dsDNA-triggered energy transfer and lanthanide sensitization processes. Luminescent probing of specific A/T sequences†‡

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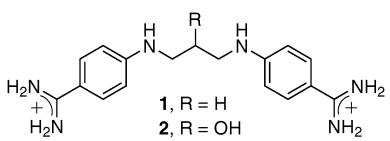
Orthogonal attachment of a DOTA[Ln^{3+}] complex or a coumarin fluorophore to appropriately functionalized bis-4-aminobenzamidines yields compounds that experience A/T-selective, dsDNA-dependent energy transfer processes, and elicit long wavelength emission of light.

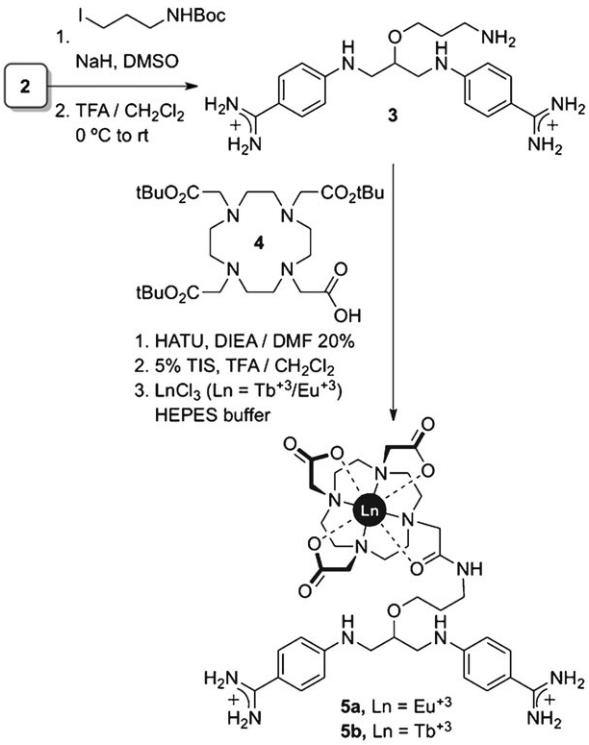
The study of DNA has unfolded over the past two decades from the initial structural and functional characterization in living organisms, to what is now known as DNA technology, encompassing the development of DNA as a tool in biological sciences, and more recently as a structural and nanotechnological scaffold.[1,2] However, the development of DNA-based functional and dynamic processes, particularly those employing double stranded DNA (dsDNA), is still in its infancy.[3] In this context, finding new ways of codifying physicochemical processes with dsDNA,[4] and in particular the development of efficient methods for selective sensing of dsDNA sequences,[5] might provide new opportunities for research and discovery at the interface of biomedical and chemical sciences. Herein, we report the first examples of small molecules displaying intramolecular energy transfer processes induced by specific dsDNA sites, in our case A/T-rich sequences. The designed probes emit long wavelength light, and might therefore be relevant for future applications in dsDNA sensing and imaging.

This project arose from our recent observation that bis-4-aminobenzamidines such as **1** or **2** (Scheme 1) exhibit a remarkable fluorescence emission enhancement upon binding to A/T-rich sites in dsDNA (λ_{exc} = 329 nm, λ_{em} = 387 nm).[6,7]

We envisaged that appropriate attachment of an acceptor fluorophore to the parent benzamidine might allow the implementation of dsDNA-dependent energy transfer processes. We first focused on lanthanide ions as it is well known that they can exhibit long-lived excited states and narrow, long wavelength emission bands,[8,9] and are therefore very attractive for luminescent biosensing applications.[10] Towards this end we designed the DOTA[Eu^{3+}] and [Tb^{3+}] conjugates **5a** and **5b**, which were assembled as detailed in Scheme 2. Coupling of bis-benzamidine **3**, which features an alkoxyaminopropane handle, with the tri-^{t}Bu protected DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) monoacid **4**, followed by deprotection and complexation with the appropriate lanthanide trichloride in buffer solution, afforded the desired macrocyclic conjugates **5a**/**5b**.



Scheme 1 Structure of fluorescent bis-aminobenzamidines **1** and **2**.



Scheme 2 Synthesis of amino derivative **3**, and lanthanide conjugates **5a**/**5b** (Ln = Eu^{+3}, Tb^{3+}).

As expected, irradiation at 329 nm of a 0.5 μM solution of compounds **5a** or **5b** in buffer did not result in significant emission from the metal ions. Likewise, addition of a hairpin oligonucleotide featuring a non-target *GGCCC* site, or a sequence featuring a short A/T tract (*AAT*) did not affect the luminescence intensity of the lanthanide complexes (see Fig. S1 in ESI‡). However, addition of the dsDNA hairpin containing the consensus *AATTT* sequence induced an increase in the emission bands of lanthanide ions in both **5a** (Fig. 1, top), and **5b** (Fig. 1, bottom). Despite the similar

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† This work is dedicated to Prof. Luis Castedo on the occasion of his 70th birthday.

‡ Electronic supplementary information (ESI) available: Synthesis and characterization, DNA binding, and detailed spectroscopic experiments. See DOI: 10.1039/b927305k

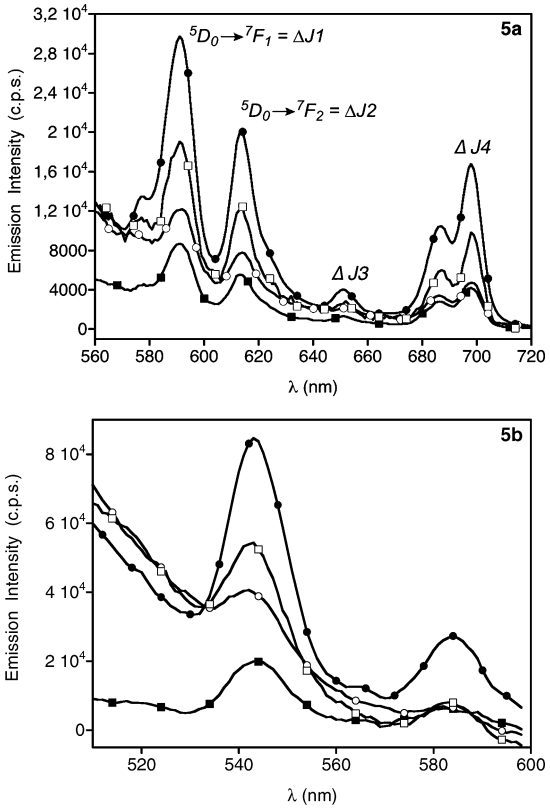
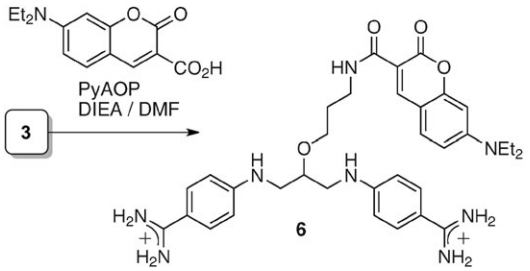


Fig. 1 **Top**. Emission spectra of DOTA[Eu^{3+}] **5a** showing the corresponding electronic transitions. **Bottom**. Emission spectra of DOTA[Tb^{3+}] **5b**. In both graphs: spectra in the absence of DNA (■), with 9 equiv. of non-target dsDNA (**GGCCC**, ○), with 9 equiv. of suboptimal dsDNA (**AATTC**, □), and with 9 equiv. of target dsDNA (**AATTT**, ●). Hairpin oligonucleotide sequences (binding sites in italics): **AATTT**: 5′-GGCG-*AATTT*-CGCTTTTTGCG-*AAATT*-CGCC-3′; **AATTC**: 5′-GGCG-*AATTC*-AGCTTTTTGCT-*GAATT*-CGCC-3′; **GGCCC**: 3′-GGCA-*GGCCC*-AGCTTTTTGCT-*GGGCC*-TGCC-3′.

relative variation in the emission intensity of both conjugates, the shorter emission wavelengths of terbium at 488, and 545 nm are significantly overlapped with the tail of the bis-benzamidine band, yielding less clear spectra. Likewise, incubation of **5a** with a hairpin dsDNA containing an *AATT* binding site also induced a response, albeit weaker than for the consensus *AATTT* site (Fig. 1, top).[11]

For **5a** we observed at least a four-fold increase in the emission intensity of the typical Eu^{3+} bands. The luminescence emission profile of Eu^{3+} in **5a** was as expected for a square-antiprismatic structure, typical of DOTA[Eu^{3+}] complexes, which is mainly characterized by a ΔJ_2 ($5D_0 \rightarrow 7F_2$) transition, known as the hypersensitive band, weaker than the ΔJ_1 band (Fig. 1).[12] To our knowledge, this is the first demonstration of a sequence-selective dsDNA-promoted environment-sensitive fluorescence enhancement coupled to lanthanide sensitization, and sets the basis for further developments of lanthanide-based dsDNA probes.

Having demonstrated the application of the bis-benzamidine as lanthanide antenna for the DNA-triggered energy transfer, we reasoned that other organic acceptors such as a coumarin, with a large spectral overlap with the emitting bis-benzamidine,



Scheme 3 Synthesis of the coumarin conjugate **6**.

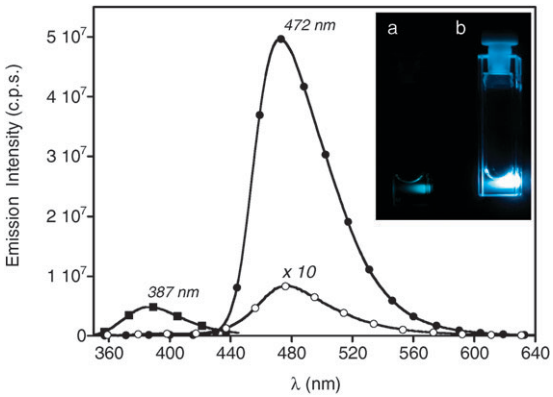


Fig. 2 Fluorescence emission of **1** (0.5 μM in Tris-HCl buffer) with 9 equiv. of target dsDNA (**AATTT**, ■); fluorescence emission of hybrid **6** 0.5 μM in Tris-HCl buffer (○; the intensity of this spectrum is presented multiplied by 10 for clarity), and in the presence of 9 equiv. of target dsDNA (**AATTT**, ●). Insert shows fluorescence cuvettes with 500 nM solutions of **6**: (a) no dsDNA added; (b) 2 equiv. of dsDNA **AATTT**. Excitation at 329 nm.

might make for a more efficient process.[13] We therefore prepared conjugate **6** by direct coupling of parent amino-benzamidine **3** with PyAOP-activated 7-(diethylamino)-coumarin-3-carboxylic acid (Scheme 3).

As shown in Fig. 2, the fluorescence emission of **6** after excitation at 329 nm is very weak, and is dominated by the coumarin emission band at 472 nm. More importantly, addition of the target dsDNA oligonucleotide containing the *AATTT* target sequence induced a large increase (~60-fold) in the fluorescence emission intensity of coumarin. The bright emission of the coumarin fluorophore even allows the visual detection of small quantities of DNA (less than 3 ng), as shown in the inset.

Therefore, appropriate conjugation of bis-benzamidine with coumarin results in a bright fluorescent probe that displays a remarkable fluorescence change upon binding to specific dsDNA sites (*AATTT*), and an increased Stokes shift (145 nm) relative to the parent compound **1**. Moreover, addition of the dsDNA containing the truncated target sequence *AAT* induced significantly smaller increases in the emission of the coumarin conjugate at 472 nm (Fig. 3 and ESI‡).

In summary, we have described a sequence specific, dsDNA-induced lanthanide sensitization process, with concomitant long-wavelength luminescence, as well as a very efficient Förster intramolecular energy transfer promoted by specific

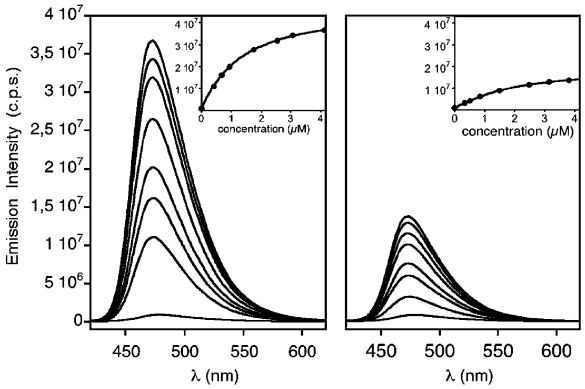


Fig. 3 Emission spectra of a 0.5 μM solution of **6** with increasing amounts of dsDNAs. **Left**. with hairpin with the target sequence *AATTT*; **Right**, with dsDNA with a truncated target site (*AAT*). Concentrations are 0, 0.4, 0.7, 1, 1.8, 2.5, 3.2 and 4 μM. Both graphs are on the same scale for comparison. Insets show the best fit at 472 nm to a 1 : 1 model. Hairpin oligonucleotide sequence (binding site in italics): **AATGC**: 5′-GGCG-*AATGC*-AGCTTTTTGCT-*GCATT*-CGCC-3′; (see ESI for details‡).

A/T rich dsDNA sequences. Studies to develop optimized versions of these compounds that can lead to efficient and practical dsDNA sequence-specific optical probes are underway.

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