A metallo base-pair incorporating a terpyridyl-like motif: bipyridylpyrimidinone·Ag(I)·4-pyridine[†]

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The design and characterization of a geometrically unique, metallo base-pair motif is reported.

DNA is an attractive target for molecular engineering as its structural alternatives are relevant to therapeutics,¹ materials chemistry,² synthetic biology³ and molecular evolution.⁴ Alteration of base-pair structure has been a repeated object of study,⁵ and has led to an expanded set of base-pairs, including those that interact *via* hydrogen-bonds or hydrophobically.⁶ Metal ion coordination has emerged as a third means of inducing DNA double strand interactions.⁷ We report the design and characterization of the first metallo base-pair incorporating a terpyridyl-like motif, Pyr^{*bipy*}.Ag⁺.4-Py (Fig. 1).



Fig. 1 Top left: 4-(2"-Bipyridyl)pyrimidinone·Ag⁺·4-pyridine base-pair; top right: B3LYP/SDD,6-31G* geometry of Pyr^{bipy}·Ag⁺·Py comparing the N–C distances of optimal 4-pyridyl and suboptimal 3-pyridyl geometries; bottom: superposition of the Pyr^{bipy}·Ag⁺·Py B3LYP/SDD,6-31G* structure on an A·T pair.

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The starting point for the design of the terpyridine-like motif was our recently reported Ni(II) base-pairs incorporating symmetrical bipyridine-like ligands.7h,i These Ni(II) pairs incorporate pyridyl-pyrimidinone or -purine bases. Formal migration of the pyridyl group from one side of a bis-bipyridine-like base-pair motif results in the $Pyr^{bipy} \cdot M^{n+} \cdot Py$ pair displayed in Fig. 1. Due to its asymmetry, the latter has the advantage of greater information content over self-pairs. A density functional calculation[‡] was performed initially using Ni(II) as the metal, but later with Ag(I), to ascertain the geometric properties of $Pyr^{bipy} \cdot M^{n+} \cdot Py$. The results are shown in Fig. 1 (top right and bottom). The calculation predicts that a motif incorporating a 4-pyridyl group more closely approximates the 9.05 Å N1···N1 distance found in a natural DNA helix than a motif bearing a 3-pyridyl group, where the corresponding distances are 7.7 and 6.4 Å, respectively. Superposition of the calculated geometry for Pyrbipy Ag+ Py on an A·T DNA base-pair (bottom of Fig. 1) provided further support for the choice of a 4-pyridyl over a 3-pyridyl group.

Independent of design considerations, both 4- and 3-pyridyl substituted nucleosides were prepared as described below to allow their properties in a helix to be compared. Syntheses of the pyridyl deoxyriboside phosphoramidites are shown in Fig. 2. Several syntheses are known for the 3- and 4-pyridyl deoxyribosides.⁸ However, we were attracted to methodology reported by Reese and Wu⁹ for the synthesis of 4-amino-3-pyridyl deoxyribosides as it provided a unified approach to the synthesis of our 3- and 4-pyridyl targets, and gave the prospect of improved efficiency. Thus, 2'-deoxyribonolactone 1 was transformed into 2 and 3 by a reaction with the appropriate lithio-pyridine followed by reduction of the resulting keto-lactol mixture with L-selectride. Cyclization of 2 and 3 under Mitsunobu conditions gave 2'-deoxyribofuranosides 4 and 5 with >98 and 87% diastereometric excess, respectively, in favor of the beta isomers. The remaining transformations to arrive at phosphoramidites 10 and 11 were uneventful.

Bipyridylpyrimidinone phosphoramidite **14** was obtained from **12** in five steps as shown in Fig. 3. Bipyridylpyrimidinone nucleoside **13** was accessed through a Stille coupling between the 4-chloro-2-pyrimidinone derived from **12** and tributylstannyl bipyridine.

The oligonucleotides listed in Table 1 bearing Pyr^{*bipy*}, 4-Py, 3-Py and natural nucleobases were prepared on a DNA synthesizer using standard protocols. Purification was accomplished *via* polyacrylamide gel electrophoresis. Oligonucleotide identity was confirmed by MALDI-TOF mass spectrometry.

Melting temperatures of the oligonucleotide duplexes are listed in Table 1. Only Ag^+ was found to significantly stabilize the duplex



Fig. 2 Synthesis of 2'-deoxyribofuranosyl 3- and 4-pyridyl nucleoside phosphoramidites. *Reagents and conditions*: (a) 3- or 4-pyridyl bromide, *n*-butyllithium, diethyl ether, -78 °C; (b) L-Selectride, THF, -78 °C; (c) DIAD, Ph₃P, THF, 0 °C; (d) Et₃N·(HF)₃, THF, 0 °C; (e) DMTCl, pyridine, RT; (f) bis(diisopropyl)aminocyanoethoxychlorophosphine, Et(Pr^j)₂N, CH₂Cl₂, 0–25 °C.

bearing a Pyr^{*bipy*}/4-Py site, yielding a $T_{\rm m}$ of 35.2 °C. This result equates to a 12.9 °C stabilization relative to the duplex in the absence of Ag⁺ ($T_{\rm m} = 22.3$ °C). Notable is the lack of appreciable stabilization of the Pyr^{*bipy*}/4-Py containing duplex by Ni²⁺, the metal ion found to have a strong affinity for the bis-bipyridyl-like structure (*e.g.*, Pyr^{*py*}/Pyr^{*py*}).^{7*h*,*i*}

To ascertain the importance of the geometrical features of the $Pyr^{bipy} \cdot Ag^+ \cdot 4 \cdot Py$ pair for stability, the alternative $Pyr^{bipy} \cdot Ag^+ \cdot 3 \cdot Py$ pair was examined (lower left of Table 1). Here the stabilization in the presence *vs.* the absence of Ag^+ was reduced to 4.5 °C. Thus, the $Pyr^{bipy} \cdot Ag^+ \cdot 4 \cdot Py$ pair has enhanced stabilization in comparison to $Pyr^{bipy} \cdot Ag^+ \cdot 3 \cdot Py$, in keeping with modeling studies (*vide supra*).

Exploration of mispairs of Pyr^{*bipy*} with both natural nucleobases and itself in the presence and absence of Ag⁺ showed diminished

Table 1 DNA duplex melting temperatures in the presence and absence of metal ions in addition to Na⁺. Samples contained 2.5 μ M of each DNA strand, 5 μ M non-sodium metal ion where indicated, 50 mM NaNO₃, and 10 mM HEPES, pH 7

5'-d-CTTTCTYTCCCT 3'-d-GAAAGAXAGGGA					
X/Y	М	$T_{\rm m}/^{\circ}{\rm C}$	X/Y	М	$T_{\rm m}/^{\circ}{\rm C}$
Pyr ^{bipy} /4-Py	$\begin{array}{c} & - & \\ & Ag^{+} & \\ Co^{2+} & \\ Zn^{2+} & \\ Cu^{2+} & \\ Ni^{2+} & \\ Mn^{2+} & \\ Mg^{2+} & \\ Tl^{+} & \\ \end{array}$	22.3 35.2 25.3 25.2 23.4 23.1 22.1 21.8 21.4	Pyr ^{bipy} /T Pyr ^{bipy} /C Pyr ^{bipy} /A Pyr ^{bipy} /G A/T		23.4 25.9 25.1 28.6 22.3 27.3 22.6 28.0 34.1
Pyr ^{bipy} /3-Py	Fe ²⁺ — Ag ⁺	21.1 22.0 26.5	G/C Pyr ^{bipy} / Pyr ^{bipy}	$Ag^{+ a}$ $$ $Ag^{+ a}$ $$ Ag^{+}	38.7 38.0 41.0 31.9 30.8

stability in comparison to the matched $Pyr^{bipy} \cdot Ag^+ \cdot 4$ -Py pair (Table 1). Control experiments with natural duplexes (X/Y = G/C or A/T) in the presence of Ag^+ showed stability enhancements consistent with the well-known interaction of this metal ion with DNA helices.¹⁰

One possible explanation of the metal ion binding preference of Pyr^{*bipy*}/4-Py considers two factors: (i) the stacking/unstacking of the terpyridyl-like group upon release/binding of a metal ion, and (ii) the enthalpy associated with metal ion dehydration. In connection to the first point, the geometry of the metal pair in the bottom of Fig. 1 suggests that metal ion binding places the bipyridyl moiety of Pyr^{*bipy*} outside of the base stack in B-DNA,§ and, *inter alia*, that metal ion release might permit the pyridyl groups sufficient freedom to move within the base stack, possibly forming a zipper motif.¹¹ On balance, Ag⁺ appears to overcome both considerations better in that its ability to π -coordinate¹² may recover some of the stacking energy lost upon metal ion binding, and its enthalpy of hydration is lower in comparison to divalent ions.¹³

In conclusion, Pyr^{*bipy*} pairs selectively with 4-Py over 3-Py and natural nucleobases in the presence of Ag⁺. This result demonstrates the viability of a terpyridyl-like base-pair in DNA,



Fig. 3 Synthesis of 2'-deoxyribosyl-1-(4-(2",6",2"'-bipyridyl))pyrimidinone phosphoramidite. *Reagents and conditions*: (a) SOCl₂, DMF, CHCl₃, reflux; (b) 2-tributylstannyl-6-pyridylpyridine, Pd(Ph₃P)₄, toluene, reflux; (c) ammonia, methanol; (d) DMTCl, pyridine, RT; (e) bis(diisopropyl)aminocyanoethoxy chlorophosphine, Et($Pr^{i}_{2}N$, CH₂Cl₂, 0–25 °C.

and suggests $Pyr^{bipy} \cdot Ag^+ \cdot 4$ -Py may be orthogonal to metallo-pairs incorporating the 3-Py group.^{7a,c,e}

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Notes and references

[‡] Gaussian 03, Revision B.03, M. J. Frisch *et al.*, Gaussian, Inc., Pittsburgh PA, 2003.

A circular dichroism spectrum of the $Pyr^{bipy}\cdot Ag^+\cdot 4\text{-}Py$ bearing duplex is included in the ESI.†

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