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ENERGY TRANSFER IN LUMINESCENT LANTHANOID CHELATES

Markus Räsänen



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I dedicate this work to my wife and offspring

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ABSTRACT

Luminescent lanthanoid complexes are widely utilized, especially in the medical diagnostics. The complexes have been much studied and ligands with high quantum efficiency have been developed for different lanthanoid ions and for different applications. However, the present ligands are not applicable for all applications, for example for biological imaging, which would require excitation by visible light, water solubility and stability in the aqueous environments. The development of ligands for these applications would require a better knowledge of the fundamental processes in lanthanoid luminescence. In spite of numerous studies, additional information is still needed, at least regarding the energy transfer in chelates. The role of different energy states in the energy transfer processes, especially, would require more studies.

The aim of this thesis is to provide new information about the energy transfer phenomenon. The results show that the roles of the ligand energy states in the energy transfer processes are depended only on their relative positions with respect to the receiving Ln(III) states and the energy transfer rates between the states but not on the nature of the energy states. The determination of the singlet and intraligand charge transfer state energies with the help of absorption and emission spectra is an important result, which helps to show that the energy back-transfer takes place from Ln(III) to the emissive intraligand CT state. In addition, the method for the determination of the stability constant utilizing the luminescence intensity was developed. According to this work, it seems that the absolute values of the ligand energy states should not be taken into consideration in the ligand development but rather the information provided by the (deconvoluted) fluorescence and phosphorescence emission bands of the ligands.

In addition, new efficient ligands and their complexes with Eu(III) and Tb(III) have been studied. These can be excited by visible light and used in aqueous media.

KEYWORDS: energy transfer, lanthanoid, complex, luminescence, europium, terbium

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TIIVISTELMÄ

Luminoivat lantanoidikompleksit ovat laajalti hyödynnettyjä erityisesti diagnostiikassa. Komplekseja on tutkittu paljon ja tehokkaita ligandeja on kehitetty eri lantanoidi-ioneille eri sovelluksiin, mutta vieläkään ei kaikkiin sovelluksiin ole sopivia kelaatteja. Erityisesti biologisiin näytteisiin soveltuville kelaateille olisi tarvetta. Näiden vaatimuksina ovat virittyminen näkyvän valon aallonpituudella sekä vesiliukoisuus ja pysyvyys vesiliuksissa. Ligandien kehittäminen näihin sovelluskohteisiin on mielekästä, kun luminesenssin muodostumisen perusteet ovat hyvin tiedossa. Perusteet ovatkin pääosin hyvin tutkitut, mutta erityisesti energiansiirron ymmärtämisessä on vielä puutteita. Kirjallisuuden perusteella on epäselvää, mitkä ovat erityyppisten energiatilojen roolit energiansiirrossa.

Tämän väitöstyön tarkoituksena oli tutkia tietoa lantanoidikompleksien sisäistä energiansiirtoa. Lopputulema on, että eri energiatilojen rooli on riippuvainen vain energiatilojen suhteellisista energiatasoista ja energiansiirtonopeuksista riippumatta energiatilojen luonteesta. Väitöstyön merkittävimmät annit ovat (i) singletti- ja varauksensiirtotilojen energioiden määrittäminen spektreistä, (ii) sen osoittaminen, että energia virtaa takaisin varauksensiirtotilalle sekä (iii) luminesenssiin perustuvan kompleksivakioiden määrittäminen julkaisemiseksi. Erityisesti ensimmäinen kohta saattaa muodostua tärkeäksi anniksi, jos menetelmä saa laajan hyväksynnän. Tämän työn perusteella näyttää siltä, että ligandikehityksessä ei tarvitse ottaa huomioon absoluuttisia liganditilojen energioita vaan vain dekonvoloitujen luminesenssi- ja fosforesenssiemissiospektrien maksimien energiat.

Lisäksi väitöstyössä julkaistiin useita uusia ligandeja eri sovelluskohteisiin sekä kompleksoitumisvakioiden määrittäminen.

ASIASANAT: energian takaisinvirtaus, lantanoidikompleksi, fotoluminesenssi, europium, terbium

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Abbreviations

A	acceptor
ACN	acetonitrile
aq	aqueous solution, solvated in water
CT	charge transfer
D	donor
DPA (dpa)	dipicolinic acid
DPAD	dipicolinic acid derivatives
ED	electric dipole
EDTA	ethylenediaminetetraacetic acid
E_{ILCT}	energy of ILCT state
E_{s}	energy of singlet state
E_{T} or $E_{\text{Triplet state}}$	energy of triplet state
ϵ_{max}	molar absorption coefficient at absorption maximum
$\epsilon_{\text{Exmax}}\Phi$	molar absorption coefficient at excitation maximum
FRAG	freely rotating amino group
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (buffer)
HOMO	highest occupied molecular orbital
$(\text{H})_{\text{n}}\text{Lx}$	ligand
ILCT	intra-ligand charge transfer
ISC	intersystem crossing
$I/A_{285\text{nm}}$	relative intensity
LMCT	ligand-to-metal charge transfer
Ln(III)	lanthanoid(III) ion
LUMO	lowest unoccupied molecular orbital
$\lambda_{\text{max}}^{\text{abs}}$	absorption maximum
$\lambda_{\text{max}}^{\text{ex}}$	excitation maximum
M	metal atom
M^{3+}	metal(III) ion
MD	magnetic dipole
PCR	polymerase chain reaction

PET	photoinduced electron transfer
phen	phenanthroline
RT	room temperature
$^1S_x^*$	excited singlet state
$^3T^*$	excited triple state
UV	ultraviolet
Y	shorthand notation for EDTA in complexes
z	atomic number

Mathematical symbols

A	constant
a	proportionality constant
$A_{x \text{ or } y}$	amplitude
$A_x(\lambda_{ex})$	absorbance of sample at the excitation wavelength
$A_s(\lambda_{ex})$	absorbance of standard at the excitation wavelength
α	combination of the temperature-independent high-frequency component and the diamagnetic contribution
B	amplitude
β	constant
C	constant or concentration
$C_{ref}(t)$	reference signal
ΔE	energy difference
E_s	integrated area of emission spectrum of standard
E_x	integrated area of emission spectrum of sample
ε	molar extinction coefficient / molar absorptivity
$\varepsilon_A(\lambda)$	extinction coefficient of the acceptor at wavelength λ
$\varepsilon\Phi$	brightness
f	frequency
$F_D(\lambda)$	normalized (total area = 1) emission spectrum of the donor at wavelength λ to $\lambda + \Delta\lambda$
g	Landé splitting factor
h	amplitude
\hbar	Dirac constant
$\hbar\omega$	energy of phonon
θ	phase shift
I_{tot}	total intensity
J	total angular momentum quantum number
K	constant or stability constant
$k (= \tau^{-1})$	decay rate constant
k_0	decay rate constant at $T = 0$
k'	pre-exponential factor

k_B	Boltzmann constant
k^{vibr}	decay rate in presence of vibrational quencher
κ^2	orientational factor
L	Bohr radius
$\log \beta$ or K	stability constant
λ	wavelength
μ_B	magnetic moment
μ_{ef}	Bohr magneton
n	number of unpaired electrons
N_A	Avogadro's constant
n_r	refractive index of medium
n_s	refractive index of medium of standard
n_x	refractive index of medium of sample
PL	photoluminescence
q	number of coordinated water molecule
Q_D	quantum yield of donor in the absence of the acceptor
r	distance between donor and acceptor
S	spin states
$S_{in}(t)$	emission signal
$S_{ref}(t)$	reference signal
S_x	in-phase signal
S_y	out-of-phase signal
σ_p^+	substituent constant
T	temperature
t	time
τ	luminescence lifetime
τ_D	luminescence lifetime of donor in the absence of the acceptor
τ_{D2O}	luminescence lifetime in the absence of water molecules
τ_{H2O}	luminescence lifetime in the presence of water molecules
Φ	quantum yield
χ_M	molar susceptibility
ω	angular frequency

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

- I Markus Räsänen, Harri Takalo, Jaana Rosenberg, Joonas Mäkelä, Keijo Haapakka ja Jouko Kankare; Study on photophysical properties of Eu(III) complexes with aromatic β -diketones - role of charge transfer states in energy migration; *J. Lumin.*, 2014, 146, 211 – 217.
- II Markus Räsänen, Harri Takalo, Jaana Rosenberg, Tero Soukka, Keijo Haapakka ja Jouko Kankare; Photophysical study of blue-light excitable ternary Eu(III) complexes and their encapsulation into polystyrene nanoparticles; *J. Lumin.*, 2015, 160, 128 – 133.
- III Markus Räsänen, Jukka Lukkari, Keijo Haapakka, Jouko Kankare ja Harri Takalo; Study on luminescent ternary EuEDTA complexes with a set of substituted 4-phenylethynyl and 4-aryl pyridine-2,6-dicarboxylic acids; *J. Lumin.*, 2017, 187, 471 – 478.
- IV Markus Räsänen, Harri Takalo, Jaana Rosenberg, Keijo Haapakka, Jukka Lukkari ja Jouko Kankare; Energy transfer in ternary TbEDTA chelates with a series of dipicolinic acid derivatives; *J. Lumin.*, 2020, 220, p – p.

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1 Introduction

This thesis deals with the energy transfer in luminescent lanthanoid chelates. The luminescence is emission of light by a substance without thermal excitation. The light is emitted from the material by the transition of electrons from a higher electronic energy state to a lower one. The higher electronic energy state, the 'excited state', can be produced by a chemical reaction (chemiluminescence), crystallization (crystalloluminescence), electric current (electroluminescence), mechanical stress (mechanoluminescence), ionizing radiation (radioluminescence), heat (thermoluminescence), flame (candoluminescence) and light (photoluminescence). This thesis concentrates on photoluminescence, which is conventionally divided into two categories: fluorescence and phosphorescence. In the fluorescence, the spin is not changed in the relaxation process (a singlet–singlet transition), whereas it is changed in phosphorescence (a singlet–triplet–singlet transition). Depending on the transition, the multiplicity may or may not change in the electronic transition of a lanthanoid ion, and so only the term 'luminescence' is used in this thesis in the case of emitting lanthanoid ions. The main luminescent transitions in lanthanoid ions are fluorescent in Pr(III), Nd(III), Ho(III), Er(III) and Yb(III) and phosphorescent in Sm(III), Eu(III), Gd(III), Tb(III), Dy(III) and Tm(III). Lanthanoids from La to Lu are metallic.

Lanthanoid chelates consist of an emitting central ion and one or more light harvesting organic ligands. The luminescence efficiency of the chelates is dependent mainly to three factors: i) the harvesting efficiency of the organic ligand, ii) the efficiency of energy transfer and iii) the shielding of the ions from quenching species. The first and last points are quite clear but the topic ii) is still not fully understood. This thesis tries to shed light on the topic of the energy transfer. The distance between the ligand and Ln(III) ion is typically so short that the energy transfer is very efficient and the knowledge of the energy transfer phenomenon brings probably additional value only to the shifting of the excitation maximum towards visible wavelength. Later, the terms 'chelate' and 'complex' are used as synonyms because the studied ligands are at least bidentate.

1.1 Historical background

The luminescence has occurred “always” in nature, *e.g.* the glow of glowworms (*Lampyris noctiluca*) is bioluminescence caused by the oxidation of the compound named luciferin[1], but humans have utilized the luminescence only for little more than hundred years. In 1859, Becquerel invented a fluorescent lamp.[2] This lamp consisted of a partially evacuated glass tube whose wall was coated with certain luminescent substances. When electric current passed through the tube it excited the gas molecules which relaxed emitting UV-light (electroluminescence). This light was absorbed by the luminescent material of the wall which in turn emitted light in the visible region (photoluminescence). These early luminescent lamps turned out to be very short-lived and thus they did not have commercial use. However, their operating principle was same as that of the modern fluorescent lamps. Nowadays, studies and applications of luminescence are rapidly increasing. This can be illustrated by Google.scholar search using ‘Luminescence’ as a key word. The search gives 350 000 results prior to 2000 and 644 000 results for the interval 2000 – 2019.

The lanthanoids were discovered in the turn of the 19th and 20th centuries. The lanthanoids are also known as “rare earths” although they are not so rare. For example lanthanum, which is the first element in the lanthanoid series and which has given the name for the whole series, exists in the Earth’s crust at a concentration of 29 ppm which is over 400-fold the concentration of silver.[3] The name “lanthanum” is of Greek origin and means “lying hidden”. The name of dysprosium is also derived from a Greek word, namely “dysprositos”, which means “hard to get”. These names may reflect the earlier impression that these naturally abundant elements are rare and they also imply why the lanthanoids were discovered relatively late. In 1787, C. A. Arrhenius discovered heavy black mineral at Ytterby in Sweden. A Finnish chemistry professor at University of Åbo (Turku), J. Gadolin, isolated an oxide from this mineral in 1794 and named it “ytterbia”. Half a century later, in 1843, Swedish C. G. Mosander further separated “ytterbia” by oxalate and hydroxide precipitation and named three fractions “yttria”, “erbia” and “terbia”. These “erbia” and “terbia” were further separated into many lanthanoid elements. The other branch of discovery of the lanthanoids is the separation of elements from “cerite”, a mineral found in 1751. Figure 1 shows the outlines of the discovery of the lanthanoids. An interesting detail is that gadolinium is named after mineral gadolinite discovered by J. Gadolin and named after discoverer.

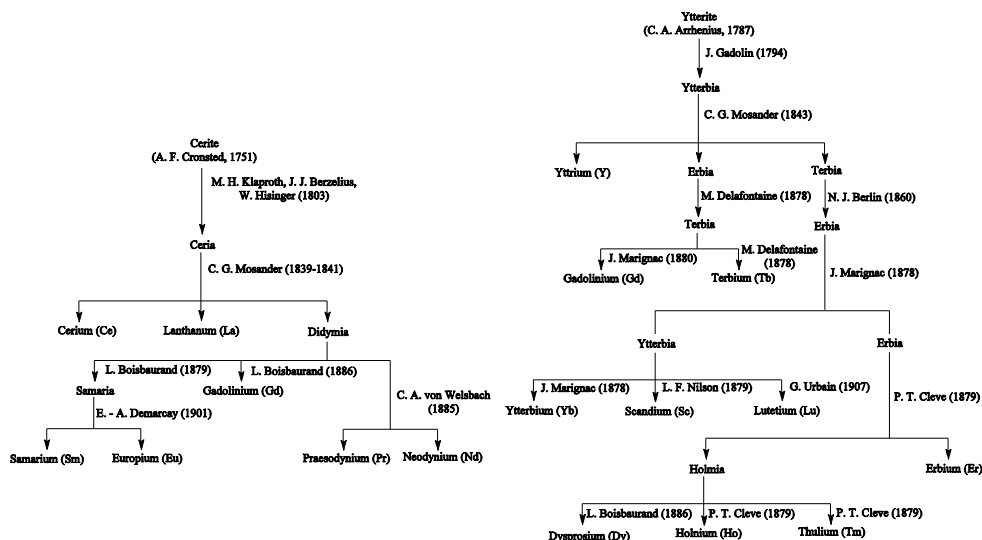


Figure 1. Outlines of discovery of lanthanoids.[4]

The first application of lanthanoids in history was the use of lanthanum in gas lantern mantles since 1885. The gas mantles operate partly using candoluminescence.[5]

The history of lanthanoid chelates stems from 1897 when G. Urbain prepared the first lanthanoid β -diketone chelate.[6] The history of the luminescence of the lanthanoid chelates stems from 1942 when S. I. Weissman discovered that lanthanoid ions (especially Eu(III) but also Tb(III) and Sm(III)) emit their typical luminescence when organic ligands complexed to them were excited.[7] At the beginning of the 1960's G. Crosby *et al.* investigated intramolecular energy transfer processes in these complexes[8,9] and during this decade the Ln(III) chelates were studied intensively because of their applicability as active material in liquid lasers.[10] The chelates suffered from weak operating efficiency and did not find wide use. At the beginning of the 1980's the study of the lanthanoid chelates saw a new rise because they were found to be suitable as labels in fluoroimmunoassays.[11] At this time, the development of Ln(III) probes with long luminescence lifetime and in time-resolved photoluminescence (PL) spectroscopy together made a time-resolved PL-immunoassays possible. This is again connected to Turku because a Finnish company Wallac from Turku has a big role in this advancement. The time-resolved PL-immunoassay is a powerful technique because the background fluorescence and the scattering of the excitation light can be effectively excluded in this technique.[10] This technique is still widely used[12] but it is not currently optimal *e.g.* for fluorescence imaging which can be disturbed by UV light. Therefore, there is still demand for new chelates.

1.2 Lanthanoid ions

In aqueous solutions the lanthanoid ions exist in their trivalent state, Ln(III), due to the balance between the hydration enthalpy of trivalent cations and the sum of the first three ionization energies.[13] The trivalent lanthanoid ions have their electronic configurations of the form $[\text{Xe}]4f^n$ (see Table 1). The electronic wavefunctions of the $4f$ orbitals lie spatially (but not energetically) below the occupied $5s$ and $5p$ orbitals and are thus shielded from environment. The ionic radii of the lanthanoid ions decrease (lanthanoid contraction) and the hydration enthalpies increase linearly in the series.[13] The lanthanoid contraction is a result of the imperfect shielding of one $4f$ electron by another $4f$ electrons. As the nuclear charge, and thus the $4f$ electron population, increases, the imperfect shielding due to the geometry of these orbitals causes each $4f$ electron to experience added electrostatic attraction by the nucleus.[14]

Table 1. Symbols, atomic numbers, M and M^{3+} configurations, M^{3+} radius and standard reduction potentials (E°) of lanthanoids.

ELEMENT	SYM-BOL	Z	M CONFIGURATION	M^{3+} CONFIGURATION	M^{3+} RADIUS [Å] ^A	$E^\circ (\text{Ln}^{3+}(\text{aq}) + e^- \rightarrow \text{Ln}^{2+}(\text{aq}))[\text{V}]^b$
LANTHANUM	La	57	$[\text{Xe}]5d^16s^2$	$[\text{Xe}]4f^0$	1,061	-3,74
CERIUM	Ce	58	$[\text{Xe}]4f^15d^16s^2$	$[\text{Xe}]4f^1$	1,034	-3,76
PRASEODYNIUM	Pr	59	$[\text{Xe}]4f^36s^2$	$[\text{Xe}]4f^2$	1,013	-3,03
NEODYMIUM	Nd	60	$[\text{Xe}]4f^46s^2$	$[\text{Xe}]4f^3$	0,995	-2,62
PROMETHIUM	Pm	61	$[\text{Xe}]4f^56s^2$	$[\text{Xe}]4f^4$	0,979	-2,67
SAMARIUM	Sm	62	$[\text{Xe}]4f^66s^2$	$[\text{Xe}]4f^5$	0,964	-1,57
EUROPIUM	Eu	63	$[\text{Xe}]4f^76s^2$	$[\text{Xe}]4f^6$	0,950	-0,35
GADOLINIUM	Gd	64	$[\text{Xe}]4f^75d^16s^2$	$[\text{Xe}]4f^7$	0,938	-3,82
TERBIUM	Tb	65	$[\text{Xe}]4f^96s^2$	$[\text{Xe}]4f^8$	0,923	-3,47
DYSPROSIUM	Dy	66	$[\text{Xe}]4f^{10}6s^2$	$[\text{Xe}]4f^9$	0,908	-2,42
HOLMIUM	Ho	67	$[\text{Xe}]4f^{11}6s^2$	$[\text{Xe}]4f^{10}$	0,894	-2,80
ERBIUM	Er	68	$[\text{Xe}]4f^{12}6s^2$	$[\text{Xe}]4f^{11}$	0,881	-2,96
THULIUM	Tm	69	$[\text{Xe}]4f^{13}6s^2$	$[\text{Xe}]4f^{12}$	0,869	-2,25
YTTERBIUM	Yb	70	$[\text{Xe}]4f^{14}6s^2$	$[\text{Xe}]4f^{13}$	0,858	-1,04
LUTETIUM	Lu	71	$[\text{Xe}]4f^{14}5d^16s^2$	$[\text{Xe}]4f^{14}$	0,848	-

^a From ref. [15]

^b From ref. [13]

It is remarkable that the reduction potential of the reaction $\text{Eu}^{3+}(\text{aq}) + \text{e}^- \rightarrow \text{Eu}^{2+}(\text{aq})$ is quite high (see Table 1). This is due to the thermodynamic stability of the half-filled ($4f^7$) subshell which $\text{Eu}(\text{III})$ (electronic configuration $4f^6$) achieves by accepting one electron.

1.2.1 Physical properties of lanthanoid ions

Magnetic and photophysical properties of lanthanoid ions will be discussed in this chapter. The magnetic properties of $\text{Ln}(\text{III})$ ions are not at the centre of this thesis but they are strongly connected to the photophysical properties *e.g.* their effect on organic chromophores is discussed in the section **1.3.3**.

1.2.1.1 Magnetic properties of lanthanoid ions

The magnetism can be divided into diamagnetism, paramagnetism and ferromagnetism. The electrons in atoms have magnetic orbital and spin dipole moments. The electrons are paired in diamagnetic species and thus the sum of the magnetic dipole moments is zero. The diamagnetic substances repel the magnetic field. Paramagnetic substances contain unpaired electrons and thus they have the magnetic moment. The magnetic field attracts the paramagnetic substances, but they are not permanently magnetized. Their magnetic regions are randomly ordered and thus they revoke each other. Ferromagnetic substances have the organized magnetic domains and thus they have permanent magnetization.

The strength of magnetization can be described with the permanent magnetic moment, μ_B , or molar susceptibility, χ_M . The magnetic moment is related to the spin-only formulation

$$\mu_B = 2\sqrt{[S(S+1)]} = \sqrt{n(n+2)} \quad (1)$$

where S represents the total spin quantum number, n the number of unpaired electrons and μ_B is the moment in Bohr magnetons (B.M.). The above equation (1) is valid whenever the contribution of the orbital angular momentum can be ignored. This is not a case with lanthanoid ions but the calculation of their magnetic moments demands the second order Zeeman terms.[16] The value of χ_M can be calculated according to the equation

$$\chi_M = N_A \left(\frac{g^2 \mu_{ef}^2 J(J+1) + \alpha}{3k_B T} \right) \quad (2)$$

where N_A is Avogadro's constant, g is the Landé splitting factor, μ_{ef} is the magnetic moment in Bohr magnetons (μ_B from eq. 1 if it is valid), J is the total angular

momentum quantum number, k_B is the Boltzmann constant, T is the temperature in Kelvin and α is a combination of the temperature-independent high-frequency component and the diamagnetic contribution.

The lanthanoid ions are paramagnetic except the La(III) and Lu(III) ions, which are diamagnetic, because the other lanthanoid ions have unpaired $4f$ electrons and the La(III) and Lu(III) ions have closed-shell electronic configurations and no unpaired electrons. Table 2 shows the permanent magnetic moments (μ_B) for the Ln(III) ions.

The rather good correlation between calculated and measured (in the different environments) μ_B values suggest either minor or no involvement of the $4f$ orbitals in bonding. Unexpectedly, the $4f^7$ species, Gd(III), with the maximum number of the unpaired electrons is not the most strongly paramagnetic. Obviously the outer $5s^25p^6$ arrangement does not shield the $4f^7$ electrons like other $4f^n$ contributions.[14]

Table 2. Permanent magnetic moments (μ_B) of Ln(III) ions at 20-30 °C.

Ln(III)	CALCULATED μ_B (B.M.) ^A	MEASURED μ_B (B.M.) (Ln ₂ (SO ₄) ₃ •8H ₂ O) ^b	MEASURED μ_B (B.M.) ([Ln(EDTA)]) ^c
La	0,00	diam.	diam.
Ce	2,56	2,37	-
Pr	3,62	3,47	3,6
Nd	3,68	3,52	3,6
Pm	2,83	-	-
Sm	1,55-1,65	1,53	1,7
Eu	3,40-3,51	-	3,6
Gd	7,94	7,81	7,9
Tb	9,7	9,4	-
Dy	10,6	-	-
Ho	10,6	10,3	-
Er	9,6	9,6	-
Tm	7,6	-	-
Yb	4,5	4,4	-
Lu	0,00	diam.	diam.

^a From ref. [16]

^b From ref. [17]

^c From ref. [18]

All of the Ln(III) ions except Sm(III) and Eu(III) obey the Curie law

$$\chi_M = C/T \quad (3)$$

where C is a constant and T the temperature. The lowest J states of Sm(III) and Eu(III) are near in energy (see Figure 2) resulting in a population of the upper J states even at room temperature. This causes these species to significantly depart from the Curie law in their magnetic behavior.

1.2.1.2 Photophysics of lanthanoid ions

The photophysics of the lanthanoid ions is characterized by the sharp emission lines and long luminescence lifetimes. These properties are based on the environmentally shielded $4f$ orbitals and the forbidden $f-f$ transitions due to the Laporte rule, respectively. The forbidden nature of these transitions causes also another property of lanthanoid ions, low molar absorption coefficient ($\epsilon < 1 \text{ M}^{-1}\text{cm}^{-1}$). This makes the direct excitation of the ions very inefficient, but this drawback can be overcome by using strongly absorbing chromophores.

1.2.1.2.1 *Electronic energy levels*

Figure 2 shows the energy levels of Pr(III) – Yb(III) in energetic order. The term symbols are shown only for few lowest states of Eu(III) and Tb(III). The luminescence lifetime of the $^5\text{D}_1$ state of complexed Eu(III) is of the order of $1 \mu\text{s}$ whereas that of the $^5\text{D}_0$ state is of the order of 1 ms . The luminescence lifetime of the $^5\text{D}_4$ state of complexed Tb(III) is also of the order of 1 ms . The energy difference between the $^5\text{D}_1$ and $^5\text{D}_0$ states of Eu(III) is 1740 cm^{-1} and these can be easily bridged even by the low frequency vibrations, and thus, the $^5\text{D}_1$ state relaxes quite fast to $^5\text{D}_0$ states.

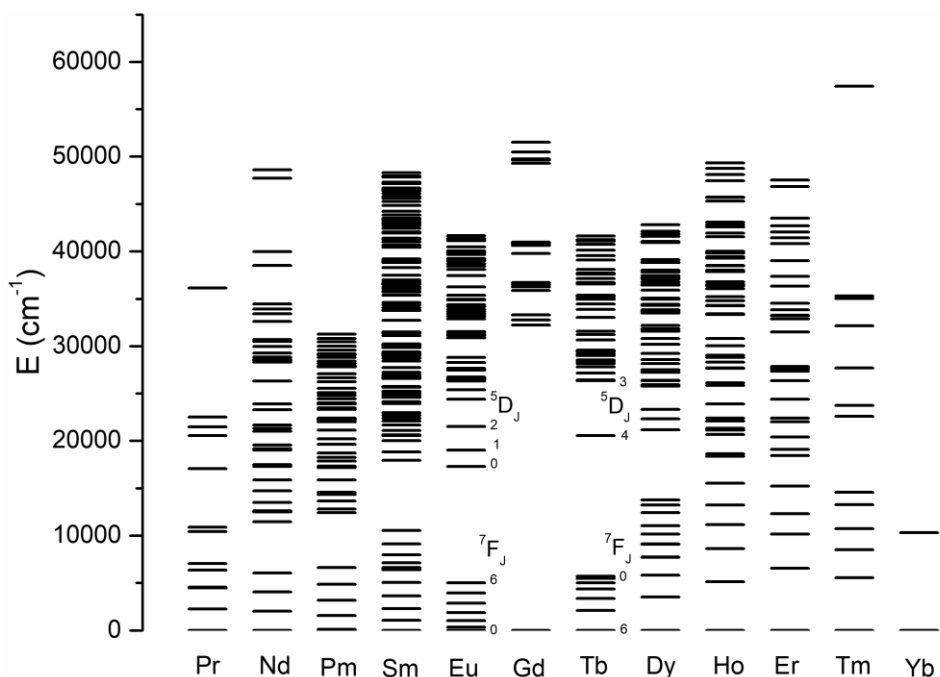


Figure 2. The energy level scheme of Ln(III) ions.[19–22]

The term symbols $^{2S+1}L_J$ give three pieces of information[23]:

- I. The letter indicates the total orbital angular momentum quantum number, L . Letters correspond to the quantum numbers as follows: S, P, D, F, G, H, I... \leftrightarrow 0, 1, 2, 3, 4, 5, 6..., respectively.
- II. The left superscript gives the multiplicity ($2S + 1$) of the term.
- III. The right subscript is the value of the total angular momentum quantum number, J .

As Table 2 shows, even Pr(III) has two f -electrons which give 7 total orbital angular momenta from S to I, let alone the Ln(III) species with more f -electrons. In addition to this electrostatic splitting, the electronic states are further split by spin correlation. For two unpaired electrons (*e.g.* in Pr(III)), this gives multiplets 1 and 3. These states are further split by spin-orbit –coupling that is expressed by the total angular momentum quantum number. Although the lanthanoid ions reasonably high atomic masses and thus they would require the use of an intermediate coupling scheme between Russel-Saunders and jj -coupling schemes, the Russel-Saunders scheme is usually used. It gives for example for the 3S state of Pr(III) two states 3S_0 and 3S_1 . All calculated states are not allowed according to the Pauli Exclusion Principle but still Ln(III) ions have very many energy levels.[23]

1.2.1.2.2 Radiative transitions

The Ln(III) ions which have incompletely filled $4f$ orbitals absorb electromagnetic radiation in the ultraviolet, visible and near-infrared regions. The absorption can be caused by the $4f^n \rightarrow 4f^{n-1}5d^1$, charge transfer or intra- $4f^n$ transitions. The $4f^n \rightarrow 4f^{n-1}5d^1$ transitions are allowed and therefore they have a reasonably high intensity ($\epsilon = 200 - 1000 \text{ M}^{-1}\text{cm}^{-1}$). Because the d orbitals experience more strongly the ligand field than the f orbitals, their energies much depend upon the surroundings of the metal-ion being order of $> 50\,000 \text{ cm}^{-1}$ ($\lambda < 200 \text{ nm}$). Thus they are rarely observed in coordination compounds except for quite easily oxidized Ce(III), Pr(III) and Tb(III) (see Table 1).[24,25] The excited $4f^{n-1}5d^1$ state can be relaxed radiatively to the ground state either by the $4f^{n-1}5d^1 \rightarrow 4f^n$ or intra- $4f^n$ transitions. The charge transfer (CT) transitions can occur whenever an easily oxidized ligand is bound to Sm(III), Eu(III) and Yb(III).[26,27] The CT transition bands are usually broad.

The most common transitions are intra- $4f^n$ transitions. As mentioned above, the f - f transitions are the Laporte (or parity) forbidden. However, the magnetic dipole (MD) transitions are allowed according to $\Delta L = 0, \pm 1$ and $\Delta J = 0, \pm 1$ (but $J = 0 \leftrightarrow J = 0$ and $L = 0 \leftrightarrow L = 0$ are forbidden) selection rules. This is because a selection rule $\Delta S = 0$ is partly broken since the wave functions obey an intermediate coupling scheme instead of the Russell-Saunders coupling. These selection rules stem from the conservation of angular momentum during a transition and from the spin 1 for the photon. The intensities of the MD transitions are weak and practically independent on the surrounding matrix. For free ion, only the MD transitions are allowed but situation is changed when Ln(III) is in coordinative environment. In that case, the ligand field mixes odd-parity configurations slightly into the $[\text{Xe}]4f^n 5d^0$ configuration allowing the electric dipole (ED) transitions.[28] The forced ED transitions are much weaker than allowed transitions (e.g. $4f^n \rightarrow 4f^{n-1}5d^1$ transitions) but of the same order of magnitude than MD transitions. Because the ED transitions are induced by the ligand field their strengths are quite sensitive to it. Strongly asymmetric or interacting ligand fields lead to the relatively intense ED transitions. Also mixed MD/ED transitions occur and, e.g. the emission spectra of the Tb(III) complexes are dominated by the mixed MD/ED transitions. Some ED transitions are extremely sensitive to coordination environment (hypersensitive transitions).[29]

Table 3 shows the emissive transitions of Eu(III) from the 5D_0 state to the 7F_J ($J = 0 - 6$) states and their sensitivity to environmental factors. The $^5D_0 \rightarrow ^7F_{1,3,5}$ transitions are forbidden by both ED and MD mechanisms but they frequently appear in the emission spectra of the Eu(III) complexes. A detailed analysis has shown that these transitions 'borrow' intensity from the $^5D_0 \rightarrow ^7F_2$ transition through higher order perturbations by the crystal field.[30,31] The $^5D_0 \rightarrow ^7F_2$ transition is represented usually by a main peak at 615 nm in the emission spectra of the Eu(III) complexes. Usually, when the energy transferring state of the ligand is above the 5D_1

state of Eu(III), the emission spectrum of the complex can also contain the transitions from this state, and even transitions from the 5D_2 state can be seen sometimes. The peak of the $^5D_0 \rightarrow ^7F_1$ MD transition at 590 nm is not sensitive to environment and it can be used as the internal standard *e.g.* for the calculation of the radiative rate constants.[29]

Table 3. Emission transitions of Eu(III) from 5D_0 state to 7F_J states.

$^5D_0 \rightarrow ^7F_J, J =$	WAVELENGTH (nm)	ED/MD	SENSITIVITY TO COORDINATION ENVIRONMENT
0	580	-	quite sensitive
1	590	MD	not sensitive
2	615	ED	hypersensitive
3	650	-	
4	690	ED	sensitive
5	760	-	
6	810	ED	

All of the emissive $^5D_4 \rightarrow ^7F_J$ ($J = 0 - 6$) transitions of Tb(III) are the ED transitions but at least the $^5D_4 \rightarrow ^7F_J$ ($J = 3 - 5$) transitions are also the MD transitions.[32] The energy of the 5D_3 state of Tb(III) is so high that it is usually not involved.

1.2.1.2.3 Nonradiative transitions

The main mechanism of the nonradiative deactivation in the excited lanthanoid ions in crystal lattice is a process known as multiphonon relaxation.[33] In the multiphonon relaxation the electronic excitation energy is dissipated by the vibrations of the surrounding matrix. The similar processes occur also in the solutions of the Ln(III) complexes with organic ligands.[34] The efficiency of the vibrational quenching [$k^{vibr}(T)$] is proportional to the number of the vibrational quanta (i) needed to bridge the energy gap between two electronic levels (ΔE). This can be described by the equation[29]:

$$k^{vibr}(T) = k^{vibr}(0) \cdot \left(1 - e^{-\hbar\omega/k_B T}\right)^{-i} \quad \text{with } i = \frac{\Delta E}{\hbar\omega}. \quad (4)$$

Here $\hbar\omega$ is the energy of phonon, k_B Boltzmann's constant ($1,38 \times 10^{-23}$ J/K ≈ 0.695 cm $^{-1}$ /K), T temperature in Kelvin and $k^{vibr}(0)$ the spontaneous rate at 0 K.

The most common vibrational quenchers are the OH ($\nu \approx 3600 \text{ cm}^{-1}$), NH ($\nu \approx 3400 \text{ cm}^{-1}$) and CH ($\nu \approx 2900 \text{ cm}^{-1}$) groups. In the case of the class 3) (see 1.2.1.2.4), mainly only OH vibrations play a role, but in class 2), also other groups have a remarkable role.

1.2.1.2.4 Classification of lanthanoid ions on the basis of luminescing capability

The lanthanoid ions can be divided in three groups according to their ability to luminesce in the complexes[9,35]:

- 1) The La(III) ($4f^0$), Gd(III) ($4f^7$) and Lu(III) ($4f^{14}$) ions do not give the ion luminescence. The intra- $4f$ transitions are, of course, impossible with La(III) and Lu(III) and the lowest-lying excited term of the Gd(III) ion is so high in energy, that its excitation is not feasible.
- 2) The Pr(III), Nd(III), Ho(III), Er(III), Tm(III) and Yb(III) ions display only weak ion luminescence as a consequence of small energy differences between the terms (see Figure 2). This makes the nonradiative transitions efficient. These ions emit in the near-infrared wavelength region.
- 3) The Sm(III), Eu(III), Tb(III) and Dy(III) ions exhibit strong ion luminescence because they have suitable energy differences between the terms with respect to the energy levels of the ligands and nonradiative quenching routes (see Figure 2). These ions emit in the visible wavelength region.

1.2.2 Chemical properties of lanthanoid ions

The lanthanoid ions have very similar chemical properties. The small differences in their complex formation and solubility properties are based on their decrease in size along the series, *i.e.*, on the lanthanide contraction.

1.2.2.1 Coordination chemistry of lanthanoid ions

The lanthanoid ions can be classified as “hard” Lewis acids. Therefore, they prefer “hard” Lewis bases and their binding preference is $\text{O} > \text{N} > \text{S}$. It is also generally agreed that the Ln(III)-ligand coordination occurs predominantly via ionic bonding interactions leading to a strong preference for negatively charged groups that are also “hard” bases. Therefore, water molecules and hydroxide ions are particularly strong Ln(III) ligands, and in aqueous solution only ligands with negatively charged oxygen atoms can bind strongly. In aqueous solutions, ligands containing neutral oxygen or

nitrogen atoms generally bind if they contain also at least one negatively charged oxygen donor group. The predominantly ionic character of Ln(III)-ligand interactions and the relatively low charge-to-ionic radius ratios of the Ln(III) ions result in little or no directionality in the Ln(III)-ligand interactions. Consequently, complex geometries are determined almost entirely by ligand characteristic (conformational properties and the number, sizes and charge of donor groups). The Ln(III) ions exhibit coordination numbers ranging from six to twelve with eight and nine being the most common.[36]

1.2.2.2 Solubility of lanthanoid ions

The Ln(III) ions are soluble in aqueous media but under basic conditions they start to precipitate as hydroxides. For example, the cumulative stability constants for the complexes between Eu(III) and OH⁻ are $\log \beta_{1-1} = 7,4$, $\log \beta_{1-3} = 19,3$, $\log \beta_{1-4} = 26,2$ and $\log \beta_{2-3} = 15,4$ [37] and the distribution diagram shown in Figure 3 can be drawn by using these values. As figure shows, Eu(III) can exist as a free below pH8. Thus, the preferred pH:s of the lanthanoid complex solutions is below pH8. The lanthanoid oxides, Ln₂O₃, are soluble only in very acidic aqueous solutions but the lanthanoid chlorides, LnCl₃, are easily soluble in aqueous solutions.

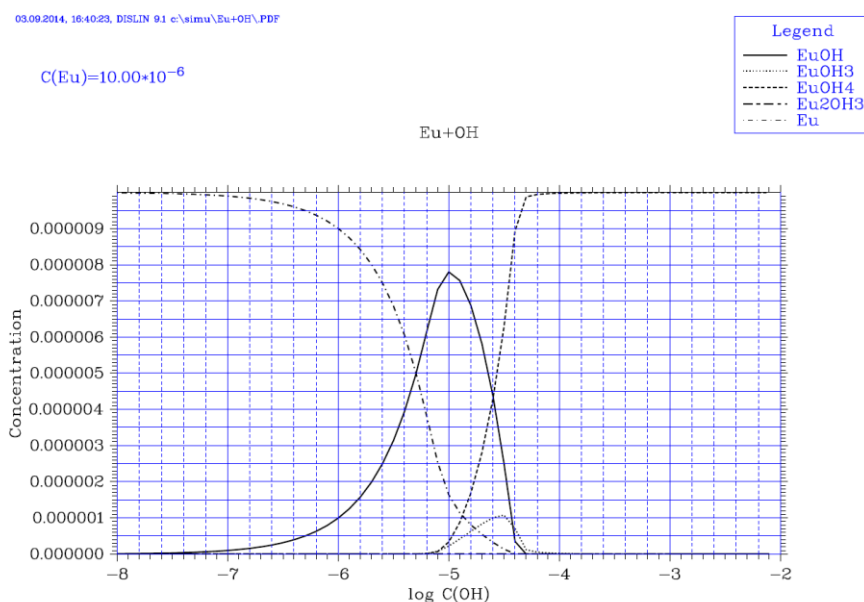


Figure 3. Distributions of the complexes between Eu(III) and OH⁻ as a function of log C_{OH⁻}. The log C(OH) value -2 corresponds to the pH value 12 and -8 to the pH value 6.

1.3 Organic ligands for lanthanoid ion complexes

A wide variety of ligands can be used for sensitizing the lanthanoid ions, for example cryptans[38–40], podans[40–43], calixarenes[44,45], macrocyclic ligands[41,46,47], heterobiaryl ligands[48–50], multidentate pyridine[49,51–53], terpyridine[54,55], β -diketones and dipicolinic acid derivatives. Among these, β -diketones form the most studied group, together with dipicolinic acid and its derivatives. This thesis concentrates on these groups. Figure 4 shows the general structures of these ligands.

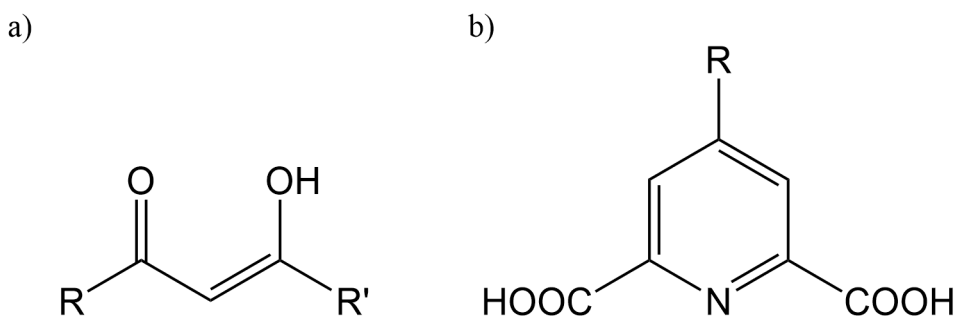


Figure 4. Structures of β -diketones (a) and dipicolinic acids (b).

1.3.1 β -Diketones

β -Diketones are chelated at charge -1 and are bidentate ligands. However, they form usually tris-complexes ($Ln(III)$:ligand ratio is 1:3) with $Ln(III)$ ions, maybe because tris- β -diketone- $Ln(III)$ complexes are neutral. However, tris- β -diketone- $Ln(III)$ complexes have three free coordination sites which will be filled if there are coordinative molecules available. The complex formation of the β -diketones with the lanthanide ions is suppressed in aqueous solutions because the water molecules significantly compete with β -diketones. This is reason why the tris- β -diketone- $Ln(III)$ complexes are not extremely bright in aqueous solutions. Ternary tris- β -diketone- $Ln(III)$ complexes that contain a Lewis base in the free coordination sites dissociate extremely easily in aqueous solutions. An exception is 1,10-phenanthroline that coordinates to aromatic tris- β -diketone-Eu(III) complexes via synergistic effect.[56] In addition to the filling of the free coordination sites, the Lewis base may also bring about two additional advantages: It may enhance the brightness of the complex [57] and allow to extend the excitation wavelength towards visible wavelength region [58–63].

The lanthanoid complexes of β -diketones are quite stable but they may undergo the electrophilic substitution reaction to the 2-position.[64–66] Moreover, the β -

diketones may undergo the photochemical DeMayo reaction with molecules containing C=C bond [67], which must be taken into consideration if the lanthanoid complex solutions contain impurities. The β -diketones exhibit keto-enol tautomerism. In the enol form (Figure 4a), the H-atom of the alcohol function is hydrogen-bonded to the carbonyl O-atom. The position of the keto-enol equilibrium depends on a variety of factors *e.g.* substituent in the α -carbon, the fluorination of the molecule and the polarity of the solvent. When the β -diketone is deprotonated, the proton is removed from the α -carbon (in the keto form) or from the alcohol alcohol group (in the enol form). The acidity of the β -diketone depends on the substituent. Electron-withdrawing groups increase the acidity whereas electron-donating groups decrease it. The negative charge of the β -diketone ligand in lanthanoid β -diketonates partly delocalize in the lanthanoid ion. Many β -diketones are commercially available at reasonably low prices. If new β -diketones are needed, the synthesis can usually be carried out by the Claisen condensation reaction between a deprotonated methylketone and an ethyl or methyl ester. The lanthanoid ion β -diketonate complexes are kinetically labile, which results in the fast exchange of ligands in solutions. The lanthanoid β -diketones easily dimerize in non-polar solvents. More information about β -diketones can be found in an excellent review of K. Binnemans.[68]

The tris- β -diketone-Ln(III) complexes can be applied to electroluminescent devices [69,70], sensors[63], lasers [71] and bioassays [12]. The ternary tris- β -diketone-Ln(III) complexes expand the applicability of the β -diketone-Ln(III) complexes to the bioimaging [72,73] and make possible the use of glass in measurements.

1.3.2 Dipicolinic acids

Dipicolinic acid derivatives (dipicolinic acids) are tridentate ligands with charge -2 in chelates. They form also tris-complexes (1:3) with Ln(III) ions. All forms, 1:1, 1:2 and 1:3 forms, exist in relatively dilute solutions. The cumulative stability constants for the complexes between dipicolinic acid (dpa) and Eu(III) are $\log \beta_1=8,83$, $\log \beta_2=15,98$, and $\log \beta_3=21,03$. [74] The pK_a values for dpa are $pK_{a1} = 5,14$ and $pK_{a2} = 2,29$. [75] The complexes dissociate at pH below pK_{a1} and also in very basic conditions.

Dipicolinic acid is a common reagent in organic chemistry. It exists also in nature, *e.g.* many bacteria contain dipicolinic acid. Dipicolinic acids form also the ternary complexes. For example, Choppin *et.al.* have studied the EuEDTA complexes with nitriloacetate [76], oxalate[77] and imido diacetic acid[78]. The stability constants for these complexes were determined to be $\log \beta = 25,96$, $\log \beta =$

20,30 and $\log \beta = 22,95$, respectively. The stability constant between Eu(III) and EDTA is determined to be $\log K = 17,29$. [79]

The dipicolinic acids have applications *e.g.* in the time-resolved dissociative enhancement spectroscopy [80,81], luminescent nanoparticle assays [82,83], NMR studies [84] and bioimaging [85–90].

1.3.3 Ligand energy states

In this context, only the vibrational and electronic energy states must be taken into account. The vibrational and electronic energy level differences are of the order of 1000 cm^{-1} and $10\,000 \text{ cm}^{-1}$, respectively. The vibrational states of the ligands serve mainly to quench the luminescence and only the electronic states have a role in the excitation of the Ln(III) ion complexes. The excited intra ligand energy states are the singlet, triplet and an intraligand charge transfer (ILCT) states. These states may correspond either to the $\pi\text{-}\pi^*$ or $n\text{-}\pi^*$ transitions. The $\pi\text{-}\pi^*$ transition is the excitation of the electron from the lower π -orbital to an antibonding one and $n\text{-}\pi^*$ transition the excitation of the electron from the orbital with a free electron pair to an antibonding π -orbital. Generally, the energies of the $n\text{-}\pi^*$ transitions are lower than those of the $\pi\text{-}\pi^*$ transitions and their absorption bands are at higher wavelengths. In principle, the electric field of Ln(III) ions affects the absorption spectra of the ligands but the effect is not usually strong. The large changes in the absorption spectra during the complexation indicates a short distance between the ligand and Ln(III) ion. In some cases, the complexation has the extremely strong effect on the absorption spectrum of the ligand, which is discussed on 'Results and discussion' section. The triplet state, which can be formed via ISC (intersystem crossing) from the excited singlet state, is lower in energy than the feeding singlet state. ISC usually requires the presence of either a heavy or paramagnetic atom close to (in electronic contact) the chromophore. The heavy atoms promote ISC by breaking the selection rule $\Delta S = 0$ because the quantum numbers S and L became ill-quantified. The Ln(III) ions are both heavy and paramagnetic and the organic chromophores are not expected to be luminescent in the Ln(III) complexes. The paramagnetic atoms generate the inhomogeneous magnetic field which causes the mixing of the singlet and triplet states of the ligand. However, the paramagnetic atoms have the more important effect on ISC via the exchange of the electrons between the ligand and metal. [91] The triplet state is efficiently quenched in solution at room temperature (RT) by O_2 [92] unless the energy transfer to the Ln(III) ion is faster than the quenching rate. The ILCT state is normally connected to the $n\text{-}\pi^*$ transition. If the Ln(III) ion is easily reducible (Eu(III) or Sm(III)) a LMCT (ligand-to-metal charge transfer) state may form instead of the ILCT state.

1.4 Luminescence of lanthanoid ions complexes

As mentioned before, the molar absorption coefficients of the Ln(III) ions are very low but this drawback can be overcome by using the organic chromophores. The organic part of the complexes harvests light and the energy absorbed is transferred to the central ion. The luminescence is characterized by the excitation and emission spectra, emission lifetime(s) and quantum yield. The excitation spectra usually resemble the absorption spectra unless several chromophores contribute to the latter, for example absorption due to different ligands and/or both the singlet and ILCT absorption bands. The emission spectra of the lanthanide ion complexes show the sharp emission lines due to the shielded $4f$ -transitions. If measured without delay, the emission spectrum may show also the bands of the ligands due to incomplete complexation and/or to the incomplete quenching of the ligands by the Ln(III) ion induced ISC. The luminescence lifetime implies how long is the average delay between the absorption and emission of the chromophore, that is, is the average lifetime of the excited state. The quantum yield is expressed by

$$\phi = \frac{\text{number of emitted photons}}{\text{number of absorbed photons}}. \quad (5)$$

The diluted solutions should be used in the quantum yield determination in order to avoid the inner filter effect. There exists two kind of inner filter effects: i) exciting light can be absorbed before achieving the sample molecules or ii) the emitted light may be absorbed by the other molecules. The Stokes shifts of the lanthanoid complexes are large and the molar absorption coefficient of the Ln(III) ions are low so only the effect i) needs to be considered in lanthanoid chelate solutions.

The efficiency of light production by the lanthanoid complexes can be expressed by the product of the molar extinction coefficient, ϵ , and the quantum yield, Φ . This product, $\epsilon\Phi$, called brightness, describes the combined efficiency of the complexes to harvest and produce light.

1.4.1 Photosensitization of luminescence of lanthanoid complexes

Depending on the chelate, the excitation energy may be transferred from a chromophore to the Ln(III) ion by different mechanisms and pathways. The possible mechanisms are a multipolar [93], exchange [94] and step-by-step mechanisms [95]. The energy may be transferred directly from the singlet state [59,96] or the charge transfer (ILCT an LMCT) states [58,86,97], or via the triplet state [98–102].

1.4.1.1 Energy transfer pathways

Figure 5 shows the schematic representation of the energy flow in lanthanoid complexes.[103] The light energy absorbed by a ligand excites it to some vibrational level of the excited singlet state, $^1S_x^*$. The excited state ($^1S_{2,3...}^*$) relaxes rapidly (within $10^{-12} - 10^{-14}$ s) to the lowest vibrational level $^1S_1^*$ by the non-radiative vibrational mechanism.[104] In the lanthanoid complexes, the $^1S_1^*$ state may either relax by the radiative or non-radiative routes directly to the ground state or the energy may be transferred to the lanthanoid ion. The energy transfer may occur either directly from the singlet state or via triplet state, $^3T^*$. In the absence of energy transfer, $^1S_1^*$ relaxes to some vibrational levels of the ground state, 1S_0 , and is relaxed to the zero vibrational level via the thermal relaxation. The energy transfer is generally assumed to take place via the triplet state, but recent studies suggest that the routes involving the ILCT or LMCT states might also be possible. Figure 5 does not show the energy back transfer processes, but the energy may back-transfer from the lanthanoid ion to the triplet and LMCT states of the ligands.

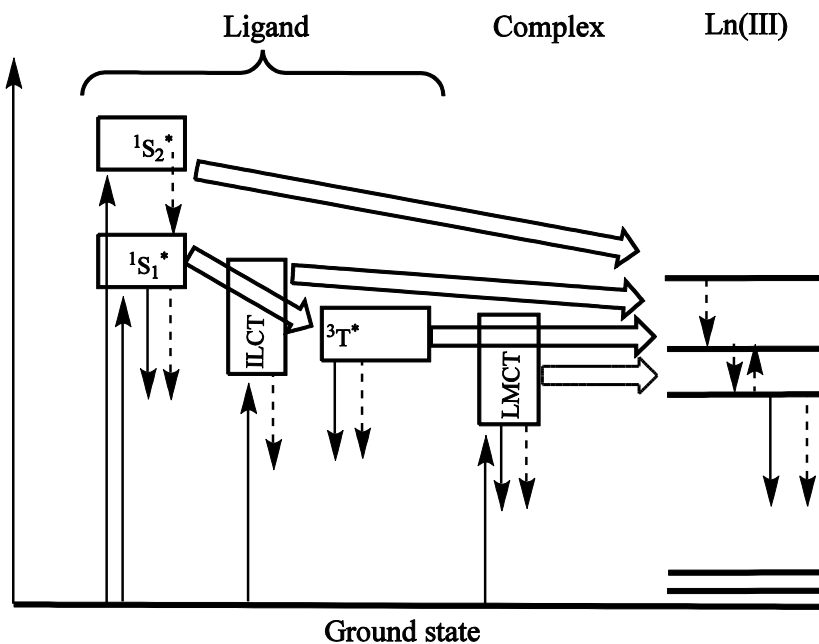


Figure 5. Schematic representation of energy flow in lanthanide complexes. Absorption and emission are shown with plain arrows and non-radiative energy dissipation with dashed arrows. Energy transfer are shown with bold arrows and back transfer processes are not drawn for the sake of clarify. $^1S_x^*$ = singlet state, $^3T^*$ = triplet state, ILCT = intra-ligand charge transfer state and LMCT = ligand-to-metal charge transfer state.[29]

1.4.1.1.1 Energy transfer involving ligand triplet state

The heavy and paramagnetic lanthanoid ions strongly enhance ISC in the ligand, and it can be assumed that the triplet state has a central role in the energy transfer processes in the lanthanoid complexes. This energy transfer route was discovered shortly after lanthanide luminescence was observed [8,98,100] and have been further studied later [101,105,106]. Crosby *et.al.* noticed that the lanthanoid complexes luminesce when the energies of the triplet states of the ligands are above the emitting level of Ln(III) ions but not if the energy is below the emitting level. Thus, they concluded that the energy transfer is triplet mediated.[8,98,100] Bhaumik and El-Sayed showed that if the lowest triplet level of EuHFA (HFA = hexafluoroacetylacetone) is excited by triplet-to-triplet intermolecular energy transfer from another donor, like benzophenone, the energy can be transferred to the lanthanoid ion excluding the energy transfer directly from the singlet state.[105] Sato and Wada [101] showed the participation of the triplet state in the energy transfer process of the lanthanoid complexes by proving that the excitation energy from the lanthanoid ion may thermally back-transfer to the triplet state. Latva *et.al.* [106] showed that the quantum yields of the complexes are dependent on the energy levels of the triplet states: the energy transfer is most efficient when the triplet state is energetically aptly above the excited energy levels of the lanthanoid ions. The triplet state should not to be too high or too close to the receiving state of Ln(III) ion because the feeding and receiving energy states must be in resonant levels but the energy back-transfer should not exist. Steemers *et.al.* [107] have pointed out that using the triplet mediated energy transfer the high-wavelength limit for the excitation maximum is 385 nm for Eu(III).

1.4.1.1.2 Energy transfer involving ligand singlet state

In 1969, the direct energy transfer from the ligand singlet state to Ln(III) was suggested by M. Kleinerman.[108] According to him, the direct energy transfer from the singlet state is predominant in many cases and the triplet state mainly acts as a quencher only. He argued proposition by the fact that the energy transfer from the singlet state (rate constant is of the order of 10^{11} s^{-1}) is usually faster than that of ISC (of the order of 10^9 s^{-1} [92]). C. Yang *et.al.* proposed in 2004 that the Eu(III) ion in a visible light excitable ternary β -diketone complex could be excited directly from the singlet state.[59] They based their conclusion on the fact that the ligand fluorescence lifetime (1,8 ns) was equal to the rise time of the $^5\text{D}_1$ of Eu(III) and that the ligand phosphorescence lifetime was six times longer than that of the $^5\text{D}_0$ state of Eu(III). However, in 2010, they re-explained the results so that the energy transfers via the CT state and the lifetime of 1,8 ns represents the energy back-transfer from the CT state to the ligand.[58]

1.4.1.1.3 Energy transfer involving charge transfer states

The discussion in literature about the CT state mediated energy transfer in the lanthanoid complexes is focused mainly on the ILCT and LMCT states.[58,97,109–112] In special cases, also metal-to-ligand CT (MLCT) and stacking-induced CT (SICT) [113] states can be taken into account but they were excluded from this thesis. According to the literature, both the ILCT [58,111] and LMCT states [112,113] can act as the energy donating or accepting states in the energy transfer in the lanthanoid complexes.

1.4.1.2 Energy transfer mechanisms

Generally accepted energy transfer mechanisms are Dexter's electron exchange [94] and Förster's multipolar [93] type mechanisms (see Figure 6 which shows very simplified models but they apply the presentation of the energy transfer mechanisms).

A newer, but not widely accepted, energy transfer mechanism is Horrocks' stepwise electron transfer mechanism (Figure 7).[95]

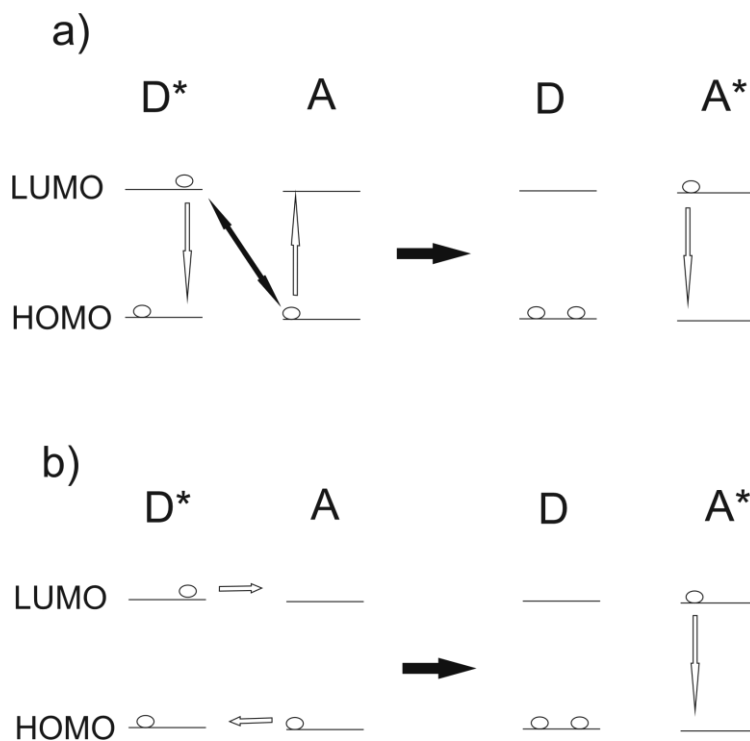


Figure 6. Schematic presentations for multipolar a) and exchange b) energy transfer mechanisms. D = donor, A = acceptor.

In the exchange mechanism, the simultaneous electron transfers take place between LUMO:s (lowest unoccupied molecular orbital) and HOMO:s (highest occupied molecular orbital) of the donor and acceptor. In the case of lanthanoid complexes, both the excited singlet and triplet states (and also the ILCT state) of the ligands are shown as LUMO in Figure 6 and LUMO of the acceptor represents the receiving state of the Ln(III) ion. The rate equation for the exchange mechanism is [94]

$$k_T = \left(\frac{2\pi}{\hbar}\right) K^2 e^{\left(-\frac{2r}{L}\right)} \int F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda, \quad (6)$$

where \hbar is Dirac constant, K is a constant independent of the spectroscopic data, r is the distance between the donor and acceptor, L is Bohr radius, $F_D(\lambda)$ is the normalized (to total area = 1) emission spectrum of the donor at a wavelength range (λ to $\lambda + \Delta\lambda$) and $\varepsilon_A(\lambda)$ is the extinction coefficient of the acceptor at wavelength λ . This equation shows that the energy transfer rate for this mechanism is exponentially dependent on the distance between the donor and acceptor and is directly proportional to the overlap of the emission spectrum of the donor and the absorption spectrum of the acceptor. The effective energy transfer distance with the exchange mechanism is $< 10 \text{ \AA}$.

In the multipolar mechanism, the electrons in LUMO of the donor and HOMO of the acceptor interact coulombically resulting the electronic excitation of the Ln(III) ion. The rate equation for the multipolar mechanism is [93]

$$k_T = \frac{Q_D \kappa^2}{\tau_D r^6} \left(\frac{9000(\ln 10)}{128\pi^2 N_A n_r^4} \right) \int F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda, \quad (7)$$

where Q_D is a quantum yield of the donor in the absence of the acceptor, n_r is the refractive index of the medium, N_A is Avogadro's constant, r is the distance between the donor and acceptor, τ_D is the luminescence lifetime of the donor in the absence of the acceptor, $F_D(\lambda)$ and $\varepsilon_A(\lambda)$ are as above and κ^2 is an orientational factor. Again, the rate equation (7) shows the direct dependency of the rate constant on overlap of the emission spectrum of the donor and the absorption spectrum of acceptor. The energy transfer distance with the multipolar mechanism is $< 100 \text{ \AA}$.

According to the theoretical treatment by De Sá *et.al.* [114], the strongest candidates for the electron transfer with the multipolar mechanism in the Eu(III) complexes are the energy states 5D_2 , 5L_6 , 5G_6 and 5D_4 of Eu(III) and with the exchange mechanism the state 5D_1 . The direct excitation of 5D_0 is forbidden in both mechanisms. In the case of the Tb(III) complexes, the favored mechanism in the energy transfer involving the 5D_4 and 5D_3 states of Tb(III) is the multipolar mechanism.

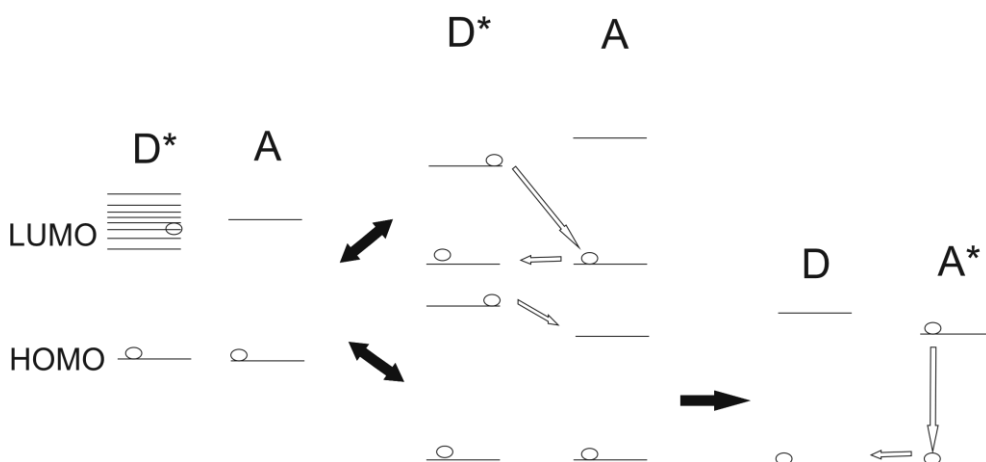


Figure 7. Schematic presentations for stepwise energy transfer mechanism. D = donor, A = acceptor.

Figure 7 tries to show the stepwise energy transfer mechanism. In this case, LUMO of the donor represents the ILCT state and the state involving the energy transfer is the LMCT state. Depending on the relative positions of LUMOs of the donor and acceptor, the electron from LUMO may be transferred either to HOMO (the upper route in Figure 7) or LUMO (the lower route) of the acceptor. The upper route represents the PET (photoinduced electron transfer) process and demands the low-lying ILCT state of the chromophore. In the upper case, the electron transfers from LUMO of the donor and HOMO of the acceptor must be quite simultaneous, and thus, the acceptor (Ln(III) ion) drops only formally to an oxidation state II. In the lower case, the electron from HOMO of the acceptor must be transferred before the electron relaxation from LUMO. In that case, the process leads to the reduction of the acceptor.

1.4.2 Energy dissipation in lanthanoid ion complexes

Commonly, distances in the Ln(III) complexes are so short that the energy transfer is the efficient process and the energy transfer is connected mainly to the shifting of the excitation maximum. Energy dissipation of the excited Ln(III) ion is the most important factor in determining the luminescence quantum yields of lanthanoid complexes. In this context, the fluorescence of the ligands can also be regarded as the energy dissipation. Usually, the ligand fluorescence is effectively quenched by the energy transfer to the Ln(III) ion and its observation implies incomplete complexation. However, the quenching of the ligand fluorescence of the Ln(III)

complexes is not always complete. Thus, the most significant energy dissipation processes in the Ln(III) complexes are the multiphonon quenching of the Ln(III) ion, the PET quenching and the energy back-transfer from the Ln(III) ion to the ligand. The former case has been discussed previously (chapter 1.2.1.2.3) but two other processes require further discussion.

PET usually takes place when the ligand with an amino group is conjugated to the vicinity of Eu(III) (or Sm(III)). The phenomenon is enhanced if a twisted molecular conformation is possible. If the free electron pair of the amino group is not directly conjugated to the vicinity of the Eu(III) PET is not possible. Apparently only amino group has an electron donating ability strong enough to lead to the PET process.[92]

The energy back-transfer can take place via the same routes and mechanisms as the excitation of the Ln(III) ion if the energy levels are close enough. The demanded condition is that the energy states are in appropriate level in relation to each other. The equation for the rate of the energy back-transfer is [115]

$$\tau^{-1} = k = k_0 + k' \exp(-\Delta E/k_B T). \quad (8)$$

Here k_0 is the decay rate constant at the low temperature limit, k' is the pre-exponential factor, ΔE the energy difference between the feeding and receiving states, and k_B is the Boltzmann's constant ($0,69503 \text{ cm}^{-1}/\text{K}$). This equation can be applied to the experimental determination of the energy difference between the feeding and receiving states.

2 Aim of Thesis

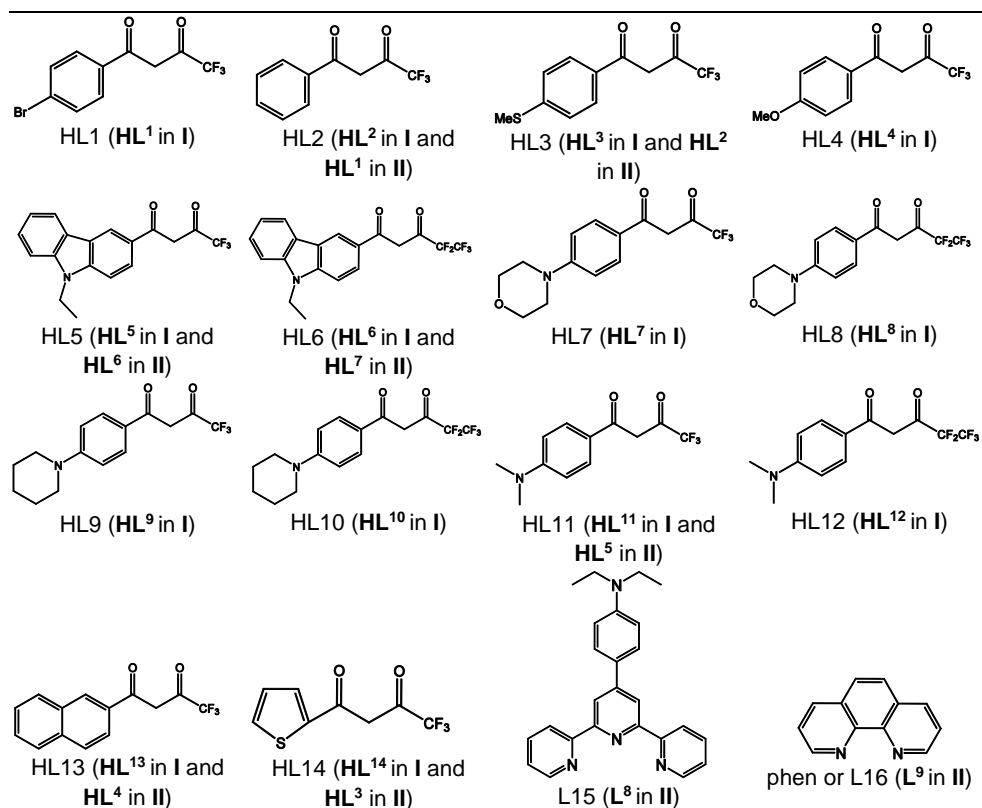
The luminescence of the lanthanoid complexes is an excessively studied topic (Google.scholar search gave 75 800 results for ‘Luminescence of lanthanide complex’) and plenty of bright complexes for different applications have been developed. However, there is still a need for new chelates that are suitable for new applications. Especially, the extension of the excitation wavelength towards longer wavelengths in the visible range is required for some applications. A few chelates suitable to the visible light excitation have been developed but they are often not stable in aqueous solutions. The rational development of chelates requires good knowledge of the fundamental processes involved in the lanthanoid luminescence, particularly understanding of the energy transfer processes, of which more detailed information is needed. The main aim of this thesis is to shed light on the energy transfer processes in the lanthanoid chelates. In addition, each publication included in the thesis have aim to produce the results of practical value for the different applications. Specified by publications, these are

- I. the study of new β -diketones for various applications,
- II. the incorporation of the visible light excitable ternary β -diketone chelates into polystyrene nanoparticles for bioimaging,
- III. the study of possible dipicolinic acid derivative candidates for Eu(III) in the homogenous polymerase chain reaction assay and
- IV. the study of the Tb(III) complexes of the dipicolinic acid derivatives as possible candidates for the homogenous polymerase chain reaction assay.

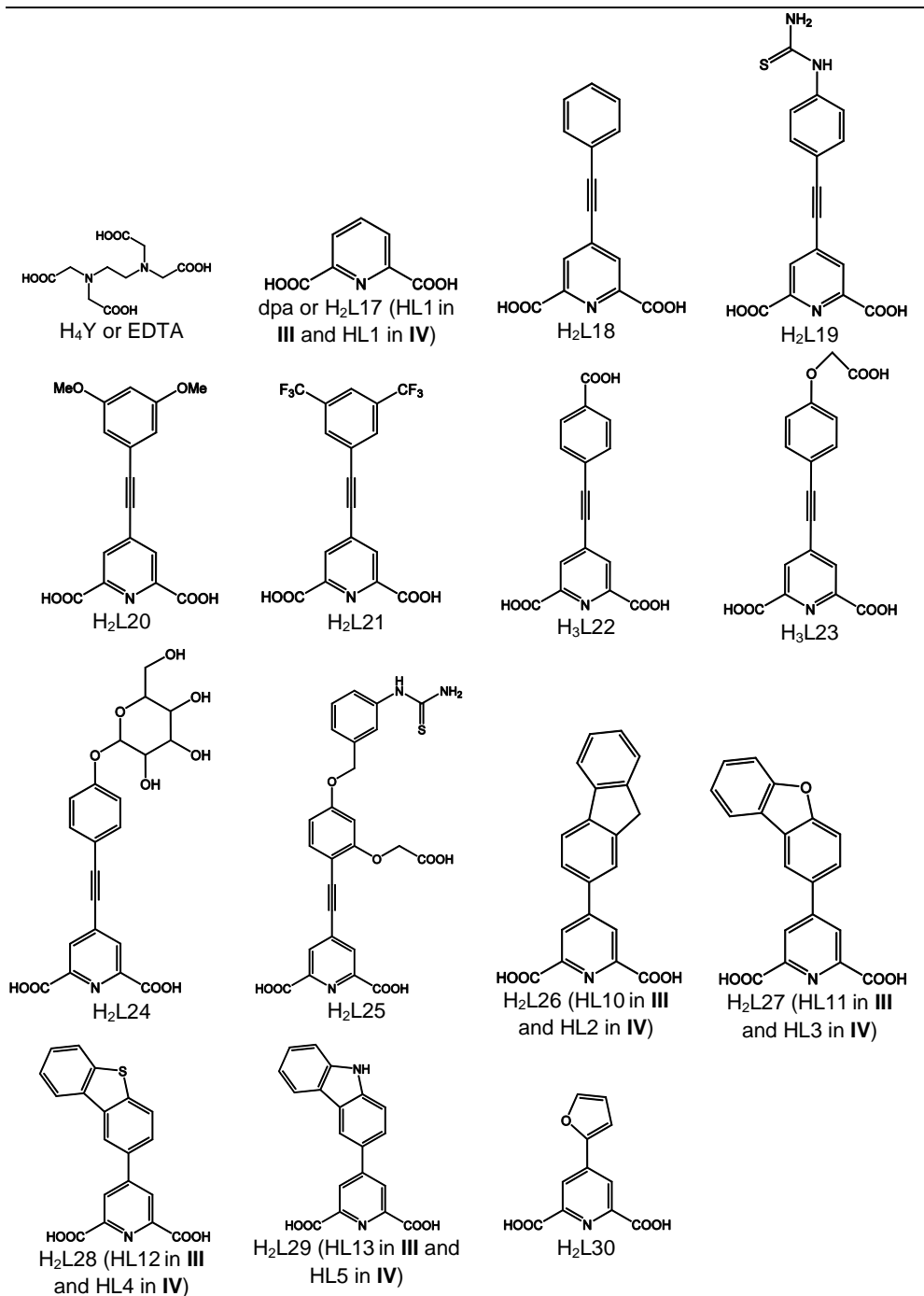
3 Materials and Methods

3.1 Materials

The materials used have been specified in the corresponding papers **I – IV**. Schemes 1 and 2 show the studied ligands.



Scheme 1. Structures and abbreviations of studied β -diketone and Lewis base ligands (in brackets abbreviations used in the corresponding papers **I** or **II**). Synthesis in paper **I**.



Scheme 2. Structures and abbreviations of studied dipicolinic acids and EDTA (in brackets abbreviations used in the corresponding papers **III** or **IV**). Synthesis in paper **III**.

The β -diketone complexes were prepared using the following procedures, depending whether the ligand contains a freely rotating amino group (FRAG):

General procedure for the preparation of FRAG complexes

An aqueous solution (1 ml) of EuCl_3 (0,1 mmol) was added into a methanol solution (5 ml) of β -diketone (0,3 mmol). After stirring for 10 minutes at RT, the pH was adjusted to 6,5 – 7,0 by 30 % aq. ammonia. After stirring overnight at RT, the product was centrifuged and dried under vacuum at 100 °C.

General procedure for the preparation of non-FRAG complexes

An aqueous solution (1 ml) of EuCl_3 (0,1 mmol) was added into a methanol solution (5 ml) of β -diketone (0,3 mmol) followed by an addition of N,N-diisopropylethylamine (0,9 mmol). After overnight stirring at RT, the solution was evaporated by an argon flow. The precipitate was washed with water, dried first in desiccator and then under vacuum at 100 °C with the exception of $\text{Eu}(\text{L}^{13})_3$ and $\text{Eu}(\text{L}^1)_3$ which did not tolerate this temperature.

The dipicolinic acids complexes were prepared directly by mixing the components in the aqueous solutions. Water used for solutions was distilled twice in quartz vessels after reverse osmosis.

3.2 Methods

Mainly absorption and luminescence spectroscopy were used in this work. An attempt was made to use thermal analysis for the determination of the water content of the β -diketone complexes but it was unsuccessful because of the volatility of the fluorinated β -diketone complexes [71]. The use of the mass spectroscopy (MALDI-TOF) for the same purpose also failed. The used apparatus are explained in the corresponding papers **I – IV**.

The triplet state energies (measured using the Gd(III) complexes at 77 K), corresponding to the 0-0 transition, were taken from the maxima of the lowest bands of the measured phosphorescence emission spectra. The phosphorescence measurements were made in HEPES:glycerol solutions (3:2 vol-%) ($C_{\text{HEPES}} = 10 \text{ mM}$ in water, pH 8) with $C_{\text{complex}} = 10 - 45 \text{ }\mu\text{M}$.

The concentrations of the Eu(III) and Tb(III) solutions were determined by EDTA titration using xylenol orange as the indicator.[116]

The concentrations of the measurement solutions were usually 15 μM unless otherwise specified in order to avoid the inner filter effect.

Special precaution was taken to avoid water in ACN (acetonitrile) in paper **I** because ACN is very hydroscopic. Neat ACN was used and all glassware was dried in oven and the solutions were made under argon atmosphere.

The most used software in the study was Origin (OriginLab, U.S.A).

3.2.1 Luminescence quantum yield determinations

The quantum yields (Φ) were determined using the equation (9).[29,117]

$$\Phi = \Phi_s \frac{E_x(1-10^{-A_s(\lambda_{ex})})n_x^2}{E_s(1-10^{-A_x(\lambda_{ex})})n_s^2}. \quad (9)$$

Here the subscripts S and x refer to the standard and the sample, E_i is the integrated area of the emission spectrum, $A_i(\lambda_{ex})$ is the absorbance at the excitation wavelength and n_i the refractive index. The standard was the $7,5 \times 10^{-5}$ M solution of a complex of Eu(III) with DPA, $[\text{Eu}(\text{DPA})_3]^{3-}$, in TRIS buffer for which $\Phi = 0,24$. [29] All the measurements were performed using the same measurement settings in the luminometer and the luminescence spectra used in the determinations were corrected. In paper **IV**, instead of quantum yields the relative intensities were used, which were calculated using the intensities from the frequency modulation measurements. The luminometer (Varian Cary Eclipse) allowed the measurements only with the integration time of 0 – 40 μs or 0,100 – ... ms. For this reason, the lifetime of sample should be near to that of the standard.

3.2.2 Luminescence lifetime measurements

Both the time and frequency domain methods were used for the luminescence lifetime measurements. The time domain method is based on the measurement of the luminescence intensity decay after the excitation pulse whereas the frequency domain method relies on the phase shift of the modulated signals of the luminescence excitation and emission. Further information of the frequency domain method can be found in ref.[92] The frequency domain method was not conventional but it was based on either the out-of-phase or in-phase signals of the lock-in-amplifier. The instrumental details and the theory are presented in the literature[118,119] and only a short introduction is given here.

These frequency domain measurements were performed using a lock-in-amplifier. It generates the modulated ac signal used for the excitation of the sample. The excitation and reference signals are identical, and the lock-in-amplifier compares the phase of the reference signal to that of the response signal from the photomultiplier tube. Due to the delay caused by the luminescence lifetime the

emission signal has a phase shift θ with respect to the excitation signal, and can be expressed by

$$S_{in}(t) = A \sin(\omega t + \theta), \quad (10)$$

where the angular frequency ω is given by frequency f

$$\omega = 2\pi f. \quad (11)$$

The reference signal is either

$$S_{ref}(t) = B \sin \omega t \text{ or } C_{ref}(t) = B \cos \omega t. \quad (12 \text{ and } 13)$$

The lock-in amplifier electronically multiplies the excitation and response signals. By using the trigonometric formulae we obtain

$$S_{in}(t)S_{ref}(t) = AB \sin(\omega t + \theta) \sin \omega t = \frac{1}{2}AB \cos \theta - \frac{1}{2}AB \cos(2\omega t + \theta) \quad (14)$$

and

$$S_{in}(t)C_{ref}(t) = AB \sin(\omega t + \theta) \cos \omega t = \frac{1}{2}AB \sin \theta - \frac{1}{2}AB \sin(2\omega t + \theta). \quad (15)$$

The main components of the lock-in-amplifier are a multiplier and low-pass filter. Depending on the cutting frequency of the low-pass filter, it removes more or less completely the time-dependent part of the signal, and thus, the lock-in-amplifier produces the signals

$$S_x = \frac{1}{\sqrt{2}} A \cos \theta \quad (16)$$

and

$$S_y = \frac{1}{\sqrt{2}} A \sin \theta. \quad (17)$$

Here S_x is the *in-phase* and S_y the *out-of-phase* signal.

Any harmonic signal of frequency ω can be described by

$$S(\omega, t) = B e^{i(\omega t + \theta)} = B e^{i\theta} e^{i\omega t} = B(\cos \theta + i \sin \theta) e^{i\omega t}. \quad (18)$$

In many cases the signal after some mathematical procedures is given in the form

$$S = (x + iy)e^{i\omega t}. \quad (19)$$

Comparing with (18), we have equations

$$x = A_x = B \cos \theta \text{ and } y = A_y = B \sin \theta \text{ (20 and 21)}$$

which gives

$$A_x^2 + A_y^2 = B^2 \cos^2 \theta + B^2 \sin^2 \theta = B^2 \quad (22)$$

and

$$\frac{A_y}{A_x} = \frac{B \sin \theta}{B \cos \theta} = \tan \theta. \quad (23)$$

Hence, inserting (22) in (18)

$$S = \sqrt{A_x^2 + A_y^2} (\cos \theta + i \sin \theta) e^{i\omega t}. \quad (24)$$

In our case, for luminescence with a single time constant[118]

$$S = \frac{h}{k+i\omega} e^{i\omega t} = \frac{h\tau}{1+i\omega\tau} e^{i\omega t} = \frac{h\tau(1-i\omega\tau)}{1+\omega^2\tau^2} e^{i\omega t}. \quad (25)$$

Here τ is the luminescence lifetime. Thus, when inserting (25) in (24) we obtain:

$$A_x = \frac{h\tau}{1+\omega^2\tau^2}, \quad (26)$$

$$A_y = -\frac{\omega h\tau^2}{1+\omega^2\tau^2}, \quad (27)$$

$$\sqrt{A_x^2 + A_y^2} = \frac{h\tau}{\sqrt{1+\omega^2\tau^2}} \quad (28)$$

and

$$\frac{A_y}{A_x} = -\omega\tau = \tan \theta. \quad (29)$$

Thus, using trigonometric formulae, for the *out-of-phase* signal (17)

$$S_y = \frac{1}{\sqrt{2}}A \sin \theta = \frac{1}{\sqrt{2}}A \sin(\arctan(-\omega\tau)) = -\left(\frac{1}{\sqrt{2}}A\right) \frac{\omega\tau}{\sqrt{1+\omega^2\tau^2}} = C \frac{2\pi f\tau^2}{1+(2\pi f)^2\tau^2}. \quad (30)$$

In this study, only the *out-of-phase* signal was used. The *out-of-phase* signal reaches the minimum at $f = (2\pi\tau)^{-1}$ (C is negative), and conversely, the luminescence lifetime can be determined from the minimum according to $\tau = (2\pi f_{min})^{-1}$. Hence $\theta = 45^\circ$. The frequency region of the lock-in-amplifier (10 – 100 000 Hz) allowed the luminescence lifetime measurements between 1 μ s – 20 ms which is appropriate for the luminescence lifetimes measurements of the Eu(III) and Tb(III) complexes.

The dependence of the luminescence lifetimes on temperature was measured in the papers **I** and **IV**. The fitting of the equation (8) to the τ vs. T data gave the ΔE value which represents the energy difference between the feeding/receiving energy state of the ligand and the emissive state of Ln(III). This information is valuable for the discussion of the energy transfer. The Peltier element enabled to use the temperature region from ~ 5 to 55 $^\circ$ C. This is an appropriate temperature region for the ΔE value over 1000 cm^{-1} .

Additional information about the energy transfer can be obtained by measuring the luminescence lifetimes before and after the argon treatment. This was done by deaerating the solutions for 20 min with argon. The molecular oxygen is an efficient triplet quencher and this method allows to study the participation of the triplet state in the energy transfer. This method is used in the paper **IV**.

The luminescence lifetimes can also be used for the determination of the number of complexed water molecules. The equation [120]

$$q = A(\tau_{H_2O}^{-1} - \tau_{D_2O}^{-1} - \beta) \quad (31)$$

where τ_i are the luminescence lifetimes in the presence ($i = \text{H}_2\text{O}$) and absence ($i = \text{D}_2\text{O}$) of the complexed water molecules and A is 1.11 and β 0.31 for Eu(III) [120] allows the determination of the number of coordinated water molecules, q . This was used in the papers **I** and **II**.

4 Results and Discussion

The main focus of thesis, the energy transfer in the lanthanoid complexes, is discussed throughout the whole chapter but the more application related special results are found in subchapters dealing with the corresponding papers.

4.1 Energy transfer in lanthanoid complexes

The articles (I – IV) shed a new light on the energy transfer processes in the lanthanoid complexes. Table 4 from I shows the photophysical properties of complexes Eu(L1)₃ – Eu(L14)₃.

Table 4. Photophysical properties and σ_p^+ substituent constants of the chelates Eu(L1)₃ – Eu(L14)₃ (1 μ M in ACN).

COMPLEX	σ_p^+ ^a	λ_{\max}^{abs} (nm)	λ_{\max}^{ex} (nm)	ϵ_{\max} (M ⁻¹ cm ⁻¹)	Φ (%)	E _t (cm ⁻¹)
Eu(L1) ₃	0,025	326	325	37 500	32	21 000
Eu(L2) ₃	0	320	320	35 300	25	21 800
Eu(L3) ₃	-0,164	340	340	63 000	28	21 600
Eu(L4) ₃	-0,648	327	326	55 600	30	21 800
Eu(L5) ₃	-	345	346	63 300	21	21 400
Eu(L6) ₃	-	346	346	70 400	47	21 300
Eu(L7) ₃	-	354	357	68 200	8,9	20 000
Eu(L8) ₃	-	358	357	55 300	13	20 000
Eu(L9) ₃	-	368	369	88 400	3,0	19 400
Eu(L10) ₃	-	374	367	73 200	13	19 400
Eu(L11) ₃	-1,7	368	375	90 400	7,2	19 400
Eu(L12) ₃	-1,7	369	374	86 800	12	19 500
Eu(L13) ₃	-	329	330	44 600	42	19 900
EU(L14) ₃	-	337	337	42 900	40	20 400

^a From ref. [121]

Table 4 shows the complexes in the order of the increasing electron donating ability of the *p*-substituent in the ligand. This is presented by the substituent constant σ_{P^+} if found, otherwise the electron donating ability is evaluated on the basicity of the amino group. The substituent constant σ_{P^+} represents a situation where the substituent is directly conjugated with a reaction center in an electron demanding transition state. Table 4 shows that the quantum yields of the complexes Eu(L2)₃ – Eu(L4)₃ and Eu(L6)₃ follow the electron donating ability of the substituent rather than the triplet state energy although the triplet states are at the appropriate levels for an efficient energy transfer. Because only one luminescence lifetime was observed for these complexes (see paper I) their dissociation can be neglected. According to Latva *et.al.*, the ligand triplet state must be 1500 cm⁻¹ above the receiving state of the Ln(III) ion in order to avoid the thermal quenching of the Ln(III) ion via the energy back-transfer to the ligand triplet state.[102] The receiving state of Eu(III), ⁵D₁, is at 19 000 cm⁻¹ [22] which means that the energy of the ligand triplet state must be > 20 500 cm⁻¹ for good quantum yield. However, the ligand triplet state must also be close enough to the receiving state of the Ln(III) ion. The energy transfer to the emissive state of Eu(III), ⁵D₀ at 17 270, is not possible.[114] Thus, the triplet state energy levels of the complexes Eu(L1)₃ – Eu(L6)₃ are ideal for a high quantum yield but the quantum yield seems to follow the electron donating ability of the *p*-substituent, that is, the energy of the ILCT state.

Paper IV shows that within similar ligand structures, the electron donating ability of the *p*-substituent correlates with the energy of the ligand ILCT state. The deviation of Eu(L1)₃ from the general quantum yield order is understandable because the electron withdrawing capability of the bromo substituent is questionable. This can be seen in the fact that the molar absorptivity of Eu(L1)₃ is higher than that of Eu(L2)₃. The surprisingly low quantum yield of Eu(L5)₃ is difficult to explain if the quantum yield of Eu(L6)₃ is not higher than can be expected. Thus, in the Eu(III) complexes, the quantum yield is more strongly dependent on the ILCT than the triplet state provided that the energy of the triplet state is not too low. This means that the ILCT state is the favored energy transfer route in the Eu(III) (and probably also in the Sm(III) in which the stepwise energy transfer also possible) complexes. This may be attributed to the involvement of the proposed stepwise mechanism of energy transfer [95], which may increase its efficiency. In general, the ILCT state is the singlet state because it often corresponds to a n-π* transition (it is electronic transition from the free electron pair (of nitrogen) to π*-orbital) and the energy transfer from it may be faster than from the triplet state also with other mechanisms (the energy transfer from the singlet state may faster than ISC as can be seen later).

The ligand/complex series in paper I shows nicely the effect of the amino group: when the amino substituent is directly conjugated to the ligand structure in the vicinity of Eu(III) ion and is freely rotating, it effectively quenches the luminescence

(via PET) (as in cases of $\text{Eu}(\text{L}7)_3$ – $\text{Eu}(\text{L}12)_3$) but if the rotation is hindered quenching does not take place even if the group were very close to the $\text{Eu}(\text{III})$ ion (as in cases of $\text{Eu}(\text{L}5)_3$ and $\text{Eu}(\text{L}6)_3$). However, the huge difference in the quantum yields between $\text{Eu}(\text{L}5)_3$ and $\text{Eu}(\text{L}6)_3$ demands an explanation. It could be thought that the CF_2CF_3 group is so strong an electron donator that it hinders the formation of the ILCT state (increase the energy of the ILCT state) but, in fact, it is an electron acceptor. The σ_P^+ values for the CF_3 and CF_2CF_3 groups are 0,54 and 0,52 [122], respectively, and the CF_3 group might lower the energy of the ILCT state because it is a stronger electron attractor. In addition, it was shown in paper **I** that the complexation partly prevents the ILCT state formation. This must be connected to the rigidity of the ligand bound to $\text{Ln}(\text{III})$ but its effect on the observed properties is not fully understood.

Paper **II** shows that the quantum yields in the series of the ternary complexes $\text{Eu}(\text{L}x)_3\text{L}15$ ($x = 2, 4 - 6, 11, 13, 14$) depend on the excitation wavelength, that is, the quantum yield is dependent on the ligand, either the β -diketone or Lewis' base (L15). This suggests that the energy transfer does not take place between the ligands in the ternary complexes. Apparently the distances between the chromophores are much larger than the distances between the chromophores and the $\text{Eu}(\text{III})$ ion. In addition, the paper shows that the complexation has a remarkable effect on the absorption spectrum of L15 (the excitation maximum redshifted about 30 nm). This kind of large effect on the absorption spectrum by complexation can hardly be explained by the polarization effect of the ligand field of the $\text{Eu}(\text{III})$ [61] but suggests the coplanarization of the pyridine rings of L15 in the ternary complex.

In addition, papers **I** and **II** together show that the tris- β -diketone complexes contain quenching water molecules in toluene but not in ACN although lanthanide ions are “hard” acids and prefer “hard” bases, *e.g.*, oxygen. It seems that in “dry” acetonitrile water molecules are replaced by acetonitrile molecules, which are “soft” bases. Interestingly, Díaz-Torres and Alvarez have determined the coordination ability of solvents toward lanthanides by analyzing the previously published data on their complexes. They found that the coordination ability index a^{Ln} is 0,6 for water, -0,7 for acetonitrile and -1,2 for toluene.[123] In spite of the much higher a^{Ln} value for water, acetonitrile molecules seem to replace the water molecules in neat acetonitrile due to their high concentration.

Table 5 from paper **III** shows the photophysical properties of $\text{Eu}(\text{L}17)_3$ – $\text{Eu}(\text{L}30)_3$. The complexes $\text{EuY-L}26$ – $\text{EuY-L}29$ are shown in the order of the ILCT state energy (the substituent constant order is $\text{Ph} > \text{O} > \text{S} > \text{N}$ [122], that is, the electron donating ability order is $\text{Ph} < \text{O} < \text{S} < \text{N}$) and it can be seen that the quantum yield again follows the order of the ILCT state energy. However, now the amino group of the carbazol quenches the luminescence via PET more strongly than in $\text{Eu}(\text{L}5)_3$ (as compared to $\text{Eu}(\text{L}6)_3$). In this case, the electron receiving part contains

two electron attracting groups (two COOH groups for which $\sigma_P^+ = 0,45$ from ref. [122]) in dpa.

Table 5. The absorption maxima, molar absorption coefficients at maxima, excitation maxima, quantum yields, brightness at excitation maxima and triplet state energy levels of the complexes EuY-Lx in TRIS or NH₃ buffer (EuY-L3 and EuY-L9 in NH₃ buffer). C_{TRIS/NH₃} = 50 mM, pH = 7,75 and emission wavelength 615 nm.

COMPLEX	λ_{max}^{Abs} (nm)	ϵ_{max} (M ⁻¹ cm ⁻¹)	λ_{max}^{Ex} (nm)	Φ (%)	$\epsilon_{Exmax}\Phi$ (M ⁻¹ cm ⁻¹)	E _{Triplet state} (cm ⁻¹)
EuY-L17	271	5280	271	13	690	25 300
EuY-L18	304	15 100	317	23	2600	21 400
EuY-L19	315	20 000	327	12	2100	20 100
EuY-L20	303	20 700	322	25	3200	20 900
EuY-L21	285	20 300	308	21	3000	21 500
EuY-L22	303	21 600	323	21	3000	20 700
EuY-L23	321	20 100	337	23	3500	20 400
EuY-L24	316	16 800	332	24	3200	20 700
EuY-L25	335	10 900	344	19	2000	20 200
EuY-L26	317	14 500	331	14	1600	20 000
EuY-L27	288	24 100	295	18	3700	22 200
EuY-L28	288	30 800	334	26	5800	21 300
EuY-L29	295	19 100	343	13	1500	21 000
EuY-L30	310	16 500	324	28	3700	19 800

Paper **III** introduces a method for the determination of the stability constant by measuring the total luminescence intensity as a function of concentration. Fitting the equation

$$I_{tot} = a \frac{2KC+1-\sqrt{4KC+1}}{2K} \quad (32)$$

to the I_{tot} vs. C data yields the stability constant K for the reaction $LnY + Lx \rightleftharpoons LnY \cdot Lx$. Here a is a proportionality constant that comprises all instrumental factors involved. The stability constant for EuY-L17 was determined with the FD-method to be $\log K = 5,38 \pm 0,16$ (paper **III**) and with the pulse excitation method $5,30 \pm 0,12$ (paper **IV**). These values are reasonable as they are within the range of stability constants previously reported for similar systems.[76–78] In addition, the stability constant of TbY-L17 was found to be $\log \beta = 5,40 \pm 0,34$ which is close to the value

of EuY-L17 (the stability constants of the Tb(III) and Eu(III) complexes of the same ligands can be assumed to be similar). This method was used for the relative luminescence intensity determination of the complex series in the paper IV.

Table 6 from paper IV shows the complex formation and σ_p^+ values for the complexes TbY-Lx (x = 17 and 26 – 29).

Table 6. Substituent constant values (σ_p^+), stability constant ($\log \beta$) for reaction TbY + Lx \rightarrow TbY-Lx (x = 17 and 26 – 29) and calculated fraction of complexed ligand in 25 μ M solutions.

TbY + Lx \rightarrow TbY-Lx	$\log \beta$	COMPLEXED FRACTION IN 25 μ M SOLUTIONS	σ_p^{+a} (value for)
L17	5,40 \pm 0,34	0,68 \pm 0,12	-
L26	4,10 \pm 0,03	0,20 \pm 0,01	-0,18 (Ph)
L27	3,85 \pm 0,27	0,15 \pm 0,07	-0,50 (OPh)
L28	5,55 \pm 0,24	0,70 \pm 0,06	-0,55 (SPh)
L29	4,83 \pm 0,15	0,46 \pm 0,04	-1,40 (NPh)

^a From ref. [122]

Table 7 from paper IV shows the singlet (E_S), ILCT (E_{ILCT}) and triplet state (E_T) energies of the ligands Lx and the I/A_{285nm} values (where I and A_{285nm} are the intensity from the FD curves and the absorbance value at 285 nm, respectively) for the complexes TbY-Lx (x = 17 and 26 – 29). The values of E_S and E_{ILCT} are determined from the deconvoluted absorption and emission spectra.

Table 7. Singlet (E_S), ILCT (E_{ILCT}) and triplet state (E_T) energies of the ligands Lx and I/A_{285nm} values for TbY-Lx (x = 17 and 26 – 29).

(TbY-)Lx	E_S (cm ⁻¹) ^a	E_{ILCT} (cm ⁻¹) ^a	E_T (cm ⁻¹) ^b	I/A_{285nm} ^c
L17	-	-	25 300	1,9
L26	27 700	26 100	20 000	0,16
L27	30 000	26 500	22 200	42
L28	29 200	26 500	21 300	0,30
L29	-	25 200	21 000	0,030

^a From convoluted spectra; ^b From the paper III; ^c Calculated from the absorbance values at 285 nm (A_{285nm} , Figure 1) and emission intensities at 545 nm (I, from FD curves) (The absorbance values were multiplied with the fraction of complexed ligand (Table 6)); - Missing value.

The E_{ILCT} , E_T , and I/A_{285nm} values in Table 7 show that this TbY-L_x ($x = 26 - 29$) series follows the triplet state energy contrary to the corresponding EuY-L_x series. This may be the result of energy back transfer to the triplet state because the receiving (and at the same time the emissive) energy state of the Tb(III) ion is relatively high in energy, at 20 545 cm^{-1} [21]. Thus, only the energy of the triplet state of H₂L27 is high enough to exclude the energy back transfer. Thus, it cannot be concluded on the ground of these results if the energy is transferred mainly via the triplet state or if the energy is only thermally back transferred to the triplet states of the ligands when it is possible. In the case of the Tb(III) complexes, the energy cannot be transferred from the ILCT state via the stepwise mechanism which might make the energy transfer less efficient than in the case of the Eu(III) complexes.

The energy transfer is further studied in the paper IV by measuring the effect of the oxygen removal on the luminescence lifetimes. The results are shown in Table 8.

Table 8. Luminescence lifetimes of 25 μM TbY-L_xs in TRIS (pH = 7,75) before and after argon treatment (solutions passed 20 min with argon flow). The values in brackets are relative intensities. Excitation wavelength was 285 nm and emission wavelength 545 nm.

COMPLEX	IN THE PRESENCE OF O ₂		DEAERATED	
	τ_1 (μs) (I)	τ_2 (μs) (I)	τ_1 (μs) (I)	τ_2 (μs) (I)
TbY-L17	1878 \pm 7 (86)	-	1724 \pm 4 (116)	-
TbY-L26	1449 \pm 11 (165)	4,4 (-43)	1150 \pm 7 (225)	3,9 (-24)
TbY-L27	1359 \pm 13 (341)	765 \pm 11 (209)	982 \pm 5 (59)	262 \pm 8 (9)
TbY-L28	358 \pm 13 (213)	25 \pm 19 (19)	885 \pm 15 (203)	233 \pm 16 (64)
TbY-L29	17 \pm 1 (3)	6,64 \pm 0.05 (25)	37 \pm 3 (4)	11,1 \pm 0,1 (24)

The luminescence of the Ln(III) complexes usually proceeds via the triplet state, and argon treatment would be expected to increase the luminescence lifetimes because O₂ is an efficient triplet quencher. The complex TbY-L17 exhibited only one lifetime, which decreased after argon treatment, although its amplitude slightly increased. This is consistent with the general fact that oxygen does not quench the luminescence of $Tb(L17)_{n(=1-3)}^{+3-2n}$. [124] It suggests that the energy is either transferred directly from the singlet state of L17 to Tb(III), or that the lifetime of the L17 triplet state in the complex is too short for O₂ quenching. Paper IV shows that the singlet energy transfer rate constant between dpa and Tb(III) can be estimated to be of the order of $k > 10^{10} s^{-1}$ and the rate constant of the ISC (inter system crossing) is of the order of $10^{10} s^{-1}$ [92]. Therefore, the direct energy transfer from the singlet state and

the triplet mediated energy transfer routes may compete with each other in the absence of other factors. The determined luminescence lifetime of L17 in the ternary complex deviates from the previously reported value (2250 μ s), measured with Tb(L17)_3 [102]. This is probably due to the symmetry effect of different coordinative environments, additional C-H quenching groups in the vicinity of Tb(III) or fast ligand exchange.[34,57,125]

The luminescence of the complex TbY-L26 exhibited two processes, a decay and rise of the luminescence intensity (a negative amplitude in the FD method can be interpreted to represent a rise time). Because the ligand triplet state is below the emitting $^5\text{D}_4$ state of Tb(III) the observed luminescence lifetime must represent emission via the ILCT state or the temperature driven upstream energy flow from the triplet state, or both. However, in the latter case, the removal of oxygen should increase the luminescence lifetime for long enough lifetimes (above several microseconds).[126] On the contrary, the observed decrease in the lifetime implies that the emissive state excitation must, at least partially, take place from the ILCT state. Therefore, we attribute the observed rise time to the upward energy flow from the triplet state, and suggest that the luminescence of Tb(III) in the TbY-L26 complex is mediated by both the ILCT and triplet state. Because the rise time represents the upward energy flow from the triplet state to the Tb(III) $^5\text{D}_4$ state, it is also the lifetime of the triplet state of L26 in TbY-L26.

Two observed luminescence lifetimes in the TbY-L27 – TbY-L29 complexes are attributed to the ternary TbY-Lx complex (longer τ) and to the intermediates introduced in paper **III** in case of EuY-Lx complexes (shorter τ). The increase of the luminescence lifetimes of TbY-L28 and TbY-L29 after oxygen removal suggests that the energy transfer in these complexes occurs mainly via the triplet state, and the triplet state of the ligands is long-lived enough for the O_2 quenching. On the contrary, oxygen increases the luminescence lifetimes in TbY-L26 and TbY-L27 and this must be connected to the involvement of the ILCT state in the energy transfer. However, the detailed description cannot be derived from these results. The shorter luminescence lifetime (τ_1) of the TbY-L28 complex can be explained by a low-lying triplet state (800 cm^{-1} above the $^5\text{D}_4$ state of Tb(III)) but it is not clear if a decrease of 300 cm^{-1} in the triplet state energy can alone cause the observed large decrease in the luminescence lifetime between complexes TbY-L28 and TbY-L29.

In order to obtain further information about the energy transfer processes, the temperature dependence of the luminescence lifetimes of the complexes were determined in paper **IV**. The measured temperature dependence is shown in Figure 8.

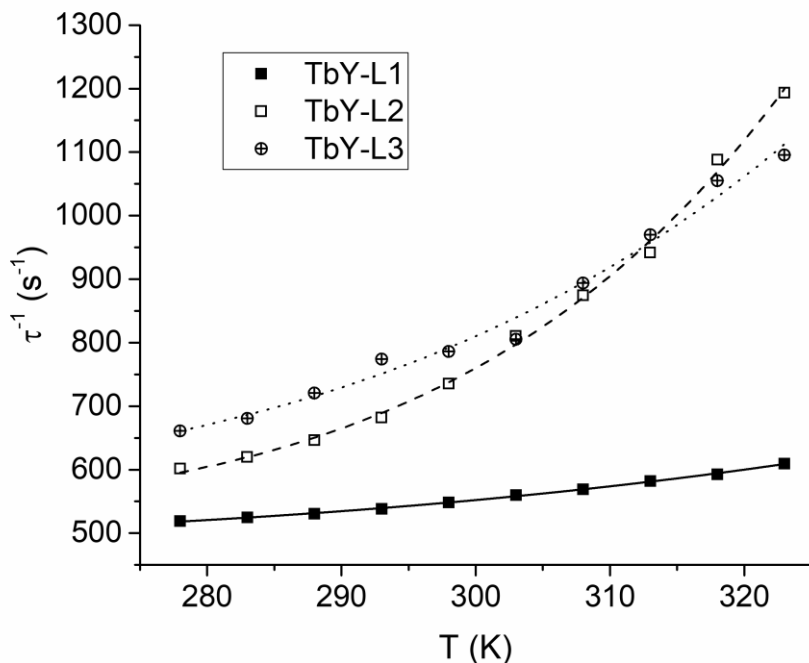


Figure 8. Temperature dependencies of luminescence lifetimes. Filled squares, TbY-L17 (τ); hollow squares, TbY-L26 (τ_1); blue crossed circles, TbY-L27 (τ_1) (all in 25 μM in TRIS at pH 7,75). Lines are the fits to the energy back-transfer equation (8).

The fitted curves in Figure 8 yield $\Delta E = 1731 \pm 141 \text{ cm}^{-1}$ for TbY-L17, $\Delta E = 3046 \pm 246 \text{ cm}^{-1}$ for TbY-L26 and $\Delta E = 2267 \pm 500 \text{ cm}^{-1}$ for TbY-L27. The anomalously low value of ΔE obtained for TbY-L17 would require an explanation, which is, however, beyond the scope of this thesis. Figure 9 shows the luminescence and phosphorescence emission spectra and the energy corresponding to the sum of the energy of the lanthanoid emissive state ($^5\text{D}_4$ for Tb(III)) and the ΔE value obtained from the fit above. Figure 9 a suggests that, in the ternary Tb(III) complex, energy can be transferred back to the emissive state of the ligand L26, and according to the deconvoluted emission spectrum in Figure 9 a, rather the emissive ILCT state than singlet state because it can be assumed that the back-transfer include a “potential” wall. This must mean that energy from the excited Tb(III) ion back-transfers to the ligand faster than the thermal relaxation occurs from the higher vibrational levels of the ground electronic state of the ligand (to which ligand ends up after the energy transfer to Tb(III)) to the zero vibrational level of the ground state. The energy back-transfer must be faster because otherwise the energy back-transfer would demand

that the vibrational level 4480 cm^{-1} ($E_{\text{ILCT}} - E_{\text{ILCT em.max}} = 26100\text{ cm}^{-1} - 22420\text{ cm}^{-1} = 4480\text{ cm}^{-1}$) above the lowest state should be populated at room temperature, which is impossible. The emissive ILCT state is a singlet state but, due to its charge transfer nature, it may increase the efficiency of the energy transfer in the Eu(III) complexes via the stepwise mechanism. However, energy can be transferred between the ligand ILCT state and $^5\text{D}_4$ state of Tb(III) by a multipolar mechanism, and the short distance between the chromophore and Tb(III) in the complex can be assumed to enhance this process.[114]

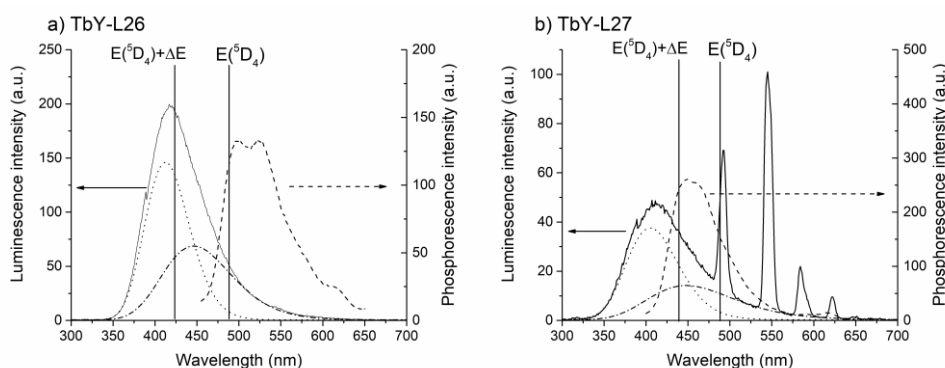


Figure 9. Luminescence spectrum (with deconvolution; singlet emission with dotted line and ILCT emission with dashed-dotted line) of TbY-L26 (solid line) and phosphorescence spectrum of Gd(L26)₃ (dashed line) (a), and those of TbY-L27 and Gd(L27)₃ (b). The energy level of the Tb(III) $^5\text{D}_4$ state and sum of $^5\text{D}_4$ and ΔE from energy back transfer equation (8) are shown for both complexes.

According to Figure 9 b, the energy back-transfers to the triplet state of the ligand L27 rather than to the ILCT state because the sum $E(^5\text{D}_4$ of Tb(III)) + ΔE is just above the phosphorescence maximum and coincides with the ILCT emission maxima. These results imply that also in the Tb(III) complexes the energy transfer depends on the relative energy of the states involved and is independent of the nature of those states.

4.2 Paper I: Study on photophysical properties of Eu(III) complexes with aromatic β -diketones

β -Diketones are probably the most studied chelating ligands for lanthanides and these luminescent chelates have plenty of applications, e.g. in electroluminescent devices[69,70], sensors[63], lasers[71] and bioassays[12]. The specific aim of paper

I was to develop a brighter and longer-wavelength excitable tris- β -diketone-Eu(III) chelate than those presently used. One of the studied chelates, Eu(L6)_3 was much brighter than Eu(L13)_3 and Eu(L14)_3 (see Table 4). The complex Eu(L5)_3 is published earlier [127] but Eu(L6)_3 is not and Eu(L6)_3 is brighter than Eu(L5)_3 , probably due to the lower σ_P^+ value for the CF_2CF_3 than for CF_3 (0,52 and 0,54, respectively). In addition, Eu(L6)_3 can be excited efficiently at 360 nm (see Figure 10).

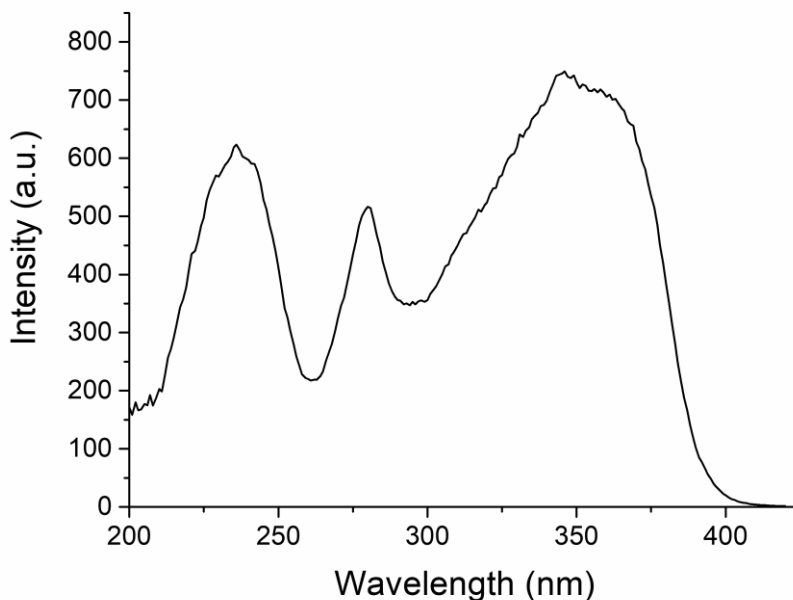


Figure 10. Excitation spectrum of 1 μM Eu(L6)_3 in ACN. Emission wavelength 615 nm.

The paper **II** shows that $\text{Eu(L6)}_3\text{L15}$ is degradable but it is unclear this is due only to the presence of the additional ligand L15.

4.3 Paper II: Photophysical study of blue-light excitable ternary Eu(III) complexes and their encapsulation into polystyrene nanoparticles

Recently, much attention has been paid to the ternary β -diketone complexes containing an additional bi- or tridentate Lewis base, *e.g.* 1,10-phenanthroline or bispyrazol-triazine.[58,59,61–63,128] The additional ligand has at least two advantages: it replaces the quenching water molecules and, therefore, increases the

luminescence intensity. Moreover, in some cases the complex can be excited at longer wavelength, even in the visible region. This broadens the application field of the lanthanide complexes for example in bioimaging[72,73] and facilitates the use of semiconductor light sources for their excitation. A drawback with the ternary complexes is their low stability and solubility in aqueous solutions. This can be overcome by incorporating them into protective environment *e.g.* inside nano- or microparticles.[129] Polystyrene nano/microparticles are commercially available with amino and carboxylic acid surface modifications, which allow covalent conjugation of proteins *e.g.* antibodies on their surface and the particles can be used in bioanalysis.[130,131] In this study, ternary tris- β -diketone-Eu(III) complexes were prepared by using 1,10-phenanthroline (L16) and 4'-(4-diethylaminophenyl)-2,2':6',2''-terpyridine (L15) as the Lewis bases and the incorporation of the ternary complexes inside the polystyrene particles was also demonstrated. The β -diketones used in this study were those found to be the most interesting in the previous work. The toluene solutions were used as a model environment for the nonpolar polystyrene cavities.

Table 9. The absorption maxima (λ_{max}^{abs}), quantum yields (Φ_{max}) and molar absorption coefficients (ϵ_{max}) of 5 μ M Eu(Lx)₃ and Eu(Lx)₃L15 at maximum wavelength in toluene.

COMPLEX	WITHOUT L15			WITH L15			
	λ_{max}^{abs} (nm)	ϵ_{max} (M ⁻¹ cm ⁻¹)	Φ_{max}	λ_{max}^{abs} (nm)	ϵ_{max} (M ⁻¹ cm ⁻¹)	Φ_{max}	($\epsilon\Phi$) _{max} (M ⁻¹ cm ⁻¹)
Eu(L2)₃	327	25800	0,016	341	39900	0,22	8800
Eu(L4)₃	338	43400	0,016	348	61400	0,16	9800
Eu(L14)₃	342	28300	0,12	352	48600	0,33	16000
Eu(L13)₃	340	33000	0,084	348	59400	0,32	19000
Eu(L11)₃	410	58700	0,010	376	57900	0,057	3300
Eu(L5)₃	381	42500	0,026	372	57100	0,16	9100
Eu(L6)₃	381	55400	0,059	372	73200	0,37	27000

Table 9 from paper **II** shows the photophysical properties of the complexes studied. As the table shows, Eu(L6)₃L15 was the brightest complex but its quantum yield decreased 54 % after standing a week in dark at room temperature, and thus its brightness decreased below the that of Eu(L13)₃L15 and Eu(L14)₃L15. An increased temperature, air flow and UV exposure (285 nm) did not affect the redshift, leaving the reason for the shift unsolved. In any case, one possible explanation is intercomplex stacking or association of ligands, which can take place in more

concentrated solutions, and changes the absorption spectrum and quantum yield, and breaks up within time. Thus, the encapsulation of the polystyrene nanoparticles was done with the complexes $\text{Eu}(\text{L14})_3\text{L15}$, and also $\text{Eu}(\text{L13})_3$ for comparison.

Table 10 shows the luminescence lifetimes of the complexes $\text{Eu}(\text{L14})_3\text{L15}$ and $\text{Eu}(\text{L13})_3$ (synthesized and commercial) in polystyrene nanoparticles. Because of the scattering in the colloidal solution the quantum yield could not be determined

Table 10. Luminescence lifetimes of $\text{Eu}(\text{L13})_3$ (synthesized and commercial) and $\text{Eu}(\text{L14})_3\text{L15}$ with the lifetime fractions in polystyrene nanoparticles in 1:5 EtOH-H₂O solution. The excitation at 335 nm and emission at 615 nm.

COMPLEX IN THE NANOPARTICLES	T ₁ (μS)	T ₂ (μS)	T ₃ (μS)
	[FRACTION (%)]	[FRACTION (%)]	[FRACTION (%)]
$\text{Eu}(\text{L13})_3$ (commercial)	800 (83)	356 (17)	-
$\text{Eu}(\text{L13})_3$	750 (51)	370 (49)	-
$\text{Eu}(\text{L14})_3\text{L15}$	1300 (33)	600 (59)	223 (6)

The luminescence lifetimes of the synthesized and commercial particles loaded with $\text{Eu}(\text{L13})_3$ are nearly equal but not their luminescence fractions. The bigger fraction of τ_1 of commercially available particles with $\text{Eu}(\text{L13})_3$ indicates that the complexes have penetrated deeper inside the particles. Polystyrene cavities are so non-polar environments that the complexes probably do not have any water molecules and, therefore, the lifetime distribution can be assumed to represent the complexes in different environments. The longest lifetime may be attributed to partially immobilized complexes and the shorter lifetime to more freely moving complexes. The long luminescence lifetimes imply good quantum yields. The luminescence lifetime of $\text{Eu}(\text{L14})_3\text{L15}$ lengthened even more, implying their potential use in particles for other applications. Figure 11 shows the excitation spectrum of $\text{Eu}(\text{L14})_3\text{L15}$. The encapsulation technique makes the visible light excitation possible in aqueous environments.

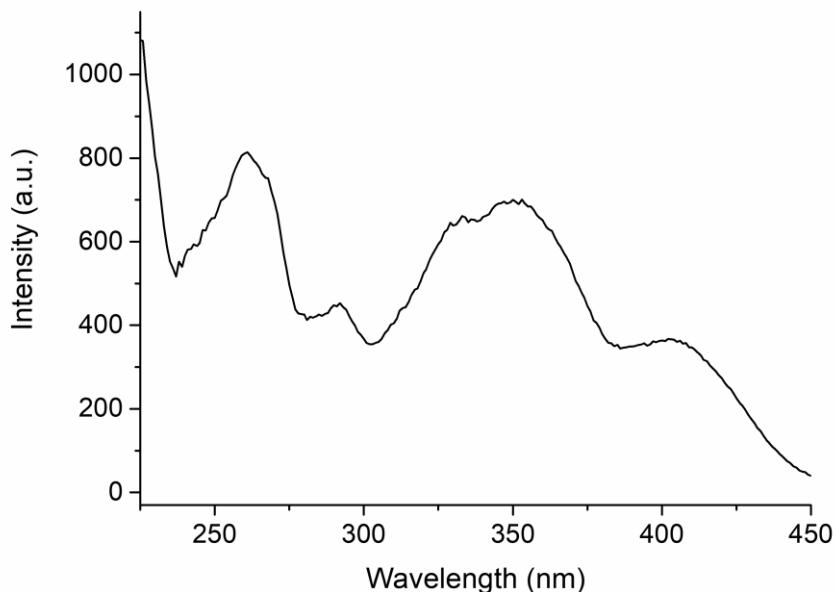


Figure 11. The excitation spectra of $\text{Eu}(\text{L14})_3\text{L15}$ in polystyrene particles. Emission wavelength 615 nm.

4.4 Paper III: Study on luminescent ternary EuEDTA complexes with dipicolinic acid derivatives

A ternary complex of terbium cation has been used in a DNA-hybridization assay for ca. 25 years.[132] In this assay a specific nucleic acid sequence is identified by using two oligonucleotide sequences, one labelled with a Tb(III) complex of diethylenetriamine pentaacetate and the other with aminosalicylate. The oligo sequences have been chosen to be complementary to the DNA strand in such a way that, when hybridized, the labels are in direct proximity to form a ternary complex where the aminosalicylate moiety functions as an antenna for Tb(III). The assay is then accomplished by measuring the intensity of the long-lived Tb(III) emission. The method has been later improved by replacing Tb(III) by Eu(III) and aminosalicylate by an aromatic β -diketone.[133] Even higher sensitivity was obtained by Karhunen *et.al.* by using a dipicolinic acid derivative as the light-harvesting antenna.[134] The method, called “switchable lanthanide probe technique”, was further successfully extended into homogeneous PCR (polymerase chain reaction) assays.[135,136]

Derivatives of dipicolinic acid (DPAD) are tridentate ligands and, together with a hexadentate carrier ligand, such as ethylenediaminetetraacetic acid (EDTA, or Y in the complexes of this work), interesting model systems for ternary complexes and their applications are formed. In this work 14 DPAD:s were studied with an aim to find brighter antennae for Eu(III) than the currently used 4-[(4-isothiocyanatophenyl)ethynyl]-pyridine-2,6-dicarboxylic acid (used as 4-[(4-thioureido)ethynyl]-pyridine-2,6-dicarboxylic acid (H₂L19)).[136] The comparisons were made using the basic form of H₂L19, H₂L18, as the reference ligand. The brighter ligand would decrease the number of required PCR cycles and thus would reduce the assay time. Table 5 (in chapter 4.1) shows the quantum yields, molar absorptivities and brightnesses at the excitation maxima of the ternary Eu(III)-EDTA complexes of the studied ligands. The table shows that most of the studied ligands form a brighter ternary Eu(III)-EDTA complex than H₂L18. Especially, the complex EuY-L28 (Y = EDTA) is more than two times brighter than EuY-L18. Moreover, its excitation maximum is redshifted compared to that of EuY-L18 (see Figure 12). However, the introduction of the linking isothiocyanato group may affect the quantum yield and be synthetically challenging.

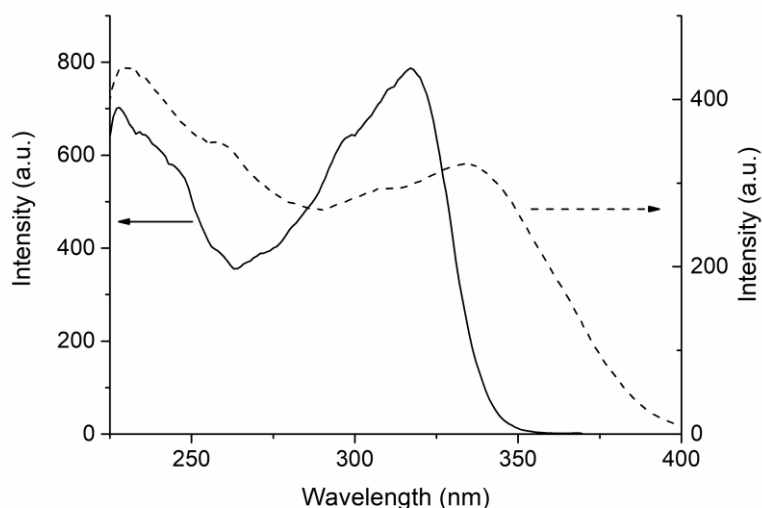


Figure 12. Excitation spectra of 25 μM EuY-L18 (solid line) and EuY-L28 (dashed line) in TRIS buffer ($C_{\text{TRIS}} = 50 \text{ mM}$, $\text{pH} = 7.75$). Emission wavelength 615 nm.

Moreover, paper **III** introduces the method for the stability constant determination. The method is based on the nonlinearity of the luminescence intensity vs. concentration curve, caused by complexation. The fitting of the equation (32) to the luminescence intensity vs. concentration data yields the stability constant. The

method is applicable to the determination of the stability constants of the 1:1 complexes, that is, either the ternary complexes or complexes with more than pentadentate ligands. The concentration range should be chosen in such a way that the bending in the curve is strong enough. The concentration range 5 – 25 μM is suitable for a stability constant around $\log K = 5$.

4.5 Paper IV: Study on luminescent ternary TbEDTA complexes with dipicolinic acid derivatives

In paper IV we tried to find a more efficient Tb(III) chelate for the DNA-hybridization assay than the currently used 4-{3-[2-(4-isothiocyanatophenyl)ethyl]-2,4,6-trimethoxyphenyl}-pyridine-2,6-dicarboxylic acid.[136] Table 7 shows the $I/A_{285\text{nm}}$ values for the complexes TbY-L x ($x = 17$ and $26 - 29$). The $I/A_{285\text{nm}}$ values suggest that H₂L27 may be a good candidate for the DNA-hybridization assay because they imply a high quantum yield. However, it is again unclear how the linking isothiocyanato group can be attached and how it would affect the quantum yield.

5 Summary and Conclusions

This thesis contributes to the development of the lanthanoid chelates by providing new information on the energy transfer processes. Especially, the advantages of deconvoluting the absorption and luminescence spectra are emphasized. The energy transfer in the lanthanoid complexes studied seems to depend only on the relative levels of the energy states involved and not on their nature. The singlet, triplet and ILCT states of the ligands, and the LMCT state, contribute to the energy transfer, depending on their relative levels. More importantly, it seems that the absolute energies of the states should not be taken into consideration in the development of the chelates but only the (deconvoluted) emission bands of ligands. In addition to the energy levels, also the energy transfer rates bring their own contribution to the energy transfer route preferences. Depending on the reduction potential of the Ln(III) ion and the energy level of the ligand ILCT state, energy transfer may take place via the LMCT state or the ILCT/LMCT state may be a quenching state (in case of PET). It is not clear if the stepwise mechanism enhances the energy transfer but it is, at least, a requirement for PET.

Paper **I** presented a new β -diketone ligand for Eu(III) with a high quantum yield. This complex, Eu(L6)₃, is both brighter and can be excited at longer wavelength than the complexes commonly used, *i.e.*, Eu(L13)₃ and Eu(14)₃.

Paper **II** demonstrated the encapsulation of visible light excitable ternary β -diketone complexes in polystyrene nanoparticles.

Paper **III** introduced many good ligand candidates for Eu(III) in the homogenous PCR assay. Especially the ligand H₂L28 is much brighter than the currently used H₂L18. Moreover, the paper presented the stability constant determination method based on the nonlinear dependence of the luminescence intensity on concentration. The method yielded the stability constants for the ternary Eu(III)-EDTA-DPAD complexes.

In paper **IV**, the ligand H₂L27 was suggested as a good ligand for Tb(III) in the DNA-hybridization assays, based on the estimated high quantum yield. However, the required isothiocyanato linker may affect the quantum yield and its introduction may be synthetically challenging.

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Markus Räsänen

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