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 \(\mu\)Bondasphere C18 5\(\mu\)m 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-\(d_6\)): 8 0.89-0.94 (6H, m, NCH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)\(_3\times2\)); 1.23-1.35 (4H, m, NCH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)\(_3\times2\)); 1.50-1.60 (4H, m, NCH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)\(_3\times2\)); 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\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)\(_3\times2\)); 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\(_4\)O\(_4\) 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. NaHCO₃ soln. The solvent was removed under reduced pressure and the residue was dissolved in CHCl₃. The resulting solution was washed with saturated aq. NaHCO₃ soln. and dried with Na₂SO₄, and concentrated. The residue was purified by column chromatography (70-100% CHCl₃ in n-hexane containing 1% Et₃N) to give 2 as a colorless foam (0.94 g, 68%).

\[ \text{1H NMR (300 MHz; CDCl}_3\text{): } \delta 0.93 (3H, t, J = 7.3 Hz, NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3); 0.94 (3H, t, J = 7.3 Hz, NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3); 1.25-1.38 (4H, m, NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3×2); 1.57-1.62 (4H, m, NCH}_2\text{CH}_2\text{CH}_2\text{CH}_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}_2\text{CH}_2\text{CH}_2\text{CH}_3); 3.30-3.38 (4H, t, J = 7.5 Hz, NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3); 3.40-3.63 (6H, s, -OCH}_3×2); 3.79 (6H, s, -OCH}_3×2); 4.06 (1H, m, H-4’); 4.52 (1H, m, H-3’); 6.74 (1H, m, 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 C₄₀H₅₁N₄O₆ ([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 CH₂Cl₂ was added diisopropylammonium tetrazolate (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. NaHCO₃ soln. and the mixture was extracted with CHCl₃. The organic layer was dried with Na₂SO₄, and concentrated. The residue was purified by column chromatography (0-2% methanol in ethyl acetate containing 1% Et₃N) to give 2 as a colorless foam (4.3 g, 92%).

\[ \text{1H NMR (300 MHz; CDCl}_3\text{): } \delta 0.92-0.98 (6H, m, NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3×2); 1.04-1.18 (12H, m, NCH(CH}_3)₂×2); 1.25-1.39 (4H, m, NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3×2); 1.54-1.67 (7H, m, 5-CH}_3), NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3×2); 2.27-2.61 (4H, m, H-2’, H-2” , -CH}_2\text{CN); 3.30-3.38 (4H, m, NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3×2); 3.40-3.63 (6H, m, H-5’, H-5”; CNCH}_2\text{CH}_2\text{O, 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
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(13H, m, aromatic); 7.66, 7.73 (1H, 2s, H-6); 8.83 (1H, s, CHN). $^{31}$P-NMR (202 MHz; CDCl$_3$): $\delta$ 148.9, 149.6.

HRMS (FAB) m/z calcld for C$_{49}$H$_{68}$N$_6$O$_7$P ([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 phosphoramidite, were purchased from Applied Biosystems Japan. Deprotection of oligomers containing 2'-deoxy-5-methylisocytidine 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 calcld 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 calcld 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 calcld for C$_{107}$H$_{136}$N$_{43}$O$_{63}$P$_{10}$ ([M+H]$^+$), 3340.61, found: 3340.15; d(GAC GTm$^5$mC CTA CG); m/z calcld 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 calcld for C$_{107}$H$_{137}$N$_{40}$O$_{65}$P$_{10}$ ([M+H]$^+$), 3331.60, found: 3331.59; d(CGT AGC ACG TC); m/z calcld for C$_{106}$H$_{135}$N$_{41}$O$_{64}$P$_{10}$ ([M+H]$^+$), 3316.60, found: 3316.47; d(CGT AGm$^5$mC ACG TC); m/z calcld 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 90°C 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 0.5°C/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$^+$ 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\(^{1}\) ion concentration on the stability of duplex 4. Samples contained 5 μM duplex, 1 M NaClO\(_4\), 10 mM MOPS, pH 7.1.

![Normalized absorbance at 270 nm vs. Temperature (°C)](image)

**Figure S3.** Effects of Ag\(^{1}\) ion concentration on the stability of duplex 5. Samples contained 5 μM duplex, 1 M NaClO\(_4\), 10 mM MOPS, pH 7.1.

![Normalized absorbance at 270 nm vs. Temperature (°C)](image)
**Figure S4.** Effects of Ag\(^{+}\) ion concentration on the stability of duplex
6. Samples contained 5 μM duplex, 1 M NaClO\(_4\), 10 mM MOPS, pH 7.1.

**References**