A metallo base-pair incorporating a terpyridyl-like motif: bipyridyl-pyrimidinone·Ag(I)·4-pyridine†

Dongwon Shin and Christopher Switzer*

Received (in Cambridge, UK) 19th June 2007, Accepted 3rd August 2007
First published as an Advance Article on the web 20th August 2007
DOI: 10.1039/b709291a

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 biology[3] 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).

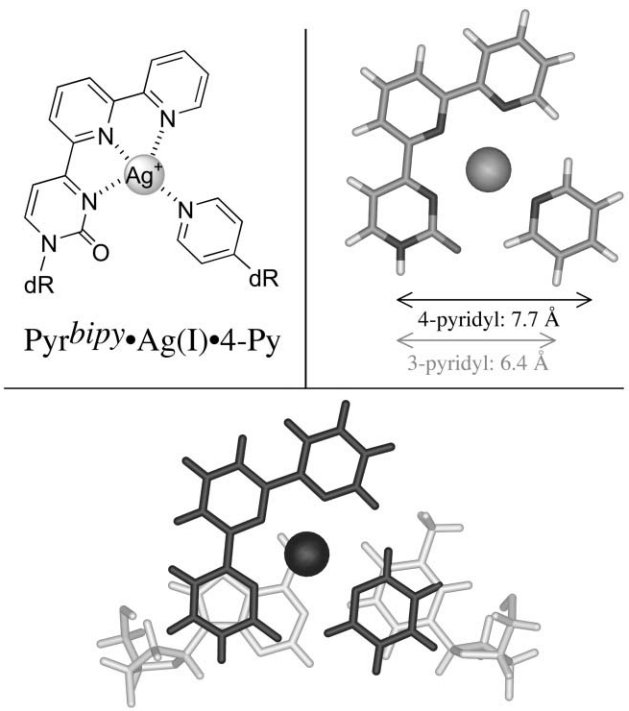

Fig. 1 *Top left*: 4-(2″-Bipyridyl)pyrimidinone·Ag^{+}·4-pyridine base-pair; *top right*: B3LYP/SDD,6-31G* geometry of Pyr^{bipy}·Ag^{+}·Py comparing the N–C distances of optimal 4-pyridyl and suboptimal 3-pyridyl geometries; *bottom*: superposition of the Pyr^{bipy}·Ag^{+}·Py B3LYP/SDD,6-31G* structure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

Department of Chemistry, University of California–Riverside, Riverside, CA, 92521, USA. E-mail: switzer@ucr.edu; Fax: 951-827-2435; Tel: 951-827-7266

† Electronic supplementary information (ESI) available: Experimental procedures and spectral information. See DOI: 10.1039/b709291a

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}·M^{n+}·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}·M^{n+}·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 Pyr^{bipy}·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 Wu[9] 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% diastereomeric 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

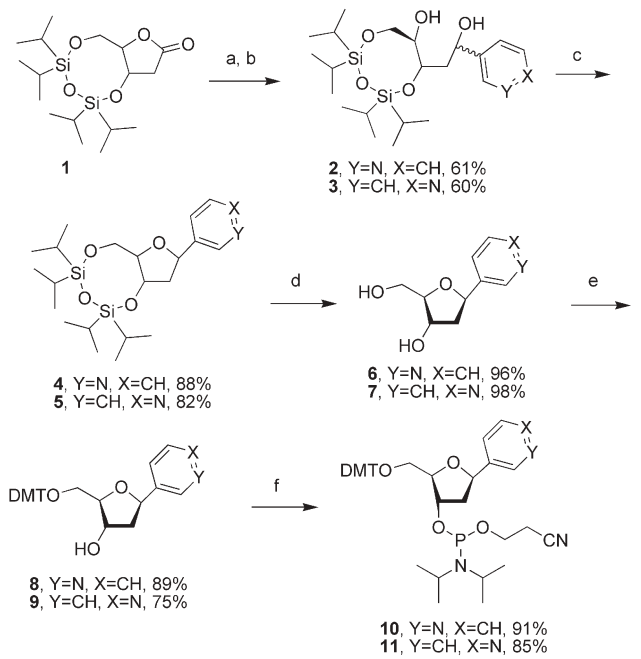

Fig. 2 Synthesis of 2′-deoxyribofuranosyl 3- and 4-pyridyl nucleoside phosphoramidites. *Reagents and conditions*: (a) 3- or 4-pyridyl bromide, *n*-butyllithium, diethyl ether, −78 °C; (b) L-Selectride, THF, −78 °C; (c) DIAD, Ph_3P, THF, 0 °C; (d) $Et_3N{\cdot}(HF)_3$, THF, 0 °C; (e) DMTCl, pyridine, RT; (f) bis(diisopropyl)aminocyanoethoxychlorophosphine, $Et(Pr^i)_2N$, CH_2Cl_2, 0–25 °C.

bearing a Pyr^{bipy}/4-Py site, yielding a T_m of 35.2 °C. This result equates to a 12.9 °C stabilization relative to the duplex in the absence of Ag^+ (T_m = 22.3 °C). Notable is the lack of appreciable stabilization of the Pyr^{bipy}/4-Py containing duplex by Ni^{2+}, the metal ion found to have a strong affinity for the bis-bipyridyl-like structure (*e.g.*, Pyr^{py}/Pyr^{py}).[7h,i]

To ascertain the importance of the geometrical features of the $Pyr^{bipy}{\cdot}Ag^+{\cdot}$4-Py pair for stability, the alternative $Pyr^{bipy}{\cdot}Ag^+{\cdot}$3-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-Py pair has enhanced stabilization in comparison to $Pyr^{bipy}{\cdot}Ag^+{\cdot}$3-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 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]

Table 1 DNA duplex melting temperatures in the presence and absence of metal ions in addition to Na^+. Samples contained 2.5 μM of each DNA strand, 5 μM non-sodium metal ion where indicated, 50 mM $NaNO_3$, and 10 mM HEPES, pH 7

5′-d-CTTTCT**Y**TCCCT
3′-d-GAAAGA**X**AGGGA

X/Y	M	T_m/°C	X/Y	M	T_m/°C
Pyr^{bipy}/4-Py	—	22.3	Pyr^{bipy}/T	—	23.4
	Ag^+	35.2		Ag^+	25.9
	Co^{2+}	25.3	Pyr^{bipy}/C	—	25.1
	Zn^{2+}	25.2		Ag^+	28.6
	Cu^{2+}	23.4	Pyr^{bipy}/A	—	22.3
	Ni^{2+}	23.1		Ag^+	27.3
	Mn^{2+}	22.1	Pyr^{bipy}/G	—	22.6
	Mg^{2+}	21.8		Ag^+	28.0
	Tl^+	21.4	A/T	—	34.1
	Fe^{2+}	21.1		Ag^+ [a]	38.7
Pyr^{bipy}/3-Py	—	22.0	G/C	—	38.0
	Ag^+	26.5		Ag^+ [a]	41.0
			Pyr^{bipy}/ Pyr^{bipy}	—	31.9
				Ag^+	30.8

[a] Silver(I) ion was added in these cases as a control.

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-coordinate[12] 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,

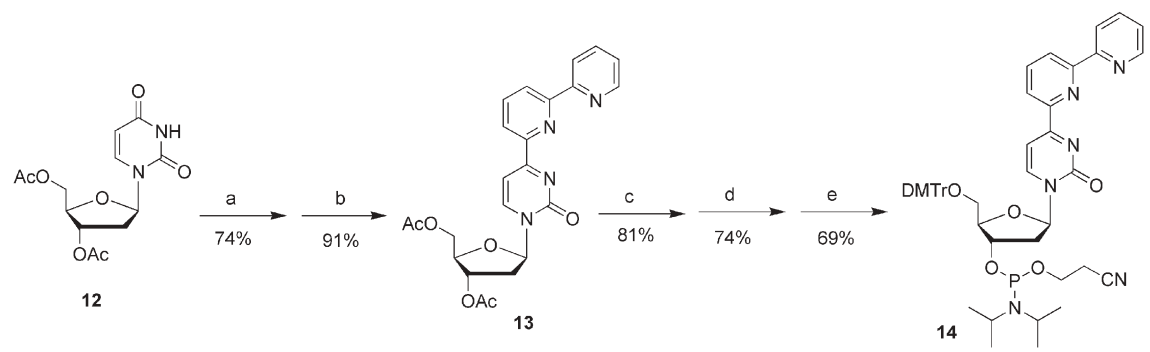

Fig. 3 Synthesis of 2′-deoxyribosyl-1-(4-(2″,6″,2‴-bipyridyl))pyrimidinone phosphoramidite. *Reagents and conditions*: (a) $SOCl_2$, DMF, $CHCl_3$, reflux; (b) 2-tributylstannyl-6-pyridylpyridine, $Pd(Ph_3P)_4$, toluene, reflux; (c) ammonia, methanol; (d) DMTCl, pyridine, RT; (e) bis(diisopropyl)aminocyanoethoxy chlorophosphine, $Et(Pr^i)_2N$, CH_2Cl_2, 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]

This work was supported by DOD/DARPA/DMEA under award no. DMEA90-02-2-0216 and the University of California–Riverside.

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-Py bearing duplex is included in the ESI.†

1 M. Nesterova and Y. S. Cho-Chung, *Curr. Drug Targets*, 2004, **5**, 683.
2 T. H. LaBean and H. Y. Li, *Nano Today*, 2007, **2**, 26.
3 A. M. Sismour and S. A. Benner, *Nat. Rev. Genet.*, 2005, **6**, 533.
4 A. Eschenmoser, *Science*, 1999, **284**, 2118.
5 (*a*) E. T. Kool, *Acc. Chem. Res.*, 2002, **35**, 936; (*b*) E. Rozners, *Lett. Org. Chem.*, 2005, **2**, 496; (*c*) K. Tanaka and M. Shionoya, *Chem. Lett.*, 2006, **35**, 694.
6 (*a*) S. R. Lynch, H. B. Liu, J. M. Gao and E. T. Kool, *J. Am. Chem. Soc.*, 2006, **128**, 14704; (*b*) B. A. Schweitzer and E. T. Kool, *J. Am. Chem. Soc.*, 1995, **117**, 1863; (*c*) D. L. McMinn, A. K. Ogawa, Y. Q. Wu, J. Q. Liu, P. G. Schultz and F. E. Romesberg, *J. Am. Chem. Soc.*, 1999, **121**, 11585.
7 (*a*) E. Meggers, P. L. Holland, W. B. Tolman, F. E. Romesberg and P. G. Schultz, *J. Am. Chem. Soc.*, 2000, **122**, 10714; (*b*) H. Weizman and Y. Tor, *J. Am. Chem. Soc.*, 2001, **123**, 3375; (*c*) K. Tanaka, Y. Yamada and M. Shionoya, *J. Am. Chem. Soc.*, 2002, **124**, 8802; (*d*) T. Tanaka, A. Tengeiji, T. Kato, N. Toyama, M. Shiro and M. Shionoya, *J. Am. Chem. Soc.*, 2002, **124**, 12494; (*e*) N. Zimmerman, E. Meggers and P. G. Schultz, *J. Am. Chem. Soc.*, 2002, **124**, 13684; (*f*) K. Tanaka, A. Tengeji, T. Kato, N. Toyama and M. Shionoya, *Science*, 2003, **299**, 1212; (*g*) C. Brotschi and C. J. Leumann, *Nucleosides, Nucleotides Nucleic Acids*, 2003, **22**, 1195; (*h*) C. Switzer and D. Shin, *Chem. Commun.*, 2005, **10**, 1342; (*i*) C. Switzer, S. Sinha, P. H. Kim and B. D. Heuberger, *Angew. Chem., Int. Ed.*, 2005, **44**, 1529; (*j*) K. Tanaka, G. H. Clever, Y. Takezawa, Y. Yamada, C. Kaul, M. Shionoya and T. Carell, *Nat. Nanotechnol.*, 2006, **1**, 190; (*k*) Y. Tanaka, S. Oda, H. Yamaguchi, Y. Kondo, C. Kojima and A. Ono, *J. Am. Chem. Soc.*, 2007, **129**, 244; (*l*) G. H. Clever and T. Carell, *Angew. Chem., Int. Ed.*, 2007, **46**, 250; (*m*) Y. Miyake, H. Togashi, M. Tashiro, H. Yamaguchi, S. Oda, M. Kudo, Y. Tanaka, Y. Kondo, R. Sawa, T. Fujimoto, T. Machinami and A. Ono, *J. Am. Chem. Soc.*, 2006, **128**, 2172; (*n*) D. Lindegaard, D. O. Wood, J. Wengel and J. S. Lee, *J. Biol. Inorg. Chem.*, 2006, **11**, 82.
8 (*a*) M. A. W. Eaton, T. A. Millican and J. Mann, *J. Chem. Soc., Perkin Trans. 1*, 1988, 545–7; (*b*) M. A. Calter and C. Zhu, *J. Org. Chem.*, 1999, **64**, 1415; (*c*) Y. Kim, A. M. Leconte, Y. Hari and F. E. Romesberg, *Angew. Chem., Int. Ed.*, 2006, **45**, 7809.
9 C. B. Reese and Q. Wu, *Org. Biomol. Chem.*, 2003, **1**, 3160.
10 M. Daune, C. A. Dekker and H. K. Schachman, *Biopolymers*, 1966, **4**, 51.
11 C. Brotschi and C. J. Leumann, *Angew. Chem., Int. Ed.*, 2003, **42**, 1655.
12 J. Y. Lee, J. Kwon, C. S. Park, J. Y. Lee, J. Kwon, C. S. Park, J. E. Lee, W. Sim, J. S. Kim, J. Seo, I. Yoon, J. H. Jung and S. S. Lee, *Org. Lett.*, 2007, **9**, 493.
13 J. Blumberger, L. Bernasconi, I. Tavernelli, R. Vuilleumier and M. Sprik, *J. Am. Chem. Soc.*, 2004, **126**, 3928.