

The present invention, which is the result of work carried out in collaboration with Professor J.M. Lehn and his research team at the Université Louis Pasteur in Strasbourg, relates to the field of
5 fluorescence and more particularly of fluorescent macropolycyclic complexes which are especially suitable as labels in immunological determinations.

It is known that detection methods using fluorescence are intrinsically very sensitive and could
10 make possible lower detection limits than those attained by radioactivity measurements, in particular through the use of laser sources (I. Wieder, Immunofluorescence and Related Staining Techniques, 1978, Elsevier).

In practice, however, these detection limits are
15 not reached because the measurement is made in a matrix which does not have the properties required to achieve this optimum. In general, the measurement medium is turbid and promotes diffusion, and other molecules fluorescing at the same wavelength can be present in the
20 measurement medium.

In some cases, the improvements made to the measuring equipment are not sufficient to substantially improve this detection limit or are very expensive (laser, monochromators, and the like).

This state of affairs is even more troublesome in
25 the field of biochemistry and immunology, where very small quantities of active molecules have to be measured in biological media which can be turbid or can contain proteins or other molecules which are themselves fluorescent (turbidity and intrinsic fluorescence of the
30 serum).

In immunological determinations using a fluorescent tracer, and when the antigen or antibody is labelled with a fluorescent molecule, the latter must
35 have the following properties:

- it must have a chemical functional group which makes possible coupling with the biological molecule without denaturing it or modifying its immunological properties;

- the molar absorption coefficient of the fluorescent molecule must be as high as possible;
- the quantum yield of fluorescence must be the highest;
- 5 - the Stokes shift must be as large as possible;
- the emission wavelength must be, if possible, greater than 500 nm;
- it must be soluble in water or buffer solutions.

These conditions are specified, for example, in the
10 article by E. Soini in Clin. Chem., 25, 353 (1979).
Now, the molecules known to date are far from possessing all these qualities.

The fluorescence of certain rare earth chelates has been known for many years by virtue of the work
15 relating to their application in the field of lasers (A.P.B. Sinha, Spectroscop. Inorg. Chem., 2, 255 (1971)).

These complexes are formed:

- on the one hand, from a chelating molecule having an electron system capable of populating the
20 triplet state T_1 by inter-system crossing after excitation of the singlet state S_1 following the absorption of light energy;

- on the other hand, from a rare earth ion which, in the case of fluorescence applications, has a strong
25 ion fluorescence intensity (Eu, Tb) or a moderate intensity (Sm, Dy) and whose resonance level is populated by the transfer of non-radiant energy from the triplet state of the complexing agent.

In order to obtain a strong fluorescence of the
30 rare earth chelate, it is necessary for the triplet energy of the complexing molecule to be greater than that of the resonance level of the ion and for it to have a sufficient lifetime (preponderance of this type of deactivation with respect to other processes, such as
35 phosphorescence and non-radiant deactivation, for example thermal deactivation).

In this case, after excitation in the absorption band of the chelate, a fluorescence characteristic of the rare earth ion is observed.

These compounds have great advantages in the field of detection by the measurement of fluorescence; for example, they are characterized by:

- a large Stokes shift,
- 5 - a molar absorption coefficient ϵ which is high relative to that of the rare earth,
- a possibility of simultaneous detection of several chelates by changing the rare earth,
- an emission spectrum characteristic of the rare
10 earth (line spectrum, λ of maximum emission > 500 nm),
- long lifetime (from microsecond to millisecond).

Patent US 4,058,732 describes a method for analytical spectroscopy by fluorescence using fluorescent
15 molecules (rare earth chelates) having a relatively long lifetime. Given that the diffusion due to the particles of the matrix, and that the fluorescence of the majority of the organic molecules of which it is composed, are short-lived phenomena (generally less than 1 μ sec), this
20 patent recommends a pulsed excitation and a functionalized rare earth chelate for labelling the biological molecules to be identified, detection taking place between each pulsation and after a sufficiently long time for the undesired phenomena to have substantially
25 decreased.

According to the prior art cited below, rare earth chelates theoretically show all the conditions for constituting a class of ideal fluorescent molecules, in particular as labels for biological molecules in immuno-
30 logical determinations, in cytology and for cell classifiers:

- Patent US 4,058,732
- Soini, Clin. Chem., 25, 353 (1979)
- L.M. Vallarino et al. in Automation of Uterine
35 Cancer Cytology 2, Proceedings of 2nd Int. Conf., Editors G.L. Wied, G.F. Gahr and P.H. Bartel, Tutorials of Cytology, Chicago, Ill, 1977.

Now, in practice, it is observed that the

chelates used hitherto do not have all the following properties demanded by these applications, which are mainly:

- 5 - a high quantum yield of fluorescence and a high molar absorption coefficient,
- a suitable triplet energy of the chelating agent,
- non-inhibition by the solvent (water or other solvent) or by molecules present in the medium where the measurement is being carried out,
- 10 - ease of functionalization for the purpose of coupling with molecules of biological interest or other molecules,
- selectivity of the chelation in favour of the rare earth and at the expense of other cations which may be found in large quantities in the measurement medium,
- 15 - solubility in water under the conditions of application of the immunological determinations,
- a high stability, in particular at low dilution.

20 The β -diketone chelates described in Patent US 4,058,732 have little or no solubility in water and biological media and their fluorescence is largely inhibited by water.

Other chelates have been proposed as labels in
25 immunological determinations; in particular, there may be mentioned the chelates derived from EDTA, HEDTA and DTPA, imidodiacetate and the like, described, for example, in: Proc. Nat. Acad. Sci. USA, 72, 4764 (1975), Patent US 4,374,120 and in Patent Applications EP-A-0,068,875
30 and DE-OS 3,033,691. Their efficacy is assumed to originate from the high value of their stability constant, which is generally greater than 10^{10} .

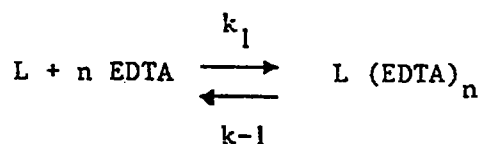
Such a criterion, based solely on thermodynamic considerations, is certainly not sufficient since it has
35 been shown that the lifetime of a Tb(III) EDTA complex is increased by addition of apotransferrin (Biochem., 19, 5057, 1980), which is characteristic of a protein/chelate bond.

It has recently been recommended, during the use

of labels of Eu(III) EDTA type, such as those described in Patent US 4,374,120, to use a buffer containing DTPA in order to remove the free europium which has dissociated from the chelate, which clearly proves the
5 instability of such chelates (Clin. Chem., 29, 60, 1983).

This state of the prior art shows that the rare earth chelates used hitherto cannot be used at low dilution in aqueous media containing other cations or in biological media, and in particular in immunological
10 determinations, and that it is advisable to take into account criteria such as kinetic stability (rate of dissociation of the complex) and the selectivity of the complexation of the rare earth.

Moreover, it will be noted that the majority of
15 the chelates of the prior art have found, by virtue of their high formation constant (in general $> 10^{10}$), an application in technical γ imaging, in which γ -emitting heavy ions of relatively short lifetime, obtained in dilute aqueous solutions, are used. The fixation of the
20 ion on the molecule of biological interest which carries the chelating agent must then take place rapidly; in particular, the rate of formation of the chelate must be high:



k_1 and $k-1$ being the rate constants of formation and
25 dissociation respectively; at equilibrium, the situation is:

$$K = \frac{k_1}{k-1} = \frac{\text{L (EDTA)}_n}{[\text{L}] [\text{EDTA}]^n}$$

For the complexes used hitherto, and for the reasons referred to above, chelates have always been used

which make it possible to obtain a high value of k_1
[$\approx 1.9 \cdot 10^{22} \text{ M}^{-1} \text{ min}^{-1}$ for Eu^{3+} EDTA - see J. Inorg. Nucl.
Chem., 1971, 33, p. 127]. The result thereof is that for
5 relatively large, which means that the rate of ion
exchange between the chelate and the solution is
relatively fast.

Other macropolycyclic compounds capable of
forming complexes containing certain metal ions have
10 already been described. To this end, there may especially
be cited Patent FR 2,088,519, the subject of which is
macropolycyclic heteroimines belonging to the "crown",
"lantern" or "dumbbell" compounds.

Macropolycyclic rare earth complexes have now
15 been found which have excellent properties of selectivity
and stability, especially of kinetic stability in aqueous
media and biological media. These macropolycyclic com-
plexes are particularly well suited as fluorescent labels
for biological substances in immunological detection or
20 determination techniques using fluorescence. They are
equally suitable as reagents in luminescence reactions.

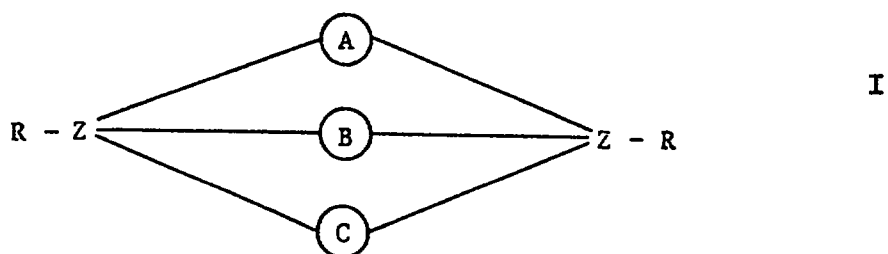
It has actually been found that it was possible
to enhance the fluorescence of a rare earth ion in
aqueous solution by complexing the latter using a
25 macropolycyclic compound having a donor unit of greater
triplet energy than that of the emission level of the
rare earth ion and by exciting the donor unit.

Whereas the excitation of a rare earth ion
produces a very weak fluorescence, given that the rare
30 earths generally have low molar absorption coefficients
 ϵ , excitation of the donor unit of the macropolycyclic
compound defined below makes it possible to enhance the
characteristic fluorescence of the rare earth ion. The
rare earth complexes thus formed are excellent
35 fluorescent labels which furthermore are compounds which
are stable in aqueous medium and which have a very high
selectivity with respect to rare earth ions.

In contrast to the chelates of the prior art,
which are characterized by a high formation constant, the

rare earth complexes according to the invention have, as an essential characteristic, a high kinetic stability (low rate of dissociation). This characteristic is all the more important as the biological solutions used in immunological detection and determination methods generally contain proteins which are also themselves capable of fixing the rare earth ion. On the other hand, the rate of formation of the complexes of the invention is not critical. The formation of the rare earth ion complex, on the one hand, and the coupling of the complex obtained with the biological molecule, on the other hand, can be carried out separately. The result thereof is that the formation of the rare earth ion complex according to the invention can be carried out under non-biological conditions (use of organic solvents, possible introductions of energy, time, and the like).

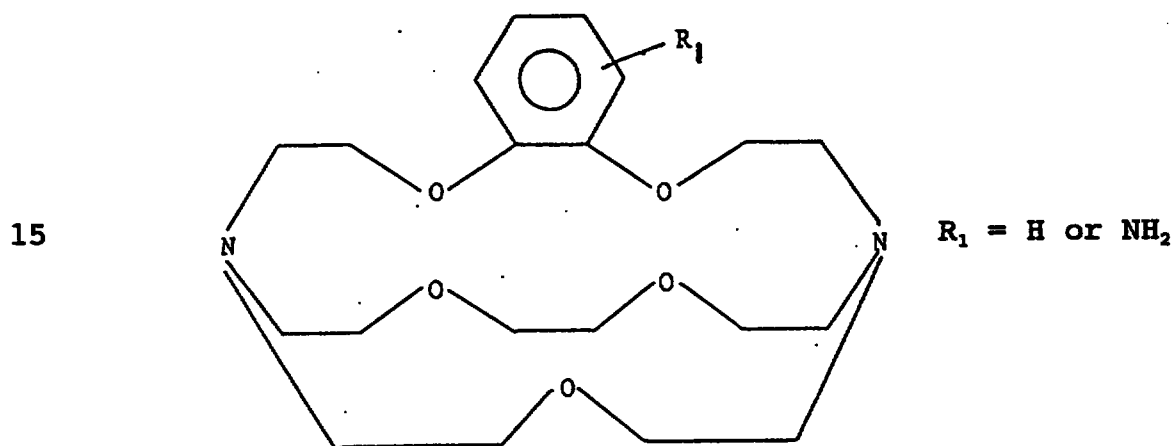
According to a first aspect, the present invention therefore relates to the use, as fluorescent labels, especially in methods of immunological detection and determination, of macropolycyclic rare earth complexes which consist of at least one rare earth salt complexed by a macropolycyclic compound of general formula:



in which Z is a trivalent or tetravalent atom, such as nitrogen, carbon or phosphorus, R does not exist or represents hydrogen, the hydroxyl group, an amino group or a hydrocarbon radical, the bivalent radicals $\textcircled{\text{A}}$, $\textcircled{\text{B}}$ and $\textcircled{\text{C}}$ are, independently from each other, hydrocarbon chains which optionally contain one or more heteroatoms and are optionally interrupted by a heteromacrocycle, at least one of the radicals $\textcircled{\text{A}}$, $\textcircled{\text{B}}$ or $\textcircled{\text{C}}$ moreover comprising at least one molecular unit or essentially consisting of a molecular unit, the said molecular unit

having a triplet energy greater than that of the emission level of the complexed rare earth ion.

According to another aspect, the invention also relates to a process for enhancing the fluorescence of a rare earth ion, which consists in complexing at least one rare earth ion with a macropolycyclic compound, such as defined above, having a donor molecular unit with a triplet energy greater than that of the emission level of the rare earth ion and in exciting the complex thus formed at the absorption wavelength of the said donor unit. The rare earth complexes defined above are new compounds except for the europium and terbium complexes obtained with the macropolycyclic compound of the formula below:



and the lanthanum and praseodymium complexes with the above compound in which R_1 is NH_2 . The latter are described in the document Chemical Abstracts, Vol. 99, No. 10 (5 September 1983), p. 676, No. 81500q.

20 Thus, the present invention also relates, by way of new compounds, to the rare earth complexes consisting of at least one rare earth salt complexed by a macropolycyclic compound of formula I above with the proviso that, when the rare earth salt is a europium or terbium salt, Z is nitrogen, (A) is $-(\text{CH}_2)_2-\text{O}-\text{C}_6\text{H}_4\text{R}_1-\text{O}-(\text{CH}_2)_2-$ with $R_1 = \text{H or NH}_2$, (B) and (C) are not simultaneously one the $-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-$ radical and the other

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$-(CH_2)_2-O-(CH_2)_2-$ radical and that, when the rare earth salt is a lanthanum or praseodymium salt, Z is nitrogen, (A) is $-(CH_2)_2-O-C_6H_5NH_2-O-(CH_2)_2-$, and (B) and (C) are not simultaneously $-(CH_2)_2-O-(CH_2)_2-$ and $-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-$.

The hydrocarbon chains which constitute the radicals (A), (B) and (C) can contain 2 or more carbon atoms and can optionally be interrupted by one or more heteroatoms chosen from oxygen, sulphur or nitrogen atoms.

The preferred hydrocarbon chains for the purposes of the invention are polyether chains, in particular oxyethylenated or polyoxyethylenated chains.

The molecular unit, which constitutes an essential component of the macropolycyclic compound according to the invention, is a triplet energy donor unit composed of molecules or groups of molecules having a triplet energy greater than that of the emission level of the complexed rare earth ion.

The energy transfer takes place from the triplet level of the donor unit to one of the emission levels of the complexed rare earth. For example, europium has the emission levels 5D_0 at $17,270\text{ cm}^{-1}$, 5D_1 at $19,030\text{ cm}^{-1}$ and possibly 5D_2 and terbium has the emission level 5D_4 at $20,480\text{ cm}^{-1}$.

The triplet energy donor units which are suitable for the purposes of the invention must have a triplet energy greater than or equal to that of the emission levels of the rare earth ion. For example, in the case of the europium and terbium complexes according to the invention, the triplet level of the donor unit must be greater than $17,270\text{ cm}^{-1}$.

As the phenomenon of phosphorescence is due to the radiant deactivation of a triplet state, a criterion of choice of the donor units can be the phosphorescence emission wavelength of the units. For example, units will be chosen which emit a phosphorescence at lower wavelengths (higher energies) than the wavelengths corresponding to the populating of the emission levels of the

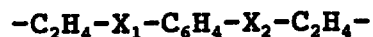
rare earth. In the present case, the phosphorescence wavelength of the donor unit will have to be less than 580 nm.

5 It will be noted that the complexes according to the invention can have one or more donor units, the said donor units constituting either part or all of the radicals (A) , (B) and (C).

10 It is possible to use by way of molecular units, in a non-limiting way, any triplet sensitizer having the required triplet energy, for example those described in Patent Application EP-A-0,068,875 or in Spectroscop. Inorg. Chem., 2, 255, 1971, these documents being cited in the present description by way of reference.

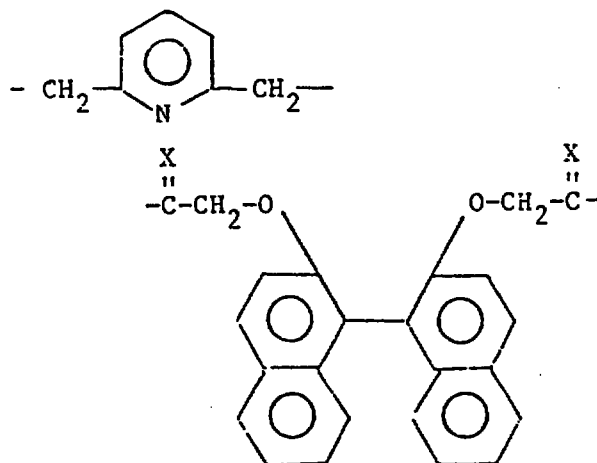
15 Particularly preferred molecular units for the purposes of the invention are phenanthroline, anthracene, benzene, naphthalene, biphenyl and terphenyl, bipyridines, biquinolines, in particular the biisoquinolines, for example 2,2'-bipyridine, azobenzene, azopyridine, pyridine or 2,2'-biisoquinoline.

20 The chains below may be mentioned in particular as examples of radicals (A) , (B) and (C) containing an energy donor unit:



25 X_1 and X_2 , which can be identical or different, represent oxygen or sulphur;





X being oxygen or hydrogen.

The macropolycyclic rare earth complexes according to the invention can be obtained by the conventional processes for the preparation of metal complexes, which consist in reacting the complexing compound with a compound which donates the cation to be complexed. Processes of this type are described especially in Patents US 3,888,377, 3,966,766 and 4,156,683, cited in the present description by way of reference.

For example, the macropolycyclic complexes can be obtained by reacting a compound which donates a rare earth cation with the macropolycyclic compound having the characteristics defined above, each compound advantageously being in solution, preferably in the same solvent or in compatible solvents which are inert with respect to complex formation. In general, acetonitrile, DMSO or ethanol is used as the solvent.

It is possible to use, as rare earth cation donor compound, any rare earth salt, advantageously a chloride, an acetylacetonate or a nitrate.

The reaction is advantageously carried out at the boiling temperature of the solvent.

When the macropolycyclic complex formed is soluble in the reaction solvent, it is isolated from the

solution by evaporation to dryness. If the macropolycyclic complex formed crystallizes in the reaction solvent, it is separated by filtration or any other suitable conventional means. The complexes thus obtained
5 can be purified by crystallization.

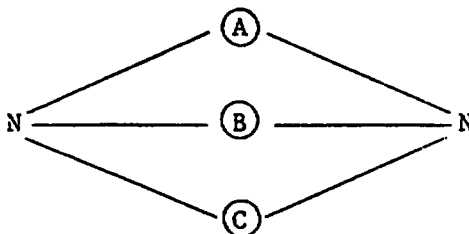
The above reaction can also be carried out by using a solution of the macropolycyclic compound and the cation donor compound in the crystalline form. A synergic agent for protecting against deactivation can also be
10 introduced into the coordination sphere of the cation, as is described in J. Chem. Phys., 40, 2790 (1964) and 41, 157 (1964).

In the remainder of the present description, the macropolycyclic complexes according to the invention are
15 also denoted by "cryptates" and the macropolycyclic compound by itself is also denoted by "cryptand". The nomenclature as defined by Lehn will be adopted hereinafter to denote the cryptates and the cryptands. For this purpose, reference may be made especially to the
20 articles by J.M. Lehn in Struct. Bonding (Berlin), 16, 1 1973 and Acc. Chem. Res., 11, 49 (1978).

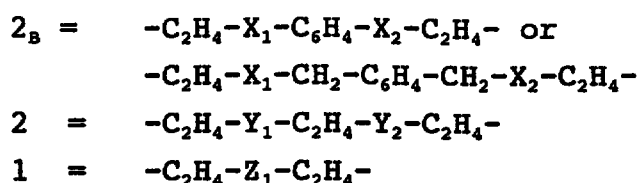
All rare earth ions are suitable for the purposes of the present invention. However, those which have the most intense ion fluorescence, i.e. terbium and europium
25 and, to a lesser degree, samarium and dysprosium, will be preferred.

It is possible to use, as macropolycyclic compounds which are suitable for the purposes of the invention, the known cryptands, for example:

30 1) the benzo-cryptands of general formula:



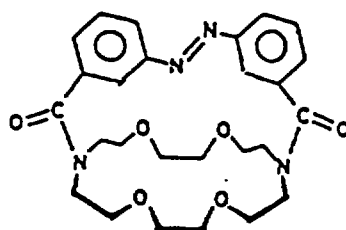
in which (A) , (B) and (C) represent, independently of one another, the groups 2_s, 2 or 1, which have the following meanings:



5 in which X_1 , X_2 , Y_1 , Y_2 and Z_1 each represent a heteroatom chosen from oxygen and sulphur, it being possible for X_1 , X_2 and Y_1 , Y_2 to be identical or different.

There may be mentioned, as examples of such cryptands, those in which the units (A), (B) and (C) are respectively composed as follows:

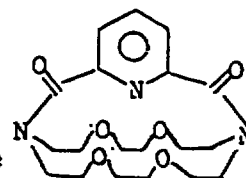
10 (A) (B) (C) = $(2_B 11)$, $(2_B 21)$, $(2_B 22)$, $(2_B 2_B 2)$ and the compound of formula:



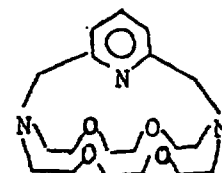
2) the cryptands containing nitrogenous heterocycles, such as those described in JACS, 99, 4683 (1977) of formula I in which:

- 15 - (A) and (B), which are identical, represent a polyoxyethylenated chain of formula $-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-O-$
- 20 - (C) is a hydrocarbon chain containing a nitrogenous heterocycle as energy donor unit. As examples of such compounds there may especially be mentioned the compounds below:

25 - (22)pyridinamide:
2,6-[N,N',N,N'-bis(3,6-dioxaocta-methylene)bis(aminocarbonyl)]pyridine

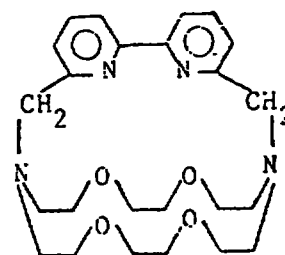


- (22)pyridinamine:
2,6-[N,N',N,N'-bis(3,6-dioxaocta-methylene)bis(aminomethyl)]pyridine



- (22)bipyridine:

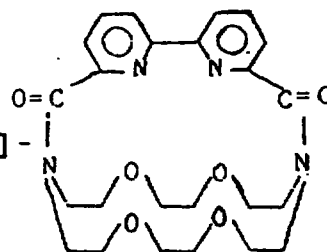
6,6'-[N,N',N,N'-bis(3,6-dioxa-
octamethylene)bis(aminomethyl)]-
2,2'-bipyridine.



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- (22)bipyridinamide:

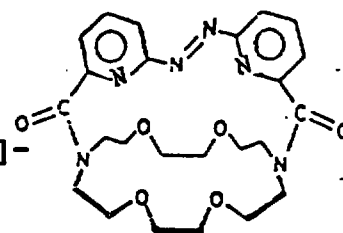
6,6'-[N,N',N,N'-bis(3,6-dioxa-
octamethylene)bis(aminocarbonyl)]-
2,2'-bipyridine.



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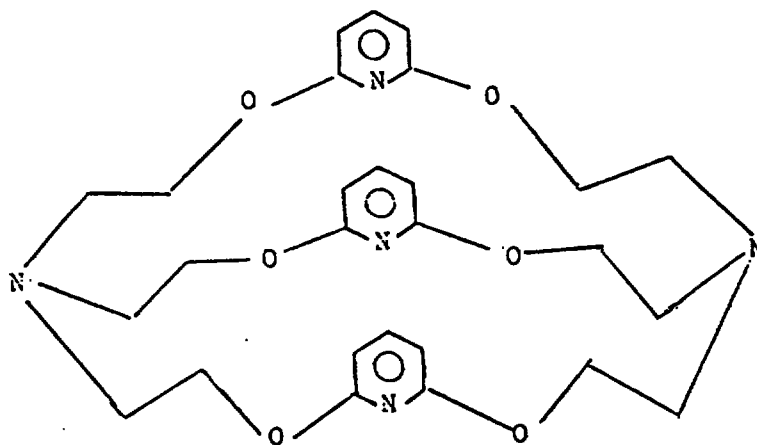
- (22)azopyridine:

6,6'-[N,N',N,N'-bis(3,6-dioxa-
octamethylene)bis(aminocarbonyl)]-
2,2'-azopyridine.



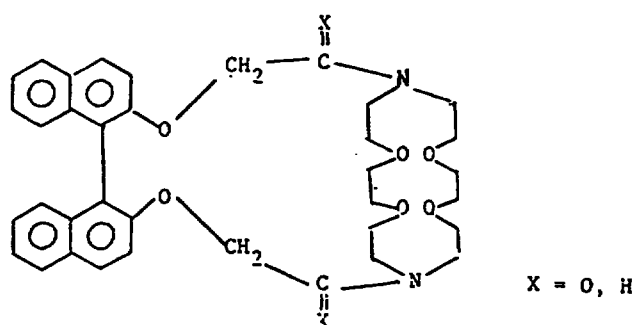
3) The cryptands containing several nitrogenous hetero-
cycles as donor units, such as the compound of the
formula below:

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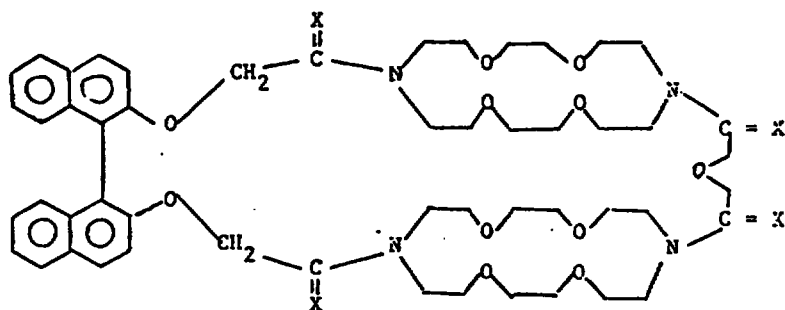


described in J. Am. Chem. Soc., 1979, 101, p. 1047.

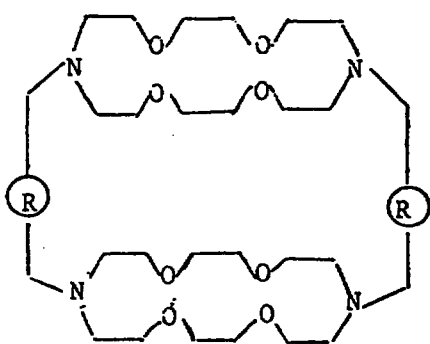
4) The polycyclic cryptands containing aromatic units,
for example the compounds corresponding to the
formula below:



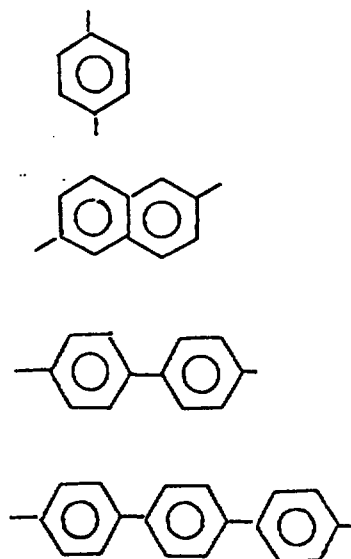
or the compounds of formula I in which the hydrocarbon chain carrying the donor unit(s) is interrupted by a heteromacrocycle, such as the compounds of the formulae below:



X = O, H



R =

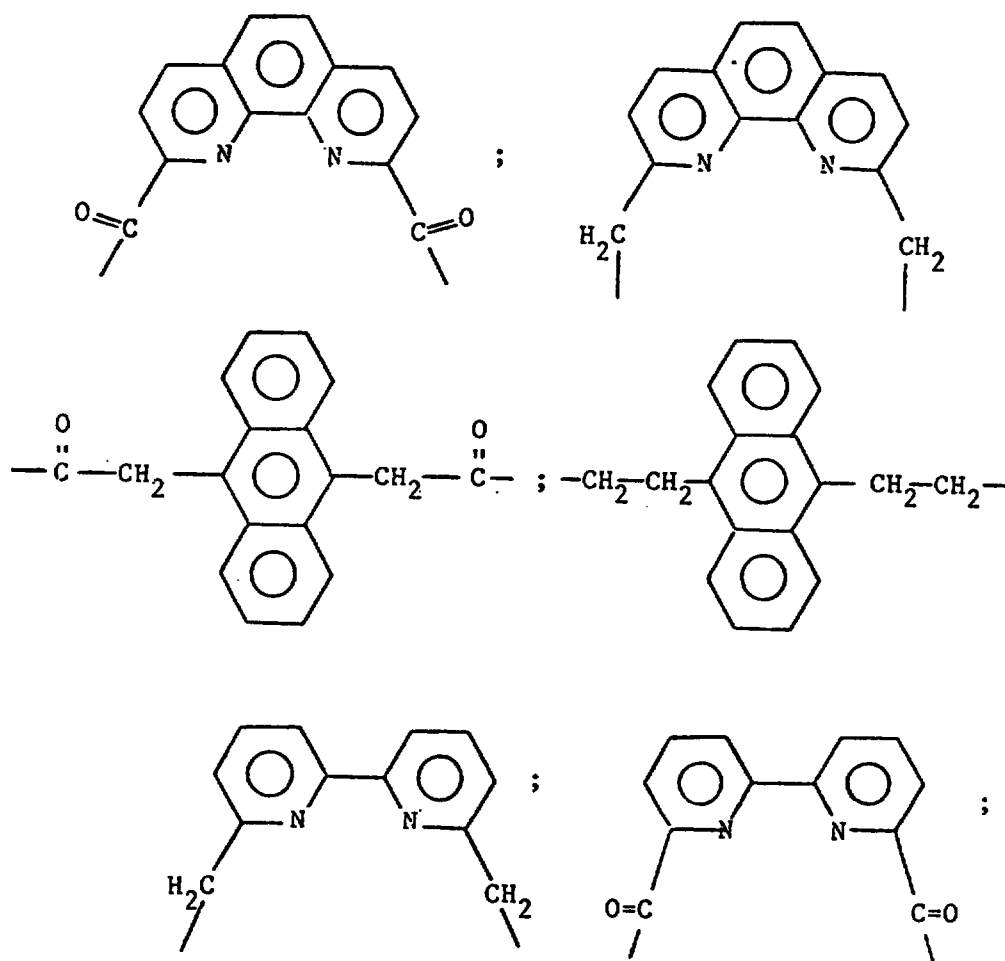


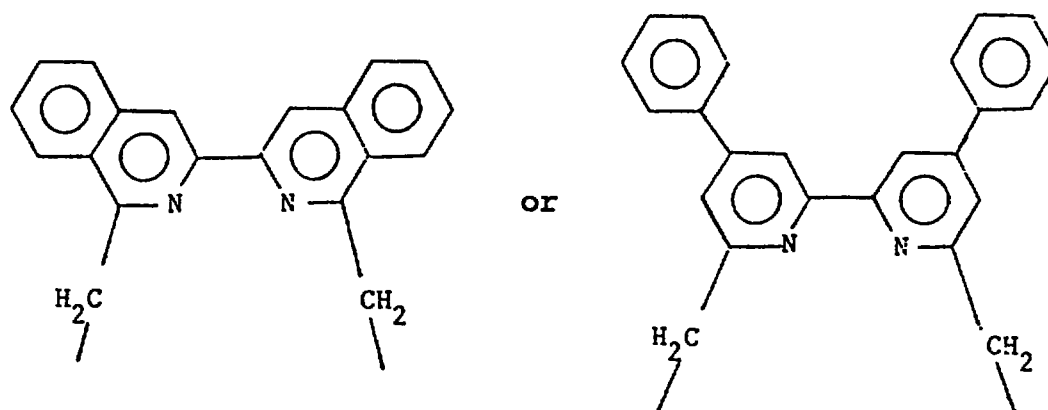
Such compounds are described especially in:

- Angew. Chemie, 86, 443 (1974)
- Tet. Letters, 21, 941 (1980) and Chem. Comm., 833, 1981.

- 5 It is also possible to use, as cryptands, the macropolycyclic compounds of formula I above in which the molecular unit(s) having a triplet energy greater than the energy of the rare earth to be cryptated are chosen from phenanthroline, anthracene, bipyridines and bi-quinolines, optionally substituted in suitable positions and by groups which are capable of increasing the efficiency of the energy transfer or of modifying the excitation spectrum of the rare earth cryptate [J. Phys. Chem., 1964, vol. 68, p. 3324]. The phenyl group may especially be mentioned as an example of such groups.

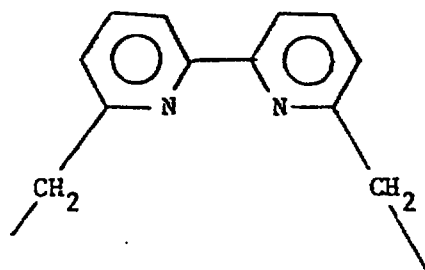
In these new macropolycyclic compounds, at least one of the radicals (A) , (B) or (C) preferably corresponds to one of the formulae below:



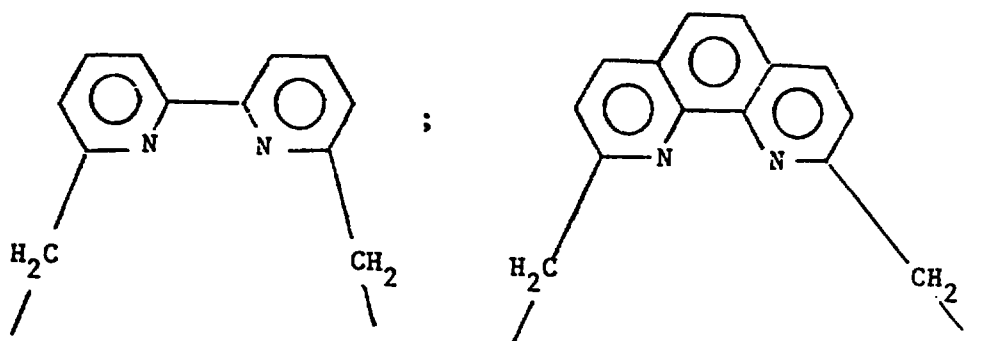


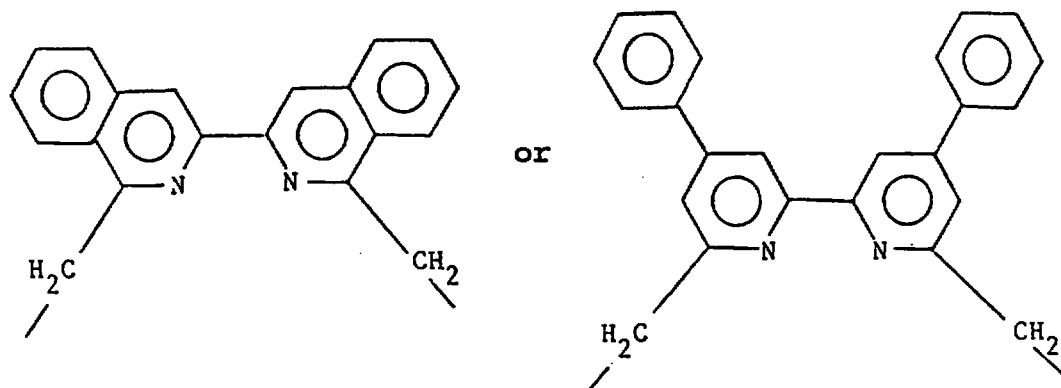
5 A specific class of these macropolycyclic compounds consists of the compounds of formula I in which Z is nitrogen, (A) and (B) are two mono- or polyoxyethylenated, preferably dioxiethylenated, chains and (C) corresponds to one of the above formulae.

These new macropolycyclic compounds also include the compounds of formula I in which (A) and (B) each represent the group



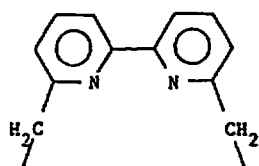
10 and (C) is one of the following groups:



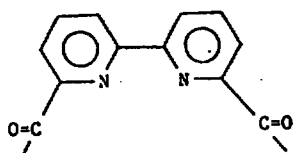


Another specific class of these new macropolycyclic compounds consists of the compounds of formula I in which Z is nitrogen and (A), (B) and (C) are identical and represent one of the above heterocycles, especially 2,2'-bipyridine and phenanthroline.

The macropolycyclic compounds of formula I, in which the molecular unit(s) are phenanthroline, anthracene, bipyridines or quinolines, are new compounds with the exception of the compounds of formula I in which (A) and (B) are each a chain of formula $-(CH_2)_2-O-(CH_2)_2-O-$ and (C) is the group of formula:



or the group of formula



which are described in Chem.

15 Ber., 111, 200-204 (1978).

The macropolycyclic compounds of formula I can be obtained by known chemical processes, mainly using condensation and/or addition reactions.

20 There may be mentioned, as examples of such processes, especially the processes described in Patent FR 70 21079 (2052947) and Patents US 3,888,877, 3,966,766 and 4,156,683. These processes are especially suitable

for producing compounds of formula I in which Z is nitrogen or phosphorus.

To obtain compounds of formula I in which Z is carbon and R is as defined above, analogous condensation processes will be used.

In all the cases, it is sufficient to use, as starting materials, chemical compounds each containing one of the radicals (A), (B) or (C) and end groups capable of being substituted or containing radicals which can easily be removed.

By way of example, it will be shown that the compound of formula I in which Z is carbon, R is the hydroxyl group and (A), (B) and (C) each represent 2,2'-bipyridine can be obtained by condensation of 6,6'-dilithiobipyridine with 6,6'-dicyano-2,2'-bipyridine, hydrolysis of the cyano group of the macrocycle thus obtained and condensation of the resulting product with 6,6'-dilithiobipyridine.

According to a preferred procedure, the new macropolycyclic compounds of formula I in which Z is nitrogen and (A) and (B) are polyoxyethylenated chains can be obtained by reaction:

- of the nitrogenous macrocycle consisting of the two polyoxyethylenated chains with
- the hydrocarbon chain containing the said molecular unit, the said chain having cleavable end groups such as, for example, halo groups.

This coupling reaction is advantageously carried out in an anhydrous solvent, for example dimethyl sulphoxide (DMSO) or acetonitrile, optionally in the presence of a reducing agent, such as sodium hydride or sodium carbonate. The macropolycyclic compound is then obtained in the form of a sodium salt.

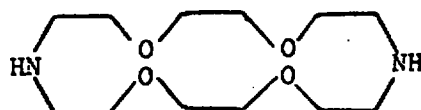
The reaction is preferably carried out at a temperature below the boiling point of the solvent, for example between 60 and 100°C.

In order to obtain the free macropolycyclic compound, the sodium salt thus obtained can be reacted with silver nitrate to form the corresponding

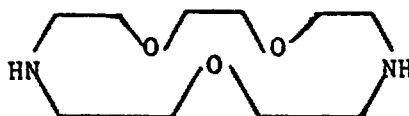
macropolycyclic silver complex which is then treated with a stream of H_2S . The precipitate formed is then neutralized with an $N(CH_3)_4OH$ solution and extracted with methylene chloride.

5 It will be noted that the macropolycyclic rare earth complex according to the invention can be obtained from the free complex or from a salt of the latter, for example the sodium salt.

10 In the above process, it is possible to advantageously use as macrocycle any one of the nitrogenous monocyclic macrocycle compounds described in Patent US 3,966,766, the preferred compounds being: 1,7,10,16-tetraoxa-4,13-diazacyclooctadecane of general formula:



15 or 1,7,16-trioxa-4,13-diazacyclodecane of general formula:



20 It will be noted that the preferred complexes according to the invention, obtained from the above macrocycles, can be regarded as derivatives of cryptates of 222 or 221 type, in which the ether units in at least one of the hydrocarbon chains have been substituted by energy donor units (in general aromatic or polyaromatic units which can contain heteroatoms).

25 The europium cryptates of 222 or 221 type have very low dissociation rates in aqueous solutions, although their formation constant is relatively low ($K = 10^{6.8}$ and $10^{5.9}$ respectively for the cryptates 221 and 222 - Inorganic Chem., 1981, 20, p. 616 and J.A.C.S., 1980, 102, p. 2278). Such cryptates do not correspond to the stability criteria defined in the prior art. On the 30 other hand, substitution of ether units by energy donor

units, which provides the preferred complexes according to the invention, does not modify the dissociation characteristic of these cryptates but makes it possible, by excitation of the energy donor unit in its absorption
5 band, to obtain a greatly enhanced fluorescence which is characteristic of the cryptated rare earth.

When the rare earth cryptates, which are the subject of the present invention, are used to specifically label biologically active molecules with the aid of
10 a covalent bond, they can be substituted on one or more of their constituent atoms by one or more substituents which are sufficiently accessible and which have one or more molecular units which make possible covalent coupling with the biological molecule under operating
15 conditions which are compatible with its biological integrity.

Among these molecular units, there may be mentioned, as non-limiting examples, alkylamino, arylamino, isothiocyano, cyano, isocyano, thioccyano, carboxyl,
20 hydroxyl, mercapto, phenol, imidazole, aldehyde, epoxide, thionyl halide, sulphonyl halide, nitrobenzoyl halide, carbonyl halide, triazo, succinimido, anhydride, haloacetate, hydrazino or dihalotriazinyl radicals and the like (Biol. Chem., 245, 3059 (1970)). The length of
25 the arm bonding the macrocyclic complex to the molecule of biological interest can vary, for example, from 1 to 20 atoms and can contain carbon atoms but also heteroatoms such as N, O, S or P. The invention therefore also relates to biological complexes consisting of a
30 biological molecule which is associated by coupling or adsorption with a macropolycyclic complex according to the invention.

In order to carry out the coupling it is possible to use any reactant described to that end in the literature and to separate the labelled molecule from the
35 unreacted macropolycyclic complex by any suitable separation means (gel filtration, for example).

Among the molecules of biological interest which can advantageously be labelled with the subject of the

present invention, there may be mentioned, in a non-limiting way, antigens, antibodies, monoclonal antibodies, fragments and antibody-fragment combinations, drugs, receptors, hormones, hormonal receptors, bacteria,
5 steroids, amino acids, peptides, vitamins, viruses, nucleotides or polynucleotides, in hybridization methods, enzymes and their substrates, lectins, nucleic acids, DNA and RNA.

The macropolycyclic rare earth complexes according to the present invention find an important application as fluorescent labels in immunological determinations, both in so-called competition and so-called excess determination methods, in the homogeneous or heterogeneous phase, described in the prior art (Landon, Ann.
10 Clin. Biochem., 1981, 18, p. 253 and Soini, Clin. Chem., 25, 353, 1979).

In the heterogeneous methods, it is possible to advantageously use:

- tubes coated with antibodies which are specific for
20 the substance to be determined and to read off the fluorescence by the methods described above, directly through the tube (Clin. Chem., 29, 60, 1983),
- or another solid phase, in particular a narrow strip
25 or a gelatinous film on which a medium containing a specific antibody has been deposited beforehand, the fluorescence being read off at an angle different from the excitation and from the reflection of the exciting wave or directly through the support, if
30 the latter is transparent.

It is also possible, because of the line spectrum of these labels, to detect several antigens simultaneously, either by using cryptates of different rare earths, whose fluorescence lines do not overlap (for
35 example, Tb and Eu), or by using conventional fluorescent labels (fluorescein or rhodamine) and labels according to the present invention.

Another application relates to immunochemistry, where the fluorescence of the labelled cell is detected

by microscopy as described by R.C. Nairn in "Fluorescent Protein Tracing", Longman Group Ltd., 1976, also with the possibility of carrying out multidetection.

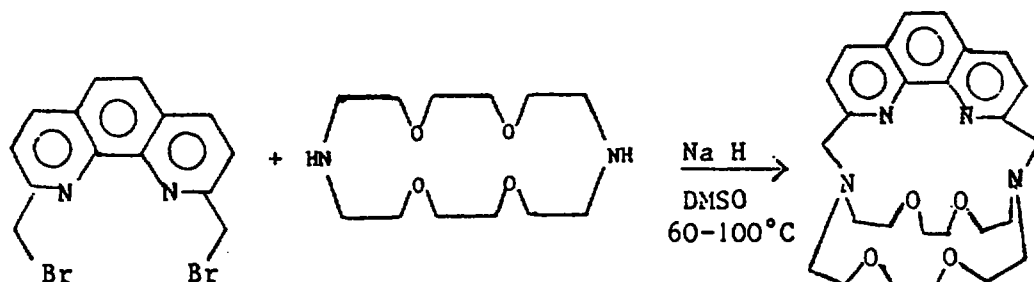
Another application of the rare earth complexes according to the invention relates to cytology and cell classifiers, where the use of a label having a line spectrum at high wavelength, coupled to that of conventional labels, makes it possible to carry out multi-parameter analyses.

Furthermore, with a given cryptand and different rare earth ions, it is possible, with a single excitation wavelength which is that of the molecular unit which transfers the energy and which can be generated with a single source (laser, for example), to obtain two fluorescence line spectra characteristic of the two cryptated ions (for example Tb and Eu) and thus disclosing the respective biological molecules to which the cryptates are fixed.

Another application relates to the use of rare earth cryptates according to the present invention in the field of genetic engineering, for example as indicators in hybridization reactions, such as those described in Patent Applications EP-A-0,070,685 and 0,070,687.

The invention will now be described in greater detail by the non-limiting illustrative examples below.

Example 1 Preparation of (22)phenanthroline: 2,9-[N,N',N,N'-bis(3,6-dioxaoctamethylene)bis(aminomethyl)]-1,10-phenanthroline (hereinafter called: (22)phen)



di(CH₂Br)phen

N₂O₄ macrocycle

(22)phen

The solvent used in this synthesis is DMSO which has been dried for several days over a 4Å molecular sieve

and optionally distilled under vacuum.

1 mmol (366 mg) of dry and freshly chromatographed dibromomethylphenanthroline compound ($\text{di}(\text{CH}_2)\text{Br-phen}$) was dissolved in 10 to 20 ml of DMSO. It was transferred to a dry dropping funnel; the volume was made up to 100 ml with dry DMSO.

100 to 200 mg of NaH in oil (Fluka) were placed in a dry round-bottomed flask; 5 ml of dry toluene (or hexane) were added to the mixture, which was left to settle. The solution was evaporated under vacuum (1 torr) for 1 h. The NaH was redissolved in 20 ml of dry DMSO (H_2 given off). Heating can optionally be carried out to 50°C .

1 mmol of the N_2O_4 macrocycle (262 mg) was dried and 10 to 20 ml of dry DMSO were added.

The NaH and N_2O_4 macrocycle solutions were each introduced into a dropping funnel and the volume was made up to 100 ml with dry DMSO.

100 ml of dry DMSO were placed in a three-necked, round-bottomed flask. This round-bottomed flask was equipped with two dropping funnels, a reflux condenser and a drying system (silica gel). The round-bottomed flask was heated to $60\text{--}100^\circ\text{C}$ with magnetic stirring.

The contents of the two dropping funnels were added dropwise and simultaneously over 1 to 2 h.

The reaction mixture was heated for 1 h after addition of the reactants. The mixture was then distilled under vacuum to remove the solvent.

It is possible to add a small quantity of water to remove the hydrides and bases.

The reaction mixture was brought to dryness; a dry or pasty red residue was obtained.

100 to 200 ml of CH_2Cl_2 were added; the paste was triturated at room temperature for at least 30 min. The residue was filtered and washed. The organic liquid phase was retained and evaporated under vacuum. The residue obtained was dried under vacuum (1 torr, at least 30 min).

100 ml of ethyl ether were added to the residue,

mixing was carried out and the mixture was left to settle; this operation was repeated 3 to 4 times.

In order to remove the remainder of the N_2O_4 monocycle more completely, 50 ml of hexane and 1 ml of CH_3OH were added to the residue; the solution was evaporated under vacuum (gentle heating) until the appearance of cloudiness; the solution was allowed to settle and the operation was repeated several times.

The red residue was tested by slab chromatography (Polygram Alox N/UV₂₅₄ from Macherey-Nagel), and migration in 9/1 CH_2Cl_2/CH_3OH medium gave the following values:

(22)Phen $R_f = 0.4-0.8$

N_2O_4 Monocycle $R_f = 0.2-0.5$

The residue was chromatographed on a column (20 cm, ϕ 15 cm) packed with neutral activated aluminium oxide 90, 70-230 mesh (Merck), the eluent consisting of the mixtures:

CH_2Cl_2/CH_3OH (5 %), $CHCl_3/C_2H_5OH$ (5 %), CH_3OH /hexane 9:1

Yields of 12 to 35 % depending on the operating conditions.

There was thus obtained the sodium salt of (22)phen of formula $[Na^+C(22phen)Br \cdot H_2O]$ whose elemental analysis was the following:

found: C 53.00 H 6.21 N 9.44

calculated: C 53.16 H 6.18 N 9.55

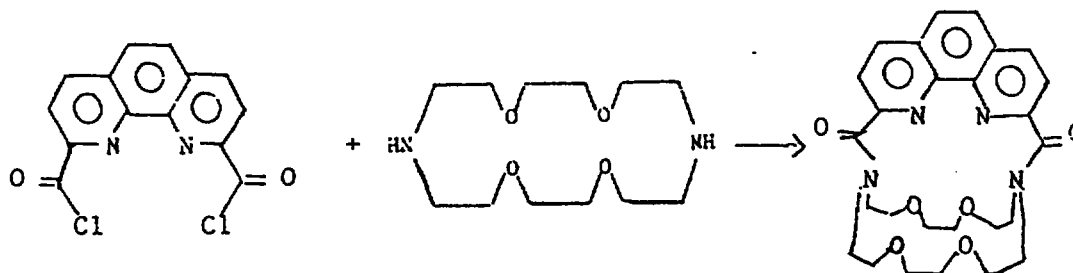
B - Formation of the $[Eu^{3+}C(22)phen]$ complex

0.07 mol (19.4 mg) of anhydrous $EuCl_3$ were dissolved in 4 ml of dry CH_3CN and the solution was heated at reflux for 1 h 30. 35 mg of the sodium salt of (22)phen obtained above, in 2.5 ml of CH_3CN , were added. The mixture was heated at reflux for 2 h 30. After having left the mixture standing overnight at 4°C, the yellow precipitate was filtered.

The precipitate collected (11 mg) shows a strong fluorescence both when it is in solution in H_2O and in CH_3OH (UV 254 nm).

No modification in the excitation and emission spectra was observed after three months in aqueous solution at a concentration below 10^{-4} mol/l.

Example 2 Preparation of (22)phenanthrolinamide: 2,9-[N,N',N,N'-bis(3,6-dioxaoctamethylene)bis(amino-carbonyl)]-1,10-phenanthroline (hereinafter called (22)phenamide)



5 di(COCl)phen (22) (22)phenamide

The solvent used in this synthesis is acetonitrile distilled over P_2O_5 . The reaction was carried out in anhydrous glass equipment.

305 mg of dry, recrystallized di(COCl)phen
10 (1 mmol) were dissolved in 50 ml of CH₃CN; after one
night, the solution was filtered and transferred to a
dropping funnel; the volume was made up to 90 ml with
CH₃CN.

524 mg of (22) (2 mmol) were dried under vacuum
15 (< 1 torr; overnight). The product was dissolved in 90 ml
of CH₃CN and transferred to a dropping funnel.

The two dropping funnels were mounted on a 2 to 3 l round-bottomed flask containing 1 litre of CH_3CN . The simultaneous addition of the two reactants was carried out over 2 h to 2 h 30 with rapid stirring (magnetic). Stirring was continued for 30 min after addition. The solvent was removed under vacuum at 50°C . The residue was dried for at least 1 h at $P < 1$ torr. The residue was mixed for 30 min in 300 ml of CH_2Cl_2 and filtered. The filtrate was evaporated to dryness. It was chromatographed on neutral activated Alox 90 (column 30 cm, ϕ 1.5 cm), the eluent being CH_2Cl_2 containing 1 % CH_3OH . The first product eluted was recovered.

310 mg of (22)phenamide were recovered with a

yield of 63 %. The product was recrystallized from a 1/1 toluene/hexane mixture.

Melting point: 300-302°C

IR amide band at 1630 cm⁻¹

5 Mass spectrum: M⁺ at 494 for MW = 494.55

Elemental analysis: C ₂₆ H ₃₀ N ₄ O ₆			
	C	H	N
Calculated	63.15	6.11	11.33
	(59.8	5.7	10.5
Found			
	(59.9	5.9	10.6

10

B - Formation of the [Eu³⁺C(22)phenamide] complex

45 mg (0.17 mmol) of anhydrous EuCl₃ were dissolved in 10 ml of dry CH₃CN. 80 mg (0.16 mmol) of (22)phenamide, dissolved in 2 ml of CH₂Cl₂, were heated at reflux for 3 h, 50 ml of CH₃CN were added and the mixture was heated at the boiling point of the CH₃CN (CH₂Cl₂ is removed by evaporation). The Eu³⁺ solution was then added.

The mixture was heated at reflux for 2 h and then was left to stand at room temperature. The white precipitate was recovered by filtration (50 mg). It showed a strong red fluorescence (λ excitation: 254 nm) as well as in solution in H₂O, CH₃OH and DMSO.

Elemental analysis for EuC(22)phenamide(OH)Cl₂·2H₂O

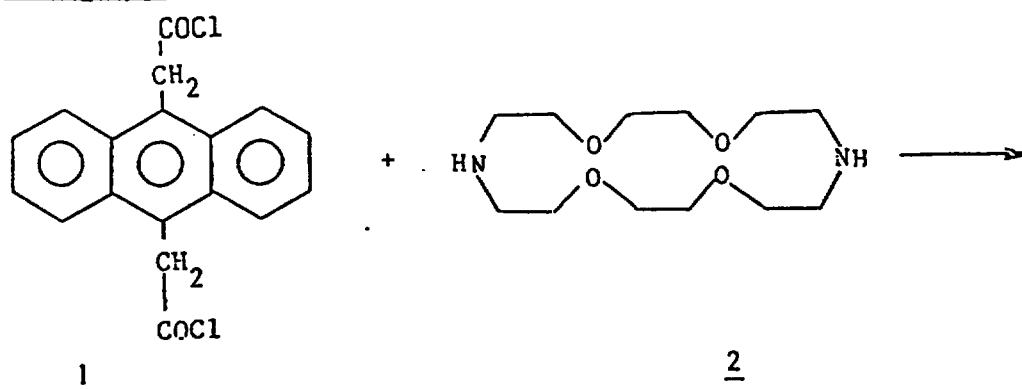
C ₂₆ H ₃₇ N ₄ O ₁₀ Cl ₂ Eu			
MW = 788.47			
	C	H	N
Calculated	39.61	4.73	7.11
Found	(40.1	4.9	7.2
	(40.3	4.8	7.1

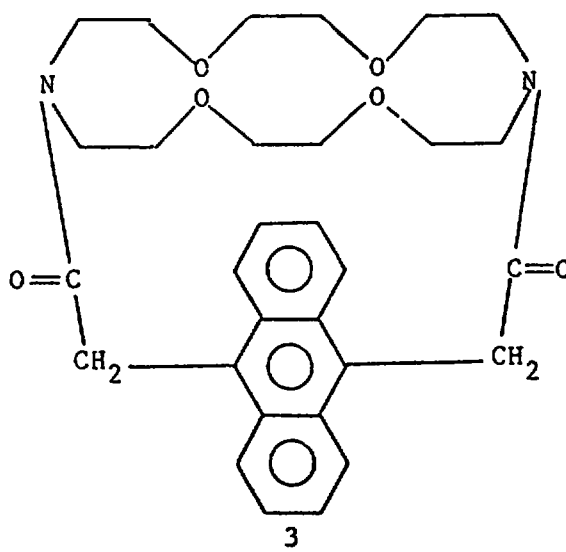
25

No modifications in the excitation and emission spectra of this complex were observed after three months in aqueous solution at a concentration below 10⁻⁴ mol/l.

30

Example 3 Preparation of (22)anthracenamide: 9,10-[N,N',N,N'-bis(3,6-dioxaoctamethylene)bis(carbamoylmethyl)]anthracene





In this example, the high-dilution method is employed.

- 0.564 g of the acid dichloride 1 (1.7 mmol) was dissolved in 70 ml of anhydrous CH_2Cl_2 and introduced into a 100 ml dropping funnel,
- 0.446 g of the (22) macrocycle, i.e. 1.7 mmol, and 0.47 ml of triethylamine (2×1.7 mmol) were also dissolved in 70 ml of anhydrous CH_2Cl_2 and introduced into a 100 ml dropping funnel.

0.4 ml of toluene and 0.15 l of anhydrous methylene chloride were introduced into a 3 l, three-necked flask. The simultaneous introduction into the three-necked flask of the two reactants prepared above in the dropping funnels was carried out over 5 h (14 ml/h).

The organic phase was collected, concentrated to a few millilitres and then passed through a SiO_2 column under pressure (column diameter 3.5 cm; height = 17 cm; eluent CH_2Cl_2 /1 % methanol).

A pale-yellow, crystalline, fluorescent product was obtained.

Yield: 45 %

Thin layer chromatography (TLC)

CH_2Cl_2 /2 % methanol solvent; SiO_2 ; $R_f = 0.6$

^1H NMR: 5/5 CDCl_3 / CD_2Cl_2 solvent at 200 MHz

	ppm	2.53 (m)	}	24 H, HCH ₂ + OCH ₂ crown
		2.93 (m)		
		3.18 (m)		
		3.42 (m)		
5		4.02 (d)	}	J _{AB} = 16 Hz, 4H, OCCH ₂ N
		δ _A = 4.62		
		δ _B = 5.03		
		7.6 (m)	}	aromatic
		8.43 (d)		
10		8.82 (d)		

microanalysis: C₃₀H₃₆O₆N₂

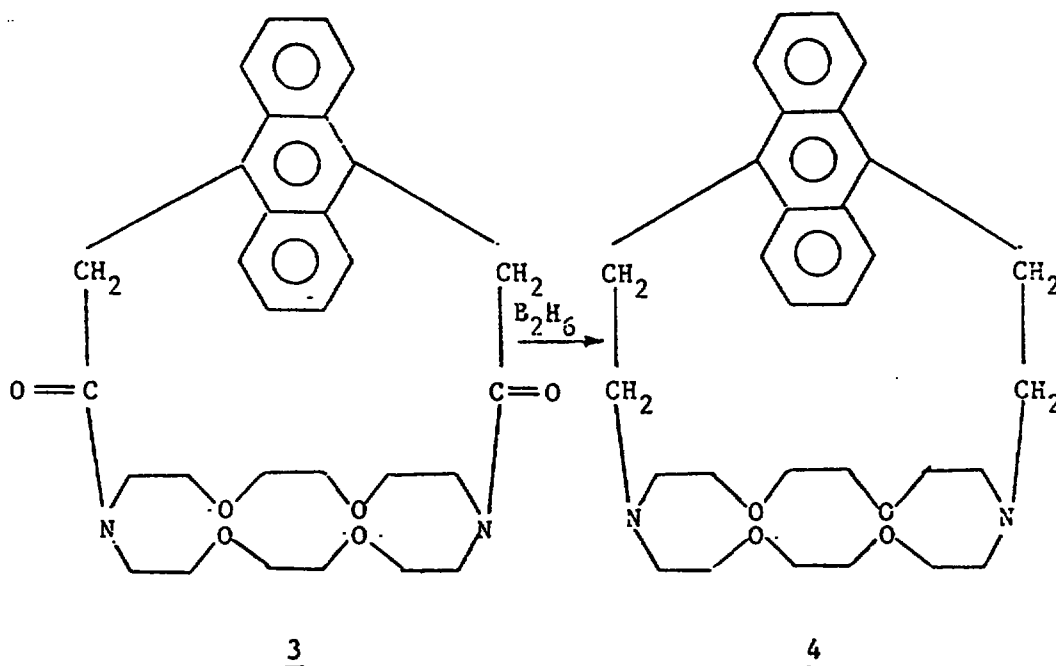
Calculated C 69.70 H 6.97 N 5.38

Found C 69.07 H 7.00 N 5.22

Molecular weight 520.55

15 The acid dichloride 1 used as starting material in this example can be obtained by the processes described by M.W. Miller et al. in R.W. Amidon and P.O. Tawney, J.A.C.S., 77, 2845 (1955) or by B.M. Mikhailov, Izvest. Akad. Nank., SS. Osdel Khim. Nank., 1948, 420-6; CA, 42, 6350.

20 Example 4 Preparation of (22)anthracene: 9,10-[N,N',N,N'-bis(3,6-dioxaoctamethylene)bis(aminoethyl)]anthracene



380 mg of the diamide 3 (0.73 mmol) were partially dissolved in 20 ml of anhydrous THF contained in a round-bottomed flask.

5 The round-bottomed flask, fitted with a reflux condenser, was placed under an argon atmosphere and 8 ml of 1.1M B₂H₆ in anhydrous THF were added at room temperature.

10 The whole reaction mixture was kept under reflux overnight and the excess diborane was destroyed at 0°C with a few drops of distilled water.

The solvents were evaporated and 40 ml of a 6N HCl solution were added to the residual white solid.

Heating was carried out at reflux for 30 h under an argon atmosphere and the solution became dark green.

15 The solution was left to cool, the water was then evaporated and the resulting solid was dried using a vane pump for 1 h and then dissolved in 50 ml of distilled water; 50 ml of CH₂Cl₂ were added and the two phases were agitated, and then the aqueous phase was separated and
20 basified at 0°C with an aqueous LiOH solution to a pH of 13; a solid precipitated. A further 50 ml of CH₂Cl₂ were added, the two phases were agitated and were left to settle. The organic phase was recovered and dried over MgSO₄.

25 The crude product was passed through an alumina column and elution was carried out with CH₂Cl₂/1 % methanol. A crystalline product was recovered which is pure on a thin layer chromatography plate.

Yield: 62-70 %

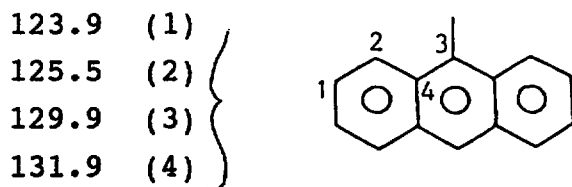
30 TLC: Al₂O₃; eluent CH₂Cl₂/6 % MeOH

R_f: 0.33

Melting point: > 260°C

¹³C NMR : CDCl₃ solvent

ppm 25.2 (aromatic CH₂)
35 54.3)
56.3) NCH₂ macrocycle + branch
69.3)
40 70.0) OCH₂ macrocycle



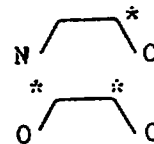
5

¹H NMR: CDCl₃ solvent

ppm 2.39 (t), 8H, NCH₂, macrocycle

2.55 and 2.76 (m) - 2 × 4H, OCH₂

2.89 and 3.19 (m) - 2 × 4H, OCH₂



3.03 and 3.82 (t) - 2 × 4H, -CH₂-CH₂

7.47 and 8.31 (AB) - 4H

7.50 and 8.36 (AB) - 4H

aromatic H

microanalysis: C₃₀H₄₀O₄N₂ (molecular weight 492.6)

Calculated C 73.13 H 8.18 N 5.6

Found C 73.06 H 8.04 N 5.2

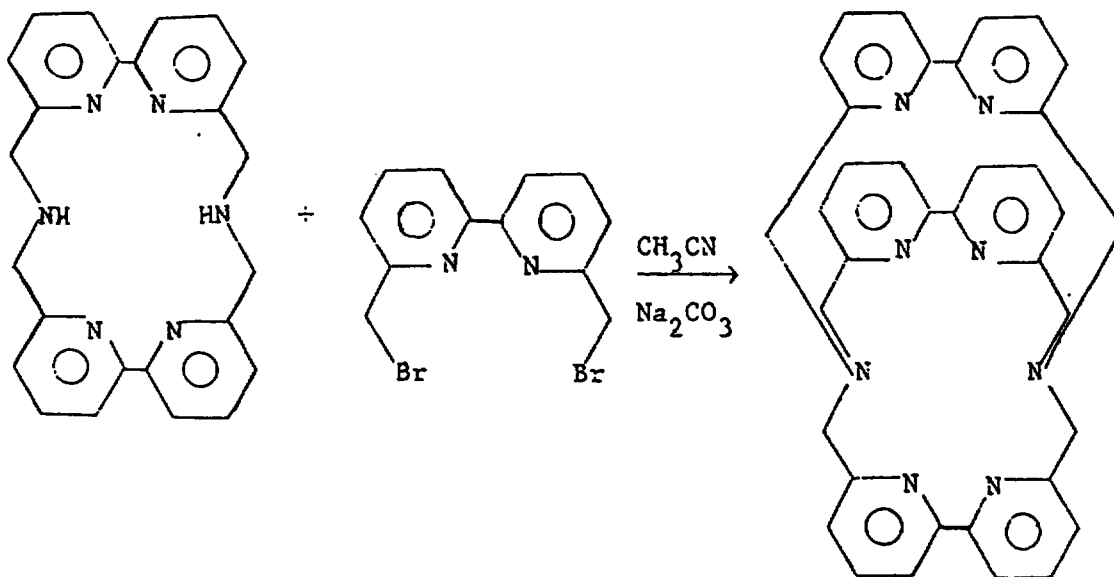
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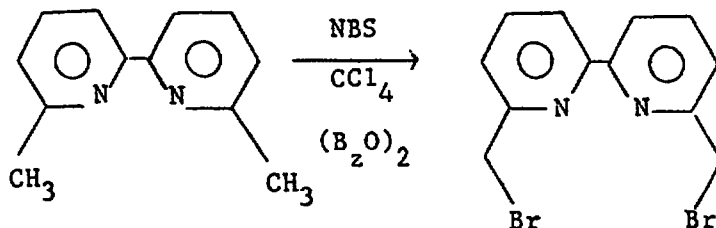
By carrying out the reaction according to the procedure described in Example 1, the [Eu³⁺C(22)anthracene] complex was prepared.

20

Example 5 Preparation of the macropolycyclic compound of formula (7): 6,6',6'',6''', 6'''', 6''''-bis[nitrilotri(methylene)]tris(2,2'-bipyridine) and of the corresponding europium complex



A - Preparation of 6,6'-bis(bromomethyl)-2,2'-bipyridine



molecular weight 184

$C_{12}H_{12}N_2$

molecular weight 342

$C_{12}H_{10}N_2Br_2$

A mixture of 6,6'-dimethyl-2,2'-bipyridine (2.76 g, 15 mmol) and of N-bromosuccinimide (5.10 g, 28.6 mmol) in CCl_4 (150 ml) is maintained at reflux for 30 min. Benzoyl peroxide (30 mg) was then added to the mixture, the mixture was again held at reflux for 2 h and the succinimide was then filtered off. The solution was cooled to 0°C and the solid filtered and washed with methanol to give the bisdibromide in the form of a white crystalline solid (1.65 g).

Yield 32 %; melting point 180-181°C.

The CCl_4 solution was concentrated and chromatographed on a column (silica gel) by elution with a 98/1 dichloromethane/methanol mixture to give:

- 6,6'-bis(dibromomethyl)-2,2'-bipyridine, 0.9 g, 12 %
- 6,6'-bis(bromomethyl)-2,2'-bipyridine, 1.38 g, 27 %
- 6-methyl-6'-bromomethyl-2,2'-bipyridine, 0.55 g, 14 %

B - Preparation of the macropolycyclic compound

A mixture of the bisbipyridine macrocycle of formula (5) (0.15 g, 0.38 mmol) (described in J. Org. Chem., 1983, 48, 4848) and of Na_2CO_3 (0.4 g) in acetonitrile (200 ml) was heated at reflux and then a solution of 6,6'-bis(bromomethyl)-2,2'-bipyridine (0.12 g, 0.38 mmol) was added over a period of 3 h. The mixture was then maintained at reflux for 20 h. The Na_2CO_3 was filtered off and the filtrate was evaporated. The residue was filtered through a short alumina column, eluting with 98/2 CH_2Cl_2 /MeOH, to produce the sodium salt

of the trisbipyridine macrobicycle in the form of a white solid (0.16 g; 73 %; melting point greater than 270°C).

microanalysis: $C_{36}H_{30}N_8 \cdot NaBr$ (677.6)

	Calculated	C	63.81	H	4.46	N	16.53
5	Found	C	63.79	H	4.48	N	16.49

1H NMR: $CDCl_3$ solvent

- 3.85 (s, 6 CH_2);
 7.33 (dd, $J = 7.2, 1.2$, 6H, H-C(5), H-C(5'))
 7.82 (t, $J = 7.2$, 6H, H-C(4), H-C(4'))
 10 7.90 (dd, $J = 7.2, 1.2$, 6H, H-C(3), H-C(3'))

A mixture of silver nitrate $AgNO_3$ (15 mg, 0.08 mmol) and of the sodium salt obtained above (20 mg, 0.03 mmol) was heated with 5 ml of CH_3OH for 30 min. The methanol was evaporated and the resulting complex was
 15 purified by passing through a silica gel column with CH_2Cl_2/CH_3OH as eluent (96/4).

The silver complex thus obtained (20 mg) was dissolved in a water/methanol mixture (1/1-5 ml) and treated with a stream of H_2S for 15 min. The precipitate
 20 formed was centrifuged, the solution was neutralized with 0.1N $N(CH_3)_4OH$ and extracted with methylene chloride (3.5 ml). The solution was dried over $MgSO_4$ and evaporated; the resulting solid was filtered through silica gel, CH_2Cl_2/CH_3OH (96/4), to give the free complex
 25 (13 mg; 86 %) having the following characteristics:

1H NMR: $CDCl_3$ solvent

- 3.82 (s, 12H, CH_2)
 7.44
 7.78 ABX system, $J = 8, 7.5$ and 0.9 Hz)
 30 7.83

microanalysis: $C_{36}H_{30}N_8$ (574.7)

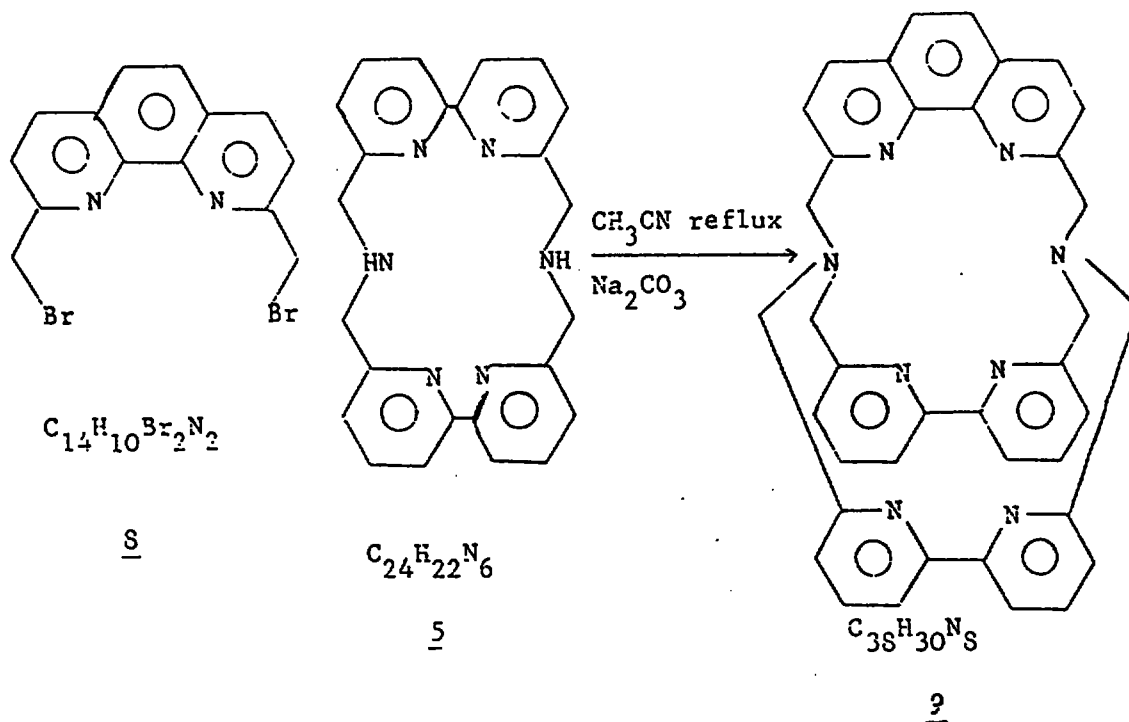
	Calculated	C	75.24	H	5.26	N	19.50
	Found	C	75.37	H	4.98	N	19.41

The $[Eu^{3+}C(\text{trisbipyridine macropolycycle})]$ complex
 35 was prepared from the macropolycyclic compound obtained, the reaction being carried out according the procedure described in Example 1.

The excitation and emission wavelengths characteristic of this complex appear in the table below.

Example 6 Preparation of the bis(bipyridine)phenanthroline macropolycyclic compound of formula (9): 2,9-[N,N',N,N'-[bis(2,2'-bipyridine-6,6'-dimethyl)]bis(amino-methyl)]-1,10-phenanthroline and of the corresponding europium complex

5



0.158 mmol of the bispyridine macrocycle (5) was weighed into a 500 ml, two-necked, round-bottomed flask. 0.75 mmol of Na_2CO_3 (approximately 5 times excess) and 105 ml of freshly distilled CH_3CN were added. The mixture was heated at reflux and with magnetic stirring for 30 min.

An equimolecular dibromophenanthroline solution in 100 ml of acetonitrile was then added dropwise.

The fairly slow (2 h 10 min) addition took place while maintaining reflux and magnetic stirring.

The mixture was then heated for a further 18 h under the same conditions. The solution obtained was evaporated to dryness. The crude product, in solution in CH_2Cl_2 , was washed with water.

(Thin layer chromatography on alumina: eluent: $CH_2Cl_2/MeOH$: 90/10).

This crude product was purified on a standardized

alumina column (activity II-III). The eluent is $\text{CH}_2\text{Cl}_2/\text{MeOH}$: 98/2.

microanalysis: $\text{C}_{38}\text{H}_{30}\text{N}_8 \cdot \text{NaBr} \cdot \text{H}_2\text{O}$ (719.6)

	Calculated	C	63.42	H	4.45	N	15.57
5	Found	C	62.77	H	4.46	N	15.31

^1H NMR: CDCl_3 solvent

- 3.91 (s, 8H, $\text{CH}_2\text{-bpy}$)
- 4.07 (s, 4H, $\text{CH}_2\text{-phen}$)
- 7.36 (dd, $J = 7.2, 1.3$, 4H, H-C(5), H-C(5') of the
10 bipyridine)
- 7.64 (d, $J = 8.2$, 2H, H-C(3), H-C(8) of the
phenantroline)
- 7.78 (s, 2H, H-C(5), H-C(6) of the phenantroline)
- 7.83 (t, $J = 7.2$, 4H, H-C(4), H-C(4') of the
15 bipyridine)
- 7.91 (dd, $J = 7.2, 1.3$, 4H, H-C(3), H-C(3') of the
bipyridine)
- 8.27 (d, $J = 8.2$, 2H, H-C(4), H-C(7) of the
phenantroline)

20 The $[\text{Eu}^{3+}\text{C}(\text{bis}(\text{bipyridine})\text{phenanthroline macro-
polycycle})]$ complex was prepared from the macropolycyclic
compound thus obtained by carrying out the reaction
according to the procedure described in Example 1.

25 The excitation and emission wavelengths charac-
teristic of this complex appear in the table below.

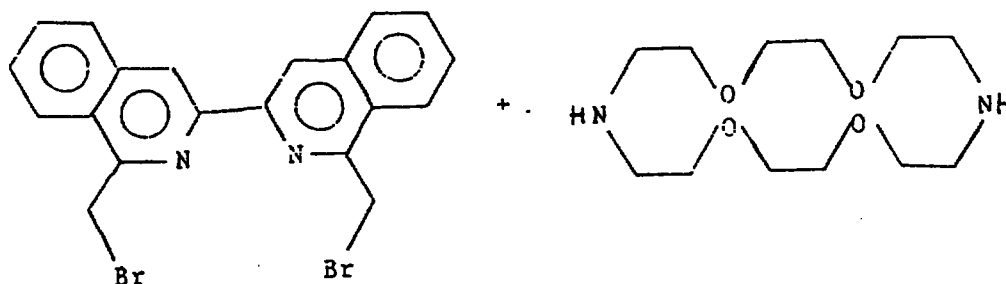
Example 7

30 By carrying out the reaction according to the
procedure of Example 1 with the macropolycyclic compounds
(22)pyridinamide and (222_B), the macropolycyclic com-
plexes below were respectively obtained:

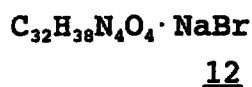
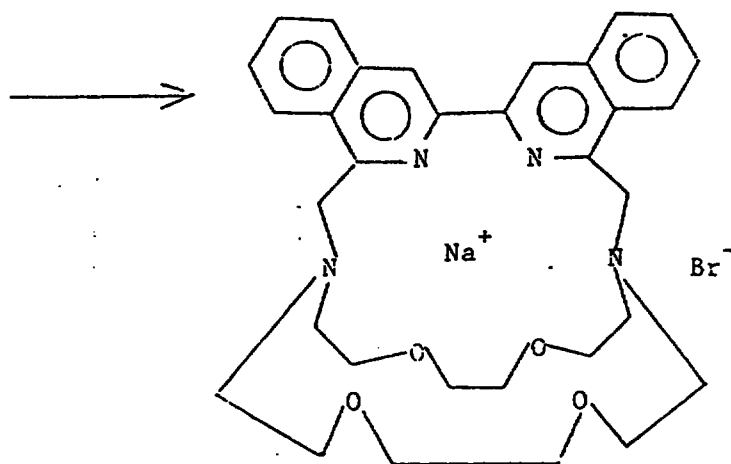
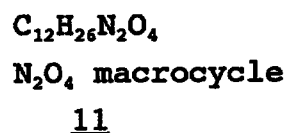
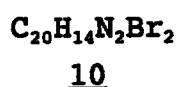
$[\text{Eu}^{3+}\text{C}(22)\text{pyridinamide}]$

$[\text{Tb}^{3+}\text{C}(222_{\text{B}})]$

Example 8 Preparation of the (22)bisisoquinoline macro-
polycyclic compound of formula (12): 1,1'-[N,N',N,N'-
bis(3,6-dioxaoctamethylene)bis(aminomethyl)-3,3'-biiso-
quinoline and of the corresponding europium complex

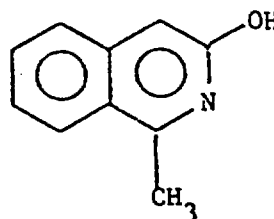


5



10 a) Preparation of 1,1'-bis(bromomethyl)-3,3'-biiso-
quinoline of formula 10

This compound was manufactured from 1-methyl-3-hydroxyisoquinoline of formula



1-Methyl-3-hydroxyisoquinoline was prepared by cyclization of N-(±)-(phenylethyl)diethoxyacetamide. 72 g of this starting material, prepared according to the procedure described by H. Fukumi et al. in "Heterocycles", 9(9), 1197 (1978) and purified by distillation under reduced pressure (140°C-0.1 mm Hg), were poured, dropwise and with stirring, over one hour, into 400 ml of concentrated H₂SO₄ cooled to 10°C. The reaction mixture was cooled during the addition so that its temperature does not exceed 10°C. The reaction mixture was then stirred for 10 h at room temperature and then poured in 600 g of ice. After filtration, the light solution was neutralized with 20 % aqueous ammonia with efficient cooling. The yellow precipitate was filtered, washed with water and dried under vacuum. 41.5 g of 1-methyl-3-hydroxyisoquinoline were obtained (yield 90 %).

The 1-methyl-3-hydroxyisoquinoline thus obtained was then tosylated.

A suspension of 41.5 g of 1-methyl-3-hydroxyisoquinoline was stirred with pyridine (250 ml) with cooling in an ice bath. p-Toluenesulphonyl chloride (70 g, 1.5 equivalent) was then progressively added over 30 min. The yellow starting material has then disappeared. The reaction was monitored by thin layer chromatography. As soon as the reaction was complete, water (50 ml) was added and stirring was carried out for a further hour. The mixture was diluted in 700 ml of water and neutralized with solid Na₂CO₃. The precipitate formed was filtered, carefully washed with water and dried. 70.5 g of a non-hygroscopic, air-stable product were obtained (yield 86 %).

The 1-methyl-3-(p-tolylsulphonyloxy)isoquinoline thus obtained was then coupled according to the procedure described by M. Tiecco et al. in Synthesis, 736, 1984.

1 l of dimethylformamide (DMF), 236.3 g of triphenylphosphine and 53.5 g of NiCl₂·6H₂O, which formed a dark blue-green solution, were introduced into a three-necked, round-bottomed flask equipped with a magnetic

stirrer and purged with argon. When the temperature of the oil bath reached 50°C, 14.64 g of zinc powder were added and the colour of the solution changed to brown-red. After one hour, the solution of the starting material (70.5 g in 200 ml of DMF), namely 1-methyl-3-(p-tolyl-sulphonyloxy)isoquinoline, was rapidly added, dropwise and with stirring, and with heating at 50°C, which heating was maintained for 6 h. After cooling to room temperature, the reaction mixture was poured into 4 l of dilute ammonia (1.5 l of H₂O and 500 ml of 20 % NH₃). A strong airstream was passed into the suspension in order to oxidize the Ni⁰(Ph₃P)₄ complex (ph = phenyl). The end of the oxidation was indicated by the disappearance of the brown colour. The suspension was filtered, washed with water and placed in 400 ml of 20 % HCl to convert the biisoquinoline into its water-insoluble hydrochloride. The suspension was stirred with two portions of 400 ml of ethyl ether to remove the triphenylphosphine and the acidic layer was filtered and washed with 100 ml of water and acetone to remove the traces of Ph₃P and of Ph₃PO. The hydrochloride was placed in a round-bottomed flask with 200 ml of 20 % aqueous ammonia and stirred overnight to release the base. The white product obtained (26 g), namely 1,1'-dimethyl-3,3'-biisoquinoline, was filtered, washed carefully with water and dried under vacuum (yield 81 %). This product had little solubility in the majority of solvents and was slightly soluble in CHCl₃ and THF.

The 1,1'-dimethyl-3,3'-biisoquinoline thus obtained was then brominated to form 1,1'-bis(bromomethyl)-3,3'-biisoquinoline.

1.20 g of 1,1'-dimethyl-3,3'-biisoquinoline were dissolved in 500 ml of CCl₄ at reflux and N-bromosuccinimide (2.26 g, 3 equivalents) was added. After 10 min, 10 g of 2,2'-azobis(2-methylpropionitrile) were added. The reaction was monitored by thin layer chromatography. Two other portions of initiator (each 10 mg) were added over 1 h. After 3 h, the solution was evaporated to dryness and the residue was treated with 150 ml of

methanol, stirred for 30 min and filtered. The solid obtained was washed with 100 ml of methanol. The filtration cake was washed under vacuum, dissolved in boiling toluene (100 ml) and quickly filtered. The product precipitated overnight in a refrigerator (1.28 g - yield 68 %).

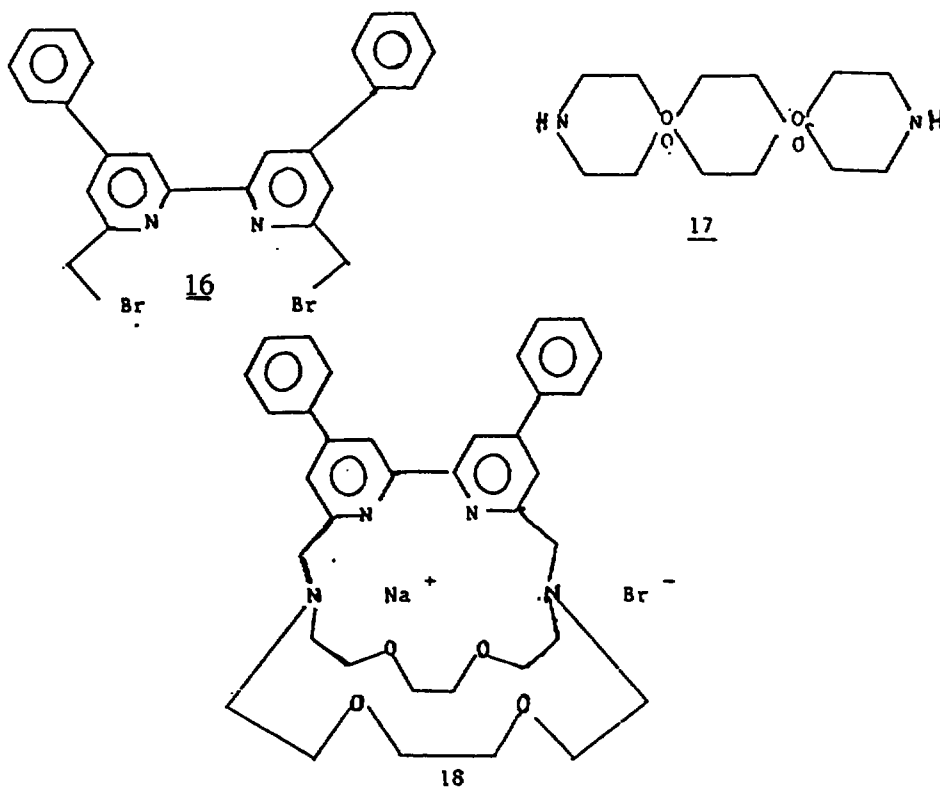
b) Preparation of the macropolycyclic compound of formula 12

A suspension of 1,1'-bis(bromomethyl)-3,3'-biisoquinoline in CH_3CN (100 ml) was added, over 3 h, to a stirred mixture of 1.416 g of the N_2O_4 macrocycle and 3.39 g of Na_2CO_3 in 250 ml of CH_3CN . Stirring was maintained for 20 h. After filtration and washing with CH_3CN and evaporation, a crude product was obtained which was chromatographed twice, first of all through alumina (eluent: 3 % CH_3OH in CHCl_3 , and then 10 % CH_3OH in CHCl_3 , volume/volume) and then through silica gel (eluent: 10 % CH_3OH in CHCl_3). The yield for the two purifications was 0.289 g, i.e. 14 %. Another purification was carried out by crystallization from an ethanol/ether mixture by vapour diffusion.

c) Preparation of the $[\text{Eu}^{3+}\text{C}(22)\text{biisoquinoline}]$ complex

24,5 mg (1 equivalent) of anhydrous europium nitrate were dissolved in 0.5 ml of anhydrous CH_3CN . The sodium complex of (22)biisoquinoline (37.0 mg) in 0.3 ml of CH_3CN was added and the resulting mixture was treated according to the procedure described in Example 2 B. The pale-yellow precipitate obtained was diluted in 3 ml of acetonitrile and heated for 2 h. The solution was left at room temperature for several days. A yellow crystalline product of Formula 12 was obtained.

Example 9 Preparation of the (22)diphenylbipyridine macropolycyclic compound of Formula 18: 6,6'-[N,N',N,N'-bis(3,6-dioxaoctamethylene)bis(aminomethyl)]-4,4'-diphenyl-2,2'-bipyridine



5 a) Preparation of 6,6'-bis(bromomethyl)-4,4'-diphenyl-2,2'-bipyridine (16)

This compound was prepared from commercially available 4,4'-diphenyl-2,2'-bipyridine according to the procedure below:

10 - 6,6'-dimethyl-4,4'-diphenyl-2,2'-bipyridine

1.5M methyllithium (3 equivalents) was added dropwise, under a nitrogen atmosphere, to a stirred suspension of 4,4'-diphenyl-2,2'-bipyridine (4 g, 13 mmol) in anhydrous tetrahydrofuran (THF), cooled with an ice bath. After this addition, the solution was stirred for a further 30 min under the same conditions. The dark red mixture obtained was then heated and stirred at 40°C for 3 h under nitrogen.

20 An excess of water was added slowly at 0°C under nitrogen. The organic phase was separated and the aqueous phase was extracted three times with dichloromethane.

Manganese dioxide (20 to 40 times the weight of the starting material) was then added to the orange organic solution. The mixture was stirred at room temperature for 30 to 50 min. The reaction was monitored by thin layer chromatography (Al₂O₃ - eluent: toluene).

Magnesium sulphate was then added to the reaction mixture, which was stirred for an additional 30 min. The reaction mixture was then filtered and the filtrate evaporated. The residue was chromatographed on alumina (eluent: toluene/hexane 1/1) and two products were obtained:

6,6'-dimethyl-4,4'-diphenyl-2,2'-bipyridine (0.85 g; 19 %)

6-monomethyl-4,4'-diphenyl-2,2'-bipyridine (0.45 g; 10.8 %)

- 6,6,'-Dimethyl-4,4'-diphenyl-2,2'-bipyridine N,N'-dioxide

A solution of m-chloroperbenzoic acid (0.57 g - 3.3 mmol) in chloroform (60 ml) was added gradually to a 250 ml round-bottomed flask containing a solution, stirred and cooled in an ice bath, of the above compound (0.28 g, 0.83 mmol) in chloroform (60 ml). Stirring was continued for 3 h, during which the mixture was left to return to room temperature.

The reaction mixture was treated with a stirred aqueous sodium bicarbonate solution (3.3 mmol) for 30 min.

The organic phase was separated and evaporated under vacuum. The residue was redissolved in dichloromethane (CH₂Cl₂) and passed through a basic alumina column. It was chromatographed a second time through standard alumina in order to entirely purify the product (0.21 g; 68 %).

- 6,6'-Bis(acetoxymethyl)-4,4'-diphenyl-2,2'-bipyridine

0.21 g (0.57 mmol) of the above compound in acetic anhydride (1.3 ml) was heated for one hour at reflux. The solution was concentrated under vacuum and toluene was added until the azeotrope of the mixture was

formed.

The resulting solid was redissolved in dichloromethane, washed with a 10 % aqueous sodium bicarbonate solution, dried and the solvent was evaporated. The crude
5 product was passed through a silica gel column and eluted with dichloromethane to give 0.126 g (49 % yield) of the expected compound.

- 6,6'-Bis(bromomethyl)-4,4'-diphenyl-2,2'-bipyridine 16

A stirred solution of the above compound
10 (0.053 g; 0.117 mmol) and of 47 % hydrobromic acid (0.9 ml) was heated for 4 h at 130°C.

The solution was then cooled in a methanol/ice bath. 15 ml of water and 40 ml of chloroform were added thereto and then, gradually, a saturated sodium carbonate
15 solution was added thereto until the pH of the solution becomes alkaline.

The organic phase was separated and the aqueous phase was extracted with chloroform (2 times 10 ml). All the organic fractions were combined and evaporated. The
20 residue was chromatographed on an alumina column with chloroform as eluent. 40 mg (yield 69 %) of the dibromide of Formula 16 were obtained.

b) Preparation of the [Na⁺C(22)diphenylbipyridine Br⁻] salt of formula 18

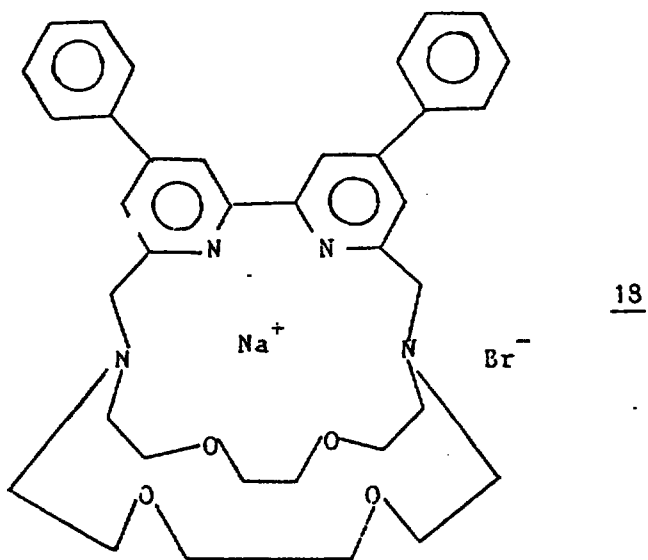
25 The reaction was carried out according to the procedure described in Example 5 B, using:

21.2 mg (0.081 mmol) of the N₂O₄ macrocycle of Formula 17

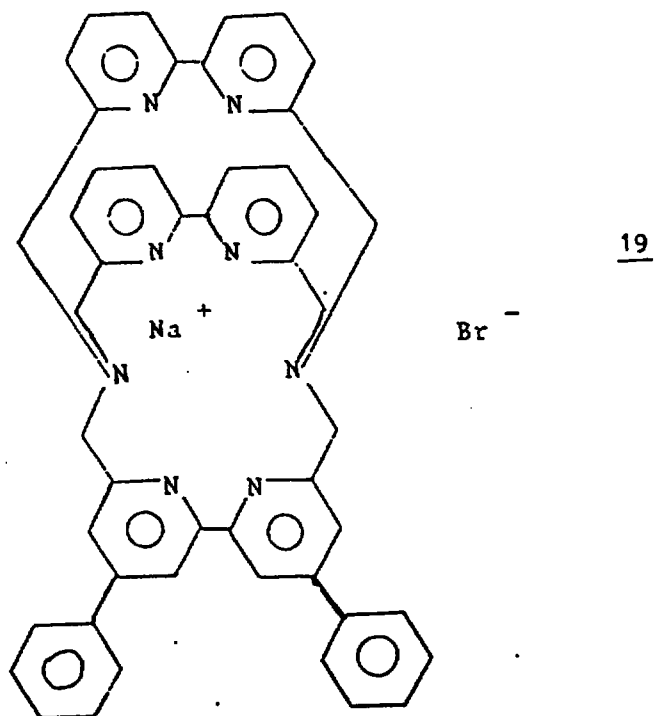
43 mg (0.40 mmol) of sodium carbonate

40 mg (0.081 mmol) of the dibromide of Formula 16.

30 29.8 mg (yield 53 %) were obtained of the complex of Formula 18.



Example 10 Preparation of the diphenylbipyridinebisbipyridine macropolycyclic complex of Formula 19: 6,6'-[N,N',N,N'-[bis(2,2'-bipyridine-6,6'-dimethyl)]bis-(aminomethyl)]-4,4'-diphenyl-2,2'-bipyridine



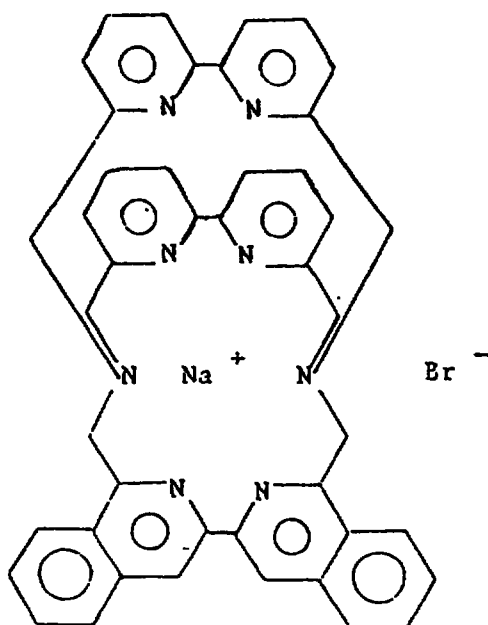
The procedure of the above example was repeated using the bispyridine macrocycle of Formula (5) (Example 5) in place of the N_2O_4 macrocycle of Formula 17.

There were used:

- 5 24.7 mg (0.063 mmol) of the bispyridine macrocycle 5
31.1 mg (0.063 mmol) of the dibromide of Formula 16
0.1 mg (0.94 mmol) of sodium carbonate.

The reaction lasted 23 h and the compound of Formula 19 was obtained with a yield of 20 %.

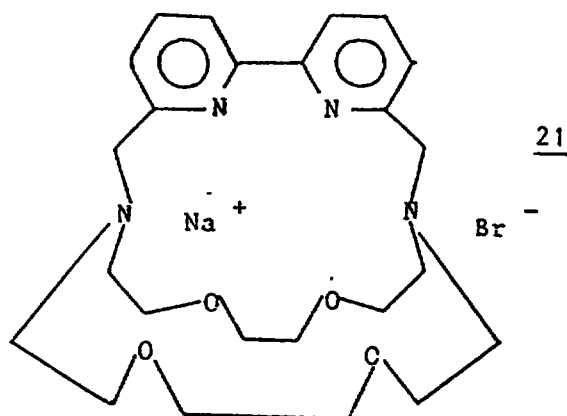
- 10 Example 11 Preparation of the isoquinolinebisbipyridine macropolycyclic compound of Formula 20: 1,1'-[N,N',N,N'-[bis(2,2'-bipyridine-6,6'-dimethyl)]bis(aminomethyl)]-3,3'-biisoquinoline



- 15 The procedure of Example 8 was repeated using the bisbipyridine macrocycle of Formula 5 in place of the N_2O_4 macrocycle of Formula 11. Equimolecular amounts of the compound of Formula 5 and of the dibromide of Formula 10 were used and the compound of Formula 20 was obtained.
- 20 Example 12 Preparation of the (22)bipyridine macropolycyclic compound

The reaction was carried out according to the procedure of Example 5, using the N_2O_4 macrocycle of

Formula 11 in place of the bisbipyridine macrocycle of Formula 5, and the macropolycyclic compound of Formula 21 was obtained:



Example 13

5 By carrying out the reaction according to the procedure of Example 1 with the macropolycyclic compounds obtained according to Examples 9 to 12, the macropolycyclic complexes below were respectively obtained:

10 [Eu³⁺C(22)bipyridine]: Eu³⁺ complex of 6,6'-[N,N',N,N'-bis(3,6-dioxaoctamethylene)bis(aminomethyl)]-2,2'-bipyridine

[Eu³⁺C(bpy.bpy.biisoq.)]: Eu³⁺ complex of 1,1'-[N,N',N,N'-(bis2,2'-bipyridine-6,6'-dimethyl)bis(aminomethyl)]-3,3'-biisoquinoline

15 [Eu³⁺C(22)diph.bpy]: Eu³⁺ complex of 6,6'-[N,N',N,N'-bis(3,6-dioxaoctamethylene)bis(aminomethyl)]-4,4'-diphenyl-2,2'-bipyridine

20 [Tb³⁺C(22)bipyridine]: Tb³⁺ complex of 6,6'-[N,N',N,N'-bis(3,6-dioxaoctamethylene)bis(aminoethyl)]-2,2'-bipyridine

Likewise, by carrying out the reaction according to the procedure of Example 5, the [Eu³⁺C(trisphenanthroline macropolycycle)] complex (Eu³⁺ complex of 2,2',2'',-9,9',9''-bis[nitrilotri(methylene)]tris(1,10-phenanthro-

line) was prepared by using 6,6'-bis(bromomethyl)-phenanthrolinediyl)diamine macrocycle. The corresponding macropolycyclic compound had the characteristics below:
microanalysis: $C_{42}H_{38}N_8 \cdot NaBr \cdot H_2O$ (767.6)

5 Calculated: C 65.71 H 4.17 N 14.6
 Found: C 65.23 H 4.26 N 13.10

1H NMR: $CDCl_3$ solvent

 4.03 4.45 (very broad, AB, 12H, CH_2)
 7.66 (d, $J = 8.1$, 6H, H-C(3), H-C(8))
10 7.78 (s, 6H, H-C(5), H-C(6))
 8.27 (d, $J = 8.1$, 6H, H-C(4), H-C(7))

Fluorescent characteristics of the cryptates of the invention

a) Excitation peaks for a given emission wavelength

15 For each complex, the excitation wavelengths were determined, for a given emission wavelength, characteristic of the rare earth ion of the complex; the excitation peaks recorded do not correspond to those of the rare earth ion on its own. Moreover, it has been
20 observed that, by exciting the complex at the wavelength corresponding to the excitation peak determined above, the fluorescence maximum was characteristic of the rare earth ion.

 The above determinations were carried out with a
25 Perkin-Elmer LS 5 spectrometer under the operating conditions shown in Table I below.

b) Measurement of the lifetime τ of the europium and terbium cryptates

 The emission spectrum of the peak situated
30 between 610 and 620 nm for europium and between 540 and 550 for terbium was recorded on an LS 5 spectrometer, the solution being excited in one of the absorption peaks. Recording was carried out in "phosphorescence" mode and the value of the window was fixed at 1 ms = t_g . The
35 recordings were carried out at several t_d (time delay) values, i.e. 0.1, 0.2, 0.3, 0.4 and 0.5 ms. The intensity of the peak I_t was measured and τ was determined by the formula:

$$I_t = I_0 e^{-\frac{t}{\tau}}$$

$$\tau = \frac{0.434t}{\log I_0 - \log I_t}$$

- 5 By recording the curve $\log I_t$ as a function of td , it is possible to determine I_0 and to calculate τ . The results obtained appear in Table II below.

TABLE I
EXCITATION AND EMISSION CHARACTERISTICS OF THE COMPLEXES OF THE INVENTION
(Perkin-Elmer LS 5)

Complex	Concentration (solvent)	Width of slits	Expansion factor	EXCITATION		EMISSION	
				Given emis- sion λ	Recorded ex- citation λ	Given ex- citation λ	Fluorescence maximum ob- served at:
[Eu ³⁺ C(22)phen]	3.7 · 10 ⁻⁶ mol/l (water)	2.5/20 nm	10	615 nm	(290 nm (peak) (312 nm (shoulder) (330 nm (")	290 nm	(615 nm (" (peak) (593 (second peak)
[Eu ³⁺ C(22)phen- amide]	2.5 · 10 ⁻⁴ mol/l (water)	2.5/10 nm	3	613 nm	290 nm (peak) 323 nm (peak) 333 nm (shoulder) 345 nm (shoulder)	290 nm	613 nm 590 nm
[Eu ³⁺ C(trisbi- pyridine mac- ropolycycle)]	7 · 10 ⁻⁷ mol/l (0.1M phosphate buffer, pH 7.5)	2.5/10 nm	15	617 nm	maximum at 300 nm	300 nm	maximum at 617 nm
[Eu ³⁺ C(phenan- throlinabis- bipyridine)]	2.8 · 10 ⁻⁵ mol/l (water)	2.5/10 nm	2			280 nm	590 nm 613 nm

TABLE I (continuation)

EXCITATION AND EMISSION CHARACTERISTICS OF THE COMPLEXES OF THE INVENTION

(Perkin-Elmer LS 5)

Complex	Concentration (solvent)	Width of slits	Expansion factor	EXCITATION		EMISSION	
				Given emis- sion λ	Recorded ex- citation λ	Given ex- citation λ	Fluorescence maximum ob- served at:
[Eu ³⁺ C(22)bipyr- idine]	10 ⁻⁵ M (water)	2.5/10 nm	10	617 nm	(237 nm (peak) (303 nm (peak)	310 nm	(617 nm (592 nm
[Eu ³⁺ C(22)biiso- quinoline]	10 ⁻⁵ 0.01M tris buffer, pH 7	2.5/10 nm	0.5	617 nm	355 nm (peak) 325 nm (peak)	325 nm	617 nm 592 nm
[Eu ³⁺ C(22)bi- phenylbi- pyridine]	10 ⁻⁵ M H ₂ O	2.5/10 nm	5	617 nm	254 (peak) 323 (peak)	320 nm	617 nm 592 nm
[Eu ³⁺ C(bisbi- pyridine bi- isoquinoline)]	0.06 mg/ 2.4 ml of water	2.5/10 nm	20	617 nm	310 (peak)	310 nm	617 nm 592 nm

TABLE I (continuation)

EXCITATION AND EMISSION CHARACTERISTICS OF THE COMPLEXES OF THE INVENTION

(Perkin-Elmer LS 5)

Complex	Concentration (solvent)	Width of slits	Expansion factor	EXCITATION		EMISSION	
				Given emis- sion λ	Recorded ex- citation λ	Given ex- citation λ	Fluorescence maximum ob- served at:
[Eu ³⁺ C(22)anthracene]	0.2 mg in 3 ml of water	2.5/10 nm	0.2	613 nm	287 nm (peak) 317 nm (shoulder)	287 nm	590 nm (peak) 613 nm (peak)
[Eu ³⁺ C(22)pyridinamide] *	2.10 ⁻⁴ mol/l (DMSO)	2.5/5 nm	40	-	-	290 nm	613 nm 590 nm
[Tb ³⁺ C(222 _B)] **	10 ⁻³ mol/l (DMSO)	2.5/20 nm	15	541 nm	290 nm (shoulder) 333 nm (peak)	290 nm	541 nm 485 nm
[Eu ³⁺ C(trisphenanthroline)]	0.3 mg/3 ml of tris buffer	2.5/10 nm	5	617 nm	272 (peak)	270 nm	617 nm 593 nm
[Tb ³⁺ C(22)bi-pyridine]	0.1 mg/2.4 ml H ₂ O	2.5/10 nm	1	617 nm	240 (peak) 305 (peak) 314 (shoulder)	320 nm	545 nm 492 nm 586 nm

- * measurement carried out in phosphorescence mode; time delay t_d = 0.1 millisecond
 t_g = 2 milliseconds
- ** measurement carried out in phosphorescence mode; time delay t_d = 0.2 millisecond
 t_g = 5 milliseconds

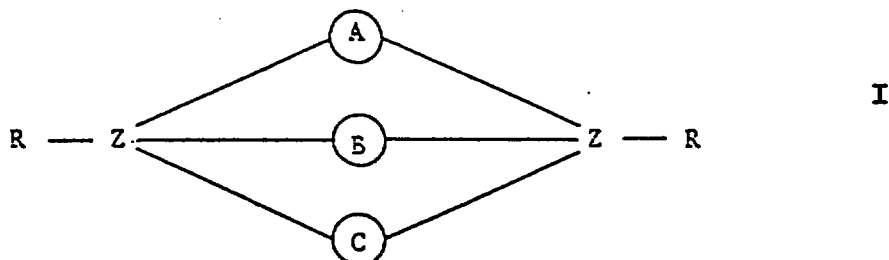
TABLE II

Macropolycyclic complex	Excitation λ nm	Medium	Concentration	τ (ms)
[Eu ³⁺ C(22)phen]	280	H ₂ O	$3.7 \times 10^{-4}\text{M}$	0.27
[Eu ³⁺ C(22)phenamide]	280	H ₂ O	$2.5 \times 10^{-6}\text{M}$	0.21
[Eu ³⁺ C(22)bipyridine]	310	H ₂ O	$1.2 \times 10^{-5}\text{M}$	0.41
[Eu ³⁺ C(22)biisoquinoline]	325	0.01M tris, pH 7	10^{-5}M	0.135
[Eu ³⁺ C(22)biphenylbipyridine]	320	H ₂ O	10^{-5}M	0.32
[Eu ³⁺ C(trisbipyridine)]	300	0.01M phosphate buffer, pH 7.4	$2 \times 10^{-7}\text{M}$	0.43
[Eu ³⁺ C(bisbipyridinebi- isoquinoline)]	311	H ₂ O	10^{-5}M	0.25
[Eu ³⁺ C(trisphenanthroline)]	272	0.01M tris, pH 7	10^{-4}M	0.32
[Tb ³⁺ C(22)bipyridine]	318	"	$4 \times 10^{-5}\text{M}$	0.72



CLAIMS for the Contracting States: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

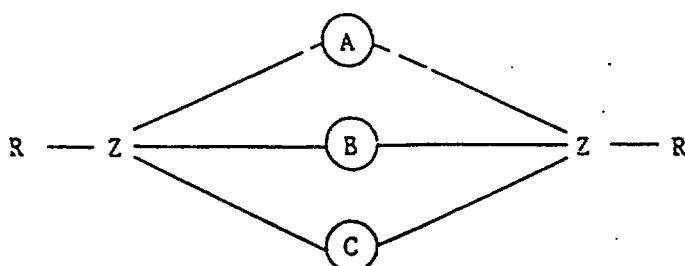
1. Application, as labels of biological products in methods of immunological detection and determination by
5 fluorescence, of macropolycyclic rare earth complexes consisting of at least one rare earth salt complexed by a macropolycyclic compound of general formula:



- 10 in which Z is an atom chosen from nitrogen, carbon or phosphorus, R does not exist or represents hydrogen, the hydroxyl group, an amino group or a hydrocarbon radical, the bivalent radicals (A), (B) and (C) are, independently from each other, hydrocarbon chains containing at least two atoms of carbon and optionally one or more
15 heteroatoms chosen from oxygen or sulphur and being optionally interrupted by a heteromacrocycle, at least one of the radicals (A), (B) or (C) moreover comprising at least one molecular unit or essentially consisting of a molecular unit, the said molecular unit having a
20 triplet energy greater than that of the emission level of the complexed rare earth ion.

2. Application according to Claim 1, characterised in that the said rare earth complexes are substituted by a radical chosen from the alkylamino, arylamino, isothio-
25 cyano, cyano, isocyano, thiocyno, carboxyl, hydroxyl, mercapto, phenol, imidazole, aldehyde, epoxide, thionyl halide, sulphonyl halide, nitrobenzoyl halide, carbonyl halide, triazo, succinimido, anhydride, haloacetate, hydrazino or dihalotriazinyl radicals.

- 30 3. As new products, the macropolycyclic rare earth complexes consisting of at least one rare earth salt complexed by a macropolycyclic compound of formula:



I

in which Z is an atom chosen from nitrogen, carbon or phosphorus, R does not exist or represents hydrogen, the hydroxyl group, an amino group or a hydrocarbon radical, the bivalent radicals (A), (B) and (C) are, independently of each other, hydrocarbon chains containing at least two atoms of carbon and optionally one or more heteroatoms chosen from oxygen and sulphur and optionally interrupted by a heteromacrocycle, at least one of the radicals (A), (B) or (C) moreover comprising at least one molecular unit or essentially consisting of a molecular unit, the said molecular unit having a triplet energy greater than that of the emission level of the complexed rare earth ion, with the proviso that, when the rare earth salt is a europium or terbium salt, Z is nitrogen, (A) is $-(CH_2)_2-O-C_6H_3R_1-O-(CH_2)_2-$ with $R_1 = H$ or NH_2 , B and C are not simultaneously one the $-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-$ radical and the other the $-(CH_2)_2-O-(CH_2)_2-$ radical, and that, when the rare earth salt is a lanthanum or praseodymium salt, Z is nitrogen, (A) is $-(CH_2)_2-O-C_6H_3NH_2-O-(CH_2)_2-$, (B) and (C) are not simultaneously $-(CH_2)_2-O-(CH_2)_2-$ and $-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-$.

4. Complexes according to Claim 3, characterised in that the hydrocarbon chains are oxyethylenated or polyoxyethylenated chains.

5. Macropolycyclic complexes according to one of Claims 3 or 4, characterised in that the rare earth ion is chosen from europium, terbium, samarium and dysprosium.

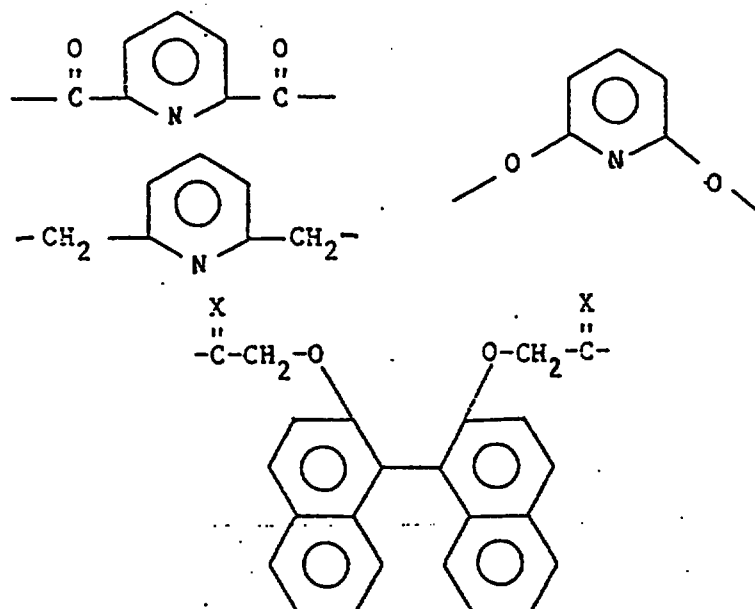
6. Macropolycyclic complexes according to any one of Claims 3 to 5, characterised in that the energy-donor unit has a phosphorescence wavelength of less than 580 nm.

7. Macropolycyclic complexes according to any one of

Claims 3 to 6, characterised in that the molecular unit is chosen from phenanthroline, anthracene, benzene, naphthalene, biphenyl and terphenyl, azobenzene, azopyridine, pyridine, the bipyridine, the biquinolines, in particular the biisoquinolines and the compounds having the formulae below:



in which X_1 and X_2 , which can be identical or different, represent oxygen or sulphur;



X being oxygen or hydrogen.

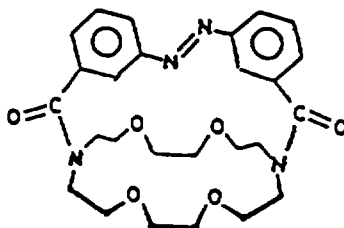
8. Macropolycyclic complex according to any one of Claims 3 to 7, characterised in that it consists of the terbium or europium ion complexed by one of the macrocyclic compounds below:

2,9-[N,N',N,N'-bis(3,6-dioxaoctamethylene)bis(aminomethyl)]-1,10-phenanthroline; 2,9-[N,N',N,N'-bis(3,6-dioxaoctamethylene)bis(aminocarbonyl)]-1,10-phenanthroline; 9,10-[N,N',N,N'-bis(3,6-dioxaoctamethylene)bis(aminoethyl)]anthracene; 9,10-[N,N',N,N',-bis(3,6-dioxaoctamethylene)bis(carbamoylmethyl)]anthracene; 1,1'-[N,N',N,N'-bis(3,6-dioxaoctamethylene)bis(aminomethyl)]-3,3'-bisisoquinoline; 6,6'-[N,N',N,N'-bis(3,6-dioxaoctamethylene)bis(aminomethyl)]-4,4'-diphenyl-2,2'-

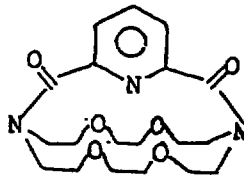
bipyridine; 6,6'-[N,N',N,N'-bis(3,6-dioxaoctamethylene)-bis(aminomethyl)]-2,2'-bipyridine; 6,6'-[N,N',N,N'-bis(3,6-dioxaoctamethylene)bis(aminocarbonyl)]-2,2'-bipyridine; 6,6',6'',6''',6'''',6'''''-bis[nitrilotri(methylene)] tris-2,2'-bipyridine; 2,2',2'',9,9',9''-bis[nitrilotri(methylene)] tris(1,10-phenanthroline); 2,9-[N,N',N,N'-bis(2,2'-bipyridine-6,6'-dimethyl)bis(aminomethyl)]-1,10-phenanthroline; 1,1'-[N,N',N,N'-bis(2,2'-bipyridine-6,6'-dimethyl)bis(aminomethyl)]-3,3'-biisoquinoline; 6,6'-[N,N',N,N'-bis(2,2'-bipyridine-6,6'-dimethyl)bis(aminomethyl)]-4,4'-diphenyl-2,2'-bipyridine.

9. Macropolycyclic complexes according to Claim 3, characterised in that they correspond to the formula (I) in which $Z = N$.

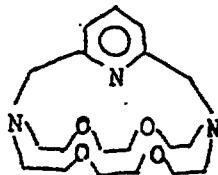
15 10. Macropolycyclic rare earth complexes consisting of at least one rare earth salt complexed by a macropolycyclic compound, characterised in that the said macropolycyclic compound is chosen from the compounds below:



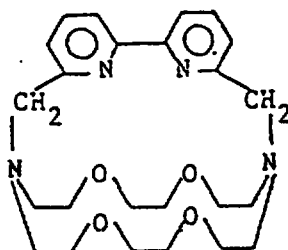
- (22)pyridinamide



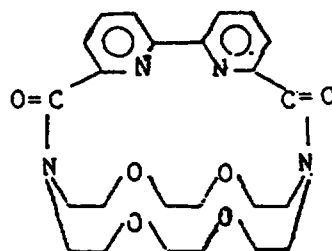
- (22) pyridinamine



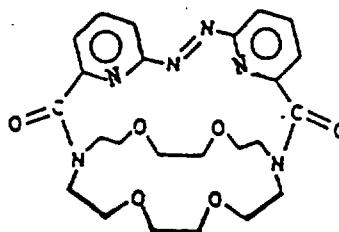
- (22)bipyridine

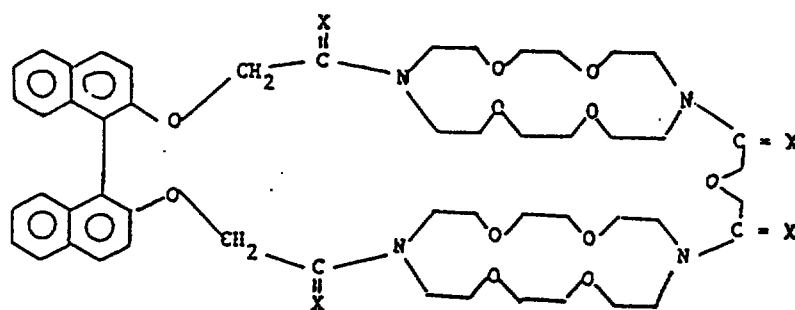
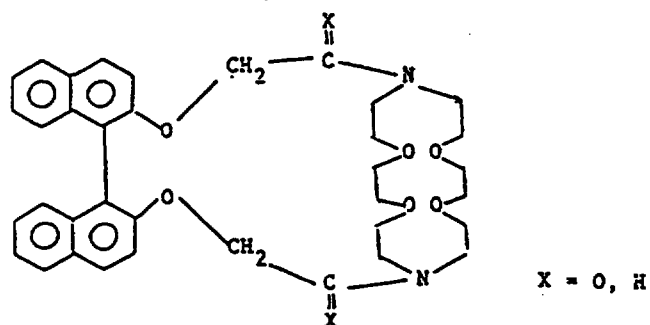
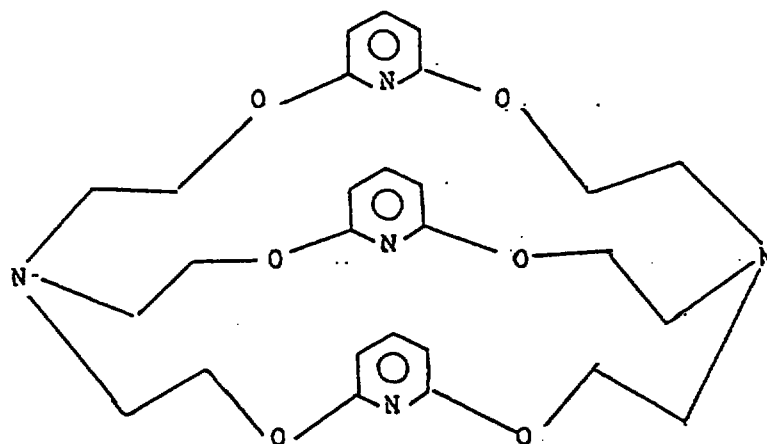


- (22)bipyridinamide



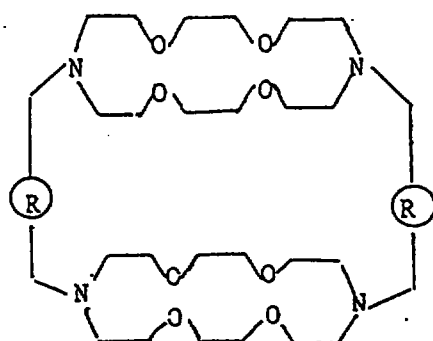
- (22)azopyridine



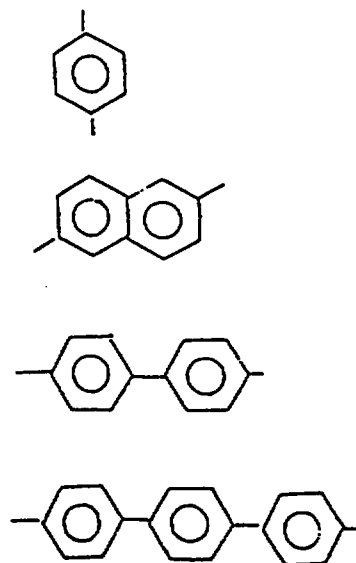




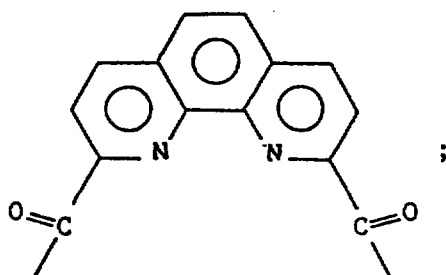
X = O, H



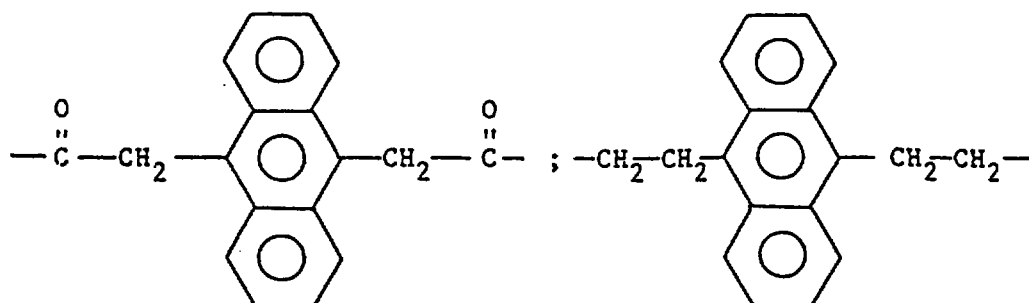
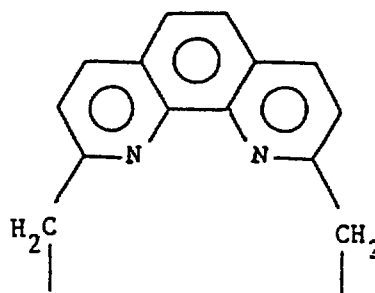
R =

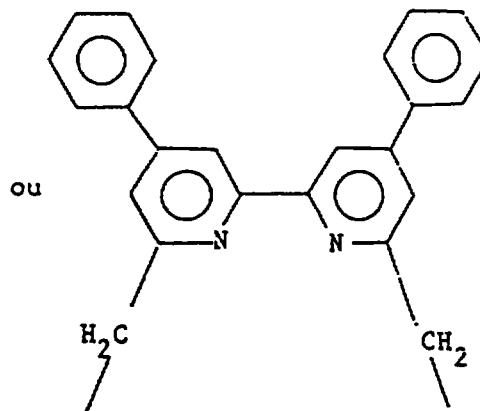
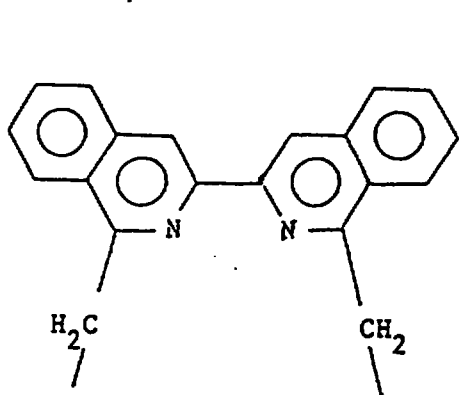
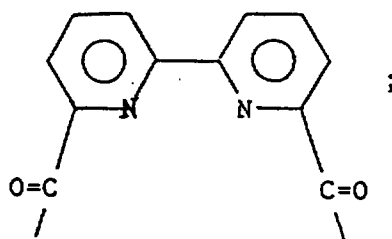
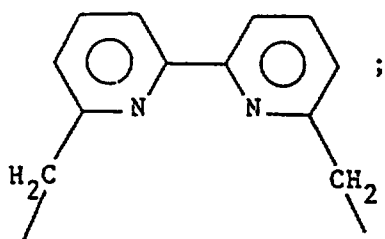


11. Macropolycyclic rare earth complexes consisting of at least one rare earth salt complexed by a macropolycyclic compound of formula I according to Claim 3, characterised in that Z = N, (A) and (B) are two chains containing one or more oxygen atoms and in that (C) corresponds to one of the formulae below:

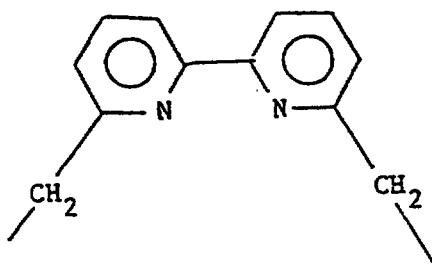


;

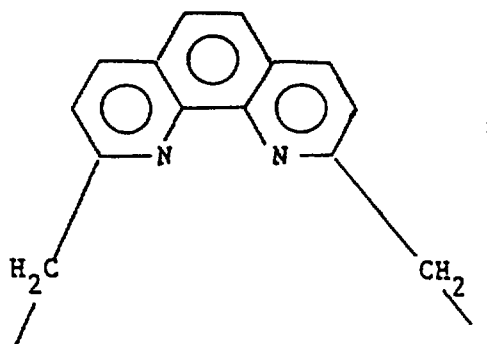
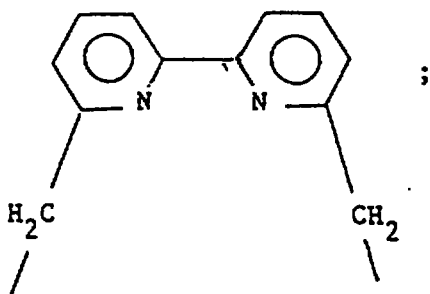


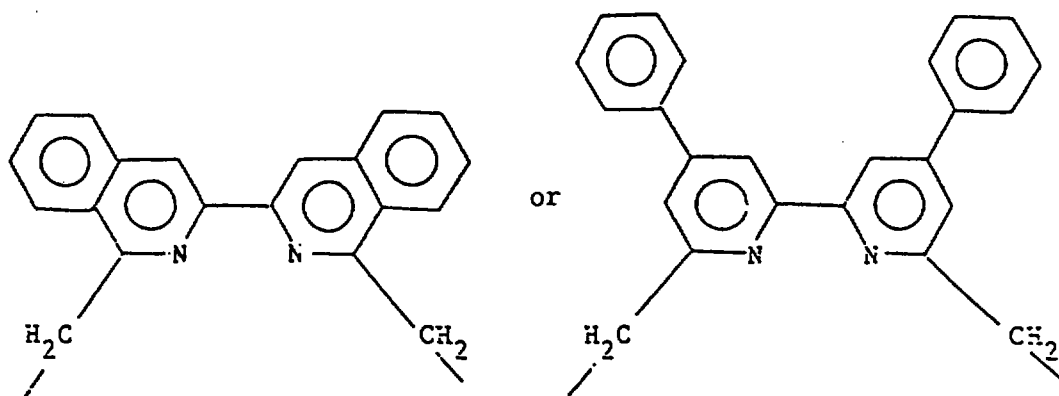


12. Macropolycyclic rare earth complexes consisting of at least one rare earth salt complexed by a macropolycyclic compound of formula I according to Claim 3, characterised in that (A) and (B) each represent the group
- 5

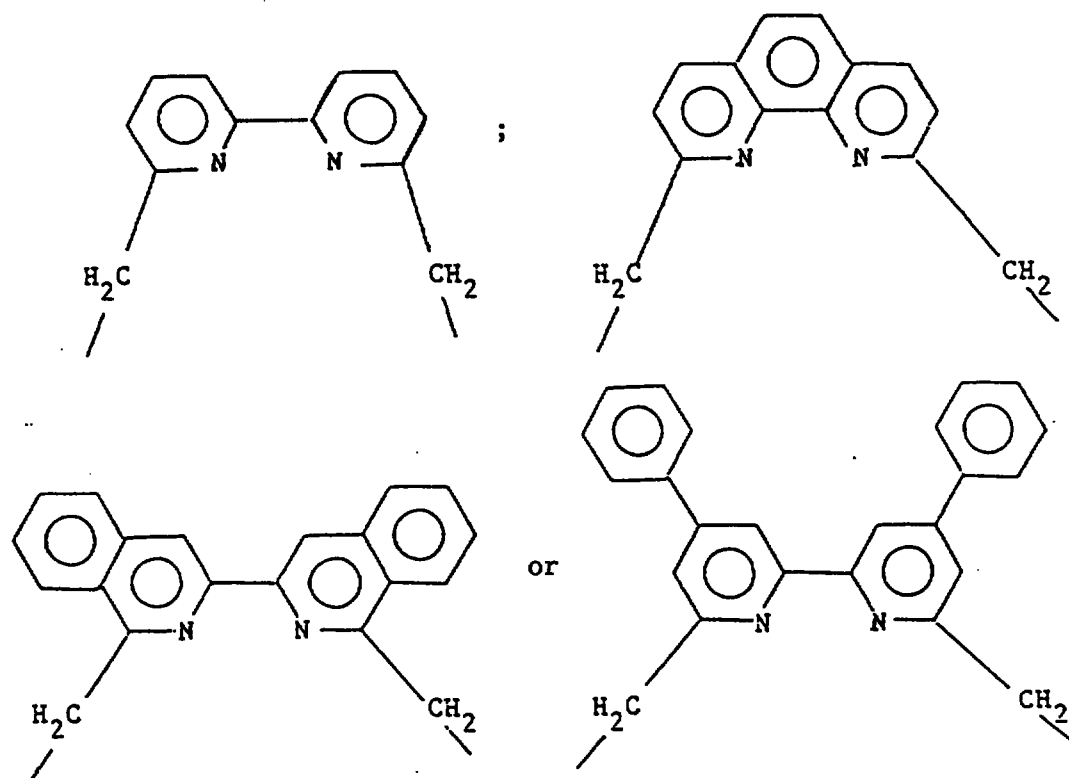


and (C) is one of the following groups:



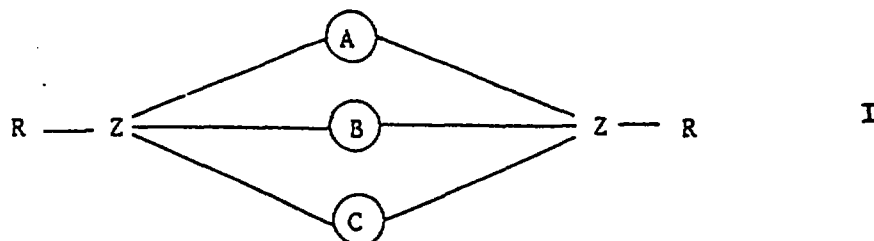


13. Macropolycyclic rare earth complexes consisting of at least one rare earth salt complexed by a macropolycyclic compound of formula I according to Claim 3, characterised in that $Z = N$; (A), (B) and (C) are identical and represent one of the heterocycles below:

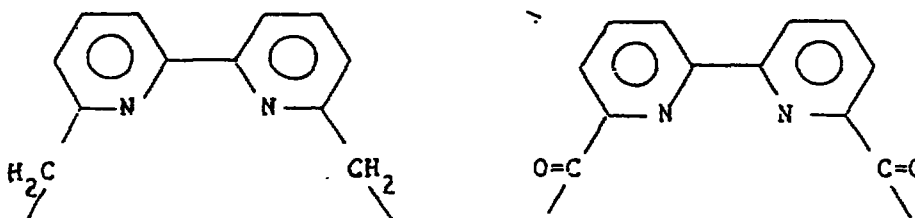




14. Macropolycyclic compound corresponding to the formula:



in which Z is an atom chosen from nitrogen, carbon or phosphorus, R does not exist or represents hydrogen, the hydroxyl group, an amino group or a hydrocarbon radical, the bivalent radicals (A), (B) and (C) are, independently of each other, hydrocarbon chains containing at least two atoms of carbon and optionally one or more heteroatoms chosen from oxygen or sulphur and optionally interrupted by a heteromacrocycle, at least one of the radicals (A), (B) or (C) comprising moreover at least one molecular unit or essentially consisting of a molecular unit, the said molecular unit having a triplet energy greater than that of the emission level of the complexed rare earth ion and being chosen from phenanthroline, anthracene, the bipyridines and the biquinolines, in particular the biisoquinolines, with the proviso that, when Z is nitrogen and (A) corresponds to one of the formulae below:



(B) and (C) are not simultaneously $-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-$.

15. Compound according to Claim 14, characterised in that Z is nitrogen and (A) and (B) are polyoxyethylenated chains.

16. Compound according to one of Claims 14 or 15,



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earth ions, characterised in that it consists in complexing at least one rare earth ion with a macropolycyclic compound such as defined in Claims 1, 3 to 13, and in exciting the molecular unit of the said compound.