A new chiral lanthanide NMR probe for the determination of the enantiomeric purity of α-hydroxy acids and the absolute configuration of α-amino acids in water

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A water-soluble, enantiopure lanthanide complex, *SSS*-[Ln·L^3], has been assessed as an effective chiral derivatising agent for the determination of the enantiomeric purity of α-hydroxy acids in aqueous solution. The complex displays superior chemical shift non-equivalence ($\Delta\Delta\delta$ ~2–11 ppm) for the diastereomeric resonances of interest compared to lanthanide shift reagents reported in the literature ($\Delta\Delta\delta$ <0.1 ppm, typically). ^{1}H NMR studies have also revealed that *SSS*-[Ln·L^3] can be used to determine the absolute configuration of α-amino acids at physiological pH, in water. The ability of *SSS*-[Ln·L^3] to signal anion binding and, in particular, to distinguish between diastereomers through optical techniques such as lanthanide luminescence and circular dichroism has also been assessed.

Introduction

The paramagnetic properties of the lanthanides have been exploited in NMR spectroscopy for many years to effect spectral simplification and resolution enhancement.[1,2] The angular and distance information gleaned from the lanthanide induced shift has found widespread application from the determination of solution structures of lanthanide chelates to the development of biological probes of protein and peptide structure.[3,4] Chiral lanthanide complexes continue to be assessed as chiral shift and derivatising agents for the NMR separation of enantiomers and determination of enantiomeric purity.[2,3,5] Although substantial developments have been reported within this field, the majority of studies have been performed in organic media. As a large number of optically active compounds are of natural origin, water is the solvent of choice and studies in aqueous media are therefore highly desirable. A few water soluble systems have been reported since Reuben first introduced a 'self-resolution' approach to separate the enantiomeric signals of α-hydroxy carboxylic acids using lanthanide ions.[6] Peters *et al*. proposed the first genuine aqueous chiral lanthanide shift reagents based on lanthanide complexes of (*S*)-[(carboxymethyl)oxy]succinic acid (CMOS) which resolved the enantiomeric nuclei of α-amino acids and carboxylates.[7] Although spectral resolution was sufficient to determine enantiomeric purity, the magnitude of shift difference between enantiomers ($\Delta\Delta\delta$) was small (<0.1 ppm) and displayed a strong pH dependence. This is typical of a number of reported systems for α-hydroxy, α-amino and carboxylic acids which are found to operate within a specific pH range and often display no resolution beyond an optimal pH.[8] There have been reports of aqueous lanthanide shift reagents for α-amino acids and *N*-acyloligopeptides operating at neutral pH without displaying significant signal broadening.[9] However, the observed limiting chemical shift non-equivalence in each of the examples is very small (<0.1 ppm, typically) as found in so many of these aqueous chiral lanthanide shift reagents. This, coupled with the broadness of the observed signals, which is often associated with chemical exchange due to competing internal exchange processes, has meant there has been little direct application for the determination of enantiomeric purity in aqueous solution.

However, the resurgence of interest in the coordination chemistry of lanthanide complexes in aqueous solution over the past few years has led to the development of well-defined, water-soluble chiral systems which offer considerable scope for the design of more effective chiral shift reagents and, where the association constant is much larger, of chiral derivatising agents. We have been involved in the selective recognition of bioactive species in aqueous solution by coordinatively unsaturated lanthanide complexes in which the bound water molecules may be readily displaced by a variety of monodentate (*e.g.* fluoride, phosphate) or chelating (*e.g.* lactate, carbonate) anions.[10,11] The rich magnetic and optical properties exhibited by the lanthanide ions has enabled the anion binding to be effectively signalled through NMR (Eu, Yb), luminescence (Eu, Tb) and chiroptical techniques (Eu, Yb). Within these studies we have reported a first step towards the development of an effective chiral derivatising agent for α-hydroxy acids in aqueous solution.[12] A large lanthanide induced shift, chemical shift non-equivalence and an apparent absence of kinetic resolution in complex formation has been observed upon addition of racemic lactic acid to [Yb·L^1]$^{3+}$. The lactate CH and CH_3 resonances are clearly resolved for the (*R*) and (*S*) diastereomers ($\Delta\Delta\delta$ = 10 ppm) and experience a large lanthanide induced shift; the CH resonating at +48 and +58 ppm (*cf*. 4.1 ppm in the free form) and the CH_3 resonating at +30 and +20 ppm for (*R*) and (*S*)-lactate respectively (*cf*. 1.3 ppm in the free form). Herein, we report the synthesis of a novel, enantiopure lanthanide complex and assess its ability to act as an effective chiral derivatising agent in aqueous solution, for a number of bioactive species.

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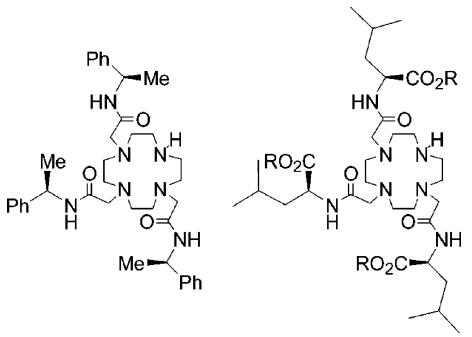


L^1 L^2 R = Me, L^3 R = H

Results and discussion

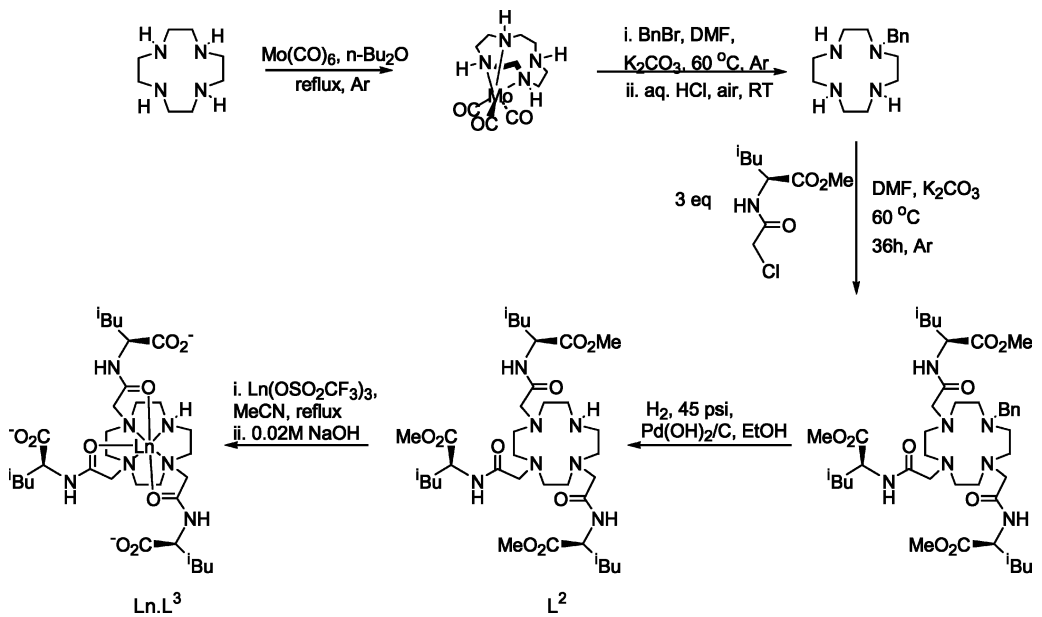
Synthesis

The synthesis of the tri-substituted ligand, L^2, was attempted by direct alkylation of tetraazacyclododecane with the appropriate chloroamide. However, a mixture of mono-, di- and tri-substituted products resulted. Although the trialkylated product was isolated *via* exhaustive extraction of the organic phase with water or by column chromatography, yields were poor. Therefore an alternative route involving the selective protection of a single macrocyclic ring nitrogen, followed by trialkylation and subsequent deprotection was employed (Scheme 1). Reaction of 1,4,7,10-tetraazacyclododecane-molybdenum tricarbonyl[13] with benzyl bromide (dry DMF, 60 °C, K_2CO_3), followed by decomplexation of the molybdenum moiety in aqueous acid, yielded the monobenzylated macrocycle. Subsequent reaction with three equivalents of the appropriate chloroamide (dry DMF, Ar, 60 °C, K_2CO_3) afforded the tetra-alkylated ligand after purification on neutral alumina. The trialkylated ligand, L^2, was isolated following reductive deprotection of the N-benzyl group (H_2, 45 psi, $Pd(OH)_2/C$, acidic EtOH, RT). Complexation was achieved by refluxing L^2 in dry acetonitrile, with the appropriate lanthanide trifluoromethanesulfonate salt. Subsequent basic hydrolysis of $[Ln{\cdot}L^2]^{3+}$ (0.02 M NaOH) followed by cation exchange chromatography, yielded the neutral *SSS*-$[Ln.L^3]$ complexes.

Solution NMR studies

The isomerism exhibited by chiral lanthanide complexes such as $[Ln{\cdot}L^1]^{3+}$–$[Ln{\cdot}L^3]$ is well documented and understood.[2] They exhibit two independent elements of chirality denoted by the NCCO torsion angle of the pendent arms (positive = Δ, negative = Λ) and the NCCN torsion angle of the macrocyclic ring (positive = δδδδ, negative = λλλλ) which gives rise to four possible isomers in solution. The remote chiral centre at the amide functionality renders each of the isomers diastereomeric. Variable temperature (295–193 K) NMR studies on lanthanide complexes of L^1 confirm the existence of two square antiprismatic isomers (Λδδδδ and Δλλλλ) at room temperature, which are in fast exchange on the NMR timescale.[11] In contrast, $[Ln{\cdot}L^3]$ complexes appear to exist solely as a single isomer at room temperature, as confirmed by VT NMR, with shifts indicative of a square antiprismatic geometry.[14] The partial ^{1}H NMR spectrum of *SSS*-$[Yb{\cdot}L^3]$ (1 mM, D_2O, pD 7, 200 MHz, 295 K) is shown in Fig. 1(a). The eight distinct resonances to high frequency (+99 to +16 ppm) can be attributed to the four most shifted axial (●) and equatorial (□) protons of the macrocyclic ring. It should be noted that the ^{1}H NMR spectrum of *SSS*-$[Yb{\cdot}L^3]$ at a concentration of 10 mM revealed the presence of many broadened peaks which disappeared upon dilution, consistent with the formation of dimers at higher concentrations, which was also confirmed by ESMS [1735 (10%), M_2Na^+].

Recently, we have reported that the binding of anions to the co-ordinatively unsaturated lanthanide complexes of L^1 is effectively signalled through ^{1}H NMR as the observed lanthanide induced shift of the macrocyclic ring protons can be directly correlated to the nature of the donor atom in the axial position.[15,16] It is



Scheme 1

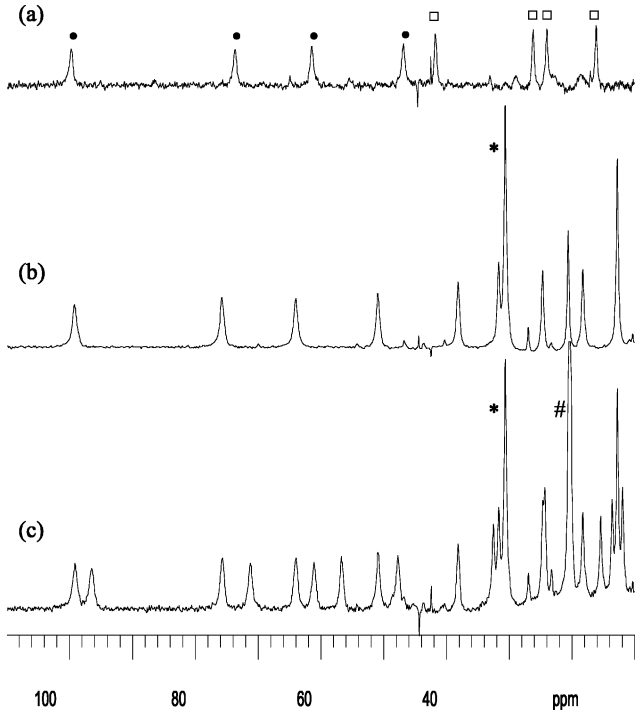


Fig. 1 Partial ^{1}H NMR (200 MHz, D_2O, 295 K) of (a) *SSS*-[Yb·L^3] (diaqua species) showing the four axial (●) and four equatorial (□) protons of the macrocyclic ring; and in the presence of (b) 10 equivalents of (*S*)-lactate [(*) (*S*)-lactate CH_3]; (c) 10 equivalents of racemic lactate [(#) (*R*)-lactate CH_3].

the polarisability of the axial donor that ranks the second order crystal field coefficient, B_0^2, and determines the magnitude of the observed shift.[17,18] Values of the mean shift of the four most shifted axial protons of the macrocyclic ring for *SSS*-[Yb·L^3] are collated in Table 1. Hard, uncharged donors such as water give rise to the largest shifts whereas the more polarisable donors (*e.g.* carbonate) give much smaller shifts. This simple correlation has proved to be invaluable in the determination of binding modes for a variety of anions. For example, the phosphorylated amino acids (*e.g.* (*S*)-OP-serine) give rise to a shift indicative of a preferred binding mode to *SSS*-[Yb·L^3] through the phosphate moiety rather than chelation through the amino acid functionality (*via* formation of a 5-ring chelate involving the NH_2 and CO_2^-). Furthermore, nuclei within the binding anion that lie within the McConnell cone [$(3\cos^2\theta - 1)r^{-3}$ dependence] will also be subjected to a lanthanide induced shift. This can give useful information into the binding mode of chelating anions. For example, lactate binds to [Yb·L^1]$^{3+}$ in a bidentate manner, as confirmed through crystallographic and hydration state studies.[12] However, there are two possible binding modes: the OH group may be axially disposed with the carboxylate occupying the equatorial position or *vice versa*. Upon the addition of (*S*)-lactate to [Yb·L^1]$^{3+}$ two additional singlet resonances were evident in the high frequency range at +58 ppm, integrating to one proton and attributed to the CH of the bound lactate, and at +20 ppm, integrating to three protons and subsequently attributed to the methyl resonance of the bound lactate.[19] The induced paramagnetic shift, which for Yb is largely dipolar in origin, can be approximated to having a $(3\cos^2\theta - 1)r^{-3}$ dependence and suggests the lactate CH is closer to the principal axis of the complex than the lactate methyl group and axial ligation therefore occurs through the OH functionality with the carboxylate group occupying the equatorial position (Fig. 2). This is consistent with solid state crystallographic studies.[20]

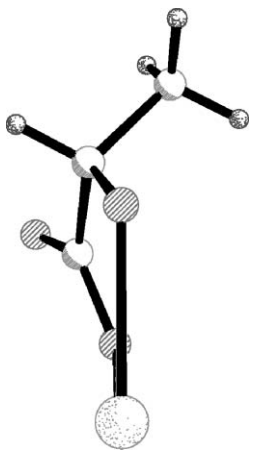


Fig. 2 Ligation of lactate to the Yb centre showing the hydroxyl group in an axial position which brings the lactate CH closer to the principal axis compared to the lactate methyl group.

α-Hydroxy acids. The ^{1}H NMR spectrum of *SSS*-[Yb·L^3] (10 mM, 200 MHz, D_2O, 295 K) in the presence of a ten-fold excess of (*S*)-lactate (Fig. 1b) is also consistent with bidentate

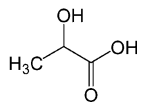
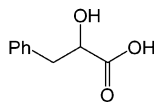
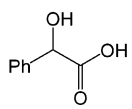
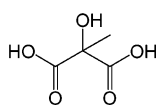
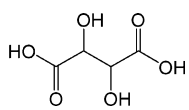
Table 1 Effect of the axial donor on the ^{1}H NMR spectral range for *SSS*-[Yb·L^3] (200 MHz, D_2O, 295 K)

Anion	Axial donor	Shift range (ppm)	Mean shift[a] δH_{ax} (ppm)
(*S*)-OP-serine	H_2O	+110 → −83	82
Phosphate	H_2O	+102 → −77	75
Water	H_2O	+100 → −120	70
(*S*)-Lactate	OH	+99 → −56	72
Acetate	$CO^{\delta-}$	+93 → −94	68
Citrate[b]	$CO^{\delta-}$	+89 → −100	64
(*S*)-Serine	NH_2	+68 → −50	43
(*S*)-Alanine	NH_2	+65 → −43	40
Oxalate	CO^-	+56 → −31	36
Carbonate	CO^-	+45 → −44	33

[a] Mean chemical shift of the four most shifted axial protons of the macrocyclic ring. [b] Minor species also present (>10%), due to additional possible binding modes for the anion.

chelation with the OH group axially disposed. An additional singlet resonance at +31 ppm was evident, integrating to three protons, which can be accounted for by the methyl resonance of the bound (*S*)-lactate. The expected methyl doublet was not resolved due to the broadening effect of the paramagnetic ion. However, the exact position of the lactate CH resonance could not be defined due to inconclusive two dimensional spectra obtained for this system,[21] although an extra singlet resonance observed in the high frequency region of the spectrum could be accounted for. Upon addition of 10 equivalents of racemic lactate to *SSS*-[Yb·L^3] a lack of enantioselectivity in adduct formation was apparent and a 1 : 1 mixture of diastereomeric complexes was observed by ^{1}H NMR (Fig. 1c). The lactate methyl resonance was clearly resolved for the (*R*) (+20 ppm) and (*S*) (+31 ppm) diastereoisomers ($\Delta\Delta\delta$ ~11 ppm) and experienced a large lanthanide induced shift (+1.3 ppm in the free form). Furthermore, the macrocyclic ring protons for the (*R*) and (*S*) diastereomeric complexes were also clearly resolved and displayed large $\Delta\Delta\delta$ values ranging from 2–10 ppm. As the complex displays a substantial improvement in the chemical shift difference observed for the diastereomeric resonances compared to reported lanthanide shift reagents ($\Delta\Delta\delta$ < 0.1 ppm, typically) a range of related derivatives were then studied to assess the potential of *SSS*-[Yb·L^3] to act as an aqueous chiral derivatising agent for α-hydroxy acids in general (Table 2). A lack of enantioselectivity in complex formation was observed in each case, even with more sterically demanding substrates, and additional resonances due to the α-CH and side chain functionality held within the McConnell cone were apparent to high frequency. Due to a lack of cross peaks being observed in ^{1}H–^{1}H COSY experiments[21] the exact assignment of the bound α-hydroxy acid resonances proved to be difficult. However, a large chemical shift non-equivalence was apparent for the macrocyclic ring protons in the (*R*) and (*S*) diastereomeric adducts. In particular, the magnitude of the shift difference for the most shifted axial ring proton in the diastereomers was of the order 2–5 ppm (Table 2). This is a huge improvement compared to $\Delta\Delta\delta$ values reported

Table 2 ^{1}H NMR data for *SSS*-[Yb·L^3] in the presence of 10 equivalents of racemic α-hydroxy acid (200 MHz, D_2O, 295 K)

α-Hydroxy acid	Ratio of (*R*) : (*S*) diastereomers	Most shifted δH_{ax} for (*R*)-diastereomer (ppm)	Most shifted δH_{ax} for (*S*)-diastereomer (ppm)	$\Delta\Delta\delta$ (ppm)
Lactate	1 : 1	+97 (δCH_3 = +20)[a]	+99 (δCH_3 = +30)[a]	2 (10)
				
Phenyllactate	1 : 1	+94	+91	3
				
Mandelate[b]	1 : 1	+94	+96	2
				
Citramalate	1 : 1 1 : 1	+66 +97	+70 +92	4 5
				
Tartrate	1 : 1	+109	+114	5
				

[a] Values in parentheses indicate the chemical shift observed for the bound lactate CH_3 resonance. [b] The resonances for the most shifted axial protons for the (*R*) and (*S*) diastereomers were broadened and overlapped slightly, hence enantioselectivity in binding was determined using the subsequently most shifted resonance occurring at +66 and +70 ppm for (*R*) and (*S*) mandelate respectively.

for aqueous lanthanide shift reagents in the literature which are generally around 1 ppm and often <0.1 ppm and enables the determination of the enantiomeric purity of α-hydroxy acids to be easily assessed in aqueous solution.

α-Amino acids. ^{1}H NMR studies have revealed that *SSS*-[Yb·L^3] binds to α-amino acids at physiological pH *via* a common chelated mode. The chemical shifts observed are indicative of axial ligation through the amine functionality[11] with the carboxylate group occupying an equatorial position (Table 1). No evidence of competing chelation through side chain functionality was apparent. There are very few examples of shift reagents capable of determining the enantiomeric purity of α-amino acids at physiological pH in aqueous media. Therefore the ability of *SSS*-[Yb·L^3] to act as a chiral derivatising agent for α-amino acids in water was also assessed (Table 3). Upon addition of 10 equivalents of racemic α-amino acid to *SSS*-[Yb·L^3] enantioselectivity in binding was apparent for the majority of the amino acids, with preferential binding occurring to the (*R*) enantiomer. This precludes the use of *SSS*-[Yb·L^3] as a chiral derivatising agent for α-amino acids. However, a large chemical shift non-equivalence was apparent for the diastereomeric macrocyclic ring protons of the (*R*) and (*S*) amino acid adducts, with $\Delta\Delta\delta$ values ranging from 4–9 ppm for the most shifted axial proton resonance. Furthermore, the most shifted axial ring proton resonance for the (*S*) diastereomer always appeared to higher frequency compared to the (*R*) diastereomeric peak. Therefore *SSS*-[Yb·L^3] may be used in the determination of absolute configuration of α-amino acids of unknown configuration. Indeed, although there have been a few reports of the use of lanthanide complexes as chiral shift reagents in aqueous media for the α-amino acids,[8,9] the assignment of the absolute configuration by such reagents is not usually reliable. Kabuto and Sasaki have reported a consistent correlation between absolute configuration of α-amino acids and their shift induced by a propylenediaminetetraacetato europium complex in aqueous solution.[22] However, the observed chemical shift differences ($\Delta\Delta\delta$) were very small (<0.2 ppm) and the studies were performed at pH = 9–11.

Luminescence and circular dichroism studies

Optical properties along with NMR spectral characteristics of the lanthanides are determined by the nature and local symmetry of the coordination environment.[23,24] Lanthanide luminescence, hydration state studies, circular dichroism and circularly polarised emission have all been reported to successfully signal anion binding at coordinatively unsaturated lanthanide complexes.[10,11] A systematic study was therefore undertaken to determine whether the (*R*) and (*S*) diastereomeric adducts formed upon addition of a racemic substrate to enantiopure *SSS*-[Ln·L^3] complexes may be distinguished by optical methods.

Emission studies. In Bleaney's theory of magnetic anisotropy[25] the dipolar NMR shift of paramagnetic lanthanide complexes is determined by the second order crystal field parameter, B_0^2. This parameter may be measured directly from the band splitting of the $\Delta J = 1$ transition in the europium emission spectrum.[26] The europium emission spectrum also provides information on the dipolar polarisability of the ligand donors by analysis of the hypersensitive $\Delta J = 2$ and $\Delta J = 4$ transitions. Indeed, there have been recent reports highlighting the sensitivity of the $\Delta J = 2$ transition to the nature of the donor in the axial position[10,18] and the ratio of the integrated emission intensities of the $\Delta J = 2/\Delta J = 1$ spectral band is regarded as a useful parameter in assessing changes in the europium coordination environment.[27]

Upon addition of a 10-fold excess of added anion to *SSS*-[Eu·L^3] (2.5 mM complex in 0.1 M MOPS buffer, pH 7.4, λ_{ex} = 397 nm, 295 K) two distinct trends in the emission spectrum were apparent (Fig. 3). The magnitude of splitting observed in the $\Delta J = 1$ band appeared to be highly dependant upon the nature of the axial donor (Table 4), with the more polarisable donors giving rise to the largest band splittings. The band splitting was found to correlate well with the most shifted axial ring proton resonance observed in the corresponding *SSS*-[Yb·L^3]

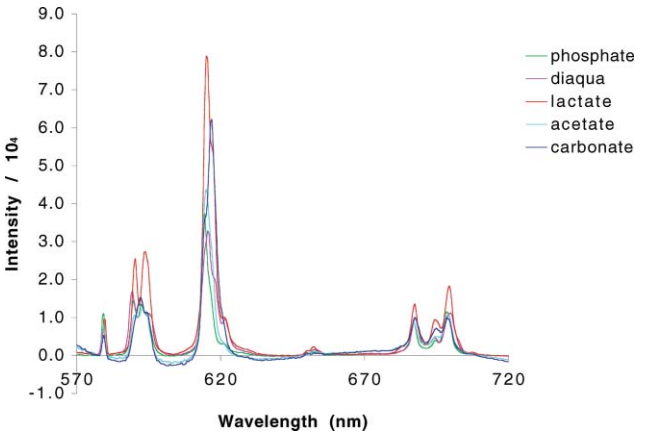


Fig. 3 Variation in the emission spectrum of *SSS*-[Eu·L^3] (2.5 mM complex in 0.1 M MOPS, pH 7.4, 295 K) following the addition of a ten-fold excess of added anion.

Table 3 ^{1}H NMR data for *SSS*-[Yb·L^3] in the presence of 10 equivalents of racemic α-amino acid (200 MHz, D_2O, 295 K, pD 7.4)

α-Amino acid	Ratio of (*R*) : (*S*) diastereomers	Most shifted δH_{ax} for (*R*)-diastereomer (ppm)	Most shifted δH_{ax} for (*S*)-diastereomer (ppm)	$\Delta\Delta\delta$ (ppm)
Alanine	1 : 1	+60	+65	5
Serine	2.2 : 1	+59	+68	9
Threonine	2 : 1	+61	+67	6
Methionine	1.7 : 1	+64	+70	6
Glutamic acid	2.6 : 1	+62	+68	6
Lysine[a]	1 : 1	+62	+66	4
Phenylalanine	2 : 1	+68	+72	4

[a] The presence of a minor species was also apparent (<7%).

Table 4 Effect of the polarisability of the axial donor on the ^{1}H NMR shift of *SSS*-[Yb·L^3], emission properties of *SSS*-[Eu·L^3] and CD spectral properties of [Yb·L^3]

Anion	Most shifted δH_{ax} (ppm)	$\Delta J = 1$ splitting/10^4 cm^{-1}	$\Delta J = 2/\Delta J = 1$ band intensity ratio	CD wavelength[a]/nm
(*S*)-OP-serine	110	286	1.4	990
Phosphate	101	222	1.5	991
Water	100	286	1.5	989
(*S*)-Lactate	98	267	2.2	988
Acetate	94	308	2.3	987
(*S*)-Alanine	64	—	—	986
Oxalate	56	667	3.1	985
Carbonate	42	800	3.1	983

[a] Wavelength refers to the peak of the intense band centred around 990 nm.

ternary complexes (Fig. 4), indicating the second order crystal field coefficient, which largely determines the pseudo-contact shift in the paramagnetic complexes is primarily affected by the ligand field in both Eu and Yb complexes. The intensity of the magnetic dipole allowed $\Delta J = 1$ transition is relatively independent of the coordination environment whereas the $\Delta J = 2$ transition, being predominantly electric dipole in character, is highly sensitive to the ligand field, in particular the nature and polarisability of the axial donor. Therefore analysis of the integrated emission intensities for the $\Delta J = 2/\Delta J = 1$ spectral bands for *SSS*-[Eu·L^3] will provide a direct measure of the polarisability of the axial donor. The more polarisable donors (*e.g.* carbonate) were found to give rise to the largest band intensity ratios (Table 4) and a linear correlation between the observed chemical shift of the most shifted axial ring proton in the corresponding *SSS*-[Yb·L^3] anion-adducts and the europium band intensity ratios was observed. Therefore anion binding to heptadentate lanthanide complexes is effectively signalled not only through observation of the induced paramagnetic shift but also through characteristics in the europium emission spectrum.

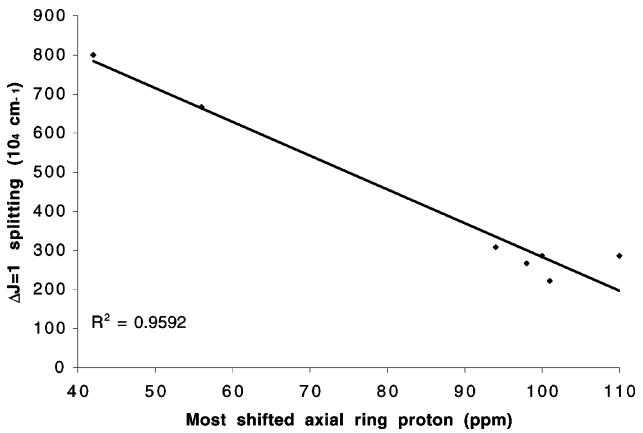


Fig. 4 Correlation between the most shifted axial ring proton for *SSS*-[Yb·L^3] and the $\Delta J = 1$ band splitting for *SSS*-[Eu·L^3] in the presence of a ten-fold excess of added anion.

Emission spectra were then recorded for *SSS*-[Eu·L^3] (2.5 mM complex in 0.1 M MOPS buffer, pH 7.4, $\lambda_{ex} = 397$ nm, 295 K) in the presence of a ten-fold excess of a variety of (*R*) and (*S*) chiral anions (*e.g.* lactate, amino acids, phosphorylated amino acids) to deduce whether diastereomeric adducts may be distinguished through emission studies. Identical spectral characteristics were observed for each of the (*R*) and (*S*) diastereomeric adducts, the emission studies therefore appear to be unsuitable in distinguishing between such diastereomers.

Circular dichroism. The local coordination environment about an ytterbium centre may be probed through near-IR CD. Ytterbium exhibits sensitive CD bands centred around 980 nm associated with the magnetic-dipole allowed $^2F_{7/2} \rightarrow {}^2F_{5/2}$ transitions.[17,28] Although spectral analysis is not straightforward due to the large number of sub-levels present, there appears to be a distinct trend between the wavelength of the observed CD and the polarisability of the axial donor.[16]

Near-IR CD spectra of *SSS*-[Yb·L^3] (20 mM complex in 0.1 M MOPS, pH 7.4, 295 K) in the presence of a ten-fold excess of added anion revealed that the band centred around 990 nm moves to shorter wavelength as the polarisability of the axial donor increases (Fig. 5). Therefore, through observation of the form of the CD, the nature of the coordinating anion may be assessed. Indeed, the variation in the CD wavelength was found to correlate well with the shift of the axial ring proton in the *SSS*-[Yb·L^3] anion adducts, suggesting the same axial ligand effect is manifest in each parameter and is probably associated with the second order crystal field coefficient, B_0^2, that determines characteristics of Eu emission and NMR spectra (Table 4).

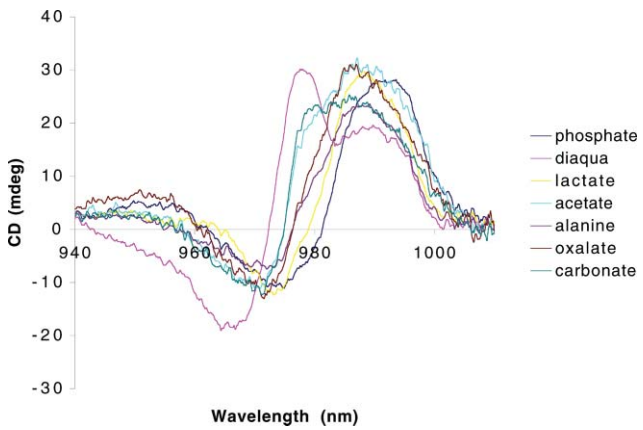


Fig. 5 Near-IR CD spectra of *SSS*-[Yb·L^3] (20 mM complex in 0.1 M MOPS, pH 7.4, 295 K) in the presence of a ten-fold excess of added anion.

Near-IR CD spectra were then recorded for *SSS*-[Yb·L^3] (20 mM complex in 0.1 M MOPS, pH 7.4, 295 K) in the presence of a ten-fold excess of a variety of (*R*) and (*S*) chiral anions (*e.g.* α-hydroxy acids, α-amino acids, phosphorylated amino acids) in

the hope that the differing chiral environment about the Yb centre in the diastereomers may be effectively signalled through CD. As in the case of the emission studies, although characteristic spectra were obtained distinguishing between the different ternary anion complexes, identical CD spectra were recorded for all the (*R*) and (*S*) diastereomeric adducts.

Conclusions

The heptadentate lanthanide complexes of L^3 are highly water soluble and appear to exist as a single isomer in aqueous solution. Such complexes are therefore highly desirable for potential use as probes for bioactive species in aqueous solution. *SSS*-[Ln·L^3] complexes have been shown to act as effective NMR, luminescent and chiroptical probes for the signalling of a variety of anions in water. The nature of the axial donor has been shown to play a prominent role in determining NMR, emission and CD spectral characteristics. By observation of the lanthanide induced shift of *SSS*-[Yb·L^3], the $\Delta J = 2/\Delta J = 1$ band intensity ratio and $\Delta J = 1$ band splitting in the emission spectrum of *SSS*-[Eu·L^3] and the wavelength of the CD band centred around 990 nm for *SSS*-[Yb·L^3] the nature of the axial donor may be easily assessed. As the polarisability of this axial donor increases the observed paramagnetic NMR shift decreased, a larger $\Delta J = 2/\Delta J = 1$ band intensity ratio and a larger $\Delta J = 1$ splitting (cm^{-1}) in the Eu emission spectra was observed and the CD band centred around 990 nm moved to shorter wavelength.

Upon the addition of (*R*) and (*S*) chiral anions to *SSS*-[Ln·L^3] no apparent changes in the form of the Eu emission or Yb near-IR CD spectra were observed. Therefore the signalling of diastereomeric adducts through optical techniques is not appropriate for such systems. However, the *SSS*-[Yb·L^3] diastereomeric ternary adducts formed upon the addition of racemic α-hydroxy acids, α-amino acids and phosphorylated amino acids were easily distinguishable by ^{1}H NMR and exhibited large chemical shift non-equivalences. In fact, an apparent absence of kinetic resolution in complex formation between *SSS*-[Yb·L^3] and racemic α-hydroxy acids was observed and augurs well for the development of such systems as aqueous chiral derivatising agents. Furthermore, the chemical shift non-equivalence ($\Delta\Delta\delta$ ~2–5 ppm) and large lanthanide induced shifts exhibited by this unique chiral derivatising agent are far superior compared to values reported in the literature for aqueous lanthanide shift reagents which are generally <0.1 ppm. Enantioselectivity in complex formation was observed with *SSS*-[Yb·L^3] in the presence of racemic α-amino acids, which precludes its use as a chiral derivatising agent for such species. However, the (*S*)-amino acid adducts always experienced a greater lanthanide induced shift compared to the (*R*)-adducts, therefore such complexes may be effectively used to determine the absolute configuration of α-amino acids in aqueous media at physiological pH.

Experimental

General procedures and characterization techniques

Reactions requiring anhydrous or inert conditions were carried out using Schlenk-line techniques under an atmosphere of dry argon. Anhydrous solvents when required were freshly distilled over the appropriate drying agent. Water was purified by the 'Purite$_{STILL}$plus' system.

Thin-layer chromatography was carried out on neutral alumina plates (Merck Art 5550) and visualised under UV (254 nm) or by staining with iodine. Preparative column chromatography was carried out using neutral alumina (Merck Aluminium Oxide 90, activity II–III, 70–230 mesh), pre-soaked in ethyl acetate. Cation and anion exchange chromatography were performed using Dowex 50 W strong ion exchange resin and Dowex 1-X8(Cl), respectively, pre-treated with hydrochloric acid (3 M).

Infrared spectra were recorded on a Graseby-Specac "Golden Gate" Diamond ATR accessory spectrometer. Melting points were recorded using a Köfler block and are uncorrected.

^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury-200 (^{1}H at 199.975 MHz, ^{13}C at 50.289 MHz), Varian Unity 300 (^{1}H at 299.908 MHz, ^{13}C at 75.412 MHz) or a Varian Inova 500 (^{1}H at 499.7 MHz, ^{13}C at 125.67 MHz) spectrometer. Two-dimensional spectra were carried out on a Varian Inova 500 spectrometer. Spectra were referenced internally relative to *tert*-butanol for paramagnetic complexes or to the residual protio-solvent resonances which are reported relative to TMS. All chemical shifts are given in ppm and coupling constants are in Hz. ^{1}H NMR spectra for the anion ternary adducts were obtained by adding an excess (~10 equivalents) of the anion, as a solid, to a solution of the complex (10 mM in D_2O). The pD was carefully adjusted to 7.5 (±0.4) using sodium deuteroxide.[29]

Electrospray mass spectra were recorded on a VG Platform II (Fisons instrument), operating in positive or negative ion mode as stated, with methanol as the carrier solvent. Accurate mass spectra were recorded at the EPSRC Mass Spectrometry Service at the University of Wales, Swansea.

Corrected emission spectra were recorded on a S.A. Fluorolog 3, operating with DataMax software using quartz fluorescence cuvettes of path length 1 cm, a 420 nm cutoff filter and an excitation wavelength of 397 nm (Eu). Eu^{3+} spectra were recorded between 570 nm and 730 nm, at 0.5 nm increments with 1 s integration time. Excitation and emission slits were 10 and 1 nm respectively. Data analysis was performed using an iterative least-squares fitting procedure, assuming a 1 : 1 binding stoichiometry, operating in Microsoft Excel. Statistical errors were estimated to be <10%, and experimental errors were within 20%. Emission spectra in the presence of added anions were obtained by adding an excess of the anion (10 equivalents), as a solid, to a solution of the complex (0.50 ml, 2.5 mM) prepared in MOPS (0.1 M, pH 7.4) buffer.

Near-IR CD spectra were recorded in four successive scans using a Jasco J-810 spectropolarimeter at 295 K using a 1 cm path length and slits of 60 μm. To the complex solution (20 mM in 0.1 M MOPS buffer, pH 7.4) was added an excess (>10 equiv.) of the anion as a solid. Spectra were recorded immediately and showed no change in form 24 h later.

Ligand synthesis

2-Chloro-*N*-[(*S*)[1-methoxycarbonyl-3-methyl] butyl] ethanamide. (*S*)-Leucinemethylester hydrochloride (3 g, 16.5 mmol) was dissolved in dry diethyl ether (100 ml) under argon and triethylamine (5.52 ml, 39.6 mmol) was added. The solution was cooled to −20 °C (acetone, CO_2 (s) bath) and chloroacetyl chloride (1.58 ml, 19.8 mmol) in dry diethyl ether (60 ml) was added

dropwise, maintaining the temperature at −20 °C. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. The resulting white precipitate was dissolved in water (60 ml) and the organic layer washed with hydrochloric acid (0.1 M, 80 ml) and water (3 × 80 ml), dried (K_2CO_3) and the solvent was removed *in vacuo* to give a brown oil (3.21 g, 88%); ν_{max} (film)/cm^{-1} 3304 (NH), 1745 (*CO*O), 1680 (*CO*NH); δ_H (200 MHz, $CDCl_3$) 6.90 (1H, br s, NH), 4.68–4.56 (1H, br m, C*H*NH), 4.04 (2H, s, $ClCH_2CO$), 3.72 (3H, s, OCH_3), 1.69–1.50 (3H, br m, CH_2CH), 0.92 (6H, d, ^3J = 5.8, CH_3); δ_C (50.29 MHz, $CDCl_3$) 172.8 (CO_2Me), 166.6 (CONH), 52.2 (OCH_3), 51.0 (CHNH), 42.4 ($ClCH_2CO$), 40.7 (CH_2CH), 24.7 (CH_2CH), 22.7 (CH_3), 21.6 (CH_3); *m*/*z* (ES^+) 244 (100%, MNa^+) (Found: MNa^+, 244.0739. $C_9H_{16}NO_3NaCl$ requires MNa^+, 244.0716).

1-Benzyl-4,7,10-tris-[(*S*)-((1-methoxycarbonyl-3-methyl)butyl)-carbamoylmethyl]-1,4,7,10-tetraazacyclododecane. 2-Chloro-*N*-[(*S*)[1-methoxycarbonyl-3-methyl] butyl] ethanamide (1.33 g, 6.00 mmol) in dry *N*,*N*-dimethylformamide (5 ml) was added to a stirred solution of 1-benzyl-1,4,7,10-tetraazacyclododecane[13] (0.526 g, 2.00 mmol) and fine mesh anhydrous potassium carbonate (0.830 g, 6.00 mmol) in dry *N*,*N*-dimethylformamide (25 ml). The reaction mixture was heated at 60 °C under an argon atmosphere for 36 h. The solvent was distilled off under vacuum and the resulting brown oil was extracted into dichloromethane (40 ml), washed with purite water (3 × 40 ml), brine (40 ml), dried (K_2CO_3) and concentrated to dryness. The mixture was purified by alumina column chromatography (gradient elution from dichloromethane to 0.5% methanol–dichloromethane) and the product was isolated as a brown oil (0.821 g, 50%); R_f (Al_2O_3; 10% CH_3OH–CH_2Cl_2; I_2 and UV detection) 0.38; ν_{max} (film)/cm^{-1} 1741 (*CO*O), 1659 (*CO*NH); δ_H (200 MHz, $CDCl_3$) 8.0–7.8 (3H, br s, NHCO), 7.3–7.1 (5H, m, Ar), 4.5 (3H, m, C*H*NH), 3.70–2.40 (33H, br m, ring-CH_2, CH_2CO, OCH_3, CH_2Ph), 1.58–1.40 (9H, br m, CH_2CH, CH_2CH), 0.87 (18H, m, CH_3); δ_C (50.29 MHz, $CDCl_3$) 173.4 (CO_2Me), 173.2 (CO_2Me), 171.3 (CONH), 170.9 (CONH), 138.0 (*ipso*-Ar), 129.0, 128.5, 127.4 (C–Ar), 59.9–59.0 (br, NCH_2Ph, NCH_2CO), 53.4–52.3 (br, ring-CH_2, OCH_3), 50.5 (CHNH), 41.3 (CH_2CH), 25.1 (CH_2CH), 23.0 (CH_3), 22.1 (CH_3); *m*/*z* (ES^+) 429 (100%, MCa^{2+}), 840 (30%, MNa^+), 440 (20%, MK^+Na^+) (Found: $(MHK)^{2+}$, 428.7514. $C_{42}H_{72}N_7O_9K$ requires $(MHK)^{2+}$, 428.7542).

1,4,7-Tris-[(*S*)-((1-methoxycarbonyl-3-methyl)butyl)carbamoyl-methyl]-1,4,7,10-tetraazacyclododecane L^2. A hydrochloric acid solution (1 M, 1 ml) and a catalytic amount of palladium hydroxide on carbon were added to 1-benzyl-4,7,10-tris-[(*S*)-((1-methoxycarbonyl-3-methyl)butyl)carbamoylmethyl]-1,4,7,10-tetraazacyclododecane (1.07 g, 1.31 mmol) in ethanol (45 ml) and the mixture was treated with hydrogen (45 psi) at room temperature for 48 h. The reaction mixture was filtered through Celite and the solvent was removed under vacuum. The residue was taken into dichloromethane (30 ml) and washed with a solution of sodium bicarbonate (30 ml) and brine (1 × 30 ml), dried (K_2CO_3) and concentrated to dryness to give the product as a yellow-orange glassy solid (0.77 g, 81%), mp 120–122 °C; ν_{max} (solid)/cm^{-1} 1739 (*CO*O), 1665 (*CO*NH); δ_H (200 MHz, $CDCl_3$) 8.0–7.84.(3H, br s, NHCO), 80–2.55 (35H, br m, ring-CH_2, CH_2CO, OCH_3, ring-NH, C*H*NH), 2.00–1.20 (9H, br m, CH_2CH, CH_2CH), 1.00–0.87 (18H, br, CH_3); δ_C (50.29 MHz, $CDCl_3$) 173.8 (CO_2Me), 173.4 (CO_2Me), 171.6 (CONH), 171.5 (CONH), 59.6 (br, NCH_2CO), 53.8–52.3 (br, ring-CH_2, OCH_3), 50.7 (CHNH), 47 (ring-CH_2), 41.3 (CH_2CH), 25.1 (CH_2CH), 23.0 (CH_3), 21.1 (CH_3); *m*/*z* (ES^+) 384 (100%, MCa^{2+}), 728 (15%, MH^+), 766 (5%, MK^+) (Found: MH^+, 728.4878. $C_{35}H_{66}N_7O_9$ requires MH^+, 728.4922).

Complex synthesis

Yb·L^2. Ytterbium(III) triflate (0.44 g, 0.72 mmol) in dry acetonitrile (1 ml) was added to a solution of L^2 (0.512 g, 0.72 mmol) in dry acetonitrile (4 ml) and the mixture was heated to reflux under argon overnight. The reaction solution was dropped onto stirring diethyl ether (50 ml) and the resulting yellow precipitate was collected by centrifugation and filtration. The solid was redissolved in the minimum amount of acetonitrile and the precipitation procedure repeated once more. A yellow solid resulted (0.70 g, 75%), mp 142–144 °C; ν_{max} (solid)/cm^{-1} 1742 (*CO*O), 1627 (*CO*NH); δ_H (200 MHz, CD_3OD) partial assignment 119.5 (1H, br s, ring-H_{ax}), 76.4 (1H, br s, ring-H_{ax}), 67.8 (1H, br, ring-H_{ax}), 53.9 (1H, br, ring-H_{ax}), 43.0 (1H, br s, ring-H_{eq}), 35.8 (1H, br s, ring-H_{eq}), 31.9 (1H, br s, ring-H_{eq}), 27.0 (1H, br, ring-H_{eq}), 12.1, −17.5, −19.1, −29.7, −33.8, −36.3, −41.1, −42.8, −48.5, −78.1, −91.0; *m*/*z* (ES^+) 450 (100%, M^{2+}) (Found: M^{2+}, 450.2109. $C_{35}H_{64}N_7O_9Yb$ requires M^{2+} 450.2077).

Eu·L^2. The title compound was prepared following a method similar to Yb·L^2, using europium(III) triflate (0.37 g, 0.62 mmol) in dry acetonitrile (3 ml) and L^2 (0.450 g, 0.62 mmol) in dry acetonitrile (3 ml). A yellow solid resulted (0.713 g, 87%), mp > 178 °C (decomp.); ν_{max} (solid)/cm^{-1} 1742 (*CO*O), 1627 (*CO*NH); δ_H (200 MHz, CD_3OD) partial assignment 24.8 (1H, br s, ring-H_{ax}), 16.0 (1H, br s, ring-H_{ax}), 13.5 (1H, br s, ring-H_{ax}), 9.28 (1H, br s, ring-H_{eq}), −1.66, −4.83, −6.40, −7.33, −9.43, −11.6, −13.6, −14.9, −19.0; *m*/*z* (ES^+) 440 (100%, M^{2+}), 514 (40%, $[M^{3+} + (CF_3SO_3^-)]^{2+}$) (Found: $[M^{3+} + (CF_3SO_3^-)_2]^+$, 1178.3163. $C_{37}H_{65}N_7O_{15}F_6S_2Eu$ requires $[M^{3+} + (CF_3SO_3^-)_2]^+$ 1178.3097).

Yb·L^3. Yb·L^2 (0.25 g, 0.18 mmol) was dissolved in the minimum amount of methanol and treated with an aqueous sodium hydroxide solution (0.02 M, 37 ml). The solution was brought to pH 6, reduced to small volume and loaded onto a cationic exchange column (Dowex, 50 WH^+), eluting with water and then aqueous ammonia solution (6%). The ammonia layer was dried giving the product as a yellow solid (0.080 g, 50%), mp > 250 °C; ν_{max} (solid)/cm^{-1} 1625 (br, *CO*NH, *CO*O); δ_H (200 MHz, CD_3OD) partial assignment 105.6 (1H, br s, ring-H_{ax}), 99.1 (1H, br s, ring-H_{ax}), 79.0 (1H, br s, ring-H_{ax}), 75.0 (1H, br s, ring-H_{ax}), 43.4 (1H, br s, ring-H_{eq}), 27.0 (3H, br s, ring-H_{eq}), 21.7, 18.7, 10.4, 8.15, −17.0, −22.8, −24.3, −33.0, −36.3, −46.7, −51.9, −54.3, −58.5, −61.7, −121.3; *m*/*z* (ES^+) after addition of CF_3COOH: 429 (100%, $[M + 2H^+]^{2+}$) (Found: MH^+, 857.3681. $C_{32}H_{57}N_7O_9Yb$ requires MH^+ 857.3607).

Eu·L^3. The title compound was prepared following a method similar to that for Yb·L^3, using Eu·L^2 (0.500 g, 0.37 mmol), dissolved in the minimum amount of methanol and an aqueous sodium hydroxide solution (0.02 M, 75 ml). A yellow solid resulted (0.028 g, 50%), mp > 250 °C; ν_{max} (solid)/cm^{-1} 1625 (br, *CO*NH, *CO*O); δ_H (200 MHz, CD_3OD) partial assignment 25.7 (1H, br s, ring-H_{ax}), 23.4 (1H, br s, ring-H_{ax}), 18.0 (1H, br s, ring-H_{ax}), 17.6

(1H, br s, ring-H_{ax}), 11.03, −1.12, −2.34, −2.78, −3.32, −5.13, −5.37, −6.89, −7.28, −8.84, −9.33, −11.1, −12.8, −14.6, −15.43; *m/z* (ES^+) after addition of CF_3COOH: 418 (100%, $[M + 2H^+]^{2+}$) (Found: MH^+, 836.3474 $C_{32}H_{57}N_7O_9Eu$ requires MH^+ 836.3430).

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