Synthesis of asymmetrically substituted cyclen-based ligands for the controlled sensitisation of lanthanides†

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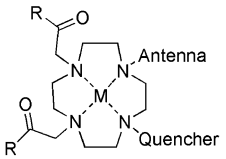
A series of unsymmetrical cyclen-based ligands incorporating an antenna and a quencher have been prepared for the complexation of the visible- (Eu, Tb) and near IR-emitting (Nd, Yb) lanthanides. Eu and Tb were sensitised with coumarin 2, and Nd and Yb with rhodamine. Both antennae were paired with nucleoside (uridine and adenosine) quenchers. The interaction between the quencher and the antenna can be regulated by the addition of the complementary base or DNA to the complexes, resulting in changes in the lanthanide luminescence intensity and lifetime.

Introduction

The luminescent lanthanide ions have been receiving considerable attention from the scientific community for a number of years. This interest stems from their excellent properties, namely their long emission wavelengths (400–1300 nm), well-defined, line-like spectra, and long emission lifetimes (μs to ms range), which make them suitable for life sciences applications.[1] As the f–f transitions involved in lanthanide luminescence are Laporte-forbidden, absorption coefficients are typically <1.[2] Effective excitation can be performed *via* a light-harvesting antenna capable of transferring the excitation energy to the metal centre. This is well established and europium and terbium have been sensitised with, among others, benzyloxyquinoline,[3] acridone derivatives,[4] phenanthridine,[5] pyridines[6] and dansyl groups.[7,8] Sensitisation of Nd(III), Yb(III) and Er(III) is possible with fluorescein,[9] pyrene,[10] Pd-porphyrins,[11] eosin,[12] Tb(III)-complexes,[13] rhenium(II)-[14] and ruthenium(II) bipyridyl moieties,[15] and quinoline.[16] However, efforts to control the degree and extent of sensitisation by the antenna are more limited. Attention has focused mainly on the visible-emitting europium and terbium complexes,[17,18] which have found applications in fields as diverse as pH-sensing,[19] DNA-,[20] oxygen-,[21] organophosphate-[22] and Cu(I)- detection.[7] Recently, complexes of the near infrared-emitting neodymium, ytterbium and erbium[23–25] have been gaining attention. Excitation of the latter group can be performed with visible light (>400 nm), avoiding interference from the medium, for example biological samples.

Although a number of examples of luminescent detection with lanthanide fluorophores have been reported,[7,19–22,26] the majority of the sensors developed to date signal the substrate presence by an increase or decrease in luminescence intensity, making unambiguous detection difficult. There are notable exceptions, a particularly elegant one being the Cu(I)-probe developed by Viguier and Hulme.[7] We have been interested in developing lanthanide-based luminescent sensors that are non-emissive in the absence of their substrates, and become emissive upon binding. We have sought to control the energy transfer between the excited state of the antenna and the lanthanide. Recently we have reported the synthesis of a series of Nd(III) and Yb(III) complexes that incorporated a quencher moiety in close proximity of a rhodamine antenna.[27] The quencher (the nucleotides adenosine and uridine) possesses a recognition unit (the hydrogen-binding motif), which enables substrate binding, *i.e.* Watson–Crick base pairing or π–π stacking. The formation of base pairs impairs the quencher's ability to disrupt energy transfer from the antenna to the lanthanide, thus turning the emission 'on'. Here we report the full characterisation of the Nd(III) and Yb(III) complexes, as well as the preparation of a series of Eu(III) and Tb(III) complexes designed according to the same principle.

The general complex design is shown in Chart 1. Cyclen was chosen as the scaffold to which the antenna, the quencher and the additional stabilising arms could be attached *via* a series of *N*-alkylations. Many traditional cyclen based ligands have identical coordinating arms on the nitrogens or are only partially substituted. Some examples exist with two types of arms. The key requirement for the successful strategy was to develop a method for three different types of pendant arms to be sequentially added to the cyclen ring. The ligands for europium and terbium complexation would be equipped with a coumarin 2 antenna, which, with an emission maximum of 432 nm, is ideally suited for the sensitisation of these lanthanides.[28] Adenosine or uridine



R = OH, OEt

Ligand	Antenna	Quencher	M
1U	Coumarin 2	Uridine	Eu Tb
1A	Coumarin 2	Adenosine	Eu Tb
2U	Rhodamine	Uridine	Nd Yb
2A	Rhodamine	Adenosine	Nd Yb

Chart 1

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† Electronic supplementary information (ESI) available: General experimental procedures for selected compounds. Excitation spectrum for **1ATb**, emission spectra for **2AYb** and **2UYb** with and without added substrates, changes in emission spectra of **1UEu** and **1ATb** with complementary bases, and the spectrum of **1UEu** with *ds*DNA. MMX calculated geometries for synthetic intermediates and an NMR titration curve for the free ligand. See DOI: 10.1039/b705757a

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serve as the quencher as it was shown that the redox couples of these nucleosides make them ideal quenchers *via* an electron transfer process.[29] These quenchers were chosen both for their known abilities to quench both rhodamine and coumarins,[29] and for their synthetic accessibility. The synthesis of the neodymium and ytterbium complexes ([**Nd(2U)**], [**Yb(2U)**], [**Nd(2A)**], [**Yb(2A)**]) have been described previously.[27]

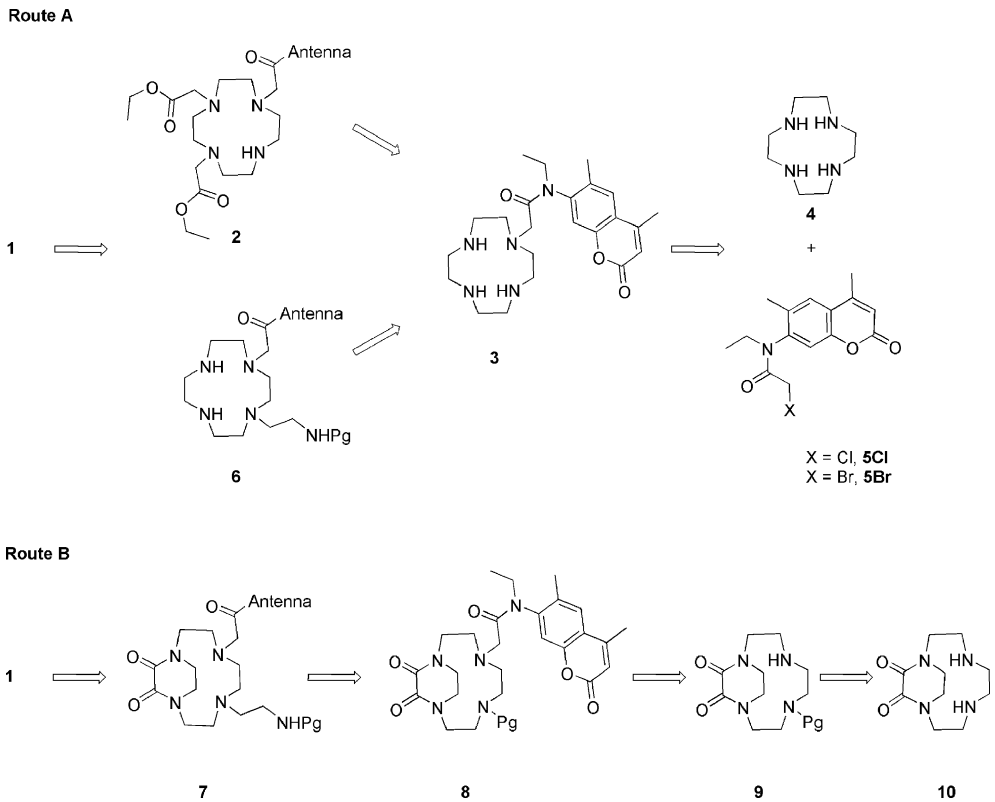
To maximise the antenna–quencher interaction it was deemed essential to introduce these functionalities at adjacent nitrogens of the cyclen. The other two nitrogens would bear carbonyl-donor substituents, thus yielding a ligand with eight donor atoms. The coordination sphere is expected to be completed with a solvent molecule.

Results and discussion

1. Synthesis

The retrosynthetic analysis is outlined in Scheme 1. Two distinct routes were considered. **Route A** does not require protection of the cyclen nitrogens, and is therefore particularly attractive. Monoalkylation of cyclen (**4**) with A-X or L-X (where X is a halogen or a pseudohalogen, A is the antenna, L is a linker) is possible by a careful choice of conditions, giving **3** as the major product.[27,30] Dialkylation of the second and third nitrogen positions with ethyl bromoacetate at high temperature and/or polar and protic solvents was expected to furnish mainly **2**. It is known from the literature that the second *N*-alkylation under such conditions yields almost exclusively *N*1,*N*7-alkylated products. Thus alkylation of the third nitrogen in the cyclen ring with the second ethylbromoacetate will always leave one of the nitrogens *cis* to the antenna free.[31] The fourth substituent can then be introduced in the final step. An alternative approach branches off at the monoalkylated cyclen derivative **3**. This route closely mimics our synthesis of the cyclen-based ligands for the sensitisation of Nd and Yb, and we were eager to test the strategy on different antenna–quencher pairs.[27] Briefly, **3** can be *cis*-alkylated in chloroform in the presence of triethylamine with L-X to give **6**. Simultaneous alkylation of the remaining two secondary nitrogens with ethyl bromoacetate introduces the stabilising arms. In the final step the quenchers can be attached *via* amide bonds through the primary amino group of the linker, yielding **1** (**1U** or **1A**).

We have considered a second approach to the desired ligands, starting from a trifold-protected cyclen derivative, bearing two orthogonal protecting groups (**Route B**). In **Route B** the stabilising arms are attached at the final stage of the synthesis to a cyclen already equipped with both the quencher and the antenna. The two secondary nitrogens (*N*7 and *N*10) are revealed after removal of the bifunctional oxalamide[32,33] protecting group in **7** or a suitable derivative. Stepwise attachment of the quencher and the antenna are possible by selective protection of one of the non-amidified nitrogens (*N*1 or *N*4). The forward sequence starts with monoprotection of the known cyclen oxamide **10** to furnish **9**.[33] Alkylation of the fourth (unprotected) nitrogen in **9** with either a suitably modified antenna, or a linker gives **8**. Removal of the protecting group is followed by the second alkylation step, this

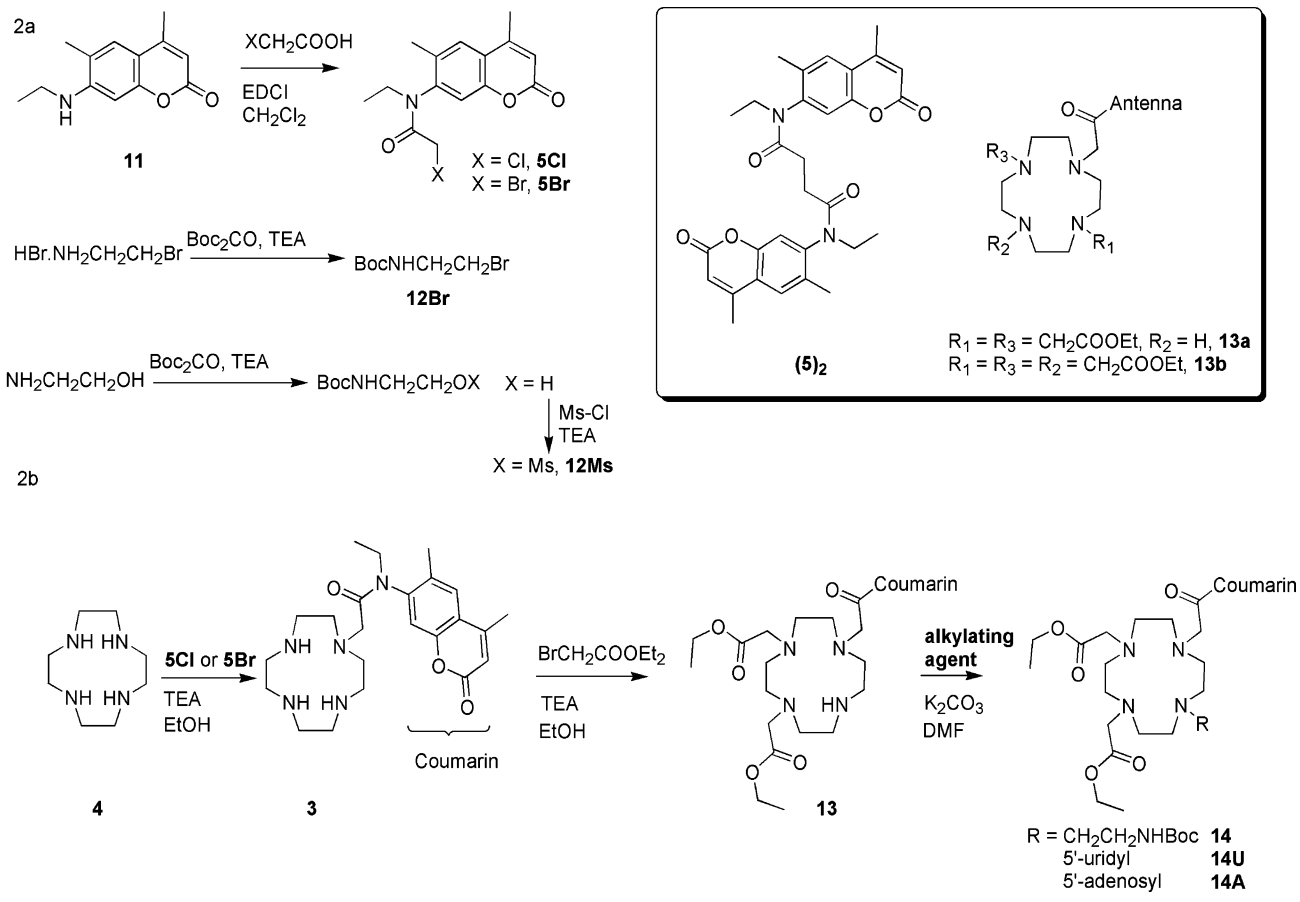


Scheme 1

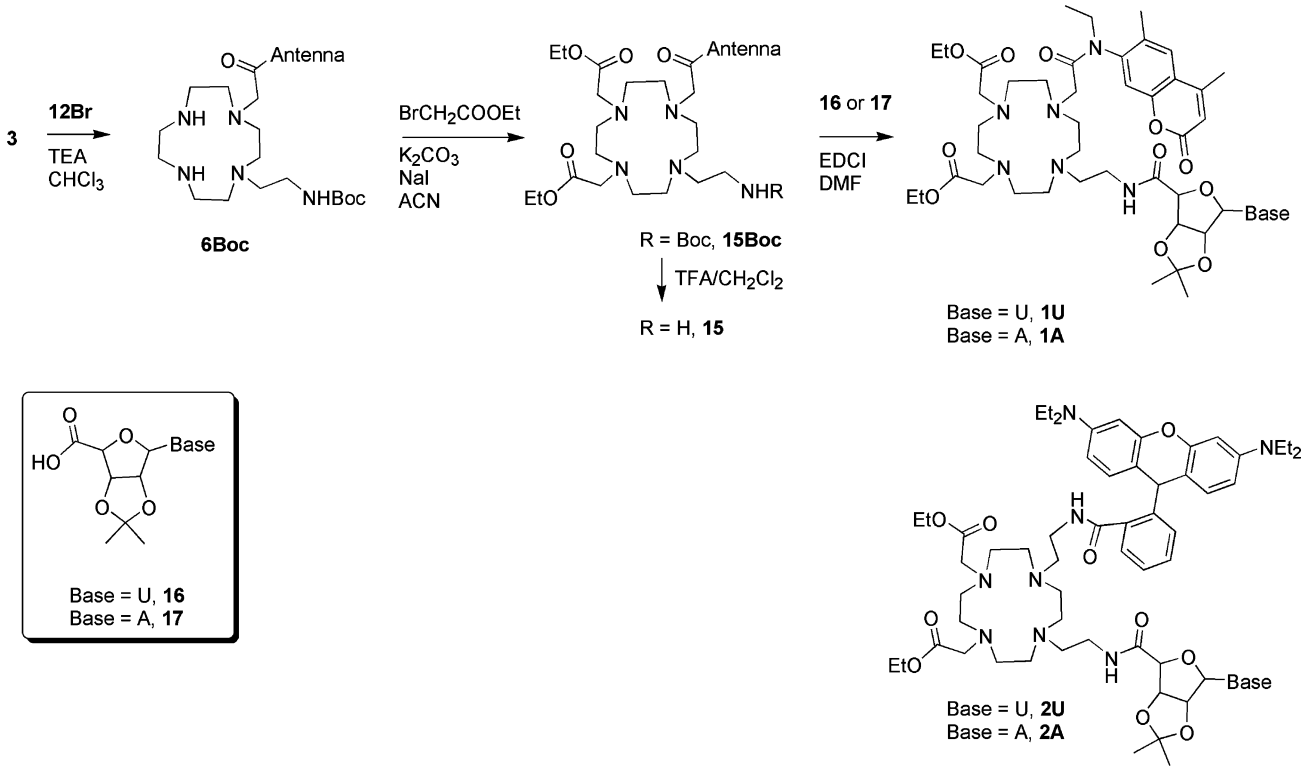
time with the quencher, or a linker to which the quencher can be attached at a later stage to yield **7**. Cleavage of the oxalamide with concentrated sodium hydroxide or hot hydrochloric acid, followed by dialkylation with ethyl bromoacetate would afford the desired *N*1,*N*4-functionalised ligand **1**.

The synthesis of the precursors is shown in Scheme 2a. Coumarin 2 (**11**) was coupled to chloro- or bromoacetic acid in EDCI-mediated amide bond-formations giving **5Cl** or **5Br** in excellent yield. Chloroacetic acid coupled to coumarin 2 without the formation of side-products. Unreacted coumarin 2 (33%) could be recovered by column chromatography. Bromoacetic acid reacted faster than chloroacetic acid and with quantitative consumption of coumarin 2, but this reaction gave rise to a small amount (13% by ^{1}H NMR spectroscopy) of another compound, tentatively identified as $(\mathbf{5})_2$ (see box in Scheme 2), which was difficult to remove. As $(\mathbf{5})_2$ could be removed in the successive steps, this was not a significant problem. Two linkers, **12Ms** and **12Br** were prepared from ethanolamine and bromoethylamine hydrochloride, respectively, by protection of the primary amino groups with a Boc protecting group under standard conditions. Two alkylating quenchers, **AMs** and **UMs** were also prepared by treating 2′,3′-isopropylidene adenosine or -uridine with Ms-Cl in the presence of TEA (see ESI†). Treatment of cyclen with **5Cl** or **5Br** in hot ethanol in the presence of triethylamine yielded mostly the monoalkylated derivative **3** after column chromatography on basic alumina. Alternatively, the alkylation could be carried out in warm DMF with potassium carbonate base. The latter method yielded sufficiently pure **3** for use in the following synthetic step after aqueous–organic work-up and trituration with diethyl ether. The major side product in this reaction was $(\mathbf{5})_2$. Dialkylation of **3** with ethyl bromoacetate yielded **13**, with the formation of some isomeric **13a** (<5%) and fully alkylated **13b** (30–35%). Reactions on the secondary nitrogen in **13** with a number of alkylating agents gave either low yields of the desired product, or no reaction at all (*e. g.* 12–15% with *N*-Boc bromoethylamine). Treatment of **13** with Ms-5′-*O*-adenosine yielded a fully equipped ligand **14A** in 3.5% yield after extensive chromatography, but the strategy could not be extended to the synthesis of **14U**. Therefore, the alternative route, based on our previous synthesis of **2U** and **2A** was implemented (Scheme 3).

cis-Alkylation on *N*4 of **3** was possible in chloroform with *N*-Boc-bromoethylamine (**12Br**). The *N*1,*N*4-alkylated **6Boc** was isolated after column chromatography. An analogous reaction with *N*-Boc-*O*-Ms-ethanolamine (**12Ms**) yielded the same dialkylated product in low yield (14%), presumably because of the increased steric demand of the mesityl group as compared to the bromide in what is likely to be an S_N2 reaction. For the same reasons, 5′-*O*-Ms nucleosides (**AMs**, **UMs**) also gave the corresponding *N*1,*N*4-functionalised cyclen derivatives in only moderate yields. Furthermore, quenchers introduced *via* 5′-*O*-Ms



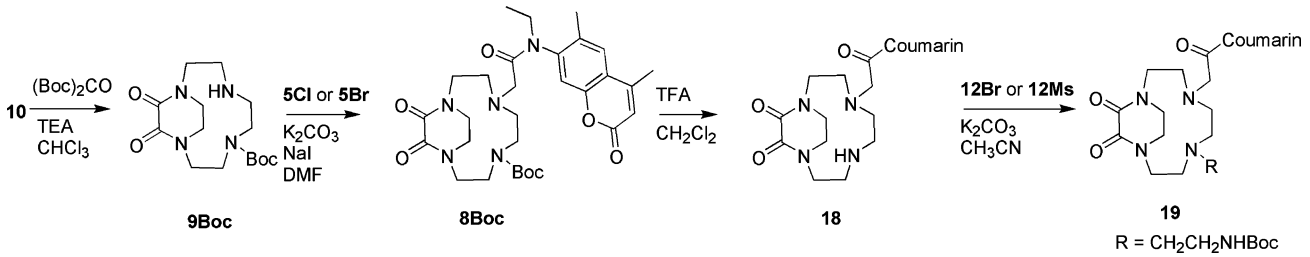
Scheme 2



Scheme 3

nucleosides would lack a carbonyl moiety necessary for an octadentate ligand. Treatment of **6Boc** with ethyl bromoacetate in the presence of K_2CO_3 and NaI in acetonitrile furnished the fully *N*-alkylated cyclen derivative **15Boc** in excellent yield. Cleavage of the Boc protection revealed the primary amino group to which 5′-carboxynucleotides **16** and **17**[34] could be coupled in an EDCI-mediated amidation in 84% and 47% yields, respectively. This short (5-step) synthesis could be applied to the preparation of several hundreds of milligrams of **1U** or **1A**. Complexation of Eu(III) and Tb(III) was accomplished by treatment of methanolic solutions of **1U** and **1A** with the corresponding anhydrous chlorides. The complexes ([**Eu**(**1U**)], [**Tb**(**1U**)], [**Eu**(**1A**)] and [**Tb**(**1A**)]) were isolated by precipitation from cold ether, followed by freeze-drying from water. A similar reaction sequence yielded [**Nd**(**2U**)], [**Yb**(**2U**)], [**Nd**(**2A**)] and [**Yb**(**2A**)].

Route B. The attempted route to synthesise the ligands **1U**,**A** from trifold-protected cyclen derivative **9Boc** is shown in Scheme 4. The known oxalamide **10**[33] could be protected on one secondary nitrogen with Boc_2CO in chloroform in the presence of TEA. After column chromatography **9Boc** was isolated in 70% yield. Treatment of **9Boc** with **5Cl** or **5Br** in the presence of potassium carbonate and sodium iodide in DMF afforded the desired product **8Boc** after standard aqueous–organic work-up and column chromatography in only moderate yields. Two side-reactions were identified: (1) decomposition of **5Cl** and **5Br** to coumarin 2 and (2) dimerisation of **5Cl** and **5Br** to yield $(\mathbf{5})_2$. A model reaction using *N*-methylaniline chloroacetate instead of **5Cl** under the same conditions proceeded with 51% yield, further supporting the theory that the low yields in the case of the coumarin 2 antenna were at least partially due to the instability of **5Cl** and **5Br**, in addition to the low reactivity of **9Boc**. Acidolysis of the Boc protecting group of **8Boc**, followed by neutralisation afforded secondary amine **18**. Treatment of **18** with either of the linkers (**12Br** or **12Ms**) gave derivatives of **19** in prohibitively low yields (10–12% yield after extensive chromatography). Therefore, **Route B** was abandoned at this point. The failure of this strategy is most likely due to a combination of several factors, among them the strongly electron-withdrawing nature of the oxalamide



Scheme 4

protection resulting in low reactivity of **9Boc** and **18**, possible steric hindrance caused by the coumarin 2, and the sensitivity of the reactants to the reaction conditions. The oxalamide is also locking the cyclen ring in a conformation that may render the lone pair of the fourth nitrogen less accessible (see MMX-calculated geometry in ESI†).

2. Characterisation

The intermediates and the ligands were characterised by ^{1}H and ^{13}C NMR spectroscopy. In most cases, to aid the assignment, ^{1}H-^{1}H COSY, ^{1}H-^{13}C correlation, DEPT and NOESY experiments were performed. Elemental compositions were confirmed by high resolution ESI-MS spectroscopy.

The lifetimes for the NIR emitting complexes were measured using a known method,[35] and were comparable to previously reported complexes.

The luminescent lifetimes of the europium and terbium complexes were determined following established procedures (Table 1).[36] The lifetimes are in good agreement with data obtained for similar compounds.[3,18,36] The number of co-ordinated solvent molecules (OH-oscillators, n) was calculated from the differences in the luminescent lifetimes of the complexes in H_2O and D_2O using eqn (1), where q stands for the number of water molecules bound to the central atom, A is an experimental value (0.525 ms^{-1} in the case of Eu per OH oscillator, 2.1 ms^{-1} for Tb). The rate constants, assuming first-order decays, are the reciprocals of the experimentally determined lifetimes. As expected, q is >1. The q values obtained for compounds [**Eu**(**1U**)], [**Tb**(**1U**)], [**Eu**(**1A**)] and [**Tb**(**1A**)] are somewhat higher than for similar octadentate ligands while being less than two as has been observed for heptadentate ligands.[1] The carbonyl group on the fourth arm bearing the quencher forms a seven-membered ring when coordinated the lanthanide ion and may not be as closely bound to the metal ion. It is probable that a second water molecule is partially coordinated at a longer distance as a result. It should be noted that the less polarisable carbonyl oxygen atoms on the ester groups makes them poorer donors for lanthanides compared to the amide groups. While we observed no decomplexation in solution, it is possible that a slight increase in lability due to the donor ester groups also allows a second water molecule to partially coordinate.

$$q = A(k_{H2O} - k_{D2O}) \quad (1)$$

Table 1 The lifetimes and the hydration states of the Eu, Tb complexes, and the lifetimes of the Nd and Yb complexes. Luminescence decay measured at 592 nm (Eu), 545 nm (Tb), 890 nm (Nd) and 980 nm (Yb)

	[Eu(1U)]	[Tb(1U)]	[Eu(1A)]	[Tb(1A)]
$\tau(D_2O)$	2.70 ms	0.35 ms	3.11 ms	0.56 ms
$\tau(H_2O)$	0.61 ms	0.27 ms	0.49 ms	0.40 ms
q	1.34	1.72	1.67	1.47
	[Nd(2U)]	**[Yb(2U)]**	**[Nd(2A)]**	**[Yb(2A)]**
τ/μs	0.09	2.61	—[a]	2.23
τ/μs[b]	0.47	0.89	0.23	1.17
τ/μs[c]	0.81	—[a]	0.16	—[a]

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

3. Luminescent properties

Neodymium and ytterbium complexes. The absorption spectra of the ligands **2U** and **2A** displayed maxima at 355 and 545 nm, indicative of the rhodamine antenna.[27] Excitation of [**Nd**(**2U**)], [**Yb**(**2U**)] and [**Yb**(**2A**)] at 355 nm resulted in characteristic near IR emission (Fig. 1 and ESI†). On the other hand, only very short-lived luminescence was observed for [**Nd**(**2A**)]. For all complexes, steady-state emission spectra were calculated by integration of each decay and plotting the values as the function of the wavelength. For **2ANd** without added substrate this was not possible due to the low signal-to-noise ratio, but a typical Nd-spectrum was obtained with emission lines centred at 890 nm ($^{4}F_{3/2} \rightarrow {}^{4}I_{9/2}$) and 1064 nm ($^{4}F_{3/2} \rightarrow {}^{4}I_{11/2}$) upon plotting the decay values for the adenosine- or uridine-treated [**Nd**(**2A**)]. Quenching of the antenna by the built-in quencher (adenosine) was almost complete. The quenching could be disrupted by engaging the quencher in base pairing with either uridine or adenosine. Thus, as proposed in the original design for these types of molecules, addition of the substrate 'turns on' the non-luminescent probe.

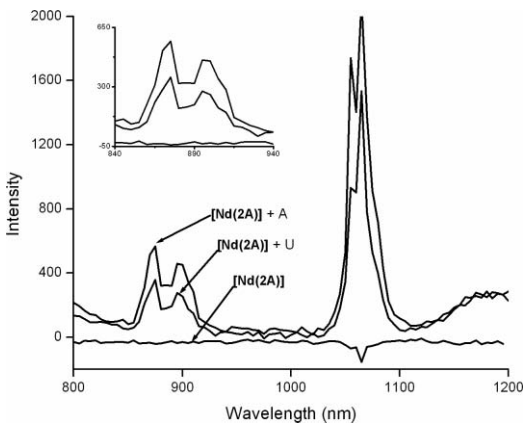


Fig. 1 Emission spectra for [**Nd**(**2A**)].

Compound [**Nd**(**2U**)] was only slightly luminescent, and was further quenched upon the addition of either its complementary base (adenosine) or its own base (uridine). The 890 nm emission of [**Nd**(**2A**)] + A, + U and [**Nd**(**2U**)] consisted of two peaks, at 875 nm and at 900 nm. A similar splitting was observed for the $^{4}F_{3/2} \rightarrow {}^{4}I_{11/2}$ transition (1055 nm and 1065 nm). The Yb-complexes were weakly luminescent with emission maxima at 980 nm ($^{2}F_{5/2} \rightarrow {}^{2}I_{7/2}$). Addition of the complementary bases to either [**Yb**(**2U**)] or [**Yb**(**2A**)] resulted in further decrease in the emission intensity. The changes in the luminescence lifetimes of [**Nd**(**2U**)], [**Nd**(**2A**)], [**Yb**(**2U**)] and [**Yb**(**2A**)] upon addition of complementary bases and solutions of the quenchers has already been reported.[27] Briefly, the Nd-complex [**Nd**(**2A**)] had a very short-lived luminescence, which increased considerably upon the addition of the complementary base uridine. In the cases of the other three compounds ([**Nd**(**2U**)], [**Yb**(**2U**)] and [**Yb**(**2A**)]), the lifetimes were comparable to those reported for similar octadentate complexes.[37] Addition of the complementary bases to [**Nd**(**2U**)], [**Yb**(**2U**)] and [**Yb**(**2A**)] resulted in a decrease of the lifetimes.

Europium and terbium complexes. The excitation spectrum of [**Tb**(**1A**)] in water (pH 7.4) displayed a maximum at 328 nm, which is consistent with the presence of the coumarin 2 antenna

(ESI†). Excitation of [**Eu(1U)**], [**Tb(1U)**], [**Eu(1A)**] and [**Tb(1A)**] is possible at 328 nm without interference from the metals or the quenchers. The emission spectra of [**Eu(1U)**] and [**Eu(1A)**] (Fig. 2) are indicative of highly asymmetrical species, with the high intensity of the hypersensitive $\Delta J = 2$ transition at 618 nm being particularly indicative of the asymmetrical environment around the europium ion. Furthermore the magnetic-dipole allowed $\Delta J = 1$ transition shows the components allowed by the low symmetry environment. The emission spectra have similar splitting patterns and intensities to heptadentate complexes reported by Parker[1] and the intensity and splitting pattern of the $\Delta J = 4$ is further indication of the unsymmetrical environment. It is also clear that the coumarin is not completely quenched by the nucleotides and is able to serve as the sensitiser. Addition of the complementary base adenosine to a solution of [**Eu(1U)**] in methanol, acetonitrile, or pH 7.4 MOPS buffer resulted in a modest increase in the luminescence intensity at $\Delta J = 1$ (588 nm) and $\Delta J = 2$ (613 nm) (ESI†). Consistent data could not be obtained for the adenosine quencher-equipped europium complex [**Eu(1A)**]. The Tb(III)-complexes were quenched by the addition of their substrates (ESI†). One equivalent of complementary base quenched [**Tb(1U)**] and [**Tb(1A)**] by approximately 37% and 20%, respectively. The quenching is presumably the result of direct quenching of the antenna by the added complementary base.

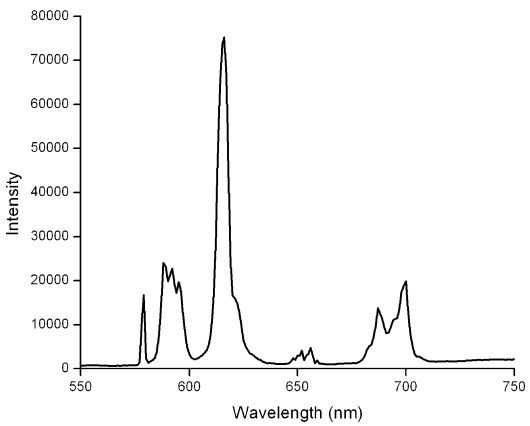


Fig. 2 Emission spectrum of [**Eu(1A)**].

When the luminescent lifetimes (τ) of [**Eu(1U)**] or [**Eu(1A)**] were monitored at 588 nm, slight (approximately 0.05 ms for both complexes) increases were observed upon addition of 1 equivalent of complementary base (Fig. 3) and this could be observed reproducibly. The τ of [**Tb(1U)**] increased and that of [**Tb(1A)**] slightly decreased upon addition of the complementary bases.

Treatment of Eu(III)-complexes [**Eu(1U)**] and [**Eu(1A)**] with *ds*DNA quenched the lanthanide emission, possibly due to intercalation of the coumarin 2 antenna with the DNA strands. A similar trend was observed for [**Tb(1A)**], while no consistent data could be obtained for [**Tb(1U)**]. The lifetimes did not show a consistent variation for [**Eu(1U)**] and [**Eu(1A)**], presumably due to the presence of a number of competing processes. A decrease was observed for the lifetime changes of [**Tb(1A)**], and a minor increase for [**Tb(1U)**] (data not shown).

The quenching of [**Eu(1U)**], [**Tb(1U)**], [**Eu(1A)**] and [**Tb(1A)**] by complementary bases and *ds*DNA were analysed by calculating

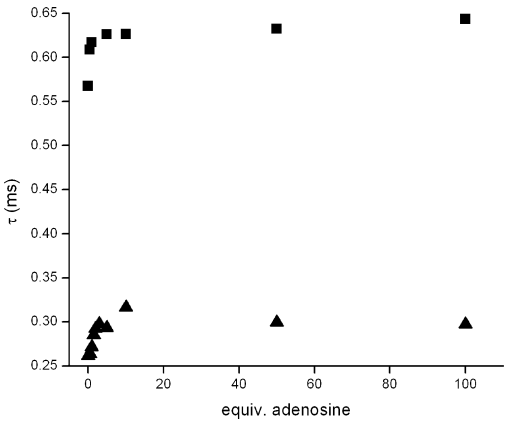


Fig. 3 Increase in luminescent lifetimes for [**Eu(1U)**] (upper) and [**Tb(1U)**] (lower) upon addition of adenosine.

Table 2 Stern–Volmer constants (K) of the quenching of the lanthanide complexes by DNA and the complementary bases

	[Eu(1U)]	[Tb(1U)]	[Eu(1A)]	[Tb(1A)]
c.b.[b]	[a]	6.8×10^3	[a]	8.4×10^3
DNA	[a]	1.0×10^4	1.8×10^3	6.8×10^3

[a] No consistent data were obtained and K could not be calculated. [b] *c.b.*: complementary base.

the Stern–Volmer constants from fitting $I(0)/I = 1 + K(SV)[Q]$ (I_0: initial intensity, I: actual intensity, $[Q]$: quencher concentration) to the initial (linear) sections of the Stern–Volmer plots.[38] The Stern–Volmer constants are summarised in Table 2. These are consistent with a dynamic quenching process occurring up until one equivalent of the substrate is added in the case of nucleosides. In this case the SV constants are a measure of K_d and are of an order for Watson–Crick interactions between nucleosides. This was confirmed by titrating the ligands with nucleoside and monitoring their ^{1}H NMR spectra in DMSO. Monitoring shifts in the NH protons of the nucleosides gave binding constants comparable to the binding of nucleoside base pairs in DMSO. The interaction with *ds*DNA is more complex with static quenching likely to play a role if intercalation is occurring.

Thus for the all the europium and terbium complexes reported in this paper the lanthanide sensitisation could not be completely switched off and intensity and lifetime changes were modest upon substrate addition. This reflects that the rate of energy transfer from the antenna is faster than the electron transfer quenching processes. The changes upon binding are therefore minimal. Adenosine, being more readily oxidised, is a superior quencher to uridine. This is similar to the behaviour reported by Seidel *et al.*[29] Thus when added as substrate it is also able to quench the antenna directly.

Conclusions

A series of donor–acceptor–quencher (**D–A–Q**) triads were synthesised and characterised. The donors are coumarin 2 or rhodamine, and the acceptors are the luminescent lanthanides Eu(III) and Tb(III), or Nd(III) and Yb(III), respectively. The **D–A–Q** moieties are held together by a cyclen scaffold. Disruption

of the lanthanide sensitisation by the antennae was attempted by the incorporation of nucleotide quenchers into the molecules. In one instance, ([**Nd**(**2A**)]), a very short-lived luminescence was observed that could be extended by the addition of the substrate to [**Nd**(**2A**)]. This highly efficient and flexible synthesis enables the straightforward introduction of other antenna–quencher pairs to fine-tune the photophysical and recognition behaviour of similar lanthanide complexes.

Experimental

General procedures

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded in $CDCl_3$ unless noted otherwise. Absorption spectra and fluorescence spectra were collected at room temperature in H_2O or D_2O, unless noted otherwise. Infrared absorption spectra were recorded as neat or paraffin-admixed thin films, or as KBr pellets. Low resolution ESI-MS spectra were recorded on VG Quattro equipment in the positive ion mode by direct infusion of methanolic solution. High resolution mass spectra were recorded at the EPSRC National Mass Spectrometry Service at Swansea. Melting points are uncorrected. Solvents were dried according to standard procedures. Preparative chromatography was performed using silica or alumina (80–200 mesh). Thin layer chromatography was performed on silica or alumina. Samples were visualized by UV-light (254 nm and 365 nm), I_2-vapor or $KMnO_4$–K_2CO_3.

The Eu and Tb complexes were titrated as solutions in pH 7.4 MOPS buffer, in HPLC-grade acetonitrile or methanol. Luminescent lifetimes were determined in water and D_2O following standard procedures.[36]

Emission spectra and excited state lifetimes were recorded on a FluoromaxP or a Fluorolog 3 Tau spectrofluorimeter. Emission intensities and luminescent lifetimes of the Nd and Yb complexes were determined analogously to a reported method.[35] Steady state luminescence spectra were obtained by recording the luminescence decay from 800 nm to 1200 nm (Nd, 5 nm intervals) and from 800 nm to 1100 nm (Yb, 2.5 nm intervals). The decays were integrated, and plotted as the function of the wavelength to give the total emission spectra.

The synthesis of the Nd and Yb complexes and ligands has been reported previously.[27]

1U and 1A. A solution of 5′-carboxylic nucleotide (2.00 mmol, 2 eq) and EDCI (0.43 g, 2.2 mmol, 2.2 eq) in DMF (5 mL) was treated with a sample of **15** (0.65 g, 1.0 mmol) in DMF (1 mL). The reaction mixture was stirred at room temperature for 24 h. The DMF was removed at reduced pressure. The sample was dissolved in a mixture of CH_2Cl_2 and water. The phases were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with water and dried ($MgSO_4$). Purification of the sample by column chromatography [silica, CH_2Cl_2–^{i}PrNH$_2$ (0 → 20%)] gave pale yellow solids:

1U. (B = U): Yield: 0.78 g, 84%; HR-ESI-MS ($C_{45}H_{64}N_8O_{13}$): calcd 947.4485 $[M + Na]^+$, obsd 947.4477 $[M + Na]^+$; ^{1}H NMR δ 1.06 (t, J = 7.1 Hz, 3H), 1.17 (t, J = 7.1 Hz, 6H), 1.28 (s, 3H), 1.51 (s, 3H), 2.23 (d, J = 1.7 Hz, 3H), 2.35 (s, 3H), 2.17–3.35 (m, 33H), 3.22 (s, 4H), 4.05 (q, J = 7.1 Hz, 4H), 4.01–4.15 (m, 1H), 4.53 (m, 1H), 4.92–4.98 (m, 2H), 5.66 (m, 1H), 5.74 (m, 1H), 6.26 (d, J = 0.9 Hz, 1H), 7.03 (d, J = 4.0 Hz, 1H), 7.48 (s, 1H), 7.66 (m, 1H), 7.80 (br s, 1H); ^{13}C NMR 12.62, 13.93, 14.10, 17.19, 17.30, 18.55, 25.00, 26.94, 37.00, 43.00, 51.82, 52.57, 53.35, 54.86, 55.48, 59.94, 60.02, 82.99, 83.97, 102.44, 113.81, 115.48, 117.20, 119.84, 127.11, 131.72, 142.27, 151.54, 151.85, 160.09, 169.00, 169.64, 169.64, 169.76, 171.27; mp: turns glassy at 87 °C, gas evolution at 110 °C, complete melting at 122 °C; IR (ν_{max}/(cm^{-1}), KBr): 2983, 2938, 2826, 1729, 1697, 1669, 1627, 1614, 1558, 1526, 1455.

1A. (B = A): Yield: 0.45 g, 47%; HR-ESI-MS ($C_{46}H_{66}N_{11}O_{11}$): calcd 948.4938 $[M + H]^+$, obsd 948.4947 $[M + H]^+$; ^{1}H NMR δ 1.05 (t, J = 7.1 Hz, 3H), 1.12–1.21 (m, 6H), 1.33 (s, 3H), 1.54 (s, 3H), 2.22 (s, 3H), 2.40 (s, 3H), 2.15–3.34 (m, 33H), 4.01–4.07 (m, 4H), 5.34–5.36 (m, 1H), 5.40 (m, 1H), 5.84 (br s, 2H), 6.15 (m, 1H), 6.25 (s, 1H), 7.10 (d, J = 11.3 Hz, 1H), 7.47 (s, 1H), 8.03 (d, J = 1.5 Hz, 1H), 8.22 (d, J = 1.5 Hz, 1H); ^{13}C NMR 12.79, 14.24, 14.27, 17.35, 18.67, 25.04, 26.83, 43.15, 51.45, 51.58, 51.80, 52.41, 55.15, 56.32, 56.51, 60.21, 83.56, 84.07, 86.78, 91.43, 113.85, 115.70, 117.48, 117.61, 119.75, 119.98, 127.05, 131.86, 140.19, 149.50, 151.54, 151.60, 152.03, 153.09, 155.54, 160.24, 168.77, 171.48; mp: turns glassy at 105 °C, gas evolution at 114 °C, complete melting at 137 °C; IR (ν_{max}/(cm^{-1}), KBr): 2981, 2828, 2361, 2341, 1654, 1577, 1559, 1541, 1522, 1507, 1498.

[Eu(1U)] and [Eu(1A)]. A solution of **1U** or **1A** (74 mg or 71 mg, 0.074 mmol) in HPLC-grade methanol (0.5 mL) was treated with anhydrous $EuCl_3$ (17.5 mg, 0.067 mmol). The solution was refluxed under Ar for 12 h. The reaction mixture was allowed to cool back to room temperature. The solution was poured into cold diethyl ether (5 mL). The mixture was centrifuged and the ether was poured off the white precipitate. The solid was suspended in ether (5 mL), and centrifuged again. The ether was decanted again. The solid residue was dissolved in distilled water (1 mL) and was filtered through a plug of cotton wool. The sample was freeze-dried. White solids were obtained:

[Eu(1U)]. (B = U): Yield: 60 mg, 74%; ESI-MS: 897 [**1U** − 2 Et]$^+$; ^{1}H NMR (CD_3OD) δ 9.23, 9.97, 12.02, 13.42, 15.81, 17.28, 18.53, 19.70, 20.94, 21.41, 22.58.

[Eu(1A)]. (B = A): Yield: 63 mg, 76%; ESI-MS: 920 [**1A** − Et]$^+$, 1043 [[**Eu(1A)**] − 2 Et]$^+$; ^{1}H NMR (CD_3OD) δ several peaks between 1.63 and 8.40, 10.84, 18.01.

[Tb(1U)] and [Tb(1A)]. A solution of **1U** or **1A** (74 mg or 71 mg, 0.074 mmol) in HPLC-grade methanol (0.5 mL) was treated with anhydrous $TbCl_3$ (19 mg, 0.67 mmol). The solution was refluxed under Ar for 12 h. The reaction mixture was allowed to cool back to room temperature. The solution was poured into cold diethyl ether (5 mL). The mixture was centrifuged and the ether was poured off the white precipitate. The solid was suspended in ether (5 mL), and centrifuged again. The ether was decanted again. The solid residue was dissolved in distilled water (1 mL) and was filtered through a plug of cotton wool. The sample was freeze-dried. White solids were obtained:

[Tb(1U)]. (B = U): Yield: 63 mg, 78%; ESI-MS: 1101 [M + H_2O]$^+$, 897 [**1U** − Et]$^+$.

[Tb(1A)]. (B = A): Yield: 66 mg, 80%; ESI-MS: 920 [**1A** − Et]$^+$; ^{1}H NMR (CD_3OD) δ 10.76, 13.85, 16.05, 18.04, 20.91.

3. Method A

Cyclen (**4**, 2.52 g, 14.7 mmol) and **5Cl** (2.87 g, 9.78 mmol) were dissolved in dry DMF (30 mL). K_2CO_3 (3 g, 22 mmol) and NaI (3 g, 20 mmol) were added to the solution. The reaction mixture was stirred at 80 °C under an Ar atmosphere for 24 h. The DMF was removed at reduced pressure. The residue was dissolved in a mixture of water and CH_2Cl_2. The phases were separated and the aqueous phase was extracted with 2 × 30 mL CH_2Cl_2. The combined organic phases were washed with 30 mL water, dried over $MgSO_4$, filtered, and evaporated to dryness. The dark yellow oily residue was dissolved in a minimum amount of methanol and triturated with ether. The crystals were filtered and washed with small portions of cold diethyl ether. The product was isolated as pale yellow crystals in 87% yield (3.65 g). For characterisation see **Method B**.

Method B

Cyclen (**4**, 2.52 g, 14.7 mmol) and **5Cl** (2.87 g, 9.78 mmol) were dissolved in 100% EtOH (25 mL), in the presence of TEA (4.12 mL, 29.34 mmol, 3 eq). The solution was heated at reflux for 24 h under an inert atmosphere. The solvents were evaporated. Chromatography on basic alumina [pH 9.5, CH_2Cl_2–MeOH (0 → 10%)] yielded a pale yellow foam (1.89 g, 45%): HR-ESI-MS ($C_{23}H_{35}N_5O_3$): calcd 430.2813 obsd 430.2814 $[M + H]^+$; ^{1}H NMR δ 1.11–1.16 (t, $J = 6.8$ Hz, 3H), 2.27 (s, 3H), 2.40 (s, 3H), 2.66–3.32 (m, 17H), 3.41 (q, $J = 6.8$ Hz, 2H), 3.99–4.11 (m, 1H), 6.26 (s, 1H), 7.04 (s, 1H), 7.50 (s, 1H); ^{13}C NMR 12.80, 15.22, 17.53, 18.69, 43.36, 45.33, 46.40, 47.65, 51.87, 65.79, 115.81, 117.27, 120.21, 127.48, 131.95, 142.23, 151.58, 152.07, 160.10, 169.88.

5Cl. Coumarin 2 (**11**, 210 mg, 1.00 mmol) was dissolved in CH_2Cl_2 (10 mL). Chloroacetic acid (0.13 g, 1.30 mmol) and EDCI (0.19 g, 1.00 mmol) were added and the solution was stirred at room temperature for three days. The solution was diluted with CH_2Cl_2 and water, the phases were separated, the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with dilute NaOH and water. The organic layer was dried ($MgSO_4$), filtered and concentrated. Chromatography [silica, ethyl acetate–hexane (1 : 1)] yielded recovered coumarin 2 (70 mg, 33%) and a white, crystalline solid (200 mg, 67%): HR-ESI-MS ($C_{15}H_{16}NO_3Cl$): calcd 294.0891 $[M + H]^+$, obsd 294.0891 $[M + H]^+$; ^{1}H NMR δ 1.11 (t, $J = 7.0$ Hz, 3H), 2.27 (s, 3H), 2.39 (s, 3H), 3.14–3.26 (m, 1H), 3.67 (s, 2H), 4.04–4.18 (m, 1H), 6.28 (s, 1H), 7.08 (s, 1H), 7.48 (s, 1H); ^{13}C NMR 13.04, 17.94, 19.17, 42.05, 44.54, 116.62, 118.16, 120.88, 127.75, 132.51, 142.55, 151.96, 152.78, 160.55, 165.86; mp: 172 °C; IR (ν_{max}/(cm^{-1}), KBr): 1722, 1678, 1626, 1615, 1558, 1502, 1444, 1402.

5Br. Coumarin 2 (**11**, 4.06 g, 18.70 mmol) and bromoacetic acid (10.40 g, 74.80 mmol, 4 eq) were dissolved in CH_2Cl_2. EDCI (14.36 g, 74.80 mmol, 4 eq) was added in small portions to the vigorously stirred solution. The reaction was allowed to proceed for 24 h at room temperature. The reaction mixture was diluted with water and CH_2Cl_2. The phases were separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic phases were washed with water and NaOH (2 M), dried ($MgSO_4$), and filtered. The sample was concentrated to dryness to yield a white solid (5.28 g, 84%), which was ~87% pure, as shown by ^{1}H NMR. This material was of good enough quality to use in the step. Analytically pure samples could be obtained by column chromatography [silica, EtOAc–CH_2Cl_2 (1 : 9)]: HR-ESI-MS ($C_{15}H_{16}NO_3Br$) calcd 338.0386 $[M + H]^+$, obsd 338.0389 $[M + H]^+$; ^{1}H NMR δ 1.10 (t, $J = 7.0$ Hz, 3H), 2.27 (s, 3H), 2.40 (s, 3H), 3.13–3.23 (m, 1H), 3.61 (s, 2H), 4.08–4.18 (m, 1H), 6.28 (s, 1H), 7.09 (s, 1H), 7.49 (s, 1H); ^{13}C NMR 12.49, 17.38, 18.64, 41.54, 43.99, 116.04, 117.58, 120.38, 127.24, 131.97, 142.17, 151.29, 152.02, 159.96, 165.45; mp: 171 °C; IR (ν_{max}/(cm^{-1}), KBr): 1723, 1676, 1626, 1614, 1558, 1502, 1444.

(5)$_2$. (Limited characterisation) ESI-MS: 513 $[M + H]^+$; ^{1}H NMR δ 1.11 (t, $J = 7.1$ Hz, 6H), 2.23 (s, 6H), 2.39 (s, 6H), 3.20–3.31 (m, 2H), 3.45 (d, $J = 15.8$ Hz, 2H), 3.74 (d, $J = 15.8$ Hz, 2H), 4.07–4.19 (m, 2H), 6.28 (s, 2H), 7.03 (s, 2H), 7.53 (s, 2H); ^{13}C NMR 12.76, 17.12, 18.65, 30.29, 43.67, 60.66, 116.18, 117.58, 120.56, 132.01, 140.39, 151.24, 152.20, 159.79, 171.14; mp: 133–134 °C; IR (ν_{max}/(cm^{-1}), KBr): 3450, 3066, 2983, 2930, 1728, 1662, 1611, 1557, 1499, 1448.

6Boc. A sample of **3** (3.10 g, 7.23 mmol) was dissolved in $CHCl_3$ (100 mL) and TEA (10.20 mL, 7.40 g, 72.3 mmol) was added to the solution. The solution was flushed with Ar for 10 min. *N*-Boc-Bromoethylamine (1.77 g, 7.95 mmol), dissolved in $CHCl_3$ (20 mL) was added dropwise (1 h). Stirring was continued for 24 h. Water was added. The phases were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with water and dried ($MgSO_4$). The solution was concentrated. The sample was subjected to column chromatography [silica, CH_2Cl_2–^{i}PrNH$_2$ (0 → 20%)] to afford a pale yellow solid (1.21 g, 29%): HR-ESI-MS ($C_{30}H_{48}N_6O_5$): calcd 573.3759 $[M + H]^+$, obsd 573.3765; ^{1}H NMR δ 1.06 (t, $J = 7.1$ Hz, 3H), 1.22 (s, 9H), 2.24 (s, 3H), 2.39 (s, 3H), 2.38–3.23 (m, 24H), 4.02 (m, 1H), 6.26 (s, 1H), 7.00 (s, 1H), 7.48 (s, 1H); ^{13}C NMR 12.72, 17.22, 18.55, 28.19, 38.79, 43.09, 45.96, 47.41, 47.63, 51.49, 51.75, 51.98, 53.16, 53.35, 77.98, 115.67, 117.07, 120.02, 127.25, 131.65, 142.70, 151.44, 152.05, 156.07, 159.96, 169.56, 172.73; mp: 55 °C; IR (ν_{max}/(cm^{-1}), KBr): 3368, 2975, 2933, 2824, 1703, 1664, 1626, 1614, 1559, 1502.

15Boc. A sample of **6Boc** (1.09 g, 1.91 mmol) was dissolved in CH_3CN (6 mL). K_2CO_3 (4.25 g, 30.6 mmol), NaI (4.58 g, 30.6 mmol) and ethyl bromoacetate (2.12 mL, 3.19 g, 19.1 mmol) were added to the solution. The mixture was refluxed under Ar for 24 h. The sample was concentrated. The residue was dissolved in a mixture of water and CH_2Cl_2. The phases were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with water and dried ($MgSO_4$). The solution was concentrated. Column chromatography [silica, CH_2Cl_2–^{i}PrNH$_2$ (0 → 5%)] yielded an off-white solid (0.99 g, 70%): ESI-MS: 743 $[M + H]^+$, 765 $[M + Na]^+$; ^{1}H NMR δ 1.12 (m, 3H), 1.25 (m, 6H), 1.38 (s, 9H), 2.32 (s, 3H), 2.45 (s, 3H), 2.00–3.21 (m, 33H), 4.15 (m, 1H), 6.29 (s, 1H), 6.95 (s, 1H), 7.62 (s, 1H); ^{13}C NMR 12.58, 14.06, 14.12, 17.53, 18.87, 28.38, 43.48, 43.71, 50.52, 53.40, 55.10, 55.73, 56.27, 61.23, 61.36, 67.74, 79.98, 105.01, 115.84, 116.78, 117.10, 120.49, 127.99, 141.48, 141.55, 151.86, 160.06, 170.64, 173.24.

15. A solution of **15Boc** (0.99 g, 1.33 mmol) in CH_2Cl_2 (10 mL) was treated with TFA (5 mL). The reaction mixture was stirred at room temperature for 30 min. The volatile components were

evaporated. The residue was redissolved in CH_2Cl_2. Concentrated aqueous $KHCO_3$ was added in small portions until pH 8 was reached. The aqueous phase was extracted with CH_2Cl_2, and the combined organic phases were washed with water. The organic phase was dried ($MgSO_4$). A pale yellow solid was obtained after evaporation of the solvent, which was used without further purification (0.72 g, 84%): ESI-MS: 643 $[M + H]^+$, 686 unassigned, ^{1}H NMR δ 1.07 (t, $J = 6.9$ Hz, 3H), 1.13–1.26 (m, 6H), 2.25 (s, 3H), 2.39 (s, 3H), 2.32–3.74 (m, 27H), 4.04–4.17 (m, 5H), 6.26 (s, 1H), 7.00 (s, 1H), 7.50 (s, 1H), 7.95 (s, 1H), 8.01 (br s, 1H); ^{13}C NMR 12.63, 14.00, 17.20, 18.64, 43.22, 49.16, 49.82, 50.95, 51.88, 53.39, 54.95, 60.42, 63.99, 115.74, 117.11, 127.47, 151.99, 160.09, 162.44, 164.67, 166.44, 171.38.

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