CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.201100390

Towards Libraries of Luminescent Lanthanide Complexes and Labels from Generic Synthons

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Abstract: A synthetic approach is developed to obtain families of luminescent lanthanide complexes and markers from a generic family of precursors built from nonadentate coordination sites. The syntheses of the precursors, based on a directed regioselective nucleophilic aromatic substitution on polyfluoropyridines, are described. Functionalisation of the synthons on the aromatic moieties allowed the introduction of labelling functions and/or the extension of the electronic delocalisation, with concomitant changes in the spectroscopic properties. The synthesis of two such families of ligands and of some of their complexes of Eu^{III} and Tb^{III} are described, and the photophysical properties of the complexes were measured, revealing excellent luminescence quantum yields reaching unity in some cases. For some of these complexes, the emphasis was further put on the preparation of an *N*-hydroxylsuccinimide (NHS) ester as activated function for labelling. The Tb and La complexes in the NHS activated form were synthesized and fully characterized. The labelling was first demonstrated on amino functionalized polymer beads and characterized by time-resolved luminescence microscopy. In a second step, the activated Tb complex was used for the labelling of GFR44 monoclonal antibody, and was applied to the detection of carcinoembryonic antigene (CEA) within the frame of a time-resolved fluoroimmunoassay. Comparison with a commercially available kit based on a europium cryptate as energy donor confirms the efficiency of Tb to act as an energy donor with an unoptimised 35% increase of the detection efficiency.

Keywords: fluoroimmunoassays · labelling · lanthanides · luminescence · terbium

Introduction

It is now clearly established that analytical techniques based on luminescence detection are among, if not the most sensitive tools, some of them having sensitivities that reach the atomic level.[1,2] Combining sensitivity, chemically induced selectivity[3] and user-friendly technologies, they have reached a major place in the daily work of most scientists and the last technological and scientific developments of the past few decades further open wide perspectives. Among those, the emergence of new tools in the luminescent pool, such as luminescent proteins,[4] semi-conducting nanocrystals,[5] or up-converting phosphors,[6] greatly extended the promises of luminescence sensing.

Nevertheless, there still remain large spaces for "older" tools that keep on being unoccupied by emerging luminophores. Among those, time-resolved or gated techniques are still dominated by the luminescent lanthanide complexes and their very specific properties.[7] The parity forbidden *f–f* transitions of lanthanide elements result in extremely long luminescence lifetimes, generally ranging from μs to ms, which is two to five orders of magnitude longer than common organic fluorophores. A long lived luminescent excited state is particularly interesting when combined to time-resolved (or gated) acquisition of the luminescence signal,[8] generally leading to large improvements in the signal-to-noise ratio and a concomitant sensitivity increase.[9] In the field of fluorescence resonance energy transfer (FRET), long lived luminescent lanthanide complexes can also bring significant sensitivity gain,[10,11] and were even shown to be the only chemical species able to transfer energy by FRET to particular acceptors that are quantum dots.[12] Among other benefits in FRET experiments, lanthanide complexes also display narrow emission bands, almost not perturbed by the ligand field, such as the typical $^5D_4 \rightarrow {}^7F_J$ ($J=6$ to 3) transitions of Tb in the visible region.[7] The wells in the emission spectra can then be fitted with the emission region of fluorescent acceptors for multiplexed

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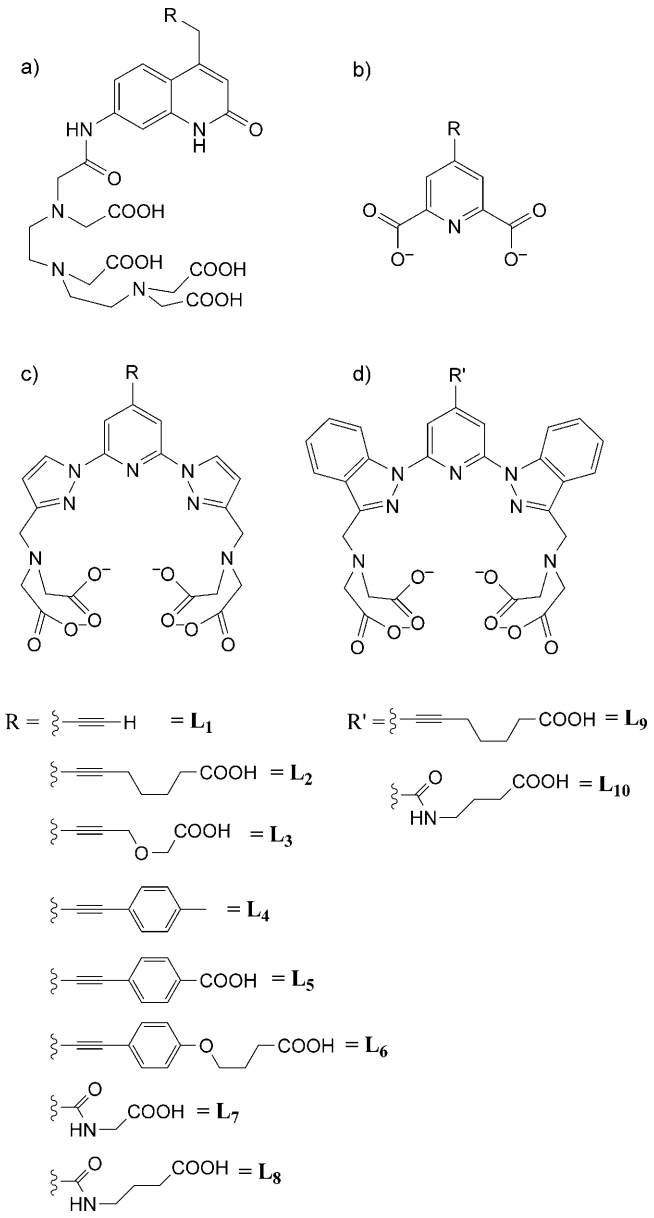
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201100390.

FRET experiments,[13] now demonstrated with up to five different acceptors simultaneously.[14]

Nevertheless, the flip side of having long lifetimes resides in time expensive synthetic protocols required to build up an efficient antenna effect[15] in these complexes. Absorption coefficients of these forbidden *f–f* transitions are extremely weak (few units $M^{-1}cm^{-1}$) and an efficient population of the lanthanide centred excited state requires powerful excitation sources or the complexation with strongly absorbing chromophoric ligands able to transfer their absorbed energy to the lanthanide cation. Despite the numerous requirements to obtain accomplished luminescent lanthanide complexes and labels, this field of research is still very fruitful with newly emerging antennas[16] and structures,[17] and applications such as biphotonic excitation,[18] near infrared emission[19] or data storage.[20]

Numerous efforts have been devoted to the understanding of the relationship between the antennas and the luminescence properties,[21] but only few of them tried to keep a constant coordination environment around the metal, while varying a peripheral part that affects the electronic properties of the ligand. Nagano and co-workers studied the influence of the substitution of an aromatic ring (Scheme 1a) on the energy and electron transfer pathways within Eu and Tb complexes.[22] But in these systems, the substituted aromatic part was not directly coupled to the antenna, thereby favouring through space processes and the DTPA based complexing ligand did not fully saturate the first coordination sphere of the lanthanide cations. More recently, Maury and co-workers[23] described a broad family of *para*-substituted dipicolinic acid ligands (Scheme 1b) and the influence of the substitution on the luminescence properties of europium complexes, with a particular emphasis on the two photon absorption properties. In that case, efficient photosensitization can only be obtained for the LnL_3 complexes, pointing to possible dissociation troubles at high dilution.

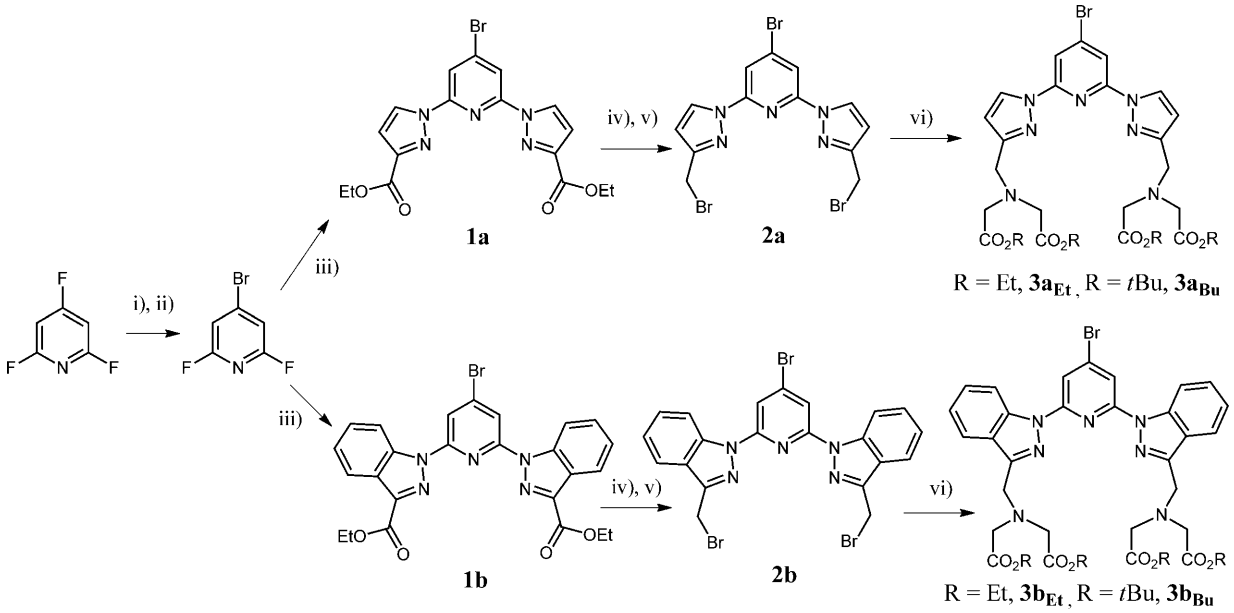
Our research groups recently demonstrated that this approach can be extended to a nonadentate coordination site able to fulfil the coordination sphere of lanthanide cations (Scheme 1c), leading to highly luminescent visible and near infrared emitting complexes, some of them displaying lyotropic properties.[24] The present study aims at demonstrating that the synthetic approach can be extended to other nonadentate precursors and that a judicious choice of the substitution allowed for the introduction of a labelling function for the grafting of the complexes on beads or biological material. Labelling of a monoclonal antibody for CEA[25] was also described and the labelled antibody was applied to time-resolved fluoro-immunoassays for the CEA detection.



Scheme 1. Examples of previous efforts devoted to the understanding of the relationship between the antennas and the luminescence properties (a and b) and ligands described in this work (c and d).

Results and Discussion

Synthesis of the complexation site precursors: Considering that our aim was to obtain a synthon that can provide a nonacoordinated complexation site with four anionic carboxylate functions by a simple deprotection step and that it has to be electronically and/or chemically modified, we focused our attention toward the introduction of a halogen atom into an aromatic framework. Using conventional metal assisted coupling reactions, the introduction of unsaturated groups or aminoacid functions would then allow to change the electronic levels on the ligand or to introduce activated functions for labelling. For that purpose, a complexation site based on a 4-bromo-2,6-bis{3-[*N*,*N*-bis(carboxymethyl)aminomethyl]pyrazol-1-yl}-pyridine (Scheme 1c; R = Br), was a target of choice considering both the spectroscopic properties[26] and the water stability[27] of the lanthanide complexes obtained with this kind of ligands.



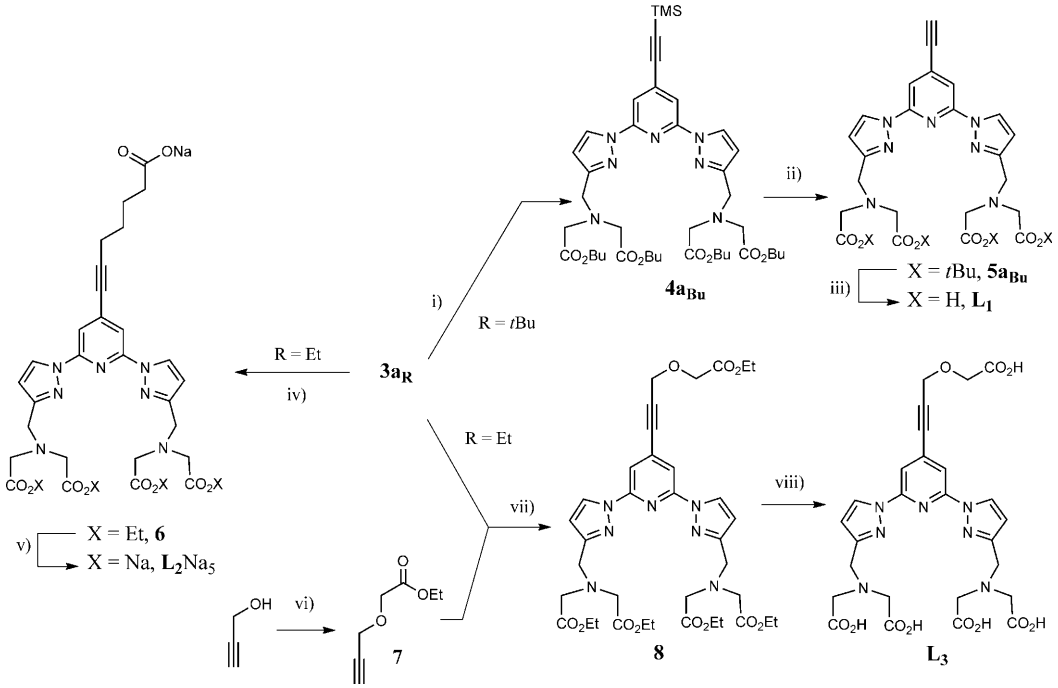
Scheme 2. Synthetic protocol for the preparation of the coordination-site precursors. i) $H_2NNH_2.H_2O$, THF, 50 °C, 2 h, 85 %.[28b] ii) Br_2, $CHCl_3$, reflux, 6 h, 38 %.[29] iii) ethylpyrazolate, NaH, DMF, 0 °C, 2 h, 47 % for **1a** or ethylindazolate, 0–40 °C, 5 h, 94 % for **1b**. iv) $LiAlH_4$, 0 °C; then v) PBr_3, DMF, RT, 12 h, 43 % for **2a** and 35 % for **2b**. vi) $HN(CH_2COOR)_2$, THF/Et_3N, 50 °C, 14 h, for $\mathbf{3a_{Et}}$ (87 %) and $\mathbf{3a_{Bu}}$ (85 %) or K_2CO_3, CH_3CN/THF, 60–80 °C, 14–15 h for $\mathbf{3b_{Et}}$ (56 %) and $\mathbf{3b_{Bu}}$ (84 %).

The synthetic protocol for the preparation of the precursor functionalized by a bromine atom at the *para* position of the pyridyl ring is described in Scheme 2. Taking advantage of the recent work of Schlosser et al. on the regioselectivity of polyhalogenated pyridine compounds,[28] the use of 4-bromo-2,6-difluoropyridine was envisaged to direct the nucleophilic aromatic substitution of pyrazolate anion at the 2 and 6 positions. Thanks to the superior reactivity of the *para* position in 2,4,6-trifluoropyridine,[28b] hydrazine was introduced at this position and subsequently reacted in a modified Sandmeyer-type reaction to obtain the *para* bromo substitution.[29] As expected, the nucleophilic aromatic substitution with the anion of ethyl pyrazolate proceeded regioselectively in very smooth experimental conditions compared to reported examples using substitution on 2,6-dibromopyridines.[26,30] The obtained diester, **1a**, was readily reduced to the corresponding alcohol using $LiAlH_4$. It is worth noting that, despite our efforts to optimize this step, the reduction is unfortunately not limited to the ester functions and a non negligible quantity of the compounds was reduced to the 4-*H*-pyridine analogue, as previously observed on similar *para*-bromo-substituted terpyridines.[31] Furthermore, chromatographic separation of the polar diol intermediate and its non-brominated side product could not be achieved in our hands, and it was decided to run the next bromination step with PBr_3 on the mixture, to allow an easier separation of the less polar **2a** with respect to the derivative non-brominated in *para* position of the pyridine. A final nucleophilic substitution with alkyl iminodiacetate gave the precursors **3a**, either in its ethyl or *tert*-butyl version, that allow final deprotection of the acetic acid function in either basic or acidic conditions, depending on the *para* substituent.

The same protocol can be used with ethylindazolate, obtained from the commercially available indazolic acid,[32] affording **1b** and a second set of precursor of the complexation site in which the absorption and emission properties will be greatly varied (see below). This second example illustrates the versatility of the method which may be enlarged to other nucleophilic moieties.

Synthesis of the ligands: To illustrate the functionalisation of the complexation precursors, two kinds of metal-catalyzed coupling reactions were investigated. The first one is a Sonogashira coupling with an acetylenic function catalyzed by Pd. As described in Scheme 3, the reaction conditions were first set up using a trimethylsilyl protected acetylene, showing conventional conditions ($[Pd(PPh_3)_2Cl_2]$ with CuI) to be adequate to obtain $\mathbf{4a_{Bu}}$ with a reasonable 73 % yield. Deprotection of TMS was performed in good yield using tetrabutylammonium fluoride in THF, yielding the intermediate compound $\mathbf{5a_{Bu}}$, which could be used for additional coupling at the acetylenic position. Ligand $\mathbf{L_1}$ was finally obtained by deprotection of the *tert*-butyl groups with TFA. In similar conditions, $\mathbf{5a_{Et}}$ can be obtained by coupling of $\mathbf{3a_{Et}}$ with TMS-acetylene yielding $\mathbf{4a_{Et}}$ (70 %) followed by TBAF deprotection in 83 % yield (see the Experimental Section).

Pd coupling can then be used to introduce a labelling function such as a carboxylic acid. In a first example, $\mathbf{3a_{Et}}$ was coupled with 6-heptynoic acid to yield compound **6**, which was subjected to a saponification of the ethyl ester functions to give ligand $\mathbf{L_2}$ as its sodium salt. To improve the water solubility of the linker, we also developed the synthesis of compound **7**, according to modifications of the literature procedure,[33] containing an ether function, and which



Scheme 3. Protocols for Sonogashira coupling reactions with alkynyl compounds. i) TMS-acethylene, $[Pd(PPh_3)_2Cl_2]$, CuI, THF/Et_3N, RT, 14 h, 73% for R = *t*Bu, 70% for R = Et. ii) TBAF, THF, 2 h, 0 °C, 81% for R = *t*Bu, 83% for R = Et. iii) R = *t*Bu, TFA, CH_2Cl_2, RT, 2 h, quant. iv) 6-heptynoic acid, $[Pd(PPh_3)_2Cl_2]$, CuI, THF/Et_3N, 50 °C, 14 h, 77%. v) NaOH, $MeOH/H_2O$, 60 °C, 3 h, 80%. vi) Ethylbromoacetate, NaH, THF, RT, 1 h, 47%. vii) $[Pd(PPh_3)_2Cl_2]$, CuI, THF/Et_3N, 50 °C, 24 h, 40%. viii) NaOH, $MeOH/H_2O$, 60 °C, 4 h, then HCl 1 M, quant.

was similarly coupled on $\mathbf{3a_{Et}}$ before saponification to yield ligand $\mathbf{L_3}$.

The coupling with ethynyl functions can also be performed with functionalized arylethynyl moieties. Scheme 4 depicts the synthesis of ligands $\mathbf{L_4}$ to $\mathbf{L_6}$, in which the precursor was coupled to a *para*-tolyl, a *para*-carboxyphenyl or a *para*-4-oxybutanoic acid phenyl group through the acetylenic spacer, these functions representing respectively electron donating or withdrawing groups (the carboxylic acid function of $\mathbf{L_5}$ playing both rules depending on the pH), electronically coupled to the chromophoric pyridyl unit of the ligand.

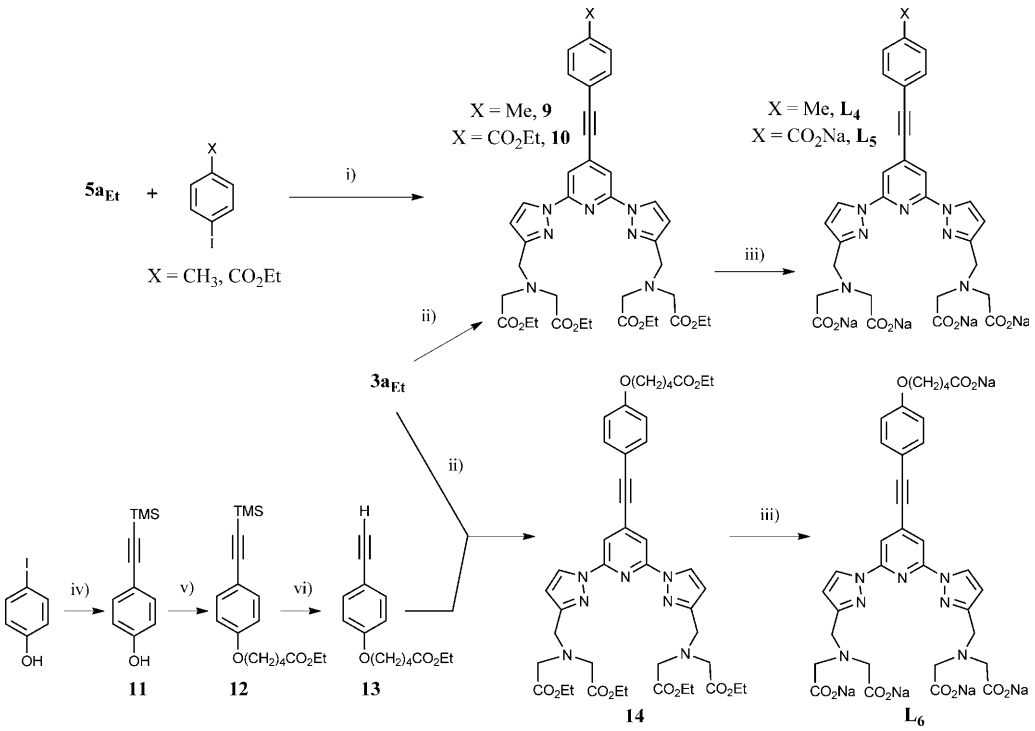
As shown in Scheme 4, the Pd coupling can be performed either with **3a** as halogenated aromatic compound or with the previously described $\mathbf{5a_{Et}}$ as the source of alkyne function. Also, the Pd catalyst used in the coupling reaction can be either Pd^0 or a combination of Pd^{II} in the presence of CuI, but the former species requires higher temperatures for the coupling to be efficient. Finally, the terminal carboxylate function of $\mathbf{L_5}$ and $\mathbf{L_6}$ should potentially be used as labelling function in the form of an activated ester, providing one can differentiate them from the iminodiacetate functions during the activation step (see below).

The second kind of metal catalyzed coupling reaction was a Pd assisted carboamidation process, in which a protocol previously used for carboalkoxylation reactions has been adapted.[34] Scheme 5 describes the protocols developed to obtain ligand $\mathbf{L_7}$ and $\mathbf{L_8}$ by this procedure.

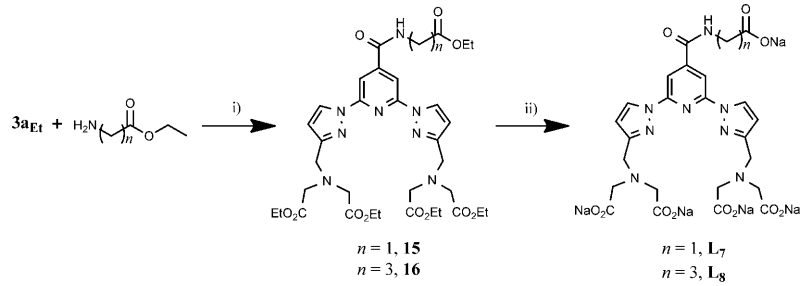
Finally, the different Pd catalyzed reactions were also carried out on compound **3b**, generating either the alkyne substituted compound **17** or the carboamide **18**, which afforded respectively ligands $\mathbf{L_9}$ and $\mathbf{L_{10}}$ after ester hydrolysis (Scheme 6).

Synthesis and characterization of the complexes: Lanthanide complexes were obtained by mixing equimolar amounts of the ligand and lanthanide salts in water, followed by thermal equilibration for few hours at 60–70 °C, after which the pH of the solution was adjusted to ca. 7.0 using diluted aqueous solutions of NaOH or HCl. Table 1 summarizes the main photophysical properties of the complexes prepared.

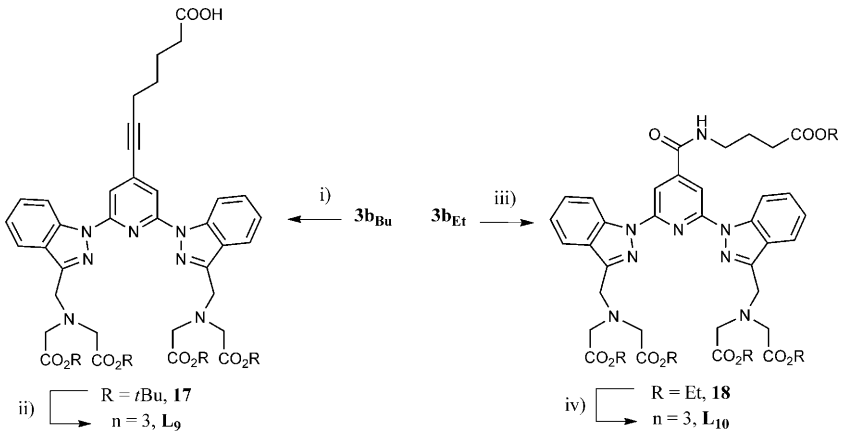
The UV/Vis absorption spectra of the complexes of $\mathbf{L_1}$ to $\mathbf{L_8}$ are dominated by intense $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions centred on the pyridyl[36] and pyrazolyl groups (Figure 1).[37] Interestingly, the absorption bands at ca. 278 nm, generally associated to a second component between 265 and 269 nm, are present in all complexes of $\mathbf{L_1}$ to $\mathbf{L_8}$ and are little perturbed by the functionalization in the *para* position of the central pyridyl ring. By analogy with other ligands,[37] these transitions were attributed to pyrazolyl centred absorption bands, and they tend to indicate that the *N*-pyrazolyl moiety does not allow for a full delocalisation of the electronic cloud over the entire aromatic core. In contrast, the low energy component was directly influenced by the substitution pattern, pointing to pyridyl centred transitions, and its intensity is almost doubled when the substitution pattern



Scheme 4. Protocols for Sonogashira coupling reactions with *para*-substituted phenyl alkynyl compounds. i) $[Pd(PPh_3)_4]$, THF/Et_3N, 60 °C, 14 h, 55 %. ii) $[Pd(PPh_3)_2Cl_2]$, CuI, THF/Et_3N: *p*-tolulacetylene, 24 h, 50 °C, 63 % for **9**; ethyl-4-iodobenzoate, 14 h, 50 °C, 78 % for **10**; and **13**, 50 °C, 14 h, 53 % for **14**. iii) NaOH, MeOH/H_2O, 60 °C, 3 h, 86 % for L_4; 86 % for L_5 and 87 % for L_6. iv) TMS-acetylene, $[Pd(PPh_3)_2Cl_2]$, CuI, THF/Et_3N, RT, 14 h, 78 %. v) NaH, THF, 0 °C then ethyl 4-bromobutyrate, NaI, RT, 50 °C, 48 h, 37 %. vi) TBAF, THF, 0 °C, 1 h, then RT, 2 h, quant.



Scheme 5. Synthesis of L_7 and L_8. i) $[Pd(PPh_3)_2Cl_2]$, Et_3N/toluene, CO flow (1 atm), 100 °C, 14 h, 81 % for **15**; 12 h, 57 % for **16**. ii) NaOH, MeOH/H_2O, 60 °C, 3 h, 94 % for L_7; 95 % for L_8.



Scheme 6. Synthesis of ligands L_9 and L_{10}. i) 6-heptynoic acid, $[Pd(PPh_3)_2Cl_2]$, CuI, THF/Et_3N, 40 °C, 72 h, 40 %. ii) TFA, CH_2Cl_2, RT, 4 h, 52 %. iii) Ethyl 4-aminobutyrate hydrochloride, $[Pd(PPh_3)_2Cl_2]$, Et_3N/tol., CO (1 atm), 100 °C, 24 h, 54 %. iv) NaOH, MeOH/H_2O, 60 °C, 4 h, 50 %.

contains a second aromatic ring such as in complexes of L_4 to L_6.

Changing the pyrazolyl groups to indazolyl resulted in a bathochromic shift of the absorption, as would be expected from the increase of the conjugation, with a maximum pointing at 360 nm for complexes of L_9 and L_{10} and extending up to 390 nm. This absorption was accordingly attributed to indazolyl centred transitions. A second absorption band is observed with maximum at 320 nm, which can likely be attributed to pyridyl centred transitions as for the pyrazolyl based complexes.

For all complexes, excitation into the UV/Vis absorption bands resulted in the observation of the characteristic emission patterns of the Eu or Tb complexes. As a typical example, Figure 2 displays the emission spectra of the Eu and Tb

Table 1. Photophysical properties of the prepared complexes.

	Absorption[a]	Emission			
	λmax [nm] (ε [M^{-1} cm^{-1}])	τ_{H_2O} [ms][e]	τ_{D_2O} [ms][e]	Φ_{H_2O} [%][e]	q[b]
Eu$\mathbf{L_1}$	332 (5540)	1.30	2.40	12	0.1
	278 (12000)				
	250 (19300)				
Tb$\mathbf{L_1}$[c]	330 (8900)	2.51		78	
	279 (18600)				
	268 (18100)				
Eu$\mathbf{L_2}$	328 (7450)	1.2	2.4	8	0
	277 (18100)				
	265 (33200)				
Tb$\mathbf{L_2}$	328 (9030)	2.6	3.0	42	0
	277 (20400)				
	265 (39700)				
Eu$\mathbf{L_3}$	318 (6500)	1.4	2.4	4.5	0
	278 (13600)				
	269 (15700)				
Tb$\mathbf{L_3}$	318 (6800)	2.5	3.0	19	0
	278 (15400)				
	269 (17500)				
Eu$\mathbf{L_4}$	318 (20300)	1.3	2.4	15	0
	280 (18300)				
	273 (18500)				
Tb$\mathbf{L_4}$	318 (19200)	1.3	2.2	3.4	–[d]
	280 (18300)				
	273 (18500)				
Eu$\mathbf{L_5}$	314 (23500)	1.1	2.3	15	0.3
	280 (23600)				
	272 (23800)				
Eu$\mathbf{L_6}$	334 (22800)	1.1	2.3	14	0.3
	277 (20600)				
	267 (22800)				
Eu$\mathbf{L_7}$	328 (8670)	1.3	2.4	8	0.1
	278 (21200)				
	271 (21100)				
Tb$\mathbf{L_7}$	325 (9950)	2.5	3.1	>90	0
	278 (23700)				
	271 (23300)				
Eu$\mathbf{L_8}$	327 (6600)	1.4	2.5	12	0.1
	279 (16300)				
	271 (16000)				
Tb$\mathbf{L_8}$	327 (6900)	2.8	3.1	>90	0.2
	279 (16500)				
	271 (16000)				
Tb$\mathbf{L_9}$	309 (11400)	0.9	1.35	35	–[d]
	360 (8800)				
Eu$\mathbf{L_{10}}$	320 (11400)	1.15	1.84	16	0
	360 (10700)				
Tb$\mathbf{L_7}$	325 (9950)	2.5	3.1	>90	0
	278 (23700)				
	271 (23300)				

[a] In 0.01 m TRIS/HCl buffer at pH 7.0. [b] According to Ref. [35a] for Eu and Ref. [35b] for Tb (see the Experimental Section for details of the calculation). [c] measured in 0.01 m TRIS/HCl buffer at pH 7.0 for a Tb/L2 = 1:1 concentration. [d] not applicable (see text). [e] estimated errors are ±15% on ϕ and ±5% on τ.

complexes of $\mathbf{L_8}$. The Tb emission spectrum is composed of three intense emission bands with maxima at 485, 545 and 578 nm, attributed to the $^5D_4 \rightarrow {}^7F_J$ transitions with $J = 6$ to 4 respectively,[38] a medium intensity band at 615 nm corresponding to $J = 3$, and three weak bands between 640 and 670 nm corresponding to $J = 2$ to 0. The emission spectra of the Eu complexes are similarly composed of the $^5D_0 \rightarrow {}^7F_J$ transitions with: i) a weak emission band at ca. 580 nm for the non-degenerated $J = 0$ forbidden transition; ii) a pattern of three distinct bands ranging from 585 to 605 nm for the $J = 1$ transition, which indicates a low symmetry of the complex (at best containing a two fold symmetry axis);[39] iii) an intense emission between 615 and 630 nm for the hypersensitive $J = 2$ transition; iv) a weak emission from 645 to 660 nm for $J = 3$; and v) a broad emission of medium intensity from 680 to 710 nm corresponding to $J = 4$. As evidenced from the corresponding excitation spectra, the excitation in the UV domain can be directly correlated with the absorption spectra of the complexes (Figure 2), as a result of an efficient ligand to metal energy transfer,[15] the so-called antenna effect.[40]

The luminescence lifetime of the Eu complexes in water are comprised between 1.1 and 1.4 ms, pointing to an excellent shielding of the metal from the environment and in particular from water molecules. Thanks to the measurements of the lifetimes in D_2O, it was possible to calculate the hydration number according to the theoretical treatments published in the literature,[35] pointing to an absence of water molecule in the first coordination sphere, in accordance with a perfect shielding of the metal by the nonadentate ligand. The luminescence quantum yields of these complexes in water are rather good with values ranging from 4.5 to 16%.

Similarly, the lifetimes of the Tb complexes are also very long, ranging from 2.5 to 2.6 ms, except for the three complexes of $\mathbf{L_4}$, $\mathbf{L_9}$ and $\mathbf{L_{10}}$. A common feature of these three compounds is the presence of an intense low energy absorption band significantly red shifted. It is suspected that the corresponding $^3\pi\pi^*$ level on the ligand becomes close in energy from the Tb $^5D_4$ level, resulting in back energy transfer.[21] This supplementary deactivation process decreased the luminescence lifetime and the theoretical treatment for the calculation of the hydration numbers[35] is no more applicable in this case. For all other complexes, the hydration number was determined to be close to zero, indicative of a saturated coordination sphere. The luminescence quantum yields are very good and quantitative when the para position is substituted with a carboxamide function, in excellent agreement with previous results of the literature.[26,41] Considering the excellent results obtained for the Tb complexes of $\mathbf{L_7}$ and $\mathbf{L_8}$, a carboxamide function was further chosen to develop the labelling chemistry. Regarding the short length of the glycine residue of $\mathbf{L_7}$, possibly leading to undesired H-bonding interaction in the peptide coupling reaction, the longer propylene spacer of the aminobutyrate function of $\mathbf{L_8}$ was preferred.

Activation and labelling experiments: Considering the efficiency obtained with the Tb complex of ligand $\mathbf{L_8}$ function-

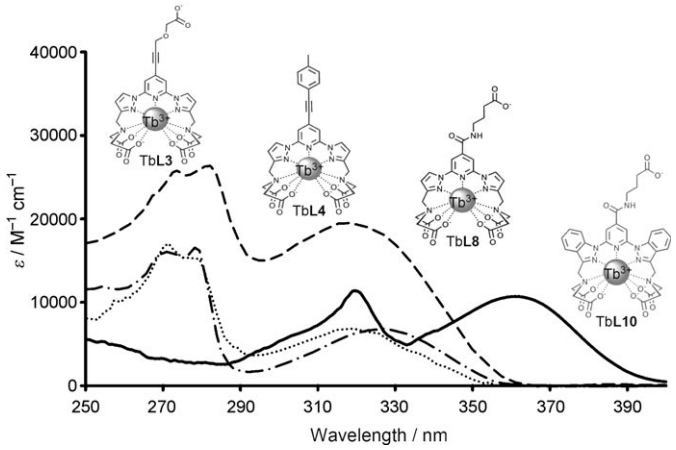


Figure 1. UV/Vis absorption spectra of the Tb complexes of $\mathbf{L_3}$ (dotted), $\mathbf{L_4}$ (dashed), $\mathbf{L_8}$ (dotted–dashed), and $\mathbf{L_{10}}$ (solid line) in 0.01 M TRIS/HCl buffer at pH 7.0.

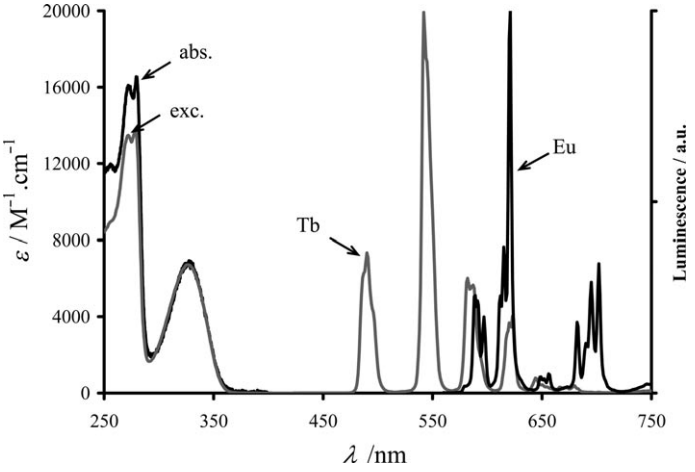
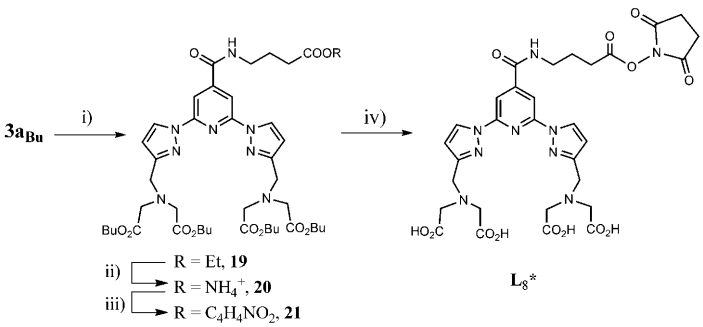


Figure 2. Absorption, excitation ($\lambda_{em}=615$ nm) and emission ($\lambda_{exc}=327$ nm) spectra of $Na_2[Eu\mathbf{L_8}]$ and emission spectrum of $Na_2[Tb\mathbf{L_8}]$ in 0.01 M TRIS/HCl, pH 7.0.

alized in *para* position by a carboxamide function, we focussed our attention towards the use of this ligand for grafting on biomolecules. Taking into account the paramagnetic contribution of the Tb cations, which excluded simple interpretation of NMR data, we first turned our attention towards the diamagnetic lanthanum complex, allowing the follow up of the activation reactions by ^{1}H NMR experiments. For that purpose, $Na[La\mathbf{L_8}]$ was synthesized and fully characterized. In a first set of experiments, the formation of the NHS ester on the dangling amino butyrate arm was attempted by coupling experiments on the complex in water with EDCI as a coupling agent. ^{1}H NMR rapidly revealed a messy spectrum with the presence of numerous singlet peaks in the 2.5–2.8 ppm region, together with a loss of symmetry in the aromatic region, attributed to a non selective activation process which also occurred on the coordinating carboxylate functions. It was thus decided to perform the activation prior to deprotection of the coordinating carboxylate functions, as depicted in Scheme 7.



Scheme 7. Synthesis of $\mathbf{L_8}$*. i) $[Pd(PPh_3)_2Cl_2]$, CO (1 atm), toluene/Et_3N, 100 °C, 12 h, 54 %. ii) NaOH, THF/H_2O, 60 °C, 4 h, then NH_4OH, 70 %. iii) DIEA, CH_2Cl_2, DSC, RT, 4 h, 52 %. iv) TFA, $CHCl_3$, 12 h, RT, 65 %.

Starting from the *tert-b*utyl protected synthon $\mathbf{3a_{Bu}}$, a carboamidation reaction afforded compound **19**, which was saponified with NaOH to selectively deprotect the dangling ester function, leading to compound **20**. The activated NHS ester was obtained by reaction of **20** with disuccinimidylcarbonyl (DSC) in the presence of the Hünig's base, affording compound **21**, which was subjected to a selective acidic hydrolysis of the *tert*-butyl esters, affording the activated form of $\mathbf{L_8}$ denoted $\mathbf{L_8}$*. The lanthanum complex of $\mathbf{L_8}$* was obtained by mixing equimolar amount of $\mathbf{L_8}$* and lanthanum chloride. Figure 3 displays the ^{1}H NMR spectra of $\mathbf{L_8}$* and

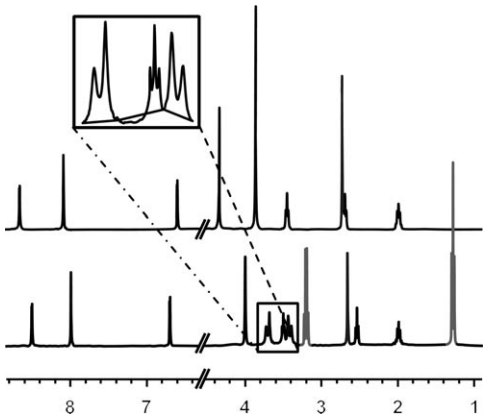


Figure 3. ^{1}H NMR spectra of $\mathbf{L_8}$* (top) and of $(Et_3NH)[La\mathbf{L_8}*]$ (bottom) in D_2O (400 MHz). Inset: enlargement of the region of the methylenic protons of $(Et_3NH)[La\mathbf{L_8}*]$.

of $(Et_3NH)[La\mathbf{L_8}*]$ in D_2O. The complexation was clearly evidenced by the appearance of an AB spin system in the 3.35-3.75 ppm region, corresponding to the diastereotopic protons of the N-CH_2-COO^- methylene groups upon coordination.[27]

The two steps of activation/complexation of $\mathbf{L_8}$ were also followed by means of IR spectroscopy in the solid state (Figure 4). The introduction of the NHS group was evidenced by the appearance of a strong absorption band at 1732 cm^{-1}, which was attributed to the C=O stretching absorption band of the succinimide cycle,[42] together with two

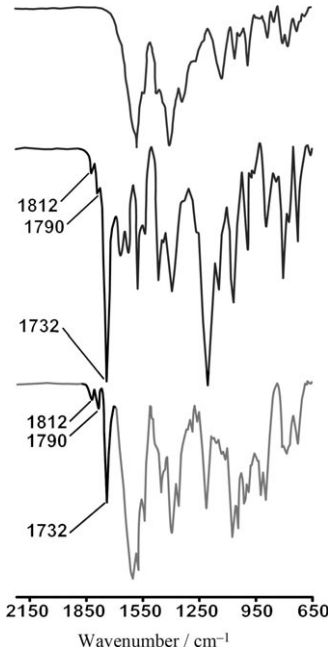


Figure 4. IR spectra of ligand $\mathbf{L_8}$ (top), its activated NHS ester form $\mathbf{L_8}$* (middle) and the terbium complex $(Et_3NH)[Tb\mathbf{L_8}^*]$ (bottom) in the solid state.

weak absorption bands at 1790 and 1812, already observed in other activated NHS esters.[43] After TFA hydrolysis of the *tert*-butyl esters of compound **21**, the activated $\mathbf{L_8}$* is obtained in its acidic form and this can be observed with a significant shift of the C=O stretching vibrations at 1660 cm^{-1} and 1620 cm^{-1} for the COOH functions of $\mathbf{L_8}$*, but at 1750 cm^{-1} for the sodium carboxylate functions of $\mathbf{L_8}$.[44] Upon complexation of Tb, the carboxylate forms are restored and the corresponding stretching band is again shifted at lower energy to 1600 cm^{-1}, while the absorption bands at 1732, 1790 and 1812 cm^{-1}, associated with the NHS ester function, remained unchanged.

Finally, the $(Et_3NH)[Tb\mathbf{L_8}^*]$ complex was further characterized by ES/MS spectrometry which displayed a molecular peak at 882.1 m/z units in the negative mode for the $[Tb\mathbf{L_8}^*]^-$ anion.

Labelling of aminofunctionalised beads and characterisation by time-resolved luminescence microscopy: To check the availability of the activated Tb complex to act as a label, it was reacted with amino functionalized polyacrylate beads. Amberzyme oxirane™ beads of 100 to 200 μm diameters were treated with diaminopropane in the presence of $LiClO_4$,[45] according to a previously published procedure.[46] The labelling process was performed in water by agitating at room temperature for 12 hours a mixture containing the beads and the activated complex in water in the presence of diisopropylethyl amine. After filtration of the beads, they were thoroughly washed with water, MeOH, THF and finally Et_2O. The isolated beads were characterized by microscopy. Figure 5 (left) displays a micrograph of a mixture of Tb labelled and unlabelled beads as observed by optical transmission microscopy. When observed in the fluorescence mode, the labelled beads displayed a strong green luminescence, while unlabelled beads appeared as pale blue as a result of the residual fluorescence of the polymer core (see graphical abstract for real colours images).

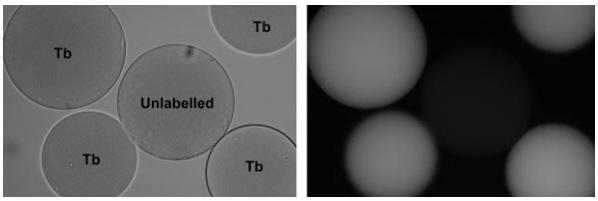


Figure 5. Transmission (left) and fluorescence (right) microscopy images of a mixture of Tb labelled and unlabelled beads.

The same region of interest was then observed in the time-resolved mode, in which a temporal delay is implemented between the pulsed excitation of the sample and integration of the emitted signal (Figure 6).[47] This method is well suited to remove undesired signals associated to light scattering in the apparatus and autofluorescence of the observed samples, thereby offering a large improvement of the signal to noise ratio.[9] The delay time was first set to zero, corresponding to the fluorescence mode. The corresponding image then reflects the observation of Figure 5 in which both labelled and unlabelled beads could be observed, even though the autofluorescence of unlabelled beads is far less intense than that of the Tb labelled ones. Upon imposition of a 0.1 ms delay, the fluorescence signal of unlabelled beads disappeared and only the long lived signal associated to Tb could be evidenced and this signal can still be observed even after very long delay time up to 10 ms. By integrating the emitted signal as a function of the delay time, it was also possible to get the luminescence lifetime of the Tb labels in the solid state (Figure 7). The emitted signal could be satisfactorily fitted with a single exponential decay, giving a lifetime of 2.2 ms in the solid state, that is slightly shorter than that observed in solution for the complex (2.8 ms, Table 1).

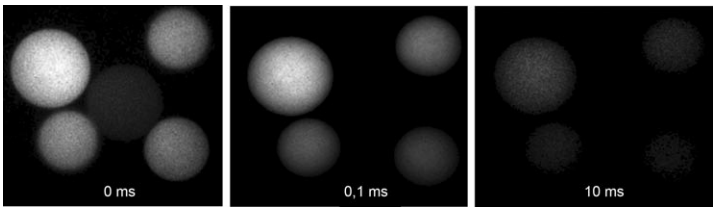


Figure 6. Time-resolved luminescence micrographs of a mixture of labelled and unlabelled beads observed after a delay of 0 (left), 0.1 (middle) and 10 (right) ms.

Biolabelling of antibodies and fluoroimmunoassay for CEA: The ability of the $[Tb\mathbf{L_8}^*]$ complex to be used as a luminescent label of biomaterial was checked within the frame of a homogeneous fluoroimmunoassay for the detection of carci-

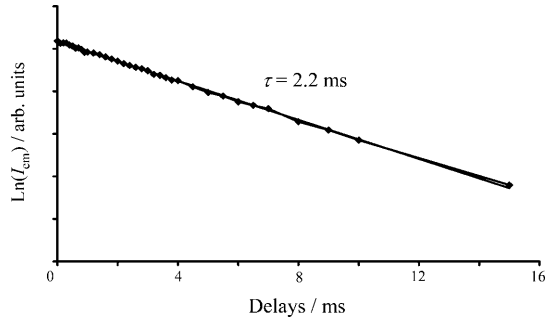


Figure 7. Evolution of the intensity of the emission signal of the Tb labelled beads as a function of the delay time.

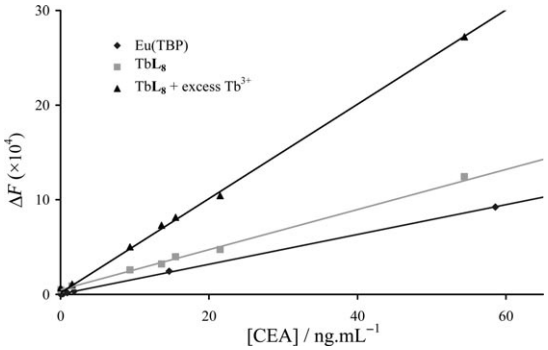


Figure 9. Evolution of the time-resolved intensity ratio (ΔF) as a function of the concentration of CEA in the assay.

noembryonic antigene (CEA), a glycoprotein which is found over-expressed in a number of cancer types, especially colorectal ones.[25] The principle of the immunoassay is depicted in Figure 8. GFR44 and G15 are two monoclonal antibodies

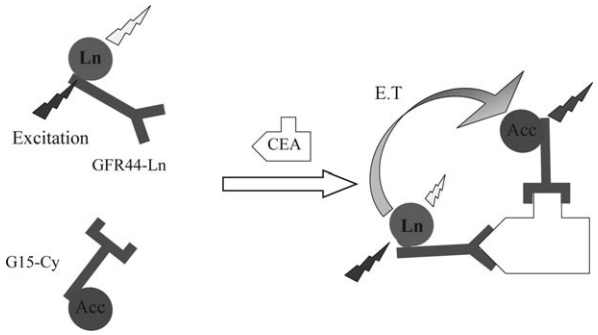


Figure 8. Schematic representation of the CEA fluoroimmunoassay.

that recognize specific epitopes of CEA in an immunogenic reaction.[48] GFR44 was labelled with a lanthanide complex as energy donor, [Tb**L**$_8$] or the europium cryptate [Eu(TBP)],[10,49] which was used as a reference in the commercially available reference kit, and G15 was labelled with cyanine5 (Cy5) acting as energy acceptor.

In a mixture containing both antibodies but no antigene, excitation of the lanthanide donor in the UV domain led to the characteristic emission of the lanthanide used, from which the spurious emission due to direct excitation of the fluorescent acceptor is removed by time resolved detection starting 50 μs after the pulsed excitation and during 400 μs. In the presence of the CEA antigene, the immunogenic reactions takes place, bringing the Ln donor and the Cy5 acceptor in close spatial proximity. Excitation of the donor then leads to an energy transfer process (ET) from the Ln donor to the cyanine acceptor. Due to the long-lived character of the donor, the energy transfer rate is generally slow, resulting in a delayed fluorescence[11b,50] of the acceptor with lifetimes reaching hundreds of μs.[51] The ratio of the time-resolved emission intensities of the acceptor over that of the donor, expressed as ΔF, is then proportional to the concentration of the CEA antigen present in solution. Figure 9 displays the evolution of the ΔF ratio obtained with the [Tb**L**$_8$] label and the reference cryptate for increasing concentrations of CEA in the assay. The results show that the emitted signal measured for the Tb**L**$_8$-Cy5 donor-acceptor system is 35% more intense than that obtained for the reference kit using the Eu(TBP) cryptate as energy donor. Nevertheless, these results are to be weighted by different factors. First, the excitation wavelength (337 nm) was fixed by the instrumental set up (KRYPTOR System ©) and was optimized for the Eu(TBP) excitation. Considering that the absorption coefficient of Tb**L**$_8$ at 337 nm is smaller than that at the maximum of absorption (327 nm) and that the excitation spectrum is perfectly superimposed with the absorption one, excitation at 327 nm should result in a theoretical enhancement of the Tb signal and of ΔF.

As a second point, the efficiency of the energy transfer in RET experiments is proportional to the overlap integral, which represent the overlap between the absorption spectrum of the energy acceptor and the emission spectrum of the energy donor.[11b,52] Cyanine5, used here as energy acceptor, has its maximum of absorption at 694 nm, well suited for overlapping with the europium emission with maximum at 615-620 nm, but far less adapted for Tb emission with maxima at 485 and 545 nm. Changing the energy acceptor with one absorbing at higher energy should thus improve the energy transfer and the detection signal. Finally, it was noticed that the Tb emission signal seemed to be affected during the purification protocol, which was attributed to a partial decomplexation of the Tb cations. To verify this hypothesis, a supplementary set of experiments was carried out in the presence of free Tb cations added as $TbCl_3$ salts into the assay. Under these conditions, the ΔF ratio was further increased by a factor 2 compared to Tb**L**$_8$ alone (Figure 9). If this large increase was a strong improvement of the sensitivity, it also emphasized some limitations due to the stability of Tb**L**$_8$ in solution that can be overcome by the addition of free terbium into the assay. Recent investigations on the thermodynamic stability of this kind of complexes[27] revealed a stability slightly weaker than EDTA, which is sufficient in aqueous solutions, but may not be enough in biological media containing large amounts of competing cations and anions. Nevertheless, it was recently demonstrated,[37b] that the replacement of carboxylate func-

tions by phosphonate ones strongly stabilize the complexes, becoming stable even in biological fluids such as serum.

Conclusion

A generic method was developed that allows the synthesis of families of luminescent lanthanide complexes, some of which being adapted to luminescence labelling. The concept is based on the synthesis of protected nonadentate coordinations sites containing pyridine bispyrazolyl and pyridine bisindazolyl chromophoric units and two iminodiacetate arms protected by ester functions. Thanks to a rerouting of the aromatic nucleophilic reactivity of polyhalogenated pyridines, a bromine atom can be introduced in the *para* position of the central pyridyl ring, allowing the introduction of various substituents at this position by palladium catalyzed coupling reactions. From these families of ligands, Tb and Eu complexes were synthesized and their photophysical properties were determined in aqueous solutions, revealing excellent luminescence properties for the Tb complex of $\mathbf{L_8}$, substituted by a carboxamido butyric acid function. A synthetic protocol was then developed to selectively activate this dangling carboxylic acid function into an activated *N*-hydroxysuccinimide ester, while leaving aminodiacetate functions free for the coordination to the Tb atom. The labelling activity of the activated Tb complex was first checked on aminopropyl functionalized beads, which were characterized by time-resolved luminescence microscopy. Finally, using the [Tb$\mathbf{L_8}$] complex as an energy donor within the frame of a fluoro-immunoassay for the detection of carcynoembryonic antigene revealed a very promising 35% increase of the response signal compared to commercially available kits, and which can be further doubled by addition of free Tb cations into the solution.

We believe that our synthetic strategy opens numerous perspectives towards the development of families of ligands for Ln complexation and their applications for time-resolved luminescence spectroscopy. Current efforts are devoted toward the extension to other complexation synthons with improved stability in aqueous solutions and biological media.

Experimental Section

Materials and methods: Column chromatography and flash column chromatography were performed on silica (0.063–0.200 mm, Merck) or silica gel (40–63 μm, Merck) or on standardized aluminium oxide (Merck, Activity II–III). Acetonitrile was filtered over aluminium oxide and distilled over P_2O_5, DMF was distilled under reduced pressure and triethylamine was refluxed over KOH and distilled prior to use. Other solvents were used as purchased. ^{1}H- and ^{13}C NMR spectra were recorded on Bruker AC 200, Avance 300 and Avance 400 spectrometers working at 200, 300 or 400 MHz respectively for ^{1}H. Chemical shifts are given in ppm, relative to residual protiated solvent.[53] IR spectra were recorded on a Perkin–Elmer Spectrum one spectrometer. Compounds **1a**, **2a**, **3a**,[24] 2,6-difluoro-4-bromopyridine[29] and ethylindazolate[32] were obtained according to literature procedures.

Synthesis of compound 1b: In a round bottom Schlenk flask under argon were dissolved 2,6-difluoro-4-bromopyridyne (680 mg, 3.5 mmol) and ethyl indazolate (1.46 g, 7.7 mmol) in 30 mL of freshly distilled DMF. The solution was cooled to 0 °C and NaH (60% in oil, 308 mg, 7.7 mmol) was added. The reaction mixture was left at RT then heated at 40 °C for 5 h, after which a white precipitate formed. The solid was isolated by centrifugation and addition of water in the liquid phase gave a second precipitate, isolated by centrifugation. The combined solids were dissolved in 40 mL of CH_2Cl_2 and the organic phase was washed with water (40 mL) and the aqueous phase was extracted with CH_2Cl_2 (3×10 mL). The combined organic phases were dried over Na_2SO_4 and concentrated to dryness to afford **1b** (1.75 g, 94%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.64 (d, J=8.5 Hz, 2H), 8.35 (d, J=8.1 Hz, 2H), 8.31 (s, 2H), 7.56–7.45 (m, 4H), 4.62 (q, J=7.1 Hz, 4H), 1.56 ppm (t, J=7.1 Hz, 6H); ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ=14.4, 61.7, 114.7, 115.8, 122.5, 124.8, 125.1, 128.8, 136.5, 139.2, 140.0, 151.9, 162.1 ppm; IR (ATR): $\tilde{\nu}$=3089 (w), 2966 (w), 2929 (w), 1748 (s), 1709 (s), 1613 (w), 1593 (s), 1574 (s), 1564 (s), 1501 (m), 1491 (m), 1476 (w), 1444 (s), 1417 (s), 1383 (m), 1370 (m), 1348 (m), 1288 (w), 1258 (s), 1208 (w), 1189 (m), 1173 (s), 1162 (s), 1149 (m), 1122 (m), 1108 (s), 1063 (m), 1029 (s), 1013 (m), 968 (w), 937 cm^{-1} (w); MS (EI): m/z (%) 537.5 (100) $[M]^+$, 535.5 (100) $[M]^+$, 533.5 (30) $[M]^+$; elemental analysis calcd (%) for $C_{25}H_{20}BrN_5O_4$: C 56.19, H 3.77, N 13.11; found: C 56.47, H 4.01, N 12.95.

Synthesis of compound 2b: In a round bottom Schlenk flask under argon **1b** (1.7 g, 3.2 mmol) was dissolved in 120 mL of anhydrous THF. The reaction mixture was cooled to −10 °C and $LiAlH_4$ (240 mg, 6.4 mmol) was added in small portion and stirred for 2 h at −10 °C. Saturated aqueous NH_4Cl was added until a white precipitate formed. The precipitate was filtered off and the solution concentrated to dryness. The residue was dissolved in CH_2Cl_2 (50 mL) and washed with water (3×25 mL). The organic phase was dried over Na_2SO_4 and concentrated to dryness to yield 1.12 g of a white solid. The solid was dissolved in freshly distilled DMF (50 mL) and PBr_3 (0.6 mL, 6.3 mmol) was added at RT. The reaction mixture was stirred at RT for 14 h. The solvent was removed under reduced pressure to yield a yellowish residue which was dissolved in 20 mL of CH_2Cl_2 and neutralised with a saturated solution of $NaHCO_3$ (10 mL). The aqueous phase was extracted and washed with CH_2Cl_2 (4×20 mL). The combined organic phases were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated to dryness. Flash chromatography over silica gel (toluene/petroleum ether=75:25) yielded **2b** (420 mg, 35%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.64 (d, J=8.5 Hz, 2H), 8.04 (s, 2H), 7.92 (d, J=8.1 Hz, 2H), 7.54–7.37 (m, 4H), 4.90 ppm (s, 4H); ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$) : δ=23.2, 113.8, 115.1, 120.7, 123.4, 124.5, 128.8, 139.1, 140.1, 145.7, 152.3 ppm; IR (ATR): $\tilde{\nu}$=3104 (w), 1612 (s), 1586 (m), 1565 (m), 1507 (m), 1437 (m), 1420 (m), 1385 (w), 1347 (m), 1259 (s), 1216 (w), 1186 (m), 1087 (s), 1069 (s), 1012 (s), 892 (w), 866 (w), 854 (w), 795 cm^{-1} (s); MS (FAB): m/z (%): 573.1 (30) $[M+H]^+$, 575.2 (100) $[M+H]^+$, 577.1 (90) $[M+H]^+$; elemental analysis calcd (%) for $C_{21}H_{14}Br_3N_5$: C 43.78, H 2.45, N 12.16; found: C 43.43, H 2.07, N 11.86.

Synthesis of compound 3b$_{Et}$: In a round bottom Schlenk flask under argon were dissolved **2b** (420 mg, 0.73 mmol), diethyl iminodiacetate (0.29 mL, 1.61 mmol) and K_2CO_3 (430 mg; 2.92 mmol) in anhydrous CH_3CN (50 mL) and THF (25 mL). The reaction mixture was heated at 60 °C for 15 h, cooled to RT and filtered. The filtrate was concentrated to dryness and the resulting oil was dissolved in 20 mL of CH_2Cl_2. The organic phase was washed with water (3×10 mL) and brine, dried over Na_2SO_4 and concentrated to dryness. Flash chromatography over silica gel (CH_2Cl_2/MeOH=100:00 to 98:2) afforded **3b$_{Et}$** (322 mg, 56%) as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ=8.62 (d, J=8.4 Hz, 2H), 8.18 (d, J=7.7 Hz, 2H), 7.97 (s, 2H); 7.50–7.26 (m, 4H), 4.40 (s, 4H), 4.19 (q, J=7.0 Hz, 8H), 3.67 (s, 8H), 1.28 ppm (t, J=7.0 Hz, 12H); ^{13}C {^{1}H} NMR (50 MHz, $CDCl_3$): δ=14.2, 50.3, 54.7, 60.5, 112.8, 114.5, 121.7, 122.7, 125.6, 128.3, 135.4, 139.7, 146.8, 152.4, 170.9 ppm; IR (ATR): $\tilde{\nu}$=2986 (w), 2905 (w), 1732 (s), 1610 (w), 1586 (w), 1569 (s), 1518 (w), 1438 (s), 1346 (m), 1264 (w), 1236 (w), 1187 (s), 1136 (s), 1070 (s), 1025 (m), 981 cm^{-1} (m); MS (EI): m/z (%): 793.0 (100) $[M]^+$, 791.0 (100) $[M]^+$; elemental analysis calcd (%) for $C_{37}H_{42}BrN_7O_8$: C 56.06, H 5.34, N 12.37, found: C 56.24, H 5.59, N 12.59.

Synthesis of compound $3b_{Bu}$: In a round bottom Schlenk flask under argon were dissolved **2b** (90 mg, 0.16 mmol), di-*tert*-butyl iminodiacetate (84.2 mg, 0.34 mmol) and K_2CO_3 (43 mg; 0.31 mmol) in anhydrous CH_3CN (10 mL) and THF (5 mL). The reaction mixture was heated at 80 °C for 14 h, cooled to RT and filtered. The filtrate was concentrated to dryness and the resulting oil was dissolved in 50 mL of CH_2Cl_2. The organic phase was washed with water (2×25 mL), dried over Na_2SO_4 and concentrated to dryness. Flash chromatography over silica gel (CH_2Cl_2/MeOH = 100:00 to 98:2) afforded $3b_{Bu}$ (100 mg, 84 %) as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.63 (d, J = 8.4 Hz, 2H), 8.21 (d, J = 7.7 Hz, 2H), 7.98 (s, 2H), 7.50–7.26 (m, 4H), 4.38 (s, 4H), 3.53 (s, 8H), 1.45 ppm (s, 36H); ^{13}C {^{1}H} NMR (50 MHz, $CDCl_3$): δ = 28.2, 51.0, 55.7, 81.2, 112.9, 114.6, 122.0, 122.9, 125.9, 128.4, 135.5, 139.8, 147.3, 152.7, 170.4 ppm; IR (ATR): $\tilde{\nu}$ = 2990 (w), 2908 (w), 1735 (s), 1612 (w), 1585 (w), 1572 (s), 1517 (w), 1442 (s), 1347 (m), 1259 (w), 1233 (w), 1190 (s), 1135 (s), 1072 (s), 1023 (m), 980 cm^{-1} (m); MS (EI): m/z (%): 905.3 (100) $[M]^+$, 903.3 (100) $[M]^+$; elemental analysis calcd (%) for $C_{45}H_{58}BrN_7O_8$: C 59.73, H 6.46, N 10.84; found: C 59.52, H 6.28, N 10.92.

Synthesis of compound $4a_{Bu}$: A mixture of compound $3a_{Bu}$ (200 mg, 0.25 mmol), trimethylsilylacetylene (0.04 mL, 0.31 mmol), $[Pd(PPh_3)_2Cl_2]$ (10.5 mg, 0.02 mmol), dry THF (20 mL) and dry Et_3N (15 mL) was degassed with argon for 30 min, and CuI (4.80 mg, 0.03 mmol) was added. The reaction mixture was stirred at RT for 14 h. The solvent was removed in vacuo and the residue was taken up in dichloromethane (100 mL), washed with water (3×100 mL) and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness under vacuum. Purification of the crude product was achieved by column chromatography on silica (CH_2Cl_2/MeOH = 100:00 to 98:2) yielding compound $4a_{Bu}$ (148 mg, 72 %) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.41 (d, J = 2.6 Hz, 2H), 7.78 (s, 2H), 6.50 (d, J = 2.6 Hz, 2H), 4.00 (s, 4H), 3.45 (s, 8H), 1.43 (s, 36H), 0.23 ppm (s, 9H); ^{13}C {^{1}H} NMR (50 MHz, $CDCl_3$): δ = 170.3, 153.3, 150.0, 136.0, 127.7, 111.1, 108.4, 101.5, 100.6, 80.8, 55.2, 50.9, 28.1, 0.51 ppm; IR (ATR): $\tilde{\nu}$ = 2167 (w; $\nu_{C\equiv C}$), 1732 (s), 1610 (s), 1553 (s), 1539 (m), 1464 (s), 1391 cm^{-1}, (s); MS (EI): m/z (%): 822.3 (100) $[M]^+$; elemental analysis calcd (%) for $C_{42}H_{63}N_7O_8Si$: C 61.36, H 7.72, N 11.93; found: C 61.28, H 7.64, N 11.89.

Synthesis of compound $5a_{Bu}$: TBAF (1 M in THF; 0.20 mL, 0.20 mmol) was added dropwise to a solution of compound **4** (140 mg, 0.17 mmol) in dry THF (4 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and neutralized with water. The solvent was removed in vacuo. The slurry residue was dissolved in dichloromethane (100 mL), washed with water (3×100 mL) and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by column chromatography on silica (CH_2Cl_2/MeOH = 100:00 to 99.4:0.6) to yield $5a_{Bu}$ (103 mg, 81 %). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.43 (d, J = 2.6 Hz, 2H), 7.83 (s, 2H), 6.54 (d, J = 2.6 Hz, 2H), 4.00 (s, 4H), 3.47 (s, 8H), 3.35 (s, 1H), 1.45 ppm (s, 36H); ^{13}C {^{1}H} NMR (50 MHz, $CDCl_3$): δ = 170.3, 153.6, 150.1, 135.1, 127.8, 111.4, 108.6, 82.4, 80.9, 80.6, 55.3, 51.0, 28.1 ppm; IR (ATR): $\tilde{\nu}$ = 2112 (w, $\nu_{C\equiv C}$), 1732 (s), 1608 (s), 1557 (s), 1536 (m), 1465 (s), 1391 cm^{-1} (s); MS (EI): m/z (%): 749.3 (100) $[M]^+$; elemental analysis calcd (%) for $C_{39}H_{55}N_7O_8$: C 62.46, H 7.39, N 13.07; found: C 62.19, H 7.18, N 12.68.

Synthesis of L_1: Compound **5** (30.3 mg, 0.04 mmol) was dissolved in dichloromethane (1 mL) and TFA (1 mL) and stirred at RT during 2 h. The solvent was removed in vacuo and the resulting oil was triturated several times in diethylether, leading to a white precipitate. Ligand $\mathbf{L_1}$ (24,6 mg) was recovered by centrifugation. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.86 (d, J = 2.6 Hz, 2H), 7.67 (s, 2H), 6.57 (d, J = 2.6 Hz, 2H), 4.80 (s, 1H), 3.94 (s, 4H), 3.47 ppm (s, 8H); ^{13}C {^{1}H} NMR (75 MHz, [D$_6$]DMSO): δ = 172.7, 154.1, 150.3, 135.5, 129.6, 110.7; 109.3, 80.9, 80.7, 55.3, 51.1 ppm; IR (ATR): $\tilde{\nu}$ = 2111 (w, $\nu_{C\equiv C}$), 1609 (s), 1558 (s), 1534 (m), 1465 (s), 1389 (s), 1275 cm^{-1}, (w); MS (ESI, H_2O, negative mode): m/z (%): 261.6 (80) $[M-2H]^{2-}$.

Synthesis of $4a_{Et}$: $4a_{Et}$ was obtained similarly to $4a_{Bu}$ starting from $3a_{Et}$ (700 mg, 1.01 mmol) and yielding $4a_{Et}$ (502 mg, 70 %) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.45 (d, J = 2.5 Hz, 2H), 7.81 (s, 2H), 6.53 (d, J = 2.6 Hz, 2H), 4.18 (q, J = 7.2 Hz, 8H), 4.04 (s, 4H), 3.62 (s, 8H), 1.27 (t, J = 7.1 Hz, 12H), 0.27 ppm (s, 9H); ^{13}C {^{1}H} NMR (50 MHz, $CDCl_3$): δ = 171.1, 153.1, 150.0, 136.2, 128.0, 111.3, 108.5, 101.5, 60.5, 54.6, 51.4, 14.2, 0.40 ppm. IR (ATR): $\tilde{\nu}$ = 2162 (w, $\nu_{C\equiv C}$), 1736 (s), 1610 (s), 1553 (s), 1464 (s), 1389 (s), 1328 cm^{-1} (w); MS (EI): m/z (%): 709.2 (100) $[M]^+$; elemental analysis calcd (%) for $C_{34}H_{47}N_7O_8Si$: C 57.53, H 6.67, N 13.81; found: C 57.32, H 6.32, N 13.62.

Synthesis of $5a_{Et}$: $5a_{Et}$: these compounds were obtained in 83 % yield according to the methodology developed for $5a_{Bu}$. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.45 (d, J = 2.6 Hz, 2H), 7.85 (s, 2H), 6.54 (d, J = 2.6 Hz, 2H), 4.18 (q, J = 7.1 Hz, 8H), 4.04 (s, 4H), 3.62 (s, 8H), 3.36 (s, 1H), 1.27 ppm (t, J = 7.2 Hz, 12H); ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ = 171.0, 153.3, 150.1, 135.2, 127.9, 111.6, 108.6, 82.5, 80.6, 60.5, 54.6, 51.4, 14.2 ppm. IR (ATR): $\tilde{\nu}$ = 2111 (w, $\nu_{C\equiv C}$), 1734 (s), 1609 (s), 1556 (s), 1536 (m), 1465 (s), 1389 cm^{-1} (s); MS (EI): m/z (%): 637.2 (100) $[M]^+$; elemental analysis calcd (%) for $C_{31}H_{39}N_7O_8$: C 58.39, H 6.16, N 15.38; found: C 58.03, H 5.95, N 15.20.

Synthesis of compound 6:[33] A Schlenk flask containing compound $3a_{Et}$ (137 mg, 0.20 mmol), 6-heptinoic acid (0.03 mL, 0.24 mmol), $[Pd(PPh_3)_2Cl_2]$ (8.40 mg, 0.01 mmol), dry THF (30 mL) and dry Et_3N (12 mL) was degassed with argon for 30 min, and CuI (3.80 mg, 0.02 mmol) was added to the solution. The mixture was heated at 50 °C for 14 h. The solvent was removed under reduced pressure, the residue was dissolved in dichloromethane (100 mL), washed with water (3× 100 mL) and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness under vacuum. The crude product was purified by column chromatography on silica (CH_2Cl_2/MeOH = 100:00 to 98:2) yielding compound **6** (113 mg, 77 %) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.44 (d, J = 2.4 Hz, 2H), 7.77 (s, 2H), 6.51 (d, J = 2.6 Hz, 2H), 4.21 (q, J = 7.2 Hz, 8H), 4.05 (s, 4H), 3.63 (s, 8H), 2.49 (t, J = 7.2 Hz, 2H), 2.44 (t, J = 7.4 Hz, 2H), 1.90–1.81 (m, 2H), 1.75–1.65 (m, 2H), 1.27 ppm (t, J = 7.1 Hz, 12H); ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ = 171.2, 171.1, 152.8, 150.0, 137.2, 127.9, 111.3, 108.4, 96.2, 79.0, 60.6, 54.6, 51.3, 33.3, 27.5, 23.9, 19.2, 14.2 ppm; IR (ATR): $\tilde{\nu}$ = 2235 (w, $\nu_{C\equiv C}$), 1733 (s), 1610 (s), 1536 (m), 1464 (s), 1388 (s), 1261 cm^{-1} (m); MS (ESI, negative mode): m/z (%): 736.5 (100) $[M-H^+]^-$; elemental analysis calcd (%) for $C_{36}H_{47}N_7O_{10}$: C 58.60, H 6.42, N 13.29; found: C 58.42, H 6.15, N 12.89.

Synthesis of ligand L_2: NaOH (41.2 mg, 1.03 mmol) dissolved in H_2O (4 mL) was added to a solution of compound **6** (126 mg, 0.17 mmol) in methanol (20 mL). The mixture was heated at 60 °C for 3 h. The solvent was removed under vacuum and the residue was dissolved in ca. 2 mL of H_2O. Successive addition of THF, MeOH and Et_2O resulted in the formation of a precipitate, which was collected by centrifugation and dried under vacuum to give ligand $\mathbf{L_2}Na_5$ (110 mg, 80 %) as a light brown solid. ^{1}H NMR (300 MHz, D_2O): δ = 8.64 (br s, 2H), 7.72 (s, 2H), 6.70 (d, J = 2.5 Hz, 2H), 3.96 (br s, 4H), 3.30 (br s, 8H), 2.59 (t, J = 6.9 Hz, 2H), 2.29 (t, J = 7.1 Hz, 2H), 1.97–1.71 ppm (m, 4H); ^{13}C {^{1}H} NMR (75 MHz, D_2O): δ = 183.9, 179.4, 153.8, 150.1, 138.1, 130.2, 112.2, 109.7, 98.9, 78.3, 58.1, 50.8, 37.4, 27.8, 25.3, 19.0 ppm; IR (ATR): $\tilde{\nu}$ = 2161 (w, $\nu_{C\equiv C}$), 1610 (s), 1410 (s), 1327 cm^{-1} (m); MS (ESI, MeOH/H_2O, negative mode): m/z (%): 222.2 (60) $[M-3Na^+]^{3-}$; elemental analysis calcd (%) for $C_{28}H_{26}N_7Na_5O_{10}.4H_2O$: C 41.64, H 4.24, N 12.14; found: C 41.52, H 4.02, N 11.79.

Synthesis of compound 7: To a solution of propargyl alcohol (1.77 mL, 30 mmol) in anhydrous THF (80 mL) under an argon atmosphere, NaH (60 % in mineral oil, 1.32 mg, 33 mmol) was added and the solution was stirred 1 h at RT. Ethyl bromoacetate (4.02 mL, 35 mmol) was added and the reaction mixture was stirred under argon atmosphere, at RT for 12 h. The solvent was distilled off to give a yellow oil which was dissolved in diethylether (40 mL) and washed with water (3×20 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated to dryness to yield colourless oil. **7** (2,0 g, 47 %) was obtained after flash chromatography on silica gel (Petroleum ether/diethylether = 100:0 to 96:4). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.27 (d, J = 2.4 Hz, 2H), 4.19 (q, J = 7.1 Hz, 2H), 4.15 (s, 2H), 2.45 (t, J = 2.4 Hz, 1H), 1.26 ppm (t, J = 7.1 Hz, 3H); ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ = 14.1, 58.2, 60.9, 66.2, 75.5, 78.5, 169.8 ppm; IR (ATR): $\tilde{\nu}$ = 3278 (m), 2984 (w), 2909 (w), 2118 (w), 1748 (s), 1448 (m), 1429 (m), 1382 (m), 1370 (m), 1349 (m), 1275 (m), 1249 (m), 1206 (s), 1116 (s), 1025 cm^{-1} (s); MS (EI): m/z (%): 142.0 (100)

$[M]^+$; elemental analysis calcd (%) for $C_7H_{10}O_3$: C 59.14, H 7.09; found: C 58.78, H 6.84.

Synthesis of compound 8: $\mathbf{3a_{Et}}$ (300 mg, 0.43 mmol) and compound **7** (148 mg, 1.04 mmol) were dissolved in anhydrous THF (20 mL) and triethylamine (15 mL) under an argon atmosphere. $[Pd(PPh_3)_2Cl_2]$ (18 mg, 0.026 mmol) was added and the solution was degassed for 30 min before CuI (8.2 mg, 0.043 mmol) was added. The solution was heated at 50°C for 24 h and the solvents were distilled off under reduced pressure to afford a dark brown oil. The residual oil was dissolved in CH_2Cl_2 (30 mL) and washed with water (3×15 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Compound **8** (130 mg, 40%) was isolated by chromatography on silica gel (CH_2Cl_2/MeOH = 100:0 to 98:2) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.43 (d, J = 2.6 Hz, 2H), 7.8 (s, 2H), 6.51 (d, J = 2.6 Hz, 2H), 4.25 (m, 4H), 4.24 (q, J = 7.2 Hz, 2H), 4.16 (q, J = 7.2 Hz, 8H), 4.0 (s, 4H), 3.6 (s, 8H), 1.29 (t, J = 7.2 Hz, 3H), 1.25 ppm (t, J = 7.2 Hz, 12H); ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ = 14.1, 14.2, 51.4, 54.6, 58.7, 60.5, 61.1, 66.5, 84.4, 89.6, 108.6, 111.1, 127.9, 135.4, 150.1, 153.3, 169.7, 171.0 ppm; IR (ATR): $\tilde{\nu}$ = 3126 (w), 2982 (w), 2935 (w), 2907 (w), 1735 (s), 1610 (m), 1555 (m), 1536 (m), 1464 (m), 1388 (m), 1269 (w), 1188 (s), 1152 (s), 1118 (s), 1027 cm^{-1} (s); MS (EI): m/z (%): 754.2 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{36}H_{47}N_7O_{11}$: C 57.36, H 6.28, N 13.01; found: C 56.94, H 5.67, N 12.74.

Synthesis of ligand L_3: Compound **8** (130 mg, 0.17 mmol) was dissolved in MeOH (20 mL) and NaOH (41 mg, 1.03 mmol) in 4 mL of water was added. The solution was stirred at 60°C for 3 h. The solvents were distilled off to afford a brown oil which was dissolved in water (10 mL) and 0.1 M HCl solution was added until pH 4–5, resulting in the formation of a white precipitate which was isolated by centrifugation and dried to give $\mathbf{L_3}$ (67 mg, 64%) as a white solid. ^{1}H NMR (300 MHz, D_2O): δ = 8.76 (s, 2H), 7.55 (s, 2H), 6.46 (s, 2H), 4.47 (s, 2H), 4.09 (s, 2H), 3.84 (s, 4H), 3.37 ppm (s, 8H); ^{13}C {^{1}H} NMR (75 MHz, D_2O): δ = 54.3, 57.1, 58.7, 66.6, 86.4, 90.3, 107.7, 110.1, 130.7, 136.4, 151.8, 153.3, 178.0, 178.2 ppm; IR (ATR): $\tilde{\nu}$ = 3410 (br), 2923 (w), 2852 (w), 1729 (s), 1611 (s), 1558 (m), 1457 (s), 1388 (s), 1217 (s), 1109 (s), 1055 cm^{-1} (s); MS (EI): m/z (%): 614.1 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{26}H_{27}N_7O_{11}\cdot(H_2O)_{0.5}$: C 50.11, H 4.53, N 15.75; found: C 49.90, H 4.04, N 15.38.

Synthesis of compound 9: $\mathbf{5a_{Et}}$ (120 mg, 0.173 mmol) and *para*-tolylacethylene (24.2 mg, 0.208 mmol) were dissolved in anhydrous THF (10 mL) and triethylamine (6 mL) under an argon atmosphere. $[Pd(PPh_3)_2Cl_2]$ (7.2 mg, 0.010 mmol) was added and the solution was degassed for 30 min before CuI (3.2 mg, 0.017 mmol) was added. The solution was heated at 50°C for 24 h. The solvents were distilled off under reduced pressure to afford a dark brown oil which was dissolved in CH_2Cl_2 (30 mL) and washed with water (3×15 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Compound **9** (80 mg, 63%) was isolated by chromatography on silica gel (CH_2Cl_2/MeOH = 99.75:0.25 to 99.5:0.5) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.45 (d, J = 2.5 Hz, 2H), 7.86 (s, 2H), 7.44 (d, J = 7.9 Hz, 2H), 7.18 (d, J = 7.9 Hz, 2H), 6.5 (d, J = 2.5 Hz, 2H), 4.17 (q, J = 7.2 Hz, 8H), 4.04 (s, 4H), 3.62 (s, 8H), 2.40 (s, 3H), 1.26 ppm (t, J = 7.2 Hz, 12H); ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ = 14.1, 21.5, 51.3, 54.5, 60.4, 86.0, 95.2, 108.4, 110.8, 118.7, 127.9, 129.2, 131.9, 136.6, 139.7, 149.9, 152.9 ppm, 171.0; IR (ATR): $\tilde{\nu}$ = 3126 (w), 2981 (w), 2936 (w), 2228 (w), 2207 (w), 1738 (s), 1606 (s), 1553 (s), 1538 (m), 1513 (w), 1464 (s), 1389 (s), 1299 (w), 1259 (w), 1188 (s), 1153 (s), 1096 (w), 1028 cm^{-1} (s); MS (EI): m/z (%): 728.3 (100) $[M]^+$; elemental analysis calcd (%) for $C_{38}H_{45}N_7O_8$: C 62.71, H 6.23, N 13.47; found: C 62.36, H 5.93, N 13.19.

Synthesis of ligand L_4: **9** (80 mg, 0.11 mmol) was dissolved in 20 mL of a MeOH/Water (1:1) solution and NaOH (22 mg, 0.55 mmol) was added. The solution was stirred at 60°C for 3 h and the solvents were distilled under reduced pressure. The pale yellow residue was dissolved in 0.5 mL of water and 4 mL of MeOH. $\mathbf{L_4}$ (66.5 mg, 86%) was precipitated as a white powder with a dropwise addition of THF. ^{1}H NMR (300 MHz, D_2O): δ = 8.34 (s, 2H), 7.43 (s, 2H), 7.26 (d, J = 7.5 Hz, 2H), 7.02 (d, J = 7.5 Hz, 2H), 6.39 (s, 2H), 3.62 (s, 4H), 3.01 (s, 8H), 2.15 ppm (s, 3H) ^{13}C {^{1}H} NMR (75 MHz, D_2O): δ = 20.8, 25.0, 50.7, 51.3, 57.8, 85.8, 95.5, 109.3, 110.4, 117.7, 127.3, 129.2, 132.0, 136.5, 140.7, 178.9 ppm; IR (ATR): $\tilde{\nu}$ = 3365 (br), 2926 (w), 2210 (w), 1586 (s), 1553 (s), 1532 (w), 1466 (m), 1405 (s), 1327 (s), 1258 (m), 1211 (w), 1123 (m), 1049 (m), 1021 cm^{-1} (m); MS (ESI, MeOH/H_2O, negative mode): m/z (%): 680.1 (70) $[M-Na^+]^-$, 211.3 (100) $[M-3Na^+]^{3-}$; elemental analysis calcd (%) for $C_{30}H_{25}N_7Na_4O_8.4H_2O$: C 46.46, H 4.29, N 12.64; found: C 46.30, H 3.85, N 12.43.

Synthesis of compound 10: A Schlenk tube was charged with compound $\mathbf{5a_{Et}}$ (59.5 mg, 0.09 mmol), ethyl-4-iodobenzoate (25.8 mg, 0.09 mmol), dry Et_3N (4 mL) and dry THF (8 mL). The solution was degassed with argon for 30 min and $[Pd(PPh_3)_4]$ (10.5 mg, 0.01 mmol) was added. The mixture was degassed for 5 min with argon and then heated at 60°C for 14 h. After evaporation of the solvent, the oily residue was dissolved in dichloromethane (100 mL), washed with water (3×100 mL) and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness under vacuum. The crude product was purified by column chromatography on silica (CH_2Cl_2/MeOH = 100:00 to 99:1) yielding compound **10** (40.2 mg, 55%) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.48 (d, J = 2.6 Hz, 2H), 8.07 (d, J = 8.0 Hz, 2H), 7.91 (s, 2H), 7.63 (d, J = 8.0 Hz, 2H), 6.56 (d, J = 2.6 Hz, 2H), 4.40 (q, J = 7.2 Hz, 2H), 4.18 (q, J = 7.2 Hz, 8H), 4.06 (s, 4H), 3.64 (s, 8H), 1.41 (t, J = 7.1 Hz, 3H), 1.28 ppm (t, J = 7.3 Hz, 12H); ^{13}C {^{1}H} NMR (50 MHz, $CDCl_3$): δ = 171.0, 153.2, 150.1, 135.8, 131.9, 130.9, 129.6, 128.0, 126.3, 111.0, 108.6, 89.0, 61.2, 60.5, 54.6, 51.4, 14.3, 14.2 ppm; IR (ATR): $\tilde{\nu}$ = 2229 (w, $\nu_{C\equiv C}$), 1721 (s), 1608 (s), 1553 (s), 1537 (m), 1464 (s), 1389 cm^{-1} (s). MS (EI): m/z (%): 785.2 (100) $[M]^+$.

Synthesis of ligand L_5: NaOH (11.3 g, 0.28 mmol) dissolved in H_2O (2 mL) was added to a solution of compound **10** (37.0 mg, 0.05 mmol) in methanol (5 mL). The mixture was heated at 60°C for 3 h. The solvent was removed under vacuum and the residue was dissolved in H_2O. The aqueous layer was extracted with CH_2Cl_2 (3×100 mL) and reduced to ca. 1 mL. Successive addition of THF, MeOH and Et_2O resulted in the formation of a precipitate, which was collected by centrifugation and dried under vacuum to give ligand $\mathbf{L_5}$ as its pentasodic salt (30.7 mg, 86%). ^{1}H NMR (300 MHz, D_2O): δ = 8.58 (br s, 2H), 7.92–7.65 (m, 6H), 6.63 (br s, 2H), 3.88 (s, 4H), 3.28 ppm (s, 8H); ^{13}C {^{1}H} NMR (75 MHz, D_2O): δ = 178.9, 174.5, 153.1, 149.3, 137.2, 136.2, 132.0, 129.6, 128.9, 123.5, 110.7, 109.5, 94.7, 87.5, 58.0, 48.9 ppm; IR (ATR): $\tilde{\nu}$ = 2220 (w, $\nu_{C\equiv C}$), 1582 (s), 1550 (s), 1534 (w), 1465 (m), 1384 (s), 1327 (m), 1260 cm^{-1} (w). MS (ESI, MeOH/H_2O = 1:1, negative mode): m/z (%): 354.4 (80) $[M-2Na^+]^{2-}$, 228.5 (25) $[M-3Na^+]^{3-}$; elemental analysis calcd (%) for $C_{30}H_{22}N_7Na_5O_{10}.7H_2O$: C 40.87, H 4.12, N 11.12; found: C 40.52, H 3.19, N 10.82.

Synthesis of compound 11: A solution of 4-iodophenol (500 mg, 2.27 mmol), trimethylsilylacetylene (0.4 mL, 2.72 mmol), $[Pd(PPh_3)_2Cl_2]$ (95.5 mg, 0.14 mmol) in dry THF (15 mL) and dry Et_3N (8 mL) was degassed with argon for 30 min, and CuI (43.2 mg, 0.23 mmol) was added. The reaction mixture was stirred at RT for 14 h. The solvent was removed in vacuo and the residue was taken up in dichloromethane (100 mL), washed with water (3×100 mL) and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness under vacuum. Purification of the crude product was achieved by column chromatography on silica (Petroleum ether/CH_2Cl_2 = 50:50 to 00:100) yielding compound **11** (335 mg, 78%) as a brown solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.39–7.34 (m, 2H), 6.78–6.73 (m, 2H), 6.15 (s, 1H), 0.27 ppm (s, 9H); ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ = 155.6, 133.7, 115.4, 115.3, 105.5, 92.9, 0.05 ppm; IR (ATR): $\tilde{\nu}$ = 2161 (s, $\nu_{C\equiv C}$), 1882 (s), 1608 (s), 1590 (m), 1545 (w), 1507 (s), 1434 cm^{-1} (m). MS (EI): m/z (%): 190.1 (100) $[M]^+$; elemental analysis calcd (%) for $C_{11}H_{14}OSi$: C 69.42, H 7.41; found: C 69.13, H 7.22.

Synthesis of compound 12: To a suspension of NaH (60% dispersion in mineral oil; 74.8 mg, 1.87 mmol) in dry THF (10 mL) under argon at 0°C was added compound **11** (323 mg, 1.70 mmol). The mixture was allowed to warm up to RT and ethyl-4-bromobutyrate (0.49 mL, 3.40 mmol) and a catalytic amount of NaI were added. The reaction mixture was heated at 50°C for 36 h and the solvent was removed in vacuo. The resulting oily residue was taken up in dichloromethane (100 mL), washed with water (3×100 mL) and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness. The crude product was purified by column

chromatography on silica (Petroleum ether/CH_2Cl_2 = 90:10 to 00:100) affording compound **12** (194 mg, 37%) as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.37 (d, J = 8.8 Hz, 2H), 6.77 (d, J = 8.8 Hz, 2H), 4.12 (q, J = 7.1 Hz, 2H), 3.96 (t, J = 6.0 Hz, 2H), 2.48 (t, J = 7.3 Hz, 2H), 2.07 (q, J = 6.7 Hz, 2H), 1.23 (t, J = 7.1 Hz, 3H), 0.23 ppm (s, 9H); ^{13}C {^{1}H} NMR (50 MHz, $CDCl_3$): δ = 172.9, 158.9, 133.4, 115.2, 114.2, 105.1, 92.3, 66.6, 60.3, 30.6, 24.4, 14.1, 0.03 ppm. IR (ATR): $\tilde{\nu}$ = 2155 (s, $\nu_{C\equiv C}$), 1893 (w), 1733 (s), 1605 (s), 1569 (w), 1506 (s), 1473 cm^{-1} (m). MS (EI): m/z (%): 305.1 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{17}H_{24}O_3Si$: C 67.06, H 7.95; found: C 66.75, H 7.78.

Synthesis of compound 13: Compound **12** (170 mg, 0.56 mmol) was dissolved in dry THF (5 mL) and cooled to 0°C. To this solution was added dropwise a solution of TBAF (1 M in THF; 0.67 mL, 0.67 mmol). The mixture was stirred for 1 h at 0°C and 2 h at RT. The reaction mixture was neutralized with water and the solvent was evaporated under reduced pressure. The residue was taken up in dichloromethane (100 mL), washed with water (3×100 mL) and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness. The crude product was purified by column chromatography on silica (Petroleum ether/CH_2Cl_2 = 50:50 to 00:100) to afford compound **12** in quantitative yield (133 mg) as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.39 (d, J = 8.8 Hz, 2H), 6.80 (d, J = 8.8 Hz, 2H), 4.13 (q, J = 7.2 Hz, 2H), 3.97 (t, J = 6.2 Hz, 2H), 3.00 (s, 1H), 2.49 (t, J = 7.1 Hz, 2H), 2.08 (q, J = 6.7 Hz, 2H), 1.24 ppm (t, J = 7.1 Hz, 3H); ^{13}C {^{1}H} NMR (50 MHz, $CDCl_3$): δ = 172.9, 159.1, 133.4, 114.3, 114.1, 83.5, 75.8, 66.6, 60.3, 30.6, 24.4, 14.1 ppm; IR (ATR): $\tilde{\nu}$ = 2107 (w, $\nu_{C\equiv C}$), 1722 (s), 1607 (s), 1572 (m), 1511 (s), 1470 (s), 1440 cm^{-1} (m); MS (EI): m/z (%): 233.1 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{14}H_{16}O_3$: C 72.39, H 6.94; found: C 72.20, H 6.73.

Synthesis of compound 14: A Schlenk tube containing compound $\mathbf{3a_{Et}}$ (188 mg, 0.27 mmol), compound **13** (75.7 mg, 0.33 mmol), $[Pd(PPh_3)_2Cl_2]$ (11.4 mg, 0.02 mmol), dry THF (10 mL) and dry Et_3N (5 mL) was degassed with argon for 30 min, and CuI (5.20 mg, 0.03 mmol) was added. The mixture was heated at 50°C for 14 h. The solvent was removed under reduced pressure, the slurry residue was dissolved in dichloromethane (100 mL), washed with water (3×100 mL) and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness under vacuum. The crude product was purified by column chromatography on silica (CH_2Cl_2/MeOH = 100:00 to 98:2) yielding compound **14** (123 mg, 53%) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.45 (d, J = 2.6 Hz, 2H), 7.83 (s, 2H), 7.47 (d, J = 8.4 Hz, 2H), 6.87 (d, J = 8.8 Hz, 2H), 6.52 (d, J = 2.6 Hz, 2H), 4.21–3.99 (m, 16H), 3.61 (s, 8H), 2.50 (t, J = 7.2 Hz, 2H), 2.10 (q, J = 6.7 Hz, 2H), 1.28–1.20 ppm (m, 15H); ^{13}C {^{1}H} NMR (50 MHz, $CDCl_3$): δ = 173.0, 171.0, 159.7, 152.9, 149.9, 136.7, 133.6, 127.9, 114.6, 113.9, 110.7, 108.3, 95.2, 85.6, 66.8, 60.4, 60.3, 54.5, 51.3, 30.6, 24.4, 14.1 ppm; IR (ATR): $\tilde{\nu}$ = 2204 (m, $\nu_{C\equiv C}$), 1731 (s), 1599 (s), 1552 (s), 1538 (m), 1512 (m), 1464 cm^{-1} (s). MS (EI): m/z (%): 843.2 (100) $[M]^+$, 866,2 (20) $[M+Na]^+$; elemental analysis calcd (%) for $C_{43}H_{53}N_7O_{11}$: C 61.20, H 6.33, N 11.62; found: C 62.35, H 6.65, N 11.47.

Synthesis of ligand $\mathbf{L_6}$: NaOH (30.7 mg, 0.77 mmol) dissolved in H_2O (2 mL) was added to a solution of compound **14** (108 mg, 128 μmol) in methanol (5 mL). The mixture was heated at 60°C during 3 h. The solvent was removed under vacuum and the residue was dissolved in ca. 2 mL of H_2O. Successive addition of THF, MeOH and Et_2O resulted in the formation of a precipitate, which was collected by centrifugation and dried under vacuum to give ligand **L9** as its pentasodic salt (90.1 mg, 76%). ^{1}H NMR (300 MHz, D_2O): δ = 8.37 (br s, 2H), 7.37 (s, 2H), 7.31 (d, J = 7.9 Hz, 2H), 6.75 (d, J = 7.9 Hz, 2H), 6.52 (br s, 2H), 3.74 (br s, 4H), 3.19 (s, 8H), 2.36 (t, J = 7.4 Hz, 2H), 2.05–2.00 (m, 2H), 1.86–1.84 ppm (m, 2H); ^{13}C {^{1}H} NMR (75 MHz, D_2O): δ = 183.2, 179.6, 159.9, 153.7, 149.6, 137.2, 134.5, 130.0, 115.2, 113.9, 110.7, 110.0, 96.3, 86.2, 68.4, 58.6, 51.4, 34.5, 26.1 ppm; IR (ATR): $\tilde{\nu}$ = 2201 (s, $\nu_{C\equiv C}$), 1590 (s), 1554 (w), 1511 (m), 1408 (s), 1336 (m), 1248 cm^{-1} (m); MS (ESI, MeOH/H_2O, negative mode): m/z (%): 248.0 (100 $[M-3Na^+]^{3-}$), 180.3 (20) $[M-4Na^+]^{4-}$; elemental analysis calcd (%) for $C_{33}H_{34}N_7Na_5O_{14}.3H_2O$: C 45.68, H 3.95, N 11.30; found: C 45.62, H 3.75, N 11.20.

Synthesis of compound 15: A mixture of compound $\mathbf{3a_{Et}}$ (300 mg, 0.43 mmol), ethylglycinate hydrochloride (182 mg, 1.30 mmol), $[Pd(PPh_3)_2Cl_2]$ (60.8 mg, 0.09 mmol), dry Et_3N (10 mL) and dry toluene (15 mL) was heated at 100°C for 14 h under a continuous flow of bubbling CO. The solution was filtered and the filtrate was concentrated to dryness under reduced pressure. The resulting residue was dissolved in dichloromethane (100 mL), washed with water (3×100 mL) and brine, and dried over Na_2SO_4, filtered and evaporated under vacuum. The crude product was purified by column chromatography on silica (CH_2Cl_2/MeOH = 100:00 to 95:5) to yield compound **15** (262 mg, 81%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.45 (d, J = 2.6 Hz, 2H), 8.12 (s, 2H), 7.07 (t, J = 4.9 Hz, 1H), 6.55 (d, J = 2.6 Hz, 2H), 4.30–4.14 (m, 12H), 4.04 (s, 4H), 3.62 (s, 8H), 1.34–1.24 ppm (m, 15H); ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ = 171.0, 169.4, 164.7, 153.5, 150.7, 146.7, 128.0, 108.7, 106.8, 61.7, 60.5, 54.6, 51.4, 41.9, 14.2, 14.1 ppm; IR (ATR): $\tilde{\nu}$ = 1732 (s), 1674 (m), 1618 (m), 1571 (s), 1534 (s), 1463 cm^{-1} (s). MS (EI): m/z (%): 743.0 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{34}H_{46}N_8O_{11}$: C 54.98, H 6.24, N 15.09; found: C 54.70, H 5.95, N 14.65.

Synthesis of ligand $\mathbf{L_7}$: NaOH (91.6 mg, 2.29 mmol) dissolved in H_2O (4 mL) was added to a solution of compound **15** (283 mg, 0.38 mmol) in methanol (20 mL). The mixture was heated at 60°C for 3 h. The solvent was removed under vacuum and the residue was dissolved in H_2O. The aqueous layer was extracted with CH_2Cl_2 (3×100 mL) and reduced to ca. 2 mL. Successive addition of THF, MeOH and Et_2O resulted in the formation of a precipitate, which was collected by centrifugation and dried under vacuum to give a yellow precipitate of $\mathbf{L_7}$ as its pentasodic salt (260 mg, 89%). ^{1}H NMR (300 MHz, D_2O): δ = 8.70 (br s, 2H), 8.01 (br s, 2H), 6.71 (br s, 2H), 4.01 (br s, 2H), 3.95 (br s, 4H), 3.29 ppm (br s, 8H); ^{13}C {^{1}H} NMR (75 MHz, D_2O): δ = 179.9, 177.0, 167.7, 154.4, 150.9, 148.1, 130.6, 110.2, 108.1, 58.6, 51.2, 44.7 ppm; IR (ATR): $\tilde{\nu}$ = 1570 (s), 1531 (s), 1463 (s), 1389 (s), 1326 (s), 1255 cm^{-1} (s). MS (ES,. MeOH/H_2O, negative mode): m/z (%): 214.2 (80) $[M-3Na^+]^{3-}$; elemental analysis calcd (%) for $C_{24}H_{21}N_8Na_5O_{11}.3H_2O$: C 37.61, H 3.55, N 14.62; found: C 37.42, H 3.25, N 14.41.

Synthesis of compound 16: Compound $\mathbf{3a_{Et}}$ (200 mg, 0.29 mmol) and ethyl 4-aminobutyrate hydrochloride (97 mg, 0.58 mmol) were dissolved in distilled toluene (10 mL) and triethylamine (10 mL). $[Pd(PPh_3)_2Cl_2]$ (20 mg, 0.029 mmol) was added and the solution was heated at 100°C under a continuous flow of bubbling CO for 12 h. The solution was filtered over celite and the solvents evaporated to dryness to give a pale yellow oil. The oil was dissolved in CH_2Cl_2 (30 mL) and washed with water (3×15 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. **16** (126 mg, 57%) was isolated by flash chromatography on silica gel (CH_2Cl_2/MeOH = 100:0 to 98:2) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.40 (d, J = 2.6 Hz, 2H), 8.04 (s, 2H), 6.48 (d, J = 2.6 Hz, 2H), 4.13 (q, J = 7.2 Hz, 8H), 4.10 (q, J = 7.2 Hz, 2H), 3.99 (s, 4H), 3.58 (s, 8H), 3.50 (q, J = 7.0 Hz, 2H), 2.44 (t, J = 7.1, 2H), 1.96 (qt, J = 7.1 Hz, 2H), 1.22 (t, J = 7.2 Hz, 12H), 1.21 ppm (t, J = 7.2 Hz, 3H); ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ = 14.1 (2C), 24.3, 31.7, 39.7, 51.3, 54.4, 60.4, 60.5, 106.7, 108.5, 127.9, 147.5, 150.4, 153.2, 164.6, 170.9, 173.2 ppm; IR (ATR): $\tilde{\nu}$ = 3330 (br), 3126 (w), 2981 (w), 2936 (w), 1731 (s), 1671 (m), 1617 (m), 1569 (m), 1534 (m), 1463 (s), 1388 (s), 1304 (w), 1259 (w), 1187 (s), 1154 (s), 1096 (w), 1027 cm^{-1} (s); MS(EI): m/z (%): 771.2 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{36}H_{50}N_8O_{11}$: C 56.09, H 6.54, N 14.54; found: C 56.30, H 6.87, N 14.80.

Synthesis of ligand $\mathbf{L_8}$: Compound **16** (126 mg, 0.16 mmol) was dissolved in 40 mL of a MeOH/H_2O (1:1) solution and NaOH (39 mg, 0.98 mmol) was added. The solution was stirred at 60°C for 3 h. The solvents were evaporated and the residue was dissolved in water (20 mL) and washed with CH_2Cl_2 (3×10 mL). The aqueous phase was evaporated to dryness to afford a pale yellow solid. The pale yellow residue was dissolved in 0.5 mL of water and 4 mL of MeOH. $\mathbf{L_8}$ (113 mg, 95%) was precipitated as a fine yellow powder upon a dropwise addition of THF and diethylether. ^{1}H NMR (300 MHz, D_2O): δ = 8.64 (s, 2H), 7.94 (s, 2H), 6.66 (s, 2H), 3.91 (s, 4H), 3.45 (t, J = 7.2 Hz, 2H), 3.27 (s, 8H), 2.29 (t, J = 7.2 Hz, 2H), 1.91 ppm (qt, J = 7.2 Hz, 2H); ^{13}C {^{1}H} NMR (75 MHz, D_2O): δ = 25.8, 35.4, 40.5, 51.3, 58.7, 68.3, 107.3, 110.0, 130.0, 150.2, 165.0, 167.1, 179.4, 183.1 ppm; IR (ATR): $\tilde{\nu}$ = 3276 (br), 2934 (w), 1568 (s), 1462 (m), 1403 (s), 1327 (m), 1123 (m), 1051 cm^{-1} (m); MS (ESI, MeOH/H_2O, negative mode): m/z (%): 347.1 (80) $[M-2Na^+]^{2-}$, 223.6 (100) $[M-3Na^+]^{3-}$;

elemental analysis calcd (%) for $C_{26}H_{25}N_8Na_5O_{11}.5H_2O$: C 37.60, H 4.25, N 13.49; found: C 37.43, H 3.96, N 13.24.

Synthesis of compound 17: In a Schlenk tube were dissolved **3b$_{Bu}$** (100 mg, 0.11 mmol), 6-heptinoic acid (17 μL; 0.13 mmol), [Pd(PPh3)2 L2] (8.40 mg; 0.01 mmol), anhydrous THF (10 mL) and freshly distilled Et3N (6 mL). The reaction mixture was degassed for 15 min and CuI (3.4 mg; 0.02 mmol) was added. Reaction was heated at 40 °C for 72 h. The solvent was evaporated to dryness and the residue was dissolved in CH_2Cl_2 (50 mL). The organic phase was washed with water (2× 50 mL), dried over Na_2SO_4 and evaporated to dryness. Flash chromatography over silica gel (CH_2Cl_2/MeOH = 100:00 to 95:5) afforded compound **17** (41 mg, 40 %) as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.65 (d, J = 8.8 Hz, 2H), 8.16 (d, J = 7.7 Hz, 2H), 7.82 (s, 2H), 7.47–7.26 (m, 4H), 4.41 (s, 4H), 3.56 (s, 8H), 2.54–2.42 (m, 4H), 1.87–1.71 (m, 4H), 1.48 ppm (s, 36H); ^{13}C {^{1}H} NMR (50 MHz, $CDCl_3$): δ = 19.3, 24.5, 27.9, 28.3, 45.0, 50.1, 55.7, 79.3, 81.2, 95.7, 112.3, 114.7, 121.7, 122.5, 125.7, 128.1, 136.4, 139.8, 146.4, 152.3, 170.5, 175.2 ppm; IR (ATR): $\tilde{\nu}$ = 3329 (br), 3125 (w), 2988 (w), 2929 (w), 2110 (w; $\nu_{C\equiv C}$), 1734 (s), 1658 (m), 1608 (m), 1581 (s), 1558 (w), 1435 (s), 1403 (s), 1332 (w), 1192 (w), 1133 (w), 1070 cm^{-1} (w); MS (EI): m/z (%): 949.2 (100) $[M]^+$, 950.2 (30 $[M]^+$; elemental analysis calcd (%) for $C_{52}H_{67}N_7O_{10}$: C 65.73, H 7.11, N 10.32; found: C 65.88, H 7.19, N 10.43.

Synthesis of ligand L$_9$: **17** (40 mg, 42 μmol) was dissolved in CH_2Cl_2 (3 mL) and TFA (1.5 mL). The reaction mixture was stirred for 4 h at RT and evaporated to dryness. The residue was dissolved in MeOH (0.5 mL), and **L$_9$** (20.8 mg, 52 %) was precipitated by addition of diethyl ether and isolated as a pale brown powder. ^{1}H NMR (200 MHz, CD_3OD): δ = 8.47 (d, J = 8.5 Hz, 2H), 8.17 (s, 2H), 7.83 (d, J = 8.5 Hz, 2H), 7.42–7.24 (m, 4H), 4.06 (s, 4H), 3.26 (s, 8H), 2.47–2.30 (m, 4H), 1.77–1.61 ppm (m, 4H); ^{13}C {^{1}H} NMR (50 MHz, CD_3OD,): δ = 25.5, 29.0, 29.5, 34.5, 47.9, 55.5, 79.5, 98.0, 114.0, 115.9, 121.6, 124.9, 126.8, 130.0, 138.2, 140.8, 141.3, 153.1, 170.0, 177.5 ppm; IR (ATR): $\tilde{\nu}$ = 3390 (s), 1604 (s), 1580 (s), 1555 (w), 1439 (s), 1406 (s), 1328 (w), 1198 (w), 1129 (w), 1069 cm^{-1} (w); MS (ESI, Acetone/H_2O): m/z (%): 726.3 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{36}H_{35}N_7O_{10}.H_2O$: C 58.14, H 5.01, N 13.18; found: C 57.97, H 4.76, N 12.92.

Synthesis of compound 18: **3b$_{Et}$** (90 mg, 0.11 mmol) and ethyl 4-aminobutyrate hydrochloride (38 mg, 0.23 mmol) were dissolved in distilled toluene (10 mL) and triethylamine (10 mL). [Pd(PPh3)2 L2] (8 mg, 0.011 mmol) was added and the solution was heated at 100 °C under a continuous flow of CO for 24 h. The solution was filtered over celite and the solvents were evaporated to dryness to give a pale yellow oil. The oil was dissolved in CH_2Cl_2 (30 mL) and washed with water (3×15 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. **18** (98 mg, 54 %) was isolated by flash chromatography on silica gel (CH_2Cl_2/MeOH = 100:0 to 99.4:0.6) as yellowish oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.64 (d, J = 8.5 Hz, 2H), 8.17 (d, J = 8.1 Hz, 2H), 8.15 (s, 2H), 7.50–7.28 (m, 4H), 7.18 (t, J = 5.4 Hz, 1H), 4.41 (s, 4H), 4.18 (q, J = 7.2 Hz, 10H), 3.68 (s, 8H), 3.62–3.55 (m, 2H), 2.47 (t, J = 7.2 Hz, 2H), 2.03 (qt, J = 7.2 Hz, 2H), 1.26 ppm (t, J = 7.2 Hz, 15H); ^{13}C {^{1}H} NMR (300 MHz, $CDCl_3$): δ = 14.1, 24.5, 31.9, 39.8, 50.6, 54.8, 60.6, 107.6, 114.5, 121.6, 122.7, 125.6, 128.3, 139.7, 146.5, 146.7, 152.8, 165.2, 171.1, 173.5 ppm; IR (ATR): $\tilde{\nu}$ = 3329 (br), 3127 (w), 2985 (w), 2934 (w), 1732 (s), 1669 (m), 1613 (m), 1571 (m), 1533 (m), 1466 (s), 1389 (s), 1302 (w), 1261 (w), 1187 (s), 1156 (s), 1092 (w), 1030 cm^{-1} (s); MS (EI): m/z (%): 871.2 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{44}H_{54}N_8O_{11}$: C 60.68, H 6.25, N 12.87; found: C 60.55, H 6.78, N 14.64.

Synthesis of ligand L$_{10}$: Compound **18** (50 mg, 57 μmol) was dissolved in 20 mL of MeOH/water = 1:1 and NaOH (14 mg, 0.34 mmol) was added. The reaction mixture was heated at 60 °C for 4 h. The solvent were evaporated and the residue was dissolved in water (20 mL). The aqueous phase was washed with CH_2Cl_2 (3×10 mL) and evaporated to afford a pale yellow residue. The residue was dissolved in water (0.5 mL) and **L$_{10}$** (24 mg, 50 %) was obtained as a fine pale brown powder by precipitation with MeOH, THF and diethyl ether. ^{1}H NMR (300 MHz, D_2O): δ = 8.35 (s, 2H), 7.95 (s, 2H), 7.80 (s, 2H), 7.50–7.36 (m, 4H), 4.08 (s, 4H), 3.27 (m, 10H), 2.30 (m, 2H), 1.88 ppm (m, 2H); ^{13}C NMR (200 MHz, D_2O): δ = 25.7, 35.3, 40.4, 49.3, 58.7, 107.7, 114.4, 121.5, 123.4, 125.7, 129.0, 139.0, 145.8, 146.7, 152.0, 166.9, 179.2, 183.0 ppm; IR (ATR): $\tilde{\nu}$ = 3390 (s), 1604 (s), 1580 (s), 1555 (w), 1439 (s), 1406 (s), 1328 (w), 1198 (w), 1129 (w), 1069 cm^{-1} (w); MS (ESI, MeOH/H_2O, negative mode): m/z (%): 397.2 (75) $[M-2Na^+]^{2-}$, 257.1 (35) $[M-3Na^+]^{3-}$; elemental analysis calcd (%) for $C_{34}H_{29}N_8Na_5O_{11}.5H_2O$: C 43.88, H 4.22, N 12.04; found: C 43.54, H 3.88, N 11.71.

Synthesis of compound 19: **3a$_{Bu}$** (480 mg, 0.61 mmol) and ethyl 4-aminobutyrate hydrochloride salt (204 mg, 1.22 mmol) were dissolved in distilled toluene (10 mL) and triethylamine (10 mL). $[Pd(PPh_3)_2Cl_2]$ (86 mg, 0.061 mmol) was added. The solution was heated at 100 °C under CO atmosphere during 24 h. The solution was filtered over celite and the solvents were evaporated to dryness to give a pale yellow oil. The oil was dissolved in CH_2Cl_2 (30 mL) and washed with water (3×15 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. **33** (288 mg, 54 %) was isolated as a colourless oil by flash chromatography on silica gel (CH_2Cl_2/MeOH = 100:0 to 99.5:5). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.43 (d, J = 2.2 Hz, 2H), 8.1 (s, 2H), 6.53 (d, J = 2.2 Hz, 2H), 4.06 (q, J = 7.0 Hz, 2H), 3.99 (s, 4H), 3.52 (q, J = 6.3 Hz, 2H), 3.47 (s, 8H), 2.42 (t, J = 7.2 Hz, 2H), 1.97 (qt, J = 7.1 Hz, 2H), 1.44 (s, 36H), 1.19 ppm (t, J = 6.8 Hz, 3H); ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ = 14.3, 24.5, 28.2, 31.7, 39.8, 51.3, 55.4, 60.5, 81.1, 106.8, 108.8, 128.0, 147.7, 150.7, 153.8, 164.9, 170.5, 173.5, 173.7 ppm; IR (ATR): $\tilde{\nu}$ = 3330 (br), 2978 (w), 2930 (w), 2851 (w), 1731 (m), 1659 (m), 1618 (m), 1570 (m), 1535 (m), 1463 (m), 1391 (m), 1368 (m), 1312 (w), 1282 (w), 1552 (w), 1217 (m), 1142 (s), 1045 (m), 978 (m), 912 cm^{-1} (m); MS (FAB): m/z (%): 883.3 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{44}H_{66}N_8O_{11}$: C 59.85, H 7.53, N 12.69; found: C 59.67, H 7.33, N 12.53.

Synthesis of compound 20: **19** (96 mg, 0.11 mmol) was dissolved in THF (10 mL) and 1 mL of aqueous solution of NaOH (10 mg, 0.24 mmol) was added. The solution was stirred at 60 °C during 3 h. The solvents were distilled off and the residue was dissolved in CH_2Cl_2 (10 mL), and washed with an NH_4Cl saturated solution in water (3×5 mL). The aqueous phase was washed with CH_2Cl_2 (3×10 mL). The combined organic phases were evaporated to dryness to afford a white solid mixed with colourless oil. The residue was purified by flash chromatography over silica gel (CH_2Cl_2/MeOH = 100:00 to 98:2) to afford **20** (65 mg, 70 %) as a white solid after drying under reduced pressure. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.47 (d, J = 2.5 Hz, 2H), 8.22 (s, 2H), 6.53 (d, J = 2.5 Hz, 2H), 4.01 (s, 4H), 3.54 (m, 2H), 3.50 (s, 8H), 2.54 (t, J = 6.0 Hz, 2H), 2.00 (qt, J = 6.0 Hz, 2H), 1.46 ppm (s, 36H); ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ = 28.3, 33.3, 40.1, 51.5, 55.8, 81.5, 107.3, 108.9, 128.3, 147.9, 150.6, 153.7, 164.4, 170.8, 178.3 ppm; IR (ATR): $\tilde{\nu}$ = 3330 (w), 2979 (w), 2931 (w), 2850 (w), 1731 (s), 1659 (m), 1618 (m), 1570 (s), 1535 (m), 1463 (s), 1391 (s), 1311 (w), 1252 (m), 1216 (s), 1142 (s), 1045 (m), 978 cm^{-1} (m); MS (ESI, CH_3CN/H_2O/TFA 1 %): m/z (%): 853.3 (100) $[M-NH_4^+]^-$; elemental analysis calcd (%) for $C_{42}H_{61}N_8O_{11}.NH_4.3H_2O$: C 55.56, H 7.44, N 12.34; found: C 54.47, H 7.73, N 12.69.

Synthesis of compound 21: In a Schlenk tube under argon, **20** (85 mg, 0.097 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL). Disuccinimidylcarbonyl (30 mg, 0.12 mmol) and DIEA (34 μL, 0.195 mmol) were added. The reaction mixture was stirred at RT during 4 h and 10 mL of CH_2Cl_2 were added. The organic phase was washed with water (2×10 mL), HCl 1N (2×10 mL) and brine (2×10 mL). The combined organic phase were dried over Na_2SO_4 and concentrated to dryness. Flash chromatography over silica gel (CH_2Cl_2/MeOH = 100:00 to 95:5) afforded pure **21** (47.7 mg, 52 %) as a white solid. ^{1}H NMR(300 MHz, $CDCl_3$): δ = 8.47 (d, J = 2.6 Hz, 2H), 8.12 (s, 2H), 6.57 (d, J = 2.6 Hz, 2H), 4.04 (s, 4H), 3.61 (q, J = 6.4 Hz, 2H), 3.51 (s, 8H), 2.85 (s, 4H), 2.75 (t, J = 7.2 Hz, 2H), 2.15 (qt, J = 7.2 Hz, 2H), 1.46 ppm (s, 36H); ^{13}C NMR (300 MHz, $CDCl_3$): δ = 24.4, 25.6, 28.2, 28.5, 38.9, 51.2, 55.3, 81.1, 106.7, 108.8, 128.0, 147.2, 150.7, 153.7, 165.0, 168.2, 169.2, 170.4 ppm. IR (ATR): $\tilde{\nu}$ = 2979 (w), 2926 (w), 2851 (w), 1812 (w), 1785 (w), 1733 (s), 1666 (w), 1618 (w), 1570 (m) 1534 (m), 1462 (m), 1391 (m), 1367 (m), 1310 (w), 1284 (w), 1249 (w), 1208 (s), 1141 (s), 1067 (m), 1045 (m), 978 cm^{-1} (m); MS (EI): m/z (%): 952.3 (100) $[M]^+$; elemental analysis calcd for $C_{46}H_{65}N_9O_{13}.H_2O$: C 56.95, H 6.95, N 12.99; found: C 56.68, H 6.53, N 12.67.

Synthesis of ligand L_8*: 21 (40 mg, 42 μmol) was dissolved in CH_3Cl (5 mL) and TFA (0.3 mL) was added. The reaction mixture was stirred at RT resulting in the formation of pale yellow immiscible drops. The solvents were pipetted off, and the residual oil was triturated with diethyl ether resulting in the formation of a white precipitate of **L_8*** as its bis triflate salt (26 mg, 65%). ^{1}H NMR (400 MHz, D_2O): $\delta = 8.68$ (s, 2H), 8.25 (s, 2H), 6.61 (s, 2H), 4.35 (s, 4H), 3.87 (s, 8H), 3.45 (t, $J = 7.0$ Hz, 2H), 2.74 (s, 4H), 2.70 (t, $J = 7.0$ Hz, 2H), 2.2 ppm (qt, $J = 7.0$ Hz, 2H); IR (ATR): $\tilde{\nu} = 2977$ (w), 1812 (w), 1777 (w), 1733 (s), 1659 (w), 1620 (w), 1571 (m), 1530 (m), 1459 (m), 1429 (w), 1387 (m), 1325 (m), 1251 (w), 1200 (s), 1139 (m), 1064 (m), 989 cm^{-1} (m); MS (ESI, acetone/MeOH 10%): *m/z* (%): 362.5 (100) $[M-2H^+-2(CF_3COOH)]^{2-}$; elemental analysis calcd (%) for $C_{30}H_{35}N_9O_{13}.C_4F_6O_4.3H_2O$: C 40.44, H 4.09, N 12.48; found: C 40.35, H 4.14, N 12.54.

Synthesis of the complexes: Full experimental details and characterization of the complexes are described in the Supporting Information .

Absorption, emission spectroscopy and fluorescence microscopy: UV/Vis absorption spectra were recorded on a Uvikon 933 or a Shimadzu UV3600 spectrometers. Emission and excitation spectra were recorded on a Perkin–Elmer LS50B (working in the phosphorescence mode with 0 μs delay time) or an Edimburgh Instrument FL920 spectrometer equipped with a Hamamatsu R928 photomultiplier. Luminescence decays were obtained on the later instrument over temporal windows covering at least five decay times. Luminescence quantum yields were measured according to conventional procedures,[54] with diluted solutions (optical density < 0.05), using $[Ru(bipy)_3]Cl_2$ in non degassed water ($\Phi = 2.8\%$)[55] and rhodamine 6G in ethanol ($\Phi = 88\%$)[56] as references for Eu and Tb compounds, with the necessary correction for refractive index of the media used.[50] Estimated error are ±15%. Hydration numbers, *q*, were obtained using Equation (1), in which τ_{H2O} and τ_{D2O} respectively refer to the measured luminescence decay lifetime (in ms) in water and deuterated water, using A = 1.11 and B = 0.31,[35a] or A = 1.2 and B = 0.25[35b] for Eu, and A = 5 and B = 0.06[35b] or A = 4.2 and B = 0[35c] for Tb (estimated error ±0.2 water molecules).

$$q = A \times (1/\tau_{H2O} - 1/\tau_{D2O} - B) \quad (1)$$

Fluorescence microscopy images were recorded on a Leica DMLB microscope equipped with a continuous 100 W mercury lamp. Time-resolved acquisition was obtained on the same microscope using a home made set up previously described.[47c]

Labelling experiment and time-resolved fluoroimmunoassay: [Tb**L8***] dissolved in DMF at a concentration of 3.8 μmol/mL was coupled with GFR44 antibody in carbonate buffer at pH 8.3 for one hour at RT with a [Tb**L8***]/[GFR44] ratio of 30. Purification of the conjugate was performed by size-exclusion chromatography on a Sephadex G25 (GE Healthcare) with TRIS/HCl buffer at pH 7.5. The final labelling ratio was determined by absorption spectroscopy using the following procedure. The Tb**L_8** concentration was obtained by $[Tb] = d.o._{.327}/\varepsilon_{Tb327}$, in which $d.o._{.327}$ is the absorption of the labelled antibody at 327 nm and ε_{Tb327} the absorption coefficient of [Tb**L_8***] at 327 nm. The labelled antibody concentration was determined by: $[GFR44] = (d.o._{.280} - d.o._{.327} \times (\varepsilon_{Tb280}/\varepsilon_{Tb327})/210000$; in which $d.o._{.280}$ is the absorption of the labelled antibody at 280 nm, and ε_{Tb280} is the absorption coefficient of [Tb**L_8***] at 280 nm. The labelling ratio is then the ratio of [Tb]/[GFR44] and was determined to be 11.8 Tb**L8** complex per GFR44 with a final yield of 44%. The stock solutions of G15-Cy5 and Tb**L_8**-GFR44 conjugates were diluted to respectively 6.25 and 0.5 μg mL with assay buffer (0.1 M TRIS buffer, 0.1% bovin serum albumin, 0.3 mg/mL non-specific mouse igG, pH 7.1) prior to use. Other standards used were taken from the commercial kit (BRAHMS) and the coupling reactions with the europium cryptate were performed according to the manufacturer's coupling protocol. The immunoassay was performed by incubating 70 μL of sample or calibrator, 40 μL of acceptor labelled antibody solution and 40 μL donor conjugated antibody solutions at 37 °C on a BRAHMS Kryptor automate (Cezanne SAS), according to manufacturer's instructions. The reaction time of the assay was 39 min. For measurements in the presence of free Tb^{3+} a 40 μL aliquot of a 5 μg mL $TbCl_3$ solution was added in the assay. The excitation of the sample was performed with a pulsed LASER at 337 nm and the time-resolved emission signal was recorded 50 μs after the pulse and for a duration of 400 μs. The emitted intensity was recorded at 560 nm for Tb, 620 nm for the Eu cryptate and 707 nm for Cy5. Finally, ΔF was calculated using the equation (2):

$$\Delta F = (R_{tf} - R_{t0})/R_{t0} \quad (2)$$

where R_{tf} and R_{t0} are respectively the ratio of the donor intensity (Tb or Eu) over acceptor intensity (Cy5) at the end of incubation and before the addition of the antigene.

Acknowledgements

Dr Frédéric Boschetti (Chematech, Dijon, France) is gratefully acknowledged for fruitful discussions. We would like to thank the European Commission for financial support of MS through a grant for Specific Targeted Research Project (POC4 Life-LSHB-CT-2007–037933).

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Received: February 3, 2011
Published online: July 1, 2011

