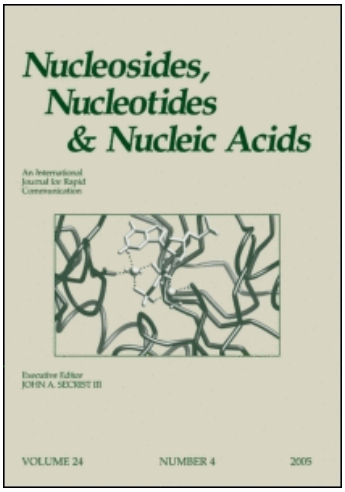

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Silver(I)-Mediated Cytosine Self-Pairing is Preferred Over Hoogsteen-Type Base Pairs with the Artificial Nucleobase 1,3-Dideaza-6-Nitropurine

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SILVER(I)-MEDIATED CYTOSINE SELF-PAIRING IS PREFERRED OVER HOOGSTEEN-TYPE BASE PAIRS WITH THE ARTIFICIAL NUCLEOBASE 1,3-DIDEAZA-6-NITROPURINE

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□ *A 2′-deoxyribonucleoside containing 1,3-dideaza-6-nitropurine was synthesized and incorporated into oligonucleotides. The acid-base properties of this nucleoside and the corresponding N9-methylated derivative were investigated by pD-dependent ^{1}H NMR spectroscopy. A possible formation of Hoogsteen-type base pairs with cytosine was studied by ultraviolet (UV) and circular dichroism (CD) spectroscopy in the presence and absence of Ag(I) and under neutral and acidic conditions, respectively. In each case, no indication for the formation of Hoogsteen-type base pairs was obtained, which is attributed to the higher affinity of cytosine to form self-complementary hemi-protonated base pairs under acidic conditions and metal-mediated homo base pairs in presence of Ag(I), respectively.*

Keywords Bioinorganic chemistry; metal-mediated base pairs; cytosine; Hoogsteen; silver(I)

INTRODUCTION

During the last years many novel base pairs mediated by metal-binding have been reported.[1–4] Most of these display a high thermal stability, resulting in a significant increase of the melting temperature, T_m, even if just one artificial base pair is present within the oligonucleotide double helix. Recently, we have reported a Hoogsteen-type base pair between 1-deazaadenine and a deprotonated thymine, mediated by hydrogen bonding and metal-ion binding.[5] In this case, the binding of Ag(I) leads to an

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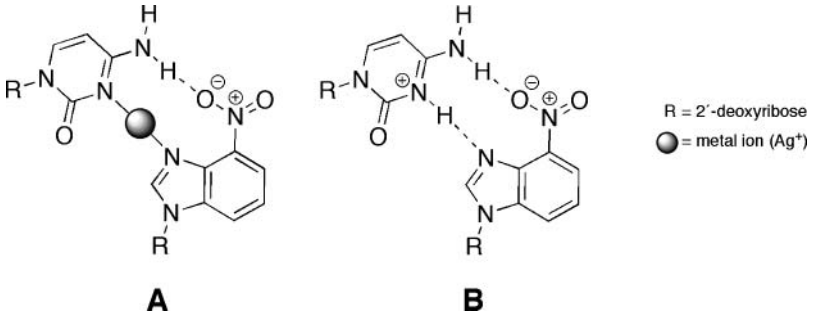


FIGURE 1 Proposed Hoogsteen-type base pairs between cytosine and 1,3-dideaza-6-nitropurine.

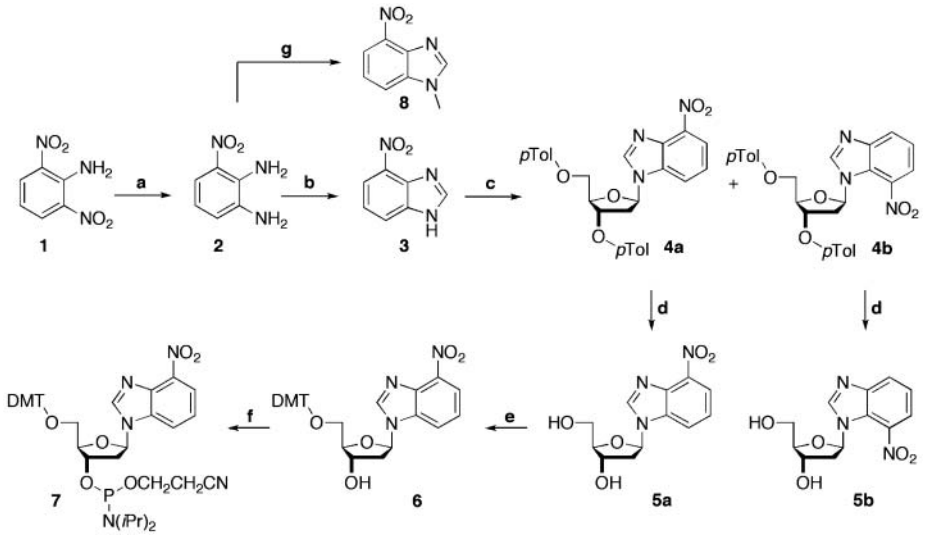
increase of T_m of around 2°C per base pair, thus resembling the stability of a natural base pair. As a result, a large number of metal ions can be incorporated inside the DNA double helix without loss of the reversibility of the hybridization process. We, therefore, set out to devise an analogous base pair incorporating cytosine instead of thymine to create a pair of orthogonal metal-mediated base pairs. This should expand our possibilities to fine-tune duplex stability and metal-ion binding properties of artificial DNA constructs.

Seela and coworkers had previously investigated the use of 1,3-dideaza-6-nitropurine as a universal nucleobase.[6] In their study, the natural nucleobases in Watson-Crick base pairs were successively exchanged by 1,3-dideaza-6-nitropurine, and the melting temperatures of the resulting Watson-Crick duplexes were determined. Contrary to that work, we report here an approach to use 1,3-dideaza-6-nitropurine as an artificial nucleobase complementary to cytosine in a Hoogsteen-type metal-mediated base pair. In this prospective base pair, one oxygen atom of the nitro group acts as a hydrogen bond acceptor, and the N7 position acts as the metal-ion binding site (Figure 1A). In principle, the metal ion in this base pair could also be replaced by a proton (Figure 1B).

RESULTS AND DISCUSSION

Syntheses

Starting from 2,6-dinitroaniline, the DMT-protected phosphoramidite **7** was isolated after 6 steps with an overall yield of 25% (Scheme 1) according to modified literature procedures.[7–10] The modifications included the glycosylation of 1,3-dideaza-6-nitropurine **3** (reaction **c**) and the deprotection of the *p*-toluoyl-protected nucleoside (reaction **d**): NaH was used to deprotonate **3** to afford the desired N9 glycosylated β-anomer **4a** in 64% yield, whereas the use of KOH/TDA-1 as reported in the literature[10] resulted in a poor stereo- and regioselectivity. The deprotection of **4a** was



SCHEME 1 Synthesis of 1,3-dideaza-6-nitropurine 2′-deoxyribonucleoside **5a** and conversion into a DMT-protected phosphoramidite **7**. (a) Na_2S and $NaHCO_3$ in H_2O, 1.5 hours at 70°C, 86%;[7] (b) formic acid, 20 hours reflux, 87%;[8] (c) NaH, Hoffer's chloro sugar, in MeCN, 30 minutes at 0°C, 3 hours at 20°C, 65%;[9] (d) $NH_{3\,(aq.)}$ in MeOH, 24 hours at ambient temperature, 91%; (e) DMT-Cl, DMAP, in pyridine, 2 hours at ambient temperature, 90%; (f) 2-cyanoethyl diisopropyl chloro phosphoramidite, DIPEA, in CH_2Cl_2, 30 minutes at ambient temperature, 80%; (g) formaldehyde in EtOH/HCl (3:1), 30 minutes, reflux, 40%.[11]

carried out with a solution of aqueous NH_3 in methanol instead of sodium methoxide. The N9-methylated derivative **8** was synthesized following literature procedures.[11]

Different oligonucleotides containing cytosine (C) and 1,3-dideaza-6-nitropurine (X) were synthesized (Table 1). Oligonucleotides **I—IV** are self-complementary and should be able to form antiparallel-stranded double helices with CH^+–X or C–Ag^+–X base pairs. Oligonucleotides **II** and **IV** were designed to investigate the alternative formation of parallel-stranded duplexes in combination with **I** and **III**, respectively.

Oligonucleotide **V** represents one half of the strands **III** and **IV** and was used as a reference.

TABLE 1 Oligonucleotides investigated in this study (X = 1,3-dideaza-6-nitropurine)

Oligonucleotide	Sequence
I	5′-d(TACXCXCXTA)-3′
II	5′-d(ATXCXCXCAT)-3′
III	5′-d(XXXXXXXXCCCCCCCC)-3′
IV	5′-d(CCCCCCCCXXXXXXXX)-3′
V	5′-d(CCCCCCCC)-3′

Acid-Base Properties

Prior to performing experiments with the oligonucleotides, the pK_a value of the protonated artificial nucleoside **5a** was determined by pD-dependent ^{1}H NMR spectroscopy. The pK_a value of 3.86 ± 0.03 indicates that under physiological conditions and even in the slightly acidic pH range, a protonation at the N7 position can be excluded. The pK_a value of the N7 glycosylated side product **5b** could not be determined because an acidification of the sample to approximately pD 4 led to a depurination of the nucleoside. The protonated N9-methyl derivative **8**, acting as a model nucleobase, has a pK_a value of 4.03 ± 0.02, obeying the empirical rule that nucleosides are less basic than the corresponding methylated nucleobases.[12,13]

pH-Dependent Hybridization Experiments in the Absence of Silver(I)

To investigate the possible formation of a Hoogsteen-type base pair between CH^+ and X, ultraviolet (UV) melting curves and circular dichroism (CD) spectra were recorded at pH 6.8 and pH 5.5, respectively. As cytosine is not protonated at pH 6.8,[12] the resulting potential C–X base pair would be held together by one hydrogen bond only and therefore be extremely unstable. In fact, no cooperative melting was observed for any of the oligonucleotides under investigation (**I** and **III**) at pH 6.8, and the CD spectra showed no features characteristic of double helices. After decreasing the pH to 5.5, neither cooperative melting nor changes in the CD spectrum were observed in the case of **I** (data not shown). However, in the case of **III**, a positive Cotton effect at 289 nm and a concomitant negative one at 267 nm were observed under acidic conditions (Figure 2A). The spectral

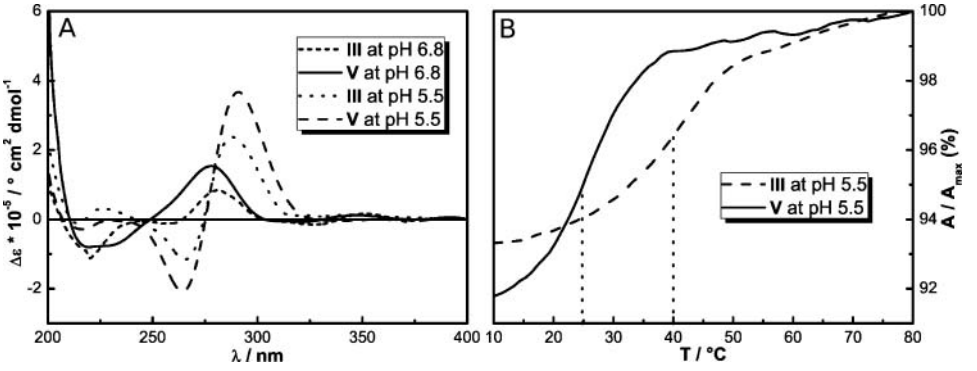


FIGURE 2 (A) CD spectra of **III** and **V** at different pH values. (B) Melting curves of the i-motif structures formed by **III** and **V**. Conditions: 1 μM oligonucleotide (5 μM for the CD spectra of **III** at pH 5.5), 150 mM $NaClO_4$, 5 mM MOPS (pH 6.8), or 5 mM MES (pH 5.5).

properties observed are characteristic for an i-motif structure[14, 15] with CH^+–C instead of the desired CH^+–X base pairs, as shown by control experiments with oligonucleotide **V**. In the latter case, the minimum and maximum are more distinct though (Figure 2A). The different spectral intensities as well as the differences below 240 nm are probably a result of the fact that the tetrameric i-motif structure of **III** contains four overhanging $d(X_8)$ sequences that additionally influence the CD spectra.

The T_m values of the i-motif structures formed by **III** and **V** at pH 5.5, determined by temperature-dependent UV spectroscopy at 260 nm, amount to 40°C and 25°C, respectively. Additionally, hyperchromicity values of around 7% for both i-motif structures were observed (Figure 2B). The fact that the T_m of **III** is higher by 15°C compared to that of **V** might be explained by further stabilization of the cytosine-based i-motif structure via additional weak interactions (π stacking and/or other hydrophobic interactions) involving the overhanging $d(X_8)$ regions.

Titrations with $AgNO_3$

The oligonucleotides were titrated with $AgNO_3$ to investigate the potential formation of metal-mediated base pairs in the presence of a linearly coordinating metal ion. The CD spectrum of oligonucleotide **I** remains unaffected by the addition of Ag(I) (data not shown), suggesting that the desired C–Ag^+–X base pairs are not formed. In contrast, the CD spectrum of oligonucleotide **III** shows strong changes upon the addition of Ag(I): two intense minima at 217 and 270 nm and a weak one at 357 nm appear until eight Ag(I) ions per duplex are present (Figures 3A and 3B). However, 16 metal ions per duplex should be expected if only C–Ag^+–X base pairs were formed. The observed stoichiometry could be explained in two ways: Either only every other binding site is occupied by a Ag(I) ion (similar to the neighbor-exclusion principle[16] found for nucleic acid intercalators), or the resulting duplex contains one sort of metalated homo base pairs only, that is, either C–Ag^+–C or X–Ag^+–X base pairs. Inspection of a series of CD spectra clearly points to the second possibility: The CD spectra of oligonucleotide **V** (comprising only cytosine) with Ag(I) display the same spectral features as those of **III** with Ag(I) (Figure 3C), except for the weak minimum at 357 nm, which can most probably be attributed to an interaction of the overhanging $d(X_8)$ regions (see below), strongly suggesting that C–Ag^+–C base pairs are formed in both cases. This conclusion is further corroborated by the fact that oligonucleotide **V** can bind eight metal ions per duplex, indicative of the formation of eight C–Ag^+–C base pairs (Figure 3D).

However, no cooperative melting was observed by means of UV spectroscopy (at 260, 271 and 314 nm, with the latter two wavelengths representing the maxima of the UV spectra) for the duplexes of **III** and **V**. Possibly, there are only weak π-stacking effects in a metalated poly-d(C) sequence

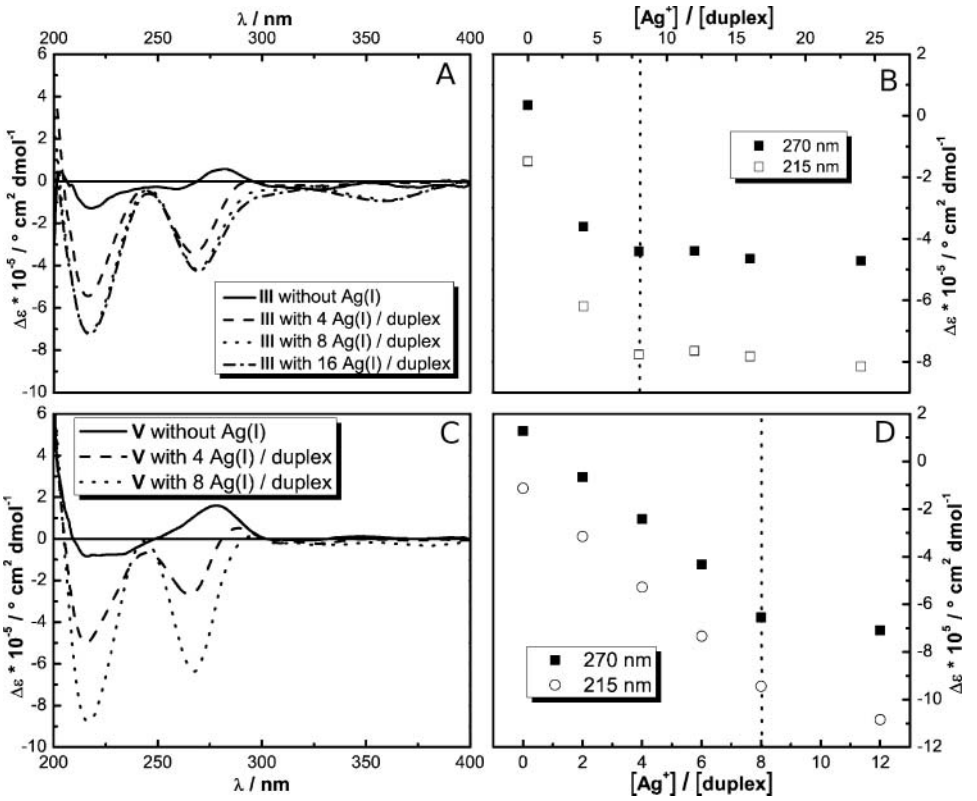


FIGURE 3 (A and C) CD spectra of **III** and **V** in the absence and presence of various amounts of $AgNO_3$. (B and D) Changes of molar ellipticity of **III** and **V** upon the addition of $AgNO_3$. Conditions: 1 μM oligonucleotide, 150 mM $NaClO_4$, 5 mM MOPS (pH 6.8).

that in turn lead to a small hyperchromicity. Therefore, temperature-dependent CD spectra of the oligonucleotides **III** and **V** were recorded in the presence of Ag(I) to obtain information on the thermal stability of the metalated species. The spectra display their largest temperature dependence at 270 and 280 nm. At the latter wavelength, cooperative melting can be observed, revealing melting temperatures of T_m(**III**) = 63°C and T_m(**V**) = 73°C (both with eight Ag(I) per duplex; Figure 4A). A possible explanation for the higher stability of **V** as compared with **III** will be discussed in the following chapter. It should be noted that—contrary to the behavior of **III** and **V** under acidic conditions (see above)—the formation of a tetra-stranded structure like the i-motif is unlikely in the presence of Ag(I), and therefore the different effect of the single-stranded overhang in **III** on the thermal stability of the resulting helices is not unexpected. The assumption that each nucleobase of the overhanging d(X_8) region might act as a competitive potential binding site for the added metal ions appears to be an unlikely explanation for the higher stability of **V** as compared with **III**.

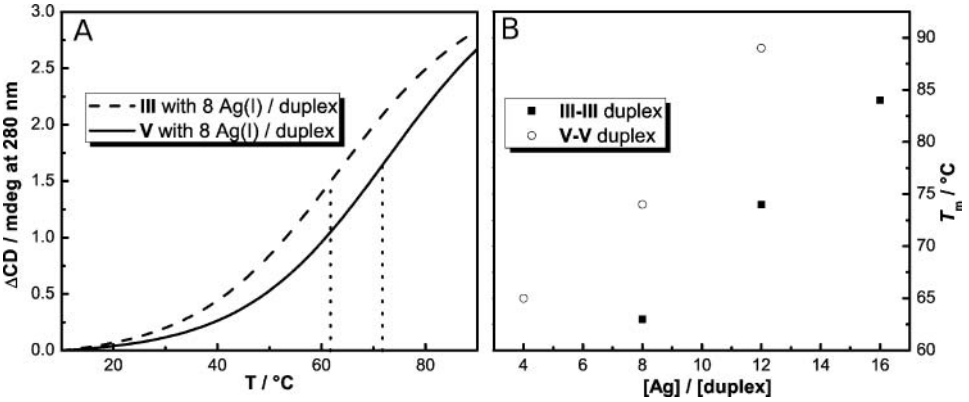


FIGURE 4 (A) CD melting curves of **III** and **V** in the presence of eight Ag(I) per duplex, and (B) T_m values upon addition of various amounts of $AgNO_3$ (B). Conditions: 1 μM oligonucleotide, 150 mM $NaClO_4$, 5 mM MOPS (pH 6.8).

Under these conditions, different slopes for the plot of T_m versus number of Ag(I) ions should be expected for **III** and **V**. However, identical slopes are observed (Figure 4B).

Investigation of a Parallel-Stranded Duplex Formation

To examine the potential formation of parallel-stranded duplexes with C–Ag^+–X base pairs, the above-mentioned titrations and hybridization experiments were repeated with equimolar mixtures of **I**+**II** and **III**+**IV** instead of **I** only and **III** only. For both mixtures, results similar to those reported above for oligonucleotides **I** and **III** alone were obtained, revealing that no parallel-stranded double helices with C–Ag^+–X or CH^+–X base pairs are formed either. Instead, formation of C–Ag^+–C base pairs appears to be predominant also in the cases of **III** and **III**+**IV**. Oligonucleotides **I** and **I**+**II**, not being able to form C–Ag^+–C base pairs as a result of their sequences, do not adopt any double helical structure (data not shown).

Nonetheless, these investigations helped to shed light onto the question of the strand-orientation of duplexes with consecutive C–Ag^+–C base pairs: The CD spectra of **III** and **III**+**IV** display analogous changes upon the addition of Ag(I) (data not shown) with the exception of the region of 350–370 nm. In this wavelength region, the CD spectrum of **III**+**IV** does not change when adding Ag(I) whereas the spectrum of **III** exhibits a small yet significant negative Cotton effect (Figure 5A). This differential behavior could be conveniently explained by the formation of a parallel-stranded duplex: A parallel duplex of C–Ag^+–C base pairs formed by **III**+**IV** would locate the overhanging $d(X_8)$ region on opposite ends of the helix whereas an analogous duplex formed by **III** would result in overhanging $d(X_8)$ regions located in close vicinity to each other (Figure 5D), probably for

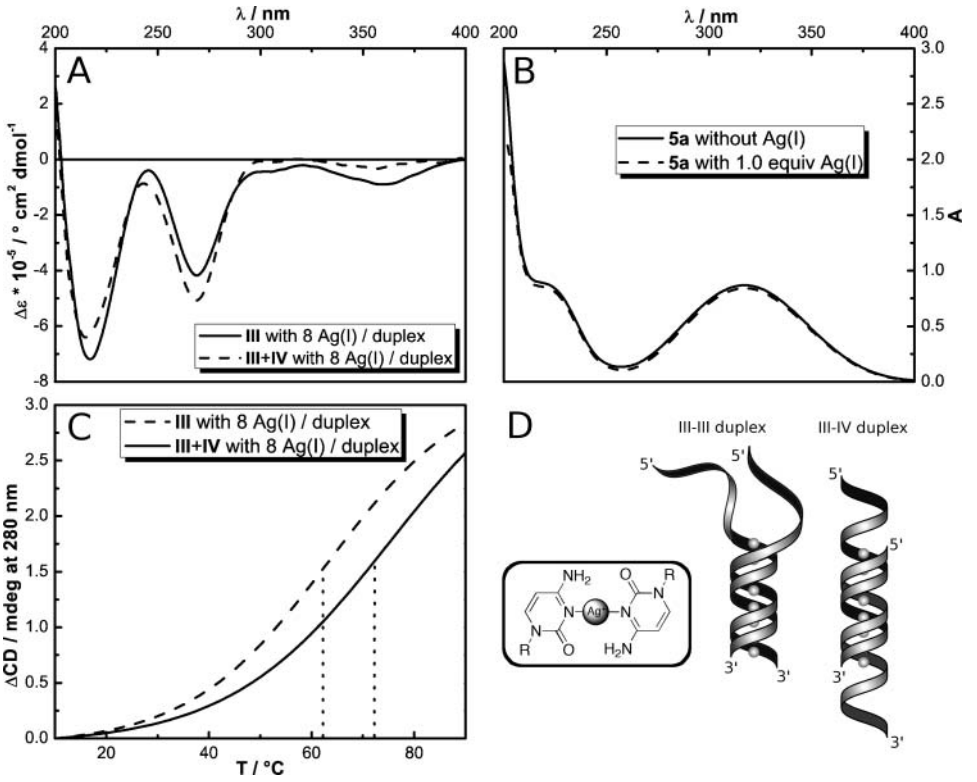


FIGURE 5 (A) CD spectra of **III** and **III**+**IV** in presence of eight Ag(I) per duplex. (B) UV spectra of **5a** (0.1 mM in water) in the absence and presence of $AgNO_3$. (C) CD melting curves of **III** and **III**+**IV** in presence of eight Ag(I) per duplex. (D) Schematic representation of the proposed parallel-stranded double helices formed by **III** and **III**+**IV**, respectively. Conditions: 1 μM oligonucleotide, 150 mM $NaClO_4$, 5 mM MOPS (pH 6.8).

steric reasons adopting some preferred non-random conformation and thereby evoking the negative Cotton effect. The following observations are supportive of such an interpretation:

1. A comparison with the CD spectra of **V** shows (Figure 3C) the negative Cotton effect around 360 nm cannot be attributed to cytosine. It must, therefore, originate from the artificial nucleobases.
2. It cannot be caused by a mere binding of excess Ag(I) to the d(X_8) region because in this case no differences would be expected for **III** and for **III**+**IV**, respectively. Furthermore, such binding should also influence the UV spectrum of the nucleoside **5a** which does not show any significant changes upon the addition of $AgNO_3$ (Figure 5B).
3. The thermal stabilities of the duplexes with C–Ag^+–C base pairs formed by **III** and **III**+**IV** vary significantly: In the presence of comparable amounts of Ag(I), both **III**+**IV** (with the d(X_8) regions on opposite ends

of the duplex) and **V** (with no $d(X_8)$ region) have melting temperatures of 73°C, whereas **III** displays a T_m of only 63°C (Figures 4A and 5C). Assuming a parallel strand orientation, this would suggest that the duplex formed by **III** is destabilized by the above-mentioned interaction of the neighboring overhanging $d(X_8)$ regions.

Taken together, this evidence is supportive of a parallel-stranded orientation of duplexes with contiguous C–Ag^+–C base pairs, contrary to what is expected for single C–Ag^+–C base pairs surrounded by regular Watson-Crick base pairs.[17] Provided that the glycosidic bond angles are in the usual *anti* range, the metal-mediated base pairs are comprised of nucleobases with transoid glycosidic bonds (Figure 5D).

CONCLUSIONS

Oligonucleotides containing the artificial nucleobase 1,3-dideaza-6-nitropurine were synthesized and characterized. By UV and CD spectroscopic experiments the potential formation of a Hoogsteen-type base pair with cytosine was investigated. Under none of the conditions investigated experimentally (decreased pH, presence of Ag(I) or complementary parallel strands) the formation of a stable duplex with CH^+–X base pairs was detected. For the alternating sequences d(TACXCXCXTA) (**I**) and d(ATXCXCXCAT) (**II**), no indication was found for the formation of a stable double-helical structure at all. On the contrary, the oligonucleotide sequences $d(X_8C_8)$ (**III**) and $d(C_8X_8)$ (**IV**), respectively, revealed the formation of C–Ag^+–C base pairs in presence of Ag(I) as well as the formation of an i-motif structure with CH^+–C under slightly acidic conditions. It can, therefore, be concluded that cytosine self-pairing is preferred over the formation of Hoogsteen-type base pairs between cytosine and 1,3-dideaza-6-nitropurine, both in the presence and in the absence of Ag(I). The experiments are supportive of a parallel-stranded orientation of the duplexes containing consecutive C–Ag^+–C base pairs.

EXPERIMENTAL

Nucleoside and Phosphoramidite Synthesis

The nucleobase 1,3-dideaza-6-nitropurine **3**,[7,8] the N9-methylated derivative **8**,[11] Hoffer's chloro sugar,[9] the DMT-protected nucleoside **6**,[10] and phosphoramidite **7**[10] were synthesized following literature procedures. For the glycosylation of **3** and the deprotection of **4** these procedures were modified as follows.

1,3-dideaza-6-nitropurine-N7/N9-β-[2′-deoxy-3′,5′-di-(p-toluoyl)-ribonucleoside] 4

To a suspension of 1,3-dideaza-6-nitropurine **3** (1.522 g, 9.330 mmol) in acetonitrile (40 mL), NaH (60% suspension in mineral oil, 511.0 mg, 12.78 mmol) was added at 0°C. After stirring the reaction mixture for 1 hour at 0°C, Hoffer's chloro sugar (2.467 g, 6.345 mmol) was added in 4 portions every 20 minutes. The suspension was stirred for further 4 hours (0°C → room temperature), filtered, and the resulting solution evaporated to dryness. The resulting brownish syrup was purified by column chromatography (SiO_2, cyclohexane (100) : ethylacetate (60) : CH_2Cl_2 (5)) yielding the desired N9-glycosylated β-anomer **4a** as a colorless foam (2.108 g, 4.082 mmol, 64%) as well as the N7-glycosylated β-anomer **4b** (148.8 mg, 288.1 μmol, 5%). **4a**: ^{1}H NMR (500 MHz, $CDCl_3$): δ/ppm = 8.33 (s, 1H, H8); 8.12 (d, 1H, H1); 7.98 (d, 2H, PG); 7.90 (d, 1H, H3); 7.83 (d, 2H, PG); 7.30 (d, 2H, PG); 7.24 (m, 1H, H2); 7.23 (d, 2H, PG); 6.45 (pt, 1H, H1′); 5.77 (m, 1H, H3′); 4.78 (m, 1H, H5′); 4.67 (m, 1H, H4′); 4.65 (m, 1H, H5″); 2.96 (m, 1H, H2′); 2.85 (m, 1H, H2″); 2.45 (s, 3H, CH_3); 2.42 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ/ppm = 166.4 (PG); 166.3 (PG); 145.1 (PG); 144.8 (PG); 143.9 (C8); 137.9 (C6); 135.6 (C4); 130.1 (C5); 129.6 (PG); 126.6 (PG); 126.5 (PG); 122.8 (C2); 120.1 (C1); 117.7 (C3); 86.0 (C1′); 83.2 (C4′); 74.6 (C3′); 63.7 (C5′); 38.5 (C2′); 22.0 (CH_3); 21.9 (CH_3). Anal. Calcd for $C_{28}H_{25}N_3O_7$: C, 65.2; H, 4.9; N, 8.2; found: C, 65.3; H, 4.9; N, 7.9%. HRMS (ESI): Calcd for $C_{28}H_{26}N_3O_7$ $(M+H)^+$: 516.17653, found 516.17592. **4b**: ^{1}H NMR (200 MHz, $CDCl_3$): δ/ppm = 8.51 (s, 1H, H8); 8.07 (d, 1H, H1); 8.01 (d, 1H, H3); 7.96 (d, 2H, PG); 7.80 (d, 2H, PG); 7.35 (t, 1H, H2); 7.27 (d, 2H, PG); 7.18 (d, 2H, PG); 6.79 (dd, 1H, H1′); 5.62 (m, 1H, H3′); 4.64 (m, 3H, H4′, H5′, H5″); 3.00 (m, 1H, H2′); 2.68 (m, 1H, H2″); 2.43 (s, 3H, CH_3); 2.38 (s, 3H, CH_3).

1,3-dideaza-6-nitropurine-N9-β-2′-deoxyribonucleoside 5a

To a solution of **4a** (984.4 mg, 1.910 mmol) in methanol (100 mL), aqueous NH_3 (25%, 100 mL) was added, and the mixture stirred for 24 hours at room temperature. The solvent was evaporated, and the oily residue treated with CH_2Cl_2 (50 mL). The resulting suspension was sonicated for 10 minutes and filtered. The white solid was dried at 40°C to yield the deprotected deoxyribonucleoside **5a** (483. 8mg, 1.733 mmol, 91%). ^{1}H NMR (500 MHz, DMSO-d_6): δ/ppm = 8.78 (s, 1H, H8); 8.25 (d, 1H, H1); 8.21 (d, 1H, H3); 7.47 (t, 1H, H2); 6.47 (pt, 1H, H1′); 5.39 (d, 1H, 3′-OH); 5.02 (t, 1H, 5′-OH); 4.42 (m, 1H, H3′); 3.90 (m, 1H, H4′); 3.57 (m, 2H, H5′, H5″); 2.63 (m, 1H, H2′); 2.38 (m, 1H, H2″). ^{13}C NMR (125 MHz, DMSO-d_6): δ/ppm = 145.3 (C8); 138.6 (C6); 136.7 (C4); 135.5 (C5); 122.1 (C2); 118.6 (C1); 118.3 (C3); 87.8 (C4′); 85.0 (C1′); 70.2 (C3′); 61.2 (C5′); 39.7 (C2′). Anal. Calcd for $C_{12}H_{13}N_3O_5$: C, 51.6; H, 4.7; N, 15.1%; found:

C, 51.7; H, 5.0; N, 15.1. HRMS (ESI): Calcd for $C_{12}H_{14}N_3O_5$ $(M+H)^+$: 280.09280, found: 280.09288.

1,3-dideaza-6-nitropurine-N7-β-2′-deoxyribonucleoside 5b

Following the above-mentioned procedure, **4b** (573.6 mg, 1.112 mmol) was deprotected to yield **5b** as a brown solid (236.9 mg, 848.3 μmol, 76%). ^{1}H NMR (500 MHz, DMSO-d_6): δ/ppm = 8.83 (s, 1H, H8); 8.10 (d, 1H, H1); 7.97 (d, 1H, H3); 7.42 (t, 1H, H2); 6.43 (pt, 1H, H1′); 5.33 (d, 1H, 3′-OH); 4.92 (t, 1H, 5′-OH); 4.32 (m, 1H, H3′); 3.82 (m, 1H, H4′); 3.43 (m, 2H, H5′, H5″); 2.58 (m, 1H, H2′); 2.45 (m, 1H, H2″). ^{13}C NMR (125 MHz, DMSO-d_6): δ/ppm = 144.5 (C8); 136.35 (C6); 126.2 (C5); 124.6 (C4); 121.5 (C2); 120.1 (C1); 117.7 (C3); 87.4 (C4′); 86.8 (C1′); 69.6 (C3′); 60.7 (C5′); 50.0 (C2′). Anal. Calcd for $C_{12}H_{13}N_3O_5$: C, 51.6; H, 4.7; N, 15.1; found: C, 52.1; H, 4.3; N, 15.1%. LRMS (MALDI): Calcd for $C_{12}H_{13}N_3O_5Na$ $(M+Na)^+$: 302, found: 302.

Oligonucleotide Synthesis, Purification, and Characterization

Oligonucleotides **I-IV** were synthesized on an Expedite 8909 DNA synthesizer (PerSeptive Biosystems, Framingham, MA, USA) in DMT-off mode and purified by HPLC with a Nucleogen 60–7 DEAE column (Macherey-Nagel, Düren, Germany). The following linear gradients were used during HPLC purification: **I** and **II**: 0–10 minutes: 100–80% A, 10–40 minutes: 80–75% A, 40–45 minutes: 75–0% A; **III** and **IV**: 0–10 minutes: 100–80% A, 10–40 minutes: 80–50% A, 40–45 minutes: 50–0% A (1.5 mL/min, solvent A: 20 mM sodium acetate (pH 6) in H_2O/acetonitrile (4:1), solvent B: solvent A + 2 M LiCl). After desalting via NAP 10 columns, the oligonucleotides were characterized by MALDI-TOF mass spectrometry on a Bruker Autoflex II instrument (**I**: Calcd for $(M-H)^-$: 3062, found: 3063; **II**: Calcd for $(M-H)^-$: 3062, found: 3063; **III**: Calcd for $(M-H)^-$: 4979, found: 4977; **IV**: Calcd for $(M+Na-2H)^-$: 5000, found: 4999). The oligonucleotide **V** was purchased from Eurogentec (Seraing, Belgium).

Titrations and Hybridization Experiments

For the hybridization experiments, oligonucleotide solutions (1–5 μM) containing 150 mM $NaClO_4$ and 5 mM MOPS (adjusted to pH 6.8) or 5 mM MES (adjusted to pH 5.5) were prepared. UV melting curves were recorded on a Varian CARY BIO 100 spectrometer at 260 nm (heat rate: 1°C/min, data interval: 0.2°C). CD spectra and melting curves were recorded on a Jasco J-815 (CD spectra: 10°C, scan rate: 100 nm/min, 5 accumulations; CD melting curves: 10–90°C, heat rate: 1°C/min, data interval: 1°C). Melting temperatures were determined as the maxima of the first derivatives of the melting curves.

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