A Metal-Coordinating DNA Hairpin Mimic

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A self-complementary oligodeoxynucleotide containing a 6,6″-substituted terpyridine was found to adopt a highly stable, hairpin-like structure. In addition to serving as a hairpin-loop mimic, the terpyridine can act as a coordination site for metals. Thus, the binding of several divalent transition metals (Zn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Pd^{2+}) to the terpyridine hairpin mimic was investigated. The terpyridine-modified hairpin mimic forms a stable secondary structure in the presence of these metals. The stability of the metal-coordinated hairpin mimic was found to be lower than in the absence of metal. Furthermore, the T_m of the metallo-hairpin is strongly influenced by the type of the bound metal, with T_m's increasing in the order $Co^{2+} \sim Ni^{2+} < Zn^{2+} < Cu^{2+} < Pd^{2+}$. Model considerations suggest that a conformational change of the terpyridine ligand is required to allow coordination of the metal.

Introduction

The hairpin belongs to the most common secondary structural motifs found in nucleic acids.[1] In RNA, it is an essential element for the assembly of higher-order structures. Extrastable hairpins containing four bases in the loop (tetraloops) have emerged as a distinct class of hairpin that forms highly specific interactions with tetraloop receptor sites. By enabling the proper folding, the hairpin contributes to the many functional properties of RNA. The hairpin motif is also found in DNA, though to a much lesser extent, due to the intrinsically double-stranded nature of DNA. The requirements for the formation of DNA hairpins[2] and cruciform structures[3] in palindromic DNA sequences have been investigated in detail, and the involvement of such structures in the regulation of gene expression has been discussed.[4,5] Furthermore, the hairpin loop also acts as a site of specific metal coordination.[6–8] The central role of the hairpin as a structural and functional element has stimulated the design and synthesis of chemically modified hairpin analogues. Thus, the hairpin loop has been replaced with flexible oligo(ethylene glycol) linkers in DNA[9] and RNA[10,11] as well as with more rigid aromatic derivatives.[12–16] Furthermore, metal-bridged hairpins have been prepared and investigated for their structural and spectroscopic properties. Lewis et al.[17] reported on Ru^{II}-brigded DNA hairpins and, very recently, Czlapinski and Sheppard described the synthesis of nickel and manganese metallosalen-derived DNA hairpins.[18] In addition to serving as a structural scaffold, ligand-based loop replacements offer the possibility of metal coordination. The importance of metal ions to the structure and function of nucleic acids is well documented.[19–21] In this light, metal-coordinating hairpins are of particular interest. Here, we report the synthesis and properties of a terpyridine-based, metal-coordinating hairpin mimic.

Results and Discussion

Preparation of terpyridine-modified oligonucleotides

The synthesis of the required terpyridine phosphoramidite building block is shown in Scheme 1. Thus, we started from the known 6,6″dibromo-4′-phenyl-2,2′:6′,2″-terpyridine (**1**),[22,23] which was transformed into the symmetrical diol **2** by treatment with an excess of 3-aminopropan-1-ol. Monoprotection with 4,4′-dimethoxytrityl chloride (→**3**) followed by phosphitylation with cyanoethyl-bis-(*N*,*N*-diisopropylamino)phosphine in the presence of *N*,*N*-diisopropylammonium tetrazolide yielded the desired terpyridine building block **4**. Phosphoramidite **4** was subsequently incorporated into the terpyridine-modified, self-complementary oligonucleotide **5** (Scheme 2) by automated solid-phase synthesis. To ensure a high coupling efficiency of the terpyridine building block, a coupling time of 45 min was used for that cycle. Final deprotection was performed with concentrated aqueous ammonia for 40 h at room temperature. Purification involved 20% polyacrylamide gel electrophoresis followed by electroelution. In addition, the terpyridine-modified oligomer **5** was treated with a metal-binding solid support (Chelex®)[24,25] to ensure complete removal of potentially present trace metals (see Experimental Section). Oligodeoxynucleotides **6** and **7**, prepared as reference oligomers, contained an identical stem sequence and either a dA_4 or a dT_4 loop. The self-complementary oligonucleotide **8** served as a control oligonucleotide for an interstrand duplex.

Properties of the terpyridine-modified hairpin

We first investigated the influence of the terpyridine on the stability of the hairpin. Thermal denaturation experiments were carried out at a 100 mM NaCl concentration, pH 7.5 and a 1.5 μM concentration of the self-complementary oligomers **5**–**8** (Figure 1 A). All three hairpin-forming oligomers (**5**–**7**) show transitions at considerably higher temperature than the analogous duplex composed of two nonlinked self-complementary

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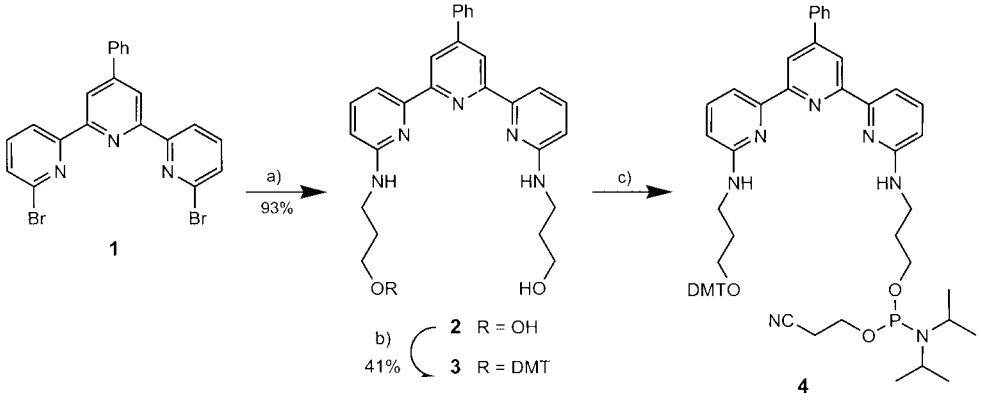
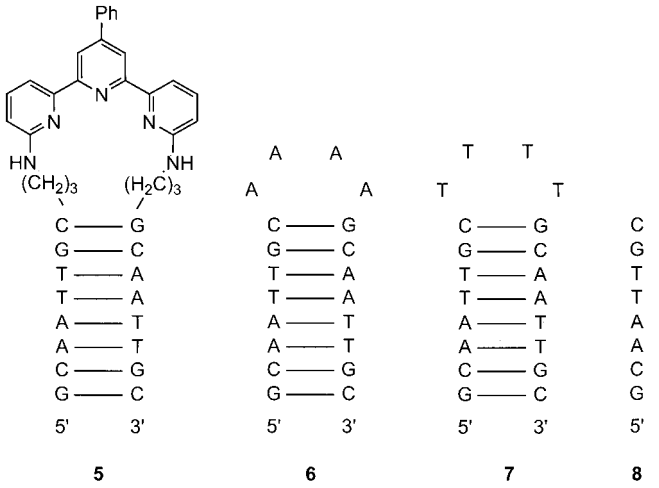


Scheme 1. *a) 3-Aminopropan-1-ol, reflux, 90 min. b) Dimethoxytrityl chloride, Py, 24 h, RT. c) (iPr$_2$N)$_2$P(OCH$_2$CH$_2$CN), N,N-diisopropylammonium 1H-tetrazolide, CH$_2$Cl$_2$, RT, 2 h.*



Scheme 2. *Oligonucleotides used in this study. Terpyridine-modified hairpin **5**, reference oligonucleotides **6** and **7**, containing a dA$_4$ and a dT$_4$ loop, respectively, and a self-complementary, interstrand duplex-forming control oligonucleotide (**8**).*

strands of **8** (Table 1). This finding is consistent with the literature,[2] and the data correspond well with the T_m's calculated for oligomers **6**–**8** (calculated values are 64.8, 70.1 and 27.5 °C, respectively[26]). The terpyridine-modified hairpin (**5**) shows a T_m (83.6 °C) that is approximately 10 °C higher than the T_m's of the corresponding dA$_4$ and dT$_4$ hairpins (**6** and **7**, respectively).

Table 1. *T$_m$-values of the terpyridine hairpin mimics **5** in comparison to those of the reference hairpins **6** and **7** and the control oligonucleotide **8**.*[a]

oligomer	loop	T_m [°C]
5	terpyridine	83.6
6	A_4	73.6
7	T_4	74.6
8	–	29.0

[a] Conditions: 1.5 μM oligonucleotides, 100 mM NaCl, 10 mM NaH_2PO_4, pH 7.5.

Effect of divalent transition metals

The ability of terpyridine to coordinate transition metals is well documented in the literature.[27] Thus, we next investigated the influence of different metals on the stability of the terpyridine-modified hairpin **5**. Initial thermal denaturation experiments showed that Zn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Pd^{2+} had a pronounced influence on the melting curves. No effect was observed with La^{3+} or Eu^{3+}; Pt^{2+} had only a marginal effect. These metals were therefore not further investigated. To ensure complete saturation of the terpyridine, five equivalents of metal were used throughout the studies, although the use of one equivalent of metal resulted in almost the same quantitative effect (see Supporting Information). All curves showed a highly cooperative melting behaviour (Figure 1 B) in the presence of the transition metals. The data are summarised in Table 2. T_m values varied from 58 °C to 64.3 °C in the presence of Zn^{2+}, Co^{2+}, Ni^{2+} and Cu^{2+}. For Pd^{2+} a value of 70.8 °C was observed. The T_m value of the control hairpin **7**, containing a T$_4$-nucleotide loop, was not influenced by any of the investigated metals within the concentration range used in these experiments (see Supporting Information). The observed effect of the metals on the T_m was reversible. This was illustrated by an experiment involving several cycles of metal addition followed by addition of EDTA. Thus, addition of an excess of EDTA to the Zn^{2+}- or Ni^{2+}-containing sample of hairpin **5** completely restored the original T_m value of 83.5 ± 0.5 °C (see illustration in Scheme 3). Alternative rounds of metal and EDTA additions resulted in T_m values alternating between the two values (see Supporting Information).

Table 2. *T$_m$ values of terpyridine hairpin mimics **5** in the presence of different metals (Zn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Pd^{2+}).*[a]

Metal	–	Zn	Co	Ni	Cu	Pd
T_m [°C]	83.6	61.0	58.0	58.5	64.3	70.8
ΔT_m [°C]		−22.6	−25.6	−25.1	−19.3	−12.8

[a] Conditions: 1.5 μM oligonucleotides, 100 mM NaCl, 10 mM NaH_2PO_4, 7.5 μM metal, pH 7.5.

Model of the terpyridine-derived metallohairpin

The circular dichroism (CD) spectra for all four oligomers (shown in Figure 2) are consistent with a B-DNA structure. Furthermore, the T_m values of the hairpin mimic **5** were—in the presence or absence of any of the metals—independent of the

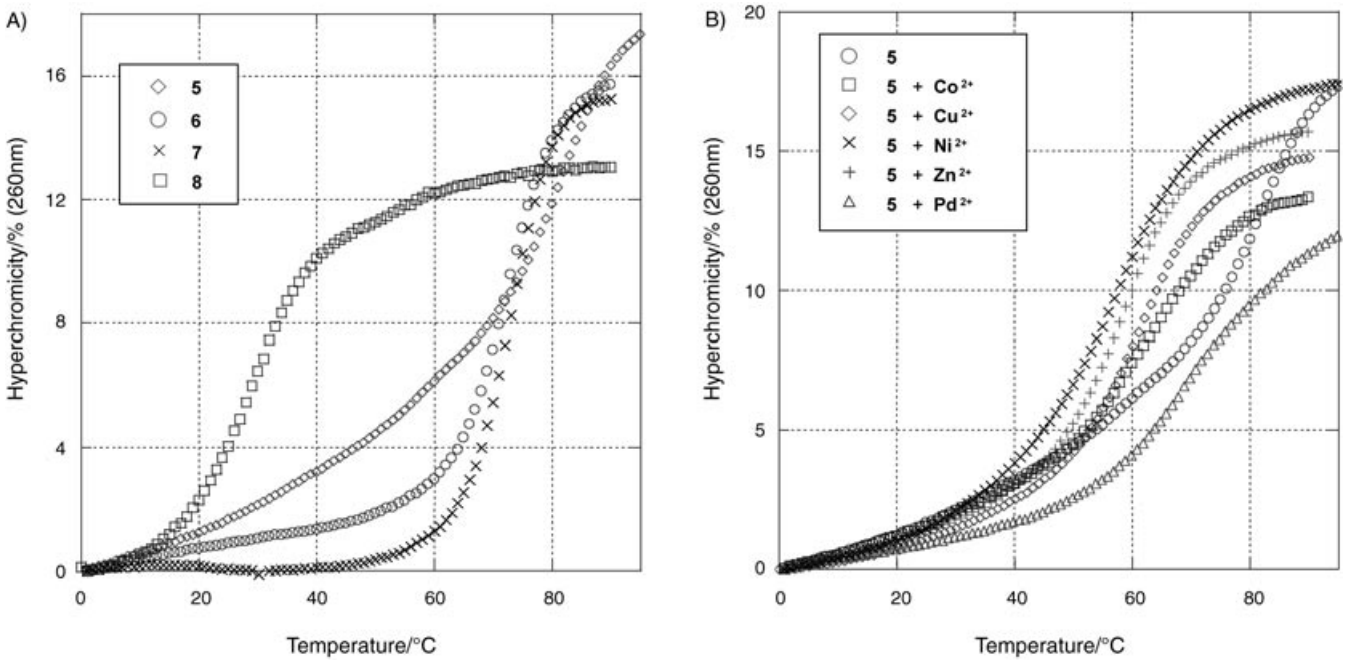
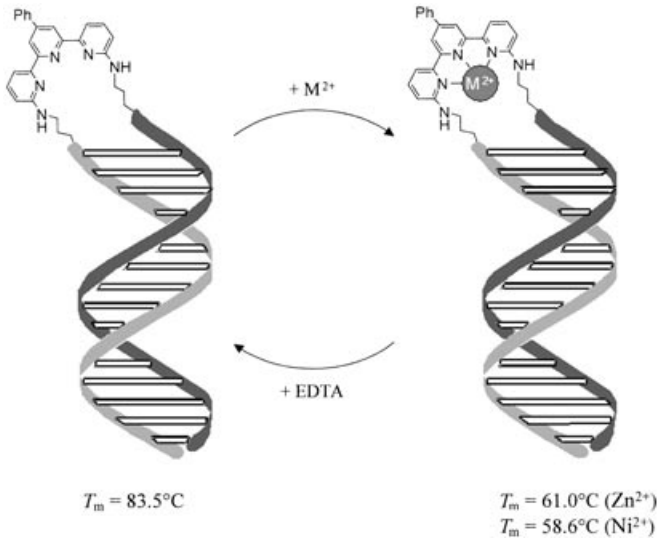


Figure 1. *Thermal denaturation curves. A) Terpyridine hairpin mimic* ***5*** *in comparison with the reference oligonucleotides* ***6***–***8****. B) Terpyridine hairpin mimic* ***5*** *in the presence of 5 equiv of different metals (Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pd^{2+}). Conditions: 1.5 μM oligonucleotides, 100 mM NaCl, 10 mM NaH_2PO_4, 7.5 μM metal, pH 7.5.*



Scheme 3. *Effect on the* T_m *of hairpin* ***5*** *of alternating additions of metal and EDTA.*

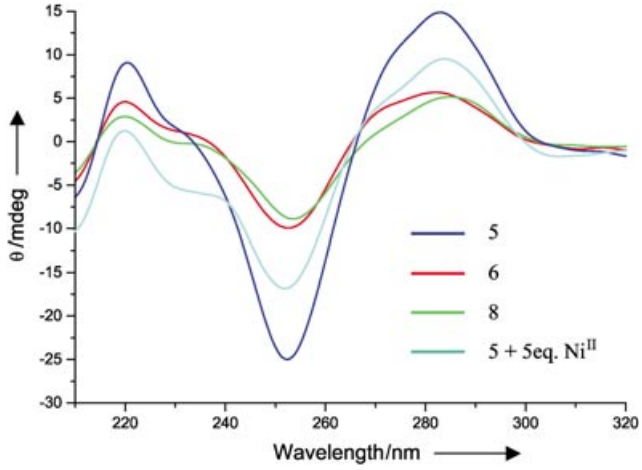


Figure 2. *CD spectra of oligonucleotides* ***5***–***8****. Conditions: 5 μM oligonucleotides; 100 mM NaCl; 10 mM NaH_2PO_4, pH 7.5 at 10 °C.*

concentration of the oligonucleotides over a range from 0.5 to 5 μM; this indicated a monomolecular process (see Supporting Information). It has been shown previously that self-complementary oligonucleotides connected through aromatic linkers form stable, hairpin-like structures. These linkers include *cis*- and *trans*-stilbene[13,28] and phenanthrene derivatives.[16] The unusual thermal stability of these hairpin analogues is due to favourable stacking effects between the aromatic linker and the adjacent DNA base pair. A model of the terpyridine-containing hairpin **5** is shown in Figure 3. The conformation represents a local-minimum structure obtained with *Hyperchem* by starting from B-form DNA and using the *amber force field*. This model suggests, as with the above-mentioned non-natural linkers, a stacking interaction between the terpyridine and the top base pair of the duplex stem. The terpyridine linker adopts a *trans*/*cis* conformation[29] that does not correspond to the preferred *trans*/*trans* conformation normally observed for uncoordinated 2,2′:6′,2″-terpyridines.[30,31] The adoption of this unusual conformation may well be a result of the geometrical constraints given by the sites of attachment to the two nucleotide strands. The observed decrease in the T_m, which results upon addition of a divalent metal, is at least partly rationalised

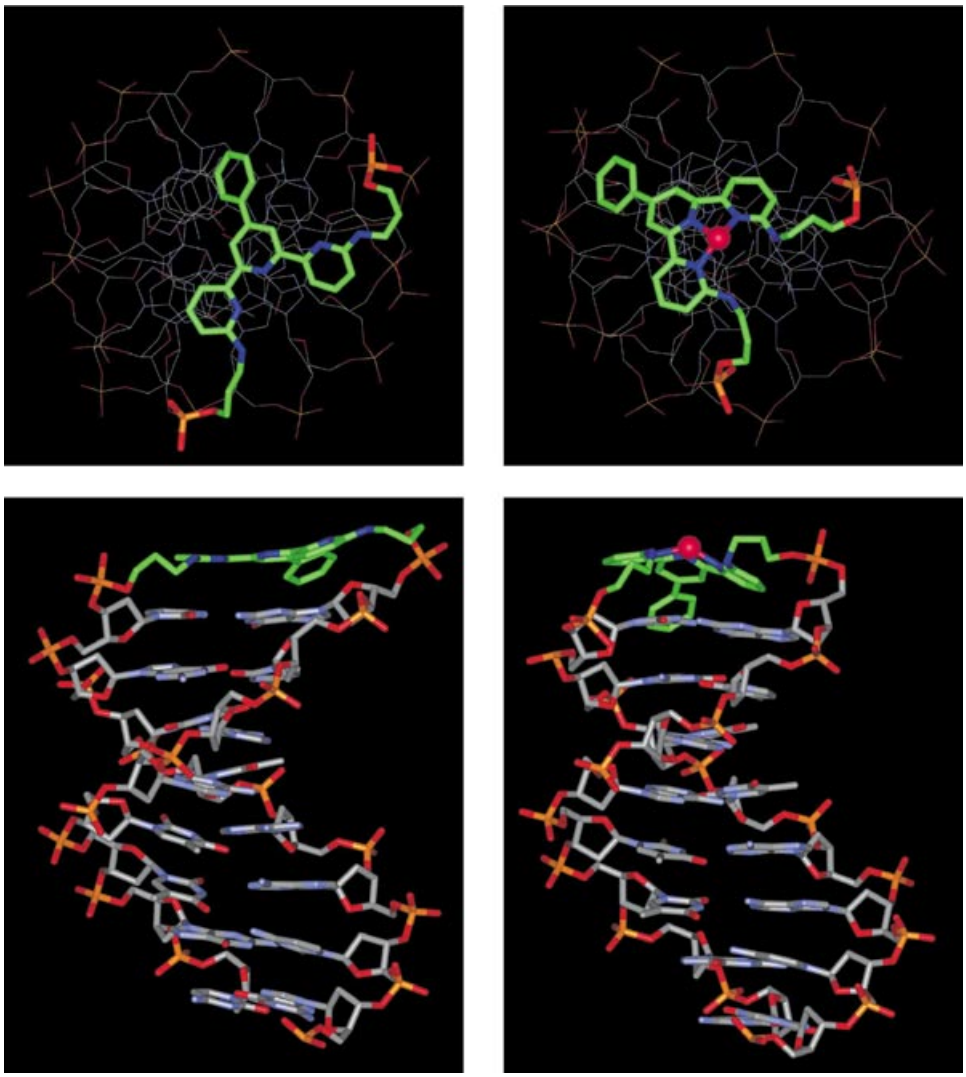


Figure 3. *Amber-minimized structures of hairpin mimic* ***5*** *containing a terpyridine linker (carbon: green, nitrogen: blue); left: no metal; right: in the presence of* Cu^{2+} *(red sphere). Top: top view along to the helical axis. Bottom: view perpendicular to the helical axis.*

in this model by the necessary conformational change (to *cis*/*cis*) of the terpyridine in order to allow coordination of the metal. As can be seen from Figure 3 (top), the coordination of Cu^{2+} requires rotation around the C2–C2′ bond. It is likely that this rotation, along with further conformational changes in the linker, leads to a less-favourable stacking between the terpyridine and the adjacent base pair. Of course, additional (electrostatic) effects of the metal itself cannot be ruled out and might well play a significant role. A direct influence by the metal is supported by the observation that different metals have a different effect on the stability of the hairpin.

Conclusion

A self-complementary oligodeoxynucleotide containing a 6,6″-linked terpyridine adopts a highly stable, hairpin-like structure. The thermodynamic stability of this hairpin mimic is considerably higher than the one of analogous hairpins containing a dT_4 or dA_4 loop ($\Delta T_m = 9\,°C$ or $10\,°C$, respectively). The remarkable stability is most likely a result of a favourable stacking interactions between the terpyridine and the adjacent base pair. In addition, the terpyridine can act as a coordination site for divalent metals. Several metals (Zn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Pd^{2+}) were found to bind to the hairpin. Metal coordination to the hairpin can be reversed by addition of EDTA. The stability of the metal-coordinated hairpin mimic was found to be lower than in the absence of metal. Furthermore, the T_m is strongly influenced by the type of the bound metal, increasing in the order $Co^{2+} \sim Ni^{2+} < Zn^{2+} < Cu^{2+} < Pd^{2+}$.

Experimental Section

General: 6,6″Dibromo-4′-phenyl-2,2″;6′,2″-terpyridine (**1**),[32] and (cyanoethoxy)bis(*N*,*N*-diisopropylamino)phosphine[33] were prepared as described in the literature. If not indicated otherwise, chemicals and solvents for reactions were purchased from Fluka, Acros or Aldrich and were used without further purification. TLC was performed on silica gel SIL G-25 UV_{254} glass plates (Macherey–Nagel), followed by UV detection and/or dipping in a solution of 5% phosphomolybdic acid hydrate in EtOH, followed by heating. Flash column chromatography was performed on silica gel 60 (63–32 μM, Chemie Brunschwig AG). When the compound was acid-sensitive, the silica was pretreated with a solvent containing 2% Et_3N. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC-300 with solvent signals as internal references; ^{31}P NMR spectra were recorded on a Bruker AMX 400 with 85% H_3PO_4 as external reference. ESI-MS was performed on a VG Platform single quadrupole ESI mass spectrometer.

Synthesis of terpyridine-derived phosphoramidite building block

*3-[6″-(3-hydroxy-propylamino)-4′-phenyl-[2,2′:6′,2″]terpyridin-6-ylamino]-propan-1-ol (**2**).* A mixture of **1** (1.5 g, 3.2 mmol) and 3-aminopropanol (20 mL, 26 mmol) was heated under reflux for 90 min, after which the reaction mixture was concentrated under vacuum. The residue was taken up in HCl (2 M) and extracted three times with EtOAc. The organic phase was dried over Na_2SO_4 and evaporated to yield **2** as a yellow solid (1.31 g, 90%). TLC (EtOAc): $R_f = 0.16$; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.83$ (m, $J = 5.84$ Hz, 4H), 3.70 (m, 8H), 4.73 (s, 2H, amine), 6.44 (d, $J = 8.1$ Hz, 2H), 7.46 (m, 1H), 7.54 (m, 4H), 7.85 (t, $J = 0.025$ Hz, 4H), 8.43 ppm (s, 2H); ^{13}C NMR

(300 MHz, $CDCl_3$): 33.07, 38.28, 59.48, 108.42, 110.91, 118.64, 127.41, 128.80, 129.03, 138.48, 150.25, 158.47 ppm; ESI-MS (positive mode): $m/z = 455.26$ (calcd: 455.23).

*3-(6″-3(3-(Bis-(4-methoxyphenyl)phenylmethoxy)propylamino)-4′-phenyl-(2,2′;6′,2″)terpyridin-6-ylamino)-propan-1-ol (**3**)*: Compound **2** (685 mg, 1.5 mmol) was dissolved in dry pyridine (5 mL) and concentrated (twice) to remove any traces of water. The residue was then dissolved in dry pyridine (15 mL) under a nitrogen atmosphere. 4,4′-Dimethoxytrityl chloride (610 mg, 1.8 mmol) was added, and the mixture was stirred at RT for 24 h. After this time, methanol (750 μL) was added, and the mixture was stirred for another 5 min. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2 and washed three times with sat. aqueous $NaHCO_3$ and water. The organic phase was dried over $MgSO_4$, and the solvent was evaporated. The residue was purified by flash chromatography (silica gel, hexane/EtOAc 1:2 + 2% Et_3N) to afford **3** (462 mg, 41%) as a slightly yellow solid. TLC (EtOAc): $R_f = 0.38$; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.88$ (m, 2H), 1.99 (m, 2H), 3.30 (t, $J = 5.7$ Hz, 2H), 3.55 (q, $J = 6.25$ Hz, 2H), 3.70 (m, 3H), 3.75 (s, 8H), 6.45 (d, $J =$ 8.45, H), 6.55 (d, $J = 8.09$ Hz, 2H), 6.81 (d, $J = 8.82$ Hz, 4H), 7.20 (d, $J = 7.35$ Hz, 1H), 7.33–7.37 (m, 8H), 7.43–7.51 (m, 6H), 7.61 (m, 2H), 7.84 (d, $J = 6.61$ Hz, 2H), 7.91 (m, 2H), 8.46 (d, $J = 1.4$ Hz, 1H), 8.60 ppm (d, $J = 1.47$ Hz, 1H); ^{13}C NMR (300 MHz, CD_3CN): 29.7, 55.1, 59.4, 86.1113.0, 126.7, 127.3, 127.8, 128.1, 128.3, 129.0, 130.0, 136.2, 158.3 ppm; ESI-MS (positive mode): $m/z = 758.19$ (calcd: 758.36).

*Isopropylphosphoramidous acid 3-(6″-(3-(bis-(4-methoxyphenyl)phenylmethoxy)-propylamino)-4′-phenyl-(2,2′:6′,2″)terpyridin-6-ylamino)-propyl ester 2-cyanoethyl ester (**4**)*: Diisopropylammonium cyanoethoxyphosphine (125 μL, 0.40 mmol) was added to a mixture of **3** (250 mg, 0.33 mmol) and diisopropylammonium tetrazolide (56.3 mg, 0.33 mmol) in dry CH_2Cl_2 (10 ml) under a nitrogen atmosphere. The mixture was stirred at RT for 2 h. Evaporation of the solvent gave a slight yellow foam, which was purified by flash chromatography (hexane/EtOAc 2:1 + 2% Et_3N) to afford **4** (266 mg, 84%) as a yellow foam. TLC (hexane/EtOAc 2:1): $R_f = 0.48$; ^{1}H NMR (300 MHz, CD_3CN): $\delta = 1.03$ (t, $J = 6.15$ Hz, 12H), 1.11 (m, 5H), 2.50 (t, $J = 5.9$ Hz, 2H), 3.09 (t, $J = 5.85$ Hz, 2H), 3.48 (m, 6H), 3.59 (s, 6H), 3.68 (m, 4H), 3.98 (q, $J = 7.3$ Hz, 2H), 5.18 (t, $J = 5.3$ Hz, H), 5.30 (t, $J = 5$ Hz, H), 6.4 (d, $J = 8$ Hz, H), 6.46 (d, $J = 8.3$ Hz, H), 6.66 (d, $J =$ 8.3 Hz, 4H), 7.05 (m, H), 7.14 (m, 6H), 7.33 (m, 5H), 7.5 (q, $J =$ 4.8 Hz, 2H), 7.7 (s, 2H), 7.8 (d, $J = 7$ Hz, 2H), 8.5 ppm (d, $J = 13.6$ Hz, 2H); ^{13}C NMR (300 MHz, CD_3CN): 20.3, 24.5, 24.6, 29.7, 39.6, 40.1, 42.9, 43.1, 61.6, 61.9, 86.1, 113.0, 118.3, 126.7, 127.2, 127.8, 128.1, 128.6, 128.8: 130.0, 136.3, 138.1, 139.3, 145.1, 149.6, 154.5, 145.6, 156.2, 158.3 ppm; ^{31}P NMR (161.9 MHz, CD_3CN): 148.22 ppm; ESI-MS (positive mode): $m/z = 958.42$ (calcd: 958.47).

Synthesis and purification of oligonucleotides: Oligonucleotides were synthesized on a 392 DNA/RNA Synthesizer (Applied Biosystems) according to phosphoramidite chemistry.[34,35] The nucleoside phosphoramidites were from ChemGenes (Ashland, MA). The standard synthetic procedure ("trityl-off" mode) was used to synthesize the oligonucleotides. For the non-natural phosphoramidite **4**, the coupling time was increased to 45 min. Detachment from the solid support and final deprotection was achieved by treatment with 30% ammonium hydroxide for 40 h at room temperature. The crude oligonucleotides were purified by PAGE (20% polyacrylamide, denaturing conditions) and recovered by electroelution (1× TBE buffer, 100 W, 4 V, 80 mA, 90 min) by using equipment from Pharmacia Biotech AB (Sweden). The unmodified oligonucleotides were purified by reverse-phase HPLC (X Terra RP_{18} 3.5 μm column from Waters) and desalted over Sep-Pak cartridges (Waters, Milford, USA). The masses of the purified oligonucleotides were determined by electrospray mass spectroscopy: VG Platform single quadrupole ESI-MS.

Thermal denaturation experiments: Prior to all experiments, the oligonucleotides were treated with a chelating agent (Chelex-100 resin, from Sigma) in order to avoid any trace-metal contamination.[24] If not indicated otherwise, all experiments were carried out under the following conditions: oligonucleotide concentration 1.5 μM; 10 mM phosphate buffer, pH 7.5, 23 °C; 100 mM NaCl. For experiments with metals, stock solutions (100 μM) of the following metals were used: $Zn^{II}Cl_2$, $Co^{II}Cl_2{\cdot}6H_2O$, $Ni^{II}Cl_2{\cdot}6H_2O$, $Cu^{II}Cl_2{\cdot}2H_2O$, $Pd^{II}Cl_2$.

UV Melting Curves: A Varian Cary 3e UV/Vis spectrophotometer equipped with a Peltier block temperature-controller and Varian WinUV software were utilized to determined the melting curves at 260 nm, a heating–cooling–heating cycle in the temperature range of 0–90 °C or 20–90 °C was applied with a temperature gradient of 0.5° min^{-1}. To avoid H_2O condensation on the UV cells at temperatures above 20 °C, the cell compartment was flushed with N_2. Data were collected and analysed with Kaleidagraph® software from Synergy Software. T_m values were determined as the maximum of the first derivative of the melting curve.

T_m calculations: Calculated T_m values were obtained by using the program RNA mfold[26] (http://www.bioinfo.rpi.edu/applications/mfold/).

Molecular modelling: The calculations were performed with Hyperchem® (Release 7) from Hypercube, Inc., Waterloo, Ontario. Oligonucleotide duplexes were generated with the parameters for B-DNA. The terpyridine moiety was premodelled and built into a standard B-DNA duplex. The whole construct was then minimized in the presence of water molecules (periodic boundary conditions) by using the Polak–Ribiere algorithm and 0.1 kcal mol^{-1} as RMS gradient. A dielectric constant of 4 was used. Both the electrostatic and van der Waals 1–4 scale factors were set to 0.5. The structure was calculated with the Amber force field. The minimized structures were warmed from 100 to 300 K within 0.1 ps.

CD Spectra: A Jasco J-715 spectropolarimeter with a 150 W Xe high-pressure lamp was used. A Jasco PDF-350S-Peltier unit, coupled with a Colora K5 ultrathermostat, controlled the temperature of the cell holder. The temperature was determined directly in the sample.

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Keywords: complexes · DNA · hairpin mimics · oligonucleotides · terpyridine · transition metals

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