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Rigid 5′-6-locked phenanthroline-derived nucleosides chelated to ruthenium and europium ions

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ABSTRACT

We describe complexes of ruthenium and europium with rigid, 5′-6-locked 1,10-phenanthroline-containing nucleosides. Both nucleosides were synthesized from condensation of 5-amino-2′-deoxycytidine with the corresponding diketone. The ruthenium nucleoside displayed fluorescence characteristic of polypyridine ruthenium complexes with a maximum at 616 nm and a quantum yield of 0.011. Binding of europium to the 1,10-phenanthroline-2,9-diacid moiety of the lanthanide binding nucleoside showed formation of a 1:1 complex with emission at 570–630 nm, whose emission was enhanced by addition of two phenanthroline ligands. The lanthanide-binding nucleoside was incorporated into DNA oligonucleotides and shown to selectively bind one equivalent of europium ions.

Complexes of metal ions with organic ligands are of interest as site-specific probes in biophysical studies of nucleic acids.[1] Transition metal complexes that contain polypyridine ligands are among the most extensively applied for such studies due to their interesting electrochemical and photophysical properties.[2,3] Tor and coworkers have, for example, reported the use of DNA hairpins labeled with polypyridine complexes of ruthenium and osmium as hybridization probes[4] and studied energy transfer in DNA containing a Ru(II)–Os(II) dyad.[5] Osmium complexes have also been applied in redox labeling of DNA and for DNA minisequencing.[6] Covalently attached ruthenium complexes have also been used as photocatalysts in nucleic acid-templated reductive uncaging of fluorophores.[7]

Another class of interesting metal ion complexes are those containing lanthanides. Certain lanthanide ions, such as Ln^{3+}, Eu^{3+} and Tb^{3+}, exhibit luminescence at high wavelengths as well as large Stokes shifts and have been applied as luminescent probes in nucleic acid studies.[8–11] Moreover, lanthanide ions are paramagnetic and can, therefore, act both as partial-alignment agents for residual dipolar coupling experiments and as pseudocontact-shift agents in NMR studies of biomolecules.[12] These advantageous magnetic properties, as well as using bound lanthanide ions for phasing in X-ray crystallography,[13,14] have mainly been applied to proteins.[11] However, with proper anchoring of the lanthanide ions, these techniques should be applicable for nucleic acid research.

The method of attachment between the metal complex and the biomolecule to be studied is a critical component of the experimental design when using metal ions for biophysical studies. If the complex is attached through a flexible linker, movement of the probe independent of the biomolecule to which it is attached can influence the outcome of the experiment, for example decrease accuracy in distance-based measurements. The method of attaching the metal complex through an alkyne linker to the nucleoside base was a substantial improvement that reduced the independent movement of the metal to a large extent and has become the prevalent method for linking complexes to nucleic acids.[10,15–17] However, rotation of the complex is still possible around the single-bonds flanking the triple-bond.

With the aim of minimizing movement of the metal ions attached to nucleic acids, we have previously reported the synthesis of a rigid 1,10-phenanthroline-containing 5′-6-locked nucleoside **1** (Fig. 1), in which the metal–ion ligand is fused to the nucleobase through a pyrazine ring.[18] Thus, a metal ion that would chelate to the phenanthroline moiety is not able to move relative to the nucleic acid. Nucleoside **1** is a T-analog, forming a base-pair with A, and was readily accommodated at the end of DNA duplexes.[18] Here, we report the synthesis and preliminary characterization of two derivatives of **1**, the ruthenium complex **2** (Fig. 1) and the europium complex **3** (Fig. 1) and incorporation of the latter into DNA oligonucleotides.

The initial synthetic approach to obtain complex **2** was to directly insert nucleoside **1** as a ligand by a reaction with a ruthenium diphenanthroline complex, similar to what was reported for the synthesis of 1,10-phenanthroline-5,6-dione containing

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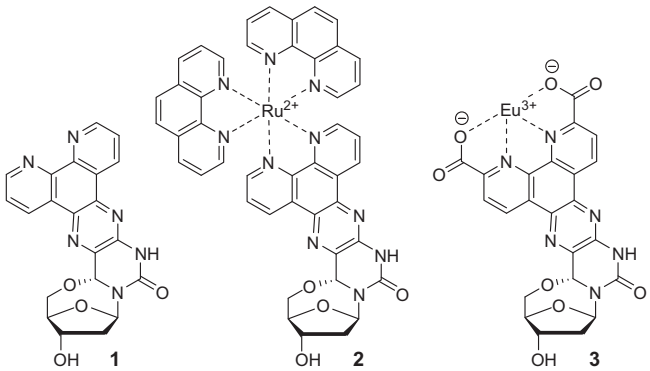
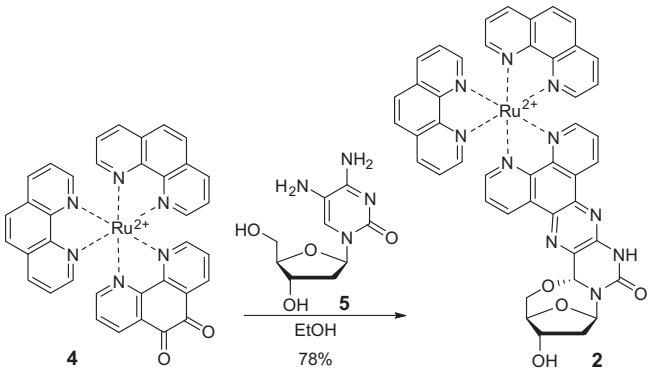


Figure 1. Structures of 5′-6-locked nucleoside **1** and its derivatives **2** and **3**.



Scheme 1. Synthesis of nucleoside **2**, isolated as its $(PF_6)_2$-salt.

complex **4** (Scheme 1).[19] However, this approach gave **2** in very low yields in spite of several attempts, possibly due to limited solubility of **1** under the condition of complex formation.[19] Instead, complex **4** was synthesized and subsequently coupled to 5-amino-2′-deoxycytidine (**5**) by heating in ethanol,[18] yielding nucleoside **2** in good yields**,** as a mixture of the Δ and Λ diastereomers. A UV–Vis spectrum of the nucleoside in water showed an intense π–π^* band at appr. 260 nm and three weaker bands at higher wavelengths (Fig. 2, appr. 360, 420 (shoulder) and 450 nm). Excitation at the metal-to-ligand charge-transfer (MLCT) band at 450 nm (ε = 10,369) lead to a characteristic ruthenium fluorescence at 616 nm (Fig. 2) with a quantum yield of Φ_F = 0.011.

For lanthanide ion chelation, we chose 1,10-phenanthrolin-2,9-diacid as a ligand (**3**), but its derivatives are well known chelators/sensitizers for lanthanide ions.[20–23] With the eventual aim of incorporating the 1,10-phenanthroline-2,9-diacid-derived nucleoside

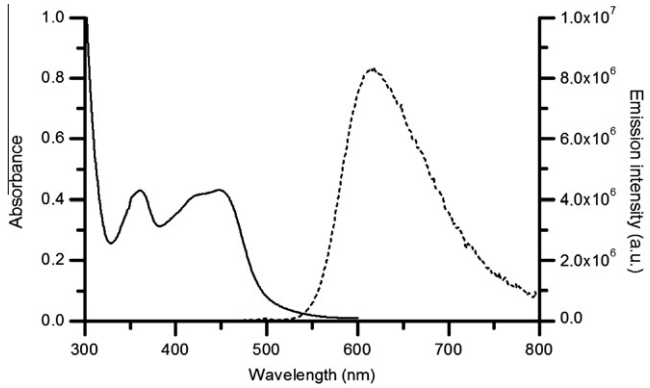
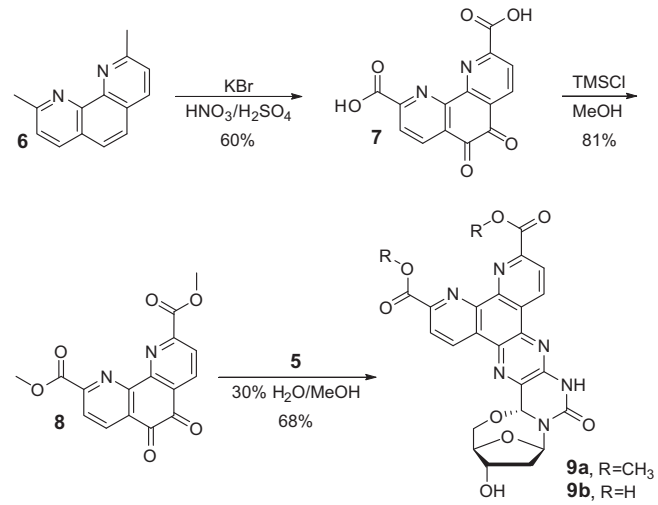


Figure 2. UV–Vis (solid) and fluorescence (dotted) spectra of nucleoside **2**.



Scheme 2. Synthesis of nucleoside **9**.

into oligonucleotides using automated oligonucleotide synthesis, the two carboxylic acid groups were protected as methyl esters, since they can be hydrolyzed in situ for metal ion binding studies.[10] The synthesis of the protected nucleoside **9** is shown in Scheme 2. Initially, the methyl groups of neocuproine (**6**) were trichlorinated using N-chlorosuccinimide, or oxidized to aldehyde groups with selenium dioxide, prior to oxidizing the 5,6-double bond of the phenanthroline. However, we discovered that a solution of potassium bromide in a mixture of concd nitric- and sulfuric acids effected oxidation of both the methyl groups to carboxylates and the 5,6-double bond, directly yielding compound **7** in moderate yield (Scheme 2). Initial attempts to esterify compound **7** in methanol under acidic conditions, resulted in simultaneous formation of a dimethyl acetal at one of the ketone groups. However, reaction of compound **7** with trimethylsilyl chloride in methanol[24] selectively methylated the carboxylic acid groups, yielding compound **8**, which was subsequently coupled to **5** to give the desired nucleoside **9a** in good yield.

For studying lanthanide binding, the protected nucleoside **9a** was first deprotected in situ with aqueous sodium hydroxide, yielding a solution of **9b** in water. Eu^{3+} ions were added to this solution to form **3**, but europium is one of the most commonly used of the emissive lanthanides. The formation of **3** was conveniently monitored by fluorescence, since binding of lanthanides to 1,10-phenanthroline-2,9-diacid derivatives results in almost complete quenching of the phenanthroline fluorescence, along

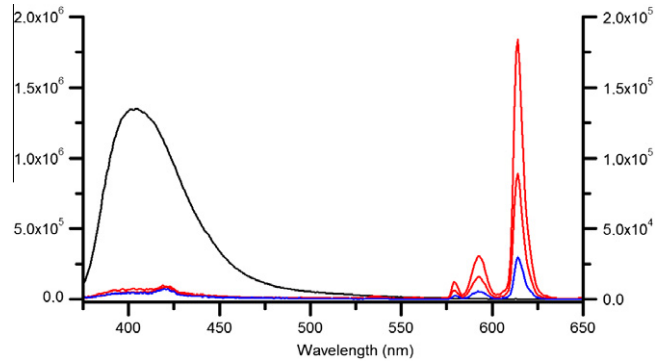


Figure 3. Fluorescence spectra of nucleoside **9b** (black), **3** (a 1:1 [Eu:**9b**] complex, blue) and solutions of **3** with 1 and 2 equiv of 1,10-phenanthroline (red). The intensity of the Eu^{3+} emissions are shown on the right *y*-axis.

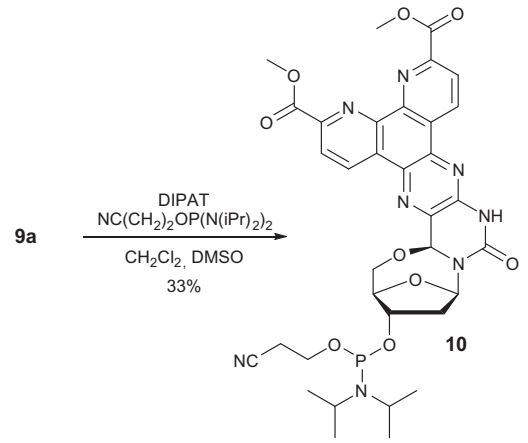
with a concomitant appearance of lanthanide emission at a higher wavelength.[21,22] Indeed, Figure 3 shows that **3** (1:1 solution of Eu^{3+} and **9b**) has negligible fluorescence around 400 nm where free **9b** fluoresces. Furthermore, fluorescence peaks for **3** appeared at 570–630 nm, resulting from complexed Eu^{3+}, which is not luminescent in the absence of a sensitizing ligand. The fluorescence pattern of **3** is characteristic for 1:1 europium–sensitizer complexes.[21] The combined results indicate quantitative formation of **3**.

The weak emission of complex **3** is due to water molecules coordinated to the metal ion.[21,22] This causes the excited complex to relax to the ground state through vibration of the O–H bonds. Therefore, additional ligands that replace water molecules in the coordination sphere of the lanthanide increase the quantum yield of such complexes.[21] In an attempt to increase the fluorescence of **3,** we applied 1,10-phenanthroline as a shielding ligand. When titrating phenanthroline into a solution of **3**, the fluorescence increased until two equivalents of phenanthroline had been added. Figure 3 shows the spectra for addition of 1 equiv and 2 equiv; further addition of up to 10 equiv of phenanthroline showed no additional increase (data not shown). This data indicates that the 1:2 [**3**:phenanthroline] complex is readily formed in solution, where the phenanthroline replaces water molecules and thereby increases the fluorescence. The results also illustrate the difference in binding strength between the 1,10-phenanthroline-2,9-diacid moiety and phenanthroline. Even with a 10-fold excess of phenanthroline present, no increase was observed in the fluorescence of free **9b**, showing clearly that phenanthroline does not replace **9b** in the complex.

To verify that nucleoside **9b** is able to complex europium after incorporation into oligonucleotides, **9a** was phosphitylated to yield phosphoramidite **10** (Scheme 3). After standard automated DNA oligonucleotide synthesis using **10**, the **9a**-containing oligomers were first treated with NaOH (aq) to hydrolyze the esters, followed by standard deprotection with NH_4OH (Supplementary data).

Binding of europium ions coordinated to a **9b**-containing oligomer was first investigated by a MALDI-ToF experiment, where the mass spectrum of an oligomer containing **9b** was recorded with and without Eu-ions present. The addition of 2 equiv of europium ions nearly quantitatively shifted the peaks of the oligonucleotide by the mass of a single europium ion (Fig. S1), consistent with europium binding to the chelating moiety of **9b** in the oligomer.

The fluorescence spectrum of the oligonucleotide 5′-d(**9b**AC CTC GCA TCG TG) showed a single broad peak with a maximum at ~400 nm (Fig. S2), similar to nucleoside **9b**. For the binding study, europium was titrated into a solution of the oligomer, while monitoring the emission of complexed europium (Figs. S2 and 4).



Scheme 3. Synthesis of phosphoramidite **10**.

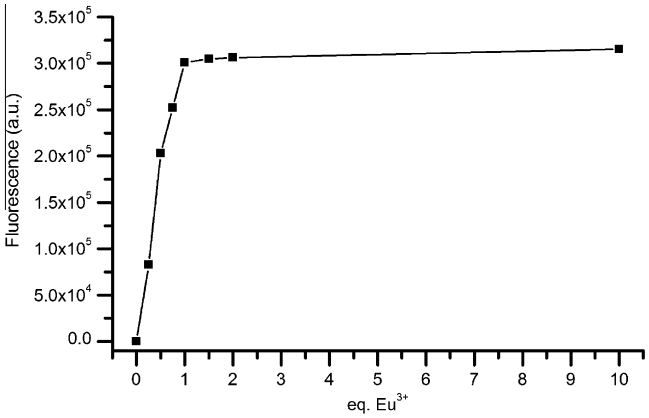


Figure 4. The emission of europium ions bound to 5′-d(**9b**AC CTC GCA TCG TG) plotted against equivalents of added europium ions.

Indeed, europium fluorescence emission emerged and reached a maximum after adding 1 equiv of europium (Fig. 4), while europium emission was not detected when adding europium to an unmodified oligonucleotide (data not shown). Our combined results clearly show that **9b** is able to selectively bind europium after incorporation into oligonucleotides.

In summary, 5′-6-locked nucleosides chelated to both ruthenium and europium ions have been synthesized and are to our knowledge the first examples of rigid metal–ion labels for incorporation into nucleic acids. The two derivatives are readily prepared by condensation of diketones with 5-amino-2′-deoxycytidine and can be incorporated at the 5′-end of oligonucleotides.[18] Nucleoside **9a** was incorporated into oligonucleotides and shown to selectively bind one equivalent of europium. Results obtained with fluorescent 5′-6-locked nucleosides indicate that modified nucleosides, such as **2** and **3**, could also be incorporated at internal nicked sites in duplex DNA (unpublished data). Therefore, 5′-6-locked nucleosides **2** and **3** could be used for a variety of applications in nucleic acids.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.10.104.

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