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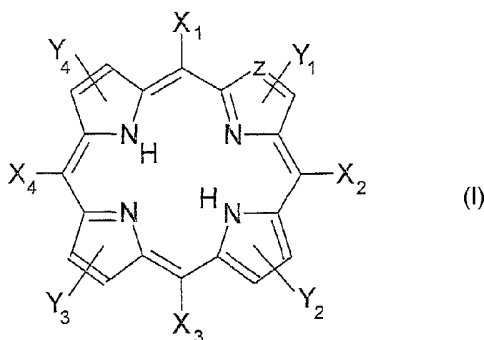
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(54) Title: NOVEL USES



(57) **Abstract:** The invention provides the use of a compound of Formula (I), or metallated derivative thereof, in the preparation of a medicament for killing or attenuating the growth of microorganisms by a method which does not comprise exposing the compound to a photodynamic therapy light source or a sonodynamic therapy ultrasound source Formula (I) wherein X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, Y<sub>1</sub>, Y<sub>2</sub>, Y<sub>3</sub>, Y<sub>4</sub> and Z have meanings given in the description. Preferably, the microorganisms are selected from the group consisting off bacteria, mycoplasmas, yeasts, fungi and viruses.

## NOVEL USES OF PORPHYRIN COMPOUNDS

5

**Field**

The present invention relates to new uses of porphyrin compounds and, in particular, the use of such compounds in the curative or prophylactic treatment of microbial colonisation and infection.

**Background**

15 The resistance to antibiotics developed by an increasing number of microorganisms is recognised to be a worldwide health problem (Tunger *et al.*, 2000, *Int. J. Microb. Agents* **15**:131-135; Jorgensen *et al.*, 2000, *Clin. Infect. Dis.* **30**:799-808). As a consequence, the development of new approaches for killing microorganisms is urgently required.

20

The treatment of microbial infections by photodynamic therapy (PDT) represents a valuable recent method for eradicating bacteria since it involves a mechanism which is markedly different from that typical of most antibiotics. Thus, PDT is based on the use of a photosensitising molecule that, once activated by light, generates oxygen reactive species that are toxic for a large variety of prokaryotic and eukaryotic cells including bacteria, mycoplasmas and yeasts (Malik *et al.*, 1990, *J. Photochem. Photobiol. B Biol.* **5**:281-293; Bertoloni *et al.*, 1992, *Microbios* **71**:33-46). Importantly, the photosensitising activity of many photodynamic agents against bacteria is not impaired by the resistance to

30

antibiotics but, instead, depends mainly on their chemical structure (Malik *et al.*, 1992, *J. Photochem. Photobiol. B Biol.* **14**:262-266).

Various types of neutral and anionic photosensitising agents exhibit a pronounced phototoxic activity against Gram positive bacteria. However, such photosensitising agents exert no appreciable cytotoxic activity against Gram negative bacteria unless the permeability of the outer membrane is altered by treatment with ethylene diamine tetra-acetic acid (EDTA) or polycations (Bertoloni *et al.*, 1990, *FEMS Microbiol. Lett.* **71**: 149-156; Nitzan *et al.*, 1992, *Photochem. Photobiol.* **55**:89-97). It is believed that the cellular envelope of Gram negative bacteria, which is more complex and thicker than that of Gram positive bacteria, prevents an efficient binding of the photosensitising agent or intercepts and deactivates the cytotoxic reactive species photogenerated by the photosensitising agent (Ehrenberg *et al.*, 1985, *Photochem. Photobiol.* **41**:429-435; Valduga *et al.*, 1993, *J. Photochem. Photobiol. B. Biol.* **21**:81-86).

In contrast, positively charged (cationic) photosensitising agents, including porphyrins and phthalocyanines, promote efficient inactivation of Gram negative bacteria without the need for modifying the natural structure of the cellular envelope (Merchat *et al.*, 1996, *J. Photochem. Photobiol. B. Biol.* **32**:153-157; Minnock *et al.*, 1996, *J. Photochem. Photobiol. B. Biol.* **32**:159-164). It appears that the positive charge favours the binding of the photosensitising agent at critical cellular sites that, once damaged by exposure to light, cause the loss of cell viability (Merchat *et al.*, 1996, *J. Photochem. Photobiol. B. Biol.* **35**:149-157). Thus, it has been reported that *Escherichia coli* is efficiently inactivated by visible light after incubation with the cationic 5,10,15,20-tetrakis-(4-*N*-methylpyridyl)-porphine (T<sub>4</sub>MPyP) (Valduga *et al.*, 1999, *Biochem.*

*Biophys. Res. Commun.* **256**:84-88). The phototoxic activity of this porphyrin is mainly mediated by the impairment of the enzymic and transport functions of both the outer and cytoplasmic membranes, rather than by binding to DNA.

5

However, the utility of known porphyrin-based antimicrobial agents is limited due to their toxicity against mammalian host tissue cells, *i.e.* the compounds are unable to differentiate between target microbial cells and host cells. In addition, the utility of known porphyrin-based antimicrobial  
10 agents is further limited by their relatively low potency for target microbial cells.

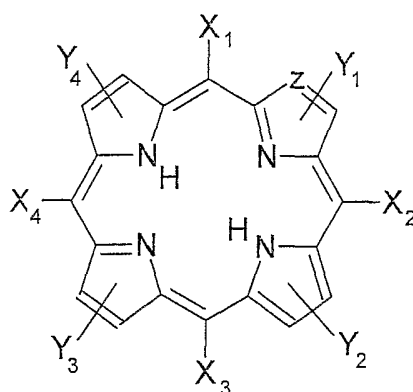
Furthermore, not all microbial infections are suitable for treatment using photodynamic therapy, *e.g.* the site of infection may not be accessible to  
15 light.

Hence, there is a need for new methods of killing and attenuating the growth of microbial agents.

20

## Summary

According to a first aspect of the invention, there is provided use of a compound of formula I in the preparation of a medicament for killing or  
 5 attenuating the growth of microorganisms by a method which does not comprise exposing the compound to a photodynamic therapy light source or a sonodynamic therapy ultrasound source



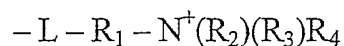
*I*

wherein:

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$X_1$ ,  $X_2$ ,  $X_3$  and  $X_4$  independently represent (*i.e.* are the same or different) a hydrogen atom, a lipophilic moiety, a phenyl group, a lower alkyl, alkaryl or aralkyl group, or a cationic group of the following formula;

15



wherein:

20

$L$  is a linking moiety or is absent;

$R_1$  represents lower alkylene, lower alkenylene or lower alkynylene, which is optionally substituted by one or more

substituents selected from lower alkyl, lower alkylene (optionally interrupted with oxygen), fluoro, OR<sub>5</sub>, C(O)R<sub>6</sub>, C(O)OR<sub>7</sub>, C(O)NR<sub>8</sub>R<sub>9</sub>, NR<sub>10</sub>R<sub>11</sub> and N<sup>+</sup>R<sub>12</sub>R<sub>13</sub>R<sub>14</sub>; and

5 R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> independently represent (*i.e.* are the same or different) H, aryl, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which are optionally substituted by one or more substituents selected from lower alkyl, lower alkylene (optionally interrupted with oxygen), aryl,  
 10 OR<sub>5</sub>, C(O)R<sub>6</sub>, C(O)OR<sub>7</sub>, C(O)NR<sub>8</sub>R<sub>9</sub>, NR<sub>10</sub>R<sub>11</sub> and N<sup>+</sup>R<sub>12</sub>R<sub>13</sub>R<sub>14</sub>

Z is -CH or N;

15 Y<sub>1</sub>, Y<sub>2</sub>, Y<sub>3</sub> and Y<sub>4</sub> are absent or independently represent aryl, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which are optionally substituted by one or more substituents selected from lower alkyl, lower alkylene (optionally interrupted with oxygen), aryl, OR<sub>5</sub>, C(O)R<sub>6</sub>, C(O)OR<sub>7</sub>, C(O)NR<sub>8</sub>R<sub>9</sub>, NR<sub>10</sub>R<sub>11</sub>, N<sup>+</sup>R<sub>12</sub>R<sub>13</sub>R<sub>14</sub>,  
 20 or, taken in conjunction with the pyrrole ring to which they attach, may form a cyclic group; and

R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub>, R<sub>9</sub>, R<sub>10</sub>, R<sub>11</sub>, R<sub>12</sub>, R<sub>13</sub> and R<sub>14</sub> independently represent H or lower alkyl

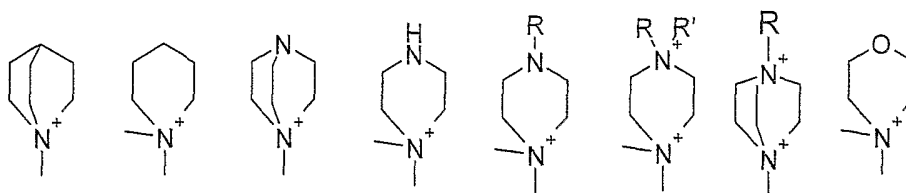
25 provided that at least one of X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub> and X<sub>4</sub> is a cationic group as defined above and at least one of X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub> and X<sub>4</sub> is a hydrogen atom, a phenyl group, a lipophilic moiety, or a lower alkyl, alkaryl or aralkyl group.

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The term "lower alkyl" is intended to include linear or branched, cyclic or acyclic, C<sub>1</sub>-C<sub>20</sub> alkyl which may be interrupted by oxygen (preferably no more than five oxygen atoms are present in each alkyl chain). Lower alkyl groups which R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub>, R<sub>9</sub>, R<sub>10</sub>, R<sub>11</sub>, R<sub>12</sub>, R<sub>13</sub> and R<sub>14</sub> may represent include C<sub>1</sub>-C<sub>18</sub> alkyl, C<sub>1</sub>-C<sub>16</sub> alkyl, C<sub>1</sub>-C<sub>14</sub> alkyl, C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>1</sub>-C<sub>10</sub> alkyl, C<sub>1</sub>-C<sub>9</sub> alkyl, C<sub>1</sub>-C<sub>8</sub> alkyl, C<sub>1</sub>-C<sub>7</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>5</sub> alkyl, C<sub>1</sub>-C<sub>4</sub> alkyl, C<sub>1</sub>-C<sub>3</sub> alkyl and C<sub>1</sub>-C<sub>2</sub> alkyl. Preferred lower alkyl groups which R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub>, R<sub>9</sub>, R<sub>10</sub>, R<sub>11</sub>, R<sub>12</sub>, R<sub>13</sub> and R<sub>14</sub> may represent include C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub>, C<sub>6</sub>, C<sub>7</sub>, C<sub>8</sub>, C<sub>9</sub>, C<sub>10</sub>, C<sub>11</sub>, C<sub>12</sub>, C<sub>13</sub>, C<sub>14</sub>, C<sub>15</sub> and C<sub>16</sub> alkyl.

Thus, any one or more of N<sup>+</sup>R<sub>2</sub>R<sub>3</sub>R<sub>4</sub> and/or N<sup>+</sup>R<sub>12</sub>R<sub>13</sub>R<sub>14</sub> may represent cyclic amine/ammonium groups, for example:

15



It will be appreciated that the cyclic amine/ammonium groups may also comprise fewer or greater than six members, for example such groups may comprise 4-, 5-, 7-, 8-, 9- or 10-membered rings.

The term "lower alkylene" is to be construed accordingly.

The terms "lower alkenyl" and "lower alkynyl" are intended to include linear or branched, cyclic or acyclic, C<sub>2</sub>-C<sub>20</sub> alkenyl and alkynyl, respectively, each of which may be interrupted by oxygen (preferably no more than five oxygen atoms are present in each alkenyl or alkynyl chain).

The term "lower alkenyl" also includes both the *cis* and *trans* geometric isomers. Lower alkenyl groups which R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub>, R<sub>9</sub>, R<sub>10</sub>, R<sub>11</sub>, R<sub>12</sub>, R<sub>13</sub> and R<sub>14</sub> may represent include C<sub>2</sub>-C<sub>18</sub> alkenyl, C<sub>2</sub>-C<sub>17</sub> alkenyl, C<sub>2</sub>-C<sub>16</sub> alkenyl, C<sub>2</sub>-C<sub>14</sub> alkenyl, C<sub>2</sub>-C<sub>12</sub> alkenyl, C<sub>2</sub>-C<sub>10</sub> alkenyl, C<sub>2</sub>-C<sub>8</sub> alkenyl, C<sub>2</sub>-C<sub>7</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>5</sub> alkenyl, C<sub>2</sub>-C<sub>4</sub> alkenyl, C<sub>2</sub>-C<sub>3</sub> alkenyl and C<sub>3</sub>-C<sub>4</sub> alkenyl. Preferred lower alkenyl groups which R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub>, R<sub>9</sub>, R<sub>10</sub>, R<sub>11</sub>, R<sub>12</sub>, R<sub>13</sub> and R<sub>14</sub> may represent include C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub>, C<sub>6</sub>, C<sub>7</sub>, C<sub>8</sub>, C<sub>9</sub>, C<sub>10</sub>, C<sub>11</sub>, C<sub>12</sub>, C<sub>13</sub> and C<sub>14</sub> alkenyl.

The term "lower alkenylene" is to be construed accordingly.

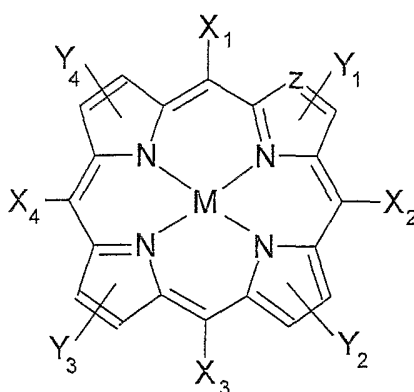
"Lower alkynyl" groups which R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub>, R<sub>9</sub>, R<sub>10</sub>, R<sub>11</sub>, R<sub>12</sub>, R<sub>13</sub> and R<sub>14</sub> may represent include C<sub>2</sub>-C<sub>18</sub> alkynyl, C<sub>2</sub>-C<sub>16</sub> alkynyl, C<sub>2</sub>-C<sub>14</sub> alkynyl, C<sub>2</sub>-C<sub>12</sub> alkynyl, C<sub>2</sub>-C<sub>10</sub> alkynyl, C<sub>2</sub>-C<sub>9</sub> alkynyl, C<sub>2</sub>-C<sub>8</sub> alkynyl, C<sub>2</sub>-C<sub>7</sub> alkynyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>2</sub>-C<sub>5</sub> alkynyl, C<sub>2</sub>-C<sub>4</sub> alkynyl, C<sub>2</sub>-C<sub>3</sub> alkynyl and C<sub>3</sub>-C<sub>4</sub> alkynyl. Preferred lower alkynyl groups which R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub>, R<sub>9</sub>, R<sub>10</sub>, R<sub>11</sub>, R<sub>12</sub>, R<sub>13</sub> and R<sub>14</sub> may represent include C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub>, C<sub>6</sub>, C<sub>7</sub>, C<sub>8</sub>, C<sub>9</sub>, C<sub>10</sub>, C<sub>11</sub>, C<sub>12</sub>, C<sub>13</sub> and C<sub>14</sub> alkynyl.

The term "lower alkynylene" is to be construed accordingly.

The term "aryl" includes six to ten-membered carbocyclic aromatic groups, such as phenyl and naphthyl, which groups are optionally substituted by one or more substituents selected from fluoro, cyano, nitro, lower alkyl (*i.e.* alkaryl), OR<sub>5</sub>, C(O)R<sub>6</sub>, C(O)OR<sub>7</sub>, C(O)NR<sub>8</sub>R<sub>9</sub> and NR<sub>10</sub>R<sub>11</sub>.

The term “aralkyl” includes aryl groups joined to the porphyrin ring via a lower alkyl group.

A second aspect of the invention provides use of a compound of formula  
 5 II in the preparation of a medicament for killing or attenuating the growth of microorganisms by a method which does not comprise exposing the compound to a photodynamic therapy light source or a sonodynamic therapy ultrasound source:



II

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wherein M is a metallic element or a metalloid element and X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, Y<sub>1</sub>, Y<sub>2</sub>, Y<sub>3</sub>, Y<sub>4</sub> and Z are as defined above.

Preferably, in the first and second aspects of the invention the  
 15 medicament is for killing or attenuating the growth of microorganisms by a method which does not comprise exposing the compound to a stimulus which activates antimicrobial activity.

By “a stimulus which activates antimicrobial activity” we mean a  
 20 stimulus which increases the ability of the compound to kill or attenuate the growth of microbial agents, such as irradiation with a photodynamic therapy light source or an ultrasound source. In other words, the medicament exhibits *innate* antimicrobial activity, *i.e.* the medicament

(and specifically the active compound therein) is intrinsically active. Such activity may be determined by methods well known in the art; for example, see Example B.

- 5 Hence, the medicament is for killing or attenuating the growth of microorganisms by a method other than photodynamic or sonodynamic therapy. However, it will be appreciated that methods for killing or attenuating the growth of microorganisms wherein the medicament is exposed to normal ambient light (*i.e.* sunlight or artificial ambient light)  
10 are not excluded.

Preferably, the medicament is exposed to light/radiation of intensity less than  $10 \text{ mW/cm}^2$ , for example less than  $20 \text{ mW/cm}^2$ , less than  $25 \text{ mW/cm}^2$ , less than  $30 \text{ mW/cm}^2$  (*i.e.* less than  $300 \text{ W/m}^2$ ) less than  
15  $40 \text{ mW/cm}^2$ , less than  $50 \text{ mW/cm}^2$ , less than  $60 \text{ mW/cm}^2$ , less than  $70 \text{ mW/cm}^2$ , less than  $80 \text{ mW/cm}^2$ , less than  $90 \text{ mW/cm}^2$  or less than  $100 \text{ mW/cm}^2$ .

Advantageously, the medicament is exposed to light/radiation dose of less  
20 than  $100 \text{ J/cm}^2$ , for example less than  $90 \text{ J/cm}^2$ , less than  $80 \text{ J/cm}^2$ , less than  $70 \text{ J/cm}^2$ , less than  $60 \text{ J/cm}^2$ , less than  $50 \text{ J/cm}^2$ , less than  $40 \text{ J/cm}^2$ , less than  $30 \text{ J/cm}^2$ , less than  $20 \text{ J/cm}^2$  or less than  $10 \text{ J/cm}^2$ .

It will be further appreciated by persons skilled in the art that the  
25 medicament may be for use in a treatment regime that exploits both its innate activity *and* its photodynamic and/or sonodynamic activity. For example, the medicament may first be used in the absence of an activating stimulus, such that its innate antimicrobial activity is exploited, and subsequently exposed to an activating stimulus such that its  
30 photodynamic and/or sonodynamic activity is exploited.

The term "metallic element" is intended to include a divalent or trivalent metallic element. Preferably, the metallic element is diamagnetic. More preferably, the metallic element is selected from Zn (II), Cu (II), La (III),  
5 Lu (III), Y (III), In (III) Cd (II), Mg (II), Al(III), Ru, Ni(II), Mn(III), Fe(III) and Pd(II). Most preferably, the metallic element is Ni(II), Mn(III), Fe(III) or Pd(II).

The term "metalloid" is intended to include an element having physical  
10 and chemical properties, such as the ability to conduct electricity, that are intermediate to those of both metals and non-metals. The term "metalloid element" includes silicon (Si) and germanium (Ge) atoms which are optionally substituted with one or more ligands.

15 It will be appreciated that the terms metallic element and metalloid element include a metal element or a metalloid element having a positive oxidation state, all of which may be substituted by one or more ligands selected from fluoro, OH, OR<sub>15</sub> wherein R<sub>15</sub> is lower alkyl, lower alkenyl, lower alkynyl, aralkyl, aryl or alkaryl as defined above (wherein aryl and  
20 alkaryl are mono-substituted).

The compounds of formulae I and II comprise at least one cationic group. Thus, the compounds of the invention may carry a net positive charge, for example a charge of +1, +2, +3, +4, +5, +6 or more. In a preferred  
25 embodiment, the compounds carry a net charge of less than +4, for example +1, +2 or +3. In a particularly preferred embodiment, the compounds carry a net charge of +2.

It will be appreciated by persons skilled in the art that compounds of  
30 formulae I and II may be counterbalanced by counter-anions. Exemplary

counter-anions include, but are not limited to, halides (*e.g.* fluoride, chloride and bromide), sulfates (*e.g.* decylsulfate), nitrates, perchlorates, sulfonates (*e.g.* methane sulfonate) and trifluoroacetate. Other suitable counter-anions will be well known to persons skilled in the art. Thus,  
5 pharmaceutically, and/or veterinarily, acceptable derivatives of the compounds of formulae I and II, such as salts and solvates, are also included within the scope of the invention. Salts which may be mentioned include: acid addition salts, for example, salts formed with inorganic acids such as hydrochloric, hydrobromic, sulfuric and  
10 phosphoric acid, with carboxylic acids or with organo-sulfonic acids; base addition salts; metal salts formed with bases, for example, the sodium and potassium salts.

It will be further appreciated by skilled persons that the compounds of  
15 formula I may exhibit tautomerism. All tautomeric forms and mixtures thereof are included within the scope of the invention.

Compounds of formulae I and II may also contain one or more asymmetric carbon atoms and may therefore exhibit optical and/or  
20 diastereoisomerism. Diastereoisomers may be separated using conventional techniques, *e.g.* chromatography or fractional crystallisation. The various stereoisomers may be isolated by separation of a racemic or other mixture of the compounds using conventional, *e.g.* fractional crystallisation or HPLC, techniques. Alternatively, the  
25 desired optical isomers may be made by reaction of the appropriate optically active starting materials under conditions which will not cause racemisation or epimerisation, or by derivatisation, for example with a homochiral acid followed by separation of the diastereomeric esters by conventional means (*e.g.* HPLC, chromatography over silica). All  
30 stereoisomers are included within the scope of the invention.

In a preferred embodiment of the first and second aspects of the invention, Z is  $-\text{CH}$ .

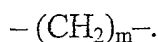
- 5 A characterising feature of the first and second aspects of the invention is that at least one of substituent groups  $X_1$ ,  $X_2$ ,  $X_3$  and  $X_4$  is a quaternary ammonium cationic group of the formula  $-\text{L}-\text{R}_1-\text{N}^+(\text{R}_2)(\text{R}_3)\text{R}_4$ , as defined above. Preferably, none of  $X_1$ ,  $X_2$ ,  $X_3$  and  $X_4$  is an anilinium or a pyridinium cationic group.

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In a preferred embodiment,  $\text{R}_1$  is an unsubstituted lower alkylene, lower alkenylene or lower alkynylene group.

Advantageously,  $\text{R}_1$  is a straight-chain lower alkylene group of formula:

15



- Preferably, 'm' is an integer between 1 and 20. More preferably, 'm' is an integer between 1 and 10, for example between 1 and 6, between 1 and 5, between 1 and 4 or between 1 and 3. Preferred straight-chain lower alkylene groups which  $\text{R}_1$  may represent include groups of the above formula wherein m is 2, 3, 4, 5, 6, 7, 8, 9 or 10. Most preferably, 'm' is 2 or 3.

- 25 The remaining three substituent groups of the quaternary ammonium moiety, *i.e.*  $\text{R}_2$ ,  $\text{R}_3$  and  $\text{R}_4$ , may be the same or different and are selected from H, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which are optionally substituted by one or more substituents selected from lower alkyl,  $\text{OR}_5$ ,  $\text{C(O)R}_6$ ,  $\text{C(O)OR}_7$ ,  $\text{C(O)NR}_8\text{R}_9$ ,  $\text{NR}_{10}\text{R}_{11}$  and  
30  $\text{N}^+\text{R}_{12}\text{R}_{13}\text{R}_{14}$ .

In a preferred embodiment,  $R_2$ ,  $R_3$  and/or  $R_4$  are lower alkyl, lower alkenyl or lower alkynyl group.

- 5 Preferably,  $R_2$ ,  $R_3$  and/or  $R_4$  are unsubstituted lower alkyl groups.

Optionally, at least one of  $R_2$ ,  $R_3$  and  $R_4$  is an alkyl group which is substituted with a primary, secondary or tertiary amine group or a quaternary ammonium group.

10

In a preferred embodiment of the first and second aspects of the invention,  $R_1$  is  $-(CH_2)_3-$ ,  $R_2$  and  $R_3$  are  $CH_3$  and  $R_4$  is  $-(CH_2)_3-N(CH_3)_2$ .

- 15 In an alternative preferred embodiment of the first and second aspects of the invention,  $R_1$  is  $-(CH_2)_3-$ , and  $R_2$ ,  $R_3$  and  $R_4$  are each  $CH_3$ .

In a further alternative preferred embodiment of the first and second aspects of the invention,  $R_1$  is  $-(CH_2)_3-$ , and  $R_2$ ,  $R_3$  and  $R_4$  are each  $C_2H_5$ .

20

Advantageously, at least one of  $X_1$ ,  $X_2$ ,  $X_3$  and  $X_4$  is a cationic group as defined above and at least one of  $X_1$ ,  $X_2$ ,  $X_3$  and  $X_4$  is a hydrogen atom.

- 25 Preferably, each of  $X_1$ ,  $X_2$ ,  $X_3$  and  $X_4$  is a hydrogen atom or a cationic group as defined above.

Conveniently, the pK values of any primary, secondary or tertiary amine groups, if present in the compounds of the invention, is greater than 8 to ensure that the group is protonated when in a physiological environment.

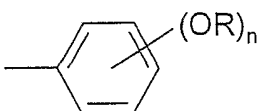
30

The quaternary ammonium cationic group is optionally joined to the porphyrin ring via a linking moiety, L.

- 5 Preferred linking moieties, L, include phenoxy, phenylene, sulfonyl amido, aminosulfonyl, sulfonylimino, phenylsulfonylamido, phenyl-aminosulfonyl, urea, urethane and carbamate linking moieties.

In a preferred embodiment, the quaternary ammonium cationic group is  
10 joined to the porphyrin ring via a phenoxy linker.

Thus,  $X_1$ ,  $X_2$ ,  $X_3$  and/or  $X_4$  may have the following formula:

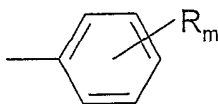


- 15 wherein R is  $R_1 - N^+(R_2)(R_3)R_4$ , as defined above, and 'n' is an integer between 1 and 3.

In an alternative preferred embodiment, the quaternary ammonium cationic group is joined to the porphyrin ring via a phenylene linker.

20

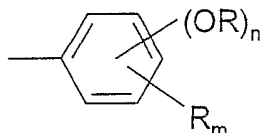
Thus,  $X_1$ ,  $X_2$ ,  $X_3$  and/or  $X_4$  may have the following formula:



- wherein R is  $R_1 - N^+(R_2)(R_3)R_4$ , as defined above, and 'm' is an integer  
25 between 1 and 3.

Preferably, 'm' is 2, and most preferably 1.

In an alternative preferred embodiment,  $X_1$ ,  $X_2$ ,  $X_3$  and/or  $X_4$  may have the following formula:



5

wherein R is  $R_1 - N^+(R_2)(R_3)R_4$ , 'n' and 'm' are as defined above, and 'n + m' is between 1 and 3.

Advantageously, L comprises a benzene ring (*e.g.* phenoxy, phenylene, phenylsulfonylamido or phenylamino-sulfonyl) mono-substituted at the *para*-position. Alternatively, L may be mono- or di-substituted at *meta*- or *ortho*-positions. L may also be both *para*- and *ortho*-substituted.

In an alternative preferred embodiment, the quaternary ammonium cationic group is joined directly to the porphyrin ring, *i.e.* L is absent.

In a preferred embodiment of the first and second aspects of the invention, the compound comprises two cationic groups, as defined above, on opposite sides of the porphyrin ring, *i.e.* at ring positions 5 and 15 or ring positions 10 and 20. For example,  $X_1$  and  $X_3$  may be a hydrogen atom, a lipophilic moiety, a phenyl group, a lower alkyl, alkaryl or aralkyl group, and  $X_2$  and  $X_4$  may be cationic groups, or *vice versa*. Preferably,  $X_1$  and  $X_3$  are both a hydrogen atom and  $X_2$  and  $X_4$  are both a cationic group, or *vice versa*.

25

Alternatively, the compound may comprise two cationic groups, as defined above, on neighbouring positions of the porphyrin ring, *i.e.* at

ring positions 5 and 10, or ring positions 10 and 15, or ring positions 15 and 20 or ring positions 20 and 5. For example,  $X_1$  and  $X_2$  may be hydrogen and  $X_3$  and  $X_4$  may be cationic groups, or  $X_2$  and  $X_3$  may be hydrogen and  $X_4$  and  $X_1$  may be cationic groups, *etc.*

5

It will be appreciated by persons skilled in the art that additional isomeric structural possibilities arise when  $Z$  represents nitrogen. Such possibilities are included within the scope of the present invention.

- 10 In a further preferred embodiment of the first and second aspects of the invention, the compound is substituted on one or more of its constituent pyrrole rings. Thus,  $Y_1$ ,  $Y_2$ ,  $Y_3$  and  $Y_4$  may be absent or independently represent aryl, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which are optionally substituted by one or more substituents selected
- 15 from lower alkyl, lower alkylene (optionally interrupted with oxygen), aryl,  $OR_5$ ,  $C(O)R_6$ ,  $C(O)OR_7$ ,  $C(O)NR_8R_9$ ,  $NR_{10}R_{11}$  and  $N^+R_{12}R_{13}R_{14}$ . It will be appreciated by skilled persons that  $Y_1$ ,  $Y_2$ ,  $Y_3$  and/or  $Y_4$  may comprise cyclic groups, which may be saturated or aromatic. For example, one or more of the pyrrole rings may be substituted to form an
- 20 iso-indole group, *i.e.*  $Y_1$ ,  $Y_2$ ,  $Y_3$  and/or  $Y_4$  together with the pyrrole ring to which they are attached may be cyclic.

In an alternative preferred embodiment of the first and second aspects of the invention,  $Y_1$ ,  $Y_2$ ,  $Y_3$  and  $Y_4$  are absent. Thus, the porphyrin ring is

25 preferably substituted only at one or more of positions 5, 10, 15 or 20.

In a further preferred embodiment of the first and second aspects of the invention, at least one of  $X_1$ ,  $X_2$ ,  $X_3$  and  $X_4$  is or comprises a lipophilic moiety.

30

By 'lipophilic moiety' we include moieties having a partition coefficient between 1-*n*-octanol and water expressed as log P of greater than 1.0 at physiological pH and 25°C.

- 5 Conveniently, the lipophilic moiety is a saturated, straight-chain alkyl group of formula  $-(CH_2)_pCH_3$ , or an equivalent alkylene group of formula  $-(CH_2)_p-$ , wherein 'p' is an integer between 1 and 22, for example between 1 and 18. Preferably, 'p' is between 1 and 18, more preferably between 2 and 16, between 4 and 16, between 6 and 18,  
10 between 8 and 16 or between 4 and 12. Most preferably, 'p' is between 10 and 12.

It will be appreciated that  $X_1$ ,  $X_2$ ,  $X_3$  and/or  $X_4$  may be a cationic group, as defined above, which also comprises a lipophilic moiety.

15

In an alternative preferred embodiment of the first and second aspects of the invention, none of  $X_1$ ,  $X_2$ ,  $X_3$  and  $X_4$  is a lipophilic moiety.

- Advantageously, the compounds used in the first and second aspects of  
20 the invention are soluble in water. Preferably, the compounds may be dissolved in water to a concentration of at least 5 µg/l, for example at least 10 µg/l, 15 µg/l or 20 µg/l. More preferably, the compounds may be dissolved in water to a concentration of at least 100 µg/l, for example 200 µg/l, 300 µg/l, 400 µg/l, 500 µg/l, 1 mg/ml, 5 mg/ml, 10 mg/ml,  
25 20 mg/ml, 50 mg/ml or 100 mg/ml.

- Conveniently, the compounds used in the first and second aspects of the invention exhibit selective toxicity to microbial agents. By 'selective' we mean the compound is preferentially toxic to one or more  
30 microorganisms (such as bacteria, mycoplasmas, yeasts, fungi and/or

viruses) compared to mammalian, *e.g.* human, host cells. Preferably, the toxicity of the compound to a target microorganism is at least two-fold greater than the toxicity of that compound to mammalian cells, more preferably at least three-fold, at least four-fold, at least five-fold, at least  
5 six-fold, at least eight-fold, at least ten-fold, at least fifteen-fold or at least twenty fold. Most preferably, the compound of the invention is substantially non-toxic to mammalian cells.

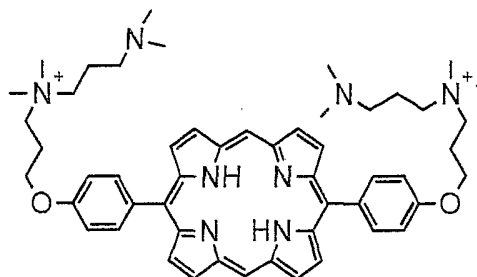
In this way, when the compounds are used to treat bacterial infections, for  
10 example, dosing regimes can be selected such that bacterial cells are destroyed with minimal damage to healthy host tissue. Thus, the compounds for use in the first and second aspects of the invention preferably exhibit a 'therapeutic window'.

15 In a preferred embodiment, the compound is toxic to the target microorganism (*e.g.* bacterial cells) at low doses. Preferably, the compound is toxic to the target microorganism at a concentration of less than 10  $\mu\text{M}$ , for example less than 1  $\mu\text{M}$ , less than 0.1  $\mu\text{M}$ , less than 0.01  $\mu\text{M}$ , less than 0.005  $\mu\text{M}$  or less than 0.001  $\mu\text{M}$  (see Example B).

20

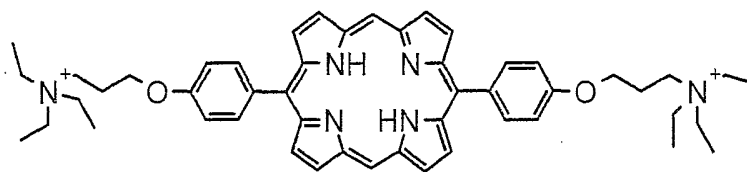
Preferred compounds for use in the first and second aspects of the invention include the following:

- (a) 5,15-bis-(4-{3-[(3-Dimethylamino-propyl)-dimethyl-ammonio]-propyloxy}-phenyl)-porphyrin dichloride ("*Compound 8*")



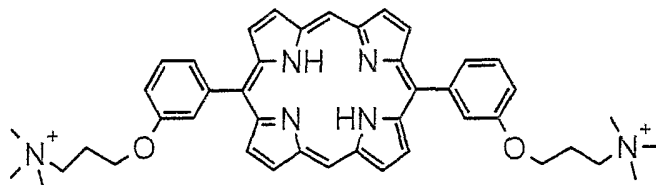
Preferably, this compound is provided as a dichloride or tetrachloride salt.

- (b) 5,15-bis-[4-(3-Triethylammonio-propyloxy)-phenyl]-porphyrin dichloride ("*Compound 9*");



Preferably, this compound is provided as a dichloride salt.

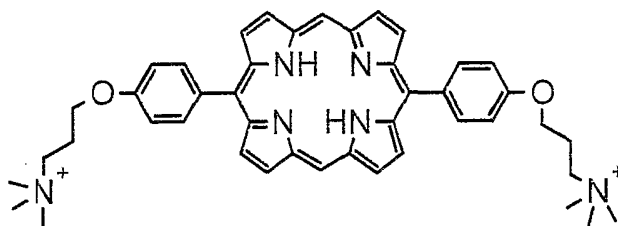
- (c) 5,15-bis-[3-(3-Trimethylammonio-propyloxy)-phenyl]-porphyrin dichloride ("*Compound 12*");



5

Preferably, this compound is provided as a dichloride salt.

- (d) 5,15-bis-[4-(3-Trimethylammonio-propyloxy)-phenyl]-porphyrin dichloride ("*Compound 10*");

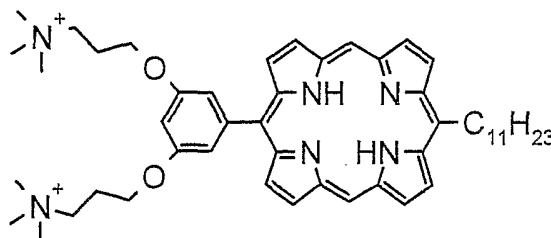


10

Preferably, this compound is provided as a dichloride salt.

- (e) 5-[3,5-bis-(3-Trimethylammonio-propyloxy)-phenyl]-15-undecyl-porphyrin dichloride ("*Compound 6*");

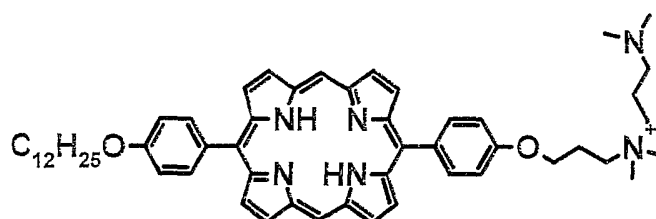
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Preferably, this compound is provided as a dichloride salt.

- (f) 5-{4-[3-Dimethyl-(3-dimethylaminopropyl)-ammonio-propyloxy]phenyl}-15-(4-dodecyloxy-phenyl)-porphyrin chloride  
 ("Compound 23");

5

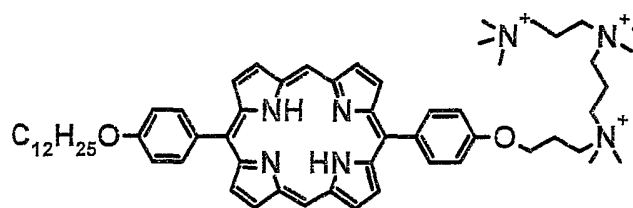


Preferably, this compound is provided as a chloride or dichloride salt.

10

- (g) 3-[(3-[(3-{4-[15-(4-Dodecyloxy-phenyl)-porphyrin-5-yl]-phenoxy}-propyl)-dimethyl-ammonio]-propyl)-dimethyl-ammonio)-propyl]-trimethyl-ammonium trichloride  
 ("Compound 25");

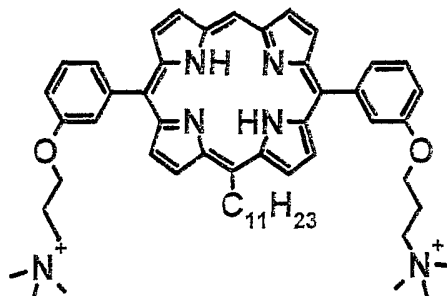
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Preferably, this compound is provided as a trichloride salt.

20

- (h) 5,15-bis-[3-(3-Trimethylammonio-propyloxy)-phenyl]-10-undecyl-porphyrin dichloride ("*Compound 28*");

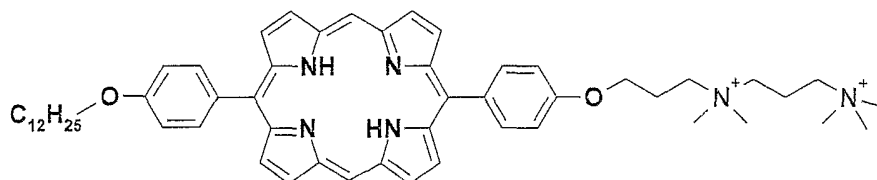


5

Preferably, this compound is provided as a dichloride salt.

- (i) 5-{4-[3-Dimethyl-(3-trimethylammonio-propyl)-ammonio-propyloxy]-phenyl}-15-(4-dodecyloxy-phenyl)-porphyrin dichloride ("*Compound 31*"); and

10

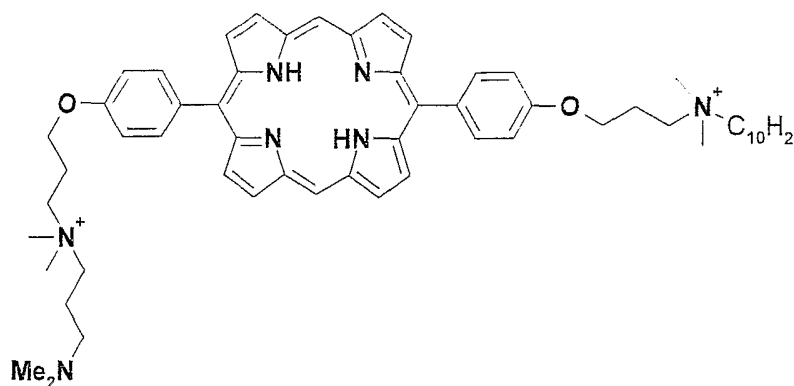


Preferably, this compound is provided as a dichloride salt.

15

- (j) 5-[4-(3-Dimethyldecyl-ammoniopropoxy)-phenyl]-15-{4-[3-dimethyl-(3-dimethylaminopropyl)-ammoniopropoxy]-phenyl}-porphyrin dichloride ("*Compound 32*").

5



Preferably, this compound is provided as a dichloride salt.

- 10 It will be appreciated that the above compounds may alternatively be in a metallated form, *i.e.* they may comprise a chelated metallic element or metalloid element within the porphyrin ring.

The medicament as prepared according to the first or second aspects of  
 15 the invention may be formulated at various concentrations, depending on the efficacy/toxicity of the compound being used and the indication for which it is being used. Preferably, the medicament comprises the compound at a concentration of between 0.1  $\mu$ M and 1 mM, more preferably between 1  $\mu$ M and 100  $\mu$ M, between 5  $\mu$ M and 50  $\mu$ M,  
 20 between 10  $\mu$ M and 50  $\mu$ M, between 20  $\mu$ M and 40  $\mu$ M and most preferably about 30  $\mu$ M. For *in vitro* applications, formulations may comprise a lower concentration of a compound, for example between 0.0025  $\mu$ M and 1  $\mu$ M.

It will be appreciated by persons skilled in the art that the compound used in the first or second aspects of the invention will generally be administered in admixture with a suitable pharmaceutical excipient diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice (for example, see 5 *Remington: The Science and Practice of Pharmacy*, 19<sup>th</sup> edition, 1995, Ed. Alfonso Gennaro, Mack Publishing Company, Pennsylvania, USA). Suitable routes of administration are discussed below, and include topical, intravenous, oral, pulmonary, nasal, aural, ocular, bladder and CNS 10 delivery.

For example, for application topically, *e.g.* to the skin or a wound site, the compounds can be administered in the form of a lotion, solution, cream, gel, ointment or dusting powder (for example, see *Remington, supra*, 15 pages 1586 to 1597). Thus, the compounds can be formulated as a suitable ointment containing the active compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, 20 they can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, e-lauryl sulphate, an alcohol (*e.g.* ethanol, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol) and water.

25

In a preferred embodiment, the medicament (*e.g.* lotion, solution, cream, gel or ointment) is water-based.

Formulations suitable for topical administration in the mouth further include 30 lozenges comprising the active ingredient in a flavoured basis, usually

sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

- 5 The medicament as prepared according to the first or second aspects of the invention may also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurised container, pump, spray or nebuliser with the use of a suitable propellant,
- 10 *e.g.* dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A<sup>3</sup> or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA<sup>3</sup>), carbon dioxide or other suitable gas. In the case of a pressurised aerosol, the dosage unit may be determined by providing a valve to deliver a metered
- 15 amount. The pressurised container, pump, spray or nebuliser may contain a solution or suspension of the active compound, *e.g.* using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, *e.g.* sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be
- 20 formulated to contain a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains at least 1 mg of a compound for delivery

25 to the patient. It will be appreciated that the overall dose with an aerosol will vary from patient to patient and from indication to indication, and may be administered in a single dose or, more usually, in divided doses throughout the day.

Alternatively, other conventional administration routes known in the art may also be employed; for example the medicament as prepared according to the first or second aspects of the invention may be delivered orally, buccally or sublingually in the form of tablets, capsules, ovules,  
5 elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed- or controlled-release applications. The medicament may also be administered intra-ocularly (see below), intra-aurally or *via* intracavernosal injection.

10 The medicament may also be administered parenterally, for example, intravenously, intra-arterially, intraperitoneally, intrathecally, intraventricularly, intrasternally, intracranially, intra-muscularly or subcutaneously (including via an array of fine needles or using needle-free *Powderject*® technology), or they may be administered by infusion  
15 techniques. They are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is  
20 readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants,  
25 buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried  
30 (lyophilised) condition requiring only the addition of the sterile liquid

carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

- 5 The medicament may also be administered by the ocular route, particularly for treating diseases of the eye. For ophthalmic use, the compounds can be formulated as micronised suspensions in isotonic, pH adjusted, sterile saline, or, preferably, as solutions in isotonic, pH adjusted, sterile saline, optionally in combination with a preservative such  
10 as a benzylalkonium chloride. Alternatively, they may be formulated in an ointment such as petrolatum.

For veterinary use, a compound is administered as a suitably acceptable formulation in accordance with normal veterinary practice and the  
15 veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular animal.

In a preferred embodiment of the first and second aspects of the invention, the medicament is for oral or parenteral administration. Thus,  
20 the medicaments are preferably for treating systemic microbial infections.

The medicaments may be stored in any suitable container or vessel known in the art. It will be appreciated by persons skilled in the art that the container or vessel should preferably be airtight and/or sterilised.  
25 Advantageously, the container or vessel is made of a plastics material, such as polyethylene.

It will be appreciated that the medicaments as prepared according to the first or second aspects of the invention may be used for killing a number  
30 of types of microorganism, including bacteria, mycoplasmas, yeasts,

fungi and/or viruses. It will be further appreciated that the medicaments may be used to prevent and/or treat infection with such microorganisms, *i.e.* the medicaments are suitable for prophylactic and/or therapeutic treatment. For example, the medicament may be used to prevent or  
5 reduce the spread or transfer of a pathogen to other subjects, *e.g.* patients, healthcare workers, *etc.*

Preferably, the medicaments as prepared according to the first or second aspects of the invention are for use in the curative and/or prophylactic  
10 treatment of bacterial infections such as Gram positive cocci (*e.g.* *Streptococcus*), Gram negative cocci (*e.g.* *Neisseria*), Gram positive bacilli (*e.g.* *Corynebacterium* species), Gram negative bacilli (*e.g.* *Escherichia coli*), acid-fast bacilli (*e.g.* a typical *Mycobacterium*) and including infections causing abscesses, cysts, blood infection  
15 (bacteraemia), dermatological infections, wound infections, arthritis, urinary tract infections, pancreatitis, pelvic inflammatory disease, peritonitis, prostatitis, infections of the vagina, oral cavity (including dental infections), eye and/or ear, ulcers and other localised infections; actinomyces infections; fungal infections such as *Candida albicans*,  
20 *Aspergillus* and *Blastomyces*; viral infections such as HIV, encephalitis, gastro-enteritis, haemorrhagic fever, hantavirus, viral hepatitis, herpesvirus (*e.g.* cytomegalovirus, Epstein-Barr, herpesvirus simiae, herpes simplex and varicella-zoster); protozoal infections such as amoebiasis, babesiosis, coccidiosis, cryptosporidiosis, giardiasis,  
25 Leishmaniasis, Trichomoniasis, toxoplasmosis and malaria; helminthic infections such as caused by nematodes, cestodes and trematodes, *e.g.* ascariasis, hookworm, lymphatic filariasis, onchocerciasis, schistosomiasis and toxocariasis; prion diseases; and inflammatory diseases such as soft-tissue rheumatism, osteoarthritis, rheumatoid  
30 arthritis and spondyloarthropathies.

More preferably, the medicaments are for use in the curative and/or prophylactic treatment of infections by Gram positive bacteria and/or Gram negative bacteria. Most preferably, the compounds of the invention  
5 are for use in the curative and/or prophylactic treatment of infections by Gram positive bacteria.

The medicaments are preferably used to kill microorganisms, e.g. bacteria, mycoplasmas, yeasts, fungi and viruses. The medicaments  
10 are particularly suitable for killing bacteria which have developed resistance to conventional antibiotic treatments, such as methicillin-resistant *Staphylococcus aureus* (MRSA).

It will be appreciated by persons skilled in the art that the medicaments  
15 are suitable to treat all microbial infections, regardless of whether the site of infection is light accessible or not. Hence, such medicaments may have utility to treat infections which are not able to be treated by conventional photodynamic therapy agents. Preferably, the microbial infection is on a light-inaccessible surface or in a light-inaccessible area.

20

Dosages of the compound in the medicaments as prepared according to the first or second aspects of the invention will depend on several factors; including the particular compound used, the formulation, route of administration and the indication for which the compound is used.  
25 Typically, however, dosages will range from 0.01 to 20 mg of compound per kilogram of body weight, preferably from 0.1 to 15 mg/kg, for example from 1 to 10 mg/kg of body weight.

In a preferred embodiment, the medicaments as prepared according to the  
30 first or second aspects of the invention are used in combination with

conventional antimicrobial agents. For example, the compounds may be used in combination with one or more of the following conventional antibiotics: anti-bacterial agents, for example natural and synthetic penicillins and cephalosporins, sulphonamides, erythromycin, kanomycin, 5 tetracycline, chloramphenicol, rifampicin and including gentamicin, ampicillin, benzypenicillin, benethamine penicillin, benzathine penicillin, phenethicillin, phenoxy-methyl penicillin, procaine penicillin, cloxacillin, flucloxacillin, methicillin sodium, amoxicillin, bacampicillin hydrochloride, ciclacillin, mezlocillin, pivampicillin, talampicillin 10 hydrochloride, carfecillin sodium, piperacillin, ticarcillin, mecillinam, pirmecillinan, cefaclor, cefadroxil, cefotaxime, cefoxitin, cefsulodin sodium, ceftazidime, ceftizoxime, cefuroxime, cephalixin, cephalothin, cephamandole, cephalolin, cephradine, latamoxef disodium, aztreonam, chlortetracycline hydrochloride, clomocycline sodium, demeclocycline 15 hydrochloride, doxycycline, lymecycline, minocycline, oxytetracycline, amikacin, framycetin sulphate, neomycin sulphate, netilmicin, tobramycin, colistin, sodium fusidate, polymyxin B sulphate, spectinomycin, vancomycin, calcium sulphaloxate, sulfametopyrazine, sulphadiazine, sulphadimidine, sulphaguanidine, sulphaurea, 20 capreomycin, metronidazole, tinidazole, cinoxacin, ciprofloxacin, nitrofurantoin, hexamine, streptomycin, carbenicillin, colistimethate, polymyxin B, furazolidone, nalidixic acid, trimethoprim-sulfamethoxazole, clindamycin, lincomycin, cycloserine, isoniazid, ethambutol, ethionamide, pyrazinamide and the like; anti-fungal agents, for example 25 miconazole, ketoconazole, itraconazole, fluconazole, amphotericin, flucytosine, griseofulvin, natamycin, nystatin, and the like; and anti-viral agents such as acyclovir, AZT, ddI, amantadine hydrochloride, inosine pranobex, vidarabine, and the like.

In a further preferred embodiment, the medicaments comprise and/or are co-administered with penetration enhancing agents, such as poly-(ethyleneimine), or antibiotic agents which exhibit such penetration-enhancing capability (*e.g.* polymyxin or colistin).

5

The medicaments as prepared according to the first or second aspects of the invention are particularly suited for use in the curative or prophylactic treatment of one or more of the following indications:

10 Impetigo

Impetigo is a highly communicable infection. It is the most common infection in children.

- 15 Impetigo have two classic forms nonbullous and bullous. The nonbullous impetigo, also named impetigo contagiosa accounts for approximately 70% of cases. Lesions normally resolve in 2 to 3 weeks without treatment. Impetigo also may complicate other skin diseases such as scabies, varicella, atopic dermatitis, and Darier's disease.

20

(a) Nonbullous Impetigo

*Type of bacteria*

- 5 Nonbullous is an infection caused principally by Group A beta-haemolytic streptococci (*Streptococcus pyogenes*), *Staphylococcus aureus*, or a combination of these two organisms (see Andrews' diseases of the skin: clinical dermatology 9th ed. (2000) edited by Odom RB editor Saunders p.312-4). Non-Group A (Group B, C, and G)
- 10 streptococci may be responsible for rare cases of impetigo, and Group B streptococci are associated with impetigo in the newborn.

*Type of wounds*

- 15 Nonbullous is a superficial, intraepidermal, unilocular vesiculopustular infection.

Lesions of nonbullous impetigo commonly begin on the skin of the face or extremities following trauma. As a rule, intact skin is resistant to

20 impetiginization.

The clinical presentation of impetigo evolves in an orderly fashion from a small vesicle or pustule, which progresses into honey-coloured crusted plaque. Lesions usually are less than 2 cm in diameter. Lesions tend to

25 dry, leaving fine crusts without cicatrization. Lesions are usually minimally symptomatic. Rarely, erythema associated with mild pain or slight pruritus may be present. The infection spreads to contiguous and distal areas through the inoculation of other wound from scratching.

*Site of bacteria*

Nonbullous impetigo is a superficial streptococcal or staphylococcal infection which is localised to the subcorneal (just beneath the stratum  
5 corneum) layer of the skin (see Figure 1). More particularly, infection in impetigo is confined histopathologically to highly differentiated, upper epidermal keratinocytes. Once the bacteria invade a break in the skin, they begin to multiply.

10 The histopathology is that of an extremely superficial inflammation about the funnel-shaped upper portion of the pilosebaceous follicles. A subcorneal vesicopustule is formed, containing a few scattered cocci, together with debris of polymorphonuclear leukocytes and epidermal cells. In the dermis, there is a mild inflammatory reaction - vascular  
15 dilatation, oedema, and infiltration of polymorphonuclear leukocytes (Andrews' diseases of the skin, *supra.*, p.312-4).

*(b) Bullous impetigo**20 Type of bacteria*

Bullous impetigo is caused primarily by strains of *Staphylococcus aureus* which produce exfoliative toxins (Sadick *et al.*, 1997, *Dermatologic Clinics* **15**(2): 341-9).

25

*Type of wounds*

Bullous impetigo is histologically characterised by subcorneal cleavage and infiltrate with polymorphonuclear leucocytes migrating through the  
30 epidermis and accumulating between granular and stratum corneum skin

layers. Small or large superficial fragile bullae are present on the trunk and extremities.

5 Flaccid bullae and moist erosions with surrounding erythema are characteristic of this subcorneal infections. Often, only the remnants of ruptured bullae are seen at the time of presentation. The separation of the epidermis is due to an exotoxin produced by *Staphylococcus aureus*.

#### *Sites of bacteria*

10

Bullous impetigo is a superficial staphylococcal infection that occurs in and just beneath the stratum corneum (see figure 1). Bullous impetigo is considered due to exfoliative toxin produced by some *Staphylococcus aureus* attached to stratum corneum cells.

15

#### Atopic dermatitis (AD)

Atopic dermatitis, also named atopic eczema, is a chronic inflammation of the skin resulting in an itchy rash, especially in the flexures *i.e.* behind  
20 the knees, in front of the elbows, wrists, neck, and eyelids. Infection of the rash is common, and causes further inflammation and itch.

Eczema typically manifests in those aged 1-6 months. Approximately 60% of patients have their first outbreak by 1 year and 90% by 5 years.  
25 Onset of atopic dermatitis in adolescence or later is uncommon and should prompt consideration of another diagnosis. Disease manifestations vary with age.

*Type of bacteria*

Bacteria and their superantigens contribute to the pathogenesis of AD.

- 5    *Staphylococcus aureus* colonises the skin of 90% of AD patients (chronic eczematous lesions) and only 5% of non-atopic patients. The colonisation density of *Staphylococcus aureus* can reach up to  $10^7$  colony forming units  $\text{cm}^{-2}$  without clinical signs of infection in patients with AD. In addition, the apparently normal non-lesional skin of atopic patients  
10   contains increased numbers of *Staphylococcus aureus*.

- The reason for the overgrowth of *Staphylococcus aureus* in atopic dermatitis, though much less severely or not at all in diseases such as psoriasis, is not known. Protein A elicits a much less vigorous response  
15   in atopics than in normals or psoriatics, but this may be the result rather than a cause of colonisation. Attention has recently turned to the skin lipids and there is some evidence that fatty acids which may control staphylococcal colonisation are deficient in atopics.

- 20   Superantigens are a unique group of proteins produced by bacteria and viruses that bypass certain elements of the conventional, antigen-mediated immune sequence. Whereas conventional antigens activate approximately 0.01% to 0.1% of the body's T cells, a superantigen has the ability to stimulate 5% to 30% of the T-cell population. *S. aureus* may  
25   exacerbate or maintain skin inflammation in AD by secreting a group of exotoxins that act as superantigens. AD patients possess an altered skin barrier secondary to an insufficiency of ceramides within the stratum corneum. It has been proposed that penetration of the skin by these exotoxins may cause activation of T cells, macrophages, LCs, and mast  
30   cells, thereby leading to the release of cytokines and mast cell mediators.

It is conceivable that these events may provide the basis for inflammation in chronic AD. Speculation remains whether *S. aureus* colonisation and local superantigen secretion is a primary or secondary phenomenon in AD (Andrews' diseases of skin, Chap. 5, Atopic Dermatitis, Eczema, and  
5 non-infectious immunodeficiency disorders, p.69-76).

Cutaneous viral, fungal, and bacterial infections occur more commonly in AD patients. Viral infections are consistent with a T cell defect and include herpes simplex (local or generalised, *i.e.* eczema herpeticum),  
10 molluscum contagiosum, and human papilloma virus. Superficial fungal infections with *Trichophyton rubrum* and *Pityrosporon ovale* also occur frequently. Bacterial infections, specifically those with *S. aureus*, are extremely common. Superinfection results in honey-coloured crusting, extensive serous weeping or folliculitis.

15

#### *Type of wounds*

Acute lesions appear as erythematous papules, vesicles, and erosions; chronic disease consists of fibrotic papules and thickened, lichenified  
20 skin.

A finding of increasing numbers of pathogenic staphylococci is frequently associated with weeping, crusting, folliculitis and adenopathy. Secondary staphylococcal infection is frequent and local oedema and  
25 regional adenopathy commonly occur during atopic dermatitis. Impetigo can be a sort of secondary infection of atopic dermatitis.

The histology of atopic dermatitis ranges from acute spongiotic dermatitis to lichen simplex chronicus, depending on the morphology of the skin  
30 lesion biopsied.

### *Sites of bacteria*

*Staphylococcus aureus* cell walls exhibit receptors, the so-called  
5 adhesins, for epidermal and dermal fibronectin and fibrinogen. It has  
been demonstrated that the binding of *Staphylococcus aureus* was  
mediated by fibrinogen and fibronectin in AD patients. As the skin of  
AD patients lacks an intact *stratum corneum*, dermal fibronectin might be  
uncovered and increase the adherence of *Staphylococcus aureus*. Fibrillar  
10 and amorphous structures have been traced between *Staphylococcus*  
*aureus* cells and corneocytes and may results in a bacterial biofilm. It has  
been observed that *Staphylococcus aureus* penetrates into intracellular  
spaces suggesting that the skin surface lipids are deteriorated in AD  
patients (see Breuer K *et al.*, 2002, *British Journal of Dermatology* **147**:  
15 55-61) .

### Ulcers

Skin ulcers, such as diabetic foot ulcers, pressure ulcers, and chronic  
20 venous ulcers, are open sores or lesions of the skin characterised by the  
wasting away of tissue and sometimes accompanied by formation of pus.  
Skin ulcers may have different causes, and affect different populations,  
but they all tend to heal very slowly, if at all, and can be quite difficult  
and expensive to treat.

25

### *Type of bacteria*

Superficial pressure ulcers are not associated with major infection  
problems. Aerobic microorganisms at low levels will contaminate  
30 pressure ulcers, but will not impede timely healing. However, deep full-

thickness pressure ulcers can become secondarily infected, and osteomyelitis can occur. Those pressure ulcers with necrotic tissue contain high levels of aerobic and anaerobic microorganisms as compared to non-necrotic ulcers; foul smell is usually present when anaerobes  
5 invade the tissues. Thus, a treatment strategy is to clear necrotic tissue from the wound, producing a decrease in anaerobe presence.

The infections of pressure ulcers are typically polymicrobial and can contain *Streptococcus pyogenes*, enterococci, anaerobic streptococci,  
10 *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Bacteroides fragilis* and *Staphylococcus aureus*.

#### *Type of wounds*

15 Stage I pressure ulcer: Nonblanchable erythema of intact skin, considered to be heralding lesion of skin ulceration.

Stage II pressure ulcer: Partial thickness skin loss involving the epidermis and/or dermis. The ulcer is superficial and presents clinically as an  
20 abrasion, blister, or shallow crater. Because the epidermis may be interrupted by an abrasion, blister, or shallow crater, the ulcer should be evaluated for signs of secondary infections.

Stage III: Full thickness skin loss involving damage or necrosis of  
25 subcutaneous tissue which may extend down to, but not through, underlying fascia. The ulcer presents clinically as a deep crater with or without undermining of adjacent tissue.

Stage IV: Full thickness skin loss with extensive destruction, tissue necrosis, or damage to muscle, bone, or supporting structures, such as tendons or joint capsules.

#### 5     *Sites of bacteria*

There are three microbiological states that are possible in a wound: contamination, colonisation and infection. Contamination is characterised as the simple presence of microorganisms in the wound but without  
10 proliferation. It is generally accepted that all wounds, regardless of aetiology, are contaminated. Colonisation is characterised as the presence and proliferation of microorganisms in the wound but without host reaction. Colonisation is a common condition in chronic wounds such as venous ulcers and pressure ulcers and does not necessarily delay the  
15 healing process. When bacteria invade healthy tissues and continue to proliferate to the extent that their presence and by-products elicit or overwhelm the host immune response, this microbial state is known as infection. The classic signs and symptoms of infection include local redness, pain and swelling, fever and changes in the amount and character  
20 of wound exudates.

#### Lung infections

The medicaments of the invention are also suitable for treating a patient  
25 having an infectious disease of the lung. Lung infection can occur with a variety of bacterial genera and species, which include *Mycobacterium tuberculosis* (tuberculosis), *Pseudomonas* (primary cause of death of cystic fibrosis patients), *Streptococcus*, *Staphylococcus pneumoniae*, *Klebsiella*, *Toxoplasma*, etc. Lung infection can also occur with a variety  
30 of virus strains and opportunistic pathogens (fungi, parasites). As

pathogens of the lung are increasingly resistant to classical antibiotic therapies, photodynamic therapy offers an alternative method for eliminating these harmful organisms.

- 5 The medicaments of the invention can be administered to the lung in a variety of ways. For example the compound can be administered by the respiratory tract (*i.e.* intra-tracheally, intra-bronchially, or intra-alveolarly) or through the body wall of the chest.

10 Further indications

The medicaments of the invention are also suitable for the curative and/or prophylactic treatment of the following:

- 15 Systemic infections, bacteraemia (blood infection), periodontitis and other dental infections, treatment of tooth decay and against plaque, urinary tract infections, vaginal infections, treatment of all microorganism diseases including prions, viral infections, yeast infections, throat infections, stomach ulcers (caused by *Helicobacter pylori*), infections of  
20 burn sites and skin grafts, otitis (ear infection), bacterial conjunctivitis and other eye infections, infected bones exposed during surgical procedures, and bioterrorism attacks.

- Suitable veterinary applications include the curative and/or prophylactic  
25 treatment of foot-and-mouth disease, BSE and animal parasite infestations.

Thus, further aspects of the invention provide the following:

- 5 (i) Use of a compound as described above in the preparation of a medicament for the curative and/or prophylactic treatment of a dermatological infection;
- (ii) Use of a compound as described above in the preparation of a medicament for the curative and/or prophylactic treatment of an infection of the lungs;
- 10 (iii) Use of a compound as described above in the preparation of a medicament for the curative and/or prophylactic treatment of a wound infection and/or an ulcer;
- 15 (iv) A method for treating a patient in need of treatment with a antimicrobial agent comprising administering to the patient a compound as described above, wherein the method does not comprise irradiating the compound with a stimulus which activates antimicrobial activity; and
- 20 (v) A method for treating a patient in need of treatment with an antimicrobial agent comprising administering to the patient a compound as described above, wherein the method comprises a first treatment phase during which the compound is not irradiated with a stimulus which activates antimicrobial activity, followed by a
- 25 second treatment phase when the compound is irradiated with a stimulus which activates antimicrobial activity (such as ultrasound and/or light). Preferably, the first treatment phase lasts at least 10 minutes, for example at least 20 minutes, 30 minutes, 40 minutes,

50 minutes, 1 hour, 2 hours, 3. hours. 5 hours, 12 hours and 24 hours.

The medicaments prepared according to the first and second aspects of the invention may also be used to kill microorganisms *in vitro*. For example, the medicament may also be used in the form of a sterilising solution or wash to prevent the growth of microorganisms on a surface or substrate, such as in a clinical environment (*e.g.* surgical theatre) or a domestic environment (*e.g.* a kitchen work surface, washing clothes such as bed linen).

Preferably, such a medicament comprises the antimicrobial compound in solution at a concentration of 1 to 100 µg/ml.

Preferably, the solution further comprises a surface-active agent or surfactant. Suitable surfactants include anionic surfactants (*e.g.* an aliphatic sulphonate), amphoteric and/or zwitterionic surfactants (*e.g.* derivatives of aliphatic quaternary ammonium, phosphonium and sulfonium compounds) and nonionic surfactants (*e.g.* aliphatic alcohols, acids, amides or alkyl phenols with alkylene oxides)

Conveniently, the surface-active agent is present at a concentration of 0.5 to 5 weight percent.

The sterilising solutions are particularly suited for use in hospital environments. For example, the sterilising solutions may be used to sterilise surgical instruments and surgical theatre surfaces, as well as the hands and gloves of theatre personnel. In addition, the sterilising solutions may be used during surgery, for example to sterilise exposed bones. In all cases, the solution is applied to the surface to be sterilised.

The medicament may also be used to disinfect blood and blood products and in the diagnosis of bacterial contamination or infection.

- 5 In both *in vitro* and *in vivo* uses, the medicament prepared according to the first and second aspects of the invention is preferably exposed to the target microorganisms (or surface/area to be treated) for at least five minutes. For example, the exposure time may be at least 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 1 hour, 2 hours, 3, hours, 5  
10 hours, 12 hours and 24 hours.

Preferred, non-limiting embodiments of the invention will now be described by way of example, with reference to the accompanying drawings in which:

15

Figure 1 shows a schematic diagram of the structure of skin.

Figure 2 shows cell toxicity of normal human dermal fibroblasts after 5 minutes, 1 hour and 4 hours incubation with Compound 10.

20

NHDF were incubated with different concentrations of Compound 10 for 5 min, 1 h and 4 h (0  $\mu$ M, 0.01  $\mu$ M, 0.1  $\mu$ M, 1.0  $\mu$ M, 10  $\mu$ M). Cells were then incubated for 24 h in the dark. Toxicity was tested by standard MTT-assay. Cell viability was normalised to one, which means, the values of  
25 control cells were normalised to one. Grey dotted line: 5 min incubation; black dotted: 1 h incubation; black line: 4 h incubation; (n=3, mean  $\pm$  SD).

Figure 3 shows cell toxicity of normal human epidermal keratinocytes  
30 after 5 minutes, 1 hour and 4 hours incubation with Compound 10.

NHEK were incubated with different concentrations of Compound 10 for 5 min, 1 h and 4 h (0  $\mu$ M, 0.01  $\mu$ M, 0.1  $\mu$ M, 1.0  $\mu$ M, 10  $\mu$ M). Cells were then incubated for 24 h in the dark. Toxicity was tested by standard MTT-  
5 assay. Cell viability was normalised to one, which means, the values of control cells were normalised to one. Red dotted line: 5 min incubation; black dotted: 1 h incubation; blue dotted: 4 h incubation only; (n=3, mean  $\pm$  SD).

10 Figure 4 shows the chemical stability of Compound 10 formulated (A) as a solid, (B) in water and (C) in PBS.

Figure 5 shows a 3D plot of the stability (measured by HPLC) of Compound 10 after 21 days in PBS buffer.

15

Figure 6 shows the stability over 8 weeks of various formulations of (A) Compound 1, (B) Compound 8, (C) Compound 12 and (D) Compound 10.

20 Figure 7 shows the extended stability over 17 weeks of various formulations of (A) Compound 10 and (B) Compound 8.

25

## EXAMPLES

## EXAMPLE A: SYNTHESIS OF EXEMPLARY COMPOUNDS

5 *Materials and Methods*NMR-measurements

Proton NMR spectra were recorded on a Bruker B-ACS60 (300 MHz)  
10 instrument using TMS as internal standard. The chemical shifts are given  
in ppm and coupling constants in Hz in the indicated solvent. Some  
abbreviation for NMR: singlet (s), broad singlet (bs), doublet (d), triplet  
(t), quartet (q), quintet (quint), multiplet (m).

15 Chemicals

All solvents and reagents were purchased from Aldrich, Fluka, Merck and  
Lancaster and used without further purification.

20 Dipyrrrolemethane was prepared as described by C. Brücker *et al.*, *J.*  
*Porphyrins Phthalocyanines*, **2** 455 (1998).

Chromatography

25 Column chromatography was carried out using silica gel (Merck Silicagel  
60, Fluka 60, 0.040-0.063 mm) and Sephadex LH-20 (Pharmacia). All  
solvents (Synopharm) for chromatography were technical pure grade.

Abbreviations

- DDQ: 2,3-dichloro-5,6-dicyano-p-benzoquinone  
DMF: *N,N*-dimethylformamide  
5 TFA: trifluoroacetic acid

*Synthesis routes for test compounds*

The following test compounds were synthesised:

10

*Exemplary compounds for use in the invention*

Compounds 6, 8 to 10, 12, 23, 25, 28, 31 and 32.

15 *Reference compounds (for use as comparative controls)*

Compounds 1, 3, 16, 19, 26, 29, 33, 36, 37, 39, 41 and 46 to 51.

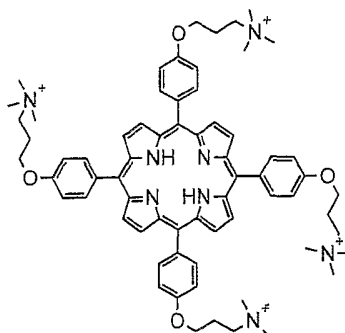
*Chemical intermediates*

20

Compounds 2, 4, 5, 7, 11, 13 to 15, 17, 18, 20 to 22, 24, 27, 30, 34, 35,  
38, 40 and 42 to 45.

COMPOUND 1

5,10,15,20-tetrakis-[4-(3-Trimethylammonio-propyloxy)-phenyl]-  
porphyrin tetrachloride



5

To a vigorously-stirred suspension of 5,10,15,20-tetrakis-(4-hydroxy-phenyl)-porphyrin (50 mg, 0.07 mmol) and  $K_2CO_3$  (230 mg, 1.7 mmol) in DMF (20 mL), a solution of (1-bromopropyl)-trimethylammonium bromide (0.27 g, 1.05 mmol) in DMF (5 mL) is added dropwise at 50°C during 30 mins. The mixture is stirred at 50°C for 15 h. After removal of DMF under reduced pressure, the residue obtained is dissolved in methanol (5 mL) and filtered through a pad of silica gel (depth 2 cm) supported on a steel frit (diameter 3.5 cm). After washing with methanol (1 L), the pad is eluted with acetic acid. After evaporation of solvent from the eluate, the residue obtained is purified by chromatography on a column (2.5 x 40 cm) of Sephadex LH20 eluting with n-butan-  
 10  
 15  
 20  
 ol:water:acetic acid (4:5:1, by vol., upper phase). The recovered material is dissolved in the minimum volume of methanol and the solution is passed through a short column (3.5 x 20 cm) of anion exchange resin (Amberlite IRA 400, chloride form). The recovered tetrachloride salt is dried under high vacuum and obtained as a violet solid.

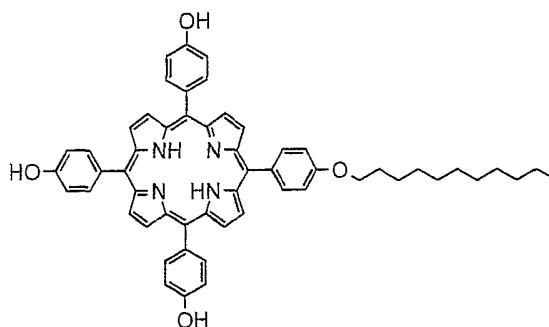
<sup>1</sup>H-NMR:

$\delta_H$  (300MHz, CD<sub>3</sub>OD): 2.35-2.50 (bs, 8 H), 3.25-3.35 (bs, 36 H), 3.65-3.75 (bs, 8 H), 4.35 (m, 8 H), 7.30, 8.10 (2 x d, <sup>3</sup>J 8.5 Hz, 16 H), 8.80-9.00 (bs, 8 H).

5

## COMPOUND 2

5,10,15-tris-(4-Hydroxy-phenyl)-20-(4-undecyloxy-phenyl)-porphyrin



10

To a vigorously-stirred suspension of 5,10,15,20-tetrakis-(4-hydroxy-phenyl)-porphyrin (400 mg, 0.59 mmol) and K<sub>2</sub>CO<sub>3</sub> (1.0 g, 7.1 mmol) in DMF (75 mL), a solution of 1-bromoundecane (0.1 mL, 0.45 mmol) in DMF (10 mL) is added dropwise at 50°C during 30 mins and the mixture is stirred at the same temperature for 1.5 h. After removal by filtration of K<sub>2</sub>CO<sub>3</sub> and removal under reduced pressure of DMF, the residue obtained is dissolved in dichloromethane (200 mL), washed with water (3x150 mL) and the solution dried (Na<sub>2</sub>SO<sub>4</sub>). The solvent is evaporated under reduced pressure and the residue obtained is dissolved in toluene:ethanol (5:1 by vol., ca. 10 mL) and purified by chromatography using a column (5 X 50 cm) of silica gel (Merck 60). The column is eluted with toluene followed by toluene:ethyl acetate (2:1 by vol.) and the desired material recovered by evaporation of solvent from the appropriate fractions is dried under high vacuum. The product is obtained as a violet solid.

25

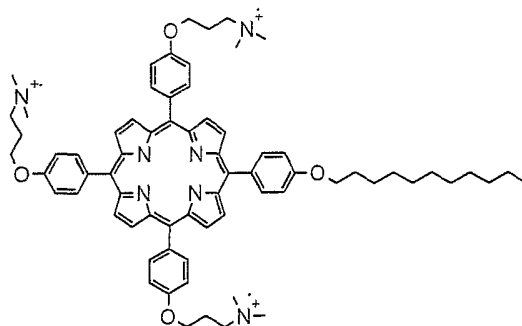
$^1\text{H-NMR}$ :

$\delta_{\text{H}}$  (300Mz, d6-acetone): 0.95 (t,  $^3J$  7.5 Hz, 3 H), 1.25-1.55 (m, 14 H),  
 1.58 (quint,  $^3J$  7.5 Hz, 2 H), 1.85 (quint,  $^3J$  7.5 Hz, 2 H), 4.16 (t,  $^3J$  7.5  
 Hz, 2 H), 7.20 (d,  $^3J$  8.1 Hz, 2 H), 7.25 (d,  $^3J$  8.2 Hz, 6 H), 8.00-8.15 (m,  
 5 8 H), 8.80-9.10 (m, 8 H).

### COMPOUND 3

5,10,15-tris-[4-(3-Trimethylammonio-propyloxy)-phenyl]-20-(4-  
 undecyloxy-phenyl)-porphyrin trichloride

10



To a vigorously-stirred suspension of Compound 2 (100 mg, 0.12 mmol)  
 and  $\text{K}_2\text{CO}_3$  (230 mg, 1.7 mmol) in DMF (30 mL), a solution of (1-  
 15 bromopropyl)-trimethylammonium bromide (0.3 g, 16.6 mmol) in DMF  
 (10 mL) is added at  $50^\circ\text{C}$  and the mixture is stirred at this temperature for  
 12 h. After removal of the DMF under reduced pressure, the residue  
 obtained is dissolved in methanol (5 mL) and filtered through a pad of  
 silica gel (depth 2 cm) supported on a steel frit (diameter 3.5 cm). After  
 20 washing with methanol (ca. 1L), the pad is eluted with acetic  
 acid:methanol:water (3:2:1, by vol.). After evaporation of the solvent  
 from the eluate under reduced pressure, the residue obtained is purified by  
 chromatography on a column (2.5 x 40 cm) of Sephadex LH-20 eluting  
 with n-butanol:water:acetic acid (5:4:1, by vol., upper phase). After  
 25 removal of the solvent from appropriate fractions of the eluate under

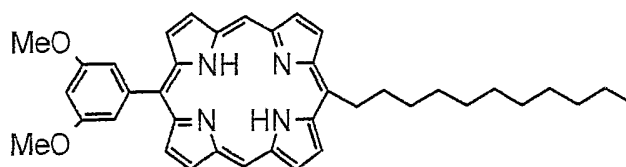
reduced pressure, the residue obtained is dissolved in methanol (5 mL) and the solution is passed through a short column (3.5 x 20 cm) of anion exchange resin (Amberlite IRA 400, chloride form). The final product is obtained as the trichloride salt, after removal of solvent and drying under high vacuum, as a violet solid.

<sup>1</sup>H-NMR:

$\delta_H$  (300MHz, CD<sub>3</sub>OD): 0.80 (t, <sup>3</sup>J 7.5 Hz, 3 H), 1.15-1.45 (m, 16 H), 1.50-1.60 (bs, 2 H), 2.25-2.45 (bs, 6 H), 3.25-3.35 (bs, 27 H), 3.75-3.85 (bs, , 6 H), 4.18 (t, <sup>3</sup>J 7.5 Hz, 2 H), 4.40-4.45 (bs, 6 H), 7.20-7.40, 7.95-8.15 (2 x m, 16 H), 8.60-9.00 (bs, 8 H).

#### COMPOUND 4

5-(3,5-Dimethoxy-phenyl)-15-undecyl-porphyrin



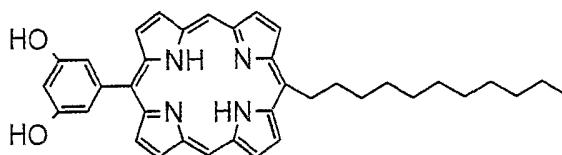
To a stirred solution of dipyrrolemethane (0.62 g, 4.2 mmol) in dichloromethane (5 mL) is added 3,5-dimethoxybenzaldehyde (0.35 g, 2.1 mmol) and dodecanal (0.464 g, 2.52 mmol) in degassed dichloromethane (1L). TFA (0.07 mL, 3.0 mmol) is added dropwise. The solution is stirred at room temperature in the dark for 17 h under argon. After addition of DDQ (2.7 g, 12 mmol), the mixture is stirred at room temperature for a further hour. Purification of material recovered after removal of solvent under reduced pressure by chromatography on a column (400 g) of silica gel (Merck 60) with toluene for elution yields the product as a violet solid.

<sup>1</sup>H-NMR:

$\delta_H$  (300Mz, CDCl<sub>3</sub>): 0.80 (t, <sup>3</sup>J 7.5 Hz, 3 H), 1.10-1.25 (m, 12 H), 1.40 (m, 2H), 1.75 (quint, <sup>3</sup>J 7.5 Hz, 2 H), 2.45 (quint, <sup>3</sup>J 7.5 Hz, 2 H), 3.90 (s, 6H), 4.90 (t, <sup>3</sup>J 7.5 Hz, 2 H), 6.80 (m, 1 H), 7.35 (m, 2 H), 9.00, 9.25, 9.30, 9.50 (4 x d, <sup>3</sup>J 4.7 Hz, 4 x 2 H), 10.15 (s, 2H).

#### COMPOUND 5

5-(15-Undecyl-porphyrin-5-yl)-benzene-1,3-diol



10

To a solution of Compound 4 (80 mg, 0.133 mmol) in anhydrous dichloromethane (80 mL) under an argon atmosphere, BBr<sub>3</sub> (5 mL, 1M in dichloromethane) is added dropwise at -70°C and the mixture is stirred for 1 h at this temperature and then warmed to room temperature and stirred overnight. The mixture is cooled to -10°C and hydrolysed by the addition of water (2 mL) and stirring for 1 h. NaHCO<sub>3</sub> (3 g) is added directly for neutralisation. The mixture is stirred for a further 12 h and after filtration of NaHCO<sub>3</sub> and removal of dichloromethane under vacuum the residue obtained is purified by column chromatography using silica gel eluting with dichloromethane. After evaporation of solvent from appropriate combined fractions and drying of the residue obtained under high vacuum the product is obtained as a violet solid

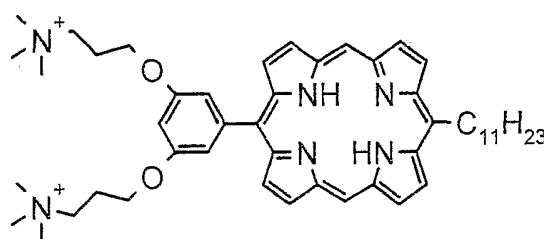
<sup>1</sup>H-NMR:

$\delta_H$  (300Mz, d<sub>6</sub>-acetone): 0.75 (t, <sup>3</sup>J 7.5 Hz, 3 H), 1.05-1.25 (m, 12 H), 1.30-1.40 (m, 2H), 1.45-1.50 (m, 2 H), 2.40 (quint, <sup>3</sup>J 7.5 Hz, 2 H), 4.90

(t,  $^3J$  7.5 Hz, 2 H), 6.65 (m, 1 H), 7.18 (m, 2 H), 8.60-8.65, 9.00-9.05, 9.35-9.40, 9.55-9.60 (4 x m, 8 H), 10.25 (s, 2H).

#### COMPOUND 6

- 5 5-[3,5-bis-(3-Trimethylammonio-propyloxy)-phenyl]-15-undecyl-porphyrin dichloride



- 10 To a vigorously-stirred suspension of Compound 5 (80 mg, 0.14 mmol) and K<sub>2</sub>CO<sub>3</sub> (230 mg, 1.7 mmol) in DMF (30 mL) is added (1-bromopropyl)-trimethylammonium bromide (0.3 g, 16.6 mmol) at 50 °C. The mixture is stirred at this temperature for 18 h. After removal of the DMF under reduced pressure, the residue obtained is dissolved in
- 15 methanol (5 mL) and filtered through a pad of silica gel (depth 2 cm) supported on a steel frit (diameter 3.5 cm). After washing the pad with methanol (ca. 1L) the crude product is eluted with acetic acid:methanol:water (3:2:1, by vol.). Appropriate fractions are collected and, after evaporation of the solvent under reduced pressure, the residue
- 20 obtained is purified by chromatography on a column (2.5 x 40 cm) of Sephadex LH-20 eluting with n-butanol:water:acetic acid (5:4:1, by vol., upper phase). After removal of the solvent from appropriate fractions under reduced pressure, the residue obtained is dissolved in methanol (5 mL) and the solution is passed through a short column (3.5 x 20 cm) of
- 25 anion exchange resin (Amberlite IRA 400, chloride form). After collection of the eluate, solvent is removed under reduced pressure and

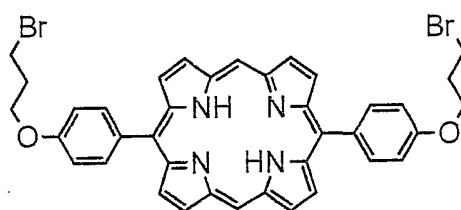
the residue obtained is dried under high vacuum to yield the dichloride salt as a violet solid.

<sup>1</sup>H-NMR:

- 5     $\delta_{\text{H}}$  (300Mz, CD<sub>3</sub>OD): 0.75 (t, <sup>3</sup>J 7.5 Hz, 3 H), 1.05-1.20 (m, 14 H), 1.45-1.50 (m, 2 H), 2.05-2.15 (m, 4 H), 2.15-2.20 (m, 2 H), 2.95 (s, 18 H), 3.35-3.45 (m, 4 H), 3.95 (t, <sup>3</sup>J 7.5 Hz, 4 H), 4.55 (t, <sup>3</sup>J 7.5 Hz, 2 H), 6.85 (m, 1 H), 7.35 (m, 2 H), 8.85-8.90, 9.15-9.20, (3 x m, 8 H), 10.10 (s, 2 H).

10    COMPOUND 7

5,15-bis-[4-(3-Bromo-propyloxy)-phenyl]-porphyrin



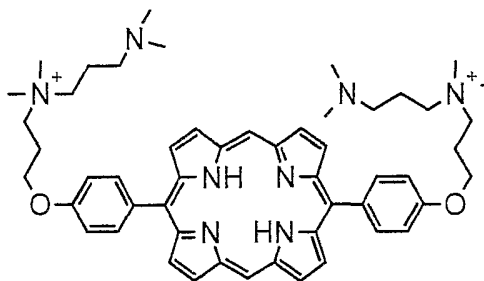
<sup>1</sup>H-NMR:

$\delta_H$  (300Mz, C<sub>6</sub>D<sub>6</sub>): -3.15 (2 H, s), 2.00 (quint, <sup>3</sup>J 7.5 Hz, 4 H), 3.30 (t, <sup>3</sup>J 7.5 Hz, 4 H), 3.90 (t, <sup>3</sup>J 7.5 Hz, 4 H), 7.15-7.18, 7.95-8.15 (2 x m, 2 x 4 H), 9.15-9.20, (m, 8 H), 10.05 (s, 2H).

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#### COMPOUND 8

5,15-bis-(4-{3-[(3-Dimethylamino-propyl)-dimethyl-ammonio]-propyloxy}-phenyl)-porphyrin dichloride



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Compound 7 (200 mg, 0.27 mmol) is dissolved in absolute DMF (40 mL) with N,N,N',N'-tetramethyl-1,3-propanediamine (5 mL, 13.9 mmol) and the solution is stirred at 50°C under argon overnight. After evaporation of the solvent under reduced pressure, the residue obtained is dissolved in methanol (5 mL) and the solution is filtered through a pad of silica gel (depth 2 cm) supported on a steel frit (diameter 3.5 cm). The pad is eluted with methanol (ca. 1L) followed by acetic acid:methanol:water (3:2:1, by vol.). After evaporation of the solvent from appropriate fractions, the raw product obtained is dissolved in methanol (5 mL) and further purified by chromatography on a column (2.5 x 40 cm) of Sephadex LH-20 using n-butanol:water:acetic acid (4:5:1, by vol., upper phase) as the developing phase. The first fraction eluted is the desired product. After removal of solvent under reduced pressure the residue obtained is dissolved in methanol (5 mL) and passed through a short column (3.5 x 20 cm) of anion exchange resin (Amberlite IRA 400,

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chloride form). After removal of solvent under reduced pressure from the eluate, the residue is treated with diethylether and dried under high vacuum to give the product as a violet solid.

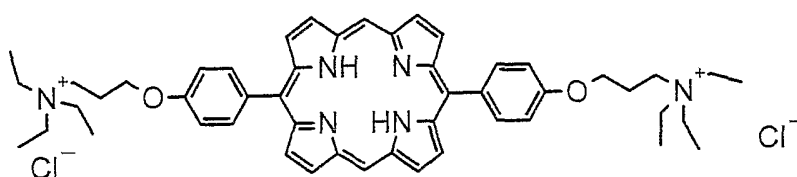
5 <sup>1</sup>H-NMR:

$\delta_H$  (300MHz, CD<sub>3</sub>OD): 2.20-2.35 (m, 4 H), 2.40-2.50 (m, 4 H), 2.80 (s, 12 H), 3.05 (4 H, t, <sup>3</sup>J 7.8, 2 H), 3.25 (s, 12 H), 3.45-3.55 (bs, 4 H), 3.65-3.75 (m, 4 H), 4.30 (t, <sup>3</sup>J 4.2 Hz, 4 H), 7.40, 8.10 (2 x d, <sup>3</sup>J 7.5 Hz, 2 x 4 H), 8.95, 9.45 (2x d, <sup>3</sup>J 4.2 Hz, 8 H), 10.40 (s, 2 H).

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COMPOUND 9

5,15-bis-[4-(3-Triethylammonio-propyloxy)-phenyl]-porphyrin dichloride



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To a solution of Compound 7 (50 mg, 0.068 mmol) in absolute DMF (20 mL) is added triethylamine (4.7 mL, 0.034 mol, 500 eq.). The mixture is stirred at 60°C for 24 h. The solvent is removed under reduced pressure and the residue obtained is dissolved in methanol (5 mL) and filtered  
 20 through a pad of silica gel (depth 2 cm) supported on a steel frit (diameter 3.5 cm). After washing with methanol (ca. 1L) the pad is eluted with acetic acid:methanol:water (3:2:1, by vol.). After evaporation of the solvent from the eluted fraction, the raw product obtained is dissolved in methanol (5 mL) and purified by chromatography on a column (2.5 x 40  
 25 cm) of Sephadex LH-20 eluting with n-butanol:water:acetic acid (4:5:1, by vol., upper phase). The solvents are removed under reduced pressure from appropriate fractions, the residue obtained is dissolved in methanol (5 mL) and the solution is passed through a short column (3.5 x 20 cm) of

anion exchange resin (Amberlite IRA 400, chloride form) to yield the product as a violet solid after evaporation of solvent.

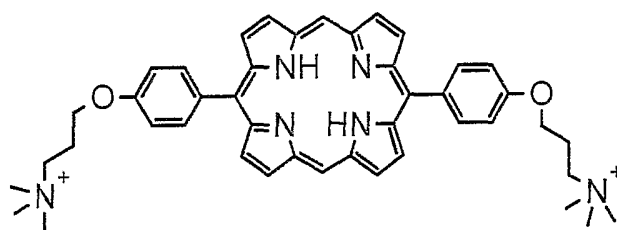
$^1\text{H-NMR}$ :

- 5  $\delta_{\text{H}}$  (300Mz,  $\text{CD}_3\text{OD}$ ): 1.25 (m, 18H), 2.13 (m, 4H), the signals for  $-\text{CH}_2\text{NCH}_2-$  (16H) are in the area 3.00-3.40 as a part of the multiplet covered by the solvent signals, 4.15 (t, 4H,  $^3J = 7.5$  Hz), 7.36 (d, 4H,  $^3J = 7.5$  Hz), 8.15 (d, 4H,  $^3J = 7.5$  Hz), 9.05 (d, 4H,  $^3J = 7.5$  Hz), 9.54 (d, 4H,  $^3J = 7.5$  Hz), 10.45 (s, 2H)

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#### COMPOUND 10

5,15-bis-[4-(3-Trimethylammonio-propyloxy)-phenyl]-porphyrin  
dichloride



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- A solution of Compound 7 (300 mg, 0.41 mmol) in absolute DMF (50 mL) is transferred into a 100 mL autoclave. After addition of trimethylamine (4.5 g), the mixture is stirred at 50°C for 16 h. After evaporation of the solvent, the residue obtained is dissolved in methanol (5 mL) and the solution is filtered through a pad of silica gel (depth 2 cm) supported on a steel frit (diameter 3.5 cm). After washing with methanol (ca. 1L) the pad is eluted with acetic acid:methanol:water (3:2:1, by vol.). After evaporation of the solvent from appropriate fractions, the residue obtained is dissolved in methanol (5 mL) and purified by chromatography on a column (2.5 x 40 cm) of Sephadex LH-20, eluting with n-butanol:water:acetic acid (4:5:1, by vol., upper phase). Two fractions are
- 20
- 25

obtained, the first-eluting of which is the desired product. The solvent is removed under reduced pressure and the residue obtained is redissolved in methanol (5 mL) and the solution is passed through a short column (3.5 x 20 cm) of anion exchange resin (Amberlite IRA 400, chloride form).  
5 After evaporation of the solvent under reduced pressure, the residue is treated with methanol:diethylether and dried under high vacuum to give the product as a violet solid.

<sup>1</sup>H-NMR:

10  $\delta_H$  (300Mz, CD<sub>3</sub>OD): 2.40-2.60 (m, 4 H), 3.30-3.25 (bs, 18 H), 3.75-3.80 (m, 4 H), 4.40(t, <sup>3</sup>J 7.5 Hz, 4 H), 7.40, 8.20 (2 x d, <sup>3</sup>J 8.5 Hz, 8 H), 9.05, 9.50 (2 x d, <sup>3</sup>J 4.5 Hz, 8 H), 10.45 (s, 2 H).

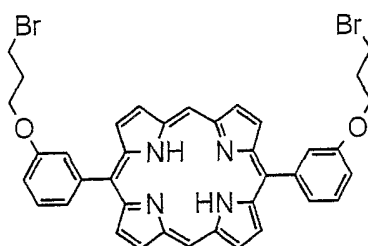
*Alternative synthesis route for Compound 10*

15 Compound 42 (100mg, 0.2mMol; see below) is dissolved and potassium carbonate (230mg 1.7mMol) is suspended in DMF (30mL) and to the vigorously-stirred mixture is added a solution of (1-bromopropyl)-trimethylammonium bromide (350mg, 1.3mMol) in DMF (5mL)  
20 dropwise at 50°C during 30 mins. The mixture is heated for 15h. DMF is removed by rotary evaporation and the residue obtained is dissolved in methanol and the solution is filtered through a pad of silica gel (depth 2 cm) supported on a steel frit (diameter 3.5 cm). After washing with methanol (ca. 1L) the pad is eluted with acetic acid:methanol:water  
25 (3:2:1, by vol.). After evaporation of the solvent from appropriate fractions, the residue obtained is dissolved in methanol (5 mL) and purified by chromatography on a column (2.5 x 40 cm) of Sephadex LH-20, eluting with n-butanol:water:acetic acid (4:5:1, by vol., upper phase). Two fractions are obtained, the first-eluting of which is the desired  
30 product. The solvent is removed under reduced pressure and the residue

obtained is redissolved in methanol (5 mL) and the solution is passed through a short column (3.5 x 20 cm) of anion exchange resin (Amberlite IRA 400, chloride form). After evaporation of the solvent under reduced pressure, the residue is treated with methanol:diethylether and dried under high vacuum to give the product as a violet solid.

COMPOUND 11

5,15-bis-[3-(3-Bromo-propyloxy)-phenyl]-porphyrin



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To a stirred solution of dipyrrolemethane (1.22 g, 8.2 mmol) and 3-(3-bromo-propyloxy)- benzaldehyde (2.06 g, 8.2 mmol) in degassed dichloromethane (2 L), TFA (0.14 mL, 3 mmol) is added dropwise. The solution is stirred at room-temperature in the dark for 17 h under argon. After addition of DDQ (5.4 g, 0.024 mol), the mixture is stirred at room temperature for a further 1h. After removal of solvents under reduced pressure, the residue obtained is dissolved in dichloromethane (5 mL) and passed through a column (300 g) of silica (Fluka 60) using dichloromethane as eluent to give raw product which is treated with dichloromethane:methanol to yield pure material as a violet solid.

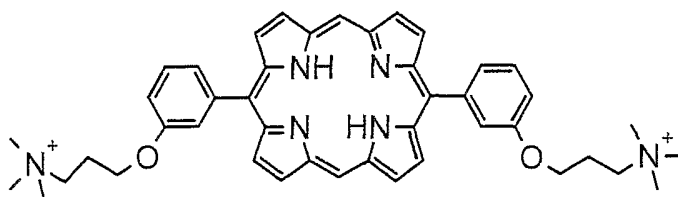
<sup>1</sup>H-NMR:

$\delta_H$  (300Mz, CDCl<sub>3</sub>): -3.20 (2 H, s), 2.40 (quint, <sup>3</sup>J 7.5 Hz, 4 H), 3.65 (t, <sup>3</sup>J 7.5 Hz, 4 H), 4.25 (t, <sup>3</sup>J 7.5 Hz, 4 H), 7.20-7.25, 7.60-7.65, 7.75-7.80 (3 x m, 8 H), 9.05, 9.25, (2 x d, <sup>3</sup>J 4.2 Hz, 8 H), 10.25 (s, 2 H).

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#### COMPOUND 12

5,15-bis-[3-(3-Trimethylammonio-propyloxy)-phenyl]-porphyrin  
dichloride



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A solution of Compound 11 (400 mg, 0.543 mmol) in DMF (50 mL) is transferred into a 100 mL autoclave. After addition of trimethylamine (6.3g), the mixture is stirred at 50°C for 8 h. After evaporation of the solvent under reduced pressure, the residue obtained is dissolved in methanol (5 mL) and the solution is filtered through a pad of silica gel (depth 2 cm) supported on a steel frit (diameter 3.5 cm). After washing the pad with methanol (ca.1L), elution with acetic acid:methanol:water (3:2:1, by vol.) affords fractions which, after evaporation of the solvent under reduced pressure, gives a solid residue. This is dissolved in methanol (5 mL) and purified by chromatography on a column (2.5 x 40 cm) of Sephadex LH-20 eluting with n-butanol:water:acetic acid (4:5:1, by vol., upper phase). Two fractions are eluted from the column, the first of which is the desired product. After removal of the solvent under reduced pressure, the residue obtained is dissolved in methanol (5 mL). The solution is passed through a short column (3.5 x 20 cm) of anion exchange resin (Amberlite IRA 400, chloride form), the solvent is removed under reduced pressure and the raw product is treated with

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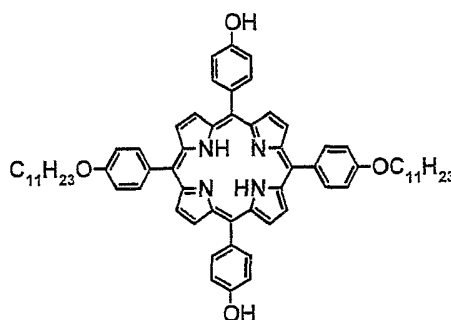
methanol:diethylether to give a violet solid which is dried under high vacuum.

$^1\text{H}$ -NMR:

- 5  $\delta_{\text{H}}$  (300Mz,  $\text{CD}_3\text{OD}$ ): 2.30-2.35 (m, 4 H), 3.15 (s, 18 H), 3.95-4.05 (m, 4 H), 4.20-4.25 (m, 4 H), 7.40-7.45, 7.65-7.70, 7.80-7.85 (3 x m, 8 H), 9.00-9.05, 9.40-9.45, (2 x m, 8 H), 10.40 (m, 2 H).

### COMPOUND 13

- 10 5,15-bis-(4-Hydroxy-phenyl)-10,20-bis-(4-undecyloxy-phenyl)-porphyrin



- The third fraction eluted from the column during the chromatographic separation described for the synthesis of Compound 2 is characterised as  
15 5,15-bis-(4-hydroxy-phenyl)-10,20-bis-(4-undecyloxy-phenyl)-porphyrin

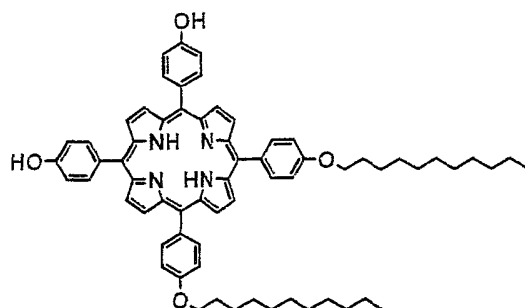
$^1\text{H}$ -NMR:

- 20  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ): -2.88 (2 H, s), 0.85 (t,  $^3J$  7.5 Hz, 6 H), 1.20-1.40 (m, 28 H), 1.55 (br m, 4 H), 1.80 (quint,  $^3J$  7.5 Hz, 4 H), 4.15 (t,  $^3J$  7.5 Hz, 4 H), 6.65, 7.15 (d,  $^3J$  8.1 Hz, 8 H), 7.80, 8.00 (d,  $^3J$  8.1 Hz, 8 H), 8.75-8.80 (m, 8 H).

*trans*-Regioisomer geometry is assigned by  $^1\text{H}$ - $^{13}\text{C}$ -2D-NMR in d-acetic acid.

COMPOUND 14

5,10-bis-(4-Hydroxy-phenyl)-15,20-bis-(4-undecyloxy-phenyl)-porphyrin



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The fourth fraction eluted from the column during the chromatographic separation described for the synthesis of Compound 2 is characterised as 5,10-bis-(4-hydroxyphenyl)-15,20-bis-(4-undecyloxy-phenyl)-porphyrin

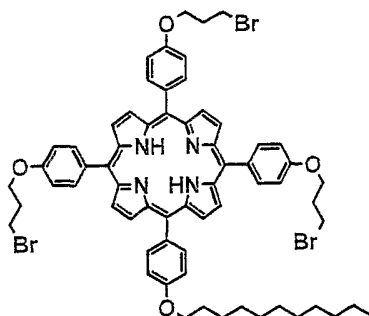
10  $^1\text{H-NMR}$ :

$\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ): -2.80 (2 H, s), 0.90 (t,  $^3J$  7.5 Hz, 6 H), 1.20-1.60 (m, 28 H), 1.65 (quint,  $^3J$  7.5 Hz, 4 H), 2.00 (quint,  $^3J$  7.5 Hz, 4 H), 4.22 (t,  $^3J$  7.5 Hz, 4 H), 7.15 (d,  $^3J$  8.1 Hz, 4 H), 7.25 (d,  $^3J$  8.2 Hz, 4 H), 8.10 (d,  $^3J$  8.2 Hz, 4 H), 8.15 (d,  $^3J$  8.2 Hz, 4 H), 8.80-8.90 (m, 8 H).

15 *cis*-Regioisomer geometry is assigned by  $^1\text{H-}^{13}\text{C-2D-NMR}$  in d-acetic acid.

COMPOUND 15

5,10,15-tris-[4-(3-Bromo-propyloxy)-phenyl]-20-(4-undecyloxy-phenyl)-  
porphyrin



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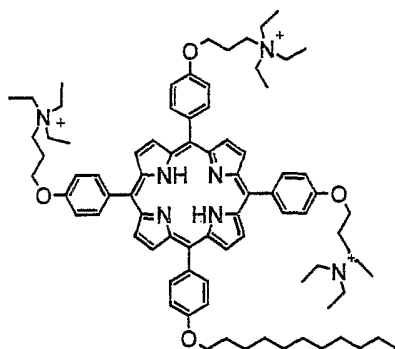
Under an argon atmosphere, Compound 2 (200 mg, 0.24 mmol) is dissolved in absolute DMF (40 mL) in the presence of  $K_2CO_3$  (500 mg) and 1,3-dibromopropane (1.02 mL, 10 mmol). The mixture is heated overnight at 80°C. Work-up is as the procedure given for Compound 2 described above. The product is purified by column chromatography on silica gel (Merck 60) eluting with hexane:ethyl acetate (5:1, by vol.).

$^1H$ -NMR:  
 $\delta_H$  (300MHz,  $CDCl_3$ ): -2.75 (2 H, s), 0.85 (t,  $^3J$  7.5 Hz, 3 H), 1.20-1.45 (m, 14 H), 1.50 (quint,  $^3J$  7.5 Hz, 2 H), 1.90 (quint,  $^3J$  7.5 Hz, 2 H), 2.40 (quint,  $^3J$  7.4 Hz, 6 H), 3.65 (t,  $^3J$  7.4 Hz, 6 H), 4.16 (t,  $^3J$  7.5 Hz, 2 H), 4.25 (t,  $^3J$  7.5 Hz, 6 H), 7.18-7.20 (m, 8 H), 8.00-8.05 (m, 8 H), 8.75-8.85 (m, 8 H).

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COMPOUND 16

5,10,15-tris-[4-(3-Triethylammonio-propyloxy)-phenyl]-20-(4-undecyloxy-phenyl)-porphyrin trichloride



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Compound 15 (200 mg, 0.17 mmol) is dissolved in absolute DMF (40 mL) with triethylamine (5 mL, 34.5 mmol, 208 eq.). The mixture is heated to 50°C for 48 h. After removal of DMF under vacuum, the residue obtained is dissolved in methanol and purified by column chromatography using silica gel (Merck, 60) eluting with methanol:water:acetic acid (2:1:3, by vol.) and then acetic acid:pyridine (1:1, by vol.). Removal of solvent from appropriate fractions under vacuum affords raw product which is dissolved in methanol:aqueous NaCl (1M) (5 mL, 1:1, by vol.). The mixture is stirred for 30 mins and filtered through a pad of silica gel (depth 2 cm) supported on a steel frit (diameter 3.5 cm). After washing the pad with methanol (200 mL) it is eluted with methanol:water:acetic acid (2:1:3, by vol.). After evaporation of solvent from appropriate combined fractions, the residue obtained is dissolved in methanol (2mL) and dichloromethane (5 mL) is added dropwise. The precipitated white gel is collected by filtration and the solvent is removed under high vacuum.

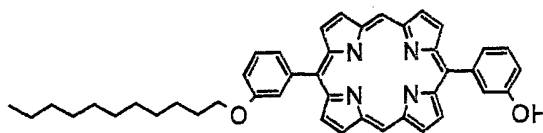
20

<sup>1</sup>H-NMR:

$\delta_H$  (300MHz, CD<sub>3</sub>OD): 0.90 (t, <sup>3</sup>J 7.5 Hz, 3 H), 1.20-1.45 (m, 43H), 1.45-1.65 (bs, 2 H), 2.25-2.40 (bs, 6 H), 3.35-3.45 (bs, 24 H), 3.50-3.60 (bs, , 6 H), 4.25 (t, <sup>3</sup>J 7.5 Hz, 2 H), 4.40-4.45 (bs, 6 H), 7.25-7.40, 8.10-8.20 (m, 16 H), 8.80-9.10 (bs, 8 H).

#### COMPOUND 17

5-[4-(3-Hydroxy-phenyl)]-15-(3-undecyloxy-phenyl)-porphyrin



10

5-15-bis-(3-Hydroxy-phenyl)-porphyrin (Wiehe, A., Simonenko, E. J., Senge, M. O. and Roeder, B. *Journal of Porphyrins and Phthalocyanines* **5**, 758-761 (2001)) (86 mg, 0.17 mmol) is dissolved and K<sub>2</sub>CO<sub>3</sub> (250 mg, 7.1 mmol) is suspended in DMF (40 mL). To the vigorously-stirred mixture a solution of 1-bromoundecane (0.04 mL, 0.17 mmol) in DMF (5 mL) is added dropwise at 50 °C during 30 mins and the mixture is heated at that temperature for 1 h. After removal by filtration of K<sub>2</sub>CO<sub>3</sub>, DMF is removed under high vacuum. The residue obtained is purified by column chromatography using silica gel (Merck 60) eluting with n-hexane:ethyl acetate (10:1, by vol.). The 2nd fraction is collected and dried under high vacuum to give the product.

<sup>1</sup>H-NMR:

δ<sub>H</sub> (300Mz, CDCl<sub>3</sub>): -3.15 (2 H, s), 0.75 (t, <sup>3</sup>J 7.5 Hz, 3 H), 1.10-1.30 (m, 14 H), 1.35 (m, 2 H), 1.80 (quint, <sup>3</sup>J 7.5 Hz, 2 H), 4.05 (t, <sup>3</sup>J 7.5 Hz, 2 H), 6.85-6.90, 7.20-7.25, 7.35-7.45, 7.50-7.65, 7.75-7.80 (5 x m, 8 H), 8.85, 8.95, 9.10, 9.20 (4 x d, <sup>3</sup>J 4.9 Hz, 4 x 2 H), 10.15 (s, 2 H).

#### COMPOUND 18

5,10,15-tris-(3-Hydroxy-phenyl)-20-(3-dodecyloxy-phenyl)-porphyrin

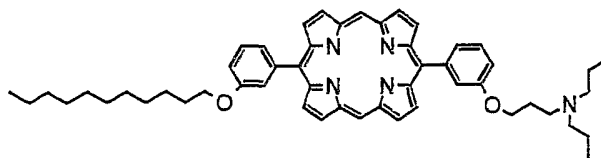
3-Hydroxybenzaldehyde (1.8 g, 14.8 mmol, 3 eqv.) and 3-dodecyloxybenzaldehyde (1.35 g, 4.9 mmol, 1 eqv.) are dissolved in a mixture of acetic acid (145 mL) and nitrobenzene (98 mL, 960 mmol) and heated to 120°C. Pyrrole (1.35 mL, 19.6 mmol, 4 eqv.) is added in one portion and the mixture is stirred at 120°C for 1h. After cooling to room temperature, solvents are removed *in vacuo* at 50°C. The product is isolated by chromatography on a column (500 g) of silica using toluene as eluent. The desired product is obtained as the fifth fraction from the column and is re-chromatographed using a smaller (200 g) silica coulumn eluted with toluene. The product is obtained as a violet solid after evaporation of the solvent.

<sup>1</sup>H-NMR:

δ<sub>H</sub> (300 MHz, CDCl<sub>3</sub>): 0.64 (t, 3 H, <sup>3</sup>J 6.8 Hz), 0.94-1.15 (m, 16 H), 1.25 (bs, 2 H), 1.62 (bs, 2 H), 3.90 (bs, 2 H), 6.33-6.95 (m, 8 H), 7.08-7.60 (m, 8 H), 8.20-8.47 (m, 4 H), 8.51-8.70 (m, 4 H)

COMPOUND 19

5- $\{3$ -[bis-(2-Diethylamino-ethyl)-aminopropoxy]-phenyl}-15-(3-undecyloxy-phenyl)-porphyrin



5

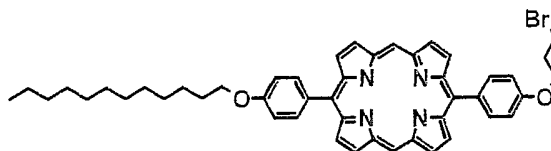
Compound 17 (50 mg, 0.065 mmol) is dissolved with N,N,N',N'-tetraethyldiethylenetriamine (1mL, 39 mmol) in THF(10 mL) and the mixture is stirred at room temperature for 4 days. After evaporation of the solvent, the residue is dissolved in diethyl ether (20mL) and the solution is washed with water (5 x 30 mL). The organic phase is dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under high vacuum. The mixture is purified by column chromatography (silica gel, Merck 60) eluting with n-hexane:ethyl acetate (5:1, by vol.) followed by n-hexane:ethyl acetate:triethyl amine (10:10:1, by vol.). After collection of appropriate fractions and removal of solvent under reduced pressure, pure product is obtained by treatment of the residue with diethyl ether:methanol.

<sup>1</sup>H-NMR:  
 $\delta_H$  (300Mz, CDCl<sub>3</sub>): 0.80 (t, <sup>3</sup>J 7.5 Hz, 3 H), 0.9 (t, <sup>3</sup>J 7.5 Hz, 12 H), 1.20-1.40 (m, 14 H), 1.45 (quint, <sup>3</sup>J 7.5 Hz, 2 H), 1.80 (quint, <sup>3</sup>J 7.5 Hz, 2 H), 1.95 (quint, <sup>3</sup>J 7.5 Hz, 2 H), 2.40-2.60 (m, 16 H), 2.65 (t, <sup>3</sup>J 7.5 Hz, 2 H), 4.10 (t, <sup>3</sup>J 7.5 Hz, 2 H), 4.20 (t, <sup>3</sup>J 7.5 Hz, 2 H), 7.30-7.40, 7.55-7.65, 7.75-7.80 (3 x m, 8 H), 9.10-9.15, 9.20-9.25 (2 x m, 2 x 4 H), 10.15 (s, 2 H).

25

COMPOUND 20

5-[4-(3-Bromo-propyloxy)-phenyl]-15-(4-dodecyloxy-phenyl)-porphyrin



5

To a stirred solution of dipyrrolemethane (0.31 g, 2.1 mmol), 4-(3-bromo-propyloxy)-benzaldehyde (0.27 g, 1.1 mmol) and 4-dodecyloxy-benzaldehyde (0.32 g, 1.1 mmol) in degassed dichloromethane (500 mL). TFA (0.035 mL, 1.5 mmol) is added dropwise. The solution is stirred at  
 10 room temperature in the dark for 17 h under argon. After addition of DDQ (1.38 g, 6 mmol), the mixture is stirred at room temperature for a further hour. Purification by column chromatography using silica gel (Merck 60, 400 g) with toluene as eluent affords the product (2<sup>nd</sup> fraction) together with Compound 7 (3<sup>rd</sup> fraction).

15

<sup>1</sup>H-NMR:

$\delta_H$  (300MHz, CDCl<sub>3</sub>): -3.15 (2 H, s), 0.90 (t, <sup>3</sup>J 7.5 Hz, 3 H), 1.20-1.40 (m, 16 H), 1.55 (quint, <sup>3</sup>J 7.5 Hz, 2 H), 1.90 (quint, <sup>3</sup>J 7.5 Hz, 2 H), 2.40 (quint, <sup>3</sup>J 7.5 Hz, 2H), 3.75 (t, <sup>3</sup>J 7.5 Hz, 2 H), 4.20 (t, <sup>3</sup>J 7.5 Hz, 2 H),  
 20 4.35 (t, <sup>3</sup>J 7.5 Hz, 2 H), 7.20-7.30, 8.10-8.15 (2 x m, 8 H), 9.10-9.15, 9.25-9.30 (2 x m, 2 x 4 H), 10.20 (s, 2 H).

COMPOUND 21

5,10,15,20-tetrakis-(3-Hydroxy-phenyl)-porphyrin

25

3-Hydroxybenzaldehyde (0.910 g, 7.45 mmol) is dissolved in propionic acid (50 mL) and heated to 140°C. Pyrrole (0.52 mL, 7.45 mmol) is added in one portion and the mixture heated at reflux for 2h. Stirring is

continued for an additional 12 h at room temperature. Propionic acid is removed *in vacuo* and the residue dissolved in acetone and purified by chromatography on a column (250 g) of silica which is eluted with toluene containing a continuously increasing proportion of ethyl acetate.

5 The product is eluted with toluene:ethyl acetate (6:1 by vol.). Solvent is removed *in vacuo* to afford the product as a violet solid.

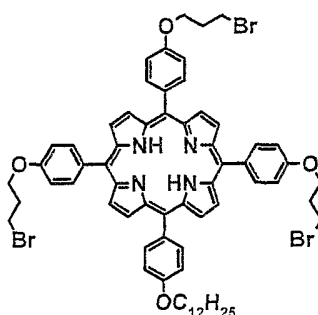
$^1\text{H-NMR}$ :

$\delta_{\text{H}}$  (300 MHz, d6-acetone): 7.18 (d, 4H,  $^3J = 8.25$  Hz), 7.49 (t, 4H,  $^3J =$   
10 8.25 Hz), 7.56-7.62 (m, 8H), 8.81 (m, 8 H)

#### COMPOUND 22

5,10,15-tris-[4-(3-Bromo-propyloxy)-phenyl]-20-(4-dodecyloxy-phenyl)-  
porphyrin

15



To a stirred solution of pyrrole (0.7 ml, 10 mmol), 4-(3-bromopropoxy)-benzaldehyde (1.8 g, 7.5 mmol) and 4-(n-dodecyloxy)-benzaldehyde  
20 (0.725 g, 2.5 mmol) in degassed dichloromethane (1 L) is added TFA (0.085 ml, 10 mmol) dropwise. The reaction solution is stirred under argon at room temperature in the dark for 17 h. After addition of DDQ (6.9 g, 30 mmol), the reaction mixture is stirred at room temperature for a further 1h. The solvents are removed under reduced pressure and the  
25 residue re-dissolved in toluene. Chromatographic purification on a

column (3.5 x 30 cm ) of silica gel (Merck 60) using toluene:n-hexane (1:4 by vol.) as eluent gives crude product which is purified by treatment with methanol:dichloromethane, giving a violet solid.

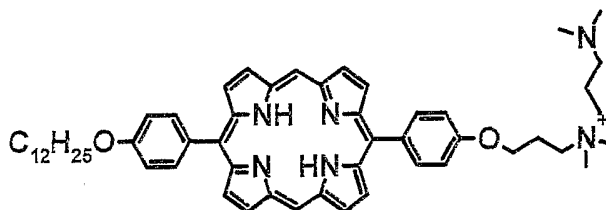
5 <sup>1</sup>H-NMR:

$\delta_H$  (300MHz, CDCl<sub>3</sub>): 0.90 (t, <sup>3</sup>J 7.5 Hz, 3 H), 1.20-1.45 (m, 16 H), 1.60 (quint, <sup>3</sup>J 7.5 Hz, 2 H), 1.90 (quint, <sup>3</sup>J 7.5 Hz, 2 H), 2.50 (quint, <sup>3</sup>J 7.4 Hz, 6 H), 3.75 (t, <sup>3</sup>J 7.4 Hz, 6 H), 4.20 (t, <sup>3</sup>J 7.5 Hz, 2 H), 4.35 (t, <sup>3</sup>J 7.5 Hz, 6 H), 7.25-7.30 (m, 8 H), 8.15-8.30 (m, 8 H), 8.80-8.85 (m, 8 H).

10

### COMPOUND 23

5-{4-[3-Dimethyl-(3-dimethylaminopropyl)-ammonio-propyloxy]phenyl}-15-(4-dodecyloxy-phenyl)-porphyrin chloride



15

Compound 20 (30 mg, 0.038 mmol) is dissolved with N,N,N',N'-tetramethyl-1,3-propanediamine (156 mg, 1.2 mmol) in THF:DMF(1:1 by vol., 20 mL) and stirred at 50 °C for 18 h. After evaporation of the solvent under reduced pressure, the residue is dissolved in dichloromethane and purified by column chromatography (silica gel Merck 60) eluting with acetic acid:methanol:water (3:2:1, by vol.). After combining appropriate fractions and removal of solvent under reduced pressure, the residue is treatment with dichloromethane:hexane to afford the product as a violet solid.

20

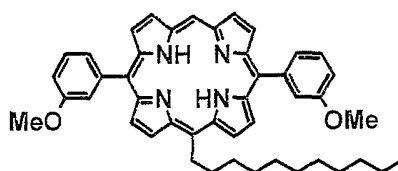
25

<sup>1</sup>H-NMR:

$\delta_H$  (300Mz, CDCl<sub>3</sub>+1 % acetic acid ): 0.85 (m, 3 H), 1.20-1.40 (m, 18 H),  
1.55-1.60 (m, 2 H), 1.60-1.65 (m, 4H), 2.10-2.20 (bs, 8 H), 3.15-3.25 (m,  
8 H), 3.75 (bs, 2 H), 4.20 (bs, 2 H), 4.35 (bs, 2 H), 7.15-7.20, 8.10-8.15 (2  
5 x m, 8 H), 8.95-9.00, 9.10-9.15, 9.25-9.30 (3 x bs, 8 H), 10.20 (s, 2H).

#### COMPOUND 24

5,15-bis-(3-Methoxy-phenyl)-10-undecyl-porphyrin



10

Into a 50 mL flask containing lithium (500 mg, 71 mmol) is added freshly distilled diethyl ether (15 mL) under an argon atmosphere. The suspension is refluxed for 1 hour, cooled to 15 °C and treated with a  
15 solution of n-undecylbromide (6.58 g, 71 mmol) in ether (6 mL) added dropwise via syringe. The mixture is cooled to 7-10 °C and, after 5 min, when the suspension becomes slightly cloudy and bright spots appear on the lithium metal, the remainder of the n-undecylbromide solution is added at an even rate over a period of 30 min while the internal  
20 temperature is maintained at below 10 °C. Upon completion of addition, the mixture is stirred further for 1 h at 10 °C. The suspension is filtered under argon to remove excess lithium and lithium bromide.

5,15-bis-(3-Methoxy-phenyl)-porphyrin (100 mg, 0.19 mmol) is  
25 dissolved in anhydrous THF (30 mL) at -50 °C under an argon atmosphere. The organolithium reagent described above (5 mL) is added dropwise to the mixture. After 5 min the cooling bath is removed and the mixture is warmed to room temperature. After stirring at room

temperature for 15 min the reaction is quenched by slow addition of water (2 mL). After 15 min the mixture is oxidized by the addition of DDQ (4 mL, 0.4 mmol, 0.1 M in THF) and stirred for a further 15 min. The mixture is filtered through alumina (neutral, Brockman grade +) and  
 5 purified by column chromatography on silica gel eluting with hexane:dichloromethane (4:1 by vol.). The first fraction is collected and treated with methanol:dichloromethane to give a solid product.

<sup>1</sup>H-NMR:

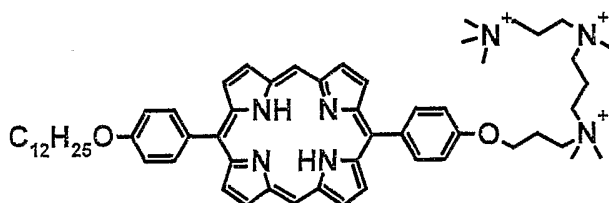
10  $\delta_H$  (300Mz, CDCl<sub>3</sub>): -3.05 (bs, 2 H, s), 0.80 (t, <sup>3</sup>J 7.5 Hz, 3 H), 1.10-1.20 (m, 12 H), 1.25 (m, 2 H), 1.70 (quint, <sup>3</sup>J 7.5 Hz, 2 H), 2.40 (quint, <sup>3</sup>J 7.5 Hz, 2 H), 3.85 (s, 6H), 4.95 (t, <sup>3</sup>J 7.5 Hz, 2 H), 7.20-7.23, 7.50-7.60, 7.65-7.75 (3x m, 8 H), 8.85-8.90, 9.10-9.15, 9.35-9.40 (3 x m, 8 H), 9.95 (s, 1H).

15

#### COMPOUND 25

3-[(3-[(3-{4-[15-(4-Dodecyloxy-phenyl)-porphyrin-5-yl]-phenoxy}-propyl)-dimethyl-ammonio]-propyl]-dimethyl-ammonio)-propyl]-trimethyl-ammonium trichloride

20

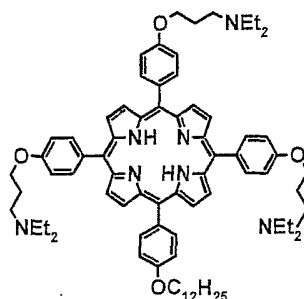


Compound 23 (20 mg, 0.022 mmol) and (1-bromopropyl)-trimethyl-ammonium bromide (26 mg, 0.1 mmol) are dissolved in DMF(15 ml) and  
 25 stirred overnight at 50°C. After evaporation of the solvent under reduced pressure, the residue is dissolved in methanol (5 ml) and applied to a pad (3 cm deep) of silica gel which is washed with methanol (500 ml)

followed by acetic acid:methanol:water (3:2:1 by vol.). After evaporation of the solvent the residue is purified by column chromatography (silica gel Merck 60) using at first acetic acid:methanol:water (3:2:1 by vol.) and then pyridine:acetic acid (1:1 by vol.). The second fraction eluted is  
5 collected and dried under vacuum. The residue is dissolved in methanol (2 ml) and purified by chromatography on a column (2.5 x 40 cm) of Sephadex LH-20 which is eluted with n-butanol:acetic acid:water (5:1:4 by vol., upper phase). After removal of solvent under reduced pressure, the residue is dried under vacuum at 80 °C. NMR spectroscopy indicates  
10 the product is contaminated with a small proportion of elimination products.

#### COMPOUND 26

5,10,15-tris-[4-(3-Diethylamino-propyloxy)-phenyl]-20-(4-dodecyloxy-  
15 phenyl)-porphyrin



Compound 22 (50 mg, 0.06 mmol) and freshly distilled diethylamine (5  
20 ml) are dissolved in absolute DMF (30 ml) under argon. The reaction mixture is stirred at room temperature for 20 h and poured into ethyl acetate (50 ml). The mixture is washed with water (4 x 50 ml) and, after drying the combined organic phases (Na<sub>2</sub>SO<sub>4</sub>), evaporation of solvent affords a residue which is purified by chromatography on a column (2.5 x  
25 30 cm) of silica (Merck 60) which is eluted with ethyl acetate:n-hexane:triethyl amine (10: 10: 1, by vol.). Fractions are combined as

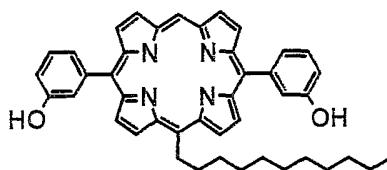
appropriate, the solvent evaporated under reduced pressure and the residue dried under high vacuum. Treatment with dichloromethane:n-hexane affords pure product.

5  $^1\text{H-NMR}$ :

$\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ): 0.85 (t,  $^3J$  7.5 Hz, 3 H), 1.05 (m, 18 H), 1.20-1.45 (m, 18 H), 1.55 (quint,  $^3J$  7.5 Hz, 2 H), 2.15 (quint,  $^3J$  7.5 Hz, 6 H), 2.75 (quint,  $^3J$  7.4 Hz, 6 H), 3.15-3.25 (m, 12 H), 4.15 (t,  $^3J$  7.5 Hz, 2 H), 4.25 (t,  $^3J$  7.5 Hz, 6 H), 7.15-7.20 (m, 8 H), 8.00-8.05 (m, 8 H), 7.95-8.05 (m, 8 H).

#### COMPOUND 27

5,15-bis-(3-Hydroxy-phenyl)-10-undecyl-porphyrin



15

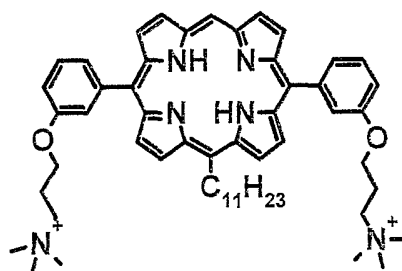
To a solution of Compound 24 (95 mg, 0.14 mmol) in anhydrous dichloromethane (80 mL) under an argon atmosphere  $\text{BBr}_3$ , (6 mL, 1M in dichloromethane) is added dropwise at  $-70^\circ\text{C}$  and the mixture is stirred for 1 h. The mixture is warmed to room temperature and stirred overnight then cooled to  $-10^\circ\text{C}$  and hydrolysed by addition of 2 mL water during 1 h.  $\text{NaHCO}_3$  (3 g) is added directly to neutralisation. The mixture is stirred for a further 12 h. After removal of  $\text{NaHCO}_3$  by filtration and of dichloromethane under vacuum, the residue obtained is purified by column chromatography using silica gel eluting with dichloromethane. After removal of solvent from appropriate combined fractions and drying under high vacuum the product is obtained as a violet solid.

$^1\text{H}$ -NMR:

$\delta_{\text{H}}$  (300Mz,  $\text{CDCl}_3$ ): -3.05 (bs, 2 H, s), 0.85 (t,  $^3J$  7.5 Hz, 3 H), 1.20-1.40 (m, 12 H), 1.50 (m, 2 H), 1.80 (quint,  $^3J$  7.5 Hz, 2 H), 2.55 (quint,  $^3J$  7.5 Hz, 2 H), 5.00 (t,  $^3J$  7.5 Hz, 2 H), 7.15-7.25, 7.50-7.60, 7.80-7.90 (3x m, 8 H), 8.95-9.00, 9.20-9.25, 9.50-9.60 (3 x m, 8 H), 10.15 (s, 1H).

#### COMPOUND 28

5,15-bis-[3-(3-Trimethylammonio-propyloxy)-phenyl]-10-undecyl-  
 10 porphyrin dichloride



To a solution of Compound 27 (50 mg, 0.08 mmol) in DMF (20 mL)  
 15 under an argon atmosphere  $\text{K}_2\text{CO}_3$  (100 mg, 0.72 mmol) and (3-bromopropyl)-trimethylammonium bromide (300 mg, 1.2 mmol) are added and the mixture is stirred at  $50^\circ\text{C}$  for 18 h. After removal of solvent under high vacuum the residue obtained is dissolved in methanol (5 mL) and filtered through a pad of silica gel (depth 2 cm) supported on a steel frit (diameter 3.5 cm). After washing the pad with methanol (500 mL) it is eluted with acetic acid:methanol:water (3:2:1, v:v). After drying of appropriate combined fractions under high vacuum the residue is dissolved in methanol and purified by column chromatography on Sephadex LH-20 eluting with n-butanol:acetic acid:water (5:1:4, by vol.,  
 25 upper phase). After evaporation of solvent the residue obtained from the first fraction eluted is dissolved in methanol and passed through a short

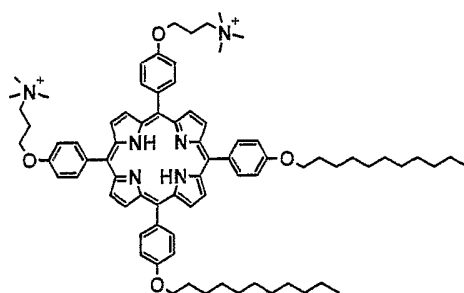
column of anion exchange resin (Amberlite IRA 400, chloride form) to give, after evaporation of solvent, the pure product.

<sup>1</sup>H-NMR:

5  $\delta_H$  (300Mz, CD<sub>3</sub>OD): 0.85 (t, <sup>3</sup>J 7.5 Hz, 3 H), 1.20-1.40 (m, 12 H), 1.50 (m, 2 H), 1.80 (m, 2 H), 2.40 (bs, 4 H), 2.55 (m, 2 H), 3.20 (bs, 18 H), 3.65 (bs, 4 H), 4.35 (bs, 4 H), 5.10 (m, 2 H), 7.50-7.55, 7.70-7.85 (2 x m, 8 H), 8.95-9.00, 9.25-9.24, 9.50-9.70 (3 x bs, 8 H), 10.15 (bs, 1H).

# 10 COMPOUND 29

5,10-bis-[4-(3-Trimethylammonio-propyloxy)-phenyl]-15,20-bis-(4-undecyloxy-phenyl)-porphyrin dichloride



15

Compound 14 (50 mg, 0.05 mmol) is dissolved and K<sub>2</sub>CO<sub>3</sub> (150 mg, 1.1 mmol) is suspended in DMF (30 mL). To the vigorously-stirred mixture a solution of (1-bromopropyl)-trimethylammonium bromide (0.3 g, 16.6 mmol) in DMF (10 mL) is added dropwise at 50°C and the mixture is heated for 18 h. After removal of DMF under high vacuum, the residue obtained is dissolved in methanol (5 mL) and filtered through a pad of silica gel (depth 2 cm) supported on a steel frit (diameter 3.5 cm). After washing the pad with methanol (ca. 500 mL) it is eluted with acetic acid:methanol:water (3:2:1, by vol.). After evaporation of solvent from appropriate combined fractions the residue obtained is purified by chromatography on a column (2.5 x 40 cm) of Sephadex LH-20 eluting

25

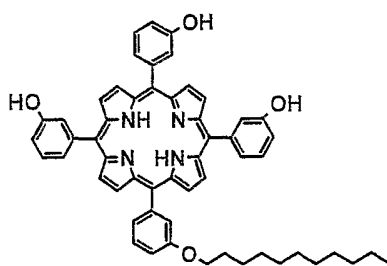
with n-butanol:water:acetic acid (5:4:1, by vol., upper phase) for further separation from the excess ammonium salt and other by-products. After removal of solvent under reduced pressure the residue obtained is dissolved in methanol and passed through a short column (3.5 x 20 cm) of anion exchange resin (Amberlite IRA 400, chloride form). After evaporation of solvent under reduced pressure, the product is dried under high vacuum.

<sup>1</sup>H-NMR:

$\delta_H$  (300MHz, CD<sub>3</sub>OD): 0.80 (t, <sup>3</sup>J 7.5 Hz, 6 H), 1.15-1.35 (m, 28 H), 1.35-1.45 (bs, 4 H), 1.70-1.80 (bs, 4 H), 2.30-2.40 (bs, 4 H), 3.15-3.30 (bs, 18 H), 3.65-3.75 (bs, 4 H), 4.00-4.05 (m, 4 H), 4.30-4.40 (bs, 4 H), 7.00-7.15, 7.20-7.30, 7.80-95, 7.95-8.15 (4 x m, 4 x 4 H), 8.60-9.00 (bs, 8 H).

#### COMPOUND 30

5,10,15-tris-(3-Hydroxy-phenyl)-20-(3-undecyloxy-phenyl)-porphyrin



20

Pyrrole (1.31 g, 19.6 mmol) is added in one portion to a mixture of 3-hydroxybenzaldehyde (1.8 g, 14.8 mmol) and 3-undecyloxybenzaldehyde (1.36 g, 4.9 mmol) in acetic acid (145 mL) and nitrobenzene (118 g, 960 mmol) preheated to 130°C and the mixture is stirred for 1 hour at 120°C. The mixture is cooled and solvent removed under high vacuum. The residue is dissolved in dichloromethane (5 mL) and purified by column

chromatography using silica gel (Merck 60) eluting with hexane:toluene (4:1, by vol.). The product is obtained after removal of solvent from the eluate under reduced pressure and drying the obtained residue under vacuum.

5

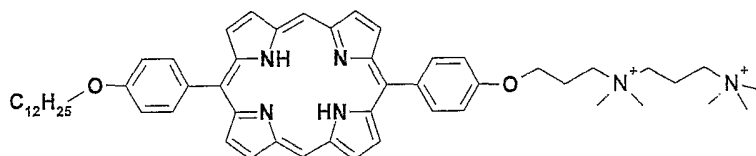
$^1\text{H-NMR}$ :

$\delta_{\text{H}}$  (300Mz,  $\text{CDCl}_3$ ): 0.75-0.80 (m, 3 H), 1.05-1.35 (m, 14 H), 1.40-1.50 (m, 2 H), 1.75-1.85 (m, 2 H), 3.90-4.10 (m, 2 H), 6.90- 7.70 (m, 16 H), 8.45-8.80 (m, 8 H).

10

### COMPOUND 31

5-{4-[3-Dimethyl-(3-trimethylammonio-propyl)-ammonio-propyloxy]-phenyl}-15-(4-dodecyloxy-phenyl)-porphyrin dichloride



15

Compound 23 (50 mg, 0.055 mmol) is dissolved with methyl iodide (5 mL, 80 mmol) in absolute DMF(30 mL) and the mixture is stirred at 40°C for 3h. After evaporation of solvent the residue obtained is dissolved in methanol (5 mL) and filtered through a pad of silica gel (depth 2 cm) supported on a steel frit (diameter 3.5 cm). After washing the pad with methanol (ca. 1 L) it is eluted with dichloromethane:methanol (2:3 by vol., 500 mL) and then acetic acid:water:methanol (3:1:2, by vol.). After removal of solvent from appropriate pooled fractions the residue obtained is dissolved in acetic acid and purified by column chromatography on Sephadex LH-20 eluting with acetic acid. After evaporation of solvent from appropriate pooled fractions and drying the residue obtained under high vacuum, the residue is dissolved in methanol and passed through a

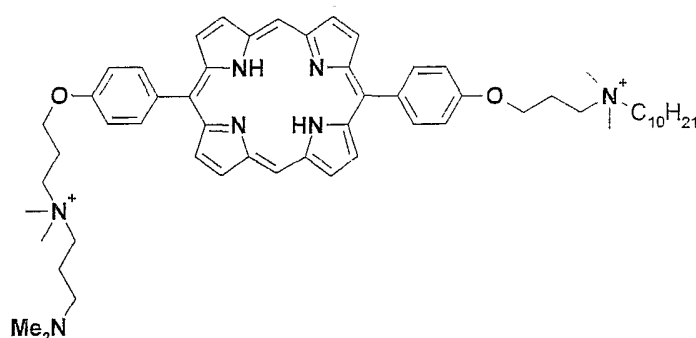
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small column (3.5 x 20 cm) of anion exchange resin (Amberlite IRA 400, chloride form). After evaporation of solvent from the eluate, the product is dried under high vacuum.

# 5 COMPOUND 32

5-[4-(3-Dimethyldecyl-ammoniopropoxy)-phenyl]-15-{4-[3-dimethyl-(3-dimethylaminopropyl)-ammoniopropoxy]-phenyl}-porphyrin dichloride



10

Compound 23 (50 mg, 0.068 mmol) is dissolved with N,N,N',N'-tetramethyl-1,3-propanediamine (354 mg, 1.36 mmol) and N,N-dimethyldecylamine (1 g, 2.72 mmol) in DMF:THF(30 mL, 1:1, by vol.) and the mixture is stirred at 50°C overnight. After evaporation of the solvent under reduced pressure the residue obtained is dissolved in methanol (10 mL) and filtered through a pad of silica gel (depth 2 cm) supported on a steel frit (diameter 3.5 cm). After washing the pad with methanol (ca. 500 mL) it is eluted with acetic acid:methanol:water (3:2:1, by vol.). The first two fractions eluted are combined and after evaporation of the solvent under reduced pressure the residue obtained is dissolved in methanol and purified by chromatography on a column (2.5 x 40 cm) of Sephadex LH-20 eluting with n-butanol:water:acetic acid (4:5:1, by vol.). After removal of solvent under reduced pressure from the second fraction eluted, the residue is dissolved in methanol (5 mL)

25

and passed through a short column (3.5 x 20 cm) of anion exchange resin (Amberlite IRA 400, chloride form). The eluate is evaporated to dryness and the residue obtained is dried under high vacuum to afford the product.

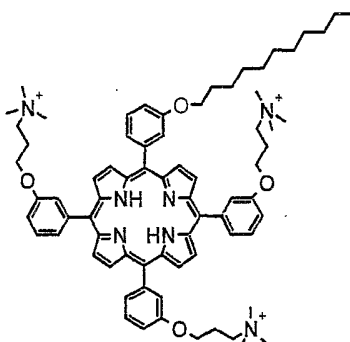
5  $^1\text{H}$ -NMR:

$\delta_{\text{H}}$  (300MHz,  $\text{CD}_3\text{OD}$ ): 0.80 (m, 3 H), 1.05-1.25 (m, 10 H), 1.25-1.40 (bs, 2 H), 1.80-1.90 (bs, 4 H), 2.15-2.30 (bs, 2 H), 2.80-3.60 (m, 20 H), 3.80-3.95 (bs, 4 H), 7.05-7.15, 7.85-8.00 (2 x m, 2 x 4 H), 8.75-8.90, 9.20-9.35 (2 x bs, 2 x 4 H), 10.15 (bs, 2 H).

10

### COMPOUND 33

5,10,15-tris[3-(3-Trimethyl-ammoniopropoxy)-phenyl]-20-(3-undecyloxy-phenyl)-porphyrin trichloride



15

Compound 30 (100 mg, 0.12 mmol) is dissolved and  $\text{K}_2\text{CO}_3$  (230 mg, 1.7 mmol) is suspended in DMF (30 mL). To the vigorously-stirred mixture a solution of (1-bromopropyl)-trimethylammonium bromide (0.3 g, 16.6 mmol) in DMF (10 mL) is added dropwise at  $50^\circ\text{C}$  during 30 mins and the mixture is heated for 18 h. After removal of DMF under reduced pressure, the residue obtained is dissolved in methanol (5 mL) and filtered through a pad of silica gel (depth 2 cm) supported on a steel frit (diameter 3.5 cm). After washing the pad with methanol (ca. 500 mL) it is eluted with acetic acid:methanol:water (3:2:1, by vol.). After

20

25

evaporation of solvent from appropriate combined fractions under reduced pressure. the residue is purified by chromatography on a column (2.5 x 40 cm) of Sephadex LH-20 eluting with n-butanol:water:acetic acid (5:4:1, by vol., upper phase). After removal of solvent under reduced pressure from the eluate, the residue obtained is dissolved in methanol and the solution is passed through a short column (3.5 x 20 cm) of anion exchange resin (Amberlite IRA 400, chloride form). Evaporation of solvent from the eluate gives the product which is dried under high vacuum.

10

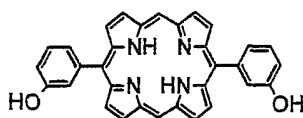
<sup>1</sup>H-NMR:

$\delta_H$  (300MHz, CD<sub>3</sub>OD): 0.75-0.80 (m, 3 H), 1.00-1.40 (m, 18 H), 1.60-1.80 (bs, 2 H), 2.25-2.40 (bs, 6 H), 3.29 (bs, 27 H), 3.40-3.60 (m, 6 H), 3.90-4.00 (m, 2 H), 4.05-4.25 (m, 6 H), 7.10-7.20, 7.25-7.40, 7.60-7.80, 7.80-7.90 (4 x m, 16H), 8.70-9.00 (bs, 8 H).

15

#### COMPOUND 34

5,15-bis-(3-Hydroxy-phenyl)-porphyrin



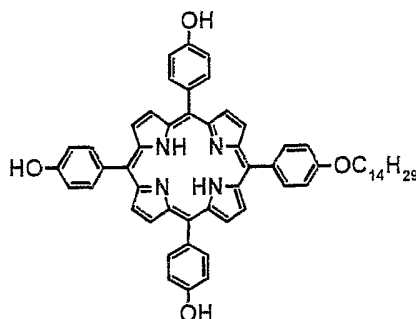
20

This is prepared as described by Wiehe, A., Simonenko, E. J., Senge, M. O. and Roeder, B. *Journal of Porphyrins and Phthalocyanines* **5**, 758-761 (2001).

25

COMPOUND 35

5,10,15-tris-(4-Hydroxy-phenyl)-20-(4-tetradecyloxy-phenyl)-porphyrin



5

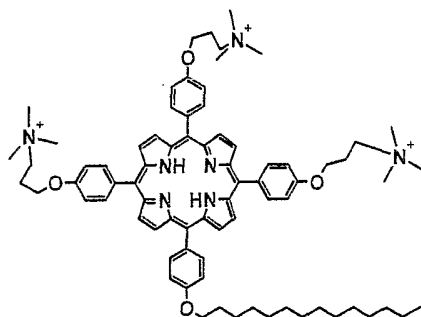
5,10,15,20-tetrakis-(4-Hydroxy-phenyl)-porphyrin (170 mg, 0.25 mmol) is dissolved and  $K_2CO_3$  (0.65 g, mmol) is suspended in DMF (30 mL). To the vigorously stirred reaction mixture a solution of 1-bromotetradecane (0.1 mL, 0.45 mmol) in DMF (10 mL) is added dropwise at 50°C during 30 mins and the mixture is heated for 1.5h. After evaporation of solvent, the residue is dissolved in toluene:ethanol (1:1 by vol., ca. 5 mL) and purified by chromatography using a column (5 x 25 cm) of silica gel (Merck 60) which is washed with toluene. After the elution of the first 3 fractions, elution is continued using toluene:ethyl acetate (2:1 by vol.). The fifth compound eluted is collected, the solvent evaporated and the residue dried under high vacuum to afford product as a violet solid.

$^1H$ -NMR:  
 $\delta_H$  (300MHz,  $d_6$ -acetone): 0.85 (t,  $^3J$  7.5 Hz, 3 H), 1.15-1.55 (m, 20 H), 1.45 (quint,  $^3J$  7.5 Hz, 2 H), 1.75 (quint,  $^3J$  7.5 Hz, 2 H), 4.10 (t,  $^3J$  7.5 Hz, 2 H), 7.20 (d,  $^3J$  8.5 Hz, 2 H), 7.25 (d,  $^3J$  8.5 Hz, 6 H), 8.00-8.15 (m, 8 H), 8.80-9.10 (m, 8 H).

25

COMPOUND 36

5,10,15-tris-[4-(3-Trimethyl-ammoniopropoxy)-phenyl]-20-(4-tetradecyloxy-phenyl)-porphyrin trichloride



5

The n-tetradecyloxy-analogue of Compound 2, prepared similarly as described above for Compound 2 but using 1-bromotetradecane in place of 1-bromoundecane, (50 mg, 0.057 mmol) and (1-bromopropyl)-trimethylammonium bromide (210 mg, 0.8 mmol) are dissolved and

10  $K_2CO_3$  (230 mg, 1.7 mmol) is suspended in DMF (20 mL). The vigorously stirred mixture is stirred at this temperature for 18 h. After removal of DMF under reduced pressure the residue obtained is dissolved in methanol (5 mL) and filtered through a pad of silica gel (depth 2 cm)

15 supported on a steel frit (diameter 3.5 cm). After washing the pad with methanol (ca. 500 mL) it is eluted with acetic acid:methanol:water (3:2:1, by vol.). After evaporation of the solvent from appropriately combined fractions, the residue obtained is purified by chromatography on a column (2.5 x 40 cm) of Sephadex LH-20 eluting with n-butanol:water:acetic acid

20 (4:5:1, by vol., upper phase) for separation from the excess of ammonium salt and other contaminating materials. After elution and removal of the solvent from appropriate fractions, the residue obtained is dissolved in methanol (5 mL) and passed through a short column (3.5 x 20 cm) of anion exchange resin (Amberlite IRA 400, chloride form). Solvent is

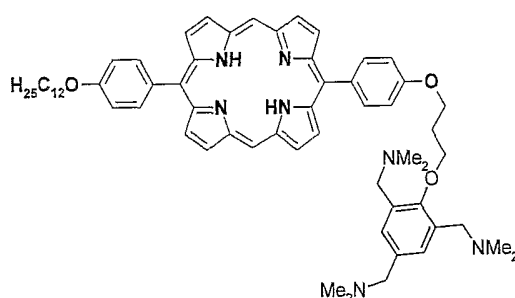
removed under reduced pressure and the residue obtained is dried under high vacuum to afford the product as a violet solid.

<sup>1</sup>H-NMR:

- 5  $\delta_H$  (300MHz, CD<sub>3</sub>OD): 0.75 (t, <sup>3</sup>J 7.5 Hz, 3 H), 0.95-1.25 (m, 22 H), 1.50-1.65 (bs, 2 H), 2.20-2.40 (bs, 6 H), 3.05-3.15 (bs, 27 H), 3.45-3.60 (bs, 6 H), 3.60-3.80 (bs, 2 H), 4.05-4.25 (bs, 6 H), 6.80-7.25, 7.65-8.05, (2 x m, 16 H), 8.45-8.95 (bs, 8 H).

10 COMPOUND 37

5-(4-{3-[2,4,6-tris-(Dimethylaminomethyl)-phenyloxy]-propyloxy}-phenyl)-15-(4-dodecyloxy-phenyl)-porphyrin



15

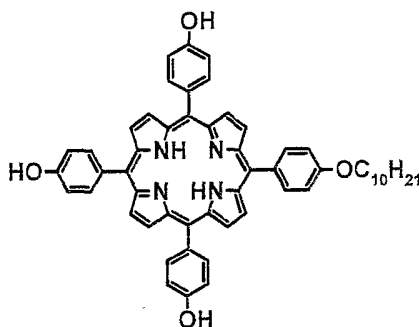
- Compound 20 (50 mg, 0.063 mmol) is dissolved in DMF (20 mL) in the presence of 2,4,6-tris-(dimethylaminomethyl)-phenol (1 mL, 3.7 mmol) and stirred at 50°C overnight. After evaporation of the solvent, the residue is solidified by treatment of the residue with dichloromethane:methanol to remove the excess of amine. After filtration, the porphyrins are re-dissolved in dichloromethane and purified by chromatography on a column of silica gel (Merck 60) which is washed with dichloromethane. Evaporation of solvent under reduced pressure and treatment of the residue with dichloromethane:methanol gives the product as a violet solid.
- 20
- 25

<sup>1</sup>H-NMR:

$\delta_H$  (300Mz, CDCl<sub>3</sub>): -3.15 (2 H, s), 0.85 (t, <sup>3</sup>J 4.5 Hz, 3 H), 1.20-1.40 (m, 18 H), 1.55 (quint, <sup>3</sup>J 4.5 Hz, 2 H), 1.90 (quint, <sup>3</sup>J 4.5 Hz, 2 H), 2.20 (s, 18 H), 2.55 (t, <sup>3</sup>J 5.2 Hz, 2 H), 3.45 (s, 6 H), 4.15 (t, <sup>3</sup>J 5.5 Hz, 2 H), 4.20  
 5 (t, <sup>3</sup>J 5.5 Hz, 2 H), 4.35 (t, <sup>3</sup>J 7.5 Hz, 2 H), 6.85 (2 x s, 2 H), 7.20-7.30, 8.10-8.15 (2 x m, 8 H), 9.00-9.05, 9.25-9.30 (2 x m, 2 x 4 H), 10.20 (s, 2 H).

### COMPOUND 38

10 5,10,15-tris-(4-Hydroxy-phenyl)-20-(4-decyloxy-phenyl)-porphyrin



5,10,15,20-tetrakis-(4-Hydroxy-phenyl)-porphyrin (100 mg, 0.15 mmol)  
 15 is dissolved and K<sub>2</sub>CO<sub>3</sub> (230 mg) is suspended in DMF (30 mL). To the vigorously stirred reaction mixture a solution of 1-bromodecane (0.016 mL, 0.11 mmol) in DMF (10 mL) is added dropwise at 70°C during 30 mins and the mixture is stirred for 1.5h. After evaporation of solvent, the residue is dissolved in toluene:ethanol (1:1 by vol., ca. 3 mL) and purified  
 20 by chromatography on a column (150 g) of silica gel (Merck 60) using toluene as eluent. After elution of the first 3 fractions, the column is eluted with toluene:ethyl acetate (2:1 by vol.) and the 5<sup>th</sup> fraction eluted is collected, the solvent removed and the residue dried under high vacuum to give the product as a violet solid.

25

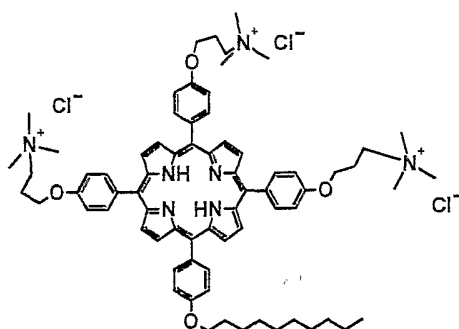
<sup>1</sup>H-NMR:

δ<sub>H</sub> (300Mz, d6-acetone): 0.95 (t, <sup>3</sup>J 7.5 Hz, 3 H), 1.25-1.55 (m, 12 H),  
 1.55 (quint, <sup>3</sup>J 7.5 Hz, 2 H), 1.85 (quint, <sup>3</sup>J 7.5 Hz, 2 H), 4.15 (t, <sup>3</sup>J 7.5  
 Hz, 2 H), 7.20 (d, <sup>3</sup>J 8.5 Hz, 2 H), 7.25 (d, <sup>3</sup>J 8.5 Hz, 6 H), 8.00-8.15 (m,  
 5 8 H), 8.80-9.10 (m, 8 H).

### COMPOUND 39

5,10,15-tris-[4-(3-Trimethylammonio-propyloxy)-phenyl]-20-(4-  
 decyloxy-phenyl)-porphyrin trichloride

10



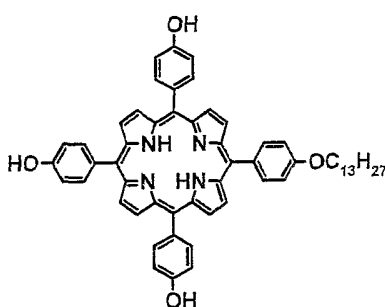
Compound 38 (50 mg, 0.061 mmol) and (1-bromopropyl)-  
 trimethylammonium bromide (210 mg, 0.8 mmol) are dissolved and  
 15 K<sub>2</sub>CO<sub>3</sub> (230 mg, 1.7 mmol) is suspended in DMF (20 mL). The  
 vigorously stirred reaction mixture is heated at 50°C for 18 h. After  
 evaporation of solvent, the raw product is dissolved in methanol and  
 purified by chromatography on a column (2.5 x 40 cm) of Sephadex,  
 eluting with n-butanol:water:acetic acid (4:5:1, by vol., upper phase).  
 20 After removal of the solvent, the residue is dissolved in methanol and  
 passed through a column (3.5 x 20 cm) of Amberlite IRA-400 (chloride  
 form). After evaporation of solvent, the product is dried under high  
 vacuum and yields a violet solid.

<sup>1</sup>H-NMR:

$\delta_H$  (300MHz, CD<sub>3</sub>OD): 0.90 (t, <sup>3</sup>J 7.5 Hz, 3 H), 1.20-1.40 (m, 12 H),  
1.45-1.60 (bs, 2 H), 1.80-1.90 (bs, 2 H), 2.45-2.55 (bs, 6 H), 3.25-3.35  
(bs, 27 H), 3.75-3.85 (bs, , 6 H), 4.05-4.25 (m, 2 H), 4.35-4.40 (bs, 6 H),  
5 7.10-7.40, 7.95-8.15 (2 x m, 16 H), 8.60-9.00 (bs, 8 H).

#### COMPOUND 40

5,10,15-tris-(4-Hydroxy-phenyl)-20-(4-tridecyloxy-phenyl)-porphyrin



10

5,10,15,20-tetrakis-(4-Hydroxy-phenyl)-porphyrin (400 mg, 0.59 mmol)  
is dissolved and K<sub>2</sub>CO<sub>3</sub> (1.0 g, 7.1 mmol) is suspended in DMF (75 mL).  
To the vigorously stirred reaction mixture a solution of 1-bromotridecane  
15 (0.1 mL, 0.45 mmol) in DMF (10 mL) is added dropwise at 50°C during  
30 mins and the mixture is then heated for 1.5h. The reaction mixture is  
cooled to room temperature and poured into water (150 mL). The  
porphyrins are extracted with ethyl acetate (100 mL) and the extract  
washed with brine (3 x 50 mL) and dried (Na<sub>2</sub>SO<sub>4</sub>). After evaporation of  
20 solvent, the residue is dissolved in toluene:ethanol (1:1, by vol., ca. 10  
mL) and purified by chromatography using a column (200g) of silica gel  
(Merck 60) with toluene as the eluent. After the elution of the first three  
compounds, the eluent is changed to toluene:ethyl acetate (2:1, by vol.).  
The fifth compound eluted is collected and dried under high vacuum to  
25 yield product as a violet solid.

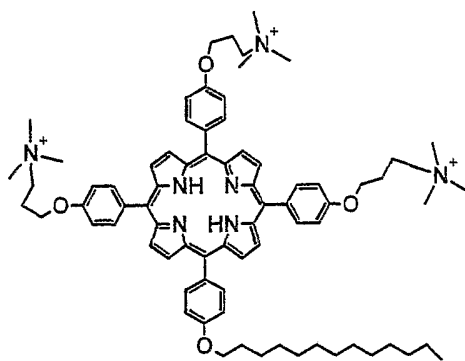
$^1\text{H}$ -NMR:

$\delta_{\text{H}}$  (300Mz, d6-acetone): 0.85 (t,  $^3J$  7.5 Hz, 3 H), 1.20-1.60 (m, 18 H),  
 1.50 (quint,  $^3J$  7.5 Hz, 2 H), 1.80 (quint,  $^3J$  7.5 Hz, 2 H), 4.14 (t,  $^3J$  7.5  
 Hz, 2 H), 7.20 (d,  $^3J$  8.5 Hz, 2 H), 7.25 (d,  $^3J$  8.5 Hz, 6 H), 8.00-8.15 (m,  
 5 8 H), 8.80-9.10 (m, 8 H).

#### COMPOUND 41

5-(4-Tridecyloxy-phenyl)-10,15,20-tris-[4-(3-trimethylammonio-  
 propyloxy)-phenyl]-porphyrin trichloride

10



Compound 40 (50 mg, 0.057 mmol) and (1-bromopropyl)-  
 trimethylammonium bromide (210 mg, 0.8 mmol) are dissolved and  
 15  $\text{K}_2\text{CO}_3$  (230 mg, 1.7 mmol) is suspended in DMF (20 mL). The  
 vigorously stirred reaction mixture is heated at  $50^\circ\text{C}$  for 18 h. After  
 removal of DMF, the residue is dissolved in methanol (5mL) and applied  
 to a pad (2 cm thick) of silica gel which is washed with methanol (ca.  
 1000 mL) and then eluted with acetic acid:methanol:water (3:2:1 by vol.).  
 20 After evaporation of the solvent the residue is dissolved in methanol and  
 further purified by chromatography on a column (2.5 x 40 cm) of  
 Sephadex LH-20 which is eluted with n-butanol:water:acetic acid (4:5:1  
 by vol., upper phase). After removal of solvent, the residue is dissolved  
 in methanol and passed through a short column (3.5 x 20 cm) of anion

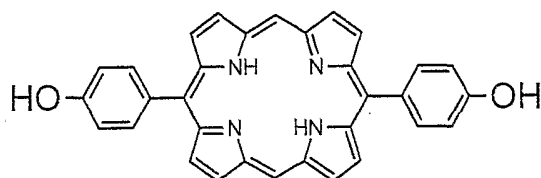
exchange resin (Amberlite IRC 400, chloride form). After evaporation of solvent, the product is dried under high vacuum to afford a violet solid.

<sup>1</sup>H-NMR:

- 5  $\delta_H$  (300MHz, CD<sub>3</sub>OD): 0.90 (t, <sup>3</sup>J 7.5 Hz, 3 H), 1.20-1.40 (m, 18 H), 1.45-1.60 (m, 2 H), 1.80-1.90 (bs, 2 H), 2.40-2.55 (bs, 6 H), 3.25-3.35 (bs, 27 H), 3.75-3.85 (bs, 6 H), 4.05-4.25 (m, 2 H), 4.35-4.40 (bs, 6 H), 7.10-7.40, 7.90-8.15 (2 x m, 16 H), 8.60-9.00 (bs, 8 H).

10 COMPOUND 42

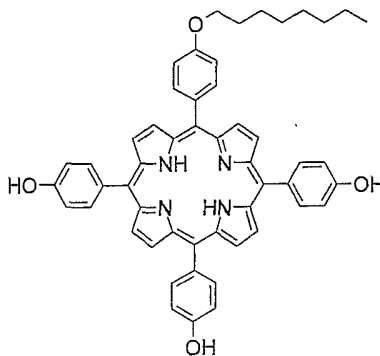
5,15-bis-(4-Hydroxy-phenyl)-porphyrin



- 15 This is prepared as described by Mehta, Goverdhan; Muthusamy, Sengodagounder; Maiya, Bhaskar G.; Arounaguiri, S., *J.Chem.Soc.Perkin Trans.1*; 2177 – 2182 (1999).

COMPOUND 43

- 20 5,10,15-tris-(4-Hydroxy-phenyl)-20-(4-octyloxy-phenyl)-porphyrin



5,10,15,20-tetrakis-(4-Hydroxy-phenyl)-porphyrin (200 mg, 0.294 mmol) is dissolved and potassium carbonate (487 mg, 3.53 mmol, 12 eqv.) is suspended under argon in absolute DMF (50 mL) and the mixture is heated to 55°C. A solution of octyl bromide (35.8 µl, 0.206 mmol, 0.7 eqv.) in absolute DMF (10 mL) is added dropwise during 30 min. and the mixture is stirred at 55°C for 2 h. The solvent is removed *in vacuo* at 50°C, water (80 mL) is added and the mixture is extracted with ethyl acetate (3 x 40 mL). The combined organic fraction is dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent evaporated. The residue is purified by chromatography on a column (300g) of silica gel. Tetra-alkylated and tri-alkylated compounds are eluted with toluene:ethyl acetate (30:1 by vol.). The third fraction (di-substituted compound, trans-isomer) is eluted with toluene:ethylacetate (15:1 by vol.). The fourth fraction (di-substituted compound, cis-isomer) is eluted with toluene:ethyl acetate (10:1 by vol.) and the desired product (mono-alkylated compound) is eluted with toluene:ethylacetate (5:1 by vol.). The solvent is removed under reduced pressure and the residue dried under high vacuum to give the product as a violet solid.

20

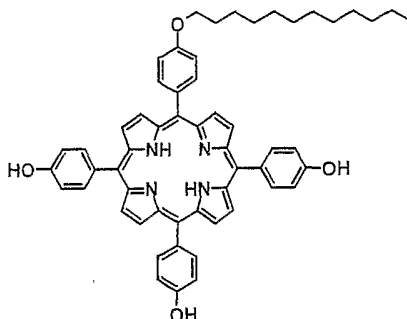
<sup>1</sup>H-NMR:

δ<sub>H</sub> (300 MHz, d<sub>6</sub>-acetone): 0.75 (t, 3H, <sup>3</sup>J = 6.8 Hz), 1.13-1.25 (m, 8H), 1.43 (quint, 2H, <sup>3</sup>J = 7.5 Hz), 1.73 (quint, 2H, <sup>3</sup>J = 7.5 Hz), 3.50 (t, 2H, <sup>3</sup>J = 8 Hz), 7.11 (d, 2H, <sup>3</sup>J = 7.5 Hz), 7.16 (d, 6H, <sup>3</sup>J = 7.5 Hz), 7.90-7.94 (m, 8H), 8.80-8.90 (m, 8H)

25

COMPOUND 44

5-(4-Dodecyloxy-phenyl)-10,15,20-tris-(4-hydroxy-phenyl)-porphyrin



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5,10,15,20-tetrakis-(4-Hydroxy-phenyl)-porphyrin (200 mg, 0.294 mmol) is dissolved and potassium carbonate (487 mg, 3.53 mmol, 12 eqv.) in suspended under argon in absolute DMF (50 mL) and the mixture is heated to 55°C. A solution of dodecyl bromide (49.4 μl, 0.206 mmol, 0.7 eqv.) in absolute DMF (10 mL) is added dropwise during 30 min. The mixture is stirred at 55°C for 2 h. The solvent is removed *in vacuo* at 50°C, water (80 mL) is added and the mixture extracted with ethyl acetate (3 x 40 mL). The combined organic fractions are dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent evaporated. The product is isolated by chromatography on a column (300g) of silica. Tetra-alkylated and tri-alkylated compounds are eluted with toluene:ethyl acetate (30:1 by vol.), di-substituted compound (trans-isomer) with toluene:ethyl acetate (15:1 by vol.), di-substituted compound (cis-isomer) with toluene:ethyl acetate (10:1 by vol.) and the desired product (mono-alkylated compound) with toluene:ethyl acetate (5:1 by vol). Solvent is removed *in vacuo* and the residue dried at high vacuum to give product as a violet solid.

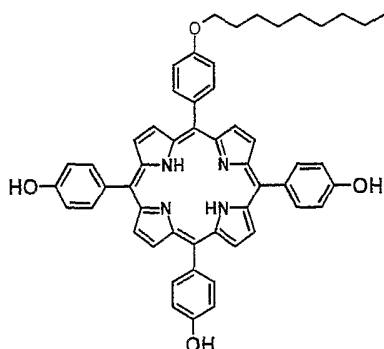
<sup>1</sup>H-NMR:

δ<sub>H</sub> (300 MHz, d<sub>6</sub>-acetone): 0.75 (t, 3H, <sup>3</sup>J = 6.8 Hz), 1.13-1.25 (m, 16H), 1.41 (quint, 2H, <sup>3</sup>J = 7.5 Hz), 1.63 (quint, 2 H, <sup>3</sup>J = 7.5 Hz), 3.89 (t, 2H, <sup>3</sup>J

= 6 Hz), 7.11 (d, 2H,  $^3J = 7.5$  Hz), 7.16 (d, 6H,  $^3J = 7.5$  Hz), 7.9-7.94 (m, 8H), 8.78-8.83 (m, 8 H)

#### COMPOUND 45

##### 5 5,10,15-tris-(4-Hydroxy-phenyl)-20-(4-nonyloxy-phenyl)-porphyrin



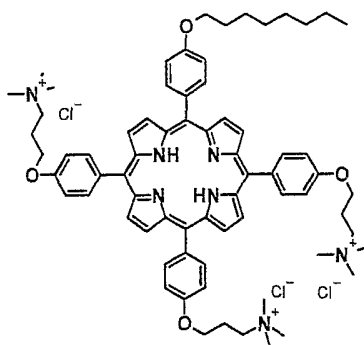
5,10,15,20-tetrakis-(4-Hydroxy-phenyl)-porphyrin (200 mg, 0.294 mmol)  
 10 is dissolved and potassium carbonate (487 mg, 3.53 mmol, 12 eqv.) is  
 suspended under argon in absolute DMF (50 mL) and the mixture heated  
 to 55°C. A solution of nonyl bromide (49.4μl, 0.206 mmol, 0.7 eqv.) in  
 absolute DMF (10 mL) is added dropwise during 30 min. The mixture is  
 stirred at 55°C for 2 h. The solvent is removed *in vacuo* at 50°C, water  
 15 (80 mL) is added and the mixture extracted with ethyl acetate (3 x 40  
 mL). The combined organic extracts are dried (Na<sub>2</sub>SO<sub>4</sub>) and solvent  
 removed under reduced pressure. The product is isolated by  
 chromatography on a column (300g) of silica. Tetra-alkylated and tri-  
 alkylated compounds are eluted with toluene:ethyl acetate (30:1 by vol.),  
 di-substituted compound (trans-isomer) with toluene:ethyl acetate (15:1  
 20 by vol.). di-substituted compound (cis-isomer) with toluene:ethyl acetate  
 (10:1 by vol.) and the desired product (mono-alkylated compound) is  
 eluted with toluene:ethyl acetate (5:1 by vol.). The solvent is removed  
 under reduced pressure and the residue dried at high vacuum to afford the  
 25 product as a violet solid.

$^1\text{H}$ -NMR:

$\delta_{\text{H}}$  (300 MHz,  $\text{d}_6$ -acetone): 0.87 (t, 3H,  $^3J = 7.5$  Hz), 1.14-1.26 (m, 10H),  
 1.41 (quint, 2H), 1.70 (quint, 2H,  $^3J = 7.5$  Hz), 3.92 (t, 2H,  $^3J = 7.5$  Hz),  
 5 7.02 (d, 2H,  $^3J = 8.25$  Hz), 7.15 (d, 6H,  $^3J = 7.5$  Hz), 7.85 (d, 2H,  $^3J =$   
 8.25 Hz), 7.91 (d,  $^3J = 7.5$  Hz), 8.76-8.84 (m, 8 H)

#### COMPOUND 46

5-(4-Octyloxy-phenyl)-10,15,20-tris-[4-(3-trimethylammonio-  
 10 propyloxy)-phenyl]-porphyrin trichloride



Compound 43 (50 mg, 0.063 mmol) and (3-bromopropyl)-  
 trimethylammonium bromide (164mg, 0.63 mmol, 10eqv.) are dissolved  
 15 and potassium carbonate (130 mg, 0.95 mmol, 15 eqv.) is suspended  
 under argon in absolute DMF (30 mL) and the mixture is stirred at 55°C  
 for 12 h. The solvent is removed *in vacuo* at 50°C and the residue  
 applied to a pad (2 cm deep) of silica. The unreacted ammonium salts are  
 washed off with methanol (1000mL) and the product is eluted with acetic  
 20 acid:methanol:water (3:2:1 by vol.). The solvent is removed under  
 reduced pressure and the residue further purified by chromatography on a  
 column (100g) of Sephadex LH-20 using n-butanol:water:acetic acid  
 (4:5:1 by vol., upper phase) as the eluent. The solvents are removed  
 under reduced pressure and the residue dissolved in methanol and passed

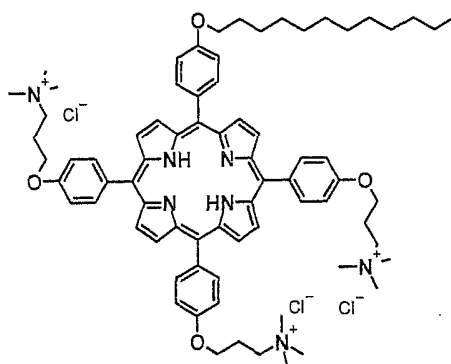
through a small column of anion exchange resin (Amberlite IRA 400, chloride form) using methanol as eluent. After evaporation of solvent, the crude product is dissolved in the minimum amount of methanol and diethylether (50 mL) added. The solution is centrifuged for 15 min. The  
 5 supernatant liquid is evaporated to dryness and the residue dried at high vacuum to give the product as a violet solid.

$^1\text{H-NMR}$ :

$\delta_{\text{H}}$  (300MHz,  $\text{CD}_3\text{OD}$ ): 0.90 (t, 3H,  $^3J = 7.5$  Hz), 1.25-1.41 (m, 8H), 1.45  
 10 (bs, 2H), 1.87 (bs, 2H), 2.38 (bs, 6H), 3.29 (bs, 27H), 3.67 (t, 6H,  $^3J = 7.5$  Hz), 4.01 (t, 2H,  $^3J = 7.5$  Hz), 4.30 (t, 6H,  $^3J = 7.5$  Hz), 7.11 (d, 2H,  $^3J = 7.5$  Hz), 7.38 (d, 6H,  $^3J = 7.5$  Hz), 7.95 (d, 2H,  $^3J = 7.5$  Hz), 8.11 (d, 6H,  $^3J = 7.5$  Hz), 8.93 (bs, 8H)

15 COMPOUND 47

5-(4-Dodecyloxy-phenyl)-10,15,20-tris-[4-(3-trimethylammonio-propyloxy)-phenyl]-porphyrin trichloride



20

Compound 44 (50 mg, 0.059 mmol) and (3-bromopropyl)-trimethylammonium bromide (154mg, 0.59 mmol, 10eqv.) are dissolved and potassium carbonate (122 mg, 0.885 mmol, 15 eqv.) is suspended under argon in absolute DMF (30 mL) and the mixture is stirred at 55°C  
 25 for 12 h. The solvent is removed *in vacuo* at 50°C and the residue re-

dissolved in a little methanol and applied to a pad of silica (2 cm deep). The unreacted ammonium salts are washed off with methanol (1000mL). The product is eluted with acetic acid:methanol:water (3:2:1 by vol.). The solvents are removed under reduced pressure and the crude product  
5 further purified by chromatography on a column (100g) of Sephadex LH-20 using n-butanol:water:acetic acid (4:5:1 by vol., upper phase) as eluent. The solvents are removed under reduced pressure, the residue re-dissolved in a little methanol and the solution passed through a short column of anion exchange resin (Amberlite IRC 400, chloride form)  
10 using methanol as eluent. After removal of solvent the crude product is re-dissolved in the minimum amount of methanol and diethyl ether (50 mL) added. The solution is centrifuged for 15 min. The supernatant liquid is evaporated to dryness and the product dried at high vacuum to give a violet solid.

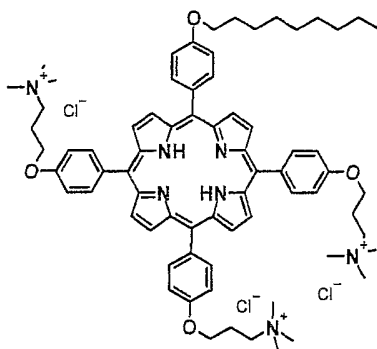
15

<sup>1</sup>H-NMR:

$\delta_H$  (300MHz, CD<sub>3</sub>OD): 0.88 (t, 3H, <sup>3</sup>J = 7.5 Hz), 1.25-1.37 (m, 16H), 1.48 (bs, 2H), 1.93 (bs, 2H), 2.42 (bs, 6H), 3.28 (bs, 27H), 3.68-3.75 (m, 6H), 4.05 (t, 2H), 4.33 (t, 6H), 7.17 (d, 2H, <sup>3</sup>J = 7.5 Hz), 7.33 (d, 6H, <sup>3</sup>J =  
20 7.5 Hz), 7.99 (d, 2H, <sup>3</sup>J = 7.5 Hz), 8.08 (d, 6H, <sup>3</sup>J = 7.5 Hz), 8.85 (bs, 8H)

COMPOUND 48

5-(4-Nonyloxy-phenyl)-10,15,20-tris-[4-(3-trimethylammonio-propyloxy)-phenyl]-porphyrin trichloride



5

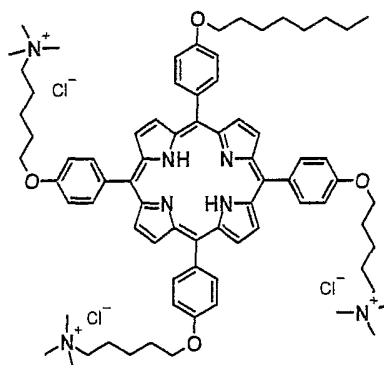
Compound 45 (50 mg, 0.062 mmol) and (3-bromopropyl)-trimethylammonium bromide (162mg, 0.62 mmol, 10eqv.) are dissolved and potassium carbonate (128 mg, 0.93 mmol, 15 eqv.) is suspended  
 10 under argon in absolute DMF (30 mL) and the mixture is stirred at 55°C for 12 h. The solvent is removed *in vacuo* at 50°C and the residue re-dissolved in a little methanol and applied to a pad of silica (2 cm deep). The unreacted ammonium salts are washed off with methanol (1000mL). The product is eluted with acetic acid:methanol:water (3:2:1 by vol.).  
 15 The solvents are removed under reduced pressure and the product further purified by chromatography on a column (100g) of Sephadex LH-20 eluting with n-butanol:water:acetic acid (4:5:1 by vol., upper phase). The solvents are removed under reduced pressure, the residue re-dissolved in a little methanol and the solution is passed through a short column of  
 20 anion exchange resin (Amberlite IRC 400, chloride form) using methanol as eluent. After removal of solvent, the product is dried at high vacuum to give a violet solid.

$^1\text{H-NMR}$ :

$\delta_{\text{H}}$  (300MHz,  $\text{CD}_3\text{OD}$ ): 0.89 (t, 3H,  $^3J = 7.5$  Hz), 1.18-1.34 (m, 10H),  
 1.41 (bs, 2H), 1.73 (quint, 2H,  $^3J = 7.5$  Hz), 2.30-2.44 (m, 6H), 3.31 (bs,  
 27H), 3.65-3.73 (m, 6H), 3.93 (t, 2H,  $^3J = 7.5$  Hz), 4.25-4.42 (m, 6H),  
 5 7.08 (d, 2H,  $^3J = 7.5$  Hz), 7.30 (d, 6H,  $^3J = 7.5$  Hz), 7.93 (d, 2H,  $^3J = 7.5$   
 Hz), 8.05 (d, 6H,  $^3J = 7.5$  Hz), 8.94 (bs, 8H)

#### COMPOUND 49

5-(4-Octyloxy-phenyl)-10,15,20-tris-[4-(5-trimethylammonio-pentyloxy)-  
 10 phenyl]-porphyrin trichloride



Compound 43 (23 mg, 0.03 mmol) and (5-bromopentyl)-  
 15 trimethylammonium bromide (84 mg, 0.3 mmol, 10eqv.) are dissolved  
 and potassium carbonate (62 mg, 0.45 mmol, 15 eqv.) is suspended under  
 argon in absolute DMF (15 mL) and the mixture is stirred at 55°C for 12  
 h. The solvent is removed *in vacuo* at 50°C and the residue re-dissolved  
 in a little methanol and applied to a pad (2 cm deep) of silica. The  
 20 unreacted ammonium salts are washed off with methanol (1000mL). The  
 product is eluted with acetic acid:methanol:water (3:2:1 by vol.). The  
 solvents are removed under reduced pressure and the product further  
 purified by chromatography on a column (100g) of Sephadex LH-20  
 using n-butanol:water:acetic acid (4:5:1 by vol., upper phase) as eluent.  
 25 The solvents are removed under reduced pressure, the residue re-

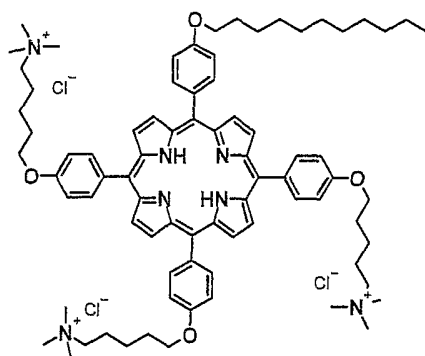
dissolved in a little methanol and the solution passed through a short column of anion exchange resin (Amberlite IRC 400, chloride form) with methanol as eluent. The complete purification process is repeated if impurities remain in the product. After removal of solvent, the residue is  
 5 dried at high vacuum to give the product as a violet solid.

$^1\text{H}$ -NMR:

$\delta_{\text{H}}$  (300MHz,  $\text{CD}_3\text{OD}$ ): 0.78 (bs, 3H), 1.08-1.35 (m, 10H), 1.45-1.59 (m, 6H), 1.63-1.93 (m, 14H), 3.17-3.32 (m, 6H), 3.31 (bs, 33H), 3.84 (bs,  
 10 2H), 4.07 (bs, 6H), 6.93 (bs, 2H), 7.09 (d, 2H,  $^3J = 7.5$  Hz), 7.74 (bs, 2H),  
 7.88 (d, 2H,  $^3J = 7.5$  Hz), 8.71 (bs, 8H)

#### COMPOUND 50

5,10,15-tris-[4-(5-Trimethylammonio-pentyloxy)-phenyl]-20-(4-  
 15 undecyloxy-phenyl)-porphyrin trichloride



Compound 2 (50 mg, 0.06 mmol) and (5-bromopentyl)-  
 20 trimethylammonium bromide (174 mg, 0.6 mmol, 10eqv.) are dissolved  
 and potassium carbonate (124 mg, 0.9 mmol, 15 eqv.) is suspended under  
 argon in absolute DMF (30 mL) and the mixture is stirred at 55°C for 12  
 h. The solvent is removed *in vacuo* at 50°C and the residue re-dissolved  
 in a little methanol and applied to a pad (2 cm deep) of silica. The  
 25 unreacted ammonium salts are washed off with methanol (1000mL). The

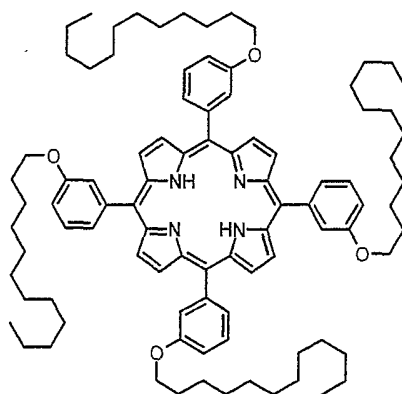
product is eluted with acetic acid:methanol:water (3:2:1 by vol.). Solvents are removed under reduced pressure and the product further purified by chromatography on a column (100g) of Sephadex LH-20 eluting with n-butanol:water:acetic acid (4:5:1 by vol., upper phase).  
5 Solvents are removed under reduced pressure, the residue re-dissolved in the minimum of methanol and the solution passed through a short column of anion exchange resin (Amberlite IRC 400) with methanol as eluent. The complete purification process is repeated if impurities remain in the product. After removal of solvent, the residue is dried at high vacuum to  
10 give the product as a violet solid.

$^1\text{H}$ -NMR:

$\delta_{\text{H}}$  (300MHz, MeOD): 0.71-0.88 (m, 13H), 0.91-1.38 (m, 14H), 1.48-1.81 (m, 12H), signals for  $-\text{CH}_2\text{NCH}_2-$  and  $\text{OCH}_2$ -long alkyl chain are part  
15 of the multiplet together with the signals for solvent in the area 2.8 – 3.3, 3.91 (bs, 6H), 6.33 (bs, 2H), 6.86 (bs, 6H), 7.35 (bs, 2H), 7.70 (bs, 6H), 8.65 (bs, 8H)

#### COMPOUND 51

20 5,10,15,20-tetrakis-(3-Dodecyloxy-phenyl)-porphyrin



Pyrrole (0.7 mL, 10 mmol) and 3-dodecyloxybenzaldehyde (2.91 g, 10 mmol) are dissolved in degassed dichloromethane (1000 mL) and TFA (0.77 mL, 10 mmol) is added dropwise. The mixture is stirred for 17h at room temperature in the dark. DDQ (6.81 g, 30 mmol) is added in one  
5 portion and the mixture is stirred for a further 1h at room temperature. The mixture is filtered through a column (400g) of silica using dichloromethane as eluent followed by dichloromethane to which triethylamine is added to adjust the pH value to 8. This purification process is repeated if impurities remain in the product until the pure  
10 product is obtained.

<sup>1</sup>H-NMR:

$\delta_H$  (300 MHz, d6-acetone): 0.80 (bs, 12H), 1.03-1.45 (m, 80H), 1.78 (quint., 8H,  $^3J = 7.5$  Hz), 4.05 (t, 8H,  $^3J = 7.5$  Hz), 7.24 (d, 4H,  $^3J = 7.5$   
15 Hz), 7.49-7.55 (m, 4H), 7.68-7.71 (m, 8H), 8.80 (m, 8 H)

EXAMPLE B: INNATE ANTI-BACTERIAL ACTIVITY OF COMPOUND 10 –  
DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)  
AND MINIMUM BACTERIOCIDAL CONCENTRATION (MBC)

5

The minimum inhibitory concentration (MIC) for an antimicrobial agent against a specific microorganism is defined as the minimum concentration of an antibacterial agent where no apparent visible growth of the organism is observed (FDA definition of Minimum Inhibitory  
10 Concentration). MIC's are typically determined using concentrations derived traditionally from serial twofold dilutions (National Committee for Clinical Laboratory Standards (NCCLS) Handbook M7-A5: "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard – 5<sup>th</sup> Edition" Volume 20 Number 2.  
15 January 2000). The MIC for Compound 10 in the absence of light was investigated, using a protocol based on the MIC protocol produced by the NCCLS (National Committee for Clinical Laboratory Standards (NCCLS) Handbook M7-A5, *supra*).

20 The minimum bacteriocidal concentration (MBC) is defined as the minimal concentration of drug needed to kill most (99.9%) of the viable organisms after incubation for a fixed length of time (generally 24 hours) under a given set of conditions (National Committee for Clinical Laboratory Standards (NCCLS) Handbook M26-A; "Methods for  
25 determining Bactericidal Activity of Antimicrobial Agents; Approved Guidelines" Volume 19 number 18, September 1999).

*Methodology*

30 *Staphylococcus aureus* BAA-44, a multi-drug resistant Methicillin Resistant *Staphylococcus aureus* (MRSA) strain obtained from the ATCC

catalogue, was used in this study. The following concentrations of Compound 10 were investigated: 0.764 ; 0.382; 0.191; 0.0955; 0.0478; 0.0239, 0.0119, 0.00597, 0.00298, 0.00149, 0.00075 & 0.00037  $\mu\text{g/mL}$ . Stock solutions were made up in distilled water and serial dilutions  
5 undertaken of this to produce the required concentrations immediately prior to use

At least 3 to 5 well-isolated colonies of the same morphological type were selected from an agar plate culture and the growth transferred to a  
10 tube containing 100 mL of Isosensitest Broth and the broth culture is incubated at 37°C overnight. The culture was then be diluted to a final density of  $10^4$  cells/mL with fresh Isosensitest Broth and incubated with shaking at 37°C until the cells entered exponential growth.

15 0.09 mL of the adjusted inoculum was transferred into each of 24 wells of a polystyrene 96 well microtiter plate. A control well of bacteria alone in the presence of growth medium alone was included (as a positive control).

20 0.09 mL of the Compound 10 stock solutions from the dilution series were pipetted into the relevant well for the microtiter plates and incubated in the dark at 37°C and the plates examined after 24 hours incubation to determine the turbidity in each well. These data are used to determine the MIC.

25

After 24 hours incubation at 37°C, 25 $\mu\text{L}$  samples of the fluid from the wells without visible bacterial growth (four wells up) were inoculated onto nutrient agar plates as spots and incubated at 37°C for a further 24 hours to determine the MBC.

30

### Results

The results demonstrated that the MIC for Compound 10 in the absence of light was 0.0955  $\mu\text{g/mL}$ , and that the MBC was 0.382  $\mu\text{g/mL}$  (Table 1).

**Table 1**

MIC and MBC data for Compound 10

	MIC ( $\mu\text{g/mL}$ )	MBC ( $\mu\text{g/mL}$ )
Series 1	0.0955	0.382*
Series 2	0.0955	Not determined

\* growth on sub of 0.191 much reduced from initial inoculum to about  $10^3/\text{ml}$

### Conclusions

The results demonstrate that in the absence of light Compound 10 has low MIC and MBC values. These data indicate that Compound 10 is considerably more potent as an antibiotic than some traditional antibiotics (see Table 2):

**Table 2**

MIC and MBC values for compound 10 and conventional antibiotics

Compound	MIC Values ( $\mu\text{g/mL}$ )	MBC Values ( $\mu\text{g/mL}$ )
Compound 10	0.0955	0.382
Vancomycin	1 <sup>a</sup>	4 – 16 <sup>b</sup>
Zyvox® (Linezolid)	4 <sup>a</sup>	4 – >64 <sup>c</sup>

- 5
- (a) Critchley LA *et al.* Baseline study to determine in vitro activities of daptomycin against gram-positive pathogens isolated in the United States in 2000-2001. *Antimicrobial Agents and Chemotherapy* (2003); **47**(5): 1689-93
  - (b) Biavasco F *et al.* *In vitro* antibacterial activity of LY333328, a new semi-synthetic glycopeptide. *Antimicrobial Agents and Chemotherapy* (1997); **41**(10): 2165-72
  - (c) Fuchs PC *et al.* *In vitro* bactericidal activity of daptomycin against staphylococci. *Journal of Antimicrobial Chemotherapy* (2002); **49**: 467-70

EXAMPLE C: INNATE ANTI-BACTERIAL ACTIVITY OF COMPOUND 10 –  
ACTIVITY OVER A RANGE OF REFERENCE STRAINS AND CLINICAL  
ISOLATES

- 5 The Minimum Inhibitory Concentrations (MIC's) for Compound 10, over a range of reference strains and clinical isolates, were determined using IsoSensitest® broth and Minimum Bactericidal concentrations (MBC's) determined by subculture onto Columbia blood agar.

10 *Methodology*

1. A 5 mg/ml stock solution of Compound 10 was made up in water
2. A series of dilutions were undertaken to produce a range of  
15 concentrations between 32 – 0.001 mg/L
3. The test microorganisms were grown up overnight in IsoSensitest®  
broth
- 20 4. The cultures were then diluted with fresh broth to a final concentration of  $10^4$  organisms/ml and placed on a shaker for 90 minutes at 37°C
5. 90 µl of the broth culture containing the microorganisms were transferred to each of 12 wells in a row in a microtitre tray and  
25 repeated in a control tray - four organisms per tray.
6. 90 µL of the appropriate Compound 10 dilution was then added to each well containing organisms to give a final dilution series from 16 mg/L to 0.0005 mg/L

7. The solutions were mixed well and incubated in the dark for 24 hours

8. The MIC was recorded and 25 $\mu$ L from wells showing no growth was subcultured onto blood agar for MBC determination

5

9. The MBC values were recorded after overnight incubation of the subcultures.

10. Controls of uninoculated broth and broth plus inoculum were undertaken for each organism in each tray

10

### Results

The results are shown in Table 3.

15

**Table 3**

MIC and MBC values for compound 10 and conventional antibiotics

<i>Organism</i>	<i>Strain</i>	<i>Cpd 10 MIC (mg/L)</i>	<i>Cpd 10 MBC (mg/L)</i>
<i>(a) Staphylococcus aureus (methicillin resistant)</i>			
	ATCC BAA-44 Experiment 1	0.5	0.5
	Experiment 2	0.5	1
	Experiment 3	2	2
	Experiment 4	0.5	1
	Experiment 5	0.5	>1
	Experiment 6	0.5	1

<i>Organism</i>	<i>Strain</i>	<i>Cpd 10 MIC (mg/L)</i>	<i>Cpd 10 MBC (mg/L)</i>
	NCTC 11939 (EMRSA-1)	0.5	0.5
	EMRSA-15*	1	1
	EMRSA-16*	0.5	0.5
<i>(b) Staphylococcus aureus (methicillin sensitive)</i>			
	NCTC 6571	0.5	0.5
	ATCC 25923	0.5	1
<i>(c) Staphylococcus epidermidis (methicillin resistant)</i>			
	38808*	0.5	0.5
	33759*	0.5	1
	33659*	0.5	1
	36572*	0.25	0.25
<i>(d) Staphylococcus epidermidis (methicillin sensitive)</i>			
	37453*	0.5	0.5
<i>(e) Enterococcus faecium</i>			
	NCTC 12204	1	1
	E1*	0.5	1
	E5*	0.5	1
	E19*	0.5	0.5
	E44*	0.5	0.5
<i>(f) Enterococcus faecalis</i>			
	ATCC 29212	1	>1
	E3*	0.5	1

<i>Organism</i>	<i>Strain</i>	<i>Cpd 10 MIC (mg/L)</i>	<i>Cpd 10 MBC (mg/L)</i>
	E4*	0.5	0.5
	E10*	0.5	1
	E37*	0.5	1

\* = Clinical isolates

### *Conclusions*

5

The results demonstrate that Compound 10 has very low MIC and MBC values for a range of gram-positive bacterial strains. The MIC and MBC values are almost identical within the limitations of the methodology, suggesting that the mode of antimicrobial activity is bacteriocidal as

10 opposed to bacteriostatic.

## EXAMPLE D: TOXICITY TESTING OF COMPOUND 10 AGAINST HUMAN CELLS

### *Methodology*

5

Test compounds were screened for toxicity against cultured human skin cells using normal human epidermal keratinocytes (NHEK) and normal human dermal fibroblasts (NHDF), purchased from CellSystems Biotechnologie GmbH, Germany.

10

The NHEK and NHDF cells were used between passages 3 and 10. The cells were seeded with 7.5 and/or  $15 \times 10^4$  cells/ well (microtitre plate) and were allowed to attach overnight in an incubator (37°C, 5% CO<sub>2</sub>). After incubation with different concentrations of the selected  
15 photosensitisers for various times, the cells were incubated for 24 hours in the dark.

Toxicity was tested by standard MTT-assay (Mossman et al., 1983 *J. Immunological Methods* **65**: 55 – 63). MTT is an indicator of  
20 metabolically active cells. Dependent on enzyme activity in mitochondria a colour reaction can be visualised, which can be measured by ELISA reader (540 nm). The cell viability was normalised to one, which means, the OD values of cells after incubation in the absence of a test compound were normalised to one. Each experiment was repeated three times.

25

### *Results*

Results of the toxicity studies in keratinocytes and fibroblasts are shown in Figures 2 and 3. The data demonstrate that Compound 10 does not  
30 demonstrate an innate toxicity for either normal human epidermal

keratinocytes or normal human dermal fibroblasts at doses which are known to have an anti-bacterial effect.

5

#### EXAMPLE E: BINDING OF EXEMPLARY COMPOUNDS WITH BACTERIAL CELLS

##### *Binding of Compounds 8, 10 and 12 with E. coli*

10

*E. coli* cells were incubated for 5 min with Compound 8, 10 or 12 at various concentrations (1-7.5  $\mu$ M). At the end of the incubation period, the cells were sedimented by centrifugation to remove the fraction of unbound test compound and the cell pellet was resuspended in 2 ml of 2% SDS to obtain cell lysates. After overnight incubation with SDS, the amount of cell-bound test compound was estimated by spectrofluorimetric analysis of the cell lysates. The concentration of the compounds in the cell lysates was calculated by measuring the intensities at the maximum of the emission fluorescence spectrum and interpolating the data on a calibration plot. The amount of cell-bound test compound was expressed as nmoles of compound per mg of cell protein. The protein concentration was determined by the method of Lowry (Lowry *et al.*, 1951, *J. Biol. Chem.* **193**:265-275).

25 All experiments were run in triplicate and the results represent the average of 3 determinations with standard deviations.

The amount of porphyrin recovered from the cells is shown in Table 4.

30

Table 4

<i>Concentration of compound (<math>\mu M</math>)</i>	<i>Bound compound (nmoles/mg cell proteins)</i>		
(a) <u>0 washings</u>			
	<i>Compound 8</i>	<i>Compound 12</i>	<i>Compound 10</i>
<b>0.01</b>	0.024 $\pm$ 0.01	0.041 $\pm$ 0.02	0.026 $\pm$ 0.005
<b>0.1</b>	0.056 $\pm$ 0.02	0.151 $\pm$ 0.02	0.274 $\pm$ 0.05
<b>0.5</b>	0.522 $\pm$ 0.2	0.806 $\pm$ 0.14	1.542 $\pm$ 0.350
<b>1</b>	3.670 $\pm$ 0.7	2.70 $\pm$ 0.30	2.70 $\pm$ 0.354
(b) <u>3 washings</u>			
	<i>Compound 8</i>	<i>Compound 12</i>	<i>Compound 10</i>
<b>0.01</b>	0.009 $\pm$ 0.001	0.021 $\pm$ 0.005	0.015 $\pm$ 0.0004
<b>0.1</b>	0.030 $\pm$ 0.02	0.089 $\pm$ 0.02	0.078 $\pm$ 0.02
<b>0.5</b>	0.274 $\pm$ 0.15	0.622 $\pm$ 0.10	0.334 $\pm$ 0.092
<b>1</b>	2.230 $\pm$ 0.8	1.930 $\pm$ 0.20	1.278 $\pm$ 0.102

5

The results shown in Table 3. show that the three test compounds bind to *E. coli* with similar efficiency and that about 50% of the compound that is associated to the cells at the end of the incubation period (5 min) is removed by 3 washings with PBS.

10

## EXAMPLE F: STABILITY STUDIES

*Chemical stability*

- 5 The following HPLC methodology was established for the analysis of the exemplary compounds of the invention.

The method involves detection by UV at a wavelength of 420 nm, which is very specific for these compounds. In order to monitor  
10 impurities not related to the porphyrin structure (and therefore not absorbing at 420 nm) UV spectra of the whole chromatograms were also recorded between 200 nm and 700 nm by DAD (diode array detector) in certain experiments.

- 15 Column: Zorbax Phenyl, 250 x 4.6 mm, 5  $\mu$ m  
Eluent A: 1.5 g sodium dodecylsulfate + 1 mL formic acid in 1000 mL water  
Eluent B: 1.5 g sodium dodecylsulfate + 1 mL formic acid in 200 mL water + 800 mL  
20 tetrahydrofuran

Gradient:

<i>Time</i> <i>[min]</i>	<i>Eluent B</i> <i>[%]</i>
0	50
31	65
32	90
33	50
43	50

Flow rate: 0.4 mL/min  
Detection: 420 nm  
Column temperature: 25 °C  
5 Injection volume: 10 µl

Solutions: Porphyrin derivatives were dissolved in eluent A to give a final concentration of approximately 0.3 mg/ml.

10

Typical retention time of the exemplary compounds was approximately 8 minutes (18 minute runtime).

Qualitative stress tests were undertaken on the exemplary compounds of the invention. Analysis was undertaken by HPLC & LC-MS. The  
15 compounds were stress tested in solid form, in an aqueous solution and a solution made up in phosphate-buffered saline buffer. The samples were initially incubated for 7 days at 50 °C and a sample removed for testing. The samples were then incubated for a further 7 days at 70 °C, samples  
20 removed as before and the samples incubated further for 7 days at 90 °C. HPLC analysis of freshly prepared solutions was undertaken and compared to the samples after 7, 14 and 21 days incubation. A visual comparison of the chromatograms was then undertaken and the content of the main products and by-products as area percentage values determined  
25 (see Figure 4).

30

The 3D plots of the chromatograms show no indications for additional formation of fragments (no signals at lower wavelengths)

The plot in Figure 5 shows the sample after 21 days in PBS buffer, which showed the largest degradation effect. The results demonstrated minimal degradation on analysis of solid drug and drug in solution heated to 80°C for a number of weeks.

5

### Conclusions

Compounds 10 and 12 were both found to exhibit good stability and were very stable even under the stressed conditions of the test protocol.

10 Although Compound 8 was less stable than Compounds 10 and 12, the stability demonstrated was found to be sufficient for practical use.

### *Stability of exemplary compounds in formulations*

15 The stability of three exemplary compounds (Compounds 8, 10 and 12) and one reference compound (Compound 1), stored at 40°C in the dark over 8 weeks in polyethylene vials in various aqueous-based formulations, was evaluated as follows:

- 20
- Sodium laureth sulphate (SLES) + water
  - 9:1 water:ethanol
  - SLES + 9:1 water:ethanol

UV spectra were recorded over the range 350-700 nm over a period of  
25 7 weeks and a visual evaluation of the samples made at 8 weeks.

The results indicate that all compounds tested exhibited good stability over an eight-week period (see Figure 6).

For Compounds 8 and 10, the stability study was extended to 17 weeks (see Figure 7).

## 5 EXAMPLE G: ACUTE TOXICITY TESTING OF COMPOUND 10

Compound 10 was tested at 3.2 mM in a topical formulation in a standard acute dermal toxicity test to determine if any clinical or histological toxicity for the compound could be detected.

10

The acute toxicity protocol was based on OECD Guidelines for the testing of chemicals /Section 4 - Health Effects Test Number 402: Acute Dermal Toxicity.

## 15 Results and Conclusions

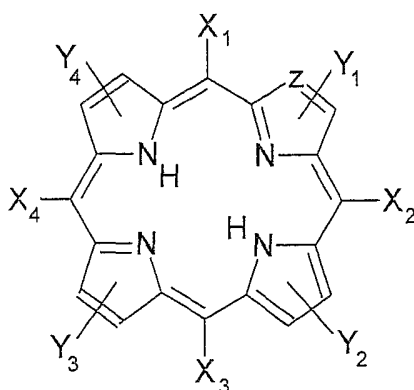
After clinical, macroscopic and microscopic observation, no clinical toxicology was observed. No histological toxicology of any major organ (including the skin) was observed.

20

In conclusion, Compound 10 does not result in any acute toxic effect: in fact, no significant clinical or pathological signs related to the substance or its vehicle application were observed.

# Claims

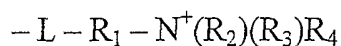
1. Use of a compound of formula I below in the preparation of a medicament for killing or attenuating the growth of microorganisms by a method which does not comprise exposing the compound to a photodynamic therapy light source or a sonodynamic therapy ultrasound source



*I*

wherein:

$X_1$ ,  $X_2$ ,  $X_3$  and  $X_4$  independently represent a hydrogen atom, a lipophilic moiety, a phenyl group, a lower alkyl, alkaryl or aralkyl group, or a cationic group of the following formula;



wherein:

$L$  is a linking moiety or is absent;

5 R<sub>1</sub> represents lower alkylene, lower alkenylene or lower alkynylene, which is optionally substituted by one or more substituents selected from lower alkyl, lower alkylene (optionally interrupted with oxygen), fluoro, OR<sub>5</sub>, C(O)R<sub>6</sub>, C(O)OR<sub>7</sub>, C(O)NR<sub>8</sub>R<sub>9</sub>, NR<sub>10</sub>R<sub>11</sub> and N<sup>+</sup>R<sub>12</sub>R<sub>13</sub>R<sub>14</sub>; and

10 R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> independently represent H, aryl, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which are optionally substituted by one or more substituents selected from lower alkyl, lower alkylene (optionally interrupted with oxygen), aryl, OR<sub>5</sub>, C(O)R<sub>6</sub>, C(O)OR<sub>7</sub>, C(O)NR<sub>8</sub>R<sub>9</sub>, NR<sub>10</sub>R<sub>11</sub> and N<sup>+</sup>R<sub>12</sub>R<sub>13</sub>R<sub>14</sub>

15 Z is -CH or N; and

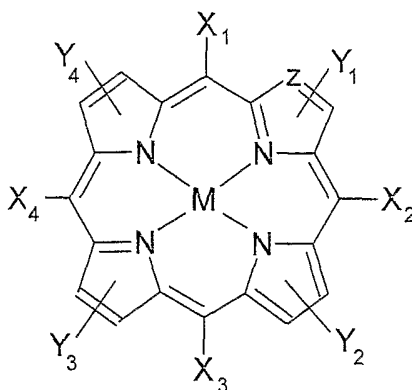
20 Y<sub>1</sub>, Y<sub>2</sub>, Y<sub>3</sub> and Y<sub>4</sub> are absent or independently represent aryl, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which are optionally substituted by one or more substituents selected from lower alkyl, lower alkylene (optionally interrupted with oxygen), aryl, OR<sub>5</sub>, C(O)R<sub>6</sub>, C(O)OR<sub>7</sub>, C(O)NR<sub>8</sub>R<sub>9</sub>, NR<sub>10</sub>R<sub>11</sub>, N<sup>+</sup>R<sub>12</sub>R<sub>13</sub>R<sub>14</sub>, or, taken in conjunction to the pyrrole ring to which they attach, forms a cyclic group; and

25 R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub>, R<sub>9</sub>, R<sub>10</sub>, R<sub>11</sub>, R<sub>12</sub>, R<sub>13</sub> and R<sub>14</sub> independently represent H or lower alkyl

provided that at least one of X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub> and X<sub>4</sub> is a cationic group as defined above and at least one of X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub> and X<sub>4</sub> is a hydrogen atom.

30

2. Use of a compound of formula II below in the preparation of a medicament for killing or attenuating the growth of microorganisms by a method which does not comprise exposing the compound to a photodynamic therapy light source or a sonodynamic therapy ultrasound source



II

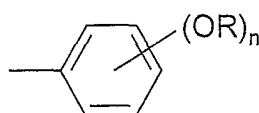
wherein M is a metallic element or a metalloid element

and X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, Y<sub>1</sub>, Y<sub>2</sub>, Y<sub>3</sub>, Y<sub>4</sub> and Z are as defined in Claim 1.

3. A use according to Claim 1 or 2 wherein the medicament is for killing or attenuating the growth of microorganisms by a method which does not comprise exposing the compound to a stimulus which activates antimicrobial activity.
4. The use according to any one of the preceding claims wherein the compound exhibits anti-microbial activity in the absence of irradiation with a photodynamic therapy light source or an ultrasound source.
5. A use according to any one of Claims 2 to 4 wherein M is a divalent or trivalent metallic element.

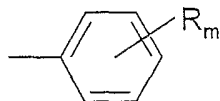
6. A use according to any one of Claims 2 to 5 wherein M is selected from Zn (II), Cu (II), La (III), Lu (III), Y (III), In (III) Cd (II), Mg (II), Al(III), Ru, Ni(II), Mn(III), Fe(III) and Pd(II).
- 5 7. A use according to any one of Claims 2 to 4 wherein M is a metalloid element, for example silicon (Si) or germanium (Ge).
8. A use according to any one of the preceding claims wherein  $Y_1$ ,  $Y_2$ ,  $Y_3$  and  $Y_4$  are absent.
- 10 9. A use according to any one of the preceding claims wherein Z is –CH.
10. A use according to any one of the preceding claims wherein  $R_1$  is an unsubstituted lower alkylene, lower alkenylene or lower alkynylene group.
- 15 11. A use according to any one of the preceding claims wherein  $R_1$  is – $(CH_2)_m$ – and ‘m’ is an integer between 1 and 20.
- 20 12. A use according to Claim 11 wherein ‘m’ is an integer between 1 and 10, for example between 1 and 6, between 1 and 5, between 1 and 4 or between 1 and 3.
- 25 13. A use according to Claim 12 wherein ‘m’ is 3.
14. A use according to any one of the preceding claims wherein  $R_2$ ,  $R_3$  and/or  $R_4$  are lower alkyl, lower alkenyl or lower alkynyl groups.

15. A use according to Claim 14 wherein  $R_2$ ,  $R_3$  and/or  $R_4$  are unsubstituted lower alkyl groups.
16. A use according to Claim 14 or 15 wherein at least one of  $R_2$ ,  $R_3$  and  $R_4$  is an alkyl group which is substituted with a primary, secondary or tertiary amine group or a quaternary ammonium group.
17. A use according to any one of the preceding claims wherein  $R_1$  is  $-(CH_2)_3-$ ,  $R_2$  and  $R_3$  are  $CH_3$  and  $R_4$  is  $-(CH_2)_3-N(CH_3)_2$ .
18. A use according to any one of the preceding claims wherein  $R_1$  is  $-(CH_2)_3-$ , and  $R_2$ ,  $R_3$  and  $R_4$  are each  $CH_3$ .
19. A use according to any one of the preceding claims wherein  $R_1$  is  $-(CH_2)_3-$ , and  $R_2$ ,  $R_3$  and  $R_4$  are each  $C_2H_5$ .
20. A use according to any one of the preceding claims wherein L is selected from the group consisting of phenoxy, phenylene, sulfonyl amido, aminosulfonyl, sulfonylimino, phenylsulfonyl-amido, phenylaminosulfonyl, urea, urethane and carbamate linking moieties.
21. A use according to Claim 20 wherein  $X_1$ ,  $X_2$ ,  $X_3$  and/or  $X_4$  are



wherein R is  $-R_1-N^+(R_2)(R_3)R_4$ , as defined in Claim 1 and 'n' is an integer between 1 and 3.

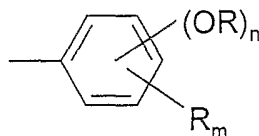
22. A use according to Claim 20 wherein  $X_1$ ,  $X_2$ ,  $X_3$  and/or  $X_4$  are



wherein R is  $-R_1-N^+(R_2)(R_3)R_4$ , as defined in Claim 1 and 'm' is an integer between 1 and 3.

5

23. A use according to Claim 20 wherein  $X_1$ ,  $X_2$ ,  $X_3$  and/or  $X_4$  are



wherein each R independently is  $-R_1-N^+(R_2)(R_3)R_4$ , as defined in Claim 1 and 'n' and 'm' are integers between 1 and 3 and wherein the sum of 'n' and 'm' is an integer between 1 and 3.

10

24. A use according to any one of Claims 21 to 23 wherein 'n' or 'm' is 3.

- 15 25. A use according to any one of Claims 21 to 23 wherein 'n' or 'm' is 2.

26. A use according to any one of Claims 21 to 23 or 25 wherein 'n' and/or 'm' is 1.

20

27. A use according to any one of Claims 21 to 23 wherein L is mono-substituted at the *para*-position.

28. A use according to any one of Claims 21 to 23 wherein L is mono-  
or di-substituted at a *meta*-position(s).
29. A use according to any one of Claims 21 to 23 wherein L is mono-  
5 or di-substituted at an *ortho*-position(s).
30. A use according to any one of the preceding claims wherein the  
compound comprises two cationic groups, as defined in Claim 1,  
on opposite sides of the porphyrin ring, *i.e.* at ring positions 5 and  
10 15 or ring positions 10 and 20.
31. A use according to Claim 30 wherein X<sub>1</sub> and X<sub>3</sub> are a hydrogen  
atom, a lipophilic moiety, a phenyl group, a lower alkyl, alkaryl or  
aralkyl group and X<sub>2</sub> and X<sub>4</sub> are cationic groups, or *vice versa*.  
15
32. A use according to any one of Claims 1 to 30 wherein the  
compound comprises two cationic groups, as defined in Claim 1,  
on neighbouring positions of the porphyrin ring, *i.e.* at ring  
positions 5 and 10, or ring positions 10 and 15, or ring positions 15  
20 and 20 or ring positions 20 and 5.
33. A use according to Claim 32 wherein X<sub>1</sub> and X<sub>2</sub> are hydrogen and  
X<sub>3</sub> and X<sub>4</sub> are cationic groups, or X<sub>2</sub> and X<sub>3</sub> are hydrogen and X<sub>4</sub>  
and X<sub>1</sub> are cationic groups.  
25
34. A use according to any one of the preceding claims wherein at  
least one of X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub> and X<sub>4</sub> is a lipophilic moiety.

35. A use according to Claim 34 wherein the lipophilic moiety is a saturated, straight-chain alkyl group of formula  $-(CH_2)_pCH_3$  wherein 'p' is an integer between 1 and 22.
- 5 36. A use according to Claim 35 wherein 'p' is between 1 and 18, for example between 2 and 16 or between 4 and 12.
37. A use according to any one of Claims 1 to 33 wherein none of  $X_1$ ,  $X_2$ ,  $X_3$  and  $X_4$  is a lipophilic moiety.
- 10 38. A use according to any one of the preceding claims wherein none of  $X_1$ ,  $X_2$ ,  $X_3$  and  $X_4$  is a phenyl group.
39. A use according to any one of the preceding claims wherein the  
15 compound is water-soluble.
40. A use according to Claim 1 wherein the compound is 5,15-bis-(4-{3-[(3-Dimethylamino-propyl)-dimethyl-ammonio]-propyl-oxy}-phenyl)-porphyrin dichloride.
- 20 41. A use according to Claim 1 wherein the compound is 5,15-bis-[4-(3-Triethylammonio-propyloxy)-phenyl]-porphyrin di-chloride.
42. A use according to Claim 1 wherein the compound is 5,15-bis-[3-  
25 (3-Trimethylammonio-propyloxy)-phenyl]-porphyrin dichloride.
43. A use according to Claim 1 wherein the compound is 5,15-bis-[4-(3-Trimethylammonio-propyloxy)-phenyl]-porphyrin dichloride.

44. A use according to Claim 1 wherein the compound is 5-[3,5-bis-(3-Trimethylammonio-propyloxy)-phenyl]-15-undecyl-porphyrin dichloride.
- 5 45. A use according to Claim 1 wherein the compound is 5-{4-[3-Dimethyl-(3-dimethylaminopropyl)-ammonio-propyl-oxy]-phenyl}-15-(4-dodecyloxy-phenyl)-porphyrin chloride.
46. A use according to Claim 1 wherein the compound is 3-[(3-  
10 {4-[15-(4-Dodecyloxy-phenyl)-porphyrin-5-yl]-phenoxy} - propyl)-dimethyl-ammonio]-propyl}-dimethyl-ammonio)-propyl] - trimethyl-ammonium trichloride.
47. A use according to Claim 1 wherein the compound is 5,15-bis-[3-  
15 (3-Trimethylammmonio-propyloxy)-phenyl]-10-undecyl-porphyrin dichloride.
48. A use according to Claim 1 wherein the compound is 5-{4-[3-Dimethyl-(3-trimethylammonio-propyl)-ammonio-propyloxy]-  
20 phenyl}-15-(4-dodecyloxy-phenyl)-porphyrin dichloride.
49. A use according to Claim 1 wherein the compound is 5-[4-(3-Dimethyldecyl-ammoniopropyloxy)-phenyl]-15-{4-[3-di-methyl-(3-dimethylaminopropyl)-ammoniopropyloxy]-phenyl}-porphyrin  
25 dichloride.
50. A use as defined in any one of Claims 40 to 49 wherein the compound is in a metallated form.

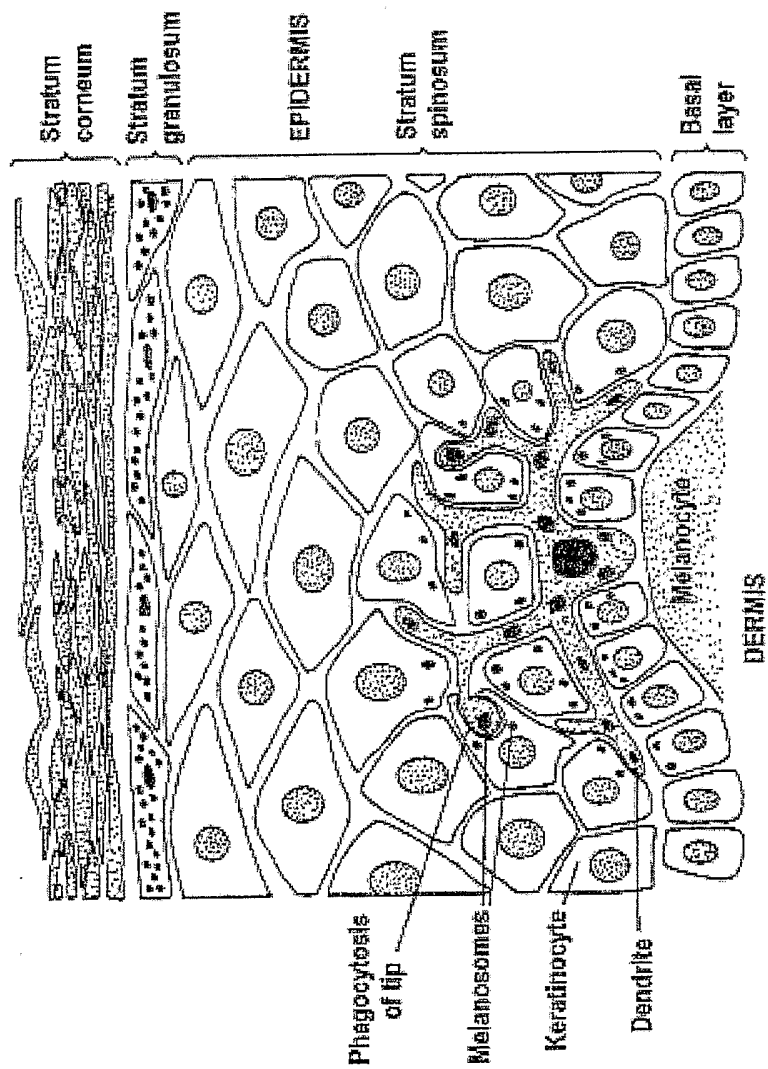
51. A use according to any one of the preceding claims wherein the compound is substantially non-toxic to mammalian cells.
52. A use according to any one of the preceding claims wherein the medicament is for oral administration.
53. A use according to any one of the preceding claims wherein the medicament is for parenteral administration.
54. A use according to any one of the preceding claims wherein the medicament is for topical administration.
55. A use according to any one of the preceding claims wherein the microorganisms are selected from the group consisting of bacteria, mycoplasmas, yeasts, fungi and viruses.
56. A use according to any one of the preceding claims wherein the microorganisms are bacteria which are resistant to one or more conventional antibiotic agents.
57. A use according to any one of the preceding claims wherein the microorganisms are on a light-inaccessible surface or in a light-inaccessible area.
58. A use according to any one of the preceding claims wherein the medicament is for use in the curative and/or prophylactic treatment of microbial infections.
59. A use according to Claim 58 wherein the microbial infection is a systemic infection.

60. A use according to any one of the preceding claims wherein the medicament is for preventing and/or treating dermatological infection.
- 5
61. A use according to any one of the preceding claims wherein the medicament is for preventing and/or treating an infection of the lungs.
- 10 62. A use according to any one of the preceding claims wherein the medicament is for preventing and/or treating wound infection and/or ulcers.
- 15 63. A method for treating a patient in need of treatment with an antimicrobial agent comprising administering to the patient a compound as described in any one of Claims 1 to 52, wherein the method does not comprise irradiating the compound with a stimulus which activates antimicrobial activity.
- 20 64. A method according to Claim 63 wherein the compound is administered orally.
65. A method according to Claim 63 wherein the compound is administered parenterally.
- 25 66. A method according to Claim 63 wherein the compound is administered topically.
- 30 67. A method according to any of Claims 63 to 66 wherein the patient has a dermatological infection or lung infection.

68. A method according to any of Claims 63 to 66 wherein the patient has a wound infection.
- 5 69. A method for killing microorganisms *in vitro* comprising contacting the microorganisms with a compound as described in any one of Claims 1 to 51, wherein the method does not comprise exposing the compound to a stimulus which activates antimicrobial activity.
- 10 70. A method for treating a patient in need of treatment with an antimicrobial agent comprising administering to the patient a compound as described in any one of Claims 1 to 51, wherein the method comprises a first treatment phase during which the  
15 compound is not irradiated with a stimulus which activates antimicrobial activity, followed by a second treatment phase when the compound is irradiated with a stimulus which activates antimicrobial activity
- 20 71. A method according to Claim 70 wherein the stimulus which activates antimicrobial activity is ultrasound and/or light.
72. A method according to Claim 70 or 71 wherein the first treatment phase lasts at least 10 minutes, for example at least 20 minutes, 30  
25 minutes, 40 minutes, 50 minutes, 1 hour, 2 hours, 3, hours, 5 hours, 12 hours or 24 hours.
73. A method according to Claim 72 wherein the method does not comprise irradiating the compound with an amount of light  
30 sufficient to cause photoactivation of the compound.

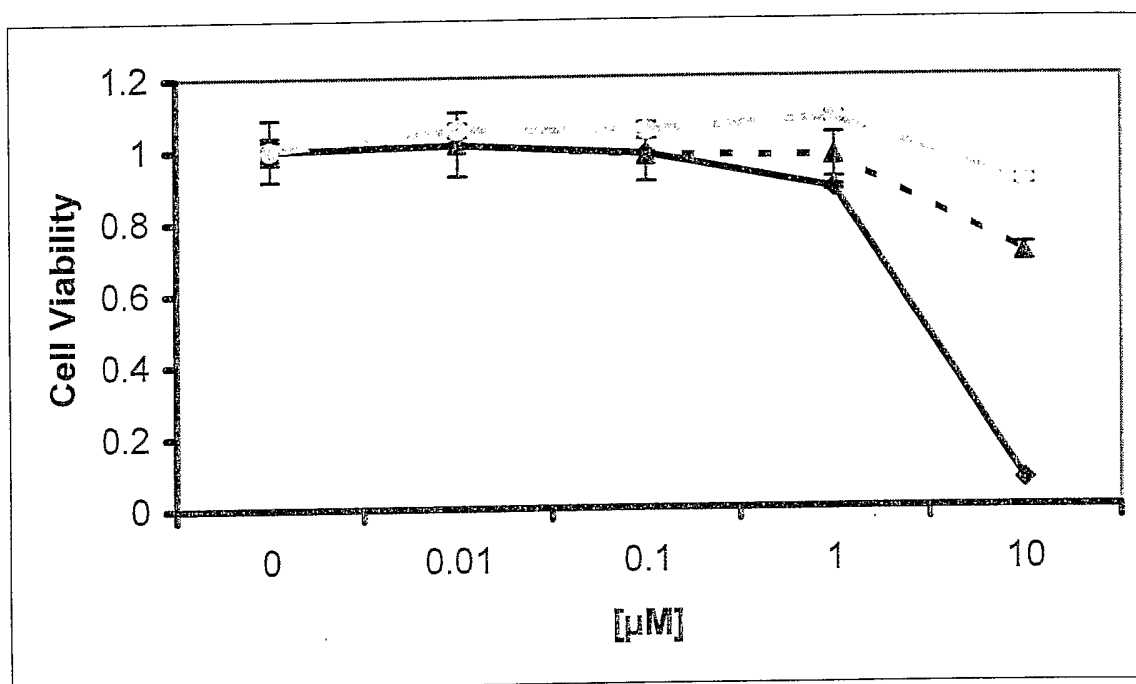
74. A method according to Claim 72 wherein the method does not comprise irradiating the compound with ultrasound.
- 5 75. Use of a compound in the preparation of a medicament substantially as hereinbefore described with reference to the description.
- 10 76. A method for killing microorganisms substantially as hereinbefore described with reference to the description.

FIGURE 1



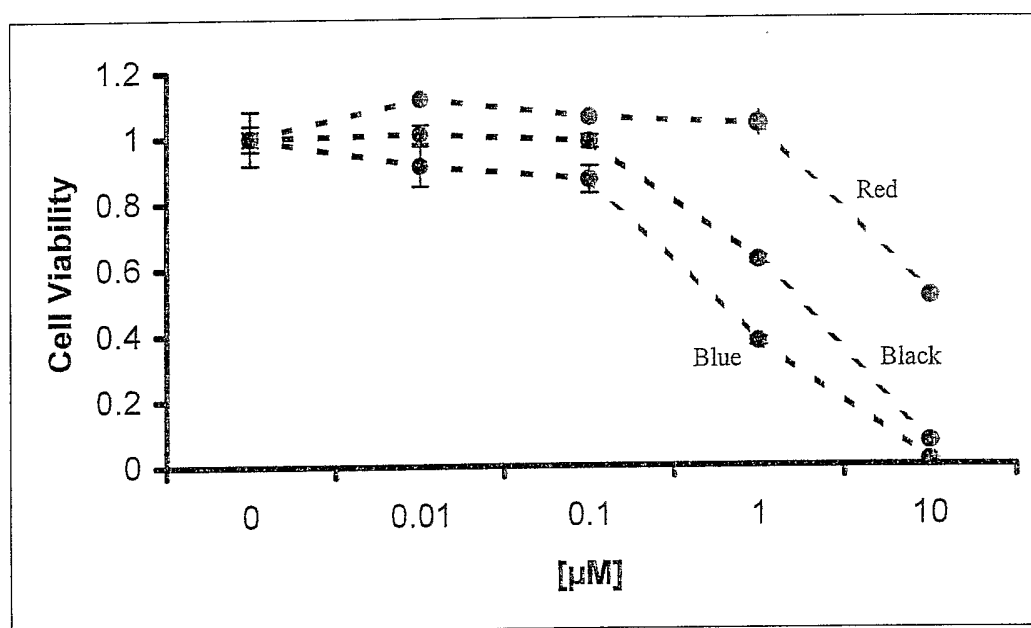
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FIGURE 2



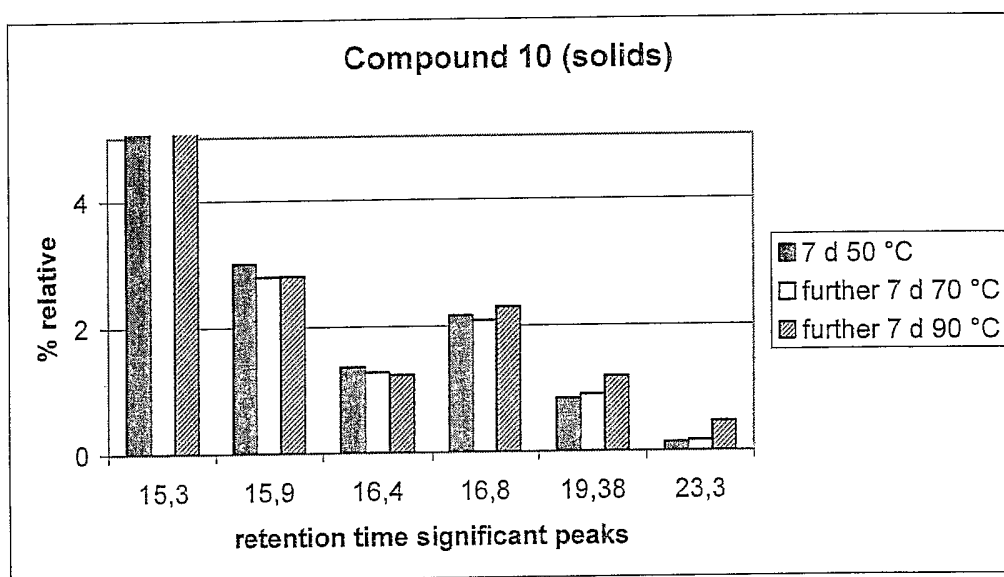
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FIGURE 3



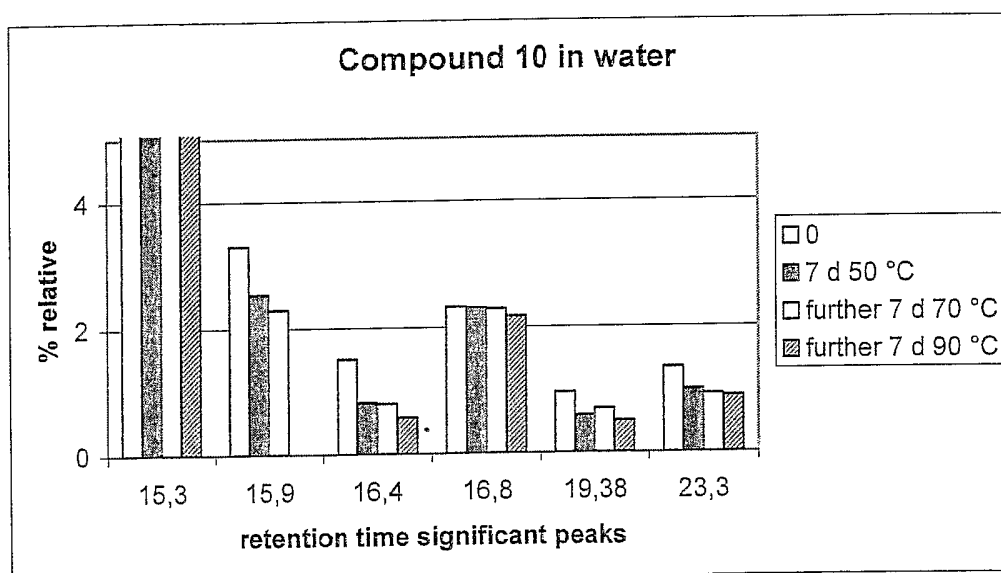
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FIGURE 4(A)



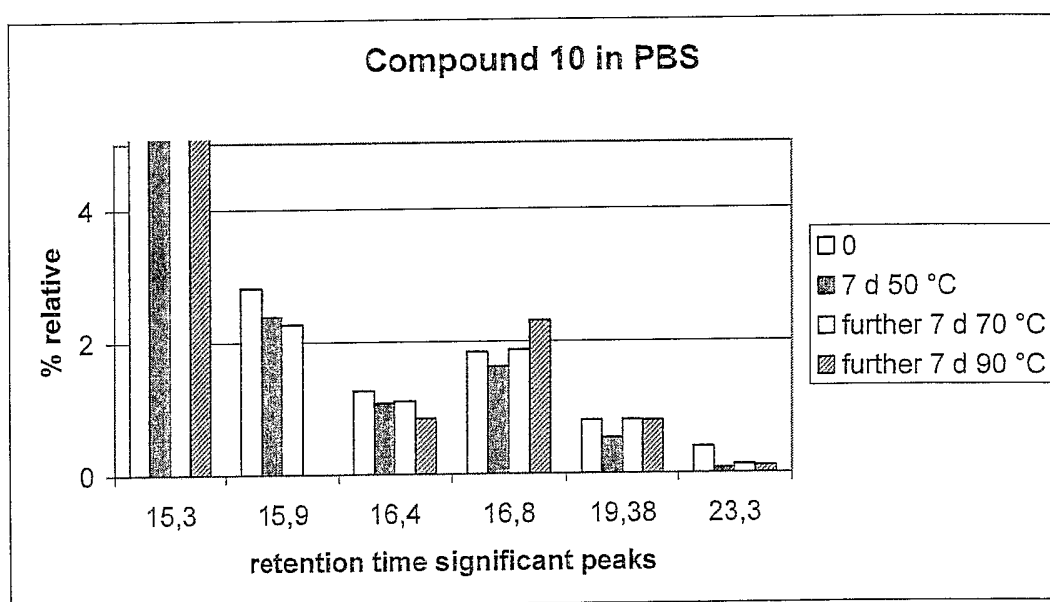
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FIGURE 4(b)



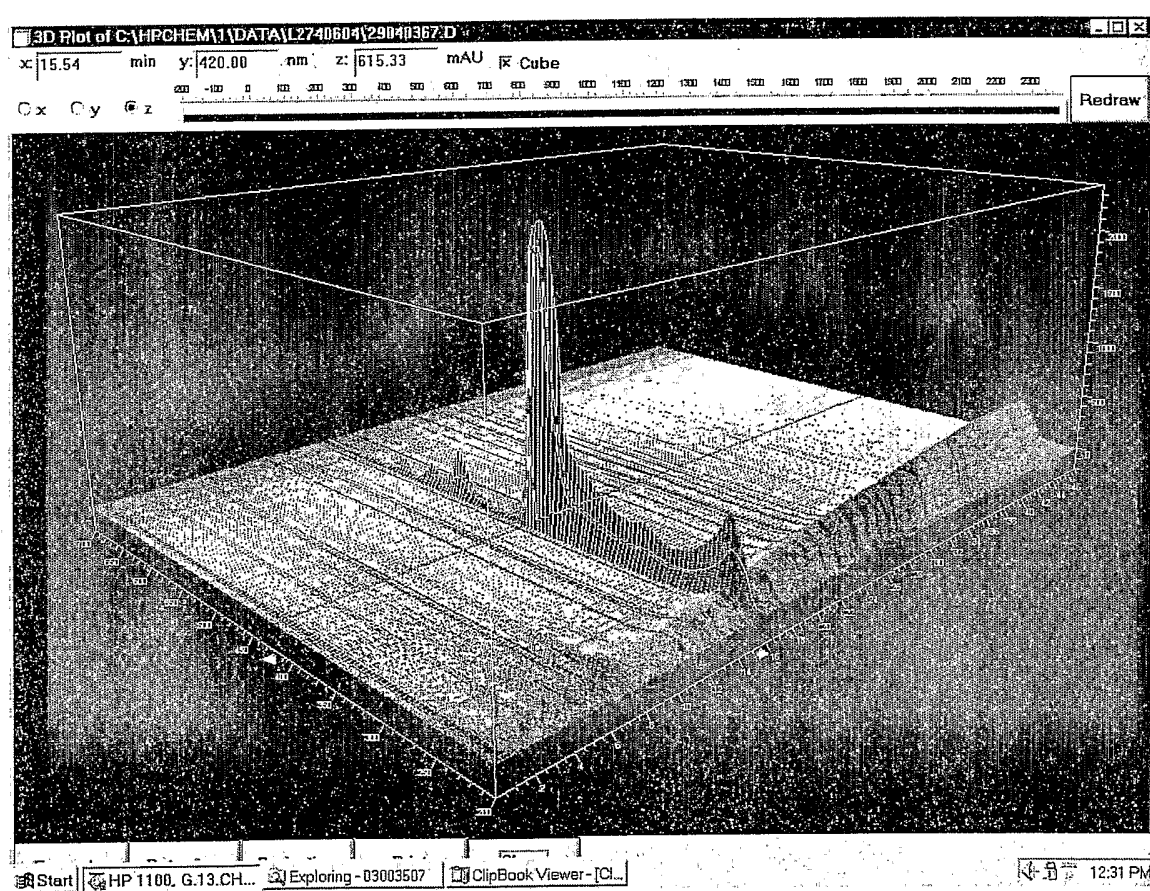
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FIGURE 4(C)



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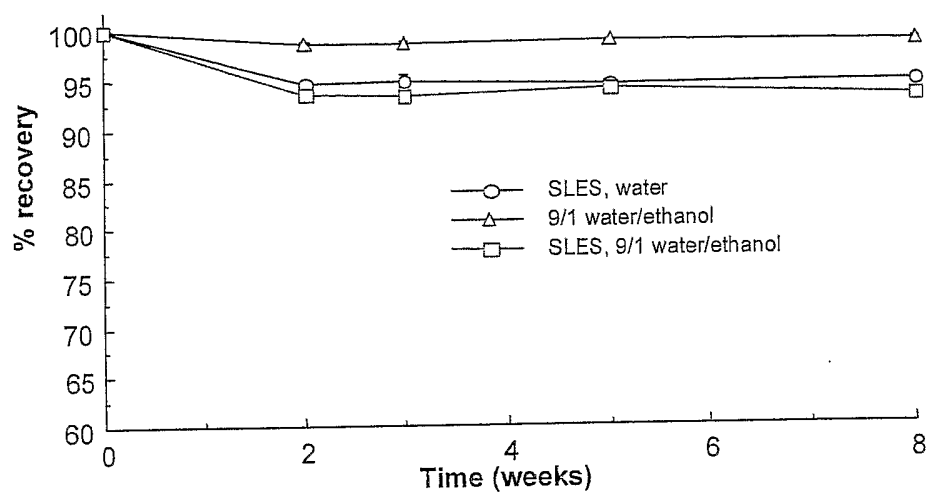
FIGURE 5



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FIGURE 6(A)

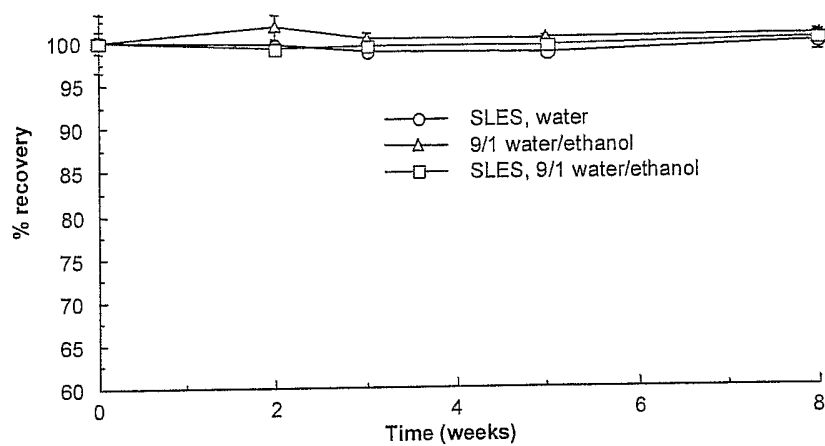
## Compound 1



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FIGURE 6(B)

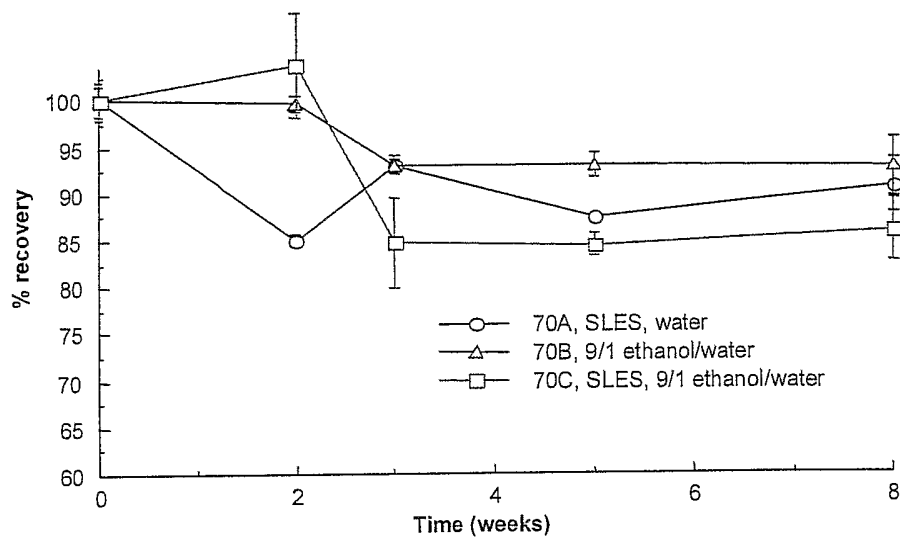
## Compound 8



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FIGURE 6(C)

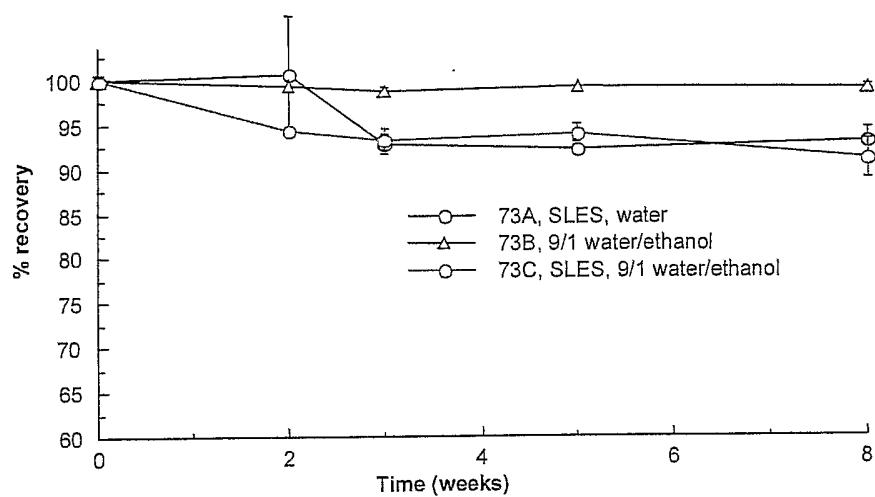
## Compound 12



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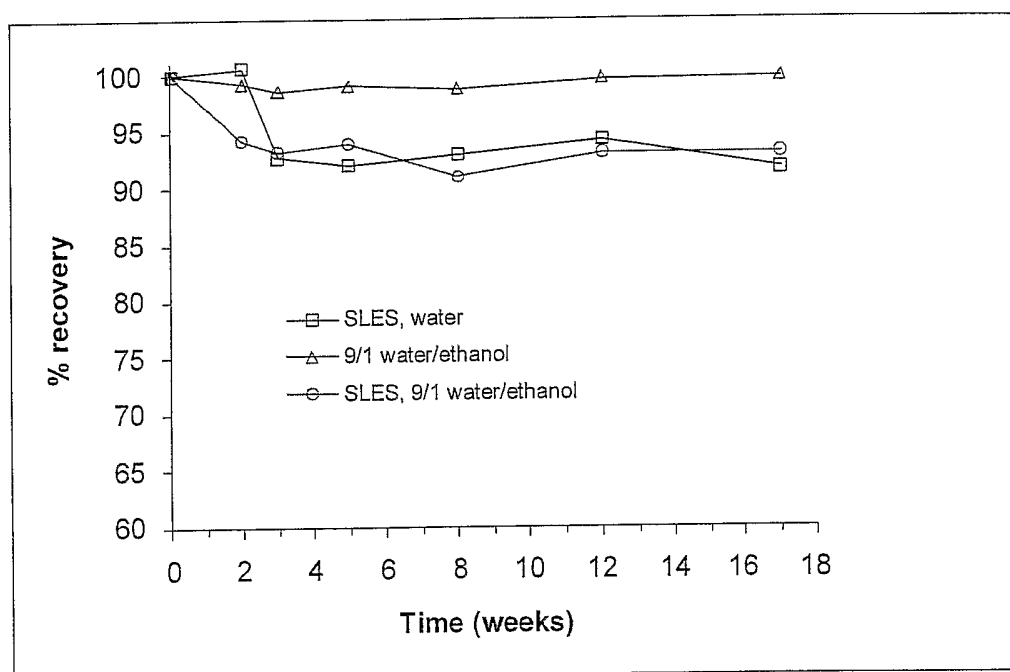
FIGURE 6(D)

## Compound 10



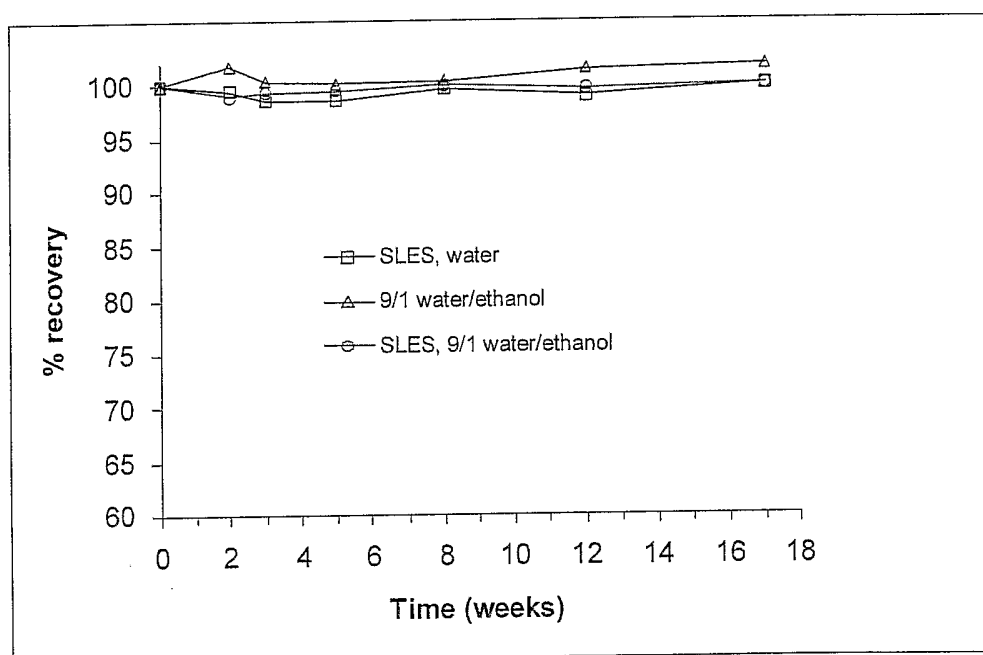
*12/13*

FIGURE 7(A)



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FIGURE 7(B)



# INTERNATIONAL SEARCH REPORT

PCT/GB2005/002457

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/409 A61P31/00

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)

IPC 7 A61K A61P

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 practical, search terms used)

EPO-Internal, WPI Data, PAJ, EMBASE, BIOSIS, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	WO 2004/035590 A (L. MOLteni & C. DEI FRATELLI ALITTI SOCIETA DI ESERCIZIO S.P.A; RONCUC) 29 April 2004 (2004-04-29)	1-74
P,X	WO 2004/056828 A (DESTINY PHARMA LIMITED; SOLVIAS AG; BRUNDISH, DEREK; FENG, XIANG, DONG) 8 July 2004 (2004-07-08) the whole document page 16, line 17 - line 27 page 27, line 4 - page 28, line 8 compounds 8,6,28,31,32 table 1 table 2 claims 55-61 figure 2/28	1-74

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

21 September 2005

Date of mailing of the international search report

30/09/2005

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

PCT/GB2005/002457

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