

PLASMALOGEN COMPOUNDS, PHARMACEUTICAL COMPOSITIONS
CONTAINING THE SAME AND METHODS FOR TREATING DISEASES OF THE
AGING

FIELD OF INVENTION

5 The present invention relates to the synthesis and utility of novel chemical entities with useful biochemical, physiochemical, and clinical properties. More specifically, a series of 1-alkyl, 2-acyl glycerol derivatives are provided which can be used for the treatment or prevention of disease. The invention also relates to pharmaceutical compositions and kits incorporating such compounds.

10 BACKGROUND OF THE INVENTION

It is well known that many diverse human diseases such as cancer, dementia, or decreased cognitive functioning increase in incidence with age. From an epidemiological and statistical perspective, these diseases often look very similar. However, from a clinical perspective, each of the cancers, dementias, and decreased cognitive functioning are very different.

15 Currently, the largest risk factor for these disorders is the subject's age. Furthermore, it is well established that most cancers, dementias, and decreased cognitive functioning have a long prodromal phase (5-15 years) in which the disease is present but at a sub-clinical manifestation. Age-associated increases in membrane cholesterol¹⁻³ and increased mitochondrial membrane cholesterol⁴⁻⁶ have been reported. These increases in membrane
20 cholesterol result in decreased membrane fluidity², decreased ion channel function⁶⁻⁸, decreased activities of some membrane-bound enzymes like 5'-nucleotidase⁹ and α -secretase¹⁰, and altered diffusion properties for signaling molecules like nitric oxide¹¹.

Subjects suffering from increased membrane cholesterol demonstrate an increased prevalence of neurodegenerative diseases (e.g. Alzheimer's, Parkinson's, multiple sclerosis and age-
25 related macular degeneration), cognitive impairment, dementia, cancers (e.g. prostate, lung, breast, ovarian, and kidney), osteoporosis, bipolar disorder and vascular diseases (atherosclerosis, hypercholesterolemia).

With respect to specific diseases, cholesterol accumulates in the brain membranes of Alzheimer's patients in a severity-dependent manner¹²⁻¹⁴. In this regard, lowering membrane

cholesterol has been shown to decrease the activities of beta- and gamma-secretases, blocking the pathogenic processing of beta-amyloid¹⁵⁻¹⁶. At the molecular level, cholesterol binds to the transmembrane domain of amyloid precursor protein (APP), activating the trafficking of APP to cholesterol-rich membrane domains rich in beta- and gamma-secretases, resulting in amyloid-beta production¹⁷. Synaptic membrane changes resulting from increased cholesterol may also be an important factor in the utilization of membrane phospholipids to support cholinergic neurotransmission (autocannibalism concept)¹⁸. Early utilization of statins have also been suggested to reduce the incidence or delay the onset of Alzheimer's and Parkinson's diseases¹⁹. Cholesterol accumulation also occurs in the drusen associated with age-related macular degeneration²⁰. Membrane cholesterol is also increased in cancer²¹ and increases in mitochondrial membrane cholesterol have been hypothesized to be the defect that leads to the Warburg effect that is associated with most cancer cells²². The Warburg effect is a defining feature of cancer cells in that, unlike normal cells, which rely almost entirely upon respiration for energy, cancer cells can utilize both respiration and glycolysis for energy.

In addition to complex negative membrane effects of cholesterol accumulation, there is also increased oxysterol production²³. These oxysterols are cytotoxic (apoptosis and necrosis), pro-inflammatory, deplete GSH and induce phospholipidosis²³⁻²⁶. Diseases in which these toxic oxysterols might be involved include neurodegeneration (both neuronal and demyelinating), osteoporosis, age-related macular degeneration and cardiovascular diseases, particularly atherosclerosis²³.

Current clinical therapies to reduce cholesterol consist mainly of inhibiting cholesterol synthesis with statins or blocking cholesterol absorption from the gastrointestinal tract with ezetimibe. To the inventors' knowledge, there are currently no drugs to mobilize cholesterol trafficking out of membranes.

SUMMARY OF THE INVENTION

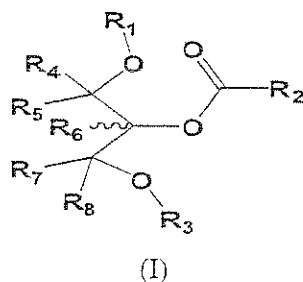
The present disclosure relates to compounds and methods for treating diseases of aging associated with abnormal membrane cholesterol levels. The described compounds include novel plasmalogen precursors which decrease membrane free cholesterol levels and augment cholesterol esterification for transport from cell membranes. These compounds therefore are useful for reducing membrane cholesterol levels in subjects suffering from elevated membrane cholesterol levels. The compounds can also be used to treat or prevent diseases

associated with increased membrane cholesterol such as neurodegenerative diseases (including but not limited to Alzheimer's disease, Parkinson's disease, multiple sclerosis and age-related macular degeneration), cognitive impairment, dementia, cancers (including but not limited to prostate, lung, breast, ovarian, and kidney cancer), osteoporosis, bipolar disorder and vascular diseases (including but not limited to atherosclerosis and hypercholesterolemia). Furthermore, these compounds are useful in the treatment of disorders resulting from abnormal genetic expression of cholesterol transport proteins, such as apolipoprotein E.

These plasmalogen precursors contain a glycerol backbone with an alkyl or alkenyl lipid substitution at sn-1 and an acyl lipid substitution at sn-2. A polar substituent is provided at sn-3 to improve pharmaceutical properties (such as to improve stability and/or bioavailability, or for formulation as a salt).

Without wishing to be bound by theory, it is believed that in certain embodiments the sn-3 substituent is cleaved by lipases and the resulting 1-alkyl, 2-acyl glycerol or 1-alkenyl, 2-acyl glycerol is then converted to plasmalogens in the endoplasmic reticulum, thereby bypassing the peroxisomal compartment which can demonstrate decreased function with ageing.

In one aspect, there is provided a compound according to the structure of formula I:

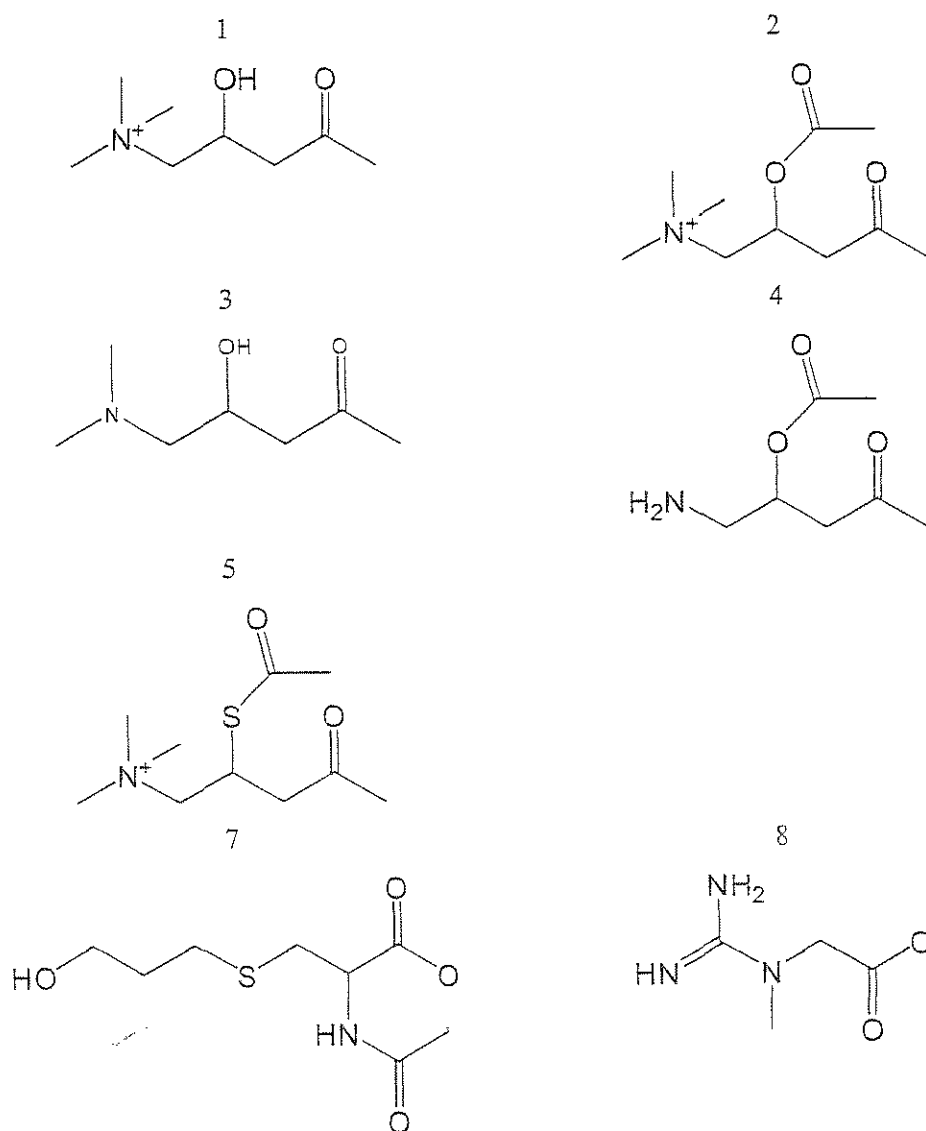


wherein:

R_1 and R_2 are the same or different and selected from an alkyl or alkenyl hydrocarbon chain selected from the group consisting of: $\text{CH}_3(\text{CH}_2)_3-$, $\text{CH}_3(\text{CH}_2)_5-$, $\text{CH}_3(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_9-$, $\text{CH}_3(\text{CH}_2)_{11}-$, $\text{CH}_3(\text{CH}_2)_{13}-$, $\text{CH}_3(\text{CH}_2)_{15}-$, $\text{CH}_3(\text{CH}_2)_{17}-$, $\text{CH}_3(\text{CH}_2)_{19}-$, $\text{CH}_3(\text{CH}_2)_{21}-$, $\text{CH}_3(\text{CH}_2)_{23}-$, $\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3\text{CH}_2(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_3(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_7(\text{CH}=\text{CH})-$,

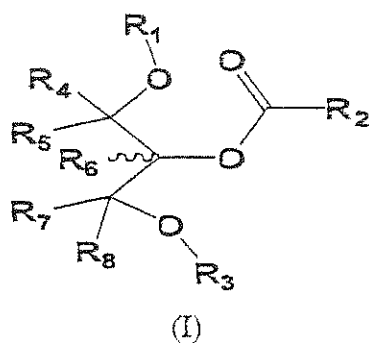
$\text{CH}_3(\text{CH}_2)_9(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{11}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{13}(\text{CH}=\text{CH})-$,
 $\text{CH}_3(\text{CH}_2)_{15}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{17}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{19}(\text{CH}=\text{CH})-$,
 $\text{CH}_3(\text{CH}_2)_{21}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$,
 $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$,
 $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})$,
 $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_7-$,
 $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_2(\text{CH}_2)_6-$,
 $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_3(\text{CH}_2)_6-$, $\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_4(\text{CH}_2)_2-$,
 $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_5(\text{CH}_2)_2-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_{11}$, and $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_6\text{CH}_2-$;

R_3 is selected from the group consisting of carnitine, acetyl-D/L-carnitine, thiocarnitine, acetyl-D/L-thiocarnitine, creatine, norcarnitine, lipoic acid, dihydrolipoic acid, N-acetylcysteine, substituted or unsubstituted amino acid groups and groups of the structures shown below:



R_4 and R_5 are independently hydrogen or lower alkyl;
 R_6 is hydrogen or lower alkyl; and
 R_7 and R_8 are independently hydrogen or lower alkyl,
 including racemates or isolated stereoisomers and pharmaceutically acceptable salts
 or esters thereof.

Accordingly, with respect to composition, there are provided compounds of formula I:



wherein:

R_1 and R_2 can be the same or different; and are an alkyl or alkenyl hydrocarbon chain
 selected from Table 1 or 2;

Table 1: Alkyl and alkenyl hydrocarbon groups

<u>No.</u>	<u>Chemical Structure</u>	<u>Abbr.</u>
1	CH ₃ (CH ₂) ₃ -	C4:0
2	CH ₃ (CH ₂) ₅ -	C6:0
3	CH ₃ (CH ₂) ₇ -	C8:0

4	CH ₃ (CH ₂) ₉ -	C10:0
5	CH ₃ (CH ₂) ₁₁ -	C12:0
6	CH ₃ (CH ₂) ₁₃ -	C14:0
7	CH ₃ (CH ₂) ₁₅ -	C16:0
8	CH ₃ (CH ₂) ₁₇ -	C18:0
9	CH ₃ (CH ₂) ₁₉ -	C20:0
10	CH ₃ (CH ₂) ₂₁ -	C22:0
11	CH ₃ (CH ₂) ₂₃ -	C24:0
12	CH ₃ (CH ₂) ₃ CH=CH(CH ₂) ₇ -	C14:1
13	CH ₃ (CH ₂) ₅ CH=CH(CH ₂) ₇ -	C16:1
14	CH ₃ (CH ₂) ₇ CH=CH(CH ₂) ₇ -	C18:1
15	CH ₃ (CH ₂) ₄ CH=CHCH ₂ CH=CH(CH ₂) ₇ -	C18:2
16	CH ₃ CH ₂ CH=CHCH ₂ CH=CHCH ₂ CH=CH(CH ₂) ₇ -	C18:3
17	CH ₃ CH ₂ (CH=CH)-	C4:1
18	CH ₃ (CH ₂) ₃ (CH=CH)-	C6:1
19	CH ₃ (CH ₂) ₅ (CH=CH)-	C8:1
20	CH ₃ (CH ₂) ₇ (CH=CH)-	C10:1
21	CH ₃ (CH ₂) ₉ (CH=CH)-	C12:1
22	CH ₃ (CH ₂) ₁₁ (CH=CH)-	C14:1
23	CH ₃ (CH ₂) ₁₃ (CH=CH)-	C16:1
24	CH ₃ (CH ₂) ₁₅ (CH=CH)-	C18:1
25	CH ₃ (CH ₂) ₁₇ (CH=CH)-	C20:1
26	CH ₃ (CH ₂) ₁₉ (CH=CH)-	C22:1
27	CH ₃ (CH ₂) ₂₁ (CH=CH)-	C24:1
28	CH ₃ (CH ₂) ₃ CH=CH(CH ₂) ₅ (CH=CH)-	C14:2
29	CH ₃ (CH ₂) ₅ CH=CH(CH ₂) ₅ (CH=CH)-	C16:2
30	CH ₃ (CH ₂) ₇ CH=CH(CH ₂) ₅ (CH=CH)-	C18:2
31	CH ₃ (CH ₂) ₄ CH=CHCH ₂ CH=CH(CH ₂) ₅ (CH=CH)-	C18:3
32	CH ₃ CH ₂ CH=CHCH ₂ CH=CHCH ₂ CH=CH(CH ₂) ₅ (CH=CH)-	C18:4

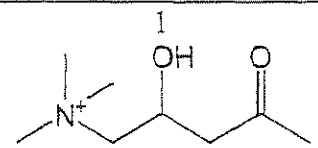
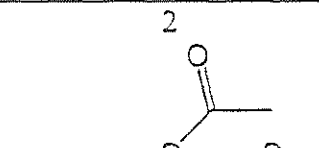
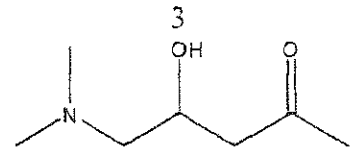
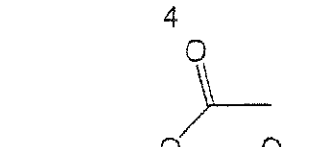
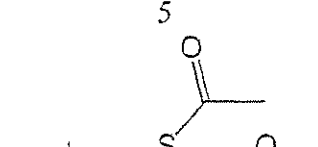
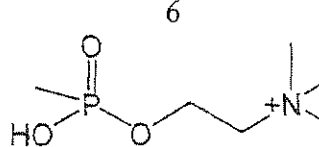
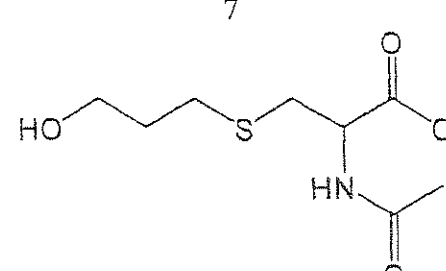
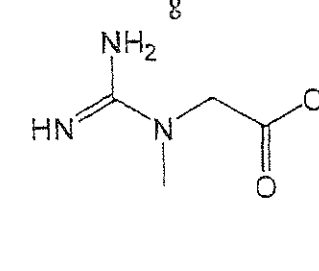
Table 2: Unsaturated cis fatty acid side chains

No.	Name of acid	Chemical Structure
1	Myristoleic (14:1)	CH ₃ (CH ₂) ₃ CH=CH(CH ₂) ₇ -
2	Palmitoleic (16:1)	CH ₃ (CH ₂) ₅ CH=CH(CH ₂) ₇ -
3	Oleic (18:1)	CH ₃ (CH ₂) ₇ CH=CH(CH ₂) ₇ -
4	Linoleic (18:2)	CH ₃ (CH ₂) ₄ (CH=CHCH ₂) ₂ (CH ₂) ₆ -
5	Linolenic (18:3)	CH ₃ CH ₂ (CH=CHCH ₂) ₃ (CH ₂) ₆ -
6	Arachidonic (20:4)	CH ₃ (CH ₂) ₄ (CH=CHCH ₂) ₄ (CH ₂) ₂ -
7	Eicosapentaenoic (20:5)	CH ₃ CH ₂ (CH=CHCH ₂) ₅ (CH ₂) ₂ -
8	Erucic (22:1)	CH ₃ (CH ₂) ₇ CH=CH(CH ₂) ₁₁ -
9	Docosahexaenoic (22:6)	CH ₃ CH ₂ (CH=CHCH ₂) ₆ CH ₂ -

5 R₃ is a group selected from fatty acids, carnitine, acetyl-D/L-carnitine, thiocarnitine, acetyl-D/L-thiocarnitine, creatine, norcarnitine, phosphocholine, lipoic acid, dihydrolipoic acid, phosphoethanolamine, phosphoserine, N-acetylcysteine, substituted or unsubstituted

amino acids and groups of the structures shown below in Table 3.

Table 3

1 	2 
3 	4 
5 	6 
7 	8 

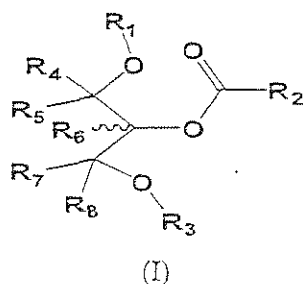
5 R_4 and R_5 are either the same or different and may be hydrogen or lower alkyl, for example methyl or ethyl;

R_6 is hydrogen or lower alkyl, for example methyl or ethyl; and

R_7 and R_8 are either the same or different and may be hydrogen or lower alkyl, for example methyl and ethyl,

10 and also including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof.

In yet another aspect, there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of formula I:

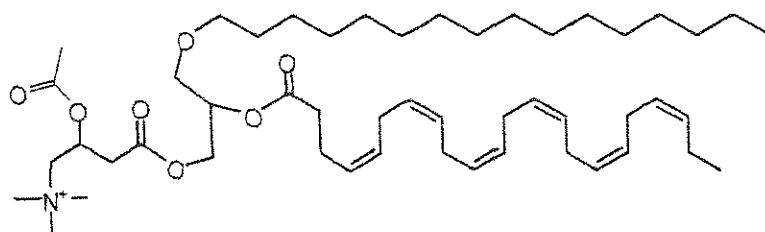


wherein R_1 to R_8 are as defined above, and including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof.

Another example is directed to pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a compound as described above.

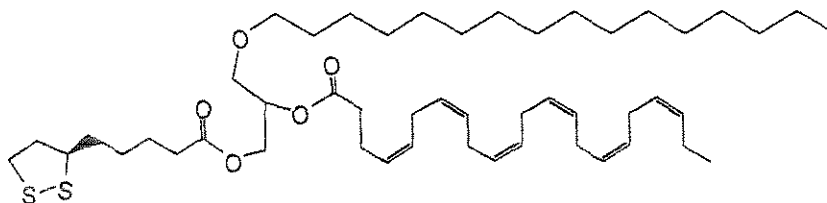
In one example of the present disclosure R_2 may be a docosahexaenoic acid (DHA) side chain, or $CH_3CH_2(CH=CHCH_2)_6CH_2-$.

In another example, the compound can be 2-acetoxy-4-(2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyloxy)-3-(hexadecyloxy)propoxy)-N,N,N-trimethyl-4-oxobutan-1-aminium.



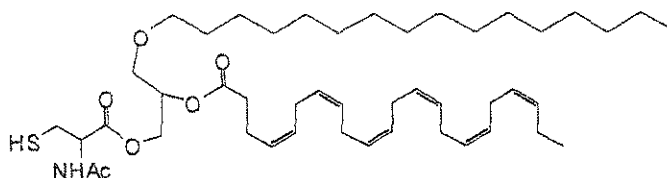
PPI-1009

In other examples the compound may be (4Z,7Z,10Z,13Z,16Z,19Z)-1-(5-((R)-1,2-dithiolan-3-yl)pentanoyloxy)-3-(hexadecyloxy)propan-2-yl docosa-4,7,10,13,16,19-hexaenoate:



PPI-1011

or (4Z,7Z,10Z,13Z,16Z,19Z)-1-(2-acetamido-3-mercaptopropanoyloxy)-3-(hexadecyloxy)propan-2-yl docosa-4,7,10,13,16,19-hexaenoate:



PPI-1014.

The present disclosure also includes pharmaceutical compositions comprising PPI-1009, PPI-1011, PPI-1014, or combinations thereof.

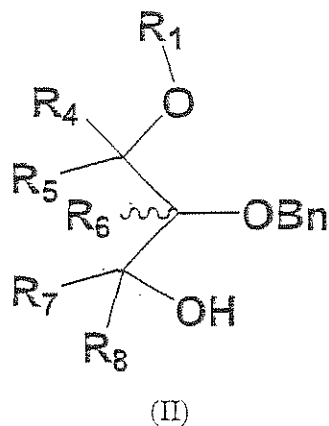
Without wishing to be bound by theory, certain examples of the compounds described herein are believed to increase plasmalogen levels and the hydrolysis of acetyl-L-carnitine from sn-3 position, and may participate in potential molecular mechanisms that include: (i) acetylation of $-NH_2$ and $-OH$ functional groups in amino acids and N-terminal amino acids in peptides and proteins resulting in modification of their structure, dynamic, function and turnover; and/or (ii) acting as a molecular chaperone to larger molecules resulting in a change in the structure, molecular dynamics, and function of the larger molecule.

Carnitine is important in the beta oxidation of fatty acids and the acetyl moiety can be used to maintain acetyl-CoA levels. Acetyl-L-carnitine (ALCAR) actions include modulation of: (i) brain energy and phospholipid metabolism; (ii) cellular macromolecules, including neurotrophic factors and hormones; (iii) synaptic morphology; and (iv) synaptic transmission of multiple neurotransmitters.

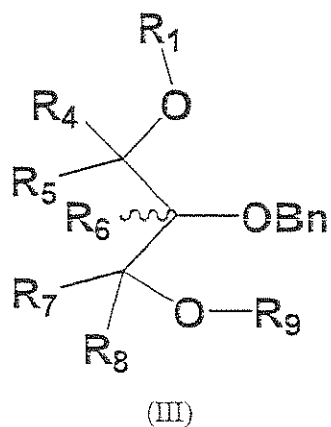
In one example there is provided a method of treating or preventing diseases of aging mediated by plasmalogen deficiency, comprising administering to a patient in need thereof an effective amount of a compound or composition as described above.

In another example there is provided a method of treating diseases of aging associated with increased membrane cholesterol, increased amyloid and decreased plasmalogen levels comprising administering to a patient in need thereof, an effective amount of a compound or composition as described above.

In yet another aspect, there is provided a process for preparing a compound according to the structure of formula II:

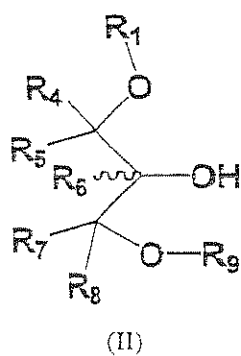


wherein R_1 and R_4 to R_8 are as defined above, and including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof, said process comprising reacting a compound of formula III:



wherein R_9 is a blocking group, in a suitable solvent with tetrabutylammonium fluoride (TBAF) under conditions to form said compound of formula II, and optionally purifying said compound of formula II.

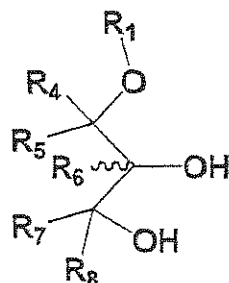
The present disclosure also relates to a process for preparing a compound according to the structure of formula II:



wherein R_1 and R_4 to R_8 are as described above and R_9 is a blocking group, and including

racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof.

The process involves reacting a compound of formula III:



(III)

in a suitable solvent with a nucleophilic catalyst, a base and a blocking agent under conditions to form said compound of formula II, and optionally purifying the resulting compound.

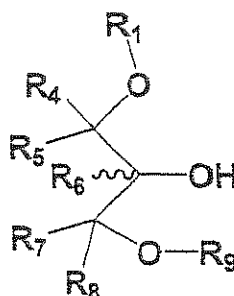
In certain non-limiting examples the blocking agent may be silane blocking agent, for instance a tert-butyldimethylsilyl halide which then gives rise to R₉ being a tert-

butyldimethylsilyl group.

In other non-limiting examples the nucleophilic catalyst may be DMAP, the base may be triethylamine, and the solvent may comprise dimethylformamide (DMF) and CH₂Cl₂.

Without wishing to be limiting, it may in certain examples be preferred to prepare a solution including the compound of formula III, the nucleophilic catalyst, and the base in the appropriate solvent at from 0 to 5 °C prior to addition of the blocking agent, followed by addition of the blocking agent and then carrying out the reaction at a temperature of from about 15 to about 25 °C, for example at about 20 °C. The reaction will then preferably be allowed to proceed to completion, which may be up to 20 hours.

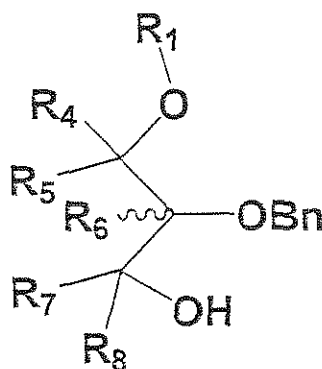
Also provided is an intermediate compound according to the structure of formula II:



(II)

wherein R_1 and R_4 to R_9 are as described above, including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof.

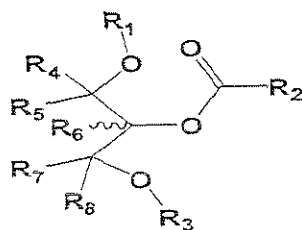
In yet another aspect, there is provided a compound according to the structure of formula II:



(II)

wherein R_1 and R_4 to R_8 are as defined above, including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof.

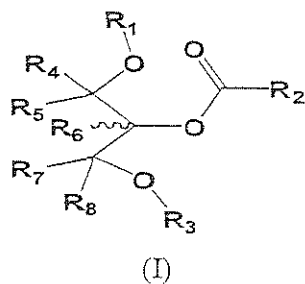
In yet another aspect, there is provided the use of a compound of formula I:



(I)

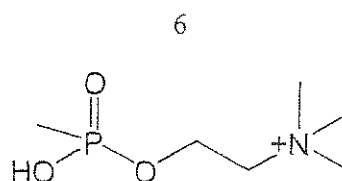
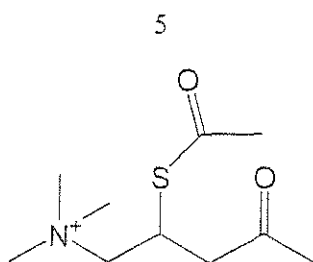
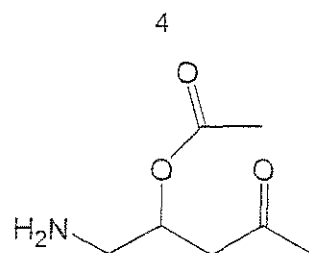
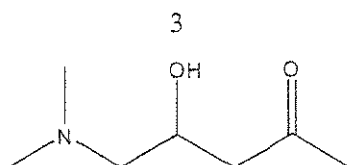
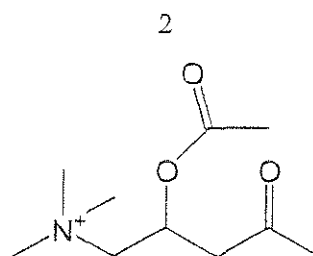
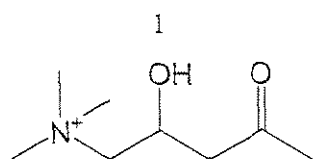
wherein R_1 to R_8 are as defined above, including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof, or a pharmaceutical composition comprising said compound admixed with a pharmaceutically acceptable carrier, for the manufacture of a medicament for treating or preventing diseases of aging associated with increased membrane cholesterol, increased amyloid or decreased plasmalogen levels, wherein the diseases of aging are selected from the group consisting of: neurodegeneration, cognitive impairment, dementia, cancer, and vascular disease.

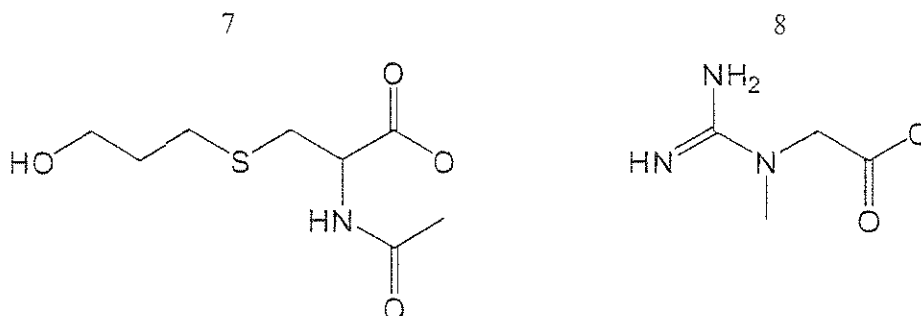
In yet another aspect, there is provided the use of a compound of formula I:



wherein

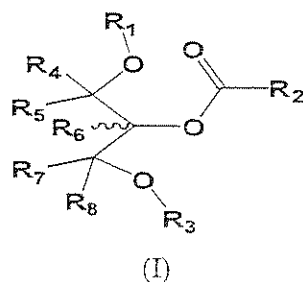
R₃ is selected from the group consisting of fatty acids having a chain of 4 to 28 carbons, carnitine, acetyl-D/L-carnitine, thiocarnitine, acetyl-D/L-thiocarnitine, creatine, norcarnitine, phosphocholine, lipoic acid, dihydrolipoic acid, phosphoethanolamine, phosphoserine, N-acetylcysteine, substituted or unsubstituted amino acid groups and groups of the structures shown below:





and R_1 , R_2 and R_4 to R_8 are as defined above, including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof, or a pharmaceutical composition comprising said compound admixed with a pharmaceutically acceptable carrier, for the manufacture of a medicament for treating or preventing diseases of the aging mediated by plasmalogen deficiency, wherein the diseases of aging are selected from the group consisting of: neurodegeneration, cognitive impairment, dementia, cancer, and vascular disease, by increasing PlsEtn levels to above control levels in either PlsEtn deficient or PlsEtn sufficient systems.

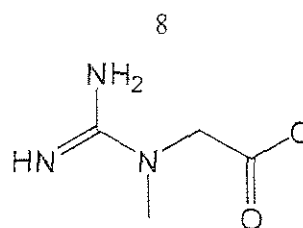
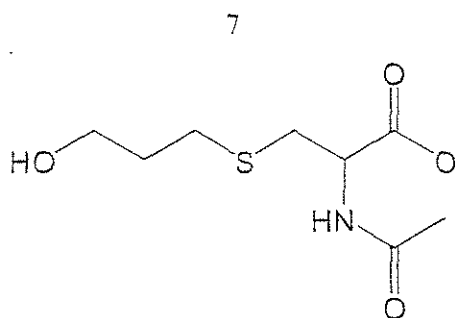
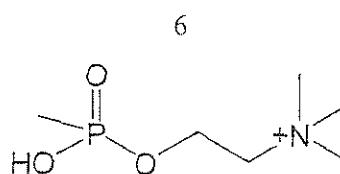
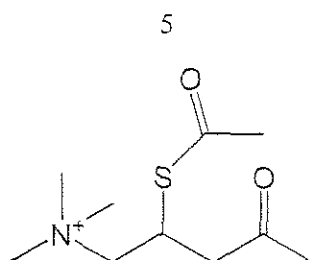
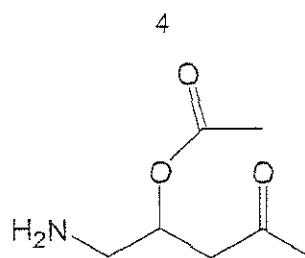
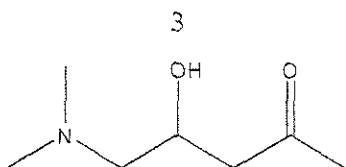
In yet another aspect, there is provided the use of a compound of formula I:



wherein

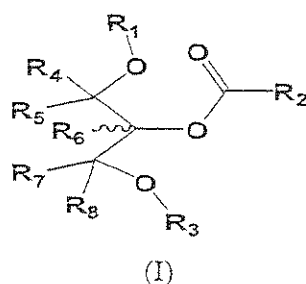
R_3 is selected from the group consisting of fatty acids having a chain of 4 to 28 carbons, carnitine, acetyl-D/L-carnitine, thiocarnitine, acetyl-D/L-thiocarnitine, creatine, norcarnitine, phosphocholine, lipoic acid, dihydrolipoic acid, phosphoethanolamine, phosphoserine, N-acetylcysteine, substituted or unsubstituted amino acid groups and groups of the structures shown below:





and R_1 , R_2 and R_4 to R_8 are as defined above, including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof, or a pharmaceutical composition comprising said compound admixed with a pharmaceutically acceptable carrier, for the manufacture of a medicament for treating or preventing a disorder resulting from abnormal genetic expression of cholesterol transport proteins, wherein the disorder is selected from the group consisting of: neurodegeneration, cognitive impairment, dementia, cancer, and vascular disease.

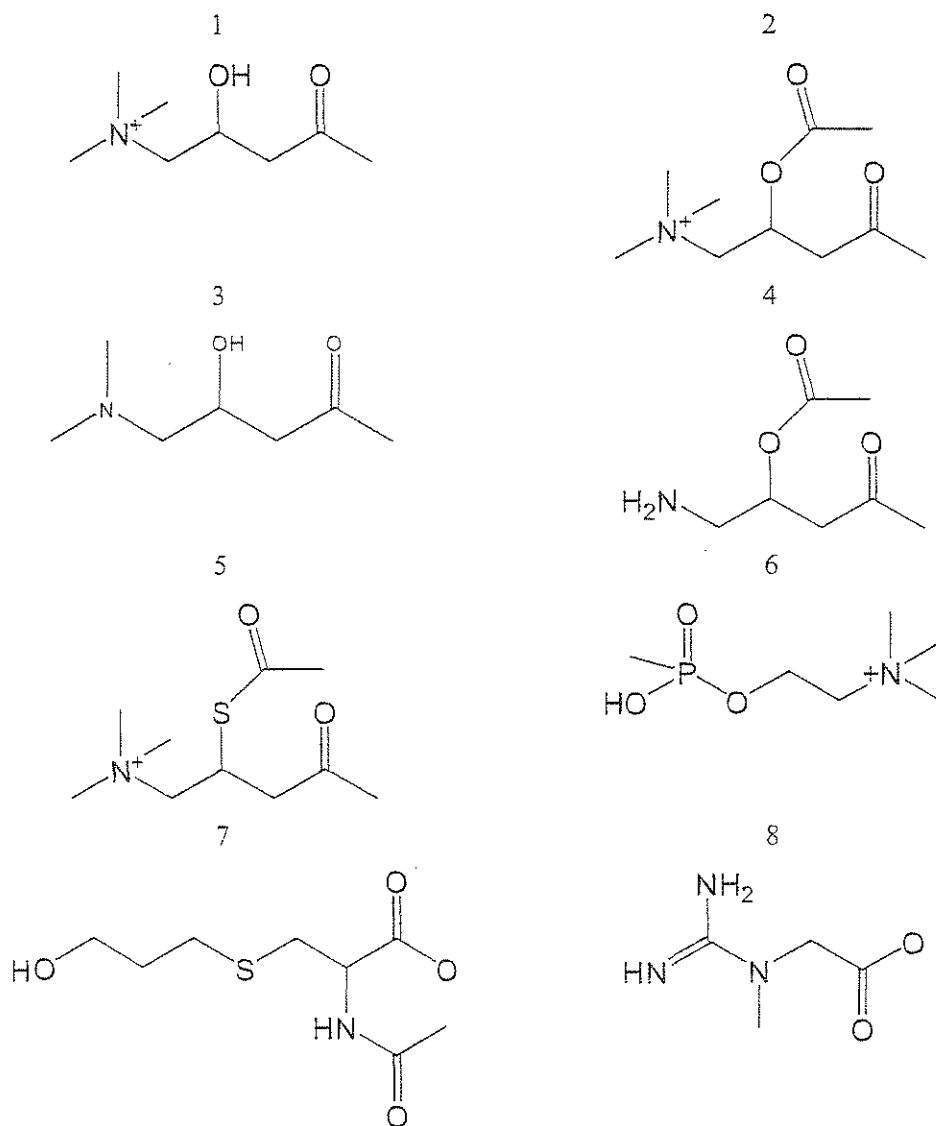
In yet another aspect, there is provided the use of a compound of formula I:



for the manufacture of a medicament for treating or preventing a neurodegeneration disease selected from the group consisting of Alzheimer's disease, Parkinson's disease and age-related macular degeneration, wherein

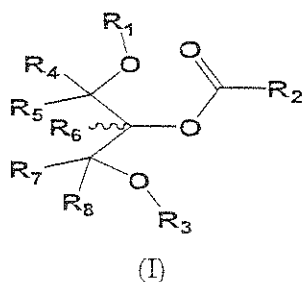
R_1 and R_2 are the same or different and selected from an alkyl or alkenyl hydrocarbon chain selected from the group consisting of: $\text{CH}_3(\text{CH}_2)_3-$, $\text{CH}_3(\text{CH}_2)_5-$, $\text{CH}_3(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_9-$, $\text{CH}_3(\text{CH}_2)_{11}-$, $\text{CH}_3(\text{CH}_2)_{13}-$, $\text{CH}_3(\text{CH}_2)_{15}-$, $\text{CH}_3(\text{CH}_2)_{17}-$, $\text{CH}_3(\text{CH}_2)_{19}-$, $\text{CH}_3(\text{CH}_2)_{21}-$, $\text{CH}_3(\text{CH}_2)_{23}-$, $\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3\text{CH}_2(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_3(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_7(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_9(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{11}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{13}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{15}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{17}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{19}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{21}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})$, $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_2(\text{CH}_2)_6-$, $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_3(\text{CH}_2)_6-$, $\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_4(\text{CH}_2)_2-$, $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_5(\text{CH}_2)_2-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_{11}$, and $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_6\text{CH}_2-$;

R_3 is selected from the group consisting of fatty acids having a chain of 4 to 28 carbons, carnitine, acetyl-D/L-carnitine, thiocarnitine, acetyl-D/L-thiocarnitine, creatine, norcarnitine, phosphocholine, lipoic acid, dihydrolipoic acid, phosphoethanolamine, phosphoserine, N-acetylcysteine, substituted or unsubstituted amino acid groups and groups of the structures shown below:



R_4 and R_5 are independently hydrogen or lower alkyl;
 R_6 is hydrogen or lower alkyl; and
 R_7 and R_8 are independently hydrogen or lower alkyl,
including racemates or isolated stereoisomers and pharmaceutically acceptable salts
or esters thereof.

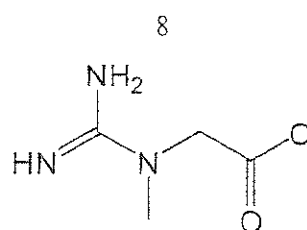
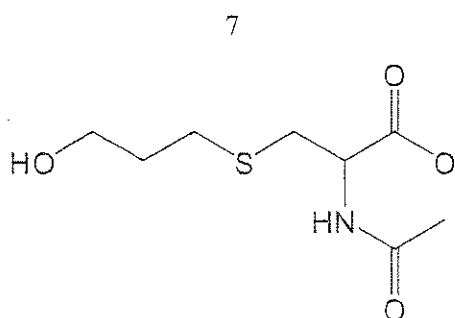
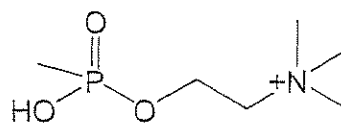
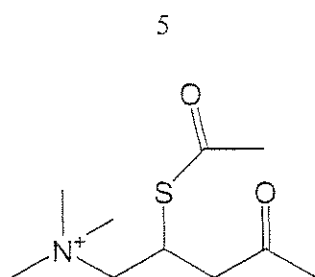
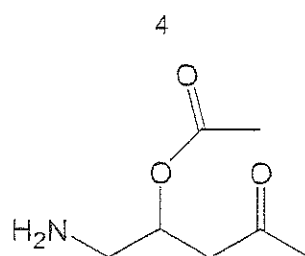
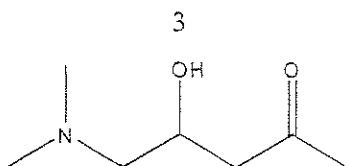
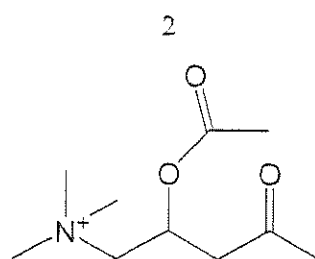
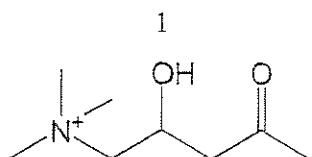
In yet another aspect, there is provided the use of a compound of formula I:



for the manufacture of a medicament for treating or preventing a vascular disease selected from the group consisting of atherosclerosis and hypercholesterolemia, wherein

R_1 and R_2 are the same or different and selected from an alkyl or alkenyl hydrocarbon chain selected from the group consisting of: $\text{CH}_3(\text{CH}_2)_3-$, $\text{CH}_3(\text{CH}_2)_5-$, $\text{CH}_3(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_9-$, $\text{CH}_3(\text{CH}_2)_{11}-$, $\text{CH}_3(\text{CH}_2)_{13}-$, $\text{CH}_3(\text{CH}_2)_{15}-$, $\text{CH}_3(\text{CH}_2)_{17}-$, $\text{CH}_3(\text{CH}_2)_{19}-$, $\text{CH}_3(\text{CH}_2)_{21}-$, $\text{CH}_3(\text{CH}_2)_{23}-$, $\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3\text{CH}_2(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_3(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_7(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_9(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{11}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{13}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{15}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{17}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{19}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{21}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})$, $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_2(\text{CH}_2)_6-$, $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_3(\text{CH}_2)_6-$, $\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_4(\text{CH}_2)_2-$, $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_5(\text{CH}_2)_2-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_{11}$, and $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_6\text{CH}_2-$;

R_3 is selected from the group consisting of fatty acids having a chain of 4 to 28 carbons, carnitine, acetyl-D/L-carnitine, thiocarnitine, acetyl-D/L-thiocarnitine, creatine, norcarnitine, phosphocholine, lipoic acid, dihydrolipoic acid, phosphoethanolamine, phosphoserine, N-acetylcysteine, substituted or unsubstituted amino acid groups and groups of the structures shown below:



R_4 and R_5 are independently hydrogen or lower alkyl;

R_6 is hydrogen or lower alkyl; and

R_7 and R_8 are independently hydrogen or lower alkyl,

including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

FIGURE 1 shows the general experimental procedure for preparation of PPI-1009: 2-acetoxy-4-(2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4, 7,10, 13, 16, 19-hexaenoyloxy)-3-(hexadecyloxy)propoxy)-N,N,N-trimethyl-4-oxobutan-1-aminium, in accordance with an embodiment of the present invention.

FIGURE 2 shows decreased levels of total DHA plasmalogens in N-Rel cells, relative to control CHO cells, are restored in a concentration-dependent manner by PPI-1005 ($R_1 = 16:0$; $R_2 = \text{DHA}$; $R_3 = \text{OH}$). 72 hr incubation. * $p < 0.05$ vs. vehicle (veh).

FIGURE 3 shows (A) a time course of PPI-1009 (10 μM) incorporation into plasmalogens and lack of effect on cellular levels of chimyl alcohol or the associated alkenyl-acyl-glycerol (B) of N-Rel cells (0, 6, 12, 24, 48 and 72 hr). Cellular plasmalogens and docosahexaenoic (DHA) acid were quantitated by LC-MS/MS while chimyl alcohol was quantitated by GC-MS.

FIGURE 4 shows (A) the concentration-dependent (72 hr) increase of DHA plasmalogens in CHO cells with PPI-1005 and PPI-1009. While chimyl alcohol increased DHA-plasmalogens in N-Rel cells there was no effect in CHO cells (B). Cellular plasmalogens were quantitated by LC-MS and expressed relative to CHO controls or N-Rel controls.

FIGURE 5 shows the concentration response for the decrease in membrane cholesterol in N-Rel cells after a 48 hr incubation with 16:0(sn-1 alkyl)/DHA(sn-2 acyl) glycerol (PLM-05). Note that cholesterol levels are significantly elevated ($p < 0.05$) in NRel cells relative to CHO cells and that at 20 μM (PLM-05) decreases membrane free cholesterol and increases membrane cholesterol esters ($p < 0.05$).

FIGURE 6 shows structure-specific and concentration-dependent incorporation (72 hr) into the associated plasmalogen ($R_1=16:0$, $R_2=22:6$, R_3 =phosphoethanolamine) of N-Rel cells with PPI-1005 (A; 1 and 5 μM) and PPI-1009 (B; 0.5, 1, 2, 3 & 10 μM). The fatty acid substitutions at sn-2 also underwent deacylation and reacylation to form the associated plasmalogens with 20:4, 18:3, 18:2 and 18:1 at sn-2. Cellular plasmalogens were quantitated by LC-MS/MS and normalized relative to N-Rel cells treated with vehicle.

FIGURE 7 shows that membrane cholesterol is increased in mutant N-Rel cells relative to control CHO cells. Membrane cholesterol in N-Rel cells is decreased ($p<0.05$) after a 48 hr incubation (20 μM) with plasmalogen precursors possessing either palmitic (16:0) or stearic (18:0) acid at sn-1 in combination with unsaturated fatty acids, particularly DHA, at sn-2. At 20 μM , free DHA was ineffective in altering membrane free cholesterol levels. In contrast to the activity of analogs with an alkyl linkage at sn-1, the diacyl analog (16:0*:DHA glycerol) was inactive. PPI-1009 (16:0/DHA/ALCAR) produced the most robust decreases in free cholesterol and augmentation of esterified cholesterol. V, vehicle.

FIGURE 8 shows the decrease ($p < 0.04$) in membrane cholesterol in HEK293 cells after a 48 hr incubation with 20 μM 16:0(sn-1 alkyl)/DHA(sn-2 acyl) glycerol, 18:0/DHA glycerol, or 16:0/18:3 glycerol. At 20 μM , 16:0/18:1 glycerol, 16:0/18:2 glycerol, 16:0/20:4 glycerol and free DHA were ineffective in altering membrane free cholesterol levels. In contrast to the activity of analogs with an alkyl linkage at sn-1, the diacyl analog (16:0*:DHA glycerol) was inactive.

FIGURE 9 shows the concentration response for the decrease in membrane cholesterol in HEK293 cells after a 48 hr incubation with 16:0(sn-1 alkyl)/DHA(sn-2 acyl)/acetyl-L-carnitine (sn-3 acyl) glycerol (PPI-1009).

FIGURE 10 shows PPI-1005 (5a, 20 μM) decreases basal and cholesterol (25.8 μM) – stimulated A β 42 secretion by HEK293 cells (A). PPI-1009 (PLM09, 10 μM) acted in a similar manner (B).

FIGURE 11 illustrates the effect of PPI-1011 on plasmalogens in rabbit plasma. PPI-1011 was incorporated into plasma ethanolamine plasmalogens (PlsEtn) and phosphatidylethanolamines (PtdEtn) 1, 3, 6, and 12 hours after a 200 mg/kg dose orally in a gelatin capsule. The release of DHA (free 22:6) from sn-2, via deacylases, was also monitored. Groups consisted of 3 to 5 rabbits.

FIGURE 12 shows a plot of the timecourse of incorporation of plasmalogen precursor PPI-1011 into circulating Pls 16:0/22:6, DHA, Pls 18:0/22:6, Pls 18:1/22:6 and Ptd 16:0/22:6. Incorporation of PPI-1011 in plasma ethanolamine plasmalogens (Pls) and phosphatidylethanolamines (Ptd) was measured 1, 3, 6, 12, 18, 24 and 48 hours after a 200 mg/kg dose orally in a gelatin capsule. The release of DHA from sn-2, via deacylases, was also monitored. Groups consisted of 3 to 5 rabbits except the 12 hour timepoint which includes 7 rabbits from 2 separate experiments.

FIGURE 13 illustrates the dose-dependent incorporation of PPI-1011 into plasma plasmalogens and phosphatidylethanolamines. Incorporation of PPI-1011 in plasma ethanolamine plasmalogens (Pls) and phosphatidylethanolamines (Ptd) was measured 6 hours after doses of 10, 75, 200, 500, and 1000 mg/kg orally in a gelatin capsule. The release of DHA from sn-2, via deacylases, was also monitored. Groups consisted of 3 to 5 rabbits.

FIGURE 14 shows augmentation of tissue plasmalogens and DHA by PPI-1011 in rabbit kidney. Incorporation of PPI-1011 in kidney ethanolamine plasmalogens (PlsEtn) and phosphatidylethanolamines (PtdEtn) was measured 1, 3, 6, and 12 hours after a 200 mg/kg dose orally in a gelatin capsule. The release of DHA (free 22:6) from sn-2, via deacylases, was also monitored. Groups consisted of 3 to 5 rabbits.

FIGURE 15 shows a timecourse of the tissue plasmalogens and DHA augmentation by PPI-1011 in rabbit kidney. Incorporation of PPI-1011 in kidney ethanolamine plasmalogen (16:0/22:6) was measured 1, 3, 6, 12, 18, 24 and 48 hours after a 200 mg/kg dose orally in a gelatin capsule. Groups consisted of 3 to 5 rabbits.

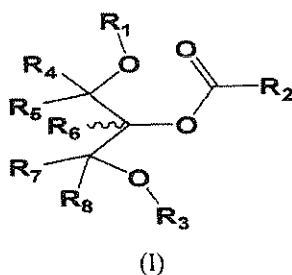
FIGURE 16 shows a timecourse of the tissue plasmalogens and DHA augmentation by PPI-1011 in rabbit liver. Incorporation of PPI-1011 in liver ethanolamine plasmalogen (16:0/22:6) was measured 12, 18, 24 and 48 hours after a 200 mg/kg dose orally in a gelatin capsule. Groups consisted of 3 to 5 rabbits.

FIGURE 17 shows structure-specific and concentration-dependent incorporation of PPI-1014 in N-Rel ethanolamine plasmalogens (PlsEtn) and phosphatidylethanolamines (PtdEtn) after 72 hours (5, 10 and 20 μ M). Cellular plasmalogens were quantitated by LC-MS/MS and normalized relative to N-Rel cells treated with vehicle. Groups consisted of three 10 cm² plates.

FIGURE 18 shows (A) effects of increasing PPI-1005 concentration on membrane resident proteins in cholesterol loaded HEK293 cells, (B) effects of PPI-1005 on membrane resident proteins in wild-type HEK293 cells, and (C) effects of pravastatin on membrane-resident proteins ADAM10 and SOAT1. β -actin was used as a loading control.

10 DETAILED DESCRIPTION

Described herein are compounds according to structure of formula I:

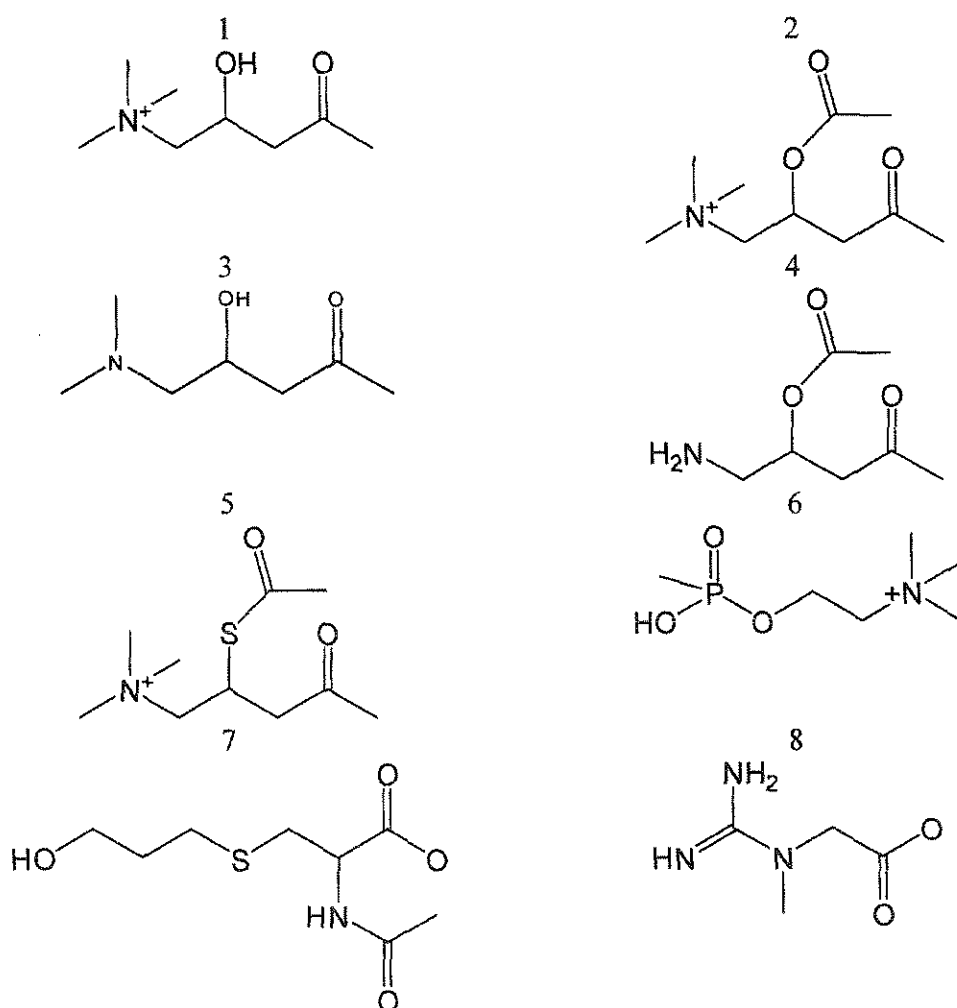


wherein:

R_1 and R_2 are the same or different and selected from an alkyl or alkenyl hydrocarbon chain selected from the group consisting of: CH₃(CH₂)₃-, CH₃(CH₂)₅-, CH₃(CH₂)₇-, CH₃(CH₂)₉-, CH₃(CH₂)₁₁-, CH₃(CH₂)₁₃-, CH₃(CH₂)₁₅-, CH₃(CH₂)₁₇-, CH₃(CH₂)₁₉-, CH₃(CH₂)₂₁-, CH₃(CH₂)₂₃-, CH₃(CH₂)₃CH=CH(CH₂)₇-, CH₃(CH₂)₅CH=CH(CH₂)₇-, CH₃(CH₂)₇CH=CH(CH₂)₇-, CH₃(CH₂)₄CH=CHCH₂CH=CH(CH₂)₇-, CH₃CH₂CH=CHCH₂CH=CHCH₂CH=CH(CH₂)₇-, CH₃CH₂(CH=CH)-, CH₃(CH₂)₃(CH=CH)-, CH₃(CH₂)₅(CH=CH)-, CH₃(CH₂)₇(CH=CH)-, CH₃(CH₂)₉(CH=CH)-, CH₃(CH₂)₁₁(CH=CH)-, CH₃(CH₂)₁₃(CH=CH)-, CH₃(CH₂)₁₅(CH=CH)-, CH₃(CH₂)₁₇(CH=CH)-, CH₃(CH₂)₁₉(CH=CH)-, CH₃(CH₂)₂₁(CH=CH)-, CH₃(CH₂)₃CH=CH(CH₂)₅(CH=CH)-, CH₃(CH₂)₅CH=CH(CH₂)₅(CH=CH)-, CH₃(CH₂)₇CH=CH(CH₂)₅(CH=CH)-, CH₃(CH₂)₄CH=CHCH₂CH=CH(CH₂)₅(CH=CH), CH₃CH₂CH=CHCH₂CH=CHCH₂CH=CH(CH₂)₅(CH=CH)-, CH₃(CH₂)₃CH=CH(CH₂)₇-,

$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_2(\text{CH}_2)_6-$,
 $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_3(\text{CH}_2)_6-$, $\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_4(\text{CH}_2)_2-$,
 $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_5(\text{CH}_2)_2-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_{11}$, and $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_6\text{CH}_2-$
 ;

- 5 R_3 is a group selected from fatty acids, carnitine, acetyl-D/L-carnitine, thiocarnitine, acetyl-D/L-thiocarnitine, creatine, norcarnitine, phosphocholine, lipoic acid, dihydrolipoic acid, phosphoethanolamine, phosphoserine, N-acetylcysteine, substituted or unsubstituted amino acids and groups of the structures shown below:



10

R_4 and R_5 are independently hydrogen or lower alkyl;

R_6 is hydrogen or lower alkyl; and

R_7 and R_8 are independently hydrogen or lower alkyl, and

including racemates or isolated stereoisomers and pharmaceutically acceptable salts

or esters thereof.

Such compounds are useful for treating or preventing diseases of aging associated with increased membrane cholesterol, increased amyloid or decreased plasmalogen levels.

Such compounds are also useful for treating or preventing diseases of aging mediated by
5 plasmalogen deficiency.

Such compounds can also be used to treat neurodegenerative diseases (including but not limited to Alzheimer's disease, Parkinson's disease and age-related macular degeneration), cognitive impairment, dementia, cancer (including but not limited to prostate, lung, breast, ovarian, and kidney cancers), osteoporosis, bipolar disorder and vascular diseases (including
10 but not limited to atherosclerosis and hypercholesterolemia).

For the purposes of this invention, the hydroxy groups at the *sn*-1, *sn*-2, and *sn*-3 positions of the glycerol back-bone of the compounds of formula I are named using conventional plasmalogen nomenclature, i.e., the oxygen atom of the glycerol bonded to the carbonyl group -C=O(acetyl), -C (form ether bond) and -P (Phosphoryl) is designated in the formula I.

15 In certain non-limiting embodiments, compounds as described herein may contain one or more chiral centers. Typically, such compounds will be prepared as a racemic mixture. If desired, however, such compounds can be prepared or isolated as pure stereoisomers, i.e., as individual enantiomers or diastereomers, or as stereoisomer-enriched mixtures. All such stereoisomers (and enriched mixtures) of the compounds of formula I are included within the
20 scope of this invention. Pure stereoisomers (or enriched mixtures) may be prepared using, for example, optically active starting materials or stereoselective reagents well known in the art. Alternatively, racemic mixtures of such compounds can be separated using, for example, chiral column chromatography, chiral resolving agents and the like.

Definitions:

25 When describing the alkyl/acyl fatty acids (Table 1, Table 2) and biologically active compounds (Table 3), pharmaceutical compositions and methods of this invention, the following terms have the following meanings unless otherwise specified.

"Fatty acids" are aliphatic monocarboxylic acids, derived from, or contained in esterified

form in an animal or vegetable fat, oil or wax. Natural fatty acids commonly have a chain of 4 to 28 carbons (usually unbranched and even numbered), which may be saturated or unsaturated. These are known as acyclic aliphatic carboxylic acids.

Within the meaning of saturated fatty acids, the term "saturated" refers to carbons (apart from the initial carboxylic [-COOH] group) containing as many hydrogens as possible. In other words, the omega (ω) end contains 3 hydrogen atoms (CH_3 -), and carbon within the chain contains 2 hydrogen atoms.

Unsaturated fatty acids (including but not limited to the examples described in Table 2) are of similar form to saturated fatty acids, except that one or more alkenyl functional groups exist along the chain, with each alkene substituting a single bonded $-\text{CH}_2-\text{CH}_2-$ part of the chain with a double-bonded $-\text{CH}=\text{CH}-$ portion (that is a carbon double-bonded to another carbon). These are named as CIS/TRANS and C:D where C is known as number of carbon atoms and D known as double bond.

"Unsubstituted and substituted amino acids" refers to an optionally substituted amino acid moiety containing an amino group, a carboxylic acid group and a variable side chain, which side chain may include common amino acid side chains, i.e., those used in forming proteins, or others as are known in the art. Protein forming amino acid moieties are particularly preferred, and include alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine amino acid groups. Substitutions on the amino acid moieties are also possible, including substitutions with functional groups including but not limited to lower alkyl, acetate, phosphate, lipids and carbohydrates.]

The term "lower alkyl" refers to a cyclic, branched or straight chain monovalent alkyl radical of one to seven carbon atoms (C_1-C_7), and in certain non-limiting embodiments from one to four carbon atoms (C_1-C_4). This term is further exemplified by such radicals as methyl, ethyl, n-propyl, i-propyl, n-butyl, t-butyl, i-butyl (or 2-methylpropyl), cyclopropylmethyl, i-amyl, n-amyl, hexyl and heptyl. Lower alkyl groups can also be unsubstituted or substituted, where a specific example of a substituted alkyl is 1,1-dimethyl heptyl.

"Hydroxyl" refers to $-\text{OH}$.

"Pharmaceutically-acceptable salt" refers to any salt of a compound of this invention which retains its biological properties and which is not biologically or otherwise undesirable. Such salts may be derived from a variety of organic and inorganic counter-ions well known in the art and include, by way of example, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like; and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, oxalate and the like. The term "pharmaceutically- acceptable cation" refers to a pharmaceutically acceptable cationic counter-ion of an acidic functional group. Such cations are exemplified by sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium cations, and the like.

"Pharmaceutically acceptable ester" refers to a conventionally esterified compound of Formula I having a carboxyl group, which esters retain the biological effectiveness and properties of the compounds of Formula I and are cleaved *in vivo* (in the organism) to the corresponding active carboxylic acid. Information concerning esters and the use of esters for the delivery of pharmaceutical compounds is available in Design of Prodrugs. Bundgaard H ed. (Elsevier 1985). See also, H. Ansel et. al., Pharmaceutical Dosage Forms and Drug Delivery Systems (6th Ed. 1995) at pp. 108-109; Krogsgaard-Larsen, et. al. Textbook of Drug Design and Development (2d Ed. 1996) at pp. 152-191.

A "pharmaceutical agent" or "drug" refers to a chemical compound or composition capable of inducing a desired therapeutic or prophylactic effect when properly administered to a subject.

The term "effective amount" means that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system, animal, or human that is being sought, for instance, by a researcher or clinician. Furthermore, the term "therapeutically effective amount" means any amount which, as compared to a corresponding subject who has not received such amount, results in improved treatment, healing, prevention, or amelioration of a disease, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder. The term also includes within its scope amounts effective to enhance normal physiological function.

All chemical compounds include both the (+) and (-) stereoisomers, as well as either the (+) or (-) stereoisomer.

Other chemistry terms herein are used according to conventional usage in the art, as exemplified

by The McGraw-Hill Dictionary of Chemical Terms (1985) and The Condensed Chemical Dictionary (1981).

The compounds described herein, which include plasmalogen precursors derived from a glycerol back-bone with substitution at sn-1 and sn-2 with fatty acids, and sn-3 with fatty acids or endogenous metabolic intermediate compounds, can be prepared from readily available starting materials using the following general methods and procedures shown in Figure 1. It will be appreciated that where typical or preferred process conditions (i.e., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are given, other process conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

Additionally, as will be apparent to those skilled in the art, conventional protecting groups may be necessary to prevent certain functional groups from undergoing undesired reactions. The choice of a suitable protecting group for a particular functional group as well as suitable conditions for protection and de-protection are well known in the art. For example, numerous protecting groups, and their introduction and removal, are described in T. W. Greene and G. M. Wuts, *Protecting Groups in Organic Synthesis*, Second Edition, Wiley, New York, 1991, and references cited therein.

In a preferred method of synthesis of the compounds described herein, which include glycerol substitutions at sn-1 and sn-2 with fatty acids and sn-3 with fatty acids or endogenous metabolic intermediate compounds as described herein, are prepared by protection/deprotection of hydroxyl groups of the glycerol back-bone with suitable protecting groups, followed by O-alkylation and O-acylation of the compound; for example PPI-1009, PPI-1011 and PPI-1014.

When employed as pharmaceuticals, compounds as described herein are typically administered in the form of a pharmaceutical composition. Such compositions can be prepared using procedures well known in the pharmaceutical art and comprise at least one active compound.

Generally the compounds of this invention are administered in a pharmaceutically effective amount. The amount of the compound actually administered will typically be determined by

a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

The compounds and compositions described herein can be administered to a subject, preferably a mammal, more preferably a human, to treat and/or prevent disease by any suitable routes including, by way of illustration, oral, topical, rectal, transdermal, subcutaneous, intravenous, intramuscular, intranasal, and the like. Depending on the intended route of delivery, the compounds of this invention are preferably formulated as either oral, topical or injectable compositions.

Pharmaceutical compositions for oral administration can take the form of bulk liquid solutions or suspensions, or bulk powders. More commonly, however, such compositions are presented in unit dosage forms to facilitate accurate dosing. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Typical unit dosage forms include prefilled, premeasured ampoules or syringes of the liquid compositions or pills, tablets, capsules or the like in the case of solid compositions.

Liquid forms suitable for oral administration may include a suitable aqueous or non aqueous vehicle with buffers, suspending and dispensing agents, colorants, flavors and the like. Solid forms may include, for example, any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

Topical compositions are typically formulated as a topical ointment or cream containing the active ingredient(s), generally in an amount ranging from about 0.01 to about 20% by weight, preferably from about 0.1 to about 10% by weight, and more preferably from about 0.5 to about 15% by weight. When formulated as an ointment, the active ingredients will typically be combined with either a paraffinic or a water-miscible ointment base. Alternatively, the

active ingredients may be formulated in a cream with, for example, an oil-in-water cream base. Such topical formulations are well-known in the art and generally include additional ingredients to enhance the dermal penetration or stability of the active ingredients or the formulation. All such known topical formulations and ingredients are included within the scope of this invention.

The compounds of this invention can also be administered by a transdermal device. Accordingly, topical administration can be accomplished using a patch either of the reservoir or porous membrane type or of a solid matrix variety.

Injectable compositions are typically based upon injectable sterile saline or phosphate-buffered saline or other injectable carriers known in the art. As before, the alkyl nitron compound in such compositions is typically a minor component, often being from about 0.05 to 10% by weight with the remainder being the injectable carrier and the like.

The above-described components for orally and topically administrable or injectable compositions are merely representative. Other materials as well as processing techniques and the like are set forth in Part 8 of Remington's Pharmaceutical Sciences, 18th edition, 1990, Mack Publishing Company, Easton, Pennsylvania, 18042, which is incorporated herein by reference.

The compounds of this invention can also be administered in sustained release forms or from sustained release drug delivery systems. A description of representative sustained release materials can be found in the incorporated materials in Remington's Pharmaceutical Sciences.

The pharmaceutical compositions of this invention can be formulated into tablets, capsules, liquid, injection formulation, or an ointment. The present invention, however, is not limited to the following pharmaceutical compositions. For example, yet without wishing to be limiting in any way, the compound of formula I can be dissolved in a buffered sterile saline injectable aqueous medium to a appropriate concentration of approximately 5 mg/mL.

The invention also encompasses kits that can simplify the administration of a pharmaceutically active agent to an animal. A typical kit of the invention comprises a unit dosage form of a pharmaceutical composition according to the invention. In one embodiment, the unit dosage form is a container (such as a vial, a pouch, a tube, a syringe, or the like),

which can advantageously be sterile, containing a pharmaceutical composition of the invention. The kit can further comprise a label or printed instructions instructing the use of the pharmaceutically active agent to treat or prevent a condition. In another embodiment, the kit comprises a unit dosage form of a pharmaceutical composition of the invention and a
5 dropper, syringe, or other applicator for administering the pharmaceutical composition. Typically, the components of the kit, for example, the unit dosage form and instructions, are contained within a suitable packaging material.

Herein it is shown that bioavailable plasmalogen precursors with docosahexaenoic acid (22:6) substitution at sn-2 decreased membrane cholesterol levels. In contrast, stearic acid (18:0),
10 oleic acid (18:1), linoleic acid (18:2), arachidonic acid (20:4) or linolenic (18:3), at sn-2, were much less active and free DHA was inactive. The fatty acid substitution at sn-1 demonstrated an absolute requirement for an alkenyl linkage, with an acyl linkage completely eliminating cholesterol-lowering activity. The alkenyl linkage can be generated in the endoplasmic reticulum from the alkyl precursor (e.g. PPI-1009); however, the synthetic alkenyl form could
15 also be a potential therapeutic molecule. The pharmaceutical properties of these targeted plasmalogen precursors can also be improved with the addition of a polar substituent to sn-3. This substitution provides improved pharmaceutical properties including: i) stabilization of the sn-2 substitution from migration to sn-3²⁷⁻²⁸ ii) the ability to generate a pharmaceutically-acceptable salt to improve formulation and drug dissolution and absorption; and iii) the
20 ability of sn-3 substituents to be readily removed by lipases²⁹ such that the precursor can be converted to the corresponding endogenous plasmalogen.

Accordingly, administering compounds of the present invention to mammalian biological systems results in increased cellular concentrations of specific sn-2 substituted ethanolamine plasmalogens independent of the ether lipid synthesis capacity of the system. The elevated
25 levels of these specific sn-2 substituted species give rise to lowering of membrane cholesterol levels and lowering of amyloid secretion, therefore making these compounds useful for the treatment or prevention of diseases of aging associated with increased membrane cholesterol, increased amyloid, and decreased plasmalogen levels.

Without wishing to be bound by theory, it is believed that the compounds described herein
30 are capable of bypassing peroxisomal ether lipid biosynthesis pathways enabling both the restoration of plasmalogen levels in plasmalogen deficient subjects, as well as the delivery of

pharmaceutically effective cholesterol lowering levels of specific cholesterol lowering plasmalogens. Accordingly, these molecules can be used to treat or prevent diseases associated with either decreased levels of plasmalogens, increased levels of membrane cholesterol or increased amyloid levels. These factors are believed to be causal in a wide variety of human diseases such as neurodegeneration (including without limitation Alzheimer's disease, Parkinson's disease and age-related macular degeneration), cognitive impairment, dementia, cancer (including without limitation prostate, lung, breast, ovarian, and kidney cancer), osteoporosis, bipolar disorder and vascular diseases (including but not limited to atherosclerosis and hypercholesterolemia). Accordingly, the present invention relates to the treatment of these diseases using the described plasmalogen precursors. Furthermore, these derivatives have utility in the treatment of disorders resulting from abnormal genetic expression of cholesterol transport proteins such as apolipoprotein E.

It is also shown herein that the administration of 1-alkyl-2-alkyl glycerols result in the stereoselective elevation of PlsEtn levels in both PlsEtn normal and PlsEtn deficient systems.

These data also demonstrate for the first time that 1-alkyl, 2-acylglycerols reduce membrane cholesterol levels and amyloid levels and that these effects require specific fatty acid substitutions at the sn-2 position.

The inventors also demonstrate herein that:

- 1) the ether bond at sn-1 is stable and is further processed by a desaturase (endoplasmic reticulum) to generate the essential alkenyl bond at sn-1 characteristic of plasmalogens but that this desaturation occurs after the addition of phosphoethanolamine by CDP-ethanolamine transferase (endoplasmic reticulum);
- 2) the charged substitution at sn-3 stabilizes the fatty acid substitution at sn-2 from migrating²⁷⁻²⁸ to sn-3;
- 3) the charged substitution at sn-3 is readily cleaved by cellular lipases and provides the free hydroxyl group for the addition of phosphoethanolamine by CDP-ethanolamine transferase (endoplasmic reticulum);
- 4) the fatty acid substituent at sn-2 is able to undergo deacylation and reacylation by other fatty acids in cells;

- 5) DHA substitution at sn-2 is optimal for lowering membrane cholesterol, and
- 6) the charged substitution at sn-3 improves the pharmaceutical properties (stability, bioavailability and formulation as a salt) of the novel presented plasmalogen precursors.

The compounds of the present invention are effectively converted to PlsEtn species in cells with impaired plasmalogen biosynthesis capacity and in cells with unimpaired plasmalogen biosynthesis capacity. These results are in direct contrast to the prior art regarding other plasmalogen precursors. 1-alkyl, 2-hydroxy glycerols (chimyl, batyl, salachyl alcohols) have been shown to increase PlsEtn levels in PlsEtn deficient systems to control levels but not to above control levels in either PlsEtn deficient or PlsEtn sufficient systems. Therefore, the compounds of the present invention can be useful in the prevention of diseases of the aging mediated by plasmalogen deficiency, but increasing PlsEtn levels to above control levels in either PlsEtn deficient or PlsEtn sufficient systems.

As discussed above, the compounds described herein are suitable for use in a variety of drug delivery systems; however, without wishing to be limiting, the compounds are especially useful for oral delivery in a capsule or tablet. In such embodiments the maximum total dose is not expected to exceed 2 g/day for a 40 to 80 kg human patient.

In the following examples the following abbreviations have the following meanings.

Abbreviations not defined below have their generally accepted meaning.

	bd	=	broad doublet
20	bs	=	broad singlet
	d	=	doublet
	dd	=	doublet of doublets
	dec	=	decomposed
	dH ₂ O	=	distilled water
25	ELISA	=	enzyme-linked immuno-sorbent assay
	EtOAc	=	ethyl acetate
	EtOH	=	ethanol
	g	=	grams
	h	=	hours
30	Hz	=	hertz
	ip	=	intraperitoneal
	L	=	liter
	m	=	multiplet
	min	=	minutes
35	M	=	molar
	MeOH	=	methanol

	mg	=	milligram
	MHz	=	megahertz
	mL	=	milliliter
	mmol	=	millimole
5	m.p.	=	melting point
	N	=	normal
	po	=	per os, oral
	q	=	quartet
	quint.	=	quintet
10	s	=	singlet
	t	=	triplet
	THF	=	tetrahydrofuran
	tlc	=	thin layer chromatography
	μg	=	microgram
15	μL	=	microliter
	UV	=	ultraviolet

In the examples below, all temperatures are in degrees Celsius unless otherwise indicated.

The following synthetic and biological examples are offered to illustrate this invention and are not to be construed in any way as limiting the scope of this invention.

20 **EXAMPLES**

EXAMPLE 1: Chemical Synthesis

PPI-1009: 2-acetoxy-4-(2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4, 7,10, 13, 16, 19-hexaenoyloxy)-3-(hexadecyloxy)propoxy)-N,N,N-trimethyl-4-oxobutan-1-aminium was prepared according to the following general experimental procedure.

25 **Step -1**

At 0 °C, to a solution of unlabelled **PPI-1001** (50 g, 158 mmol) in dry DMF (20 mL), was added imidazole (21.5 g, 316 mmol), the resulting mixture stirred for 10 min. A solution of TBDMS-Cl (26.2 g, 174 mmol) in DMF (50mL) was added drop wise and the resulting solution was stirred at rt for 4 h. The reaction mixture was diluted with water (50ml),
 30 extracted with EtOAc (2 × 250mL). The organic layer washed with ice water (2 x 100mL), brine (50mL), dried (Na₂SO₄) and evaporated to obtain crude compound **4** that was purified by column chromatography (neutral alumina, EtOAc-Pet ether (0.5:9.5) to obtain compound **3** (45 g, 66%). *R_f* = 0.65 (EtOAc-Pet ether (1-9))

Step-2

At 0 °C, to a solution of compound **3** (75 g, 174 mmol) in DMF (750mL) NaH (60% dispersion in oil, 5g, 209 mmol) was added portion wise, stirred for 30 min, benzyl bromide (44.8g, 262 mmol) added drop wise over a period of 1h, slowly allowed to rt and stirred overnight until complete consumption of compound **3** as evidenced by tlc analysis. The reaction mixture was cooled to 0 °C, methanol (5mL), ice cold water (50mL) added, extracted with Et₂O (2 × 250mL), organic layer washed with water (2 x 50mL), brine (50mL), dried (Na₂SO₄) and evaporated to obtain crude compound **4** (90 g) as yellow oil (*R_f* = 0.7; EtOAc-Pet ether [5-95]), that was used as such for the next step without further purification.

Step-3

At -15 °C, to a solution of compound **4** (1.7 g, 3.26 mmol) in dry THF (15 mL), a solution of TBAF (1.7 g, 6.53 mmol) in dry THF (5mL) added and the reaction mixture slowly warmed to rt and stirred overnight until complete consumption of compound **4** as evidenced by tlc analysis. The reaction mixture was quenched with water (10mL), extracted with EtOAc (2 × 50mL), organic layer washed with brine (20mL), dried (Na₂SO₄) and evaporated to obtain crude compound **6** that was purified by flash column chromatography (100-200 mesh silica gel, EtOAc-Pet ether(3:7) to obtain compound **5** (850 mg, 65%) as a light yellow oil. *R_f* = 0.54 (EtOAc-Pet ether (3:7)).

Step-4

At 0 °C, to a solution of compound **7** (8g, 39 mmol) in dry CH₂Cl₂(50mL)-DMF (3 drops) oxalyl chloride (4.2 mL, 40 mmol) was added and resulting mixture slowly warmed to rt stirred for 5 h. Excess oxalyl chloride was evaporated and residue dissolved in toluene and solvent evaporated to obtain compound **6**. A solution of compound **6** in dry CH₂Cl₂ (25mL) was added dropwise to a solution of compound **5** (11g, 20mmol) in dry CH₂Cl₂ (50mL) and the resulting solution purged with Argon gas till complete consumption of compound **5** as evidenced by tlc analysis. The reaction mixture was concentrated to obtain crude compound **8** (14g) that was used as such for the next step without further purification.. *R_f* = 0.45 (MeOH-CHCl₃ (1:4)).

Step-5

A solution of compound **8** (14g crude) in EtOH (50 mL), was hydrogenated over 10% Pd/C at 40psi, until complete consumption of compound **8** as indicated by TLC analysis. The reaction mixture was filtered and evaporated to obtain crude compound **9**, which was purified by column chromatography over neutral alumina to give compound **9** (6g, 6g, 61% over two steps) as a light brown oil $R_f = 0.25$ (MeOH-CHCl₃ (2:3)).

Step-6

At 0 °C, to a solution of compound **9** (8.2 mg, 16 mmol) in THF (150mL), catalytic amount of DMAP (1.9g, 20 mmol), pyridine (7.6 mL, 90 mmol) were added and stirred at rt for an hour. To this solution, a solution of DHA-Cl (prepared by addition of a solution of oxalyl chloride (2.5 mL, 28 mmol) in dry CH₂Cl₂ at 0 °C to DHA-acid (7.7g, 20 mmol), evaporating excess oxalyl chloride to give DHA-Cl) in CH₂Cl₂ (50mL) was added drop wise to the reaction mixture at 0 °C and stirred for 0.5 h. The reaction mixture was slowly warmed to rt and stirred for 24 h. The reaction mixture was diluted with water (50mL), extracted with EtOAc (2 × 200mL), washed with brine (50mL) dried (Na₂SO₄) and evaporated to obtain crude that was purified by column chromatography (neutral alumina MeOH-CHCl₃(5:95)) to obtain **PPI-1009** (1.2g, ~10%, HPLC purity 97% along with 1.7g of impure material) as a light brown semisolid $R_f = 0.45$ (MeOH-CHCl₃ (15:85)).

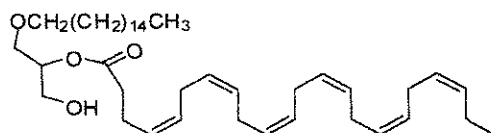
EXAMPLE 2: Biological Testing

Chinese hamster ovary (CHO), N-Rel³⁰ (a mutant CHO cell line deficient in the peroxisomal enzyme dihydroxyacetonephosphate acyltransferase), and human embryonic kidney (HEK293) cells were grown in 10 cm² dishes in DMEM/Ham's F12 (1:1) containing 10% FBS. Cells were incubated with plasmalogen precursors dissolved in ethanol (final ethanol concentration of 0.1%) and harvested for plasmalogen analysis by LC-MS-MS³¹, and cholesterol and cholesterol ester analyses utilizing a commercial colorimetric kit (BioVision #K613).

1-alkyl, 2-acyl glycerols with 16:0 at sn-1, DHA at sn-2 and either OH or acetyl-L-carnitine at sn-3, **PPI-1005** or **PPI-1009**, respectively were effectively converted to PlsEtn species in cells with impaired plasmalogen biosynthesis capacity (N-Rel, Figures 2, 3A) and in cells with unimpaired plasmalogen biosynthesis capacity (CHO, Figure 4A). These results are in direct contrast to the prior art regarding other plasmalogen precursors. 1-alkyl, 2-hydroxy glycerols

(chimyl, batyl, salachyl alcohols) has been shown to increase PlsEtn levels in PlsEtn deficient systems to control levels but not to above control levels in either PlsEtn deficient or PlsEtn sufficient systems (Figure 4B). In addition the bioconversion of the 1-alkyl, 2-acyl glycerols described in this invention did not result in increased levels of either chimyl alcohol or 1-alkenyl, 2-acyl glycerols (Figure 3B) indicating that neither chimyl alcohol nor 1-alkenyl, 2-acyl glycerols are intermediates in the biotransformation pathway of the described molecules to PlsEtn.

The compound PPI-005 is described in applicant's co-pending application PCT/CA2007/001472, and is shown below:



(4Z,7Z,10Z,13Z,16Z,19Z)-1-(hexadecyloxy)-3-hydroxypropan-2-yl docosa-4,7,10,13,16,19-hexaenoate

5a

Treatment of cells with 1-alkyl, 2-acyl glycerols with 16:0 at sn-1, DHA at sn-2 and either OH or acetyl-L-carnitine at sn-3 resulted in the structure-specific enrichment of PlsEtn with DHA at sn-2 (Figure 6). This is the first description of the structure-specific enrichment of PlsEtn.

PPI-1009 is a plasmalogen precursor with improved pharmaceutical properties. This molecule exists at room temperatures as a salt and represents the first non-oil based plasmalogen precursor ever reported.

Biological systems with a pre-existing deficiency in plasmalogen synthesis and subsequent low plasmalogen levels (N-Rel vs. CHO) (Figure 1) have elevated membrane cholesterol levels (Figure 5). Elevation of DHA-PlsEtn to 80% of control values by PPI-1005 (Figure 2) resulted in a significant reduction in membrane cholesterol (Figure 5).

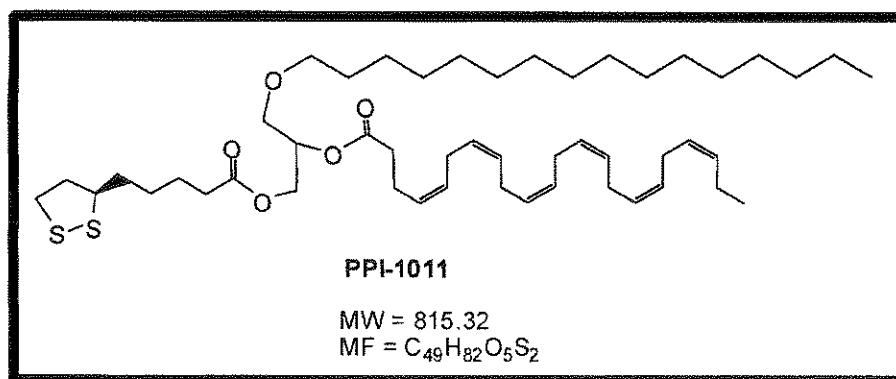
The cholesterol lowering effect of elevated PlsEtn levels was discovered to be dependent upon the sn-2 substituent (Figures 7 and 8). Only polyunsaturated fatty acids (DHA, 18:3) were observed to have cholesterol lowering activity in HEK293 cells whereas saturated, mono and diunsaturated fatty acids had no effect. Only the restoration of DHA-PlsEtn levels

resulted in a robust attenuation of the elevated membrane cholesterol levels observed in N-Rel cells as a result of decreased plasmalogen levels (Figure 7). These results indicate that poly-unsaturated fatty acid containing PlsEtn are selectively involved in membrane cholesterol homeostasis. PPI-1009 was also demonstrated to concentration-dependently decrease membrane cholesterol levels in NRel (Figure 7) and HEK293 cells (Figure 9). These results indicate that the membrane lowering effect of elevated PlsEtn levels requires the administration of plasmalogen precursor capable of selectively elevating PlsEtn levels with specific sn-2 substitutions.

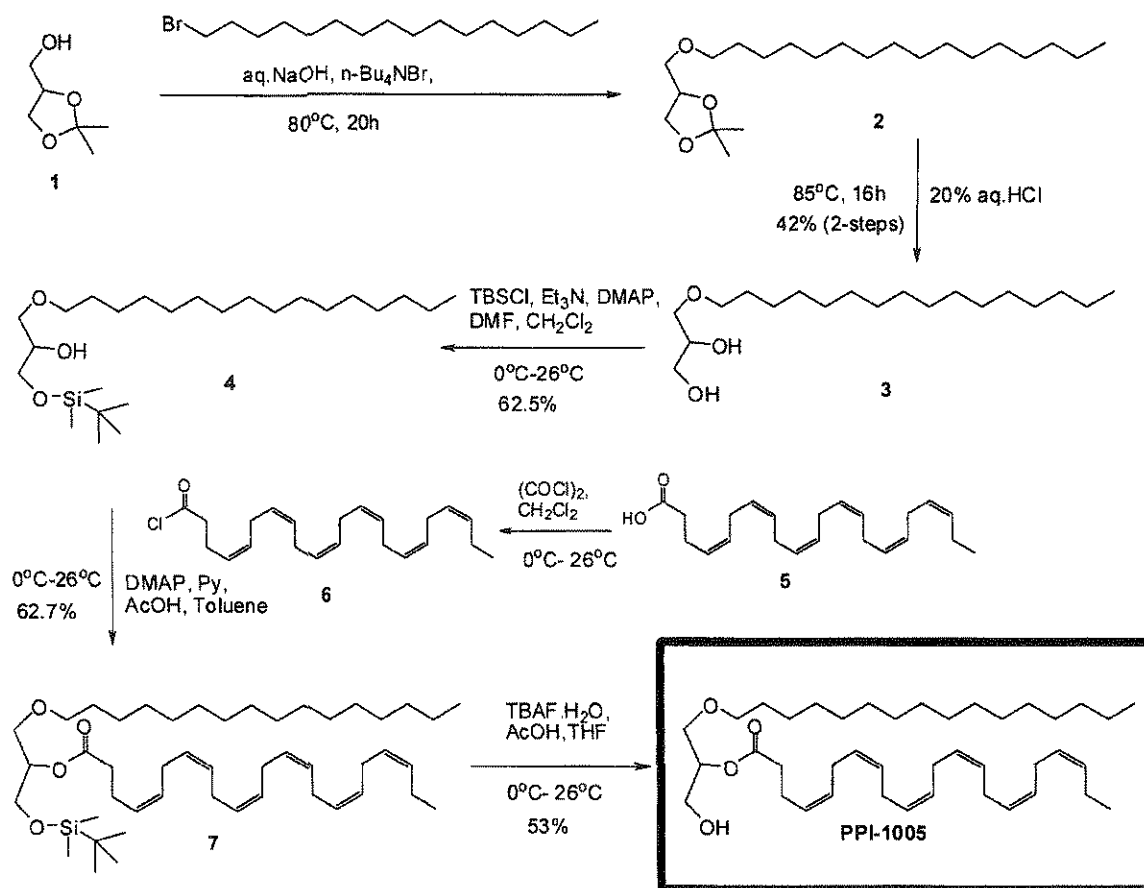
The treatment of HEK293 cells with either PPI-1005 or PPI-1009 resulted in decreased levels of both AB-40 and AB-42 in both normal cells and cholesterol loaded cells (Figure 10). These results further exemplify the functional utility of the plasmalogen precursors by their ability to positively modulate membrane protein function. In this regard, decreased membrane cholesterol levels are known to negatively modulate amyloid peptide production¹⁵. In addition to lowering membrane cholesterol in HEK293 cells, PPI-1005 and PPI-1009 were also observed to decrease the secretion of amyloid peptides (Figure 10).

A structure-specific and concentration-dependent incorporation of PPI-1014 in N-Rel ethanolamine plasmalogens (PlsEtn) and phosphatidylethanolamines (PtdEtn) was also observed after 72 hours using 5, 10 and 20 μ M concentrations of PPI-1014 (Figure 17).

EXAMPLE 3: Preparation of PPI-1011

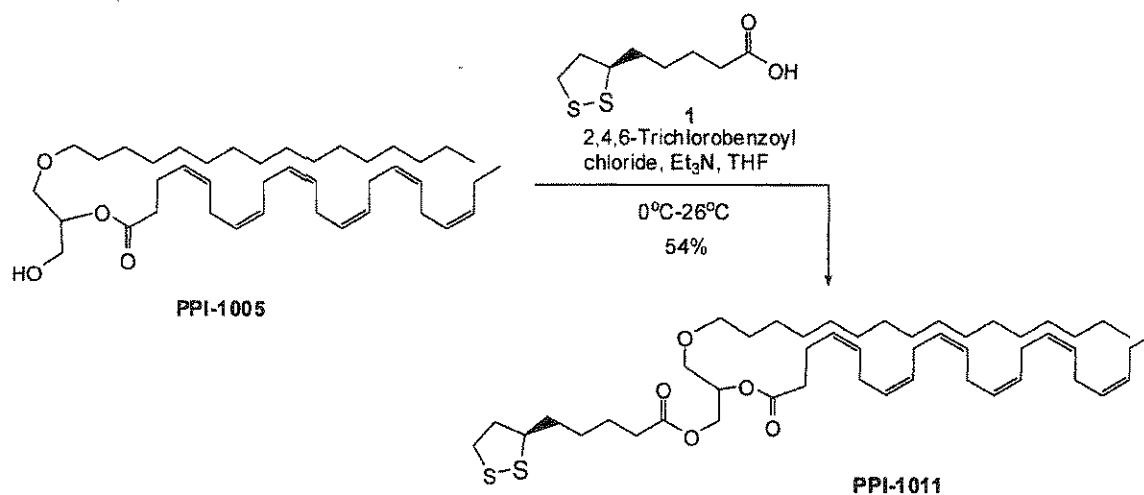


Synthetic scheme:

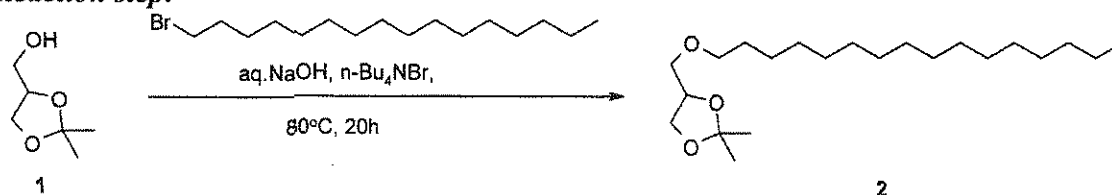


Synthetic scheme for PPI-1011

5



Reaction step:

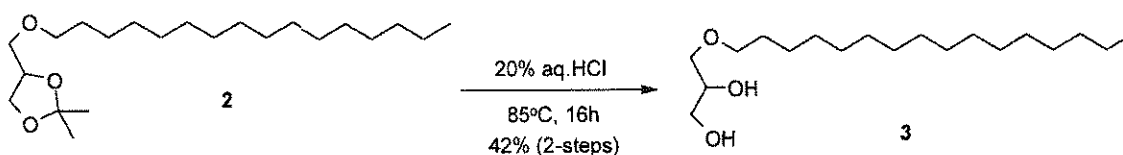


Brief procedure: A solution of compound **1** (2.0 Kg, 15.15 mol) in 10% NaOH solution (10.0 L) was stirred at 80°C for 1h, TBAB (992.0g, 3.03 mol) added and stirred for 15 min. Cetyl bromide (5.5 kg, 18.18 mol) was added slowly and the reaction mixture was stirred at 80°C for 20h. *(The reaction progress was monitored by diluting small aliquots with water, extracting with ethyl acetate and spotting over an analytical silica gel TLC plate (30 % Ethyl acetate in pet-ether) and visualizing respective spots using Mo stain and KMnO₄ solution).*

The following are the R_fs of the components of the mixture: compound **1** (0.1), compound **2** (0.7).

Work up and purification: The reaction mixture was cooled to RT, extracted with CH₂Cl₂ (3 x 3.0 L). The combined CH₂Cl₂ layer was washed with NaHCO₃ solution (1.0 L), water (2 x 2.0 L), brine solution (1.0 L), dried over anhydrous Na₂SO₄ and concentrated to afford crude compound **2** (2.9 Kg, crude) as pale yellow liquid, which was used in next step.

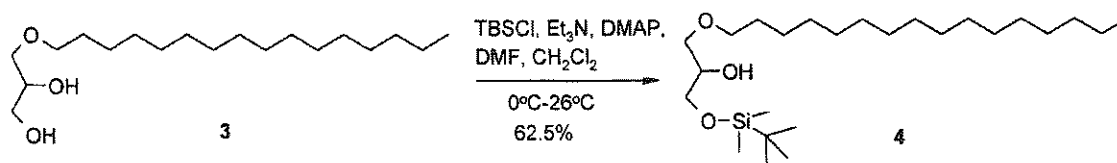
Reaction step:



Brief procedure: A solution of compound **2** (2.9 Kg, 81.23 mol) in 20% aq HCl (14.5 L) was stirred at 85°C for 16h. *(The reaction progress was monitored by diluting small aliquots with water, extracting with ethyl acetate and spotting over an analytical silica gel TLC plate (40% ethyl acetate in pet ether) and visualizing spots using Mo stain).* The following are the R_fs of the components of the mixture: compound **2** (0.8), compound **3** (0.2).

Work up and purification: The reaction mixture was cooled to RT, extracted with CH₂Cl₂ (3 x 4.0 L). The combined organic layer was washed with NaHCO₃ solution (1.0 L), water (2 x 2.0 L), brine solution (1.0 L), dried over anhydrous Na₂SO₄ and concentrated to afford crude compound, which was triturated with 5% ethyl acetate in pet-ether (2 x 1.0 L) and dried to afford compound **3** (2.0 Kg, 42% from 2-steps) obtained as off white solid.

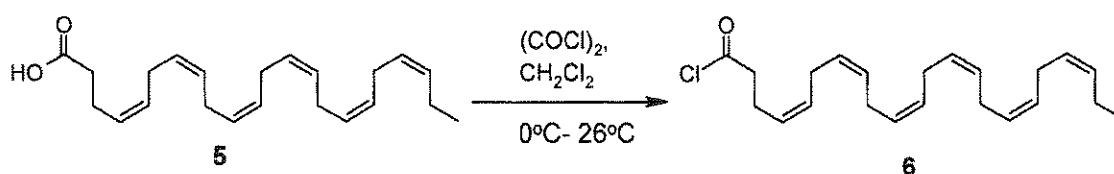
Reaction step:



Brief procedure: To a cooled solution of compound **3** (1.0 Kg, 3.16 mol) in DMF (640.0 mL) and CH_2Cl_2 (400.0 mL) at 0°C , added DMAP (38.6 g, 0.32 mol) followed by triethylamine (735.0 g, 7.27 mol). After addition the reaction mixture was stirred at 0°C for 30 min and added TBSCl (572.0 g, 3.79 mol) in equal portions (3 portions) for 1h and the reaction mixture was stirred for 20h at RT. *(The reaction progress was monitored by quenching small aliquots with water, extracting with CH_2Cl_2 and spotting over an analytical silica gel TLC plate (20% Ethyl acetate in pet ether) and visualizing spots using Mo stain and KMnO_4).* The following are the R_f s of the components of the mixture: compound **3** (0.15), compound **4** (0.7).

Work up and purification: The reaction mixture was diluted with CH_2Cl_2 (2.0 L), washed with water (3 x 3.0 L), brine solution (1.0 L), dried over anhydrous Na_2SO_4 and concentrated. The obtained crude compound was purified by column chromatography (silica gel 100-200 mesh) using 5 % ethyl acetate in pet ether as eluent to afford compound **4** (850 g, 90%) obtained as pale yellow oil.

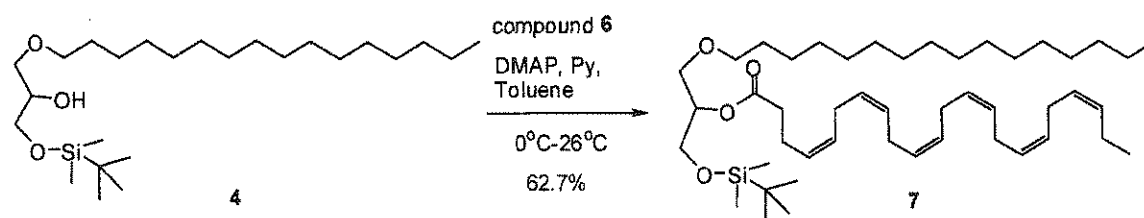
Reaction step:



Brief procedure: To a cooled solution of compound **5** (160.0 g, 0.487 mol), DMF (1.0 mL) in CH_2Cl_2 (500.0 mL) at 0°C , Oxalyl chloride (105.0 g, 0.828 mol) was added slowly for 30 min. After addition the reaction mixture was stirred at 26°C for 4h. *(The reaction progress was monitored by quenching small aliquots with MeOH and spotting over an analytical silica gel TLC plate (10% Ethyl acetate in pet ether) and visualizing spots using KMnO_4 solution).* The following are the R_f s of the components of the mixture: compound **5** (0.3), compound **6** (0.8).

Work up and purification: The reaction mixture was concentrated under N₂ atmosphere to afford crude compound **6** (175 g, crude).

Reaction step:

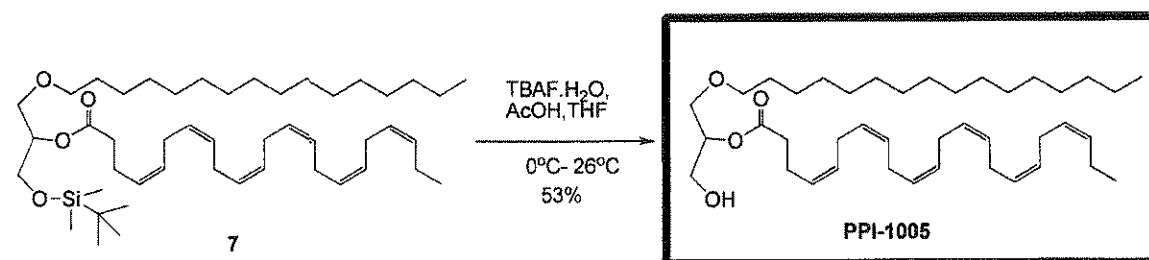


5

Brief procedure: To a cooled solution of compound **4** (150.0 g, 0.348 mol) in Toluene (1.25 L) at 0°C, pyridine (110.0 g, 1.39 mol) was added followed by DMAP (122.2g, 0.348 mol) and stirred for 10 min. Added a solution of crude compound **6** (175.0 g, 0.504 mol) in Toluene (250.0 mL) for 15 min. After addition the reaction mixture was stirred at RT for 20h.
 10 (The reaction progress was monitored by quenching small aliquots with water, extracting with EtOAc and spotting over an analytical silica gel TLC plate (5% Ethyl acetate in pet ether) and visualizing spots using KMnO₄ solution). The following are the R_fs of the components of the mixture: compound **4** (0.2), compound **7** (0.5).

Work up and purification: The reaction mixture was diluted with ethyl acetate (3.0 L),
 15 washed with water (1.0 L), 0.05 N HCl (500.0 mL), water (2 x 1.0 L), brine solution (500.0 mL) dried over anhydrous Na₂SO₄ and concentrated to afford crude compound **7**, which was purified by column chromatography (silica gel 100-200 mesh) using 2 % ethyl acetate in pet ether as eluent to afford compound **7** (162 g, 62.7%) obtained as pale yellow oil.

Reaction step:

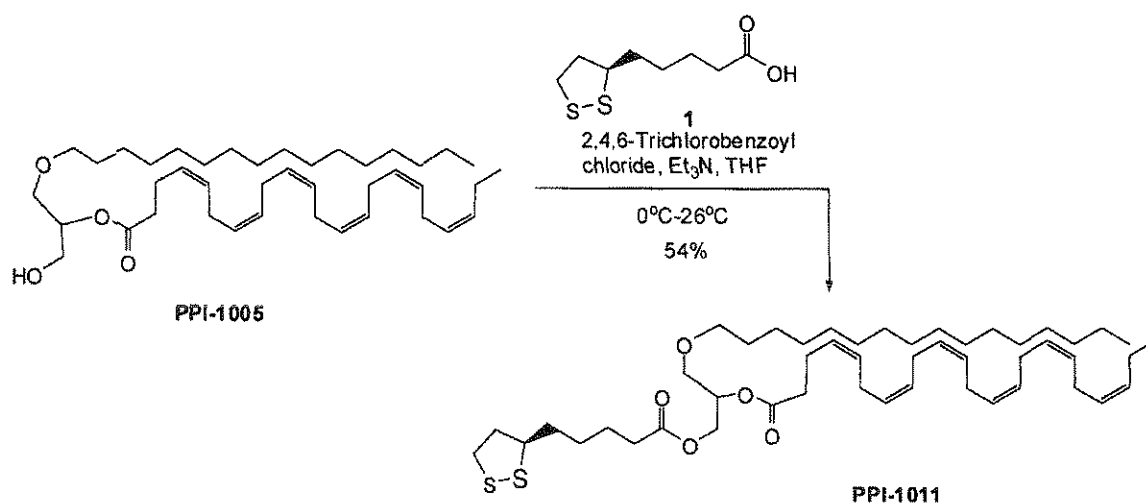


20

Brief procedure: To a cooled solution of compound **7** (160.0 g, 216.21 mmol) in THF (10.0mL) and acetic acid (52.0 g) at 0°C, TBAF (226.0 g, 864.86 mmol) was added in equal portions for 30min. After addition the reaction mixture was stirred at RT for 6h. *(The reaction progress was monitored by spotting over an analytical silica gel TLC plate (20% Ethyl acetate in pet ether) and visualizing spots using Mo stain and KMnO₄ solution).* The following are the R_fs of the components of the mixture: compound **7** (0.9), compound **PPI-1005** (0.4).

Work up and purification: The reaction mixture was diluted with ethyl acetate (3.0 L), washed with water (2 x 2.0 L), brine (500.0 mL), dried over anhydrous Na₂SO₄ and concentrated to afford crude compound, which was purified by column chromatography (silica gel 100-200 mesh) using 7 % ethyl acetate in pet ether as eluent to afford compound **PPI-1005** (72 g, 53%) obtained as pale yellow oil.

Reaction step:



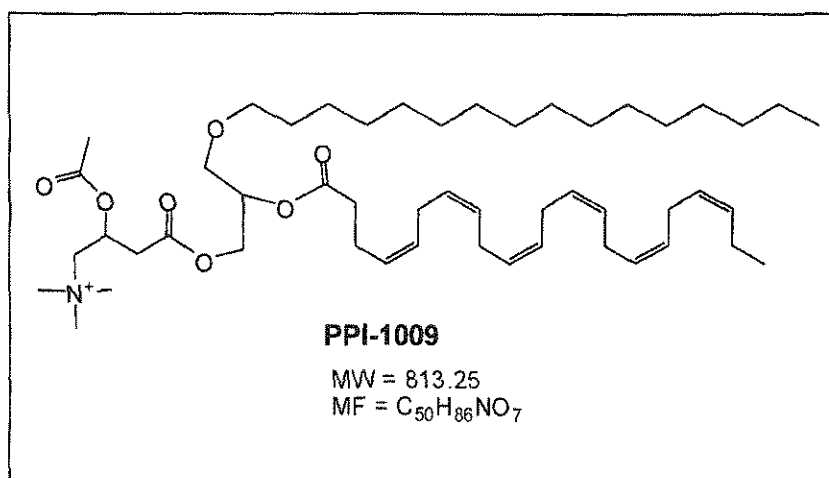
Brief procedure: To a cooled solution of α-Lipoic acid **1** (12.0 g, 58.25 mmol) in THF (500.0 mL) at 0°C, triethylamine (8.1 mL, 58.25 mmol) was added slowly for 10 min, followed by 2,4,6-Trichlorobenzoyl chloride (14.2 g, 58.25 mmol). After addition the reaction mixture was allowed to RT and stirred for 18h. *(The reaction progress was monitored by spotting on an analytical silica gel TLC plate (20% EtOAc in pet-ether), and visualizing spots using 254 nm UV light and Hanessian's stain).* The following are the R_fs of the components of the mixture: compound **1** (0.2), intermediate (0.6).

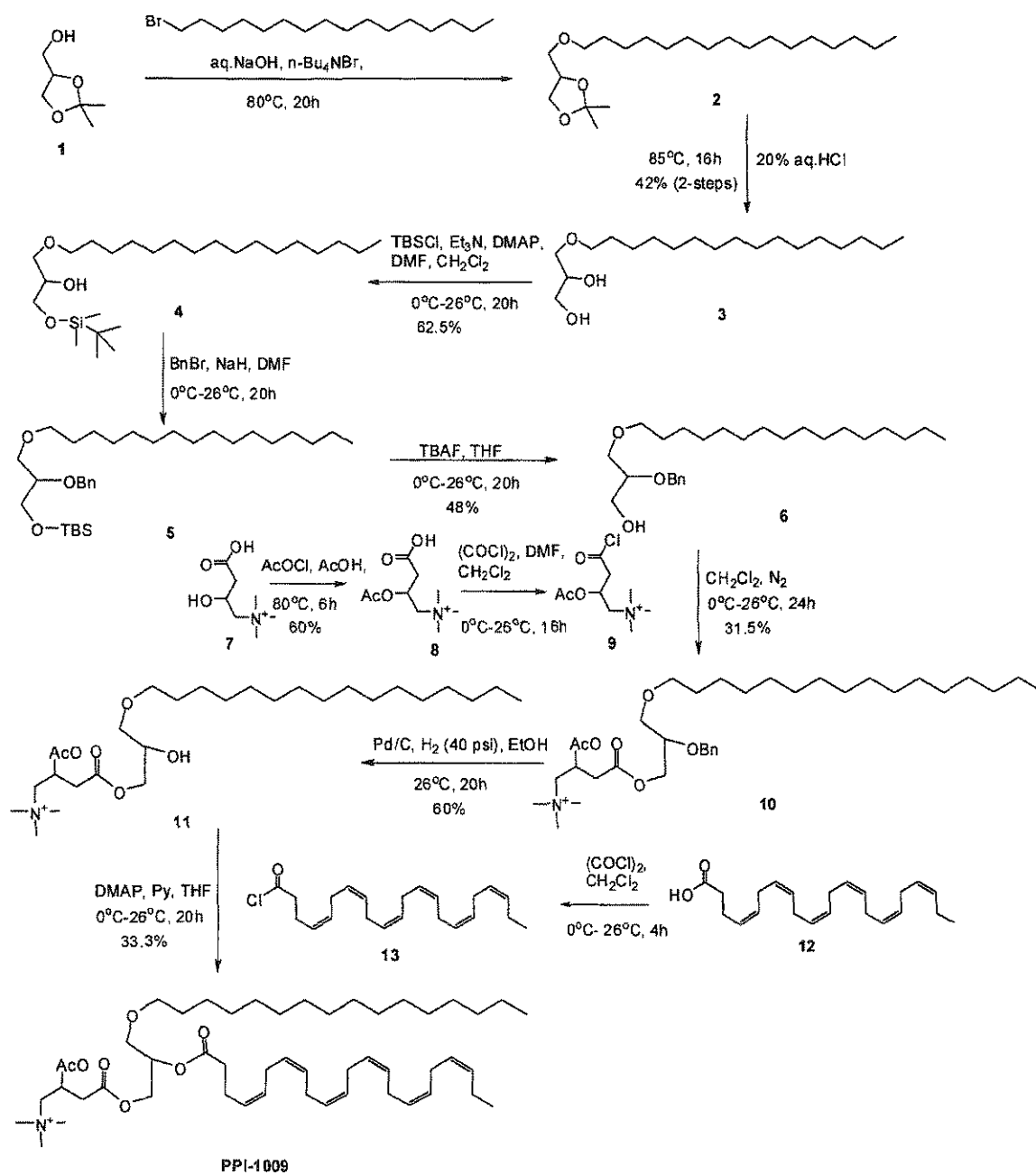
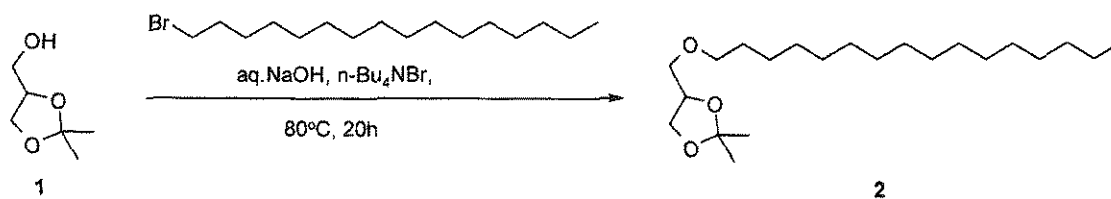
The reaction mixture was filtered off, the solid was washed with THF (25.0 mL), the combined filtrate was concentrated using reduced pressure under N₂ atmosphere to obtain the crude anhydride, which was dissolved in benzene (500.0 mL, cooled to 0°C and added DMAP (7.1 g, 58.25 mmol), stirred for 10 min. To the reaction mixture added a solution of **PPI-1005** (40.1 g, 64.07 mmol) in benzene (100.0 mL) slowly at 0°C. After addition the reaction mixture was allowed to RT and stirred for 24h. *(The reaction progress was monitored by quenching small aliquots with H₂O, extracting with ethyl acetate and spotting on an analytical silica gel TLC plate (15% THF in pet-ether), and visualizing spots using 254 nm UV light and Hanessian's stain).* The following are the R_fs of the components of the mixture: **PPI-1005** (0.3), **PPI-1011** (0.5).

Work up and purification: The reaction mixture was diluted with ethyl acetate (2000mL), washed with saturated NaHCO₃ solution (1 x 400 mL), 0.05N HCl (1 x 400 mL), water (1 x 400 mL), brine (1 x 400 mL) and dried over anhydrous Na₂SO₄ and concentrated to obtain the crude compound, which was purified by column chromatography (neutral silica gel 100-200 mesh) using 2.5 to 5% THF in pet ether as eluent.

The title compound **PPI-1011** (28.0 g, 54%) was obtained as pale brown oil.

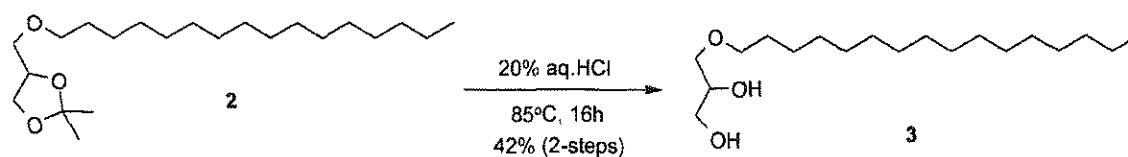
EXAMPLE 4: Alternate method for preparation of PPI-1009



Synthetic scheme:**5 Reaction step:**

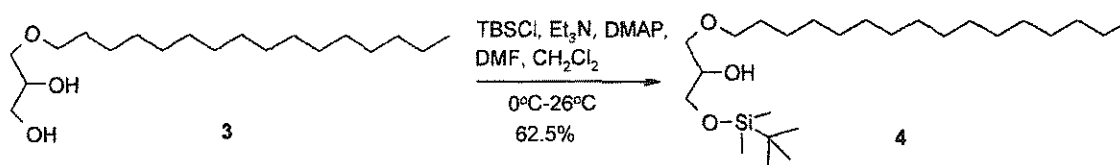
Procedure: A solution of compound **1** (2.0 Kg, 15.15 mol, Alfa Aesar) in 10% NaOH solution (10.0 L) was stirred at 80°C for 1h, added TBAB (992.0g, 3.03 mol, Rajdhani scientific) and stirred for 15 min. Added cetyl bromide (5.5 kg, 18.18 mol, Alfa Aesar) slowly and the reaction mixture was stirred at 80°C for 20h. *(The reaction progress was monitored by diluting small aliquots with water, extracting with ethyl acetate and spotting over an analytical silica gel TLC plate (30 % Ethyl acetate in pet-ether) and visualizing respective spots using Mo stain and KMnO₄ solution).* The following are the R_fs of the components of the mixture: compound **1** (0.1), compound **2** (0.7). The reaction mixture was cooled to RT, extracted with CH₂Cl₂ (3 x 3.0 L). The combined CH₂Cl₂ layer was washed with NaHCO₃ solution (1.0 L), water (2 x 2.0 L), brine solution (1.0 L), dried over anhydrous Na₂SO₄ and concentrated to afford crude compound **2** (2.9 Kg, crude) as pale yellow liquid, which was used in next step.

Reaction step:



Procedure: A solution of compound **2** (2.9 Kg, 81.23 mol) in 20% aq HCl (14.5 L) was stirred at 85°C for 16h. *(The reaction progress was monitored by diluting small aliquots with water, extracting with ethyl acetate and spotting over an analytical silica gel TLC plate (40% ethyl acetate in pet ether) and visualizing spots using Mo stain).* The following are the R_fs of the components of the mixture: compound **2** (0.8), compound **3** (0.2). The reaction mixture was cooled to RT, extracted with CH₂Cl₂ (3 x 4.0 L). The combined organic layer was washed with NaHCO₃ solution (1.0 L), water (2 x 2.0 L), brine solution (1.0 L), dried over anhydrous Na₂SO₄ and concentrated to afford crude compound, which was triturated with 5% ethyl acetate in pet-ether (2 x 1.0 L) and dried to afford compound **3** (2.0 Kg, 42% from 2-steps) obtained as off white solid.

Reaction step:

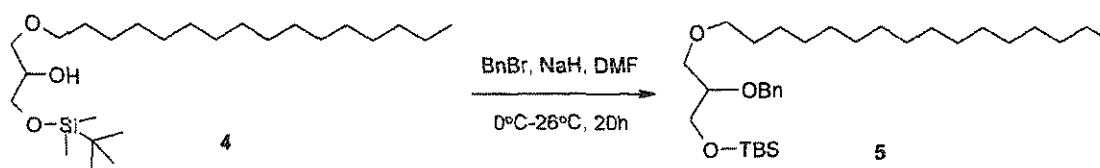


Procedure: To a cooled solution of compound **3** (1.0 Kg, 3.16 mol) in DMF (640.0 mL) and CH_2Cl_2 (400.0 mL) at 0°C , added DMAP (38.6 g, 0.32 mol) followed by triethylamine (735.0 g, 7.27 mol, Rankem). After addition the reaction mixture was stirred at 0°C for 30 min and

5 added TBSCl (572.0 g, 3.79mol, Fluoro chem 3.0 kg) in equal portions (3 portions) for 1h and the reaction mixture was stirred for 20h at RT. *(The reaction progress was monitored by quenching small aliquots with water, extracting with CH_2Cl_2 and spotting over an analytical silica gel TLC plate (20% Ethyl acetate in pet ether) and visualizing spots using Mo stain and KMnO_4).* The following are the R_f s of the components of the mixture: compound **3** (0.15),

10 compound **4**(0.7). The reaction mixture was diluted with CH_2Cl_2 (2.0 L), washed with water (3 x 3.0 L), brine solution (1.0 L), dried over anhydrous Na_2SO_4 and concentrated. The obtained crude compound was purified by column chromatography (silica gel 100-200 mesh) using 5 % ethyl acetate in pet ether as eluent to afford compound **4** (850 g, 90%) obtained as pale yellow oil.

15 **Reaction step:**



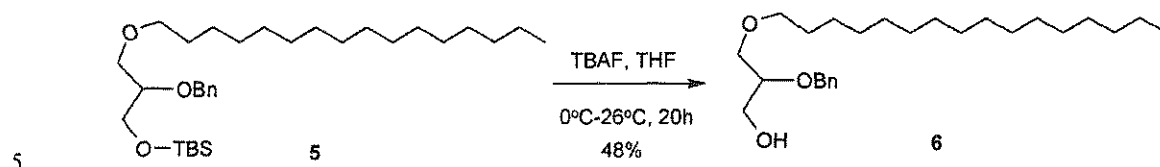
Procedure: To a cooled solution of compound **4** (734 g, 1.706 mol) in DMF (2.5 L) at 0°C , added 60% NaH (204.0 g, 5.12 mol) in portions for 30min. After addition the reaction mixture was stirred at 0°C for further 30 min and added benzyl bromide (438.0 g, 2.56 mol, S.D fine chemicals) drop wise for 1h. The reaction mixture allowed to RT was stirred for 20h.

20 *(The reaction progress was monitored by quenching small aliquots with water, extracting with EtOAc and spotting over an analytical silica gel TLC plate (5% Ethyl acetate in pet ether) and visualizing spots using Mo stain and KMnO_4).* The following are the R_f s of the components of the mixture: compound **4** (0.2), compound **5**(0.5). The reaction mixture was

25 quenched with methanol (250 mL), cold water (1500 mL) and extracted using ethyl acetate (2

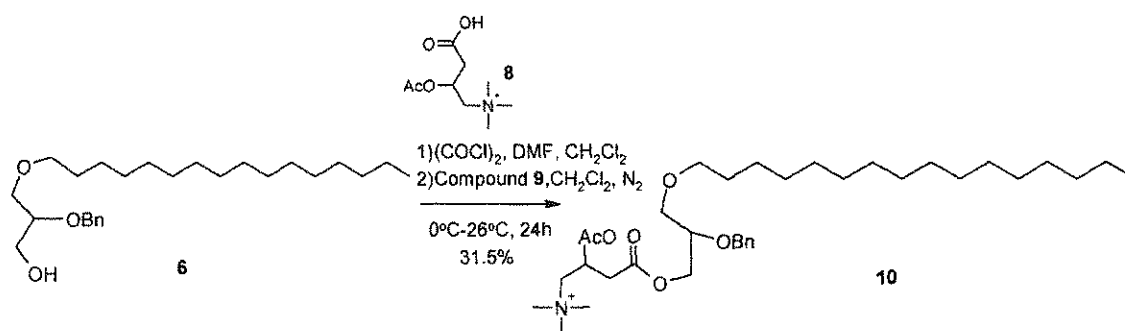
x 2.0 L). The combined ethyl acetate layer was washed with water (3 x 1.0 L), brine solution (1.0 L), dried over anhydrous Na₂SO₄ and concentrated. The obtained crude compound **5** (887 g) was used in the next step without further purification.

Reaction step:



Procedure: To a cold solution of crude compound **5** (887.0 g, 1.702 mol) in THF (2.0 L) at 0°C, a solution of TBAF (1.3 Kg, 5.107 mol, Chemrich fine chemicals) in THF (1.0 L) was added slowly for 1h. After addition the reaction mixture was stirred at RT for 16h. *(The reaction progress was monitored by quenching small aliquots with water, extracting with EtOAc spotting over an analytical silica gel TLC plate (30% Ethyl acetate in pet ether) and visualizing spots using Mo stain and KMnO₄ solution).* The following are the R_fs of the components of the mixture: compound **5** (0.9), compound **6** (0.3). The reaction mixture was diluted with ethyl acetate (2.5 L), washed with water (2 x 1.0 L), brine (1.0 L), dried over anhydrous Na₂SO₄ and concentrated to afford crude compound, which was purified by column chromatography (silica gel 100-200 mesh) using 7 % ethyl acetate in pet ether as eluent to afford compound **6** (330 g, 48% from 2 steps) obtained as pale yellow oil.

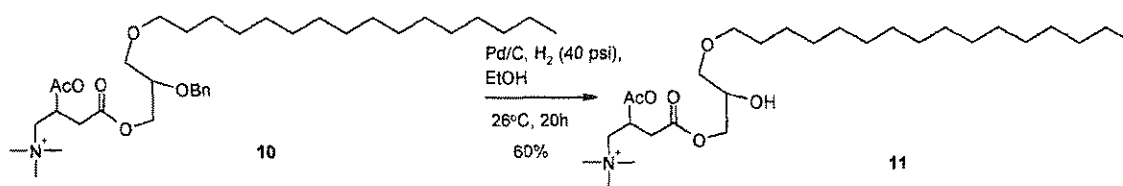
Reaction step:



Procedure: To a cold solution of N-Acetyl carnitene **8** (239.0 g, 1.177 mol, Molecula life Sciences) in CH₂Cl₂ (500.0 mL) and DMF (5.0 mL) at 0°C, added oxalyl chloride (179.4 g, 1.412 mol) slowly for 30 min and stirred at RT for 4h. The solvent from the reaction mixture

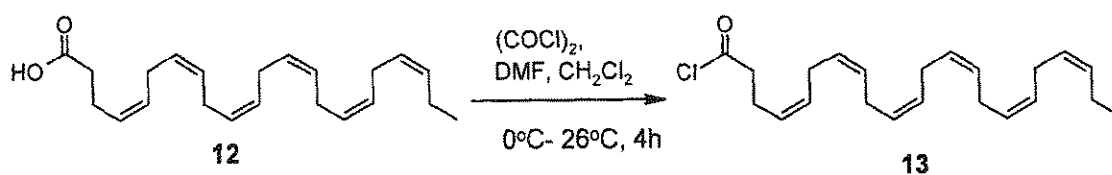
was removed by distillation under reduced pressure and traces of oxalyl chloride by co-distilling with CH_2Cl_2 . The obtained crude compound **9** (250g) was dissolved in CH_2Cl_2 (500.0 mL) and added slowly to a cold solution of compound **6** (334.0 g, 0.823 mol) in CH_2Cl_2 (500.0 mL) at 0°C with bubbling N_2 gas. After addition the reaction mixture was stirred for 20h with continuous bubbling of N_2 at RT. *(The reaction progress was monitored by quenching small aliquots with water, extracting with CH_2Cl_2 and spotting over an analytical silica gel TLC plate (25% MeOH in chloroform) and visualizing spots using Mo stain and KMnO_4).* The following are the R_f s of the components of the mixture: compound **6** (0.8) and compound **10** (0.3). The reaction mixture was diluted with CH_2Cl_2 (2.0 L), washed with brine solution (250 mL), dried over anhydrous Na_2SO_4 and concentrated to obtain crude compound which was purified by column chromatography (silica gel 100-200 mesh) using 5 % MeOH in chloroform as eluent to afford compound **10** (153.0 g, 31.5%) obtained as pale yellow oil.

Reaction step:



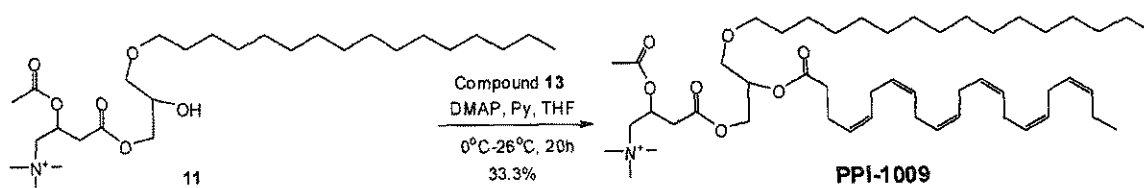
Brief procedure: To a suspension of 10% Pd/C (40.0 g, 25% w/w, AlfaAesar) in ethanol (1.2 L), compound **10** (150 g, 0.298 mol) was added and hydrogenated (H_2 , 40 psi pressure) at RT for 20h. *(The reaction progress was monitored by spotting over an analytical silica gel TLC plate (25% MeOH in chloroform) and visualizing spots using Mo stain and Ninhydrin solution).* The following are the R_f s of the components of the mixture: compound **10** (0.4), compound **11** (0.2). The reaction mixture was filtered off through celite bed, washed the cake with ethanol (2 x 200 mL), the combined filtrate was concentrated to obtain crude compound, was purified by column chromatography (silica gel 100-200 mesh) using 10 % Methanol in chloroform as eluent to afford compound **11** (75 g, 60%) obtained as pale yellow oil.

Reaction step:

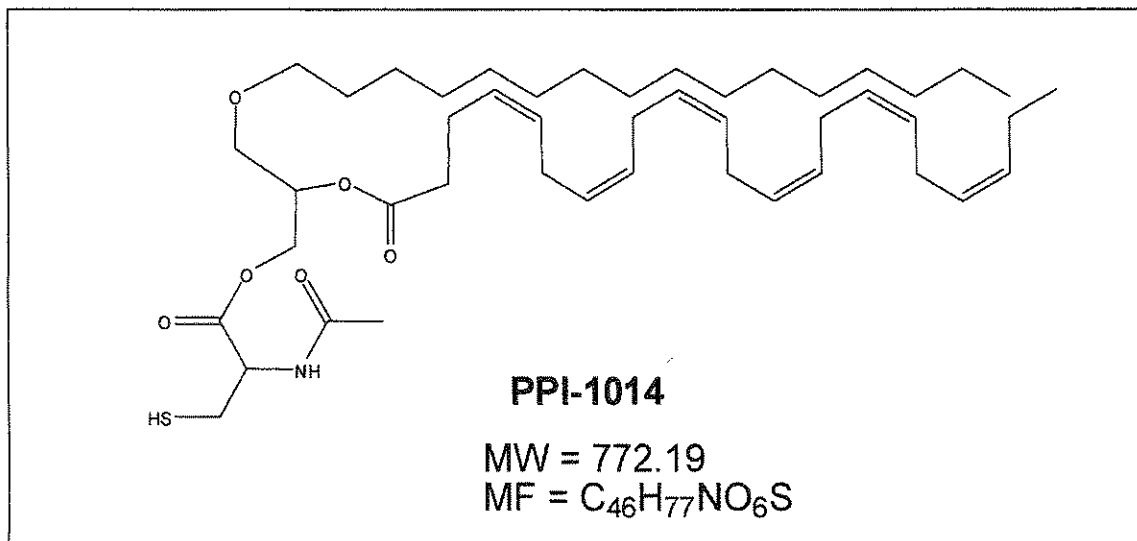


Brief procedure: To a cooled solution of compound **5** (48.0 g, 0.146 mol, Nu-Chek-Prep Inc), DMF (1.0 mL) in CH₂Cl₂ (300.0mL) at 0°C, Oxalyl chloride (22.3 g, 0.175 mol, Molecula Lifesciences) was added slowly for 30 min. After addition the reaction mixture was stirred at 26°C for 4h. *(The reaction progress was monitored by quenching small aliquots with MeOH and spotting over an analytical silica gel TLC plate (10% Ethyl acetate in pet ether) and visualizing spots using KMnO₄ solution).* The following are the R_fs of the components of the mixture: compound **12** (0.3), compound **13** (0.8). The reaction mixture was concentrated under N₂ atmosphere to afford crude compound **6** (57 g, crude).

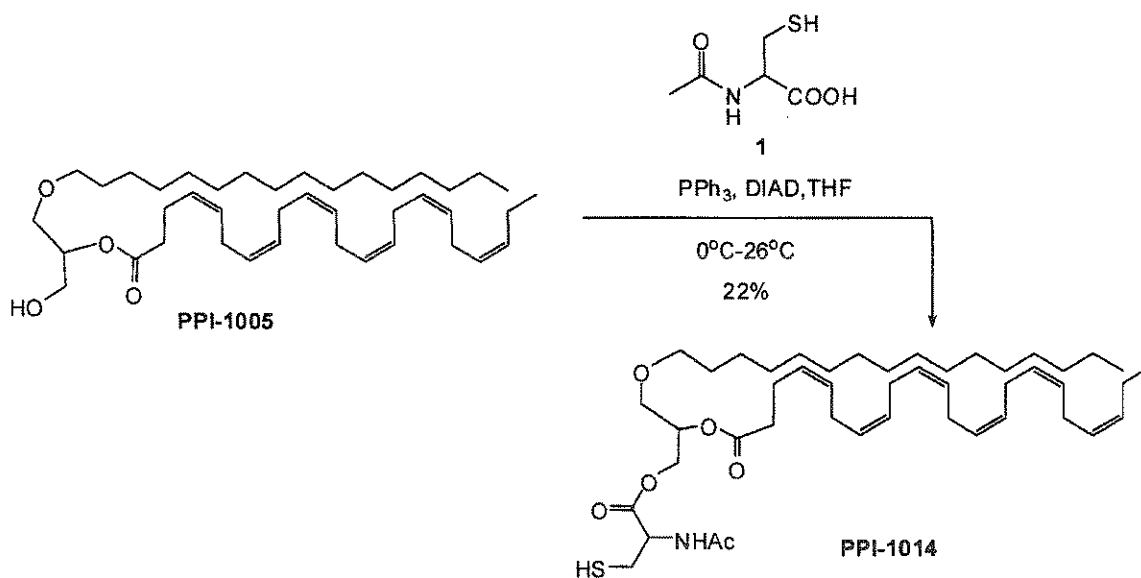
10 **Reaction step:**



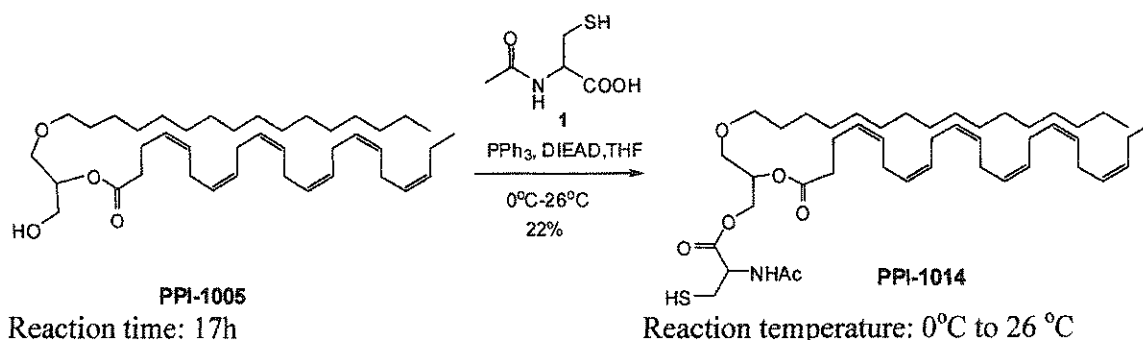
Brief procedure: To a cooled solution of compound **11** (50.0 g, 0.102 mol) in THF (1.0 L) at 0°C, pyridine (32.3 g, 0.409 mol) was added followed by DMAP (12.5g, 0.102 mol) and stirred for 10 min. Added a solution of crude compound **13** (57.0 g, 0.163 mol) in Toluene (1.0 mL) for 15 min. After addition the reaction mixture was stirred at RT for 20h. *(The reaction progress was monitored by quenching small aliquots with water, extracting with EtOAc and spotting over an analytical silica gel TLC plate (20% Methanol in chloroform) and visualizing spots using Mo stain and Ninhydrin solution).* The following are the R_fs of the components of the mixture: compound **11** (0.2) and **PPI-1009** (0.5). The reaction mixture was diluted with ethyl acetate (2.0 L), washed with 0.5N HCl (250 mL), brine solution (250.0 mL) dried over anhydrous Na₂SO₄ and concentrated to afford crude compound, which was purified by column chromatography (silica gel 100-200 mesh) using 20 % Methanol in chloroform as eluent to afford compound **PPI-1009** (27 g, 33.3%) obtained as pale yellow oil.

EXAMPLE 5: Preparation of PPI-1014**Target molecule**

5

Synthetic scheme:

Reaction step:



Reaction temperature: 0°C to 26 °C

5 **Brief procedure:** To a cooled solution of **PPI-1005** (12.0 g, 19.16 mmol) in THF (600 mL), added Triphenyl phosphene (7.5 g, 28.75 mmol) and stirred at 0°C for 10 min, followed by slow addition of DIEAD (5.8g, 28.75 mmol). After stirring at 0°C for 30 min the reaction mixture was added N-Acetyl cystine (4.6 g, 28.75 mmol) and allowed to stir at RT for 16h. (The reaction progress was monitored by extracting with ethyl acetate and spotting on an analytical silica gel TLC plate (30% Ethyl acetate in pet-ether), and visualizing spots using Mo stain). The following are the R_fs of the components of the mixture: **PPI-1005** (0.8), **PPI-1014** (0.4).

Work up and purification: The reaction mixture was diluted with water (75 mL) and extracted using ethyl acetate (3 x200mL). The combined ethyl acetate layer was washed with 15 brine (50 mL), dried over anhydrous Na₂SO₄ and concentrated to afford the crude compound, which was purified by column chromatography (100-200 mesh silica gel) using 0 to 13% ethyl acetate in pet ether as eluent.

The title compound **PPI-1014** (3.2 g, 22%) was obtained as pale yellow oil exhibited the following properties. ¹H NMR (300 MHz, CDCl₃): δ 6.35 (bs, 1H), 5.39-5.22 (m, 13H), 4.92-4.88 (m, 1H), 4.55-4.22 (m, 3H), 3.55-3.41 (m, 5H), 3.02-2.97 (m, 2H), 2.85-2.77 (m, 10H), 2.40 (s, 5H), 2.11-2.04 (m, 5H), 1.57-1.52 (m, 2H), 1.36-1.25 (m, 30H), 0.97 (t, *J* = 7.66Hz; 3H), 0.88 (t, *J* = 6.63Hz; 3H). Mass (M+H): 772.3, HPLC purity~93.68%.

EXAMPLE 6: Animal Studies

Male New Zealand rabbits (1.8-2.5 kg) were orally dosed with PPI-1011, neat in hard gelatin capsules (size 3). For time course studies, rabbits were dosed with 200 mg/kg of PPI-1011 and animals sacrificed via euthenol overdose at 1, 3, 6, 12, 18, 24 and 48 hr. Blood was

collected by cardiac puncture and plasma frozen at -70°C for later analysis. Kidney and liver also were harvested and stored at -70°C for later analysis. These studies were conducted as 2 experiments with overlapping groups at 12 hrs (Exp. 1: 1, 3, 6, and 12 hrs; Exp. 2: 12, 18, 24 and 48 hrs). Controls were harvested at each timepoint. Plasmalogens and lipids were
5 extracted and analyzed by LC-MS/MS as previously reported (Goodenowe et al., 2007).

As seen in Figure 11, using an oral dose of 200 mg/kg the plasmalogen precursor PPI-1011 was incorporated into circulating plasmalogens. Deacylation at sn-2, releasing docosahexaenoic acid (DHA), also was observed. The greatest incorporation was into the 16:0/22:6, 18:0/22:6 and 18:1/22:6 ethanolamine plasmalogens and
10 phosphatidylethanolamines. No change in the reference phosphatidylethanolamine 16:0/18:0 was observed.

Further examination of the timecourse (Figure 12) of incorporation in plasma revealed that maximum incorporation was at 12 hours and that this level of incorporation was maintained over the remaining observation period (48 hr). This was the case for both the
15 phosphatidylethanolamines and the ethanolamine plasmalogens. In contrast, circulating DHA levels peaked at 6 hours and fell to a lesser steady-state by 18 hours.

Studies of dose-dependent incorporation (Figure 13) of PPI-1011 into plasma plasmalogens and phosphatidylethanolamines demonstrated that a new steady-state level in these circulating phospholipids was attained in a dose-dependent manner from 10 to 200 mg/kg. However,
20 further increases in dose did not increase the steady-state levels of plasmalogens and phosphatidylethanolamines above that obtained with 200 mg/kg. In contrast, the highest steady-state levels of circulating DHA were obtained at 500 mg/kg and did not further increase at a dose of 1000 mg/kg.

Augmentation of tissue plasmalogens and DHA was also observed in kidney (Figures 4 and
25 5) and liver (Figures 6) tissues.

These results indicate that PPI-1011 is orally bioavailable in the rabbit and is converted to DHA-containing ethanolamine plasmalogens and phosphatidylethanolamines via deacylation/re-acylation reactions. In addition, the results suggest that endogenous metabolic systems may limit the maximal increase that that can be pharmacologically augmented.

EXAMPLE 7: Modulating membrane protein abundance *in vitro* with plasmalogen precursors

The following studies demonstrate the effectiveness of a plasmalogen precursor (1-alkyl-2 acyl glycerol) in altering the abundance of membrane-resident proteins. The cellular effects of the compound are demonstrated in wild-type cells, as well as in conditions of artificially elevated membrane cholesterol.

In wild-type cells, an elevation of amyloid precursor protein (APP) modulating enzyme ADAM10 and cholesterol esterification protein SOAT1 was observed with an increase in plasmalogen precursor concentration. Similar effects were observed in a cholesterol-loaded model. Additionally, a different APP processing enzyme, BACE1, showed a decrease in abundance only in cholesterol loaded cells. Without wishing to be bound by theory, this evidence supports a method of reducing the amyloid load in the context of Alzheimer's disease and simultaneously re-equilibrating the membrane cholesterol content of the system, thereby offering potential benefits in the treatment of diseases like atherosclerosis and hypercholesterolemia, in addition to Alzheimer's disease.

APP is predominantly processed by a canonical pathway comprised of sequential cleavage by γ -secretases (encoded by presenilin1/2 genes) and α -secretase (encoded by ADAM10). This non-pathological processing of APP results in the formation of a neurotrophic peptide (sAPP α) which exhibits protection against glutamate toxicity and hypoglycemia (Araki et al., 1991; Mattson et al., 1993; Postina et al., 2004; Fahrenholz, 2007). An alternate APP processing pathway manifests itself in Alzheimer's disease, wherein APP is cleaved by γ - and β -secretases in cholesterol-rich lipid rafts. This "non-canonical" pathway results in the formation of A β peptides 38-43 amino acids long which tend to aggregate into plaques in the extracellular matrix, a hallmark of AD (Selkoe, 2002; Walsh et al., 2002; Selkoe, 2003; Meyer-Luehmann et al., 2008). While early-onset familial AD is explained by genetic lesions in APP or APP processing enzymes (PSEN1/2, BACE, ADAM), the underlying cause of late-onset sporadic AD (switch from non-pathogenic to pathogenic APP processing) remains unclear.

The importance of cholesterol homeostasis in the etiology of AD has been investigated in humans (Corder et al., 1993; Saunders et al., 1993; Blacker et al., 1997; Hofman et al., 1997)

and in animal models (Joyce et al., 2002; Singaraja et al., 2002; Van Eck et al., 2006; Wahrle et al., 2008). Altering the cholesterol content of the plasma membrane has been shown to affect the function of membrane resident proteins (Scanlon et al., 2001; Lange et al., 2004). Elevated brain cholesterol was shown in subjects with AD (Mori et al., 2001), while rabbits
5 fed on a cholesterol-rich diet have been shown to develop plaques in the brain (Ghribi et al., 2006). *In vitro* data showed that plasmalogen deficient cells possess elevated free-cholesterol in the membrane, while in humans, serum-plasmalogen deficiency has been shown to correlate with a decline in cognition status (Goodenowe et al., 2007). Based on these observations, we investigated the interplay between cholesterol and plasmalogens, and
10 identify how the balance between the two affects the pathological manifestations of AD, measured in terms of secreted A β .

Proposed mode of action: A shift in APP processing via membrane lipid modulation

Human embryonic kidney (HEK293) cells express APP and the membrane-bound machinery required to process APP, making it a good model to study APP processing. The present *in*
15 *vitro* investigation corroborated previous studies in that modulating membrane fluidity via cholesterol loading and/or plasmalogen precursor supplementation altered the extracellular A β 42 content. Cholesterol loading of HEK293 cells increases the amount of free cholesterol by 17% (compared with Control) in the cells following a 48 hour incubation period. This elevation is accompanied with a parallel and significant increase ($p < 0.05$) in the A β 42
20 content in the conditioned medium compared with control. The 65% increase in amyloid is primarily due to 22 % increase in β -secretase concentration (Figure 18A, lane 2); the basal APP levels remain unchanged with cholesterol loading. Treatment of the cholesterol loaded HEK293 system with plasmalogen precursor PPI-1005 significantly reduced the free cholesterol content of the cell membrane ($p < 0.05$). The A β 42 content in the conditioned
25 medium dropped 70% below cholesterol-loaded levels at the 20 μ M concentration ($p = 0.0001$), while the sAPP α content in the conditioned medium was elevated. The effects on APP processing do not appear to be due to a change in APP expression, rather it appears to be due to a shift in the APP processing pathway by virtue of changes in the abundance of APP processing enzymes. While β -secretase was restored to normal levels at 20 μ M concentration
30 of PPI-1005, a 73% increase in sAPP α species was detected in the conditioned medium. This

elevation was explained by 63% increase in ADAM10 (Figure 18A, lane 3), the enzyme responsible for sAPP α formation.

The change in cholesterol profile of the cells following plasmalogen supplementation can be explained by the observation that SOAT1, the enzyme responsible for esterification of free cholesterol is approximately 25% upregulated (compared with the cholesterol loaded condition) with increasing plasmalogen concentration (Figure 18A, lane 5).

In a separate study, the effects of PPI-1005 were investigated in wild-type HEK293 cells, not loaded with cholesterol. Figure 18B shows a concentration-dependent increase in abundance of ADAM10 (35%) and SOAT1 (50%); No change in BACE1 or APP abundance was observed. Although when HEK293 cells were depleted of cholesterol by HMGCoA reductase inhibition by pravastatin, ADAM10 and SOAT1 protein levels remained constant (Figure 18C). While plasmalogen and pravastatin treatments both significantly reduce the fraction of free cholesterol in the cells, it is the plasmalogen treatment alone that alters ADAM10 and SOAT levels in the cell. This suggests that the effects on ADAM10 and SOAT1 abundance is an effect of membrane plasmalogen content rather than membrane cholesterol content.

Accordingly, the abundance of membrane-resident proteins can be altered *in vitro* by modulating the cellular plasmalogen content, which is achieved by treating cells with plasmalogen precursors as described herein.

Materials and Methods

Cholesterol loading

HEK 293 cells, cultured in DMEM, 10% FBS at 37°C, 5% CO₂, were seeded the day before the treatment. The following day, cells membranes were loaded with exogenous cholesterol at a concentration of 10 μ g/ml media using methyl- β -cyclodextrin as the carrier to deliver cholesterol as described (Rong et al., 2003).

Cholesterol Assay

Cells were treated with the plasmalogen precursor PPI-1005 or with ethanol as the control. Cells were harvested after 48 hours using Versene: TryPLE express cocktail, washed with PBS. Lipids were extracted with chloroform containing 1% Triton X-100. The organic

fraction was recovered and dried under a stream of nitrogen. The dried lipids were resuspended in cholesterol reaction buffer (Biovision, Mountain View, CA), and the total, free and esterified fractions of cholesterol were quantified using the cholesterol quantification kit (Biovision, Mountain View, CA) as per the manufacturer's recommendations. Cholesterol was initially calculated as $\mu\text{g}/\text{million cells}$, and reported as a percentage of control conditions for each experiment.

Amyloid Assay

HEK293 cells were loaded with exogenous cholesterol as described, and treated with a PPI 1005 or with ethanol as a control. Conditioned media from the treated cells was collected at the end of a 48 hour incubation period. For assaying $\text{A}\beta_{1-42}$ content, the conditioned media was enriched using Amicon ultracentrifugal filter devices (Millipore, Billerica, MA) prior to loading into the microplate. ELISA was carried out as per the manufacturer's recommendations (Covance Labs, Princeton, NJ). The reactions were quenched 25 minutes after addition of the substrate, and the absorbance was read at 495nm. The experiment was carried out in triplicate. Values were calculated as pg/ml of conditioned media, and were normalized to the amount of $\text{A}\beta$ detected in the conditioned media from untreated, cholesterol loaded control HEK293 cells.

Immunoblotting and Immunoprecipitation

HEK293 cells were treated as described in the amyloid assay. The cell pellet was washed in PBS and lysed in a RIPA buffer containing a protease inhibitor cocktail (Sigma, St. Louis, MI). Protein in the cell lysate was quantified using the Bio-Rad Protein Assay (Bio-Rad, Hercules, CA). The following antibodies were used for western analyses: APP (Calbiochem, Darmstadt, Germany), BACE1 and ADAM10 (Millipore, Temecula, CA), sAPP α (IBL, Gunma, Japan), SOAT1 (Santa Cruz Biotechnology Inc., CA), and β -actin (Sigma, St. Louis, MI). Immunoprecipitation was carried out to estimate sAPP α in the conditioned medium. Briefly, antibody to sAPP α was added to conditioned media and incubated for 16 hours at 4°C. IP was carried out by incubating with protein A/G agarose beads for 6 hours at 4°C. Beads were washed with PBS and the eluted proteins were detected by immunoblotting with anti-sAPP α antibody. Band intensities were quantified using Image Processing and Analysis in Java (ImageJ) software (National Institutes of Health, Bethesda, MD)

Statistical analysis

Statistical Analysis of the data was performed using MicrosoftTM Office Excel 2007 and JMP version 8. Multiple comparison Dunnett's tests were applied to analyze the differences between the treatments and the control.

- 5 It will be apparent to persons skilled in the art that a number of variations and modifications can be made without departing from the scope of the invention as defined in the claims.

The following references, as well as those references provided above, are hereby incorporated by reference.

References

- 5 1. Calderini G, Bonetti AC, Battistella A, Crews FT, Toffano G (1983) Biochemical changes of rat brain membranes with aging. *Neurochem Res.* 8:483-92.
2. Hashimoto M, Hossain S, Masumura S (1999) Effect of aging on plasma membrane fluidity of rat aortic endothelial cells. *Exp Gerontol.* 34:687-98.
3. Kessler AR, Kessler B, Yehuda S (1985) Changes in the cholesterol level, cholesterol-to-
10 phospholipid mole ratio, and membrane lipid microviscosity in rat brain induced by age and a plant oil mixture. *Biochem Pharmacol.* 34:1120-1.
4. Lewin MB, Timiras PS (1984) Lipid changes with aging in cardiac mitochondrial membranes. *Mech Ageing Dev.* 24:343-51.
5. Modi HR, Katyare SS, Patel MA (2008) Ageing-induced alterations in lipid/phospholipid
15 profiles of rat brain and liver mitochondria: implications for mitochondrial energy-linked functions. *J Membr Biol.* 221:51-60.
6. Wu CC, Su MJ, Chi JF, Wu MH, Lee YT (1997) Comparison of aging and hypercholesterolemic effects on the sodium inward currents in cardiac myocytes. *Life Sci.* 61:1539-51.
- 20 7. Guo J, Chi S, Xu H, Jin G, Qi Z (2008) Effects of cholesterol levels on the excitability of rat hippocampal neurons. *Mol Membr Biol.* 25:216-23.
8. Santiago J, Guzmàn GR, Rojas LV, Marti R, Asmar-Rovira GA, Santana LF, McNamee M, Lasalde-Dominicci JA (2001) Probing the effects of membrane cholesterol in the Torpedo californica acetylcholine receptor and the novel lipid-exposed mutation alpha
25 C418W in *Xenopus* oocytes. *J Biol Chem.* 276:46523-32.
9. Hashimoto M, Hossain S, Tanabe Y, Shido O (2005) Effects of aging on the relation of adenylyl purine release with plasma membrane fluidity of arterial endothelial cells. *Prostaglandins Leukot. Essent. Fatty Acids.* 73 :475-83.
10. Xiu J, Nordberg A, Qi X, Guan ZZ (2006) Influence of cholesterol and lovastatin on
30 alpha-form of secreted amyloid precursor protein and expression of alpha7 nicotinic receptor on astrocytes. *Neurochem Int.* 49:459-65.
11. Miersch S, Espey MG, Chaube R, Akarca A, Tweten R, Ananvoranich S, Mutus B (2008) Plasma membrane cholesterol content affects nitric oxide diffusion dynamics and signaling. *J Biol Chem.* 283:18513-21.

12. Cultler RG, Kelly J, Storie K, Pedersen WA, Tammara A, Hatanpaa K, Troncoso JC, Mattson MP (2004) Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. *Proc Natl Acad Sci U S A*. 101:2070-5.
- 5 13. Corrigan FM, Horrobin DF, Skinner ER, Besson JA, Cooper MB (1998) Abnormal content of n-6 and n-3 long-chain unsaturated fatty acids in the phosphoglycerides and cholesterol esters of parahippocampal cortex from Alzheimer's disease patients and its relationship to acetyl CoA content. *Int J Biochem Cell Biol*. 30:197-207.
14. Distl R, Meske V, Ohm TG (2001) Tangle-bearing neurons contain more free cholesterol
10 than adjacent tangle-free neurons. *Acta Neuropathol*. 101:547-54.
15. Grimm MO, Grimm HS, Tomic I, Beyreuther K, Hartmann T, Bergmann C (2008) Independent inhibition of Alzheimer disease beta- and gamma-secretase cleavage by lowered cholesterol levels. *J Biol Chem*. 283:11302-11.
16. Simons M, Keller P, De Strooper B, Beyreuther K, Dotti CG, Simons K (1998)
15 Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. *Proc Natl Acad Sci U S A*. 95:6460-4.
17. Beel AJ, Mobley CK, Kim HJ, Tian F, Hadziselimovic A, Jap B, Prestegard JH, Sanders CR (2008) Structural studies of the transmembrane C-terminal domain of the amyloid precursor protein (APP): does APP function as a cholesterol sensor? *Biochemistry*.
20 47:9428-46.
18. Sigle JP, Zander J, Ehret A, Honegger J, Jackisch R, Feuerstein TJ (2003) High potassium-induced activation of choline-acetyltransferase in human neocortex: implications and species differences. *Brain Res Bull*. 60:255-62.
19. Wolozin B, Wang SW, Li NC, Lee A, Lee TA, Kazis LE (2007) Simvastatin is
25 associated with a reduced incidence of dementia and Parkinson's disease. *BMC Med*. 5:20.
20. Li CM, Clark ME, Rudolf M, Curcio CA (2007) Distribution and composition of esterified and unesterified cholesterol in extra-macular drusen. *Exp Eye Res*. 85:192-201.
21. Hager MH, Solomon KR, Freeman MR (2006) The role of cholesterol in prostate cancer.
30 *Curr Opin Clin Nutr Metab Care*. ;9:379-85.
22. Campbell AM, Chan SH (2008) Mitochondrial membrane cholesterol, the voltage dependent anion channel (VDAC), and the Warburg effect. *J Bioenerg Biomembr*.

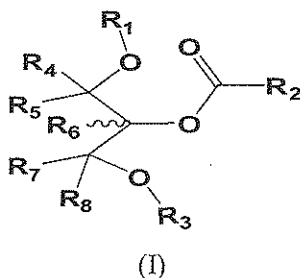
23. Vejux A, Malvitte L, Lizard G (2008) Side effects of oxysterols: cytotoxicity, oxidation, inflammation, and phospholipidosis. *Braz J Med Biol Res.* 41:545-56.
24. Streit WJ, Sparks DL (1997) Activation of microglia in the brains of humans with heart disease and hypercholesterolemic rabbits. *J Mol Med.* 75:130-8.
- 5 25. Diestel A, Aktas O, Hackel D, Hake I, Meier S, Raine CS, Nitsch R, Zipp F, Ullrich O (2003) Activation of microglial poly(ADP-ribose)-polymerase-1 by cholesterol breakdown products during neuroinflammation: a link between demyelination and neuronal damage. *J Exp Med.* 198:1729-40.
26. Thirumangalakudi L, Prakasam A, Zhang R, Bimonte-Nelson H, Sambamurti K, Kindy
10 MS, Bhat NR (2008) High cholesterol-induced neuroinflammation and amyloid precursor protein processing correlate with loss of working memory in mice. *J Neurochem.* 106:475-85.
27. Shin J, Thompson DH (2003) Direct synthesis of plasmenylcholine from allyl-substituted glycerols. *J Org Chem.* 68:6760-6.
- 15 28. Gupta CM, Radhakrishnan R, Khorana HG (1977) Glycerophospholipid synthesis: improved general method and new analogs containing photoactivable groups. *Proc Natl Acad Sci U S A.* 74:4315-9.
29. Watt MJ, Steinberg GR (2008) Regulation and function of triacylglycerol lipases in cellular metabolism. *Biochem J.* 414:313-25.
- 20 30. Nagan N, Hajra AK, Larkins LK, Lazarow P, Purdue PE, Rizzo WB, Zoeller RA. (1998) Isolation of a Chinese hamster fibroblast variant defective in dihydroxyacetonephosphate acyltransferase activity and plasmalogen biosynthesis: use of a novel two-step selection protocol. *Biochem J.* 332:273-9.
31. Goodenowe DB, Cook LL, Liu J, Lu Y, Jayasinghe DA, Ahiahonu PW, Heath D,
25 Yamazaki Y, Flax J, Krenitsky KF, Sparks DL, Lerner A, Friedland RP, Kudo T, Kamino K, Morihara T, Takeda M, Wood PL (2007) Peripheral ethanolamine plasmalogen deficiency: a logical causative factor in Alzheimer's disease and dementia. *J Lipid Res.* 48:2485-98.
32. Araki W, Kitaguchi N, Tokushima Y, Ishii K, Aratake H, Shimohama S, Nakamura S and
30 Kimura J (1991) Trophic effect of beta-amyloid precursor protein on cerebral cortical neurons in culture. *Biochem Biophys Res Commun* 181:265-271.

33. Blacker D, Haines JL, Rodes L, Terwedow H, Go RC, Harrell LE, Perry RT, Bassett SS, Chase G, Meyers D, Albert MS and Tanzi R (1997) ApoE-4 and age at onset of Alzheimer's disease: the NIMH genetics initiative. *Neurology* **48**:139-147.
34. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW,
5 Roses AD, Haines JL and Pericak-Vance MA (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**:921-923.
35. Fahrenholz F (2007) Alpha-secretase as a therapeutic target. *Curr Alzheimer Res* **4**:412-417.
36. Ghribi O, Larsen B, Schrag M and Herman MM (2006) High cholesterol content in
10 neurons increases BACE, beta-amyloid, and phosphorylated tau levels in rabbit hippocampus. *Exp Neurol* **200**:460-467.
37. Hofman A, Ott A, Breteler MM, Bots ML, Slooter AJ, van Harskamp F, van Duijn CN, Van Broeckhoven C and Grobbee DE (1997) Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam Study. *Lancet* **349**:151-
15 154.
38. Joyce CW, Amar MJ, Lambert G, Vaisman BL, Paigen B, Najib-Fruchart J, Hoyt RF, Jr., Neufeld ED, Remaley AT, Fredrickson DS, Brewer HB, Jr. and Santamarina-Fojo S (2002) The ATP binding cassette transporter A1 (ABCA1) modulates the development of aortic atherosclerosis in C57BL/6 and apoE-knockout mice. *Proc Natl Acad Sci U S A*
20 **99**:407-412.
39. Lange Y, Ye J and Steck TL (2004) How cholesterol homeostasis is regulated by plasma membrane cholesterol in excess of phospholipids. *Proc Natl Acad Sci U S A* **101**:11664-11667.
40. Mattson MP, Cheng B, Culwell AR, Esch FS, Lieberburg I and Rydel RE (1993)
25 Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the beta-amyloid precursor protein. *Neuron* **10**:243-254.
41. Meyer-Luehmann M, Spires-Jones TL, Prada C, Garcia-Alloza M, de Calignon A, Rozkalne A, Koenigsknecht-Talboo J, Holtzman DM, Bacskai BJ and Hyman BT (2008) Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of
30 Alzheimer's disease. *Nature* **451**:720-724.
42. Mori T, Paris D, Town T, Rojiani AM, Sparks DL, Delledonne A, Crawford F, Abdullah LI, Humphrey JA, Dickson DW and Mullan MJ (2001) Cholesterol accumulates in senile

- plaques of Alzheimer disease patients and in transgenic APP(SW) mice. *J Neuropathol Exp Neurol* **60**:778-785.
43. Postina R, Schroeder A, Dewachter I, Bohl J, Schmitt U, Kojro E, Prinzen C, Endres K, Hiemke C, Blessing M, Flamez P, Dequenue A, Godaux E, van Leuven F and Fahrenholz F (2004) A disintegrin-metalloproteinase prevents amyloid plaque formation and hippocampal defects in an Alzheimer disease mouse model. *J Clin Invest* **113**:1456-1464.
44. Rong JX, Shapiro M, Trogan E and Fisher EA (2003) Transdifferentiation of mouse aortic smooth muscle cells to a macrophage-like state after cholesterol loading. *Proc Natl Acad Sci U S A* **100**:13531-13536.
45. Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL, Gusella JF, Crapper-MacLachlan DR, Alberts MJ and et al. (1993) Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* **43**:1467-1472.
46. Scanlon SM, Williams DC and Schloss P (2001) Membrane cholesterol modulates serotonin transporter activity. *Biochemistry* **40**:10507-10513.
47. Selkoe DJ (2002) Alzheimer's disease is a synaptic failure. *Science* **298**:789-791.
48. Selkoe DJ (2003) Folding proteins in fatal ways. *Nature* **426**:900-904.
49. Singaraja RR, Fievet C, Castro G, James ER, Hennuyer N, Clee SM, Bissada N, Choy JC, Fruchart JC, McManus BM, Staels B and Hayden MR (2002) Increased ABCA1 activity protects against atherosclerosis. *J Clin Invest* **110**:35-42.
50. Van Eck M, Singaraja RR, Ye D, Hildebrand RB, James ER, Hayden MR and Van Berkel TJ (2006) Macrophage ATP-binding cassette transporter A1 overexpression inhibits atherosclerotic lesion progression in low-density lipoprotein receptor knockout mice. *Arterioscler Thromb Vasc Biol* **26**:929-934.
51. Wahrle SE, Jiang H, Parsadanian M, Kim J, Li A, Knoten A, Jain S, Hirsch-Reinshagen V, Wellington CL, Bales KR, Paul SM and Holtzman DM (2008) Overexpression of ABCA1 reduces amyloid deposition in the PDAPP mouse model of Alzheimer disease. *J Clin Invest* **118**:671-682.
52. Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ and Selkoe DJ (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* **416**:535-539.

CLAIMS

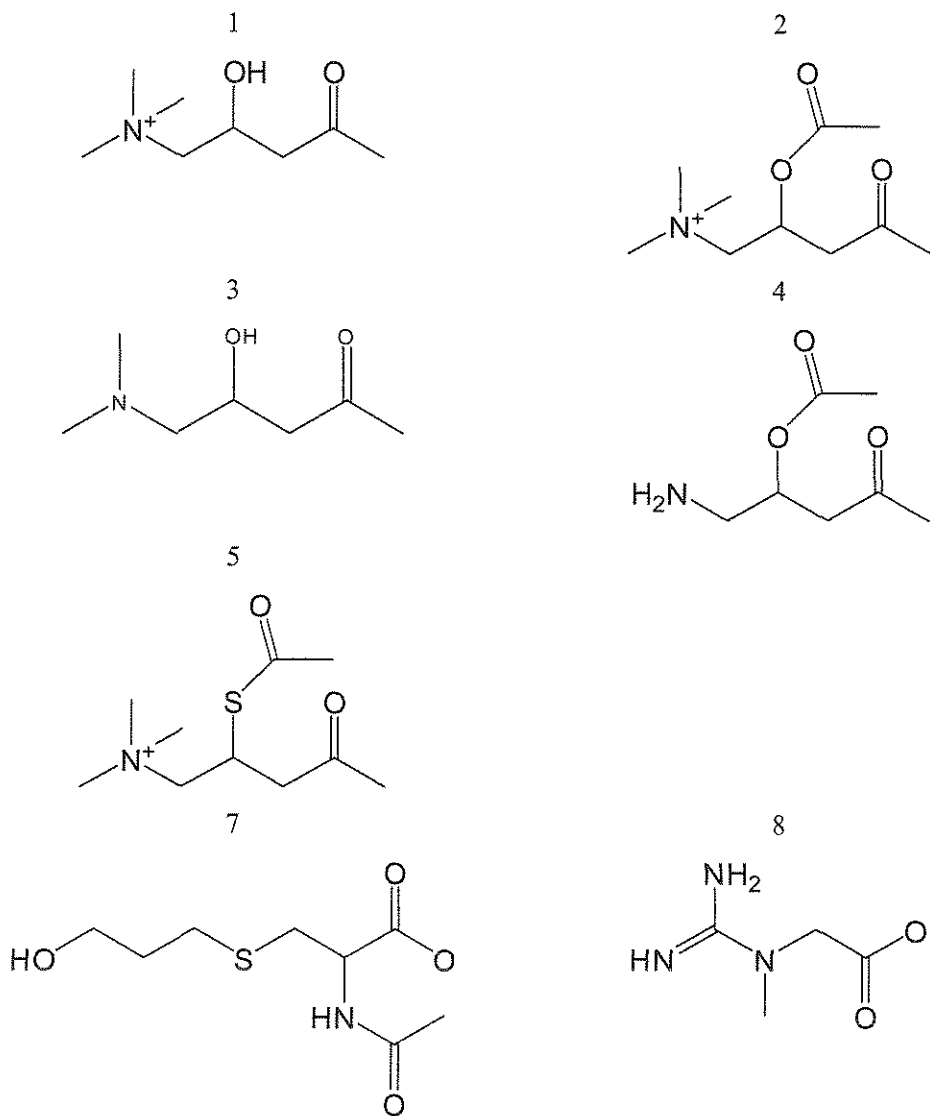
1. A compound according to the structure of formula I:



wherein:

R_1 and R_2 are the same or different and selected from an alkyl or alkenyl hydrocarbon chain selected from the group consisting of: $\text{CH}_3(\text{CH}_2)_3-$, $\text{CH}_3(\text{CH}_2)_5-$, $\text{CH}_3(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_9-$, $\text{CH}_3(\text{CH}_2)_{11}-$, $\text{CH}_3(\text{CH}_2)_{13}-$, $\text{CH}_3(\text{CH}_2)_{15}-$, $\text{CH}_3(\text{CH}_2)_{17}-$, $\text{CH}_3(\text{CH}_2)_{19}-$, $\text{CH}_3(\text{CH}_2)_{21}-$, $\text{CH}_3(\text{CH}_2)_{23}-$, $\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3\text{CH}_2(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_3(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_7(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_9(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{11}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{13}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{15}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{17}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{19}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{21}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})$, $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_2(\text{CH}_2)_6-$, $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_3(\text{CH}_2)_6-$, $\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_4(\text{CH}_2)_2-$, $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_5(\text{CH}_2)_2-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_{11}$, and $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_6\text{CH}_2-$;

R_3 is selected from the group consisting of carnitine, acetyl-D/L-carnitine, thiocarnitine, acetyl-D/L-thiocarnitine, creatine, norcarnitine, lipoic acid, dihydrolipoic acid, N-acetylcysteine, substituted or unsubstituted amino acid groups and groups of the structures shown below:



R_4 and R_5 are independently hydrogen or lower alkyl;

R_6 is hydrogen or lower alkyl; and

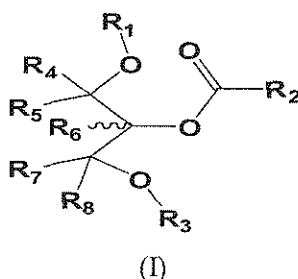
R_7 and R_8 are independently hydrogen or lower alkyl,

including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof.

2. The compound of claim 1, wherein R_2 is $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_6\text{CH}_2-$.

3. The compound of claim 1, wherein said compound is selected from the group consisting of 2-acetoxy-4-(2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4, 7,10, 13, 16, 19-hexaenoyloxy)-3-(hexadecyloxy)propoxy)-N,N,N-trimethyl-4-oxobutan-1-aminium (PPI-1009), (4Z,7Z,10Z,13Z,16Z,19Z)-1-(5-((R)-1,2-dithiolan-3-yl)pentanoyloxy)-3-(hexadecyloxy)propan-2-yl docosa-4, 7, 10, 13, 16, 19-hexaenoate (PPI-1011) and (4Z,7Z,10Z,13Z,16Z,19Z)-1-(2-acetamido-3-mercaptopropanoyloxy)-3-(hexadecyloxy)propan-2-yl docosa-4, 7, 10, 13, 16, 19-hexaenoate (PPI-1014).

4. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of formula I:

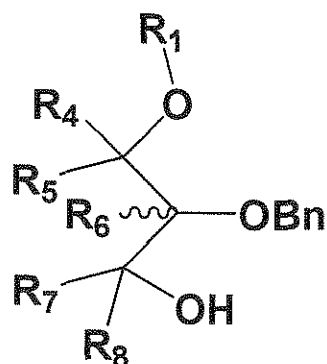


wherein R_1 to R_8 are as defined in claim 1, and including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof.

5. The pharmaceutical composition of claim 4, wherein R_2 is $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_6\text{CH}_2-$.

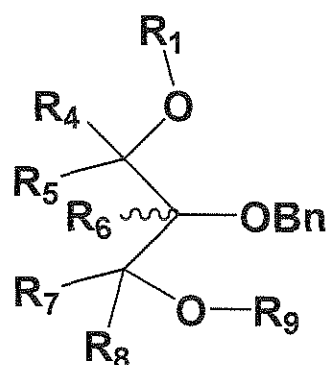
6. The pharmaceutical composition of claim 4, wherein the compound is selected from the group consisting of 2-acetoxy-4-(2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4, 7, 10, 13, 16, 19-hexaenoyloxy)-3-(hexadecyloxy)propoxy)-N,N,N-trimethyl-4-oxobutan-1-aminium (PPI-1009), (4Z,7Z,10Z,13Z,16Z,19Z)-1-(5-((R)-1,2-dithiolan-3-yl)pentanoyloxy)-3-(hexadecyloxy)propan-2-yl docosa-4, 7, 10, 13, 16, 19-hexaenoate (PPI-1011) and (4Z,7Z,10Z,13Z,16Z,19Z)-1-(2-acetamido-3-mercaptopropanoyloxy)-3-(hexadecyloxy)propan-2-yl docosa-4, 7, 10, 13, 16, 19-hexaenoate (PPI-1014).

7. A process for preparing a compound according to the structure of formula II:



(II)

wherein R_1 and R_4 to R_8 are as defined in claim 1, and including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof,
said process comprising reacting a compound of formula III:

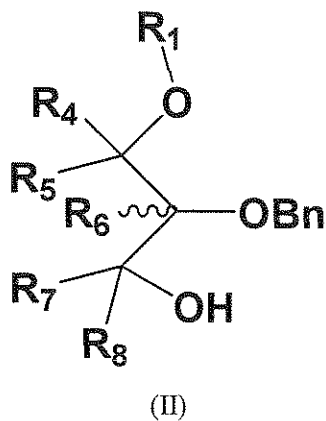


(III)

wherein R_9 is a blocking group, in a suitable solvent with tetrabutylammonium fluoride (TBAF) under conditions to form said compound of formula II, and optionally purifying said compound of formula II.

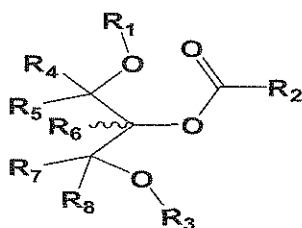
8. The process of claim 7, wherein said blocking group is a silane blocking group.
9. The process of claim 8, wherein the silane blocking group is a tert-butyldimethylsilyl halide.
10. The process of claim 7, wherein said solvent comprises dimethylformamide (DMF).

11. The process of claim 7, wherein said reaction is carried out at a temperature of 0 to 26 °C.
12. The process of claim 11, wherein said reaction is carried out at about 20 °C.
13. The process of claim 11, wherein said reaction is allowed to proceed for up to 20 hours.
14. A compound according to the structure of formula II:



wherein R₁ and R₄ to R₈ are as defined in claim 1, including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof.

15. Use of a compound of formula I:



(I)

wherein R₁ to R₈ are as defined in claim 1, including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof, or a pharmaceutical composition comprising said compound admixed with a pharmaceutically acceptable carrier, for the manufacture of a medicament for treating or preventing diseases of aging associated with increased membrane cholesterol, increased amyloid or decreased plasmalogen levels, wherein the diseases of aging are selected from the group consisting of: neurodegeneration, cognitive impairment, dementia, cancer, and vascular disease.

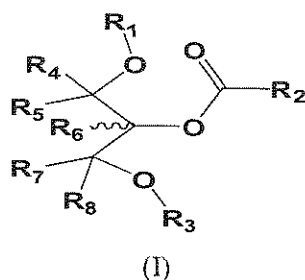
16. The use of claim 15, wherein R₂ is CH₃CH₂(CH=CHCH₂)₆CH₂-.

17. The use of claim 15 or 16, wherein the neurodegeneration disease is selected from the group consisting of: Alzheimer's disease, Parkinson's disease and age-related macular degeneration.

18. The use of claim 15 or 16, wherein the cancer is selected from the group consisting of: prostate, lung, breast, ovarian, and kidney cancer.

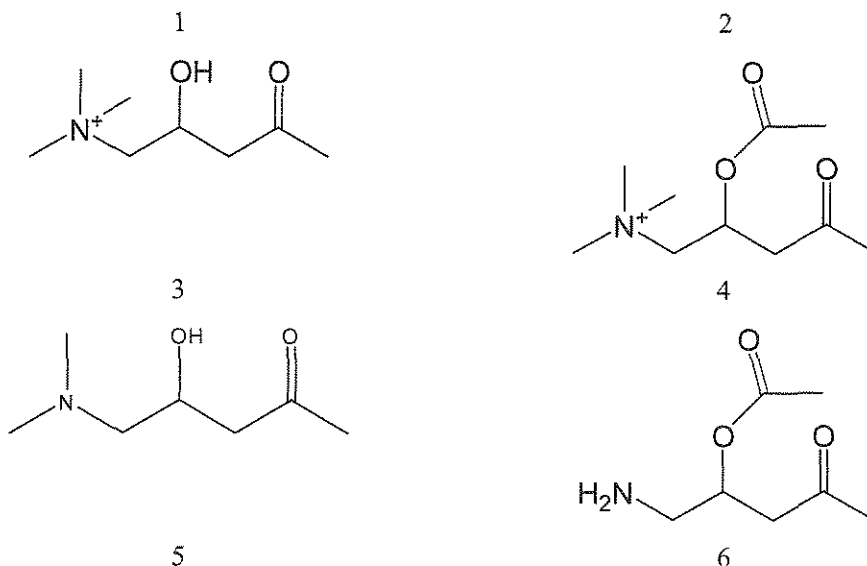
19. The use of claim 15 or 16, wherein the vascular disease is selected from the group consisting of: atherosclerosis and hypercholesterolemia.

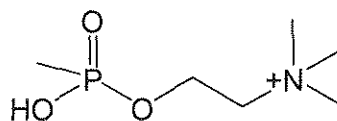
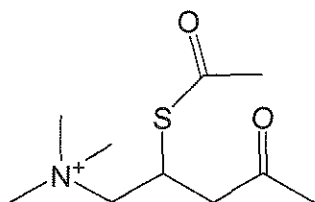
20. Use of a compound of formula I:



wherein

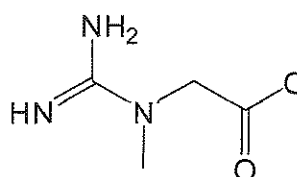
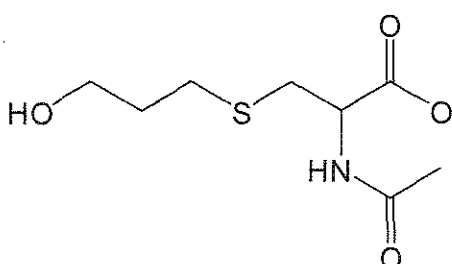
R₃ is selected from the group consisting of fatty acids having a chain of 4 to 28 carbons, carnitine, acetyl-D/L-carnitine, thiocarnitine, acetyl-D/L-thiocarnitine, creatine, norcarnitine, phosphocholine, lipoic acid, dihydrolipoic acid, phosphoethanolamine, phosphoserine, N-acetylcysteine, substituted or unsubstituted amino acid groups and groups of the structures shown below:





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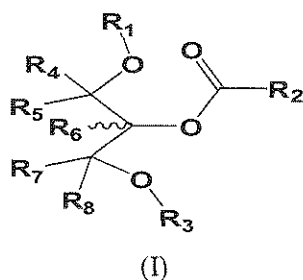


and R_1 , R_2 and R_4 to R_8 are as defined in claim 1, including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof, or a pharmaceutical composition comprising said compound admixed with a pharmaceutically acceptable carrier, for the manufacture of a medicament for treating or preventing diseases of the aging mediated by plasmalogen deficiency, wherein the diseases of aging are selected from the group consisting of: neurodegeneration, cognitive impairment, dementia, cancer, and vascular disease, by increasing PlsEtn levels to above control levels in either PlsEtn deficient or PlsEtn sufficient systems.

21. The use of claim 20, wherein R_2 is $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_6\text{CH}_2-$.
22. The use of claim 20 or 21, wherein the neurodegeneration disease is selected from the group consisting of: Alzheimer's disease, Parkinson's disease and age-related macular degeneration.
23. The use of claim 20 or 21, wherein the cancer is selected from the group consisting of: prostate, lung, breast, ovarian, and kidney cancer.

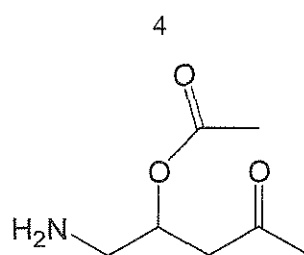
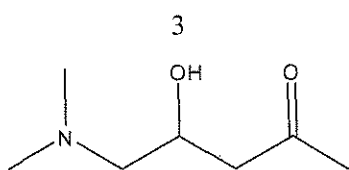
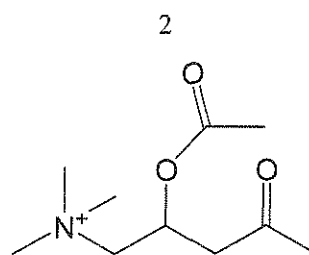
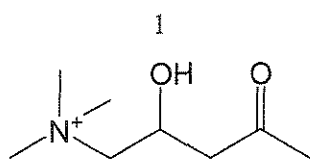
24. The use of claim 20 or 21, wherein the vascular disease is selected from the group consisting of: atherosclerosis and hypercholesterolemia.

25. Use of a compound of formula I:

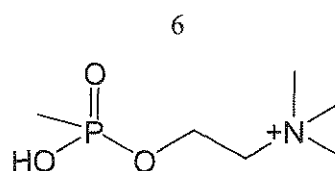


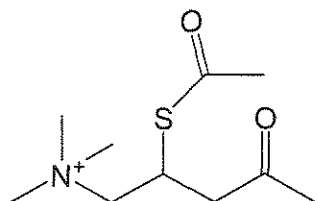
wherein

R₃ is selected from the group consisting of fatty acids having a chain of 4 to 28 carbons, carnitine, acetyl-D/L-carnitine, thiocarnitine, acetyl-D/L-thiocarnitine, creatine, norcarnitine, phosphocholine, lipoic acid, dihydrolipoic acid, phosphoethanolamine, phosphoserine, N-acetylcysteine, substituted or unsubstituted amino acid groups and groups of the structures shown below:

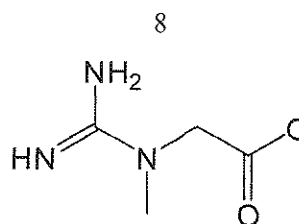
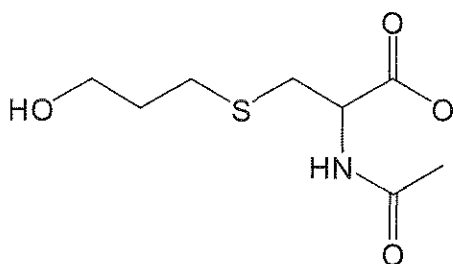


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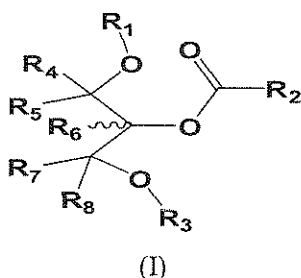
and R₁, R₂ and R₄ to R₈ are as defined in claim 1, including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof, or a pharmaceutical composition comprising said compound admixed with a pharmaceutically acceptable carrier, for the manufacture of a medicament for treating or preventing a disorder resulting from abnormal genetic expression of cholesterol transport proteins, wherein the disorder is selected from the group consisting of: neurodegeneration, cognitive impairment, dementia, cancer, and vascular disease.

26. The use of claim 25, wherein R₂ is CH₃CH₂(CH=CHCH₂)₆CH₂-.
27. The use of claim 25 or 26, wherein the cholesterol transport protein is apolipoprotein E.
28. The use of any one of claims 25 to 27, wherein the neurodegeneration disease is selected from the group consisting of: Alzheimer's disease, Parkinson's disease and age-related macular degeneration.
29. The use of any one of claims 25 to 27, wherein the cancer is selected from the group consisting of: prostate, lung, breast, ovarian, and kidney cancer.

30. The use of any one of claims 25 to 27, wherein the vascular disease is selected from the group consisting of: atherosclerosis and hypercholesterolemia.

31. The use of any one of claims 15 to 30, wherein the compound is selected from the group consisting of 2-acetoxy-4-(2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4, 7, 10, 13, 16, 19-hexaenoyloxy)-3-(hexadecyloxy)propoxy)-N,N,N-trimethyl-4-oxobutan-1-aminium (PPI-1009), (4Z,7Z,10Z,13Z,16Z,19Z)-1-(5-((R)-1,2-dithiolan-3-yl)pentanoyloxy)-3-(hexadecyloxy)propan-2-yl docosa-4, 7, 10, 13, 16, 19-hexaenoate (PPI-1011) and (4Z,7Z,10Z,13Z,16Z,19Z)-1-(2-acetamido-3-mercaptopropanoyloxy)-3-(hexadecyloxy)propan-2-yl docosa-4, 7, 10, 13, 16, 19-hexaenoate (PPI-1014).

32. Use of a compound of formula I:

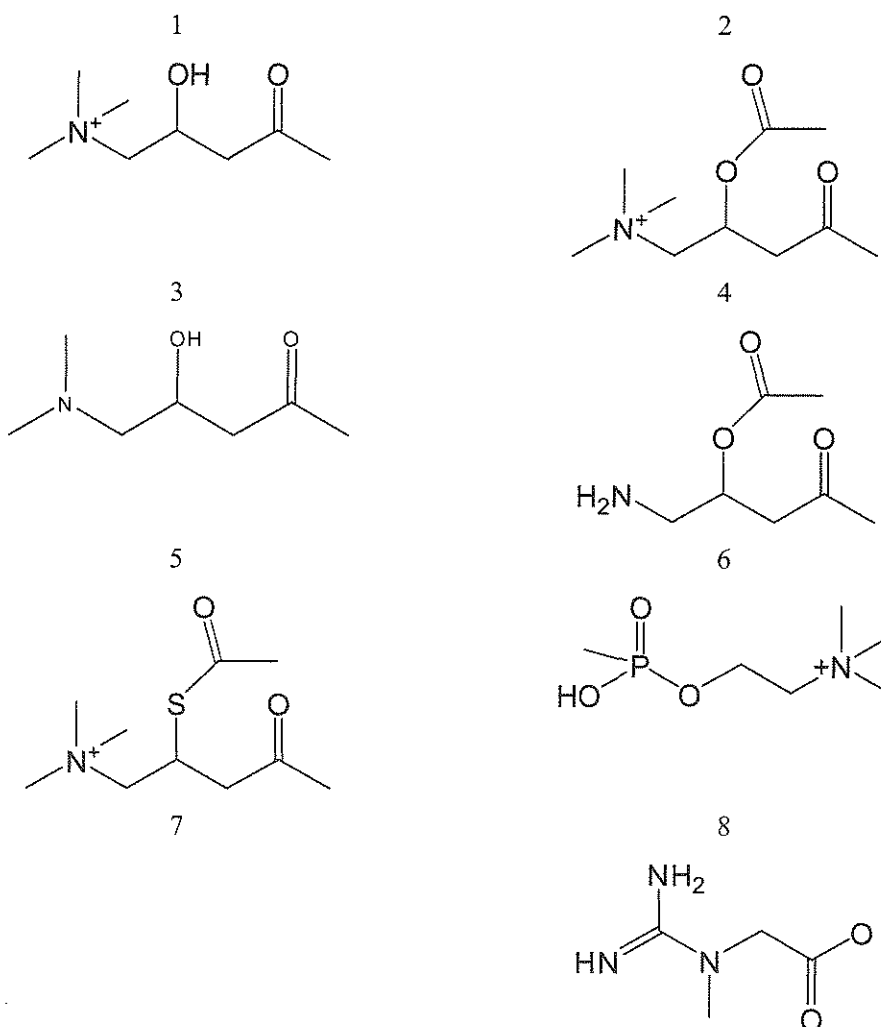


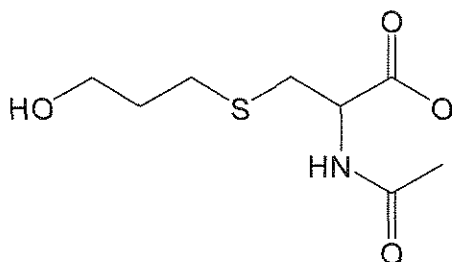
for the manufacture of a medicament for treating or preventing a neurodegeneration disease selected from the group consisting of Alzheimer's disease, Parkinson's disease and age-related macular degeneration, wherein

R₁ and R₂ are the same or different and selected from an alkyl or alkenyl hydrocarbon chain selected from the group consisting of: CH₃(CH₂)₃-, CH₃(CH₂)₅-, CH₃(CH₂)₇-, CH₃(CH₂)₉-, CH₃(CH₂)₁₁-, CH₃(CH₂)₁₃-, CH₃(CH₂)₁₅-, CH₃(CH₂)₁₇-, CH₃(CH₂)₁₉-, CH₃(CH₂)₂₁-, CH₃(CH₂)₂₃-, CH₃(CH₂)₃CH=CH(CH₂)₇-, CH₃(CH₂)₅CH=CH(CH₂)₇-, CH₃(CH₂)₇CH=CH(CH₂)₇-, CH₃(CH₂)₄CH=CHCH₂CH=CH(CH₂)₇-, CH₃CH₂CH=CHCH₂CH=CHCH₂CH=CH(CH₂)₇-, CH₃CH₂(CH=CH)-, CH₃(CH₂)₃(CH=CH)-, CH₃(CH₂)₅(CH=CH)-, CH₃(CH₂)₇(CH=CH)-, CH₃(CH₂)₉(CH=CH)-, CH₃(CH₂)₁₁(CH=CH)-, CH₃(CH₂)₁₃(CH=CH)-, CH₃(CH₂)₁₅(CH=CH)-, CH₃(CH₂)₁₇(CH=CH)-, CH₃(CH₂)₁₉(CH=CH)-, CH₃(CH₂)₂₁(CH=CH)-, CH₃(CH₂)₃CH=CH(CH₂)₅(CH=CH)-, CH₃(CH₂)₅CH=CH(CH₂)₅(CH=CH)-, CH₃(CH₂)₇CH=CH(CH₂)₅(CH=CH)-,

$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})$,
 $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})$ -, $\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_7$ -,
 $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7$ -, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7$ -, $\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_2(\text{CH}_2)_6$ -,
 $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_3(\text{CH}_2)_6$ -, $\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_4(\text{CH}_2)_2$ -,
 $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_5(\text{CH}_2)_2$ -, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_{11}$, and $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_6\text{CH}_2$ -;

R_3 is selected from the group consisting of fatty acids having a chain of 4 to 28 carbons, carnitine, acetyl-D/L-carnitine, thiocarnitine, acetyl-D/L-thiocarnitine, creatine, norcarnitine, phosphocholine, lipoic acid, dihydrolipoic acid, phosphoethanolamine, phosphoserine, N-acetylcysteine, substituted or unsubstituted amino acid groups and groups of the structures shown below:





R₄ and R₅ are independently hydrogen or lower alkyl;

R₆ is hydrogen or lower alkyl; and

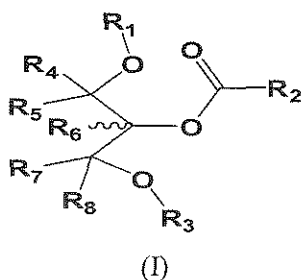
R₇ and R₈ are independently hydrogen or lower alkyl,

including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof.

33. The use of claim 32, wherein R₂ is CH₃CH₂(CH=CHCH₂)₆CH₂-.

34. The use of claim 32, wherein the compound is selected from the group consisting of 2-acetoxy-4-(2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4, 7, 10, 13, 16, 19-hexaenoyloxy)-3-(hexadecyloxy)propoxy)-N,N,N-trimethyl-4-oxobutan-1-aminium (PPI-1009), (4Z,7Z,10Z,13Z,16Z,19Z)-1-(5-((R)-1,2-dithiolan-3-yl)pentanoyloxy)-3-(hexadecyloxy)propan-2-yl docosa-4, 7, 10, 13, 16, 19-hexaenoate (PPI-1011) and (4Z,7Z,10Z,13Z,16Z,19Z)-1-(2-acetamido-3-mercaptopropanoyloxy)-3-(hexadecyloxy)propan-2-yl docosa-4, 7, 10, 13, 16, 19-hexaenoate (PPI-1014).

35. Use of a compound of formula I:



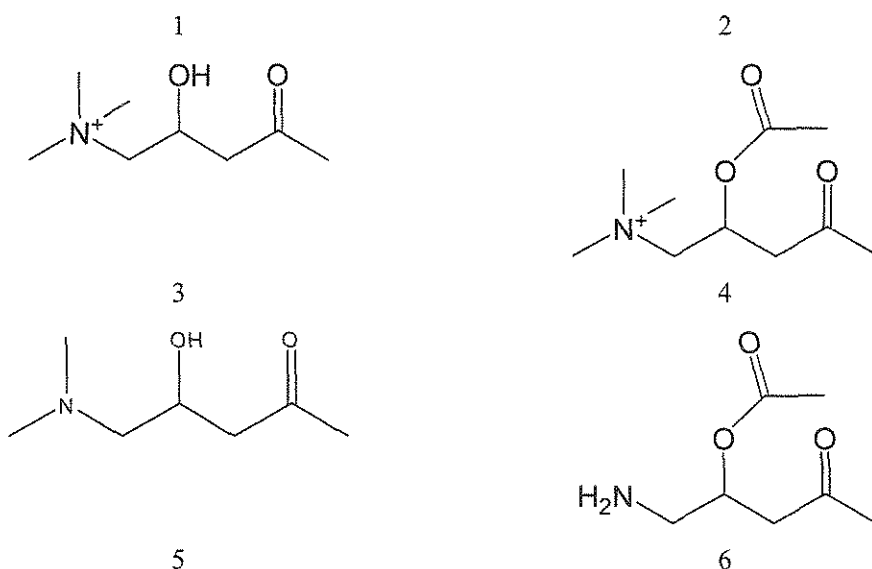
(I)

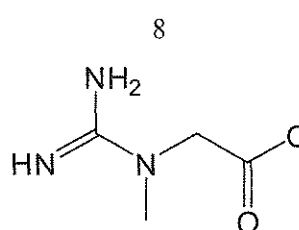
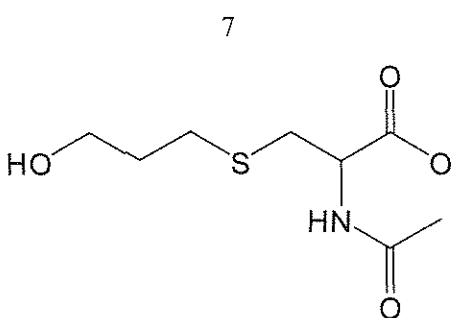
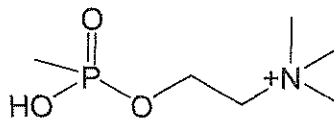
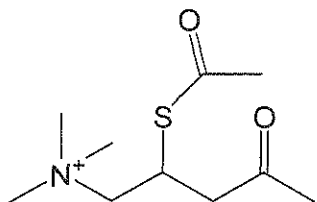
for the manufacture of a medicament for treating or preventing a vascular disease selected from the group consisting of atherosclerosis and hypercholesterolemia, wherein

R₁ and R₂ are the same or different and selected from an alkyl or alkenyl hydrocarbon chain selected from the group consisting of: CH₃(CH₂)₃-, CH₃(CH₂)₅-,

$\text{CH}_3(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_9-$, $\text{CH}_3(\text{CH}_2)_{11}-$, $\text{CH}_3(\text{CH}_2)_{13}-$, $\text{CH}_3(\text{CH}_2)_{15}-$, $\text{CH}_3(\text{CH}_2)_{17}-$,
 $\text{CH}_3(\text{CH}_2)_{19}-$, $\text{CH}_3(\text{CH}_2)_{21}-$, $\text{CH}_3(\text{CH}_2)_{23}-$, $\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_7-$,
 $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7-$,
 $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7-$,
 $\text{CH}_3\text{CH}_2(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_3(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_7(\text{CH}=\text{CH})-$,
 $\text{CH}_3(\text{CH}_2)_9(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{11}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{13}(\text{CH}=\text{CH})-$,
 $\text{CH}_3(\text{CH}_2)_{15}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{17}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_{19}(\text{CH}=\text{CH})-$,
 $\text{CH}_3(\text{CH}_2)_{21}(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$,
 $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$,
 $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})$,
 $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_5(\text{CH}=\text{CH})-$, $\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_7-$,
 $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7-$, $\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_2(\text{CH}_2)_6-$,
 $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_3(\text{CH}_2)_6-$, $\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_4(\text{CH}_2)_2-$,
 $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_5(\text{CH}_2)_2-$, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_{11}$, and $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_6\text{CH}_2-$;

R_3 is selected from the group consisting of fatty acids having a chain of 4 to 28 carbons, carnitine, acetyl-D/L-carnitine, thiocarnitine, acetyl-D/L-thiocarnitine, creatine, norcarnitine, phosphocholine, lipoic acid, dihydrolipoic acid, phosphoethanolamine, phosphoserine, N-acetylcysteine, substituted or unsubstituted amino acid groups and groups of the structures shown below:





R_4 and R_5 are independently hydrogen or lower alkyl;

R_6 is hydrogen or lower alkyl; and

R_7 and R_8 are independently hydrogen or lower alkyl,

including racemates or isolated stereoisomers and pharmaceutically acceptable salts or esters thereof.

36. The use of claim 35, wherein R_2 is $\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_6\text{CH}_2-$.

37. The use of claim 35, wherein the compound is selected from the group consisting of 2-acetoxy-4-(2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4, 7, 10, 13, 16, 19-hexaenoyloxy)-3-(hexadecyloxy)propoxy)-N,N,N-trimethyl-4-oxobutan-1-aminium (PPI-1009), (4Z,7Z,10Z,13Z,16Z,19Z)-1-(5-((R)-1,2-dithiolan-3-yl)pentanoyloxy)-3-(hexadecyloxy)propan-2-yl docosa-4, 7, 10, 13, 16, 19-hexaenoate (PPI-1011) and (4Z,7Z,10Z,13Z,16Z,19Z)-1-(2-acetamido-3-mercaptopropanoyloxy)-3-(hexadecyloxy)propan-2-yl docosa-4, 7, 10, 13, 16, 19-hexaenoate (PPI-1014).