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Enantiopure, Octadentate Ligands as Sensitizers for Europium and Terbium Circularly Polarized Luminescence in Aqueous Solution

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Circularly polarized luminescence (CPL) is the emission analogue of circular dichroism (CD). While CD spectroscopy has been widely used to investigate the configurational as well as conformational changes in biological systems, CPL also has great, albeit currently under-developed, potential due to the general sensitivity of luminescence measurements combined with the high specificity of the signal for the chiral environment.[1] Lanthanide luminescence (especially Eu(III) and Tb(III)) with its advantageous characteristics (large Stokes shift, long lifetimes, narrow emission bands) is an ideal candidate for the development of chiral CPL probes.[2]

We have earlier reported the 2-hydroxyisophthalamide (IAM) motif as a highly efficient sensitizer for the luminescence of four different Ln(III) cations (Sm, Eu, Tb, Dy).[3] In an extension of this work, enantiopure versions were recently successfully developed for use as CPL probes.[4] While these species retain the excellent brightness of their nonchiral analogues, the insolubility in physiologically relevant media remains a limitation for analytical applications. In order to address this problem, enantiopure, octadentate ligands with decreased hydrophobicity have now been developed. As an additional feature of this new approach, the stereogenic centers are introduced in the ligand backbone instead of incorporating them into the sensitizer units, thus separating the chiral information from the chromophore and allowing for a much more generally applicable, modular synthesis of chiral ligands for CPL applications. 1-Hydroxy-2-pyridinone (1,2-HOPO), which has recently proven to be a good sensitizer for Eu(III) luminescence,[5] and IAM (for Tb(III)) were chosen as model chromophores (Figure 1).

The chiral information in H_4**1** (the first chiral ligand with the 1,2-HOPO motif) and H_4**2** is easily accessible from either enantiopure amino acids or by resolution of chiral diamines. In the case of H_4**1**, the four stereogenic centers are located in the tetrapodal arms, whereas H_4**2** displays vicinal stereocenters in the central portion of the oligoamine backbone. The synthesis of the ligands is outlined in Scheme 1.[6] The hexamine backbone of H_4**1** was prepared by selective ring opening of enantiopure (*R*)-2-ethyl-*N*-tosylaziridine[7] with ethylene diamine, followed by deprotection of the tosyl groups, and ion exchange chromatography in analogy to previous reports.[8] Similarly, the backbone of H_4**2** was prepared from enantiopure (*R*,*R*)-1,2-diaminocyclohexane[9] and *N*-tosylaziridine.[10] The optical purity of both hexamines was confirmed by ^{1}H NMR spectroscopy after in situ transformation to the corresponding tetrakis(urea) derivatives with commercially available, enantiopure (*R*)-1-phenylethylisocyanate (Sigma-Aldrich, >98% ee). In each case, only one set of signals was observed with all four arms being equivalent on the NMR time scale, consistent with complete regio- and diastereoselectivity of the aziridine ring-opening reaction.

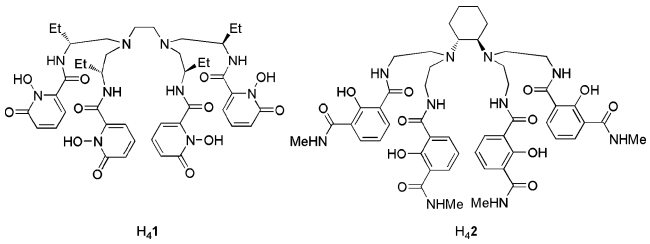
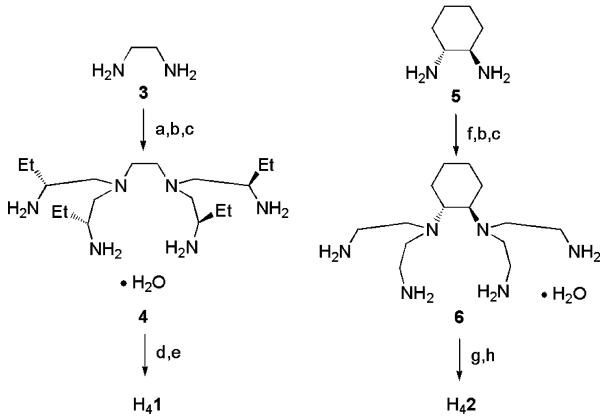


Figure 1. Enantiopure, octadentate ligands.

Scheme 1. Synthesis of Ligands H_4**1** and H_4**2**[a]



[a] Reaction conditions: (a) (*R*)-2-ethyl-*N*-tosylaziridine,[7] benzene, 68%; (b) HBr (48%)/HOAc, phenol; (c) ion exchange (DOWEX 1 × 8, OH^- form), 46−56% (over two steps); (d) benzyl-protected 1,2-HOPO acid chloride,[11] CH_2Cl_2, NEt_3; 39%; (e) HCl concd/HOAc, 89%; (f) *N*-tosylaziridine,[10] benzene, 69%; (g) protected IAM activated carboxylic acid derivative,[6,12] CH_2Cl_2, NEt_3, 31%; (h) BBr_3, CH_2Cl_2, 80%.

Coupling of the two backbones to protected, activated carboxylic acid derivatives[11,12] of the respective chelating moieties, followed by deprotection, afforded the free ligands H_4**1** and H_4**2** in reasonable yields.[6]

The lanthanide complexes [Eu(H**1**)(H_2O)] and [Tb(H**2**)][13] were synthesized by standard procedures[6] and were isolated in analytically pure form.[14] Upon complexation to the lanthanide, a new stereogenic element is usually introduced in addition to the fixed chirality at the asymmetric carbon centers of the ligand backbone.[15] This often times occurs in the form of a helical twist of the ligand, with the potential result of diastereomeric species. In order to assess the diastereopurity of the complex formation, ^{1}H NMR spectroscopy was performed on [Eu(H**1**)(H_2O)] in CD_3OD (Figure 2).[16] The terbium complex [Tb(H**2**)] could not be investigated in this way due to its much stronger paramagnetic nature. This issue will be addressed in more detail in an upcoming full paper.

The aromatic region of the spectrum at ambient temperature (293 K, Figure 2, bottom) shows only the expected three signals of a

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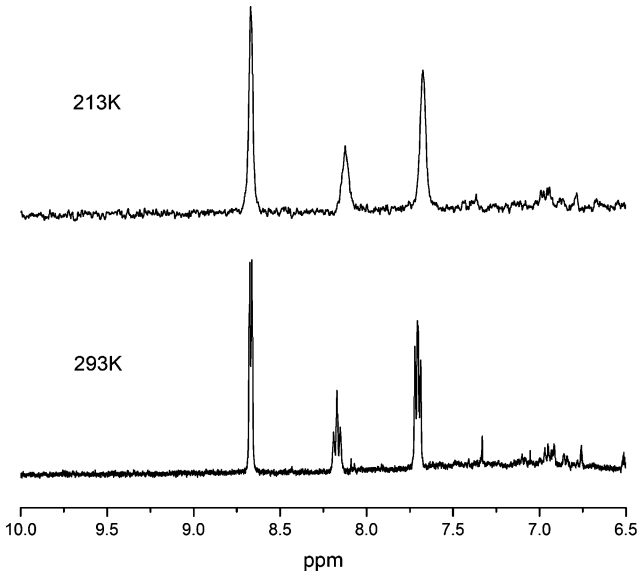

Figure 2. Aromatic region of the ^{1}H NMR spectra (500 MHz) of a saturated solution of [Eu(H**1**)(H$_2$O)] in CD$_3$OD at 293 K (bottom) and 213 K (top).

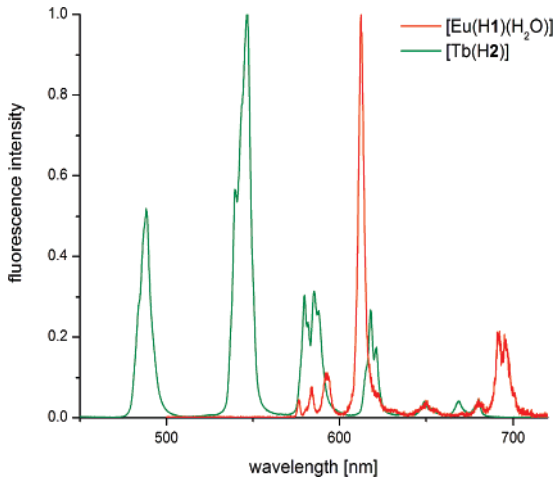

Figure 3. Normalized steady-state emission spectra (λ_{exc} = 340 nm, ca. 10^{-5} M in 0.1 M Tris buffer, pH 7.4).

6-substituted 1,2-HOPO derivative. These resonances do not exhibit a strong paramagnetic shift. At low temperature (213 K, Figure 2, top), the peaks broaden, presumably due to the increase in viscosity of CD$_3$OD, and shift very slightly but do not show any additional peaks, suggesting the absence of a fast equilibrium (at least on the NMR time scale) of interconverting diastereomeric species at 293 K.

[Eu(H**1**)(H$_2$O)] and [Tb(H**2**)] are soluble in aqueous media and show strong luminescence at physiological pH (Figure 3). The photophysical properties of the lanthanide complexes show distinct features (Table 1). The location of the UV absorption maximum for the n$-\pi^*$ transition in [Eu(H**1**)(H$_2$O)] is identical to the complex with the analogous achiral ligand,[17] whereas in [Tb(H**2**)] a slight blue shift of ca. 11 nm can be seen relative to other octadentate IAM analogues.[3,4] The CD spectra show strong Cotton effects for the two complexes ([Eu(H**1**)(H$_2$O)]: $-$330 nm, +360 nm; [Tb(H**2**)]: $-$326 nm, +353 nm).[6]

Measurements of the luminescence lifetimes gave monoexponential decays in both cases, like the NMR investigations (vide supra), also supporting the presence of one dominant emitting species and therefore highly diastereoselective complex formation in aqueous solution in each case. Determination of the number of solvent molecules (water, MeOH) present in the inner coordination sphere of the lanthanide (q) using Parker's extension[18] of the Horrock's relationship[19] revealed a value of almost 1 (q = 0.84) for the Eu(III) complex of H$_4$**1**. The absence of H$_2$O (q = $-$0.04) in the immediate proximity of the Tb(III) center in [Tb(H**2**)] results in an increase of the luminescence lifetime (τ = 2.28 ms in H$_2$O) relative to the previously reported enantiopure, octadentate IAM ligand (in MeOH: q = 1, τ = 1.27 ms).[4]

Table 1. Photophysical Properties of the Lanthanide Complexes (ca. 10^{-5} M in 0.1 M Tris Buffer, pH 7.4)

complex	λ_{max}, nm (ϵ, M^{-1} cm^{-1})	λ_{exc}, nm	quantum yield Φ^a	lifetime τ, ms^b	q
[Eu(H**1**)(H$_2$O)]	341 (19000^c)	340	0.077	0.48 (0.88)	0.84
[Tb(H**2**)]	339 (28200)	340	0.57	2.28 (2.59)	$-$0.04

^{a} Determined relative to quinine sulfate (Φ = 0.546) in 0.5 M sulfuric acid as standard. ^{b} In H$_2$O (in D$_2$O). ^{c} Saturated solution, estimated ϵ.

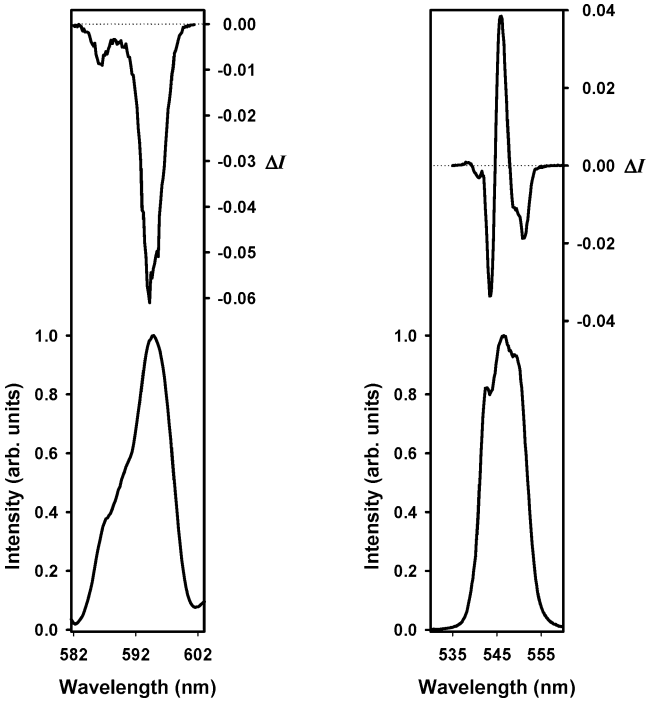

Figure 4. Circularly polarized luminescence (upper curves) and total luminescence (lower curves) spectra of the ^{5}D$_0$ → ^{7}F$_1$ transition of [Eu(H**1**)(H$_2$O)] (left) and ^{5}D$_4$ → ^{7}F$_5$ transition of [Tb(H**2**)] (right) in saturated aqueous solutions at pH 7.4 (0.1 M Tris buffer) and 295 K, upon excitation at 360 and 350 nm, respectively.

The quantum yield for [Tb(H**2**)] (Φ = 0.57) is remarkably high in aqueous media and similar to the best octadentate IAM ligands reported so far (Φ = 0.59).[3] For [Eu(H**1**)(H$_2$O)], the value of 0.077 is within the range of comparable, commercially available Eu(III) luminescent probes (Φ = 0.1$-$0.02) for use in water.[20] It is more than twice as bright as the complex with the achiral, octadentate analogue of H$_4$**1** (q = 1, Φ = 0.036).[17]

Finally, the CPL spectra of saturated aqueous solutions at pH 7.4 (0.1 M Tris buffer) of [Eu(H**1**)(H$_2$O)] and [Tb(H**2**)] are plotted in Figure 4 in the spectral range of the ^{5}D$_0$ → ^{7}F$_1$ and ^{5}D$_4$ → ^{7}F$_5$ transitions, which are particularly well-suited for CPL measurements since they satisfy the magnetic dipole selection rule, ΔJ = 0, $\pm$1 (except 0 ↔ 0), respectively. The detection of a CPL signal for both complexes confirms the presence of stable chiral emitting

Table 2. CPL Results for Lanthanide Complexes (Saturated Aqueous Solutions in 0.1 M Tris Buffer, pH 7.4)

complex	electronic transition	λ, nm	g_{lum}
[Eu(H**1**)(H_2O)]	$^5D_0 \rightarrow {}^7F_1$	586.6	−0.046
		594.2	−0.12
[Tb(H**2**)]	$^5D_4 \rightarrow {}^7F_5$	543.6	−0.083
		545.8	+0.078
		551.0	−0.051

species on the luminescence time scale. We follow the practice of reporting the degree of CPL in terms of the luminescence dissymmetry factor, $g_{lum}(\lambda)$, which is defined as follows:

$$g_{lum} = \frac{\Delta I}{1/2I} = \frac{I_L - I_R}{1/2(I_L - I_R)}$$

Here I_L and I_R refer to the intensity of left and right circularly polarized emissions, respectively.

As indicated in Table 2, these water-soluble Eu- and Tb-containing compounds exhibit a relatively strong CPL activity (g_{lum} = −0.12 {Eu($^5D_0 \rightarrow {}^7F_1$)} and −0.083 {Tb($^5D_4 \rightarrow {}^7F_5$)} at the maximum peak). It should be noted that most of the CPL studies in aqueous media have been reported for lanthanide(III) complexes with chiral DOTA ligand derivatives.[1e−h,21] For instance, absolute g_{lum} values of 0.25 ({Tb($^5D_4 \rightarrow {}^7F_5$)}) and 0.12 ({Eu($^5D_0 \rightarrow {}^7F_1$)}) have been reported for these water-soluble systems,[21] while absolute g_{lum} values of 0.29 and 0.04 have been recorded for Eu(III) and Tb(III) complexes with chiral 2-hydroxyisophthalamide-based ligand derivatives in MeOH solution.[4]

In conclusion, we have developed highly emissive, enantiopure, and water-soluble Eu(III) and Tb(III) complexes using a modular ligand design which allows for rapid changes in the chiral information, located in the hexamine backbone, and sensitizer properties independently. The solubility in biologically relevant media, the complete exclusion of water molecules from the inner coordination sphere in the terbium complex, and the more than two-fold increase in quantum yield for the europium complex in comparison to the achiral analogue constitute major advances. The general luminescence characteristics and the significant CPL activity in physiologically relevant media make both complexes very promising candidates for the development of practical CPL probes. Research in this direction is currently underway.

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Supporting Information Available: Experimental procedures for the synthesis of H_4**1** and [Eu(H**1**)(H_2O)], H_4**2**, and [Tb(H**2**)]. Full analytical characterization for H_4**1** and [Eu(H**1**)(H_2O)], H_4**2**, and [Tb(H**2**)]. Extended spectral characterization for [Eu(H**1**)(H_2O)] and [Tb(H**2**)], as well as experimental details for the spectroscopic measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Reviews: (a) Richardson, F. S.; Riehl, J. P. *Chem. Rev.* **1977**, *77*, 773−792. (b) Riehl, J. P.; Richardson, F. S. *Chem. Rev.* **1986**, *86*, 1−16. (c) Riehl, J. P.; Richardson, F. S. *Methods Enzymol.* **1993**, *226*, 539−553. (d) Riehl, J. P.; Muller, G. In *Handbook on the Physics and Chemistry of Rare Earths*; Gschneidner, K. A., Bünzli, J.-C. G., Pecharsky, V. K., Eds.; North-Holland Publishing: Amsterdam, 2005; Vol. 34. Selected examples: (e) Dickins, R. S.; Howard, J. A. K.; Maupin, C. L.; Moloney, J. M.; Parker, D.; Riehl. J. P.; Siligardi, G.; Williams, J. A. G. *Chem.—Eur. J.* **1999**, *5*, 1095−1105. (f) Di Bari, L.; Pintacuda, G.; Salvadori, P.; Dickins, R. S.; Parker, D. *J. Am. Chem. Soc.* **2000**, *122*, 9257−9264. (g) Bruce, J. I.; Parker, D.; Lopinski, S; Peacock, R. D. *Chirality* **2002**, *14*, 562−567. (h) Poole, R. A.; Bobba, G.; Cann, M. J.; Frias, J.-C.; Parker, D.; Peacock, R. D. *Org. Biomol. Chem.* **2005**, *3*, 1013−1024.
(2) (a) Bünzli, J.-C. G. *Acc. Chem. Res.* **2006**, *39*, 53−61. (b) Bünzli, J.-C. G.; Piguet, C. *Chem. Soc. Rev.* **2005**, *34*, 1048−1077. (c) Parker, D. *Chem. Soc. Rev.* **2004**, *33*, 156−165.
(3) Petoud, S.; Cohen, S. M.; Bünzli, J.-C. G.; Raymond, K. N. *J. Am. Chem. Soc.* **2003**, *125*, 13324−13325.
(4) Petoud, S.; Muller, G.; Moore, E. G.; Xu, J.; Sokolnicki, J.; Riehl, J. P.; Le, U. N.; Cohen, S. M.; Raymond, K. N. *J. Am. Chem. Soc.* **2007**, *129*, 77−83.
(5) Moore, E. G.; Xu, J.; Jocher, C. J.; Werner, E. J.; Raymond, K. N. *J. Am. Chem. Soc.* **2006**, *128*, 10648−10649.
(6) For more details, see the Supporting Information.
(7) Pei, Y.; Brade, K.; Brule, E.; Hagberg, L.; Lake, F.; Moberg, C. *Eur. J. Org. Chem.* **2005**, 2835−2840.
(8) Wagnon, B. K.; Jackels. S. C. *Inorg. Chem.* **1989**, *28*, 1923−1927.
(9) Galsbøl, F.; Steenbøl, P.; Sørensen, B. S. *Acta Chem. Scand.* **1972**, *26*, 3605−3611.
(10) Nelles, J.; Tietze, E. Patent DE698597, 1940.
(11) Benzyl-protected 1,2-HOPO acid chloride: Xu, J.; Durbin, P. W.; Kullgren, B.; Ebbe, S. N.; Uhlir, L. C.; Raymond, K. N. *J. Med. Chem.* **2002**, *45*, 3963−3971.
(12) Methyl-protected IAM monoamide 2-mercaptothiazolide: Xu, J.; Raymond, K. N. Unpublished results.
(13) H**1** and H**2** are the triply deprotonated ligands H_4**1** and H_4**2**, respectively.
(14) The assignment of water molecules to the inner coordination sphere is based on the luminescence measurements in H_2O and D_2O (see Table 1).
(15) (a) Knof, U.; von Zelewsky, A. *Angew. Chem., Int. Ed.* **1999**, *38*, 303−322. (b) Aspinall, H.; *Chem. Rev.* **2002**, *102*, 1807−1850. (c) Di Bari, L.; Salvadori, P. *Coord. Chem. Rev.* **2005**, *249*, 2854−2879.
(16) While the solubility in aqueous media for the reported lanthanide complexes is high enough to measure luminescence properties, meaningful NMR spectra could only be obtained at higher concentrations in MeOH.
(17) Moore, E. G.; Jocher, C. J.; Xu, J.; Werner, E. J.; Raymond, K. N. *Inorg. Chem.* **2007**, *46*, 5468−5470.
(18) Beeby, A.; Clarkson, I. M.; Dickins, R. S.; Faulkner, S.; Parker, D.; Royle, L.; de Sousa, A. S.; Williams, J. A. G.; Woods, M. *J. Chem. Soc., Perkin Trans. 2* **1999**, *3*, 493−504.
(19) Horrocks, W. D., Jr.; Sudnick, D. R. *Acc. Chem. Res.* **1981**, *14*, 384−392.
(20) For example: Perkin-Elmer LANCE, Cisbio HTRF, Amersham LRET, Brahms TRACE.
(21) Parker, D.; Dickins, R. S.; Puschmann, H.; Crossland, C.; Howard, J. A. K. *Chem. Rev.* **2002**, *102*, 1977.

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