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Highly emissive, nine-coordinate enantiopure lanthanide complexes incorporating tetraazatriphenylenes as probes for DNA†

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The interaction of $q = 0$ Δ- and Λ-Tb and Eu complexes with poly(dAdT), poly(dGdC) and calf-thymus DNA has been examined by absorption, emission and chiroptical spectroscopy and is sensitive to complex helicity, base-pair type and the nature of the lanthanide excited state.

Several d-block transition metal complexes with well-defined square planar or octahedral geometries have been studied as probes of nucleic acid structure and site-specific recognition.[1] More recently, a new family of nucleic acid probes based on chiral square-antiprismatic complexes of f-block metals has been devised, in which an intercalating moiety, remote from the metal centre, was incorporated as a sensitising group.[2] Herein, we report preliminary binding studies of the polynucleotides poly(dGdC) and poly(dAdT) with new cationic, enantiopure and nine-coordinate lanthanide complexes, [Ln·**1**]$^{3+}$ and [Ln·**2**]$^{3+}$, in which the configuration of the chiral centre on the amide arms determines the helicity of the overall complex. The tetraazatriphenylene chromophore is an established and efficient sensitiser for lanthanide luminescence.[3] Here, it has been integrated into the complex structure and acts as a bidentate ligand, preventing the coordination of water molecules that quench the lanthanide excited state.[4] Ruthenium complexes containing tetraazatriphenylene and related derivatives as ligands have been studied intensively and shown to intercalate DNA from the minor groove.[5]

Reaction of 1.4 eq. of methyllithium with 1,10-phenanthroline afforded the 2-methyl derivative. Oxidation of the 5,6-double bond and condensation with the appropriate 1,2-diamine, following literature procedures,[6] gave the tetraazatriphenylene aromatic systems. The chloromethyl derivatives were obtained by sequential mild oxidation with SeO_2, reduction of the aldehyde group with $NaBH_4$ and chlorination with PCl_3.[7] Monoalkylation of triBoc-cyclen[8] with the chloromethyl derivatives was followed by N-deprotection and trialkylation with (*R*)- or (*S*)-*N*-2-chloroethanoyl-1-phenylethylamine.[9] The Λ- and Δ-lanthanide complexes were prepared by reaction of the ligands with the appropriate trifluoromethanesulfonate salts.

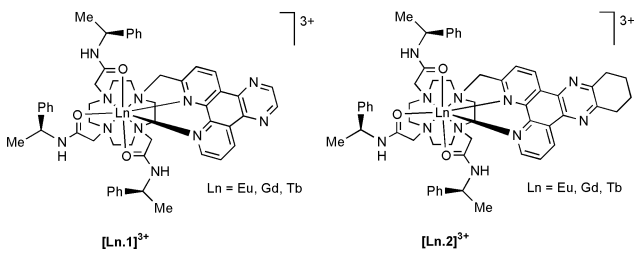
VT ^{1}H NMR spectra of both Δ- and Λ-[Eu·**1**]$^{3+}$ and [Eu·**2**]$^{3+}$ in D_2O and CD_3OD revealed the presence of one major isomer in solution, as found for the parent tetraamide complexes.[9] Circularly polarised luminescence spectra were also similar in form to those found for the tetraamide complexes, confirming the absolute configuration: mirror-image spectra were measured for the Δ- and Λ-complexes.† As X-ray crystallography and detailed ^{1}H NMR studies had revealed that related tetraamide complexes adopted a square-antiprismatic coordination geometry, the same geometry can be assumed for [Eu·**1**]$^{3+}$ and [Eu·**2**]$^{3+}$.

Rate constants for depopulation of the lanthanide excited state were measured in H_2O and D_2O; in each case the complex was shown to possess no bound water molecules. This, to a considerable extent, explains the very high overall absolute quantum yields measured in water (Table 1). These values also reflect the known facility of intersystem crossing and energy transfer[3] with this chromophore. The triplet energy of the heteroaromatic chromophore in [Gd·**1**]$^{3+}$ at 77K (4 : 1 MeOH–EtOH) was measured to be 24 000 cm^{-1} (unchanged in the presence of excess nucleic acid), significantly higher than the Eu (17 200 cm^{-1}) and Tb (20 400 cm^{-1}) emissive states, precluding back energy transfer and explaining the insensitivity of listed Φ and k values to sample de-oxygenation.

Table 1 Absolute emission quantum yield (±15%) and rate constants (k/ms^{-1}, ±10%) for depopulation of the lanthanide excited state (295 K, λ_{exc} 340 nm for [Ln·**1**] and 350 nm for [Ln·**2**], pH 7.4, 10 mM HEPES, 10 mM NaCl)

Complex	k_{H2O}	k_{D2O}	φ_{H2O}	φ_{H2O}
[Eu·**1**]$^{3+}$	0.95	0.61	0.21	0.27
[Eu·**2**]$^{3+}$	0.96	0.63	0.16	0.20
[Tb·**1**]$^{3+}$	0.54	0.42	0.36	0.46
[Tb·**2**]$^{3+}$	0.64	0.58	0.40	0.48

Changes in the absorption spectra of Δ- and Λ-[Eu·**1**]$^{3+}$ and [Eu·**2**]$^{3+}$ complexes were monitored as a function of added polynucleotide (Fig. 1). Incremental addition of poly(dAdT) was characterised by formation of a well-defined isosbestic point at 345.5 nm. This was observed for both Δ- and Λ-[Eu·**1**]$^{3+}$ and was accompanied by a marked hypochromism in the band at 340 nm and a red shift. These features are consistent with a charge-transfer interaction between the metal-bound chromophore and the DNA bases, suggesting an intercalative binding mode.[10] For Δ- and Λ-[Eu·**2**]$^{3+}$, hypochromism at 350 nm and an absorption tail to the red were also apparent, but no



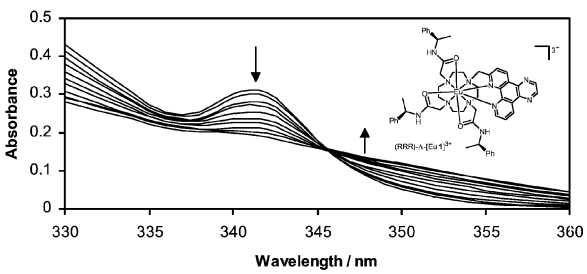


Fig. 1 Changes in the absorption spectra of Λ-[Eu·**1**]$^{3+}$ (0.1 mM) following addition of poly(dAdT) (zero to 2.3 base-pairs per complex, pH 7.4, 10 mM HEPES, 10 mM NaCl, 295 K).

† Electronic supplementary information (ESI) available: CPL spectra of [Eu·**2**]$^{3+}$ and [Tb**1**]$^{3+}$ as well as CD spectra of poly(dAdT) upon addition of Δ/Λ-[Eu·**1**]$^{3+}$ and of poly(dGdC) upon addition of Λ-·[Eu·**1**]$^{3+}$ and in the presence of added I$^-$. see http://www.rsc.org/suppdata/cc/b2/b201451n/

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isosbestic points were defined. For [Eu·**1**]$^{3+}$ and [Eu·**2**]$^{3+}$ with poly(dGdC), changes in absorption intensity were smaller and isosbestic points were absent.

The binding interaction was also examined by circular dichroism difference spectroscopy. Addition of the Λ-complexes to a solution of poly(dAdT) resulted in a change in the intensity of the nucleic acid CD spectrum at 246 and 264 nm, but not in the overall form of the spectrum, ruling out any B⟶Z transition (see ESI†). With Λ-[Eu·**1**]$^{3+}$, the negative band showed a 65% reduction in intensity and the positive band a 200% increase, together with a 7 nm shift to the red. Furthermore, the CD spectra of the dpq chromophore for Λ-[Eu·**1**]$^{3+}$, upon addition of poly(dAdT), showed a decrease in the intensity at 340 nm and a red shift of 5 nm. No significant changes were observed upon interaction of the Δ-complexes with poly(dAdT) nor with either enantiomer for poly(dGdC).†

Changes in the emission spectra of the europium complexes were recorded as a function of added polynucleotide following excitation at 345.5 nm for [Eu·**1**]$^{3+}$ and at 350 nm for [Eu·**2**]$^{3+}$. In general, quenching of the lanthanide emission was observed, which was more efficient for the Δ-isomers. Addition of poly(dAdT) to Λ- and Δ-complexes resulted in enhanced resolution of the $\Delta J = 1$ band ($^5D_0 \rightarrow {}^7F_1$) and a greater decrease in the intensity at 681 nm compared to 687 nm, within the $\Delta J = 4$ manifold (Fig. 2). These changes can be tentatively ascribed to a perturbation of the polarisability of the axially-positioned aryl N-donor, associated with its charge-transfer interaction with the DNA base-pairs.[11] The same limiting value is obtained by plotting the observed hypochromism *vs.* base-pairs per complex and is consistent with the 'neighbour exclusion' principle[2] that characterises intercalative binding. Addition of poly(dGdC) to both isomers produced significantly more quenching than poly(dAdT); addition of calf-thymus DNA (42% GC) mirrored the behaviour of poly(dGdC). The quenching process was associated with a decrease in the lifetime of the lanthanide emission (Table 2), consistent with direct quenching of the metal-based excited state. The effect was greater for Tb than Eu and was insensitive to the degree of sample deoxygenation.

Competitive quenching experiments with I^- and $Fe(CN)_6^{4-}$ were performed in the absence and presence of polynucleotide.† Δ- and Λ-[Eu**1**]$^{3+}$ displayed Stern–Volmer constants of 9.5 (±0.6) 10^{-3} M^{-1} for iodide quenching and K_{SV}^{-1} = 0.35

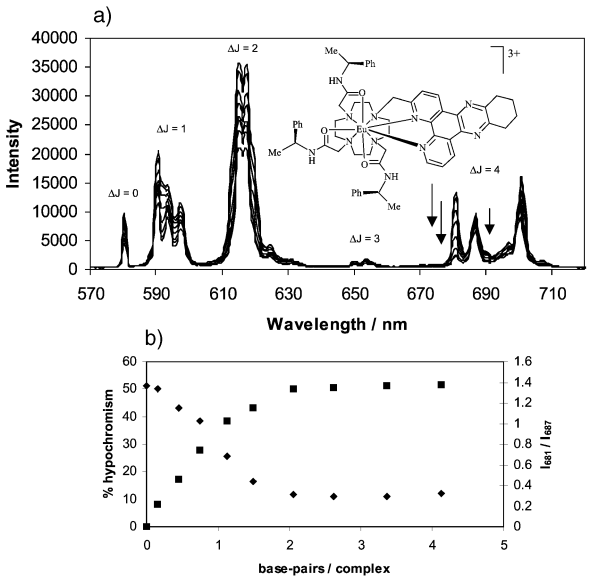

Fig. 2 (a) Emission spectra of Λ-[Eu·**2**]$^{3+}$ (20 μM) upon addition of poly(dAdT) (zero to 5.4:1 base-pairs per complex, pH 7.4, 10 mM HEPES, 10 mM NaCl, 295 K). (b) Degree of hypochromism (■) and changes in the ratio of the intensity at 681 and 687 nm (◆) as a function of poly(dAdT) base-pairs per Λ-[Eu·**2**]$^{3+}$ complex.

Table 2 Rate constants (k/ms^{-1} ±10%) for europium and terbium complexes with poly(dAdT) and poly(dGdC) at saturation state (295 K, λ_{exc} 340 nm for **1** and 350 nm for **2**, pH 7.4, 10 mM HEPES, 10 mM NaCl)

	Complex	+ poly(dGdC)	+ poly(dAdT)
Δ-Eu·**1**	0.95	1.27	0.96
Λ-Eu·**2**	0.96	1.46	0.83
Δ-Eu·**2**	0.89	> 1.50	
Δ-Tb·**1**	0.49	1.18	0.63
Λ-Tb·**2**	0.64	2.16	0.56

10^{-3} M^{-1} for ferrocyanide quenching. Addition of poly(dAdT) reduced these K_{SV}^{-1} values by about 50%, independent of complex helicity. In the presence of poly(dGdC) iodide quenching was less evident, probably as a consequence of the dominant charge-transfer quenching caused by the polynucleotide itself. Moreover, the Δ-isomers, which were quenched by poly(dGdC) to a greater extent than the Λ-series, were much less affected than the Λ-complexes by competitive iodide quenching.

In conclusion, a new family of strongly luminescent probes for nucleic acids has been defined, based on nine-coordinate lanthanide complexes bearing an efficient sensitising group. Absorption, CD and luminescent studies revealed a similar pattern of behaviour for Λ-[Eu·**1**]$^{3+}$ and Λ-[Eu·**2**]$^{3+}$ in their interaction with poly(dAdT), which is consistent with a predominantly intercalative binding mode. The Δ-isomer forms diastereoisomeric complexes of a markedly different nature. Quenching of the lanthanide excited state by GC base pairs occurs by a charge-transfer mechanism, more efficient for Tb than Eu. This will be examined in further detail by time-resolved spectroscopy.

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