- - - - - - - - - **Supporting Information** - - - - - - - - - -

Synthesis of Covalently Linked Parallel and Antiparallel DNA Duplexes Containing the Metal-Mediated Base Pairs T–Hg(II)–T And C–Ag(I)–C

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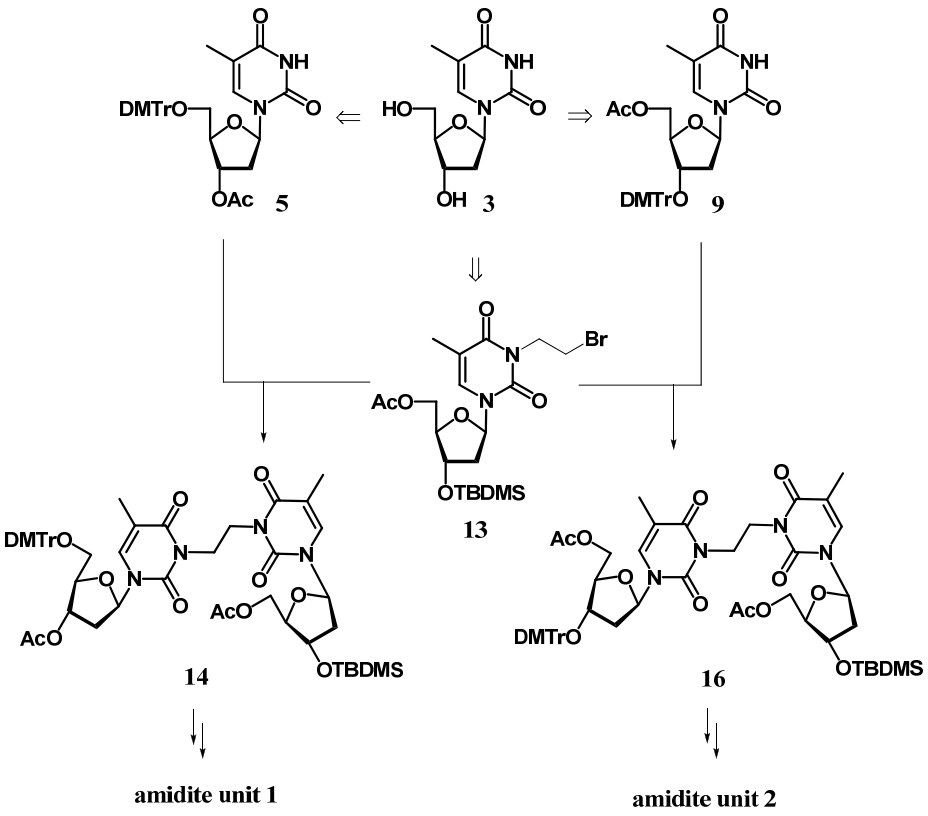
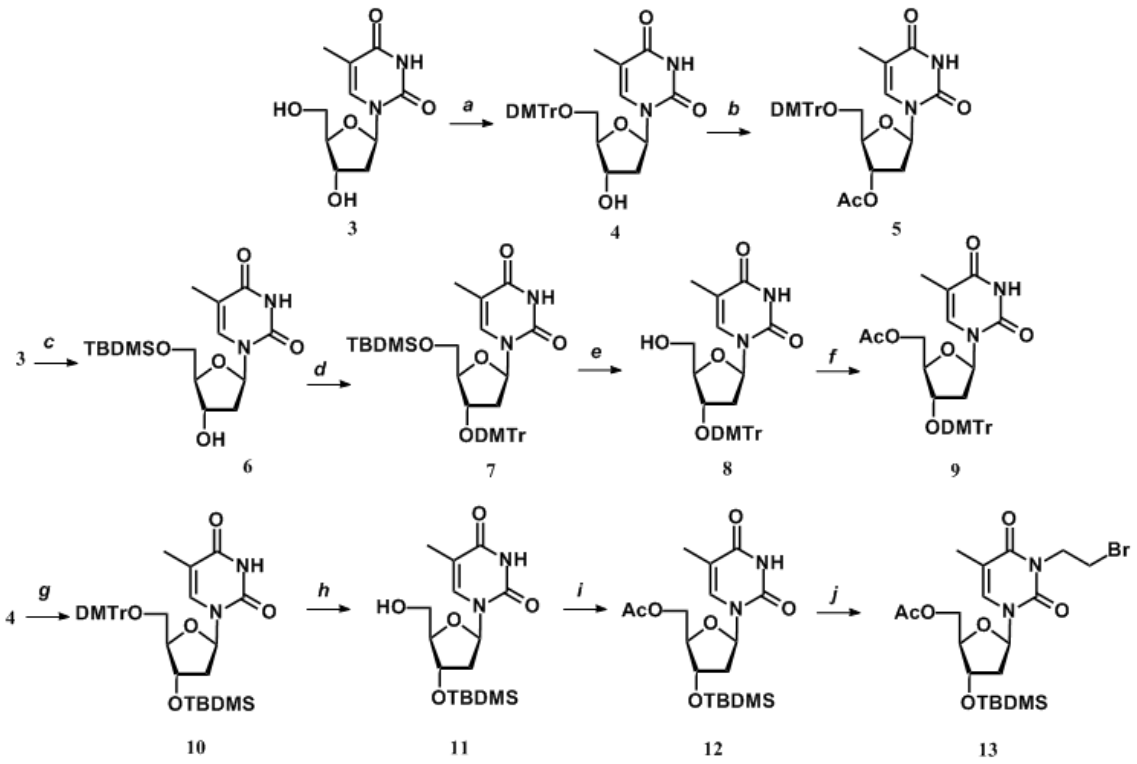
Synthesis of the phosphoramidite units of thymidine dimers 1 and 2

A strategy for the synthesis of phosphoramidite units **1** and **2** is shown in Scheme S1. 5'-*O*-DMTr derivative **5**, 3'-*O*-DMTr derivative **9**, and 3N-(2-bromoethyl) derivative **13** were prepared from thymidine as the starting material. Coupling of the bromoethyl derivative with the protected thymidines **5** or **9** gave the protected thymidine dimers **14** and **15**, which were converted into amidite units by the established method.



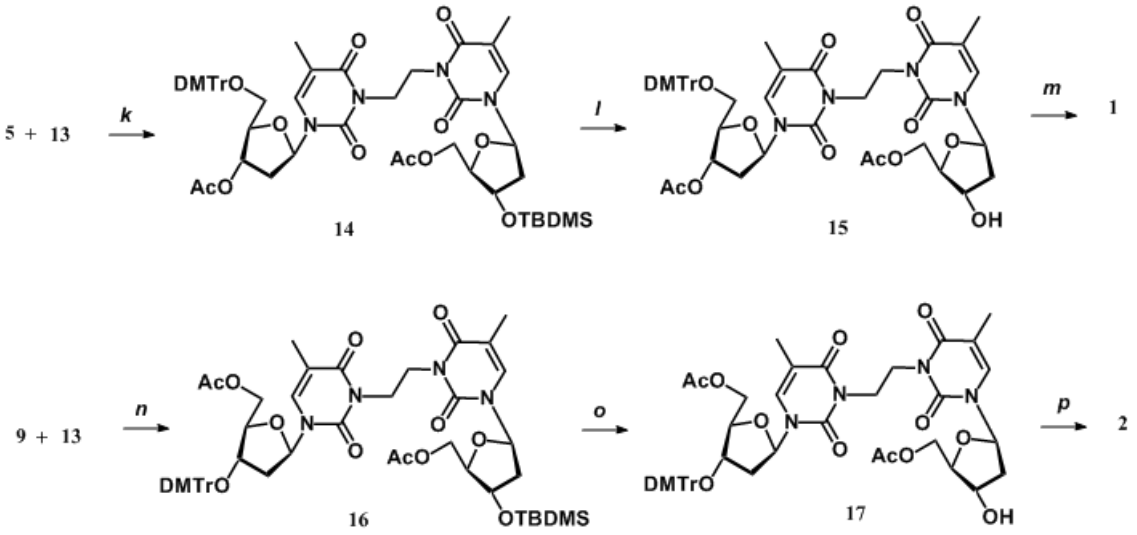
Scheme S1. An illustrative representation of the strategy used to synthesize amidite units **1** and **2**.

Reactions used in the preparation of amidite units **1** and **2** are shown in Scheme S2-1 and Scheme S2-2. The reaction conditions were carefully selected and adjusted to maintain the acetyl groups during the formation of the thymidine dimers. Protected thymidines **5** and **9** were synthesized by established methods.[S1] Also, a sequential protection and de-protection of the hydroxyl groups of thymidine gave **12**, which was directly converted to the N3-(2-bromoethyl) derivative **13** by treating with an excess of 1,2-dibromoethane in the presence of Cs_2CO_3. This method proved much simpler that the previous technique in which a halogenoethyl derivative was synthesized via a hydroxyethyl derivative.[S2] Since halogenoethyl derivatives are not fully stable, the reaction was kept anhydrous and stopped after 30 min. Of the bases evaluated, Cs_2CO_3 provided the best yield.



Scheme S2-1. Synthesis of thymidine derivatives. (a) DMTrCl, pyridine, r.t., 92%. (b) Ac_2O, pyridine, r.t., 88%. (c) *tert*-Butyldimethylsilyl chloride, imidazole, DMF, r.t., 86%. (d) DMTrCl, pyridine, r.t., 95%. (e) Tetrabutylammonium fluoride, THF, r.t., 82%. (f) Ac_2O, pyridine, r.t., 81%. (g) *tert*-Butyldimethylsilyl chloride, imidazole, DMF, r.t., 84%. (h) 1.4% Trichloroacetic acid solution ($MeOH:CH_2Cl_2$ = 3:7), 0 ^oC, 4 h, 76%. (i) Ac_2O, pyridine, r.t., 97%. (j) 1,2-Dibromoethane (20 eq.), Cs_2CO_3 (2 eq.), DMF, r.t., 30 min., 78%.

In the presence of a base, combination reactions of the bromoethyl derivative **13** and the protected thymidine (**5** or **9**) gave the protected thymidine dimers **14** or **16**, which were treated with TBAF to give **15** or **17**, respectively. The thymidine dimers were then purified by silica gel chromatography. Dimers **15** and **17** were converted to phosphoramidite units **1** and **2**, respectively, using the established method.[S3]



Scheme S2-2. Synthesis of thymidine derivatives. (k) DBU (1.2 eq.), CH_3CN, r.t. (l) Tetrabutylammonium fluoride, THF, r.t., 59% (2 steps). (m) *N*,*N*-diisopropylethylamine (2 eq.), 2-Cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (1.4 eq.), CH_2Cl_2, r.t., 74%. (n) DBU (1.2 eq.), CH_3CN, r.t. (o) Tetrabutylammonium fluoride, THF, r.t., 50% (2 steps). (p) 2-Cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (1.4 eq.), *N*,*N*-diisopropylethylamine (2 eq.), CH_2Cl_2, r.t., 88%.

Oligonucleotide synthesis

ODNs were synthesized on a DNA synthesizer (model 392; Applied Biosystems, Foster City, CA) using the phosphoramidite method employing the general protocol recommended by the manufacturer with slight modifications. A longer time (600 s) was allowed for the coupling steps of **1** and **2**. Fully protected oligonucleotides were de-protected and purified by the same procedures as those used for the purification of natural oligonucleotides.[S3]

Structures of synthesized oligonucleotides were confirmed by MALDI-TOF mass spectrometry.

	Calcd. [M-H]	found
3'-AAAAA**T-T**TTTTT-5'	3594.7	3594.2
3'-AAAAA**T-T**TTTTT-3'	3594.7	3594.6
3'-AAAAATAAAAA**T-T**TTTTTTTTTTT-5'	7288.3	7288.5
3'-AAAAATAAAAA**T-T**TTTTTTTTTTT-3'	7288.3	7288.2
3'-AAAAACAAAAA**T-T**TTTTTCTTTTT-5'	7258.3	7258.1
3'-AAAAACAAAAA**T-T**TTTTTCTTTTT-3'	7258.3	7258.1

Thermal denaturation. Prior to the preparation of ODN solutions for thermal denaturation studies, the buffers were degassed *in vacuo* for 20 min and the ODNs were dissolved in appropriate buffers. Each solution was heated at 90°C for 10 min and slowly cooled to room temperature. A thermally induced transition profile of each mixture was obtained by measuring the optical density of the mixture on a UV-1650PC spectrophotometer equipped with the T_m Analysis system (TMSPC-8; Shimadzu, Kyoto, Japan).

Electrospray ionization mass spectroscopy

ESI mass spectra of the linked *ps* duplexes are shown in Figures S1 and S2. ESI mass spectra of complexes consisting of the metal ions and *aps* duplexs containing T-T and C-C pairs have been reported (ref. 8a and 8d). ESI-MS measurements were performed on a time-of-flight mass spectrometer (JMS-T100; JEOL, Tokyo, Japan). The measurement conditions were as follows: needle voltage –1.5 kV; orifice voltage –50 V; desolvation temperature 80–100°C; resolution (10% valley definition) 2000; and sample flow rate 20 μL min^{-1}. For each sample preparation, the aqueous solution containing DNA and Ag(I) ions in 62.5 mM NH_4OAc (pH 6.9) was diluted with MeOH. The duplex concentration was 10 μM, the buffer (NH_4OAc) concentration was 50 mM, and the solvent was H_2O/MeOH (4:1).

In the spectrum of the linked *ps* duplex containing a T–T pair with Hg(II) ions, peaks corresponding to the 1:1 duplex–mercury ion complex were observed (Fig. S1). As no peak corresponding to the 1: 2 duplex–mercury complex was seen in the presence of 2 equivalent Hg(II) ions, the Hg(II) ion and T–T pair were suggested to bind in a 1:1 stoichiometry. In the presence of a 1.5 equivalent of mercury ions, a peak corresponding to the linked *ps* duplex with no Hg(II) ions was detected. These results indicate that the binding of Hg(II) ions and the T–T pair in the *ps* duplex may not be tight. In contrast, in the presence of 1 equivalent silver ion, a large peak corresponding to the 1:1 duplex–silver complex, together with a small peak corresponding to a free linked *ps* duplex containing a C–C pair, was observed. In the presence of a 1.5 equivalent of Ag(I) ions, a peak for the free duplex was absent (Fig. S2). Taken together, the results in the ESI-MS spectroscopy may indicate that the silver ion–C–C pair binding in the *ps* duplex is tighter than the binding of the mercury ion–T–T pair. This is consistent with data obtained from thermal denaturation studies (Fig. 5 in the main text) demonstrating the *ps* duplex–silver complex (T_m = 54ºC) to be more stable than the *ps* duplex–mercury complex (T_m = 47ºC).

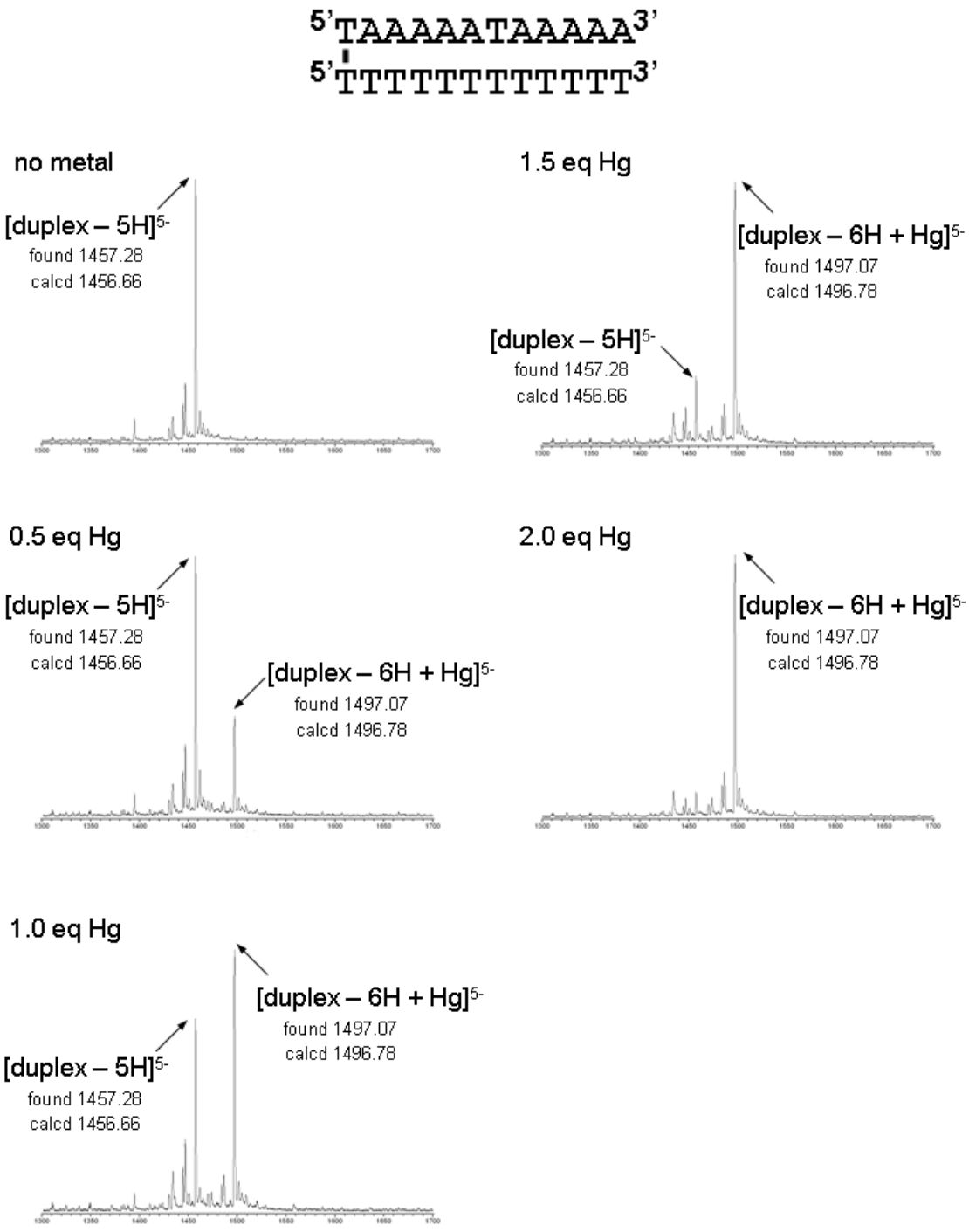


Figure S1. ESI-TOF MS spectra of the linked *ps* duplex containing a T–T pair in the presence or absence of Hg(II) ions.

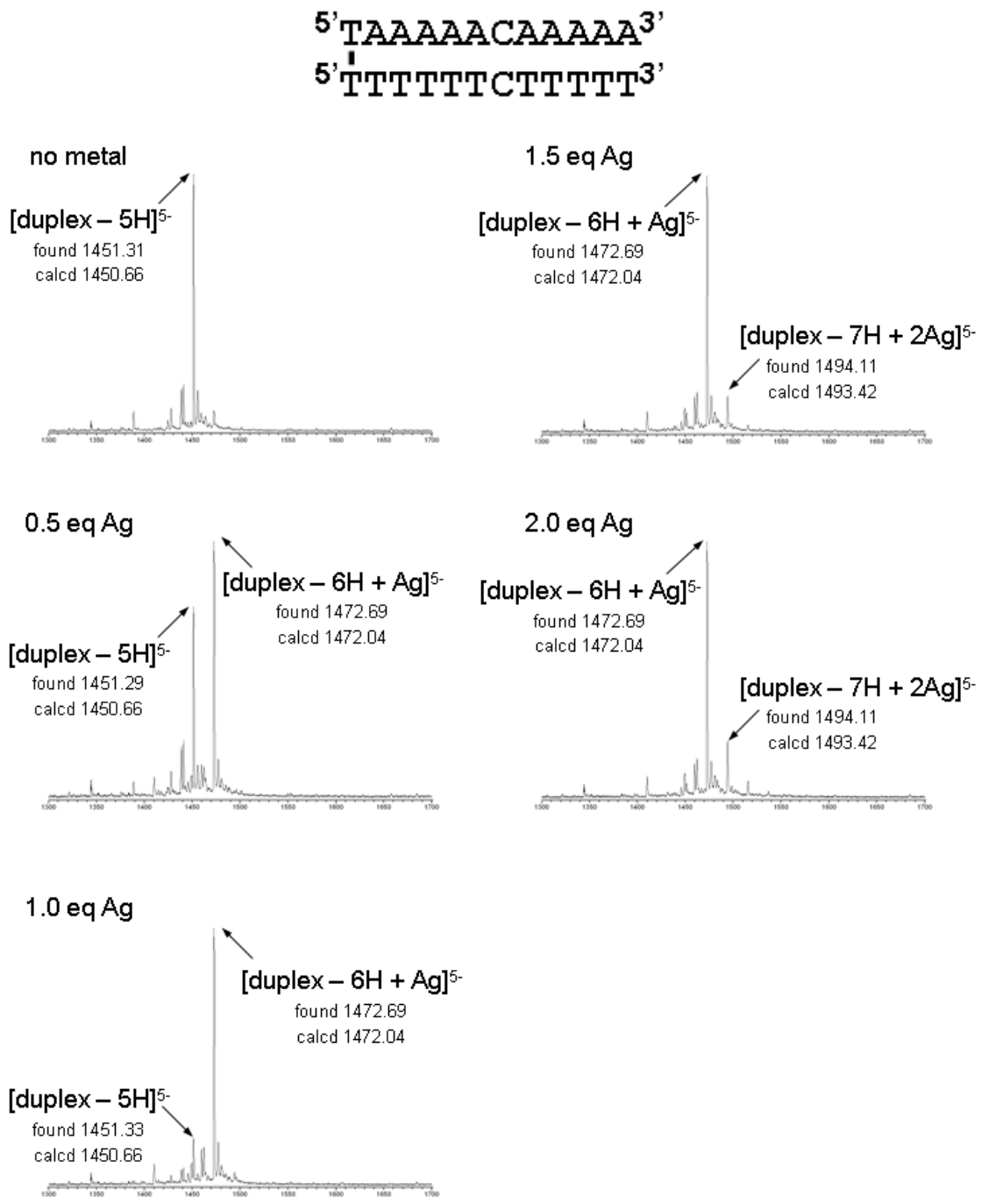


Figure S2. ESI-TOF MS spectra of the linked *ps* duplex containing a C–C pair in the presence or absence of Ag(I) ions.

Experimental Section

General

TLC was performed on Kieselgel F254 pre-coated plates (Merck, Darmstadt, Germany). The silica gel used for column chromatography was Silica Gel 60 (Kanto Chemical Co., Inc., Tokyo, Japan), or Chromatorex NH (Fuji Silysia Chemical Ltd., Kasugai, Japan). ^{1}H-NMR spectra were recorded on an ECA 500 (500 MHz) spectrometer (JEOL, Tokyo, Japan). Chemical shifts were reported in parts per million (δ) and signals were expressed as s (singlet), d (doublet), t (triplet), m (multiplet), or br (broad). All exchangeable protons were identified by their disappearance upon the addition of D_2O. ODNs were synthesized on a DNA synthesizer (model 392; Applied Biosystems) by the phosphoramidite method. Nucleoside-attached control pore glass (Genoglass; Wako, Osaka Japan) was used for a solid support. UV absorption spectra were recorded with a UV 1650PC spectrophotometer (Shimadzu). A system composed of SPD-10A VP, LC-10ATVP, C-R6A (Shimadzu) was used for HPLC analysis and purification. Thermally induced transition profiles of each mixture were obtained by measuring the optical density of each mixture on a UV-1650PC spectrophotometer equipped with the T_m Analysis system (TMSPC-8; Shimadzu). Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectra were recorded on an AXIMA-CFR plus (Shimadzu). The MALDI matrix was prepared by mixing a saturated aqueous 3-hydroxy-2-picolinic acid, an aqueous 5% 2-picolinic acid (w/v), and an aqueous 5% ammonium citrate (w/v) solution at a ratio of 10:1:1.

Synthesis of 13

After dehydration by three co-evaporation cycles with anhydrous 1,4-dioxane, **12** (0.8 g, 2.0 mmol) was dissolved in anhydrous DMF (10 mL). Cs_2CO_3 (1.3 g, 4.1 mmol) was added to the solution and the mixture was stirred at room temperature for 10 min. 1,2-Dibromoethane (3.5 mL, 40 mmol) was then added and the mixture was stirred at room temperature for 30 min. The mixture was then concentrated and the residue was dissolved in ethyl acetate. This solution was washed three times with brine, dried over $MgSO_4$, and concentrated. The residue was separated over a silica gel column (50 g) with an eluent of *n*-hexane and ethyl acetate (7:1). Fractions were combined and concentrated to give **13** (0.8 g, 1.6 mmol, 78%) as a colorless gum.

^{1}H-NMR (500 MHz, DMSO–d_6) 0.00 (6H, s, CH_3–TBDMS), 0.78 (9H, s, *t*-Bu–TBDMS), 1.77 (3H, s, CH_3–5), 1.97 (3H, s, CH_3–Ac), 2.01–2.25 (2H, m, H–2' and H–2"), 3.49 (2H, t, J = 7.5 Hz, –CH_2–Br), 3.84 (1H, dd, J = 4.0 Hz, 4.6 Hz, H–4'), 4.09 (4H, m, N–CH_2– and H–5' and H–5"), 4.33 (1H, m, H–3'), 6.12 (1H, t, J = 6.9 Hz, H–1'), 7.45 (1H, s, H–6).

ESI-MS calcd. for $C_{20}H_{33}BrN_2O_6Si$ ($M + Na^+$) 527.12, found 526.96.

Synthesis of 15

After dehydration by three co-evaporation cycles with anhydrous 1,4-dioxane, **5** (0.7 g, 1.2 mmol) and **13**

(0.7 g, 1.4 mmol) were dissolved in anhydrous CH_3CN (10 mL). DBU (0.2 mL, 1.3 mmol) was added to the solution and the mixture was stirred for 16 min at 50°C. The reaction mixture was concentrated and the residue was dissolved in ethyl acetate. This solution was successively washed with brine and saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated. The residue was separated over a silica gel column (50 g) with an eluent of *n*-hexane and ethyl acetate (1:1). Fractions were combined and concentrated to give **14** as a white foam containing a small amount of **5**.

Bu_4NF (1 M, 1.1 mL, THF solution) was added to a solution containing **14** in THF (10 mL) and the mixture was kept at room temperature for 10 min. The reaction mixture was concentrated and the residue was dissolved in ethyl acetate. The solution was washed with brine, dried over $MgSO_4$, and concentrated. The residue was separated over a silica gel column (30 g) with an eluent of *n*-hexane and ethyl acetate (1:3). Fractions were combined and concentrated to give **15** (0.6 g, 0.7 mmol, 58% from **5**) as a white foam.
^{1}H-NMR (600 MHz, $CDCl_3$) 1.33 (3H, s, CH_3–5), 1.81 (3H, s, CH_3–5), 2.00 (3H, s, CH_3–Ac), 2.04 (3H, s, CH_3–Ac), 2.08 (1H, m, H–2'), 2.32 (1H, m, H–2'), 2.34 (1H, m, H–2'), 2,38 (1H, m, H–2'), 3.32 (1H, dd, *J* = 2.7 Hz, 10.5 Hz, H–5'), 3.42 (1H, dd, *J* = 3.0 Hz, 10 Hz, H–5'), 3.72 (6H, s, OCH_3–DMTr), 4.03–4.30 (9H, m, H–3', H–4' × 2, H–5' × 2 and N–CH_2– and –CH_2–N), 6.08 (1H, t, *J* = 6.6 Hz, H–1'), 5.31 (1H, t, *J* = 2.7 Hz, H–3'), 6.09 (1H, t, *J* = 6.6, H–1'), 6.28 (1H, dd, *J* = 5.7 Hz, 8.1 Hz, H–1'), 6.76–7.37 (13H, m, Ph–DMTr), 7.31 (1H, s, H–6), 7.33 (1H, s, H–6).
^{13}C-NMR, 150 MHz, $CDCl_3$
δ 12.5, 13.4, 14.2, 20.8, 21.0, 29.7, 38.1, 39.3, 39.6, 40.7, 55.2, 60.4, 63.4, 63.7, 71.2, 74.9, 83.6, 84.1, 85.2, 86.1, 87.0, 109.6, 110.2, 113.3, 113.3, 127.1, 128.0, 128.1, 130.1, 130.1, 130.1, 133.4, 144.3, 151.0, 151.2, 158.7, 163.5, 163.7, 170.6, 170.6.
ESI-MS calcd. for $C_{47}H_{51}N_4O_{14}$ ($M–H^+$) 895.34, found 895.40.

Synthesis of 1

After dehydration by successive co-evaporation cycles with anhydrous pyridine (twice) followed by anhydrous toluene, **15** (0.6 g, 0.7 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL). *N*,*N*-diisopropylethylamine (0.4 mL, 2.0 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.3 mL, 1.4 mmol) were added to the solution and the mixture was stirred at room temperature under an Ar atmosphere. After 30 min, EtOH (0.5 mL) was added to the reaction mixture and the whole was kept at room temperature for 5 min. The mixture was concentrated and the residue was dissolved in $CHCl_3$. This solution was washed with saturated aqueous $NaHCO_3$, and the inorganic component was extracted twice with $CHCl_3$. The organic layers were combined, dried over $MgSO_4$, and concentrated. The residue was separated over a silica gel column (N–H type, 21 g) with an eluent of *n*-hexane and ethyl acetate (7:3). Fractions were combined and concentrated to give **1** (0.6 g, 0.5 mmol, 74%) as a white foam.
^{31}P-NMR (172 MHz, $CDCl_3$) δ 149.73 (A single peak was observed even **1** was a mixture of diastereomeres)

ESI-MS calcd. For $C_{56}H_{69}N_6O_{15}P$ ($M + Na^+$) 1119.45, found 1119.67.

Synthesis of 17

Compound **17** was synthesized via the procedure outlined for **15** to afford the desired product in 50% yield (2 steps).

^{1}H-NMR (600 MHz, $CDCl_3$) 1.63 (1H, m, H–2'), 1.72 (3H, s, CH_3–5), 1.78 (3H, s, CH_3–5), 1.92 (3H, s, CH_3–Ac), 2.03 (3H, s, CH_3–Ac), 2.08 (1H, m, H–2'), 2,17 (1H, m, H–2'), 2.33 (1H, m, H–2'), 2.96 (1H, d, J = 4.2 Hz, OH–3'), 3.56 (1H, dd, J = 4.2 Hz, 12 Hz, H–5'), 3.71 (6H, s, OCH_3–DMTr), 3.80 (1H, m, H–5'), 3.84 (1H, dd, J = 2.4 Hz, 12 Hz, H–5'), 4.04 (1H, m, H–5'), 4.14–4.26 (8H, m, H–3'×2, H–4' × 2, and N–CH_2– and –CH_2–N), 6.08 (1H, t, J = 6.6 Hz, H–1'), 6.23 (1H, dd, J = 6.0 Hz, 8.4 Hz, H–1'), 6.76–7.37 (13H, m, Ph–DMTr), 7.38 (1H, s, H–6), 7.38 (1H, s, H–6).

^{13}C-NMR, 150 MHz, $CDCl_3$

δ 13.3 13.3, 20.8, 39.2, 39.4, 39.6, 40.6, 55.2, 55.2, 63.8, 63.9, 71.2, 74.1, 83.5, 84.1, 86.1, 86.2, 87.4, 109.4, 109.5, 113.3, 127.1, 128.0, 128.2, 130.2, 133.1, 133.2, 136.0, 136.0, 144.9, 150.9, 150.9, 158.8, 158.8, 163.5, 163.6, 170.3, 170.6

ESI-MS calcd. for $C_{47}H_{51}N_4O_{14}$ ($M-H^+$) 895.34, found 895.38.

Synthesis of 2

Compound **2** was synthesized via the procedure outlined for **1** to afford the desired product in 88% yield.

^{31}P-NMR (172 MHz, $CDCl_3$) δ 149.0, 149.2 (diastereomers).

ESI-MS calcd. for $C_{56}H_{69}N_6O_{15}P$ ($M + Na^+$) 1119.45, found 1119.47.

MALDI-TOF mass spectrometry

MALDI-TOF mass spectrometry was performed on an AXIMA-CFR plus (Shimadzu). The MALDI matrix was prepared by mixing a saturated aqueous 3-hydroxy-2-picolinic acid, an aqueous 5% 2-picolinic acid (w/v), and an aqueous 5% ammonium citrate solution (W/V) at a ratio of 10:1:1. Negative and reflectron modes were used and the acquired values were corrected using the values for the two ODNs, 5'–T_{10}–3' (M–H; 2977.50) and 5'–T_{20}–3' (M–H; 6017.96), as external controls.

Preparation of samples for thermal denaturation

Oligonucleotides with or without metals were dissolved in appropriate buffer solutions, which were heated at 90°C in a hot water bath for 10 min. The bath temperature was gradually decreased to 4°C and kept at this temperature for 2 h in a refrigerator prior to thermal denaturation. The thermally induced transition profile of each mixture was obtained by measuring the optical density of each mixture on a UV-1650PC spectrophotometer equipped with the T_m analysis system (TMSPC-8; Shimadzu). The temperature ramp was 1ºC min^{-1}. $Hg(ClO_4)_2$ and $AgNO_3$ (Wako Co. LTD, Japan) were used for sample preparations.

References

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