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Description

SEQUENCE LISTING

[0001] This application incorporates by reference in its entirety the sequence listing.

FIELD

[0002] The present invention relates to the transport and delivery of therapeutic molecules to their sites of action via parenteral administration. More particularly, the present invention concerns a formulation technology enabling the incorporation of drugs into nanoparticles that can be readily administered parenterally for the safe and effective delivery of the incorporated drugs to their therapeutic targets.

BACKGROUND

[0003] Many therapeutic substances that operate systemically are not suitable for the relative simplicity of oral dosing and must be administered parenterally instead. However, often the same factors that complicate oral dosing impede the parenteral dosing process. Such factors include poor water solubility due to hydrophobicity or other properties, drug instability in the digestive tract, insufficient absorption or enhanced clearance after absorption, resulting in inadequate plasma concentrations and/or exposure times.

[0004] Options for dealing with hydrophobic drugs requiring parenteral dosing usually involve the addition of various excipients to obtain stable suspensions, dispersions or solutions suitable for injection. The types of excipients used include detergents, polymers of various types, oil emulsions, phospholipids and albumin. In many cases the excipients used to obtain the necessary drug solubilization are detergent-like substances. These include deoxycholate; Cremophor EL®, a polyethyloxated derivative of castor oil; and polysorbate 80. The latter two are typically used in tandem with ethanol. These agents solve the solubilization problem but they have noxious properties which introduce a high risk of hypersensitivity reactions. It is a common requirement that patients injected with solutions containing Cremophore EL® or polysorbate 80 are pretreated with anti-inflammatory drugs to subdue formulation-dependent inflammation. The most serious consequences of hypersensitivity reactions are reduced tolerance to treatment and increased risk of death.

[0005] Examples of approved drug formulations containing Cremophore EL® or polysorbate 80 for solubilization include the hydrophobic anti-cancer drugs paclitaxel (Taxol®), docetaxel (Taxotere®), cabazitaxel (Jevtana®) and ixabepilone (Ixempra®). The immnuosuppresants cyclosporine (Sandimmune®), tacrolimus (Prograf®) and temsirolimus (Torisel®) also depend on these agents for formulation. In the case of paclitaxel, some progress has been made through the substitution of albumin for Cremophore EL® and ethanol as a dispersing agent (i.e. Abraxane®). The antifungal agent amphotericin B is also quite hydrophobic and requires measures to obtain a stable suspension for infusion.

[0006] Other dosing hurdles many drugs face include rapid clearance and inactivation by metabolic pathways. For example, the anti-cancer agent gemcitabine must be infused at high doses to overwhelm its breakdown by cytidine deaminase in order to obtain therapeutic levels. A further problem limiting drug administration is unintended exposure of non-target tissues to the effects of those drugs. This is particularly significant for cytotoxic anti-cancer drugs.

[0007] Descriptions of attempts to discover superior parenteral formulations including emulsions, micelles, liposomal formulations, polymers, and solid-lipid nanoparticles, are numerous but many efforts have been hindered by problems of low entrapment efficiency, drug instability, payload leakage and poor storage stability. Some success has been obtained with liposomal formulations. Examples include cytarabine in liposomes (DepoCyt®) to reduce clearance rates, doxorubicin in liposomes (Doxil®, Myocet®) to reduce cardiotoxicity, and amphotericin-B in liposomes (e.g. AmBisome®) to improve solubilization.

[0008] Given the limited number of current treatments, and inadequacy of options, there is a clear need to provide formulation alternatives for parenterally administered drugs with improved safety profiles and therapeutic indexes; and/or therapies which at least provide the public with a useful choice.

SUMMARY

[0009] The present disclosure addresses this need by providing novel nanoparticle formulations of lipid and peptide and methods to form them that allow incorporation of molecules, e.g., drugs and are stable in infusion or injection solutions. The formulations of the invention provide one or more improvements, including but not limited to, improved pharmacokinetic parameters, increased half-life, targeted delivery, diminished toxicity or an improved therapeutic index for parenterally-administered drugs, in particular, anti-cancer drugs. The present disclosure provides amphiphilic, alpha-helical peptides that comprise an amino acid sequence of SEQ ID NO:1, SEQ ID NO:24, SEQ ID NO:36 or SEQ ID NO:59.

[0010] Further, the present disclosure provides peptide amphiphile lipid micelles (PALM) which comprise a peptide

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comprising an amino acid sequence of the disclosure, sphingomyelin and one or more additional phospholipids. The PALM of the present disclosure optionally comprise one or more cargo molecules, such as imaging agents and drugs.

[0011]   The present disclosure also provides for processes for preparing PALM and PALM composition formulated with cargo molecules.

[0012]   Additionally the present disclosure provides for compound conjugates and methods of preparing compound conjugates suitable for use with PALM.

[0013]   Further the present disclosure provides for methods of treating disorders by administering PALM-drug conjugates.

BRIEF DESCRIPTION OF THE FIGURES

[0014]

Figure 1A and 1B are Edmundson Wheel depictions of the peptides of SEQ ID NOs: 3 and 25 respectively showing their amphiphilic conformation. Figures 1A and 1B further show the axial positions of the constituent amino acids (identified by standard single letter abbreviations) around the long axis of the alpha-helix. The letter "B" represents 2-amino-isobutyric acid. The dashed lines indicate the approximate boundaries between hydrophilic amino acids (shaded) forming the polar faces of the peptides and the hydrophobic amino acids forming the non-polar faces. Figures 1C and 1D are helical net depictions of the peptides of SEQ ID NOs: 3 and 25 respectively.

Figure 2. The size exclusion chromatogram of PALM containing miriplatin (solid line) compared to human HDL(dashed line). PALM was composed of peptide of SEQ ID NO:25 and POPC, SM and miriplatin at a 2.5:3:7 :0.75 mole ratio.

Figure 3. Shows the size exclusion chromatograph of PALM containing XC and prepared with the peptide of SEQ ID NO:25 at a peptide:phospholipid:XC mole ratio of 1:4:0.4 . The elution positions of protein standards of various Stokes diameters are marked.

Figure 4. Comparison of the size exclusion chromatograms for PALM containing XT3 and prepared with peptide of SEQ ID NO:25 (dashed line) or with R4F peptide (solid line). The composition of both was peptide:POPC:SM:XT3 at a mole equivalent ratio of 1:2.8:1.2:0.4.

Figure 5. Depicts the size exclusion chromatogram of PALM prepared with the peptide of SEQ ID NO:25 and containing fenretinide. The PALM composition was peptide:POPC:SM:fenretinide at a mole equivalent ratio of 2.5:3:7:2.

Figure 6. Inhibition of PC3 prostate cancer cell growth by PALM(MP) compared to inhibition by cisplatin

Figure 7. Effect of SR-BI antibody on inhibition of PC3 prostate cancer cell growth by PALM(MP). The lines indicate fits of the data to the logistic equation.

Figure 8. Inhibition of SKOV3 ovarian cancer cell growth by PALM/(XC) (square, dotted line) or PALM(XT3) (diamond, solid line) compared to inhibition by paclitaxel (circle, dashed line) The lines indicate fits of the data to the logistic equation.

Figure 9. PALM prepared with various peptides, as indicated, and containing DiI, were incubated with BHK(SR-BI) cells that were stably transfected with a mifepristone inducible, human SR-BI gene. The incubations were performed with un-induced (Control) or induced cells. Human HDL, labeled with DiI, was tested for comparison. The amount of DiI taken up by cells over 4 hours of incubation was detected by fluorescence.

Figure 10. BHK(SR-BI) cells with a mifepristone inducible, human SR-BI gene, which was either induced (SR-BI+) or un-induced (Control), were incubated with the indicated concentrations of PTX or PALM(XT3) for 12 hours Cells were incubated further in the absence of test agents for an additional 36 hours before detection of % growth by MTT assay.

Figure 11. SR-BI antibody blocks XT3 uptake from PALM(XT3) (arrow).

DETAILED DESCRIPTION

Definitions

[0015]   "Nanoparticle" means a particle having no dimension greater than 100 nm.

[0016]   As used herein, the singular forms "a", "an" and "the" include plural references unless the context clearly dictates otherwise.

[0017]   It is noted that in this disclosure, terms such as "comprises", "comprised", "comprising", "contains", "containing" and the like have the meaning attributed in United States Patent law; they are inclusive or open-ended and do not exclude additional, un-recited elements or method steps. Terms such as "consisting essentially of' and "consists essentially of' have the meaning attributed in United States Patent law; they allow for the inclusion of additional ingredients or steps

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that do not materially affect the basic and novel characteristics of the claimed invention. The terms "consists of" and "consisting of" have the meaning ascribed to them in United States Patent law; namely that these terms are close ended.

[0018] The antecedent "about" indicates that the values are approximate. For example the range of "about 1 mg to about 50 mg" indicates that the values are approximate values. The range of "about 1 mg to about 50 mg" includes approximate and specific values, e.g., the range includes about 1 mg, 1 mg, about 50 mg and 50 mg.

[0019] When a range is described, the range includes both the endpoints of the range as well as all numbers in between. For example, "between 1 mg and 10 mg" includes 1 mg, 10 mg and all amounts between 1 mg and 10 mg. Likewise, "from 1 mg to 10 mg" includes 1 mg, 10 mg and all amounts between 1 mg and 10 mg.

[0020] As used herein, "alkyl" refers to a saturated aliphatic hydrocarbon group containing from 7-21 carbon atoms. As used herein, the terminology (C_1-Cn) alkyl refers to an alkyl group containing 1-n carbon atoms. For example, (C_8-C_{12}) alkyl refers to an alkyl group containing 8, 9, 10, 11, or 12 carbon atoms. An alkyl group can be branched or unbranched.

[0021] As used herein, "alkenyl" refers to an aliphatic carbon group that contains from 7-21 carbon atoms and at least one double bond. As used herein, the terminology (C_1-C_n) alkenyl refers to an alkenyl group containing 1-n carbon atoms. An alkenyl group can be branched or unbranched.

[0022] "Consisting essentially of" when used to describe the lipid component means that the lipid component includes less than 0.1 mol% of any additional lipid other than those specified.

[0023] "XC" is an abbreviation for paclitaxel 2'-cholesteryl carbonate.

[0024] XT3 is an abbreviation for paclitaxel 2'-δ-tocotrienyl carbonate.

[0025] "MP" is an abbreviation for miriplatin.

[0026] "PTX" is an abbreviation for paclitaxel

[0027] "POPC" is an abbreviation for 1-palmitoyl-2-oleoyl phosphatidylcholine

[0028] "SM" is an abbreviation for sphingomyelin

[0029] "HDL" is an abbreviation for high density lipoprotein.

[0030] "SR-BI" is an abbreviation for scavenger receptor class B, type 1.

[0031] "BHK" is an abbreviation for baby hamster kidney.

[0032] "DiI" is an abbreviation for 1,1'-dioctadecyl- 3,3,3',3'-tetramethylindocarbocyanine

[0033] "PALM" is an acronym used to identify the peptide amphiphile lipid micelles formed from a combination of amphiphilic peptide with phospholipids and optionally other hydrophobic molecules, in aqueous suspension.

[0034] "Amphiphilic" describes a molecule or polymer (e.g. peptide) with affinity for both lipid and aqueous phases due to a conformation in which hydrophilic (water seeking) substituents and hydrophobic (water avoiding) substituents in the molecule or polymer are structurally segregated from one another.

[0035] "Lipophilic" describes a substance that distributes preferentially to lipid domains of lipid-rich particles in aqueous suspension. The lipid-rich particles include lipid micelles, liposomes, lipoproteins, cell membranes and lipid emulsions.

[0036] "Peptide" is a polymer produced from alpha-amino acid monomers joined together by amide bonds formed between the carboxylic group of one amino acid and the alpha-amine group of the next amino acid in the polymer. "Peptide" also includes a polymer of amino acid monomers joined together. Both L-optical isomers and the D-optical isomers of amino acids can be used. Amino acids making up the polymer may be either those found in nature (i.e. natural amino acids) or un-natural amino acids. The term "residue" or "amino acid residue" includes reference to an amino acid that is incorporated into a peptide, polypeptide, or protein.

[0037] Peptide sequences according to convention, and as used herein, are written N-terminus to C-terminus, left to right.

[0038] "Micelle" is a multi-molecular structure organized by non-covalent interactions in an aqueous phase. The micelle is composed of amphiphilic and hydrophobic molecules which aggregate in such a manner that the hydrophobic domains of molecules are shielded from the water and the hydrophilic constituents are at the micelle-water interface.

[0039] "Cargo molecules" are hydrophobic or amphiphilic molecules with pharmaceutical, therapeutic or diagnostic properties that are stably incorporated into PALM and do not disrupt the stability of PALM.

[0040] "siRNA" are small, interfering ribonucleic acids created to control cellular gene expression as part of the RNA-induced gene silencing complex.

[0041] "Aib" is the three letter code for the amino acid alpha-amino isobutyric acid.

[0042] "Aba" is the three letter code for the amino acid alpha-amino butyric acid.

[0043] "Amv" is the three letter code for the unnatural amino acid alpha-methyl valine.

[0044] "Orn" is the three letter code for the amino acid ornithine.

[0045] "SEC" is size exclusion chromatography

[0046] "DLS" is dynamic light scattering

[0047] A first aspect of the present disclosure provides "amphiphilic peptides". Amphiphilic peptides are able to adopt an alpha helical conformation in which the helix has opposing polar and non-polar faces oriented along the long axis of the helix. Techniques of synthesizing peptides are well known in the art. The peptides of the present disclosure can be

synthesized by any technique known in the art.

[0048] Table 1 shows the charge distribution of specific amphiphilic peptides of the present disclosure compared with several prior art sequences. The charge distribution of the peptides of the present invention are novel in view of the prior art shown below.

TABLE 1

Charges of Residues in Apolipoprotein A-I Mimetic Peptides at Neutral pH

Peptide	N-Term Charge	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	C-Term Charge
SEQ ID[a]	+	-	o	o	o	o	o	+	-	o	o	o	o	o	o	o	-	+	o	+	o	o				-
SEQ ID[b]	+	-	o	o	o	+	o	o	-	o	o	o	o	o	o	-	+	o	+	o	o					-
A-I_con[c]	+	o	o	o	-	-	o	+	-	+	o	o	-	o	o	-	o	o	+	o	+	o	+			-
LAP642[d]	+	o	o	o	-	o	o	+	-	o	o	o	-	o	o	-	o	o	+	o	+	o	+			-
18A[e]	+	-	o	o	+	o	o	o	-	+	o	o	-	+	o	+	-	o	o							-
2F[f]	o	-	o	o	+	o	o	o	-	+	o	o	-	+	o	+	-	o	o							o
R4F[g]	o	o	o	-	+	o	+	-	o	o	+	-	o	o	o	+	o	o	-							o
FAMP[h]	+	o	o	-	o	o	o	o	o	o	-	+	o	o	+	o	o	-	-	o	o	+	+	o	o	-

[a] SEQ ID NOs: 1-23

[b] SEQ ID NOs: 24-35

[c] Anantharamaiah et al. (1990) Arteriosclerosis 10:95-105

[d] Homan et al. (2013) Anal. Biochem. 441:80-86

[e] Anantharamaiah et al. (1985) J. Biol. Chem. 260:10248-10255

[f] Datta et al. (2001) J. Lipid Res. 42:1096-1104

[g] Zhang et al. (2009) Angew. Chem. Int. Ed. 48:9171-9175

[h] Uehara et al. (2013) J Am Heart Assoc. 2(3):e000048. doi: 10.1161/JAHA.113.000048

"o" indicates zero charge at the indicated position.

"+" indicates a positive charge at the indicated position.

"-" indicates a negative charge at the indicated position.

[0049] One embodiment of the first aspect of the disclosure provides a peptide that comprises the amino acid sequence: X_1- X_2 -X_3- X_4 -X_5 -X_6 -X_7 -X_8 -X_9 -X_{10} -X_{11} -X_{12} -X_{13} -X_{14} -X_{15} -X_{16} -X_{17} -X_{18}- X_{19} -X_{20} wherein: X_1 is the amino acid D ; X_2 and X_{20} are each the amino acid V; X_3, X_6, X_{10} and X_{13} are each an amino acid independently selected from the group consisting of L and F; X_4, X_{12} and X_{19} are each the amino acid Q; X_5 is an amino acid A or Aib; X_7, X_{16} and X_{18} are each the amino acid K; X_8 and X_{15} are each the amino acid E; X_9 and X_{14} are each an amino acid independently selected from the group consisting of A, L, F and Aib; X_{11} is an amino acid selected from the group consisting of A, Aib and N; and X_{17} is an amino acid selected from the group consisting of W, F and L, (SEQ ID NO: 1) wherein the peptide is from 20 to 24 amino acid in length.

[0050] Another embodiment of the first aspect provides a peptide that consists essentially of the amino acid sequence: X_1- X_2 -X_3- X_4 -X_5 -X_6 -X_7 -X_8 -X_9 -X_{10} -X_{11} -X_{12} -X_{13} -X_{14} -X_{15} -X_{16} - X_{17} -X_{18}- X_{19} -X_{20} wherein: X_1 is the amino acid D ; X_2 and X_{20} are each the amino acid V; X_3, X_6, X_{10} and X_{13} are each an amino acid independently selected from the group consisting of L and F; X_4, X_{12} and X_{19} are each the amino acid Q; X_5 is an amino acid A or Aib; X_7, X_{16} and X_{18} are each the amino acid K; X_8 and X_{15} are each the amino acid E; X_9 and X_{14} are each an amino acid independently selected from the group consisting of A, L, F and Aib; X_{11} is an amino acid selected from the group consisting of A, Aib and N; and X_{17} is an amino acid selected from the group consisting of W, F and L, (SEQ ID NO:1) wherein the peptide is from 20 to 24 amino acid in length.

[0051] Still another embodiment of the first aspect provides a peptide that consists of the amino acid sequence: X_1- X_2 -X_3- X_4 -X_5 -X_6 -X_7 -X_6 -X_9 -X_{10} -X_{11} -X_{12} -X_{13} -X_{14} -X_{15} -X_{16} -X_{17} -X_{18}- X_{19} -X_{20} wherein: X_1 is the amino acid D ; X_2 and X_{20} are each the amino acid V; X_3, X_6, X_{10} and X_{13} are each an amino acid independently selected from the group consisting of L and F; X_4, X_{12} and X_{19} are each the amino acid Q; X_5 is an amino A or Aib; X_7, X_{16} and X_{18} are each the amino acid K; X_8 and X_{15} are each the amino acid E; X_9 and X_{14} are each an amino acid independently selected from the group consisting of A, L, F and Aib; X_{11} is an amino acid selected from the group consisting of A, Aib and N; and X_{17} is an amino acid selected from the group consisting of W, F and L. (SEQ ID NO:1)

[0052] Yet another embodiment of the first aspect provides a peptide that comprises the amino acid sequence: X_1- X_2 -X_3- X_4 -X_5 -X_6 -X_7 -X_8 -X_9 -X_{10} -X_{11} -X_{12} -X_{13} -X_{14} -X_{15} -X_{16} -X_{17} -X_{18}- X_{19} -X_{20} wherein: X_1 is an amino acid selected from the group consisting of the amino acids D and E; X_2 and X_{20} are each an amino acid independently selected from the group consisting of V, I, and L; X_3, X_6, X_{10} and X_{13} are each an amino acid independently selected from the group consisting of L, I, V, W, Y, Aib, Amv and F; X_4, X_{12} and X_{19} are each an amino acid independently selected from the group consisting of Q and N; X_5, X_{16} and X_{18} are each an amino acid independently selected from the group consisting of K, R, H and Orn; X_7 is selected from the group consisting of A, G, S, V, Aib and Amv; X_8 and X_{15} are independently selected from the group consisting of the amino acids E and D; X_9 and X_{14} are an amino acid independently selected from the group consisting of A, G, S, L, F, V, Amv, and Aib; X_{11} is an amino acid selected from the group consisting of A, G, S, Aib, Amv, V and N; and X_{17} is an amino acid selected from the group consisting of W, F, Y, I, V and L (SEQ ID NO:24) wherein the peptide is optionally acylated at the N-terminus, amidated at the C-terminus, or both acylated at the N-terminus and amidated at the C-terminus wherein the peptide is from 20 to 24 amino acid in length.

[0053] Another embodiment of the first aspect provides a peptide that consists essentially of the amino acid sequence: X_1- X_2 -X_3- X_4 -X_5 -X_6 -X_7 -X_8 -X_9 -X_{10} -X_{11} -X_{12} -X_{13} -X_{14} -X_{15} -X_{16}-X_{17} -X_{18}- X_{19} -X_{20} wherein: X_1 is an amino acid selected from the group consisting of D and E; X_2 and X_{20} are each an amino acid independently selected from the group consisting of V, I, and L; X_3, X_6, X_{10} and X_{13} are each an amino acid independently selected from the group consisting of L, I, V, W, Y, Aib, Amv and F; X_4, X_{12} and X_{19} are each an amino acid independently selected from the group consisting of Q and N; X_5, X_{16} and X_{18} are each an amino acid independently selected from the group consisting of K, R, H and Orn; X_7 is selected from the group consisting of A, G, S, V, Aib and Amv; X_8 and X_{15} are independently selected from the group consisting of the amino acid E and D; X_9 and X_{14} are each an amino acid independently selected from the group consisting of A, G, S, L, F, V, Amv, and Aib; X_{11} is an amino acid selected from the group consisting of A, G, S, Aib, Amv, V and N; and X_{17} is an amino acid selected from the group consisting of W, F, Y, I, V, and L, (SEQ ID NO:24) and the peptide is from 20 to 24 amino acid in length.

[0054] Still another embodiment of the first aspect provides a peptide that consists of the amino acid sequence: X_1- X_2 -X_3- X_4 -X_5 -X_6 -X_7 -X_8 -X_9 -X_{10} -X_{11} -X_{12} -X_{13} -X_{14} -X_{15} -X_{16} -X_{17} -X_{18}- X_{19} -X_{20} wherein: X_1 is an amino acid selected from the group consisting of D and E; X_2 and X_{20} are each an amino acid independently selected from the group consisting of V, I, and L; X_3, X_6, X_{10} and X_{13} are each an amino acid independently selected from the group consisting of L, I, V, W, Y, Aib, Amv and F; X_4, X_{12} and X_{19} are each an amino acid independently selected from the group consisting of Q and N; X_5, X_{16} and X_{18} are each an amino acid independently selected from the group consisting of K, R, H and Orn; X_7 is selected from the group consisting of A, G, S, V, Aib and Amv; X_8 and X_{15} are independently selected from the group consisting of the amino acid E and D; X_9 and X_{14} are each an amino acid independently selected from the group consisting of A, G, S, L, F, V, Amv, and Aib; X_{11} is an amino acid selected from the group consisting of A, G, S, Aib, Amv, V and N; and X_{17} is an amino acid selected from the group consisting of W, F, Y, I, V, and L. (SEQ ID NO:24)

[0055] It is contemplated that any of the disclosed embodiments of the peptides according to the first aspect are optionally acylated at the alpha-amine of the N-terminal amino acid of the peptide, optionally amidated at the terminal carboxyl group of the peptide, or optionally acylated at the alpha-amine of the N-terminal amino acid and amidated at the terminal carboxyl group of the peptide. Peptides can be acylated or amidated by methods known in the art.

[0056] Particular peptides of the present invention are provided in Table 2 below.

TABLE 2

SEQ ID NO:	Peptide Sequence	Mean Hydrophobic Moment [a]	Mean Hydrophobicity [b]
2	DVFQALKELFAQLLEKWKQV	0.846	-1.043
3	DVFQ{AIB}LKELFNQLLEKWKQV	0.908	-1.135
4	DVFQ{AIB}LKELLAQLLEKFKQV	0.885	-0.995
5	DVFQ{AIB}LKELLNQLLEKFKQV	0.948	-1.092
6	DVFQ{AIB}LKELLNQL{AIB}EKFKQV	0.940	-1.120
7	DVFQ{AIB}LKELLNQL{AIB}EKWKQV	0.910	-1.151
8	DVFQALKELLAQLLEKFKQV	0.887	-1.000
9	DVFQALKELLNQLLEKFKQV	0.950	-1.097
10	DVFQ{AIB}LKELFAQLLEKWKQV	0.845	-1.038
11	DVFQ{AIB}LKELFNQLLEKWKQV	0.908	-1.135
12	DVFQ{AIB}LKELFNQLLEKFKQV	0.938	-1.104
13	DVFQALKELFAQL{AIB}EKWKQV	0.836	-1.071
14	DVFQALKELFNQL{AIB}EKWKQV	0.902	-1.168
15	DVFQALKELFNQL{AIB}EKFKQV	0.932	-1.137
16	DVFQAFKEAFAQLFEKWKQV	0.821	-1.099
17	DVFQAFKE{AIB}FAQLFEKWKQV	0.822	-1.094
18	DVFQ{AIB}FKE{AIB}FAQLFEKWKQV	0.820	-1.089
19	DVFQAFKEAF{AIB}QLFEKWKQV	0.818	-1.094
20	DVFQAFKE{AIB}F{AIB}QLFEKWKQV	0.819	-1.089
21	DVFQ{AIB}FKE{AIB}F{AIB}QLFEKWKQV	0.817	-1.084
22	DVFQALKELFNQLLEKWKQV	0.910	-1.140
23	DVFQ{AIB}LKELLNQLLEKLKQV	0.959	-1.081
25	DVFQKL{AIB}ELFNQLLEKWKQV	0.976	-1.135
26	DVFQKLVELFNQLLEKWKQV	0.979	-1.119
27	DV{AIB}QKLFELFNQLLEKWKQV	0.966	-1.135
28	DVFQKL{AIB}ELFNQLLEKFKQV	1.007	-1.104
29	DVFQKLVELFNQLLEKFKQV	1.010	-1.088
30	DV{AIB}QKLFELFNQLLEKFKQV	0.997	-1.104
31	DVLQKF{AIB}ELFNQLLEKWKQV	0.974	-1.135
32	DV{AIB}QKFLELFNQLLEKWKQV	0.958	-1.135
33	DVFQKLLE{AIB}FNQLLEKWKQV	0.979	-1.135
34	DVFQKL{AIB}ELFNQ{AIB}LEKWKQV	0.955	-1.163
35	DVFQKL{AIB}ELFNQL{AIB}EKWKQV	0.961	-1.163

[a] Calculated from amino acid hydrophobicity (Hessa et al. Nature 433:377-381 (2005)) according to Pownall et al. (FEBS Letters 159:17-23 (1983)).
[b] Calculated as the sum of amino acid hydrophobicities divided by the number of residues (kcal/mol/residue).

[0057]   One embodiment of the first aspect of the disclosure is a peptide comprising any one of the amino acid sequences of SEQ ID NOs:1-23 where the peptide is from 20 to 24 amino acid in length. Yet another embodiment is a peptide

consisting essentially of any one of the amino acid sequences of SEQ ID NOs: 1-23 where the peptide is from 20 to 24 amino acid in length. Yet another embodiment is a peptide consisting of any one of the amino acid sequences of SEQ ID NOs: 1-23. In any of the above embodiments of the disclosed peptides, optionally the alpha-amine of the N-terminal amino acid of the peptide is acylated; the terminal carboxyl group is amidated; or the alpha-amine of the N-terminal amino acid is acylated and the terminal carboxyl group of the peptide is amidated.

[0058] One embodiment of the first aspect of the disclosure is a peptide comprising any one of the amino acid sequences of SEQ ID NOs:25-35 where the peptide is from 20 to 24 amino acid in length. Still another embodiment is a peptide consisting essentially of any one of the amino acid sequences of SEQ ID NOs: 25-35 where the peptide is from 20 to 24 amino acid in length. Another embodiment is a peptide consisting of any one of the amino acid sequences of SEQ ID NOs: 25-35. In any of the above embodiments of the peptides, optionally the alpha-amine of the N-terminal amino acid of the peptide is acylated; the terminal carboxyl group is amidated; or the alpha-amine of the N-terminal amino acid is acylated and the terminal carboxyl group of the peptide is amidated.

[0059] Embodiments of the present disclosure further include peptides that have the reverse sequence of the peptides generically defined by SEQ ID NOs: 1 and 24.

[0060] One embodiment of the first aspect of the disclosure provides peptides that are the reverse of SEQ ID NO:1 and the peptides comprise the amino acid sequence: X1-X2-X3- X4-X5-X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-X20, wherein X1 and X19 are each the amino acid V; X2, X9 and X17 are each the amino acid Q; X3, X5, and X14 are each the amino acid K; X4 is an amino acid selected from the group consisting of W, F, and L; X6 and X_{13} are each the amino acid E; X7 and X_{12} are each an amino acid independently selected from the group consisting of A, L, F, and Aib; X8, X11, X15 and X18 are each an amino acid independently selected from the group consisting of L and F; X10 is an amino acid selected from the group consisting of A, Aib and N; X16 is an amino acid selected from the group consisting of A and Aib; and X20 is the amino acid D. (SEQ ID NO:36), wherein the peptide is from 20 to 24 amino acid in length.

[0061] Another embodiment of the first aspect of the disclosure provides peptides that are the reverse of SEQ ID NO:1 and the peptides comprise the amino acid sequence X1-X2-X3- X4-X5-X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-X20, wherein X1 and X19 are each the amino acid V; X2, X9 and X17 are each the amino acid Q; X3, X5, and X14 are each the amino acid K; X4 is an amino acid selected from the group consisting of W, F, and L; X6 and X13 are each the amino acid E; X7 and X12 are each an amino acid independently selected from the group consisting of A, L, F, and Aib; X8, X11, X15 and X18 are each an amino acid independently selected from the group consisting of L and F; X10 is an amino acid selected from the group consisting of A, Aib and N; X16 is an amino acid selected from the group consisting of A and Aib; and X20 is the amino acid D. (SEQ ID NO:36), wherein the peptide is from 20 to 24 amino acid in length.

[0062] One embodiment of the first aspect of the disclosure provides peptides that are the reverse of SEQ ID NO:1 and the peptides consist of the amino acid sequence: X1-X2-X3- X4-X5-X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-X20, wherein X1 and X19 are each the amino acid V; X2, X9 and X17 are each the amino acid Q; X3, X5, and X14 are each the amino acid K; X4 is an amino acid selected from the group consisting of W, F, and L; X6 and X13 are each the amino acid E; X7 and X12 are each an amino acid independently selected from the group consisting of A, L, F, and Aib; X8, X11, X15 and X18 are each an amino acid independently selected from the group consisting of L and F; X10 is an amino acid selected from the group consisting of A, Aib and N; X16 is an amino acid selected from the group consisting of A and Aib; and X20 is the amino acid D. (SEQ ID NO:36).

[0063] Another embodiment of the first aspect of the disclosure provides peptides that are the reverse of SEQ ID NO:24 and the reverse peptides comprise the amino acid sequence: X1-X2-X3- X4-X5-X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-X20 wherein X1 and X19 are each an amino acid independently selected from the group consisting of V, I and L; X2, X9 and X17 are each an amino acid independently selected from the group consisting of Q and N; X3, X5, and X16 are each an amino acid independently selected from the group consisting of K, R, H, and Orn; X4 is an amino acid selected from the group consisting of W, F, Y, I, V, and L; X6, X13 and X20 are each an amino acid independently selected from the group consisting of E and D; X7 and X12 are each an amino acid independently selected from the group consisting of A, G, S, L, F, V, Amv and Aib; X8, X11, X15, and X18 are independently selected from the group consisting of the amino acid L, I, V, W, Aib, Amv and F; X10 is an amino acid selected from the group consisting of A, G, S, Aib, Amv, V and N; and X14 is an amino acid selected from the group consisting of A, G, S, V, Aib and Amv. (SEQ ID NO:59), wherein the peptide is from 20 to 24 amino acid in length.

[0064] Another embodiment of the first aspect of the disclosure provides peptides that are the reverse of SEQ ID NO:24 and the reverse peptides consist essentially of the amino acid sequence: XI-X2-X3- X4-X5-X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-X20 wherein X1 and X19 are each an amino acid independently selected from the group consisting of V, I and L; X2, X9 and X17 are each an amino acid independently selected from the group consisting of Q and N; X3, X5, and X16 are each an amino acid independently selected from the group consisting of K, R, H, and Orn; X4 is an amino acid selected from the group consisting of W, F, Y, I, V, and L; X6, X13 and X20 are each an amino acid independently selected from the group consisting of E and D; X7 and X12 are each an amino acid

independently selected from the group consisting of A, G, S, L, F, V, Amv and Aib; X8, X11, X15, and X18 are independently selected from the group consisting of the amino acid L, I, V, W, Aib, Amv and F; X10 is an amino acid selected from the group consisting of A, G, S, Aib, Amv, V and N; and X14 is an amino acid selected from the group consisting of A, G, S, V, Aib and Amv. (SEQ ID NO:59), wherein the peptide is from 20 to 24 amino acid in length.

[0065] Another embodiment of the first aspect of the disclosure provides peptides that are the reverse of SEQ ID NO:24 and the reverse peptides consist of the amino acid sequence: X1-X2-X3- X4-X5-X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-X20 wherein X1 and X19 are each an amino acid independently selected from the group consisting of V, I and L; X2, X9 and X17 are each an amino acid independently selected from the group consisting of Q and N; X3, X5, and X16 are each an amino acid independently selected from the group consisting of K, R, H, and Orn; X4 is an amino acid selected from the group consisting of W, F, Y, I, V, and L; X6, X13 and X20 are each an amino acid independently selected from the group consisting of E and D; X7 and X12 are each an amino acid independently selected from the group consisting of A, G, S, L, F, V, Amv and Aib; X8, X11, X15, and X18 are independently selected from the group consisting of the amino acid L, I, V, W, Aib, Amv and F; X10 is an amino acid selected from the group consisting of A, G, S, Aib, Amv, V and N; and X14 is an amino acid selected from the group consisting of A, G, S, V, Aib and Amv. (SEQ ID NO:59).

[0066] Provided in Table 3 are additional peptides of the present invention. The sequences of the amino acids in these peptides are the reverse of the amino acid sequences of SEQ ID NOs:2-23 and 25-35.

TABLE 3

SEQ ID NO:	Peptide Sequence
37	VQKWKELLQAFLEKLAQFVD
38	VQKWKELLQNFLEKL{AIB}QFVD
39	VQKFKELLQALLEKL{AIB}QFVD
40	VQKFKELLQNLLEKL{AIB}QFVD
41	VQKFKE{AIB}LQNLLEKL{AIB}QFVD
42	VQKWKE{AIB}LQNLLEKL{AIB}QFVD
43	VQKFKELLQALLEKLAQFVD
44	VQKFKELLQNLLEKLAQFVD
45	VQKWKELLQAFLEKL{AIB}QFVD
46	VQKWKELLQNFLEKL{AIB}QFVD
47	VQKFKELLQNFLEKL{AIB}QFVD
48	VQKWKE{AIB}LQAFLEKLAQFVD
49	VQKWKE{AIB}LQNFLEKLAQFVD
50	VQKFKE{AIB}LQNFLEKLAQFVD
51	VQKWKEFLQAFAEKFAQFVD
52	VQKWKEFLQAF{AIB}EKFAQFVD
53	VQKWKEFLQAF{AIB}EKF{AIB}QFVD
54	VQKWKEFLQ{AIB}FAEKFAQFVD
55	VQKWKEFLQ{AIB}F{AIB}EKFAQFVD
56	VQKWKEFLQ{AIB}F{AIB}EKF{AIB}QFVD
57	VQKWKELLQNFLEKLAQFVD
58	VQKLKELLQNLLEKL{AIB}QFVD
60	VQKWKELLQNFLE{AIB}LKQFVD
61	VQKWKELLQNFLEVLKQFVD
62	VQKWKELLQNFLEFLKQ{AIB}VD
63	VQKFKELLQNFLE{AIB}LKQFVD

(continued)

SEQ ID NO:	Peptide Sequence
64	VQKFKELLQNFLEVLKQFVD
65	VQKFKELLQNFLEFLKQ{AIB}VD
66	VQKWKELLQNFLE{AIB}FKQLVD
67	VQKWKELLQNFLELFKQ{AIBIVD
68	VQKWKELLQNF{AIB}ELLKQFVD
69	VQKWKEL{AIB}QNFLE{AIB}LKQFVD
70	VQKWKE{AIB}LQNFLE{AIB}LKQFVD

[0067] One embodiment of the first aspect of the disclosure is a peptide comprising any one of the amino acid sequences of SEQ ID NOs:36-58 where the peptide is from 20 to 24 amino acid in length. Yet another embodiment is a peptide consisting essentially of any one of the amino acid sequences of SEQ ID NOs: 36-58. Yet another embodiment is a peptide consisting of any one of the amino acid sequences of SEQ ID NOs: 36-58. In any of the above embodiments of the peptides, optionally the alpha-amine of the N-terminal amino acid of the peptide is acylated; the terminal carboxyl group is amidated; or the alpha-amine of the N-terminal amino acid is acylated and the terminal carboxyl group of the peptide is amidated.

[0068] One embodiment of the first aspect of the disclosure is a peptide comprising any one of the amino acid sequences of SEQ ID NOs: 59-70 where the peptide is from 20 to 24 amino acids in length. Still another embodiment is a peptide consisting essentially of any one of the amino acid sequences of SEQ ID NOs: 59-70. Yet another embodiment is a peptide consisting of any one of the amino acid sequences of SEQ ID NOs: 59-70. In any of the above embodiments of the peptides, optionally the alpha-amine of the N-terminal amino acid of the peptide is acylated; the terminal carboxyl group is amidated; or the alpha-amine of the N-terminal amino acid is acylated and the terminal carboxyl group of the peptide is amidated.

[0069] Where a peptide of the disclosure comprises an amino acid sequence of any one of SEQ ID NOs: 1-70 and from 1-4 additional amino independently added to either the N-terminus or C-terminus of the amino acid sequence, the additional amino acids are selected such that the addition of the amino acids does not negatively affect the amphilicity of the peptide.

[0070] A second aspect of the disclosure provides peptide amphiphile lipid micelles (PALM) formed from a combination of amphiphilic peptide with phospholipids. PALM of the second aspect of the disclosure comprise one or more peptides of the first aspect of the disclosure complexed with a lipid component where the lipid component comprises sphingomyelin and one or more additional phospholipids. PALM according to the present disclosure may be passively or actively delivered to a target cell population. In one embodiment of the second aspect of the disclosure, PALM comprises one or more peptides of the present disclosure where the lipid component consists essentially of sphingomyelin and one or more additional phospholipids. In one embodiment PALM comprises a peptide of the present disclosure and a lipid component wherein the lipid component comprises sphingomyelin and one or more additional phospholipids where the additional phospholipid is selected from the group consisting of phosphatidylcholine, polyethylene glycol- phosphatidylethanolamine (PEG-PE), phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, phosphatidylinositol, cardiolipin, and any combination thereof. In another embodiment the PALM comprises a peptide of the disclosure and the lipid component comprises sphingomyelin, and phosphatidylcholine. In another embodiment the PALM comprises a peptide of the disclosure, sphingomyelin, and 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC). In yet another embodiment the PALM comprises a peptide of the disclosure and the lipid component comprises sphingomyelin, and phosphatidylethanolamine. In yet another embodiment the PALM comprises a peptide of the disclosure, and the lipid component comprises sphingomyelin, and poly(ethylene glycol)phosphatidyl-ethanolamine. In still another embodiment the PALM comprises a peptide of the disclosure and the lipid component comprises sphingomyelin, and phosphatidyl-serine. In another embodiment the PALM comprises a peptide of the disclosure and the lipid component comprises sphingomyelin and cardiolipin.

[0071] In still another embodiment of the second aspect of the disclosure, PALM comprises a peptide of the disclosure and the lipid component consists essentially of sphingomyelin and one or more additional phospholipid where the one or more additional phospholipid is selected from the group consisting of phosphatidylcholine, polyethylene glycol- phosphatidylethanolamine (PEG-PE), phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, phosphatidylinositol, cardiolipin, and any combination thereof. In still another embodiment the PALM comprises a peptide of the disclosure and the lipid component consists essentially of sphingomyelin and phosphatidylcholine. In another embodi-

ment the PALM comprises a peptide of the disclosure and the lipid component consists essentially of sphingomyelin and 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC).

[0072] In some embodiments of the second aspect of the disclosure, PALM comprises a peptide of the disclosure and the lipid component consists essentially of sphingomyelin and one or more additional phospholipid where the one or more additional phospholipid is selected from the group consisting of phosphatidylcholine, polyethylene glycol- phosphatidylethanolamine (PEG-PE), phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, phosphatidylinositol, cardiolipin, and any combination thereof where the molar ratio of phospholipid to sphingomyelin is from about 95:5 to about 10:90. In another embodiment the molar ratio of phospholipid to sphingomyelin is from about 90:10 to about 20:80. In still another embodiment the molar ratio of phospholipid to sphingomyelin is from about 25:75 to about 35:65. In another embodiment the molar ratio of phospholipid to sphingomyelin is about 30:70. In another embodiment the molar ratio of phospholipid to sphingomyelin is from about 80:20 to about 60:40. In yet another embodiment the molar ratio of phospholipid to sphingomyelin is from about 75:25 to about 65:35. In still another embodiment the molar ratio of phospholipid to sphingomyelin is about 70:30.

[0073] The fatty acid constituents of the phospholipids include fatty acids according to the formula: R-COOH, wherein R is a (C_7-C_{21}) alkyl group or a (C_7-C_{21}) alkenyl group wherein the alkenyl group can have from one to six double bonds. Examples of suitable fatty acids include, but are not limited to, phytanic acid, linolenic acid, linoleic acid, docosatetraenoic acid, oleic acid, caprylic acid, lauric acid, arachidic acid, myristic acid and palmitic acid. The pair of fatty acids esterified to the glycerol backbone of a particular phospholipid may be identical or each may be a different type of fatty acid.

[0074] The molar ratio of the lipid component to peptide is from about 10:1 to about 2:1. In one embodiment the ration is from about 9:1 to about 2:1. In one embodiment the molar ratio of the lipid component to peptide is from about 8:1 to about 2:1. In still another embodiment the molar ratio of the lipid component to peptide is from about 7:1 to about 3:1. In another embodiment the molar ratio of the lipid component to peptide is from about 6:1 to about 4:1.

[0075] Complexes of phosphatidylcholine with amphiphilic peptides are known. One method to produce these complexes is by initial co-lyophilization from a common solvent phase followed by rehydration of the dry lyophilizate to form complexes in aqueous suspension.

[0076] Particle size is measured by DLS and is expressed as the hydrodynamic mean diameter ("mean diameter"). PALM according to the second aspect of the disclosure are nanometer-sized particles having a mean diameter of 30 nm or less. In one embodiment the mean particle diameter is from about 5 nm to about 30 nm. In yet another embodiment the mean particle diameter is from about 7.5 nm to about 30 nm. In still another embodiment the mean particle diameter is from about 10 nm to about 30 nm. In another embodiment the mean particle diameter is from about 5 nm to about 25 nm. In another embodiment the mean particle diameter is from about 7.5 nm to about 25nm. In yet another embodiment the mean particle diameter is from about 10 nm to about 25 nm. In another embodiment the mean particle diameter is from about 5 nm to about 20 nm. In another embodiment the mean particle diameter is from about 7.5 nm to about 20 nm. In yet another embodiment the mean particle diameter is from about 10 nm to about 20 nm. In still another embodiment the mean particle diameter is from about 5 nm to about 15 nm. In another embodiment the mean particle diameter is from about 7.5 nm to about 15 nm. In yet another embodiment the mean particle diameter is from about 10 nm to about 15 nm. In still another embodiment the mean particle diameter is from about 7.5 nm to about 10 nm.

[0077] A third aspect of the disclosure provides for PALM-cargo molecule compositions which comprise any one of the PALM embodiments of the second aspect of the disclosure and a cargo molecule. Cargo molecules include, but are not limited to, molecules having pharmaceutical or therapeutic properties. Non-limiting examples of cargo molecules include anti-cancer compounds such as all-trans retinoic acid, alcohol esters of all-trans retinoic acid including methyl-, ethyl-, and longer chain fatty alkyl chain alcohol esters of retinoic acid and cholesteryl esters of retinoic acid; retinoic acid amides such as fenretinide; retinol and carboxylic acid esters of retinol including methyl-, ethyl-, and longer chain fatty alkyl chain alcohol esters of retinoic acid; lipophilic anti-fungal agents such as amphotericin B or nystatin; steroids such as progesterone, testosterone, prednisolone, hydrocortisone, dexamethasone and estradiols; analgesics such as propofol and haloperidol; antipsychotics such as fluphenazine decanoate and aripiprazole; the vitamin D analogs cholecalciferol and ergocalciferol; and the isomers of vitamin E, either collectively or individually.

[0078] Cargo molecules also include molecules enabling diagnostic or imaging procedures such as fluorescent imaging agents, radiolabeled imaging agents, and agents used for MRI, PET, CT, SPECT/CT and x-ray studies. MRI imaging agents include, but are not limited to, contrast agents such as a phosphatidylethanolamine with a diethylenetriamine pentaacetic acid moiety that is chelated with a gadolinium ion or similar lanthanide ion or indium-111 or gallium-67 or lutetium-177 or samarium-153.

[0079] Cargo molecules may also be various types and lengths of RNA or DNA that have been linked to cholesterol or other polycyclic fatty alcohols by known methods.

[0080] In one embodiment of the third aspect, the cargo molecule is miriplatin which has the chemical name: cis-[((1R, 2R)-1,2-cyclohexanediamine-N,N')bis(myristato)] platinum(II).
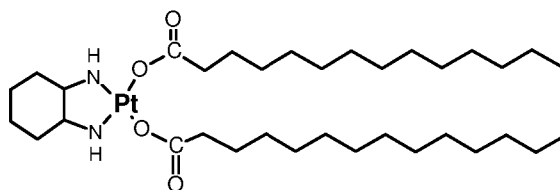
[0081] Yet another embodiments of the third aspect of the disclosure, is a PALM-cargo molecule complex wherein the cargo molecule is a compound conjugate of formula I

[0082] A-R-L-X (formula I) wherein A is an agent having an hydroxy or amine group; R is a hydroxyl or an amine group of the agent; L is a linker, and X is an anchor moiety.

[0083] Another embodiment of the third aspect of the disclosure is a PALM-cargo molecule complex wherein the cargo molecule is a compound conjugate of formula I:

[0084] A-R-L-X (formula I) wherein A is an agent having a hydroxy or amine group; R is the hydroxyl or the amine group of the agent; L is carbonic acid, succinic acid or diglycolic acid; and X is cholesterol, α-tocotrienol, β-tocotrienol, γ-tocotrienol, δ-tocotrienol, cholesterol, coprostanol, plant sterols, (β-sitosterol, sitostanol, stigmasterol, stigmastanol, campesterol, brassicasterol), ergosterol, retinol, cholecalciferol, ergocalciferol, tocopherol, or tocotrienol.

[0085] Another embodiment of the third aspect of the disclosure is a PALM-cargo molecule complex wherein the cargo molecule is a compound conjugate of formula I:
wherein A is an agent having a hydroxy or amine group; R is the hydroxyl or the amine group of the agent; L is selected from the group consisting of carbonic acid, succinic acid or diglycolic acid; and X is selected from the group consisting of cholesterol, α-tocotrienol, β-tocotrienol, γ-tocotrienol, δ-tocotrienol, cholesterol, coprostanol, plant sterols, (β-sitosterol, sitostanol, stigmasterol, stigmastanol, campesterol, brassicasterol), ergosterol, retinol, cholecalciferol, ergocalciferol, α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol,

[0086] Another embodiment of the third aspect of the disclosure is a PALM-cargo molecule complex wherein the cargo molecule is a compound conjugate of formula I:
wherein A is an agent having a hydroxy or amine group; R is a hydroxyl or an amine group of the agent; L is a linker; and X is an anchor moiety selected from the group consisting of cholesterol, cholecalciferol and δ-tocotrienol.

[0087] In one embodiment of a compound conjugate of formula (1), R is a hydroxy group of the agent, and the anchor moiety is covalently bonded to agent by a carbonate ester bond. In another embodiment of a compound conjugate of formula (1), R is an amine group of the agent, and the anchor moiety is covalently bonded to agent by a carbamate ester bond.

[0088] In another embodiment of a compound conjugate of formula (1), the anchor moiety is cholesterol. In still another embodiment of a compound conjugate of formula (1), the anchor moiety is cholesterol, with the proviso that if the anchor moiety is cholesterol, then the compound is not paclitaxel.

[0089] In yet another embodiment of a compound conjugate of formula (1) the anchor moiety is α -tocotrienol. In another embodiment of a compound conjugate of formula (1) the anchor moiety is β -tocotrienol. In still another embodiment of a compound conjugate of formula (1) the anchor moiety is γ -tocotrienol. In yet another embodiment of a compound conjugate of formula (1) the anchor moiety is δ -tocotrienol.

[0090] In some embodiments of the compound conjugate of formula (1) the agent is a drug.

[0091] In some embodiments of the compound conjugate of formula (1) the agent is an anti-cancer drug. In one embodiment of the compound conjugate of formula (1) the agent is an anti-cancer drug and the anti-cancer drug is covalently bonded to the anchor by a carbonate ester bond.
In one embodiment of the compound conjugate of formula (1) the agent is an anti-cancer drug and the anti-cancer drug is covalently bonded to the anchor by a carbamate ester bond.

[0092] Non-limiting examples of anti-cancer drugs having a hydroxyl group available to form the carbonate ester bond include AZD2811, a hydroxy camptothecin, doxorubicin, troxacitabine, vincristine, sirolimus, tubulysin A, docetaxel, or paclitaxel

[0093] Non-limiting examples of anti-cancer drugs having an amine available for forming the carbamate ester bond include, doxorubicin, daunorubicin, gemcitabine, cytarabine and troxacitabine.

[0094] In some embodiments of the PALM-cargo molecule compositions of the third aspect of the disclosure, the cargo molecule is paclitaxel 2'-cholesteryl carbonate. In another embodiment the cargo molecule is paclitaxel 2'-δ-tocotrienyl carbonate.
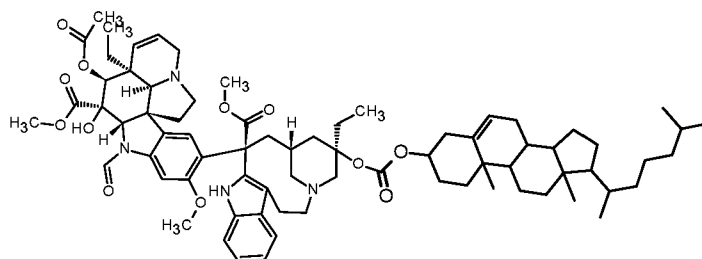
[0095] In yet other embodiments, the cargo molecule is docetaxel 2'-cholesteryl carbonate. In other embodiments, the cargo molecule is the cholesteryl carbonate ester of 10-hydroxycamptothecin. In still other embodiments, the cargo molecule is the cholesteryl carbonate ester of 7-ethyl-10-hydroxycamptothecin, which is the active metabolite of irinotecan. In another embodiment, the cargo molecule is the cholesteryl carbonate ester of sirolimus. In other embodiments,

the cargo molecule is the cholesteryl carbamate ester of gemcitabine. In other embodiments, the cargo molecule is the cholesteryl carbonate ester of tubulysin A. In other embodiments, the cargo molecule is a cholesteryl carbonate ester of morphine, a cholesteryl carbonate ester of hydromorphone, or a cholesteryl carbonate ester of codeine.

[0096] In other embodiments of the PALM-cargo molecule compositions of the third aspect of the disclosure, the cargo molecule is the cholesteryl carbamate ester of gemcitabine (Cholesteryl (N^4)-Gemcitabine Carbamate). In yet other embodiments, the cargo molecule is the cholesteryl carbamate ester of adenosine. In yet other embodiments, the cargo molecule is the cholesteryl carbonate ester of doxorubicin, the structure of which is:
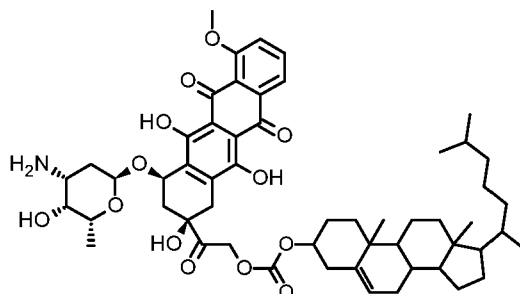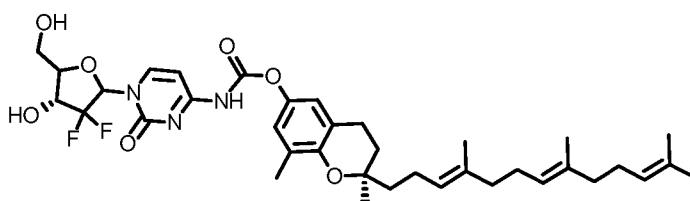

[0097] In yet another embodiments, the cargo molecule is the cholesteryl carbonate ester of vincristine, the structure of which is:


[0098] In still another embodiment the cargo molecule is the delta-tocotrienyl carbonate ester of paclitaxel, the structure of which is:
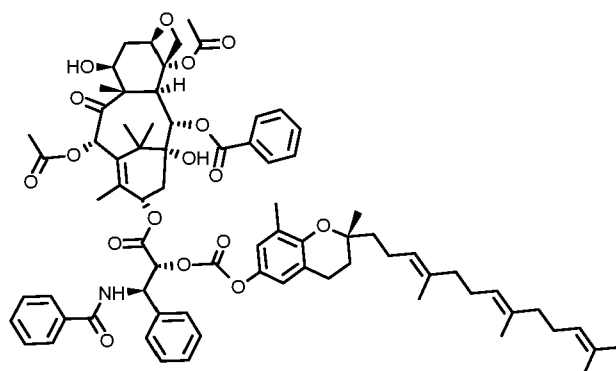
[0099] In still another embodiment the cargo molecule is the gemcitabine delta-tocotrienyl carbamate ester, the structure of which is:
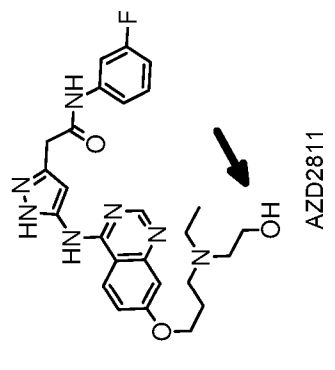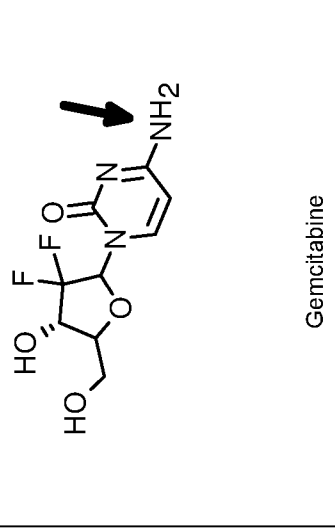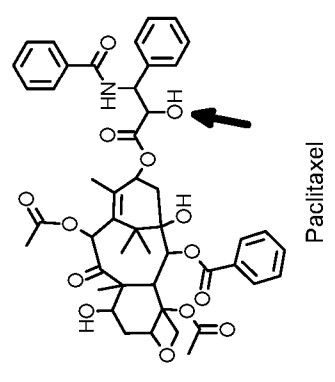
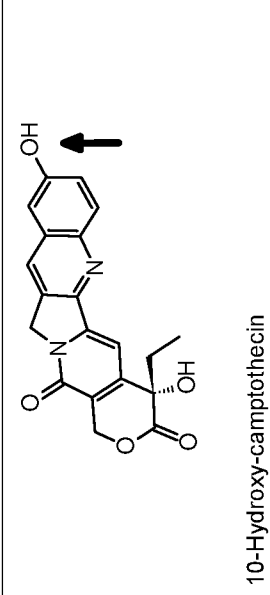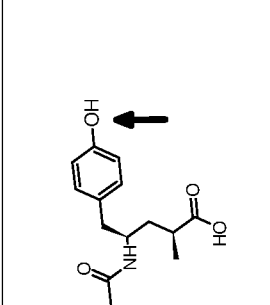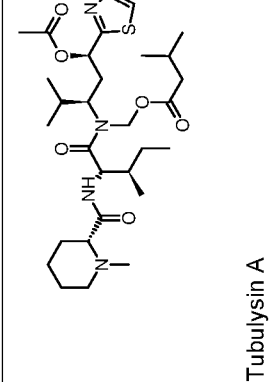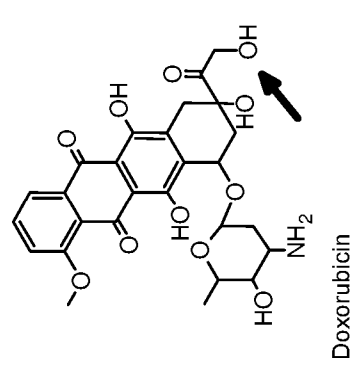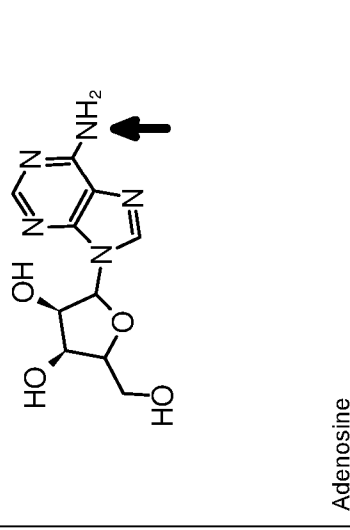**[0100]** In yet another embodiment the cargo molecule is the Doxorubicin delta-tocotrienlyl carbonate ester, the structure of which is:
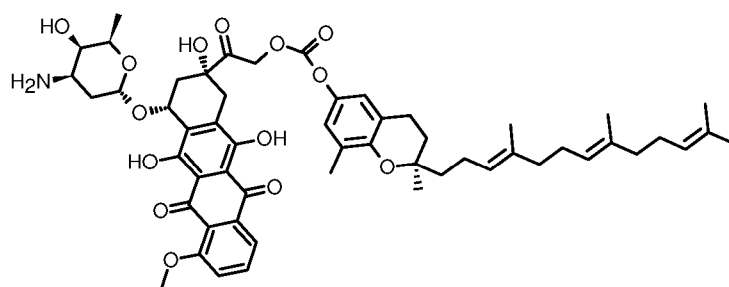
[0101] Table 4 provides the structure of non-limiting examples of agents (A) useful in the present invention with the hydroxyl or amine group (R) indicated by an arrow.

Table 4

AZD2811	10-Hydroxy-camptothecin	Doxorubicin
Gemcitabine	Adenosine	
Paclitaxel	Tubulysin A	Daunorubicin

[0102]   Table 5 provides non-liming examples of PALM-cargo compositions of formula A-R-L-X.

Table 5

Compound	A	R	L	X
1	Paclitaxel	OH	Carbonic Acid	γ-Tocotrienol
2	Paclitaxel	OH	Carbonic Acid	δ-Tocotrienol
3	Paclitaxel	OH	Carbonic Acid	Cholecalciferol
4	Paclitaxel	OH	Carbonic Acid	Ergocalciferol
5	Paclitaxel	OH	Succinic Acid	Cholesterol
6	Paclitaxel	OH	Succinic Acid	γ-Tocotrienol
7	Paclitaxel	OH	Succinic Acid	δ-Tocotrienol
8	Paclitaxel	OH	Succinic Acid	Cholecalciferol
9	Paclitaxel	OH	Succinic Acid	Ergocalciferol
10	Paclitaxel	OH	Diglycolic Acid	Cholesterol
11	Paclitaxel	OH	Diglycolic Acid	γ-Tocotrienol
12	Paclitaxel	OH	Diglycolic Acid	δ-Tocotrienol
13	Paclitaxel	OH	Diglycolic Acid	Cholecalciferol
14	Paclitaxel	OH	Diglycolic Acid	Ergocalciferol
15	Gemcitabine	NH_2	Carbonic Acid	Cholesterol
16	Gemcitabine	NH_2	Carbonic Acid	γ-Tocotrienol
17	Gemcitabine	NH_2	Carbonic Acid	δ-Tocotrienol
18	Gemcitabine	NH_2	Carbonic Acid	Cholecalciferol
19	Gemcitabine	NH_2	Carbonic Acid	Ergocalciferol
20	Gemcitabine	NH_2	Succinic Acid	Cholesterol
21	Gemcitabine	NH_2	Succinic Acid	γ-Tocotrienol
22	Gemcitabine	NH_2	Succinic Acid	δ-Tocotrienol
23	Gemcitabine	NH_2	Succinic Acid	Cholecalciferol
24	Gemcitabine	NH_2	Succinic Acid	Ergocalciferol
25	Gemcitabine	NH_2	Diglycolic Acid	Cholesterol
26	Gemcitabine	NH_2	Diglycolic Acid	γ-Tocotrienol
27	Gemcitabine	NH_2	Diglycolic Acid	δ-Tocotrienol
28	Gemcitabine	NH_2	Diglycolic Acid	Cholecalciferol
29	Gemcitabine	NH_2	Diglycolic Acid	Ergocalciferol
30	AZD2811	OH	Carbonic Acid	Cholesterol
31	AZD2811	OH	Carbonic Acid	γ-Tocotrienol
32	AZD2811	OH	Carbonic Acid	δ-Tocotrienol
33	AZD2811	OH	Carbonic Acid	Cholecalciferol
34	AZD2811	OH	Carbonic Acid	Ergocalciferol
35	AZD2811	OH	Succinic Acid	Cholesterol
36	AZD2811	OH	Succinic Acid	γ-Tocotrienol
37	AZD2811	OH	Succinic Acid	δ-Tocotrienol

(continued)

Compound	A	R	L	X
38	AZD2811	OH	Succinic Acid	Cholecalciferol
39	AZD2811	OH	Succinic Acid	Ergocalciferol
40	AZD2811	OH	Diglycolic Acid	Cholesterol
41	AZD2811	OH	Diglycolic Acid	γ-Tocotrienol
42	AZD2811	OH	Diglycolic Acid	δ-Tocotrienol
43	AZD2811	OH	Diglycolic Acid	Cholecalciferol
44	AZD2811	OH	Diglycolic Acid	Ergocalciferol
45	Daunorubicin	NH_2	Carbonic Acid	Cholesterol
46	Daunorubicin	NH_2	Succinic Acid	γ-Tocotrienol
47	Daunorubicin	NH_2	Carbonic Acid	δ-Tocotrienol
48	Daunorubicin	NH_2	Carbonic Acid	Cholecalciferol
49	Daunorubicin	NH_2	Carbonic Acid	Ergocalciferol
50	Daunorubicin	NH_2	Succinic Acid	Cholesterol
51	Daunorubicin	NH_2	Succinic Acid	γ-Tocotrienol
52	Daunorubicin	NH_2	Succinic Acid	δ-Tocotrienol
53	Daunorubicin	NH_2	Succinic Acid	Cholecalciferol
54	Daunorubicin	NH_2	Succinic Acid	Ergocalciferol
55	Daunorubicin	NH_2	Diglycolic Acid	Cholesterol
56	Daunorubicin	NH_2	Diglycolic Acid	γ-Tocotrienol
57	Daunorubicin	NH_2	Diglycolic Acid	δ-Tocotrienol
58	Daunorubicin	NH_2	Diglycolic Acid	Cholecalciferol
59	Daunorubicin	NH_2	Diglycolic Acid	Ergocalciferol
60	10-Hydroxy-camptothecin	OH	Carbonic Acid	Cholesterol
61	10-Hydroxy-camptothecin	OH	Carbonic Acid	γ-Tocotrienol
62	10-Hydroxy-camptothecin	OH	Carbonic Acid	δ-Tocotrienol
63	10-Hydroxy-camptothecin	OH	Carbonic Acid	Cholecalciferol
64	10-Hydroxy-camptothecin	OH	Carbonic Acid	Ergocalciferol
65	10-Hydroxy-camptothecin	OH	Succinic Acid	Cholesterol
66	10-Hydroxy-camptothecin	OH	Succinic Acid	γ-Tocotrienol
67	10-Hydroxy-camptothecin	OH	Succinic Acid	δ-Tocotrienol
68	10-Hydroxy-camptothecin	OH	Succinic Acid	Cholecalciferol
69	10-Hydroxy-camptothecin	OH	Succinic Acid	Ergocalciferol
70	10-Hydroxy-camptothecin	OH	Diglycolic Acid	Cholesterol
71	10-Hydroxy-camptothecin	OH	Diglycolic Acid	γ-Tocotrienol
72	10-Hydroxy-camptothecin	OH	Diglycolic Acid	δ-Tocotrienol
73	10-Hydroxy-camptothecin	OH	Diglycolic Acid	Cholecalciferol
74	10-Hydroxy-camptothecin	OH	Diglycolic Acid	Ergocalciferol
75	Adenosine	NH_2	Carbonic Acid	Cholesterol

(continued)

Compound	A	R	L	X
76	Adenosine	NH_2	Carbonic Acid	γ-Tocotrienol
77	Adenosine	NH_2	Carbonic Acid	δ-Tocotrienol
78	Adenosine	NH_2	Carbonic Acid	Cholecalciferol
79	Adenosine	NH_2	Carbonic Acid	Ergocalciferol
80	Adenosine	NH_2	Succinic Acid	Cholesterol
81	Adenosine	NH_2	Succinic Acid	γ-Tocotrienol
82	Adenosine	NH_2	Succinic Acid	δ-Tocotrienol
83	Adenosine	NH_2	Succinic Acid	Cholecalciferol
84	Adenosine	NH_2	Succinic Acid	Ergocalciferol
85	Adenosine	NH_2	Diglycolic Acid	Cholesterol
86	Adenosine	NH_2	Diglycolic Acid	γ-Tocotrienol
87	Adenosine	NH_2	Diglycolic Acid	δ-Tocotrienol
88	Adenosine	NH_2	Diglycolic Acid	Cholecalciferol
89	Adenosine	NH_2	Diglycolic Acid	Ergocalciferol
90	Tubulysin A	OH	Carbonic Acid	Cholesterol
91	Tubulysin A	OH	Carbonic Acid	γ-Tocotrienol
92	Tubulysin A	OH	Carbonic Acid	δ-Tocotrienol
93	Tubulysin A	OH	Carbonic Acid	Cholecalciferol
94	Tubulysin A	OH	Carbonic Acid	Ergocalciferol
95	Tubulysin A	OH	Succinic Acid	Cholesterol
96	Tubulysin A	OH	Succinic Acid	γ-Tocotrienol
97	Tubulysin A	OH	Succinic Acid	δ-Tocotrienol
98	Tubulysin A	OH	Succinic Acid	Cholecalciferol
99	Tubulysin A	OH	Succinic Acid	Ergocalciferol
100	Tubulysin A	OH	Diglycolic Acid	Cholesterol
101	Tubulysin A	OH	Diglycolic Acid	γ-Tocotrienol
102	Tubulysin A	OH	Diglycolic Acid	δ-Tocotrienol
103	Tubulysin A	OH	Diglycolic Acid	Cholecalciferol
104	Tubulysin A	OH	Diglycolic Acid	Ergocalciferol
105	Doxorubicin	OH	Carbonic Acid	Cholesterol
106	Doxorubicin	OH	Carbonic Acid	γ-Tocotrienol
107	Doxorubicin	OH	Carbonic Acid	δ-Tocotrienol
108	Doxorubicin	OH	Carbonic Acid	Cholecalciferol
109	Doxorubicin	OH	Carbonic Acid	Ergocalciferol
110	Doxorubicin	OH	Succinic Acid	Cholesterol
111	Doxorubicin	OH	Succinic Acid	γ-Tocotrienol
112	Doxorubicin	OH	Succinic Acid	δ-Tocotrienol
113	Doxorubicin	OH	Succinic Acid	Cholecalciferol

(continued)

Compound	A	R	L	X
114	Doxorubicin	OH	Succinic Acid	Ergocalciferol
115	Doxorubicin	OH	Diglycolic Acid	Cholesterol
116	Doxorubicin	OH	Diglycolic Acid	γ-Tocotrienol
117	Doxorubicin	OH	Diglycolic Acid	δ-Tocotrienol
118	Doxorubicin	OH	Diglycolic Acid	Cholecalciferol
119	Doxorubicin	OH	Diglycolic Acid	Ergocalciferol

[0103] A fourth aspect of the disclosure provides for a surprisingly effective co-lyophilization techniques to produce PALM or PALM-cargo molecule compositions from a homogenous solvent phase composed of tert-butyl alcohol and water. The advantages of this approach are: 1) all PALM constituents including peptide, phosphopholipid and optional lipophilic cargo (e.g. paclitaxel-2'-cholesteryl carbonate), are co-solubilized in a single solvent phase, 2) the solvent components are totally miscible and well-suited to removal by standard lyophilization procedure, 3) the procedures avoids potentially toxic substances because tert-butyl alcohol is a low toxicity, class 3 solvent and 4) the resultant dried lyophilizate enables opportunities for greater stability during storage than is possible with aqueous preparations.

[0104] The solvent mixture used to prepare PALM is preferably a mixture of tert-butyl alcohol (TBA) and water. In one embodiment the percent ration of TBA to water is between about 70%:30% to about 90%:10%. In another embodiment the ratio is between about 75%:25% and about 85%:15%. In yet another embodiment the ratio is 80%:20%.

[0105] One embodiment of the fourth aspect provides a process for preparing PALM comprises the steps:

i) solubilizing an amphiphilic peptide in a first solvent mixture to provide a peptide solution;
ii) solubilizing a sphingomyelin in a second solvent mixture to provide a sphingomyelin solution
iii) solubilizing an additional phospholipid in a third solvent mixture to provide a phospholipid solution;
iv) combining the peptide solution, the sphingomyelin solution and the phospholipid solution to form a peptide/sphingomyelin /phospholipid solution; and
v) lyophilizing the peptide/sphingomyelin/phospholipid solution,

wherein steps i), ii), and iii) are performed in any order; and wherein the first, second, and third solvent mixture comprises tert-butyl alcohol and water.

[0106] Another embodiment of the fourth aspect of the disclosure provides a process for preparing PALM comprises the steps:

i) combining an amphiphilic peptide, sphingomyelin and an additional phospholipid, to form a peptide/sphingomyelin/phospholipid mixture;
ii) solubilizing the peptide/sphingomyelin/phospholipid mixture in a solvent mixture to form a peptide sphingomyelin/phospholipid solution; and
iii) lyophilizing the peptide/phospholipid solution,

wherein the solvent mixture comprises tert-butyl alcohol and water.

[0107] The forth aspect of the present disclosure additionally provides a process for preparing PALM comprising a cargo molecule to form a PALM-cargo molecule complex. To prepare a PALM-cargo molecule complex, the peptide, sphingomyelin, one or more additional phospholipid and a cargo molecule are each separately prepared in a solvent mixture and, depending on the desired formulation, are combined in specific molar ratios. Alternately, the peptide, sphingomyelin, one or more additional phospholipid and a cargo molecule can be combined directly, without prior solubilization, and then brought into solution with the desired solvent mixture prior to lyophilization.

[0108] One embodiment of the fourth aspect of the disclosure provides a process for preparing a PALM-cargo molecule complex comprising the steps:

i) solubilizing an amphiphilic peptide in a first solvent mixture to provide a peptide solution;
ii) solubilizing a sphingomyelin in a second solvent mixture to provide a sphingomyelin solution
iii) solubilizing an additional phospholipid in a third solvent mixture to provide a phospholipid solution;
iv) solubilizing a cargo molecule in a fourth solvent mixture to provide a cargo molecule solution;
v) combining the peptide solution, the sphingomyelin solution, the phospholipid solution and the cargo molecule

solution to form a peptide/ sphingomyelin /phospholipid/cargo molecule solution; and

vi) lyophilizing the peptide/sphingomyelin/phospholipid/cargo molecule solution, wherein steps i) ii), iii) and iv) are performed in any order; and wherein the first, second, third and fourth solvent mixture comprise tert-butyl alcohol and water.

[0109] Another embodiment of preparing a PALM-cargo molecule complex comprises the steps:

i) combining an amphiphilic peptide, sphingomyelin, an additional phospholipid and a cargo molecule, to form a peptide/sphingomyelin/phospholipid/cargo molecule mixture;

ii) solubilizing the peptide/sphingomyelin/phospholipid/cargo molecule mixture in a solvent mixture to form a peptide/phospholipid solution; and

iii) lyophilizing the peptide/sphingomyelin/phospholipid/cargo molecule solution, wherein the solvent mixture comprises tert-butyl alcohol and water

[0110] The resultant lyophilized cake can be stored for long periods of time and will remain stable. The lyophilized product is rehydrated by adding any suitable aqueous solution, e.g., water or saline, followed by gentle swirling of the contents. Reconstitution of PALM lyophilizates can be enhanced by incubation of the PALM solution at 50° C for from 5 to 30 minutes. The solution is then filter sterilized (0.2 μm) and stored at 4° C. Alternately, the solvent mixture comprising the peptide, phospholipid and the cargo molecule is filter sterilized prior to lyophilization.

[0111] A fifth aspect of the present disclosure provides methods for treating a disorder comprising administering to a subject in need thereof, an effective amount of a PALM-cargo composition according to any one the embodiments of the third aspect of the disclosure.

[0112] Scavenger receptor B-1 (SR-B1) is a membrane receptor that binds apolipoprotein A-I, the principle protein component of HDL, to facilitate cellular transport of cholesterol. Cholesterol is an essential nutrient for proliferating cells like those found in malignant tumors. SR-B1 is highly expressed in many tumor cells, including but not limited to breast, prostate, colorectal, pancreatic, adrenal, skin, nasopharyngeal and ovarian cancers. Some amphiphilic peptides are also recognized and bound by SR-BI. PALM are formed from combinations of phospholipid and amphiphilic peptides designed to bind to SR-BI and thereby to selectively deliver cargo molecules to SR-BI-positive cells.

[0113] Accordingly one embodiment of the fifth aspect of the present disclosure provides for methods of treating disorders associated with the overexpression of SR-B1 receptors comprising administering a PALM-drug to a subject in need thereof. In one embodiment the method is a method of treating cancer by administering a PALM-cargo molecule composition to a subject in need thereof.

[0114] For pharmaceutical use, lyophilized PALM may be provided in single dose or multiple dose containers that can be conveniently reconstituted at the point of use, e.g., hospital or doctor's office using standard diluents such as sterile water for injection, normal sterile saline or sterile 5% dextrose solution. Suitable containers are then aseptically filled with the sterilized mixture, lyophilized and sealed appropriately to maintain sterility of the lyophilized material. Suitable containers include but are not limited to a vial comprising a rubber seal, or the equivalent, that allows for introduction of a diluent for reconstitution, e.g., via a syringe. Such PALM preparations are suitable for parenteral administration including intravenous, subcutaneous, intramuscular, intraperitoneal injection.
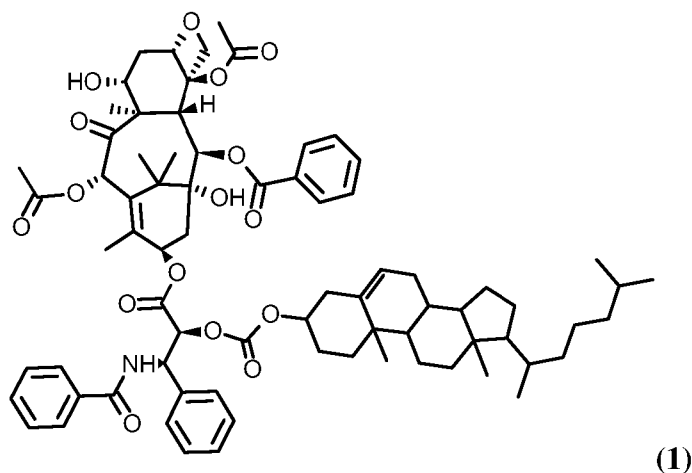
EXAMPLES

Example 1 Peptide Synthesis and Purification

[0115] Peptides were produced by standard Fmoc solid-phase synthesis techniques at GenScript USA, Inc. (Piscataway, NJ). Certain peptides were modified at the terminal amino acids by acetylation of the N-terminus and amidation of the C-terminus by standard procedures. Peptides were chromatographically purified to greater than 90% purity by a standard high-performance liquid chromatography method for peptide purification. Purity was confirmed by HPLC and mass spectroscopic analysis.

Example 2 Paclitaxel 2'-Cholesteryl Carbonate (XC) Synthesis

[0116] Fifty milligrams of paclitaxel was dissolved in 2 ml of chloroform and then combined with 1.5 molar excess of cholesterol chloroformate in 2 ml of chloroform plus 4 ml of N,N-diisopropylethylamine and 2 ml acetonitrile. The mixture was stirred overnight at ambient temperature and then dried on a rotary evaporator. The resulting off-white precipitate was then dissolved in ethyl acetate/ hexane (3:1) and extracted with water, dried, and then redissolved in chloroform. The formation of the product was confirmed by thin-layer chromatography using ethyl acetate/ hexane (3:1) as the mobile phase (Rf of paclitaxel 0.4, Rf of Tax-Chol 0.92). Further purification of the product was then carried out on a silica gel

column using ethyl acetate/hexane (3:1) as the mobile phase to yield the titled compound (1). The structure was confirmed by mass spectrometry and NMR analysis.


(1)

Example 3 Paclitaxel 2'-δ-Tocotrienyl Carbonate (XT3) Synthesis

[0117]   Step 1. Synthesis of p-nitrophenyl carbonate of delta-tocotrienol

(2)
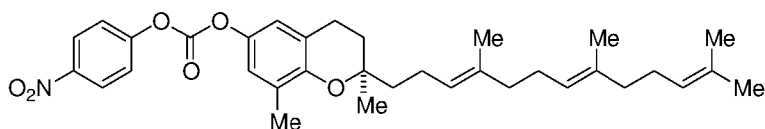
[0118]   To the solution of delta-tocotrienol (25 Mg, 0.0629 mmol) in anhydrous methylene chloride (1.5 mL), was added 4-nitrophenyl chloroformate (51 Mg, 0.25 mmol) and triethylmine (35 μL, 0.25 mmol) at room temperature. The reaction mixture was stirred at room temperature for 24 h and then concentrated and then the desired product (2) was obtained using preparative TLC using ethyl acetate/heptanes (10:90) as eluent. The desired product was obtained as yellow powder (18 Mg). ^{1}H NMR (CDCl$_3$): δ 8.30 (d, 2H), 7.45 (d, 2H), 6.80 (dd, 2H), 5.05-5.20 (m, 3H), 2.72-2.78 (t, 2H), 2.18 (s, 4H), 1.95 - 2.15 (m, 4H), 1.72 - 1.85 (m, 4H), 1.68 (s, 3H), 1.55-1.62 (br s, 12 H), 1.30 (br s, 5H).
[0119]   Step 2. Synthesis of Delta-tocotrienol carbonate of Paclitaxel (3)

(3)
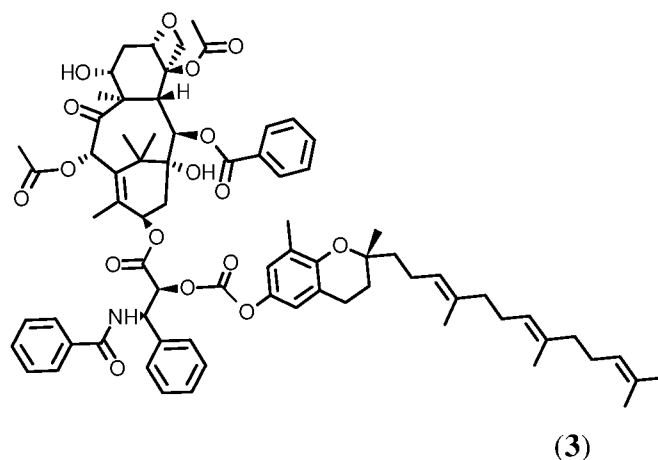
[0120]   A solution of compound (2) (18 mg, step 1 product) in methylene chloride (2 mL), paclitaxel (28 mg) and DMAP (10 Mg) are combined at room temperature. The mixture was stirred at room temperature for 24 h. The mixture was concentrated and purified by using preparative TLC using ethyl acetate/ heptanes (50:50) as eluent. The desired product

(3) (17 Mg) was obtained as colorless solid. TLC Analysis (Rf 0.25, EA/ Hexanes: 1:1). ^{1}H NMR (CDCl$_3$): δ 8.20 (d, 2H), 7.75 (d, 2H), 7.60-7.62 (m, 1H), 7.30-7.52 (m, 9H), 6.90 - 6.95 (d, 1H), 6.60 - 6.75 (dd, 2H), 6.20 - 6.30 (m, 2H), 6.00 - 6.05 (m, 1H), 5.70 -5.75 (d, 1H), 5.50 (s, 1H), 5.10 - 5.20 (br s, 2H), 4.95 - 5.00 (d, 1H), 4.30 - 4.35 (br s, 1H), 4.20-4.30 (dd, 2H), 3.75-3.80 (d, 1H), 2.70-2.75 (m, 2H), 2.30-2.60 (m, 7H), 2.23-2.27 (m, 11H), 1.50-2.20 (m, 26H), 1.25 (m, 9H), 1.15 (s, 3H)

Example 4 Peptide Amphiphile Lipid Micelle (PALM) Preparation

[0121] Separate stock solutions of peptide and phospholipids were prepared in a solvent mixture composed of 80% tert-butyl alcohol (TBA) and 20% water to obtain separate solutions of 10mM peptide, 20mM 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) or 20mM 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) and 20mM egg SM. Aliquots of stock solution were combined to obtain a final solution containing 10 mole equivalents of peptide, 42 mole equivalents of phosphatidylcholine and 18 mole equivalents of SM. The solutions were combined in a 1.5 ml glass vial, frozen (-70 °C), and lyophilized at -5 to -10° C overnight. The resultant lyophilized cakes were rehydrated by addition of Dulbeco's phosphate buffered saline followed by gentle swirling of the contents. Formation of PALM was completed by incubating the PALM solution at 50° C for 10 minutes. Some peptide complexes remained turbid upon heating and were also subjected to one cycle of freezing to -80° C followed by thawing to room temperature in an attempt to obtain a clear solution. The qualities of the PALM preparations were evident in their appearance. A visually clear preparation indicated any nanoparticles that had formed were less than approximately 20nm diameter. Results are shown in Table 5.

TABLE 5

Stability of Peptide/Phospholipid Complexes by Visual Inspection

Phospholipid Content[a]	Mole Ratio PC/SM	Mole Ratio C$_{16}$PTX/PL	Peptide			
			SEQ ID No. 25[b]	SEQ ID No. 26	SEQ ID No. 27	SEQ ID No. 32
POPC, SM	70/30	0	Clear	Clear	Clear	Clear
POPC, SM	70/30	0.1	Clear	Turbid	Turbid	Turbid
DOPC, SM	70/30	0	Clear		Turbid	Turbid
DOPC, SM	70/30	0.1	Clear			Turbid

[a] 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC), 1,2-dioleoyl phosphatidylcholine (DOPC), egg sphingomyelin (SM), phosphatidylcholine (PC), phospholipids (PL), paclitaxel 2'-palmitate (C$_{16}$PTX)
[b] Mole ratio of peptide to phospholipids was 1/4.

Example 5 Preparation of PALM containing the Fluorescent Dye Dil

[0122] A 40µl aliquot of 10mM or peptide was combined with 56µl of 20mM POPC, 24µl 20mM SM(egg) and 16µl 2.5mM Dil in a small glass vial. The peptide and lipid solutions were prepared in 80% TBA/20% water. The Dil stock was prepared in 92% TBA/8% water. The solution was lyophilized and the resultant cake was rehydrated by addition of 0.2 ml of Dulbeco's phosphate buffered saline. The solution was briefly swirled, water bath sonicated (for approx. 15 sec.) and placed in a 50 °C heating block for 20 minutes.

Example 6 Preparation of PALM Containing Miraplatin

[0123] A 50 µl aliquot of 10 mM SEQ ID NO:25 in 80% TBA/20% water corresponding to 2.5 mole equivalents of peptide was combined with 3 mole equivalents of POPC and 7 mole equivalents of egg SM from 40 mM and 20 mM stock solutions, respectively, made up of the same solvent mixture. To this was added 0.75 mole equivalents of miriplatin (MedKoo Biosciences, Raleigh, NC) from a 1 mM stock solution prepared with 100% TBA. The solution was lyophilized and the resultant cake was rehydrated by addition of 0.4 ml of 5% dextrose in water. The solution was briefly swirled, water bath sonicated (for approx. 15 sec.) and placed in a 50°C heating block for 20 minutes. The resultant clear solution was passed through a 0.2 µm pore size, polyethersulfone, sterilization filter and stored at 4 °C. Particle size analysis (Example 11) by DLS indicated a hydrodynamic mean diameter of 8 nm. SEC confirmed a single particle population comparable to HDL in size. The SEC chromatogram is shown in figure 2 (miriplatin (solid line), human HDL(dashed line)).

Example 7 Preparation of PALM Containing Paclitaxel Cholesteryl Carbonate (XC)

[0124] The preparation of PALM containing XC was essentially as described in Example 6 with the following exceptions. A total of 1.5 mole equivalents of XC from a 10 mM stock solution in 92% TBA/8% water were combined with the other PALM components. The solution was lyophilized and the resultant cake was rehydrated with 0.4 ml of Dulbeco's phosphate buffered saline. The hydrodynamic mean diameter of this preparation, determined by DLS, was 9nm (Example 11). Size analysis by SEC indicated a single particle population principally 10nm in diameter (Figure 3).

Example 8 Preparation of PALM Containing Paclitaxel δ-Tocotrienyl Carbonate (XT3)

[0125] A 50 μl aliquot of 10 mM of the peptide of SEQ ID NO:25 in 80% TBA/20% water corresponding to 2.5 mole equivalents of peptide was combined with 7 mole equivalents of POPC and 3 mole equivalents of egg SM from 20 mM stock solutions, made up of the same solvent mixture. To this was added 1 mole equivalent of XT3 from a 10 mM stock solution in 92% TBA/8% water. The lyophilized cake was rehydrated with 0.4 ml of Dulbeco's phosphate buffered saline.

Example 9 R4F is Unsuitable for Preparation of PALM Containing Paclitaxel δ-Tocotrienyl Carbonate (XT3)

[0126] PALM preparation was conducted as in Example 9 with SEQ ID NO:25 and with R4F (Table 1). Unlike PALM made with the peptide of SEQ ID NO:25, which remained a clear solution at room temperature and 4 °C, PALM containing R4F was a clear solution at room temperature but became a hazy gel at 4 °C. The gel returned to clear liquid upon warming to room temperature. The PALM preparations were analyzed for size (Example 11). Dynamic light scattering indicated the PALM with the peptide of SEQ ID NO:25 had a mean hydrodynamic diameter of 8nm (volume intensity). The same analysis for PALM with R4F showed 94% of particle population at a mean hydrodynamic diameter of 11nm with the remainder at 32nm. SEC confirmed the uniform size distribution of the PALM with the peptide of SEQ ID NO:25 (Figure 4). In contrast, the PALM with R4F showed a range of peaks eluting at sizes larger than the SEQ ID NO:25 PALM to sizes smaller than 8nm. The lack of smaller particle detection by DLS is not surprising since sensitivity for particles below 7 nm is quite weak. These results indicate R4F is not a suitable peptide for PALM preparation.

Example 10 Fenretinide is Loaded in PALM Prepared with the peptide of SEQ ID NO:25

[0127] A 35 μl aliquot of 10 mM the peptide of SEQ ID NO:25 in 80% TBA/20% water corresponding to 2.5 mole equivalents of peptide was combined with 3 mole equivalents of POPC and 7 mole equivalents of egg SM from 40 mM and 20 mM stock solutions, respectively, made up in the same solvent mixture. Two mole equivalents of 20 mM fenretinide, in the same solvent mixture, were also added. The solution was lyophilized and the resultant cake was rehydrated with 0.325 ml phosphate buffered saline. The solution became clear within 20 min at 50 °C. Analysis by SEC (Example 11) indicated all components eluted as a single peak in the 8nm-10nm diameter range (Figure 5).

Example 11 Determination of PALM Size

[0128] The size and size uniformity of the PALM preparations was determined by DLS and SEC. Sizes based on hydrodynamic mean diameters were determined by DLS with a Nicomp 370 particle size analyzer. The analyzer was calibrated with latex standards. Particle sizes referred to herein and in the claims are calculated by DLS as described above unless clearly indicated otherwise.

[0129] The relative hydrodynamic size of PALM particles was also determined by SEC with a GE Superose 6 Increase column, (10x300 mm) connected to a Beckman/Coulter Model 126 pump and a Model 128 diode array detector. The mobile phase (150 mM NaCl, 6 mM NaPO4 (pH 7.4)) flow rate was 0.5 ml/min. The eluent was monitored at 215 and 280 nm wavelengths. System performance was confirmed by injection of protein molecular weight standards (Figure 3).

Example 12 SR-BI Selectivity of PALM in BHK(SR-BI) Cells

[0130] SR-BI interaction studies are done with BHK(SR-BI) cells obtained from the NIH. The cells were stably transfected with an inducible human SR-BI gene by means of the GeneSwitch™ System (Invitrogen). The cells were plated (96-well plate) (8000 cells/well) in growth medium (Dulbeco's modified Eagle medium containing 10% fetal bovine serum) containing 200 ug/ml each of zeocin and hygromycin. After 24 hours incubation, the growth medium was removed and replaced with 0.2% bovine serum albumin in Dulbeco's modified Eagle medium. The medium of cells to be induced for SR-BI expression also contained 10nM mifepristone, added from a DMSO stock solution. DMSO alone was added to the medium of uninduced cells. The induction medium was removed after 24 hours and replaced with medium containing Dil-labeled PALM (32 μg peptide/ml) or Dil-labeled HDL (19 μg protein/ml) (Kalen Biomedical, Montgomery Village,

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MD). The test media were prepared by diluting an aliquot of Di-I-labeled PALM (Example 5) or the DiI-labeled HDL in 0.2% bovine serum albumin in Dulbeco's modified Eagle medium. The solutions were passed through 0.2 μm pore size, polyethersulfone, sterilization filters before use. The cells were incubated for 4 hours. Next, the cells were washed 3 times with 0.1% albumin in Dulbeco's phosphate buffered saline (with calcium and magnesium). The last wash was replaced with 200 ul/well of t-butanol/ water (95%/5%). The covered plate was left to stand at ROOM TEMPERATURE for 30 min with occasional shaking. The fluorescence in each well was detected at 520nm excitation and 580nm emission with a 550 nm cutoff filter on a Molecular Dynamics Gemini fluorescence plate reader (Figure 9).

Table 6

Dil Uptake from HDL and from PALM Prepared with Various Peptides by BHK(SR-BI) Cells Depends on SR-BI Expression

| | Un-induced | Induced | |
	Dil Uptake [a] (pmol/ug/ml)	Dil Uptake (pmol/ug/ml)	Increase Over Un-induced
HDL[b]	0.13 ± 0.02	0.86 ± 0.03	561%
SEQ ID NO:3	0.16 ± 0.01	0.29 ± 0.03	88%
SEQ ID NO:5	0.54 ± 0.03	0.93 ± 0.03	73%
SEQ ID NO:25	0.24 ± 0.01	0.83 ± 0.02	245%
SEQ ID NO:26	0.49 ± 0.04	0.81 ± 0.04	65%
SEQ ID NO:27	0.34 ± 0.01	0.66 ± 0.02	90%
SEQ ID NO:32	0.28 ± 0.02	0.90 ± 0.03	221%
SEQ ID NO:35	0.35 ± 0.01	1.26 ± 0.06	264%

[a] Amount of Dil taken up by cells relative to protein (HDL) or peptide (PALM) concentration. The average (n=4) and standard error of the mean are shown.
[b] HDL Dil content was 21 pmol/ug protein. PALM Dil content was 40 pmol/ug peptide. HDL concentration was 19 μg/ml. PALM peptide concentrations were 32 μg/ml.

Example 13 Quantification of Paclitaxel

[0131] Paclitaxel, XT3 and XC are extracted from aqueous samples by mixing 1 volume aqueous sample with 4 volumes of ethyl acetate/acetone/methanol (70/30/5 v/v). The upper organic layer, obtained after shaking and centrifugation, is collected, dried by solvent evaporation and vacuum, and re-dissolved in HPLC mobile phase (methanol/water (65/35 v/v)). A 20 μL aliquot of reconstituted sample is injected on an HPLC at a flow rate of 1.2 ml/minute through a Macherey-Nagel column (4 x 250 mm with Nucleosil 10-5 C18) and detected with a UV detector at 230nm wavelength.

Example 14 PALM Containing Miriplatin Inhibits PC-3 Cell Growth as Well as Cisplatin

[0132] PC-3cells (American Type Culture Collection, CRL-1435) were seeded in 96-well plates at a density of 5x10^{3} cells per well (100 μL) and grown till approximately 70% confluence (24 hour) in growth medium composed of F-12K medium supplemented with 10% fetal bovine serum. Next, growth medium was replaced by either 100 μL fresh growth medium (control) or by growth medium supplemented with various concentrations of cisplatin (e.g. 0 μM and 0.1 to 100 μM final concentration in medium) added from 100-fold concentrated stock solutions prepared in 5% dextrose or with equivalent amounts of MP in PALM, as prepared in Example 6. Each condition was tested in triplicate. Plates were incubated for 48 hours. Cell viability was assayed with the thiazolyl blue tetrazolium bromide (MTT) assay by adding 20 μl of 5 mg/ml MTT in Dulbeco's phosphate buffered saline (with calcium and magnesium) and incubating for 3 hours. Next, media were carefully removed and replaced by 200 μL of dimethylsulfoxide (DMSO). The plates were agitated gently for 15 minutes on an orbital shaker. The absorbance of each well was read at 570 nm. The concentration resulting in 50% growth inhibition (IC50) was determined by non-linear regression fit of the data to the logistic equation. The average absorbance of the control wells represented 100% growth (Figure 6).

Example 15 SR-BI Antibody Attenuates PC-3 Cell Growth Inhibition by PALM Containing Miriplatin

[0133] PC-3cells were grown as in Example 14. Cells to be tested in the presence of SR-BI antibody (Novus Biologics, NB400-113) were preincubated for 1hr in growth medium containing a 1/400 dilution of stock antibody solution. Next, all media were removed and replaced with growth medium containing the indicated amounts of platinum compounds, prepared as in Example 13. The growth media with PALM(MP) for the antibody-treated cells contained antibody at a

1/400 dilution of stock antibody solution. The cells were incubated for 5hr. Next, all media were removed; the cells were washed one time with medium and then incubated for a further 43 hours in growth medium. Cell survival was determined by MTT assay as in Example 14 (Figure 7).

Example 16 XT3 in PALM is More Active Than XC in PALM in Blocking SKOV-3 Cell Growth XC

[0134]   SKOV-3 ovarian cancer cells (American Type Culture Collection, HTB-77) were seeded in 96-well plates at a density of $5x10^3$ cells per well (100 μL) and grown till approximately 70% confluence (24 hour) in growth medium composed of McCoy's medium supplemented with 10% fetal bovine serum. Next, growth medium was replaced by either 100 μL fresh growth medium (control) or by growth medium supplemented with various concentrations of paclitaxel, PALM(XC) or PALM(XT3). A test solution of 20μM paclitaxel was prepared by dilution of a 5mM stock solution of paclitaxel in DMSO into growth medium followed by filter sterilization (0.2 μm filter). An aliquot of the 20μM solution was diluted 5-fold in growth medium to obtain 4μM paclitaxel. The 5-fold dilution process was continued with the 4μM to obtain an 800nM paclitaxel solution. This process was continued until a concentration of 0.051nM paclitaxel in growth medium had been obtained. Four 100μL aliquots of each of the 9 solutions thus obtained were applied to separate wells containing cells. A similar but modified process was used for preparation of PALM(XC) and PALM(XT3) test solutions. The highest concentration tested was 50μM, which was prepared by dilution of 1mM preparations (Examples 7, 8) of PALM(XC) and PALM(XT3) in growth medium followed by filter sterilization. The lowest concentration obtained in the process of 5-fold dilution of each newest dilution repeated 8 times was 0.13 nM. Cells were incubated with the test solutions for 72 hours. At the end of this period cell viability was determined by MTT assay, as in Example 14. (Figure 8).

Example 17 Inhibition of BHK(SR-BI) Cell Growth by PALM(XT3) is SR-BI-Dependent

[0135]   BHK(SR-BI) cells were plated (3000 cells/well) in 96-well plates with growth medium (Dulbeco's modified Eagle medium containing 10% fetal bovine serum and 200 ug/ml each of zeocin and hygromycin) and incubated 24 hours. Growth medium was replaced with 0.2% bovine serum albumin in Dulbeco's modified Eagle medium containing either 10 nM mifepristone (induced), added from a DMSO stock solution, or the equivalent amount of only DMSO (Control). Cells were incubated for 24 hours. Next, media were replaced with PTX or PALM(XT3) in 0.2% bovine serum albumin in Dulbeco's modified Eagle medium, at the indicated concentrations, and the cells were incubated for 12 hours. Those media were then replaced by normal growth medium and the cells were incubated for 36hr more. Percent cell growth relative to cells without test agent was determined by MTT assay (Figure 10).

Example 18 SKOV-3 Ovarian Cell Xenograft Suppression in Nude Mice

[0136]   The maximum tolerated dose is determined in female mice (Foxnlnu) obtained from commercial suppliers. The efficacy of PALM(XT3) is tested in comparison to Taxol®, which consists of paclitaxel formulated with cremophor EL and ethanol. Each agent is tested at 3 dose levels using 8 mice per level. The Taxol® levels to be tested are 9, 15, 25 mg/kg. Mice are dosed Q2Dx4 intravenously. Separate vehicle control groups are treated with the appropriate volume of saline or cremophor EL/ethanol vehicle. All mice are weighed daily and observed for clinical signs. All mice are held for 14 days after the last dose to detect any delayed toxicity

[0137]   For the efficacy studies, mice are implanted subcutaneously with 30 to 60mg tumor fragments collected from donor animals. Mice with tumors in excess of 1g or with ulcerated tumors are euthanized, as are those found in obvious distress or in a moribund condition. Dosing (Q2Dx4) begins when the mean estimated tumor mass for all groups in the experiment is 100-150mg. There are 10 treatment groups (8 mice per group) comprising a vehicle control group for each of the 2 vehicles, 3 treated groups for Taxol® at 0.6X, 1X, and 1.67X the maximum tolerated dose and 5 groups for PALM(XT3) at 0.3X, 0.6X, 1X, 1.7X and 2.8X the maximum tolerated dose. All mice are observed for clinical signs at least once daily. Body weights and tumor measurements are recorded thrice weekly. Mice continue evaluation for 60 days after the last dose to obtain tumor growth delay data.

[0138]   Tumor burden (mg) is estimated by the formula for the volume of a prolate ellipsoid assuming unit density as: Tumor burden (mg) = (L x W2)/2, where L and W are the respective orthogonal tumor length and width measurement with calipers (mm). The primary endpoints used to evaluate efficacy are: 1) %T/C which is defined as the median tumor mass of the Treated Group divided by the median tumor mass of the Control Group x 100, 2) complete and partial tumor response, 3) tumor growth delay, and 4) the number of tumor-free survivors at the end of the study. The median times to evaluation size for all study groups are analyzed by the log rank test (Kaplan-Meier) to determine if any significant differences exist between groups.

Example 19 δ-Tocotrienyl (N⁴)-Gemcitabine Carbamate

[0139] The hydroxyl groups in gemcitabine are protected by conversion to tert-butoxycarbonyl (BOC) esters with di-tert-butyl dicarbonate following the procedure of Guo and Gallo (J. Org. Chem. 1999, 64, 8319) to yield **(1)**


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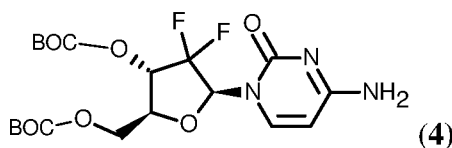
[0140] Compound **4** is dissolved in anhydrous dichloromethane to a final concentration of 0.2M compound **(4)**. For every mole of compound **(4)** in solution, 1.2 mole equivalents of compound **(2)** at 0.5M concentration in methylene chloride and 3 mole equivalents of DMAP are combined at rt. The mixture is stirred at room temperature for 24 h. The resultant product is deprotected with trifluoracetic acid, as referenced. Pure compound is obtained by flash column chromatography using dichloromethane and methanol eluent, beginning with 100% dichloromethane and gradually increasing the concentration to 10% methanol to yield the titled compound **(5)**.

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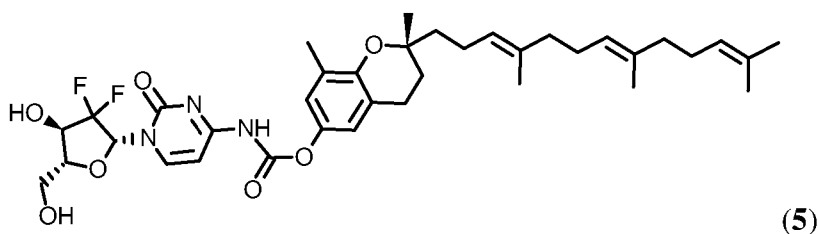
Example 20 Synthesis of (N⁴)-gemcitabine carbamates with the α, β or γ-tocotrienol isomers is performed similarly to Example 19.

Example 21 Cholesteryl (N⁴)-Gemcitabine Carbamate

[0141] The synthesis of cholesteryl (N⁴)-gemcitabine carbamate **(6)** is performed in the same manner as described in Example 19 with the exception that compound **(4)** is reacted with cholesterol chloroformate (commercially available) and deprotected as in Example 19 to yield the titled compound **(6)**.

(6)
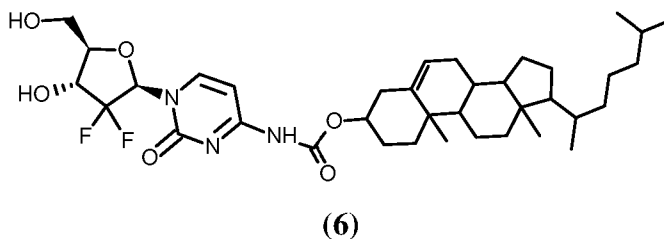
Example 22 Paclitaxel linked to fatty alcohols via succinic and diglycolic acids

[0142] Synthesis of paclitaxel linked to fatty alcohol via a succinate or diglycolate diester link is accomplished by reacting fatty alcohol with 4-(dimethylamino)pyridine and succinic anhydride or diglycolic anhydride in anhydrous pyridine with constant stirring for 24 h at room temperature. The reaction is quenched with 0.1 N HCl in dichloromethane. The product is obtained by preparative TLC or flash column chromatography with ethyl acetate in petroleum ether. The alcohol-succinic acid or -diglycolic acid conjugate is combined with 4-(dimethylamino)pyridine and N-(3-dimethylamino-propyl)-N'-ethylcarbodiimide in dry dichloromethane. Paclitaxel is added into the reaction mixture. After 24 h, the reaction is quenched with water and extracted with dichloromethane. The product is obtained by preparative TLC using ethyl acetate/ heptanes (50:50) as eluent.

Example 23 Effect of SR-BI antibody on PALM(XT3) cytotoxicity in SKOV-3 cells

[0143] SKOV-3 were plated and incubated for 24 hour, as in Example 16. Next, growth medium was replaced with serum-free medium containing 0.5% albumin and the indicated concentrations of test agents, with or without anti-SRBI (1/250 dilution) (NB400-113, Novus Biologicals). The cells were incubated 12 hr. Next, the cells were washed with serum-free medium containing 0.5% albumin and grown a further 60 hour in growth medium. Cell growth was detected by MTT assay (Figure 11).

[0144] While a number of embodiments of this disclosure are described, it is apparent that the basic examples may be altered to provide other embodiments that use or encompass methods and processes of this invention. The embodiments and examples are for illustrative purposes and are not to be interpreted as limiting the disclosure, but rather, the appended claims define the scope of this invention.

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<400> 24

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1                   5                   10                  15

Xaa Xaa Xaa Xaa
            20
```

<210> 25
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<222> (7)..(7)
<223> Aib

<400> 25

Asp Val Phe Gln Lys Leu Xaa Glu Leu Phe Asn Gln Leu Leu Glu Lys
1   5   10   15

Trp Lys Gln Val
                20

<210> 26
<211> 20
<212> PRT
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<220>
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<400> 26

Asp Val Phe Gln Lys Leu Val Glu Leu Phe Asn Gln Leu Leu Glu Lys
1   5   10   15

Trp Lys Gln Val
                20

<210> 27
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<222> (3)..(3)
<223> Aib

<400> 27

Asp Val Xaa Gln Lys Leu Phe Glu Leu Phe Asn Gln Leu Leu Glu Lys
1   5   10   15

Trp Lys Gln Val
                20

<210> 28
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<223> Aib

<400> 28

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Asp Val Phe Gln Lys Leu Xaa Glu Leu Phe Asn Gln Leu Leu Glu Lys
1               5               10                  15

Phe Lys Gln Val
            20
```

<210> 29
<211> 20
<212> PRT
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<220>
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<400> 29

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Asp Val Phe Gln Lys Leu Val Glu Leu Phe Asn Gln Leu Leu Glu Lys
1               5               10                  15

Phe Lys Gln Val
            20
```

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<223> Aib

<400> 30

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Asp Val Xaa Gln Lys Leu Phe Glu Leu Phe Asn Gln Leu Leu Glu Lys
1               5               10                  15

Phe Lys Gln Val
            20
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<210> 31
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<223> Xaa can be any naturally occurring amino acid

<400> 31

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Asp Val Leu Gln Lys Phe Xaa Glu Leu Phe Asn Gln Leu Leu Glu Lys
1               5                   10                  15

Trp Lys Gln Val
            20
```

<210> 32
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<223> Aib

<400> 32

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Asp Val Xaa Gln Lys Phe Leu Glu Leu Phe Asn Gln Leu Leu Glu Lys
1               5                   10                  15

Trp Lys Gln Val
            20
```

<210> 33
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<223> Aib

<400> 33

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        Asp Val Phe Gln Lys Leu Leu Glu Xaa Phe Asn Gln Leu Leu Glu Lys
        1               5                   10                  15
```

5
```
        Trp Lys Gln Val
                    20
```

<210> 34
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20
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25
<400> 34

```
        Asp Val Phe Gln Lys Leu Xaa Glu Leu Phe Asn Gln Xaa Leu Glu Lys
        1               5                   10                  15
```
30

```
        Trp Lys Gln Val
                    20
```

35   <210> 35
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<223> Aib

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50   <222> (14)..(14)
<223> Aib

<400> 35

55

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    1               5                   10                  15


    Trp Lys Gln Val
                20
```

<210> 36
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<223> A, L, F, or Aib

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<223> A or Aib

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44

<223> L or F

<400> 36

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        Val Gln Lys Xaa Lys Glu Xaa Xaa Gln Xaa Xaa Xaa Glu Lys Xaa Xaa
        1               5               10              15

        Gln Xaa Val Asp
                    20
```

<210> 37
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide

<400> 37

```
        Val Gln Lys Trp Lys Glu Leu Leu Gln Ala Phe Leu Glu Lys Leu Ala
        1               5               10              15

        Gln Phe Val Asp
                    20
```

<210> 38
<211> 20
<212> PRT
<213> Artificial Sequence

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<222> (16)..(16)
<223> Aib

<400> 38

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        Val Gln Lys Trp Lys Glu Leu Leu Gln Asn Phe Leu Glu Lys Leu Xaa
        1               5               10              15

        Gln Phe Val Asp
                    20
```

<210> 39
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<212> PRT
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45

5

<210> 39 <220>
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<223> Aib

<400> 39

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Val Gln Lys Phe Lys Glu Leu Leu Gln Ala Leu Leu Glu Lys Leu Xaa
1               5                   10                  15

Gln Phe Val Asp
            20
```

15   <210> 40
<211> 20
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20   <220>
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<223> Aib

<400> 40

30

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Val Gln Lys Phe Lys Glu Leu Leu Gln Asn Leu Leu Glu Lys Leu Xaa
1               5                   10                  15

              Gln Phe Val Asp
                          20
```

35

<210> 41
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<223> Aib

<400> 41

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         1               5               10                  15

         Gln Phe Val Asp
                     20
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<223> Aib

<400> 42

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         1               5               10                  15

         Gln Phe Val Asp
                     20
```

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<400> 43

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         Val Gln Lys Phe Lys Glu Leu Leu Gln Ala Leu Leu Glu Lys Leu Ala
         1               5               10                  15

         Gln Phe Val Asp
                     20
```

<210> 44
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<400> 44

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        Val Gln Lys Phe Lys Glu Leu Leu Gln Asn Leu Leu Glu Lys Leu Ala
        1               5               10              15

        Gln Phe Val Asp
                    20
```

<210> 45
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<223> Aib

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        1               5               10              15

        Gln Phe Val Asp
                    20
```

<210> 46
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<223> Aib

<400> 46

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        1               5               10              15

        Gln Phe Val Asp
                    20
```

<210> 47
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<223> Aib

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        1               5                   10                  15

        Gln Phe Val Asp
                    20
```

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<400> 48

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        1               5                   10                  15

                        Gln Phe Val Asp
                                    20
```

<210> 49
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<223> Aib

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                1               5               10                  15

                Gln Phe Val Asp
                            20
```

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<223> Aib

<400> 50

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                1               5               10                  15

                Gln Phe Val Asp
                            20
```

<210> 51
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<220>
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<400> 51

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                1               5               10                  15

                Gln Phe Val Asp
                            20
```

<210> 52
<211> 20
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<400> 52

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        1               5               10              15

        Gln Phe Val Asp
                    20
```

<210> 53
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<223> Aib

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<222> (16)..(16)
<223> Aib

<400> 53

```
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        1               5               10              15

        Gln Phe Val Asp
                    20
```

<210> 54
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<223> Aib

<400> 54

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        1               5               10              15

        Gln Phe Val Asp
                    20
```

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<223> Aib

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<222> (12)..(12)
<223> Aib

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1               5                   10                  15

Gln Phe Val Asp
            20
```

<210> 56
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<222> (16)..(16)
<223> Aib

<400> 56

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Val Gln Lys Trp Lys Glu Phe Leu Gln Xaa Phe Xaa Glu Lys Phe Xaa
1               5                   10                  15

Gln Phe Val Asp
            20
```

<210> 57
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide

<400> 57

Val Gln Lys Trp Lys Glu Leu Leu Gln Asn Phe Leu Glu Lys Leu Ala
1   5   10   15

Gln Phe Val Asp
            20

<210> 58
<211> 20
<212> PRT
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<220>
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<222> (16)..(16)
<223> Aib

<400> 58

Val Gln Lys Leu Lys Glu Leu Leu Gln Asn Leu Leu Glu Lys Leu Xaa
1   5   10   15

Gln Phe Val Asp
            20

<210> 59
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55   <220>
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<220>
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<223> D or E

<400> 59

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1   5   10   15

Xaa Xaa Xaa Xaa
            20

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<223> Aib

<400> 60

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Val Gln Lys Trp Lys Glu Leu Leu Gln Asn Phe Leu Glu Xaa Leu Lys
1               5                   10                  15

Gln Phe Val Asp
            20
```

<210> 61
<211> 20
<212> PRT
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<220>
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<400> 61

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Val Gln Lys Trp Lys Glu Leu Leu Gln Asn Phe Leu Glu Val Leu Lys
1               5                   10                  15

Gln Phe Val Asp
            20
```

<210> 62
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<222> (18)..(18)
<223> Aib

<400> 62

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Val Gln Lys Trp Lys Glu Leu Leu Gln Asn Phe Leu Glu Phe Leu Lys
1               5                   10                  15

Gln Xaa Val Asp
            20
```

<210> 63
<211> 20
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<220>
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<222> (14)..(14)
<223> Aib

<400> 63

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Val Gln Lys Phe Lys Glu Leu Leu Gln Asn Phe Leu Glu Xaa Leu Lys
1               5                   10                  15

Gln Phe Val Asp
            20
```

<210> 64
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<212> PRT
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<220>
<223> synthetic peptide

<400> 64

```
Val Gln Lys Phe Lys Glu Leu Leu Gln Asn Phe Leu Glu Val Leu Lys
1               5                   10                  15

Gln Phe Val Asp
            20
```

<210> 65
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<213> Artificial Sequence

<220>
<223> synthetic peptide

<220>
<221> MISC_FEATURE
<222> (18)..(18)
<223> Aib

<400> 65

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Val Gln Lys Phe Lys Glu Leu Leu Gln Asn Phe Leu Glu Phe Leu Lys
1               5                   10                  15

Gln Xaa Val Asp
            20
```

<210> 66
<211> 20
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<220>
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<220>
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<222> (14)..(14)
<223> Aib

<400> 66

      Val Gln Lys Trp Lys Glu Leu Leu Gln Asn Phe Leu Glu Xaa Phe Lys
      1   5   10   15

      Gln Leu Val Asp
                20

<210> 67
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<213> Artificial Sequence

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<222> (18)..(18)
<223> Aib

<400> 67

      Val Gln Lys Trp Lys Glu Leu Leu Gln Asn Phe Leu Glu Leu Phe Lys
      1   5   10   15

      Gln Xaa Val Asp
                20

<210> 68
<211> 20
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<220>
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<222> (12)..(12)
<223> Aib

<400> 68

      Val Gln Lys Trp Lys Glu Leu Leu Gln Asn Phe Xaa Glu Leu Leu Lys
      1   5   10   15

      Gln Phe Val Asp
                20

<210> 69
<211> 20

<212> PRT
<213> Artificial Sequence

<220>
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<223> Aib

<220>
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<222> (14)..(14)
<223> Aib

<400> 69

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Val Gln Lys Trp Lys Glu Leu Xaa Gln Asn Phe Leu Glu Xaa Leu Lys
1               5                   10                  15

Gln Phe Val Asp
            20
```

<210> 70
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<223> Aib

<220>
<221> MISC_FEATURE
<222> (14)..(14)
<223> Aib

<400> 70

```
Val Gln Lys Trp Lys Glu Xaa Leu Gln Asn Phe Leu Glu Xaa Leu Lys
1               5                   10                  15

                Gln Phe Val Asp
                            20
```

REFERENCES CITED IN THE DESCRIPTION

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Non-patent literature cited in the description

- **HESSA et al.** *Nature,* 2005, vol. 433, 377-381 **[0056]**
- **POWNALL et al.** *FEBS Letters,* 1983, vol. 159, 17-23 **[0056]**
- **GUO ; GALLO.** *J. Org. Chem.,* 1999, vol. 64, 8319 **[0139]**

Patentkrav

1. Peptid-amfifilt lipid-micelle-last(PALM-last)-
sammensætning, som omfatter:
a) et peptid, der omfatter aminosyresekvensen: X1-X2-X3-X4-X5-
X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-X20, hvor:
X1 er aminosyren D; X2 og X20 hver er aminosyren V; X3, X6,
X10 og X13 hver er en aminosyre valgt uafhængigt fra gruppen,
der består af L og F; X4, X12 og X19 hver er aminosyren Q; X5
er en aminosyre A eller Aib; X7, X16 og X18 hver er aminosyren
K; X8 og X15 hver er aminosyren E; X9 og X14 hver er en
aminosyre valgt uafhængigt fra gruppen, der består af A, L, F
og Aib; X11 er en aminosyre valgt fra gruppen, der består af
A, Aib og N; og X17 er en aminosyre valgt fra gruppen, der
består af W, F og L (SEQ ID NO: 1); eller
X1-X2-X3-X4-X5-X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-
X18-X19-X20, hvor: X1 er en aminosyre valgt fra gruppen, der
består af D og E; X2 og X20 hver er en aminosyre valgt
uafhængigt fra gruppen, der består af V, I og L; X3, X6, X10
og X13 hver er en aminosyre valgt uafhængigt fra gruppen, der
består af L, I, V, W, Y, Aib, Amv og F; X4, X12 og X19 hver er
en aminosyre valgt uafhængigt fra gruppen, der består af Q og
N; X5, X16 og X18 hver er en aminosyre valgt uafhængigt fra
gruppen, der består af K, R, H og Orn; X7 er valgt fra
gruppen, der består af A, G, S, V, Aib og Amv; X8 og X15 er
valgt uafhængigt fra gruppen, der består af aminosyren E og D;
X9 og X14 er en aminosyre valgt uafhængigt fra gruppen, der
består af A, G, S, L, F, V, Amv og Aib; X11 er en aminosyre
valgt fra gruppen, der består af A, G, S, Aib, Amv, V og N; og
X17 er en aminosyre valgt fra gruppen, der består af W, F, Y,
I, V og L (SEQ ID NO: 24);
hvor peptidet eventuelt er acyleret i N-terminalen, amideret i
C-terminalen eller både acyleret i N-terminalen og amideret i
C-terminalen, og peptidet er fra 20 til 24 aminosyrer langt;
b) en lipidbestanddel, der omfatter sphingomyelin og et eller
flere yderligere phospholipider; og
c) et lastmolekyle, som er en forbindelseskonjugat med formlen
(I):

A-R-L-X (formel I)

hvor A er et middel, der har en hydroxyl- eller amingruppe; R er en hydroxyl- eller en amingruppe i midlet; L er en linker, og X er en forankringsdel.

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2.   PALM-last-sammensætning ifølge krav 1, hvor peptidet omfatter en aminosyresekvens valgt fra gruppen, der består af SEQ ID NO: 2, SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID

10   NO: 10; SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 13; SEQ ID NO: 14; SEQ ID NO: 15; SEQ ID NO: 16; SEQ ID NO: 17; SEQ ID NO: 18; SEQ ID NO: 19; SEQ ID NO: 20; SEQ ID NO: 21; SEQ ID NO: 22; SEQ ID NO: 23; SEQ ID NO: 25; SEQ ID NO: 26; SEQ ID NO: 27; SEQ ID NO: 28; SEQ ID NO: 29; SEQ ID NO: 30; SEQ ID

15   NO: 31, SEQ ID NO: 32; SEQ ID NO: 33; SEQ ID NO: 34; og SEQ ID NO: 35, hvor peptidet eventuelt er acyleret i N-terminalen, amideret i C-terminalen eller både acyleret i N-terminalen og amideret i C-terminalen, og peptidet er fra 20 til 24 aminosyrer langt.

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3.   PALM-last-sammensætning ifølge krav 1 eller krav 2, hvor peptidet ikke er eventuelt acyleret i N-terminalen, amideret i C-terminalen eller både acyleret i N-terminalen og amideret i C-terminalen.

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4.   PALM-last-sammensætning ifølge et hvilket som helst af kravene 1-3, hvor mindst ét lastmolekyle er et forbindelseskonjugat med formlen (I):
A-R-L-X (formel I)

30   hvor X er valgt fra gruppen, der består af kolesterol, α-tocotrienol, β-tocotrienol, γ-tocotrienol og δ-tocotrienol.

5.   PALM-last-sammensætning ifølge et hvilket som helst af kravene 1-4, hvor det ene eller de flere yderligere

35   phospholipider er valgt fra gruppen, der består af phosphatidylcholin, polyethylenglycol- phosphatidylethanolamin (PEG-PE), phosphatidylethanolamin, phosphatidylglycerol, phosphatidylserin, phosphatidylinositol, cardiolipin eller en

hvilken som helst kombination deraf.

6. PALM-last-sammensætning ifølge et hvilket som helst af kravene 1-5, hvor peptidet er valgt fra gruppen, der består af
5   SEQ ID NO: 25, SEQ ID NO: 28, SEQ ID NO: 34 og SEQ ID NO: 35.

7. PALM-last-sammensætning ifølge et hvilket som helst af kravene 1-6, hvor midlet er et lægemiddel.

10   8. PALM-last-sammensætning ifølge krav 7, hvor lægemidlet er et anticancerlægemiddel.

9. PALM-last-sammensætning ifølge krav 8, hvor anticancerlægemidlet er et hydroxycamptothecin; daunorubicin;
15   paclitaxel; eller docetaxel.

10. PALM-last-sammensætning ifølge krav 8, hvor ARLX er: paclitaxel-2'-δ-tocotrienylcarbonat; paclitaxel-2'-cholesterylcarbonat; docetaxel-2'-cholesterylcarbonat;
20   daunorubicin-δ-tocotrienylcarbonat; eller gemcitabin-cholesterylcarbamat.

11. Peptid, som består af aminosyresekvensen valgt fra gruppen, der består af SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID
25   NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 13; SEQ ID NO: 14; SEQ ID NO: 15; SEQ ID NO: 16; SEQ ID NO: 17; SEQ ID NO: 18; SEQ ID NO: 19; SEQ ID NO: 20; SEQ ID NO: 21; SEQ ID NO: 22; og SEQ ID NO: 23, hvor peptidet eventuelt er
30   acyleret i N-terminalen, amideret i C-terminalen eller både acyleret i N-terminalen og amideret i C-terminalen, og peptidet er 20 aminosyrer langt.

12. Peptid, som omfatter aminosyresekvensen: X1-X2-X3-X4-X5-
35   X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-X20, hvor: X1 er en aminosyre valgt fra gruppen, der består af D og E; X2 og X20 hver er en aminosyre valgt uafhængigt fra gruppen, der består af V, I og L; X3, X6, X10 og X13 hver er en aminosyre

valgt uafhængigt fra gruppen, der består af L, I, V, W, Y, Aib, Amv og F; X4, X12 og X19 hver er en aminosyre valgt uafhængigt fra gruppen, der består af Q og N; X5, X16 og X18 hver er en aminosyre valgt uafhængigt fra gruppen, der består af K, R, H og Orn; X7 er valgt fra gruppen, der består af A, G, S, V, Aib og Amv; X8 og X15 er valgt uafhængigt fra gruppen, der består af aminosyren E og D; X9 og X14 er en aminosyre valgt uafhængigt fra gruppen, der består af A, G, S, L, F, V, Amv og Aib; X11 er en aminosyre valgt fra gruppen, der består af A, G, S, Aib, Amv, V og N; og X17 er en aminosyre valgt fra gruppen, der består af W, F, Y, I, V og L, (SEQ ID NO: 24), hvor peptidet eventuelt er acyleret i N-terminalen, amideret i C-terminalen eller både acyleret i N-terminalen og amideret i C-terminalen, og peptidet er fra 20 til 24 aminosyrer langt.

13. Peptid ifølge krav 12, som består af aminosyresekvensen valgt fra gruppen, der består af SEQ ID NO: 25; SEQ ID NO: 26; SEQ ID NO: 27; SEQ ID NO: 28; SEQ ID NO: 29; SEQ ID NO: 30; SEQ ID NO: 31, SEQ ID NO: 32; SEQ ID NO: 33; SEQ ID NO: 34; og SEQ ID NO: 35, hvor peptidet eventuelt er acyleret i N-terminalen, amideret i C-terminalen eller både acyleret i N-terminalen og amideret i C-terminalen, og peptidet er 20 aminosyrer langt.

14. Peptid ifølge et hvilket som helst af kravene 12-13, hvor peptidet er valgt fra gruppen, der består af SEQ ID NO: 25, SEQ ID NO: 28, SEQ ID NO: 34 og SEQ ID NO: 35.

15. Peptid ifølge et hvilket som helst af kravene 11-14, hvor peptidet ikke er eventuelt acyleret i N-terminalen, amideret i C-terminalen eller både acyleret i N-terminalen og amideret i C-terminalen.
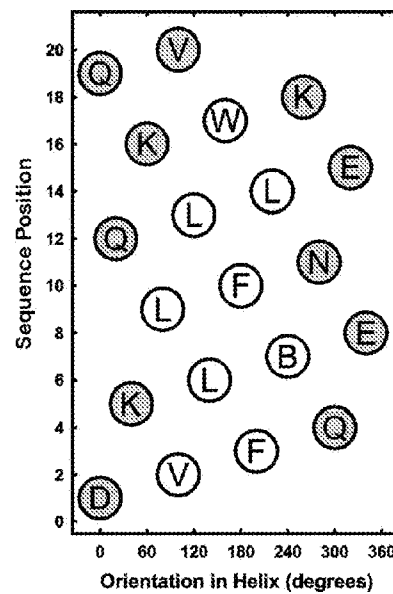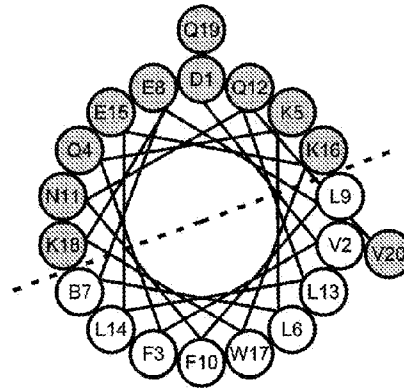
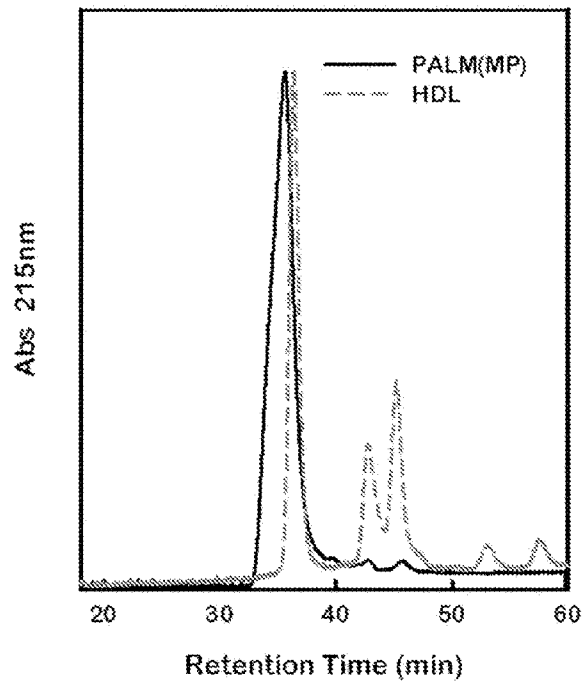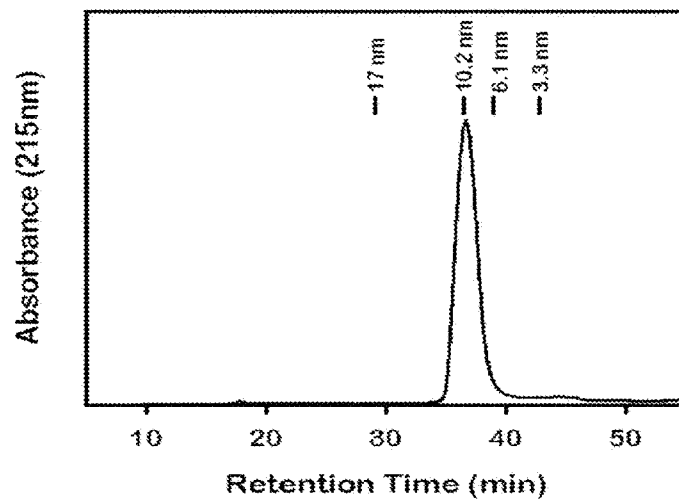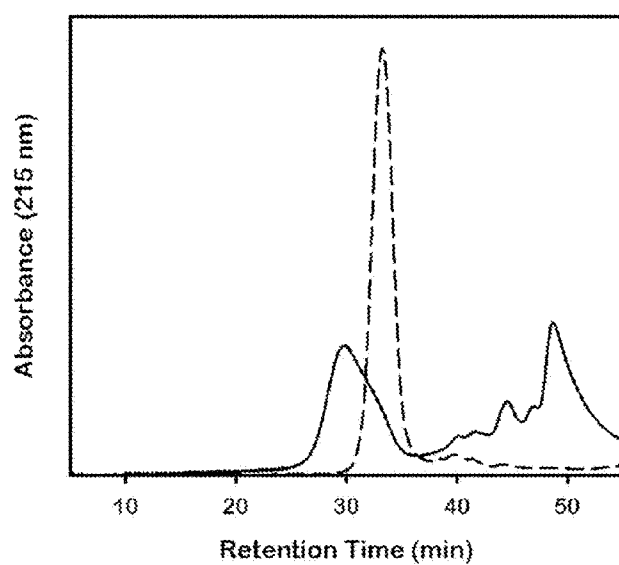# DRAWINGS

FIG. 1A
SEQ ID NO: 3
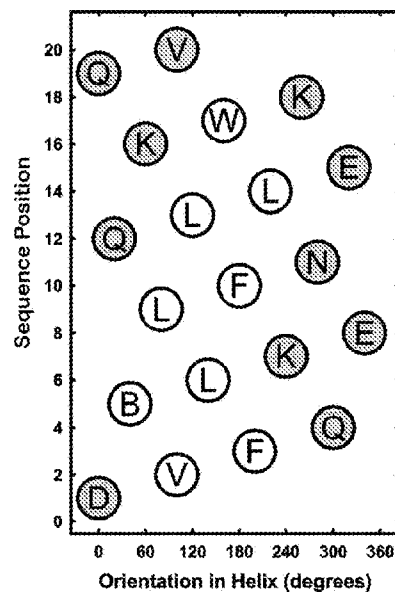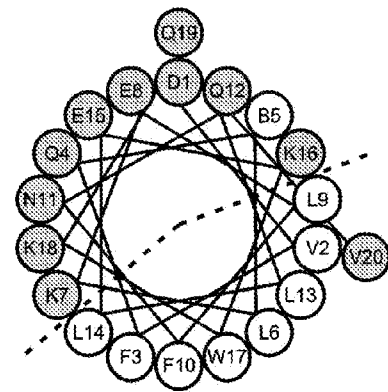
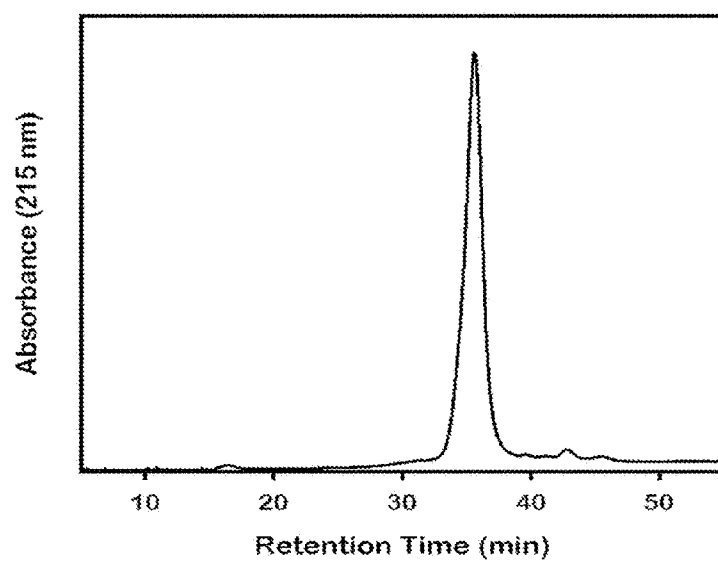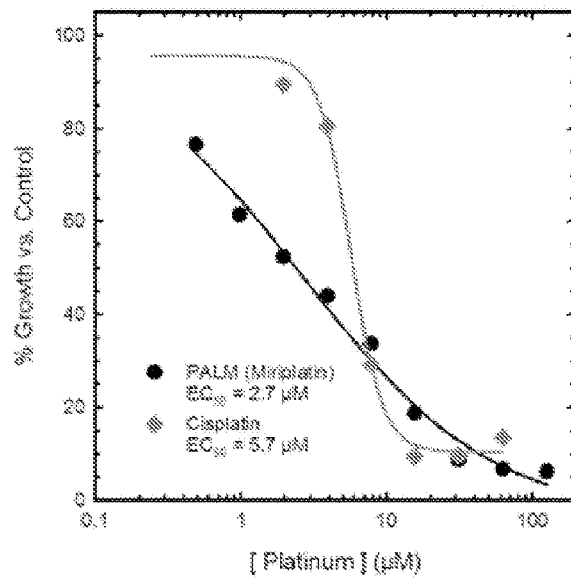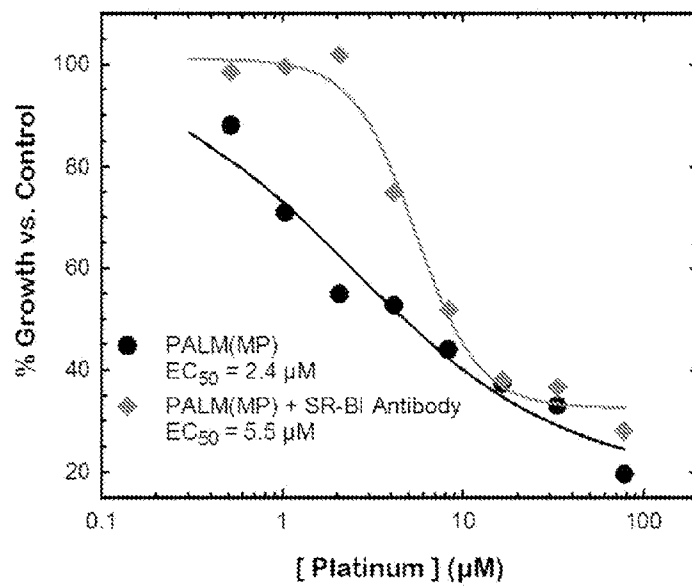FIG. 1B
SEQ ID NO: 25

FIG. 1C

FIG. 1D

FIG. 2

FIG. 3

FIG. 4

FIG. 5

FIG. 6

FIG. 7

FIG. 8

FIG. 9

FIG. 10

FIG. 11