LIPO- OR AMPHIPHILIC SCINTILLATORS AND THEIR USE IN ASSAYS

The present invention provides novel scintillator compounds, the production of these novel scintillator compounds and their use in assay systems. The invention also provides liposomes or micelles containing the novel scintillator compounds, methods for preparing the liposomes or micelles containing the novel scintillator compounds and the use of the liposomes or micelles as delivery vehicles to insert the scintillator compounds into the cell membranes of cells. In addition, the invention provides cells containing the novel scintillator compounds and the use of the cells in assay systems.

The development of new drugs and therapies is dependent upon the identification of biological targets. Once identified, a target receptor, enzyme or protein can be used to identify new ligands leading ultimately to the development of new drugs. One method of monitoring molecular interactions in chemical or biological systems involves the use of scintillators.

Scintillators are molecules, which are or comprise a phosphor. A phosphor is a chemical structure, which is capable of luminescence following excitation by a number of different species (e.g. a photon, an electron or a chemical reaction). Interaction of a phosphor with an ionising particle such as a beta particle, an auger electron or a photon results in the excitation of the phosphor. After a short delay (usually in the region of 10^{-10} to 10^{-4} seconds) the phosphor returns to its ground (non-excited) state with the release of photons of electromagnetic energy usually in the form of visible light or other forms of detectable radiation. The light emitted by the relaxation of a scintillator can be detected and measured quantitatively in an appropriate scintillation counter.

For the purposes of the present invention the term scintillant molecule or scintillator is used to define a molecule which is or comprises a phosphor and scintillation is used to

define the electromagnetic radiation (usually in the form of light) produced by such a scintillant molecule.

As discussed above, scintillation occurs as a result of the interaction between a phosphor and an ionising particle. Ionising particles are produced by sources of ionising radiation such as radioactive compounds. In order for excitation of the scintillator and resulting scintillation to occur, the ionising particle must interact directly, or indirectly via solvent molecules, with the scintillator. An example of a radioactive compound is the species tritium which is routinely used to radiolabel compounds. Tritium emits ionising radiation with a very short path length, e.g. in water the average path length of tritium is 1.5 micrometres. If the distance between the triturated molecule and the scintillator molecule is less than 1.5 micrometres, scintillation will occur. At distances significantly greater than 1.5 micrometres, no significant scintillation will occur between the scintillator molecule and the radiolabelled compound in water.

As the amount of light emitted by the scintillator is proportional to the proximity of the source of ionising particles, scintillation measurements can be used to make a quantitative assessment of the proximity and potentially the extent of binding of two species.

Previous applications of scintillant molecules have involved the use of solid supports such as polymeric resins or beads incorporating the scintillators. Scintillation Proximity Assay (SPA) involves beads, for example Sepharose beads, impregnated with fluorescent molecules. The beads are coated with a biological receptor molecule through either non-specific, non-covalent interactions or covalent bonds. The beads are then incubated with a radiolabelled ligand. The radiolabel is chosen to have a short wavelength in water. If the receptor binds to the ligand, a significant portion of the radioactivity is brought into close proximity with the impregnated fluorescent molecules, which become activated and emit light.

One problem associated with this method is that the beads can only be used in aqueous systems, as the use of organic solvents dissolves the fluorescent molecules removing them from the bead. This problem was addressed in WO00/20475 wherein the scintillator molecules were incorporated directly into the solid support by polymerisation.

A further problem is associated with the scintillation proximity assay. In order to attach a target molecule, such as a receptor, protein or enzyme to a solid support, it is necessary initially to isolate the target and then purify it. Some target molecules will lose activity as a result of this isolation procedure. Proteins which exhibit strongly hydrophobic characteristics such as intrinsically or extrinsically membrane bound proteins (enzymes, receptors or channels) are notoriously difficult to purify and lose activity when they are removed from the hydrophobic membrane. In addition, such proteins will denature when exposed to the aqueous conditions necessary for SPA. For multisubunit complexes, isolation of the protein may result in the loss of one or more of the subunits thereby inactivating the protein.

In addition, in order to attach the protein to the solid support it is necessary to form an attachment from the protein to the solid support. This attachment can be in the form of a covalent, ionic or hydrogen bond. In addition the attachment may be formed by a polar, non-polar, hydrophobic or hydrophilic interaction. In some cases, the formation of this attachment may distort or block the binding site for a particular ligand.

An alternative application of scintillant molecules is in scintillation plate technology (as discussed in WO94/26413) where a plate composed of a mixture of scintillant molecules and plastic is prepared and sealed with a coating. Cells are cultured on the plate and incubated with radioactive ligands.

One major disadvantage of scintillation proximity assays and the scintillation plate technology is the need to use ^{125}Iodine as the source of ionising radiation in some applications. ^{125}Iodine is principally a gamma and X-ray emitter whose use requires stringent safety measures.

The present invention relates to scintillator molecules containing a phosphor head and a lipophilic or amphiphilic tail and methods for their manufacture. In addition, the invention relates to liposomes incorporating one or more scintillator molecules inserted into the lipid bilayer of the liposome or micelles incorporating one or more scintillator molecule inserted into the monolayer of the micelle. Furthermore, the invention relates to methods of inserting the scintillator molecules into the liposome or micelle. In addition, the invention relates to cells incorporating one or more scintillator molecules inserted into the cell membrane of the cell and methods of inserting such scintillator molecules into the cell. The invention further relates to the use of the scintillator molecules in assay systems. One such use is to identify target molecules in their cellular environment without the need for purification or isolation of the targets and therefore to develop new drugs and therapies. These assays will allow ligands to be produced for targets, which could not previously be assayed due to their instability or high degree of hydrophobicity.

The first aspect of the present invention provides a scintillator molecule which comprises one or more moieties X, which is a phosphor, covalently attached to one or more moieties R^1, which is a lipophilic or amphiphilic group wherein the scintillator molecule is capable of integrating into a cell, a cell membrane, a biological membrane, an artificial membrane or a delivery vehicle and when integrated can act as a phosphor.

In the context of the present invention, the term "membrane" encompasses cell membranes, synthetic or artificial membrane preparations, including liposomes, micelles and the like. A delivery vehicle for the purposes of this invention is any

agent or carrier (for example a liposome or micelle) which allows the insertion of a scintillator molecule of the invention into a membrane.

The number of X and R^1 moieties will depend on the size of the scintillator molecule required, and may be such that the scintillator molecule is a oligomer of X and R^1 groups. Mixtures of different X and R^1 moieties may also be used. The oligomer may be composed of equal numbers of X and R^1 groups. Alternatively, different numbers of X and R^1 moieties may be used. The skilled person will appreciate that the size of the scintillator molecule is limited by the requirement that the scintillator molecule can be incorporated into the cell membrane and that the presence of the scintillator molecule is tolerated by the cell.

In a preferred embodiment, the scintillator molecule has the formula (I)

$$(X)_m\text{-}(R^1)_n \qquad (I)$$

or a salt thereof wherein m is 1 to 20 and n is 1 to 20, preferably m and n are independently 1 to 10, more preferably m and n are independently 1 to 4, most preferably m and n are both 1.

For the purposes of this invention, the lipophilic moiety is a chemical group, which exhibits lipophilic (or hydrophobic) properties.

Lipophilicity represents the affinity of a molecule or a moiety for a lipophilic environment. It is commonly measured by the distribution behaviour of the molecule in a biphasic system, either liquid-liquid (partition coefficient in 1-octanol/water) or solid-liquid (retention on reversed-phase high-performance liquid chromatography (RP-HPLC) or thin-layer chromatography (TLC) system).

For the purposes of this invention, the amphiphilic moiety is a chemical structure that exhibits both lipophilic and lipophobic properties. Thus the structure usually

comprises different regions which can behave in a discrete fashion in aqueous or organic solvents.

It will be appreciated that the R^1 group may contain one or more sites for the attachment of the group X.

Preferably R^1 is an unbranched or branched alkyl, alkoxy, alkenyl, alkenyoxy, alkynyl, alkynyloxy, aryl, heteroaryl, amide, amine, alkylaryl, alkyoxyaryl, reduced arylalkyl, reduced alkoxyaryl, alkenylaryl, alkenyloxyaryl, alkylheteroaryl, alkyoxyheteroaryl, alkenylheteroaryl, alkenyloxyheteroaryl, reduced alkylheteroaryl, reduced alkoxyheteroaryl or a substituted derivative of any of the foregoing groups, substituted by one or more groups independently selected from halogen, alkyl, alkoxy arylalkyl, arylalkoxy, cyano, nitro, $-OC(O)R^2$, $-CO_2R^2$, $-NR^3R^4$, $-OR^2$, $-SR^2$, $-C(O)NR^3R^4$, - or a lipid (natural or synthetic)

wherein R^2 is selected from the group comprising H, alkyl, aryl or a group as defined for R^1

and wherein R^3 and R^4 are independently selected from the group comprising H, alkyl, aryl or a group as defined from R^1.

In a preferred embodiment, R^1 is an unbranched or branched alkyl, alkoxy, alkenyl, alkenyloxy or alkylaryl or a substituted derivative of any of the foregoing groups, substituted by one or more groups independently selected from $-CO_2R^2$, $-OR^2$ or $-OC(O)R^2$.

wherein R^2 is selected from H, alkyl or aryl.

The R^1 group should confer sufficient lipophilicity to allow the scintillator molecule to incorporate or become associated with the membrane of a cell. It will be appreciated

that where a scintillator molecule is composed of one X group and one R^1, if R^1 is methyl or ethyl, these groups are unlikely to confer sufficient lipophilicity onto the scintillator molecule. However, if a scintillator group contains one X group and multiple R^1 groups (i.e. more than one R^1 group) where R^1 is methyl or ethyl, the presence of multiple methyl or ethyl groups would be sufficient to allow the scintillator molecule to incorporate or become associated with the cell membrane.

Preferably alkyl is from 1 to 30 carbon atoms, more preferably from 6 to 30 carbon atoms, most preferably from 8 to 20 carbon atoms. Alkoxy is an hydrocarbon chain from 1 to 30 carbon atoms, preferably from 1 to 20 carbon atoms most preferably 1 to 10 carbon atoms, optionally interrupted with one or more oxygen atom. In a preferred embodiment, an oxygen atom within the alkoxy moiety is separated from the phosphor group X by at least one carbon atom (preferably saturated). Alkenyl is a hydrocarbon chain containing one or more double bond wherein the alkenyl group is from 2 to 30 carbons atoms, preferably from 8 to 30 carbon atoms. Alkynyl is a hydrocarbon chain containing one or more triple bonds wherein the alkynyl group is from 2 to 30 carbon atoms, preferably from 8 to 30 carbon atoms. Aryl is an aromatic ring e.g. phenyl or a six-membered aromatic ring or a fused ring system e.g. napthyl, anthracyl. Heteroaryl is a five or six membered aromatic ring containing one or more heteroatom selected from O, S or N or a fused ring system containing two or more fused rings wherein one or more of the fused rings contain a heteroatom. Examples of such groups include pyridyl, pyrimidyl, pyridazinyl, pyrazinyl, thiophenyl, oxazolyl, pyrazolyl, quinolinyl, quinazolinyl and indolyl.

For the purpose of this invention, a lipid is an organic molecule, which can be isolated from cells and tissues by extraction with non-polar organic solvent or a synthetic equivalent or derivative of such a molecule.

Group X for the purposes of this invention comprises any chemical group which is capable of luminescence following excitation by a number of different species such as a photon, an electron or a chemical reaction, preferably a source of ionising radiation.

Examples of group X for the purposes of this invention include benzotriazoles and their derivatives, coumarins and their derivatives, aromatic hydrocarbons such as p-terphenyl, p-quaterphenyl and their derivatives, derivative of oxazoles and 1,3,4 oxadiazoles such as 2,4-(biphenylyl-6-phenylbenzoxaole, 2,5-bis-(5'-tert-butylbenzoxazolyly-[2']thiophene, 2-(4-t-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxadiazole, 2-(1-naphthyl)-5-phenyl-oxazole, 2-phenyl-5-(4-biphenylyl)-1,3,4-oxadiazole, 1,4-bis[5-phenyl-2-oxazolyl]-benzene, and 2,5-diphenyloxazole. Further examples of group X include 1,4-bis(5-pheny-2-oxazolyl)benzene, 9,10-diphenylanthracene, 1,6-diphenyl-1,3,4-hexatriene, trans-p,p'diphenylstilbene, 1,14,4-tetraphenyl-1,3butadiene, 3-hydroxyflavone, and 1,4-bis(2-methylstyryl)benzene.

It will be appreciated the head group may contain one or more sites for the attachment of the lipophilic R^1 group.

In a further preferred embodiment of the first aspect of the invention, the scintillator molecule contains one head group (i.e. m = 1). The head group may contain two or more sites for the attachment of the lipophilic R^1 group. Thus, a compound of the formula

$$X\text{-}(R^1)_n \qquad \text{(II(a))}$$

where n is an integer equal to or greater than 2.

In yet another preferred embodiment, the scintillator molecule contains one lipophilic R^1 group (i.e. n = 1). The lipophilic R^1 group may contain two or more sites for the attachment of the head group X resulting in a compound of the formula

$$(X)_m\text{-}R^1 \qquad \text{(II(b))}$$

where m is an integer equal to or greater than 2.

In the most preferred embodiment of the invention, there is provided a scintillator molecule of the formula

$$X\text{-}R^1 \qquad \text{(III)}$$

wherein one X group is attached to one lipophilic group R^1 (i.e. m and n = 1).

The scintillator molecules of the first aspect of the invention may comprise one or more X groups attached to one or more R^1 groups via a linker moiety to produce a scintillator molecule of formula (V);

$$(X)_m\text{-(linker)-}(R^1)_n \qquad \text{(V)}$$

For the purposes of this invention, the linker moiety can be alkoxy, aminoalkyl, aryloxy, arylamino, alkylacid, carbonyl amidyl, ester, amine, silyl, imidyl, urea, alkylthio, arylthio.

The linker may provide multiple sites for the attachment of one or more X group and one or more R^1 group. Examples of such linkers include amino acids such as lysine, glutamic acid, aspartic acid, polyalcohols such as glycerol, polythiols, polyacids or polyamines.

Suitably, the scintillator molecule consists of one X group linked via a linker to two or more R^1 groups. Alternatively the scintillator molecule consists of one lipophilic R^1

group linked via a linker to two or more head groups. Preferably the molecule consists of one R^1 group and one X group linked by a suitable linker group.

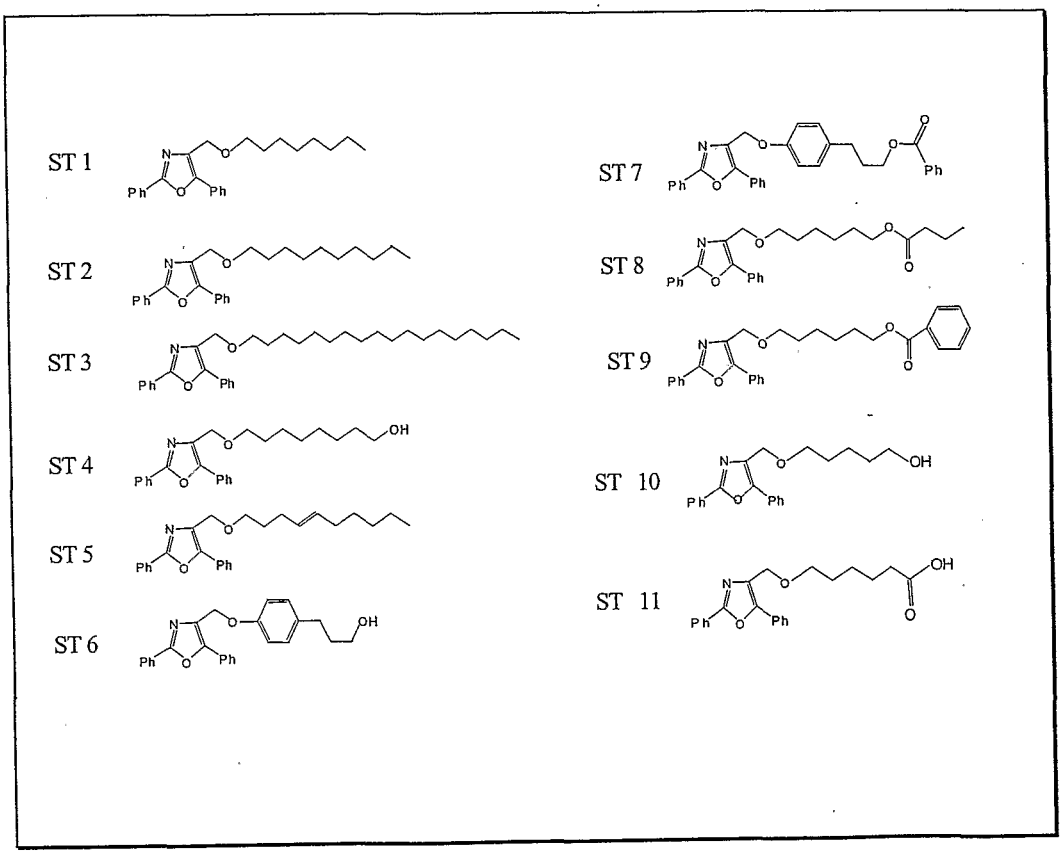
In a preferred embodiment of the invention, the phosphor head is 2,5-diphenyloxazole (PPO) or a derivative of 2,5-diphenyloxazole. More preferably the 2,5-diphenyloxazole is derivatised at the 4-carbon with a linker group or directly with the lipophilic tail.

Examples of salts of the compounds of formulae (I) to (VI) include those derived from organic acids such as methanesulphonic acid, benzenesulphonic acid and p-toluenesulphonic acid, mineral acids such as hydrochloric and sulphuric acid and the like, giving methanesulphonate, benzenesulphonate, p-toluenesulphonate, hydrochloride and sulphate, and the like, respectively or those derived from bases such as organic and inorganic bases. Examples of suitable inorganic bases for the formation of salts of compounds for this invention include the hydroxides, carbonates, and bicarbonates of ammonia, lithium, sodium, calcium, potassium, aluminium, iron, magnesium, zinc and the like. Salts can also be formed with suitable organic bases. Such bases suitable for the formation of base addition salts with compounds of the present invention include organic bases that are non-toxic and strong enough to form salts. Such organic bases are already well known in the art and may include amino acids such as arginine and lysine, mono-, di-, or trihydroxyalkylamines such as mono-, di-, and triethanolamine, choline, mono-, di-, and trialkylamines, such as methylamine, dimethylamine, and trimethylamine, guanidine; N-methylglucosamine; N-methylpiperazine; morpholine; ethylenediamine; N-benzylphenethylamine; tris(hydroxymethyl) aminomethane; and the like.

Salts may be prepared in a conventional manner using methods well known in the art. Acid addition salts of said basic compounds may be prepared by dissolving the free base compounds according to the first or second aspects of the invention in suitable solvents containing the required acid. Where a compound of formula (I) contains an

acidic function a base salt of said compound may be prepared by reacting said compound with a suitable base. The acid or base salt may separate directly or can be obtained by concentrating the solution e.g. by evaporation.

Examples of preferred compounds of the invention include



A second aspect of the invention provides a process for the manufacture of a compound or derivative according to the first aspect of the invention.

In general, the compounds of the invention are provided by coupling one or more group X to one or more group R^1. Such coupling reactions can be carried out using

conventional coupling techniques known to the person skilled in the art. The particular coupling reaction used will depend on the functionality present on the X and R^1 group and on the bond to be formed.

More preferably, the preferred compounds of the first aspect of the invention are produced according to the following processes;

Alkylation of 4-alcohol derivative of 2,5-diphenyloxazole with a haloalkyl group in the presence of a base. It should be appreciated that the haloalkyl group may contain additional functional groups that may require protection prior to the alkylation step. If protection is required then this can be achieved by any conventional protecting reagents. After the alkylation step, the protecting groups can be removed using conventional deprotection techniques or one or more protecting groups can remain on the scintillator compound.

or

Alkylation of 4-halogen derivative of 2,5-diphenyloxazole with an alcohol, in the presence of a base. Again, the alcohol may contain additional functional groups that may require protection prior to the alkylation step. Such protection can be carried out using conventional protecting reagents. Removal of the protecting groups can be achieved using conventional deprotecting techniques however one or more protecting groups can remain on the scintillator compound.

or

Conversion of one scintillator molecule of the invention into a different scintillator molecule of the invention. Examples of processes by which this could be achieved include;

Removal of protecting groups

Addition of protecting groups

Oxidation (e.g. an alcohol to an aldehyde or ketone, or an aldehyde to an acid group)

Esterification of an acid or an alcohol

Amidation of an acid or an amine

Reduction (e.g. of an alkene to an alkane, or an aldehyde to an alcohol)

The production of a salt of a particular compound

Other functional group interconversions (for example bromination of an alcohol)

Functionalisation of the 2,5-diphenyloxazole group at the 4-position can be carried out using techniques known to the skilled person. Preferably, the 2,5-diphenyloxazole is initially converted to ethyl 2,5-diphenyl-4-oxazolecarboxylate (Tanaka et al *Chem Abs.* (1963), *58*, 3407f)) and then reduced using an ethanolic solution of sodium borohydride (Clapham et al *Tetrahedron Lett.*, (1997), 52, 9061). Interconversion of an alcohol to a halide can be carried out using reagents known in the art.

All preferred features of the first aspect of the invention apply to the second aspect of the invention.

A third aspect of this invention provides a liposome incorporating a scintillator molecule of the invention.

For the purposes of this invention, a liposome is a spherical structure composed of a bilayer of lipid molecules. It is proposed that the scintillator molecule is inserted into the lipid bilayer via its lipid tail. The lipid molecules making up the liposome can be any naturally occurring or synthetic lipid. More preferably these lipid molecules are composed of a positively charged head group with a lipid tail. Preferably the liposomes comprise lipids selected from one or more of phosphatidyl choline (PC),

dioleoyltrimethylammonium propane (DOTAP), dieoleoylphosphatidylethanolamine (DOPE) or dioleoyloxypropyl-trimethylamine ammonium chloride (DOTMA).

There are a large variety of other lipids that may be suitable for forming liposomes and for delivering the scintillator molecules of the invention into the cell membrane, these include cationic lipids having single chain hydrocarbons, double chain hydrocarbons or cholesterol as the hydrophobic anchor. A comprehensive review of this subject can be found in Chesnoy S. and Huang L. Structure and function of lipid-DNA complexes for gene delivery. *Annu. Rev. Biophys. Biomol. Struct.* **29.** 27-47; 2000.

In a preferred feature of this invention the liposome-scintillator conjugate is provided as a delivery system which allows the insertion of the scintillator molecule into the cell membrane of a cell.

Alternatively, the third aspect of the invention provides a micelle incorporating a scintillator molecule of the invention.

All preferred features of the first and second aspects of the invention also apply to the third aspect.

A fourth aspect of the invention provides a method for inserting the scintillator molecules into the liposome. In one example of this method, the lipids and the scintillator molecules are incubated together in an organic solvent, which is then removed, and the resulting mixture resuspended in water to form the liposomes. The resuspended liposome-scintillator molecule complex can then be freeze-dried and provided as a lyophilised powder. Alternatively, the liposome-scintillator molecule complex is provided as a suspension in aqueous medium (for example, water or aqueous buffer or a solution of detergent).

In an alternative method it is necessary to warm the liposome suspension in order to form the liposomes. Preferably the liposome suspension is warmed to a temperature between 80 and 30°C, more preferably between 70 and 50°C, most preferably at 60°c or above.

The lipids and scintillator molecules are incubated in a ratio of between 1:1 and 10:1 scintillator molecule to lipid, more preferably from 2:1 to 8:1 scintillator molecule to lipid and more preferably 4:1 scintillator molecule to lipid.

In an alternative method, the scintillator molecules are incubated with preformed liposomes.

It will be appreciated that the formation of liposomes is well known in the art. Therefore, the skilled person will be able to produce liposomes of different sizes, compositions etc, incorporating the scintillator molecules.

The use of micelles in delivering the scintillator molecules to the cell membranes has been investigated as an alternative to liposomes. Micelles are aggregates of lipophilic molecules such as surfactants and have been used widely in drug delivery. In one embodiment of the invention, the micelles were formed using the surfactant dodecyltrimethylammonium bromide (DTAB) which was dissolved in phosphate-buffered saline (PBS) at either 0.1%, 0.5% or 1.0%, preferably at 0.1%. In a further embodiment, from 0.1mg to 25 mg of scintillator molecule, preferably from 0.5 mg to 15mg of scintillator molecule, more preferably 10mg or above of scintillator molecule was dispersed into 10 mls of a 1% DTAB solution.

Therefore the fourth aspect of the invention also provides a method for inserting the scintillator molecules into a micelle.

All preferred features of the first, second and third aspects of the invention also apply to the fourth aspect.

A fifth aspect of the invention provides a cell incorporating a scintillator molecule where the scintillator molecule is inserted into the cell membrane of the cell without affecting the normal cellular functioning. Preferably, the cell contains a scintillator molecule inserted into the membrane and a membrane bound protein or receptor of interest.

The cell can be one or more prokaryotic (e.g. bacteria) or eukaryotic (e.g. fungi, mammals, reptiles, insects, fish) cells. Preferably the cells are mammalian cells.

The cell of the fifth aspect of the invention may be taken from any cell-line expressing a protein of interest. The protein may be native or introduced into the cells and may be constitutive (i.e. expressed at all times) or induced (produced in response to certain stimuli). The protein may be membrane bound (integral or extrinsic) or may be located in the cytosol. Alternatively, a cytosolic protein may be engineered so that it is expressed on the internal or external cell surface. The cell may be modified so that the level of protein expressed by the cell is increased above its normal level.

The cell can be a germ line cell or a stem cell. In addition, the cells can be provided as mixed populations of cells (i.e. of more than one type or from more than one source).

The scintillator molecule loaded cells could be used in a variety of cell-based assays where biological or drug activity is monitored by use of radiolabelled molecules. Assay systems incorporating these scintillator molecules can be used to investigate cellular processes in real time. Assay systems where these scintillator molecules can be used include (i) incorporation assays (ii) transport assays, (iii) binding assays and (iv) signal transduction studies.

(i) Incorporation assays are used to monitor intracellular biochemical processes such as protein synthesis ($^{14}C/^{3}H/^{35}S$-methionine incorporation), cell proliferation and DNA repair/damage ($^{14}C/^{3}H$-thymidine uptake/incorporation) and lipid metabolism (incorporation of $^{14}C/^{3}H$ labelled precursors).

(ii) Membrane transport assays, measuring the transport of a wide variety of radiolabelled ($^{3}H/^{14}C/^{35}S/^{45}Ca/^{125}I$) substrates into (influx) or out (efflux) of cells can be used to monitor the activity of channels and carriers. In addition to kinetic studies of membrane transport proteins inhibitor/activator studies can also be carried leading to the identification of transport inhibitors that may be potential therapeutic agents.

(iii) Binding assays including receptor binding assays require binding of a radiolabelled ($^{3}H/^{14}C/^{35}S/^{45}Ca/^{125}I$) ligand from which both receptor number and affinity can be calculated. Competition studies can also be used to assess potential inhibitors/activators of target receptors. A wide range of ligands are commercially available covering all types of receptor including purinergic, adrenergic and nicotinic receptors amongst many others.

(iv) Signal transduction activity can also be investigated with radiolabelled second messengers and substrates. G-protein activity can be monitored using ^{35}S-GTP gamma-S. [^{33}P]-ATP binding can be used to monitor protein-phosphorylation. Calcium plays a major role in signal transduction and ^{45}Ca can be used to measure calcium uptake. Radiolabelled IP3, IP4 and cAMP are also commercially available and can be used to investigate signal transduction.

In general, measuring the activity of radioisotopes requires cell disruption or isolation of cell membranes; real time measurements are also not possible. With the use of the

scintillator molecules of the first aspect of the invention incorporated into cell membranes these problems can be overcome.

An alternative embodiment of the fifth aspect of the invention provides a liposome or micelle incorporating one or more scintillator molecules of the first aspect and one or more proteins or receptors of interest. For the purposes of the invention, the protein or receptor is purified, partially purified or recombinant. As discussed above, the protein may be cytosolic (e.g. protein kinase) or membrane bound (e.g. seven transmembrane receptors or ion channels). Such liposomes or micelles can be used in the binding assay systems as discussed above.

All preferred features of the first, second, third and fourth aspects of the invention also apply to the fifth aspect.

A sixth aspect of the invention provides a method of inserting the scintillator molecules of the first aspect into the cell. In one method, the scintillator molecule is initially inserted into a liposome or micelle. The scintillator molecule-micelles or scintillator molecule-liposomes are incubated with the cells preferably at 20 – 40°C, preferably 37°C for between 30 and 120 minutes. In an alternative method the scintillator molecule is incubated directly with the cell.

All preferred aspects of the first, second, third, fourth and fifth aspects of the invention, also apply to the sixth aspect.

A seventh aspect of the invention provides a kit comprising a liposome or micelle and the scintillator molecule of the invention. In an alternative embodiment, the kit comprises one or more reagents for the formation of liposomes or micelles e.g. detergent molecules or lipids and a scintillator molecule of the invention.

All preferred features of the first, second, third, fourth, fifth and sixth aspect of the invention also apply to the seventh aspect.

An eighth aspect of the invention provides an assay system comprising a cell, one or more scintillator molecules of the invention and a protein or receptor of interest.

In one embodiment of the invention, the scintillator molecules are inserted into the cell membrane of a cell containing a particular receptor. Radiolabelled drug molecules bind to the receptors and trigger scintillation as a result of their interaction with the membrane anchored scintillator molecules. The technique can then be used to identify new drugs (competing molecules) that bind to receptors through their ability to displace the radiolabelled ligand. The identification of a new drug will be identified when the radiolabelled ligand is displaced and no scintillation is observed.

In an alternative embodiment the scintillator molecules are used in conjunction with antisense molecules to ascertain whether a particular molecule can bind to a given cellular protein. Antisense molecules prevent expression of a given protein. Thus when no scintillation occurs following application of a radiolabelled molecule, the true target for the drug has been identified.

One embodiment of the eighth aspect of the invention is the use of scintillator molecules to determine whether a particular species is being taken up by a cell. Amongst these applications are measurement of radiolabelled-methionine incorporation into cells to monitor protein synthesis and uptake and incorporation of radiolabelled-thymidine to monitor cell proliferation. In addition, the influx and efflux of radiolabelled substrates could be used to assess activity of membrane transport systems such as channels and the inhibition of these pathways by potential therapeutic drugs.

Further uptake experiments have indicated that the scintillator molecules can be successfully used when the source of ionising radiation is a compound labelled with ^{14}carbon, ^{45}calcium, ^{35}sulphur and ^{33}phosphorous. Thus, this scintillation assay has an advantage over the previously discussed scintillation proximity assays as it allows the use of radioisotopes other than ^{125}Iodine.

All preferred features of the first, second, third, fourth, fifth, sixth and seventh aspects of the invention also apply to the eighth aspect.

An ninth aspect of the invention provides a composition comprising one or more scintillator molecule of the invention. The composition may comprise one or more scintillator molecule of the first aspect of the invention in combination with a further ingredient including a stabilising agent, a detergent and/or a dye. The composition may be provided as a liquid, a solution, as a solid, a powder, a lyophilised powder, one or more granules or pellets.

In a further feature of the ninth aspect of the invention, the composition may comprise two or more scintillator molecules that have complementary or synergistic properties. For example, each of the scintillator molecules may scintillate with different radioisotopes allowing greater sensitivity to be achieved.

All preferred aspects of the first, second, third, fourth, fifth, sixth, seventh and eighth aspects of the invention also apply to the ninth aspect.

A tenth aspect of the invention provides a kit comprising a scintillator molecule for insertion into a particular cell line and a radiolabelled ligand. In an alternative embodiment, the scintillator molecule is provided inserted into a liposome or micelle delivery vehicle.

All preferred aspects of the first, second, third, fourth, fifth, sixth, seventh, eighth and ninth aspects of the invention also apply to the tenth aspect.

A eleventh aspect of the invention provides the use of a scintillator molecule of the first aspect of the invention in an assay system. Preferably the assay system involves the use of one or more prokaryotic or eukaryotic cells. More preferably the cells are mammalian.

In a preferred embodiment of the invention, the scintillator molecule is inserted into the cell membrane of the cell. The insertion of the scintillator molecule occurs either directly or via a delivery vehicle such as a liposome or micelle.

Preferably the scintillator molecules are used in combination with a radiolabelled moiety, wherein the moiety is radiolabelled with ^{14}C, ^{3}H, ^{35}S, ^{33}P, ^{45}Ca, ^{125}I.

The assay system described in the eleventh aspect of the invention can be used to identify cellular targets within cells.

All preferred aspects of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth aspects of the invention also apply to the eleventh aspect.

The invention is now illustrated by reference to the following non-limiting examples. These examples included are further illustrated by the drawings, which are summarised below.

Brief Description of the drawings

Figure 1 is a schematic representation of a cell containing inserted scintillator molecules and a transport channel of interest. Radiolabelled substrate can pass through this channel, bringing the substrate into proximity with the scintillator molecules and resulting in scintillation. Incubation with a transport inhibitor will

prevent the transport of the radioactive substrate through the channels thereby preventing scintillation.

Figure 2 is a comparison of the degree of scintillation of the scintillator molecules ST1-ST11 compared with the commercially available scintillant "Microscint".

Figure 3 shows the scintillant activity of the scintillator molecule-liposome preparations on the addition of 0.1 microCi ^{14}C-taurine to 0.5ml liposome solution.

Figure 4 shows the effect of increasing the ratio of scintillator molecule to liposome (DOTAP) on liposome formation.

Figure 5 indicates the linear response of the liposomes containing scintillant molecules observed when the activity of taurine is increased from 0.1 microCi to 1.0 microCi.

Figure 6 indicates the uptake of the fluorescent probe N-678 into HeLa cells after incubation with cationic liposomes (a) and 0.1% micelle preparations (b).

Figure 7 indicates the uptake of the scintillator molecules ST4, ST5 and ST6 after 30 and 120 minutes by HeLa cells

Figure 8 indicates the uptake of ^{14}C-methionine by HeLa cells containing inserted scintillator molecules. The insert shows methionine uptake in HeLa cells measured using a commercially available scintillation cocktail.

Figure 9 indicates the uptake of ^{14}C-methionine by rat C6 glioma containing inserted ST4 scintillator molecules. The insert shows methionine uptake in C6 glioma cells measured using a commercially available scintillation cocktail.

Figure 10 indicates the uptake of ^{35}S-methionine by HeLa cells. The insert shows methionine uptake in HeLa cells measured using a commercially available scintillation cocktail.

Figure 11 shows the results for ST4, initially there was no uptake of ^{14}C-methionine but after approximately 72 hours accumulation of ^{14}C-methionine began to occur. There was no significant difference between cells pre-incubated with ST4/DOTAP or ST4/DOTAP/ DOPE.

Figure 12 shows the results for compound ST4 with the inset showing thymidine uptake in HeLa cells measured using a commercially available scintillant cocktail.

Figure 13 shows the results of a glucocorticoid receptor binding assay carried out on HTC cells.

Figure 14 shows results of an experimentshowing displacement of ^{3}H-dexamethasone carried out on HTC cells.

Figure 15 shows the results of a β-adrenergic receptor binding assay was carried out on C6 glioma cells incubated with scintilipid.

Figure 16 shows the results of a Na^+/K^+ ATPase binding assay carried out on HeLa cells grown using ^{3}H-ouabain as the radioligand.

Examples

Example 1: Comparison of scintillator molecules with commercially available scintillants

The scintillant activity of the synthesised scintillator molecules was compared with a commercially available scintillant, Microscint 20 (Packard) using a method based on that described by Clapham, et al. (1997) *Tetrahedron Lett.* 38, 52. Serial dilutions of the scintilipids were prepared in toluene to give final concentration concentrations of 10 mM, 1 mM, 0.1 mM and 0.01 mM. A ^{14}C-hexadecane stock containing 0.1 μCi per 100 μl toluene was also prepared and in a 96 well plate format 100 μl of radiolabel was added to 100 μl of scintilipid per well, mixed and counted. For comparison, 100 μl of radiolabel was added to 100 μl of Microscint 20. The results are illustrated in figure 2.

For each scintilipid at 10 mM, the percentage efficiency of scintillation relative to Microscint 20 has been calculated and tabulated (Table 1).

Table 1.

Scintilipid	ST 1	ST 2	ST 3	ST 4	ST 5	ST 6	ST 7	ST 8
% Efficiency	34	33	30	15	13	10	8	12

Scintilipid	ST 9	ST 10	ST 11
% Efficiency	9	0.6	65

Additional experiments performed by the Inventors have found that none of the commercially available scintillants 3-hydroxyflavone, POPOP (1,4-bis[5-phenyl-2-oxazoyl]-benzene) or butyl-PBD (2-(4-t-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxadiazole) could be incorporated into liposomes.

Example 2: Incorporation of scintillator molecules into liposomes.

The lipids (a) phosphatidyl choline, b) dioleoyltrimethylammonium propane and c) dioleoyltrimethylammonium propane/dioleoylphosphatidylethanolamine) are dissolved in chloroform/methanol along with the scintilipid under investigation (1:1; lipid:scintilipid) and then evaporated to dryness under a stream of N_2. To ensure complete dehydration the lipids are finally dried by vacuum for at least two hours. The liposomes are then formed by resuspension in H_2O to give a final lipid content of 10 mg/ml. In some cases warming the liposome suspension to 60°C was required. The liposomes were examined by microscopy to check for stable liposome structures and ^{14}C-taurine added to an aliquot of the liposome preparation to check for scintillation (Figure 3).

Scintillator molecules ST3 to ST7 and ST10 exhibited significant scintillant activity. Examination of the liposome preparations under a microscope give a qualitative measure of the incorporation of the scintilipid into the liposome membrane. The results indicate that ST4, ST5 and ST10 form the more stable liposomes and preparations with ST3, ST6 and ST7 have crystal deposits of the scintilipid. Therefore, further experiments examining the uptake of scintilipids into cells and the subsequent use in cell-based assays were carried out with ST4, ST5 and ST10.

Formulation of liposomes with increasing scintilipid content indicated that the optimum liposome preparation was 8mg scintilipid to 2mg DOTAP per ml of liposome suspension (Figure 4) and for subsequent experiments this is the liposome

composition used.

A linear response was observed when ^{14}C-taurine activity was increased from 0.1μCi to 1.0 μCi (Figure 5).

Example 3: Incorporation of scintillator molecules into cell membranes

Two approaches were used to incorporate scintilipid compounds into cell membranes. (A) Cationic liposomes containing DOTAP (as described above) and (B) micelles. Micelles are aggregates of lipophilic molecules such as surfactants and have been used widely in drug delivery. In this investigation we used the surfactant DTAB (dodecyltrimethylammonium bromide) which was dissolved in PBS at either 0.1%, 0.5% or 1.0%. Preliminary work indicated that up 10 mg scintilipid could be dispersed into 10 ml of a 1% DTAB solution.

Radiolabelled liposomes containing the scintilipid molecule were produced and the uptake by a widely available epithelial cell line (HeLa) was investigated. The radiolabelled liposomes were composed of 8mg scintilipid, 2mg DOTAP and 0.5 μCi ^{14}C-DOPE resuspended in 1 ml H_2O. The liposomes were then diluted 50% in phosphate-buffered saline (PBS) prior to incubation with the *HeLa cells*. *HeLa cells* were grown to confluence in DMEM in a 75 cm^2 flask, the cells trypsinised and seeded on to 96 well plates and incubated for approximately 48 hours to confluence at 37°C in an O_2/CO_2 incubator. The cells were then washed in PBS to remove the growth medium (presence of serum disrupts liposome uptake) and incubated with the radiolabelled liposome preparations (100 μl/well) for either 30 or 120 minutes at 37°C. After incubation the 96 well plates were washed thoroughly in PBS to remove any unbound liposomes and the plates counted for β-scintillation (Figure 7).

Scintillation counting indicated that the HeLa cells have taken up the scintilipid and that the proximity of the ^{14}C-DOPE that is also taken up produces scintillation.

Example 4: ^{14}C-Methionine uptake assay

HeLa cells were grown to confluence in DMEM in 75cm^2 flasks and then seeded onto 96 well plates and incubated overnight at 37°C. At various time points the DMEM was replaced with DMEM having ^{14}C-methionine added in PBS and the liposomes added. The composition of the liposomes being 8mg scintilipid, 2mg DOTAP resuspended in 1 ml H_2O and finally diluted in 1 ml PBS. The cells were incubated in the presence of liposomes for 120, 60 and 30 minutes.

Figure 8 shows the results for ST4, ST5 and ST10. The inset shows methionine uptake in HeLa cells measured using a commercially available scintillant cocktail (Microscint 20) and this mirrors the results for the ST4 and ST5 loaded cells. The scintillant activity of ST4 loaded cells is greater than ST5 or ST10 loaded cells, as would be predicted from the results in figure 4 and figure 7.

The methionine uptake assay was repeated in a rat neuronal cell line (C6 glioma). The C6 glioma cells were grown in Hams F12 medium but apart from this the experimental protocol was identical. The results are shown in figure 9 for ST4.

Example 5: ^{35}S-Methionine Uptake

The uptake of methionine into HeLa was repeated using ^{35}S-methionine. The results obtained were consistent with uptake of ^{14}C-methionine and are shown in figure 10.

Example 6: 'Real time' 14C-Methionine uptake assay

^{14}C methionine uptake was also shown in 'real time'. HeLa cells were grown to confluence in DMEM in 75 cm^2 flasks and the seeded onto 96 well plates and incubated overnight at 37°C. The HeLa cells were then incubated with scintilipid containing liposomes. The composition of the liposomes being 8mg scintilipid, 2mg DOTAP or 8mg scintilipid, 1mg DOTAP, 1mg DOPE, resuspended in 1ml H_2O and finally diluted in 1ml PBS. The cells were incubated in the presence of liposomes for 120 minutes at 37°C. DMEM containing ^{14}C-methionine was then added to and the uptake measured over time. Figure 11 shows the results for ST4, initially there was no uptake of ^{14}C-methionine but after approximately 72 hours accumulation of ^{14}C-methionine began to occur. There was no significant difference between cells pre-incubated with ST4/DOTAP or ST4/DOTAP/ DOPE.

Example 7: ^{14}C-Thymidine uptake assay

HeLa cells were grown to confluence in supplemented DMEM in 75cm^2 flasks and the seeded onto 24 well plates and incubated overnight at 37°C. The medium was then replaced with serum-free DMEM and incubated for 24 hours at 37°C. The serum-free DMEM was then replaced with DMEM containing 14C-thymidine (0.2 μCi/well) and incubated at 37°C for upto 24 hours. To terminate the uptake assay the cells were washed in PBS and liposomes added. The composition of the liposomes being 8mg scintilipid, 2mg DOTAP resuspended in 1ml H_2O and finally diluted in 1ml PBS. The cells were incubated in the presence of liposomes for 120, 60 and 30 minutes at 37°C. Figure 12 shows the results for ST4 with the inset showing thymidine uptake in HeLa cells measured using a commercially available scintillant cocktail.

Example 8: Radioligand binding assays

Three radioligand binding assays have been carried out using scintilipid technology, ^3H-dexamethasone binding to glucocorticoid receptors in HTC cells, ^3H-

dihydroalprenolol binding to β-adrenergic receptors in C6 glioma cells and ^{3}H-ouabain binding to the Na/K pump in HeLa cells.

Much of the experimental detail is common to all three assays carried out. Each of the cell lines were grown to confluence in 75cm^{2} flasks before being seeded into 96 well plates or 24 well plates. HTC cells were grown in minimum essential medium supplemented with essential amino acids, C6 glioma cells grown in Ham's F12 medium and HeLa cells were grown in DMEM. Once the cells were confluent they were washed in PBS and cells loaded with scintilipid for 2 hours at 37°C. The composition of the scintilipid being 8mg scintilipid, 2mg DOTAP resuspended in 1ml H_2O and finally diluted in 1ml PBS. The cells were then washed again in PBS and medium added and the cells incubated at 37°C
for 24 to 48 hours.

Example 9: Glucocorticoid receptor assay

The glucocorticoid receptor binding assay was carried out on HTC cells grown in 96 wells and incubated with scintilipid. The cells were washed three times in a HEPES-buffered saline (HBS) (155 mM NaCl, 5 mM HEPES, pH 7.4) then incubated with various concentrations of ^{3}H-dexamethasone (5 x 10^{-10} M to 1 x 10^{-8} M) for 30 mins at 37°C. The cells were then washed three times with ice-cold HBS and the plates then counted to ascertain total binding. To ascertain non-specific binding the same concentrations of ^{3}H-dexamethasone were used but with the addition of 20 x 10^{-6} M 'cold' dexamethasone, specific binding was calculated as the difference between total binding and non-specific binding. The results are shown in Figure 13.

An experiment showing displacement of ^{3}H-dexamethasone was also carried out. 1 x 10^{-9} M ^{3}H-dexamethasone was added to the cells in the presence of increasing concentrations of 'cold' dexamethasone (5 x 10^{-10} M to 1 x 10^{-8} M) the cells were

incubated for 30 minutes at 37°C before being washed and counted, the results of this assay are shown in Figure 14..

Example 10: β-adrenergic receptor assay

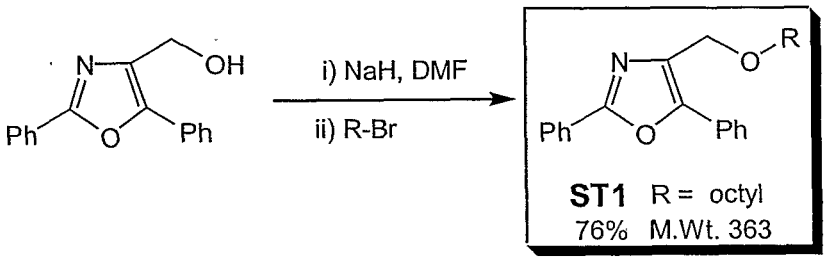
The β-adrenergic receptor binding assay was carried out on C6 glioma cells grown in 96 wells and incubated with scintilipid. The cells were washed three times in a MOPS-buffered saline (MBS) (125 mM NaCl, 25 mM MOPS, pH 8.2) then incubated with various concentrations of ^{3}H-dihydroalprenolol (1 x 10^{-8} M to 1 x 10^{-4} M) for 30 mins at 37°C. The cells were then washed three times with ice-cold MBS and the plates then counted to ascertain total binding. To ascertain non-specific binding the same concentrations of ^{3}H-dihydroalprenolol were used but with the addition of 10 x 10^{-3} M 'cold' alprenolol, specific binding was calculated as the difference between total binding and non-specific binding. The results are shown in Figure 15.

Example 11: Na^{+}/K^{+} ATPase binding assay

Binding to the Na^{+}/K^{+} ATPase was carried out on HeLa cells grown in 24 well plates using ^{3}H-ouabain as the radioligand. The cells were washed three times in a HEPES-buffered saline (HBS) (140 mM NaCl, 5 mM glucose, 0.5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 10 mM HEPES, ph 7.4) then incubated with various concentrations of ^{3}H-ouabain (5 x 10^{-8} M to 1 x 10^{-5} M) for 40 mins at 37°C. The cells were then washed three times with ice-cold MBS and the plates then counted to ascertain total binding. To ascertain non-specific binding the same concentrations of ^{3}H-ouabain were used but with the addition of 15 mM KCl to the incubation medium, specific binding was calculated as the difference between total binding and non-specific binding. The results are shown in Figure 16.

Example 12: Preparation of scintillator molecules

ST1

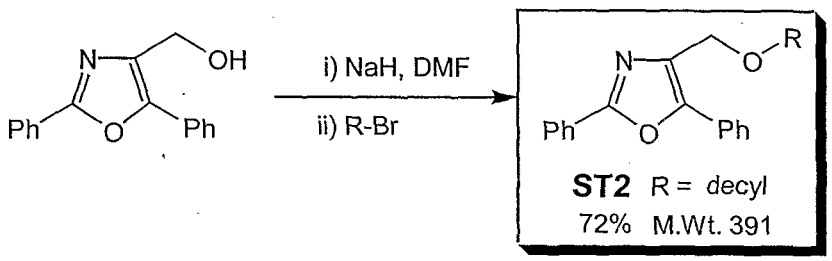


Alcohol	= 0.50g	= 2 mmol.		
Sodium hydride (60% in oil)	= 0.160g (60%)	= 0.096g	= 4 mmol	= 2 equivs.
DMF	= 10cm^3			
Bromooctane	= 0.346cm^3	= 0.386g	= 2 mmol	= 1 equiv.

Sodium hydride and the alcohol were placed in a 50cm^3 round bottomed flask. DMF was added and the resultant grey/black slurry stirred at room temperature for fifteen minutes. Bromooctane was then added slowly and the reaction mixture left to stir overnight. The mixture was poured into water (40cm^3) and extracted with ethyl acetate (2x 50cm^3). The combined organic phases were washed with brine (5x 50cm^3), dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to furnish the crude ether as a yellow oil (1.058g). Purification by flash column chromatography (gradient elution: 100cm^3 hexane followed by 200cm^3 5% ethyl acetate in hexane) furnished pure ether as a colourless oil (0.5474g, 83%). The majority of the oil was placed in a sample tube and over the period of several hours, the oil solidified to yield a waxy white solid (0.5528 g, 76%). $\square_{max}$ / cm^{-1} (thin film, NaCl plate) 3060 (m, C-H), 2925 (s, C-H), 2854 (s, C-H); $\square_H$ ($CDCl_3$, 300 MHz)

0.85 (3 H t *J* 6.9), 1.20-1.40 (10 H bs), 1.65 (2 H pentet *J* 7.5), 3.61 (2 H t *J* 6.7), 4.65 (2 H s), 7.34-7.50 (6 H m), 7.79 (2 H d *J* 7.3), 8.10 (2 H dd J 5.3, 1.8); □ $_C$ ($CDCl_3$, 75 MHz, Pendant) 14 (CH_3), 22.7 (CH_2), 26.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.8 (CH_2), 31.9 (CH_2), 65.5 (CH_2), 71.1 (CH_2), 126.9 (CH), 127.1 (CH), 129.2 (CH), 129.4 (CH), 129.5 (CH), 131.0 (CH), remaining quaternary carbon signals too small to be resolved; ^{M}/z (APCI) 364 M+H$^+$ ($C_{24}H_{29}NO_2$ requires M$^+$ 363), 234 (100%).

ST2

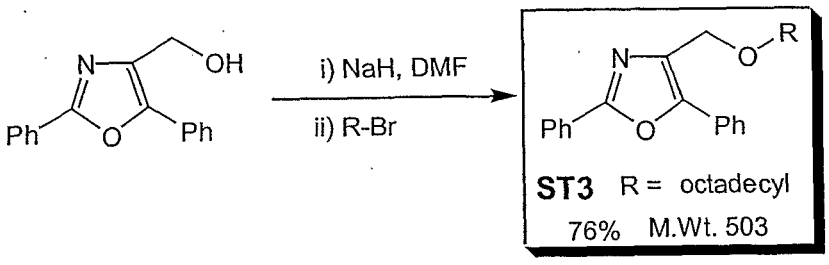


Alcohol	= 0.50g	= 2 mmol.		
Sodium hydride (60% in oil)	= 0.160g (60%)	= 0.096g	= 4 mmol	= 2 equivs.
DMF	= 5cm^3			
Bromodecane	= 0.415cm^3	= 0.442g	= 2 mmol	= 1 equiv.

Sodium hydride and the alcohol were placed in a 50cm^3 round bottomed flask. DMF was added and the resultant grey/black slurry stirred at room temperature for fifteen minutes. Bromodecane was then added slowly and the reaction mixture left to stir overnight. The mixture was poured into water (40cm^3) and extracted with ethyl acetate (2x 50cm^3). The combined organic phases were washed with brine (5x 50cm^3), dried over anhydrous magnesium sulfate, filtered and concentrated under

reduced pressure to furnish the crude ether as a yellow oil (1.173g). Purification by flash column chromatography (gradient elution: 300cm^3 hexane followed by 200cm^3 7% ethyl acetate in hexane) furnished pure ether as a white solid (0.5619g, 72%). □$_{max}$ / cm^{-1} (thin film, NaCl plate) 3059 (w, C-H), 2924 (s, C-H), 2852 (s, C-H); □$_H$ ($CDCl_3$, 300 MHz) 0.88 (3 H t *J* 6.9), 1.20-1.40 (14 H bs), 1.66 (2 H pentet *J* 7.1), 3.62 (2 H t *J* 6.5), 4.65 (2 H s), 7.33-7.50 (6 H m), 7.80 (2 H d *J* 7.7), 8.11 (2 H dd J 5.0, 1.6); □ $_C$ ($CDCl_3$, 75 MHz, Pendant) 14.1 (CH_3), 22.7 (CH_2), 26.3 (CH_2), 29.4 (CH_2), 29.6 (CH_2), 29.70 (CH_2), 29.72 (CH_2), 29.9 (CH_2), 32.0 (CH_2), 65.6 (CH_2), 71.1 (CH_2), 126.9 (CH), 127.1 (CH), 128.1 (quaternary C), 129.02 (quaternary C), 129.04 (CH), 129.46 (CH), 129.54 (CH), 131.0 (CH), 135.1 (quaternary C), 149.7 (quaternary C), 160.5 (quaternary C); ^{M}/z (APCI) 392 M+H$^+$ ($C_{26}H_{33}NO_2$ requires M$^+$ 391), 234 (100%).

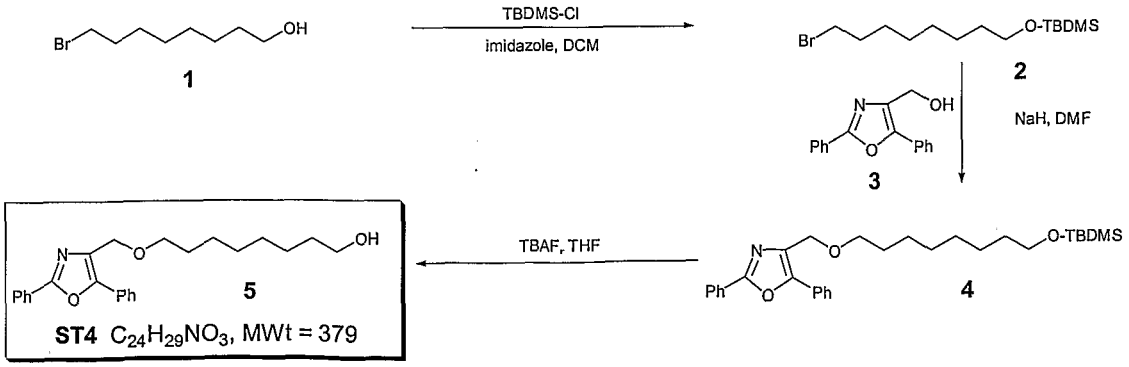
ST3



Alcohol	= 0.40g	= 1.6 mmol		
Sodium hydride (60% in oil)	= 0.128g (60%)	= 0.077g	= 3.2 mmol	= 2 equivs.
DMF	= 5cm^3			
Bromooctadecane	= 0.531g	= 1.6 mmol	= 1 equiv.	

Sodium hydride and the alcohol were placed in a 50cm^3 round bottomed flask. DMF was added and the resultant grey/black slurry stirred at room temperature for fifteen minutes. Bromooctadecane was then added slowly and the reaction mixture left to stir overnight. The mixture was poured into water (40cm^3) and extracted with ethyl acetate (2x 50cm^3). The combined organic phases were washed with brine (5x 50cm^3), dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to furnish the crude ether as a yellow solid (1.0772g). Purification by flash column chromatography (gradient elution: 400cm^3 hexane followed by 200cm^3 7% ethyl acetate in hexane) furnished pure ether as a white solid (0.6146g, 76%). $\square_{max}$ / cm^{-1} (thin film, NaCl plate) 3059 (w, C-H), 2915 (s, C-H), 2848 (s, C-H); $\square_H$ ($CDCl_3$, 300 MHz) 0.87 (3 H t *J* 6.9), 1.15-1.40 (30 H bs), 1.65 (2 H pentet *J* 7.3), 3.61 (2 H t *J* 6.5), 4.63 (2 H s), 7.32-7.50 (6 H m), 7.79 (2 H d *J* 7.3), 8.10 (2 H dd J 7.7, 1.8); $\square_C$ ($CDCl_3$, 75 MHz, Pendant) 14.1 (CH_3), 22.7 (CH_2), 26.3 (CH_2), 29.4 (CH_2), 29.6 (CH_2), 29.68 (CH_2), 29.72 (CH_2), 29.9 (CH_2), 32.0 (CH_2), remaining 8 CH_2 groups under the preceeding 8 CH_2 signals, 65.6 (CH_2), 71.1 (CH_2), 126.9 (CH), 127.1 (CH), 128.1 (quaternary C), 129.04 (quaternary C), 129.19 (CH), 129.46 (CH), 129.54 (CH), 131.0 (CH), 135.1 (quaternary C), 149.7 (quaternary C), 160.5 (quaternary C); ^{M}/z (APCI) 504 M+H$^+$ ($C_{34}H_{49}NO_2$ requires M$^+$ 503), 234 (100%).

ST4 – Method 'A'



8-Bromo-1-octanol **1**	= 1.0g	= 4.8 mmol.	
TBDMS-Cl	= 0.993g	= 6.6 mmol	= 1.4 equivs.
Imidazole	= 0.458g	= 6.7 mmol	= 1.4 equivs.
DCM	= 30cm^3		

The above reagents were combined and stirred under an atmosphere of nitrogen at room temperature for 20 hours. The mixture was poured into water (100cm^3) and extracted with DCM (2x 50cm^3). The combined organic extracts were washed with brine (2x 50cm^3), dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to furnish the crude product as a yellow oil (1.955g). Purification by flash column chromatography (10% ethyl acetate in hexane with 2% triethylamine) furnished pure silyl ether **2** as a colourless oil (1.512g, 98%). □$_{max}$ / cm^{-1} (thin film, NaCl plate) 2928 (s, C-H), 2855 (s, C-H); □$_H$ ($CDCl_3$, 300 MHz) 0.02 (6 H s), 0.87 (9 H s), 1.29 (6 H s), 1.40 (2 H t *J* 6.4), 1.48 (2 H t *J* 6.4), 1.85 (2 H pentet *J* 7.1), 3.37 (2 H t *J* 6.93) 3.55 (2 H t *J* 7);□ $_C$ ($CDCl_3$, 75 MHz, Pendant) -5.5 (2x CH_3), 18 (C), 25.5 (CH_2), 26 (3x CH_3), 28 (CH_2), 29 (CH_2), 29.5 (CH_2), 32.8 (CH_2), 34 (CH_2), 63.2 (CH_2), 71.5 (CH_2).

Silyl ether **2**	= 1.211 g	= 3.75 mmol	
Oxazole **3**	= 0.988 g	= 3.94 mmol	= 1.05 equivs
Sodium hydride	= 0.900g	= 37.49 mmol	= 10 equivs
DMF	= 30cm^3		

The above reagents were combined and stirred under an atmosphere of nitrogen at room temperature for 2 hours. The reaction was quenched cautiously with water (50cm^3) and the aqueous phase extracted with ethyl acetate (3x 30cm^3). The

combined organic extracts were washed with brine (2x 50cm^3), dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to furnish the crude product **4** as a yellow oil (2.997g, 162%). This product (containing some ethyl acetate) was reacted on without further purification. δ_H ($CDCl_3$, 300 MHz) 0.00 (6 H s), 0.85 (9 H s), 1.21 (8 H m), 1.45 (2 H t *J* 6.6), 1.62 (2 H t *J* 6.8), 3.51-4.60 (4 H m), 4.59 (2 H s), 7.3 – 8.2 (10 H m).

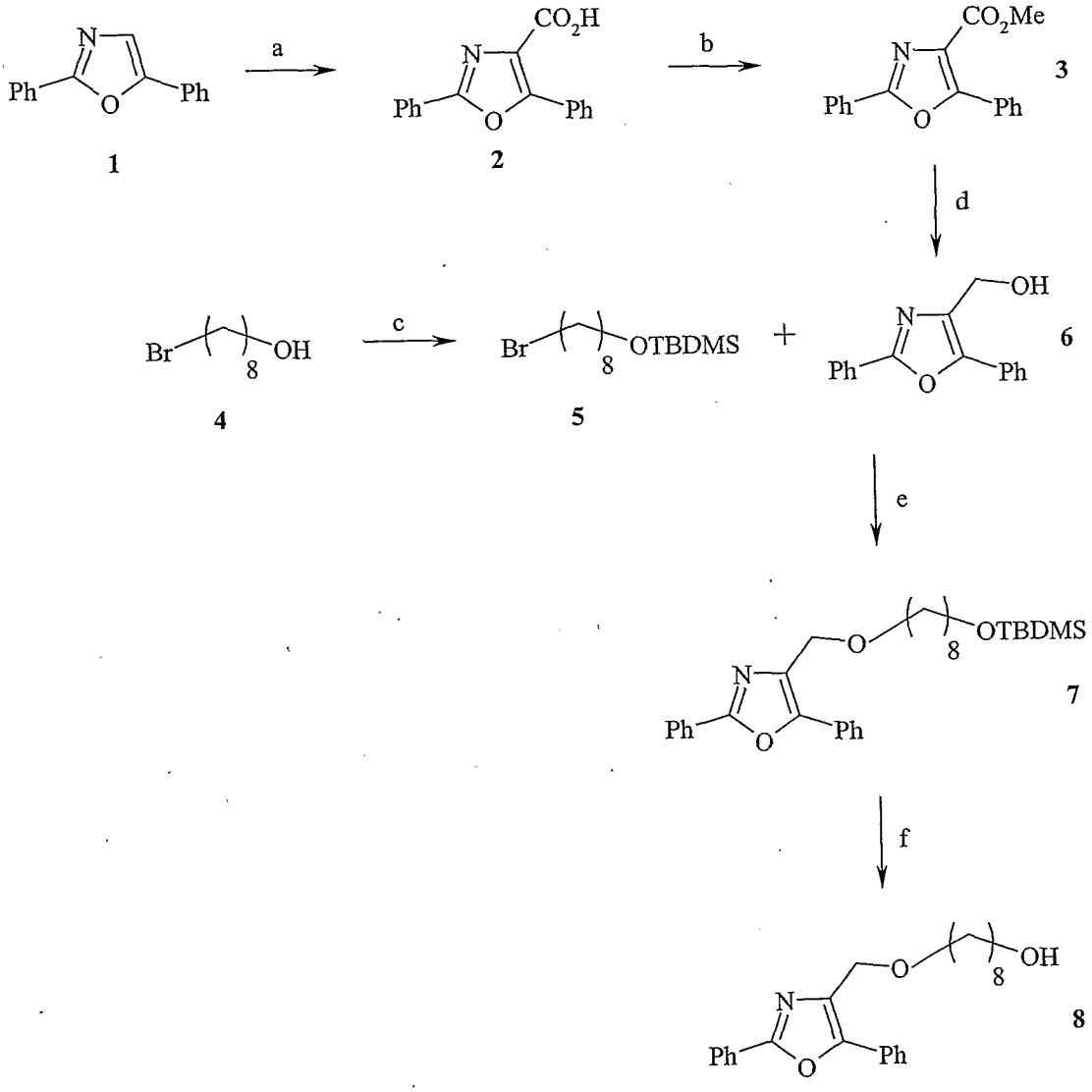
Oxazole silyl ether **4**	= 2.838 g	= 3.75 mmol (maximum theoretical quantity)
TBAF	= 1.656 g	= 6.33 mmol = 1.7 equivs
THF	= 30 cm^3	

The above reagents were combined and stirred at room temperature under an atmosphere of nitrogen for 16 hours. The reaction mixture was poured into water (50 cm^3) and extracted with ethyl acetate (2x 40 cm^3). The combined organic extracts were washed with a saturated aqueous ammonium chloride solution (2x 40cm^3), brine (2x 40cm^3), dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to furnish the crude product as a yellow oil (1.83g). Purification by flash column chromatography (gradient elution 20% ethyl acetate in hexane (150 cm^3) followed by 40% ethyl acetate in hexane (150 cm^3), isolated product R_f = 0.21 in 20 % ethyl acetate in hexane) furnished pure alcohol as a yellow oil (0.737g, 52% for the two steps from silyl ether **2**). The yellow oil was dissolved in 40% ethyl acetate in hexane (20 cm^3) and warmed gently for 10 minutes with activated charcoal (1g as a decolourising agent). The mixture was filtered through a pad of celite and concentrated under reduced pressure to furnish the pure product **5** as a pale yellow oil (0.397g, 28% for the two steps from silyl ether **2**). ν_{max} / cm^{-1} (KBr disk) 3425 (m, O-H), 3057 (w, C-H), 2928 (s, C-H), 2850 (s, C-H), 1549 (m, C=C); δ_H ($CDCl_3$, 300 MHz) 1.30 (8 H br s), 1.53-1.67 (4 H m), 3.60 (4 H t *J* 6.5), 4.63 (2 H s), 7.30-7.60 (6

H m), 7.78 (2 H d *J* 8), 8.09 (2 H dd *J* 4, 7.9); $^{m}/_{z}$ (APCI) 380 ($M+H^{+}$) ($C_{24}H_{29}NO_3$ requires M+ 379), 234 (100%).

ST4 – Method 'B'

Schematic Summary



a. i. *s*-BuLi, THF, -78°C. ii. CO_2 (s), 73% yield.

b. MeOH, H_2SO_4, reflux, 42% yield.

c. TBDMS-Cl, Imidazole, DCM, 98% yield.

d. $NaBH_4$, EtOH, 93% yield.

e. NaH, DMF.

f. THF, TBAF, 28% from **6**.

Experimental

2,5-Diphenyloxazole-4-carboxylic acid, **2**

2,5-Diphenyloxazole **1**	= 94.0 g	= 0.425 mol
s-Butyl lithium	= 360 mL @ 1.3 M (in hexanes)	=1.1 equivs
Carbon dioxide (solid)	≈ 50 g	
Anhydrous tetrahydrofuran	= 130 mL	

The diphenyloxazole was dissolved in tetrahydrofuran and cooled to –75°C. To this mixture was added the *s*-butyl lithium dropwise and the mixture stirred mechanically under nitrogen causing the colourless solution to turn deep red. After 30 minutes, crushed solid carbon dioxide was carefully added portionwise leading to a vigorous exothermic reaction. The addition was controlled so that the reaction temperature did not exceed –60°C. After complete addition the mixture was left to stir overnight and allowed to warm to room temperature. On acidification to pH 1 with 3M aqueous hydrochloric acid a white solid precipitated. This was filtered off, washed with water (3 x 30 mL) and dried under vacuum to yield 2,5-diphenyloxazole-4-carboxylic acid, **2** as an off-white solid (82.7 g, 73%). $_H$ ($CDCl_3$, 300 MHz) 7.47-7.56 (6H, m), 8.12-8.17 (2H, m), 8.28-8.33 (2H, m); $_C$ ($CDCl_3$, 75 MHz) 125.80, 126.81, 128.30, 128.67, 129.03, 130.84, 131.52, 171.58; MS (EI) m/z = 265 (M^+) ($C_{16}H_{11}NO_3$ requires M^+ 265), 105 (100%).

Methyl 2,5-diphenyloxazole-4-carboxylate, **3**

2,5-Diphenyloxazole-4-carboxylic acid, **2**	= 50.0 g	= 0.189 mol
Methanol	= 300 mL	
Concentrated sulphuric acid	= 2 mL	

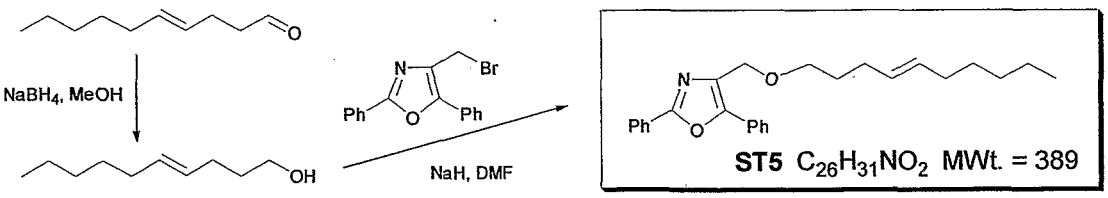
The above reagents were combined and heated overnight under reflux with the exclusion of atmospheric moisture. The solvent was then evaporated under reduced pressure and the oil redissolved in ethyl acetate (200 mL). This was then washed with 10% aqueous sodium carbonate, brine and water (3 x 100 mL each). The organic solvent was dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo* to yield methyl 2,5-diphenyloxazole-4-carboxylate, **3** as a yellow oil (22.0 g, 42%) $_H$ ($CDCl_3$, 300 MHz) 3.98 (3H, s), 7.47-7.54 (6H, m), 8.12-8.18 (4H, m); $_C$ ($CDCl_3$, 75 MHz) 52.43, 126.28, 126.85, 126.95, 128.46, 128.48, 128.85, 130.13, 130.40, 131.15, 155.32, 159.81, 162.70; MS (EI) m/z = 279 (M^+) ($C_{17}H_{13}NO_3$ requires M^+ 279), 105 (100%).

3-Hydroxymethyl-2,5-diphenyloxazole, **6**

Methyl 2,5-diphenyloxazole-4-carboxylate, **3**	= 15.1 g	= 0.054 mol	
Powdered sodium borohydride	= 10.3 g	= 0.27 mol	= 5
equivs			
Absolute ethanol	= 200 mL		

Methyl 2,5-diphenyloxazole-4-carboxylate, **3** was dissolved in ethanol and cooled to 0°C. To this was added sodium borohydride portionwise and the mixture stirred for 1.5 hours, allowing to warm to room temperature. It was then acidified to pH 1 with aqueous 3M hydrochloric acid and extracted with dichloromethane (3 x 100 mL). The organic layer was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo* to yield a yellow solid which was crystallised from chloroform. The resultant precipitate was filtered off, washed with ether and dried under vacuum to yield 3-hydroxymethyl-2,5-diphenyloxazole, **6** as pale yellow crystals (12.6 g, 93%). $_H$ ($CDCl_3$, 300 MHz) 4.40 (1H, bs), 4.86 (2H, s), 7.34-7.49 (6H, m), 7.70-7.75 (2H, m), 8.01-8.07 (2H, m); $_C$ ($CDCl_3$, 75 MHz) 56.85, 126.10, 126.35, 126.85, 128.00, 128.56, 128.78, 128.89, 130.48, 136.17, 147.39, 159.88; MS (EI) m/z = 251 (M^+) (100%) ($C_{16}H_{13}NO_2$ requires M^+ 251).

ST5



trans-4-Decenal	= 1.0cm^3	= 5.5 mmol.	
Sodium borohydride	= 1.033g	= 27.3 mmol	= 5 equivs.
Methanol	= 10cm^3		

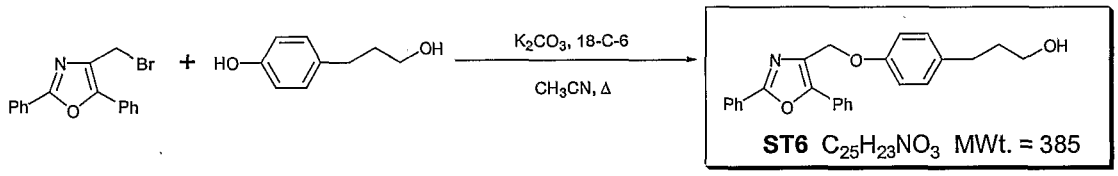
Methanol was added to the sodium borohydride under a nitrogen atmosphere. To this stirred mixture, *trans*-4-decenal was added and stirring was continued at room temperature for 16 hours. The mixture was poured into water ($30cm^3$) and extracted with ethyl acetate (2x $25cm^3$). The combined organic extracts were washed with brine (2x $50cm^3$), dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to furnish crude *trans*-4-decen-1-ol as a yellow oil (0.947g, 110%). □$_{max}$ / cm^{-1} (thin film, NaCl plate) 3332 (m, O-H), 2925 (s, C-H), 2855 (s, C-H); □$_H$ ($CDCl_3$, 300 MHz) 0.9 (3 H t *J* 7), 1.20-1.40 (6 H m), 1.61 (2 H pentet J 9.1), 1.90-2.10 (4 H m0, 3.65 (2 H t J 7), 5.44 (2 H m).

trans-4-Decen-1-ol	= 0.947g	= 6.07 mmol.	
4-Bromomethyl-2,5-diphenyloxazole	= 1.906g	= 6.07 mmol	= 1 equiv.
Sodium hydride	= 1.457g	= 60.7 mmol	= 10 equivs
DMF	= $20cm^3$		

Sodium hydride and *trans*-4-decen-1-ol were stirred in DMF under a nitrogen atmosphere at room temperature for 10 minutes. To the resultant grey slurry, a solution of 4-bromomethyl-2,5-diphenyloxazole in DMF was added. The resultant mixture was stirred at room temperature for 16 hours. The reaction was quenched cautiously with water ($50cm^3$) and extracted with diethyl ether (3x $30cm^3$). The combined organic extracts were washed with brine ($20cm^3$), dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to furnish a yellow solid. Trituration with diethyl ether caused the unreacted oxazole to precipitate and this was removed by filtration through a pad of celite. The filtrate was concentrated under reduced pressure to furnish the crude product as a yellow oil. Purification by flash column chromatography (gradient elution: hexane ($100cm^3$), 20%

ethyl acetate in hexane (200cm^3)) furnished the pure product as a colourless solid (0.284g, 12%). $\square_{max}$ / cm^{-1} (KBr disk) 2916 (s, C-H), 2852 (s, C-H), 1545 (m, C=C); $\square_H$ ($CDCl_3$, 300 MHz) 0.86 (3 H t *J* 7.1), 1.20-1.40 (6 H m), 1.71 (2 H pentet *J* 6.9), 1.93 (2 H q *J* 5.1), 2.08 (2 H q *J* 5.6), 3.63 (2 H t *J* 6.5), 4.64 (2 H s), 5.39 (2 H m), 7.30-7.50 (6 H m), 7.79 (2 H d *J* 8), 8.10 (2 H dd *J* 4, 7.7); $^m/_z$ (APCI) 390 ($M+H^+$) ($C_{26}H_{31}NO_2$ requires M^+ 389), 234 (100%).

ST6

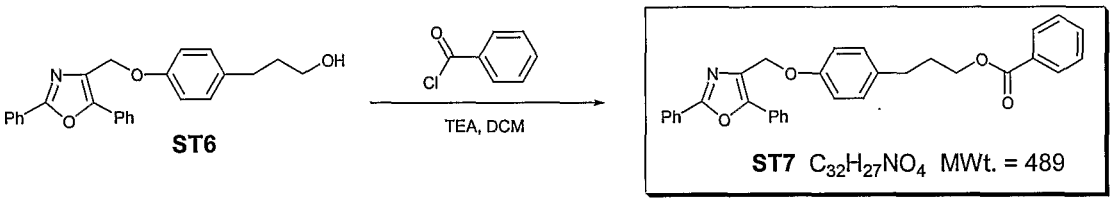


4-Bromomethyl oxazole	= 0.492g	= 1.57 mmol.	
3-(4-hydroxyphenyl)-propan-1-ol	= 0.248g	= 1.63 mmol	= 1.04 equivs.
Potassium carbonate	= 1.273g	= 16.3 mmol	= 10.4 equivs.
18-Crown-6	= catalytic amount		
Acetonitrile	= 25cm^3		

The above reagents were combined and refluxed for 16 hours under an atmosphere of nitrogen. The mixture was poured into water (30cm^3) and extracted with diethyl ether (3x 30cm^3). The combined organic extracts were washed with an aqueous solution of dilute hydrochloric acid (20%, 2x 30cm^3), dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to furnish the crude product as an

off-white solid (0.651g). Trituration with diethyl ether furnished the product as a white solid (0.330g, 55%). □$_{max}$ / cm^{-1} (KBr disk) 3444 (m, O-H), 3055 (w, C-H), 2927 (s, C-H); . $_H$ ($CDCl_3$, 300 MHz) 1.87 (2 H pentet *J* 6.5), 2.66 (2 H t *J* 7.5), 3.67 (2 H t *J* 6.2), 5.15 (2 H s), 7.00 (2 H d *J* 8.5), 7.13 (2 H d *J* 8.7), 7.30-7.60 (6 H m), 7.77 (2 H d *J* 7), 8.10 (2 H dd J 4, 7.7); $^m/_z$ (APCI) 386 ($M+H^+$) ($C_{25}H_{23}NO_3$ requires M^+ 385), 234 (100%).

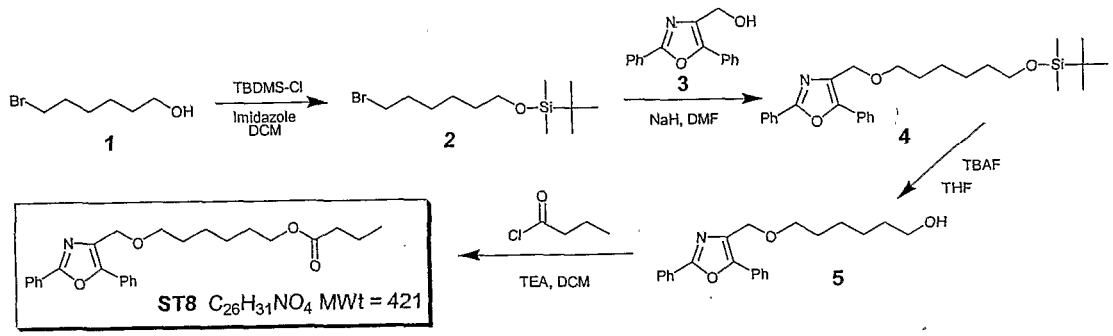
ST7



ST6		= 0.40g	= 1.04 mmol.
Benzoyl chloride	= 0.13cm^3	= 0.153g	= 1.09 mmol = 1.05 equivs.
Triethylamine	= 0.29cm^3	= 0.210g	= 2.08 mmol = 2.0 equivs.
DCM	= 10cm^3		

ST6 was dissolved in DCM and placed under an atmosphere of nitrogen. Benzoyl chloride was added and the mixture stirred for ten minutes at room temperature. Triethylamine was then added and the resultant mixture was stirred at room temperature for 3 hours. The mixture was poured into water (20cm^3) and extracted with ethyl acetate (3x 20cm^3). The combined organic extracts were dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to

furnish the crude product as a yellow oil. This oil was allowed to stand for 16 hours after which time a yellow solid had formed (0.511g). Trituration of this solid with hexane gave a yellow solution and a white solid which was collected by filtration and dried to furnish the pure product, **ST7**, as a white solid (0.330g, 65%). $\square_{max}$ / cm^{-1} (thin film, NaCl plate) 3051 (w, C-H), 2927 (s, C-H), 1716 (s, C=O); $\square_H$ ($CDCl_3$, 300 MHz) 2.07 (2 H pentet *J* 6.9), 2.73 (2 H t *J* 7.9), 4.33 (2 H t *J* 6.2), 5.14 (2 H s), 7.00 (2 H d *J* 8.5), 7.14 (2 H d *J* 8.5), 7.30-7.60 (9 H m), 7.76 (2 H d *J* 7), 8.03 (2 H d *J* 7), 8.10 (2 H m); $^m/_z$ (APCI) 490 ($M+H^+$) (100%) ($C_{32}H_{27}NO_4$ requires M^+ 489), 234.



ST8

6-Bromo-1-hexanol **1**	= 1.486g	= 8.21 mmol.
TBDMS-Cl	= 1.361g	= 9.03 mmol = 1.1 equivs.
Imidazole	= 0.615g	= 9.03 mmol = 1.1 equivs.
DCM	= 20cm^3	

The above reagents were combined and stirred under an atmosphere of nitrogen at room temperature for 48 hours. The white precipitate of imidazole hydrochloride was removed by filtration and the filtrate was extracted with ethyl acetate (2x 50cm^3). The

combined organic extracts were dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to furnish the crude product **2** as an orange oil (2.361g, 98%). This product was used directly without any further purification.

Silyl ether **2**	= 2.361 g	= 8.03 mmol	
Oxazole **3**	= 1.818 g	= 7.24 mmol	= 0.905 equivs
Sodium hydride	= 1.921g	= 80.3 mmol	= 10 equivs
DMF	= 30cm^3		

The above reagents were combined and stirred under an atmosphere of nitrogen at room temperature for 3 hours. The reaction was quenched cautiously with water (50cm^3) and the aqueous phase extracted with ethyl acetate (3x 30cm^3), the addition of brine was required to break up emulsions that formed during this extraction procedure. The combined organic extracts were washed with brine (2x 20cm^3), dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to furnish the crude product **4** as a yellow oil (3.88g, 104%). This product (containing some ethyl acetate) was reacted on without further purification.

Oxazole silyl ether **4**	= 3.88 g	= 8.03 mmol (maximum theoretical quantity)
TBAF	= 4.63g	= 17.7 mmol = 2.2 equivs
THF	= 30 cm^3	

The above reagents were combined and stirred at room temperature under an atmosphere of nitrogen for 16 hours. The reaction mixture was poured into water (50 cm^3) and extracted with ethyl acetate (2x 30 cm^3). The combined organic extracts were

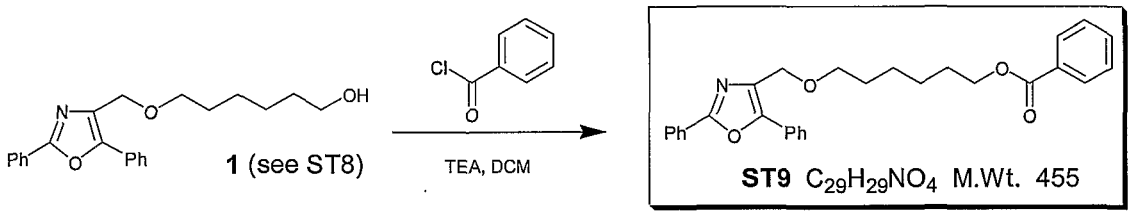
washed with a saturated aqueous ammonium chloride solution (2x 20cm^3), brine (2x 20cm^3), dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to furnish the crude product as a yellow oil (3.209g). Purification by flash column chromatography (gradient elution 20% ethyl acetate in hexane (400 cm^3) followed by 40% ethyl acetate in hexane (500 cm^3), furnished pure alcohol **ST10** as a pale yellow waxy solid (1.127g, 40% for the two steps from silyl ether **2**) ν_{max}/cm^{-1} (thin film, NaCl plate) 3386 (s, O-H), 3059 (w, C-H aromatic), 2930 (s, C-H), 2857 (s,C-H), 1594 (w, C=C aromatic), 1551 (m, C=C aromatic), 1485, 1447, 1377, 1346, 1279, 1090, 1070, 1026, 920, 775, 764, 706, 690; δ_H ($CDCl_3$, 300MHz) 1.35-1.45 (4H m), 1.54 (2H pentet *J* 6.7), *1.66 (*2H pentet *J* 6.7), 3.61 (4H hexet *J* 3.2), 4.63 (2H s), 7.36-7.48 (6H m), 7.79 (2H d *J* 7.3), 8.10 (2H dd *J* 5.3 7.9); δ_C ($CDCl_3$, 75MHz,Pendant) 25.3(CH2),25.8 (CH2), 29.4 (CH2), 32.4 (CH2), 62.4 (CH2), 64.9 (CH2), 70.3 (CH2), 126.0 (CH), 126.2 (CH), 128.6 (CH), 128.7 (CH), 129.3 (CH), 130.2 (CH); $^M/_Z$ (APCI) 352 M+H$^+$ ($C_{22}H_{25}O_3N$ requires M$^+$ 351), 234 (100%).

Alcohol **ST10**	= 0.580g	1.65 mmol	
Butyryl chloride	= 0.176g	1.65 mmol	= 1 equiv.
Triethylamine	= 0.334g	3.30 mmol	= 2 equivs.
DCM	= 20cm^3		

Alcohol **ST10** was dissolved in DCM and the resultant yellow solution was placed under a nitrogen atmosphere. To this stirred solution, butyryl chloride followed by triethylamine was added and stirring at room temperature was continued for 16 hours. The reaction mixture was poured into water (20cm^3) and extracted with ethyl acetate (3x 20cm^3). The combined organic extracts were washed with brine (20cm^3), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to furnish

crude ester **ST8** as a yellow oil (0.747g, 107%). Activated charcoal and diethyl ether ($30cm^3$) were added to this product and the resultant mixture gently warmed. The solution was filtered through a pad of celite and concentrated under reduced pressure to yield pure ester **ST8** as a pale yellow oil (0.312g, 45%). $\square_{max}$ / cm^{-1} (thin film, NaCl plate) 3059 (w, C-H), 2934 (s, C-H), 2859 (s, C-H), 1732 (s, C=O), 1549 (m, C=C); $\square_H$ ($CDCl_3$, 300 MHz) 0.92 (3 H t J 7.3), 1.35 (4 H br s), 1.61 (6 H m), 2.25 (2 H t J 7.5), 3.60 (2 H t J 6.5), 4.03 (2 H t J 6.7), 4.63 (2 H s), 7.30-7.60 (6 H m) 7.78 (2 H d *J* 7.2), 8.10 (2 H dd *J* 3, 8); $^m/_z$ (APCI) 422 $M+H^+$ ($C_{26}H_{31}NO_4$ requires M^+ 421), 234 (100%).

ST9

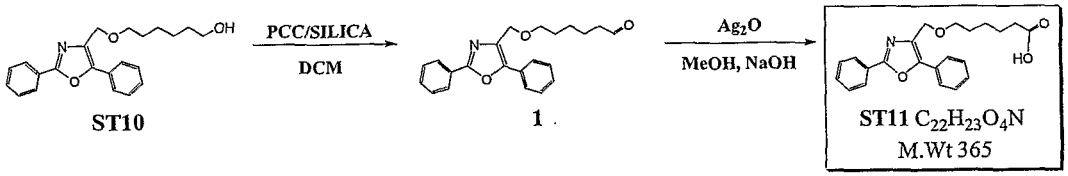


Alcohol **1**		= 0.501g	= 1.43 mmol	
Benzoyl chloride	= $0.17cm^3$	= 0.201g	= 1.43 mmol	= 1 equiv.
Triethylamine	= $0.3cm^3$	= 0.217g	= 2.15 mmol	= 1.5 equivs.
DCM	= $10cm^3$			

Alcohol **1** was dissolved in DCM and placed under an atmosphere of nitrogen. Benzoyl chloride was added and the mixture stirred for ten minutes at room temperature. Triethylamine was then added and the resultant mixture was stirred at

room temperature for 16 hours. The mixture was poured into water (20cm^3) and extracted with diethyl ether (2x 20cm^3). The combined organic extracts were dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to furnish the crude product as a yellow oil. This oil was allowed to stand for 16 hours after which time a yellow solid had formed (0.599g). Purification by flash column chromatography (20% ethyl acetate in hexane (200 cm^3)), furnished pure ester **ST9** as a colourless oil (0.340g, 52%). $\square_{max}$ / cm^{-1} (thin film, NaCl plate) 3059 (w, C-H), 2934 (m, C-H), 2858 (m, C-H), 1716 (s, C=O); $\square_H$ ($CDCl_3$, 300 MHz) 1.46 (4 H pentet *J* 3.5), 1.71 (4 H m), 3.62 (2 H t *J* 6.5), 4.28 (2 H t *J* 6.7), 4.64 (2 H s), 7.30-7.60 (9 H m), 7.80 (2 H m), 8.02 (2 H d *J* 7.3), 8.09 (2 H dd *J* 4.2, 7.7); $^m/_z$ (APCI) 456 $M+H^+$ (100%) ($C_{29}H_{29}NO_4$ requires M^+ 455), 234.

ST11



ST10	= 2.5g	= 7.12mmol	= 1eq
Pyridinium chlorochromate/silica gel (1:1)	= 4.6g	= 10.67mmol	= 1.5eq
Dichloromethane	= 30cm^{-3}		

PCC/silica was added to a solution of **ST10** in dichloromethane (30cm^3) and left to stir overnight. The reaction mixture was diluted with ether and filtered through celite and

silica before being concentrated under reduced pressure to furnish the oxazole aldehyde as a crude yellow oil (2.087g 84%). This product was used directly without further purification.

A sample, purified by eluting through a silica column (20% ethyl acetate in hexane) and combining fractions ($R_f = 0.47$) (40% ethyl acetate in hexane) was isolated as a slightly yellow oil. Spectroscopic analysis of this oil gave the following data. ν_{max} / cm^{-1} (thin plate, NaCl plate) 3059 (m, C-H aromatic), 2933 (s, C-H), 2860 (s, C-H), 1722 (s, C=O), 1594 (w, C=C aromatic), 1551 (m, C=C aromatic), 1486, 1447, 1377, 1346, 1319, 1279, 1214, 1177, 1133, 1091, 1069, 1025, 1011, 950, 921, 776, 765, 707, 691, 675, 660; δ_H ($CDCl_3$, 300MHz) 1.40-1.66 (6H m), 2.31-2.41 (2H m), 3.61 (2H t *J* 6.15), 4.63 (2H s), 7.35-7.49 (6H m), 7.77 (2H d *J* 7.44), 8.10 (2H d *J* 3.75), 9.72 (1H s);

Aldehyde **1**	= 1.580g	= 4.527mmol	= 1eq
Silver (I) oxide	= 1.574g	= 6.791mmol	= 1.5eq
$NaOH_{(aq)}$ (2M)	= 30cm^{-3}		
Methanol	= 30cm^{-3}		

Silver (I) oxide was added to a solution of aldehyde **1** in methanol followed by the dropwise addition of $NaOH_{(aq)}$ (2M). The reaction mixture was placed under a nitrogen atmosphere, to prevent solvent evaporation, and stirred at room temperature for 6 hours. The reaction mixture was filtered through celite, and acidified (20% $HCl_{(aq)}$) which turned the yellow solution cloudy. The solution was extracted with diethyl ether (3x50 cm^3), dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to give a yellow oil which solidified on standing open to the atmosphere. Trituration of the yellow solid with hot diethyl ether (50 cm^3)

gave a yellow solution to and a white solid. The white solid was isolated by filtration and washed with cold diethyl ether ($30cm^3$). The filtrate was basified (2M $NaOH_{(aq)}$) and the aqueous phase separated then acidified (20% $HCl_{(aq)}$) and extracted with diethyl ether ($2x30\ cm^3$).Charcoal was added to the diethyl ether extracts and the resultant mixture gently warmed before being filtered through celite and concentrated under reduced pressure to give an opaque oil which solidified to a white solid on standing open to the atmosphere overnight. The two solids were identical and thus were combined to give a single pure product (1.27g, 77%). ν_{max} / cm^{-1} (KBr disc) 3060 (w, C-H aromatic), 2930 (s, C-H), 2856 (s, C-H), 2581 (br, O-H), 1716 (s, C=0), 1596 (w, C=C aromatic), 1546 (m, C=C aromatic), 1488, 1450,1420, 1350, 1326, 1307, 1217, 1183, 1090, 1014, 911, 780, 761, 707, 688; δ_H ($CDCl_3$, 300MHz) 1.41-1.49 (2H m), 1.65 (4H pp J 6.7, 7.5), 2.33 (2H t *J* 7.3), 3.61 (2H t *J* 6.5), 4.64 (2H s), 7.34-7.51 (6H m), 7.77 (2H d *J* 7.5), 8.1 (2H dd); δ_C ($CDCl_3$, 75MHz, Pendant) 24.5 (CH2), 25.7 (CH2), 29.3 (CH2), 33.9 (CH2), 65.1 (CH2), 70.2 (CH2), 126.2 (CH), 126.5 (CH), 128.4 (CH), 128.6 (CH), 128.8 (CH), 128.8 (CH), remaining quarternary carbon signals remain too small to be resolved; $^M/_Z$ (APCI) 366 $M+H^+$ ($C_{22}H_{23}O_4N$ requires M^+ 365), 234 (100%).

CLAIMS

1. A scintillator molecule, consisting of one or more X moieties, which is a phosphor, covalently attached to one or more R^1 moieties which is a lipophilic or amphiphilic group, wherein the scintillator molecule is capable of integrating into a cell, a cell membrane, a biological membrane, an artificial membrane or a delivery vehicle and when integrated can act as a phosphor.

2. A scintillator molecule as claimed in claim 1 of formula (I)

$$(X)_m\text{-}(R^1)_n \qquad (I)$$

or a salt thereof wherein group m and n are independently 1 to 20.

3. A scintillator molecule as claimed in claim 2 wherein m and n are independently 1 to 4

4. A scintillator molecule as claimed in any one of claims 1 to 3 wherein m and n are of equal value.

5. A scintillator molecule as claimed in claim 4 wherein m and n are both 1.

6. A scintillator molecule as claimed in claims 1 and 2 consisting of one X moiety and two or more R^1 moieties.

7. A scintillator molecule as claimed in claims 1 and 2 consisting of one R^1 moiety and two or more X moieties.

8 A scintillator molecule as claimed in any one of claims 1 to 7 wherein the X and R^1 groups are linked via a linker moiety.

9 A scintillator molecule as claimed in any one of claims 1 to 8 wherein R^1 is independently one or more of an unbranched or branched alkyl, alkoxy, alkenyl, alkenyoxy, alkynyl, alkynyloxy, aryl, heteroaryl, amide, amine, alkylaryl, alkyoxyaryl, reduced arylalkyl, reduced alkoxyaryl, alkenylaryl, alkenyloxyaryl, alkylheteroaryl, alkyoxyheteroaryl, alkenylheteroaryl, alkenyloxyheteroaryl, reduced alkylheteroaryl, reduced alkoxyheteroaryl or a substituted derivative of any of the foregoing groups, substituted by one or more groups independently selected from halogen, alkyl, alkoxy arylalkyl, arylalkoxy, cyano, nitro, $-OC(O)R^2$, $-CO_2R^2$, $-NR^3R^4$, $-OR^2$, $-SR^2$, $-C(O)NR^3R^4$, or a lipid (natural or synthetic);

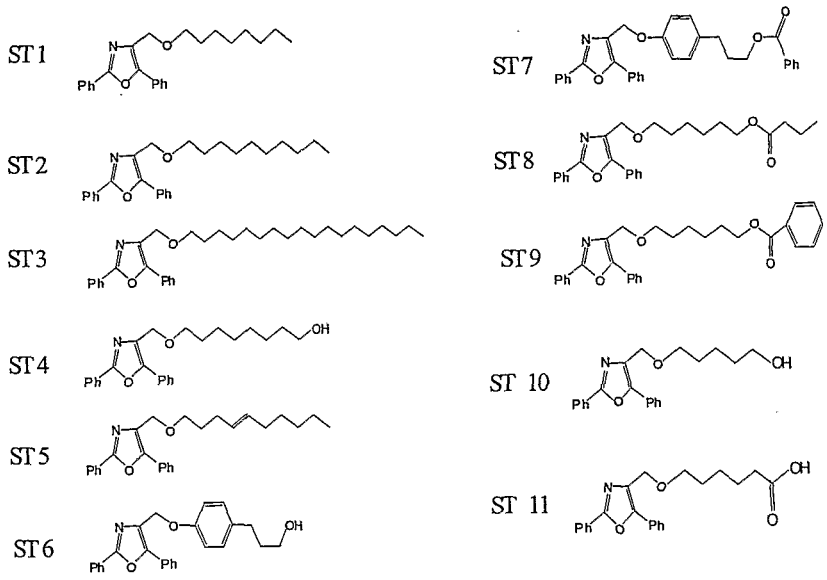
R^2 is H, alkyl, aryl, or a group as defined for R^1;

and R^3 and R^4 are independently H, alkyl and aryl or a group as defined for R^1.

10 A scintillator molecule as claimed in any one of claims 1 to 9 wherein X is independently one or more of a benzotriazole, a coumarin, an aromatic hydrocarbon, an oxazole, a 1,3,4 oxadiazole or a derivative thereof.

11 A scintillator molecule as claimed any one of claims 1 to 9 wherein X is independently one or more of p-terphenyl, p-quaterphenyl 2,4-(biphenylyl-6-phenylbenzoxaole, 2,5-bis-(5'-tert-butylbenzoxazolyly-[2']thiophene, 2-(4-t-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxadiazole, 2-(1-naphthyl)-5-phenyl-oxazole, 2-phenyl-5-(4-biphenylyl)-1,3,4-oxadiazole, 1,4-bis[5-phenyl-2-oxazolyl]-benzene, 2,5-diphenyloxazole, 1,4-bis(5-pheny-2-oxazolyl)benzene, 9,10-diphenylanthracene, 1,6-diphenyl-1,3,4-hexatriene, trans-p,p'diphenylstilbene, 1,14,4-tetraphenyl-1,3-butadiene, 3-hydroxyflavone, 1,4-bis(2-methylstyryl)benzene or derivatives thereof.

12 A scintillator molecule selected from



or a salt thereof.

13 A scintillator molecule as claimed in claim 8 wherein the linker is alkoxy, aminoalkyl, aryloxy, arylamino, alkylamino, carbonyl, amidyl, ester, aminyl silyl, imidyl, urea alkylthio or arylthio.

14 A process for the preparation of a scintillator molecule as claimed in anyone of claims 1 to 13 wherein one or more X moieties is coupled to one or more R^1 moieties.

15 A process for the preparation of a scintillator molecule as claimed in claim 8 wherein one or more X moieties is coupled to one or more R^1 moieties via a linker molecule.

16 A process for the preparation of a compound as claimed in anyone of claims 1 to 13, which process comprises:

(i) alkylation of 4-alcohol derivative of 2,5-diphenyloxazole with a haloalkyl group in the presence of a base or

(ii) alkylation of 4-halogen derivative of 2,5-diphenyloxazole with an alcohol, in the presence of a base or

(iii) converting a compound of formula (I) into a different compound of formula (I), by, for example,

removal of protecting groups
addition of protecting groups
oxidation (e.g. an alcohol to an aldehyde or ketone, or an aldehyde to an acid group)
esterification of an acid or an alcohol
amidation of an acid or an amine
reduction (e.g. of an alkene to an alkane, or an aldehyde to an alcohol)
production of a salt of a particular compound or
other functional group interconversions (for example bromination of an alcohol)

17 A liposome incorporating one or more scintillator molecules as claimed in anyone of claims 1 to 13.

18 A micelle incorporating one or more scintillator molecule as claimed in anyone of claims 1 to 13.

19 A method for inserting one or more scintillator molecule as claimed in anyone of claims 1 to 13 into a liposome, comprising

- incubating the scintillator molecule(s) and lipids in an organic solvent
- removing the organic solvent
- resuspending the scintillator molecule(s) and lipid in aqueous solution.

20 A method as claimed in claim 19 wherein the lipid scintillator suspension is warmed to a temperature of between 80 and 30°C.

21 A method as claimed in claims 19 or 20 wherein the lipid scintillator suspension is warmed to a temperature of 60°C or above.

22 A method as claimed in any one of claims 19 to 21 wherein the lipid and scintillator molecules are incubated in a ratio of between 1:1 and 10:1.

23 A method as claimed in any one of claims 19 to 22 wherein the lipid and scintillator molecules are incubated in a ratio of between 2:1 and 8:1

24 A method as claimed in any one of claims 19 to 23 wherein the lipid and scintillator molecules are incubated in a ratio of around 4:1.

25 A cell incorporating a scintillator molecule as claimed in any one of claims 1 to 13.

26 A method of inserting one or more scintillator molecules as claimed in any one of claims 1 to 13 into a cell comprising incubating the cells with a liposome as claimed in claim 17 or a micelle as claimed in claim18.

27 A method as claimed in claim 26 wherein the cells are incubated at 20 to 40°C.

28 A method as claimed in claim 27 wherein the cells are incubated at 37°c.

29 A kit comprising lipid molecules and one or more scintillator molecules as claimed in any one of claims 1 to 13.

30 An assay system comprising a cell containing a protein or receptor of interest, and one or more scintillator molecules as claimed in any one of claims 1 to 13.

31 A composition comprising one or more scintillator molecules as claimed in any one of claims 1 to 13.

32 A kit comprising one or more scintillator molecules as claimed in any one of claims 1 to 13 and a source of radiation.

33 A kit as claimed in claim 32 wherein the source of radiation is a radiolabelled ligand.

34 The use of a scintillator molecule as claimed in claims 1 to 13 in an assay.

35 The use as claimed in claim 34 wherein the assay is a incorporation assay, a transport assay, a receptor binding assay or a signal transduction assay.

36 A liposome or micelle as claimed in claims 17 or 18 further comprising a protein or receptor of interest.

37 A scintillator molecule as substantially hereinbefore discussed with reference to or as shown in the examples or figures.

38 A process as substantially hereinbefore discussed with reference to or as shown in the examples or figures.

39 A liposome as substantially hereinbefore discussed with reference to or as shown in the examples or figures.

40 A micelle as substantially hereinbefore discussed with reference to or as shown in the examples or figures.

41 A method as substantially hereinbefore discussed with reference to or as shown in the examples or figures.

42 A kit as substantially hereinbefore discussed with reference to or as shown in the examples or figures.

43 A cell as substantially hereinbefore discussed with reference to or as shown in the examples or figures.

44 An assay system as substantially hereinbefore discussed with reference to or as shown in the examples or figures.

45 A composition as substantially hereinbefore discussed with reference to or as shown in the examples or figures.