

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 patterns¹ 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 Cu²⁺ 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

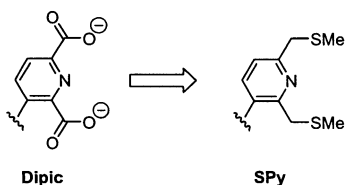
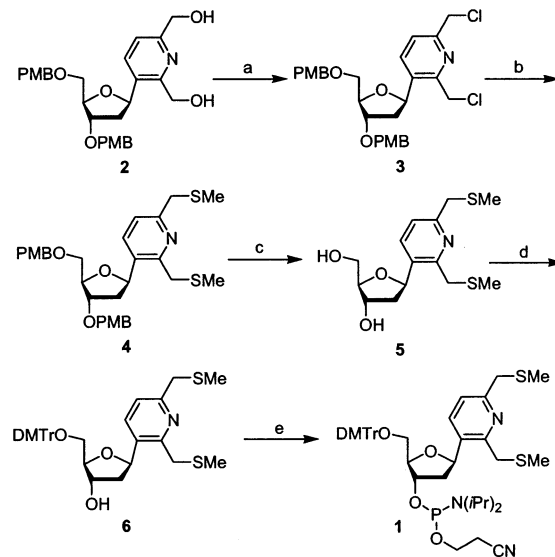


Figure 1. Replacement of the **Dipic** nucleobase with a 2,6-bis(ethylthiomethyl)pyridine (**SPy**) nucleobase.

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 = dSPy, dPy, dA, dT, dC, and dG) in the presence of different transition metals (Ni²⁺, Pd²⁺, Pt²⁺, Cu²⁺, Ag⁺, Au³⁺) 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) SOCl₂, THF (86%). (b) NaSCH₃, EtOH/CH₂Cl₂ (10:1) (97%). (c) TFA, CH₂Cl₂ (83%). (d) DMTr-Cl, NEt₃, py (67%). (e) (iPr)₂NP(Cl)CH₂CH₂CN, (iPr)₂NEt, CH₂Cl₂ (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'-CACATTAXTGTTGTA-3' 3'-GTGTAATYACAACAT-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	n.d. ^c
Py	35.0 (23.5) ^b	25.4 (24.6) ^b	25.0	30.6	26.1	<i>d</i>	
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 (39.6) ^b	
G	30.1	n.d. ^c	28.1	30.1	41.1	<i>d</i>	

^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 dSPy:dSPy 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 dSPy:dSPy, dC:dG, and dA:dT, respectively, Table 1) (Figure 2). The mixed

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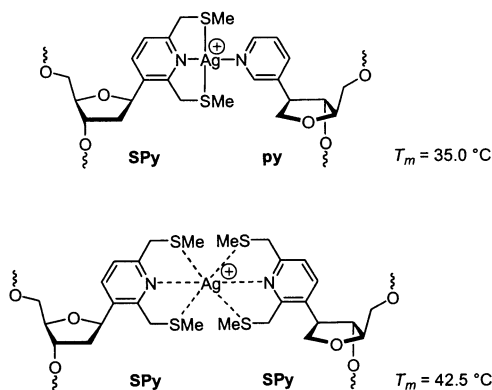


Figure 2. Ag(I)-mediated base pairs between dSPy:dPy and dSPy:dSPy.

dSPy:dPy 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 dSPy:dPy and dA:dT, respectively). It is noteworthy that the dPy:dPy 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 dPy:dPy base pair in a 21-nucleotide dT₁₀PyT₁₀/dA₁₀PyA₁₀ 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 dPy:dPy, dSPy:dPy, and dSPy:dSPy 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 dSPy:dSPy 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 dSPy nucleobase are destabilized by 12.0–13.1 °C relative to dSPy:dSPy, and by 4.5–5.6 °C relative to dSPy:dPy (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 dSPy:dPy. These data suggest that the dSPy:dSPy self-pair exhibits pairing stability and selectivity rivaling that of the natural base pairs, whereas the dSPy:dPy 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 dSPy:dSPy and the dSPy:dPy metallo-base pair, oligonucleotides containing multiple metallo-base pairs were synthesized (Figure 3). UV-melting experiments revealed that the dSPy:dSPy 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 dSPy:dPy 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'-CACASTACTGSGTA-3'	8	39.1 °C
3'-GTGTSATGACSACAT-5'		
5'-CACSTTASTGTS GTA-3'	9	44.6 °C
3'-GTGSAATSACASCAT-5'		
5'-CACASTACTGSGTA-3'	10	37.0 °C
3'-GTGTPATGAC PACAT-5'		
5'-CACSTTAPTGTSGTA-3'	11	25.6 °C
3'-GTGPAATSACAPCAT-5'		

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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- T_m -data for all other metal ions except Ag⁺ is not shown.
- In contrast to ref 5b there is no further significant increase in T_m for the dPy:dPy self-pair using more than one equivalent of Ag(I) within the sequence context of duplex 7.
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