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Ag^+-Mediated DNA Base Pairing: Extraordinarily Stable Pyrrolo-dC–Pyrrolo-dC Pairs Binding Two Silver Ions

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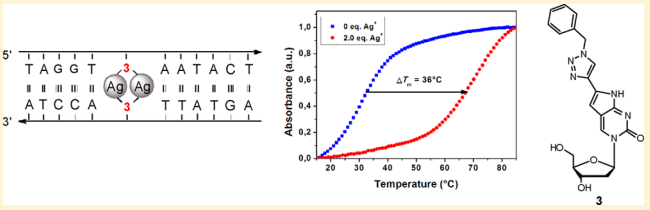
Supporting Information

ABSTRACT: 6-Substituted pyrrolo-dC–pyrrolo-dC mismatches selectively capture silver ions to form extraordinarily stable metal-mediated base pairs. One single modification in a 12-mer duplex causes a T_m increase of 36.0 °C relative to the metal-free mismatched duplex. Spectrophotometric titrations as well as ESI mass spectra confirmed the binding of two silver ions per base pair. The Ag^+-mediated base pairs may permit the construction of metal-responsive DNA with a very high silver loading.



Metal-mediated base pairs represent a high-tech alternative to Watson–Crick base pairing stabilized by hydrogen bonds. Already in 1952 Katz reported on binding of $HgCl_2$ to DNA,[1a] and Eichhorn later performed detailed investigations.[1b] Likewise, Lee observed that other divalent metal ions such as Zn^{2+} interact with duplex DNA.[2] Our laboratory utilized modified 6-azauridine as a nucleobase substitute to shift Zn^{2+} binding and duplex stabilization to neutral pH.[3] Interactions of silver ions with DNA have been studied extensively by UV–vis and CD spectroscopy and potentiometric titrations.[4]

Recently, metal ions have been utilized for homo-base-pair formation by conversion of base-pair mismatches into metal-coordinated matching pairs. Accordingly, the groups of Marzilli and Ono transformed the dT–dT mismatch into a stable metal base pair in the presence of Hg^{2+} ions (Figure 1).[5] In a similar way, a dC–dC mismatch was converted into a metal base pair using Ag^+ ions.[6] Artificial metal base pairs have been constructed with chelating heterocycles.[7,8] For some metal base pairs, replication by the polymerase chain reaction has been demonstrated.[9]

6-Methylpyrrolo-dC (^{me}PyrdC, **1**) can serve as fluorescent substitute for dC as it possesses the same Watson–Crick binding face as the canonical nucleoside. It has been widely used to explore the structure and dynamics of nucleic acids.[10,11] Recently, it was demonstrated that a **1**–dG base pair stabilizes the DNA duplex structure, and Ag^+-mediated base pairing between **1** and dC has been used for Ag^+ sensing.[12] Hence, we envisioned that Ag^+-mediated homo base pairs of ^{me}PyrdC might be exceptionally stable, considering that this base pair might bind two silver ions. Further stabilization was expected by functionalization of the pyrrole skeleton. Herein we report on various pyrrolo-dC derivatives such as **1**, 6-(2-pyridyl)-pyrrolo-dC (^{py}PyrdC, **2**), and 6-(1-benzyl-1*H*-1,2,3-triazolyl)-pyrrolo-dC (^{triaz}PyrdC, **3**) as components of duplex DNA (Figure 1) and the formation of extraordinarily stable Ag^+-mediated homo base pairs stabilized by two silver ions.

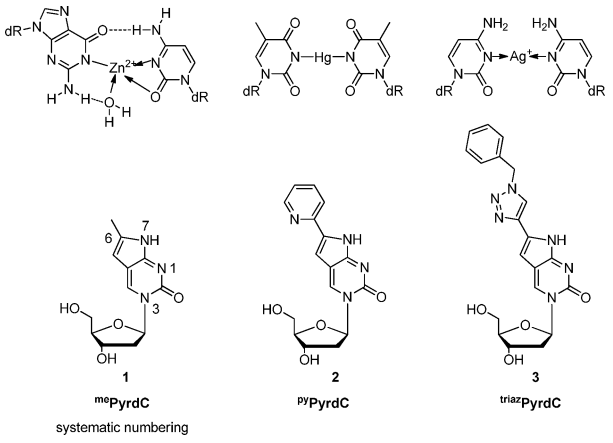


Figure 1. Structures of metal base pairs[2,6,7] and PyrdC derivatives.

While compounds **1** and **3** have already been described in the literature,[10g] compound **2** was synthesized as depicted in Scheme 1. Sonogashira cross-coupling of **4** to give **6** has been reported previously.[13] Next, the 4,4′-dimethoxytrityl group was introduced at the 5′-position and the amino group was blocked with a benzoyl residue, resulting in intermediate **8**. Intramolecular cyclization (CuI and Et_3N) resulted in the formation

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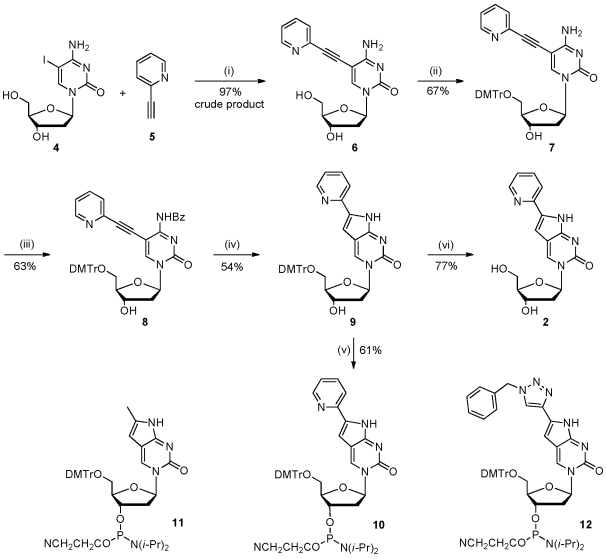
Scheme 1. Synthesis of Phosphoramidite 10[a]



[a]Reagents and conditions: (i) CuI, $Pd(PPh_3)_2Cl_2$, Et_3N, 100 °C, 4 h. (ii) 4,4′-Dimethoxytriphenylmethyl chloride, anhydrous pyridine, rt, 8 h. (iii) (a) Trimethylsilyl chloride, pyridine, 20 min; (b) benzoyl chloride, rt, 15 h. (iv) CuI, DMF, Et_3N, 60 °C. (v) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $(i\text{-}Pr)_2EtN$, rt. (vi) 2.5% Dichloroacetic acid, Et_3N.

of the pyrrole system, affording nucleoside **9**. Compound **9** was either deprotected to give nucleoside **2** or treated with 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidochloridite to yield phosphoramidite **10**. Phosphoramidite **11** is commercially available, while phosphoramidite **12** was described earlier by our laboratory.[10g] All of the compounds were characterized by ^1H, ^{13}C, $^1H-^{13}C$ gated-decoupled, and DEPT-135 NMR spectroscopy as well as elemental analysis or ESI mass spectrometry [see the Experimental Section and the Supporting Information (SI)].

Oligonucleotides were prepared by solid-phase synthesis utilizing phosphoramidites **10**–**12**. Compounds **1**, **2**, and **3** were incorporated at the center of the 12-mer duplex 5′-d(TAGGTXAATACT)·3′-d(ATCCAXTTATGA), where **X** = C, G, **1**, **2**, **3**. After cleavage from the solid support and deprotection under standard conditions, the oligonucleotides were purified before and after detritylation. The masses of the silver-free oligonucleotides were confirmed by MALDI-TOF mass spectrometry (Table S1 in the SI).

The most obvious feature of metal base pairs is their significant contribution to the stabilization of DNA duplexes, which goes beyond Watson–Crick base pairing. In a first series of experiments, the stability of oligonucleotide duplexes incorporating compounds **1**–**3** was determined in the absence of Ag^+ ions (for melting profiles, see Figure 2 and the SI). The T_m values are displayed in Table S2 in the SI. The three modified PyrdC derivatives showed similar T_m values when the modified bases were paired with dG (T_m = 49.0–50.0 °C) or formed mismatches with dC (T_m = 27.5–30.5 °C). Significant differences were observed when PyrdC–PyrdC homo base pairs were introduced. Most strikingly, the duplex ODN-**6**·ODN-**7** containing ^{py}PyrdC derivative **2** exhibited a high T_m value similar to that for duplex ODN-**1**·ODN-**2** with the canonical dG–dC base pair, possibly as a result of pyridine–pyridine interactions.[14]

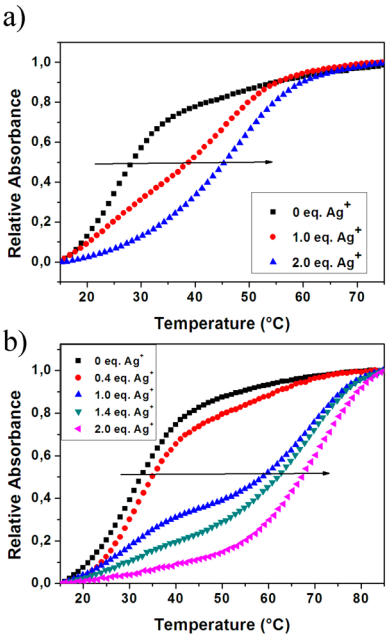


Figure 2. Thermal denaturation experiments were performed at a duplex concentration of 5 μM in buffer [100 mM NaOAc, 10 mM $Mg(OAc)_2$, pH 7.4] at 260 nm in the presence of various concentrations of Ag^+ (0–2.0 equiv). (a) ODN-**4**·ODN-**5**; relative absorbance[5a] = $[(A_{t°C} - A_{15°C})/(A_{75°C} - A_{15°C})]$ at 260 nm. (b) ODN-**8**·ODN-**9**; relative absorbance[5a] = $[(A_{t°C} - A_{15°C})/(A_{85°C} - A_{15°C})]$ at 260 nm.

Subsequently, the T_m values for DNA duplex melting were determined in the presence of 1 or 2 equiv of Ag^+ ions (Table 1 and Table S2 in the SI). For duplexes in which dG was located opposite to the PyrdC derivatives **1**–**3**, the addition of Ag^+ ions had no effect on the duplex stability, and thus, formation of metal base pairs can be excluded. On the contrary, duplexes containing PyrdC–dC pairs were stabilized by silver ions. Already the addition of 1 equiv of $AgNO_3$ caused a notable increase in duplex stability (ΔT_m = +6.5 to +9.0 °C). Increasing the amount of Ag^+ to 2 equiv did not lead to further changes (Table S2 in the SI).

Next, duplexes with PyrdC–PyrdC homo base pairs were studied. The addition of 1 equiv of silver ions induced biphasic melting for duplexes incorporating **1** (ODN-**4**·ODN-**5**) or **3** (ODN-**8**·ODN-**9**), while an overlap of the two transitions occurred with **2** (ODN-**6**·ODN-**7**). Two species coexisted: duplexes without silver ions (lower T_m) and duplexes binding two silver ions (higher T_m) (Table 1). When 2 equiv of Ag^+ was added, only the high T_m values were observed (monophasic melting). The most significant T_m increase (ΔT_m = +36.0 °C) was observed for the duplex ODN-**8**·ODN-**9** incorporating a ^{triaz}PyrdC–^{triaz}PyrdC base pair, representing an unique example of duplex stabilization by a Ag^+-mediated base-pair complex. Such a T_m increase (ΔT_m = +40.0 °C) was previously observed for a copper ion–salen base pair.[7g] CD spectra of the duplexes containing PyrdC–PyrdC homo base pairs confirmed the formation of B-type DNA (Figure S19 in the SI).

Then, the stoichiometry of the Ag^+-mediated PyrdC–PyrdC base pairs was determined. UV absorption curves were measured as functions of the $AgNO_3$ concentration. Titration of the duplexes ODN-**8**·ODN-**9** (Figure 3a and Figure S5 in the SI) and ODN-**6**·ODN-**7** (Figure S4a,b in the SI) with Ag^+ led to significant UV–vis spectral changes. In both cases, three

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Table 1. T_m Values for PyrdC (1−3)-Modified DNA Duplexes in the Presence or Absence of Silver Ions[a]

duplex	T_m (°C) with n equiv of Ag^+			ΔT_m (°C)[b]
	n = 0	n = 1	n = 2	
5′-d(TAG GT**C** AAT ACT) ODN-**1** 3′-d(ATC CA**G** TTA TGA) ODN-**2**	46.5	47.0	46.5	0
5′-d(TAG GT**C** AAT ACT) ODN-**1** 3′-d(ATC CA**C** TTA TGA) ODN-**3**	26.0	32.5	34.5	+8.5
5′-d(TAG GT**1** AAT ACT) ODN-**4** 3′-d(ATC CA**1** TTA TGA) ODN-**5**	27.5	~25/46.0[c]	46.0	+18.5
5′-d(TAG GT**2** AAT ACT) ODN-**6** 3′-d(ATC CA**2** TTA TGA) ODN-**7**	42.0	56.0	63.5	+21.5
5′-d(TAG GT**3** AAT ACT) ODN-**8** 3′-d(ATC CA**3** TTA TGA) ODN-**9**	33.0	~30/68.0[c]	69.0	+36.0

[a]Measured at 260 nm at a heating rate of 1.0 °C/min with single-strand concentrations of 5 μM in buffer [100 mM NaOAc, 10 mM $Mg(OAc)_2$, pH 7.4] in the presence of various concentrations of $AgNO_3$ (0−2.0 equiv). T_m values were calculated from the cooling curves. [b]ΔT_m = (T_m with 2 equiv of Ag^+) − (T_m without Ag^+). [c]Biphasic melting.

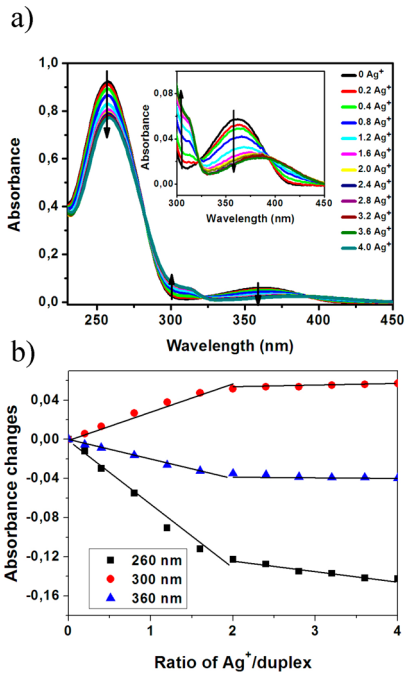

Figure 3. (a) Spectrophotometric titration of 5 μM ODN-**8**·ODN-**9** with increasing Ag^+ concentration in buffer [100 mM NaOAc, 10 mM $Mg(OAc)_2$, pH 7.4]. (b) Determinations of the silver/duplex ratio at different wavelengths.

isosbestic points were observed, indicating two-state transitions. The UV measurements were performed at different wavelengths, and the ratios of silver ions to duplexes were plotted (Figure 3b and Figures S3c and S4c in the SI). UV changes were detected during the addition of 2 equiv of Ag^+. Additional Ag^+ did not lead to further significant changes. As Ag^+ ion binding was detected beyond 300 nm, selective monitoring of the UV changes of PyrdC was possible. The UV titration studies were supported by ESI mass spectra (Figures S6 and S7 in the SI). Both ODN-**6**·ODN-**7** and ODN-**8**·ODN-**9** displayed masses of duplexes with two silver ions. Additional small peaks were detectable for duplexes with two silver ions plus one sodium ion or even with three silver ions. The binding position of the third metal ion is not clear. However, base pairs with three silver ions cannot be excluded (Figure S8 in the SI). Possibly because of the low T_m value of ODN-**4**·ODN-**5** (Table 1), the mass of its Ag^+ complex was not detectable.

Other metal ions were analyzed and showed almost no effect on the duplex stability (Table S3 in the SI). The ability of Ag^+ to bind through the PyrdC−PyrdC mismatches appears to be highly specific and surpasses that of the other tested metal ions.

Only a very few examples of DNA homo-base-pair binding of two silver ions have been described in the literature.[15−17] In one example reported by Ono, DNA duplexes bearing $^{5\text{-fluoro}}$dT−$^{5\text{-fluoro}}$dT mismatches were significantly stabilized by two silver ions at pH 9.0.[15] In another context, Hoogsteen base pairs formed by aminobenzimidazole-dT that were slightly stabilized by two silver ions have been described.[16] Also, a base pair of 4-thio-dT capturing two silver ions has been reported.[17] Our results discussed above indicate that the PyrdC−PyrdC base pairs instantly bind two silver ions in a cooperative manner. It seems that a paucity of silver ions in solution (1 equiv of Ag^+) leads to the coexistence of two species, one without Ag^+ and the other with two Ag^+, resulting in the observation of biphasic melting (Figure 2).

On the basis of our results, we propose structures of the PyrdC−Ag_2−PyrdC base pairs (Figure 4, motifs III and IX). We anticipate that the strong binding capability with two silver ions results from the special features of the pyrrolo[2,3-*d*]pyrimidine moiety, which can form tautomeric structures (motifs I and II) and bind silver ions by salt bridges as well as by metal coordination. Silver ions replace the protons of the pyrrolo[2,3-*d*]pyrimidine heterocycles and coordinate to the electron pairs of the nearby nitrogens. Similar proximal positioning of silver ions has been reported before[18] and is a prerequisite for motif III. Other motifs under participation of heterocyclic substituents are conceivable.

In conclusion, new PyrdC−PyrdC homo base pairs with heteroaromatic 6-substituents that bind Ag^+ ions strongly and specifically in a cooperative way have been designed. These metal-mediated base pairs are among the rare examples where each base pair has the capability to bind two Ag^+ ions. The incorporation of only one ^{triaz}PyrdC−Ag_2−^{triaz}PyrdC pair increases the T_m by 36.0 °C relative to the silver-free mismatched duplex. Further DNA duplex stabilization can be expected when multiple metal-mediated base pairs are

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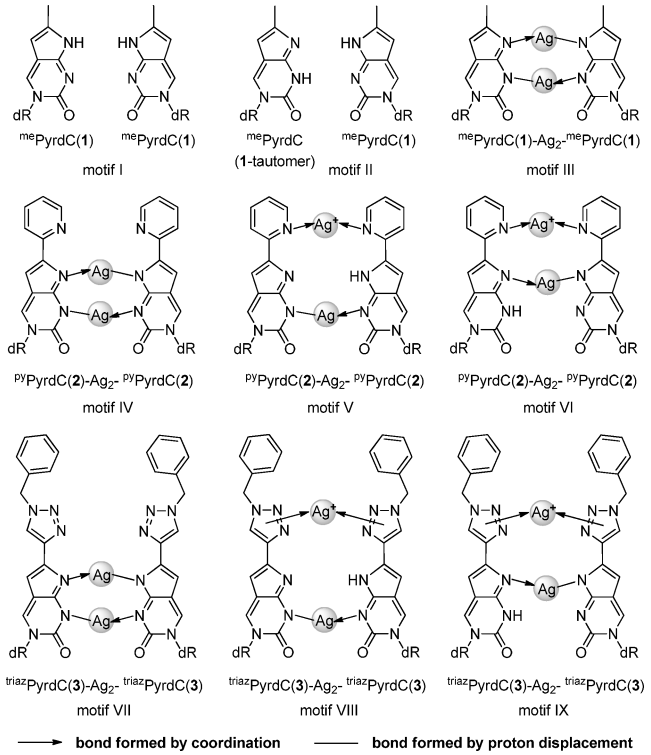

Figure 4. Selected tautomeric structures and proposed Ag^+-mediated base pairs. For additional base pairs, see Figure S8 in the SI.

incorporated.[19a−e] By this means, metal-containing DNA with a very high silver loading (two silver units per base pair) may be constructed.

EXPERIMENTAL SECTION

General Methods and Materials. All of the chemicals and solvents were of laboratory grade as obtained from commercial suppliers and were used without further purification. Thin-layer chromatography (TLC) was performed on TLC aluminum sheets covered with silica gel 60 F254. Flash column chromatography (FC) employed silica gel 60 at 0.4 bar. UV-spectra were recorded on a U-3200 UV−vis spectrometer. ^{1}H, ^{13}C, and ^{31}P NMR spectra were measured at 300.15 MHz for ^{1}H, 75.48 MHz for ^{13}C, and 121.52 MHz for ^{31}P. The reported δ values are in parts per million relative to Me_4Si as an internal standard (^{1}H and ^{13}C) or external 85% H_3PO_4 (^{31}P). The J values are given in hertz. For NMR spectra measured in DMSO-d_6, the chemical shift of the solvent peak was set to 2.50 ppm for ^{1}H NMR spectra and 39.50 ppm for ^{13}C NMR spectra. DEPT-135 and ^{1}H−^{13}C gated-decoupled spectra were used to assign the ^{13}C signals (Table 2). The ^{1}H−^{13}C coupling constants are given in Table 3. Reversed-phase HPLC was carried out on a 4 mm × 250 mm RP-18 (10 μm) LiChrospher 100 column with an HPLC pump connected with a variable-wavelength monitor, a controller, and an integrator. The molecular masses of the oligonucleotides were determined by MALDI-TOF mass spectrometry in the linear positive-ion mode with 3-hydroxypicolinic acid (3-HPA) as a matrix. CD spectra were recorded on a CD spectrometer at 20 °C. The thermal melting curves were measured with a UV−vis spectrophotometer equipped with a thermoelectrical controller. The temperature was measured continuously in the reference cell with a Pt-100 resistor at a heating rate of 1 °C min^{-1}. The calculation of thermodynamic data was performed with the program MeltWin (version 3.0) using curve fitting of the melting profiles according to a two-state model.

4-Amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-(2-pyridylethynyl)pyrimidin-2(1*H*)-one (6).[13] To a suspension of 5-iodo-2′-deoxycytidine[20] (**4**) (3.0 g, 8.5 mmol) and CuI (162 mg, 0.85 mmol) in anhydrous DMF (20 mL) were added $[Pd(PPh_3)_2Cl_2]$ (298 mg, 0.42 mmol), anhydrous Et_3N (20 mL), and 2-ethynylpyridine (**5**) (1.9 g, 18.4 mmol). The reaction mixture was refluxed under a nitrogen atmosphere for 4 h. The solvent was evaporated, affording a gum. Upon addition of CH_2Cl_2 (100 mL) to the gummy residue, compound **6** precipitated as crude brown solid (2.7 g, 97%). This material was directly used for the next step. A small amount of pure compound **6** was obtained by FC (silica gel, column 15 cm × 3 cm, 8:1 CH_2Cl_2/MeOH). TLC (10:1 CH_2Cl_2/MeOH): R_f = 0.21. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.99−2.08 (m, 1H, 2′-H$_\alpha$), 2.15−2.23 (m, 1H, 2′-H$_\beta$), 3.55−3.67 (m, 2H, 5′-H), 3.82 (m, 1H, 4′-H), 4.23 (m, 1H, 3′-H), 5.14 (m, 1H, 5′-OH), 5.24 (d, J = 3.6 Hz, 1H, 3′-OH), 6.13 (t, J = 6.6 Hz, 1H, 1′-H), 7.08 (s, 1H, NH$_a$), 7.35−7.39 (m, 1H, pyridyl-H), 7.77−7.86 (m, 3H, NH$_b$, pyridyl-H), 8.38 (s, 1H, 6-H), 8.56 (d, J = 4.8 Hz, 1H, pyridyl-H). The spectroscopic data correspond to the literature values.[13]

4-Amino-1-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]-5-(2-pyridylethynyl)pyrimidin-2(1*H*)-one (7). Compound **6** (1.36 g, 4.14 mmol) was coevaporated with anhydrous pyridine (2 × 20 mL) before dissolution in anhydrous pyridine (30 mL). Four drops of *N*,*N*-diisopropylethylamine were added. Next, 4,4′-dimethoxytrityl chloride (1.82 g, 5.37 mmol) was added in three portions, and the reaction mixture was stirred at room temperature for 8 h. Finally, MeOH (2 mL) was added, and the mixture was stirred for another 30 min. The reaction mixture was diluted with CH_2Cl_2 (2 × 80 mL) and extracted with 5% aqueous $NaHCO_3$ solution (100 mL) followed by H_2O (80 mL). The organic layer was dried over Na_2SO_4 and then concentrated. Purification by FC (silica gel, column 15 cm × 3 cm, 20:1:0.05 CH_2Cl_2/MeOH/Et_3N) gave **7** as a light-yellow foam (1.74 g, 67%). TLC (10:1 CH_2Cl_2/MeOH): R_f = 0.25. UV (MeOH) λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}): 274.5 (15000), 322 (21500). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.13−2.22 (m, 1H, 2′-H$_\alpha$), 2.27−2.34 (m, 1H, 2′-H$_\beta$), 3.14−3.28 (m, 2H, 5′-H), 3.66 (s, 6H, 2 × OCH$_3$), 3.97−3.99 (m, 1H, 4′-H), 4.21−4.25 (m, 1H, 3′-H), 5.31 (d, J = 4.2 Hz, 1H, 3′-OH), 6.12 (t, J = 6.6 Hz, 1H, 1′-H), 6.84−6.88 (m, 4H, DMTr-H), 7.05 (s, 1H, NH$_a$), 7.12−7.42 (m, 11H, DMTr-H, pyridyl-H), 7.72−7.78 (m, 1H, pyridyl-H), 7.89 (s, 1H, NH$_b$), 8.11 (s, 1H, 6-H), 8.54−8.56 (m, 1H, pyridyl-H). Anal. Calcd for $C_{37}H_{34}N_4O_6$: C, 70.46; H, 5.43; N, 8.88. Found: C, 70.40; H, 5.42; N, 8.88.

4-Benzoylamino-1-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]-5-(2-pyridylethynyl)-pyrimidin-2(1*H*)-one (8). To a solution of **7** (1.37 g, 2.17 mmol) in pyridine (20 mL) was added trimethylsilyl chloride (386 μL, 3.04 mmol) at rt. The mixture was stirred for 20 min and then cooled to 0 °C (ice bath), and benzoyl chloride (303 μL, 2.63 mmol) was added dropwise using a syringe. The ice bath was removed, and the reaction mixture was stirred overnight. Water (5 mL) was added, and the reaction mixture was stirred for an additional 20 min. Finally, concentrated 28% aq. NH_3 (1 mL) was added, and the mixture was stirred for another 10 min. The solution was diluted with CH_2Cl_2 (80 mL) and extracted with 5% aqueous $NaHCO_3$ solution (100 mL) followed by H_2O (80 mL). The organic layer was dried over Na_2SO_4 and then concentrated. Purification by FC (silica gel, column 15 cm × 3 cm, 30:1:0.05 CH_2Cl_2/MeOH/Et_3N) gave **8** as a light-yellow foam (1.0 g, 63%). TLC (10:1 CH_2Cl_2/MeOH): R_f = 0.53. UV (MeOH) λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}): 236.5 (38900), 310.0 (22100). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.36 (m, 2H, 2′-H), 3.23−3.24 (m, 2H, 5′-H), 3.66 (s, 6H, 2 × OCH$_3$), 4.04 (m, 1H, 4′-H), 4.28−4.31 (m, 1H, 3′-H), 5.39 (d, J = 4.5 Hz, 1H, 3′-OH), 6.13 (t, J = 6.3 Hz, 1H, 1′-H), 6.84−7.65 (m, 19H, DMTr-H, Bz-H, pyridyl-H), 8.33−8.36 (m, 2H, 6-H, pyridyl-H), 8.52 (s, 1H, NH), 8.53 (m, 1H, pyridyl-H). Anal. Calcd for $C_{44}H_{38}N_4O_7$: C, 71.92; H, 5.21; N, 7.62. Found: C, 71.78; H, 5.25; N, 7.62.

6-(2-Pyridyl)-3-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]pyrrolo[2,3-*d*]pyrimidin-2(3*H*)-one (9). A mixture of compound **8** (594 mg, 0.81 mmol) and CuI (230 mg, 1.21 mmol) in Et_3N (8 mL) and DMF (8 mL) was heated at 60 °C for 24 h. The solvent was evaporated, and the remaining residue was dissolved in CH_2Cl_2 (80 mL). The organic phase was washed several times with 5% Na_2EDTA solution (4 × 100 mL), dried over

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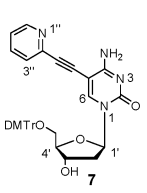
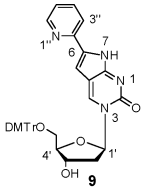
Table 2. ^{13}C NMR Chemical Shifts (δ) of Pyrrolo[2,3-*d*]pyrimidine Derivatives[a]

cmpd	C2[b]/ C2[c]	C6[b]/ C4[c]	C5[b]/ C4a[c]	C5[c]	C6[c]	C4[b]/ C7a[c]	C1′	C2′	C3′	C4′	C5′	OCH_3	C≡C	pyridyl
6	153.3	145.8	93.6			163.8	85.6	40.9	70.0	87.5	60.9		81.1, 88.6	149.8, 142.5, 136.5, 127.4, 123.2
7	153.2	145.0	93.7			163.8	85.8	40.9	70.6	85.9	63.7	54.9	80.7, 88.8	149.7, 142.4, 136.2, 127.3, 123.1
8	158.0[d]	149.9	93.5			157.9[d]	86.7	–	70.2	86.4	63.5	54.9	81.0, 85.7	149.9, 142.2, 136.3, 127.6, 123.2
9	153.6	137.2	108.7	99.3	148.7	159.4	85.6	41.4	69.0	86.7	62.6	54.9		149.5, 138.9, 137.0, 122.9, 119.5
2	153.7	137.7	108.8	99.7	148.8	159.4	87.1	41.4	69.8	87.9	60.9			149.4, 138.9, 137.0, 122.8, 119.8



Pyrimidine numbering



Systematic numbering

[a]Measured in DMSO-d_6 at 298 K. [b]Pyrimidine numbering. [c]Systematic numbering. [d]Tentative.

Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by FC (silica gel, column 10 cm × 3 cm, 20:1:0.05 CH_2Cl_2/MeOH/Et_3N), affording compound **9** (275 mg, 54%) as a yellow foam. TLC (10:1 CH_2Cl_2/MeOH): R_f = 0.29. UV (MeOH) λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}): 233.5 (33500), 273.0 (18400), 369.5 (11300). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.24 (m, 1H, 2′-H$_\alpha$), 2.39–2.46 (m, 1H, 2′-H$_\beta$), 3.28–3.41 (m, 2H, 5′-H), 3.70, 3.71 (2s, 6H, 2 × OCH_3), 3.99–4.01 (m, 1H, 4′-H), 4.39–4.42 (m, 1H, 3′-H), 5.41 (d, J = 4.8 Hz, 1H, 3′-OH), 6.21–6.25 (m, 2H, 1′-H, 5-H), 6.85–6.91 (m, 4H, DMTr-H), 7.22–7.90 (m, 12H, DMTr-H, pyridyl-H), 8.59–8.60 (m, 1H, pyridyl-H), 8.71 (s, 1H, 6-H), 11.79 (s, 1H, NH). Anal. Calcd for $C_{37}H_{34}N_4O_6$: C, 70.46; H, 5.43; N, 8.88. Found: C, 70.29; H, 5.45; N, 8.88.

6-(2-Pyridyl)-3-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]pyrrolo[2,3-*d*]pyrimidin-2(3*H*)-one 3′-(2-Cyanoethyl)-*N*,*N*′-diisopropyl phosphoramidite (10). A stirred solution of **9** (0.34 g, 0.54 mmol) in anhydrous CH_2Cl_2 (10 mL) was preflushed with nitrogen and treated with (*i*-Pr)$_2$NEt (146 μL, 0.86 mmol) followed by 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidochloridite (180 μL, 0.81 mmol). The solution was stirred for 35 min at room temperature and then diluted with CH_2Cl_2 (40 mL) and extracted with 5% aq. $NaHCO_3$ solution (30 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by FC (silica gel, column 10 cm × 2 cm, 20:1:0.05 CH_2Cl_2/MeOH/Et_3N), giving **10** (275 mg, 61%) as a light-yellow foam. TLC (25:1:0.05 CH_2Cl_2/MeOH/Et_3N): R_f = 0.26. ^{31}P NMR ($CDCl_3$, 121 MHz): δ 148.94, 149.80. ESI-TOF *m*/*z*: calcd for $C_{46}H_{51}N_6O_7P$ [M + H$^+$] 831.3630, found 831.3617; [M + Na$^+$] 853.3449, found 853.3442.

6-(2-Pyridyl)-3-[2-deoxy-β-D-*erythro*-pentofuranosyl]-pyrrolo[2,3-*d*]pyrimidin-2(3*H*)-one (2). A solution of compound **9** (190 mg, 0.30 mmol) in CH_2Cl_2 (5 mL) was cooled to 0 °C (ice bath). Next, 2.5% dichloroacetic acid (4 mL) was added, and the reaction mixture was stirred at 0 °C for 40 min and then neutralized with triethylamine (4 mL). The solvent was removed under vacuum, and the remaining residue was adsorbed on silica gel and purified by FC (silica gel, column 10 cm × 3 cm, 9:1 CH_2Cl_2/MeOH) to afford compound **2** (76 mg, 77%) as slightly yellow solid. TLC (4:1 CH_2Cl_2/MeOH): R_f = 0.35. UV (MeOH) λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}): 257.0 (20700), 270.0 (20300), 366.5 (13300). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.00–2.09 (m, 1H, 2′-H$_\alpha$), 2.35–2.42 (m, 1H, 2′-H$_\beta$), 3.60–3.74 (m, 2H, 5′-H), 3.90–3.93 (m, 1H, 4′-H), 4.23–4.29 (m, 1H, 3′-H), 5.15 (t, J = 5.4 Hz, 1H, 5′-OH), 5.28 (d, J = 3.6 Hz, 1H, 5′-OH), 6.25 (t, J = 6.3 Hz, 1H, 1′-H), 6.98 (s, 1H, 5-H), 7.31–7.35 (m, 1H, pyridyl-H), 7.84–7.94 (m, 2H, pyridyl-H), 8.62 (d, J = 4.2 Hz, 1H, pyridyl-H), 8.81 (s, 1H, 4-H), 11.79 (s, 1H, NH). Anal. Calcd for $C_{16}H_{16}N_4O_4$: C, 58.53; H, 4.91; N, 17.06. Found: C, 58.28; H, 4.96; N, 17.00.

^{13}C NMR Chemical Shift Assignments and ^{1}H–^{13}C Coupling Constants. The ^{13}C NMR chemical shift assignments and ^{1}H–^{13}C NMR coupling constants are summarized in Tables 2 and 3, respectively.

Table 3. Values of Selected ^{1}H–^{13}C Coupling Constants (J, in Hz) for the Nucleoside Derivatives[a]

coupling constant	6	7	8	9	2
^1J(C6, H-C6),[b] ^1J(C4, H-C4)[c]	179.8	181.6	179.9	187.3	183.8
^3J(C2, H-C6),[b] ^3J(C2, H-C1′),[b] ^3J(C2, H-C4)[c]	6.0	5.8	4.4[d]	4.9	6.1
^2J(C5, H-C6),[b] ^2J(C4a, H-C4)[c]	2.5	3.1	2.2	3.5	2.9
^3J(C4, H-C6),[b] ^3J(C7a, H-C4)[c]	7.5	7.6	4.6[d]	7.4	7.5
^3J(C7a, H-C5)[c]				7.4	7.5
^1J(C5, H-C5)[c]				179.6	179.1
^2J(C6, H-C5)[c]				10.4	8.2
^1J(C1′, H-C1′)	171.9	170.5	185.6	173.0	173.4
^1J(C3′, H-C3′)	148.7	150.0	148.2	144.6	149.7
^1J(C4′, H-C4′)	147.3	150.4	149.8	145.3	147.9
^1J(C5′, H-C5′)	141.4	142.2	141.4	142.0	140.1

[a]^{1}H–^{13}C gated-decoupled NMR spectra were measured in DMSO-d_6 at 298 K. [b]Pyrimidine numbering. [c]Systematic numbering. [d]Tentative.

Oligonucleotide Syntheses. The oligonucleotide syntheses were performed on an ABI 392-08 synthesizer on a 1 μmol scale (trityl-on mode) employing the standard phosphoramidites and the modified building blocks **10**, **11**, and **12** following the synthesis protocol for 3′-cyanoethyl phosphoramidites. The average coupling yield was always higher than 95%. After cleavage from the solid support, the oligonucleotides were deprotected in 25% aq. NH_3 for 16 h at 60 °C. The 4,4′-dimethoxytrityl (DMT)-containing oligonucleotides were purified by reversed-phase HPLC (RP-18) with the following solvent gradient system (gradient I): 0–3 min, 10–15% B in A; 3–15 min, 15–50% B in A; 15–20 min, 50–10% B in A; flow rate = 0.8 mL min^{-1}, where A is 95:5 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN and B is MeCN. The mixture was then evaporated to dryness, and the residue was treated with 2.5% $Cl_2CHCOOH/CH_2Cl_2$ for 3 min at 0 °C to

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remove the DMT residues. The detritylated oligomers were purified by reversed-phase HPLC using gradient II: 0−20 min, 0−20% B in A; 20−25 min, 20% B in A; 25−30 min, 20−0% B in A; flow rate = 0.8 mL min^{-1}. The oligomers were desalted on a short column (RP-18, silica gel) using H_2O for elution of the salt, while the oligomers were eluted with 3:2 MeOH/H_2O. The oligonucleotides were lyophilized on a Speed Vac evaporator to yield colorless solids, which were stored frozen at −24 °C. The extinction coefficients ε_{260} (H_2O) of the nucleosides (in dm^3 mol^{-1} cm^{-1}) were dA 15400, dG 11700, dT 8800, dC 7300, **1** 4000 (MeOH), **2** 19900 (MeOH), and **3** 23800 (MeOH). The extinction coefficients of the oligonucleotides were calculated as the sum of the extinction coefficients of the nucleoside constituents. The extinction coefficients ε_{260} (H_2O) of ODN-**1** to ODN-**9** were 107800 (ODN-**1**), 107800 (ODN-**2**), 104300 (ODN-**3**), 105200 (ODN-**4**), 101700 (ODN-**5**), 117900 (ODN-**6**), 114400 (ODN-7), 121000 (ODN-**8**), and 117500 (ODN-**9**). The concentrations of single-stranded oligonucleotides were determined at 260 nm at 20 °C with corrections for hyperchromicity.

Electrospray Ionization Mass Spectrometry of Silver−DNA Complexes. ESI-MS measurements were performed on a quadrupole-time-of-flight mass spectrometer (Q-ToF). The measurement conditions were as follows: end plate offset, −500 V; capillary voltage, 4200 V; desolvation temperature, 180 °C. The resolution based on peak width definition using the full width at half-maximum (fwhm) was at least 28000. For sample preparation, an aqueous solution containing duplex ODN-**4**·ODN-**5**, ODN-**6**·ODN-7, or ODN-**8**·ODN-**9** and Ag^+ ions was diluted with 100 mM ammonium acetate (pH 7.0) to a final duplex concentration of 5 μM in 50 mM ammonium acetate.[15]

The sample solution was delivered via a syringe pump at a flow rate of 10 μL min^{-1}. Before infusion into the ESI-MS instrument, the sample solution was mixed in a ratio of 1:9 with a solution of 16 mM triethylammonium acetate and 100 mM hexafluoroisopropanol in 1:1 (v/v) H_2O/MeOH using a T-mixing piece. The resulting total sample flow rate to the ESI source was 100 μL min^{-1}. For more details and ESI mass spectra, see Figures S6 and S7 in the SI.

■ ASSOCIATED CONTENT

Supporting Information

Molecular masses of oligonucleotides; HPLC profiles of oligonucleotides; additional T_m values and UV titration curves; ESI mass spectra of silver complexes; additional proposed Ag^+-mediated base pairs; melting curves and CD spectra of DNA duplexes; and ^1H, ^{13}C, ^{31}P, DEPT-135, and $^1H-^{13}C$ gated-decoupled NMR spectra of compounds **2** and **6**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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