HETEROCYCLES, Vol. 97, No. 2, 2018, pp. 1149 - 1156. © 2018 The Japan Institute of Heterocyclic Chemistry
Received, 14th February, 2018, Accepted, 18th May, 2018, Published online, 30th May, 2018
DOI: 10.3987/COM-18-S(T)53

DESIGN AND SYNTHESIS OF A DNA-LIKE STRUCTURE COMPOSED OF ALKYNYL *C*-NUCLEOTIDE WITH 2-AMINOPYRIMIDIN-4-ONE AS A NUCLEOBASE

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Abstract – A heterocyclic compound, 2-aminopyrimidin-4-one, was found to be a new candidate of a non-natural nucleobase that forms a novel base pair by self-dimerization. 2-Aminopyrimidin-4-one was connected to a deoxyribose through an acetylene linkage. The non-natural nucleoside was derivatized to its phosphoramidite that could be subjected to a conventional solid-phase DNA synthesis. The solid-phase auto-synthesis successfully afforded a non-natural DNA-like oligomer bearing 2-aminopyrimidin-4-one as a nucleobase. The synthetic oligomer exhibited reversible formation of a higher-order structure that would be a duplex like natural DNA. The present study means creation of a novel artificial DNA-like system with unordinary odd numbers of genetic alphabets.

Pyrimidine derivatives are DNA-related important heterocyclic compounds not only as canonical nucleobases such as thymine, uracil, and cytosine but also as non-natural bases.[1] We previously reported 6-alkynyl-2-aminopyrimidin-4-one derivatives that self-dimerized by multiple hydrogen-bonding.[2] DFT calculations and X-ray structural analysis revealed that the self-dimerization was based on the ADD•DAA-type triple hydrogen bonds between its two tautomers (Figure 1). Recently, we successively developed artificial DNAs made exclusively of non-natural alkynyl *C*-nucleotides with four types of heterocyclic compounds as non-natural nucleobases.[3] Together with these two previous studies, we imagined that a *C*-nucleotide from 6-alkynyl-2-aminopyrimidin-4-one might be a self-associated non-natural base pair in our artificial DNA system. Incorporation of this self-dimerized base pair into natural base pairs would afford a novel non-natural DNA-like structure

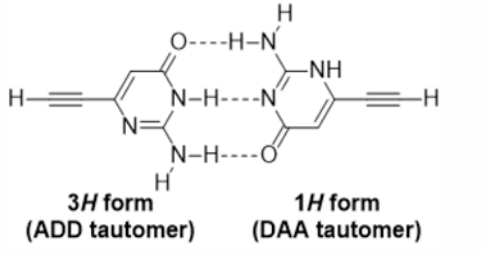
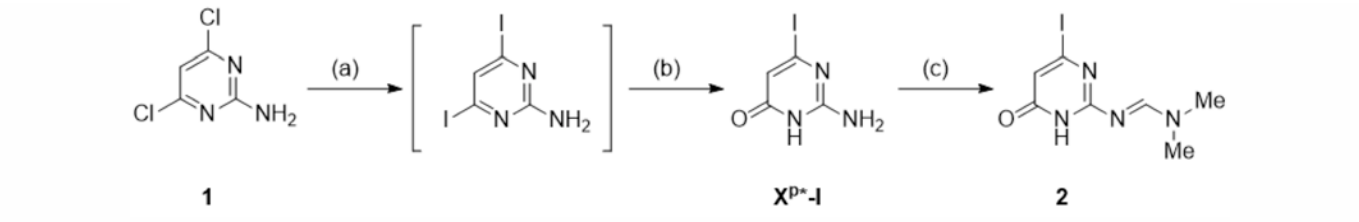
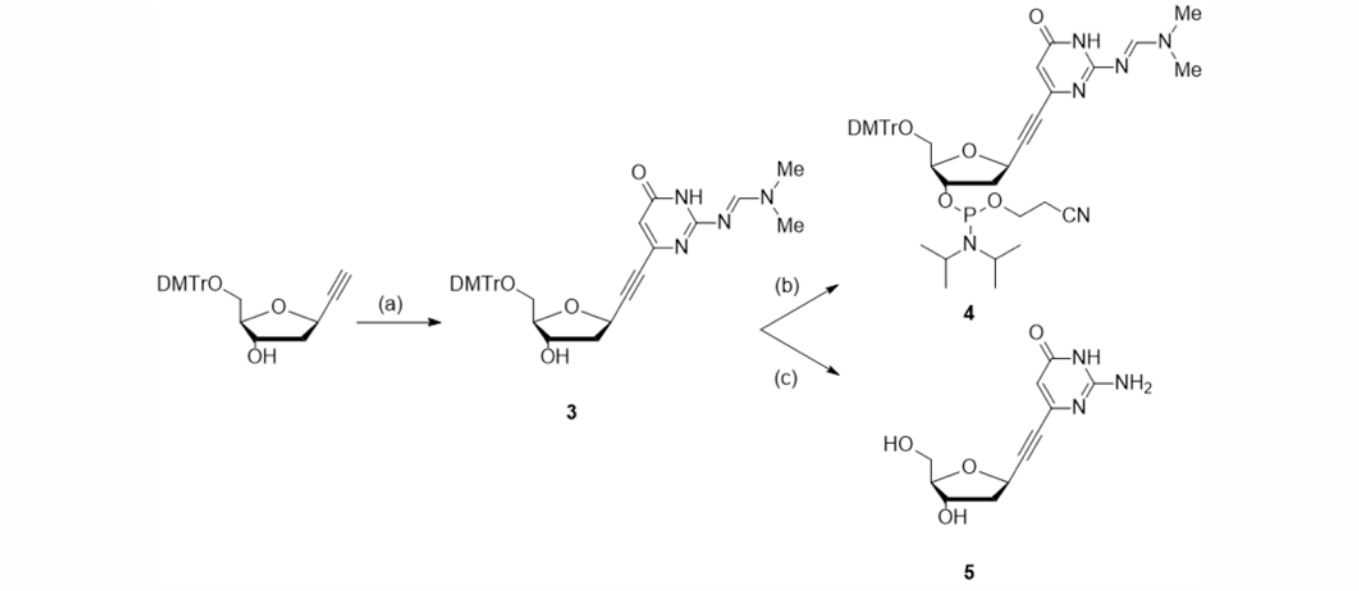


Figure 1. Self-dimerization of 6-alkynyl-2-aminopyrimidin-4-one

composed of unordinary odd numbers of nucleobases. Herein, we report the synthesis of an alkynyl *C*-nucleoside with 2-aminopyrimidin-4-one as a non-natural nucleobase **X^{p}*** and its phosphoramidite derivative. Furthermore, a DNA-like oligomer containing the **X^{p}*** nucleotide residue was found to form a novel DNA-like duplex with an odd number of genetic letters.



Scheme 1. Synthesis of iodine-attached X^{p}* base with dmf-protection. (a) NaI, 55% aq. HI, acetone; (b) aq. NaOH, 32% in 2 steps; (c) $Me_2NCH(OMe)_2$, MeOH, 97%.



Scheme 2. Synthesis of X^{p}* phosphoramidite **4** and X^{p}* nucleoside **5**. (a) $PdCl_2(PPh_3)_2$, CuI, **2**, DMF, *i*-Pr_2NH, 68%; (b) *i*-$Pr_2NP(Cl)O(CH_2)_2CN$, DIPEA, CH_2Cl_2, 76%; (c) (i) NH_3, MeOH, (ii) CCl_3CO_2H, CH_2Cl_2, 68% in 2 steps.

While the halogen-attached non-natural base **X^{p}*-I** was commercially available, it takes long time over 2 weeks to arrive and was expensive. Therefore, we synthesized **X^{p}*-I** from easily available 2-amino-4,6-dichloropyrimidine **1** by performing a Finkelstein reaction first followed by treatment of the crude product in an aqueous sodium hydroxide solution (Scheme 1). The amino group in **X^{p}*-I** was protected with *N*′,*N*′-dimethylaminomethylene group for being subjected to DNA auto-synthesis, affording **2**. A Sonogashira coupling reaction of **2** with 4,4'-dimethoxytrityl- (DMTr-) protected ethynyl *C*-2-deoxy-β-D-ribofuranoside, a key intermediate in our artificial DNA system reported previously,[4]

gave a stable *C*-nucleoside **3** in a good isolated yield (Scheme 2).[5] A conventional phosphoramidation of **3** furnished **4** for DNA auto-synthesis, whereas complete deprotection of dmf and DMTr groups in **3** afforded X^p*-nucleoside **5** for the determination of its molar extinction coefficient ε.

Next, the phosphoramidite **4** was subjected to a solid-phase DNA synthesis to give a chimera DNA oligomer containing the X^p* nucleotide residue successfully. The non-natural DNA has a palindromic 17-mer sequence, 5′-d(A_8X^p*T_8). After having been released from the solid support, the DNA-like oligomer was deprotected with an NH_3-contained basic solution, purified by reverse-phase HPLC, and identified by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectroscopy (see Experimental).

Duplex formation was evaluated for 5′-d(A_8X^p*T_8) on the basis of circular dichroism (CD) and UV-vis measurements in a buffer solution (10 mM HEPES (pH 7.0), 10 mM $MgCl_2$ and 100 mM NaCl). Strong positive-negative type Cotton effects emerged around 260 nm as the center at 20 ºC in CD spectra and then began to weaken with several isodichroic points upon gradual heating to 70 ºC. The CD change means the transition between two CD-active higher-order structures in the oligomer. In the UV-vis spectra, hyperchromisity was observed by 21% at 262 nm, suggesting the reduction of π-stacking interactions between the nucleobases (Figure 2a,b). Cooling of the solution to 20 ºC took back the CD and UV-vis

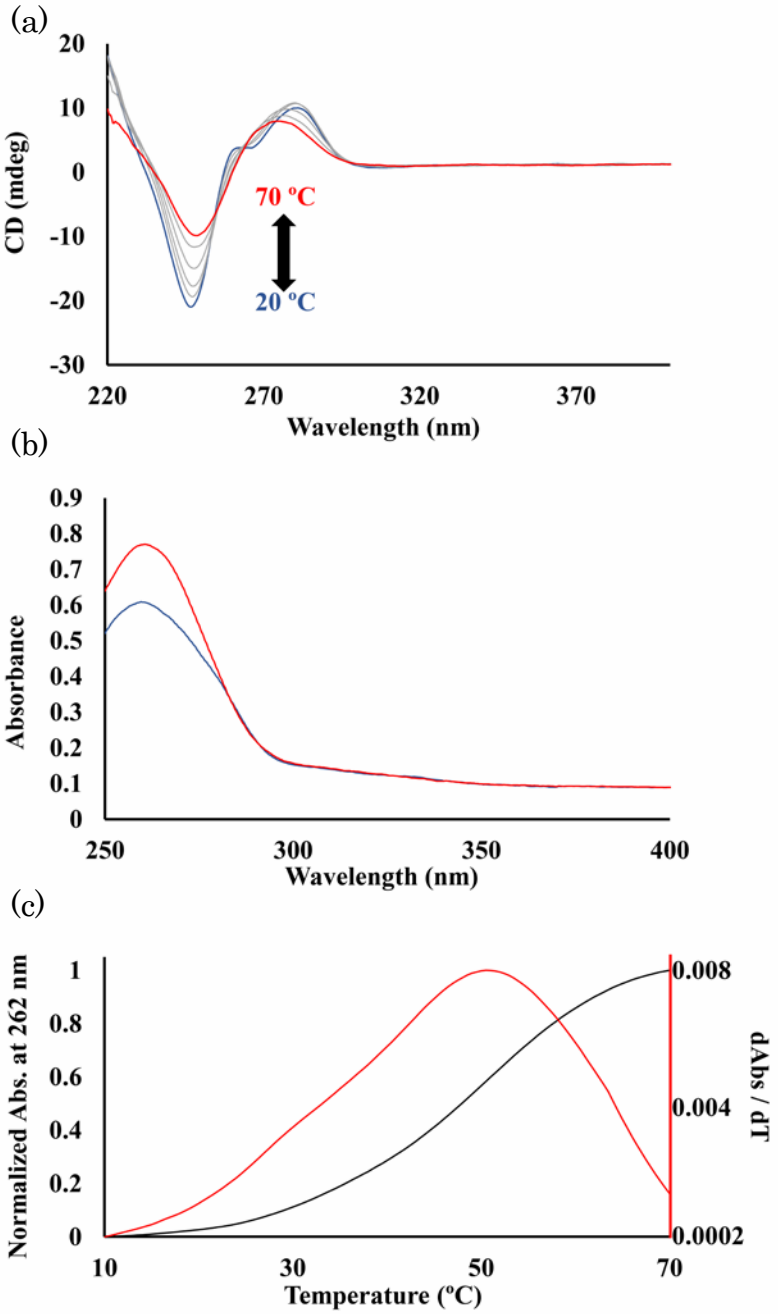


Figure 2. UV-vis and CD data for 5′-d(A_8X^p*T_8) (4.0 μM) in 10 mM HEPES (pH 7.0), 10 mM $MgCl_2$ and 100 mM NaCl. (a) CD spectra at 20 (blue), 30, 40, 50, 60 and 70 ºC (red) (b) UV spectra at 10 (blue) and 70 ºC (red) (c) UV melting curve (black) monitored at 262 nm and its first derivative profile (red).

spectra to the same intensities before the heating. A cooperative, sigmoidal transition was confirmed at 50.5 ºC for the chimeric, palindromic d($A_8X^p*T_8$) in a thermal denaturation experiment (Figure 2c and Table 1). These findings illustrate that the non-natural oligomer containing the X^p* nucleotide residue reversibly formed a higher-order structure, possibly resembling a duplex of natural DNA. Table 1 shows T_m values for non-natural 5′-d($A_8X^p*T_8$), natural fully-matched 5′-d(A_9T_8)/3′-d(T_9A_8), and natural mismatched 5′-d(A_8GT_8). The chimeric 5′-d($A_8X^p*T_8$) exhibited a slightly lower T_m than that for a native duplex with the same oligomer lengths probably because of the structural difference between ethynyl and natural deoxyriboses (entries 1 and 2). While the chimera showed significant stability compared to a natural duplex containing the G-G mismatched base pair (entry 3). The X^p* base would participate in the duplex formation without flipping out like G base in the G-G mis-pair. Further study is now underway to clarify the detailed structural confirmation and to construct another type of DNA-like architecture with odd numbers of genetic alphabets.

Table 1. T_m values of duplexes

	Sequences	T_m [a] (ºC)
1.	5′-d($A_8X^p*T_8$)	50.5
2.	5′-d(A_9T_8) / 3′-d(T_9A_8)	51.5
3.	5′-d(A_8GT_8)[b]	48.0

[a] T_m values were obtained from the maxima of the first derivatives of the melting curves measured in a buffer solution: 2.0 μM duplex, 10 mM HEPES (pH 7.0), 10 mM $MgCl_2$, and 100 mM NaCl. Errors were estimated at ±1.0 ºC. [b] The italicized letter *G* indicates a mismatched base.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR spectra of compounds **4** and **5** were obtained at 400 and 100 MHz, respectively, on a JEOL ECX400 spectrometer. Those of compound **3** were obtained at 300 and 75 MHz on a Varian GEMINI300 spectrometer. IR spectra were measured on a JASCO-FT/IR-460 plus spectrometer. Molar extinction coefficient was obtained on a JASCO V-560 UV/VIS spectrophotometer. ESI-HRMS analyses were carried out on a Thermo LTQ Orbitrap XL ETD mass spectrometer. Melting points were determined with Yanako MP-500D and not corrected.

Materials. 2-Amino-6-iodopyrimidin-4-one (X^p*-I)[6,7] and (2*R*,3*S*,5*R*)-2-(4,4'-dimethoxytrityloxymethyl)-5-ethynyl-3-hydroxytetrahydrofuran[4] were synthesized according to the procedures previously reported. Other materials were all commercially available.

2-*N*-(*N′*,*N′*-Dimethylaminomethylene)amino-6-iodo-4(1*H*)-pyrimidinone (2). A mixture of X^p*-I (11.9 g, 50 mmol) and $Me_2NCH(OMe)_2$ (50 mL, 375 mmol) in MeOH (300 mL) was stirred at room temperature for 3 h and then concentrated. The residue was dried under reduced pressure and used in the next step without further purification. Isolation of compound **2** was pursued as follows; the residue was

diluted with few drops of EtOAc and dropped in *n*-hexane. The resulting precipitate was filtered and dried under vacuum to give **2** (14.2 g, 97%) as a pale yellow solid. Mp > 250 ºC; IR (KBr) 2361, 1656, 1623, 1520 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.81 (br s, 1 H), 8.52 (s, 1 H), 6.29 (s, 1 H), 3.17 (s, 3H), 3.03 (s, 3H) ppm; ^{13}C NMR (DMSO-d_6) δ 160.1, 158.6, 158.3, 128.7, 116.0, 40.9, 34.9 ppm; HRMS calcd for MNa^+, $C_7H_9IN_4NaO$: 314.9719; found 314.9695.

(2*R*,3*S*,5*R*)-2-(4,4'-Dimethoxytrityloxymethyl)-5-[2-*N*-(*N*′,*N*′-dimethylaminomethylene)aminopyrimidin-4-on-6-ylethynyl]-3-hydroxytetrahydrofuran (3). A mixture of (2*R*,3*S*,5*R*)-2-(4,4'-dimethoxytrityloxymethyl)-5-ethynyl-3-hydroxytetrahydrofuran[4] (500 mg, 1.1 mmol), **2** (481 mg, 1.65 mmol), $PdCl_2(PPh_3)_2$ (78 mg, 0.12 mmol), and CuI (11.4 mg, 0.06 mmol) in *i*-Pr_2NH (5 mL) and DMF (5 mL) was stirred under argon atmosphere for 4 h at 80 °C. After removal of the solvent under reduced pressure, the residue was diluted with EtOAc, and washed with saturated $NaHCO_3$ and saturated NaCl aqueous solutions subsequently. The organic phase was dried over Na_2SO_4, evaporated, and chromatographed (SiO_2; eluent, CH_2Cl_2/MeOH = from 50:1 to 30:1) to give **3** (450 mg, 68%) as a colorless foam. Mp 123–125 °C; IR (KBr) 2836, 2361, 2234, 1662, 1524, 1511 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.64 (br s, 1 H), 8.51 (s, 1 H), 7.43-7.41 (m, 2 H), 7.31-7.19 (m, 7 H), 6.88-6.85 (m, 4 H), 5.83 (s, 1 H), 5.17-5.16 (m, 1 H), 4.92 (t, J = 7.5 Hz, 1 H), 4.18-4.08 (m, 1 H), 3.89-3.80 (m, 1 H), 3.71 (s, 6 H), 3.05-2.95 (m, 2 H), 2.11-2.06 (m, 2 H) ppm; ^{13}C NMR (DMSO-d_6) δ 162.5, 159.4, 158.2, 157.6, 146.8, 144.5, 135.3, 135.1, 129.4, 129.3, 127.4, 126.2, 112.8, 106.9, 90.6, 85.6, 85.1, 83.2, 78.9, 71.9, 66.9, 64.1, 54.7, 41.5, 34.5 ppm; HRMS calcd for MH^+, $C_{35}H_{37}N_4O_6$: 609.2713; found 609.2719.

X^p*-Phosphoramidite 4. To a dry CH_2Cl_2 (5 mL) solution of **3** (300 mg, 0.49 mmol) were added *i*-$Pr_2NP(Cl)O(CH_2)_2CN$ (0.4 mL, 1.69 mmol) and *i*-Pr_2NEt (5 mL) at room temperature under argon atmosphere. The reaction mixture was stirred for 90 min at the same temperature, and then to the reaction mixture was added several drops of MeOH. After removal of the solvent, the residue was diluted with EtOAc. The organic solution was washed with saturated $NaHCO_3$ and saturated NaCl aqueous solutions subsequently, dried over Na_2SO_4, and concentrated. The residue was chromatographed (SiO_2; eluent EtOAc/Hexane = from 1:2 to 2:1) to give a diastereomer mixture of **4** (300 mg, 76%) as a colorless foam. Further purification was performed by reverse phase HPLC (eluent; MeOH). Mp 91–95 °C; IR (KBr) 3432, 2966, 2931, 2837, 2251, 1670, 1630, 1523, 1342, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.79 (br s, 1 H), 8.45 (d, J = 7.3 Hz 1 H), 7.54-7.45 (m, 2 H), 7.43-7.33 (m, 4 H), 7.31-7.23 (m, 2 H), 7.22-7.14 (m, 1 H), 6.87-6.74 (m, 4 H), 6.14 (s, 1 H), 5.00 (dd, J = 8.7, 6.4 Hz, 1 H), 4.54-4.43 (m, 1 H), 4.17-4.09 (m, 1 H), 3.87-3.45 (m, 4 H), 3.77 (s, 6 H), 3.29-3.19 (m, 2 H), 3.16- 3.09 (m, 1 H), 3.04 (s, 3 H), 2.94 (d, J = 7.3 Hz, 3 H), 2.6 (t, J = 6.4 Hz, 1 H), 2.45 (t, J = 6.6 Hz, 1 H), 2.43-2.28 (m, 2 H), 1.23-0.97 (m, 12 H) ppm;

^{13}C NMR ($CDCl_3$) δ 163.3, 158.9, 158.4, 148.2, 144.9, 136.11, 136.08, 135.9, 130.2, 128.33, 128.29, 127.8, 126.7, 113.1, 111.02, 110.99, 90.9, 86.21, 86.19, 75.9, 75.4, 68.2, 68.1, 58.2, 55.2, 43.3, 43.2, 43.1, 41.2, 35.1, 24.6, 24.53, 24.48, 24.4 ppm; HRMS calcd for MH^+, $C_{44}H_{54}N_6O_7P$: 809.3792; found 809.3755.

X^{p*} Nucleoside; (2*R*,3*S*,5*R*)-2-Hydroxymethyl-5-(2-aminopyrimidin-4-on-6-ylethynyl)-3-hydroxy-tetrahydrofuran (5). A solution of **3** (250 mg, 0.41 mmol) in 2M NH_3/MeOH (50 mL) was stirred at room temperature for 3 days. After removal of the solvent, the residue was diluted with MeOH (45 mL). To the solution was added 3% CCl_3CO_2H in CH_2Cl_2 (30 mL), and the mixture was stirred for 3 h at room temperature. After quenched by adding a few drops of Et_3N, the solvent was removed under reduced pressure. The residue was suspended in CH_2Cl_2 and filtrated. The filtrate was again suspended in MeOH and refluxed. The suspension was cooled to room temperature, and the resulting precipitate was filtrated and dried under reduced pressure to give **5** (69 mg, 68%) as a white solid. Mp > 264 °C (decomp); IR (KBr) 3468, 3322, 3198, 3083, 2918, 2732, 2237, 1688, 1638, 1417 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.96 (br s, 1 H), 6.65 (br s, 2 H), 5.63 (s, 1 H), 5.11 (d, J = 4.1 Hz, 1 H), 4.79 (dd, J = 10.8, 5.3 Hz, 2 H), 4.18-4.06 (m, 1 H), 3.7-3.61 (m, 1 H), 3.44-3.25 (m, 2 H), 3.17 (d, J = 5.0 Hz, 1 H) 2.06-1.99 (m, 2 H) ppm; ^{13}C NMR (DMSO-d_6) δ 162.2, 156.1, 148.4, 105.2, 90.3, 87.7, 83.1, 71.7, 66.6, 62.0, 41.6 ppm; HRMS calcd for MH^+, $C_{11}H_{14}N_3O_4$: 252.0984; found 252.0982; UV (H_2O, 25 °C) ε_{260} = 1700 $Lmol^{-1}cm^{-1}$.

Synthesis of DNA oligomer. The artificial DNA oligomer was synthesized by use of the phosphoramidite **4** on an Applied Biosystems 392 synthesizer using standard β-cyanoethyl phosphoramidite chemistry with the coupling reaction time of 10 min. The solid support (Glen UnySupport™ FC) was purchased from Glen Research. After solid-phase auto-synthesis, the oligomer was removed from the solid support with concentrated NH_4OH at 30 ºC for 30 min and deprotected with concentrated NH_4OH at 40 ºC for 8 h. The oligomer was then purified by reverse-phase HPLC using a 5C_{18}-MS-II column (4.6 x 150 mm) with an eluent of 100 mM triethylammonium acetate (TEAA) and the following MeCN percentages of linear gradient (0–60 min, 5–50%) at a flow rate of 1.0 mL/min under 40 ºC using a column oven.

MALDI-TOF mass measurements. MALDI-TOF mass spectra were recorded on a Bruker-Daltonics-Autoflex mass spectrometer operating under the negative ion mode with 3-hydroxypicolinic acid as a matrix. $d(A_8X^{p*}T_8)$: calcd for $[M\text{-}H^+]^-$, $C_{171}H_{212}N_{59}O_{100}P_{16}$: 5186.9 ; found 5190.1.

UV and T_m Measurements. UV-vis spectra and T_m melting curves (1.0 ºC/1.0 min) were obtained by JASCO V-560 UV/vis spectrophotometer with a peltier and a temperature controller in a temperature range from 10 to 70 °C. The T_m values were determined from the maxima of the first derivatives of the melting curves measured in a buffer solution: 2 μM duplexes, 10 mM Hepes (pH 7.0), 10 mM $MgCl_2$, 100 mM NaCl. Errors were estimated at ± 1.0 ºC. Concentrations of the solutions containing artificial DNAs were determined based on the molar extinction coefficients at 260 nm (ε_{260}) of the X^p*nucleoside **5**, and natural nucleoside monomers.

CD measurements. CD spectra were recorded using a JASCO-J-720WI spectropolarimeter with a temperature controller at 20, 30, 40, 50, 60, and 70 °C in a buffer solution: 4 μM 5′-d(A_8X^p*T_8), 10 mM Hepes (pH 7.0), 10 mM $MgCl_2$, 100 mM NaCl.

ACKNOWLEDGEMENTS

This work was supported by "Dynamical ordering of biomolecular systems for creation of integrated functions" of Japan Society for the Promotion of Science (JSPS) Grant-in Aid for Scientific Research on Innovative Areas Grant Number JP16H00757. This work was also supported by JSPS Core-to-Core Program, B. Asia-Africa Science Platforms.

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