

## METHODS FOR THE SYNTHESIS OF SPHINGOMYELINS AND DIHYDROSPHINGOMYELINS

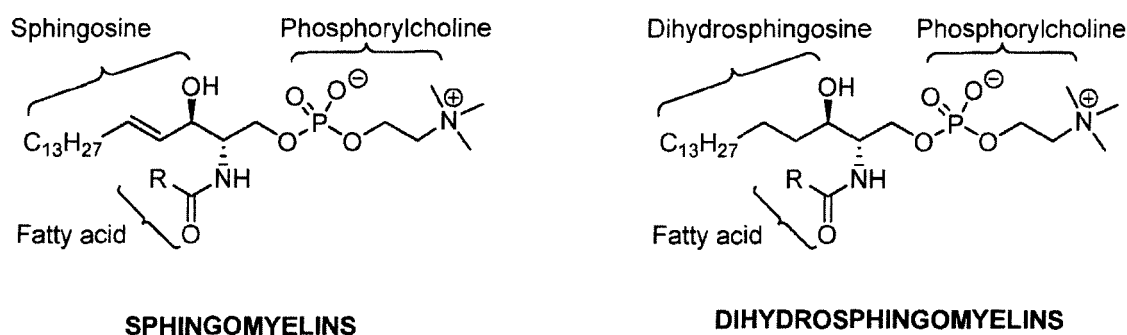
### REFERENCE TO PRIOR APPLICATIONS

[001] This application claims the benefit of U.S. Provisional Application No. 61/801,641, filed March 15, 2013 and European Patent Application No. 13306056.6, filed July 23, 2013, each of which is incorporated by reference herein in its entirety.

### BACKGROUND OF THE INVENTION

[002] Sphingomyelins are the major phospholipid components of biological membranes and plasma lipoproteins. Sphingomyelins consist of a ceramide core (sphingosine bound to a fatty acid *via* an amide linkage) and a phosphorylcholine head group (Formulae 1, left). Dihydrosphingomyelins are the saturated homologues of sphingomyelins, and have a saturated ceramide core, namely dihydrosphingosine bound to a fatty acid *via* an amide linkage (Formulae I, right).

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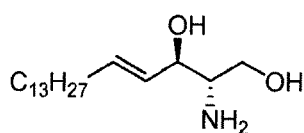
Formulae 1

[003] The sphingosine typically found in naturally occurring sphingomyelin is *D-erythro*-sphingosine an 18-carbon amino alcohol with an unsaturated hydrocarbon chain having a stereo chemical configuration of *D-erythro*. The IUPAC name for this sphingosine is (2*S*,3*R*,*E*)-2-amino-octadec-4-ene-1,3-diol (Compound A). The dihydrosphingosine, *D-erythro*-dihydrosphingosine, is its saturated homologue with the IUPAC name (2*S*,3*R*)-2-amino-octadecane-1,3-diol (Compound B).

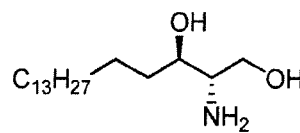
[004] Commercially available sphingomyelins are usually naturally products that comprise mixtures of naturally occurring sphingomyelins. The actual composition of this mixture

varies depending on the biological source and contains various fatty acid chain lengths. The *N*-palmitoyl-sphingomyelin is a major component in the natural sphingomyelins.

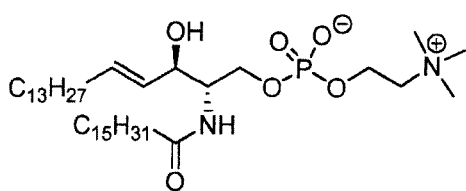
[005] *N*-Palmitoyl-*D*-erythro-sphingomyelin (Compound C), one of the isomers of palmitoyl sphingomyelin, which has the IUPAC name *N*-((2*S*, 3*R*, *E*)-1,3-dihydroxyoctadec-4-en-2-yl)palmitamide, is believed to be the main naturally-occurring isomer. Its corresponding dihydrosphingomyelin, *N*-palmitoyl-*D*-erythro-dihydrosphingomyelin (Compound D), has the IUPAC name (2*S*,3*R*)-3-hydroxy-2-palmitamidooctadecyl (2-(trimethylammonio)ethyl) phosphate.



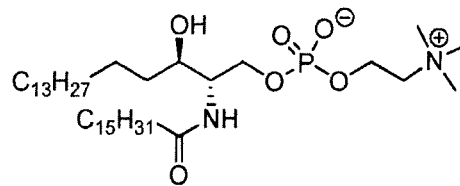
Compound A



Compound B



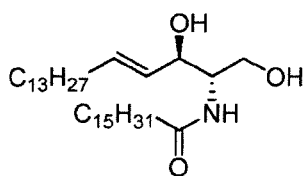
Compound C



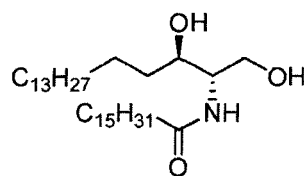
Compound D

[006] Industrially and economically-relevant synthetic alternatives of this natural source of sphingomyelin have yet to be developed. Synthetic pathways known in the art have not been useful for the large scale synthesis of sphingomyelins, particularly those with fatty acids having 12 to 25 carbons.

[007] The ceramides *N*-palmitoyl-*D*-erythro-sphingosine (Compound E) and *N*-palmitoyl-*D*-erythro-dihydrosphingosine (Compound F) are intermediates in the synthesis of *N*-palmitoyl-*D*-erythro-sphingomyelin and *N*-palmitoyl-*D*-erythro-dihydrosphingomyelin, respectively.



Compound E

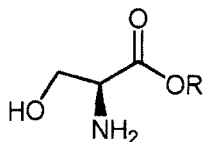


Compound F

### SUMMARY OF THE INVENTION

[008] In one embodiment, the invention provides methods for synthesizing *D-erythro*-sphingosine, comprising the steps of:

- 5 a) protecting the amino group of an *L*-serine ester having the following structure:



wherein R is a C1-5 alkyl group, or a salt thereof with a *tert*-butoxycarbonyl group, resulting in a Boc-protected *L*-serine ester;

- 10 b) allowing the Boc-protected *L*-serine ester to react with 2,2-dimethoxypropane in the presence of benzenesulfonic acid under conditions effective to yield the corresponding C1-C5 alkyl ester of (*S*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-oxazolidinocarboxylic acid;

- 15 c) allowing the corresponding C1-C5 alkyl ester of (*S*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-oxazolidinocarboxylic acid to react with dimethyl methylphosphonate in the presence of *n*-butyllithium under conditions effective to yield (*S*)-3-(*tert*-butoxycarbonyl)-4-(2-(dimethoxy-phosphoryl)-1-oxo-ethyl)-2,2-dimethyloxazolidine;

- 20 d) allowing (*S*)-3-(*tert*-butoxycarbonyl)-4-(2-(dimethoxy-phosphoryl)-1-oxo-ethyl)-2,2-dimethyloxazolidine to react with 1-tetradecanal under conditions effective to yield (*S*)-3-(*tert*-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidine;

- e) allowing (*S*)-3-(*tert*-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidine to react with sodium borohydride and cerium trichloride under

conditions effective to yield (2*S*,3*R*,4*E*)-3-(*tert*-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidine; and

f) removing the *tert*-butoxycarbonyl (Boc) protecting group of (2*S*,3*R*,4*E*)-3-(*tert*-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidine under conditions effective to yield *D*-erythro-sphingosine.

[009] In a further embodiment, the invention provides methods for synthesizing *N*-palmitoyl-*D*-erythro-sphingosine, comprising the steps of:

a) allowing (1*R*, 2*R*, 5*R*)-(+)-2-hydroxy-3-pinanone to react with ethylglycinate under conditions effective to yield (1*R*, 2*R*, 5*R*)-ethyl-((2-hydroxypinan-3-ylene)amino)acetate;

b) allowing (1*R*, 2*R*, 5*R*)-ethyl-((2-hydroxypinan-3-ylene)amino)acetate (Compound IIIb) to react with 2-(*E*)-hexadecen-1-al in the presence of chlorotitanium triisopropoxyde and triethylamine under conditions effective to yield one or both of (2*S*,3*R*,*E*)-ethyl 3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate and (2*S*,3*R*,*E*)-isopropyl 3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate;

c) allowing the one or both of (2*S*,3*R*,*E*)-ethyl 3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate and (2*S*,3*R*,*E*)-isopropyl 3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate to react with hydrochloric acid under conditions effective to yield one or both of (2*R*,3*R*,*E*)-ethyl 2-amino-3-hydroxyoctadec-4-enoate and (2*R*,3*R*,*E*)-propyl 2-amino-3-hydroxyoctadec-4-enoate;

d) allowing the one or both of (2*R*,3*R*,*E*)-ethyl 2-amino-3-hydroxyoctadec-4-enoate and (2*R*,3*R*,*E*)-propyl 2-amino-3-hydroxyoctadec-4-enoate to react with sodium borohydride under conditions effective to yield *D*-erythro-sphingosine; and

e) allowing *D*-erythro-sphingosine to react with palmitic acid under conditions effective to yield *N*-palmitoyl-*D*-erythro-sphingosine.

[0010] In yet another embodiment, the invention provides methods for synthesizing an *N*-acyl-*D*-erythro-sphingomyelin comprising the steps of:

a) allowing *D*-erythro-sphingosine to react with a fatty acid under conditions effective to yield a *D*-erythro-ceramide;

5           b) allowing *D*-erythro-ceramide to react with a tritylating reagent under conditions effective to yield 1-O-trityl-*D*-erythro-ceramide;

c) allowing 1-O-trityl-*D*-erythro-ceramide to react with a benzoylating reagent under conditions effective to yield 1-O-trityl-3-O-benzoyl-*D*-erythro-ceramide;

10           d) removing the trityl group of 1-O-trityl-3-O-benzoyl-*D*-erythro-ceramide to yield *D*-erythro-3-O-benzoyl-ceramide;

e) allowing 3-O-benzoyl-*D*-erythro-ceramide to react with with 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) under conditions effective to yield 3-O-benzoyl-*D*-erythro-1-O-(2-oxo-1,3,2-dioxaphospholane) ceramide;

15           f) allowing 3-O-benzoyl-*D*-erythro-1-O-(2-oxo-1,3,2-dioxaphospholane) ceramide to react with trimethylamine under conditions effective to yield the *N*-acyl-3-O-benzoyl-*D*-erythro -sphingomyelin; and

g) removing the benzoyl group of *N*-acyl-3-O-benzoyl-*D*-erythro-sphingomyelin under conditions effective to yield the *N*-acyl-*D*-erythro-sphingomyelin.

20 [0011] In a particular embodiment, the invention provides methods for synthesizing *D*-erythro-3-O-benzoyl-ceramide, comprising the steps of

a) allowing *D*-erythro-ceramide to react with a tritylating reagent under conditions effective to yield 1-O-trityl-*D*-erythro-ceramide;

25           b) allowing 1-O-trityl-*D*-erythro-ceramide to react with a benzoylating reagent under conditions effective to yield 1-O-trityl-3-O-benzoyl-*D*-erythro-ceramide; and

c) removing the trityl group of 1-O-trityl-3-O-benzoyl-*D*-erythro-ceramide under conditions effective to yield 3-O-benzoyl-*D*-erythro-ceramide.

[0012] In yet another embodiment, the invention provides methods for synthesizing an *N*-acyl-*D*-erythro-sphingomyelin comprising the steps of:

a) allowing 3-O-benzoyl-*D*-erythro-ceramide to react with 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) under conditions effective to yield 3-O-benzoyl-*D*-erythro-1-O-(2-oxo-1,3,2-dioxaphospholane)ceramide;

b) allowing 3-O-benzoyl-*D*-erythro-1-O-(2-oxo-1,3,2-dioxaphospholane)ceramide to react with trimethylamine to yield the 3-O-benzoyl-*D*-erythro-ceramide; and

c) removing the benzoyl group of 3-O-benzoyl-*D*-erythro-ceramide under conditions effective to yield the *N*-acyl-*D*-erythro-sphingomyelin.

[0013] In still another embodiment, the invention provides methods for synthesizing an *N*-acyl-*D*-erythro-dihydrosphingomyelin comprising the steps of:

a) allowing a *D*-erythro-dihydrosphingosine to react with a fatty acid under conditions effective to yield a *D*-erythro-dihydroceramide;

b) allowing the *D*-erythro-dihydroceramide to react with a tritylating agent under conditions effective to yield a 1-O-trityl-*D*-erythro-dihydroceramide;

c) allowing the 1-O-trityl-*D*-erythro-dihydroceramide to react with a benzoylating agent under conditions effective to yield a 1-O-trityl-3-O-benzoyl-*D*-erythro-dihydroceramide;

d) removing the trityl group of 1-O-trityl-3-O-benzoyl-*D*-erythro-dihydroceramide under conditions effective to yield a 3-O-benzoyl-*D*-erythro-ceramide;

e) allowing the 3-O-benzoyl-*D*-erythro-dihydroceramide to react with 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) under conditions effective to yield a 3-O-benzoyl-*D*-erythro-1-O-(2-oxo-1,3,2-dioxaphospholane) dihydroceramide;

f) allowing the 3-O-benzoyl-*D*-erythro-1-O-(2-oxo-1,3,2-dioxaphospholane) dihydroceramide to react with trimethylamine under conditions effective to yield an *N*-acyl-3-O-benzoyl-*D*-erythro-dihydrosphingomyelin; and

g) removing the benzoyl group of the *N*-acyl-3-*O*-benzoyl-*D*-erythro-sphingomyelin under conditions effective to yield the *N*-acyl-*D*-erythro-dihydrosphingomyelin.

[0014] In yet another embodiment, the invention provides methods for synthesizing *N*-palmitoyl-*D*-erythro-sphingomyelin, comprising the steps of:

a) allowing *N*-palmitoyl-*D*-erythro-sphingosine to react with ethylene halophosphite under conditions effective to yield *N*-((2*S*,3*R*,*E*)-1-((1,3,2-dioxaphospholan-2-yl)oxy)-3-hydroxyoctadec-4-en-2-yl)palmitoylamide;

b) allowing *N*-((2*S*,3*R*,*E*)-1-((1,3,2-dioxaphospholan-2-yl)oxy)-3-hydroxyoctadec-4-en-2-yl)palmitoylamide to react with bromine under conditions effective to yield 2-bromoethyl ((2*S*,3*R*,*E*)-3-hydroxy-2-palmitamidooctadec-4-en-1-yl)phosphorobromidate; and

c) allowing 2-bromoethyl ((2*S*,3*R*,*E*)-3-hydroxy-2-palmitamidooctadec-4-en-1-yl)phosphorobromidate to react with trimethylamine under conditions effective to yield *N*-palmitoyl-*D*-erythro-sphingomyelin.

[0015] In a particular embodiment, the invention provides methods for synthesizing *N*-palmitoyl-*D*-erythro-dihydrosphingomyelin, comprising the steps of:

a) allowing *N*-palmitoyl-*D*-erythro-dihydrosphingosine to react with ethylene chlorophosphite under conditions effective to yield *N*-((2*S*,3*R*)-1-((1,3,2-dioxaphospholan-2-yl)oxy)-3-hydroxyoctadecan-2-yl)palmitamide;

b) allowing *N*-((2*S*,3*R*)-1-((1,3,2-dioxaphospholan-2-yl)oxy)-3-hydroxyoctadecan-2-yl)palmitamide to react with bromine under conditions effective to yield 2-bromoethyl ((2*S*,3*R*)-3-hydroxy-2-palmitamidooctadecyl)phosphorobromidate; and

c) allowing 2-bromoethyl ((2*S*,3*R*)-3-hydroxy-2-palmitamidooctadecyl)phosphorobromidate to react with trimethylamine under conditions effective to yield *N*-palmitoyl-*D*-erythro-dihydrosphingomyelin.

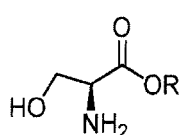
[0016] In a further embodiment, the invention provides methods for synthesizing an *N*-acyl-*D*-erythro-sphingomyelin, comprising the steps of:

a) reacting 3-O-benzoyl-*D*-erythro-ceramide with 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) to under conditions effective to yield 3-O-benzoyl-*D*-erythro-1-O-(2-oxo-1,3,2-dioxaphospholane)ceramide;

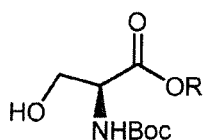
b) reacting 3-O-benzoyl-*D*-erythro-1-O-(2-oxo-1,3,2-dioxaphospholane)ceramide with trimethylamine under conditions effective to yield an *N*-acyl-3-O-*D*-erythro-benzoyl-sphingomyelin; and

c) removing the benzoyl group of 3-O-*D*-erythro-benzoyl-sphingomyelin under conditions effective to yield the *N*-acyl-*D*-erythro-sphingomyelin.

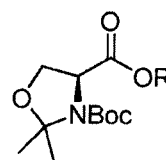
[0017] In yet another embodiment, the invention provides the following compounds, which are useful as intermediates for the methods of the present invention:



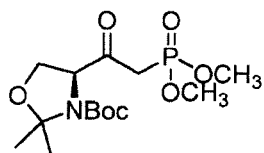
L-Serine Ester  
Compound Ia, and salts thereof;



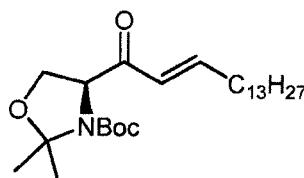
Boc-L-Ser-OR  
Compound Ib ;



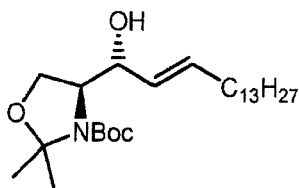
Compound Ic ;



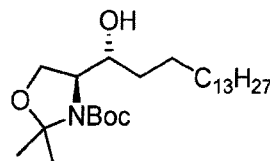
(S)-3-(tert-butoxycarbonyl)-4-(2-(dimethoxyphosphoryl)-1-oxo-ethyl)-2,2-dimethyloxazolidin  
Compound Id ;



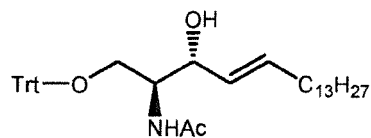
(S)-3-(tert-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidin  
Compound Ie ;



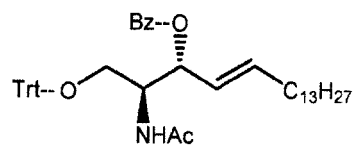
(2S,3R,4E)-3-(tert-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidin  
Compound If ;



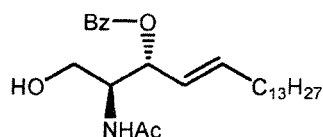
((S)-tert-butyl 4-((R)-1-hydroxyhexadecyl)-2,2-dimethyloxazolidine-3-carboxylate  
Compound IIa ;



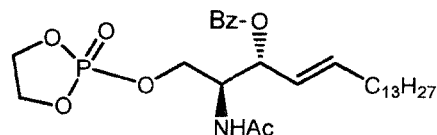
1-O-Trityl-*D*-erythro-ceramide  
Compound Vc-Trt ;



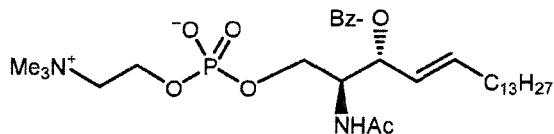
1-O-Benzoyl-3-O-Trityl-*D*-erythro-ceramide  
Compound Vd-Bz-Trt ;



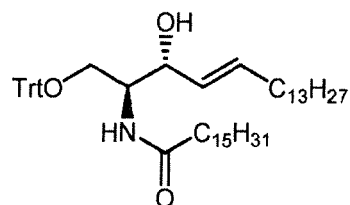
1-O-Benzoyl-*D*-erythro-ceramide  
Compound Ve-Bz ;



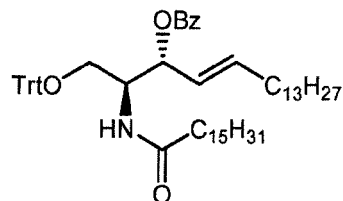
3-O-Benzoyl-*D*-erythro-1-O-(2-oxo-1,3,2-dioxaphospholan)-ceramide  
Compound Vf-Bz



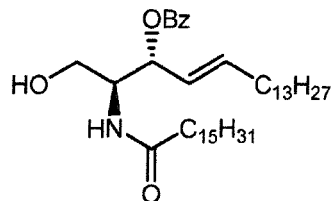
Compound Vg - Bz ;



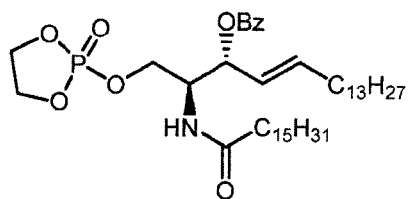
N-Palmitoyl-1-O-Trityl-*D*-erythro-sphingosine  
Compound VIa ;



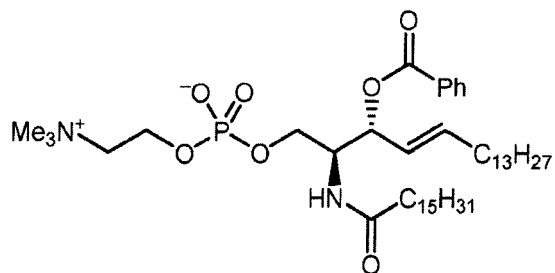
N-Palmitoyl-1-O-Trityl-3-O-Benzoyl-*D*-erythro-sphingosine  
Compound VIb ;



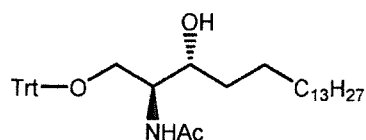
N-Palmitoyl-3-O-Benzoyl-*D*-erythro-sphingosine  
Compound VIc ;



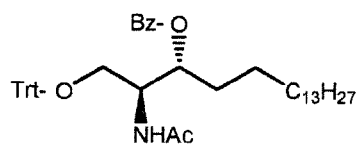
N-Palmitoyl 3-O-Benzoyl-*D*-erythro-1-O-(2-oxo-1,3,2-dioxaphospholan)-sphingosine  
Compound VIId ;



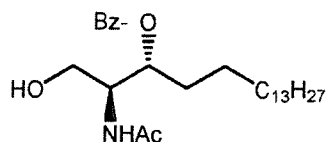
N-Palmitoyl 3-O-Benzoyl-*D*-erythro-Sphingomyelin  
Compound VIe ;



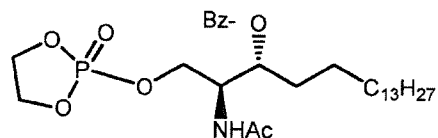
1-O-Trityl-*D*-erythro-dihydroceramide  
Compound VIIc -Trt ;



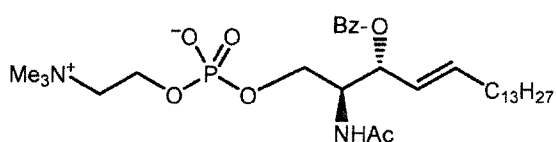
1-O-Trityl-3-O-Benzoyl-*D*-erythro-  
dihydroceramide  
Compound VIId ;



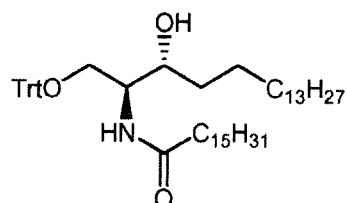
3-O-Benzoyl-*D*-erythro-dihydroceramide  
Compound VIIe ;



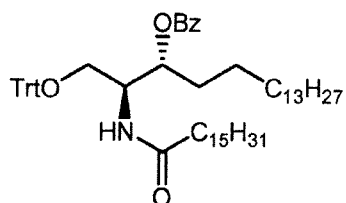
3-O-Benzoyl-*D*-erythro-1-O-  
(2-oxo-1,3,2-dioxaphospholan)-dihydroceramide  
Compound VIIf ;



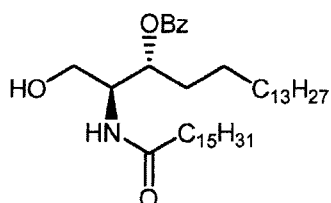
Compound VIIg ;



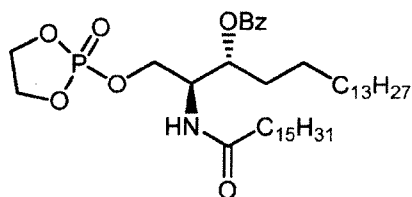
N-palmitoyl 1-O-Trityl-  
D-erythro-dihydrosphingosine  
Compound VIIla ;



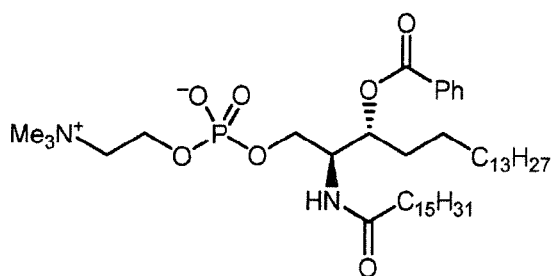
N-palmitoyl-1-O-Trityl-3-O-Benzoyl-  
D-erythro-dihydrosphingosine  
Compound VIIlb ;



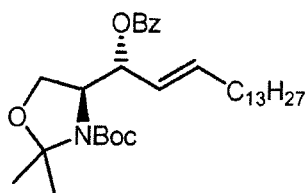
N-palmitoyl 3-O-Benzoyl-  
D-erythro-dihydrosphingosine  
Compound VIIlc ;



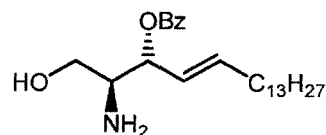
N-palmitoyl 3-O-Benzoyl-  
1-O-(2-oxo-1,3,2-dioxaphospholan)-  
D-erythro-dihydrosphingosine  
Compound VIIId ;



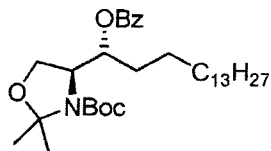
N-Palmitoyl-3-O-Benzoyl-*D*-erythro-  
dihydrosphingomyelin  
Compound VIIle ;



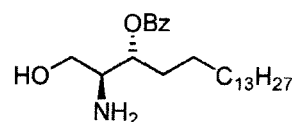
(2S,3R,4E)-3-(tert-butoxycarbonyl)-4-(1-benzoyl-hexadec-2-enyl)-2,2-dimethyloxazolidine  
Compound IXa



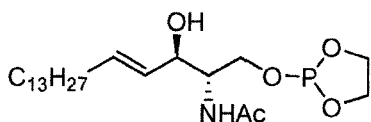
D-erythro-3-O-Benzoyl-Sphingosine  
Compound IXb



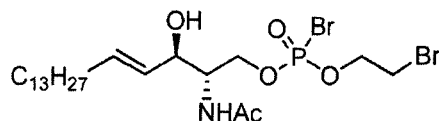
(S)-tert-butyl 4-((R)-1-(benzoyloxy)hexadecyl)-2,2-dimethyloxazolidine-3-carboxylate  
Compound XIa



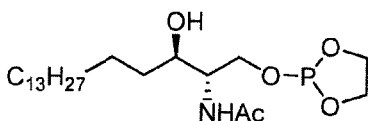
3-O-Benzoyl-D-erythro-dihydrosphingosine  
Compound XIb



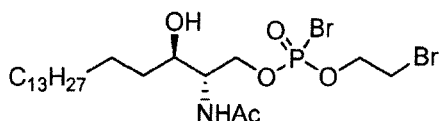
Compound XIIIa



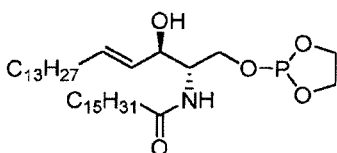
Compound XIIIb



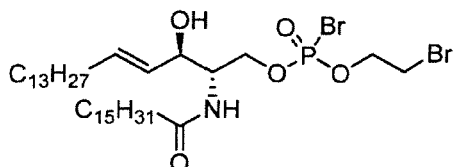
Compound XIVa



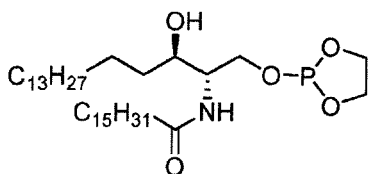
Compound XIVb



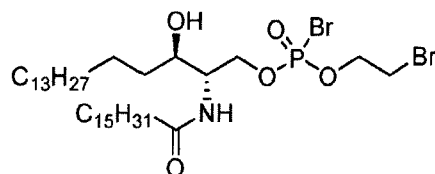
5 N-((2S,3R,E)-1-((1,3,2-dioxaphospholan-2-yl)oxy)-3-hydroxyoctadec-4-en-2-yl)palmitamide ;



2-bromoethyl ((2S,3R,E)-3-hydroxy-2-palmitamido-octadec-4-en-1-yl) phosphorobromidate ;



N-((2S,3R)-1-((1,3,2-dioxaphospholan-2-yl)oxy)-3-hydroxyoctadecan-2-yl)palmitamide



2-bromoethyl ((2S,3R)-3-hydroxy-2-palmitamido-octadecyl) phosphorobromidate ;

; and

where Ac is a fatty acid residue having 3 to 36 carbons and zero to six carbon-carbon double bonds; and  
R is a C1-5 alkyl group.

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## BRIEF DESCRIPTION OF DRAWINGS

[0018] Fig. 1 is a  $^1\text{H}$  NMR spectrum of naturally occurring egg sphingomyelin.

[0019] Fig.2 is a  $^1\text{H}$  NMR spectrum of *N*-palmitoyl-*D*-*Erythro*-sphingomyelin synthesized according to methods of the invention.

10 [0020] Fig. 3 is a  $^1\text{H}$  NMR spectrum of *N*-palmitoyl-*D*-*Erythro*-sphingomyelin synthesized according to methods of the invention.

[0021] Fig. 4 is a  $^1\text{H}$  NMR spectrum of a sample taken from a reaction mixture of *R*-methoxyphenylacetic acid and naturally occurring egg sphingomyelin.

[0022] Fig. 5 is a  $^1\text{H}$  NMR spectrum of a sample taken from a reaction mixture of *R*-methoxyphenylacetic acid and *N*-palmitoyl-*D*-*Erythro*-sphingomyelin synthesized according to methods of the invention.

[0023] Fig. 6 is a  $^1\text{H}$  NMR spectrum of a sample taken from a reaction mixture of *R*-methoxyphenylacetic acid and *N*-palmitoyl-*D*-*Erythro*-sphingomyelin synthesized according to methods of the invention.

20 [0024] Fig. 7 is a  $^1\text{H}$  NMR spectrum of a sample taken from a reaction mixture of one equivalent of egg sphingomyelin and 1.2 equivalents of a racemic mixture of *R*-(-)-MPA methoxyphenylacetic acid (*R*-MPA) and *S*-(+)-methoxyphenylacetic acid (*S*-MPA), 1.2 equivalents of dicyclohexylcarbodiimide (DCC), and a catalytic amount of 4-dimethylaminopyridine (DMAP) (bottom) and 1D-TOCSY (1 Dimensional-Total Correlation Spectroscopy) spectra showing selective excitation of the H2 hydrogen peak in the *R*-MPA (top) and *S*-MPA (middle) esters.

[0025] Fig. 8 is a photograph of a thin-layer chromatography plate of crude *N*-palmitoyl-3-*O*-benzoyl-*D*-*erythro*-sphingosine (Compound VIc). In the column marked "Crude Product" the spot identified as "A" is *N*-palmitoyl-*D*-*erythro*-sphingosine (Compound E), the spot identified as "B" is *N*-palmitoyl-3-*O*-benzoyl-*D*-*erythro*-sphingosine (Compound VIc), the spot identified as "C" is *N*-palmitoyl-1-*O*-benzoyl-*D*-*erythro*-sphingosine (a product of

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benzoyl-group migration), the spot identified as “D” is triphenylmethanol (trityl-OH), and the spot identified as “E” is *N*-palmitoyl-1,3-O,O-dibenzoyl-*D*-erythro-sphingosine.

## DETAILED DESCRIPTION OF THE INVENTION

[0026] The invention provides methods for synthesizing *N*-palmitoyl-*D*-erythro-

5 sphingomyelin. The invention also provides methods for the synthesis of *D*-erythro-sphingosine. The invention also provides methods for synthesizing *N*-Palmitoyl-*D*-erythro-dihydrosphingomyelin. The invention also provides methods for the synthesis of *D*-erythro-dihydrosphingosine. The invention also provides methods for synthesizing *N*-palmitoyl-*D*-erythro-sphingosine and *N*-palmitoyl-*D*-erythro-dihydrosphingosine.

10 [0027] The invention also provides for methods for synthesizing *D*-erythro-sphingosines on a kilogram scale. In a particular embodiment, the *D*-erythro-sphingosine is *N*-palmitoyl-*D*-erythro-sphingosine.

[0028] The invention also provides for methods for synthesizing *D*-erythro-dihydrosphingosines on a kilogram scale. In a particular embodiment, the *D*-erythro-sphingosine is *N*-palmitoyl-*D*-erythro-dihydrosphingosine.

[0029] The present invention provides methods for synthesizing sphingomyelin. The methods allow for large-scale synthesis of substantially enantiomerically pure compounds and use of substantially enantiomerically pure intermediates. A compound that is “substantially enantiomerically pure” contains no more than about 10 mol % of its corresponding opposite enantiomer, in another embodiment no more than about 5 mol % of its corresponding opposite enantiomer, in another embodiment no more than about 2 mol % of its corresponding opposite enantiomer, in another embodiment no more than about 1 mol % of its corresponding opposite enantiomer, and in another embodiment no more than about 0.1 mol % of its corresponding opposite enantiomer. In some embodiments, the invention provides methods for preparing sphingomyelins having a fatty acid chain length of 12 to 25 carbons.

[0030] The present invention provides methods for synthesizing an *N*-acyl-*D*-erythro-sphingomyelin. In certain embodiments of the invention, the *D*-erythro-sphingomyelin can be synthesized at a large, commercially relevant scale using a suitable *L*-serine ester (methyl, ethyl, *i*-propyl, *n*-butyl, etc.). The methods of the present invention are useful for the synthesis of *D*-erythro-sphingomyelin.

[0031] The present invention also provides methods useful for the synthesis of *D-erythro*-sphingosine.

[0032] The invention also provides compounds synthesizable using the methods described herein, including compounds useful as intermediates.

5 [0033] The present invention further provides each individual step of the methods disclosed herein, which is useful for synthesizing an intermediate or product of the methods disclosed herein.

[0034] As described herein, sphingomyelins have a ceramide core bound to a polar head group.

10 [0035] The ceramide core includes a sphingosine bound to a fatty acid *via* an amide linkage. Where the term “ceramide-C<sub>n</sub>” is used, n is an integer and refers to the number of carbons (C) in the fatty acid residue, e.g., ceramide-C16 refers to a ceramide core having a 16-carbon fatty acid residue, such as palmitoyl, and ceramide-C18 refers to a ceramide core having a 18-carbon fatty acid residue, such as stearyl.

15 [0036] Where “ceramide” or “ceramide core” is used without specifying the length of the fatty acid carbon chain, it is to be understood that the fatty acid chain carbon can be any suitable length.

[0037] As used herein, the term “sphingomyelin” describes a ceramide core bound to a phosphorylcholine functional group.

20 [0038] A fatty acid is a carboxylic acid having a long aliphatic tail that can be either saturated or unsaturated. Unsaturated fatty acids have one or more carbon-carbon double bonds, and each carbon-carbon double bond can occur in a *cis* or *trans* configuration. A fatty acid residue is a fatty acid less the –OH group of the fatty acid’s carboxyl group. As used herein, the term “Ac” refers to a fatty acid residue.

25 [0039] In certain embodiments of the invention the fatty acid or fatty acid residue has 3 to 36 carbons and zero to six carbon-carbon double bonds. In particular embodiments of the invention the fatty acid or fatty acid residue has 4 to 28 carbons and zero to six carbon-carbon double bonds. In further embodiments of the invention, the fatty acid or fatty acid residue has 11 to 25 carbons and zero to six carbon-carbon double bonds. In still further embodiments of  
30 the invention, the fatty acid or fatty acid residue has 11 to 25 carbons and one or two carbon-carbon double bonds. In further embodiments of the invention, the fatty acid or fatty acid

residue has 14 to 20 carbons and zero to six carbon-carbon double bonds. In yet further embodiments of the invention, the fatty acid or fatty acid residue has 15 to 17 carbons and zero to six carbon-carbon double bonds. In a particular embodiment of the invention, the fatty acid is palmitic acid and the fatty acid residue is palmitoyl.

5 [0040] Suitable fatty acids also include, but are not limited to, omega fatty acids such as  $\omega$ -3, or  $\omega$ -6, or  $\omega$ -9 fatty acids; and essential fatty acids, such as, but not limited to, linoleic acid (LA),  $\alpha$ -linolenic acid (ALA), an n-3 fatty acid, e.g., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

[0041] Suitable fatty acids useful in the present invention include, but are not limited to,  
 10 propionic acid, butyric acid, valeric acid, caproic acid, enanthic acid, caprylic acid, pelargonic acid, capric acid, undecylic acid, lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid, margaric acid, stearic acid, nonadecylic acid, arachidic acid, heneicosylic acid, behenic acid, tricosylic acid, lignoceric acid, pentacosylic acid, cerotic acid, heptacosylic acid, montanic acid, nonacosylic acid, melissic acid, henatriacontylic acid, lacceroic acid, psyllic  
 15 acid, geddic acid, ceroplastic acid, hexatriacontylic acid, myristoleic acid, palmitoleic acid, sapienic acid, oleic acid, elaidic acid, vaccenic acid, linoleic acid, linoelaidic acid,  $\alpha$ -linolenic acid, and erucic acid.

[0042] If the fatty acid is a monounsaturated fatty acid, it can be a *cis*- or *trans*-  
 monounsaturated fatty acid such as, but not limited to, oleic acid, elaidic acid, myristoleic  
 20 acid, palmitoleic acid, sapienic acid, oleic acid, elaidic acid, vaccenic acid, and erucic acid.

[0043] As used herein, " $C_{13}H_{27}$ -" and " $C_{15}H_{31}$ -" mean  $CH_3(CH_2)_{12}$ - and  $CH_3(CH_2)_{14}$ - , respectively.

[0044] As used herein, the term "acyl" refers to a radical of general formula  $-C(O)R$ , where R is an alkyl group having 2 to 35 carbons and zero to six carbon-carbon double bonds.

25 [0045] Certain compounds of the invention can be in the form of a salt. In some embodiments, the salt is a pharmaceutically acceptable salt. Pharmaceutically acceptable salts include, for example, acid-addition salts and base-addition salts. The acid that forms an acid-addition salt can be an organic acid or an inorganic acid. A base that forms a base-addition salt can be an organic base or an inorganic base. In some embodiments, a pharmaceutically  
 30 acceptable salt is a metal salt. In some embodiments, a pharmaceutically acceptable salt is an ammonium salt.

[0046] Acid-addition salts can arise from the addition of an acid to the free-base form of a compound of the invention. In some embodiments, the acid is organic. In some embodiments, the acid is inorganic. Non-limiting examples of suitable acids include hydrochloric acid, hydrobromic acid, hydroiodic acid, nitric acid, nitrous acid, sulfuric acid, sulfurous acid, a phosphoric acid, nicotinic acid, isonicotinic acid, lactic acid, salicylic acid, 4-aminosalicylic acid, tartaric acid, ascorbic acid, gentisinic acid, gluconic acid, glucaronic acid, saccharic acid, formic acid, benzoic acid, glutamic acid, pantothenic acid, acetic acid, propionic acid, butyric acid, fumaric acid, succinic acid, citric acid, oxalic acid, maleic acid, hydroxymaleic acid, methylmaleic acid, glycolic acid, malic acid, cinnamic acid, mandelic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, embonic acid, phenylacetic acid, *N*-cyclohexylsulfamic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, *p*-toluenesulfonic acid, 2-hydroxyethanesulfonic acid, ethane-1,2-disulfonic acid, 4-methylbenzenesulfonic acid, naphthalene-2-sulfonic acid, naphthalene-1,5-disulfonic acid, 2-phosphoglyceric acid, 3-phosphoglyceric acid, glucose-6-phosphoric acid, and an amino acid.

[0047] Non-limiting examples of suitable acid-addition salts include a hydrochloride salt, a hydrobromide salt, a hydroiodide salt, a nitrate salt, a nitrite salt, a sulfate salt, a sulfite salt, a phosphate salt, a hydrogen phosphate salt, a dihydrogen phosphate salt, a carbonate salt, a bicarbonate salt, a nicotinate salt, an isonicotinate salt, a lactate salt, a salicylate salt, a 4-aminosalicylate salt, a tartrate salt, an ascorbate salt, a gentisinate salt, a gluconate salt, a glucaronate salt, a saccharate salt, a formate salt, a benzoate salt, a glutamate salt, a pantothenate salt, an acetate salt, a propionate salt, a butyrate salt, a fumarate salt, a succinate salt, a citrate salt, an oxalate salt, a maleate salt, a hydroxymaleate salt, a methylmaleate salt, a glycolate salt, a malate salt, a cinnamate salt, a mandelate salt, a 2-phenoxybenzoate salt, a 2-acetoxybenzoate salt, an embonate salt, a phenylacetate salt, an *N*-cyclohexylsulfamate salt, a methanesulfonate salt, an ethanesulfonate salt, a benzenesulfonate salt, a *p*-toluenesulfonate salt, a 2-hydroxyethanesulfonate salt, an ethane-1,2-disulfonate salt, a 4-methylbenzenesulfonate salt, a naphthalene-2-sulfonate salt, a naphthalene-1,5-disulfonate salt, a 2-phosphoglycerate salt, a 3-phosphoglycerate salt, a glucose-6-phosphate salt, and an amino acid salt.

[0048] Metal salts can arise from the addition of an inorganic base to a compound of the invention having a carboxyl group. The inorganic base consists of a metal cation paired with a basic counterion, such as, for example, hydroxide, carbonate, bicarbonate, or phosphate. The metal can be an alkali metal, alkaline earth metal, transition metal, or main group metal. Non-

limiting examples of suitable metals include lithium, sodium, potassium, cesium, cerium, magnesium, manganese, iron, calcium, strontium, cobalt, titanium, aluminum, copper, cadmium, and zinc.

5 [0049] Non-limiting examples of suitable metal salts include a lithium salt, a sodium salt, a potassium salt, a cesium salt, a cerium salt, a magnesium salt, a manganese salt, an iron salt, a calcium salt, a strontium salt, a cobalt salt, a titanium salt, an aluminum salt, a copper salt, a cadmium salt, and a zinc salt.

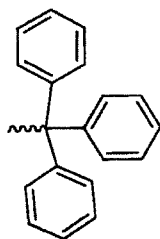
10 [0050] Ammonium salts can arise from the addition of ammonia or an organic amine to a compound of the invention having a carboxyl group. Non-limiting examples of suitable organic amines include triethyl amine, diisopropyl amine, ethanol amine, diethanol amine, triethanol amine, morpholine, *N*-methylmorpholine, piperidine, *N*-methylpiperidine, *N*-ethylpiperidine, dibenzyl amine, piperazine, pyridine, pyrrazole, imidazole, pyrazine, piperazine, ethylenediamine, *N,N'*-dibenzylethylenediamine, procaine, chlorprocaine, choline, dicyclohexyl amine, and *N*-methylglucamine.

15 [0051] Non-limiting examples of suitable ammonium salts include is a triethylammonium salt, a diisopropylammonium salt, an ethanolammonium salt, a diethanolammonium salt, a triethanolammonium salt, a morpholinium salt, an *N*-methylmorpholinium salt, a piperidinium salt, an *N*-methylpiperidinium salt, an *N*-ethylpiperidinium salt, a dibenzylammonium salt, a piperazinium salt, a pyridinium salt, a pyrrolizium salt, an imidazolium salt, a pyrazinium salt, an ethylenediammonium salt, an *N,N'*-dibenzylethylenediammonium salt, a procaine salt, 20 a chlorprocaine salt, a choline salt, a dicyclohexylammonium salt, and a *N*-methylglucamine salt.

[0052] The term “about” when used in connection with a referenced numeric indication means the referenced numeric indication plus or minus up to 10% of that referenced numeric 25 indication. For example, the language “about 50” covers the range of 45 to 55.

### **General Methods**

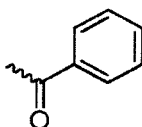
[0053] “Trt” represents the trityl (triphenylmethyl) protecting group, having the structure:



[0054] As used herein, tritylating reagents include, but are not limited to, trityl halides such as trityl chloride and trityl bromide

[0055] Removal of the trityl protecting group typically proceeds as follows: the trityl-protected sphingomyelin is dissolved in an organic solvent and an acid is added. The reaction proceeds at a temperature of about 22°C for 1 to 16 hours. The reaction mixture is neutralized by the addition of a base. The organic solvent can be a protic polar solvent, an aprotic polar solvent, or a mixture thereof. In one embodiment the organic solvent is a protic polar solvent and is methanol, ethanol, *n*-propanol, or isopropanol. In one embodiment the organic solvent is an aprotic polar solvent. In one embodiment, the aprotic organic solvent is chlorinated and is methylene chloride, chloroform, or carbon tetrachloride. In another embodiment, the aprotic organic solvent is nonchlorinated and is diethyl ether, tetrahydrofuran, or ethyl acetate. The acid can be any acid known by one of skill in the art to be suitable for removal of the trityl protecting group, e.g., acetic acid, trifluoroacetic acid, hydrochloric acid and p-toluenesulfonic acid. In certain embodiments of the invention the acid is p-toluenesulfonic acid. In particular embodiments the base is an organic base, such as triethylamine or pyridine

[0056] "Bz" represents the benzoyl protecting group, having the structure:



[0057] As used herein, benzoylating reagents include, such as, but are not limited to, benzoyl halides such as benzoyl chloride and trityl bromide

[0058] Removal of the benzoyl protecting group typically proceeds as follows: the benzoyl-protected sphingomyelin is dissolved in a protic polar solvent and a base is added. The reaction proceeds for 8 to 24 hours at about 22°C. In one embodiment the protic polar solvent is methanol, ethanol, *n*-propanol, isopropanol, or mixtures thereof. In yet another embodiment the base is sodium methoxide, potassium carbonate, lithium hydroxide. In a particular embodiment, the base is sodium methoxide.

***D-erythro-sphingosine***

[0059] In a particular embodiment, the invention provides methods for synthesizing *D-erythro*-sphingosine using an *L*-serine ester, such as that of Compound Ia in Scheme I, where R is C1-5 alkyl group. The term "alkyl," as used herein unless otherwise defined, refers to a straight, branched, or cyclic saturated group derived from the removal of a hydrogen atom from an alkane. Representative straight chain alkyl groups include -methyl, -ethyl, -n-propyl, -n-butyl, and -n-pentyl. Representative branched alkyl groups include -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl, -neopentyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl, 1,1-dimethylpropyl and 1,2-dimethylpropyl. Representative cyclic alkyl groups include cyclopentyl, and cyclopropyl.

[0060] In certain embodiments of the invention, the *L*-serine ester is *L*-serine methyl ester. In another embodiment, the *L*-serine ester is *L*-serine ethyl ester. In yet another embodiment, the *L*-serine ester is *L*-butyl ester.

[0061] In one embodiment of the invention *D-erythro*-sphingosine is synthesized by the method shown in Scheme I, which comprises the following steps:

a) protecting the amino group of a *L*-serine ester (Compound Ia) with *tert*-butoxycarbonyl group, resulting in a Boc-protected *L*-serine ester (Compound Ib);

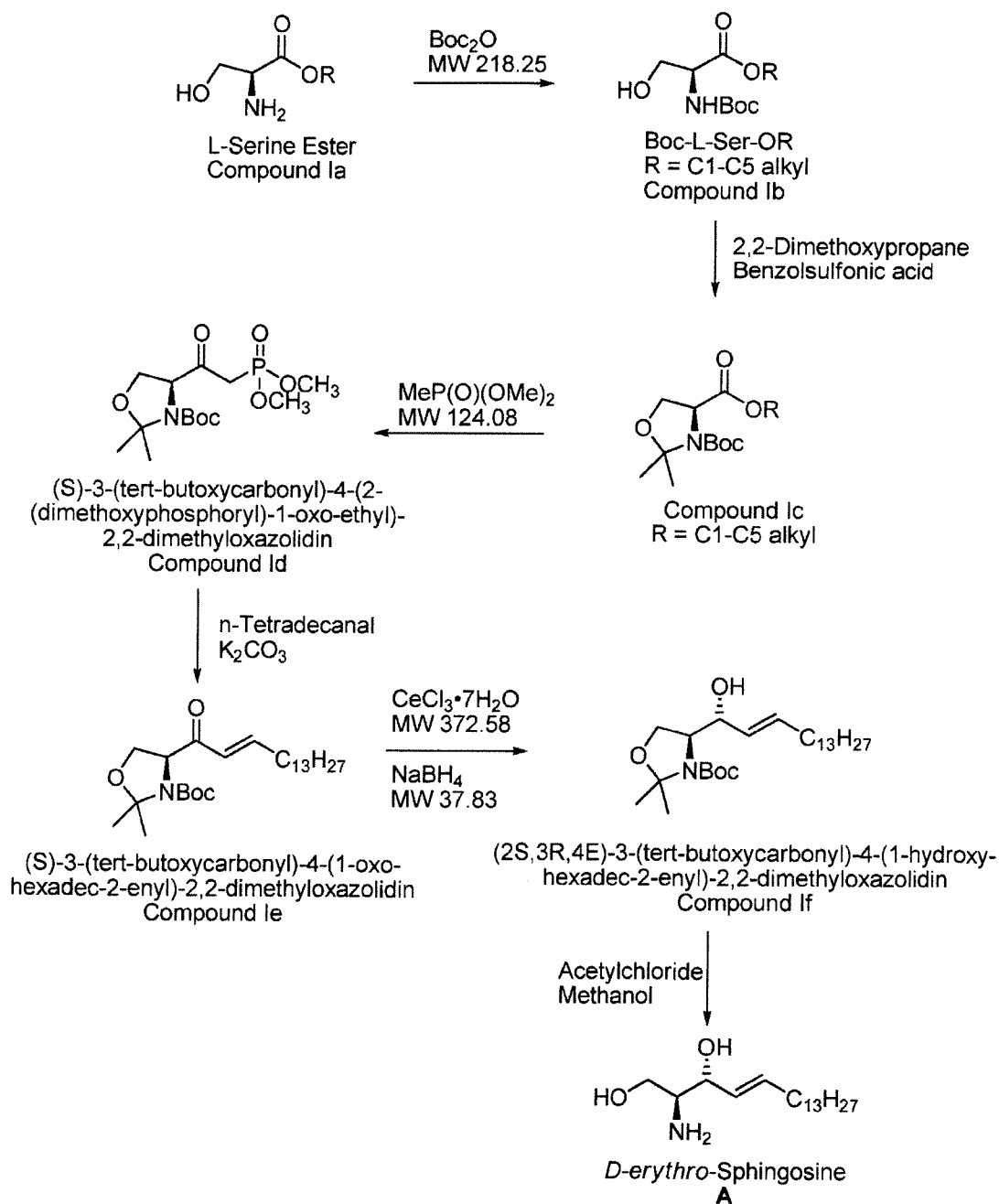
b) allowing the Boc-protected *L*-serine ester to react with 2,2-dimethoxypropane in the presence of benzenesulfonic acid under conditions effective to yield the corresponding C1-C5 alkyl ester of (*S*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-oxazolidincarboxylic acid (Compound Ic);

c) allowing the corresponding C1-C5 alkyl ester of (*S*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-oxazolidincarboxylic acid (Compound Ic), to react with dimethyl methylphosphonate in the presence of *n*-butyllithium under conditions effective to yield (*S*)-3-(*tert*-butoxycarbonyl)-4-(2-(dimethoxy-phosphoryl)-1-oxo-ethyl)-2,2-dimethyloxazolidine (Compound Id);

d) allowing (*S*)-3-(*tert*-butoxycarbonyl)-4-(2-(dimethoxy-phosphoryl)-1-oxo-ethyl)-2,2-dimethyloxazolidine to react with 1-tetradecanal under conditions effective to yield (*S*)-3-(*tert*-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidine (Compound Ie);

e) allowing (*S*)-3-(*tert*-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidine to react with sodium borohydride and cerium trichloride under conditions effective to yield (2*S*,3*R*,4*E*)-3-(*tert*-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidine (Compound If); and

- 5            f) removing the *tert*-butoxycarbonyl (Boc) protecting group of (2*S*,3*R*,4*E*)-3-(*tert*-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidine to yield *D*-erythro-sphingosine (Compound A)



Scheme I

[0062] In one aspect of the invention, the amino group of the *L*-serine ester (Compound Ia of Scheme I) is protected with a *tert*-butoxycarbonyl (Boc) group in the presence of a base, such as triethylamine or pyridine, to yield a Boc-protected *L*-serine ester (Compound Ib). The reaction can then be quenched with the addition of water and the reaction product, Compound Ib, recovered from the organic layer.

[0063] The above addition of the Boc protecting group can proceed in an aprotic organic solvent at a temperature of about 22°C for about 6 to 24 hours. In one embodiment, the aprotic organic solvent is a chlorinated hydrocarbon, e.g., methylene chloride, chloroform or carbon tetrachloride. In another embodiment, the aprotic organic solvent is nonchlorinated and is, e.g., diethyl ether, tetrahydrofuran, or ethyl acetate. The base is typically an organic base, such as triethylamine or pyridine. The reaction product, Compound Ib, can be extracted from the organic layer with an organic solvent, including, but not limited to, an aprotic organic solvent described above.

[0064] In another aspect of the invention the Boc-protected *L*-serine ester (Compound Ib) is reacted with 2,2-dimethoxypropane in the presence of benzenesulfonic acid to yield the corresponding C1-C5 alkyl ester of (*S*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-oxazolidincarboxylic acid (Compound Ic). The reaction can proceed at reflux temperature for 1-3 hours in an organic solvent. In one embodiment, organic solvent is non-polar and is toluene, benzene or hexane. In another embodiment, organic solvent is a polar organic and is diethyl ether, tetrahydrofuran, or ethyl acetate. The reaction can then be neutralized with a base and the solvent evaporated. Water and an organic solvent can then be added to the remaining residue and the reaction product can be extracted from the organic layer using an organic solvent. The base is typically an organic base, such as triethylamine or pyridine. The reaction product can be extracted from the organic layer with an organic solvent, including, but not limited to the organic solvents described above.

[0065] In another aspect of the invention, (*S*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-oxazolidincarboxylic acid ester (Compound Ic) is reacted with *n*-butyllithium in the presence of dimethyl methylphosphonate to yield (*S*)-3-(*tert*-butoxycarbonyl)-4-(2-(dimethoxyphosphoryl)-1-oxo-ethyl)-2,2-dimethyloxazolidin (Compound Id). The reaction can be quenched with water and the pH adjusted by the addition of an organic acid. The product can be recovered from the organic phase. The reaction can proceed in an organic

solvent at a temperature of about -70 to -80°C for about 2 to 4 hours. In certain embodiments, the organic solvent is a polar organic solvent and is diethyl ether, tetrahydrofuran, or ethyl acetate. In certain embodiments the acid is citric acid or acetic acid.

[0066] In a further embodiment of the invention, (S)-3-(*tert*-butoxycarbonyl)-4-(2-(dimethoxyphosphoryl)-1-oxo-ethyl)-2,2-dimethyloxazolidin (Compound Id) reacts with tetradecanal in the presence of base to yield (S)-3-(*tert*-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidin (Compound Ie). In one embodiment, the base is potassium carbonate. The reaction can proceed at room temperature, e.g., at about 22°C, and in the presence of an organic solvent and water. The reaction can proceed for 8-14 hours with stirring. The product, (S)-3-(*tert*-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidin (Compound Ie), can be recovered from the organic phase. In certain embodiments, the organic solvent is a polar organic solvent and is acetonitrile, tetrahydrofuran, or ethyl acetate.

[0067] In yet another embodiment of the invention, (S)-3-(*tert*-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidin (Compound Ie) is reduced in the presence of sodium borohydride and cerium chloride heptahydrate to yield (2S,3R,4E)-3-(*tert*-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidin (Compound If). For example, Compound Ie and cerium chloride heptahydrate are stirred in an organic solvent and the mixture is cooled to -20 to -15°C. Sodium borohydride is added to the mixture over 1 to 6 hours. After addition of the sodium borohydride, the reaction can proceed for 15 to 90 minutes, at which point it is warmed to about 22°C over 1 to 3 hours. After reaching 22°C, the mixture can be stirred for 30 to 90 minutes. In one embodiment the organic solvent is a protic polar solvent and is methanol, ethanol, *n*-propanol, or isopropanol. In certain embodiments the sodium borohydride is added as a solid. In other embodiments, the sodium borohydride is added as an aqueous solution. In certain embodiments of the invention at least some of the solvent is removed through evaporation and the precipitated salts are filtered and washed with an organic solvent. The product, Compound If, can be recovered from the organic phase of the resulting filtrate.

[0068] In a certain embodiment of the invention (2S,3R,4E)-3-(*tert*-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidin (Compound If) is deprotected to yield *D*-erythro-sphingosine. The reaction can proceed as follows: methanol is cooled to about 0°C and acetylchloride is added over the course of about 15 to 60 minutes. The solution is then

warmed to produce a methanolic hydrochloride solution. (2S,3R,4E)-3-(tert-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidin) is dissolved in methanol and the methanolic hydrochloride solution is added over the course of about 15 to 60 minutes. The reaction can be neutralized with the addition of a base. The solvent can then  
 5 be removed and the resulting *D-erythro*-sphingosine can be recovered from the residue. The base can be an organic base, such as, but not limited to, triethylamine or pyridine.

[0069] In certain embodiments of the invention the *D-erythro*-sphingosine (Compound A) can be purified by recrystallization, silica gel chromatography, high performance liquid chromatography or other methods known to those skilled in the art.

10 [0070] A particular embodiment of the invention also provides for a method of synthesizing *D-erythro*-sphingosine using *L*-serine methyl ester.

[0071] In a particular embodiment of the invention *L*-serine methyl ester (Compound Ia, R = methyl) is suspended in ethyl acetate and cooled to about 2°C, about 1.15 molar equivalents of triethylamine is added, followed by about 1.15 molar equivalents of di-*tert*-butyl  
 15 dicarbonate in ethyl acetate. The reaction mixture is warmed to about 22°C and stirred for 8 to 12 hours. Purified water is added and the phases separated. The reaction product Boc-*L*-Ser-OMe (Compound Ib, R = methyl) can be extracted with ethyl acetate from the organic layer and the resulting fractions dried *in vacuo*.

[0072] In a particular embodiment of the invention Boc-*L*-Ser-OMe (Compound Ib, R = methyl) is dissolved in tetrahydrofuran and 3-4 equivalents of 2,2-dimethoxypropane is  
 20 added, followed by a solution of about 0.10 equivalents of benzenesulfonic acid in tetrahydrofuran, and the reaction heated to reflux while some of tetrahydrofuran is distilled off. The reaction is neutralized to pH 6.5 with triethylamine at about 22 C. The solvent is distilled off and water and hexane are added. The reaction product, (*S*)-3-(*tert*-  
 25 butoxycarbonyl)-2,2-dimethyl-4-oxazolidincarboxylic acid methylester (Compound Ic, R = methyl), can be isolated from the hexane layer.

[0073] In another aspect of the invention, 2 equivalents of dimethyl methylphosphonate are dissolved in tetrahydrofuran and the resultant mixture is cooled to about -70 to -80°C. About 2 equivalents of *n*-butyllithium in heptane are added over the course of 1 to 3 hours while the  
 30 mixture is kept at about -70 to -80°C. After stirring for about 1 hour, 1 equivalent of (*S*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-oxazolidincarboxylic acid methylester in tetrahydrofuran is added over the course of 30 to 90 minutes while the mixture is kept at

about -70 to -80°C. The mixture is warmed to about 0°C over the course of 30 to 60 minutes and then stirred for 15 to 60 minutes. The reaction is quenched with the addition of water in tetrahydrofuran and the pH adjusted to pH 6-7 with the addition of a citric acid solution. An organic solvent, such as but not limited to ethyl acetate or diethyl ether, is added and the product, (S)-3-(tert-butoxycarbonyl)-4-(2-(dimethoxyphosphoryl)-1-oxo-ethyl)-2,2-dimethyloxazolidin (Compound Id), recovered from the organic layer.

[0074] In a further aspect of the invention, 1 equivalent of (S)-3-(tert-butoxycarbonyl)-4-(2-(dimethoxyphosphoryl)-1-oxo-ethyl)-2,2-dimethyloxazolidin (Compound Id) and about 2 equivalents of potassium carbonate are stirred in acetonitrile at about 22°C, followed by the addition of about 0.5 equivalents of 1-tetradecanal and water. The reaction proceeds for 8-14 hours with stirring. The salts are filtered off and washed with hexane and the product, (S)-3-(tert-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidin (Compound Ie) is recovered from the organic phase.

[0075] In a still further aspect of the invention, 1 equivalent of (S)-3-(tert-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidin (Compound Ie) and about 1.1 to 1.5 equivalents of cerium chloride heptahydrate are stirred in methanol and the mixture is cooled to -20 to -15°C. An aqueous solution of about 1.5 equivalents of sodium borohydride and about 0.01 equivalents of NaOH is cooled to about 0°C and added to the (S)-3-(tert-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidin mixture over the course of about 4 to 6 hours. After about 15 to 60 minutes of additional stirring the mixture is heated to about 22 °C over the course of 1 to 3 hours, followed by stirring for 30 to 90 minutes. Methanol is removed under vacuum and the resulting aqueous suspension is filtered. The resulting solids are washed with an organic solvent, such as toluene. The aqueous layer is extracted at least twice with an organic solvent, such as toluene. The organic layers are combined and the product, (2S,3R,4E)-3-(tert-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidin (Compound If), is recovered from the organic layer.

[0076] In a certain embodiment of the invention (2S,3R,4E)-3-(tert-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidin (Compound If) is converted to *D-erythro*-sphingosine. The reaction proceeds as follows: methanol is cooled to about 0°C and about 2 equivalents of acetylchloride are added over the course of about 15 to 60 minutes, generating a methanolic hydrochloric acid solution. The solution is then warmed to room temperature. (2S,3R,4E)-3-(tert-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidin is

dissolved in methanol and the methanolic hydrochloride solution is added over the course of about 15 to 60 minutes. The reaction is neutralized with the addition of triethylamine. The solvent can then be removed and the resulting *D-erythro*-sphingosine can be recovered from the residue.

5 [0077] In a particular embodiment, the invention provides methods for synthesizing *D-erythro*-sphingosine, comprising the steps of:

a) protecting the amino group of an *L*-serine methyl ester or a salt thereof with a *tert*-butoxycarbonyl group, to yield Boc-*L*-Ser-OMe;

10 b) allowing Boc-*L*-Ser-OMe to react with 2,2-dimethoxypropane in the presence of benzenesulfonic acid under conditions effective to yield (*S*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-oxazolidincarboxylic acid methylester;

15 c) allowing (*S*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-oxazolidincarboxylic acid methylester to react with dimethyl methylphosphonate in the presence of *n*-butyllithium under conditions effective to yield (*S*)-3-(*tert*-butoxycarbonyl)-4-(2-(dimethoxy-phosphoryl)-1-oxo-ethyl)-2,2-dimethyloxazolidine;

d) allowing (*S*)-3-(*tert*-butoxycarbonyl)-4-(2-(dimethoxy-phosphoryl)-1-oxo-ethyl)-2,2-dimethyloxazolidine to react with 1-tetradecanal under conditions effective to yield (*S*)-3-(*tert*-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidine;

20 e) allowing (*S*)-3-(*tert*-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidine to react with sodium borohydride and cerium trichloride, under conditions effective to yield (2*S*,3*R*,4*E*)-3-(*tert*-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidine; and

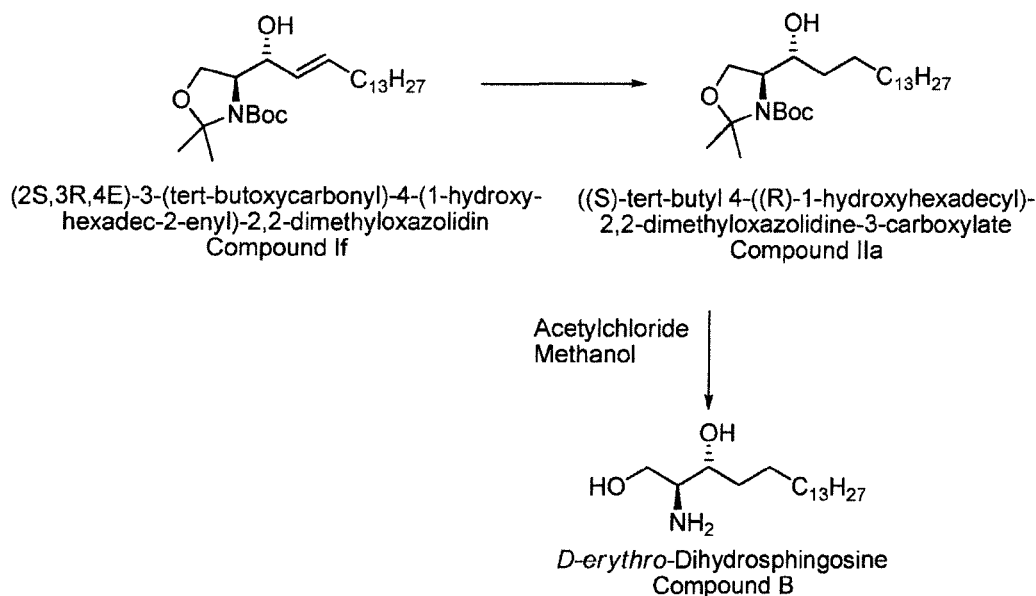
25 f) removing the *tert*-butoxycarbonyl (Boc) protecting group of (2*S*,3*R*,4*E*)-3-(*tert*-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidine to yield *D-erythro*-sphingosine.

***D-erythro-dihydrosphingosine***

[0078] In a particular embodiment, the invention provides methods for synthesizing *D-erythro*-dihydrosphingosine using a suitable *L*-serine ester, such as that of Compound Ia. In a certain embodiment of the invention R is an alkyl group having 1 to 5 carbons. The term “alkyl,” as used herein unless otherwise defined, refers to a straight, branched, or cyclic saturated group derived from the removal of a hydrogen atom from an alkane. Representative straight chain alkyl groups include -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, and -n-heptyl. Representative branched alkyl groups include -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl, -neopentyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl, 1,1-dimethylpropyl and 1,2-dimethylpropyl. Representative cyclic alkyl groups include cyclohexyl, cyclopentyl, and cyclopropyl.

[0079] In certain embodiments of the invention, the *L*-serine ester is *L*-serine methyl ester. In another embodiment, the *L*-serine ester is *L*-serine ethyl ester. In yet another embodiment, the *L*-serine ester is *L*-serine butyl ester.

[0080] In one embodiment of the invention *D-erythro*-dihydrosphingosine is synthesized by the method shown in Scheme II. The reaction comprises the following steps:



Scheme II

[0081] Compound If is reacted with a reducing agent to afford its corresponding saturated compound, Compound IIa. The reaction is performed in an organic solvent such as, but not limited to, a primary or secondary alcohol, THF, or 2-methyl-THF, in the presence of H<sub>2</sub> and a catalyst, such as, but not limited to a palladium(0) on carbon catalyst ruthenium(II) catalyst, e.g., Ru(OAc)<sub>2</sub>(BINAP), [{RuCl(μ-Cl)(η<sup>6</sup>-C<sub>6</sub>Me<sub>6</sub>)}<sub>2</sub>], or Ru(OH)<sub>x</sub>/Al<sub>2</sub>O<sub>3</sub>.

[0082] In a particular embodiment, Compound If is reacted with H<sub>2</sub> in isopropyl alcohol at about 80°C in the presence of [{RuCl(μ-Cl)(η<sup>6</sup>-C<sub>6</sub>Me<sub>6</sub>)}<sub>2</sub>] at reflux. The reaction mixture is quenched and subjected to work-up when no more starting allyl alcohol, Compound If, is detected by using a method well known to a person skilled in the art, such as, but not limited to, HPLC, thin-layer chromatography, or IR. In a certain embodiment, H<sub>2</sub> is added as a gas and the reaction is performed in a hydrogenation vessel under pressure.

[0083] Compound IIa thus obtained, either crude or purified, is dissolved in methanol at about 0°C, and acetylchloride is added over about 15 to 60 minutes, generating methanolic hydrochloric acid. When no more starting material (or no more conversion) is detected by a method such as a chromatography method, the reaction is treated with a base, which can be an organic base, such as, but not limited to, triethylamine or pyridine, or an inorganic base in aqueous solution, such as bicarbonates or carbonates of sodium, potassium, calcium, magnesium and ammonium. Further, the reaction mixture is extracted with a solvent, such as a chlorinated solvent, ethyl acetate or an ether, such as diethyl ether, THF, t-butyl methyl ether, isopropyl ether, etc. The solvent is then removed and the resulting *D-erythro*-dihydrosphingosine is recovered as a free base. The base can be treated with hydrochloric acid to produce the corresponding hydrochloride salt.

### ***D-erythro-sphingosine***

[0084] In another embodiment, the invention provides methods for synthesizing *N*-palmitoyl-*D-erythro*-sphingosine as shown in Scheme III.

[0085] In yet another embodiment, the invention methods for synthesizing *N*-palmitoyl-*D-erythro*-sphingosine, comprising the steps of:

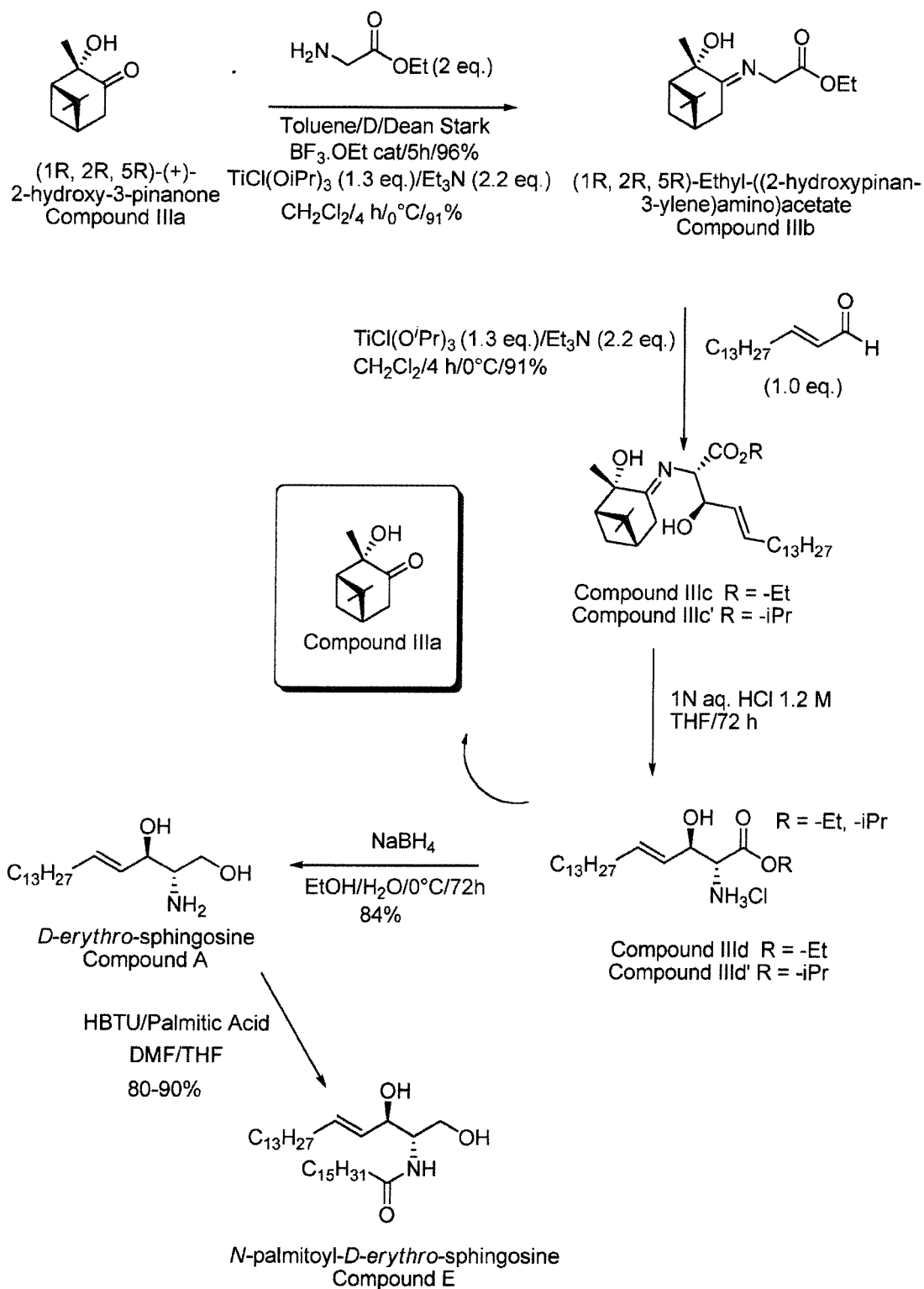
- a) allowing (1*R*, 2*R*, 5*R*)-(+)-2-hydroxy-3-pinane (Compound IIIa) to react with ethylglycinate under conditions effective to yield (1*R*, 2*R*, 5*R*)-Ethyl-((2-hydroxypinan-3-ylene)amino)acetate (Compound IIIb);

b) allowing (1*R*, 2*R*, 5*R*)-Ethyl-((2-hydroxypinan-3-ylene)amino)acetate (Compound IIIb) to react with 2-(*E*)-hexadecen-1-al presence of chlorotitanium triisopropoxyde and triethylamine to yield one or both of (2*S*,3*R*,*E*)-ethyl-3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate (Compound IIIc) and (2*S*,3*R*,*E*)-isopropyl-3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate (Compound IIIc');  
 5

c) allowing the one or both of (2*S*,3*R*,*E*)-ethyl-3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate (Compound IIIc) and (2*S*,3*R*,*E*)-isopropyl-3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate (Compound IIIc') to react with hydrochloric acid under conditions effective to yield one or both of (2*R*,3*R*,*E*)-ethyl 2-amino-3-hydroxyoctadec-4-enoate (Compounds IIId) and (2*R*,3*R*,*E*)-isopropyl 2-amino-3-hydroxyoctadec-4-enoate (Compound IIId');  
 10

d) allowing the one or both of (2*R*,3*R*,*E*)-ethyl 2-amino-3-hydroxyoctadec-4-enoate and (2*R*,3*R*,*E*)-isopropyl 2-amino-3-hydroxyoctadec-4-enoate to react with sodium borohydride under conditions effective to yield *D*-erythro-sphingosine (Compound A); and  
 15

e) reacting *D*-erythro-sphingosine with palmitic acid under conditions effective to afford *N*-palmitoyl-*D*-erythro-sphingosine (Compound E).  
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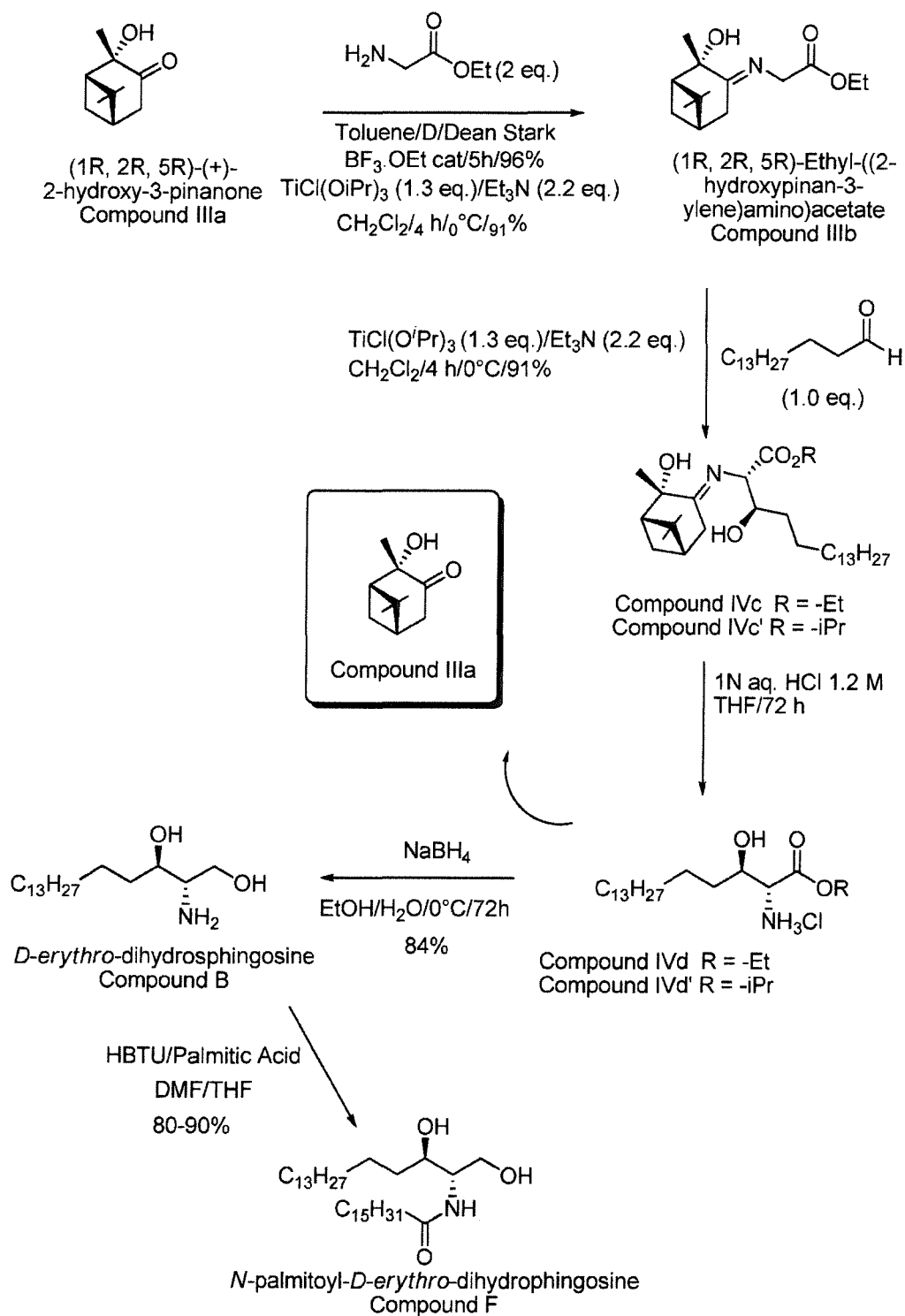


Scheme III

*D-erythro-dihydrosphingosine*

[0086] In yet another embodiment, the invention provides methods for synthesizing *D-erythro*-dihydrosphingosine as shown in Scheme IV, and comprises the following steps:

- 5                   a) allowing (1*R*, 2*R*, 5*R*)-(+)-2-hydroxy-3-pinanone (Compound IIIa) to react with ethylglycinate under conditions effective to yield (1*R*, 2*R*, 5*R*)-Ethyl-((2-hydroxypinan-3-ylene)amino)acetate (Compound IIIb);
- 10                  b) allowing (1*R*, 2*R*, 5*R*)-Ethyl-((2-hydroxypinan-3-ylene)amino)acetate (Compound IIIb) to react with hexadecanal in the presence of chlorotitanium triisopropoxyde to under conditions effective yield one or both of (2*S*,3*R*,*E*)-ethyl 3-hydroxy-2-(((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadecanoate (Compound IVc) and (2*S*,3*R*,*E*)-isopropyl 3-hydroxy-2-(((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadecanoate (Compound IVc');
  - 15                  c) allowing the one or both of (2*S*,3*R*,*E*)-ethyl 3-hydroxy-2-(((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadecanoate (Compound IVc) and (2*S*,3*R*,*E*)-isopropyl 3-hydroxy-2-(((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadecanoate (Compound IVc') to react with hydrochloric acid under conditions effective to yield one or both of (2*R*,3*R*,*E*)-ethyl 2-amino-3-hydroxyoctadecanoate (Compound IVd) and (2*R*,3*R*,*E*)-isopropyl 2-amino-3-hydroxyoctadecanoate (Compound IVd');
    - 20                  d) allowing the one or both of (2*R*,3*R*,*E*)-ethyl 2-amino-3-hydroxyoctadecanoate (Compound IVd) and (2*R*,3*R*,*E*)-[isopropyl 2-amino-3-hydroxyoctadecanoate (Compound IVd') to react with sodium borohydride under conditions effective to yield *D-erythro*-dihydrosphingosine (Compound B);
      - 25                  e) allowing *D-erythro*-dihydrosphingosine (Compound B) to react with palmitic acid under conditions effective to yield *N*-palmitoyl-*D-erythro*-dihydrosphingosine (Compound F).

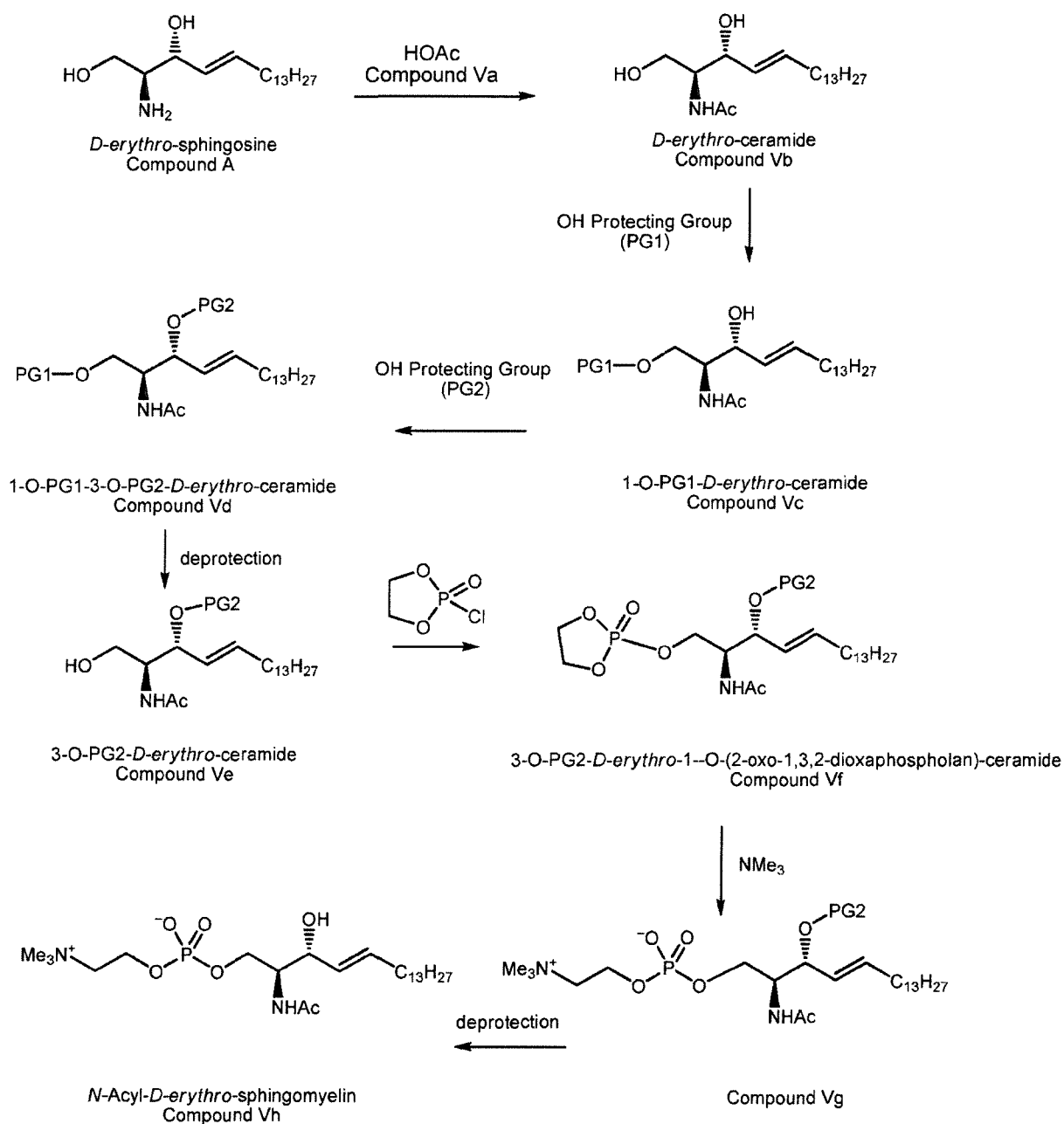


Scheme IV

***Synthesis of N-acyl-D-erythro-sphingomyelin***

[0087] In a further embodiment, the invention provides methods for synthesizing an *N*-acyl-*D*-erythro-sphingomyelin as shown in Scheme V comprising the steps of:

- 5           a) allowing *D*-erythro-sphingosine to react with a fatty acid (Compound Va) under conditions effective to yield a *D*-erythro-ceramide (Compound Vb);
- b) protecting the primary hydroxyl group of the *D*-erythro-ceramide (Compound Vb) with a first protection group to yield Compound Vc;
- c) protecting the secondary hydroxyl group of Compound Vc with a second protection group to yield Compound Vd;
- 10          d) removing the first protecting group of Compound Vd to yield Compound Ve;
- e) allowing Compound Ve to react with 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) under conditions effective to yield Compound Vf;
- f) allowing Compound Vf to react with trimethylamine under conditions  
15       effective to yield Compound Vg;
- g) removing the second protecting group of Compound Vg with sodium methoxide to yield the *N*-acyl-*D*-erythro-sphingomyelin (Compound Vh).



Scheme V

HOAc is a fatty acid

Ac is a fatty acid residue having 3 to 36 carbons and zero to six carbon-carbon double bonds

PG1 and PG2 are any suitable protecting groups known in the art.

In certain embodiments PG1 is triphenylmethyl (Trt). In certain embodiments PG2 is benzoyl (Bz)

### Synthesis of *N*-acyl-*D*-erythro-ceramide

[0088] In certain embodiments of the invention, the *N*-acylation of the *D*-erythro-sphingosine with fatty acid to yield *N*-acyl-*D*-erythro-ceramide comprises the steps shown in Scheme V. The steps are as follows: *D*-erythro-sphingosine (Compound A), the fatty acid (Compound Va), and an amide-forming agent are suspended in an aprotic organic solvent and the mixture is cooled at a temperature of about 0-5°C. In one embodiment, the aprotic organic solvent is tetrahydrofuran, dimethylformamide, 2-methyltetrahydrofuran, or mixtures thereof. In particular embodiments of the invention, the amide-forming agent is O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluorophosphate (HBTU).

[0089] An organic base, such as, but not limited to triethylamine or pyridine, is added to the mixture of *D*-erythro-sphingosine (Compound A), fatty acid (Compound Va), and amide-forming agent. In certain embodiments, the organic base is in an aprotic organic solvent and is tetrahydrofuran, dimethylformamide, 2-methyltetrahydrofuran, or mixtures thereof. In further embodiments of the invention, the organic base is added over the course of about 15 to 90 minutes. The mixture is then stirred for 1 to 15 hours at a temperature of about 0-22°C. In certain embodiments of the invention, the reaction proceeds at a temperature of about 0-5°C. In other embodiments of the invention, the reaction proceeds at about 22°C. In yet other embodiments of the invention, the reaction proceeds at about room temperature.

[0090] After stirring for about 1 to 15 hours, the product, Compound Vb, is precipitated by the addition of an acid. In certain embodiments of the invention, the acid is an organic acid, such as citric acid, acetic acid, or oxalic acid. The acid can be in an aqueous solution when added. The reaction can be at about 22°C when the acid is added. The resulting suspension can be stirred for 30 to 120 minutes at a temperature of about 0-5°C. In certain embodiments of the invention, the suspension is stirred at about 22°C.

[0091] After stirring, the suspension is filtered. The resulting product, Compound Vb, can then be resuspended in water, after which it can be filtered and washed. The resuspension can be reiterated at least one more time. The resulting product, *D*-erythro-ceramide (Compound Vb), can be washed with water, acetone, or a mixture thereof.

### Synthesis of protected *D*-erythro-ceramide

[0092] In certain embodiments of the invention, the primary hydroxyl group of the *D*-erythro-ceramide is protected, followed by protection of the secondary hydroxyl, and then

deprotection of the primary hydroxyl. In further embodiments of the invention, the protection and deprotection steps proceed without isolation or purification of the primary hydroxyl protected *D-erythro*-ceramide.

[0093] In particular embodiments of the invention, the primary hydroxyl group is protected with a trityl group by reacting the *D-erythro*-ceramide with a tritylating reagent, such as, but not limited to, trityl halides such as trityl chloride and trityl bromide. In certain embodiments of the invention, the secondary hydroxyl group is protected with a benzoyl group by reacting the 1-O-protected *D-erythro*-ceramide with a benzoylating reagents include, such as, but are not limited to, benzoyl halides such as benzoyl chloride and trityl bromide. In further  
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10  
embodiments of the invention the primary hydroxyl group is protected with a trityl group and the secondary hydroxyl is protected with a benzoyl group.

[0094] In certain embodiments of the invention, the protection of the primary hydroxyl group proceeds as follows: *D-erythro*-ceramide (Compound Vb) and trityl chloride are suspended in an organic solvent in the presence of a base. The reaction proceeds at a temperature of  
15  
about 25 -55°C for about 10 to 60 hours to yield the trityl protected *D-erythro*-ceramide (1-O-trityl-*D-erythro*-ceramide Compound Vc, where PG1 = -Trt) . The organic solvent can be a nonpolar or polar solvent. In one embodiment of the invention, the organic solvent is a nonpolar solvent and is toluene, benzene, hexane or mixtures thereof. In one embodiment the organic solvent is an aprotic polar solvent. In one embodiment, the aprotic organic solvent is  
20  
methylene chloride, chloroform or carbon tetrachloride. In another embodiment, the aprotic organic solvent is nonchlorinated and is diethyl ether, tetrahydrofuran, or ethyl acetate. The base is typically an organic base, such as triethylamine or pyridine.

[0095] In certain embodiments of the invention, the protection of the primary hydroxyl group, e.g., using trityl chloride, yields no more than about 10 mol% of 1,3-O,O-ditrityl-*D-erythro*-ceramide of the crude reaction products. In further embodiments of the invention, the  
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protection of the primary hydroxyl group, e.g., using trityl chloride, yields no more than about 7 mol% of 1,3-O,O-ditrityl-*D-erythro*-ceramide of the crude reaction products. In still further embodiments of the invention, the protection of the primary hydroxyl group, e.g., using trityl chloride, yields no more than about 5 mol% of 1,3-O,O-ditrityl-*D-erythro*-ceramide of the  
30  
crude reaction products. In still further embodiments of the invention, the protection of the primary hydroxyl group, e.g., using trityl chloride, yields no more than about 1 mol% of 1,3-O,O-ditrityl-*D-erythro*-ceramide of the crude reaction products.

[0096] In further embodiments of the invention, the protection of the secondary hydroxyl group proceeds directly as follows: the above reaction mixture is cooled to about 0-5°C and benzoyl chloride and a base are added. The reaction proceeds at a temperature of about 0-5°C for about 1 to 16 hours. The reaction product, 3-benzoyl and 1-trityl protected *D-erythro*-ceramide (Compound Vd; PG1 = -Trt; PG2 = -Bz), can be extracted from the organic layer with an organic solvent, including, but not limited to, an aprotic organic solvent described above. The organic solvent is then removed by a suitable method known to one of skill in the art, including, but not limited to, evaporation, e.g., concentration *in vacuo*. In particular embodiments the base is an organic base, such as triethylamine or pyridine.

[0097] In yet further embodiments of the invention, the deprotection of the primary hydroxyl group proceeds directly as follows: the residue from the above reaction is dissolved in an organic solvent and an acid is added. The reaction proceeds at a temperature of about 22°C for 1 to 16 hours. The reaction mixture is neutralized by the addition of a base. The organic solvent can be a protic polar solvent, an aprotic polar solvent, or a mixture thereof. In one embodiment the organic solvent is a protic polar solvent and is methanol, ethanol, *n*-propanol, or isopropanol. In one embodiment the organic solvent is an aprotic polar solvent. In one embodiment, the aprotic organic solvent is chlorinated and is methylene chloride, chloroform, or carbon tetrachloride. In another embodiment, the aprotic organic solvent is nonchlorinated and is diethyl ether, tetrahydrofuran, or ethyl acetate. The acid can be any acid known by one of skill in the art to be suitable for removal of the trityl protecting group, e.g., acetic acid, trifluoroacetic acid, hydrochloric acid and p-toluenesulfonic acid. In certain embodiments of the invention the acid is p-toluenesulfonic acid. In particular embodiments the base is an organic base, such as triethylamine or pyridine.

[0098] In certain embodiments of the invention the above deprotection product (Compound Ve; PG2 = -Bz) can be purified by recrystallization, silica gel chromatography, high performance liquid chromatography or other methods known to those skilled in the art.

Phosphorylation and amination of 3-benzoyl-protected *D-erythro*-ceramide to yield benzoyl-protected *N*-acyl-*D-erythro*-sphingomyelin

[0099] In further embodiments of the invention the 3-benzoyl-protected *D-erythro*-ceramide, (Compound Ve; PG2 = -Bz), is phosphorylated as follows: The 3-benzoyl-protected *D-erythro*-ceramide is dissolved in an organic solvent and an organic base is added. After cooling to about 4-9 °C, a solution of 2-Chloro-2-oxo-1,3,2-dioxaphospholane in an organic

solvent is added. The reaction proceeds at a temperature from about 4-22 °C for about 2 to 6 hours to produce 3-O-Benzoyl-1-O-(2-oxo-1,3,2-dioxaphospholan)-ceramide (Compound Vf, where PG2 = Bz). In certain embodiments of the invention, the reaction proceeds at about 4-9 °C for about 15 minutes to 2 hours and is then warmed to about 22 °C and proceeds for an additional 2 to 4 hours. The organic solvent can be a nonpolar solvent, a polar solvent, or mixtures thereof. In one embodiment of the invention, the organic solvent is a nonpolar solvent and is toluene, benzene, hexane or mixtures thereof. In one embodiment the organic solvent is an aprotic polar solvent such as acetonitrile, ethyl acetate, tetrahydrofuran, or mixtures thereof. The organic base is typically tetramethylethylenediamine or triethylamine.

In certain embodiments of the invention, the organic base is tetramethylethylenediamine.

[00100] In certain embodiments, amination of the 3-O-Benzoyl-1-O-(2-oxo-1,3,2-dioxaphospholan)-ceramide (Compound Vf, PG2 =-Bz) occurs without purification or isolation of the phosphorylated benzoyl-protected *D-erythro*-cceramide starting material. After the above reaction has proceeded for about 2 to 6 hours, additional organic solvent and trimethylamine are added, the reaction mixture is heated to 60 – 70°C and the reaction is allowed to proceed for 10 to 16 hours to yield the 3-benzoyl-protected *N*-acyl-*D-erythro*-sphingomyelin (Compound Vg, PG2 =-Bz). The organic solvent can be a nonpolar solvent, a polar solvent or mixtures thereof. In one embodiment of the invention, the organic solvent is a nonpolar solvent and is toluene, benzene, hexane or mixtures thereof. In one embodiment the organic solvent is an aprotic polar solvent such as acetonitrile, ethyl acetate, tetrahydrofuran, or mixtures thereof. In certain embodiments, trimethylamine is added as a liquid. In other embodiments, trimethylamine is added in a gaseous form. In particular embodiments, the liquid trimethylamine is anhydrous. In certain embodiments, the trimethylamine is cooled to below its boiling point and added as a liquid. In certain embodiments the reaction is cooled to about -10°C to 0°C prior to addition of liquid trimethylamine. In further embodiments, the reaction is cooled to about -10°C prior to addition of liquid trimethylamine.

[00101] In certain embodiments of the invention the 3-benzoyl-protected *N*-acyl-*D-erythro*-sphingomyelin can be purified by recrystallization, silica gel chromatography, high performance liquid chromatography or other methods known to those skilled in the art.

Deprotection of 3-O-benzoyl-protected *N*-acyl-*D-erythro*-sphingomyelin to yield *N*-acyl-*D-erythro*-sphingomyelin

[00102] The removal of the benzoyl protecting group from the 3-O-benzoyl-protected *N*-acyl-*D*-erythro-sphingomyelin proceeds as follows: the 3-O-benzoyl-protected sphingomyelin is dissolved in a protic polar solvent and a base is added. The reaction proceeds for 8 to 24 hours at about 22°C. In certain embodiments of the invention an aprotic solvent and water are added to the reaction mixture and the *N*-acyl-*D*-erythro-sphingomyelin (Compound Vh) is recovered from the organic layer. In one embodiment the protic polar solvent is methanol, ethanol, *n*-propanol, isopropanol, or mixtures thereof. In yet another embodiment the base is sodium methoxide.

[00103] In further embodiments of the invention the *N*-acyl-*D*-erythro-sphingomyelin can be purified by recrystallization, silica gel chromatography, high performance liquid chromatography or other methods known to those skilled in the art.

[00104] In certain embodiments of the invention, the resulting *N*-acyl-*D*-erythro-sphingomyelin has an enantiomeric purity of at least about 85% and contains no more than about 15% of its corresponding opposite enantiomer. In further embodiments of the invention, the *N*-acyl-*D*-erythro-sphingomyelin has an enantiomeric purity of at least about 90% and contains no more than about 10% of its corresponding opposite enantiomer. In yet further embodiments *N*-acyl-*D*-erythro-sphingomyelin has an enantiomeric purity of at least about 95% and contains no more than about 5% of its corresponding opposite enantiomer. In still further embodiments *N*-acyl-*D*-erythro-sphingomyelin has an enantiomeric purity of at least about 98% and contains no more than about 2% of its corresponding opposite enantiomer.

[00105] In another embodiment, the invention provides methods for synthesizing an *N*-acyl-*D*-erythro-sphingomyelin comprising the steps of:

a) allowing *D*-erythro-sphingosine to react with a fatty acid under conditions effective to yield a *D*-erythro-ceramide;

b) allowing *D*-erythro-ceramide to react with a tritylating reagent under conditions effective to yield 1-O-trityl-*D*-erythro-ceramide;

c) allowing 1-O-trityl-*D*-erythro-ceramide to react with a benzoylating reagent under conditions effective to yield 1-O-trityl-3-O-*D*-erythro-benzoyl-ceramide;

d) removing the trityl group of 1-O-trityl-3-O-*D-erythro*-benzoyl-ceramide to yield *D-erythro*-3-O-benzoyl-ceramide;

e) allowing 3-O-benzoyl-*D-erythro*-ceramide to react with with 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) under conditions effective to yield 3-O-benzoyl-*D-erythro*-1-O-(2-oxo-1,3,2-dioxaphospholane) ceramide;

f) allowing 3-O-benzoyl-*D-erythro*-1-O-(2-oxo-1,3,2-dioxaphospholane) ceramide to react with trimethylamine under conditions effective to yield the *N*-acyl-*D-erythro*-3-O-benzoyl-sphingomyelin; and

g) removing the benzoyl group of *N*-acyl-*D-erythro*-3-O-benzoyl-sphingomyelin with sodium methoxide to yield *N*-acyl-*D-erythro*-sphingomyelin.

### ***Synthesis of N-palmitoyl-D-erythro-sphingomyelin***

[00106] In still another embodiment, the invention provides methods for synthesizing *N*-palmitoyl-*D-erythro*-sphingomyelin comprising the steps of:

a) allowing *D-erythro*-sphingosine to react with palmitic acid in the presence of O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate and triethylamine under conditions effective to yield *N*-palmitoyl-*D-erythro*-sphingosine

b) protecting *N*-palmitoyl-*D-erythro*-sphingosine to react with a trityl group to yield *N*-palmitoyl-1-O-trityl-*D-erythro*-sphingosine;

c) protecting *N*-palmitoyl-1-O-trityl-*D-erythro*-sphingosine with a benzoyl group to yield *N*-palmitoyl-1-O-trityl-3-O-benzoyl-*D-erythro*-sphingosine ;

d) removing the trityl group of *N*-palmitoyl-1-O-trityl-3-O-benzoyl-*D-erythro*-sphingosine in the presence of *para*-toluenesulfonic acid to yield *N*-palmitoyl-3-O-benzoyl-*D-erythro*-sphingosine;

e) allowing *N*-palmitoyl-3-O-benzoyl-*D-erythro*-sphingosine to react with 2-chloro-2-oxo-1,3,2-dioxaphospholane under conditions effective to yield *N*-palmitoyl-3-O-benzoyl-*D-erythro*-1-O-(2-oxo-1,3,2-dioxaphospholane)-sphingosine;

f) allowing *N*-palmitoyl-3-O-benzoyl-*D*-erythro-1-O-(2-oxo-1,3,2-dioxaphospholane)-sphingosine to react with trimethylamine under conditions effective to yield *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingomyelin; and

5 g) removing the benzoyl group of *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingomyelin with sodium methoxide to yield *N*-palmitoyl-*D*-erythro-sphingomyelin.

[00107] In a particular embodiment of the invention *N*-palmitoyl-*D*-erythro-sphingomyelin is synthesized as shown in Scheme VI:

[00108] One equivalent of palmitic acid, one equivalent of *D*-erythro-sphingosine (Compound  
10 A), and 1.10 equivalents of O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluorophosphate (HBTU) are suspended in tetrahydrofuran and dimethylformamide and cooled to about 0-5°C. Two to three equivalents of triethylamine are added and the mixture is stirred for about one to twelve hours at about 0-5°C. The mixture is warmed to about 22°C. An aqueous solution of citric acid is added and the mixture is stirred for 15 to 90 min at about  
15 22°C. The resulting suspension is filtered and the cake is suspended in water, at room temperature. The suspension is filtered and washed with water and acetone. The resulting product, *N*-palmitoyl-*D*-erythro-sphingosine (Compound E), can then be dried.

[00109] For the first hydroxyl protection, one equivalent of *N*-palmitoyl-*D*-erythro-sphingosine (Compound E) is suspended in pyridine and methylene chloride. A solution of  
20 about 1.05 equivalents of trityl chloride in methylene chloride is added followed by additional methylene chloride. The reaction mixture is stirred at about 25°C for 50-60 hours.

[00110] In certain embodiments of the invention, the protection of the primary hydroxyl group yields less than 10 mol% of *N*-palmitoyl-1,3-O,O-ditrityl-*D*-erythro-sphingosine of the crude reaction products. In further embodiments of the invention, the protection of the primary  
25 hydroxyl yields no more than about 7 mol% of *N*-palmitoyl-1,3-O,O-ditrityl-*D*-erythro-sphingosine of the crude reaction products. In still further embodiments of the invention, the protection of the primary hydroxyl yields no more than about 5 mol% of *N*-palmitoyl-1,3-O,O-ditrityl-*D*-erythro-sphingosine of the crude reaction products. In still further  
30 embodiments of the invention, the protection of the primary hydroxyl yields no more than about 1 mol% of *N*-palmitoyl-1,3-O,O-ditrityl-*D*-erythro-sphingosine of the crude reaction products.

[00111] For the second hydroxyl group protection, the reaction mixture comprising Compound VIa is cooled to about 2°C. *N,N*-Dimethylaminopyridine at about 0.10 equivalent, benzoyl chloride at about 1.50 equivalents and additional methylene chloride are added. The reaction is allowed to proceed at about 2°C with stirring until thin layer chromatography (TLC)

5 analysis shows the presence of starting material *N*-palmitoyl-1-*O*-trityl-sphingosine of less than about 5%. Ethyl acetate and an aqueous citric acid and sodium chloride solution are added to the reaction mixture, and *N*-palmitoyl-1-*O*-trityl-3-*O*-benzoyl-*D*-*erythro*-sphingosine (Compound VIb) is recovered from the organic phase.

[00112] To remove the trityl protecting group, *N*-palmitoyl-1-*O*-trityl-3-*O*-benzoyl-*D*-*erythro*-sphingosine (Compound VIb) is dissolved in methanol and methylene chloride and cooled to 10 2°C. The pH is adjusted to 2.5 with a solution of 0.57 equivalents of *para*-toluene sulfonic acid monohydrate in methanol. The reaction is allowed to proceed at about 22°C with stirring until TLC analysis shows the presence of starting material 1-*O*-trityl-3-*O*-benzoyl-sphingosine of less than 5%. Triethylamine is added to adjust the pH to about 7.0. The 15 reaction mixture is evaporated to dryness and the resulting crude *N*-palmitoyl-3-*O*-benzoyl-*D*-*erythro*-sphingosine is suspended in hexane at about 40°C and cooled down to about 0°C. After about 30 to 60 minutes the solid is isolated by filtration and washed with hexane. The resulting product, *N*-palmitoyl-3-*O*-benzoyl-*D*-*erythro*-sphingosine (Compound VIc), can then be purified by an appropriate method, such as silica gel chromatography.

[00113] One equivalent of *N*-palmitoyl-3-*O*-benzoyl-*D*-*erythro*-sphingosine (Compound VIc) is dissolved in toluene, about 0.6 to 1 equivalents of tetramethylethylenediamine (TMEDA) is added and the mixture is cooled to about 4-9 °C. About 1 to 2 equivalents of 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) in acetonitrile is added, followed by additional acetonitrile. The reaction is warmed to about 22 °C and stirring continued for 1-3 hours. Additional 25 acetonitrile is added and the temperature is decreased to about -10 to 0°C. Gaseous trimethylamine is cooled to below its boiling point, and about 40 to 60 equivalents of this liquid trimethylamine are added. The reaction mixture is heated to about 60 – 70°C and proceeds for 10 to 16 hours to yield the *N*-palmitoyl-3-*O*-benzoyl-*D*-*erythro*-sphingomyelin (Compound VIe). The reaction is cooled to about -30°C and the resulting suspension is 30 filtered. The crude *N*-palmitoyl-3-*O*-benzoyl-*D*-*erythro*-sphingomyelin is further purified by silica gel chromatography.

[00114] One equivalent of *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingomyelin is dissolved in methanol, about 0.2 equivalents of sodium methoxide are added and the mixture is stirred for 20-26 hours at about 22 °C. Methylene chloride and water are added and the pH is adjusted to about 7 with the addition of hydrochloric acid. *N*-palmitoyl-*D*-erythro-sphingomyelin

5 (Compound C) is recovered from the organic layer.

[00115] In further embodiments of the invention the *N*-palmitoyl-*D*-erythro-sphingomyelin can be purified by recrystallization, silica gel chromatography, high performance liquid chromatography or other methods known to those skilled in the art.

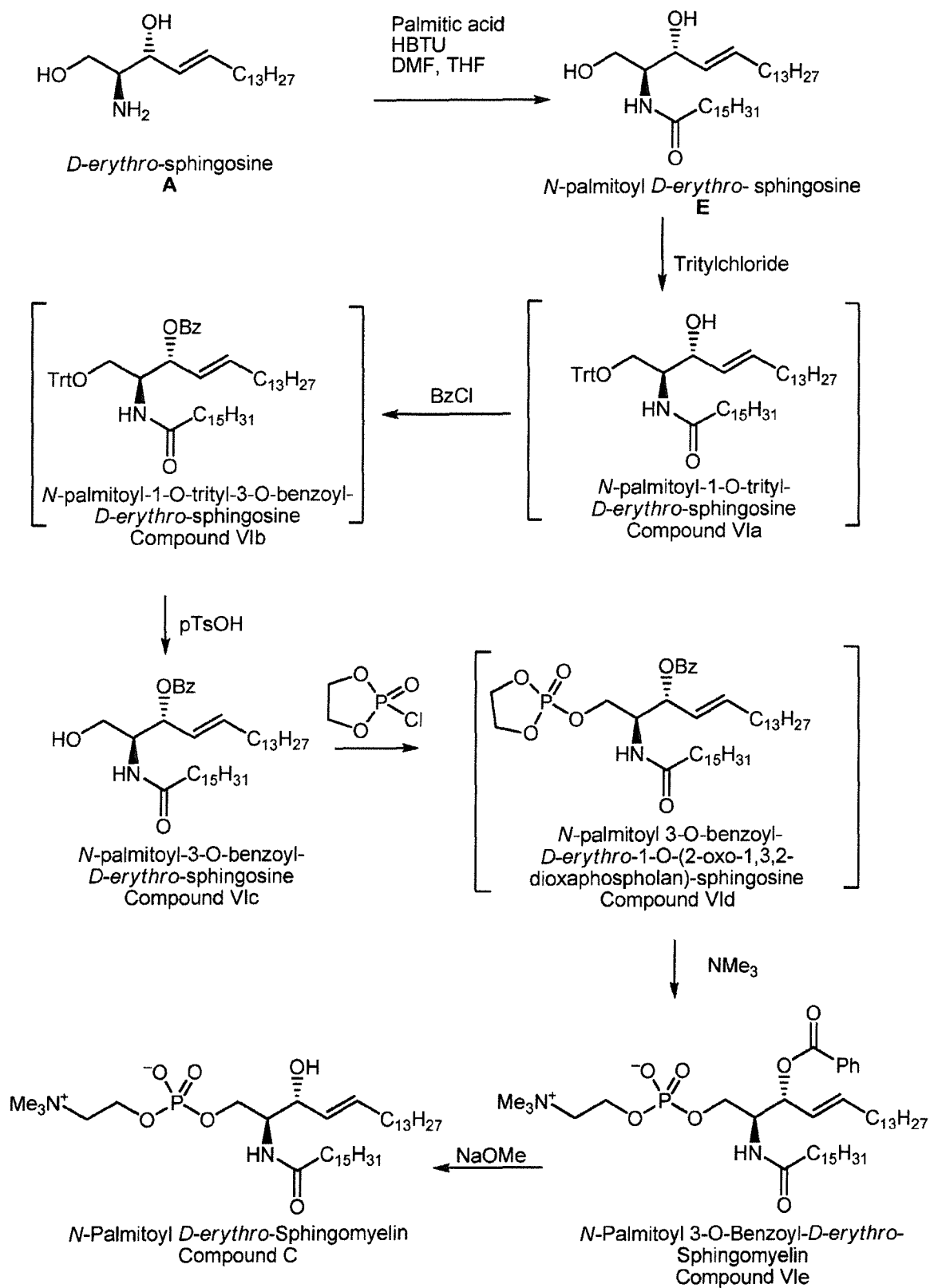
[00116] In certain embodiments of the invention, the resulting *N*-palmitoyl-*D*-erythro-sphingosine has an enantiomeric purity of at least about 85% and contains no more than about 15% of its corresponding opposite enantiomer. In further embodiments of the invention, the *N*-palmitoyl-*D*-erythro-sphingomyelin has an enantiomeric purity of at least about 90% and contains no more than about 10% of its corresponding opposite enantiomer. In yet further  
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15  
embodiments *N*-palmitoyl-*D*-erythro-sphingomyelin has an enantiomeric purity of at least about 95% and contains no more than about 5% of its corresponding opposite enantiomer. In still further embodiments *N*-palmitoyl-*D*-erythro-sphingomyelin has an enantiomeric purity of at least about 98% and contains no more than about 2% of its corresponding opposite enantiomer.

[00117] It has been found that the use of a benzoyl group to protect the secondary alcohol (3-OH) in connection with the methods of the present invention provides the surprising and  
20  
unexpected benefit of minimizing the extent of protecting group migration from the secondary alcohol (3-OH) to the primary alcohol (1-OH).

[00118] Fig. 8 is a photograph of a thin-layer chromatography plate of crude *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingosine (Compound VIc). *N*-palmitoyl-1-O-benzoyl-*D*-erythro-sphingosine is less than about 1% by weight of *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingosine (Compound VIc) and less than about 0.5% by weight of the crude reaction  
25  
products. In further embodiments of the invention, the weight ratio of the *N*-palmitoyl-1-O-benzoyl-*D*-erythro-sphingosine to the *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingosine obtained from the present methods is about 10:90. In still further embodiments of the  
30  
invention, the weight ratio of the *N*-palmitoyl-1-O-benzoyl-*D*-erythro-sphingosine to the *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingosine obtained from the present methods is about 5:95. In still further embodiments of the invention, the weight ratio of the *N*-palmitoyl-1-O-

benzoyl-*D-erythro*-sphingosine to the *N*-palmitoyl-3-O-benzoyl-*D-erythro*-sphingosine obtained from the present methods is about 2:98. In still further embodiments of the invention, the weight ratio of the *N*-palmitoyl-1-O-benzoyl-*D-erythro*-sphingosine to the *N*-palmitoyl-3-O-benzoyl-*D-erythro*-sphingosine obtained from the present methods is about 1:99.

[00119] Additional embodiments of the invention enable large-scale synthesis of an *N*-acyl-*D-erythro*-sphingomyelin, in particular, *N*-palmitoyl-*D-erythro*-sphingomyelin. In certain embodiments of the invention, the present methods enable the synthesis of an *N*-acyl-*D-erythro*-sphingomyelin at an about 1 kilogram scale. In certain embodiments of the invention, the present methods enable the synthesis of an *N*-acyl-*D-erythro*-sphingomyelin at an about 1 to about 5 kilogram scale. In further embodiments of the invention, the present methods enable the synthesis of an *N*-acyl-*D-erythro*-sphingomyelin at an about 1 to about 10 kilogram scale. In yet further embodiments of the invention, the present methods enable the synthesis of *N*-acyl-*D-erythro*-sphingomyelin at an about 1 to about 50 kilogram scale. In still further embodiments of the invention, the present methods enable the synthesis of *N*-palmitoyl-*D-erythro*-sphingomyelin at an about 1 kilogram scale. In still further embodiments of the invention, the present methods enable the synthesis of *N*-palmitoyl-*D-erythro*-sphingomyelin at an about 1 to about 5 kilogram scale. In other embodiments of the invention, the present methods enable the synthesis of *N*-palmitoyl-*D-erythro*-sphingomyelin at an about 1 to about 10 kilogram scale. In particular embodiments of the invention, the present methods enable the synthesis of *N*-palmitoyl-*D-erythro*-sphingomyelin at an about 1 to about 50 kilogram scale.



Scheme VI

***Synthesis of N-acyl-D-erythro-dihydrosphingomyelin***

[00120] In a further embodiment, the invention provides methods for synthesizing a *D-erythro*-dihydrosphingomyelin as shown in Scheme VII comprising the steps of:

a) allowing *D-erythro*-dihydrosphingosine to react with a fatty acid  
 5 (Compound VIIa) under conditions effective to yield a *D-erythro*-dihydroceramide  
 (Compound VIIb);

b) protecting the primary hydroxyl group of the *D-erythro*-dihydroceramide  
 VIIb with a first protection group to yield Compound VIIc;

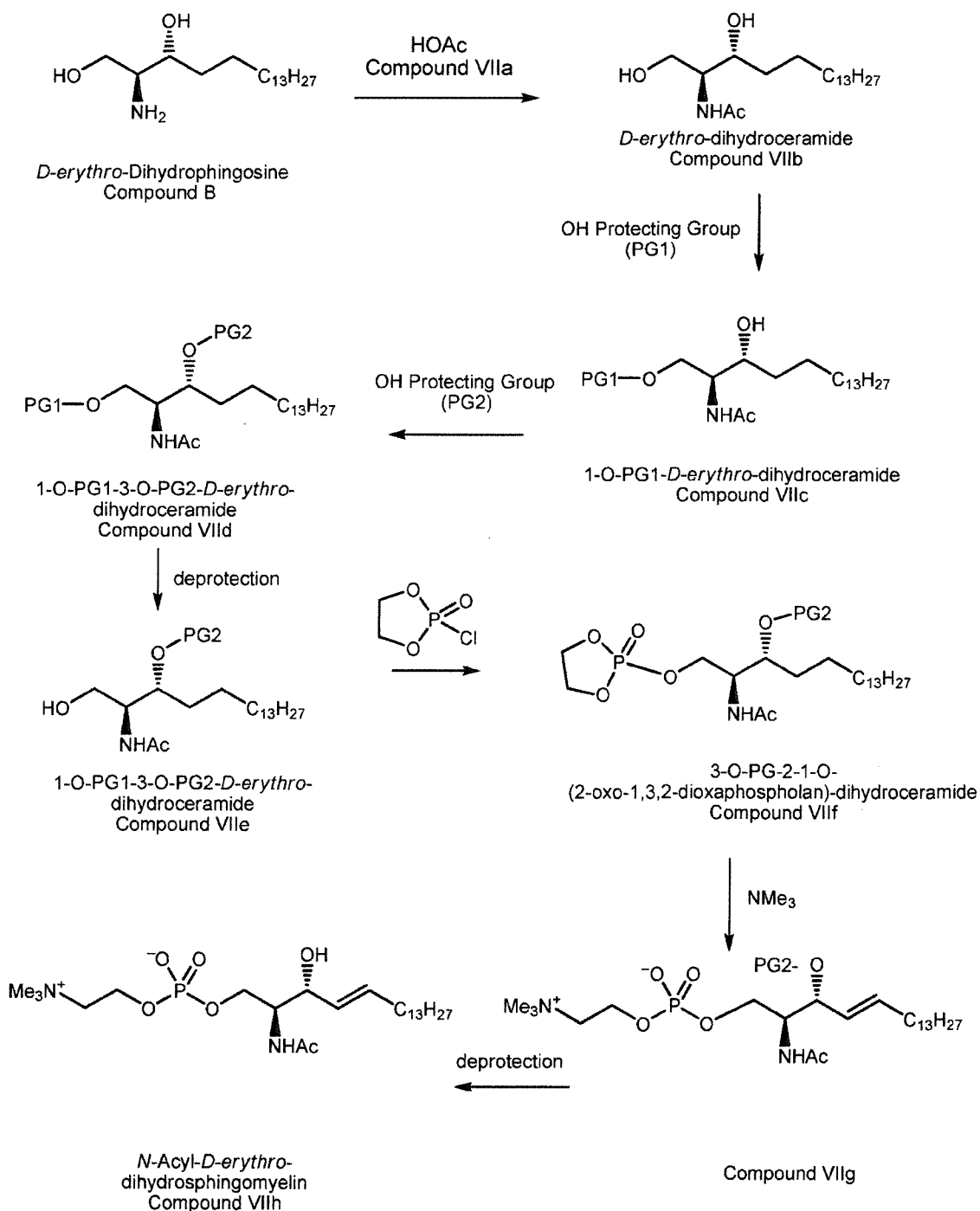
c) protecting the secondary hydroxyl group of the *D-erythro*-dihydroceramide  
 10 with a second protection group yield to yield Compound VIId;

d) removing the first protecting group of Compound VIId to yield Compound  
 VIIe;

e) allowing Compound VIIe to react with 2-chloro-2-oxo-1,3,2-  
 dioxaphospholane (CCP) under conditions effective to yield Compound VIIf;

f) allowing Compound VIIf to react with trimethylamine under conditions  
 15 effective to yield Compound VIIg; and

g) removing the second protecting group of Compound VIIg with sodium  
 methoxide to yield the *D-erythro*-dihydrosphingomyelin (Compound VIIh).



Scheme VII

HOAc is a fatty acid

Ac is a fatty acid residue having 3 to 36 carbons and zero to six carbon-carbon double bonds

PG1 and PG2 are any suitable protecting groups known in the art.

In certain embodiments PG1 is triphenylmethyl (Trt). In certain embodiments PG2 is benzoyl (Bz)

### Synthesis of *D-erythro*-dihydroceramide

[00121] In certain embodiments of the invention, the *N*-acylation of the *D-erythro*-dihydrosphingosine with fatty acid to yield *D-erythro*-dihydroceramide proceeds as shown in Scheme VII. The steps are as follows: *D-erythro*-dihydrosphingosine (Compound B), the fatty acid (Compound VIIa), and an amide forming agent are suspended in an aprotic organic solvent and the mixture is cooled at a temperature of about 0-5°C. In one embodiment, the aprotic organic solvent is tetrahydrofuran, dimethylformamide, 2-methyltetrahydrofuran, or mixtures thereof. In particular embodiments of the invention, the amide forming agent is O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU).

[00122] An organic base, such as, but not limited to triethylamine or pyridine, is added to the mixture of *D-erythro*-dihydrosphingosine, fatty acid, and amide forming agent. In certain embodiments, the organic base is in an aprotic organic solvent and is tetrahydrofuran, dimethylformamide, 2-methyltetrahydrofuran, or mixtures thereof. In further embodiments of the invention, the organic base is added over the course of about 15 to 90 minutes. The mixture is then stirred for 1 to 15 hours at a temperature of about 0-22°C. In certain embodiments of the invention, the reaction proceeds at a temperature of about 0-5°C. In other embodiments of the invention, the reaction proceeds at about 22°C. In yet other embodiments of the invention, the reaction proceeds at about room temperature.

[00123] After stirring for about 1 to 15 hours, the product, Compound VIIb, is precipitated by the addition of an acid. In certain embodiments of the invention, the acid is an organic acid, such as citric acid, acetic acid, or oxalic acid. The acid can be in an aqueous solution when added. The reaction can be at about 22°C when the acid is added. The resulting suspension can be stirred for 30 to 120 minutes at a temperature of about 0-5°C. In certain embodiments of the invention, the suspension is stirred at about 22°C.

[00124] After stirring, the suspension is filtered. The resulting product, Compound VIIb, can then be resuspended in water, after which it can be filtered and washed. The resuspension can occur at least one more time. The resulting product, *D-erythro*-dihydroceramide (Compound VIIb), can be washed with water, acetone, or a mixture thereof.

### Synthesis of benzoyl-protected *D-erythro*-dihydroceramide

[00125] In certain embodiments of the invention, the primary hydroxyl group of the *D-erythro*-ceramide is protected, followed by protection of the secondary hydroxyl, and then deprotection of the primary hydroxyl. In further embodiments of the invention, the protection and deprotection steps proceed without isolation or purification of the primary hydroxyl protected *N*-acyl-*D-erythro*-ceramide.

[00126] In particular embodiments of the invention, the primary hydroxyl group is protected with a trityl group by reacting the *D-erythro*-dihydroceramide with a tritylating reagent, such as, but not limited to, trityl halides such as trityl chloride and trityl bromide. In certain embodiments of the invention, the secondary hydroxyl group is protected with a benzoyl group by reacting the 1-O-protected *D-erythro*-dihydroceramide with a benzoylating reagents include, such as, but are not limited to, benzoyl halides such as benzoyl chloride and trityl bromide. In further embodiments of the invention the primary hydroxyl group is protected with a trityl group and the secondary hydroxyl is protected with a benzoyl group.

[00127] In certain embodiments of the invention, the protection of the primary hydroxyl group proceeds as follows: *D-erythro*-dihydroceramide (Compound VIIb) and trityl chloride are suspended in an organic solvent in the presence of a base. The reaction proceeds at a temperature of about 25 -55°C for about 10 to 60 hours to yield the trityl protected *D-erythro*-dihydroceramide (1-O-trityl-*D-erythro*-ceramide Compound VIIc, where PG1 = -Trt) . The organic solvent can be a nonpolar or polar solvent. In one embodiment of the invention, the organic solvent is a nonpolar solvent and is toluene, benzene, hexane or mixtures thereof. In one embodiment the organic solvent is an aprotic polar solvent. In one embodiment, the aprotic organic solvent is methylene chloride, chloroform or carbon tetrachloride. In another embodiment, the aprotic organic solvent is nonchlorinated and is diethyl ether, tetrahydrofuran, or ethyl acetate. The base is typically an organic base, such as triethylamine or pyridine.

[00128] In certain embodiments of the invention, the protection of the primary hydroxyl group, c.g., using trityl chloride, yields no more than about 10 mol% of *N*-palmitoyl-1,3-O,O-ditrityl-*D-erythro*-sphingosine of the crude reaction products. In further embodiments of the invention, the protection of the primary hydroxyl group, e.g., using trityl chloride, yields no more than about 7 mol% of *N*-palmitoyl-1,3-O,O-ditrityl-*D-erythro*-sphingosine of the crude reaction products. In still further embodiments of the invention, the protection of the primary

hydroxyl group, e.g., using trityl chloride, yields no more than about 5 mol% of *N*-palmitoyl-1,3-O,O-ditrityl-*D*-erythro-sphingosine of the crude reaction products. In still further embodiments of the invention, the protection of the primary hydroxyl group, e.g., using trityl chloride, yields no more than about 1 mol% of *N*-palmitoyl-1,3-O,O-ditrityl-*D*-erythro-sphingosine of the crude reaction products.

[00129] In further embodiments of the invention, the protection of the secondary hydroxyl group proceeds directly as follows: the above reaction mixture is cooled to about 0-5°C and benzoyl chloride and a base are added. The reaction proceeds at a temperature of about 0-5°C for about 1 to 16 hours. The reaction product, 1-O-trityl-3-O-benzoyl-*D*-erythro-dihydroceramide (Compound Vd; PG1 = -Trt; PG2 = -Bz), can be extracted from the organic layer with an organic solvent, including, but not limited to, an aprotic organic solvent described above. The organic solvent is then removed by a suitable method known to one of skill in the art, including, but not limited to, evaporation, e.g., concentration *in vacuo*. In particular embodiments the base is an organic base, such as triethylamine or pyridine.

[00130] In yet further embodiments of the invention, the deprotection of the primary hydroxyl group proceeds directly as follows: the residue from the above reaction is dissolved in an organic solvent and an acid is added. The reaction proceeds at a temperature of about 22°C for 1 to 16 hours. The reaction mixture is neutralized by the addition of a base. The organic solvent can be a protic polar solvent, an aprotic polar solvent, or a mixture thereof. In one embodiment the organic solvent is a protic polar solvent and is methanol, ethanol, *n*-propanol, or isopropanol. In one embodiment the organic solvent is an aprotic polar solvent. In one embodiment, the aprotic organic solvent is chlorinated and is methylene chloride, chloroform, or carbon tetrachloride. In another embodiment, the aprotic organic solvent is nonchlorinated and is diethyl ether, tetrahydrofuran, or ethyl acetate. The acid can be any acid known by one of skill in the art to be suitable for removal of the trityl protecting group, e.g., acetic acid, trifluoroacetic acid, hydrochloric acid and *p*-toluenesulfonic acid. In certain embodiments of the invention the acid is *p*-toluenesulfonic acid. In particular embodiments the base is an organic base, such as triethylamine or pyridine.

[00131] In certain embodiments of the invention the above deprotection product (Compound VIIe; PG2 = -Bz) can be purified by recrystallization, silica gel chromatography, high performance liquid chromatography or other methods known to those skilled in the art.

Phosphorylation and amination of benzoyl-protected *D-erythro*-dihydroceramide to yield benzoyl-protected *N*-acyl-*D-erythro*-dihydrosphingomyelin

[00132] In further embodiments of the invention the 3-benzoyl-protected *D-erythro*-dihydroceramide, (Compound VIIe; PG2 = -Bz), is phosphorylated as follows: the 3-benzoyl-protected *D-erythro*-dihydroceramide is dissolved in an organic solvent and an amine is added, after cooling to about 4-9 °C, a solution of 2-Chloro-2-oxo-1,3,2-dioxaphospholane in an organic solvent is added. The reaction proceeds at a temperature from about 4-22 °C for about 2 to 6 hours to produce 3-O-Benzoyl-1-O-(2-oxo-1,3,2-dioxaphospholan)-dihydroceramide (Compound VIIf; PG2 = -Bz). In certain embodiments of the invention, the reaction proceeds at about 4-9 °C for about 15 minutes to 2 hours and is then warmed to about 22 °C and proceeds for an additional 2 to 4 hours. The organic solvent can be a nonpolar solvent, a polar solvent, or mixtures thereof. In one embodiment of the invention, the organic solvent is a nonpolar solvent and is toluene, benzene, hexane or mixtures thereof. In one embodiment the organic solvent is an aprotic polar solvent such as acetonitrile, ethyl acetate, tetrahydrofuran, or mixtures thereof. The amine is typically tetramethylethylenediamine or triethylamine. In certain embodiments of the invention, the amine is tetramethylethylenediamine.

[00133] In certain embodiments, amination of the 3-O-Benzoyl-1-O-(2-oxo-1,3,2-dioxaphospholan)-dihydroceramide (Compound VIIf, PG2 = Bz) occurs without purification or isolation of the phosphorylated benzoyl-protected *N*-acyl-*D-erythro*-dihydroceramide. After the above reaction has proceeded for about 2 to 6 hours, additional organic solvent is added and the reaction is cooled to about -10 to 0°C. Gaseous trimethylamine is cooled to below its boiling point, and about 40 to 60 equivalents of this liquid trimethylamine are added. The reaction mixture is heated to about 60 – 70°C and proceeds for 10 to 16 hours to yield the benzoyl-protected *N*-acyl-*D-erythro*-dihydrosphingomyelin (Compound VIIg, PG2 = Bz). The organic solvent can be a nonpolar solvent, a polar solvent or mixtures thereof. In one embodiment of the invention, the organic solvent is a nonpolar solvent and is toluene, benzene, hexane or mixtures thereof. In one embodiment the organic solvent is an aprotic polar solvent such as acetonitrile, ethyl acetate, tetrahydrofuran, or mixtures thereof. In certain embodiments, trimethylamine is added as a liquid. In other embodiments, triethylamine is added in a gaseous form. In particular embodiments, the liquid trimethylamine is anhydrous. In certain embodiments the reaction is cooled to about -10°C -

0°C prior to addition of liquid trimethylamine. In other embodiments the reaction is cooled to about -10°C prior to addition of liquid trimethylamine.

[00134] In certain embodiments of the invention the benzoyl-protected *N*-acyl-*D*-erythro-dihydrosphingomyelin can be purified by recrystallization, silica gel chromatography, high performance liquid chromatography or other methods known to those skilled in the art.

Deprotection of benzoyl-protected *N*-acyl-*D*-erythro-dihydrosphingomyelin to yield *N*-acyl-*D*-erythro-dihydrosphingomyelin

[00135] The removal of the benzoyl protecting group from the benzoyl-protected *N*-acyl-*D*-erythro-dihydrosphingomyelin proceeds as follows: the benzoyl-protected

dihydrosphingomyelin is dissolved in a protic polar solvent and a base is added. The reaction proceeds for 8 to 24 hours at about 22°C. In certain embodiments of the invention an aprotic solvent and water are added to the reaction mixture and the *N*-acyl-*D*-erythro-dihydrosphingomyelin (Compound VIIh) is recovered from the organic layer. In one embodiment the protic polar solvent is methanol, ethanol, *n*-propanol, isopropanol, or mixtures thereof. In yet another embodiment the base is sodium methoxide.

[00136] In further embodiments of the invention the *N*-acyl-*D*-erythro-dihydrosphingomyelin can be purified by recrystallization, silica gel chromatography, high performance liquid chromatography or other methods known to those skilled in the art.

[00137] In certain embodiments of the invention, the resulting *N*-acyl-*D*-erythro-dihydrosphingomyelin has an enantiomeric purity of at least about 85% and contains no more than about 15% of its corresponding opposite enantiomer. In further embodiments of the invention, the *N*-acyl-*D*-erythro-dihydrosphingomyelin has an enantiomeric purity of at least about 90% and contains no more than about 10% of its corresponding opposite enantiomer. In yet further embodiments *N*-acyl-*D*-erythro-dihydrosphingomyelin has an enantiomeric purity of at least about 95% and contains no more than about 5% of its corresponding opposite enantiomer. In still further embodiments *N*-acyl-*D*-erythro-dihydrosphingomyelin has an enantiomeric purity of at least about 98% and contains no more than about 2% of its corresponding opposite enantiomer.

[00138] In yet another embodiment, the invention provides methods for synthesizing an *N*-acyl-*D*-erythro-dihydrosphingomyelin comprising the steps of:

a) allowing *D-erythro*-dihydrosphingosine to react with a fatty acid under conditions effective to yield a *D-erythro*-dihydroceramide;

b) allowing the *D-erythro*-dihydroceramide to react with a tritylating reagent under conditions effective to yield a 1-*O*-trityl-*D-erythro*-dihydroceramide;

5 c) allowing the 1-*O*-trityl-*D-erythro*-dihydroceramide to react with a benzoylating reagent under conditions effective to yield a 1-*O*-trityl-3-*O*-benzoyl-*D-erythro*-dihydroceramide;

d) removing the trityl group of the 1-*O*-trityl-3-*O*-benzoyl-*D-erythro*-dihydroceramide to yield a 3-*O*-benzoyl-*D-erythro*-dihydroceramide;

10 e) allowing the 3-*O*-benzoyl-*D-erythro*-dihydroceramide to react with 2-chloro-2-oxo-1,3,2-dioxaphospholane under conditions effective to yield an 3-*O*-benzoyl-*D-erythro*-1-*O*-(2-oxo-1,3,2-dioxaphospholane)-dihydroceramide;

f) allowing the 3-*O*-benzoyl-*D-erythro*-1-*O*-(2-oxo-1,3,2-dioxaphospholane)-dihydroceramide to react with trimethylamine under conditions effective to yield an *N*-acyl-3-*O*-benzoyl-*D-erythro*-dihydrosphingomyeline; and

15 g) removing the benzoyl group of *N*-acyl-3-*O*-benzoyl-*D-erythro*-dihydrosphingomyelin with sodium methoxide to yield an *N*-acyl-*D-erythro*-dihydrosphingomyelin.

### ***Synthesis of N-palmitoyl-D-erythro-dihydrosphingomyelin***

20 [00139] In yet another embodiment, the invention provides methods for synthesizing *N*-palmitoyl-*D-erythro*-dihydrosphingomyelin comprising the steps of:

a) allowing *D-erythro*-dihydrosphingosine to react with palmitic acid under conditions effective to yield *N*-palmitoyl-*D-erythro*-dihydrosphingosine;

25 b) allowing *N*-palmitoyl-*D-erythro*-dihydrosphingosine to react with a tritylating reagent under conditions effective to yield *N*-palmitoyl-1-*O*-trityl-*D-erythro*-dihydrosphingosine;

c) allowing *N*-palmitoyl-1-*O*-trityl-*D*-erythro-dihydrosphingosine to react with a benzoylating reagent under conditions effective to yield *N*-palmitoyl-1-*O*-trityl-3-*O*-benzoyl-*D*-erythro-dihydrosphingosine;

d) removing the trityl group of *N*-palmitoyl-1-*O*-trityl-3-*O*-benzoyl-*D*-erythro-dihydrosphingosine to yield *N*-palmitoyl-3-*O*-benzoyl-*D*-erythro-dihydrosphingosine;

e) allowing *N*-palmitoyl-3-*O*-benzoyl-*D*-erythro-dihydrosphingosine to react with with 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) under conditions effective to yield *N*-palmitoyl-3-*O*-benzoyl-*D*-erythro-1-*O*-(2-oxo-1,3,2-dioxaphospholane)-dihydrosphingosine;

f) allowing *N*-palmitoyl-3-*O*-benzoyl-*D*-erythro-1-*O*-(2-oxo-1,3,2-dioxaphospholane)-dihydrosphingosine to react with trimethylamine under conditions effective to yield *N*-palmitoyl-3-*O*-benzoyl-*D*-erythro-dihydrosphingomyelin; and

g) removing the benzoyl group of *N*-palmitoyl-3-*O*-benzoyl-*D*-erythro-dihydrosphingomyelin with sodium methoxide to yield *N*-palmitoyl-*D*-erythro-dihydrosphingomyelin.

[00140] In a particular embodiment of the invention *N*-palmitoyl-*D*-erythro-dihydrosphingomyelin is synthesized as shown in Scheme VIII.

[00141] One equivalent of palmitic acid, one equivalent of *D*-erythro-dihydrosphingosine (Compound B), and 1.10 equivalents of *O*-benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU) are suspended in tetrahydrofuran and dimethylformamide and cooled to about 0-5°C. Two to three equivalents of triethylamine are added and the mixture is stirred for about one to twelve hours at about 0-5°C. The mixture is warmed to about 22°C. An aqueous solution of citric acid is added and the mixture is stirred for 15 to 90 min at about 22°C. The resulting suspension is filtered and the cake is suspended in water, at room temperature. The suspension is filtered and washed with water and acetone. The resulting product, *N*-palmitoyl-*D*-erythro-dihydrosphingosine (Compound F), can then be dried.

[00142] For the first hydroxyl protection, one equivalent of *N*-palmitoyl-*D*-erythro-dihydrocceramide (Compound F) was suspended in pyridine and methylene chloride. A solution of about 1.05 equivalents of trityl chloride in methylene chloride is added followed

by additional methylene chloride. The reaction mixture was stirred at about 25°C for 50-60 hours.

[00143] In certain embodiments of the invention, the protection of the primary hydroxyl yields less than 10 mol% of *N*-palmitoyl-1,3-O,O-ditrityl-*D*-erythro-dihydrosphingosine of the crude reaction products. In further embodiments of the invention, the protection of the primary hydroxyl yields less than 7 mol% of *N*-palmitoyl-1,3-O,O-ditrityl-*D*-erythro-dihydrosphingosine of the crude reaction products. In still further embodiments of the invention, the protection of the primary hydroxyl yields less than 5 mol% of *N*-palmitoyl-1,3-O,O-ditrityl-*D*-erythro-dihydrosphingosine of the crude reaction products. In yet further  
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[00144] For the second hydroxyl protection, the reaction mixture from the first hydroxyl protection comprising *N*-palmitoyl-1-O-trityl-*D*-erythro-dihydrosphingosine (Compound VIIIA) is cooled to about 2°C. *N,N*-Dimethylaminopyridine at about 0.10 equivalent, benzoyl chloride at about 1.50 equivalents and additional methylene chloride are added. The reaction is allowed to proceed at about 2°C with stirring until thin layer chromatography (TLC) analysis shows a content of starting material *N*-palmitoyl-1-O-trityl-*D*-erythro-dihydrosphingosine (Compound VIIIA) of less than about 5%. Ethyl acetate and an aqueous citric acid and sodium chloride solution are added to the reaction and the *N*-palmitoyl-1-O-Trityl-3-O-benzol-*D*-erythro-dihydrosphingosine (Compound VIIIB) is recovered from the organic phase.

[00145] To remove the trityl protecting group, *N*-palmitoyl-1-O-Trityl-3-O-benzol-*D*-erythro-dihydrosphingosine (Compound VIIIB) is dissolved in methanol and methylene chloride and cooled to 2°C. The pH is adjusted to 2.5 with a solution of 0.57 equivalents of *para*-toluene sulfonic acid monohydrate in methanol. The reaction is allowed to proceed at about 22°C with stirring until TLC analysis showed a content of starting material, *N*-palmitoyl-1-O-Trityl-3-O-benzol-*D*-erythro-dihydrosphingosine (Compound VIIIB) of less than 5%. Triethylamine is added to adjust the pH to about 7.0. The reaction mixture is evaporated to dryness and the resulting crude *N*-palmitoyl-3-O-Benzoyl-*D*-erythro-dihydrosphingosine (Compound VIIIC) is suspended in hexane at about 40°C and cooled down to about 0°C. After about 30 to 60 minutes the solid is isolated by filtration and washed with hexane. The

resulting product can then be purified by an appropriate method, such as silica gel chromatography.

[00146] One equivalent of *N*-palmitoyl-3-O-Benzoyl-*D*-erythro-dihydrosphingosine (Compound VIIIc) is dissolved in toluene and about 0.6 to 1 equivalents of

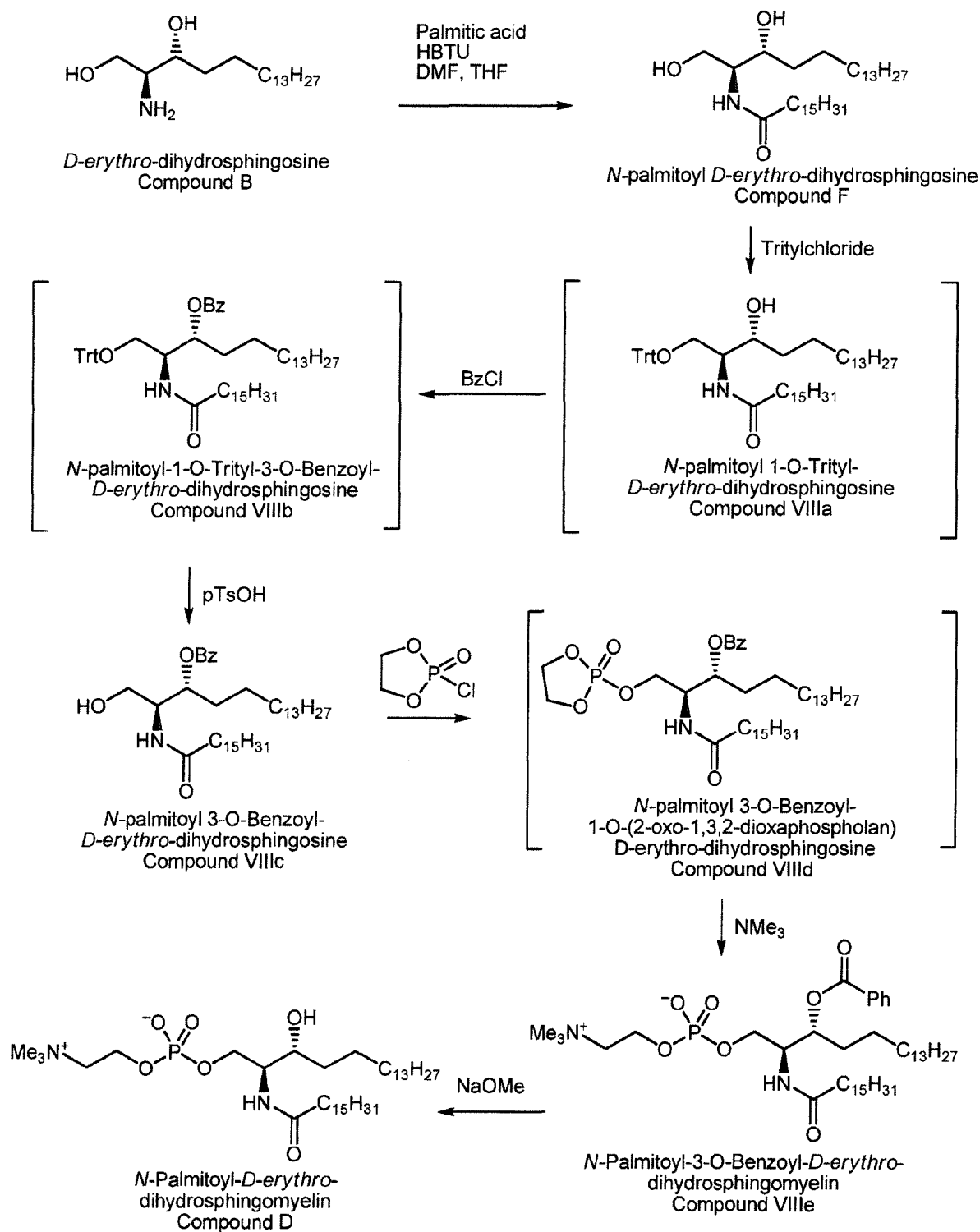
5 tetramethylethylenediamine is added and the mixture is cooled to about 4-9 °C. About 1 to 2 equivalents of 2-chloro-2-oxo-1,3,2-dioxaphospholane in acetonitrile is added, followed by additional acetonitrile. The reaction is warmed to about 22 °C and stirring continued for 1-3 hours. After which additional acetonitrile is added and the temperature decreased to about -10 to 0°C. Gaseous trimethylamine is cooled to below its boiling point, and about 40 to 60  
10 equivalents of this liquid trimethylamine are added. The reaction is heated to about 60 – 70°C and proceeds for 10 to 16 hours to yield the *N*-palmitoyl-3-O-benzoyl *D*-erythro-dihydrosphingomyelin (Compound VIIIe). The reaction is cooled to about -30°C and the resulting suspension is filtered. The crude *N*-palmitoyl-3-O-benzoyl *D*-erythro-dihydrosphingomyelin is further purified by silica gel chromatography.

15 [00147] One equivalent of *N*-palmitoyl-3-O-benzoyl *D*-erythro-dihydrosphingomyelin (Compound VIIIe) is dissolved in methanol and about 0.2 equivalents of sodium methoxide is added and the mixture is stirred for 20-26 hours at about 22 °C. Methylene chloride and water are added and the pH is adjusted to about 7 with the addition of hydrochloric acid. *N*-palmitoyl-*D*-erythro-dihydrosphingomyelin (Compound D) is recovered from the organic  
20 layer.

[00148] In further embodiments of the invention the *N*-palmitoyl-*D*-erythro-dihydrosphingomyelin can be purified by recrystallization, silica gel chromatography, high performance liquid chromatography or other methods known to those skilled in the art.

[00149] In certain embodiments of the invention, the resulting *N*-palmitoyl-*D*-erythro-dihydrosphingosine has an enantiomeric purity of at least about 85% and contains no more  
25 than about 15% of its corresponding opposite enantiomer. In further embodiments of the invention, the *N*-palmitoyl-*D*-erythro-dihydrosphingomyelin has an enantiomeric purity of at least about 90% and contains no more than about 10% of its corresponding opposite enantiomer. In yet further embodiments *N*-palmitoyl-*D*-erythro-dihydrosphingomyelin has an  
30 enantiomeric purity of at least about 95% and contains no more than about 5% of its corresponding opposite enantiomer. In still further embodiments *N*-palmitoyl-*D*-erythro-

dihydrosphingomyelin has an enantiomeric purity of at least about 98% and contains no more than about 2% of its corresponding opposite enantiomer.



Scheme VIII

***Protection of (2S,3R,4E)-3-(tert-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidine (Compound If)***

[00150] In another embodiment of the invention, the secondary hydroxyl group of (2S,3R,4E)-3-(tert-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidine (Compound If) is protected and the synthesis of the sphingomyelin proceeds as shown in Scheme IX.

[00151] In a particular embodiment of the invention, the secondary hydroxyl group of (2S,3R,4E)-3-(tert-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidine (Compound If) is protected with a benzoyl group to yield (2S,3R,4E)-3-(tert-butoxycarbonyl)-4-(1-benzoyl-hexadec-2-enyl)-2,2-dimethyloxazolidine (Compound IXa) as shown in Scheme IX. The (2S,3R,4E)-3-(tert-butoxycarbonyl)-4-(1-benzoyl-hexadec-2-enyl)-2,2-dimethyloxazolidine is then dissolved in methanol and 2 to 3 equivalents of acetyl chloride, generating hydrochloric acid and removing the tert-butoxycarbonyl (Boc) protecting group, resulting in 3-O-benzoyl-*D-erythro*-sphingosine (Compound IXb).

[00152] In certain embodiments of the invention, *N*-acylation of 3-O-benzoyl-*D-erythro*-sphingosine with palmitic acid to yield *N*-palmitoyl-3-O-benzoyl-*D-erythro*-sphingosine (Compound IXc) proceeds as shown in Scheme IX. The steps are as follows: 3-O benzoyl-*D-erythro*-sphingosine (Compound IXb), palmitic acid, and an amide forming agent are suspended in an aprotic organic solvent and the mixture is cooled at a temperature of about 0-5°C. In one embodiment, the aprotic organic solvent is tetrahydrofuran, dimethylformamide, 2-methyltetrahydrofuran, or mixtures thereof. In particular embodiments of the invention, the amide forming agent is O-benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluorophosphate (HBTU).

[00153] An organic base, such as, but not limited to triethylamine or pyridine, is added to the mixture of 3-O-benzoyl-*D-erythro*-sphingosine, palmitic acid, and amide forming agent. In certain embodiments, the organic base is in an aprotic organic solvent and is tetrahydrofuran, dimethylformamide, 2-methyltetrahydrofuran, or mixtures thereof. In further embodiments of the invention, the organic base is added over the course of about 15 to 90 minutes. The mixture is then stirred for 1 to 15 hours at a temperature of about 0-22°C. In certain embodiments of the invention, the reaction proceeds at a temperature of about 0-5°C. In other embodiments of the invention, the reaction proceeds at about 22°C. In yet other embodiments of the invention, the reaction proceeds at about room temperature.

[00154] After stirring for about 1 to 15 hours, the product is precipitated by the addition of an acid. In certain embodiments of the invention, the acid is an organic acid, such as citric acid, acetic acid, or oxalic acid. The acid can be in an aqueous solution when added. The reaction can be at about 22°C when the acid is added. The resulting suspension can be stirred for 30 to 120 minutes at a temperature of about 0-5°C. In certain embodiments of the invention, the suspension is stirred at about 22°C.

[00155] After stirring, the suspension is filtered. The resulting product can then be resuspended in water, after which it can be filtered and washed. The resuspension can occur at least one more time. The resulting product, *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingosine (Compound IXc), can be washed with water, acetone, or a mixture thereof.

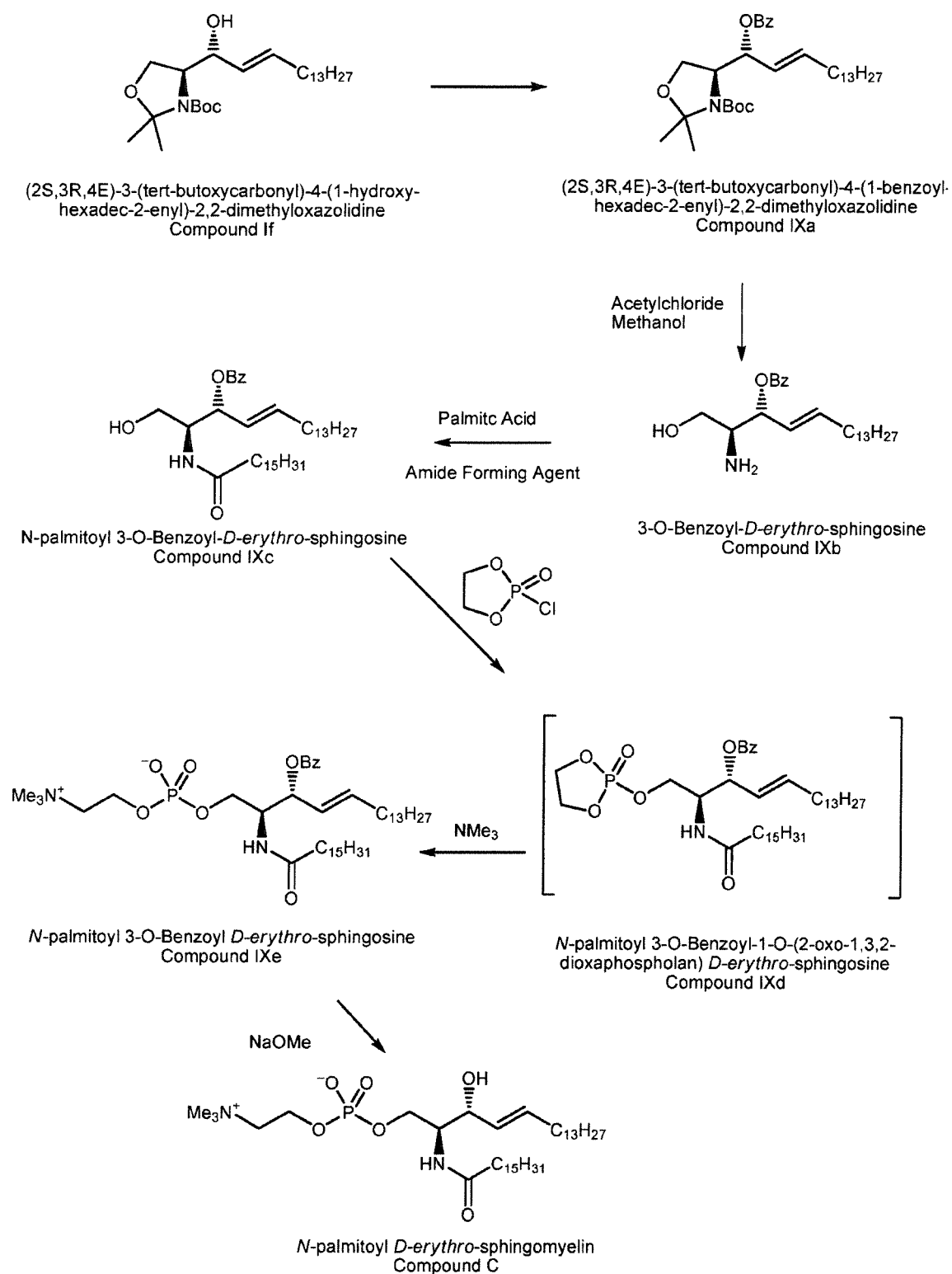
[00156] In certain embodiments of the invention *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingosine or one or more of its intermediates can be purified by recrystallization, silica gel chromatography, high performance liquid chromatography or other methods known to those skilled in the art.

[00157] One equivalent of *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingosine (Compound IXc) is dissolved in toluene, about 0.6 to 1 equivalents of tetramethylethylenediamine (TMEDA) is added and the mixture is cooled to about 4-9 °C. About 1 to 2 equivalents of 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) in acetonitrile is added, followed by additional acetonitrile. The reaction is warmed to about 22 °C and stirring continued for 1-3 hours. Additional acetonitrile is added and the temperature is decreased to about -10 to 0°C. Gaseous trimethylamine is cooled to below its boiling point, and about 40 to 60 equivalents of this liquid trimethylamine are added. The reaction mixture is heated to about 60 – 70°C and proceeds for 10 to 16 hours to yield the *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingomyelin (Compound IXe). The reaction is cooled to about -30°C and the resulting suspension is filtered. The crude *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingomyelin (Compound IXe) is further purified by silica gel chromatography.

[00158] One equivalent of *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingomyelin (Compound IXe) is dissolved in methanol, about 0.2 equivalents of sodium methoxide are added and the mixture is stirred for 20-26 hours at about 22 °C. Methylene chloride and water are added and the pH is adjusted to about 7 with the addition of hydrochloric acid. *N*-palmitoyl-*D*-erythro-sphingomyelin (Compound C) is recovered from the organic layer.

[00159] In further embodiments of the invention the *N*-palmitoyl-*D*-erythro-sphingomyelin (Compound C) can be purified by recrystallization, silica gel chromatography, high performance liquid chromatography or other methods known to those skilled in the art.

[00160] In certain embodiments of the invention, the resulting *N*-palmitoyl-*D*-erythro-sphingosine has an enantiomeric purity of at least about 85% and contains no more than about 15% of its corresponding opposite enantiomer. In further embodiments of the invention, the *N*-palmitoyl-*D*-erythro-sphingomyelin has an enantiomeric purity of at least about 90% and contains no more than about 10% of its corresponding opposite enantiomer. In yet further embodiments *N*-palmitoyl-*D*-erythro-sphingomyelin has an enantiomeric purity of at least about 95% and contains no more than about 5% of its corresponding opposite enantiomer. In still further embodiments *N*-palmitoyl-*D*-erythro-sphingomyelin has an enantiomeric purity of at least about 98% and contains no more than about 2% of its corresponding opposite enantiomer.



Scheme IX

[00161] In certain embodiments of the invention, an *N*-acyl-*D*-erythro-sphingomyelin is prepared as described below.

[00162] In one aspect of the invention the secondary hydroxyl group of (2*S*,3*R*,4*E*)-3-(tert-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidine (Compound If) can be protected with a protecting group, such as, but not limited to an ester or an ether. In certain embodiments of the invention, the protecting group is an ester, such as, but not limited to, benzoyl ester or fluorenylmethyloxycarbonyl ester. In further embodiments of the invention, the protecting group is an ether, such as, but not limited to, *t*-butyldiphenylsilyl ether.

[00163] In a particular embodiment of the invention, the secondary hydroxyl group of (2*S*,3*R*,4*E*)-3-(tert-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidine (Compound If) is protected with a benzoyl group to yield (2*S*,3*R*,4*E*)-3-(tert-butoxycarbonyl)-4-(1-benzoyl-hexadec-2-enyl)-2,2-dimethyloxazolidine (Compound Xa) as shown in Scheme X. Compound Xa is then dissolved in methanol and 2 to 3 equivalents of acetyl chloride, generating hydrochloric acid and removing the tert-butoxycarbonyl (Boc) protecting group, resulting in 3-*O*-benzoyl-*D*-erythro-sphingosine (Compound Xb). 3-*O*-benzoyl-*D*-erythro-sphingosine can then be *N*-acylated by the addition of a suitable fatty acid and an amide forming agent. The steps are as follows: 3-*O*-benzoyl-*D*-erythro-sphingosine (Compound Xb), a fatty acid, and an amide forming agent are suspended in an aprotic organic solvent and the mixture is cooled at a temperature of about 0-5°C. In one embodiment, the aprotic organic solvent is tetrahydrofuran, dimethylformamide, 2-methyltetrahydrofuran, or mixtures thereof. In particular embodiments of the invention, the amide forming agent is *O*-benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU).

[00164] An organic base, such as, but not limited to triethylamine or pyridine, is added to the mixture of 3-*O*-benzoyl-*D*-erythro-sphingosine (Compound Xb), fatty acid, and amide forming agent. In certain embodiments, the organic base is in an aprotic organic solvent and is tetrahydrofuran, dimethylformamide, 2-methyltetrahydrofuran, or mixtures thereof. In further embodiments of the invention, the organic base is added over the course of about 15 to 90 minutes. The mixture is then stirred for 1 to 15 hours at a temperature of about 0-22°C. In certain embodiments of the invention, the reaction proceeds at a temperature of about 0-5°C. In other embodiments of the invention, the reaction proceeds at about 22°C. In yet other embodiments of the invention, the reaction proceeds at about room temperature.

[00165] After stirring for about 1 to 15 hours, the product (Compound Xc) is precipitated by the addition of an acid. In certain embodiments of the invention, the acid is an organic acid, such as citric acid, acetic acid, or oxalic acid. The acid can be in an aqueous solution when added. The reaction can be at about 22°C when the acid is added. The resulting suspension  
 5 can be stirred for 30 to 120 minutes at a temperature of about 0-5°C. In certain embodiments of the invention, the suspension is stirred at about 22°C.

[00166] After stirring, the suspension is filtered. The resulting product can then be resuspended in water, after which it can be filtered and washed. The resuspension can occur at least one more time. The resulting product, 3-O-benzoyl-*D*-erythro-ceramide (Compound  
 10 Xc), can be washed with water, acetone, or a mixture thereof.

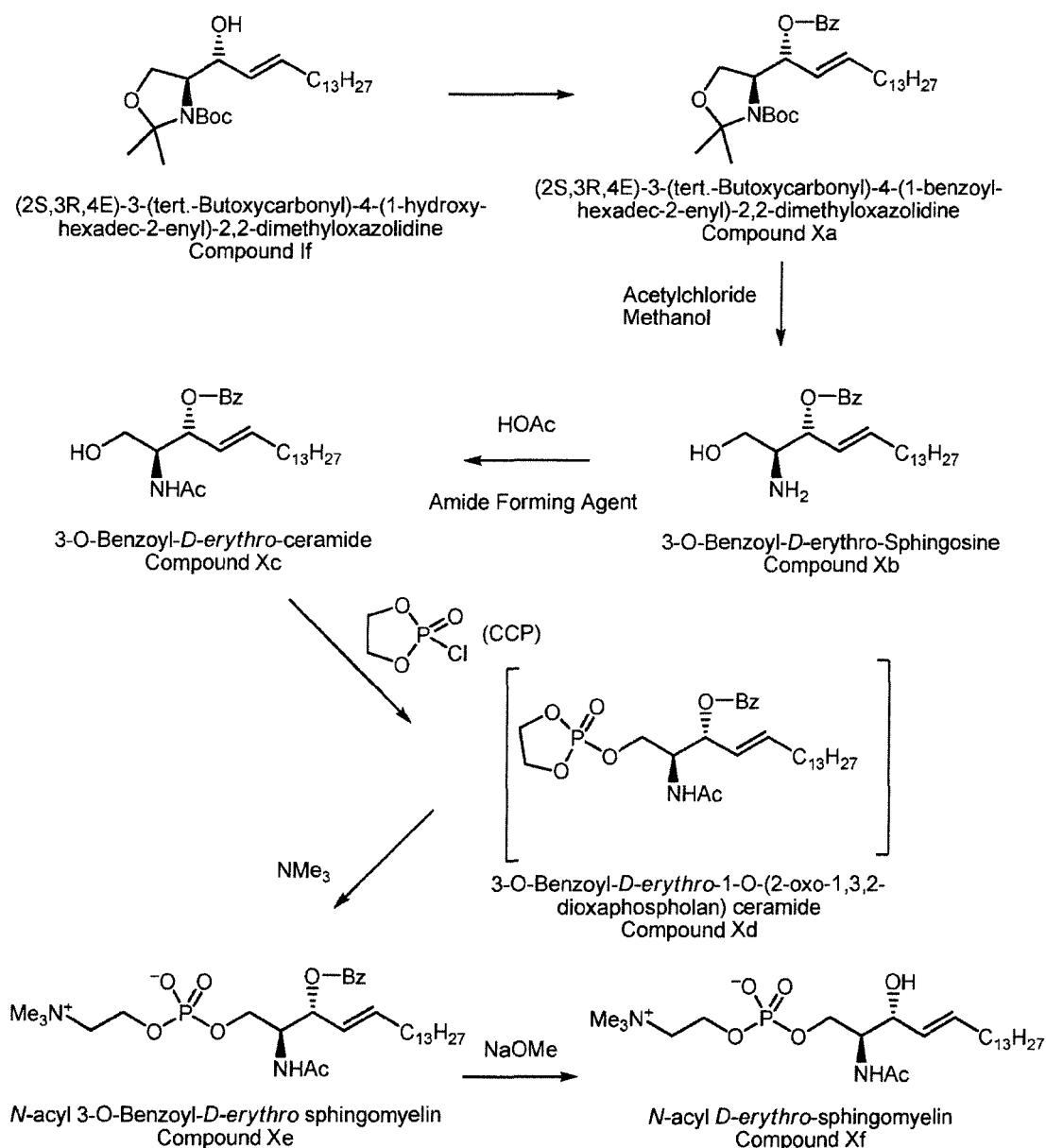
[00167] In certain embodiments of the invention Compound Xc or one or more of its intermediates can be purified by recrystallization, silica gel chromatography, high performance liquid chromatography or other methods known to those skilled in the art.

[00168] One equivalent of Compound Xc is dissolved in toluene, about 0.6 to 1 equivalents of  
 15 tetramethylethylenediamine (TMEDA) is added and the mixture is cooled to about 4-9 °C. About 1 to 2 equivalents of 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) in acetonitrile is added, followed by additional acetonitrile. The reaction is warmed to about 22 °C and stirring continued for 1-3 hours. Additional acetonitrile is added and the temperature is decreased to about -10 to 0°C. Gaseous trimethylamine is cooled to below its boiling point, and about 40  
 20 to 60 equivalents of this liquid trimethylamine are added. The reaction mixture is heated to about 60 – 70°C and proceeds for 10 to 16 hours to yield the *N*-acyl-O-benzoyl-*D*-erythro-sphingomyelin (Compound Xe). The reaction is cooled to about -30°C and the resulting suspension is filtered. The crude *N*-acyl-*D*-erythro-sphingomyelin (Compound Xe) is further purified by silica gel chromatography.

25 [00169] One equivalent of *N*-acyl-*D*-erythro-sphingomyelin (Compound Xe) is dissolved in methanol, about 0.2 equivalents of sodium methoxide are added and the mixture is stirred for 20-26 hours at about 22 °C. Methylene chloride and water are added and the pH is adjusted to about 7 with the addition of hydrochloric acid. *N*-acyl-*D*-erythro-sphingomyelin (Compound Xf) is recovered from the organic layer.

30 [00170] In further embodiments of the invention the *N*-acyl-*D*-erythro-sphingomyelin (Compound Xf) can be purified by recrystallization, silica gel chromatography, high performance liquid chromatography or other methods known to those skilled in the art.

[00171] In certain embodiments of the invention, the resulting *N*-acyl-*D*-*erythro*-sphingosine has an enantiomeric purity of at least about 85% and contains no more than about 15% of its corresponding opposite enantiomer. In further embodiments of the invention, the *N*-acyl-*D*-*erythro*-sphingomyelin has an enantiomeric purity of at least about 90% and contains no more than about 10% of its corresponding opposite enantiomer. In yet further embodiments *N*-acyl-*D*-*erythro*-sphingomyelin has an enantiomeric purity of at least about 95% and contains no more than about 5% of its corresponding opposite enantiomer. In still further embodiments *N*-acyl-*D*-*erythro*-sphingomyelin has an enantiomeric purity of at least about 98% and contains no more than about 5% of its corresponding opposite enantiomer.



Scheme X

HOAc is a fatty acid

Ac is a fatty acid residue having 3 to 36 carbons and zero to six carbon-carbon double bonds

**Protection of (S)-tert-butyl 4-((R)-1-hydroxyhexadecyl)-2,2-dimethyloxazolidine-3-carboxylate (Compound IIa)**

[00172] In another embodiment of the invention, the secondary hydroxyl of the sphingosine precursor (S)-tert-butyl 4-((R)-1-hydroxyhexadecyl)-2,2-dimethyloxazolidine-3-carboxylate (Compound IIa in Scheme II) is protected and the synthesis of the dihydrosphingomyelin proceeds as shown in Scheme XI.

[00173] In a particular embodiment of the invention, the secondary hydroxyl group of (*S*)-*tert*-butyl 4-((*R*)-1-hydroxyhexadecyl)-2,2-dimethyloxazolidine-3-carboxylate (Compound IIa) is protected with a benzoyl group to yield (*S*)-*tert*-butyl 4-((*R*)-1-(benzoyloxy)hexadecyl)-2,2-dimethyloxazolidine-3-carboxylate (Compound XIa) as shown in Scheme XI. Compound

5 XIa is then dissolved in methanol and 2 to 3 equivalents of acetyl chloride, generating hydrochloric acid and removing the *tert*-butoxycarbonyl (Boc) protecting group, resulting in 3-O-benzoyl-*D*-*erythro*-dihydrosphingosine (Compound XIb).

[00174] In certain embodiments of the invention, *N*-acylation of 3-O-benzoyl-*D*-*erythro*-dihydrosphingosine with palmitic acid to yield *N*-palmitoyl-3-O-benzoyl-*D*-*erythro*-

10 dihydrosphingosine (Compound XIc) proceeds as follows: 3-O benzoyl-*D*-*erythro*-dihydrosphingosine (Compound XIb), palmitic acid, and an amide forming agent are suspended in an aprotic organic solvent and the mixture is cooled at a temperature of about 0-5°C. In one embodiment, the aprotic organic solvent is tetrahydrofuran, dimethylformamide, 2-methyltetrahydrofuran, or mixtures thereof. In particular embodiments of the invention, the

15 amide forming agent is O-benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluorophosphate (HBTU).

[00175] An organic base, such as, but not limited to triethylamine or pyridine, is added to the mixture of 3-O-benzoyl-*D*-*erythro*-dihydrosphingosine, palmitic acid, and amide forming agent. In certain embodiments, the organic base is in an aprotic organic solvent and is

20 tetrahydrofuran, dimethylformamide, 2-methyltetrahydrofuran, or mixtures thereof. In further embodiments of the invention, the organic base is added over the course of about 15 to 90 minutes. The mixture is then stirred for 1 to 15 hours at a temperature of about 0-22°C. In certain embodiments of the invention, the reaction proceeds at a temperature of about 0-5°C. In other embodiments of the invention, the reaction proceeds at about 22°C. In yet other

25 embodiments of the invention, the reaction proceeds at about room temperature.

[00176] After stirring for about 1 to 15 hours, the product is precipitated by the addition of an acid. In certain embodiments of the invention, the acid is an organic acid, such as citric acid, acetic acid, or oxalic acid. The acid can be in an aqueous solution when added. The reaction can be at about 22°C when the acid is added. The resulting suspension can be stirred for 30 to

30 120 minutes at a temperature of about 0-5°C. In certain embodiments of the invention, the suspension is stirred at about 22°C.

[00177] After stirring, the suspension is filtered. The resulting product can then be resuspended in water, after which it can be filtered and washed. The resuspension can occur at least one more time. The resulting product, *N*-palmitoyl-3-O-benzoyl-*D*-erythro-dihydrosphingosine (Compound XIc), can be washed with water, acetone, or a mixture thereof.

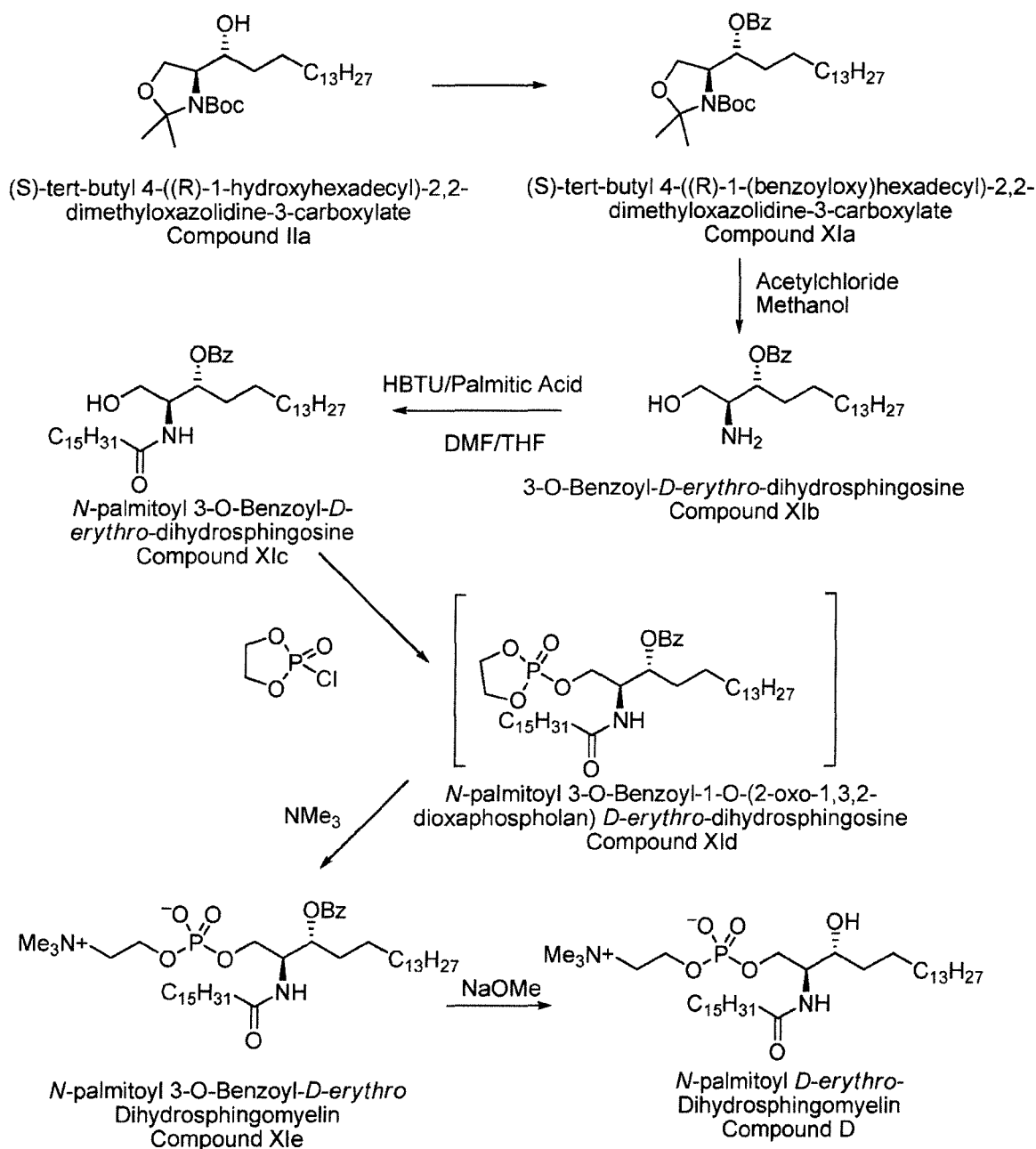
[00178] In certain embodiments of the invention *N*-palmitoyl-3-O-benzoyl-*D*-erythro-dihydrosphingosine or one or more of its intermediates can be purified by recrystallization, silica gel chromatography, high performance liquid chromatography or other methods known to those skilled in the art.

[00179] One equivalent of *N*-palmitoyl-3-O-benzoyl-*D*-erythro-dihydrosphingosine (Compound XIc) is dissolved in toluene, about 0.6 to 1 equivalents of tetramethylethylenediamine (TMEDA) is added and the mixture is cooled to about 4-9 °C. About 1 to 2 equivalents of 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) in acetonitrile is added, followed by additional acetonitrile. The reaction is warmed to about 22 °C and stirring continued for 1-3 hours. Additional acetonitrile is added and the temperature is decreased to about -10 to 0°C. Gaseous trimethylamine is cooled to below its boiling point, and about 40 to 60 equivalents of this liquid trimethylamine are added. The reaction mixture is heated to about 60 – 70°C and proceeds for 10 to 16 hours to yield the *N*-palmitoyl-3-O-benzoyl-*D*-erythro-dihydrosphingomyelin (Compound XIe). The reaction is cooled to about -30°C and the resulting suspension is filtered. The crude *N*-palmitoyl-3-O-benzoyl-*D*-erythro-dihydrosphingomyelin (Compound XIe) is further purified by silica gel chromatography.

[00180] One equivalent of *N*-palmitoyl-3-O-benzoyl-*D*-erythro-dihydrosphingomyelin (Compound XIe) is dissolved in methanol, about 0.2 equivalents of sodium methoxide are added and the mixture is stirred for 20-26 hours at about 22 °C. Methylene chloride and water are added and the pH is adjusted to about 7 with the addition of hydrochloric acid. *N*-palmitoyl-*D*-erythro-dihydrosphingomyelin (Compound D) is recovered from the organic layer.

[00181] In further embodiments of the invention the *N*-palmitoyl-*D*-erythro-dihydrosphingomyelin (Compound D) can be purified by recrystallization, silica gel chromatography, high performance liquid chromatography or other methods known to those skilled in the art.

[00182] In certain embodiments of the invention, the resulting *N*-palmitoyl-*D*-erythro-dihydrosphingosine has an enantiomeric purity of at least about 85% and contains no more than about 15% of its corresponding opposite enantiomer. In further embodiments of the invention, the *N*-palmitoyl-*D*-erythro-dihydrosphingomyelin has an enantiomeric purity of at least about 90% and contains no more than about 10% of its corresponding opposite enantiomer. In yet further embodiments *N*-palmitoyl-*D*-erythro-dihydrosphingomyelin has an enantiomeric purity of at least about 95% and contains no more than about 5% of its corresponding opposite enantiomer. In still further embodiments *N*-palmitoyl-*D*-erythro-dihydrosphingomyelin has an enantiomeric purity of at least about 98% and contains no more than about 2% of its corresponding opposite enantiomer.



Scheme XI

5 [00183] In certain embodiments of the invention, an *N*-acyl-*D*-erythro-dihydrosphingomyelin is prepared as described below.

[00184] In one aspect of the invention the secondary hydroxyl group of (*S*)-*tert*-butyl 4-((*R*)-1-hydroxyhexadecyl)-2,2-dimethyloxazolidine-3-carboxylate (Compound IIa) can be protected with a protecting group, such as, but not limited to an ester or an ether. In certain embodiments of the invention, the protecting group is an ester, such as, but not limited to,

benzoyl ester or fluorenylmethyloxycarbonyl ester. In further embodiments of the invention, the protecting group is an ether, such as, but not limited to, *t*-butyldiphenylsilyl ether.

[00185] In a particular embodiment of the invention, the secondary hydroxyl group of Compound IIa is protected with a benzoyl group to yield Compound XIIa as shown in

5 Scheme XII. Compound XIIa is then dissolved in methanol and 2 to 3 equivalents of acetyl chloride, generating hydrochloric acid and removing the tert-butoxycarbonyl (Boc) protecting group, resulting in 3-O-benzoyl-*D*-erythro-dihydrosphingosine (Compound XIIb). 3-O-benzoyl-*D*-erythro-dihydrosphingosine can then be *N*-acylated by the addition of a suitable fatty acid and an amide forming agent. The steps are as follows: 3-O-benzoyl-*D*-  
10 *erythro*-dihydrosphingosine (Compound XIIb), a fatty acid, and an amide forming agent are suspended in an aprotic organic solvent and the mixture is cooled at a temperature of about 0-5°C. In one embodiment, the aprotic organic solvent is tetrahydrofuran, dimethylformamide, 2-methyltetrahydrofuran, or mixtures thereof. In particular embodiments of the invention, the amide forming agent is O-benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-  
15 phosphate (HBTU).

[00186] An organic base, such as, but not limited to triethylamine or pyridine, is added to the mixture of 3-O-benzoyl-*D*-erythro-dihydrosphingosine (Compound XIIb), fatty acid, and amide forming agent. In certain embodiments, the organic base is in an aprotic organic solvent and is tetrahydrofuran, dimethylformamide, 2-methyltetrahydrofuran, or mixtures  
20 thereof. In further embodiments of the invention, the organic base is added over the course of about 15 to 90 minutes. The mixture is then stirred for 1 to 15 hours at a temperature of about 0-22°C. In certain embodiments of the invention, the reaction proceeds at a temperature of about 0-5°C. In other embodiments of the invention, the reaction proceeds at about 22°C. In yet other embodiments of the invention, the reaction proceeds at about room temperature.

25 [00187] After stirring for about 1 to 15 hours, the product (Compound XIIc) is precipitated by the addition of an acid. In certain embodiments of the invention, the acid is an organic acid, such as citric acid, acetic acid, or oxalic acid. The acid can be in an aqueous solution when added. The reaction can be at about 22°C when the acid is added. The resulting suspension can be stirred for 30 to 120 minutes at a temperature of about 0-5°C. In certain embodiments  
30 of the invention, the suspension is stirred at about 22°C.

[00188] After stirring, the suspension is filtered. The resulting product can then be resuspended in water, after which it can be filtered and washed. The resuspension can occur

at least one more time. The resulting product, 3-O-benzoyl-*D-erythro*-dihydroceramide (Compound XIIc), can be washed with water, acetone, or a mixture thereof.

[00189] In certain embodiments of the invention Compound XIIc or one or more of its intermediates can be purified by recrystallization, silica gel chromatography, high performance liquid chromatography or other methods known to those skilled in the art.

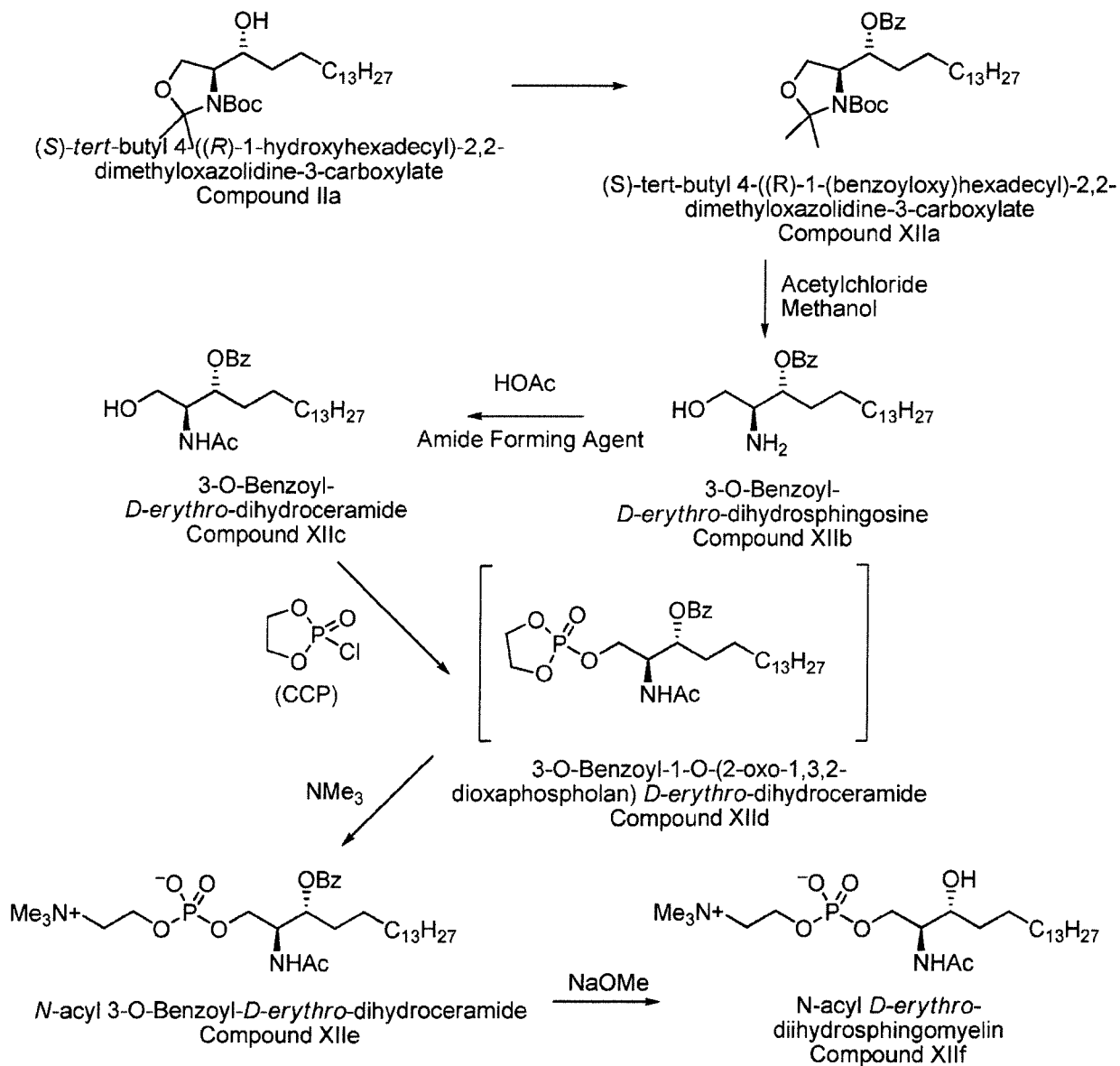
[00190] One equivalent of Compound XIIc is dissolved in toluene, about 0.6 to 1 equivalents of tetramethylethylenediamine (TMEDA) is added and the mixture is cooled to about 4-9 °C. About 1 to 2 equivalents of 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) in acetonitrile is added, followed by additional acetonitrile. The reaction is warmed to about 22 °C and stirring continued for 1-3 hours. Additional acetonitrile is added and the temperature is decreased to about -10 to 0°C. Gaseous trimethylamine is cooled to below its boiling point, and about 40 to 60 equivalents of this liquid trimethylamine are added. The reaction mixture is heated to about 60 – 70°C and proceeds for 10 to 16 hours to yield the *N*-acyl-O-benzoyl-*D-erythro*-dihydrosphingomyelin (Compound XIIe). The reaction is cooled to about -30°C and the resulting suspension is filtered. The crude *N*-acyl-*D-erythro*-dihydrosphingomyelin (Compound XIIe) is further purified by silica gel chromatography.

[00191] One equivalent of *N*-acyl-*D-erythro*-dihydrosphingomyelin (Compound XIIe) is dissolved in methanol, about 0.2 equivalents of sodium methoxide are added and the mixture is stirred for 20-26 hours at about 22 °C. Methylene chloride and water are added and the pH is adjusted to about 7 with the addition of hydrochloric acid. *N*-acyl-*D-erythro*-dihydrosphingomyelin (Compound XIIIf) is recovered from the organic layer.

[00192] In further embodiments of the invention the *N*-acyl-*D-erythro*-dihydrosphingomyelin (Compound XIIIf) can be purified by recrystallization, silica gel chromatography, high performance liquid chromatography or other methods known to those skilled in the art.

[00193] In certain embodiments of the invention, the resulting *N*-acyl-*D-erythro*-dihydrosphingosine has an enantiomeric purity of at least about 85% and contains no more than about 15% of its corresponding opposite enantiomer. In further embodiments of the invention, the *N*-acyl-*D-erythro*-dihydrosphingomyelin has an enantiomeric purity of at least about 90% and contains no more than about 10% of its corresponding opposite enantiomer. In yet further embodiments *N*-acyl-*D-erythro*-dihydrosphingomyelin has an enantiomeric purity of at least about 95% and contains no more than about 5% of its corresponding opposite enantiomer. In still further embodiments *N*-acyl-*D-erythro*-dihydrosphingomyelin

has an enantiomeric purity of at least about 98% and contains no more than about 2% of its corresponding opposite enantiomer.



Scheme XII

HOAc is a fatty acid

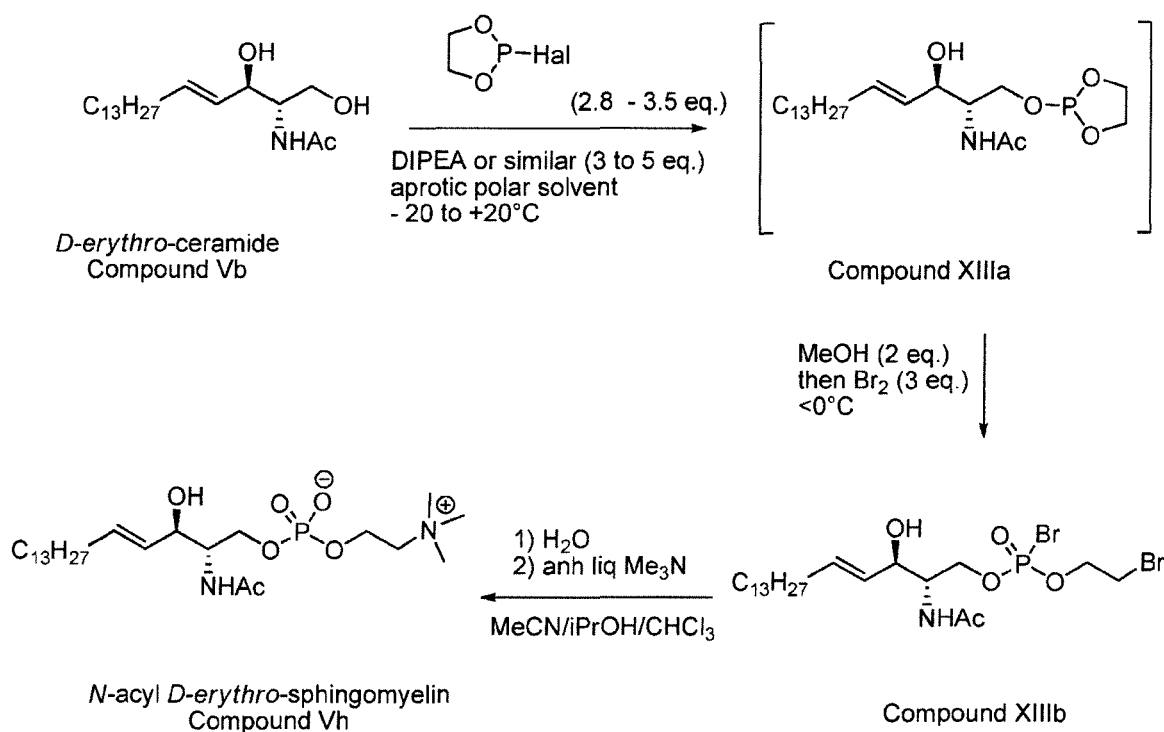
Ac is a fatty acid residue having 3 to 36 carbons and zero to six carbon-carbon double bonds

***Synthesis of an N-acyl-D-erythro-sphingomyelin without protection of the secondary hydroxyl group***

[00194] In certain embodiments of the invention, an D-erythro-ceramide (Compound Vb) is directly phosphorylated with ethylene halophosphite as shown in Scheme XIII. The reaction proceeds in the presence of about 2.5-3.5 equivalents of ethylene halophosphite in the presence of about 4 - 10 equivalents of a base in an aprotic polar solvent having a large dielectric constant ( $>20$ ) and a large dipole moment. In certain embodiments, the reaction proceeds at about  $-20$  to  $+20$  °C. In certain embodiments, the aprotic polar solvent has a dielectric constant greater than 20. In certain embodiments, the aprotic polar solvent is chloroform, nitromethane, acetonitrile, acetone, dimethyl sulfoxide, or mixtures thereof. In certain embodiments, the halophosphite is chlorophosphite. In further embodiments, the reaction proceeds with 3 equivalents of ethylene chlorophosphite. In other embodiments, the base is *N,N*-diisopropylethylamine. In yet other embodiments the reaction proceeds with 5 eq *N,N*-diisopropylethylamine.

[00195] Without being bound to any particular mechanism, it is believed that such a solvent hinders, for example, by solvation of the hydroxyl moieties, the intramolecular hydrogen bonding in the ceramide. Suitable solvents include, but not limited to: chloroform, nitromethane, acetonitrile, acetone, or dimethyl sulfoxide.

[00196] After quenching the unreacted ethylene halophosphite with an alcohol, the cyclic phosphate (Compound XIIIa) is oxidized and the ring opened in the presence of bromine at temperatures between about  $-50$  to  $10^{\circ}\text{C}$ , in one embodiment about  $-20^{\circ}\text{C}$ , to produce ceramide-bromide derivative (Compound XIIIb), whose P-Br bond is hydrolyzed by the addition of water. In particular embodiments of the invention the alcohol is methanol or ethanol. Compound XIIIb is quaternized with anhydrous liquid trimethylamine to afford the *N*-acyl-D-erythro-sphingomyelin (Compound Vh). In further embodiments of the invention the *N*-acyl-D-erythro-sphingomyelin is *N*-palmitoyl-D-erythro-sphingomyelin.



Scheme XIII

Ac is a fatty acid residue having 3 to 36 carbons and zero to six carbon-carbon double bonds

[00197] In yet another embodiment, the invention provides methods for synthesizing *N*-

5 palmitoyl-*D*-erythro-sphingomyelin comprising the steps of:

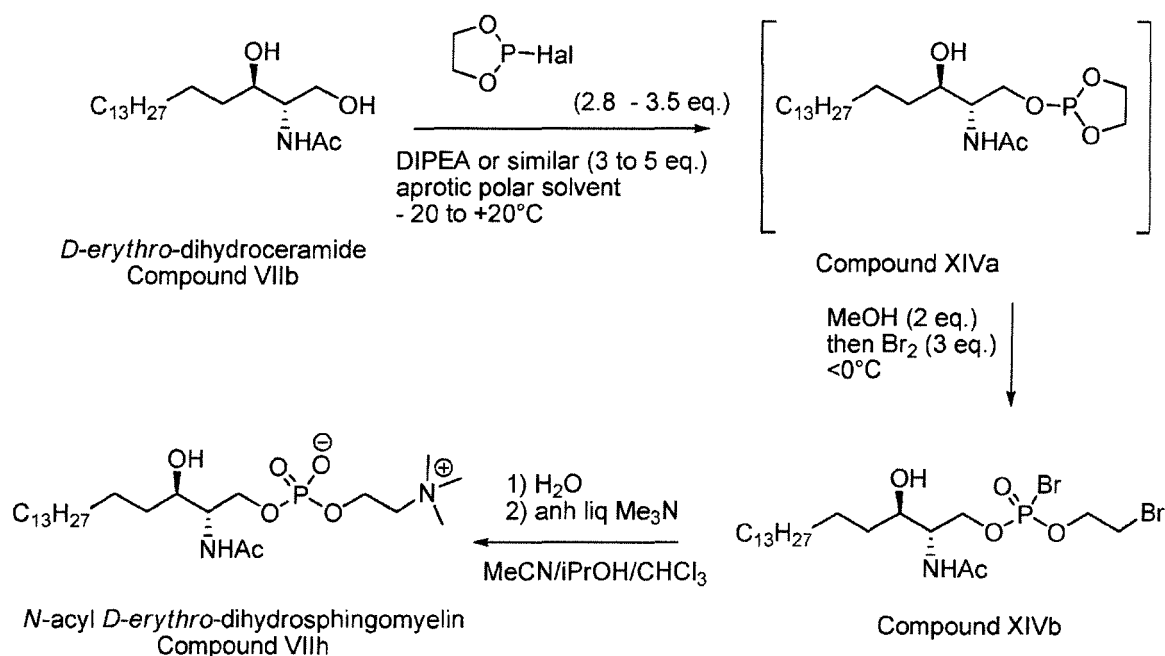
a) allowing *N*-palmitoyl-*D*-erythro-sphingosine to react with ethylene chlorophosphite under conditions effective to yield *N*-((2*S*,3*R*,*E*)-1-((1,3,2-dioxaphospholan-2-yl)oxy)-3-hydroxyoctadec-4-en-2-yl)palmitoylamide;

10 b) allowing *N*-((2*S*,3*R*,*E*)-1-((1,3,2-dioxaphospholan-2-yl)oxy)-3-hydroxyoctadec-4-en-2-yl)palmitoylamide to react with bromine under conditions effective to yield 2-bromoethyl ((2*S*,3*R*,*E*)-3-hydroxy-2-palmitamidooctadec-4-en-1-yl)phosphorobromidate; and

15 c) allowing 2-bromoethyl ((2*S*,3*R*,*E*)-3-hydroxy-2-palmitamidooctadec-4-en-1-yl)phosphorobromidate to react with trimethylamine under conditions effective to yield *N*-palmitoyl-*D*-erythro-sphingomyelin.

***Synthesis of N-acyl-D-erythro-dihydrosphingomyelin without protection of the secondary hydroxyl group***

- [00198] In certain embodiments of the invention, D-erythro-dihydroceramide (Compound VIIb) is directly phosphorylated with ethylene halophosphite as shown in Scheme XIV. The reaction proceeds in the presence of about 2.5-3.5 equivalents of ethylene halophosphite in the presence of about 4 - 10 equivalents of a base in an aprotic polar solvent having a large dielectric constant (>20) and a large dipole moment. In certain embodiments, the reaction proceeds at about -20 to +20 °C. In certain embodiments, the aprotic polar solvent has a dielectric constant greater than 20. In certain embodiments, the aprotic polar solvent is chloroform, nitromethane, acetonitrile, acetone, dimethyl sulfoxide, or mixtures thereof. In particular embodiments, the halophosphite is ethylene chlorophosphite. In further embodiments, the reaction proceeds with 3 equivalents of ethylene chlorophosphite. In other embodiments, the base is N,N-diisopropylethylamine. In yet other embodiments the reaction proceeds with 5 eq N,N-diisopropylethylamine.
- [00199] Without being bound to any particular mechanism, it is believed that such a solvent hinders, by solvation of the hydroxyl moieties, the intramolecular hydrogen bonding in the acyl dihydroceramide. Suitable solvents include, but not limited to: chloroform, nitromethane, acetonitrile, acetone, or dimethyl sulfoxide.
- [00200] After quenching the unreacted ethylene halophosphite with an alcohol the cyclic phosphite (Compound XIVa) is simultaneously oxidized and the ring opened in the presence of bromine at temperatures between about -50 to 10°C (preferably about -20°C) to produce a dihydroceramide bromide derivative (Compound XIVb) , whose P-Br bond is hydrolyzed by the addition of water. In particular embodiments of the invention the alcohol is methanol or ethanol. Compound XIVb is quaternized with anhydrous liquid trimethylamine to afford the N-acyl-D-erythro-dihydrosphingomyelin (Compound VIIh). In further embodiments of the invention the N-acyl-D-erythro-dihydrosphingosine is N-palmitoyl-D-erythro-dihydrosphingosine.

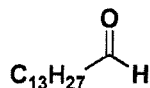


Scheme XIV

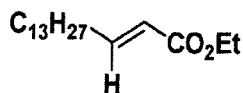
Ac is a fatty acid residue having 3 to 36 carbons and zero to six carbon-carbon double bonds

[00201] In yet another embodiment, the invention provides methods for synthesizing *N*-palmitoyl-*D*-erythro-dihydrosphingomyelin comprising the steps of:

- 5                   a) allowing *N*-palmitoyl-*D*-erythro-dihydrosphingosine to react with ethylene chlorophosphite under conditions effective to yield *N*-((2*S*,3*R*)-1-((1,3,2-dioxaphospholan-2-yl)oxy)-3-hydroxyoctadecan-2-yl)palmitamide;
- b) allowing *N*-((2*S*,3*R*)-1-((1,3,2-dioxaphospholan-2-yl)oxy)-3-hydroxyoctadecan-2-yl)palmitamide to react with bromine under conditions effective to yield 2-bromoethyl ((2*S*,3*R*)-3-hydroxy-2-palmitamidooctadecyl) phosphorobromidate; and
- 10               c) allowing 2-bromoethyl ((2*S*,3*R*)-3-hydroxy-2-palmitamidooctadecyl) phosphorobromidate to react with trimethylamine under conditions effective to yield the *N*-palmitoyl-*D*-erythro-dihydrosphingomyelin.

**Examples****Example 1: Synthesis of *N*-Palmitoyl-*D*-erythro-sphingomyelin*****Step 1 : Synthesis of 2-(E)-Hexadecenal***5 **Step 1.1: 1-Tetradecanal**

[00202] To a solution of 1-tetradecanol (110.0 g ; 0,513 mol) and trichloroisocyanuric acid (178.1 g ; 0,77 mol) in methylene chloride (1500 ml) at -30°C was added (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl (TEMPO) (800 mg ; 0.051 mol). The reaction mixture was stirred for 1.5 h at 0°C and filtered on celite. The organic phase collected was then washed with a saturated solution of Na<sub>2</sub>CO<sub>3</sub> (800 ml) followed by HCl 1N (800 mL). It was then dried over MgSO<sub>4</sub>, filtered and concentrated under vacuum to give rise to the title compound, 1-tetradecanal, as white solid (99.6 g ; 91%).

**Step 1.2: Ethyl-2-Hexadecenoate**

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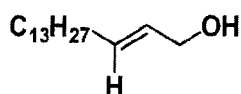
[00203] To a suspension of NaH (2.4 g, 0,059 mol) in anhydrous tetrahydrofuran (40 mL) triethylphosphonoacetate (9.4 mL, 0,047 mol) was added, dropwise, at 0 °C. After stirring for 30 minutes at 0°C, a solution of tetradecanal (10.0 g, 0,047 mol) in tetrahydrofuran (40 mL) was added and the reaction mixture was warmed to room temperature and stirred for an additional 3 h. A saturated solution of NaCl (50 mL) was then added and the aqueous layer was extracted with Et<sub>2</sub>O (3 X 200 mL). The organic layers were gathered, dried over MgSO<sub>4</sub> and filtered, and the solvents were evaporated. The resulting product was purified by flash chromatography on silica gel (heptane-ethyl acetate: 95/5) to give the title compound, ethyl-2-hexadecenoate, as a colorless liquid (12 .3 g ; 92%).

25 [00204] **R<sub>f</sub>** = 0.24 (Hexane/Et<sub>2</sub>O : 95/5).

[00205] **GC** : tr = 13.13 min (triethylphosphonoacetate) ; tr = 15.80 min (1-tetradecanal) ; tr = 20.60 min (ethyl 2-Hexadecenoate)

[00206]  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) : 0.87 (t, 3H,  $\text{CH}_3$ ) ; 1.25 (br, 20H) ; 1.28 (t, 3H,  $\text{CH}_3$ ) ; 1.44 (m, 2H,  $\text{CH}_2$ ) ; 2.18 (qd, 2H,  $\text{CH}_2$ ,  $^3J = 6.5$  Hz,  $^4J = 1.5$  Hz) ; 4.18 (q, 2H,  $\text{CH}_2$ ) ; 5.80 (dt, 1H,  $^3J = 15.5$  Hz,  $^4J = 1.5$  Hz) ; 6.96 (dt, 1H,  $\text{CH}_2$ ,  $^3J = 15.5$  Hz,  $^3J = 6.5$  Hz).

Step 1.3: 2-(*E*)-Hexadecen-1-ol



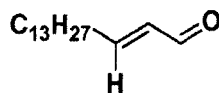
[00207] Diisobutylaluminium hydride (54.5 mL, 1 M in cyclohexane, 0,054 mol) was added, drop-wise at 0 °C, to a solution of ethyl-2-hexadecenoate (6.4 g, 0,023 mol) in tetrahydrofuran (20 mL). The reaction mixture was stirred at 0°C until complete consumption of the starting material as monitored by TLC.  $\text{Et}_2\text{O}$  (50 mL) and a saturated solution of sodium tartrate (50 mL) were successively added while stirring until 2 separate layers were distinctly visible. The aqueous layer was extracted with  $\text{Et}_2\text{O}$  (2 x 50 mL). The organic layers were combined and dried over  $\text{MgSO}_4$ , and the solvents removed under vacuum to give the title compound, 2-(*E*)-hexadecen-1-ol, as a white waxy solid (5.3 g ; 97%).

[00208]  $R_f = 0.31$  (Hexane/ $\text{Et}_2\text{O}$  : 1/1).

[00209] GC : tr = 19.1 min

[00210]  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) : 0.87 (t, 3H,  $\text{CH}_3$ ) ; 1.25 (br, 22H) ; 2.03 (q, 2H,  $\text{CH}_2$ ,  $^3J = 6.0$  Hz) ; 4.09 (d, 1H,  $^3J = 5$  Hz) ; 5.66 (m, 2H).

Step 1.4: 2-(*E*)-Hexadecen-1-al



[00211] To a solution of 2-(*E*)-Hexadecen-1-ol (5.2 g ; 0,022 mol) in methylene chloride (30 mL) under argon at 0°C a suspension of pyridinium chlorochromate (PCC) (16,3 g ; 0,043 mol) in methylene chloride (30 mL) followed by celite (20 g) was added. After stirring for 3h at 0°C, the reaction mixture was diluted with 20 mL of diethyl ether, and filtered over a pad of silica. Solvents were evaporated and the crude product was purified by flash chromatography on silica gel (heptane – ethyl acetate: 95/5) to give the title compound, 2-(*E*)-hexadecen-1-al, as a white solid (2.2 g ; 43%).

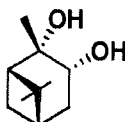
[00212]  $R_f = 0.14$  (Hexane/ $\text{Et}_2\text{O}$  : 95/5)

[00213] GC : tr = 18.9 min

[00214] <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) : 0.88 (t, 3H, CH<sub>3</sub>) ; 1.26 (br, 20H) ; 1.50 (m, 2H, CH<sub>2</sub>) ; 2.35 (qd, 2H, <sup>3</sup>J = 7 Hz, <sup>4</sup>J = 1.5 Hz) ; 6.1 (ddt, 1H, <sup>3</sup>J = 15.5 Hz, <sup>3</sup>J = 8 Hz, <sup>4</sup>J = 1.5 Hz) ; 6.85 (td, 1H, <sup>3</sup>J = 15.5 Hz, <sup>3</sup>J = 7 Hz) ; 9.5 (d, 1H, <sup>3</sup>J = 8 Hz).

## 5 Step 2: Synthesis of (1R, 2R, 5R)-(+)-2-Hydroxy-3-Pinanone

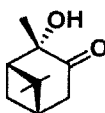
### Step 2.1: (1R, 2R, 3S, 5R)-(-)-Pinanediol



[00215] S-(-)-α-pinene (24.3 g ; 0,18 mol), potassium osmate dihydrate (0,13 g), N-methylmorpholine-N-oxide (60% in water ; 0.21 mol ; 41.7 g) dissolved in 17.3 mL of pyridine, 107 mL of acetone and 11.9 mL of deionized water were combined in a 250 mL three-necked flask . The reaction mixture was refluxed for 60 hours and then diluted with methyl tert-butyl ether (MTBE) (300 mL) and hexane (60 mL). Water (200 mL) was then added and the organic layer was decanted, washed successively with 10% citric acid (3x100 mL), a saturated solution of NaHCO<sub>3</sub> (100 mL), brine (100 mL), and then dried over MgSO<sub>4</sub> and filtered. The solvents were removed under vacuum to give the title compound, 1R, 2R, 3S, 5R)-(-)-pinanediol, as a dark orange oil (24.5 g).

[00216] GC : tr = 12.0 min (diol) ; tr = 10.9 min (1R,2R,5R)-(+)-2-hydroxy-3-pinanone ; (5 - 10%)

### Step 2.2: (1R, 2R, 5R)-(+)-2-Hydroxy-3-Pinanone



20

[00217] Triethylamine (Et<sub>3</sub>N) (80.2 mL; 0,58 mol) was added to a solution of (1R, 2R, 3S, 5R)-(-)-Pinanediol (24.5 g; 143.9 mmol) in a dimethyl sulfoxide/ methylene chloride solvent mixture (154 mL; 1/1) at 10°C. SO<sub>3</sub>·Pyridine (68.7 g ; 0,43 mol) was then added portion-wise over 30 minutes while the temperature was maintained below 20°C. The reaction mixture was stirred for 2 hours at 10°C then diluted with ethyl acetate (300 mL). The organic layer was washed with HCl 0.5 N (2\*150 mL), brine (150 mL), then dried over MgSO<sub>4</sub> and filtered.

The solvents were removed under vacuum to give a brown oil. The crude product was purified by flash chromatography on silica gel (methylcyclohexane-ethyl acetate: 9/1) to give the title compound, (*1R, 2R, 5R*)-(+)-2-Hydroxy-3-Pinanone, as a yellow oil (19.2 g ; 63% over two steps).

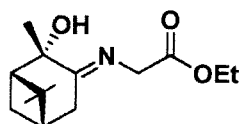
5 [00218] **GC** : tr = 10.9 min ;

[00219] **Distillation: B.p** = 100-104°C ( 3-4 mmHg)

[00220] **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) : 0.90 (s, 3H) ; 1.30 (s, 3H) ; 1.40 (s, 3H) ; 1.70 (d, 1H, *J* = 12.0 Hz) ; 2.10 (m, 2H) ; 2.30 (s, 1H) ; 2.50 (m, 1H) ; 2.60 (brs, 2H).

**Step 3: D-erythro-Sphingosine hydrochloride**

10 Step 3.1 : (*1R, 2R, 5R*)-Ethyl-((2-hydroxypinan-3-ylene)amino)acetate



[00221] NH<sub>3</sub> gas was bubbled through a suspension of ethylglycinate hydrochloride (16.6 g ; 0,13 mol) in toluene (100 mL) for 1 h. The ammonium chloride formed was filtered off and (*1R,2R,5R*)-(+)-2-hydroxy-3-pinanone (Step 2.2) (10.0 g ; 0,59 mol) was added to the

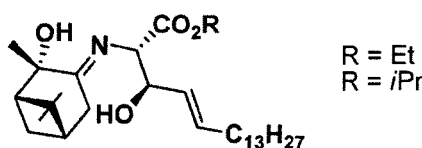
15 solution of free base ethylglycinate with few drops of BF<sub>3</sub>.OEt<sub>2</sub>. The reaction mixture was then refluxed for 5 hours with a Dean-Stark apparatus. After completion of the reaction, the solvents were evaporated. The resulting product was purified by flash chromatography on silica gel impregnated with Et<sub>3</sub>N (5% in ether) and the title compound, (*1R, 2R, 5R*)-ethyl-((2-hydroxypinan-3-ylene)amino)acetate, was eluted with Et<sub>2</sub>O.

20 [00222] **R<sub>f</sub>** = 0,35 (Cyclohexane – ethyl acetate: 1/1)

[00223] **<sup>1</sup>H NMR** (CDCl<sub>3</sub>): 0.88 (s, 3H, CH<sub>3</sub>) ; 1.30 (t, 3H, CH<sub>3</sub>, *J* = 7.0 Hz) ; 1.34 (s, 3H, CH<sub>3</sub>) ; 1.53 (s, 3H, CH<sub>3</sub>) ; 1.57 (d, 1H, *J* = 10.0 Hz) ; 2.07 (m, 2H) ; 2.36 (dtt, 1H, *J* = 10.0 Hz ; *J* = 6.0 Hz ; *J* = 1.5 Hz) ; 2.50 (d, 2H, *J* = 1.5 Hz ; *J* = 1.0 Hz) ; 2.61 (s, 1H, OH) ; 4.17 (s, 2H, =N-CH<sub>2</sub>) ; 4.23 (q, 2H, CH<sub>2</sub>CH<sub>3</sub>, *J* = 7.0 Hz).

25 [00224] **NMR <sup>13</sup>C** (CDCl<sub>3</sub>) δ (ppm): 180,0 (C-1 quat. Ester) ; 170,2 (C-1' quat. amide) ; 76,5 (C-2' quat.) ; 60,9 (CH<sub>2</sub>-CH<sub>3</sub>) ; 52,6 (C-2) ; 50,4 (C-3') ; 38,6 (C quat) ; 38,3 (C-5) ; 33,7 (C-6) ; 28,2 (CH<sub>3</sub>) ; 28,1 (C-4') ; 27,3 (CH<sub>3</sub>) ; 22,8 (CH<sub>3</sub>) ; 14,2 (CH<sub>2</sub>-CH<sub>3</sub>).

Step 3.2 (2*S*,3*R*,*E*)-Ethyl 3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate and (2*S*,3*R*,*E*)-isopropyl 3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate



5

[00225] A solution of chlorotitanium triisopropoxyde (5.2 g ; 0,02 mol) in methylene chloride (15 mL), a solution of 2-(*E*)-Hexadecen-1-al (4,35 g ; 0,0018) in methylene chloride (8 mL) and triethylamine (6.1 mL ; 0,044 mol) was added to a solution of (1*R*, 2*R*, 5*R*)-Ethyl-((2-hydroxypinan-3-ylidene)amino)acetate (5.0 g ; 0,020 mol) in methylene chloride (9.6 mL) under argon at 0°C. After stirring the reaction mixture for 4h at 0°C, it was then quenched with brine (25 mL). The aqueous layer was extracted with ethyl acetate and dried over MgSO<sub>4</sub>; the solvents were removed under vacuum to give rise to a yellowy-orange oil (9.7 g), mixture of the 73/27 isopropyl and ethyl esters, (2*S*,3*R*,*E*)-Ethyl 3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate and (2*S*,3*R*,*E*)-isopropyl 3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate.

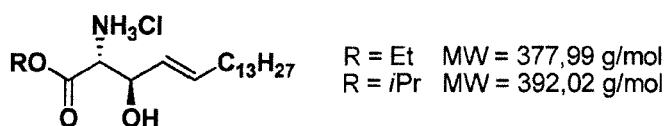
15

[00226] **R<sub>f</sub>** = 0,7 (Cyclohexane – ethyl acetate: 1/1)

[00227] **<sup>1</sup>H NMR (CDCl<sub>3</sub>)** δ (ppm) : 0.88 (t, 3H, *J* = 6.5 Hz) ; 1.50-1.10 (m, 28H, (CH<sub>2</sub>)<sub>12</sub> + 2CH<sub>3</sub>) ; 1.50 (s, 3H, CH<sub>3</sub>) ; 1.53 (d, 1H) ; 2.13 (q, 2H) ; 2.18-1.95 (m, 2H) ; 2.34 (dtd, 1H) ; 2.51 (m, 1H) ; 3.25 (s, 1H) ; 3.75 (s, 1H) ; 4.15 (d, 1H, *J* = 6.7 Hz) ; 4.20 (dt, 1H, *J* = 7.0 Hz ; *J* = 4.0 Hz) ; 4.55 (t, 1H, *J* = 6.7 Hz) ; 5.05 (hept, 1H, *J* = 6.3 Hz, CH(CH<sub>3</sub>)<sub>2</sub>) ; 5.55 (dd, 1H, *J* = 15.4 Hz ; *J* = 7.1 Hz) ; 5.70 (dt, 1H, *J* = 15.4 Hz ; *J* = 6.5 Hz).

20

Step 3.3: (2*R*,3*R*,*E*)-ethyl 2-amino-3-hydroxyoctadec-4-enoate and (2*R*,3*R*,*E*)-isopropyl 2-amino-3-hydroxyoctadec-4-enoate

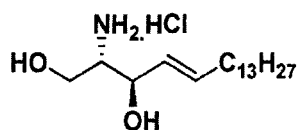


25

[00228] HCl 1.2 M (203 mL) was added dropwise to the crude mixture of the isopropyl and ethyl esters, (2*S*,3*R*,*E*)-Ethyl 3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate and (2*S*,3*R*,*E*)-isopropyl 3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate, from the previous step (14.8 g ; 0,030 mol) in tetrahydrofuran (51 mL). The mixture was then stirred for 72 h at room temperature. Tetrahydrofuran was evaporated and the aqueous layer was then extracted with ethyl acetate. The organic layer contained, after removal of the solvents and (+)-2-hydroxy-3-pinane (6.8g). The aqueous layer was dried to give rise to (2*R*,3*R*,*E*)-ethyl 2-amino-3-hydroxyoctadec-4-enoate and (2*R*,3*R*,*E*)-isopropyl 2-amino-3-hydroxyoctadec-4-enoate as their hydrochloride salts (5.7 g).

[00229]  $R_f=0,45$  (Et<sub>2</sub>O-MeOH : 96/4)

Step 3.4: *D*-Erythro-sphingosine hydrochloride



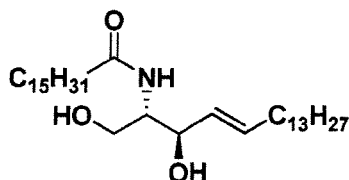
[00230] Sodium borohydride (4.40 g; 0,12 mol) was added to a suspension of the aminoester hydrochloride from the step above (2.2 g ; 0,0058 mol) in 40 mL of a solvent mixture of EtOH/H<sub>2</sub>O (3/1). The mixture was stirred for 72h at 0°C before a saturated solution of NH<sub>4</sub>Cl (40 mL) was added. The aqueous layer was extracted with methylene chloride (4\*100 mL), washed with brine, dried over MgSO<sub>4</sub>, and filtered. The solvents were removed under vacuum to give rise to the title compound, *D*-erythro-sphingosine hydrochloride, as a white solid (1.5 g ; 86%).

[00231]  $R_f = 0,3$  (CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O : 13/6/1)

[00232] <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ (ppm) 0.90 (t, 3H,  $J = 6.5$  Hz) ; 1.50-1.20 (m, 22H, (CH<sub>2</sub>)<sub>12</sub>) ;

2.00 (q, 2H,  $J = 7.8$  Hz) ; 3.15 (s, 1H, OH) ; 3.70 (m, 4H) ; 4.30 (s, 1H, OH) ; 5.40 (dd, 1H,  $J = 15.5$  Hz ;  $J = 6.3$  Hz) ; 5.80 (dt, 1H,  $J = 15.5$  Hz ;  $J = 7.8$  Hz) ; 8.46 (brs, 3H).

**Step 4: *N*-Palmitoyl-*D*-erythro-sphingosine**

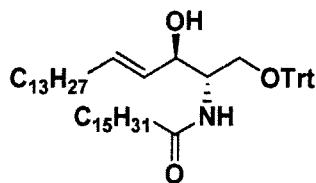


[00233] Palmitic acid (1.9 g ; 0,074 mol) and a solution of *D-erythro*-sphingosine (2.2 g ; 0,074 mol) in tetrahydrofuran (99 mL) were successively added to a suspension of O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (3.1 g ; 8.1 mmol) in dimethylformamide (15 mL). The white suspension obtained was cooled to 0°C and triethylamine (2.5 mL ; 0,018 mmol) was added. The reaction mixture was stirred for 12 h at room temperature. A solution of 5% citric acid (400 mL) was then added and the suspension was filtered off. The white solid was mixed with water (60 mL) at rt, filtered off and washed with water. It was then dried under vacuum at 40°C to give the title compound, *N*-Palmitoyl-*D-erythro*-sphingosine (3.5 g ; 80 %).

[00234] <sup>1</sup>H NMR (CDCl<sub>3</sub>): 0.97 (6H, t) ; 1.10-1.40 (m, 46H) ; 1.62 (2H, m) ; 2.04 (2H, m, CH<sub>2</sub>-CH) ; 2.21 (t, 2H, *J* = 8.2 Hz, CH<sub>2</sub>CONH) ; 2.71 (m, 2H) ; 3.69 (m, 1H) ; 3.80-4.00 (m, 2H) ; 4.28 (m, 1H, CH(OH)CH) ; 5.52 (ddt, 1H, *J* = 15.4 Hz ; *J* = 6.4 Hz ; *J* = 1.0 Hz, CH(OH)CH) ; 5.77 (dtd, 1H, *J* = 15.4 Hz ; *J* = 6.7 Hz ; *J* = 1.1 Hz, CH<sub>2</sub>CH) ; 6.22 (d, 1H, *J* = 6.8 Hz, NH).

#### Step 5: *N*-Palmitoyl-3-O-benzoyl-*D-erythro*-sphingosine

##### Step 5.1: *N*-Palmitoyl-1-O-trityl-*D-erythro*-sphingosine



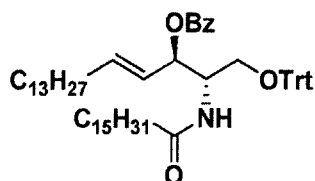
[00235] A suspension of *N*-palmitoyl-*D-erythro*-sphingosine (0.58 g, 1.08 mmol), triethylamine (1.2 mL), 4-dimethylaminopyridine (5 mg), and trityl chloride (0.45 g, 1.62 mmol) in methylene chloride (14 mL) was heated at reflux for 60 h. Volatile materials were evaporated, the residue was re-dissolved in ethyl acetate and the mixture was washed successively with 1 M hydrochloric acid, aq. NaHCO<sub>3</sub> and brine. The organic phase was dried over MgSO<sub>4</sub> and evaporated. The residue was chromatographed on silica gel in heptane/ethyl

acetate (7:3) to afford *N*-palmitoyl-1-*O*-trityl-*D*-erythro-sphingosine (0.39 g, 46%) as a waxy solid.

[00236]  $R_f = 0.49$  ( $\text{CH}_2\text{Cl}_2$ / ethyl acetate / $\text{Et}_3\text{N}$  : 97/3/0.1)

[00237]  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.88 (6H, t), 1.40–1.15 (46H, m), 1.64 (2H, m), 1.91 (2H, m), 2.20 (2H, t,  $J = 8.2$  Hz), 3.28 (1H, dd,  $J = 9.6$  Hz,  $J = 4.0$  Hz), 3.40–3.35 (2H, m), 4.04 (1H, m), 4.17 (1H, m), 5.24 (1H, dd,  $J = 15.4$  Hz,  $J = 6.2$  Hz), 5.62 (1H, dt,  $J = 15.4$  Hz,  $J = 6.6$  Hz), 6.06 (1H, d,  $J = 7.5$  Hz, NH), 7.35–7.20 (9H, m), 7.35–7.45 (6H, m).

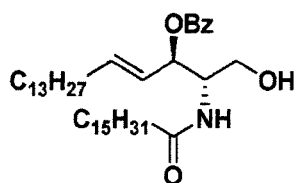
Step 5.2: *N*-Palmitoyl-1-*O*-trityl-3-*O*-benzoyl-*D*-erythro-sphingosine



[00238] 4-Dimethylaminopyridine (10 mg) and benzoyl chloride (0.1 ml, 0.85 mmol) was added to a solution of *N*-Palmitoyl-1-*O*-trityl-*D*-erythro-sphingosine (0.39 g, 0.50 mmol) in pyridine (5 ml) under nitrogen were added and the mixture was stirred for 20 h. Solvent was removed under reduced pressure and the residue was partitioned between aq.  $\text{NaHCO}_3$  and ethyl acetate. The organic phase was washed with brine, dried ( $\text{MgSO}_4$ ), and evaporated, and the residue chromatographed on silica gel in Heptane/Ethyl acetate-hexane (85 : 15 to 1 : 1) to give the title compound, *N*-palmitoyl-1-*O*-trityl-3-*O*-benzoyl-*D*-erythro-sphingosine (207 mg, 60%), as a waxy solid.

[00239]  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.88 (6H, t), 1.31–1.23 (46H, m), 1.56 (2H, m), 1.99 (2H, m), 2.08 (2H, t), 3.17 (1H, dd,  $J = 7.4$  Hz,  $J = 3.9$  Hz,  $\text{CH}(\text{H}')\text{OH}$ ), 3.43 (1H, dd,  $J = 9.7$  Hz,  $J = 3.9$  Hz,  $\text{CH}(\text{H}')\text{OH}$ ), 4.47 (1H, m,  $\text{CH}-(\text{NHCOR})$ ), 5.43 [1H, dd,  $J = 15.3$  Hz,  $J = 7.3$  Hz,  $\text{CH}(\text{OCOPh})\text{CH=}$ ], 5.75–5.60 [2H, m, NH,  $\text{CH}(\text{OCOPh})$ ], 5.86 (1H, dt,  $J = 15.3$  Hz,  $J = 7.9$  Hz,  $\text{CH}_2\text{CH=}$ ), 7.25–7.10 (9H, m), 7.40–7.30 (8H, m), 7.54 (1H, t,  $J = 7.5$  Hz), 7.92 (2H, d,  $J = 7.3$  Hz).

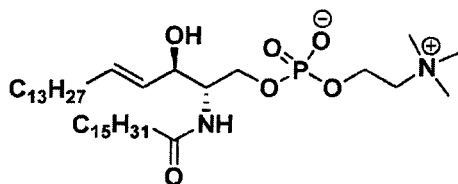
Step 5.3: *N*-Palmitoyl-3-*O*-benzoyl-*D*-erythro-sphingosine



[00240] A solution of *N*-Palmitoyl-1-O-trityl-3-O-benzoyl-*D*-erythro-sphingosine (1.10 g, 1.24 mmol) and toluene-*p*-sulfonic acid monohydrate (0.23 g, 1.36 mmol) in methylene chloride (18 ml) and methanol (18 ml) was stirred under nitrogen for 3 h. Solvent was evaporated and the residue was partitioned between aq. NaHCO<sub>3</sub> and ethyl acetate. The organic phase was washed with brine, dried over MgSO<sub>4</sub>, and evaporated to dryness. The residue was chromatographed on silica gel and eluted with Heptane/Ethyl acetate (1 : 1) to give the title compound, *N*-Palmitoyl-3-O-benzoyl-*D*-erythro-sphingosine (0.64 g ; 80%).

[00241] <sup>1</sup>H NMR (CDCl<sub>3</sub>/CD<sub>3</sub>OD) δ 0.87 (6H, t), 1.30-1.10 (46H, m), 1.54 (2H, m), 1.96 (2H, m), 2.14 (2H, m), 2.77 (2H, br s), 3.71 (2H, m, CH<sub>2</sub>O), 4.24 (1H, m, CHN), 5.60-5.40 [2H, m, CH(OCOPh)CH=], 5.79 (1H, dt, J = 15.0 Hz, J = 6.8 Hz, CH<sub>2</sub>CH=), 6.18 (1H, d, J = 9.6 Hz, NH), 7.38 (2H, dd, J = 7.6 Hz, J = 7.2 Hz), 7.52 (1H, dd, J = 7.6, J = 7.6 Hz), 7.96 (1H, d, J = 7.2 Hz).

**Step 6: *N*-Palmitoyl-*D*-erythro-sphingomyelin**



[00242] A solution of *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingosine (0.2 g, 0.31 mmol) and tetramethylethylenediamine (TMEDA) (51 μL, 0.53 mmol) in dry toluene (5 ml) was cooled to about 8°C. To this solution 2-chloro-2-oxo-1,3,2 dioxaphospholane (82 mg, 0.57 mmol) in 0.1 mL of acetonitrile was added dropwise. The mixture was then warmed to room temperature and stirred for 4 h. Acetonitrile (5 mL) was added, followed by anhydrous trimethylamine. The flask was heated to 65-70°C for 14 h. The system was then cooled to room temperature and the flask was opened. The solvents were removed under reduced pressure.

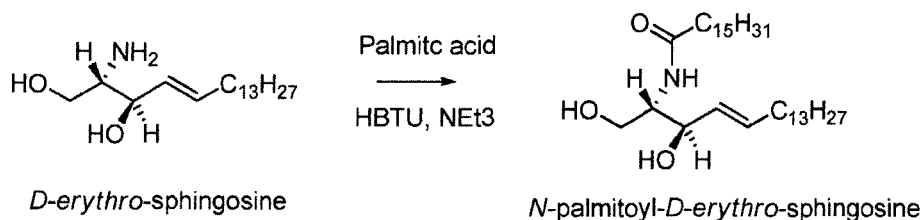
[00243] The resulting product, *N*-palmitoyl-3-*O*-benzoyl-*D*-erythro-sphingomyelin, was dissolved in methanol (1.5 mL). Sodium methoxide (30% in methanol, 15 μL) was added to

the solution. After stirring overnight, methylene chloride and water were added. The pH was adjusted to and the organic layer was evaporated to dryness. The crude material was purified by column chromatography to give the title compound, *N*-palmitoyl-*D*-erythro-sphingomyelin (66 mg, 30% over 3 steps).

- 5 [00244]  $^1\text{H}$  NMR ( $\text{CDCl}_3/\text{CD}_3\text{OD}$ )  $\delta$ : 0.90 (t,  $J = 7.1$  Hz, 6H), 1.26 (m, 46H), 1.56 (m,  $(\text{C}=\text{O})\text{CH}_2\text{CH}_2$ , 2H), 1.99 (m,  $\text{CH}=\text{CHCH}_2$ , 2H), 2.14 (t,  $(\text{C}=\text{O})\text{CH}_2$ , 2H), 3.24 (s,  $\text{N}(\text{CH}_3)_3$ , 9H), 3.68 (m,  $\text{POCH}_2\text{CH}_2\text{N}$ , 2H), 3.91 (m,  $\text{POCH}_2\text{CH}$ , 2H), 4.04 (t,  $\text{CHO}$ , 4H,  $J = 7.7$  Hz), 4.14 (m,  $\text{CHN}$ , 2H), 4.28 (m,  $\text{POCH}_2\text{CH}_2\text{N}$ , 2H), 5.44 (ddt,  $J = 15.4$  Hz,  $J = 7.6$  Hz,  $J = 1.5$  Hz, 2H), 5.71 ddt,  $J = 15.4$  Hz,  $J = 6.6$  Hz,  $J = 0.5$  Hz, 1H).

## 10 Example 2 : Lab-Scale Synthesis of *N*-palmitoyl-*D*-erythro-sphingomyelin

### Step 1: *N*-palmitoyl-*D*-erythro-sphingosine

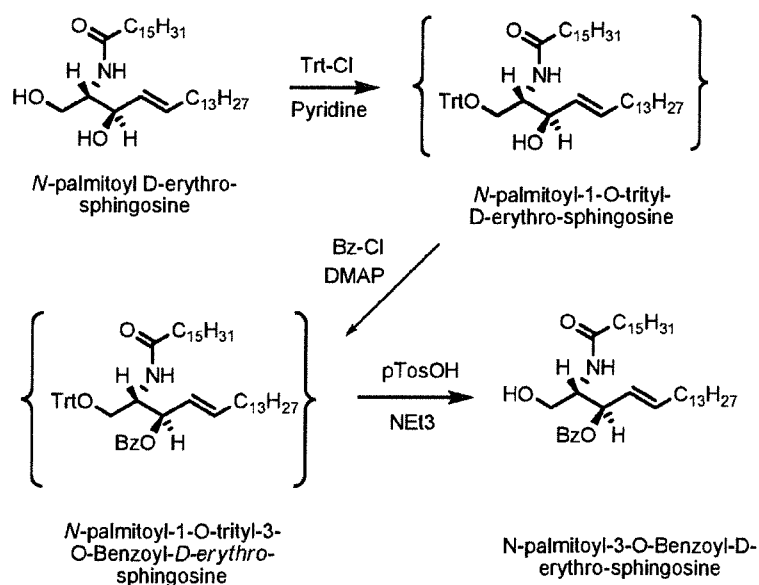


- [00245] Palmitic acid (17.12 g, 66.8 mmol) and *D*-erythro-sphingosine (20g, 66.8 mmol) in tetrahydrofuran (890 ml) were added to a suspension of O-Benzotriazole- $\text{N,N,N',N'}$ -tetramethyl-uronium-hexafluoro-phosphate (27.84 g, 73.4 mmol) in 140 ml dimethylformamide. The obtained white suspension was cooled at 0-5°C, 22.5 ml (160.7 mmol) triethylamine was added over the course of 30-60 min, and the mixture was stirred at room temperature for 12 h. After this time the thin layer chromatography (TLC) analysis indicated >99% conversion to *N*-palmitoyl-*D*-erythro-sphingosine. Citric acid 5% (400 ml) was added, the mixture was stirred for 30 min at 0-5°C and the obtained suspension was filtered. The white cake was suspended in water (600 ml) at room temperature. The suspension was filtered and washed with water. Drying for 12 hours at reduced pressure at 40°C gave 32.4 g (yield 90%) of *N*-palmitoyl-*D*-erythro-sphingosine. Purity by HPLC was 98.1% and by HPTLC 99.2%.

[00246]  $^1\text{H}$  NMR ( $\delta$  ppm,  $\text{CDCl}_3$ ): 0.97 (6H, t), 1.1–1.4 (46H, m), 1.62 (2H, m), 2.04 (2H, m,  $\text{CH}_2\text{CH}$ ), 2.21 (2H, t,  $J$  8.2 Hz,  $\text{CH}_2\text{CONH}$ ), 2.71 (2H, m), 3.69 (1H, m), 3.8–4.0 (2H, m),

4.28 (1H, m, CH(OH)CH), 5.52 (1H, ddt, J 15.4, 6.4, 1.0 Hz, CH(OH)CH)), 5.77 (1H, dtd, J 15.4, 6.7, 1.1 Hz, CH<sub>2</sub>CH), 6.22 (1H, d, J 6.8 Hz, NH).

**Step 2: N-Palmitoyl-3-O-Benzoyl-D-erythro-sphingosine**



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Step 2.1 N-palmitoyl-1-O-trityl-D-erythro-sphingosine

[00247] A suspension of *N*-palmitoyl-*D*-erythro-sphingosine (16.30 g, 30.3 mmol), pyridine (250 ml), and trityl chloride (10.15 g, 36.4 mmol) in toluene (150 ml) was heated at 52°C for 12 h. TLC analysis after the 12 hours indicated a greater than 90 % conversion to *N*-palmitoyl-1-O-trityl-*D*-erythro-sphingosine, with about 5% unreacted *N*-palmitoyl-*D*-erythro-sphingosine and about 2-5% *N*-palmitoyl-1-O-Trityl-3-O-trityl-*D*-erythro-sphingosine. The suspension was cooled to 0-5°C and filtered to remove some salts.

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Step 2.2: N-Palmitoyl-1-O-trityl-3-O-benzoyl-D-erythro-sphingosine

[00248] 4-Dimethylaminopyridine (560 mg, 4.54 mmol) and benzoyl chloride (5.8 ml, 50 mmol) was added to a solution of the crude reaction products from the above step and the mixture was stirred at 0-5°C for 15 h. After 15 hours the TLC analysis indicated a greater than 97 % conversion to *N*-palmitoyl-1-O-trityl-3-O-benzoyl-*D*-erythro-sphingosine. The reaction mixture was partitioned between water (130 ml) and ethyl acetate (530 ml). The organic phase was washed 4 times with water (160 ml) to reach pH 7. The organic phase was evaporated at reduced pressure and the resulting the oily yellow residue (34 g) was dissolved

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in methylene chloride (300 ml) and methanol (300 ml) at 5°C and used directly for the subsequent detritylation step.

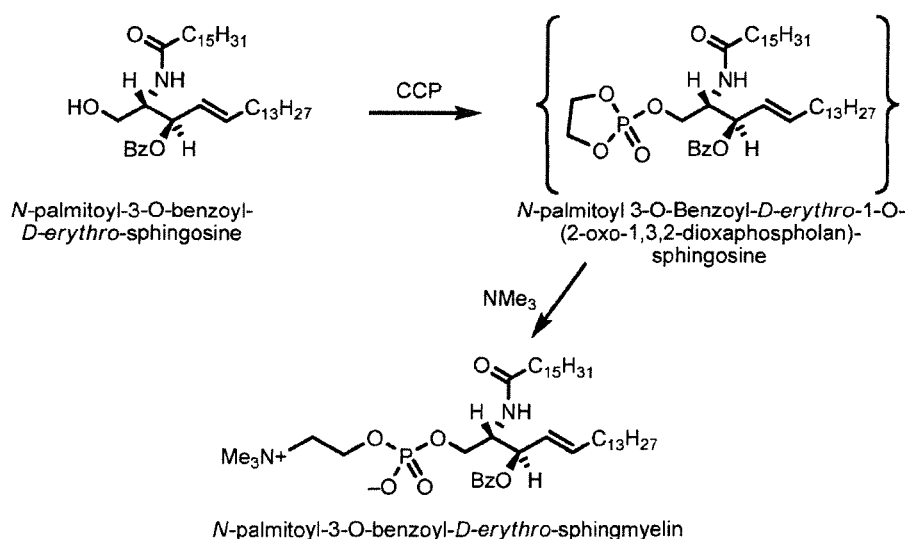
Step 2.3: *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingosine

[00249] Toluene-p-sulfonic acid monohydrate (2.88 g, 15.15 mmol) was added to the above  
 5 solution of *N*-palmitoyl-1-O-trityl-3-O-benzoyl-*D*-erythro-sphingosine in methylene chloride/methanol. The mixture was stirred at 18-22°C for 3 h. After 3 hours the TLC analysis indicated greater than 97 % conversion to *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingosine. The reaction mixture was neutralized at 0-5°C with 2.58 ml triethylamine, and the methylene chloride was evaporated (40°C/340 mbar). The obtained residue was stirred at  
 10 0-5°C for 1 h. The suspension was filtered off, washed with methanol and dried at 35°C for 12h, yielding 21.9 g (113%) of crude product (*N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingosine).

[00250] Recrystallization was performed with methanol (500 ml) and methylene chloride (5 ml) at 42°C, the solution was stirred at 20-22°C for 1 h and then cooled to 0-5°C for 1 h.  
 15 After filtration, the resulting cake was washed with methanol (2 x 50 ml) and dried for 12 h at 35°C under reduced pressure yielding 13.6 g of *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingosine contaminated with 13% of the unprotected *N*-palmitoyl-*D*-erythro-sphingosine. The crystallized material was further purified by column chromatography on 185 g silica gel. It was eluted with 2.2 L hexane/ethyl acetate 5/1, 2.2 L hexane/ethyl acetate 3/1 and finally  
 20 4.4 L hexane/ethyl acetate 2/1. The product containing fractions were combined and evaporated to dryness at 40°C resulting in 12.1 g (yield 62%) of *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingosine. Purity by HPLC was 97.7% and by HPTLC 99.6%.

[00251] <sup>1</sup>H NMR (δ ppm, CDCl<sub>3</sub>): 0.87 (6H, t), 1.1–1.3 (46H, m), 1.54 (2H, m), 1.96 (2H, m), 2.14 (2H, m), 2.77 (2H, br s), 3.71 (2H, m, CH<sub>2</sub>O), 4.24 (1H, m, CHN), 5.4–5.6 (2H, m, CH(OCOPh)CH=), 5.79 (1H, dt, J 15.0, 6.8 Hz, CH<sub>2</sub>CH=), 6.18 (1H, d, J 9.6 Hz, NH), 7.38  
 25 (2H, dd, J 7.6, 7.2 Hz), 7.52 (1H, dd, J 7.6, 7.6 Hz), 7.96 (1H, d, J 7.2 Hz)

**Step 3: *N*-Palmitoyl-3-*O*-benzoyl-*D*-erythro-sphingomyelin**



Step 3.1: *N*-Palmitoyl-3-*O*-benzoyl-*D*-erythro-1-*O*-(2-oxo-1,3,2-dioxaphospholan)-sphingosine

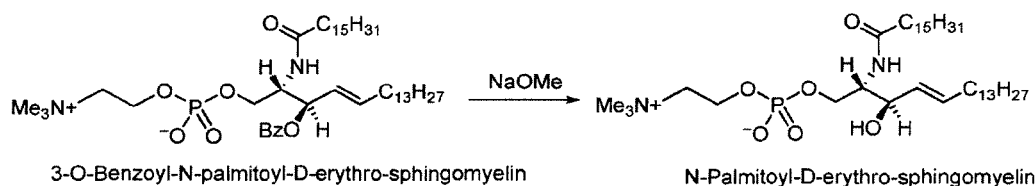
- 5 [00252] *N*-palmitoyl-3-*O*-benzoyl-*D*-erythro-sphingosine (9.49 g, 14.8 mmol) was nearly completely dissolved in toluene (200 ml) and charged into a pressure reactor. Tetramethylethylenediamine (2.4 ml, 15.8 mmol) was added to the mixture. The mixture was cooled to 7°C followed by the addition of a solution of 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) ( 3.90 g, 27.3 mmol) in acetonitrile (5 ml). After two hours at 7°C
- 10 the reaction was warmed to 21°C for two hours. TLC analysis indicated greater than 97 % conversion of *N*-palmitoyl-3-*O*-benzoyl-*D*-erythro-sphingosine to the intermediate *N*-palmitoyl-3-*O*-benzoyl-*D*-erythro-1-*O*-(2-oxo-1,3,2-dioxaphospholan)-sphingosine.

Step 3.2: *N*-palmitoyl-3-*O*-benzoyl-*D*-erythro-sphingomyelin

- [00253] Acetonitrile (200 ml) was introduced into the reactor followed by gaseous
- 15 trimethylamine until a constant pressure of 0.5 – 0.7 bar was reached. During heating to 67°C the pressure rose to 1.8 – 2.0 bar. After 14 hours TLC analysis indicated nearly complete reaction of the intermediate *N*-Palmitoyl-3-*O*-benzoyl-*D*-erythro-1-*O*-(2-oxo-1,3,2-dioxaphospholan)-sphingosine. After cooling down to –5°C the suspension was filtered. Crude 3-*O*-Benzoyl-*N*-palmitoyl-*D*-erythro-sphingomyelin was dried at 35°C, yielding 9.1 g
- 20 (76%). The crude material was further purified by column chromatography on 90 g silica gel. It was eluted with 1.1 L methylene chloride /methanol 6/1; 1.1 L methylene chloride /methanol 4/1; and finally 4.3 L methylene chloride /methanol 3/1. The product-containing

fractions were combined and evaporated to dryness at 35°C resulting in 8.0 g (yield 88%) of 3-O-Benzoyl-*N*-palmitoyl-*D*-erythro-sphingomyelin. Purity by HPLC was 94.8%.

**Step 4: *N*-Palmitoyl-*D*-erythro-sphingomyelin**



5 [00254] 3-O-Benzoyl-*N*-palmitoyl-*D*-erythro-sphingomyelin (8.0 g, 9.9 mmol) was dissolved in methanol (40 ml). Sodium methoxide (30% in methanol, 5.4 M, 367  $\mu$ l, 2.0 mmol) was added to the solution to result in pH 11. After stirring overnight, TLC analysis indicated nearly complete reaction of the benzoylester. Methylene chloride (80 ml) was added followed by 38 ml water and the pH was adjusted to 6-7 with 1.8 ml 1M hydrochloric acid. The lower layer was evaporated to dryness at 35°C and the residue was taken up in 8.5 ml methanol and 8.5 ml methylene chloride. Acetone (95mL) was added to the clear solution. The suspension was stirred at 0°C a few hours and filtered. The resulting residue was dried at 30°C, yielding 4.3 g of the title product, *N*-Palmitoyl-*D*-erythro-sphingomyelin, (62%). Purity by HPLC was 98.9%. Based on NMR analysis the content of the *cis* isomer and the *L*-*threo* isomer was lower than 1% respectively.

[00255]  $^1\text{H}$  NMR ( $\delta$  ppm,  $\text{CDCl}_3/\text{CD}_4\text{OD}$ ): 0.88 (6H, t, 2 x  $\text{CH}_3$ ), 1.25 (46H, m, 23 x  $\text{CH}_2$ ), 1.56 (2H, m, ( $\text{C}=\text{O}$ ) $\text{CH}_2\text{CH}_2$ ), 1.99 (2H, m,  $\text{CH}=\text{CHCH}_2$ ), 2.14 (2H, t, ( $\text{C}=\text{O}$ ) $\text{CH}_2$ ), 3.24 (9H, br s,  $\text{N}(\text{CH}_3)_3$ ), 3.68 (2H, m,  $\text{POCH}_2\text{CH}_2\text{N}$ ), 3.91 (2H, m,  $\text{POCH}_2\text{CH}$ ), 4.04 (1H, t,  $\text{CHO}$ , J 7.7 Hz), 4.14 (1H, m,  $\text{CHN}$ ), 4.28 (2H, m,  $\text{POCH}_2\text{CH}_2\text{N}$ ), 5.42–5.46 (2H, dd,  $\text{CHCH}=\text{CHCH}_2$ , J 15.3, 7.4 Hz), 5.65–5.70 (1H, dt,  $\text{CHCH}=\text{CHCH}_2$ , J 14.6, 7.2 Hz)

**Example 3 : Pilot-Scale Synthesis of Palmitoyl Sphingomyelin**

**Step 1: *Boc-L-Ser-OMe***

[00256] 32.2 kg *L*-Ser-OMe•HCl (206.97 mol) was suspended in 288 kg ethyl acetate in a 630 L vessel and cooled to 2°C. Liquid triethylamine at about 2°C (24.1 kg, 238.17 mol, 1.15 eq.) was added followed by a solution of  $\text{Boc}_2\text{O}$  (51.9 kg, 237.80 mol, 1.15 eq.) in 24 kg ethyl acetate. The reaction mixture was warmed to 22°C and stirred overnight. TLC analysis showed a content of *L*-Ser-OMe•HCl of less than 1%. 114 L purified water was added and the phases were separated. Washing was repeated twice with 114 L purified water. The three

aqueous phases were combined and extracted with 102 kg ethyl acetate. TLC analysis indicated absence of product in the aqueous phase. The two organic phases were combined and evaporated to dryness at 60°C. 88 kg toluene was added to the residue and distilled off at 60°C. This procedure was repeated. Purity of crude Boc-L-Ser-OMe was 95-97% by TLC analysis.

**Step 2: (S)-3-(Tert-butoxycarbonyl)-2,2-dimethyl-4-oxazolidinocarboxylic acid methylester**

[00257] Crude Boc-L-Ser-OMe (about 45.4 kg, 206.97 mol) was dissolved in 256 kg tetrahydrofuran at 22°C in a 630 L vessel. 71.8 kg 2,2-dimethoxypropane (689.39 mol, 3.33 eq.) were added followed by a solution of 3.3 kg benzenesulfonic acid (20.86 mol, 0.10 eq.) in 20 kg tetrahydrofuran and washing with 20 kg tetrahydrofuran. The reaction mixture was heated to reflux, 210 L tetrahydrofuran were distilled off in three hours. TLC analysis showed a content of Boc-L-Ser-OMe of 1-2%. Neutralization to pH 6.5 was performed with 1.0 kg triethylamine (9.88 mol, 0.05 eq) at 22°C. The reaction mixture was evaporated to dryness at 60°C, followed by addition of 82 kg hexane and 26 L purified water at 25°C. The organic phase was washed with 45 L purified water. TLC analysis indicated absence of product in the aqueous phases. The organic phase was evaporated to dryness at 60°C. Toluene (88 kg) was added to the residue and distilled off at 60°C twice. The product, (S)-3-(tert-butoxycarbonyl)-2,2-dimethyl-4-oxazolidinocarboxylic acid methylester, (48.89 kg, 188.55 mol, 91% yield from L-Ser-OMe•HCl) was isolated as a yellow liquid. Purity was found to be 97% by TLC analysis. Loss on drying was 4.2% and the water content 0.1%. Identity was confirmed by MS and <sup>1</sup>H NMR.

**Step 3: (S)-3-(tert-butoxycarbonyl)-4-(2-(dimethoxyphosphoryl)-1-oxo-ethyl)-2,2-dimethyloxazolidine**

[00258] A 100 L vessel was conditioned with 15 kg tetrahydrofuran and dried under vacuum at 50°C. Dimethyl methylphosphonate (4.6 kg, 37.07 mol, 2.00 eq) was introduced into the vessel and dissolved in 29 kg tetrahydrofuran. The mixture was cooled down to -75°C and 9.4 kg of a solution of 25% n-butyllithium in heptane (2.34 kg n-butyllithium, 36.58 mol, 1.98 eq) was added over two hours while the mixture was kept at -70 to -75°C, followed by washing with 5 L heptane. After stirring for one hour a solution of 4.8 kg crude (S)-3-(tert-butoxycarbonyl)-2,2-dimethyl-4-oxazolidinocarboxylic acid methylester (18.51 mol) in 4 kg tetrahydrofuran was added over one hour while the mixture was kept at -70 to -75°C,

followed by washing with 5 L tetrahydrofuran. The reaction mixture was warmed to 0°C over 40 minutes and stirred for 30 minutes.

[00259] TLC analysis showed a content of (S)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-oxazolidincarboxylic acid methylester of 10-15%. Quenching was performed with a solution of 600 mL purified water in 4.8 kg tetrahydrofuran below 20°C. The pH was adjusted to 6-7 with 13 L of a solution of 20% citric acid monohydrate in purified water below 20°C. After addition of 10 L ethyl acetate, the phases were separated. The aqueous phase was extracted with 13 kg ethyl acetate. TLC analysis indicated absence of product in the aqueous phase. The two organic phases were combined and evaporated to a volume of 20 L at 60°C. Final drying in a rotary evaporator at 40°C yielded 7.2 kg (20.49 mol, 111%) of the title compound, (S)-3-(*tert*-butoxycarbonyl)-4-(2-(dimethoxyphosphoryl)-1-oxo-ethyl)-2,2-dimethyloxazolidine, as a yellow oil. Purity was approximately 70% by TLC analysis. Loss on drying was 14.4% and the water content 1.4%. Identity was confirmed by MS.

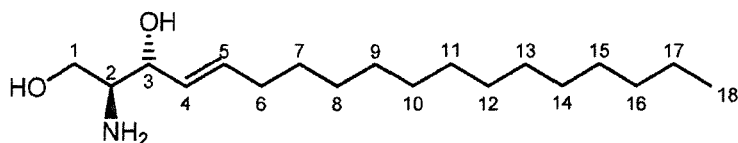
***Step 4: (S)-3-(tert-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidine***

[00260] (S)-3-(*tert*-butoxycarbonyl)-4-(2-(dimethoxyphosphoryl)-1-oxo-ethyl)-2,2-dimethyloxazolidin (21.6 kg, 61.48 mol) and 17.0 kg potassium carbonate (123.0 mol, 2.00 eq.) in added to 239 kg acetonitrile at 22°C in a 250 L vessel while stirring. 1-Tetradecanal (6.53 kg, 30.75 mol, 0.50 eq) and 3.1 L purified water were added to give a pH of 9.0. After the reaction was allowed to proceed over night, TLC analysis showed a content of 1-tetradecanal of 5-10% and phosphonate of 2%. The salts were filtered off and washed with 270 L hexane in portions. The combined organic phases were evaporated to dryness at 60°C. The residue was dissolved in 48 kg hexane and washed twice with a solution of 0.9 kg sodium chloride in 18 L purified water. TLC analysis indicated absence of product in the aqueous phases. The organic phase was evaporated to dryness at 60°C and dissolved in 75 kg hexane. Final drying in a rotary evaporator at 40°C yielded 16.9 kg (38.61 mol, 63% based on Phosphonate) of the title compound, (S)-3-(*tert*-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidin, as a brown oil. Purity was approximately 50-86% by TLC depending on detection method and 81% by HPLC analysis. Loss on drying was 2.2% and the water content 0.05%. Identity was confirmed by MS.

**Step 5: (2*S*,3*R*,4*E*)-3-(*tert*-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidine**

[00261] Crude (S)-3-(*tert*-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidin (16.9 kg, 38.61 mol) and 15.8 kg cerium chloride heptahydrate (42.49 mol, 1.10 eq.) were stirred in 305 kg methanol in a 1000 L vessel and cooled to  $-18^{\circ}\text{C}$ . A solution of 2.19 kg sodium borohydride (57.89 mol, 1.50 eq.) and 58 g 30% caustic soda (0.44 mol) in 8.8 L purified water (resulting in a 0.2% caustic soda) was cooled to  $0^{\circ}\text{C}$  and then added to the ketone over five hours. After additional 30 minutes of stirring, TLC analysis showed a content of ketone of less than 1%. Excess sodium borohydride was deactivated by warming the reaction mixture to  $22^{\circ}\text{C}$  over two hours, followed by stirring for one hour. Methanol (320 L) was distilled off at  $60^{\circ}\text{C}$ . The precipitated salts were filtered and washed with 44 kg toluene in two portions. The filtrate separated into two phases and the aqueous phase was extracted twice with 33 kg toluene. TLC analysis indicated absence of product in the aqueous phase and the filter residue. The combined organic phases were diluted with 77 L ethyl acetate and washed with a mixture of 39 L purified water, 3.9 kg EDTA, and 1.9 L 30% caustic soda followed by 1.9 kg sodium chloride in 39 L purified water. TLC analysis indicated absence of product in the aqueous phases. The organic phase was evaporated to dryness at  $60^{\circ}\text{C}$  and dissolved in 18 kg toluene. Final drying in a rotary evaporator at  $60^{\circ}\text{C}$  yielded 15.95 kg (36.28 mol, 94%) of the title compound, (2*S*,3*R*,4*E*)-3-(*tert*-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidin, as a yellow oil. Purity was approximately 70-90% by TLC depending on detection method and 90% by HPLC analysis. Loss on drying was 5.2% and the water content 0.05%. Identity was confirmed by MS.

**Step 6: D-Erythro-sphingosine**

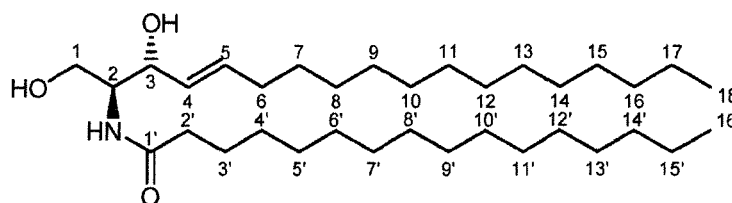


D-erythro-sphingosine

[00262] In a 60 L vessel 24 kg methanol was cooled to  $0^{\circ}\text{C}$ . Over the course of 30 minutes 5.69 kg acetylchloride (72.48 mol, 2.00 eq.) was introduced. This was followed by warming to  $22^{\circ}\text{C}$  to produce a methanolic hydrochloride solution. Crude (2*S*,3*R*,4*E*)-3-(*tert*-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidin (15.95 kg, 36.28 mol)

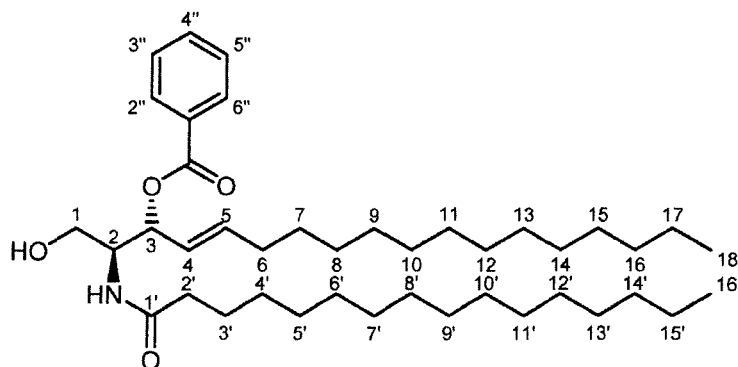
was dissolved in 31 kg methanol in a 160 L vessel at 22°C. The methanolic hydrochloride solution was added over 30 minutes. After seven hours, TLC analysis showed a less than 1% of the starting material. The reaction was neutralized with a solution of 7.34 kg triethylamine (72.54 mol, 2.00 eq.) in 10 kg methanol. The reaction mixture was evaporated to dryness at 5 60°C and dissolved in 105 kg methylene chloride, 31 kg 2-propanol, and 40 L purified water. After phase separation the organic phase was washed with 40 L purified water and subsequently with 40 L demineralized water and 7 kg 2-propanol. TLC analysis indicated absence of product in the two first aqueous phases but product in the third aqueous phase. The product was extracted with 40 L methylene chloride. The combined organic phases were 10 evaporated to dryness at 60°C. The residue was suspended in 33 kg ethyl acetate and again evaporated to dryness. Crystallization was performed from a mixture of 36 L ethyl acetate and 7.2 L hexane at -20°C. The resultant solid was filtered and washed in portions with a mixture of 7.2 L ethyl acetate and 1.4 L hexane followed by 7.2 L pure ethyl acetate. After drying at 30°C the resulting 6.45 kg were recrystallized from a mixture of 24 L ethyl acetate 15 and 8 L hexane at -20°C. The solid was filtered and washed in portions with a mixture of 4.8 L ethyl acetate and 1.6 L hexane followed by 6.4 L pure ethyl acetate. After drying at 30°C the resulting 5.90 kg were again recrystallized from a mixture of 16 L ethyl acetate and 16 L hexane at -20°C. The solid was filtered and washed in portions with a mixture of 3.2 L ethyl acetate and 3.2 L hexane followed by 6.4 L pure ethyl acetate. Final drying at 30°C yielded 20 5.60 kg (18.71 mol, 52%) of the title compound, D-erythro-sphingosine, as a brown solid. Purity was 89.2% by HPTLC and 95.6% by HPLC analysis with 0.61% L-threo-sphingosine. Loss on drying was 0.4% and the water content 0.6%. Identity was confirmed by MS and 1H NMR.

[00263] <sup>1</sup>H NMR (600 MHz, δ ppm, CDCl<sub>3</sub>), position: 0.88 (3H, t) 18, 1.2–1.3 (20H, m) 8, 9, 25 10, 11, 12, 13, 14, 15, 16, 17; 1.37 (2H, m) 7; 2.04 (2H, m, CH<sub>2</sub>CH) 6; 3.17 (1H, m, CHNH<sub>2</sub>) 2; 3.77 (2H, m, CH<sub>2</sub>OH) 1; 4.34 (1H, m, CH(OH)CH) 3; 5.46 (1H, ddt, J 15.4, 6.4, 1.0 Hz, CH(OH)CH) 4; 5.79 (1H, dtd, J 15.4, 6.7, 1.1 Hz, CH<sub>2</sub>CH) 5.

**Step 7: N-Palmitoyl-D-erythro-sphingosine***N*-palmitoyl-*D*-erythro-sphingosine

[00264] 5.50 kg *D*-erythro-sphingosine (18.36 mol), 4.71 kg palmitic acid (18.37 mol, 1.00 eq.) and 7.66 kg O-Benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate (20.20 mol, 1.10 eq.) were suspended in 36 kg dimethylformamide and 118 kg tetrahydrofuran in a 250 L vessel and cooled to 2°C. Triethylamine (5.06 kg, 50.00 mol, 2.72 eq.) in 5 L tetrahydrofuran was added resulting in pH 9.0. After 90 minutes TLC analysis showed a content of *D*-erythro-sphingosine of less than 1% and a content of palmitic acid of less than 1.5%. The reaction mixture was warmed to 22°C. The product was precipitated by addition of a solution of 4.7 kg citric acid in 89 kg purified water. After one hour at 22°C the reaction mixture was filtered. The crude product was suspended in 154 L purified water for one hour at 22°C. Filtration was followed by washing with three times 28 L purified water and three times 28 L acetone. Suspension was repeated in 122 kg acetone, washing with three times 28 L acetone. Final drying at 35°C yielded 6.08 kg (11.31 mol, 62%) of the title compound, *N*-palmitoyl-*D*-erythro-sphingosine, as a slightly yellow solid. Purity was 96.2% by HPTLC and 99.2% by HPLC analysis. Loss on drying was 0.2% and the water content 0.3%. Identity was confirmed by MS and <sup>1</sup>H NMR.

[00265] <sup>1</sup>H NMR (600 MHz, δ ppm, CDCl<sub>3</sub>): 0.88 (6H, t) 18, 16'; 1.2–1.4 (44H, m) 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 4', 5', 6', 7', 8', 9', 10', 11', 12', 13', 14', 15'; 1.37 (2H, m) 7; 1.64 (2H, m) 3'; 2.04 (2H, m, CH<sub>2</sub>CH) 6; 2.23 (2H, t, CH<sub>2</sub>CO) 2'; 3.71 (1H, dd, CHNH<sub>2</sub>) 2; 3.93 (2H, m, CH<sub>2</sub>OH) 1; 4.31 (1H, m, CH(OH)CH) 3; 5.53 (1H, ddt, J 15.4, 6.4, 1.0 Hz, CH(OH)CH) 4; 5.79 (1H, dtd, J 15.4, 6.7, 1.1 Hz, CH<sub>2</sub>CH) 5; 6.25 (1H, d) NH.

**Step 8: N-Palmitoyl-3-O-benzoyl-D-erythro-sphingosine***N*-Palmitoyl-3-O-Benzoyl-*D*-erythro-sphingosine

[00266] For the first protection, 6.08 kg of *N*-palmitoyl-*D*-erythro-sphingosine (11.31 mol) was suspended in 11.91 kg pyridine and 2.5 kg methylene chloride in a 60 L vessel. A

- 5 solution of trityl chloride (3.31 kg, 11.87 mol, 1.05 eq.) in 9.5 kg methylene chloride was added followed by 2.5 kg methylene chloride. The reaction mixture was stirred at 25°C for 56 hours. TLC analysis showed a content of *N*-palmitoyl-*D*-erythro-sphingosine of 3-5%.

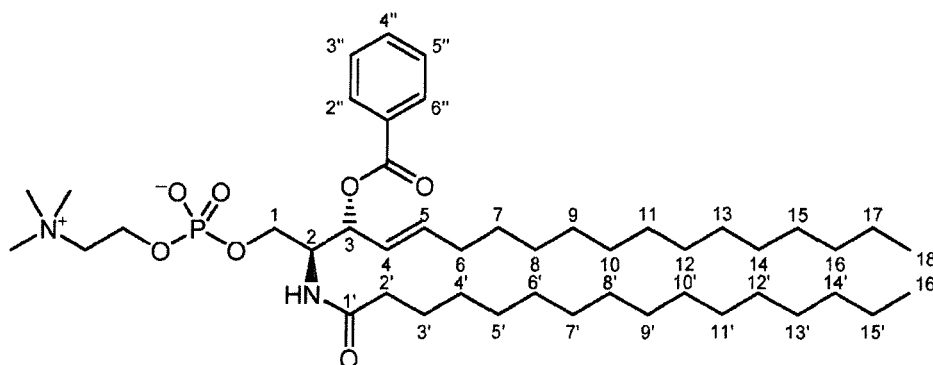
[00267] For the second step the reaction mixture was cooled to 2°C. *N,N*-

- 10 Dimethylaminopyridine (0.139 kg, 1.14 mol, 0.10 eq.) and 2.38 kg benzoyl chloride (16.93 mol, 1.50 eq.) was added to the mixture, followed by 5 kg methylene chloride. After 90 minutes at 2°C TLC analysis showed a content of intermediate *N*-palmitoyl-1-O-Trityl *D*-erythro-sphingosine of less than 1%. Work up was performed with 55 kg ethyl acetate and a solution of 1.7 kg citric acid and 3.0 kg sodium chloride in 33 L purified water. The organic phase was washed again with a solution of 1.7 kg citric acid and 3.0 kg sodium chloride in 33
- 15 L purified water and twice with a solution of 3.5 kg sodium chloride in 30 L purified water. TLC analysis indicated absence of product in the aqueous phases. The organic phase was evaporated to dryness at 50°C. The residue which contained the product, *N*-palmitoyl-1-O-Trityl-3-O-benzoyl-*D*-erythro-sphingosine, was dissolved in 27 kg toluene and subsequently evaporated to dryness at 50°C. This procedure was repeated twice.

- 20 [00268] For the third step the residue from the previous reaction was dissolved in 67 kg methanol and 161 kg methylene chloride and cooled to 2°C. pH was adjusted to 2.5 with a solution of para-toluene sulfonic acid mono hydrate (6.41 mol 0.57 eq.) in 23 kg methanol. After warming to 22°C and stirring for 14 hours TLC analysis showed a content of intermediate *N*-palmitoyl-1-O-Trityl-3-O-benzoyl-*D*-erythro-sphingosine of less than 1%.

The addition of 969 g Triethylamine (9.58 mol, 0.85 eq.) raised the pH to 7.0. The reaction mixture was evaporated to dryness at 50°C. Crude *N*-palmitoyl-3-*O*-benzoyl-*D*-erythro-sphingosine was suspended in 69 kg hexane at 40°C and cooled down to 0°C. After 40 minutes the solid was isolated by filtration and washed with 20 kg hexane. Drying at 35°C yielded 5.40 kg which was dissolved in 38 kg methylene chloride. This solution was purified by chromatography on 76 kg silica gel which was conditioned with a mixture of 175 kg hexane and 49 kg ethyl acetate. Elution with 12 kg methylene chloride, a mixture of 502 kg hexane and 137 kg ethyl acetate and a mixture of 482 kg hexane and 647 kg ethyl acetate was performed. The collected fractions contained no product. The product was eluted with a mixture of 451 kg hexane and 205 kg ethyl acetate and a mixture of 802 kg hexane and 547 kg ethyl acetate. Solvents were distilled off at 50°C. The resulting residue was suspended in 24 L hexane at 40°C and cooled down to 0°C. After 45 minutes the title product, *N*-palmitoyl-3-*O*-benzoyl-*D*-erythro-sphingosine, was isolated as a solid by filtration and washed with 4.8 L hexane in portions. Drying at 35°C yielded 3.15 kg (4.91 mol, 43%) of the title compound as a white solid. Purity was 100.0% by HPTLC and 96.3% by HPLC analysis. Loss on drying was 0.05% and the water content 0.2%. Identity was confirmed by MS and <sup>1</sup>H NMR.

[00269] <sup>1</sup>H NMR (600 MHz, δ ppm, CDCl<sub>3</sub>): 0.88 (6H, t) 18, 16'; 1.2–1.4 (44H, m) 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 4', 5', 6', 7', 8', 9', 10', 11', 12', 13', 14', 15'; 1.35 (2H, m) 7; 1.61 (2H, m) 3'; 2.05 (2H, m, CH<sub>2</sub>CH) 6; 2.19 (2H, m, CH<sub>2</sub>CO) 2'; 3.71 (2H, m, CH<sub>2</sub>OH) 1; 4.27 (1H, m, CHNH) 2; 5.54 (1H, t, CH(OCOPh)CH)) 3; 5.62 (1H, ddt, J 15.4, 6.4, 1.0 Hz, CH(OCOPh)CH)) 4; 5.85 (1H, dtd, J 15.4, 6.7, 1.1 Hz, CH<sub>2</sub>CH) 5; 6.05 (1H, d) NH; 7.46 (2H, dd, J 7.6, 7.2 Hz) 3'', 5''; 7.59 (1H, dd, J 7.6, 7.6 Hz) 4''; 8.04 (2H, d, J 7.2 Hz) 2'', 6''.

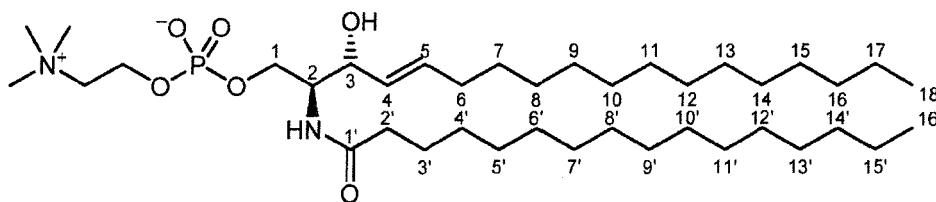
**Step 9: N-Palmitoyl-3-O-benzoyl-D-erythro-sphingomyelin***N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingomyelin

[00270] 1.60 kg *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingosine (2.50 mol) and 0.20 kg tetramethylethylenediamine (TMEDA) (1.73 mol, 0.69 eq.) were dissolved in 38 L toluene in a 100 L vessel at 35°C. After cooling to 6°C a solution of 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) (0.47 kg, 3.30 mol, 1.32 eq.) in 1 L acetonitrile was added during 15 minutes followed by 3 L acetonitrile. The reaction mixture was warmed to 22°C. Stirring was continued for two hours. TLC analysis showed a content of *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingosine of less than 0.5%. After addition of 32 L acetonitrile temperature was decreased to –10°C. Gaseous trimethylamine was cooled to below its boiling point, and the resulting liquid trimethylamine (7.42 kg, 125.53 mol, 50.21 eq.) was introduced. The next reaction step was started by heating to 65°C for 15 hours. TLC analysis showed a content intermediate ring of less than 0.5%. Product was crystallized by cooling to –30°C and isolated by filtration with subsequent washing with 13 L acetonitrile. By drying at 35°C yielded 1.85 kg of an off-white solid. The reaction was repeated with 1.58 kg *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingosine (2.45 mol) yielding another 1.82 kg crude *N*-palmitoyl-3-O-benzoyl-*D*-erythro-sphingomyelin. Both crude materials were combined and dissolved in 29 L methylene chloride and 14.5 L methanol. This solution was purified by chromatography on 72 kg silica gel which was conditioned with a mixture of 337 kg methylene chloride and 33 kg methanol. Elution with a mixture of 966 kg methylene chloride and 95 kg methanol, a mixture of 1866 kg methylene chloride and 223 kg methanol, a mixture of 328 kg methylene chloride and 82 kg methanol, a mixture of 1345 kg methylene chloride and 268 kg methanol, a mixture of 530 kg methylene chloride and 158 kg methanol and a mixture of 371 kg methylene chloride and 221 kg methanol was performed. The volume of the collected fractions was 140 L. Solvents of fractions 17-38 were distilled off at 50°C. Final drying in a

rotary evaporator at 40°C yielded 3.36 kg (2.92 kg on dry basis, 3.61 mol, 73%) of the title compound, *N*-palmitoyl-3-*O*-benzoyl-*D*-erythro-sphingomyelin, as a slightly yellow solid. Purity was 99.5% by HPTLC and 98.7% by HPLC analysis. Loss on drying was 11.5% and the water content 1.7%. Identity was confirmed by MS and <sup>1</sup>H NMR.

- 5 [00271] <sup>1</sup>H NMR (600 MHz, δ ppm, CDCl<sub>3</sub>): 0.88 (6H, t) 18, 16'; 1.2–1.3 (46H, m) 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 4', 5', 6', 7', 8', 9', 10', 11', 12', 13', 14', 15'; 1.56 (2H, m) 3'; 1.99 (2H, m, CH<sub>2</sub>CH) 6; 2.16 (2H, m, CH<sub>2</sub>CO) 2'; 3.21 (9H, s, N(CH<sub>3</sub>)<sub>3</sub>) N(CH<sub>3</sub>)<sub>3</sub>; 3.65 (2H, m, POCH<sub>2</sub>CH<sub>2</sub>N(CH<sub>3</sub>)<sub>3</sub>) CH<sub>2</sub>N; 3.97 (2H, m, CH<sub>2</sub>OP) 1; 4.21 (2H, br s, POCH<sub>2</sub>CH<sub>2</sub>N(CH<sub>3</sub>)<sub>3</sub>) POCH<sub>2</sub>; 4.45 (1H, m, CHNH) 2; 5.50 (1H, m, CH(OCOPh)CH) 3; 5.54 (1H, m, CH(OCOPh)CH) 4; 5.82 (1H, dt, CH<sub>2</sub>CH) 5; 7.39 (1H, d) NH; 7.43 (2H, t, J 7.2 Hz) 3'', 5''; 7.55 (1H, t, J 7.2 Hz) 4''; 7.99 (2H, d, J 7.2 Hz) 2'', 6''.
- 10

**Step 10: *N*-Palmitoyl-*D*-erythro-sphingomyelin**



*N*-Palmitoyl-*D*-erythro-sphingomyelin

- [00272] 3.36 kg *N*-Palmitoyl-3-*O*-benzoyl-*D*-erythro-sphingomyelin (2.92 kg on dry basis, 3.61 mol) was dissolved in 10 L methanol in a rotary evaporator at 22°C and transferred into a 70 L vessel with 5 L methanol. 138 mL of a solution of sodium methoxide in methanol (30%, 0.75 mol, 0.21 eq.) was used to adjust the pH to 11.5. Stirring was continued for 23 hours at 22°C. TLC analysis showed a content of *N*-palmitoyl-3-*O*-benzoyl-*D*-erythro-sphingomyelin of less than 0.5%. Phase separation occurred after introduction of 31 L methylene chloride and 13 L purified water. The organic phase was neutralized to pH 7.0 with 8 L methanol, 8 L purified water and 55 mL 1M hydrochloric acid. TLC analysis indicated absence of product in the aqueous phases. The organic phase was evaporated to dryness at 35°C. The residue was co-evaporated twice with 6 L 2-propanol and twice with 12 L methylene chloride. Crude product was dissolved in 2.6 L methanol and 2.6 L methylene chloride and filtered through a 0.2 μm filter with washing with 1.2 L methanol and 1.2 L methylene chloride. Crystallization was induced by addition of 42 L acetone and cooling to 0°C. After 15 hours the precipitate was isolated and washed with 24 L acetone in four portions. The wet product was suspended in 19 L acetone at 22°C for 2.5 hours. After isolation and washing with 12 L acetone in four
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- 20
- 25

portions process was finalized by drying at 30°C for 46 hours. 2.29 kg (3.25 mol, 90%) of *N*-palmitoyl-*D*-erythro-sphingomyelin as a white powder was obtained. Purity was 99.2% by HPTLC and 99.0% by HPLC analysis. Water content was 0.7%. Identity was confirmed by MS and <sup>1</sup>H NMR.

- 5 [00273] <sup>1</sup>H NMR (600 MHz, δ ppm, CDCl<sub>3</sub>): 0.88 (6H, t) 18, 16'; 1.2–1.3 (46H, m) 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 4', 5', 6', 7', 8', 9', 10', 11', 12', 13', 14', 15'; 1.57 (2H, m) 3'; 1.99 (2H, m, CH<sub>2</sub>CH) 6; 2.15 (2H, m, CH<sub>2</sub>CO) 2'; 3.23 (9H, s, N(CH<sub>3</sub>)<sub>3</sub>) N(CH<sub>3</sub>)<sub>3</sub>; 3.65 (2H, m, POCH<sub>2</sub>CH<sub>2</sub>N(CH<sub>3</sub>)<sub>3</sub>) CH<sub>2</sub>N; 3.91 (2H, m, CH<sub>2</sub>OP) 1; 4.05 (1H, t, J 7.7 Hz, CH(OH)CH) 3; 4.15 (1H, m, CHNH) 2; 4.26 (2H, m, POCH<sub>2</sub>CH<sub>2</sub>N(CH<sub>3</sub>)<sub>3</sub>) POCH<sub>2</sub>; 5.45 (1H, dd, J 15.3 Hz, 7.4 Hz, CH(OH)CH) 4; 5.69 (1H, dt, J 14.6 Hz, 7.2 Hz, CH<sub>2</sub>CH) 5.

[00274] Table 1 shows yields and /intermediate/product characteristics at a 120-g scale (Example 2). Table 2 displays the results for a 2-kg scale (Example 3).

**Table 1**

| Reaction Starting Material                                       | Reaction Product                                                 | Yield | HPLC  | HPTLC | LoD  | KF   |
|------------------------------------------------------------------|------------------------------------------------------------------|-------|-------|-------|------|------|
| <i>D</i> -erythro-sphingosine                                    | <i>N</i> -palmitoyl- <i>D</i> -erythro-sphingosine               | 91.6% | 98.4% | 99.0% | 0.1% | 0.2% |
| <i>N</i> -palmitoyl- <i>D</i> -erythro-sphingosine               | <i>N</i> -palmitoyl-3-O-benzoyl- <i>D</i> -erythro-sphingosine   | 57.9% | 97.1% | -     | 0.2% | 0.2% |
| <i>N</i> -palmitoyl-3-O-benzoyl- <i>D</i> -erythro-sphingosine   | <i>N</i> -palmitoyl-3-O-benzoyl- <i>D</i> -erythro-sphingomyelin | 14.2% | 99.0% | 98.8% | 3.2% | 2.8% |
| <i>N</i> -palmitoyl-3-O-benzoyl- <i>D</i> -erythro-sphingomyelin | <i>N</i> -palmitoyl- <i>D</i> -erythro-sphingomyelin             | 68.5% | 98.6% | 99.4% | -    | 4.4% |

LoD = loss on drying, KF = water content

15 **Table 2**

| Reaction Starting Material                                       | Reaction Product                                                 | Yield | HPLC  | HPTLC  | LoD       | KF   |
|------------------------------------------------------------------|------------------------------------------------------------------|-------|-------|--------|-----------|------|
| <i>D</i> -erythro-sphingosine                                    | <i>N</i> -palmitoyl- <i>D</i> -erythro-sphingosine               | 61.6% | 99.2% | 96.2%  | 0.2%      | 0.3% |
| <i>N</i> -palmitoyl- <i>D</i> -erythro-sphingosine               | <i>N</i> -palmitoyl-3-O-benzoyl- <i>D</i> -erythro-sphingosine   | 43.4% | 96.3% | 100.0% | 0.05%     | 0.2% |
| <i>N</i> -palmitoyl-3-O-benzoyl- <i>D</i> -erythro-sphingosine   | <i>N</i> -palmitoyl-3-O-benzoyl- <i>D</i> -erythro-sphingomyelin | 72.7% | 99.0% | 99.5%  | 11.5%     | 1.7% |
| <i>N</i> -palmitoyl-3-O-benzoyl- <i>D</i> -erythro-sphingomyelin | <i>N</i> -palmitoyl- <i>D</i> -erythro-sphingomyelin             | 90.1% | 99.0% | 99.2%  | < 250 ppm | 0.7% |

LoD = loss on drying, KF = water content

**Example 4: Confirmation of optical purity, identity with natural product egg sphingomyelin and absolute configuration**

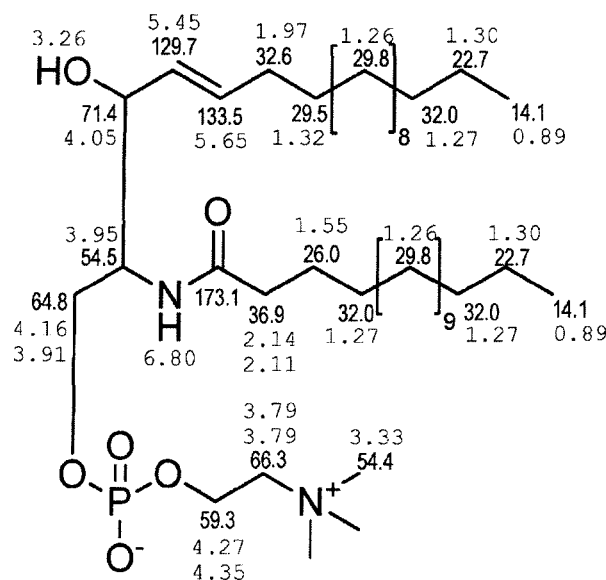
[00275] The NMR spectra were obtained using a Varian Inova spectrometer, operating at 500 MHz for  $^1\text{H}$  and 125 MHz for  $^{13}\text{C}$ , equipped with a 5 mm triple resonance probe and  $z$ -axis gradients. The solvent was chloroform- $d$  and the temperature 25 °C. The chemical shifts for  $^1\text{H}$  and  $^{13}\text{C}$  were reference to the residual solvent signal, 7.27 ppm for  $^1\text{H}$  and 77 ppm for  $^{13}\text{C}$ , on the tetramethylsilane scale.

[00276] The proton spectrum was taken in 4 transients, with a 90 ° pulse, on a spectral window from 18 to -1 ppm. The acquisition time was 5 s and the relaxation delay 5 s. 94842 points in the FID were transformed into 131072 points in the spectrum, with no apodization.

[00277] The  $^1\text{H}$ - $^{13}\text{C}$  gHMBCAD spectrum was acquired with the standard Varian pulse sequence, using an adiabatic pulse on  $^{13}\text{C}$ , and it was optimized for a coupling constant of 8 Hz. In the proton dimension 4096 points were acquired over a spectral window of 3755 Hz, from 1.24 to 8.74 ppm and transformed into the same number of points in the spectrum, weighting with a shifted Gaussian function (gf=0.277, gfs=0.126). In the carbon dimension 2\*512 increments were taken in one transient each, over a spectral window from 10 to 190 ppm and transformed into 4096 points, using a shifted Gaussian function (gf=0.019, gfs=0.005). The relaxation delay was 1 s.

Chemical shifts assignment in *N*-palmitoyl-*D*-erythro-sphingomyelin.

[00278] The chemical shifts assignment was based on the  $^1\text{H}$ - $^1\text{H}$  couplings seen in the DQCOSY spectra and the  $^1\text{H}$ - $^{13}\text{C}$  couplings, one-bond and long-range seen in the  $^1\text{H}$ - $^{13}\text{C}$  gHSQC and gHMBC spectra. The assignments are presented in Scheme XV.



Scheme XV. Assignment of the  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts in *N*-Palmitoyl-*D*-erythro-sphingomyelin, in chloroform-*d* at 25 °C.

[00279] The assignment begun with the proton at 6.80, bound to no carbon, which has to be the  
 5 amide proton. The gDQCOSY spectrum revealed the sequence 6.80 – 3.95 – 4.05 – 5.45 – 5.65 – 1.97, of the sphingosine backbone. Of the three methylene groups seen in the gHSQC spectrum, one has a carbon at 66.3 which couples with the trimethylamino protons at 3.33. Its protons, at 3.79, couple with the protons at 4.27 and 4.53. One of the protons of the remaining methylene group, 4.16, displays indeed a coupling with 3.95. The amide carbon displays a  
 10 cross-peak with the protons at 2.14 and 2.11.

#### Comparison of the egg and synthetic sphingomyelin.

[00280] In order to maximize the accuracy of the integrals, proton spectra for the three samples were taken in 64 transients, with a 45 ° pulse, on a spectral window from 14 to -1 ppm. The acquisition time was 5 s and the relaxation delay 15 s. 79872 points in the FID were  
 15 transformed into 131072 points in the spectrum, with no apodization.

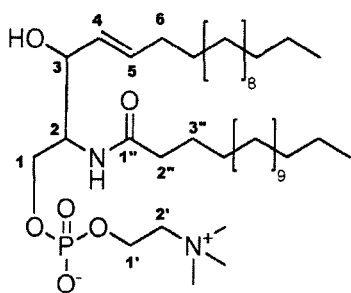
[00281] The  $^1\text{H}$  spectra for *N*-palmitoyl-*D*-erythro-sphingomyelins from egg and synthesized by methods of the present invention are presented in Figs. 1-3, correspondingly. The integral was referenced to the signal of the trimethylamonium group at 3.33 ppm (9H). The signal at 2.58 in Fig. 1 and 3.00 in Figs. 2 and 3 is water. The two synthetic samples are identical,

within the precision of the integral, about 1%. The natural sample has a shorter average alkyl chain, and some impurities are noticeable for the NH and the alkene signals.

[00282] The sphingosine backbone has two chiral carbons, hence the possibility of 4 stereoisomers. The synthetic samples do not show the doubling of the signals expected for a mixture of diastereomers. No such assessment can be made for the egg sphingomyelin, since it is a mixture. To confirm the enantiomeric purity, the samples of *N*-palmitoyl-*D*-erythro-sphingomyelins from egg and synthesized by methods of the invention were treated in tube with an excess of *R*-methoxyphenylacetic acid (*R*-MPA), dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP), and the region 5.20 – 5.95 ppm was examined and are presented in Figs. 4-6, correspondingly. The triplet at 5.42 (Fig. 5) is H3 (see caption to Table 3), the doublet of doublets at 5.49 is H4 and the doublet of triplets at 5.73 is H5. Fig. 4 shows the rise of a doublet at 5.53 as the reaction mixture matures, and this doublet is also visible in Fig. 6. Other these signals, it was determined that there are no signals above 5% of the signals of the ester, therefore the enantiomeric purity of the sample was determined to appear to be at least 95%, i.e., that the sample contains no more than about 5% of its corresponding opposite enantiomer. The absolute configuration of all three samples is the same, since the signals of H3-H5 in their *R*-MPA esters have the same chemical shifts.

#### Determination of the absolute configuration.

[00283] In order to verify the absolute configuration, one equivalent of palmitoyl sphingomyelin from egg was treated in the NMR tube with 1.2 equivalents of a racemic mixture of *R*-(-)- $\alpha$ -methoxyphenylacetic acid (*R*-MPA) and *S*-(+)-  $\alpha$ -methoxyphenylacetic acid (*S*-MPA), 1.2 equivalents of dicyclohexylcarbodiimide (DCC), and a catalytic amount of 4-dimethylaminopyridine (DMAP). Fig. 7 (bottom) shows the  $^1\text{H}$  NMR spectrum of this reaction mixture. Fig. 7 also shows the 1D-TOCSY (1 Dimensional-Total Correlation Spectroscopy) spectra showing selective excitation of the H2 hydrogen peak in the *R*-MPA (top) and *S*-MPA (middle) esters are in the top. The *DdRS* measured in the 1D-TOCSY spectra are given in Table 3.



**Table 3. DdRS in egg sphingomyelin.**

| position | 1a    | 1b    | 2     | 3    | 4    | 5    | 6    | NH   |
|----------|-------|-------|-------|------|------|------|------|------|
| alcohol  | 4.16  | 3.91  | 3.95  | 4.05 | 5.45 | 5.65 | 1.97 | 6.8  |
| R-MPA    | 3.85  | 3.45  | 4.21  | 5.39 | 5.47 | 5.73 | 1.97 | 7.54 |
| S-MPA    | 3.97  | 3.97  | 4.3   | 5.39 | 5.26 | 5.44 | 1.82 | 7.4  |
| DdRS     | -0.12 | -0.52 | -0.09 | 0    | 0.21 | 0.29 | 0.15 | 0.14 |

[00284] Positive DdRS for H4-H6 and negative DdRS for H1a, H1b and H2 indicate that the absolute configuration at C3 is *R*.

[00285] The present invention is not to be limited in scope by the specific embodiments disclosed in the examples which are intended as illustrations of a few aspects of the invention and any embodiments which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art and are intended to fall within the appended claims.

[00286] Each reference disclosed in this application is incorporated by reference herein in its entirety.

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d) allowing (*S*)-3-(*tert*-butoxycarbonyl)-4-(2-(dimethoxy-phosphoryl)-1-oxo-ethyl)-2,2-dimethyloxazolidine to react with 1-tetradecanal under conditions effective to yield (*S*)-3-(*tert*-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidine;

d) allowing (*S*)-3-(*tert*-butoxycarbonyl)-4-(2-(dimethoxy-phosphoryl)-1-oxo-ethyl)-2,2-dimethyloxazolidine to react with 1-tetradecanal under conditions effective to yield (*S*)-3-(*tert*-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidine;

e) allowing (*S*)-3-(*tert*-butoxycarbonyl)-4-(1-oxo-hexadec-2-enyl)-2,2-dimethyloxazolidine to react with sodium borohydride and cerium trichloride under conditions effective to yield (2*S*,3*R*,4*E*)-3-(*tert*-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidine; and

f) removing the *tert*-butoxycarbonyl (Boc) protecting group of (2*S*,3*R*,4*E*)-3-(*tert*-butoxycarbonyl)-4-(1-hydroxy-hexadec-2-enyl)-2,2-dimethyloxazolidine under conditions effective to yield *D*-*erythro*-sphingosine.

2. The method of claim 1, wherein R is a methyl group.
3. A method for synthesizing *N*-palmitoyl-*D*-erythro-sphingosine, comprising the steps of:
  - a) allowing (1*R*, 2*R*, 5*R*)-(+)-2-hydroxy-3-pinanone to react with ethylglycinate under conditions effective to yield (1*R*, 2*R*, 5*R*)-ethyl-((2-hydroxypinan-3-ylene)amino)acetate;
  - b) allowing (1*R*, 2*R*, 5*R*)-ethyl-((2-hydroxypinan-3-ylene)amino)acetate (Compound IIIb) to react with 2-(*E*)-hexadecen-1-al in the presence of chlorotitanium triisopropoxyde and triethylamine under conditions effective to yield one or both of (2*S*,3*R*,*E*)-ethyl 3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate and (2*S*,3*R*,*E*)-isopropyl 3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate;
  - c) allowing the one or both of (2*S*,3*R*,*E*)-ethyl 3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate and (2*S*,3*R*,*E*)-isopropyl 3-hydroxy-2-((*E*)-((1*S*,2*S*,5*S*)-2-hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-ylidene)amino)octadec-4-enoate to react with hydrochloric acid under conditions effective to yield one or both of (2*R*,3*R*,*E*)-ethyl 2-amino-3-hydroxyoctadec-4-enoate and (2*R*,3*R*,*E*)-propyl 2-amino-3-hydroxyoctadec-4-enoate;
  - d) allowing the one or both of (2*R*,3*R*,*E*)-ethyl 2-amino-3-hydroxyoctadec-4-enoate and (2*R*,3*R*,*E*)-propyl 2-amino-3-hydroxyoctadec-4-enoate to react with sodium borohydride under conditions effective to yield *D*-erythro-sphingosine; and
  - e) allowing *D*-erythro-sphingosine to react with palmitic acid under conditions effective to yield *N*-palmitoyl-*D*-erythro-sphingosine.
4. A method for synthesizing an *N*-acyl-*D*-erythro-sphingomyelin comprising the steps of:
  - a) allowing *D*-erythro-sphingosine to react with a fatty acid under conditions effective to yield a *D*-erythro-ceramide;

b) allowing *D-erythro*-ceramide to react with a tritylating reagent under conditions effective to yield 1-O-trityl-*D-erythro*-ceramide;

c) allowing 1-O-trityl-*D-erythro*-ceramide to react with a benzoylating reagent under conditions effective to yield 1-O-trityl-3-O-benzoyl-*D-erythro*-ceramide;

5 d) removing the trityl group of 1-O-trityl-3-O-benzoyl-*D-erythro*-ceramide to yield *D-erythro*-3-O-benzoyl-ceramide;

e) allowing 3-O-benzoyl-*D-erythro*-ceramide to react with with 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) under conditions effective to yield 3-O-benzoyl-*D-erythro*-1-O-(2-oxo-1,3,2-dioxaphospholane) ceramide;

10 f) allowing 3-O-benzoyl-*D-erythro*-1-O-(2-oxo-1,3,2-dioxaphospholane) ceramide to react with trimethylamine under conditions effective to yield the *N*-acyl-3-O-benzoyl-*D-erythro* -sphingomyelin; and

15 g) removing the benzoyl group of *N*-acyl-3-O-benzoyl-*D-erythro*-sphingomyelin under conditions effective to yield the *N*-acyl-*D-erythro*-sphingomyelin.

5. A method for synthesizing *D-erythro*-3-O-benzoyl-ceramide, comprising the steps of

a) allowing *D-erythro*-ceramide to react with a tritylating reagent under conditions effective to yield 1-O-trityl-*D-erythro*-ceramide;

20 b) allowing 1-O-trityl-*D-erythro*-ceramide to react with a benzoylating reagent under conditions effective to yield 1-O-trityl-3-O-benzoyl-*D-erythro*-ceramide; and

c) removing the trityl group of 1-O-trityl-3-O-benzoyl-*D-erythro*-ceramide under conditions effective to yield 3-O-benzoyl-*D-erythro*-ceramide.

6. The method of claim 4 wherein the fatty acid is palmitic acid.

7. The method of claim 5 wherein in the *D-erythro*-ceramide is *N*-palmitoyl-*D-erythro*-sphingosine.

25

8. The method of claim 4 wherein the trimethylamine is added as a liquid.

9. The method of claim 4 wherein step b yields less than about 5% of 1,3-O,O-ditrityl-*D-erythro*-ceramide.
10. The method of claim 9 wherein step a yields less than about 5% of 1,3-O,O-ditrityl-*D-erythro*-ceramide.
- 5 11. A method for synthesizing an *N*-acyl-*D-erythro*-sphingomyelin, comprising the steps of:
  - a) allowing 3-O-benzoyl-*D-erythro*-ceramide to react with 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) under conditions effective to yield 3-O-benzoyl-*D-erythro*-1-O-(2-oxo-1,3,2-dioxaphospholane)ceramide;
  - 10 b) allowing 3-O-benzoyl-*D-erythro*-1-O-(2-oxo-1,3,2-dioxaphospholane)ceramide to react with trimethylamine to yield the 3-O-benzoyl-*D-erythro*-ceramide; and
  - c) removing the benzoyl group of 3-O-benzoyl-*D-erythro*-ceramide under conditions effective to yield the *N*-acyl-*D-erythro*-sphingomyelin.
- 15 12. The method of claim 11 wherein in the *D-erythro*-3-O-benzoylceramide is *N*-palmitoyl-*D-erythro*-sphingosine.
13. The method of claim 11 wherein the trimethylamine is added as a liquid.
14. The method of claim 11 wherein the trimethylamine is cooled to below its boiling point prior to addition.
- 20 15. A method for synthesizing an *N*-acyl-*D-erythro*-dihydrosphingomyelin, comprising the steps of:
  - a) allowing a *D-erythro*-dihydrosphingosine to react with a fatty acid under conditions effective to yield a *D-erythro*-dihydroceramide;
  - b) allowing the *D-erythro*-dihydroceramide to react with a tritylating agent
  - 25 under conditions effective to yield a 1-O-trityl-*D-erythro*-dihydroceramide;

c) allowing the *D-erythro*-1-O-trityl-dihydroceramide to react with a benzoylating agent under conditions effective to yield a 1-O-trityl-3-O-benzoyl-*D-erythro*-dihydroceramide;

d) removing the trityl group of 1-O-trityl-3-O-benzoyl-*D-erythro*-dihydroceramide under conditions effective to yield a 3-O-benzoyl-*D-erythro*-ceramide;

e) allowing the 3-O-benzoyl-*D-erythro*-dihydroceramide to react with 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) under conditions effective to yield a 3-O-benzoyl-*D-erythro*-1-O-(2-oxo-1,3,2-dioxaphospholane) dihydroceramide;

f) allowing the 3-O-benzoyl-*D-erythro*-1-O-(2-oxo-1,3,2-dioxaphospholane) dihydroceramide to react with trimethylamine under conditions effective to yield an *N*-acyl-3-O-benzoyl-*D-erythro*-dihydrosphingomyelin; and

g) removing the benzoyl group of the *N*-acyl-3-O-benzoyl-*D-erythro*-sphingomyelin under conditions effective to yield the *N*-acyl-*D-erythro*-dihydrosphingomyelin.

16. A method for synthesizing *N*-palmitoyl-*D-erythro*-sphingomyelin comprising the steps of:

a) allowing *N*-palmitoyl-*D-erythro*-sphingosine to react with ethylene halophosphite under conditions effective to yield *N*-((2*S*,3*R*,*E*)-1-((1,3,2-dioxaphospholan-2-yl)oxy)-3-hydroxyoctadec-4-en-2-yl)palmitoylamide;

b) allowing *N*-((2*S*,3*R*,*E*)-1-((1,3,2-dioxaphospholan-2-yl)oxy)-3-hydroxyoctadec-4-en-2-yl)palmitoylamide to react with bromine under conditions effective to yield 2-bromoethyl ((2*S*,3*R*,*E*)-3-hydroxy-2-palmitamidooctadec-4-en-1-yl)phosphorobromidate; and

c) allowing 2-bromoethyl ((2*S*,3*R*,*E*)-3-hydroxy-2-palmitamidooctadec-4-en-1-yl)phosphorobromidate to react with trimethylamine under conditions effective to yield *N*-palmitoyl-*D-erythro*-sphingomyelin.

17. The method of claim 16 wherein the ethylene halophosphite is ethylene chlorophosphite.

18. A method for synthesizing *N*-palmitoyl-*D*-*erythro*-dihydrosphingomyelin, comprising the steps of:

5 a) allowing *N*-palmitoyl-*D*-*erythro*-dihydrosphingosine to react with ethylene chlorophosphite under conditions effective to yield *N*-((2*S*,3*R*)-1-((1,3,2-dioxaphospholan-2-yl)oxy)-3-hydroxyoctadecan-2-yl)palmitamide;

b) allowing *N*-((2*S*,3*R*)-1-((1,3,2-dioxaphospholan-2-yl)oxy)-3-hydroxyoctadecan-2-yl)palmitamide to react with bromine under conditions effective to yield 2-bromoethyl ((2*S*,3*R*)-3-hydroxy-2-palmitamidooctadecyl)phosphorobromidate; and

10 c) allowing 2-bromoethyl ((2*S*,3*R*)-3-hydroxy-2-palmitamidooctadecyl)phosphorobromidate to react with trimethylamine under conditions effective to yield *N*-palmitoyl-*D*-*erythro*-dihydrosphingomyelin.

19. The method of claim 20 wherein the ethylene halophosphite is ethylene chlorophosphite.

20. A method for synthesizing an *N*-acyl-*D*-*erythro*-sphingomyelin, comprising the steps of:

15 a) allowing 3-O-benzoyl-*D*-*erythro*-ceramide to react with 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) to under conditions effective to yield 3-O-benzoyl-*D*-*erythro*-1-O-(2-oxo-1,3,2-dioxaphospholane)ceramide;

b) allowing 3-O-benzoyl-*D*-*erythro*-1-O-(2-oxo-1,3,2-dioxaphospholane)ceramide to react with trimethylamine under conditions effective to yield an *N*-acyl-3-O-benzoyl-*D*-*erythro*-sphingomyelin; and

20

c) removing the benzoyl group of the *N*-acyl-3-O-benzoyl-*D*-*erythro*-sphingomyelin under conditions effective to yield the *N*-acyl-*D*-*erythro*-sphingomyelin.

21. The method according to claim 20 wherein in the 3-O-benzoyl-*D*-*erythro*-ceramide is *N*-palmitoyl-3-O-benzoyl-*D*-*erythro*-sphingosine.

25

22. The method according to claim 1, further comprising, prior to step a:

1) allowing *D*-*erythro*-sphingosine to react with a fatty acid under conditions effective to yield *D*-*erythro*-ceramide;

2) allowing *D-erythro*-ceramide to react with a tritylating agent under conditions effective to yield *D-erythro*-1-O-trityl-ceramide;

3) allowing 1-O-trityl-*D-erythro*-ceramide to react with a benzoylating agent under conditions effective to yield 1-O-trityl-3-O-benzoyl-*D-erythro*-ceramide; and

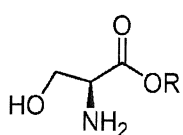
5 4) removing the trityl group of 1-O-trityl-3-O-benzoyl-*D-erythro*-ceramide under conditions effective to yield *D-erythro*-3-O-benzoyl-ceramide.

23. The method according to claim 23 wherein the fatty acid is palmitic acid.

10 24. The method according to claim 20 wherein the trimethylamine is added as a liquid.

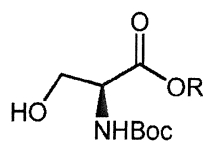
25. The method according to claim 22 wherein step 2) yields less than about 5% of 1,3-O,O-ditrityl-*D-erythro*-ceramide..

15 26. A compound having the structure:

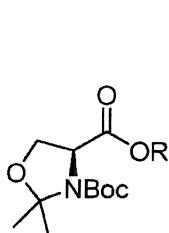


L-Serine Ester  
Compound Ia

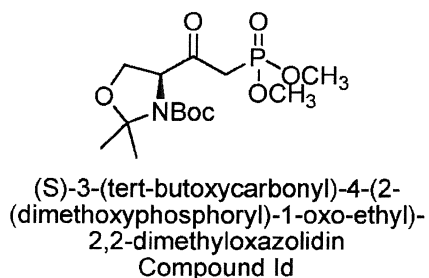
, or a salt thereof;



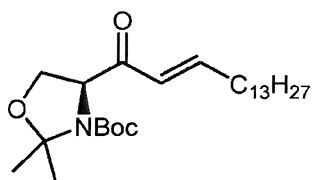
Boc-L-Ser-OR  
Compound Ib ;



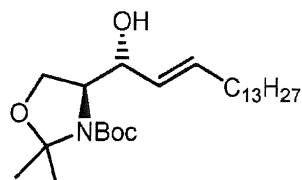
Compound Ic ;



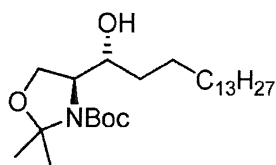
(S)-3-(tert-butoxycarbonyl)-4-(2-(dimethoxyphosphoryl)-1-oxo-ethyl)-  
2,2-dimethyloxazolidin  
Compound Id ;



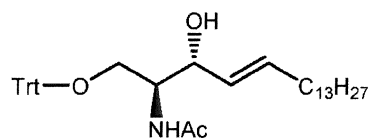
(S)-3-(tert-butoxycarbonyl)-4-(1-oxohexadec-2-enyl)-2,2-dimethyloxazolidin  
Compound 1e



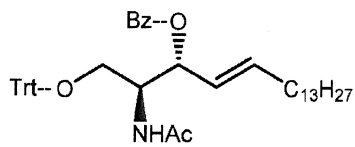
(2S,3R,4E)-3-(tert-butoxycarbonyl)-4-(1-hydroxyhexadec-2-enyl)-2,2-dimethyloxazolidin  
Compound 1f



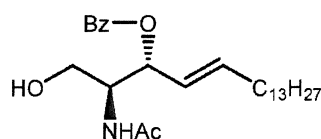
((S)-tert-butyl 4-((R)-1-hydroxyhexadecyl)-2,2-dimethyloxazolidine-3-carboxylate  
Compound 1la



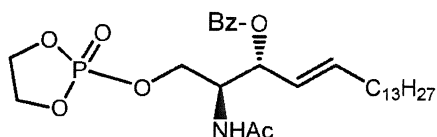
1-O-Trityl-D-erythro-ceramide  
Compound 1c-1



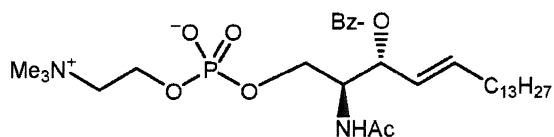
1-O-Benzoyl-3-O-Trityl-D-erythro-ceramide  
Compound 1d-1



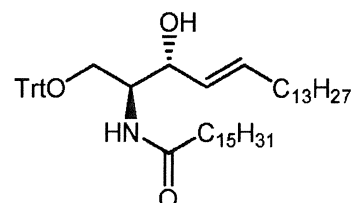
1-O-Benzoyl-D-erythro-ceramide  
Compound 1e-1



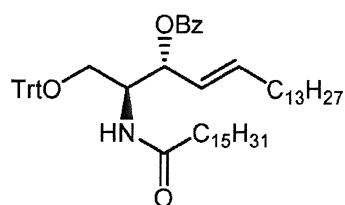
3-O-Benzoyl-D-erythro-1-O-(2-oxo-1,3,2-dioxaphospholan)-ceramide  
Compound 1f-1



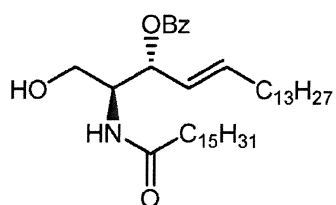
Compound 1g - Bz



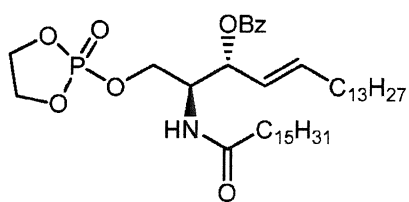
N-Palmitoyl-1-O-Trityl-D-erythro-sphingosine  
Compound 1h-1



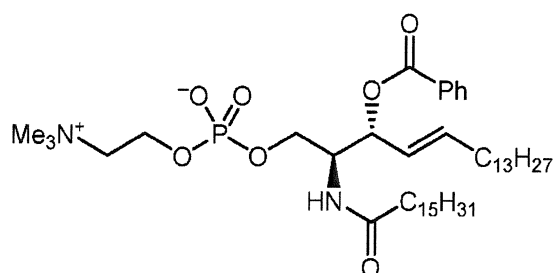
N-Palmitoyl-1-O-Trityl-3-O-Benzoyl-  
D-erythro-sphingosine  
Compound VIb ;



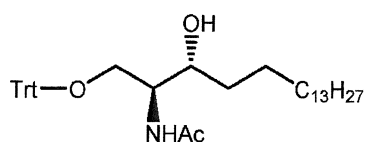
N-Palmitoyl-3-O-Benzoyl-  
D-erythro-sphingosine  
Compound VIc ;



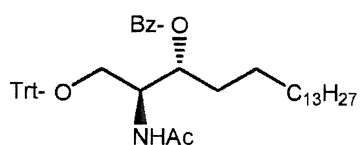
N-Palmitoyl 3-O-Benzoyl-  
D-erythro-1-O-(2-oxo-1,3,2-  
dioxaphospholan)-sphingosine  
Compound VIId ;



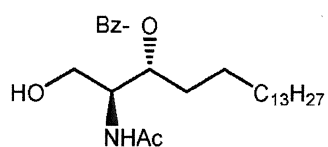
N-Palmitoyl 3-O-Benzoyl-D-erythro-  
Sphingomyelin  
Compound VIe ;



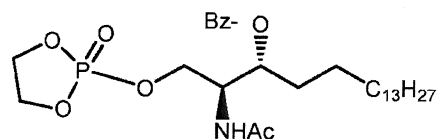
1-O-Trityl-D-erythro-dihydroceramide  
Compound VIIf -Trt ;



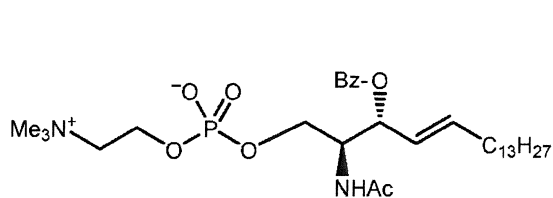
1-O-Trityl-3-O-Benzoyl-D-erythro-  
dihydroceramide  
Compound VIId ;



3-O-Benzoyl-D-erythro-dihydroceramide  
Compound VIIf ;

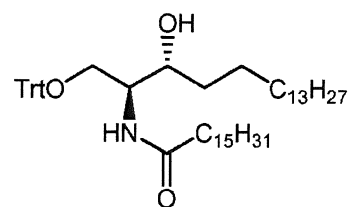


3-O-Benzoyl-D-erythro-1-O-  
(2-oxo-1,3,2-dioxaphospholan)-dihydroceramide  
Compound VIIf ;

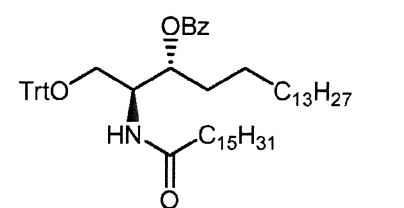


Compound VIIg

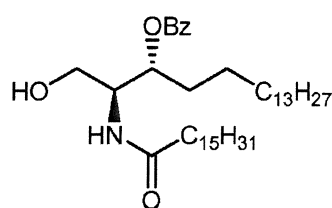
;

N-palmitoyl 1-O-Trityl-D-erythro-dihydrosphingosine  
Compound VIIla

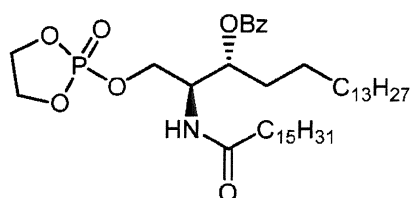
;

N-palmitoyl-1-O-Trityl-3-O-Benzoyl-D-erythro-dihydrosphingosine  
Compound VIIlb

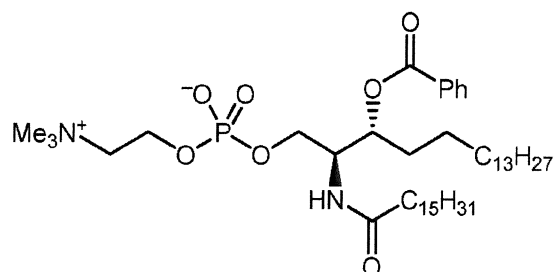
;

N-palmitoyl 3-O-Benzoyl-D-erythro-dihydrosphingosine  
Compound VIIlc

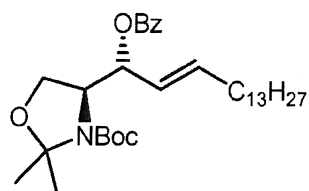
;

N-palmitoyl 3-O-Benzoyl-1-O-(2-oxo-1,3,2-dioxaphospholan)-D-erythro-dihydrosphingosine  
Compound VIIId

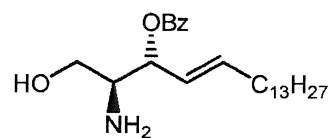
;

N-Palmitoyl-3-O-Benzoyl-D-erythro-dihydrosphingomyelin  
Compound VIIle

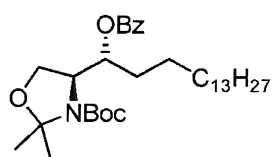
;



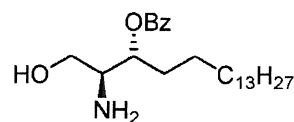
(2S,3R,4E)-3-(tert.-Butoxycarbonyl)-4-(1-benzoyl-hexadec-2-enyl)-2,2-dimethyloxazolidine  
Compound IXa ;



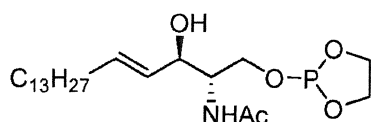
D-erythro-3-O-Benzoyl-Sphingosine  
Compound IXb ;



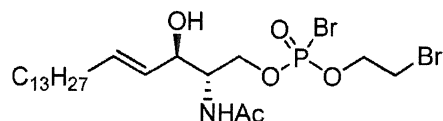
(S)-tert-butyl 4-((R)-1-(benzoyloxy)hexadecyl)-2,2-dimethyloxazolidine-3-carboxylate  
Compound XIa ;



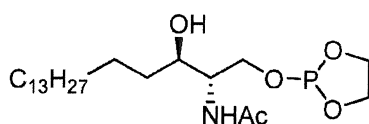
3-O-Benzoyl-  
D-erythro-dihydrosphingosine  
Compound XIIb



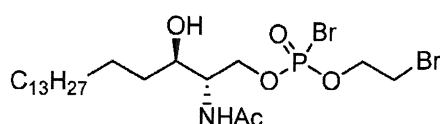
Compound XIIIa ;



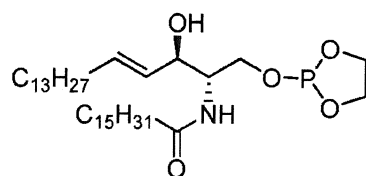
Compound XIIIb ;



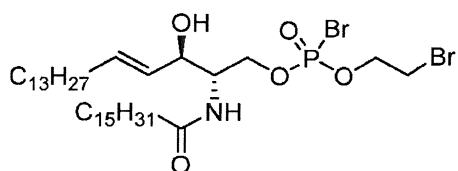
Compound XIVa ;



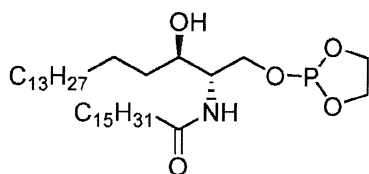
Compound XIVb ;



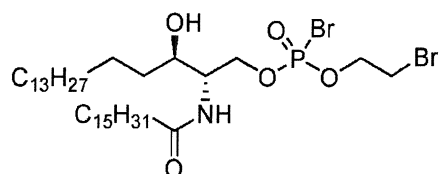
5 N-((2S,3R,E)-1-((1,3,2-dioxaphospholan-2-yl)oxy)-3-hydroxyoctadec-4-en-2-yl)palmitamide ,



2-bromoethyl ((2S,3R,E)-3-hydroxy-2-palmitamidooctadec-4-en-1-yl) phosphorobromidate ;



N-((2S,3R)-1-((1,3,2-dioxaphospholan-2-yl)oxy)-3-hydroxyoctadecan-2-yl)palmitamide



2-bromoethyl ((2S,3R)-3-hydroxy-2-palmitamidooctadecyl) phosphorobromidate ;

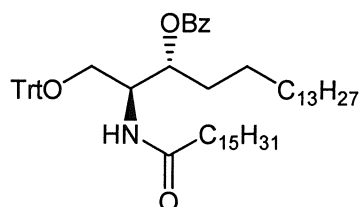
; or

where Ac is a fatty acid residue having 3 to 36 carbons and zero to six carbon-carbon double bonds; and

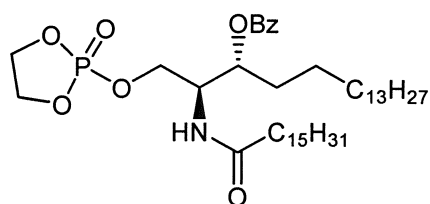
5 R is a C1-5 alkyl group.

## CLAIMS

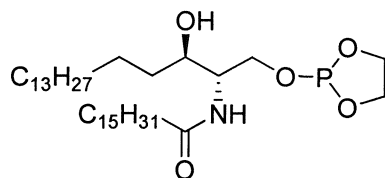
1. A compound having the structure:



2. A compound having the structure:



3. A compound having the structure:



4. A compound having the structure:

