solution was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (3.87 mL, 12.4 mmol) and the mixture was stirred at room temperature for 12h. The solution was concentrated and purified by chromatography with $CHCl_3$-MeOH to afford compound **22a**. (5.35 g, 91%)

^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.98-1.15 (28H, m), 2.01 (3H, s), 2.95 (1H, d, *J* = 1.0), 4.00 (1H, dd, *J* = 2.7, 13.2), 4.07-4.11(1H, d, *J* = 4.9), 4.15 (1H, d, *J* = 5.0), 4.21 (1H, dd, *J* = 1.3, 13.2), 4.38 (1H, dd, *J* = 4.9, 8.8), 5.73 (1H, s), 7.77 (1H, d), 8.36 (1H, s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 4.7, 12.5, 12.7, 13.0, 13.4, 16.8, 16.9, 17.0, 17.1, 17.2, 17.3, 17.4, 60.1, 68.9, 70.2, 75.2, 82.1, 90.8, 91.0, 100.7, 141.5, 148.7, 161.6; MS (ESI) calcd. for $C_{24}H_{41}N_2O_7Si_2^+$ $[M+H]^+$ 525.2447, found 525.2427.

3´,5´-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-4-*O*-(2,6-dimethylphenyl)-5-propynyluridine (22b)

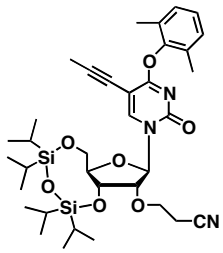


22b

3´,5´-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-5-propynyluridine **22a** (8.1 g, 15.4 mmol) was dissolved in CH_2Cl_2 (308 mL). To the solution were added 0.025M aq. Na_2CO_3 (616 mL), tetrabutylammonium bromide (1.99 g, 6.2 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (7.01 g, 23.2 mmol). The resulting two-phase solution was stirred vigorously at room temperature for 14 h. The organic phase was collected and the aqueous phase was washed twice with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was rendered anhydrous by coevaporated with dry PhMe and dry MeCN. Then the residue was dissolved in dry MeCN (154 mL). Dimethylphenol (2.82 g, 23.1 mmol), 1,4-diazabicyclo[2,2,2]octane (0.17 g, 1.5 mmol) and triethylamine (6.43 mL, 4.62 mmol) were added to the solution. After being stirred at room temperature for 3h, the reaction mixture was concentrated. The residue was diluted with $CHCl_3$ and washed with sat. $NaHCO_3$. The organic phase was concentrated and purified by chromatography with hexane-ethylacetate to afford compound **22b**. (7.1 g, 73%)

^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.94-1.16 (28H, m), 2.04 (3H, s), 2.14 (6H, s), 2.89-2.94 (1H, br d, *J* = 4.9), 4.02 (1H, dd, *J* = 2.4, 13.2), 4.14-4.18 (1H, br d, *J* = 8.8), 4.22 (1H, d, *J* = 4.4), 4.24-4.29 (1H, br d, *J* = 13.2), 4.38 (1H, dd, *J* = 4.9, 8.8), 5.74(1H, s) 7.03 (3H, s), 8.13 (1H, s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 4.8, 12.7, 12.9, 13.1, 13.6, 16.6, 17.0, 17.1, 17.2, 17.2, 17.4, 17.4, 17.6, 60.4, 69.1, 69.8, 75.2, 82.3, 91.0, 92.2, 94.1, 126.0, 128.8, 130.2, 146.1, 149.6, 153.9, 169.5; MS (ESI) calcd. for $C_{32}H_{49}N_2O_7Si_2^+$ $[M+H]^+$ 629.3073, found 629.0646.

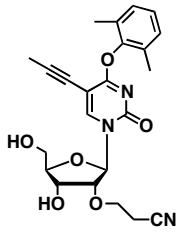
2′-*O*-(2-Cyanoethyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-*O*-(2,6-dimethylphenyl)-5-propynyluridine (23)



23

3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-*O*-(2,6-dimethylphenyl)-5-propynyluridine **22b** (7.1 g, 11.3 mmol) was dissolved in dry *t*-BuOH (113 mL). Acrylnitrile (14.79 mL, 225.8 mmol) and Cs_2CO_3 (3.68 g, 11.3 mmol) were added to the solution. After being stirred at room temperature for 13 h, the reaction mixture was filtrated through celite pad. The filtrate was concentrated under reduced pressure. The residue was purified by chromatography with hexane-$CHCl_3$ to afford compound **23**. (7.44 g, 97%) ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.92-1.19 (28H, m), 2.03 (3H, s), 2.13 (6H, s), 2.62-2.77 (2H, m), 3.93 (1H, d, *J* = 2.4), 3.96-4.02 (1H, br d, *J* = 13.7), 4.02-4.11 (2H, m), 4.14-4.24 (2H, m), 4.26-4.33 (1H, br d, *J* = 13.7), 5.67 (1H, s), 7.03 (3H, s), 8.28 (1H, s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 4.7, 14.7, 12.8, 13.0, 13.6, 17.0, 17.1, 17.3, 17.3, 17.3, 17.4, 17.6, 19.1, 59.3, 65.9, 67.8, 69.7, 82.0, 82.2, 90.2, 91.2, 94.1, 117.7, 126.0, 128.7, 145.5, 149.5, 154.1, 169.7; MS (ESI) calcd. for $C_{35}H_{52}N_3O_7Si_2^+$ $[M+H]^+$ 682.3338, found 682.3083.

2′-*O*-(2-Cyanoethyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-*O*-(2,6-dimethylphenyl)-5-propynyl-uridine (23a)

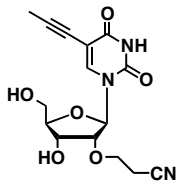


23a

Compound **23** (1.5 g, 2.2 mmol) was dissolved in dry THF (22 mL). To the solution were added $3HF{\cdot}NEt_3$ (1.1 mL, 6.6 mmol) and triethylamine (0.61 mL, 4.4 mmol). After being stirred at room temperature for 3 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by chromatography with $CHCl_3$-MeOH to afford compound **23a**. (0.93 g, 96%) ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.06 (3H, s), 2.12-2.18 (6H, brs), 2.51-2.57 (1H, brs), 2.63-2.76 (3H, m) 3.86-3.98 (2H, m), 4.03-4.12 (3H, m), 4.16-4.24 (1H, td, *J*=5.5, 10.0), 4.24-4.31 (1H, m), 5.76 (1H, s), 7.04(3H, s), 8.47 (1H, s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 4.7, 16.4, 19.0, 60.1, 65.3, 67.5, 69.4, 82.0,

84.2, 90.1, 91.1, 94.2, 117.7, 125.9, 128.6, 129.9, 130.0, 146.7, 149.3, 154.1, 169.6; MS (ESI) calcd. for $C_{23}H_{25}N_3NaO_6^+$ $[M+Na]^+$ 462.1636, found 462.1686.

2´-*O*-(2-Cyanoethyl)-5-propynyl-uridine (24)

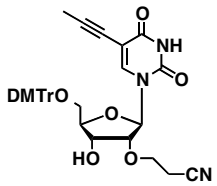


24

Compound **23a** (1.70 g, 3.9 mmol) was dissolved in dry MeCN (39 mL). To the solution was added 1,1,3,3-tetramethylguanidine (1.45 mL, 11.6 mmol) and *syn*-*o*-nitrobenzaldoxime (1.93 g, 11.6 mmol), and the resulting mixture was stirred at 50°C for 5 h. The mixture was concentrated and purified by chromatography with $CHCl_3$-MeOH to afford compound **24**. (1.07 g, 82%)

^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.97 (3H, s), 2.71-2.82 (2H, m), 3.56 (1H, dd, *J* =2.0, 7.0), 3.65-3.81 (3H, m), 3.83-3.87 (1H, m), 3.99 (1H, t, *J* = 4.5), 4.10 (1H, t, *J* = 5.0), 5.79 (1H, d, *J* = 4.0), 8.21 (1H, s), 11.61 (1H, s); ^{13}C NMR (DMSO-d_6, 126 MHz): δ 4.1, 18.1, 59.8, 64.8, 67.8, 72.0, 81.5, 84.7, 86.7, 89.2, 99.1, 119.0, 142.4, 149.5, 161.9; MS (ESI) calcd. for $C_{15}H_{18}N_3O_6^+$ $[M+H]^+$ 336.1190, found 336.1121.

2´-*O*-(2-Cyanoethyl)-5´-*O*-(4,4´-dimethoxytrityl)-5-propynyl-uridine (24a)

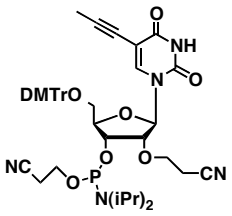


24a

Compound **24** (1.07 g, 3.2 mmol) was dissolved in dry pyridine (32 mL). To the solution was added 4,4´-dimethoxytrityl chloride (1.19 g, 3.5 mmol), and the resulting mixture was stirred at room temperature for 3.5 h. The mixture was concentrated under reduced pressure. The residue was purified by chromatography with $CHCl_3$-MeOH containing 1.0% triethylamine to afford compound **24a**. (2.04 g, 70%)

^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.61 (3H, s), 2.67-2.79 (2H, m), 3.48 (1H, d, *J* = 11.0), 3.52 (1H, dd, *J* = 3.0, 11.0), 3.78 (6H, s), 3.96 (1H, dt, *J* = 10.0, 6.0), 4.07-4.14 (2H, m), 4.21 (1H, dt, *J* =10.0, 6.0), 4.51 (1H, dd, *J* = 5.6, 7.8), 5.84 (1H, s), 6.84 (4H, d, *J* = 7.7), 7.17-7.49 (9H, m), 8.03(1H, s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 4.3, 19.0, 55.2, 61.1, 65.5, 68.4, 69.8, 82.7, 83.2, 86.7, 91.3, 101.1, 113.2, 113.2, 117.7, 126.8, 127.8, 128.0, 129.9, 135.3, 135.5, 140.8, 144.5, 149.8, 158.5, 158.5, 162.1; MS (ESI) calcd. for $C_{36}H_{35}N_3NaO_8^+$ $[M+Na]^+$ 660.2316, found 660.2314.

2′-*O*-(2-Cyanoethyl)-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite)- 5-propynyl-uridine (25)

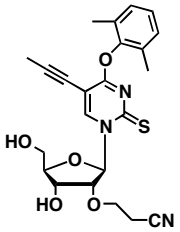


25

Compound **24a** (0.55 g, 0.86 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry CH_2Cl_2 (8.6 mL). To the solution were added diisopropylamine (73 μL, 0.52 mmol), 1*H*-tetrazole (36 mg, 0.52 mmol) and (2-cyanoethoxy)-bis-(*N*,*N*-diisopropylamino)phosphine (0.3 mL, 1.03 mmol). The resulting mixture was stirred at room temperature for 10.5 h. The mixture was washed with sat. $NaHCO_3$ and dried over Na_2SO_4. The organic phase was concentrated and purified by chromatography with $CHCl_3$-MeOH containing 1.0% triethylamine to afford compound **25**. (0.43 g, 60%)

^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.05-1.27 (12H, m), 1.48-1.53 (3H, m), 2.36-2.80 (4H, m), 3.40-4.69 (17H, m), 5.78-5.83 (1H, m), 6.78-6.90 (4H, m), 7.15-7.58 (9H, m), 8.03-8.14 (1H, m); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 4.1, 18.8, 20.1, 20.2, 20.2, 20.3, 24.3, 24.4, 24.5, 42.9, 43.0, 43.0, 55.0, 57.5, 57.5, 57.7, 57.8, 60.2, 60.9, 65.3, 65.5, 68.9, 69.0, 69.5, 69.6, 70.0, 70.1, 81.1, 81.8, 82.1, 82.4, 86.3, 86.5, 89.0, 89.2, 90.5, 100.6, 100.7, 113.0, 117.6, 117.9, 126.6, 127.7, 129.9, 135.2, 135.2, 135.3, 135.4, 140.8, 140.9, 144.3, 144.5, 149.8, 150.0, 158.3, 162.1, 162.2; ^{31}P NMR ($CDCl_3$) δ 149.9, 151.6; MS (ESI) calcd. for $C_{45}H_{51}N_5O_9P^-$ $[M-H]^-$ 836.3430, found 836.3423.

2′-*O*-(2-Cyanoethyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-*O*-(2,6-dimethylphenyl)-5-propynyl-2-thiouridine (26)



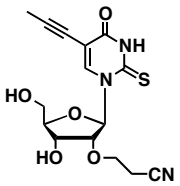
26

Compound **23** (0.5 g, 0.7 mmol) was dissolved in dry PhMe (7 mL). To the solution, Lawesson Reagents (0.36 g, 0.8 mmol) was added. The resulting mixture was refluxed for 2 h and then cooled to room temperature. The mixture was concentrated under reduced pressure. The residue was dissolved in THF (7 mL). $3HF{\cdot}NEt_3$ (0.34 mL, 2.1 mmol) and triethylamine (0.2 mL, 1.4 mmol) were added to the solution. After being stirred at room temperature for 1h, the reaction mixture was concentrated under reduced

pressure. The residue was purified by chromatography with $CHCl_3$-MeOH to afford compound **26**. (0.16 g, 49%)

^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.08 (3H, s), 2.14 (6H, s), 2.66-2.79 (2H, m), 4.00-4.05 (1H, br d, *J* = 12.0), 4.05-4.13 (2H, m), 4.15 (1H, d, *J* = 4.6), 4.19-4.25 (1H, br d, *J* = 12.0), 4.28-4.35 (1H, m), 4.60 (1H, td, *J* = 4.9, 10.0), 6.53 (1H, s), 7.05 (3H, s), 9.00 (1H, s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 5.0, 16.7, 19.4, 59.4, 66.4, 66.7, 69.4, 82.5, 84.0, 93.0, 94.0, 98.9, 118.0, 126.2, 128.9, 130.1, 147.6, 149.4, 163.7, 178.8; MS (ESI) calcd. for $C_{23}H_{26}N_3O_5S^+$ $[M+H]^+$ 456.1588, found 456.1470.

2′-*O*-(2-Cyanoethyl)-5-propynyl-2-thiouridine (27)

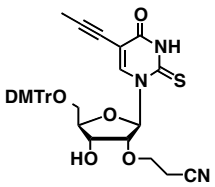


27

Compound **26** (1.49 g, 3.3 mmol) was dissolved in dry MeCN (33 mL). To the solution was added 1,1,3,3-tetramethylguanidine (1.23 mL, 9.8 mmol) and *syn*-*o*-nitrobenzaldoxime (1.63 g , 9.8 mmol), and the resulting mixture was stirred at room temperature for 4 h. The mixture was concentrated and purified by chromatography with $CHCl_3$-MeOH to afford compound **27**. (0.97 g, 84%)

^{1}H NMR (DMSO-d_6, 500 MHz): δ 2.05 (3H, s), 2.65-2.95 (2H, m), 3.95-4.04 (2H, m), 4.05-4.10 (2H, m), 4.20 (1H, d, J = 12.0), 4.31-4.38 (1H, br s), 4.45 (1H, td, J = 5.5, 11.0), 6.48 (1H, s), 8.53 (1H, s), 9.23-9.41 (1H, br s); ^{13}C NMR (DMSO-d_6, 126 MHz): δ 4.2, 18.4, 58.5, 65.7, 67.2, 71.7, 82.3, 84.2, 91.6, 91.7, 103.8, 119.1, 142.7, 158.7, 174.1; MS (ESI) calcd. for $C_{15}H_{18}N_3O_5S^+$ $[M+H]^+$ 352.0962, found 352.0965.

2′-*O*-(2-Cyanoethyl)-5′-*O*-(4,4′-dimethoxytrityl)- 5-propynyl-2-thiouridine (27a)

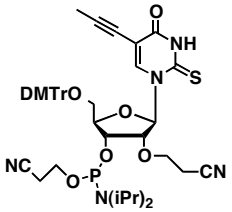


27a

Compound **27** (0.97 g, 2.8 mmol) was dissolved in dry pyridine (28 mL). To the solution was added 4,4′-dimethoxytrityl chloride (1.02 g, 3.0 mmol), and the resulting mixture was stirred at room temperature for 4 h. The mixture was concentrated under reduced pressure. The residue was diluted with $CHCl_3$ and washed with sat. $NaHCO_3$. The organic phase was concentrated and purified by chromatography with $CHCl_3$-MeOH to afford compound **27a**. (1.61 g, 90%)

^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.45 (3H, s), 2.59-2.73 (2H, br s), 3.45-3.58 (2H, br s), 3.76 (6H, s), 3.92-4.05 (1H, br s), 4.10-4.25 (2H, m), 4.30-4.43 (1H, br s), 4.53-4.65 (1H, br s), 6.40 (1H, s), 6.75-6.91 (4H, m), 7.13-7.56 (9H, m), 8.24 (1H, s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 4.2, 19.0, 55.1, 60.7, 66.4, 68.3, 69.7, 82.6, 83.1, 86.6, 92.2, 94.3, 105.6, 113.1, 113.1, 117.7, 126.7, 127.7, 127.8, 129.8, 129.8, 135.3, 135.4, 141.4, 144.4, 158.3, 158.4, 158.9, 173.0; MS (ESI) calcd. for $C_{36}H_{36}N_3O_7S^+$ $[M+Na]^+$ 676.2088, found 676.1976.

2´-*O*-(2-Cyanoethyl)-5´-*O*-(4,4´-dimethoxytrityl)-3´-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite)- 5-propynyl-2-thiouridine (28)



28

Compound **27a** (95 mg, 0.15 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry CH_2Cl_2 (1.5 mL). To the solution were added diisopropylamine (12 µL, 0.09 mmol), 1*H*-tetrazole (6 mg, 0.09 mmol) and (2-cyanoethoxy)-bis-(*N*,*N*-diisopropylamino)phosphine (50 µL, 0.17 mmol). The resulting mixture was stirred at room temperature for 4 h. The mixture was washed with sat. $NaHCO_3$. The organic phase was concentrated and purified by chromatography with $CHCl_3$-MeOH containing 0.5% triethylamine to afford compound **28**. (70 mg, 53%)

^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.02-1.37 (15H, m), 2.57-2.78 (4H, m), 3.33-3.44 (1H, m), 3.50-3.73 (4H, m), 3.73-3.91 (7H, m), 4.01-4.62 (5H, m), 6.27-6.40 (1H, m), 6.74-6.90 (4H, m), 7.18-7.51 (9H, m), 8.27-8.40 (1H, m); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 4.2, 19.1, 19.2, 20.5, 20.6, 24.5, 24.6, 24.6, 24.7, 43.2, 43.3, 55.2, 55.3, 57.7, 57.9, 61.0, 66.4, 69.9, 70.1, 70.2, 82.0, 82.0, 82.8, 86.8, 93.4, 93.5, 94.2, 105.6, 113.2, 117.6, 117.7, 126.9, 127.9, 128.1, 130.2, 130.2, 135.4, 135.5, 141.5, 141.7, 144.6, 158.6, 158.6, 158.8, 173.3; ^{31}P NMR ($CDCl_3$) δ 149.5, 151.9; MS (ESI) calcd. for $C_{45}H_{53}N_5O_8PS^+$ $[M+H]^+$ 854.3347, found 854.2712.

Synthesis of oligonucleotides

The synthesis of 2′-*O*-methyl-oligoribonucleotides (CGUUUXUUUGC) containing 2′-*O*-cyanoethyluridine (X = U_{OCE}), 2′-*O*-cyanoethyl-2-thiouridine (X = $^{2}sU_{OCE}$), 2′-*O*-cyanoethyl-5-methyluridine (X = $^{5}mU_{OCE}$), 2′-*O*-cyanoethyl-5-bromouridine (X = $^{5}bU_{OCE}$), 2′-*O*-cyanoethyl-5-propynyluridine (X = $^{5}pU_{OCE}$), 2′-*O*-cyanoethyl-5-methyl-2-thiouridine (X = $^{5}m^{2}sU_{OCE}$), 2′-*O*-cyanoethyl-5-bromo-2-thiouridine (X = $^{5}b^{2}sU_{OCE}$) and 2′-*O*-cyanoethyl-5-propynyl-2-thiouridine (X = $^{5}p^{2}sU_{OCE}$) residue was performed in an ABI 392 DNA synthesizer by the standard 1.0-μmol-scale RNA phosphoramidite approach, which consists of detritylation, coupling, capping, and iodine oxidation steps. Then, the synthesized oligomers were released from the resin by treatment with a solution of aq. NH_3-NH_4OAc (10:1, v/w) solution, at room temperature for 1 h. The polymer supports were removed by filtration and washed with 0.1 M ammonium acetate buffer (1 mL × 3). The filtrates were purified by anion-exchange HPLC to give oligoribonucleotides.

Oligonucleotide: X = U_{OCE}

MALDI-TOF Mass (M + H) calcd for $C_{114}H_{150}N_{31}O_{82}P_{10}^{+}$: 3574.6; found: 3575.1.

Oligonucleotide: X = $^{2}sU_{OCE}$

MALDI-TOF Mass (M + H) calcd for $C_{114}H_{150}N_{31}O_{81}P_{10}S^{+}$: 3590.6; found: 3589.6.

Oligonucleotide: X = $^{5}mU_{OCE}$

MALDI-TOF Mass (M + H) calcd for $C_{115}H_{152}N_{31}O_{82}P_{10}^{+}$: 3588.6; found: 3588.5.

Oligonucleotide: X = $^{5}bU_{OCE}$

MALDI-TOF Mass (M + H) calcd for $C_{114}H_{149}BrN_{31}O_{82}P_{10}^{+}$: 3652.5; found: 3652.3.

Oligonucleotide: X = $^{5}pU_{OCE}$

MALDI-TOF Mass (M + H) calcd for $C_{117}H_{152}N_{31}O_{82}P_{10}^{+}$: 3612.6; found: 3612.5.

Oligonucleotide: X = $^{5}m^{2}sU_{OCE}$

MALDI-TOF Mass (M + H) calcd for $C_{115}H_{152}N_{31}O_{81}P_{10}S^{+}$: 3604.6; found: 3604.4.

Oligonucleotide: X = $^{5}b^{2}sU_{OCE}$

MALDI-TOF Mass (M + H) calcd for $C_{114}H_{149}BrN_{31}O_{81}P_{10}S^{+}$: 3668.5; found: 3668.5.

Oligonucleotide: X = $^{5}p^{2}sU_{OCE}$

MALDI-TOF Mass (M + H) calcd for $C_{117}H_{152}N_{31}O_{81}P_{10}S^{+}$: 3628.6; found: 3627.8.

Computational methods

The simulations were carried out using the AMBER 9.0 program package with the parmBSC0 revision of the parm99 Cornell *et al.* force field[1]. The initial structures of each duplex were derived from NUCGEN module embedded in AMBER[2]. The charge of the 2´-*O*-methyl group (2´-OMe) and the 2´-*O*-cyanoethyl (2´-OCE) was taken from the Auffinger's parameter[3] and our previous report[4]. Parameterization of the modified nucleobase (2-thiouracil, 5-methyluracil, 5-bromouracil, 5-propynyluracil, 5-methyl-2thiouracil, 5-bromo-2-thiouracil, 5-propynyl-2-thiouracil) was done using the RESP/6-31G(d) charges (Figure S1)[5]. The additional force field parameters of the nucleobase were taken from the gaff force-field parameters (Figure S2)[6].

The sequence of calculated modified-RNA/RNA hetero duplexes were CGUUUXUUUGC where C or G or U denoted the 2´-*O*-methyl nucleotides and X denoted modified or unmodified nucleotides with complementary unmodified RNA. The RNAs were solvated in a periodic box with a 10 Å buffer of water molecules, explicitly described by the SPC/E model and neutralized, resulting in a concentration of added NaCl of approximately 0.1 M by using ions08.lib[7]. An initial optimization of 1000 cycles, the first 500 cycles by steepest descent and the rest with a conjugate gradient method, was performed with the duplex constrained 500 kcal/(mol·Å^2) to relax the solvent. Then, a further optimization of 5000 cycles with no constraints on the whole system was carried out to lead to a final relaxed geometry. The first equilibrations were carried out with a 10 kcal/(mol·Å^2) constraint on the duplex for 100 ps at constant volume, constantly increasing the temperature from 0 to 300 K. Next, the equilibrations were continued to 200 ps at a constant pressure of 1 atm, and the temperature was kept constant with the Langevin algorithm. The production simulations were performed for 100 ns with the Berendsen algorithm to maintain the temperature[8]. During the MD calculation, hydrogen vibrations were removed using SHAKE bond constraints, allowing a longer time step of 2 fs[9]. Long range electrostatic interactions were treated using the particle mesh Ewald approach and a 10 Å cutoff[10].

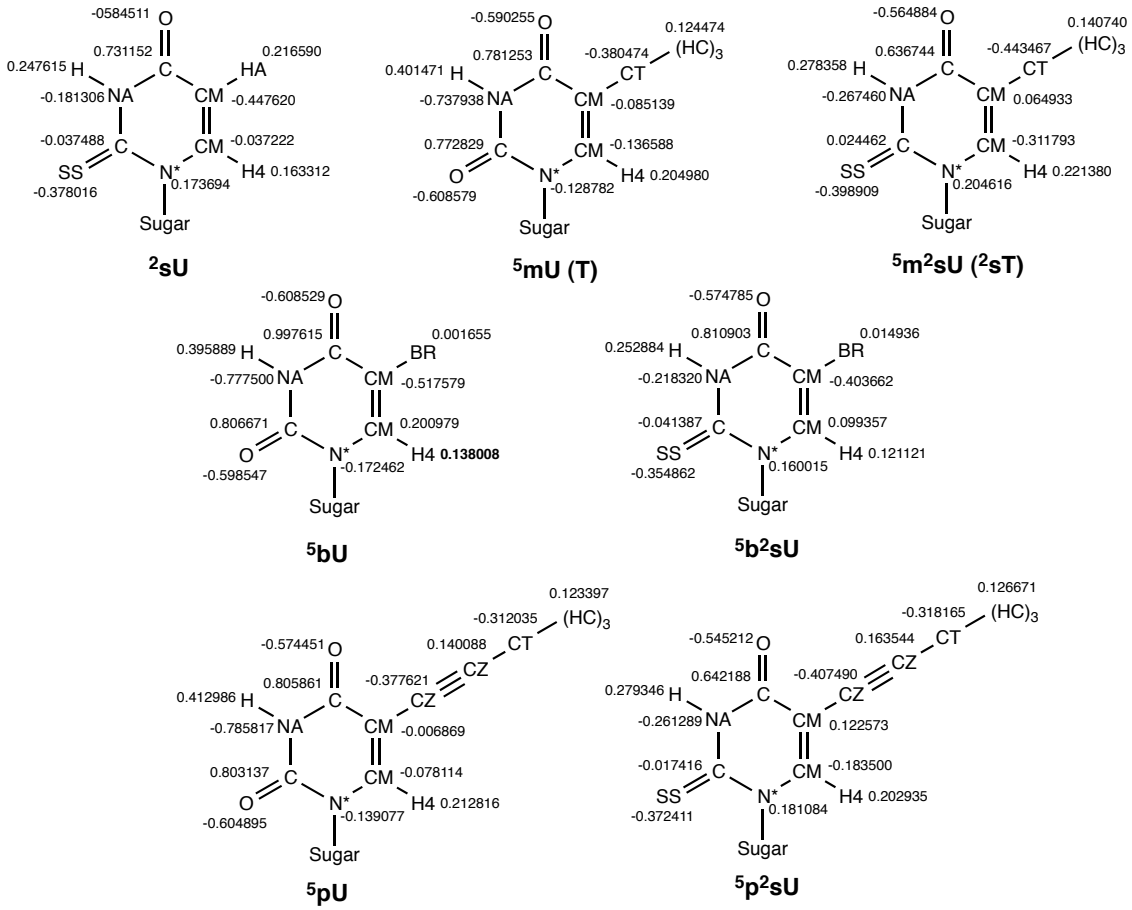


Figure S1. Resp charge distributions and atom types of the modified nucleobases.

```
MASS
CY 12.010       0.360             nitrile C (Howard et al JCC,16,243,1995)
NY 14.010       0.530             nitrile N (Howard et al JCC,16,243,1995)
CZ 12.010       0.360             same as c1
SS 32.060       2.900             same as SS (JTCT 2007 2SU)
BR 79.900       2.880             same as br from GAFF

BOND
CT-CY  400.00   1.458       Howard et al JCC,16,243,1995
CY-NY  600.00   1.150       Howard et al JCC,16,243,1995
C -SS  280.00   1.669       same as CA-SS (JTCT 2007 2SU)
CM-CZ  625.00   1.307       same as c1-c2
CZ-CZ  986.20   1.181       same as c1-c1
CZ-CT  368.30   1.470       same as c1-c3
CM-BR  269.60   1.897       same as br-ca from GAFF

ANGLE
CT-CT-CY   63.000     110.000   Junmei et al, 1999
CT-CY-NY   80.000     180.000   Junmei et al, 1999
CY-CT-H1   50.000     110.000   Junmei et al, 1999
N*-C -SS   67.370     123.932   same as N*-CA-SS (JTCT 2007 2SU)
NA-C -SS   67.370     120.744   same as SS-CA-NA (JTCT 2007 2SU)
C -CM-CZ   67.966     117.825   Calculated with empirical approach
CM-CZ-CZ   60.800     180.000   same as c1-c1-c2
CM-CM-CZ   70.300     121.620   same as c1-c2-c2
CZ-CZ-CT   56.400     177.990   same as c1-c1-c3
CZ-CT-HC   48.300     109.750   same as c1-c3-hcn
C -CM-BR   63.500     118.130   same as br-ca-ca from GAFF
CM-CM-BR   63.500     118.130   same as br-ca-ca from GAFF

DIHE
CT-CT-CY-NY   3    0.000        0.000          1.000      Junmei et al, 1999
H1-CY-CT-NY   3    0.000        0.000          1.000      Junmei et al, 1999
C -CM-CZ-CZ   1    0.000      180.000          2.000      same as X -c1-c2-X
CM-CZ-CZ-CT   1    0.000      180.000          2.000      same as X -c1-c1-X
CM-CM-CZ-CZ   1    0.000      180.000          2.000      same as X -c1-c2-X
CZ-CZ-CT-HC   1    0.000      180.000          2.000      same as X -c1-c3-X

IMPROPER
N*-NA-C -SS        1.1         180.0        2.0         Using default value
C -C -NA-H         1.0         180.0        2.0         General improper torsional angle (2 general atom types)
CM-NA-C -O        10.5         180.0        2.0         General improper torsional angle (2 general atom types)
C -CM-CM-CZ        1.1         180.0        2.0         Using default value
CM-H4-CM-N*        1.1         180.0        2.0         General improper torsional angle (2 general atom types)
BR-C -CM-CM        1.1         180.0        2.0         Using default value

NONBON
  NY          1.8240  0.1700          N in nitrile
  CZ          1.9080  0.0860          same as c1
  SS          2.0000  0.2500          same as SS (JTCT 2007 2SU)
  BR          2.2200  0.3200          same as br from GAFF
```

Figure S2. Additional force field parameters for the modified nucleobases.

Harmonic stiffness analysis

Harmonic stiffness analysis for the helical parameters of nucleic acids, such as twist, roll, tilt, rise, shift and slide, was introduced by Lankas *et al.* and Olson *et al.* to quantitatively analyze the deformability of nucleic acid duplexes[11]. The harmonic stiffness analysis is based on the identical form of the Gauss distribution function (Eq. 1) and Einstein's formula of a harmonic oscillator (Eq. 2).

$$P(x) = N\exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right) \quad (1)$$

$$P(x) = N\exp\left(-\frac{f(x-\mu)^2}{2}\cdot\frac{1}{k_BT}\right) \quad (2)$$

where x: any structural parameter, μ: the average of x, σ: the standard deviation, f: the elastic constant, N: the normalization constant, k_B: Boltzmann's constant, and T: the absolute temperature, respectively. In the simple case, by comparing these two formulas, one can calculate the f value as (Eq. 3) from the standard deviation (σ) obtained by analyzing the distribution of parameter x throughout the molecular dynamic simulation.

$$f = \frac{k_BT}{\sigma^2} \quad (3)$$

In complex molecules, such as DNA duplexes, each of the structural parameters, such as roll, rise, shift, slide, tilt, and twist, are correlated. Therefore, a covariance-variance matrix (***C***) of six parameters (twist, roll, tilt, rise, shift, and slide) was used instead of the σ^2 value.

$$F = k_BTC^{-1} \quad (4)$$

where ***F*** is the stiffness matrix associated with helical deformation at the base pair step.

A global view of helical deformability can be obtained by defining the global translational (F_{trans}), rotational (F_{rot}), and their product (F_{prod}) deformability indexes[12].

$$F_{trans} = f_{rise}f_{shift}f_{slide} \quad (5)$$

$$F_{rot} = f_{twist}f_{roll}f_{tilt} \quad (6)$$

$$F_{prod} = F_{trans}F_{rot} \quad (7)$$

Our assumption here is that the deformability (F_{total}) of a certain sequence could be expressed by simple sum of the deformability (F_{prod}) of each base-pair step like nearest neighbor parameters.

$$F_{total} = \Sigma F_{prod} \quad (8)$$

In this study, the molecular dynamics simulations were carried out using AMBER 9.0, Ptraj modules of AMBER 9.0 and X3DNA[13]. The elastic constants (f) could be calculated under harmonic approximation, as described above.

The stiffness matrix (***F***) associated with helical deformation at the base pair step was determined as the inverse of the covariance-variance matrix (***C***) of six parameters (twist, roll, tilt, rise, shift, and slide),

which were extracted by X3DNA. The diagonal elements of the stiffness matrix were used as the elastic constants of the helical parameters.

We analyzed the parameters of only the central base pairs (omitting terminal 3 base pairs) and averaged same base pair steps to provide more reliable values (Figure S3). We used the last 90 ns of 100.3 trajectory and check the convergence of F_{total} values (Figure S4).

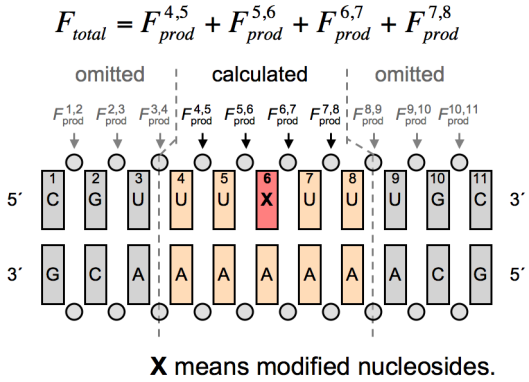


Figure S3. Definition of F_{total} value.

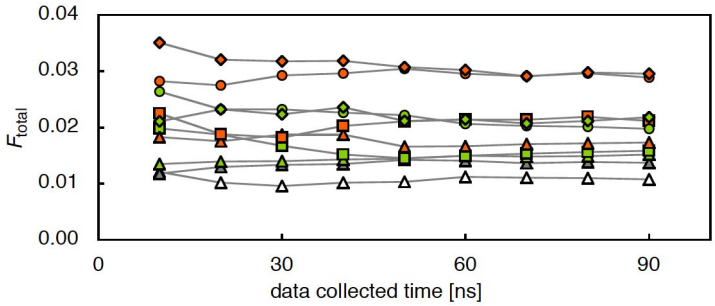


Figure S4. Convergence of F_{total} value during MD simulation. white triangle: $\mathbf{U_{OH}}$, gray triangle: $\mathbf{U_{OMe}}$, green triangle: $\mathbf{U_{OCE}}$, orange triangle: $^{2}\mathbf{sU_{OCE}}$, green square: $^{5}\mathbf{mU_{OCE}}$, orange square: $^{2}\mathbf{s}^{5}\mathbf{mU_{OCE}}$, green circle: $^{5}\mathbf{bU_{OCE}}$, orange circle: $^{2}\mathbf{s}^{5}\mathbf{bU_{OCE}}$, green diamond: $^{5}\mathbf{pU_{OCE}}$, and orange diamond $^{2}\mathbf{s}^{5}\mathbf{pU_{OCE}}$.

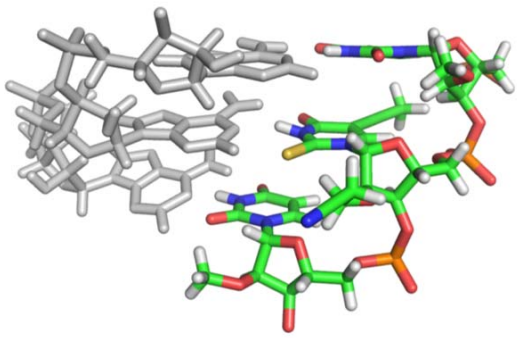


Figure S5. Last snapshot of the $^{2}\mathbf{s}^{5}\mathbf{pU_{OCE}}$ duplex ($\underline{\mathrm{U}}^{2}\mathrm{s}^{5}\mathrm{pU}_{\mathrm{OCE}}\underline{\mathrm{U}}$/AAA). The 2-thiocarbonyl moiety overlapped the 3′-downstream base and the 5-propynyl moiety overlapped the 5′-upstream base.

2´-*O*-modified or unmodified uridine derivatives (90 ns)

U_{OH} 5´ 3´	base pair steps	f_{twist} [kcal·mol^{-1}·deg^{-2}]	f_{roll} [kcal·mol^{-1}·deg^{-2}]	f_{tilt} [kcal·mol^{-1}·deg^{-2}]	f_{rise} [kcal·mol^{-1}·Å^{-2}]	f_{shift} [kcal·mol^{-1}·Å^{-2}]	f_{slide} [kcal·mol^{-1}·Å^{-2}]	F_{rot} [(kcal·mol^{-1}·deg^{-2})^3]	F_{trans} [(kcal·mol^{-1}·Å^{-2})^3]	F_{prod} [(kcal·mol^{-1}·deg^{-1}·Å^{-1})^6]
C-G										
G-C	1-2	0.039	0.013	0.023	3.3	1.1	3.1	0.000011	12	0.0001
U-A	2-3	0.060	0.024	0.036	12.1	1.8	3.1	0.000051	66	0.0034
U-A	3-4	0.065	0.020	0.034	9.4	1.9	3.8	0.000043	66	0.0028
U-A	4-5	0.063	0.020	0.033	9.9	2.0	3.7	0.000041	75	0.0031
X-A	5-6	0.056	0.021	0.030	10.1	1.8	4.2	0.000035	78	0.0027
U-A	6-7	0.066	0.020	0.036	9.4	1.2	4.4	0.000048	50	0.0024
U-A	7-8	0.063	0.019	0.034	8.8	1.6	4.3	0.000041	61	0.0025
U-A	8-9	0.067	0.019	0.035	9.2	1.8	3.1	0.000044	51	0.0023
G-C	9-10	0.058	0.017	0.031	5.8	2.7	4.9	0.000030	77	0.0023
C-G	10-11	0.033	0.022	0.027	10.9	2.4	2.4	0.000019	61	0.0012

U_{OMe} 5´ 3´	base pair steps	f_{twist} [kcal·mol^{-1}·deg^{-2}]	f_{roll} [kcal·mol^{-1}·deg^{-2}]	f_{tilt} [kcal·mol^{-1}·deg^{-2}]	f_{rise} [kcal·mol^{-1}·Å^{-2}]	f_{shift} [kcal·mol^{-1}·Å^{-2}]	f_{slide} [kcal·mol^{-1}·Å^{-2}]	F_{rot} [(kcal·mol^{-1}·deg^{-2})^3]	F_{trans} [(kcal·mol^{-1}·Å^{-2})^3]	F_{prod} [(kcal·mol^{-1}·deg^{-1}·Å^{-1})^6]
C-G										
G-C	1-2	0.020	0.013	0.023	3.3	1.1	3.0	0.000006	11	0.0001
U-A	2-3	0.056	0.024	0.035	12.1	1.8	3.0	0.000048	65	0.0031
U-A	3-4	0.064	0.020	0.035	9.3	1.9	3.9	0.000046	68	0.0031
U-A	4-5	0.067	0.021	0.035	10.2	1.8	4.1	0.000050	78	0.0039
X-A	5-6	0.065	0.021	0.035	10.1	1.9	4.4	0.000049	84	0.0041
U-A	6-7	0.065	0.021	0.034	9.5	1.6	4.5	0.000047	68	0.0032
U-A	7-8	0.064	0.020	0.033	9.0	1.7	4.1	0.000042	62	0.0026
U-A	8-9	0.063	0.020	0.034	9.1	1.8	3.0	0.000043	50	0.0021
G-C	9-10	0.057	0.017	0.031	5.8	2.7	4.7	0.000029	74	0.0022
C-G	10-11	0.032	0.021	0.026	10.7	2.0	2.4	0.000018	51	0.0009

U_{OCE} 5´ 3´	base pair steps	f_{twist} [kcal·mol^{-1}·deg^{-2}]	f_{roll} [kcal·mol^{-1}·deg^{-2}]	f_{tilt} [kcal·mol^{-1}·deg^{-2}]	f_{rise} [kcal·mol^{-1}·Å^{-2}]	f_{shift} [kcal·mol^{-1}·Å^{-2}]	f_{slide} [kcal·mol^{-1}·Å^{-2}]	F_{rot} [(kcal·mol^{-1}·deg^{-2})^3]	F_{trans} [(kcal·mol^{-1}·Å^{-2})^3]	F_{prod} [(kcal·mol^{-1}·deg^{-1}·Å^{-1})^6]
C-G										
G-C	1-2	0.027	0.012	0.014	3.2	0.9	2.9	0.000005	8	0.0000
U-A	2-3	0.061	0.024	0.035	12.3	1.7	3.0	0.000051	65	0.0033
U-A	3-4	0.067	0.021	0.035	9.9	1.8	4.0	0.000050	72	0.0036
U-A	4-5	0.067	0.021	0.036	10.3	1.8	4.3	0.000049	80	0.0039
X-A	5-6	0.065	0.021	0.035	10.1	2.0	4.6	0.000047	90	0.0043
U-A	6-7	0.068	0.021	0.036	10.0	1.9	4.3	0.000052	80	0.0041
U-A	7-8	0.064	0.020	0.033	9.1	1.7	4.1	0.000043	64	0.0028
U-A	8-9	0.067	0.020	0.035	9.3	1.7	3.2	0.000046	51	0.0023
G-C	9-10	0.060	0.017	0.031	5.9	2.9	5.2	0.000032	89	0.0028
C-G	10-11	0.034	0.022	0.027	11.0	2.4	2.3	0.000020	61	0.0012

5-modified uridine derivatives (90 ns)

^{5}mU$_{OCE}$ 5′ 3′	base pair steps	f_{twist} [kcal·mol^{-1}·deg^{-2}]	f_{roll} [kcal·mol^{-1}·deg^{-2}]	f_{tilt} [kcal·mol^{-1}·deg^{-2}]	f_{rise} [kcal·mol^{-1}·Å^{-2}]	f_{shift} [kcal·mol^{-1}·Å^{-2}]	f_{slide} [kcal·mol^{-1}·Å^{-2}]	F_{rot} [(kcal·mol^{-1}·deg^{-2})^{3}]	F_{trans} [(kcal·mol^{-1}·Å^{-2})^{3}]	F_{prod} [(kcal·mol^{-1}·deg^{-1}·Å^{-1})^{6}]
C-G										
G-C	1-2	0.037	0.013	0.022	3.3	1.1	2.9	0.000010	11	0.0001
U-A	2-3	0.061	0.023	0.036	12.1	1.7	3.2	0.000050	66	0.0033
U-A	3-4	0.068	0.019	0.036	9.9	1.9	4.0	0.000048	76	0.0036
U-A	4-5	0.061	0.020	0.036	10.2	2.2	4.6	0.000044	101	0.0044
X-A	5-6	0.065	0.024	0.043	9.4	1.7	4.8	0.000066	75	0.0050
U-A	6-7	0.069	0.022	0.036	10.1	1.7	4.2	0.000054	70	0.0038
U-A	7-8	0.063	0.020	0.033	9.0	1.8	4.0	0.000041	64	0.0026
U-A	8-9	0.063	0.020	0.034	9.4	1.9	3.2	0.000044	57	0.0025
G-C	9-10	0.058	0.018	0.031	6.1	2.9	5.2	0.000031	92	0.0029
C-G	10-11	0.033	0.021	0.026	10.9	2.2	2.4	0.000019	56	0.0010

^{5}bU$_{OCE}$ 5′ 3′	base pair steps	f_{twist} [kcal·mol^{-1}·deg^{-2}]	f_{roll} [kcal·mol^{-1}·deg^{-2}]	f_{tilt} [kcal·mol^{-1}·deg^{-2}]	f_{rise} [kcal·mol^{-1}·Å^{-2}]	f_{shift} [kcal·mol^{-1}·Å^{-2}]	f_{slide} [kcal·mol^{-1}·Å^{-2}]	F_{rot} [(kcal·mol^{-1}·deg^{-2})^{3}]	F_{trans} [(kcal·mol^{-1}·Å^{-2})^{3}]	F_{prod} [(kcal·mol^{-1}·deg^{-1}·Å^{-1})^{6}]
C-G										
G-C	1-2	0.000	0.003	0.005	0.4	1.2	1.5	0.000000	1	0.0000
U-A	2-3	0.058	0.024	0.035	12.3	1.7	3.4	0.000050	69	0.0035
U-A	3-4	0.068	0.020	0.037	9.7	2.2	4.2	0.000051	91	0.0047
U-A	4-5	0.069	0.021	0.037	10.4	2.2	4.9	0.000054	113	0.0060
X-A	5-6	0.067	0.024	0.045	9.5	1.8	5.5	0.000071	92	0.0065
U-A	6-7	0.073	0.023	0.037	10.4	1.8	4.5	0.000062	84	0.0053
U-A	7-8	0.063	0.019	0.033	8.7	1.4	3.9	0.000041	47	0.0019
U-A	8-9	0.066	0.020	0.034	9.1	1.7	2.9	0.000044	45	0.0020
G-C	9-10	0.056	0.017	0.030	5.8	2.8	4.5	0.000029	72	0.0021
C-G	10-11	0.033	0.022	0.026	10.8	2.3	2.3	0.000019	56	0.0011

^{5}pU$_{OCE}$ 5′ 3′	base pair steps	f_{twist} [kcal·mol^{-1}·deg^{-2}]	f_{roll} [kcal·mol^{-1}·deg^{-2}]	f_{tilt} [kcal·mol^{-1}·deg^{-2}]	f_{rise} [kcal·mol^{-1}·Å^{-2}]	f_{shift} [kcal·mol^{-1}·Å^{-2}]	f_{slide} [kcal·mol^{-1}·Å^{-2}]	F_{rot} [(kcal·mol^{-1}·deg^{-2})^{3}]	F_{trans} [(kcal·mol^{-1}·Å^{-2})^{3}]	F_{prod} [(kcal·mol^{-1}·deg^{-1}·Å^{-1})^{6}]
C-G										
G-C	1-2	0.033	0.013	0.020	3.2	0.8	2.9	0.000008	8	0.0001
U-A	2-3	0.056	0.024	0.035	12.0	1.5	3.0	0.000046	53	0.0025
U-A	3-4	0.068	0.020	0.037	9.7	1.7	4.1	0.000049	68	0.0033
U-A	4-5	0.071	0.021	0.037	10.1	2.2	4.8	0.000055	105	0.0057
X-A	5-6	0.076	0.025	0.048	10.0	1.6	5.6	0.000091	92	0.0083
U-A	6-7	0.071	0.023	0.037	10.7	1.9	4.4	0.000060	88	0.0053
U-A	7-8	0.062	0.020	0.033	9.0	1.5	4.4	0.000040	61	0.0024
U-A	8-9	0.068	0.019	0.034	9.2	1.6	3.5	0.000045	53	0.0024
G-C	9-10	0.058	0.017	0.031	5.9	2.9	5.2	0.000031	88	0.0027
C-G	10-11	0.033	0.021	0.026	10.7	2.3	2.3	0.000019	57	0.0011

2-thio-5-modified or unmodified uridine derivatives (90 ns)

^{2}sU$_{OCE}$ 5′ 3′	base pair steps	f_{twist} [kcal·mol^{-1}·deg^{-2}]	f_{roll} [kcal·mol^{-1}·deg^{-2}]	f_{tilt} [kcal·mol^{-1}·deg^{-2}]	f_{rise} [kcal·mol^{-1}·Å^{-2}]	f_{shift} [kcal·mol^{-1}·Å^{-2}]	f_{slide} [kcal·mol^{-1}·Å^{-2}]	F_{rot} [(kcal·mol^{-1}·deg^{-2})^3]	F_{trans} [(kcal·mol^{-1}·Å^{-2})^3]	F_{prod} [(kcal·mol^{-1}·deg^{-1}·Å^{-1})^6]
C-G										
G-C	1-2	0.040	0.013	0.023	3.3	1.1	3.0	0.000012	11	0.0001
U-A	2-3	0.062	0.024	0.036	12.3	1.7	3.2	0.000054	68	0.0037
U-A	3-4	0.067	0.021	0.035	9.7	1.9	4.0	0.000049	73	0.0036
U-A	4-5	0.067	0.021	0.036	10.5	1.9	4.3	0.000050	86	0.0043
X-A	5-6	0.064	0.022	0.035	10.0	2.0	4.7	0.000049	94	0.0046
U-A	6-7	0.068	0.024	0.037	10.7	2.2	4.2	0.000059	102	0.0060
U-A	7-8	0.063	0.020	0.033	9.0	1.6	4.2	0.000041	59	0.0024
U-A	8-9	0.066	0.019	0.034	9.1	1.7	3.4	0.000043	53	0.0023
G-C	9-10	0.055	0.017	0.030	5.7	2.5	4.8	0.000028	69	0.0019
C-G	10-11	0.034	0.021	0.026	10.8	2.2	2.2	0.000019	54	0.0010

^{2}s^{5}mU$_{OCE}$ 5′ 3′	base pair steps	f_{twist} [kcal·mol^{-1}·deg^{-2}]	f_{roll} [kcal·mol^{-1}·deg^{-2}]	f_{tilt} [kcal·mol^{-1}·deg^{-2}]	f_{rise} [kcal·mol^{-1}·Å^{-2}]	f_{shift} [kcal·mol^{-1}·Å^{-2}]	f_{slide} [kcal·mol^{-1}·Å^{-2}]	F_{rot} [(kcal·mol^{-1}·deg^{-2})^3]	F_{trans} [(kcal·mol^{-1}·Å^{-2})^3]	F_{prod} [(kcal·mol^{-1}·deg^{-1}·Å^{-1})^6]
C-G										
G-C	1-2	0.007	0.014	0.022	3.7	0.7	2.7	0.000002	7	0.0000
U-A	2-3	0.058	0.024	0.035	12.3	1.8	3.2	0.000049	69	0.0034
U-A	3-4	0.071	0.020	0.036	9.5	1.9	4.2	0.000051	75	0.0038
U-A	4-5	0.070	0.019	0.038	10.6	2.3	4.8	0.000052	115	0.0060
X-A	5-6	0.066	0.025	0.044	9.7	2.1	5.1	0.000071	102	0.0072
U-A	6-7	0.071	0.024	0.037	10.7	2.1	3.9	0.000063	89	0.0056
U-A	7-8	0.061	0.019	0.033	8.8	1.7	3.9	0.000039	60	0.0024
U-A	8-9	0.067	0.020	0.035	9.3	1.8	3.1	0.000047	54	0.0025
G-C	9-10	0.059	0.019	0.030	5.9	3.0	5.2	0.000033	91	0.0030
C-G	10-11	0.029	0.021	0.026	10.6	2.4	2.4	0.000016	60	0.0010

^{2}s^{5}bU$_{OCE}$ 5′ 3′	base pair steps	f_{twist} [kcal·mol^{-1}·deg^{-2}]	f_{roll} [kcal·mol^{-1}·deg^{-2}]	f_{tilt} [kcal·mol^{-1}·deg^{-2}]	f_{rise} [kcal·mol^{-1}·Å^{-2}]	f_{shift} [kcal·mol^{-1}·Å^{-2}]	f_{slide} [kcal·mol^{-1}·Å^{-2}]	F_{rot} [(kcal·mol^{-1}·deg^{-2})^3]	F_{trans} [(kcal·mol^{-1}·Å^{-2})^3]	F_{prod} [(kcal·mol^{-1}·deg^{-1}·Å^{-1})^6]
C-G										
G-C	1-2	0.031	0.009	0.014	3.1	0.8	2.7	0.000004	7	0.0000
U-A	2-3	0.057	0.025	0.036	12.2	1.8	3.0	0.000050	67	0.0033
U-A	3-4	0.067	0.021	0.037	9.7	2.1	4.2	0.000051	88	0.0044
U-A	4-5	0.074	0.021	0.038	10.4	2.3	5.0	0.000060	120	0.0072
X-A	5-6	0.070	0.025	0.046	9.7	2.2	5.9	0.000082	127	0.0104
U-A	6-7	0.076	0.026	0.039	11.4	2.4	4.3	0.000076	116	0.0088
U-A	7-8	0.064	0.020	0.033	9.0	1.5	4.3	0.000041	59	0.0024
U-A	8-9	0.067	0.019	0.035	9.1	1.7	3.5	0.000045	54	0.0024
G-C	9-10	0.058	0.017	0.031	5.9	2.8	5.2	0.000031	87	0.0027
C-G	10-11	0.033	0.020	0.025	9.0	2.3	1.8	0.000016	38	0.0006

^{2}s^{5}pU$_{OCE}$ 5′ 3′	base pair steps	f_{twist} [kcal·mol^{-1}·deg^{-2}]	f_{roll} [kcal·mol^{-1}·deg^{-2}]	f_{tilt} [kcal·mol^{-1}·deg^{-2}]	f_{rise} [kcal·mol^{-1}·Å^{-2}]	f_{shift} [kcal·mol^{-1}·Å^{-2}]	f_{slide} [kcal·mol^{-1}·Å^{-2}]	F_{rot} [(kcal·mol^{-1}·deg^{-2})^3]	F_{trans} [(kcal·mol^{-1}·Å^{-2})^3]	F_{prod} [(kcal·mol^{-1}·deg^{-1}·Å^{-1})^6]
C-G										
G-C	1-2	0.033	0.012	0.020	3.3	0.9	2.9	0.000008	8	0.0001
U-A	2-3	0.061	0.025	0.035	12.3	1.7	3.1	0.000053	65	0.0035
U-A	3-4	0.064	0.020	0.037	9.8	1.7	4.2	0.000048	69	0.0033
U-A	4-5	0.074	0.021	0.038	10.4	2.1	4.7	0.000061	104	0.0063
X-A	5-6	0.081	0.027	0.049	10.5	2.1	5.9	0.000106	133	0.0142
U-A	6-7	0.073	0.025	0.038	11.2	2.2	4.1	0.000069	103	0.0071
U-A	7-8	0.063	0.019	0.032	8.9	1.5	3.7	0.000039	50	0.0020
U-A	8-9	0.067	0.020	0.034	9.3	1.9	2.7	0.000045	48	0.0022
G-C	9-10	0.057	0.018	0.031	6.1	3.1	4.9	0.000031	93	0.0029
C-G	10-11	0.034	0.022	0.026	10.8	2.4	2.4	0.000019	63	0.0012

Results of the sum of each value (excluded terminal 3 base-pairs)

	Σf_{twist} [kcal·mol^{-1}·deg^{-2}]	Σf_{roll} [kcal·mol^{-1}·deg^{-2}]	Σf_{tilt} [kcal·mol^{-1}·deg^{-2}]	Σf_{rise} [kcal·mol^{-1}·Å^{-2}]	Σf_{shift} [kcal·mol^{-1}·Å^{-2}]	Σf_{slide} [kcal·mol^{-1}·Å^{-2}]	ΣF_{rot} [(kcal·mol^{-1}·deg^{-2})^3]	ΣF_{trans} [(kcal·mol^{-1}·Å^{-2})^3]	F_{total} [(kcal·mol^{-1}·deg^{-1}·Å^{-1})^6]
U_{OH}	0.247	0.080	0.134	38.2	6.7	16.7	0.00017	266	0.011
U_{OMe}	0.261	0.083	0.138	38.8	7.0	17.1	0.00019	291	0.014
U_{OCE}	0.265	0.083	0.140	39.5	7.4	17.3	0.00019	315	0.015
$^5mU_{OCE}$	0.257	0.085	0.149	38.6	7.3	17.6	0.00021	310	0.016
$^5bU_{OCE}$	0.273	0.087	0.152	39.0	7.2	18.9	0.00023	337	0.020
$^5pU_{OCE}$	0.280	0.088	0.155	39.8	7.2	19.2	0.00025	345	0.022
$^2sU_{OCE}$	0.262	0.086	0.140	40.2	7.7	17.5	0.00020	341	0.017
$^2s^5mU_{OCE}$	0.268	0.087	0.153	39.8	8.2	17.7	0.00023	365	0.021
$^2s^5bU_{OCE}$	0.284	0.092	0.157	40.5	8.4	19.6	0.00026	421	0.029
$^2s^5pU_{OCE}$	0.292	0.092	0.157	41.1	8.0	18.4	0.00027	391	0.030

References

(1) a) Cheatham, T. E. III; Cieplak, P.; Kollman, P. A. *J. Biomol. Struct. Dyn.* **1999**, *16*,845–862. b) Perez, A.; Marchan, I.; Svozil, D.; Sponer, J.; Cheatham, T. E. III; Laughton, C. A.; Orozco, M. *Biophys. J.* **2007**, *92*, 3817–3829.

(2) Case, D. A.; Darden, T. A.; Cheatham, T. E. III; Simmerling, C. L.; Wang, J.; Duke, R. E.; Luo, R.; Merz, K. M.; Pearlman, D. A.; Crowley, M.; Walker, R. C.; Zhang, W.; Wang, B.; Hayik, S.; Roitberg, A.; Seabra, G.; Wong, K. F.; Paesani, F.; Wu, X.; Brozell, S.; Tsui, V.; Gohlke, H.; Yang, L.; Tan, C.; Mongan, J.; Hornak, V.; Cui, G.; Beroza, P.; Mathews, D. H.; Schafmeister, C.; Ross, W. S.; Kollman P. A. (2006), AMBER 9, University of California, San Francisco.

(3) Auffinger, P.; Westhof, E. *Angew. Chem. Int. Ed.* **2001**, *40*, 4648-4650.

(4) Masaki, Y.; Miyasaka, R.; Ohkubo, A.; Seio, K.; Sekine, M. *J. Phys. Chem. B* **2010**, *114*, 2517-2524.

(5) a) Bayly, C. I.; Cieplak, P.; Cornell, W.; Kollman, P. A. *J. Phys. Chem.* **1993**, *97*, 10269–10280. b) Aduri, R.; Psciuk, B. T.; Saro, P.; Taniga, H.; Schlegel, H. B.; Santa Lucia, J. Jr. *J. Chem. Theory and Comput.* **2007**, *3*, 1464 -1475. c) Zhang, Q.; Schlick, T. *Biophys. J.* **2006**, *90*, 1865-1877.

(6) Wang, J.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A. *J. Comput. Chem.* **2004**, *25*, 1157-1174.

(7) Joung, I. S.; Cheatham, T. E. III *J. Phys. Chem. B* **2008**, 112, 9020-9041.

(8) Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; DiNola, A.; Haak, J. R. *J. Chem. Phys.* **1984**, *81*, 3684-3690.

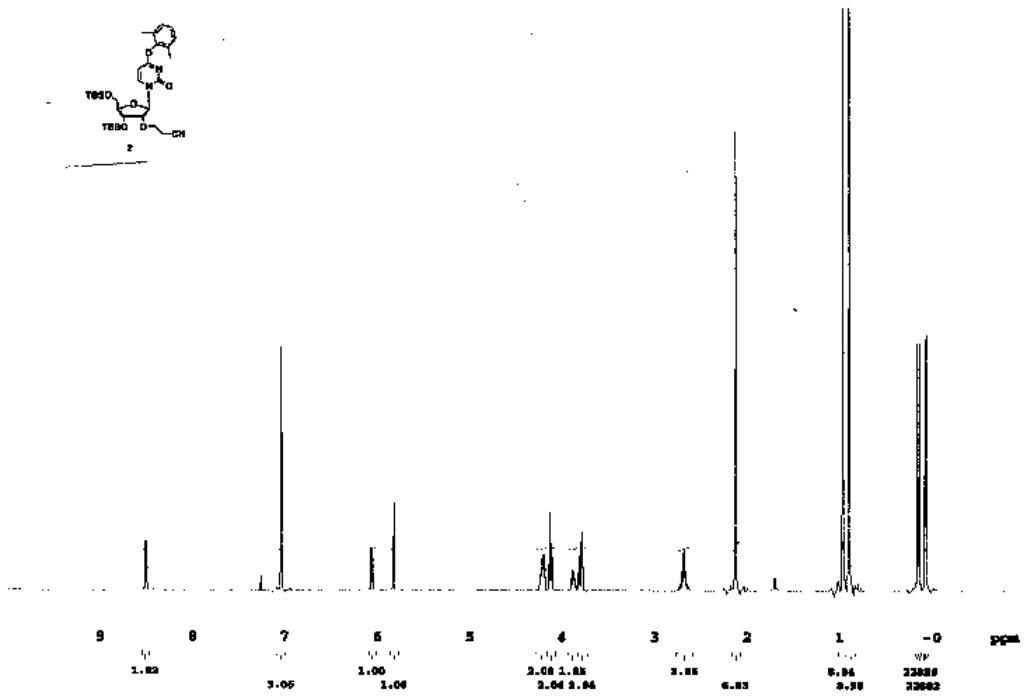
(9) Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C. *J. Comput. Phys.* **1977**, *23*, 327-341.

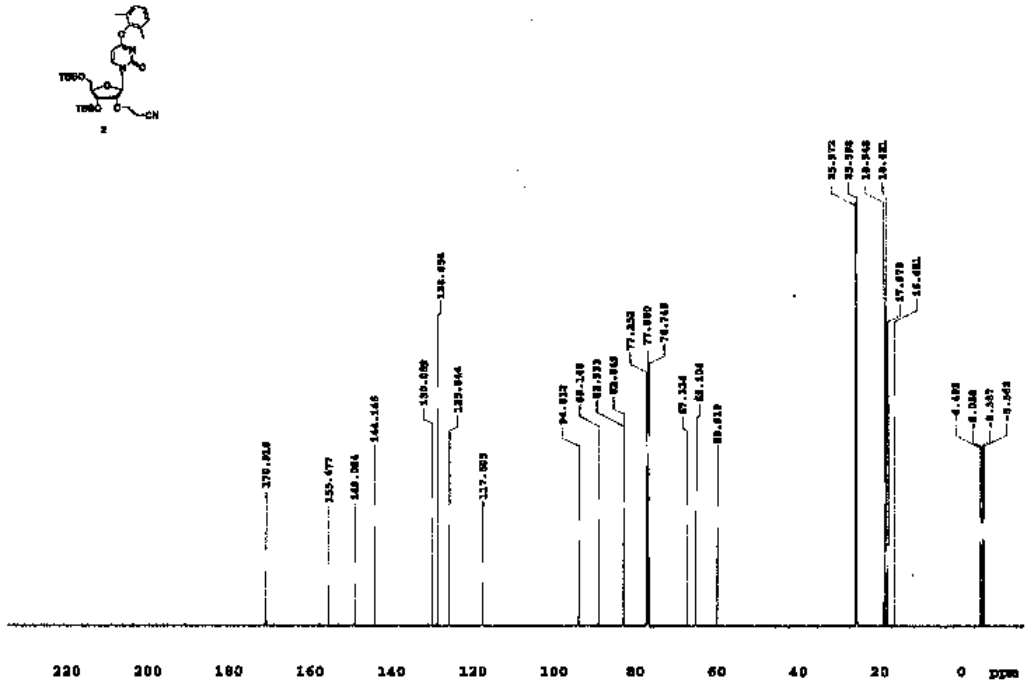
(10) Darden, T.; York, D.; Pedersen, L. *J. Chem. Phys.* **1993**, *98*, 10089-10092.

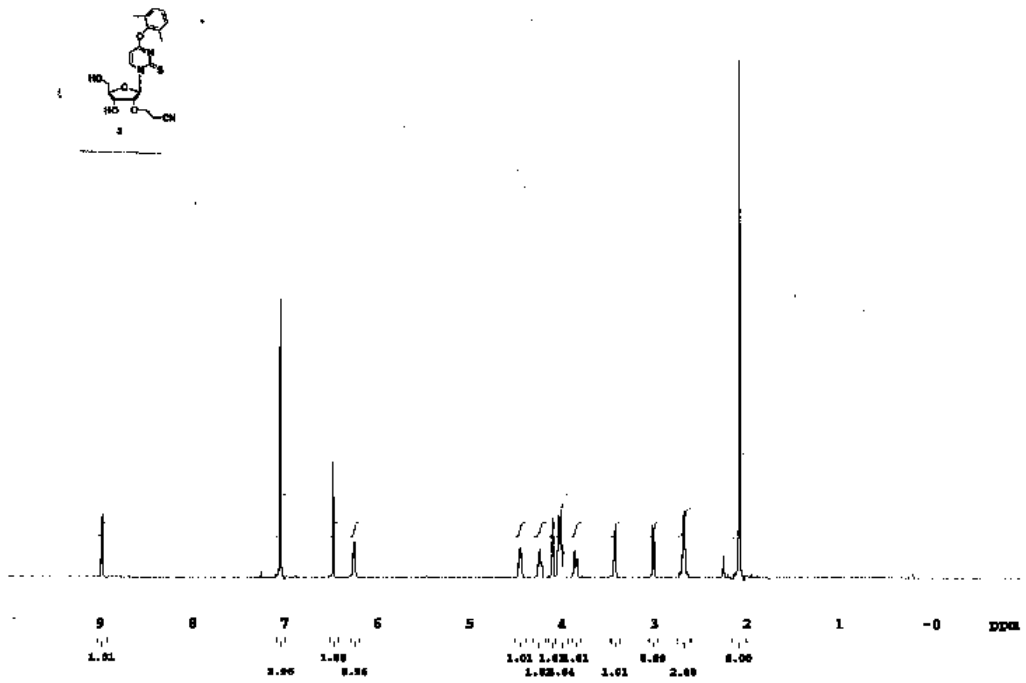
(11) a) Olson, W. K.; Gorin, A. A.; Lu, X. J.; Hock, L. M.; Zhurkin, V. B. *Proc. Natl. Acad. Sci. USA.* **1998**, *95*, 11163-11168. b) Lankas, F.; Sponer, J.; Langowski, J.; Cheatham, T. E. III. *Biophys. J.* **2003**, *85***,** 2872-2883. c) Lankas, F. *Biopolymers* **2004**, *73*, 327-339.

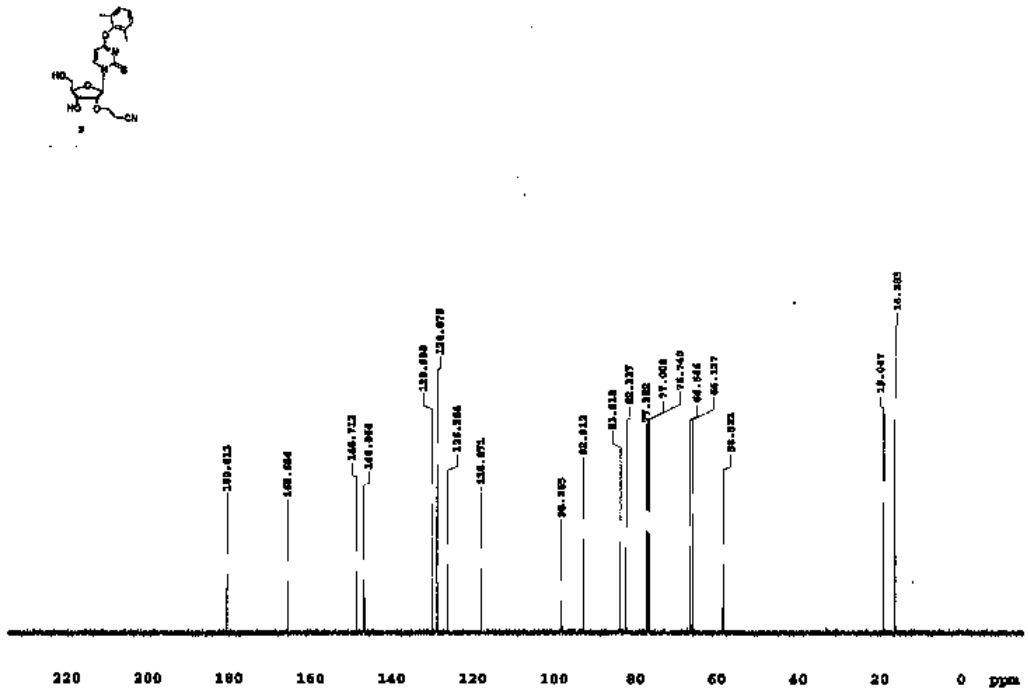
(12) Noy, A.; Luque, F. J.; Orozco, M. *J. Am. Chem. Soc.* **2008**, *130*, 3486-3496.

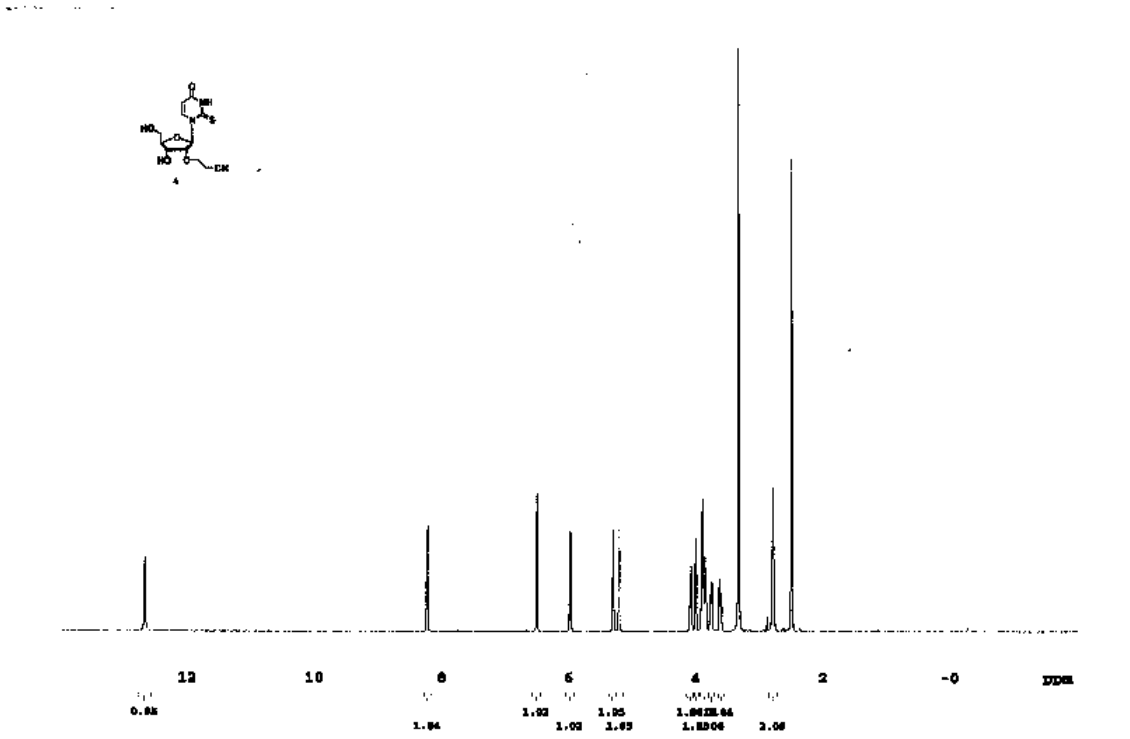
(13) Lu, X. J. ; Olson, W. K. *Nucleic Acids Res.* **2003**, *31*, 5108-5121.

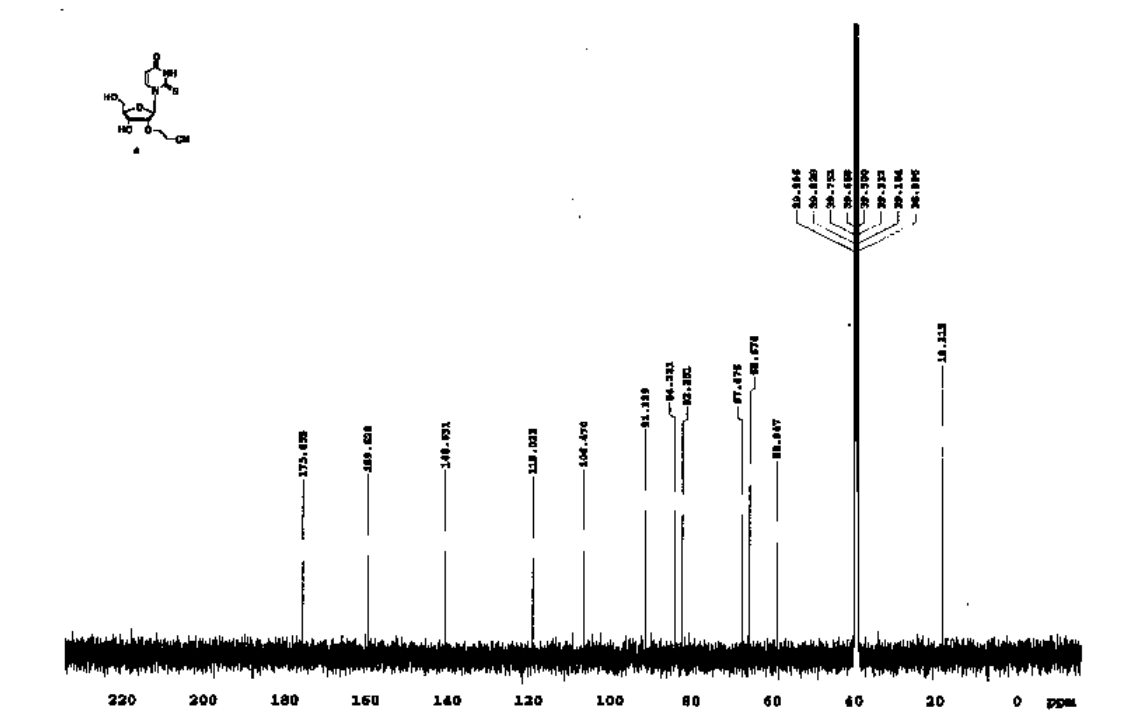

9
8
7
6
5
4
3
2
1
0
ppm

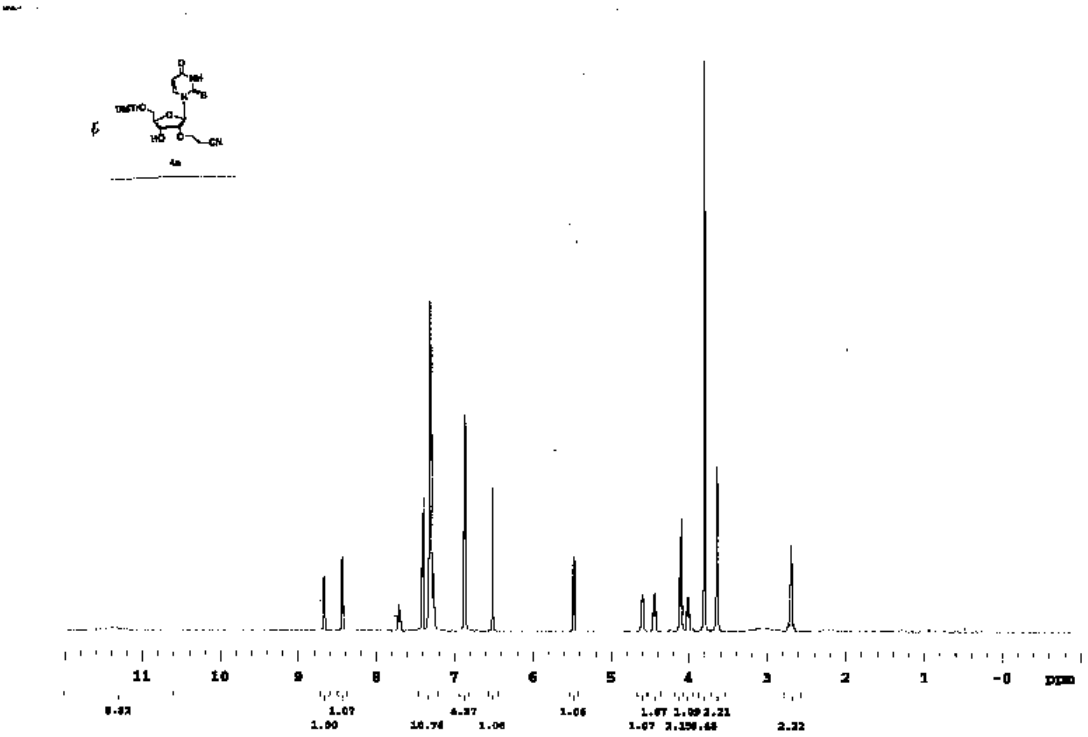

220
200
180
160
140
120
100
80
60
40
20
0
ppm

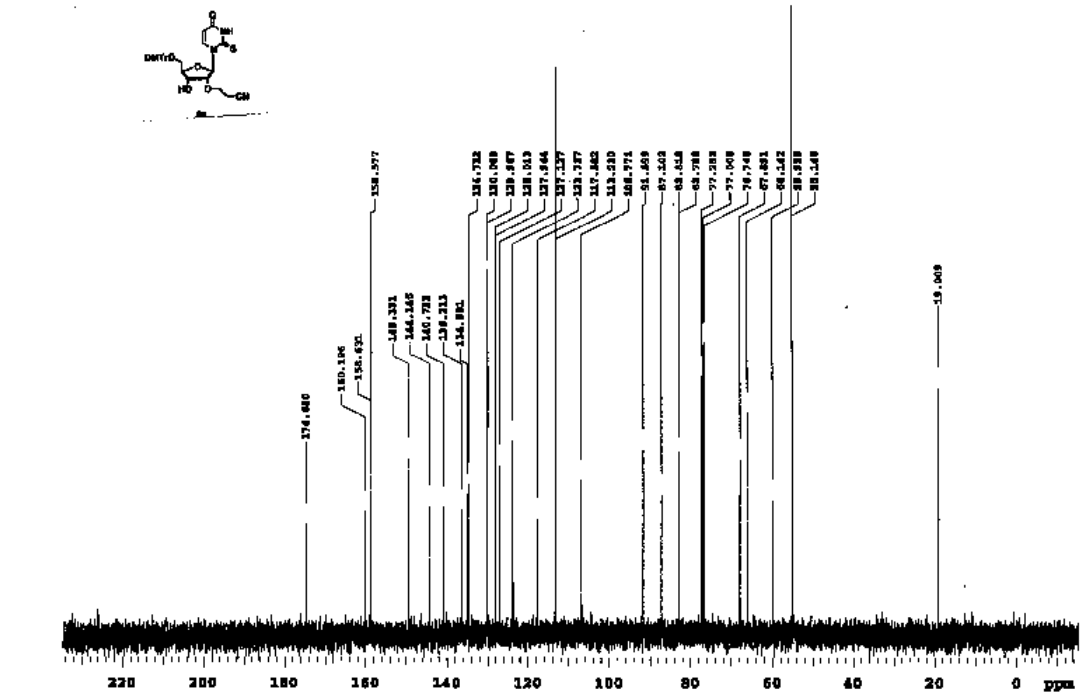

9
8
7
6
5
4
3
2
1
-0
ppm

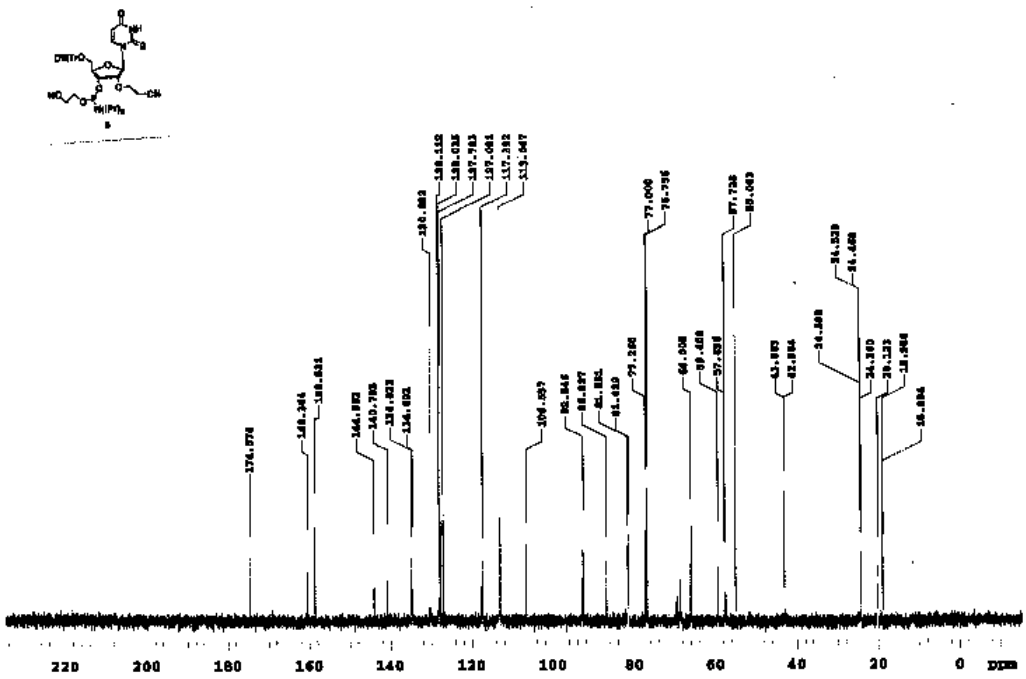

220
200
180
160
140
120
100
80
60
40
20
0
ppm

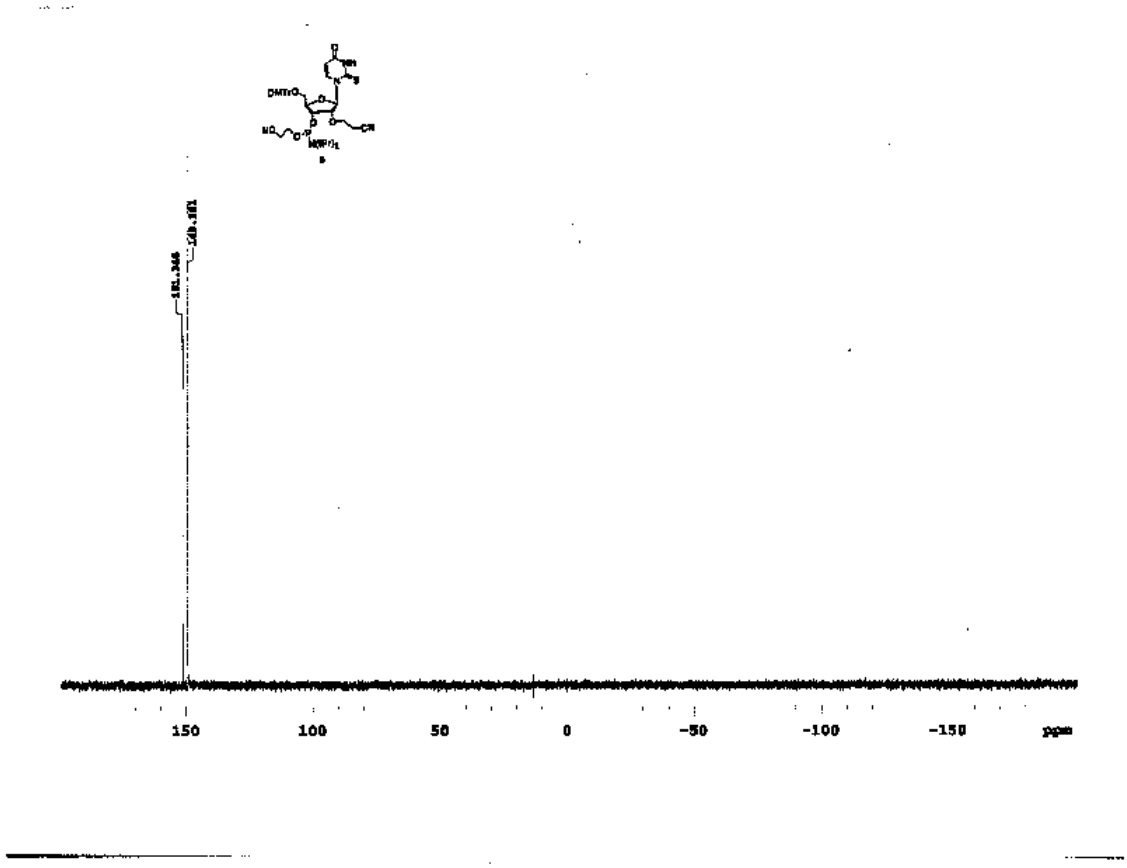

12
10
8
6
4
2
-0
ppm

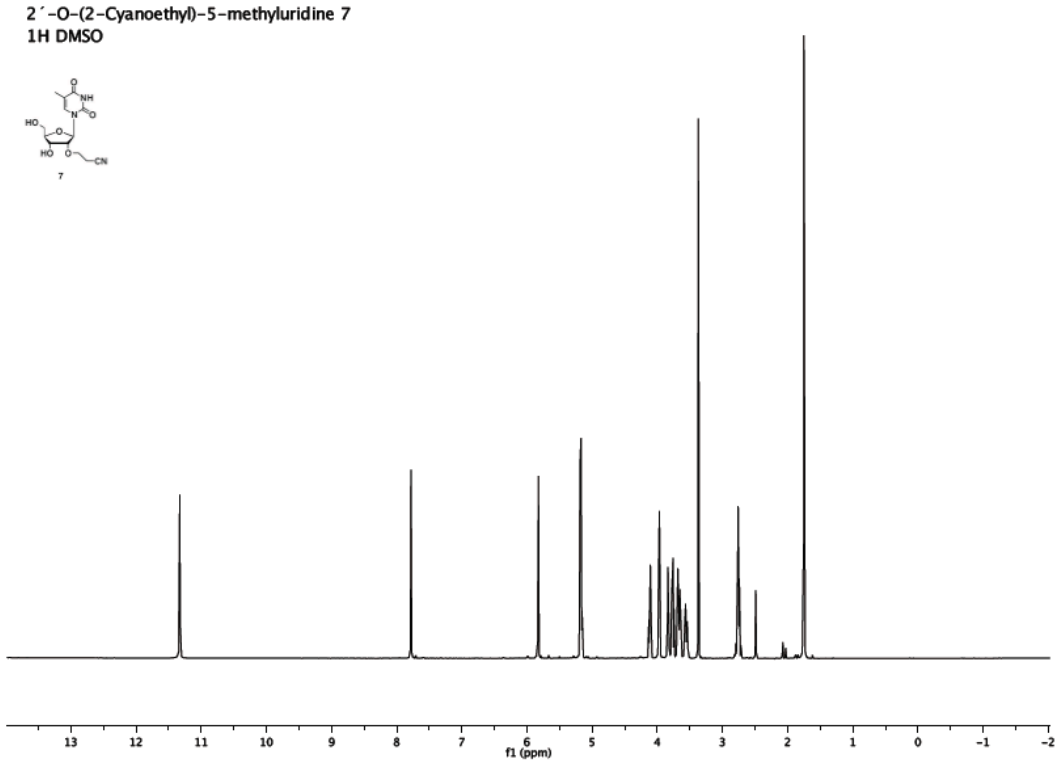

220
200
180
160
140
120
100
80
60
40
20
0
ppm

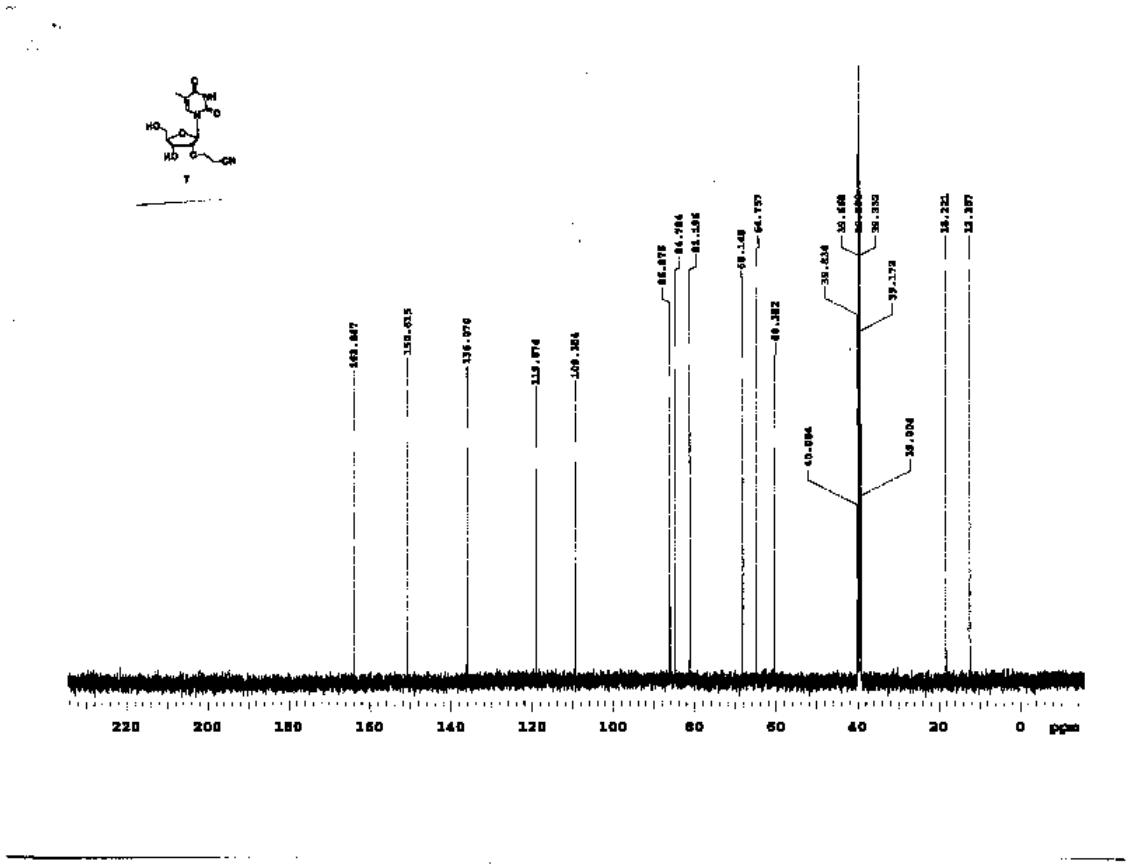

11
10
9
8
7
6
5
4
3
2
1
-0
ppm


220
200
180
160
140
120
100
80
60
40
20
0
ppm

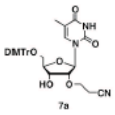

220
200
180
160
140
120
100
80
60
40
20
0
ppm

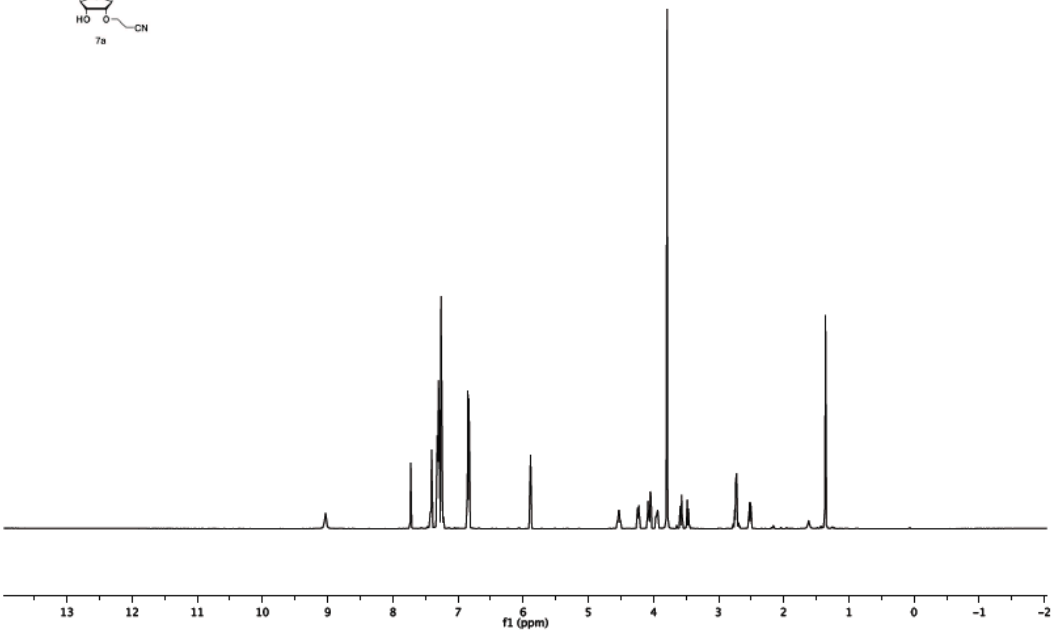



2´-O-(2-Cyanoethyl)-5-methyluridine 7
1H DMSO
f1 (ppm)

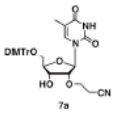

ppm

2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-5-methyluridine 7a
1H $CDCl_3$

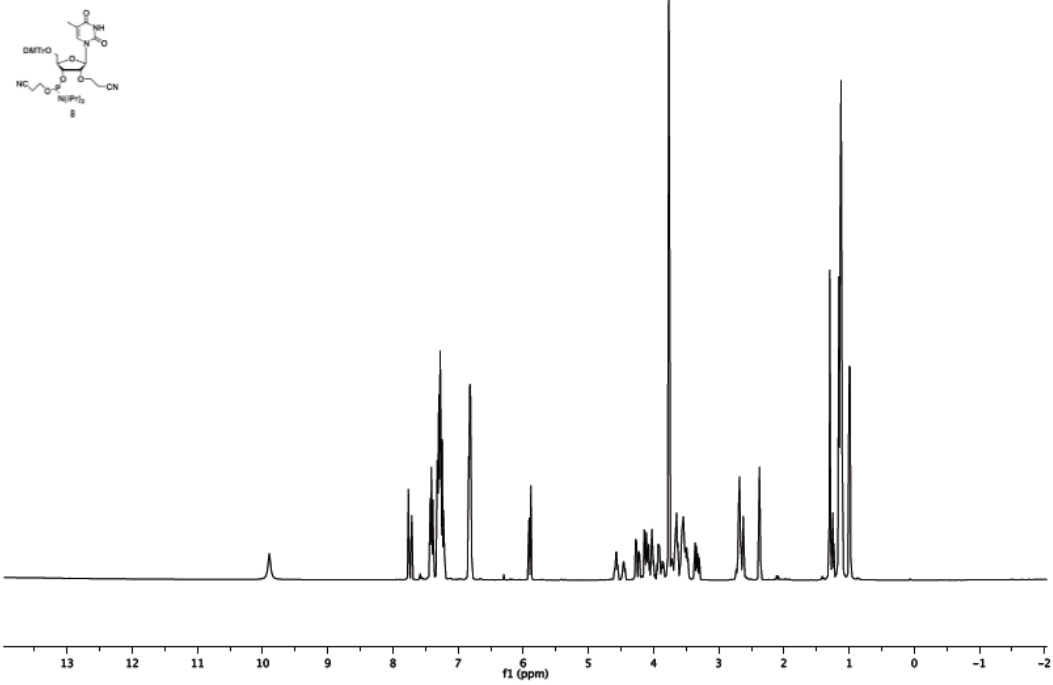




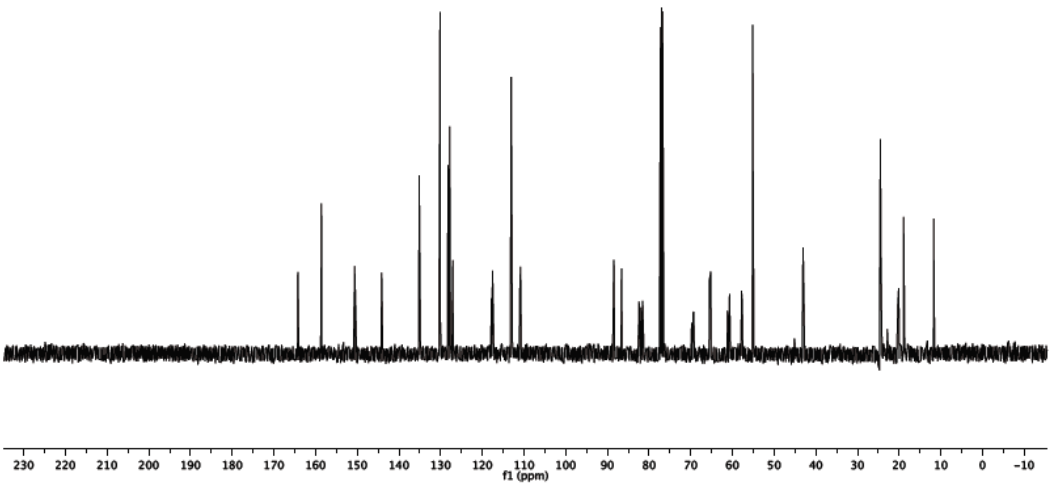
2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-5-methyluridine 7a
13C $CDCl_3$



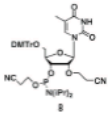
2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-3´-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-5-methyluridine 8
1H $CDCl_3$

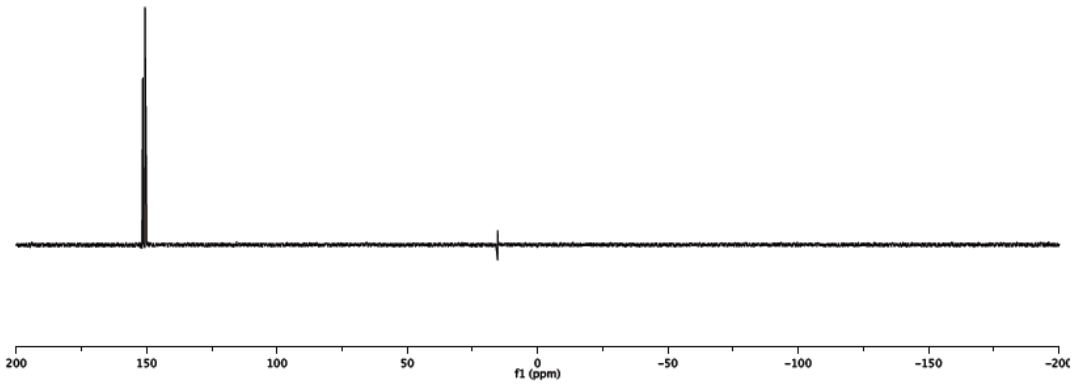


2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-3´-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-5-methyluridine 8
13C $CDCl_3$

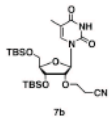


2′-O-(2-Cyanoethyl)-5′-O-(4,4′-dimethoxytrityl)-3′-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-5-methyluridine 8
31P $CDCl_3$



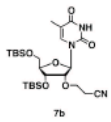


2´-O-(2-Cyanoethyl)-3´,5´-O-bis-(tert-butyldimethylsilyl)-5-methyluridine 7b
1H $CDCl_3$

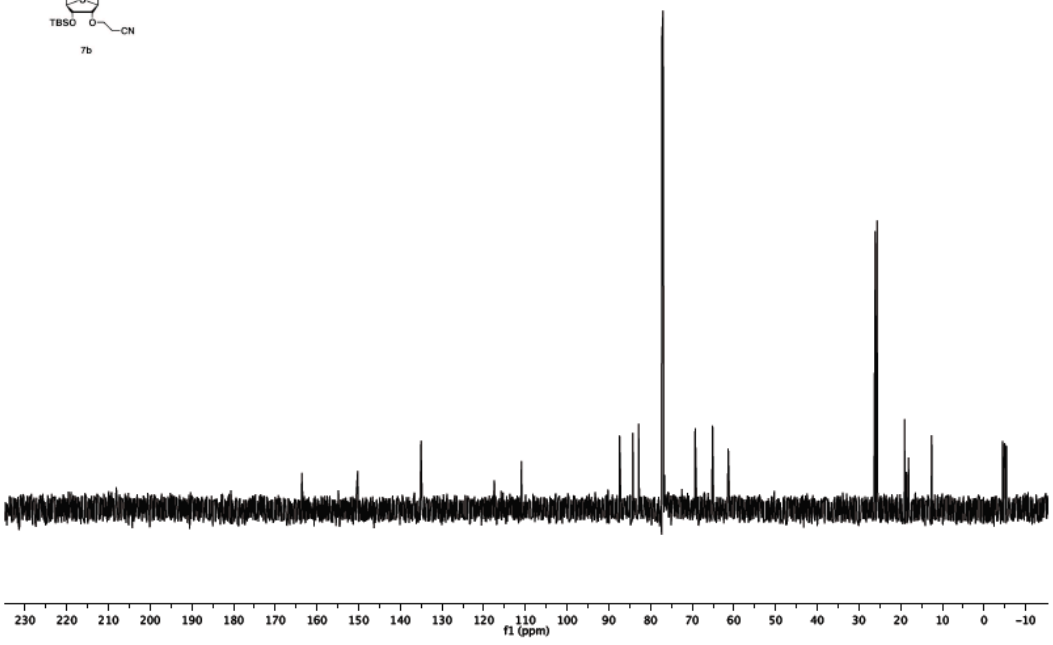


7b

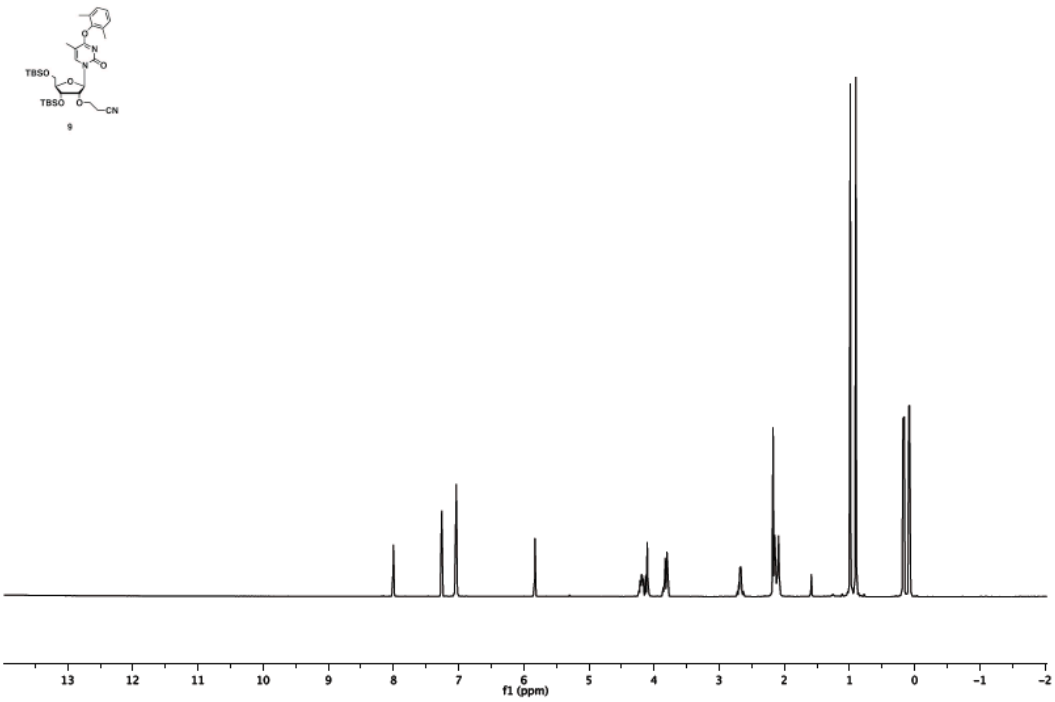
2´-O-(2-Cyanoethyl)-3´,5´-O-bis-(tert-butyldimethylsilyl)-5-methyluridine 7b
13C $CDCl_3$



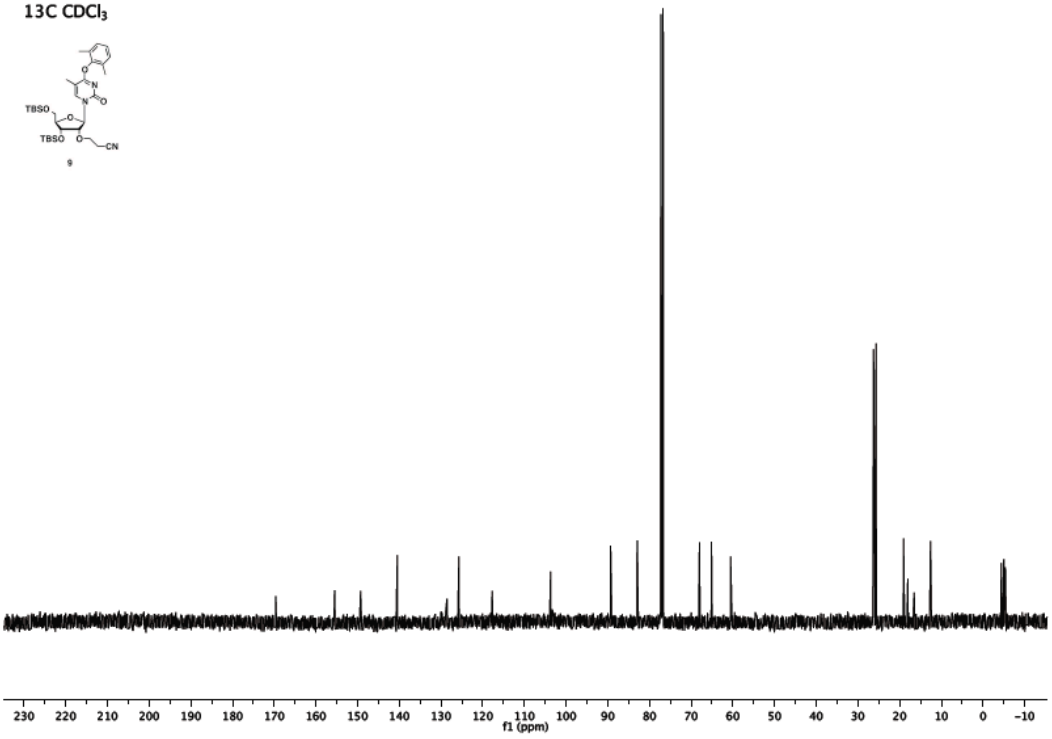
7b



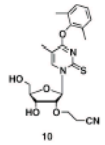
2´-O-(2-Cyanoethyl)-3´,5´-O-bis-(tert-butyldimethylsilyl)-4-O-(2,6-dimethylphenyl)-5-methyluridine 9
1H $CDCl_3$

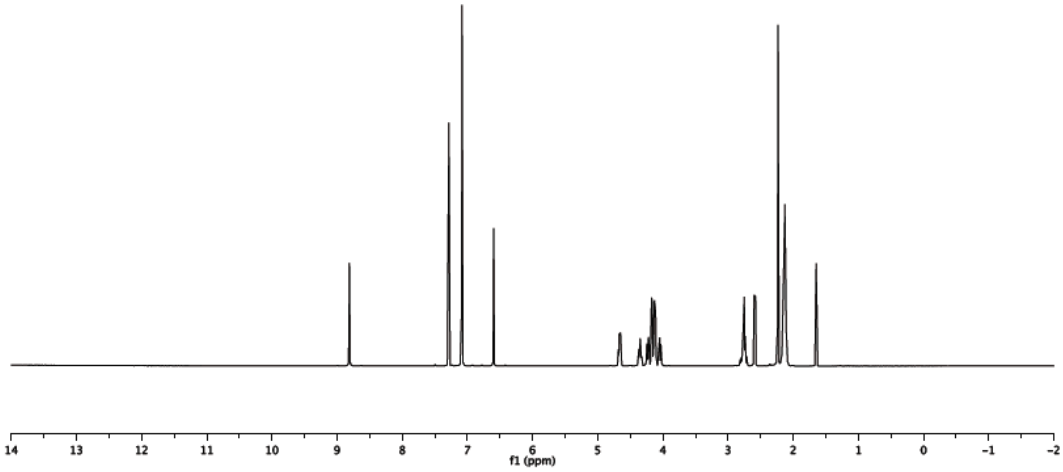


2´-O-(2-Cyanoethyl)-3´,5´-O-bis-(tert-butyldimethylsilyl)-4-O-(2,6-dimethylphenyl)-5-methyluridine 9
13C $CDCl_3$

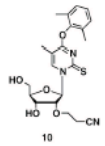


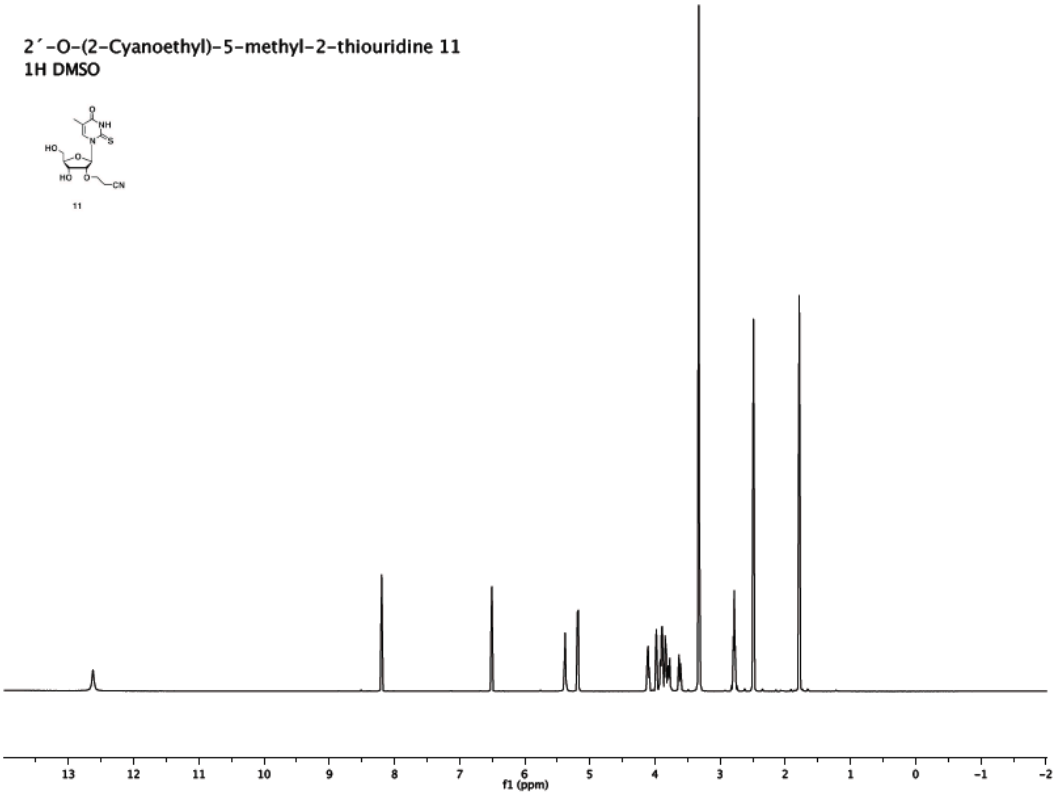
2´-O-(2-Cyanoethyl)-4-O-(2,6-dimethylphenyl)-5-methyl-2-thiouridine 10
1H $CDCl_3$

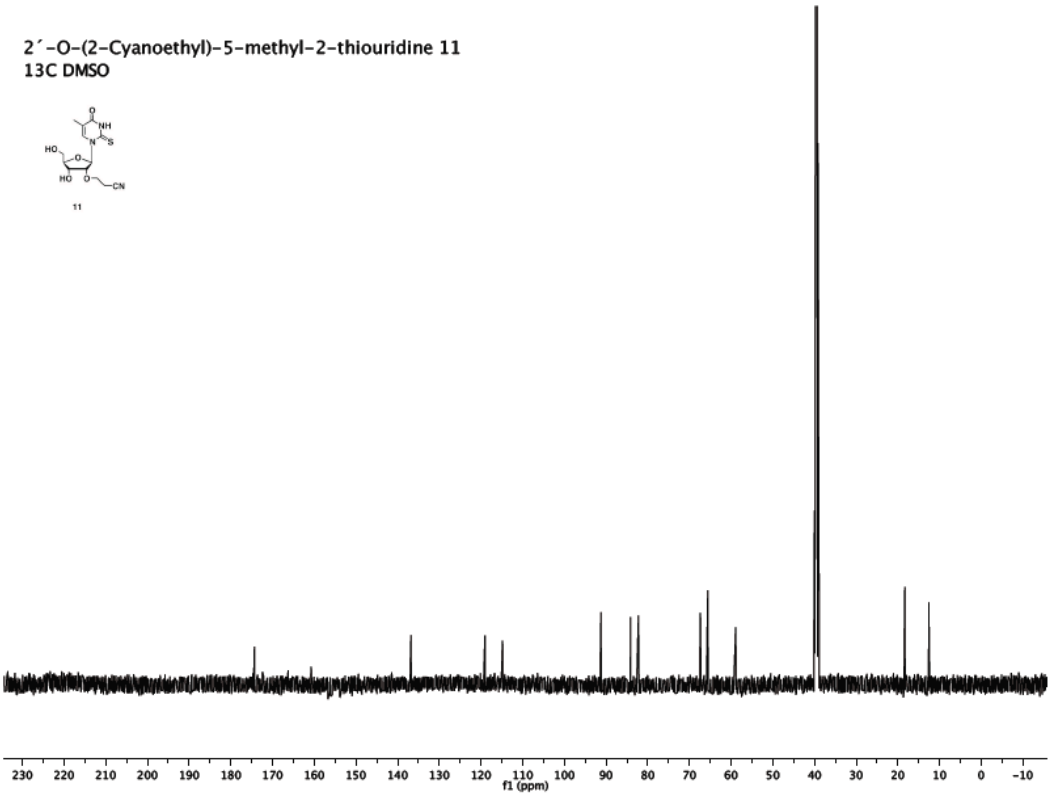




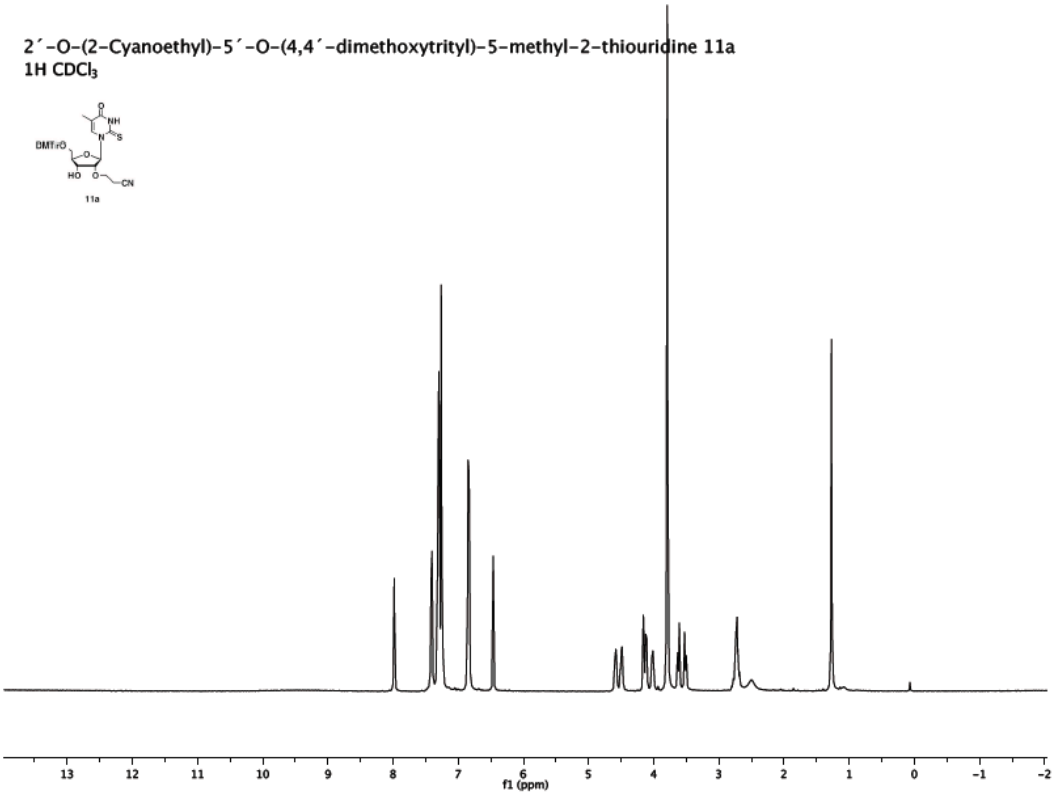
2´-O-(2-Cyanoethyl)-4-O-(2,6-dimethylphenyl)-5-methyl-2-thiouridine 10
13C $CDCl_3$



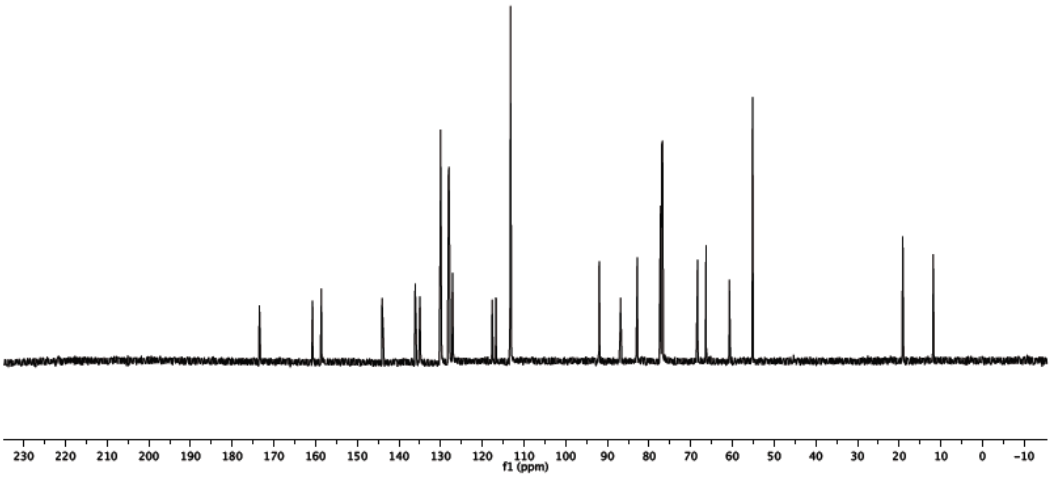

2´-O-(2-Cyanoethyl)-5-methyl-2-thiouridine 11
1H DMSO
11
13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)


2´-O-(2-Cyanoethyl)-5-methyl-2-thiouridine 11
13C DMSO
11
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

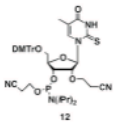
2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-5-methyl-2-thiouridine 11a
1H $CDCl_3$



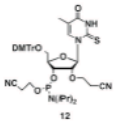
2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-5-methyl-2-thiouridine 11a
13C $CDCl_3$



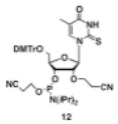
2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-
3´-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-5-methyl-2-thiouridine 12
1H $CDCl_3$

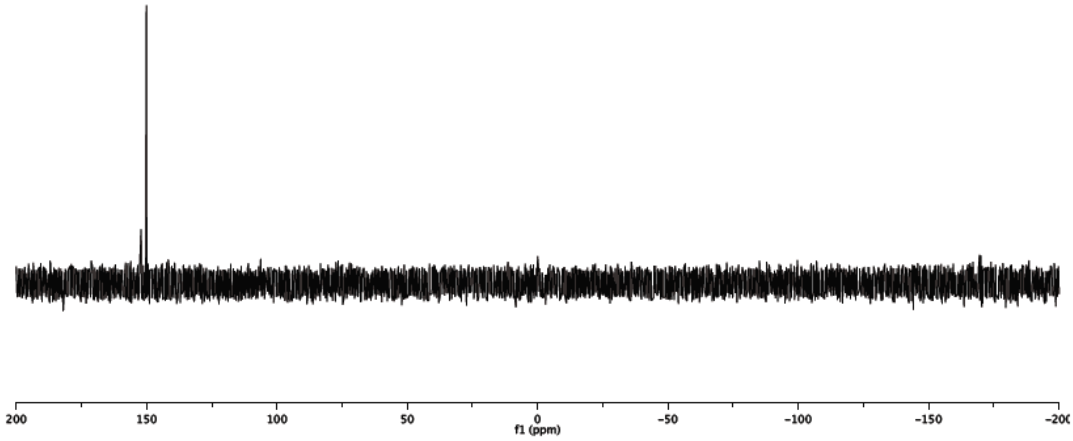


2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-
3´-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-5-methyl-2-thiouridine 12
13C $CDCl_3$

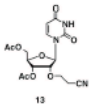


2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-
3´-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-5-methyl-2-thiouridine 12
31P $CDCl_3$

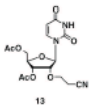


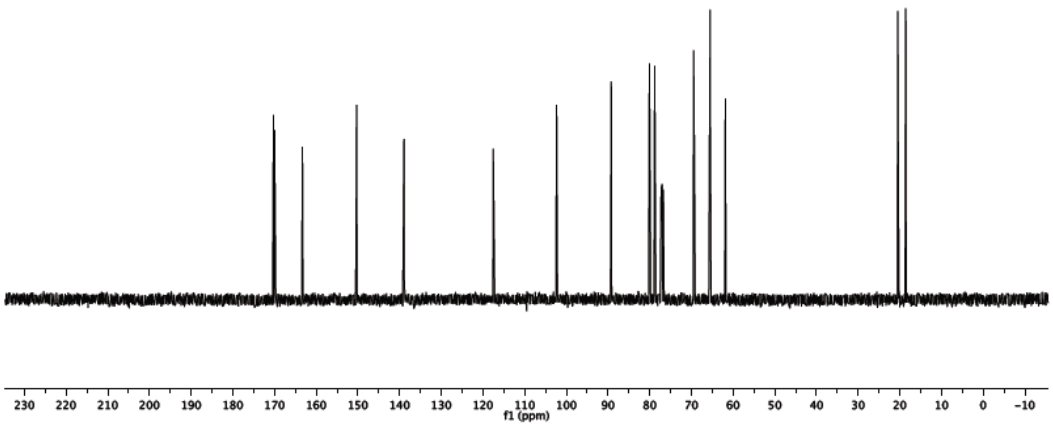


2´-O-(2-Cyanoethyl)-3´,5´-O-diacetyl-uridine 13
1H $CDCl_3$

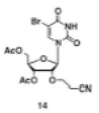


2´-O-(2-Cyanoethyl)-3´,5´-O-diacetyl-uridine 13
13C $CDCl_3$

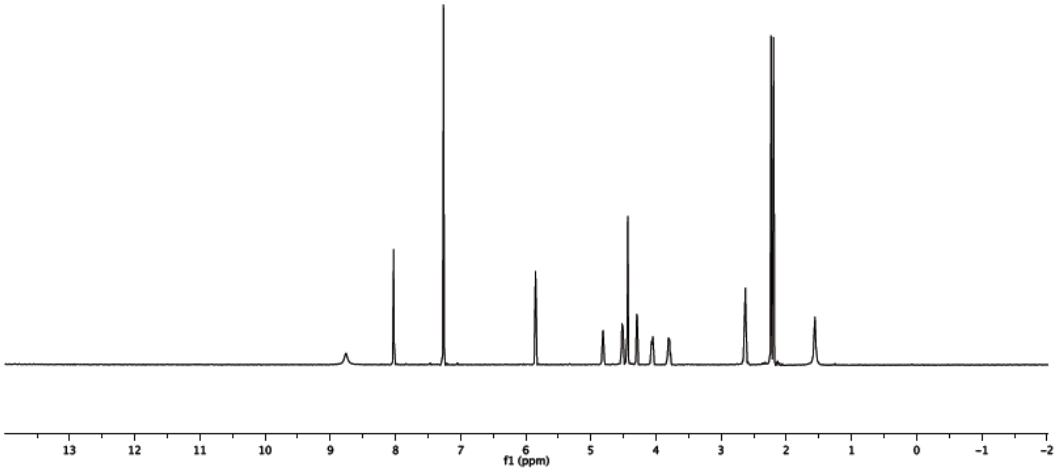




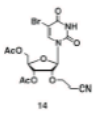
2′-O-(2-Cyanoethyl)-3′,5′-O-diacetyl-5-bromouridine 14
1H $CDCl_3$

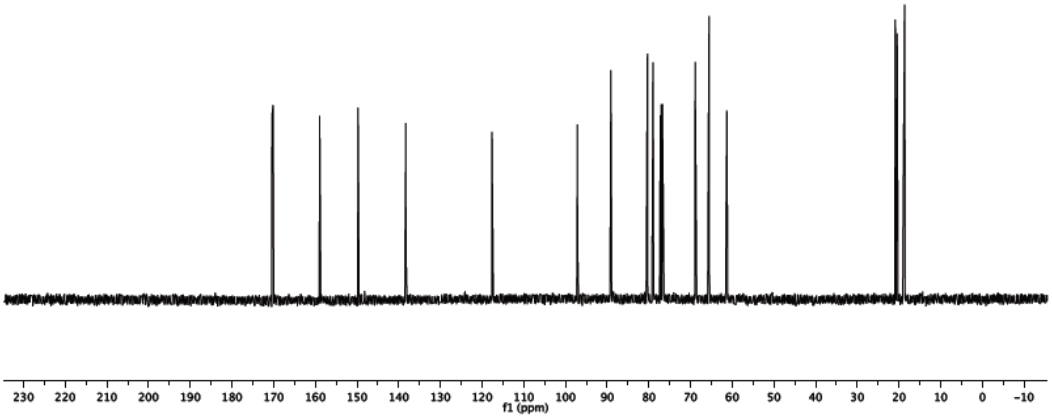


14

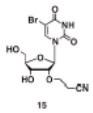


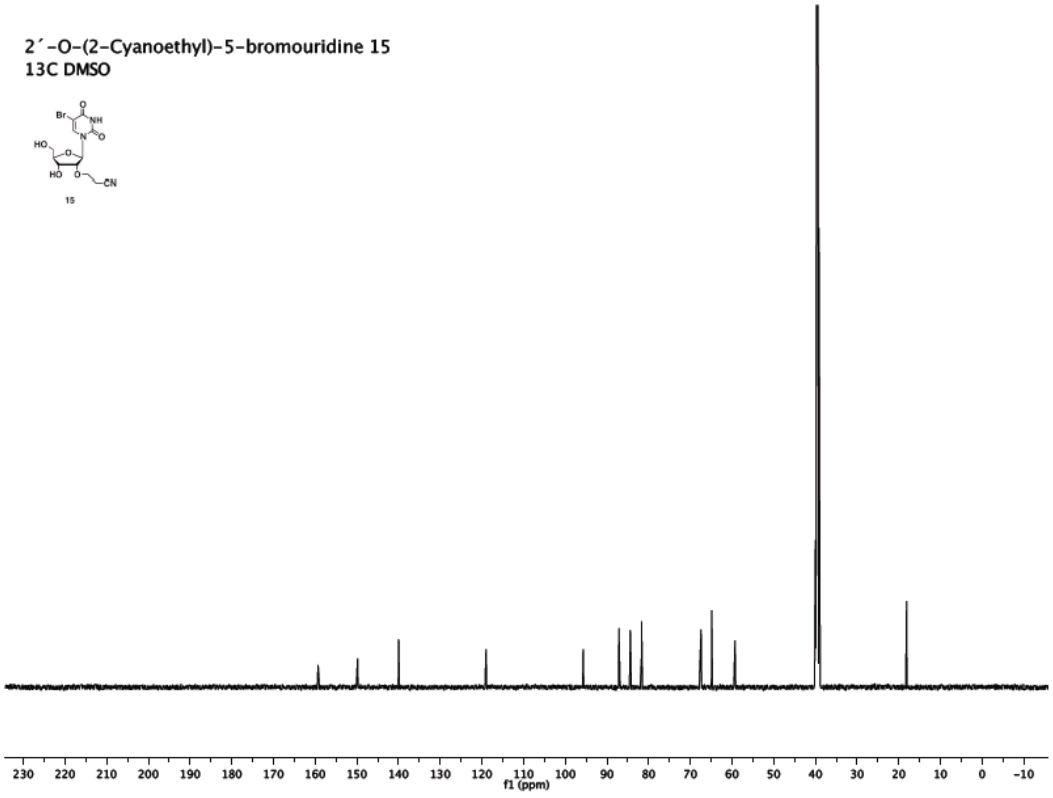
2´-O-(2-Cyanoethyl)-3´,5´-O-diacetyl-5-bromouridine 14
13C $CDCl_3$



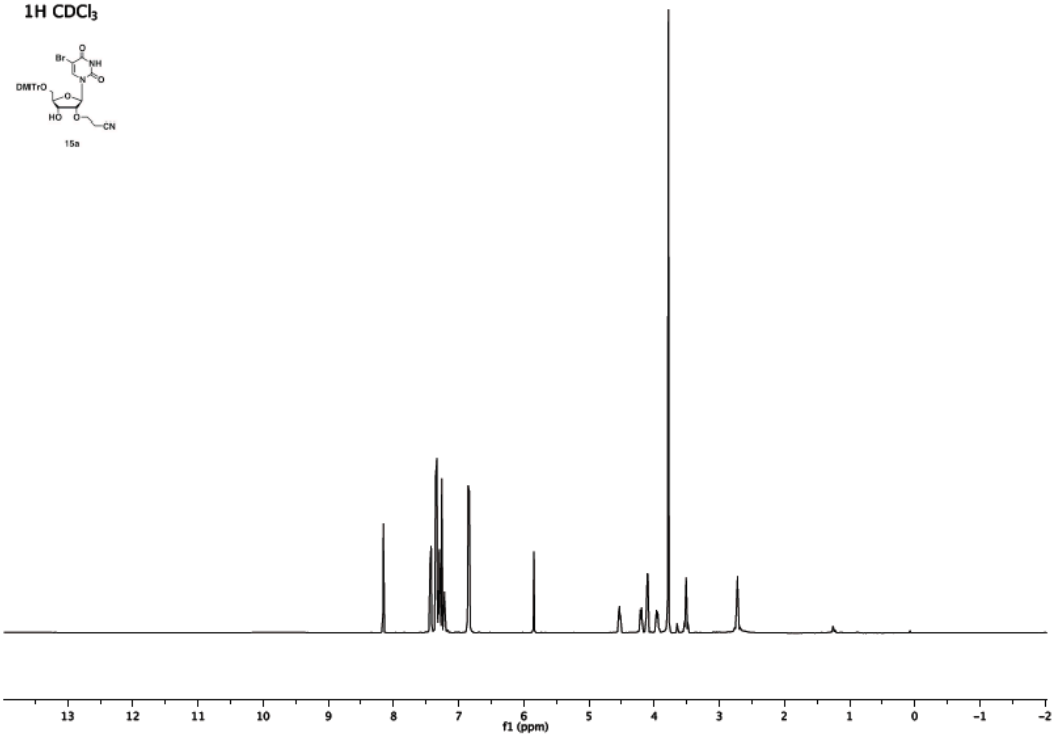


2´-O-(2-Cyanoethyl)-5-bromouridine 15
1H DMSO

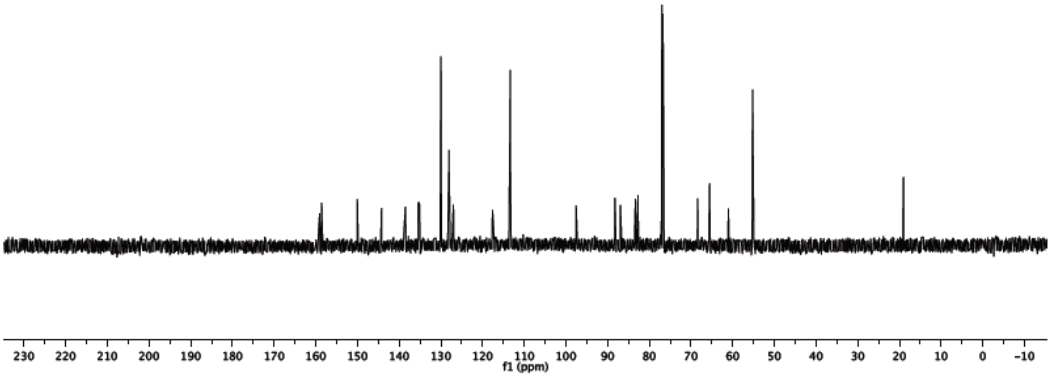




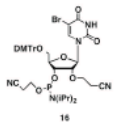
2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-5-bromo-uridine 15a
1H $CDCl_3$



2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-5-bromo-uridine 15a
13C $CDCl_3$

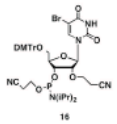


2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-
3´-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)- 5-bromo-uridine 16
1H $CDCl_3$

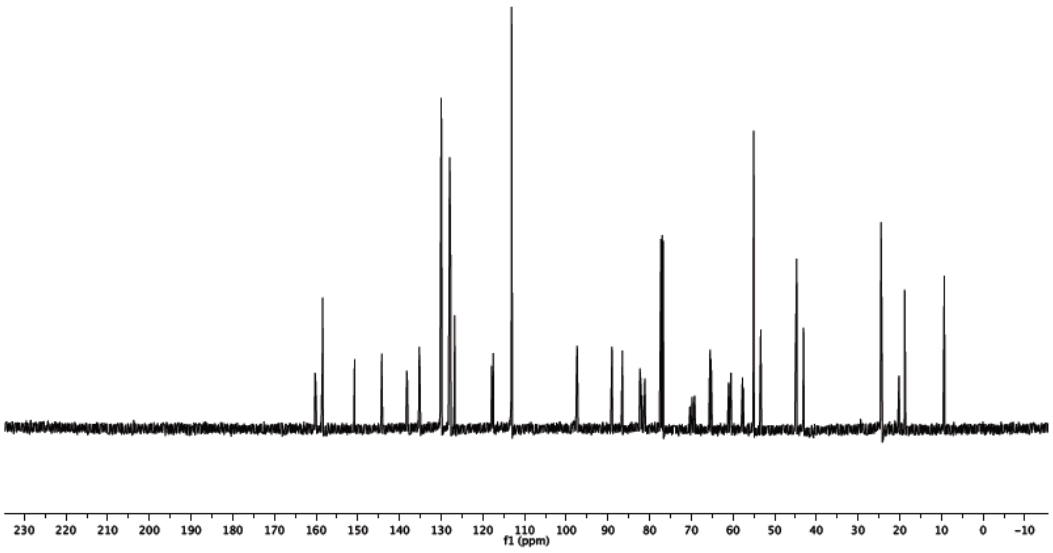


16

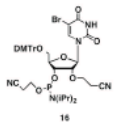
2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-
3´-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)- 5-bromo-uridine 16
13C $CDCl_3$



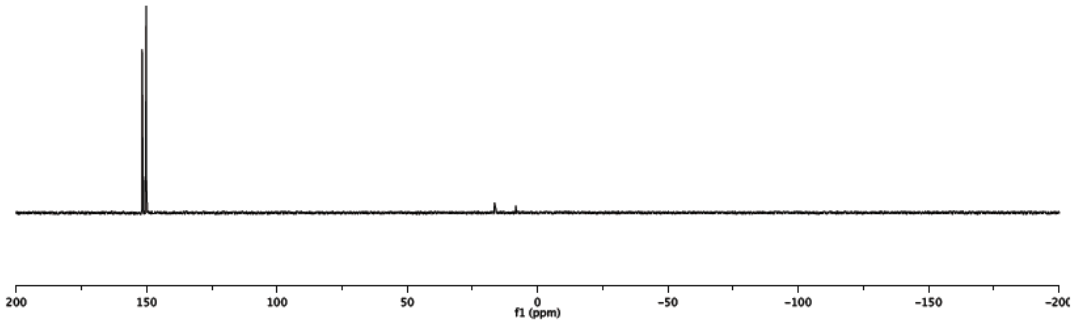
16

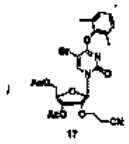


2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-
3´-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)- 5-bromo-uridine 16
31P $CDCl_3$

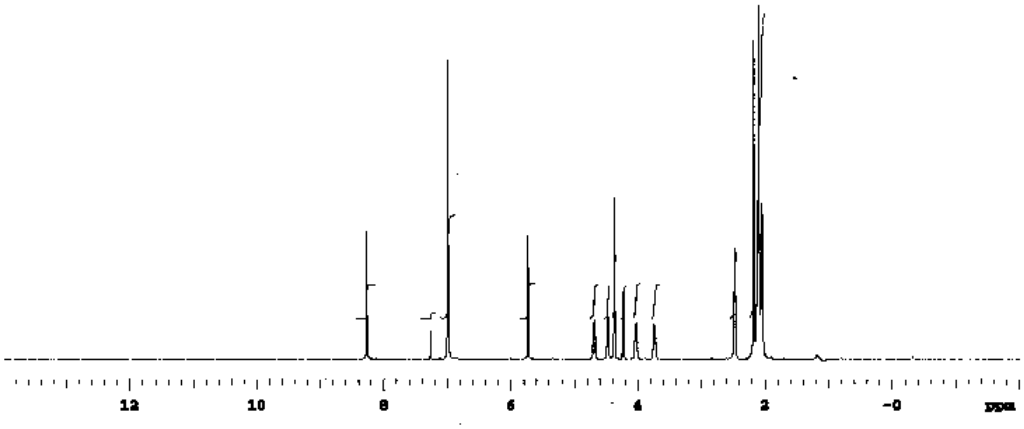


16

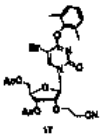




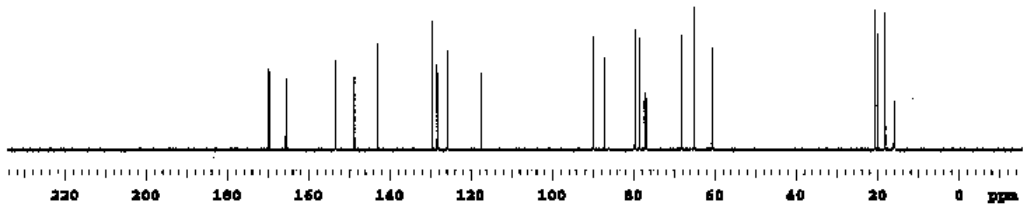
^1H. $CDCl_3$



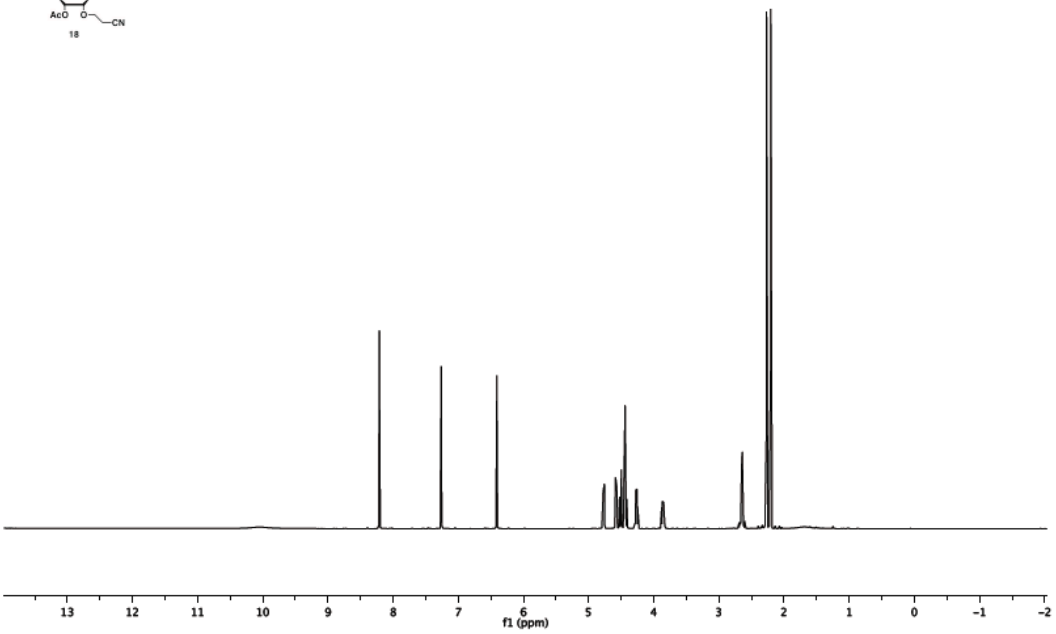
A



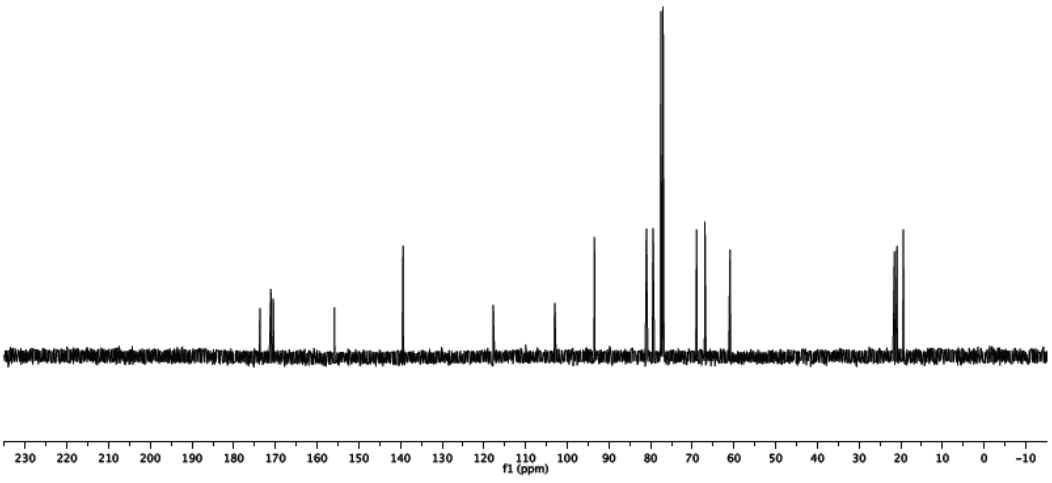
^{13}C. $CDCl_3$



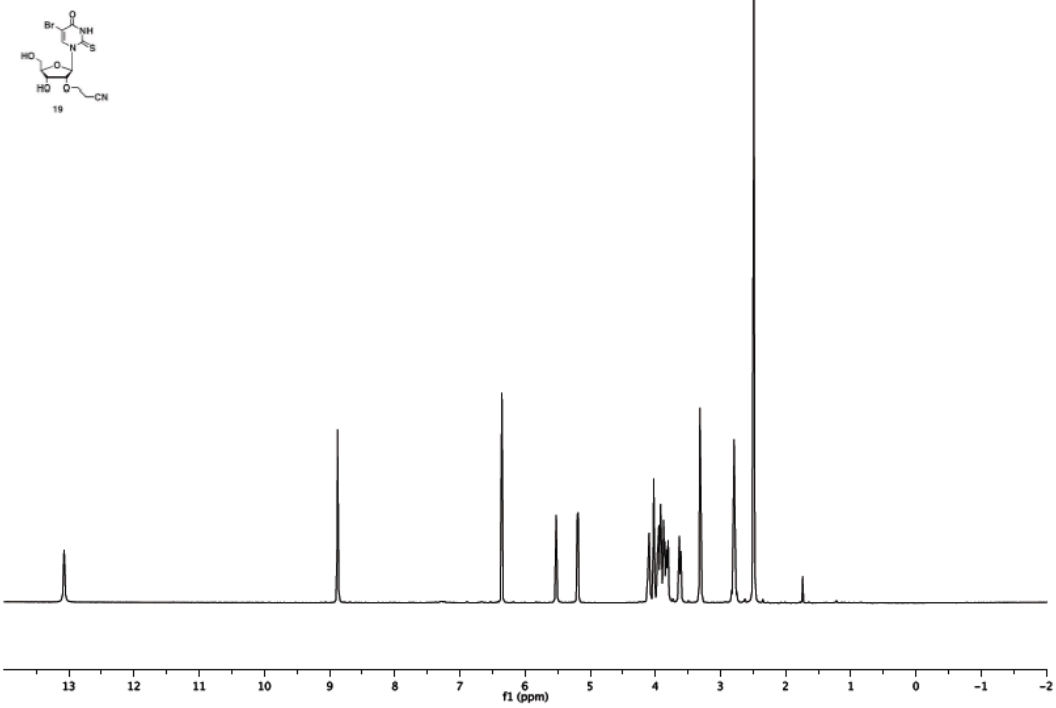
2´-O-(2-Cyanoethyl)-3´,5´-O-diacetyl-5-bromo-2-thiouridine 18
1H $CDCl_3$



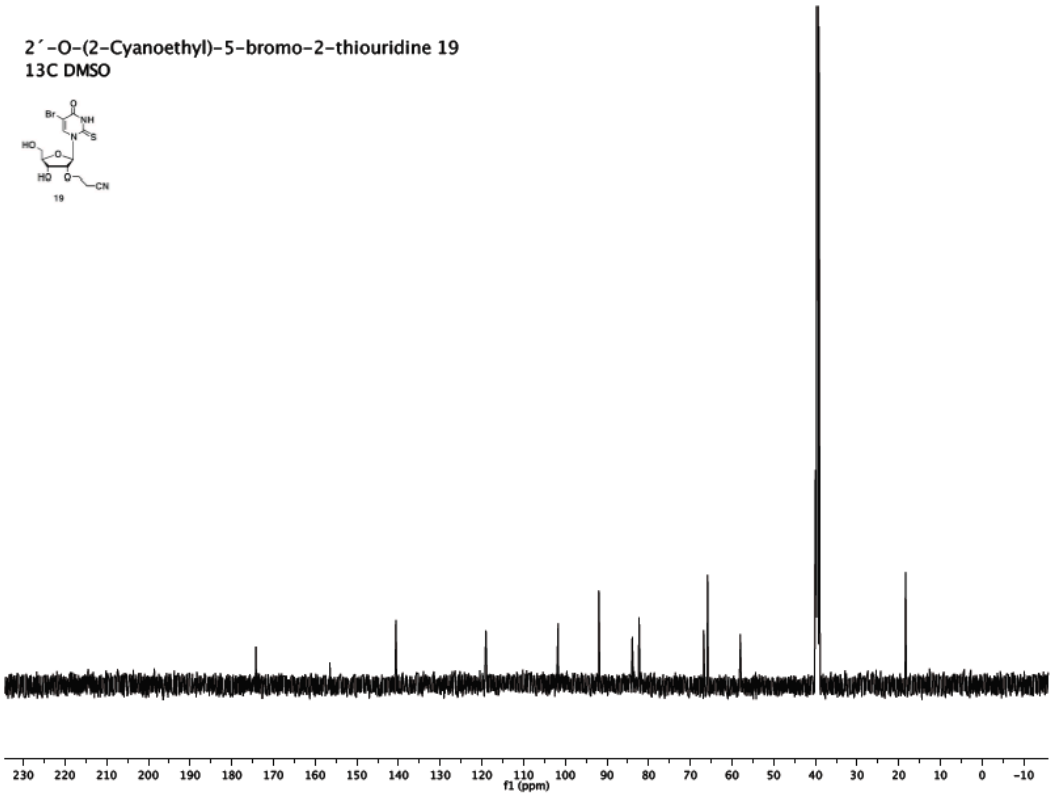
2´-O-(2-Cyanoethyl)-3´,5´-O-diacetyl-5-bromo-2-thiouridine 18
13C $CDCl_3$



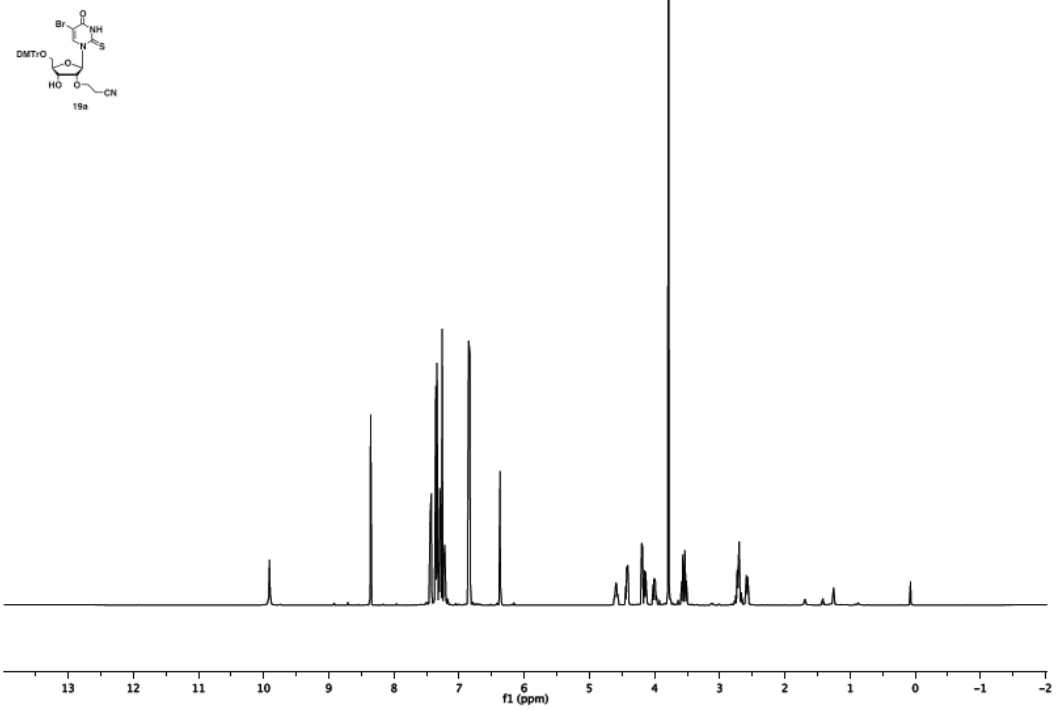
2´-O-(2-Cyanoethyl)-5-bromo-2-thiouridine 19
1H DMSO



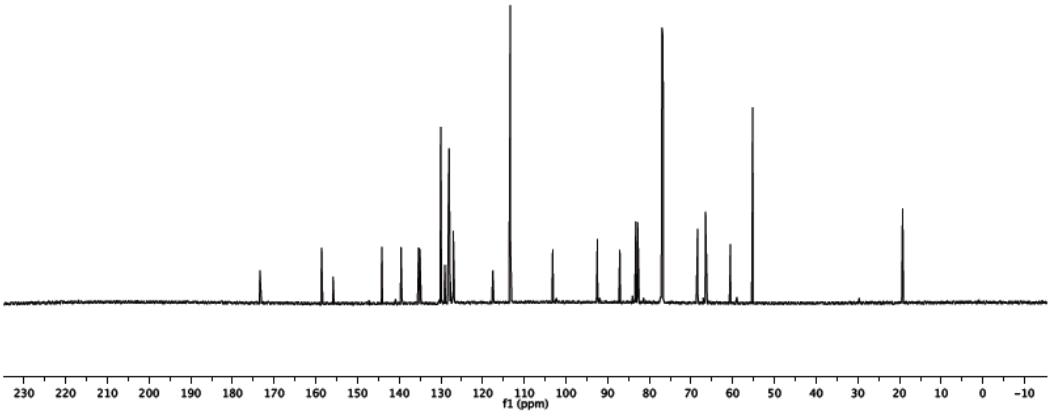
2´-O-(2-Cyanoethyl)-5-bromo-2-thiouridine 19
13C DMSO



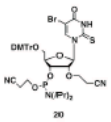
2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-5-bromo-2-thiouridine 19a
1H $CDCl_3$

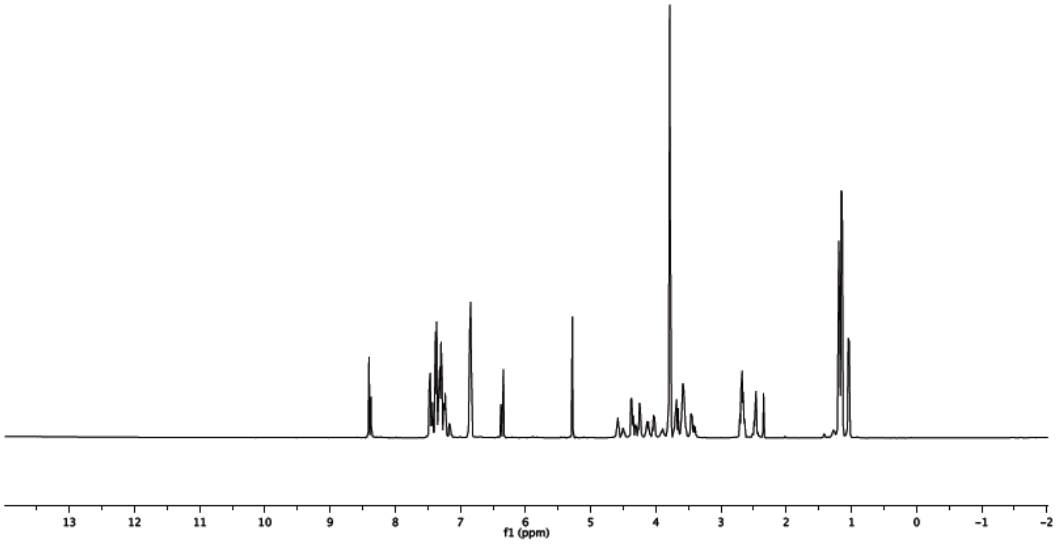


2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-5-bromo-2-thiouridine 19a
13C $CDCl_3$

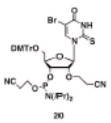


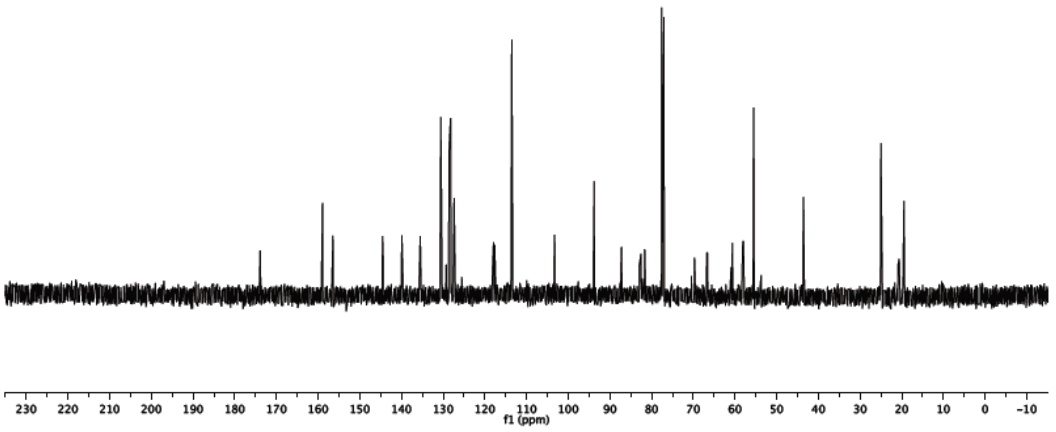
2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-
3´-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-5-bromo-2-thiouridine 20
1H $CDCl_3$



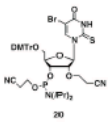


2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-
3´-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-5-bromo-2-thiouridine 20
13C $CDCl_3$

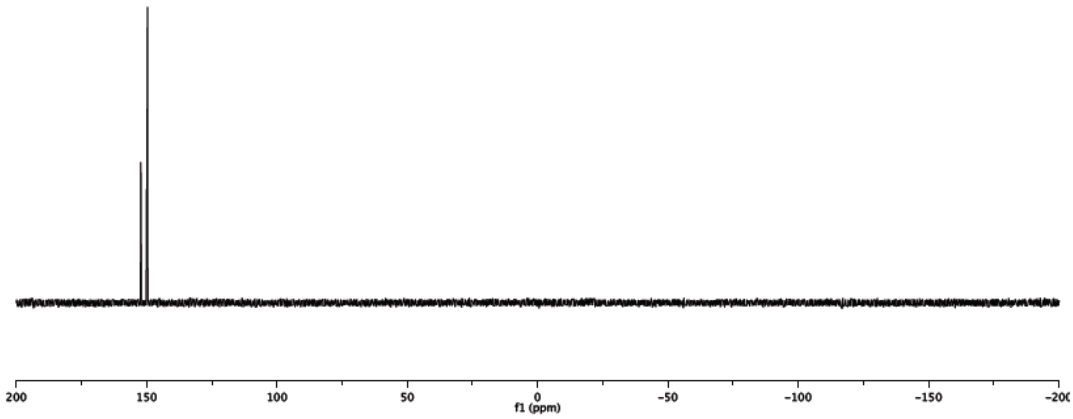




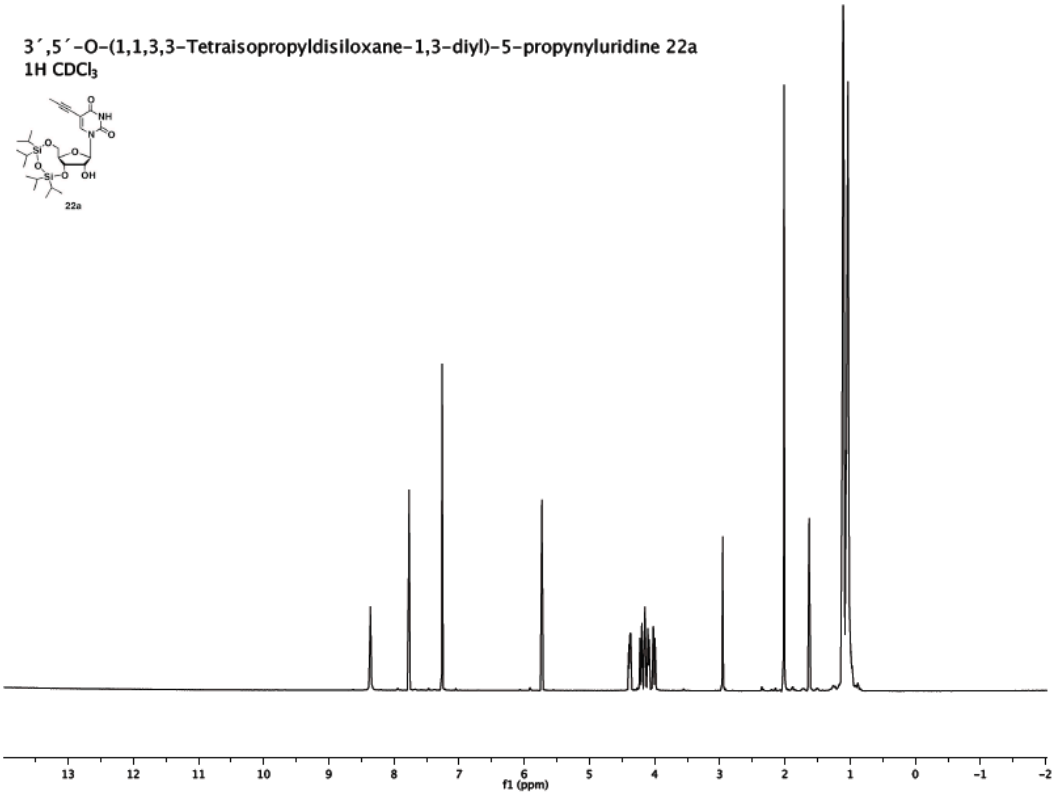
2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-
3´-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-5-bromo-2-thiouridine 20
31P $CDCl_3$



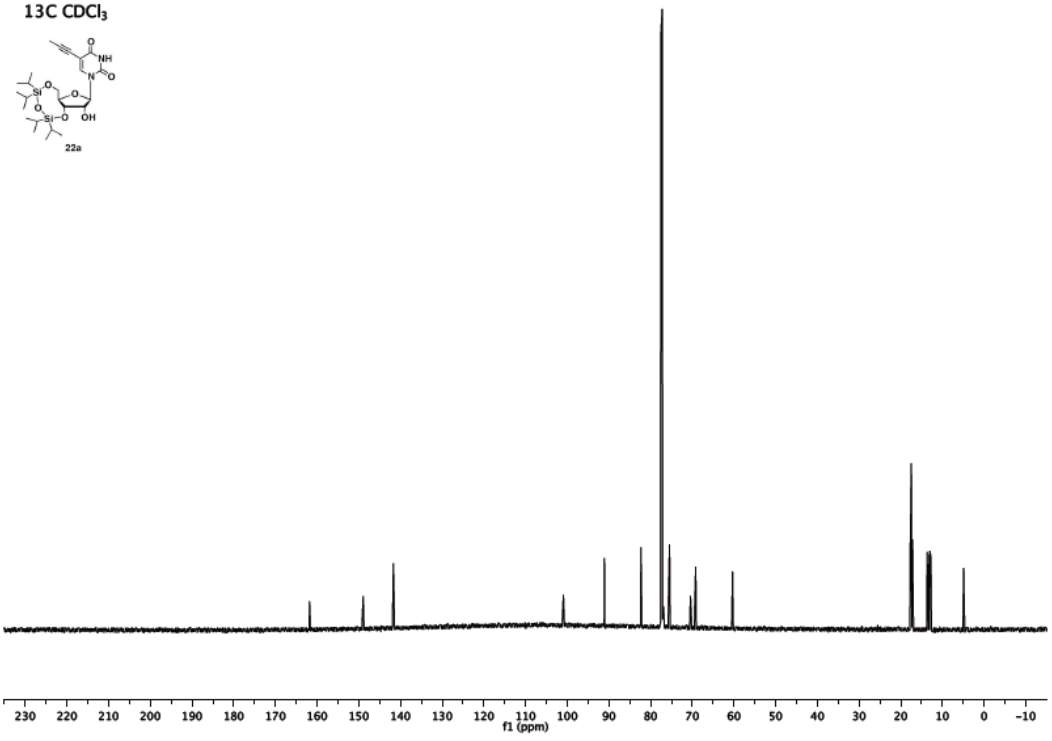
20



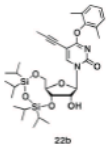
3´,5´-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-5-propynyluridine 22a
1H $CDCl_3$



3´,5´-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-5-propynyluridine 22a
13C $CDCl_3$

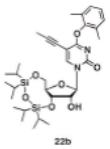


3´,5´-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-4-O-(2,6-dimethylphenyl)-5-propynyluridine 22b
1H $CDCl_3$



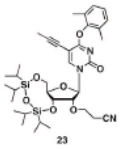
22b

3´,5´-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-4-O-(2,6-dimethylphenyl)-5-propynyluridine 22b
13C $CDCl_3$

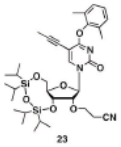


22b

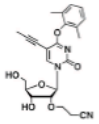
2´-O-(2-Cyanoethyl)-3´,5´-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-O-(2,6-dimethylphenyl)-5-propynyluridine 23
1H $CDCl_3$



2´-O-(2-Cyanoethyl)-3´,5´-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-O-(2,6-dimethylphenyl)-5-propynyluridine 23
13C $CDCl_3$

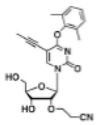


2´-O-(2-Cyanoethyl)-4-O-(2,6-dimethylphenyl)-5-propynyl-uridine 23a
1H $CDCl_3$



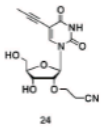
23a

2´-O-(2-Cyanoethyl)-4-O-(2,6-dimethylphenyl)-5-propynyl-uridine 23a
13C $CDCl_3$

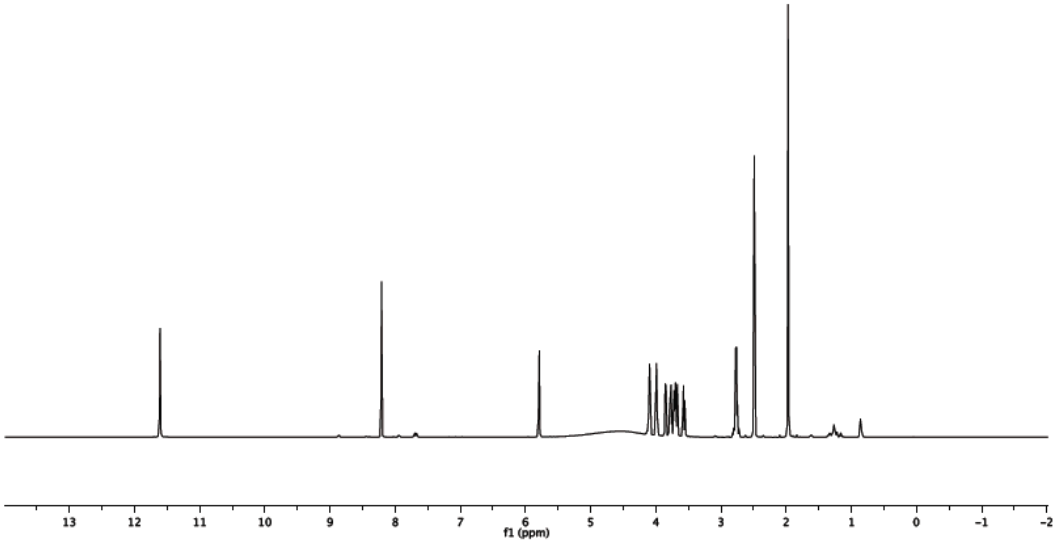


23a

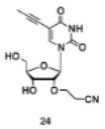
2´-O-(2-Cyanoethyl)-5-propynyl-uridine 24
1H DMSO



24

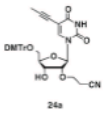


2´-O-(2-Cyanoethyl)-5-propynyl-uridine 24
13C DMSO

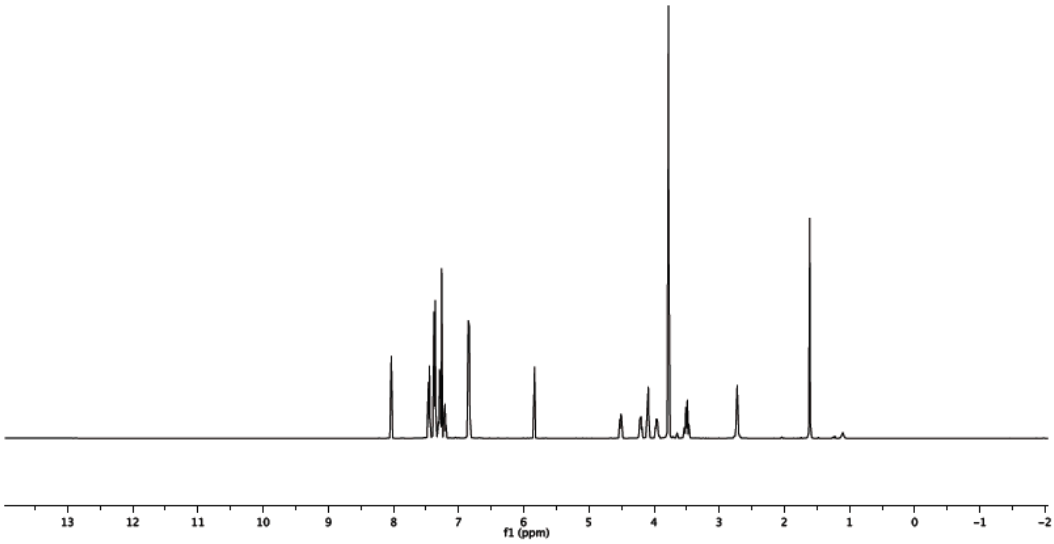


24

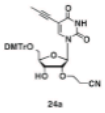
2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-5-propynyl-uridine 24a
1H $CDCl_3$



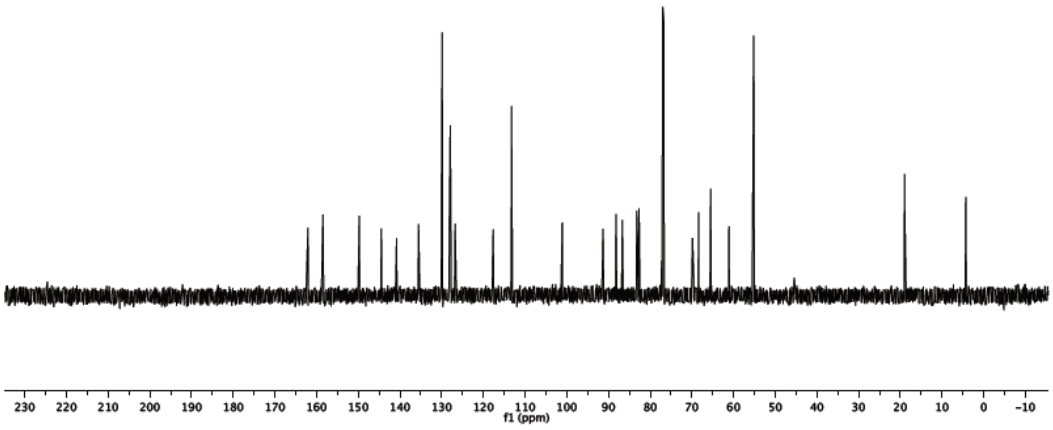
24a



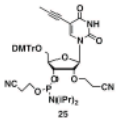
2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-5-propynyl-uridine 24a
13C $CDCl_3$



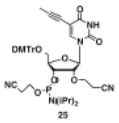
24a

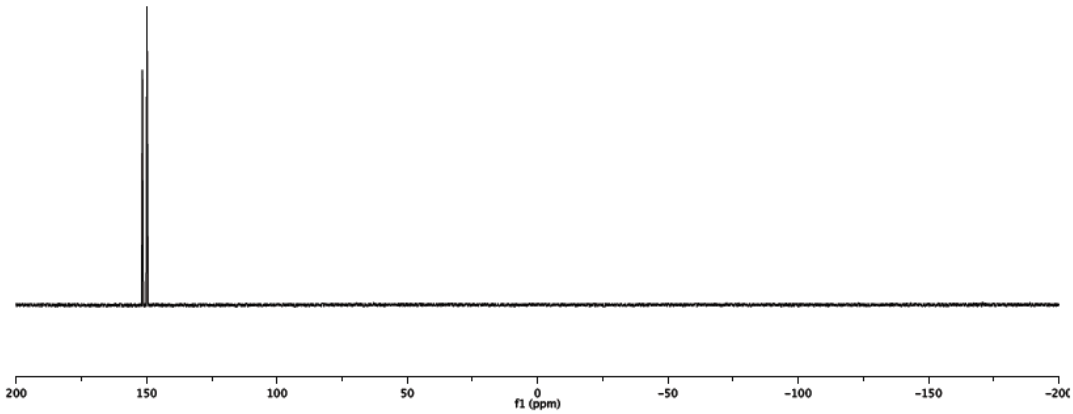


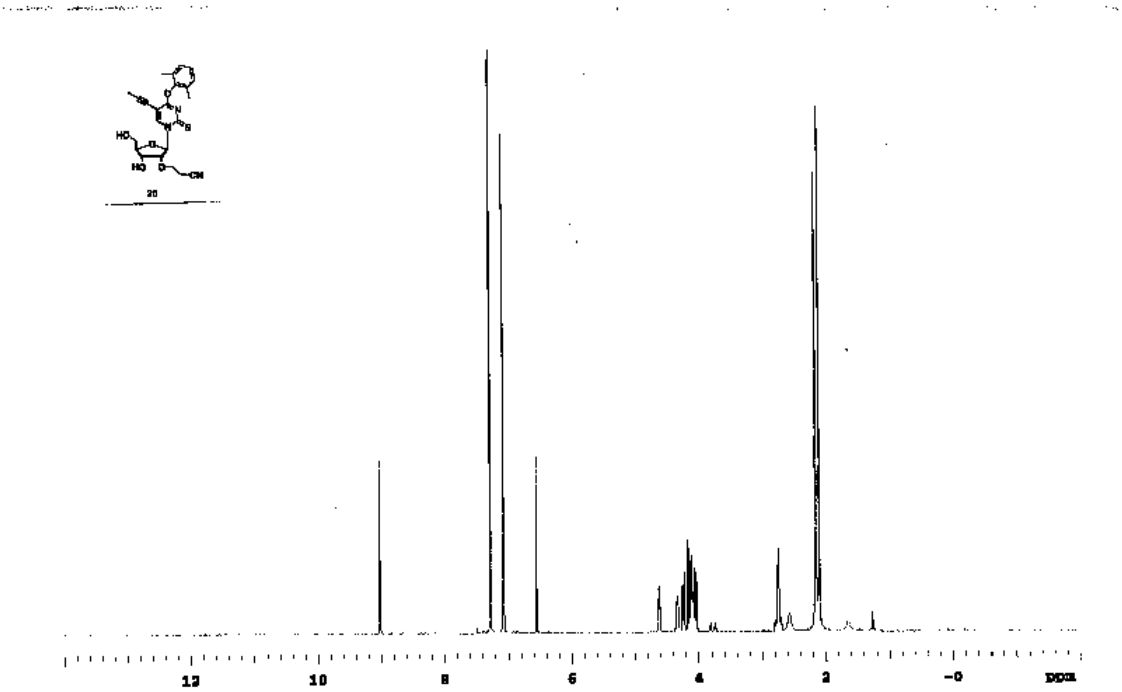
2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-
3´-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)- 5-propynyl-uridine 25
1H $CDCl_3$

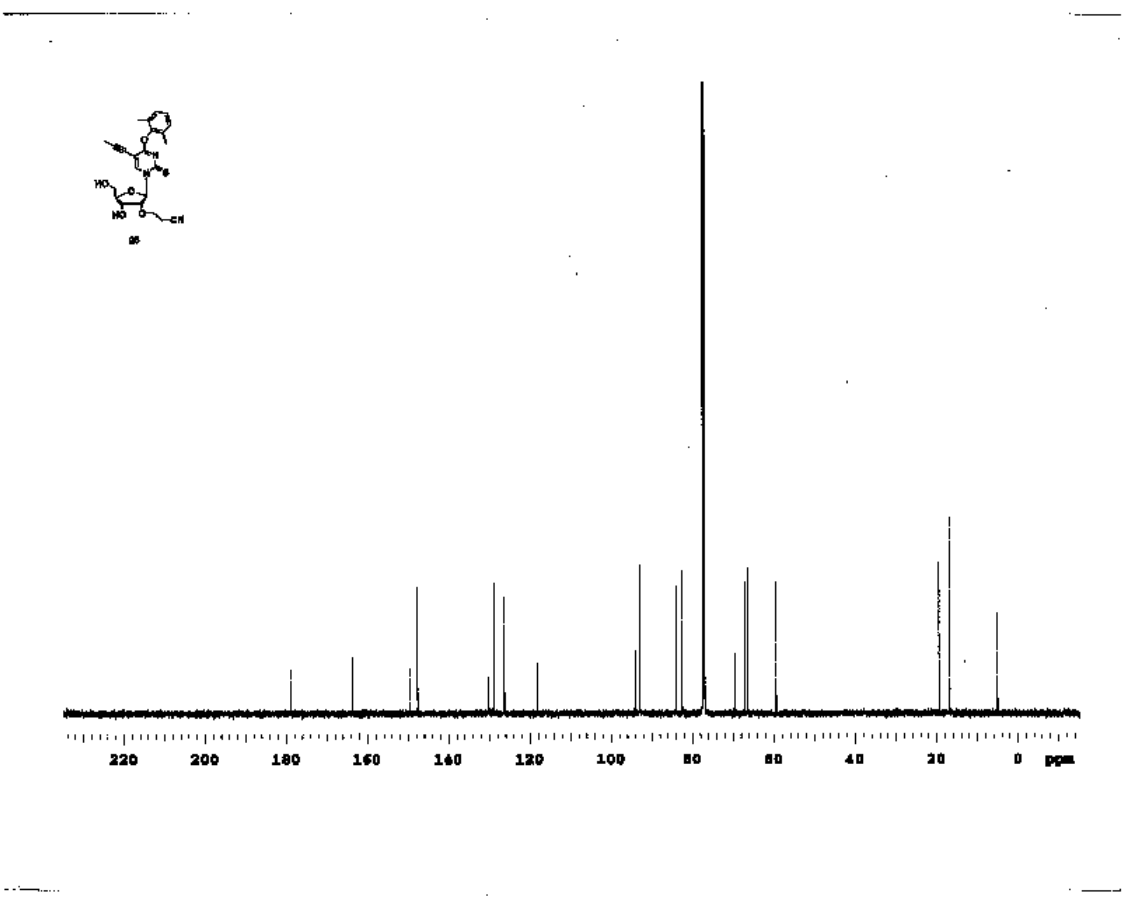


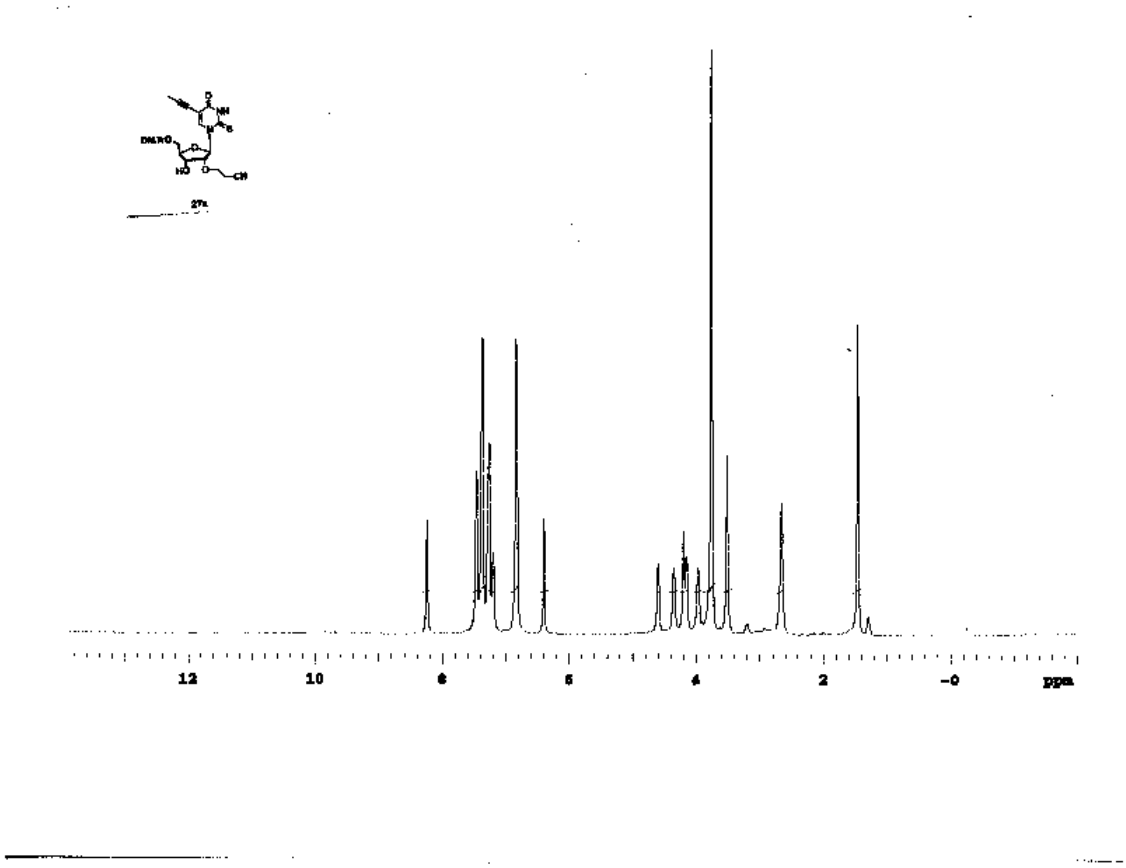
2´-O-(2-Cyanoethyl)-5´-O-(4,4´-dimethoxytrityl)-
3´-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)- 5-propynyl-uridine 25
31P $CDCl_3$

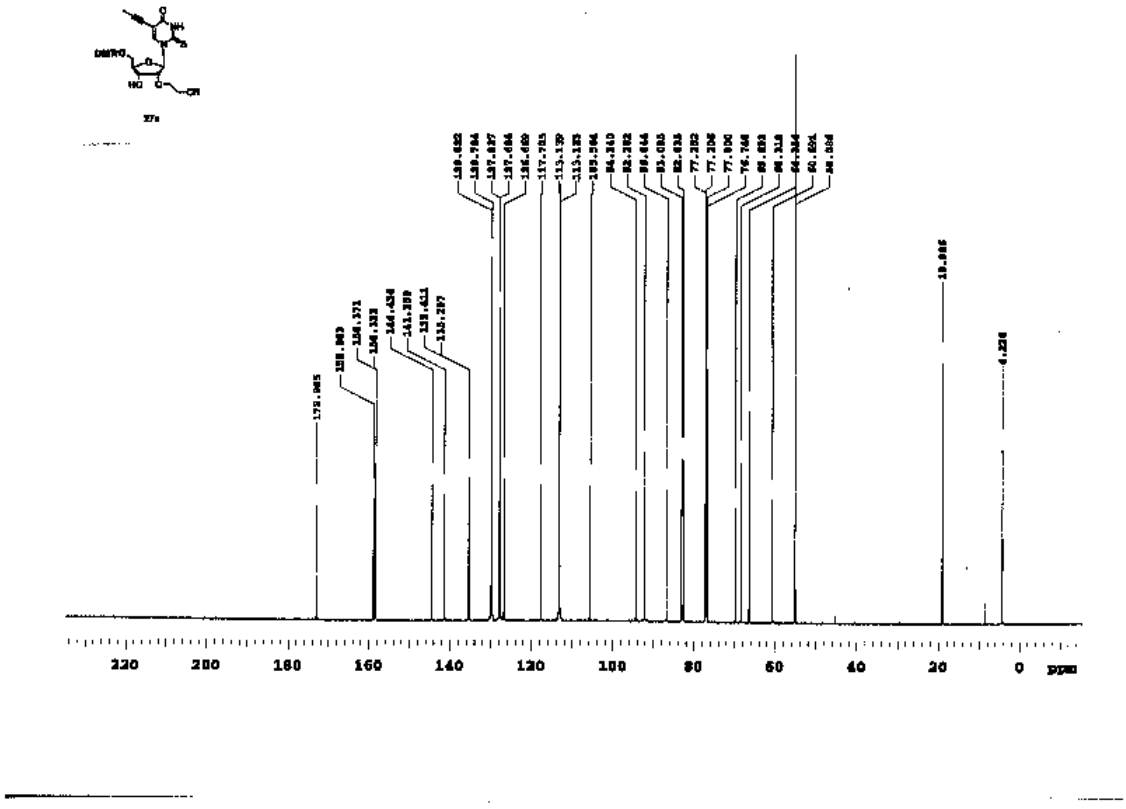


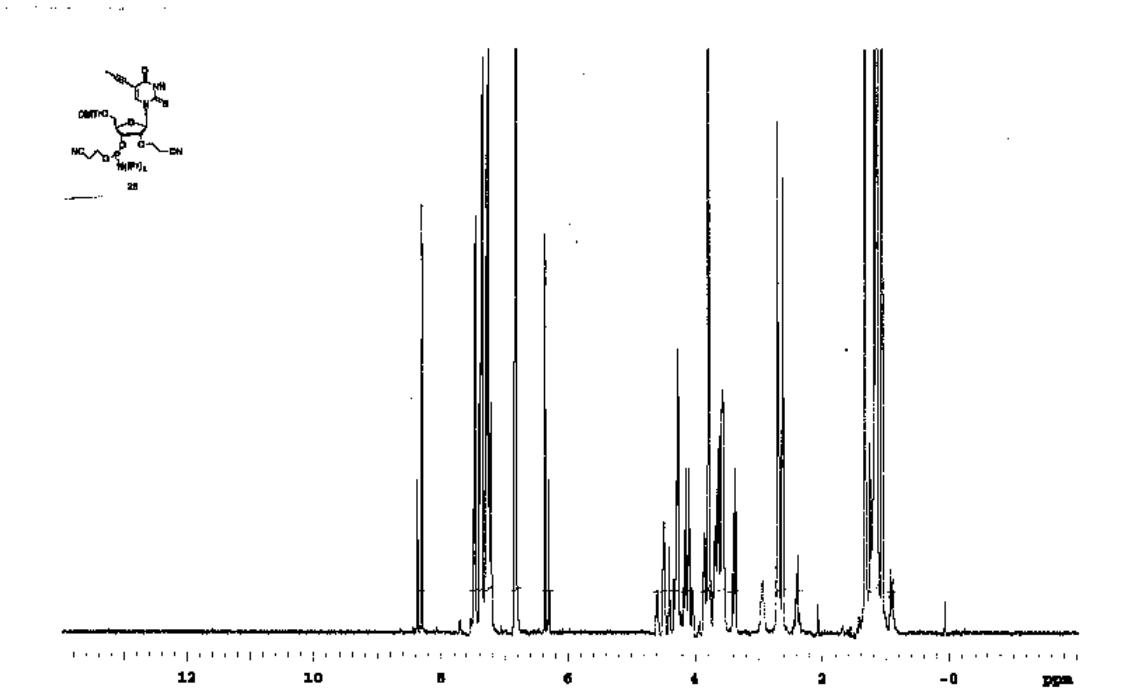


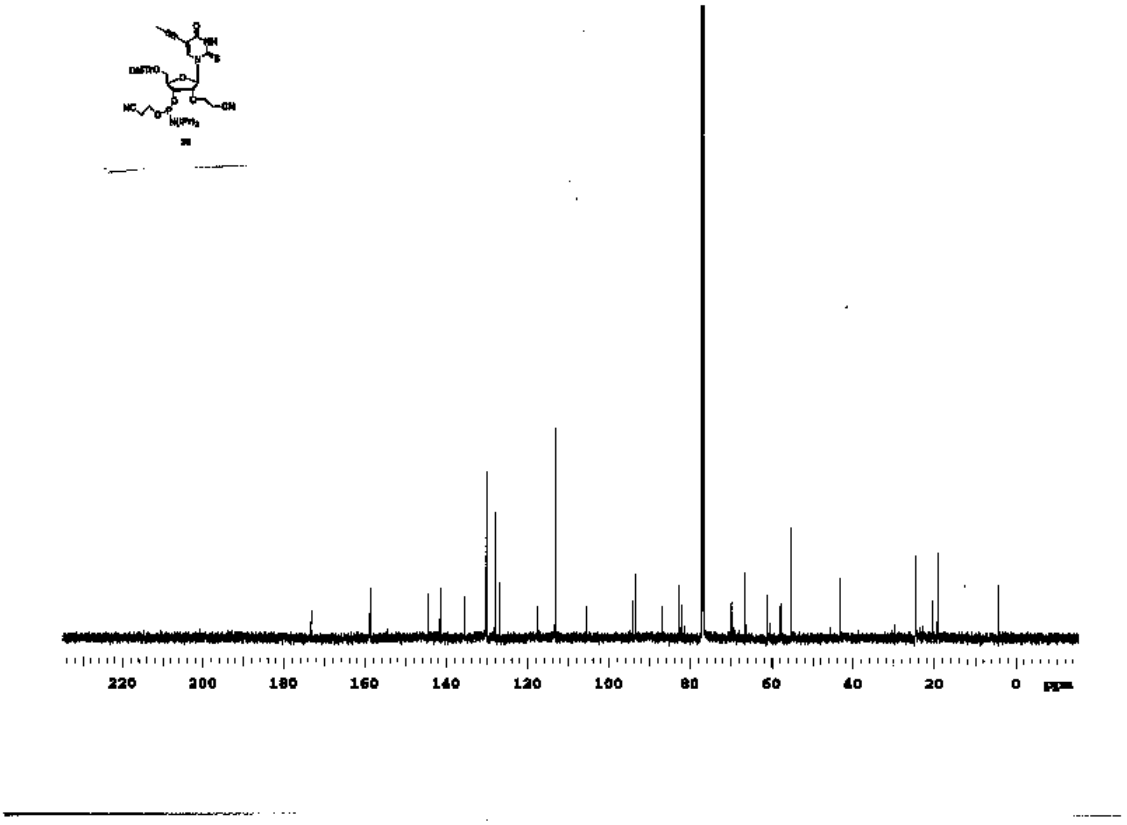

12
10
8
6
4
2
0
ppm


220
200
180
160
140
120
100
80
60
40
20
0
ppm


12
10
8
6
4
2
0
ppm


220
200
180
160
140
120
100
80
60
40
20
0
ppm


12
10
8
6
4
2
-0
ppm


220
200
180
160
140
120
100
80
60
40
20
0
ppm