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A Novel Silver(I)-Mediated DNA Base Pair

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Selective Watson−Crick hydrogen bonding between the natural base pairs dA:dT and dC:dG is the key element for information storage and retrieval in DNA. Over the years efforts to find additional base pairs have focused on bases that pair through altered hydrogen-bonding patterns[1] or hydrophobic interactions.[2,3] We and others recently reported a third strategy which involves metal-dependent pairing of two nucleobases.[4,5] Our first-generation metallo-base pair consisted of a planar tridentate pyridine-2,6-dicarboxylate (**Dipic**) nucleobase and a monodentate pyridine (**Py**) nucleobase.[4] When these bases are introduced into the middle of a 15-nucleotide duplex, the duplex displays thermal stability in the presence of equimolar Cu^{2+} comparable to that of a duplex with a dA:dT base pair at the same site. However, this metallo-base pair has only modest pairing selectivity. To further explore the scope of metallo-base pairing in DNA and to improve the properties of our first-generation metallo-base pair, we are investigating additional metal−ligand complexes. According to the principle of hard and soft acids and bases (HSAB-principle), replacement of the *O*-atoms of the **Dipic**-ligand with softer donor atoms such as *S* or *N* should improve the stability of complexes with softer, late transition metals.[6] We therefore decided to substitute the **Dipic** ligand with the *S,N,S*-ligand 2,6-bis(ethylthiomethyl)pyridine (**SPy**) which is known to form stable complexes with a variety of different transition metals (Figure 1).[7]

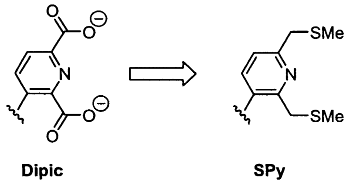
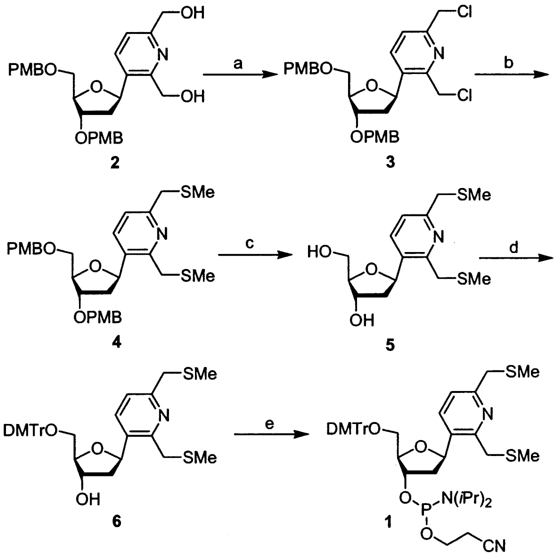


Figure 1. Replacement of the **Dipic** nucleobase with a 2,6-bis(ethylthiomethyl)pyridine (**SPy**) nucleobase.

The corresponding **SPy** phosphoramidite **1** was synthesized from diol **2,** an intermediate in the synthesis of the **Dipic** nucleobase (Scheme 1).[4] After conversion to the dichloride **3**, treatment with sodium thiomethoxide afforded the 2,6-bis(ethylthiomethyl)pyridine nucleoside **4**. Tritylation followed by phosphitylation provided phosphoramidite **1**. Incorporation of **1** into the 15-nucleotide **7** was achieved by standard protocols for automated DNA synthesis, and identities of all oligonucleotides were confirmed by MALDI-TOF MS. The thermal stability of the duplexes **7** (X, Y = d**SPy**, d**Py**, dA, dT, dC, and dG) in the presence of different transition metals (Ni^{2+}, Pd^{2+}, Pt^{2+}, Cu^{2+}, Ag^{+}, Au^{3+}) was evaluated by determining their melting temperatures (T_m) using UV spectroscopy.[8] Of these metals only Ag(I) shows a metal-dependent base pairing, and the corresponding T_m-values are reported in Table 1.

Scheme 1. Synthesis of the **SPy** Phosphoramidite **1**[a]



[a] Conditions: (a) SOCl$_2$, THF (86%). (b) NaSCH$_3$, EtOH/CH$_2$Cl$_2$ (10:1) (97%). (c) TFA, CH$_2$Cl$_2$ (83%). (d) DMTr-Cl, NEt$_3$, py (67%). (e) (iPr)$_2$NP(Cl)CH$_2$CH$_2$CN, (iPr)$_2$NEt, CH$_2$Cl$_2$ (76%).

Table 1. Sequence of the 15-mer DNA Duplex **7** and Denaturation Temperatures (T_m) for Various Combinations of X and Y[a]

5′-CACATTA**X**TGTTGTA-3′
3′-GTGTAAT**Y**ACAACAT-5′ **7**

	X =					
Y =	SPy	Py	A	T	C	G
SPy	**42.5** (23.4)[b]	n.d.[c]	n.d.[c]	n.d.[c]	n.d.[c]	n.d.[c]
Py	**35.0** (23.5)[b]	**25.4** (24.6)[b]	25.0	30.6	26.1	*d*
A	30.5	n.d.[c]	26.5	**38.6**	26.6	27.5
T	29.4	n.d.[c]	**39.4** (39.1)[b]	31.4	34.2	30.5
C	29.6	n.d.[c]	25.3	32.5	25.5	**39.1**
G	30.1	n.d.[c]	28.1	30.1	**41.1** (39.6)[b]	*d*

[a] Melting temperature experiments were carried out on a Cary 300-Bio UV/vis spectrophotometer. The heating rates were 0.5 °C min^{-1}. Conditions: 5 mM sodium phosphate, 50 mM sodium perchlorate, pH 7 with 1 μM silver(I) nitrate and 1 μM DNA duplex. [b] Melting temperature in the absence of silver(I) nitrate. [c] Not determined. [d] No sigmoidal melting curve was observed in the temperature range between 15 and 65 °C.

The d**SPy**:d**SPy** base-pair shows a very high pairing stability in the sequence context of duplex **7**. In the presence of one equivalent of Ag(I) this self-pair is even more stable than the natural dC:dG and dA:dT base pairs (T_m = 42.5, 41.1, and 39.4 °C for d**SPy**:d**SPy**, dC:dG, and dA:dT, respectively, Table 1) (Figure 2). The mixed

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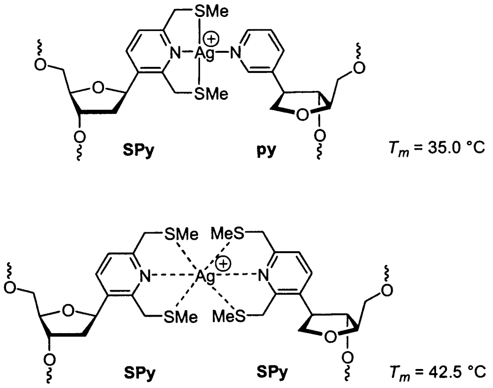

Figure 2. Ag(I)-mediated base pairs between d**SPy**:d**Py** and d**SPy**:d**SPy**.

d**SPy**:d**Py** base-pair is stabilized by 11.5 °C in the presence of Ag(I) and is only 4.4 °C less stable than a dA:dT base pair (T_m = 35.0 and 39.4 °C for d**SPy**:d**Py** and dA:dT, respectively). It is noteworthy that the d**Py**-d**Py** self-pair is only stabilized by 0.8 °C in the presence of Ag(I) in this sequence context. For comparison, T_m-values of the natural base pairs dA:dT and dC:dG increase by 0.3 and 1.5 °C, respectively, in the presence of one equivalent Ag(I). Shionoya and co-workers recently reported a much higher increase in T_m of 6.8 °C for exactly the same d**Py**-d**Py** base pair in a 21-nucleotide dT$_{10}$**Py**T$_{10}$/dA$_{10}$**Py**A$_{10}$ duplex, indicating that the stabilization is context-dependent.[5b,9] The relationship between thermal stability and increasing number of *S*-donor atoms in the series d**Py**:d**Py**, d**SPy**:d**Py**, and d**SPy**:d**SPy** clearly shows the stabilizing effect of additional thioether substituents on the stability of the metallo-base pair. Ag(I) generally has a high affinity for sulfur ligands but normally prefers a linear, trigonal, or tetrahedral metal coordination.[10] The coordination geometry of the d**SPy**:d**SPy** self-pair with six potential donor atoms is therefore not obvious, and efforts are underway to obtain a structure of a DNA duplex containing this metallo-base pair.

To store genetic information, an unnatural base pair should not only be highly stable but also highly selective against mispairing with the natural nucleobases. Mispairs with the d**SPy** nucleobase are destabilized by 12.0−13.1 °C relative to d**SPy**:d**SPy**, and by 4.5−5.6 °C relative to d**SPy**:d**Py** (Table 1). For comparison, mispairs between the natural nucleobases are 6.9−15.6 °C less stable than natural base pairs in this sequence context. Mispairs with the **Py** nucleobase are destabilized by 4.4−10.0 °C relative to d**SPy**:d**Py**. These data suggest that the d**SPy**:d**SPy** self-pair exhibits pairing stability and selectivity rivalling that of the natural base pairs, whereas the d**SPy**:d**Py** shows a moderate pairing stability and selectivity.

Automated DNA synthesis offers a convenient method for constructing DNA duplexes containing metal ions in defined locations. Encouraged by the high pairing stabilities of both the d**SPy**:d**SPy** and the d**SPy**:d**Py** metallo-base pair, oligonucleotides containing multiple metallo-base pairs were synthesized (Figure 3). UV-melting experiments revealed that the d**SPy**:d**SPy** self-pair can replace natural base pairs with these duplexes at multiple sites and still form stable duplexes (T_m = 39.1 and 44.6 °C for **8** and **9**, respectively).[11] The stability of the mixed d**SPy**:d**Py** base pair is apparently too low to allow the substitution of natural base pairs at multiple sites (T_m = 37.0 and 25.6 °C for **10** and **11**, respectively).

5'-CACA**S**TACTG**S**TGTA-3'
3'-GTGT**S**ATGAC**S**ACAT-5' **8** 39.1 °C

5'-CAC**S**TTA**S**TGT**S**GTA-3'
3'-GTG**S**AAT**S**ACA**S**CAT-5' **9** 44.6 °C

5'-CACA**S**TACTG**S**TGTA-3'
3'-GTGT**P**ATGAC**P**ACAT-5' **10** 37.0 °C

5'-CAC**S**TTA**P**TGT**S**GTA-3'
3'-GTG**P**AAT**S**ACA**P**CAT-5' **11** 25.6 °C

Figure 3. Fifteen-nucleotide duplexes containing multiple metallo-base pairs (**S** = **SPy**, **P** = **Py**).

In summary we have generated a novel silver(I)-mediated metallo-base pair consisting of two 2,6-bis(ethylthiomethyl)-pyridine **SPy** nucleobases. This self-pair has a remarkably high pairing stability and selectivity which rivals that of the natural base pairs dA:dT and dC:dG. Efforts are underway to determine the exact coordination geometry of this new base pair by X-ray analysis. In addition, we plan to investigate potentially novel electronic properties of DNA duplexes containing multiple metallo-base pairs.

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Supporting Information Available: Experimental procedures and characterization data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) T_m-data for all other metal ions except Ag^+ is not shown.
(9) In contrast to ref 5b there is no further significant increase in T_m for the d**Py**:d**Py** self-pair using more than one equivalent of Ag(I) within the sequence context of duplex **7**.
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(11) Conditions: 5 mM sodium phosphate, 50 mM sodium perchlorate, pH 7, 1 μM DNA duplex and 2 μM (**8**/**10**)/3 μM (**9**/**11**) silver(I) nitrate.

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