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— *with international search report*
- (88) Date of publication of the international search report:
10 April 2003
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



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(54) Title: ECL LABELS HAVING IMPROVED NON-SPECIFIC BINDING PROPERTIES, METHODS OF USING AND KITS CONTAINING THE SAME

(57) Abstract: Bipyridine or phenanthroline ligands presenting functional groups that prevent non-specific binding (in particular, negatively charged functional groups that are unaffected by standard conditions for conjugating biological reagents through amide bonds) are described as are luminescent metal complexes comprising these ligands. The use of luminescent ruthenium and osmium complexes comprising these ligands in electrochemiluminescence assays shows that the use of these labels can significantly reduce the amount of non-specific binding observed relative to assays carried out using reagents labeled with analogous labels that don't present functional groups that decrease non-specific binding.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/19788

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07D 213/22; G01N 33/532, 33/533; C12Q 1/68; C07K 1/13
 US CL : 546/88, 257; 436/544, 546; 435/6, 7.72; 530/402

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 U.S. : 546/88, 257; 436/544/546; 435/6, 7.72; 530/402

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---------------|--|--|
| X --- A | U.S. 5,597,910 A (GUDIBANDE et al) 28 January 1997 (28.01.1997), col. 3, line 1 - col. 4, line 39; claims 1 and 4. | 1, 2, 5, 8-13, 17, 62-67, 69-72, 76-78, 80-81 ----- 3, 4, 14-16, 18, 20-24, 29-38, 44-48, 54-61, 82-91 |
| A | US 5,310,687 A (BARD et al) 10 May 1994 (10.05.1994), col. 7, line 50 - col. 8, line 44; claims 1 and 16. | 1-6, 8-18, 20-24, 29-38, 44-48, 54-67, 69-72, 77-78, 80-91 |
| X --- A | US 5,695, 890 A (THOMPSON et al) 09 December 1997 (09.12.1997), Example 1; col. 13, lines 5-17; Example 20. | 1, 2, 5 ----- 3, 4, 6, 8-18, 20-24, 29-38, 44-48, 54-67, 69-72, 76-78, 80-91 |



Further documents are listed in the continuation of Box C.



See patent family annex.

| | |
|---|--|
| * Special categories of cited documents: | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" document defining the general state of the art which is not considered to be of particular relevance | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" earlier application or patent published on or after the international filing date | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" document member of the same patent family |
| "O" document referring to an oral disclosure, use, exhibition or other means | |
| "P" document published prior to the international filing date but later than the priority date claimed | |

Date of the actual completion of the international search

03 September 2002 (03.09.2002)

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INTERNATIONAL SEARCH REPORT

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---------------|--|--|
| X --- A | WILLNER, I. et al. Photosensitized Reduction of CO ₂ to CH ₄ and H ₂ Evolution in the Presence of Ruthenium and Osmium Colloids: Strategies To Design Selectivity of Products Distribution. Journal of the American Chemical Society. 1987, Vol. 109, No. 20, pages 6080-6086, formula (4). | 1-4, 10 ----- 5, 6, 8, 9, 11-18, 20-24, 29-38, 44-48, 54-67, 69-72, 76-78, 80-91 |
| X --- A | OKURA, I. et al. Photoinduced Hydrogen Evolution from Water Using Bipyridinium Salts as Electron Carriers. Inorganica Chimica Acta. 1985, Vol. 101, pages L25-L26, structure PVS. | 1-4 ----- 5, 6, 8-18, 20-24, 29-38, 44-48, 64-67, 69-72, 76-78, 80-91 |
| X --- A | TAKEYAMA, N. et al. Photo-induced Charge Separation in Carbazole-containing Bilayer Membrane in the Presence of Various Electron Acceptors. Chemical Society of Japan. 1985, Vol. 11, pages 1735-1738, Fig. 1. | 1-4 ----- 5-6, 8-18, 20-24, 29-38, 44-48, 54-67, 69-72, 76-78, 80-91 |
| X | PENICAUD, V. et al. New Water-soluble Diamine Complexes as Catalysts for the Hydrogenation of Ketones Under Hydrogen Pressure. European Journal of Organic Chemistry. 1999, Vol. 7, pages 1745-1748, structure 1. | 1, 5 |
| A | ZABAN, A. et al. Relative Energetics at the Semiconductor/Sensitizing Dye/Electrolyte Interface. Journal of Physical Chemistry B. 1998, Vol. 102, pages 452-460, Dye 4. | 1, 2, 5 |
| X | YAN, S. et al. Evaluating Interfacial Charge Transfer Kinetics and Energetics Via Laser Based Spectroelectrochemical Methods. Proceedings-Electrochemical Society. 1996, Vol. 96-9, pages 53-64, page 54, second full paragraph. | 1, 2, 5 |
| A | YAN, S. et al. Semiconductor-based Interfacial Electron-transfer Reactivity: Decoupling Kinetics from pH-Dependent Band Energetics in a Dye-Sensitized Titanium Dioxide/Aqueous Solution System, Journal of Physical Chemistry. 25 April 1996, Vol. 100, No. 17, pages 6867-6870, formula 1. | 1, 2, 5, 10 |
| A | YAN, S. et al. Energetics of Electron Transfer at the Nanocrystalline Titanium Dioxide Semiconductor/Aqueous Solution Interface: pH Invariance of the Metal-based Formal Potential of a Representative Surface-attached Dye Couple. Journal of Physical Chemistry B. 1997, Vol. 101, pages 1493-1495, formula (1). | 1, 2, 5, 10 |
| A | WILL, G. et al. Potentiostatic Modulation of the Lifetime of Light-Induced Charge Separation in a Heterosupermolecule. Journal of Physical Chemistry B. 1999, Vol. 103, pages 8067-8079, Scheme 7a. | 1, 2, 5, 10 |

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/19788

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

- 1. Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

- 2. Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

- 3. Claim Nos.: 7, 19, 25-28, 39-43, 49-53, 68, 73-75, 79
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- 1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
- 2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
- 3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

- 4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

- Remark on Protest**
- The additional search fees were accompanied by the applicant's protest.
 - No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

PCT/US02/19788

Continuation of B. FIELDS SEARCHED Item 3:

APS, CAS ONLINE: structure searches; search terms: osmium, ruthenium, luminesc?, electrochemiluminesc? ECL, bipyridine, phenanthroline, metal complex, ligand, phenanthrolyl, bipyridyl, label?