## Electronic Supplementary Information

# Oxy-peptide nucleic acid with a pyrrolidine ring that is configurationally optimized for hybridization with DNA

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### Experimental procedures for synthesis of POPNA(A) monomers and oligomers

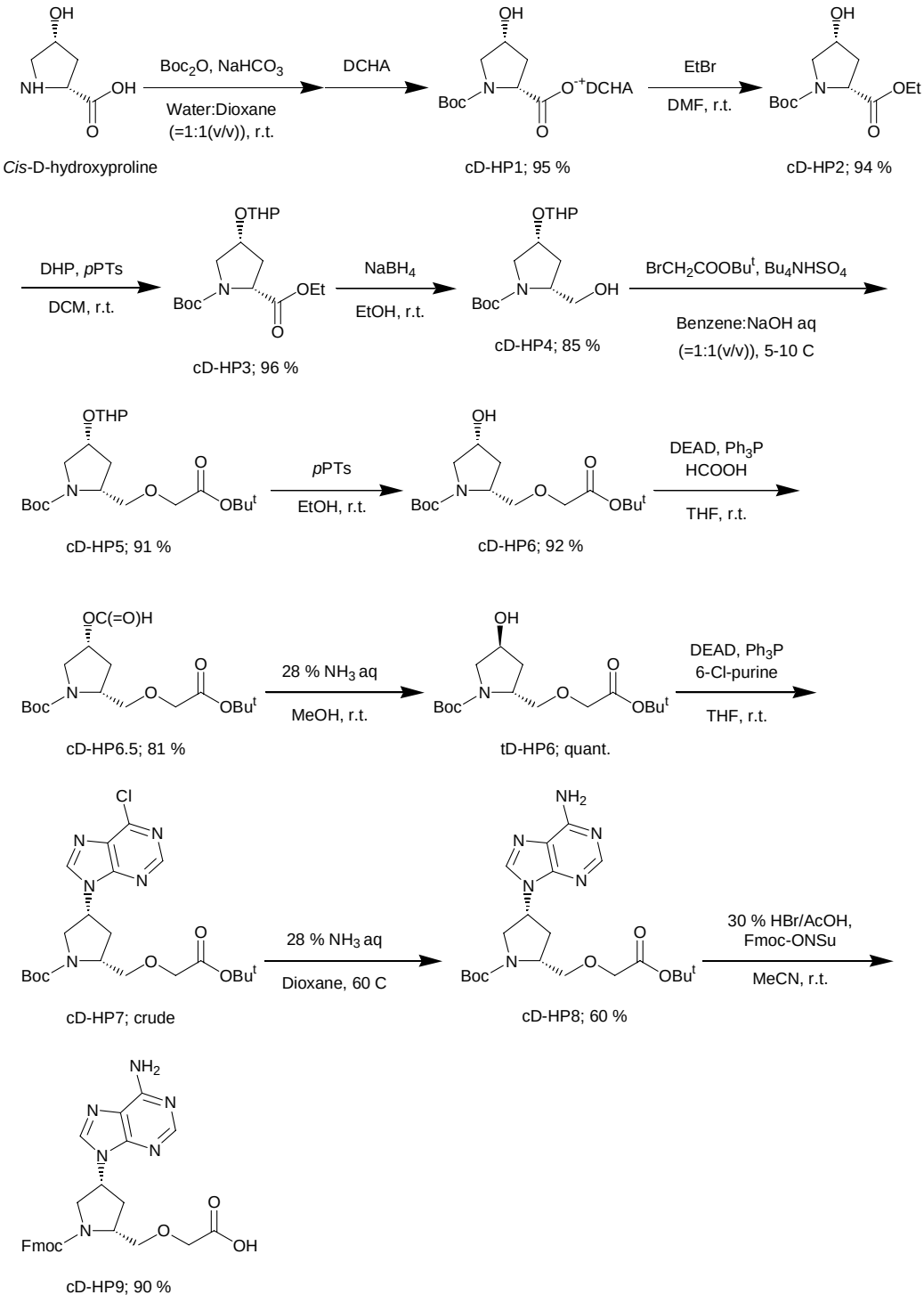
**General** H-D-*cis*-Hyp-OH (*cis*-4-Hydroxy-D-proline) was purchased from Bachem and used without further purification. H-L-*trans*-Hyp-OH (*trans*-4-Hydroxy-L-proline), di-*tert*-butyldicarbonate and 9-fluorenylmethyl succinimidyl carbonate was purchased from Watanabe Chemicals (Hiroshima, Japan) and used without further purification. Formic acid was purchased from Merck and used without further purification. 1,4-Dioxane, $KHSO_4$, $MgSO_4$, $NaBH_4$, bromoethane and 28% aq. $NH_3$ were purchased from Wako Chemicals (Tokyo, Japan) and used without further purification. NaCl, $NaHCO_3$, ethanol, 1,2-dichloroethane, dicyclohexylamine, benzene and NaOH was purchased from Nakarai tesque (Kyoto, Japan) and used without further purification. 3,4-Dihydro-2H-pyran, pyridinium-*p*-toluenesulfonate, tetra-*n*-butylammonium hydrogensulfate, *tert*-butyl bromoacetate and 6-chloropurine were purchased from Tokyo Kasei (Tokyo, Japan) and used without purification. Diethyl azodicarboxylate (DEAD) was purchased from Sigma and used as received. Triphenylphosphine was purchased from Aldrich and used as received. Ethyl acetate, hexane, diethyl ether, DMF, THF and conc. aq. HCl was purchased from Kanto Chemicals (Tokyo, Japan) and used as received. Distilled water was used throughout the synthesis. $^1$H NMR spectra were recorded on a Varian Mercury 300 spectrometer. High-resolution mass spectroscopy was measured on Nihon Denshi MS 700.

Oligo DNAs were purchased from Invitrogen Japan (Tokyo, Japan) and used without purification.

### Synthesis of POPNA(A) monomers

A common synthetic route for the POPNA(A) monomers of different stereoisomers is

shown in **Scheme S1**.




**Scheme S1.** A common synthetic route for POPNA monomers

***N*-α-*tert*-Butyloxycarbonyl-*cis*-4-hydroxy-D-proline dicyclohexylammonium salt cD-HP1:** To a solution of di-*tert*-butyldicarbonate (9.98 g, 45.8 mmol) in 1,4-dioxane (20 mL), H-D-*cis*-Hyp-OH (5.00 g, 38.2 mmol) dissolved in water (20 mL) was added. $NaHCO_3$ (3.53 g, 42.0 mmol) was added to the mixture under stirring at ice temperature. The reaction mixture was then stirred for 15 h at room temperature and condensed *in vacuo*. Water (20 mL) was added to the condensed solution and the aqueous layer was washed three times with diethyl ether (each 30 mL), acidified to pH 2 with 5% aq $KHSO_4$ in an ice bath, and extracted 7 times with ethyl acetate (each 50 mL). The extract was dried over $MgSO_4$. The ethyl acetate solution was filtrated and dicyclohexylamine (13.9 g, 76.4 mmol) was added to the solution. The mixture was concentrated until white crystals began to appear. The latter was washed with diethyl ether. **cD-HP1** was obtained as a white powder (14.9 g, 94.9 %): $^1$H NMR (300MHz, $CDCl_3$) δ 1.2-2.0 (m, 22H, DCHA), 1.45 (s, 9H, $CH_3$- Boc), 2.9-3.0 (m, 2H, -$CH_2$- pyrrolidine ring), 3.4-3.7 (m, 2H, N-$CH_2$- pyrrolidine ring), 4.09 (t, 1H, $C_\alpha$-H), 4.25 (m, 1H, C-CH(OH)–C pyrrolidine ring).

***N*-α-*tert*-Butyloxycarbonyl-*cis*-4-hydroxy-D-proline ethyl ester cD-HP2:** Bromoethane (15.7 g, 144 mmol) was added to a solution of compound **cD-HP1** (14.9 g, 36.2 mmol) in anhydrous DMF (20mL) and the mixture was stirred for 39 h at room temperature. Insoluble product was removed by filtration and the resultant solution was evaporated to dryness. The residue was dissolved in water (50 mL) and extracted three times with ethyl acetate (each 50 mL). The ethyl acetate layer was washed with water (100 mL) and brine (100 mL), then dried over $MgSO_4$. Filtration followed by solvent evaporation gave a crude viscous oil. The crude oil was chromatographed over silica gel (0.04-0.06 mm) with 50:50 ethyl acetate:hexane mixture as eluting solvent. **cD-HP2** was obtained as a colorless viscous oil (8.81 g, 94.0 %) : $^1$H NMR (300MHz, $CDCl_3$) δ 1.44, 1.47 (s+s, 9H, $CH_3$- Boc), 1.32 (t+t, 3H, $CH_3$- ethyl), 2.1-2.4 (m, 2H, -$CH_2$- pyrrolidine ring), 3.3-3.7 (m, 3H, N-$CH_2$- pyrrolidine ring + -OH), 4.23 (q, 2H, O-$CH_2$- ethyl), 4.2-4.4 (m, 2H, C-CH(OH)–C pyrrolidine ring + $C_\alpha$-H).

***N*-α-*tert*-Butyloxycarbonyl-*cis*-4-(2-tetrahydropyranyloxy)-D-proline ethyl ester cD-HP3:** 3,4-Dihydro-2H-pyran (4.54 g, 54.0 mmol) and a solution of pyridinium-*p*-toluenesulfonate (3.39 g, 13.5 mmol) in 1,2-dichloroetahane (8 mL) was added to a solution of **cD-HP2** in 1,2-dichloroetahane (7 mL) under ice cooling and the

mixture was stirred for 5 h at room temperature. The mixture was evaporated to dryness. The residue was dissolved in water (50 mL) and extracted three times with ethyl acetate (each 50 mL). The ethyl acetate layer was washed with water (100 mL) and brine (150 mL), then dried over $MgSO_4$. Filtration followed by solvent evaporation gave a crude viscous oil. The crude oil was chromatographed over silica gel with 10:90 ethyl acetate:hexane mixture as eluting solvent. **cD-HP3** was obtained as a colorless viscous oil (8.57 g, 95.8 %): $^1$H NMR (300MHz, $CDCl_3$) δ 1.27 (t+t, 3H, $CH_3$ methyl), 1.43, 1.44 (s+s, 9H, $CH_3$ Boc), 1.5-1.9 (m, 6H, C-$(CH_2)_3$-C THP ring), 2.1-2.5 (m, 2H, -$CH_2$- pyrrolidine ring), 3.4-3.9 (m, 4H, N-$CH_2$- pyrrolidine ring + O-$CH_2$- THP ring), 4.1-4.4 (m, 4H, C-CH(OH)-C pyrrolidine ring + $C_\alpha$-H pyrrolidine ring + O-$CH_2$- ethyl), 4.65 (s, br, 1H, O-CH-O THP ring).

***N*-α-*tert*-Butyloxycarbonyl-*cis*-4-(2-tetrahydropyranyloxy)-D-prolinol cD-HP4:** **cD-HP3** (7.21 g, 21.0 mmol), anhydrous ethanol (50 mL) and $NaBH_4$ (3.18 g, 84.0 mmol) were mixed under argon atmosphere at ice temperature and the mixture was stirred for 24 h at room temperature. The resultant mixture was evaporated and added to water (30 mL). The aqueous layer was acidified to pH 7 with 5% aq $KHSO_4$ and extracted three times with ethyl acetate (each 50 mL). The ethyl acetate layer was washed with water (100 mL) and brine (100 mL), then dried over $MgSO_4$. Filtration followed by solvent evaporation gave a crude viscous oil. The crude oil was chromatographed over silica gel with 30:70 ethyl acetate:hexane mixture as eluting solvent. **cD-HP4** was obtained as a colorless viscous oil (5.34 g, 84.5 %): $^1$H NMR (300MHz, $CDCl_3$) δ 1.48 (s, 9H, $CH_3$ Boc), 1.5-1.9 (m, 6H, C-$(CH_2)_3$-C THP ring), 2.2-2.4 (m, 1H, -$CH_2$- pyrrolidine ring), 3.3-3.9 (m, 6H, -$CH_2$- pyrrolidine ring + $C_\alpha$-H + N-$CH_2$- pyrrolidine ring + O-$CH_2$- THP ring), 4.1-4.3 (m, 2H, -$CH_2$-OH), 4.5-4.7 (m, 2H, C-CH(OH)-C pyrrolidine ring + O-CH-O THP ring).

***N*-α-*tert*-Butyloxycarbonyl-(2*R*,4*R*)-2-*tert*-butyloxycarbonylmethoxymethyl-4-(2'-tetrahydropyranyloxy)-pyrrolidine cD-HP5:** To a solution of **cD-HP4** (6.85 g, 22.8 mmol) in benzene were added 50% aq NaOH (50 mL), *tert*-butyl bromoacetate (11.1 g, 57.0 mmol) and tetra-*n*-butylammonium hydrogensulfate (1.94 g, 5.70 mmol). The mixture was vigorously stirred for 10 h under ice temperature. To the resultant mixture was added 2N aq. HCl (200 mL), then the aqueous layer was removed. The benzene layer was washed with water (50 mL) and with brine (50 mL), then dried over $MgSO_4$.

Filtration followed by solvent evaporation gave a crude viscous oil. The crude oil was chromatographed over silica gel with 20:80 ethyl acetate:hexane mixture as eluting solvent. **cD-HP5** was obtained as a colorless viscous oil (8.60 g, 90.9 %): $^1$H NMR (300MHz, $CDCl_3$) δ 1.47, 1.48 (s+s, 18H, $CH_3$- Boc + $CH_3$- *tert*-butyl), 1.5-1.9 (m, 6H, C-$(CH_2)_3$-C THP ring), 2.0-2.3 (m, 2H, -$CH_2$- pyrrolidine ring), 3.3-4.0 (m, 9H, N-$CH_2$- pyrrolidine ring + $C_\alpha$-H + $C_\alpha$-$CH_2$-O + O-$CH_2$-CO + O-$CH_2$ THP ring), 4.4 (s, br, C-CH(OH)-C pyrrolidine ring), 4.7 (d, br, O-CH-O THP ring).

***N*-α-*tert*-Butyloxycarbonyl-(2R,4R)-2-*tert*-butyloxycarbonylmethoxymethyl-4-hydroxy-pyrrolidine cD-HP6:** A solution of pyridinium-*p*-toluenesulfonate (2.17 g, 8.65 mmol) in ethanol (18 mL) was added to a solution of **cD-HP5** (7.20 g, 17.3 mmol) in ethanol (17 mL) and the mixture was stirred for 21 h at room temperature. The resultant mixture was evaporated to dryness. The residue was dissolved in water (50 mL) and extracted three times with ethyl acetate (each 50 mL). The ethyl acetate layer was washed with water (50 mL) and with brine (50 mL), then dried over $MgSO_4$. Filtration followed by solvent evaporation gave a crude viscous oil. The crude oil was chromatographed over silica gel with 30:70 ethyl acetate:hexane mixture as eluting solvent. **cD-HP6** was obtained as a white powder (4.54 g, 79.2 %): $^1$H NMR (300MHz, $CDCl_3$) δ 1.47, 1.48 (s+s, 18H, $CH_3$- Boc + $CH_3$- *tert*-butyl), 2.0-2.4 (m, 2H, -$CH_2$- pyrrolidine ring), 3.4-3.6 (m, 3H, N-$CH_2$- pyrrolidine ring + OH), 3.9-4.1 (m, 4H, C-CH(OH)-C, pyrrolidine ring + O-$CH_2$-CO + $C_\alpha$-H), 4.3-4.8 (m, 2H, $C_\alpha$-$CH_2$-O).

***N*-α-*tert*-Butyloxycarbonyl-(2R,4S)-2-*tert*-butyloxycarbonylmethoxymethyl-4-(6'-chloropurin-9'-yl)-pyrrolidine tD-HP7: cD-HP6** (1.00 g, 3.02 mmol), triphenylphosphine (1.98 g, 7.55 mmol) and 6-chloropurine (0.93 g, 6.04 mmol) were added to THF (10 mL). To the mixture was added DEAD (1.32 g, 7.55 mmol) under argon atmosphere at –15 ˚C and then stirred at room temperature overnight. The resultant mixture was evaporated to dryness. The residue was chromatographed over silica gel with 50:50 ethyl acetate:hexane mixture as eluting solvent. **tD-HP7** was obtained as a yellow powder (1.06 g, 75.2 %): $^1$H NMR (300MHz, $CDCl_3$) δ 1.47, 1.49 (s+s, 18H, $CH_3$- Boc + $CH_3$- *tert*-butyl), 2.7 (m, 2H, -$CH_2$- pyrrolidine ring), 3.7-4.3 (m, 7H, N-$CH_2$- pyrrolidine ring + $C_\alpha$-H + $C_\alpha$-$CH_2$-O + O-$CH_2$-CO), 5.4 (m, 1H, C-CH(N)-C pyrrolidine ring), 8.14, 8.76 (s+s, 2H, adenine).

***N*-α-*tert*-Butyloxycarbonyl-(2R,4S)-2-*tert*-butyloxycarbonylmethoxymethyl-4-(adenin-9'-yl)-pyrrolidine tD-HP8:** To a solution of **tD-HP7** (1.06 g, 2.22 mmol) in dioxane (3 mL) was added 28% aq $NH_3$ (3 mL) and the mixture was stirred for 24 h at 60 ˚C. The resultant mixture was evaporated to dryness. The residue was chromatographed over silica gel with methanol as eluting solvent. **tD-HP8** was obtained as a yellow foam (0.52 g, 52 %): $^1$H NMR (300MHz, $CDCl_3$) δ 1.46, 1.49 (s+s, 18H, $CH_3$- Boc + $CH_3$- tert-butyl), 2.7 (m, 2H, -$CH_2$- pyrrolidine ring), 3.7-4.3 (m, 7H, N-$CH_2$- pyrrolidine ring + $C_\alpha$-H + $C_\alpha$-$CH_2$-O + O-$CH_2$-CO), 5.3 (m, 1H, C-CH(N)-C pyrrolidine ring), 5.6 (s, 2H, $NH_2$- adenine), 7.8, 8.4 (s+s, 2H, adenine).

***N*-α-9-Fluorenylmethoxycarbonyl-(2R,4S)-2-hydroxycarbonylmethoxymethyl-4-(adenin-9'-yl)-pyrrolidine tD-HP9: tD-HP8** (0.49g, 1.09 mmol) was treated with 30% HBr in acetic acid (10 mL) for 1 h at room temperature. The resultant mixture was evaporated to dryness. The residue was dissolved in 5% aq $NaHCO_3$ (30 mL) to bring the pH to 8. A solution of 9-fluorenylmethyl succinimidyl carbonate (0.40 g, 1.20 mmol) in acetonitrile (60 mL) was added to the aqueous solution with stirring under ice cooling. The reaction mixture was stirred at room temperature overnight and evaporated to dryness. The residue was dissolved in water and the aqueous layer was washed three times with diethyl ether (each 30 mL), acidified to pH 7 with 5% aq $KHSO_4$, and the resultant precipitate was collected by filtration. The residue was washed several times with water and dried *in vacuo*. **tD-HP9** was obtained as a white powder (0.40 g, 71.3 %): $^1$H NMR (300MHz, DMSO-$d_6$) δ 2.4-2.8 (m, 2H, -$CH_2$- pyrrolidine ring), 3.6-4.6 (m, 7H, N-$CH_2$- pyrrolidine ring + $C_\alpha$-H + $C_\alpha$-$CH_2$-O + O-$CH_2$-CO), 4.8-5.0 (m, 1H, C-CH(N)-C pyrrolidine ring), 7.2-7.8 (m, 12H, Fmoc- + adenine). HRMS: obsd; 515.2050, calcd for (M+H); 515.2042.

***N*-α-*tert*-Butyloxycarbonyl-(2R,4S)-2-*tert*-butyloxycarbonylmethoxymethyl-4-formyloxy-pyrrolidine tD-HP6.5: cD-HP6** (2.00 g, 6.04 mmol) and triphenylphosphine (1.90g, 7.25mmol) were dissolved in THF (10 mL). To the mixture was added formic acid (300 μL, 7.85 mmol) and diethyl azodicarboxylate (DEAD: 1.2 mL, 7.25 mmol) at –15 ˚C and then the mixture was stirred at room temperature overnight. The resultant mixture was evaporated to dryness. The residue was chromatographed over silica gel with 25:75 ethyl acetate:hexane mixture as eluting solvent. **tD-HP6.5** was obtained as a colorless viscous oil (1.83 g, 84.5 %): $^1$H NMR (300MHz, $CDCl_3$) δ 1.48 (s, 18H, $CH_3$-

Boc + $CH_3$- *tert*-butyl), 2.2-2.5 (m, 2H, -$CH_2$- pyrrolidine ring), 3.6-4.1 (m, 7H, N-$CH_2$- pyrrolidine ring + $C_\alpha$-H + $C_\alpha$-$CH_2$-O + O-$CH_2$-CO), 5.4 (s, 1H, C-CH(OH)-C pyrrolidine ring), 8.0 (s, 1H, COH).

***N*-α-*tert*-Butyloxycarbonyl-(2R,4S)-2-*tert*-butyloxycarbonylmethoxymethyl-4-hydroxy-pyrrolidine tD-HP6: tD-HP6.5** (1.50 g, 4.18 mmol) was dissolved in methanol (10 mL) and 28 % aq $NH_3$ (0.5 mL) was added to the solution. The mixture was stirred for 1 h at room temperature. The resultant mixture was evaporated to dryness. The residue was chromatographed over silica gel with 40:60 ethyl acetate:hexane mixture as eluting solvent. **tD-HP6** was obtained as a white powder (1.38 g, 100 %): $^1$H NMR (300MHz, $CDCl_3$) δ 1.47, 1.48 (s+s, 18H, $CH_3$- Boc + $CH_3$- *tert*-butyl), 2.0-2.3 (m, 2H, -$CH_2$- pyrrolidine ring), 3.5-3.7 (m, 4H, N-$CH_2$- pyrrolidine ring + $C_\alpha$-$CH_2$-O), 3.9-4.1 (m, 3H, O-$CH_2$-CO + $C_\alpha$-H), 4.49 (s, br, 1H, C-CH(OH)-C pyrrolidine ring).

***N*-α-*tert*-Butyloxycarbonyl-(2R,4R)-2-*tert*-butyloxycarbonylmethoxymethyl-4-(adenin-9'-yl)-pyrrolidine cD-HP8: tD-HP6** (1.00 g, 3.02 mmol), triphenylphosphine (1.19 g, 4.53 mmol) and 6-chloropurine (0.47 g, 3.02 mmol) were added to THF (10 mL). To the mixture was added DEAD (0.72 mL, 4.53 mmol) under Ar atmosphere at –15 ˚C and then stirred at room temperature overnight. The resultant mixture was evaporated to dryness and the residue was washed several times with 50:50 ethyl acetate:hexane mixture and dried *in vacuo*. The crude **cD-HP7** (1.50 g) was dissolved in dioxane (10 mL) and 28% aq $NH_3$ (10 mL) was added. The mixture was stirred for 24 h at 60 ˚C. The resultant mixture was evaporated to dryness. The residue was chromatographed over silica gel with methanol as eluting solvent. **cD-HP8** was obtained as a yellow foam (0.81 g, 60.0 %): $^1$H NMR (300MHz, $CDCl_3$) δ 1.4-1.5 (s+s, 18H, $CH_3$- Boc + $CH_3$- *tert*-butyl), 2.7 (m, 2H, -$CH_2$- pyrrolidine ring), 3.5-4.3 (m, 7H, N-$CH_2$- pyrrolidine ring + $C_\alpha$-H + $C_\alpha$-$CH_2$-O + O-$CH_2$-CO), 5.1 (m, 1H, C-CH(N)-C pyrrolidine ring), 6.0 (s, 2H, $NH_2$- adenine), 8.2, 8.4 (s+s, 2H, adenine).

***N*-α-9-Fluorenylmethoxycarbonyl-(2R,4R)-2-hydroxycarbonylmethoxymethyl-4-(adenin-9'-yl)-pyrrolidine cD-HP9: cD-HP8** (1.00 g, 2.23 mmol) was treated with 30% HBr in acetic acid (10 mL) for 1 h at room temperature. The resultant mixture was evaporated to dryness. The residue was dissolved in 5% aq $NaHCO_3$ (30 mL) to adjust the pH to 8. A solution of 9-fluorenylmethyl succinimidyl carbonate (0.82 g, 2.45

mmol) in acetonitrile (30 mL) was added to the aqueous solution with stirring under ice cooling. The reaction mixture was stirred at room temperature overnight and evaporated to dryness. The residue was dissolved in water and the aqueous layer was washed three times with diethyl ether (each 30 ml), acidified to pH 7 with 5% aq $KHSO_4$, and the resultant precipitate was collected by filtration. The residue was washed several times with water and dried *in vacuo*. **cD-HP9** was obtained as a white powder (1.03 g, 90 %): $^1$H NMR (300MHz, DMSO-$d_6$) δ 2.4-2.8 (m, 2H, -$CH_2$- pyrrolidine ring), 3.6-4.6 (m, 7H, N-$CH_2$- pyrrolidine ring + $C_\alpha$-H + $C_\alpha$-$CH_2$-O + O-$CH_2$-CO), 4.8-5.0 (m, 1H, C-CH(N)-C pyrrolidine ring), 7.2-7.8 (m, 12H, Fmoc- + adenine). HRMS: obsd; 515.2029, calcd for (M+H); 515.2042.

*Cis* L-POPNA momomer with an adenine base was synthesized from *trans* L-hydroxyproline through 9 steps, as reported previously. The synthetic route was similar to that of the *trans* D-POPNA monomer with adenine. HRMS: obsd; 515.2037, calcd for (M+H); 515.2042.

***N*-α-*tert*-Butyloxycarbonyl-(2S,4S)-2-*tert*-butyloxycarbonylmethoxymethyl-4-formyloxy-pyrrolidine cL-HP6.5: tL-HP6** (2.00 g, 6.04 mmol) and triphenylphosphine (1.90 g, 7.25 mmol) were dissolved in THF (10 mL). To the mixture was added formic acid (300 μL, 7.85 mmol) and diethyl azodicarboxylate (DEAD: 1.2 mL, 7.25 mmol) at –15 ˚C and then the mixture was stirred at room temperature overnight. The resultant mixture was evaporated to dryness. The residue was chromatographed over silica gel with 25:75 ethyl acetate:hexane mixture as eluting solvent. **cL-HP6.5** was obtained as a colorless viscous oil (1.53 g, 70.5 %): $^1$H NMR (300MHz, $CDCl_3$) δ 1.47, 1.48 (s, 18H, $CH_3$- Boc + $CH_3$- *tert*-butyl), 2.2-2.4 (m, 2H, -$CH_2$- pyrrolidine ring), 3.4-4.2 (m, 7H, N-$CH_2$- pyrrolidine ring + $C_\alpha$-H + $C_\alpha$-$CH_2$-O + O-$CH_2$-CO), 5.4 (s, 1H, C-CH(OH)-C pyrrolidine ring), 8.0 (s, 1H, COH).

***N*-α-*tert*-Butyloxycarbonyl-(2S,4S)-2-*tert*-butyloxycarbonylmethoxymethyl-4-hydroxy-pyrrolidine cL-HP6: cL-HP6.5** (1.50 g, 4.18 mmol) was dissolved in methanol (10 mL) and 28% aq $NH_3$ (0.5 mL) was added to the solution. The mixture was stirred for 1 h at room temperature. The resultant mixture was evaporated to dryness. The residue was chromatographed over silica gel with 40:60 ethyl acetate:hexane mixture as eluting solvent. **cL-HP6** was obtained as a white powder (1.33 g, 94.2 %): $^1$H NMR

(300MHz, $CDCl_3$) δ 1.47, 1.48 (s+s, 18H, $CH_3$- Boc + $CH_3$- *tert*-butyl), 2.0-2.4 (m, 2H, -$CH_2$- pyrrolidine ring), 3.4-3.6 (m, 3H, N-$CH_2$- pyrrolidine ring + OH), 3.9-4.1 (m, 4H, C-CH(OH)-C, pyrrolidine ring + O-$CH_2$-CO + Ca-H), 4.3-4.8 (m, 2H, $C_\alpha$-$CH_2$-O).

***N*-α-*tert*-Butyloxycarbonyl-(2S,4R)-2-*tert*-butyloxycarbonylmethoxymethyl-4-(adenin-9'-yl)-pyrrolidine tL-HP8: cL-HP6** (1.33 g, 4.02 mmol), triphenylphosphine (1.58 g, 6.03 mmol) and 6-chloropurine (0.62 g, 4.02 mmol) were added to THF (10 mL). To the mixture was added DEAD (0.96 ml, 6.03 mmol) under argon atmosphere at –15 ˚C and then stirred at room temperature overnight. The resultant mixture was evaporated to dryness and the residue was washed several times with 50:50 ethyl acetate:hexane mixture and dried *in vacuo*. The crude **tL-HP7** (2.00 g) was dissolved in dioxane (10 mL) and 28% aq $NH_3$ (10 mL) was added. The mixture was stirred for 24 h at 60 ˚C. The resultant mixture was evaporated to dryness. The residue was chromatographed over silica gel with methanol as eluting solvent. **tL-HP8** was obtained as a yellow foam (0.90 g, 50.0 %): $^1$H NMR (300MHz, $CDCl_3$) δ 1.46, 1.49 (s+s, 18H, $CH_3$- Boc + $CH_3$- tert-butyl), 2.7 (m, 2H, -$CH_2$- pyrrolidine ring), 3.7-4.3 (m, 7H, N-$CH_2$- pyrrolidine ring + $C_\alpha$-H + $C_\alpha$-$CH_2$-O + O-$CH_2$-CO), 5.3 (m, 1H, C-CH(N)-C pyrrolidine ring), 5.6 (s, 2H, $NH_2$- adenine), 7.8, 8.4 (s+s, 2H, adenine).

***N*-α-9-Fluorenylmethoxycarbonyl-(2S,4R)-2-hydroxycarbonylmethoxymethyl-4-(adenin-9'-yl)-pyrrolidine tL-HP9: tL-HP8** (0.90 g, 2.01 mmol) was treated with 30% HBr in acetic acid (10 mL) for 1 h at room temperature. The resultant mixture was evaporated to dryness. The residue was dissolved in 5% aq $NaHCO_3$ (30 mL) to bring the pH to 8. A solution of 9-fluorenylmethyl succinimidyl carbonate (0.74 g, 2.21 mmol) in acetonitrile (30 mL) was added to the aqueous solution with stirring under ice cooling. The reaction mixture was stirred at room temperature overnight and evaporated to dryness. The residue was dissolved in water and the aqueous layer was washed three times with diethyl ether (each 30 mL), acidified to pH 7 with 5% aq $KHSO_4$, and the resultant precipitate was collected by filtration. The residue was washed several times with water and dried *in vacuo*. **tL-HP9** was obtained as a white powder (1.03 g, 100 %): $^1$H NMR (300MHz, DMSO-$d_6$) δ 2.4-2.8 (m, 2H, -$CH_2$- pyrrolidine ring), 3.6-4.6 (m, 7H, N-$CH_2$- pyrrolidine ring + $C_\alpha$-H + $C_\alpha$-$CH_2$-O + O-$CH_2$-CO), 5.1-5.3 (m, 1H, C-CH(N)-C pyrrolidine ring), 7.2-7.8 (m, 12H, Fmoc- + adenine). HRMS: obsd; 515.2023, calcd for (M+H); 515.2042.

**Proton nmr Spectra**

Proton nmr spectra of the four POPNA monomers were measured (**Figure S1**). As expected, each enantiomeric pair gave identical spectrum, i.e., the spectrum of *cis*-D-POPNA(A) was identical to that of *cis*-L-POPNA(A) and the spectrum of *trans*-D-POPNA(A) was identical to that of *trans*-L-POPNA(A). But the two diastereomeric pairs gave different spectra.

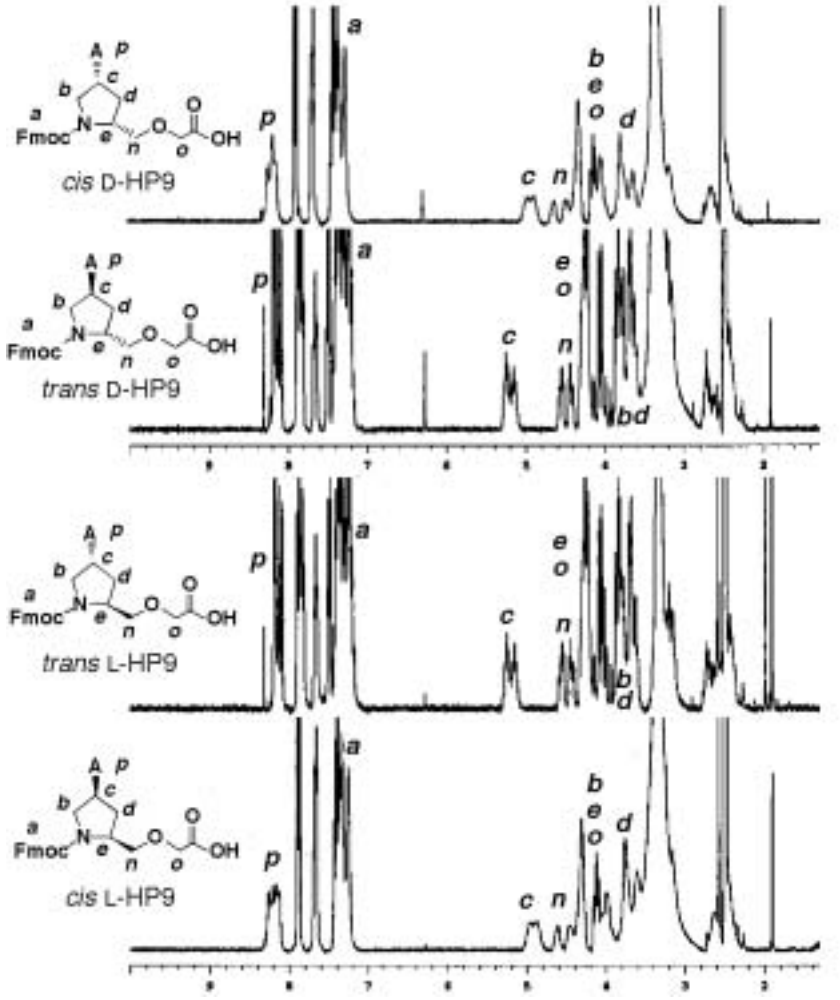



**Figure S1.** H-Nmr spectra of **HP-9s** in DMSO-$d_6$ at room temperature.

**HPLC Analysis**

The purity of POPNA(A) monomers was checked by HPLC (Figures S2-S5). Each

monomer showed a single peak, except for the case of *trans*-D monomer where a small amount of impurity was found. Single peaks in HPLC charts suggest that no diastereomeic counterpart is contained in each sample. To confirm this, HPLC charts for mixtures of diastereomeric pairs were taken. Figure S6 shows an HPLC chart of a diastereomeric mixture of *trans*-D and *cis*-D-POPNA(A). Figure S7 shows that of *trans*-L- and *cis*-L-POPNA(A). Both show two peaks (29.0 min and 29.8 min), indicating that the diastereomeric pairs can be well resolved under the HPLC conditions. On the other hand, the mixture of *trans*-D and *trans*-L monomers shows a single peak (Figure S 8) and that of *cis*-L and *cis*-D-POPNA(A) also shows a single peak (Figure S9). These results show that the optical purity of each POPNA(A) monomer is satisfactory.

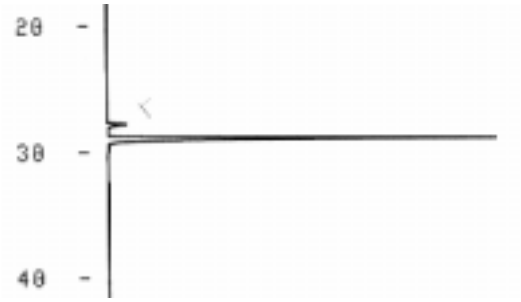



**Figure S2.** Analytical reverse-phase HPLC of the **tD-HP9** on C18 column monitored at 260 nm. Buffer A, 0.1 % aq. TFA; buffer B, acetonitrile. Linear gradient of 0-100 % buffer B over 50 min at flow rate 0.6 mL/min.


**Figure S3.** Analytical reverse-phase HPLC of the **cD-HP9** on C18 column monitored at 260 nm. Buffer A , 0.1 % aq. TFA; buffer B, acetonitrile. Linear gradient of 0-100 % buffer B over 50 min at flow rate 0.6 mL/min.


**Figure S4.** Analytical reverse-phase HPLC of the **cL-HP9** on C18 column monitored at 260 nm. Buffer A , 0.1 % aq. TFA; buffer B, acetonitrile. Linear gradient of 0-100 % buffer B over 50 min at flow rate 0.6 mL/min.


**Figure S5.** Analytical reverse-phase HPLC of the **tL-HP9** on C18 column monitored at 260 nm. Buffer A , 0.1 % aq. TFA; buffer B, acetonitrile. Linear gradient of 0-100 % buffer B over 50 min at flow rate 0.6 mL/min.


**Figure S6.** Analytical reverse-phase HPLC of the **tD-HP9** and **cD-HP9** mixture on C18 column monitored at 260 nm. Buffer A , 0.1 % aq. TFA; buffer B, acetonitrile. Linear gradient of 0-100 % buffer B over 50 min at flow rate 0.6 mL/min.


**Figure S7.** Analytical reverse-phase HPLC of the **cL-HP9** and **tL-HP9** mixture on C18 column monitored at 260 nm. Buffer A, 0.1 % aq. TFA; buffer B, acetonitrile. Linear gradient of 0-100 % buffer B over 50 min at flow rate 0.6 mL/min.


**Figure S8.** Analytical reverse-phase HPLC of the **tD-HP9** and **tL-HP9** mixture on C18 column monitored at 260 nm. Buffer A, 0.1 % aq. TFA; buffer B, acetonitrile. Linear gradient of 0-100 % buffer B over 50 min at flow rate 0.6 mL/min.

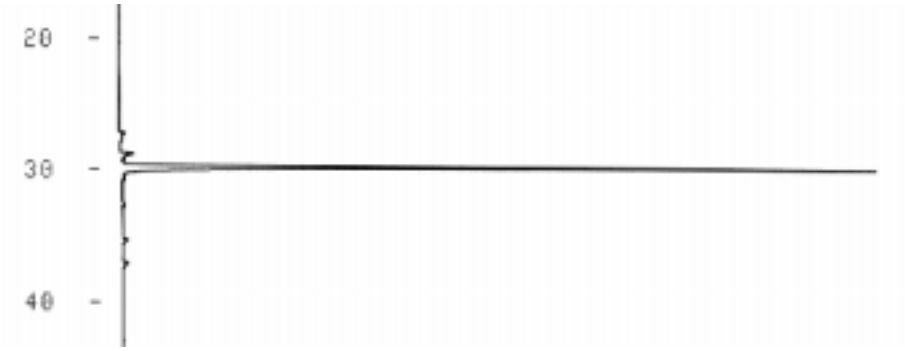



**Figure S9.** Analytical reverse-phase HPLC of the **cL-HP9** and **cD-HP9** mixture on C18 column monitored at 260 nm. Buffer A, 0.1 % aq. TFA; buffer B, acetonitrile. Linear gradient of 0-100 % buffer B over 50 min at flow rate 0.6 mL/min.

**Synthesis of POPNA 9-mers, POPNA($A_9$)**

Syntheses of POPNA($A_9$) of four different configurations (*cis*-L, *cis*-D, *trans*-L, *trans*-D configurations) were carried out on the solid phase. Single lysine amide unit was added at the C-terminal to increase water solubility. The Fmoc-δ-amino acids with unprotected adenine bases were used as the monomers. Virtually no side reaction took place during the peptide synthesis with the unprotected adenine monomers.[1] The solid phase synthesis was performed on an Fmoc-SH-SAL-PEG resin (super acid-labile polyethyleneglycol resin from Watanabe Chemicals, Hiroshima, Japan), with a single lysine residue (Fmoc-Lys(Boc)-OH) being incorporated as the first unit into the growing chain. Chain elongation was achieved by using bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP)/1-hydroxy- benzotriazole(HOBt) as the coupling agent in the presence of *N*-methylmorpholine (Kanto Chemicals) in dimethylacetamide (DMAA). The coupling time was set to 1 h at room temperature. In general, coupling efficiency at each elongation step was >95 %, as estimated from UV absorption of the fulvene-adduct formed upon removal of the $N_\delta$-Fmoc protecting group with 20% piperidine/DMF for 7 min at room temperature ($\varepsilon_{290}$= 4950 $M^{-1}$ $cm^{-1}$). The resin-bound POPNA($A_9$) was cleaved off from the resin with *m*-cresol/TFA (20/80 v/v) for 90 min at room temperature. The crude POPNA($A_9$) was purified by a reversed-phase HPLC (C18 column) eluting with acetonitrile-0.1% aq TFA gradient mixture (a linear gradient of 13-15 % acetonitrile over 60 min at flow rate 10.0 mL/min was used). The purity of the peptide was checked by C18 reversed-phase analytical HPLC and was found to be >99 %. Finally, final products were identified by MALDI-TOF mass spectroscopy (Figure S11).

*trans*-L-POPNA($A_9$): Found, 2612.73; Calcd for $C_{114}H_{142}N_{57}O_{19}$ $(M+H)^+$, 2613.18

*cis*-D-POPNA($A_9$): Found, 2612.37; Calcd for $C_{114}H_{142}N_{57}O_{19}$ $(M+H)^+$, 2613.18

*trans*-D-POPNA($A_9$): Found, 2613.23; Calcd for $C_{114}H_{142}N_{57}O_{19}$ $(M+H)^+$, 2613.18

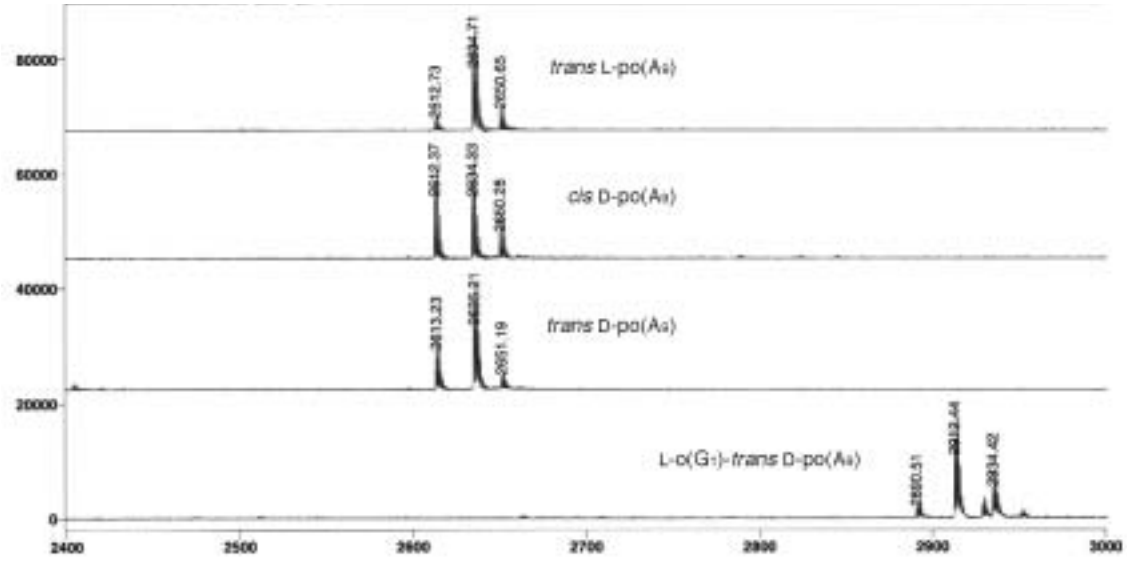



**Figure S11.** MALDI-TOF mass spectra of D-OPNA(A9), *trans*-L-POPNA(A9), *cis* -D-POPNA(A9), *trans*-D-POPNA(A9).

To examine the chirality of POPNA oligomers, CD spectra were measured (Figure S12). Mirror images were confirmed for the enantiomeric pairs of *cis*-L-POPNA($A_9$) and *cis*-D-POPNA($A_9$) and of *trans*-L-POPNA($A_9$) and *trans*-D-POPNA($A_9$).

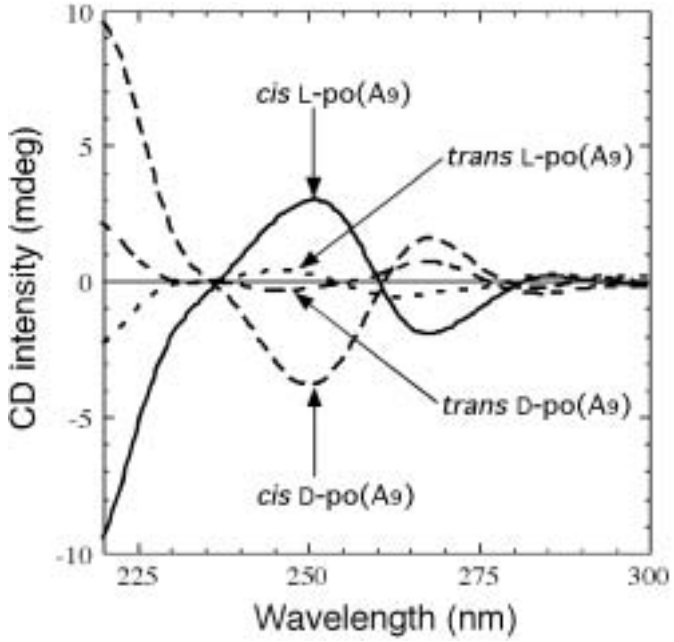



**Figure S12.** CD spectra for POPNA($A_9$) oligomers at 5 ˚C in 100 mM NaCl, 10 mM $NaH_2PO_4$ and 0.1 mM EDTA, pH 7.0. Concentration = 10 μM. Optical path length = 1.0 cm.

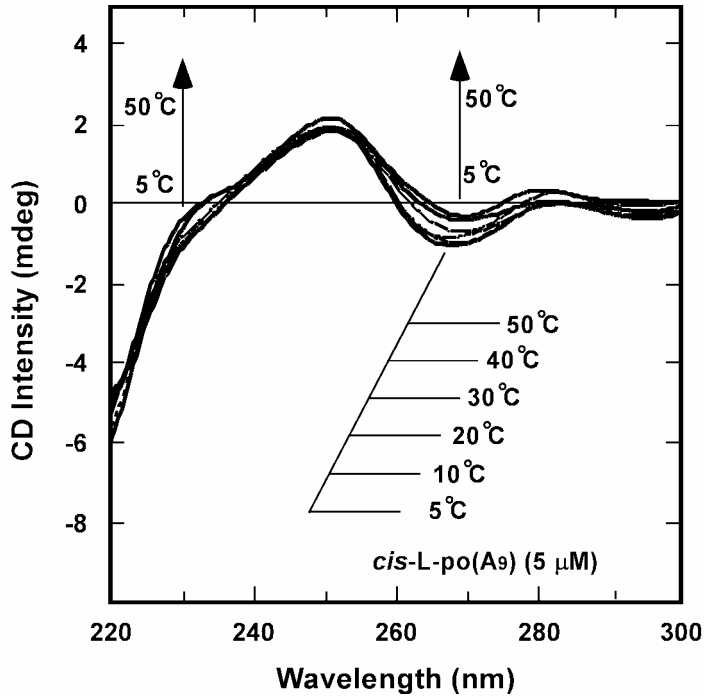



**Figure S13.** CD spectra for *cis*-L-po($A_9$) at various temperature in phosphate buffer (pH 7). [POPNA] = 5 μM.

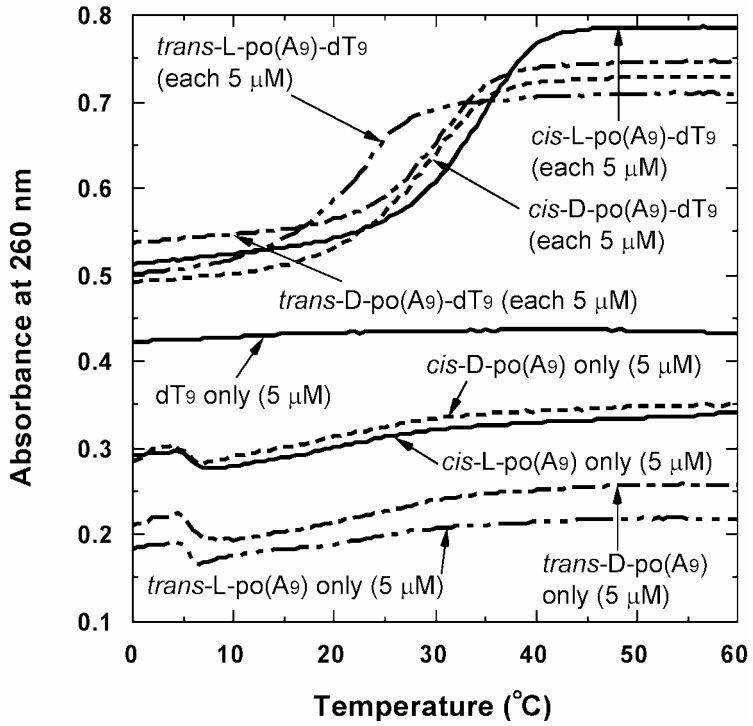



**Figure S14.** Melting curves of *cis*-L-po($A_9$), *cis*-D-po($A_9$), *trans*-L-po($A_9$), *trans*-D-po($A_9$) and $dT_9$. each concentration is 5 μM.

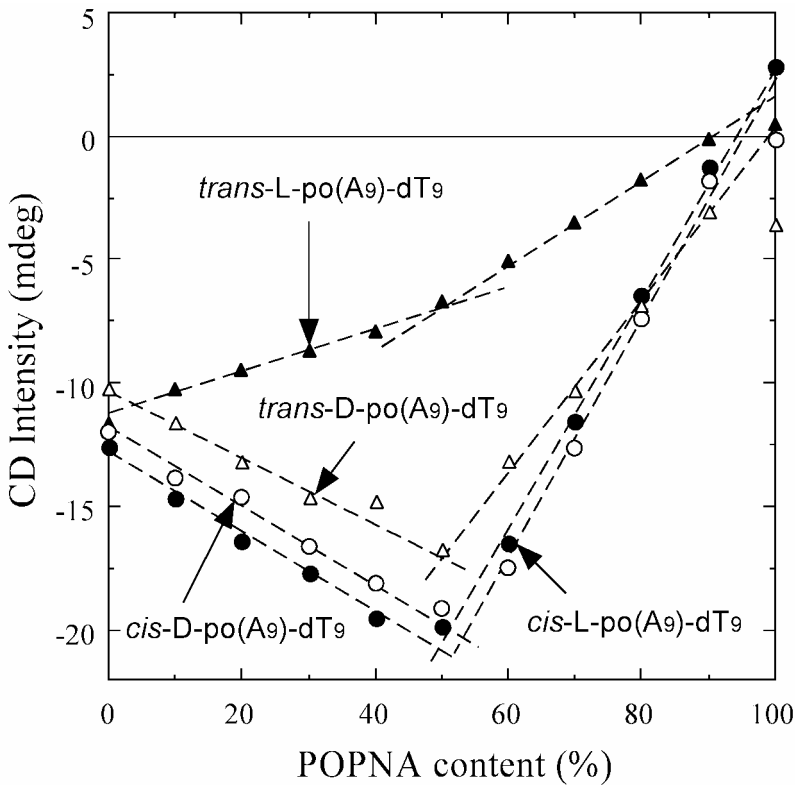



**Figure S15.** CD job plots of *cis*-L-po($A_9$), *trans*-L-po($A_9$), *cis*-D-po($A_9$) and *trans*-D-po($A_9$) with $dT_9$ at 5 ˚C. Total strand concentrations are 10 μM.

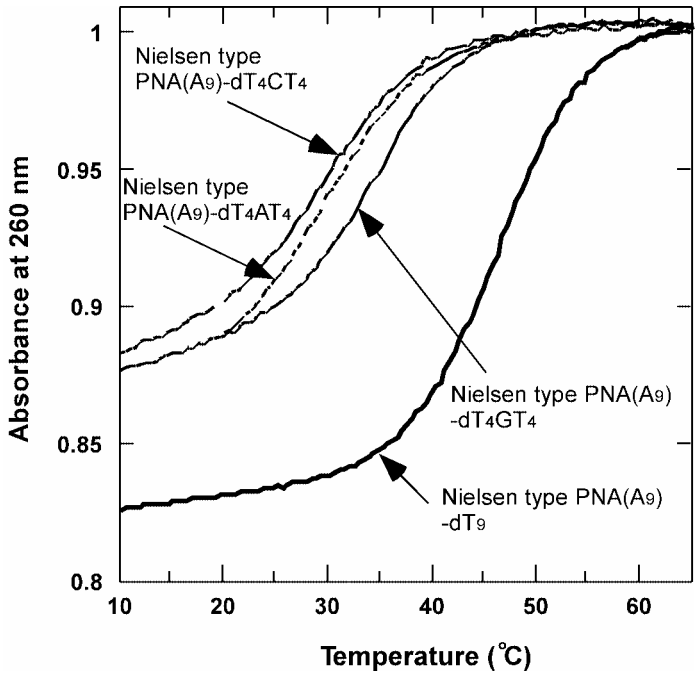



**Figure S16.** Melting curves of Nielsen type PNA($A_9$) and DNA in phosphate buffer (pH

7). [PNA] = [DNA] = 5 μM.