A pyrimidine-like nickel(II) DNA base pair

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4-(2′-Pyridyl)-pyrimidinone deoxyriboside is synthesized and characterized as a DNA metallo base-pair; this novel nucleoside forms a self-pair in the presence of Ni(II) and stabilizes double helical DNA to the same extent as a G·C pair.

All natural nucleobases support some level of self-pairing. In a genomic context, A, G, C and T self-pairs are undesirable and can lead to mutations. Nevertheless, from the standpoint of *de novo* design, bases capable of high fidelity self-recognition hold a theoretical advantage of fewer possible mispairs and less synthetic overhead, balanced against their diminished informational capacity. Synthetic base-pairs have been devised whose recognition depends on van der Waals interactions,[1] metal-coordination[2] and hydrogen-bonds,[3] some of which rely on self-recognition. Here we report the successful realization of the most improbable of naturally inspired self-pairs, one based on a pyrimidine scaffold. 4-(2′-Pyridyl)-pyrimidinone (Pyr$^{\text{P}}$, Fig. 1) is found to bind nickel(II) selectively over other divalent ions, forming a Pyr$^{\text{P}}$·Ni·Pyr$^{\text{P}}$ base-pair with stability and mismatch discrimination rivaling natural Watson–Crick pairs.

Pyr$^{\text{P}}$ (Fig. 1) is formally derived from the natural nucleobase cytosine by replacement of its 4-amino group with pyridine. This transformation leads to Lewis basic nitrogen atoms in an optimal 1,4 relationship for metal ion coordination. The synthesis of Pyr$^{\text{P}}$ is summarized in Fig. 2. The critical step was a modified Negishi coupling[4] of pyridyl zinc bromide with the chloropyrimidinone deoxyriboside derived from **1** to provide pyridylpyrimidinone deoxyriboside **2**. Nucleoside **2** was then converted in three steps to phosphoramidite **3**. Two complementary DNA dodecamer strands bearing single Pyr$^{\text{P}}$ residues, 5′-d-CTTTCTPyr$^{\text{P}}$TCCCT (**4**) and 5′-d-AGGGAPyr$^{\text{P}}$AGAAAG (**5**), were prepared using an ABI 394 synthesizer and phosphoramidite **3**. Oligonucleotides were purified by PAGE, and their identities confirmed by MALDI-TOF mass spectrometry.

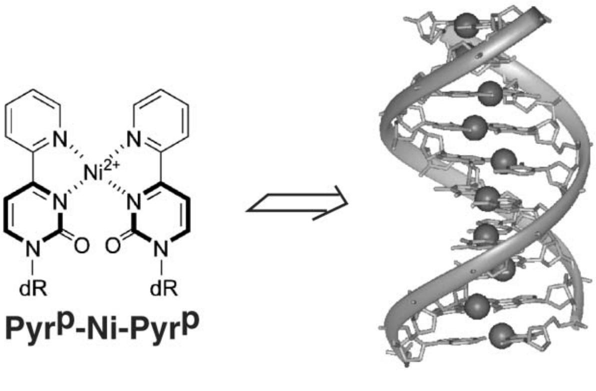

Fig. 1 4-(2′-Pyridyl)-pyrimidinone (Pyr$^{\text{P}}$) metallo base-pair.

Pyr$^{\text{P}}$ metallo base-pair formation was assayed by UV monitored thermal denaturation of the **4/5** duplex in the presence of divalent metal ions (Table 1). Denaturation profiles are displayed in Fig. 3. Specifically, the assay consisted of comparing T_m's of Pyr$^{\text{P}}$/Pyr$^{\text{P}}$ containing duplex **4/5** in the presence of the various divalent metal ions to the T_m obtained in the absence of any divalent ion (bottom of first column, Table 1). Of the six divalent metal ions screened, Ni^{2+} led to far and away the greatest duplex stabilization—a dramatic increase in T_m of 16.5 °C relative to the metal free control. Also significant, the data in Table 1 indicate Pyr$^{\text{P}}$·Ni^{2+}·Pyr$^{\text{P}}$ is as stabilizing to a double helix as C·G (41.2 *versus* 40.2 °C). Finally, in control experiments, essentially no effect was observed on T_m values of the T/A or C/G duplexes when denatured in the presence or absence of Ni^{2+} (bottom of second region, Table 1), or the Pyr$^{\text{P}}$·Ni^{2+}·Pyr$^{\text{P}}$ duplex when denatured at pH 8 rather than 7. The latter result when taken with a mixing curve determined in earlier work[5] on the parent **11/9** duplex supports a duplex (as opposed to a triplex) structure for **4/5**.

Mismatch discrimination of Pyr$^{\text{P}}$ was assessed by measuring the stability of the four natural bases against Pyr$^{\text{P}}$ in the presence of Ni^{2+} (top of second region, Table 1). These data show Pyr$^{\text{P}}$·Ni^{2+} is a mismatch against all four natural bases as ΔT_m values of the mismatched pairs relative to Pyr$^{\text{P}}$·Ni^{2+}·Pyr$^{\text{P}}$ ranged from 18.3–21.9 °C (these values are distinct from the Δ values in Table 1 that are rooted to the C/G pair). As a reference, natural T/G and C/A mismatches of the parent duplex under the same conditions show ΔT_m values of 7.4 and 18.5 °C.[5] Therefore, all four Pyr$^{\text{P}}$·Ni^{2+} mismatches are similar to severe natural nucleobase mismatches in their instability.

Three coordination geometries are possible in principle for Pyr$^{\text{P}}$·Ni^{2+}·Pyr$^{\text{P}}$: square planar, $D_2{}^{d}$, and tetrahedral. All other factors being equal, a square planar geometry should be preferred to minimize disruption of base-stacking in the DNA double helix. Square planar geometries are predicted to be accessible for Ni^{2+}, Co^{2+}, and Cu^{2+}, three of the metal ions screened for their ability to stablize the Pyr$^{\text{P}}{}_2$ bearing helix. *Ab initio* geometry optimization of Pyr$^{\text{P}}$·Ni^{2+}·Pyr$^{\text{P}}$ at the B3LYP/6-31G*(CHN)/LACVP*(Ni) level of theory led to a square-planar geometry as a (local) minimum as depicted in Fig. 4a. Remarkably, the N1–N1′ (pyrimidine numbering) distance in the optimized Pyr$^{\text{P}}$·Ni^{2+}·Pyr$^{\text{P}}$ structure spans only 4.9 Å (also depicted in Fig. 4a). In contrast, the corresponding N9–N1′, Pur–Pyr, distance in natural B-DNA helices for both G/C and A/T base-pairs is 9.1 Å (structure not shown). Thus, despite a predicted base to base distance of approximately half (*i.e.*, 54%) of the corresponding distance of a natural base-pair, Pyr$^{\text{P}}$·Ni^{2+}·Pyr$^{\text{P}}$ nonetheless confers stability to a helix equivalent to a G·C base-pair. Bipyridyl-2′-deoxyriboside[2g,6]

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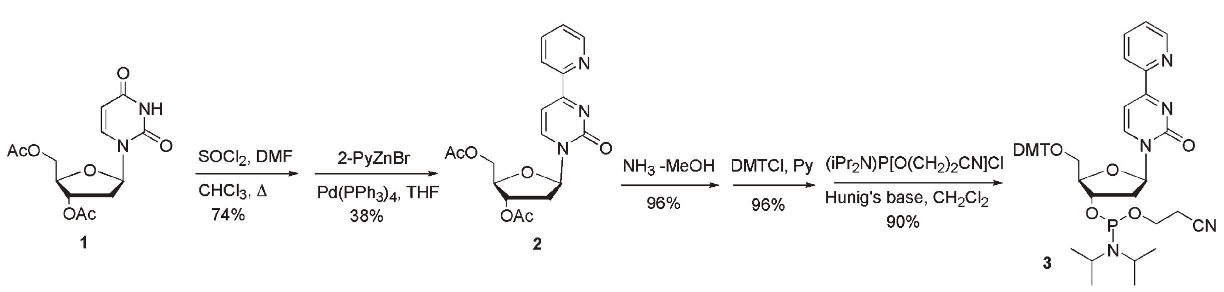

Fig. 2 Synthesis of 2′-deoxyribosyl-1-[6-(2″-pyridyl)-pyrimidinone].

Table 1 DNA duplex melting temperatures in the presence and absence of divalent ions. Samples contained 2.5 μM of each DNA strand, 10 μM divalent ion where indicated, 50 mM NaCl, and 10 mM NaH_2PO_4, pH 7

5′-d-CTTTCT**X**TCCCT

3′-d-GAAAGA**Y**AGGGA

X/Y	No.	M	T_m	Δ[a]	X/Y	No.	M	T_m	Δ[a]
Pyr^P/Pyr^P	**4/5**	Ni^{2+}	41.2	+1.1	Pyr^P/T	**4/6**	Ni^{2+}	19.5	−20.6
Pyr^P/Pyr^P	**4/5**	Co^{2+}	29.9	−10.2	Pyr^P/C	**4/7**	Ni^{2+}	19.3	−20.8
Pyr^P/Pyr^P	**4/5**	Cu^{2+}	26.8	−13.3	Pyr^P/A	**4/8**	Ni^{2+}	19.7	−20.4
Pyr^P/Pyr^P	**4/5**	Zn^{2+}	24.1	−16.0	Pyr^P/G	**4/9**	Ni^{2+}	22.9	−17.2
Pyr^P/Pyr^P	**4/5**	Fe^{2+}	24.0	−16.1	T/A	**10/8**	—	36.8	−3.3
Pyr^P/Pyr^P	**4/5**	Mn^{2+}	23.9	−16.2	T/A	**10/8**	Ni^{2+b}	37.4	−2.7
Pyr^P/Pyr^P	**4/5**	—	24.7	−15.4	C/G	**11/9**	—	40.2	+0.1
					C/G	**11/9**	Ni^{2+b}	40.1	0.0

[a] Difference in T_m compared to X/Y = C/G. [b] Divalent ion was added in these cases as a control.

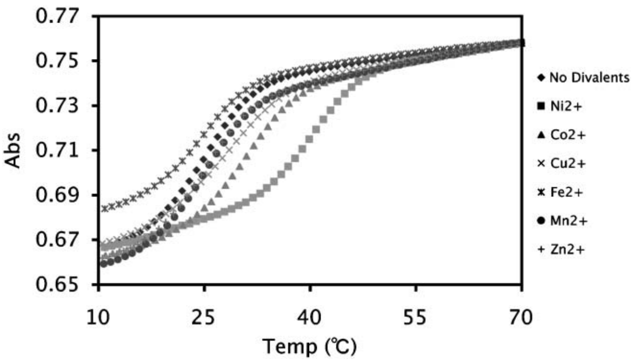

Fig. 3 Absorbance *versus* temperature denaturation profiles. Conditions as reported in the legend to Table 1.

is structurally similar to Pyr^P, but to date self-pairing has been reported only in the absence of metal ions.

The remarkable stability of $Pyr^P{\cdot}Ni^{2+}{\cdot}Pyr^P$ in the face of its predicted dimensions is best appreciated in the context of natural pyrimidine–pyrimidine mismatches. The C·T mismatch is particularly illustrative as a comparison since it is in effect a hydrogen-bonding counterpart to $Pyr^P{\cdot}Ni^{2+}{\cdot}Pyr^P$. Where C·T forms two hydrogen bonds, $Pyr^P{\cdot}Ni^{2+}{\cdot}Pyr^P$ forms two coordination bonds (per base), and both pairs have opposing 2-carbonyl groups (Fig. 4b *vs.* 4a). Despite outward similarities, the properties of $Pyr^P{\cdot}Ni^{2+}{\cdot}Pyr^P$ and C·T are divergent as C·T is among the most destabilizing mismatches to a DNA helix.[7] Structural studies of C·T(U) mismatches in DNA and RNA helices indicate a single direct hydrogen bond between the bases (N4H–O4) and a water mediated hydrogen bond (N3–H_2O–N3H) (Fig. 4c) rather than two direct hydrogen bonding interactions between the two sets of complementary donor/acceptor groups (Fig. 4b).[8] *Ab initio* optimization of both these latter structures provided the N1–N1′ interatomic distances shown (Fig. 4b/c). It is apparent from a comparison of these distances that recruiting a water molecule into the C·T base-pairing motif causes a favorable increase in the interaction distance of the bases relative to the distance in a natural helix (glycosidic N–N distances of 9.6 Å/C·H_2O·T, Fig. 4c, *vs.* 9.1 Å/A·T & G·C, data not shown). However, there is at least one report that the C·H_2O·T structure disrupts stacking interactions.[8c] In the case of $Pyr^P{\cdot}Ni^{2+}{\cdot}Pyr^P$ we suggest its high stability derives from two unique features: (i) stacking interactions provided by the pyridyl groups, and (ii) the greater strength of coordination bonds

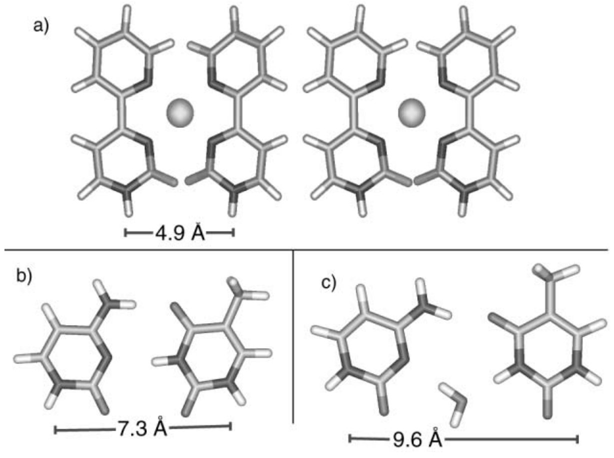

Fig. 4 Optimized geometries (B3LYP/6-31G*(CHN)LACVP*(Ni)) using Jaguar 3.5 (Schrodinger Inc.) of: a) $Pyr^P{\cdot}Ni^{2+}{\cdot}Pyr^P$ (stereo view), b) C·T, and c) C·H_2O·T.

in comparison to hydrogen bonds. As a result, $\mathrm{Pyr^{p}{\cdot}Ni^{2+}{\cdot}Pyr^{p}}$ is able to surmount its acute dimensional shortfall and still strongly stabilize a double helix.

In summary, the $\mathrm{Pyr^{p}{\cdot}Ni^{2+}{\cdot}Pyr^{p}}$ metallo base-pair has fidelity and stability on a par with natural Watson–Crick base-pairs despite assuming a non-natural dimension. We are actively pursuing materials incorporating this motif with applications in nano-electronics and artificial biology.

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Notes and references

1 (*a*) B. A. Schweitzer and E. T. Kool, *J. Am. Chem. Soc.*, 1995, **117**, 1863; (*b*) 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.

2 (*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 and Nucleic Acids*, 2003, **22**, 1195.

3 (*a*) C. Switzer, S. E. Moroney and S. A. Benner, *J. Am. Chem. Soc.*, 1989, **111**, 8322; (*b*) J. A. Piccirilli, T. Krauch, S. E. Moroney and S. A. Benner, *Nature*, 1990, **343**, 33.

4 M. Hocek, A. Hol, I. Vortuba and H. Dvoáková, *Collect. Czech. Chem. Commun.*, 2001, **66**, 483.

5 H. Hashimoto, M. G. Nelson and C. Switzer, *J. Am. Chem. Soc.*, 1993, **115**, 7128.

6 C. Brotschi, A. Haberli and C. J. Leumann, *Angew. Chem., Int. Ed.*, 2001, **40**, 3012.

7 (*a*) F. Aboul-ela, D. Koh, I. Tinoco and F. H. Martin, *Nucleic Acids Res.*, 1985, **13**, 4811; (*b*) H. Werntges, G. Steger, D. Riesner and H.-J. Fritz, *Nucleic Acids Res.*, 1986, **14**, 3773.

8 (*a*) Y. Boulard, J. A. H. Cognet and G. V. Fazakerly, *J. Mol. Biol.*, 1997, **268**, 331; (*b*) Y. Tanaka, C. Kojima, T. Yamazaki, T. S. Kodama, K. Yasuno, S. Miyashita, A. M. Ono, A. S. Ono, M. Kainosho and Y. Kyogoku, *Biochemistry*, 2000, **39**, 7074; (*c*) W. B. T. Cruse, P. Saludjian, E. Biala, P. Strazewski, T. Prangé and O. Kennard, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 4160; (*d*) S. R. Holbrook, C. Cheong, I. Tinoco and S. H. Kim, *Nature*, 1991, **353**, 579.