

Synthesis of asymmetrically substituted 1,4,7,10-tetraazacyclododecanes for the triggered near infrared emission from lanthanide complexes†

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A synthetic strategy to prepare asymmetrically substituted 1,4,7,10-tetraazadodecane derivatives was developed to prepare a novel series of photoactive donor–acceptor quencher triads based on Yb and Nd complexes; a nucleoside quencher is used to regulate the extent of energy transfer between the donor and the acceptor.

The antenna effect supplied by coordinating ligands is extensively exploited to overcome the low molar extinction coefficients of the luminescent trivalent lanthanides.¹ While most of the work has focused on those lanthanides such as europium(III) and terbium(III)^{2,3} that emit in the visible region, recently the focus has switched to the NIR emitting lanthanides such as neodymium(III)⁴ and ytterbium(III).⁵ Luminescent probes emitting in the near IR region have particular application in imaging in biological systems. Tissue penetration of light increases with the wavelength, which means that near IR-emitting probes are better suited for *in vivo* imaging than those emitting in the UV-Vis region. Similarly the low energy sensitisation of NIR-emitting lanthanides means longer excitation wavelengths can be used.

This offers a wide possibility of antenna chromophores and to date, sensitization using fluorescein,⁶ eosin,⁷ porphyrins,⁸ pyrene,⁹ tetrazines¹⁰ transition metal complexes¹¹ and other lanthanides¹² has been described.

There is already a variety of NIR emitting lanthanide complexes reported, however the effect of added analytes on the emission is limited to a few examples.^{8,13} In these cases changes in an existing emission intensity was used to monitor the interaction. We sought to increase the sensitivity of such probes by incorporating a quencher unit in close proximity to the sensitizer. In this way, the emission from the lanthanide is retarded until the binding of the target substrate to the quencher switches the emission on. This is manifest in changes in the observed excited state lifetimes for the lanthanides.

Cyclen-based ligands for the sensitisation of near IR emitting lanthanides (Nd or Yb) were designed: the cyclen scaffold holding together an antenna (rhodamine B)–quencher (nucleoside) pair and serving as a chelator for the metal. The key requirement was to obtain a N1,N4 substitution pattern with respect to the

rhodamine and nucleoside base to ensure the donor and quencher are in sufficient proximity to interact. The remaining two nitrogens are alkylated with coordinating arms to enhance the stability of the complexes and reduce the quenching caused by OH-oscillators in the solvent molecules¹⁴ (Scheme 1). 2-Bromoethylamine was attached to Rhodamine B *via* an amide bond to furnish **2** as a pale pink solid after silica column chromatography. Monosubstitution of cyclen was possible by treatment of a small excess of cyclen with **2** in refluxing ethanol in the presence of TEA. The N1-alkylated main product could be easily separated from the higher-substituted side-products by chromatography on basic alumina. The required N1,N4 substitution pattern was selectively achieved over the more favoured N1,N7 substitution by treating the monosubstituted cyclen with a solution of N-Boc-bromoethylamine in chloroform at room temperature. This procedure is a modification of a previously reported method.¹⁵ There cyclen was selectively N1,N4-bis-substituted with *tert*-butylbromoacetate in chloroform in the presence of TEA. We found that it was possible to perform the second substitution step on the previously isolated monosubstituted cyclen derivative under similar conditions, affording *cis*-disubstituted cyclen derivatives bearing two different N-substituents. Separation of the desired product **4** was facilitated by the fact that the only side-products were threefold-substituted and monosubstituted cyclen derivatives with very different chromatographic properties. None of the isomeric N1,N7-disubstituted derivative was observed upon either NMR or TLC analysis.

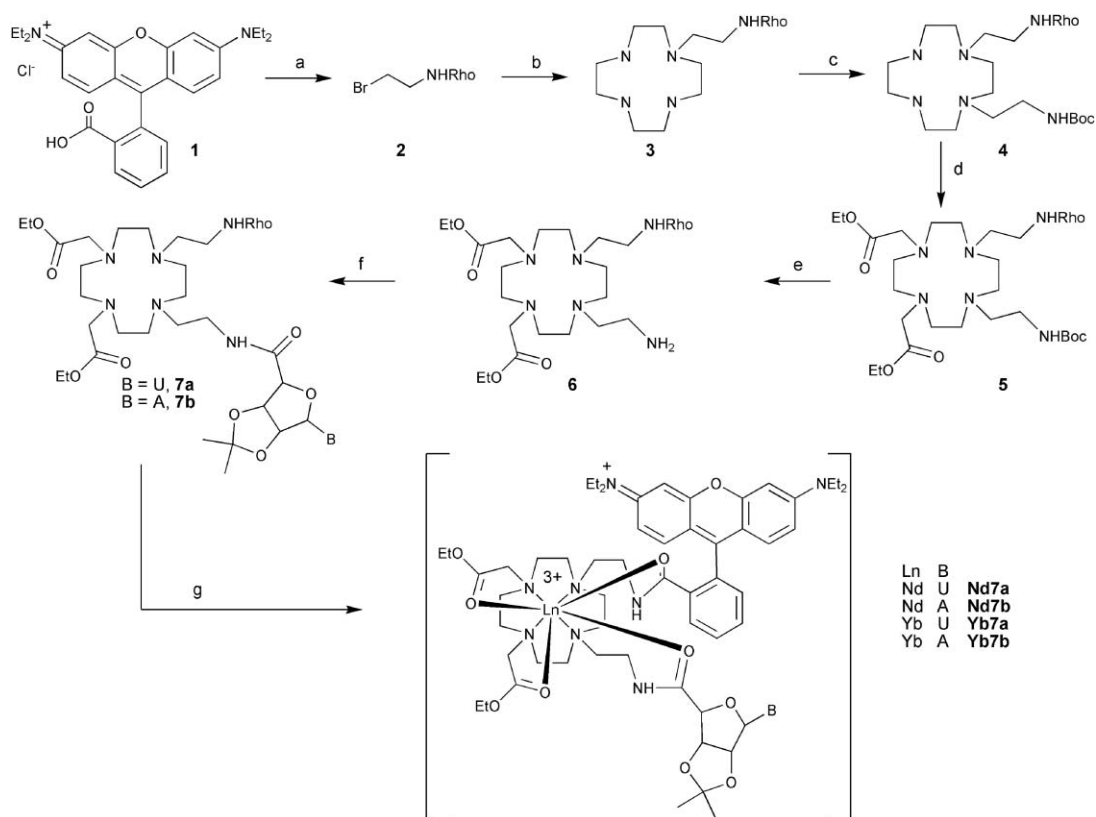
Compound **4** was then treated with excess ethyl bromoacetate in anhydrous acetonitrile in the presence of potassium carbonate and sodium iodide to afford the tetrasubstituted compound **5** in good yields. The Boc protection in **5** could be cleaved quantitatively with 33% TFA in CH₂Cl₂ to reveal a reactive primary amino group to which 5'-oxidised nucleosides¹⁶ could be attached by conventional peptide synthesis methods. Thus, treatment of a solution of oxidised 5'-uridine or -adenosine with EDCI, followed by the addition of a solution of **6** afforded the unsymmetrical ligands **7a** and **7b** in good yields after column chromatography.

This synthetic strategy (Scheme 1) is quite broadly applicable and we have been able to prepare unsymmetrically substituted cyclen based ligands in good yields. Ligands **7a** and **7b** both utilise the rhodamine chromophore with a uridine and an adenosine quencher, respectively. These nucleosides were selected, in part, for their synthetic accessibility and their ability to quench rhodamine fluorescence.¹⁷ The hydrogen bonding motif provides the point at which substrates, namely the complementary bases can interact and alter the quenching process. It is worth noting that in aqueous solutions the hydrogen bonding between the base pairs is likely to

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Scheme 1 Synthesis of NIR ligands and complexes. a, $\text{Br}(\text{CH}_2)_2\text{NH}_2\cdot\text{HBr}$, TEA, EDCI, DCM, R.T.; b, cyclen, TEA, 100% EtOH, reflux, Ar; c, $\text{Br}(\text{CH}_2)_2\text{NHBoc}$, TEA, CHCl_3 , R.T., Ar; d, $\text{BrCH}_2\text{COOEt}$, K_2CO_3 , NaI, CH_3CN , reflux, Ar; e, 33% TFA, DCM, R.T.; f, nucleoside-5' carboxylic acid, EDCI, DMF (B = U or A), R.T.; g, LnCl_3 , CH_3OH , reflux, Ar (Ln = Nd or Yb).

play a lesser role to π - π stacking and hydrophobic interactions between the nucleoside bases.

The ligands **7a** and **7b** and their intermediates were characterised by ^1H and ^{13}C NMR spectroscopy, UV-Vis absorption and emission spectroscopy, and high resolution ESI-MS.

Treatment of **7a** with anhydrous NdCl_3 or YbCl_3 in refluxing HPLC-grade methanol gave the complexes **Nd7a** and **Yb7a** respectively. These were characterised by ESI-MS, absorption spectroscopy and elemental analysis. In a similar manner, the complexation of **7b** with YbCl_3 and NdCl_3 gave the complexes **Nd7b** and **Yb7b**.

The absorption spectrum (Fig. 1) shows that the direct excitation of the rhodamine at 355 nm allows the selective excitation of the chromophore unit in the presence of both the

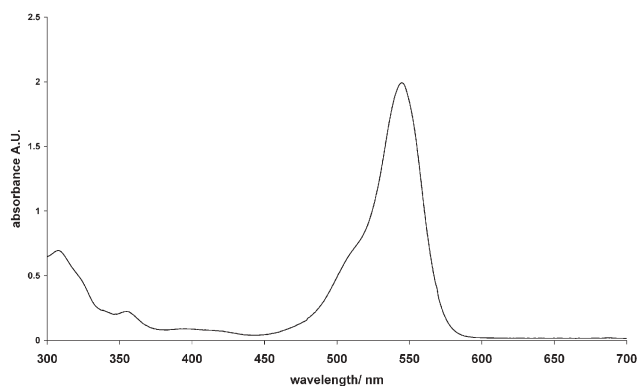


Fig. 1 Absorption spectrum of **7a** in CH_3CN .

quencher and the acceptor. It is noteworthy that excitation can be performed at any wavelength in the 350–700 nm (rhodamine absorption) region but the experimental setup available for us made excitation at 355 nm convenient for the method used as reported.¹⁸ Any NIR emission from the Yb (980 nm) and the Nd (890 nm and 1064 nm) arises exclusively from the population of the $^4\text{F}_{3/2}$ excited state for the Nd^{3+} and $^2\text{F}_{5/2}$ for Yb^{3+} by energy transfer from the excited triplet state of the rhodamine antenna. Quenching of this rhodamine excited state by the adjacent nucleoside will retard the sensitization of the lanthanide. This is the case for the Nd complex **Nd7b** which has a very short-lived luminescence (Fig. 2). While steady state emission from the neodymium could be observed it was too short lived to measure the lifetime. Upon addition of the complementary uridine base, the interaction between the quencher and donor was perturbed and longer lived luminescent emission from the Nd was observed. The lifetime observed for the complex **Nd7b** when adenosine or uridine is added is comparable to that of previously reported complexes.¹⁹

This is, we believe, one of the first examples of chemically induced switching of NIR emission lifetime. The effect was more modest for the remaining three complexes **Nd7a**, **Yb7a** and **Yb7b**. In all of these examples the antenna was not completely quenched and some NIR emission was observed from the complexes in the absence of added base. For all these complexes the lifetimes were comparable to values reported in the literature.^{4,5,18}

For the complex **Nd7a** with a uridine quencher, the lifetime is also quite short-lived and is increased upon the addition of adenosine or uridine

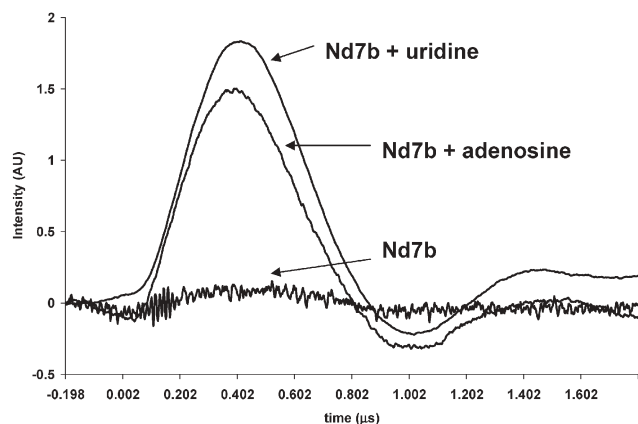


Fig. 2 Time resolved luminescence profiles of **Nd7b** in methanol ($\lambda_{\text{ex}} = 355 \text{ nm}$, $\lambda_{\text{em}} = 890 \text{ nm}$); (i) no added nucleoside, (ii) 1 equiv. uridine added, (iii) 1 equiv. adenosine added.

Table 1 Luminescent lifetimes of the near IR-emitting lanthanide complexes in methanol. Luminescence decay measured at 890 nm (Nd) and 980 nm (Yb)

	Nd7a	Yb7a	Nd7b	Yb7b
$\tau/\mu\text{s}$	0.09	2.61	— ^c	2.23
$\tau^a/\mu\text{s}$	0.47	0.89	0.23	1.17
$\tau^b/\mu\text{s}$	0.81	—	0.16	—

^a 1 equiv. complementary base added. ^b 1 equiv. own base added.
^c Lifetime could not be calculated.

Interestingly the changes in lifetime are more marked when uridine is present. In comparing **Nd7a** and **Nd7b** (Table 1), the free host had a measurable lifetime when uridine was present but not when adenosine was included as the quencher. The increases in lifetime observed were greater when uridine was added to both complexes compared to when adenosine was added as the guest. This seems to imply that the adenosine is a more effective quencher and that even free adenosine is able to quench the antenna directly.

For the Yb complexes the opposite trend was observed. Upon addition of uridine for complex **Yb7a**, the lifetime of the complex was significantly decreased. Similarly complex **Yb7b** with the adenosine quencher also showed a decrease in lifetime when the complimentary uridine was added. This may be due to the susceptibility of Yb(III) to reduction directly by the added nucleoside.²⁰

Overall, the addition of a nucleoside base (either complementary or identical) to the complexes can quench the antenna directly as well as bind to the nucleoside quencher. The observed lifetime changes are a balance between the removal of the bound quencher enhancing the luminescence and the direct quenching of the added base on the antenna decreasing the luminescence. Adenosine seems to be more effective quencher than uridine based on the observed changes though this needs to be clarified using electrochemical measurements and time resolved spectroscopic measurement of the energy transfer rates. This will be the subject of further photophysical studies.

In conclusion we have developed a new synthetic strategy for unsymmetrically substituting cyclen and applied this in

the preparation of ligands for the NIR emitting lanthanides neodymium and ytterbium. The ligands are able to regulate to some degree the energy transfer process between the antenna chromophore and the lanthanide and therefore it is possible to regulate the extent of energy transfer between the antenna and the lanthanide by an external chemical stimulus.

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