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Synthesis and spectroscopy of Ru(II)-bridged DNA hairpins

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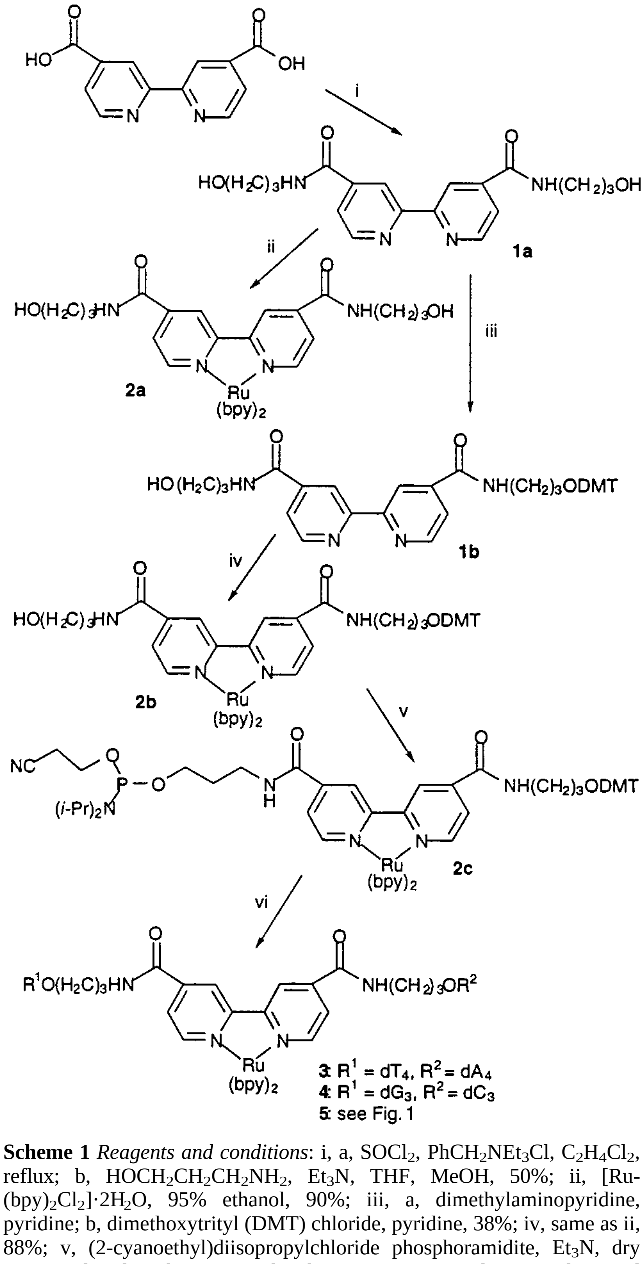
A difunctional Ru(II) complex has been prepared which can be incorporated into synthetic oligonucleotides by means of standard solid-phase methodology and the properties of several oligonucleotide conjugates capable of forming Ru(II)-bridged hairpins have been investigated.

There is continuing interest in the development of oligonucleotide conjugates containing luminescent or redox-active transition metal complexes.[1] These conjugates have been investigated as alternatives to the use of radioactive isotopes for gene sensing, as site specific photonucleases, and as luminescent probes for the study of energy transfer and electron transfer in duplex DNA. In most of the conjugates investigated to date the metal complex is attached post-synthetically to the 5′-end of an oligonucleotide by means of a flexible tether.[2] Flexible tethers[3] and rigid linkers[4] with metal-binding ligands have also been used to modify nucleosides which can be incorporated using standard solid-phase methodology at a specific position in a synthetic oligonucleotide. Hybridization of conjugates with flexible tethers results in the formation of duplexes in which the metal complex may be intercalated, groove bound, or only weakly associated with the duplex. Uncertainty about the location of the metal complex can complicate the interpretation of electron and energy transfer measurements. Our interest in photoinduced electron transfer in duplex DNA[5] led us to undertake the synthesis of oligonucleotide conjugates in which a difunctional metal complex forms a linker connecting two oligonucleotides. We report here the synthesis and spectral properties of the first Ru(II)-linked oligonucleotides which possess complementary sequences capable of forming hairpin structures.

Preparation of synthetic oligonucleotides with a linking metal complex by standard solid state synthesis required the development of a difunctional ligand in which one functional group can be protected and the other activated. Our solution to this problem is outlined in Scheme 1. The difunctional diamidobipyridine ligand dabp (**1a**[‡]) was prepared from 2,2′-bipyridine-4,4′-dicarboxylic acid following the method developed for the synthesis of analogous arenedicarboxamides.[6] Reaction of this ligand with $[Ru(bpy)_2Cl_2]\cdot 2H_2O$[7] afforded the complex $[Ru(bpy)_2(dabp)]^{2+}$ **2a**, isolated as the PF_6^- salt.[8] Synthesis of the mono-protected complex $[Ru(bpy)_2(DMT\text{-}dabp)][PF_6]_2$ **2b** was best accomplished *via* reaction of the monoprotected dabp ligand **1b** with $[Ru(bpy)_2Cl_2]\cdot 2H_2O$. Repeated efforts to convert **2b** to **2c** using standard conditions led to recovery of **2b**. We found that **2c** is moderately stable when prepared in a glove box, but is rapidly hydrolyzed to **2b** upon exposure to moisture.

The instability of **2c** necessitated a modified protocol for oligonucleotide synthesis. The 3′-segment of the oligonucleotide was prepared according to standard solid phase procedures.[9] After removal of its 5′-DMT protecting group, the column was brought into a dry box and the oligonucleotide coupled with **2c**, using tetrazole as the activator. The column was returned to the automatic synthesizer to complete the synthesis of the oligonucleotide. The coupling efficiency for Ru incorporation, as determined by monitoring release of the dimethoxytrityl cation, was > 95%. Obtained by this procedure were the Ru-linked oligonucleotides $dT_4\text{–}Ru(bpy)_2(dabp)^{2+}\text{–}dA_4$, $dG_3\text{–}Ru(bpy)_2(dabp)^{2+}\text{–}dC_3$, and $dGCAATTGC\text{–}Ru(bpy)_2(dabp)^{2+}\text{–}dGCAATTGC$, **3**–**5**.[10] After removal from the solid support and deprotection the oligonucleotides were purified by HPLC and appear as a single peak on both ion exchange and reverse phase HPLC.[6]

The absorption spectrum of $[Ru(bpy)_2(dabp)]^{2+}$ **2a** displays maxima at 468 nm (ε = 8538 $dm^3\ mol^{-1}\ cm^{-1}$) and 288



Scheme 1 *Reagents and conditions*: i, a, $SOCl_2$, $PhCH_2NEt_3Cl$, $C_2H_4Cl_2$, reflux; b, $HOCH_2CH_2CH_2NH_2$, Et_3N, THF, MeOH, 50%; ii, $[Ru(bpy)_2Cl_2]\cdot 2H_2O$, 95% ethanol, 90%; iii, a, dimethylaminopyridine, pyridine; b, dimethoxytrityl (DMT) chloride, pyridine, 38%; iv, same as ii, 88%; v, (2-cyanoethyl)diisopropylchloride phosphoramidite, Et_3N, dry acetonitrile, glove box, not isolated; vi, see text, Expidite™ nucleic acid synthesis system.

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(43 550) which are red-shifted with respect to those of $[Ru(bpy)_3]^{2+}$, as previously observed for Ru(II) complexes with a single bipyridine-4,4′-dicarboxamide ligand.[11] The spectra of **3**–**5** have maxima below 300 nm for the nucleobases which overlap the shorter wavelength Ru^{2+} band. The 260 nm bands of **3**–**5**, but not the 468 nm bands exhibit hypochromism. The complex **2a** and conjugates **3**–**5** display a single broad emission band with a maximum at 665 nm, similar to that reported for other Ru(II) complexes possessing a single bipyridine-4,4′-dicarboxamide ligand.[11] The luminescence quantum yields and decay times are summarized in Table 1. The neighboring nucleobases have little effect on the photophysical behavior of the Ru(II) complex, in accord with previous observations for $[Ru(bpy)_3]^{2+}$ covalently attached to oligonucleotides.[3]

Table 1 Emission lifetimes and quantum yields for complexes **2a**, **3**, **4** and **5**

Complex	τ/ns	Φ_e[a]
2a	850	0.013
3	815	0.018
4	790	0.016
5	608	0.019

[a] Values are reported relative to $[Ru(bpy)_3][PF_6]_2$ in water (Φ_e = 0.042) and calculated according to published procedures (J. N. Demas and G. A. Crosby, *J. Phys. Chem.*, 1971, **75**, 991).

Molecular modeling indicates that the conjugates **3**–**5** can adopt low energy hairpin conformations as shown schematically in Fig. 1; however, these conjugates also might form duplexes in which the Ru(II) complexes occupy bulges on opposite strands. In the case of **5** a value of T_M = 50 °C is obtained from the thermal dissociation profile in 0.1 M NaCl. This value is independent of concentration (1.0–5.0 μM). In the case of **4** the observed value of T_M = 50 °C in 1.0 M NaCl is higher than that calculated for either a GGG/CCC duplex [T_M(calc.) = −42 °C] or for two such duplex segments [T_M(calc.) = 20 °C] with no contribution from the Ru(II) linkers and no cooperativity in melting of the two segments.[12] This evidence supports the tentative assignment of a hairpin *vs.* duplex structures for **4** and **5**. The conjugates **3** and **4** have broad thermal dissociation profiles (not shown) and values of T_M (< 20 and 50 °C, respectively, in 1.0 M NaCl) lower than those of the analogous stilbene dicarboxamide-bridged hairpins (T_M = 49 and > 80 °C, respectively, in 1.0 M NaCl).[7] This may reflect a better fit for the stilbene *vs.* Ru(II) linker across the double helix. The broad thermal dissociation profiles for **3**–**5** may reflect multiple conformations for the hairpin loop region as well as the presence of two diastereomeric octahedral complexes.

The preliminary results reported here provide a potentially versatile method for the introduction of a bipyridyl-complexed metal ion at a specific location in a synthetic conjugate. The three conjugates prepared in this study have complementary arms and thus are capable of forming hairpin structures with a bridging metal complex. Whereas the precise structures of these conjugates remain to be established, the metal complex is likely located at the end of a duplex region of a hairpin structure. The excited Ru(II) complex selected for this study is not quenched by nucleobases, however excited state redox potentials can be tuned over a wide range by variation of the nonbridging ligands or metal.[13] Similarly, the use of nucleobase analogs such as 6-oxoguanine or 7-deazaguanine which have low oxidation potentials should increase the driving force for photoinduced electron transfer.[14] In addition, hybridization of metal linked conjugates possessing noncomplementary arms with unlabelled oligonucleotides should position the metal center near a specific location in the unlabelled strand. Thus the availability of difunctional metal complexes which can be introduced into oligonucleotides *via* automated phosphoramidite chemistry serves to extend structural diversity currently available with monofunctional metal complexes.[2–4] The long lifetimes and moderately large fluorescence quantum yields make these Ru(II) complexes particularly well-suited for studies of long range energy and electron transfer.

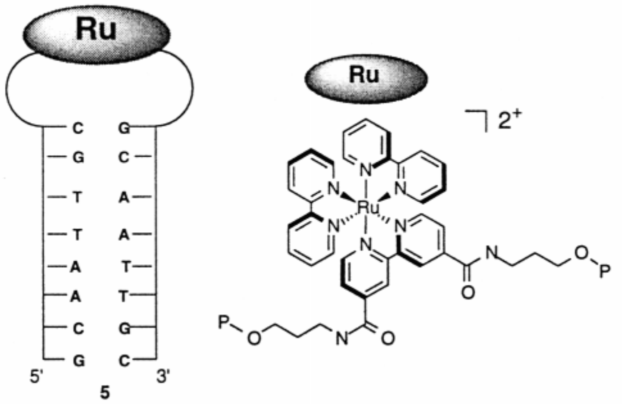

Fig. 1 Schematic structure for the hairpin conformation of **5**.

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Notes and references

‡ *Selected data* for **1a**: ES/MS (DMSO–H_2O): *m/z* 359 NMR (DMSO-d_6, ^1H): δ 8.95 (t, 2H), 8.85 (d, 2H), 8.75 (s, 2H), 7.8 (d, 2H), 4.5 (t, 2H), 3.5 (t, 4H), 3.3 (t, 4H), 1.7 (q, 4H). For **1b**: NMR (DMSO-d_6, ^1H): δ 9.9 (t, 2H), 9.85 (d, 2H), 9.75 (d, 2H); 7.8 (dd, 2H), 7.35 (d, 2H), 7.2 (m, 5H), 6.8 (d, 4H), 3.65 (s, 6H), 3.4 (m, 8H), 1.85 (q, 2H), 1.7 (q, 2H). For **2a**: ES/MS (MeCN): *m/z* 917 ($[Ru(bpy)_2dabp][PF_6]^+$), 386 ($[Ru(bpy)_2dabp]^{2+}$). UV–VIS (MeCN): 468 nm (ε = 8538 $dm^3\ mol^{-1}\ cm^{-1}$); 350 (6847), 288 (43550), 236 (37107). For **2b**: ES/MS (MeCN): *m/z* 1219 ($[Ru(bpy)_2(DMT\text{-}dabp)][PF_6]^+$); 537 ($[Ru(bpy)_2(DMT\text{-}dabp)]^{2+}$). UV–VIS (MeCN): 468, 350, 288, 236 nm. For **2c**: NMR (MeCN, ^{31}P): δ +140 (two singlets).

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