Communication

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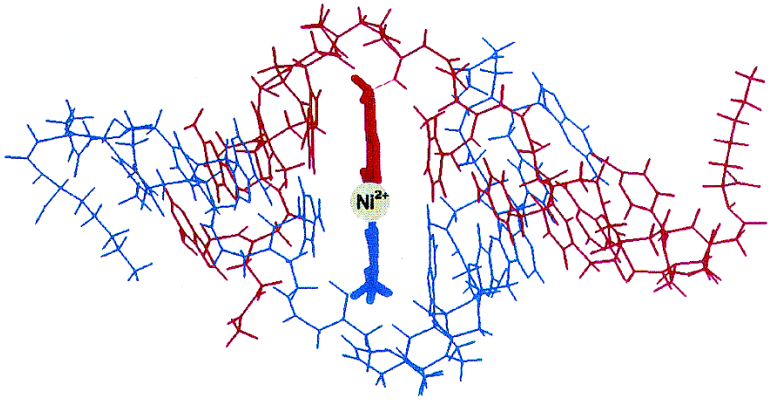

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Metal Incorporation in Modified PNA Duplexes

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Recently, ligands have been introduced in DNA strands, and metal ions have been used to bridge these modified DNA strands into duplexes or triplexes. Incorporation of metal-bridged base pairs in DNA represents a means to expand the scope of information storage and retrieval by nucleic acids and to organize transition metal ions in nanostructures. Cu^{2+} and Ag^{+} have been shown to bridge ligands such as pyridine, bipyridine, pyridine 2,6-dicarboxylate, 2,6-bis(ethylthiomethyl)pyridine, and hydroxypyridone into metal-bridged alternative base pairs.[1] Substitution of a natural base pair by a pair of ligands leads to DNA duplex destabilization comparable to the destabilization induced by the presence of a mismatch. Incorporation of metal ions into the ligand-modified DNA duplexes leads to formation of coordinative bonds that act in a manner analogous to that of hydrogen bonds in DNA.

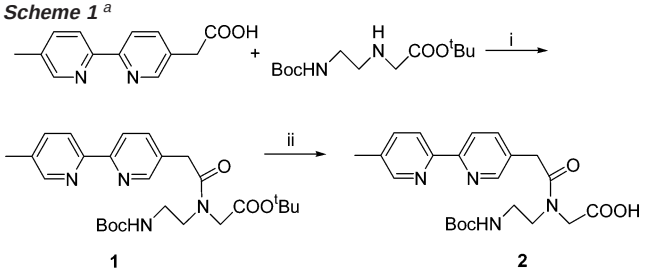
Herein, we demonstrate that metal incorporation can be expanded to peptide nucleic acid (PNA), a structural analogue of DNA.[2] PNA contains nucleobases linked to a pseudo-peptide backbone based commonly on *N*-(2-aminoethyl)-glycine (Aeg). Some of the advantages of using PNA as a scaffold for metal ions are the greater chemical stability of amide bonds of the PNA backbone as compared to the phosphate and glycosidic bonds of DNA and the fact that the neutral PNA backbone offers more flexibility for controlling the overall charge of metal-containing structures than does the anionic DNA backbone. Also, to create PNA monomers, ligands can be attached to the secondary amino group of Aeg by acylation, a synthetic method simpler than the ones for making ligand-containing phosphoramidites for DNA synthesis.

A design consideration for a metallo-base pair in PNA is the possibility that the amide groups in the backbone and the nucleobases might act as competing chelating sites for metal ions. Therefore, we selected bipyridine, which has a high affinity for metal ions, as the ligand to be introduced in PNA. 5-Acetic acid-5′-methyl-2,2′-bipyridine, obtained by lithiation followed by reaction with CO_2,[3] was coupled to Boc-protected Aeg *tert*-butyl ester using the peptide coupling agent TOTU in DMF in the presence of DIEA (Scheme 1).[4] Hydrolysis of the butyl ester **1** using NaOH afforded a Boc-protected PNA monomer derivatized with bipyridine **2** (5-Me-bipy).

The monomer was incorporated in PNA oligomers using solid-phase peptide synthesis and Boc-protection strategy.[5] We replaced two nucleobases situated in complementary positions in the middle of a 10-bp PNA duplex ($\mathbf{P_1}$) that was extensively investigated ($\mathbf{P_1}$, M = A, N = T in Chart)[7] with bipyridine ($\mathbf{P_2}$, M = N = bipy).[6] PNA strands were purified by HPLC and characterized by MALDI-TOF mass spectrometry.

We have investigated the interaction of $\mathbf{P_1}$ and $\mathbf{P_2}$ with Ni^{2+}, Pd^{2+}, and Pt^{2+} because they can form square-planar complexes. Formation of tetrahedrally distorted square-planar $[M(bipy)_2]^{2+}$ complexes with the latter two ions is extensively documented,[8] and molecular dynamics simulations indicate that incorporation of such a metal-bridged fragment in PNA duplexes has little effect on the position of adjacent nucleobase pairs.[9]

Scheme 1 [a]



1 **2**

[a] Reagents and conditions: (i) TOTU, DIEA, DMF, 4 h, 44%; (ii) NaOH, EtOH, H_2O, 4 h, 60%.

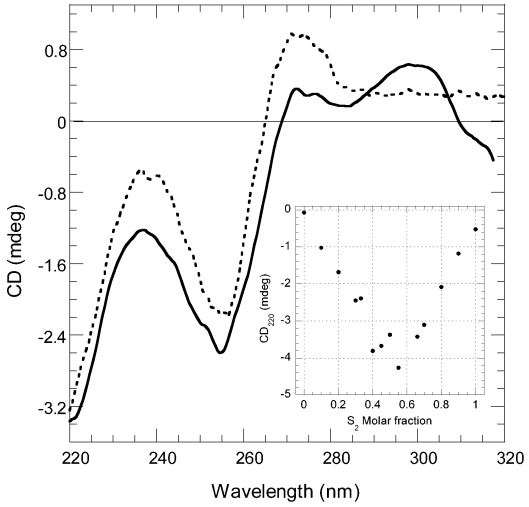


Figure 1. CD spectra for $\mathbf{P_2}$ (- - -) and Ni^{2+}−$\mathbf{P_2}$ (—). Spectra were recorded at 20 °C in 10 mM sodium phosphate buffer at pH 7.1. $\mathbf{P_2}$ and Ni^{2+} concentrations were 5 μM. Inset: Job plot for CD intensity at 220 nm of complementary strands at a total concentration of 10 μM, in the presence of 10 μM Ni^{2+}. Abscissa is molar fraction of $\mathbf{S_2}$ (N = bipy).

Chart 1. PNA Sequence

H-GTAG**M**TCACT-LysNH$_2$	$\mathbf{S_1}$
H$_2$N-Lys-CATC**N**AGTGA-H	$\mathbf{S_2}$

Figure 1 shows CD spectra for $\mathbf{P_2}$ and Ni^{2+}−$\mathbf{P_2}$.[6] Between 220 and 280 nm, these spectra are similar to the previously reported CD spectrum of $\mathbf{P_1}$,[7b] indicating that a duplex forms from strands that contain bipyridine in the absence and in the presence of Ni^{2+}. A continuous variation experiment following CD intensity at 220 nm (Figure 1, inset) supports the inference that the two strands form a duplex in the presence of Ni^{2+}. These data also confirm the occurrence of ordinary Watson−Crick base pairs and hence antiparallel arrangement of the two strands. The CD spectrum of Ni^{2+}−$\mathbf{P_2}$ has two extra features at 300 and ∼320 nm, which are assigned to π−π* transitions of the coordinated bipyridine in $[Ni(5\text{-Me-bipy})_2]^{2+}$. This assignment is corroborated by the results of UV absorption experiments. Thus, titration of $\mathbf{P_2}$ with Ni^{2+} leads to the appearance of two absorption bands at 305 and 320 nm, for

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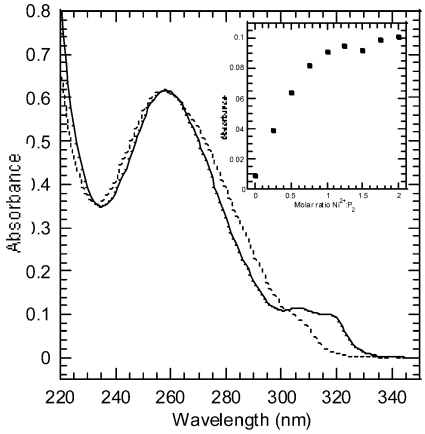

Figure 2. UV−vis spectra for $\mathbf{P_2}$ (- - -) and $\mathbf{P_2}$ in the presence of Ni^{2+} (Ni^{2+}:$\mathbf{P_2}$= 1:1). Spectra were recorded at room temperature, in 10 mM phosphate buffer at pH 7.1. $\mathbf{P_2}$ concentration was 5 μM. Inset: Absorbance at 320 nm as a function of Ni^{2+}:$\mathbf{P_2}$ molar ratio.

Table 1. Melting Temperatures of (Modified-)PNA Duplexes[a]

M	A	T	C	G	bipy
N = T	66.5	50.2	51.3	56.8	47.3
N = T, $+Ni^{2+}$	66.5	50.3	51.0	56.7	46.0
N = bipy	50.6	47.9	48.3	49.5	48
N = bipy, $+Ni^{2+}$	46.5	45.5	45.5	47.2	59

[a] Solutions in 10 mM sodium phosphate pH = 7.1 buffer were 5 μM in each strand and in Ni^{2+} where this is the case. Uncertainties in T_m values are given in the Supporting Information and are typically less or equal to ±1 °C.

which the intensity increases linearly with the concentration of Ni^{2+} up to a Ni^{2+}:$\mathbf{P_2}$ ratio of 1:1, beyond which there is no change in intensity (Figure 2).

To investigate the stability of the PNA duplexes, melting temperatures of $\mathbf{P_1}$ and $\mathbf{P_2}$ in the absence and in the presence of transition metal ions were determined from the temperature dependence of UV absorption at 260 nm (Figure S1). Table 1 shows the melting temperatures for PNA duplexes that contain natural base pairs or bipyridine moieties situated in the same sequence context, in the absence and in the presence of Ni^{2+}.

As seen in the first row of Table 1, TT and TC mismatches lead to a lowering of the melting point by ~16 and 15 °C, respectively, relative to the control duplex $\mathbf{P_1}$ (T_m = 66.5 °C). The GT mismatch has a significantly smaller effect ($\Delta T \approx -10$ °C). These results are in agreement with the ones previously observed for DNA/PNA duplexes.[10] The small effect of the GT mismatch on the duplex stability is due to the formation of a wobble pair.[10] Substitution of 5-Me-bipyridine for one or two of the bases in the duplex leads to a destabilization larger than that for a mismatch ($\Delta T = -16$ to 19 °C) (third row of Table 1). Addition of Ni^{2+} has practically no effect on the stability of PNA duplexes containing natural bases, but, as can be seen by comparing data in rows 3 and 4, it reduces the stability of duplexes that contain one bipyridine moiety. In contrast to the latter effect, addition of Ni^{2+} to the duplex that contains bipyridine in complementary positions leads to a considerable increase in stability over duplexes that contain one bipyridine or mismatches (T_m = 59 °C). The stability is slightly superior to that of the PNA containing a GT mismatch. These results indicate that Ni^{2+} coordination to $\mathbf{P_2}$ is quite selective. The effect on PNA duplex stability of ligand substitution and of Ni^{2+} coordination is larger than the effects observed for DNA duplexes with similar modifications, in the same way in which the effect of mismatches in PNA is larger than that in DNA.

The melting temperature of $\mathbf{P_2}$ does not change in the presence of $K_2[PdCl_4]$ or $K_2[PtCl_4]$. We attribute this to the fact that, in the presence of Cl^- anions, $[M(bipy)_2]^{2+}$ (M = Pd or Pt) transforms to $[M(bipy)Cl_2]$.[8] Alternative starting materials and conditions for incorporation of these metal ions are presently being pursued.

Our results demonstrate that metal incorporation in PNA duplexes can be achieved and that the helical structure of the duplex is preserved upon incorporation of one alternative metal-bridged base pair.

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Supporting Information Available: Experimental procedures and characterization data for **1**, **2**, and PNA oligomers, and melting curves for $\mathbf{P_1}$, $\mathbf{P_2}$, and $Ni^{2+}-\mathbf{P_2}$ (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Shionoya, M.; Tanaka, K. *Bull. Chem. Soc. Jpn.* **2000**, *73*, 1945−1954. (b) Tanaka, K.; Tengeiji, A.; Kato, T.; Toyama, N.; Shiro, M.; Shionoya, M. *J. Am. Chem. Soc.* **2002**, *124*, 12494−12498. (c) Tanaka, K.; Yamada, Y.; Shionoya, M. *J. Am. Chem. Soc.* **2002**, *124*, 8802−8803. (d) Tanaka, K.; Tengeiji, A.; Kato, T.; Toyama, N.; Shionoya, M. *Science* **2003**, *299*, 1212−1213. (e) Meggers, E.; Holland, P. L.; Tolman, W. B.; Romesberg, F. E.; Schultz, P. G. *J. Am. Chem. Soc.* **2000**, *122*, 10714−10715. (f) Atwell, S.; Meggers, E.; Spraggon, G.; Schultz, P. G. *J. Am. Chem. Soc.* **2001**, *123*, 12364−12367. (g) Zimmermann, N.; Meggers, E.; Schultz, P. G. *J. Am. Chem. Soc.* **2002**, *124*, 13684−13685. (h) Weizman, H.; Tor, Y. *J. Am. Chem. Soc.* **2001**, *123*, 3375−3376.

(2) (a) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497−1500. (b) Nielsen, P. E.; Haaima, G. *Chem. Soc. Rev.* **1997**, 73−78. (c) Uhlman, E.; Peyman, A.; Breipohl, G.; Will, W. D. *Angew. Chem., Int. Ed.* **1998**, *37*, 2796−2823.

(3) Beyeler, A.; Belser, P.; De Cola, L. A. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2779−2780.

(4) Breipohl, G.; Will, W. D.; Peyman, A.; Uhlman, E. *Tetrahedron* **1997**, *53*, 14671−14686.

(5) (a) Dueholm, K. L.; Egholm, M.; Behrens, C.; Christensen, L.; Hansen, H. F.; Vulpius, T.; Petersen, K. H.; Berg, R. H.; Nielsen, P. E.; Buchardt, O. *J. Org. Chem.* **1994**, *59*, 5767−5773. (b) Christensen, L.; Fitzpatrick, R.; Gildea, B.; Petersen, K. H.; Hansen, H. F.; Koch, T.; Egholm, M.; Buchardt, O.; Nielsen, P. E. *J. Pept. Sci.* **1995**, *1*, 175−183.

(6) Hybridization of complementary strands was accomplished by slow cooling from 95 °C of equimolar solutions of the strands.

(7) (a) Wittung, P.; Eriksson, M.; Lyng, R.; Nielsen, P. E.; Nordén, B. *J. Am. Chem. Soc.* **1995**, *117*, 10167−10173. (b) Wittung, P.; Nielsen, P. E.; Buchardt, O.; Egholm, M.; Nordén, B. *Nature* **1994**, *368*, 561−563. (c) Ratilainen, T.; Holmen, A.; Tuite, E.; Haaima, G.; Christensen, L.; Nielsen, P. E.; Nordén, B. *Biochemistry* **1998**, *37*, 12331−12342.

(8) Constable, E. C. *Adv. Inorg. Chem.* **1989**, *34*, 1−62.

(9) Preliminary molecular dynamics simulations indicate that substitution of the AT pair by $[Pt(bipy)_2]^{2+}$ leads to small changes in the position of the nucleobase pairs adjacent to the metal-bridged one. Popescu, D.; Madrid, M.; Achim, C., manuscript in preparation.

(10) Ratilainen, T.; Holmen, A.; Tuite, E.; Nielsen, P. E.; Nordén, B. *Biochemistry* **2000**, *39*, 7781−7791.

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