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Supporting Information for:

Synthesis of Thymine Glycol-containing Oligonucleotides Using a Building Block with the Oxidized Base

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General methods

All solvents and reagents were obtained from Wako Pure Chemical Industries (Osaka, Japan), except for *tert*-butyldimethylchlorosilane (Shin-Etsu Chemical Co., Tokyo, Japan), (2-cyanoethyl)-*N*,*N*-diisopropylchlorophosphoramidite (Digital Specialty Chemicals, Ontario, Canada), and tetrabutylammonium fluoride (Tokyo Chemical Industry Co., Tokyo, Japan). Reagents for the DNA synthesis were purchased from PE Biosystems Japan (Tokyo, Japan). TLC analyses were carried out on Merck Silica gel 60 F_{254} plates, which were visualized by UV illumination at 254 nm and spraying with an anisaldehyde-sulfuric acid solution, followed by heating. For silica gel column chromatography, either Wakogel C-200 or C-300 (Wako Pure Chemical Industries) was used.

^{1}H NMR spectra were measured on a Bruker DPX300 spectrometer, using tetramethylsilane (TMS) as an internal standard. COSY and NOESY spectra were used for the signal assignment and the configuration determination, respectively. ^{31}P NMR spectra were measured on the same spectrometer at 121.5 MHz, using trimethyl phosphate as an internal standard. Mass spectra were obtained on a ThermoQuest TSQ-700, JEOL HX-110, or Hitachi M-4000H spectrometer.

HPLC analyses were carried out on a Gilson gradient-type analytical system equipped with a Waters 996 photodiode array detector. A µBondasphere C18 5µm 300 Å column (3.9 × 150 mm, Waters Corporation, Milford, MA, USA) was used with a linear gradient of acetonitrile in 0.1 M triethylammonium acetate (pH 7.0).

5'-*O*-(4,4'-Dimethoxytrityl)-3'-*O*-benzoyl-(5*R*,6*S*)-5,6-dihydro-5,6-dihydroxythymidine (3)

A solution of 5'-*O*-(4,4'-dimethoxytrityl)-3'-*O*-benzoylthymidine (**2**, 2.55 g, 3.93 mmol) and osmium tetroxide (1.0 g, 3.93 mmol) in pyridine (15 ml) was stirred at room temperature for 2 h. To this solution, sodium hydrogen sulfite (1.8 g) dissolved in water (30 ml) and pyridine (20 ml) was added, and the mixture was stirred for 30 min. The product was extracted with chloroform (150 ml in total), and the organic layer was dried with Na_2SO_4. After evaporation, the pyridine was removed by coevaporation with toluene, and the residue was chromatographed on silica gel (70 g). The minor isomer, which was eluted faster on silica gel, was separated at this step. The product was eluted with 0.5% methanol in chloroform and was obtained as a foam after evaporation. Yield: 2.03 g (2.97 mmol, 76%). TLC ($CHCl_3$/MeOH, 10/1): R_f: 0.57. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 8.06 (d, ^3J(H,H) = 7 Hz, 2H; Bz), 7.88 (s, 1H; -NH-), 7.59 (t, ^3J(H,H) = 7 Hz, 1H; Bz), 7.50–7.43 (m, 4H; Bz, DMT), 7.40–7.15 (m, 7H; DMT), 6.85 (d, ^3J(H,H) = 9 Hz, 4H; DMT), 6.34 (dd, ^3J(H,H) = 9 Hz, 5 Hz, 1H; H1'), 5.72 (d, ^3J(H,H) = 6 Hz, 1H; H3'), 5.26 (s, 1H; H6), 4.22 (br, 1H; H4'), 3.78 (s, 6H; -OCH_3), 3.62 (br, 1H; 5-OH), 3.57 (dd, ^3J(H,H) = 10 Hz, 4 Hz, 1H; H5'), 3.32 (dd, ^3J(H,H) = 10 Hz, 3 Hz, 1H; H5'), 2.98 (br, 1H; 6-OH), 2.83–2.69 (m, 1H; H2'), 2.49 (dd, ^3J(H,H) = 14 Hz, 6 Hz, 1H; H2"), 1.35 (s, 3H; -CH_3). FABHRMS: *m*/*z*: 681.2451 (M^-, $C_{38}H_{37}N_2O_{10}$: 681.2448).

5*S*,6*R*-Isomer ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 8.04 (d, ^3J(H,H) = 7 Hz, 2H; Bz), 7.87 (s, 1H; -NH-), 7.61 (t, ^3J(H,H) = 7 Hz, 1H; Bz), 7.50–7.13 (m, 11H;

Bz, DMT), 6.84 (d, ^3J(H,H) = 9 Hz, 4H; DMT), 6.49 (t, ^3J(H,H) = 7 Hz, 1H; H1'), 5.65–5.60 (m, 1H; H3'), 5.02 (s, 1H; H6), 4.24 (br, 1H; H4'), 3.78 (s, 6H; -OCH_3), 3.68 (br, 1H; 5-OH), 3.50 (dd, ^3J(H,H) = 11 Hz, 4 Hz, 1H; H5'), 3.39 (dd, ^3J(H,H) = 11 Hz, 3 Hz, 1H; H5'), 3.32 (br, 1H; 6-OH), 2.47–2.39 (m, 2H; H2', H2"), 1.39 (s, 3H; -CH_3).

5'-*O*-(4,4'-Dimethoxytrityl)-3'-*O*-benzoyl-(5*R*,6*S*)-5,6-dihydro-5,6-di[(*tert*-butyl)dimethylsilyloxy]thymidine (4)

A solution of 5'-*O*-(4,4'-dimethoxytrityl)-3'-*O*-benzoyl-(5*R*,6*S*)-5,6-dihydro-5,6-dihydroxythymidine (**3**, 1.80 g, 2.64 mmol), imidazole (1.80 g, 26.4 mmol), and *tert*-butyldimethylchlorosilane (1.99 g, 13.2 mmol) in *N*,*N*-dimethylformamide (15 ml) was stirred at 37°C for 24 h. This mixture was diluted with chloroform (100 ml) and was washed with 0.5 M sodium phosphate (pH 5.0). The organic layer was dried with Na_2SO_4, and after evaporation and coevaporation with toluene, the residue was chromatographed on silica gel (60 g) with a step gradient of ethyl acetate in hexane. The product was eluted with 15% ethyl acetate in hexane and was obtained as a foam after evaporation. Yield: 1.98 g (2.17 mmol, 82%). TLC (hexane/ethyl acetate, 3/2): R_f: 0.71. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 8.05 (d, ^3J(H,H) = 7 Hz, 2H; Bz), 7.60 (t, ^3J(H,H) = 7 Hz, 1H; Bz), 7.51–7.41 (m, 4H; Bz, DMT), 7.37–7.18 (m, 7H; DMT), 7.17 (s, 1H; -NH-), 6.80 (d, ^3J(H,H) = 9 Hz, 4H; DMT), 6.10 (dd, ^3J(H,H) = 10 Hz, 5 Hz, 1H; H1'), 5.64–5.57 (m, 1H; H3'), 4.78 (s, 1H; H6), 4.13 (dd, ^3J(H,H) = 7 Hz, 4 Hz, 1H; H4'), 3.77 (s, 6H; -OCH_3), 3.42 (dd, ^3J(H,H) = 10 Hz, 5 Hz, 1H; H5'), 3.27 (dd, ^3J(H,H) = 10 Hz, 5 Hz, 1H; H5'), 2.53–2.39 (m, 1H; H2'), 2.28 (dd, ^3J(H,H) = 13 Hz, 5 Hz, 1H; H2"), 1.42 (s, 3H; -CH_3), 0.85, 0.80 (s, 18H; TBDMS), 0.23, 0.22 (s, 6H; TBDMS), 0.12, 0.10 (s, 6H; TBDMS). FABHRMS: *m*/*z*: 909.4207 (M^-, $C_{50}H_{65}N_2O_{10}Si_2$: 909.4178).

5'-*O*-(4,4'-Dimethoxytrityl)-(5*R*,6*S*)-5,6-dihydro-5,6-di[(*tert*-butyl)dimethylsilyloxy]thymidine (5)

5'-*O*-(4,4'-Dimethoxytrityl)-3'-*O*-benzoyl-(5*R*,6*S*)-5,6-dihydro-5,6-di[(*tert*-butyl)dimethylsilyloxy]thymidine (**4**, 1.98 g, 2.17 mmol) was dissolved in a 50 mM solution of potassium carbonate in methanol (90 ml). This mixture was stirred for 2h, and after the solution was cooled in an ice bath, 0.5 M sodium phosphate (pH 5.0, 100 ml) was added. The mixture was extracted with chloroform (150 ml in total). The organic layer was dried with Na_2SO_4, and after evaporation, the residue was chromatographed on silica gel (50 g) with a step gradient of ethyl acetate in hexane. The product was eluted with 25–30% ethyl acetate in hexane and was obtained as a foam after evaporation. Yield: 1.70 g (2.10 mmol, 97%). TLC (hexane/ethyl acetate, 3/2): R_f: 0.34. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 7.41 (d, ^3J(H,H) = 7 Hz, 2H; DMT), 7.33–7.17 (m, 7H; DMT), 7.15 (s, 1H; -NH-), 6.82 (d, ^3J(H,H) = 9 Hz, 4H; DMT), 5.97 (dd, ^3J(H,H) = 8 Hz, 6 Hz, 1H; H1'), 4.64 (s, 1H; H6), 4.40 (dd, ^3J(H,H) = 6 Hz, 3 Hz, 1H; H3'), 3.88–3.80 (m, 1H; H4'), 3.79 (s, 6H; -OCH_3), 3.36 (dd, ^3J(H,H) = 10 Hz, 5 Hz, 1H; H5'), 3.14 (dd, ^3J(H,H) = 10 Hz, 7 Hz, 1H; H5'), 2.32–2.19 (m, 1H; H2'), 2.14–2.04 (m, 1H; H2"), 1.92 (d, ^3J(H,H) = 3 Hz, 1H; 3'-OH), 1.37 (s, 3H; -CH_3), 0.84, 0.82 (s, 18H; TBDMS), 0.22, 0.21 (s, 6H; TBDMS), 0.08, 0.05 (s, 6H; TBDMS). FABHRMS: *m*/*z*: 805.3909 (M^-, $C_{43}H_{61}N_2O_9Si_2$: 805.3916).

5'-*O*-(4,4'-Dimethoxytrityl)-(5*R*,6*S*)-5,6-dihydro-5,6-di[(*tert*-butyl)dimethylsilyloxy]thymidine 3'-[(2-cyanoethyl)-*N*,*N*-diisopropyl]phosphoramidite (6)

To a solution of 5'-*O*-(4,4'-dimethoxytrityl)-(5*R*,6*S*)-5,6-dihydro-5,6-di[(*tert*-butyl)dimethylsilyloxy]thymidine (**5**, 155 mg, 192 μmol) in tetrahydrofuran (1.9 ml), *N*,*N*-diisopropylethylamine (134 μl, 768 μmol) and (2-cyanoethyl)-*N*,*N*-

diisopropylchlorophosphoramidite (86 µl, 384 µmol) were added. This mixture was stirred for 30 min, diluted with ethyl acetate, and washed with 2% $NaHCO_3$ and water. The organic layer was dried with Na_2SO_4, and after evaporation, the residue was chromatographed on silica gel (5 g) with a step gradient of ethyl acetate in hexane containing 0.1% pyridine. The product was eluted with 15% ethyl acetate, and after evaporation, the pyridine was removed by coevaporation with acetonitrile. Yield: 168 mg (167 µmol, 87%). TLC (hexane/ethyl acetate, 3/2): R_f: 0.60. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 8.17 (s, 1H; -NH-), 7.48–7.15 (m, 9H; DMT), 6.87–6.77 (m, 4H; DMT), 6.07–5.98 (m, 1H; H1'), 4.72, 4.69 (s, 1H; H6), 4.57–4.46 (m, 1H; H3'), 4.07–3.99 (m, 1H; H4'), 3.85–3.70 (m, 7H; -OCH_3, -OC$H_2$$CH_2CN$ × 1/2), 3.68–3.48 (m, 3H; -OC$H_2$$CH_2CN$ × 1/2, -C$H$$(CH_3)_2$), 3.30–3.12 (m, 2H; H5'), 2.58 (t, ^3J(H,H) = 6 Hz, 1H; -OCH_2CH_2CN × 1/2), 2.43 (t, ^3J(H,H) = 6 Hz, 1H; -OCH_2CH_2CN × 1/2), 2.30–2.08 (m, 2H; H2', H2"), 1.43, 1.41 (s, 3H; -CH_3), 1.23–1.04 (m, 12H; -CH(C$H_3$$)_2$), 0.84, 0.83, 0.81, 0.80 (s, 18H; TBDMS), 0.23, 0.22 (s, 6H; TBDMS), 0.09, 0.07 (s, 6H; TBDMS). ^{31}P NMR (121.5 MHz, $CDCl_3$, 25°C, trimethyl phosphate): δ = 146.63, 146.15. SIHRMS: *m*/*z*: 1005.4985 (M^-, $C_{52}H_{78}N_4O_{10}PSi_2$: 1005.4990).

Oligonucleotide synthesis

Oligonucleotides were synthesized on a 0.2 µmol scale on a PE Biosystems Model 394 DNA/RNA synthesizer. The thymine glycol building block (**6**) was dissolved in anhydrous acetonitrile at a concentration of 0.1 M. Phosphoramidites bearing the tBPA group for the protection of the exocyclic amino function were used for dA, dG, and dC. The reaction time for the coupling of **6** was prolonged to 5 min. After chain assembly and removal of the 5'-terminal DMT group on the synthesizer, the solid supports containing the oligonucleotides were treated with 28% aqueous ammonia (2 ml) at room temperature for 2 h. The resulting ammoniac solutions were concentrated to dryness on a rotary evaporator equipped with a vacuum pump. The residues were

dissolved in a 1.0 M solution of tetrabutylammonium fluoride in tetrahydrofuran (0.1 ml), and the mixtures were left at room temperature. After 16 h, 0.1 M triethylammonium acetate (pH 7.0, 0.4 ml) was added, and the solutions were desalted, either by dialysis using a Spectra/Por CE (cellulose ester) membrane MWCO: 500 (Spectrum, Houston, TX, USA) or by gel filtration on a NAP 10 column (Amersham Pharmacia Biotech, Uppsala, Sweden). After the analysis shown in Figures 1 and 2, the oligonucleotides were purified under similar HPLC conditions. The yields of the purified 6-, 13-, and 30-mers were 1.0, 2.6, and 3.4 A_{260} units, respectively.

MALDI-TOF MS analysis

Purified oligonucleotides (10 pmol) were dissolved in 10 mM triethylammonium acetate (pH 7.0, 2 µl) and were mixed with a solution (0.7 µl) of 22.5 mg/ml 3-hydroxypicolinic acid and 2.5 mg/ml picolinic acid (F. Kirpekar, E. Nordhoff, L. K. Larsen, K. Kristiansen, P. Roepstorff, F. Hillenkamp, *Nucleic Acids Res.* **1998**, *26*, 2554–2559). The mixtures were put on target wells, and the solvent was allowed to evaporate. Measurements were performed on a PerSeptive Biosystems Voyager Elite mass spectrometer.