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(54) **Title:** PEPTIDE-LIPID CONSTRUCTS AND THEIR USE IN DIAGNOSTIC AND THERAPEUTIC APPLICATIONS

(57) **Abstract:** Peptide-lipid constructs of the structure L-S-F are disclosed, where F is a peptide, S is a spacer covalently linking F to L via an oligomer of ethylene glycol, and L is a diacyl- or dialkyl-glycerolipid (including glycerophospholipids). The spacer ideally has 6 to 14 ethylene glycol repeats, corresponding to PEG with a molecular weight of approximately 250 to 600. Also disclosed is a method of detecting reactive antibodies in serum by contacting serum with cells modified to incorporate a peptide-lipid construct, where the peptide is an epitope of the antibody, and determining the degree of agglutination of the cells.

PEPTIDE-LIPID CONSTRUCTS AND THEIR USE IN DIAGNOSTIC AND THERAPEUTIC APPLICATIONS

TECHNICAL FIELD

The invention relates to methods for effecting qualitative and quantitative changes in the levels of peptide expressed at the surface of cells and multi-cellular structures, and constructs for use in such methods.

In particular, the invention relates to peptide-lipid constructs for use in diagnostic and therapeutic applications, including serodiagnosis.

BACKGROUND ART

The ability to effect qualitative and quantitative changes in the level of peptides expressed at the surface of cells and multi-cellular structures provides for a range of diagnostic and therapeutic applications.

Qualitative and quantitative changes in the level of peptides expressed at the surface may modify trans-membrane transport, cell-solute and cell-cell interactions, and thus the functionality of the modified cell or multi-cellular structure.

Known methods of effecting such changes include gene manipulation, chemical modification of endogenous membrane peptides, and "cell surface painting" using lipid anchors such as GPI.

The specification accompanying international application number PCT/NZ2005/000052 (publication number WO 2005/090368) describes the preparation of water soluble carbohydrate-lipid constructs for use in methods of effecting qualitative and quantitative changes in the level of carbohydrates expressed at the surface of cells and multicellular structures.

The specification accompanying international application number PCT/NZ2006/000245 (publication number WO 2007/035116) describes another method for the preparation of water soluble carbohydrate-lipid constructs where the carbohydrate is the polymer hyaluronic acid. Use of the construct to modify embryos and promote association with endometrial cells is described.

Relatively little work has been performed on the site-directed coupling of peptides to phospholipids as individual components prior to their incorporation in self assembling lipid structures, such as liposomes, or as would be required to provide peptide-lipid constructs for use in methods of effecting qualitative and quantitative changes in the level of peptide expressed at the surface of cells and multicellular structures.

A variety of standard techniques have been described for the covalent coupling of peptides to liposomes surfaces.

Martin *et al* (1990) has reviewed methods of attaching moieties including peptides, to the surface of liposomes.

Blume *et al* (1993) describes the coupling of the water soluble Glu-plasminogen to liposomes by the method described by Kung and Redemann (1986). The chemical ECDI (1-ethyl-(3-

dimethylaminopropyl) carbodiimide hydrochloride) is used to activate the liposomes prior to incubation of the activated liposome suspension with Glu-plasminogen. Proteo-PEG-coated liposomes with Glu-plasminogen covalently attached to the ends of the distearylyphosphatidylethanolamine (DSPE)-PEG-COOH are provided.

Haselgrűbler *et al* (1995) describes a heterobifunctional crosslinker used to facilitate the preparation of immunoliposomes. The crossllinker is synthesised from a diamine derivative of poly(ethylene glycol) (PEG, average molecular weight 800 dalton (18*mer*)). The crosslinker has 2-(pyridylthio)propronyl (PDP) and N-hydroxysuccinimide ester (NHS) as functional groups.

Ishida *et al* (2001) describes the preparation of liposomes bearing polyethylene glycol-coupled transferrin. Transferrin was conjugated via the terminal carboxyl residue of DSPE-PEG-COOH. The liposomes were proposed as having utility in *in vivo* cytoplasmic targeting of chemotherapeutic agents or plasmid DNAs to target cells.

Massaguer *et al* (2001) describes the incorporation of a peptide sequence (GGRGRS) and hydrophobic derivatives to the surface of chemically activated liposomes. The incorporation was carried out through the carboxyl group of N-glutaryl dipalmitoyl phosphatidyl choline (NGPE).

Massaguer *et al* (2001) noted that considering potential *in vivo* applications, where sterility and simplicity would be some of the most important requirements, processes based on chemical reactions on the surface of liposomes involving extra steps would be more difficult to be scaled up at the

industrial level. A hydrophobic derivative of the peptide sequence was identified as providing optimal properties for incorporation to the surface of liposomes.

Chung *et al* (2004) describe the antigenic determinant shielding effect of DOPE-PEG incorporated into the membranes of cells and speculated concerning the potential of lipid-PEG(n)(s) to regulate biological cell responses and the extension of this concept to the introduction of functional molecules at the end of the PEG chain.

Kato *et al* (2004) describe a method for anchoring of macromolecular proteins into the membranes of living mammalian cells. A dioleylphosphatidylethanolamine (DOPE) derivative coupled with hydrophilic poly(ethylene glycol) (PEG80) was used as the synthetic membrane anchor. Peptides were conjugated at the distal terminal of the PEG moiety via an amino-reactive N-hydroxysuccinimide derivative of the synthetic membrane anchor.

The PEG80 moiety facilitated solublisation of the synthetic membrane anchor in water. As noted by Kato *et al* (2004) if the anchor is insoluble in water, undesirable and complicated processes such as liposome preparation and the fusion of liposomes with the cell membrane may be required to anchor the conjugates into the cell membrane.

An additional advantage noted by Kato *et al* (2004) was that synthetic membrane anchors with high hydrophile-lipophile balance values (attributable to PEG spacer with a high number of oxyethylene units) were concluded to have no cytolytic activity. However, difficulties arise in the use of synthetic

membrane anchors including a PEG spacer with a high number of oxyethylene units.

Firstly, the expression of the conjugative peptide or other endogenous cell surface peptides may be masked by the PEG spacer. Secondly, a PEG spacer with a high number of oxyethylene units may elicit non-specific adherence of protein (including antibodies in certain individuals) and/or the non-specific activation of the complement cascade.

Winger *et al* (1996) describes the conjugation of bromoacetylated DSPE with a thiol terminated decapeptide comprising at its C-terminus the minimal human thrombin-receptor peptide agonist (HS---SerPheLeuLeuArgAsn).

Hashimoto *et al* (1986) describes the conjugation of iodoacetylated DSPE with thiolated compounds.

A need exists for peptide-lipid constructs that can be used to effect qualitative and quantitative changes in the level of peptides expressed at the surface of cells and multi-cellular structures.

It is an object of this invention to provide peptide-lipid constructs that satisfy this need or at least provide a useful choice.

DISCLOSURE OF INVENTION

In a **first** aspect the invention provides a method of detecting reactive antibody in the serum of a subject including the steps of:

- Contacting a sample of the serum with a suspension of cells modified to incorporate a peptide-lipid construct of the structure $(L\text{-}S\text{-})_iF(\text{-}S\text{-}L)_j$ to provide a mixture;

- Incubating the mixture for a time and at a temperature sufficient to allow agglutination; and

- Determining the degree of agglutination of the cells in the mixture;

where:

F is a peptide comprising an epitope for the reactive antibody;
S is a spacer covalently linking F to L; and
L is a lipid selected from the group consisting of diacyl- and dialkyl-glycerolipids, including glycerophospholipids; and
i and j are independently 0 or 1,

Optionally, the method includes the preliminary step of:

- Adding an amount of the peptide to the sample of the serum;

where the amount of the peptide is sufficient to neutralize non-specific agglutination or confirm specificity of the reactive antibody.

Optionally, the method includes the intermediate step of:

- Adding an anti-subject globulin antibody to the mixture prior to determining the degree of agglutination of the cells of the mixture.

Preferably, the subject is a human.

Preferably, the cells are red blood cells.

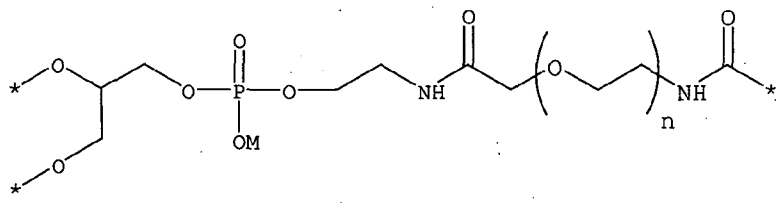
Preferably, the anti-subject globulin antibody is anti-human globulin (AHG) antibody.

Preferably, the reactive antibody is reactive to an antigen selected from the group consisting of: Glycophorin A, Glycophorin B, or mutations thereof (including the MNS blood group system).

The spacer (S) is selected to provide a water soluble peptide-lipid construct.

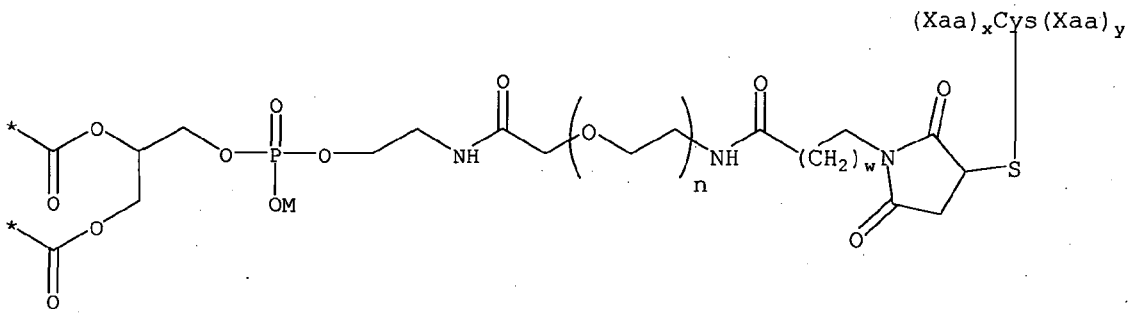
Preferably, S is a spacer covalently linking F to L via an oligomer of ethylene glycol.

Preferably, the structure of the peptide-lipid construct includes the substructure:

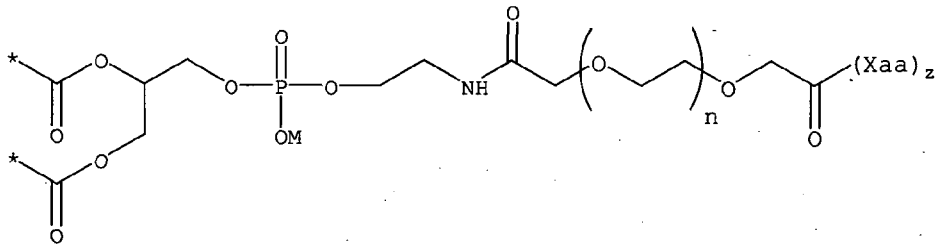


where M is a monovalent cation (M^+), n is 6 to 14 and * is other than H.

More preferably, the structure of the peptide-lipid construct is either:



or



where M is a monovalent cation (M^+), n is 6 to 14, w is 1 or 2, the sum of x and y is greater than 5, z is greater than 5, and * is other than H.

Preferably, the sum of i and j is 1.

Optionally, F is a peptide including a proximal terminal sequence (PTS) selected to promote solubility of the peptide.

In a preferment of this option, the PTS of the peptide is selected from the group consisting of:

SerLysLysLysLysGly

AlaAlaAlaAla

GlySerGlySerGly

Preferably, F is a peptide comprising an epitope of antigens selected from the group consisting of: Glycophorin A, Glycophorin B, or mutations thereof (including the MNS blood group system).

More preferably, F is a peptide selected from the *List of Peptides*.

Most preferably, F is a peptide selected from the group consisting of:

GlnThrAsnAspLysHisLysArgAspThrTyrAlaAlaAlaAlaAlaCys

GlnThrAsnAspLysHisLysArgAspThrTyrGlySerGlySerGlyCys

GlnThrAsnAspMetHisLysArgAspThrTyrGlySerGlySerGlyCys

SerSerGlnThrAsnAspLysHisLysArgAspThrTyrCys

ThrTyrProAlaHisThrAlaAsnGluValCys

ProAlaHisThrAlaAsnGluValCys

SerGlnThrAsnAspLysHisLysArgAspCys

AlaAlaAlaAlaValMetTyrAlaSerSerGly

GlySerGlySerGlyValMetTyrAlaSerSerGly

Preferably, L is a glycerophospholipid. More preferably, L is a glycerophospholipid selected from the group consisting of: 1,2-O-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine (DOPE)

and 1,2-O-distearyl-*sn*-glycero-3-phosphatidylethanolamine (DSPE).

Preferably, the peptide-lipid construct is an exemplifying embodiment of the second or third aspect of the invention.

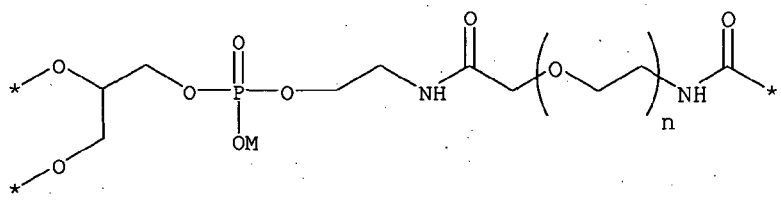
In a **second** aspect the invention provides a peptide-lipid construct of the structure:

L-S-F

where

F is a peptide;
S is a spacer covalently linking F to L via an oligomer of ethylene glycol; and
L is a lipid selected from the group consisting of diacyl- and dialkyl-glycerolipids, including glycerophospholipids.

Preferably, the structure of the peptide-lipid construct includes the substructure:



where M is a monovalent cation (M^+), n is 6 to 14 and * is other than H.

Optionally, F is a peptide including a proximal terminal sequence (PTS) selected to promote solubility of the peptide.

In a preferment of this option, the PTS of the peptide is selected from the group consisting of:

SerLysLysLysLysGly

AlaAlaAlaAla

GlySerGlySerGly

Preferably, the terminal sequence of the peptide is selected from the group consisting of:

GlyLysLysLysLysSerCys

AlaAlaAlaAlaCys

GlySerGlySerGlyCys

CysSerLysLysLysLysGly

CysAlaAlaAlaAla

CysGlySerGlySerGly

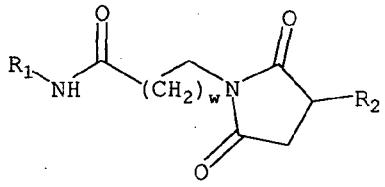
Preferably, S is covalently linked to F via a sulphide bond formed with the Cys residue of the peptide.

More preferably, S is covalently linked to F via a sulphide bond formed with a Cys residue of the peptide at or proximal to a terminus of the peptide.

Most preferably, S is linked to F via a sulphide bond formed with a Cys residue of the peptide at the carboxy-terminus of the peptide.

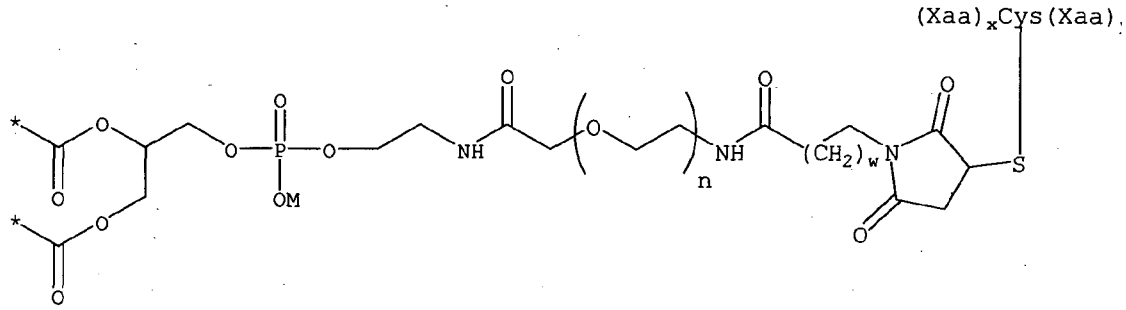
The spacer (S) is of the structure S_1-S_2-S_3 and selected to provide a water soluble peptide-lipid construct. S_1 is an oligomer of ethylene glycol.

Preferably, S_2-S_3 is selected from the group consisting of:



where R_1 is a terminal carbon of S_1, R_2 is the sulphur of the Cys residue and w is 1 or 2.

Preferably, the structure of the peptide-lipid construct is:



where M is a monovalent cation (M^+), n is 6 to 14, w is 1 or 2, the sum of x and y is greater than 5, and * is other than H. More preferably, n is 6. Most preferably, y is 0.

Preferably, F is a peptide comprising an epitope of antigens selected from the group consisting of: Glycophorin A, Glycophorin B, or mutations thereof (including the MNS blood group system).

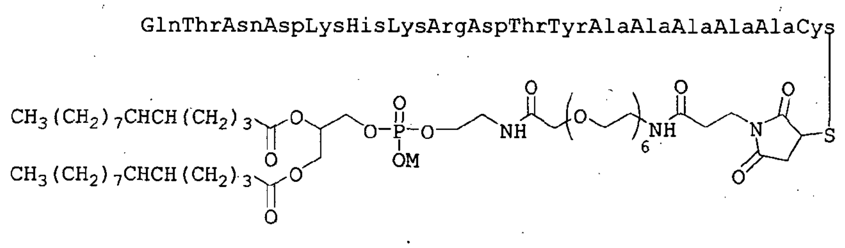
More preferably, F is a peptide selected from the *List of Peptides*.

Most preferably, F is a peptide selected from the group consisting of:

GlnThrAsnAspLysHisLysArgAspThrTyrAlaAlaAlaAlaAlaCys
GlnThrAsnAspLysHisLysArgAspThrTyrGlySerGlySerGlyCys
GlnThrAsnAspMetHisLysArgAspThrTyrGlySerGlySerGlyCys
SerSerGlnThrAsnAspLysHisLysArgAspThrTyrCys
ThrTyrProAlaHisThrAlaAsnGluValCys
ProAlaHisThrAlaAsnGluValCys
SerGlnThrAsnAspLysHisLysArgAspCys

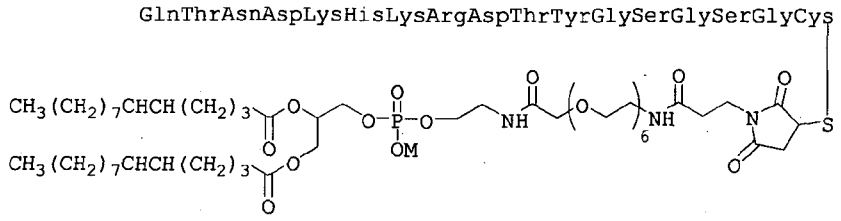
Preferably, L is a glycerophospholipid. More preferably, L is a glycerophospholipid selected from the group consisting of: 1,2-O-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine (DOPE) and 1,2-O-distearyl-*sn*-glycero-3-phosphatidylethanolamine (DSPE).

In an exemplifying **first** embodiment of the second aspect the invention provides a peptide-lipid construct of the structure:



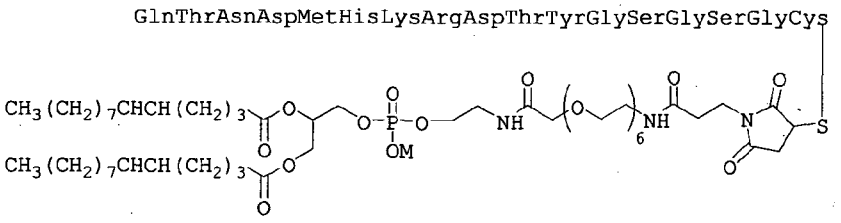
where M is a monovalent cation (M^+) and designated DOPE-PEG$_6$-βAla-Mal-PTS-1MUTK) (**M1**).

In an exemplifying **second** embodiment of the second aspect the invention provides a peptide-lipid construct of the structure:



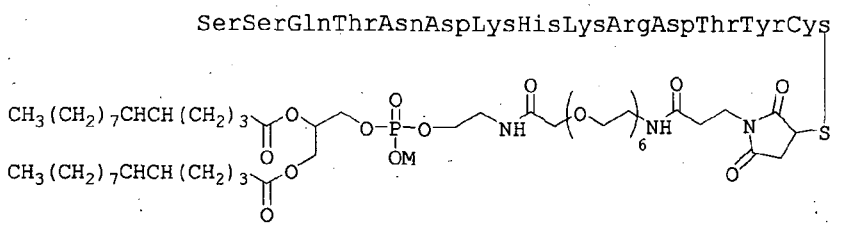
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-PTS-2MUTK)(**M2**).

In an exemplifying **third** embodiment of the second aspect the invention provides a peptide-lipid construct of the structure:



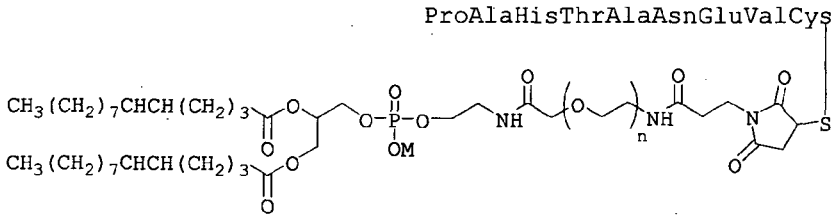
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-PTS-3MUTM(**M3**).

In an exemplifying **fourth** embodiment of the second aspect the invention provides a peptide-lipid construct of the structure:



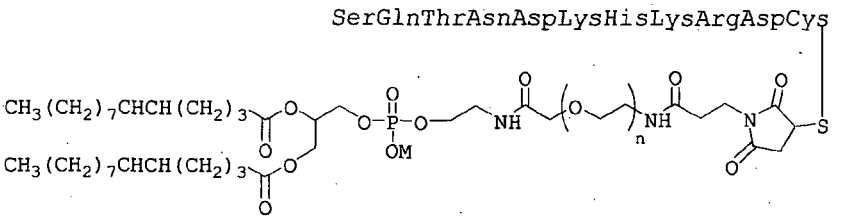
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-13MUTK(**M13**).

In an exemplifying **fifth** embodiment of the second aspect the invention provides a peptide-lipid construct of the structure:



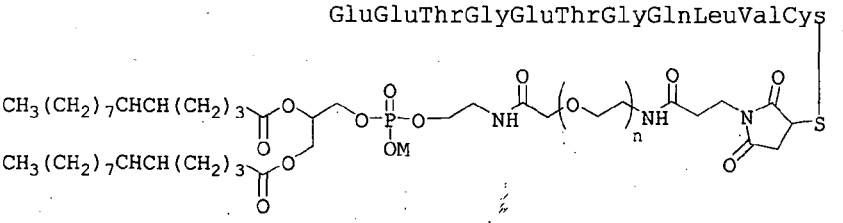
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-18Mur (**M18**) (n=6).

In an exemplifying **sixth** embodiment of the second aspect the invention provides a peptide-lipid construct of the structure:



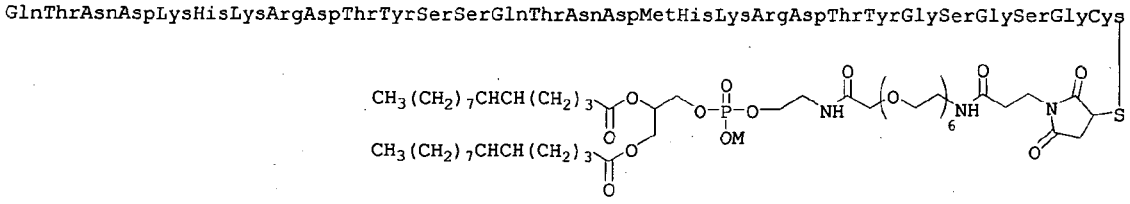
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-21MUTK(**M21**) (n=6).

In an exemplifying **seventh** embodiment of the second aspect the invention provides a peptide-lipid construct of the structure:



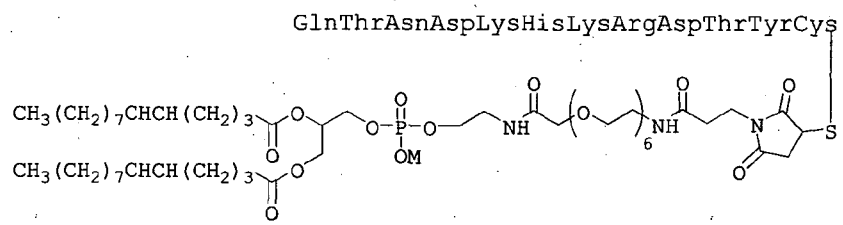
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-Hil3(**M23**) (n=6).

In an exemplifying **eighth** embodiment of the second aspect the invention provides a peptide-lipid construct of the structure:



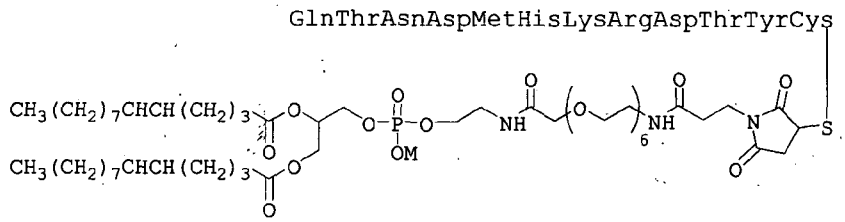
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-PTS-Milt(K,M).

In an exemplifying **ninth** embodiment of the second aspect the invention provides a peptide-lipid construct of the structure:



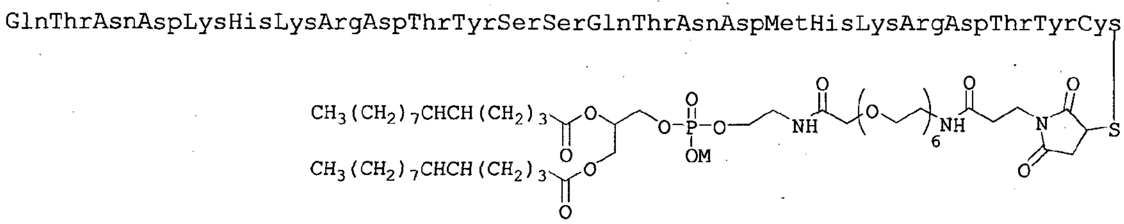
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-Milt(K) (**M00**).

In an exemplifying **tenth** embodiment of the second aspect the invention provides a peptide-lipid construct of the structure:



where M is a monovalent cation (M^+) and designated DOPE-PEG$_6$-βAla-Mal-Milt(M).

In an exemplifying **eleventh** embodiment of the second aspect the invention provides a peptide-lipid construct of the structure:



where M is a monovalent cation (M^+) and designated DOPE-PEG$_6$-βAla-Mal-Milt(K,M).

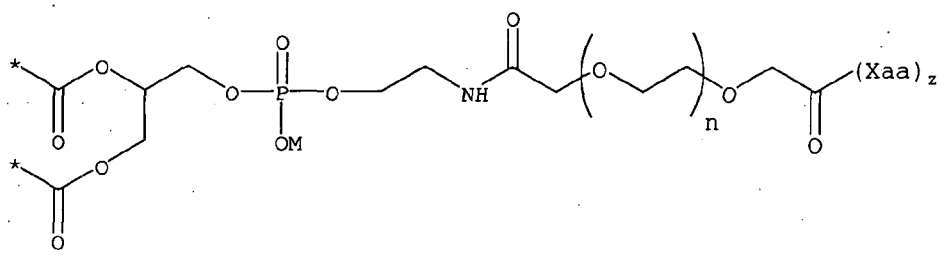
In a **third** aspect the invention provides a peptide-lipid construct of the structure:

L-S-F

where

F is a peptide;
S is a spacer covalently linking F to L via an oligomer of ethylene glycol; and
L is a lipid selected from the group consisting of diacyl- and dialkyl-glycerolipids, including glycerophospholipids.

Preferably, the structure of the peptide-lipid construct is:



where M is a monovalent cation (M^+), n is 6 to 14, z is greater than 5, and * is other than H. More preferably, n is 14.

Optionally, F is a peptide including a terminal sequence selected to promote solubility of the peptide.

In a preferment of this option, the terminal sequence of the peptide is selected from the group consisting of:

SerLysLysLysLysGly

AlaAlaAlaAla

GlySerGlySerGly

Preferably, F is a peptide selected from the group consisting of:

$(Xaa)_z$ValMetTyrAlaSerSerGly;

where z is the integer 4, 5 or 6.

Preferably, F is a peptide selected from the group consisting of:

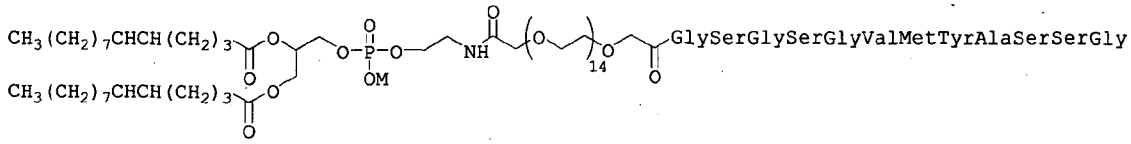
SerLysLysLysLysGlyValMetTyrAlaSerSerGly

AlaAlaAlaAlaValMetTyrAlaSerSerGly

GlySerGlySerGlyValMetTyrAlaSerSerGly

Preferably, L is a glycerophospholipid. More preferably, L is a glycerophospholipid selected from the group consisting of: 1,2-O-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine (DOPE) and 1,2-O-distearyl-*sn*-glycero-3-phosphatidylethanolamine (DSPE).

In an exemplifying **first** embodiment of the third aspect the invention provides a peptide-lipid construct of the structure:



where M is a monovalent cation (M^+) and designated DOPE-PEG_{14}-Syph.

In a **fourth** aspect the invention provides a method of preparing a peptide-lipid construct (F-S-L) of the second aspect of the invention including the steps of:

- Preparing a maleimido-derivative of a precursor construct by reacting a maleimido-donating reagent with a precursor construct of the structure L-S_1-NH_2; and

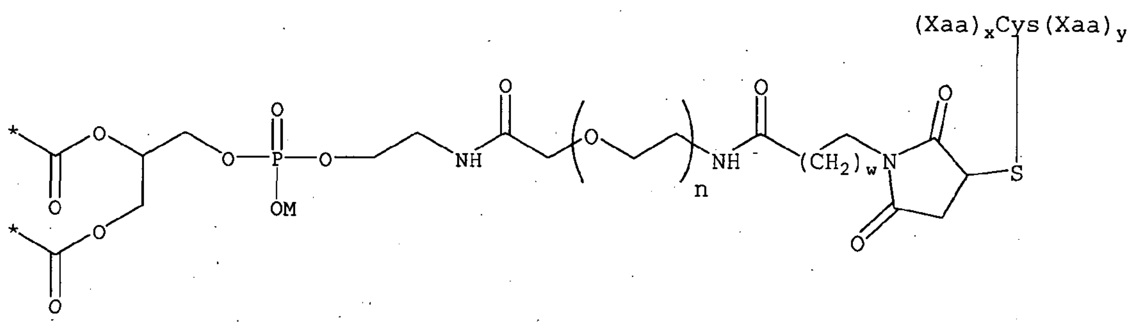
- Reacting the maleimido-derivative of the precursor construct with a peptide (F) including a Cys residue and solubilised in a solvent.

where:

L is a lipid selected from the group consisting of diacyl- and dialkyl-glycerolipids, including glycerophospholipids; and

S_1 is selected from the group consisting of oligomers of ethylene glycol.

Preferably, the structure of the peptide-lipid construct is:



where n is 6 to 14, w is 1 or 2, the sum of x and y is greater than 5, and * is other than H.

Preferably the maleimido-donating reagent is selected from the group consisting of: N-oxysuccinimid ester of maleimidobutyric acid; and N-oxysuccinimid ester of maleimidopropionic acid

Preferably, S_1 is an oligomer of ethylene glycol selected from the group consisting of 6 to 14 *mer* PEG (PEG_6 to PEG_{14}). Most preferably, S_1 is PEG_6.

Preferably, the solvent is selected from the group consisting of: trifluoroethanol; DMSO; or mixtures thereof.

Preferably, the Cys residue is a terminal Cys residue.

Optionally, F is a peptide including a proximal terminal sequence (PTS) selected to promote solubility of the peptide in the reaction solvent.

In a preferment of this option, the PTS of the peptide is selected from the group consisting of:

SerLysLysLysLysGly

AlaAlaAlaAla

GlySerGlySerGly

Preferably, the terminal sequence of the peptide is selected from the group consisting of:

GlyLysLysLysLysSerCys

AlaAlaAlaAlaCys

GlySerGlySerGlyCys

CysSerLysLysLysLysGly

CysAlaAlaAlaAla

CysGlySerGlySerGly

Preferably, F is a peptide selected from the *List of Peptides*.

Preferably, F is a peptide selected from the group consisting of:

GlnThrAsnAspLysHisLysArgAspThrTyrAlaAlaAlaAlaAlaCys

GlnThrAsnAspLysHisLysArgAspThrTyrGlySerGlySerGlyCys

GlnThrAsnAspMetHisLysArgAspThrTyrGlySerGlySerGlyCys

SerSerGlnThrAsnAspLysHisLysArgAspThrTyrCys

ThrTyrProAlaHisThrAlaAsnGluValCys

ProAlaHisThrAlaAsnGluValCys

SerGlnThrAsnAspLysHisLysArgAspCys

Preferably, L is a glycerophospholipid. More preferably, L is a glycerophospholipid selected from the group consisting of: 1,2-O-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine (DOPE) and 1,2-O-distearyl-*sn*-glycero-3-phosphatidylethanolamine (DSPE).

In a **fifth** aspect the invention provides a method of effecting qualitative and quantitative changes in the levels of peptide expressed at the surface of cells and multi-cellular structures including the step of:

- contacting the cells or multi-cellular structures with a solution of a peptide-lipid construct of the second or third aspects of the invention at a concentration and for a time and temperature sufficient to allow the construct to incorporate into the surface.

Preferably, the peptide-lipid construct is a construct of the second aspect of the invention.

Preferably the cells or multicellular structures are selected from the group consisting of: red blood cells; and embryos. More preferably, the cells or multicellular structures are human cells or multicellular structures.

Preferably, the time and temperature is no greater than 2 hours at 37 °C or 24 hours at 4 °C.

In all aspects of the invention M is typically H, but may be replaced by another monovalent cation such as Na^+, K^+ or NH_4^+.

In the description and claims of the specification the following acronyms, phrases and terms have the meaning provided:

"Diagnostic marker" means a molecule, the presence of which in a body fluid of a subject is diagnostic of a phenotype or pathological condition of the subject.

"MNS blood group system " means blood group antigens or epitopes of those antigens and mutations which are present on either glycophorin A, glycophorin B or mutations which result in glycophorin A/B hybrids.

"Proximal terminal sequence" means that portion of the peptide sequence proximal to the amino- or carboxy- terminus of the peptide (F).

"RBC" means red blood cells.

"Reactive antibody" means an immunoglobulin, the presence of which in a body fluid of a subject is diagnostic of a phenotype or pathological condition of the subject.

"Via an oligomer of ethylene glycol" means a polymer of ethylene glycol consisting of 2 to 32 *mer* and specifically excludes via a polymer of ethylene glycol consisting of greater than 32 *mer*.

"Water soluble" means a stable, single phase system is formed when the construct is contacted with water or saline (such as PBS) at a concentration of at least 100 µg/ml and in the absence of organic solvents or detergents. The phrase is used synonymously with the term "water dispersible".

Exemplifying embodiments of the invention are claimed and will now be described in detail with reference to the Figures of the accompanying drawings pages.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1. ^{1}H-NMR spectrum of the peptide-lipid construct designated DOPE-PEG_6-βAla-Mal-Milt(K)(**M13**) (5 mg/ml in $CD_3OD/CDCl_3/D_2O$/0.5M CF_3COOD 60/20/10/1, 600 MHz, 30 °C, δ ppm).

Figure 2. MALDI TOF mass-spectrum of the peptide-lipid construct designated DOPE-PEG_6-βAla-Mal-Milt(K)(**M13**) (2856:Peptide-DOPE (M+H); 2878: Peptide-DOPE (M+Na); 2894:Peptide-DOPE (M+K); 2900:Peptide-DOPE (M+Na, Na salt); 2916:Peptide-DOPE (M+K, Na salt)).

Figure 3. ESI mass-spectrum and analytical HPLC of the peptide SerSerGlnThrAsnAspLysHisLysArgAspThrTyrGlySerGlySerGlyCys of the peptide-lipid construct designated DOPE-PEG_6-βAla-Mal-Milt(K)(**M13**).

Figure 4. ^{1}H-NMR spectrum of the peptide SerSerGlnThrAsnAspLysHisLysArg AspThrTyrGlySerGlySerGlyCys of the peptide-lipid construct designated DOPE-PEG_6-βAla-Mal-Milt(K)(**M13**) (4.5 mg/ml in D_2O, 600 MHz, 30 °C, δ ppm).

Figure 5. Photomicrographs of zona free embryos modified to incorporate the M2 peptide-lipid construct by contacting the embryos with a dispersion of the construct at a concentration of 1 mg/mL for 2 hours. The upper photomicrograph is the DIC image. The lower photomicrograph is the fluorescent image showing 3.0+ fluorescence.

DETAILED DESCRIPTION

In general terms the invention provides peptide-lipid constructs of the structure $(L\text{-}S\text{-})_iF(\text{-}S\text{-}L)_j$ where:

F is a peptide;
S is a spacer covalently linking F to L;
L is a lipid selected from the group consisting of diacyl- and dialkyl-glycerolipids, including glycerophospholipids;
i and j are independently 0 or 1;

and the use of these peptide-lipid constructs in diagnostic and therapeutic applications.

Where i is 0 and j is 1 the peptide-lipid constructs are of the structure:

F-S-L

Where i is 1 and j is 0 the peptide-lipid constructs are of the structure:

L-S-F

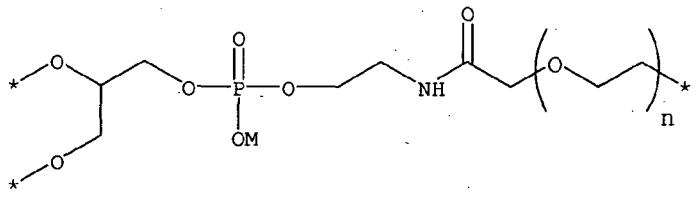
Where S is linked to F via the amino terminus of the peptide the construct is represented by the structure or substructure L-S-F.

Where S is linked to F via the carboxyl terminus of the peptide the construct is represented by the structure or substructure F-S-L.

Where S is linked to F via a sulphide bond formed via the sulfhydryl group of a Cys residue of the peptide the residue is identified with an underscore (Cys).

Where S is linked to F via a sulphide bond formed with one or more Cys residues of the peptide, the representation of the peptide-lipid construct by the structure L-S-F-S-L, L-S-F or F-S-L is not intended to imply the sulphide bond is formed exclusively with terminal Cys residues.

The use of the peptide-lipid constructs in diagnostic applications is illustrated with reference to the use of constructs including the substructure:



where M is a monovalent cation (M^+), n is 6 to 14, * is other than H, and the peptide is selected from the group of peptides consisting of peptides included in the *List of Peptides* provided on the following pages where z is an integer from 0 to 6.

[followed by pages 27 & 28]

List of Peptides
Cys(Xaa)$_z$TrpThrProProArgAlaGlnIleThrGlyTyrLeuThrValGlyLeuThrArgArg
Cys(Xaa)$_z$TrpThrProProArgAlaGlnIleThrGlyTyrArgLeuThrValGlyLeuThrArgArg
Cys(Xaa)$_z$ValMetTyrAlaSerSerGly
ValMetTyrAlaSerSerGly(Xaa)$_z$Cys
AspTyrHisArgValMetTyrAlaSerSerGly(Xaa)$_z$Cys
ThrAsnGlyGluThrGlyGlnLeuValHisArgPhe(Xaa)$_z$Cys
ThrAsnGlyGluMetGlyGlnLeuValHisArgPhe(Xaa)$_z$Cys
AspThrTyrProAlaHisThrAlaAsnGluValSerGlu(Xaa)$_z$Cys
ThrTyrProAlaHisThrAlaAsnGluVal(Xaa)$_z$Cys
ProAlaHisThrAlaAsnGluVal(Xaa)$_z$Cys
TyrProAlaHisThrAlaAsnGlu(Xaa)$_z$Cys
ThrTyrProAlaHisThrAlaAsn(Xaa)$_z$Cys
ThrTyrProAlaHisThrAlaAsnGlu(Xaa)$_z$Cys
TyrProAlaHisThrAlaAsnGluVal(Xaa)$_z$Cys
ProAlaHisThrAlaAsnGluValSer(Xaa)$_z$Cys
AspThrTyrProAlaHisThrAlaAsnGlu(Xaa)$_z$Cys
TyrProAlaHisThrAlaAsnGluValSer(Xaa)$_z$Cys

List of Peptides
SerGlnThrAsnAspLysHisLysArgAsp(Xaa)$_z$Cys
GlnThrAsnAspLysHisLysArgAspThrTyr(Xaa)$_z$Cys
GlnThrAsnAspLysHisLysArgAspThrTyrSerSerGlnThrAsnAspMetHisLysArgAspThrTyr(Xaa)$_z$Cys
GlnThrAsnAspMetHisLysArgAspThrTyr(Xaa)$_z$Cys
SerSerGlnThrAsnAspLysHisLysArg(Xaa)$_z$Cys
SerSerGlnThrAsnAspLysHisLysArgAspThrTyr(Xaa)$_z$Cys
SerSerGlnThrAsnAspMetHisLysArgAspThrTyr(Xaa)$_z$Cys
SerSerGlnThrAsnAspLysHisLysArgAspThrTyrSerSerGlnThrAsnAspMetHisLysArgAspThrTyr(Xaa)$_z$Cys
GlnThrAsnAspLysHisLysArgAspThr(Xaa)$_z$Cys
SerGlnThrAsnAspLysHisLysArgAspThr(Xaa)$_z$Cys
ThrAsnAspLysHisLysArgAspThrTyrPro(Xaa)$_z$Cys
GluGluThrGlyGluThrGlyGlnLeuVal(Xaa)$_z$Cys
GluGluGluThrGlyGluThrGlyGlnLeu(Xaa)$_z$Cys
GluThrGlyGluThrGlyGlnLeuValHis(Xaa)$_z$Cys
SerProProArgArgAlaArgValThr(Xaa)$_z$Cys
TyrArgTyrArgTyrThrProLysGluLysThrGlyProMetLysGlu(Xaa)$_z$Cys
TrpGlnProProArgAlaArgIle(Xaa)$_z$Cys
ThrIleThrGlyLeuGluProGlyThrGlu(Xaa)$_z$Cys

The amino acid residues of peptides are identified according to Table 3 of Appendix 2 of Annex C of the *Administrative Instructions under the Patent Cooperation Treaty* dated 7 February 2007 and in accordance with the convention:

H_2N-XaaXaaXaa.....XaaXaaXaa-COOH

There is a need for inexpensive and low level sensitivity test systems for a range of diagnostic markers in donated blood, in transfusion recipients, or in antenatal patients (where the unborn child may be at risk of haemolytic disease), e.g. syphilis markers and markers of the MNS blood group system. A particular advantage provided by the invention is the opportunity to employ established blood typing platforms to detect a range of peptide antigen-antibody interactions. The capital costs associated with establishing a new diagnostic assay may therefore be avoided.

Some clinically significant blood group antigens are rare (or rare in some populations). For example mutations of the MNS blood group system resulting in Miltenberger antigens are rare in Europeans, but common in Asians. Being able to create antibody detection and identification panels requires that these antigens be present on the diagnostic screening cells. Obtaining ells suitable for antibody screening/identification having rare antigens is therefore problematic. Being able to add to cells rare antigens prepared exogenously is therefore a major advantage.

According to the method of the invention epitope containing peptide sequences for a range of diagnostic markers, such as specific reacting antibodies, can be localized to the surface of red blood cells (RBCs). These modified RBCs may then be

used on existing blood typing platforms to detect blood antobodies or pathologies.

Although the invention is illustrated with reference to the modification of red blood cells and embryos the outer surface of other cells and multi-cellular structures is contemplated. However, red blood cells are preferred for use in diagnostic assays because of the facility with which these modified cells could be used in blood typing laboratories.

The level of peptide-lipid construct incorporated into the cell membrane of red blood cells is controlled by the concentration of the construct in the dispersion contacted with the suspension. The presence of diagnostic markers may then be assessed by agglutination whether direct (induced by centrifugation of cells) or indirect (induced by adding an antibody directed against the immunoglobulins of the subject). Other methods of assessment may be employed including, for example, rosetting (Indiveri *et al* 1979) and enzyme linked immunosorbant assays (ELISA).

In contrast with the preparation of constructs where the function (F) is a carbohydrate, the preparation of constructs where F is a peptide presents a combination of technical difficulties.

Firstly, it is desirable for the peptide (F) ligated to the L-S or S-L moiety to be dispersible in the solvents used for the ligation chemistry. Overcoming this difficulty may require the selection of a proximal terminal sequence (PTS) to promote solubility without modifying the desired biological properties of the construct.

Secondly, it is r for the construct (L-S-F-S-L, L-S-F or F-S-L) to be dispersible in water, or at least a biocompatible medium such as buffered saline, according to the requirements of the proposed application (i.e. it is desirable for the construct to be "water soluble" as defined herein). Overcoming this difficulty requires the selection of a spacer (S) to promote solubility of the construct.

Thirdly, where the proposed application is the modification of cells such as red blood cells (RBCs) for use in diagnostic applications, including use as quality controls in blood group typing or detection of diagnostic antibodies present in patient serum, it is required for the construct to be dispersible without participating in antigen-antibody cross reactivity not specific to the diagnostic marker. Satisfying this requirement requires the identification of suitable structural motifs for the spacer (S) and proximal terminal sequence (PTS) when the latter is present, or the development of sample preparation procedures that neutralize or at least substantially mitigate the undesired cross reactivity and likelihood of false positives.

It should also be noted that where the application is for use in the modification of the surface of cells or multi-cellular structures (e.g. an embryo) with a view to promoting the association of the modified cell or modified multi-cellular structure with a target surface (e.g. the endometrium) exposing the cell or multi-cellular structure to solvents is incompatible with maintaining the cells or multicellular structures in a viable state.

The ability to localise peptides to the surface of cells or multi-cellular structures via a residue proximal to either the

N- or C- terminus of the peptide may also allow the naturally occurring configuration of the peptide sequence relative to the cell surface to be approximated. The presentation of the peptide sequence in the tertiary (or quaternary) structure of the parent polypeptide (or protein) may therefore be mimicked.

Although not demonstrated here it is contemplated that peptides may be localised to the surface of cells via multiple residues. For example, where both a residue proximal to the amino terminus and a residue proximal to the carboxyl terminus are used to localize the peptide, a "looped" configuration of the peptide may be promoted at the surface.

The use of polyethylene glycol (PEG) as a spacer to promote solubility is known. However, polymers of PEG may interfere with the expression and function of the peptide at the surface. In the peptide-lipid constructs of the invention an oligomer of ethylene glycol (6 to 14 *mer*) is selected as a component (S_1) of the spacer (S) linking the lipid (L) and peptide (F).

Oligomers of ethylene glycol impart less solubility to peptide-lipid constructs of the structure L-S-F than polymers of PEG. The difficulty referred to above therefore arises when it is desired to obtain peptide-lipid constructs that are dispersible in biocompatible solvents and can be used in methods of effecting qualitative and quantitative changes in the levels of peptide expressed at the surface of cells and multi-cellular structures.

The properties of the peptide-lipid constructs must be such that they can be readily dispersed in biologically compatible media in the absence of solvents or detergents, but

incorporate into the lipid bilayer of a membrane when a solution of the construct is contacted with a suspension of cells or multi-cellular structures.

Peptide-lipid constructs with these potentially conflicting properties are prepared by adopting the combination of structural motifs described here. The preparation of the peptide-lipid constructs where S is linked to F via a sulphide bond formed with a terminal Cys residue of the peptide at the carboxy-terminus of the peptide is preferred as the peptide is less prone to oxidation.

Adopting the combinations of structural motifs in accordance with the description provided here a range of peptides may be prepared as peptide-lipid constructs for use in methods of effecting qualitative and quantitative changes in the levels of peptide expressed at the surface of cells and multi-cellular structures.

It will be understood that for a non-specific interaction, such as the interaction between diacyl- or dialkyl-glycerolipids or glycerophospholipids and a membrane, structural and stereo-isomers of naturally occurring lipids can be functionally equivalent. For example, it is contemplated that diacylglycerol 2-phosphate could be substituted for phosphatidate (diacylglycerol 3-phosphate). Furthermore it is contemplated that the absolute configuration of phosphatidate can be either R or S.

Preparation of DOPE-PEG_6-NH_2 (7)

DOPE-PEG_6-NH_2 (L-S_1-NH_2) (**7**, 800 mg) was prepared by the method of SCHEME 1. To a stirred solution of DOPE (**5**) (36 mg, 0.0484

mmol) in dry $CHCl_3$ (3 ml) a solution of Fmoc-PEG-NOS (**4**)(237 mg, 0.0697 mmol (containing about 80% of active *N*-oxysuccinimide ester)) in dry $CHCl_3$ (1 ml) and Et_3NH (30 ml) was added.

The solution was stirred for 15 h at 20 °C, then Et_3NH (3 ml) was added, and the mixture was maintained for at 8 h at 20°C. The solution was then diluted with toluene (10 ml), evaporated under reduced pressure (10 to 15 torr) and dried under vacuum.

The crude residue was dissolved in H_2O/MeOH/AcOH mixture (10:5:1 (v/v/v), 3 ml) and the solution was slowly applied to a reverse phase C_{16} column (15 ml, water). Salts, *N*-hydroxysuccinimide and H_2N-PEG-DOPE (**7**) were eluted from the column with MeOH/H_2O 1:2 (v/v) (30 ml), 1:1 (v/v) (15 ml) and 2:1 (v/v) (15 ml). Target H_2N-PEG-DOPE (**7**) was eluted from the column with MeOH (30 ml) and then with MeOH to MeOH/$CHCl_3$ mixtures (4:1 (v/v), 3:1 (v/v), 2:1 (v/v) and 1:1 (v/v); 30 ml each). Fractions containing H_2N-PEG-DOPE (**7**) were combined, evaporated under reduced pressure (10 to 15 torr) and dried under vacuum.

The residue obtained as a thin film on the flask walls was extracted twice with hexane (2 x 5 ml) and dried under vacuum to yield 143 mg of H_2N-PEG-DOPE (**7**) (78% on DOPE) as a white solid. TLC: R_f= 0.62 (ethanol/water/pyridine/AcOH; 3:1:1:1 (v/v/v/v)).

^{1}H-NMR (500 MHz, CD_3OD, 30 °C): δ = 5.541 (m, 4H; 2 -C<u>H</u>=C<u>H</u>-), 5.416 (m, 1H; $OCH_2C\underline{H}CH_2O$), 4.624 (dd, *J* = 12 Hz, *J* = 3.2 Hz, 1H; CO-OC<u>H</u>$CHCH_2$), 4.373 (dd, *J* = 12 Hz, *J* = 6.6 Hz, 1H; CO-OC<u>H</u>$CHCH_2$), 4.195 (t, *J* = 5.6 Hz, 2H; $POC\underline{H}_2CH_2N$), 4.117 (m, 2H; POC<u>H</u>$CHCH_2$), 3.968 (m, 4H; $OC\underline{H}_2CH_2O$, $OC\underline{H}_2CH_2N$), 3.932 (t, *J* =

6.2 Hz, 2H; $OC\underline{H}_2CH_2CO$), 3.827 (m, 272 H; $(-OC\underline{H}_2CH_2-)_n$, n = 68), 3.683 (m, 2H; $OC\underline{H}_2CH_2O$), 3.622 (t, *J* = 5.6 Hz, 2H; $OCH_2C\underline{H}_2N$), 3.397 (t, J = 5.0 Hz, 2H; OCH_2CH_2N), 2.678 (t, J = 6.2 Hz, 2H; $OCH_2C\underline{H}_2CO$), 2.519 (m, 4H; 2 CH_2CO), 2.228 (m, 8H; 2 $CH_2CH=CHCH_2$), 1.801 (m, 4H; 2 CH_2CH_2CO), 1.508 (m, 40H; $-CH_2-$), 1.096 (~t, 6H; 2 CH_3) ppm.

Preparation of peptide-lipid constructs

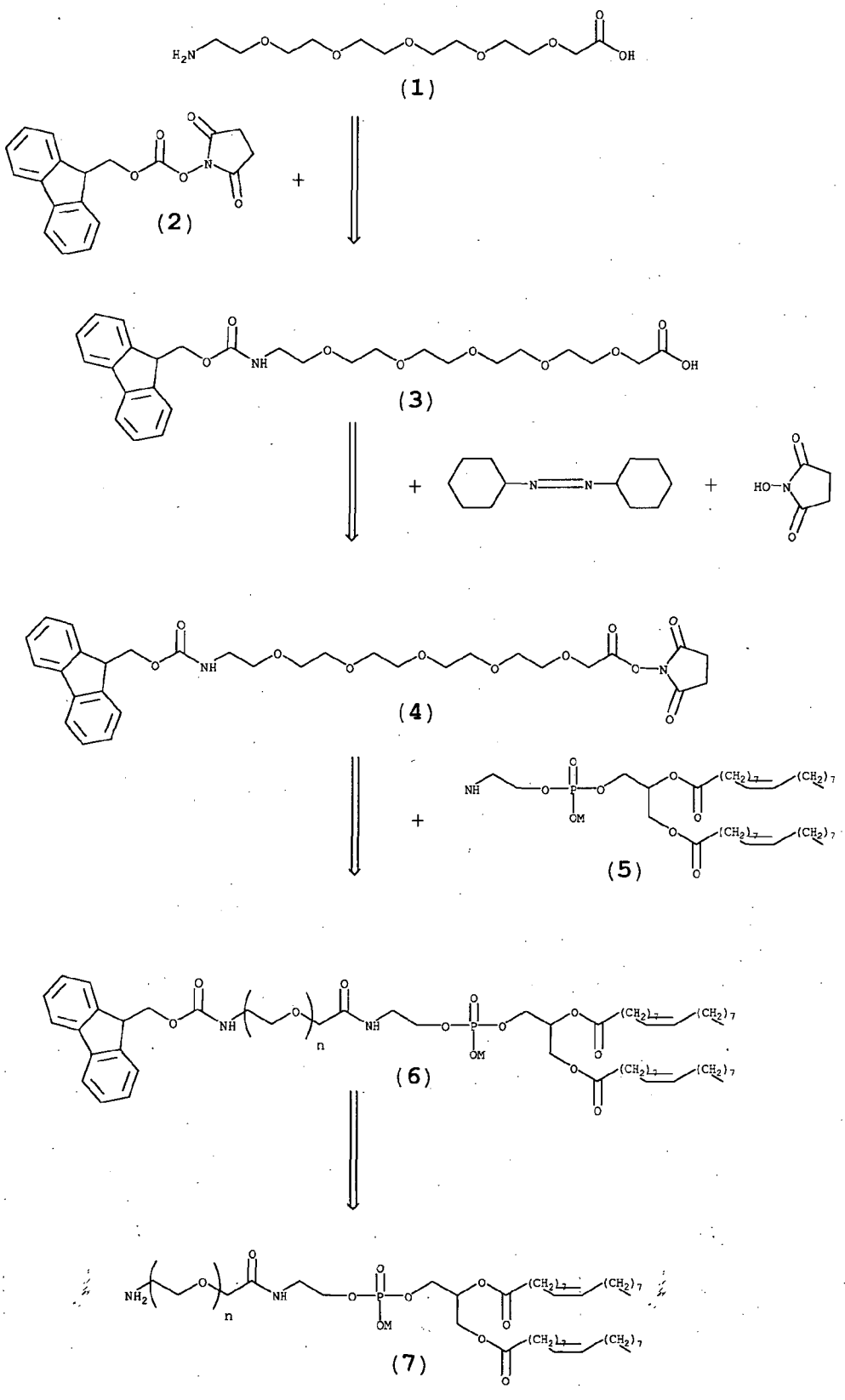
Maleimido-derivatives of DOPE-PEG_6-NH_2 were used for the preparation of peptide-lipid constructs (L-S-F) by the method of SCHEME 2 via the maleimide-thiol Michael addition reaction.

Synthesis via the maleimido-derivatives of DOPE-PEG_6-NH_2 has particular advantages over synthesis via iodoacetate derivatives as difficulties and low yields as a consequence of oxidation of the sulfhydryl residues of the peptide and subsequent dimer formation. Reducing agents (e.g. tertiary phosphines) may be used during conjugation.

Maleimido-derivatives were synthesized with 65 to 70% yields starting from N-oxysuccinimid esters of maleimidobutyric and maleimidopropionic acids (**8a**, **8b**). An unexpected complication arose due to the presence of excess Bu_3P which appeared to be highly reactive towards the maleimide function. Phosphine was therefore used only in sub-equivalent amounts (0.1 to 0.2 equivalents).

[followed by page 36]

SCHEME 1



Trifluoroethanol used as a co-solvent in the preparation of **10bC** where the peptide was GlnThrAsnAspMetHisLysArgAspThrTyr-GlySerGlySerGlyCys appeared to be highly efficient for solubilization of both reactants. However, the solvent also caused unwanted acidification of the reaction medium which may inhibit the Michael reaction. The isolated yield of **10bC** in this experiment was ~25%. Preparation of **10aC** where the peptide was GlnThrAsnAspMetHisLysArgAspThrTyrGlySerGlySerGly-Cys (DOPE-PEG_6-βAla-Mal-3MUTM (**M3**)) carried out using DMSO as co-solvent was more successful and provided a 43% yield.

The same solvent strategy in the preparation of **10bC** where the peptide was GlnThrAsnAspLysHisLysArgAspThrTyrSerSerGlnThrAsn-AspMetHisLysArgAspThrTyrAlaAlaAlaAlaCys (DOPE-PEG_6-βAla-Mal-PTS-Milt(K,M)) failed because the peptide supplied appeared to be very acidic and caused solubilization problems. The yield of **10bC** in this experiment was only 23% and about half of the peptide was recovered.

Molecular weights for the peptide lipid constructs were determined to be:

DOPE-PEG_6-βAla-Mal-Milt(M) - 3029.48

DOPE-PEG_6-βAla-Mal-Milt(K,M) - 4591.12

As expected for peptides bearing the glutamine residue at the N-terminus, all preparations contain variable amounts of related pyroglytamyl derivatives, M-17 in MS, due to loss of NH_3. The formation of related pyroglytamyl derivatives was mitigated in peptides with N-terminal Ser residues.

The use of the peptide-lipid constructs in methods for effecting qualitative and quantitative changes in the levels of peptide expressed at the surface of cells and multi-cellular structures is illustrated with reference to the serodiagnosis.

Modification of red blood cells with peptide-lipid constructs (general method)

Red blood cells are modified by mixing 1 part by volume of washed packed red blood cells with 1 part by volume of peptide-lipid construct dispersed at a concentration of 10 to 1000 µg/ml in cell media (Celpresol™).

The suspensions are either:

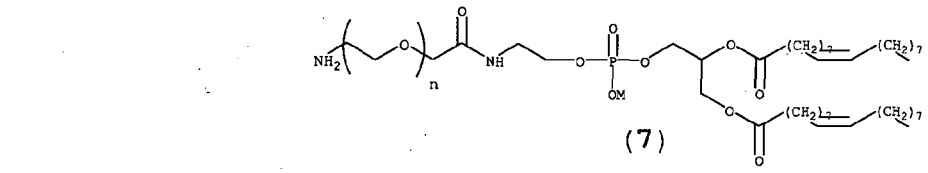
1. incubated for 2 hours at 37 °C before being washed and suspended in a cell medium for serological analysis at a concentration of 0.8 to 3% (Method 1); or

2. incubated for 3 to 4 hours at room temperature (*circa* 25 °C) followed by 18 hours at 4 °C before being washed and suspended in a cell medium for serological analysis at a concentration of 0.8 to 3% (Method 2).

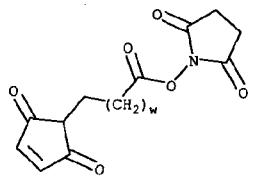
*Modification of red blood cells with DOPE-PEG_6-βAla-Mal-Milt(K) (**M00**)*

4.7 mg of the lipid-peptide construct DOPE-PEG_6-βAla-Mal-Milt(K) (**M00**)was reconstituted in 0.47 ml of Celpresol™ by sonicating for 10 min and allowing to stand for 1 hour to provide a clear 10 mg/ml stock solution.

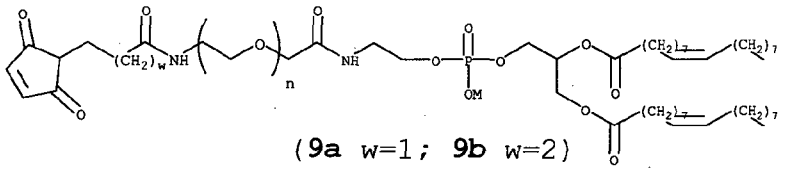
SCHEME 2



(7)

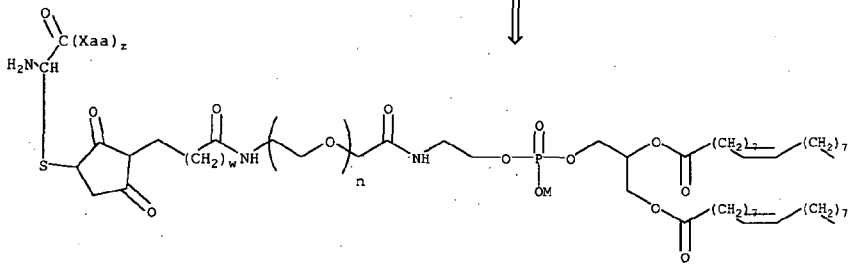


(8a w=1; 8b w=2)



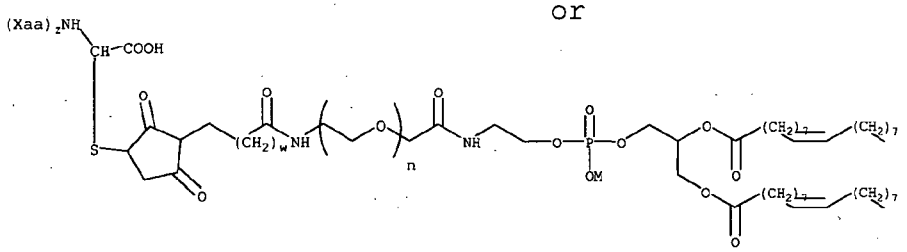
(9a w=1; 9b w=2)

+ Cys(Xaa)$_z$ or (Xaa)$_z$Cys



(10aN w=1; 10bN w=2)

or



(10aC w=1; 10bC w=2)

The stock solution was diluted two-fold to provide a solution of 5 mg/ml and a dilution series then prepared for the peptide-lipid construct at the following concentrations:

1 mg/ml (1:5 dilution in Celpresol™)

0.5 mg/ml (1:10 dilution in Celpresol™)

0.25 mg/ml (1:20 dilution in Celpresol™)

200 µl of Miltenberger negative red blood cells (*Milt*$^-$ RBCs) were washed two times with PBS and one time with Celpresol™. 40 µl of a washed packed volume of *Milt*$^-$ RBCs were mixed with 40 µl of a dilution of the peptide-lipid construct and incubated for 2 hours at 37 °C.

The modified RBCs were then washed with Celpresol™ and stored in Celpresol™ until used in tube serology testing (3 days and 24 days).

Tube serology testing of modified red blood cells

Serological reactions are graded or scored by either of two established systems (0 or '-' = no agglutination, 1+ or 3 = very weak agglutination, 2+ or 5 = weak agglutination, 3+ or 8 = moderate strong agglutination, 4+ or 10/12 = strong agglutination)

Serological platforms used are Tube (addition of reagents and reactants into plastic or glass serology tubes and after appropriate incubations, washing and centrifugation observing reactions macroscopically by eye and a 10X magnification eyepiece and scoring) and BioVue™ (addition of reactants into

cassettes containing beads (including some reactants) and after appropriate incubations and centrifugation observing the reaction patterns traped within the Gel matrix). BioVue is the serological column agglutination platform of Ortho-Clinical Diagnostics.

Serum samples were available from 47 blood donors of negative antibody screen status. These samples were designated "negative samples", but not determined not to have anti-Miltenberger antibodies).

Three serum samples known to have Miltenberger related antibodies T217, T6025, T5896. These samples were designated "positive samples", but not determined to have anti-antibodies against the peptide of the peptide of the construct designated DOPE-PEG_6-βAla-Mal-Milt(K) (**M00**).

A suspension of 3 % modified RBCs was prepared in PBS and 30 µl of the suspension mixed with 30 µl serum sample. The mixtures were then incubated for 45 min at 37 °C. Following incubation the RBCs were centrifuged for 10 s in an Immufuge™ (setting: "high") and observed for agglutination before being washed 3 times with PBS.

After washing one drop of Epiclone™ anti-human globulin (AHG) was added and the tubes then centrifuged for 10 s in an Immufuge™ (setting: "high"). Tubes were then read and serology scores recorded.

[followed by page 42]Age of modified RBCs (days)		Concentration of DOPE-PEG_6-βAla-Mal-Milt(K)(**M00**) (mg/ml)					
	Serum	1.0 (n = 47)		0.5 (n = 21)		0.25 (n = 21)	
3	Negative samples	AHG+	AHG-	AHG+	AHG-	AHG+	AHG-
		1	46	0	21	0	21

Table 1. Summary of reactivity of samples of serum from 47 blood donors not expected to have anti-Miltenberger activity ("negative samples"). AHG+ means sample reacted by the anti-human globulin test. AHG- means sample is unreactive. RBCs were modified with the peptide-lipid construct designated DOPE-PEG_6-βAla-Mal-Milt(K) at the concentrations indicated. Sera were tested against modified RBCs following 3 days storage.

Age of modified RBCs (days)		Concentration of DOPE-PEG_6-βAla-Mal-Milt(K)(**M00**) (mg/ml)		
	Serum	1.0	0.5	0.25
3	T217	2+	1+	-
3	T6025	4+	4+	4+
3	T5896	-	-	-
24	T217	-	-	n.t.
24	T6025	2+	2+	n.t.
24	T5896	-	-	n.t.

Table 2. Results by tube serology of 3 serums known to contain antibodies against antigens of the Miltenberger complex. Score results show sample reactivity by the anti-human globulin test, 1+ = weak, 2+ = medium, 3+ = medium/strong, 4+ = strong, - means sample is unreactive. RBCs were modified with the peptide-lipid construct at the concentrations indicated. Sera were tested against modified RBCs following 3 days and 24 days storage. (n.t. - not tested).

Age of modified RBCs (days)		Concentration of DOPE-PEG_6-βAla-Mal-Milt(K)(M00) (mg/ml)		
	Serum	1.0	0.5	0.25
3	T217	-	-	1+
3	T6025	1+	2+	1+
3	T5896	-	-	-
24	T217	-	-	-
24	T6025	2+	2+	1+
24	T5896	-	-	-

Table 3. Results by Diamed column serology of 3 serums known to contain antibodies against the Miltenberger complex. Score results show sample reactivity by the anti-human globulin test, 1+ = weak, 2+ = medium, 3+ = medium/strong, 4+ = strong, - means sample is unreactive. RBCs were modified with the peptide-lipid construct at the concentrations indicated. Sera were tested against modified RBCs following 3 days and 24 days storage.

Peptide inhibition

A 5 mg/ml stock solution of the peptide GlnThrAsnAspLysHisLys-ArgAspThrTyrCys dissolved in Celpresol™ was prepared. A 4 µl (20 µg peptide) volume of the stock solution was added to a 30 µl volume of each serum sample (Test). A 4 µl volume of Celpresol™ was added to 30 µl of each serum sample (Control). Serum samples (Test and Control) were then incubated at room temperature (RT) for 10 min.

A 30 µl volume of a 5% suspension of the modified RBCs was added to each sample and incubated at 37 °C for 45 min. The incubated RBCs were then washed 3 times with PBS in an Immufuge™. One drop of Epiclone™ anti-human globulin (AHG)

reagent was then added to each sample and the tubes centrifuged for 10 s in an Immufuge™ (setting: "high"). Tubes were read and serology scores recorded.

		Concentration of DOPE-PEG_6-βAla-Mal-Milt(K)(**M00**) (mg/ml)	
Peptide	Serum	1.0	0.5
CONTROL	T217	3+	2+
	T6025	4+	4+
	T5896	-	-
TEST	T217	-	-
	T6025	-	-
	T5896	-	-

Table 4. Results by tube serology of 3 serums known to contain antibodies against the Miltenberger complex and inhibited with peptide. Recorded scores show sample reactivity by the anti-human globulin test, 1+ = weak, 2+ = medium, 3+ = medium/strong, 4+ = strong, - means sample is unreactive. RBCs were modified with the peptide-lipid construct at the concentrations indicated.

[followed by page 45]

Reagent	ID	Type	EIA/Miltenberger Specificity
2	T217	Human group AB serum	Reactive with MUT-T peptides by EIA
3	T165	Human group O serum	Reactive with MUR peptides by EIA
4	T7202	Human group B serum	Reactive with MUT-M peptides by EIA
6	T6025	Human group A serum	Reactive with MUT-T peptides by EIA
7	T8445	Human group O serum	Uncertain
8	T5896	Human group O serum	Uncertain
9	MIII	Monoclonal antibody	Reactive with Mi III red cells
10	Mia	Monoclonal antibody	Reactive with Mi III red cells
11	Mur	Monoclonal antibody	Reactive with Mur positive red cells
12	Gam	IgG monoclonal antibody	Reactive with Mi III red cells
13	BoxH	Human serum	Uncertain
14	TAP1	Human group O serum	Presumed MUT-K specificity
15	TAP2	Human serum	Presumed MUR specificity

Table 5. Identification of polyclonal sera and monoclonal antibodies employed as reagents.

Cell ID	Antigen	Polyclonal sera								Monoclonal antibodies			
		2	3	4	6	7	8	14	15	9	10	11	12
		T217	T165	T7202	T6025	T8445	T5896	TAP1	TAP2	MIII	Mia	Mur	Gam
9422184	Vw	8	5	3	0	8	0	5	0	0	10	0	12
11297161	MiIII	12	10	12	12	10	10	10		10	10	12	12
4131850	MiIV	12			12				10	0	10	12	12
1523	MiVI	12			12				8	0	10	12	10
T1569	MiVII	0	0	0	0	10	0	0		0	0	0	0
C.BR	Mi?X	12	10	12	12	8	12	12	8	0	10	10	10

Table 6. Identification of naturally occurring Miltenberger antigen positive (Milt$^+$) human red cells as determined in BioVue AHG cards. The specificity of C.BR is uncertain.

Designation		Peptide sequence													Terminal sequence	S_1
M	Antigen	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*		
1	MUTK			Gln	Thr	Asn	Asp	Lys	His	Lys	Arg	Asp	Thr	Tyr	AlaAlaAlaAlaAla*	PEG_6
2	MUTK			Gln	Thr	Asn	Asp	Lys	His	Lys	Arg	Asp	Thr	Tyr	GlySerGlySerGlyCys	PEG_6
3	MUTM			Gln	Thr	Asn	Asp	**Met**	His	Lys	Arg	Asp	Thr	Tyr	GlySerGlySerGlyCys	PEG_6
13	MUTK	Ser	Ser	Gln	Thr	Asn	Asp	Lys	His	Lys	Arg	Asp	Thr	Tyr	Cys	PEG_6
16	Mur		Thr	Tyr	Pro	Ala	His	Thr	Ala	Asn	Glu	Val			Cys	PEG
18	Mur				Pro	Ala	His	Thr	Ala	Asn	Glu	Val			Cys	PEG
21	MUTK		Ser	Gln	Thr	Asn	Asp	Lys	His	Lys	Arg	Asp			Cys	PEG
23	Hil		Glu	Glu	Thr	Gly	Glu	Thr	Gly	Gln	Leu	Val			Cys	PEG

Table 7. Identification of peptide-lipid constructs. Cys denotes the cysteine residue via the sulfhydryl residue of which the spacer (S) is covalently linked to the peptide or PTS-peptide (F). * Where Cys is absent the spacer (S) is covalently linked to the peptide (F) via the terminal amino residue. All peptide-lipid constructs (F-S-L or L-S-F) were prepared as the DOPE (L) variant.

Identity of constructs used in modification of RBCs (see Table 7)		Identity of polyclonal sera and monoclonal antibodies (see Table 5)												
		4	8	2	6	3	14	7	9	10	11	12	13	15
M	µg/ml	T7202	T5896	T217	T6025	T165	TAP1	T8445	MIII	Mia	Mur	Gam	BoxH	TAP2
1	500	5	0	3	8	0	nt	0	0	5	0	8	nt	nt
2	500	8	8	8	8	5	nt	0	0	5	0	8	nt	nt
3	1000	8	10	0		5	nt	0	0	0	0	nt	5	nt
13	250	8	3	8	8	0	nt	0	0	0	0	0	8	nt
21	200	0	0	0	8	8	nt	0	0	0	0	3	nt	5

Table 8. Analysis of sorted data for cells modified to incorporate MUT peptide constructs and reactivity against the Miltenberger Antibody Positive Panel. 'nt' denotes not tested.

Identity of constructs used in modification of RBCs (see Table 7)		Identity of polyclonal sera and monoclonal antibodies (see Table 5)											
		3	6	7	4	8	2	15	9	10	11	12	13
M	ug/ml	T165	T6025	T8445	T7202	T5896	T217	TAP2	MIII	Mia	Mur	Gam	BoxH
16	100	10	5	12	5	0	0	nt	0	0	0	0	0
18	100	10	10	8	0	0	0	nt	0	0	0	0	nt

Table 9. Analysis of sorted data for cells modified to incorporate MUR peptide constructs and reactivity against the Miltenberger Antibody Positive Panel. 'nt' denotes not tested.

The majority of polyclonal sera demonstrated cross reactivity with one or more modified red blood cell populations (Tables 8 and 9).

Where false positives were observed these could be substantially eliminated by pre-treatment of the sample of serum with the peptide of the peptide-lipid constructs (Table 10 and 11).

	M1 modified cells			M2 cells vs serum		
Identity of sera	#4	#5	#6	#2	#6	#8
Serum alone	5	5	10	8	8	8
Serum + peptide	0	0	0	0	2	0

Table 10. Sera reactive with RBCs modified to incorporate the M1 peptide-lipid construct or M2 peptide-lipid construct constructs by contacting the cells with a 500 µg/ml dispersion of the construct (Method 1) were "neutralised" with the peptide QTNDKHKRDTY and retested against the modified cells. Sera were neutralized by adding 10 µL of 1 mg/ml solution of peptide to a 50 µL volume of sera and incubating for 30 minutes at 37 °C. Testing was performed using BioVue™ cards.

	M13 modified cells			
Identity of sera	#3	#42	#37	#34
Serum alone	8	8	8	8
Serum + peptide	0	0	0	0

Table 11. Sera reactive with RBCs modified to incorporate the M13 peptide-lipid construct by contacting the cells with a 500 µg/ml dispersion of the construct (Method 1) were "neutralised" with the peptide SSQTNDKHKRDTY and retested against the modified cells. Sera were neutralized by adding 10 µL of 1 mg/ml solution of peptide to a 50 µL volume of sera and incubating for 30 minutes at 37 °C. Testing was performed using BioVue™ cards.

*Modification of embryos with the peptide-lipid construct designated DOPE-PEG6-βAla-Mal-PTS-Milt(K) (**M2**)*

The zona pellucida of day 3.5 embryos prepared as microdrops were removed by incubation in 0.5% pronase solution for circa 5 minutes at 37 °C. The zona pellucida removed embryos were transferred to microdrops containing media alone and contacted with a dispersion of the peptide-lipid construct designated DOPE-PEG6-βAla-Mal-PTS-Milt(K)(**M2**) at a concentration of 1 mg/ml for 2 hours. The dispersion of the peptide-lipid construct contained azide as an anti-microbial agent.

The incubated embryos were washed four times in handling media and transferred to microdrops containing the Gam monoclonal antibody (see Table 8) and incubated at 37 °C for 40 min. The embryos were then washed four times in handling media and transferred to microdrops containing secondary antibody (FITC anti-mouse)at a 1:50 dilution.

The microdrops were incubated at room temperature in the dark for 30 minutes before being washed four times in handling media, placed on microscope slides, and overlaid with mineral oil. The embryos were visualized using an Olympus™ BX51 fluorescent microscope at 200 x magnification with WIB filter 550 nm emission wavelength. The scale used for grading fluorescence was 0 to 4+, where 0 is no fluorescence and 4+ is very bright fluorescence. The mean fluorescence of the modified embryos was 2+ versus zero for unmodified embryos. The grading of fluorescence is recorded in Table 12.

Mean Fluorescence*	
M2 FSL-peptide	**Media alone**
2.0+	0

Table 12. Fluorescence of embryos modified by contacting with the peptide-lipid construct designated DOPE-PEG6-βAla-Mal-PTS-Milt(K)(**M2**) (10 embryos per group; scale is 0 to 4+).

A mean fluorescence of 2+ was observed for the zona pellucida removed embryos modified to incorporate the peptide-lipid construct designated DOPE-PEG6-βAla-Mal-PTS-Milt(K)(**M2**). No fluorescence was observed for control embryos. The de-compaction of treated embryos was attributed to the presence of azide in the dispersion of the peptide-lipid construct.

Although the invention has been described by way of exemplifying embodiments it should be appreciated that variations and modifications may be made without departing from the scope of the invention. Furthermore where known equivalents exist to specific features, such equivalents are incorporated as if specifically referred to in this specification.

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CLAIMS

1) A **method** of detecting reactive antibody in the serum of a subject including the steps of:

- Contacting a sample of the serum with a suspension of cells modified to incorporate a peptide-lipid construct of the structure $(L\text{-}S\text{-})_iF(\text{-}S\text{-}L)_j$ to provide a mixture;
- Incubating the mixture for a time and at a temperature sufficient to allow agglutination; and
- Determining the degree of agglutination of the cells in the mixture;

where:

F is a peptide comprising an epitope for the reactive antibody;
S is a spacer covalently linking F to L; and
L is a lipid selected from the group consisting of diacyl- and dialkyl-glycerolipids, including glycerophospholipids; and
i and j are independently 0 or 1,

2) The method claim 1 where the method includes the preliminary step of:

- Adding an amount of the peptide to the sample of the serum;

where the amount of the peptide is sufficient to neutralize non-specific agglutination or confirm specificity of the reactive antibody.

3) The method claim 1 where includes the intermediate step of:

- Adding an anti-subject globulin antibody to the mixture prior to determining the degree of agglutination of the cells of the mixture.

4) The method claim 1 where the subject is a human.

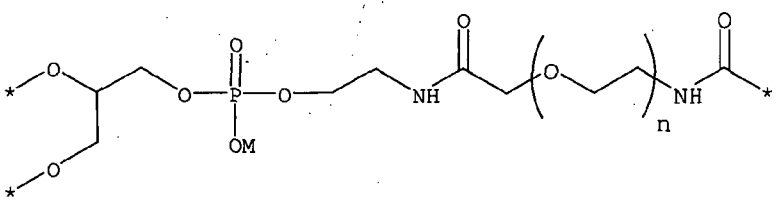
5) The method claim 1 where the cells are red blood cells.

6) The method claim 1 where the anti-subject globulin antibody is anti-human globulin (AHG) antibody.

7) The method claim 1 where the reactive antibody is reactive to an antigen selected from the group consisting of: Glycophorin A, Glycophorin B, or mutations thereof (including the MNS blood group system).

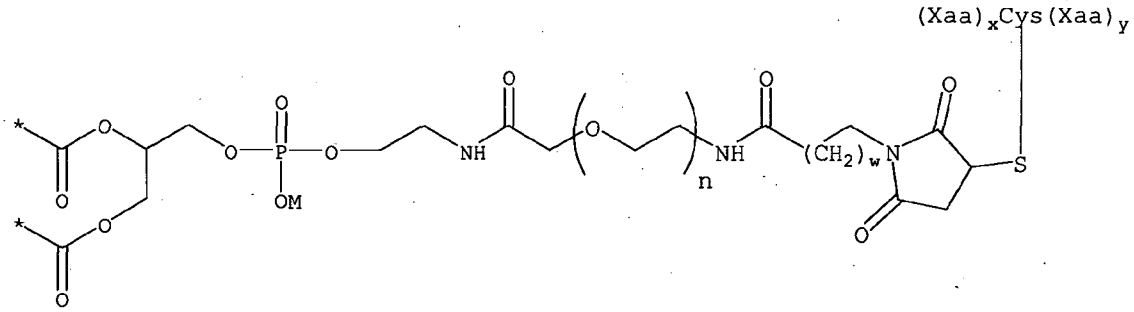
8) The method claim 1 where S is a spacer covalently linking F to L via an oligomer of ethylene glycol.

9) The method claim 1 where the structure of the peptide-lipid construct includes the substructure:

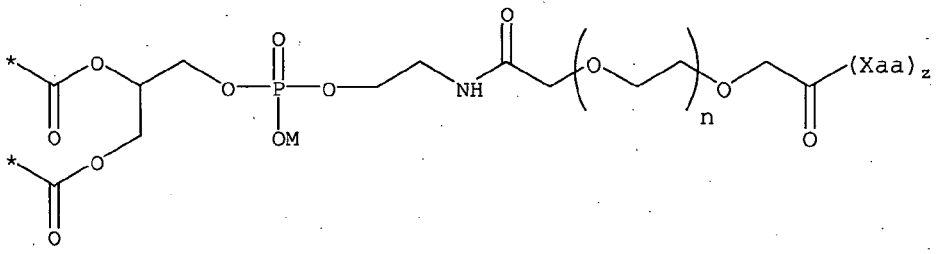


where M is a monovalent cation (M^+), n is 6 to 14 and * is other than H.

10) The method claim 1 where the structure of the peptide-lipid construct is either:



or



where M is a monovalent cation (M^+), n is 6 to 14, w is 1 or 2, the sum of x and y is greater than 5, z is greater than 5, and * is other than H.

11) The method claim 1 where the sum of i and j is 1.

12) The method claim 1 where F is a peptide including a proximal terminal sequence (PTS) selected to promote solubility of the peptide.

13) The method claim 12 where the PTS of the peptide is selected from the group consisting of:

SerLysLysLysLysGly
AlaAlaAlaAla
GlySerGlySerGly

14) The method claim 1 where F is a peptide comprising an epitope of antigens selected from the group consisting of: Glycophorin A, Glycophorin B, or mutations thereof (including the MNS blood group system).

15) The method claim 1 where F is a peptide selected from the *List of Peptides*.

16) The method claim 1 where F is a peptide selected from the group consisting of:

GlnThrAsnAspLysHisLysArgAspThrTyrAlaAlaAlaAlaAlaCys
GlnThrAsnAspLysHisLysArgAspThrTyrGlySerGlySerGlyCys
GlnThrAsnAspMetHisLysArgAspThrTyrGlySerGlySerGlyCys
SerSerGlnThrAsnAspLysHisLysArgAspThrTyrCys
ThrTyrProAlaHisThrAlaAsnGluValCys
ProAlaHisThrAlaAsnGluValCys
SerGlnThrAsnAspLysHisLysArgAspCys
AlaAlaAlaAlaValMetTyrAlaSerSerGly
GlySerGlySerGlyValMetTyrAlaSerSerGly

17) The method claim 1 where L is a glycerophospholipid.

18) The method claim 1 where L is a glycerophospholipid selected from the group consisting of: 1,2-O-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine (DOPE) and 1,2-O-distearyl-*sn*-glycero-3-phosphatidylethanolamine (DSPE).

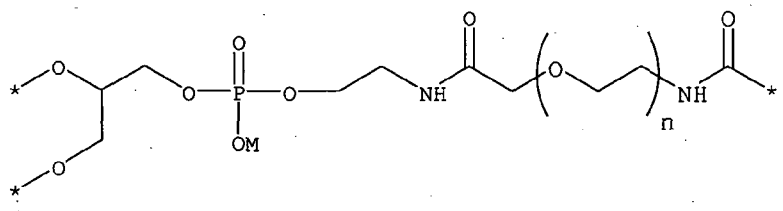
19) A **peptide-lipid construct** of the structure:

L-S-F

where

F is a peptide;
S is a spacer covalently linking F to L via an oligomer of ethylene glycol; and
L is a lipid selected from the group consisting of diacyl- and dialkyl-glycerolipids, including glycerophospholipids.

20) The peptide-lipid construct of claim 19 where the structure of the peptide-lipid construct includes the substructure:



where M is a monovalent cation (M^+), n is 6 to 14 and * is other than H.

21) The peptide-lipid construct of claim 19 where F is a peptide including a proximal terminal sequence (PTS) selected to promote solubility of the peptide.

22) The peptide-lipid construct of claim 21 where the PTS of the peptide is selected from the group consisting of:

SerLysLysLysLysGly

AlaAlaAlaAla

GlySerGlySerGly

23) The peptide-lipid construct of claim 19 where the terminal sequence of the peptide is selected from the group consisting of:

GlyLysLysLysLysSerCys

AlaAlaAlaAlaCys

GlySerGlySerGlyCys

CysSerLysLysLysLysGly

CysAlaAlaAlaAla

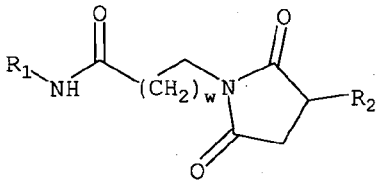
CysGlySerGlySerGly

24) The peptide-lipid construct of claim 19 where S is covalently linked to F via a sulphide bond formed with the Cys residue of the peptide.

25) The peptide-lipid construct of claim 19 where S is covalently linked to F via a sulphide bond formed with a Cys residue of the peptide at or proximal to a terminus of the peptide.

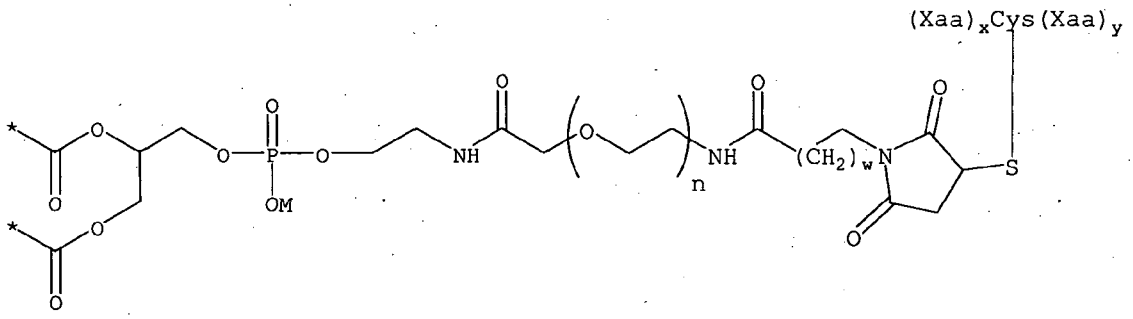
26) The peptide-lipid construct of claim 19 where S is linked to F via a sulphide bond formed with a Cys residue of the peptide at the carboxy-terminus of the peptide.

27) The peptide-lipid construct of claim 19 where S is of the structure S_1-S_2-S_3, S_1 is an oligomer of ethylene glycol and S_2-S_3 is selected from the group consisting of:



where R_1 is a terminal carbon of S_1, R_2 is the sulphur of the Cys residue and w is 1 or 2.

28) The peptide-lipid construct of claim 19 where the structure of the peptide-lipid construct is:



where M is a monovalent cation (M+), n is 6 to 14, w is 1 or 2, the sum of x and y is greater than 5, and * is other than H.

29) The peptide-lipid construct of claim 28 where n is 6.

30) The peptide-lipid construct of claim 28 where y is 0.

31) The peptide-lipid construct of claim 19 where F is a peptide comprising an epitope of antigens selected from the group consisting of: Glycophorin A, Glycophorin B, or mutations thereof (including the MNS blood group system).

32) The peptide-lipid construct of claim 19 where F is a peptide selected from the *List of Peptides*.

33) The peptide-lipid construct of claim 19 where F is a peptide selected from the group consisting of:

GlnThrAsnAspLysHisLysArgAspThrTyrAlaAlaAlaAlaAlaCys

GlnThrAsnAspLysHisLysArgAspThrTyrGlySerGlySerGlyCys

GlnThrAsnAspMetHisLysArgAspThrTyrGlySerGlySerGlyCys

SerSerGlnThrAsnAspLysHisLysArgAspThrTyrCys

ThrTyrProAlaHisThrAlaAsnGluValCys

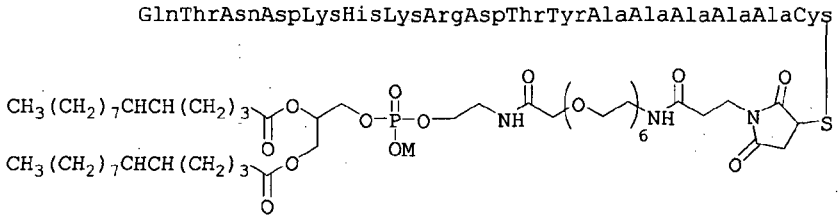
ProAlaHisThrAlaAsnGluValCys

SerGlnThrAsnAspLysHisLysArgAspCys

34) The peptide-lipid construct of claim 19 where L is a glycerophospholipid.

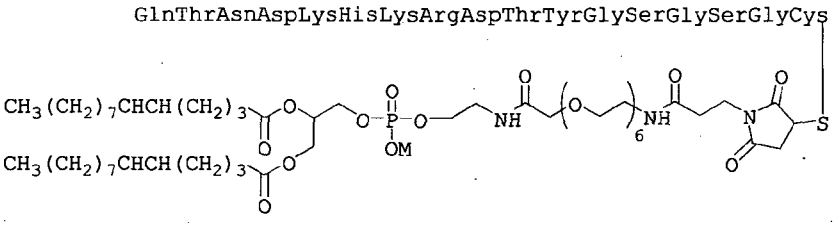
35) The peptide-lipid construct of claim 19 where L is a glycerophospholipid selected from the group consisting of: 1,2-O-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine (DOPE) and 1,2-O-distearyl-*sn*-glycero-3-phosphatidylethanolamine (DSPE).

36) A **peptide-lipid construct** of the structure:



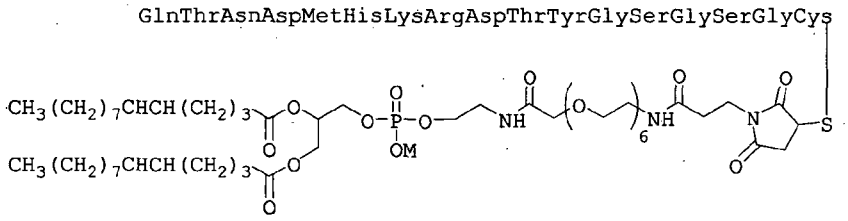
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-PTS-1MUTK) (**M1**).

37) A **peptide-lipid construct** of the structure:



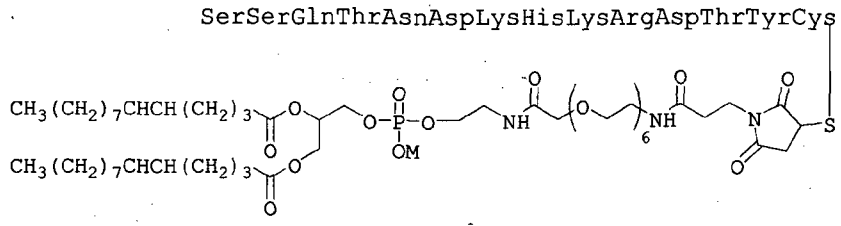
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-PTS-2MUTK) (**M2**).

38) A **peptide-lipid construct** of the structure:



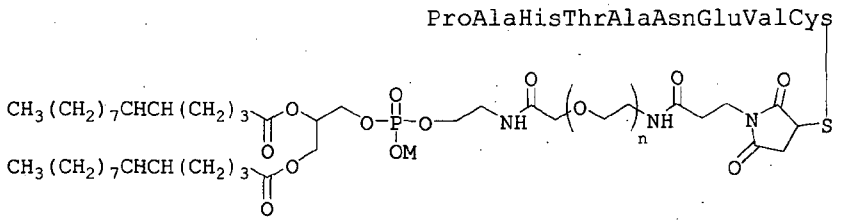
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-PTS-3MUTM (**M3**).

39) A **peptide-lipid construct** of the structure:



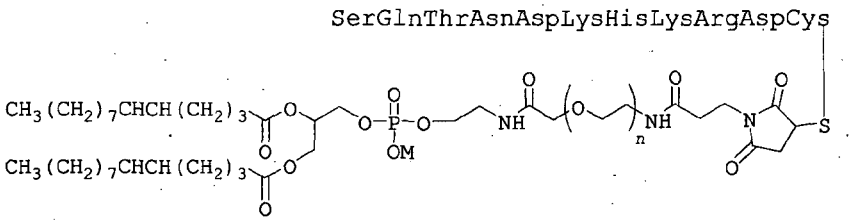
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-13MUTK(**M13**).

40) A **peptide-lipid construct** of the structure:



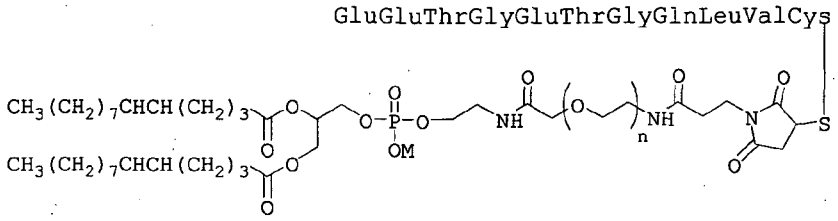
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-18Mur (**M18**) (n=6).

41) A **peptide-lipid construct** of the structure:



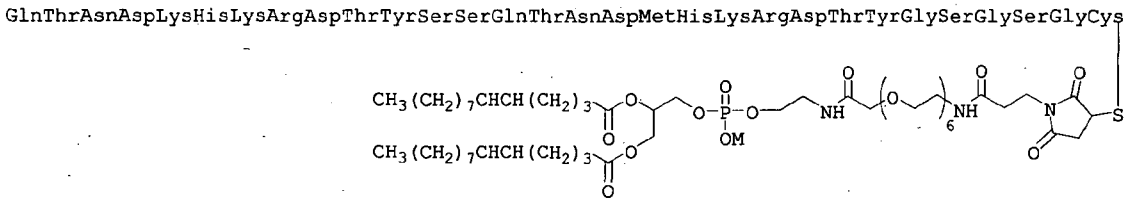
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-21MUTK(**M21**) (n=6).

42) A **peptide-lipid construct** of the structure:



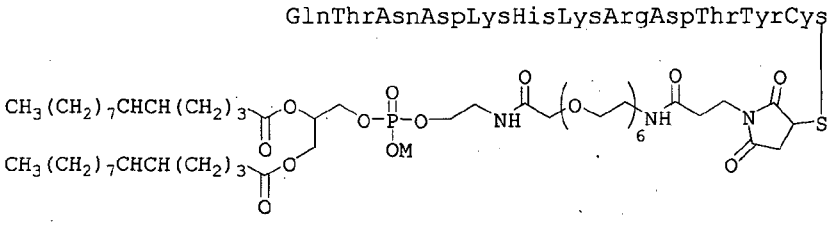
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-Hil3(**M23**) (n=6).

43) A **peptide-lipid construct** of the structure:



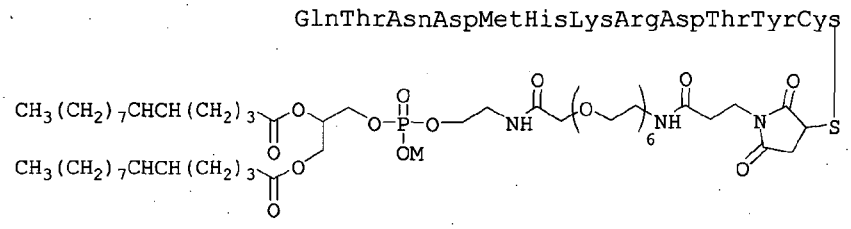
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-PTS-Milt(K,M).

44) A **peptide-lipid construct** of the structure:



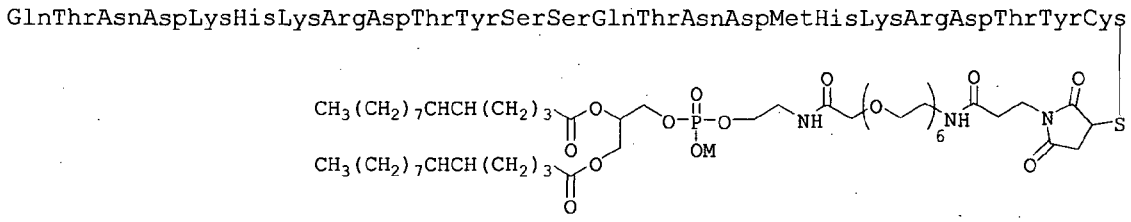
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-Milt(K) (**M00**).

45) A **peptide-lipid construct** of the structure:



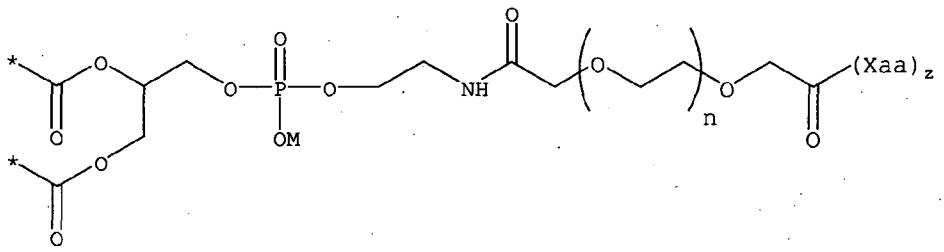
where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-Milt(M).

46) A **peptide-lipid construct** of the structure:



where M is a monovalent cation (M^+) and designated DOPE-PEG_6-βAla-Mal-Milt(K,M).

47) The peptide-lipid construct of claim 19 where the structure of the peptide-lipid construct is:



where M is a monovalent cation (M^+), n is 6 to 14, z is greater than 5, and * is other than H.

48) The peptide-lipid construct of claim 45 where n is 14.

49) The peptide-lipid construct of claim 45 where F is a peptide including a terminal sequence selected to promote solubility of the peptide.

50) The peptide-lipid construct of claim 47 where the terminal sequence of the peptide is selected from the group consisting of:

SerLysLysLysLysGly

AlaAlaAlaAla

GlySerGlySerGly

51) The peptide-lipid construct of claim 45 where F is a peptide selected from the group consisting of:

$(Xaa)_{z-7}$ValMetTyrAlaSerSerGly;

where z is the integer 4, 5 or 6.

52) The peptide-lipid construct of claim 45 where F is a peptide selected from the group consisting of:

SerLysLysLysLysGlyValMetTyrAlaSerSerGly

AlaAlaAlaAlaValMetTyrAlaSerSerGly

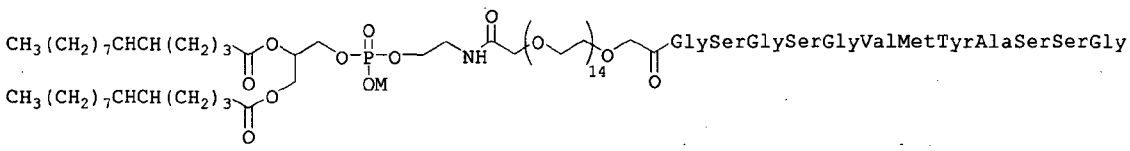
GlySerGlySerGlyValMetTyrAlaSerSerGly

53) The peptide-lipid construct of claim 45 where L is a glycerophospholipid.

54) The peptide-lipid construct of claim 45 where L is a glycerophospholipid selected from the group consisting

of: 1,2-O-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine (DOPE) and 1,2-O-distearyl-*sn*-glycero-3-phosphatidylethanolamine (DSPE).

55) A **peptide-lipid construct** of the structure:



where M is a monovalent cation (M^+) and designated DOPE-PEG_{14}-Syph.

56) A **method** of preparing a peptide-lipid construct (F-S-L) of claim 19 to 47 including the steps of:

- Preparing a maleimido-derivative of a precursor construct by reacting a maleimido-donating reagent with a precursor construct of the structure L-S_1-NH_2; and

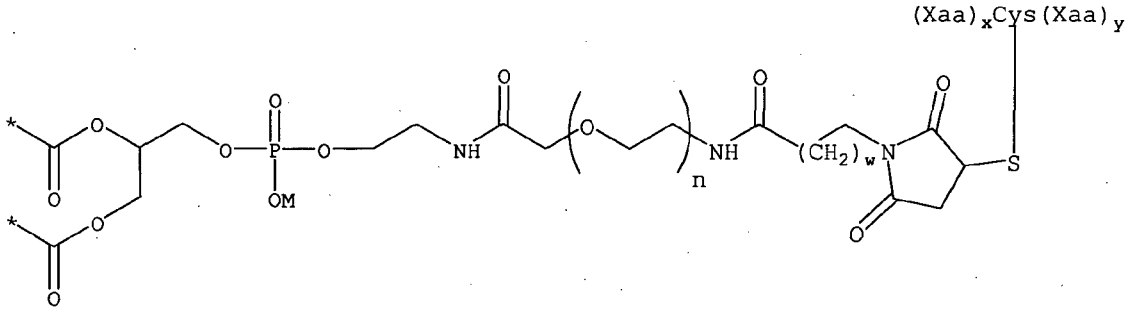
- Reacting the maleimido-derivative of the precursor construct with a peptide (F) including a Cys residue and solubilised in a solvent.

where:

L is a lipid selected from the group consisting of diacyl- and dialkyl-glycerolipids, including glycerophospholipids; and

S_1 is selected from the group consisting of oligomers of ethylene glycol.

57) The method of claim 54 where the structure of the peptide-lipid construct is:



where n is 6 to 14, w is 1 or 2, the sum of x and y is greater than 5, and * is other than H.

58) The method of claim 54 where the maleimido-donating reagent is selected from the group consisting of: N-oxysuccinimid ester of maleimidobutyric acid; and N-oxysuccinimid ester of maleimidopropionic acid.

59) The method of claim 54 where S_1 is an oligomer of ethylene glycol selected from the group consisting of 6 to 14 *mer* PEG (PEG_6 to PEG_{14}).

60) The method of claim 54 where S_1 is PEG_6.

61) The method of claim 54 where the solvent is selected from the group consisting of: trifluoroethanol; DMSO; or mixtures thereof.

62) The method of claim 54 where the Cys residue is a terminal Cys residue.

63) The method of claim 54 where F is a peptide including a proximal terminal sequence (*PTS*) selected to promote solubility of the peptide in the reaction solvent.

64) The method of claim 61 where the PTS of the peptide is selected from the group consisting of:

SerLysLysLysLysGly

AlaAlaAlaAla

GlySerGlySerGly

65) The method of claim 54 where the terminal sequence of the peptide is selected from the group consisting of:

GlyLysLysLysLysSerCys

AlaAlaAlaAlaCys

GlySerGlySerGlyCys

CysSerLysLysLysLysGly

CysAlaAlaAlaAla

CysGlySerGlySerGly

66) The method of claim 54 where F is a peptide selected from the *List of Peptides*.

67) The method of claim 54 where F is a peptide selected from the group consisting of:

GlnThrAsnAspLysHisLysArgAspThrTyrAlaAlaAlaAlaAlaCys

GlnThrAsnAspLysHisLysArgAspThrTyrGlySerGlySerGlyCys

GlnThrAsnAspMetHisLysArgAspThrTyrGlySerGlySerGlyCys

SerSerGlnThrAsnAspLysHisLysArgAspThrTyrCys

ThrTyrProAlaHisThrAlaAsnGluValCys

ProAlaHisThrAlaAsnGluValCys

SerGlnThrAsnAspLysHisLysArgAspCys

68) The method of claim 54 where L is a glycerophospholipid.

69) The method of claim 54 where L is a glycerophospholipid selected from the group consisting of: 1,2-O-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine (DOPE) and 1,2-O-distearyl-*sn*-glycero-3-phosphatidylethanolamine (DSPE).

70) A **method** of effecting qualitative and quantitative changes in the levels of peptide expressed at the surface of cells and multi-cellular structures including the step of:

- contacting the cells or multi-cellular structures with a solution of a peptide-lipid construct of any one of claims 19 to 55 at a concentration and for a time and temperature sufficient to allow the construct to incorporate into the surface.

71) The method of claim 54 where the peptide-lipid construct is a construct of any one of claims 19 to 47.

72) The method of claim 54 where the cells or multicellular structures are selected from the group consisting of: red blood cells; and embryos.

73) The method of claim 54 where the cells or multicellular structures are human cells or multicellular structures.

74) The method of claim 54 where the time and temperature is no greater than 2 hours at 37 °C or 24 hours at 4 °C.

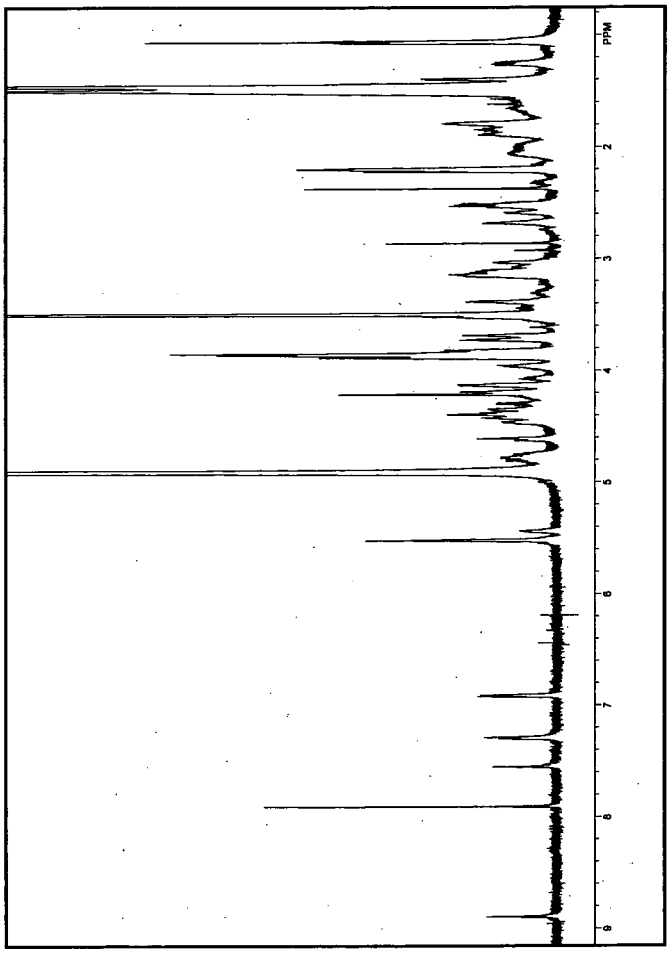


FIGURE 1

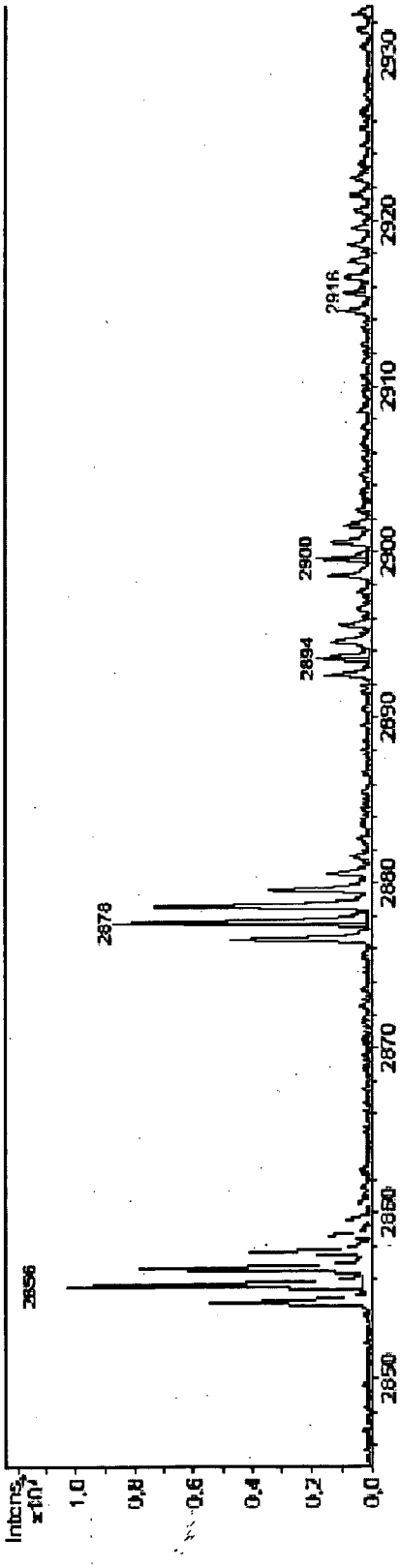

Intens.
x10⁴
1,0
0,8
0,6
0,4
0,2
0,0
2850
2860
2870
2880
2890
2900
2910
2920
2930
2856
2878
2894
2900
2916

FIGURE 2

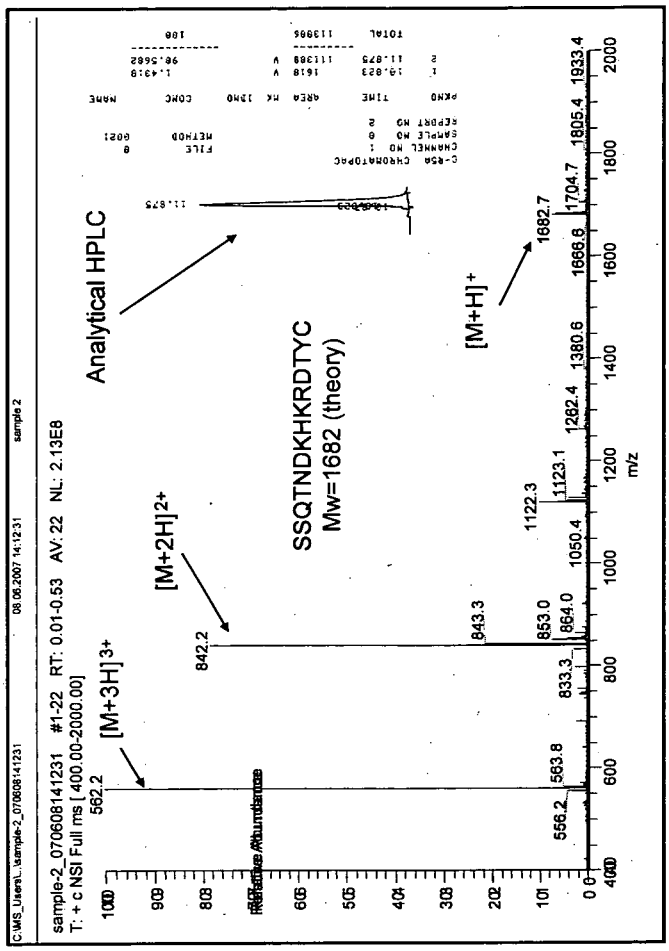

Analytical HPLC
SSQTNDKHKRDTYC
Mw=1682 (theory)
[M+3H]3+
[M+2H]2+
[M+H]+
562.2
842.2
843.3
1682.7
m/z

FIGURE 3

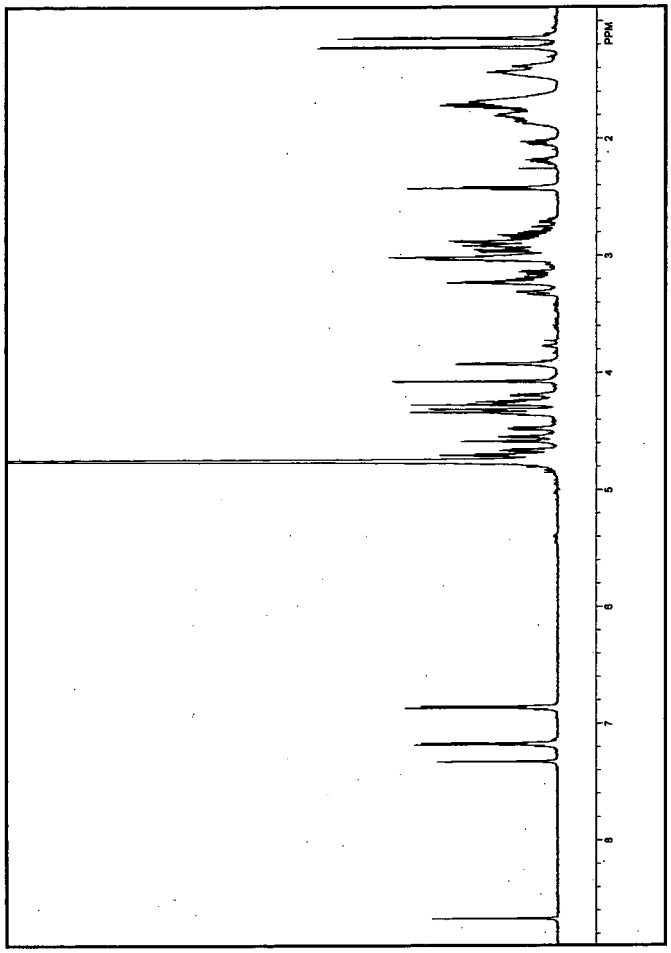
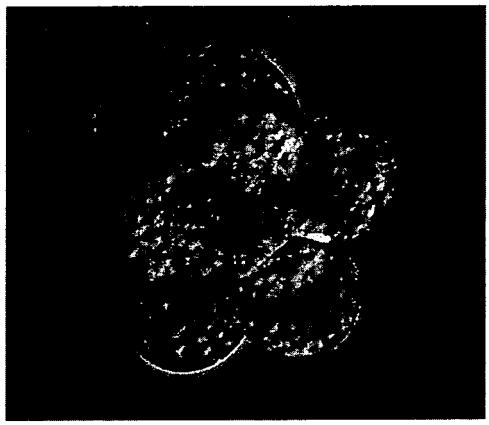


FIGURE 4



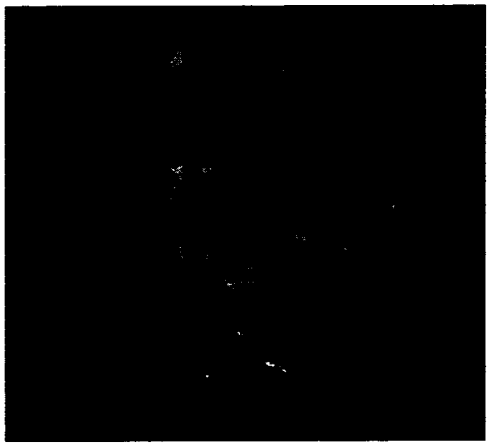


FIGURE 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ2008/000239

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

G01N 33/539 (2006.01)	***C07K 1/04*** (2006.01)	***G01N 33/536*** (2006.01)
A61K 39/385 (2006.01)	***C07K 17/08*** (2006.01)	***G01N 33/564*** (2006.01)
C07F 9/10 (2006.01)	***G01N 33/53*** (2006.01)	***G01N 33/68*** (2006.01)
C07K 1/02 (2006.01)	***G01N 33/531*** (2006.01)	***G01N 33/92*** (2006.01)

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)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Chemical Abstracts Registry: Substructure search based on a generalisation of the structures in Claim 10
WPI, EPODOC: conjugate, peptide, lipid, detect

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 7,153,933 B2 (Wu *et al.*), 26 December 2006 See Abstract; Example 6 and 8 at column 26	19-69
A	US 6,521,211 B1 (Unger *et al.*), 18 February 2003 See column 8, lines 2-3; Column 41; Example 14	1-74

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 November 2008	02 DEC 2008
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. +61 2 6283 7999	Authorized officer **ANDREW BRYCE** AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : +61 2 6283 3132

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ2008/000239

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2000/045856 A2 (IMARX PHARMACEUTICAL CORP.), 10 August 2000 See Examples 4-7, 11, 14, 32, 44, 60	1-74
A	Schelté P. *et al.* "Differential Reactivity of Maleimide and Bromoacetyl Functions with Thiols: Application to the Preparation of Liposomal Diepitope Constructs" Bioconjugate Chemistry (2000) 11 (1): 118-123 See Figure 2; Page 121, right-hand column, second paragraph	1-74

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/NZ2008/000239

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	7153933	CA	2413629	CN	1453293	EP	1319667
		US	2003229013	US	2003229017	US	2007106064
US	6521211	AU	33131/97	AU	35866/00	AU	61885/01
		AU	62703/96	AU	77432/00	AU	93830/98
		AU	2005200059	CA	2218541	CA	2256592
		CA	2362200	CN	1187137	CN	1397348
		EP	0831932	EP	0930844	EP	0959908
		EP	1146911	EP	1444991	US	6033645
		US	6139819	US	6231834	US	7329402
		US	2001051131	US	2003012735	US	2003157025
		WO	0045856	WO	0124705	WO	9640285
		WO	9748337	WO	9913919		
WO	0045856	AU	33131/97	AU	35866/00	AU	61885/01
		AU	62703/96	AU	77432/00	AU	93830/98
		AU	2005200059	CA	2218541	CA	2256592
		CA	2362200	CN	1187137	CN	1397348
		EP	0831932	EP	0930844	EP	0959908
		EP	1146911	EP	1444991	US	6033645
		US	6139819	US	6231834	US	6521211
		US	7329402	US	2001051131	US	2003012735
		US	2003157025	WO	0124705	WO	9640285
		WO	9748337	WO	9913919		

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX

专利名称(译)	肽 - 脂质构建体及其在诊断和治疗应用中的用途		
公开(公告)号	EP2198301A1	公开(公告)日	2010-06-23
申请号	EP2008830156	申请日	2008-09-11
[标]申请(专利权)人(译)	科德生物工程有限公司		
申请(专利权)人(译)	KODE BIOTECH LIMITED		
当前申请(专利权)人(译)	KODE BIOTECH LIMITED		
[标]发明人	WEINBERG CRISTINA SIMONO BOVIN NICOLAI HENRY STEPHEN		
发明人	WEINBERG, CRISTINA-SIMONO BOVIN, NICOLAI HENRY, STEPHEN		
IPC分类号	G01N33/539 C07K1/04 G01N33/536 A61K39/385 C07K17/08 G01N33/564 C07F9/10 G01N33/53 G01N33/68 C07K1/02 G01N33/531 G01N33/92		
CPC分类号	G01N33/92 A61K47/543 A61K47/544 C07F9/091 C07F9/572 C07K5/1008 C07K14/47 C07K17/08 C12N5/0006 C12N5/0641 C12N2503/00 G01N33/5304 G01N33/554 G01N33/555 G01N33/6854 G01N33/80 G01N2405/00		
优先权	569023 2008-06-06 NZ 561381 2007-09-11 NZ 562476 2007-10-12 NZ		
其他公开文献	EP2198301A4 EP2198301B1		
外部链接	Espacenet		

摘要(译)

公开了结构L-S-F的肽 - 脂质构建体，其中F是肽，S是通过乙二醇的低聚物将F与L共价连接的间隔基，L是二酰基 - 或二烷基 - 甘油脂（包括甘油磷脂）。间隔物理想地具有6至14个乙二醇重复，对应于分子量为约250至600的PEG。还公开了通过使血清与经修饰以掺入肽 - 脂质构建体的细胞接触来检测血清中的反应性抗体的方法，其中肽是抗体的表位，并决定细胞的凝集程度。