Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Post-Synthetic Approach for the Synthesis of 2′-*O*-Methyldithiomethyl-Modified Oligonucleotides Responsive to Reducing Environment

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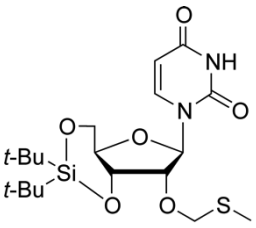
Contents

General Methods.

All reagents and solvents except 2,4,6-trimethoxybenzyl mercaptane were obtained from commercial sources and used without purification. Flash chromatography was performed using Wakogel C-400HG (20-40 μm; Wako Pure Chemical Industries, Japan). TLC was performed on Merck silica gel 60 F_{254} and compounds were visualized under UV light (254 nm). ^{1}H, ^{13}C, ^{19}F and ^{31}P NMR spectra were measured on a Agilent Mercury-300, Agilent UNITY INOVA-500, Agilent 400-MR-DD2, and Agilent NMR System 600-DD2 NMR spectrometers. ^{1}H NMR spectra were referenced to the internal TMS signal in the samples, $\delta = 0$ ppm. ^{13}C NMR spectra were referenced to the internal $CHCl_3$ signal in the samples, $\delta = 77$ ppm. ^{19}F NMR chemical shifts were referenced to external trifluoroacetic acid (TFA), $\delta = -76.55$ ppm. ^{31}P NMR chemical shifts were referenced to an external 85% H_3PO_4 standard, $\delta = 0$ ppm. 0.25 M 5-Ethylthio-1*H*-tetrazole (ETT) in acetonitrile, anhydrous acetonitrile, and 0.02 M iodine in THF/pyridine/H_2O were obtained from Glen Research. Thymidine-CPG 500 Å was obtained from Applied Biosystems. Snake venom phosphodiesterase (SVPDE) was purchased from Boehringer Mannheim. Reversed-phase HPLC was performed on columns of Nacalai COSMOSIL 5C18-MS-II ϕ 4.6 × 250 mm (analytical column) and ϕ 10.0 × 250 mm (purification column) with a linear gradient of acetonitrile in 50 mM TEAA (pH 7). Oligonucleotides were synthesized on an Applied Biosystems Model 392 DNA/RNA Synthesizer (Perkin-Elmer Applied Biosystems). The mass spectra of nucleosides and oligonucleotides were measured on a JMS-700 mass spectrometer (JEOL) in the positive-ion mode and a Voyager-DE STR MALDI-TOF mass spectrometer (AB SCIEX) in the negative-ion mode, respectively.

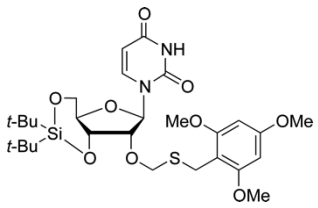
3′,5′-*O*-Di-*tert*-butylsilanediyl-2′-*O*-methylthiomethyluridine (10).



To a suspension of uridine (**9**) (4.89 g, 20 mmol) in anhydrous DMF (40 mL) was added di(*tert*-butyl)silyl ditriflate (7.18 mL, 22 mmol). After the reaction mixture was stirred at 0 °C for 1 h, methanol (5.0 mL) and Et_3N (7.0 mL) were added. The mixture was poured into sat. $NaHCO_3$ aqueous solution and extracted with AcOEt. The organic layer was washed with distilled water and brine, and then dried over anhydrous Na_2SO_4, filtered and concentrated. To the resulting crude 3′,5′-*O*-di-*tert*-butylsilanediyluridine were added DMSO (62 mL), acetic acid (63 mL) and acetic anhydride (41 mL). The reaction mixture was stirred at room temperature for 24 h. After the reaction, the volatile materials were evaporated and the resulting solution was poured into sat. $NaHCO_3$ aqueous solution. The mixture was extracted with ethyl acetate. The organic layer was washed with distilled water and brine, and then dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography. Desired compound **10** was obtained as a colorless foam (6.62 g, 14.9 mmol, 74% from **9**). ^{1}H NMR (300 MHz, $CDCl_3$): δ

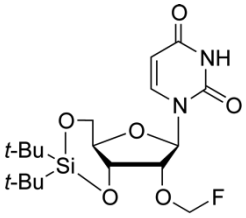
8.65 (br s, 1 H, N3-H), 7.27 (d, *J* = 8.2 Hz, 1 H, H-6), 5.76 (dd, *J* = 2.3 Hz, 8.3 Hz, 1 H, H-5), 5.74 (s, 1 H, H-1′), 4.98 (d, *J* = 11.5 Hz, 1 H, OC*H*$_2$S), 4.93 (d, *J* = 11.5 Hz, 1 H, OC*H*$_2$S), 4.53-4.48 (m, 2 H, H-2′, 5′), 4.18-4.10 (m, 1 H, H-3′), 4.02-3.95 (m, 2H, H-4′, 5′), 2.19 (s, 3 H, SC*H*$_3$), 1.06 (s, 9 H, *t*Bu), 1.02 (s, 9 H, *t*Bu). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 163.7, 150.1, 139.5, 102.8, 91.5, 76.9, 76.4, 75.1, 74.8, 67.6, 27.5, 27.3, 23.0, 20.5, 13.5. HRMS (FAB): *m/z* 445.1828 ([M+H]$^+$, $C_{19}H_{33}N_2O_6SSi^+$ Calcd 445.1828).

3′,5′-*O*-Di-*tert*-butylsilanediyl-2′-*O*-(2,4,6-trimethoxybenzylthiomethyl)uridine (4b).



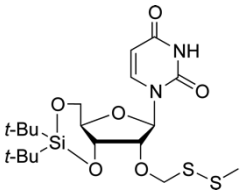
To a solution of 3′,5′-*O*-di-*tert*-butylsilanediyl-2′-*O*-methylthiomethyluridine (**10**) (0.76 g, 1.7 mmol) in anhydrous dichloromethane (7.5 mL) was added dropwise a solution of sulfuryl chloride (138 μL, 1.7 mmol) in anhydrous dichloromethane (1.6 mL) for 1 min. After the reaction mixture was stirred at room temperature for an additional 45 min, the volatile materials in the mixture were evaporated. The residue was dissolved with anhydrous DMF. After dissolution of 2,4,6-trimethoxybenzyl mercaptane[18] (0.91 g, 4.2 mmol) with anhydrous DMF (7.5 mL) in another flask, 60% NaH (0.16 g, 4.0 mmol) was added to the solution at 0 °C. To the thiolate solution was added the DMF solution (5 mL) of the sulfuryl-chloride-treated residue. The reaction mixture was stirred for 2 h and then poured into 0.5 M KH_2PO_4 aqueous solution. The mixture was extracted with ethyl acetate. The organic layer was washed with distilled water and brine, and then dried over anhydrous sodium sulfate, filtered and concentrated. The crude mixture was purified by silica gel column chromatography. Desired compound **4b** was obtained as a colorless foam (0.75 g, 1.2 mmol, 72% from **10**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25 (br s, 1 H, N3-H), 7.16 (d, *J* = 8.1 Hz, 1 H, H-6), 6.12 (s, 2 H, Ar-H), 5.72 (dd, *J* = 8.1 Hz, 2.1 Hz, 1 H, H-5), 5.59 (s, 1 H, H-1′), 5.07 (d, *J* = 11.7 Hz, 1 H, OC*H*$_2$S), 4.95 (d, *J* = 11.9 Hz, 1 H, OC*H*$_2$S), 4.53 (d, *J* = 4.8 Hz, 1 H, H-5′), 4.48-4.44 (m, 1 H, H-2′), 4.16-3.94 (m, 3 H, H-3′, 4′, 5′), 3.86 (d, *J* = 3.1 Hz, 2 H, SC*H*$_2$Ar), 3.81 (s, 9 H, OC*H*$_3$), 1.05 (s, 9 H, *t*-Bu-C*H*$_3$), 1.02 (s, 9 H, *t*-Bu-C*H*$_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 163.4, 160.2, 158.7, 149.5, 140.9, 108.3, 102.2, 92.7, 90.5, 76.6, 74.6, 74.0, 67.2, 55.7, 55.3, 36.4, 27.2, 27.0, 22.6, 22.5, 20.2. HRMS (FAB): *m/z* 633.2276 ([M+Na]$^+$, $C_{28}H_{42}N_2O_9SSiNa^+$ Calcd 633.2278).

3′,5′-*O*-Di-*tert*-butylsilanediyl-2′-*O*-fluoromethyluridine (8).



To a solution of 3′,5′-*O*-di-*tert*-butylsilanediyl-2′-*O*-(4-methoxybenzylthiomethyl)uridine (**4a**) (0.20 g, 0.36 mmol) in anhydrous THF (4 mL) was added dimethyldisulfide (0.92 mL, 10 mmol). A suspension of DMTSF (0.18 g, 0.90 mmol) in anhydrous THF (24 mL) was added dropwise to the solution for 10 min. After the reaction mixture was stirred at room temperature for 20 min, sat. $NaHCO_3$ aqueous solution (2 mL) was added. The mixture was poured into sat. $NaHCO_3$ aqueous solution and extracted with ethyl acetate. The organic layer was washed with distilled water and brine, and then dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by silica gel column chromatography. Compound **8** was obtained as a colorless foam (88 mg, 0.21 mmol, 58% from **4a**). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.59 (br s, 1 H, N3-H), 7.23 (d, *J* = 8.2 Hz, 1 H, H-6), 5.76 (dd, *J* = 2.3 Hz, 8.0 Hz, 1 H, H-5), 5.62 (d, *J* = 0.5 Hz, 1 H, H-1′), 5.51 (dd, *J* = 2.7 Hz, 58 Hz, 1 H, OC*H*$_2$F), 5.47 (d, *J* = 2.7 Hz, 53 Hz, 1 H, OC*H*$_2$F), 4.54 (d, *J* = 5.0 Hz, 1 H, H-2′), 4.47 (dd, *J* = 4.4 Hz, 8.8 Hz, 1 H, H-5′), 4.20 (dd, *J* = 4.4 Hz, 9.4 Hz, 1 H, H-3′), 4.07-4.03 (m, 1 H, H-4′), 3.99 (dd, *J* = 8.8 Hz, 10.6 Hz, 1 H, H-5′), 1.07 (s, 9 H, *t*Bu), 1.01 (s, 9 H, *t*Bu). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 162.5, 149.4, 140.8, 103.7, 102.7, 102.0, 93.1, 79.3, 75.7, 74.5, 67.1, 27.3, 27.0, 22.7, 20.3. ^{19}F-NMR (564.4 MHz, $CDCl_3$) δ: -150.00 (dd, *J* = 53 Hz, 58 Hz). HRMS (FAB): *m/z* 417.1874 ([M+H]$^+$, $C_{18}H_{29}N_2O_6FSi^+$ Calcd 417.1857).

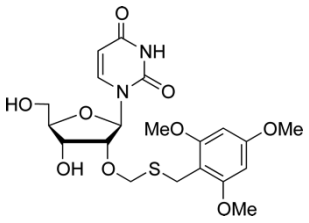
3′,5′-*O*-Di-*tert*-butylsilanediyl-2′-*O*-methyldithiomethyluridine (6).



To a solution of 3′,5′-*O*-di-*tert*-butylsilanediyl-2′-*O*-(2,4,6-trimethoxybenzylthiomethyl)uridine (**4b**) (0.12 g, 0.21 mmol) in anhydrous THF (4 mL) was added dimethyldisulfide (0.50 mL, 5.5 mmol). A suspension of DMTSF (0.10 g, 0.52 mmol) in anhydrous THF (12 mL) was added dropwise to the solution for 5 min. After the reaction mixture was stirred at room temperature for 25 min, sat. $NaHCO_3$ aqueous solution (2 mL) was added. The mixture was poured into sat. $NaHCO_3$ aqueous solution and extracted with ethyl acetate. The organic layer was washed with distilled water and brine, and then dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography. Desired compound **6** was obtained as a colorless foam (91 mg, 0.19 mmol, 91% from **4b**). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.84 (br s, 1 H, N3-H), 7.25 (d, *J* = 8.2 Hz, 1 H, H-6), 5.77 (dd, *J* = 2.2 Hz, 8.2 Hz, 1 H, H-5), 5.71 (s, 1 H, H-1′), 5.21 (d, *J* = 11.7 Hz, 1 H, OC*H*$_2$S), 5.15 (d, *J* = 11.8 Hz, 1 H, OC*H*$_2$S), 4.51-4.46 (m, 2 H, H-2′, 5′), 4.15-4.11 (m, 1 H, H-4′), 4.08-4.05 (m, 1 H, H-3′), 4.01-3.98 (m, 1 H, H-5′), 2.52 (s, 3 H, SC*H*$_3$), 1.05 (s, 9 H, *t*Bu), 1.03 (s, 9 H, *t*Bu). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 163.4, 149.7, 139.7, 102.7,

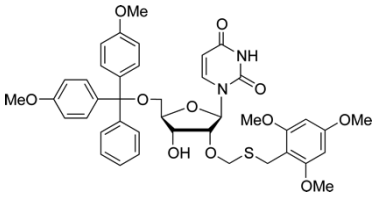
91.4, 80.1, 77.5, 76.4, 74.8, 67.2, 27.2, 27.0, 23.7, 22.7, 20.3. HRMS (FAB): *m/z* 477.1551 ($[M+H]^+$, $C_{19}H_{33}N_2O_6S_2Si^+$ Calcd 477.1549).

2′-*O*-(2,4,6-Trimethoxybenzylthiomethyl)uridine (11).



To a solution of 3′,5′-*O*-di-*tert*-butylsilanediyl-2′-*O*-(2,4,6-trimethoxybenzylthiomethyl)uridine (**4b**) (0.35 g, 0.57 mmol) in THF (7.5 mL) was added $Et_3N{\cdot}3HF$ (131 μL, 0.81 mmol). The reaction mixture was stirred at room temperature for 20 min. The mixture was added to sat. $NaHCO_3$ aqueous solution and extraction was carried out with ethyl acetate. The organic layer was washed with distilled water and brine, and then dried over anhydrous sodium sulfate, filtered, and concentrated. The crude mixture was purified by silica gel column chromatography. Desired compound **11** was isolated as a colorless foam (0.24 g, 0.51 mmol, 89% from **4b**). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.59 (br s, 1 H, N3-H), 7.61 (d, *J* = 8.0 Hz, 1 H, H-6), 6.14 (s, 2 H, Ar-H), 5.71 (dd, *J* = 2.4 Hz, 7.9 Hz, 1 H, H-5), 5.69 (d, *J* = 4.7 Hz, 1 H, H-1′), 4.94 (d, *J* = 12.3 Hz, 1 H, OCH_2S), 4.77 (d, *J* = 12.2 Hz, 1 H, OCH_2S), 4.41-4.40 (m, 1 H, H-2′), 4.37 (dd, *J* = 5.3 Hz, 10.0 Hz, 1 H, H-3′), 4.07-4.06 (m, 1 H, H-4′), 3.99-3.96 (m, 1 H, H-5′), 3.88 (s, 2 H, SCH_2Ar), 3.84-3.80 (m, 1 H, H-5′), 3.83 (s, 6 H, OCH_3), 3.81 (s, 3 H, OCH_3), 3.05 (d, *J* = 4.3 Hz, 1 H, OH-3′), 2.70-2.69 (m, 1 H, OH-5′). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 163.7, 160.5, 158.7, 150.3, 141.8, 106.6, 102.2, 90.6, 89.9, 85.1, 79.9, 74.5, 68.8, 61.4, 55.8, 55.3, 23.4. HRMS (FAB): *m/z* 470.1360 ($[M]^+$, $C_{20}H_{26}N_2O_9S^+$ Calcd 470.1359).

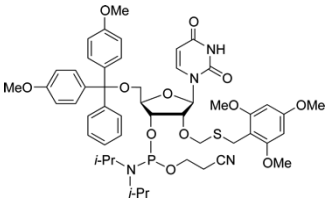
2′-*O*-(2,4,6-Trimethoxybenzylthiomethyl)-5′-*O*–(4,4′-dimethoxytrityl)uridine (12).



To a solution of 2′-*O*-(2,4,6-trimethoxybenzylthiomethyl)-uridine (**11**) (0.24 g, 0.52 mmol) in anhydrous pyridine (7.5 mL) was added 4,4′-dimethoxytrityl chloride (0.21 g, 0.62 mmol). The reaction mixture was stirred at room temperature for 1.5 h. After the addition of ethanol (2 mL), the volatile materials were evaporated. The residue was dissolved with chloroform and then washed with sat. $NaHCO_3$ aqueous solution. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated. The crude mixture was purified by silica gel column chromatography. 2′-*O*-(2,4,6-trimethoxybenzylthiomethyl)-5′-*O*-(4,4′-dimethoxytrityl)uridine (**12**) was obtained as a colorless foam (0.38 g, 0.48 mmol, 95% from **11**). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.24 (br d, *J* = 1.8 Hz, 1 H, N3-H), 7.91 (d, *J* = 8.2 Hz, 1 H, H-6), 7.39-7.38 (m, 2 H, Ar-H), 7.32-7.23 (m, 7 H, Ar-H), 6.87-6.82 (m, 4 H, Ar-H), 6.13 (s, 2 H, Ar-H), 5.95 (d, *J* = 3.3 Hz, 1 H, H-1′), 5.26 (dd, *J* = 2.4 Hz, 8.3 Hz, 1 H, H-5), 4.98 (d, *J* = 12.1

Hz, 1 H, OCH_2S), 4.80 (d, *J* = 12.0 Hz, 1 H, OCH_2S), 4.47 (dd, *J* = 6.3 Hz, 11.9 Hz, 1 H, H-3′), 4.37 (dd, *J* = 3.2 Hz, 5.3 Hz, 1 H, H-2′), 4.05-4.03 (m, 1 H, H-4′), 3.89 (d, *J* = 12.6 Hz, 1 H, SCH_2Ar), 3.83 (d, *J* = 12.6 Hz, 1 H, SCH_2Ar), 3.809 (s, 6 H, 2,6-OCH_3 × 2), 3.801 (s, 3 H, 4-OCH_3), 3.798 (s, 6 H, 4-OCH_3 × 2), 3.50 (d, *J* = 2.4 Hz, 2 H, H-5′), 2.95 (d, *J* = 6.8 Hz, 1 H, O*H*-3′). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 163.3, 160.5, 158.8, 158.6, 150.1, 144.3, 140.1, 135.3, 135.0, 130.2, 130.1, 128.1, 128.0, 127.1, 113.2, 106.7, 102.0, 90.6, 87.2, 87.0, 83.7, 79.6, 73.8, 68.8, 61.9, 55.8, 55.3, 55.2, 23.0. HRMS (FAB): *m/z* 772.2670 ($[M]^+$, $C_{41}H_{44}N_2O_{11}S^+$ Calcd 772.2665).

3′-*O*-[(2-Cyanoethyl)-(*N*,*N*-diisopropylamino)phosphoramidyl]-2′-*O*-(2,4,6-trimethoxybenzylthiomethyl)-5′-*O*-(4,4′-dimethoxytrityl)uridine (13).



To a solution of compound **12** (0.65 g, 0.83 mmol) in anhydrous dichloromethane (8 mL) were added 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylaminophosphorodiamidite (0.50 μL, 1.58 mmol) and diisopropylammonium tetrazolide (71.1 mg, 0.41 mmol). After the reaction mixture was stirred for 15 h at room temperature, the mixture was extracted with chloroform. The organic layer was washed sat. $NaHCO_3$ aqueous solution and then dried over anhydrous sodium sulfate, filtered, and concentrated. The crude mixture was purified by silica gel column chromatography. Desired compound **13** was obtained as a colorless foam (0.75 g, 0.77 mmol, 92% from **12**). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.95 (br s, 1 H), 7.839 (d, *J* = 8.2 Hz, 0.5 H), 7.836 (d, *J* = 8.2 Hz, 0.5 H), 7.42-7.37 (m, 2 H), 7.33-7.22 (m, 7 H), 6.87-6.82 (m, 4 H), 6.16-6.07 (m, 3 H), 5.25 (t, *J* = 8.1 Hz, 1 H), 5.00 (t, *J* = 12.6 Hz, 1 H), 4.82-4.74 (m, 1 H), 4.72-4.69 (m, 1 H), 4.53-4.47 (m, 1 H), 4.30 (br d, *J* = 2.5 Hz, 0.5 H), 4.20 (br d, *J* = 2.7 Hz, 0.5 H), 3.99-3.67 (m, 19 H), 3.67-3.35 (m, 4 H), 2.67 (t, *J* = 6.4 Hz, 1 H), 2.42 (t, *J* = 6.4 Hz, 1 H), 1.18 (dd, *J* = 6.7 Hz, 13.8 Hz, 10 H), 1.03 (d, *J* = 6.9 Hz, 2 H). ^{31}P NMR (161.9 MHz, $CDCl_3$) δ: 150.6, 150.3. HRMS (FAB): *m/z* 973.3828 ($[M+H]^+$, $C_{50}H_{62}N_4O_{12}SP^+$ Calcd 973.3822).

Synthesis of oligonucleotides.

Oligonucleotides were synthesized on a 1 μmol scale on an ABI Model 392 DNA/RNA synthesizer with the trityl-on mode. The standard ABI CE DNA synthesis protocol was employed except for the following points. The coupling wait time was extended to 900 sec for the coupling of compound **13** with 5-ethylthio-1*H*-tetrazole (ETT) as an activator. 0.02 M iodine solution was used for phosphite triester oxidation. Synthesized oligonucleotides were treated with concentrated aqueous ammonia at 55 °C for 10 h. After removal of ammonia in the suspensions, CPGs were removed by filtration with Millex-LG 0.20 μm (Millipore). After the addition of TEAA, the crude oligonucleotides were analyzed and purified by RP-HPLC. The obtained oligonucleotide solutions were concentrated in a Speed-Vac. After the addition of 2.0 M TEAA, the oligonucleotides were applied to a Sep-Pak C18 plus (Waters), and then washed with 100 mM TEAA. Subsequently, the 5′-DMTr group of the oligonucleotides was removed by adding 2% TFA solution and the oligonucleotides were desalted with water.

Conversion of TMBTM-oligonucleotides into REDUCT-oligonucleotides.

Reactions contained 0.1 mM oligonucleotide (**14a**, **15a**, **16a**, or **17a**) and 30 mM dimethyl(methylthio)sulfonium tetrafluoroborate (DMTSF, 300 eq) in 200 mM sodium acetate buffer (pH 4) and were left to stand at room temperature. After the reactions were monitored by RP-HPLC (3～4 h), the excess DMTSF and buffer were removed with a gel filtration column (GE Healthcare NAP-25). Finally, oligonucleotides **14b**, **15b**, **16b**, and **17b** were purified by RP-HPLC.

Evaluation of resistance of REDUCT-oligonucleotides to SVPDE.

Each oligonucleotide (5 nmol) was dissolved in 50 mM Tris-HCl buffer (at pH 8.0, 10 mM $MgCl_2$) (395 μL). To the solution was added SVPDE (8 μg/mL) (5 μL) to adjust the final concentration of each oligonucleotide and SVPDE to 12.5 μM and 0.1 μg/mL. The solution was incubated at 37 °C. Aliquots (40 μL each) were removed from the reaction mixture at 0, 1, 10, 20, 30, 60, 90, and 180 min and each sample was heated at 90 °C for 2 min. After cooling to room temperature, the samples were analyzed by RP-HPLC.

Evaluation of resistance of REDUCT-oligonucleotides to fetal bovine serum.

Each oligonucleotide (5 nmol) was incubated at 37 °C in 5% fetal bovine serum (400 μL). Aliquots (20 μL) were removed from the reaction mixtures at 0, 10, 20, 40, 60, 90, 120, 180 min and the samples were mixed with formamide (20 μL) to terminate the reactions. The samples were analyzed by RP-HPLC.

Conversion of REDUCT-oligonucleotides into 2′-hydroxyoligonucleotides under DTT conditions.

Reactions contained 0.1 mM oligonucleotide (**14b, 15b, 16b,** or **17b**) and 10 mM 1,4-dithiothreitol (DTT) in 100 mM Tris-HCl buffer (pH 8) and were left to stand at room temperature. After the reactions were monitored by RP-HPLC (1～5 h), DTT was removed from the solution with a gel filtration column (GE Healthcare NAP-25).

Conversion of REDUCT-oligonucleotides into 2′-hydroxyoligonucleotides under glutathione conditions.

Reactions contained 0.1 mM oligonucleotide (**14b or 16b**) and 10 mM glutathione in 50 mM sodium phosphate buffer (pH 7) and were left to stand at room temperature. After the reactions were monitored by RP-HPLC, glutathione was removed from the solution with a gel filtration column (GE Healthcare NAP-25).

Table S1. Sequences and Results of MALDI-TOF Mass Analysis of Synthesized Oligonucleotides

ODN	Sequence (5′-3′)	MALDI-TOF Mass	
		calcd. $[M-H]^-$	found
14a	d(GCGTT**X**TTTGCT)[a]	3860.6	3859.1
14b	d(GCGTT**Y**TTTGCT)[b]	3726.5	3727.9
14c	d(GCGTT**Z**TTTGCT)[c]	3634.3	3634.6
15a	d(GCGTT**X**T**X**TGCT)[a]	4088.9	4090.0
15b	d(GCGTT**Y**T**Y**TGCT)[b]	3820.7	3820.2
15c	d(GCGTT**Z**T**Z**TGCT)[c]	3636.3	3636.9
16a	d(GCG**X**T**X**T**X**TGCT)[a]	4317.2	4316.4
16b	d(GCG**Y**T**Y**T**Y**TGCT)[b]	3914.8	3914.4
16c	d(GCG**Z**T**Z**T**Z**TGCT)[c]	3638.3	3637.1
17a	d(GCGTT**XXX**TGCT)[a]	4317.2	4318.4
17b	d(GCGTT**YYY**TGCT)[b]	3914.8	3914.4
17c	d(GCGTT**ZZZ**TGCT)[c]	3638.3	3638.6
18	d(TTTTTTTT**Y**T)[b]	3073.1	3073.4

[a] **X**: 2′-*O*-TMBTM-uridine, [b] **Y**: 2′-*O*-methyldithiomethyl-uridine, [c] **Z**: uridine.

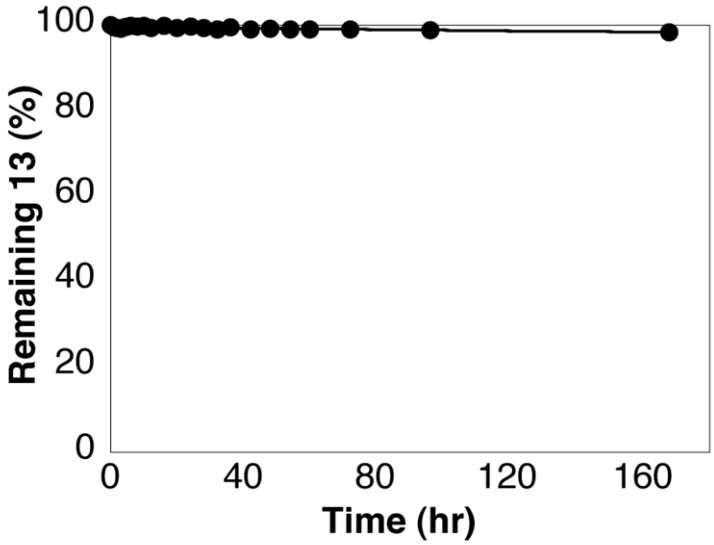


Figure S1. Stability of 2'-*O*-TMBTM amidite **13** in acetonitrile-d_3 (0.1 M) at 25 °C. ^{31}P NMR spectra were periodically measured at 25 °C and the ratio of the remaining amidite **13** was plotted.

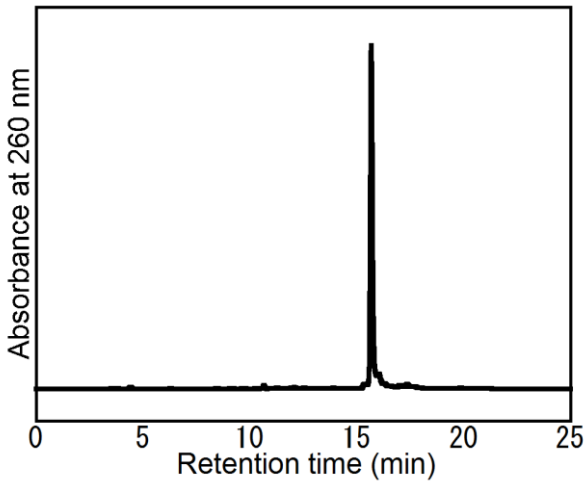


Figure S2. HPLC analysis of crude 5'-*O*-DMTr oligonucleotide after ammonia treatment, which was deprotected by acid treatment to afford **15a**. A COSMOSIL 5C18-MS-II ϕ 4.6 × 250 mm HPLC reversed-phase column was used at the flow rate of 1 mL/min. The solvent system was 50 mM TEAA (pH 7.0) and acetonitrile, and the oligonucleotide was eluted with a linear gradient from 10 to 50 % acetonitrile in 20 min (detection at 260 nm).

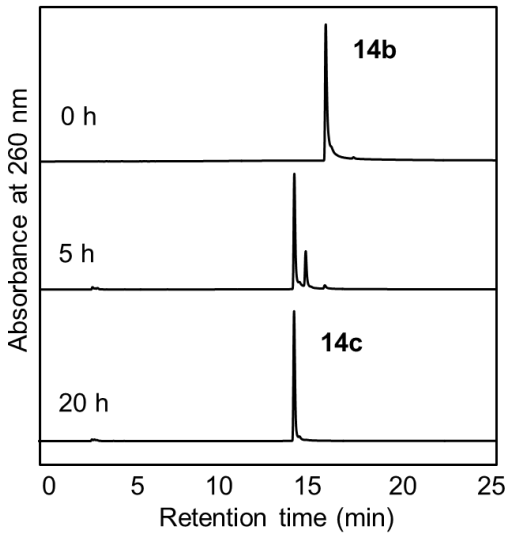


Figure S3. HPLC analysis of the reductive conversion of **14b** into **14c** by treatment with 10 mM glutathione (pH 7.0). A COSMOSIL 5C18-MS-II φ 4.6 × 250 mm HPLC reversed-phase column was used at the flow rate of 1 mL/min. The solvent system was 50 mM TEAA (pH 7.0) and acetonitrile, and the oligonucleotide was eluted with a linear gradient from 5 to 20% acetonitrile in 20 min (detection at 260 nm).

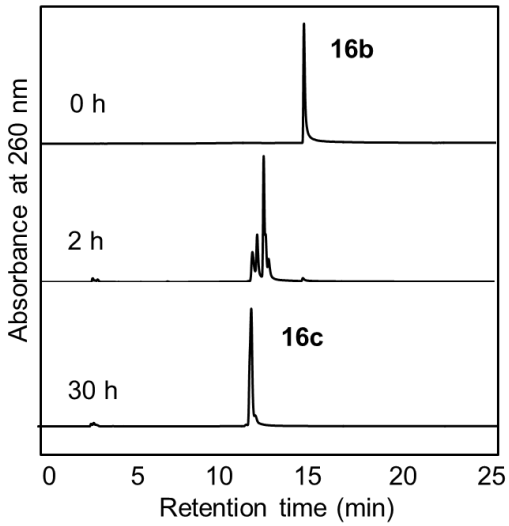


Figure S4. HPLC analysis of the reductive conversion of **16b** into **16c** by treatment with 10 mM glutathione (pH 7.0). A COSMOSIL 5C18-MS-II φ 4.6 × 250 mm HPLC reversed-phase column was used at the flow rate of 1 mL/min. The solvent system was 50 mM TEAA (pH 7.0) and acetonitrile, and the oligonucleotide was eluted with a linear gradient from 5 to 30% acetonitrile in 20 min (detection at 260 nm).

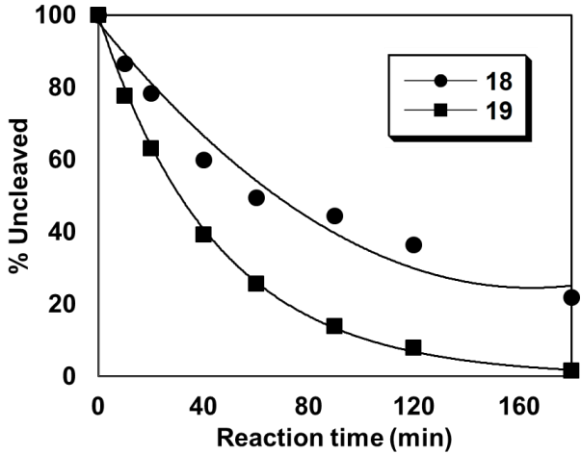


Figure S5. Kinetics of degradation of oligonucleotides 5′-d(TTTTTTTTU$_{MDTM}$T)-3′ **18** and 5′-d(TTTTTTTTTT)-3′ **19** with 5% fetal bovine serum at 37 °C.