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Enantiopure lanthanide complexes incorporating a tetraazatriphenylene sensitiser and three naphthyl groups: exciton coupling, intramolecular energy transfer, efficient singlet oxygen formation and perturbation by DNA binding †

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In cationic nine-coordinate chiral terbium and europium complexes incorporating exciton-coupled naphthyl groups and a tetraazatriphenylene sensitising chromophore, efficient intramolecular energy transfer occurs leading to population of the naphthyl triplet state. With the terbium complex, the absolute quantum yield of singlet oxygen formation is 51% (λ_{exc} 355 nm), and for the Eu complex the intensity of metal-based emission increases by up to 350% on binding to poly(dGdC) or calf-thymus DNA, and was greater for the Δ-isomer.

Following the definition of square planar and octahedral 'd' block complexes as DNA probes,[1] significant progress has been made in establishing the utility of chiral, cationic complexes of the lanthanide ions as probes for biomolecular structure.[2] In particular, a new family of chiral square-antiprismatic complexes has been devised,[3] in which an intercalating tetraazatriphenylene[4] group serves as an effective sensitiser for lanthanide emission, *e.g.* $[Ln\cdot\mathbf{1}]^{3+}$. Moreover, such complexes seem to be efficiently taken into cells and quickly localise in the cell nucleus. Following photoirradiation at 350 nm, cell death occurs by a mechanism that has been postulated to involve apoptosis, rather than necrosis.[5] Here, we report the properties of an analogue of $[Ln\cdot\mathbf{1}]^{3+}$, in which the three remote phenyl rings are replaced by 1-naphthyl groups $[Ln\cdot\mathbf{2}]^{3+}$. Earlier, it had been established that the related tetranaphthylamide lanthanide complexes, *e.g.* $[Ln\cdot\mathbf{3}]^{3+}$, were characterised by efficient exciton coupling between adjacent naphthyl chromophores.[6] In addition, the terbium complex served as an effective precursor for formation of singlet oxygen, formed *via* collisional quenching of the long-lived naphthyl triplet.[6,7] The naphthyl triplet state ($E_T \sim 20\ 900\ cm^{-1}$) underwent thermally activated reversible energy transfer with the long-lived emissive $^5D_4$ excited state of the terbium ion (20 400 cm^{-1}), leading to the establishment of a photo-equilibrium.[7]

The (*SSS*)-Δ and (*RRR*)-Λ $[Ln\cdot\mathbf{2}]^{3+}$ complexes were synthesised by analogous methods to those used in the preparation of $[Ln\cdot\mathbf{1}]^{3+}$. Complexes were isolated as their triflate salts (Ln = Eu, Gd, Tb) and were characterised by ESMS (accurate mass) and NMR. The Δ- and Λ-europium complexes exhibited exciton coupling (298 K, H_2O) between the 1-naphthyl chromophores, characterised by a Davydov splitting of 10 nm (Fig. 1). The mirror image CD spectra revealed $g_{abs}^{219} = -1.8 \times 10^{-3}$ for the Λ isomer and $g_{abs}^{229} = +0.9 \times 10^{-3}$, consistent with exciton coupling of positive chirality. These values are very similar to those observed with Λ-(*RRRR*)-$[Na\cdot\mathbf{3}]^+$ ($g_{abs}^{219} = -2.2 \times 10^{-3}$), for which X-ray crystallography had revealed an average dihedral angle between adjacent naphthyl planes of 81°,[6] but rather less than that observed with Λ-$[Eu\cdot\mathbf{3}]^{3+}$ ($g_{abs}^{219} = -7.8 \times 10^{-3}$).

Analysis of emission spectra and decay constants revealed significant differences between $[Ln\cdot\mathbf{1}]^{3+}$ and $[Ln\cdot\mathbf{2}]^{3+}$. The complex $[Eu\cdot\mathbf{2}]^{3+}$ was 70 times less emissive than $[Eu\cdot\mathbf{1}]^{3+}$ (λ_{exc} 340 nm), following excitation of the heteroaromatic chromophore, yet revealed only a slightly longer excited state lifetime, that was insensitive to sample deoxygenation. The form of the emission spectrum was almost identical in the range 575–670 nm, differing only in the relative intensity of the 680/702 nm bands within the $\Delta J = 4$ manifold. The terbium complex, $[Tb\cdot\mathbf{2}]^{3+}$ was only weakly emissive with a lifetime in aerated aqueous solution of 4 μs, that increased to 114 μs upon deoxygenation (Table 1). In ethanol solution the lifetime of $[Tb\cdot\mathbf{2}]^{3+}$ increased significantly at low temperature ($T < 180$ K) and analysis of the temperature dependence (295–120 K) of the

Table 1 Absolute emission quantum yields, rate constants (k/ms^{-1}, ±10%) for depopulation of the lanthanide excited state (Ln = Eu, Tb, 295 K, λ_{exc} 340 nm, pH 7.4) and relaxivities of the corresponding gadolinium complexes (r_{1p}/$mM^{-1}\ s^{-1}$, 65 MHz, 295 K)

Complex	k_{H_2O}	k_{D_2O}	r_{1p} [c]	ϕ_{H_2O}
$[Eu\cdot\mathbf{1}]^{3+}$ [a]	0.95	0.61	2.1	0.21
$[Tb\cdot\mathbf{1}]^{3+}$ [a]	0.54	0.42	—	0.36
$[Eu\cdot\mathbf{2}]^{3+}$ [a]	1.66	0.79	2.4	0.003
$[Tb\cdot\mathbf{2}]^{3+}$ [b,d]	250	250	—	< 0.0004

[a] Unchanged on deoxygenation. [b] $k_{H_2O} = 8.8\ ms^{-1}$ in the absence of oxygen (295 K) reducing to 0.95 ms^{-1} in the absence of oxygen at 120 K (EtOH). [c] The relaxivity values are typical of those expected for a $q = 0$ complex.[8] [d] The absolute quantum yield for singlet oxygen formation was measured to be 51% ($\phi_{MeOH} = 0.51$), following excitation at 355 nm using a pulsed laser (standard used was perinaphthenone $\phi_{MeOH} = 0.97$).[10]

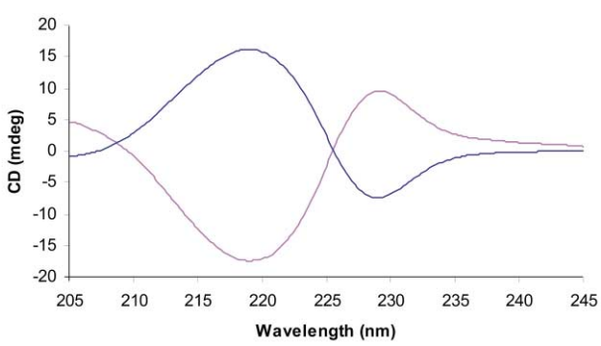

Fig. 1 CD spectra for Δ- (positive at 219 nm) and Λ-$[Eu\cdot\mathbf{3}]^{3+}$ showing exciton coupling between adjacent naphthyl chromophores (298 K, H_2O).

† Electronic supplementary information (ESI) available: Eyring plot for $[Tb\cdot\mathbf{3}]^{3+}$; agarose gel electrophoresis images following the irradiation of plasmid-DNA at 340 nm in the presence of Δ/Λ-$[Ln\cdot\mathbf{2}]^{3+}$ (Ln = Eu/Tb). See http://www.rsc.org/suppdata/ob/b3/b303085g/

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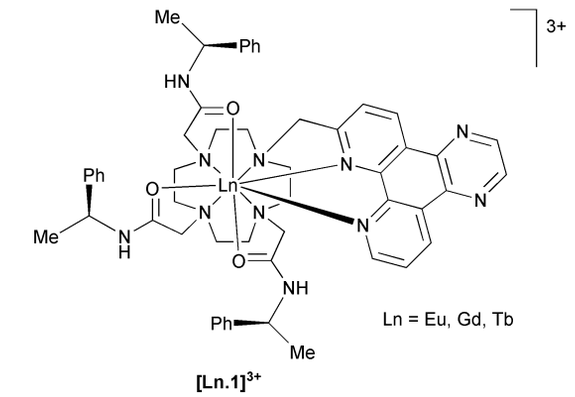
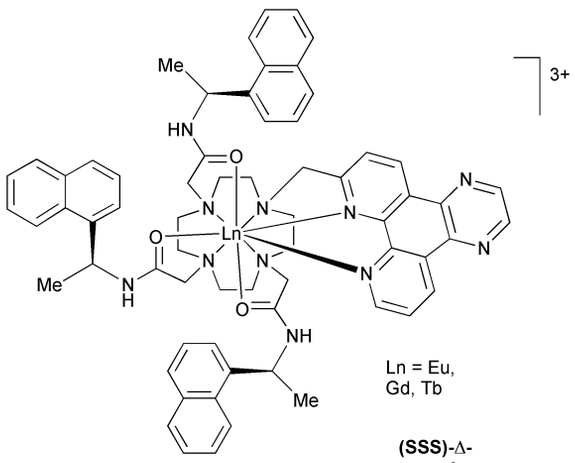
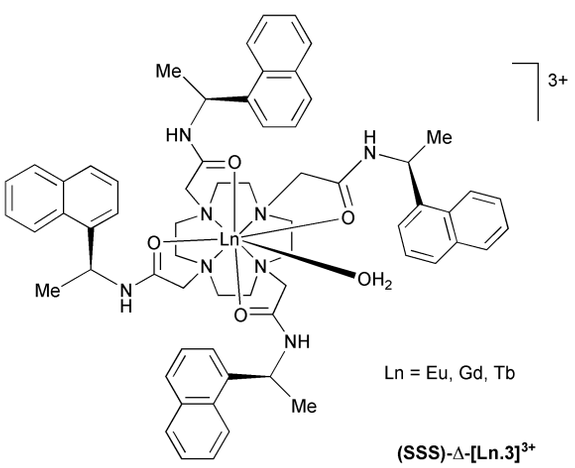


[Ln.1]$^{3+}$

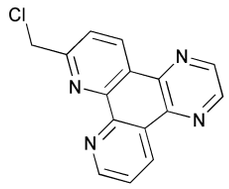


(SSS)-Δ-[Ln.2]$^{3+}$



(SSS)-Δ-[Ln.3]$^{3+}$

rate of decay of the $^5D_4$ excited state revealed a linear Eyring plot with $\Delta H^{\ddagger} = 9.6$ kJ mol^{-1} and $\Delta S^{\ddagger} = -100$ J mol^{-1} K^{-1}.† The sensitivity of the terbium emission to dissolved oxygen and temperature suggested the naphthyl triplet excited state was involved in the photochemical process. Indeed, the apparent rate of decay of the $^5D_4$ Tb excited state in [Tb·**2**]$^{3+}$ (2×10^5 s^{-1}, 295 K) was the same as that observed for [Tb·**3**]$^{3+}$ and related tetranaphthyl complexes.[6,7] In support of this hypothesis, examination of ligand-based phosphorescence spectra (77 K, MeOH–EtOH glass) of the 'non-emissive' complexes [Gd·**1**]$^{3+}$ *vs.* [Gd·**2**]$^{3+}$ revealed marked differences. With [Gd·**2**]$^{3+}$, a relatively weak series of bands was observed in the range 420–500 nm corresponding to emission from the tetraazatriphenylene triplet state. In addition, a series of more intense bands was observed in the range 470–580 nm consistent with emission from the naphthyl triplet state. Comparative spectra for the tetraazatriphenylene **4** and the naphthyl model **5** accord with this interpretation (Fig. 2). Taken together, this information is



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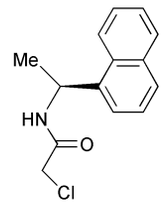

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consistent with the presence of an intramolecular energy transfer process from the tetraazatriphenylene triplet (23 500 cm^{-1}) to the naphthyl acceptor (20 900 cm^{-1}). Such triplet–triplet energy transfer processes are generally fast with a long r_o value (20 Å).[9] For both the Eu and Tb complexes of **3**, this process will compete with efficient energy transfer from the heteroaryl triplet state to the Ln^{3+} ion. The competitive formation of the naphthyl triplet limits the emissive quantum yield and sets up the oxygen/T dependence of the Tb complex (Tb $^5D_4$ = 20 400 cm^{-1}) *via* a reversible energy transfer process which only becomes relatively slow as $kT < 120$ cm^{-1}. The overall efficiency of singlet oxygen formation with [Tb·**2**]$^{3+}$, following laser excitation at 355 nm was measured to be 51% (295 K) using established methods,[10] in accord with the facility of the Tb–naphthyl back energy transfer process that repopulates the naphthyl triplet state (Fig. 3).

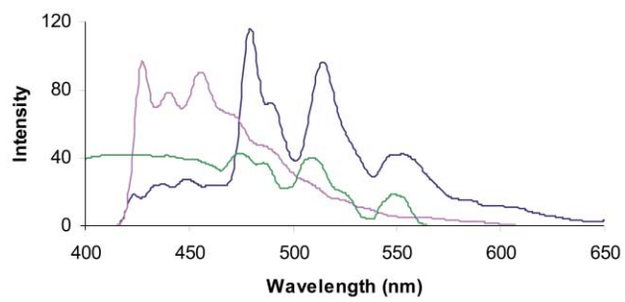

Fig. 2 Phosphorescence spectra for [Gd·**2**]$^{3+}$ (most intense at 550 nm) and the tetraazatriphenylene **4** (least intense at 550 nm) and naphthyl, **5**, model chromophores (77 K, EtOH–MeOH; 3 : 1).

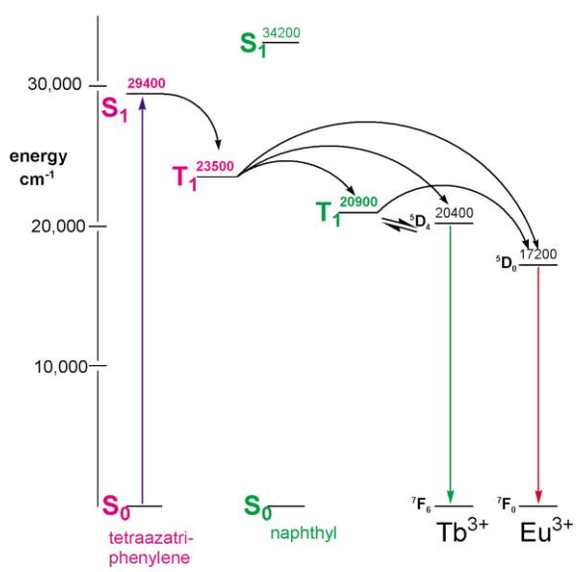

Fig. 3 Schematic representation of the ligand and metal centred excited states that may be populated following excitation of the tetraazatriphenylene chromophore (sometimes termed dpq), in [Ln·**2**]$^{3+}$ (Ln = Eu, Tb).

Changes in the absorption and CD spectra for Δ- and Λ-[Eu·**2**]/[Tb·**2**]$^{3+}$ were monitored (pH 7.4, 10 mM NaCl, 10 mM HEPES) as a function of added poly(dAdT), poly-

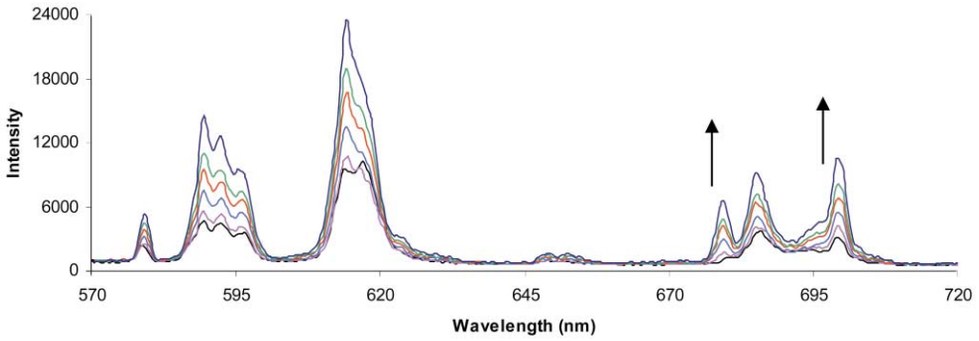

Fig. 4 Changes in europium emission spectra for Λ-[Eu·**2**]$^{3+}$ (45 μM), following incremental addition of poly(dGdC), 0.8 mM, from 0 to 1 base-pair per complex (λ_{exc} 340 nm, pH 7.4, 10 mM HEPES, 10 mM NaCl, 295 K).

(dGdC) and calf-thymus DNA (42% GC). In marked contrast to the behaviour of [Ln·**1**]$^{3+}$, little or no change was discerned in the absorption spectra; only for Λ-[Eu·**2**]$^{3+}$–poly(dAdT) was there any evidence of hypochromism (10% at 340 nm) with a small red shift. Circular dichroism difference spectra, examining the nature of the DNA chromophores, also revealed no significant change on addition of DNA.

However, there was a marked change in the CD spectrum of the *complex* in the near-UV region. Some qualitative observations have been made: addition of poly(dAdT) to Λ-[Eu·**2**]$^{3+}$ caused a limiting 140% reduction in the intensity of the exciton coupled bands at 219 and 229 nm, following addition of only 0.1 base-pairs of DNA per complex. For the Λ-isomer, the limiting reduction was 70% and this was achieved only at a much higher ratio of close to 1.5 base-pairs/complex—corresponding to the charge neutralisation limit. Addition of poly (GC) to the Δ- and Λ-isomers gave rise to a limiting CD intensity reduction of 75% in each case, but with no significant stereoselectivity. The remarkable behaviour of the Λ-Eu isomer in its reversible binding to poly(dAdT) suggests that in the presence of poly(dAdT), the Λ-complex adopts a conformation in which the naphthyl groups are unable to undergo exciton coupling. In control experiments with Δ/Λ-[Eu·**3**]$^{3+}$, addition of poly(dGdC) caused a 30–40% decrease in CD intensity at 219 nm (Δ ~ Λ), whilst for poly(dAdT), the Δ-complex gave a 20% decrease and the Λ-isomer a 90% decrease, again with a higher apparent binding affinity (limit *ca.* 1.5 bp/complex). Thus, it seems to be the differing helicity of the chiral complexes which is responsible for the striking differences in the binding of the Δ and Λ isomers to poly(dAdT).

Addition of each nucleic acid to [Eu·**2**]$^{3+}$ led to an *increase* in the intensity and slight changes in the form of the metal-based emission (Fig. 4). Again, this behaviour is in contrast to that observed for [Eu·**1**]$^{3+}$ for which DNA binding led to quenching of the Eu emission. Addition of poly(dGdC) and calf-thymus DNA, for example, led to a limiting 350% increase in emission intensity at 590 nm (Δ ~ Λ, limit was *ca.* 1.5 bp/complex), whereas addition of poly(dAdT) gave a 200% increase (Λ > Δ). The Eu emission lifetime was measured at the end of each titration and revealed a 41% increase in lifetime for Λ-[Eu·**2**]$^{3+}$ (+9% for Δ) with poly(dAdT), an 83% rise for Λ-[Eu·**2**]$^{3+}$ with CT-DNA (+58% Δ isomer), and a 29% increase following addition of the Λ isomer to poly(dGdC) (+2% Δ isomer). Given the complexity of the photophysical processes preceding and competing with Eu emission, it is imprudent to attempt to rationalise this behaviour further,[11] without further experimentation. However, the increase in lifetime on DNA binding is indicative of the deactivation of a process that quenches the Eu excited state, a process requiring the presence of both the tetra-aza chromophore and the naphthyl group.

Finally, the interaction of [Ln·**2**]$^{3+}$ with super-coiled (pBlue Script) plasmid DNA has been monitored, by gel electrophoresis, following irradiation (5 ⟶ 45 min) at 340 nm. Separation of the product DNA species revealed evidence for formation of first nicked DNA (Tb > Eu; Δ > Λ) and then linear DNA—whose formation was most clearly defined with Δ-[Tb·**2**]$^{3+}$ following 45 minutes irradiation. † Such a pattern of behaviour contrasts with that observed for [Ln·**1**]$^{3+}$, for which DNA damage was greater for the Λ-isomer. Further experimentation is clearly required in seeking to define the mechanistic details of the processes involved.

Acknowledgements

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References and notes

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11 Addition of DNA to Δ or Λ to [Tb·**2**]$^{3+}$ caused a *reduction* (up to 60%, for poly(dGdC)) in the Tb emission intensity, indicating that the bound complex remains susceptible to oxygen quenching of the naphthyl triplet.