DOI: 10.1002/ange.201106748

Lanthanide Complexes

A Single Sensitizer for the Excitation of Visible and NIR Lanthanide Emitters in Water with High Quantum Yields**

*Ga-Lai Law, Tiffany A. Pham, Jide Xu, and Kenneth N. Raymond**

In recent years the use of lanthanide luminescence for biological applications has been of increasing importance.[1] The development of such luminescent compounds has been due to the burgeoning demand for multifunctional and efficient luminescent markers to probe signal transduction, neurobiology, cancer, stem cell biology, and infectious diseases.[2] This has initiated a focus on multiplexing assays, for which the multifunctional design is still problematic. Approaches using nanoparticles and quantum dots,[3] have been somewhat successful, but the limitations of these materials are still significant.[4]

Herein we present a versatile, multidentate ligand that has been found to sensitize both visible and near-infrared (NIR) emitters by using the same excitation wavelength, with significantly high quantum yields. This has been a persistent challenge, as different lanthanides have different emitting states that are easily quenched by nonradiative decay processes.[5] We have previously described the properties of several emissive terbium complexes with high quantum yields that feature the 2-hydroxyisophthalamide binding unit (IAM).[6,7] Past studies have shown that similar tetradentate and octadentate ligands[7] form Tb^{III} complexes with comparable photophysical properties. We ascribe this to the fact that modifying the functional groups of the sensitizer does not play a major role in changing the electron distribution of the ligand chromophore and its chelating oxygen atoms, based on investigation of different electron-withdrawing and electron-donating groups on the para position of the aromatic ring on the chromophore. Using TD-DFT calculations and screening studies with tetradentate ligands,[8] the inclusion of an additional amide group on the para position of the ring was thought to be favorable to the electronics of the system. It is further hypothesized that large molecular appendages will result in better shielding of the metal center. The TIAM binding moiety was introduced in the ligand design to investigate both of these hypotheses.

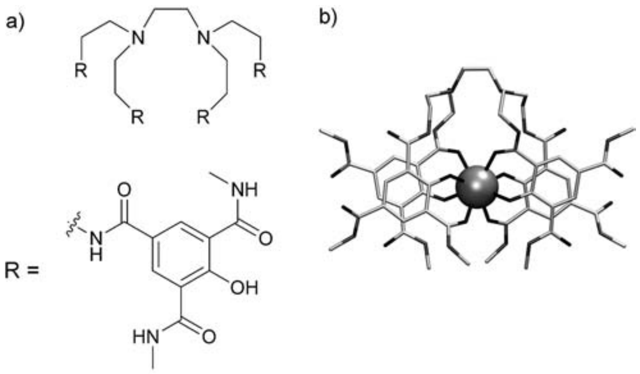

Figure 1. a) Chemical structure of the H(2,2) scaffold (above) and the TIAM binding moiety (below) in H_4L. b) View of the X-ray crystal structure of $[HoL]^-$ (see also Figure 4 a).

The octadentate ligand (H_4L) has four TIAM chromophores attached to an H(2,2) backbone (Figure 1 a), and has been found to sensitize a range of visible emitters, namely Sm, Eu, Tb, Dy, and Ho, which emit in both the visible and NIR regions. The sensitization of the metal occurs through the ligand, which is simultaneously the chromophore and chelator.[9] These emitters display uncommonly high luminescent properties in water, given that they are extremely sensitive to O-H vibronic quenching.

The $[Ln^{III}L]^-$ complexes (Ln = Sm, Eu, Tb, Dy, Ho) were characterized by the crystal structure of the holmium complex (Figure 1 b) and by HT-ESI mass spectrometry. The synthesis and characterization data is shown in the Supporting Information and the Experimental Section.

In general, emission from holmium complexes is rare, especially in the solution state, as their electronic structure is susceptible to non-radiative deactivation. There have been fewer than ten reports on holmium complexes with documented solution state luminescence and photophysics to date.[11] We show herein the emission spectra of holmium in water with emission in both the visible and NIR regions (Figure 2). The observed peaks are assigned at about 640 nm and 990 nm with a slight shoulder at 1010 nm (the most prominent band), and the weaker transitions are observed at 1210 and 1450 nm, which correspond to the transition bands of $^5F_5 \rightarrow {}^5I_8$, $^5F_5 \rightarrow {}^5I_7$, $^5I_6 \rightarrow {}^5I_8$, and $^5F_5 \rightarrow {}^5I_6$, respectively. These bands are observed owing to the relaxation of the photons from the multiple upper 4f levels to the $^5F_5$ and $^5I_6$ first excited states of the Ho^{3+} ion before decaying to the $^5I_6$, $^5I_7$, $^5I_8$ (Figure 2). This is the first time that the weak transition bands at 1210 and 1450 nm have been reported in aqueous solution. In most cases, these bands are not observed due to strong reabsorption of the weakly emitted NIR radiation by the solvent, which has an absorption coefficient of nearly two orders of magnitude higher than the holmium transitions.[12]

---

[*] Dr. G.-L. Law, T. A. Pham, Dr. J. Xu, Prof. K. N. Raymond
Department of Chemistry, University of California Berkeley
Berkeley, CA 94720-1460 (USA)
E-mail: raymond@socrates.berkeley.edu

[**] The lanthanide luminescence research is supported by the Director, Office of Science, Office of Basic Energy Sciences, Division of Chemical Sciences, Geosciences, and Biosciences of the U.S. Department of Energy at LBNL under Contract No. DE-AC02-05CH11231, and related support is from NIH Grant HL069832 for other aspects of this research.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201106748.

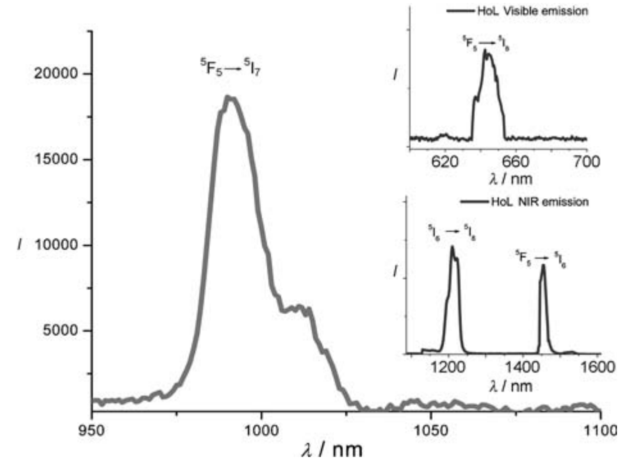

Figure 2. HoL emission spectra in water and less than 5%DMSO at $\lambda_{exc} = 330$ nm. The strongest emission in the NIR is at 940/1010 nm. Upper inset: $^5F_5 \rightarrow {}^5I_8$, very weak emission in the visible region at 640 nm; lower inset: the $^5I_6 \rightarrow {}^5I_8$ and $^5F_5 \rightarrow {}^5I_8$ transitions bands at 1210 and 1455 nm in the NIR region.

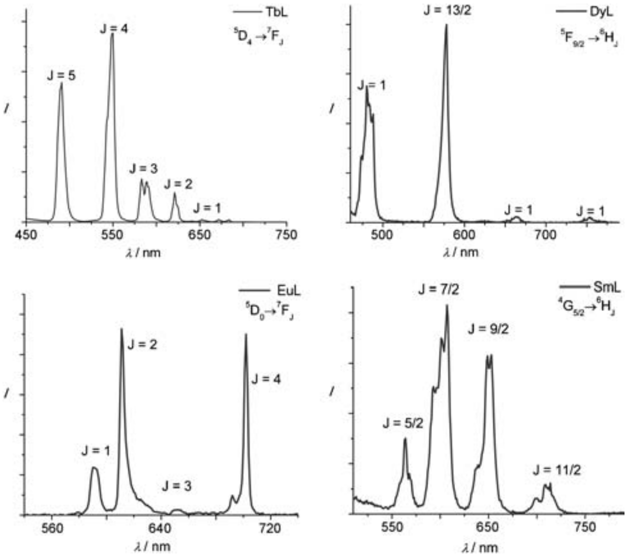

Figure 3. Room-temperature emission spectra of a) TbL b) DyL, c) EuL, and d) SmL at $\lambda_{exc} = 350$ nm in water with less than 5% DMSO.

The only measurable lifetime was at 1010 nm and gave an monoexponential curve ($\tau_{obs} = 11$ ns; Table 1). In aqueous solution the other transition bands were too weak to give conclusive values; however, aqueous emission spectra, quantum yields, and lifetimes have been collected for the other four visible emitters, namely SmL, EuL, TbL, and DyL (Table 2).The emission spectra with their assigned f–f transitions are shown in Figure 3.

Table 1: Photophysical data of HoL in water.

HoL	Transitions	λ_{em}(max) [nm]	τ_{H_2O}
Ho (f–f)	$^5F_5 \rightarrow {}^5I_8$	ca. 645	–
	$^5F_5 \rightarrow {}^5I_7$	990/1010	11 ns
	$^5I_6 \rightarrow {}^5I_8$	1210	–
	$^5F_5 \rightarrow {}^5I_8$	1450	–
L (fluorescence)	$S_1 \rightarrow S_0$	407	48 ns
L (phosphorescence)	$T_1 \rightarrow S_0$	432	58 μs

The Sm^{III} complex produced four characteristic bands, peaking at 562.5, 605.0, 650.0, and 710.0 nm, which are responsible for the transitions from the emitting $^4G_{5/2}$ state to the ^6H_J ($J = 5/2$, 7/2, 9/2, and 11/2, respectively). For DyL, the characteristic bands associated with the emitting $^4F_{9/2}$ state of Dy^{III} were observed at 481.0, 575.6, 664.1, and 752.5 nm, where the hypersensitive $^5F_{9/2} \rightarrow {}^6H_{13/2}$ is the most dominant. For EuL and TbL, these were the two most emissive complexes as expected as they have fewer excited states and ground states in contrast to Sm and Dy. TbL was the most luminescent, giving the highest quantum yield ($\Phi \approx 47\%$) For EuL, it should be noted that from the spectra, the ratio of the $^7F_4$ transition of the europium is unusually high relative to the $^7F_2$ transition. This is uncommonly observed for europium but has been previously reported,[13] and can be an attributing factor for the low quantum yield obtained as it indicates a different coordination geometry. Another possible cause of the quenching could be by electron transfer processes.

The triplet state of the gadolinium complex was observed from studies at 77 K at 23148 cm^{-1}, which is masked at room temperature. This is shown by the predominant ligand fluorescence of the complexes, centered around 432 nm in water (Supporting Information, Figure S3), which also indicates inefficient energy transfer. Microsecond lifetimes (58 μs) confirm the phosphorescence observed which is promoted by the heavy metal effect of the Gd center.[14] The high emitting states of Gd ($^6P_{7/2}$) prevent energy transfer to the metal ion. The general energy transfer mechanism of these complexes involves population of the ligand singlet state followed by intersystem crossing to the triplet state and finally to the nearest excited states of the metal.

Table 2: Photophysical data of LnL complexes in aqueous media.

LnL	ΔE[a] [cm^{-1}] $T_1 \rightarrow Ln_{(f-f)}$	λ_{em}(max) [nm]	τ_{H_2O} [μs]	τ_{D_2O} [μs]	q[b,d]	q[c,e]	Φ_{H_2O}[f] [%]	Φ_{HEPES}[f] [%]
Tb	2659	545	2050	2610	0.2	0.4	47.2	48.0
Eu	5909	614	840	1320	−0.1	0.1	11.6	10.8
Dy	2469	574	25.0	–	–	−0.5[e]	0.09[c]	0.07[c]
Sm	2659	605	21.0	226.0	−0.4[d]	0.3[e]	0.35	0.36

[a] Tb (f–f = $^5D_5$), Eu (f–f = $^5D_1$), Dy (f–f = $^6H_1$), Sm (f–f = $^6H_{5/2}$). [b] Parker's equation. [c] Horrocks' equation. [d] Hakala. [e] Kimura. [f] Approximately Φ for visible region of the f–f emission shown in Figure 3; integration extrapolated to baseline.

These complexes all show relatively high quantum yields in water, with extinction coefficients of about 23000 L mol^{-1} cm^{-1}. The quantum yield of terbium is around 47 %, while europium was poorly sensitized relative to higher values cited in the literature; however, its quantum yield is still higher than that of the commercially used europium cryptand assay of about 2 %.[15] Thus, all these complexes show potential for multiplexing applications, as they can all be excited with the same wavelength. The lifetimes are relatively long, and from both Horrocks' and Parker's corrected q equations,[16] the calculated q value is approximately zero, which suggests that there are no coordinating water molecules in the inner sphere. This is also supported by the crystal structure of HoL.

From the photophysical measurements it can be concluded that the triplet state of the ligand alone cannot account for the remarkable luminescence properties, as all five lanthanide emitters have different excited states. The energy differences between the triplet level of the ligand and the lowest emitting states of the five different lanthanides account for the different sensitization efficiencies. For example, with terbium complexes, an energy gap of 3500 cm^{-1} or higher is necessary to facilitate efficient and irreversible energy transfer.[17] Here the energy gap is much smaller (ca. 2659 cm^{-1}), whereas for EuL the gap of about 5909 cm^{-1} is much greater than the ideal of 1800 cm^{-1}, a prerequisite to prevent back energy transfer. For the europium system, the high triplet of the ligand can facilitate relaxation to the $^5D_2$ transition (ca. 21 500 cm^{-1}) instead of to the $^5D_1$ state.

We believe that along with the well-matched triplet state, the strong luminescent properties were attributed to the coordination geometry and the protruding amide groups on the exterior of the ligand that limit solvent access to the metal center. These factors contribute to the reduction in non-radiative decay processes, which is especially important for lanthanides with numerous closely emitting states and hypersensitive transitions. The crystal structure of the holmium complex supports this hypothesis, as the metal center is well encapsulated by the ligand, while the para amide groups extend from the complex acting like a secondary shield from solvent molecules.

Rhomboid single crystals suitable for X-ray diffraction of HoL as the pyridinium salt were grown by layering diethyl ether onto a 5 % aqueous DMF/MeOH 1:1 (v/v) solution of the complex. The HoL complex spontaneously resolves to crystallize in the chiral orthorhombic space group $C222_1$ (Figure 1 b).[10] The crystallographic data (Table S1) and selected bond distances and angles (Table S2) are in the Supporting Information. Interestingly, the holmium atoms are not equivalent and do not lie on the eight general positions of this space group, but rather on two different special positions with C_2 symmetry axes. The unit cell is comprised of two $[HoL]^-$ complexes (with metal centers denoted as Ho1 and Ho2) with a coordination number of eight (Figure 4 a) and significantly different coordination environments (Figure 4 b,c).

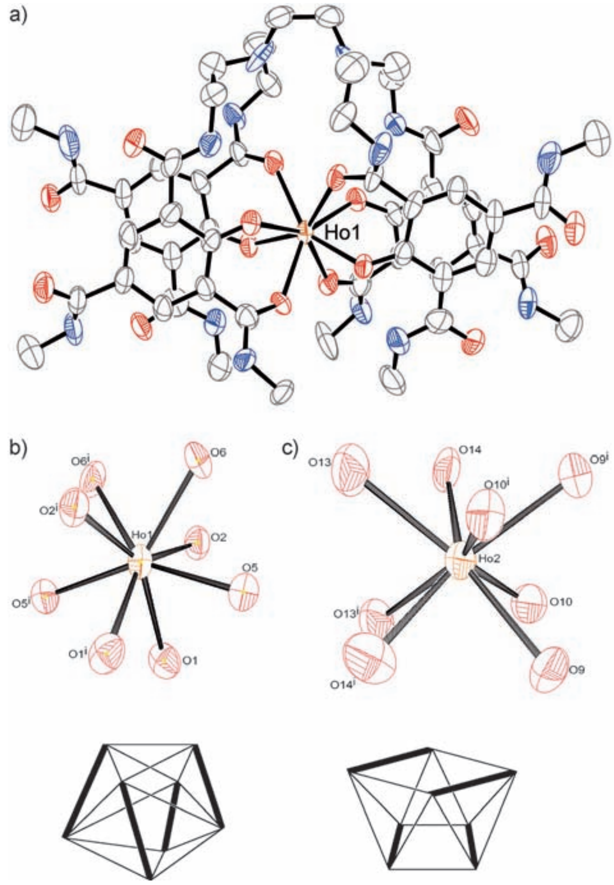

Figure 4. a) ORTEP diagram of the X-ray crystal structure of the Ho1 anionic complex. Ellipsoids are set at 50 % probability; hydrogen atoms are omitted for clarity. Ho orange, C black, O red, N blue. b),c) Inner coordination environments of the Ho1 (b) and Ho2 (c) cations. The bold edges of the corresponding idealized polyhedra are those spanned by the binding moieties.

The three most common polyhedra describing the coordination geometry for an eight-coordinate complex are the bicapped trigonal prism (C_{2v}), square antiprism (D_{4d}), and trigonal dodecahedron (D_{2d}). The shape-measure (SM) parameter is a reference to the agreement between these idealized polyhedra and the observed structure.[18] Shape analysis of the coordination environment around Ho1 reveals that it is best described as a dodecahedron (SM = 7.04 (D_{2d}), 11.96 (C_{2v}), and 15.64 (D_{4d})), while Ho2 is closest to a square antiprism (SM = 7.47 (D_{4d}), 8.34 (D_{2d}), and 10.19 (C_{2v}); Figure 4 b,c), similar to several other eight-coordinate holmium complexes.[11c,19] Both structures are intermediate between the ideal D_{2d} and D_{4d} geometries, with similarly seen D_2 symmetry in each case. For Ho1, the bidentate ligands are twisted off the D_{2d} mirror planes, while for Ho2 the top and bottom faces of the antiprism are rectangular rather than square.

The TIAM ligands alternate in binding through the amide carbonyl connected to the backbone and the pendant one. As expected, the average length of the (hydroxy) O−Ho bond is significantly smaller than that of the (keto) O−Ho bond (2.30 Å vs. 2.38 Å). The aromatic rings pendant from the same side of the bridge engage in notable π stacking, while hydrogen bonding is observed between the amide protons and the coordinating hydroxy oxygen atoms. These interactions most likely contribute to the stability of the complex.

In conclusion, we have reported a new holmium luminescent complex and its emission spectra in both the visible and

NIR region. This work shows that the incorporation of external groups on the ligand can form a secondary shield to reduce external quenching effects and thus is a significant factor to consider in ligand design. An unmet need in the field of biological imaging applications is for multiplex assays using a single excitation source. The H(2,2)TIAM ligand offers a promising approach, as it forms highly emissive complexes in water with a variety of lanthanides.

Experimental Section

All chemicals and solvents were used without further purification unless otherwise stated. The Ln^{III} salts utilized were of the highest available purity (> 99.99 % or (> 99.999 %). The synthesis of H_4L is shown in the Supporting Information. Mass spectra were obtained by the QB3/Chemistry Mass Spectrometry Facility at the University of California, Berkeley, CA.

X-ray diffraction data collection was performed at the X-Ray Facility in the College of Chemistry at the University of California, Berkeley, using procedures detailed in the Supporting Information. Resulting drawings of molecules were produced with ORTEP-3 for Windows.[20] CCDC 797480 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Received: September 22, 2011
Revised: November 2, 2011

Keywords: holmium · lanthanide sensitization · octadentate ligands · Vis/NIR emitters · X-ray diffraction

---

[1] a) H. E. Rajapakse, D. R. Reddy, S. Mohandessi, N. G. Butlin, L. W. Miller, *Angew. Chem.* **2009**, *121*, 5090 – 5092; *Angew. Chem. Int. Ed.* **2009**, *48*, 4990 – 4992; b) S. H. Kim, J. R. Gunther, J. A. Katzenellenbogen, *J. Am. Chem. Soc.* **2010**, *132*, 4685 – 4692; c) E. G. Moore, A. P. S. Samuel, K. N. Raymond, *Acc. Chem. Res.* **2009**, *42*, 542 – 552.

[2] K. L. Haas, K. J. Franz, *Chem. Rev.* **2009**, *109*, 4921 – 4960.

[3] a) F. Morgner, D. Geisler, N. G. Butlin, H.-G. Lohmannsroben, N. Hidebrandt, *Angew. Chem.* **2010**, *122*, 7732 – 7736; *Angew. Chem. Int. Ed.* **2010**, *49*, 7570 – 7574; b) S. Chen, L. Wang, S. L. Duce, S. Brown, S. Lee, A. Melzer, A. Cuschieri, P. Andre, *J. Am. Chem. Soc.* **2011**, *132*, 15022 – 15029.

[4] K. Binnemans, *Chem. Rev.* **2009**, *109*, 4283 – 4374.

[5] J.-C. G. Bünzli, A.-S. Chauvin, H. K. Kim, E. Deiters, S. V. Eliseeva, *Coord. Chem. Rev.* **2010**, *254*, 2623 – 2633.

[6] a) M. K. Johansson, R. M. Cook, J. Xu, K. N. Raymond, *J. Am. Chem. Soc.* **2004**, *126*, 16451 – 16455; b) S. Petoud, S. M. Cohen, J.-C. G. Bünzli, K. N. Raymond, *J. Am. Chem. Soc.* **2003**, *125*, 13324 – 13325.

[7] a) A. P. S. Samuel, E. G. Moore, M. Melchior, J. Xu, K. N. Raymond, *Inorg. Chem.* **2008**, *47*, 7535 – 7544; b) A. P. S. Samuel, J. L. Lunkley, G. Muller, K. N. Raymond, *Eur. J. Inorg. Chem.* **2010**, 3343 – 3347.

[8] A. P. S. Samuel, J. Xu, K. N. Raymond, *Inorg. Chem.* **2009**, *48*, 687 – 698.

[9] A. E. V. Gorden, J. Xu, K. N. Raymond, P. Durbin, *Chem. Rev.* **2003**, *103*, 4207 – 4282.

[10] Crystal data: $C_{54}H_{64}N_{14}O_{16}Ho$ ($M_r = 1330.12$), light-pink rhomboid, $1.04 \times 0.52 \times 0.27$ mm^3, orthorhombic, space group $C222_1$ (No. 20), $a = 17.455(5)$, $b = 47.542(13)$, $c = 27.414(8)$ Å, $V = 22750(11)$ Å^3, $Z = 8$, $\rho_{calcd} = 0.777$ Mg m^{-3}, $\mu = 0.737$ mm^{-1}, Mo X-ray radiation source $\lambda = 0.71073$ Å, $T = 163(2)$ K, 293944 measured reflections, 20707 independent reflections, R_1 ($I > 2\sigma(I)) = 0.0367$, wR_2 (all data) $= 0.0930$, GOF $= 1.079$.

[11] a) S. Quici, M. Cavazzini, G. Marzanni, G. Accorsi, N. Armaroli, B. Ventura, F. Barigelletti, *Inorg. Chem.* **2005**, *44*, 529 – 537; b) J. Zhang, P. D. Badger, S. J. Geib, S. Petoud, *Angew. Chem.* **2005**, *117*, 2564 – 2568; *Angew. Chem. Int. Ed.* **2005**, *44*, 2508 – 2512; c) E. G. Moore, G. Szigethy, J. Xu, L.-O. Pålsson, A. Beeby, K. N. Raymond, *Angew. Chem.* **2008**, *120*, 9642 – 9645; *Angew. Chem. Int. Ed.* **2008**, *47*, 9500 – 9503.

[12] K. F. Palmer, D. Williams, *J. Opt. Soc. Am.* **1974**, *64*, 1107 – 1110.

[13] R. A. Sá Ferreira, S. S. Nobre, C. M. Granadeiro, H. I. S. Nogueira, L. D. Carlos, O. L. Malta, *J. Lumin.* **2005**, *121*, 561 – 567.

[14] G. G. Giachino, D. R. Kearns, *J. Chem. Phys.* **1970**, *52*, 2964 – 2974.

[15] B. Alpha, J.-M. Lehn, G. Mathis, *Angew. Chem.* **1987**, *99*, 259 – 261; *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 266 – 267.

[16] a) R. M. Supkowski, W. de W. Horrocks, Jr., *Inorg. Chim. Acta* **2002**, *340*, 44 – 48; b) A. Beeby, I. M. Clarkson, R. S. Dickins, S. Faulkner, D. Parker, L. Royle, A. S. De Sousa, J. A. G. Williams, M. Woods, *J. Chem. Soc. Perkin Trans. 2* **1999**, 493 – 502; c) H. Hakala, P. Litti, J. Peuralahti, J. Karvinen, V.-M. Mukkala, J. Hovinen, *J. Lumin.* **2005**, *113*, 17 – 26.

[17] F. J. Steemers, W. Verboom, D. N. Reinhoudt, E. B. Van der Tol, J. W. Verhoeven, *J. Am. Chem. Soc.* **1995**, *117*, 9408 – 9414.

[18] a) J. Xu, E. Radkov, M. Ziegler, K. N. Raymond, *Inorg. Chem.* **2000**, *39*, 4156 – 4164; b) D. L. Kepert, *Prog. Inorg. Chem.* **1978**, *24*, 179 – 249.

[19] a) M. Gonzáles-Lorenzo, C. Platas-Iglesias, F. Avecilla, S. Faulkner, S. J. A. Pope, A. de Blas, T. Rodríguez-Blas, *Inorg. Chem.* **2005**, *44*, 4254 – 4262; b) P. Miranda, Jr., J. Sukerman-Schpector, P. C. Isolani, G. Vicentini, L. B. Zinner, *J. Alloys Compd.* **2001**, *323 – 324*, 13 – 17.

[20] ORTEP-3 for Windows: L. J. Farrugia, *J. Appl. Crystallogr.* **1997**, *30*, 565.