2,2′-Bipyridine Ligandoside: A Novel Building Block for Modifying DNA with Intra-Duplex Metal Complexes

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The structure, dynamics, and recognition properties of the DNA double helix continue to fascinate chemists. While ingenious and diverse approaches have been devised to probe the various surfaces of DNA (e.g., groove binders, metal complexes),[1] little has been done to explore the interior of the helix. This partially exposed domain is comprised of stacked heterocycles that project H-bond donors and acceptors into the interior as well as the two distinct grooves. The ability of this aromatic π-stack to mediate charge-transfer processes has recently taken center stage[2] and prompted us to consider modification to the DNA core.[3] We have envisioned the placement of charged metal complexes at the center of the DNA helix as illustrated in Figure 1.[4,5]

Nucleoside mimics, hereby coined *ligandosides*, where the heterocyclic base is replaced by a strong chelator, are key to our approach (Figure 1).[6,7] A ligandoside should meet the following requirements: (a) Be compatible with standard DNA synthesis; (b) have higher affinity for metal ions than the heterocyclic bases; (c) form complexes with comparable dimensions to a DNA base pair. 2,2′-Bipyridine has been selected as the ligand due to its high stability and affinity to numerous metal ions.[8,9] Modeling reveals that direct attachment of the bpy to the sugar results in compression of the duplex upon metal complexation. A methylene group has therefore been introduced between position 5 of the ligand and the 2′-deoxy-D-ribose. The saturated methylene bridge relaxes the ligandoside and results in better fitting of the complex within the DNA duplex.

While numerous methods for synthesizing aromatic *N*- and *C*-glycosides have been developed,[10] synthetic approaches to aliphatic *C*-nucleosides are scarce.[11] Our approach is based on the lithiation of 5-methyl-2,2′-bipyridine followed by coupling with 3,5-Di-*O*-benzyl-2-deoxy-D-ribofuranose (Scheme 1).[12] The resulting diol **1** is then cyclized by a Mitsunobu reaction[13] to give a 1:1 diastereomeric mixture of the protected ligandoside **2**. Removal of the benzyl groups of **2** by hydrogenolysis failed under various conditions, but was successfully accomplished using BCl_3 to give the free ligandoside **3**. Selective protection of the 5′ hydroxyl with 4,4′-dimethoxytrityl facilitated the chromatographic resolution of the two diastereoisomers. The assignment of their absolute configuration was based on NOE experiments.[14] Phosphitylation of the β-anomer provided the ligandoside phosphoramidite **5**.

The ligandoside (**3**, X^{bpy}) was incorporated into DNA oligonucleotides using solid-phase DNA synthesis.[14] A self-complementary 11-mer sequence **6** was synthesized and purified by polyacrylamide gel electrophoresis in the presence of EDTA. Enzymatic digestion followed by HPLC analysis confirmed the presence of the intact ligandoside in the anticipated amount.[14] A 10-mer oligonucleotide **7** that has an identical base sequence but lacks the modified nucleoside was synthesized as a control (Figure 2).

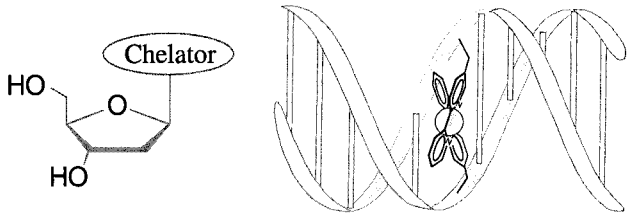
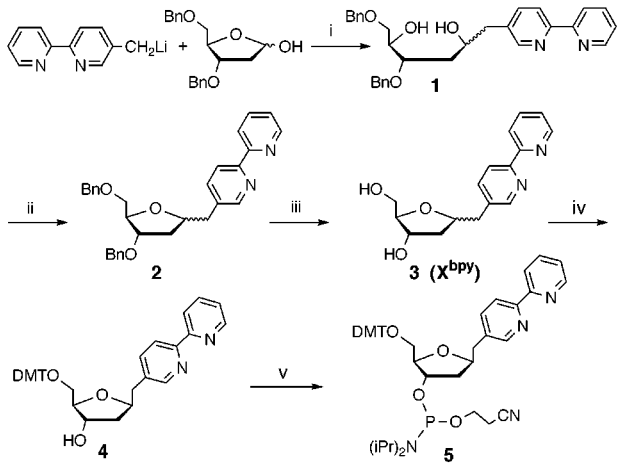


Figure 1. Schematic representation of an interstrand metal complex within a DNA double helix (right) and a ligandoside (left).

Scheme 1. Synthesis of 2,2′-bipyridine Ligandoside and Its Phosphoramidite[a]



[a] Reagents and conditions: (i) THF, −78 °C, rt 1 h, 72%; (ii) diethylazodicarboxylate, Ph_3P, THF, 3 h, 87%; (iii) BCl_3, THF, −78 °C, 1.5 h, 58%; (iv) DMT-Cl, DMAP, Py, 1 h, 80% (mixture of two anomers), chromatographic resolution; (v) 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropyl-phophordiamidite, 1*H*-tetrazole, CH_3CN, 1.5 h, 66%.[14]

(1) Erkkila, K. E.; Odom, D. T.; Barton, J. K. *Chem. Rev.* **1999**, *99*, 2777−2795. Jamieson, E. R.; Lippard, S. J. *Chem. Rev.* **1999**, *99*, 2467−2498. Kool, E. T. *Chem. Rev.* **1997**, *97*, 1473−1488. Nielsen, P. E. *Chem. Eur. J.* **1997**, *3*, 505−508. Waring, M. J. Bailly, C. *J. Pharm. Belg.* **1997**, *52*, 70−78. Trauger, J. W.; Baird, E. E.; Dervan, P. B. *Nature* **1996**, *382*, 559−561. Kahne, D. *Chem. Biol.* **1995**, *2*, 7−12.

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(4) Backbone phosphates may then be envisioned to serve as counteranions.

(5) Hydrophobic nucleoside mimics form surrogate non-hydrogen-bonded base pairs. See: Kool, E. T.; Morales, J. C.; Guckian, K. M. *Angew. Chem., Int. Ed.* **2000**, *39*, 991−1009. Ogawa, A. K.; Wu, Y. Q.; McMinn, D. L.; Liu, J. Q.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2000**, *122*, 3274−3287.

(6) The synthesis of *o*-phenylenediamine furanoside, a potential metal binder, has been reported: Tanaka, K.; Shionoya, M. *J. Org. Chem.* **1999**, *64*, 5002−5003.

(7) A selective, neutral, and complementary copper-mediated base pair has been reported: Meggers, E.; Holland, P. L.; Tolman, W. B.; Romesberg, F. E.; Schultz, P. G. *J. Am. Chem. Soc.* **2000**, *122*, 10714−10715.

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(9) The low nucleophilicity of the heterocyclic bpy nitrogens precludes the need for protecting groups during DNA synthesis.

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(14) See Supporting Information for experimental details.

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6 5′ AGT-CG**X**^{bpy}-CGA-CT 3′
3′ TCA-GC**X**^{bpy}-GCT-GA 5′

7 5′ AGT-CGC-GAC-T 3′
3′ TCA-GCG-CTG-A 5′

Figure 2. Sequences of a ligandoside-containing oligonucleotide (**6**) and its control sequence (**7**).

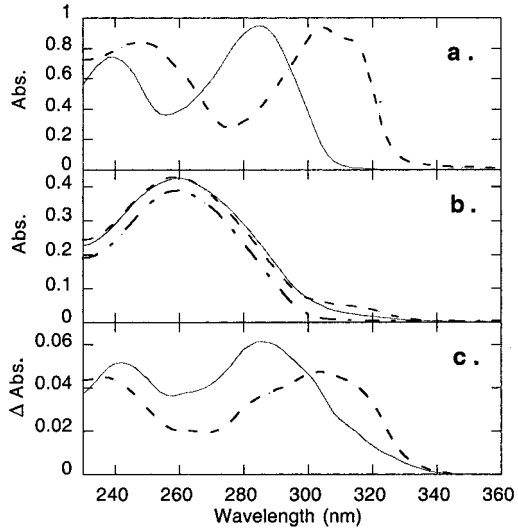

Figure 3. Absorption spectra of ligandoside **3** and the modified DNA **6** upon addition of Cu^{2+}. (a) Unattached ligandoside (— **3**, - - - **3**+Cu^{2+}); (b) Modified DNA and a control sequence (— **6**, - - - **6**+Cu^{2+}, · — · — **7**); (c) Difference spectra (— **6**−**7**, - - - **6**+Cu^{2+}−**7**).

The UV spectrum of the free ligandoside **3** displays two bands at 240 and 284 nm, characteristic to the bpy $\pi-\pi^*$ transitions (Figure 3a).[15] Upon addition of 0.5 equiv of $Cu(OAc)_2$ the absorptions bands are shifted to 248 and 304 nm, respectively. The UV spectrum of the ligandoside-containing sequence **6**, is predominated by the 260 nm absorption of the DNA heterocycles (Figure 3b). Subtracting the spectrum of the control DNA sequence **7** (Figure 3b) from that of the ligandoside-containing DNA **6** reveals the characteristics transitions of the ligandoside (Figure 3c). Upon addition of 1.0 equiv of Cu^{2+} to the modified DNA duplex **6**, a new band appears as a shoulder around 305 nm (Figure 3b).[16] Subtracting the spectrum of the control DNA sequence **7** from that of the metalated ligandoside-containing DNA **6**-Cu^{2+} unveils a shift in the transitions associated with the chelator, indicative of metal complex formation (Figure 3c). Addition of Cu^{2+} to the control sequence **7** results in no spectral changes.

Thermal denaturation experiments have been utilized to compare the relative stability of the various duplexes (Figure 4).[14]

(15) Due to the high affinity of bpy to numerous metal ions, all solvents and reagents were purified over Chelex 100 resin prior to use.

(16) Note that since oligonucleotides **6** and **7** are self-complementary, the amount of Cu^{2+} added is given per duplex and not per strand.

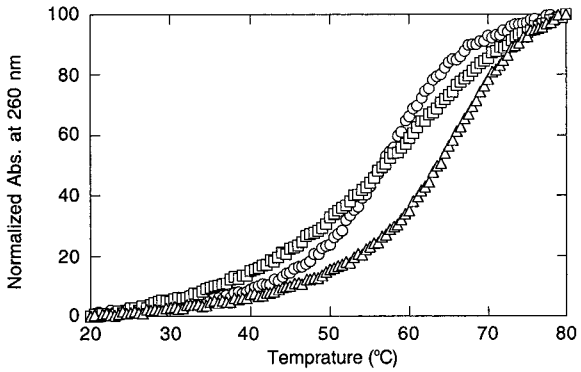

Figure 4. Thermal denaturation curves for ligandoside modified duplex **6** (□), control duplex **7** (○), and duplex **6** in the presence of Cu^{2+} (△). Measurements were performed in 400 mM Tris-acetate pH 7.8, in the presence of 2×10^{-6} M oligonucleotide for **6** and **7**, and 2×10^{-6} M oligonucleotide + 1×10^{-6} M $Cu(OAc)_2$ for **6**+Cu^{2+}.

The observed melting temperature of the ligandoside-containing DNA **6** was the same as the 10-mer control ($T_m = 56.5$ °C), yet the slope of the melting curve was less steep, suggesting lower cooperativity of the melting process (Figure 4). A priori, the bpy ligands can be either stacked inside the DNA duplex or exposed to the solvent. At neutral and basic pHs, the bpy ligands are uncharged and are therefore expected to minimize interactions with water. The shape of the melting curve suggests that the free ligands are likely to be buried within the helix, causing disruption of the melting process. Upon addition of 1.0 equiv of Cu^{2+} to the ligandoside-containing duplex **6**, a significant increase in the T_m value is observed ($T_m = 64$ °C), and a cooperative melting curve is restored (Figure 4). This stabilization is in agreement with an interstrand, intraduplex tetracoordinated complex formation. It is likely that, within the duplex, solvent molecules are excluded and the backbone phosphate groups serve as the counterions. It is important to note that the melting curve of the control duplex **7** dose not change upon addition of 1 equiv of Cu^{2+}.

These results demonstrate the utilization of a ligandoside, an unnatural nucleoside mimic, for the incorporation of a charged homoleptic metal complex inside a DNA duplex. This strategy represents a unique approach for metal-mediated interstrand binding. Such noncovalent interactions can be viewed as tunable cross-linking. Metal coordination is typically stronger than hydrogen bonds and, unlike covalent bonds, can be easily "denatured" in the presence of competing ligands.

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Supporting Information Available: Additional data regarding synthesis and structural assignment of the ligandoside, thermal denaturation measurements, HPLC, and enzymatic digestions of the oligonucleotides (PDF). This material is available free of charge via the Internet at http://pubs.acs.org

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