



(86) Date de dépôt PCT/PCT Filing Date: 2011/07/06
(87) Date publication PCT/PCT Publication Date: 2012/01/12
(85) Entrée phase nationale/National Entry: 2013/01/03
(86) N° demande PCT/PCT Application No.: PL 2011/000070
(87) N° publication PCT/PCT Publication No.: 2012/005615
(30) Priorité/Priority: 2010/07/07 (PL P.391776)

(51) Cl.Int./Int.Cl. *C09K 11/06* (2006.01),
C07F 3/06 (2006.01), *H01L 51/50* (2006.01),
H05B 33/14 (2006.01), *H05B 33/22* (2006.01)
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(54) Titre : COMPOSES LUMINESCENTS, LEUR PROCEDE DE PREPARATION ET LEURS APPLICATIONS
(54) Title: LUMINESCENT COMPOUNDS, METHOD OF PREPARATION OF LUMINESCENT COMPOUNDS AND
APPLICATIONS THEREOF

(57) **Abrégé/Abstract:**

This invention relates to compounds of general formula $(R')_xZn_y(L)_z(A)_n(XR)_m$, where L is a bi- or multifunctional organic neutral ligand or its deprotonated form, containing at least two heteroatoms selected from N, O, S, wherein at least one of the functional groups of the ligand is selected from -OH, -SH, -NH₂, -NHR, -COOH, -CONH₂, -CONRH or their deprotonated equivalents; A is an inorganic anion, X is oxygen or sulfur, R' is C1-C10 alkyl, straight or branched, benzyl, phenyl cyclohexyl or halogen, R is hydrogen, alkyl or aryl, x is a number from 0 to 6, y is a number from 1 to 12, z is a number from 1 to 12, n is a number from 0 to 6, m is a number from 0 to 6, wherein $x \neq 0$, $n \neq 0$ and $m \neq 0$ at the same time and if n and m = 0, then $x \neq z$. The invention also relates to the method of preparation of these compounds and applications thereof.



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
12 January 2012 (12.01.2012)

PCT

(10) International Publication Number
WO 2012/005615 A1

(51) International Patent Classification:

C09K 11/06 (2006.01) *C07F 3/06* (2006.01)
H05B 33/14 (2006.01) *H01L 51/50* (2006.01)
H05B 33/22 (2006.01)

(21) International Application Number:

PCT/PL2011/000070

(22) International Filing Date:

6 July 2011 (06.07.2011)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

P.391776 7 July 2010 (07.07.2010) PL

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with amended claims and statement (Art. 19(1))

(54) Title: LUMINESCENT COMPOUNDS, METHOD OF PREPARATION OF LUMINESCENT COMPOUNDS AND APPLICATIONS THEREOF

(57) Abstract: This invention relates to compounds of general formula $(R')_xZn_y(L)_z(A)_n(XR)_m$, where L is a bi- or multifunctional organic neutral ligand or its deprotonated form, containing at least two heteroatoms selected from N, O, S, wherein at least one of the functional groups of the ligand is selected from -OH, -SH, -NH₂, -NHR, -COOH, -CONH₂, -CONRH or their deprotonated equivalents; A is an inorganic anion, X is oxygen or sulfur, R' is C1-C10 alkyl, straight or branched, benzyl, phenyl cyclohexyl or halogen, R is hydrogen, alkyl or aryl, x is a number from 0 to 6, y is a number from 1 to 12, z is a number from 1 to 12, n is a number from 0 to 6, m is a number from 0 to 6, wherein $x \neq 0$, $n \neq 0$ and $m \neq 0$ at the same time and if n and m = 0, then $x \neq z$. The invention also relates to the method of preparation of these compounds and applications thereof.



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Luminescent compounds, method of preparation of luminescent compounds and applications thereof

The invention relates to luminescent compounds based on complexes of organic ligands with zinc, with potential application in electronics, biology and medicine and, in particular, in the design of organic light emitting diodes. The invention also relates to the method of preparation of these compounds and applications thereof.

8-hydroxyquinoline (Hq) chelates of type Mq_n (where M is metal) are widely used in analytical chemistry. As of 1980s, extensive research has started regarding the usefulness of Hq chelates and their derivatives in the design of emission and conduction layers in the production of organic light emitting diodes (OLEDs). A true milestone in material chemistry that led to preparation of first stable electroluminescent diodes was the use of trichelate complex of 8-Hq with aluminum (Alq_3). [C. W. Tang, S. A. VanSlyke *Appl. Phys. Lett.*, **1987**, *51*, 913 and patents no. US 1985/4539507 and US 1988/4720432]. In recent years, extensive research was also conducted on electroluminescence properties of bischelate 8-Hq complexes of zinc (Znq_2) [L. S. Sapochak, F. E. Benincasa, R. S. Schofield, J. L. Baker, K. K. C. Riccio, D. Fogarty, H. Kohlmann, K. F. Ferris, P. E. Burrows *J. Am. Chem. Soc.*, **2002**, *124*, 6119]

In the structure of Alq_3 , the aluminum atom is bound to three deprotonated hydroxyquinoline ligands. Thermal stability of Alq_3 makes it possible to deposit the compound in thin layers without decomposition by vacuum planting at 350 °C. Quantum yield of Alq_3 in solutions was 11% with maximum fluorescence at 532 nm. Znq_2 is characterized by comparable quantum yield. [T. A. Hopkins, K. Meerholz, S. Shaheen, M. L. Anderson, A. Schmidt, B. Kippelen, A. B. Padias, H. K. Hall, Jr., N. Peyghambarian, N. R. Armstrong *Chem. Mater.*, **1996**, *8*, 344]

Various attempts are made to obtain materials with predefined color of the emitted light. This is particularly important in the design of OLEDs of potential use e.g., in TV panels. Currently, extensive studies are conducted to obtain fluorescent systems with a wide range of colors, with blue and white emitters being probably the most attractive.

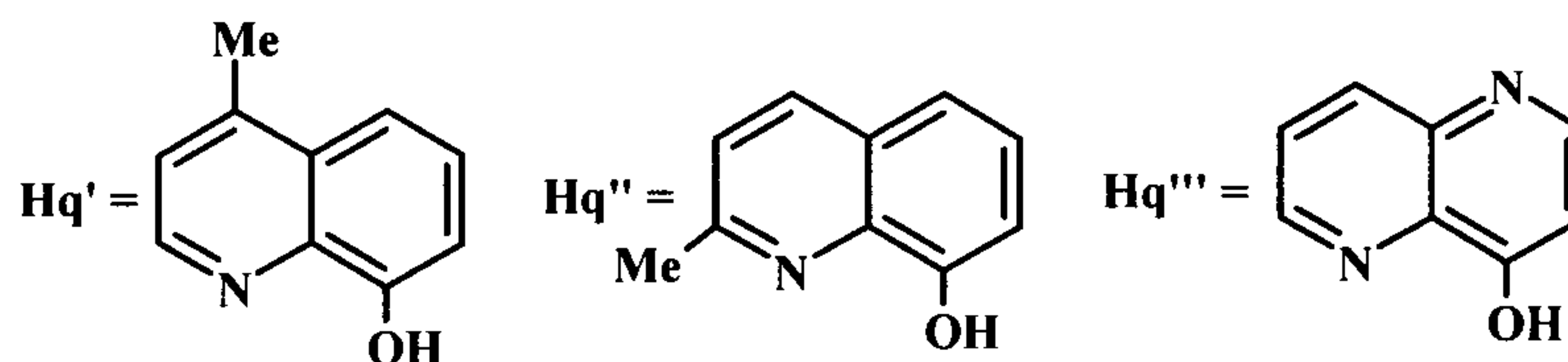
There is also a need to search for novel possibilities of fluorescent tagging, widely used in imaging and physicochemical examinations in biology and medicine. The area of applicability of fluorescent tags is very wide and diverse. They are used in studying and imaging of cell components: the membrane, cytoskeletal proteins, organelles: nuclei, mitochondria, lysosomes, endoplasmic reticula, Golgi apparatus; tags are used to stain proteins for various purposes, including staining antibodies and enzymes, peptides, oligonucleotides and nucleic acids; fluorescent tags of suitable designs are used as chemical sensors for the measurement of

concentrations of important intracellular substances, such as O_2 , K^+ , H^+ and for the measurement of electric potentials of cellular membranes; tagged substances are used in both *in vitro* and *in vivo* studies, both in fixed materials and in living models. Tagged substances are used in many areas of biology and medicine, including genetics, biochemistry, e.g. in studying enzymatic activity, immunology, pathology, neurology, medical diagnostics, etc. In cellular function studies, tagging is used to study cell viability, cellular cycle, adhesion, apoptosis, substance cytotoxicity tests, etc. Numerous modern study techniques and technologies widely used in the above areas make use of compounds containing fluorescent tags, and the advances in the development of these techniques is determined by the advances in the development of tags, in particular in their sensitivity and stability. These techniques include: flow cytometry; biochips; DNA sequencing or nucleic acid synthesis by polymerase chain reaction (PCR); fluorescence correlation spectroscopy (FCS) used for studying intermolecular interactions, including interbiomolecular interactions, where high intensity excitation radiation is also used and where the intensity of the luminescence of the tag following the capture of individual photons; Fluorescence Resonance Energy Transfer (FRET) -based biological sensors, which are widely used in cellular biology for studying signaling pathways and for imaging of biological processes using confocal fluorescence lifetime imaging microscopy (FLIM) – an imaging technique based on measuring the differences in the lifetimes of fluorescence used to study protein-protein interactions and limited by the low quantum yield of currently used fluorophores; or super-resolution stimulated emission depletion (STED) microscopy used for do studying subcellular location of proteins (resolution of ca. 70 nm).

The market of fluorescent tags is comprised of fluorescent proteins, small organic molecules and quantum dots, which have been introduced in recent years and which are still in the implementation stage [1, 2, 4, 5]. In addition, literature contains reports on attempted preparation of tags based on phosphorescent lanthanide complexes [10], carbon nanoparticles [9] and complexes of heavy metals [11]. The predominant and the most versatile group are small organic molecules. They belong to various classes of compounds, their molecular mass usually does not exceed 1000 Da, and their size allows them to be inscribed within a sphere of the diameter of 1-1.5 nm. Quantum dots (QDs), or semiconductor-based nanoparticles, have photoluminescent properties, and their diameter usually does not exceed 10 nm. An example of QDs are nanoparticles of cadmium selenide coated in a zinc sulphide layer: CdSe/ZnS QDs. Organic tags are small, but not resistant to photobleaching. Quantum dots are optically stable, but too large for many applications.

In the case of complexes based on Hq and its derivatives, the shift of maximum fluorescent emission may be achieved by introduction of a ligand with modified electronic properties, a change in the metal center or in the geometry of complex coordination zone. For

example, maximum fluorescence for Alq'_3 (i.e., a complex consisting of monoanions of 8-hydroxyquinoline substituted with $-\text{CH}_3$ at C-4) is 515 nm and is shifted hypsochromatically by 17 nm compared to Alq_3 .



On the other hand, the use of 8-hydroxyquinoline substituted with $-\text{CH}_3$ at C-2 led to formation of a poorly stable oxoaluminum complex of the type $[(q''\text{Al})_2\text{O}]$, with the maximum light emission at 490 nm. When a Hq''' proligand was used, an $\text{Al}(q''')_3$ compound was obtained with fluorescence spectrum peak at 440 nm. [C. H. Chen, J. Shi *Coordin. Chem. Rev.*, **1998**, 171, 161]

Fluorescent properties of complexes of the type Mq_n depend also on the nature of the central ion:

- i) chelate complexes with paramagnetic metal ions do not show any fluorescence (e.g., Cr, Ni complexes);
- ii) quantum yield drops are usually observed along with the increase in the atomic number of metal ions;
- iii) fluorescence maximum is shifted toward longer wavelengths along with the increasingly covalent character of the metal-ligand bond; for instance, Al, Ga, In chelates emit light at 532, 545 and 558 nm, respectively, while Mgq_2 emits light of shorter wavelengths (500 nm) than that of its zinc analog Znq_2 (557 nm).

[D. C. Bhatnagar, L. S. Forster *Spectrochim. Acta*, **1965**, 21, 1803; R. Ballardini, G. Varani, M. Y. Indelli, F. Scandola *Inorg. Chem.*, **1986**, 25, 3858]

The shift in the emission bands is also dependent on the geometry of the molecule, as well as relative locations of molecules in the crystalline lattice and intermolecular interactions. For instance, differences in spectroscopic properties are observed for different polymorphic variants of Alq_3 . [M. Cölle, R. E. Dinnebier, W. Brütting *Chem. Commun.*, **2002**, 2908]

The described examples pertain mostly to mononuclear chelate compounds containing ligands of one type, which leads to small diversity in the molecular geometries and crystal packing and, thus, in their spectroscopic properties.

The aim of the invention was to obtain a novel class of fluorescent materials expanding the possibilities for the design of modern fluorescent systems.

The subject matter of the invention are novel compounds of general formula $(R')_xZn_y(L)_z(A)_n(XR)_m$, where L is a bi- or multifunctional organic neutral ligand or its deprotonated form, containing at least two heteroatoms selected from N, O, S, wherein at least one of the functional groups of the ligand is selected from -OH, -SH, -NH₂, -NHR, -COOH, -CONH₂, -CONRH or their deprotonated equivalents; A is an inorganic anion, R' is C1-C10 alkyl, straight or branched, benzyl, phenyl, cyclohexyl or halogen, X is oxygen or sulfur, R is hydrogen, alkyl or aryl, x is a number from 0 to 6, y is a number from 1 to 12, z is a number from 1 to 12, n is a number from 0 to 6, m is a number from 0 to 6, wherein $x \neq 0$, $n \neq 0$ and $m \neq 0$ at the same time and if n and m = 0, then $x \neq z$.

Preferably, the inorganic anion A is an anion originating from an oxyacid, a binary acid, an acid anhydride, oxygen, sulfur, selenium, or tellurium.

More preferably, the inorganic anion A is O^{2-} , S^{2-} , Se^{2-} , Te^{2-} , CO_3^{2-} , SO_4^{2-} , SO_3^{2-} , CS_2O^{2-} , CS_3^{2-} , BO_3^{3-} , NO_2^- , NO_3^- .

Most preferably, the inorganic anion A is O^{2-} , S^{2-} , Se^{2-} , CO_3^{2-} , CS_2O^{2-} , BO_3^{3-} , NO_3^- .

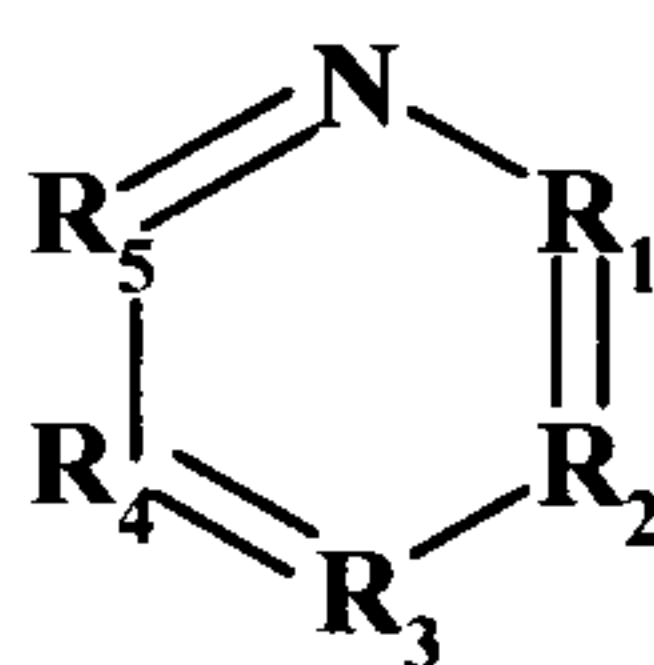
Preferably, the multifunctional ligand L consists of a neutral organic compound or its deprotonated equivalent containing at least one Lewis base center and at least one functional group selected from -OH, -SH, -NH₂, -NHR, -COOH, -CONH₂, -CONRH or their deprotonated equivalents.

More preferably, the multifunctional ligand L consists of an organic compound containing at least one Lewis base center and at least one functional group selected from $-O^-$, $-S^-$, $-NH^-$, $-NR^-$, $-COO^-$, $-CONH^-$, $-CONR^-$.

Preferably, the multifunctional ligand L consists of an organic compound in which the Lewis base center is separated from the -OH, -SH, -NH₂, -NHR, -COOH, -CONH₂, -CONRH group by a saturated or unsaturated carbon chain of 1-3 carbon atoms.

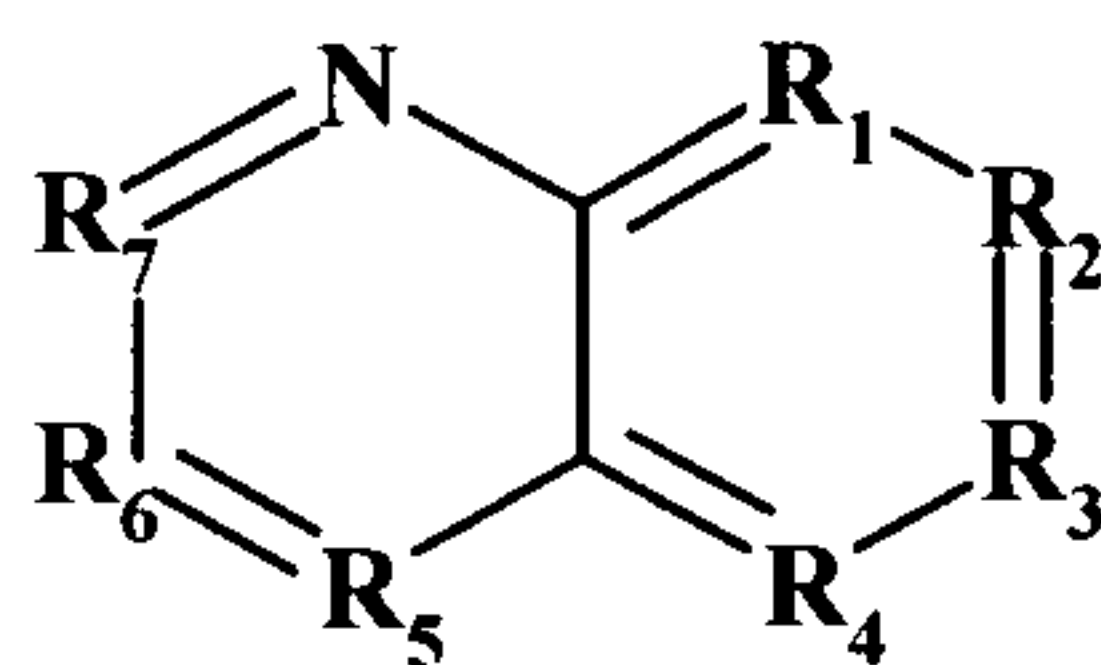
More preferably, the multifunctional ligand L consists of an organic compound in which the Lewis base center is separated from the $-O^-$, $-S^-$, $-NH^-$, $-NR^-$, $-COO^-$, $-CONH^-$, $-CONR^-$ group by a saturated or unsaturated carbon chain of 1-3 carbon atoms.

Preferably, the ligand L is the organic compound of formula 1 or of formula 2 or of formula 3 or of formula 4 or of formula 5 or of formula 6 or of formula 7 or of formula 8 or of formula 9 or of formula 10 or of formula 11 or of formula 12 or of formula 13:

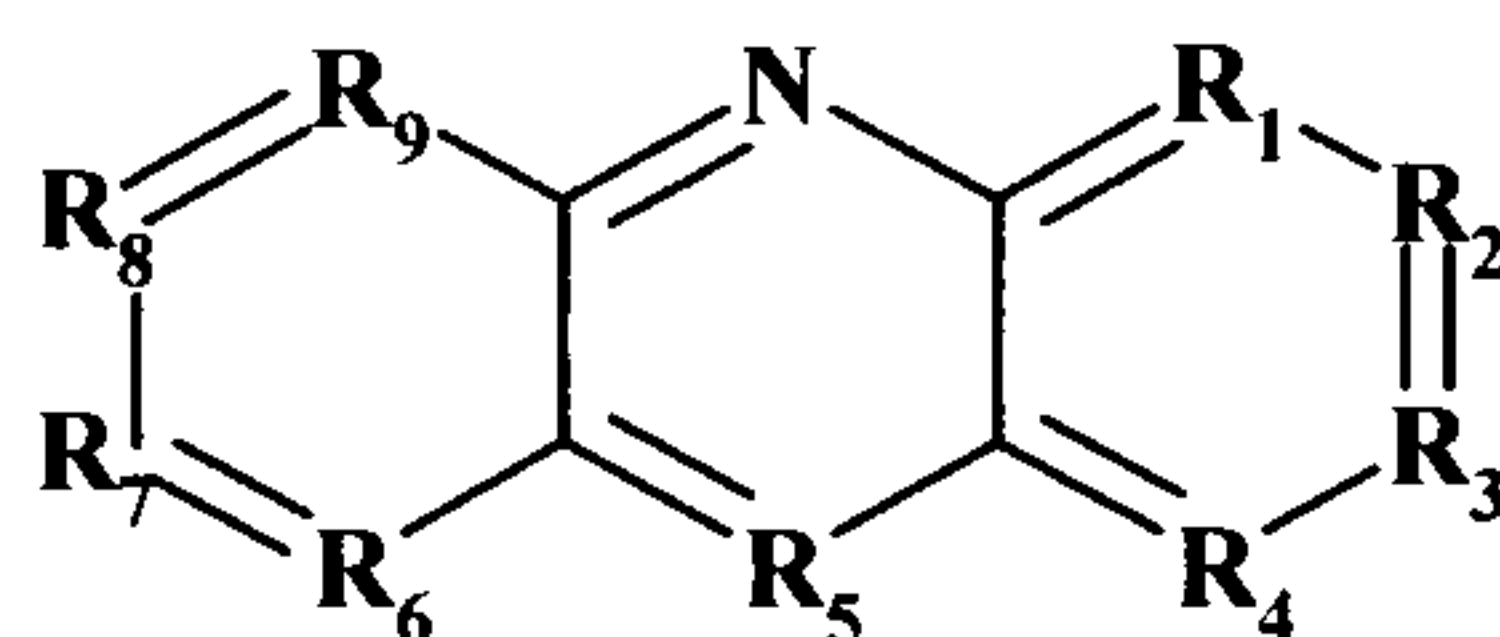


Formula 1

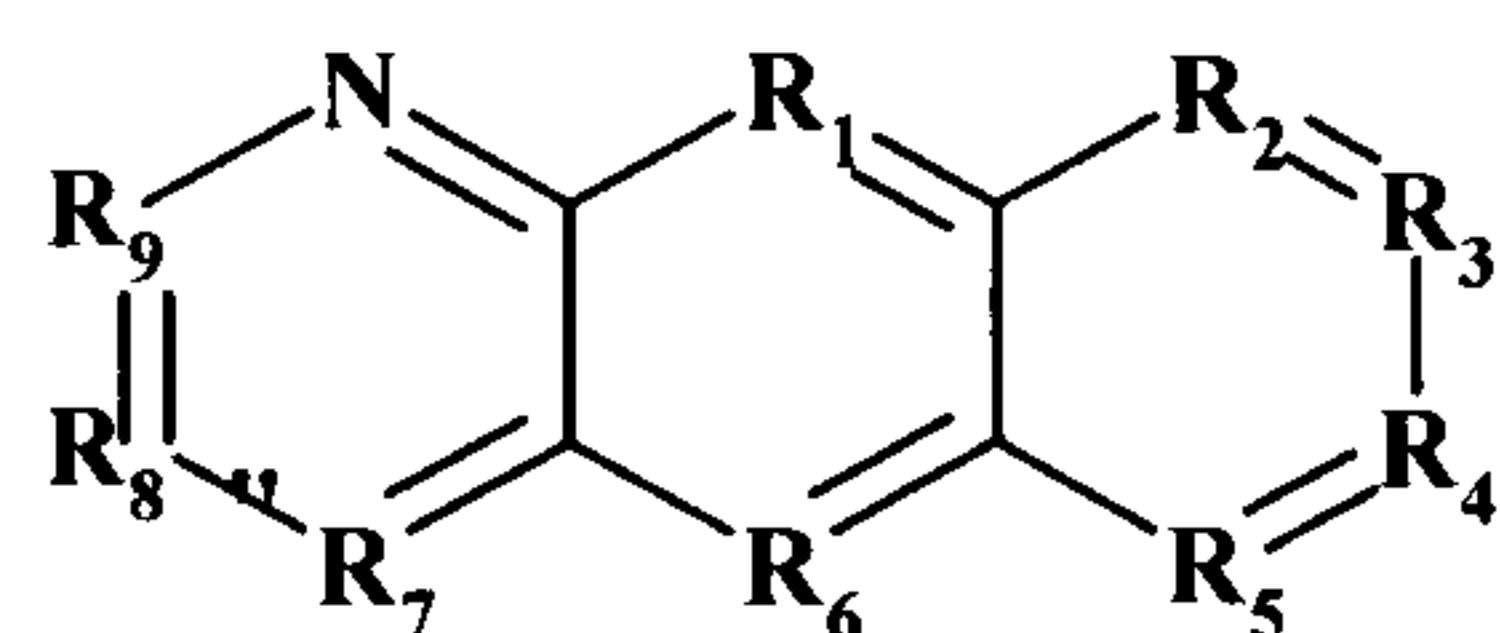
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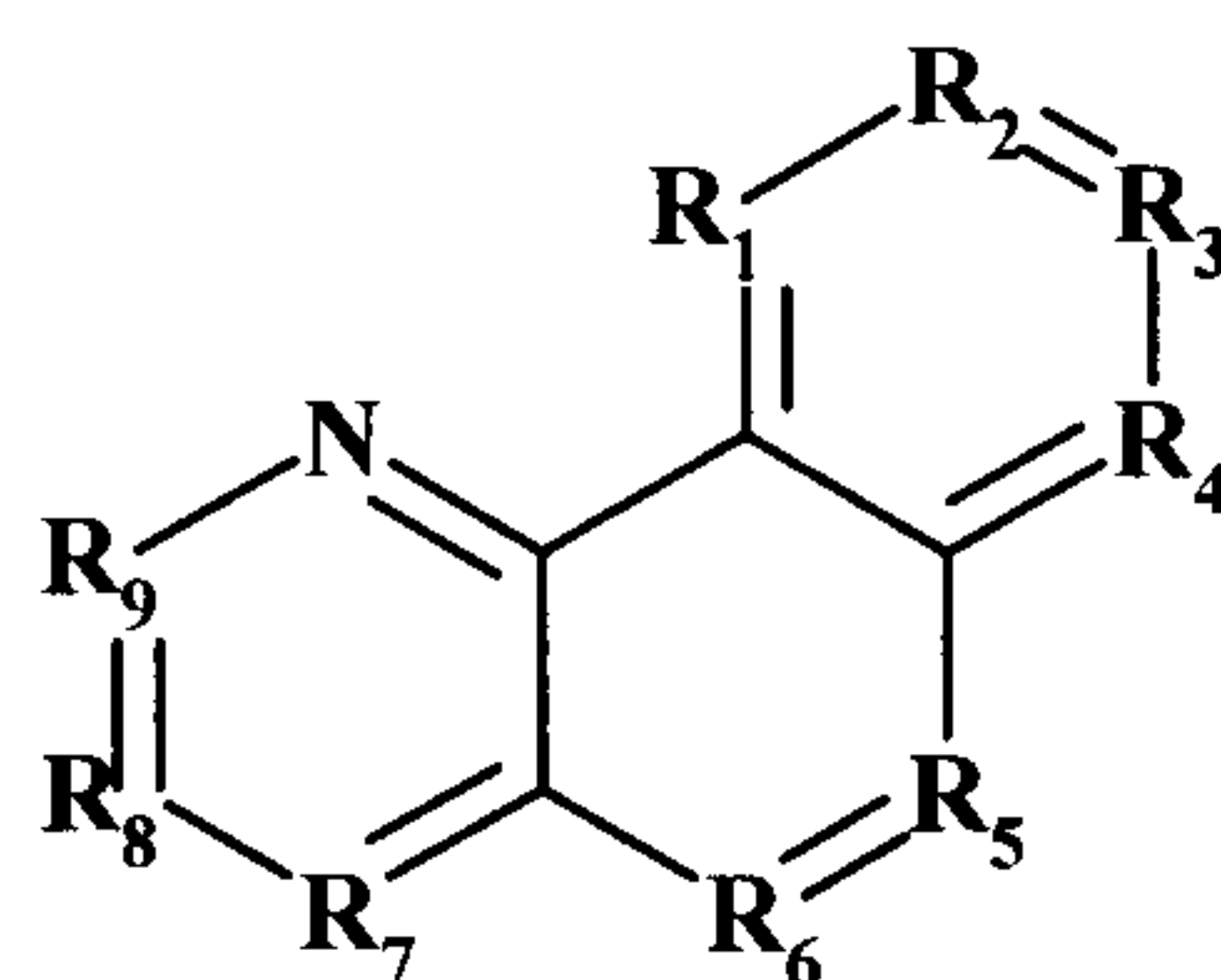
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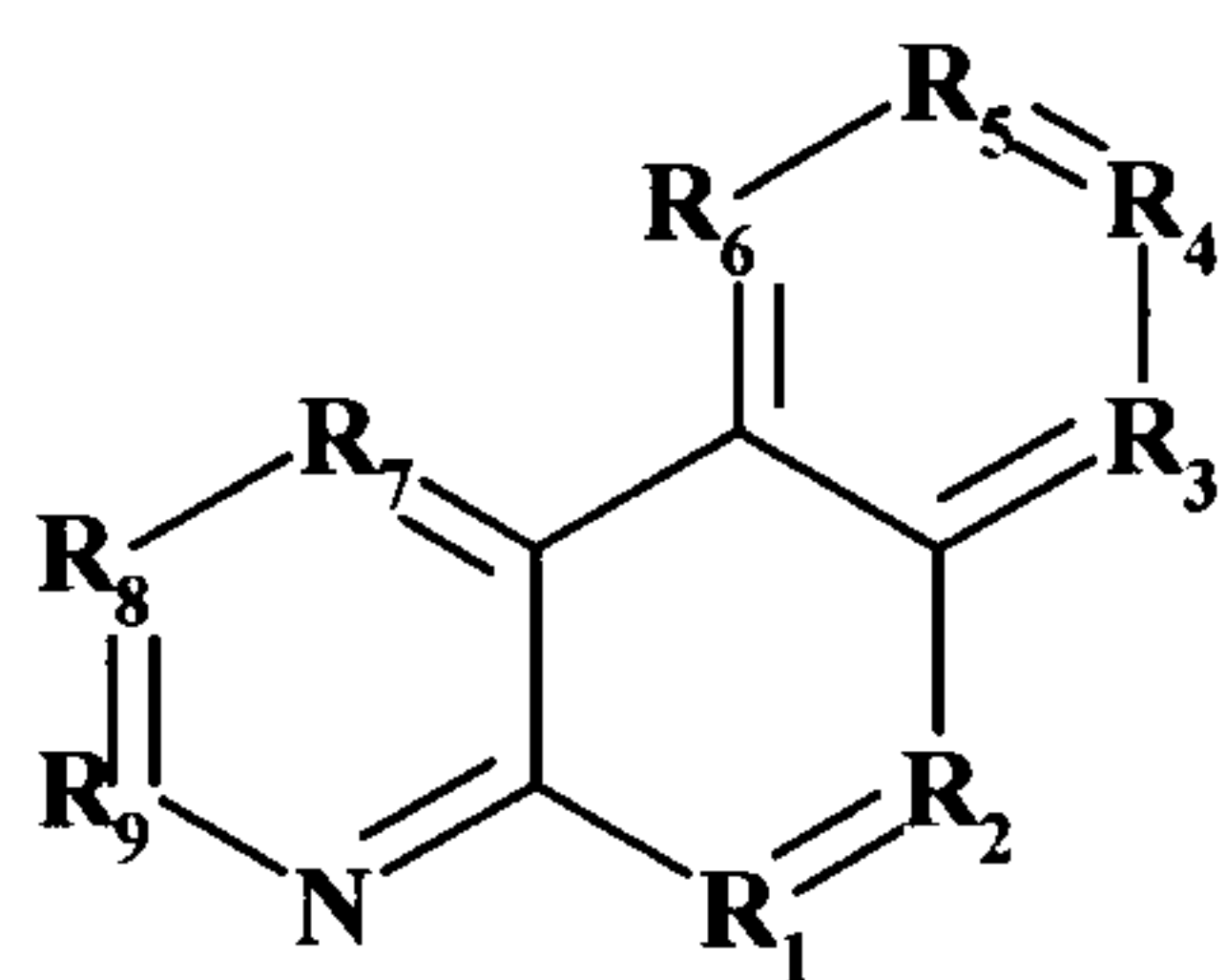
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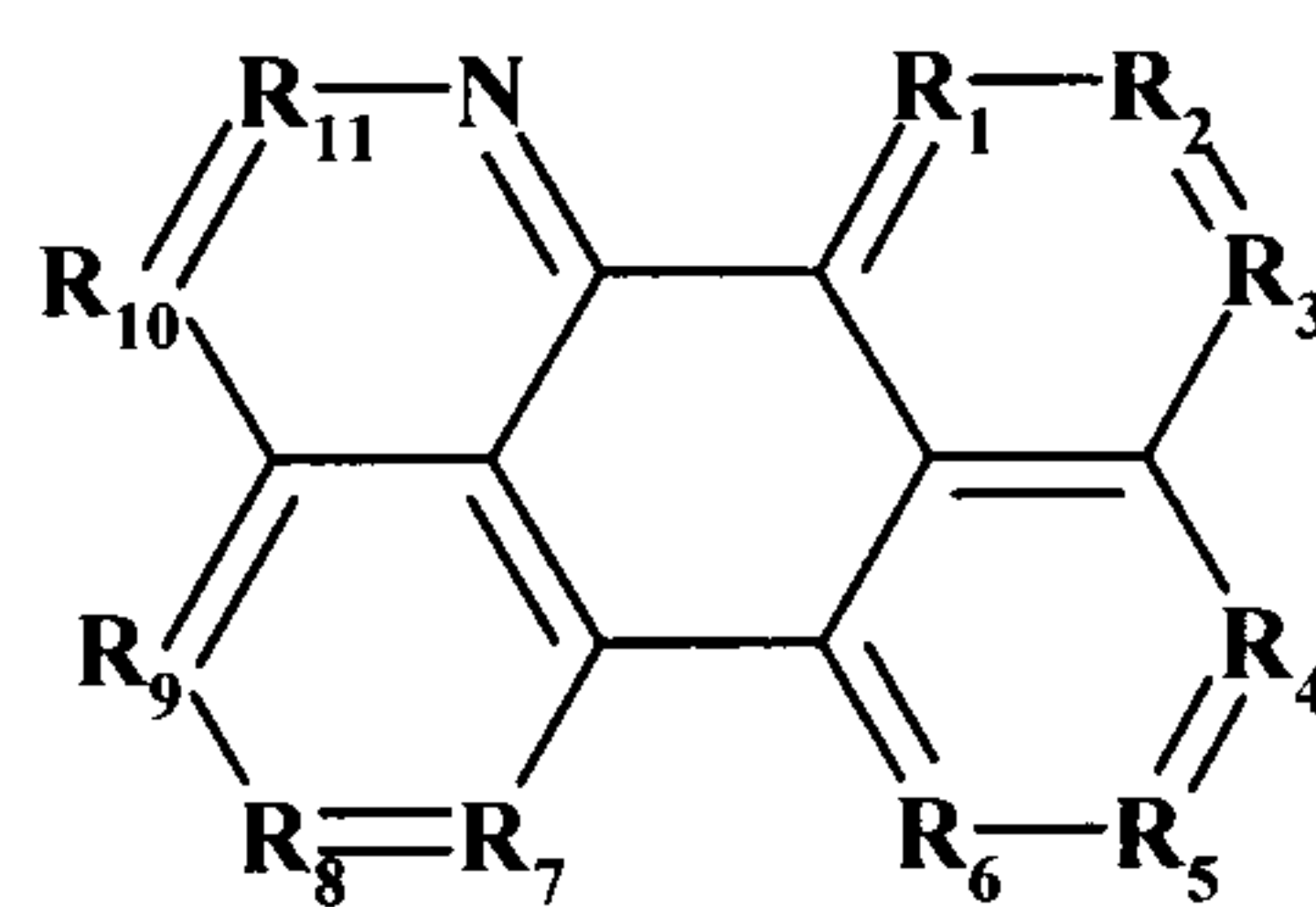
Formula 4



Formula 5

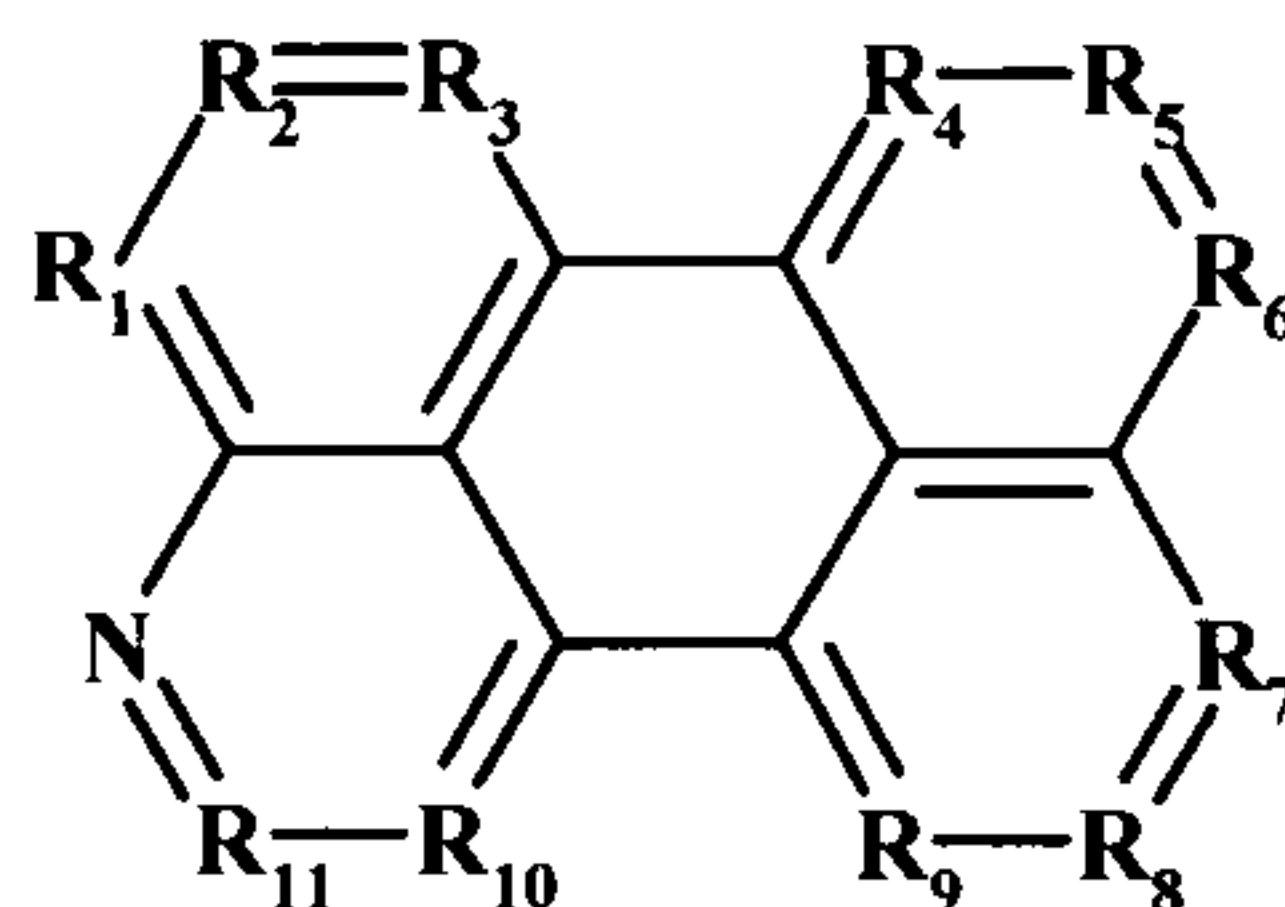


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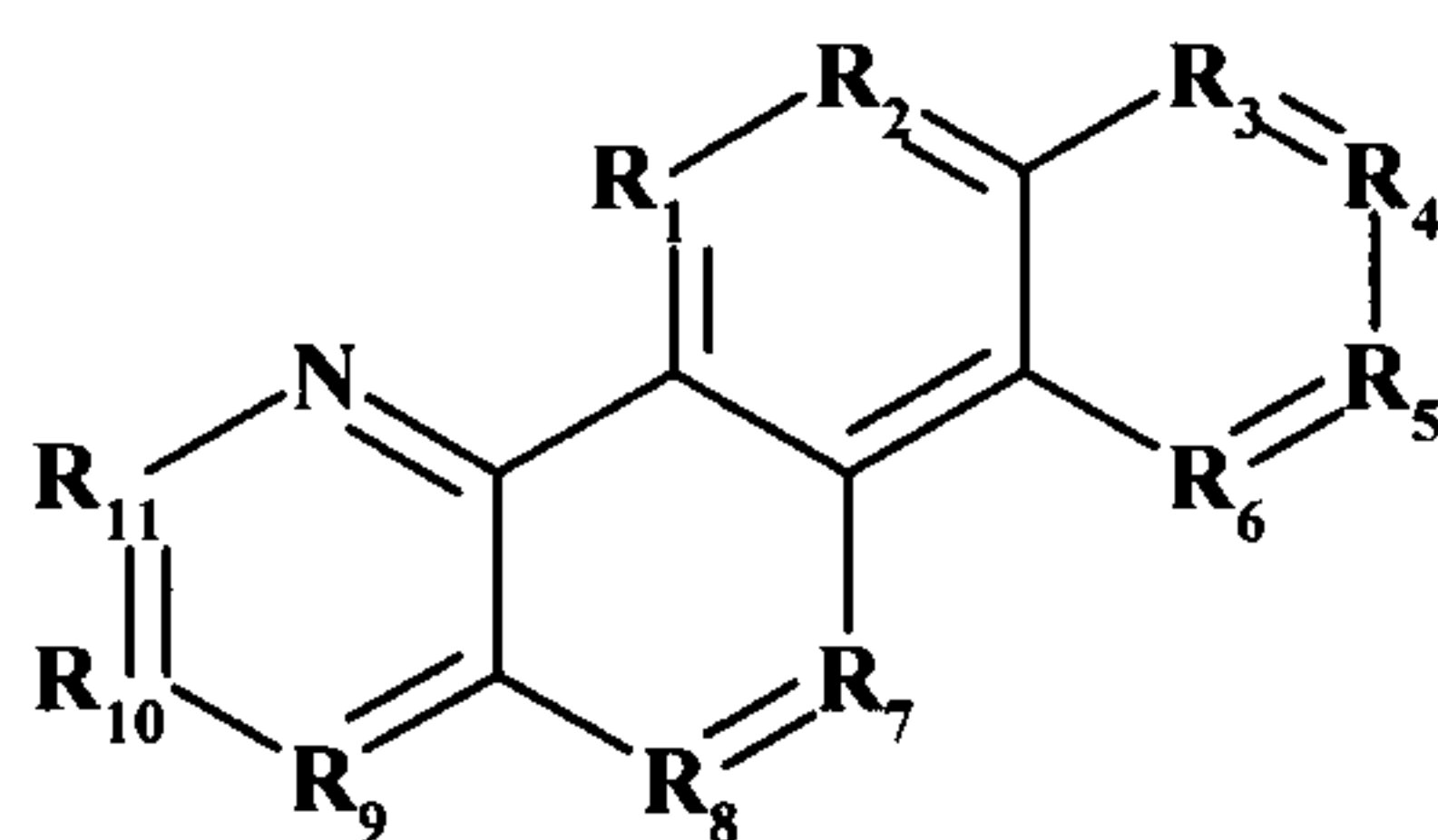


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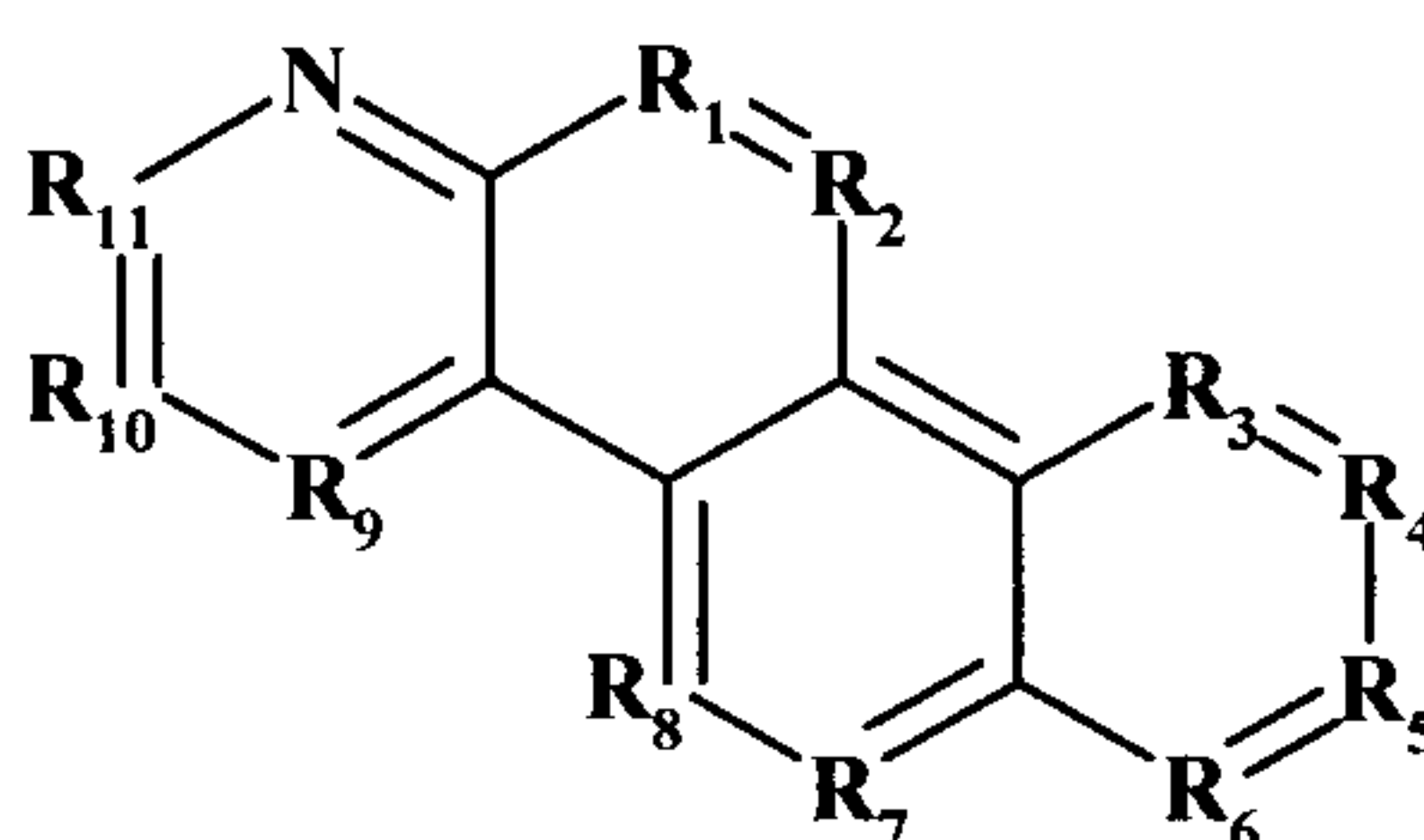
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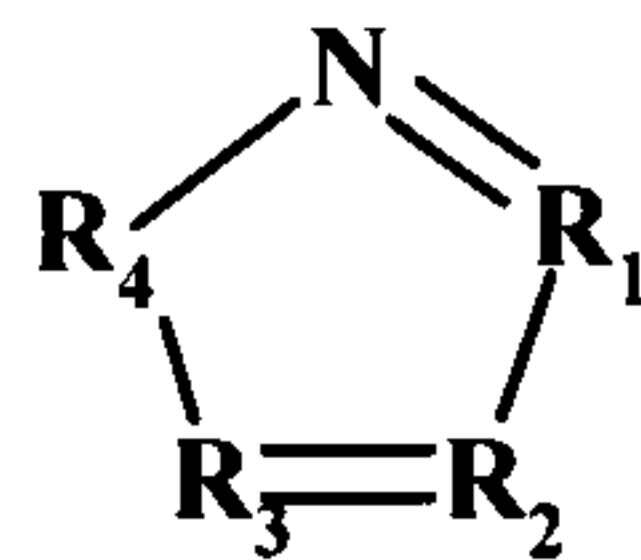
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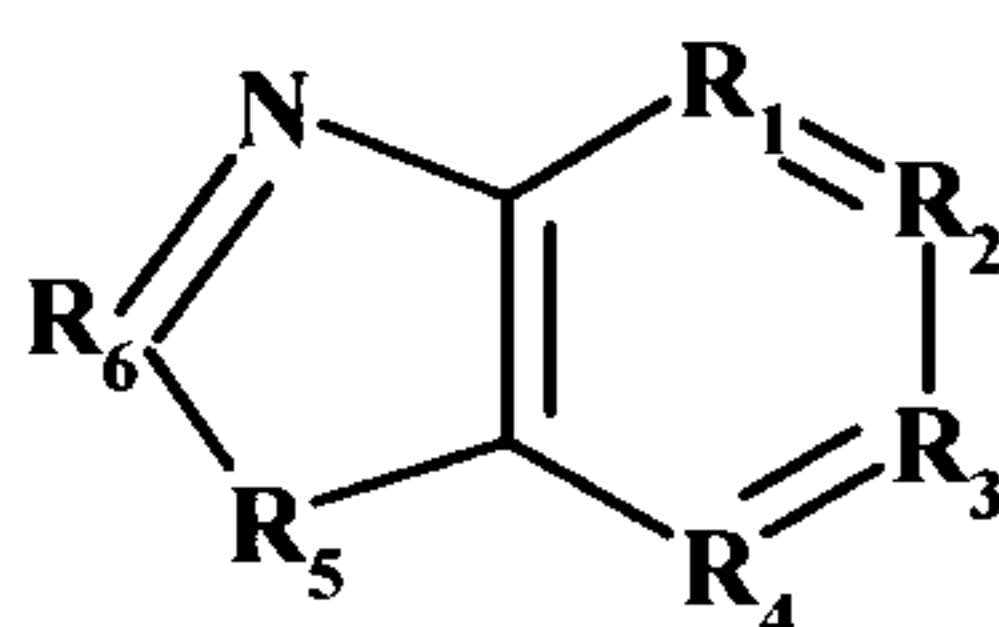
Formula 9



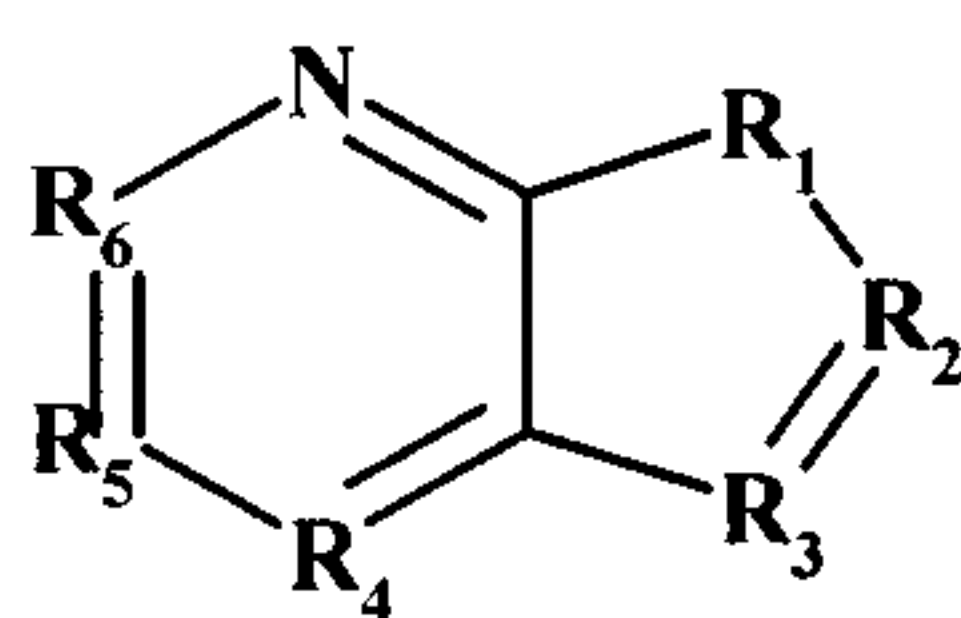
Formula 10



Formula 11



Formula 12



Formula 13

wherein R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 are heteroatoms selected from nitrogen, sulfur, oxygen or carbon atom attached to a hydrogen, straight or branched C1-C10 alkyl (possibly substituted), phenyl (possibly substituted) benzyl (possibly substituted), ether group (possibly substituted), ketone group (possibly substituted), halogen, -OH, -SH, -NH₂, -NHR, -COOH, -CONH₂, -CONRH, -O⁻, -S⁻, -NH⁻, -NR⁻, -COO⁻, -CONH⁻, -CONR⁻ wherein R1 is preferably a carbon atom

attached to one of the groups: -OH, -SH, -NH₂, -NHR, -COOH, -CONH₂, -CONRH, more preferably a carbon atom attached to one of the groups: -O⁻, -S⁻, -NH⁻, -NR⁻, -COO⁻, -CONH⁻, -CONR⁻.

The invention also relates to the method for preparation of compounds of general formula (R')_xZn_y(L)_z(A)_n(XR)_m, where the precursor R'_xZn_y(L)_z(X_kR)_m, where L is a bi- or multifunctional organic neutral ligand or its deprotonated form, containing at least two heteroatoms selected from N, O, S, wherein at least one of the functional groups of the ligand is selected from -OH, -SH, -NH₂, -NHR, -COOH, -CONH₂, -CONRH or their deprotonated equivalents; X is oxygen or sulfur, R' is C1-C10 alkyl, straight or branched, benzyl, phenyl cyclohexyl or halogen, R is hydrogen, alkyl or aryl, x is a number from 0 to 6, y is a number from 1 to 12, z is a number from 1 to 12, m is a number from 0 to 6, k is 1 or 2, is subjected to reaction with oxygen or water or elemental sulfur or selenium, or tellurium, or oxyacids or binary acids, or acid anhydrides and/or inorganic acid salts in a solvent or to thermal transformation.

The method of the invention allows for using anhydrous organic solvents, organic solvents containing water as well as inorganic solvents, preferably water.

Preferably, the organic solvent is toluene, tetrahydrofuran, hexane, methylene chloride, dimethylsulfoxide, acetonitrile as well as an alcohols, phenol or acids in which the precursor is well soluble or a mixture of these compounds.

Reactions with oxygen may be conducted with oxygen, atmospheric air or mixtures of both as oxidating agents.

Preferably, an acid anhydride is used in the reaction.

Preferably, the acid anhydrides used include CO₂, SO₂, CS₂, B₂O₃, NO, NO₂.

Preferably, inorganic acid salts are used in the reaction.

Preferably, the inorganic salts used include the salts of carbonic acid, sulfuric (IV) acid sulfuric (VI) acid, thiocarbonic acid, boric acid, nitric (III) acid, nitric (V) acid, hydrosulphuric acid, hydroselenic acid, hydrotelluric acid.

Preferably, oxyacids used include carbonic acid, sulfuric (IV) acid sulfuric (VI) acid, thiocarbonic acid, boric acid, nitric (III) acid, nitric (V) acid.

Preferably, the binary acids used include H₂S, H₂Se, H₂Te.

Preferably, elemental sulfur, selenium or tellurium is used in the reaction.

Preferably, the reaction is conducted in the temperature range of -70-200⁰C, more preferably -70-100⁰C and in the pressure range of 0.1-100 bar, more preferably 1-100 bar, and most preferably 1-20 bar.

Preferably, thermal transformation is conducted in the temperature range of 60-700⁰C, more preferably 60-400⁰C.

The invention also relates to the use of compounds of general formula $(R')_xZn_y(L)_z(A)_n(XR)_m$ for manufacture of emission and conduction layers for use in organic electroluminescent diode production technology as well as in other devices making use of luminophores.

Compounds of the invention may also be used as fluorescent tags in cellular and tissue imaging.

The method of the invention allows for convenient preparation of novel classes of fluorescent materials characterized by the presence of at least two types of ligands, various nuclearity and various quantitative metal-ligand ratios, which in turn affects relative locations of ligands, geometry of complexes and thus the packing of the molecules in the crystal lattice. Such changes lead to significant changes towards more desirable spectroscopic properties of the obtained materials, allowing for rational design of novel fluorescent systems. For example, peak fluorescence of the $Zn_{10}Q_{12}(CO_3)_4$ complex is located at 490 nm and is blue-shifted by 60 nm compared to classic chelates of aluminum and zinc. In addition, a 6-fold increase in quantum yield (11% to 64%) is observed for $Zn_{10}Q_{12}(CO_3)_4$ compared to classic compounds. The new method extends the possibilities for preparing fluorescent compounds with predefined crystallographic structures and unique spectroscopic properties.

Compounds according to the invention will find their use in the imaging of cells and tissues in biology and medicine, particularly as fluorescent tags. This application makes use of their capability to register the emission of light with very high intensity, sometimes down to a single emitted photon. Such level of sensitivity is unattainable in case of light absorption-based spectroscopic techniques. Compounds of the invention used as fluorescent tags are significantly smaller than quantum dots and, at the same time, much more resistant to photobleaching than organic dyes.

Before or upon use, compounds of the invention used for the imaging of cells and tissues will be tethered, either covalently or by physical interactions, to molecules originating from cells, their synthetic copies or analogs, in particular to proteins, peptides, nucleic acids, nucleosides, nucleotides, polysaccharides, hormones, amino acids or with other molecules, in particular drugs or toxins, where said tethering of the compounds of the invention will be performed with a view to the proven or suspected interaction of these compounds with one of the aforementioned molecules, i.e. molecules originating from cells, their synthetic copies or analogs, in particular to proteins, peptides, nucleic acids, nucleosides, nucleotides, polysaccharides, hormones, amino acids. As part of this application, compounds of the invention will also be used in qualitative or quantitative analysis of chemical substances in tissues or cells performed *in vitro* or *in vivo*, either

in living models or in fixed materials. Finally, the compounds of the invention will be used due to their ability to accumulate in tissues, cells or cell fragments.

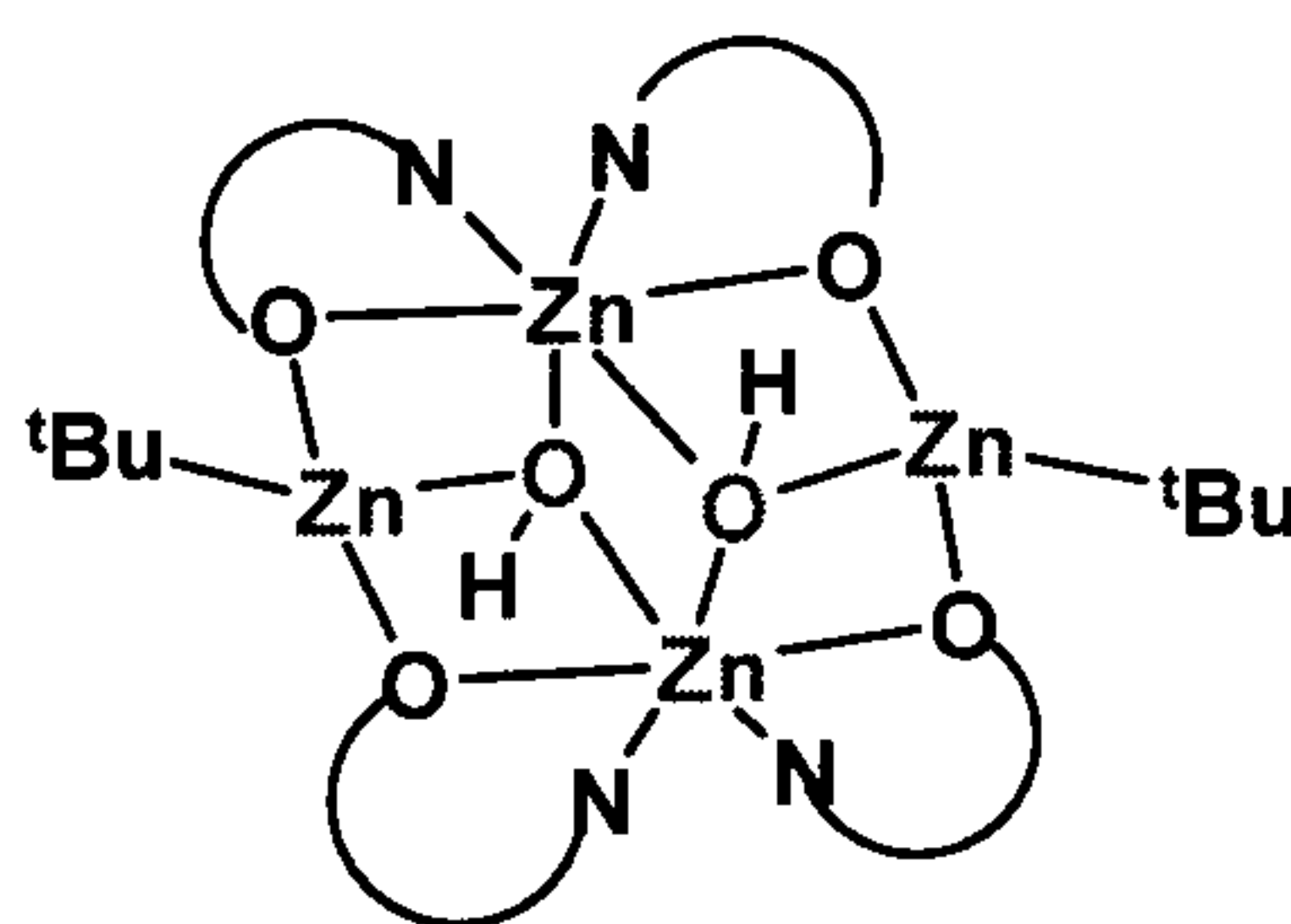
The comparison of the known parameters of the compound according to the invention of the formula $[\text{Zn}(\text{CO}_3)]_4[\text{Znq}_2]_6$, such as its size, quantum yield, Stokes shift, and optical stability with the parameters of fluorescent tags currently available in the market, listed in Table 1 suggests that the compound according to the invention has a significant advantage over the tags of current art and that it may significantly expand the area of applicability of fluorescent tags.

The subject matter of the invention is presented in more detail in the following examples.

Example 1

Preparation of a fluorescent compound of formula $[(\text{Znq}_2)_2(\text{}^t\text{BuZnOH})_2]$

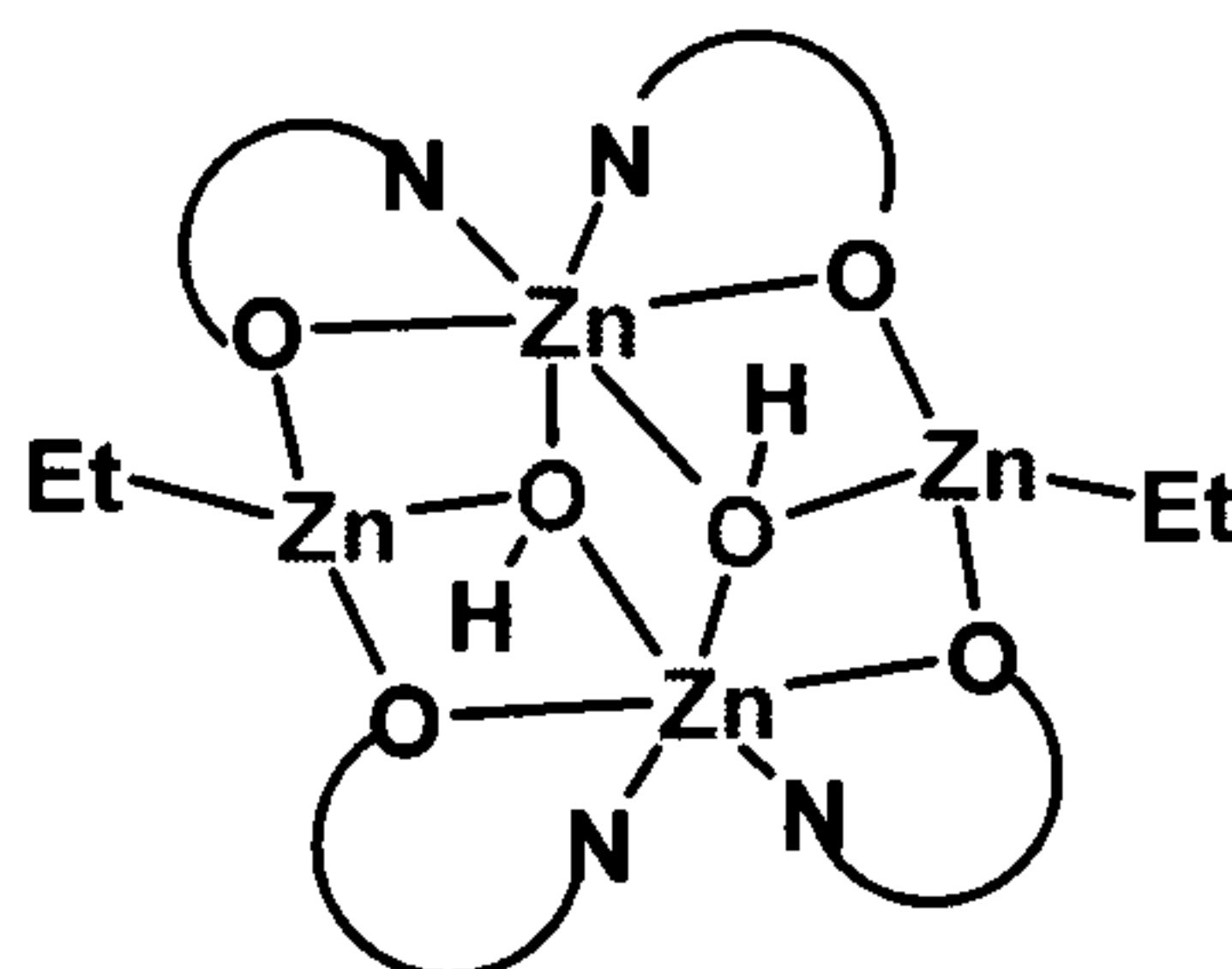
10 μL of water (0.56 mmol H_2O) was added to 5 mL of a solution containing 0.15 g (0.56 mmol) of *tert*-butylzinc derivative of $(\text{}^t\text{BuZnq})_3$ in tetrahydrofuran. The reaction was carried out for 12 hours at room temperature. Crystallization yielded monocrystals suitable for x-ray structural studies. The x-ray structural studies showed that the product of the reaction was the $[(\text{Znq}_2)_2(\text{}^t\text{BuZnOH})_2]$ adduct of structural formula presented below.



Example 2

Preparation of a fluorescent compound of formula $[(\text{Znq}_2)_2(\text{EtZnOH})_2]$

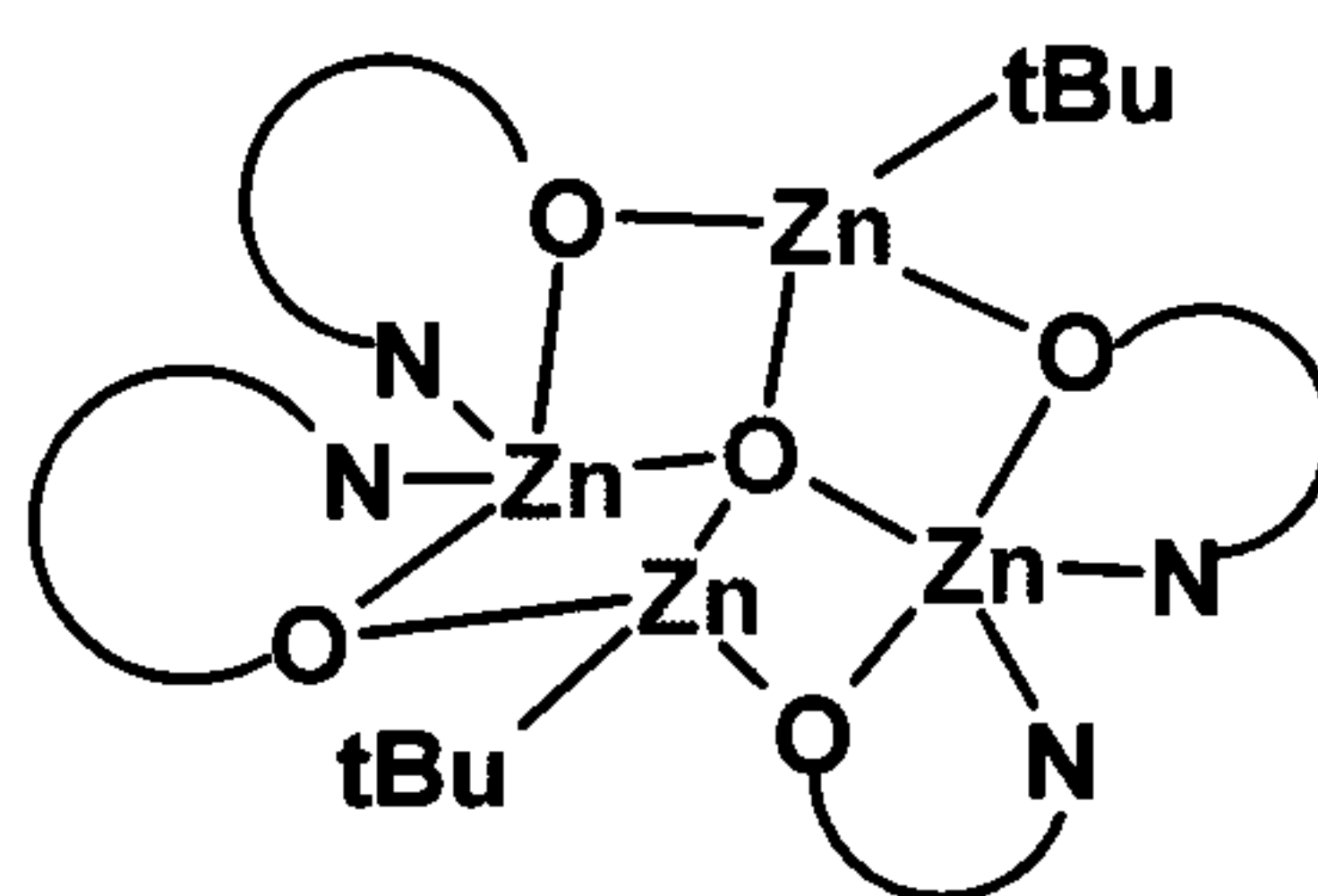
10 μL of water (0.56 mmol H_2O) was added to 5 mL of a solution containing 0.135 g (0.56 mmol) of ethylzinc derivative of $(\text{EtZnq})_2$ in tetrahydrofuran. The reaction was carried out for 4 hours at room temperature. The x-ray structural and spectral studies showed that the product of the reaction was the $[(\text{Znq}_2)_2(\text{EtZnOH})_2]$ adduct of structural formula presented below.



Example 3

Preparation of a fluorescent compound of formula $[(^t\text{Bu})_2\text{Zn}_4(\mu_4\text{-O})(\text{Meq})_4]$

0.1 mL of 0.5M solution of water in THF (0.05 mmol H_2O) was added to 5 mL of a solution containing 0.150 g (0.5 mmol) of *tert*-butylzinc derivative of 5,7-dimethyl-8-hydroxyquinoline $(^t\text{BuZnMeq})_3$ in tetrahydrofuran. The reaction was carried out for 4 hours at room temperature. Crystallization yielded monocrystals suitable for x-ray structural studies. The x-ray structural studies showed that the product of the reaction was the $[(^t\text{Bu})_2\text{Zn}_4(\mu_4\text{-O})(\text{Meq})_4]$ adduct of structural formula presented below



Example 4

Preparation of a fluorescent compound of formula $[(^t\text{Bu})_2\text{Zn}_4(\mu_4\text{-O})(\text{Meq})_4]$

The monocrystals of the $[(\text{ZnMeq}_2)_2(^t\text{BuZnOH})_2]$ adducts were heated at 200°C for 60 min. After this time, crystalline powder was obtained and characterized using an x-ray powder diffractometer. The spectrum of the tested compound corresponds to the reference spectrum of the $[(^t\text{Bu})_2\text{Zn}_4(\mu_4\text{-O})(\text{Meq})_4]$ adduct of structural formula presented in Example 3.

Example 5

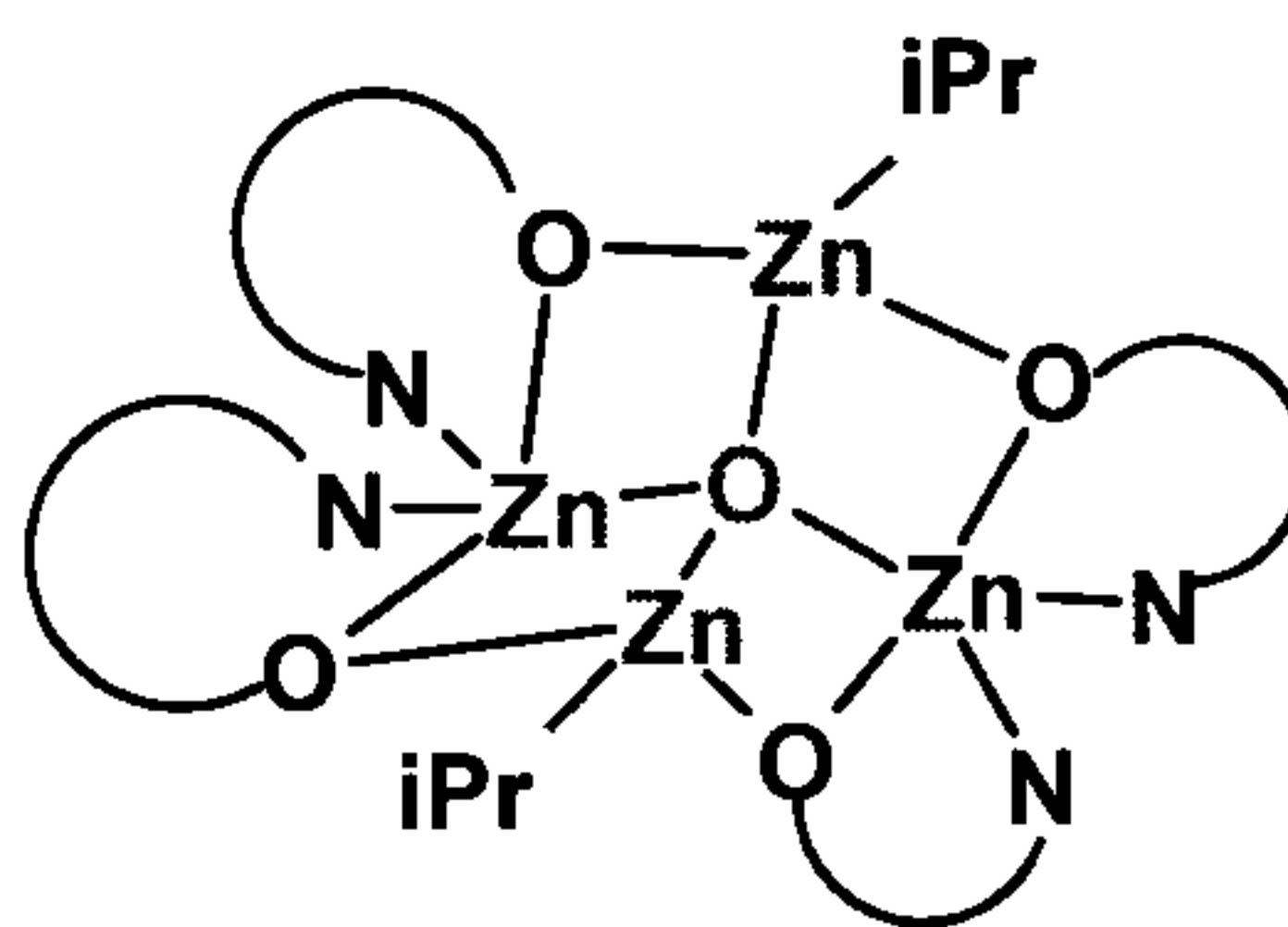
Preparation of a fluorescent compound of formula $[(^t\text{Bu})_2\text{Zn}_4(\mu_4\text{-O})(\text{Meq})_4]$

The monocrystals of the $[(\text{ZnMeq}_2)_2(^t\text{BuOOZnMeq})_2]$ adducts were heated at 200°C for 30 min. After this time, crystalline powder was obtained and characterized using an x-ray powder diffractometer. The spectrum of the tested compound corresponds to the reference spectrum of the $[(^t\text{Bu})_2\text{Zn}_4(\mu_4\text{-O})(\text{Meq})_4]$ adduct of structural formula presented in Example 3.

Example 6

Preparation of a fluorescent compound of formula $[(^i\text{Pr})_2\text{Zn}_4(\mu_4\text{-O})(\text{Meq})_4]$

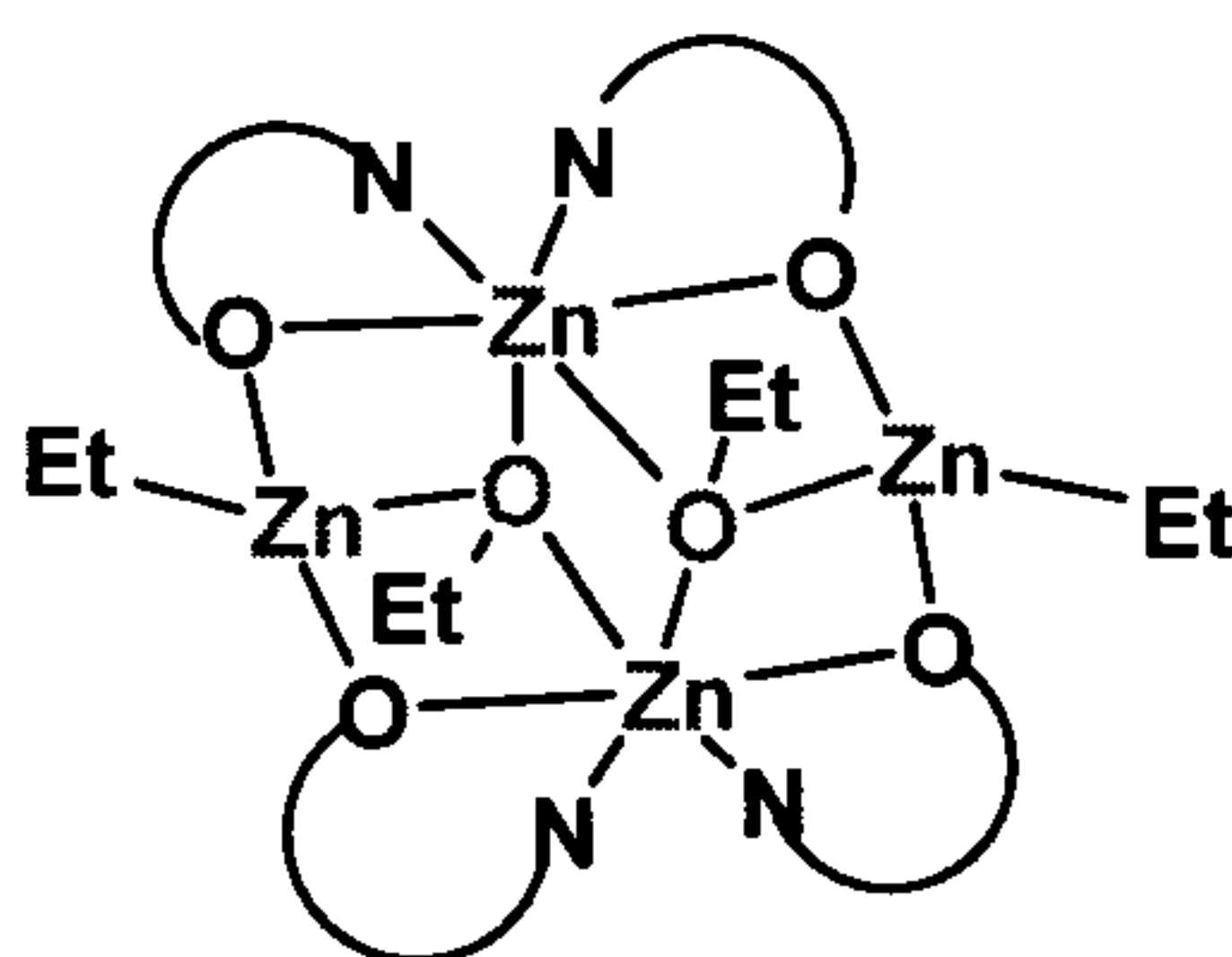
0.1 mL of 0.5M solution of water in THF (0.05 mmol H_2O) was added to 5 mL of a solution containing 0.135 g (0.5 mmol) of isopropyl derivative of 5,7-dimethyl-8-hydroxyquinoline $(^i\text{PrZnMeq})_3$ in tetrahydrofuran. The reaction was carried out for 4 hours at room temperature. The x-ray structural and spectral studies showed that the product of the reaction was the $[(^i\text{Pr})_2\text{Zn}_4(\mu_4\text{-O})(\text{Meq})_4]$ adduct of structural formula presented below.



Example 7

Preparation of a fluorescent compound of formula $[(Et)_2Zn_4(OEt)_2(Bq)_4]$

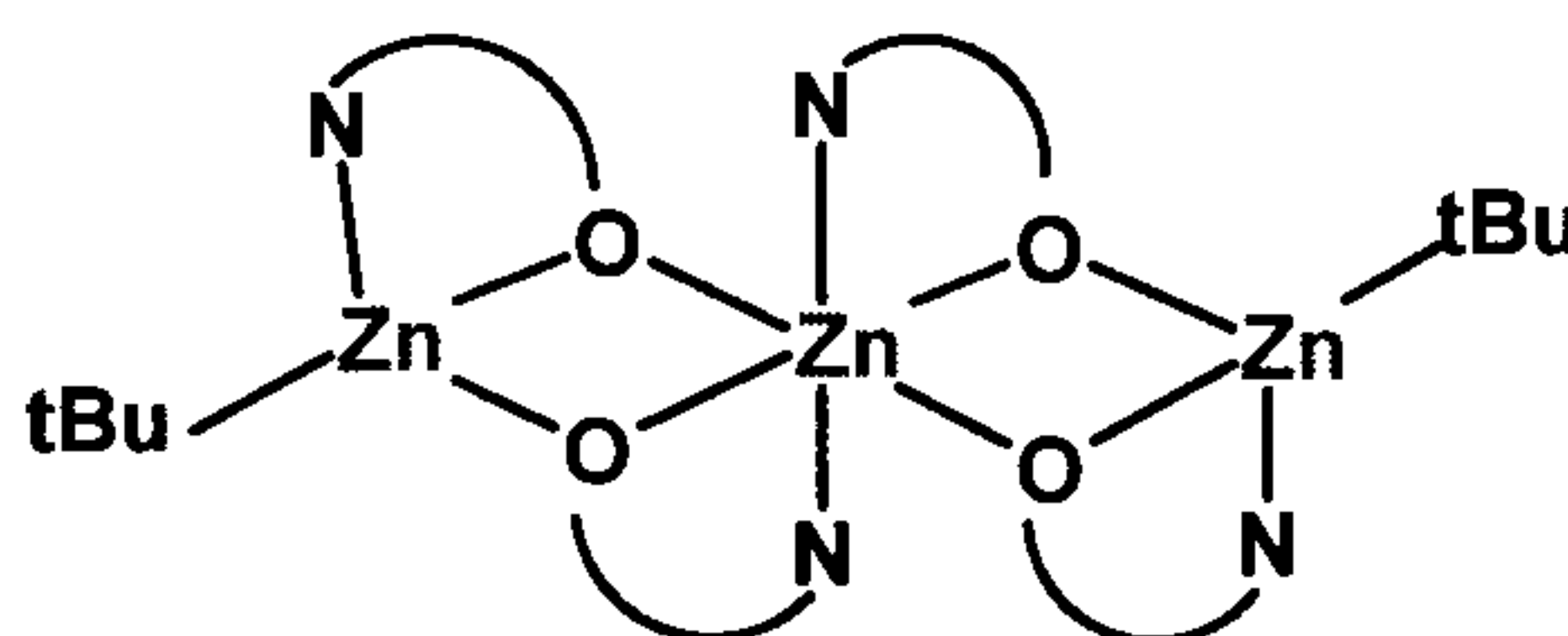
2 mL of the solution containing 0.1 g of the ethylzinc derivative of 10-hydroxybenzoquinoline ($tBuZnBq$)₃ in tetrahydrofuran was submitted to reaction with oxygen at -70°C for 5 minutes. Crystallization yielded monocrystals suitable for x-ray structural studies. The x-ray structural studies showed that the oxidation product was the $[(Et)_2Zn_4(OEt)_2(Bq)_4]$ adduct of structural formula presented below.



Example 8

Preparation of a fluorescent compound of formula $\{[Zn(BTZ)_2] (tBuZnBTZ)_2\}$

An equimolar amount of water was added to 5 mL of the solution containing 0.1 g of *tert*-butylzinc derivative of 2-(2-hydroxyphenyl)benzothiazole (BTZ) in toluene at -78°C. The reaction mixture was left to reach the room temperature, after which the reaction was conducted for 4 hours. Crystallization yielded monocrystals suitable for x-ray structural studies. The x-ray structural studies showed that the product of the reaction was the $\{[Zn(BTZ)_2] (tBuZnBTZ)_2\}$ adduct of structural formula presented below.

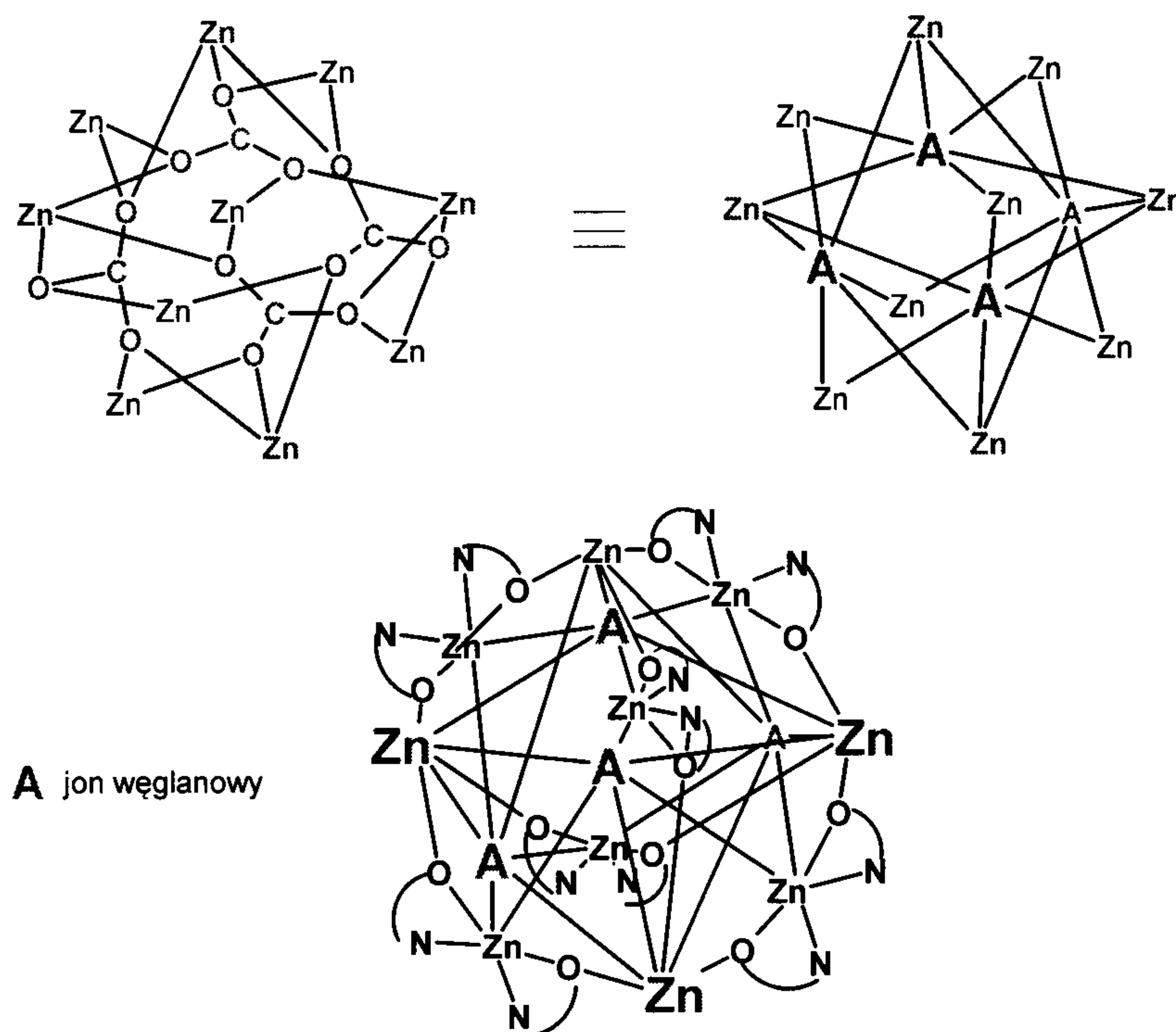


Example 9

Preparation of a fluorescent compound of formula $Zn_{10}q_{12}(CO_3)_4$

5 mL of the solution containing 0,1 g of the precursor $[(Znq_2)_2(tBuZnOH)_2]$ in tetrahydrofuran was submitted to reaction with carbon dioxide at 0°C under 1 atm. Crystallization yielded

monocrystals suitable for x-ray structural studies. The x-ray structural studies showed that the product of the reaction was the $\text{Zn}_{10}\text{q}_{12}(\text{CO}_3)_4$ adduct of structural formula presented below.



Example 10

Preparation of a fluorescent compound of formula $\text{Zn}_{10}\text{q}_{12}(\text{CO}_3)_4$

5 mL of the solution containing 0,1 g of the precursor $[(\text{Znq}_2)_2(\text{tBuZnOH})_2]$ in tetrahydrofuran was submitted to reaction with carbon dioxide at 25°C under 70 atm for 2 h. The product was obtained as a crystalline powder characterized using an x-ray powder diffractometer. The x-ray diffraction studies showed that the product of the reaction was the $\text{Zn}_{10}\text{q}_{12}(\text{CO}_3)_4$ adduct of structural formula presented in Example 9.

Example 11

Preparation of a fluorescent compound of formula $\text{Zn}_{10}\text{q}_{12}(\text{CO}_3)_4$

0.042 g (0.4 mmol) of Na_2CO_3 was added to 5 mL of a solution containing 0.355 g (1 mmol) Znq_2 precursor in toluene. The reaction was carried out at 25°C for 24 h. Crystallization yielded monocrystals suitable for x-ray structural studies. The x-ray diffraction studies showed that the product of the reaction was the $\text{Zn}_{10}\text{q}_{12}(\text{CO}_3)_4$ adduct of structural formula presented in Example 9.

Spectroscopic properties of $\text{Zn}_{10}\text{q}_{12}(\text{CO}_3)_4$ obtained according to Examples 9., 10 and 11 are presented in Fig. 1 and Fig. 2. Fig. 1 shows the increase in fluorescence intensity as measured in the course of transformations of the $[(\text{Znq}_2)_2(\text{tBuZnOH})_2]$ precursor in the presence of carbon

dioxide, while Fig. 2 shows the fluorescence spectra of $\text{Zn}_{10}\text{q}_{12}(\text{CO}_3)_4$ (solid line) and quinine sulphate as the reference compound (dashed line) in toluene. Quantum yield of $\text{Zn}_{10}\text{q}_{12}(\text{CO}_3)_4$ is 64% (Examples 9., 10 and 11).

Table 2 presents crystallographic data of $[(^t\text{Bu})_2\text{Zn}_4(\mu_4\text{-O})(\text{Meq})_4]$, $[(^t\text{Bu})_2\text{Zn}_4(\mu_4\text{-O})(\text{Meq})_4]$, $[(\text{Et})_2\text{Zn}_4(\text{OEt})_2(\text{Bq})_4]$, $\{[\text{Zn}(\text{BTZ})_2] (^t\text{BuZnBTZ})_2\}$ and $\text{Zn}_{10}\text{q}_{12}(\text{CO}_3)_4$.

Example 12

Cell staining

$\text{Zn}_{10}\text{q}_{12}(\text{CO}_3)_4$, solubilized in water using appropriate double- and triple-block polymers, such as polyethylene-polypropylene glycol and poloxameres, was used for staining of a line of human fibroblasts. Microscopic specimens were prepared following three hours of incubation of cells in a phosphate buffer solution containing $\text{Zn}_{10}\text{q}_{12}(\text{CO}_3)_4$. Microscopic analysis revealed efficient migration of the fluorophore into the cytoplasm, resulting in cell staining. No drop of fluorescence intensity over time was observed during irradiation with excitation wavelengths. Polymer-coated molecules dissolved in water had the average size of ca. 5 nm. Fig. 3 shows a fluorescence microscopy image of stained fibroblasts, while Fig. 4 shows an image of the control sample, i.e. cells treated with polymer alone.

Example 13

Thin layers of $[\text{RZn}(\text{q})]_3$ (**1**), $[(\text{R})_3\text{Zn}_5(\mu_4\text{-O})(\text{q})_5]$ (**2**) and $[\text{Zn}(\text{q})_2]_2[\text{RZn}(\text{OH})]_2$ (**3**) were obtained. The compounds have different spectroscopic properties. Maximum luminescence bands cover a wide range of 470 nm to 555 nm (Fig. 5), which makes it possible to obtain emission layers generating blue, green, or yellow light.

Table 1

Parameter	Fluorescent organic dyes	Quantum dots (QDs)	$[\text{Zn}(\text{CO}_3)]_4[\text{Znq}_2]_6$
Absorption spectrum	Band spectrum with half-width ranging from 20 to over 100 nm.	Continuous spectrum, with intensities increasing towards shorter wavelengths (UV), which makes excitation of QDs possible over a wide range of the spectrum.	Band spectrum with half-width ranging from 80 to 200 nm which makes excitation possible over a wide range of the spectrum.
Emission spectrum	Asymmetric bands with half-widths of 30-100 nm	Symmetric bands with half-widths of 30-90 nm	Symmetric bands with half-widths of 50-200 nm
Stokes shift	Usually below 50 nm	Below 50 nm upon excitation with visible wavelengths.	Above 100 nm, which makes the emitted light have a different color than the excitation light.
Quantum yield (QY)	0.5–1.0	0.1–0.5	Above 0,5
Size	Ca. 0.5- 10 nm	10-60 nm, (hydrodynamic radius)	1.5-5 nm, (hydrodynamic radius)
Optical stability	Insufficient for methods making use of high-intensity light or near-infrared tags Application in long-lasting tests is impossible. Photobleaching occurs.	Orders of magnitude higher than in case of organic tags. Blinking occurs.	Very high optical stability allowing to conduct tests lasting many hours. No photobleaching or blinking.

Table 2. Crystallographic data of $[(^t\text{Bu})_2\text{Zn}_4(\mu_4\text{-O})(\text{Meq})_4]$, $[(^t\text{Bu})_2\text{Zn}_4(\mu_4\text{-O})(\text{Meq})_4]$, $[(\text{Et})_2\text{Zn}_4(\text{OEt})_2(\text{Bq})_4]$, $\{[\text{Zn}(\text{BTZ})_2] (^t\text{BuZnBTZ})_2\}$ and $\text{Zn}_{10}\text{q}_{12}(\text{CO}_3)_4$.

	$[(^t\text{Bu})_2\text{Zn}_4(\mu_4\text{-O})(\text{Meq})_4]$ Example 1	$[(^t\text{Bu})_2\text{Zn}_4(\mu_4\text{-O})(\text{Meq})_4]$ Example 3
molecular formula	$\text{C}_{44}\text{H}_{42}\text{N}_4\text{O}_6\text{Zn}_4$	$\text{C}_{52}\text{H}_{58}\text{N}_4\text{O}_5\text{Zn}_4$
M_r	984.30	1080.50
crystallographic system	Triclinic	triclinic
space group	$P-1$ (no. 2)	$P-1$ (no. 2)
temperature [K]	100 (2)	100 (2)
a [Å]	11.3576(7)	13.4530(13)
b [Å]	11.7735(6)	13.7190(15)
c [Å]	12.1176(7)	18.322(2)
α [°]	96.619(3)	97.862(5)
β [°]	106.962(3)	96.991(7)
γ [°]	109.971(3)	98.680(7)
unit cell volume [Å ³]	1414.43(14)	3276.2(6)
Number of molecules per unit cell	1	2
calculated density [g cm ⁻³]	1.156	1.095
radiation applied	$\mu(\text{Mo-K}\alpha) \lambda = 0.71073$	
Angular range 2θ [°]	2.92-21.26	2.04-22.72
Number of counted reflexes	12035	14564
Number of data / parameters	3138 / 262	8074 / 586
GOOF fit index	1.062	1.064
Divergence coefficients R for $I > 2\sigma(I)$	R1 = 0.0671 wR2 = 0.1493	R1 = 0.0595 wR2 = 0.1509
Divergence coefficients R for all reflexes	R1 = 0.0926 wR2 = 0.1620	R1 = 0.0762 wR2 = 0.1608

	[(Et) ₂ Zn ₄ (OEt) ₂ (Bq) ₄] Example 7	{[Zn(BTZ) ₂] (^t BuZnBTZ) ₂ } Example 8
molecular formula	C ₆₀ H ₅₂ N ₄ O ₆ Zn ₄	C ₆₀ H ₅₀ N ₄ O ₄ S ₄ Zn ₃
Mr	1186.54	1215.39
crystallographic system	Monoclinic	monoclinic
space group	<i>P</i> 2 ₁ /c (no. 14)	<i>P</i> 2 ₁ (no. 4)
temperature [K]	100 (2)	100 (2)
a [Å]	12.7490(7)	11.4520(5)
b [Å]	11.1180(6)	14.4330(9)
c [Å]	23.9520(12)	18.2980(10)
α [°]	90.00	90.00
β [°]	102.192(3)	100.66(3)
γ [°]	90.00	90.00
unit cell volume [Å ³]	3318.5(3)	2972.3(3)
Number of molecules per unit cell	2	2
calculated density [g cm ⁻³]	1.187	1.358
radiation applied	μ(Mo-Kα) λ = 0.71073	
Angular range 2θ [°]	2.03-24.71	1.81-23.81
Number of counted reflexes	8150	8848
Number of data / parameters	4848 / 334	7848 / 677
GOOF fit index	1.113	1.080
Divergence coefficients R for <i>I</i> > 2σ(<i>I</i>)	R1 = 0.0520 wR2 = 0.1310	R1 = 0.0782 wR2 = 0.1552
Divergence coefficients R for all reflexes	R1 = 0.0662 wR2 = 0.1385	R1 = 0.1022 wR2 = 0.1686

Zn ₁₀ q ₁₂ (CO ₃) ₄	
Example 9, 10 and 11	
molecular formula	C ₁₁₂ H ₇₂ N ₁₂ O ₂₄ Zn ₁₀
<i>M_r</i>	2623.52
crystallographic system	Trigonal
space group	<i>R</i> -3 (no. 148)
temperature [K]	100 (2)
<i>a</i> [Å]	22.3630(19)
<i>b</i> [Å]	22.3630(19)
<i>c</i> [Å]	54.763(3)
<i>α</i> [°]	90.00
<i>β</i> [°]	90.00
<i>γ</i> [°]	120
unit cell volume [Å ³]	23718(3)
Number of molecules per unit cell	6)
calculated density [g cm ⁻³]	1.102
radiation applied	μ(Mo-Kα) λ = 0.71073
Angular range 2θ [°]	2.14-23.23
Number of counted reflexes	7487
Number of data / parameters	6679 / 475
GOOF fit index	1.027
Divergence coefficients <i>R</i> for <i>I</i> > 2σ(<i>I</i>)	<i>R</i> 1 = 0.0542 <i>wR</i> 2 = 0.1398
Divergence coefficients <i>R</i> for all reflexes	<i>R</i> 1 = 0.0797 <i>wR</i> 2 = 0.1535

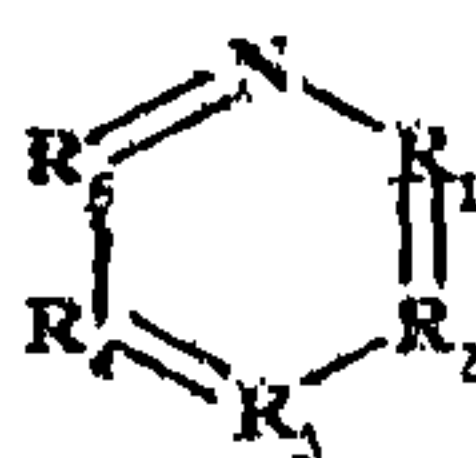
AMENDED CLAIMS
received by the International Bureau on 16 December 2011 (16.12.2011)

1. Compounds of general formula

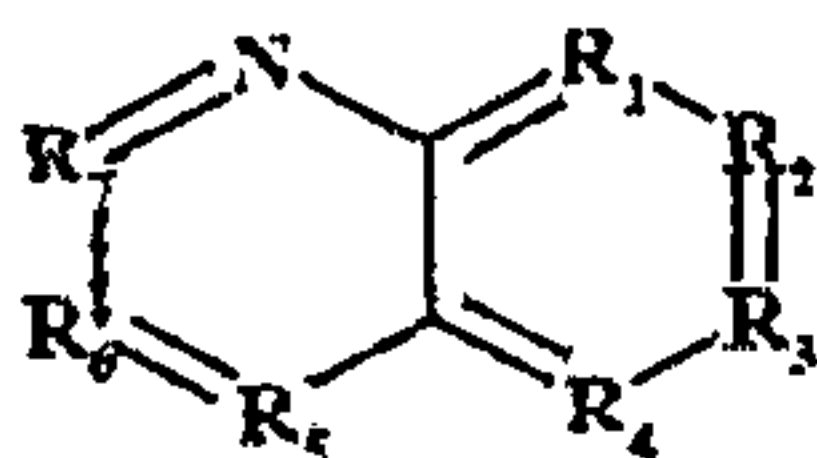


wherein

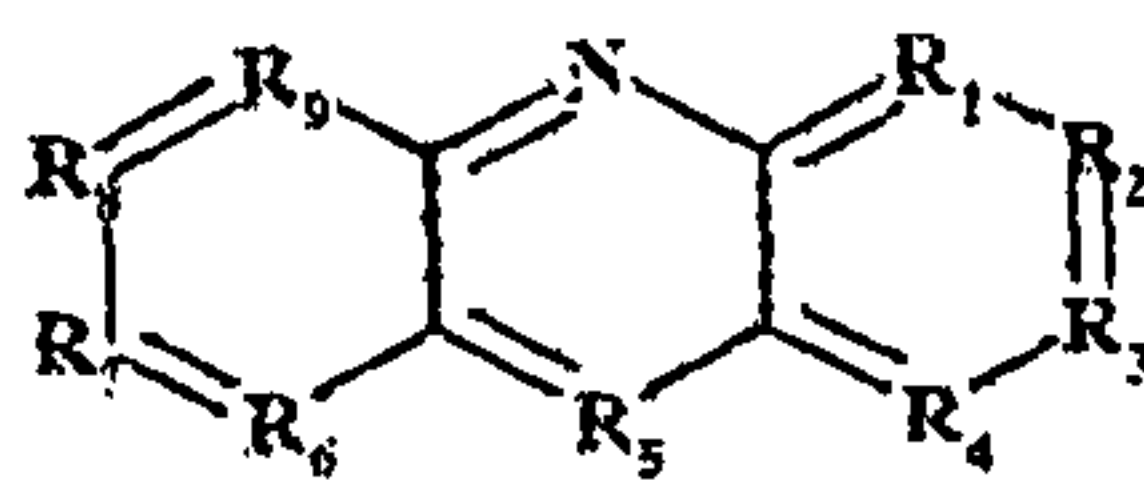
L is a bi- or multifunctional organic neutral ligand or its deprotonated form, represented by the structure of formula 1 or formula 2 or formula 3 or formula 4 or formula 5 or formula 6 or formula 7 or formula 8 or formula 9 or formula 10 or formula 11 or formula 12 or formula 13:



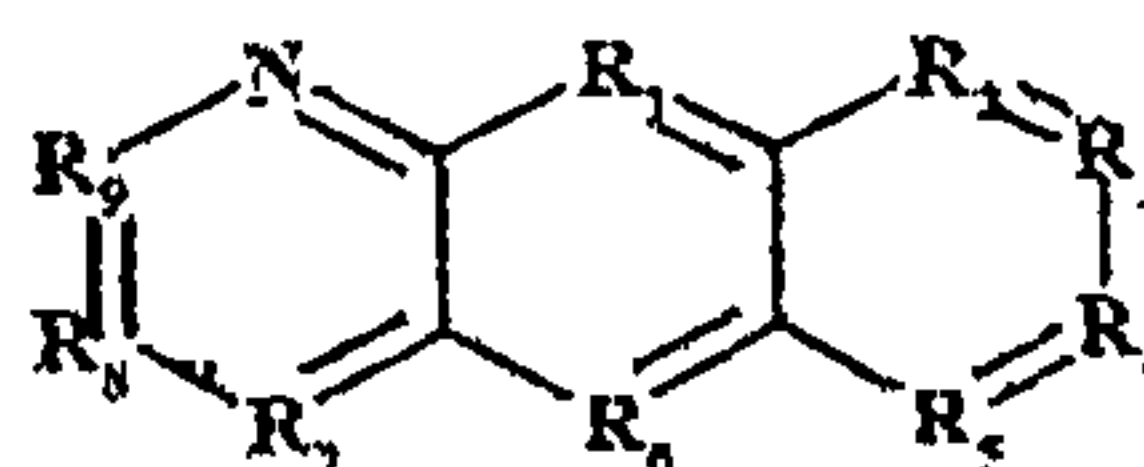
Formula 1



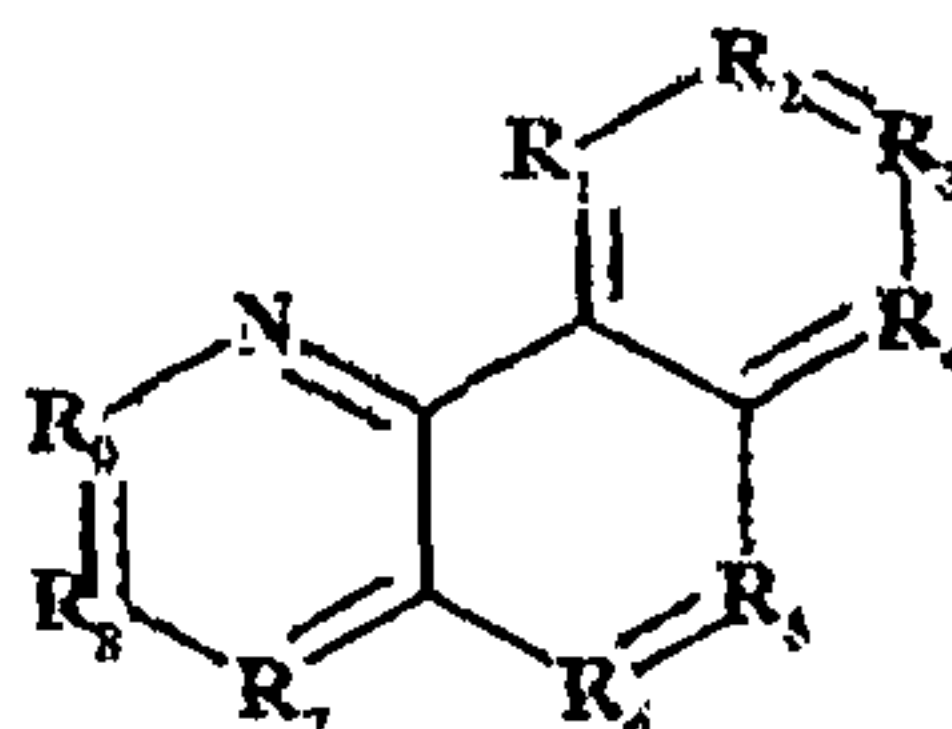
Formula 2



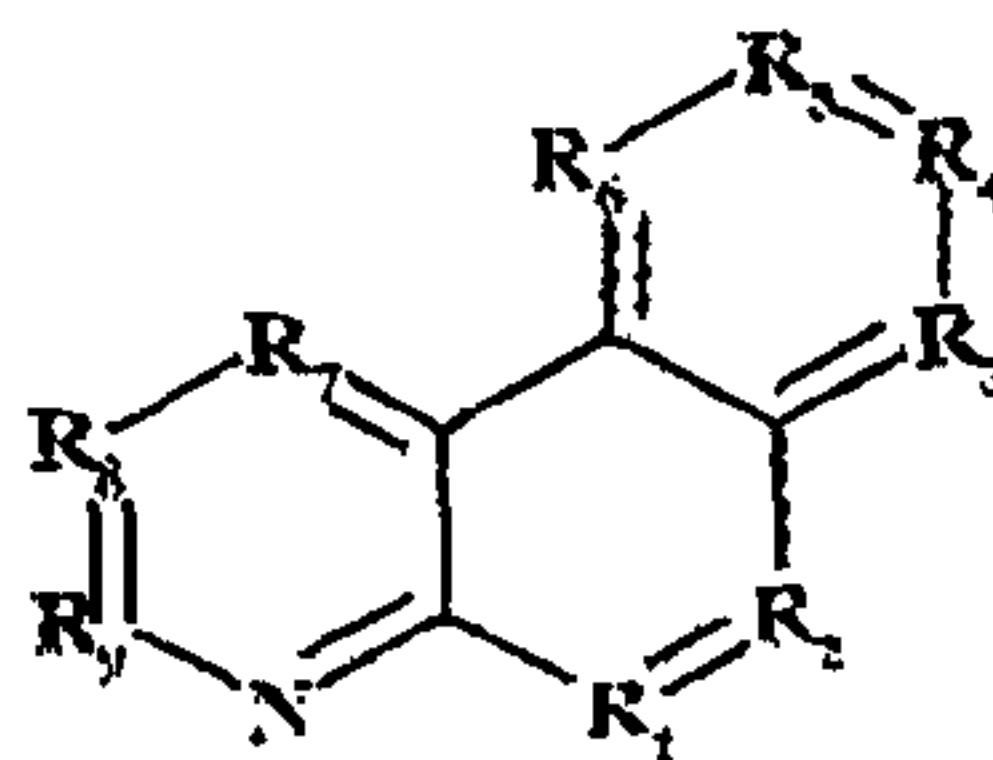
Formula 3



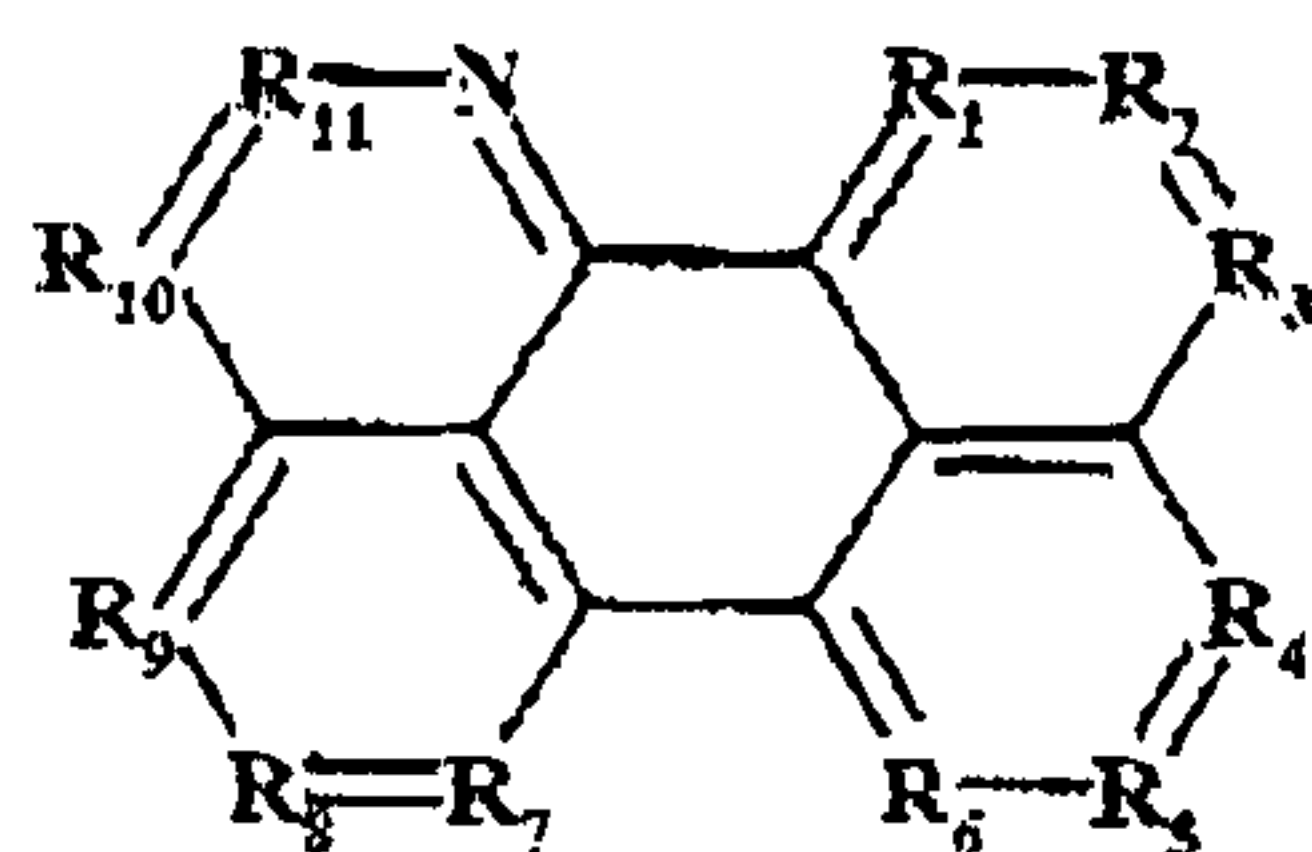
Formula 4



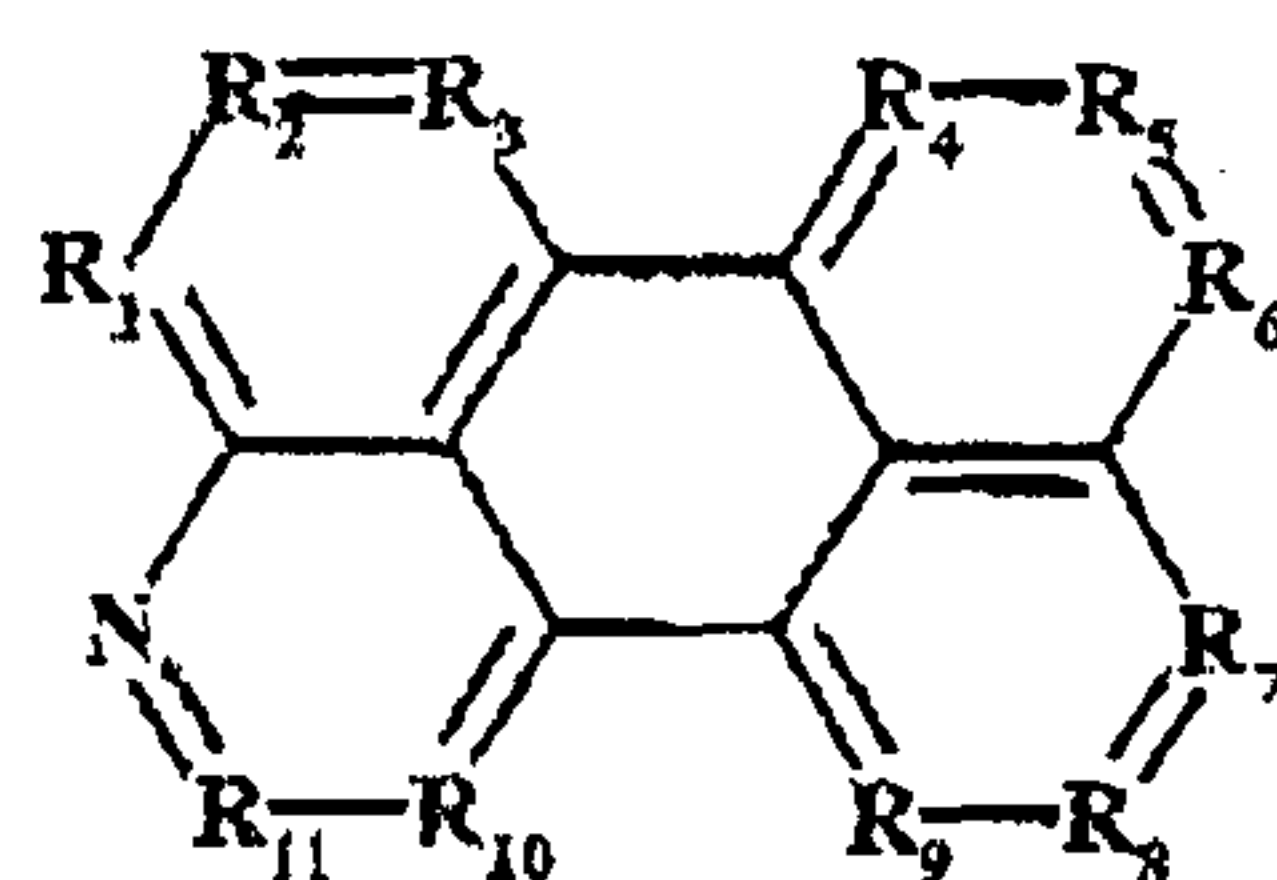
Formula 5



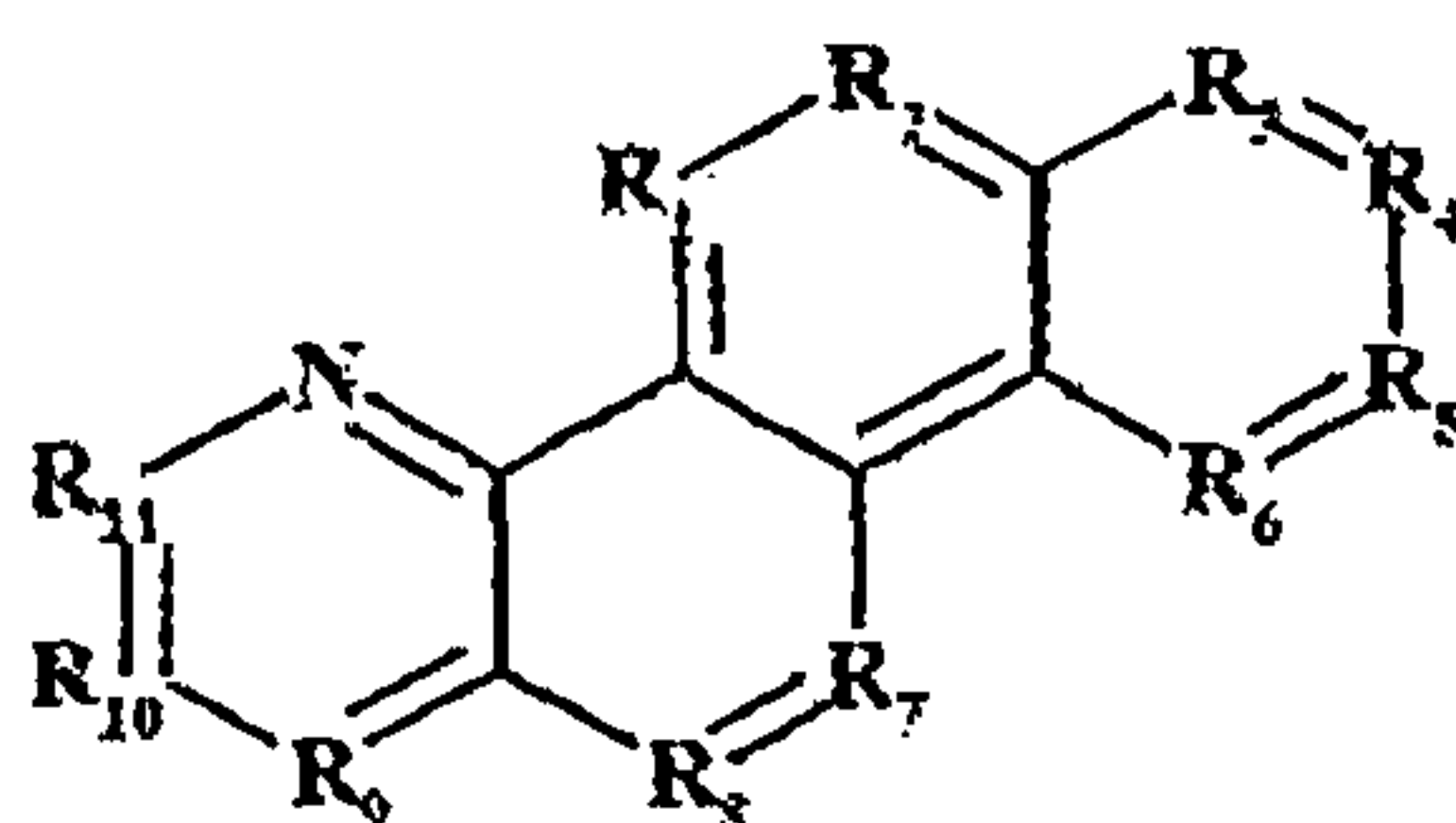
Formula 6



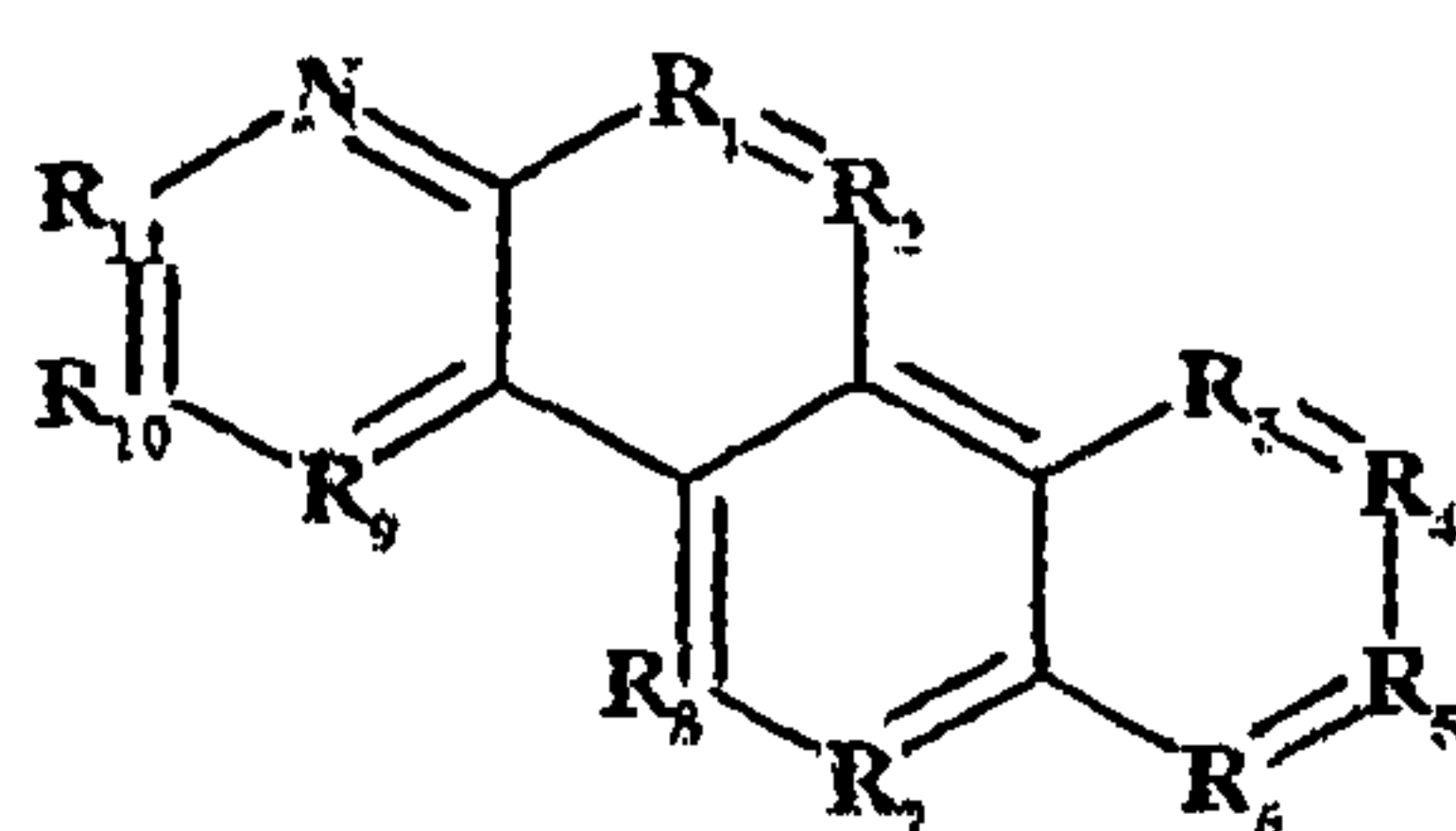
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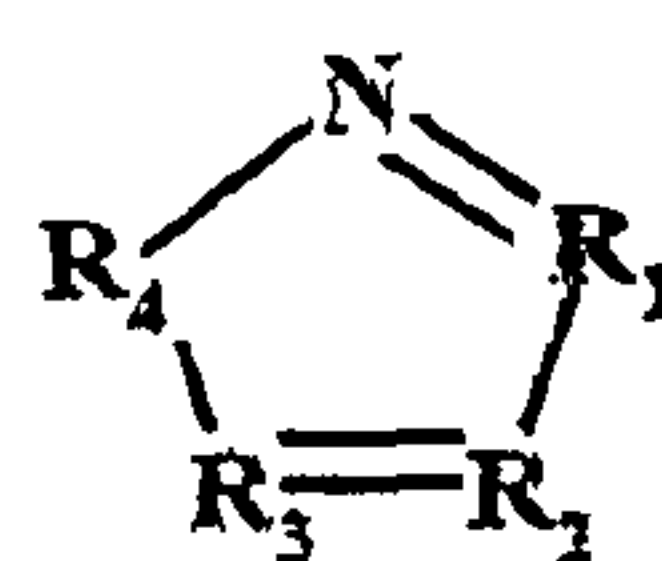
Formula 8



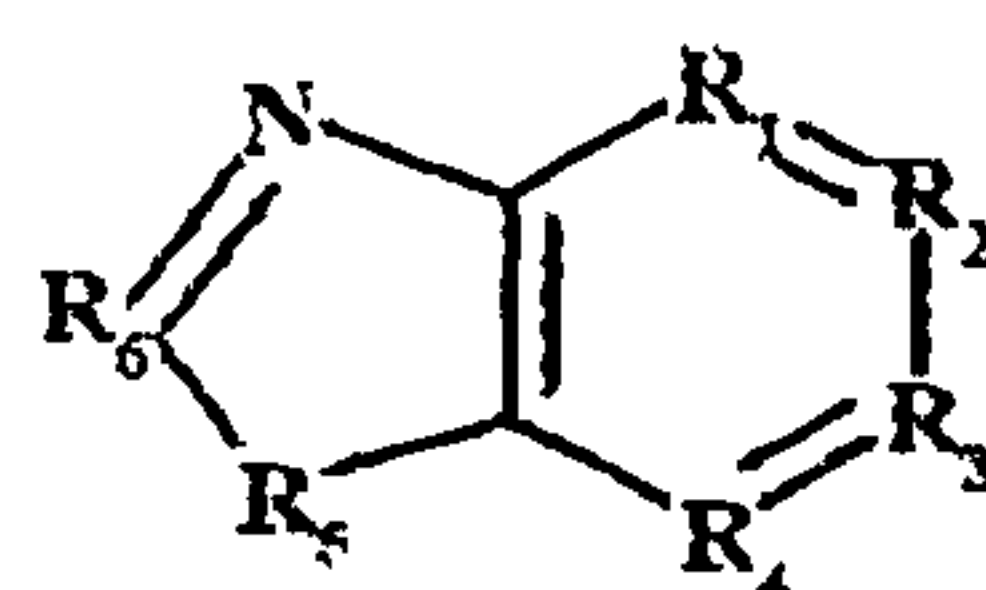
Formula 9



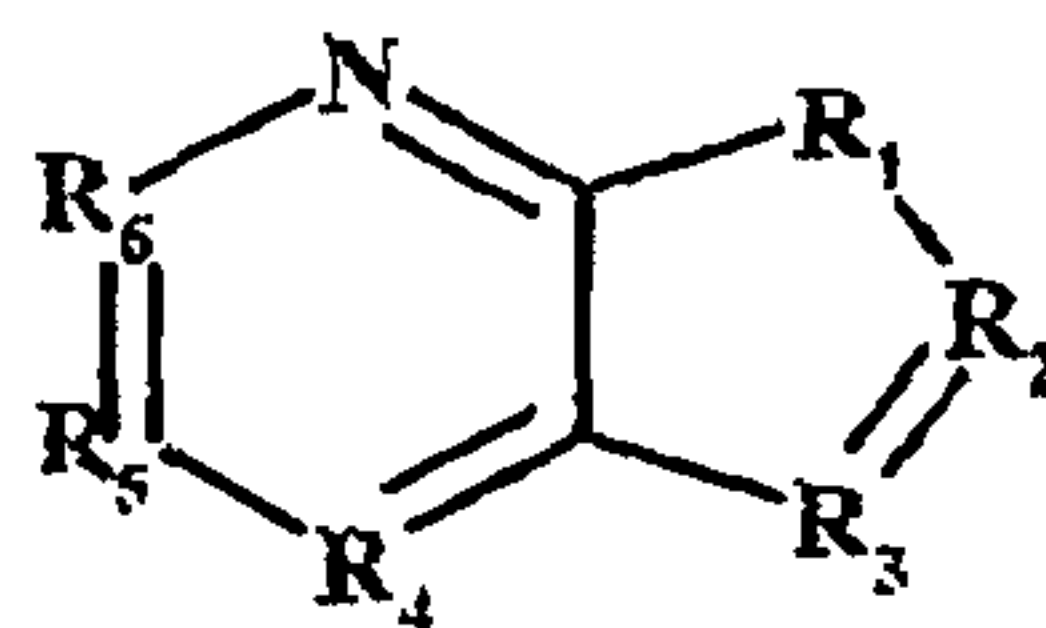
Formula 10



Formula 11



Formula 12



Formula 13

wherein R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 are heteroatoms selected from nitrogen, sulfur, oxygen and carbon attached to: a hydrogen, straight or branched C1-C10 alkyl, possibly substituted, phenyl, possibly substituted, benzyl, possibly substituted, ether group, possibly substituted, ketone group, possibly substituted, halogen, -OH, -SH, -NH₂, -NHR, -COOH, -CONH₂, -CONRH, -O⁻, -S⁻, -NH-, -NR⁻, -COO⁻, -CONH⁻, or -CONR⁻;

wherein the ligand comprises at least one functional group selected from -OH, -SH, -NH₂, -NHR, -COOH, -CONH₂, -CONRH and their deprotonated equivalents -O⁻, -S⁻, -NH-, -NR⁻, -COO⁻, -CONH⁻, -CONR⁻;

A is an inorganic anion selected from O²⁻, S²⁻, Se²⁻, Te²⁻, CO₃²⁻, SO₄²⁻, SO₃²⁻, CS₂O²⁻, CS₃²⁻, BO₃³⁻, NO₂⁻, and NO₃⁻,

X is oxygen or sulfur,

R' is C1-C10 alkyl, straight or branched, benzyl, phenyl, cyclohexyl or halogen,

R is hydrogen, alkyl or aryl,

x is a number from 0 to 6,

y is a number from 1 to 12,

z is a number from 1 to 12,

n is a number from 0 to 6,

m is a number from 0 to 6,

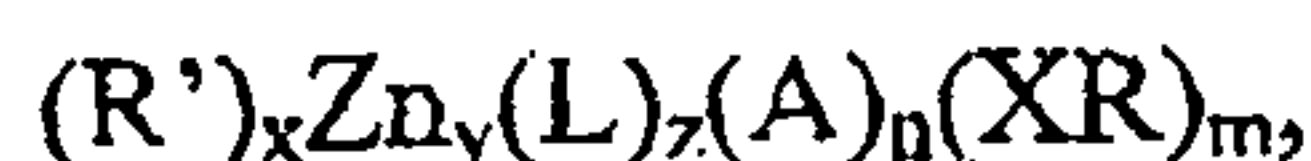
wherein x≠0, n≠0 and m≠0 at the same time and if n and m = 0, then x ≠ z.

2. Compounds according to Claim 1, wherein the inorganic anion A is selected from O²⁻, S²⁻, Se²⁻, CO₃²⁻, CS₂O²⁻, BO₃³⁻, and NO₃⁻.

3. Compounds according to claim 1, wherein the functional group is separated from the Ligand L by a saturated or unsaturated carbon chain of 1-3 carbon atoms.

4. Compounds according to Claim 1, wherein R1 in ligand L is bound to one of groups selected from: -OH, -SH, -NH₂, -NHR, -COOH, -CONH₂, -CONRH, -O⁻, -S⁻, -NH⁻, -NR⁻, -COO⁻, -CONH⁻, and -CONR⁻.
5. Compounds according to Claim 1, wherein R1 in ligand L is bound to one of the groups selected from: -O⁻, -S⁻, -NH⁻, -NR⁻, -COO⁻, -CONH⁻, and -CONR⁻.
6. Compounds according to claim 1, wherein L is selected from the group consisting of 8-hydroxyquinoline, 10-hydroxybenzoquinoline and 2-(2-hydroxyphenyl)benzothiazole.
7. Compounds according to claim 1, wherein x is a number from 1 to 6.
8. Compounds according to claim 1, wherein m is a number from 1 to 6.
9. Compounds according to claim 1, wherein L is represented by the structure of formula 2 or formula 3 or formula 5 or formula 8 or formula 10 or formula 12.
10. A compound according to claim 1, wherein the compound is selected from the group consisting of
 - (a) [(Znq₂)₂(^tBuZnOH)₂];
 - (b) [(Znq₂)₂(EtZnOH)₂];
 - (c) [(^tBu)₂Zn₄(μ₄-O)(Meq)₄];
 - (d) [(ⁱPr)₂Zn₄(μ₄-O)(Meq)₄];
 - (e) [(Et)₂Zn₄(OEt)₂(Bq)₄];
 - (f) {[Zn(BTZ)₂](^tBuZnBTZ)₂};
 - (g) Zn₁₀q₁₂(CO₃)₄; and
 wherein:
 q is 8-hydroxyquinoline;
 Meq is 5,7-dimethyl-8-hydroxyquinoline;
 BZT is 2-(2-hydroxyphenyl)benzothiazole;
 Et is ethyl;
ⁱPr is isopropyl; and
^tBu is t-butyl;

11. Method for preparation of compounds of general formula



wherein A, L, X, R, R', x, y, z, n, and m are as defined in Claim 1,

wherein a precursor represented by the structure $(R')_xZn_y(L)_z(X_kR)_m$, wherein k is 1 or 2, is subjected to reaction with any one or more of:

- (a) oxygen;
 - 5 (b) water;
 - (c) elemental sulfur;
 - (d) selenium;
 - (e) tellurium;
 - (f) oxyacids selected from carbonic acid, sulfuric (IV) acid sulfuric (VI) acid, thiocarbonic acid, boric acid, nitric (III) acid and nitric (V) acid;
 - 10 (g) binary acids selected from H_2S , H_2Se , and H_2Te ;
 - (g) acid anhydrides selected from CO_2 , SO_2 , CS_2 , B_2O_3 , NO , and NO_2 ; or
 - (h) inorganic acid salts selected from salts of carbonic acid, sulfuric (IV) acid, thiocarbonic acid, boric acid, nitric (III) acid, nitric (V) acid, hydrosulphuric acid, hydroselenic acid, and hydrotelluric acid,
 - 15 in a solvent or to thermal transformation.
12. Method according to Claim 11, wherein an anhydrous organic solvent is used.
 13. Method according to Claim 11, wherein a water-containing organic solvent is used.
 14. Method according to Claim 11, wherein water is used as the solvent.
 - 20 15. Method according to Claim 12 or 13, wherein the organic solvent is toluene, tetrahydrofuran, hexane, methylene chloride, dimethylsulfoxide, acetonitrile as well as alcohols, phenol or acids in which the precursor is well soluble or a mixture of these compounds.
 16. Method according to Claim 11, wherein the reaction is carried out at the temperature of -70°C to 200°C.
 - 25 17. Method according to Claim 16, wherein the reaction is carried out at a temperature of -70°C to 100°C.
 18. Method according to Claim 11, comprising the step of reacting the precursor with an acid anhydride selected from CO_2 , SO_2 , CS_2 , B_2O_3 , NO , and NO_2 .
 - 30 19. Method according to Claim 11, comprising the step of reacting the precursor with an inorganic acid salt selected from salts of carbonic acid, sulfuric (IV) acid, thiocarbonic acid, boric acid, nitric (III) acid, nitric (V) acid, hydrosulphuric acid, hydroselenic acid, and hydrotelluric acid.

20. Method according to Claim 11, comprising the step of reacting the precursor with anoxyacid selected from carbonic acid, sulfuric (IV) acid sulfuric (VI) acid, thiocarbonic acid, boric acid, nitric (III) acid, and nitric (V) acid.
21. Method according to Claim 11, comprising the step of reacting the precursor with a
5 binary acid selected from H_2S , H_2Se , and H_2Te .
22. Method according to Claim 11, comprising the step of reacting the precursor with elemental sulfur, selenium or tellurium.
23. Method according to Claim 11, wherein the reaction is carried out under a pressure range of 0.1-100 bar.
- 10 24. Method according to Claim 11 or 23, wherein the reaction is carried out under a pressure range of 1-100 bar.
25. Method according to Claim 11 or 23, wherein the reaction is carried out under a pressure range of 1-20 bar.
26. Method according to Claim 11, wherein the precursor is subjected to thermal
15 transformation at a temperature range of 60-700°C.
27. Method according to Claim 11 or 26, wherein the precursor is subjected to thermal transformation at a temperature range of 60-400°C.
28. The use of compounds according to Claim 1 in generation of emission and conduction layers.
- 20 29. Use according to Claim 28, wherein the emission and conduction layers are used in production of electroluminescent diodes and devices making use of luminophores.
30. The use of compounds according to Claim 1 as fluorescent tags in cellular and tissue imaging.

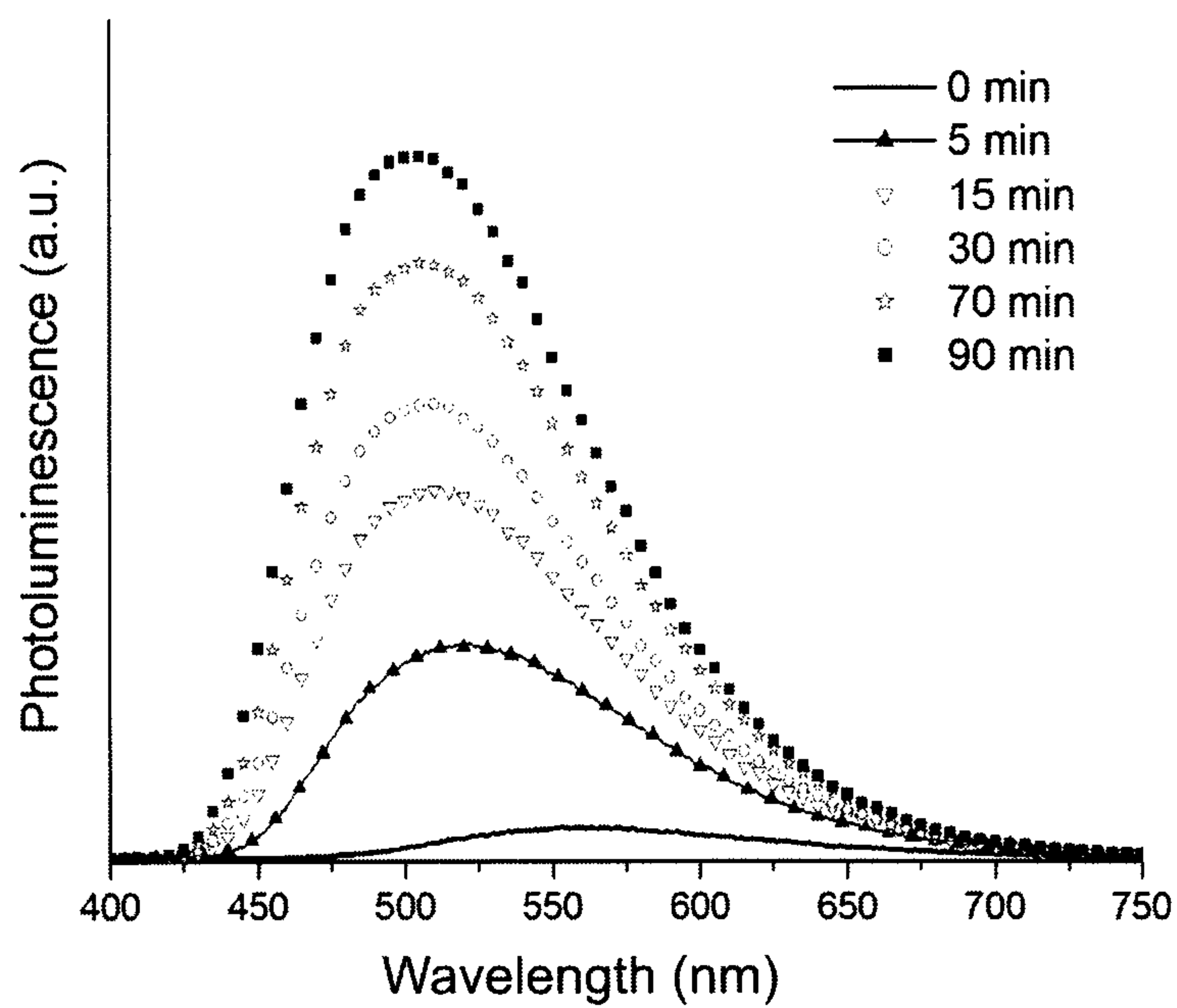


Fig. 1

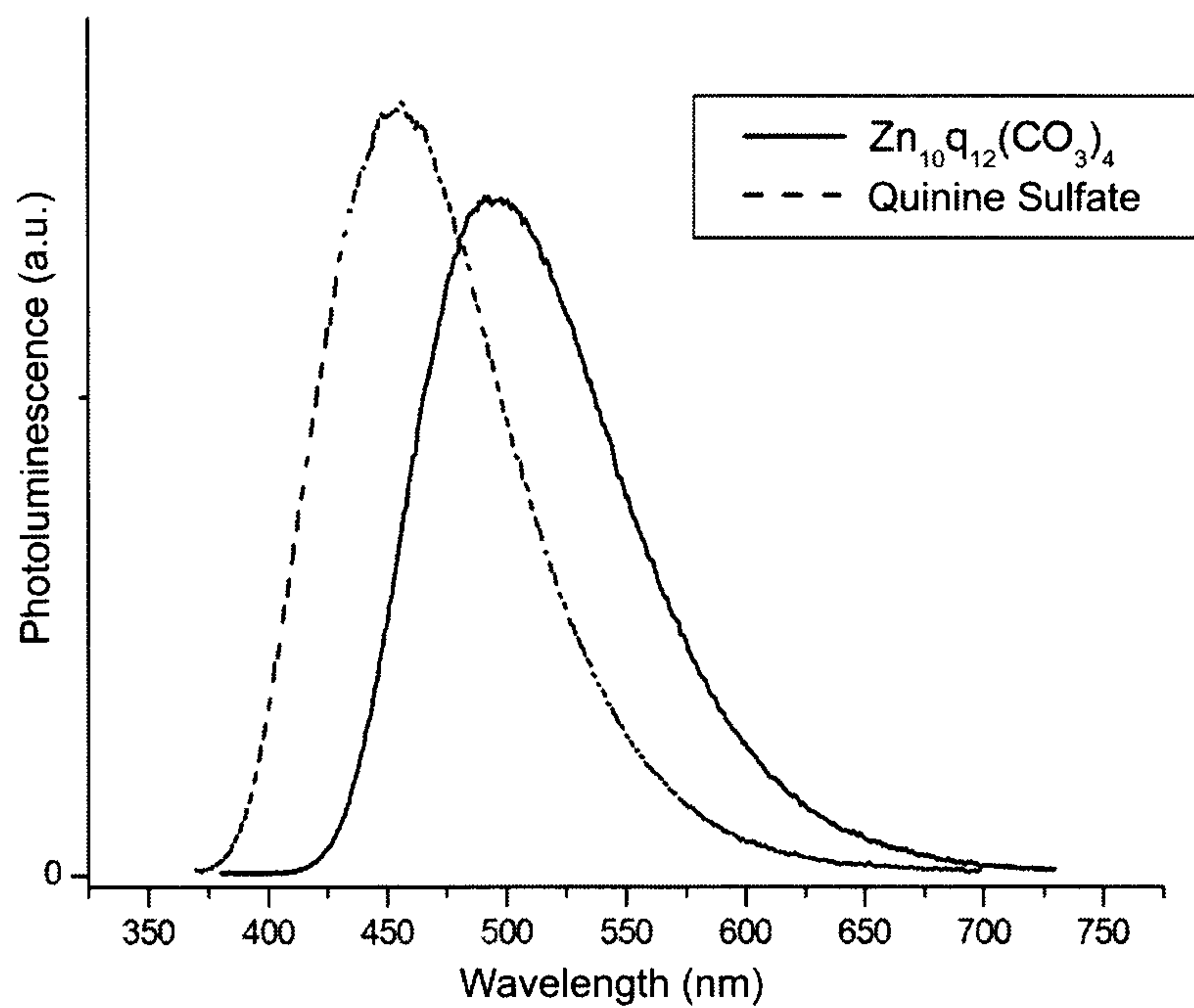


Fig. 2

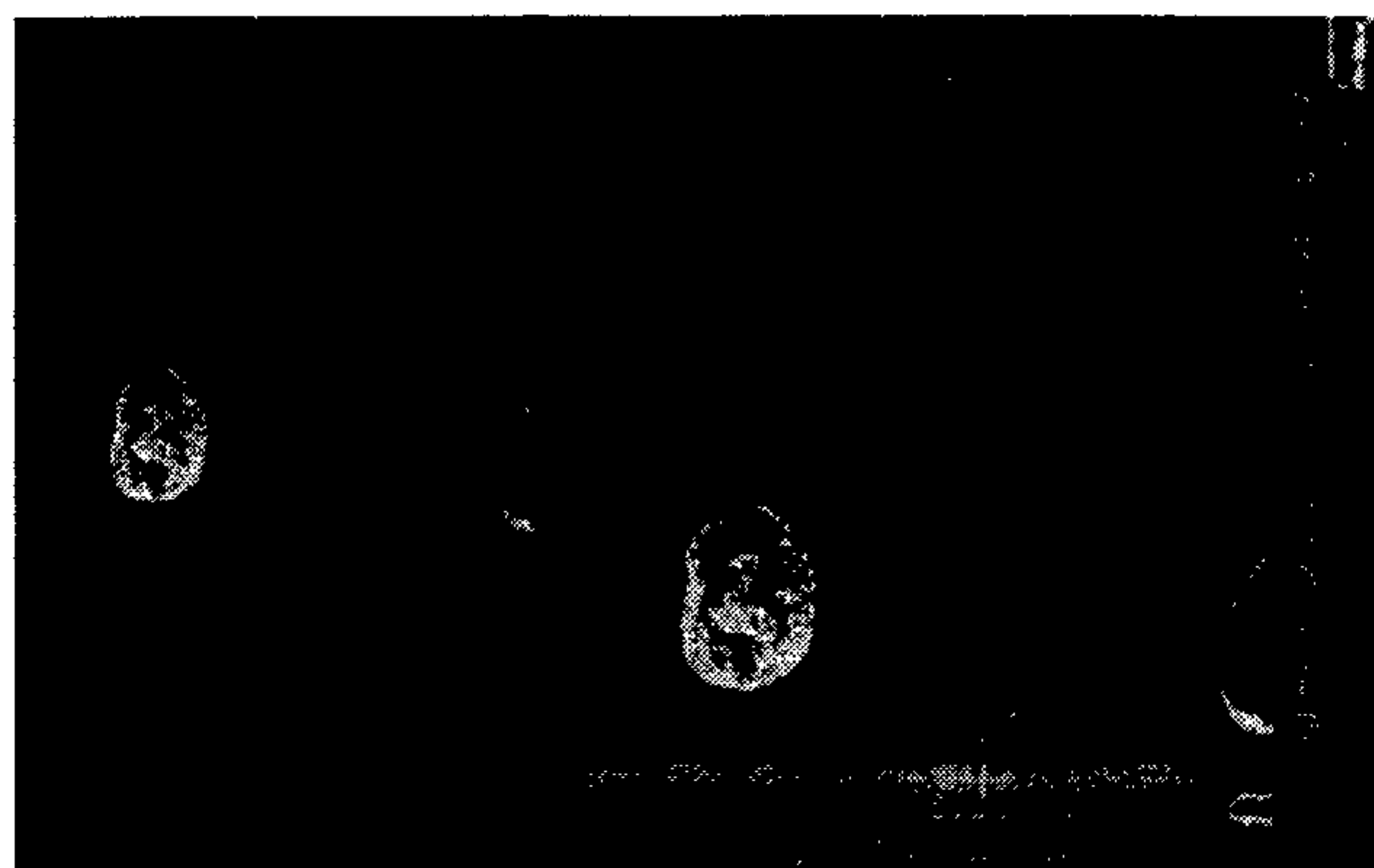


Fig. 3

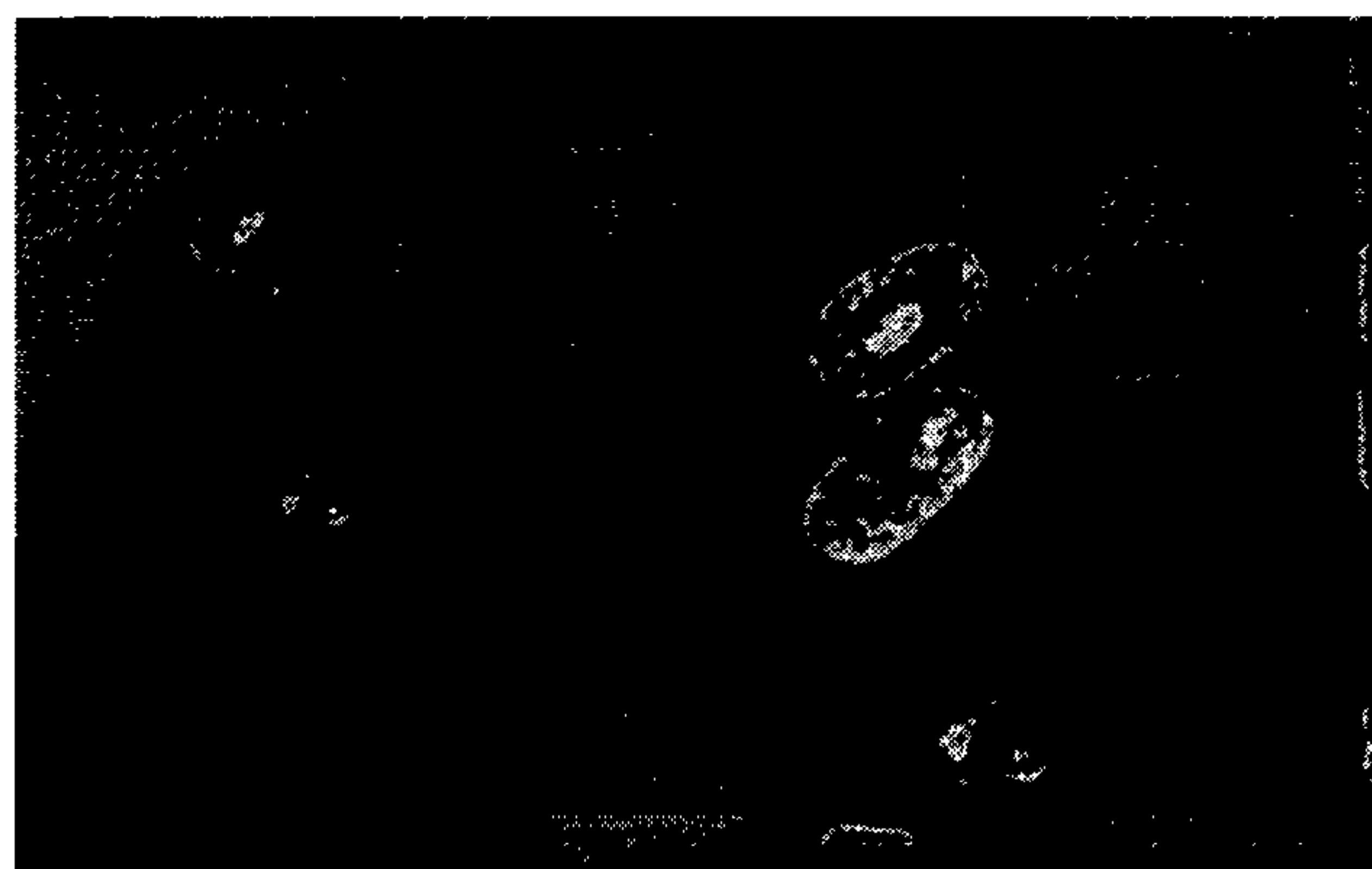


Fig. 4

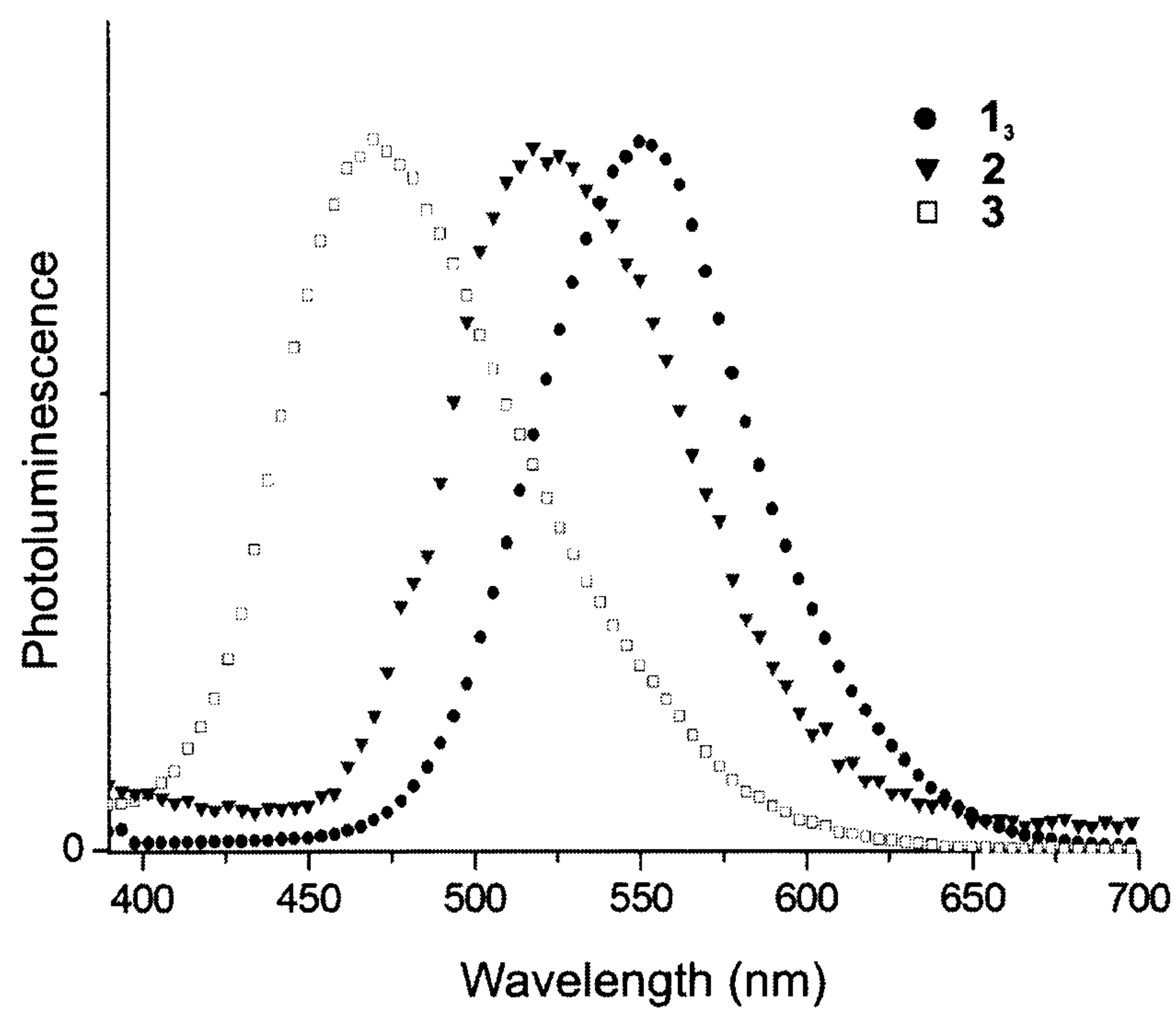


Fig. 5