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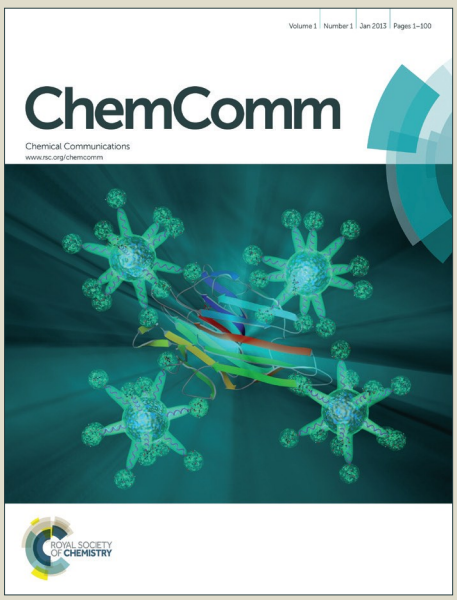

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Robust silver-mediated imidazolo-dC base pairs in metal DNA: dinuclear silver bridges with exceptional stability in double helices with parallel and antiparallel strand orientation†

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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 imidazolo-dC units decorated with furan residues. T_m measurements and spectroscopic studies reveal that each silver-mediated furano-imidazolo-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 Hg^{2+} 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]

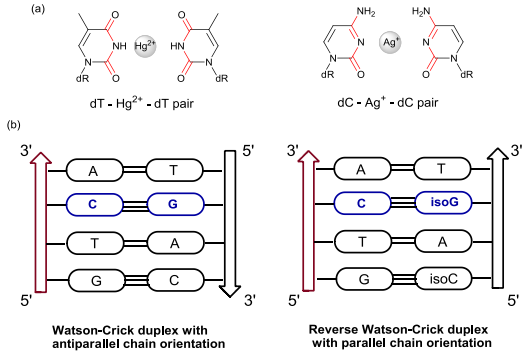

Fig. 1 (a) Proposed structure for metal-mediated base pairs.[3] (b) Schematic presentation of ps and aps duplex DNA. isoG = 2'-deoxyisoguanosine, isoC = 2'-deoxy-5-methylisocytidine, dR = 2'-deoxyribofuranosyl. A, T, G and C refer to 2'-deoxyribonucleosides.

Recently, it was reported that the pyrrolo-dC-dC base pair binds one silver ion.[3c] Our laboratory discovered that pyrrolo-dC pyrrolo-dC base pairs can bind two silver ions.[4c,8] Then, for aps DNA it was observed that the imidazolo-dC (ImidC) system functionalized with a phenyl residue at the 8-position (^{ph}ImidC, **2**, Fig. 2) is even a better silver ion binder.[9] 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 imidazolo-dC system might add extra coordination forces for silver ions to the system and as a side effect, it might improve the fluorescence properties.[10] 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 imidazolo-dC (^{H}ImidC, **1**) and 8-furyl imidazolo-dC (^{fur}ImidC, **3**) and disclose their effect on ps and aps duplexes in the presence of silver ions. Pleasingly, we observed that ^{fur}ImidC forms exceptional strong silver-mediated imidazolo-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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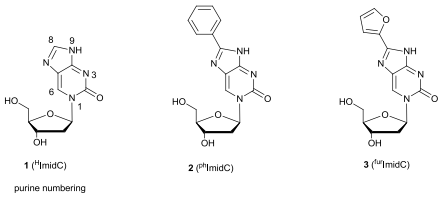
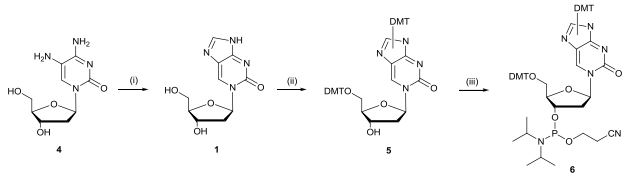
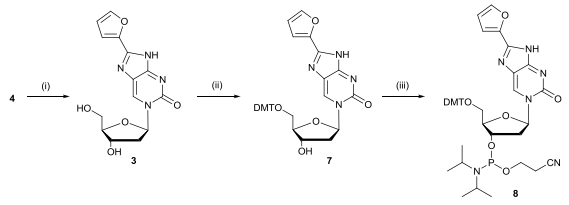


Fig. **2** The structures of ImidC nucleosides **1**-**3**.

HImidC nucleoside **1** was synthesized following a procedure described for the corresponding ribonucleoside.[11] To this end, 5-amino-dC (**4**) was treated with diethoxymethyl acetate to give **1** (Scheme 1). Treatment of **1** with excess of 4,4'-dimethoxy-triphenylmethyl 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, Et_3N, rt, 16 h, 44%; (iii) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $(i\text{-}Pr)_2EtN$, 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) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $(i\text{-}Pr)_2EtN$, rt, 10 min, 79%.

For the synthesis of furImidC **3** initially a Traube condensation was employed which was used earlier for the synthesis of **2**.[9,12] 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 $PhI(OAc)_2$, $Cu(OH)_2$, $Cu(OAc)_2$ and ceric ammonium nitrate (CAN) at different conditions. The desired furImidC **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 imidazolo-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_d 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 us... the phosphoramidites of canonical nucleosides, iG_d (2'-deox... isoguanosine),[5b,13] and $^{5me}iC_d$ (2'-deoxy-5-methyl-isocytidine)[13], th... phosphoramidite of **2**[9], 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 ΔT_m 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 referenc... duplexes (ODN-**1** • ODN-**2** and ODN-**1** • ODN-**9**) caused no significant T_m 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 (ΔT_m = +8.5 °C), but still shows lower strength than the canonical one.

However, the situation is entirely different when ImidC residues ... present and facing each other. Now, the T_m increased tremendously for ps and aps DNA by addition of 2 equiv. of silver ions (Table 1). The ΔT_m values relative to mismatches amount to 27.0-38.0 °C fo... ps DNA and to 38.5-48.0°C for aps DNA. The T_m increase is unique for compound **3** bearing a furan residue; the phenyl residue of **2** di... not stabilize the silver-mediated base pair over that of the non-functionalized nucleoside **1** (Table 1). For ps DNA two differe... sequence motifs (series I and II) were studied. This positioned the silver-mediated base pairs in different environments and change... the interaction with neighboring base pairs. However, the T_m increase was almost the same for both series. For aps DNA, the T_m values were 10 °C higher than for ps DNA. Also in this case, th... stabilization of the furan residue of **1** is exceptional high while th... phenyl residue of **2** does not add stability. The silver-mediated bas... pairs of **1**-**3** are much stronger than the dG-dC or iG_d-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 ba... pair in ps and aps DNA has never been observed before.

Typical T_m curves of ImidC modified ps and aps duplexes in th... absence and presence of silver ions are shown in Fig. 3. Biphasic T_m curves with a low and a high T_m value were observed by adding on... one equivalent of silver ions. This indicates the existence of two species; duplexes without silver ions (low T_m) and duplexes wit... two silver ions (high T_m).[8,9] Addition of two equiv. of silver ion... leads to monophasic melting curves showing only the high T_m value. From that it is obvious that the ImidC-ImidC base pair captures tw... silver ions instantaneously in a cooperative way.[9] To verify th... stoichiometry of silver ion binding, fluorescence and UV titration experiments were performed (see ESI†). ImidC derivatives s... ow strong fluorescence around 390 nm (Φ = 0.03 for HImidC (**1**), Φ = 0.33 for phImidC (**2**), and Φ = 0.39 for furImidC (**3**); for details se... ESI†). These experiments confirm that each duplex with an ImidC-ImidC pair captures two silver ions. Coordination forces betwee... the furan oxygen and silver ions might be the central reason for th... exceptional stability of this silver ion mediated base pa... Nonetheless, pK_a changes induced by the substituents have to be considered as it is documented that the ease of base deprotonatio... plays a key role in the formation of silver-mediated base pairs.[14]

Table 1 T_m Values of DNA duplexes containing ImidC nucleosides **1**-**3** in the presence or absence of silver ions[a,f]

Duplexes	T_m [°C] with n equiv of $AgNO_3$			ΔT_m [°C] relative to match[b]	ΔT_m [°C] relative to mismatch[c]
	n = 0	n = 1	n = 2		
ps duplexes series I					
5′-d(TAG GT**C** AAT ACT) ODN-**1** 5′-d(ATiCiCA**iG** TTATiGA) ODN-**2**	34.5	n.d.	33.5	-	-
5′-d(TAG GT**1** AAT ACT) ODN-**3** 5′-d(ATiCiCA**1** TTATiGA) ODN-**4**	28.0	n.d./55.0[d]	55.0	+20.5	+27.0
5′-d(TAG GT**2** AAT ACT)[9] ODN-**5** 5′-d(ATiCiCA**2** TTATiGA) ODN-**6**	31.0	n.d./58.0[d]	58.0	+23.5	+27.0
5′-d(TAG GT**3** AAT ACT) ODN-**7** 5′-d(ATiCiCA**3** TTATiGA) ODN-**8**	29.0	27.0/67.0[d]	67.0	+32.5	+38.0
ps duplexes series II					
5′-d(AGTATT**G**AC CTA) ODN-**9** 5′-d(TiCATAA**iC** TiGiGAT) ODN-**13**	42.0	42.0	42.0	0	-
5′-d(AGTATT**1**AC CTA) ODN-**10** 5′-d(TiCATAA**1** TiGiGAT) ODN-**14**	28.0	n.d./57.0[d]	57.0	+15.0	+29.0
5′-d(AGT ATT **2**AC CTA) ODN-**11** 5′-d(TiCATAA **2**TiGiGAT) ODN-**15**	32.5	n.d.	61.0	+19.0	+28.5
5′-d(AGTATT**3**AC CTA) ODN-**12** 5′-d(TiCATAA**3** TiGiGAT) ODN-**16**	28.0	27.0/66.0[d]	66.0	+24.0	+38.0
aps duplexes					
5′-d(TAGGT**C**AATACT) ODN-**1** 3′-d(ATCCA**G**TTATGA) ODN-**9**	46.5	47.0	46.5	-	-
5′-d(TAGGT**C**AATACT) ODN-**1** 3′-d(ATCCA**C**TTATGA)[8b] ODN-**1a**	26.0	32.5[e]	34.5	+12.0	+8.5
5′-d(TAGGT**1**AATACT) ODN-**3** 3′-d(ATCCA**1**TTATGA) ODN-**10**	32.0	32.0/71.0[d]	71.0	+24.5	+39.0
5′-d(TAGGT**2**AATACT)[9] ODN-**5** 3′-d(ATCCA**2**TTATGA) ODN-**11**	35.5	32.0/72.0[d]	74.0	+27.5	+38.5
5′-d(TAGGT**3**AATACT) ODN-**7** 3′-d(ATCCA**3**TTATGA) ODN-**12**	30.0	25.0/75.0[d]	78.0	+31.5	+48.0

[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]$\Delta T_m = T_m$ 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 $^{5me}iC_d$. n.d. not determined.

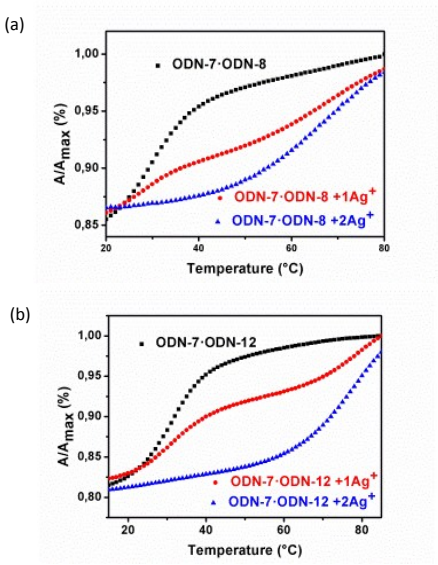


Fig. 3 Thermal melting (T_m) curves measured at 260 nm in 100 mM NaOAc, 10 mM $Mg(OAc)_2$ at pH 7.5 in presence of 0-2 equivalents of Ag^+. (a) ps ODN-**7** • ODN-**8**, (b) aps ODN-**7** • ODN-**12**.

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 ^{ph}ImidC-^{ph}ImidC base pair in aps DNA.[9] 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).[17] 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.

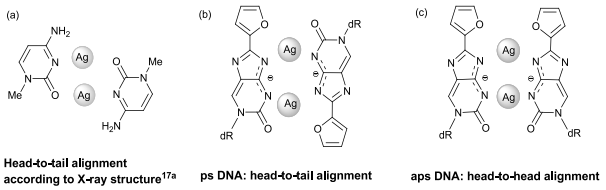


Fig. **4** Proposed silver-mediated base pairs. (a) Dimeric 1-methylcytosine silver complex. ^{fur}ImidC-^{fur}ImidC pair with two silver ions in (b) ps DNA and (c) in aps DNA. dR = 2'-deoxyribofuranosyl. Ag corresponds to Ag^+.

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 T_m 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 (ΔT_m = 48.0 °C). Almost no sequence dependent stability increase was observed when the silver-mediated base pairs were located in different helical environment 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.[9]

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.[8a] Strong and robust functional materials can be constructed with possible applications in metal-ion based nanomechanical devices[18] 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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