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Journal of Alloys and Compounds 300–301 (2000) 141–146

Journal of ALLOYS AND COMPOUNDS

www.elsevier.com/locate/jallcom

Macrocyclic and macropolycyclic heteroaromatic *N*-oxides: powerful sensitizers for the lanthanide ions emission

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Abstract

Heterocyclic ligands are common sensitizers for the lanthanide ions emission upon UV irradiation. Of particular interest are Tb(III) and Eu(III) ions in combination with acyclic, macrocyclic and macropolycylic ligands incorporating heterobiaryls, such as 2,2′-bipyridyl, 1,10-phenanthroline, 1,1′-biquinoline, 3,3′-biisoquinoline, and their derivatives. Much more powerful sensitizers are the same systems bearing *N*-oxide functions, furthermore their complexes are more stable than the parent heterocycles. Such luminescent materials are the basis for time-resolved fluoroimmunoassays displaying very high sensitivity, and serve as materials for advanced medical diagnostics. In this short review, a brief presentation will focus on state-of-the-art photochemistry and photophysics of the lanthanide complexes of heterocyclic and *N*-oxide-incorporating heterocyclic systems.

Keywords: Cryptates; Luminescent complexes; Lanthanides; *N*-oxides; Fluoroimmunoassay; Fluorescence

1. Introduction

Heterocyclic ligands have been known for decades as sensitizers for the lanthanide ions emission in their complexes, upon UV irradiation. There was a need in the past to replace the present radioimmunoassays that are dangerous due to the handling of radioactive substances, disposal and production, by equally sensitive methods that are safe by all means. Fluorimetric methods deserve a good example for highly sensitive analytical tools for monitoring the analytes with the sensitivity of several orders of magnitude higher than routine spectroscopic methods. Thus the attention was placed on luminescent lanthanide complexes, whose luminescence life-times are much longer than any organic, short lived fluorescence of biological materials. Therefore, the idea was to develop so-called time-resolved fluorimetric methodology with gated counting of the luminescence of lanthanide ions embedded in the heterocylic ligands. The lanthanide complex thus might serve as a label for biological materials, such as monoclonal antibodies that were crucial in developing time-resolved immunoassays based on luminescent markers [1,2].

The mechanism that governs the lanthanide ion emission is called ‘Absorption-Energy Transfer-Emission’. The excitation of the aromatic (or heteroaromatic) electron system leads to the singlet state S_1 that is deactivated in a radiationless process to the triplet state of the ligand, and further energy transfer to the resonance level of the lanthanide ion (for instance $^5D_0$ of Eu(III)) results in visible emission of the lanthanide ion).

There are two general approaches to the synthesis of efficient luminescent materials based on lanthanide complexes: acyclic systems, and macrocylic and macropolycyclic ligands. Both approaches have been used successfully in practice, although they have some drawbacks.

1.1. The principle of time-resolved fluoroimmunoassay

The lanthanide complex bearing a spacer with the reactive group is linked with the monoclonal antibody covalently to form a bioconjugate. The antibody-based bioconjugate targets a specific antigen to be detected. The separated whole assembly-marker–antibody–antigen is irradiated with short UV pulses in the range of nanoseconds. The heterocyclic system is excited, and the radiationless energy transfer via triplet state occurs to the resonance level of the lanthanide ion, and luminescence from the lanthanide ion is observed. The counting of the lanthanide luminescence is gated to filter the short-lived fluorescence originating from the biological material within tens of nanoseconds. Long-lived lanthanide lumines-

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0925-8388/00/$ – see front matter
PII: S0925-8388(99)00745-8

cence is counted within 0.4–0.8 ms. The cycle can be repeated 1000 times in 1 s to enhance the noise-to-signal ratio. The choice of the sensitizer has very strict rules. It should possess the following features: high absorbance coefficient in the near UV region, high photostability, strong coordination properties for the lanthanide ions, high efficiency in energy transfer, pH-insensitivity; the lanthanide complex should be kinetically stable in aqueous solution, with a high quantum yield for lanthanide emission, pH-insensitive, shielding the lanthanide ion from water in solution.

1.2. Design of the ligands for lanthanide ions

The crucial point is to achieve a high quantum yield for lanthanide emission and high stability in aqueous media. Both account for the high sensitivity of the method. The high quantum yield can be achieved in several ways: selection of the ligand with high absorbance coefficients, array of ligands around the lanthanide ion (antenna effect), two-lanthanide ion system (co-fluorescence effect), the presence of sensitizing anions instead of chlorides, perchlorates, etc., (synergistic effect).

1.3. Acyclic ligands

There are several groups active in this area. The group of Mukkala and co-workers has been interested in designing acyclic heterobiaryl systems possessing polycarboxylic functions [3–11]. Although nitrogen-containing ligands are not the best ligands for lanthanide ion complexation, the presence of several carboxylic groups led to an enhancement of the overall stability of the complex and several examples of the acyclic ligands are shown in the Fig. 1.

Another group (Bünzli and Piguet) was interested in acyclic heterocyclic ligands for lanthanide ion complexation and luminescence studies [12–18].

1.4. Macrocyclic and macropolycyclic ligands

An alternative route to highly luminescent materials based on encapsulated lanthanide ions has been developed by Lehn and co-workers [19–30]. Their approach consisted of biheteroaromatic units, such as 2,2′-bipyridine, bipyrimidine, 1,10-phenanthroline, 3,3′-biisoquinoline, and later their *N*-oxides [31,32] harnessed into macrocyclic, or macropolycyclic structures. Some structural variations involved incorporation of sensitizing units as pendent arms to collect more light and to bind lanthanides more tightly. Although heteroaromatic ligands are not the best binders for lanthanide ions while in acyclic structure, their cooperative binding effect known as the ‘macrocyclic’ or ‘macrobicyclic’ effect led to remarkable stabilisation of the complex and satisfactory sensitizing properties of the ligands.

Alternative ligand design involved calixarenes as a framework on which photoactive groups have been attached. [33–35]. In this case good luminescence properties were observed for selected lanthanide ions.

1.5. Macrocyclic and macropolycyclic ligands bearing N-oxide functions

We have found that heteroaromatic *N*-oxides are much more powerful binding ligands for lanthanide ions, due to the hard Lewis base–Lewis acid strong interaction, when compared to initial *N*-heterocycles, and at the same time they are much more excellent sensitizers for lanthanide emission [36–43]. In several papers we have demonstrated the usefulness of these systems based mainly on 3,3′-biisoquinoline-2,2′-dioxide units. The formulae of the discussed ligands are presented in Figs. 2–4.

2. Experimental

All ligands and their Eu(III) and Tb(III) complexes have been prepared by published methods [36–43]. Spectral properties of the complexes were measured in water triply distilled from quartz vessels. For all quantum yield measurements, we have used a custom-made spectrofluorimeter [44]. A nitrogen laser (IGT 50, ZWG, GDR, pulse at 337 nm), or an excimer laser (Lambda Physics, pulse at 308 nm) were applied for luminescence life-time measurements. The luminescence decay was observed at 618 nm. The signal from the photomultiplier (RCA 1P28), after

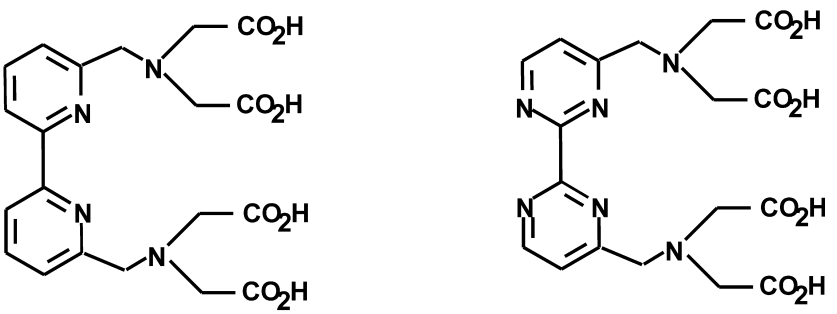


Fig. 1. Examples of acyclic ligands for lanthanide complexation.

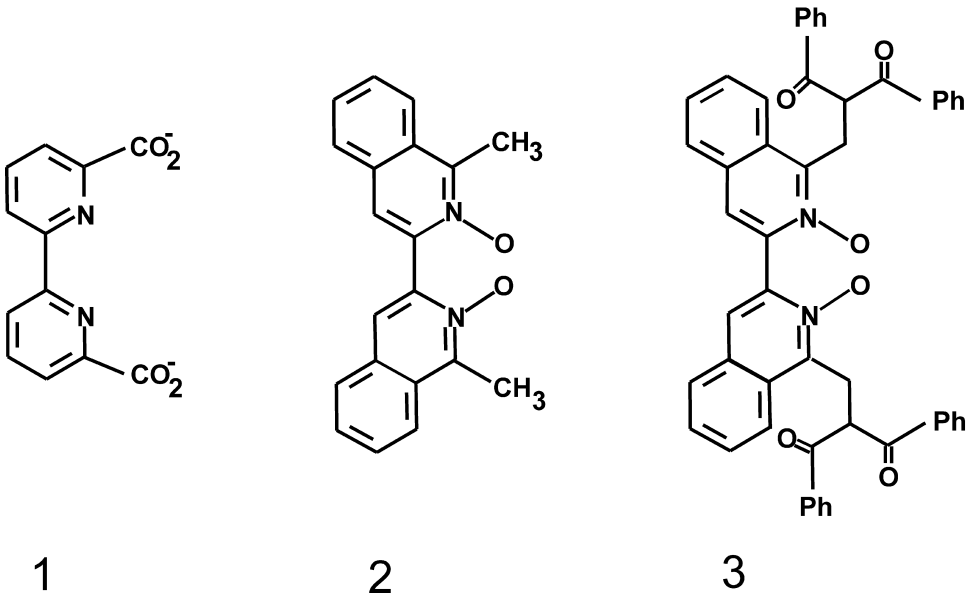


Fig. 2. Acyclic ligands.

passing a delay line, was processed by a BCI-280 boxcar (ZWG) and PC computer. For quantum yield measurements we have used the $[Ru(bipy)_3]Cl_2$ complex in water solution deaerated with a stream of Ar. The error for the quantum yield determination was ±20%.

3. Results and discussion

3.1. Acyclic ligands

Several of our compounds are acyclic, bearing the 3,3′-biisoquinoline-2,2′-dioxide unit as a very efficient sensitizer for Eu(III) ion emission. With this unit in all further combinations, only Eu(III) could have been taken into account, since the energy of the resonance level for Tb(III) is higher than the energy of the triplet state of this unit. Ligand **2** turned out to be a very potent sensitizer for the Eu(III) emission. The quantum yield was found to be 0.25 with suitable luminescence lifetimes (0.65 ms) in the 1:2 complex [37]. Unfortunately, the complex was not stable in aqueous solution, thus its practical usefulness was highly limited. Our attempts to enhance the efficiency of the ligand both in stabilising the complex and improving the quantum yield led us to the design of ligand **3** possessing two pendent arms consisting of dibenzoylmethane. These units are known to form highly luminescent chelates with lanthanides. Furthermore, dibenzoylmethane arms can be ionised to form even stronger complexes with Eu(III) than with **2**. The complexes for

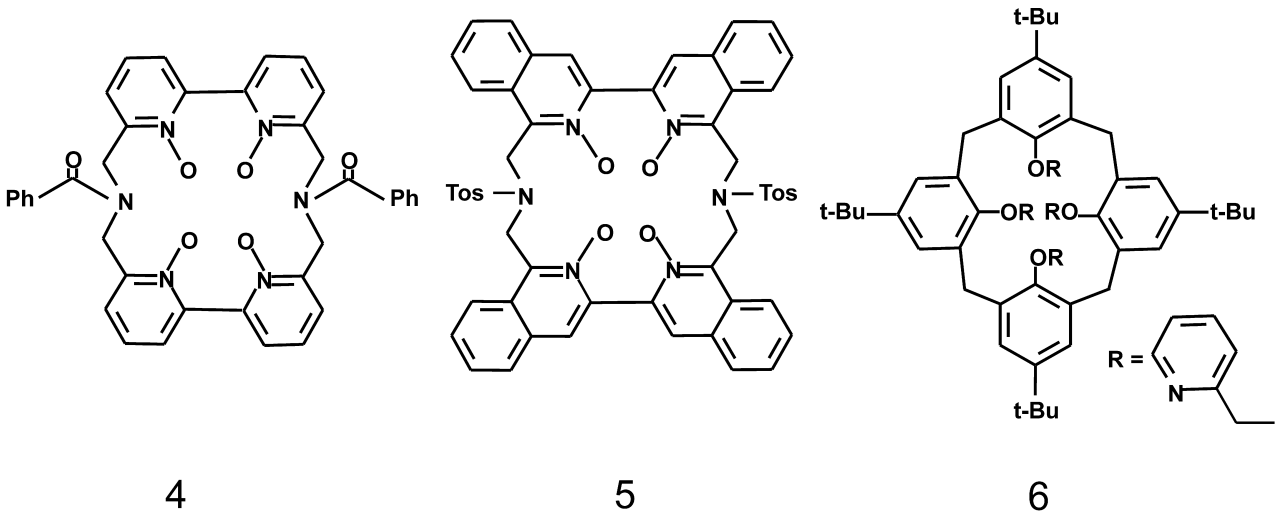
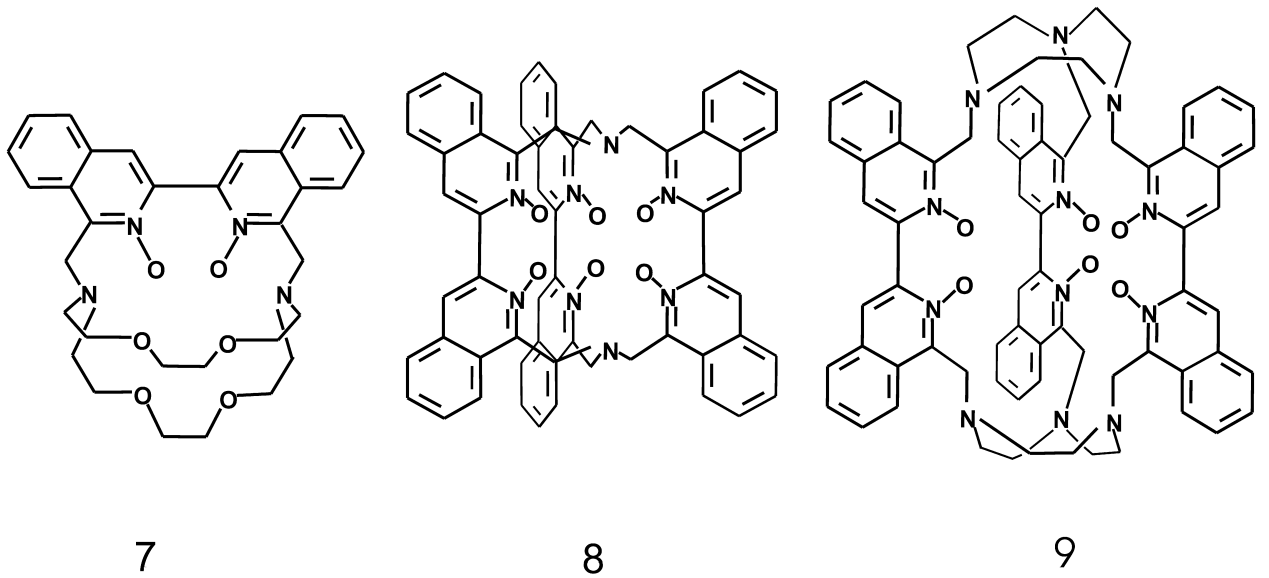


Fig. 3. Monomacrocyclic ligands; Ph=Phenyl; Tos=*p*-toluenesulphonyl.



7 8 9

Fig. 4. Polymacrocyclic ligands.

ligand **3** were not as good as **2**, all their photophysical properties were not very impressive, probably as a result of mismatch between the electronic states of the biisoquinoline unit and dibenzoylmethane [42]. Also their stability in water was low.

3.2. Macrocyclic ligands

Initially we have devised the macrocyclic ligand incorporating two 2,2′-bipyridine-1,1′-dioxide units and its Eu(III) complex **4** [36]. Its luminescent properties were remarkable, particularly the stability in aqueous solution was very high; the intensity of luminescence has not changed within one year. The quantum yield was not very high, however (0.016 in water).

The 3,3′-biisoquinoline-2,2′-dioxide is a more attractive ligand than bipyridine, and we have incorporated this unit into macrocyclic structure **5**. The ligand formed a luminescent complex with Eu(III), but its luminescence quantum yield was surprisingly low (0.064 in acetonitrile). This fact may be interpreted in a way that only four oxide functions may interact strongly with the Eu(III) ion, thus the coordination sphere of the lanthanide ion can be complemented by residual water present in the solvent, and OH oscillators quench the luminescence to a high degree. Furthermore, the complex was not stable in aqueous media. A total loss of luminescence was observed after 3 h.

The *t*-butylcalix[4]arene was used as the basic unit to incorporate pyridine units on oxygen atoms by simple alkylation with 2-chloromethylpyridine [33]. The ligand **6** formed luminescent complexes with Eu(III) and Tb(III) ions in aprotic solvents. Also in this case the quantum yields for the emission were not high, probably due to complementary complexation of few water molecules by the lanthanide ions. It was important to have as many ligating groups as possible to encapsulate lanthanide ions tightly in order to avoid a residual water complexation.

3.3. Macrobicyclic and macropolycyclic ligands

The ‘Cryptate Effect’ was used in further experiments to enhance the stability of the complexes in solution and to apply the ‘antenna effect ’ as well. Initially we incorporated one 3,3′-biisoquinoline-2,2′-dioxide unit into the bicyclic structure **7** [37]. More heteroatoms involved in Eu(III) binding turned out to be very promising. The complex was stable in water indefinitely, and the quantum yield for the emission was very satisfactory, depending also on the counter ion present in the Eu(III) salt used in the experiment. It turned out that the anion may attune the luminescence properties to a great extent not only in quantum yields, but also in luminescence lifetimes. Thus, this compound might have been considered for further studies to commercialise it.

The logical approach to have more sensitizing units forming an ‘antenna array’ prompted us to incorporate more 3,3′-biisoquinoline-2,2′-dioxide units. In this way we have synthesised two macropolycyclic ligands **8** and **9** [41]. These ligands formed luminescent Eu(III) complexes. Surprisingly, their emission quantum yields were lower than in the case of **7**. The ligand **8** might be too tight for efficient encapsulation of the Eu(III) ion, since its six oxygen atoms point towards the interior of the cavity. Thus, the complex that is formed can be conceived as a so-called ‘exclusive’ complex, in which the inorganic ion is not fully buried in the cavity, thus still accessible to co-ligands, such as the solvent, and particularly residual water. This may account for a low quantum yield. The more expanded ligand **9**, on the contrary, may have too

much space available inside the cavity, and therefore still the coordination sphere of the Eu(III) ion can be available to residual water penetration. In both cases the 'antenna effect' was not observed. Furthermore, these complexes are not stable in aqueous media, and fluorescence disappeared within several hours in water. Therefore, the complex of **7** turned out to be the most promising for further studies.

Table 1 contains all the spectral, lifetime and quantum yields data for the discussed complexes. The analysis of them led to the conclusion that the number of sensitizing units is not essential to achieve the required luminescence properties. The adjustment can be made on the basis of anions surrounding the Eu(III) ion.

For practical purposes in time-resolved fluoroimmunoassays, the lanthanide complexes must follow several strict criteria: they have to be kinetically and thermodynamically stable in aqueous media, to have high quantum yield for emission and suitable luminescence lifetime, and insensitive towards organic and inorganic ions present in body fluids. Of particular importance is their insensitivity in the presence of calcium and phosphate ions. Calcium may replace the lanthanide ion, due to their comparable ion radii, phosphate, on the other hand, may precipitate insoluble lanthanide phosphates. In both cases there is a loss of luminescence.

We have compared the model compound: 2,2′-bipyridine-6,6′-dicarboxylic acid (**1**) salt of Eu(III), and the Eu(III) complex of **7** [43]. Addition of phosphates and calcium ions in great excess to solutions of $\mathbf{1}_3{\cdot}Eu_2$ and $\mathbf{7}{\cdot}Eu(CF_3SO_3)_3$ did not affect the luminescent properties of the complex **7**, whereas complex **1** was remarkably affected. The luminescence data are collected in Tables 2 and 3.

Table 2
Relative quantum yields and luminescence lifetimes for Eu^{3+} emission in water in the presence of interfering ions for $Eu_2[2,2'\text{-bipy-}6,6'\text{-}(CO_2)_2]_3$[a]

Ion concentration (M/l)	Ion	Φ	τ_1 (μS)	τ_2 (μS)
–	–	1.0	780	200
10^{-4}	Ca^{2+}	0.55	790	190
10^{-3}	Ca^{2+}	0.34	800	200
10^{-4}	PO_4^{3-}	0.71	780	185
10^{-3}	PO_4^{3-}	0.23	790	200

[a] Concentration of the complex: 2×10^{-5} M/l.

Table 3
Relative quantum yields and luminescence lifetimes for Eu^{3+} emission in water in the presence of interfering ions for $BiquiO_2(2.2)Eu(O_3SCF_3)_3$[a]

Ion concentration (M/l)	Ion	Φ	τ (μS)
–	–	1.0	400
10^{-3}	Ca^{2+}	1.33	410
10^{-3}	PO_4^{3-}	1.33	406

[a] Complex concentration: 2×10^{-5} M/l.

4. Conclusions

It turns out that bicyclic lanthanide complexes are advantageous over acyclic lanthanide complexes in aqueous media containing the affecting ions, both inorganic cations and anions. Proper design of the ligand possessing the cavity compatible with the size of the lanthanide ion is crucial to achieve high stability in water and prevention of the lanthanide ion from water penetration in the first coordination sphere. It will be important to introduce heteroaromatic *N*-oxides into macrobicyclic structures to

Table 1
Luminescent characteristics of the Eu(III) and Tb(III) complexes 2–9 in acetonitrile solution at room temperature (λ_{exc}=337 nm)

Ligand (L)	Lanthanide salt (S)	L/S ratio	λ_{em} (nm)	τ (ms)	Φ
7	$EuCl_3$	1:1	580, 590, 600, 611, 618, 650	0.62	0.57
7	$Eu(OTf^a)_3$	1:1	580, 590, 600, 611, 619, 650	0.70	0.17
7	$Eu(OTf)_2Br$	1:1	580, 590, 600, 611, 619, 650	0.52	0.138
7	$Eu(ClO_4)_2Br$	1:1	579, 589, 598, 611, 618, 682	0.64	0.04
7	$Eu(DBM^b)_3$	1:1	585, 613, 653, 703	0.26	0.023
4[c]	$Eu(ClO_4)_3$	1:1	582, 594, 618, 700	–	0.016
2	$EuCl_3$	2:1	592, 618, 700	0.65	0.25
3	$EuCl_3$	1:1	579, 594, 615, 622, 653, 703	0.64	0.05
3^{2-}	EuCl	1:1	592, 615, 653, 703	0.60	0.027
3^{2-}	EuOTf	1:1	580, 594, 615, 653, 702	0.38	0.028
5	$Eu(ClO_4)_3$	1:1	580, 590, 595, 614, 618, 650	0.25	0.064
8	$Eu(OTf)_3$	1:1	580, 607, 613, 653, 703	0.25	0.0022
8	$Eu(OTf)_3$	1:1	580, 594, 607, 652, 703	0.24	0.0015
9	$Eu(OTf)_3$	1:1	594, 616, 653, 703	0.14	0.001
6	$EuCl_3$	1:1	580, 592, 616, 700	–	0.003
6	$TbCl_3$	1:1	490, 544, 587, 618	–	0.002

[a] OTf=CF_3SO_3.
[b] DBM=dibenzoylmethane.
[c] Data in water solution.

achieve all desired properties of the ligands suitable for modern time-resolved fluoroimmunoassays.

Acknowledgements

Grant No. 02 0796 91 01 from the Polish State Committee for Scientific Research is gratefully acknowledged.

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