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A new unprecedented metal-mediated base pair was designed that stabilizes reverse Watson-Crick DNA (parallel strand orientation, ps) as well as canonical Watson-Crick DNA (antiparallel strand orientation, aps). This base pair contains two imidazo-dC units decorated with furan residues. Tm measurements and spectroscopic studies reveal that each silver-mediated furano-imidazo-dC forms exceptionally stable duplexes with ps and aps chain orientation. This stability increase by a silver-mediated base pair is the highest reported so far for ps and aps DNA helices.

Metal-mediated base pairs have generated significant attention as substitutes of Watson-Crick pairs.1 These base pairs not only expand the existing genetic coding system but also allow the construction of extremely stable DNA structures. Thus, metal-mediated base pairs are considered as a high-tech alternative to canonical base pairs. Nevertheless, transition metals have been associated with the oxidative damage of DNA.2 Metal base pairs between canonical nucleobases have been developed in which Hg2+ or Ag+ take over the function of hydrogen bonds (Fig. 1a).3 Generally, metal-mediated pairing systems bind only one metal ion per base pair. Depending on the type of ligand, a metal-mediated base pair can even bind two transition metal ions.4

DNA is known to adopt a wide range of structures. Among those parallel-stranded (ps) DNA represents an unique DNA assembly characterized by a sugar-phosphate backbone pointing in the same direction while canonical DNA forms duplexes with antiparallel chains (aps DNA) (Fig. 1b).5 Although, ps DNA is rather stable, it is less stable than aps DNA.6 As chemical modification of ps DNA is more restricted, its stabilization remains a challenge.7

Recently, it was reported that the pyrrolo-dC-dC base pair binds one silver ion.8 Our laboratory discovered that pyrrolo-dC pyrrolo-dC base pairs can bind two silver ions.9,10 Then, for aps DNA it was observed that the imidazo-dC (imidC) system functionalized with a phenyl residue at the 8-position (8imidC, Fig. 2) is even a better silver ion binder.11 This positive effect has to be caused by the purine skeleton replacing the pyrrolo[2,3-d]pyrimidine system. Thus, we envisaged that a furyl moiety decorating the imidazo-dC system might add extra coordination forces for silver ions to the system and as a side effect, it might improve the fluorescence properties.12 We expected that the modified purine nucleobases provide flexibility to the system and act as silver ion binder on both ps as well as on aps DNA structures. Herein we describe the synthesis of imidazo-dC (imidC, 1) and 8-furyl imidazo-dC (furimidC, 3) and disclose their effect on ps and aps duplexes in the presence of silver ions. Pleasingly, we observed that furimidC forms exceptional strong silver-mediated imidazo-dC base pairs that stabilize both parallel and antiparallel DNA far beyond the stability of canonical base pairs in natural DNA.

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1Schematic presentation of ps and aps duplex DNA (Fig. 1a).

2Schematic presentation of ps and aps duplex DNA (Fig. 1b).

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The structures of ImidC nucleosides 1-3.

ImidC nucleoside 1 was synthesized following a procedure described for the corresponding ribonucleoside. To this end, 5-amino-dC (4) was treated with diethoxy methyl acetate to give 1 (Scheme 1). Treatment of 1 with excess of 4,4'-dimethoxytriphosphoryl chloride (DMT-Cl) (2.2 equiv) leads to the bis-DMT derivative 5 with one protecting group at the 5'-position and the other at the base. Phosphitylation under standard conditions afforded phosphoramidite 6.

Scheme 1 Synthesis of phosphoramidite 6: (i) (EtO)₂CHOAc, 100 °C, 3 h, 29%; (ii) DMT-Cl, pyridine, Et₂N, rt, 16 h, 44%; (iii) NC(CH₃)₂OP(Cl)N[(i-Pr)₂], (i-Pr)₂EtN, rt, 20 min, 29%.

Scheme 2 Synthesis of phosphoramidite 8: (i) furfural, CAN, DMF, 55 °C, 24 h, 57%; (ii) DMT-Cl, pyridine, rt, 16 h, 47%; (iii) NC(CH₃)₂OP(Cl)N[(i-Pr)₂], (i-Pr)₂EtN, rt, 10 min, 79%.

For the synthesis of ²¹⁵ImidC 3 initially a Traube condensation was employed which was used earlier for the synthesis of ²¹⁵²¹⁶²¹⁷²¹⁸²¹⁹²²⁰. However, condensation between 5-amino-dC (4) with furfural in the presence of para-toluenesulfonic acid led to the formation of only 4% of desired 3. Thus, we examined several oxidants including Ph(OAc)₂, Cu(OH)₂, Cu(OAc)₂ and ceric ammonium nitrate (CAN) at different conditions. The desired ²¹⁵²¹⁶²¹⁷²¹⁸²¹⁹²²⁰ImidC 3 was obtained in 57% yield using CAN and DMF as solvent at 55 °C. Protection of 5'-OH with DMT-Cl followed by phosphitylation yielded phosphoramidite 8 (Scheme 2). Next, the imidazo-dC derivatives 1-3 were incorporated near the center of the two parallel stranded duplexes ODN-1 • ODN-2 and ODN-9 • ODN-13 (Table 1) thereby replacing strongly bonded central dC:iG base pairs. For comparison aps duplexes were designed in which one strand is identical to the ps duplexes and the central dG-dC pair was replaced by compounds 1-3. Oligonucleotide synthesis was performed on solid phase using the phosphoramidites of functionalized nucleosides, iG₄ (2'-deoxyisoguanosine), 3b,3c and iC₉ (2'-deoxy-5-methyl-isocytidine), 3d, as well as phosphoramidites 6 and 8.

The most obvious feature of metal-mediated base pairs is their significant contribution to the stabilization of DNA duplexes, since the strength of the coordinative bond is much stronger than that of hydrogen bonds. Table 1 summarizes the ∆Tm values in the presence and absence of silver ions with regard to duplexes with a dC-dC pair, ImidC pairs as well as relative to the unmodified reference duplexes. Addition of silver ions to the reference duplexes (ODN-1 • ODN-2 and ODN-1 • ODN-9) caused no significant Tm changes showing that silver ions are not involved in base pairing. Only one silver ion was captured by a dC-dC pair. This base pair caused a notable increase in aps duplex stability (ΔTm = +8.5 °C), but still shows lower strength than the canonical one.

However, the situation is entirely different when ImidC residues are present and facing each other. Now, the Tm increased tremendously for ps and aps DNA by addition of 2 equiv. of silver ions (Table 1). The ∆Tm values relative to mismatches amount to 27.0-38.0 °C for ps DNA and to 36.3-48.0 °C for aps DNA. The Tm increase is unique for compound 3 bearing a furan residue; the phenyl residue of 2 does not stabilize the silver-mediated base pair over that of the nonfunctionalized nucleoside 1 (Table 1). For ps DNA two different sequence motifs (series i and ii) were studied. This positioned the silver-mediated base pairs in different environments and changed the interaction with neighboring base pairs. However, the Tm increase was almost the same for both series. For aps DNA, the Tm values were 10 °C higher than for ps DNA. Also in this case, the stabilization of the furan residue of 1 is exceptional high while the phenyl residue of 2 does not add stability. The silver-mediated base pairs of 1-3 are much stronger than the dG-dC or iG₄-dC pair. The enhanced stability probably results from additional interactions of the furan ligands with silver ions. To the best of our knowledge, such a strong stabilization induced by only one silver-mediated base pair in ps and aps DNA has never been observed before.

Typical Tm curves of ImidC modified ps and aps duplexes in the absence and presence of silver ions are shown in Fig. 3. Biphasic Tm curves with a low and a high Tm value were observed by adding only one equivalent of silver ions. This indicates the existence of two species; duplexes without silver ions (low Tm) and duplexes with two silver ions (high Tm). Addition of two equiv. of silver ions leads to monophasic melting curves showing only the high Tm value. From that it is obvious that the ImidC-imidC base pair captures two silver ions instantaneously in a cooperative way. To verify the stoichiometry of silver ion binding, fluorescence and UV titration experiments were performed (see ESI†). ImidC derivatives show strong fluorescence around 390 nm (Φ = 0.03 for ²¹⁵ImidC (1), φ = 0.33 for ³²¹⁵ImidC (2), and Φ = 0.39 for ³²¹⁵²¹⁶²¹⁷²¹⁸²¹⁹²²⁰ImidC (3); for details see ESI†). These experiments confirm that each duplex with an ImidC-imidC pair captures two silver ions. Coordination forces between the furan oxygen and silver ions might be the central reason for the exceptional stability of this silver ion mediated base pair. Nonetheless, pKₐ changes induced by the substituents have to be considered as it is documented that the ease of base deprotonation plays a key role in the formation of silver-mediated base pairs.
Table 1  $T_m$ Values of DNA duplexes containing ImidC nucleosides 1-3 in the presence or absence of silver ions$^{a/b}$.  

<table>
<thead>
<tr>
<th>Duplexes</th>
<th>$T_m$ [°C] with $n$ equiv of AgNO$_3$</th>
<th>$\Delta T_m$ [°C] relative to match$^b$</th>
<th>$\Delta T_m$ [°C] relative to mismatch$^c$</th>
</tr>
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<tr>
<td></td>
<td>$n = 0$</td>
<td>$n = 1$</td>
<td>$n = 2$</td>
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<tr>
<td>ps duplexes series I</td>
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<td></td>
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<tr>
<td>5'-d(TAG GT C AAT ACT)</td>
<td>34.5</td>
<td>n.d.</td>
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<td>5'-d(ATICACAG TTATIGA)</td>
<td>ODN-1</td>
<td>ODN-2</td>
<td></td>
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<tr>
<td>5'-d(TAG GT T AAT ACT)</td>
<td>28.0</td>
<td>n.d./55.0$^d$</td>
<td>55.0</td>
</tr>
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<td>5'-d(ATICAC A TTATIGA)</td>
<td>ODN-3</td>
<td>ODN-4</td>
<td></td>
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<tr>
<td>5'-d(TAG GT T2 AAT ACT)$^f$</td>
<td>31.0</td>
<td>n.d./58.0$^d$</td>
<td>58.0</td>
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<tr>
<td>5'-d(ATICACA2 TTATIGA)</td>
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<td>ODN-6</td>
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<tr>
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<td>29.0</td>
<td>27.0/67.0$^d$</td>
<td>67.0</td>
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<td>ps duplexes series II</td>
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<td>ODN-13</td>
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<tr>
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<td>n.d./57.0$^d$</td>
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<td>5'-d(AGT ATT 2AC CTA)</td>
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<td>ODN-15</td>
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<td>27.0/66.0$^d$</td>
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<tr>
<td>aps duplexes</td>
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<td>5'-d(TAG GT CAAT A CT)</td>
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<td>47.0</td>
<td>46.5</td>
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<tr>
<td>5'-d(TA AT C TTAGTA)</td>
<td>ODN-1</td>
<td>ODN-9</td>
<td></td>
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<tr>
<td>5'-d(TAG GT TAAT A CT)$^b$</td>
<td>26.0</td>
<td>32.5$^e$</td>
<td>34.5</td>
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<tr>
<td>5'-d(TAG GT T2 AAT ACT)$^f$</td>
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<td>32.0/71.0$^d$</td>
<td>71.0</td>
</tr>
<tr>
<td>5'-d(TiCATAA2 TTATIGAT)</td>
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<td>ODN-11</td>
<td></td>
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<tr>
<td>5'-d(AGT ATT3AC A CT)</td>
<td>35.5</td>
<td>32.0/72.0$^d$</td>
<td>74.0</td>
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<td>5'-d(ATICACA3 TTATIGA)</td>
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<td>ODN-12</td>
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</tr>
<tr>
<td>3'-d(ATICAC TTTA TA)</td>
<td>ODN-10</td>
<td></td>
<td></td>
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</table>

$^a$ Measured at 260 nm with 5 μM + 5 μM single-strand concentration in 100 mM NaOAc, 10 mM Mg(OAc)$_2$, pH 7.5 in the presence of AgNO$_3$ (0-2 equivalents).  
$^b$ $T_m$ after the addition of 2.0 equivalents AgNO$_3$ – $T_m$ of reference duplex.  
$^c$ $\Delta T_m = T_m$ after the addition of 2.0 equivalents AgNO$_3$ – $T_m$ before the addition of AgNO$_3$.  
$^d$ Biphasic melting.  
$^e$ Binds only one silver ion [ESI†, Fig. S11].  
$^f$ For thermodynamic data see ESI†, Table S6.  
$^iC_d$ corresponds to $^{35}$SiC$_d$, n.d. not determined.

Fig. 3  Thermal melting ($T_m$) curves measured at 260 nm in 100 mM NaOAc, 10 mM Mg(OAc)$_2$ at pH 7.5 in presence of 0-2 equivalents of Ag$^+$.  
(a) ps ODN-7 • ODN-8, (b) aps ODN-7 • ODN-12.

Indeed, the $pK_a$ value of compound 3 ($pK_a = 7.3$) is the lowest compared to 2 ($pK_a = 7.9$) and 1 ($pK_a = 8.8$) (see ESI†).  
In accordance with the $pK_a$ values, the furano derivative 3 forms the most stable silver-mediated base pair while the non-functionalized derivative 1 shows the lowest stability.

Earlier, we proposed a structure for the silver-mediated $^{35}$ImidC–$^{35}$ImidC base pair in aps DNA.$^8$  
Herein, a structure for the ps duplex is suggested which aligns the purine bases head-to-tail instead of head-to-head as suggested for canonical DNA which is consistent with the observation for dC residues in silver-mediated dC–dC base pairs made by Ono$^{15}$ and by the computational studies of Marino$^{16}$ (Fig. 4).  
Strong support for the head-to-tail alignment in ps DNA comes from the single crystal X-ray structure of the dimeric 1-methylcytosine silver nitrate...
complex by Marzilli in which the two silver ions connect the heterocyclic ligands with bonds between the atoms N-3 and O-2 in that way that the bases are aligned head-to-tail (Fig. 4). Connectivity between silver ions and purine bases should be different in ps and aps DNA. According to the size of the silver ions, the base connectivity is not strictly linear but bent by 132°. A similar spacing of the Ag-Ag repeat length between columnar stacks (3.642 Å) and the base stacking distance in duplex DNA (about 3.5 Å) suggests that this dimeric unit provides a model for the cross-linking of DNA.

![Fig. 4 Proposed silver-mediated base pairs. (a) Dimeric 1methylcytosine silver complex. furImidC:furImidC pair with two silver ions in (b) ps DNA and (c) in aps DNA. dR = 2'-deoxyribofuranosyl. Ag corresponds to Ag⁺.](image)

In summary, we designed an exceptional strong silver-mediated imidazolo-dC base pair decorated with furan residues. The purine nucleobases act as silver ion binder and provide flexibility to form base pairs in ps and aps DNA. Two silver ions are bound to a single imidazolo-dC pair. The incorporation of only one silver-mediated furano-imidazolo-dC pair increased the Tm value of a 12-mer duplex with parallel strand orientation by 38.0 °C relative to the silver-free duplex. An even higher increment was observed for canonical DNA with antiparallel chains (ΔTm ≈ 48.0 °C). Almost no sequence dependent stability increase was observed when the silver-mediated base pairs were located in different helical environments as shown for the ps duplexes (series I and II; Table 1). The extremely high stability of these base pairs probably superimposes other possible changes in the double helix structure. For silver-mediated base pairs of lower stability, sequence dependent stability changes were detected.

In recent years, various new base pairs have been developed to increase duplex stability. In this context, the silver-mediated base pair of 1 is an unique example. Incorporation of multiple base pairs with two silver ions per base pair is feasible. Strong and robust functional materials can be constructed with possible applications in metal-ion based nanomechanical devices and biomedical science. To the best of our knowledge, the furan functionalized imidazolo-dC (3) pair is the most stable Ag⁺ mediated base pair in ps and aps DNA reported so far.

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### Notes and references


