Supplementary Material (ESI) for Chemical Communications

Supporting Information

Pyrimidine-Pyrimidine Base Pairs Stabilized by Silver(I) Ions

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1. General methods

Commercially available reagents were used without further purification. *N*,*N*-di-*n*-butylformamide dimethyl acetal was synthesized from *N*,*N*-dimethylformamide dimethyl acetal according to the literature procedure.[1] 2'-Deoxy-5-methylisocytidine was synthesized by the method of Tor et al.[2] Thin-layer chromatography was carried out on Merck coated plates $60F_{254}$. Silica gel column chromatography was performed with Wako gel C-400 HG silica gel. ^{1}H- and ^{31}P-NMR spectra were obtained by a Varian mercury 300 or Varian UNITY INOVA-500 spectrometer. Chemical shifts were measured relative to internal tetramethylsilane for $CDCl_3$ and d_6-DMSO for ^{1}H-NMR and to external 85% phosphoric acid for ^{31}P-NMR, and are given in ppm. Coupling constants (*J*) are given in Hz. FAB mass spectra were recorded on a JEOL JMS-700 spectrometer. HPLC analyses were performed on a Shimadzu LC-10A system. A µBondasphere C18 5µm 100Å column (3.9×150 mm, Waters) was used with a linear gradient of acetonitrile in 50 mM triethylammonium acetate (TEAA, pH 7.0). Matrix-assisted laser desorption/ionization time-of-flight (MALDI TOF) mass spectra were acquired on a Voyager-DE™ STR (Applied Biosystems) with 3-hydroxypicolinic acid as the matrix.

2. Synthesis of 2'-deoxy-5-methylisocytidine phosphoramidite

2'-Deoxy-2-{[*N*,*N*-di(*n*-butyl)amino]methylidene}amino-5-methylisocytidine (1).

To a solution of 2'-deoxy-5-methylisocytidine (1.13 g, 5 mmol) in dry DMF (24 mL) was added *N*,*N*-di-*n*-butylformamide dimethyl acetal (1.76 mL, 7.5 mmol). After the mixture was stirred for 45 min at r.t., the reaction was quenched with methanol. The solvent was removed under reduced pressure and the residue was purified by column chromatography (0-6% methanol in $CHCl_3$) to give **1** as a colorless solid (1.58 g, 83%).

^{1}H NMR (300 MHz; DMSO-*d*6): δ 0.89-0.94 (6H, m, $NCH_2CH_2CH_2CH_3$×2); 1.23-1.35 (4H, m, $NCH_2CH_2CH_2CH_3$×2); 1.50-1.60 (4H, m, $NCH_2CH_2CH_2CH_3$×2); 1.78 (3H, d, *J* = 1.1 Hz, 5-CH_3); 2.02-2.11 (2H, m, H-2', H-2"); 3.43 (4H, t, *J* = 7.2 Hz, $NCH_2CH_2CH_2CH_3$×2); 3.60 (2H, m, H-5', H5"); 3.79 (1H, m, H-4'); 4.23 (1H, m, H-3'); 5.08 (1H, t, *J* = 5.2 Hz, 5'-OH); 5.23 (1H, d, *J* = 4.0 Hz, 3'-OH); 6.59 (1H, t, *J* = 6.6 Hz, H-1'); 7.77 (1H, d, *J* = 1.3 Hz, H-6); 8.58 (1H, s, N=CH).

HRMS (FAB) *m*/*z* calcd for $C_{19}H_{33}N_4O_4$ 381.2501 ($[M+H]^+$), found: 381.2505 ($[M+H]^+$).

2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-2-{[*N*,*N*-di(*n*-butyl)amino]methylidene}amino-5-methylisocytidine ***(2)***.

Compound **1** (0.77 g, 2 mmol) was co-evaporated with dry pyridine and dissolved in dry pyridine. To the solution was added 4,4'-dimethoxytrityl chloride (0.61 g, 1.8 mmol) in three portions (every 30 min) at 0°C under stirring. After the mixture was stirred for 6 h at 0°C, the reaction was quenched with saturated aq. $NaHCO_3$ soln. The solvent was removed under reduced pressure and the residue was dissolved in $CHCl_3$. The resulting solution was washed with saturated aq. $NaHCO_3$ soln. and dried with Na_2SO_4, and concentrated. The residue was purified by column chromatography (70-100% $CHCl_3$ in *n*-hexane containing 1% Et_3N) to give **2** as a colorless foam (0.94 g, 68%).

^{1}H NMR (300 MHz; $CDCl_3$): δ 0.93 (3H, t, *J* = 7.3 Hz, $NCH_2CH_2CH_2CH_3$); 0.94 (3H, t, *J* = 7.3 Hz, $NCH_2CH_2CH_2CH_3$); 1.25-1.38 (4H, m, $NCH_2CH_2CH_2CH_3$×2); 1.57-1.62 (4H, m, $NCH_2CH_2CH_2CH_3$×2); 1.67 (3H, s, 5-CH_3); 2.22-2.42 (2H, m, H-2', H-2"); 3.32 (4H, t, *J* = 7.5 Hz, $NCH_2CH_2CH_2CH_3$); 3.38-3.55 (4H, m, H-5', H-5", $NCH_2CH_2CH_2CH_3$); 3.78 (6H, s, -OCH_3×2); 4.06 (1H, m, H-4'); 4.52 (1H, m, H-3'); 6.74 (1H, t, *J* = 6.6 Hz, H-1'); 6.81-7.44 (13H, m, aromatic); 7.62 (1H, s, H-6); 8.83 (1H, s, N=CH).

HRMS (FAB) m/z calcd for $C_{40}H_{51}N_4O_6$ ($[M+H]^+$), 683.3808, found: 683.3795 ($[M+H]^+$).

2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-2-{[*N*,*N*-di(*n*-butyl)amino]methylidene}amino-5-methylisocytidine 3'-*O*-(2-cyanoethyl diisopropylphosphoramidite) (3).

To a solution of compound **2** (3.6 g, 5.3 mmol) in dry CH_2Cl_2 was added diisopropylammonium tetrazolide (0.45 g, 2.6 mmol) and 2-cyanoethyl *N*,*N*,*N*',*N*'-tetraisopropylphosphordiamidite (2 mL, 6.4 mmol). The mixture was stirred for 1 h at r.t. The reaction was quenched with saturated aq. $NaHCO_3$ soln. and the mixture was extracted with $CHCl_3$. The organic layer was dried with Na_2SO_4, and concentrated. The residue was purified by column chromatography (0-2% methanol in ethyl acetate containing 1% Et_3N) to give **2** as a colorless foam (4.3 g, 92%).

^{1}H NMR (300 MHz; $CDCl_3$): δ 0.92-0.98 (6H, m, $NCH_2CH_2CH_2CH_3$×2); 1.04-1.18 (12H, m, $NCH(CH_3)_2$×2); 1.25-1.39 (4H, m, $NCH_2CH_2CH_2CH_3$×2); 1.54-1.67 (7H, m, 5-CH_3, $NCH_2CH_2CH_2CH_3$×2); 2.27-2.61 (4H, m, H-2', H-2", -CH_2CN); 3.30-3.38 (4H, m, $NCH_2CH_2CH_2CH_3$×2); 3.40-3.63 (6H, m, H-5', H-5", $CNCH_2CH_2O$, $NCH(CH_3)_2$×2); 3.79 (6H, s, -OCH_3×2); 4.18 (1H, m H-4'); 4.61 (1H, m, H-3'); 6.74 (1H, m, H-1'); 6.81-7.44

(13H, m, aromatic); 7.66, 7.73 (1H, 2s, H-6); 8.83 (1H, s, CHN). ^{31}P-NMR (202 MHz; $CDCl_3$): δ 148.9, 149.6.
HRMS (FAB) m/z calcd for $C_{49}H_{68}N_6O_7P$ ($[M+H]^+$), 883.4887, found: 883.4885 ($[M+H]^+$).

3. Oligonucleotide synthesis and characterization with MALDI-TOF mass

Oligodeoxyribonucleotides were synthesized on an Applied Biosystems model 392 automated DNA/RNA synthesizer. Reagents for the synthesizer, other than 5-methylisocytosine phosphramidite, were purchased from Applied Biosystems Japan. Deprotection of oligomers containing 2'-deoxy-5-methyisocytidine were performed by prolonged treatment with concentrated aqueous ammonia at 55°C for 16 h for completion of deprotection of the di(*n*-butyl)formamidine group.
d(GAC GTT CTA CG); m/z calcd for $C_{107}H_{137}N_{40}O_{65}P_{10}$ ($[M+H]^+$), 3331.60, found: 3330.97; d(GAC GTC CTA CG); m/z calcd for $C_{106}H_{136}N_{41}O_{64}P_{10}$ ($[M+H]^+$), 3316.60, found: 3316.66; d(GAC GTA CTA CG); m/z calcd for $C_{107}H_{136}N_{43}O_{63}P_{10}$ ($[M+H]^+$), 3340.61, found: 3340.15; d(GAC GTm^{5}iC CTA CG); m/z calcd for $C_{107}H_{138}N_{41}O_{64}P_{10}$ ($[M+H]^+$), 3330.62, found: 3330.60; d(CGT AGT ACG TC); m/z calcd for $C_{107}H_{136}N_{40}O_{65}P_{10}$ ($[M+H]^+$), 3331.60, found: 3331.59; d(CGT AGC ACG TC); m/z calcd for $C_{106}H_{135}N_{41}O_{64}P_{10}$ ($[M+H]^+$), 3316.60, found: 3316.47; d(CGT AGm^{5}iC ACG TC); m/z calcd for $C_{107}H_{137}N_{41}O_{64}P_{10}$ ($[M+H]^+$), 3330.62, found: 3330.09.

4. UV melting experiments

Duplex solutions (5 μM) in 1 M $NaClO_4$, 10 mM MOPS, pH 7.1 were heated at 90ºC and cooled gradually to room temperature. Melting curves were measured at least twice at 270 nm on a JASCO V-560 spectrophotometer equipped with a programmable temperature control unit. The temperature was raised at a rate of 0.5ºC/min. To obtain T_m values, melting curve data were fitted using the Meltwin program[3] (version 3.5), assuming a two-state transition of two non-self-complementary oligonucleotides.

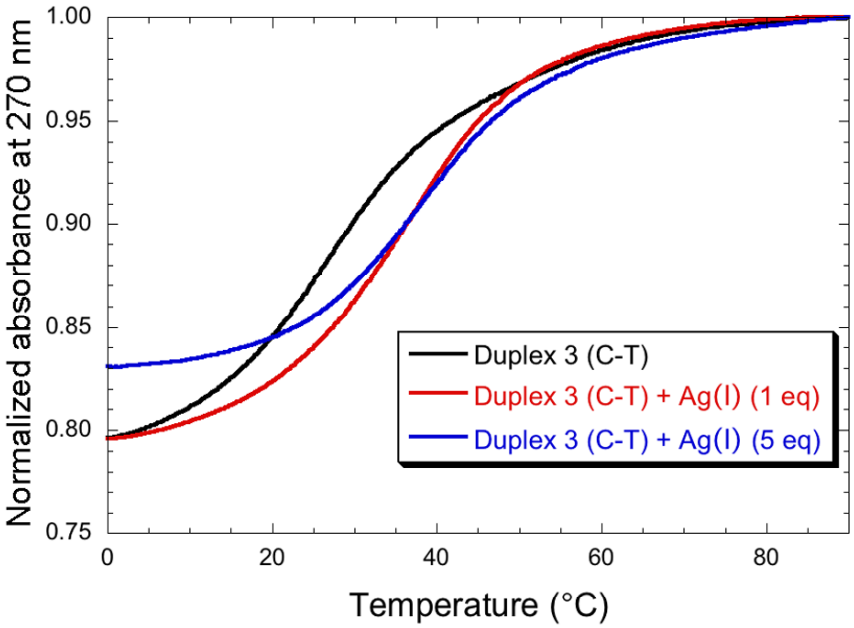

Figure S1. Effects of Ag^{I} ion concentration on the stability of duplex 3. Samples contained 5 μM duplex, 1 M $NaClO_4$, 10 mM MOPS, pH 7.1.

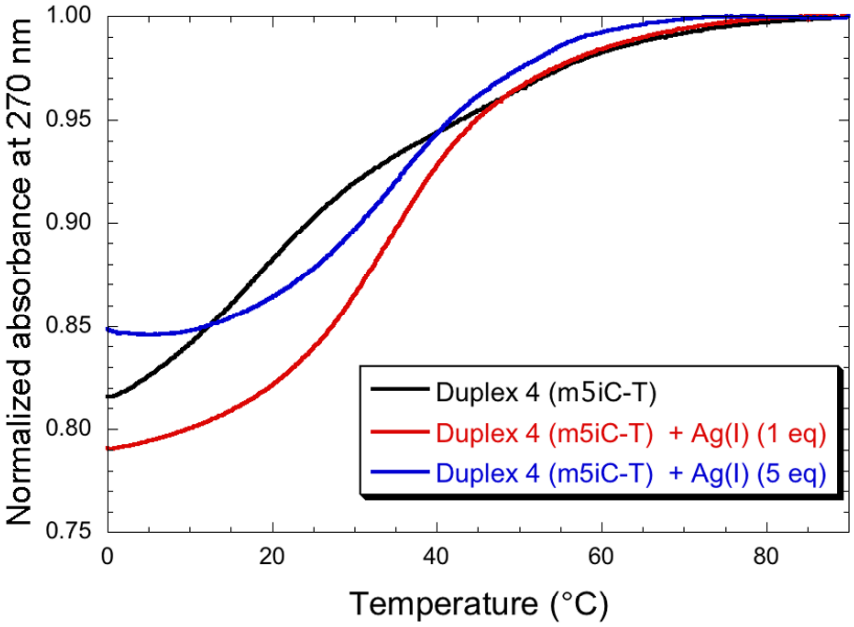

Figure S2. Effects of Ag^{I} ion concentration on the stability of duplex 4. Samples contained 5 μM duplex, 1 M $NaClO_4$, 10 mM MOPS, pH 7.1.

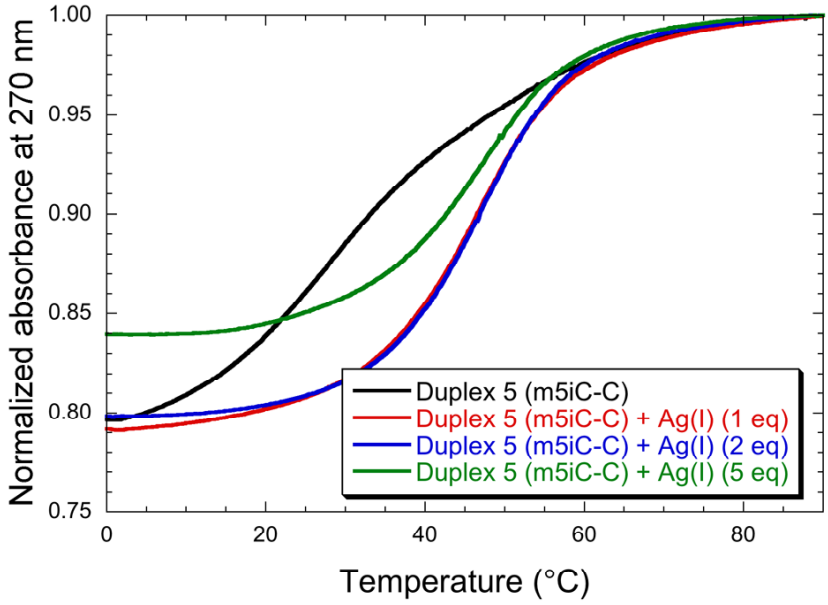

Figure S3. Effects of Ag^{I} ion concentration on the stability of duplex 5. Samples contained 5 μM duplex, 1 M $NaClO_4$, 10 mM MOPS, pH 7.1.

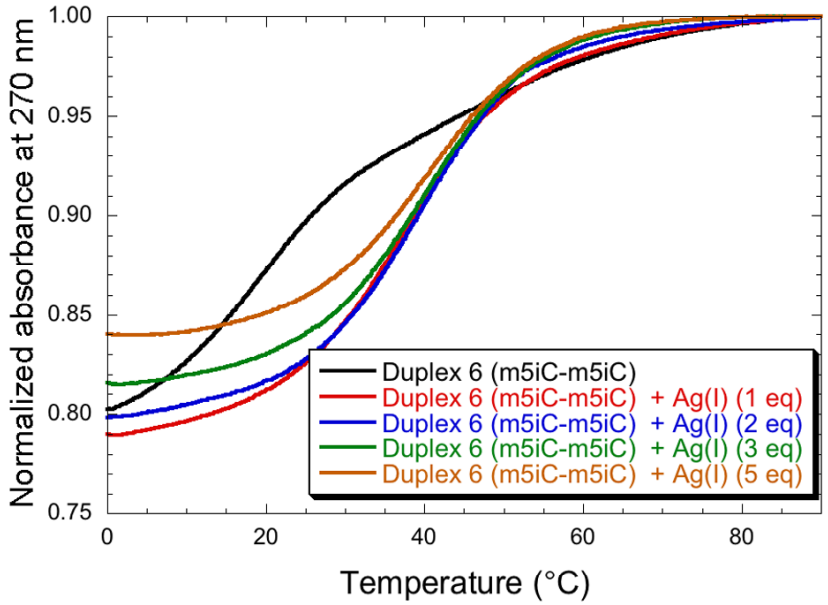

Figure S4. Effects of Ag^{I} ion concentration on the stability of duplex 6. Samples contained 5 μM duplex, 1 M $NaClO_4$, 10 mM MOPS, pH 7.1.

References

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